

# **Frontiers in Water Biophysics**

**23-26 September 2012**  
**Perugia, Italy**

**Book of Abstracts**

Front cover picture  
Fontana Maggiore – Perugia  
*Courtesy of Lanfranco Sportolari*

## COMMITTEES

### *Scientific Committee*

**Attilio Cesàro**, University of Trieste - Italy (Chair)  
**Ruggero Bettini**, University of Parma - Italy  
**John Brady**, Cornell University - USA  
**John Crowe**, University of California - USA  
**Harmen De Jongh**, TI Food and Nutrition - The Netherlands  
**Letizia Lombardini**, National Italian Transplant Centre - Italy  
**Salvatore Magazù**, University of Messina - Italy  
**Roberto Righini**, LENS and University of Firenze - Italy  
**Alberto Schiraldi**, University of Milano - Italy  
**Francesco Sciortino**, University of Rome 'La Sapienza' - Italy  
**Juergen Siepmann**, University of Lille - France

### *Organizing Committee*

**Daniele Fioretto**, University of Perugia - Italy (Chair)  
**Paolo Blasi**, University of Perugia - Italy  
**Lucia Comez**, University of Perugia - Italy  
**Silvia Corezzi**, University of Perugia - Italy  
**Laura Lupi**, University of Perugia - Italy  
**Assunta Morresi**, University of Perugia - Italy  
**Alessandro Paciaroni**, University of Perugia - Italy  
**Marco Paolantoni**, University of Perugia - Italy  
**Paola Sassi**, University of Perugia - Italy  
**Massimiliano Borgogna**, University of Trieste - Italy  
**Claudio Masciovecchio**, ELETTRA Trieste - Italy

# GENERAL PROGRAMME

## **Monday 24**

- 9.00      **Opening**
- 9.15      **Stephen R. Meech** - invited  
Ultrafast OKE Spectroscopy for the THz Raman Spectra of Aqueous Solutions
- 9.55      **Renato Torre**  
Time-Resolved Laser Spectroscopy on Interfacial and Nanoconfined Water
- 10.10     **Andrea Orecchini**  
Collective Modes and Amorphous Dynamical Character of Hydration Water in Biomolecules
- 10.25     **Polycarpus Pissis**  
Water and Protein Dynamics in Hydrated Globular Proteins
- 10.40     **coffee break / posters**
- 11.10     **Dmitry Matyushov** – invited  
Theory of Dielectric Response of Proteins in Solution from MHz to THz
- 11.50     **Riccardo Cucini**  
Broadband Time - Frequency Resolved Spectroscopy: The MARTELLO Project
- 12.05     **Francesco D'Amico**  
Hydration Shells of Macromolecules Studied by High-Resolved Resonant Raman Spectroscopy
- 12.20     **Simone Capaccioli**  
Relaxation in Aqueous Mixtures: Local Solute Dynamics vs. Water Specific Motions
- 12.35     **Silvia Caponi**  
Cluster Phases of Decorated Micellar Solutions with Macrocyclic Ligands
- 13.00     **Lunch**

## **Monday 24**

14.00 **POSTER SESSION**

15.00 **Kia L. Ngai** - keynote

A Unified View of the Dynamics of Water, Aqueous Mixtures, and Hydrated Proteins Derived from Experiments and Theory

15.20 **Maria Grazia Ortore**

Detailing Protein Solvation Shell in Binary Mixtures

15.35 **Simone Wiegand**

Transport in Temperature Gradients: Aqueous Solutions

15.50 **Marc Descamps** – invited

Pharmaceutical Processing: A Physical Perspective on Induced Transformations and Water Involvement

16.30 **coffee break / posters**

17.00 **Julien Michel** – invited

Water in Protein-Ligand Interactions: Challenges and Opportunities for Drug Design

17.40 **Raffaele Lamanna**

Anisotropic Enhanced Water Diffusion in Scleroglucan/Borax Swelled Tablets Studied by PGSE NMR

17.55 **Maria Letizia Barreca**

Accounting for Water Molecules in HCV Drug Discovery

18.10 **Matteo Ceccarelli**

Which Role of Water in Determining the Residence Time of Drugs?

18.25 **Paul Wan Sia Heng**

Tablet Disintegration – Role of Pressure and Moisture

## ***Tuesday 25***

- 9.00     **Damien Laage** – invited  
Magnitude and Molecular Origin of Water Slowdown Next to a Protein
- 9.40     **Francesco Aliotta**  
Water is not so Anomalous
- 9.55     **Dino Leporini**  
Molecular Probe Dynamics Reveals Suppression of Ice-Like Regions in Strongly Confined Supercooled Water
- 10.10    **Laurence Noirez**  
Identification of Low Frequency Shear-Elasticity in Liquid Water
- 10.25    **Oswaldo Chara**  
Disclosing the Role of Hydrophobic Interactions Through Aggregation of non-Polar Solutes in Water: a Molecular Dynamics Study
- 10.40    **coffee break / posters**
- 11.10    **Valeria Molinero** – invited  
Crystallization of Water
- 11.50    **J. Raúl Grigera** – keynote  
Water Structure and its Relevance in Protein Stability
- 12.10    **Valeria Conti Nibali**  
On the Coupling Between the Dynamics of Proteins and that of their Hydration Water
- 12.25    **Barbara Hribar-Lee**  
Ion Specific Effects in Polyelectrolyte Solutions: an Example of Ionene Salts in Water
- 12.40    **Marie Plazanet**  
Low Temperature Behaviour of Hydrated Nafion Membranes
- 13.00    **Lunch**

