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Frontiers in Water Biophysics www.waterbiophysics.eu

BOOK OF ABSTRACTS AND CONFERENCE PROGRAMME

Welcome

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Conference Programme

Abstracts: **Invited Lectures**

Oral Presentations

Poster Presentations

List of Presentations

List of Participants

V 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

We warmly welcome all the participants and contributors to the third Conference on “Frontiers in Water Biophysics”.

So many people and independent events contributed to FWB2015 that it would be quite impossible to list. All previous meetings and colleagues which dealt with water properties, water in food, pharmaceutical matter and biophysical properties of living systems and biomolecules, have contributed to some extent in developing the ideas merged in the present event. Some special thanks are deserved to the members of the Scientific Committee for their past, present and future advice.

The challenge and the difficulties of putting down the organization of such a composite meeting were always clear; it has been only after the enthusiastic response received from many colleagues and friends that our dream took the final shape of the expected “melting pot of a science broth”.

The lightening words of our mission were “Fostering mixing, learning and empathy between science cultures was and remains the main purpose of any project". As in every scientific endeavour, these concepts have supported us in pursuing ahead with the strongest consideration of achieving a fantastic goal.

We sincerely hope to see new contacts and collaborations, new ideas and new work, soon generated from the intense program set for “Frontiers in Water Biophysics”.

Attilo Cesaro, *Daniele Fioretto*, *Giancarlo Franzese*

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PROGRAM

	MON 7	TUE 8	WED 9	THU 10	FRI 11	SAT 12
9:00-9:30		HENG	SOPER	BALDWIN	SCIORTINO	ROOS
9:30-10:00						
10:00-10:30						
10:30-11:00		coffee/poster	coffee/poster	coffee/poster	coffee/poster	coffee/poster
11:00-11.30		Oral 7	Oral 14	Oral 20	Oral 22	Oral 28
11.30-12.00		Oral 8	Oral 15	Oral 21	Oral 23	Oral 29
12:00-12.30		Oral 9	Oral 16	Mezzenga	Oral 24	Blasi
12:30-13:00		Oral 10	Oral 17		Oral 25	
13:00-16:00	opening (15:30)	Lunch	Lunch	Lunch	Lunch	
16:00-16:30	Oral 1	Drusch	Righini		Weik	
16:30-17:00	Oral 2					
17:00-17:30	coffee/poster	coffee/poster	coffee/poster		coffee/poster	
17:30-18:00	Oral 3	Oral 11	Oral 18		Oral 26	
18:00-18:30	Oral 4	Oral 12	Oral 19		Oral 27	
18:30-19:00	Oral 5	Oral 13	Round Table		Round Table	
19:00-19:30	Oral 6					

Wednesday evening: social dinner
Thursday afternoon: excursion to Selinunte (Greek town of about 650 BC)

MONDAY 7

16.00 Oral 1
P. Gallo "Microscopic dynamics of hydration water in aqueous solutions of lysozyme and trehalose upon cooling"

16.30 Oral 2
L. Comez "Hydration dynamics of biosystems: from small hydrophobes to proteins"

17.30 Oral 3
J. Savolainen "New insights into the role of water in biological function: studying solvated biomolecules using terahertz spectroscopy"

18.00 Oral 4
Y. Kurzweil-Segev "Hydration shell dynamics driven by protein interface"

18.30 Oral 5
C. Calero "Dynamics of water at stacked phospholipid bilayers: a molecular dynamics simulations study"

19.00 Oral 6
R. Gregor Weiß "Anomalies of hydrophobic solvation extend into water dynamics"

TUESDAY 8

9.00: Invited
P.W.S. Heng "Usefulness of water activity in understanding the stability of pharmaceutical tablets"

11.00: Oral 7
J. Michel "Exploring biomolecular hydration thermodynamics with grid cell theory"

11.30: Oral 8
L. Pusztai "Neutron diffraction of hydrogenous materials: measuring incoherent and coherent intensities separately. The example of liquid water"

12.00: Oral 9
B. Rossi "Hydrophobic/hydrophilic effects in water dynamics as probed by UV Raman scattering"

12.30: Oral 10
L. Lupi "Rate determing step for homogeneous nucleation of ice"

16.00 Invited
S. Drusch "Water and its role in microencapsulation of food ingredients"

17.30: Oral 11
M. Faieta "Role of saccharides on colour stability of phycocyanin extracts in aqueous solutions"

18.00: Oral 12
F. Bruni "Structure-Activity relationships in carbohydrates: A water mediated business?"

18.30: Oral 13
M. Malferrari "Protein-matrix coupling in photosynthetic reaction centers embedded in trehalose and sucrose glasses: the effect of protein concentration"

WEDNESDAY 9

9.00: Invited
A.K. Soper "Pore waters – how did we muddy them so badly?"

11.00: Oral 14
R. Torre "Nanoconfined water: network structures and dynamics processes"

11.30: Oral 15
J.W. Brady "The structuring of water by solutes in aqueous solutions"

12.00: Oral 16
C. Olsson "The molecular origin of the cryoprotective properties of trehalose on proteins"

12.30: Oral 17
M.A. Ricci "Local structure of temperature and pH-sensitive colloidal microgels"

16.00: Invited
R. Righini "Water in confined and bulk liquid phases: a time resolved infrared study"

17.30: Oral 18
M. Paolantoni "Hydrogen bond dynamics in aqueous solutions of formamide probed by extended depolarized light scattering (EDLS)"

18.00: Oral 19
S. Perticaroli "Elastic and dynamical properties of elastin hydrogels"

18.30: *Round Table*

THURSDAY 10

9.00: Invited
A.J. Baldwin "Membraneless organelles destabilise the double helix"

11.00: Oral 20
O. Vilanova "Predicting the kinetics of protein-nanoparticle corona formation in a simplified plasma using water-mediated bionanointeractions."

11.30: Oral 21
J. Nickels "Using neutrons to investigate the structure and dynamics of monodisperse dendrimeric polysaccharide nanoparticle dispersions"

12.00: Invited
R. Mezzenga "Self-assembly of amyloid fibrils in 1, 2 and 3 dimensions: fundamentals and applications"

FRIDAY 11

9.00: Invited
F. Sciortino "The liquid-liquid critical point in water"

11.00: Oral 22
A. Lerbret "Molecular dynamics simulation study of the interaction of the divalent cations Ca^{2+} and Zn^{2+} with polygalacturonic acid"

11.30: Oral 23
V. Vlachy "Explicit solvent theory for protein-salt mixtures in water"

12.00: Oral 24
D.Z. Caralampio "Hydration of lanthanide cations in acidic aqueous solution"

12.30 Oral 25
E. Sánchez Marcos "The hydration of cisplatin and its aqua-derivatives by molecular dynamics simulations using first principles intermolecular potentials"

16.00: Invited:
M. Weik "Coupling between protein and hydration water dynamics"

17.30: Oral 26
V. Bianco "Design and stability of proteins and biopolymers in explicit water: a coarse-grain approach"

18.00: Oral 27
F. Mallamace "The energy landscape in protein folding and unfolding: a ^{1}H NMR study"

18.30: *Round Table*

SATURDAY 12

9.00: Invited
Y.H. Roos "Glass transitions, structural relaxation times and physicochemical properties of complex food systems"

11.00: Oral 28
S. Corezzi "Solute, solvent and solvation dynamics in water-carbohydrate solutions revealed by extended depolarized light scattering"

11.30: Oral 29
F. Sterpone "Biomolecular hydration without water. Multi-scale modelling of biomolecules including hydrodynamics"

12.00: Invited
P. Blasi "Studying water in hydrophobic polymers: a drug delivery perspective"

INVITED LECTURES

Usefulness of Water Activity in Understanding the Stability of Pharmaceutical Tablets

Paul W S Heng & Tze Ning Hiew

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Introduction

Tablet is one of the most common solid dosage forms available in the market, as it is generally preferred by patients due to the ease of administration and transportation. Tablets generally consist of an active pharmaceutical ingredient (API) and other functional additives or excipients. Some APIs are moisture sensitive and product quality could be adversely affected if the dosage forms are subjected to high moisture conditions. The stability of moisture-sensitive APIs and functionality of certain type of excipients become a concern when they are exposed to humid conditions during production, especially during wet granulation when water is added as binder solution or when supposedly dry products are exposed to high humidity conditions. While the effect of moisture on many APIs and excipients are well studied, the mechanisms underlying these water-solid interactions remain unclear. The aim of this study was to investigate the potential of water activity (a_w) in explaining the effect of moisture on API during wet granulation, with aspirin as the model drug. This study also aimed to investigate the effect of storage relative humidity levels on disintegrant functionality and efficiency.

Methods

Aspirin granules were produced by wet granulation, with different amounts of water added. The granules were stored at 40 °C and 75 % RH and the extent of drug degradation was determined by UV spectrophotometerically over various time points. The moisture content and a_w were also measured at the respective time points.

Moisture sorption isotherms for six disintegrants were obtained using a vapour sorption analyser. Nonlinear regression analysis of the Guggenheim-Anderson-de Boer (GAB) equation was used to analyse the isotherms from 0.03 to 0.90 a_w. The water clustering functions of the disintegrants were also calculated using the parameters obtained from the GAB model. These disintegrants were also stored in chambers pre-equilibrated at 11, 33, 53 and 75 % RH maintained by saturated salt slurries. Water activity and moisture content measurements were obtained after 60 days of storage. Tablets comprising the disintegrants with lactose as the diluent were also prepared and evaluated.

Results

It was found that water activity showed a consistent correlation with drug stability but there was no clear correlation between moisture content and drug stability.

All the isotherms obtained were sigmoidal in shape, characteristic of Type II isotherms. All the disintegrants examined in this study showed marked increase in disintegration time with increasing water activity apart from sodium starch glycolate and crospovidone. Tablets prepared using these two disintegrants also showed reduced tensile strength at high water activity.

Conclusion

Water activity shows promise in explaining the role of moisture in affecting drug stability of solid dosage form. Besides, water activity of the disintegrants was found to impact tablet quality in terms of tensile strength and disintegration time.

WATER AND ITS ROLE IN MICROENCAPSULATION OF FOOD INGREDIENTS

Drusch, S.

Technische Universität Berlin, Berlin, Germany

A variety of food ingredients like aroma-active compounds, nutritional oils, natural food colorants, vitamins or antimicrobial extracts are encapsulated prior to their use in food production. All these food ingredients have in common, that they are isolated from their natural matrix and are susceptible against environmental factors like light or oxygen. As a consequence changes in the chemical structure of key components may occur, which result in a loss of functionality. Thus, encapsulation is a common strategy to protect food ingredients and to control their release.

Water, on one hand, is the solvent or the continuous phase in dispersed encapsulation systems and enables the formation of structural elements in the encapsulating matrix. Incompatibility of polymers in aqueous solution is the driving force in complex coacervation for encapsulation. In the case of a solvent-activated release mechanism, contact with water is the trigger for release of the encapsulate.

On the other hand, dissolution upon contact with water frequently limits the range of applications for the encapsulated food ingredient. Resistance against dissolution and swelling might be desirable to obtain sensorical effects after ingestion of foods or to control delivery of functional ingredients *in vivo*. Like for a variety of foods, water needs to be removed from encapsulation systems to increase the stability against microbial spoilage. Materials science aspects as well as process engineering parameters need to be taken into consideration to maintain the desired physical structure and functionality of the encapsulation system. Finally, water is responsible for adverse changes like phase transition of the capsule matrix components and structural changes, which affect molecular mobility and as a consequence limit the stability of the encapsulate.

PORE WATERS – HOW DID WE MUDDY THEM SO BADLY?

Alan K Soper[a]

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The discussion of what makes water water goes back to at least 1880[1]. The earliest ideas appear to have centred on the notion that water is a mixture of two or more components, although opinions on what those components actually are differed quite markedly. Somehow one had to reconcile the concept of distinct components with the fact that water diffusion, as derived from the viscosity, was clearly rapid, which inevitably would lead to the components becoming mixed and therefore indistinguishable, or at least inseparable, on a very short time scale. In late 1960s and early 1970s Monte Carlo and molecular dynamics computer simulation of water[2] re-introduced an existing paradigm, perhaps originally due to Bernal and Fowler[3], that water is a single molecule liquid with a complex hydrogen-bonding pattern between molecules: it was this hydrogen bonding pattern that gave water its unique properties. In spite of the initial success of computer simulation at capturing, qualitatively, some of the more important properties of water, it is however clear that such models, even though they have become increasingly sophisticated[4], never quite make it to the status of a "standard model" for water which readily explains all its properties.

A putative standard model for water *did* emerge in 1992[5].This was the idea that water might have a second, liquid-liquid critical point, in the metastable region of the phase diagram, where water can only stably exist as crystalline ice. However, as with other concepts about water, this idea is, to the present day, still hotly debated, with some critics roundly opposed[6]. Yet, arguably the single biggest obstacle to the idea of a liquid-liquid transition, is the fact that so far it has proved completely impossible to verify experimentally, in a convincing manner. One such claim appeared recently, in which water absorbed into MCM41 porous silica, was cooled and pressurised to several thousand atmospheres, using He as a pressuring medium[7]. The evidence for a liquid-liquid transition was based primarily on the changes in height of a single Bragg diffraction peak as a function of temperature and pressure. Yet in other work[8] I have argued such simple-minded interpretations of a single feature in the diffraction pattern can lead to quite spurious interpretations about what might be going on at the molecular level. I will attempt to make some rigorous delineation between what is known for sure about pore water in such circumstances, and what is likely to be, at this stage, only speculation.

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WATER IN CONFINED AND BULK LIQUID PHASES: A TIME RESOLVED INFRARED STUDY

Roberto Righini

Department of Chemistry "Ugo Schiff" and
European Laboratory for Non-Linear Spectroscopy (LENS)
University of Florence

Water is the most abundant compound on the Earth surface, ruling life and being central to most of the chemical, biological, geological, and atmospheric processes. It is well known, since a long time, that many kinetic and thermodynamic properties of liquid water exhibit an anomalous dependence on physical parameters. One of the main points is that of the relation between structural and dynamical properties, what implies reconciling the microscopic picture with a collective approach. We show here that different time-domain spectroscopic experiments, performed in a broad range of temperature and pressure conditions, supported by computer simulations, provide important information on both aspects. In particular, here we consider the results obtained from time resolved spectroscopic experiments in the infrared region on water segregated in organic and inorganic matrices, on water solvating ions and biomolecules, on bulk liquid water in different conditions (at temperatures from 245 K to 365 K and pressure from 0 to ~1 GPa).

The results provide quantitative estimate of the characteristic time scales of the dynamics in different conditions and shed light on the relation between local structure and collective properties.

MEMBRANELESS ORGANELLES DESTABILISE THE DOUBLE HELIX

Timothy J. Nott[a], *Timothy D. Craggs*[b], *Parick Farber*[c], *Julie D. Forman-Kay*[c], *Andrew J. Baldwin*[a]

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[b]Clarendon Laboratory, University of Oxford, Oxford OX1 3PU, UK
[c]Hospital for Sick Children, 686 Bay Street, Toronto, ON M5G 0A4, Canada

Cells chemically isolate molecules in compartments to both facilitate and regulate their interactions. In addition to membrane-encapsulated compartments, cells can form proteinaceous and membraneless organelles, including nucleoli, Cajal and PML bodies, and stress granules. The principles that determine when and why these structures form have remained elusive.

I will discuss recent results looking at organelles formed from the disordered tails of Ddx4, a primary constituent of nuage or germ granules. These proteins form phase-separated organelles both in live cells and in vitro. We show that these bodies are stabilized by patterned electrostatic interactions that are highly sensitive to temperature, ionic strength, arginine methylation, and splicing. We used this information to produce an algorithm that looks at sequence determinants of Ddx4, to predict other disordered protein sequences that may phase separate under physiological conditions. This algorithm is able to identify a number of proteins found in both membraneless organelles and cell adhesion. We demonstrate that a number of these proteins do in fact form liquid blobs.

Finally, we show that these bodies provide an alternative solvent environment that can concentrate single-stranded DNA and RNA but largely exclude double-stranded DNA. Moreover, the double stranded DNA that goes inside the organelles is effectively melted once it goes inside.

It would appear that phase separation of disordered proteins containing weakly interacting blocks is a general mechanism for forming regulated, membraneless organelles, and that the interior of these bodies present a solvent environment capable of inducing significant changes in commonly encountered biomolecules.

References

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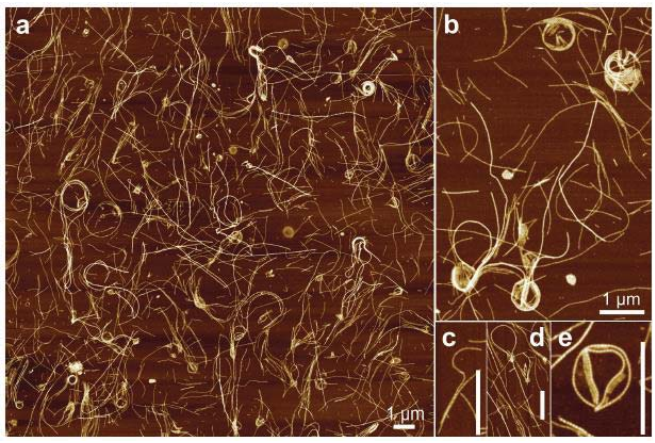
SELF-ASSEMBLY OF AMYLOID FIBRILS IN 1, 2 AND 3 DIMENSIONS: FUNDAMENTALS AND APPLICATIONS

Raffaele Mezzenga

ETH Zurich, Food & Soft Materials,
Department of Health Science & Technology & Department of Materials
Schmelzbergstrasse 9, LFO, E23, 8092 Zürich, SWITZERLAND

Protein fibrils are protein aggregates, which can be generated from food-grade proteins by unfolding and hydrolysis. The resulting protein fibrils can be used in a broad context of applications. In food applications, they may serve as texture enhancers, gelling agents, thickeners as well as surfaces and interfaces stabilizers. To fully exploit their potential, it is necessary to understand these systems at the most fundamental level. At length scales above the well-established atomistic fingerprint of amyloid fibrils, these colloidal aggregates exhibit mesoscopic properties comparable to those of natural polyelectrolytes, yet with persistence lengths several orders of magnitude beyond the Debye length. This intrinsic rigidity, together with their chiral, polar and charged nature, provides these systems with some unique physical behavior in one, two and three dimensions. In this talk I will discuss our current understanding on the mesoscopic properties of amyloid fibrils at the single molecule level, the implication of their semiflexible nature on their liquid crystalline properties, and I will illustrate how this information prove useful in understanding their collective behavior in bulk and when adsorbed at liquid interfaces. By the careful exploitation of the physical properties of amyloid fibrils, the design of advanced materials with unprecedented physical properties become possible, and I will give a few examples on how these systems can ideally suit the design not only of complex food systems, but also of biosensors and biomaterials.



