# CSGI

## *Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase*

## *Report 2009*

# SUMMARY



## 1A: Hard Matter (Nanomaterials and Solid Interfaces)



## 1B: Soft Matter (Nanomaterials and Liquid Interfaces)

## 1C: Theory and Modelling



## 2A: Art Restoration, Environmental Remediation and Textile Chemistry



## 2B: Metals and Ceramics



## 2C: Biotechnology

# CSGI Research Activity

## *Outline*

CSGI (Research Center for Colloids and Nanoscience) was established in Firenze, in December 1993. It has been officially recognized by the Italian Government in 1994, and is under the supervision and control of the Italian Ministry for University and Scientific Research (MIUR). Since 1995 CSGI began its scientific activity, devoted to basic research and to the development of high-tech new processes, and is supporting the activities of the small and medium size business industrial companies, that cannot afford the financial costs of an independent research activity.

In the last 14 years, CSGI has sponsored several different research programs, mainly supported by European Union grants, and partly also by other international and national Institutions, such as the Italian "Articolo 10, Law 46/1982", PRIN, PNR, FISR, FIRB, CNR, and so forth.

CSGI has signed numerous contracts that involve about 75 national and international industrial companies, and some highly qualified research Centers, such as Procter & Gamble, Siemens, Tecnotessile SpA, Massachusetts Institute of Technology, Pharmacia-Upjon, Elf-Atochem, Ansaldo, Glaxo-Wellcome, Sintech, Inver, Cover, Tooling International Ltd, Industrial Materials Technology GmbH, MBN SpA, Inteti, Icmese, Comune di Firenze, VTT, etc. Such lively activity has brought to several International Patents and research agreements.

CSGI has reached a very qualified standard, and its level has been acknowledged abroad, in several fields. For example, CSGI is a leader in a number of applications of Nanotechnology, in the conservation of cultural heritage, and in the production of nanophasic powders (with MBN) for the production of special materials for aeronautics, high resistance coatings, etc. CSGI supports the local authorities for the safeguard and conservation of works or art ("Sovrintendenze Artistiche") in Tuscany and other Italian districts, with a set of technologies that have been developed for this aim. Similar actions have been promoted in agreement with the Mexican Federal Government for the conservation of monuments (Puebla Cathedral, Maya and Aztec heritage, the archaelogical site of Calakmul, Campeche).

CSGI is active also in the training of specialized researchers, has granted several fellowships, PhD supporting programs, post-doc grants, and other education projects, and has organized several national and international Meetings. In particular, during the year 2008, CSGI has issued 10 PhD scholarships, 42 fellowships, and 7 post-doc grants, and is actively participating in two European Master Programs: EMASCO-COSOM (European Master in Supramolecular and Colloidal Chemistry) and IMES (International Master on Bioenergy and Environment). CSGI has co-sponsored national and international congresses (International RILEM Symposium on Photocatalysis, Environment and Construction Materials and CSGI National Meeting).

The CSGI financial plan is solid, with a strong growth of its financial assets, mainly due to EU funding.
The main topics of CSGI research activity are:

1) development of processes for the production of nanophasic systems, for the production of innovative textiles, for the synthesis of nanophasic alloys, ceramics and nanophasic or nanostructured composites (low temperature and low energy costs)
2) setup of new additives for cement products. These projects are mainly carried out in collaboration with Italcementi and MIT, and are aimed at investigating and optimizing the cement hydration process and the production of new, ceramics-like materials for the cement-related industry
3) formulation of dispersions in fluids, emulsions and inverted emulsions (paints, adhesives, sealing materials, detergents, etc.)
4) development of systems for the confinement of proteins and for the controlled release of pharmaceutics
5) development of food-related industrial processes (for example the treatment of milk and milk derivatives in supercritical phase)
6) development of innovative procedures for the conservation and restoration of works of art (paintings, frescoes and stone-based materials). CSGI is a world leader in this research activity, and is involved in a significant campaign for the recover of archaelogical treasures in Mexico (Calakmul), in the largest Maya site, and with the Maritime Museum in Stockholm for the conservation of the Vasa ship. New methods are under development for the removal of polymer and grime from oil paintings (relining) in collaboration with the Louvre Museum (Paris). CSGI is currently involved in the conservation of Annunciation Grotto (Nazareth).

## *Fields of Interest*

- Nanostructured and ultrafine materials.
- Structure and dynamics of supramolecular assemblies (monolayers, micelles, liposomes, microemulsions, Langmuir-Blodgett films, host-guest systems).
- Nanophasic ternary oxides.
- Structural analysis of biomolecules in solution, interaction processes, recognition of ligands with macromolecular surfaces, theoretical and experimental analysis of cellular metabolism, interactions between metals and ligands, characterization of the interaction sites.
- Formulation of nanophasic systems.
- Innovative processes for the conservation and restoration of cultural heritage (stone materials, wood materials, paintings, frescoes, paper, photographic material).

## *Structure and Organization of CSGI*

Management Offices
President, Council, Director, Audit Council, Technical-Scientific Board.

Director of CSGI
Prof. Piero Baglioni, Department of Chemistry, University of Florence.

President of CSGI
Prof. Giovanni Marletta, Department of Chemical Sciences, University of Catania.

Website
http://www.csgi.unifi.it/

Foundation
December 21st, 1993

Official recognition by the Italian Government
November 15th, 1994 (G.U. Nr. 267)

## *Academic Units and Associated Centers*

University of Florence (headquarter)
University of Siena
University of Udine
University of Pavia
University of Cagliari
University of Molise (Campobasso)
University of Catania
University of Bergamo
University of Naples "Federico II"
University of Milan, Bicocca
Polytechnic Institute of Milan
University of Perugia
University of Rome, La Sapienza
University of Bologna

## Personnel

CSGI gathers about 300 researchers including Full Professors, Associate Professors, University Researchers, that belong to the academic members.
Moreover, CSGI employs 75 researchers and 3 administration employees on its own. Several PhD and post-doc students are financially supported through CSGI fellowships. CSGI hosts researchers hired by industrial companies for training and specific research activities, in the framework of particular projects.
CSGI owes two research Laboratories, located in Vascon di Carbonera (Nanophases Laboratory) and in Prato (Laboratory for the refinement and surface modification of textiles). These plants collaborate closely with the local industrial activities.

## Current Academic Collaborations

ANU (Australian National University)
Argonne National Laboratory
Aston University (Birmingham)
Brookhaven National Laboratory
California Institute of Technology (CalTech)
Centro di Istochimica del CNR di Pavia
Collège de France
Columbia University
CNIC (Cuba)
CSIC (Sevilla)
East China Normal University (Shangai)
École Normale Supérieure (Lion)
Escuela Superior Politecnica del Chimborazo
ETH (Zurich)
Hahn-Meitner Institut (Berlin)
Hull University
Inst. Science des Matériaux
Institut Laser Technology
Inst. Nat. Polytechnique de Lorraine (Nancy)
Inst. Scientific Instruments (Czech Rep.)
ITER
Laboratoire Leon Brillouin (Saclay)
Lehstul Fertigungstechnologie
Massachusetts Institute of Technology
Max Planck Institut (Berlin)
Museum of Fine Arts (Boston)
Nuclear Research Institute (Prague)
Oak Ridge National Laboratory
Oklahoma State University
Risø National Laboratory
Technical University di Budapest
Tekniska Hogskolan I Luleå
The Getty Conservation Institute
Università degli Studi Bari
Università degli Studi Camerino
Università degli Studi Chieti
Università degli Studi della Calabria
Università degli Studi di Parma
Università degli Studi Genova
Università degli Studi Padova
Università degli Studi Palermo
Università degli Studi Pisa
Università degli Studi Salerno
Università degli Studi Torino
Università degli Studi Trento
Università degli Studi Urbino
Universitad de Santiago de Compostela
Universitad del Salvador
Universitad Estadual de Campinas
Universität Gesamthoschule Kassel
Universität Heidelberg
Université de Bourgogne
Université de Grenoble
Université “Louis Pasteur” (Strasbourg)
Université de Montpellier II
University College (London)
University of Berkeley
University of Bristol
University of Cambridge
University of Detroit
University of East Anglia
University of Houston
University of Leiden
University of Lund
University of South Florida
University of York
Weizmann Institute (Israel)

# *Previous and Current Industrial Partners*

Alcea
Alfa Test
Alfa Wasserman
Ansaldo
Aprilia
Ascor chimici
Ausimont
Bigagli
Biokimica S.p.A
Bioscreen Technology srl
BTG-Holland
Bitossi
Comune di Firenze
Consorzio delle Buone Idee
Chemia
Cover
D'Appolonia
Dynamotive
Elf-Atochem
ENEA (Energy Department – Casaccia)
Eniricerche
Enitecnologie
EUBIA (Bruxelles)
Flory's
Getty Conservation Institute
Glaxo-Wellcome
Icmese
INASCO-Hellas (Int. Aerosp. Sci. Corp.)
Industrial Materials Technology GmbH
Industrie Casearie Podda
Ineti
Institute for the Care of Hystorical Monuments (Prague)
International Broker
Inver
IRBM
Italcementi S.p.A.
Italfarmaco
Lamberti S.p.A.
Lima
JRC (Joint Research Centre of the European Commission)
Mapei
Mariplast
Martelli S.p.A.
MBN Nanomaterialia
Merk
Microtec (Germany)
National Museum of Denmark
Nicox
Novuspharma Omrod Diesel (UK)
Philips
Pharmacia-Upjon (USA and Sweden)
Pharmaness
Procter & Gamble
Rifiniture BP
Sem
Siemens AG
S.I.F.I.
Sintech
SIR Industriale
Sirio Panel
Solvay
Soprintendenze ai Beni Artistici e Storici di: FI-PO-PT, SI-GR, PI-LU-MS
Soprintendenze ai Beni Ambientali ed Architettonici di:
Arezzo
Firenze-Prato-Pistoia
Roma
Veneto Orientale
Lombardia
Reggio Calabria
Tecnotessile SpA
TIL (Tooling International Ltd UK)
TNO (Netherlands)
3M
Transfergomma (Padova)
WIP (Germany)
VTT (Finland).

# CSGI owned Patents

1) Baglioni Piero, Dei Luigi, Ferroni Enzo, Giorgi Rodorico – "Sospensioni stabili di idrossido di calcio". Italian Patent FI/96/A000255 deposit date 31/10/1996.

2) Matteazzi Paolo, Baglioni Piero, Basset Diego - "Process for Recycling, by Milling, Solid Industrial Waste and Materials at the end of their Service Life" European Patent Application 97203735.2, Priority IT96 FI96A000280.

3) Grassi Giuliano, Chiaramonti David, Baglioni Piero – "Apparato a combustione di etanolo o miscele etanolo per cucine, stufe e illuminazione a uso domestico". Italian Patent FI/98/A42 deposit date 24/ 02/ 98.

4) Ambrosone Luigi, Ceglie Andrea – "Software per l'analisi grafica e numerica di dati di Risonanza Magnetica Nucleare per la determinazione della polidispersità di emulsioni". Italian Patent FI99A000044 deposit date 09/03/1999.

5) Baglioni Piero, Fratini Emiliano, Ricceri Riccardo, Sarti Giuseppe, Chiaramonti David – "Engine fuels consisting of an emulsion comprising mineral and/or natural oils, their preparation and use in internal combustion engine". PCT International Application WO n. 99936473.0 del 02.07.99

6) Baglioni Piero, Carretti Emiliano, Dei Luigi – "Microemulsioni ed emulsioni di olio in acqua, loro uso per la solubilizzazione di resine polimeriche e impacchi contenenti detti microemulsioni o emulsioni". Italian Patent FI99A000071 deposit date 02/ 04/ 99

7) Baglioni Piero, Bardi Ugo, Bonini Massimo -New method for the production of solid powder and films by compartimentalised solution thermal spraying (CSTS). European Patent Application EP 00-105673.8 deposit date 17.03.2000

8) Baglioni Piero, Dei Luigi, Giorgi Rodorigo, Claudio Vinicius Schettino – "Basic Suspensions their Preparation and Use in Processes for Paper Deacidification", European Patent Application EP 02714088.8 deposit date 15/01/02

9) Baglioni Piero, Dei Luigi, Fratoni Laura, Lo Nostro Pierandrea, Moroni Michelangelo - "Processo per la preparazione di nano e microparticelle di ossidi e idrossidi di metalli del secondo gruppo e di transizione, nano e microparticelle così ottenute e loro impiego in campo ceramico, tessile e cartario", Patent Query N. FI2002A000052, deposit date 28/03/2002 – EP 03745367.7

10) Baglioni Piero, Dei Luigi, Fratoni Laura, Lo Nostro Pierandrea, Moroni Michelangelo – "Preparation of nano- and micro-particles of group II and transition metals oxides and hydroxides and their use in the ceramic, textile and paper industries". *PCT Int. Appl.* (2003), 10 pp. CODEN: PIXXD2 WO 2003082742 A2 20031009 CAN 139:278604 AN 2003:796605

11) Baglioni Piero, Dei Luigi, Giorgi Rodorico, Ninham Barry W. – "Process for preparing nano- and micro-sized particles of inorganic compounds" European Patent Application EP 04101822.7, deposit date 29/04/2004.

12) Angelico Ruggero, Ceglie Andrea, Hochoeppler Alejandro, Palazzo Gerardo, Stefan Alessandra – "Macroemulsioni acqua-in olio a lunga stabilità, loro preparazione ed uso" – Patent Query N. FI2001A000016 deposit date 29/01/01.

13) Ambrosone Luigi, Ceglie Andrea – "Materiale assorbente e suoi usi nei processi di bonifica di falde acquifere inquinate da prodotti chimici" – Patent Query FI2003A000236 deposit date 11/09/03.

14) Ambrosone Luigi, Ceglie Andrea – "Gel stabili contenenti gelatina" – Patent Query N. FI2003A000237 deposit date 11/09/03.

15) Fratoni Laura, Lo Nostro Pierandrea – "Composizione detergente a base di un estere dell'acido L-ascorbico" – Patent Query N. TO2003A001032 deposit date 22/12/03.

16) Baglioni Piero, Ambrosi Moira, Dei Luigi, Faneschi Mauro, Mancioli Luciano, Santoni Sergio – "Ceramic products comprising nanoparticles of zirconium hydroxide and/or glass frits" – RIF. Patent Query 7303 PTEP/2006 EP06112439.2 deposit date 10/04/06

17) Ceglie Andrea, Venditti Francesco, Lopez Francesco, Palazzo Gerardo, Colafemmina Giuseppe, Angelico Ruggero, Ambrosone Luigi – "Materiale adsorbente contenente tensioattivo cationico, sua preparazione ed uso per la rimozione di metalli da soluzioni acquose" – Patent Query N. FI 2006 A000113 – RIF. 7490 PTIT deposit date 10/05/2006

18) Ambrosi Moira, Baglioni Piero, Bonini Massimo, Fratini Emiliano – "Nanoparticelle monodisperse di ossidi ed idrossidi metallici e loro applicazione nei settori tessile, cartario e ceramico" – Patent Query FI 2006A000313 – RIF. 7845 PTIT deposit date 11/12/06.

## *CSGI Registered Trade Marks*

Nanorestore® International Class 01,37,40 FI2008C00067527508 RIF. 19558

## *Prospective CSGI Activity in 2009-2011*

CSGI is involved in 7 European programs, in several national projects, and in collaborations with small and medium size industrial companies.
CSGI has activated a triennial program with CNR on "Functionalities in surfaces and at interfaces (FUSINT)".
CSGI is developing its own research activity in order to optimize the application of research projects inspired by the urging demands of small and medium size companies.
CSGI is actively working in order to offer a valid support to the Italian industrial system in the setting up and development of projects and pre-industrial processes.

# List of Publications 2007-2009


1. Fluorescence spectroscopy of synthetic melanin in solution. Perna, G., Frassanito, M.C., Palazzo, G., Gallone, A., Mallardi, A., Biagi, P.F., Capozzi, V. *Journal of Luminescence* 129, 44–49 (2009).
2. Hofmeister Effects in enzymatic activity, colloid stability and pH measurements: ion dependent specificity of intermolecular forces. Salis, A., Monduzzi, M., Ninham, B.W., In Nanoscience and Nanotechnology. Ed S. Bellucci, Springer. In Press.
3. Nanoparticles of calcium hydroxide for wood deacidification: decreasing the emissions of organic acid vapors in church organ environments, Giorgi, R., Baglioni, P., Chelazzi, D., Fratini, E., Langer, S., Niklasson, A., Rådemar, M., Svensson, J.E., *Journal of Cultural Heritage* (2008). Accepted.
4. Biodiesel from microalgae in Microbiology of Hydrocarbons, oils, lipids and derived compounds Salis, A., Nicolò M., Guglielmino, S., Solinas, V. Ed. Timmis, K., Springer. Accepted.
5. Soft condensed matter for the conservation of cultural heritage. Baglioni, P., Giorgi, R., Dei, L. *C. R. Chimie* In Press.
6. Recent advances in swollen-state NMR spectroscopy in the study of drying oil. Cipriani, G., Salvini, A., Dei, L., Macherelli, A., Cecchi, F. S., Giannelli, C. *Journal of Cultural Heritage* In press.
7. Bacterial community analysis on the antique stained glass window "Natività" of Florence Cathedral. Marvasi M., Vedovato E., Balsamo C., Macherelli A., Dei L., Mastromei G., Perito B. *Journal of Cultural Heritage* In press.
8. Nanocontainer aqueous systems for removing polymeric materials from marble surfaces: a new and promising tool in cultural heritage conservation. Grassi, S., Favaro, M., Tomasin P., Dei, L. *Journal of Cultural Heritage* In press.
9. A new class of gels for the conservation of painted surfaces. Carretti, E., Dei, L., Weiss, R. G., Baglioni, P. *Journal of Cultural Heritage* In press.
10. Closed Nanoconstructs Assembled by step-by-step ss-DNA Coupling Assisted by Phospholipid Membranes. Betti, F., Baldelli Bombelli, F., Gambinossi, F., Caminati, G., Brown, T., Baglioni, P., Berti, D. *Soft Matter*. In press.
11. Intercalation of Single Strand Oligonucleotides between Nucleolipid Anionic Membranes: A Neutron Diffraction Study. Milani, S., Berti, D., Dante, S., Hauβ, T., Baglioni, P. *Langmuir*. In press.
12. Mg – Ni – Cu mixtures for hydrogen storage: phase analysis and kinetic sorption performances. Milanese, C., Girella, A., Bruni, G., Cofrancesco, P., Berbenni, V., Marini, A., Villa, M., Matteazzi, P. *International Journal of Hydrogen Energy*. In press.
13. Morandi, S., Focardi, S., Nocentini, M., Puggelli, M., Caminati G., Development and Validation of Europium-Sensitized Luminescence (ESL) Method for the Determination of Tetracycline Residues in Milk *Food Anal. Methods* In press.
14. Gambinossi, F., Viitala, T., Caminati, G. Step-wise formation of supported Lipid Bilayers on gold substrates studied by Dissipative Quartz Crystal Microbalance and Imaging Ellipsometry, *Langmuir*. Submitted.
15. F. Gambinossi, T. Viitala and G. Caminati, "Step-by-Step formation of hybrid LbL/LB nanodevices followed by QCM with dissipation monitoring and ellispometric mapping" *J. Phys. Chem*. Submitted.
16. Numerical simulation of dielectric spectra of aqueous suspensions of non-spheroidal differently shaped biological cells. Di Biasio, A., Ambrosone, L., Cametti, C. *Journal of Physics D*, iop.org/JPhysD/41. In press.
17. Soft condensed matter for the conservation of cultural heritage, Baglioni, P., Giorgi, R., Dei, L., *Compte Rendus Chimie*, 2009, XX, 1-9. In press.
18. Identification of provenance of obsidian samples analyzing elemental composition by INAA. Seccaroni, C., Volante, N., Rosada, A., Ambrosone, L., Bufalo, G., Avino, A. *Journal of Radioanalytical and Nuclear Chemistry*. DOI: 10.1007/s10967-088-0502. In press.
19. Structural Studies In Solution And In The Solid State On The Zinc Chelate Of 2-Hydroxy-(4-Methylthio) Butanoic Acid, An Effective Mineral Supplement In Animal Feeding. Predieri, G., Feltrami, D., Pattacini, R., Parisi, M.L., Sinicropi, A., Valensin, D., Basosi, R., *Inorg. Chim. Acta* (2008) doi: 10.1016/J.Ica.2008.05.027.
20. Effect of headgroup chirality in nanoassemblies. Part 1. Self-assembly of D-isoascorbic acid

derivatives in water. Ambrosi, M., Lo Nostro, P., Fratini, E., Giustini L., Ninham, B. W., Baglioni, P. *Journal of Physical Chemistry B*. In press.

21. Quenching Efficiency of Pyrene Fluorescence by Nucleotide Monophosphates in Cationic Micelles. Cuomo, F., Palazzo, G., Ceglie, A., Lopez F. *J. Photochem. Photobiol. A*, DOI: 10.1016/j.jphotochem.2008.10.028. In press.
22. Reaction Mixtures Based on the CTAB - Dodecyl Epoxide – Water Microemulsion for the Synthesis of Novel Nucleo-Lipids. Angelico, R., Ceglie, A., Cuomo, F. *Colloids and Surfaces B*. In press.
23. Nucleolipid Membranes: Structure and Molecular Recognition. Milani, S., Baldelli Bombelli, F., Berti, D., Dante, S., Hauβ T., Baglioni P. *J. Phys.: Condens. Matter* 20, 104212 (2008).
24. Cloning, polymorphism, and inhibition of beta-carbonic anhydrase of Helicobacter pylori. Morishita, S., Nishimori, I., Minakuchi, T., Onishi, S., Takeuchi, H., Sugiura, T., Vullo, D., Scozzafava, A., Supuran, C. T. *Journal of Gastroenterology* 43, 849-857 (2008).
25. Carbonic anhydrase inhibitors. Inhibition of the beta-class enzyme from the yeast Saccharomyces cerevisiae with anions. Isik, S., Kockar, F., Arslan, O., Guler, O. O., Innocenti, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 6327-6331 (2008).
26. Carbonic anhydrase inhibitors: 2-Substituted-1,3,4-thiadiazole-5-sulfamides act as powerful and selective inhibitors of the mitochondrial isozymes VA and VB over the cytosolic and membrane-associated carbonic anhydrases I, II and IV. Smaine, F. Z., Pacchiano, F., Rami, M., Barragan-Montero, V., Vullo, D., Scozzafava, A., Winum, J. Y., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 6332-6335 (2008).
27. Carbonic anhydrase activators: Activation of the archaeal beta-class (Cab) and gamma-class (Cam) carbonic anhydrases with amino acids and amines. Innocenti, A., Zimmerman, S. A., Scozzafava, A., Ferry, J. G., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 6194-6198 (2008).
28. Carbonic anhydrase inhibitors: Inhibition of Plasmodium falciparum carbonic anhydrase with aromatic/heterocyclic sulfonamides-in vitro and in vivo studies. Krungkrai, J., Krungkrai, S. R., Supuran, C. T. Bioorganic & Medicinal Chemistry Letters 18, 5466-5471 (2008).
29. In vitro inhibition of salicylic acid derivatives on human cytosolic carbonic anhydrase isozymes I and II. Bayram, E., Senturk, M., Kufrevioglu, O. I., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 9101-9105 (2008).
30. Carbonic anhydrase inhibitors: Synthesis and inhibition studies against mammalian isoforms I-XV with a series of 2-(hydrazinocarbonyl)-3-substituted-phenyl-1H-indole-5-sulfonamides. Guzel, O., Innocenti, A., Scozzafava, A., Salman, A., Parkkila, S., Hilvo, M., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 9113-9120 (2008).
31. Biochemical characterization of CA IX, one of the most active carbonic anhydrase isozymes. Hilvo, M., Baranauskiene, L., Salzano, A. M., Scaloni, A., Matulis, D., Innocenti, A., Scozzafava, A., Monti, S. M., Di Fiore, A., De Simone, G., Lindfors, M., Janis, J., Valjakka, J., Pastorekova, S., Pastorek, J., Kulomaa, M. S., Nordlund, H. R., Supuran, C. T., Parkkila, S. *Journal of Biological Chemistry* 283, 27799-27809 (2008).
32. External pH influences the transcriptional profile of the carbonic anhydrase, CAH-4b in Caenorhabditis elegans. Hall, R. A., Vullo, D., Innocenti, A., Scozzafava, A., Supuran, C. T., Klappa, P., Muhlschlegel, F. A. *Molecular and Biochemical Parasitology* 161, 140-149 (2008).
33. Carbonic anhydrase inhibitors: Inhibition of the beta-class enzymes from the fungal pathogens Candida albicans and Cryptococcus neoformans with simple anions. Innocenti, A., Muhlschlegel, F. A., Hall, R. A., Steegborn, C., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 5066-5070 (2008).
34. Carbonic anhydrase activators: Kinetic and X-ray crystallographic study for the interaction of D- and L-tryptophan with the mammalian isoforms I-XIV. Temperini, C., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 8373-8378 (2008).
35. Carbonic anhydrase in the scleractinian coral Stylophora pistillata - Characterization, localization, and role in biomineralization. Moya, A., Tambutte, S., Bertucci, A., Tambutte, E., Lotto, S., Vullo, D., Supuran, C. T., Allemand, D., Zoccola, D. *Journal of Biological Chemistry* 283, 25475-25484 (2008).
36. Anticancer steroid sulfatase inhibitors: synthesis of a potent fluorinated second-generation agent, in vitro and in vivo activities, molecular modeling, and protein crystallography. Woo, L. W. L., Fischer, D. S., Sharland, C. M., Trusselle, M., Foster, P. A., Chander, S. K., Di Fiore, A., Supuran, C. T., De Simone, G., Purohit, A., Reed, M. J., Potter, B. V. L. *Molecular Cancer Therapeutics* 7, 2435-2444 (2008).

37. Muscarinic acetylcholine receptors as therapeutic targets for obesity. Maresca, A., Supuran, C. T. *Expert Opinion on Therapeutic Targets* 12, 1167-1175 (2008).
38. Inhibition of human mitochondrial carbonic anhydrases VA and VB with para-(4-phenyltriazole-1-yl)-benzenesulfonamide derivatives. Poulsen, S. A., Wilkinson, B. L., Innocenti, A., Vullo, D., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 4624-4627 (2008).
39. Carbonic anhydrase inhibitors: Inhibition of mammalian isoforms I-XIV with a series of substituted phenols including paracetamol and salicylic acid. Innocenti, A., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 7424-7428 (2008).
40. Carbonic anhydrase inhibitors. Interaction of the antitumor sulfamate EMD 486019 with twelve mammalian carbonic anhydrase isoforms: Kinetic and X-ray crystallographic studies. Temperini, C., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 4282-4286 (2008).
41. Carbonic anhydrase activators: Activation of the human cytosolic isozyme III and membrane-associated isoform IV with amino acids and amines. Vullo, D., Nishimori, I., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 4303-4307 (2008).
42. Tumor-associated carbonic anhydrase 9 spatially coordinates intracellular pH in three-dimensional multicellular growths. Swietach, P., Wigfield, S., Cobden, P., Supuran, C. T., Harris, A. L., Vaughan-Jones, R. D. *Journal of Biological Chemistry* 283, 20473-20483 (2008).
43. Carbonic anhydrase inhibitors. Sulfonamide diuretics revisited - old leads for new applications. Temperini, C., Cecchi, A., Scozzafava, A., Supuran, C. T. *Organic & Biomolecular Chemistry* 6, 2499-2506 (2008).
44. Carbonic anhydrase inhibitors: Thioxolone versus sulfonamides for obtaining isozyme-selective inhibitors? Innocenti, A., Maresca, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 3938-3941 (2008).
45. Carbonic anhydrase inhibitors as emerging drugs for the treatment of obesity. Supuran, C. T., Di Fiore, A., De Simone, G. *Expert Opinion on Emerging Drugs* 13, 383-392 (2008).
46. Recent developments of carbonic anhydrase inhibitors as potential anticancer drugs. Thiry, A., Supuran, C. T., Masereel, B., Dogne, J. M. *Journal of Medicinal Chemistry* 51, 3051-3056 (2008).
47. Carbonic anhydrase inhibitors: Bioreductive nitro-containing Sulfonamides with selectivity for targeting the tumor associated isoforms IX and XII. D'Ambrosio, K., Vitale, R. M., Dogne, J. M., Masereel, B., Innocenti, A., Scozzafava, A., De Simone, G., Supuran, C. T. *Journal of Medicinal Chemistry* 51, 3230-3237 (2008).
48. Carbonic anhydrase inhibitors: Design of spin-labeled sulfonamides incorporating TEMPO moieties as probes for cytosolic or transmembrane isozymes. Cecchi, A., Ciani, L., Winum, J. Y., Montero, J. L., Scozzafava, A., Ristori, S., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 3475-3480 (2008).
49. Carbonic anhydrase inhibitors: Inhibition of the new membrane-associated isoform XV with phenols. Innocenti, A., Hilvo, M., Scozzafava, A., Parkkila, S., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 3593-3596 (2008).
50. Structure and biological properties of first row d-transition metal complexes with NI-substituted sulfonamides. Chohan, Z. H., Supuran, C. T. *Journal of Enzyme Inhibition and Medicinal Chemistry* 23, 240-251 (2008).
51. Carbonic anhydrase IX: A new druggable target for the design of antitumor agents. Winum, J. Y., Rami, M., Scozzafava, A., Montero, J. L., Supuran, C. *Medicinal Research Reviews* 28, 445-463 (2008).
52. Carbonic anhydrase inhibitors. Interaction of indapamide and related diuretics with 12 mammalian isozymes and X-ray crystallographic studies for the indapamide-isozyme II adduct. Temperini, C., Cecchi, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 2567-2573 (2008).
53. Carbonic anhydrase inhibitors: The X-ray crystal structure of ethoxzolamide complexed to human isoform II reveals the importance of thr200 and gln92 for obtaining tight-binding inhibitors. Di Fiore, A., Pedone, C., Antel, J., Waldeck, H., Witte, A., Wurl, M., Scozzafava, A., Supuran, C. T., De Simone, G. *Bioorganic & Medicinal Chemistry Letters* 18, 2669-2674 (2008).
54. Carbonic anhydrase activators: Activation of the human tumor-associated isozymes IX and XII with amino acids and amines. Pastorekova, S., Vullo, D., Nishimori, I., Scozzafava, A., Pastorek, J., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 3530-3536 (2008).

55. Carbonic anhydrase inhibitors: Inhibition of human cytosolic isozymes I and II and tumor-associated isozymes IX and XII with S-substituted 4-chloro-2-mercapto-5-methyl-benzenesulfonamides. Saczewski, F., Innocenti, A., Slawinski, J., Kornicka, A., Brzozowski, Z., Pomarnacka, E., Scozzafava, A., Temperini, C., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 16, 3933-3940 (2008).
56. Cancer-associated, hypoxia-inducible carbonic anhydrase IX facilitates CO2 diffusion. Swietach, P., Wigfield, S., Supuran, C. T., Harris, A. L., Vaughan-Jones, R. D. *Bju International* 101, 22-24 (2008).
57. Development of small molecule carbonic anhydrase IX inhibitors. Supuran, C. T. *Bju International* 101, 39-40 (2008).
58. Investigations of the esterase, phosphatase, and sulfatase activities of the cytosolic mammalian carbonic anhydrase isoforms I, II, and XIII with 4-nitrophenyl esters as substrates. Innocenti, A., Scozzafava, A., Parkkila, S., Puccetti, L., De Simone, G., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 18, 2267-2271 (2008).
59. Supramolecular Mimetic Peroxidase Based On Hemin And Pamam Dendrimers. Castriciano, M.A., Romeo, A., Baratto, M. C., Pogni, R., Monsù S. *Chemical Communications*, 6, 688-690, (2008).
60. Structural characterization of Di-$C_{12}$P-Uridine wormlike micelles: ionic strength dependence. Baldelli Bombelli, F., Betti F., Berti, D., Pini, F., Heinrich, M., Baglioni, P. *J. Phys.: Condens. Matter* 20, 104213 (2008).
61. DNA Self-Assembled Ring Nanostructures: A Structural and Monte Carlo Simulation Study. Baldelli Bombelli, F., Gambinossi, F., Lagi, M., Berti D., Caminati G., Brown, T., Sciortino, F., Nordén, B., Baglioni, P. *J. Phys. Chem. B* 112, 48, 15283–15294 (2008).
62. Caratterizzazione chimico fisica non invasiva di due ferrotipi inglesi del XIX secolo, Carretti, E., Milano, M., Poggi, G., Giorgi, R., Dei, L., Baglioni, P., *Atti del VI Congresso Nazionale IGIIC - Lo Stato dell'Arte* - Spoleto, 2-4 Ottobre 2008, Nardini editore, 77-82 (2008).
63. Deacidificazione di documenti cartacei mediante dispersioni non acquose di nanoparticelle alcaline, Giorgi, R., Poggi, G., Baglioni, P., *Atti del VI Congresso Nazionale IGIIC - Lo Stato dell'Arte* - Spoleto, 2-4 Ottobre 2008, Nardini editore, 69-76 (2008).
64. G-Protein Binding Features And Regulation Of The Ralgds Family Member Rgl2. Ferro E., Magrini, D., Guazzi, P., Fischer, T. H., Pistolesi S., Pogni, R., White Ii, G. C., Trabalzini, L., *J. Biochem.* 415(1), 145-154, (2008).
65. Phospholipid membranes decorated by cholesterol-based oligonucleotides as soft hybrid nanostructures. Banchelli, M., Betti, F., Berti, D., Caminati, G., Baldelli Bombelli, F., Brown, T., Wilhelmsson, L. M., Nordén, B., Baglioni, P. *J. Phys. Chem. B* 112, 35, 10942–10952 (2008).
66. Collective Headgroup Conformational Transition in Twisted Micellar Superstructures. Baldelli Bombelli, F., Berti, D., Milani, S., Lagi, M., Barbaro, P., Karlsson, G., Brandt, A., Baglioni, P. *Soft Matter* 4, 1102–1113 (2008).
67. The triazine ring as a scaffold for the synthesis of new organogelators. Cicchi, S., Ghini G., Fallani S., Brandi, A., Berti, D., Betti, F., Baglioni, P. *Tetrahedron Letters* 49, 1701–1705 (2008).
68. Rimozione selettiva di mowilith DM5 da pitture maya di epoca post-classica mediante sistemi micellari, Giorgi, R., Chelazzi, D., Carretti, E., Baglioni, M., Solis, C.G., Castro Barrera, M.C., Arroyo, M.C., Baglioni, P., *Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metrodi, Compatibilità, Cantieri"*, Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 208-218 (2008).
69. Eliminazione di residui di colle usate per la rintelatura di dipinti mediante sistemi chimici detergenti, Pizzorusso, G., Giorgi, R., Chelazzi, D., Carretti, E., Arroyo, M.C., Chevalier, A., Menu, M., Baglioni, P., *Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metodi, Compatibilità, Cantieri"*, Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 517-526 (2008).
70. Nanotecnologie per il recupero di materiali lapidei e pitture murali: la Casa di Maria nella chiesa dell'Annunciazione di Nazareth, Baglioni, P., Giorgi, R., Arroyo, M.C., Pratesi, G., Malesani, P., *Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metrodi, Compatibilità, Cantieri"*, Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 163-172 (2008).
71. Rimozione di polimeri acrilici da pitture murali mediante sistemi detergenti e gel chimici magnetici, Baglioni, M., Giorgi, R., Chelazzi, D., Carretti, E., Falletta, E., Pizzorusso, G., Baglioni, P., *Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i*

*Restauri – Metrodi, Compatibilità, Cantieri"*, Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 477-484 (2008).

72. Microemulsions for the cleaning of wall paintings, Giorgi, R., Chelazzi, D., Carretti, E., Falletta, E., Baglioni, P., *Proceedings of the 15th Triennal Conference ICOM Committee for Conservation*, New Delhi, September 22-26, 2008, vol.I, 527-533 (2008).
73. Extraction d'adhésifs de rentoilage en peinture de chevalet: nouvelle approche, Chevalier, A., Chelazzi, D., Baglioni, P., Giorgi, R., Carretti, E., Stuke, M., Menu, M., Duchamp, R., *Proceedings of the 15th Triennal Conference ICOM Committee for Conservation*, New Delhi, September 22-26, 2008, vol.II, 581-589 (2008).
74. Clusters of Poly(acrylates) and Silver Nanoparticles: Structure and Applications for Antimicrobial Fabrics. Falletta, E., Bonini, M., Fratini, E., Lo Nostro, A., Pesavento G., Becheri, A., Lo Nostro, P., Canton, P., Baglioni, P. *Journal of Physical Chemistry C* 112, 11758-11766 (2008).
75. Hydrogen storage in magnesium–metal mixtures: Reversibility, kinetic aspects and phase analysis. Milanese, C., Girella, A., Bruni, G., Berbenni, V., Cofrancesco, P., Marini, A., Villa, M., Matteazzi, P. *Journal of Alloys and Compounds* 465, 396-405 (2008).
76. Reactivity and Hydrogen Storage Performances of Magnesium – Nickel – Copper Ternary Mixtures prepared by reactive mechanical grinding. Milanese, C., Girella, A., Bruni, G., Cofrancesco, P., Berbenni, V., Villa, M., Matteazzi, P., Marini, A. *International Journal of Hydrogen Energy* 33, 4593-4606 (2008).
77. Acrylamide-Based Magnetic Nanosponges: A New Smart Nanocomposite Material. Bonini, M., Lenz, S., Falletta, E., Ridi, F., Carretti, E., Fratini, E., Wiedenmann, A., Baglioni, P. *Langmuir* 24, 12644-12650 (2008).
78. Catanionic Systems from Conversion of Nucleotides into Nucleo-Lipids. Angelico, R., Ceglie, A., Cuomo, F., Cardellicchio, C., Mascolo, G., Colafemmina, G. *Langmuir* 24, 2348 -2355 (2008).
79. Basak, A.K., Achanta, S., Celis, J.P., Vardavoulias, M., Matteazzi, P. Structure and mechanical properties of plasma sprayed nanostructured alumina and FeCuAl-alumina cermet coatings, *Surface & Coatings Technology* 202, 2368- 2373 (2008).
80. Physico-chemical characterization and conservation issues of photographs dated between 1890 and 1910, Cattaneo, B., Chelazzi, D., Giorgi, R., Serena, T., Merlo, C., Baglioni, P., *Journal of Cultural Heritage,* 9, 277-284 (2008).
81. Caratterizzazione chimico-fisica di positivi fotografici del periodo 1890-1910: degrado e conservazione, Cattaneo, B., Chelazzi, D., Giorgi, R., Serena, T., Merlo, C., Baglioni, P., *Atti del V Congresso Nazionale IGIIC - Lo Stato dell'Arte* - Cremona, 4-6 Ottobre 2007, Nardini editore, 573-582 (2008).
82. Does thermophoretic mobility depend on particle size? Braibanti, M., Vigolo, D., Piazza, R. *Physical Review Letters* 100, 108303 (2008).
83. Dynamic Crossover Phenomenon in Confined Supercooled Water and its Relation to the Existence of a Liquid-Liquid Critical Point in Water. Chen, S. H., Mallamace, F., Liu, L., Chu, X., Zhang, Y., Faraone, A., Fratini, E., Baglioni, P. *AIP Conference Proceedings* 982, 39-52 (2008).
84. Interaction between Alzheimer's Aß(25–35) peptide and phospholipid bilayers: The role of cholesterol. D'Errico, G., Vitiello, G., Ortona, O., Tedeschi, A. M., Ramunno, A., D'Ursi, A.M. *Biochimica et Biophysica Acta-Biomembranes* 1778 (12), 2710-2716 (2008).
85. Interaction of a Peptide Derived from Glycoprotein gp36 of Feline Immunodeficiency Virus and Its Lipoylated Analogue with Phospholipid Membranes. D'Errico, G., D'Ursi, A.M., Marsh, D. *Biochemistry* 47 (19), 5317-5327 (2008).
86. Interconnected networks: Structural and dynamic characterization of aqueous dispersions of dioctanoylphosphatidylcholine. Lo Nostro, P., Murgia, S., Lagi, M., Fratini, E., Karlsson, G., Almgren, M., Monduzzi, M., Ninham, B. W., Baglioni, P. *Journal of Physical Chemistry B* 112, 12625-12634 (2008).
87. Micelles by self-assembling peptide-conjugate amphiphile: synthesis and structural characterization. Accardo, A., Tesauro, D., Del Pozzo, L., Mangiapia, G., Paduano, L. *Journal of peptide science* 14 (8), 903-910 (2008).
88. Mutual and intradiffusion coefficients for the binary system n-decyl dimethyl phosphine oxide + water at 25C. D'Errico, G., Mangiapia, G., Ortona, O. *Journal of Chemical and Engineering Data* 53 (7), 1651-1654 (2008).
89. Nucleotides and nucleolipids derivatives interaction effects during multi-lamellar vesicles formation. Cuomo, F., Lopez, F., Angelico, R., Colafemmina, G., Ceglie, A. *Colloids and*

*Surfaces B* 64, 184-193 (2008).

90. Observation of a dynamic crossover in RNA hydration water which triggers a dynamic transition in the biopolymer. Chu, X., Fratini, E., Baglioni, P., Faraone, A., Chen, S. H. *Physical Review E* 77, 011908 (2008).
91. Observation of dynamic crossover and dynamic heterogeneity in hydration water confined in aged cement paste. Zhang, Y., Lagi, M., Ridi, F., Fratini, E., Baglioni, P., Mamontov, E., Chen, S. H. *Journal of Physics: Condensed Matter* 20, 502101 (2008).
92. Peptide-containing aggregates as selective nanocarriers for therapeutics. Accardo, A., Tesauro, D., Aloj, L., Tarallo, C., Arra, C., Mangiapia, G., Vaccaro, M., Pedone, C., Paduano, L., Morelli, G. *ChemMedChem* 3 (4), 594-602 (2008).
93. Polymerized mixed aggregates containing gadolinium complex and CCK8 peptide. Vaccaro, M., Mangiapia, G., Accardo, A., Tesauro, D., Gianolio, E., Frielinghaus, H., Morelli, G., Paduano, L. *Colloid & Polymer Science* 286 (14-15), 1643-1652 (2008).
94. Protein-Matrix Coupling/Uncoupling in "Dry" Systems of Photosynthetic Reaction Center Embedded in Trehalose/Sucrose: The Origin of Trehalose Peculiarity. Francia, F., Dezi, M., Mallardi, A., Palazzo, G., Cordone, L., Venturoli, G. *Journal of American Chemical Society* 130, 10240-10246 (2008).
95. Quenching and Dequenching of Pyrene Fluorescence by Nucleotide Monophosphates in Cationic Micelles. Lopez, F., Cuomo, F., Ceglie, A., Ambrosone, L., Palazzo, G. *Journal of Physical Chemistry B* 112, 7338–7344 (2008).
96. Structural and Mechanical Properties of UV-Photo-Cross-Linked Poly(N-vinyl-2-pyrrolidone) Hydrogels. D'Errico, G., De Lellis, M., Mangiapia, G., Tedeschi, A. M., Ortona, O., Fusco, S., Borzacchiello, A., Ambrosio, L. *Biomacromolecules* 9(1), 231-240 (2008).
97. Structures and Micelle Locations of the Nonlipidated and Lipidated C-Terminal Membrane Anchor of 2',3'-Cyclic Nucleotide-3'-phosphodiesterase. Esposito, C., Scrima, M., Carotenuto, A., Tedeschi, A. M., Rovero, P., D'Errico, G., Malfitano, A.M., Bifulco, M., D'Ursi, A.M. *Biochemistry* 47 (1), 308-319 (2008).
98. Studies of phononlike low-energy excitations of protein molecules by inelastic X-ray scattering. Chu, X., Lagi, M., Zhang, Y., Fratini, E., Baglioni, P., Alatas, A., Said, A., Alp, E., Chen, S. H. *Physical Review Letters* 101, 135501 (2008).
99. The aggregative behavior of hydrophobically modified chitosans with high substitution degree in aqueous solution. Ortona, O., D'Errico, G., Mangiapia, G., Ciccarelli, D. *Carbohydrate Polymers* 74 (1), 16-22 (2008).
100. The $Fe^{2+}$ site of photosynthetic reaction centers probed by multiple scattering x-ray absorption fine structure spectroscopy: Improving structure resolution in dry matrices. Veronesi, G., Giachini, L., Francia, F., Mallardi, A., Palazzo, G., Boscherini, F., Venturoli, G. *Biophysical Journal* 95, 814-822 (2008).
101. Thermophoresis in colloidal suspensions. Piazza, R., Parola, A. *Journal of Physics: Condensed Matter* 20, 153102 (2008).
102. Threading, Growth, and Aggregation of Pseudopolyrotaxanes. Lo Nostro, P., Giustini L., Fratini, E., Ninham, B. W., Ridi, F., Baglioni, P. *J. Phys. Chem. B* 112, 1071-1081 (2008).
103. Tetra-2,3-pyrazinoporphyrazines with externally appended pyridine rings. 6. Chemical and redox properties and highly effective photosensitizing activity for singlet oxygen production of penta- and monopalladated complexes in dimethylformamide solution. Donzello, M., Viola, E., Bergami, C., Dini, D., Ercolani, C., Giustini, M., Kadish, K.M., Meneghetti, M., Monacelli, F., Rosa, A., Ricciardi, G. *Inorganic Chemistry* 47, 8757-8766 (2008).
104. Circular dichroism of polyncleotides: Interactions of $NiCl_2$ with poly(dA-dT)center dot poly(dA-dT) and poly(dG-dC)center dot poly(dG-dC) in a water-in-oil microemulsion. Airoldi, M.; Gennaro, G., Giomini, M., Giuliani, A. M., Giustini, M. *Chirality* 20, 951-960 (2008).
105. Use of chitosan for chromium removal from exhausted tanning baths. Cesaro, R., Fabbricino, M., Lanzetta, R., Mancino, A., Naviglio, B., Parrilli, M., Sartorio, R., Tomaselli, M., Tortora, G. *Water Science & Technology* 58 (3), 735-739 (2008).
106. Water Activity Regulates the QA- to QB Electron Transfer in Photosynthetic Reaction Centers from Rhodobacter sphaeroides. Palazzo, G., Francia, F., Mallardi, A., Giustini, M., Lopez, F., Venturoli, G. *Journal of American Chemical Society* 130, 9353–9363 (2008).
107. An Artificial Molecular Switch that Mimics the Visual Pigment and Completes its Photocycle in Picoseconds. Sinicropi, A., Martin, E, Helbing, J., Briand, J., Sharma, D., Leonard, J., Haacke, S., Canizzo, A., Chergui, M., Zanirato, V., Fusi, S., Santoro, F., Basosi, R., Ferré, N., Olivucci, M. *PNAS* in press (2008).

108. Interaction of Quercetin and its conjugate Quercetin 3-b-D-glucoside with Albumin Determined by NMR Relaxation Data. Martini, S., Bonechi, C., Rossi, C. *Journal Natural Products* 71, 175-178 (2008).
109. Recent Applications of a QM/MM scheme at the CASPT2//CASSCF/AMBER (or CHARMM) level of theory in Photochemistry and Photobiology. Sinicropi, A., Basosi, R., Olivucci, M. *Journal of Physics: Conference Series* 101, 1-11 (2008).
110. Stacking interaction study of resveratrol (trans-3,5,4'-trihydroxystilbene) in solution by Nuclear Magnetic Resonance and Fourier Transform Infrared Spectroscopy. Bonechi, C., Martini, S., Magnani, A., Rossi, C. *Magnetic Resonance in Chemistry* 46, 625-629 (2008).
111. The fluorescence of tryptophane in monellin and parvalbunin resolved at the ab-initio multiconfigurational perturbation theory level. Sinicropi, A., Pistolesi, S., Pogni, R., Basosi, R., Ferré, N., Olivucci, M. *Journal of the American Chemical Society* in press (2008).
112. Variability in factors causing light attenuation in Lake Victoria. Loiselle, S., Azza, N., Cozar, A., Bracchini, L., Dattilo, A. M., Rossi, C., Tognazzi, A. *Freshwater Biology* 53, 535-545 (2008).
113. A Thermodynamic and material flow analysis of the italian highway and railway transport systems. Federici, M., Ulgiati, Basosi, R. *Energy* 33, 760-775 (2008).
114. Air versus terrestrial transport modalities: an environmental comparison. Federici, M., Basosi, R. *Advances in Energy Studies* in press (2008).
115. Energy conservation policy: should public administration give the good example? Ruzzenenti, F., Pagni, M., Federici, M., Bruni, C., Basosi, R. *Advances in Energy Studies* in press (2008).
116. Energy efficiency and structural change in production: an analysis of long-term impacts in the road freight transport sector. Ruzzenenti, F., Federici, M., Basosi, R. *Advances in Energy Studies* in press (2008).
117. Environmental and economical advatanges of the local scale: a pilot biodiesel production line in the Province of Siena, Italy. Federici, M., Basile, Pogni, R., Basosi, R. in *First European Workshop on biotechnology for lignocellulose biorefinerie* , 47 (2008).
118. On the relationship between energy efficiency and complexity: insight on the causality chain. Ruzzenenti, F., Basosi, R. *International Journal of Design & Nature and Ecodynamics* in press (2008).
119. Satellite based indices in the analysis of land cover for municipalities in the Province of Siena, Italy. Focardi, S., Loiselle, S., Mazzuoli, S., Bracchini, L., Dattilo, A. M., Rossi, C. *Journal of Environmental Management* 86, 383-389 (2008).
120. Synthesis and Characterization of Zinc Oxide Nanoparticles. Application to Textiles as UV-Absorbers. Becheri, A., Dürr, M., Lo Nostro, P., Baglioni, P. *Journal of Nanoparticle Research* 10, 679-689 (2008).
121. The Rebound Effect: an evolutionary perspective. Ruzzenenti, F., Basosi, R. *Ecological Economics* (2008).
122. The role of the power/efficiency misconception in the rebound effect's size debate: Does efficiency actually lead to a power enhancement?". Ruzzenenti, F., Basosi, R. *Energy Policy* 36(9), 3626-3632 (2008).
123. Cations distribution and valence states in Mn-substituted $Li_4Ti_5O_{12}$ structure. Capsoni, D., Bini, M., Massarotti, V., Mustarelli, P., Chiodelli, G., Azzoni, C. B., Mozzati, M. C., Linati, L., Ferrari, S. *Chemistry of Materials* 20 [13], 4291-4298 (2008).
124. Solid state formation of calcium ferrites from thermal decomposition of mixtures $Ca_3(C_6H_5O_7)_2{\cdot}4H_2O$ - $Fe_2(C_2O_4)_3{\cdot}\ 6H_2O$. Berbenni, V., Marini, A., Bruni, G., Milanese, C. *Journal of Analytical and Applied Pyrolysis* 82 [2], 255-259 (2008).
125. The combined effect of mechanical and thermal energy on the solid-state formation of $NiFe_2O_4$ from the system $2NiCO_3{\cdot}3Ni(OH)_2{\cdot}4H_2O$–$FeC_2O_4{\cdot}2H_2O$. Berbenni, V., Milanese, C., Bruni, G., Marini, A. *Thermochimica Acta* 469 [1-2], 86-90 (2008).
126. The Effect of Mechanical Activation on the Synthesis of $MgFe_2O_4$ from Mixtures of $MgCO_3{\cdot}Mg(OH)_2{\cdot}\ xH_2O$ and $FeC_2O_4{\cdot}\ 2H_2O$. Berbenni, V., Marini, A., Milanese, C., Bruni, G. *Zeitschrift Fur Naturforschung Section B. A Journal of Chemical Sciences* 63B, 1052-1056 (2008).
127. Antioxidant dispersions in emulsified olive oils. Mosca, M., Ceglie, A., Ambrosone, L. *Food Research International* 41, 201-207 (2008).
128. Artificial antisense RNAs silence lacZ in Escherichia coli by decreasing traget mRNA concentration. Stefan, A., Tonelli, A., Schwarz, F., Hochkoeppler, A. *Biochemistry and Molecular Biology Reports* 41, 568-574 (2008).
129. Biocompatible water-in-oil emulsion as a model to study ascorbic acid effect on lipid

oxidation. Mosca, M., Ceglie, A., Ambrosone, L. *Journal of Physical Chemistry B* 112, 4635-4641 (2008).

130. Characterization of radical intermediates in the laccase-mediator systems. A multifrequency EPR, ENDOR, and DFT/PCM investigation. Brogioni, B., Biglino, D., Sinicropi, A., Reijerse, E. J., Giardina, P., Sannia, G., Lubitz, W., Basosi, R., Pogni, R. *Physical Chemistry Chemical Physics* (2008).
131. An Artificial Molecular Switch That Mimics The Visual Pigment And Completes Its Photocycle In Picoseconds. Sinicropi, A., Martin, E., Ryasantsev, R., Helbing, J., Briand, J., Sharma, D., Leonard, J., Haacke, S., Canizzo, A., Chergui, M., Zanirato, V., Fusi, S., Santoro, F., Basosi, R., Ferrè, N., Olivucci, M., *Proc. Natl. Acad. Sci. USA* 105, 17642-17647, (2008).
132. Gambinossi, F., Lorenzelli, L., Baglioni, P., Caminati, G., Silicon oxide surface functionalization by self-assembled nanolayers for microcantilever transducers, *Colloids and Surfaces A: Physicochem. Eng. Aspects* 321, 87–93 (2008).
133. Morandi, S., Puggelli, M., Caminati, G., Antibiotic association with phospholipid nano-assemblies: A comparison between Langmuir–Blodgett films and supported lipid bilayers *Colloids and Surfaces A: Physicochem. Eng. Aspects* 321, 125–130 (2008).
134. Comparison among immobilised lipases on macroporous polypropylene toward biodiesel synthesis. Salis, A., Pinna, M., Monduzzi, M., Solinas, V. *Journal of Molecular Catalysis B: Enzymatic* 54, 19-26 (2008).
135. Role of the support surface on the loading and the activity of Pseudomonas fluorescens lipase used for biodiesel synthesis. Salis, A., M.S. Bhattacharyya, Monduzzi, M., Solinas, V. *Journal of Molecular Catalysis B: Enzymatic* (2008) [in press] (doi:10.1016/j.molcatb.2008.09.015).
136. Selenite transiently represses transcription of photosynthesis-related genes in potato leaves. Poggi, V., Del Vescovo, V., Di Sanza, C., Negri, R., Hochkoeppler, A. *Photosynthesis Research* 95, 63-71 (2008).
137. Characterization Of Radical Intermediates In The Laccase-Mediator Systems. A Multifrequency Epr, Endor, And Dft/Pcm Investigation. Brogioni, B., Biglino, D., Sinicropi, A., Reijerse, E.J., Giardina, P., Sannia, G., Lubitz, W., Basosi, R., Pogni, R., *PCCP*, 10, 7284-7292, (2008).
138. Specific Anion Effects On Enzymatic Activity in Non Aqueous Media. Bilanicova, D., Salis, A., Ninham, B. W., Monduzzi, M. *Journal of Physical Chemistry B* 112, 12066-12072 (2008).
139. The Rebound Effect: An Evolutionary Perspective, Ruzzenenti, F., Basosi, R., *Ecological Economics* 526-537, (2008).
140. Enzyme, protein, carbohydrate, and phenolic contaminants in commercial tyrosinase preparations: potential problems affecting tyrosinase activity and inhibition studies. Flurkey, A., Cooksey, J., Reddy, A., Spoonmore, K., Rescigno, A., Inlow, J., Flurkey, W.H., *Journal of Agricultural and Food Chemistry* 56, 4760-4768 (2008).
141. Degradation of Alizarin Red S under mild experimental conditions by immobilized 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine-Mn(III) as a biomimetic peroxidase-like catalyst. Zucca, P., Vinci, C., Sollai, F., Rescigno, A., Sanjust, E., *Journal of Molecular Catalysis A: Chemical* 288, 97-102 (2008).
142. On The Relationship Between Energy Efficiency And Complexity: Insight On The Causality Chain, Ruzzenenti, F., Basosi, R., *Int. J. Design Nature Ecodynamics* 3, 95-108 (2008).
143. Spectroscopic investigation on hydrophobic and hydrophilic fractions of dissolved organic matter extracted from soils at different salinities. Provenzano, M. R., Cilenti, A., Gigliotti, G., Senesi, N. *Clean-Soil Air Water* 36, 748-753 (2008).
144. Mediterranean shrubs as potential antioxidant sources. Sanjust, E., Mocci, G., Zucca, P., Rescigno, A., *Natural Product Research*, 22(8), 689-708 (2008).
145. Structural studies in solution and in the solid state on the zinc chelate of 2-hydroxy-(4-methylthio) butanoic acid, an effective mineral supplement in animal feeding. Predieri, G., Beltrami, Pattacini, Parisi, M. L. , Sinicropi, A., Valensin, Basosi, R. *Inorg. Chim. Acta* , 1-22 (2008).
146. Umbelliferone and esculetin: inhibitors or substrates for polyphenol oxidases? Sollai, F., Zucca, P., Sanjust, E., Steri, D., Rescigno, A., *Biol. Pharm. Bull.* 31(12), 2187-2193 (2008).
147. The olive oil oxygen radical absorbance capacity (DPPH assay) as a quality indicator. Zullo B.A., Ciafardini, G. *European Journal of Lipid Science and Technology* 110, 428-434 (2008).
148. Interaction of Quercetin and its conjugate Quercetin 3-b-D-glucoside with Albumin Determined by NMR Relaxation Data. Martini, S., Bonechi, C., Rossi, C. *Journal of natural products* 71(2), 175-178 (2008).

149. Stacking Stacking interaction study of resveratrol *(trans*-3,5,4'-trihydroxystilbene) in solution by Nuclear Magnetic Resonance and Fourier Transform Infrared Spectroscopy. Bonechi, C., S. Martini, S., Magnani, A., Rossi, C. *Magnetic Resonance Chemistry* 46(7), 625-629 (2008).
150. Satellite based indices in the analysis of land cover for municipalities in the Province of Siena, Italy, Focardi, S., Loiselle, S., Mazzuoli, S., Bracchini, L., Dattilo, A. M., Rossi, C. *Journal of Environmental Management* 86, 383-389 (2008).
151. Variability in factors causing light attenuation in Lake Victoria. Loiselle, S., Azza, N., Cozar, A., Bracchini L., Dattilo, A. M., Rossi, C., Tognazzi A. *Freshwater Biology* 53, 535-545 (2008).
152. Anionic Polymerization of a Benzofulvene Monomer Leading to a Thermoreversible π - Stacked Polymer. Studies in Macromolecular and Aggregate Structure. Cappelli, A., Galeazzi, S., Giuliani, G., Anzini, M., Aggravi, M., Donati, A., Zetta, L., Boccia, A., Mendichi, R., Giorgi, G., Paccagnini, E., Vomero, S. *Macromolecules* 41, 2324-2334 (2008).
153. TOF-SIMS investigation of FIB patterning of Lactoferrin using Self-Assembled Monolayers of Iron Complexes. Tuccitto, N., Giamblanco, N., Marletta, G., Licciardello, A. *Appl. Surf. Sci.* 255, 1075-1078 (2008).
154. Confined Protein Adsorption into Nanopore Arrays Fabricated Colloidal-Assisted Polymer Patterning. Satriano, C., Messina, M.G., Marletta, G. *Chem. Comm.* 40, 5031-5033 (2008).
155. Enhancement of fibroblastic proliferation on chitosan surfaces immobilized epidermal growth factor. Karakecili, A.G., Cristina, S., Gumusderelioglu, M., Marletta, G. *Acta Biomaterialia* 4, 989-96 (2008).
156. A theoretical and experimental study of self-assembling polysaccharide forming nanochannels: static and dynamic effects induced a *soft*-confinement. Bocchinfuso, G., Palleschi, A., Mazzuca, C., Coviello, T., Alhaique, F., Marletta, G. *J. Phys. Chem. B* 112, 6473-6483 (2008).
157. Self-Organizing Models of Bacterial Aggregation States. Caratozzolo, M., Carnazza, S., Fortuna, L., Frasca, M., Guglielmino, S., Gurrieri, G., Marletta, G. *Math. Biosci. Eng.* 5, 75-83 (2008).
158. Oxygen Plasma Induced Conversion of Polysiloxane into Hydrophilic and Smooth $SiO_x$ Surfaces. Satriano, C., Marletta, G., Kasemo, B. *Surf. Interf. Anal.* 40, 649-656 (2008).
159. UV-O3-treated and protein-coated polymer surfaces facilitate endhotelial cell adhesion and proliferation mediated the PKCα/ERK/cPLA2 pathway. Formosa, F., Anfuso, C.D., Satriano, C., Lupo, G., Giurdanella, G., Ragusa, N., Marletta, G., Alberghina, M. *Microvasc. Res.* 75, 330-342 (2008).
160. Thermoresponsive and bioactive poly(vinyl-ether)-based hydrogels synthesized radiation copolymerization and photochemical immobilization. Karakecili, A.G., Satriano, C., Gumusderelioglu, M., Marletta, G. *Radiat. Phys. Chem.* 77, 154-161 (2008).
161. Zirconium phosphate/phosphonate multilayered films based on push-pull stilbazolium salt: synthesis, characterization and second harmonic generation. Morotti, T., Calabrese, V., Cavazzini, M., Pedron, D., Cozzuol, M., Licciardello, A., Tuccitto, N., Quici, S., *Dalton Trans.* 22, 2974-2982 (2008).
162. Pulsed radiofrequency glow discharge time of flight mass spectrometer for the direct analysis of bulk and thin coated glasses. Muñiz, A.C., Pisonero, J., Lobo, L., Gonzalez, C., Bordel, N., Pereiro, N., Tempez, N., Chapon, P., Tuccitto, N., Licciardello, A., Sanz-Medel, A. *JAAS* 9, 1239-1246 (2008).
163. Monoatomic and Cluster beam effect on ToF-SIMS Spectra of Self-Assembled Monolayers on Gold. Tuccitto, N., Torrisi, V., Delfanti, I., Licciardello, A. *Appl. Surf. Sci.* 255, 874-876 (2008).
164. ToF-SIMS characterization of fluoroalkyl derivatives of possible interest as water repellent for stone protection. Torrisi, A., Tuccitto, N., Maccarrone, G., Licciardello, A. *Appl. Surf. Sci.* 255, 1527-1530 (2008).
165. Soft-Landed Protein Voltammetry: A Tool for Redox Protein Characterization. Mazzei, F., Favero, G., Frasconi, M., Tata, A., Tuccitto, N., Licciardello, A., Pepi, F. *Anal. Chem.* 80, 5937–5944 (2008).
166. Nano-and microstructured polymer LB layers: a combined AFM/SIMS study. Torrisi, V., Tuccitto, N., Delfanti, I., Audinot, J.N., Zhavnerko, G., Migeon, H.N., Licciardello, A. *Appl. Surf. Sci.* 255, 1006-1010 (2008).
167. Covalent surface modification of titanium oxide with different adhesive peptides: Surface characterization and osteoblast-like cell adhesion. Dettin, M., Bagno, A., Gambaretto, R., Iucci, G., Conconi, M.T., Tuccitto, N., Menti, A.M., Grandi, C., Di Bello, C., Licciardello, A., Polzonetti, G. *J. Biomed. Mat. Res. A* (2008). doi:10.1002/JBM.A.32064.

168. Radiofrequency Glow Discharge Time Of Flight Mass Spectrometry For Thin Films Analysis: Pulse Mode Versus Continuous Mode. Bordel, N., Muñiz, A. C., Pisonero, J., Lobo, L., Gonzalez, C., Pereiro, R., Tempez, A., Chapon, P., Tuccitto, N., Licciardello, A. Sanz-Medel, A. in: *Metallurgical Analysis*. 11/04/2007. (Vol. 2, 2008, pages 1182-1189). Beijing: (China).
169. Nanostructured morphologies of complexes containing porphyrin bridges between Pt(II) acetylide tethers. Amato, M.E., Licciardello, A., Torrisi, V., Ugo, L., Venditti, I., Russo, M.V. *Mat. Sci. Eng. C*. (2009) In press
170. Highly Conductive Extra-Long Molecular Wires. Tuccitto, N. Ferri, N., Cavazzini, M., Quici, S., Zhavnerko, G., Licciardello, A., Rampi, M.A. *Nature Materials*. (2008) doi:10.1038/nmat2332.
171. Micro-Contact-Printing ($\mu$CP) Langmuir-Blodgett Strategies for Surface Patterning in Biotechnology. Zhavnerko, G., Marletta, G. *Mat. Sci. Eng. C* (2009). In press.
172. Molecular Dynamics Simulation of Surface-Driven Primary Self-organisation Events of EAK 16 peptides. Forte, G., Grassi, A., Marletta, G. *J. Phys. Chem. B* (2008) in press.
173. Ion Beam Modification of Polymer Surfaces for Biological Applications. Marletta, G. in *Materials Science with Ion Beams*, Ed. H.Bernas, Springer-Verlag, Heidelberg (Germany), 2008, in press.
174. The greenhouse gas balance of the Province of Siena. Ridolfi, R., Kneller, M., Donati, A., Pulselli, R.M. *Journal of Environmental Management* 86, 365-372 (2008).
175. Homology modeling and receptor-based 3D-QSAR study of carbonic anhydrase IX. Tuccinardi, T., Ortore, G., Rossello, A., Supuran, C. T., Martinelli, A. *Journal of Chemical Information and Modeling* 47, 2253-2262 (2007).
176. Design, synthesis, and docking studies of new 1,3,4-thiadiazole-2thione derivatives with carbonic anhydrase inhibitory activity. Abdel-Hamid, M. K., Abdel-Hafez, A. A., El-Koussi, N. A., Mahfouz, N. M., Innocenti, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 6975-6984 (2007).
177. Saccharin inhibits carbonic anhydrases: Possible explanation for its unpleasant metallic aftertaste. Kohler, K., Hillebrecht, A., Wischeler, J. S., Innocenti, A., Heine, A., Supuran, C. T., Klebe, G. *Angewandte Chemie-International Edition* 46, 7697-7699 (2007).
178. Carbonic anhydrase inhibitors: The inhibition profiles of the human mitochondrial isoforms VA and VB with anions are very different. Nishimori, I., Innocenti, A., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 6742-6747 (2007).
179. QSAR studies on the activation of the human carbonic anhydrase cytosolic isoforms I and II and secretory isozyme VI with amino acids and amines. Singh, J., Shaik, B., Singh, S., Sikhima, S., Agrawal, V. K., Khadikar, P. V., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 6501-6509 (2007).
180. Reconstitution of carbonic anhydrase activity of the cell-surface-binding protein of vaccinia virus. Ohradanova, A., Vullo, D., Kopacek, J., Temperini, C., Betakova, T., Pastorekova, S., Pastorek, J., Supuran, C. T. *Biochemical Journal* 407, 61-67 (2007).
181. Exploration of the binding mode of indanesulfonamides as selective inhibitors of human carbonic anhydrase type VII by targeting Lys 91. Thiry, A., Masereel, B., Dogne, J. M., Supuran, C. T., Wouters, J., Michaux, C. *Chemmedchem* 2, 1273-1280 (2007).
182. Inhibition of carbonic anhydrase isozymes I, II and IX with benzenesulfonamides containing an organometallic moiety. Salmon, A. J., Williams, M. L., Innocenti, A., Vullo, D., Supuran, C. T., Poulsen, S. A. *Bioorganic & Medicinal Chemistry Letters* 17, 5032-5035 (2007).
183. Carbonic anhydrase inhibitors. Inhibition of cytosolic isoforms I and II, and extracellular isoforms IV, IX, and XII with sulfamides incorporating sugar moieties. Colinas, P. A., Bravo, R. D., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 5086-5090 (2007).
184. Carbonic anhydrase inhibitors: Selective inhibition of the extracellular, tumor-associated isoforms IX and XII over isozymes I and II with glycosyl-thioureido-sulfonamides. Smaine, F. Z., Winum, J. Y., Montero, J. L., Regainia, Z., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 5096-5100 (2007).
185. Carbonic anhydrase activators: Activation of the human isoforms VII (cytosolic) and XIV (transmembrane) with amino acids and amines. Vullo, D., Innocenti, A., Nishimori, I., Scozzafava, A., Kaila, K., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 4107-4112 (2007).
186. Carbonic anhydrase inhibitors: Inhibition of human, bacterial, and archaeal isozymes with benzene-1,3-disulfonamides - Solution and crystallographic studies. Alterio, V., De Simone,

G., Monti, S. M., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 4201-4207 (2007).