**Tuesday 25**

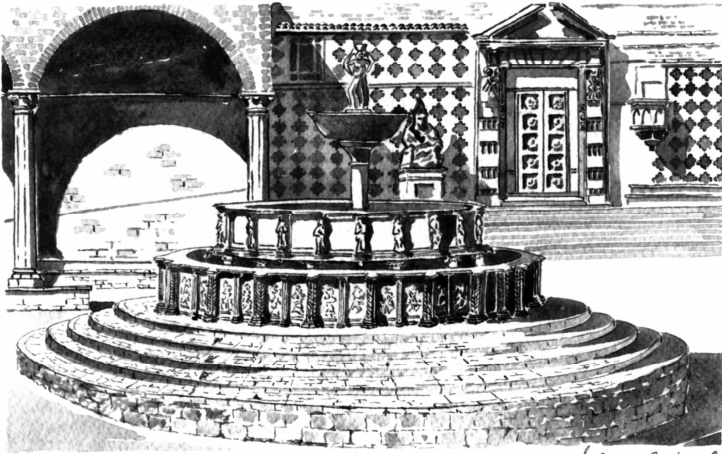
- 14.00     **POSTER SESSION**
- 15.00     **John Brady** - keynote  
The Hydration of Solutes with Extended Planar Surfaces
- 15.20     **Paola D'Angelo**  
On the Solvation of Ions in High-Density Water
- 15.35     **Rosaria Mancinelli**  
About Emulsion Water Structure
- 15.50     **Richard D. Ludescher** – invited  
Protein Mobility and Dynamic Coupling in Amorphous Glassy Sugars
- 16.30     **coffee break / posters**
- 17.00     **Paola Pittia** – invited  
Effects of Sugars on Flavour Release in Solution and Real Food Matrices at Different Hydration Degree
- 17.40     **Gaylon Campbell**  
Modeling Changes in Food Polymer Properties using Hygro-Thermal Time
- 17.55     **Dominique Champion**  
Different Material Behaviors During the Protein Powder Ageing
- 18.10     **Guo Chen**  
States and Motions of Water in Wheat Bread upon Freezing and Frozen Storage
- 18.25     **Mohammad Shafiur Rahman**  
Challenges in Multi-Hurdles Food Preservation and its Stability
- 20.30     **Social Dinner**

## **Wednesday 26**

- 9.00 **Riccardo Saccardi** – invited  
Cryopreservation of Cord Blood-Derived Haematopoietic Stem Cells: an Internal Bio-Banks Network
- 9.40 **Teena Goel**  
Solvation Dynamics of BIV TAR RNA with and without TAT Peptide
- 9.55 **Giuseppe Bellavia**  
Analysis of Bulk and Hydration Water During Thermal Lysozyme Denaturation
- 10.10 **Paola Gallo**  
Aqueous Solutions of Lysozyme and Disaccharides: Focus on Cryoprotection
- 10.25 **Adrien Lerbret**  
How Strongly does Trehalose Interact with Lysozyme at Low Water Contents? Insights from Molecular Dynamics Simulation and Inelastic Neutron Scattering
- 10.40 **coffee break / posters**
- 11.10 **Willem F. Walkers** – invited  
Water Transport Processes During Freezing of Cells
- 11.50 **Giancarlo Franzese** - keynote  
Cooperative Rearranging Regions in Water at Biological and Inorganic Interfaces
- 12.10 **Rodolphe Heyd**  
Do we Understand the Dehydration Process?
- 12.25 **Francesca Natali**  
Anomalous Proton Dynamics of Water in Neutral Tissue as Seen by Quasi-Elastic Neutron Scattering. Impact on Medical Imaging Techniques
- 12.40 **David Porter**  
Water Mobility, Denaturation and the Glass Transition in Proteins
- 12.55 **Fabio Sterpone**  
Temperature and Composition Effects on Protein Hydration. Insight on Thermostability.
- CLOSING**



**INVITED LECTURES**



*Stefano Albi Cordioli*

Fontana Maggiore – Perugia (Watercolour)  
*Courtesy of the Artist Stefano Alunni Cardinali*  
[WWW.STUDIOARTEAPPIA.IT](http://WWW.STUDIOARTEAPPIA.IT)

## ULTRAFAST OKE SPECTROSCOPY FOR THE THZ RAMAN SPECTRA OF AQUEOUS SOLUTIONS

*Stephen R. Meech, Kamila Mazur, Ismael A. Heisler and Francesca Palombo*

School of Chemical Sciences, University of East Anglia, Norwich Research Park,  
Norwich NR4 7TJ, UK. [S.Meech@uea.ac.uk](mailto:S.Meech@uea.ac.uk)

### Introduction

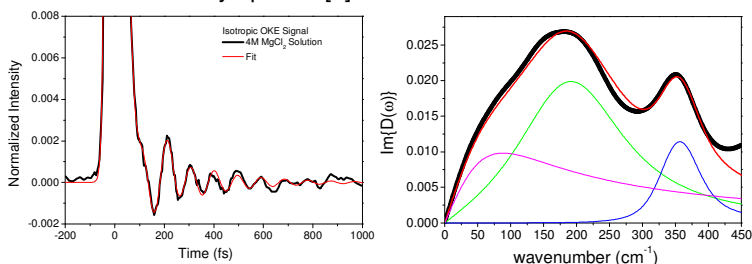
The structure and dynamics of water in the vicinity of a solute continue to attract widespread interest. This is of course because of its importance in a wide variety of topics, from the function of proteins to the stability of solutions. In this presentation the application of time domain optical Kerr effect measurements to probe the polarisability relaxation dynamics of a series of aqueous solutions will be described. The information content of the method will be outlined, with reference to the special problems associated with polarisability relaxation measurements on aqueous solutions. Next results will be presented on a series of solutions starting with 'simple' salt solutions (alkali halides), moving on to small molecular solutes (including both hydrophilic and amphiphilic molecules), then a series of di-peptides and finally protein solutions.