AFM images of amyloid fibrils made of β-lactoglobulin adsorbed at the air-water interface after horizontal transfer onto mica using a modified Langmuir-Schaefer method. Scale bar is 1 µm. Images show spontaneous formation of rings and loops. From Jordens et al. ACS Nano 2014, 8, 11071.

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2. "*Biodegradable Nanocomposites of Amyloid Fibrils and Graphene with Shape Memory and Enzyme Sensing Properties*" Li, C. et al. *Nature nanotechnology*, **2012**, *7*, 421.
3. "*Adsorption at Liquid Interfaces Induces Amyloid Fibril Bending and Ring Formation*" Jordens S. et al. *ACS Nano*. **2014**, 8, 1171
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THE LIQUID-LIQUID CRITICAL POINT IN WATER

Francesco Sciortino

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The thermodynamic behavior of water at low temperatures, and even more in supercooled states, is unconventional. Several response functions are characterized by a non monotonic temperature or pressure dependence. Well known examples are the isobaric density, the isothermal compressibility, the constant-pressure specific heat. The anomalous behavior of these quantities has attracted the attention of several researchers and several propositions have been put forward to explain it. In this lecture, I will describe the evolution of an idea, germinated while interpreting results of one of the early simulation study of supercooled water[1], which has permeated a large part of the water community. The idea of a liquid-liquid critical point in a one-component system, despite can not be possibly experimentally proved in water due to fast crystallization, has been extremely fruitful to understand the behavior of several different compounds.

The debate on the existence of a liquid-liquid transition in supercooled liquids is not limited to water. The mechanism underlying such a transition is expected to be a genuine feature shared by all atoms or molecules promoting tetrahedral order at atomic or molecular level. Such a phenomenon should also affect the collective behavior of particles dissolved in a solvent (colloids) when the effective interparticle interaction similarly promotes tetrahedrality. In the final part of the lecture I will discuss some recent numerical studies[2-3] which are paving the way for an experimental test of the liquid-liquid critical point scenario, in the absence of crystallization.

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COUPLING BETWEEN PROTEIN AND HYDRATION WATER DYNAMICS

Schiro Giorgio[a], *Fichou Yann*[a], *Gallat François-Xavier*[a], *Zaccai Giuseppe*[b], *Weik Martin*[a]

[a]Institut de Biologie Structurale, Grenoble, France; [b]Institut Laue-Langevin, Grenoble, France

As molecular workhorses, proteins fulfil a multitude of tasks that keep the complex machinery in biological cells alive. In order to be biologically active, most soluble proteins require their surface to be covered with water. This so-called hydration water is generally acknowledged to enable a protein to undergo the internal motions that are so fundamental for its capacity to fulfil a specific biological function. Incoherent neutron scattering (INS) in combination with selective deuterium labelling is a powerful tool that puts the focus either on protein or on water motions on the ns-ps time scale and allows their dynamic coupling to be studied. This coupling proves to decrease in *tightness* from intrinsically disordered, *via* globular-folded to membrane proteins and is modified upon protein aggregation (Fichou *et al*., 2015). Thus, there exists a gradient of coupling with hydration-water motions across different protein classes and different aggregation states. In particular, it is the translational diffusion of hydration-water molecules on the protein surface that enables functionally-relevant motions (Schiro, Fichou, Gallat *et al*., 2015). Surprisingly, hydration-water is not the only matrix within which proteins are biologically active. The polymer-coating of protein surfaces in solvent-free protein-polymer hybrids dynamically replaces hydration-water and allows the protein moiety to undergo motions necessary for biological activity (Gallat, Brogan *et al*., 2012). Molecular details of the coupling between polymer and protein motions remain elusive and are currently being studied by the powerful combination of INS and selective deuterium labelling.

These pieces of research are the fruit of collaborations with colleagues whose names appear in references 1 - 3. We are deeply indebted to them.

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GLASS TRANSITIONS, STRUCTURAL RELAXATION TIMES AND PHYSICOCHEMICAL PROPERTIES OF COMPLEX FOOD SYSTEMS

Yrjö H. Roos

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Food components often form noncrystalline molecular assemblies in most intermediate and low moisture foods. We have investigated noncrystalline structures in sugars, carbohydrates and proteins. Typical methods for the determination of glass transitions included thermal analysis and particularly differential scanning calorimetry (DSC). On the other hand, structural relaxation times were followed using dynamic mechanical analysis (DMA) and dielectric analysis (DEA). Small sugars and carbohydrates formed noncrystalline solids in cooling from melt and in dehydration from solutions. Their noncrystalline solids exhibited clear, water-content dependent glass transition behaviour and state diagrams were established for most common sugars and carbohydrates. DMA and DEA were used to determine their structural relaxation times and shown that relaxation times above the glass transition temperature followed the Williams-Landel-Ferry (WLF) relationship. Structural relaxations and conformational transformations of proteins were more complex. We identified structural relaxations of protein hydration water which was antiplasticised by the protein. Ternary state diagrams were established based on carbohydrate-protein systems transitions and their water plasticization. Carbohydrate and proteins are phase separated systems and the knowledge of their complex transition behaviour is useful in characterisation of various biological materials. We also introduced a strength parameter for the measurement of solids behaviour in plasticization above the glass transition. Such parameters are of significant importance to materials processing and stabilisation.

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STUDYING WATER IN HYDROPHOBIC POLYMERS: A DRUG DELIVERY PERSPECTIVE

Paolo Blasi

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Polymers have today an unquestionable central role in the medical and pharmaceutical fields, especially in drug delivery science and technology. In fact, an active pharmaceutical ingredient (API) *per se* does not possess the characteristics required to be administered and has to be formulated, employing adequate excipients, in a dosage form. Excipients have specific functions, such as to assist in the processing of the drug delivery system during its manufacture, enhance bioavailability, and enhance other attributes of the overall safety, effectiveness, or delivery of the drug during storage or use. Many of the excipients used to formulate conventional (e.g., powder, tablet) as well as advanced dosage forms (e.g., microparticles, transdermal patches) are natural, semi-synthetic or synthetic macromolecules.[1,2] Obviously, their physico-chemical characteristics strongly influence the device performances and the therapy outcome. Actually, the precise and predictable control of the drug release from a delivery system is fundamental to improve pharmacological therapies. Water, as a contaminant or an excipient, is present during the whole life span of a drug product. And, independently of the release mechanism/s (e.g., dissolution, diffusion, swelling, erosion), water molecules trigger the release of the API from the dosage form. The significant effects of water when interacting with hydrophilic amorphous polymers of pharmaceutical interest has been deeply studied and a basic understanding of these phenomena is now available.[3-5] On the contrary, the interaction between water and hydrophobic polymers has been more or less ignored by the scientific community. Due to the fact that these macromolecules absorb very tiny amounts of water its effects may be underestimated. However, being amorphous or partially crystalline materials, their glass transition temperature (Tg) becomes a fundamental parameter to monitor and to control. A modification in Tg value during manufacturing or usage may drastically change the tensile strength, the release kinetics, and the release mechanisms of the device.[6,7] Indeed, the diffusion coefficients of small molecules may increase of several orders of magnitude going from the glassy to the rubbery state.[8] In this lecture, I will give a critical overview of the present knowledge on the effects of water on macromolecules of pharmaceutical interest. I will emphasize the importance of studying and understanding the behaviour of water in hydrophobic macromolecules as well as the consequences of its presence. The attention will be focused on biodegradable polymers obtained by condensation of lactic and/or glycolic acid, namely poly(lactic acid) and poly(lactic-co-glycolic acid), largely employed in drug delivery.

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ORAL PRESENTATIONS

MICROSCOPIC DYNAMICS OF HYDRATION WATER IN AQUEOUS SOLUTIONS OF LYSOZYME AND TREHALOSE UPON COOLING

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Aqueous solutions of carbohydrates have an extraordinary cryopreservation ability and among them trehalose is particularly effective [1]. I will present results from molecular dynamics simulations of two aqueous solutions, water and lysozyme, and water, lysozyme and trehalose. The dynamics of the system is studied upon cooling. In particular relaxation times are analyzed and a comparison between the two system is done. Water in the ternary system is slowed down, especially around the lysozyme and this is due to the influence of trehalose [2].
We show here a detailed study of hydration water around lysozyme. Hydration water shows two distinct slow relaxations in both solutions. One is the alpha relaxation typical of glass formers, the longer one is specific of hydration water.
When trehalose is added to the aqueous solution the relaxation times of hydration water molecules become substantially higher especially at lower temperatures and expecially for the longer relaxation time [3]. These results point to a cryoprotective action exerted by packing slow water between the protein and a layer of trehalose molecules.

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HYDRATION DYNAMICS OF BIOSYSTEMS: FROM SMALL HYDROPHOBES TO PROTEINS

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The collective dynamics of water in diluted aqueous solutions of biosystems with different chemical topologies has been studied by extended frequency range depolarized light scattering (EDLS). Relaxation times and hydration numbers have been obtained as a function of temperature and solute concentration in solutions of small hydrophobes [1], amino acids [2], dipeptides [2-4] and model proteins [5-6]. The key result is that the more complex is the solute, the greater is the dynamical slowing down and the spatial extent of the perturbation induced on the collective dynamics of surrounding water [2]. This is in favour of the view that hydration dynamics of complex macromolecules, due to its collective character, cannot be trivially predicted based on the effects observed in simple model systems.

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NEW INSIGHTS INTO THE ROLE OF WATER IN BIOLOGICAL FUNCTION: STUDYING SOLVATED BIOMOLECULES USING TERAHERTZ SPECTROSCOPY

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In life science, water is the ubiquitous solvent, sometimes even referred to as the "matrix of life". An increasing experimental and theoretical evidence suggests that solvation water is not merely a passive spectator in biomolecular processes. New experimental techniques can quantify how water interacts with biomolecules and, in doing so, differs from "bulk" water. Terahertz (THz) absorption spectroscopy has turned out to be a powerful tool to study (bio)molecular hydration [1]. The main concepts that have been developed in the recent years to investigate and describe the underlying solute-induced dynamics of the hydration shell will be discussed [2].

Protein folding, one of the most fundamental biological processes, involves large amplitude skeletal motions described by low frequency modes. Here we introduce a method to directly probe changes in the collective, low frequency modes of the protein-solvent system associated with large amplitude conformational changes of the protein in real time. As we record the THz absorption transient of the solvated protein following a rapid T-jump. Three distinct time scales emerge: Thermal relaxation of the solution, changes in the low frequency spectrum of the protein itself and the "imprinted" low frequency spectrum of the hydration water. In combination with molecular simulations, the transient THz signal monitoring the evolution of the low-frequency spectrum provides information about both dynamic and thermodynamic changes of the solvated protein during folding.

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HYDRATION SHELL DYNAMICS DRIVEN BY PROTEIN INTERFACE

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In bio- molecular systems water forms an hydration shell near the proteins, which plays a major factor for their structure, dynamics and functionality[1,2]. The hydration shell and its influence on the protein were studied extensively during last several decades with variety of experimental techniques. However, despite the broad discussion, the effect of the protein structure on its hydration shell has almost not been taken into account. Nevertheless, the unique physical and chemical properties of water in the close vicinity of different materials depends on the structure and electrical properties of the close environment [3–6].

One of the experimental methods capable to study directly the dynamic and structure of the hydration shell is the Broadband Dielectric Spectroscopy (BDS). To exploit the delicate interplay between protein and water , a new approach has recently been presented, which is based on the fractal nature of the time set of the interaction of the relaxing water dipoles with their encompassing matrix[7]. It demonstrates a fundamental connection between the relaxation time,τ, the broadening parameter, α, and the Kirkwood-Froehlich correlation function B, itself depending on $\Delta\varepsilon$. The parameters B, τ and α were chosen as basis of the coordinates of the new 3D space, wherein the evolution of the relaxation process, as a result of the variation of an external macroscopic parameter (temperature, concentration, pressure, etc.), will depict a trajectory. This trajectory is a result of the connection between the kinetic and the structural properties of water in the hydration environment.
In this work, the dependence of water dynamics on the protein structure will be examined using the 3D trajectories tool.

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OP5

Dynamics of Water at Stacked Phospholipid Bilayers: a Molecular Dynamics Simulations Study

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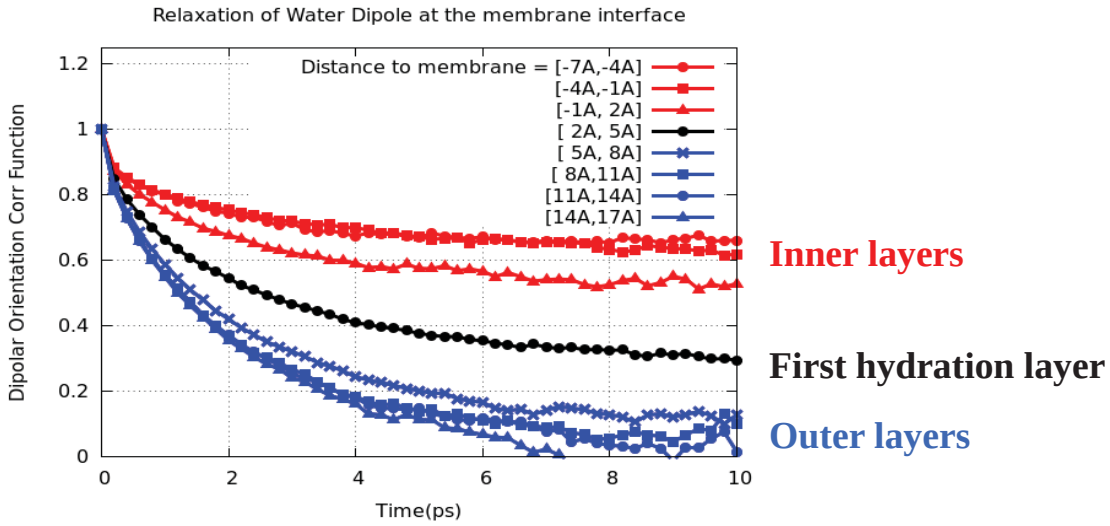
An accurate description of interfacial water at phospholipid membranes is essential for a complete understanding of the functions of biological membranes. In particular, the dynamical and structural properties of water under membrane confinement is closely related to phenomena of great importance such as membrane fusion or drug transport [1].

In this contribution we have investigated the dynamics of water molecules confined between DMPC phospholipid bilayers with different hydration levels using all-atom molecular dynamics (MD) simulations. Our results show the existence of three types of water dynamical behavior —fast, bulk-like, slow— and determine their relative importance as a function of the hydration level of the membrane, in agreement with recent experiments [2]. We interpret our findings with the help of a layered model of the membrane's interface using a local definition of distance. We demonstrate that such dynamical behavior is consequence of the different capabilities of water molecules to create hydrogen bonds and of their different dynamics across the membrane.



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ANOMALIES OF HYDROPHOBIC SOLVATION EXTEND INTO WATER DYNAMICS

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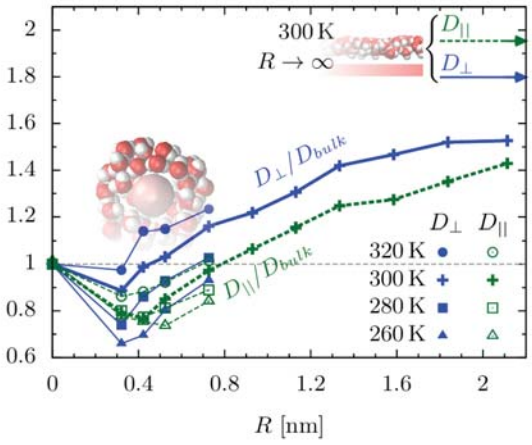
In recent decades experimental as well as theoretical research hinted that water dynamics couples to kinetics of biochemical and biophysical processes [1]. In the course of this the solvent exhibits length-scale dependent thermodynamic anomalies as polypeptides enter an aqueous environment [2]. For small hydrophobic solutes, on a sub-nanometer length-scale, structural changes dominate the solvation free energy which thus mainly comprises entropic contributions. As solute size increases beyond a critical crossover length-scale structural changes are negligible and enthalpic costs dominate due to water-vapor interface formation.

In a molecular dynamics simulation study we show that the yet known thermodynamic and structural anomalies of hydrophobic solvation extend into solvation water dynamics entering through a pronounced non-monotonicity in single molecule dynamics [Phys. Rev. Lett. **2015**, 114, 187802]. At highly curved hydrophobic surfaces mobility of hydrating water at ambient conditions is inhibited by down to -20% than in bulk water.



