Frontiers in Water Biophysics www.waterbiophysics.eu

ERICE 21-26 July 2019

# BOOK OF ABSTRACTS AND CONFERENCE PROGRAMME




**XVII Course of the International School of Statistical Physics**
**ETTORE MAJORANA FOUNDATION AND CENTRE FOR SCIENTIFIC CULTURE**
**http://www.ccsem.infn.it/**
**Directors: Peter Hanggi, Fabio Marchesoni**

## Welcome

A very warm welcome is addressed to all the participants and contributors to this fifth 2019 Conference on “Frontiers in Water Biophysics”, located for the third time in the superb location of Erice, hosted by the Ettore Majorana Foundation and Centre for Scientific Culture.

We wish to pinpoint this Conference within two very different events.
The first one is the great scientific rumor raised fifty years ago (1969) around the extremely anomalous properties of “polywater” (see “Polywater” by F. Franks, The MIT Press, 1981), resulted from undesired pitfalls of experimental design but immediately supported by several theories advanced to explain the phenomenon.
The second event is the death of the Sicilian writer Andrea Camilleri (passed away the 17$^{th}$ of July, this year), the author of Inspector Salvo Montalbano, who had in mind the rule that “everything has to follow a certain logic and everything has to be in a certain place.” The first result of this successful experiment was “The Shape of Water,” published in Italy in 1994.

Thus, this conference is dedicated to Andrea Camilleri and to all the scientists in the field of “water biophysics” and in particular to those gathered in Erice to discuss about the interplay of water molecules and relevant biological surfaces as those of hydrogels, membranes, nucleic acids and, last but not least, proteins.

As for the past editions, many peoples contributed in theory and in practice to the preparation and the organization of this 2019 event. The continuous advice of the Ettore Majorana Center and of the colleagues involved in the previous events has been highly appreciated, and special thanks are deserved to the members of the Scientific Committee for their past and future suggestions.

We believe that our scientific endeavor in setting down the Frontiers in Water Biophysics Conference will generate new contacts and collaboration, new ideas and new relevant results. This objective supported and will support us in pursuing ahead with the strongest consideration of achieving a fantastic socio-scientific goal.

Lucia Comez, Attilio Cesàro, Giancarlo Franzese

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# Programme

| | SUN 21 | MON 22 | TUE 23 | WED 24 | THU 25 | FRI 26 |
|---|---|---|---|---|---|---|
| 08:45-09:00 | | Opening | | | | DEPARTURE |
| CHAIR | | Cesàro | Franzese | Brady | Cottone | |
| 09:00-10:30 | | STOKKE | LORENZ | MONTESARCHIO | SPINOZZI | |
| 10:30-11:00 | | Coffee Break | Coffee Break | Coffee Break | Coffee Break | |
| CHAIR | | Paolantoni | Allen | Comez | Paciaroni | |
| 11:00-11:30 | | Graziano | Martelli | De Michele | Schirò | |
| 11:30-12:00 | | Orecchini | Fusco | Pagano | Othon | |
| 12:00-12:30 | | Rossi | Calero | Webba Da Silva | Matyushov | |
| 12:30-12:55 | | Alfarano | Chiricotto | Bottari | Pusztai | |
| 13:00-15:00 | | Lunch | Lunch | Lunch | Lunch | |
| CHAIR | | De Simone | Gomila | Giancola | Stokke | |
| 15:00-16:00 | Arrival and registration | CLAESSENS | ALLEN | SISSI | RUZICKA | |
| 16:00-16:30 | | Sancini | Ortore | Di Fonzo | Closure | |
| 16:30-17:00 | | Coffee Break | Coffee Break | Coffee Break | SOCIAL EVENT | |
| CHAIR | | Lorenz | Gallo | Corezzi | | |
| 17:00-17:30 | | D'Angelo | Gomila | Gallo | | |
| 17:30-17:55 | | Sikk | Sebastiani | Lupi | | |
| 17:55-18:20 | | De Simone | Coronas | POSTER SESSION / PRIZE | | |
| 18:20-19:00 | | POSTER SESSION | POSTER SESSION | | | |
| 20:00-22:00 | | | | | SOCIAL DINNER | |

## MONDAY 22

09:00 TU1
**B. T. Stokke** "*Molecular Responsive Hydrogels – An Overview of Mechanisms"*
11:00 IL1
**G. Graziano** *"Pnipam Cononsolvency in Water-Methanol Solutions"*
11:30 IL2
**A. Orecchini** *"Low-Temperature Dynamical Transition in Concentrated Microgels"*
12:00 IL3
**B. Rossi** *"Swelling Behaviour in Hydrogels: A Structural and Molecular point of view"*
12:30 OP1
**S.R. Alfarano** *"Does Hydrated Glycine Act as Crystallization Nucleus at Multi-Kilobar Conditions?"*

15:00 PL1
**M. Classens***"(Dis)Functional Membrane Remodeling with the Intrinsically Disordered Protein $\alpha$-Synuclein"*
16:00 IL4
**G. Sancini***"From the Lungs to the Brain: The Fantastic Voyage of Nanoparticles Targeting $\beta$-Amyloid ($\alpha\beta$)"*
17:00 IL5
**G. D'Angelo***"Update on Waters at the Membrane Interface and Collective Dynamics in Phospholipid Bilayers"*
17:30 OP2
**L. Sikk** *"Qsar Modelling of Blood-Brain Barrier Permeability"*
17:55 OP3
**A. De Simone** *"Water Displacement During Macromolecular Assembly: From Supramolecular Chemistry to Neurodegenerative Diseases"*

**POSTER SESSION**

## TUESDAY 23

09:00 TU2
**C. D. Lorenz** *"Molecular Dynamics Simulations of Biological Lipid Membranes"*
11:00 IL6
**F. Martelli** *"The Hydrogen-Bond Network of Water Confined by Phospholipid Memberanes"*
11:30 IL7
**G. Fusco** *"Membrane Interaction by $\alpha$-Synuclein: A key player in Neuronal Communication and Parkinson's Disease"*
12:00 IL8
**C. Calero** *"Local Structural and Dynamical Properties of Water at the Interface with Phospholipid Membranes"*
12:30 OP4
**M. Chiricotto** *"Hydrodynamic Effects on $\beta$-Amyloid Peptide Aggregation: From Disordered Coagulation and Lateral Branching to Amorphous Prefibrils"*

15:00 PL2
**H. Allen** "*Aqueous nanoscale systems: from long range interactions in water to membrane hydration and water droplets"*
16:00 IL9
**G. Ortore** *"Amyloid $\beta$-Peptides Interaction with Membranes"*
17:00 IL10
**G. Gomila** *"Mapping the Electric Polarization of dry and hydrated Biomembranes at the Nanoscale"*
17:30 OP5
**F. Sebastiani** *"Confined Water: Same Solvent, Different Properties "*
17:55 OP6
**L. E. Coronas** *"Phase Transition Analysis between Low Density and High Density Nanoconfined Water"*

**POSTER SESSION**

## WEDNESDAY 24

09:00 TU3
**D. Montesarchio** *"Aptamers and G-Quadruplexes: A Fruitful Marriage (Celebrated with Water!)"*
11:00 IL11
**C. De Michele** *"Nematic, Cholesteric and Smectic Ordering of Dna Nanoparticles"*
11:30 IL12
**B. Pagano** *"Insights into Noncanonical Dna Structures and their binding properties in Aqueous Environments"*
12:00 IL13
**M. Webba Da Silva** *"On Encoding the Fold Of G-Quadruplexes"*
12:30 OP7
**C. Bottari** *"Biophysical Properties of Dna in Ionic Liquid Probed by Uv-Resonance Raman Scattering"*

15:00 PL3
**C. Sissi** *"The Fluid Friendship Between Water and Nucleic Acids"*
16:00 IL14
**S. Di Fonzo** *"Uv Resonant Raman Spectroscopy of G-Quadruplex Dna different Topology Structures with Drug Ligands "*
17:00 IL15
**P. Gallo** *"Hydration Water in biosolutions for Cryopreservation and the Role of the Hydrogen Bond Network"*
17:30 OP8
**L. Lupi** *"Role of Stacking Disorder on the Barrier and Pathway of Ice Nucleation"*

**POSTER SESSION**

## THURSDAY 25

09:00 TU4
**F. Spinozzi** *"Exploring Water Shell of Proteins Through Cosolvents: The Lesson of SAXS and SANS"*
11:00 IL16
**G. Schirò** *"Water-Protein Dynamical Coupling in Functional and Pathological Protein States"*
11:30 IL17
**C. Othon** *"Hydration Dynamics in Osmolyte Biopreservation"*
12:00 IL18
**D. Matyushov** *"Many Faces of the Protein-Water Interface: From Wetting of Active Sites to Protein Mobility"*
12:30 OP9
**L. Putzai** *"On the Possibility of (Micro)Phase Separation in Methanol-Water Mixtures: First Direct Experimental Evidence"*
15:00 PL4
**B. Ruzicka** "*PCS/XPCS Tools for Studying the Dynamical Behaviour of Colloidal Dispersions. Applications to Gels/Glasses"*

**EXCURSION TO ERICE ART&FOOD (INCLUD. DINNER)**

# TUTORIAL

# MOLECULAR RESPONSIVE HYDROGELS – AN OVERVIEW OF MECHANISMS

*Bjørn Torger Stokke*

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Hydrogels are polymer networks imbibed with water. There are numerous examples of this material group either existing naturally or synthesized. Responsive hydrogels being a class within the overall soft material group, are coined for such gels that adopt their state to changes in environmental conditions. Stimuli triggers can be generic (e.g., solvent conditions, physical variables) or specific molecules depending on the molecular compositions of network. In the presentation, an overview of our efforts within molecular stimuli responsive hydrogels will be provided. These include various networks custom designed to recognize various molecules and where the recognition lead to a change in equilibrium swelling (thus supporting a transduction of the recognition to a readable signal). In this line of research, we apply a fiber-optic interferometric platform for precise monitoring of changes in hydrogel swelling response to provide insight into fundamental and applied issues of responsive hydrogels. Hemispherical hydrogels with ~60 μm radius was synthesized covalently linked at the end of the optical fibers. The optical length of the hydrogels was monitored with a resolution of 2 nanometer thus providing a highly sensitive method for characterization their swelling. The hydrogels investigated encompasses various recognition moieties, and include glucose responsive hydrogels,[1] oligonucleotide based recognition[2-4] as well as examples where hydrophobic[5] and electrostatic interactions[6,7] are dominating. The oligonucleotide-based recognition hydrogel comprises hybridized di-oligonucleotides grafted to the polymer network as network junctions in addition to the covalent crosslinks. This supports detection of complementary oligonucleotides or other biological molecules based on their aptamer sequences. Insight into the coupled processes of transport, binding, competitive displacement and swelling in this hybrid hydrogels was obtained using time-lapse confocal imaging. Furthermore, changes in swelling properties of anionic hydrogels following impregnation of polycations that either penetrate the network or preferentially deposit at the surface of the hydrogel show that the distribution of the polycationic component strongly affect the swelling behaviour. Despite of the importance of changes in fraction of water in the molecular swelling processes, the standard entry into the interpretation of the results is based on the non-aqueous components. For these reasons, some aspects of the aqueous perspective will also be alluded to in the overview. In conclusion, the responsive hydrogels are versatile materials that can be designed to display recognition – transducing behaviour that can be exploited in different applications.