**Results:** Measurements of the polarisability anisotropy relaxation are made utilizing the conventional optical Kerr effect, which may be regarded as a time domain analogue of depolarized light scattering. The two methods differ only in a thermal occupation factor. To extract more information from the experiments it is necessary to measure the isotropic relaxation, which requires polarization resolved measurements, and can be achieved using a diffractive optic transient grating scattering geometry (Figure 1) [1].

Measurements of the polarized response of alkali halide solution are particularly informative. In pure water the polarized response is negligible because of the low polarisability anisotropy of water. However, in the solutions strong oscillation is observed. This can be assigned to a halide – water H-bond mode. Analysis of the concentration and halide dependence of the mode frequency yields information of solvation and solution structure in salt solutions [2].

Studies of the polarisability anisotropy in aqueous solutions suggest that all solutes slow down the relaxation dynamics compared to bulk water, but that hydrophilic solutes have a larger effect than hydrophobic groups. The OKE

also probes the H-bond structure in the solutions, with some unexpected results, in particular that hydrophobic groups leave the H-bond structure rather unperturbed. These conclusions are more or less carried over into solutions of peptides and proteins, where the picosecond dynamics scale with the hydrophobic area of the solute, although the trend is not quantitative and other factors may operate [3].



**Figure 1.** Polarized time domain response (left) of  $\text{MgCl}_2$  solution in water. Red line is a fit to three damped oscillators. The frequency domain equivalent is shown on the right side, with the oscillators separated. The high frequency mode is the cation – water stretch, while the lower frequency one is the chloride water H-bond.

### References

1. N. A. Smith and S. R. Meech, *International Reviews in Physical Chemistry* 21 (1), 75 (2002); N. T. Hunt, A. A. Jaye, and S. R. Meech, *Physical Chemistry Chemical Physics* 9 (18), 2167 (2007); I. A. Heisler and S. R. Meech, *Journal of Physical Chemistry B* 112 (41), 12976 (2008).
2. I. A. Heisler and S. R. Meech, *Science* 327 (5967), 857 (2010); I. A. Heisler, K. Mazur, and S. R. Meech, *Journal of Physical Chemistry B* 115 (8), 1863 (2011).
3. K. Mazur, I. A. Heisler, and S. R. Meech, *Journal of Physical Chemistry B* 114 (32), 10684 (2010); K. Mazur, I. A. Heisler, and S. R. Meech, *Journal of Physical Chemistry B* 115 (11), 2563 (2011); K. Mazur, I. A. Heisler, and S. R. Meech, *Journal of Physical Chemistry A* 116 (11), 2678; K. Mazur, I. A. Heisler, and S. R. Meech, *Physical Chemistry Chemical Physics* 14 (18), 6343.

# THEORY OF DIELECTRIC RESPONSE OF PROTEINS IN SOLUTION FROM MHZ TO THZ

*Dmitry Matyushov*

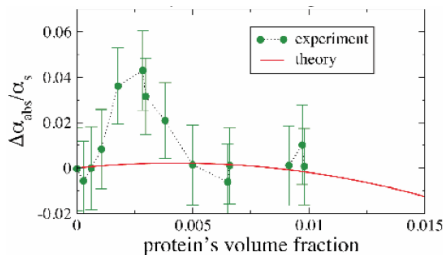
Department of Physics/Chemistry, Arizona State University,  
PO Box 871504, Tempe, AZ 85287-1504, USA. [dmitrym@asu.edu](mailto:dmitrym@asu.edu)

## **Problem**

Hydrated proteins present a system intermediate in size between polar molecules forming polar liquids and large dielectric bodies. They also usually carry significant dipole moments, 200-300 D. The combination of a large dipole moment with extended interface creates theoretical vacuum since standard theories of polar liquids do not recognize the existence of an extended polarized shell of the solvent, while dielectric models, operating with dielectric constants, do not correctly incorporate the molecular dipole. The new theoretical formalism [1-3] aims at filling the gap.

## **Results**

The theory combines the molecular dipole moment of the protein with the polarization of a large sub-ensemble of solvent molecules at the solute-solvent interface. It represents the dielectric response of the solution as a mutually compensating combination of the response of the protein dipole and the response of its hydration layer. The formulation is applied to experimental frequency-dependent dielectric spectra of lysozyme in solution [2]. The analysis of the data in the broad range of frequencies up to 700 GHz shows that the cavity field susceptibility, critical for the theory formulation, is consistent with the prediction of Maxwell's electrostatics in the frequency range of 10–200 GHz, but deviates from it outside this range. In particular, it becomes much smaller than the Maxwell result and shifts to negative values at small frequencies. The latter observation implies a dia-electric response, or negative dielectrophoresis, of hydrated lysozyme. The effective protein dipole recorded by dielectric spectroscopy is much smaller than the value calculated from protein's charge distribution.



**Figure 1** Relative absorption of an aqueous solution of the lambda repressor protein vs protein's volume fraction [3].

The theory is also applied to terahertz (THz) absorption of protein solutions [3]. In this range of frequencies, experiment reports both positive and negative slopes of the absorption coefficient vs the protein concentration. The slope is negative for sugars and positive for proteins (Figure 1) and polar amino acids. The theory is combined with molecular dynamics simulations to show that the polarization of the hydration shells of sugars is mostly consistent with the standard dielectric models, but deviates from them in case of hydrated proteins. The highly heterogeneous protein-water interface, combining patches of water of different polarization, allows a broader spectrum of outcomes for the interfacial susceptibility. We also show that the correlation between the protein dipole and the dipole moment of the hydration shell is long-ranged, propagating 20-40 Å into the bulk [1,3].

### References

1. D. V. Matyushov, Dipolar response of hydrated proteins, *J. Chem. Phys.* 136, 085102 (2012).
2. D. V. Matyushov, On the theory of dielectric spectroscopy of protein solutions, arXiv:1203.3005.
3. M. Heyden, D. J. Tobias, and D. V. Matyushov, Terahertz absorption by dilute aqueous solutions, unpublished.