187. Carbonic anhydrase inhibitors. Interaction of the antiepileptic drug sulthiame with twelve mammalian isoforms: Kinetic and X-ray crystallographic studies. Temperini, C., Innocenti, A., Mastrolorenzo, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 4866-4872 (2007).
188. Novel targets against Helicoblacter pylori: a bioinformatic approach. Supuran, C. T. *Future Microbiology* 2, 111-114 (2007).
189. An update in the development of HIV entry inhibitors. Rusconi, S., Scozzafava, A., Mastrolorenzo, A., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 1273-1289 (2007).
190. Imaging the hypoxia surrogate marker CA IX requires expression and catalytic activity for binding fluorescent sulfonamide inhibitors. Dubois, L., Douma, K., Supuran, C. T., Chiu, R. K., van Zandvoort, M., Pastorekova, S., Scozzafava, A., Wouters, B. G., Lambin, P. *Radiotherapy and Oncology* 83, 367-373 (2007).
191. Synthesis and antimalarial activity of novel chiral and achiral benzenesulfonamides bearing 1,3,4-oxadiazole moieties. Zareef, M., Iqbal, R., De Dominguez, N. G., Rodrigues, J., Zaidi, J. H., Arfan, M., Supuran, C. T. *Journal of Enzyme Inhibition and Medicinal Chemistry* 22, 301-308 (2007).
192. QSAR studies for the inhibition of the transmembrane isozymes XII and XIV of human carbonic anhydrase with a series of sulfonamides. Tarko, L., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 5666-5671 (2007).
193. Carbonic anhydrase inhibitors: The beta-carbonic anhydrase from Helicobacter pylori is a new target for sulfonamide and sulfamate inhibitors. Nishimori, I., Minakuchi, T., Kohsaki, T., Onishi, S., Takeuchi, H., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 3585-3594 (2007).
194. Carbonic anhydrase activators: The first activation study of the human secretory isoform VI with amino acids and amines. Nishimori, I., Onishi, S., Vullo, D., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 5351-5357 (2007).
195. Carbonic anhydrases as targets for medicinal chemistry. Supuran, C. T., Scozzafava, A. *Bioorganic & Medicinal Chemistry* 15, 4336-4350 (2007).
196. Carbonic anhydrase inhibitors. Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 823-824 (2007).
197. Carbonic Anhydrases as drug targets - An overview. Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 825-833 (2007).
198. Metal binding functions in the design of carbonic anhydrase inhibitors. Winum, J. Y., Scozzafava, A., Montero, J. L., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 835-848 (2007).
199. The development of topically acting carbonic anhydrase inhibitors as antiglaucoma agents. Mincione, F., Scozzafava, A., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 849-854 (2007).
200. Carbonic anhydrase inhibitors as anticonvulsant agents. Thiry, A., Dogne, J., Supuran, C. T., Masereel, B. *Current Topics in Medicinal Chemistry* 7, 855-864 (2007).
201. Antiobesity carbonic anhydrase inhibitors. De Simone, G., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 879-884 (2007).
202. Dual carbonic anhydrase - Cyclooxygenase-2 inhibitors. Dogne, J. M., Thiry, A., Pratico, D., Masereel, B., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 885-891 (2007).
203. Characterization and inhibition of the recently discovered carbonic anhydrase isoforms CA XIII, XIV and XV. Hilvo, M., Supuran, C. T., Parkkila, S. *Current Topics in Medicinal Chemistry* 7, 893-899 (2007).
204. Inhibition of the archaeal beta-class (Cab) and gamma-class (Cam) carbonic anhydrases. Zimmerman, S. A., Ferry, J. G., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 901-908 (2007).
205. Malarial parasite carbonic anhydrase and its inhibitors. Krungkrai, J., Krungkrai, S. R., Supuran, C. T. *Current Topics in Medicinal Chemistry* 7, 909-917 (2007).
206. Molecular modeling study for the binding of zonisamide and topiramate to the human mitochondrial carbonic anhydrase isoform VA. Vitale, R. M., Pedone, C., Amodeo, P., Antel, J., Wurl, M., Scozzafava, A., Supuran, C. T., De Simone, G. *Bioorganic & Medicinal Chemistry* 15, 4152-4158 (2007).

207. Carbonic anhydrase inhibitors. Inhibition of isoforms I, II, IV, VA, VII, IX, and XIV with sulfonamides incorporating fructopyranose-thioureido tails. Winum, J. Y., Thiry, A., El Cheikh, K., Dogne, J. M., Montero, J. L., Vullo, D., Scozzafava, A., Masereel, B., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 2685-2691 (2007).
208. Carbonic anhydrase inhibitors: The X-ray crystal structure of the adduct of N-hydroxysulfamide with isozyme II explains why this new zinc binding function is effective in the design of potent inhibitors. Temperini, C., Winum, J. Y., Montero, J. L., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 2795-2801 (2007).
209. Phosph(on)ate as a zinc-binding group in metalloenzyme inhibitors: X-ray crystal structure of the antiviral drug foscarnet complexed to human carbonic anhydrase I. Temperini, C., Innocenti, A., Guerri, A., Scozzafava, A., Rusconi, S., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 2210-2215 (2007).
210. Membrane-bound carbonic anhydrases in osteoclasts. Riihonen, R., Supuran, C. T., Parkkila, S., Pastorekova, S., Vaananen, H. K., Laitala-Leinonen, T. *Bone* 40, 1021-1031 (2007).
211. Carbonic anhydrase inhibitors. Inhibition of transmembrane isozymes XII (cancer-associated) and XIV with anions. Innocenti, A., Vullo, D., Pastorek, J., Scozzafava, A., Pastorekova, S., Nishimori, I., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 1532-1537 (2007).
212. Carbonic anhydrase inhibitors: Inhibition of cytosolic/tumor-associated isoforms I, II, and IX with iminodiacetic carboxylates/hydroxamates also incorporating benzenesulfonamide moieties. Santos, M. A., Marques, S., Vullo, D., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 1538-1543 (2007).
213. Carbonic anhydrase inhibitors: Binding of an antiglaucoma glycosyl-sulfanilamide derivative to human isoform II and its consequences for the drug design of enzyme inhibitors incorporating sugar moieties. Di Fiore, A., Scozzafava, A., Winum, J. Y., Montero, J. L., Pedone, C., Supuran, C. T., De Simone, G. *Bioorganic & Medicinal Chemistry Letters* 17, 1726-1731 (2007).
214. Carbonic anhydrase inhibitors: Inhibition of isozymes I, II, and IX with triazole-linked O-glycosides of benzene sulfonamides. Wilkinson, B. L., Bornaghi, L. F., Houston, T. A., Innocenti, A., Vullo, D., Supuran, C. T., Poulsen, S. A. *Journal of Medicinal Chemistry* 50, 1651-1657 (2007).
215. Analysis of human carbonic anhydrase II: Docking reliability and receptor-based 3D-QSAR study. Tuccinardi, T., Nuti, E., Ortore, G., Supuran, C. T., Rossello, A., Martinelli, A. *Journal of Chemical Information and Modeling* 47, 515-525 (2007).
216. Carbonic anhydrase activators: An activation study of the human mitochondrial isoforms VA and VB with amino acids and amines. Vullo, D., Nishimori, I., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 1336-1340 (2007).
217. Carbonic anhydrase and matrix metalloproteinase inhibitors. Inhibition of human tumor-associated isozymes IX and cytosolic isozyme I and II with sulfonylated hydroxamates. Nuti, E., Orlandini, E., Nencetti, S., Rossello, A., Innocenti, A., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry* 15, 2298-2311 (2007).
218. Inhibition of membrane-associated carbonic anhydrase isozymes IX, XII and XIV with a library of glycoconjugate benzenesulfonamides. Wilkinson, B. L., Bornaghi, L. F., Houston, T. A., Innocenti, A., Vullo, D., Supuran, C. T., Poulsen, S. A. *Bioorganic & Medicinal Chemistry Letters* 17, 987-992 (2007).
219. Carbonic anhydrase inhibitors. Inhibition studies of the human secretory isoform VI with anions. Nishimori, I., Innocenti, A., Vullo, D., Scozzafava, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 1037-1042 (2007).
220. The sexually transmitted papillomavirus infections: clinical manifestations, current and future therapies. Mastrolorenzo, A., Supuran, C. T., Zuccati, G. *Expert Opinion on Therapeutic Patents* 17, 173-211 (2007).
221. Carbonic anhydrase activators: L-Adrenaline plugs the active site entrance of isozyme II, activating better isoforms I, IV, VA, VII, and XIV. Temperini, C., Innocenti, A., Scozzafava, A., Mastrolorenzo, A., Supuran, C. T. *Bioorganic & Medicinal Chemistry Letters* 17, 628-635 (2007).
222. Carbonic anhydrase inhibitors. DNA cloning, characterization, and inhibition studies of the human secretory isoform VI, a new target for sulfonamide and sulfamate inhibitors. Nishimori, I., Minakuchi, T., Onishi, S., Vullo, D., Scozzafava, A., Supuran, C. T. *Journal of Medicinal Chemistry* 50, 381-388 (2007).

223. ESI mass spectrometry and X-ray diffraction studies of adducts between anticancer platinum drugs and hen egg white lysozyme. Casini, A., Mastrobuoni, G., Temperini, C., Gabbiani, C., Francese, S., Moneti, G., Supuran, C. T., Scozzafava, A., Messori, L. *Chemical Communications* 156-158 (2007).
224. Nanoscience contribution to preservation of acidic shipwrecks, *Proceedings of the 10th ICOM-CC conference on Wet Organic Archaeological Materials - WOAM 2007, Amsterdam, September 10-15, 2007,* Giorgi, R., Chelazzi, D., Baglioni, P. Accepted.
225. Competitive Surface Adsorption of Solvent Molecules and Compactness of Agglomeration in Calcium Hydroxide Nanoparticles. Fratini, E., Giorgi, R., Cölfen H., Baglioni, P., Deme B., Zemb Th. *Langmuir* 23, 2330-2338 (2007).
226. Porous silicon-based potentiometric biosensor for triglycerides. Setzu, S., Salis, S., Demontis, V., Salis, A., Monduzzi, M., Mula G. *Physica Status Solidi (A)* 204, 1434-1438 (2007).
227. Surface characteristics of ionically crosslinked chitosan membranes. Karakecili, A.G., Satriano, C., Gumusderelioglu, M., Marletta, G. *J. Appl. Polym. Sci.* 106, 3884-3888 (2007).
228. Le nanoparticelle per la conservazione ed il restauro del patrimonio culturale, Baglioni, P., Giorgi, R., *Giornate Lincee della Chimica, VII edizione "Contributi della chimica alla soluzione di alcuni problemi della societa' moderna"*, Accademia Nazionale dei Lincei, Roma 2007, Bardi Editore-Editore Commerciale, 81-97 (2007).
229. Self-Assembly of β-Cyclodextrin in Water. 2. Electron Spin Resonance. Rossi, S., Bonini, M., Lo Nostro, P., Baglioni, P. *Langmuir* 23, 10959-10967 (2007).
230. A new organogelator based on an enantiopure C2 symmetric pyrrolidine. Cicchi, S., Ghini, G., Lascialfari, L., Brandi, A., Betti, F., Berti, D., Ferrati, S., Baglioni, P. *Chemical Communications* 14, 1424-1426 (2007).
231. Evaluation of L929 fibroblast attachment and proliferation on Arg-Gly-Asp-Ser (RGDS)-immobilized chitosan in serum-containing/ serum-free cultures. Karakecili A.G., Demirtas, T.T., Satriano, C., Gumusderelioglu, M., Marletta, G. *J. Biosc. Bioeng.* 104, 69-77 (2007).
232. Molecular Modelling of Oligopeptide Adsorption onto Functionalised Quartz Surfaces. Forte, G., Grassi, A., Marletta, G. *J. Phys. Chem. B* 111, 11237- 11243 (2007).
233. Self-assembled peptide monolayers on interdigitated gold microelectrodes. Gatto, E., Venanzi, M.., Palleschi, A., Stella, L., Pispisa, B., Lorenzelli, L., Toniolo, C., Formaggio, F., Marletta, G. *Mat. Sci. Eng. C* 27, 1309-1312 (2007).
234. Identification and discrimination between some contaminant enzyme activities in commercial preparations of mushroom tyrosinase. Rescigno, A., Zucca, P., Flurkey, A., Inlow, J., Flurkey, W.H., *Enzyme and Microbial Technology*, 41, 620-627 (2007).
235. Nanocalce per il consolidamento di pitture murali Maya in area sub-tropicale: un esempio di internazionalizzazione del know-how del CSGI, Giorgi, R., Chelazzi, D., Baglioni, P., *Atti del XXIII Convegno Internazionale Scienza e Beni Culturali – Il consolidamneto degli apparati architettonici e decorative – Conoscenze, Orientamenti, Esperienze*, Bressanone, 10-13 Luglio 2007, Ed. Arcadia Ricerche, 559-566 (2007).
236. Il ruolo degli inchiostri metallo-gallici nei processi degradativi di manoscritti cartacei, Giorgi, R., Chelazzi, D., Baglioni, P., *Atti del V Congresso Nazionale IGIIC* - Lo Stato dell'Arte - Cremona, 4-6 Ottobre 2007, Nardini editore, 273-280 (2007).
237. Addressable high-information-density DNA nanostructures. Baglioni, P. *Chemical Physics Letters* 440, 125-129 (2007).
238. Addressable molecular node assembly - A generic platform of nano-scale functionalised surfaces based on a "Digitally addressable" molecular grid. Baglioni, P. *Journal of Biomolecular Structure & Dynamics* 24, Conversation 15 (2007).
239. Alkaline titrations of poly(dG-dC).poly(dG-dC): Microemulsion versus solution behavior. Airoldi, M., Gennaro, G., Giomini, M., Giuliani, A. M., Giustini, M. *Journal of Biomolecular Structure & Dynamics* 24, 561-570 (2007).
240. Irreversible affinity immobilization of lentil seedling amine oxidase with activity retention. Sollai, F., Noli, B., Floris, G., Sanjust, E., *Environ. Eng. Manage. J.*, 6, 31-35 (2007).
241. Amphiphilic Self-assemblies Decorated by Nucleobases. Berti, D., Baldelli Bombelli, F., Fortini, M., Baglioni, P. *Journal of Physical Chemistry B* 111, 11734-11744 (2007).
242. Supercritical $CO_2$ extract of *cinnamomum zeylanicum*: chemical characterization and antityrosinase activity. Marongiu, B., Piras, A., Porcedda, S., Tuveri, E., Sanjust, E., Meli, M., Sollai, F., Zucca, P., Rescigno, A., *Journal of Agricultural and Food Chemistry* 55, 10022-10027 (2007).
243. Diffusion coefficients for the ternary system water + chloroform + acetic acid at 25 C. Buzatu, D., Buzatu, F., Paduano, L., Sartorio, R. *Journal of Solution Chemistry* 36 (11/12), 1373-1384.

(2007).
244. Effect of the Preparation Procedure on the Structural Properties of a Oligonucleotide/Cationic Liposome Complex (lipoplex) Studied by Electron Spin Resonance and Zeta Potential. Ciani, L., Ristori, S., Bonechi, C., Rossi, C., Martini, G. *Biophysical Chemistry* 131, 80-87 (2007).
245. 5,10,15,20-Tetrakis(4-sulfonato-phenyl)porphine-Mn(III) immobilized on imidazole-activated silica as a novel lignin-peroxidase-like biomimetic catalyst. Zucca, P., Mocci, G., Rescigno, A., Sanjust, E., *Journal of Molecular Catalysis, A: Chemical*, 278, 220-227 (2007).
246. High-relaxivity supramolecular aggregates containing peptides and Gd complexes as contrast agents in MRI. Accardo, A., Tesauro, D., Morelli, G., Gianolio, E., Aime, S., Vaccaro, M., Paduano, L., Schillen, K. *Journal of Biological Inorganic Chemistry* 12 (2), 267-276 (2007).
247. Hofmeister Effects in Enzymatic Activity: Weak and Strong Electrolyte Influences on the Activity of Candida rugosa Lipase. Salis, A., Bilanicova, D., Ninham, B. W., Monduzzi, M. *Journal of Physical Chemistry B* 111, 1149-1156 (2007).
248. Hybrid LbL/LB films as molecular OLEDs: An acoustic shear wave attenuation and Brewster angle microscopy study. Gambinossi, F., Baglioni, P., Caminati, G. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* 27, 1056-1060 (2007).
249. Effect of ascorbic acid based amphiphiles on human erythrocytes membrane. Rasia, M.; Spengler, M. I.; Palma, S.; Manzo, R.; Lo Nostro, P.; Allemandi, D. *Clinical Hemorheology and Microcirculation* 36(2), 133-140 (2007).
250. Indagini preliminari ToF-SIMS su Manufatti Lignei Provenienti dal Museo Diocesano di Palermo. Tognazzi, A., Maccotta, A., Sebastianelli, M., Rossi, C. *Atti del IV Congresso di Archeometria, Pisa 1-3 Febbraio 2006, Pàtron Editore, Bologna*. , 641-652 (2007).
251. Insights into Hofmeister Mechanisms: Anion and Degassing Effects on the Cloud Point of Dioctanoylphosphatidylcholine/Water Systems. Lagi, M., Lo Nostro, P., Fratini, E., Ninham, B. W., Baglioni, P. *Journal of Physical Chemistry B* 111, 589-597 (2007).
252. Interaction between pentaethylene glycol n-octyl ether and poly(acrylic acid): Effect of the polymer molecular weight. D'Errico, G., Ciccarelli, D., Ortona, O., Paduano, L., Sartorio, R. *Journal of Colloid and Interface Science* 314 (1), 242-250 (2007).
253. Interaction of the alternating double stranded copolymer poly(dA-dT)center dot poly(dA-dT) with $NiCl_2$ and $CdCl_2$: Solution behavior. Airoldi, M., Gennaro, G., Giomini, M., Giuliani, A. M., Giustini, M. *Journal of Biomolecular Structure & Dynamics* 25, 77-84 (2007).
254. Investigation of the Adsorption of PEG1500-12-Acyloxystearate Surfactants onto Phospholipid Bilayers: An Ellipsometry and Cryo-TEM Study. Vaccaro, M., von Corswant, C., Söderman, O. *Biophysical Journal* 93, 4300-4306 (2007).
255. Nanoassemblies from Vitamin C Derivatives. Lo Nostro, P., Ambrosi, M., Fratini, E., Ridi, F., Carretti, E., Alfredsson, V., Ninham, B.W., Baglioni, P. *Technical Proceedings 2007 NSTI Nanotechnology Conference and Trade Show*, Santa Clara (CA), May 20-24, 2007, (2007), 2, 9-12.
256. Poly(acrylic) acid-coated Silver Nanoparticles for Antibacterial Textile Finishing. Falletta, E., Bonini, M., Fratini, E., Lo Nostro, A., Becheri, A., Lo Nostro, P., Baglioni, P. *Technical Proceedings 2007 NSTI Nanotechnology Conference and Trade Show*, Santa Clara (CA), May 20-24, 2007, (2007), 4, 412-414.
257. Mesoscopic and Microscopic Investigation on Poly(vinyl alcohol) Hydrogels in the Presence of Sodium Decylsulfate. Mangiapia, G., Ricciardi, R., Auriemma, F., De Rosa, C., Lo Celso, F., Triolo, R., Hennan, R. K., Radulescu, A., Tedeschi, A. M., D'Errico, G., Paduano, L. *Journal of Physical Chemistry B* 111 (9), 2166-2173 (2007).
258. Microstructure of ternary system di-lauroyl-phosphatidyl-adenosine/water/cyclohexane. Betti, F., Baldelli Bombelli, F., Berti, D., Bonini, M., Brandt, A., Baglioni, P. *Journal of Applied Crystallography* 40, s240-s244 (2007).
259. Molecular Recognition Drives Oligonucleotide Binding to Nucleolipid Self-Assemblies. Banchelli M., Berti, D., Baglioni, P. *Angewandte Chemie International Edition* 46, 3070-3073 (2007).
260. Multicomponent Diffusion of Lysozyme in Aqueous Calcium Chloride. The Role of Common-Ion Effects and Protein-Salt Preferential Interactions. Annunziata, O., Albright, J. G., Paduano, L. *Journal of Physical Chemistry B* 111 (35), 10591-10598 (2007).
261. Nanostructures by Self-Assembling Peptide Amphiphile as Potential Selective Drug Carriers. Accardo, A., Tesauro, D., Mangiapia, G., Pedone, C., Morelli, G. *Biopolymers* 88 (2), 115-121 (2007).
262. Nanostructures from alkyl vitamin C derivatives (ASCn): Properties and potential platform for drug delivery. Palma, S., Manzo, R. H., Lo Nostro, P., Allemandi, D. *Int. J. Pharm.* 345, 26-34

(2007).
263. NMR of Liquid Crystals and Micellar Solutions - V. Monduzzi, M., Murgia, S. in *Nuclear Magnetic Resonance* 36, 397-418 Ed. G. A. Webb, The Royal Society of Chemistry (2007).
264. Nucleolipoplexes: A New Paradigm for Phospholipid Bilayer-nucleic Acid Interactions. Milani, S., Baldelli Bombelli, F., Berti, D., Baglioni, P. *Journal of American Chemical Society* 129, 11664-11665 (2007).
265. Real-time PCR and enzyme-linked fluorescent assay methods for detecting Shiga-toxin-producing *Escherichia coli* in mincemeat samples. Stefan, A., Scaramagli, S., Bergami, R., Mazzini, C., Barbanera, M., Perelle, S., Fach, P. *Canadian Journal of Microbiology* 53, 337-342 (2007).
266. Organogels from a Vitamin C-Based Surfactant. Lo Nostro, P., Ramsch, R., Fratini, E., Lagi, M., Ridi, F., Carretti, E., Ambrosi, M., Ninham, B. W., Baglioni, P. *Journal of Physical Chemistry B* 111, 11714-11721 (2007).
267. Peptide derivatized lamellar aggregates as target-specific MRI contrast agents. Tesauro, D., Accardo, A., Gianolio, E., Paduano, L., Teixeira, J., Schillen, K., Aime, S., Morelli, G. *ChemBioChem* 8 (8), 950-955 (2007).
268. Peptides and Gd complexes containing colloidal assemblies as tumor-specific contrast agents in MRI: physicochemical characterization. Vaccaro, M., Accardo, A., D'Errico, G., Schillen, K., Radulescu, A., Tesauro, D., Morelli, G., Paduano, L. *Biophysical Journal* 93 (5), 1736-1746 (2007).
269. Physico-chemical and structural properties of hydrogels formed by chitosan, in the presence and absence of poly(vinylpyrrolidone) and sodium decylsulfate. Mangiapia, G., Frielinghaus, H., D'Errico, G., Ortona, O., Sartorio, R., Paduano, L. *Physical Chemistry Chemical Physics* 9, 6150-6158 (2007).
270. Preparation of Nanosize Silica in Reverse Micelles: Ethanol Produced during TEOS Hydrolysis Affects the Microemulsion Structure. Venditti, F., Angelico, R., Palazzo, G., Colafemmina, G., Ceglie, A., Lopez, F. *Langmuir* 23, 10063-10068 (2007).
271. Dinelli, M., Bonini, M., Giorgetti, A., Casucci, F., Ridi, R., Fratini, E., Baldanzini, N., Citti, P., Baglioni, P. Flame Spraying Of $TiO_2$ Nanoparticles: Deposition Onto Glass Substrate. *RILEM Proceedings PRO055: Photocatalysis, Environment and Construction Materials,* TDP2007, 281 (2007).
272. Role of the solvent in the dynamical transitions of proteins: the case of the lysozyme-water system. Mallamace, F., Chen, S. H., Broccio, M., Baglioni, P., Fratini, E., Vannucci, C. *Journal of Chemical Physics* 127, 045104-045106 (2007).
273. SAXS study of chain-like structures formed by magnetic nanoparticles. Bonini, M., Fratini, E., Baglioni, P. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* 27, 1377-1381 (2007).
274. SDS micelles with propylene carbonate as cosolvent: a PGSE-NMR and SAXS study. Colafemmina, G., Fiorentino, D., Ceglie, A., Carretti, E., Fratini, E., Dei, L., Baglioni, P., Palazzo, G. *Journal of Physical Chemistry B* 111, 7184-7193 (2007).
275. Small angle scattering and zeta potential of Liposomes loaded with octa(carboranyl)porphyrazine. Salvati, A., Ristori, S., Oberdisse, J., Spalla, O., Ricciardi, G., Pietrangeli, D., Giustini, M., Martini, G. *Journal of Physical Chemistry B* 111, 10357-10364 (2007).
276. Small-Angle Neutron Scattering of Mixed Ionic Perfluoropolyether Micellar Solutions. Gambi, C. M. C., Giordano, R., Chittofrati, A., Pieri, R., Laurati, M., Baglioni, P., Teixeira, J. *J.Phys. Chem. B* 111, 1348-1353 (2007).
277. Sodium Chloride Molar Conductance in Different Poly(ethylene glycol)–Water Mixed Solvents. Capuano, F., Mangiapia, G., Ortona, O., D'Errico, G., Sartorio, R. *Journal of Solution Chemistry* 36 (5), 617-629 (2007).
278. Specific Anion Effects on the Optical Rotation of α-Amino Acids. Rossi, S., Lo Nostro, P., Lagi, M., Ninham, B. W., Baglioni, P. *Journal of Physical Chemistry B* 111, 10510-10519 (2007).
279. Stabilization of charge separation and cardiolipin confinement in antenna-reaction center complexes purified from Rhodobacter sphaeroides. Dezi, M., Francia, F., Mallardi, A., Colafemmina, G., Palazzo, G., Venturoli, G. *Biochimica et Biophysica Acta-Bioenergetics* 1767, 1041-1056 (2007).
280. Structural and Relaxometric Characterization of Peptide Aggregates Containing Gadolinium Complexes as Potential Selective Contrast Agents in MRI. Vaccaro, M., Mangiapia, G., Paduano, L., Gianolio, E., Accardo, A., Tesauro, D., Morelli, G. *ChemPhysChem* 8 (17), 2526-

2538 (2007).
281. Study of ferrite ferrofluids by small-angle scattering of polarized neutrons. Bonini, M., Wiedenmann, A., Baglioni, P. *Journal of Applied Crystallography* 40, s254-s258 (2007).
282. The 2Y model and its applications: the cases of charged proteins and copolymer micellar solutions. Chen, S. H., Broccio M., Liu, Y.C., Fratini, E., Baglioni, P. *Journal of Applied Crystallography* 40, s321-s326 (2007).
283. Use of lipases for the production of biodiesel. Salis, A., Monduzzi, M., Solinas, V. in *Industrial enzymes*, 315-337 Ed. J. Polaina, A. McCabe, Springer (2007).
284. Determination of the Modified "Affinity Index" of Small Ligands and Macromolecular Receptors from NMR Spin-lattice Relaxation Data. Martini, S., Bonechi, C., Corbini, G., Rossi, C. *Chemical Physics Letters* 447, 147-153 (2007).
285. Fibrinogen-catecholamines interaction as observed by NMR and Fourier Transform Infrared Spectroscopy. Martini, S., Consumi, M., Bonechi, C., Rossi, C., Magnani, A. *Biomacromolecules* 8, 2689-2696 (2007).
286. Quantum Chemical Modelling and preparation of a Biomimetic Photochemical Switch. Lumento, F., Zanirato, V., Fusi, S., Busi, E., Latterini, L., Elisei, F., Sinicropi, A., Andruniow, T., Ferré, N., Basosi, R., Olivucci, M. *Angewandte Chemie International Edition* 31, 471-482 (2007).
287. The cis/trans isomerisation of Cu(II)-bis-(glycinato) complex in solution: a computer aided Multifrequency EPR and DFT/PCM calculations study. Pezzato, M., Della Lunga, G., Baratto, M. C., Sinicropi, A., Pogni, R., Basosi, R. *Magnetic Resonance in Chemistry* 45, 846-849 (2007).
288. Chimica e Ambiente. Basosi, R. in *Pianeta Galileo 2007* 1, 467-481 (2007).
289. Efficienza problematica. Basosi, R., Ruzzenenti, F. *Quale Energia* 4, 61-64 (2007).
290. Examining the dynamics of phytoplankton biomass in Lake Tanganyika using Empirical Orthogonal Functions. Bergamino, N., Loiselle, S., Cozar, A., Dattilo, A. M., Bracchini, L., Rossi, C. *Ecological Modelling* 204, 156-162 (2007).
291. Light limitations to algal growth in tropical ecosystems. Loiselle, S., Cozar, A., Dattilo, A. M., Bracchini, L., Galvez, J. A. *Freshwater Biology* 52, 305-312 (2007).
292. Nanomagnetic Sponges for the Cleaning of Works of Art. Bonini, M., Lenz, S., Giorgi, R., Baglioni, P. *Langmuir* 23, 8681-8685 (2007).
293. Membrane Insertion And Bilayer Perturbation By Antimicrobial Peptide Cm15. Pistolesi, S., Pogni, R., Feix J. B., *Biophysical Journal* 93, 1651-1660, (2007).
294. On the contribution of nanotechnology to the conservation of Cultural Heritage, Baglioni, P., Giorgi, R., Chelazzi, D., *Proceedings of The International Congress on Particle Technology - PARTEC 2007*, Nuremberg, Germany, 27–29.3.2007, published on CD-ROM (2007).
295. Novel surfactant-based adsorbent material for groundwater remediation. Venditti, F., Angelico, R., Ceglie, A., Ambrosone, L. *Env. Sci. and Technology* 41, 6836-6840 (2007).
296. Oil-in-water nanocontainers as low environmental impact cleaning tools for works of art: two case studies. Carretti, E., Giorgi, R., Berti, D., Baglioni, P. *Langmuir* 23, 6396-6403 (2007).
297. Removal of chromate from water by a new CTAB–silica gelatin composite. Venditti, F., Ceglie, A., Palazzo, G., Colafemmina, G., Lopez, F. *Journal of Colloid and Interface Science* 310, 353-361 (2007).
298. The conservation of the Vecchietta's wall paintings in the Old Sacristy if Santa Maria in Siena: the use of nanotechnological cleaning agents. S., Carretti, E., Pecorelli, P., Iacopini, F., Baglioni, P., Dei, L. *Journal of Cultural Heritage* 8, 119-125 (2007).
299. The optical qualities of shallow wetland lined bays il Lake Victoria. Bracchini, L., Loiselle, S., Tognazzi, A., Dattilo, A. M., Focardi, S., Cozar, A., Rossi, C. *Wetlands Ecology and Management* 15, 509-519 (2007).
300. Effect of high-energy milling on the solid state formation of zinc manganites ($Zn_xMn_{3-x}O_4$, 0.5 = x = 1.5) from the system $ZnC_2O_4 \cdot 2H_2O$ - n $MnCO_3$ (n = 1, 1.5 and 2). Berbenni, V., Milanese, C., Bruni, G., Marini, A. *Zeitschrift Fur Naturforschung Section B. A Journal of Chemical Sciences* 62, 663-668 (2007).
301. New insights into the magnetic properties of the $Ca_2Fe_2O_5$ ferrite. Azzoni, C. B., Mozzati, M. C., Massarotti, V., Capsoni, D., Bini, M. *Solid State Science* 9 [6], 515-520 (2007).
302. Preparation of NiO-$LiFeO_2$ solid solutions: the role of mechanical and thermal treatments. Berbenni, V., Milanese, C., Bruni, G., Cofrancesco, P., Marini, A., Chiodelli, G. *International Journal of Materials Research* 98 [3], 205-208 (2007).

303. Tungsten bronzes framework as a glasslike host for transition cations: the case of $Ba_6Zr_2Ta_8O_{30}$. Massarotti, V., Capsoni, D., Bini, M., Azzoni, C. B., Mozzati, M. C., Galinetto, P. *Journal of Physical Chemistry C* 111 [18], 6857-6861 (2007).
304. A new tetrahydrated form of sodium naproxen. Di Martino, P., Barthélémy, C., Joiris, E., Capsoni, D., Masic, A., Massarotti, V., Gobetto, R., Bini, M., Martelli, S. *Journal of Pharmaceutical Sciences* 96 [1], 156-167 (2007).
305. Driving Forces in the Delivery of Penetratin Conjugated G Protein Fragment. Albrizio, S., Giusti, L., D'Errico, G., Esposito, C., Porchia, F., Caliendo, G., Novellino, E., Mazzoni, M.R., Rovero, P., D'Ursi, A.M. *Journal of Medicinal Chemistry* 50 (7), 1458-1464 (2007).
306. Effect of Al and Cr addition on tribological behaviour of HVOF and APS nanostructured WC–Co coatings. Basak, A.K., Achanta, S., De Bonte, M., Celis, J.P., Vardavoulias, M., Matteazzi, P. *Transactions of the Institute of Metal Finishing* 85, 310-315 (2007).
307. Efficient silencing of the gene coding for the epsilon subunit of DNA polymerase III in Escherichia coli is triggered by antisense RNAs featuring stability in vivo. Stefan, A., Tabler, M., Hochkoeppler, A. *FEMS Microbiol Lett*. 270, 277-283 (2007).
308. Quantum Chemical Modelling and preparation of a Biomimetic Photochemical Switch. Lumento, F., Zanirato, V., Fusi, S., Busi, E., Latterini, L., Elisei, F., Sinicropi, A., Andruniow, T., Ferrè, N., Basosi, R., Olivucci, M., *Angew. Chem. Int. Ed*. 47, 3, 414-420, (2007).
309. Evidence for a Radical Mechanism in Biocatalytic degradation of synthetic dyes by Fungal Laccases mediated by violuric acid. Pogni, R., Brogioni, B., Baratto, M. C., Sinicropi, A., Giardina, P., Sannia, G., Basosi, R., Pezzella, C. *Bicatalysis and Biotransformations* 25, 269-275 (2007).
310. Functionality of Photosynthetic Reaction Centers in Polyelectrolyte Multilayers: Toward an Herbicide Biosensor. Mallardi, A., Giustini, M., Lopez, F., Dezi, M., Venturoli, G., Palazzo, G. *Journal of Physical Chemistry B* 111, 3304-3314 (2007).
311. Identification and Structural Characterization of a Transient Radical Species in the Uricase Reaction Mechanism. Busi, E., Sinicropi, A., Terzuoli, L., Marinello, E., Basosi, R. *Applied Magnetic Resonance* 31, 471-482 (2007).
312. Impact of edible surfactants on the oxidation of olive oil in water-in-oil emulsions. Ambrosone, L., Mosca, M., Ceglie, A. *Food Hydrocolloids* 21, 1163-1171 (2007).
313. Influence of solvent and crystallization method on the crystal habit of metronidazole. Di Martino, P., Censi, R., Malaj, L., Capsoni, D., Massarotti, V., Martelli, S. *Crystal Research and Technology* 42 [8], 800-806 (2007).
314. Photosynthetic Reaction Centers Embedded in Polyelectrolyte Multilayer as a Tool in the Determination of PSII Herbicides. Lopez, F., Mennuni, M., Giustini, M., Giomini, M., Dezi, M., Venturoli, G., Mallardi, A., Palazzo, G. *2nd IEEE International Workshoop On Advances in Sensors and Interfaces* , 237-241 (2007).
315. Quantification of drug amorphous fraction by DSC. Bruni, G., Milanese, C., Bellazzi, G., Berbenni, V., Cofrancesco, P., Marini, A., Villa, M. *Journal of Thermal Analysis and Calorimetry* 89, 761-766 (2007).
316. Tryptophan Radicals as Reaction Intermediates in Versatile Peroxidases: Multifrequency EPR, ENDOR and Density Functional Theory Studies. Pogni, R., Teutloff, F., Lendzian, F., Basosi, R. *Applied Magnetic Resonance* 31, 509-526 (2007).
317. Tyrosine83 is essential for the activity of E. coli galactoside transacetylase. Radeghieri, A., Bonoli, M., Parmeggianti, F., Hochkoeppler, A. *Biochimica et Biophysica Acta* 1774, 243-248 (2007).
318. Structural And Dynamic Characterization Of Copper(Ii) Binding Of The Human Prion Protein Outside The Octarepeat Region. Berti, F., Gaggelli, E., Guerrini, R., Janicka, A., Kozlowski, H., Legowska A. Miecznikowska, H., Migliorini, C., Pogni, R., Remelli, M., Rolka, K., Valensin, G., Valensin, D., *Chemistry-A European Journal* 13, 1991-2001, (2007).
319. Consolidation of Tuff: in Situ Polymerization or Traditional Methods ?. Camaiti, M., Dei L., Errico, V. In *Proceedings of 5th Int. Conference on Structural Analysis of Historical constructions* (New Delhi, India Nov. 6-8, 2006), Edited by Paulo B. Lourenco, Pere Roca, Claudio Modena, Shailesh Agrwal, Ed. Macmillan India Ltd., New Delhi, 2007, pp. 1339-1346.
320. Pre-consolidation of pictorial layers in frescoes; the high performance of CSGI's method based on nanolime evaluated by OPD team in Agnolo Gaddi's *La Leggenda della Vera Croce*, Santa Croce, Firenze, Dei, L., Bandini, F., Felici, A., Lanfranchi, M., Lanterna, G., Macherelli, A., Salvadori, B. In *Proceedings of the 23rd International Congress 'Il consolidamento degli apparati architettonici e decorativi. Conoscenze, Orientamenti, Esperienze'*, Arcadia Ricerche,

Venezia, 2007, pp. 217-224.

321. Metodologie di consolidamento di superfici architettoniche interessate da sali solubili, Croveri, P., Dei L., Cassar, J. In *Proceedings of the 23rd International Congress 'Il consolidamento degli apparati architettonici e decorativi. Conoscenze, Orientamenti, Esperienze'*, Arcadia Ricerche, Venezia, 2007, pp. 549-558.
322. Una nuova metodologia di pulitura delle superfici murali esterne basata sulle nanotecnologie: il caso della Scuola di Santa Maria dei Battuti a Conegliano. Grassi, S., Carretti, E., Macherelli, A., Mauro, M., Salvadori, B., Baglioni, P., Dei, L. In *Atti della Giornata di Studio "Facciate dipinte. Verifiche sui protettivi, metodologie di pulitura a Feltre e nel Veneto Orientale"*, A. M. Spiazzi, V. Fascina, F. Magani Eds., Feltre (BL), 25 Novembre 2005, Il Prato Edizioni, Padova (2007) pp. 67-71.
323. Studio di consolidanti nanometrici per il consolidamento della pellicola pittorica dei dipinti murali. Costa, E., Dei, L., Gaetani, M. C., Giovagnoli, A., Ioele, M. In *'Lo Stato dell'Arte, Atti del 5° Congresso Nazionale IGIIC'*, Cremona 2007, pp. 193-198.
324. Solution Behavior of a Sugar-based Carborane for Boron Neutron Capture Therapy: a Nuclear Magnetic Resonance Investigation. Bonechi, C., Donati, A., Ristori, S., Martini, S., Panza, L., Martini, G., Rossi C. *Biophysical Chemistry* 125, 320–327 (2007).
325. Determination of the Modified "Affinity Index" of Small Ligands and Macromolecular Receptors from NMR Spin-lattice Relaxation Data. Martini, S., Bonechi, C., Corbini, G., Rossi, C. *Chemical Physics Letters* 447 147-153 (2007).
326. Fibrinogen-catecholamines interaction as observed by NMR and Fourier Transform Infrared Spectroscopy, Martini, S., Consumi, M., Bonechi, C., Rossi, C., Magnani, A. *Biomacromolecules* 8, 2689-2696 (2007).
327. Effect of the Preparation Procedure on the Structural Properties of a Oligonucleotide/Cationic Liposome Complex (lipoplex) Studied by Electron Spin Resonance and Zeta Potential. Ciani, *L.,* Ristori, S., Bonechi, C., Rossi, C., Martini, G. *Biophysical Chemistry* 131, 80-87 (2007).
328. Examining the dynamics of phytoplankton biomass in Lake Tanganyika using Empirical Orthogonal Functions. Bergamino, N., Loiselle, S., Cozar, A., Dattilo, A. M., Bracchini, L., Rossi, C. *Ecological Modelling* 204, 156-162 (2007).
329. Light limitations to algal growth in tropical ecosystems. Loiselle, S., Cozar, A., Dattilo, A. M., Bracchini, L., Galvez, J. A., *Freshwater Biology* 52, 305-312 (2007).
330. The optical qualities of shallow wetland lined bays in Lake Victoria. Bracchini, L., Loiselle, S., Tognazzi, A., Dattilo, A. M., Focardi, S., Cozar, A., Rossi, C. *Wetlands Ecology and Management* 15, 509-519 (2007).
331. Structural Manipulation of Benzofulvene Derivatives Showing Spontaneous Thermoreversible Polymerization. Role of the Substituents in the Modulation of Polymer Properties. Cappelli, A., Galeazzi, S., Giuliani, G., Anzini, M., Donati, A., Zetta, L., Mendichi, R., Aggravi, M., Giorgi, G., Paccagnini, E., Vomero, S. *Macromolecules* 40, 3005-3014 (2007).
332. With Arsenic on The Etruscans' Footprints. Donati, A.,. Pulselli, F.M., Protano, G., Dallai, L., Francovich, R., Tiezzi, E. *International Journal of Ecodynamics* 2, 24-26 (2007). Patterning of Lactoferrin Using Functional SAMs of Iron Complexes. Tuccitto, N., Giamblanco, N., Licciardello, A., Marletta, G. *Chem. Comm.* 25, 2621-2623 (2007).
333. Expression of cell adhesion receptors in human osteoblasts cultured on biofunctionalized poly-(ε-caprolactone) surfaces. Amato, I., Ciapetti, G., Pagani, S., Marletta, G., Baldini, N., Granchi, D. *Biomaterials* 28, 3668-3678 (2007).
334. Scanning Force Microscopy and optical spectroscopy of phase-segregated thin films of poly-(9,9'-dioctylfluorene-*alt*-benzothiadiazole) and poly(ethylene oxide). Sardone, L., Sabatini, C., Latini, G., Barigelletti, F., Marletta, G., Cacialli, F., Samorì, P. *J. Mater. Chem.* 17, 1387 – 1391 (2007).
335. Relationship between the Fibroblastic Behaviour and the Surface Properties of RGD-immobilized PCL Membranes. Karakecili, A.G., Satriano, C., Gümüşderelioğlu, M., Marletta, G. *J. Mater. Sci.: Mater. in Medic.* 18 317-319 (2007).
336. Phase Segregation in Thin Films of Conjugated Polyrotaxane-Poly(ethylene oxide) Blends: A Scanning Force Microscopy Study L. Sardone, C. C. Williams, H. L. Anderson, G. Marletta, F. Cacialli , P. Samorì; *Adv. Funct. Mater.* 17, 927-932 (2007).
337. Improved osteogenic differentiation of human marrow stromal cells cultured on ion-induced chemically structured poly-ε-caprolactone. Marletta, G., Ciapetti, G., Satriano, C., Perut, F., Salerno, M., Baldini, N. *Biomaterials* 28, 1132-1140 (2007).
338. Focused Ion Beam Treatment of Self-Assembled Monolayers for Protein Patterning. Giamblanco, N., Tuccitto, N., Licciardello, A., Marletta, G. *European Cells & Materials*. 14,

32 (2007).

339. Stepwise Formation of Ruthenium(II) Complexes Direct Reaction on Organized Assemblies of Thiol-Terpyridine Species on Gold: a Surface Study. Tuccitto, N., Torrisi, V., Cavazzini, M., Morotti, T., Puntoriero, F., Quici, S., Campagna, S., Licciardello, A. *ChemPhysChem* 8, 227-230 (2007).

# Conferences 2007-2008

1. M. Mosca, L. Ambrosone, A. Ceglie. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Antioxidant dispersion in biocompatibile oils". Oral Presentation.
2. M. Mosca, L. Ambrosone, A. Ceglie. Workshop-Applicazioni della risonanza magnetica nella scienza degli alimenti, Università degli Studi del Molise, Campobasso 22-23/05/2008. "Caratterizzazione con tecniche di NMR di formulazioni biocompatibili da usare come modelli per lo studio di attività di antiossidanti". Oral Presentation.
3. M. Mosca, L. Ambrosone, A. Ceglie. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Lipid oxidation in food emulsions initiated by radical gene rating azo-compounds". Oral Presentation.
4. F. Cuomo, F. Lopez, A. Ceglie. 7th Symposium on Polyelectrolytes, Coimbra (Portugal) 16-19/06/2008. "Liposomal formulation for polynucleotides delivery". Poster.
5. L. Ambrosone, R. Angelico, G. Bufalo, F. Lopez, F. Venditti, A. Ceglie. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008 "Nanostructured materials for environmental remediation". Oral Presentation.
6. R. Angelico, A. Ceglie, F. Cuomo, F. Lopez, I. Losito, S. Diomede, F. Palmisano. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Effect of base-pairing upon the reaction of alkylation of a pair of complementary ribonucleotides mediated by a micellar interface". Oral Presentation.
7. L. Ambrosone. Medoliva, Arezzo 17-19/10/2008. "Le nanoscienze per il miglioramento della stabilità ossidativa degli oli di oliva". Oral Presentation.
8. L. Ambrosone. Qualicibi, Positano (SA) 28-30/05/2008. "Dispersioni colloidali come amplificatori naturali delle capacità antiossidanti degli oli di oliva". Oral Presentation.
9. I. Husu, M. Giustini, M.Giomini, A. Mallardi, G. Palazzo. Acta Biophysica Romana 2008 Università degli Studi di Roma Tre, Roma 10-11/04/2008. "Influence of anionic phospholipids on the functionality of QA and QB sites of the photosynthetic Reaction Centre from Rb. Sphaeroides". Poster.
10. M. Autullo, M. Giomini, M Giustini, A. Mallardi, M. Mennuni, G. Palazzo. III Convegno Giovani del Dipartimento di Chimica de "La Sapienza", Roma 18-19/05/2008. Poster.
11. M. Giustini, M. Mennuni, M. Autullo, M. Giomini, G. Venturoli, M. Dezi, A. Mallardi, G. Palazzo. 2nd EuChems Chemistry Congress, Torino 16-20/09/2008. "An optical biosensor for herbicide detection in water". Poster.
12. M. Giustini, M. Airoldi, G. Gennaro, M. Giomini, A.M. Giuliani. 2nd EuChems Chemistry Congress, Torino 16-20/09/2008. "Cadmium interactions with polynucleotides in w/o microemulsion". Poster.
13. G. Palazzo, A. Mallardi, G. Venturoli, M. Giustini. Sensori per il monitoraggio on-line e sul campo GS2008, Bari 6-7/11/2008. "Photosynthetic Reaction Centers Embedded in Polyelectrolyte Multilayer as an Optical Biosensor for Herbicide Detection". Oral Presentation.
14. G. Mangiapia, H. Frielinghaus, E. Paparo, M. Vaccaro, G. D'Errico, L. Paduano. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Kinetic investigation of the vesicle-micelle transition". Poster.
15. M. Vaccaro, G. Mangiapia, A. Costantino, R. Del Litto, F. Ruffo, G. D'Errico, L. Paduano. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Lipid based nanovectors containing ruthenium complexes: a new approach in cancer treatment". Poster.
16. G. D'Errico, A.M. D'Ursi, D. Marsh, L. Paduano. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Interaction of a peptide deriving from the glycoprotein gp36 of the Feline Immunodeficiency Virus and its lipoylated analogue with phospholipid membranes: an ESR study". Oral Presentation.
17. D. Capsoni, M.C. Mozzati, C.B. Azzoni, M. Bini, V. Massarotti, P. Mustarelli. Convegno dell'UdR di Pavia del CNISM (Consorzio Interuniversitario per le Scienze Fisiche della Materia), Pavia 25/01/2008. "Studio spettroscopico e strutturale di $Li_4Ti_5O_{12}$ sostituito manganese". Oral Presentation.
18. V. Massarotti, D. Capsoni, M. Bini, M.C. Mozzati, C.B. Azzoni, C.B., P. Mustarelli, S. Ferrari, G. Chiodelli. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-

29/02/2008. "Cation distribution and valence states in Mn substituted $Li_4Ti_5O_{12}$". Oral Presentation.

19. C. Milanese, A. Girella, G. Bruni, P. Cofrancesco, V. Berbenni, A. Marini. Convenzione Quadro di Cooperazione Generale tra le Università di Sassari e di Pavia, IV Meeting Scientifico–Culturale, Sassari 23–24/05/2008. "Stoccaggio di idrogeno per applicazioni veicolari non inquinanti". Oral Presentation.
20. C. Milanese, A. Girella, G. Bruni, P. Cofrancesco, V. Berbenni, A. Marini, M. Villa, A. Colella, P. Matteazzi. P. International Symposium on Metal-Hydrogen Systems – MH2008, Reykjavík (Islanda) 24–28/06/2008. "Ball-milled Mg – Ni – Cu mixtures: phase analysis and sorption performances". Poster.
21. C. Milanese. An Introduction to High Performance Gas Sorption Instruments for Hydrogen Storage Applications – One day seminar, Lion (France) 4/07/2008. "Hydrogen storage in Mg–based materials". Invited Lecture.
22. C. Milanese, A. Girella, G. Bruni, P. Cofrancesco, V. Berbenni, M. Villa, P. Matteazzi, A. Marini. A. 2nd EuChems Chemistry Congress, Torino 16-20/09/2008. "Magnesium-Nickel-Copper mixtures for hydrogen storage: phase analysis and sorption performances". Poster.
23. P. Galinetto, M.C. Mozzati, D. Capsoni, M. Bini, S. Ferrari, V. Massarotti. XCIV Congresso Nazionale della Società Italiana di Fisica, Genova 22–27/09/2008. "Studio spettroscopico di $Li_4Ti_5O_{12}$ drogato con ioni di transizione". Oral Presentation.
24. A. Girella, C. Milanese, G. Bruni, P. Cofrancesco, V. Berbenni, P. Matteazzi, A. Marini. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Hydrogen sorption performances of Mg–Ni and Mg–Ni–C (graphite) nanocomposites". Oral Presentation.
25. V. Berbenni, G. Bruni, C. Milanese, A. Girella, A. Marini. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Solid state synthesis and thermal characterization of $CuFe_2O_4$ prepared from milled mixtures $Cu(OH)_2 \cdot CuCO_3$–4 $FeC_2O_4 \cdot 2H_2O$". Oral Presentation.
26. D. Capsoni, M. Bini, V. Massarotti, S. Ferrari, M.C. Mozzati, P. Galinetto. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Synthesis and characterization of undoped and Mn doped $LiFePO_4$". Oral Presentation.
27. G. Bruni, V. Berbenni, C. Milanese, A. Girella, P. Cofrancesco, A. Cardini, A. Marini. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Looking for the polymorph stable at room temperature". Oral Presentation.
28. C. Milanese, A. Girella, G. Bruni, P. Cofrancesco, V. Berbenni, P. Matteazzi, A. Marini. 3rd International Conference on Surfaces, Coatings and Nanostructured Materials (NanoSmat 2008), Barcellona (Spain) 21–24/10/2008. "Ball-milled Mg – Ni – Cu mixtures: phase analysis and sorption performances". Poster.
29. C. Milanese, A. Girella, G. Bruni, V. Berbenni, P. Matteazzi, A. Marini. Workshop on nanomaterials: production, characterization and industrial applications, Milano 3/12/2008. "Mg-based nanomaterials for on-board $H_2$ storage". Poster.
30. A. Salis, M.S. Bhattacharyya, V. Solinas, M. Monduzzi. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "The surface chemistry of the support affects the loading and the catalytic activity of an immobilised lipase". Oral Presentation.
31. M. Monduzzi, R. Angius, S. Lampis, S. Murgia, A. Falchi, G. Diaz, M. Mano, M. Giacca. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Lipid Based Nanostructures for Drug Delivery Formulations". Poster.
32. A. Salis, D. Bilanicova, M.C. Pinna, B.W. Ninham, M. Monduzzi, M. Ion Specific Phenomena in Physics, Chemistry and Biology, Garching (Germany) 15-17/09/2008. "Ion Specific Effectrs in Biocatalysis". Invited Lecture.
33. M. Monduzzi. Workshop Nanomedicine ETP, Madrid (Spain) 25-26/11/2008. "Nanostructures disign for molecular recognition, and nanosize hazard". Invited Lecture.
34. D. Berti, P. Baglioni, F. Baldelli Bombelli, M. Banchelli, F. Betti, S. Milani. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Self-assembly of Nucleolipids". Oral Presentation.
35. F. Baldelli Bombelli, F. Betti, F. Gambinossi, M. Lagi, D. Berti, G. Caminati, T. Brown, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Self-assembled DNA nanostructures supported by liposome surface: a comparison between different building strategies". Poster.
36. M. Banchelli, D. Berti, G. Caminati, F. Betti, F. Baldelli Bombelli, T. Brown, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008.

"Phospholipids membranes decorated by cholesterol-based oligonucleotides as addressable soft nanostructures". Poster.

37. M. Banchelli, S. Milani, D. Berti, F. Baldelli Bombelli, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Can molecular recognition between complementary bases drive the interaction between nucleolipid assemblies and polynucleotides?". Poster.
38. F. Betti, F. Baldelli Bombelli, D. Berti, A. Brandt, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Microstructure of ternary systems of phospholiponucleosides". Poster.
39. D. Berti, P. Luciani, M. Fortini, L. Di Cesare Mannelli, D. Manetti, C. Gherardini, F. Gualtieri, A. Bartolini, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Direct modulation of Ga protein in a receptor-independent manner: role of liposomes as membrane models and drug delivery systems". Poster.
40. E. Carretti, R. Giorgi, D. Chelazzi, D. Berti, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Soft Matter for the cleaning of wall paintings". Poster.
41. E. Carretti, L. Dei, E. Fratini, D. Berti, J. Teixeira, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Oil-in-water microemulsions based gels as low environmental impact cleaning tools for painted surfaces". Poster.
42. D. Berti. AAPS, Toronto (Canada), 22-25/06/2008. "Nucleolipoplexes: a New Paradigm for Phospholipid Bilayer-Nucleic Acid Interactions". Invited Lecture.
43. D. Berti. XXII Conference of the European Colloid and Interface Society (ECIS), Cracow (Poland) 31/08-5/09/2008. "Hybrid Lipid/DNA Self-Assemblies". Oral Presentation.
44. P. Lo Nostro, L. Giustini, E. Fratini, B.W. Ninham, F. Ridi, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Threading, growth and aggregation of pseudopolyrotaxanes". Oral Presentation.
45. M. Ambrosi, P. Lo Nostro, R. Ramsch, E. Fratini, E. Carretti, V. Alfredsson, B.W. Ninham, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Nanostructures from vitamin C derivatives". Poster.
46. L. Tattini, P. Lo Nostro, E. Falletta, M. Bonini, F. Weichelt, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Synthesis and characterization of PZT nanoparticles". Poster.
47. P. Lo Nostro. Internal Seminar of the CNR "Istituto di Chimica del Riconoscimento Molecolare", Milano 12/03/2008. "Self-assembly di derivati della Vitamina C". Invited Lecture.
48. P. Lo Nostro, P. Baglioni. Workshop of Cost Action D43 "Functionalized Materials and Interfaces", Technical University Berlin (Germany) 2-4/04/2008. "From Nanosystems to the Real World". Invited Lecture.
49. P. Lo Nostro, P. Baglioni. Workshop "Nanotechnology and Nanomaterials", Oporto (Portugal) 14/05/2008. "Nanosystems for textile finishing". Invited Lecture.
50. P. Lo Nostro, B.W. Ninham. XXII Conference of the European Colloid and Interface Society (ECIS), Cracow (Poland) 31/08-5/09/2008. "Effect of chirality on nanoassemblies". Oral Presentation.
51. R. Basosi. Governo e sviluppo del territorio, Siena 11/01/2008. "L'uso razionale ed efficiente dell'energia e le potenzialità delle fonti rinnovabili come volano dello sviluppo sostenibile del territorio senese". Invited Lecture.
52. M.L. Parisi, A. Sinicropi, R. Basosi, E. Busi, S. Fusi, L. Latterini, A. Melloni, V. Zanirato, M. Olivucci. Winter School on Physical Organic Chemistry - WISPOC 2008, Bressanone (BZ) 27/01-1/02/2008. "An experimental and Computational investigation in solution of a Zwitterionic bio-mimetic and photochemical Switch". Oral Presentation.
53. A. Sinicropi, M.L. Parisi, R. Basosi, E. Busi, S. Fusi, L. Zatterini, A. Melloni, V. Zanirato, M. Olivucci. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "An Experimental and Computational Investigation in solution of a Zwitterionic bio-mimetic and photochemical Switch". Poster.
54. S. Pistolesi, L. Rossigni, E. Ferro, R. Basosi, L. Trabalzini, R. Pogni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "The aggregation state of humanin in solution studied by site directed spin labelling". Oral Presentation.
55. M.C. Baratto, D.A. Lipscomb, C.C.R. Allen, M.J. Larkin, R. Basosi, R. Pogni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "EPR Characterization of a Novel Naphthalene Dioxygenase". Oral Presentation.

56. S. Giansanti, C. Teutloff, R. Basosi, F. Lendzian, R. Pogni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. “Fast Freeze-Quench Multifrequency EPR Investigation of Factoperoxidase Catalytic Intemediates”. Poster.
57. M. Federici, M. Basile, R. Pogni, R., Basosi. First European Workshop on biotechnology for lignocellulose biorefineries, Copenhagen (Denmark) 27-28/03/2008. “Environmental and economical advantages of the local scale: a pilot biodiesel production line in the Province of Siena, Italy”. Poster.
58. R. Basosi, B. Brogioni, A. Sinicropi, D. Biglino, E.J. Reijerse, W. Lubitz, R. Pogni. 41st Annual International Meeting of the Electron Spin Resonance Group of the Royal Society of Chemistry, London (United Kingdom) 6-10/04/2008. “Advanced Techniques and Applications of EPR. Characterization of radical intermediates in the laccase-mediator systems involved in eco-sustainable processes by a multifrequency EPR and DFT/PCM approach”. Poster.
59. S. Vanhulle, R. Basosi, B. Francois, K. Grieder, I. Jager, A. Jarosz-Wilkolazka, L. Martins, T. Keshavarz, C. Purity, A. Sandrelli, G. Sannia, K. Scheibner, M. Senner, C. Tamerler, T. Tron, C.M. Bols. 4th European Meeting in Oxizymes, Helsinki (Finland) 16-18/06/2008. “Toward the development of novel sustainable bioprocesses for the colour industries”. Invited Lecture.
60. S. Giansanti, C. Teutloff, R. Basosi, F. Lendzian, R. Pogni. 4th European Meeting in Oxizymes, Helsinki (Finland) 16-18/06/2008. “Fast Freeze-quench Multifrequency EPR Investigation of Lactoperoxidase Catalytic Intermediates”. Oral Presentation.
61. R. Pogni, S. Giansanti, R. Basosi. 4th European Meeting in Oxizymes, Helsinki (Finland) 16-18/06/2008. “Bleaching of textile dyes in wastewaters: effect of laccases with and without mediators”. Oral Presentation.
62. R. Pogni, B. Brogioni, S. Giansanti, R. Basosi. Last Annual Meeting European Project Sophied, Malaga (Spain) 28-30/05/2008. “Insights on the Catalytic mechanism of Laccases: Blue Enzymes for Green Chemistry”. Invited Lecture.
63. S. Forte, R. Basosi, R. Pogni. Last Annual Meeting European Project Sophied, Malaga (Spain) 28-30/05/2008. “Structural Characterization of dyes obtained by enzymatic synthesis”. Oral Presentation.
64. M. Federici, R. Pogni, S. Giansanti, R. Basosi. Last Annual Meeting European Project Sophied, Malaga (Spain) 28-30/05/2008. “Introduction to Life Cycle Assessment Analysis of bio-synthetic dyes”. Oral Presentation.
65. R. Basosi. Firenze Duemilaventi: l’economia salvata dalle energie rinnovabili, Firenze 19/06/2008. “Fonti rinnovabili ed uso razionale dell’energia come pilastri dello sviluppo sostenibile”. Invited Lecture.
66. F. J. Ruiz-Duenas, R. Pogni, M. Morales, S. Giansanti, M.J. Martìnez, R. Basosi, A.T. Martìnez. 8th International Peroxidase Symposium, FESB 2008, Tampere (Finland) 17-22/08/2008. “Tailoring catalytic protein-radicals in *Pleurotus eryngii* versatile peroxidase”. Oral Presentation.
67. P. Fulini, E. Cecchini, A. Baldini, M. Federici, R. Basosi. II Congresso Nazionale AIGE, Pisa 4-5/09/2008. “An Hydrogen-solar system for zero emission heating and cooling”. Oral Presentation.
68. R. Pogni, S. Giansanti, R. Basosi. 4th European BioRemediation Conference, Chania (Greece) 3-6/09/2008. “Bleaching of textile dyes in wastewaters: effect of laccases with and without mediators”. Poster.
69. S. Bani, I. Cioni, V. Millarini, I. Ciullini, E. Fatarella, F. Briganti, A. Scozzafava, S. Giansanti, R. Pogni, R. Basosi. 4th European BioRemediation Conference, Chania (Greece) 3-6/09/2008. “Bio-based advanced oxidative process for textile wastewater treatment”. Poster.
70. F. Lendzian, R. Pogni, C. Jung, V. Schunemann, C. Teutloff, M.C. Baratto, S. Giansanti, F.J. Ruiz-Duenas, A.T. Martìnez, R. Basosi. International COST Meeting on Advanced Paramagnetic Resonance Methods in Molecular Biophysics, COST P15 Action, Siena 24-26/09/2008. “Amino Acid Radicals and High-Valent Metal Centers in Enzymatic Catalysis Studied by Freeze-Quench High-Field EPR and ENDOR Spectroscopy”. Invited Lecture.
71. S. Pistolesi, L. Rossini, E. Ferro, R. Basosi, L. Trabalzini, R. Pogni. International COST Meeting on Advanced Paramagnetic Resonance Methods in Molecular Biophysics, COST P15 Action, Siena 24-26/09/2008. “Insights into humanin translocation across model neuronal membranes: a SDSL-EPR study”. Poster.

72. A. Sinicropi, M.L. Parisi, E. Busi, G. Predieri, R. Basosi. International COST Meeting on Advanced Paramagnetic Resonance Methods in Molecular Biophysics, COST P15 Action, Siena 24-26/09/2008. "Structural and Magnetic Properties Characterization of metal chelates of 2-hydroxy-4-methylthiobutanoic acid by EPR and DFT calculations". Poster.
73. E. Busi, V. Travagli, I. Zanardi, A. Gabbrielli, R. Basosi. 24-26 settembre 2008, Siena. International COST Meeting on Advanced Paramagnetic Resonance Methods in Molecular Biophysics, COST P15 Action. "Role of the molecular oxygen and iron on the degradation pattern of hyaluronan". Poster.
74. R. Pogni. International COST Meeting on Advanced Paramagnetic Resonance Methods in Molecular Biophysics, COST P15 Action, Siena 24-26/09/2008. "Characterization of transient radical intermediates in the catalytic mechanism of oxidative enzymes: the central role of EPR Spectroscopy". Invited Lecture.
75. F. Ruzzenenti, R. Basosi. I International Workshop on Constructural Theory Shape and Thermodynamics, Firenze 25-26/09/2008. "Complexity change in system's structure as a result of space symmetry rupture: an example from transport system". Oral Presentation.
76. R. Basosi. ZeroEmission Rome 2008 La sfida al $CO_2$: dal Protocollo di Kyoto al 2020, Roma 3/10/2008. "Il ruolo della finanza nella dimensione regionale per il raggiungimento degli obiettivi del protocollo di Kyoto". Invited Lecture.
77. R. Basosi. FEI, Forum Energetico Internazionale, Efficienza energetica, Pisa 22-24/10/2008. "Basi teoriche e termodinamiche dell'efficienza energetica". Invited Lecture.
78. R. Basosi. Pianificazione Energetica su scala locale e strumenti di supporto decisionale: risultati dello studio FORMEZ, Roma 27/11/2008. "La pianificazione energetica su scala locale". Invited Lecture.
79. R. Basosi. Presentazione del 2° rapporto di sostenibilità della Provincia di Firenze, Firenze 11/12/2008. "Il Piano Energetico Provinciale nel quadro della Programmazione regionale". Invited Lecture.
80. F. Gambinossi, M. Banchelli, F. Baldelli Bombelli, D. Berti, T. Brown, P. Baglioni, G. Caminati. 11th European Conference on Organized Films (ECOF11), Potsdam (Germany) 09-11/07/2008. "Anchoring of DNA self-assembled nanostructures on supported lipid bilayers". Oral Presentation.
81. S. Ciappelli, F. Gambinossi, P. Baglioni, G. Caminati. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Solvent-memory effect for tuning the conformational properties of conjugated polymers in Langmuir monolayers". Poster.
82. F. Gambinossi, L. Lorenzelli, S. Ciappelli, P. Baglioni, G. Caminati. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Silicon oxide surface functionalization by self-assembled nanolayers for micro-cantilever transducers". Poster.
83. F. Gambinossi, T. Viitala, S. Ciappelli, M. J. Swann, P. Baglioni, G. Caminati. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Comparative study of the step-wise formation of supported phospholipid bilayers: dissipative quartz crystal microbalance, ellipsometry and dual polarization interferometry". Poster.
84. F. Baldelli Bombelli, F. Betti, F. Gambinossi, M. Lagi, D. Berti, G. Caminati, T. Brown, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Self-assembled DNA nanostructures supported by liposome surface: a comparison between different building strategies". Poster.
85. S. Ciappelli, F. Gambinossi, P. Baglioni, G. Caminati. 11th European Conference on Organized Films (ECOF11), Potsdam (Germany) 09-11/07/2008. "Novel Langmuir-Blodgett/Layer-by-layer assemblies for organic photovoltaic devices". Poster.
86. F. Gambinossi, A.M. Stadler, J.M. Lehn, P. Baglioni, G. Caminati. 11th European Conference on Organized Films (ECOF11), Potsdam (Germany) 09-11/07/2008. "Metallosupramolecular architectures at the liquid-air interface". Poster.
87. F. Ridi, P. Luciani, E. Fratini, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Low temperature spectroscopic and calorimetric investigation of water confined in cement pastes". Poster.
88. M. Ambrosi, E. Fratini, P. Baglioni. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Small-angle X-ray scattering study of hydrous zirconia nanopowder dispersions". Poster.
89. A. Faraone, E. Fratini, P. Baglioni. 4th Biennal American Conference on Neutron Scattering (ACNS), Santa Fe (USA) 11-15/05/2008. "Dynamics of Hydration Water in Nanosized Polyoxomolybdate Clusters". Poster.

90. E. Fratini. IX School of Neutron Scattering, S. Margherita di Pula (CA) 22/09-3/10/2008. "Dynamics of Hydration Water by QENS". Invited Lecture.
91. C. Bonechi, S. Ristori, S. Martini, G. Martini, C. Rossi. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Nuclear Magnetic Resonance studies on the conformational structures of Bradykinin with model membrane" Oral Presentation.
92. C. Bonechi, S. Martini, A. Magnani, C. Rossi. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Stacking interactions studies of resveratrol (trans-3,5,4'-trihydroxystilbene) in solution by nuclear magnetic resonance and infrared spectroscopy". Oral Presentation.
93. M. Aggravi, C. Bonechi, N. Marchettini, E. Tiezzi, A. Donati. XXXVII Congresso Nazionale di Chimica Fisica, Camogli (GE) 24-29/02/2008. "Complexation of As(III) and As(V) in rat and human erythrocytes". Poster.
94. C. Rossi, S. Martini, C. Bonechi. 7th International Conference Polymer-Solvent Complexes & Intercalates, Marrakech (Morocco) 21-23/05/2008. "Nuclear Spin Relaxation Studies Of Polymer-Solvent Interactions". Oral Presentation.
95. S. Martini, C. Bonechi, C. Rossi. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Nuclear Spin Relaxation Studies of Water-Protein Interactions". Oral Presentation.
96. S. Martini, C. Bonechi, C. Rossi. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Aggregation behaviour of Resveratrol in solution and its interaction with proteins". Oral Presentation.
97. C. Bonechi, S. Martini, C. Rossi. VII Convegno annuale del C.S.G.I., Vallombrosa (FI) 16-17/10/2008. "Studio dei processi di interazione quercetina-albumina e quercetina-3-O-β-D-glucopiranoside-albumina mediante NMR". Poster.
98. L. Bracchini, D.A. Massimo, S.A. Loiselle, S. Chiara, S. Focardi, C. Rossi. Oceans Science Meeting, Orlando (USA) 2-7/03/2008. "The optical properties of Cdom in the mediterranean basin. Exploring the role of physical and biological forcing". Oral Presentation.
99. C. Rossi, F. Bartolini, A. Magnani, A. Foletti, S. Martini, M. Ricci. First Biomedical Electronics and Biomedical Informatics, Rodhes (Greece) 20-22/08/2008. "*Saccharomyces cerevisiae* metabolic process by mathematical modellingand in vivo 13C NMR". Invited Lecture.
100. F. Lopez, M. Mennuni, M. Giustini, M. Giomini, M. Dezi, G. Venturoli, A. Mallardi, G. Palazzo. 2nd IEEE International Workshoop On Advances in Sensors and Interfaces, Bari 26-27/06/2007. "Photosynthetic Reaction Centers embedded in polyelectrolyte multilayer as a tool in the determination of PSII herbicides". Oral Presentation.
101. F. Cuomo, F. Lopez, R. Angelico, G. Colafemmina, A. Ceglie. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Nucleotides and nuclelipids interactions during Multi Lamellar Vesicles formation". Oral Presentation.
102. F. Cuomo, F. Lopez, R. Angelico, G. Colafemmina, A. Ceglie. 7th Annual Surface and Colloid Symposium, Lund (Sweden) 14-16/11/2007. "Nucleotides and nuclelipids interactions during Multi Lamellar Vesicles formation". Poster.
103. F. Cuomo, A. Ceglie, L. Ambrosone, G. Palazzo, F. Lopez. 7th Annual Surface and Colloid Symposium. P 22. Lund (Sweden) 14-16/11/2007. "Fluorescence studies on the interaction properties between CTAB micelles and monophosphate-nucleotides". Poster.
104. M. Mosca, L. Ambrosone, A. Ceglie. 21st Conference of the European Colloid and Interface Society, Ginevra, (Svizzera), 10-14/09/2007. "Effect of ascorbic acid dispersion on the oxidation of biocompatible W/O emulsions". Poster.
105. M. Mosca, L. Ambrosone, A. Ceglie. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Antioxidant dispersions in biocompatible oils". Poster.
106. R. Angelico, A. Ceglie, F. Cuomo. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Nucleotide-based Ionic Surfactants". Oral Presentation.
107. R. Angelico, K. Mortensen, U. Olsson, and G. Palazzo. 4$^{th}$ European Conference on Neutron Scattering, Lund Sweden, 25-29/06/2007. "Rheo-SANS study of lecithin wormlike reverse micelles: Tumbling dynamics during shear-induced isotropic-to-nematic transition". Poster.
108. G. Mangiapia, L. Paduano, H. Frielinghaus, G. D'Errico, O. Ortona, R. Sartorio. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Physico-Chemical and Structural Properties of hydrogels formed by Chitosan, in the Presence and Absence of Poly(vinylpyrrolidone) and Sodium decylsulfate". Poster.