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## MOLECULAR DYNAMICS SIMULATIONS OF BIOLOGICAL LIPID MEMBRANES

*Christian D. Lorenz*

Department of Physics, King's College London, London, UK

In this presentation, I will give an introduction to molecular dynamics simulations, and then provide an insight into how they are used to investigate a variety of interesting problems within biophysics. Specifically I will demonstrate how we have used molecular dynamics to investigate how water and a variety of other solvents interact with different commonly found lipid types (e.g. phosphocholine, phosphoethanolamine). I will also discuss the work that we (and others) have done to model increasingly complex lipid membranes (including models of red blood cell and brain cell membranes) in order to attempt to better represent true biological membranes. Then I will give a description of how molecular dynamics simulations are used to investigate drug-lipid and protein-lipid interactions (in particular antimicrobial peptides). Throughout this talk, I will attempt to provide comparison to the work of the various experimental groups with whom we have collaborated in these studies.

## APTAMERS AND G-QUADRUPLEXES: A FRUITFUL MARRIAGE (CELEBRATED WITH WATER!)

*Daniela Montesarchio*

Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Via Cintia 21, 80126 Napoli, Italy

Nucleic acid aptamers are single-stranded DNA or RNA molecules, generally identified by an *in vitro* selection process called SELEX (Systematic Evolution of Ligands by EXponential enrichment) [1],[2]. Thanks to their unique three-dimensional folding, aptamers can recognize a wide range of molecular targets including proteins, small molecules, ions, whole cells or even entire organisms, such as viruses or bacteria [3],[4]. Each aptamer typically adopts a unique subset of conformations so to recognize the target with high affinity and selectivity, thus resulting into a valid alternative to antibodies in a wide number of applications, from therapeutic agents to diagnostic and sensing devices [5],[6],[7],[8].
Among the combinatorially selected aptamers endowed with significant bioactivity, many are G-rich oligonucleotides sharing a common structural feature, i.e. the ability to fold into stable G-quadruplex (G4) structures under physiological conditions [9],[10],[11]. This apparent oddity can be explained considering the extremely high polymorphism of G-quadruplex structures [12]. Indeed, the apparently rigid G4 structure is instead rather plastic and can fit into widely different nucleic acid architectures; therefore, these unusual DNA structures, even if with very similar sequences, can result into a large variety of different conformations [13]. In the last two decades, the discovery of several G4-forming aptamers able to recognize proteins playing crucial roles in different pathologies opened the way to the development of DNA or RNA G4-based therapeutic agents [14],[15],[16],[17].
Here we will focus on these particular aptamers, the most popular of which proved to be potential drugs against cancer, human immunodeficiency virus (HIV) and coagulation-related diseases.

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# EXPLORING WATER SHELL OF PROTEINS THROUGH COSOLVENTS: THE LESSON OF SAXS AND SANS

*Francesco Spinozzi*

Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

Proteins live in a watery crowed environment or confined in lipid bilayers. In biological solutions, structure, dynamics and then stability of a protein molecule is strongly affected by water and, simultaneously, it is the protein that controls the behaviour of the surrounding water. This profound interplay between protein conformation and water structure is modified by the presence of cosolvents, which are always ubiquitous in crowed biological fluids. Small-angle scattering (SAS) techniques based on X-rays (SAXS) or neutrons (SANS) are optimum tools to study the structural and thermodynamic properties of soluble proteins dissolved in binary solvent mixtures [1-4]. However, to access to the structural information behind SAS curves recorded at different compositions of the binary solvent and at several protein concentrations, it is necessary to adopt a proper thermodynamic model, able to describe the exchange between water and cosolvent molecules occurring at the protein surface [5-6]. In this way, it can be established if there is a preferential interaction of the protein with water molecules or with cosolvent molecules, and so to get a picture, at molecular level, of the stabilizing or de-stabilizing role of the cosolvent. With respect to SAXS, SANS experiments, performed in different combinations of heavy and light water as well as by using fully o partially deuterated cosolvents, allow to emphasise the difference in the composition of bulk solvent and solvation shell around the protein. To consolidate the interpretation of SAXS or SANS data very often Differential Scanning Calorimetry (DSC) experiments performed on similar samples are exploited [7].

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# PLENARY LECTURES

**PL1**

# (DIS)FUNCTIONAL MEMBRANE REMODELING WITH THE INTRINSICALLY DISORDERED PROTEIN α-SYNUCLEIN

*Mireille Claessens*

Nanobiophysics, Faculty of Science and Technology, University of Twente, the Netherlands

Proteins are the workhorse molecules of life and their function has long been thought to be reflected in their folded 3D structure. However, it turns out that a considerable fraction of proteins evades this structure function paradigm. These intrinsically disordered proteins (IDPs) do not possess a unique and persistent structure. It starts to become clear that nature uses these proteins for multiple parallel functions. The versatility and responsiveness of IDPs has a lot of merits. It however comes at a cost, the aggregation of IDPs has been implicated in cell death in neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

Here I will discuss our current understanding of the function and toxic interactions of one of these IDPs; alpha-synuclein (aS). The exact function of this protein is still largely unknown. *In vitro*, it is able to bind membranes via an amphipathic α-helix which possibly contributes to the remodeling of cellular membranes. The interaction of aS with cellular membranes is however not only functional, it is thought to be critical in the development of Parkinson's disease. In Parkinson's disease aS aggregates into oligomeric structures and amyloid fibrils. The binding of oligomeric aS aggregates to membranes has been associated with pore formation and the (membrane associated) aggregation of aS into amyloid fibrils has been reported to disrupt membranes. The mechanisms by which monomeric, oligomeric and fibrillar aS remodel membranes or disrupt membrane integrity are not well understood. I will show how we used a broad repertoire of quantitative single molecule and ensemble biophysical techniques, to obtain insight into aS function and possible disease mechanisms.

# ELECTRIC FIELDS, ION PAIRING, LIPIDS, AND SALTY AQUEOUS SURFACES

*Heather C. Allen*

Professor of Chemistry and Biochemistry
The Ohio State University, Department of Chemistry and Biochemistry

Our oceans are the largest generators of highly saline and organic-rich atmospheric aerosol. Biological breakdown products provide the organic fat of marine surfaces and aerosols while some generation mechanisms of aerosol selectively enrich or deplete the droplets in the organic fat. Research in the Allen lab investigates the air-aqueous interface. We study proxy and relatively simple chemical systems to understand interfacial speciation and organization to then inform on atmospheric aerosol, cloud, and marine surface reactivity, correlating to climate change and its contributing uncertainties. Yet, we are also highly interested in biomembranes, lung surfactant, bioinsipired ion capture, ion pairing, and hydration of such, noting that water is the persistent solvent in all of these systems, and is of great interest itself. Surface selective experiments reveal surface propensity of hydrated ions and ion pairs, and generation of electric fields, the surface potential, inherent to the ordering of ions in the electrical double layers at the aqueous surface. At low and high salt concentrations, these experiments reveal ion specific ordering. Magnesium solvent shared ion pairing with sulfate is one example where relatively few ion pairs produce a significant electric field at these surfaces. In current work, we also investigate iron (III) hydration and speciation where centrosymmetric Fe(III) complexes appear to outcompete other common Fe(III) species for surface sites. Hydration structure is revealed and discussed. Lipid – ion binding and surface domain formation is also studied and will be presented. The surface potential is measured using radioactive Americium, a method being refined in our laboratory. Surface tensiometry, Brewster angle microscopy (BAM), and surface vibrational probes of sum frequency generation (SFG), and infrared reflection absorption spectroscopy (IRRAS) are also discussed.

# THE FLUID FRIENDSHIP BETWEEN WATER AND NUCLEIC ACIDS

*Claudia Sissi*

Department of Pharmaceutical and Pharmacological Sciences, Via Marzolo 5, 35131 Padova, Italy

The resolution of the crystal structure of the double helix represents a milestone in the human heritage. Among the multiple cultural and technical advancements that the understanding of this unique structure produced, the correlation between the double helix structural features and the nucleic acids biological functions is simply amazing. Here, we will start from a simple analysis of the energetic contribution that supports this worldwide known model to understand the role of water in driving DNA conformational changes. Intriguingly, this will drive us to the function of this small molecule when the macromolecule must be preserved as well as when it must be degraded.

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# PCS/XPCS TOOLS FOR STUDYING THE DYNAMICAL BEHAVIOUR OF COLLOIDAL DISPERSIONS. APPLICATIONS TO GELS/GLASSES

*Barbara Ruzicka*

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Photon Correlation Spectroscopy (PCS) and X-ray Photon Correlation Spectroscopy (XPCS) are respectively a conventional and a synchrotron radiation technique very powerful for the investigation of the dynamical behaviour of different colloidal dispersions. The combination of PCS and XPCS gives access to the microscopic dynamics of the systems in a very large range of time and length-scales. In very dilute conditions from the measured relaxation time one can also derive the hydrodynamic radius of the particles. Moreover these techniques are very efficient also to follow the system far from equilibrium, for example in the changeover from a liquid phase towards an arrested state (gel and/or glass). During the formation of these states, obtained for example by increasing packing fraction or waiting time (aging), the dynamics is not stationary but is progressively slowed down and its evolution can be monitored through combined PCS and XPCS.

The use of these techniques, together with structural studies, have permitted to follow the formation of gel/glass states in dilute aqueous dispersions of a synthetic clay (Laponite®) that dispersed in water originates a charged colloidal system of nanometer-sized discotic platelets with inhomogeneous charge distribution and directional interactions. The system spontaneously evolves with waiting time from a liquid phase to an arrested equilibrium gel for low concentration ($C_w$ < 2.0 %) [1] and to a Wigner glass for high concentrations ($C_w$ ≥ 2.0 %) [2] with a glass-glass transition [3] for very long waiting times.

The same techniques have been also applied to the investigation of the dynamical behaviour of Interpenetrated Polymer Networks (IPN) microgels of poly(Nisopropylacrylamide) (PNIPAM) and poly(acrylic acid) (PAAc) that have independent sensitivity to both temperature and pH. This novel class of responsive microgels has recently become very popular since their smart responsivity to external stimuli makes them very attractive for industrial applications and excellent model systems for exploring exotic behaviours due to their softness. For this system the slowing down of the dynamics approaching the arrested state is obtained by increasing particle concentration [4] and can be tuned by changing PAAc concentration [5].

Both systems discussed are good candidates for pharmaceutics and medicine applications like drug delivery and tissue repairing thanks to the biocompatibility of Laponite® [6] and to the multiresponsivity of IPN microgels [7].