# PHARMACEUTICAL PROCESSING: A PHYSICAL PERSPECTIVE ON INDUCED TRANSFORMATIONS AND WATER INVOLVEMENT

Marc Descamps

UMET (UMR CNRS 8207), University Lille1 - 59655 Villeneuve d'Ascq (FR).  
[marc.descamps@univ-lille1.fr](mailto:marc.descamps@univ-lille1.fr)

About 80% of drugs are formulated in the solid state which can be crystalline or amorphous (glassy). The molecular nature of these solids - either active substances or excipients - gives them specific properties: low melting point, low molecular and crystalline symmetry, easy vitrification (difficulty of recrystallization)... and also a great sensitivity to external disturbances. Industrial formulation processes impose strong constraints, which involve dynamic aspects in addition to temperature or pressure variations. These specific perturbations are, for example, the frequency of the shocks during grinding or the rate of desolvation during atomizations (spray drying) or freeze-drying. The dynamic aspects are also clearly important during extrusions. These materials, which are driven by dynamic stresses, often undergo modifications of their physical state. One can observe amorphizations or on the contrary recrystallizations. In other circumstances, one can observe phase transformations between polymorphic crystalline varieties - transformations which can generate either metastable phases or more stable phases. All these modifications, which affect properties such as solubility, can have a strong impact on the bioavailability. In addition, the physical stability of these compounds becomes dubious and can involve modification of chemical stability and reactivity. The identification of the relevant physical parameters that it is advisable to control during the driving processes is a requirement for the industrial formulation. It is also a challenge in condensed matter physics. The wealth of situations provided by the pharmaceutical science offers new possibilities to progress in the understanding of this type of non equilibrium phase transformations.

In this presentation, we will consider the transformations induced by grinding and desolvation. The role of water will be exemplified by showing examples of grinding and dehydration of hydrates. Examples will be taken among excipients (lactose, trehalose, sorbitol, mannitol...) and active substances like indomethacine. We will show how it is possible to identify the driving parameters of the non equilibrium phase transformations. Time permitting we will present possibilities offered by mechanical alloying of molecular compounds.

## **References**

1. Journ of Pharm. Sc. Vol 96 n<sup>o</sup>5, 1398-1406 (2007).
2. Mol Pharm. 5(6), 905 (2008).

# WATER IN PROTEIN-LIGAND INTERACTIONS: CHALLENGES AND OPPORTUNITIES FOR DRUG DESIGN

*Julien Michel*

EaStCHEM School of Chemistry, University of Edinburgh, EH9 3JJ, Edinburgh, United Kingdom. [mail@julienmichel.net](mailto:mail@julienmichel.net)

An important problem in preclinical drug discovery is the design of ligands, small drug-like molecules, which bind with high affinity and specificity to a protein involved in a disease. Water makes an important contribution to the binding affinity of a ligand because binding usually requires partial desolvation of the ligand and protein. In addition, protein-ligand interactions are frequently mediated by ordered water molecules. Atomistic simulations of protein-ligand complexes can provide a rich source of information on water behaviour near protein surfaces that is difficult to obtain by experimental methods.

This presentation will discuss the use of the molecular simulation algorithm JAWS to predict the location and stability of water molecules in protein binding sites. We will show that the methodology has a broad range of applications in molecular modelling and enables ligand optimisation strategies to displace favourably ordered water molecules from binding sites. Finally we will describe applications of JAWS to structure-based drug design programs.

## **References**

1. Michel, J. ; Tirado-Rives, J. ; Jorgensen W. L. J. Phys. Chem. B, 113 (40), 13337 -13346, 2009
2. Michel, J. ; Tirado-Rives, J. ; Jorgensen W. L. J. Am. Chem. Soc., 131 (42), 15403 -15411, 2009
3. Lucarelli, J. ; Michel, J. ; Tirado-Rives, J. ; Jorgensen W. L. J. Chem. Theory Comput., 6 (12), 3850 -3856, 2010



## MAGNITUDE AND MOLECULAR ORIGIN OF WATER SLOWDOWN NEXT TO A PROTEIN

*Damien Laage, Fabio Sterpone, Guillaume Stirnemann and Aoife Fogarty*

Department of Chemistry, Ecole Normale Supérieure, UMR ENS-CNRS-UPMC 8640–24 rue Lhomond, 75005 Paris, France. [damien.laage@ens.fr](mailto:damien.laage@ens.fr)

Hydration shell dynamics plays a critical role in protein folding and biochemical activity [1] and has thus been actively studied through a broad range of techniques. While all observations concur with a slowdown of water dynamics relative to the bulk, the magnitude and molecular origin of this retardation remain unclear. Via numerical simulations and theoretical modeling, we establish a molecular description of protein hydration dynamics and identify the key protein features that govern it. Through detailed microscopic mapping of the water reorientation and hydrogen-bond (HB) dynamics around lysozyme, we first determine that most of the hydration layer waters experience a moderate slowdown factor of 2–3, while the slower residual population is distributed along a power-law tail, in quantitative agreement with recent NMR results [2]. We then establish that the water reorientation mechanism at the protein interface is dominated by large angular jumps similar to the bulk situation [3]. A theoretical extended jump model is shown to provide the first rigorous determination of the two key contributions to the observed slowdown: a topological excluded-volume factor resulting from the local protein geometry, which governs the dynamics of the fastest waters, and a free energetic factor arising from the water–protein HB strength, which is especially important for the remaining waters in confined sites at the protein interface. These simple local factors are shown to provide a nearly quantitative description of the hydration shell dynamics [4]. Extensions to other proteins including ubiquitin and subtilisin will also be presented.