109. A. Molisso, O. Ortona, G. D'Errico, G. Mangiapia, L. Paduano. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "The aggregative behavior of hydrophobically modified chitosans of 10% level substitution". Poster.
110. M. Vaccaro, C. von Corswant, O. Söderman. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Investigation of the adsorption of PEG1500-12-acyloxystearate surfactants into phospholipids bilayers: An Ellipsometry and Cryo-TEM study". Poster.
111. V. Massarotti, D. Capsoni, M. Bini, M.C. Mozzati, C.B. Azzoni, P. Galinetto. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Sostituzione cationica e proprietà in bronzi di tungsteno". Oral Presentation.
112. S. Morandi, G. Ghiotti, M.G. Paganini, E. Giamello, M. Bini, D. Capsoni, V. Massarotti. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Structural and spectroscopic characterisation of $Mo_{1-x}W_xO_{3-\delta}$ oxides". Poster.
113. C. Milanese, A. Girella, V. Berbenni, G. Bruni, P. Cofrancesco, A. Marini. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Nanomateriali a base Mg per lo stoccaggio di idrogeno". Poster.
114. C. Milanese, A. Girella, V. Berbenni, G. Bruni, P. Cofrancesco, A. Marini. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Caratterizzazione di nanomateriali per lo stoccaggio di idrogeno mediante analisi manometrica". Poster.
115. G. Bruni, V. Berbenni, C. Milanese, P. Cofrancesco, A. Girella, A. Marini. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Polimorfismo del D-mannitolo". Poster.
116. V. Berbenni, C. Milanese, G. Bruni, A. Marini. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Effetto Combinato di Energia Meccanica e Termica sulla formazione allo stato solido di $NiFe_2O_4$ dal sistema $2NiCO_3{\cdot}3Ni(OH)_2{\cdot}4H_2O$ – $FeC_2O_4{\cdot}2H_2O$". Poster.
117. V. Berbenni, G. Bruni, C. Milanese, A. Marini. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Calibrazione di un Calorimetro Differenziale a Scansione (DSC) per misure di capacità termica". Poster.
118. D. Capsoni, M. Bini, V. Massarotti. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Proprietà di $Li_4Ti_5O_{12}$: il ruolo del drogante". Poster.
119. M.C. Mozzati, C.B. Azzoni, V. Massarotti, D. Capsoni, M. Bini. XCIII Congresso Nazionale della Società Italiana di Fisica, Pisa 24–29/09/2007. "Proprietà magnetiche della calcioferrite $Ca_2Fe_2O_5$: nuove evidenze". Oral Presentation.
120. C. Milanese, V. Berbenni, G. Bruni, A. Marini. 1ª Conferenza Nazionale del Programma NIC (Nanotecnologie nell'Industria Chimica), Fiera RICHMAC 2007, Milano 2–5/10/2007. "Nanomateriali a base Mg per lo stoccaggio di idrogeno". Poster.
121. A. Salis, M.F. Casula, D. Meloni, M.S. Bhattacharyya, M. Monduzzi, E. Dumitriu, V. Solinas. VII International Conference of the Romanian Catalysis Society (ROMCAT2007), Bucharest (Romania) 21-23/06/2007. "Synthesis, characterization and chemical modification of silica based mesoporous materials for lipases immobilization". Oral Presentation.
122. D. Berti, F. Baldelli Bombelli, F. Betti, S. Milani, P. Baglioni. 4th European Conference on Neutron Scattering, Lund (Sweden) 25-29/06/2007. "Self-assembly of Nucleolipids: Insights from Neutron Scattering". Oral Presentation.
123. F. Baldelli Bombelli, F. Betti, D. Berti, F. Pini, M. Henrich, P. Baglioni P. 4th European Conference on Neutron Scattering, Lund (Sweden) 25-29/06/2007. "Small Angle Neutron Scattering Investigation of the Self-Assembling Behavior of Di-$C_{12}$P-Nucleosides in Solution". Poster.
124. D. Berti, S. Milani, F. Baldelli Bombelli, P. Baglioni. 234th ACS National Meeting, Boston (USA) 19-23/08/2007. "Nucleolipoplexes: A new paradigm for phospholipid bilayer-nucleic acid interactions". Oral Presentation.
125. P. Baglioni, D. Berti, M. Banchelli, F. Baldelli, F. Betti. 234th ACS National Meeting, Boston (USA) 19-23/08/2007. "Amphiphilic self-assemblies decorated by nucleobases". Oral Presentation.
126. P. Luciani, M. Fortini, D. Berti, L. Di Cesare Mannelli, D. Manetti, C. Gherardini, F. Gualtieri, A. Bartolini, P. Baglioni. XXI Conference of the European Colloid and Interface Society (ECIS), Geneva (Switzerland) 10-14/09/2007. "Direct modulation of Gi protein in a receptor-independent manner: role of liposomes as membrane models and drug delivery systems". Poster.

127. S. Milani, F. Baldelli Bombelli, D. Berti, P. Baglioni. XXI Conference of the European Colloid and Interface Society (ECIS), Geneva (Switzerland) 10-14/09/2007. "Nucleolipoplexes: complementary polynucleotides intercalation in nucleolipid lamellar phases". Poster.
128. S. Cicchi, G. Ghini, L. Lascialfari, D. Berti, F. Betti. VIII Congresso Nazionale di Chimica Supramolecolare, Trieste 19-22/09/2007. "New organogelators based on a 3,4-dihydroxy-pyrrolidine nucleus and their applications". Poster.
129. P. Lo Nostro. Spring Seminar of the Dept. of Chemical Engineering, Oklahoma University, Norman (USA) 17/05/2007. "Supramolecular nanoassemblies from cyclodextrins: effects of temperature, salts and dissolved gases on the formation of pseudopolyrotaxanes". Invited Lecture.
130. P. Lo Nostro. Internal Seminar GeorgiaTech, Atlanta (USA) 29/05/2007. "Effects of temperature, salts and dissolved gases on the formation of pseudopolyrotaxanes". Invited Lecture.
131. P. Lo Nostro, M. Ambrosi, E. Fratini, F. Ridi, E. Carretti, V. Alfredsson, B.W. Ninham, P. Baglioni. Nanotech2007, Santa Clara (USA) 20-24/05/2007. "Nanoassemblies from Vitamin C Derivatives". Oral Presentation.
132. E. Falletta, M. Bonini, E. Fratini, A. Lo Nostro, A. Becheri, P. Lo Nostro, P. Baglioni. Nanotech2007, Santa Clara (USA) 20-24/05/2007. "Poly(acrylic) acid coated Silver Nanoparticles for Antimicrobial Textile Finishing". Poster.
133. L. Tattini, E. Falletta, M. Bonini, F. Weichelt, P. Lo Nostro, P. Baglioni. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Synthesis and characterization of PZT nanoparticles". Poster.
134. L. Tattini, S. Rossi, N. Ritter, P. Lo Nostro, P. Baglioni. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Aggregation of semifluorinated n-alkanes in selective solvents". Poster.
135. P. Lo Nostro, L. Giustini, E. Fratini, B.W. Ninham, F. Ridi, P. Baglioni. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Threading, Growth and Aggregation of Pseudopolyrotaxanes". Poster.
136. R. Basosi. Follonica Ecocity: risparmio energetico ed energie rinnovabili, Follonica (GR) 26/04/2007. "Uso razionale dell'energia e fonti rinnovabili: i pilastri della sostenibilità ambientale". Invited Lecture.
137. R. Basosi. XII Conferenza Regionale sull'ambiente, Terra Futura 2007, Firenze 18/05/2007. "La pianificazione energetica tra uso razionale e sviluppo delle rinnovabili". Invited Lecture.
138. R. Basosi. XII Conferenza Regionale sull'ambiente, Terra Futura 2007, Firenze 18/05/2007. "Il Piano Energetico: Il contributo del mondo accademico". Invited.
139. R. Basosi. Fiera dell'Energia, Firenze 2/06/2007. "Il programma energetico Provinciale tra uso razionale e sviluppo delle rinnovabili". Invited Lecture.
140. F.J. Ruiz-Duenas, M. Morales, M. Perez-Boada, R. Pogni, R. Basosi, M.J. Martinez, K. Piontek, A. T. Martinez. International Conference on Biotechnology in the Pulp and Paper Industry, Madison (USA) 10-14/06/2007. "Structure-function characterization of different substrate oxidation sites in *Pleurotus* versatile peroxidise". Invited Lecture.
141. A. Sinicropi, R. Basosi, M. Olivucci. RADAM 2007-Radiation Damage in Biomolecules Conference, Dublin (Ireland) 19-22/06/2007. "Recent Applications of a QM/MM scheme at the CASPT2//CASSCF/AMBER (or CHARMM) level of theory in Photochemistry and Photobiology". Invited Lecture.
142. R. Pogni, B. Brogioni, A. Sinicropi, M. C. Baratto, P. Giardina, G. Sannia, R. Basosi. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Evidence for different routes of oxidation in the laccase-mediator system: a multifrequency EPR and DFT study". Oral Presentation.
143. A. Sinicropi, S. Pistolesi, R. Pogni, R. Basosi, M. Olivucci. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "The fluorescence of Tryptophan in Monellin and Parvalbumin Resolved at the ab inizio Multiconfigurational Perturbation Theory Level". Oral Presentation.
144. R. Basosi. Energia in Armonia con l'Ambiente, Forum Provinciale Agenda 21 Terre di Siena, Siena 27/06/2007. "La pianificazione energetica tra uso razionale e sviluppo delle rinnovabili". Invited Lecture.
145. R. Basosi, M. Federici. Energie Rinnovabili: risparmio opportunità, soluzioni, Sovicille (SI) 18/11/2007. "L'energia tra necessità e limitatezza delle risorse: Il ruolo del risparmio

energetico e delle energie rinnovabili". Invited Lecture.

146. F. Ruzzenenti, M. Pagni, M. Federici, C. Bruni, R. Basosi. Advances in Energy Studies. Perspectives on Energy Future, Porto Venere (SP) 12/09/2007. "Energy conservation policy: should public administration give the good example?". Poster.
147. M. Federici, R. Basosi. Advances in Energy Studies. Perspectives on Energy Future, Porto Venere (SP) 12/09/2007. "Air versus terrestrial transport modalities: an environmental comparison". Poster.
148. F. Ruzzenenti, M. Federici, R. Basosi. Advances in Energy Studies. Perspectives on Energy Future, Porto Venere (SP) 12/09/2007. "Energy efficiency and structural change in production: an analysis of long-term impacts in the road freight transport sector". Invited Lecture.
149. R. Basosi, R. Pogni, M. C. Baratto, A. Sinicropi, S. Giansanti, S. Pistolesi, B. Brogioni. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. "Structure-activity relationships in Oxizymes". Oral Presentation.
150. B. Brogioni, D. Biglino, A. Sinicropi, E.J. Reijerse, W. Lubitz, R. Basosi, R. Pogni. 29th Annual discussion Meeting- Magnetic Resonance in Biophysical Chemistry, Göttingen (Germany) 26-29/09/2007. "Structure determination of radical mediators involved in the catalytic activity of laccase-like enzymes in eco-sustainable processes: a multifrequency EPR study". Oral Presentation.
151. A. Sinicropi, M.L. Parisi, E. Busi, G. Predieri, R. Basosi. Gruppo Italiano di Risonanza di Spin Elettronico-GIRSE07, Vietri sul Mare (SA) 30/09-3/10/2007. "Characterization of metal chelates of 2-hydroxy-4-methylthiobutanoic acid in animal feeding by EPR and DFT calculations". Poster.
152. R. Pogni, B. Brogioni, A. Sinicropi, M.C. Baratto, P. Giardina, G. Sannia, W. Lubitz, R. Basosi. Gruppo Italiano di Risonanza di Spin Elettronico-GIRSE07, Vietri sul Mare (SA) 30/09-3/10/2007. "Evidence for different routes of oxidation in the laccase-mediator system: a multifrequency EPR and DFT study". Poster.
153. R. Basosi. MEC: XI International Utilities Forum, Venezia 9-11/05/2007. "Fonti rinnovabili tra vecchie diffidenze e nuove opportunità". Invited Lecture.
154. G. Caminati, F. Gambinossi, D. Berti, M. Banchelli, P. Baglioni, T. Brown, B. Norden. XXXVI Congresso Nazionale di Chimica Fisica, Gallipoli (LE), 17-22/06/2007. "Addressable DNA architectures anchored to Supported Lipid Bilayers". Oral Presentation.
155. G. Caminati, F. Gambinossi, D. Berti, M. Banchelli, P. Baglioni, T. Brown, B. Norden. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Controlled grafting of oligonucleotide architectures on supported lipid bilayers". Oral Presentation.
156. S. Ciappelli, F. Gambinossi, P. Baglioni, G. Caminati. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Confocal Laser Scanning Microscopy (CLSM) and Brewster Angle Microscopy (BAM) studies of Hybrid Nanostructures for OLED applications". Oral Presentation.
157. S. Ciappelli, F. Gambinossi, P. Baglioni, G. Caminati. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Solvent-memory effect for tuning the conformational properties of conjugated polymers in Langmuir monolayers". Poster.
158. F. Gambinossi, L. Lorenzelli, P. Baglioni, G. Caminati. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Fabrication of self-assembled nanolayers for microcantilever-based sensors". Poster.
159. S. Morandi, M. Puggelli, G. Caminati. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Antibiotic penetration in supported phospholipid nano-assemblies: comparison between LB films and Supported Lipid Bilayers (SLB)". Poster.
160. F. Gambinossi, T. Viitala, F. Betti, P. Baglioni, G. Caminati. 12th International Conference on Organized Molecular Films (LB-12), Cracow (Poland) 01-05/07/2007. "Step-wise formation of supported Lipid Bilayers on gold substrates studied by Dissipative Quartz Crystal Microbalance and Imaging Ellipsometry". Poster.
161. F. Ridi, P. Luciani, E. Fratini, P. Baglioni. XXI Conference of the European Colloid and Interface Society (ECIS), Geneva (Switzerland) 10-14/09/2007. "Hydration water and microstructure in calcium silicate hydrates". Poster.
162. A.K. Basak, J.P. Celis, P. Ponthiaux, F. Wenger, P. Matteazzi, M. Varvadoulias. 7th International Symposium on Electrochemical Impedance Spectroscopy, Argelès-sur-Mer

(France) 3-8/06/2007. “Electrochemical behavior of HVOF sprayed WC-Co based nanostructured coatings”. Poster.

163. N. Petsas, G. Papapanos, J.P. Celis, P. Matteazzi, A. Moutsatsou, M. Vardavoulias. 21st International Conference on Surface Modifiaction Technologies (SMT 21), Paris (France) 24-26/09/2007. “Study on the Development and Optimization of Nanophased FeCu/Ceramic Thermal Spray Coatings”. Poster.
164. P. Matteazzi. Icaleo® 2007, Orlando (USA) 29/10-1/11/2007. “Micro laser sintering using aggregated nanopowders”. Poster.
165. C. Bonechi, S. Ristori, S. Martini and C. Rossi. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. ”Nuclear Magnetic Resonance study of the conformational structures of Bradykinin interacting with model membrane". Poster.
166. C. Bonechi, S. Martini, M. Ricci, C. D’Addario, A. Magnani, C. Rossi. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. “Stacking interactions studies of resveratrol (trans-3,5,4’-trihydroxystilbene) in solution by Nuclear Magnetic Resonance and Infrared Spectroscopy”. Oral Presentation.
167. C. D’Addario, S. Martini, C. Bonechi, N. Figura, C. Rossi. VI Convegno annuale del C.S.G.I., Santa Vittoria in Matenano (AP) 21-22/09/2007. “Determinazione delle Proprieta’ Antiossidanti e Battericide di Estratti Vegetali”. Poster.
168. S. Martini, M. Consumi, C. Bonechi, C. Rossi, A. Magnani: “Fibrinogen-Catecholamine Interaction as Observed by NMR and Fourier Transform Infrared Spectroscopy”. Oral Presentation.

# *Theses (undergraduate, PhD, master, post-doc)*

U.O. Bologna

1. F. Guiduzzi: Laurea Magistrale "Selezione di mutanti di *Escherichia coli* tolleranti nei confronti di alcoli a corta catena" 2007.
2. E. Martelli: Laurea Magistrale "Sovraespressione in *Escherichia coli* e purificazione dell'inibitore tripsinico di senape MTI-2" 2007.
3. A. Merulla: Laurea Magistrale "Individuazione dei fattori molecolari determinanti l'espressione di interferoni in *Escherichia coli*" 2007.

U.O. Cagliari

4. P. Fadda: PhD Thesis "Microemulsioni innovative per formulazioni biocompatibili" 2008.
5. C. Vinci: Laurea Magistrale "Degradazione ossidativa biomimetica di alcuni coloranti di interesse industriale" 2008.
6. M.I. Marras: Laurea Magistrale "Caratterizzazione biochimica del lievito nitrato assimilatore *Sporobolomyces salmonicolor*" 2008.
7. C. Meloni, : Laurea Magistrale "Purificazione e caratterizzazione funzionale di una variante emoglobinica fetale: HbF Columbus GA (Ggamma94 (FG1) Asp-Asn" 2008.
8. M. Mamusa: Laurea Triennale "Le Nanostrutture del Tensioattivo AOT in Acqua e Liquido Ionico" 2008.
9. R. Angius: PhD Thesis "Nanostrutture Funzionalizzate per il Riconoscimento Molecolare e Valutazione della Biocompatibilità Cellulare" 2007.
10. S. Lampis: Laurea Magistrale "Effetto di Nucleotidi Inseriti nei Cristalli Liquidi del Sistema Monooleina-Acqua" 2007.
11. F. Cugia: Laurea Magistrale "Adsorbimento fisico di lipasi su Silicio mesoporoso per la realizzazione di un biosensore" 2007.
12. R. Piras: Laurea Magistrale "Immobilizzazione del lievito nitrito assimilatore *Sakaguchia dacryoidea*" 2007.
13. R. Lixi: Laurea Magistrale "Demolizione ossidativa di un colorante antrachinonco industriale in presenza di un catalizzatore biomimetico" 2007.
14. L. Grassellini: Laurea Magistrale "Immobilizzazione di una laccasi blu indotta in *Pleurotus sajor caju* dall'acido ferulico" (2007).
15. G. Cireddu: Laurea Magistrale "Caratterizzazione preliminare di una laccasi non-blu indotta in *Pleurotus sajor caju* da acido 4-idrossi-benzen-solfonico" (2007).

U.O. Campobasso

16. F. Cuomo: PhD Thesis "Riconoscimento Molecolare in sistemi Biomimetici di Membrana" 2008.
17. Colalillo: Laurea Triennale "Ossidazione di Lipidi in sistemi colloidali" 2008 (Univ. Molise)
18. R. Recchia: Laurea Quinquennale "Caratterizzazione strutturale di soluzioni di tensioattivi per l'igiene personale" 2008 (Univ. Bari).
19. S. Fischetti: Laurea Magistrale "Meccanismi di denaturazione termica del centro di reazione di Rhodobacter Sphaeroides" 2008 (Univ. Bari).
20. P. Sacco: Laurea Triennale "Separazione di sostanze Xenobiotiche mediante compositi nanostrutturati" 2008 (Univ. Molise).
21. F. Venditti: PhD Thesis "Risanamento e biorisanamento ambientale mediante l'uso di sistemi dispersi" 2007.
22. F. Sarlo: Laurea Triennale "Effetto della cardiolipina sulla stabilità termica del centro di reazione batterico" 2007 (Univ. Bari).
23. M. Trillo: Laurea Triennale "Effetto del trealosio sulla stabilità termica del centro di reazione batterico" 2007 (Univ. Bari).
24. P. Flocco: Laurea Triennale "Ossidazione di oli di oliva extravergini emulsionati con additivi biocompatibili" 2007 (Univ. Molise).

U.O. Catania

25. F. Formosa: PhD Thesis "Polymer Thin Film Processing for Advanced Biomimetic Materials" 2008. G.M.L. Messina: PhD Thesis "Molecular Self-Assembling Processes at Surfaces and Nanopatterning" 2008.
26. M.C. Curatolo: PhD Thesis "Physico-Chemical Properties of Polymeric Biomaterials for Ophtalmic Applications" 2008.
27. V. Torrisi: PhD Thesis "Application of secondary ion mass spectrometry to the study of molecular and supramolecular films" 2008.
28. V. Spampinato: Laurea Magistrale "Assemblaggio di oligonucleotidi su superfici mediante preparazione di strati molecolari di complessi bis-terpidinici" 2008.
29. S. Crifò: Laurea Triennale "Nanostrutturazione di proteine fibrillari su superfici di interesse biologico" 2008.

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# *RESEARCH PROJECTS*

# 1A – Porous air electrodes for energy conversion

*M. Villa, P. Nelli*

## Aims

Developing Ni-based air electrodes for alkaline electrolysis and fuel cells

## Results

We are developing non-precious metal catalysts and electrodes for alkaline fuel cells and electrolysers. We are focusing upon the secondary electrode reactions which cause slow modifications of electrode composition and morphology, and are responsible for processes of "activation" and "ageing". A major role is played by the electrode oxidation processes, and in particular by an initial progressive increase in thickness of the $Ni(OH)_2$/NiOOH layers (activation) followed by formation of increasingly stable oxides which lead to passivation and higher resistive losses (ageing).

A little understood property of Ni cathods in alkaline solution is the capability of absorbing hydrogen, or forming $NiH_x$ ($x$<1) hydrides. The reason is that hydriding and de-hydriding processes are masked by the concurrent processes of hydrogen evolution and Ni oxidation, respectively. They may be studied with electrochemical techniques which determine the charge released after cathodic treatments which are short or long relative to the expected times of hydriding. In Figure 1 we compare the potential evolution of a Ni-foam electrode in 6M KOH under constant anodic current (10 mA/cm$^2$) after one short (1000 s) and one long (20000 s) treatment at −1.3 V. Both charging curves display the expected plateau of $Ni^{II} \rightarrow Ni^{III}$ oxidation around 0.3 V, with different amplitudes due to the "activation" process mentioned above. However, after a long cathodisation we have also a large feature at −0.85 V, which is assigned to Ni and H oxidation occurring in a reaction layer which is reduced by hydrogen diffusing out of the bulk while it is oxidized at the electrolyte side.

The performances of an air electrode depend upon the catalytic properties of active material, which we have found to be affected by treatments, but also upon its morphology, which determines the extension of the "triple phase boundary" where the fluids containing reactants and products meet over the solid catalyst. We have developed a new method, which may be called **electrochemical pressure spectroscopy**, to characterize distribution and dynamic evolution of the triple phase boundary under different current regimes, and following pressure steps applied across the air electrode. Figure 2 shows, as a function of time, the potential of the oxygen electrode of an alkaline fuel cell at a constant current of 160 mA/cm$^2$ when a train of pressure pulses is applied at the electrolyte side. Here, we have slowed down on purpose the approach of pressure to its top value to put into evidence that the cell potential improves significantly up to a 120 mbar value; then it drops rapidly.

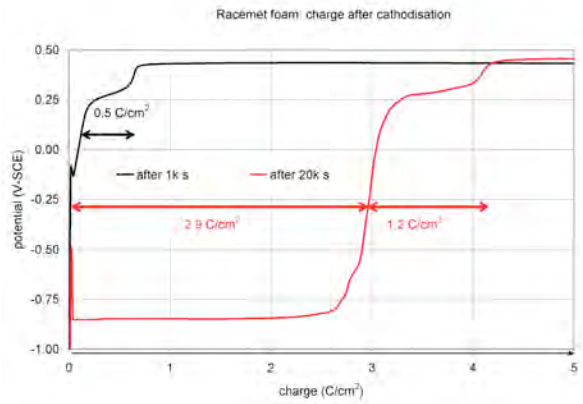


Figure 1. Charging curves of a Ni-foam electrode after short (1ks) and long (20 ks) cathodisation

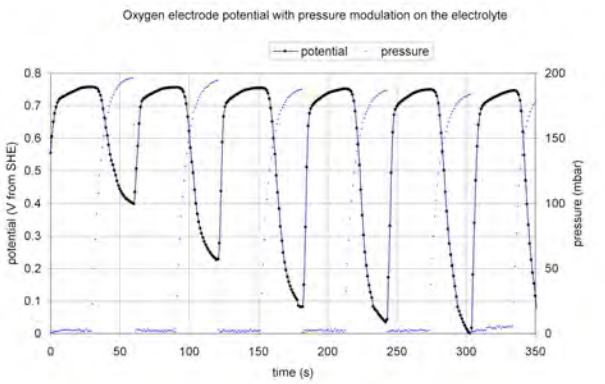


Figure 2. Potential of the oxygen electrode of an alkaline fuel cell during application of a train of pressure pulses on the electrolyte (30% KOH)

The voltage increase is due to an expanded active area, and concomitant lower losses; the voltage drop is due to the electrolyte penetrating the hydrophobic pores previously occupied by $O_2$, and dislodging this reagent. From the pressure where the voltage drop begins and the Laplace law we obtain an effective radius of the hydrophobic pores of 13 μm, which is consistent with the electrode's morphology. We call attention to the different rates at which oxygen flows in and out of the pores, and to the "learning" effect which causes a progressively larger drop of voltage at each subsequent pressure pulse until the cell fully shuts off. Notice, however, that the voltage always rebounds to about the same value when the pressure drops. The relatively slow drop of voltage after the critical pressure as been attained is due to exhaustion of the oxygen reservoir contained in small pores, which are isolated when flooding cuts off the main $O_2$ supply. This reservoir is consumed much faster than it is replenished, which explains the learning effect and the increasingly faster voltage drop with subsequent cycles. The experiment suggests that improved performances at high currents may be achieved with a better interconnected structure, rather than with smaller pores and larger surface area.

## References

Villa M., Nelli P., Shao W., Zangari G., *Electrochemical Behavior of Nickel in Acidic Sulfate Electrolytes*, ECS Transactions, 6 (25) 297-305 (2008).

Villa M., Lorenzi M., Nelli P., Kohnke H-J, Zangari G., *Effects of pressure modulation on porous gas electrodes*, ECS Transactions, 6 (25) 351-359 (2008).

Villa M., Verardi E., Salvi P., Nelli P., Zangari G., *Electrochemical activation of Raney Nickel air electrodes*, ECS Transactions, 11 (32) 105-113 (2008).

Villa M., Salvi P., Verardi E., Zangari G., *Reactions at a porous Ni electrode concurrent with hydrogen evolution,* ECS Transactions 2008, in press.

# 1A – Mesoporous silica and silicon as matrices for enzyme immobilization

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## Aims

Synthesis and characterisation and surface modification of nanostructured materials as host for biological macromolecules

## Results

Recent advances in colloid and material sciences have led to the synthesis of new highly ordered mesoporous materials (OMMs). The synthesis uses the self-assembly properties of surfactants and polymers. They act as templates for the polymerization of a silica precursor (i.e. TEOS) that are removed by calcination or solvent extraction. The resulting materials have uniform pore size in the mesopores range (2-50 nm), high surface area (about 1000 $m^2/g$), and large pore volume (about 1$cm^3/g$). Porous silicon (PSi) is an emerging material for biomedical devices applications. PSi layers are realized by electrochemical etching in the dark of $n^+$-type bulk crystalline (100) Si wafers using a HF solution. Similarly to OMMs also PSi has very large surface are (up to 500 $m^2/g$). For both materials the range of pore size is comparable to that of most proteins, thus OMMs and PSi have attracted researchers attention as viable supports for enzyme immobilization.
In this project mesoporous silica (SBA-15) and silicon (PSi) were used as matrices for enzyme immobilisation.

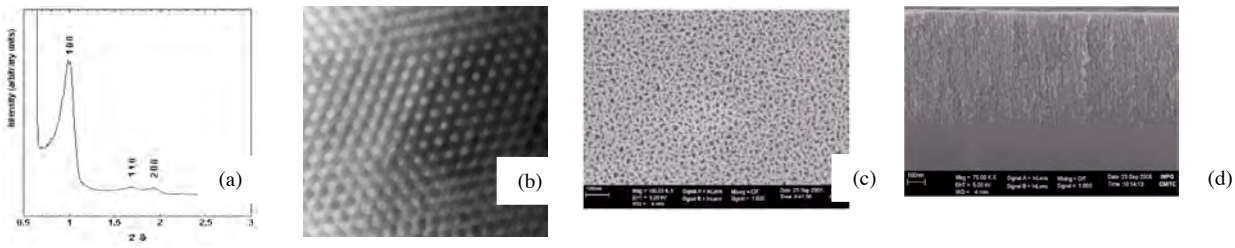


Figure 1. (a): X-ray powder diffractogram in the low angular range and (b) transmission electron images of the SBA-15 silica sample. SEM image of $n^+$-type porous silicon (c) top and (d) lateral views.

The XRD pattern of the SBA-15 sample shows the presence of well-defined peaks in the low angular range which can be associated to the presence of a highly ordered porous structure made out of an hexagonal array of pore channels (Figure 1a). These results are confirmed by TEM observations as shown by a representative image of the

silica surface (Figure 1.b) in which a top view of the hexagonal array of pore channels is clearly visible. The SEM images of a PSi sample show a columnar mesoporous layer with pores oriented perpendicularly to the surface (Fig 1c and 1d). The textural features of the original SBA-15 support and the effect of surface modification on the porosity were investigated by $N_2$-physisorption measurements. Table 1 summarizes the textural parameters of the SBA-15 support at the different stages as derived from $N_2$-physisorption isotherms: the surface area progressively decreases during the surface modification. The significant decrease of all parameters after the reaction with glutaraldehyde should be noticed. These results could be either ascribed to the formation of supramolecular aggregates which may be formed by glutaraldehyde as a result of aldol condensation or to partial occlusion of the pore channels/openings leading to a decrease in the apparent surface area and available pore volume.

Table 1 Textural properties of the SBA-15 support at the different stages of chemical modification as obtained by $N_2$ Physisorption measurements. [a] Determined by the B.E.T. method. [b] Determined by the BJH method.

| SBA-15 modification steps | $S_{BET}$ $(m^2 \cdot g^{-1})^a$ | Pore Volume $(cm^3 \cdot g^{-1})$ | Pore Diameter $(nm)^b$ |
|---|---|---|---|
| SBA-15 | 845 | 1.4 | 6.9 |
| SBA-15····· $NH_2$ | 620 | 1.4 | 5.4 |
| SBA-15····· N=CH···CHO | 213 | 0.4 | 3.6 |

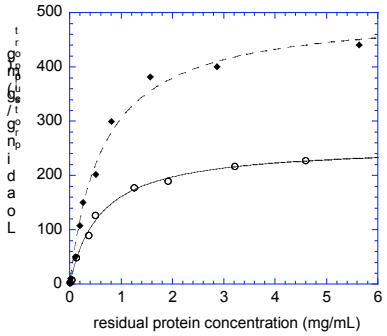


Figure 2: Adsorption isotherms of Pfl on original and functionalized SBA-15

The immobilization of *Pseudomonas fluorescens* lipase (Pfl) on the original and on the chemically modified SBA-15 support through adsorption was performed. Figure 2 shows the adsorption isotherms of Pfl on SBA-15 (original and functioalized). Both the isotherms can be fitted with the Langmuir model. The functionalized SBA-15 achieved a higher loading compared to the original SBA-15. These results point out that chemisorption is more efficient than physisorption in the immobilization process. This result is expected on the basis of the support-enzyme interactions responsible for Pfl immobilization in the two biocatalysts, which are covalent bonds and the sum of ionic and dipolar interactions for the chemisorbed and physisorbed lipase, respectively. The same kind of study was also performed using PSi as immobilization matrix. The immobilized enzymes on the mesoporous materials can find application in biocatalysis (SBA-15) and for the realization of reduced-size devices as biosensors (PSi).

## References

Salis, A., Meloni, D., Ligas, S., Casula, M. F., Monduzzi, M., Solinas, V., Dumitriu, E., *Physical and Chemical Adsorption of Mucor javanicus Lipase on SBA-15 Mesoporous Silica. Synthesis, Structural Characterization, and Activity Performance*, Langmuir 2005, 21, 5511-5516.

# 1A – Supported metalloporphines as heterogeneous catalysts for biomimetic oxidative degradation of industrial organic pollutants

*E. Sanjust, A. Rescigno, F. Sollai, P. Zucca*

## Aims

Synthetic metalloporphines are the obvious emulators of natural metalloporphyrins such as ferriheme, as they could feature chemical and operational robustness and high catalytic efficiency owing to their comparatively high redox potentials. Therefore, a huge number of articles have appeared dealing with this subject, ranging from synthetic and theoretical aspects to a wide variety of potential and real applications. We have recently obtained some preparations of selected metalloporphines immobilized on to properly modified, commercial silica gel, and have assessed their ability to efficiently degrade some organic pollutants of very different chemical nature (such as aromatic alcohols and related ligninoids, and an anthraquinone dye, alizarin red S).

## Results

Bioinspired catalytic systems more or les strictly resembling peroxidases are well known since many years, and are commonly based on redox-active metalloporphines (usually containing Fe(III) or Mn(III) as the coordinated metal, but also Ru(III)), possibly supported on solid supports to afford heterogeneous (and therefore recoverable and recyclable) catalysts. To more closely emulating true peroxidases, the linkages between the support and the metalloporphine should be of the same nature of those found within the enzymes, i.e. axial coordination to a specific nitrogen atom of a histidine residue. To this purpose, some imidazole-bearing supports have been prepared and described. More recently, we have obtained modified silicas, where the imidazolyl functionality is linked to the silica by means of a flexible, hydrophilic chemical bridge, so ensuring both minimized sterical hindrance and non-specific adsorption of hydrophobic compounds (leading to catalyst fouling). In particular, 5,10,15,20-tetrakis(pentafluorophenyl)porphine-iron(III) chloride (FeTFPP) and 5,10,15,20-tetrakis(p-sulfonatophenyl)porphine-Mn(III) chloride (MnTSPP) have been chosen for their stability and catalytic activity. Since now, the chemistry of immobilized MnTSPP has been explored with concern to its reaction with diluted hydrogen peroxide in aqueous media (without any added organic solvent) under mild pH and temperature conditions, and subsequent oxidative degradation of some organic compounds by the high-valent manganese porphine so obtained. Firstly, we tested the catalyst for its ability to degrade some aromatic compounds, (bio)chemically related to lignin. Lignin is a 3-D, chemically inert, hydrophobic polymer, typical for vascular

plants, whose monomeric units are linked by means of C–C, phenylether or diphenylether bonds, so rendering its hydrolytic (bio)degradation not feasible. Only some selected basidiomycetes (the so-called 'white-rot fungi') are capable of oxidatively degrade lignin by virtue of their peculiar enzyme outfit, based on extracellular peroxidases and laccases. Our catalyst was not tested directly on lignin, but proved to be capable of mediate the oxidation of 3,4-dimethoxybenzyl (veratryl) alcohol, which is widely recognized as a suitable model for ligninoids. Its oxidation led to veratraldehyde, veratric acid, and above all the unstable 2-hydroxymethyl-5-methoxy-1,4-benzoquinone. Also other related compounds such as 4-methoxybenzyl alcohol, vanillyl alcohol, vanillic acid, ferulic acid, 1,2-dimethoxy- and 1,2,3-trimethoxy-benzene were oxidized by hydrogen peroxide in the presence of the catalyst (unpublished). The catalys efficiency was later tested on alizarin red S (ARS) degradation. ARS is a slowly biodegradable industrial dye, whose efficient degradation could open a new way for the treatment of other polluting anthraquinone dyes. Fortunately, our catalyst was able to mediate the degradation of ARS by hydrogen peroxide; phthalic acid was the main degradation product.
Work is in progress towards a deeper insight in the catalytic power of the catalyst, to find new application fields; moreover the due attention will be soon paid to the alternative metalloporphine, FeTFPPP, immobilized on the same (or on similar) support.

## *References*

Zucca P., Mocci G., Rescigno A., Sanjust E., *5,10,15,20-Tetrakis(4-sulfonato-phenyl)porphine-Mn(III) immobilized on imidazole-activated silica as a novel lignin-peroxidase-like biomimetic catalyst*. Journal of Molecular Catalysis, A: Chemical 2007, 278, 220-227

Zucca P., Vinci C., Sollai F., Rescigno A., Sanjust E., *Degradation of Alizarin Red S under mild experimental conditions by immobilized 5,10,15,20-tetrakis(4-sulfonatophenyl) porphine-Mn(III) as a biomimetic peroxidase-like catalyst*. Journal of Molecular Catalysis A: Chemical 2008, 288, 97–102

# 1A – Water Confined in Cement Pastes as a Probe of Cement Microstructure Evolution

*F. Ridi, P. Luciani, E. Fratini, P. Baglioni*

## Aims

The calorimetric and spectroscopic properties of the confined water have been used as a probe of the evolution of the cement microstructure. The low temperature investigation enabled to identify a High Density to Low Density crossover of the water confined in the fine nanometric porosity.

## Results

The properties of the water confined in hydrating white cement paste have been investigated using Low Temperature Differential Scanning Calorimetry (LT-DSC) and Low Temperature Near Infrared Spectroscopy (LT-NIR)[1]. LT-DSC thermograms show, upon cooling, several exothermic peaks in the temperature range -10/-42 °C, whose position and area depend on the hydration process, as a consequence of the cement microstructure evolution. The peaks have been interpreted in terms of the Jennings' Colloidal Model-II[2] for the hydrated calcium silicate microstructure.

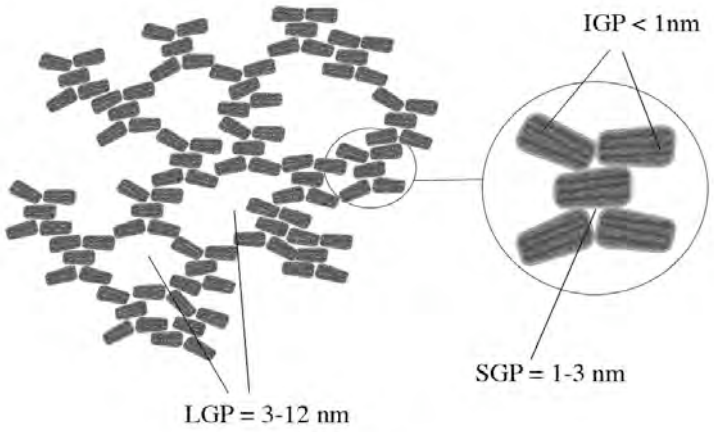


Figure 1. Representation of the *Jennings Colloidal Model- II*

In particular, the *Small Gel Pores* (*SGP*) and *Large Gel Pores* (*LGP*) evolution during the hydration have been monitored and connect to the -42 and -20 °C, respectively. As time passes only the exothermic peak at -42 °C is still present (i.e. water confined only in the *SGP*), concurrently LT-NIR spectroscopy did not show any crystallization associated with this calorimetric transition even freezing to -150 °C. This evidence confirms that the water confined in the SGP spaces of a hydrating cement paste presents the same HD-LD liquid-liquid crossover, as already described for other systems (i.e. zeolites, Vycor and proteins). The average relaxation time accounting for

the hydrogenated mobile species (i.e. confined water) as extracted by Quasi-Elastic Neutron Scattering[3] performed on the very same cement paste shows clear evidence of a super-Arrhenius (non-linear behavior) to Arrhenius (linear behavior) crossover (see Figure 2). This dynamic feature, usually referred as a fragile to strong crossover, is present at -42 ± 5 °C and marks the change in structure from a HDL to a LDL phase upon cooling. If taken together with the other presented evidences it strongly confirms the conclusion that the water constrained in the Small Gel Pores in a cured cement paste undergoes a liquid-liquid crossover.

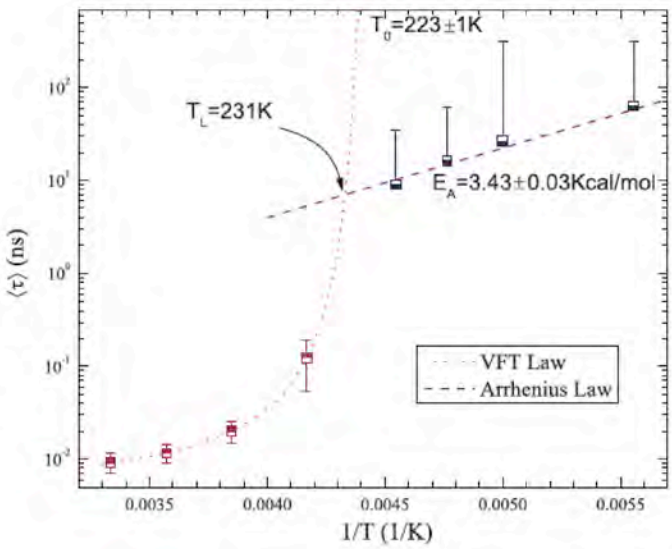


Figure 2. Arrhenius plot of experimentally extracted relaxation time versus 1/T . An evidence of a Super-Arrhenius (non-linear behavior) to Arrhenius (linear behavior) dynamic crossover is observed upon cooling at about $T_L$=-42 ± 5 °C [3].

## References

[1] Ridi, F.; Luciani, P.; Fratini, E.; Baglioni, P., *Water Confined in Cement Pastes as a Probe of Cement Microstructure Evolution*, Journal of Physical Chemistry B, December 2008, *accepted with minor revision*.

[2] Jennings, H. M., *Refinements to colloid model of C-S-H in cement: CM-II,* Cement and Concrete Research 2008, 38, 275-289.

[3] Zhang, Y.; Lagi, M.; Ridi, F.; Fratini, E.; Baglioni, P.; Mamontov, E.; Chen, S. H., *Observation of dynamic crossover and dynamic heterogeneity in hydration water confined in aged cement paste*, Journal of Physics: Condensed Matter 2008, 20, 502101-502106

# 1A – AFM and SIMS-SS: an integrated approach to obsidian hydration dating

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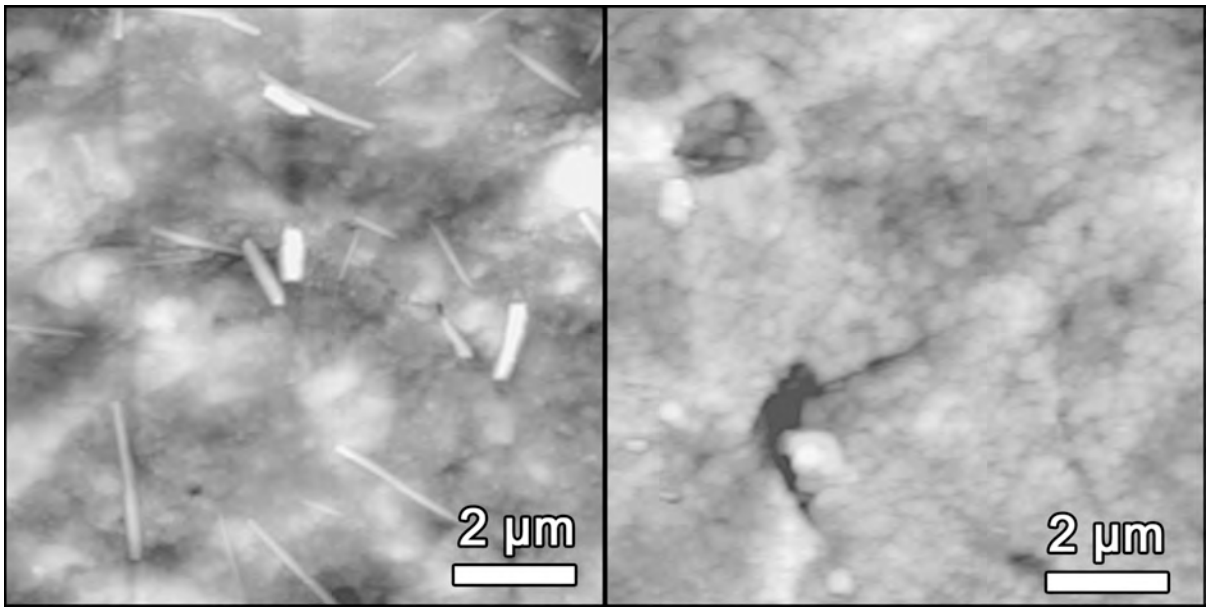
## Aims

Nano- and Micro- scale resolution in ancient obsidian artefacts surfaces: The impact of AFM on the obsidian hydration dating by SIMS-SS

## Results

The prehistoric tools made by natural glass of obsidian can be dated by the recent method of the secondary ion mass spectrometry using the surface saturation approach (SIMS-SS). The obsidian hydration dating (OHD) method is based upon modeling the rate of water diffusion into a natural glass surface and establishing a diffusion coefficient for this process. It is accepted that the rate of water diffusion, the diffusion coefficient, is exponentially dependent on temperature and exhibits an Arrhenius type behavior. A variety of strategies have been developed over the years to calibrate the movement of ambient water into a glass. Many of these approaches have developed procedures for controlling the chemical composition of the glass and modeling the environmental history of the artifact context (e.g., temperature, humidity). However, the development of calibrations to compensate for variation in external variables has proven to be difficult. Recently a reviving of OHD has been made especially by the two leading groups – Oak Ridge National Laboratory and Tennessee University,[1] and the Laboratory of Archaeometry of the University of the Aegean, Rhodes, Greece.[2-3] In this framework, surface irregularities represent a serious problem towards an accurate dating. Cavities, holes and other surface anomalies could distort the SIMS profiles including the surface saturation (SS) layer. Moreover, these surface anomalies are also reflected on the diffusion coefficient, which in turn depends on the composition of the obsidian, and on the physical nature of the material e.g. pores etc.
Therefore having an exact picture of the surface to be investigated by SIMS-SS is of crucial importance to evaluate the reliability of dating results based upon this approach. Atomic force microscopy (AFM) is the technique of choice when targeting the topological characterization of a solid surface with nanometric resolution. Therefore we integrated the SIMS-SS approach with a preventive AFM analysis of the samples, in order to validate this dating approach.[4-6] AFM shows that surface of obsidians consists of micron-scaled features (cracks and voids, mainly) together with spheroidal and acycular nanostructures (an example is shown in Figure 1). Dating results on the same samples show that meaningful results are obtained as long as the spot selected for the SIMS profile acquisition is chosen avoiding micron-scaled inhomogeneities of the samples. Furthermore, an indication of the linear correlation

between SIMS-diffused profile properties and surface roughness measured by AFM reconfirms the interdependence. This aids selection of appropriate obsidian samples/surfaces for dating.




Examples of AFM images of obsydians.

## References

[1] Anovitz, L.M., Elam, J.M., Riciputi, L.R., Cole, D.R., *The failure of obsidian hydration dating: sources, implications and new directions*, Journal of Archaeological Science 1999, 26, 735-752.

[2] Liritzis, I., Diakostamatiou. M., Stevenson, C.M., Novak, S.W. Abdelrehim, I., *The dating of hydrated obsidian surfaces by SIMS-SS*, Journal of Radioanalytical & Nuclear Chemistry 2003, 261, 51-60.

[3] Liritzis, I., *SIMS-SS, a new obsidian hydration dating method: analysis and theoretical principles*, Archaeometry 2006, 48, 533-547.

[4] Liritzis, I., Bonini, M., Laskaris, N.; *Obsidian hydration dating by SIMS-SS: surface suitability criteria from atomic force microscopy*, Surface & Interface Analysis 2008, 40, 458-463.

[5] Liritzis, I., Stevenson, C., Novak, S., Abdelrehim, I., Perdikatsis, V., Bonini, M.; *New prospects in obsidian hydration dating: An integrated approach*, Proceedings of the Hellenic Archaeometry Society (In Press).

[6] Liritzis,I., Laskaris, N., and Bonini, M.; *Nano- and Micro-scale resolution in ancient obsidian artefacts surfaces: The impact of AFM on the obsidian hydration dating by SIMS-SS*, Physica Status Solidi (In Press).

# 1A – Multilayered Nanofilms with tunable functionality

*G. Caminati, F. Gambinossi, S. Ciappelli, P. Baglioni*

## Aims

The target of the project is the design and realization of novel nano-architectures with tunable functionality. In particular, we focused on organic photovoltaic nano-devices (OPC, *Organic Photovoltaic Cell*) for the conversion of light to electrical energy and on OLED (*Organic Light Emitting Devices*).

## Results

The field of nanotechnology for molecular electronics ties two areas together: the search for compounds with the desirable function and the assembly of the individual molecules into new devices with superior properties. The previous research activity has shown that stable assemblies of controlled thickness and known in-plane structure can be successfully fabricated by means of a combination of different nano-techniques: Langmuir-Blodgett (LB) method, chemical Self-Assembly and Layer-by-Layer (LbL) polyelectrolyte deposition. This same approach was adopted for the fabrication of tunable nanodevices.

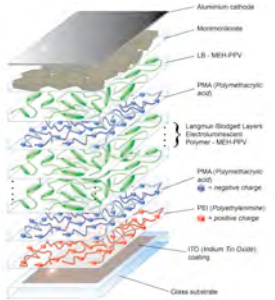



Figure 1. Schematic representation of a multi-functional molecular device: OLED and OPC

**OLED nano-devices**. The OLED hetero-structure was based on poly(2-methoxy-5-(2-ethylhexyloxy)-p-phenylenevinylene) (MEH–PPV), on poly(methacrylic acid) (PMA) and on poly(ethylenimine) (PEI). The film-forming properties of MEH–PPV were investigated at the water–air interface by recording surface pressure–area and surface potential–area isotherms. Stable MEH–PPV monolayers were transferred by means of the LB technique on solid supports. The assembling process of the LbL layers was followed by quartz crystal microbalance (QCM) measurements. The final multilayered structure was characterized by means of ellipsometric thickness determination (see also 1B. Surface Imaging of Nanostructures), static contact angle and conductivity measurements as a function of experimental variables such as: spreading solvent, transfer pressure, layers sequence as well as the presence of protective layers. The morphology of the outer surface of the LbL/LB/LbL structure

was analyzed by means of Brewster Angle Microscopy (BAM) studies and Confocal Laser Scanning Microscopy combined with spatially resolved fluorescence.

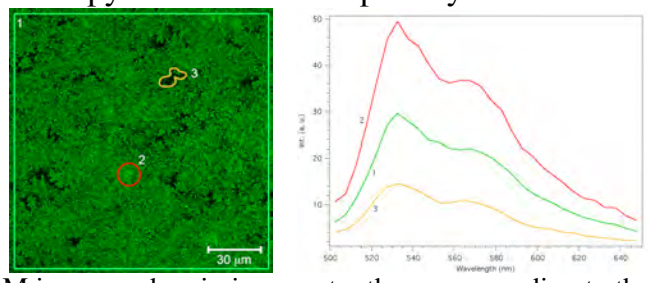



Figure 2. CSLM image and emission spectra the corresponding to the circled domain

The optical response of the hybrid multilayer was tested measuring the electronic absorption and the emission spectra.

**OPC nano-devices**. Photovoltaic architectures were built using as redox partners PPV derivatives (donor) and differently functionalized C60 molecules (acceptor). A variety of strategies were planned to assemble the redox couple in an efficient way in the active layers of the nanodevices. Besides LB transfer of well-defined sequences of PPV and C60 containing layers, we also explored the possibility to use LbL methodology or LbL/nanoparticles combination.


Figure 3. Typical redox partners for OPC: water-soluble derivatives of PPV and C60.

The preliminary studies showed that the LbL strategy produces devices with better performances compared to conventional LB methods.

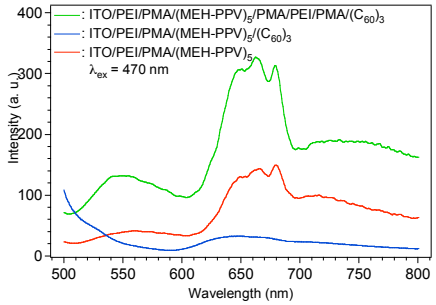



Figure 4. Fluorescence emission intensity as a function of multilayer sequence

Moreover, insertion of a variable number of polyelectrolyte layers permits to control the molecular distance between acceptor and donor to optimize the OPC efficiency.

## *References*

Gambinossi, G., Baglioni, P., Caminati, G., Hybrid LbL/LB films as molecular OLEDs: An acoustic shear wave attenuation and Brewster angle microscopy study, Materials Science and Engineering 2007 C 27 1056–1060.

# 1A – Nanosensors based on ultrathin organic films

*G. Caminati, F. Gambinossi, M. Puggelli, D. Salvatori, S. Morandi*

## Aims

The research is focused on the preparation of new molecular devices for sensor applications by means of different nanotechniques tailored to meet the specific requirements of several detection systems. In particular we explored hybrid architectures formed by a combination of Supported Lipid Bilayers (SLB), Langmuir-Blodgett (LB), Self-Assembly (SA), Layer-by-Layer (LbL) systems.

## Results

Supported layers of a dipalmitoyl phosphatidylglycerol (DPPG) were prepared on solid surfaces by means of two different approaches: by Langmuir–Blodgett (LB) and by direct adsorption of a liposomal dispersion onto gold surfaces (SLB).

The penetration behaviour of an ansamycin antibiotic Rifaximin (Rfx) in the two different supported DPPG layers was monitored by a variety of techniques: quartz crystal microbalance (QCM) with dissipation monitoring, UV–Vis absorption spectroscopy and cyclic voltammetry (CV) investigation. Experiments were run as a function of time and of the antibiotic concentration in solution to clarify the kinetics and the mechanism of Rfx association with the phospholipid layer.


Figure 1. Formation of SLB followed by Rfx adsorption (left); QCM mass density of adsorbed Rfx: comparison between LB and SLB systems (right).

The results evidenced differences in the extent of Rfx penetration depending on the film fluidity and on the type of outer layer exposed to the water environment and demonstrate that the investigated supported layers can be successfully used as sensing surfaces for the detection of food-contaminants such as Rfx for concentrations lower than the allowed maximum residue limit in food.

We also developed a functionalization procedure for silicon oxide surfaces used in microcantilever-based sensors dedicated to the detection of food contaminants in fluid matrices. In particular we focused on the determination of heavy metal ions and of agricultural pesticides. The surface functionalization was obtained by direct self-assembly of long chain molecules bearing at one end a complexing moiety for metal ions. The selected chelating molecule, the nitrilotriacetic acid (NTA), was immobilized onto silicon oxide surfaces using a three-step process involving the

consecutive addition of an organosilane, glutaraldehyde and a NTA derivative solutions. The formation of the self-assembled nanostructure (SAN) at the surface was traced by means of quartz crystal microbalance with dissipation monitoring (QCM) measurements as a function of time.

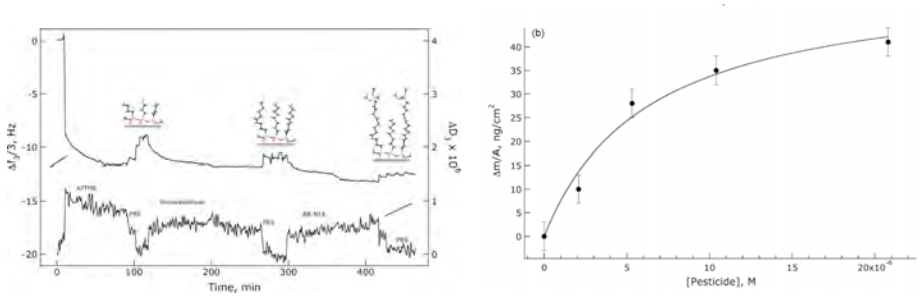



Figure 2. QCM monitoring of SAN formation and QCM titration curve for a carbamate pesticide.

The results indicated that the functionalized molecule forms a rigid self-assembled film on silicon dioxide. Data analysis provided the layer thickness and the molecular orientation of the chemisorbed layers at the interface. The optimized procedure was tentatively applied to functionalize the silicon oxide outer surface of an array of microwells each containing four microcantilevers.

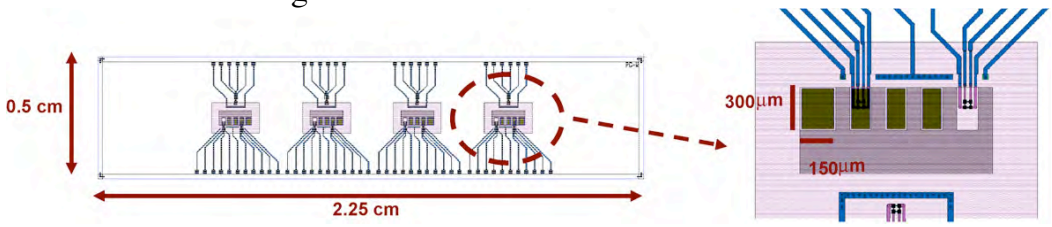



Figure 3. Microcantilever array layout

Quantitative determination of the metal ions complexation at the surface was achieved adding the desired solution in the QCM measuring chamber and recording the adsorbed mass change as a function of concentration.

The above self-assembled system was further exploited for the detection of pesticides in fluid matrices monitoring the variation in the QCM signal upon addition of the analyte in the measuring chamber.

## References

1. Gambinossi, F., Lorenzelli, L., Baglioni, P., Caminati, G., *Silicon oxide surface functionalization by self-assembled nanolayers for microcantilever transducers*, Colloids and Surfaces A: Physicochem. Eng. Aspects 2008 321 87–93.
2. Morandi, S., Puggelli, M., Caminati, G., *Antibiotic association with phospholipid nano-assemblies: A comparison between Langmuir–Blodgett films and supported lipid bilayers* Colloids and Surfaces A: Physicochem. Eng. Aspects 2008 321 125–130.
3. Morandi, S., Focardi, S., Nocentini, M., Puggelli, M. and Caminati, G., *Development and Validation of Europium-Sensitized Luminescence (ESL) Method for the Determination of Tetracycline Residues in Milk* Food Anal. Methods 2009 in press.

# 1A – Tunable Gold Nanostructures

*E. Falletta, E. Fratini, F. Ridi, C. Vannucci, P. Baglioni*

## *Aims*

Gold nanostructures synthesized in the presence of a novel tri-block copolymer can be modulated by varying the gold/polymer molar ratio.

## *Results*

Tunable gold nanostructures have attracted a lot interest from the scientific community in the recent years [1]. In the present study, gold nanoparticles have been synthesized in water in the presence of an amphiphilic tri-block copolymer (poly(OEGMA-b-MAA-b-OEGMA) or shortly BMB) [2] reducing $HAuCl_4$ by $NaBH_4$. In order to obtain a control over the final particles' size, shape and monodispersity, different gold/BMB molar ratios have been explored. The nanosols have been investigated by means of UV-Visible Absorption, Small Angle X-Ray Scattering (SAXS), Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM).

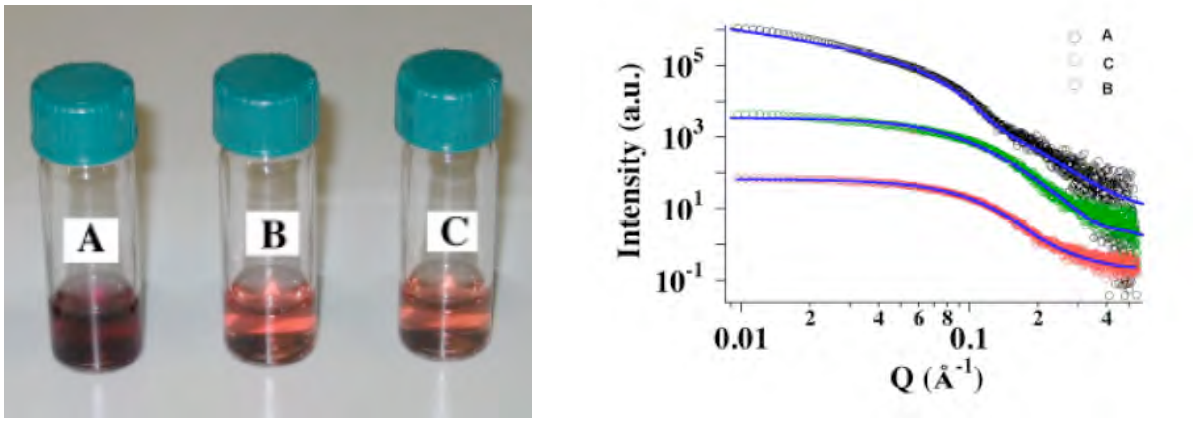



Figure 1. Left: Investigated samples having gold/polymer molar ratios A=1000, B=215 and C=4.6. Right: Characteristic SAXS intensity distributions.

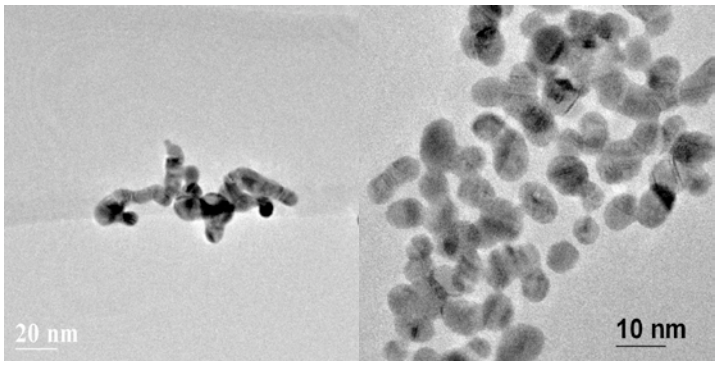



Figure 2. Representative TEM micrographs for sample A (left panel) and C (right panel).

The results clearly indicate that the gold nanostructures can be simply tuned from elongated bundles of several tenths of nanometers to monodisperse spherical particles with a mean radius of 1.5 nm by varying the Au/BMB molar ratio from 1000 (sample A) to about 5 (sample C). [3].

## *References*

[1] M.-C. Daniel et al., Chem. Rev. 104 (2004) 293.
[2] C. Vannucci et al., Manuscript in preparation.
[3] Falletta, E.; Fratini, E.; Ridi, F.; Vannucci, C.; Canton, P.; Bianchi, S.; Castelvetro, V.; Baglioni, P. *Submitted to J. Phys. Chem. C.*

# 1A – Nanostore: $H_2$ production and storage in nanomaterials

*C. Milanese, A. Girella, G. Bruni, P. Cofrancesco, V. Berbenni, A. Marini*

## Aims

This project focuses on the preparation and characterization of Mg-based binary and ternary nanocomposites, with the final aim to find systems meeting the technical targets for on – board applications fixed by the U.S. Department of Energy (DOE) for the year 2010.
The best promising composite will be employed as storage material in an integrated $H_2$ fuelling system/1 kW polymer electrode membrane FC prototype to be realized at the end of the project.

## Results

In the framework of this four - year FISR project, the effect of many metals (Al, Cu, Fe, Mo, Mn, Ni, Sn, Ti, Zn, Zr) as catalyzing agents towards the sorption reactions of the pure Mg – $MgH_2$ system was tested. Mixtures with metal contents up to 63% were prepared by high energy ball milling (by MBN Nanomaterialia S.p.A.) and deeply characterized as regards their kinetic storage performances. The best results were obtained for the mixtures containing Ni, the optimum ratio Mg - Ni being 85 wt% - 15 wt%: these composites showed high gravimetric capacity (about 6 %), good reversibility and sorption rates appreciably higher than that of pure $MgH_2$. Moreover, the desorption temperatures of these systems are lower (40 °C) than the value recorded for pure $MgH_2$ dissociation. All the other binary systems showed lower gravimetric capacity, too slow kinetics, no full sorption reversibility and worsening of the performance upon cycling.
In the present year we focused on the study of the effect of C (graphite) addition towards the $H_2$ sorption kinetics and the reactivity of the Mg - Ni system. To this aim, ternary Mg – Ni – C (graphite) mixtures (with Mg 85%: Ni15 % ratio and C amount increasing from 5 to 30 wt%) were prepared by high energy ball milling (BM) in Ar for two different processing times ($t_{BM}$ = 2 h and 8 h) and their performance compared with that of binary Mg – Ni mixtures processed in identical way.
The sorption properties of the mixtures were characterized by kinetic measurements and thermal programmed desorptions. Moreover, X-ray powder diffraction analyses (XRPD) were performed on the samples both after milling and at the end of each absorption/desorption step.
BM did not lead to any reaction among the components of the mixtures. Samples activation was performed at 350 °C by charging/discharging runs at starting $H_2$ pressure of 50 bar/1 bar. Concerning the binary Mg – Ni mixtures, both the sorption

kinetics and the storage capacity increased noticeably with the activation cycle number, and the effective sorption performances were reached after four activation cycles independently on $t_{BM}$. For the mixtures containing C the activation was easier and faster even if, surprisingly, the number of needed activation cycles increased (from two to three) by raising $t_{BM}$ and the 2 h BM mixtures showed better kinetic performances than the corresponding 8 h BM samples. On the other hand, no appreciably differences could be seen in their sorption capacity. In all the mixtures, the thermal treatment induced the reaction between Mg and Ni, with quantitative formation of $Mg_2Ni$. By charging, $MgH_2$ and $Mg_2NiH_4$ were obtained. On the contrary, C reacted neither with Mg and Ni nor with $H_2$.
In the binary samples, a fast $H_2$ absorption stage (taking place in the first 20 s and showing a linear profile) was followed by a gradual $H_2$ intake (about one order of magnitude slower). After 2 h charging the samples had not reached an equilibrium content yet, even if their sorption yield[1] was 90%. The presence of C had a strong catalyzing effect on the charging kinetics: the ternary systems showed a very quick absorption step, whose profile is nearly perpendicular to the time axis, after which constant mass was reached within 2 min and with an $H_2$ sorption yield close to 100%. The starting dissociation temperatures of both $MgH_2$ and $Mg_2NiH_4$ (280 °C and 300 °C at 1 bar $H_2$, respectively) did not appreciably change due to C addition, but unfortunately desorption became a little slower.
The best promising composition of the ternary system is Mg 85 wt % - Ni 15 wt % - C 5%: at T = 320°C it exchanges 6.5 wt % $H_2$ (the theoretical capacity is 6.7%; the target DOE is 6.0%) in a full reversible way, with a refuelling rate higher than the DOE target up to 96% of its filling up and a desorption rate higher than 200 g $H_2$/min (i.e. enough to feed a 160 kW FC) during all the discharge process. The only limitation to the on-board application of this Mg-based nanomaterial is represented by the high temperature needed for full desorption.

## *References*

Milanese, C., Girella, A., Bruni, G., Cofrancesco, P., Berbenni, V., Marini, A.,Villa, M., Matteazzi, P., *Hydrogen Storage in Magnesium – Metal Mixtures: Reversibility, Kinetic Aspects and Phase Analysis,* Journal of Alloys and Compounds 2008, 465, 396–405.

Milanese, C., Girella, A., Bruni, G., Cofrancesco, P., Berbenni, V., Villa, M., Matteazzi, P., Marini, A. *Reactivity and Hydrogen Storage Performances of Magnesium – Nickel – Copper Ternary Mixtures prepared by reactive mechanical grinding,* International Journal of Hydrogen Energy 2008, 33, 4593–4606.

[1] The sorption yield of the mixtures was obtained as the ratio between their experimental $H_2$ intake and the theoretical storage capacity. This last value was calculated in the hypothesis that the $H_2$ active species Mg and $Mg_2Ni$ reacted quantitatively with $H_2$ upon charging.

# 1A – $H_2$ storage for on - board applications by complex hydrides nanocomposites

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## Aims

This project - in the framework of a wider collaboration program between Italy and Spain - will focus on the preparation and the characterization of novel nanostructured metal hydrides composites, in the search for materials with kinetic and thermodynamic characteristics meeting the technical targets for on-board applications fixed by the U.S. Department of Energy (DOE) for the year 2010.