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# INVITED LECTURES

# PNIPAM CONONSOLVENCY IN WATER-METHANOL SOLUTIONS

*Andrea Pica*[a], *Giuseppe Graziano*[b]

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Cononsolvency refers to the experimental finding that a polymer is swollen in both solvent A and solvent B, but collapses in their mixtures. In particular, poly(N-isopropylacrylamide), PNIPAM, has a coil conformation in both pure water and pure methanol, at 20 °C and 1 atm, but assumes a globule conformation in methanol-water solutions, over the $0.1 \leq X(MeOH) \leq 0.4$ methanol molar fraction.[1] This strange phenomenon has recently been rationalized by claiming that: (a) MeOH molecules are able to bind two distant monomers in the chain, driving collapse;[2] (b) the preferential binding of MeOH stabilizes globule conformations due to a conformational entropy gain of the chain.[3] In the present work a self-consistent application of the approach already used to rationalize the effect of sodium salts,[4] urea and tetramethylurea[5] on PNIPAM collapse leads to a different explanation. The emerging scenario is that cononsolvency is caused by the fact that, on adding methanol, the competition between water and methanol molecules to make attractive interactions with PNIPAM surface causes a decrease in the magnitude of attractive energy with respect to the pure water situation, for basic geometric reasons. Polymer chains collapse to reduce this geometric frustration.[6,7] Recent MD simulations[8,9] confirm this scenario.

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## LOW-TEMPERATURE DYNAMICAL TRANSITION IN CONCENTRATED MICROGELS

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Since long, hydrated protein powders are known to undergo a low-temperature dynamical transition, i.e. the onset of anharmonic atomic flexibility, connected with the activation of biological functionality. We provide the first observation of a "protein-like" dynamical transition in nonbiological aqueous environments. To this aim, we exploit the popular colloidal system of poly-Nisopropylacrylamide (PNIPAM) microgels, extending their investigation to unprecedentedly high concentrations, in a regime comparable to those of hydrated protein powders. Owing to the heterogeneous architecture of the microgels, water crystallization is avoided in concentrated samples, allowing to monitor atomic dynamics at low temperatures. By elastic incoherent neutron scattering and atomistic molecular dynamics simulations, we find that a dynamical transition occurs at a temperature of about 250 K in all water-containing samples, whereas the transition is smeared out on approaching dry conditions. The quantitative agreement between experiments and simulations provides evidence that the transition occurs simultaneously for PNIPAM and water dynamics. In addition, the numerical simulations provide a detailed description of the molecular origin of the dynamical transition in these systems, showing how it crucially depends on the water-macromolecule coupling.
The similarity of these results with hydrated protein powders supports the idea that the dynamical transition is a generic feature of complex macromolecular systems, independently from their biological function.

## SWELLING BEHAVIOUR IN HYDROGELS: A STRUCTURAL AND MOLECULAR POINT OF VIEW

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During the past decades, hydrogels have found many uses as novel materials for a variety of applications in the field of biomedical engineering, food products, agriculture, etc. Thanks to their high water content, elastic texture and biocompatibility, many hydrogel-based networks have been designed and fabricated as intelligent carriers of drugs and they find important applications also in regenerative medicine. Water plays an important role in hydrogels since the physical properties and state of the water confined in the polymer network can significantly affect the hydrogel stability and function. For example, the water content and therefore the volume of hydrogels can vary in response to external conditions of temperature, pH and ionic strength through swelling/shrinking phenomenon. Moreover, the rate and degree of hydrogel swelling are the most important parameters which control the diffusion and release of active pharmaceutical ingredients or drugs inside the polymer network of hydrogels.
Despite the wide literature concerning the development of formulations of hydrogels, a thorough knowledge of the complex structural and molecular arrangements leading to the superior properties exhibited by these materials is still lacking. In this contribution, we will propose a multi-scale approach for investigating the complex swelling phenomena in polymeric hydrogels from a molecular and structural point of view. We will combine the information extracted by different and complementary experimental techniques, i.e. synchrotron-based UV Resonant Raman scattering, Brillouin Light Scattering and Small Angles Neutron Scattering, in order to provide insights on the properties of both polymeric and water species, with the final goal to clarify how the microscopic behaviour of the system is reflected in the water uptake of hydrogels.

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# FROM THE LUNGS TO THE BRAIN: THE FANTASTIC VOYAGE OF NANOPARTICLES TARGETING BETA-AMYLOID (αβ)

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The brain is always confronted with the dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites. Endothelial cells, forming together with other cells the blood-brain barrier (BBB), are known as the "gatekeepers" of this trafficking. Recent applications in nanomedicine focus on nanoparticles and liposomes as they are promising tools for site-specific delivery of drugs and diagnostic agents, through the possibility to functionalize their surface with target-specific ligands. Treatment options for Alzheimer's disease (AD) are limited because of the inability of drugs to cross the BBB. We disclosed that intraperitoneal administration of liposomes functionalized with phosphatidic acid and an ApoE-derived peptide (mApoE-PA-LIP) reduces brain beta-amyloid (Aβ) burden and ameliorates impaired memory in AD mice. Among the different administration routes, pulmonary delivery is a field of increasing interest not only for the local treatment of airway diseases but also for the systemic administration. Here we proved that mApoE-PA-LIP are able to cross the pulmonary epithelium in vitro and reach the brain following in vivo intratracheal instillations. Lung administration of mApoE-PA-LIP to AD mice significantly decreases total brain Aβ (–60%; $p < 0.05$) compared to untreated mice. These results suggest that pulmonary administration could be exploited for brain delivery of nanoparticles designed for AD therapy.

# UPDATE ON WATERS AT THE MEMBRANE INTERFACE AND COLLECTIVE DYNAMICS IN PHOSPHOLIPID BILAYERS

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The cell membrane is now known to be highly complex and specialized molecular machine that performs a wide variety of roles in cellular function. Its major structural components are phospholipids, that are organized in bilayer, as a consequence of the hydrophobic interaction with the water surrounding them. The structure and dynamics of water at the interface were extensively studied and found to be strongly dependent on the hydration level and specific interaction with phospholipids. Despite the intense studies, the details in the hydration processes of lipid bilayers are still lacking, and this hinders the design of biomimetic systems for specific medical and biotechnological applications.

In this talk I will discuss the results of an in-depth FTIR analysis of DMPC phospholipid bilayers at different hydration levels, showing the existence of four different hydrogen-bonded populations. In this study, intensities of the water stretch absorptions were used to determine the amount of bound water, whereas hydration dependent changes of the bands corresponding to the vibrations of the lipid moieties at the interface were examined to detail the water-lipid interactions.

Furthermore, the results of a recent Brillouin neutron spectroscopy investigation of the picosecond collective dynamics in DMPC will be also presented, showing the existence of multiple low energy optical modes that are dynamically coupled with acoustic modes, displaying typical avoided crossing. These optical vibrational modes are believed to be potentially involved in the vibrational energy transfer and in the hydrogen-bond dynamics at the membrane-water interface.

# THE HYDROGEN-BOND NETWORK OF WATER CONFINED BY PHOSPHOLIPID MEMBERANES

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When water is confined by phospholipid membranes, the dynamical properties and the density of bulk water are recovered at relatively short distances, i.e., ~1.0 nm. On the other hand, we have recently shown that, using classical molecular dynamics simulations and an highly sensitive order parameter, the intermediate range order of bulk water is recovered only at distances above 3.5 nm from the fluctuating surfaces. Here I will show that further insights on the effects of confinement on the properties of water can be accessed by probing the hydrogen-bond network of liquid water, confirming that the phospholipid membranes perturb the properties of confined water at distances of up to 4.0 nm

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# MEMBRANE INTERACTION BY α-SYNUCLEIN: A KEY PLAYER IN NEURONAL COMMUNICATION AND PARKINSON'S DISEASE

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The aggregation of α-synuclein (αS), a neuronal protein that is abundant at the pre-synaptic terminals, is associated with a range of highly debilitating neurodegenerative conditions including Parkinson's Disease. Fibrillar aggregates of αS have been identified as major constituents of proteinacious inclusions known as Lewy bodies that form in dopaminergic neurons of patients suffering from these conditions. The function of αS, however, is currently unknown, with evidences suggesting a role in the regulation of the trafficking of synaptic vesicles.

We have studied the interactions of αS with neuronal membranes, as these appear to influence the biological behaviour of both monomeric and aggregated species of the protein. We elucidated the membrane binding properties of the normal form of αS [1-3] and the interactions that drive the neurotoxicity of its pathological oligomers [4]. Our studies enabled to identify the fundamental structural characteristics driving such toxicity, including a highly lipophilic element that promotes strong interactions with biological membranes and a fibrillar region that inserts into lipid bilayers and disrupts their integrity.

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# LOCAL STRUCTURAL AND DYNAMICAL PROPERTIES OF WATER AT THE INTERFACE WITH PHOSPHOLIPID MEMBRANES

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The structure and function of biological membranes is greatly determined by the properties of hydration water. In addition to establishing its bilayer structure, interfacial water also plays an important role in the tasks that the cell membrane performs, mainly related to transport and signalling functions, since it mediates the interaction between membranes and solutes such as ions, proteins, DNA and other membranes. For these reasons it is essential to have a proper description of the structural and dynamical properties of water at the interface with membranes.

In this contribution we discuss the properties and role of interfacial water. We investigate using all-atom molecular dynamics simulations the properties of confined water in between interacting phospholipid membranes [1]. We show that both the translational and rotational dynamics of water molecules exhibit a monotonic dependence with the distance between the confining membranes. We interpret the results using a layering model of water molecules with the help of a local definition of distance to the membrane. We demonstrate that both the rotational and translational dynamics of water molecules depend monotonically on the local distance to the membrane, defining three regions with distinctive water behavior: inner region, first hydration shell and outer region [2]. In addition, we evaluate how the local tetrahedral arrangement of liquid water is affected by the proximity to the membrane [3]. Finally, we also use this decomposition to evaluate the dependence of water-water and water-lipid interactions on the local distance to the membrane.

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# AMYLOID β-PEPTIDES INTERACTION WITH MEMBRANES

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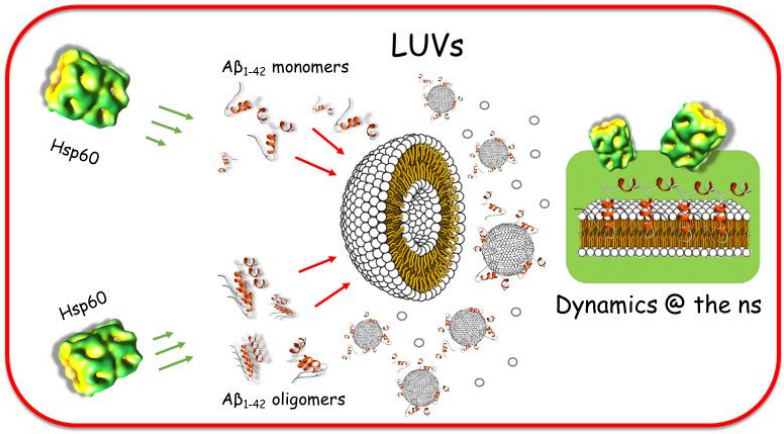
A remarkable biological issue concerns the specificity of lipid–protein interactions. The first question is related to the way intrinsic membrane proteins interact with the lipids that surround them in a membrane and the topic extends until the interaction between membranes and extracellular peptides. This is the case of the interplay between amyloid β-peptide (Aβ), associated with Alzheimer's disease (AD), and membranes of cells in the human brain. The understanding of Aβ influence on the structure [1] and on the dynamical features of model membranes is clearly a crucial molecular challenge [2].