For decreasing curvatures beyond the crossover length-scale bulk diffusivity is restored and even further becomes enhanced to values faster than in bulk. Additionally, the temperature dependence of the dynamic anomaly obeys a similar trend as the thermodynamic and structural crossover length scale [3]. Thus taking into account existing entropy scaling laws for diffusion [4, 5], which scale exponentially with excess entropy, suggests it to be a constitutive measure for the presented dynamic behavior. Ultimately, water mobility is initmatly linked to its hydrogen bond kinetics which was described by reaction-diffusion equations [6]. Translational diffusion is accompanied by processes breaking, forming and re-forming hydrogen bonds. Hence the crossover in mobility persists within the correlation times of hydrogen bonds consistent with the underlying reaction-diffusion model.

Our findings advance the understanding of impact and implications of hydrophobic solvation in processes involving topologically heterogeneous (bio)molecules. With that it benefits the interpretation of dynamic heterogeneities found from experiments.

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EXPLORING BIOMOLECULAR HYDRATION THERMODYNAMICS WITH GRID CELL THEORY

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Grid cell theory (GCT) is a discretised variant of cell theory models of liquids.[1,2] The GCT methodology has been used in this work to post-process molecular simulation trajectories and spatially resolve the excess thermodynamic properties of water molecules at the interface of organic and biomolecules. GCT has been shown to yield predictions of excess hydration free energies of small molecules with accuracy comparable to established simulation methodologies such as thermodynamic integration. In addition spatial resolution of variations in excess enthalpy and entropy of water in an inhomogeneous system enables insightful computational analyses of hydration thermodynamics.

The GCT method has been implemented in the software Nautilus (http://siremol.org/Sire/Apps.html) to enable simple post processing of molecular simulation trajectories in popular file formats. Applications of GCT will be presented to highlight the versatility of the approach. Some of the topics that have been investigated thus far include: the identification of solvent regions that drive hydration of common organic functional groups;[1] the elucidation of the influence of water on the thermodynamic signature of host-guest association in model cavities;[2] the evaluation of energetics of water network perturbations upon ligand modification in protein binding sites;[3] the statistical analysis of correlations in local water thermodynamics with structural descriptors for a large dataset of protein hydration sites.

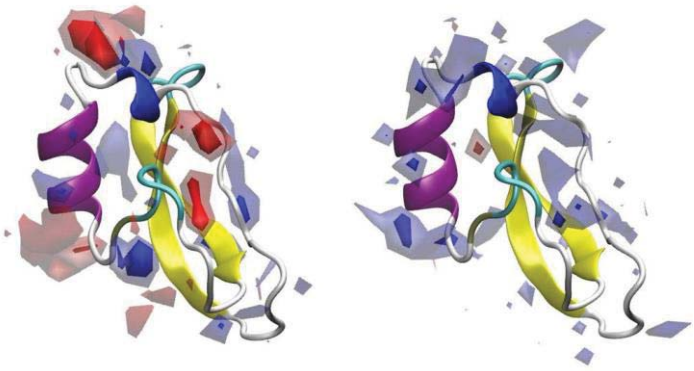

Figure 1. Spatial decomposition of the excess enthalpy (left) and entropy (right) of water molecules at the surface of the protein BPTI. Blue/red isocontours indicate regions that are disfavoured/favoured with respect to bulk conditions.

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NEUTRON DIFFRACTION OF HYDROGENOUS MATERIALS: MEASURING INCOHERENT AND COHERENT INTENSITIES SEPARATELY. THE EXAMPLE OF LIQUID WATER

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Accurate determination of the coherent static structure factor of any disordered material containing substantial amounts of proton nuclei has proven to be rather problematic by neutron diffraction, due to the large incoherent cross section of ^{1}H. This problem has continued to set severe obstacles to the reliable determination of liquid structures of hydrogenous materials up to this day, by introducing large uncertainties whenever a sample with a ^{1}H content larger than about 20 % had to be measured by neutron diffraction. Huge theoretical efforts over the past 40 years that had been aimed at estimating the 'incoherent background' of such data have not resulted in any practical solution to the problem.

Here we present the first accurate separate measurements, by polarized neutron diffraction, of the coherent and incoherent contributions to the total static structure factor of mixtures of light and heavy water, over a wide momentum transfer range, and over the entire composition range, i.e. for light water contents between 0 and 100 %. We show that the measured incoherent scattering can be approximated by a Gaussian function. The separately measured coherent intensities exhibit signs of small inelastic contributions. Out of several possible approaches, we have chosen to subtract a cubic background using the Reverse Monte Carlo algorithm, which has the advantage of requiring an actual physical model (thousands of realistic water molecules at the correct density) behind the corrected data.

Finally, coherent static structure factors for 5 different compositions of liquid H_2O and D_2O mixtures are presented for which the huge incoherent background could actually be measured and separated, instead of being approximated as it has been done every time so far. These experimental results provide a strong hope that determining the structure of hydrogenous materials, including, e.g., protein solutions, may become feasible in the near future.

HYDROPHOBIC/HYDROPHILIC EFFECTS IN WATER DYNAMICS AS PROBED BY UV RAMAN SCATTERING

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The behaviour of water in the vicinity of a biomolecule plays a fundamental role in determining several mechanisms such as solvation, gelation phenomena and self-assembly of macromolecules. On the other hand, the hydrogen-bond (HB) dynamics of water molecules that surround a solute or that are confined in nano-cavities is strongly affected by the different interactions established with the hydrophobic and hydrophilic groups that could be present in the chemical structure of biomolecules.
Here we report the results of wide investigation performed on different systems of biological interest, aimed to demonstrate the high capability of UV Raman scattering experiments for exploring the influence of hydrophobic/hydrophilic effects in the HB dynamics of water. The great advantage of the experimental approach exploited in our study, is that the line shape analysis of UV Raman spectra separately provides information on both hydrophobic and hydrophilic chemical groups of the system by measuring the vibrational relaxation dynamics of specific molecular probes.
Particular attention will be devoted to presentation of the results obtained on the polysaccharide hydrogel based on cyclodextrins, namely cyclodextrin nanosponges (NS). The case example of NS hydrogel is a good model system for the study of water-water and water-polymer interactions in hydrogel phases, due to the presence of both hydrogen-bond donor/acceptor groups in polymer skeleton. We will show as in this case study the combination of UV Raman scattering experiments with IR spectroscopy measurements allows to safely disentangle the spectral response arising from the two main components of the hydrogel systems, i.e. polymer matrix and water solvent. The separate analysis of the vibrational dynamics associated to confined water molecules and polymer skeleton will provide new insights on the interplay of different types of HB interactions that contribute to determine the co-existence of liquid-like and gel phases in hydrogel systems.

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RATE DETERMING STEP FOR HOMOGENEOUS NUCLEATION OF ICE.

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Homogeneous nucleation of ice is one of the most common processes in the atmosphere. Given the importance of clouds formation on the Earth's climate it is extremely important to predict at which temperature homogeneous freezing will occur in a cloud of supercooled water droplets. With the advent of advanced computer simulation a large number of studies with different water models have been devoted to nucleation of ice. However, the microscopic mechanism of ice nucleation is still unraveled. Many processes preceding ice nucleation have been observed, including formation of clusters of 4-coordinated water molecules, formation of stacking disordered nuclei and of polymorphs of ice different from ice I. The possible participation of these processes in the reaction coordinate of ice nucleation, however, is at odds with classical nucleation theory which assumes the reaction coordinate to be the size of the crystalline nucleus and which has been widely used to interpret homogeneous nucleation of ice. The assumption that the barrier-determining step in homogeneous nucleation of ice is the size of the crystalline nucleus has never been tested. Also, the role of different metric size, of other possible variables such as internal structure, energy, and shape of the nucleus in the reaction coordinate and the extent to which early stages processes in supercooled liquid water contribute to determine the free energy to form a crystalline nucleus have never been assessed. Here we investigate for the first time a large set of candidate reaction coordinates for homogeneous nucleation of ice using aimless shooting version of transition path sampling and likelihood maximization of the committor probability. We find that, irrespective of the method used to identify crystalline order, the size of the crystalline nucleus is consistently a better reaction coordinate than the aspect ratio and stacking disorder of the nucleus. We find that the reaction coordinate for homogeneous nucleation is the size of the nucleus consisting of a crystalline core and a wetting layer of four-coordinate water molecules (n_4). The enrichment in n_4 close to the nucleus is twice as much the enrichment far from the nucleus at 205K and it goes up to 88% at 230K. We suggest that the wetting must be decreasing the nucleation barrier. Committor (pB) probability histogram at the transition state surface predicted by our reaction coordinate quantitatively accounts for almost all (97% of the standard deviation) of the exact binomial distribution. This implies that the processes observed in early stages might be important precursors of ice nucleation but are not the rate-determining step of homogeneous nucleation. Critical nuclei from unbiased simulations are stacking disordered with cubic and hexagonal ice in a 50:50 proportion, consistently with experiments and previous computational investigations. Simulations seeded with a sphere of pure hexagonal ice, predict similar barriers for nucleation of stacking disordered and pure hexagonal nuclei and show that pure hexagonal nuclei are able to restructure during AS run. Results are used to propose a mechanism for nucleation and growth of stacking disordered ice nuclei. Finally, the finding of the reaction pathway along the gradient of potential energy for homogeneous nucleation of ice open the way to the calculation of reliable values of free energy barriers and rates using umbrella sampling techniques.

ROLE OF SACCHARIDES ON COLOUR STABILITY OF PHYCOCYANIN EXTRACTS IN AQUEOUS SOLUTIONS

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Colouring foodstuffs are gaining importance in food formulation due to the increasing need to substitute E-number colours and produce more 'natural' foods.
Spirulina extract, admitted in EU as colouring foodstuff rely its blue colour to the presence of phycocyanin, a phycobiliprotein of approximately 20 kDa part of the phycobilisomes, large protein complexes associated with the thylakoid membrane [1].
Phycocyanin is a multi-chain holo-protein composed of an apo-protein covalently bound to phycobilins, open chair tetrapyrrole chromophores and has a maximum absorption peak at 610-620nm. Water soluble, it is characterised by a rather high instability to heat and light and this limits its application in foods production especially when their processing implies the use of relative high temperatures. Recent studies have shown the positive effects of some solute (and in particular sugars) in rather limited concentration to protect phycocyanin upon heating. Missing are, however, information about the effect of thermoprotective saccharides (e.g. trehalose) and/or of environmental factors (e.g. pH).
Aim of this study was to evaluate the role of trehalose and sucrose (as reference) on thermal stability of phycocyanin as well as the kinetics of the colour changes. The effect of pH on the protein stability were also investigated.
Two commercial *Spirulina* extracts and pure C-Phycocyanin (reference) were used and added in sugar solutions (sucrose and trehalose) at different concentration (40 - 70 % w/w) and two different pH (7 and 3.0) Heat treatments were carried out at different temperatures (50 - 100 °C) and times. Protein degradation was evaluated by colour change by spettrophotometric analysis (Abs_{620}). Additional information on the effect of the thermal treatments on protein state and kinetic degradation was obtained by spettrofluorimetric, NMR and calorimetric analysis.
In water, a decrease of the absorbance with an exponential trend upon process time was observed, of higher entity at increasing temperature. The coupling of thermic and acid stresses promote a faster degradation of the protein compared with test carried out at pH 7.0, leading, at long heat treatment times, to aggregation. In saccharides solutions a higher protein stability than water was observed [2,3] with increasing effects at increasing solute concentration. A thermoprotective effect in acid systems was exerted by trehalose while at pH 7.0, sucrose had better performances and this highlights the specific effect of the sugar moiety on thermal stability of the phycocyanin. Kinetic data of colour change were used to compute activation energy of the phycocyanin thermal degradation that resulted affected by both pH and sugar type.

Results of this study will contribute the development of new ingredients for food applications as well as to develop new strategies to stabilise phycocyanin and *Spirulina* extracts (eg. encapsulation) thus, enhancing the colouring performance.

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Structure-Activity relationships in carbohydrates: A water mediated business?

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In this presentation, we would like to link the hydration of a monosaccharide, like glucose, and that of two disaccharides, like trehalose and cellobiose, with their specific activity.
The intriguing fact is the sensible difference in sweet taste between glucose and trehalose. This is particularly intriguing for at least two reasons: first, both saccharides contain at least one glucose ring that is thought as sufficient to elicit sweet response; second, intermolecular hydrogen bonding between glycol OH groups and the taste bud receptor site is considered the primary mechanism for sweet taste response by humans. The above mentioned difference in sweetness between glucose and trehalose (made up by two glucose rings), triggered our curiosity, and recently we have performed Neutron Diffraction Experiments with isotopic Substitution (NDIS) on both carbohydrates in solution to look at the solvent structure.These results were compared with those obtained with cellobiose, made up by two glucose rings (albeit with a 1-4 bond between the two glucose units instead of the 1-1 bond in trehalose), but with no reported taste. All these three sets of NDIS data augmented by EPSR simulations, have been analyzed with a novel tool called ANGULA. This analysis has been used to characterize the position and orientation of water molecules around a central reference carbohydrate molecule, resulting in spatial density maps (SDMs) that show the probability density of water around a solute in three dimensions. An example of SDMs for the three solutes is shown in Fig. 1. The striking observation is that these water molecules are located (and likely H-bonded) close to hydroxyl and hydroxymethyl sites of cellobiose and glucose, while, in the case of trehalose, these are located between the two rings. Based on these findings, the different hydration patterns of the three solutes might suggest a possible link between the hydration of specific solute sites with the solute specific activity as a sweet substance, as building block of cellulose fiber, and as a bioprotective agent, respectively.

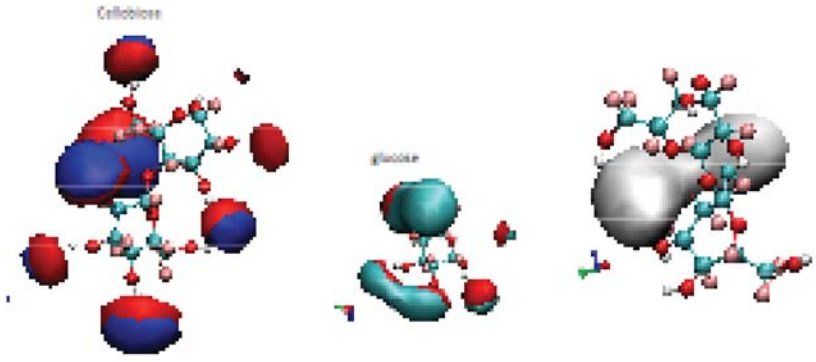

Figure 1. Spatial density maps of water molecules around (from left to right) cellobiose, glucose, and trehalose.

PROTEIN-MATRIX COUPLING IN PHOTOSYNTHETIC REACTION CENTERS EMBEDDED IN TREHALOSE AND SUCROSE GLASSES: THE EFFECT OF PROTEIN CONCENTRATION

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Anhydrobiotic organisms survive extreme drought and high temperatures by accumulating sugars, mainly trehalose and sucrose, which form biological glasses. The molecular bases of this process have been investigated by studying the room temperature dynamics of bacterial photosynthetic reaction centers (RC) embedded into sucrose or trehalose glasses. Small scale RC dynamics, in the ms timescale, have been probed by the recombination kinetics of the $P^+Q_A^-$ charged separated state induced by a ns laser pulse. To gain insight into larger scale RC dynamics up to the timescale of days, isothermal denaturation kinetics have been studied at 44 °C by NIR spectroscopy. At high sugar/protein molar ratios, conformational dynamics on both time- and space-scales were dramatically retarded in trehalose, but not in sucrose [1]. To understand this behaviour, glassy disaccharide-water binary systems of trehalose or sucrose, incorporating a nitroxide spin probe, have been characterized by FTIR and W-band EPR spectroscopies as a function of the water content [2]. In dehydrated matrices, EPR spectra showed that water and the nitroxide probe were homogeneously distributed and immobilized in trehalose, but not in sucrose. A similar analysis, which combines electron transfer and denaturation kinetics with high-field EPR of the nitroxide probe, was extended to disaccharide-water-RC ternary systems characterized by sugar to protein molar ratios of 10^4, $5{\cdot}10^3$ and 10^3. It was found that at low molar ratios a tight protein-matrix dynamical coupling occurs also in sucrose. These results emphasize the role of the protein in the structural and dynamical organization of glassy disaccharide-protein matrices and can explain the preference for sucrose rather than trehalose in some anhydrobiotic organisms.

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NANOCONFINED WATER: NETWORK STRUCTURES AND DYNAMICS PROCESSES

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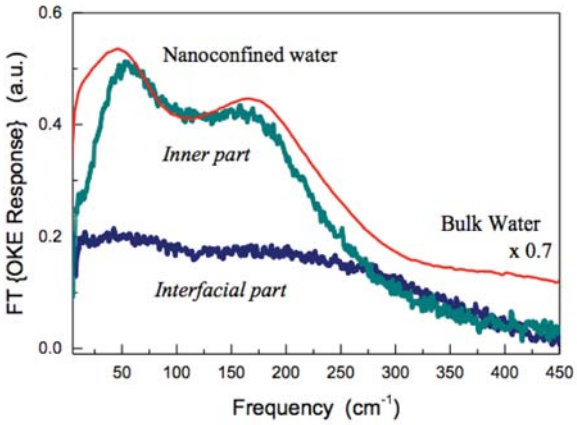
We investigate the vibrational dynamics and the structural relaxation of water nanoconfined in Vycor porous silica samples (pore size: 4 nm) at different levels of hydration and temperatures. We use as spectroscopic technique the time-resolved optical Kerr effect.

At low levels of hydration, corresponding to two complete superficial water layers, no freezing occurs and the water remains mobile at all the investigated temperatures with dynamic features similar, but not equal, to the bulk water. The fully hydrated sample shows the formation of ice at about 248 K. This process does not involve all the contained water; a part of it remains in a supercooled phase.