### **References**

1. P. Ball, Chem. Rev. 108, 74-108 (2008).
2. C. Mattea, J. Qvist, B. Halle, Biophys. J. 95, 2951-2963 (2008).
3. D. Laage, J. T. Hynes, Science 311, 832-835 (2006).
4. F. Sterpone, G. Stirnemann, D. Laage, J. Am. Chem. Soc. 134, 044513 (2012).

## CRYSTALLIZATION OF WATER

Valeria Molinero

Department of Chemistry, University of Utah, Salt Lake City, Utah, 84112, USA.  
[Valeria.Molinero@utah.edu](mailto:Valeria.Molinero@utah.edu)

One of water's puzzles is the question of what determines the lowest temperature to which it can be cooled before freezing to ice. The heat capacity and compressibility of liquid water increases anomalously in the supercooled region, apparently following a power law that would diverge at 225 K, just below the experimental temperature of homogeneous nucleation of ice  $T_H$ . This suggests that there may be a relation between the anomalous thermodynamics of liquid water and the rate at which it crystallizes. We use molecular simulations with an efficient coarse-grained model to investigate the relationship between structure and anomalies of liquid water and the rate of crystallization of ice. We find that a structural transformation of liquid water into a mostly four-coordinated liquid determines the rate of crystallization of ice in bulk water [1,2], water confined in nanopores [1,3] and nanoparticles [4], and in solutions of salts [5]. I will discuss the relationship between liquid structure, thermodynamics and crystallization rate, and the structure of the ice crystallized from deeply supercooled water [3,4,6].

### **References**

1. E. B. Moore and V. Molinero, Nature 479, 506 (2011).
2. E. B. Moore and V. Molinero, J Chem Phys 132, 244504 (2010).
3. E. B. Moore, E. de La Llave, K. Welke, D. A. Scherlis and V. Molinero, Phys Chem Chem Phys 12, 4124 (2010).
4. J. C. Johnston and V. Molinero, J. Am. Chem. Soc., 134, 6650 (2012)
5. G. Bullock and V. Molinero, to be submitted.
6. E. B. Moore and V. Molinero, Phys. Chem. Chem. Phys. 13, 20008 (2011)

## PROTEIN MOBILITY AND DYNAMIC COUPLING IN AMORPHOUS GLASSY SUGARS

*Richard D. Ludescher<sup>a</sup>, Andrew Draganski<sup>a</sup> and Joel M. Friedman<sup>b</sup>*

<sup>a</sup>Department of Food Science, Rutgers University – 65 Dudley Road,  
New Brunswick, NJ, USA 08901. [ludescher@aesop.rutgers.edu](mailto:ludescher@aesop.rutgers.edu)

<sup>b</sup>Department of Physiology and Biophysics, Albert Einstein College of Medicine – 1300  
Morris Park Ave., Bronx, NY, USA 10461

### Problem

The stability of dried and frozen foods and pharmaceuticals and the viability of seeds, spores and even entire organisms during anhydrobiosis depends largely, perhaps even primarily, upon the physical properties of mixtures of biomolecules in the amorphous glassy state. Although macroscopically solid, the glassy matrix of biomolecules undergoes complex modes of molecular mobility that include large scale  $\alpha$ -relaxations that support macroscopic flow above the glass transition temperature ( $T_g$ ) as well as a complex array of more localized  $\beta$ -relaxations that have appreciable rates and amplitudes below  $T_g$ . A complete biophysical analysis of the glassy state and its role in stability and viability involves not only a map of the various modes of mobility and their dependence upon temperature and matrix composition, especially including the effects of water, but also the rules that control dynamic coupling between the matrix and labile macromolecules and cellular structures: proteins (enzymes), nucleic acids, membranes, etc.

Biomolecular glasses of anhydrobiotic organisms are primarily formed from sugars, sucrose in plants and trehalose in animals; such sugars also form the matrix of many glassy foods and are used as excipients in pharmaceutical preparations. This project investigates the biophysical mechanisms underlying the stability of proteins embedded in amorphous glassy sugars. We hypothesize that a fundamental requirement of long-term stability is a true dynamic coupling between the rigid, glassy matrix and the protein. Such coupling is crucially dependent upon the amount and location of water within the complex matrix, water difficult to eliminate completely given its high affinity for both sugars and proteins. In an effort to map out the mechanisms of dynamic coupling we use time-resolved phosphorescence spectroscopy of tryptophan.

### Methodology

The triplet state of tryptophan, with a radiative lifetime of 6.0 s, is readily quenched by molecular motions within the matrix surrounding the indole

ring; analysis of tryptophan phosphorescence lifetimes thus provide a sensitive indicator of matrix mobility. We use measurements of the lifetime of the single tryptophan of the protein human serum albumin (HSA) or of single tryptophans in select polypeptides to monitor local protein mobility when embedded in a matrix of amorphous solid sugar; comparable measurements of tryptophan free amino acid dispersed within pure sugar provide a measure of matrix mobility. Tryptophan phosphorescence emission transients (collected over a range from ms to 10's of s) are analysed for a distribution of lifetimes using Maximum Entropy Methods; non-radiative decay rates as a function of temperature ( $k_{NR}(T)$ ) are calculated from the lifetimes. Analysis of the emission spectra of pyranine bound to HSA provide an estimate of the amount of water bound to the protein surface within the various matrixes.