The results of a structural investigation by Small Angle X-ray Scattering [1] and of a dynamic study by neutron-scattering [3] on the interaction of large unilamellar vesicles, as cell membrane models, with both freshly dissolved Aβ and early toxic prefibrillar oligomers, will be presented. By Small Angle Scattering the structural features of model membranes in presence of Aβ species in different aggregation states were compared, and by Neutron Spin Echo their dynamical behaviour was investigated. Also, the effect of coincubating the Aβ-peptide with the chaperonin Hsp60, which is known to strongly interact with it in its aggregation pattern, was described. Neutron Spin Echo results proved the existence of a noticeable interaction between model membranes and both freshly dissolved and aggregate Aβ species. Membrane stiffness increases after Aβ is released in solution. On the other side, the presence of even very low amounts of Hsp60 maintains unaltered the elastic properties of the membrane bilayer. These results can be related to the ability of the chaperonin to interfere with Aβ aggregation, by the specific recognition of an Aβ-reactive transient species. Hsp60, according to our results, is capable to assist to misfolding events, playing the suitable role of a chaperonine escaping the onset of the aggregation cascade and the consequent insult on the cellular membrane.

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# MAPPING THE ELECTRIC POLARIZATION OF DRY AND HYDRATED BIOMEMBRANES AT THE NANOSCALE

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The electric polarization properties of biomembranes play an important role in various areas of science and technology. For instance, cell membrane dielectric properties are key in bioelectric phenomena such as membrane potential formation, action potential propagation or ion membrane transport. On the other side, the main electro-transducing mechanisms used in electrical and electrochemical capacitance or impedance biosensors are based on changes in the dielectric properties of biolayers. Here I will present an overview of a scanning probe microscopy technique, referred to as Scanning Dielectric Microscopy, we developed to measure the dielectric properties at the nanoscale [1,2], and detail its application to supported biomembranes [3-5]. Examples will be shown for bacteriorhodopsin layers, lipid bilayers, cholesterol layers, and mixed lipid bilayers, on both dry [3] and hydrated [4,5] conditions. The results presented will be compared with those obtained on single bacterial cells on dry and hydrated conditions [6,7] and discussed in terms of the level of hydration of the biomembranes and on the dielectric properties of biological water [8].

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# NEMATIC, CHOLESTERIC AND SMECTIC ORDERING OF DNA NANOPARTICLES

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Self-assembly is the spontaneous formation through free energy minimization of reversible aggregates of basic building blocks. The size of the aggregating units can vary from a few angstroms to microns, thus making self-assembly ubiquitous in nature and of interest in several fields, including material science, soft matter and biophysics. Through self-assembly it is possible to design new materials whose physical properties are controlled by tuning the interactions of the individual building blocks. A relevant self-assembly process is the formation of filamentous aggregates (i.e. linear chains) induced by the anisotropy of attractive interactions. Examples are provided by micellar systems, formation of fibers and fibrils, solutions of long duplex B-form DNA, filamentous viruses, chromonic liquid crystals (LCs) as well as inorganic nanoparticles. If linear aggregates possess sufficient rigidity, the system may exhibit liquid crystal phases above a critical concentration. In order to grasp a physical understanding of this complex behavior, building on the venerable Onsager theory, we developed few years ago a novel theoretical approach for these self-assembly-driven LCs. Noticeably, our theory contains no adjustable nor fitting parameters. Predictions for the isotropic-nematic transition have been carefully tested in two simple model systems, namely bifunctional polymerizing hard cylinders [1] and bent-cylinders [2] by using Monte Carlo simulations. Theoretical results for isotropic-cholesteric transition and for helical ordering in the cholesteric phase have been tested in a real system – i.e. a water suspensions of short DNA duplexes – where the chirality of the constituent building blocks induces the formation of a chiral nematic (cholesteric) phase [3]. Among all LC phases observed in self-assembly-driven LCs based on DNA, the smectic one was elusive so far. Building on DNA versatility in creating novel constructs and our former theoretical understanding of self-assembly-driven LC phases we designed three DNA sequences which self-assemble at room temperature into a nanoparticle about 50 nm long comprising of two double-stranded DNA duplexes linked together by a DNA filament 13 nm long. As shown in Figure 1a, this nanoparticle resembles a nunchaku (see Fig. 1b), which is the traditional weapon of several martial arts, such as kung-fu and ju-jitsu, their size being 30 millions times smaller though. We have provided unambiguous and clear evidence through experiments and numerical simulations that a water suspension of these synthetic DNA nanonunchakus form smectic phases. In addition, computer simulations of a suitable DNA coarse-grained model (Fig. 1c) allow us to afford some insight into the physical mechanism underlying the formation of such smectic phase [4].

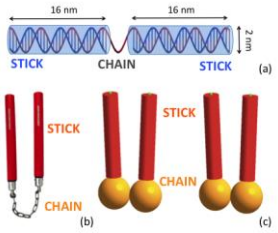


Figure 1 DNA nanonunchaku (chain-stick): (a) molecular structure (b) real nunchaku (c) coarse-grained model for computer simulations.

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# INSIGHTS INTO NONCANONICAL DNA STRUCTURES AND THEIR BINDING PROPERTIES IN AQUEOUS ENVIRONMENTS

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Based on sequence composition and environmental conditions, DNA can adopt different conformations which delineate its function [1]. Indeed, aside from double helix, DNA is capable of forming alternative structures such as G-quadruplex and i-motif. These so-called noncanonical DNA structures are involved in many relevant biological processes, and their formation has also been associated with a number of human diseases [2]. In addition, their unique nanoscale geometry, biocompatibility, biodegradability, and molecular recognition capacity, have made noncanonical DNA motifs promising candidates for the development of novel functional nanomaterials and nanodevices [3].

Actually, most biological and aqueous systems contain cations and other cosolutes that strongly influence such DNA structures. For example, the presence of crowding and/or dehydrating agents, used to mimic the cellular environment, affects the stability of noncanonical structures, their folding/unfolding kinetics, as well as their binding processes with proteins or small ligands [4,5].

In this frame, here I will discuss some aspects concerning the factors affecting the conformational stability and binding properties of noncanonical DNA structures in aqueous environments. Understanding of these aspects is extremely important in view of biomedical and nanotechnological applications of such nucleic acid molecules.

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## ON ENCODING THE FOLD OF G-QUADRUPLEXES

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Biomolecule folding is a central puzzle of biophysics. In DNA G-quadruplexes, a guanine-rich nucleotide sequence quickly and reliably winds its way into a preordained three-dimensional shape. Yet despite over a decade of research, it's not fully understood just how that happens, nor is it possible to predict a G-quadruplex final structure from its nucleotide sequence. Solving the mystery could enable the design of G-quadruplexes from scratch for therapeutics, diagnostics, and other biotechnology applications. It could also facilitate understanding mechanisms of G-quadruplex functions in genomes.

In attempting to gain some understanding on how the folding of G-quadruplexes can be controlled, we have examined the folding of over 200 primary sequences, to identify and parametrize key structural elements. These features were then used to demonstrate control of design of G-quadruplex architectures through primary sequence and choice of environmental conditions. Additionally, we identified common causes for instances of failure to form the target topology we term G-quadruplex "collapse". This understanding marks a significant step in prediction of G-quadruplex folding.

An added bonus in these studies has been the fact that the large scale of this study has required the development of methods for rapid biophysical characterization including near- and far-UV, fluorescence and solution NMR spectroscopy. These methods are expected to expand the scale of future G-quadruplex studies by enabling rapid accurate and time-efficient characterization of structural features.

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# UV RESONANT RAMAN SPECTROSCOPY OF G-QUADRUPLEX DNA DIFFERENT TOPOLOGY STRUCTURES WITH DRUG LIGANDS

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The aim of this project is to study, by means of UV resonant Raman (UVRR) spectroscopy, the interaction between several different topologies of G-quadruplex DNA with two well-known ligands, like Braco-19 and pyridostatin. The preliminary results concern five different sequences able to form parallel, hybrid, and anti-parallel G4 conformations. Three sequences are all known to form parallel G4 structures: the intermolecular quadruplex formed by four units of d(TGGGT), the 22-mer sequence from the promoter regions of the human oncogenes MYC (PDB: 1XAV) and the promoter region of the KRAS human oncogene (PDB: 5I2V). Two additional sequences, the mutant 24-nucleotide human telomeric sequence m-tel24 (PDB: 2GKU), and the thrombin binding aptamer, TBA (PDB: 1C35), are able to form hybrid and antiparallel G4s, respectively.

The detailed procedure for detecting structural changes in the Raman spectra that identify binding sites will be described and results compared with other experimental and computational findings. In particular, CD-melting experiments and preliminary MD simulations have been carried out on two different G4 complexes for which the structures bound to drug molecules have been reported from experiment. Both these results show the stability of the complex with Braco-19, although the loss of one of the K+ ions from the simulated G4 complexes is a significant deviation from the experimental results.

# HYDRATION WATER IN BIOSOLUTIONS FOR CRYOPRESERVATION AND THE ROLE OF THE HYDROGEN BOND NETWORK

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I will show results from molecular dynamics simulations on the slow dynamics of hydration water in different biological solutions upon cooling.
In all these solutions hydration water shows the presence of two different slow relaxations, each one with its dynamic crossovers upon cooling. One of the two relaxations is the alpha-relaxation related to water glassy behavior and present also in the bulk. The other one is characteristic only of hydration water, extremely slow, and it is coupled to the slow protein motions. I will more extensively focus on recent results of water and trehalose, a disaccharide with cryopreserving ability, and I will show that for concentrations for which trehalose molecules form a cluster in the solution hydration water behaves upon cooling similar to protein hydration water. Trehalose clusters show a Trehalose Dynamical Transition (TDT) similar to the Protein Dynamical Transition (PDT).
This TDT is also coupled to the long relaxation that shows a crossover from strong to strong similar to protein hydration water. In the region of mild supercooling, before the well-known fragile to strong crossover takes place, the dynamics of the beta and alpha relaxations of hydration water follows the predictions of the Mode Coupling Theory (MCT). I will in particular focus on results that clearly show the role and the importance of the dynamics of the hydrogen bonds formed between water molecules and between water and trehalose.

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# WATER-PROTEIN DYNAMICAL COUPLING IN FUNCTIONAL AND PATHOLOGICAL PROTEIN STATES

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Hydration water surrounds biological macromolecules such as soluble proteins. Rather than being a mere bystander, hydration water actively partakes in macromolecular biological activity. The first hydration layer on the protein surface is of particular importance for biological activity that is lost in most completely dry proteins. A protein together with its first hydration layer thus forms the biologically active entity. Protein and water molecules are connected by an extended hydrogen-bonded network, the fluctuations of which lead to breakage and formation of water–protein hydrogen bonds that eventually allow for functionally important protein motions at physiological temperatures. The coupling between water and protein dynamics has been, and remains, a matter of extensive debate.
The complex ensemble of water–protein motions can be teased apart by extending experiments and simulations down to cryo-temperatures. I will show how a combination of protein perdeuteration, quasi-elastic neutron scattering, MD simulations and other complementary techniques provides evidence for a general connection between the diffusive behavior of water molecules on a protein surface and the promotion of the large amplitude motions of proteins required for their biological activity. When applied to proteins in aggregated pathological states (so-called amyloid fibers), this approach reveals how dynamics and entropy of hydration water play a key role in promoting protein aggregation and suggests possible strategies for detecting/controlling the evolution of protein pathological degeneration.