## Results

The current research on solid state hydrogen storage materials is focused on complex ternary hydrides such as alanates, amides and borohydrides. Recently, some research groups have proposed the preparation of the so called *reactive hydrides composites* (RHC), that are hydrogen storage systems based on the interaction and the reciprocal destabilizing effects between the light metal hydride $MgH_2$ (up to now the best promising hydrogen storage material) and a complex hydride. The interaction between the two hydrogen storage materials seems leading to lower desorption temperatures with respect to the pure systems, to an improvement of their sorption kinetics and above all to a better reversibility of the sorption processes. The research field is new and systematic studies have not been published yet.

Our project is focused on the preparation and characterization of the following RHC:

I. $NaBH_4 – MgH_2$ and $LiBH_4 – MgH_2$ binary mixtures;
II. $M'(BH_4)_x$ - $M'(AlH_4)_x$ - $MgH_2$ [M' = Na, Li, Ca]; $M'(BH_4)_x$ - $M''(AlH_4)_x$ - $MgH_2$ [M' and M'' = Na, Li, Ca; M'≠M'']; SS - $M''(AlH_4)_x$- $MgH_2$ ternary mixtures.

The mixtures are prepared by high energy ball milling. The mechanical treatment is performed under different conditions (milling atmosphere, duration, impact energy, rotation speed of the bowls), in order to find the combination of parameters that allows to obtain nanocomposites with improved sorption properties.

The first results regard mixtures with composition 2 $NaBH_4$ – $MgH_2$ milled 8 h at 1050 rounds per minutes under inert atmosphere (Ar) or under reactive atmosphere ($H_2$). X-ray powders diffraction analysis shows that milling does not lead to the formation of any new phase, but it appreciably reduces the average size of the powders (from 325 μm down to about 115 nm and even 65 nm for the treatment in Ar and in $H_2$ respectively). Independently on the processing atmosphere, the mixtures desorbed about 9.4 wt % $H_2$. This value is much higher than the DOE target (6.0 %) and it is close to the $H_2$ amount released during the reaction:

$$2NaBH_4 + MgH_2 \rightarrow MgB_2 + 2Na + 5H_2$$

that is the fully dissociation of both the hydrides. The first tests suggests that the sorption processes are fully reversible in our mixtures.

The desorption profile of the mixtures milled in Ar clearly shows three steps. The experimental amounts of released $H_2$ allow to draw the following desorption pathway:

1st step: $MgH_2 \rightarrow Mg + H_2$

2nd step: $2\ NaBH_4 + Mg \rightarrow MgB_2 + 2\ NaH + 3\ H_2$

3rd step: $2\ NaH \rightarrow 2\ Na + H_2$

The third step has never been observed in literature before.

For the mixtures processed in $H_2$, only two desorption steps are distinguishable, with $MgH_2$ dissociating first and $NaBH_4$ dehydrogenating gradually in one only stage. The starting desorption temperatures for the dissociation of the two hydrides are 315 °C and 390 °C, independently on the milling atmosphere. On the contrary, the average dissociation rates are much higher if the mechanical treatment is performed in $H_2$ ($5.60 \cdot 10^{-2}$ wt % $H_2$/min vs $3.30 \cdot 10^{-2}$ % $H_2$/min for $MgH_2$ and $1.5 \cdot 10^{-2}$ % $H_2$/min vs $0.9 \cdot 10^{-2}$ % $H_2$/min for the full decomposition of the borohydride). This suggests that milling in $H_2$ leads to a strong improvement in the dehydrogenation kinetics of our nanocomposites. Unfortunately, 10 h are required to obtain full desorption at 450 °C, i.e. both a too high time and a too high temperature for on – board applications. Work is in progress to find suitable catalyzing/destabilizing agents (such as transition metals chlorides) able to appreciably reduce both the parameters.

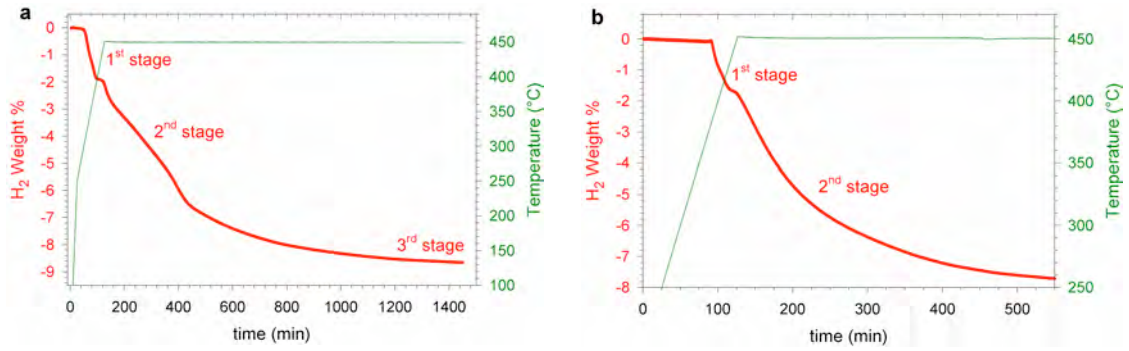



Figure 1: Desorption profiles recorded for the mixtures 2$NaBH_4$ – $MgH_2$ after milling in Ar (a); in $H_2$ (b). The samples are heated from room temperature to 450 °C (β = 2 °C / min) at a starting pressure of 0.1 bar and then kept in isothermal conditions till desorption is completed.

# 1B – Lipid-Based Nanostructured Systems for Drug Encapsulation and Sustained Release

*M. Monduzzi, S. Murgia, S. Lampis, P. Hiwale*

## Aims

Glycerol monooleate-based nanostrucured drug carriers have been developed and investigated for structural features, controlled release and *in vitro* cytotoxicity.

## Results

A growing effort in the discovery of innovative therapies has lead to an increasing demand for drug delivery vehicles, the capability of which should not be limited to a simple drug encapsulating and transporting affair. Indeed, protecting and selectively releasing the drug (bioadhesion) and, eventually, overcoming biological barriers that prevent the drug from reaching the receptor represent novel, strict requirements. The remarkable self-assembly ability of lipids, and particularly of glycerol monooleate (GMO), promotes these systems as candidates in producing functionalized formulations having significant similarities with and affinity towards biological membranes. The potentialities of GMO/water (W) formulations as drug delivery systems were explored and GMO/W/model drug pseudo-binary systems were investigated via optical microscopy, NMR and SAXRD.

A hydrosoluble drug, the 1-amino-adamantane, was included in a GMO/W cubic liquid-crystalline (LC) phase, then its release in water from the bulk cubic phase was followed through conductivity measurements. The good performance observed validate the use of the investigated system as suitable nanocontainer.[1]

Mononucleotides (XMP) were also solubilized in the aqueous phase of GMO/W cubic LC phases. The rationale behind the choice of these molecules is that nucleosides, nucleotides and modified nucleotides, oligonucleotides and aptamers represent a class of innovative antiviral and antitumoral drugs. These types of new drugs need to be protected since they can be easily recognized and degraded by different extracellular nucleases. As evidenced mainly by $^{31}$P NMR measurements XMP experience strong interactions with the lipid interface which at first induce XMP hydrolysis, followed by cubic-to-hexagonal phase transition. A model that explain both hydrolysis and phase transition is proposed.[2]

Protocols to evaluate the impact of GMO-based nanoparticles on biological systems were presented. Such nanoparticles, newly obtained through fragmentation of bulk cubic LC phases, and stabilized by two different emulsifiers, namely Pluronic F127 (PF127) and lauroylcholine chloride (LCh), were investigated for structural features and for in-vitro cytotoxicity. Results show that nanoparticles stabilized by PF127 display a relevant toxicity towards different cell lines whereas those stabilized by LCh do not affect cell viability significantly (see fig. 1a). GMO, being a membrane lipid, favors molecular recognition and internalization. The presence of PF127 induces

adverse interactions; conversely, LCh, acting as a cell-penetrating peptide, is not toxic and reinforces the molecular recognition action of the monoglyceride.[3]
Another fundamental goal is to prepare and evaluate transdermal/topical formulations in order to achieve controlled release of protein and peptides. The formulations selected for our study were water-in-oil emulsions stabilized by LC phases prepared using glycerol trioleate and GMO as apolar lipid and as surfactant, respectively. The *in vitro* release studies through ethyl vinyl acetate membrane of these formulations containing model proteins (BSA and lysozyme) were carried out by means of Franz diffusion cell. Some preliminary results are shown in fig. 1b. Further, the formulations will be evaluated for their physical and structural properties. Such properties will be then correlated with the *in vitro* release of proteins and peptides.

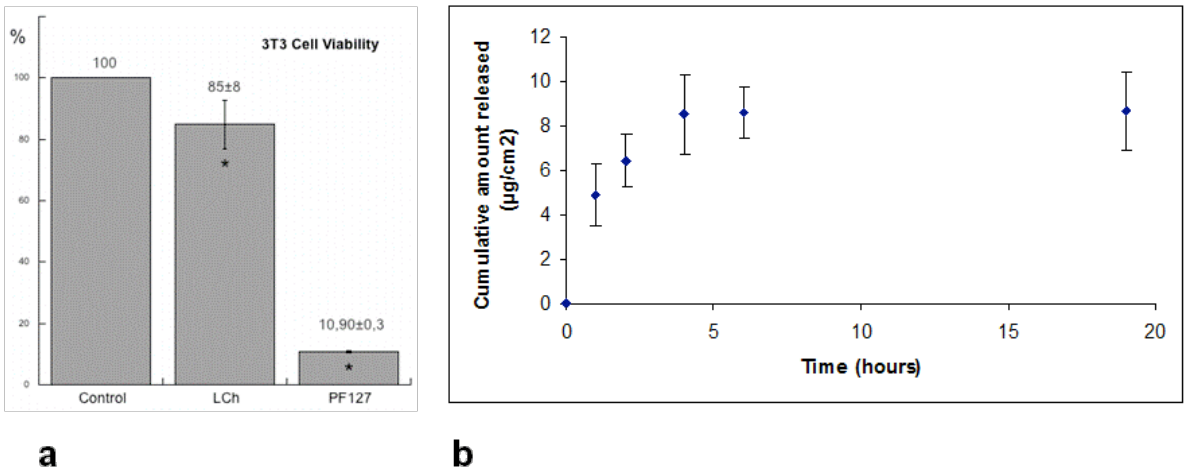



Fig.1 a) Effect of the MO/W dispersions stabilized by PF127 and LCh on the cell viability of 3T3 cells. b) Average cumulative amount of lysozyme released per unit area.

## References

1. Angius, R., Murgia, S., Berti, D., Baglioni, P., Monduzzi, M., *Molecular recognition and controlled release in drug delivery systems based on nanostructured lipid surfactants*, Journal of Physics: Condensed Matter 2006, 18, S2203-S2220.
2. Murgia, S., Lampis, S., Angius, R., Berti, D., Monduzzi, M., *Mononucleotides Interactions and Hydrolysis in Monoolein-Based Liquid Crystals*, manuscript in preparation.
3. Angius, R., Falchi, A. M., Lampis, S., Mano, M., Murgia, S., Diaz, G., Giacca, M., Monduzzi, M., *Nanoparticles from Lipid-Based Liquid Crystals: Emulsifier Influence on Molecular Recognition and Cytotoxicity*, Chemical Research in Toxicology 2008, submitted.

# 1B – Self-Assembly in Ionic Liquids

*S. Murgia, A. Salis, S. Lampis, M. Mamusa, M. Monduzzi*

## Aims

Exploitation of ionic liquids peculiar properties for the preparation of novel microemulsion systems useful in catalysis and nanoparticles synthesis.

## Results

Ionic liquids (ILs) are a class of tunable solvents composed of mismatched ions that hinder crystal formation. Due to their potential environmentally-benign nature and their unusual properties ILs are receiving great attention from the academic community. It is worth noticing that the electrolytic/surfactant nature of ILs may induce alterations in the polymorphism typically expressed by surfactant molecules when dissolved in a suitable solvent. This would be particularly useful in expanding the applications of both aqueous and non-aqueous surfactant systems.

The main goals of this project are to design and describe under the physical-chemistry profile original micellar and microemulsion systems whose qualities primarily conform a) to the explication of the catalytic activity of proteins and b) to the nanoparticles synthesis.

Our investigation on the potentialities of IL in favorably altering the characteristics of common surfactants in solution started from the sodium bis-(2-ethylhexyl)-sulfosuccinate (NaAOT) and the 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), a popular hydrophilic IL. The NaAOT/Water (W)/$BMIMBF_4$ ternary diagram was explored via optical microscopy, SAXRD and NMR methods. Results show that an unexpected broad, normal micellar region having a percolative behavior forms and extends from the W/$BMIBF_4$ binary axis towards the center of the ternary diagram. Interactions and self-assembly properties of NaAOT and $BMIMBF_4$ in this region were studied mainly by means of $^1H$ NMR self-diffusion experiments. The swelling ability of the micellar phase was also tested by adding p-Xilene, a non-penetrating oil, thus producing a microemulsion system. At low IL content the liquid-crystalline nanostructures found in the NaAOT/W binary system (lamellar, cubic and reverse hexagonal) are retained (see figure 1). Further researches are in progress to evaluate the role of the IL counterion.

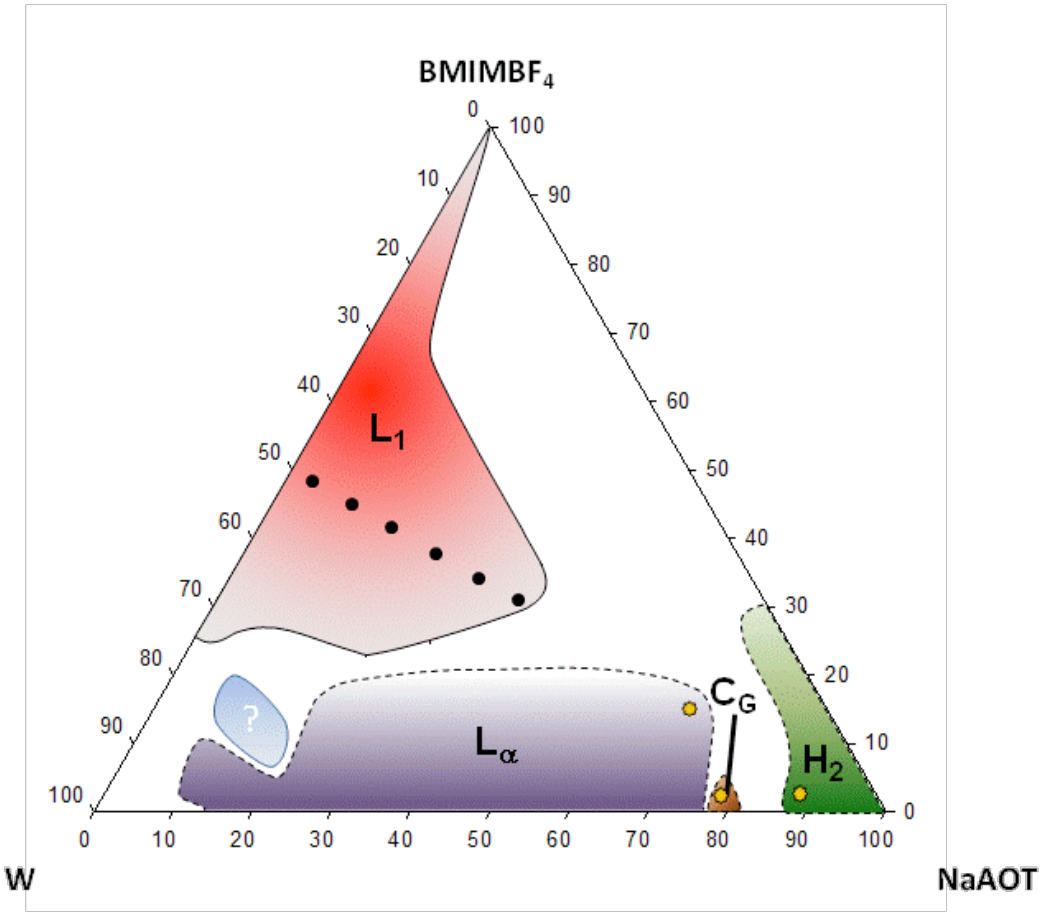



Figure 1. NaAOT/W/$BMIMBF_4$ ternary diagram at 25 °C.

## *References*

Murgia, S., Mamusa, M., Lampis, S., Monduzzi, M., *Self-Assembly in the $BMIMBF_4$/W/NaAOT Ternary System*, manuscript in preparation.

# 1B – Molecular interactions between positively charged CTAB micelles and nucleotide-monophosphates

*F. Cuomo, A. Ceglie, G. Palazzo, F. Lopez*

## Aims

Investigation on the interaction between monophosphate-nucleotides and the cationic interface of micellar solution based on hexadecyltrimethylammonium bromide (CTAB).

## Results

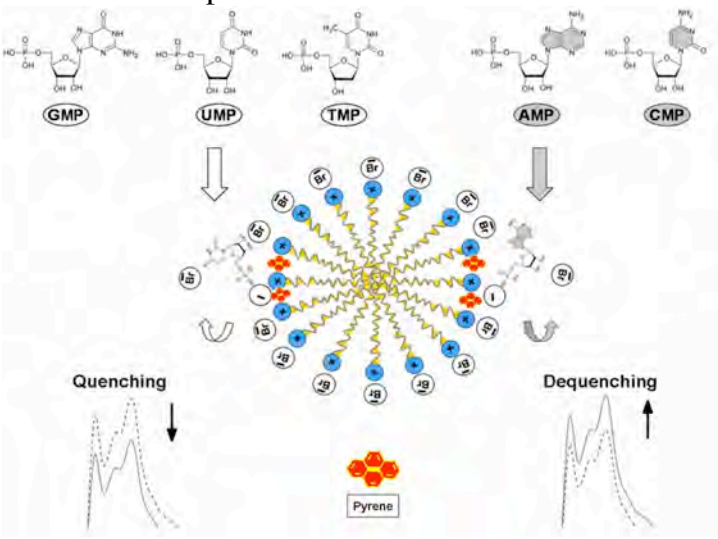
The studies on the interaction between charged surfactant and macromolecules have been object of considerable efforts. Molecular interaction in biological systems is achived through combined noncovalent interactions, such as electrostatic interaction, hydrogen bonding and hydrophobic interaction. Molecular interaction investigation in restricted geometry systems such as micelles, reverse micelles, and vesicles attracts a great deal of interest because of the simplicity of the systems.

In this project the interaction between monophosphate-nucleotides and the cationic interface of micellar solution based on hexadecyltrimethylammonium bromide (CTAB) were studied by fluorescence, conductivity light scattering and Z-potential measurements. Binding of nucleotides to micellar system at different compositions was evaluated by following the fluorescence behaviour of pyrene in CTAB micelle. The variations of fluorescence signal were evaluated by considering the quenching of steady-state fluorescence. We demonstrate that although akin in chemical structure, AMP and UMP were found to influence the fluorescence of pyrene secluded in CTAB micelles oppositely. UMP acts as quencher, while AMP acts as dequencher. Both the effects saturate at high NMP concentration (about 40 mM)[1].

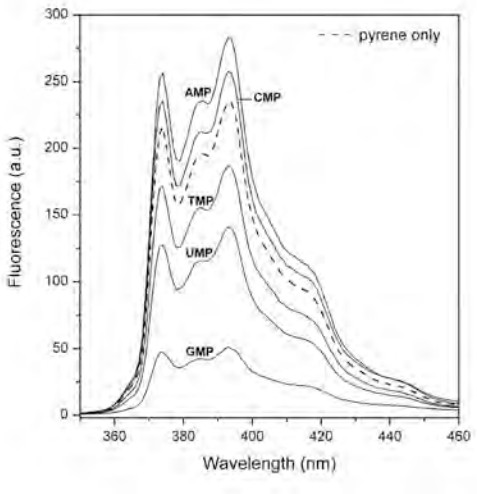
The obtained results indicate that UMP, AMP and orthophosphate have similar affinities for the cationic surface of cetyltrimethylammonium micelles.

Consequently the quenching and dequenching ability of the following series of NMPs (AMP, GMP, UMP, CMP and TMP) was analyzed by following the fluorescence of pyrene solubilized in CTAB micelles. The data of this study clearly indicate that NMPs influence in different way the fluorescence of micellized pyrene. Remarkably, GMP, UMP and TMP act as quenchers and AMP and CMP act as dequenchers. The quenching efficiency follows the scale GMP>UMP>TMP while the dequenching efficiency follows the scale AMP>CMP. As a whole, the data collected were successfully accounted for by assuming that the NMPs compete with the surfactant counterion (bromide) for the surface of the micelle[2]. These results could also be highly valuable for sensing applications allowing the use of simple spectrofluorimetric assays

for the determination of species that do not have any influence on the fluorescence of the micellized probe.




Schematic representation of the photophysical response due to counter ion switch. The scheme shows that all nucleotides concentrate on the micellar interface replacing the bromide counter ions. GMP, UMP and TMP lead to a reduction in the pyrene fluorescence intensity because have a quenching efficiency higher than bromide counter ion. AMP and CMP induced an increment in the pyrene fluorescence because they are less effective than the bromide counter ion. The aromatic rings of NMPs with quenching abilities are reported in white while the aromatic rings of NMPs with dequenching abilities are reported in grey.




Fluorescence emission spectra of pyrene (5 x $10^{-7}$ M) solubilized in 2 mM CTAB micellar solution at pH 7.5 (10mM Tris/HCl) upon addition of 10 mM AMP, GMP, UMP, CMP, TMP (excitation wavelength 334 nm).

## References

1. Lopez, F., Cuomo, F., Ceglie, A., Ambrosone, L., Palazzo, G. *Quenching and dequenching of pyrene fluorescence by nucleotide-monophosphates in cationic micelles*. Journal of Physical Chemistry B, 2008, 112, 7338-7344.

2. Cuomo, F. Palazzo, G., Ceglie, A., Lopez, F. *Quenching efficiency of pyrene fluorescence by nucleotide monophosphates in cationic micelles*. Journal of Photochemistry Photobiology A, 2008. DOI: 10.1016/j.jphotochem.2008.10.028.

# 1B – Liposomal formulations containing nucleolipid derivatives

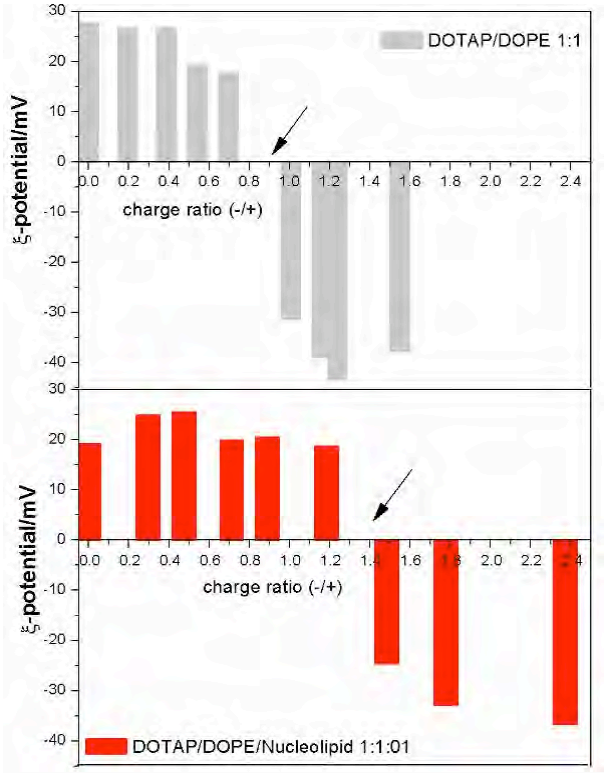
*F. Cuomo, F. Lopez, A. Ceglie*

## Aims

Investigation on the ability to form lipoplexes with liposomes containing nucleolipids to highlight an eventual crucial role of the nucleobase's derivatives surfactants.

## Results

The formulation of complexes obtained with liposomes and nucleic acid (lipoplexes) has been in the last few years and still is of major concern, because of their enormous utilization for items related to the investigation of biotechnological aspects.
In general, the future of non-viral delivery of nucleic acids depends on the understanding of the barriers to delivery. Since, these naked macromolecules are often prone to degradation and do not reach their target site, their formulation needs special care. For instance to produce a successful genetic material delivery the vectors should have nanometric size, the particles should be stable and resistant to non-specific uptake in the circulation.
Since recently a new category of anionic and non ionic lipids with a nucleotide in the headgroup, the so called nucleolipids, has been synthesized[1,2,3], a possible use of nucleolipids associated with conventional lipids (DOTAP and DOPE) in the formulations of new categories of lipoplexes seems to be an attractive target.
In the current investigation we perform ζ-potential and DLS measurements on liposomes containing the lipidic nucleobase's derivatives to investigate on their interaction with anionic polyelectrolytes i.e. oligonucleotides and polynucleotides.
The tritration performed with the addition of DNA onto liposomes cationic surface results in a decreasing of zeta potential. We verify that the zeta potential decreasing allows us to check for the isoelectric point. The point of maximum instability of assemblies corresponds to the charge inversion point where the whole surface charge is neutralized. The positive zeta potentials' region is considered the most efficient one concerning the transfection's capabilities[4]. The negative zeta-potential values correspond to the whole cationic vesicles' surface wrapped with the polyanion. Complexes with negative surface charge have reduce tranfection efficiency because of the repulsion interactions with the negative cellular membranes. The graphs reported, as an example, show the charge inversion points on liposomes formulation with and without the nucleolipid and subsequently that the useful amount of adsorbed DNA is shifted toward an higher value in nucleolipid enriched liposomes.




ζ-potential values vs. charge ratio. The figure report the surface charge inversion with the addition of DNA on liposomal formulation without the nucleolipid (upper panel) and with nucleolipid (lower panel). Liposomes enriched with the nuclebases' derivative allow the binding of higher amount of DNA.

## *References*

1. Angelico R., Ceglie A., Cuomo F., Cardellicchio C., Mascolo G., Colafemmina G., *Catanionic Systems from Conversion of Nucleotides into Nucleo-Lipids,* Langmuir 2008, 24, 2348-2355.

2. Cuomo F., Lopez F., Angelico R., Colafemmina G., Ceglie A., *Nucleotides and nucleolipids derivatives interaction effects during multi-lamellar vesicles formation,* Colloids and Surfaces B: Biointerfaces 2008, 64, 184–193.

3. Baglioni P., Berti D., *Self assembly in micelles combining stacking and H-bonding*, Current Opinion in Colloid & Interface Science 2003, 8, 55-61.

4. Salvati, A., Ciani, L., Ristori, S., Martini, G., Masi, A., and Arcangeli, A. *Physico-chemical characterization and transfection efficacy of cationic liposomes containing the pEGFP plasmid*, Biophysical Chemistry 2006, 121, 21-29.

# 1B – Phase equilibria and dynamic investigations in the three-component system Lecithin-$H_2O$-Organic oil

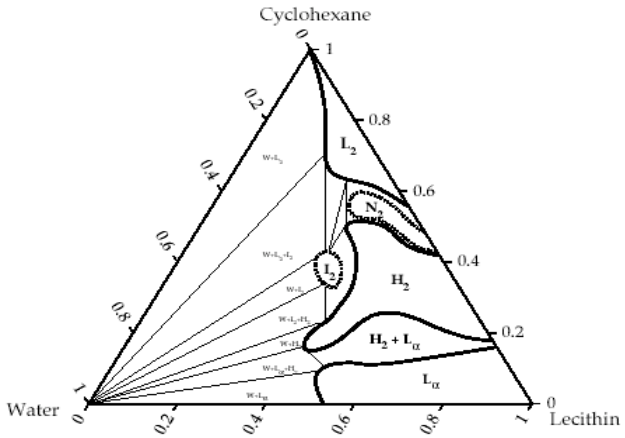
*R. Angelico, A. Ceglie, L. Ambrosone, G. Palazzo, G. Colafemmina, U. Olsson (Chemical Center, Lund Univ., Sweden)*

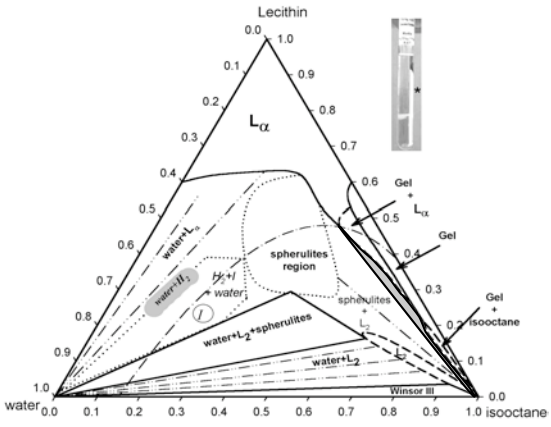
## Aims

Phase behavior of lecithin-water-oil ternary systems. Microstructure of micellar networks. Dynamic investigations on shear-induced phase transitions.

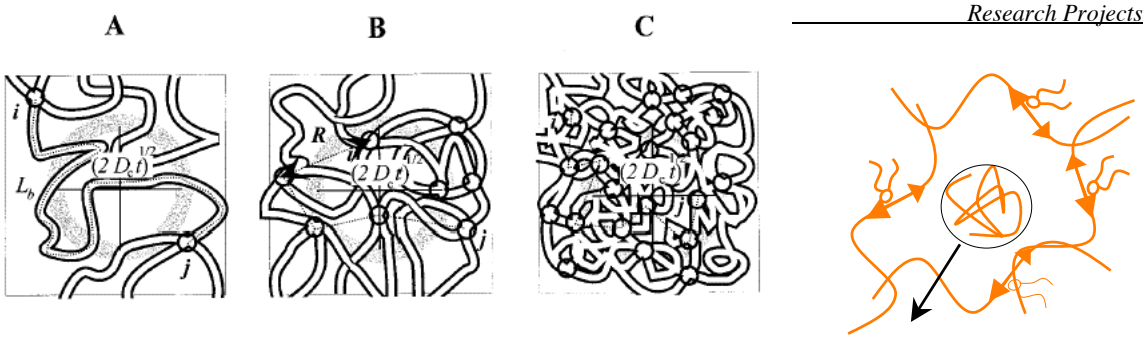
## Results

We investigated in details the phase behaviours of pseudo-ternary systems formed by soybean phosphatidylcholine (lecithin), water and low molecular weight organic solvents. We found a considerable effect of solvent structure – Cyclohexane and Isooctane – on the overall phase behaviour. With the former, this is governed essentially by the local curvature energy. The absence of bicontinuous intermediate phases and the larger presence of phases of reverse structure indicates a negative spontaneous curvature, H0, of the lipidic film (*left diagram*). In the latter, the lower ability of Isooctane to penetrate the surfactant palisade gives rise to a large portion of the phase diagram occupied by lamellar and bicontinuous structures (*right diagram*).

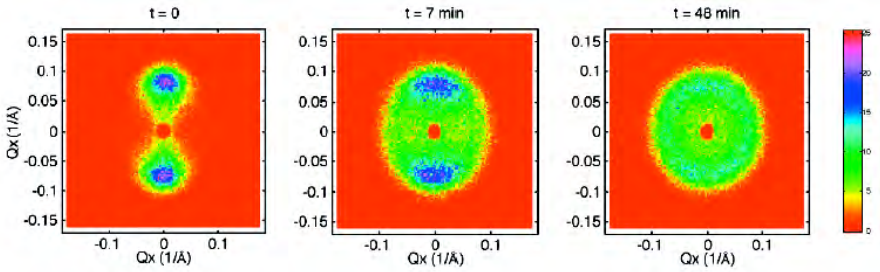



Detailed characterisation has been acquired, through self-diffusion NMR technique, about the dependence of the morphology of the micellar network in the reverse isotropic $L_2$ phase on type of oil. In fact, in addition to the dependence of the extent of the $L_2$ phase on the solvent structure, our results indicate that the topology of the microstructure can be tuned from *disconnected unbranched living polymers* (Cyclohexane) to *connected branched living network* (Isooctane). For the latter, the




number of branches can be opportunely controlled by varying the water-to-lecithin molar ratio $W_0$. Microstructure of lecithin organogels in isooctane as deduced by surfactant self-diffusion NMR experiments. (**A**) Network of long worm-like micelles with few junction points (branches), whose mean density increases with increasing both micellar volume fraction and water content (**B** and **C**). Cyclohexane organogels are characterised by *anomalous* diffusion such as *subdiffusion*, where the dominating mechanism corresponds to a lateral diffusion along the contour of the wormlike micelles, and *superdiffusion*, found near the overlap concentration from dilute to semi-dilute regime where the center of mass diffusion of smaller polymer-like micelles contributes to the transport of surfactant molecules. The shear-induced Isotropic to Nematic transition in Lecithin organogels in Cyclohexane presents the unusual property – for a micellar system – to have a very long relaxation time for the process of re-entanglement back to the disordered phase. This phenomenon, which is reminiscent of a mechanism where nematic state slowly re-melts to isotropic, has been recorded by performing time-resolved SANS experiments as shown below:




## References

Angelico, R., Ceglie, Olsson, U. and Palazzo, G. "Anomalous surfactant diffusion in a living polymer system", Phys. Rev. E, 74, 031403-031411, (2006).

Angelico, R., Ceglie, A., Colafemmina, G., Lopez, F., Olsson, U. and Palazzo, G. "Biocompatible lecithin organogels: microstructure and phase equilibria", Langmuir 21, 140-148, (2005).

Angelico, R., Ceglie, A., Olsson, U., Palazzo, G., Colafemmina, G. and Delfine, F. "Phase behaviour of the lecithin/water/isooctane and lecithin/water/decane systems", Langmuir 20, 619-631 (2004).

# 1B – Nanostructured media for the synthesis of bio-inspired anionic lipids

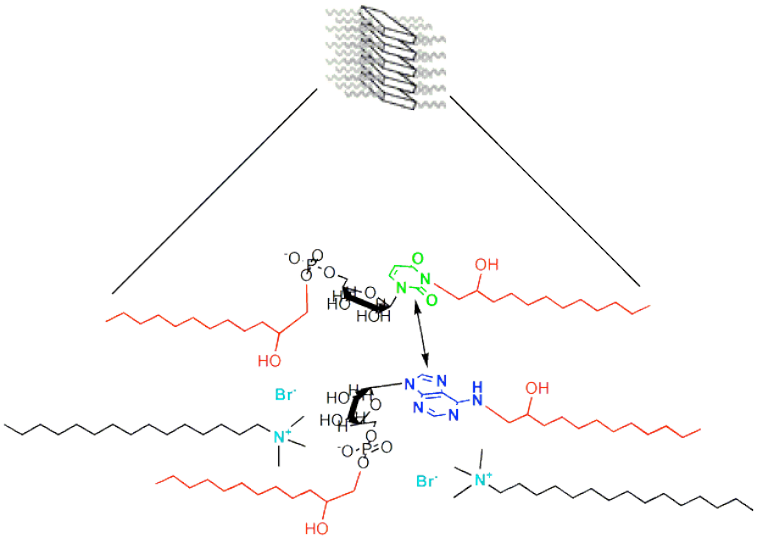
*R. Angelico, A. Ceglie, F. Cuomo, G. Colafemmina, I. Lo Sito, F. Palmisano (Dip. Chimica, Univ. Bari)*

## Aims

Oil-in-Water microemulsions as nanoreactors to produce alkylated nucleobases.
Effect of base-pairing on the reaction of alkylation of complementary ribonucleotides.

## Results

Evidences of a subtle role of base-base interaction upon a reaction of alkylation of complementary ribonucleotides – giving rise to their amphiphilic derivatives – have been experimentally obtained in a model ternary microemulsion. The aliphatic epoxide 1,2-Dodecyl-Epoxide (DE) has been used as alkylating agent, which can be finely dispersed in a microemulsion of Oil-in-Water spherical micelles, provided by the cationic surfactant Cetyl-Trimethyl-Ammonium-Bromide (CTAB).
The presence of a charged interface not only overcomes the mutual immiscibility between the apolar epoxide and aqueous solutions of AMP and UMP, but also provides a suitable domain where both reactants meet and react. The corresponding reaction products have been identified through HPLC-ESI-MS and $MS^n$ as mixtures of mono- and di-chained (α or β hydroxyl) derivatives of the nucleobases, with some peculiar differences depending on whether the starting microemulsions were incubated with AMP, UMP or their equimolar mixture.
In particular, MS/MS data gave indication about the sites of alkylation occurring, e.g., at level of uracil/phosphate OH groups in doubly-alkylated UMP, whereas in the case of alkylated derivatives of AMP, adenine $NH_2$ group and phosphate or ribose OH groups were found to be involved as such (single alkylation) or in combination (di-chained products). In the system incubated with both complementary nucleotides, the absence of α-hydroxyl-derivatives has been considered as a signature of a weak base-base preferential interaction between uridine and adenine nucleobases occurring at micellar interface.
After several days, the initial isotropic microemulsions, incubated with both nucleotides and DE, transformed into milky suspensions identified by optical microscopy as a system of Multi-Layered-Vesicular (MLV) structures.
The interface of these aggregates is constituted presumably by cationic CTAB monomers and the novel anionic mono- and di-chained $C_{12}$-amphiphiles, bearing nucleobases in their head polar groups.



Pictorial representation of a catanionic bilayer composed by anionic di-alkylated nucleolipids and cationic CTAB monomers.

NMR spectroscopy and UV–Vis measurements performed on MLVs showed strong aryl interactions. Interestingly, NMR spectra revealed prevailing stacking interactions between complementary nucleolipids. The assembly of complementary nucleotides has been found to affect the course of the reaction during the MLVs formation. Moreover the MLVs supramolecular stability has been tested by means of turbidity and UV–vis measurements. In particular, an enhanced stability has been recorded in systems prepared with complementary nucleotides, confirming that in these systems the self-assembly process is influenced by nucleolipids interactions. Furthermore by following the hypocromic effect during the micellar catalysis, we showed that even in the earlier stages of the reaction significant differences are detectable.

## *References*

Angelico, R., Ceglie, A., Cuomo, F., Cardellicchio, C., Mascolo, G., Colafemmina, G., *Catanionic Systems from Conversion of Nucleotides into Nucleo-Lipids*, Langmuir 2008, 24, 2348-2355.

Cuomo, F., Lopez, F., Angelico, R., Ceglie, A., Colafemmina, G., *Nucleotides and nucleolipids derivatives interaction effects during multi-lamellar vesicles formation*, Colloids and Surfaces B: Biointerfaces 2008, 64, 184-193.

Angelico, R., Ceglie, A., Cuomo, F. *Reaction Mixtures Based on the CTAB - Dodecyl Epoxide – Water Microemulsion for the Synthesis of Novel Nucleo-Lipids*. Colloid and Surfaces B. *Accepted*.

# 1B – Photosynthetic Reaction Centers Embedded in Polyelectrolyte Multilayer as an Optical Biosensor for Herbicide Detection

*G. Palazzo, M. Giustini, F. Lopez, A. Mallardi (Dip. Chimica, Univ. Bari), G. Venturoli (Dip. BES, Univ. Bologna)*

## Aims

Development of an herbicide biosensor based the bacterial reaction center (a photosynthetic protein) immobilized on glass substrates through layer-by-layer adsorption.

## Results

Herbicides are widely used on a variety of crops for the control of broadleaf weeds but can be highly toxic for human and animal health. Their wide use in agriculture has resulted often in the herbicide pollution of water and the level of herbicides allowed in drinking water is subject to regulation, at least in the industrialized countries. Different attempts have been made to introduce biological detection systems in order to overcome the high cost of time consuming HPLC analysis.
Here we present an optical biosensor based on a photosynthetic protein deposited on a quartz surface. The protein is the Reaction Center (RC) purified from *Rhodobacter sphaeroides* and for the preparation of the solid specimen its deposition is alternated with the cationic polymer poly(dimethyl diallyl) ammonium chloride (PDDA). The RC-herbicides interaction can be easily monitored since the steady-state attained by RCs under continuous light is different in the presence and in the absence of an inhibitor as shown in Fig. 1.

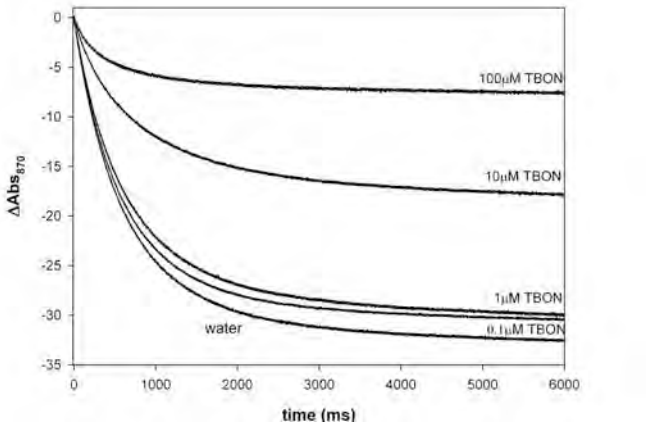



Figure 1. Time course of RC photobleaching induced by continuous illumination at 870 nm and measured on the same specimen immersed in solutions of herbicide (TBON) at different concentration.

The bound herbicide can be easily removed from PEM, thus allowing the reuse of the same sample.
The composite material developed has been previously characterized from a physico-chemical point of view.[1] The full characterization of their analytical properties regarding the broad family of PSII herbicides will be here presented together with the critical evaluation of costs and benefits of their use.
Among the most striking features are the long lifetime of the specimen (> 12 months) and the high reproducibility. The RC layered on quartz can be also used to test potentially active herbicides of new synthesis.
Its long time stability, easiness of handling, low demanding instrumental setup, let the RC/quartz assembly particularly appealing for the realization of a stand alone analytical apparatus.

## *References*

Lopez F., Mennuni M., Giustini M., Giomini M., Dezi M., Venturoli G., Mallardi A., Palazzo G. *Photosynthetic reaction centers embedded in polyelectrolyte multilayers as a tool in the determination of PSII herbicides*. Proceedings of 2nd IEEE International Workshop on Advances in Sensors and Interfaces, Bari 26-27 June 2007, p.237-242. ISBN: 1-4244-1244-7.

Mallardi A., Giustini M., Lopez F., Dezi M., Venturoli G., Palazzo G. *Functionality of Photosynthetic Reaction Centers in Polyelectrolyte Multilayers: Towards an Herbicide Biosensor*. J. Phys.Chem. B 2007, 111, 3304-3314.

# 1B – Protein-Matrix Coupling: the Photosynthetic Reaction Center embedded in Solid Matrices

*G. Palazzo, M. Giustini, F. Lopez, A. Mallardi (Dip. Chimica, Univ. Bari), G. Venturoli (Dip. BES, Univ. Bologna)*

## Aims

Study of the coupling of intra-protein electron transfer to protein dynamics in bacterial reaction centers embedded in glassy or polymer matrix.

## Results

Proteins are soft materials. Indeed, a protein is not rigid, rather it can undergo a variety of (fast) vibrations and (slower) structural rearrangements, these latter being called *protein-specific motions*. A protein share with supercooled liquids and glasses the existence of a very large number of non-identical conformation (substates)and separated by free-energy barriers. All possible substates together form the energy landscape, where a substate is pictured as a valley in the landscape. We have investigated the coupling of protein motions to intra-protein long-range electron transfer and to inter-protein interactions. All the investigations were carried out on a given protein: the *bacterial photosynthetic reaction center* of purple bacterium *Rhodobacter sphaeroide*s.

This protein provides an excellent model system for the detailed study of reaction substates and the free-energy barriers between them. The reactions can be probed by means of spectroscopical techniques, and it is possible to initiate electron-transfer in reaction centers (RC) with a pulse of light. This allows synchronized, single turnover measurements over a wide range of time scales. Reactions are reversible, allowing signal averaging.

Hindering of protein specific motions coupled to long-range electron transfer has been detected in RC embedded in trehalose glasses at room temperature. According to our investigations, conformational relaxations occurring in response to primary charge separation decrease both the free energy gap and the electronic coupling between acceptor and donor states, resulting in the observed stabilisation of the charge-separated state. Fig 1 shows the decay kinetics of laser induced charge separated state (panel A) and the corresponding rate distribution functions (panel B) obtained in solution (a) and in trehalose glass (b). Incorporation of the protein into a rigid the trehalose-water matrix leads to a substantial increase of mean rate of charge recombination and of the distribution width. Both the acceleration and the spreading of the recombination kinetics observable at room temperature in extremely dried trehalose-water matrices are quite comparable with those detected upon cooling the RC in the dark below 60 K. The coupling between relaxation and electron transfer is shown schematically in Panel C, which depicts simplified free energy surfaces of the

electronic states of neutral and charge-separated RC as a function of a generic conformational coordinate q. Within the Franck-Condon approximation, transitions between the $P^+Q_A^-$ and $PQ_A$ electronic states are vertical in this diagram. The electron transfer rate is controlled by the energy gap ε, i.e. by the vertical separation between the two surfaces. Since ε varies with q, the charge recombination kinetics reflects the evolution of the protein ensemble on the lower or upper energy surfaces. In trehalose glass, the RC relaxation from the *dark-adapted* to the *light-adapted* conformation, which stabilises primary charge separation in solution at room temperature (see transition *a* in Fig2C), is prevented over the time scale of $P^+Q_A^-$ recombination. This results in an accelerated charge recombination, occurring in a structurally inhomogeneous population of essentially non-relaxed *dark-adapted* proteins (see transition *b* in Fig.2C). In moderately 'soft' matrices, a partial relaxation takes place over the time scale of charge recombination, leading to intermediate recombination kinetics (see transition *c* in Fig. 2C).

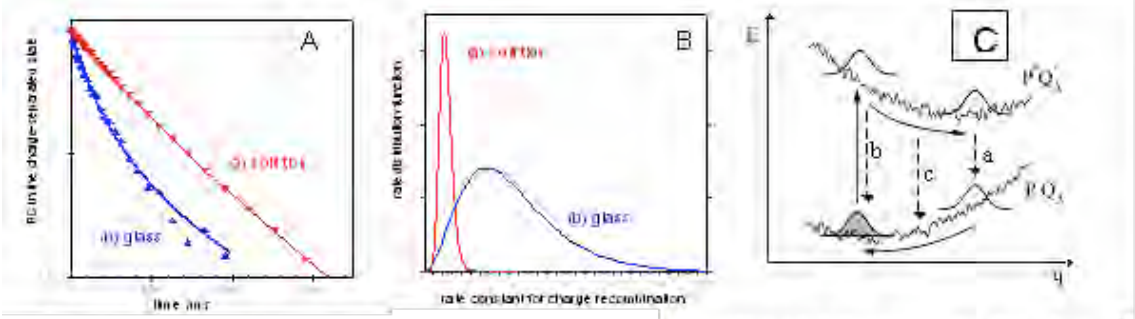



**Fig. 1** *Kinetic analysis of charge recombination following laser flash excitation of RC in a trehalose solution and glass. (A) Normalized decay of the charge separated state measured in a 0.4 M trehalose solution (trace a), and in a dry glass (water/trehalose mole ratio of 0.47; trace b). (B) The corresponding distribution functions. (C) The corresponding evolution of the protein ensembles in a simplified diagram showing relaxation on the energy surfaces associated with the neutral ($PQ_A$) and the charge separated state ($P^+Q_A^-$); the abscissa q represents a generic conformational coordinate.*

## References

Palazzo, G.; Francia, F.; Mallardi, A.; Giustini, M.; Lopez, F.; Venturoli, G. *Water Activity Regulates the QA- to QB Electron Transfer in Photosynthetic Reaction Centers from Rhodobacter sphaeroides*. Journal of American Chemical Society 2008, 130, 9353- 9363.

Veronesi, G., Giachini, L., Francia, F., Mallardi, A., Palazzo, G., Boscherini, F., Venturoli G. *The Fe2+ site of photosynthetic reaction centers probed by multiple scattering x-ray absorption fine structure spectroscopy: Improving structure resolution in dry matrices.,* G. Biophysical Journal 2008, *95*, 814-822.

Francia, F., Dezi, M.; Mallardi, A., Palazzo, G., Cordone, L., Venturoli, G. *Protein-Matrix Coupling/Uncoupling in "Dry" Systems of Photosynthetic Reaction Center Embedded in Trehalose/Sucrose: The Origin of Trehalose Peculiarity*. Journal of American Chemical Society 2008, 130, 10240-10246.

Cordone L., Cottone G., Giuffrida S., Palazzo G., Venturoli G., Viappiani C., *Internal Dynamics and Protein-Matrix Coupling in Trehalose Coated Proteins*, Biochim. Biophys. Acta 2005, *1749*, 252-281.

# 1B – Self-Assembly of Biologically Inspired Surfactants

*D. Berti, F. Baldelli Bombelli, M. Banchelli, S. Milani, P. Baglioni*

## Aims

Design, preparation, strucutral and dynamical investigation of nanostructured materials built by self-assembly of functional bio-inspired amphiphiles

## Results

We have designed, prepared and studied some nucleolipid derivatives, whose aggregates potentially represent alternative and less toxic lipid-based delivery systems for DNA. These lipids belong to the class of functional amphiphiles, where a chemical group with precise functional properties or responsivity to external stimuli is covalently attached to a hydrophobic assembler. In nucleolipids this chemical motif is a nucleic base, which impart molecular recognition capability to self-assemblies. We have shown that molecular recognition is triggered by self-assembly and affects structural features of the aggregates: amphiphilic systems composed of mixed "complementary" nucleolipids show deviations from ideal behavior that have been ascribed to selective molecular recognition. These assemblies provide negatively charged interfaces decorated with nucleic motifs that can complex complementary nucleic acid strands using, as driving force, molecular recognition instead of electrostatic interactions. We have shown that globular micelles composed of 1,2-dioctanoyl-phosphatidyl-adenosine interact with complementary RNA single strands (ss-polyUridylic acid) forming a new hexagonal phase where the nucleic acids is confined amongst cylindrical micelles. This provides a proof-of-principle that complexation is possible notwithstanding the same charge of both partners (i.e. lipid assembly and nucleic acid). We have further extended our investigation to zero-curvature assemblies, i.e. nucleolipid membranes, formed by POPNs. Nucleolipid bilayers of POPA, when swollen with aqueous solutions containing single strand complementary polynucleotides (polyUridylic acid) show an increase of the smectic period with respect to binary POPA/water systems, and the appearance, upon annealing, of an additional SAXS peak ascribable to 1D ordering of the biopolymer in-between the lamellar stacks. None of the observed effects could be detected for identically charged membranes formed by POPG, ruling thus out any unspecific effect due to the biopolymer presence in the swelling solution. The extra-peak position scaled in the SAXS spectrum with biopolymer concentration similarly to lipoplexes.
These findings opened new perspectives in the field of nucleic acid complexation and confinement for therapeutic purposes. Such nucleolipids are non-cytotoxic and can be metabolized by phospholipases; moreover their interaction with nucleic acids is base-

specific and not aspecifically diriven by electrostatic compensation. The above outlined approach would represent a strategic shift from aspecific to tailored vectors. Currently, our research is devoted to the development of nucleolipid liposomal formualtions containing more realistic mimics for therapeutics nucleic acids.

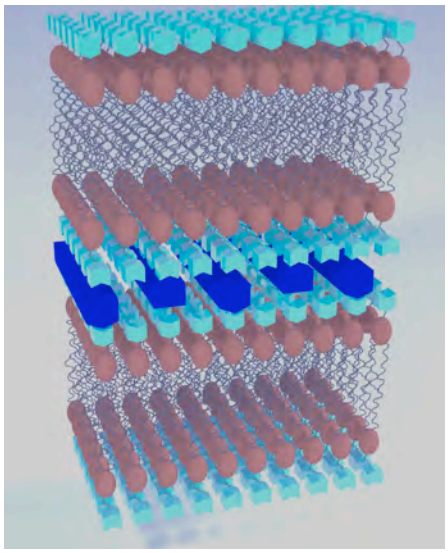


Figure 1. Sketch of the possible structural model for nucleolipoplexes, as inferred from SAXS and Neutron Diffraction.

## References


Berti, D. *Self assembly of biologically inspired amphiphiles*, Current Opinion In Colloid & Interface Science 2006, 11, 74-78.

Milani, S., Baldelli Bombelli, F., Berti, D., Hauß, T., Dante, S., Baglioni, P. *Structural Investigation of Bilayers Formed by 1-Palmitoyl-2-Oleoylphosphatidylnucleosides*, Biophys. J. 2006, 90, 1260-1269.

Baldelli Bombelli, F. Berti, D., Milani, Lagi, M., Barbaro, P., Karlsson, G., Brandt, A., Baglioni, P. *Collective Headgroup Conformational Transition in Twisted Micellar Superstructures*, Soft Matter 2008, 4, 1102–1113.

Baldelli Bombelli, F., Berti, D., Almgren, M. Karlsson, G. Baglioni, P. *Light Scattering and Cryo-TEM Investigation of the Self-Assembling Behavior of di-$C_{12}$P-Nucleosides in Solution*, J. Phys. Chem. B 2006, 110 (35) 17627.

Banchelli, M., Berti, D., Baglioni, P. *Molecular recognition drives oligonucleotide binding to nucleolipid self-assemblies,* Angew. Chem. Int. Ed. 2007, 46, 3070 –3073.

Milani, S., Baldelli Bombelli, F. Berti, D., Baglioni, P. *Nucleolipoplexes: A New Paradigm for Phospholipid Bilayer-nucleic Acid Interactions*, J. Am. Chem. Soc., 2007, 129, 11664-11665.

Berti, D. Baldelli Bombelli, F., Fortini, M., Baglioni, P. *Amphiphilic Self-assemblies Decorated by Nucleobases*, J. Phys. Chem. B 2007, 111, 40, 11734 -11744.

Milani, S., Baldelli Bombelli, F. Berti, D., Dante, S., Hauβ, T. and Baglioni, P. *Nucleolipid Membranes: Structure and Molecular Recognition*, J. Phys.:Condens. Matter 2008, 20, 104212.

# 1B – Addressable Molecular Node Assemblies

*G. Caminati, D. Berti, F. Baldelli Bombelli, F. Gambinossi, M. Banchelli, M. Lagi, P. Baglioni*

## Aims

The ultimate goal is the build-up, through a bottom-up modular approach, of a grid of addressable molecular blocks, supported onto planar or curved lipid bilayers.

## Results

The use of nucleic acids as building blocks in Nanotechnology has an increasingly prominent role, due to the fact that several characteristics render DNA an ideal material for the directed self-assembly into nanometer-scaled objects with defined properties. A unique library of single strands can be designed to contain or bind molecular components (optically or electronically active) at precise sites, and to assemble them in a 2D-array with sub-nm precision. The realization of addressable DNA architectures requires the design of complex motifs with sticky ends that assemble to form extended DNA scaffolds, possibly supported onto functionalized surfaces. The approach we are pursuing is the use of trigonal single strands, where a synthetic three-way junction serves as the central node from which three DNA "branches" lead off. Six of these trigonal units should assemble into the unit cell of an hexagonal lattice. These DNA pseudo-hexagons, whose sides consist of an integer multiple of helix turn, represent a convenient choice in terms of possible future applications in nanoelectronics and for the fact that a more rigid and defined scaffold can offer many advantages for addressability and spatial control of molecular components. As a first step towards this ultimate goal, we have recently investigated the aggregation in solution of linear ss-42-mer oligonucleotides, demonstrating their self-assembly into pseudo-hexagonal DNA subunits20 with rigid sides composed of 20-mer double strands and edges formed by non-pairing TT sequences.

Each pseudohexagon is an isolated nanostructure, with no sticky ends suitable for further directional assembly, but represents nonetheless an excellent model to test structural and steric requirements for formation and stability.

These results have been extended to the construction of a lipid membrane/pseudo-hexagonal DNA hybrid. The effects of grafting density, lipid/DNA ratio, liposome number density and preparation procedure on the final structure and yield of the resulting hybrid nanomaterial, were explicitly addressed. To this aim three different approaches of construction were illustrated and compared: two step-wise procedures, where each strand is added in a different order, and a third strategy, consisting in the immobilization of preformed DNA hexagons.

The so-built hybrid soft nanomaterial has never been reported previously and merge the unique features offered by DNA building blocks in nanotechnology, to the

characteristics of amphiphilic self-assembly, in terms of responsiveness and further hierarchical aggregation in functional array of nanounits.

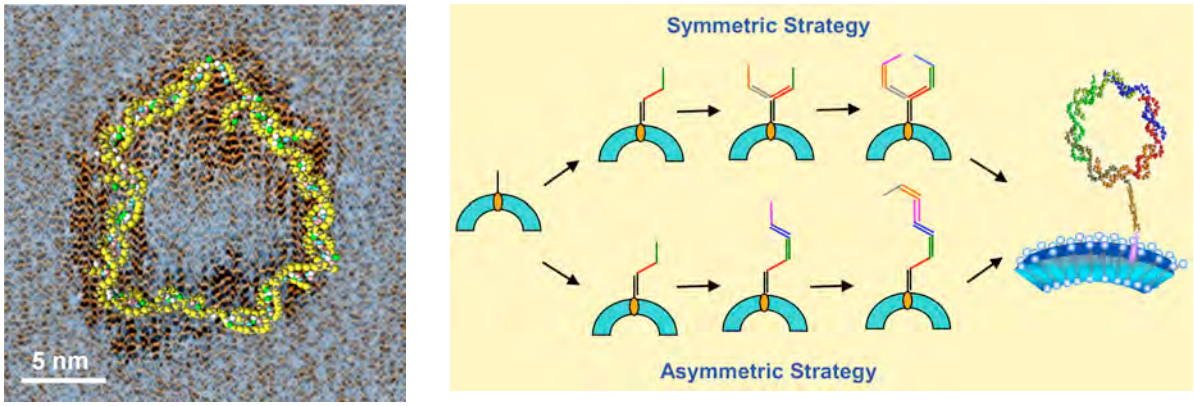



Figure 1. Left: representation of the experimental AFM image of an isolated DNA pseudo-hexagonal nanostructure overlapped with the equilibrium conformation at 20°C obtained for the same DNAsequence from Monte Carlo simulation. Right: Build-up of a DNA nanosized hexagon on a curved lipid bilayer.

The liposomes decorated with DNA pseudo-hexagons are structurally stable for weeks and can thus be further exploited or specifically addressed. The structural features of the final nanoobjects are independent on the sequence of preparation, i.e. step-wise on the membrane or addition of preformed hexagons, up to a threshold of density on the surface or vesicle number density, as sketched in Figure 1.

## *References*

Banchelli, M., Betti, F., Berti, D., Caminati, G., Baldelli Bombelli, F., Brown, T., Wilhelmsson, L. M., Nordén, B., Baglioni, P *Phospholipid membranes decorated by cholesterol-based oligonucleotides as soft hybrid nanostructures* J. Phys.Chem. B 2008, 112, 35, 10942–10952.

Baldelli Bombelli, F., Gambinossi, F., Lagi, M., Berti D., Caminati G., Brown, T., Sciortino, F., Nordén, B., Baglioni, P. *DNA Self-Assembled Ring Nanostructures: A Structural and Monte Carlo Simulation Study* J. Phys.Chem. B 2008, 112, 48, 15283–15294.

Betti, F., Baldelli Bombelli, F., Gambinossi, F., Caminati, G., Brown, T., Baglioni, P., Berti, D Closed *Nanoconstructs Assembled by step-by-step ss-DNA Coupling Assisted by Phospholipid Membranes* Soft Matter, in press.

# 1B – Fragile-to-Strong Dynamic Crossover phenomenon in Hydrated Biopolymers

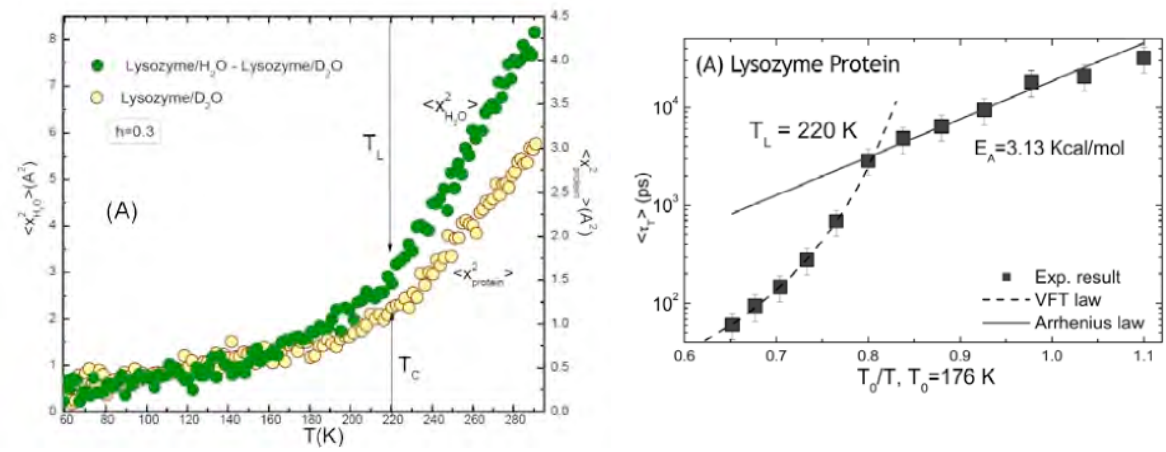
*E. Fratini, M. Lagi, C. Vannucci, P. Baglioni, S.H. Chen (MIT), F. Mallamace (Univ. Messina)*

## Aims

The Fragile-to-Strong Dynamic Crossover (FSC) phenomenon for the hydration water in a biopolymer controls its biological function.

## Results

The hydration water is behaved to have an important role in controlling the functionality of many biopolymers. As an example, the activity of many enzymes generally show a sharp slowing-down around 200-240 K. Experimental and computational results show a sharp increase of $<x^2>$ of hydrogen atoms in biopolymers at about $Tc$ = 220 K, which suggests that the dynamic transition (sometime called the glass transition) may be occurring in the biopolymers at this temperature. There is strong evidence that this dynamic transition of protein is solvent-induced, since the hydration water of a protein also shows a kind of dynamic transition around similar temperature. It was demonstrated that this dynamic transition of hydration water on lysozyme, B-DNA and RNA is, in fact, a FSC happening at 220-222 K[1-3]. These results suggest the universality of $Tc$ in biopolymers in spite of the difference in chemical backbone structure of proteins, DNA, and RNA.




Left Panel: Mean square displacement for the lysozyme and its hydration water as a function of T showing the abrupt change at about 220K. Right Panel: average relaxation time as extracted by QENS spectra confirming the FSC from a super-Arrhenius (non-linear) to the Arrhenius behavior (linear region) connected with a HDL to a LDL crossover[1]. Same trends were shown even for DNA[2] and RNA[3].

Moreover, above $T_L$ the hydration water is predominately in its high-density form (HDL), which is more fluid. But below $T_L$, it transforms to predominately the low-density form (LDL), which is less fluid. This abrupt change in the mobility of hydration water apparently induces the change in the energy landscape of biopolymer, which causes the dynamic transition (or the glass transition) in the biopolymer [4-5]. The evolution of the FCS was investigated as a function of pressure [6]. Moreover, a second FCS was evidenced in the high temperature regime [7], where the lysozyme denaturation process occurs and the population of the non-hydrogen-bonded fraction of water molecules is dominant [5]. This latter result can be considered as a strong signal that changes in hydration water accompany the well-known process of protein thermal unfolding.

## *References*

[1] Chen, S.H.; Liu, L.; Fratini, E.; Baglioni, P.; Faraone, A.; Mamontov, E., *Observation of fragile-to-strong dynamic crossover in protein hydration water*, Proc. Natl. Acad. Sci. 2006, 103, 9012-9016.
[2] Chen, S.H.; Liu, L.; Chu, X.; Zhang, Y.; Fratini, E.; Baglioni, P.; Faraone, A.; Mamontov, E., *Experimental evidence of fragile-to-strong dynamic crossover in DNA hydration water*, Journal of Chemical Physics 2006, 125, 171103.
[3] Chu, X.Q.; Fratini, E.; Baglioni, P.; Faraone, A.; Chen, S. H., *Observation of a Dynamic Crossover in RNA Hydration Water which Triggers the Dynamic Transition in the Biopolymer*, Physical Review E 2008, 77, 011908.
[4] S.-H. Chen, F. Mallamace, L. Liu, D. Z. Liu, X. Q. Chu, Y. Zhang, C. Kim, A. Faraone, C.-Y. Mou, E. Fratini, P. Baglioni, A.I. Kolesnikov, V. Garcia-Sakai, *Dynamic Crossover Phenomenon in Confined Supercooled Water and its Relation to the Existence of a Liquid-Liquid Critical Point in Water*, AIP Conf. Proc. 2008, 982, 39-52.
[5] Mallamace, F.; Chen, S.H.; Broccio, M.; Corsaro, C.; Crupi, V.; Majolino, D.; Venuti, V.; Baglioni, P.; Fratini, E.; Vannucci, C.; Stanley, H.E., *Role of the Solvent in the Dynamical Transitions of Proteins: The Case of the Lysozyme-Water System*, J. Chem. Phys. 2007, 127, 045104.
[6] Zhang, Y.; Lagi. M.; Liu, D.; Mallamace, F.; Fratini, E.; Baglioni, P.; Mamontov, E.; Chen S.-H., *Observation of high-temperature dynamic crossover in protein hydration water and its relation to reversible denaturation of lysozyme,* accepted with minor revision J. Chemical Physics, 2008.
[7] Chu, X.-Q.; Faraone, A.; Kim, C.; Fratini, E.; Baglioni, P.; Leao, J.; Chen, S.-H. *Proteins Remain Soft at Lower Temperatures under Pressure,* submitted to J. Physics Condensed Matter, 2008.

# 1B – Temperature-induced Aggregation of Odorant Binding Proteins

*P. Luciani, F. Ridi, E. Fratini, P. Baglioni, R. Ramoni (Univ. Parma)*

## Aims

Odorant binding proteins (OBPs) pertain to one of the most abundant classes of proteins found in the olfactory apparatus. Temperature induces the formation of aggregates that were characterized by means of UV, CD, fluorescence and AFM.

## Results

Odorant-binding proteins (OBPs) are a sub-class of lipocalins, defined by their property of reversibly binding volatile chemicals, called 'odorants'. Bovine odorant binding protein (bOBP), the most peculiar representative OBP [1], and two monomeric mutants [2], M3 - in which a glycine residue was inserted after Lys 121 and MB1 - obtained by introducing a Gly after position 121 and two cysteine residues at positions 64 (Trp) and 155 (His) – were investigated. In particular, the temperature effect on protein conformation was evaluated and correlated to the different the tertiary and quaternary structure.[3]

A peculiar behavior was observed: temperature induces an irreversible aggregation. Aggregate morphologies were investigated by means of AFM and characteristic features were attributed to the studied biomacromolecules.

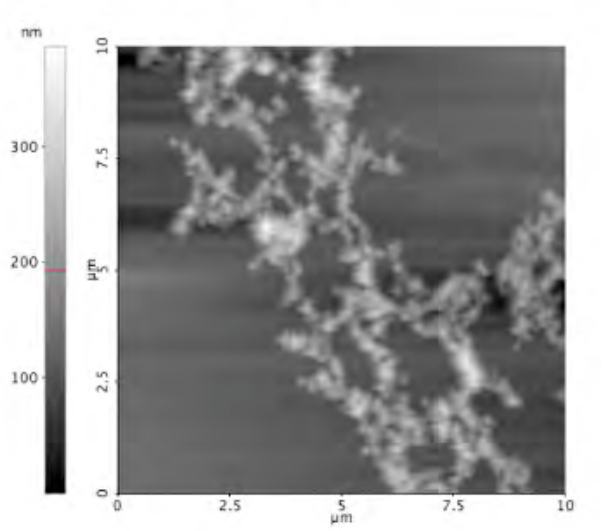



Figure 1. AFM image of bOBP solution after denaturation.

However, UV, CD and fluorescence data confirmed that for all investigated mutants the non-aggregated protein fraction retains its native conformation as well as its binding ability.

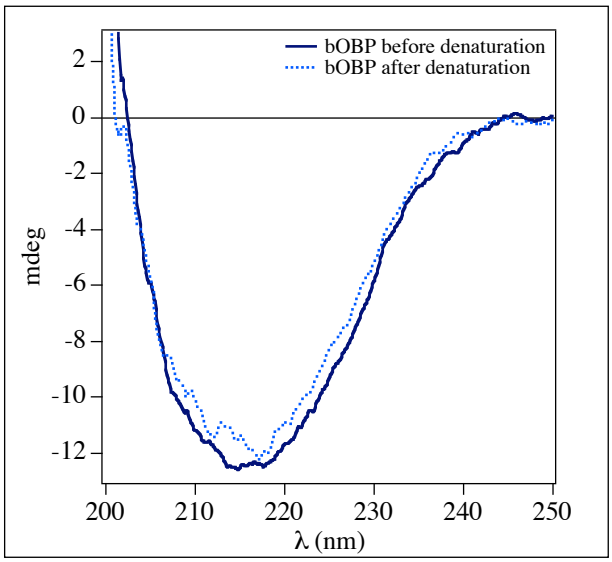



Figure 2. CD spectra of bOBP before and after denaturation (spectra acquired at 25 ºC).

A Small Angle Scattering (of both X-rays and neutrons) is currently on going in order to have a deeper insight on the solution structure of these proteins and on the temperature-induced aggregation. Standard routines developed by D.I. Svergun [4] will be applied on experimental data in order to extract characteristic dimensions and to reconstruct the molecular architecture of both the native and denatured states.