## Results

The phosphorescence lifetime of intrinsic tryptophan reports on the local matrix/protein molecular mobility at the residue site. Activation energies for triplet state deactivation are determined for both protein phosphorescence and free tryptophan dispersed in amorphous sugar matrixes from Arrhenius analysis of  $k_{NR}(T)$ ; energies vary from  $\sim 2\text{kJ/mol}$  at liquid nitrogen temperatures and up to  $\sim 150\text{kJ/mol}$  at the sugar's glass transition temperature. The increasing energies with temperature reflect thermal activation of modes of motion in the matrix ranging from  $\beta$ -relaxations in the sugar glass and in the hydration layer to perhaps include the effect of  $\alpha$ -relaxations near the sugar  $T_g$ . Despite faster dynamics in the protein than in the matrix, the similar energetics of the distinct environments provide evidence that certain protein dynamics are coupled to those of the embedding matrix. The rates of phosphorescence decay are also widely distributed, as may be expected of dynamics in a glassy system. Sites of different dynamics are shown to display unique thermal evolutions.

HSA readily binds small molecular probes which may report a variety of information at a site on the protein's surface. The water-sensitive charge-transfer probe pyranine (8-hydroxypyrene 1,3,6-trisulfonate) was bound to HSA and its fluorescence spectra analyzed to extract qualitative information on the amount of the water in the protein's hydration shell. A matrix of the monosaccharide glucose more effectively displaced water in the embedded protein's hydration shell than any disaccharide (sucrose, maltose, trehalose) matrix. Matrixes doped with magnesium ion further sequester water from the protein's hydration shell. In all cases, the removal of water from the hydration shell slowed protein dynamics.

# EFFECTS OF SUGARS ON FLAVOUR RELEASE IN SOLUTION AND REAL FOOD MATRICES AT DIFFERENT HYDRATION DEGREE

*Paola Pittia*

Dipartimento di Scienze degli Alimenti, University of Teramo, via C.R. Lerici 1  
64023 Mosciano Sant'Angelo (TE), Italy. [ppittia@unite.it](mailto:ppittia@unite.it)

## Introduction

Quality of foods is largely dependent on the presence of aroma compounds that, released in the vapour phase, stimulate the olfactive receptors and influence their sensory acceptability by the consumers. Kinetic and thermodynamic mechanisms control the rate of release and the concentration of the aroma in the head space surrounding the product that, in turn, depend on the intrinsic characteristics of the volatiles and the food matrix, environmental factors and the interactions occurring with non-volatile compounds.

The interactions of aroma compounds with food macromolecules (proteins, carbohydrates, etc.) has been largely investigated, whilst scarce attention has been given to the presence in the food matrices of small solutes like mono- and di-saccharides and their importance in affecting the liquid-vapor partition and the kinetics of aroma release even if they influence some important physico-chemical ( $a_w$ ) and physical properties (viscosity,  $T_g$ ) of the water phase.

This presentation will review the role of small carbohydrates and their concentration on the release kinetics of aroma compounds and their vapour partition in simple models at different hydration degree (from dried to diluted solutions) and real food matrices (coffee beverage, candies, custard).

In model systems the release kinetics of volatiles depend mainly on their hydrophobicity as well as type and concentration of the sugar. Viscosity affects the kinetics of the release only above a given solute concentration, different depending on the aroma type, being mainly affected at lower solute concentration by changes of water freedom [1]. Liquid-vapour partition coefficient ( $k$ ) of the volatiles is significantly affected by sugar type and concentration that, at high solute concentration could be related to chemical physico-chemical and physical changes occurring in the saccharide solution and that, in turn, affect both water state and affinity of the aroma for the condensed phase.

Aroma compounds could be considered as reliable probes of chemical and physical changes occurring in the liquid phase of saccharide solutions and the aroma release an interesting tool for their investigation.

In real complex food matrices, however, the interactions of the volatile with the non volatile compounds may become the prevalent factor affecting the partition, reducing the effects and the importance of the small saccharides [2].

1. P. Pittia, P. Piccone and M. Martuscelli, Effect of sugars on the release of aroma compounds in model systems. In *Water Stress in Biological, Chemical, Pharmaceutical and Food Systems*, Springer (2012, *in printing*)
2. P. Piccone, V. Lonzarich, L. Navarini, G.C. Fusella, P. Pittia, Effect of sugars on liquid-vapour partition of volatile compounds in ready-to-drink coffee beverages. *J. Mass Spectr.* (*accepted, in printing*).

## **CRYOPRESERVATION OF CORD BLOOD-DERIVED HAEMATOPOIETIC STEM CELLS: AN INTERNATIONAL BIO-BANKS NETWORK**

*Riccardo Saccardi<sup>a</sup> and Letizia Lombardin<sup>b</sup>*

<sup>a</sup>Tuscan Cord Blood Bank, Haematology Dept – Careggi University Hospital  
L.go Brambilla, 3 - 50134, Firenze (Italy) riccardo.saccardi@aouc.unifi.it

<sup>b</sup>Tuscan Cord Blood Bank, Haematology Dept – Careggi University Hospital  
Centro Nazionale Trapianti - Via Giano della Bella, 34 - 00162 Roma

Initially used as a salvage treatment in children with acute leukemia in advanced stage or with bone marrow failure without related or unrelated bone marrow donor, umbilical cord blood transplantation is now a therapy used for almost all indications of hematopoietic stem cell transplantation in children and, more recently, in adults [1]. Favorable initial results of related Umbilical Cord Blood Transplantation (UCBT), including a lower rate of acute and chronic Graft vs Host Disease (GvHD) when compared to bone marrow transplantation, lead to the creation of unrelated cord blood banks after 1992 [2-4]. Due to availability of a larger inventory of cord blood units (CBU), with a trend from Banks to select large size units for storage [5], the number of transplants using this source of stem cells increased significantly. According to the WMDA annual survey, more than 20,000 cord blood units were already used as source of stem cell and more than 500,000 cord blood units are currently available for transplant worldwide ([www.worldmarrow.org](http://www.worldmarrow.org)).