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# HYDRATION DYNAMICS IN OSMOLYTE BIOPRESERVATION

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Small compatible osmolytes such as polyols, sugars, free amino acids, and amine oxides are used by a wide range of plants and animals in combating environmental stress due to temperature, salinity, and hydrostatic pressure. The origin of the biopreservative nature of these solutes have often been attributed to their impact on the dynamical properties of water. Co-solute concentration can dramatically alter hydration dynamics in aqueous solutions, however it is unclear whether the water structuring capabilities directly correlate with the biopreservative nature of the osmolyte. We show, using ultrafast fluorescence spectroscopy, that the bulk hydration dynamics are retarded by increasing concentration of compatible cosolutes, but that the connection between dynamical perturbation and protein structural preservation is not simple. Using a range of cyclic polyhydroxy molecules such as monosaccharides, disaccharides, sugar alcohols, and unnatural substituted polyols we show that water structuring does not scale simply with hydroxyl number or size of the cosolute.

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# MANY FACES OF THE PROTEIN-WATER INTERFACE: FROM WETTING OF ACTIVE SITES TO PROTEIN MOBILITY

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Interfacial water at the protein-water interface significantly impacts protein's function, its interaction with the external fields, and its loss of function with lowering temperature. Water not only forms the solvation shell around an enzyme, but also penetrates into the protein interior. Changing the wetting pattern of the protein's active site in response to altering its charge (through a redox reaction) initiates a highly nonlinear structural change and non-Gaussian electrostatic fluctuations at the active site. The activation barrier goes through a minimum corresponding to Schottky's noise when water wets and de-wets the active site with equal probability [1]. Fluctuations of water in protein's hydration shell also determine absorption of radiation by protein solutions [2] and the pulling force on the protein in the electric field gradient [3]. In contrast to predictions of standard models of dielectric interfaces, the microscopic dielectrophoretic susceptibility of proteins in solution is $10^4$ times greater [3]. This finding opens the door to developing separation techniques of proteins by dielectrophoresis. One of the reasons of high dielectrophoretic response of proteins is in their high flexibility coupled to polarized interfacial water. The coupling of protein's elastic fluctuations with hydration shells is terminated at the dynamical transition of the protein when the relaxation time of the interface crosses the observation window [4,5]. This transition is characterized by restoration of the fluctuation-dissipation relation, which is broken at higher temperatures. Characterizing the strength of fluctuations, enzymes possess an effective temperature far exceeding the kinetic temperature of the bath [5]. The breakdown of the FDT leads to a substantial lowering of the activation barrier, thus allowing enzyme's catalytic function [6].

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# ORAL PRESENTATIONS

# DOES HYDRATED GLYCINE ACT AS CRYSTALLIZATION NUCLEUS AT MULTI-KILOBAR CONDITIONS?

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The investigation of aqueous solutions containing biomolecules as a function of thermodynamic parameters, such as the pressure, is crucial for understanding biological processes. Here we report the first low frequency spectra of 1.5 M aqueous glycine from ambient pressure up to 8 kbar, i.e. in the pressure range which is crucial for understanding biological processes under extreme conditions.
We observe a linear pressure dependent blue shift of the specific N-C-C-O open/close mode at ~ 320 $cm^{-1}$ indicating an increasing compression of the solvated glycine. In contrast, the characteristic peak of the hydrogen bond hydration water network centred, at ambient conditions, at ~184 $cm^{-1}$ non-linearly blue shits with increasing pressure but with a slower rate than the intramolecular glycine peak.
This indicates that the macroscopic phase transition observed at a pressure above 8 kbar is driven by hydrated glycine as crystal nucleus.

## QSAR MODELLING OF BLOOD-BRAIN BARRIER PERMEABILITY

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Passage of a drug from circulatory system to central nervous system (CNS) remains one of the biggest challenges in medicinal chemistry as it is crucial for the effective drug delivery to the brain. The blood-brain barrier (BBB) also includes various transporter proteins that can strongly influence the transport of drug to CNS. Due to high cost of experimental measurement of BBB crossing, quantitative structure-activity relationship (QSAR) methods have gained popularity as a cost-effective and rapid method for screening potential drug candidates. Earliest BBB crossing models mainly focussed on molecular descriptors related to lipophilicity of a molecule in a multilinear regression model[1]. In recent years, molecular descriptors with complementary molecular dynamics simulations as well as various machine learning algorithms have been used to predict the BBB crossing ability of drug candidates[2,3,4].

While the computational methods for predicting BBB permeability have advanced, quality of experimental data still remains one of the main causes of low prediction power of the QSAR models. In this talk, quality of available datasets and their effect on the accuracy of various QSAR models will be discussed with emphasis on offering models with wide applicability domain. As the ionization equilibrium of a molecule and other solvent effects have significant influence on its BBB permeability. Therefore, methods for taking this into account, such as modelling acid-base equilibrium, hydrophobic/hydrophilic effect etc will be covered.

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# WATER DISPLACEMENT DURING MACROMOLECULAR ASSEMBLY: FROM SUPRAMOLECULAR CHEMISTRY TO NEURODEGENERATIVE DISEASES

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Water molecules are active players in many macromolecular processes occurring in aqueous solutions. Waters can directly mediate molecular interactions by acting as bridges between partnering surfaces. A less evident, yet crucial, contribution can arise from the dehydration of macromolecular interfaces during binding. Such water displacement can play a key role for the spontaneous assembly of molecules into nanostructures, including host-guest interactions in supramolecular chemistry and protein self-assembly. In this context, I will overview some examples where the the study of molecular hydration has revealed the hidden energy terms that drive macromolecular assembly.

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# HYDRODYNAMIC EFFECTS ON β-AMYLOID PEPTIDE AGGREGATION: FROM DISORDERED COAGULATION AND LATERAL BRANCHING TO AMORPHOUS PREFIBRILS

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Computer simulations based on simplified representations are routinely used to explore the early steps of amyloid aggregation. However, when protein models with implicit solvent are employed, these simulations miss the effect of solvent induced correlations on the aggregation kinetics and lifetimes of metastable states. In this work, we apply the multi-scale Lattice Boltzmann Molecular Dynamics technique (LBMD) to investigate the aggregation of the amyloid Aβ16-22 peptide. LBMD includes naturally hydrodynamic interactions (HIs) via a kinetic on-lattice representation of the fluid kinetics. The peptides are represented by the flexible OPEP coarse-grained force field.

We show that HIs clearly impact the aggregation process and the fluctuations of the oligomer sizes by favouring the fusion and exchange dynamics of oligomers between aggregates. HIs also guide the growth of the leading largest cluster [1]. We follow the formation and growth of a large elongated aggregate and its slow structuring. We observe a variety of coagulation events coexisting, including lateral growth. The latter mechanism, sustained by long-range hydrodynamic correlations, can create a large branched structure and prefibril hosting annular pores [2].

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# CONFINED WATER: SAME SOLVENT, DIFFERENT PROPERTIES

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Water confined in nanometer-sized environments is known to have structural and dynamical properties different from the bulk liquid. These changes upon nanoscale confinement are relevant for a number of different fields, e.g. biological systems, flow and ion transport in organic and inorganic media, the properties of materials like cements, and nanoparticles in solution. Whether and how the interface properties of the confining medium, such as the geometry or its hydrophilic/hydrophobic character, affect the confined water is still not well understood at the molecular level. [1]
Recently, there is an increasing interest on actual chemical reactions in the aqueous phase under confinement. Weakly interacting confining environments are under investigation to avoid the strong interactions of both reactants and water with charged or highly polar groups, which make hard to separate the specific nanoconfinement effects from those depending on the nature of the interface. [2] In particular, the role of confined water in nanocatalysis still remains elusive.
THz-FTIR spectroscopy, which is able to probe the intermolecular collective dynamics of bulk and hydration water as well as the low-frequency modes of solutes in solution [3, 4], is ideally suited to tackle these challenges.
We report here results on water confined in different systems, with implications for nanoconfined chemical reactions, by broad-band linear THz-FTIR spectroscopy in the frequency range from 30 to 660 $cm^{-1}$ (1-20 THz).

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# PHASE TRANSITION ANALYSIS BETWEEN LOW DENSITY AND HIGH DENSITY NANOCONFINED WATER

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We perform Monte Carlo simulations of a coarse-grained model for confined water that includes many-body interactions associated to water cooperativity. The model is computationally efficient and allows us to consider systems with a great number of molecules at extreme conditions. Our results show the presence of a liquid-liquid phase transition (LLPT) ending in a liquid-liquid critical point (LLCP) between a low density liquid (LDL) and a high density liquid (HDL) forms of water, and a smooth structural change between HDL and very high density liquid (VHDL), recalling the structural transformation occurring among LD-amorphous, HD-amorphous and VHD-amorphous. Our results are consistent with atomistic simulations and experiments and clarify fundamental properties of water at the supercooled region.

# BIOPHYSICAL PROPERTIES OF DNA IN IONIC LIQUID PROBED BY UV-RESONANCE RAMAN SCATTERING

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Deoxyribonucleic acid (DNA) is one of the most important biomacromolecules thanks to its unique capability to contain the genetic code, essential for the growth and the functionality of living organisms. Although the DNA is considered stable in aqueous solution, slow hydrolysis can damage the double-helix structure and cause denaturation when it is stored for several months. Therefore, the design of new like-aqueous media that can stabilize and maintain DNA conformation for a long period is a challenging issue. The usage of ionic liquids (ILs) as alternative solvent for DNA biotechnology is attractive thanks to their unique properties, such as low toxicity, good solubility and high conductivity.

In this framework, we focused on understanding how the ILs influences the structural stability of DNA. More precisely, we have investigated the thermal denaturation of DNA in imidazolium-based ILs/water solutions by UV resonant Raman (UVRR) scattering performed at Elettra-Sincrotrone Trieste. We have revealed the role played by both DNA/ILs ratio and different ionic species in ILs structure in stabilizing/destabilizing the DNA natural conformation. The synchrotron-based UV source for UVRR measurements allowed us to enhance specific vibrational signals associated to nitrogenous bases, through an appropriate tuning of the excitation wavelength. Such approach permits to identify the molecular mechanism responsible to different stability induced by ILs on specific nucleotides. Moreover, Raman results have been corroborated by UV absorbance and circular dichroism analyses in order to obtain a comprehensive picture of the molecular interactions involved in DNA/ILs systems.

The results of our work may provide more insight into the studied system, allowing a better understanding the ILs-DNA binding and expanding the overall capabilities and applications of ILs in biological and biomedical applications.