## *References*

[1] Tegoni, M., Pelosi, P., Vincent, F., Spinelli, S., Campanacci, V., Grolli, S., Ramoni, R., Cambillau, C., *Mammalian odorant binding proteins*, Biochimica et Biophysica Acta 2000, 1482, 229-240

[2] Ramoni, R., Spinelli, S., Grolli, S., Conti, V., Merli, E., Cambillau, C., Tegoni, M., *Deswapping bovine odorant binding proteins*, Biochimica et Biophysica Acta 2008, 1784, 651-657.

[3] Luciani, P., Ridi, F., Fratini, E., Conti, V., Grolli, S., Ramoni, R., and Baglioni, P., *Temperature-induced aggregation on odorant binding proteins,* manuscript in preparation.

[4] http://www.embl-hamburg.de/ExternalInfo/Research/Sax/software.html

# 1B – Biological Role of Phonons in Globular Proteins

*M. Lagi, E. Fratini, P. Baglioni, S. H. Chen (MIT)*

## Aims

The existence of phonons in hydrated globular proteins is shown and linked to their biological function.

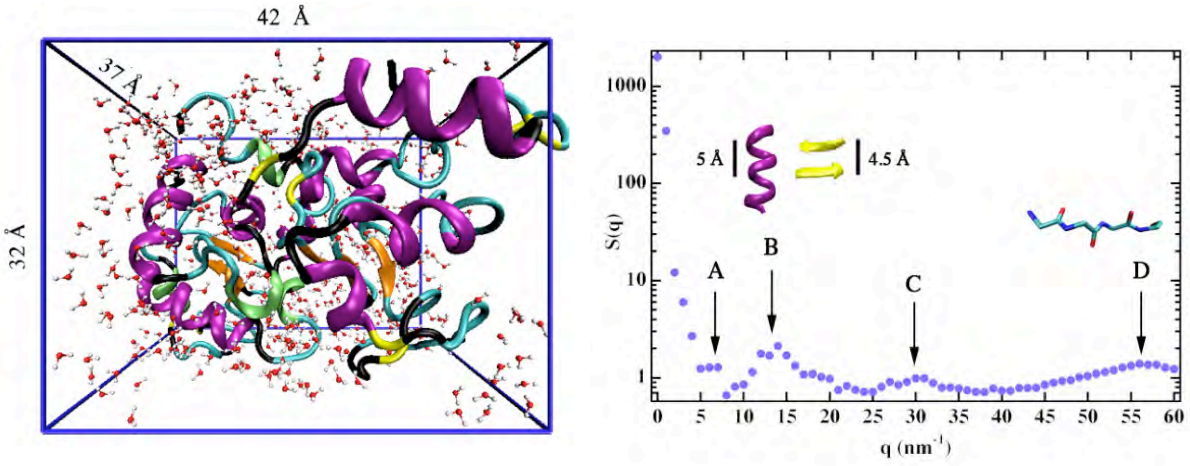
## Results

The study of phonons (the quantum mechanical vibrational motion with particle-like properties found in the atomic lattice of solids) has traditionally been the land of physicists. These phonons were found within an unusual and unexpected place: the interior of two hydrated (0.3 g of $H_2O$ per 1 g of dry powder) globular proteins, lysozyme (LYZ) and bovine serum albumin (BSA), respectively an enzyme and a transport protein. The choice of these particular proteins was driven by their quite different biological function and secondary structure, spanning different classes of globular proteins.

By using the new High Resolution Inelastic X-ray Scattering spectrometer (HERIX) at the Advanced Photon Source (Argonne, IL) coupled with a momentum-resolved method, we were able to differentiate at which length scale these biologically important modes take place as a function of temperature. We found a substantial decrease in phonon energy at a given Q-value whenever the temperature exceeded $T_D$ (i.e. the protein glass transition temperature). The slowing-down of molecular motions as represented by this softening of phonon energies provides a very strong correlation between the temperature-dependent motional behavior inside the proteins and their biological activity.

In particular, below $T_D$ the protein becomes more glass-like (i.e., solid), with considerable disorder and higher harmonic vibrational frequencies. Above $T_D$, this study has identified a significant temperature-dependence of the slowing-down and an increase in population of phonon-like collective motions, attributable to the collective vibrational motions of the atoms in the α-helices and β-sheets of the proteins. Below $T_D$, the vibrational frequency is too high and the population of the modes is too low to be able to facilitate the biological function, thus explaining why proteins do not function properly below the dynamic transition temperature. Thus, for the first time, the importance of the collective motions of the protein backbone to the biological function has been established.

The present project has confirmed indeed that phonon activity is intimately tied into the biological activity of proteins (i.e. biological function) showing that the physical concept of phonons is useful in biology, too. This study will be expanded towards different sorts of proteins possessing a quaternary structure and protein/Nucleic Acid complexes.




Left panel: The elements of LYS secondary structure are displayed with different colors: α-helices in purple, 3-10 helices in lime, β-sheets in orange, β-bridges in yellow, turns in cyan and coils in black. Right panel: LYS structure factor S(q). The phonons were measured around Peak B (that corresponds to some typical distances of the protein secondary structure: 4.5-5 Å) suggest the presence of collective motions that propagate along the α-helices and the β-sheets, which are crucial to the enzymatic function of LYS.

## *References*

Liu, D., Chu, X.-Q., Lagi, M., Zhang, Y., Fratini, E., Baglioni, P., Alatas, A., Said, A., Alp, E., Chen, S.-H., *Studies of Phononlike Low-Energy Excitations of Protein Molecules by Inelastic X-Ray Scattering,* Phys. Rev. Lett. 2008, 101, 135501 DOI: 10.1103/PhysRevLett.101.135501

# 1B – Self assembly of chiral surfactants

*P. Lo Nostro, M. Ambrosi, E. Fratini, M. Lagi, L. Giustini, E. Bocci, B.W. Ninham, P. Baglioni*

## Aims

effect of chirality on self assembly

## Results

Chirality plays a crucial role in molecular recognition, and especially in biological systems. Alkanoyl-L-(+)-Ascorbic acid esters act as radical scavengers like Vitamin C (Figure 1), and can be easily dispersed in hydrophobic or aqueous media.

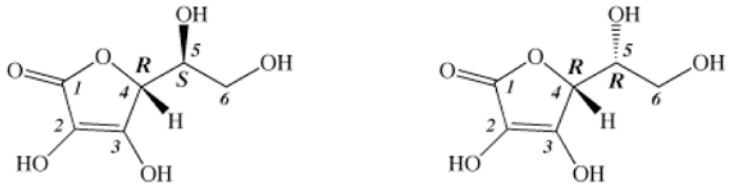



Figure 1. Molecular structures of L-ascorbic (left) and D-isoascorbic acid (right), showing the atom numbering and the configuration of the chiral centers ($C_4$ and $C_5$).

We synthesized single chained, double chained, and bolaform surfactants that bear one or two L-(+)-ascorbic acid residues as polar headgroups. Depending on their structure and temperature, these derivatives form different nanoassemblies in water. Single chain surfactants (L-ASCn) produce micellar solutions (for n=8, 10) or gels (for n≥11), and upon cooling form hydrated crystalline phases (coagels) (Figure 2) [1]. The bolaamphiphile forms monodispersed nanotubes in aqueous dispersions (Figure 3) [2], while the more hydrophobic double chain surfactant produces stable organogels [3].

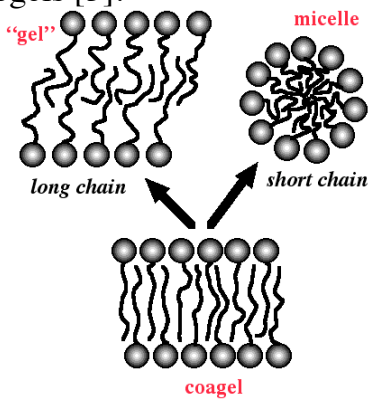



Figure 2. The coagel can form either a micelle or a gel upon heating.

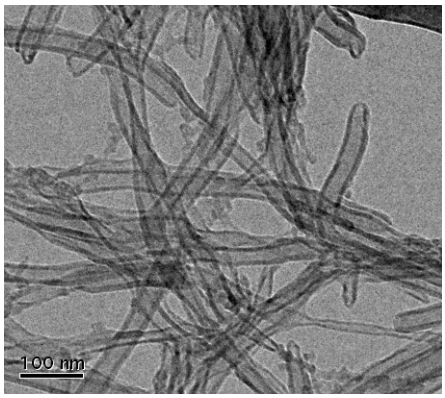



Figure 3. Cryo-TEM image of nanotubes formed by BOLA12 in water.

These nanoassemblies have been carefully studied through SAXS, XRD, DSC, SEM, surface tension isotherms, and conductivity experiments. [1-4].
More recently we explored the properties of single chain surfactants that carry a D-(-)-Isoascorbic acid moiety (Figure 1) as polar headgroup. Although the D-epimer has the same antioxidant power of L-Ascorbic acid, however its biological activity is reduced to 5%. The amphiphilic derivatives D-ASCn produce the same kinds of nanoaggregates in water, but present significantly different properties from the epimeric L-isomers, due to the different sets of inter- and intramolecular interactions that involve the surfactant molecules and the solvent [5]. Interestingly, D-ASCn and L-ASCn produce eutetics in the pure and in the coagel state [6] (Figure 4).

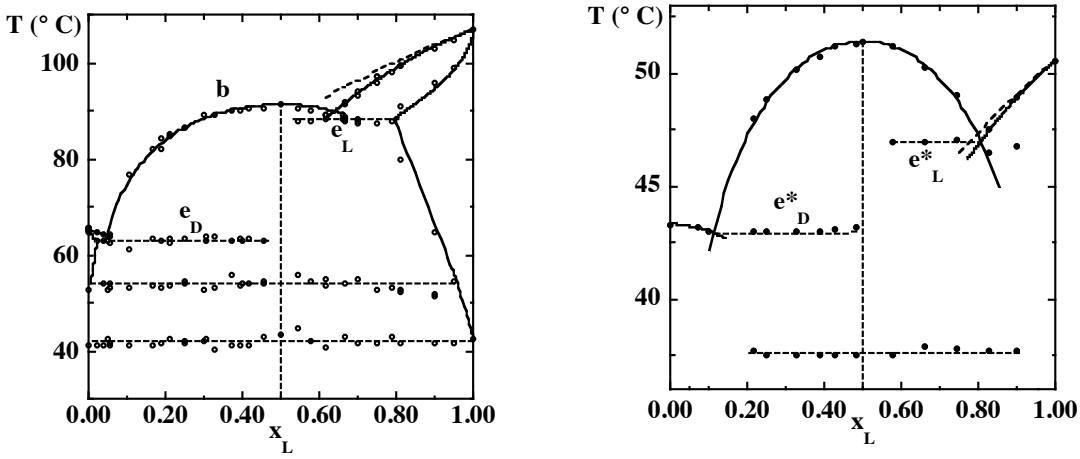



Figure 4. Phase diagrams of D-ASC12+L-ASC12 mixtures. Left: pure components; right: in the coagel state (10% w/w in water).

## References

[1] Lo Nostro, P., Ninham, B.W., Fratoni, L., Palma, S., Manzo, R., Allemandi, D., Baglioni, P. *Effect of Water Structure on the Formation of Coagels from Ascorbyl-Alkanoates*. Langmuir 2003, 19, 3222
[2] Ambrosi, M., Fratini, E., Alfredsson, V., Ninham, B.W., Giorgi, R., Lo Nostro, P., Baglioni, P. *Nanotubes from a Vitamin C-Based Bolaamphiphile*. J. Am. Chem. Soc. 2006, 128, 7209
[3] Lo Nostro, P., Ramsch, R., Fratini, E., Lagi, M., Ridi, F., Carretti, E., Ambrosi, M., Ninham, B.W., Baglioni, P. *Organogels from a Vitamin C-Based Surfactant*. J. Phys. Chem. B 2007, 111, 11714
[4] Ambrosi, M., Lo Nostro, P., Fratoni, L., Dei, L., Ninham, B.W., Palma, S., Manzo, R., Allemandi, D., Baglioni, P. *Water of hydration in coagels*. Phys. Chem. Chem. Phys. 2004, 6, 1401
[5] Ambrosi, M., Lo Nostro, P., Fratini, E., Giustini, L., Ninham, B.W., Baglioni, P. *Effect of headgroup chirality in nanoassemblies. Part 1. Self-assembly of D-isoascorbic acid derivatives in water*. J. Phys. Chem. B [in press]
[6] Lo Nostro, P., Ambrosi, M., Fratini, E., Ninham, B.W., Baglioni, P. *Effect of headgroup chirality in nanoassemblies. Part 2. Thermal behavior of Vitamin C-based surfactants*. J. Phys. Chem. B [in preparation].

# 1B – Surface Imaging of Nanostructures

*G. Caminati, F. Gambinossi, S. Ciappelli, P. Baglioni*

## Aims

A synergic combination of different imaging techniques such as Confocal Laser Scanning Microscopy (CLSM), Brewster Angle Microscopy (BAM), Ellipsometric mapping and conventional Atomic Force Microscopy (AFM) were implemented to control the structural organization and the bidimensional morphology of thin films at liquid and solid interfaces.

## Results

Brewster Angle Microscopy (BAM) offers unique possibilities to explore the bidimensional morphology of ordered nanofilms at water-air interface identifying surface phase transitions and domains with different molecular packing or orientation. The thickness of floating monolayers can be simultaneously measured with ellipsometric mapping. Mapping of the ellipsometric thickness is also an invaluable help in the characterization of functional nanodevices such Organic Light Emitting Diodes (OLED), this measurement allows the discrimination between homogenous and patchy surfaces with poor emission efficiency.

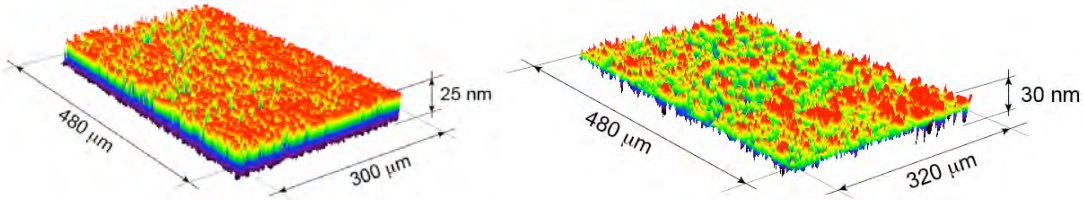



Figure 1. Ellipsometric mapping of the nanodevice deriving from chlorobenzene (left) and chloroform (right) spreading solutions.

This latter technique was also successfully used for the characterization of Supported Lipid Bilayers obtained from adsorption and rupture of phospholipid vesicles on hydrophilic surfaces.

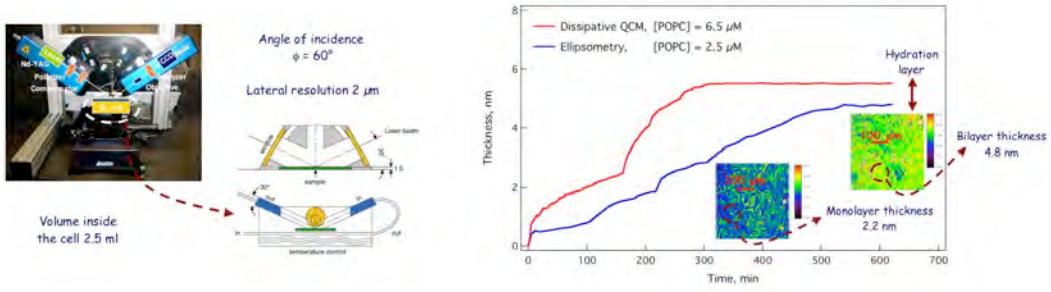



Figure 2. BAM/Ellipsometer set-up (left). Ellipsometric imaging and thickness determination for SLB on gold surfaces (right).

A parallel QCM study on the formation of SLB on gold surface was performed on the same system, the results showed unambiguously that the formation of the bilayer

proceeds in two single monolayer steps. The two sets of data evidence also the contribution of the hydration water molecules to the determination of QCM thickness. SLB can be imaged also by CLSM if a proper fluorescent label is present in the system; in the following figure we report an example of CLSM measurement for a supported phospholipids bilayer where a chol-tagged ds-oligonucleotide was anchored.

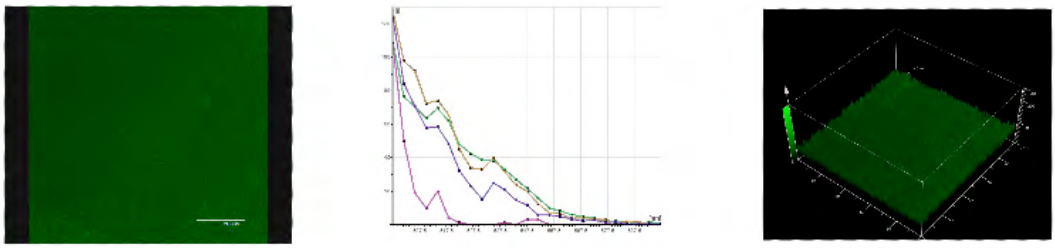


Figure 3. Schematic representation of a CLSM study: emission image, space-resolved emission spectra and thickness mapping.

Such studies clearly showed the presence of phase domain separation in the bilayer depending on the oligonucleotide concentration and preparation procedure. Although the CLSM technique is widely used in biological studies it also represents a precious tool for the investigation of nanostructures such as LB or LbL films or Supported Bilayers (see for example 1A – Multilayered Nanofilms with tunable functionality).
Surface imaging studies were performed also with conventional AFM techniques, in particular impressive results were obtained for closed DNA structures immobilized onto mica surfaces.

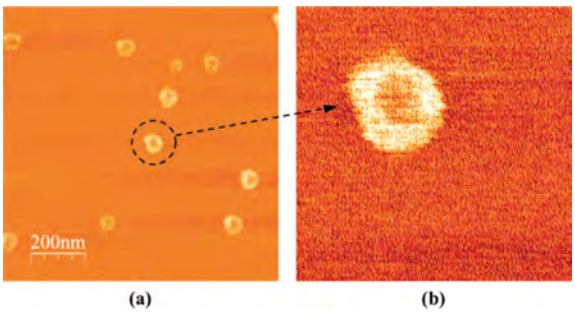



Figure 4. (a) AFM phase image (1 $\mu$m x 1 $\mu$m) on mica; (b) close-up (200 nm x 200 nm) of a single closed DNA structure.

## References

Gambinossi, F., Viitala, T. and Caminati, G. *Step-wise formation of supported Lipid Bilayers on gold substrates studied by Dissipative Quartz Crystal Microbalance and Imaging Ellipsometry*. Langmuir [submitted]

Baldelli Bombelli, F., Gambinossi, F., Lagi, M., Berti D., Caminati G., Brown, T., Sciortino, F., Nordén, B., Baglioni, P. *DNA Self-Assembled Ring Nanostructures: A Structural and Monte Carlo Simulation Study*. J. Phys.Chem. B 2008, 112, 48, 15283–15294.

# 1B – Self-Assembly of β-Cyclodextrin in Water. Formation of Pseudopolyrotaxanes

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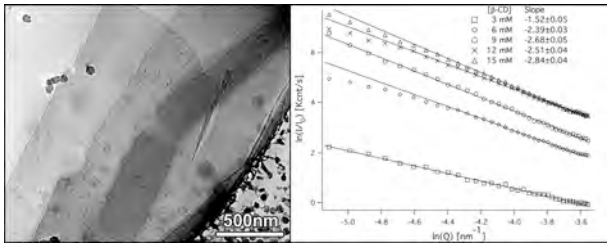
## Aims

Although cyclodextrins (CDs) have been extensively studied for more than four decades, the self-aggregation of cyclodextrins in water has been postulated only recently. The aim of this investigation is to confirm the presence of supramolecular assemblies in β-CD/water dispersions, and to evaluate the main structural and geometrical features of such mesoscopic structures, through dynamic and static light scattering, cryo-TEM, ans electron spin resonance experiments. The formation and precipitation of pseudopolyrotaxanes obtained from α- or β-CD and linear polymers were investigated through turbidity and SAXS measurements.

## Results

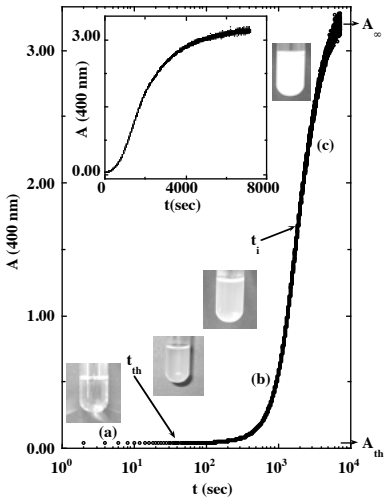
Our results definitely confirm that β-CD monomers do aggregate in water at room temperature in differently shaped particles, depending on the concentration. A critical aggregation concentration (c.a.c.) between 2 and 3 mM was determined by using Dynamic (DLS) and Static (SLS) Light Scattering. Above 3 mM, aggregates are formed in water, with a minimum hydrodynamic radius of about 90 nm. These particles are in equilibrium with larger objects at higher solute concentrations. Transmission Electron Microscopy at cryogenic temperature (Cryo-TEM) was used to detect the structural features of cyclodextrin self-aggregates.




Left: Cryo-TEM micrograph showing the planar aggregates formed by β-CD in water . Right: Static Light Scattering results of β-CD solutions in water.

The results show the occurrence of polymorphism depending on the β-CD concentration: polydisperse objects with diameters of about 100 nm are present at lower concentrations, while micrometer planar aggregates are predominant at higher concentrations. Upon sonication, the large bidimensional sheets turn into entangled long fibers and folded lamellae. Static light scattering experiments were performed to evaluate the fractal nature of the particles.

In order to explore the aggregation of β-cyclodextrin in water and its influence on the inclusive properties, the interaction of amphipilic spin labels with β-cyclodextrin has been investigated using conventional electron spin resonance (ESR) spectroscopy. Stearic acid spin probes (n-DSA) which contain a doxyl group, a cyclic nitroxide with unpaired electrons, covalently linked to the aliphatic chain carbon in position 5, 7, 12 or 16 were used. The most relevant finding from the ESR experiments is the detection of two spectral components differing in line-width, and thus in dynamics. The faster ESR component (Component 1), which was detected in all the investigated n-DSA/b-CD systems, has been simulated in order to extract the dynamic and polarity properties experienced by the spin probes (t, <A>).
The formation and precipitation of pseudopolyrotaxanes (PPR) were studied through turbidimetric and SAXS experiments. The formation and growth of the PPRs can be interpreted in terms of the Avrami model. The precipitation of large structures depends on the nature of the guest polymer. The aggregation of the PPRs was explained in terms of spatial dielectric anisotropy.




The effects of dissolved gases and electrolytes have been investigated also.

## References

Bonini, M.; Rossi, S.; Karlsson, G.; Almgren, M.; Lo Nostro, P.; Baglioni, P., *Self-Assembly of β-Cyclodextrin in Water. Part 1: Cryo-TEM and Dynamic and Static Light Scattering*, Langmuir 22, 1478-1484 (2006).
Rossi, S.; Bonini, M.; Lo Nostro, P.; Baglioni, P. *Self-Assembly of β-Cyclodextrin in Water. 2. Electron Spin Resonance*, Langmuir 23, 10959-10967 (2007).
Lo Nostro, P.; Giustini, L.; Fratini, E.; Ninham, B.W.; Ridi, F.; Baglioni, P. *Threading, Growth, and Aggregation of Pseudopolyrotaxanes*, J. Phys. Chem. B 112, 1071-1081 (2008).
Becheri, A.; Lo Nostro, P.; Ninham, B.W.; Baglioni, P. *The Curious World of Polypseudorotaxanes: Cyclodextrins As Probes of Water Structure*, J. Phys. Chem. B 107, 3979-3987 (2003).
Ceccato, M.; Lo Nostro, P.; Baglioni, P. *α-Cyclodextrin/Polyethylene Glycol Polyrotaxane: A Study of the Threading Process*, Langmuir 13, 2436-2439 (1997).

# 1B – Inhibition/Activation of Carbonic Anhydrases

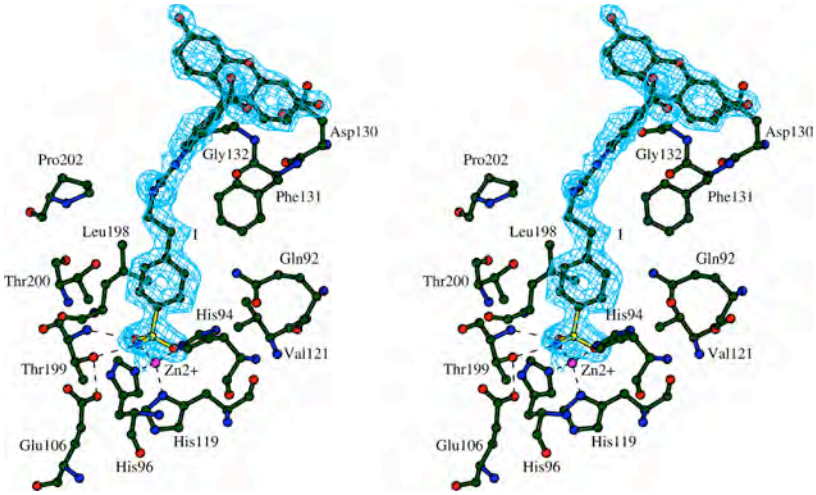
*C.T. Supuran*

## Aims

Aim of this study is the understanding of the molecular basis of inhibition/activation of the family of the carbonic as targets for the drug design.

## Results

The carbonic anhydrases (CAs, EC 4.2.1.1) are wide-spread enzymes, present in Bacteria, Archaea, and Eukarya (protosoa, plants, vertebrates, etc.). In mammals, at least 16 different isoforms have been isolated. Some of these isozymes are cytosolic (CA I, CA II, CA III, CA VII, CA XIII), others are membrane-bound (CA IV, CA IX, CA XII, CA XIV and CA XV), CA VA and CA VB are mitochondrial and CA VI is secreted in the saliva and milk. Three cytosolic acatalytic forms are also known (CA VIII, CA X and CA XI). The catalytically active isoforms, which play important physiological and patho-physiological functions, are strongly inhibited by aromatic and heterocyclic sulfonamides. Future prospects for drug design of inhibitors of these ubiquitous enzymes will be dealt with. Thus, it has emerged recently that the isozymes targeted by the antiglaucoma CA inhibitors (CAIs) are CA II and XII (and not CA IV, as previously considered). Some sulfonamide/thiazide diuretics probably target CA II, IV and XIV. The two mitochondrial isozymes CA VA and CA VB are the targets for the development of novel antiobesity agents, whereas CA VII for anticonvulsant/antiepileptic drugs. A recent discovery is connected with the involvement of CAs and their sulfonamide/sulfamate/sulfamide inhibitors in cancer: many potent CAIs were shown to inhibit the growth of several tumor cell lines in vitro and in vivo, constituting thus interesting leads for developing novel antitumor therapies or for imaging purposes (e.g., the fluorescent sulfonamide for which the X-ray crystal structure has been reported in complex with hCA II – figure below)[1]:



The isozymes involved in these processes are CA IX and XII, against which many potent inhibitors belonging to the sulfonamide, sulfamate or sulfamide classes have been reported. It has also been demonstrated the involvement of CA IX in tumor acidification in hypoxia, and the fact that the process is reverted by inhibiting the enzyme. CA XIII is highly abundant in the genital tract of both males and females and may lead to the development of contraceptives, whereas CA XIV is present in kidneys, liver and brain, where its biological role is not well understood. Some α- or β-CAs of non-vertebrate origin, such as the enzymes isolated in the malaria parasite *Plasmodium falciparum* and the ulcer producing bacteria *Helicobacter pylori* also emerged ultimately as interesting targets for the drug design.[1-4]

## *References*

[1] Alterio, V., Vitale, R. M., Monti, S. M., Pedone, C., Scozzafava, A., Cecchi, A., De Simone, G., Supuran, C. T. *Carbonic anhydrase inhibitors: X-ray and molecular modeling study for the interaction of a fluorescent antitumor sulfonamide with isozyme II and IX* J. Am. Chem. Soc. 2006, 128, 8329-35.
[2] Thiry, A., Dogne, J.M., Masereel, B., Supuran C. T., *Targeting tumor-associated carbonic anhydrase IX in cancer therapy* Trends Pharm. Sci. 2006, 27, 566-573.
[3] Supuran, C.T., *Carbonic anhydrase inhibitors* Curr. Top. Med. Chem. 2007, 7, 825-833.
[4] Supuran, C.T., *Carbonic anhydrases: novel therapeutic applications for inhibitors and activators* Nature. Rev Drug. Discov. 2008, 7, 168-81.

# 1B – Molecular Basis of Protein/Membranes Interactions

*T. Al Kayal, D. Berti, G. Caminati, P. Baglioni,*
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## Aims

Our final goal is the understanding of the physico-chemical parameters that rule protein rectruitment and unfolding at model cell membranes (i.e. Supported Lipid Bilayers or Liposomes).

## Results

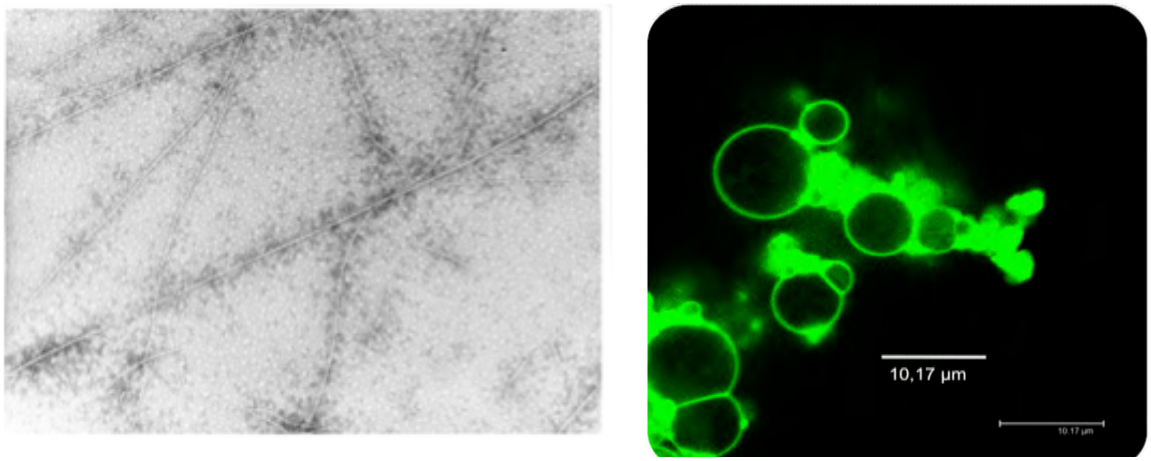
In this first part of the work, the attention was focused on the characterization of the effects of the presence of lipid membranes on protein aggregation. Lysozyme is prone to aggregation into amyloid-like prefibrillae and fibrillae when incubated at pH 2 and 60 °C for some days (Figure 1a). However this transition has been recently shown to be triggered by the presence of lipid membranes in physiological conditions [1]. Therefore the system represents an ideal model to test hypotheses on membrane effects on the unfolding and aggregation of pathologically relevant peptides. To this aim we have varied the composition of model membranes (i.e. phospholipid vesicles) by choosing appropriate combination of charged and uncharged phospholipids (PC, PS) with different degrees of unsaturation in the fatty acid portion to modulate the surface charge density and bilayer fluidity, respectively. The aggregation process of a model protein, lysozyme, was, the studied in the presence of different liposome compositions and protein/lipid ratios. Protein aggregation was detected through Dynamic Light Scattering (DLS), further information on the nature of the aggregates, in terms of the presence of an amyloid-like signature, was revealed studying the photophysical properties of two amyloid-specific fluorescent probes, i.e. Thioflavine T (ThT) and Congo Red (CR), added to the liposome/protein systems. The morphology of the aggregate Confocal Laser Scanning Microscopy (CLSM) combined with spatially-resolved emission spectroscopy. Experiments have been performed as a function of the protein/lipids ratio in various physiological buffers (HEPES, Phosphate) with different ionic strength.

Above a surface charge threshold and below a critical ionic strength, the presence of liposomes induced aggregation in the protein liposome system. DLS results show that, for a given lysozyme concentration, the aggregate size depends liposome volume fraction. Higher ionic strengths can inhibit the aggregation. Finally we observe a faster aggregation kinetic as the negative surface charge is increased.

Absorption and emission spectra of the fluorescent probes in the liposome/Lysozyme systems showed that the fluorescent probes interact both with the lipid membrane and with the aggregated protein giving rise to possible artifacts. Nevertheless, spatially

resolved spectra of elongated structures observed by CLSM disclosed the presence of beta-amyloid domains. Control experiments on lysozyme in buffer solution evidenced a time-dependent aggregation of the protein upon addition CR.

**a)** **b)**




Preliminary CLSM results on Giant Unilamellar Vesicles (GUVs) with the same phospholipid composition (see Figure 1b) showed that lysozyme aggregation induced by the probe is strongly enhanced in the presence of the phospholipid bilayer and presumably localized on the GUV surface

## *References*

[1] Zhao, H., Tuominen, E. K. J., Kinnunen P. K. J. *Formation of amyloid fibers triggered by phosphatidylserine-containing membranes* Biochemistry 2004, 43,10302–10307.

# 1B – Design and Characterization of Supramolecular Systems as Nano-devices in Cancer Diagnosis and Therapy

*G. D'Errico, O. Ortona, R. Sartorio, L. Paduano*

## Aims

This research is focused on the design and physico-chemical characterization of supramolecular aggregates (micelles, liposomes, etc.) to be used as specific nanodevices both in cancer diagnosis and therapy.

## Results

Recently, the research of the group has been devoted to the realization of two specific systems. The first one has been composed by a new amphiphilic supramolecular contrast agent, able to give good and resolved images of human tissues and organs in Magnetic Resonance Imaging. The system has been designed with the aims to display a very high relaxivity value and to be selective for cancer cells. The purposes have been simultaneously achieved by working out mixed systems formed by two different amphiphilic unimers, one containing the chelating moiety DTPAGlu, capable of forming stable complexes with $Gd^{3+}$ ions, and the other one the bioactive peptide CCK8. Both the unimers have been anchored to a double alkylic chain in order to promote the formation of vesicular aggregates. Furthermore, several attempts have been carried out for optimizing the design of the CCK8 unimer, by inserting and varying the length of a spacer situated between the double tail and the CCK8. sequence, such to assure an efficient exposure of the peptide on the external surface of the aggregate and at the same time short enough to favor the formation of vesicles. The system so formulated, containing both unimers, has been investigated with several techniques (small-angle neutron scattering, dynamic light scattering, cryo-TEM micrography) in different conditions of pH and ionic strength, due to the variety of environmental conditions the contrast agent may experience in the blood stream. The investigations have shown the presence of particularly suitable as pH-sensitive aggregates, this because of the high actual charge of the head group of the chelating agent unimer $(C18)_2$DTPAGlu. Upon decreasing the pH value in solution (from 7.4 to 3), a micelle-to-vesicle transition in three stages has been observed (rodlike micelles, threadlike micelles, vesicles) as result of the screening of the intra-aggregate electrostatic repulsions among the different head-groups. This property of the system can be applied to realize pH-responsive MRI contrast agents able to supply the in vivo pH mapping of tissues in tumor diagnosis.

The second system studied is composed by ruthenium-based nano-vectors. Recently, Ruthenium complexes have shown great potentialities in clinical use, but in spite of

that in the open literature up to date no example of nanosystem carrying Ruthenium is reported. The use of nanovectors as carriers for Ruthenium complexes can improve the therapeutic efficacy of these complexes and reduce their systemic side-effects. The basic idea behind the project developed is the synthesis of a new amphiphilic molecule constituted by two oleoyl chains and a poly(ethylene glycol)chain, PEG, bound to an uridine residue containing a pyridinium ring able to coordinate a Ruthenium (III) complex.
The molecule so designed, baptized DOPURu, has been investigated by means of the same techniques above mentioned, and it has been found that, in binary aqueous system, they can aggregate in multilamellar vesicles.
DOPURu has also been lodged in lipid bilayer formed by DOPC (1,2-Dioleoyl-sn-Glycero-3-Phosphocholine) and DOPE (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanol-amine), to reduce the commercial cost of the final formulation. DOPC and DOPE polar heads are the most abundant in plasma membranes and are known to be compatible with cells. Investigations for such systems have shown the presence of several kinds of aggregates, like uni- and oligolamellar vesicles and even the presence ofa highly ordered cubic structures (cubosomes).
The findings in the present study open up new vistas for the application of lipid based supramolecular systems in Ruthenium anti-cancer therapy. The lodgment of Ruthenium complexes in amphiphilic nanovectors allows a higher amount of drug in the bloodstream compared to the case when it is administrated as a free complex. Furthermore, the insertion of the PEG chain allows protecting Ruthenium complexes from enzymatic or environmental degradation, increasing so their circulation time in the bloodstream. A long time permanence of Ruthenium complexes in the bloodstream is advantageous from a therapeutic point of view, since the rapid uptake of colloidal drug carriers by RES (reticulo-endothelial system) would result in a fast reaching of the dose-limiting toxicity.

## *References*

Mangiapia, G., Accardo, A., Lo Celso, F., Tesauro, D., Morelli, G., Radulescu, A., Paduano, L., *Mixed Micelles Composed of Peptides and Gadolinium Complexes as Tumor-Specific Contrast Agents in MRI: A SANS Study*, Journal of Physical Chemistry B 2004, 108, 17611–17617

Vaccaro, M., Accardo, A., Tesauro, D., Mangiapia, G., Löf, D., Schillén, K., Söderman, O., Morelli, G., Paduano, L., *Supramolecular Aggregates of Amphiphilic Gadolinium Complexes as Blood Pool MRI/MRA Contrast Agents: Physicochemical Characterization*, Langmuir 2006, 22, 6635–6643

Vaccaro, M., Del Litto, R., Mangiapia, G., Carneup, A., D'Errico, G., Ruffo, F., Paduano, L. *Lipid Based Nanovectors Containing Ruthenium Complexes: A Potential Route in Cancer Therapy*, submitted to ChemComm

# 1B – Structural and dynamic properties of lipid bilayers and their interactions with biomolecules

*G. D'Errico, O. Ortona, R. Sartorio, L. Paduano*

## Aims

This research is focused on the structural determination and the study of the properties of bilayers formed by lipids and the investigation of their interactions with peptides and biomolecules.

## Results

Biomembranes are fundamental constituents of cells. They are basically formed by a lipid bilayer in which a considerable amount of proteins are intertwined. Proteins associated with membranes total approximately a third of all proteins in a typical eukaryotic cell. Membrane-associated proteins play a variety of roles, including mass transport, molecular recognition and signaling, which are fundamental for the cell functionality. Consequently, it is not surprising that membrane-protein interaction is one of the most important subjects in current scientific research. Electrostatic and hydrophobic interactions, van der Waals forces and H-bonding are all involved in the association among membrane proteins and lipids. For many years, lipids have commonly been considered a mere support for transient or permanent association of membrane proteins. However, mounting evidence demonstrates that specific lipids regulate the membrane location and activity of many proteins, often defining membrane microdomains that serve as spatio-temporal platforms for interacting proteins.

In this framework, in our labs we are performing a physico-chemical and microstructural characterization of lipid bilayers and of their interactions with peptides or proteins.

Two subjects are currently under investigation:

- membrane fusion due to peptides derived from fusion proteins of various viruses (HIV, FIV, Herpes);
- aggregation and membrane association of the β-amyloid peptide, in relation to the expression mechanism of the Alzheimer disease.

In both studies, the mesoscopic membrane properties are related to molecular feature of each single component (i.e. charged and uncharged phospholipid, cholesterol, specific lipids such as gangliosides and lipooligosaccharides) and to the interactions between them. The bilayer-protein or peptide association is also analyzed in microstructural details, identifying eventual specific interactions between lipids and the guest molecule. Experimentally, this is mainly done by electron spin resonance (ESR) spectroscopy by labeling, through a stable radical, the component (lipid, protein or peptide) under observation. By this approach, different and complementary

"viewpoints" on the same system are obtained. We are also planning to employ neutron reflectivity to obtain precise structural information on the location and the conformation of the protein interacting with the membrane.
Concerning the mechanism of membrane fusion, a key step of viral infection, we have studied the interaction between phospholipid bilayers and peptides modeled on the membrane-proximal external region (MPER) of various fusion proteins. These fragments, which usually present an unusual clustering of tryptophan residues are located on the membrane surface, exerting an evident destabilizing effect on the bilayer structure, eventually resulting in its disruption with formation of small micelles.
Concerning the interaction between the β-amyloid peptide and phospholipid bilayers, we have shown that a cytotoxic fragment of the peptide, i.e., Aβ(25-35), inserts in bilayers formed by the zwitterionic phospholipid dilauroyl phosphatidylcholine (DLPC), positioning between the outer part of the hydrophobic core and the external hydrophilic layer. Cholesterol plays a fundamental role in regulating the peptide/membrane association. This is partly ascribable to an indirect effect of the alterations in the membrane physical properties (e.g. the fluidity and heterogeneity) induced by the presence of cholesterol. However, the experimental evidences put also in evidence a specific molecular interaction between cholesterol and the peptide.

## *References*

D'Errico, G., Vitiello, G., Ortona, O., Tedeschi, A., Ramunno, A., D'Ursi, A., *Interaction between Alzheimer's A.beta.(25-35) peptide and phospholipid bilayers: The role of cholesterol*, Biochimica et Biophysica Acta, Biomembranes 2008, 1778, 2710-2716

D'Errico, G., D'Ursi, A., Marsh, D., I*nteraction of a Peptide Derived from Glycoprotein gp36 of Feline Immunodeficiency Virus and Its Lipoylated Analogue with Phospholipid Membranes* Biochemistry 2008, 47, 5317-5327.

# 1B – Study of polymer-based hydrogels in the presence of surfactants

*G. D'Errico, O. Ortona, R. Sartorio, L. Paduano*

## Aims

This research is devoted to the study of polymer-based hydrogels in the presence of surfactants, where the supramolecular structures formed by the surfactants strengthen the gel network.

## Results

Hydrogels are constituted by crosslinked polymer networks able to absorb great amounts of aqueous solutions, and can be divided into chemical and physical, depending on the nature of the junctions.

Hydrogels have attracted great research interest because of their potentiality in a wide range of applications. They have been successfully used in biomedical fields due to their high water content and the consequent biocompatibility.

In our laboratory one of the subjects developed has devoted to the realization of polymer-based gels in the presence of supramolecular aggregates (micelles, liposomes, etc.) formed by surfactant molecules. The basic idea is to form a network where junction points are represented by the surfactant aggregates.

Recently, the characterization of gels containing both normal and hydrophobically modified chitosans has been investigated, in the presence of polymer and/or surfactants like sodium decylsulfate and monoolein/sodium monooleate, respectively.

Concerning the first system, the structure of chemically crosslinked chitosan and chitosan/poly(vinylpyrrolidone) (PVP) hydrogels is investigated by means of the combined use of small angle neutron scattering (SANS), electron paramagnetic resonance spectroscopy (EPR), intradiffusion and swelling degree measurements. These hydrogels may be described in terms of an inhomogeneous structure composed by polymeric-rich and polymer-poor regions. The polymer rich regions, whose correlation distance $d$ is ranged between ~ 600 Å and ~ 850 Å, are, in turn, characterized by the presence of a network formed by the chemical crosslinks, with a mean correlation distance $\xi$ ~ 90 Å. The structure of chitosan and chitosan/PVP hydrogels has also been analyzed in the presence of sodium decylsulfate micelles that could provide a multidomain system useful, in principle, for drug delivery applications. Both SANS and EPR measurements show that sodium decylsulfate micelles do not significantly interact with both the gels. Finally, intradiffusion and sweeling degree measurements show an improved hydrophilicity of chtiosan/PVP gels, even further magnified by the presence of C10OS surfactant.

Hydrophobilcally modified chitosan has also be investigated, in combination with monoolein/sodium oleate vesicles. It is well known that the system monoolein (MO) - sodium monooleate (NaO) - water shows the spontaneous formation of unilamellar vesicles in the dilute corner of its phase diagram. These vesicles, with dimensions ranging between 1000 and 8000 Å are nowadays widely investigated because of their potential use as drug carrier. On the other hand, chitosan can be chemically modified by grafting on it a large variety of molecules with hydrophobic or hydrophilic properties. In the former case it is possible to tune its hydrophobic properties, inserting aliphatic chains of different length and operating on their crowding on the chitosan backbone.
The contemporary presence in acidic water of these vesicles and C12-chitosan, obtained by grafting a C12 aliphatic chain, gives rise to the formation of a gel.
We think that the aliphatic chains inserted on the chitosan backbone could penetrate into the vesicular double layer of the monoolein (MO) - sodium monooleate (NaO) – water vesicles, with the formation of a system with very different characteristics with respect to those shown by the aqueous vesicles and the C12-chitosan solution.
The structural characterization has been performing by means of EPR and SANS measurements allowing obtaining microscopic information about the vesicular aggregates.

## *References*

Borne, J., Nylander, T., Khan, A., *Vesicle formation and other structures in aqueous dispersions of monoolein and sodium oleate*, Journal of Colloid and Interface Science 2003, 257, 310-320

Mangiapia, G., Frielinghaus, H., D'Errico, G., Ortona, O., Sartorio, R., Paduano, L., *Physico-chemical and structural properties of hydrogels formed by chitosan, in the presence and absence of poly(vinylpyrrolidone) and sodium decylsulfate*. Physical Chemistry Chemical Physics 2007, 9, 6150-6158.

Ortona, O., D'Errico, G., Mangiapia, G., Ciccarelli, D., *The aggregative behavior of hydrophobically modified chitosans with high substitution degree in aqueous solution*. Carbohydrate Polymers 2008, 74, 16-22.

# 1B – Interaction of Bioactive Peptides with Membranes

*R. Pogni, R. Basosi, S. Pistolesi*

## Aims

The aims of this work is to study the interaction of bioactive peptides with membranes using the SDSL-EPR (Site Directed Spin Labeling – Electron Paramagnetic Resonance) technique in order to clarify the mechanism that regulates this interaction.

## Results

We have analyzed the interaction of antimicrobial peptide CM15 that is a linear peptide, synthetic hybrid AMP (Antimicrobial Peptide) composed of the first seven residues of the cecropin A and residues 2-9 of the bee venom melittin. The rationale for the use of the SDSL-EPR technique is based on the use of a spin label that is bound to a cysteine in the aminoacidic sequence of a protein (Fig. 1)

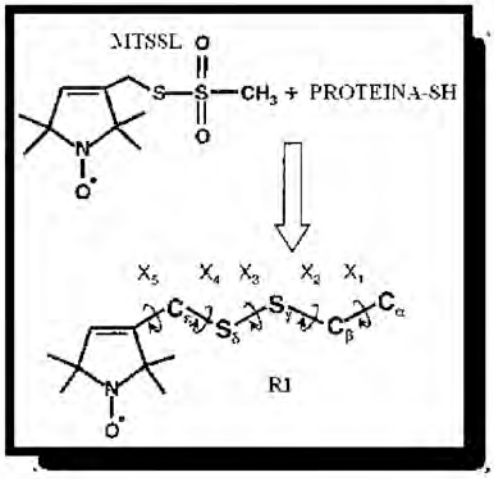



Figure 1. Structure of the MTSL (Methane-ThioSulfonate Spin Label) and the resulting side chain produced by reaction with the peptide cysteine residue.

Antimicrobial peptides (AMPs) are an essential part of innate immune defense against microbial infection. Naturally occurring AMPs are basic peptides composed of 12–50 aminoacids that are ubiquitously distributed throughout all kingdoms of life. AMPs display a broad spectrum of antimicrobial activity against both Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses (5). Importantly, they retain activity against antibiotic-resistant strains and do not readily elicit resistance. In this study we have used site-directed spin labelling (SDSL) electron paramagnetic

resonance (EPR) spectroscopy to investigate the behavior of a spin-labeled analog of CM15 as a function of increasing peptide concentration and utilized phospholipid-analog spin labels to examine the effects of CM15 binding and accumulation on physical properties of membrane lipids. We find that as the concentration of membrane-bound CM15 is increased, the N-terminal domain of the peptide becomes more deeply immersed in the lipid bilayer. Changes in the rotational dynamics of membrane lipids are minimal and confined primarily to near the membrane surface. However, peptide binding dramatically increases interaction of the lipid-analog spin labels with the polar relaxation agent NiEDDA (nickel (II) ethylenediaminediacetate), indicating that there are significant changes in the
physical state of the lipid bilayer that are not readily detected by methods that examine motional dynamics. These results are discussed in relation to the molecular mechanism of membrane disruption by CM15.

Another study is based on the interaction with membranes by Humanin (HN) a recently identified neuroprotective peptide, able to inhibit neurotoxicity induced by various insults which can be related to Alzheimer Disease as well as death induced by other stimuli. Here we apply the site directed spin labeling technique coupled to EPR spectroscopy to study the aggregation state and aggregate stability of humanin peptide in solution. This technique was previously used to study AMP's and fibrillogenic proteins behavior in aqueous environment, such as aggregation states and dynamics, and in presence of model bacterial membranes.
The purpose of this paper is the study of the conformational properties of humanin in different conditions in solution and in presence of model membranes.

## *References*

Pistolesi, S., Rossini L., Ferro, E., Basosi, R. , Trabalzini, L., Pogni R., *Humanin behavior in solution and in model neuronal membranes: a SDSL-EPR study,*
Biochemistry (submitted) 2008.

Ferro, E., Magrini, D., Guazzi, P., Fischer, T.H, Pistolesi, S., Pogni, R., White II, G.C., Trabalzini, L.,*G-Protein Binding Features and Regulation of the RALGDS Family Member RGL2*, Journal of Biochemistry , 2008, 415(1), 145-154.

Pistolesi, S. , Pogni, R. , Feix, J. B., *Membrane insertion and bilayer perturbation by antimicrobial peptide CM15*, Biophysical Journal, 2007, 93, 1651 – 1660.

Pistolesi, S. , Ferro, E., Santucci, A. , Basosi, R., Trabalzini, L. , Pogni R., *Molecular motion of spin labeled side chains in the C-terminal domain of RGL2 protein: a SDSL-EPR and MD study,* Biophysical Chemistry, 2006, 123, 49 – 57.

# 1C – Specific Ion Effects

*P. Lo Nostro, B. W. Ninham, S. Rossi, S. Pistolesi, P. Baglioni, A. Salis, M. Monduzzi, M.C. Pinna, D. Bilaničová*

## *Aims*

Hofmeister effect in bulk solutions and at interfaces

## *Results*

The effect of electrolytes in a moderate concentration range (between 0.1 and 1 M) was investigated in bulk solutions and at interfaces:

1. the kinetics of formation of pseudopseudorotaxanes (Fig. 1) [1]
2. the phase transitions in water dispersions of ascorbyl-alkanoates (Fig. 2) [2]
3. the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Fig. 3) [3]
4. the water absorbency of natural fibers (Fig. 4) [4]
5. the optical rotation of glucose and α-amino acids (Fig. 5) [5]
6. the UV-vis absorption spectra of congo red in aqueous dispersions (Fig. 6)
7. the spreading monolayers of calix[n]arenes (Fig. 7) [6]
8. the pH of buffered solutions (phosphate and cacodylate) (Fig. 8) [7]

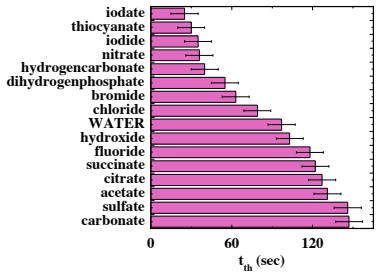
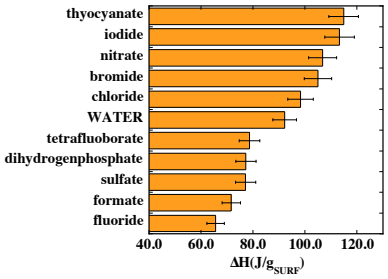



Figure 1

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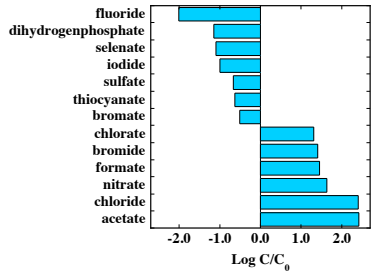



Figure 3

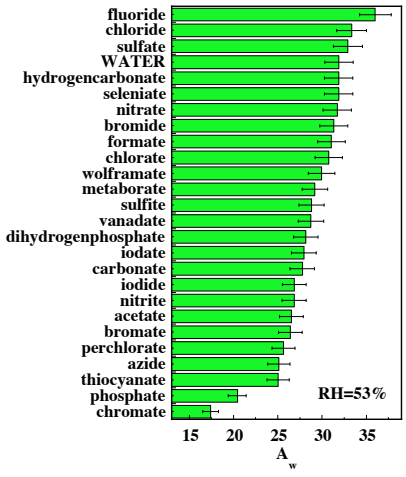



Figure 4

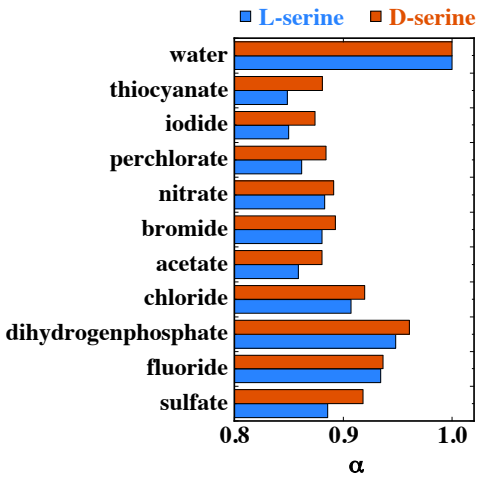



Figure 5

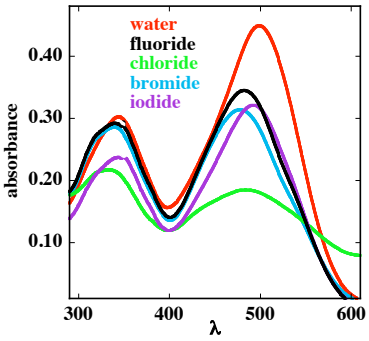



Figure 6

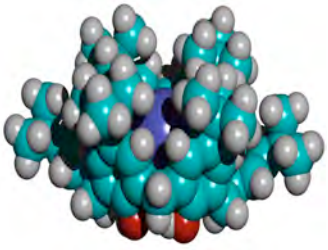


Figure 7

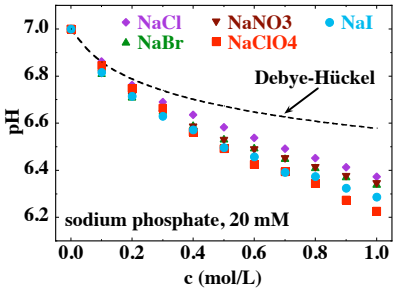



Figure 8

In each case the variation of a characteristic parameter was related to the free energy of hydration, partial molar volume, surface tension increment, polarizability and lyotropic number. The results indicate that in all these cases the phenomenon is ruled by dispersion forces, that originate from the different microscopic atomic properties of the single ions (polarizability and ionization potential).

## *References*

[1] Lo Nostro P., Lopes J.R., Ninham B.W., Baglioni P., *Effect of Cations and Anions on the Formation of Polypseudorotaxanes*, J. Phys. Chem. B 2002, 106(9), 2166-2174

[2] Lo Nostro P., Ninham B.W., Ambrosi M., Fratoni L., Palma S., Allemandi D., Baglioni P., *Hofmeister Effect in Coagels of Ascorbic Acid-based Surfactants*, Langmuir 2003, 19, 9583-9591

[3] Lo Nostro P., Ninham B.W., Lo Nostro A., Pesavento G., Fratoni L., Baglioni P. *Specific ion effects on the growth rates of Staphylococcus aureus and Pseudomonas aeruginosa*. Physical Biology 2005, 2, 1-7

[4] Lo Nostro P., Fratoni L., Ninham B.W., Baglioni P., *Water Absorbency by Wool Fibers: Hofmeister Effect*. Biomacromolecules 2002, 3, 1217-24

[5] Rossi, S., Lo Nostro P., Lagi, M., Ninham B.W., Baglioni P. *Specific Anion Effects on the Optical Rotation of α-Amino Acids*. J. Phys. Chem. B 2007, 111, 10510-19

[6] Lonetti B., Lo Nostro P., Ninham B.W., Baglioni P., *Anion Effects on Calixarene Monolayers: A Hofmeister Series Study*, Langmuir 2005, 21, 2242-2249

[7] Salis, A., Pinna, M.C., Bilanicova, D., Monduzzi, M., Lo Nostro P., Ninham, B.W. *Specific Anion Effects on Glass Electrode pH Measurements of Buffer Solutions: Bulk and Surface Phenomena*. J. Phys. Chem. B 2006, 110, 2949-56.

# 1C – Ion Specific effects in biocatalysis

*A. Salis, D. Bilaničová (STU Bratislava, Slovak Republic), B.W. Ninham (ANU Canberra, Australia), M. Monduzzi*

## Aims

Effect of weak and strong electrolytes (Hofmeister Series) on Enzymatic Activity in aqueous and non aqueous media

## Results

Ions are integral components of biological systems and are involved in several enzymatic pathways essential to life. Although known for a long time, the role of different ions in determining mechanisms of enzymatic action, has only recently started to be understood. That situation, the nature and origin of specific ion, or Hofmeister effects, is universal in physical chemistry. Lipases (triacylglycerol acyl hydrolase, EC 3.1.1.3) are surface active enzymes whose natural function is triglyceride hydrolysis. They are among the most used enzymes in biotechnology because of their high versatility mainly in non aqueous media.

In the present project the effect of the ions of the Hofmeister series on the enzymatic activity of two microbial lipases is reported. First specific ion effects in an aqueous medium, through the hydrolysis of p-nitrophenyl acetate, were studied. Both buffers and background electrolyte ions strongly affect the enzymatic activity of the lipases. Indeed, the so called 'kosmotropic' anions (i.e. sulfate) acted as activators, whereas 'chaotropic' anions (i.e. thiocianate) were strong inhibitor agents. Hofmeister neutral anions (i.e. chloride) showed a quasi-neutral behaviour.

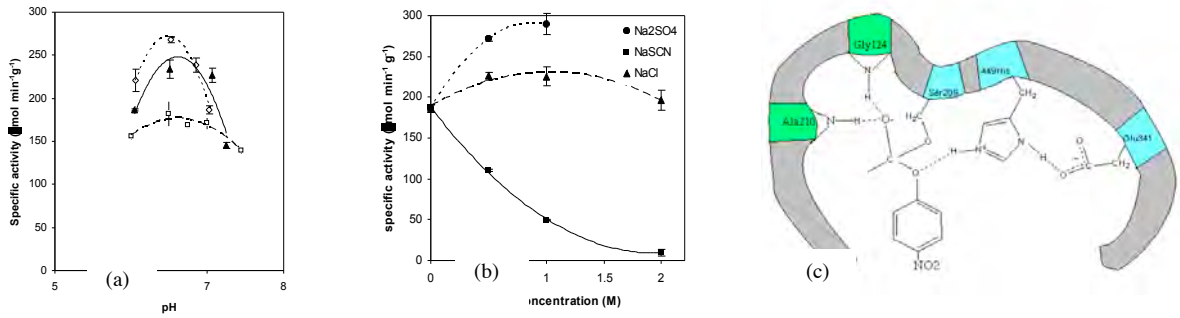



Figure 1. (a): Enzymatic activity of *Candida rugosa* lipase versus pH of different 200 mM buffers. (▲) Sodium citrate; (◊) Sodium phosphate; (□) Tris-HCl. (b): Enzymatic activity of *Candida rugosa* lipase versus salt concentration in sodium phosphate buffer 200 mM. (c): Schematic representation of the transition state (tetrahedral intermediate) of p-nitrophenyl acetate hydrolysis in the active site of *Candida rugosa* lipase.

Then, the specific anion effects on the activity of *Pseudomonas cepacia* lipase in non aqueous media (NAM) were investigated. The esterification between 1-hexyl-β-D-galactopyranoside and palmitic acid in an organic solvent was used as biocatalytic

assay. The heterogeneous biocatalysts for NAM were obtained through enzyme co-lyophilization in the presence of concentrated solutions of Hofmeister salts.

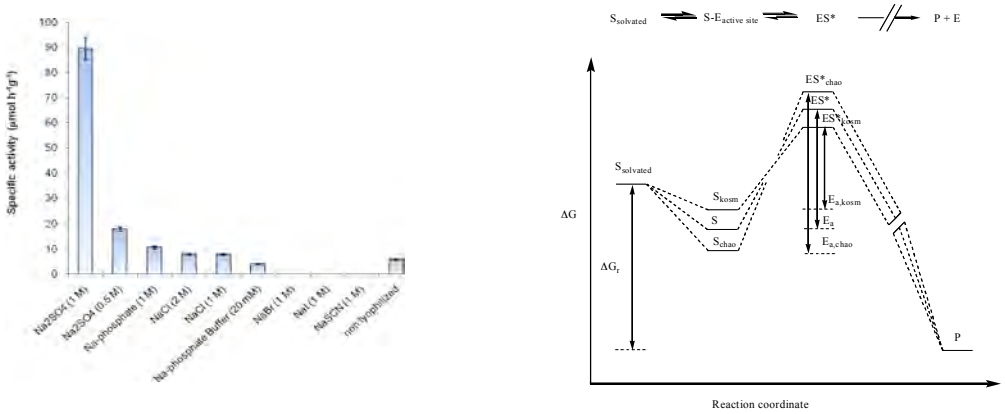



Figure 2. (left): Effect of salt type on the esterification activity of lyophilized *Pseudomonas cepacia* lipase in organic solvent. (right): Qualitative energetic diagram of the steps involved in the enzyme action in NAM: substrate and transition state stabilization/destabilization due to specific anion effects. $S_{solvated}$ is the energy level on the substrate in the solvent (2-methyl-2-butanol); S, is the energy level of the desolvated substrate bound at the active site; ES* is the energy of the transition state; $E_a$ is the activation energy; P is the energy level of products.

Salts affect enzyme activity in organic media through two mechanisms: 1. enzyme protection during lyophilization; 2. enzyme activation during the reaction. At least in our case, the latter seems to be more important than the former. The decrease of the activation energy caused by the stabilization of the transition state due to 'kosmotropic' anions might be the driving force of enzyme activation. According to the most recent findings, dispersion forces may be responsible of specific anion enzyme activation/deactivation in NAM.
Also in that case, in the presence of low amounts of water, enzyme activity followed the Hofmeister series. The decrease/increase of the activation energy caused by the stabilization/destabilization of the transition state due to kosmotropic/chaotropic anions might be the driving force of enzyme activation/deactivation. According to the most recent findings, dispersion forces may be responsible of specific anion enzyme behavior both in aqueous and non-aqueous media.

## *References*

Salis, A., Bilaniĉovà, D., Ninham, B.W., Monduzzi, M., *Hofmeister Effects in Enzymatic Activity: Weak and Strong Electrolyte Influences on the Activity of Candida rugosa Lipase*. Journal of Physical Chemistry B 2007, 111, 1149-1156.
Bilaniĉovà, D., Salis, A., Ninham B.W. Monduzzi, M., *Specific Anion Effects On Enzymatic Activity in Non Aqueous Media*. Journal of Physical Chemistry B 2008, 112, 12066-12072.

# 1C – Theoretical modeling of peptides on functinoalised surfaces

*A. Grassi, G. Forte, G. Marletta*

## Aims

Aim of the work is to study the interaction processes in biosurfaces obtained initially from the adsorption of L-lysine onto a functionalised quartz surfaces (SiOH, $SiCH_3$ and mixed $SiOH/SiCH_3$).

## Results

The modelling has been performed at static and dynamic levels, including the solvent effect. In our research, both Molecular Mechanics and semiempirical quantum mechanics techniques are used. Indeed, Molecular Mechanics method, based on the Force Field Theory, is a very suitable technique to analyse "macroscopic" properties of the model biosurface, including friction force, mechanical properties, etc... while, the strength and the nature of the molecule-surface interactions are studied using quantum-mechanics techniques, due to their nature of localized effects involving a few number of atoms. The adsorption of a EAK 16-II oligopeptide sequence in aqueous medium onto functionalized quartz surfaces has been studied by using Force Field calculations and Molecular Dynamics methods. The EAK 16-II ($C_{68}H_{118}N_{20}O_{25}$, MW 1615.8) oligopeptide, is a sequence of amino acids AEAEAKAKAEAEAKAK (- - + + - - + +), where A = alanine, E = glutamic acid, and K = lysine (see Figure 1).

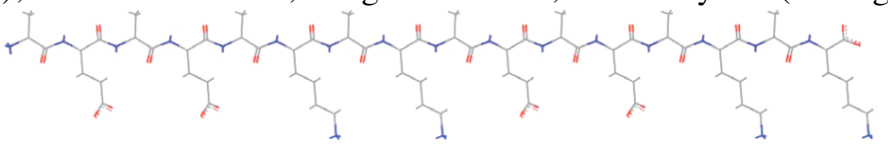


Figure 1. EAK 16-II structure: the short lateral groups (in red) represent –COOH groups, while the longer (in blue) represent $–NH_2$ groups.

Both geometry optimization and molecular dynamics simulations have been performed in the framework of the molecular mechanics methods to investigate the nature and the strength of the primary interactions in an aqueous medium between the EAK 16-II sequence and two model functionalized quartz surfaces, respectively, showing fully hydrophilic (**S1**) and fully hydrophobic (**S2**) character. The EAK sequences have been studied with three initial orientations with respect to the surfaces: (a) an orthogonal orientation with the $-NH_3^+$ terminal group pointing toward the surface (end-on, **Ia**); (b) an orthogonal orientation with the $-COO^-$ terminal group toward the surface (end-on, **Ib**); (c) a parallel orientation of the backbone with respect to the surface (side-on, **Ic**).

Static and dynamic calculations showed that the interaction process is mainly governed by the electrostatic interactions between $SiO^-$ surface groups and the charged residues of the oligopeptide sequence. In particular, for $SiO^-$ containing surfaces, we

found that strong electrostatic interactions a) prompt the parallel orientation of the oligopeptide with respect to the charged surfaces resulting in an effective physisorption process; and b) stabilize the β-sheet configuration of the physisorbed molecules.

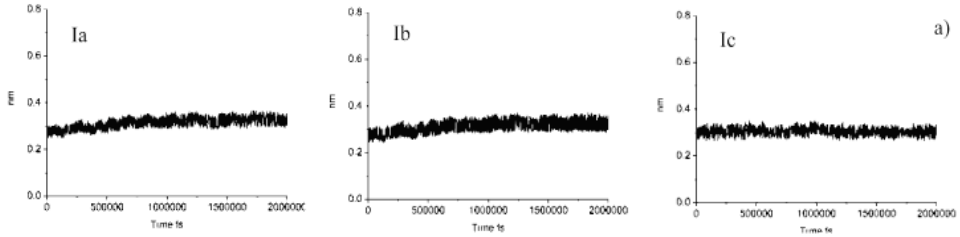



Figure 2 - Time fluctuation of the distance of Ia, Ib, and Ic forms from (a) S1.

Figure 2 shows that for the **S1** surface the molecule keeps a constant average distance of about 0.3 nm from the surface, indicating that the oligopeptide can be considered just physisorbed on the surface.

At variance with this, the continuously increasing distance with simulation time found for the oligopeptide from the **S2** surface (Figure 3) clearly indicates that the molecule freely diffuses away from the fully methylated surface, so that no effective physisorption can be considered for **Ia**, **Ib**, and **Ic**.

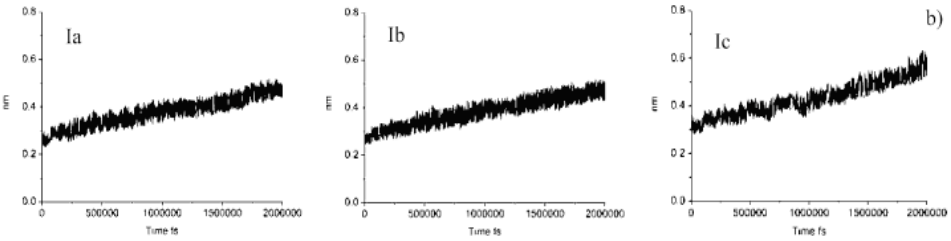



Figure 3 - Time fluctuation of the distance of Ia, Ib, and Ic forms from (b) S2 surfaces.

A related work has been performed to study the stability of scleroglucan aggregates in presence and absence of borax. The process has been experimentally studied by means of atomic force microscopy (AFM) and theoretically investigated by means of molecular dynamics (MD) simulations. The simulations indicate that the borax stabilizes nanochannel-like structures when seven triplexes are considered. The simultaneous presence of different Sclg triplexes in a narrow space strongly influences the properties of confined water molecules in a way similar, in many aspects, to that of water molecules located in the inner part of well-defined nanochannels (e.g., diffusion inside carbon nanotubes).