The structural relaxation times and the low frequency ($\nu < 500$ cm^{-1}) vibrational spectra, obtained by the HD-OKE signal is affected by confinement. At lower levels of hydration, corresponding to two complete superficial water layers or less, the H-bond bending and stretching bands, characteristic of the tetrahedral coordination of water in the bulk phase, progressively disappear, moreover the structural relaxation times are longer by a factor ~ 4.

We conclude that water dynamics of the first two hydration layers is affected by the nanoconfinement process, whereas the inner water present in the pores have dynamic features similar to the bulk water even during the supercooled phase.

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The Structuring of Water by Solutes in Aqueous Solutions

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As is well known, the behavior of water is dominated by its exceptional capacity for hydrogen bonding. Even as a liquid, hydrogen bonding imposes considerable average structure on water, and the tetrahedral structuring of water has been extensively characterized and described. The presence of solutes can significantly alter this structuring, with the detailed architecture of the solute dictating the way in which solvent water organizes around it. Hydrophobic species are those for which this structuring is sufficiently unfavorable, either because a lack of polarity disfavors hydrogen bonds, or for topological reasons, or perhaps a combination of these and other factors. Even many soluble biological species have non-polar functional groups or topological characteristics that hydrate unfavorably and significantly affect average solvent structure. Molecular dynamics simulations and neutron scattering experiments of solutions of several solutes will be described and used to characterize the structuring in these solutions. The structuring imposed by planar hydrophobic solutes will be a particular focus, including the specific cases of caffeine, guanidinium, and indole.

The molecular origin of the cryoprotective properties of trehalose on proteins

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Improvements in the ability to preserve biological matter, such as food, tissue, or organs, at low temperatures come with great benefits. This ability is often associated with different cryoprotectants that stabilize the biological molecules in their functional state. Particularly the di-saccharide trehalose is an excellent cryoprotective molecule that has been widely studied, and is currently used in the cryopreservation of a wide spectrum of materials [1]. However it is still not fully clear why trehalose possess the ability to stabilize molecules under cold stress. Several theories have been proposed to explain the mechanism behind this ability, e.g. the "water entrapment theory" [2], and the "water replacement theory" [3], but due to the lack of clear experimental evidences there is still a debate on which of these theories most accurately describes these molecular systems.

In this study we have investigated ternary systems of water, trehalose and myoglobin at different compositions and temperatures with the aim to find support for one or more of these theories. We used differential scanning calorimetry (DSC) in order to measure the glass transition temperature (T_g), and the denaturation temperature (T_{den}) simultaneously. We also performed thermal gravimetric analysis (TGA), gravimetric sorption analysis and viscosity measurements to further support our findings which will be discussed in relation to the proposed theories.

Interestingly, we also found that T_{den} decreases with increasing protein concentration, and thereby also increasing viscosity of the solution, showing that the protein stability is not only related to the viscosity, as T_g is.

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Local structure of temperature and pH-sensitive colloidal microgels

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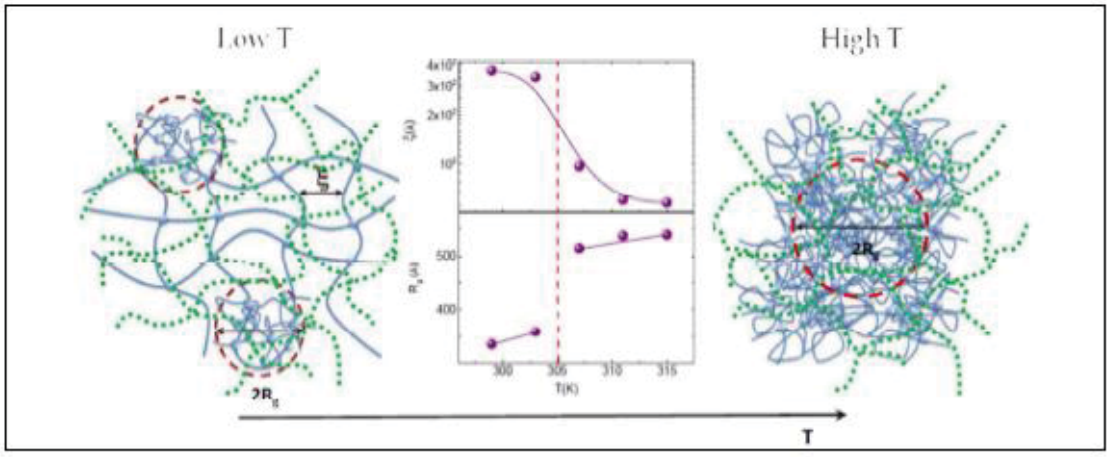
The temperature dependence of the local intra-particle structure of colloidal microgel particles, composed of Interpenetrated Polymer Networks (IPN) has been investigated by Small-Angle Neutron Scattering at different pH and concentrations, in the range 299K-315K, where a volume phase transition from a swollen to a shrunken state takes place. Data are well described by a theoretical model that takes into account the presence of both interpenetrated polymer networks and cross-linkers. Two different behaviors are found across the volume phase transition. At neutral pH and T = 307K, a sharp change of the local structure from a water rich open inhomogeneous interpenetrated polymer network to a homogeneous porous solid-like structure after expelling water is observed. Differently, at acidic pH the local structure changes almost continuously.
These fndings demonstrate that a fine control of the pH of the system allows to tune the sharpness of the volume-phase transition, according to the need for a fast or slow drug delivery.



HYDROGEN BOND DYNAMICS IN AQUEOUS SOLUTIONS OF FORMAMIDE PROBED BY EXTENDED DEPOLARIZED LIGHT SCATTERING (EDLS)

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The molecular dynamics of water has been recently subjected to numerous theoretical and experimental investigations leading to a consistent description of the molecular mechanism of hydrogen bond reorganization [1,2]. In this context, the study of formamide (FA) aqueous solutions may provide further fundamental insights on the molecular restructuring taking place in associating liquid mixtures, when different hydrogen bonding species are implicated in the formation of the overall network. Formamide ($HCONH_2$) is a simple molecule containing a peptide linkage and molecular properties FA-water solutions are strongly influenced by the formation and competition of H-bonds among H_2O molecules, C=O and N-H groups [3], also relevant in a range of biorelevant processes (i.e. protein hydration, folding and aggregation).
In the present work the molecular dynamics of FA-water mixtures is investigated at different time scales by means of depolarized light scattering experiments, which probe the relaxation of the total anisotropic polarizability of the system. The dynamical susceptibility is measured over a broad frequency range (0.01-1000 cm^{-1}) trough the combined use of dispersive and interferometric instruments. This approach, referred to as *extended frequency range depolarized light scattering* (EDLS), was proven suitable for the study of the molecular mobility in aqueous solutions of biorelevant systems at time scales ranging from fractions to hundreds of picoseconds (10^{-12} s) [4-6].
The EDLS technique will be introduced and the results obtained for FA-water solutions at different concentrations and temperatures will be presented and discussed. In particular, both relaxation dynamics (tens of picosecond) and intermolecular resonant modes (fractions of picosecond) are analyzed and interpreted in connection with the organization of the H-bonding network formed by the two species and with their dynamical coupling.

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ELASTIC AND DYNAMICAL PROPERTIES OF ELASTIN HYDROGELS

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Elastin is a major mammalian extracellular matrix protein, providing elasticity to a range of tissues including: artery, lung, skin, elastic cartilage, elastic ligament, and bladder [1]. Its mechanical properties make it a desirable biomaterial for tissue engineering. Accordingly, efforts are being made to employ elastin hydrogels as scaffolds for tissue regeneration and regrowth. Optimizing their rigidity, porosity, and hydration properties are important considerations in maximizing their potential. At present there is a lack of understanding of the molecular level origins of elastin's unique elastic behaviour. However, observations on the macroscopic scale point to a critical role of water. Elastin exhibits a counterintuitive folding known as an inverse temperature transition (ITT) [2] where at low temperatures the protein exists in an extended state, which contracts into a folded form upon heating to physiological temperature. Elastin also becomes brittle in the absence of water, a fact that is used to argue that its elastomeric properties are closely related to the entropy of hydrophobic hydration.

This presentation will provide insights gained from our recent experiments into the properties of elastin at different hydration levels and cross-linking conditions using a range of techniques. We characterized the structure, morphology and porosity of elastin samples at multiple length scales, using infrared spectroscopy and SEM. We have also explored a wide range of dynamics, from hundreds of nanoseconds to femtoseconds, below and above the ITT (280 and 300K), using neutron spectroscopy. Low-frequency collective dynamics of elastin hydrogels were observed in order to determine the stiffness of these systems on a nanometer length-scale and to compare it to that of other native proteins and other biomaterials [3-5]. These sub-picosecond dynamics were then observed using neutron scattering at different elongations under uniaxial strain. These experiments made use of a specially designed cell developed at SNS (Oak Ridge National Lab., USA). The data describe an important role of water at the heart of the elastic properties of elastin.

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PREDICTING THE KINETICS OF PROTEIN-NANOPARTICLE CORONA FORMATION IN A SIMPLIFIED PLASMA USING WATER-MEDIATED BIONANOINTERACTIONS.

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When nanoparticles (NP) interact with biological media, such as human plasma or serum, proteins and other biomolecules adsorb on the surface leading to the formation of the so-called "protein-corona". This spontaneous coating gives a biological identity to the NP determining its fate within the living systems. For this reason learning to predict the biological identities of NPs based on a partial experimental knowledge is essential to foresee a priori the safety implications of a NP for human health and, more in general, the environment. To this goal we propose a multiscale approach that allows us to predict the protein corona composition based on a partial experimental knowledge. The approach, both theoretical and computational, includes water-mediated protein-protein [1] and protein-NP interactions, accounting for the physico-chemical properties and the size of the NPs, and is implemented in an open-source software suite that we named "BUBBLES" ("Bubbles is a User-friendly Bundle for Bio-nano Large-scale Efficient Simulations") [2]. We focus on a three-component simplified plasma. We compare results obtained by different experimental techniques with two independent theoretical approaches, (a) Molecular Dynamics (MD) simulations and (b) Non-Langmuir Differential Rate Equations (NLDRE) theory. By using the experimental results for single protein solutions as an input and combining the two theoretical approaches we are able to predict the kinetics of the protein corona. We test a posteriori our predictions by direct measurements, finding an excellent agreement [3].

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OP21

USING NEUTRONS TO INVESTIGATE THE STRUCTURE AND DYNAMICS OF MONODISPERSE DENDRIMERIC POLYSACCHARIDE NANOPARTICLE DISPERSIONS

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Phytoglycogen is a highly branched polysaccharide that is very similar to the energy storage molecule glycogen. Monodisperse phytoglycogen nanoparticles can be isolated from corn and are attractive for a number of applications in the cosmetic, food and beverage, and biomedical industries. This potential is due to the properties that emerge from interaction of the nanoparticles and water, including: results in: (1) high solubility; (2) low viscosity; (3) high stability in aqueous dispersions; and (4) a remarkable capacity to sequester and retain water. To further explore the interaction of the phytoglycogen particles and water, we have performed a series of neutron scattering measurements looking at the structure and dynamics of aqueous dispersions of phytoglycogen nanoparticles as a function of concentration. Water is a hydrogen rich solvent which can be readily substituted with the deuterated form, making neutrons a uniquely useful tool.

In this presentation I will discuss the various ways that neutrons are useful in the study of water, focusing on our recent measurements on phytoglycogen dispersions. From small angle scattering we find that the nanoparticles appear to have a uniform density, consistent with our rheology measurements that indicate the nanoparticles behave like hard spheres in water. Rheology measurements also shows a divergence in the viscosity at concentrations greater than 25% (w/w), coinciding with percolation seen from the interparticle spacing. Dynamics measurements at increasing concentrations also show extra intensity emerging at low frequency relative to bulk water, indicating an increased fraction of hydration water with increased particle concentration. Because of this, aqueous suspensions of phytoglycogen provide an ideal platform for detailed testing of theories of colloidal glasses and jamming in addition to potential industrial and medical applications.

MOLECULAR DYNAMICS SIMULATION STUDY OF THE INTERACTION OF THE DIVALENT CATIONS CA^{2+} AND ZN^{2+} WITH POLYGALACTURONIC ACID

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Polygalacturonic acid (poly-Gal) is the main component of pectin, a natural polysaccharide that is often used as a gelling agent in food and pharmaceutical industries. The ionotropic gels formed by poly-Gal upon addition of the divalent cations Ca^{2+} and Zn^{2+} were found to exhibit distinct structural properties. Furthermore, solutions of poly-Gal (2.7 mM of Gal) are more viscous, but yet less turbid, in the presence of Ca^{2+} than with Zn^{2+} at a cation/Gal ratio of about 0.5. To shed some light on the molecular origin of these differences, we performed molecular dynamics (MD) simulations of aqueous solutions of the galacturonate unit (Gal) and of poly-Gal chains in the presence of either Ca^{2+} or Zn^{2+} [1]. The simulations reveal that the binding modes of the divalent cations with the carboxylate group of Gal are intimately related to their affinities with water: Zn^{2+} binds preferentially with one oxygen from the carboxylate group of Gal (monodentate coordination), whereas Ca^{2+} interacts more favorably with both carboxylate oxygens (bidentate coordination, Figure 1a). These differences arise from the stronger interaction of Zn^{2+} with water, which makes more unlikely the loss of an additional water molecule from its hydration shell when binding in a bidentate coordination with Gal (Figure 1b). Our results also evidence that the binding of divalent cations to the carboxylate groups of poly-Gal occurs before the formation of cross-links between poly-Gal chains, consistent with previous isothermal titration calorimetry measurements [2].

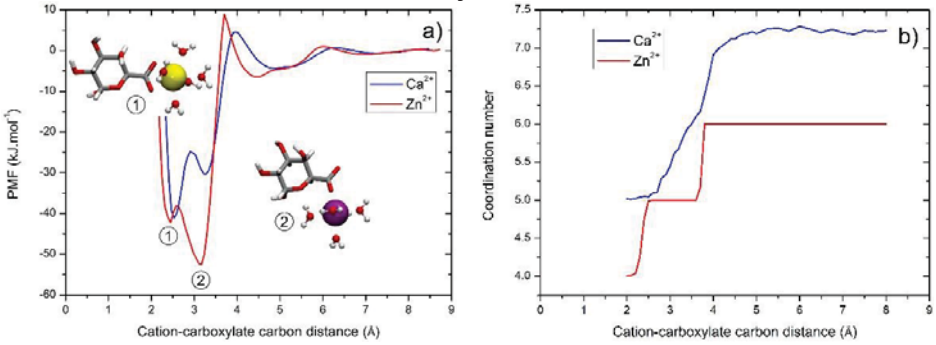

Figure 1. (a) Potentials of mean force (PMFs) for the interaction between the divalent cations Ca^{2+} and Zn^{2+} and Gal. The global minima (1) and (2) correspond to configurations where Ca^{2+} and Zn^{2+} are in contact with the carboxylate group of Gal in the bidentate or the monodentate coordination, respectively.(b) Average numbers of water molecules in the first hydration shell of Ca^{2+} and Zn^{2+} as a function of their distances from the carboxylate group of Gal.

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EXPLICIT SOLVENT THEORY FOR PROTEIN-SALT MIXTURES IN WATER

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The effects of the nature of salt on the properties of a model protein solution in water is examined theoretically and compared with experimental data. In our approach all the interacting species, proteins, ions of low-molecular-mass salt, and water molecules are accounted for explicitly. This is in contrast with the majority of other theoretical studies (see, for example, (1) and the Refs. therein), which treat the composed solvent as a structure-less continuum. The model proteins have simultaneously present positive and negative charges on the surface. The attractive square-well sites, mimicking the protein-protein van der Waals interaction, are located on the surface of the protein – all the interactions are local. The anisotropy of protein-protein interactions is crucial for the model to capture correct the physics. Water molecules are modeled as hard spheres with four off-center attractive square-well sites. These sites serve to bind either another water, or to solvate an ion. The latter are depicted as charged hard spheres of crystal size diameters. To solve numerically this complex model we utilize the associative mean spherical approximation, developed earlier for simple symmetric electrolytes (2) and extended to include protein species (3). From measurable properties we calculate the second virial coefficient, B_2, the quantity, which reflects stability of protein solutions and is closely related with the tendency of proteins to aggregate and crystalize. We show that B_2 does not depend only on the magnitude of the net charge of the protein but also on its sign, as also on the nature of the present low-molecular-mass electrolyte. We find the specific ion effects to be correlated with differences in hydration free energy between the ions in solution and charged groups on the protein. The calculations capture experimental trends with respect to pH of solution and concentration and nature of added salt, very well. This is perhaps the first attempt to calculate how the hydration of ions and charges on the protein surface shape the properties of these solutions.

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HYDRATION OF LANTHANIDE CATIONS IN ACIDIC AQUEOUS SOLUTION

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The combination of statistical mechanics and X-ray absorption spectroscopy has been proven a fruitful strategy to study structural properties of highly-charged metal cations in solution [1]. In this work will be presented theoretical studies on the Neodymium and Thulium trivalent cations in acidic aqueous solution and their comparison with experimental structural data derived from EXAFS and XANES spectra.

Ab initio calculations at the M062x/def2-TZVPP level have been employed to build a hydrated cation-water potential based on the recent extension of the hydrated ion model [2], which allows the exchange of first-shell molecules with the bulk (HIW-exchangeable potential) [3,4] by coupling the flexible and polarizable water shell model MCDHO2 [5].