## ROLE OF STACKING DISORDER ON THE BARRIER AND PATHWAY OF ICE NUCLEATION

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Ice formation, mainly consisting of ice nucleation and growth, is a ubiquitous phenomenon. The ability to predict and control the formation of ice crystals is crucial in a variety of fields from atmospheric science, to food preservation and cryopreservation of cells and organs. Accurate predictions, however, hinge on good estimates of ice nucleation rates. Such rate predictions are based on extrapolations using classical nucleation theory, which assumes that the structure of nanometer-sized ice crystallites corresponds to that of hexagonal ice, the thermodynamically stable form of bulk ice. However, simulations with various water models find that ice nucleated and grown under atmospheric temperatures is at all sizes stacking-disordered, consisting of random sequences of cubic and hexagonal ice layers. This implies that stacking-disordered ice crystallites either are more stable than hexagonal ice crystallites or form because of non-equilibrium dynamical effects, with both scenarios challenging central tenets of classical nucleation theory. Here we use rare-event sampling and free energy calculations with the mW water model to show that the entropy of mixing cubic and hexagonal layers makes stacking-disordered ice the stable phase for crystallites up to a size of at least 100,000 molecules. We find that stacking-disordered critical crystallites at 230 kelvin are about 14 kilojoules per mole of crystallite more stable than hexagonal crystallites, making their ice nucleation rates more than three orders of magnitude higher than predicted by classical nucleation theory. This effect on nucleation rates is temperature dependent, being the most pronounced at the warmest conditions, and should affect the modelling of cloud formation and ice particle numbers, which are very sensitive to the temperature dependence of ice nucleation rates. We conclude that classical nucleation theory needs to be corrected to include the dependence of the crystallization driving force on the size of the ice crystallite when interpreting and extrapolating ice nucleation rates from experimental laboratory conditions.

# ON THE POSSIBILITY OF (MICRO)PHASE SEPARATION IN METHANOL-WATER MIXTURES: FIRST DIRECT EXPERIMENTAL EVIDENCE

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Mixtures of water and simple alcohols, like methanol and ethanol, are well known in our everyday life and, most importantly from our present point of view, serving as evergreen subjects of scientific research (see, e.g., [1]). A particularly intriguing, still not completely understood, issue is the presence (or absence) of 'micro-heterogeneities' in (e.g.) methanol-water mixtures (for an influential article on this topic, see [2].) Despite the many (and many times, speculative) articles claiming that 'microphase separation' does happen, no unequivocal experimental evidence, such as a small angle scattering signal, has been presented so far for mixtures of water and the simplest alcohol.

Determining the structure of materials containing hydrogen is one of (if not) the most difficult in the area. X-ray diffraction is not a sufficiently sensitive probe for hydrogen in most of the cases (including the case of $H_2O$ and $CH_3OH$), so that neutron diffraction with H/D substitution seems to be the only feasible route for deriving more detailed information on the microscopic structure of hydrogenous (i.e., those that contain $^1H$) systems. The main difficulty in 'protonated' liquids is the huge incoherent inelastic scattering that arises due to the exceptionally high level of spin-incoherency of the proton ($^1H$). As a result, more than 90 % of the measured signal (using non-polarized neutron beams) from pure $H_2O$ (as well as from pure $CH_3OH$, methanol) is useless ('background') from the structural point of view (see, e.g., [3]). Spin-incoherence, however, can be bypassed if the neutron beam is polarized. We have previously shown that using appropriate instrumentation, it is possible to measure accurate (coherent) static structure factors of water samples, containing a varying proportion of $^1H$ [4].

A major novelty of our most recent polarised neutron diffraction experiments (instrument D7 of the ILL, France) was we have managed to change the H/D ratio so that the methyl groups (i.e., the hydrophobic parts of methanol molecules) have always clearly been (H/D) contrasted with the hydrophylic OH-groups. By determining the coherent part of scattering directly, clear features have been detected at smaller angles (low values of the scattering variable) for methanol-water mixtures in the water rich regime. (Note that this is in striking contrast with the findings of Ref. [2].) Molacular dynamics computer simulation results, when calculated for exactly the same H/D compositions as used in the experiments, are in qualitative agreement with experimental data.

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# POSTER PRESENTATIONS

# SYNTHESIS AND PROPERTIES OF SILVER NANOPARTICLE AND PARACETAMOL CONTAINING FIBROUS MESHES

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Poly(amino acids) and their derivatives are a promising new class of biomaterials, but one of their drawbacks is their relatively difficult synthesis and functionalization. Using polysuccinimide this issue can be solved, since polysuccinimide (PSI) is prone to react with nucleophiles under mild conditions therefore resulting in the water soluble and biocompatible poly(aspartic acid) (PASP) and its derivatives. Electrospinning is a versatile method for polymer processing that can be utilized to fabricate non-woven fibrous polysuccinimide meshes containing small-molecule drugs and even various nanoparticles. These so-called nanocomposites could be used as multifunctional wound dressing materials, since the incorporation of silver nanoparticles (AgNP) could provide antibacterial characteristics [1,2] while incorporating paracetamol provides analgesic properties to the fibrous system.

The aim of our research was developing a one-pot method for the synthesis of AgNPs and subsequently creating a novel antibacterial wound dressing system. AgNPs were synthesized by chemical reduction method in the presence of PSI and paracetamol then the resulting nanoparticles were characterized with DLS. By adjusting the synthesis and electrospinning parameters, the procedure was optimized for both the nanoparticle synthesis and the mesh fabrication. The fibrous structure of the meshes was examined by SEM. For the evaluation of antibacterial properties disc diffusion tests were performed using *E. coli*. Release kinetics of paracetamol was studied using UV-VIS spectroscopy as a function of the paracetamol concentration of the meshes. The release profile was investigated in different aqueous release media as well, along with the hydrolitical stability of the meshes.

Synthesis and fabrication was successfully performed resulting in drug-loaded fibrous PSI membranes containing AgNPs. The antibacterial studies confirmed that silver-content hinders the colonization of the meshes by E. coli. Based on the release kinetics study, the synthesized antibacterial meshes proved to be suitable for prolonged drug-release as well.

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# PHYSICO-CHEMICAL CHARACTERIZATION OF HALLOYSITE (HNTs)-POLYMER SYSTEMS IN AQUEOUS SOLUTION

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Studies on stability of halloysite nanotubes (HNTs) in aqueous polymer dispersions are conducted. HNTs are interesting for recent research because they are considered "green" materials and have versatile properties such as hollow tubular morphology, large specific area, tunable surface chemistry, high mechanical strength and thermal stability.[1,2] They are already used in the preparation of polymer systems as transport/drug delivery and in treatment wastewater, or as reinforcing agents. Selective absorption inside or outside nanotubes can be controlled by electrostatic force.[3] Three polymers with different surface charge, an anionic (pectin), a neutral (hydroxypropylcellulose, HPC), a cation (chitosan), are chosen. HNTs and polymers concentrations are respectively 1 and 0.1 wt% in all dispersions. Turbidimetric technique is used to evaluate the stability of functionalized nanotubes in water. Turbidimetry shows that aqueous dispersion of HNTs in presence of HPC is more stable than others. Viscosity measurements prove that dispersed particles stability in solution was not due to a kinetic effect. Polymer adsorption onto HNT surface is investigated by isothermal titration calorimetry (ITC). The experimental data are interpreted on the basis of a model of adsorption of the polymer on the nanoparticle and they allowed the calculation of the standard variation in free energy, enthalpy and entropy of the process. ITC was used to interpret turbidimetric data.□□□Potential clarifies surface charge properties of functionalized nanotubes upon polymer absorption. Measurements are conducted by varying polymer concentration or pH, respectively. Different trends are obtained for the various systems. This behaviour confirms different interaction mechanism between the components (HNTs-polymer). Chitosan, with its positive charges, could prefer to interact to negative external nanotube surface; pectin, instead, could interact with the inner nanotube surface. Both polymers, however, stabilize HNT particles in water according to electrostatic mechanism. A trend of □-potential almost constant is obtained with HPC, so the better stability of this system could be attributed to a steric rather than electrostatic stabilization.

Modified nanotubes by interactions with polymers may lead to the formation of colloidal systems with controlled stability and it offers different perspectives of new applications (area of cosmetics and medical implants) of these dispersions such as good carriers for substances to be released in response to external stimuli.

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# HYDRATION PROPERTIES OF MODEL AMPHIPHILIC MOLECULES BY RAMAN AND IR SPECTROSCOPY

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Tert-butyl alcohol (TBA) and trimethyl-amine-N-oxide (TMAO) are two biorelevant, water-soluble molecules, which share the same hydrophobic molecular portion and possess different polar moieties. Despite their similar structure, TBA acts as a denaturant towards globular proteins while TMAO is an osmolyte that protects their native state and counteracts the action of other protein destabilizers [1]. The molecular mechanisms that govern this different behavior is still unclear, but often the role of solute-induced modifications on surrounding water has been evidenced [1]. Moreover, due to the fact that a large percentage of their solvent-exposed surface area is determined three methyl groups, TBA and TMAO have been also employed as useful models to study hydrophobic hydration and interactions [1-3]. In particular, understanding the organization of water around their hydrophobic groups (hydrophobic hydration) might be relevant for an accurate comprehension of hydrophobic interactions. In this respect, even if a large number of investigations have been performed on these classical systems, their hydration properties are still under debate and there is not a univocal interpretation dealing with solute-water and solute-solute (clustering) interactions, and on their concentration dependence. Recently, a new technique called Raman-MCR has been proposed to examine the hydration shell structure of different molecules [4,5]. This methodology has been employed to analyze the Raman OH stretching spectral distribution and has proven suitable to extract shell-specific structural information. Only very recently this method has been extended to ATR-FTIR spectra to evaluate hydration properties of TBA and proteins [6]. Inspired by these approaches, we carried out a related analysis on both Raman and IR spectral profiles, based on spectral difference (SD) methods for TBA and TMAO aqueous solutions at different concentrations and temperatures. We will present this method as a simple and useful way to obtain reliable quantitative information about the hydration features of the systems considered, including, hydration numbers, aggregation properties and H-bond energy. The results will be discussed in the context of recent literature.

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## VIBRATIONAL AND ELASTIC INVESTIGATION OF LYSOZYME SELF-ASSEMBLING PROCESSES

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Many biocompatible hydrogels are formed by the self-assembly of macromolecules, among these, natural polypeptide-based hydrogels are very promising systems. They are good candidates for tissue engineering and drug delivery since they meet the majority of the design criteria for tailored biomaterials [1,2]. The balance between intramolecular and protein-solvent attractions determines the development of the molecular networks and the folding and unfolding processes are deeply involved in the self-assembly phenomena.

Lysozyme-based hydrogels show very high cyto-compatibility, suggesting that globular protein-based hydrogels may be useful as scaffolds for tissue engineering. In these hydrogels the aggregation processes are characterized by different steps in which the protein undergoes conformational rearrangements and intermolecular association to form stable structures of increasing complexity. The intermolecular β-sheet motif appears to be deeply involved in the self-assembling processes and in the development of hydrogel networks [3]. The hydrogel proprieties, including the elastic-viscous response, can be tuned using the aggregation conditions; under certain environments, lysozyme self-assembles and produces transparent thermo-reversible gels [4,5]. In this work, we have studied the unfolding, aggregation and gelation processes of highly concentrated solution of lysozyme in denaturing conditions at different temperatures. We investigate the protein structural modifications through infrared spectroscopy [6] and the elastic-viscous-thermal proprieties by transient grating spectroscopy [7], with the aim to relate micro- and macro-observables of these promising materials.