## *References*

Forte G., Grassi A., Marletta G. Molecular *Modelling of Oligopeptide Adsorption onto Functionalised Quartz Surfaces*, J. Phys. Chem. B 111, 11237- 11243 (2007).

Bocchinfuso, G., Palleschi A., Mazzuca C., Coviello T., Alhaique F., Marletta G. A theoretical and experimental study of self-assembling polysaccharide forming nanochannels: static and dynamic effects induced by a soft-confinement, J. Phys. Chem. B 112, 6473-6483 (2008).

# 1C – Bio-mimetic and Photochemical Switches and Motors

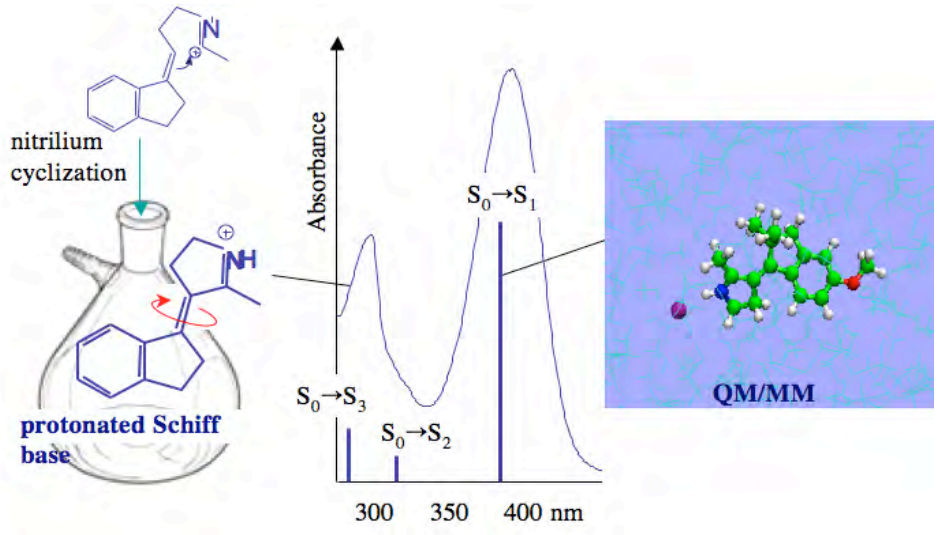
*M. Olivucci, A. Sinicropi, E. Busi, L. Parisi, C. Bernini, R. Basosi*

## Aims

Design, synthesis and characterization of novel synthetic molecules that mimic the behavior of biological photoreceptors and can be employed as bio-mimetic molecular devices

## Results

The design of components for machines on the molecular scale is a major challenge for science, in particular in the field of nanotechnology. Single molecules that act as light-energy transducers (e.g., converting the energy of a photon into atomic-level mechanical motion) are examples of minimal molecular devices. The research in this field requires high-level tools, usually involving synthetic and computational chemists. Nature offers ingenious solutions to control motion at molecular level converting chemical energy into mechanical energy. For instance, the protonated Schiff base of retinal, the chromophore of rodopsin proteins, undergoes a unidirectional photoisomerization that triggers a conformational change of the protein scaffold. Using these examples as an inspiration, we are currently working on the design of a new light-driven molecular motor, where rotary motion is produced upon a photochemical stimulus.




State-of-the-art quantum-mechanics/molecular-mechanics computations based on *ab initio* multiconfigurational perturbation theory and retrosynthetic analysis have been used to design a prototype light-driven Z/E molecular switch featuring a single

reactive double bond and the same electronic structure and photoisomerization mechanism of the chromophore of the visual pigment Rhodopsin. These molecules, containing the rigid framework of 4-(cyclopent-2'-enylidene)-3,4-dihydro-2H-pyrrolium cation, undergo a UV-visible light-driven cis-trans isomerization along the central C-C double bond.
It has been shown that the preparation of the switch can be achieved via nitrilium cation chemistry and that its photoisomerization quantum yields are 0.20 (Z→E) and 0.34 (E→Z) making the molecule a realistic basis for the development of a new class of biomimetic switches and single-molecule motors. When compared to the widely used azobenzene switch, the size and net positive charge of these compounds promise the achievement of photomodulable materials requiring functional units with these alternative features. Furthermore, complementary ab initio multiconfigurational quantum chemistry-based computations and time-resolved spectroscopy have been used to follow the light-induced isomerization of the switch in methanol. The results show that, similar to rhodopsin, the isomerization occurs on a 0.3-ps time scale and is followed by <10-ps cooling and solvation. The entire (2-photon-powered) switch cycle was traced by following the evolution of its infrared spectrum. These measurements indicate that a full cycle can be completed within 20 ps.

## *References*

Ruiz, D. S., Migani, A., Pepi, A., Busi, E., Basosi, R., Latterini, L., Elisei, F., Fusi, S., Ponticelli, F., Zanirato, V., Olivucci, M., *Design and Photochemical Characterization of a Biomimetic Light-Driven Z/E Switch*, J. Am. Chem. Soc. 2004, 126, 9349-9359

Lumento, F., Zanirato, V., Fusi, S., Busi, E., Latterini, L., Elisei, F., Sinicropi, A., Andruniów, T., Ferré, N., Basosi, R., Olivucci, M., *Quantum Chemical Modeling and Preparation of a Biomimetic Photochemical Switch,* Ang. Chem. Int. Ed. 2007, 119, 418-424

Zanirato, V., Pollini, G. P., De Risi, C., Valente, F., Melloni, A., Fusi, S., Barbetta, J., Olivucci, M., *Synthesis of bio-mimetic light-driven molecular switches via a cyclopropyl ring-opening/nitrilium ion ring-closing tandem reaction*, Tetrahedron 2007, 63, 4975-4982

Sinicropi, A., Martin, E., Ryasantsev, M., Helbing, J., Briand, J., Sharma, D., Léonard, J., Haacke, S., Canizzo, A., Chergui, M., Zanirato, V., Fusi, S., Santoro, F., Basosi, R., Ferré, N., Olivucci, M:, *An Artificial Molecular Switch that Mimics the Visual Pigment and Completes its Photocycle in Picoseconds*, Proc. Nat. Acad. Sci. 2008, 105, 17642-17647

# 1C – Multicriteria thermodynamic Analysis for Environmental Impact Assessment

*R. Basosi, M. Federici, N. Graniglia, P. Fulini*

## Aims

Using different thermodynamic methodologies (Material Flow Accounting, Energy, Exergy and LCA) the project aims to develop an integrated approach to evaluate the environmental impacts and the environmental sustainability of antropic systems.

## Results

Sustainability evaluation of antropic system can be performed by means of analytical methodologies deeply rooted on Thermodynamic laws: Material Flow Accounting, Energy, Exergy, Emergy and Life Cycle Assessment.

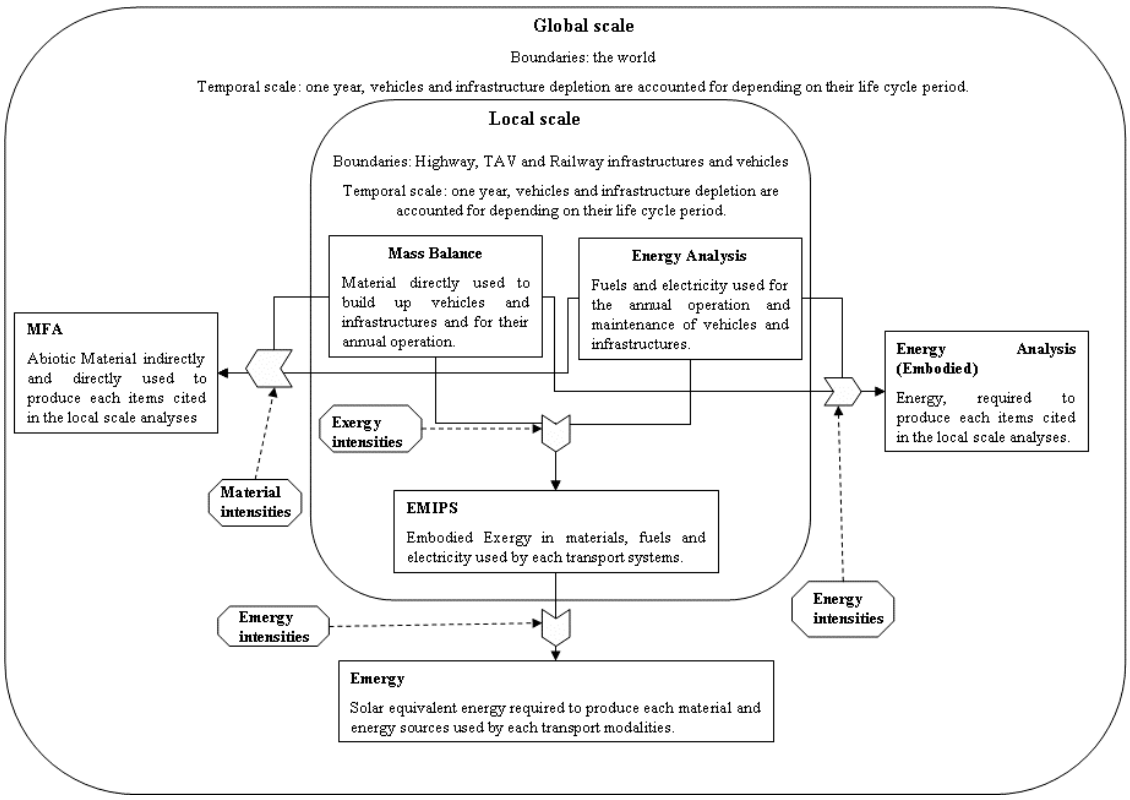



Figure 1. Local scale framework encompasses the direct inputs supporting the transport activities: Mass Balance, Energy Analysis and EMIPS are used in this context. Global scale framework takes into account the indirect and hidden material and energy flows supporting the transportation process. Specific material, energy and emergy intensities are used to shift from local to global scale.

These methodologies are based on different paradigms (for istance MFA and Energy are based on the First Law of Thermodynamic, while Exergy and Emergy are based on the second one) and different time-space windows, so the use of just one methodology lead to neglect some aspects and impacts linked to the process or the product with misleading indication about optimization policies.
In our study, we try to get a comprehensive and coherent integration among the cited methodologies in order to obtain an "investigation tool" able to encompass, virtually, all the environmental impacts. The validity of this method has been tested evaluating two kind of processes:

1. Passenger and commodity transport systems;

In this work, we perform a comparison among several transport options, not considering only the nominal performances of vehicles or modalities, but instead we focus on the useful outputs of the transport systems as a whole, namely the average demand for resources and environmental support related to the functional units, identified as one person transported per km (p-km) and one tonne of commodities transported per km (t-km). In so doing, by means of a "system approach", we are able to measure and compare the material and energy depletion required as well as the environmental impact generated per functional unit in each analysed transport systems, taking into account all the system's components.

2. Territorial Energy Planning and development of local renewable energy chains.

In this case, our multicriteria approach is used to evaluate different *energy planning* options and the environmental advantage/disadvantage coming from the development of local renewable energy chains as biomass, biofuel and wind energy.

## *References*

Federici, M., Ulgiati, S., Basosi, R., *Air versus terrestrial transport modalities: an energy and environmental comparison,* Energy, 2008, in press

Federici, M.,Ulgiati, S., Basosi, R., *A Thermodynamic and Material Flow Analysis of the Italian Highway and Railway Transport Systems,* Energy, 2008 Vol.33, 760 – 775

Federici, M., Basile, M., Pogni, R., Basosi, R., *Environmental and economical advantages of the local scale: a pilot biodiesel production line in the Province of Siena, Italy,* First European Workshop on biotechnology for lignocellulose biorefineries , 2008, 154-160

Verdesca, D., Federici, M., Torsello, L., Basosi, R., *Exergy-economic accounting for sea-coastal systems: A novel approach,* Ecological Modelling, 2006, 193, 132 – 139

Graniglia, R., Federici, M., Granigli,a N., Basosi, R., *Integration of Renewable Energy Production in Historical Downtown Centers: The Case of Siena*, World Renewable Energy Congress IX, Elsevier, 2006, 142 – 149

Federici, M., Verdesca, D., Basosi, R., *Thermodynamic and Economic Analysis of Biomass Energy Recovering Processes. Siena Case Study,* World Renewable Energy Congress IX, Elsevier , 2006, 313 – 332

# 1C – Energy Efficiency and Complexity Leap

*R. Basosi, F. Ruzzenenti, M. Federici*

## Aims

The aims of this project is to study the dynamic interplay between energy efficiency and structural complexity of macroscopic thermodynamic systems –human made or biological, on an evolutionary time-scale.

## Results

The rebound effect presents a major flaw in to energy conservation policies that aim to reduce energy consumption through energy efficiency development. Economics and energy related disciplines have thus far developed tools to measure such a phenomenon, but not enough effort has been put in explaining why. The reason why, since the very beginning of the industrial history of mankind, a new, more efficient, technology has always led to an higher energy consumption, is still a riddle. The common explanation based on price mechanisms –that higher efficiency would lower the hidden price of energy service, can account for a reduced conservative effect, but not for a full backfire (a rebound effect higher than 100%).

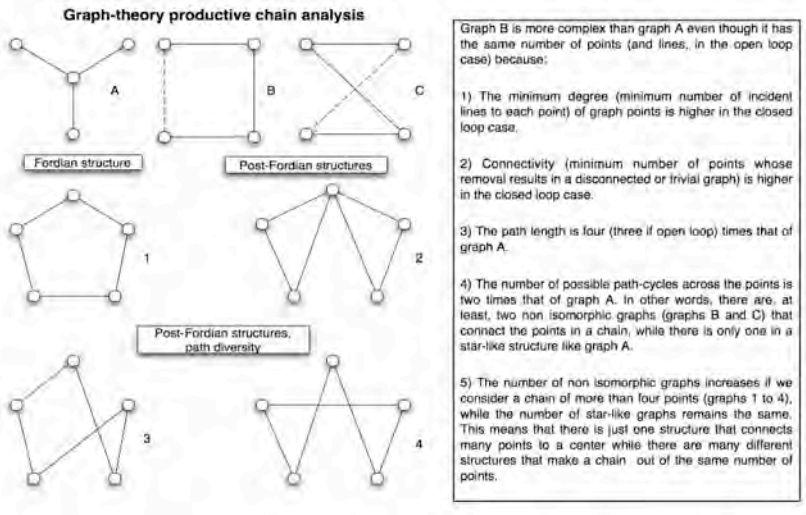



Figure 1. Complexity leap - Graph theory analysis

This first part of the analysis addressed the question of whether the rebound effect's size is bigger or smaller than one. We tackle this topic under a thermodynamic perspective, demonstrating that the dispute over the size of the rebound effect relies on a misconception of the thermodynamic nature of energy efficiency. The dichotomy, in fact, concerns the relationship between efficiency and power output rather than the scale of the economic side effects generated by energy efficiency mutations. We employed a model of finite-time thermodynamics based on the addition of the time variable to the Carnot machinery. The model presented shows

how a process of power maximization always leads to a sub-optimal efficiency level and additionally, that any efficiency improvement, in the context of low energy costs, will shift the power output of the machine instead of reducing energy consumption. The second part of the research aimed at explaining the rebound effect using a thermodynamic-evolutionary theoretical framework in addition to the traditional economic approach. We proposed that evolutionary systems, such as biological or economic systems, may rearrange themselves in a more complex fashion under the pressure of an increasing flux of energy, driven by the higher conversion rate of greater efficiency. Higher complexity, due to a greater energy density rate, counteracts the positive effects of energy efficiency. We investigated this hypothesis in the context of the road freight transport system and the productive structure. The qualitative analysis in this part of the work, further substantiated by figures (Figure 1), provides a link between the dynamics of production patterns and the effect of efficiency in the light of the macro-economic effects of increased energy demand. The analysis departs from an investigation of the actual energy efficiency evolution in the road freight transport system to develop through a survey of the subsequent worldwide economic revolution in the production system. It is then shown how outsourcing, the key feature of globalization, can be identified as the main source of traffic density growth. Finally, four paradigms are used to stress how the shift in the production system must be considered a leap in structural complexity that consequently serves to increase the frequency of components' interactions. The last part of our research program, still work-in-progress, examines to what extent complexity, on a evolutionary time scale, may evolve to counterbalance conservative effects brought about by energy efficiency. The issue of complexity leap is then addressed on a more rigorous basis. We introduce an evolutionary pattern that rests on the hypothesis that thermodynamic evolutionary systems are featured from an ever growing influx of energy driven into the system by self-catalytic processes, which must find its way through the constrains of the system. The system initially disposes of the energy by expanding, in extent and in number of components, up to saturation due to inner or outer constraints. The two counteractive forces, constraints and growing energy flux, expose the systems to new gradients. By exploring a new gradient the system imposes further restrictions on its components and increases its overall degree of freedom.

## References

Ruzzenenti, F., Basosi, R., *On the relationship between energy efficiency and complexity: Insight on the causality chain from the study of the rebound effect*. International Journal of Ecodynamics, 2008, 3, 95-108.

Ruzzenenti, F., Basosi, R., *The role of the power/efficiency misconception in the rebound effect's size debate: Does efficiency actually lead to a power enhancement?* Energy Policy, 2008, 36, 3626-3632

Ruzzenenti, F., Basosi, R., *The rebound effect: An evolutionary perspective*. Ecological Economics, 2008, 67, 526–537.

# 1C – Stacking interaction study of resveratrol in solution by NMR and FTIR Spectroscopy

*C. Bonechi, S. Martini, A. Donati, A. Magnani, C. Rossi*

## Aims

Resveratrol (*trans*-3,5,4'-trihydroxystilbene, RSV) is a phytoalexin found in *Vitis* spp. and in many other plants and food products and has received much attention due to its possible positive health benefits. In this research project the π-stacking interaction of *trans*-resveratrol was studied by nuclear magnetic resonance (NMR) and FTIR spectroscopy. In particular, the proton chemical shift dependence of the RSV concentration in the range $2x10^{-2}$-$1x10^{-5}$ M and temperature were analysed. Moreover, the dynamics of the supramolecular aggregates were studied by nuclear spin relaxation data and 2D-NMR experiments.

## Results

In order to investigate the dynamic conditions of *trans*-resveratrol, proton non-selective ($R_1^{NS}$) and selective ($R_1^{SE}$) spin-lattice relaxation rate experiments were performed. The experimental data about RSV in a DMSO:$D_2O$ solution ($R_1^{NS} = 0.52$ $sec^{-1}$ and $R_1^{SE} = 0.72$ $sec^{-1}$), $R_1^{SE} > R_1^{NS}$, suggested a slow motion regime for *trans*-resveratrol $2x10^{-2}$ M in solution.

To further verify these evidences, proton $R_1^{NS}$ were studied versus temperature, since this parameter should decrease with increasing temperature in fast motion conditions and increase with temperature in slow motion conditions. The $R_1^{NS}$ values versus temperature for all the protons, showing an increase in the relaxation rate with increasing temperature, validating the hypothesis of the existence of slow motions.

In order to explain the dynamical behaviour of *trans*-resveratrol, the presence of auto-association phenomena, much frequent in molecule containing aromatic rings and known as *stacking*, may be hypothesized. This phenomenon is characterized by non-covalent interactions between aromatic groups, also called π-π interactions. It is caused by intermolecular overlapping of p-orbitals in π-conjugated systems; so, they become stronger as the number of π-electrons increases.

The chemical shifts of aromatic protons involved in stacking interactions should change in relation to temperature and concentration. Therefore, proton NMR spectra of RSV at different concentrations and temperature have been acquired. The dependence of proton chemical shift of concentration (from $1x10^{-5}$ M to $2x10^{-2}$ M) shows that only at very low concentration ($1x10^{-4}$ M) an increase in chemical shift (~ 0.1 ppm) became observable indicating that trans-resveratrol is involved in a strong

self-association process through stacking interactions between aromatic residues of molecules. In order to check this point, we performed proton $R_1^{NS}$ in relation to temperature for $2x10^{-2}$ M and $1x10^{-5}$ M trans-resveratrol solutions. The results highlighted the dramatic change in the RSV dynamics due to the vanish of stacking processes at very low concentration.

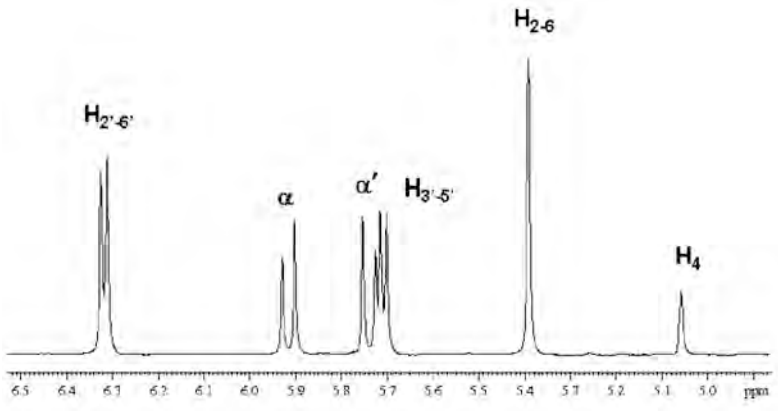



Fig. 1. Proton spectrum and structure of trans-resveratrol. Selective relaxation rate measurements refer to the $H_{2',6'}$ signal.

In order to more deeply investigate the dynamics of the aggregates, the self-diffusion coefficient of RSV was measured by DOSY (Diffusion-Ordered SpectroscopY) experiments. All the protons gave the same value of the diffusion coefficient ($1.4x10^{-10}$ $m^2/s$), indicating the absence of a distribution of aggregates with different dimensions. The radius of the aggregates, calculated using the Stokes-Einestein equation, was 6 Å.
Nuclear Magnetic Relaxation Dispersion studies indicated rotational correlation times in the order of tens of nanoseconds.

## References

S. Martini, C. Bonechi, A. Donati, A. Magnani and C. Rossi: "*Solution Structure of Rifaximin and its Synthetic Derivative Rifaximin OR determined by Experimental NMR and Theoretical Simulation Methods*". Biorg. Med. Chem. 2004, 12, 2163-2172.
S. Martini, S. Ristori, A. Pucci, C. Bonechi, A. Becciolini, G. Martini, C. Rossi: "*Boronphenylalanine Insertion in Cationic Liposomes for Boron Neutron Capture Therapy*", Biophys. Chem., 2004, 111, 27-34.
C. Bonechi, S. Martini, A. Magnani, C. Rossi: "*Stacking interaction study of resveratrol (trans-3,5,4'-trihydroxystilbene) in solution by Nuclear Magnetic Resonance and Fourier Transform Infrared Spectroscopy*" Magn. Res. Chem., 2008, 46(7), 625-9.

# 1C – Ligand-Macromolecules Interactions as observed by Nuclear Relaxation Analysis

*S. Martini, C. Bonechi, M. Ricci, A. Donati, C. Rossi*

## Aims

In this research project we propose a development of a NMR methodology to study ligand–macromolecular receptor interactions, based on the analysis of proton selective spin-lattice relaxation rate enhancements of the ligand, to calculate an affinity index, related to strength of the interaction process. In this methodology we modified this index by normalization to the relaxation rate of the free ligand, in order to take into account the effects of motional anisotropies and different proton densities. This approach also allowed the calculation of the relaxation rate of the bound ligand as well as the equilibrium constant of the interaction process.

## Results

In order to be able to compare the extent of recognition processes occurring between ligands and macromolecules, an "*Affinity Index*", representing the global affinity between the ligand and the receptor, was calculated from selective relaxation rate measurements. We applied this methodology to different systems in order to compare the strength of the interaction processes between the same ligand and different receptors, as well as between a macromolecules and different ligands. The investigation is based on the comparison of selective ($R_1^{SE}$) and non-selective ($R_1^{NS}$) proton spin-lattice relaxation rate analysis of the ligand in the presence and absence of the macromolecular receptor. The formation of intermolecular adducts affects $R_1^{NS}$ and $R_1^{SE}$ to different extents, depending on the dynamical parameters (i.e. the correlation time $t_c$), assuming fast chemical exchange between the bound and the free environments with respect to both chemical shift difference and proton relaxation rate. In particular, the slower ligand dynamics in the ligand-macromolecule complex mostly affects $R_1^{SE}$. Furthermore, the analysis of $1/\Delta R^{SE}_1$ in relation to ligand concentration gave the complex equilibrium constant K and the relaxation rate of the ligand bound to the protein. We applied this approach to the investigation of the interaction between sinapic acid and bovine serum albumin (BSA). The results show that for all the observed protons, in the absence of BSA, $R_1^{NS} > R_1^{SE}$ while with increasing protein concentration, $R_1^{SE}$ becomes greater than $R_1^{NS}$. The selective relaxation rate enhancements reveal the existence of a large contribution from the bound ligand fraction to the observed relaxation rate, which suggests the presence of an interaction between sinapic acid and BSA. In order to evaluate the strength of the binding process, the *affinity index* for sinapic acid–albumin system obtained from

different proton relaxation data was calculated from the slope of the straight line describing the dependence of proton selective relaxation rate enhancements from protein concentration. In order to remove the effects due to the existence of motional anisotropies and different proton densities on the experimental relaxation rates, the *normalized affinity index* was calculated. The methodology described above, which worked well for a "model system" as sinapic acid-albumin, has been applied to the study of the interaction processes related to ligands and macromolecules of biological interest. We investigated the interaction processes involving fibrinogen and epinephrine and norepinephrine (the two main catecholamines) by NMR and Fourier transform infrared (FT-IR) spectroscopies. The aim was to understand the role of two hormones in inducing protein conformational changes and, thus, their involvement in the platelet adhesion process. The NMR and IR data obtained for the protein-hormone systems clearly emphasized the basic role played by the ligand molecular structure in the "host-guest" molecular recognition process. Fibrinogen selectively binds epinephrine and this specific protein-hormone interaction (which is ligand-concentration dependent) is reflected in a significant structural change of the protein. Unlike epinephrine, nor-epinephrine is not selectively bound to fibrinogen, although this molecule differs from that of epinephrine only for the lack of the $CH_3$ moiety. Moreover, the weak non-specific interaction of nor-epinephrine with fibrinogen seems to be reversible, since it does not affect the protein conformation.

Due to the increasing interest in the understanding of the role of natural compounds in preventing health diseases, part of this research project was devoted to the study of phytochemical-macromolecular receptor interactions. In particular, we investigated the interaction between two flavonoids (quercetin, a major dietary flavonoid, and quercetin 3-*O*-β-D-glucopyranoside) and bovine serum albumin, the most abundant carrier protein.

The calculated values of the affinity indexes and the binding constants, K, for the two systems indicate that the binding affinity was strongest for quercetin which showed a value of the affinity index about 20 times larger than its derivative. These results provided further insights into the complex behavior of quercetin, compared to its glucosylated form. In particular all the covalent and non covalent binding sites were shown to be highly selective for quercetin. The proposed approach may represent a useful tool also for natural compound-protein recognition screening.

## References

S. Martini, M. Consumi, C. Bonechi, C. Rossi, A. Magnani. *Fibrinogen-catecholamines interaction as observed by NMR and Fourier Transform Infrared Spectroscopy*, Biomacromolecules 2007, 8, 2689-2696.

# 1C – Study of bradykinin conformation in the presence of model membrane by NMR and molecular modelling

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## Aims

The conformation of bradykinin BK was investigated by NMR spectroscopy and computer simulation in two different media, i.e. in pure aqueous solution and in the presence of phospholipid vesicles. The NMR spectra showed that lipid bilayers induced a secondary structure in the otherwise inherently flexible peptide. The results of ensemble calculations revealed conformational changes occurring rapidly on the NMR time scale and allowed for the identification of different families of conformations that were averaged to reproduce the NMR observables. These structural results supported the hypothesis of the central role played by the peptide terminal conformation in biological environments, and provided an explanation for the different biological behaviours observed for bradykinin.

## Results

The supposed interaction between BK and liposomes (DOPC/DOPE)was investigated by NOESY spectra, with the aim of pointing out the differences in the dipolar interactions that occurs in aqueous solution and in the presence of liposomes. NOESY experiments are the most useful tool to identify spatial connectivities between nuclei which interact through dipole-dipole couplings, since the size of the NOE is inversely dependent on the distance between interacting spins.
The NOESY spectrum of BK in $D_2O$ solution showed no indication of an existing secondary structure, since the pattern of NOEs did not reveal any additional significant connectivities. Furthermore, the relative sign of cross-peaks in a NOESY spectrum depends on the rotational correlation time, i.e. if the rotational correlation time of the molecules is short, the diagonal and cross-peaks have opposite signs. This result allowed to assert that the terminal amino acids of BK experienced a faster motion with respect to backbone amino acids. Both amino acids (Arg1 and Arg9) showed this behaviour in $D_2O$ solution.
In order to study the conformational modification of BK in the DOPC/DOPE:BK (5:1) system more in detail, the NOESY spectrum of the peptide plus vesicles system was analyzed and compared to the NOESY spectrum of BK without vesicles. The NOESY spectrum of DOPC/DOPE:BK (5:1) system displayed some correlations, indicating the adoption of a BK preferred conformation upon interaction with

DOPC/DOPE vesicles. The cross-peaks were also broadened with respect to those of BK without lipids, indicating that the peptide underwent slower tumbling as a result of the binding process.The comparison of NOESY spectra was also important to underline the difference between DOPC/DOPE:BK (5:1) and BK in $D_2O$ solution. The first important evidence concerned the sign of amino acids Arg1 and Arg9 cross-peaks. Contrarily to the results for BK without vesicles, the cross-peaks of Arg1 and Arg9 Hα and Hδ showed the same sign of diagonal peaks for the DOPC/DOPE:BK (5:1). This suggested the existence of a slow motion regime for the two amino acids. We thus concluded that the terminal amino acids did not show a different motional regime with respect to the backbone, and that BK molecules were associated with slowly tumbling large aggregates. The proton-proton distances were used as restraints in the Monte Carlo (MC) simulation.. For BK in $D_2O$ solution the MC protocol gave a total of 159 structures of low energy were calculated with an energy global minimum of -1042 kJ/mol. The XCluster protocol allowed to discard the molecules with relative violations of the distance restraint above 1.2-1.5 Å. Thus, we obtained 60 families of conformations.

For BK in DOPE:BK (5:1) system, the MC protocol allowed to obtain a total of 108 low energy structures, with an energy global minimum of -1086 kJ/mol. As reported above, the Xcluster analysis allowed to define 9 families of structures, where the experimental restraints were fully respected. Indeed, this amino acid showed an optimal conformation to interact with surface of liposome, whereas the Arg1 was not involved in intramolecular interactions.

The interaction between DOPC/DOPE phospholipids and bradykinin is driven by the phosphate groups, oriented toward the liposome outer surface (as suggested by the negative z potential values of BK free liposomes), which is able to induce the rearrangement of the Arg 1 residue

In conclusion in this study, we showed that BK molecules interact with zwitterionic lamellar systems and that the amino acid Arg 1 is directly involved in this process, possibly in tight contact with the polar head groups. Therefore, Arg1 act as a tethering point between the peptide and the membrane surface. The central portion of the amino acid sequence (Pro2-Pro3-Gly4-Phe5), may contributed to anchor the peptide to the membrane environment and to maintain the N- and C-termini amino acids in the optimal topological orientation. Overall, the interaction with the membrane surface provided the topological arrangement of the biologically important ligand regions. Moreover, it was found that the aromatic amino acids (Phe 5 and Phe 8) also showed a preferential conformation in the presence of liposome.

## *References*

C. Bonechi, A. Donati, S. Ristori, S. Martini, L. Panza, G. Martini, C. Rossi *Solution Behavior of a Sugar-based Carborane for Boron Neutron Capture Therapy: a Nuclear Magnetic Resonance Investigation* Biophysical Chemistry, 2007, 125, 320–327.

# 1C – Modelling the spectral shape of CDOM

*L. Bracchini, A.M. Dattilo, S.A. Loiselle, M. Ricci, A. Tognazzi, C. Rossi*

## Aims

Study the best way to modelling the spectral shape of chromophoric dissolved organic matter.

## Results

We develop two new methods to analyse the CDOM shape. The first is related to the use of an exponential function (S(λ)). The second do not use this supposition (σ(λ)).
The first method use a nonlinear fitting approach. It was possible to determine the variation of spectral slope over a wide range of wavelengths in UV and visible. The resulting spectral slope curve (S(λ)) displays some characteristics which are similar in both standard and lake samples, in particular to spectral slopes calculated near 270 and 380 nm. In lake samples, numerous degradation mechanisms will influence the spectral absorption characteristics of the CDOM produced by both allochthonous and autochthonous sources.
We show spectral slope curves for standard solutions and compare them to several lake ecosystems, as well as samples that have undergone photodegradation. Clearly, further studies are required to identify how specific sinks and sources influence the spectral slope curve in natural environments, but working from a common basis, such comparisons are greatly facilitated. We believe that this approach may help standardize the analysis of the CDOM absorption curve and provide more complete information on the chemical composition of CDOM (Figure 1).
With the second method we introduce a new approach to determine the CDOM absorption slope without assuming an exponential behaviour. The resultant $\sigma(\lambda)$ curve shows the wavelength dependent variability of spectral slope data, allowing for comparison with published values made in different wavelength intervals. Differences in $\sigma(\lambda)$ appear to be linked to changes in average chemical composition and aromaticity more specifically to different source and sink mechanisms (e.g. photobleaching).
While the $\sigma(\lambda)$ approach is facilitated by the use of the more sensitive long path spectrophotometer, we show that is can also be applied to standard spectrophotometric absorbance data, after applying the opportune signal smoothing. The resultant spectral distribution curve is consistent with that obtained using a long path spectrophotometer (Figure 2).

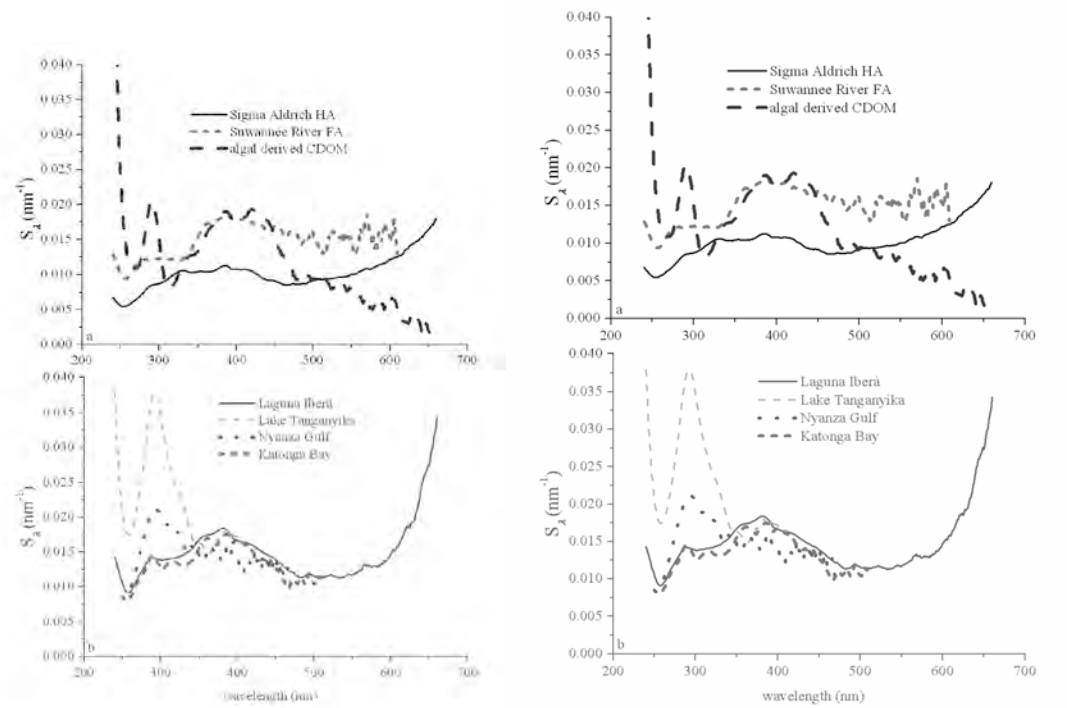



Fig. 1: The S(λ) curve generated for different aquatic ecosystems and for CDOM standards

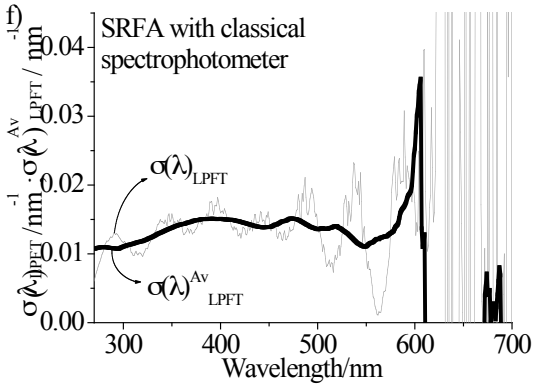



Fig. 2: The σ(λ) curve generated with theory of signals.

## References

Loiselle, S.A, Bracchini, L., Cozar, A, Ricci, M, Dattilo, A.M, Tognazzi, A, Rossi C. (in stampa). *The optical characterization of chromophoric dissolved organic matter using the wavelength distribution of absorption spectral slopes* 2009, Limnology and Oceanography. In Press.

Bracchini, L, Blough, N.V., Dattilo, A.M., Del Vecchio, R., Loiselle, S.A., Ricci, M., Tognazzi, A., Rossi, C., *An alternative way to analyze the shape of CDOM absorption curve*, 2009, submitted to Limnology and Oceanography.

# 1C – Modeling productivity

*S.A. Loiselle, F. Benetti, L. Bracchini, A.M. Dattilo, A. Tognazzi, C. Rossi*

## Aims

To study the productivity from light point of view.

## Results

Model development

The patterns of abundance of algal biomass in aquatic ecosystems depend upon on the supply of resources (both nutrients and solar irradiance) as well as upon dominant loss processes (such as grazing and sedimentation). The balance between production and losses determines where and when phytoplankton community will grow and to what extent. We develop an ecosystem specific approach for determining the algal biomass carrying capacity under light limiting conditions. The model is based on the relationship between the total solar energy available and the energy stored (algal biomass) within the ecosystem at steady state. We applied this model to describe real conditions in two disparate algal communities; the phytoplankton community in Lake Victoria, East Africa and the microphytobenthos community in lacustrine system of Esteros del Iberá (South America).

Aquatic autotrophs have nutritional and energetic requirements that are necessary for their photosynthetic carbon fixation and growth. However, the upper limits of growth in light limiting conditions remain poorly understood with respect to nutrient-controlled capacities. This bias has partly arisen due to the complexity in modelling light availability in aquatic environments. The main challenges are the spatio-temporal variability of light within the water column, the competition for light with abiotic components and the negative feedback of algal auto-shading.Attempts to describe light control of algal carrying capacities have followed two general approaches, a population dynamics approach based on the modelling of growth and loss processes and a resource based approach in which relationships between algal concentrations and available resources are directly used to estimate the supportive capacities of ecosystems. For phytoplankton in light limiting conditions, the population dynamics approach is often based upon the critical depth concept of Svedrup (1953) in which light availability and vertical mixing are related to net primary productivity. On the other hand, the resource-based approach assumes that a relationship exists between steady state algal biomass and its limiting resource and that such information can be used to examine the system carrying capacity under different resource conditions. While these two approaches seem dissimilar, we show that their results can be complimentary.

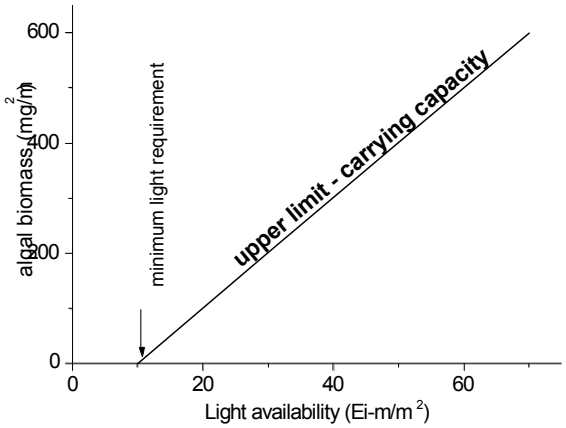



Fig. 1: Algal biomass Vs light availability. The line represent the upper limit carrying capacity

The algal concentrations at different solar irradiances for the pelagic environment of Lake Victoria and the benthic ecosystem of the lakes of Ibera wetland are shown (Fig 2). The carrying capacity for each system was derived from the upper concentration limits. For the tropical phytoplankton community, critical light requirement was determined to be 0.067 Ei/m2day/(mg/m2) (R2 = 0.9224, P<0.001). For the subtropical microphytobenthos, ψb was found to be 0.016 Ei/m2day/(mg/m2) (R2 = 0.9537, P<0.001). Minimum light requirements were found to be 1.2 Ei-m/m2day and 0.01 Ei-m/m2day respectively. As expected, benthic algae showed a higher efficiency and lower light requirement compared to pelagic algae.

## *References*

Loiselle, S. A., Bracchini, L., Rossi, C., *Modelling energy fluxes in remote wetland ecosystems with the help of remote sensing*, 2001, Ecological Modelling 145, 245-261

Loiselle, S. A., A. Cózar, A. van Dam, F. Kansiime, P. Kelderman, M. Saunders, S. Simonit, *Development of tools for wetland ecosystem resource management in Eastern Africa*, in Springer Ecological Studies; Wetlands as a Natural Resource (ed. J. T. A. Verhoeven) (in press)

Loiselle S:A., V. Hull, J. A. Galvez, C Rossi, *Qualitative modelling tools for rural ecosystem management*, 2001, International Journal of Sustainable Development and World Ecology, 8, 1-14.

# 2A – Surfactant and Silica-Surfactant composite for environmental remediation

*F. Lopez, A. Ceglie, F. Venditti, R. Angelico, L. Ambrosone, G. Bufalo (ISPESL, Napoli)*

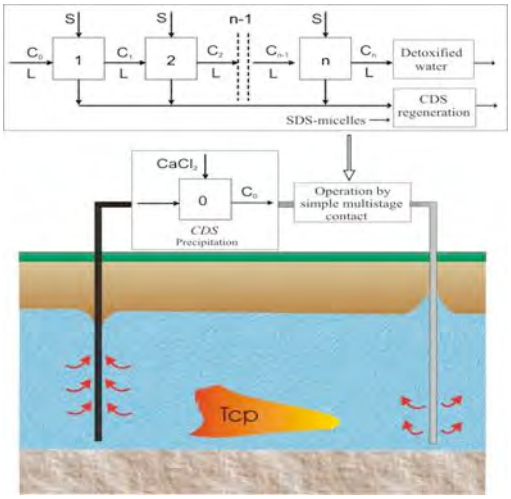
## Aims

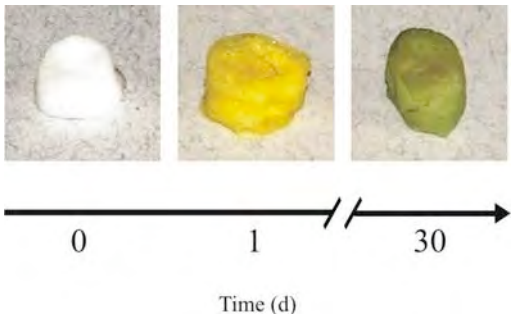
The Environmental Remediation Project focused on characterization, monitoring, and modelling of subsurface environments, with a particular emphasis on contaminant remediation and water resources. The project is both basic and applied, and includes studies that span a wide range of spatial scales (molecular studies to full-scale field investigations) and of environmental conditions, utilizing expertise in various fields.

## Results

Groundwater pollution is the loss of water quality due to waste products or others substances which alter the chemical and microbiological water properties. Traditional *pump-and-treat* techniques are very expensive and in the recent years considerable attention has been addressed towards the development of alternative technologies, such as *surfactant-enhanced aquifer remediation*. In this research project the micellar solubilization of 2,4,5 trichlorophenol was spectrophotometrically investigated and the results were used as a model of chlorinated contaminant.

A novel surfactant-based adsorbent material able to solubilize pollutants in SDS-micelles was obtained[1,2]. By means of this material the excess of the pollutant can be easily removed with a precipitation process. The adsorptive efficiency of this novel material was tested through a cocurrent multistage process and its performance was compared with those of most common adsorbents such as activated carbon and silica gel.



The experimental results are used to outline a new design to detoxify groundwater[2] (see figure). The removal of toxic ions from wastewater is another important part of this research project. We focussed our attention on the removal of chromates present in the environment. The presence of heavy metals in the environment has been in the last few years and still is of major concern, because of their toxicity to many life forms. Since the majority of heavy metals does not degrade into harmless final products, their concentrations must be reduced to acceptable levels, and therefore constantly monitored. Among these, Chromium should require treatments to public health.

Several materials have been developed and tested, ranging from low cost waste material, such as moss peat, sawdust, zeolites, clay, hazelnut shell, to more sophisticated adsorbents, such as activated carbon, modified zeolite, modified clay, modified steel slag, nanoscale magnetic material, chitosan based composite.

We proposed a novel composite able to remove hexavalent chromium Cr(VI) from aqueous solutions obtained by adding the silica precursor tetraethoxysilane (TEOS) to the hexadecyltrimethylammonium bromide (CTAB) microemulsion-based gel[3,4]. SEM and NMR analysis showed that this material is made by an interconnected network of gelatin, silicate and surfactant molecules in which water molecules manifest a high mobility. Analyses of the elemental content in the CTAB-silica gelatin composite suggest that the adsorption of chromium takes also places in the internal areas. Moreover, this adsorbent appears to be easy to handle and thus suitable for storage and manipulation processes. Interestingly, we noticed that the composite containing the adsorbed hexavalent chromium left in water for 30 days undergoes a change in colour from yellow to green (see Figure below). This evidence is an indication of the reduction in situ of Cr(VI).

## *References*

1. Ambrosone, L., Ceglie, A. Italian Patent n. 4454, 2003.

2. Venditti, F., Angelico, R., Ceglie, A., and Ambrosone L. *Novel Surfactant-Based Adsorbent Material of Groundwater Remediation*. Environ. Sci Tech., 2007, 41, 6836-684.

3. Venditti, F., Lopez, F., Palazzo, G., Colafemmina, G., Angelico, R., Ambrosone, L., Ceglie, A. Italian, Patent n. 7490, 2006.

4. Venditti, F., Ceglie, A., Palazzo, G., Colafemmina, G., Lopez, F. *Removal of chromate from water by a new CTAB–silica gelatin composite*. J. Colloid Int. Sci., 2007, 310, 353-361.

# 2A – Silver nanoparticles on fabrics: synthesis and antimicrobial effect

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## Aims

Clusters of polyacrylate-templated Silver nanoparticles can be used to transfer the antimicrobial properties of Ag to different fabrics.

## Results

Silver-poly(acrylate) clusters have been synthesized in water by reduction of $AgNO_3$ in the presence of poly(acrylates) of different molecular weights through two different methods, $NaBH_4$ reduction and UV exposure. The structure of the clusters and the effect of the synthesis parameters on the size and polydispersity of the particles were evaluated by means of small-angle X-ray scattering (SAXS). Fitting results reveal the presence of a bimodal distribution of spherical nanoparticles, showing that both the reduction method and the polymer chain length play key roles in determining the final size of the particles and the overall size of the clusters. This is confirmed by High-resolution transmission electron microscopy (HR-TEM), as shown in the example of the effect of the reduction method on the nanoparticle size and shape given in Figure 1. Nanoparticle dispersions were then used to functionalize cotton, wool, and polyester fabrics. Samples were first conditioned at constant relative humidity (RH 33%) and temperature (20 °C). They were then soaked for 15 min at room temperature in the Ag nanoparticles dispersion under magnetic stirring, squeezed to remove the excess dispersion, rinsed, and dried in an oven at 130 °C for 15 min at atmospheric pressure (dry heat). The antimicrobial activity of treated samples has been tested according to a standard procedure (agar plate diffusion test/CEN/TC 248 WG 13) against four different strains: Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, and Candida albicans. The results for Ag-treated cotton samples are shown in Figure 2. The treated textiles exhibit antimicrobial activity depending on both the strain and the fabric material.

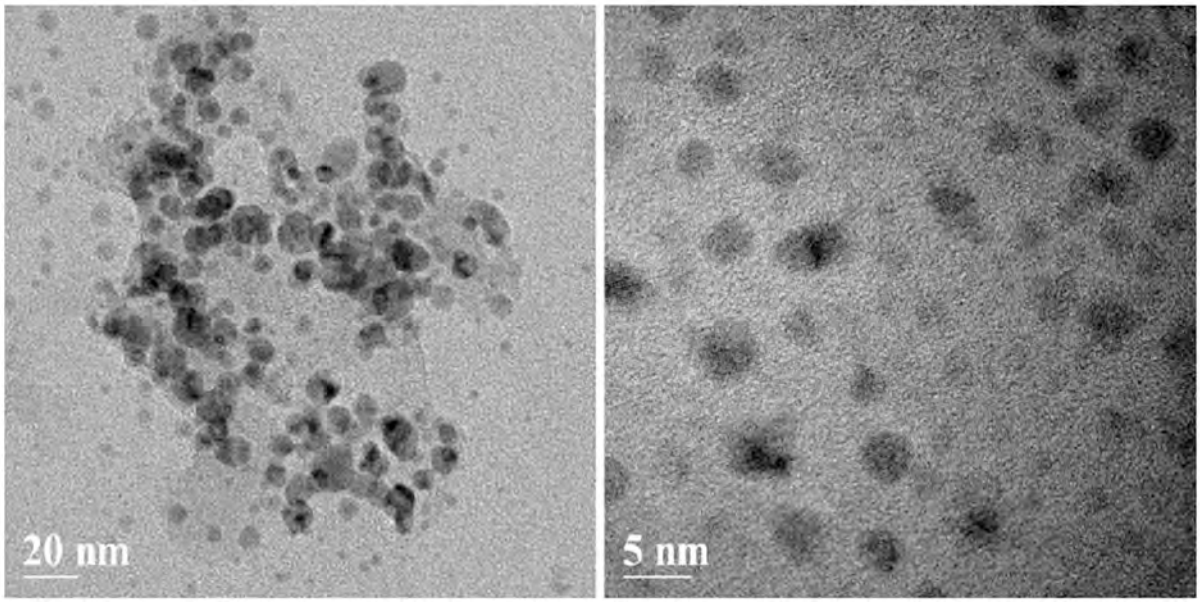



Figure 1. HR-TEM images of two Silver nanoparticle dispersions prepared in the same conditions, except for the reduction method: by UV radiation (on the *left*) and with $NaBH_4$ (on the *right*).

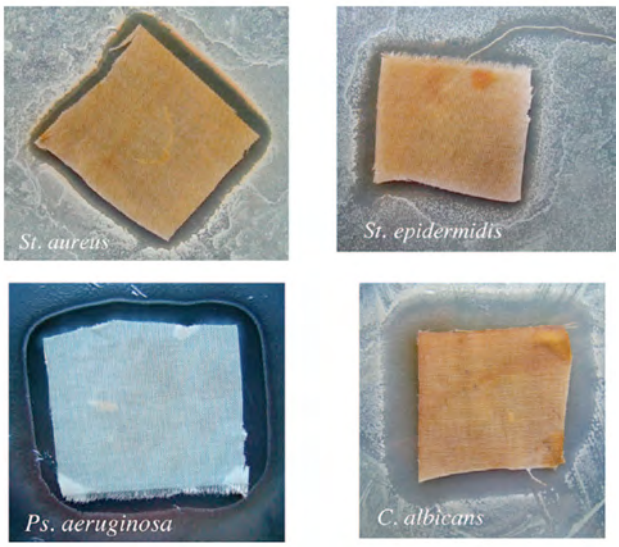



Figure 2. Effect of Ag-treated cotton samples towards the growth of the investigated strains.

## References

[1] Falletta, E., Bonini, M., Fratini, E., Lo Nostro, A., Pesavento, G., Becheri, A., Lo Nostro, P., Canton, P., Baglioni, P. *Silver Nanoclusters with Acrylates as Templating Agents: a SAXS study*, Journal of Physical Chemistry C 2008, 112, 11758-11766.

[2] Falletta, E., Bonini, M., Fratini, E., Lo Nostro, A., Becheri, A., Lo Nostro, P., Baglioni, P. *Poly(acrylic) acid-coated Silver Nanoparticles for Antibacterial Textile Finishing*, in Nanotech 2007 (Santa Clara, CA).

# 2A – Surface functionalization of textiles

*P. Lo Nostro, M. Bonini, L. Tattini, E. Falletta, E. Bocci, P. Baglioni*

## *Aims*

Modification of the surface of textile materials

## *Results*

Modification of the surface of textile materials, mainly wool and cellulosic fabrics (cotton and Tencel®), was obtained through:

- grafting of α-cyclodextrin derivative in order to endow the surface with empty hosting cavities that can be used for uptake/release of fragrances, insect repellents (such as DEET), antimicrobials, etc. (Figure 1)
- deposition of metal oxide nanoparticles (PZT, ZnO and $TiO_2$) or solar filters for anti-UV shield and comfort upgrade (Figure 2)
- flame retardancy through surface reaction of cellulosic fibers with ammonium sulfammate and urea water solutions

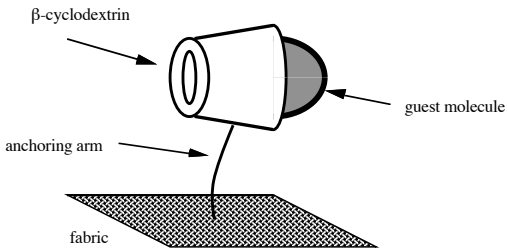


Figure 1

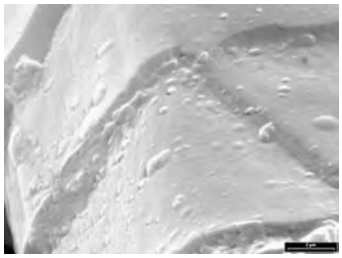


Figure 2

The formation of stable inclusion compounds at the fabrics' surface was tested with several techniques: UV-VIS spectrophotometry (with integrating sphere), back-extraction with organic solvents, calorimetry, aroma testing and insect repellency tests (in collaboration with the Italian National Institute of Health, Rome). Figures 3 and 4 show a specimen of Tencel® treated with monochlorotriazinyl-β-cyclodextrin and then with DEET, a common insect repellent.


Figure 3

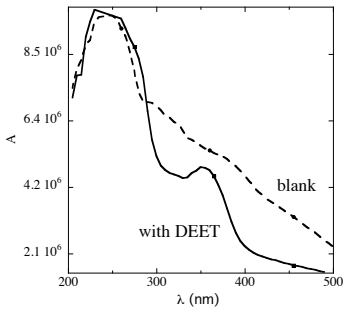



Figure 4

## *References*

Scalia S., Tursilli R., Bianchi A., Lo Nostro P., Bocci E., Ridi F., Baglioni P., *Incorporation of the sunscreen agent, octyl methoxycinnamate in a cellulosic fabric grafted with β-cyclodextrin*, International Journal of Pharmaceutics 2006, 308, 155.

Romi R., Lo Nostro P., Bocci E., Ridi F., Baglioni P., *Bioengineering of a Cellulosic Fabric for Insecticide Delivery via Grafted Cyclodextrin*, Biotechnology Progress 2005, 21, 1724.

Lo Nostro P., Fratoni L., Ridi F., Baglioni P., *Surface Treatments on Tencel® fabric: grafting with β-cyclodextrin*, Journal of Applied Polymer Science 2003, 88, 706.

Lo Nostro P., Fratoni L., Baglioni P., *Modification of a Cellulosic Fabric with β-Cyclodextrin for Textile Finishing applications*, Journal of Inclusion Phenomena and Macrocyclic Chemistry 2003, 44, 423.

Becheri, A., Dürr, M., Lo Nostro, P., Baglioni, P. *Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers*. J. Nanoparticle Res. 2008, 10, 679.

Falletta, E., Bonini, M., Fratini, E., Lo Nostro, A., Pesavento, G., Becheri, A., Lo Nostro, P., Canton, P., Baglioni, P. *Clusters of Poly(acrylates) and Silver Nanoparticles: Structure and Applications for Antimicrobial Fabrics*. J. Phys. Chem. C 2008, 112, 11758.

# 2A – Chemical gel for the restoration of canvas paintings

*M.C. Arroyo, P. Baglioni, D. Chelazzi, R. Giorgi, G. Pizzorusso*

## Aims

Removal of resins used during relining in canvas consolidation

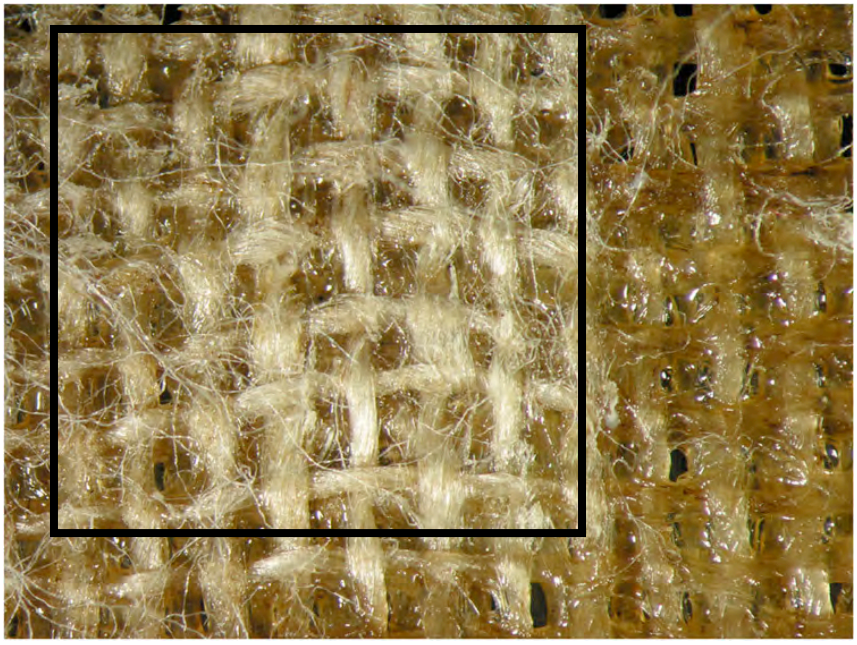
## Results

The most common cleaning method in restoration of canvas paintings is based on the usage of physical gels (called solvent gels) containing large amounts of pure solvent. This method, in spite of his high cleaning capacity, presents some drawbacks for both the object and the operator. First of all the solvent could penetrate inside of the surface and solubilize the painting binder; on the other side most of the commonly used solvents are toxic. After the application it is necessary to remove the solvent gel; to do that restorers use swabs soaked with white spirit. This step too can cause damages to the painted layer. Moreover, it has been shown that after this operation one can still find some gel residue.

This project is focused on two main points: to avoid the use of pure free solvent and to substitute physical gels with chemical gels. In this way, solubilization of painting binder and the presence of gel residues can be avoided.

Instead of pure solvents microemulsion as active cleaning agent will be tested. These systems are made of water (continuous phase), surfactant, and hydrocarbon solvent (discontinuous phase). Microemulsions solve the problem of using a large quantity of pure solvents; on the other side the presence of water could represent a problem. In fact canvas and painting layers tend to absorb water that induces swelling, deformation and mechanical stress. For this reason it was decided to load the microemulsion into a very idrophylic gel, which can reduce the water penetration. These gels are based on two idrophilic gelators: acrylamide and bis-acrylamide. Using these two substances, a gel with a tri-dimensional network can be obtained, where the polymers are covalently bounded each other. This type of gel is very different from the physical one; in fact, after the reaction of gelification an object with the same shape of the container, where reaction happens, is obtained. When the gel is formed, his shape is unchangeable. In this way, the problem of gel residue can be avoided. In fact the gel behaves like a solid; so the operation of removal is very simple and complete. Before the application, the gel must be loaded with the microemulsion; this operation is very simple: the gel is simply immersed into the microemulsion for about an hour. The nanodroplets of surfactant and solvent migrate into the gel because of a different concentration.

These systems have been already tested in some cleaning experiments. Good results were obtained in the removal of glue residues in the backside of works on canvas. In order to improve this method a water-less system, oil in fluorinated solvent microemulsion, is under investigation. The basic idea of this system is to obtain a cleaning agent more suitable than the classic microemulsion for applications on water sensitive materials. For this reason, the aim is to replace water with inerter solvents, like for example fluorocarbons.



Removal of viniyacetate-acrylate co-polymer from linen canvas by using a chemical gel soaked with o/w microemulsion

## *References*

Pizzorusso G., Giorgi R., Chelazzi D., Carretti E., Arroyo M.C., Chevalier A., Menu M., Baglioni P., *Eliminazione di residui di colle usate per la rintelatura di dipinti mediante sistemi chimici detergenti*, in Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metodi, Compatibilità, Cantieri", Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 517-526.

Chevalier A., Chelazzi D., Baglioni P., Giorgi R., Carretti E., Stuke M., Menu M., Duchamp R., *Extraction d'adhésifs de rentoilage en peinture de chevalet: nouvelle approche*, Proceedings of the 15th Triennal Conference ICOM Committee for Conservation, New Delhi, September 22-26, 2008, vol.II, p. 581-589.

# 2A – Innovative chemical gels for wall paintings cleaning

*P. Baglioni, M. Baglioni, M. Bonini, E. Fratini, R. Giorgi, G. Pizzorusso*

## Aims

Magnetic-responsive chemical gel.

## Results

Physical gel systems have been extensively used for the cleaning of artwork surfaces, especially canvas and wood paintings. Physical gels consist of a structural network, based on weak forces like hydrogen bonding, Van der Waals and dipole-dipole interactions. Due to this nature they can be easily applied, for example by gentle brushing, also over large surfaces. Unfortunately, they have the great disadvantage of leaving residues on the surface of the artwork and usually they are sticky. In order to avoid these drawbacks, different gelators were also investigated in order to obtain gels that could be loaded with microemulsions. Some preliminary findings on the use of a novel class of gels that allowed us to overcome the above-mentioned limitation and that seemed particularly suitable for the treatment of delicate surfaces, details or irregularly shaped surfaces (for example on pictorial decorations of stone) showed promising results.

Besides traditional gelators as cellulose derivatives, functionalized magnetic nanoparticles can be chemically attached to a polymer matrix to form chemical magnetic responsive gel, acting as a permanent hydrogel. These gels can be magnetically manipulated to eliminate the residue left onto the surface.

The unique properties of the magnetic responsive chemical hydrogel have been screened in order to tune the system response to physical stress (the magnetic field generated by a permanent magnet) and to optimize the cleaning properties of the oil in water/microemulsion. In particular, our work was focused on the optimization of the hydrogel properties in order to obtain an “easy-to-load” reversibile system for the incorporation of the proper cleaning media - in our case a xylene microemulsion (99% water, 0.64% surfactant, and 0.36% xylene).

The gel matrix consists of acrylamide/N,N’-bisacrylamide network polymerized in the presence of $CoFe_2O_4$ magnetic nanoparticles. As far as the problem with residues is concerned, a specific maleic anidride functionalized polyethylene-glycol cross-linker has been synthesized. Due to its chemical nature it allows the chemical bonding of the polymer chains from the cross-linker to the nanoparticles surface (through the carboxylic groups) and to the gel network (through the double bond that may

participate in the polymerization process). In this way, since the nanoparticles are chemically linked to the gel network, possible drawbacks related to nanoparticle residues left on the painted surfaces are avoided. Similar gels have been used in the removal of Paraloid® B72 from marble samples.

## *References*

Bonini M., Lenz S., Falletta E., Ridi F., Carretti E., Fratini E., Wiedenmann A., Baglioni P., *Acrylamide-based magnetic nanosponges: an approach to nanocomposite materials,* Langmuir, 2008, 24, 12644–12650.

Bonini M., Lenz S., Giorgi R., Baglioni P., *Nanomagnetic Sponges for the Cleaning of Works of Art*, Langmuir 2007, *23,* 8681-8685

Baglioni M., Giorgi R., Chelazzi D., Carretti E., Falletta E., Pizzorusso G., Baglioni P., *Rimozione di polimeri acrilici da pitture murali mediante sistemi detergenti e gel chimici magnetici,* in Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metrodi, Compatibilità, Cantieri", Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 477-484.

Giorgi R., Chelazzi D., Carretti E., Falletta E., Baglioni P., *Microemulsions for the cleaning of wall paintings*, Proceedings of the 15th Triennal Conference ICOM Committee for Conservation, New Delhi, September 22-26, 2008, vol.I, p. 527-533.

# 2A – Calcium hydroxide nanoparticles for the consolidation of wall paintings

*P. Baglioni, D. Chelazzi, L. Dei, R. Giorgi*

## Aims

Consolidation of wall paintings with compatible inorganic materials.

## Results

Criteria for conservation treatments, such as compatibility, minimal intervention or reversibility, have found only in the last years some practical applications with the emerging of new techniques based on nanotechnologies. Nanotechnology is based on the recognition that particles of size below 100 nm impart to nanostructures formed from them new behaviour and properties.

After the 1966 Florence flood, the research group directed by the CSGI co-founder Prof. Enzo Ferroni was one of the first Academic Institutions that applied a rigorous scientific approach to the investigation of Cultural Heritage conservation. In 1969, at the International ICOM Conference in Amsterdam, a new method for *in situ* wall paintings consolidation was proposed by EnzoFerroni. The method, today known as "barium or Ferroni-Dini method", inverts the chemical reactions that produce the degradation of wall paintings, stabilizing the structure of the mortar and regenerating the binder of the painted layer, that is calcium carbonate. The method is based on the application of cellulose compress soaked with ammonium carbonate and barium hydroxide solutions. The consolidation is achieved two different chemical processes: the formation of fresh portlandite or calcium hydroxide, $Ca(OH)_2$, through the action of barium hydroxide on calcium carbonate, and the slow and gradual formation of barium carbonate, $BaCO_3$. The new slaked lime ($Ca(OH)_2$), formed *in situ*, acts as the fresh binder, giving new setting for mortar.

Consolidation of mural painted surfaces (or stones) by inorganic treatments should provide the right content of carbonate binders to confer long-term preservation to the works of art. Ferroni-Dini method was the first that provided reliable results and its success was mainly related to the possibility of removing salts that threaten the paintings, reinforcing at the same time the porous structure. The evolution of Ferroni-Dini is based on calcium hydroxide that is the best binder for limestone and wall paintings. In fact, the whole physico-chemical compatibility between the original and the restoration materials can be completely achieved by using calcium hydroxide that is the 'original' binder used by artists. We were among the first able to synthesize $Ca(OH)_2$ nanoparticles. Kinetically stable dispersions can be obtained in short-chain aliphatic alcohols. The dispersions of nanoparticles are similar to an extremely concentrated solution of lime water (up to 30% volume fraction), well above the physico-chemical limit imposed by the solubility of calcium hydroxide in water.

At the moment calcium hydroxide nanoparticles application for wall painting consolidation can be considered as a revolutionary alternative to the use of synthetic polymers (i.e. acrylic). This method is currently used in several places in Italy and in other countries; in particular, among others, for the conservation of mesoamerican paintings in Calakmul, Tlatelolco, and Cholula in Mexico, and the consolidation of wall paintings in Sweden and Denmark. CSGI contribution mainly consists in a scientific co-operation with conservators and institutions for experimentation and application of this innovative methodology.
CTS company (Italy) is now distributing in several countries the product with tradename "Nanorestore®" that is produced at the CSGI laboratory. Nanorestore is the first chemical product based on nanotechnology, made available specifically to the conservator community.

*La Antigua Ciudad Maya de Calakmul*, Mexico: pyramid and wall paintings recently discovered therein and preserved by using $Ca(OH)_2$ nanoparticle dispersions

## References

Baglioni P., Giorgi R., Dei L., *Soft condensed matter for the conservation of cultural heritage*, Compte Rendus Chimie, 2008, XX, 1-9, *article in press*.
Dei L., Bandini F., Felici A., Lanfranchi M., Lanterna G., Macherelli A., Salvadori B., *Pre-consolidation of pictorial layers in frescoes; the high performance of CSGI's method based on nanolime evaluated by OPD team in Agnolo Gaddi's La Leggenda della Vera Croce, Santa Croce, Firenze*, In Proceedings of the 23rd International Congress 'Il consolidamento degli apparati architettonici e decorativi. Conoscenze, Orientamenti, Esperienze', Arcadia Ricerche, Venezia, 2007, pp. 217-224.
Baglioni P., Giorgi R., *Le nanoparticelle per la conservazione ed il restauro del patrimonio culturale*, Giornate Lincee della Chimica, VII edizione "Contributi della chimica alla soluzione di alcuni problemi della societa' moderna", Accademia Nazionale dei Lincei, Roma 2007, Bardi Editore-Editore Commerciale, 81-97.
Baglioni, P., Giorgi, R., *Soft and Hard nanomaterials for restoration and conservation of cultural heritage*, Soft Matter, 2, 2006, 293-303.
Giorgi, R., Chelazzi, D., Carrasco, R., Colon, M., Desprat, A., Baglioni, P., *The Maya site of Calakmul: "in situ" preservation of wall paintings and limestone by using nanotechnologies*, The Object in Context: Crossing Conservation Boundaries, Munich IIC Congress 2006: proceedings, Edited by David Saunders, Joyce H. Townsend and Sally Woodcock, 2006, 162-169.