Classical molecular dynamics simulations of a system including one Ln^{3+} + 1000 H_20 have been carried out. Structural and dynamical properties have been obtained from the analysis of 1 ns trajectory. Simulated EXAFS and XANES agree fairly well with the experimental ones. Features identifying the second hydration shell are associated by a shell decomposition analysis. Likewise, a novel definition of ideal angular distribution function based on ideal polyhedral structure, allows the identification of the average coordination geometry of the aquo ion in solution.

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THE HYDRATION OF CISPLATIN AND ITS AQUA-DERIVATIVES BY MOLECULAR DYNAMICS SIMULATIONS USING FIRSTPRINCIPLES INTERMOLECULAR POTENTIALS

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Pt(II) and Pd(II) aquo-complexes in solution exhibit a square-planar arrangement defining a molecular plane containing the cation and an axial region. This molecular symmetry imposes a hydration structure which has no longer a concentric-shell shape. This feature stimulated numerous theoretical and experimental studies during the last years focused on the precise description of the hydration structure and dynamics in the non-equatorial regions[1-4].The most popular Pt(II)-based anticancer metallodrug, cisplatin (cis-[PtCl2(NH3)2]) is just a typical squareplanar metal complex. It has a mechanism of action consisting of two successive hydrolysis of its chloride ligands. It yields the monoaquo-, cis-[PtCl(NH3)2(H2O)]+ (w-cisplatin) and the diaquoderivatives, cis-[Pt(NH3)2(H2O)2]2+(w2-cisplatin) followed by the formation of DNA intra-strand cross-links which ultimately produce the cancer cell death. Hydrolysis reactions have been the subject of a large number of theoretical studies aimed at correlating molecular factors to the observed kinetic parameters. Several studies evidenced the key role of explicit solvation in affecting the theoretical hydrolysis reaction rates and therefore the importance of a correct description of the hydration structure of platinum complexes [2,5]. A first work has studied cisplatin hydration by means of classical molecular dynamics (MD) simulations using a firstprinciples cisplatin-water intermolecular potential (cisplatin-W) [6]. The hydrolized cisplatin forms have only been studied in a recent work by *ab initio* MD and metadynamics [7] where the short simulation (ca. 5 ps) time and the reduced number of water molecules (ca. 50) limits a direct comparison to their solution chemistry.

This contribution reports the results on the hydration of w-cisplatin and w2-cisplatin obtained by classical MD simulations[8]. A new platinum complex-water interaction potential (wcisplatin-W) has been built and then transferred to the diaquo derivative (w2-cisplatin-W). The wcisplatin and w2-cisplatin atomic charges were specifically derived from their solute's wavefunctions. Bulk solvent effects on the complex-water interactions have been included by means of a continuum model. Angle-solved radial distribution functions and spatial distribution functions have been used to provide information about the local hydration structure. A novel definition of a multisite cavity has been applied to compute the hydration number of complexes.

Interestingly, the hydration number decreases in the order w2-cisplatin < w-cisplatin < cisplatin, just the reverse order to that of the complex net charge. The compactness of the hydration shell also increases when going from the neutral complex (cisplatin) to the doubly charged complex (w2-cisplatin). Quantum mechanics estimation of the hydration energies for the platinum complexes gives the reaction energy for the first- and second-hydrolysis of cisplatin in water. The agreement with experimental data is satisfactory.

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DESIGN AND STABILITY OF PROTEINS AND BIOPOLYMERS IN EXPLICIT WATER: A COARSE-GRAIN APPROACH

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Protein/bio-polymer design concerns the "optimization" of a sequence of residues, given an alphabet of monomers, that folds in a target structure. We present a new approach, based on a combination of water-protein coarse-grain model able, for a wide range of temperatures and pressures, to deeply explore the configurational space of a water-protein solutions.
Our model, accounting for the influence of protein interfaces on the thermodynamic properties of the hydration shell, is able to rationalize the stability of a protein against heating, cooling, and pressurization.
We show how the design of specific target structures is affected by the solvent properties. In turn, we show how the folding properties for different sequences, designed at different thermodynamic conditions, change.

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THE ENERGY LANDSCAPE IN PROTEIN FOLDING AND UNFOLDING: A ^{1}H NMR STUDY

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We use ^{1}H NMR to probe the energy landscape in the protein folding and unfolding process. Using the scheme ↔ reversible unfolded (intermediate) → irreversible unfolded (denatured) state, we study the thermal denaturation of hydrated lysozyme that occurs when the temperature is increased. Using thermal cycles in the range $295 < T < 365$K and following different trajectories along the protein energy surface we observe that the hydrophilic (the amide NH) and hydrophobic (methyl CH3 and methine CH) peptide groups evolve and exhibit different thermal behaviors. We also discuss the role of water and hydrogen bonding in the configurational stability of protein.

More precisely, the effect of water on proteins is studied in the temperature range from 180 to 370 K. By means of the NMR and the Neutron scattering we explore the protein at different hydration level h (h=0.3, 0.37, 0.42 and 0.61). The hydration level h=0.3 is equivalent to a single monolayer of water around the globular protein. We study the water role in the protein dynamical transition (glass transition or the transition from a harmonic solid like behavior to an anharmonic and liquid like motion) and the irreversible unfolding.

The thermal evolution of the spectral features allows identifying that the dynamical crossover observed for water coincides with that of the protein dynamical transition. We stress that we are able to demonstrate at a molecular level the interaction of water with the protein peptides and how via the HB it drives the protein activity. In addition on considering water thermodynamics we identify a special temperature T* that marks the crossover of water, by increasing T, from the state of a complex anomalous liquid to that of a simple conventional one. Furthermore, the combination of Scattering and NMR data allows us to clarify the role of T* in the protein properties, in particular T* is the limit of the protein native state.

At the same time we are able to clarify at microscopic level the underlying mechanisms that govern the reversibility of the folding-unfolding and irreversible denaturation processes of the protein. New NMR observations at the temperature above and below the protein irreversible unfolding (T_D) show that folding-unfolding process takes place as a function of the temperature; we observe that T acts as a control parameter of the measured nuclear magnetization M(T). Whereas far from this singular temperature, in the protein native state, the M(T) behavior is Arrhenius, approaching T_D (in a large T-interval) the system changes dramatically it energetic configurations by means a power law behavior. Hence, by following the thermal behavior of different protein-peptide metabolites we are able to explore the funneled energy landscape. By taking advantage of the polymer physics we propose this complex process (protein folding/unfolding) as a sort of sol-gel transition driven by water as the cross-linker between different protein peptides, and with T_D as the percolation threshold temperature.

SOLUTE, SOLVENT AND SOLVATION DYNAMICS IN WATER-CARBOHYDRATE SOLUTIONS REVEALED BY EXTENDED DEPOLARIZED LIGHT SCATTERING

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Carbohydrates are in a position of undisputed importance in food and biophysics. The ability to form hydrogen bonds with water through hydroxyl groups is known to play an important role in determining their hydration properties. Water-rich solutions of different carbohydrates have been studied by Extended Depolarized Light Scattering (EDLS), a technique which opens an exceptionally wide window on the dynamics of both water and solute by probing optical anisotropy fluctuations between 0.3 GHz and 40 THz [1]. Molecular motions which are active on different timescales can be revealed – such as solute rotational diffusion, collective dynamics of bulk and hydration water, H-bond stretching and bending modes – all of which help derive information about the effect of carbohydrates on surrounding water. Here we summarize recent results, concerning the magnitude of the slowdown of hydration with respect to bulk water, the spatial extent of the perturbation around solute molecules, and how the hydrogen-bond network of water is affected. Special emphasis is placed on aqueous solutions of levoglucosan, one anhydrosugar which is attracting interest due to its potential in biopreservation. EDLS reveals that the reduction from five to three OH groups per molecule and the formation of a large non-hydrogen bonding area on the surface of the molecule considerably modifies the hydration properties with respect to the parent sugar, glucose, and to other mono and disaccharides such as fructose, trehalose, and sucrose. All of these latter are found to dynamically perturb a number of water molecules in proportion to their number of hydroxyl groups (~3.3 molecules per OH), by increasing their relaxation time of a factor ~5-6 compared to bulk water [2,3], but levoglucosan does not: the average hydration number (~24) is almost twice that of glucose, and the retardation factor is lower (~3-4) [4]. Introduction of the anhydro bridge thus *decreases* the intensity of the dynamic perturbation on surrounding water, but *increases* the spatial extent of the perturbation, placing this system in a special position among small carbohydrates. These results indicate that the hydration properties of carbohydrates are related nontrivially to the number and distribution of hydroxyl groups.

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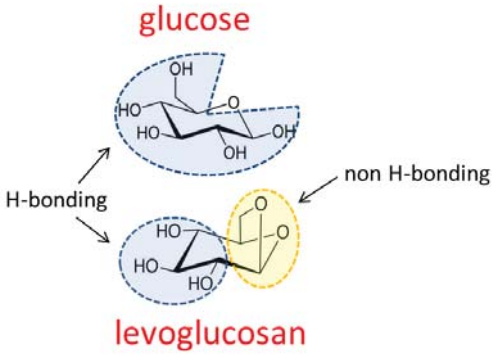

BIOMOLECULAR HYDRATION WITHOUT WATER. MULTI-SCALE MODELLING OF BIOMOLECULES INCLUDING HYDRODYNAMICS

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A novel simulation framework that integrates the OPEP coarse-grained (CG) model for proteins with the Lattice Boltzmann (LB) methodology to account for the fluid solvent at mesoscale level, is presented [1]. OPEP is a very efficient, water-free and electrostatic-free force field that reproduces at quasi-atomistic detail processes like peptide folding, structural rearrangements and aggregation dynamics [2].
The LB method is based on the kinetic description of the solvent in order to solve the fluid mechanics under a wide range of conditions, with the further advantage of being highly scalable on parallel architectures.
The capabilities of the approach are presented and it is shown that the strategy is effective in exploring the role of hydrodynamics on protein relaxation and peptide aggregation.
The end result is a strategy for modelling systems made up to thousands of proteins [3],
such as in the case of dense protein suspensions and tackle problems as misfolding/aggregation in neurodegenerative deceases [4] as well as biomolecular transport and stability in cell-like environments.

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POSTER PRESENTATIONS

PHYSICAL PROPERTIES OF CARRAGEENAN GEL FROM *HYPNEA BRYOIDES* EXTRACTED FROM OMAN USING AN ALKALINE PRETREATMENT

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Abstract: Carrageenan is a natural polysaccharide extracted from numerous marine red seaweed species of the division Rhodophyceae. This sulfated galactan is commonly used in a wide variety of applications in the food industry as a thickening, gelling, stabilizing and suspending agent in water and milk systems. A knowledge and understanding of the gel texture as well as the congealing and melting temperatures are significant in assessing their potentials in the market. In this study, rheological measurements were carried out to assess gels from the red seaweed *Hypnea bryoides*, growing along the Arabian Sea coasts of Southern area of Oman. The effect of different conditions of alkali pretreatment of extraction on physical properties of Carrageenan was evaluated. It was extracted by various concentrations of NaOH (4%, 6%, 8%), and heated at different temperatures (70, 75, 80 °C) for different durations (2, 2.75 and 3.5 hours). Under such conditions, thermal analysis and textural properties were investigated by using a modulated differential scanning calorimeter and texture Analyzer, respectively. The produced gel was prepared with 1.5% carrageenan concentration and ionic strength of 30 mM KCl in an aqueous environment. Melting and gelling temperatures were different among alkali pretreatment. Higher values of melting (65.1˚C) and gelling temperature (37.3˚C) were obtained at condition of 6%, 75˚C, and 3.5h. Furthermore, regarding gel texture, alkali pretreatment increases hardness of the gel significantly ($p < 0.05$).

Keywords: Seaweed, *Hypnea bryoides*, physical properties, Alkali pretreatment

WATER RELEASE BY DSC AS COMPLEMENTARY TO DRUG RELEASE INVESTIGATIONS

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The study of water evaporation is proposed as an alternative and complementary tool to investigate the properties of a porous matrix form the interesting viewpoint of drug release. The experimental approach is based on the calorimetric and release studies.
As a model porous matrix, calcium alginate beads were prepared by drop-wise addition of the alginate solution (2% w/v) into a calcium chloride 0.050 M solution. Lysozyme and human insulin were loaded as model proteins.
Additional components to the alginate basic formulation (such as chitosan, HPMC, ethanol) modify the lysozyme release profile, revealing that chitosan is the most effective in slowing down the release. Release curves were fitted using the general Weibull equation, the parameter n is used as an indicator of the diffusion rate. The linear form of Clausius–Clapeyron equation was used for quantitative comparison of the DSC curves; indeed the change in the slope (B) reflects changes in the "evaporation rate".
A good correlation between release and calorimetric data, supporting the hypothesis of the obstruction model, was obtained for lysozyme, except in the case of 1% HPMC, where slope B suggests obstruction but the release of LSZ is rapid.
The obstruction effect of chitosan is clear in unloaded beads, but the addition of chitosan produces different effects in case of beads loaded with lysozyme or human insulin. Chitosan decreases insulin release (high n) but the slope B changes less than in the case of LSZ.
A wide data set on other relevant systems will enforce the correlation between the release/effusion phenomena. The correlation would allow to understand whether the interaction between chitosan and insulin is predominant with respect to alginate and chitosan, thus compromising the obstruction effect, and whether the interaction of lysozyme with alginate is hampered by HPMC.

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IODINATED CONTRAST MEDIA:HYDRATED FORMS OF IOPAMIDOL. INVESTIGATIONS ON THE SOLID STATE.

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Non-ionic iodinated contrast media (NICM) are widely used for diagnostic purpose, providing satisfying quality images due to their high concentration in solution.
Although NICM solutions are characterized by a relatively low osmolality and viscosity, they are in a state of an apparent oversaturation, where a particular aggregation at the border-line between supersaturation and primary nucleation seems to occur. These molecules display several conformations in solution and in particular hindered rotations of a group(s) of the molecule give rise to metastable conformational isomers in solution, known as atropisomerism.
Thermodynamic and spectroscopic investigations carried out so far put in evidence that both intermolecular association and conformational phenomena govern the solution properties of NICM.
The major interest for the more general applications in pharmaceutical field is understanding whether atropisomeric molecules may co-crystallize with some solid state disorder or one form only is selectively crystallized from the solution mixture of atropisomers.
New results are presented on the investigation on some solid state properties of iopamidol by X-ray diffraction (XRD), IR and Raman spectroscopy, differential scanning calorimetry (DSC). X-ray diffraction experiments identified the crystallographic structures of three different polymorphs (anhydrous, monohydrate and pentahydrate), characterized also by IR and Raman spectroscopy. Calorimetric studies revealed solid state transformation occurring as a function of the temperature.
The comparison of the structural, spectroscopic and calorimetric results aim at providing potential guidelines for the implementation of atropisomerism phenomenon in the case of contrast media.

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STATIC PROPERTIES OF HYDRATION WATER AROUND LYSOZYME WITH AND WITHOUT TREHALOSE UPON COOLING

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Today aqueous solutions of sugars are widely used for food storage. Between sugars, Trehalose is the most efficient in inhibiting ice formation, which causes damage in biomolecules. Nevertheless the origin of the beneficial role of Trehalose is not completely understood.
We present structural results obtained by a molecular dynamics study of a small globular protein, the Lysozyme, and spc/e water with and without Trehalose. The two systems are studied at ambient pressure upon cooling for temperatures ranging from 300 K to 200 K.
It has been already pointed out in [1] the formation of a cage of Trehalose molecules around Lysozyme. From a dynamical point of view, this cage causes a dramatic slowing down of hydration water [2]. Here we show new results obtained from the structural characterization of Lysozyme hydration water upon cooling [3]. Particular interest is devoted to the properties of hydrogen bonds network and to the different perturbation to the tetrahedral coordination of hydration water in presence and in absence of the cryoprotectant Trehalose.

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PRINTING NANOBIOLOGY IN AQUEOUS SYSTEMS

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Water is the major constituent of living organisms and plays a central role in the daily lives of humans. From a chemical point of view, water is associated with unique physical-chemical properties which make it the universal solvent for biological systems [1]; it is therefore important to study the active role of water in the technologies aimed at the realization of functional devices in medical and biotechnological field.
Our studies in the field of printing nanobiology in aqueous solution are proposed to highlight the role of water in the processes of interaction between biomolecules in drug-screening devices fabricated by bioprinting technologies and to emphasize the influence of water evaporation on the diffusion of molecules in droplets of picoliter-scale.
In this regard, we already showed the possibility to use a low-cost and miniaturized drug screening methodology based on direct bio-printing like Dip Pen Lithography (DPL) [2], and non-contact patterning methods, such as Inkjet Printing, to dispense biomolecules under microarrays format without affecting biological activity at solid-liquid interfaces [3] or in picoliter-scale droplets [4].
Accordingly, we carried out studies by the fluorescent probe Alexa 647, dispensed as liquid droplets in picoliter-scale using Inkjet Printing methods, by finely tuning deposition parameters and aqueous ink formulation. To evaluate effects of solvent evaporation, Alexa solutions are spiked with variable quantity of glycerol from 10% to 80 % v/v. We used fluorescence confocal microscopy to quantify fluorescent probe behavior by means of fluctuation techniques that permit mapping concentration and diffusion coefficients of fluorolabeled or fluorescent molecules at nanomolar concentration.
We also showed nanoprinting of DNA oligonucleotides in order to characterize the dynamics of hybridization of DNA sequences in aqueous solution, by printing oligonucleotides with DPL in form of drops of picoliters volumes on a solid substrate.
Having previously shown the possibility to efficiently deposit oligonucleotides onto glass surfaces [2], here we deposited oligonucleotides on nylon substrate for the fabrication of low cost point of care biochips. Accordingly, we optimized oligonucleotides printing on nylon substrate, obtaining efficient deposition at 10 - 1μM concentrations, 70% relative humidity and 30% glycerol v/v. The printed oligonucleotides were then hybridized with a fluorescence-labelled complementary probe to detect and quantify DNA hybridization after DPL deposition. Remarkably, the fine tuning of the printing conditions, such as humidity, tip-surface dwell time, glycerol percentage of molecular ink, are all essential parameters to facilitate the printing process, so limiting the effect of evaporation of the water and obtain regular, circular spots.