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# A SIMPLE ANALYSIS OF BRILLOUIN SPECTRA FROM OPAQUE LIQUIDS AND ITS APPLICATION TO AQUEOUS SUSPENSIONS OF POLY-N-ISOPROPYLACRYLAMIDE MICROGEL PARTICLES

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Brillouin spectroscopy is a powerful technique to probe the viscoelastic properties of materials. However, the phenomenon of multiple scattering makes getting information from opaque liquids quite difficult, thus limiting the use of this spectroscopy. In this paper we present a new method that greatly simplifies the problem of analyzing Brillouin spectra affected by multiple scattering from samples of moderate opacity. Our approach is based on the observation that multiple-scattered contributions broaden the spectrum acquired in external backscattering geometry, while preserving in the external side the information related
to internally backscattered light. The new strategy avoids unnecessary approximations and requires minimum numerical effort to extract physical information. Here [1], we show the results of two Brillouin light scattering experiments performed on prototypical hard and soft colloidal systems. First, measurements on latex suspensions as a function of depth are used to validate the method and to derive new relations between the back-scattered and multiple-scattered components of the Brillouin spectrum. Second, measurements on poly-N-isopropylacrylamide (PNIPAM) microgels in water as a function of temperature are used as a testing ground to demonstrate the method's capabilities. Our analysis confirms that sound waves are extremely sensitive to the volume-phase transition of thermoresponsive particles. The presented approach, however, shows that a marked increase of attenuation is accompanied by only a moderate decrease of sound velocity. The study revises the viscoelastic properties of PNIPAM suspensions; more generally, it provides a new guideline in the characterization of moderately opaque media and fosters new theoretical investigations.

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# TREHALOSE-INDUCED SLOWDOWN OF LYSOZYME HYDRATION DYNAMICS PROBED BY EDLS SPECTROSCOPY

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Extended frequency range Depolarized Light Scattering (EDLS) has been demonstrated to be a powerful tool to access the fast dynamics of water in a diversity of binary systems, including sugars and proteins' aqueous solutions. EDLS enables one to disentangle solute from solvent dynamics and, into the latter, bulk from hydration water contributions [1-8]. Here, it is used for the first time in a ternary system by studying a lysozyme-trehalose aqueous solution over a broad time scale, from hundreds to fractions of ps. We provide experimental evidence that the sugar, present in the ternary solution in sufficient quantity for biopreservation, strongly modifies the solvation properties of the protein. By comparing aqueous solutions of lysozyme with and without trehalose, we show that the combined action of sugar and protein produces an exceptional slowdown of the rearrangement dynamics of a fraction of water molecules around the protein, namely more than twice the corresponding retardation in the absence of trehalose. We also demonstrate that the dynamics of these hydrating water molecules get slower and slower upon cooling. On the basis of these findings [9] and the general agreement with the results of molecular dynamics simulations on the same system [10], we hypothesize that such a hyperslow water close to lysozyme is preserved from crystallization by the presence of trehalose in the mixture, and thus it plays a key role in protecting the biomolecule.

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# THE WATER ASSOCIATION BAND IN MBCO-SACCHARIDES AMORPHOUS SYSTEMS: EFFECTS OF TEMPERATURE AND HYDRATION

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Saccharides are well known for their high efficiency in protecting biostructures against adverse environmental conditions. Bioprotective amorphous saccharide matrices have been studied with many experimental techniques and simulations, with the aim to highlight the modulation of both structure and dynamics of the embedded protein and the surrounding matrix, as well as the structural arrangement of saccharides and water in the protein domain [1]. By exploiting different conditions (type of sugar, concentration of protein and solutes, protein/saccharide ratio [2], hydration, presence of salts or osmolites, pH, temperature, pressure), various hypotheses have been proposed to explain the mechanisms at the basis of the saccharide biopreservation, often with contrasting results [3]. In this work, we report an Infrared Spectroscopy study of dry amorphous matrices of various sugars (the disaccharides trehalose, maltose and sucrose, the monosaccharide glucose, and the trisaccharide raffinose) containing Myoglobin, at different temperatures and hydrations. Water properties are inferred by the study of a peculiar infrared band, the Water Association Band [4]. A differential analysis of peak frequencies and populations of its subcomponents makes it possible to draw information on the properties of the hydrogen bonds structures in which water is involved. At low hydration, different water patterns are present in different sugar systems, independent on the matrix rigidity. In trehalose, the persistence of caotropic, cosmotropic and ice-like water classes stemming from water-protein-sugar interactions points out for a rich, friendly host enviroment.

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# MD SIMULATIONS OF THE ACTIVE AND INACTIVE STATES OF THE HUMAN α7 NICOTINIC RECEPTOR: STRUCTURE ASSESSMENT, ION TRANSLOCATION AND THE ROLE OF WATER

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Nicotinic acetylcholine receptors (nAchRs) are ligand-gated ion channels that regulate chemical transmission at the neuromuscular junction. They are responsible for signal transmission, and their function is regulated by neurotransmitters, agonists, and antagonists drugs. A detailed knowledge of their conformational transition in response to ligand binding is critical to understanding the basis of ligand-receptor interaction, in view of new pharmacological approaches to control receptor activity. The scarcity of experimental structures of human channels makes this perspective extremely challenging.
To contribute overcoming this issue, we built, via homology modelling, and assessed via MD, an all-atom structural model of the human α7 nicotinic receptor in different conformations: the open active state [1] and three different non-conductive conformations: a putative desensitized [2], a closed-locked and an apo-resting conformational state [3]. We carefully compare our structures with available experimental data and computational models of other eukaryotic LGICs, identifying key discriminators among states (in particular *pore hydration, intra protein HBonds networks, water rings in the pore*), providing a detailed structural characterization of the conformational landscape of the human α7 receptor.
We also present results on the single ion PMF in the native and mutated open channel pore and the kinetics of the ion translocation obtained by using the *Milestoning with Voronoi Tessellation* method [4,5].
The method provides a PFM profile compatible with the primary sequence and the protonation state of the protein, giving an estimate of the sodium/chloride permeability ratio in good agreement with experiments.

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# PEG HYDRATION AND CONFORMATION IN AQUEOUS SOLUTION: HINTS TO MACROMOLECULAR CROWDING

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The molecular structural dynamics of PEG in aqueous solution has been addressed with a series of experimental data correlating macroscopic solution properties of PEG with structural and hydration properties at molecular level, by using thermodynamic, UV Brillouin and Raman spectroscopy data. Water activity measured in PEG 600 solutions by a novel dynamical calorimetry approach shows that data reveal some non-equilibrium process for dilute solutions. Brillouin scattering data on aqueous solutions in the UV range made possible the measurement of the viscoelastic relaxation of the system with a characteristic temperature, T□M, as precursor of the glass transition process at lower T, while at constant temperature, the addition of water to liquid PEG 600 first lead to a slight decrease and then to an increase in the solution viscoelastic relaxation consistent with the slowest polymer dynamics observed in the concentrated solution. Specific Raman bands corresponding to trans and gauche conformations of the C–C and C–O bonds and their sequences in the PEG chain have been identified and their relative intensities as a function of concentration evidence non-monotonous variations with a rather unusual concentration dependence for the frequency of C–O bonds in the range of physiological temperatures. The heuristic result is that the extended time-space domains approach suggests an overall quasi-regular solution behavior of semidilute PEG, with opposite concentration dependence at low and at high water content, similar to discontinuities observed in excess thermodynamic properties and eutectic phase diagram.The cross-over of these distinct behaviors occurs at the concentration close to that usually employed to mimic the cellular crowding in biomolecular systems, highlighting the interplay of water molecules in solute-solute interactions.

# WATER AS A NEW MARKER FOR BIOSENSING

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Whenever the water molecules interact with either dipolar or charged systems, the main water dielectric relaxation peak broadens. If a solute is dipolar in nature, new solute-water clusters are created due to dipole-dipole interactions. It leads to the "red shift" of the dielectric loss maximum frequency. In the case of ionic solutions, another cluster structure develops, due to dipole-charge interactions and a "blue shift "is observed. In the general case when a solute molecule has both charged and dipole groups, the dielectric loss maximum demonstrates a "red" or "blue" shift, depending on the entity concentration. In all aqueous solutions, the water-solute interactions can be considered as dipole-matrix interactions in which water is the dipole subsystem. The phenomenological 3D trajectories approach was applied to the results of isothermal dielectric measurements of different concentrations of the following aqueous solutions: Hydrocarbons, NaCl and KCl, AMP and ATP, Amino Acids, Hemoglobin [1-4]. The parameters of the main water peak define a trajectory that can clarify the nature and rate, at which water interacts with the solute. We extend this approach from comparatively simple solutions to the complexity of Red Blood Cells (RBC) suspensions by monitoring the RBC cytoplasm under different external conditions [5,6]. Dielectric measurements of RBC suspensions in the frequency region of 100 MHz to 50 GHz as a function of aging or external glucose concentration also reveal a distinct time point or glucose concentration after which the spectra are radically changed. The conclusion is that the dielectric response of the cytoplasm in microwaves is due to the water therein and its interaction with physiological active components in cytoplasm. This opens a window of opportunity to exploit this for the non-invasive monitoring of diabetes or to non-invasive control of the quality of Stored RBC in a Blood bank in order to manage the inventory.

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## DYNAMICS OF PROTEINS IN GLASSY MATRICES ALONG THE PATH TOWARD UNFOLDING

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In this work, using a biophysical model, the possibility to find a relationship between the fast dynamics and the protein functionality has been explored. The main results we would like to underline are the following: **1)** We verify the view that the protein fast dynamics is dominated by the molecular environment around their surface; **2)** We extended the Lindemann criterion, recently proven for globular mesophilic proteins, to the case of a common thermophilic protein.

The first result is a further indication of the key role played by the environment in determining the protein dynamics. Comparing the MSD results obtained from the study of lysozyme in D2O and in glucose with the ones from thermolysin, it is evident how the trend obtained for both proteins is similar when they are embedded in the glucose matrix. Thermolysin is more than twice as large as lysozyme and moreover, it is a thermostable protein as it has an higher melting temperature, i.e. it has a quite large melting temperature of 370K in the physiological milieu. We quantified the extent of protein thermal fluctuations by assessing the so-called Lindemann parameter, which we have demonstrated to be the same at the melting temperature for thermolysin in glucose as well as lysozyme in water or glucose. This common behaviour is a further step toward the extension of the Lindemann criterion to thermophilic proteins. We found for the Lindemann parameter a value of 0.17 at the protein melting, in agreement with previous results for different proteins. If the Lindemann criterion is true for any protein, we could estimate the protein MSD at melting even a priori. Through this, if we know the trend of a certain protein in a determined environment, we could predict the unfolding temperature of every single protein in every environment with possible applications in all the biotechnological and biomedical fields where the knowledge of the proteome stability is needed. So far, the Lindemann criterion has been verified for the following systems in water: lysozyme (also in glucose and in glycerol), myoglobin, haemoglobin, crambin and bovine serum albumin and now for the first time to a thermophilic protein, thermolysin in glucose. In the following, on the basis of the results obtained within this thesis, it could be interesting to investigate whether the Lindemann criterion holds for different proteins and environments, i.e. if it is an universal feature. A particularly interesting case is that of intrinsic disordered proteins, where one should expect a Lindemann parameter close to that of melting temperature already at room temperature.