# 2A – Physico-chemical characterization of photographic materials and motion picture films

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## Aims

Contemporary composite materials in conservation

## Results

The present work is one of the first attempts to analyze the composition of different kinds of photographic documents:
1) Ferrotypes, ancient photographic plates realized on a support made of iron. In this case the photographic material was constituted of collodion (a solution of nitrocellulose in ether and acetone) as a support and silver halides as photosensitive grains.
2) Motion picture films made by cellulose acetate. The analytical techniques used for the morphological and physicochemical characterization were non invasive and therefore for all the investigations no samples were collected from the surface. The surface morphology was studied by means of Optical Microscopy (OM) and Environmental Scanning Electron Microscopy (ESEM) coupled with an Energy Dispersive X-rays (EDX) system for the elemental analysis. These techniques, together with microreflectance Fourier Trasformed InfraRed spectroscopy (μ-FTIR), allowed to obtain information on both the chemical composition of the materials constituting the documents and their conservation status. The study showed that the physicochemical diagnostics can be an useful tool for the study of ancient photographic plates and motion picture films in order to gain information on the materials, state of conservation, and realization techniques. Particularly, by means of both electronic and optical microscopy investigations, it is possible to study the possible damages induced by the vinegar syndrome, the most important autocatalytic degradation process of motion-picture films made by cellulose acetate.
3) Positive photographs printed or developed on paper. The characterization of the conservation status of photographic materials is usually assessed through visual analysis or optical microscopy. However, a small percentage of these materials cannot be completely characterized by the simple visual-optical inspection and needs a more quantitative investigation. In order to get to a better comprehension of this material, we adopted a characterization procedure relying both on the analysis of the photographs' materials and on the knowledge of the techniques and the materials used. For this, a survey of photographic literature, in particular Italian manuals and periodicals published around 1890-1910, is a primary source that we used together with micro-invasive and non-invasive investigations. This work was performed in order to understand the chemical and physical degradation processes of photographs

from the period around the end of the nineteenth century. We studied some sets of photographs obtained with different techniques but stored under the same environmental conditions. The different sets showed different degradation processes that can be easily attributed to the different photographic techniques used.


Positive photographs made at the end of 19th century according to different techniques

## References

Carretti E., Milano M., Natali I., Giorgi R., Dei L., Baglioni P. *Physicochemical characterization of cellulose based motion-picture film*, In Proceedings of the YOCOCU Conference, Roma, 24-25 Novembre 2008.

Cattaneo B., Chelazzi D., Giorgi R., Serena T., Merlo C., Baglioni P., *Physico-chemical characterization and conservation issues of photographs dated between 1890 and 1910*, Journal of Cultural Heritage 9 (2008) 277- 284

Cattaneo B., Chelazzi D., Giorgi R., Serena T., Merlo C., Baglioni P., *Caratterizzazione chimico-fisica di positivi fotografici del periodo 1890-1910: degrado e conservazione*, Atti del V Congresso Nazionale IGIIC - Lo Stato dell'Arte - Cremona, 4-6 Ottobre 2007, Nardini editore, p. . 573-582.

Carretti E., Milano M., Poggi G., Giorgi R., Dei L., Baglioni P., *Caratterizzazione chimico fisica non invasive di due ferrotipi inglesi del XIX secolo*, in Atti del VI Congresso Nazionale IGIIC - Lo Stato dell'Arte - Spoleto, 2-4 Ottobre 2008, Nardini editore, p. 77-82.

# 2A – Deacidification of waterlogged wood: the Vasa shipwreck case studies

*P. Baglioni, D. Chelazzi, R. Giorgi, G. Poggi*

## Aims

Calcium and magnesium hydroxide to protect wood from acidity

## Results

The promising results obtained in the deacidification of paper and books suggested that magnesium and calcium hydroxide nanoparticles could be used also for wood. Wood is a more complex system than paper. In fact, it shows a three-dimensional structure due to the arrangement of cells and contains also lignin and hemicellulose as a major component. This involves that small particles must be used in order to get a good penetration into the substrate. Moreover, the interaction of the nanoparticles (as well as the carrier solvent) with the different chemical wood components must be carefully taken into account.

The first experimentations on wood were done on some samples from the Swedish warship Vasa. The Vasa wood shows particular features that made it a unique case in archaeological wood preservation. The warship sank during its maiden voyage in 1628, in the Stockholm port, and was recovered only in 1961. Its wood shows high acidity, due to the oxidation of elemental sulfur inside the fibers, giving sulfuric acid. The elemental sulfur comes from the metabolic action of sulfate-reducing bacteria that dwell in the wasted water of the Stockholm harbor. The presence in the wood of large quantity of sulfuric acid is now threatening the preservation of Vasa, and consistent efforts are being made in order to deacidify the wood and to prevent sulfur oxidation.

We transferred the method used for paper deacidification by soaking small samples of Vasa wood into alcoholic stable dispersions of calcium and magnesium nanoparticles. Previous to the soaking with nanoparticles dispersions, the wood samples were washed with water in order to remove polyethylene glycol (PEG), used to protect the wood, whose presence in the wood porous matrix would prevent the alkaline particles from the penetration and adhesion to the cellulose fibers. In order to state the efficacy of the deacidification method, pH measurements and thermal analysis were carried out on the treated and untreated samples. Pyrolysis temperature of cellulose, determined through DTG, was monitored, and turned out to be very sensitive to the acidity of wood: acidic samples, in fact, showed lower pyrolysis temperature, whereas the deacidified ones showed higher values.

After treatment the pH of wood reverted from highly acidic values (pH=2.8) to slightly acidic ones (pH=5.5, very close to pH of native oak wood), and pyrolisis temperature, after treatment, reverted back to the typical values of fresh oak wood. It was shown that magnesium hydroxide nanoparticles penetrated inside the wood

matrix up to 1-2 cm depth. As for paper applications, nanoparticles neutralized the acidity, and the excess quickly turned to carbonate. In this way, possible (but not proved) dangerous interactions of the hydroxide excess with residual cellulose and lignin was avoided, and an alkaline reservoir was created that can neutralize the acidity that develops continuously inside the wood fibers from the sulfur oxidation.
Recent improvements of the method involve the usage of different nonaqueous solvents as fluorinated solvents (unpublished results) that allow a deeper penetration and better delivery of nanoparticles inside the wood.

Vasa shipwreck at Swedish Maritime Museum in Stockholm

## References

Giorgi R., Chelazzi D., Baglioni P., *Nanoscience contribution to preservation of acidic shipwrecks*, Proceedings of the 10th ICOM-CC conference on Wet Organic Archaeological Materials - WOAM 2007, Amsterdam on September 10-15, 2007. *accepted for publication*.
Chelazzi, D., Giorgi, R., Baglioni, P., *Nanotechnology for Vasa Wood De-Acidification*, Macromolecular Symposia (Special Issue: Macromolecules in Cultural Heritage, Issue Edited by F. Cappitelli, Y. Shashoua, E. Vassallo.) 238 (1), 2006, 30-36.
Chelazzi, D., Giorgi, R., Baglioni, P., *Nanotechnologies for the conservation of waterlogged wood: the Vasa case studies,* Heritage, Weathering and Conservation conference (HWC-2006): proceedings, edited by R.Fort, M.Alvarez de Buergo, M.Gomez-Heras & C.Vazquez-Calvo, Taylor & Francis/A.A.Balkema Publishers, London, 2006, 797-802.
Giorgi, R., Chelazzi, D., Baglioni, P., *Conservation of acid waterlogged shipwrecks: nanotechnologies for de-acidification*, Applied Physics A: Materials Science & Processing (Special issue: Materials Science and Cultural Heritage, Guest Editor(s): M.-C. Corbeil, M. Stuke, M. Menu, G. Padeletti), 83 (4), 2006, 567-571.
Giorgi, R., Chelazzi, D., Baglioni, P., *Nanoparticles of calcium hydroxide for wood conservation. The deacidification of the Vasa warship*, Langmuir, 2005, 21, 10743-10748.

# 2A – Nanotechnology for the deacidification of books and manuscripts

*M.C. Arroyo, P. Baglioni, R. Giorgi, G. Poggi*

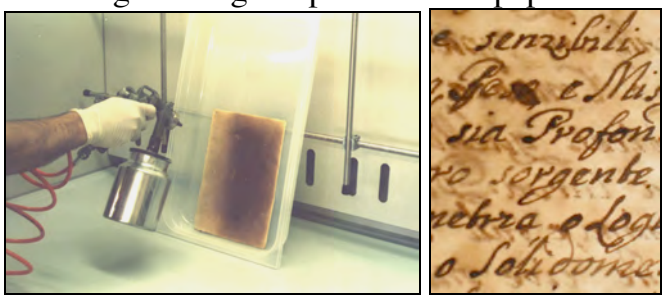
## Aims

Nanoparticles dispersions to preserve books and manuscripts

## Results

Deacidification of cellulose-based materials is universally known as a major conservation issue for the large number of items to be treated (books in libraries, newspapers, textiles, wood, and so on). It is well known that acid catalyzes the hydrolysis of cellulose, leading to depolymerization. As a consequence, the physical and mechanical properties of the cellulose polymer are compromised. For paper, this process produces brittleness and fragility, so that many ancient books and documents could be lost, if not properly treated. In the past years many methods for paper deacidification have been developed, as the Wei T'o and the Bookkeeper. New methods based on the application of magnesium (or calcium) hydroxide nanoparticles dispersed in a nonaqueous solvent are under investigation at CSGI. Nanoparticles, due to their tiny dimensions, penetrate into the network of the cellulose fibers, and adhere to them. Part of the hydroxide immediately reacts *in-situ* with the acidity, through solid-state reaction, providing deacidification. The hydroxide excess, due to the high reactivity of nanoparticles, is quickly converted into magnesium carbonate by the carbon dioxide present in the environment. In this way, an alkaline buffer is created in order to withstand the forthcoming acidity that can develop inside the fibers (for example, due to pollution). Calcium hydroxide nanoparticles could be used as well, but the smaller size of magnesium hydroxide particles involves a better penetration and faster carbonation inside the cellulose substrate. This feature is particularly useful when dealing, for example, with deacidification of wood cellulose. At this moment, research efforts are focused on synthesis routes that allow smaller size of the particles.
The first deacidification experimentations on ancient paper samples were very promising. However, in order to achieve clear evidences about the efficacy of the treatment and a better comprehension of the deacidification mechanisms, standard reference materials were investigated. Whatman filter paper, composed of pure cellulose, can be considered an ideal material for the investigations. In order to reproduce the acid degradation of the fibers, samples of Whatman no.1 paper were acidified by soaking in a sulfuric acid solution (pH 2.5). Some samples were then deacidified by soaking with an alcoholic dispersions of $Mg(OH)_2$ nanoparticles.
The particles resulted after treatment homogeneously distributed within the paper, and adhered to the cellulose fibers. In order to quantify the deacidification effect of the treatment, some parameters for degradation have been measured. Together with pH

measurements, the determination of the degree of polymerization (DP) of cellulose was chosen as a quantitative parameter of the conservation *status* of the polymer. DP is determined through viscosity measurements according to international standard protocols. Some samples of acidified paper were artificially aged (by hydrothermal aging; 90ºC and 80% R.H.) and their DP was monitored during some months of aging. The same procedure was repeated after the deacidification treatment. The investigations showed that the aging of the acidified samples promoted a strong decrease in the cellulose DP, with a loss of about 55%. On the other hand, samples acidified and then treated with magnesium hydroxide dispersion resisted very well to the aging, with a DP decrease of about 17%. Although a precise conversion of the artificial aging time to the natural aging is difficult, these data suggest that treatment with alkaline nanoparticles grants a good protection to paper cellulose.



Deacidification of paper by sprayed lime nanodispersions

## References


Giorgi R., Poggi G., Baglioni P., *Deacidificazione di documenti cartacei mediante dispersioni non acquose di nanoparticelle alcaline*, in Atti del VI Congresso Nazionale IGIIC - Lo Stato dell'Arte - Spoleto, 2-4 Ottobre 2008, Nardini editore, p. 69-76.

Giorgi R., Chelazzi D., Baglioni P., *Il ruolo degli inchiostri metallo-gallici nei processi degradativi di manoscritti cartacei*, Atti del V Congresso Nazionale IGIIC - Lo Stato dell'Arte - Cremona, 4-6 Ottobre 2007, Nardini editore, p. 273-280.

Baglioni, P., Giorgi, R., *Soft and Hard nanomaterials for restoration and conservation of cultural heritage*, Soft Matter, 2, 2006, 293-303.

Giorgi, R., Bozzi, C., Dei, L., Gabbiani, C., Ninham, B.W., Baglioni, P., *Nanoparticles of $Mg(OH)_2$: synthesis and application to paper conservation*, Langmuir, 2005, 21, 8495-8501.

Chelazzi, D., Giorgi, R., Dei, L., Baglioni, P., *Nanotecnologie per la conservazione del patrimonio culturale*, La Chimica e l'Industria, 2005, 87 (10), 78-82.

Giorgi, R., Dei, L., Ceccato, M., Schettino, C.V., Baglioni, P., *Nanotechnologies for conservation of Cultural Heritage: paper and canvas deacidification*, Langmuir, 2002, 18, 8198-8203.

Giorgi, R., Dei, L., Schettino, C., Baglioni, P., *A new method for paper deacidification based on calcium hydroxide dispersed in non-aqueous media*, Preprint of the IIC Baltimore Congress 2002, Works of Art on Paper, Books, Documents and Photographs: Techniques and Conservation, P. Smith Ed., 2002, 69.

# 2A – Innovative gels for easel paintings conservation

*P. Baglioni, E. Carretti, M. Cossalter, L. Dei, S. Grassi, I. Natali*

## Aims

Super elastic gels for easel conservation.

## Results

In the last two years the Florence unit of CSGI has developed two different classes of gels to be applied as cleaning tools for easel paintings surfaces.

First of all it has been set-up a new type of hydrogel in which various cosolvents have been added to the well-known water-PVA-borax systems. These systems can be successfully and safely used as cleaning tools for the removal of hydrophobic aged varnishes from the surface of easel paintings. The main advantages associated to the use of the PVA based gels compared to the gels commonly used for the cleaning of painted surfaces (most of them contain by cellulose and polyacrylic acid as gelators) are mainly two. 1) As in the case of classical gels, they inhibit surface spreading of the solvent (so that only a designated area is exposed to the cleaning agents), reduce solvent penetration into the original painting layers, mainly via capillary action—solvent penetration often leads to swelling and leaching of varnishes and binders constituting the work of art—and finally they favor the uptake of the solubilized materials into the liquid part within the porous matrix. 2) Respect to classical gels, PVA hydrogels do provide selective and very surface-controlled cleaning action, as well as facile and benign removal from a painting surface. Their rheological properties and mainly their very high elasticity allows them to be peeled from a surface without introducing a strong lateral force. By so doing, residues left onto the painted surface from the patina and from the gel are minimized and the mechanical action and repeated washings usually necessary for the complete removal of traditional gels can be avoided.

A new nanotechnology approach is aimed at setting up a new family of polymeric gel systems in which the continuous phase is constituted by a microemulsion. Two main formulations have been obtained. In the first the gelator is hydrophobically modified hydroxyethylcellulose (1-2% w/w) and the formation of the obtained 3D network embedding an o/w microemulsion is due to physical interactions (so we have physical gels). Macroscopical observations (the gel is completely transparent) and preliminary SAXS and rheology studies indicate that the droplet microstructure is retained into the gel. The gel seems to be not simply an additive system, but the presence of the polymer network induces some structural modifications in the geometry of the nanodroplets. This gel has been successfully used to remove acrylic and vinyl resins from Renaissance wall paints in Santa Maria della Scala (Siena), outperforming simple liquid microemulsions and further reducing the impact of the cleaning action.

# 2A – Microemulsions and micellar solution for the cleaning of wall paintings

*P. Baglioni, M. Baglioni, D. Berti, E. Carretti, L. Dei, R. Giorgi*

## Aims

Surfactant-based systems to remove synthetic polymers from painted surfaces

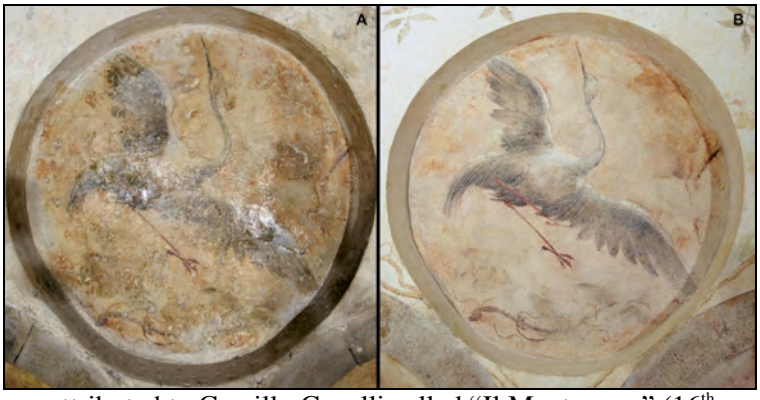
## Results

Cleaning and consolidation are important and delicate interventions potentially invasive and irreversible. Recently an important contribution, based on nanotechnology, provided new formulations that can replace the traditional methods based on organic solvents. These last are very often environmentally unsafe and usually not specific for the selective removal of degraded resins (polymers) or grime. Moreover, their application results in the undesired spreading of the dissolved materials within the porous structure of the artwork. Since the beginning of the 1990s, CSGI has been investigating the potential application of microemulsions and micellar solutions for the cleaning of artworks surfaces.

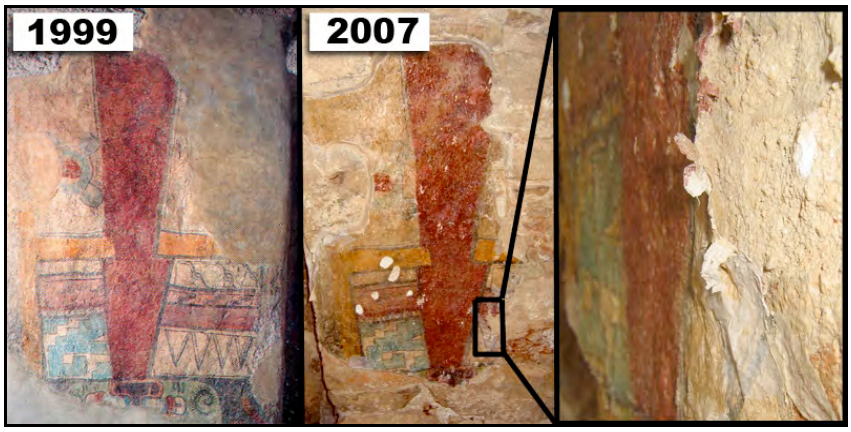
Past restoration treatments, based on the use of synthetic polymers, failed since of their degradation. In fact, they are unstable in most of the environmental conditions: drastic temperature and relative humidity changes, mechanical abrasion by dust and wind, rain and water condensation, light, and pollution promote degradation. The final effect is the oxidation of polymer end-groups or side-groups, the chemical breakdown of polymer chain (chain-scission), and cross-linking reactions. All these processes lead to the loss of pigments adhesion, yellowing of polymers, decrease of polymer solubility and loss of treatment reversibility. The only way for removing them is, at the moment, limited to the usage of toxic mixtures of solvents.

Annunciation church in Nazareth (Israel): painting dated back to the 5th-6th century AD and restored in 1973.

Unfortunately, cleaning procedures, when improperly performed, may be aggressive and invasive for the original materials. Micelle solutions, microemulsions and gels offer new tools for the cleaning of surfaces. Nano-compartimentalized systems are probably the best available cleaning methods to avoid undesired drawbacks.
The first example of microemulsion application for cleaning organic contaminants (bee wax spots) on fresco paints was performed on Masaccio, Lippi, and Masolino masterpieces in Florence (Brancacci chapel). In the last year we reported on the search of new nano-structured systems (microemulsions and micellar solutions) formulated to face new conservative challenges, like the removal of vinyl/acrylic copolymers from mesoamerican wall paintings in archeological sites of Mayapan and Cholula (Mexico), or the removal of silicone resins from wall paintings in the Annunciation grotto that is adored as the Mother Mary's house in Nazareth (Israel).
Recently, these systems have been used in several restorations for the removal of vinyl and acrylic polymers from large areas of painted surfaces. Microemulsions and micellar solution developed at the CSGI Center were used in the Sacristy of San Salvador Church in Venice (60 $m^2$), for cleaning of the mural paintings of the Conegliano (near Venice) Cathedral Façade (250 $m^2$) and also in the mural paintings of the Old Sacristy of Santa Maria della Scala in Siena (90 $m^2$).




Detail of the fresco attributed to Camillo Capelli called "Il Mantovano" (16$^{th}$ century) in the San Salvador Church in Venice before (A) and after (B) cleaning with an o/w microemulsion




Mayapan (Mexico): degradation effects caused by synthetic polymer layer applied over the wall paintings in 1999.

In the archeological site of Mayapan in Yucatan, Mexico, a few decades ago Maya wall paintings dating back to the Post-Classic Age (1200-1450) were discovered. Conservators treated the paintings with Mowilith resins in order to consolidate and protect the paint layer. Recent investigations showed problems of degradation of the polymer films and serious changes in the appearance of the paintings. Salts coming from the interior of the wall are pushing the paint layer because the natural "breathing" of the surface due to the presence of the polymer is totally lost. Methods of intervention finalized to the recovery of the original properties of the paint layer have been developed. By using innovative micelle solutions, the removal of the polymers was successful.

## References

Baglioni P., Giorgi R., Dei L., *Soft condensed matter for the conservation of cultural heritage*, Compte Rendus Chimie, 2008, XX, 1-9, *article in press*.

Giorgi R., Chelazzi D., Carretti E., Falletta E., Baglioni P., *Microemulsions for the cleaning of wall paintings*, Proceedings of the 15th Triennal Conference ICOM Committee for Conservation, New Delhi, September 22-26, 2008, vol.I, p. 527-533.

Colafemmina G., Fiorentino D., Ceglie A., Carretti E., Fratini E., Dei L., Baglioni P., Palazzo G, *Structure of SDS Micelles with Propylene Carbonate as Cosolvent: a PGSE-NMR and SAXS Study*, J. Phys. Chem. B, 111 (2007) 7184-7193.

Carretti E., Giorgi R., Berti D., Baglioni P., *Oil-in-Water Nanocontainers as Low Environmental Impact Cleaning Tools for Works of Art: Two Case Studies*, Langmuir 2007, 23, 6396-6403

Giorgi R., Chelazzi D., Carretti E., Baglioni M., Solis C.G., Castro Barrera M.C., Arroyo M.C., Baglioni P., *Rimozione selettiva di mowilith DM5 da pitture maya di epoca post-classica mediante sistemi micellari*, in Atti del XXIV Convegno Internazionale "Scienza e Beni Culturali – Restaurare i Restauri – Metrodi, Compatibilità, Cantieri", Bressanone, 24-27 Giugno 2008, Edizioni Arcadia Ricerche, 208-218.

Grassi S., Carretti E., Pecorelli P., Jacopini F., Baglioni P, Dei L., *The conservation of the Vecchietta's wall paintings in the Old Sacristy of Santa Maria della Scala in Siena: chemical characterization and innovative cleaning methodology*, Journal of Cultural Heritage, 8 (2007), 119-125.

Carretti, E., Dei, L., Weiss, R. G., *Soft matter and art conservation: rheoreversible gels and beyond*, Soft Matter, 1, 2005, 1-6.

Carretti, E., Salvadori, B., Baglioni, P., Dei, L., *Microemulsions and Micellar Solutions for Cleaning fresco Surfaces*, Studies in Conservation, 50, 2005, 1-8.

Palazzo, G., Fiorentino, D., Colafemmina, G., Ceglie, A., Carretti, E., Dei, L., Baglioni, P., *Nanostructured fluids based on propylene carbonate/water mixtures*, Langmuir, 2005, 21, 6717-6725.

# 2A – Enzymes for biocatalysis

*R. Pogni, S. Giansanti, M.C. Baratto, S. Forte, D. Spinelli, R. Basosi*

## *Aims*

Analysis of different bioprocesses for the bio-synthesis of new compounds or bioremediation using different enzymes (Peroxidases, Oxidoreductases, Mono-Di-Oxygenases) in fluidized bed reactor or supported on different materials.

## *Results*

The field of enzyme biotechnology is undergoing rapid change and diversification. It will be no doubt offer some of the most important technological revolutions in the next century, which will change the way we produce food, the way we treat or prevent diseases, and provide new ways of preserving the environment. Biotechnology requires enzymes that are functional and stable under a wide range of unnatural conditions. In this context, in our group we are analyzing different enzymes to be used as biocatalysts for synthesis of new compounds and/or for bioremediation purposes. We are analyzing the potentiality of the use of theses enzymes supported on different materials.

Laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) belong to the multicopper oxidase family, a group of enzymes that is widespread in numerous fungi, plants, and bacteria. Laccases have various functions that can be chemically related to the oxidation of a wide range of aromatic substrates with the concomitant reduction of $O_2$ to $H_2O$. The redox potentials of laccases range from +0.5 V to +0.8 V depending on the type of organism from which the enzyme has been obtained. The highest redox potential reported so far (+0.8 V) only allows the direct oxidation of high redox potential phenolic compounds and aromatic amines. The natural degradation of the complex polymer lignin by laccase has suggested the use of appropriate redox metabolites, called mediators which enable laccase to indirectly oxidize large molecules of substrate and even non-phenolic substrates. The broad substrate specificity of laccases, together with the fact that this enzyme uses molecular oxygen as the final electron acceptor, makes it particularly interesting for industrial applications in the pulp and paper industry as well as for environmental purposes and in the finishing process of the textile industry.

The synthesis of a new dye with the phenoxazine structure has been synthesized and characterized, in our laboratory, at room temperature using the laccase system.

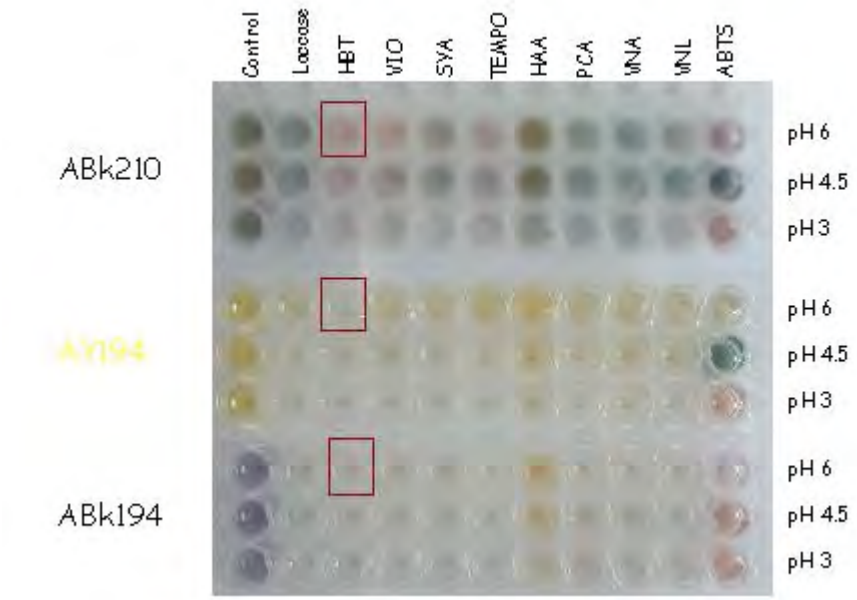



Fig.1. Bleaching percentage of Acid dye oxidation by *T. versicolor* laccase in the absence and in the presence of synthetic (1-hydroxybenzotriazole (HBT), Violuric acid (VIO), TEMPO and ABTS) and natural (vanillic acid (VNA), vanillin (VNL), p-coumaric acid (PCA), 3-hydroxyanthranilic acid(HAA) and syringaldehyde (SYA)) mediators.

## *References*

Brogioni, B., Biglino, D., Sinicropi, A., Reijerse, E. J., Giardina, P., Sannia, G. , Lubitz, W. , Basosi, R. , Pogni, R., *Characterization of Radical Intermediates in the Laccase-Mediator Systems: A Multifrequency EPR, ENDOR and DFT/PCM study*, Physical Chemistry Chemical Physics, 2008, 10, 7284-7292

Castriciano, M. A., Romeo, A., Baratto, M. C., Pogni, R. , Monsù Scolaro L. , *Supramolecular Mimetic Peroxidase Based on Hemin and PAMAM Dendrimers*, Chemical Communications, 2008, 6 , 688 – 690.

Bani, S., Cioni, I., Millarini, V., Ciullini, I., Fatarella, E., Briganti, F., Scozzafava, A., Giansanti, S.,Basosi, R., *Bio-based advanced oxidative process for textile wastewater treatment,* BioRemediation Conference, Ed. N. Kalogerakis, F. Fava, S. A. Banwart, 2008, 71-74.

Pogni, R. , Brogioni, B. , Baratto, M. C. , Sinicropi, A. , Giardina, P. , Pezzella, C. , Sannia, G. , Basosi R., *Evidence for a radical mechanism in biocatalytic degradation of synthetic dyes by fungal laccases mediated by violuric acid,* Biocatalysis and Biotransformations, 2007, 25, 269 – 275.

# 2A – ToF-SIMS Characterization of Pigments and Binders in Paint Samples From "The Martyrdom of St. Catherine" in Zejtun (Malta)

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## Aims

ToF-SIMS provides elemental data and a certain degree of molecular information whilst also allowing depth profiling, mapping or imaging to be carried out (Spoto, 2000) and was successfully applied for the characterization of inorganic and organic components of a wide range of cultural heritage objects (Keune and Boon, 2004; Adriaens and Dowsett, 2006; Mazel et al., 2006).
The aim of this paper is to characterize inorganic and organic components in paint samples, belonging to "The Martyrdom of St. Catherine", conserved in the parish Church of St. Catherine, in Zejtun (Malta), through the application of ToF-SIMS. "The Martyrdom of Saint Catherine", with size 268x204 cm, attributed to Francesco Cassarino, was painted in the period 1607-1615. It is conserved in the church of Saint Catherine, in Zejtun (Malta).

## Results

In 2007, during the diagnostic campaign, 14 microfragments (mostly smaller than 1 mm) were collected by different parts of the painting, according to the principle of minimum invasiveness, i.e. the least number of collected samples to obtain the maximum amount of information (Lapucci, 2008). For each microfragment both secondary ion spectra, positive and negative, and the images of the distribution of some of detected ions on the analyzed surface are collected. As example of secondary ion spectra, positive and negative images of spatial distribution of inorganic and organic compounds are reported in figure 1. In figure 1a, the ToF-SIMS Images of a 200x200μm$^2$ area show the distribution of the total positive ions signals (i.e. the sum of all signals in the interval 0-2000m/z), $Pb^{2+}$, $Na^+$, $Ca^{2+}$, $K^+$ and an organic fragment with mass 27 (associated to $C_2H_3^+$, one of the most common fragments coming from organic molecules, representative of the distribution of organic matter). In figure 1b, on the right, the ToF-SIMS Images of the distribution of the total negative ions signals and some negative ions ($S^{2-}$ and $Cl^-$), in the analyzed area, are reported. In the lower part of the same figure, the distributions of 253m/z, 281 and 339m/z ions, related to the presence of Palmitic acid, Oleic acid and Dioleine (or another digliceride with same weight as the linoleic stearic mixed digliceride), typical components of drying oil binders, are reported.

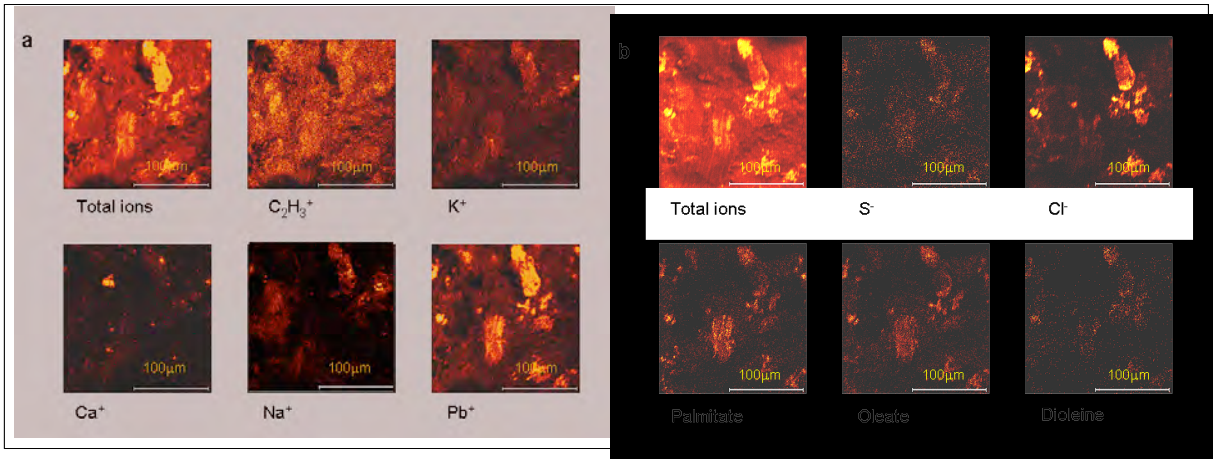


Fig. 1 – ToF-SIMS images: (a) positive ion images on a 200x200$\mu m^2$ area show the distributions of some positive ion; (b) negative ion images on a the same.

In conclusion, ToF-SIMS analyses were able to identify both pigments and binders used in paintings and evidence their distribution, by means of chemical images, on a microfragment or a microstratigraphy. In particular, in the study of the "The Martyrdom of Saint Catherine" we detected the presence of some pigments, such as Lead White, Green Earth, Red and Yellow Ochre. Additionally, a siccative oil is present as pictorial binder, namely walnut oil. Moreover, the presence of chlorine could be associate with the marine spray, compatible with the coast areas such as the maltese one is. Finally, in positive and negative ion mass spectra, the presence of an high molecular weight natural compound (perhaps a polymerized natural resin) is identified.

## *References*

Adriaens, A., Dowsett, M.G., *Applications of SIMS to cultural heritage studies*, Applied Surface Science 2006, 252: 7096-7101.

Keune, K., Boon, J.J., *Imaging secondary ion mass spectrometry of a paint cross section taken from an early Netherlandish painting by Rogier van der Weyden*, Analytical Chemistry 2004, 76: 1374-1385;

Lapucci R., *The Zejtun Painting: Shades of Caravaggio* 2008, Edifir-Edizioni Firenze, Florence;

Mazel, V., Richardin, P., Touboul, D., Brunelle, A., Walter, P., Laprévote, O., *Chemical imaging techniques for the analysis of complex mixtures: New application to the characterization of ritual matters on African wooden statuettes*, Analytica Chimica Acta 2006,570: 34-40;

Spoto, G., *Secondary ion mass spectrometry in art and archaeology*, Thermochimica Acta 2000, 365: 157-166.

# 2A – Dynamics of chromophoric dissolved organic matter and dissolved organic carbon in lakes

*F. Benetti, L. Bracchini, A.M. Dattilo, S.A. Loiselle, C. Rossi*

## Aims

Study the temporal and spatial dynamics of chromophoric dissolved organic matter (CDOM) and dissolved organic carbon in lakes. The effect of solar radiation as well as the release of CDOM of alga derived matter and the effect of bacterial activity were studied.

## Results

The results from the present study indicate that (i) CDOM in the hypolimnion undergoes little modification through the year (if compared with changes occurred in the epilimnion) and winter CDOM optical properties are similar throughout the water column; (ii) the relative composition of CDOM changes from "humic-like" to "protein-like", that is well described by the spectral slope calculated using 270-400 nm wavelength interval (Figure 1), (iii) solar UV attenuation coefficients are better correlated to CDOM absorption and Chlorophyll a concentrations than DOC concentrations. Moreover in the epilimnion, Chlorophyll a and pheopigment concentrations shows positive linear relationships with both absorption and fluorescence of CDOM. This suggest that phytoplankton contribute, together with the long term solar UV exposition, to the observed seasonal changes of the CDOM optical properties.

Salto Lake represents an interesting system where the loss in aromaticity was not followed by loss of carbon content of DOM. This promote an uncoupling between CDOM and DOC measurements (Figure 2). Multiple factors lead to an uncoupling of DOC and CDOM. Summer time conditions lead to a net loss of CDOM in the epilimnion, likely connected to processes that promote CDOM photodegradation and/or production of less (or non) chromophoric DOM. Regardless of the dominating mechanisms the results supports the idea that production of non chromophoric DOM may influence the DOC measured in the epilimnion more than CDOM photodegradation.

Finally, also the timing and duration of the physical stratification of the lake may have direct impact on CDOM/DOC dynamics. Changes in the characteristics of the stratification of the water masses (linked to climate and local changes) would have important consequences on the lake carbon budget as well as solar UV radiative transfer in the water column.

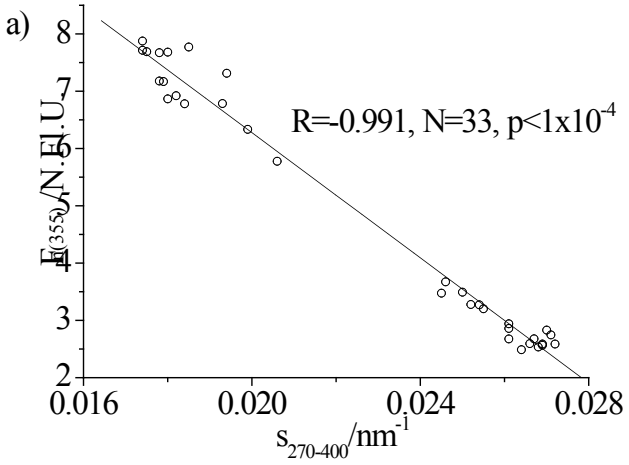


Fig. 1: relationship between spectral slope ($s_{270-400}$) and Fluorescence of humic like CDOM.

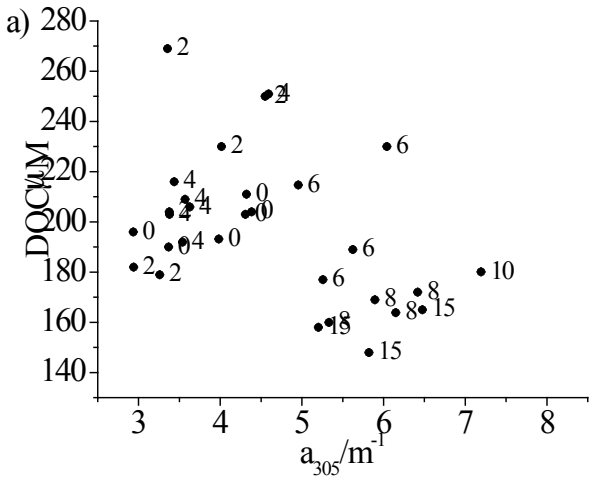


Fig. 2: Uncoupling between CDOM absorption and DOC concentrations.

## *References*

Bracchini L., Loiselle, S.A., Dattilo, A.M., Mazzuoli, S., Cózar, A., Rossi, C, *The spatial distribution of the optical properties in the UV and Visible in an aquatic ecosystem* 2004, Photochemistry and Photobiology, 80(1) 139-149.

Bracchini L., Loiselle, S.A., Dattilo, A.M., Rossi, C, The optical properties of chromophoric dissolved organic matter in the Central-Eastern Mediterranean Basin 2009, Aquatic Sciences, In press.

# 2A – Impact of solar radiation on CDOM optical properties and living systems

*L. Bracchini, A.M. Dattilo, S.A. Loiselle, A. Tognazzi, C. Rossi*

## *Aims*

Study the effect of solar ultraviolet radiation (UV, 290-400 nm) on the optical properties of the CDOM in laboratory and in situ. Moreover, in this project we study also the impact of the UV on living systems.

## *Results*

The solar radiation, in particular the UV, have an important impact on ecosystems. The UV solar radiation penetrate the water column from few centimetres to hundreds of meters (Figure 1). This is dependant by the considered wavelength and by the optical active components that are present in the water column.

We showed that the impact of solar radiation induce the phytoplankton communities to release chromophoric dissolved organic matter with different optical properties with respect to the CDOM released by planktonic communities that were not exposed to the solar UV irradiance (Figure 2).

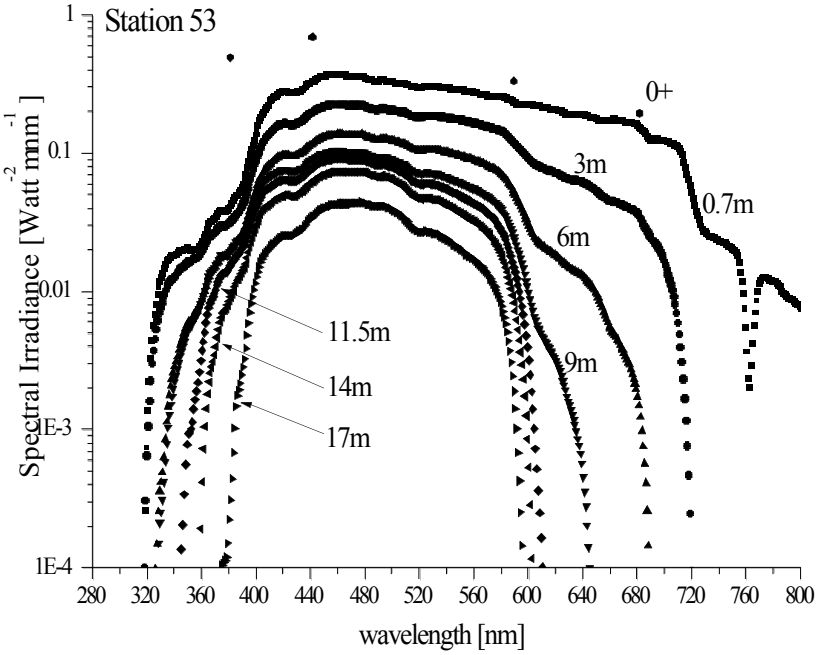


Fig. 1: The spectral irradiance in the sub surface layer of the water column.

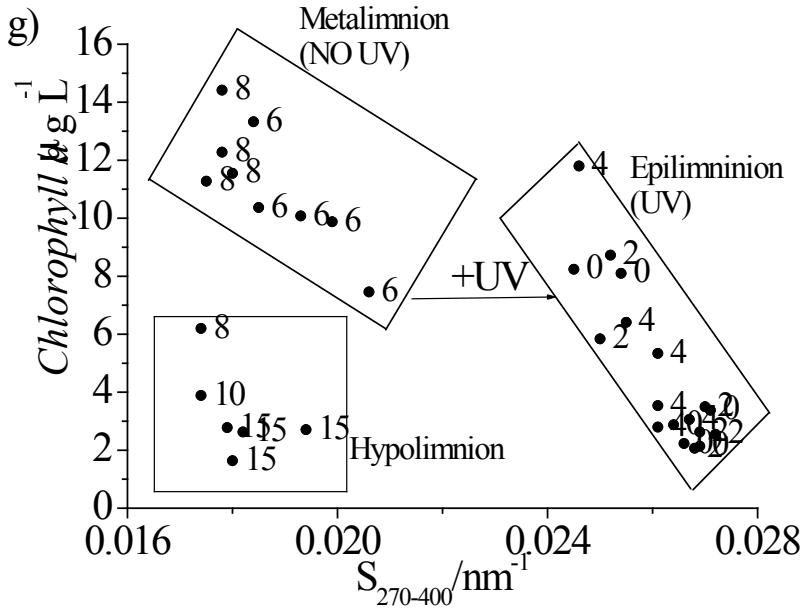


Fig. 2: The impact of solar UV irradiance on production of CDOM by phytoplankton communities.

## *References*

Bracchini L., Loiselle, S.A., Dattilo, A.M., Mazzuoli, S., Cózar, A., Rossi, C, *The spatial distribution of the optical properties in the UV and Visible in an aquatic ecosystem* 2004, Photochemistry and Photobiology, 80(1) 139-149.

Dattilo A. M., Decembrini, F., Bracchini, L., Focardi, S., Mazzuoli, S., Rossi C., *Penetration of solar radiation into waters of Messina Strait (Italy)* 2005, Annali di Chimica, 95, 177-184

Caroppo, C., Congestri, R., Bracchini, L., Albertano, P. *On the presence of Pseudo-Nitzschia calliantha Lundholm, Moestrup et Hasle and P. delicatissima (CLEVE) heiden in the southern adrietic sea (Mediterranean Sea, ITALY)* 2005, Journal of Plankton Research, , 27, 763-774.

# 2A – Ecosystems chemical and physical characterization using remote sensing approach

*S.A. Loiselle, F. Benetti, L. Bracchini, A.M. Dattilo, A. Tognazzi, C. Rossi*

## Aims

The combination of biological, chemical and optical measurements are estimated with the remote sensing approach.

## Results

*Lake Victoria, Africa*

SAC-C images were used to perform the classification of the wetland areas of the Ugandan coast and the Kenyan coast acquired on 23/06/2002 and 04/09/2002 . Images were georeferenced using the geocorrection file for each acquisition date. At sensor radiances were obtained using the published values. Path irradiance was removed by using 1% dark object subtraction. The surface reflectance was calculated by determining the sun elevation at the centre of each scene at the time and the MMRS spectral responses supplied by CONAE. Clouds present in the resultant images were then removed and those areas impacted were not considered in the analysis.

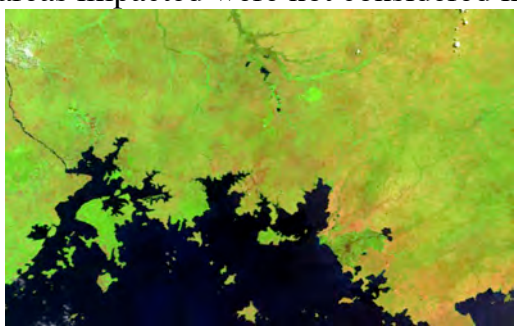

Fig. 1: The northern parts of Lake Victoria

Plots of known vegetation composition in the Kirinya and Nabugabo wetlands were then used as training areas. A classification was performed using four main classes; wetland dominated by *Cyperus papyrus* with *Tyhpa latifolia* and *Phragmites domenginensis,* wetlands dominated by *Miscanthidium violecium* with areas of *Typha latifoli* and *Phragmites domenginensis*, non wetland areas (forests and agricultural areas obtained using land use maps from 1992) and open water areas.

The results of the analysis indicate that the wetlands along the western shore have a higher density than the northeastern shore. This is most likely the result of water and air circulation in this equatorial setting as well as the increased population density

along the northern shore. The resulting impact on water quality in the Lake is presently being investigated, but the combination of lower wetland presence and increase population density will undoubtedly lead to poorer water quality in the northern bay areas where much of the lake population lives.

*Ibera wetland, Argentina*
The "Esteros del Iberá" is one of the largest pristine inland wetland ecosystems in South America. The analysis of the wetland was made to determine the seasonal changes in water quality in the large number of wetland lakes. Landsat TM and SAC-C MMRS satellite images were processed using radiometeric correction models created for each satellite system utilised. A single image of spring 2001 was used to determine the size and location of all major open water bodies (> 0.5 $km^2$) in the wetland area. Samples of permanent water bodies just beyond the wetland borders were also sampled for comparison purposes. A database of 63 lake sections of a total of 25 water bodies was obtained.

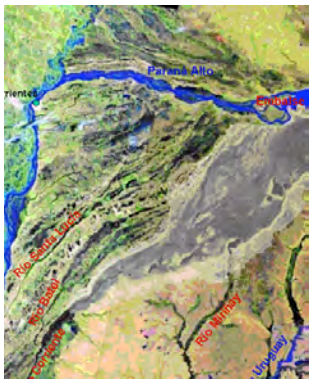


Fig. 2: The Esteros del Iberà

## References

Loiselle, S. A., Bracchini, L., Rossi, C., *Modelling energy fluxes in remote wetland ecosystems with the help of remote sensing*, 2001, Ecological Modelling 145, 245-261
Loiselle, S. A., Bracchini, L., Cozar, A., Cognetta, A., Rossi, C., *Determination of water optical parameters in a large wetland using TM bands*, In: Proceedings from the 29th International Symposium on Remote Sensing of Environment, Buenos Aires, AR
Loiselle, S. A., Canziani, G., Sabio, G., Rossi, C., "*The use of systems analysis methods in the sustainable management of wetlands*, 2001, Hydrobiologia, 458. 191-200, 2001

# 2B – New doped materials for technological applications

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## Aims

Characterization and optimization of the physico-chemical properties of materials with technological applications by putting into evidence the relationship between properties, synthesis method and doping.

## Results

Interesting results were obtained in the study of doped and undoped materials with the general formula $A_6B'_2B''_8O_{30}$ (A = Ba, B' = Ti or Zr, B'' = Nb or Ta), belonging to the tetragonal tungsten bronze-type family. These compounds display peculiar ferroelectric, electro-optical and non linear optical properties. The non monotonic trend of dielectric constant and resistivity with temperature indicates paraelectric-ferroelectric or ferroelectric-ferroelastic transitions. The combined use of spectroscopic (EPR, micro-Raman) and structural (X-ray powder diffraction and structural refinement with the Rietveld method) techniques allowed us to study $Ba_6Ti_2Nb_8O_{30}$ compound and its substituted derivatives $Ba_6Ti_{2-x}M_xNb_8O_{30}$ (M = Cr, Mn e Fe; x = 0.06 e 0.18) and $Ba_{6-x}Y_xTi_{2-x}Fe_xNb_8O_{30}$ (x = 0.18). The oxidation states of the doping ions were determined by EPR spectroscopy ($Fe^{3+}$, $Cr^{3+}$ and $Mn^{2+}$) and different charge-compensating defect equilibria, based on the creation of positive electron holes or oxygen vacancies and electrons, were proposed to explain the conductivity results and the thermoelectric power data, suggesting a negative charge transport. The results obtained for $Ba_6Zr_2Ta_8O_{30}$ compound and its substitution derivatives $Ba_6Zr_{1.94}M_{0.06}Ta_8O_{30}$ (M = Ni, Co, Fe, Mn, Cr) put into evidence the peculiar capability of the tungsten bronzes framework to behave as a glasslike host for the transition doping cations, that preferentially locate on the B'' site. The high degree of local distortion observed on the B'' site influences the oxidation state of the doping ions and can be responsible for the lower degree of substitution obtained for $Ba_6Zr_2Ta_8O_{30}$ with respect to the $Ba_6Ti_2Nb_8O_{30}$ analogue.
The study of undoped and doped $Ca_2Fe_2O_5$ ferrite puts into evidence new insights into its magnetic properties. The preparation of $Ca_2Fe_2O_5$, $Ca_{1.94}Na_{0.06}Fe_2O_5$ and $Ca_2Fe_{1.94}M_{0.06}O_5$ (M = $Mg^{2+}$, $Al^{3+}$, $Ti^{4+}$ and $Ge^{4+}$) through different synthesis routes (solid state and high energy ball milling) leads, in all cases, to the formation of $Fe_3O_4$ as impurity phase. The combined use of X-Ray powder diffraction technique, EPR

spectroscopy and magnetization measurements allowed to quantify the magnetite phase in each sample, as shown in Figure 1; the lowest magnetite amount (<1 wt%) is obtained in the undoped ball-milled and in the Mg doped samples. Our results support the hypothesis that the weak ferromagnetic component observed in $Ca_2Fe_2O_5$ ferrite can be due to the presence of $Fe_3O_4$ impurity phase.

For what concerns our research in the field of materials for applications in electrochemical devices, both anode and cathode materials were investigated. Undoped and Mn doped $Li_4Ti_5O_{12}$ anodic compounds were studied through X-ray powder diffraction, EPR and $^7$Li NMR-MAS spectroscopy techniques. It was demonstrated that Mn ions distribute in both tetrahedral and octahedral sites of the $Li_4Ti_5O_{12}$ cubic spinel framework. $Mn^{3+}$ ions are located on both the cationic sites, while $Mn^{2+}$ occupy only the tetrahedral one. The resulting cation distribution well explains the conductivity behaviour of the doped samples. $Mn^{2+}$ paramagnetic ions give rise to a through-space interaction with $Li^+$ ions of both cationic sites, as evinced by the NMR results. The investigation carried out on $LiFePO_4$ (triphylite) cathode material and on its substituted derivative $LiFe_{1-x}Mn_xPO_4$ (x = 0.03, 0.09 and 0.18) prepared via different synthesis routes (solid state, sol-gel) pointed out the best synthesis to obtain impurity free samples. X-ray powder diffraction and Rietveld structural refinement, EPR and micro-Raman spectroscopic techniques and magnetization measurements were used to verify that the sol-gel synthesis is the most suitable to obtain impurity free samples. In particular, some impurity phases that can worsen the electrochemical features of the material (oxide-based impurity phases, $Fe_xP$ or $Fe_xN$ alloys), are not present. The structural, magnetic and spectroscopic features are not influenced by Mn substitution and $Mn^{2+}$ ions successfully substitute the $Fe^{2+}$ ones in the triphylite structure. Our results suggest that the Mn substituted compound could be a suitable and potential candidate for the application in batteries technology.

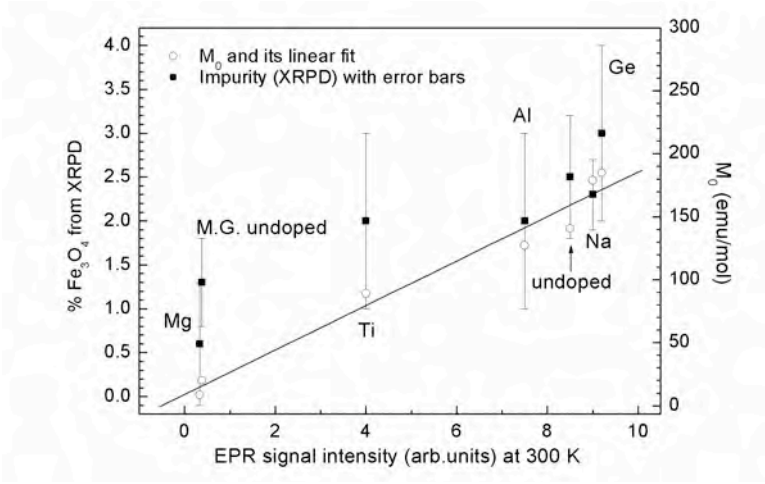


Figure 1 – Correlation between the EPR signal intensity (area), the $Fe_3O_4$ amount (wt %) from XRPD (left *y*-axis) and the $M_0$ values determined from magnetic measurements (right *y*-axis) for all the $Ca_2Fe_2O_5$ samples. A linear interpolation of the $M_0$ values *vs* the EPR signal intensity is also reported.

# 2B – Reactivity and Structure of Inorganic Compounds

*V. Berbenni, G. Bruni, P. Cofrancesco, C. Milanese, A. Marini*

## Aims

Study of the effect of mechanical activation by high energy milling on solid state reactions.

## Results

Solid state synthesis of ferrites of Ni ($NiFe_2O_4$), Ca ($CaFe_2O_4$ and $Ca_2Fe_2O_5$) and Mg ($MgFe_2O_4$) have been worked out by a combination of mechanical activation by high energy milling and by thermal annealing.
Mixtures of basic nickel carbonate [$2NiCO_3{\cdot}3Ni(OH)_2{\cdot}4H_2O$] and iron(II) oxalate dihydrate ($FeC_2O_4{\cdot}2H_2O$) have been used as precursor in the synthesis of $NiFe_2O_4$. Simultaneous TG/DSC measurements provided interesting hints on the reaction mechanism along with a reliable value of the reaction enthalpy for the formation of $NiFe_2O_4$ starting from the constituents oxides (NiO and $Fe_2O_3$). X-ray powder diffraction (XRPD) showed that the residual recovered from the thermoanalytical measurements is $NiFe_2O_4$ only when starting from the mechanically activated mixtures. In the patterns of the physical mixtures, only the peaks of $Fe_2O_3$ and NiO are present.

Samples on a mass scale of ≈ 1 g have been subjected to thermal treatments in oven. The results were as follows:

a) $NiFe_2O_4$ has been obtained by thermal treatment of 36h at 1100°C of precursors mixture not subjected to mechanical activation (XRPD evidence);
b) $NiFe_2O_4$ has been obtained from the mechanically activated mixture by annealing of 12h at 400°C (XRPD evidence);
c) Molar heat capacity has been measured and the results showed that the minimum treatment temperature to obtain $NiFe_2O_4$ starting from the milled mixtures is 450°C (DSC evidence);
d) The specific surface area of $NiFe_2O_4$ decreases by decreasing the annealing temperature from 450°C to 750°C (BET evidence)

A synthesis procedure of calcium ferrites ($CaFe_2O_4$ and $Ca_2Fe_2O_5$) has been proposed starting from the mechanically activated mixtures of organic precursors [calcium citrate tetrahydrate and iron (III) oxalate hexahydrate]. The TG analysis individuated the different stages of the mass loss process. The DSC analysis put into evidence that

the heat released is much higher than it was to be expected from the sum of the enthalpies associated to the processes occurring. Such an excess of heat released allowed to calculate the formation enthalpies of $Ca_2Fe_2O_5$ e $CaFe_2O_4$. XRPD showed that the activated mixtures give by 18h annealing at 750°C (or 800°C) $CaFe_2O_4$ (or $Ca_2Fe_2O_5$) while the same compunds can be obtained from the physical mixtures by prolonged thermal treatments (t >> 18h) at 1180°C ($Ca_2Fe_2O_5$) or at 1100°C ($CaFe_2O_4$).

The synthesis of $MgFe_2O_4$ has been performed starting from mixtures of basic Mg carbonate [$4MgCO_3{\cdot}Mg(OH)_2{\cdot}xH_2O$] and iron(II) oxalate dihydrate ($FeC_2O_4{\cdot}2H_2O$) subjected to mechanical activation by high energy milling and annealed between 400°C and 800°C.
TG measurements allowed to assess the reaction mechanism and the minimum temperature of formation of the constituent oxides (MgO e $Fe_2O_3$). XRPD evidence showed that Mg ferrite forms, though as an amorphous phase, by thermal treatment at 400°C of the activated mixture but that a thermal treatment at temperatures as high as 1200°C of a physical mixture does not result in the synthesis of $MgFe_2O_4$. DSC measurements provided molar heat capacity data and the Curie temperature of $MgFe_2O_4$ obtained from the milled mixture annealed at 600°C. Finally BET measurements allowed to establish the influence of the synthesis temperature on the specific surface area of $MgFe_2O_4$.

## *References*

Berbenni, V., Milanese, C., Bruni, G., Marini, A., *The combined effect of mechanical and thermal energy on the solid state formation of $NiFe_2O_4$ from the system $2NiCO_3{\cdot}3Ni(OH)_2{\cdot}4H_2O - FeC_2O_4{\cdot}2H_2O$*, Thermochimica Acta 2008, 469 [1-2], 86-90.

Berbenni, V., Marini, A., Bruni, G., Milanese, C., *Solid state formation of calcium ferrites from thermal decomposition of mixtures $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$-$Fe_2(C_2O_4)_3 \cdot 6H_2O$*, Journal of Analytical and Applied Pyrolysis 2008, 82 [2], 255-259.

Berbenni, V., Marini, A.., Milanese, C., Bruni, G., *The Effect of Mechanical Activation on the Synthesis of $MgFe_2O_4$ from Mixtures of $MgCO_3{\cdot}Mg(OH)_2{\cdot} xH_2O$ and $FeC_2O_4{\cdot} 2H_2O$*, Zeitschrift für Naturforschung B: Chemical Sciences 2008, 63b, 1052-1056.

# 2B – ToF-SIMS Characterization of Ancient Ceramics from the Quartaia Site (Italy)

*F. Benetti, A. Tognazzi, E. Mugnaini[1] , A. Magnani[1], C. Rossi*
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## Aims

Ceramic production is one of the oldest and extended human activities in all civilizations. The analysis of technological features (ceramic impasto and firing) and of attributes (shape, size, decoration, etc.) allows to understand the complexity of the productive process, degree of specialization and social complexity of the civilizations that create them. For example, the chemical-mineralogical composition of ceramic impasto (matrix, fillers and inclusions) is one of the parameters that contributes to characterize the technological level of a certain society that manufactured ceramic materials. There are many published papers focussed on the analysis of ceramic objects with several analytical techniques, such as AAS (Rotunno et al. 1997; Bower et al. 1975), TXRF, ICP-OES and ETAAS (Cariati et al. 2003), employed alone or integrated with other methodologies, e.g. EMPA, XRD, XRF, XAS and SEM (Feliu et al. 2004; Gliozzo et al. 2004 - I&II; Gliozzo et al. 2008).

## Results

ToF-SIMS analyses allow to estimate differences in chemical and morphologic features of a lot of kind of Cultural Heritage samples (Adams 1997, Adriens and Dowsett 2006, Spoto 2000). Moreover, through ToF-SIMS imaging, it was possible to investigate the distribution of inorganic and organic elements inside the ceramic impasto and the presence of inclusions of Ceramic samples belonging to the archaeological site of Quartaia (Tuscany, Italy)

A better visualization of ToF-SIMS data, in order to display the distribution of detected elements on the analyzed surface, was obtained by ToF-SIMS imaging. In Figure 1a the distribution of Li+, Na+, Mg+, Al+, Si+, K+, Ca+, Fe+ ions, as well as the total ion image, over an area of 200 x 200$\mu$m2 is reported. Moreover, this figure evidenced that Fe, Na, K, Mg elements hadn't an uniform distribution on the surface and they were present only in the inner of some inclusions, as shown in Figure 1b where is reported the overlay image for Fe+, Na+, K+ ions. In respect to chemical and morphologic features of ceramic impasto, we characterized two typologies of ceramics: non depurata and depurata.

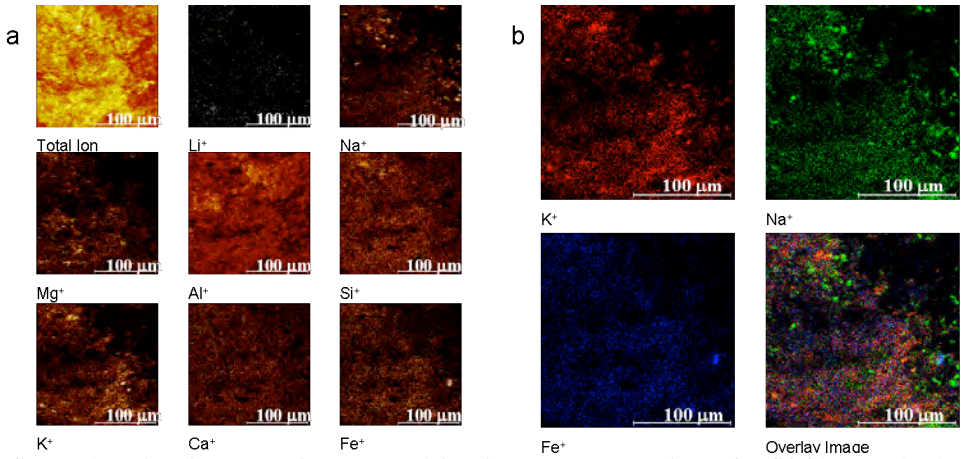



Fig. 1. a. - Secondary ion images of some positive ions over a section of US241 sample; b - Overlay image of the distribution of $K^+$, $Na^+$, $Fe^+$.

ToF-SIMS analyses result useful tool to characterize chemical and morphologic features of ceramics and, consequently, to define a classification of ceramic fragments on the basis of their production process.

## References

Adams, F., Adriaens, A., Aerts, A., De Raedt, I., Janssens, K., and Schalm, O., *Micro and Surface Analysis in Art and Archaeology Plenary Lecture*, Journal of Analytical Atomic Spectrometry 1997, 12, 257-265.

Adriaens, A. and Dowsett, M.G., *Applications of SIMS to cultural heritage studies*, Applied Surface Science 2006, 252, 7096-7101.

Cariati, F., Fermo, P., Gilardoni, S., Galli, A., and Milazzo, M., *A new approach for archaeological ceramics analysis using total reflection X-ray fluorence spectrometry*, Spectrochimica Acta Part B 2003, 58, 177-184.

Feliu, M.J., Edreira, M.C., and Martìn, J., *Application of physical-chemical analytical techniques in the study of ancient ceramics*, Analytica Chimica Acta 2004, 502, 241-250.

Gliozzo, E. and Memmi Turbanti, I., *Black gloss pottery: production sites and technology in Northern Etruria, Part I: provenance studies*, Archaeometry 2004, 46, (2) 201-225.

Gliozzo, E., Kirkman, I.W., Patos, E., and Memmi Turbanti, I., *Black gloss pottery: production sites and technology in Northern Etruria, Part II: gloss technology*, Archaeometry 2004, 46, 227-246.

Gliozzo, E., Vivacqua, P., and Turbanti Memmi, I., *Integrating archaeology, archaeometry and geology: local production technology and imports at Paola*, Journal of Archaeological Science 2008, 35, 1074-1089.

Rotunno, T., Sabbatini, L., and Corrente, M., *A provenance study of pottery from archaeological sites near Canosa, Puglia*, Archaeometry 1997, 39, (2) 343-354.

Spoto, G., *Secondary ion mass spectrometry in art and archaeology*, Thermochimica Acta 2000, 365, 157-166.

# 2B – Hydrogen Storage in nanomaterials (NANOSTORE)

*P. Matteazzi*

## Aims

The goal of the project is to work out the bottlenecks hampering hydrogen production (supply) and solid storage as fuel for a widespread mobile use.

## Results

An innovative approach to this field has been developed through the following steps:

- improvement of the efficiency of common industrial electrolyzers/fuel cells in order to reduce energy waste and commercial costs and development of a small cell/ demonstrative prototype electrolyzer;
- design and development, through combinatory methods, of innovative nanomaterials for solid hydrogen storage synthesized by mechano-chemical processing (high energy milling) and manufacturing of a prototype tank,
- manufacturing and engineering of a demonstrative device for mobile use which integrates both the tank and the cell prototypes.

CSGI (Udine unit) takes the role of project coordination and is involved mainly in material design and development as well as in activities regarding the setting-up of prototype tank. Nanomaterials compounds have been designed and produced by modifying the composition and process parameters in order to change their thermodynamic and kinetic behavior in the hydrogenation reaction and to develop innovative synthesis paths by high energy ball milling process [1-3]. In order to tailor suitable materials for hydrogen mobile storage, the following selection criteria were determined (according to European SRA targets): good reversibility, $H_2$ storage capacity, low release temperature with suitable kinetic at atmospheric pressure, fast absorption kinetic at suitable temperature.

Mg-Ni and Mg-Ni-C(graphite) based materials have shown the best properties in terms of gravimetric capacity, adsorption and desorption rates. Gravimetric capacity greater than 6.5% and desorption and absorption rates compatible with a fuel cell powered engine vehicle were achieved [4-5].
A pre industrial storage tank demonstrator was realized as well as the related control equipment [6]. Tank performances are currently under evaluation.

## References

[1] Zaluski L., Zaluska A, Strom-Olsen J.O, "*Hydrogenation properties of complex alkali metal hydrides fabricated by mechano-chemical synthesis*", J. of All. and Comp. 290 (1999) 71-78

[2] Bobet J.L, Grigorova E, "*Hydrogen sorption properties of graphite-modified magnesium nanocomposites prepared by ball-milling*", J. All. and Comp. 366 (2004)298-302:

[3] Borislav Bogdanovic, Gary Sandroc, "*Catalyzed Complex Metal Hydrides*", MRS Bullettin September 2002, 712-716.

[4] Milanese C., Girella A., Bruni G., Cofrancesco P., Berbenni V., Marini A., Villa M., Matteazzi P., *Hydrogen Storage in Magnesium – Metal Mixtures: Reversibility, Kinetic Aspects and Phase Analysis*, Journal of Alloys and Compounds; Volume 465, Issues 1-2, 6 October 2008, Pages 396-405;.

[5] Bououdina M., Grant D., Walker G. *Review on hydrogen absorbing materials—structure, microstructure, and thermodynamic properties* International Journal of Hydrogen Energy, Volume 31, Issue 2, February 2006, Pages 177-182

[6] Verga M., Armanasco F., Guardamagna C., Valli C., Bianchin A., Agresti F., Lo Russo S., Maddalena A., Principi G., S*caling up effects of Mg hydride in a temperature and pressure-controlled hydrogen storage device* International Journal of Hydrogen Energy, In Press, Corrected Proof, Available online 11 October 2008

# 2B – HYDRONANOPOL: Advancement in storage capability and Hydrogen kinetics of hydride storage alloys through nanocoating with multifunctional hybrid polymer

*P. Matteazzi*

## Aims

The objective of this project is the replacement of environmental harmful Nickel Cadmium batteries (NiCd) by Nickel metal hydride batteries (NiMH), also for high-current applications.