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TREHALOSE vs GELATIN: A COMPARISON BETWEEN CROWDING EFFECTS AND SPECIFIC BIOPROTECTION

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Preservation of biological molecules is a relevant topic both for its technological applications and for the challenges in understanding the mechanisms at its basis, either in vitro or in vivo. Saccharides, and in particular trehalose, have been widely studied for their effectiveness in biopreservation, and various hypotheses have been prompted to explain the underlying molecular mechanisms, based either on specific, water-mediated, interaction with the embedded biomolecules or on the aspecific alteration of the physical properties of the matrix. High molecular-weight polymers are also utilised as biomaterial stabilisers in pharmaceutical or food technology, mainly because of their concurrent positive effects in system rheology. However, their preservation efficiency is not likely to depend on specific interactions with the embedded biomolecules, but only on crowding effects, as studies on polysaccharides and disaccharides/polysaccharides mixtures.
Here we present a study on myoglobin (Mb) preservation in gelatin (hydrolysed collagen), in crowded Mb-only system, and gelatin/trehalose matrices. Gelatin was chosen for its simple structure, which is analogous to a simple polymer, and its great resistance to denaturation. Myoglobin was chosen as a well characterised probe molecule, both in the oxidized met-Mb form and in the reduced carboxy form (MbCO). Data from Differential scanning calorimetry (DSC) and UV-Visible Spectroscopy are reported and compared with results already reported for Mb/trehalose systems [1].
DSC measurements were performed at various water content and allowed to obtain the denaturation temperature of Mb, pointing out a difference between trehalose matrices, who always stabilise Mb, and gelatin-containing systems, in which a destabilisation at high-to-middle hydration is in contrast with a stabilisation at very low water content. Spectroscopy measurements were performed on systems dried up to 66 days at 80°C under vacuum to test the effects of sustained stress. Through the spectroscopic study of CO loss from MbCO it was also possible to differentiate massive denaturation from functional denaturation. Overall, results show that trehalose biopreservation is more effective in the long-range and against functional denaturation, while crowding-induced preservation provides a better performance against abrupt massive denaturation and/or aggregation.

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BIOPROTECTION CAN BE TUNED UP WITH PROPER PROTEIN-SACCHARIDE RATIO: THE CASE OF SOLID AMORPHOUS MATRICES

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Saccharides, and in particular trehalose, are well known for their high efficiency in protecting biostructures against adverse environmental conditions. Experiments and simulations [1] on carboxy-myoglobin (MbCO) have shown that the protein dynamics is highly inhibited in a low-water trehalose host medium, the inhibition being markedly dependent on the amount of residual water. Beside hydration, the properties of the saccharide amorphous matrices are noticeably variable also with the protein/sugar ratio.
In the work here presented, we performed an Infrared Spectroscopy (FTIR) study on the stretching band of the bound CO molecule (COB) and on the Water Association Band (WAB) in dry amorphous matrices of various sugars (the disaccharides trehalose, maltose, sucrose and lactose, and the trisaccharide raffinose), at different protein/sugar ratios. Such bands have already been successfully exploited for the simultaneous study of the thermal evolution (20-300K) of the embedded biostructure and of the matrix; indeed, the COB is one of most studied protein spectroscopical marker, and the WAB a very sensitive probe for the low-water matrix.
The results show a high dependence of the protein and matrix dynamics on the protein/sugar ratio, depicting a scenario in which the system dynamics evolves from matrix-slaved to coupled and eventully to protein slaved, with increasing protein concentration. This support the idea, already proposed [1,2], that a mutual protein↔matrix structural and dynamic influence subsists in low hydrated systems, indicating that the protein-solvent master and slave paradigm does not strictly hold, but the mutual relationship depends on the relative concentrations. Furthermore, for each sugar, an optimal protein/sugar concentration ratio, which maximizes the protein preservation minimizing the thermal dynamics, can be identified.

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PROTEINS IN TREHALOSE:

A CONSISTENT PICTURE FROM A MULTI TECHNIQUE APPROACH

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Embedding biomolecules in saccharide matrices leads to a series of peculiar properties that are relevant from the point of view of both biochemistry and biophysics, and have important implications on related fields such as food industry, pharmaceutics, and medicine. In this poster we present results from a combination of experimental (FTIR, SAXS, DSC, Light Scattering) and simulative (MD) techniques on solutions or glassy matrices of oligo- and disaccharides at different water content, rigidity and temperatures, both in the presence and in the absence of proteins. The perspective is to set up a connection between the biophysical approach, which is generally "*protein-centric*", and the pharmaceutical/applicative approach, which is traditionally "*stabilization-procedure centric*". The attention is addressed in particular to the modulation of systems dynamics, to its hydration, temperature, and composition dependence, and to the molecular origin of the trehalose peculiarity.

TEMPERATURE BEHAVIOR OF WATER SURROUNDING HYDROPHOBIC GROUPS IN SMALL PEPTIDE CHAINS

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The behavior of the hydrophobic hydration shell of organic molecules is an important area of research [1]. The reasons can be found in the intention to understand what is the real influence of hydrophobicity in free energy calculations on protein folding. It has been shown how around the hydrophobic group, a slow-dynamic hydration shell is present. It is characterized by a threshold temperature around 30-40 °C degrees, above of that the hydration shell results to be broken [1,2]. However, the complexity of such systems and the lack of appropriate investigation techniques, do not allow a quantification of the hydration shell energy contribution.

Alternatively, quantitative information can be obtained by the relaxation time of the intermolecular hydrogen bonded state, studied by means of the Kubo-Anderson analysis of the line shape of the Raman peak [3]. The bandwidth of the obtained isotropic Raman spectrum is broadened because of the environment inhomogeneity (mainly Gaussian broadening) and because of temporal perturbation effect (Lorentzian like broadening). The Kubo model allow to disentangle these two contribution by the isotropic Raman peak lineshape.

A possible way to evaluate the temperature behavior of water surrounding hydrophobic groups is to study the folding process of simplified peptides system where an α-helix folding occurs. Aqueous solutions of shorts 16-residues alanine-based peptides such e.g. 3K-I (Ac-AAAAKAAAAKAAAAKA-NH_2) shows a stable, temperature dependence, α-helix formation, with a folding temperature of about 60 °C [4]. Because of the large presence of alanine and lysine, such simplified peptides results to be valid "models" to study how the hydrophobic groups in peptides act to favour the temperature-folding process.

Following this strategy we have studied the helix formation process on 3K-I. It can be shown how the folding process in this small chain is strongly related to the behavior of water around the alanine methyl groups.

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HOPPING PHENOMENA AND FRAGILE TO STRONG TRANSITION IN SUPERCOOLED WATER

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Molecular dynamics simulations of TIP4P and TIP4P/2005 are presented upon supercooling along different isochores close to the liquid liquid critical point.
We study the MCT behaviour that holds in the region of mild supercooling, the fragile to strong transition and its connection to the Widom line, and the hopping phenomena that appear in the region of strong supercooling.
The Widom line is the line of maxima of thermodynamics response functions that emanates from a critical point. In particular we studied densities ranging from 1.03 to 0.95 gr/cm^3 and temperatures spanning from 300 K to 190 K.
We calculated the intermediate scattering functions and the translational relaxation times and we studied the onset of hopping for supercooled water through the van Hove self correlation functions. In particular hopping appears more favoured on the low density side of the Widom Line.
Besides the connection between the fragile to strong transition and the hopping phenomenon we also explore the link to the Widom line for these dynamic features [1].

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THE ROLE OF WATER IN THE SPIRAL HALLOYSITE NANOTUBES

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By means of Self-Consistent-Charge Density Functional Tight Binding calculations the structure and the role of water molecules in the inter-arms region of spiral halloysite nanotubes have been investigated [1]. Models of spiral halloysite with cavity diameter of ca. 5 nm were builded by replicating a unit cell with $Al_2Si_2O_5(OH)_4 \cdot nH_2O$ (n=0, 2) stoichiometry in the points of a spiral lattice, forming a supercell where the spiral arms overlap for 1/3 of their length.

A certain degree of disorder has been noticed in the hydrogen bond network occurring both on the outer surface of the nanotube and on the surfaces between the spiral arms. This is due to the intrinsic non periodic nature of a spiral lattice, whose curvature changes along the arms so that every [SiO] tetrahedron and [AlO] octahedron experience different small structural distortions.

The SCC-DFTB energetics of the spiralization process [2] was estimated by comparing the absolute energies of the spiral nanotubes with those of the corresponding unfolded sheets. It turned out that, in the particular case here considered, in the dehydrated system the spiral form is almost isoenergetic with the kaolinite single sheet. The effect of the water molecules present in the hydrated halloysite, on the other hand, amounts to a stabilization of ca. 300 kJ mol^{-1} following the spiralization process. This behaviour can be explained by considering that both forms are stabilized by the formation of hydrogen bonds between the overlapping arms, but the water molecules in the hydrated nanotube, in virtue of their mobility, can easily adapt in order to ensure more efficient interactions, which prevail on the tetrahedral and octahedral distortions occurring during spiralization.

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CELL BIOTHERMODYNAMICS: CAN CALORIMETRY MONITOR CYTOPLASMIC WATER ACTIVITY?

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A novel application of differential scanning calorimetry brought successfully to the determination of water activity changes in aqueous films through isothermal dehydration, especially dealing with polysaccharides solutions and gels matrices.
An extension of the technique to a wide range of substrates, cells monolayer has been tested (osteosarcoma cells U2OS). The goal concerns the correlation of the experimental calorimetric signal (heat flow) and changes in the water binding state with healthy and stressed cells. The stresses both at cytoplasmic and membrane level were achieved with solutions at different ipotonicity level, Igepal as surfactant and Arsenite as oxidative-stress grains forming agent. The several treatments required a proper set-up in order to guarantee reproducibility and to clarify the cells dehydration pattern and the analysis of the calorimetric curves.
Preliminary results proved the feasibility of the measurements on cellular substrate and the study revealed a good sensitivity of the experimental response on the textural features of the system and on its actual hydration state. This study is in line with the reinforced interest in the thermodynamic and kinetic properties of the cytoplasmic macromolecular crowding and its changes under stressing conditions.

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DSC AS OLD TOOL FOR CONTINUOUS WATER ACTIVITY DETERMINATION ON THIN AQUEOUS FILMS

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A novel microcalorimetric approach has recently been developed to investigate the kinetics and thermodynamics of the isothermal dehydration process of aqueous films, based on a theoretical description of the water evaporation process. The study reveals the great sensitivity of the experimental response on the textural features of the system and on the actual binding state of water within a system. Indeed, the main result of the approach concerns the demonstration of the correlation between the experimental heat flow and the water activity of the solution under examination. The assumption of proportionality between heat flow, rate of water evaporation and water activity was verified studying a large number of dehydration profiles of aqueous solutions of sugars, salts, surfactants and polymers, including complex mixed solutions and gels.
All the results confirmed this assumption, as long as the system is under quasi-thermodynamic conditions, namely until the water diffusion within the system is larger than the evaporation rate. Under these circumstances, the experimental heat flow curve turns into a continuous measurement of water activity as function of the water content of the system.
The capability to perform desorption isotherms in a short machine-time (from minutes to few hours) allows to project the application of the calorimetric technique to routine water activity determination in continuous for the whole water content range.

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THERMORESPONSIVE NANOMATERIALS BASED ON HALLOYSITE CLAY NANOTUBE WITH PNIPAAM SHELL

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Halloysite (HNT) is a naturally occurring nanotube. This two-layered aluminosilicate with empirical formula ($Al_2Si_2O_5(OH)_4 \cdot 2H_2O$) has a polydispersed hollow tubular structure. The HNTs external surface is composed of Si−O−Si groups conferring the negative charge while the internal surface consists of a gibbsite-like array of Al-OH groups conferring the positive charge; such a situation is present in a wide pH range.
We investigated the adsorption of Poly(N-isopropylacrylamide) (PNIPAAM) by TGA, turbidimetry, DLS and ζ-potential measurements onto this material. The PNIPAAM polymer is stimuli-responsive and it undergoes rapid changes in its microstructure from a hydrophilic to a hydrophobic state, triggered by changes in the environment including heat, pH, ionic strength, additives, etc. The selectivity towards the outer surface of the HNT was pursued by selective electrostatic interactions. The ζ-potential experiments revealed a larger net negative charge for the PNIPAAM/HNT modified (-31±1 mV) with respect to the pristine HNT (-19.4 mV) [1,2]. Turbidimetry measurements show that the obtained hybrid materials can form more stable aqueous dispersion than those obtained for pristine HNTs due to the increased electrostatic repulsions between the functionalized nanoparticles. The temperature triggered water release from the PNIPAAM shell was monitored by compressibility and apparent molar volume measurements.
We put forward an easy strategy to prepare hybrid materials with PNIPAAM modified hollow cylindrical shape of the nanotube which can be properly used into aqueous phase for external-stimuli controlled solubilization and delivery. The work was financially supported by the University of Palermo, PRIN 2010-2011 (prot. 2010329WPF), FIRB 2012 (prot. RBFR12ETL5) and PON TECLA (PON03PE_00214_1).

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THE DIELECTRIC RESPONSE OF INTERPLAY BETWEEN WATER AND SOLUTE IN AQUEOUS SOLUTIONS

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Whenever the water molecules interact with either dipolar or charged systems, a bulk water dielectric relaxation peak broadening takes place. The broadening can be described by the Cole-Cole function [1], which represented by a frequency dependent complex dielectric permittivity $\varepsilon^*(\omega)$, given by: $\varepsilon^*(\omega) = \varepsilon_\infty + \Delta\varepsilon / [1 + (i\omega\tau)^\alpha]$, where $i^2 = -1$, ω is the cyclic frequency, $\omega = 2\pi f$ and $\Delta\varepsilon = \varepsilon_s - \varepsilon_\infty$ is the dielectric strength, with ε_s and ε_∞ denoting the extrapolated low-frequency and high-frequency permittivities, respectively, τ is the relaxation time and exponent α is the broadening parameter ($0<\alpha\leq 1$).

If a solute has the dipole nature, the new clusters (compared with the bulk) are created due to the dipole-dipole interactions. It leads to the "red shift" of the dielectric loss maximum frequency. In the case of ionic solutions another cluster structure developed due to dipole-charge interactions and the "blue shift "is observed. In the general case when a solute molecule has both charged and dipole groups, the dielectric loss maximum demonstrate "red" or "blue" shifts depending on the entity concentration. In all aqueous solutions the water and solute interactions can be considered as dipole-matrix interaction in which water is the dipole subsystem. The 3D trajectories phenomenological approach[2] was applied to the results of isothermal dielectric measurements of aqueous solutions at different concentrations of:

Hydrocarbons (Glucose and Fructose) [3]
NaCl and KCl [4]
AMP and ATP [5]
Amino Acids [6]
Myoglobin.

The Cole-Cole parameters of the main water peak considered as the markers that can clarify the way and rate at which water interacts with solute, and, also indicate the solute concentration regions where dipole-dipole or dipole-charge interactions are dominated.

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DIFFUSION, MOLECULAR SEPARATION, AND DRUG DELIVERY FROM LIPID MESOPHASES WITH TUNABLE WATER CHANNELS

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Lyotropic liquid crystals (LLC), also known as mesophases have received great deal of attention in biomedical, pharmaceutical, and materials science due to their applicability ranging from active ingredients encapsulation to stimuli responsive sustained release, biomimetic membranes, membrane technology, protein crystallization, or material templating. Various types of mesophases exist depending on self-assembly of lipids in the presence of water. Bicontinuous cubic phase (BCP) which consists of two sets of non-communicating but interpenetrating three-dimensional periodic water channel networks separated by lipid bilayers has been focus of numerous studies. The control of the water channels size in these systems paves the way to their applicability in drug delivery, crystallization or membrane separation processes.

In the current work, diffusion phenomenon through aforementioned mesophases has been conducted systematically. Specifically, sucrose stearate, which has hydration-enhancing properties, is added to monolinolein/water system to tune the water channels diameter; this leads to shift in boundaries of the phase diagram. Furthermore, different molecules with varying size and conformation are selected to assess their diffusion within the water channels. The results reveal that the larger the water channel diameter is, the faster the diffusion process. It is concluded that such bicontinuous cubic phases with the ability to effectively and selectively separate nanoparticles of a target size, have the potential to be used as filtering membranes of tunable molecular cutoff.

We believe that further investigation in mesophases systems can lead to their applicability in the area of size-selective membranes for molecular separation, drug release or crystallization of large molecules such as proteins and DNA.

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CO-MILLED TREHALOSE-LIMONENE SYSTEMS: THERMAL PROPERTIES AND STABILITY

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In pharmaceutical drug formulation dry milling is widely used to reduce drug particle size and obtain nano-powders. Mechanical stresses induce physical and structural transformations from crystalline state to metastable polymorphic forms or amorphous states. Co-milling of two crystalline compounds could generate alloys with physical and physico-chemical properties different from those of the individual compounds. In the food sector milling has found until now limited applications and scarce are also the studies aimed to deep the feasibility of this process for ingredients development and functionality improvement.

This study aims at evaluating thermal and physical properties of the products of anhydrous crystalline trehalose co-milled in presence of limonene, a volatile hydrophobic aroma compound. Co-milling of trehalose and limonene was carried out on different aroma:sugar weight ratio (up to 10% limonene) and for different milling times.