A typical umbilical cord blood collection has an average 120 ml volume and an average cell content of  $0,8-3 \times 10^9$  nucleated cell. Collection is usually performed in a maternity unit linked to a cord blood bank. All other procedures (characterization, processing, freezing and storage of the cord blood unit) are performed by the bank itself. Thus, success of an UCBT is highly dependent on the quality of the cord blood unit [5]. Cryopreservation of haematopoietic Stem and Progenitor Cells (HSC) is a standard procedure of autologous transplantation and allogeneic Cord Blood transplantation (CBT), although it can seldom be used in the allogeneic transplant from adult donors too. The procedures for the cryopreservation of CB were based on that already used for cryopreservation of hematopoietic precursors derived from bone marrow or peripheral blood [6]. Currently, the methods of cryopreservation of CB are based on the use of an endogenous cryoprotectant, as DMSO, and the controlled rate freezing (CRF) by  $-1 \text{ }^\circ\text{C} / \text{min}$  through special devices.

The main clinical problem in CB transplant ,as compared to the conventional stem cell (SC) sources, is a slower engraftment kinetic, resulting in a graft failure in about 10-20% of patients [7,8]. Therefore the assessment of

engraftment potency for a selected CBU is crucial for clinical decision making: this is particularly relevant in a subset of patients who achieved a sustained complete remission after induction chemotherapy.

Quality control programs have been implemented to ensure the quality of CBU, based on a list of procedures to be followed by cord blood banks in order to provide bank accreditation through an "in site" inspection process. The accreditation systems aim to standardize all the banking steps with the final goal of achieving a better quality and homogeneity of the banks CBUs inventory, therefore improving the clinical outcomes of UCBT.

Despite the long term standardization of processing/freezing technology of HSC, there is a clear room for the improvement of both techniques and reagents, particularly in the field of cryoprotectants. With this regard, innovative technologies aimed to investigate the frozen tissue structure are mandatory in order to validate new reagents and assess their safety profile in this emerging field of transplantation medicine.

### **References**

1. Gluckman, E., et al., Milestones in umbilical cord blood transplantation. *Br J Haematol*, 2011. 154(4): p. 441-7.
2. Navarrete, C. and M. Contreras, Cord blood banking: a historical perspective. *Br J Haematol*, 2009. 147(2): p. 236-45.
3. Rubinstein, P., Cord blood banking for clinical transplantation. *Bone Marrow Transplant*, 2009. 44(10): p. 635-42.
4. Garcia, J., Allogeneic unrelated cord blood banking worldwide: an update. *Transfus Apher Sci*, 2010. 42(3): p. 257-63.
5. Wall, D.A. and K.W. Chan, Selection of cord blood unit(s) for transplantation. *Bone Marrow Transplant*, 2008. 42(1): p. 1-7.
6. Wagner, J.E., H.E. Broxmeyer, and S. Cooper, Umbilical cord and placental blood hematopoietic stem cells: collection, cryopreservation, and storage. *J Hematother*, 1992. 1(2): p. 167-73.
7. Eapen, M., et al., Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol*, 2010. 11(7): p. 653-60.
8. Barker, J.N., A. Scaradavou, and C.E. Stevens, Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood*, 2010. 115(9): p. 1843-9.



## WATER TRANSPORT PROCESSES DURING FREEZING OF CELLS

*Willem F. Wolkers*

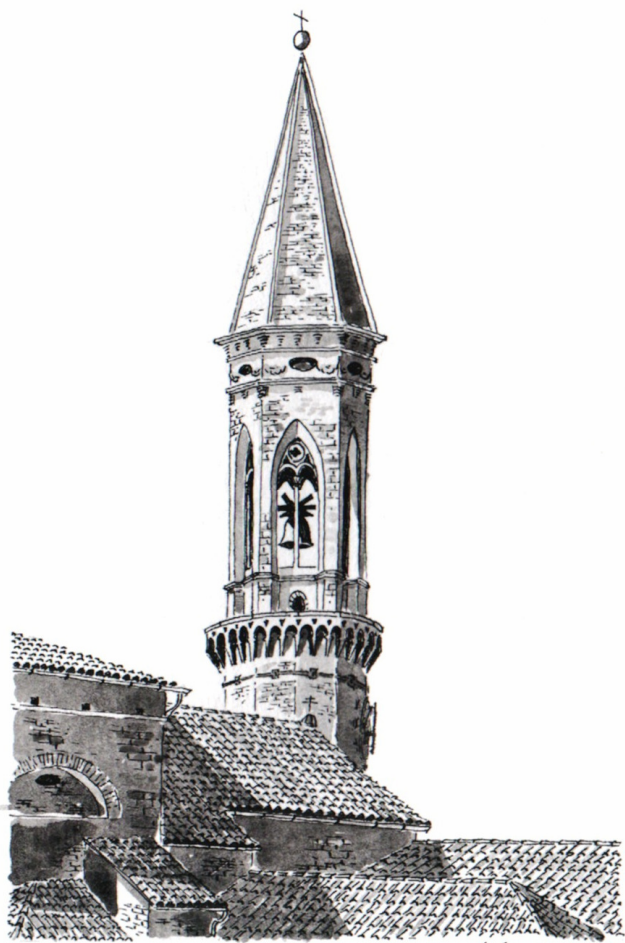
Institute of Multiphase Processes, Leibniz Universitaet Hannover – Callinstrasse 36,  
30167 Hannover, Germany. [wolkers@imp.uni-hannover.de](mailto:wolkers@imp.uni-hannover.de)