## Results

The objective of Hydronanopol is aligned with EU legislation started to ban the heavy metals like Cadmium from the batteries. Main project outputs can be summarized:

- Development of nanocoating material for hydrogen storage alloy applications;
- Basic understanding of dissolving and electrochemical processes of coating materials;
- Basic understanding of the coating behavior of new developed coating materials;
- Demonstration of NiMH batteries which are able to replace NiCd batteries in the future.

Reducing crystals down to the nano-size allows to increase interphases and to include more active sites for the electrochemical reaction, thus improving both activity and performances. Besides this a suitable hybrid polymer material (ORMOCER®) that is able to prevent oxygen access to the surface of the powder in dry state, it was employed as protective film on powder particle surfaces. Additionally, when this system is wetted with an alkaline electrolyte and gives a gel like consistency, it will improve the ionic conductivity of the hybrid polymer material.

More than twenty materials, different for composition and manufacturing process, were widely analyzed through the employment of SEM, XPS, EDS, XRD and TEM techniques. These systems were also investigated to determinate electrochemical properties like impedance, double layer capacitance, active electrode area, hydrogen transport and electrical power capacity.

“Button battery” prototypes were produced and tested demonstrating excellent performance. Electrical power capacity is resulted to be better than the existing standard NiCd batteries especially when working at very low temperature (below 0°C) and high discharge rates.

CSGI is responsible for the design, investigation and selection of nanostructured materials performing analytical measurements like XRD, SEM, particle size distribution, and hydrogen storage capacity determination.

## References

[1] Percheron-Guégan A., Achard J. C., Sarradin J. Bronoël and G., *Electrode material based on lanthanum and nickel, electrochemical use of such materials*, Fr. Pat. 75 161160, 1975; 7723812, 1977; US Pat.688537, 1978

[2] Gupta M. and Schlapbach L., E*lectronic properties in Hydrogen in Intermetallic Compounds I*, ed. L. Schlapbach, Springer-Verlag, 1988, vol. 63, p. 139

[3] Popall M., Buestrich R., Semrau G., Eichinger G., Andrei M., Parker W. O., Skaarup S. and West K., *New polymer lithium secondary batteries based on ORMOCER® electrolytes–inorganic–organic polymers* Electrochim. Acta 46 (2001) 1499

# 2B – New generation of aeronautical bearings for extreme environmental constraints. BEARINGS

*P. Matteazzi*

## *Aims*

Main objective of the project is to propose a new generation of bearings, highly reliable, able to answer the new and extreme constraints on aircraft.

## *Results*

This technological breakthrough is being achieved proposing an innovative technology approach that includes both scientific and technological aspects as:

- selection and development of new nanostructured materials tailored for the strong requirements needed, and their associated processes (high energy milling, plasma spraying and bulk consolidation) designed to keep the nanomaterials advanced properties [1,2];
- understanding of bearings degradation mechanisms of the behaviour of materials under extreme conditions (temperature, tribological in pressure + shearing conditions) through a coupling between modelling and expertises [3];
- understanding the mechanical, physical, chemical conditions leading to the Superficial Tribological Transformation within the skins of bodies in contact.

In the last year 4 different material systems were developed for both bulk and coating applications. In particular hot compaction was performed by several methods as conventional hot (indirect heating) pressing rapid induction coil heated pressing, as well as Spark Plasma Sintering which it is expected to keep nanostructures as good as possible due to short exposition of heating. All materials have been investigated in terms of density, hardness, microstructure and friction and wear properties on a specifically designed test bench to simulate the real behavior conditions of bearing part. Significant improvements on key target performances compared to the actual material have been found.

CSGI is responsible for the selection and development of nanostructured materials and it is also involved in the management of the project coordinating as work package leader in the WP3: “ Tribological material definition and transfer on sample”.

## References

[1] Ashby MF, "*Materials Selection in Mechanical Design*", 2nd edition, Butterworth Heinemann, Oxford, UK, 1999.

[2] Arcuri F., Matteazzi P. *"Mechanosynthesis of P/M Nanocomposite Hardmetals"*, International Journal of Powder Metallurgy, Vol. 39, No.1, 2003, pp. 47-52.

[3] Rocchi J., *Définition d'un problème modèle à partir d'analyses tribologiques : cas d'un contact de géométrie conforme*. Diplôme d'Etude Approfondie en Génie Mécanique, Lyon : INSA de Lyon, 2001, 80 p.

# 2B – MANUDIRECT-Direct ultraprecision manufacturing

*P. Matteazzi*

## Aims

The objective of the project is provide the manufacturing industry with an entirely new platform for manufacturing, by the way of high productivity-high resolution direct, one step, laser sintering using metals and ceramic materials.

## Results

This objective represents a key factor for future competitivity of EU based manufacturing industry. The new Direct Manufacturing Platform will integrate:

- capacity of production of different powders grades by high energy milling developed for this technology [1,2];
- new methodologies for design and manufacturing of components;
- machine equipped with highly localized powder and laser fluxes capable of high productivity rate combined with spatial resolutions in the scale of 50 mm (well beyond the state of the art) [3];
- innovative monitoring tools and control software.

The impact of the project and its success will be horizontal to many sectors and vertical in engineering methodologies. Virtual engineering concepts and design will be translated into products without the need of prototyping steps. Economic impact can be evaluated for some sectors, for example microengineering, biomedical and others. But the impact is much wider, because it open the way to direct macroscale fabrication.

A first group of activities are focused on materials and components design. Tailored nanophased powders (metal based and ceramic reinforced nano-composites) were synthesize according to industrial case studies requirements. Material composition design has been carried out by the innovative Ashby approach.

The second group of activities have been developing different components of the platform (powder feeder, powder focusing device, control SWs, geometrical monitoring systems, CAD-CNC SW) and assembling them in the first industrial machine prototype.

CSGI takes the main management role as coordinator of the project, leader of the Technical Board and of the Project Board. It is also involved directly in the research activity contributing to material design, to the building up of the material data management software (WP1) and to nanomaterials characterization (powders and consolidated materials) as leader of WP2.

## References

[1] Czerner S., Stippler P., Ostendorf A. and Matteazzi P., *Micro-laser sintering (microls) for rapid prototyping of metal-ceramics*, Proceeding of the 2nd Pacific International Conference on Application of Lasers and Optics 2006, edited by Milan Brand and Erol Harvey

[2] Matteazzi P., Czerner S., Becker H.; Stippler P., Ostendorf A.; *High Resolution Laser Sintering Using Agglomerated Nanophased Powder*s, ISNM2005, Cyprus November 3-5, 2005

[3] Becker H., Ostendorf A., Stippler P., Matteazzi P., *Direct laser micro sintering using nanophased microscale powders*, Euro-u.Rapid 2005; 10-12 May 2005.; Leipzig, Germany

# 2B – New Manufacturing Chain based on nanocomposite polymers for the Italian Textile-Footwear business field. FIRB-Nanocomp

*P. Matteazzi, P. Baglioni*

## Aims

The project is focused on the research and development of new polyamide based nanocomposites addressed to the Italian manufacturing industry.

## Results

In order to produce end products with innovative features as polyamide yarns (textile industry), plastic component (sport wear goods), the improvement of several material key properties is needed: better thermal and electrical conductivity, UV resistance and Elastic modulus.

Starting from these industry demands, the project proposes a radical step ahead towards the development of new nano-additives/fluids and nano-dispersions (to be used as precursors) and hence new polymer composites. NANOCOMP proposes two different manufacturing approaches in order to make innovation in the polymer field:

a) the thermo-chemical conventional route;

b) the mechano-chemical approach (innovative and alternative technology) based on the High energy milling approach.

Through the employment of these synthesis technologies and routes it will be possible to act at nano-scale level, improving polymer physical-chemical properties and mechanical performance (respect existing polyamide based matrixes).

The following two alternative synthesis approaches are studied and investigated in NANOCOMP:

(1) The development/modification of nanocharges/precursors followed by polimerization;

(2) the introduction and compatibilization of precursors into polymer matrixes followed by compounding.

As concerns the first synthesis approach (1), on the basis of literature data, chemical-physical properties and cost, were selected several nanocharge systems to be developed:

- nanoclays, alumina, silica (to improve mechanical properties)
- copper and silicon carbide (to improve electrical and thermal conductivity)
- zinc oxide (to improve UV shielding behaviour)

The precursor systems have been hence produced/modified at nano scale level, both by chemical synthesis (as nanoparticles produced by thermo-chemical approach) and mechano-chemical synthesis (as compatibilized nanostructured systems).

The developed precursors were hence introduced -approach (2)- in proper momomeric/polymeric matrixes (modified caprolactam and polyamides) both via direct intercalation of precursor into the caprolactam (chemical route) and via high energy milling (mechano-chemical route) obtaining two types of matrixes:

a) hybrid organic-inorganic precursors constituted by nanocharges in caprolactam matrix that are successively polymerized in a reactor;
b) hybrid nanostructured organic-inorganic composites suitable to being treated in the compounding process.

The work activity performed in this beginning stage, allowed the setting-up of the manufacturing processes and the development of the firsts sample prototypes. Outcomes, related to the improvement of mechanical properties and thermal conductivity, are already very promising.

## *References*

(1) Archie P. Smith, Richard J. Spontak, Harald Ade, *On the similarity of macromolecular responses to high-energy processes: mechanical milling vs. irradiation*, Polymer Degradation and Stability 72 (2001) 519-524

(2) J.Pan, W.J.Shaw, *Effects of Processing Parameters on Material Properties of Mechanically Processed Polyamide*; Journal of Applied Polimer Science, Vol.56, 557-566 (1995)

(3) C. M. Balik, C. Bar, C. C. Koch, R. J. Spontak, C. K. Saw, *Mechanical alloying of pet and pet/vectra blends*; Morphological Control in Multiphase Polymer Mixtures, MRS Symp. Proc. vol. 461, (1996)

(4) Chi-Ming Chan, Jingshen Wu. Jian-Xiong Li, Ying-Kit Cheung, *Polypropylene/calcium carbonate nanocomposites*, Polymer 43 (2002) 2981-2992;

(5) Andrea Sorrentino, Giuliana Gorrasi, Mariarosaria Tortora, Vittoria Vittoria, Umberto Costantino, Fabio Marmottini, Franco Padella, *Incorporation of Mg-Al hydrotalcite into a biodegradable Poly(e-caprolactone) by high energy ball milling*; Polymer 46 (2005) 1601-1608

(6) Mukul Biswas, Suprakas Sinha Ray, *Recent Progress in Synthesis and Evaluation of Polymer Montmorillonite Nanocomposites*; Advances in Polymer Science, Vol. 155 © Springer-Verlag Berlin Heidelberg 2001

(7) Mikko Karttunen and Pekka Ruuskanen, *The Microstructure and Electrical Properties of Mechanically-Alloyed Copper-Polymer Composites*; Materials Science Forum Vols. 269-272 (1998) pp 849-852

(8) La. Eastman, U.S. Choi, S.Li, L.J. Thompson, And S. Lee, *Enhanced Thermal Conductivity Through The Development Of Nanofluids,* Materials Research Society; Vol. 457 1997.

# 2C – Efficient gene silencing in Escherichia coli by means of antisense RNAs

*A. Hochkoeppler, A. Stefan*

## Aims

The antisense RNA strategy can be used in prokaryotes in order to regulate the expression of specific genes. Only few data are known about the determinants leading to effective artificial antisense molecules. In order to identify new structural elements essential for the silencing efficacy in *E. coli*, different aRNAs against the *lacZ* or the *dnaQ* mRNA have been tested *in vivo* and *in vitro*.

## Results

Silencing of *lacZ*.

We used different aRNAs to silence *lacZ* in *E. coli* in order to identify the determinants of silencing competence and the mechanism of suppression. In particular, we analyzed asRNA directed against the regulatory and/or the coding region of the target gene. Moreover, we also evaluated the size to which an effective asRNA can be shortened. The effectiveness of silencing was studied by Western blot, Northern blot and β-galactosidase assay.

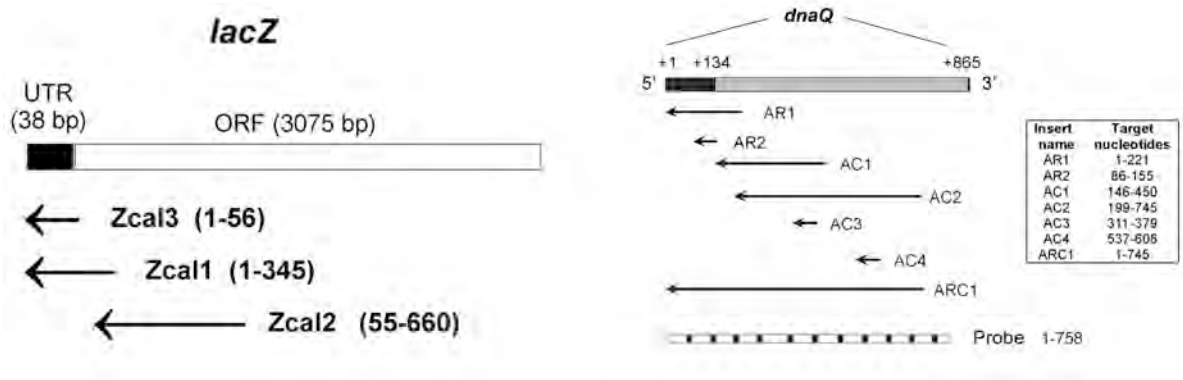
Our results can help to design antisense RNAs that can trigger conditional silencing of a target gene. We have identified some structural and functional elements conferring silencing competence: 1) a target gene can be repressed strongly with an appropriate asRNA (up to 70%); 2) the 5' UTR and the bases spanning the translational start are suitable targets for an antisense molecule; 3) silencing by aRNAs can be exerted by reducing the amount of the mRNA concentration; 4) the *in vivo* stability of an antisense RNA is a crucial parameter for determining the silencing effectiveness; 5) the interaction between antisense RNA and the ribosome can greatly enhance their stability and their performance; 6) short and effective antisense RNAs can be designed (50-60 bases).

Silencing of *dnaQ*.

The *Escherichia coli* gene *dnaQ*, coding for the epsilon subunit of DNA polymerase III, was suppressed *in vivo* via antisense RNAs. In particular, different fragments of the target gene were cloned in reverse orientation and conditionally expressed in bacterial cultures. We have studied the *in vivo* stability of the antisense molecules, showing that their concentration is positively correlated to their silencing effectiveness. Moreover, we have also analyzed *in vitro* the hybridization rate between the asRNA and the target *dnaQ* mRNA.

Our results suggest: 1) the capability of an antisense RNA to bind rapidly the cognate mRNA *in vitro* does not necessarily imply its effectiveness in silencing that gene *in*

*vivo*; 2) the performance of the same antisense transcript dipends on the 5' leader sequence conferred by the vector used for its expression; 3) the *in vivo* stability of an antisense RNA could strongly affect its silencing competence.
Our observations reported about the silencing of *dnaQ* gene are in agreement with those found with the *lacZ* gene.




Figures: aRNAs directed against different region of *lacZ* and *dnaQ* gene.

## References

Stefan, A., Tabler, M., Hochkoeppler, A., *Efficient silencing of the gene coding for the epsilon subunit of DNA polymerase III in Escherichia coli is triggered by antisense RNAs featuring stability in vivo*, FEMS Microbiology Letters 2007, 270, 277-283.

Stefan, A., Tonelli, A., Schwarz, F., Hochkoeppler, A., *Artificial antisense RNAs silence lacZ in E. coli by decreasing target mRNA concentration,* BMB Reports 2008, 41, 568 – 574.

# 2C – Over-expression of recombinant proteins in Escherichia coli

*A. Hochkoeppler, A. Stefan*

## Aims

The aim of this study is to develop an efficient expression system in bacteria in order to produce high level of target proteins. *Escherichia coli* is the most widely host used for the production of recombinant proteins. Many different expression systems are commercially available differing for the strength and the type of induction. Our study is also focused on the recovery of the target protein in a native and active form.

## Results

Production of recombinant interferons molecules.

The overexpression of four different interferons, i.e. murine interferon-$\alpha_1$ and human interferons $\alpha_1$, $\alpha_8$, and $\alpha_{21}$ was challenged in *Escherichia coli*. Synthetic genes coding for these interferons were designed, assembled, and cloned into the vector pET9a (using the NdeI and BamHI sites), placing interferon expression under the control of phage T7 promoter. We used different bacterial hosts in order to verify the production of the different proteins.

The interferon (IFN) family is a complex group of proteins, which represents the most physiologically important defence of mammalians against infections; at present, some human interferons have pharmaceutical application, including human recombinant interferon alpha 2a (Hu-rIFNα2a), human interferon alpha 2b (Hu-IFNα2b) and human natural leukocyte. A number of systems have been used to challenge the over-expression of interferons (IFNs) in *E. coli*. In particular, high-level expression of human interferon-$\alpha_{2b}$ (HuIFN-$\alpha_{2b}$) was observed while attempts to over-express HuIFN-$\alpha_1$ or HuIFN-$\alpha_8$ in *E. coli* corresponded to low yields. Since the differences observed among the expression of these HuIFNs in *E. coli* appear surprising we tried to investigate the reason(s) for the low-expression yields featured by some type of interferons. In particular, we compared the efficiency of BL21AI and BL21(DE3) expression systems.

Production of recombinant mustard trypsin inhibitor 2 (MTI2).

The mustard trypsin inhibitor II (MTI2) was identified in mustard seeds (*Sinapis alba*); the sequence of MTI2 has been determined and the protein consists of 63 residues with 8 cysteines presumably organized in 4 disulfide bridges. MTI2 is effective against Lepidoptera as resulted in experiments where insect larvae were fed on transgenic plants expressing MTI2. In our study, MTI2 was overexpressed in

different strains of *Escherichia coli* and we also studied in detail each step of the purification process in order to identify the most efficient procedure for its recovery in an active form.

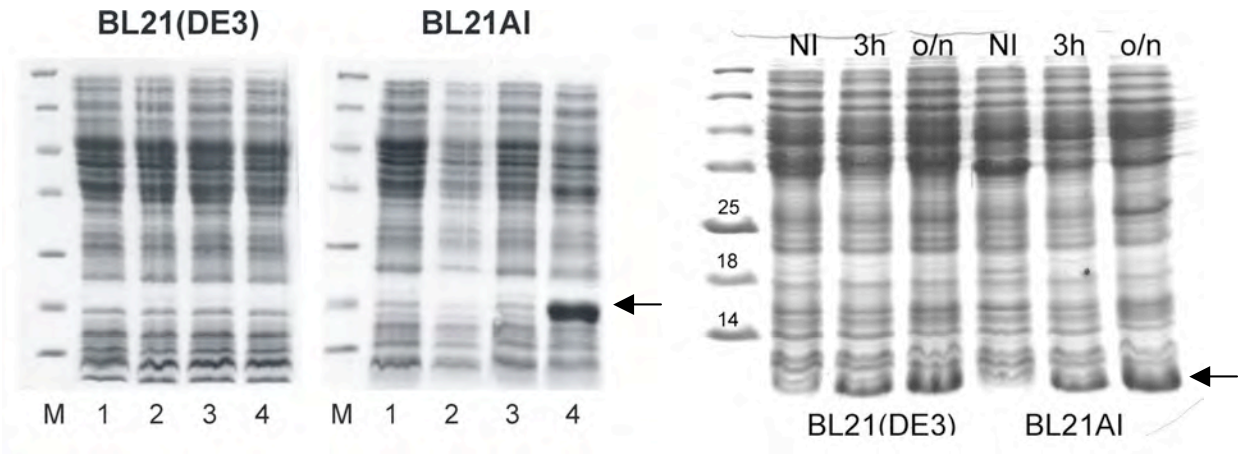



Figure. Expression of recombinant MuIFNα (left) and MTI2 (right).

## *References*

Stefan, A., Ugolini, L., Martelli, E., Palmieri, S., Hochkoeppler, A., *Expression and purification of the recombinant mustard trypsin inhibitor 2 (MTI2) in Escherichia coli*. Submitted.

# 2C – Study of the DnaQ protein (epsilon subunit of the DNA Polymerase III enzyme)

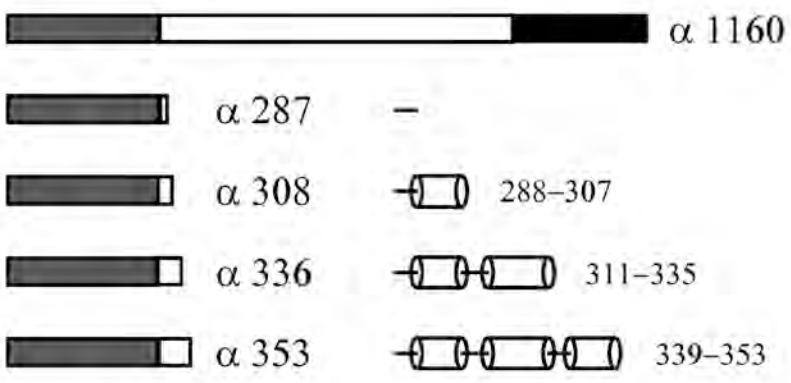
*A. Hochkoeppler, A. Stefan*

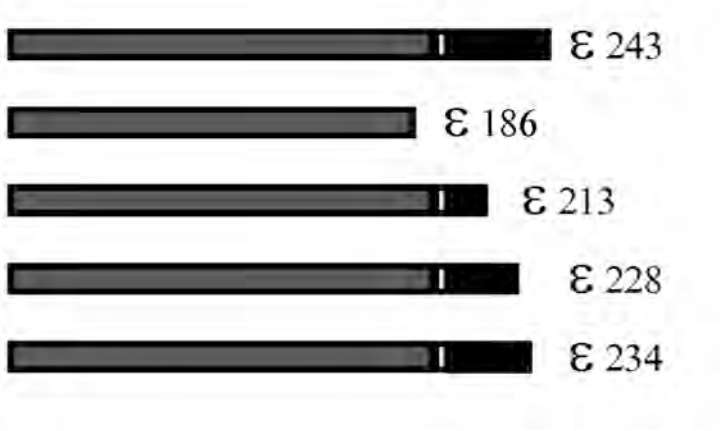
## Aims

The DnaQ protein is responsible of the proofreading activity of the bacterial replicative enzyme. Mutations on this gene lead to a strain with an increased spontaneous mutation frequency. Such strains, called mutators, represent a useful tool for the evolution of bacterial populations and for the directed evolution of target proteins.

## Results

DNA polymerase III is responsible for genome replication. This enzyme is composed of 10 subunits, of which DnaE and DnaQ (also denoted as α and ε) bear DNA extension and proofreading activities, respectively. The catalytic core of *E. coli* DNA polymerase III is composed of three different subunits, i.e α, ε, and θ, respectively coded by *dnaE*, *dnaQ* and *holE* genes. Our research is focused on the study of the assembly of the genome-replicating enzyme and, in particular, we have investigated the DnaE-DnaQ interactions and all the factors involved (chaperones, proteases, etc).
In order to study the association between DnaE and DnaQ proteins, we have designed different truncated forms of them. Upon expression in bacterial cultures, we have studied the occurrence of their interaction and the stability *in vivo* of the over-expressed proteins. In particular, protein extracts were subjected to gel filtration, activity determination, and western blotting analysis. Our observations demonstrate that the C-terminal region of *E. coli* DnaQ is sensitive to proteolysis and contains the residues essential for the association with DnaE.






Truncated forms of DnaQ (bottom) and DnaE (top) proteins.

# 2C – Biodiesel from immobilized lipases

*A. Salis, M.C. Pinna, M.S. Bhattacharyya, V. Solinas (Dip. Scienze Chimiche, Univ. Cagliari), M. Monduzzi*

## Aims

Production of biofuels through biotechnological green processes

## Results

Biodiesel is a natural substitute of diesel fuel that comes from renewable sources, such as vegetable oils thus, it does not contribute to the greenhouse effect being $CO_2$–neutral. According to the Kyoto protocol several countries have decided to partially substitute fossil fuels with biofuels, thus leading to a very fast growth of the global biodiesel industry in these years. By the chemical point of view, biodiesel is a mixture of fatty acid methyl esters (FAME) that are obtained by alcoholysis of triglycerides. The industrial reaction for biodiesel production uses homogeneous alkaline catalysis. It is highly energy consuming and gives rise to the formation of several by-products which necessitate of complicated and expensive purification processes. These and several other drawbacks go toward the direction of partially delete the benefits related to the use of biofuels.

The present project is aimed at developing a new green biocatalytic process for biodiesel production. The biocatalyst is obtained through immobilisation of a lipase on a porous support.

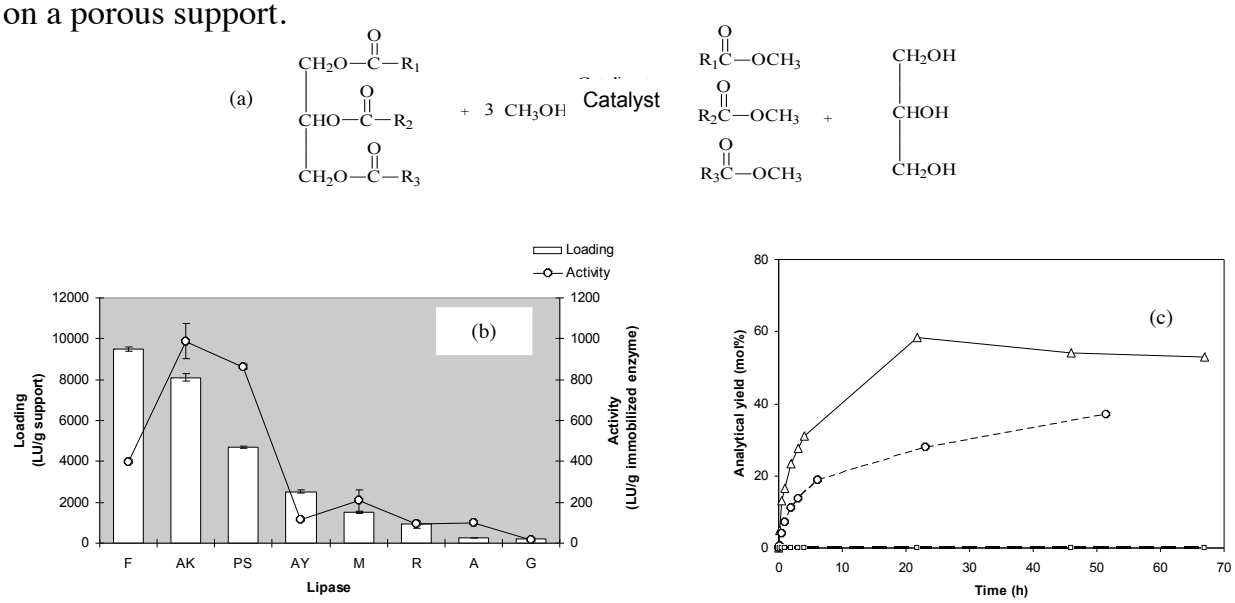



Figure 1. (a): Reaction of triglycerides methanolysis for biodiesel synthesis. (b): Loading and activity of commercial lipases immobilised onto macroporous polypropylene. (c): Screening of immobilised lipases on macroporus polypropylene toward biodiesel synthesis (t = 40°C). Lipase AK (Δ); lipase PS (□); lipase M, lipase F, lipase AY, lipase R, lipase A, and lipase G (□).

Lipases (E.C: 3.1.1.3) are able to catalyse, at low temperature and atmospheric pressure, biodiesel synthesis in solvent free media composed only by a mixture of the reagents giving exclusively FAME and glycerol. The products are easily separated due to their reciprocal immiscibility. Porous materials permits the dispersion of enzyme molecules in a high surface area, allowing to a bigger number of enzyme molecules to express their catalytic potentiality. Moreover they increase enzyme stability, permit biocatalyst reuse and facilitate its handling. Eight commercial lipases were immobilised on macroporous polypropylene via physical adsorption. The lipases showed a different level of adaptation to the support as determined by the comparison of their catalytic efficiencies (activity/loading). The immobilised lipases were compared toward methanolysis of vegetable oil to obtain biodiesel in solvent free conditions. Immobilised *Pseudomonas fluorescens* lipase (AK) was the most active biocatalyst, followed by immobilised *Pseudomonas cepacia* lipase (PS), whereas all the other microbial lipases were inactive toward biodiesel synthesis. Under the optimal conditions, i.e. t = 30°C, water content = 0.5 mg water/mg of biocatalyst, loading = 600 mg lipase AK/g support, an ester yield of 98 mol% after 70 h was obtained. Next step was the study of the influence of the support surface on the loading and the enzymatic activity of immobilised lipase AK. Different porous materials, polypropylene (Accurel), polymethacrylate (Sepabeads EC-EP), silica (SBA-15 and surface modified SBA-15), and an organosilicate (MSE), were used as supports. The immobilized biocatalysts were compared towards production of biodiesel (sunflower oil ethanolysis). Since the supports have very different structural (ordered hexagonal and disordered) and textural features (surface area, pore size, and total pore volume), in order to consider only the effect of the support surface, experiments were performed at low surface coverage. The different functional groups occurring on the support surface allowed either physical (Accurel, MSE, and SBA-15) or chemical adsorption (Sepabeads EC-EP and SBA-15--R-CHO). The surface modified SBA-15 (SBA-15--R-CHO) allowed the highest loading. The lipase immobilized on the MSE was the most active biocatalyst. However, in terms of catalytic efficiency (activity/loading) the lipase immobilized on the SBA-15, the support that allowed the lowest loading, was the most efficient.

## *References*

Salis, A., Monduzzi, M., Solinas, V., Use of lipases for the production of biodiesel, In Industrial enzymes. Eds. J. Polaina, A. McCabe, Springer (2007) 315-337.

Salis, A., Pinna, M., Monduzzi, M., Solinas, V., *Comparison among immobilised lipases on macroporous polypropylene toward biodiesel synthesis* Journal of Molecular Catalysis B: Enzymatic 2008, 54, 19-26.

Salis, A., Bhattacharyya, M.S., Monduzzi, M. and Solinas, V., *Role of the support surface on the loading and the activity of Pseudomonas fluorescens lipase used for biodiesel synthesis* Journal of Molecular Catalysis B: Enzymatic 2009, In Press.

# 2C – Formulation and utilization of Organogels in Biotecnological processes

*A. Ceglie, G. Colafemmina, F. Venditti, L. Ambrosone, F. Lopez*

## Aims

This project is aimed to the formulation of new organogels and their potential applications in biotechnological fields.

## Results

Reverse micelles have been used to solubilize a variety of biopolymers in apolar solvents with a low water content. In particular, the effect of gelatin solubilization on the rheological behavior of water-in-oil (w/o) microemulsions had a deep impact in several biotechnological processes. It was demonstrated that when solid gelatin is dispersed in reverse micelles at high enough water loading, a moderate warming followed by a careful decrease in temperature leads to the jellification of the whole sample, which remains fully transparent but becomes solidlike. These systems, often referred to as microemulsion-based gels or MBGs, were of outstanding importance in biotechnology because they can easily trap enzymes, retaining their catalytic activity, in organic solvents. In particular the new technology is dealing with supports that allow high activity and high stability of products coexisting with low costs. Inside this gels, different kinds of biomolecules or dyes may be entrapped. Furthermore, on the applied research perspective, the formulation of new biocompatible organogels could be very promising in the field of biotechnological food applications.

Almost all the reported MBGs were prepared using reverse micelles made of bis-2-ethylhexylsodiumsuccinate (AOT), an anionic surfactant. Few exceptions are represented by microemulsions based on other anionic and nonionic surfactants.

In the present project, we demonstrate that MBG can be made also in a w/o microemulsion stabilized by the cationic surfactant cetyltrimethylammonium bromide (CTAB). In particular the partial phase diagram has been determined for the CTAB/water/hexane/pentanol system stabilized by gelatine. The self diffusion coefficient of water raises proportionally with $W_0$.

The scenario confirming the idea that also this organogel based on cationic surfactant consists of a network of rigid road of water and gelatine surrounded of surfactant and cosurfactant in equilibrium with the water droplets of the microemulsion.

The novel microemulsion based organogel (MBG) prepared with cationic surfactant cetyltrimethylammonium bromide (CTAB) was used as support for the immobilization of lipase from *Candida rugosa*. In this study, we found that lipase entrapped in this system is able to catalyze the esterification reaction of pentanol with caprylic acid in hexane. The maximal pentyl caprilate production of about 94% was

reached in about 215 h. Recycling the immobilized enzyme was shown to be feasible, demonstrating that lipase retains its activity for several cycles. Remarkably, the enzymatic activity of lipase immobilized in the cationic MBG remained almost stable at 30 °C for at least 2 months.
Since highly reproducible results and quantitative production of pentyl caprylate was obtained at low costs, our data suggest that the novel CTAB microemulsion based gel represents a reliable tool for biotechnological applications and a commercially attractive system. The devised matrix may be generally applicable to other biologically interesting reaction systems, i.e. to bioconversion processes in organic solvents.

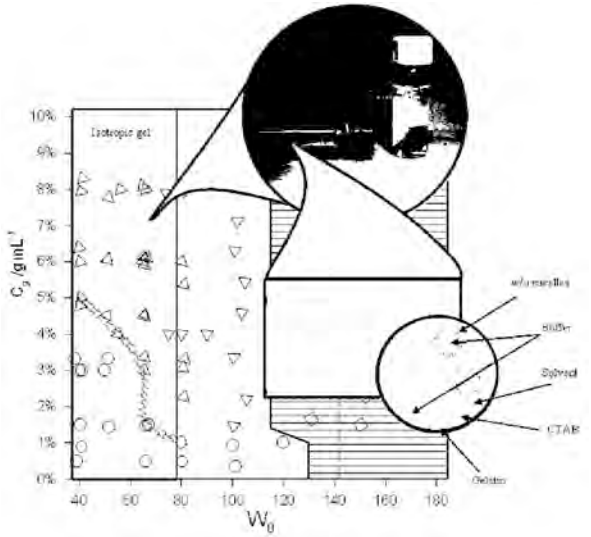



Fig.1 Schematic representation of the partial phase diagram for the CTAB MBGs. Circles, triangles and diamonds represent liquid, gel and two-phase systems, respectively. Samples at $W_0 < 78$ are optically isotropic (grey panel on the left). In the upper inset the optimal range of the gel and the aspect of the sample after preparation and before the solvent evaporation is reported. In the inset showed below the representation of the model proposed by Atkinson is reported

## *References*

LopezF., Venditti, F., Cinelli, G., Ceglie, A. *The novel hexadecyltrimethylammonium bromide (CTAB) based organogel as reactor for ester synthesis by entrapped candida rugosa lipase*. Process Biochemistry 2006, 41, 114-119.

Lopez, F., Venditti, F., Ambrosone, L., Colafemmina, G., Ceglie, A., Palazzo, P., *Gelatin Microemulsion-Based Gels with the Cationic Surfactant Cetyltrimethylammonium Bromide: A Self-Diffusion and Conductivity Study*. Langmuir 2004, 20, 9449-9452.

# 2C – Physico-Chemical Properties of Pharmaceutical Systems

*G. Bruni, V. Berbenni, C. Milanese, A. Girella, P. Cofrancesco, G. Bellazzi[1], A. Cardini [2], A. Marini*
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## Aims

The research activity in the pharmaceutical field propose developing methods for the resolution of problems related to the preformulation phase of active principles. The topics of interest are: polymorphism and stability of drugs in the solid state, host-guest systems, drug-excipient compatibility, crystallinity degree and phase diagrams characterization. In particular, in the years 2007-2008 the efforts have been directed towards the physico-chemical characterization of the solid state of pharmaceutical compounds with special care to the detection of different structural forms and to their relative stability and inter-conversion.

## Results

D-mannitol is a hexa-hydric alcohol and one of the most common excipients in pharmaceutical lyophilized products due to its tendency to crystallize from properly cooled aqueous solutions and to the relatively high melting point of the eutectic mixture mannitol/ice. These features end in obtaining a stable and good-looking solid as it is required for a pharmaceutical product. Actually some problems are still unresolved such as the generation of pressure inside the reaction vial that causes its breakage during the freeze-drying process and consequently considerable economic losses for industries. Over the years several authors individuated a number of polymorphs and differently named some of them that, later on, proved to be the same. This fact obviously has generated a confusing picture. We have carried out a physico-chemical characterization of D-mannitol with the aim to gain further knowledge on its behaviour and on the interconversion of its different polymorphs. α and β modifications can be distinguished only by XRPD and FT-IR as they show melting temperature and enthalpy that are the same within the standard deviation. Between these polymorphs exists a monotropic relationship: the α form is never thermodynamically stable. The understanding of the thermal behaviour of the δ form (obtained by re-crystallization in acetone) has required XRPD experiments performed at variable temperature. During heating, the δ form changes to α modification before undergoing melting. By cooling a melted sample, under a wide range of experimental conditions, a very fast crystallization occurs. Independently of the starting crystal form (β or δ form), the re-crystallization of D-mannitol from melt always leads to α

form. The thermal behaviour of the melted samples, i.e. the very fast crystallization and release of high amount of heat, are the most interesting points that could be at the origin of the breakage of vials during freeze-drying.
Part of the research activity falls within a scientific collaboration between the University of Pavia and the pharmaceutical factory A.M.S.A.. The research topic has been the physico-chemical characterization of an active principle with particular care to the thermodynamic relationship between the various polymorphs in which it can exists. For secrecy agreement reason we are not authorized to report here the name of the compound. At the moment, only one solid form (I) is covered by patent and it is not known if it is the form stable at room temperature. The compound may exist also as other two solid forms (II and III) but they can not be obtained pure through industrial-scale synthesis processes. Thus, the study had the final goal to identify the polymorph characterized by the lowest free energy and, in the case it is free from patent, to detect the experimental conditions which enable to obtain it. In this way there would be the chance to produce a stable dosage form which does not infringe any patent. The correlated use of thermal, diffractometric and spectroscopic techniques allowed to characterize the samples of the pure solid forms I and II received from the factory. We found that the crystalline form III may crystallize from the fuse obtained from both the solid forms I and II or after an appropriate isothermal treatment of those forms at a temperature lower than that of their melting. Measurements allowed to evaluate the relative stability of the three polymorphs through the construction of an energy-temperature diagram. The solid form III, free from any patent, proved to be the polymorph stable at room temperature.
The experimental evidences showed that heating the polymorph II (low-melting form) containing an impurity of form I (high-melting form) promotes the enrichment of the system with form I making difficult its quantification in an impure sample through DSC. Through the construction of a calibration line starting from the melting enthalpy changes of the two forms and from the nominal content of polymorph I in mixtures with known composition, we have developed a model to solve the problem of quantification of the polymorph I.

## *References*

Bruni, G., Berbenni, V., Milanese, C., Girella, A., Cofrancesco, P., Bellazzi, G., Marini A., *Physico-chemical characterization of anhydrous D-mannitol,* Journal of Thermal Analysis and Calorimetry 2008, in press.

# 2C – Models for biotechnological and environmental studies

*D. Uccelletti, E. Zanni, A. Carducci, F. Castelli, C. Palleschi*
*Dept. of Developmental and Cell Biology, Univ. Roma "La Sapienza"*

## Aims

*Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Caenorhabditis elegans*: models for biotechnological and environmental studies.

## Results

Our group is currently involved in studies on the molecular mechanisms that control the protein fate and the cell responses to environmental stimuli. We have focused our research on the secretion and glycosylation of proteins and on the cell pathways that become activated upon internal or external stress conditions; special attention is placed on the genetic networks that control such processes.
We have demonstrated that the gene KlOCH1 of Kluyveromyces lactis codes for an alfa-1,6-mannosyltransferase localized in the Golgi apparatus; the inactivation of such gene resulted in a substantial reduction of the protein glycosylation and in changes in the cell wall organization. The mutant strain, in addition, exhibited a noticeable increase in the secretory capabilities of recombinant proteins in the extracellular culture media. The described characteristics are of interest for biotechnological applications.
We have also studied the functional role of the gene *KlPMR1*, encoding for a $Ca^{2+}$-ATPase localized in the Golgi apparatus. This gene resulted involved in complex interactions controlling many cell processes: cell wall biogenesis, glycosylation, secretion, mitochondrial metabolism and defence pathways against oxidative stress. Another studied enzyme, involved in the oxidative stress responses, was the superoxide dismutase encoded by the gene KlSOD1. Such stresses may also originate by the overload of the secretory cell compartments that can be observed upon overexpression of recombinant proteins in engineered host cells. Increasing the gene dosage of KlSOD1 resulted in the relieve from such cell conditions and in the improvement of recombinant protein production.
The role and interactions of the enzyme apyrases (nucleoside triphosphate-diphosphohydrolases) have been studied both in yeast and in the nematode C.elegans. These are key enzymes in the glycosylation processes and are highly conserved from yeast to mammals. We have investigated the cell functions of the two different apyrases encoded by the KlYND1 and APY-1 genes. In both cases we have demonstrated the involvement of the apyrases in many relevant cell processes including the secretory processes and the stress response pathways. Mutants of C.elegans have been demonstrated to be highly sensitive to environmental noxious

stimuli; they could be of interest for survey and prevention programs in applied environmental sciences.
The experimental model based on a simple animal, the nematode C.elegans, has been fully implemented; our group is now therefore equipped with two of the most extensive experimental platforms, one based on a single-cell organism and one based on a multi-cellular organism.

## References

Raimondi S., Zanni E., Talora C., Rossi M., Palleschi C., Uccelletti D. *KlSOD1: a New Helper Gene for the Heterologous Protein Secretion in Kluyveromyces lactis*. Appl. Environ. Microbiol. 2008, 74, 7130-7137.

Staneva D, Uccelletti D, Venkov P, Miloshev G, Palleschi C. *The Kluyveromyces lactis CPY homologous genes - Cloning and characterization of the KlPCL1 gene*. Folia Microbiol. 2008, 53, 325-332.

Raimondi, S., Uccelletti D., Matteuzzi, D., Pagnoni, U.M., Rossi, M. and Palleschi, C. *Characterization of the superoxide dismutase SOD1 gene of Kluyveromyces marxianus L3 and improved production of SOD activity*. Appl. Microbiol. Biotechnol. 2008, 77, 1267-1277.

Uccelletti D., Pascoli, A., Farina, F., Alberti, A., Mancini, P., Hirschberg, C.B. and Palleschi, C. *APY-1, a novel Caenorhabditis elegans apyrase involved in UPR signalling and stress responses*. Mol. Biol. Cell 2008, 19, 1337-1345.

Uccelletti D., Anticoli, S. and Palleschi, C. *The apyrase KlYnd1p of Kluyveromyces lactis affects glycosylation, secretion and cell wall properties*. FEMS Yeast Res. 2007, 7, 731-739.

# 2C – Structure-Activity Relationship Studies on Enzymes of Biotechnological Interest

*R. Pogni, M.C. Baratto, S. Giansanti, A. Sinicropi, R. Basosi*

## Aims

Structure-activity relationship studies of new natural and synthetic enzymes (Peroxidases, Oxidoreductases, Mono-Di-Oxygenases) to clarify the catalytic mechanism. Possibility to obtain mutants by rationale design with increased biotechnological performances.

## Results

Different heme peroxidases are considered to be involved in the lignin biodegradation process, a key step for carbon recycling in terrestrial ecosystems. These are lignin peroxidase (LiP) and manganese peroxidase (MnP) firstly described in *Phanerochaete chrysosporium* , and the versatile peroxidase (VP) more recently described in fungi from the genera *Pleurotus* and *Bjerkandera*. VP is characterized by combining catalytic properties of the other two ligninolytic peroxidases, MnP and LiP. VP oxidizes hydroquinones and substituted phenols that are not efficiently oxidized by LiP or MnP in the absence of veratryl alcohol and Mn(II) respectively.

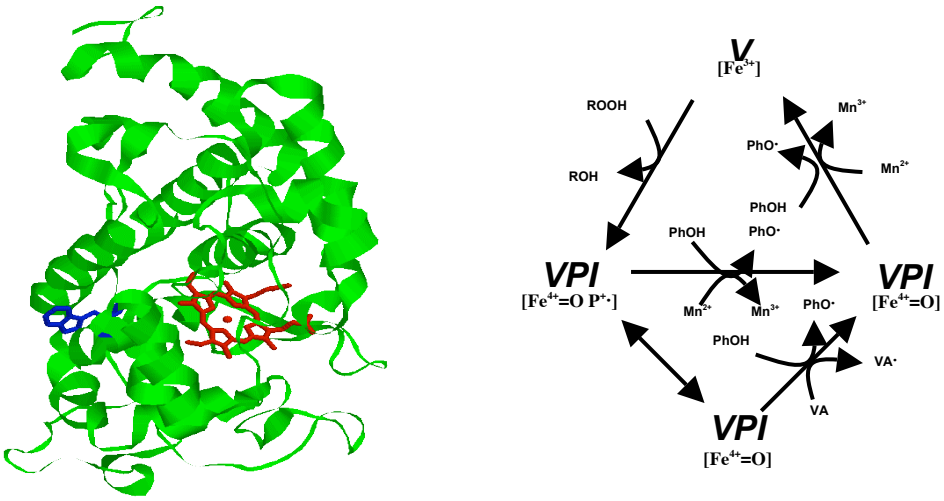



Fig. 1 Crystal structure of P. eryngii VP showing the Trp164 catalytic site (left) paired with the extended catalytic mechanism of Versatile Peroxidase (right)

VP is even able to degrade directly high redox potential dyes that can be eventually oxidized by LiP only in the presence of veratryl alcohol. For these characteristics VP enzymes can be used for bioremediation purposes. A radical intermediate, in the reaction of Versatile Peroxidase from the ligninolytic fungus *Pleurotus eryngii* with $H_2O_2$, has been characterized by multifrequency (9.4 GHz and 94 GHz) EPR and

assigned to a tryptophan residue. Comparison of experimental data and DFT (Density Functional Theory) theoretical results strongly suggests the assignment to a tryptophan neutral radical. This site, solvent exposed was demonstrated to be the real catalytic site for the VP enzymes. The crystal structure of P. Eryngii VP and the extended catalytic site are reported in Fig. 1.

## *References*

Castriciano, M. A., Romeo, A., Baratto, M. C., Pogni, R., Monsù Scolaro L., *Supramolecular Mimetic Peroxidase Based on Hemin and PAMAM Dendrimers*, Chemical Communications, 2008, 6 , 688 – 690.

Pogni, R., Teutloff, C., Lendzian, F., Basosi, R., *Tryptophan Radicals as reaction intermediates in versatile peroxidases: multifrequency EPR, ENDOR and Density Functional Theory Studies*, Applied Magnetic Resoance., 2007, 31, 509 – 526

Pogni, R., Baratto, M. C., Teutloff, C., Giansanti, S., Ruiz-Duenas, F. J., Choinowski, T., Piontek, K., Martinez, A. T., Lendzian, F., Basosi, R., *A Tryptophan Neutral radical in the Oxidized State of Versatile Peroxidase from Pleurotus eryngii: a Combined Multi-Frequency EPR and DFT Study*, Journal of Biological Chemistry, 2006, 281, 9517 – 9526

Verdin, J., Pogni, R., Baeza, A., Baratto, M. C., Basosi, R., Vazquez-Duhalt, R., *Mechanism of versatile peroxidase inactivation by calcium depletion,* Biophysical Chemistry, 2006, 121, 163 – 170

Valderrama, B., Garcia-Arellano, H., Giansanti, S., Baratto, M. C., Pogni, R., Vazquez-Duhalt, R., *Oxidative Stabilization of Iso-1-cytochrome c by Redox inspired Protein Engineering*' FASEB Journal, 2006, 20(8), 1233-1242

Pogni, R., Baratto, M. C., Giansanti, S., Teutloff, C., Verdin, J., Valderrama, B., Lendzian, F., Lubitz, W., Vazquez-Duhalt, R., Basosi, R., *Tryptophan-Based Radical in the Catalytic Mechanism of Versatile Peroxidase from Bjerkandera adusta*, Biochemistry, 2005, 44, 4267 – 4274

Perez-Boada, M., Ruiz-Duenas, F. J., Martinez, A. T., Pogni, R., Basosi, R., Choinowski, T., Piontek, K., Martinez, M. J., *Versatile Peroxidase Oxidation of High Redox Potential Aromatic compounds:Site-directed Mutagenesis, Spectroscopic and Crystallographic Investigation of three Long-Range Electron Transfer Pathways,* Journal Molecular Biology, 2005, 354, 385 – 402

# 2C – Structural and Dynamic Studies of Metal Complexes with Ligands of Biological Interest

*M.C. Baratto, R. Pogni, A. Sinicropi, M. Colombi, L. Parisi, E. Busi, R. Basosi*

## Aims

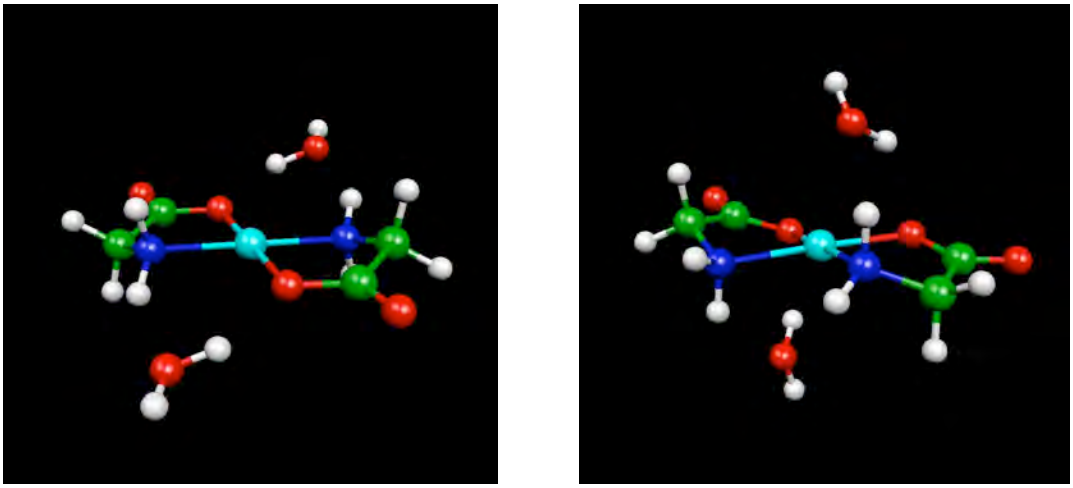
The aim of this project is a Multifrequency EPR approach aided by computational studies to investigate metal-complexes with biological applications in solution, in order to determine a structural and dynamic characterisation.

## Results

This research project proposes to increase the basic knowledge of the structures in the solid state and in solution, of the stability and in general of the physico-chemical properties of metal chelates with peptides, alpha-hydroxyacids and synthetic ligands with biological activity. In order to obtain structural and geometric information of the metal center, multifrequency EPR spectroscopy, joint to computational studies, were carried out. In this context one topic focused on Cu(II)-complexes with alpha-hydroxyacids, owing to their important role in enzymatic processes and considering their actual and potential uses in animal and human nutrition and in pharmacology.

Multifrequency EPR spectra recorded at different pH values and variable temperatures allowed to determine different species distribution in solution and obtain the magnetic properties of the analyzed systems. For the same compounds with diamagnetic metals ($Zn^{2+}$) theoretical and computational analysis was performed by means of DFT calculations to gain a deeper insight and a straightforward confirmation of the experimental data.Furthermore,

The Copper(II)complex, Cu(II)-bis-glycinato, has been used as a model system for the development and implementation of simulation programs for EPR spectra under slow motional conditions. All the spectra recorded at X-and S band at variable temperatures in the range 213-293K were simulated using the COSMOS program (realized in our laboratory), converging to a reliable and unique set of magnetic parameters. The use of a single simulation program to simulate all the spectra recorded at different motional conditions, paired to density functional theory and polarizable continuum model (DFT/PCM) computations, was synergistic in assigning the presence of different isomers of Cu(II)-bis-glycinato complex, changing the physical state of the sample.



(a) (b)

Molecular structure of t*rans* (a) and *cis* (b) isomers of Cu(II)-bis-(glycinato)·2$H_2O$ optimized at the B3LYP/6-311 + G(d,p) level.

New synthetic complexes with biological activity are under study. X and S-band EPR spectra at room and low temperatures aided by simulation and optimization programs are in progress in order to characterize such novel systems, giving information on the number and geometry of the nitrogen atoms coordinated to copper with the aim of defining a structure-activity relationship.

## *References*

Predieri, G., Beltrami, D., Pattacini, R., Parisi, M.L., Sinicropi, A.., Valensin, D., Basosi, R., *Structural studies in solution and in the solid state on the zinc chelate of 2-hydroxy-(4-methylthio) butanoic acid, an effective mineral supplement in animal feeding*, 2008, Inorg. Chim. Acta, 2008, doi: 10.1016/j.ica.2008.05.027

Pezzato, M., Della Lunga, G., Baratto, M.C., Sinicropi, A., Pogni, R., Basosi, R., *The cis/trans isomerization of Cu(II)-bis-(glycinato) complex in solution: a computer aided Multifrequency EPR and DFT/PCM calculations study*, Magn. Res. in Chem., 2008, 45, 846-849

Basosi, R., Della Lunga, G., Pogni, R., *Copper Biomolecules in Solution*, Biomedical EPR - Part A: Free Radicals, Metals, Medicine and Physiology, Kluwer Academic / Plenum Publishers, 2005, 385-416

Della Lunga, G., Pezzato, M., Basosi, R., *Recent Research Developments in Molecular Physics,* Transworld Research Network, 2003, 1, 43

Della Lunga, G., Pezzato, M., Baratto, M.C., Pogni R., Basosi R., *A New Program Based on Stochastic Liouville Equation for the Analysis of Superhyperfine Interaction in CW-ESR Spectroscopy,* J. Magn. Res., 2003, 164, 71-77

# 2C – "In vivo" NMR studies of cell metabolism

*M. Ricci, A. Donati, C. Bonechi, S. Martini, M. Aggravi, C. Rossi*

## Aims

In vivo time evolution of metabolic profile of prokaryotic and eukaryotic cells by $^{13}$C-NMR.
Development of new mathematical and statistical methods for the analysis of the metabolic profile of prokaryotic and eukaryotic cells. Development of a mathematical model for the cellular metabolism.

## Results

NMR spectroscopy in combination with statistical and mathematical methods has emerged as a suitable approach to perform metabolomic studies and to investigate biological systems. Carbon-13 NMR spectroscopy together with selective 13-C sugar substrate enrichments were used to study bacteria and yeast metabolization processes.
However, analysis of NMR spectra of biological samples containing large number of metabolites is challenging due to the high amount of information stored in the spectra itself. In order to overcome this problem and to decrypt the information hidden in the NMR spectra, we developed a suite of in-house made software based on a open-source programming environment such as SciLab. *Saccharomyces cerevisiae* fermentative metabolism was monitored using [1-$^{13}$C]glucose as substrate and 13C-NMR for measuring the concentration of the metabolites over the time
In order to average out experimental fluctuations leading to unwanted chemical shift variation and to prepare spectra for further analysis we divided the spectra in regions of equal length (bins).

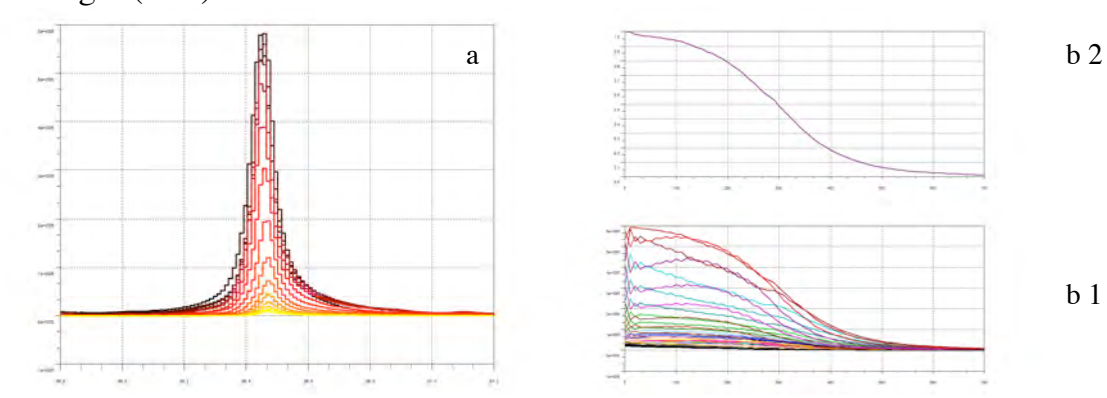



Fig. 1: a) binned peak of a glucose $^{13}$C1 b1) glucose kinetics during fermentation monitored by the peak of aglucose$^{13}$C1. b2) normalized data.

Each spectrum was reduced to bins of 0.125 ppm wide between 10 and 100 ppm; the integral within every bin was then calculated using a binning routine implemented in an specifically developed Scilab script which automatically bins the original data and

stores it in binned spectra (fig. 1a). Each curve corresponds to the time course of the integral value of a single bin forming the a glucose peak.
Thus the pseudo 2D spectrum is reduced to a matrix where the rows are the time series of the metabolite concentrations and the columns are the bins. The matrix is used as input for calculating the Pearson's correlation coefficient (***r***) between each vector time series. The calculation was carried out using a specifically developed Scilab script which builds a 3D correlation map with bins (e.g. ppm) symmetrically distribuited along the X and Z axis and the coefficient ***r*** along the Y axis. *r* value < 0.7 and > -0.7 are rejected and not included in the map.
The correlation map obtained from the original data set highlights that the methodology we propose correctly evaluates the correlations between the metabolites we monitored during yeast fermentation. Furthermore the correlation map proved to be a powerful visualization tool to identify metabolites within a mixture that are related each other. Indeed, as shown in figure 2 the script we developed correctly identifies the negative correlation between glucose and ethanol; glucose and glycerol; and the positive correlation between glycerol and ethanol.

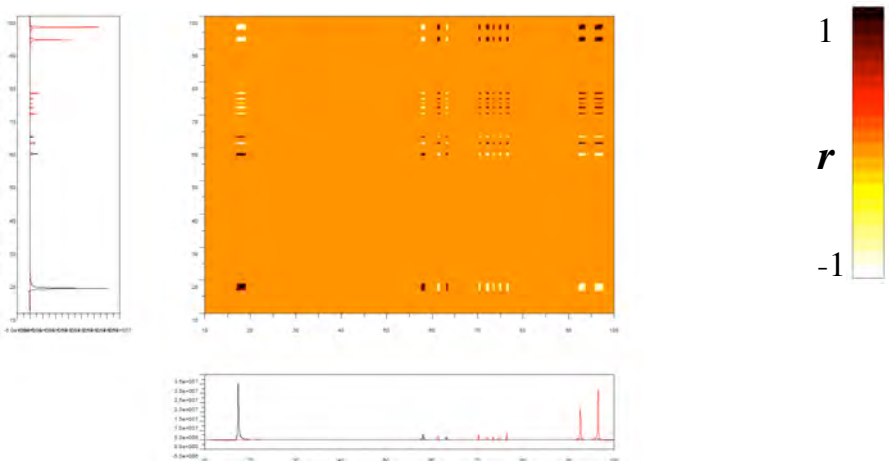



Fig. 2: A correlation matrix was built from the pair-wise linear correlation of each vector time series (representing the concentration of metabolites) using Pearson correlation coefficients (*r*). The pattern showed by the correlation map above reveals which metabolites have positive or negative correlations. Indeed the script we developed correctly identifies the negative correlation between glucose and ethanol; glucose and glycerol; and the positive correlation between glycerol and ethanol.

## *References*

S. Martini, M. Ricci, C. Bonechi, L. Trabalzini, A. Santucci, C. Rossi, *In Vivo 13C-NMR and Modelling Study of Metabolic Yield Response to Ethanol Stress in a Wild-Type Strain of Saccharomyces cerevisiae* FEBS LETTERS, 2004, 564, 63-68.

S. Martini, M. Ricci, F. Bartolini, C. Bonechi, L. Millucci, D. Braconi, A. Santucci, C. Rossi, *Metabolic response to exogenous ethanol in yeast: An in vivo NMR and mathematical modelling approach,* Biophysical Chemistry, 2005, 120, 135-142.

# 2C – Interaction between arsenic and reduce glutathione: a nuclear magnetic resonance study

*M. Aggravi, M. Ricci C. Bonechi, S. Martini, A. Cappelli, S. Burgassi, A. Donati*

## Aims

In this work several important features of the complex between As(III) and glutathione (GSH) were studied in detail by nuclear magnetic resonance as basic investigation to understand the behaviour of the toxic element inside red blood cells and other tissue.

## Results

The dynamic analysis of GSH and $As(GS)_3$ was done by measuring their $^{13}C$ relaxation time as a function of the temperature. With this kind of analysis a more accurate determination of the motional field of the molecule was obtained. The relaxation rate curves in the range 283K and 313K, indicated an intermediate correlation time for $As(GS)_3$, showing the higher relaxation efficiency at 288K.
This behaviour must be take in account for the interpretation of the data in vivo. The postulated formation of adducts of $As(GS)_3$ with other sulfhydryl groups inside RBC, which is considered fundamental for the metabolism of the toxic element, will result in subtle variation in the spectra giving a more accurate picture of the mechanism of action of As(III).

The NOESY spectra of $As(GS)_3$, recorded in $D_2O$ at different pH values (between pH 3 and pH 7.5), showed the presence of significant exchange cross-peaks involving both the free and bound molecule (Figure 1). These signals were easily discernible from the genuine NOE cross-peaks observing their relative sign with respect to the diagonal.

Apparently, two kind of exchange cross-peaks were recognizable, both concerning the Cys residues. One kind between the two diastereotopic Cys-$H\beta_{1,2}$ of $As(GS)_3$ suggesting a slow conformational inter-conversion between each other. At the same time, both Cys-Hα and Cys-Hβ showed exchange between the complex and the free molecule, suggesting a slow kinetic involving the two forms.

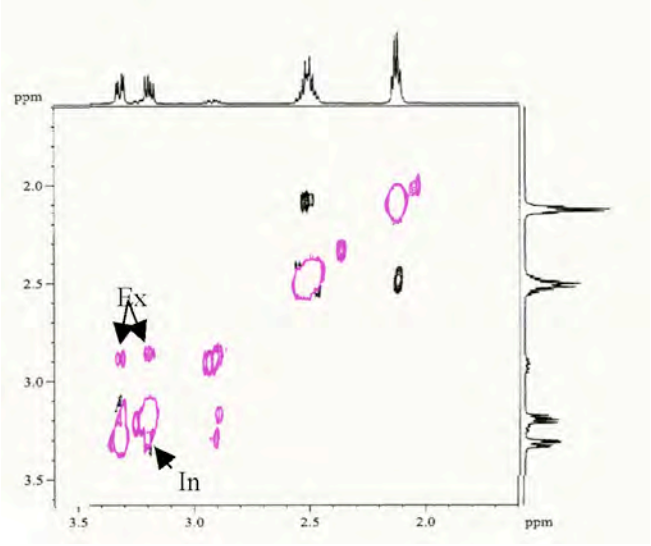



Figure 1: Portion of the NOESY spectrum of $As(GS)_3$ complex. Cross-peaks with the same sign with respect to the diagonal (same color) are exchange peaks. Exchange cross-peaks between free GSH and As(GS)3 are labelled by Ex. Diastereotopic Cys-$Hb_{1,2}$ inter-conversion is labelled by In.

The accurate comparative analysis of the $^{1}$H-NMR chemical shift together with the dynamic investigation of GSH and $As(GS)_3$ by $^{13}$C-NMR relaxation parameters allowed to develop a clear scheme to analyse spectral features which were found fundamental for the interpretation of the NMR data in vivo.
The $As(GS)_3$ complex revealed a complex dynamic motion and an exchange kinetic which were considered important for the mechanism of toxicity.

## *References*

Centeno, J. A., Mullik F. G., Martinez L., Page N. P., Gibb H., Longfellow D., Thompson C., Ladich E. R. (2002) *Pathology related to chronic arsenic exposure*. Environ. Health Perspect. 2002 *110*, 883-886.

Abernathy C. O., Liu Y., Longfellow D., Aposhian H. V., Beck B., Fowler B., Goyer R., Rossman T., Thompson C., Waalkes M. (1999) *Arsenic: health effects, mechanisms of actions, and research issues*. Environ. Health Perspect. 107, 593-597.

# 2C – Complexation of As(III) in rat and human erythrocytes

*M. Aggravi, M. Ricci, A. Donati*

## Aims

The present work examines the binding of As(III) in intact rat and human erythrocytes (RBC) by $^{1}$H-NMR spectroscopy.

## Results

In this study by $^{1}$H-NMR we recorded NMR spectra for the most abundant small molecules inside the cells using spin-echo technique to eliminate the interfering resonances from hemoglobin (Hb) and membranes. This non invasive method allowed to collect information examining interaction between arsenic and thiolic molecules inside intact cells.

Both the suspensions of human and rat RBC were incubated with increasing concentrations of arsenite until the final concentration 3mM. The resonances of intracellular glutathione were strongly modified by the presence of arsenite but we observed large differences between the two species

In human RBC, in presence of As(III), only the intensity of Cys-Hα and Cys-Hβ signals appears reduced while the resonances of the residual cytosolic molecules are unaffected by the addition of arsenite.

In rat RBC incubated with As(III) the Cys-Hα and Cys-Hβ peaks diminish in intensity as described for human RBC indicating the complexation of intracellular GSH with As(III) but in this case the resonances of glutamic acid and glycine also decrease showing a significant line broadening.

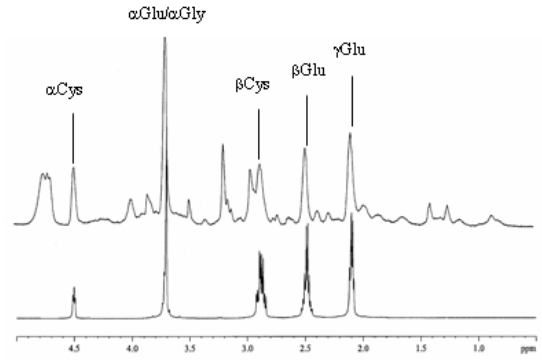



Fig 1. $^{1}$H-NMR spectrum of human RBC (a) compared with the $^{1}$H NMR spectrum of glutathione in aqueous solution.

The integrals of all GSH peaks versus the volume of arsenite obtained binning the spectra confirms that in the human cells only cysteinyl resonances vary after the addition of arsenic while in rat RBC the reduction of intensity of cysteinyl resonances versus the volume of arsenite is parallel to the diminution of glycine and glutamic acid peaks.

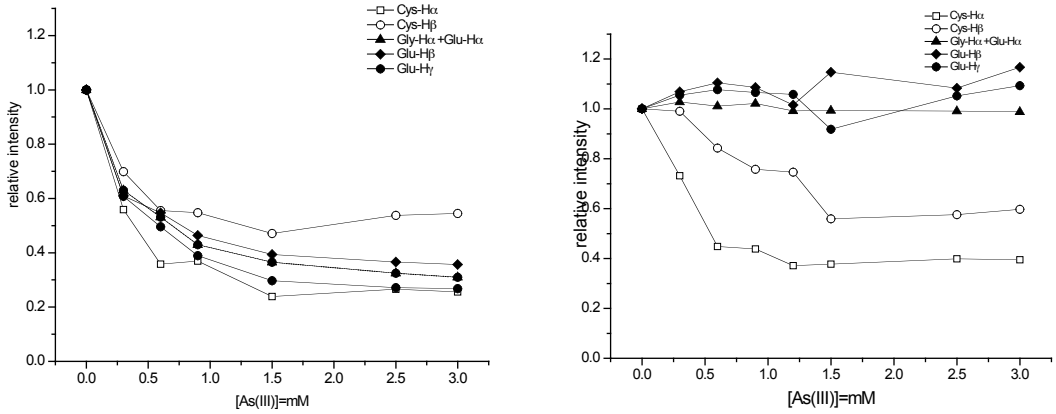



Fig 2. Volume of GSH peaks versus addition of arsenite in rat (left) and human (right) erythrocytes.

The major affinity for As(III) showed by rat Hb can be explained by observing that the molecular structure of rat Hb implies the presence of three reactive cysteines particularly exposed and accessible while in human Hb only one cysteines has a fast reacting thiol moiety.

The spectral changes observed in rat Hb resonances and the correspondent modifications in $^{1}$H-NMR signals of glutathione after the addition of As(III) suggest that the high reactivity of cysteinyl groups can act as binding site for arsenic and in the intracellular environment can origin a ternary complex Hb-As-GSH.

The results of the study confirm the formation of an intracellular As-GSH complex as observed *in vitro* in human red blood cells while the presence of high reactive cysteines in rat Hb determines the formation of a ternary adduct As-GSH-Hb.

In human erythrocytes the As-GSH complex appears not bound to other intracellular sites.

## *References*

Kala, S., Kala, G., Pater, C.I., Sartorelli, A.C., Lieberman M.W., *Formation and urinary exctretion of arsenic triglutathione and methylarsenic diglutathione*, Chem Res. Toxicol. 2004, 17, 243-249.

Hayakawa T., Kobayashi Y., Cui X., Hirano S. *A new metabolic pathway of arsenite: arsenic–glutathione complexes are substrates for human arsenic methyltransferase Cyt19*. Arch. Toxicol. 2005, 79, 183-191.

# AUTHOR INDEX