DSC, NMR and SEM experiments were carried out to characterize the differently processed co-milled sugar-limonene matrices while gas chromatographic analysis were carried out to determine limonene retention in the processed matrices. Analyses were carried out just after preparation and during storage under controlled environmental conditions (temperature and humidity).

At increasing milling time a progressive crystalline-to-amorphous state transformation occurs along with an increase of the retention volatile compound to form an anhydrous trehalose matrix entrapping limonene. While the Tg of trehalose in the co-milled systems did not change in respect to that of the pure sugar, an increased tendency towards crystallization was observed. Trehalose and limonene upon co-milling do not form alloys, although limonene, partly dispersed in the amorphous matrix, seems to exert a subtle plasticizing effect. SEM analysis showed that co-milled sugars present a powder-like but dense microstructure typical of a dried or low moisture amorphous material with micro- and nano-holes where limonene may be partly dispersed and entrapped.

Upon storage under low relative humidity (10 % RH), limonene was released from co-milled matrix even if to a limited extent and with a different extent as a function of milling time confirming the mobility of small molecules in amorphous, high viscous systems.

A comparison of these results with those obtained with other sugars will contribute to unravel the behavior of the amorphous trehalose-based systems and the feasibility of milling in the food sector for new ingredients development.

UNDERSTANDING THE COLLOIDAL PROPERTIES OF ESPRESSO COFFEE

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The peculiar sensory properties make *espresso* a unique and distinctive beverage among coffee brews. Process conditions applied during brewing enhance several surface tension-related phenomena that contribute to the development of a polyphasic system where small air bubbles and oil droplets are finely dispersed in a complex multi-component aqueous solution. Two classes of compounds of the coffee beverage (namely polysaccharides and melanoidins) are known to contribute to the development of the emulsified state during brew extraction. Presence and concentration of these compounds may vary depending on origin of the coffee and roasting degree as well as other process variables. Scarce are, moreover, the information about the role of other amphyphylic compounds present in the espresso (e.g. phenolic compounds) that may affect the dispersion state and stability.
This study investigated the role of the raw coffee cultivar and origin, roasting degree and extraction time on the properties of the o/w emulsion of espresso coffee. Beverages were made by using a domestic iper*espresso* machine (model X7.1, Illycaffè S.p.A, IT) and coffee sized in capsules. Arabica coffee from Ethiopia and India (roasting degree: medium) and a blend of Arabica coffee medium and dark degree roasting as well as a Robusta coffee were tested. Samples at different extraction times were obtained. Surface and interfacial tension, dispersion degree (by laser diffraction), melanoidins, phenolic compounds and fatty acid composition of the coffee beverages were determined. Surface properties of selected coffee compounds (chlorogenic acid and caffeine) were also evaluated.
The dispersion degree and surface tension properties of Arabica coffees resulted affected by roasting while origin did not influence significantly the parameters considered. Extraction time highly influenced the colloidal properties as the early coffee brew fractions were characterized by a lower D[4,3] of the lipidic dispersed phase and this result is related to the presence of a higher content of components that can positively affect the formation of the colloidal state of coffee during extraction. Differences in the dispersion degree between Arabica and Robusta brews were also observed and associated to the different solute composition.
Chlorogenic acid, main phenolic compound in roasted coffee brews showed a significant surface activity, that resulted affected when co-solutes like caffeine was added to the solution, likely due to the formation of complexes.

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RAMAN SPECTROSCOPY OF PEG HYDRATION AND CONFORMATION IN SOLUTION: RELEVANCE TO BIOLOGICAL WATER UNDER MACROMOLECULAR CROWDING

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Macromolecular crowding is a well-known phenomenon largely interpreted in terms of excluded volume effects, although evidence has also been accumulated in favor of attractive/repulsive interaction of the added macromolecular co-solute with the other cellular components, just as it occurs in untreated cells.
Many biological protocols require a drastic reduction of the water activity as alternative to the irreversible fixation. Among all compounds that are effective in reducing water activity, the phenomenology of PEG-water system is particularly relevant. The most practical feature of PEG seems to be the inert character of PEG based on its molecular conformation in aqueous solutions, where PEG exposes the uncharged hydrophilic ether groups and can participate in hydrogen bonding with the oxygen in ethers and hydrogen in water, having a structure similar to water [1]
In the present work, a detailed investigation of the PEG conformational/hydration characteristics is reported by using Raman Spectroscopy supported by molecular simulation studies. The wide range of concentration explored is intended to cover the pure liquid PEG and semi-dilute solutions resembling those of the crowded cellular system, while the temperature range (27-87 °C) may sufficiently disclose the changes in the energetics of the mixed interactions.

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TEMPERATURE EVOLUTION OF MOLECULAR INTERACTIONS IN SBE-B-CD/H2O SOLUTIONS A DIFFERENT CONCENTRATIONS: AN UV-RAMAN AND FTIR-ATR ANALYSIS

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Complexation is of interest in pharmaceutical field, since it increases solubility and bioavailability of drugs. In the present work, we carried out a combined FT-IR and Raman study, vs. T, of the vibrational dynamics of the inclusion complexes, in both solid and liquid phase, of beta-cyclodextrin (beta-CyD) derivatives with Coumestrol, an antioxidant phytoestrogen. The aim was to examine if and how the vibrational dynamics of the complexes is affected by the host-guest interactions, and to probe if there exists a relationship of this dynamics with the stability, as achieved by the evaluation of thermodynamic parameters. The comparative analysis among the different macrocycles allowed us to test their accessibility to Coumestrol. The information obtained for solid and liquid state are relevant to tailor new formulations for release of drugs with specific performances. In particular, the tunability of the source for the excitation of UV Raman spectra, as the one offered by the IUVS set-up, turned out to be crucial in order to achieve resonant conditions for the enhancement of the Raman signal of specific functional groups.

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DYNAMICAL CROSSOVERS IN SUPERCRITICAL WATER

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Supercritical water exhibits properties that are very different from water in normal conditions, for instance it can solvate organic solutes. There are a number of emerging new applications of supercritical water in food science, pharmaceuticals, polymers, powders, biomaterials, biotechnology, fossil and biofuels. Supercritical water is traditionally considered as an indistinct fluid, being neither a liquid nor a gas. In a recent study we have shown an important connection between thermodynamic and dynamic properties of water in the supercritical region[1]. By analysing the experimental viscosity and the diffusion coefficients obtained in simulations we found that the line of response function maxima in the one phase region, the Widom line, is connected to the crossover from a liquid-like to a gas-like behaviour of the transport coefficients. This appears to be in analogy with the crossover found in the dynamics of supercooled water at the crossing of the WL associated with the presence of a possible liquid-liquid critical point[2,3,4]. By analyzing the results of popular site models for water, we find that TIP4P, TIP4P/2005 and SPC/E models reproduce remarkably well the experimental behavior, while for other models the agreement is more qualitative[5].The coupling of thermodynamic and dynamic features in supercritical water shows that this state if more complex than what was so far thought, opening new perspectives for the characterization of supercritical fluids. In this respect the Widom line can be considered as an important unifying concept of the behaviour of water.

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ENHANCEMENT OF AMORPHOUS PRILOCAINE'S STABILITY BY WATER ADDITION

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Increasing stability of drugs is a highly sought after milestone for the pharmaceutical industry, since a more stable state implies the desirable extension of its shelf life. This way, the product becomes safer, while leaving its physical and chemical properties unaltered. However, to optimize a drug, increasing its stability is not the only goal to keep in sight: stability could also be associated with a decrease of its solubility, which is vital for drug assimilation in the body. Rapid formation of solution of a drug is sometimes desireable so as to achieve a high efficacy and a rapid absorption rate that may increase its bioavailability [1]. According to the stability hierarchy, the best solubility would be obtained in the glass state, i.e the thermodynamically unstable state, since amorphous solids have increased molecular mobility, enthalphy, and in some cases even specific volume when compared to the crystalline state [2, 3]. This situation presents then two competing effects that must be balanced in an optimal way by the use of biocompatible mechanisms, changing the original drug either by adding a component or by a physical process. The optimal solution to this two-folded problem would then be to achieve a homogeneous solution between the drug and a biocompatible compound while, when possible, avoiding crystallization of the mixture [4]. It is then of uttermost interest to study the interaction of drugs with the universal biocompatible solvent: water. Understanding the interaction of this molecule with the physiological environment is a key matter that needs to be addressed [5]. In this project the drug prilocaine has been chosen as a case study, since it is crucial for anesthetic purposes, specially in dentistry applications. The equilibrium phase diagram of prilocaine has been previously studied [6], showing that for H2O/prilocaine concentrations < Xp = 0.7 (prilocaine mole fraction), the system separates in two stable liquid phases: L1, a prilocaine-based solution saturated with water and L2, a water-based solution saturated with prilocaine. We have now established the non-equilibrium phase diagram of this system, by measuring Tg with Differential Scanning Calorimetry at different water/prilocaine concentrations for the original product and the aged material. Added to this, dielectric experiments have been done, confirming the Tg values obtained by DSC, which show the addition of water to the drug for concentrations greater than Xp = 0.7, surprisingly increase Tg while keeping the system in one unique amorphous phase.

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Water Activity in Viscous Heterogeneous Bio-systems

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Classical Thermodynamics does not treat the effects of the medium viscosity as long as it deals with equilibrium states. Unfortunately, this has consequences on the experimental practices and on the reliable characterization of the immense variety of bio-systems and bio-products that are rather viscous and heterogeneous. Since most of them are aqueous or contain aqueous phases, one is inclined to use water as an internal probe to describe their behavior and water activity, a_W, as a suitable parameter.

When the viscosity of a condensed phase is large, as in many real systems, the migration of water can be very slow. This is why any steady state can mimic the attainment of equilibrium and the detected water activity may have a value, $a_{W,app}$, smaller than the true one.

An alternative approach is therefore necessary to reconcile expectations from a thermodynamic description of an aqueous (or simply moist) system with the experimental difficulties related to the low mobility of the water molecules.

Some NMR experimental evidence was provided by Brian Hills who proposed a correlation between a_W and the normalized overall FID relaxation rate, which can be related to u_W. At least at the microscopic level, namely, the level "seen" with a NMR investigation, u_W and a_W would accordingly be someway correlated to each other, in spite of the fact that u_W and a_W belong to different realms of physics.

In present work advantage is taken from the fact that every viscous aqueous system can be imagined as obtained from a starting poorly viscous solution either by isothermal dehydration, or by cooling (or combination of these treatments), so to approach the glass transition threshold, across which viscosity and heat capacity undergo large changes.

The formal treatment of the problems starts with an expression of the chemical potential of water that includes an extra contribution to the overall potential energy of the aqueous phase(s), namely, a fraction of the “free energy” that becomes available once the viscosity drops down.

$$\mu_W = \mu_W^* + RTlna_W - \mu_{visc} = \mu_W^* + RTlna_{W,app}$$

The explicit form of the μ_{visc} comes from the one proposed by Gibbs and Di Marzio to describe the connection between the excess (with respect to the solid state) configuration part of the Helmholtz free energy F_c^{exc} to the viscosity, η, of a liquid close to its glass transition threshold, namely,

$$\ln\eta = \beta - \alpha F_c^{exc}/RT$$

$$\frac{\mu_W^{visc}}{RT} = -ln\frac{a_W}{a_{W,app}} = \alpha\frac{F_c^{exc}}{RT} = \beta - \ln\eta$$

When η approaches the viscosity of pure water, η^*, $a_{W,app}$ approaches a_W, that approaches unity: thence $\beta = ln\ \eta^*$. This allows the conclusion that, at any temperature,

$$\frac{a_W}{a_{W,app}} = \left(\frac{\eta}{\eta^*}\right) = \left(\frac{u_W^*}{u_W}\right) \geq 1$$

where the mobility has been replaced with the reverse of the viscosity, η, experienced by water molecules that migrate from the core to the surface of the system. Similar expressions are proposed to describe the effects of physical barriers that hinders water displacements across heterogeneous systems.

TOWARDS A BETTER UNDERSTANDING OF THE PLASTICIZING EFFECT OF WATER ON PLGA BY USING FT-IR

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Poly(lactic-co-glicolyc) acid (PLGA) is one of the most commonly used excipient in the formulation of modified release devices. The release mechanism of the active ingredients from such systems depends greatly on polymer features and, water, by acting as a plasticizer, can drastically change these characteristics (e.g., glass transition temperature, mechanical properties, degradation time) modifying drug release kinetics [1]. Despite the plasticizing effect of water is well known and widely studied in hydrophilic polymers [2], there are currently many open questions and a lack of knowledge on molecular interactions between water and hydrophobic polymers. The aim of this work was to use Fourier transform infrared (FTIR) spectroscopy to better understand the plasticizing effect of water on PLGA and to know how water interacts with polymer chains.

For this purpose, mixture of organic solvent with increasing structure complexity (i.e., acetone, ethyl acetate and methyl 2-methoxypropionate) and different amount of water were analyzed by FTIR and studied as models. Hydrated PLGA films on CaF_2 windows were obtained by using the *solvent casting technique* and incubating the films in hermetic chambers with different relative humidity or immersing them completely in water for 24 h at 37 °C. FTIR spectra were collected at room temperature and in the 20-70 °C temperature range.

In sample spectra, the analysis of several intramolecular modes suggested the formation of hydrogen bonds between water and carbonyl and ester groups [3]. Particular attention has been focused on the –OH stretching region (3100-3700 cm^{-1}), directly related to the water vibrational modes. In the samples with small amount of water, the –OH stretching profile is characterized by the presence of a sharp peak at 3650 cm^{-1}, probably ascribed to the oscillator OH involved in the formation of weak bonds. Increasing the amount of water, spectra showed a broad component between 3100-3400 cm^{-1}, ascribed to co-operative bonds. This suggests the presence of water molecules self-associated in clusters, confined in a hydrophobic environment [4]. The hydrated polymer film spectra collected in the 20-70 °C temperature range confirmed the presence of this unbound water.

In conclusion, the organic solvent/water mixtures seem to be good models to understand the interaction between water and polymer chains. The FTIR spectra of hydrated PLGA films showed that the water present in the samples is only weakly bound to the polymer chains. Overall, the main features of the spectrum of the "plasticizing" water can be observed by these experiments.

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DISENTANGLING CONTRIBUTIONS OF WATER FLUCTUATIONS TO THE KINETICS OF KEY-LOCK BINDING

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We investigate the contributions of the fluctuations of hydration water on the key-lock association kinetics of ligands (key) binding to hydrophobic pockets (lock) by means of stochastically coupled Langevin equations. Recent studies presented [1,2] that fluctuations in the water occupancy of hydrophobic pockets are intimately coupled to the diffusion of associating ligands, leading to an increased local spatial friction, decelerated binding kinetics, and non-Markovian (memory) effects in effectively 1D-reaction coordinate descriptions. Here we describe the fully coupled system by a minimalistic model using a 2D-reaction-coordinate system that is Markovian. The model enables the decomposition of different contributions to the kinetics, such as the fluctuating hydrophobic interaction as well as hydrodynamic interactions. With that we can elucidate the origin of the effectively enhanced friction in the process and provide design principles for a tunable model for ligand recognition kinetics. As results we present mean binding times, local diffusivities, and the characterization of the underlying stochastic processes for varying ligand-pocket interaction contributions focusing on the role of solvent.

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A 3D COARSE-GRAINED MODEL FOR PROTEIN TEMPERATURE- AND PRESSURE-DENATURATION WITH EXPLICIT WATER.

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It is generally accepted that water as the solvent of proteins plays a large role in the folding and denaturation of those. The phase diagram of water, on the other hand, is likewise affected by the surface and geometric constraints imposed by the solvate [1]. Many proteins are folded in a region which, to low temperature and high pressure, is limited by cold and pressure denaturation [1]. This two phenomena cannot be explained by accounting only protein's self-interactions in implicit water. To investigate the role of water in cold and pressure denaturation we present our preliminary results about the extension of a coarse-grained water model [2] previously employed to study the stability region of a protein in two dimensions [3] to three dimensions.

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ELECTROSPUN PAN/HALLOYSITE NANOTUBES MEMBRANES WITH HIGH WATER FILTRATION EFFICIENCY

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The necessity of benefiting a breakthrough in filtration technology has led to increasing attention in advanced functional nanosized materials such as nanofibers for filtering devices as a solution for providing water at lower energy costs. Polyacrylonitrile (PAN), a polymer with acrylonitrile as the repeating unit, gets increasing attention as precursor toward carbon materials while applications for carbon-based material are expanding rapidly due to their excellent mechanical and thermal properties (1). PAN-based nanofibers have been used in many applications such as electrically conductive nanofibers, wound dressing, biocatalyst, tissue scaffolding, and drug delivery systems (2). Furthermore, PAN nanofibers have been widely used in ultrafiltration, nanofiltration, and reverse osmosis membranes due to their high chemical resistant, thermal stability, and excellent wetability with water (3). Electrospun nanofibrous membranes were successfully fabricated by polyacrylonitrile (PAN) reinforced with halloysite nanotubes (HNTs). In order to evaluate the effect of HNTs on properties of electrospun PAN membrane, their morphology, mechanical stability, filtration performance and thermal resistance were characterized. Morphological analysis revealed the highly porous structure of nanofibrous membranes containing uniform nanofibers with diameter ranging from 350 to 650 nm. Surface area analysis signified the mesoporous structure of the membranes, while incorporation of HNTs led to increase in pore volume and BET surface area. Mechanical properties revealed that the elongation (ε) and tensile strength (σ) were initially increased by incorporation of 1% w/w HNTs while the value of elastic modulus (E) decreased with addition of HNTs. Furthermore, thermogravimetric analysis (TGA) revealed that addition of 1% w/w HNTs significantly reduced the weight loss of PAN membranes by 49%, suggesting that uniform dispersion of HNTs increases the thermal stability of the membranes. Moreover, PAN/HNTs membranes showed excellent oil/ water separation performance, while incorporation of HNTs led to increase in water flux rate, which is considered as a key point in water filtration membranes. Additionally, heavy metal ion adsorption performance of the membranes showed a significant increase by incorporation of 3% w/w HNTs. These results signified the potential of electrospun PAN/HNTs nanofibrous membranes to be used for water filtration applications.