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# THERMORESPONSIVE CHITOSAN AND POLY-N-VINYLCAPROLACTAM MICROGELS FOR DRUG EMBEDDED CHEMOEMBOLIZATION

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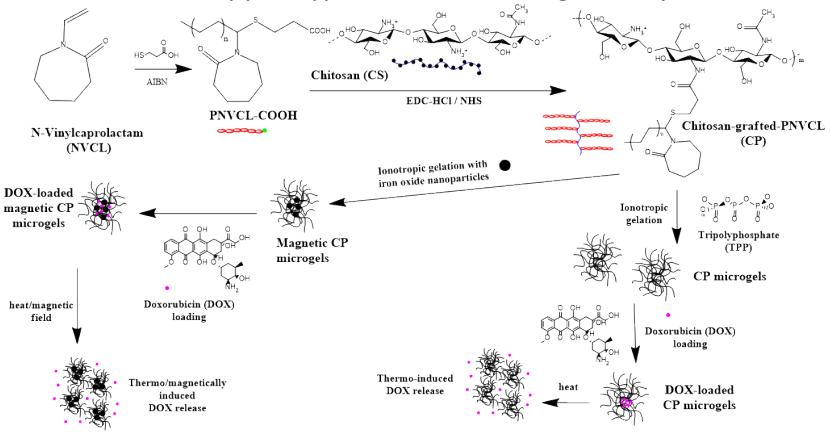
Drug-eluting bead chemoembolization (DEB-TACE) is a relatively new endovascular treatment procedure for transcatheter delivery of chemotherapic drugs to hepatocellular carcinoma (HCC)[1]. In DEB-TACE, the drug is embedded in a polymeric microsphere and slowly released into the blood vessels supplying the tumor[2]. This study focuses on the development of thermoresponsive microgels useful for the implementation of this catheter-directed procedure. A microgel is defined as a nanostructured gel with a size superior than 100 nm (microgels)[3]. By introducing a responsive monomer inside the polymeric gel structure, it can be made responsive to an external stimulus. In the present communication, we report different examples of a biocompatible thermoresponsive polymer[4], poly-N-vinyl caprolactam (PNVCL), each one showing different lower critical solubility temperature in water (LCST) in the range between 32 and 42°C. Additionally, we set-up another biocompatible system than can respond to both pH- and thermal triggers by grafting PNVCL on chitosan (CS). Microgels of both CS and CS-PNVCL (CP) were prepared through ionotropic gelation[5]. Magnetic microgels were also prepared by introducing ferrite nanoparticles (SPIONs) in the polymeric structure to create a system in which a temperature increment can be induced by a magnetic field. Our results show that, depending on the polymeric composition, CP microgels aggregate at a certain temperature in a reversible fashion. In conclusion, this system could provide a useful combinational therapy of hyperthermia and drug delivery for HCC via DEB-TACE.

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# KNUDSEN THERMOGRAVIMETRY FOR THE DETERMINATION OF WATER ACTIVITY IN $CaCO_3$ NANOPORES

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In this study we used Knudsen thermogravimetry to investigate the water evaporation from calcium carbonate porous nanoparticles (NPs). Literature reports that this approach is suitable for a comprehensive understanding of the water behaviour in minerals with nano-sized cavities. For this purpose, inorganic $CaCO_3$ NPs with different sizes, ranging from 700 nm to 3 µm in diameter length, were employed. Hence, by means of the thermal analysis we focused on water behaviour inside the nanopores and we could assess the interactions between the solid and the liquid. In particular, the resulting desorption isotherms allowed us to determine the water adsorption properties and activity onto the $CaCO_3$ nanoparticles surface, and to demonstrate the confinement of water within the NPs pores, being the water activity higher than the one of pure water and in agreement with the Gibbs-Thomson effect.
The water activity was correlated to the NPs porosity. Finally, the kinetics of decarbonatation was investigated by a model free isoconversional method that allowed us to determine the activation energy and its correlation with the surface specific area of the nanoparticles.
These findings represent a crucial step for the development of smart functional nanomaterials that could store some active molecules into their pores, acting as drug delivery systems, or that could be exploited as agents for the treatment of waterlogged archaeological woods.

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# NEW INSIGHTS ABOUT THE INTERACTION BETWEEN PROTEINS AND WATER

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Water and, in particular, the hydration shell have a critical influence on the structure, on the function and on the dynamics of the biomolecules. For example, proteins folding and their functionalities are influenced from the H-bond (HB) network of the surrounding water [1].
Despite protein-water interaction has been widely studied for long time, a deeper understanding of this topic is still lacking. THz spectroscopy [2], Neutron spectroscopy (NS) [3], MD simulations [4], Dynamic Light Scattering (DLS) [5], Raman [6] and Infrared spectroscopies [7] have been widely employed to get information about the structure and the dynamics of such hydration shell.
It has been reported that re-orientational dynamics of water molecules seems to be perturbed mainly by protein local surface topology and by the chemical nature of the solvent-exposed residues [8].
The variation of proteins environment, such as solvent pH changes, temperature jumps, presence of salts and/or alcohols and so on, are likely to provoke protein structural and functional modifications. Proteins react to these external perturbations by partially or completely losing their native state. The loss of their secondary and tertiary structures can lead proteins to adopt a misfolded conformation, where they are prone to manifest a natural tendency to self-aggregate into potentially toxic plaques. In fact, the study of protein aggregation has gained a lot of interest since it seems to be linked to the onset of several degenerative diseases [1, 4-9].
Small organic molecules, such as polyphenols, act as inhibitor and/or disaggregating agent of fibrillary protein aggregates [9]. It is of a great important to reach a deeper understanding about the mechanism of polyphenols-protein interaction and about how the water arrangement is modified by the presence both of the protein and of the antioxidant.
It has been demonstrated how UV Resonant Raman (UVRR) can be employed to study the interaction between water and biomolecules [6], investigating the spectral variations induced by modification of the HB network. By means of Raman spectroscopy we have investigated the variations of the water orientation induced by proteins, both in their native and fibrillary states, and how the water structural conformation is modified by the interaction between natural antioxidants and proteins.

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# STUDY OF THE EFFECT OF BIOLOGICALLY RELEVANT COSOLVENTS IN THERMO-RESPONSIVE PNIPAM-BASED SYSTEMS

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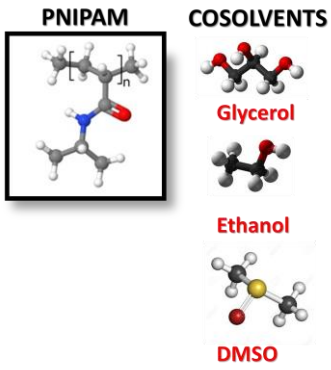
Polymers such as poly-N-isopropylacrylamide (PNIPAM) are known as *smart* materials due to their ability to respond to external stimuli like temperature, pH, pressure, and so on. PNIPAM phase behaviour, in particular, results from a highly temperature-sensitive competition between the hydrophobicity of methyl and methylene groups and the ability of amide groups to form strong hydrogen bonds, in such a way that when the temperature increases above a critical value (lower critical solution temperature, LCST) PNIPAM chains undergo a rapid and reversible coil-to-globule transition, reminiscent of the *folding transition* of proteins [1]. Not only in the room temperature regime but also at lower temperatures there are strong analogies between the behaviour of proteins and PNIPAM-based systems. In a recent work [2] the onset of anharmonic dynamics on the picosecond timescale observed in hydrated PNIPAM at ~250 K has been recognized as an analogue of the protein *dynamical transition*. Therefore, owing to its amphiphilic nature and thermoresponsive behavior, PNIPAM can be exploited as an ideal model system to get new insights into biologically relevant behaviors.




Here we focus on the effect of cosolvents, which are known to impact on the behaviour of proteins by modifying their interactions with water, but the detailed mechanisms underlying these effects are not well understood. A set of spectroscopic tools is used to study different biophysical properties of PNIPAM ternary mixtures. In particular, we investigate by vibrational spectroscopy techniques (Raman and IR) the role of protic and aprotic cosolvents (ethanol and DMSO) in the changes in hydration state of PNIPAM across the LCST, and by neutron scattering techniques the role of glycerol, a well known protein stabilizer, on the PNIPAM "dynamical transition".

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# STRUCTURE OF HUMAN TELOMERE G-QUADRUPLEX IN THEPRESENCE OF A MODEL DRUG ALONG THE THERMAL UNFOLDING PATHWAY

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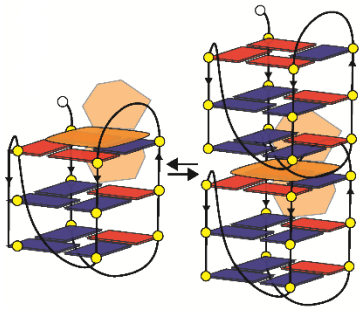
We have conceived a multi-technique approach based on the combined use of circular dichroism, ultraviolet resonance Raman spectroscopy and small angle scattering techniques, to study the interaction between the human telomeric sequence $AG_3(TTAG_3)_3$ and actinomycin D (ActD), an anticancer ligand with remarkable conformational flexibility. We find that at room temperature binding of Tel22 with ActD involves end-stacking upon the terminal G-tetrad. Structural evidence for drug-driven dimerization of a significant fraction of the G-quadruplexes is provided. When the temperature is raised, both free and bound Tel22 undergo melting through a multi-state process, from which two intermediate states emerge. At the end of the thermal cycle, the unfolded state of the free Tel22 is consistent with a self-avoiding random-coil conformation, whereas the high-temperature state of the complex is observed to assume a quite compact form. Such an unprecedented high-temperature arrangement is caused by the persistent interaction between Tel22 and ActD, which stabilizes compact conformations even in the presence of large thermal structural fluctuations [1]. The proposed method could be further applied to study properties of more selective G-quadruplex ligands.



*ActD promotes quadruplex topology changes and dimerization, and plays a key role in stabilizing high-temperature structure with residual base stacking*

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Bjørn Torger Stokke

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MOLECULAR DYNAMICS SIMULATIONS OF BIOLOGICAL LIPID MEMBRANES
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**PP5**
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**PP6**

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*S. Di Fonzo, B. Bellich, A. Gamini□, N. Quadri □, A. Cesàro*

**PP10**

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*Larisa Latypova, Anna Greenbaum, Gregory Barshtein and Yuri Feldman*

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**PP12**

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*Lorenzo Marsili, Michele Dal Bo, Federico Berti, Giuseppe Toffoli*

**PP13**

KNUDSEN THERMOGRAVIMETRY FOR THE DETERMINATION OF WATER ACTIVITY IN CACO3 NANOPORES

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**PP14**

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