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BIOCHEMICAL ISOLATION OF STRESS-GRANULES

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In response to environmental changes, the cellular response is organized to conserve energy and divert cellular resources toward survival, adaptation and eventual recovery. Given that protein translation is a highly energy-spending process, the induced response is focused on producing just the essential proteins needed for survival and adaptation currently limiting overall translation of cellular mRNAs (Lindquist, 1981) by rapidly assembling nontranslating mRNAs and their associated RNA-binding proteins into aggregate-like structures, membrane-less cytoplasmic organelles called ribonucleoprotein (RNP) granules (Anderson and Kedersha, 2008). These structures behave as liquid-like droplet phases of the cytoplasm and phase transition theory has recently been used to explain such large-scale organization (Hyman, 2014).
Cellular cytoplasm deviates markedly from ideal solution: it is heterogeneous and crowded by many different molecules, similar to a colloidal system. The manipulation of environmental conditions, such as the intracellular pH, average ion and metabolite concentrations and the macromolecular crowding, can alter its delicate equilibrium and lead to RNP-granules formation. Using semi-intact cells, a change of macromolecules concentration with the addition of further crowders lead to a change of osmotic pressure and intracellular viscosity, interfering with diffusion-controlled processes. From the physicochemical point of view, the cytoplasm behaves like a colloidal suspension, which can undergo a glass transition by extreme crowding due to sudden loss of water (Parry, 2014).
The human U2OS cell line expressing two fluorescently tagged proteins involved in the formation of RNP-granules has been used as model system. Different combinations and amounts of polymers have been tested in order to induce the granules formation, selecting 8% (w/v) PEG-6000 in combination with a detergent as suitable for a sudden formation of small stable structures. The formation of granules has been monitored using imaging-based methods and their biochemical stabilization and isolation after cell lysis has been performed by using a specific formulation of macromolecular crowders, additives and detergent.

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INVITED LECTURES

IL1
USEFULNESS OF WATER ACTIVITY IN UNDERSTANDING THE STABILITY OF PHARMACEUTICAL TABLETS
P.W.S. Heng, T.N. Hiew

IL2
WATER AND ITS ROLE IN MICROENCAPSULATION OF FOOD INGREDIENTS
S. Drusch

IL3
PORE WATERS – HOW DID WE MUDDY THEM SO BADLY?
A.K. Soper

IL4
WATER IN CONFINED AND BULK LIQUID PHASES: A TIME RESOLVED INFRARED STUDY
R. Righini

IL5
MEMBRANELESS ORGANELLES DESTABILISE THE DOUBLE HELIX
T.J. Nott, T.D. Craggs, P. Farber, J.D. Forman-Kay, A.J. Baldwin

IL6
SELF-ASSEMBLY OF AMYLOID FIBRILS IN 1, 2 AND 3 DIMENSIONS: FUNDAMENTALS AND APPLICATIONS
R. Mezzenga

IL7
THE LIQUID-LIQUID CRITICAL POINT IN WATER
F. Sciortino

IL8
COUPLING BETWEEN PROTEIN AND HYDRATION WATER DYNAMICS
G. Schirò, Y. Fichou, F.-X. Gallat, G. Zaccai, M. Weik

IL9
GLASS TRANSITIONS, STRUCTURAL RELAXATION TIMES AND PHYSICOCHEMICAL PROPERTIES OF COMPLEX FOOD SYSTEMS
Y.H. Roos

IL10
STUDYING WATER IN HYDROPHOBIC POLYMERS: A DRUG DELIVERY PERSPECTIVE
P. Blasi

ORAL PRESENTATIONS

OP1
MICROSCOPIC DYNAMICS OF HYDRATION WATER IN AQUEOUS SOLUTIONS OF LYSOZYME AND TREHALOSE UPON COOLING
P. Gallo

OP2
HYDRATION DYNAMICS OF BIOSYSTEMS: FROM SMALL HYDROPHOBES TO PROTEINS
L. Comez, M. Paolantoni, S. Corezzi, P. Sassi, A. Morresi, L. Lupi, S. Perticaroli, D. Fioretto

OP3
NEW INSIGHTS INTO THE ROLE OF WATER IN BIOLOGICAL FUNCTION: STUDYING SOLVATED BIOMOLECULES USING TERAHERTZ SPECTROSCOPY
J. Savolainen, M. Havenith

OP4
HYDRATION SHELL DYNAMICS DRIVEN BY PROTEIN INTERFACE
Y. Kurzweil-Segev, A. Greenbaum (Gutina), I. Popov, E. Levy, Y. Feldman

OP5
DYNAMICS OF WATER AT STACKED PHOSPHOLIPID BILAYERS: A MOLECULAR DYNAMICS SIMULATIONS STUDY
C. Calero, H.E. Stanley, G. Franzese,

OP6
ANOMALIES OF HYDROPHOBIC SOLVATION EXTEND INTO WATER DYNAMICS
R.G. Weiß, M. Heyden, J. Dzubiella

OP7
EXPLORING BIOMOLECULAR HYDRATION THERMODYNAMICS WITH GRID CELL THEORY
J. Michel

OP8
NEUTRON DIFFRACTION OF HYDROGENOUS MATERIALS: MEASURING INCOHERENT AND COHERENT INTENSITIES SEPARATELY. THE EXAMPLE OF LIQUID WATER
L. Temleitner, A. Stunault, G. Cuello, L. Pusztai

OP9
HYDROPHOBIC/HYDROPHILIC EFFECTS IN WATER DYNAMICS AS PROBED BY UV RAMAN SCATTERING
B. Rossi, F. D'Amico, A. Gessini, V. Venuti, A. Mele, C. Punta, L. Melone, V. Crupi, D. Majolino, C. Masciovecchio

OP10
RATE DETERMING STEP FOR HOMOGENEOUS NUCLEATION OF ICE.
L. Lupi, R.G. Mullen, A.H. Nguyen, B. Peters, V. Molinero

OP11
ROLE OF SACCHARIDES ON COLOUR STABILITY OF PHYCOCYANIN EXTRACTS IN AQUEOUS SOLUTIONS
M. Faieta, P. Pittia

OP12
STRUCTURE-ACTIVITY RELATIONSHIPS IN CARBOHYDRATES: A WATER MEDIATED BUSINESS?
L. Maugeri, S.E. Maclain, L.C. Pardo, M.A. Ricci, F. Bruni

OP13
PROTEIN-MATRIX COUPLING IN PHOTOSYNTHETIC REACTION CENTERS EMBEDDED IN TREHALOSE AND SUCROSE GLASSES: THE EFFECT OF PROTEIN CONCENTRATION
M. Malferrari, A. Nalepa, A. Savitsky, F. Francia, W. Lubitz, K. Möbius, G. Venturoli

OP14
NANOCONFINED WATER:
NETWORK STRUCTURES AND DYNAMICS PROCESSES
A. Taschin, P. Bartolini, R. Righini, R. Torre

OP15
THE STRUCTURING OF WATER BY SOLUTES IN AQUEOUS SOLUTIONS
J.W. Brady

OP16
THE MOLECULAR ORIGIN OF THE CRYOPROTECTIVE PROPERTIES OF TREHALOSE ON PROTEINS
C. Olsson, H. Jansson, J. Swenson

OP17
LOCAL STRUCTURE OF TEMPERATURE AND PH-SENSITIVE COLLOIDAL MICROGELS
V. Nigro, R. Angelini, M. Bertoldo, F. Bruni, V. Castelvetro, M.A. Ricci, S. Rogers, B. Ruzicka

OP18
HYDROGEN BOND DYNAMICS IN AQUEOUS SOLUTIONS OF FORMAMIDE PROBED BY EXTENDED DEPOLARIZED LIGHT SCATTERING (EDLS)
M. Paolantoni, S. Perticaroli, P. Sassi, L. Comez, D. Fioretto, A. Morresi

OP19
ELASTIC AND DYNAMICAL PROPERTIES OF ELASTIN HYDROGELS
S. Perticaroli

OP20
PREDICTING THE KINETICS OF PROTEIN-NANOPARTICLE CORONA FORMATION IN A SIMPLIFIED PLASMA USING WATER-MEDIATED BIONANOINTERACTIONS.
O. Vilanova, J.J. Mittag, P.M. Kelly, S. Milani, K.A. Dawson, J.O. Rädler, G. Franzese

OP21
USING NEUTRONS TO INVESTIGATE THE STRUCTURE AND DYNAMICS OF MONODISPERSE DENDRIMERIC POLYSACCHARIDE NANOPARTICLE DISPERSIONS
J. Nickels, J. Atkinson, E. Papp-Szabo, J. Katsaras, J. Dutcher

OP22
MOLECULAR DYNAMICS SIMULATION STUDY OF THE INTERACTION OF THE DIVALENT CATIONS Ca^{2+} AND Zn^{2+} WITH POLYGALACTURONIC ACID
A. Lerbret, A. Assifaoui, H.T.D. Uyen, F. Neiers, O. Chambin, C. Loupiac, F. Cousin

OP23
EXPLICIT SOLVENT THEORY FOR PROTEIN-SALT MIXTURES IN WATER
V. Vlachy, and Y.V. Kalyuzhnyi

OP24
HYDRATION OF LANTHANIDE CATIONS IN ACIDIC AQUEOUS SOLUTION
D.Z. Caralampio, N. Morales-Negrito, J.M. Martínez, R.R. Pappalardo, E. Sánchez Marcos

OP25
THE HYDRATION OF CISPLATIN AND ITS AQUA-DERIVATIVES BY MOLECULAR DYNAMICS SIMULATIONS USING FIRSTPRINCIPLES
INTERMOLECULAR POTENTIALS
E. Sánchez Marcos, A. Melchior, J.M. Martínez, R.R. Pappalardo

OP26
DESIGN AND STABILITY OF PROTEINS AND BIOPOLYMERS IN EXPLICIT WATER: A COARSE-GRAIN APPROACH
V. Bianco, G. Franzese, I. Coluzza

OP27
THE ENERGY LANDSCAPE IN PROTEIN FOLDING AND UNFOLDING: A ^{1}H NMR STUDY
F. Mallamace, C. Corsaro, D. Mallamace, S. Vasi, C. Vasi, P. Baglioni, S.V. Buldyrev, S.-H. Chen, H.E. Stanley

OP28
SOLUTE, SOLVENT AND SOLVATION DYNAMICS IN WATER-CARBOHYDRATE SOLUTIONS REVEALED BY EXTENDED DEPOLARIZED LIGHT SCATTERING
S. Corezzi, L. Comez, D. Fioretto, A. Morresi, M. Paolantoni, P. Sassi

OP29
BIOMOLECULAR HYDRATION WITHOUT WATER. MULTI-SCALE MODELLING OF BIOMOLECULES INCLUDING HYDRODYNAMICS
F. Sterpone, P. Derreumaux, S. Melchionna

POSTER PRESENTATIONS

PP1
PHYSICAL PROPERTIES OF CARRAGEENAN GEL FROM HYPNEA BRYOIDES EXTRACTED FROM OMAN USING AN ALKALINE PRETREATMENT
Z. Al-Nahdi, A. Al Alawi, I. Al-Marhobi, M.S. Rahman

PP2
WATER RELEASE BY DSC AS COMPLEMENTARY TO DRUG RELEASE INVESTIGATIONS
B. Bellich, M. Borgogna, Attilio Cesàro

PP3
IODINATED CONTRAST MEDIA: HYDRATED FORMS OF IOPAMIDOL. INVESTIGATIONS ON THE SOLID STATE.
B. Bellich, A. Cesàro, S. Di Fonzo, G. Giannini, A. Maiocchi, N. Masciocchi, L. Tavagnacco, M. Paolantoni, F. Uggeri

PP4
STATIC PROPERTIES OF HYDRATION WATER AROUND LYSOZYME WITH AND WITHOUT TREHALOSE UPON COOLING
G. Camisasca , P. Gallo

PP5
PRINTING NANOBIOLOGY IN AQUEOUS SYSTEMS
F. Cavaleri, G. Arrabito, C. Pellerito, V. Vetri, P. Cancemi, A. Desideri, S. Feo, M. Leone, B. Pignataro

PP6
TREHALOSE vs GELATIN: A COMPARISON BETWEEN CROWDING EFFECTS AND SPECIFIC BIOPROTECTION
E.F. Semeraro, S. Giuffrida, G. Cottone, A. Cupane

PP7
BIOPROTECTION CAN BE TUNED UP WITH PROPER PROTEIN-SACCHARIDE RATIO: THE CASE OF SOLID AMORPHOUS MATRICES
S. Giuffrida, G. Cottone, L. Cordone

PP8
PROTEINS IN TREHALOSE: A CONSISTENT PICTURE FROM A MULTI TECHNIQUE APPROACH
L. Cordone, G. Cottone, A. Cupane, A. Emanuele, S. Giuffrida, M. Levantino

PP9
TEMPERATURE BEHAVIOR OF WATER SURROUNDING HYDROPHOBIC GROUPS IN SMALL PEPTIDE CHAINS
F. D'Amico, B. Rossi, A. Gessini, C. Masciovecchio

PP10
HOPPING PHENOMENA AND FRAGILE TO STRONG TRANSITION IN SUPERCOOLED WATER
M. De Marzio, M. Rovere, P. Gallo

PP11
THE ROLE OF WATER IN THE SPIRAL HALLOYSITE NANOTUBES
N. Armata, G. Lazzara, F. Ferrante

PP12
CELL BIOTHERMODYNAMICS: CAN CALORIMETRY MONITOR CYTOPLASMIC WATER ACTIVITY?
E. Gurian, S. Semeraro, B. Bellich, A. Cesàro, A. Rampino, C. Schneider

PP13
DSC AS OLD TOOL FOR CONTINUOUS WATER ACTIVITY DETERMINATION ON THIN AQUEOUS FILMS
E. Gurian, B. Bellich, A. Cesàro

PP14
THERMORESPONSIVE NANOMATERIALS BASED ON HALLOYSITE CLAY NANOTUBE WITH PNIPAAM SHELL
G.Cavallaro, G. Lazzara, S. Milioto, F. Parisi

PP15
THE DIELECTRIC RESPONSE OF INTERPLAY BETWEEN WATER AND SOLUTE IN AQUEOUS SOLUTIONS
E. Levy, A. Puzenko, Y. Feldman

PP16
DIFFUSION, MOLECULAR SEPARATION, AND DRUG DELIVERY FROM LIPID MESOPHASES WITH TUNABLE WATER CHANNELS
R. Ghanbari, R. Negrini, R. Mezzenga

PP17
CO-MILLED TREHALOSE-LIMONENE SYSTEMS: THERMAL PROPERTIES AND STABILITY
P. Pittia, M. Faieta, J.-F. Villart, M. Descamps, A. Cesàro

PP18
UNDERSTANDING THE COLLOIDAL PROPERTIES OF ESPRESSO COFFEE
P. Pittia, C. Di Mattia, L. Navarini, K. Crouvisier-Urion, M. Martuscelli, D. Mastrocola

PP19
RAMAN SPECTROSCOPY OF PEG HYDRATION AND CONFORMATION IN SOLUTION: RELEVANCE TO BIOLOGICAL WATER UNDER MACROMOLECULAR CROWDING
N. Quadri, S. Di Fonzo, A. Rampino C. Schneider, A. Cesàro

PP20
TEMPERATURE EVOLUTION OF MOLECULAR INTERACTIONS IN SBE-□-CD/H2O SOLUTIONS A DIFFERENT CONCENTRATIONS: AN UV-RAMAN AND FTIR-ATR ANALYSIS
V. Crupi, F. D'Amico, A. Gessini, D. Majolino , C. Masciovecchio, B. Rossi, R. Stancanelli, V. Venuti, C.A. Ventura

PP21
DYNAMICAL CROSSOVERS IN SUPERCRITICAL WATER
P. Gallo, D. Corradini, M. Rovere

PP22
ENHANCEMENT OF AMORPHOUS PRILOCAINE'S STABILITY BY WATER ADDITION
G.N. Ruiz, M. Romanini, E. Shalaev, T. Loerting, L.C. Pardo, R. Macovez, J.L. Tamarit

PP23
WATER ACTIVITY IN VISCOUS HETEROGENEOUS BIO-SYSTEMS
A. Schiraldi

PP24
TOWARDS A BETTER UNDERSTANDING OF THE PLASTICIZING EFFECT OF WATER ON PLGA BY USING FT-IR
S. Casagrande, M. Paolantoni, M. Ricci, P. Blasi

PP25
DISENTANGLING CONTRIBUTIONS OF WATER FLUCTUATIONS TO THE KINETICS OF KEY-LOCK BINDING
R.G. Weiß, J. Dzubiella

PP26
A 3D COARSE-GRAINED MODEL FOR PROTEIN TEMPERATURE- AND PRESSURE-DENATURATION WITH EXPLICIT WATER.
A. Zantop, V. Bianco, G. Franzese

PP27
ELECTROSPUN PAN/HALLOYSITE NANOTUBES MEMBRANES WITH HIGH WATER FILTRATION EFFICIENCY
M. Makaremi, P. Pasbakhsh

PP28
BIOCHEMICAL ISOLATION OF STRESS-GRANULES
A. Rampino, M. Scarola, C. Schneider, E. Gurian, S. Semeraro, A. Cesàro