Cryopreservation is increasingly being used for biobanking of cells and tissues for reproductive, regenerative and transfusion medicine. Cells are often damaged during freezing and/or thawing resulting in variable survival rates of cryopreserved cells. A two factor hypothesis of cell damage during freezing has been proposed. Damage during slow cooling is attributed to 'solution effects injury' which is related to cell dehydration. At high cooling rates, intracellular ice formation is the main cause of cell death. Cell survival after thawing displays a maximum at an optimal cooling rate, where injury due to solution effects and intracellular ice formation is minimal. The optimal cooling rate is determined by the cell-specific membrane permeability to water, which governs the extent of cell dehydration during freezing. Transport of water across cellular membranes during freezing can be described using a water transport model, which requires the cell-specific membrane permeability to water and activation energy for water transport as input parameters. Subzero membrane hydraulic permeability measurements, however, are experimentally difficult and infrequently reported. We have used Fourier transform infrared spectroscopy (FTIR) and cryomicroscopy to study cells during freezing and used this to derive membrane hydraulic permeability parameters. FTIR studies have shown that membranes undergo pronounced fluid to gel phase changes during freezing, which has been attributed to a reduction in hydration level of the phospholipid head groups [1,2]. Combining FTIR with cryomicroscopy studies has shown that freezing-induced cellular dehydration precedes membrane dehydration. Cryoprotective agents decrease the activation energy for water transport but do not prevent the reduction in lipid hydration level. Freezing-induced cell dehydration occurs more gradually over a wider temperature range in the presence of cryoprotective agents, which may explain how cryoprotective agents protect cells during freezing. We postulate that variation in the success of cryopreservation is related to cell-specific differences in subzero membrane phase and permeability properties.

1. H. Oldenhof, K. Friedel, H. Sieme, B. Glasmacher, W.F. Wolkers, *Cryobiology* 61, 115–122 (2010).
2. M. Akhoondi, H. Oldenhof, C. Stoll, H. Sieme and W. F. Wolkers, *Biochim. Biophys. Acta* 1808, 642–648 (2011).



**KEYNOTE LECTURES**



*Stefano Ali Cardinali*

San Pietro – Perugia (Watercolour-Ink)  
*Courtesy of the Artist Stefano Alunni Cardinali*  
[WWW.STUDIOARTEAPPIA.IT](http://WWW.STUDIOARTEAPPIA.IT)

## A UNIFIED VIEW OF THE DYNAMICS OF WATER, AQUEOUS MIXTURES, AND HYDRATED PROTEINS DERIVED FROM EXPERIMENTS AND THEORY

*S. Capaccioli<sup>a</sup>, K. L. Ngai<sup>a</sup> and A. Paciaroni<sup>b</sup>*

<sup>a</sup>Dipartimento di Fisica, Università di Pisa, Italy. [ngai@df.unipi.it](mailto:ngai@df.unipi.it)

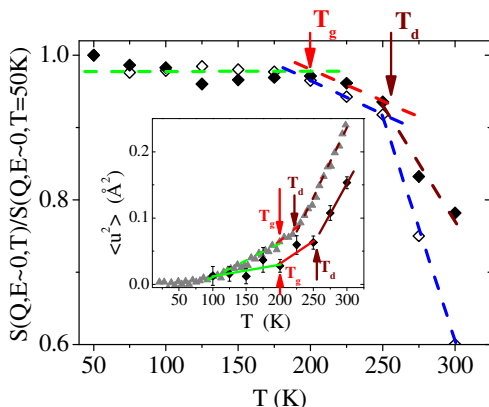
<sup>b</sup>Dip. Fisica, Università di Perugia & IOM-CNR, Perugia, Italy

Over the past six years and with collaborations with other workers we have studied the dynamics of water in the pure state [1], dynamics of water and various hydrophilic solutes in aqueous mixtures [2-5], and the dynamics of water and protein in hydrated proteins [5-8]. Experimental data from a wide range of experimental techniques, including calorimetry, broadband dielectric relaxation, Mössbauer spectroscopy, and neutron scattering, have been considered. The techniques covering a wide spectral range from pico seconds to laboratory time scales have enabled us to gain deeper understanding of the dynamics of water itself and the other component in aqueous mixtures, and hydrated proteins. Although water is unique or peculiar in its dynamic properties due to its strong tendency to crystallize and affinity for hydrogen bonding, the collection of experimental data considered together with analyses based on proven theoretical models have made possible satisfactory explanations of the dynamics observed in extant experiments, as well as predictions that were subsequently verified by experiments.

In this abstract we give two examples. One is on the dynamics of pure water and glass transition temperature deduced from the properties of the water component in many aqueous mixtures, and assisted by the properties of pure water at high temperatures and high frequencies. The collection of data is shown in Fig.7 in Ref.[1]. As will be explained in the talk, the collection of data indicates water has low degree of cooperativity as a glass-former, and its glass transition temperature  $T_g$  is in the neighbourhood of 135 K. No sign of fragile-to-strong crossover is observed.

The other example is neutron scattering experiments on maltose binding protein (MBP) performed in two spectrometers IN5 and IN16 with very different resolutions taken from Ref.[8], and shown here in Fig.1. For IN5 data in both the main figure and the inset, the lines drawn suggest the presence of two breaks in  $T$ -dependence of the MSD for H-MBP-D<sub>2</sub>O, one at  $T_g$ -200 K and another one at  $T_g$ -250-260 K. For the IN16 data in the inset, the lines are used to suggest the presence of two breaks in  $T$ -dependence of the MSD for H-MBP-D<sub>2</sub>O, one at the same  $T_g$ -200 K and another one at a lower  $T_g$ -220 K. The impact of this work is to show the existence of

crossover at  $T_g$  independent of instrument resolution, which is previously not recognized. Also the 'dynamic transition' at  $T_d$  is explained by the water specific relaxation of the hydration water coupled to the protein.



**Figure 1.** Incoherent elastic intensities measured on IN5, normalized with respect to the lowest temperature, integrated over a small  $Q$ -range ( $0.4 \text{ \AA}^{-1} < Q < 1.0 \text{ \AA}^{-1}$ ), of MBP (maltose binding protein) hydration water (empty diamonds) and H-MBP- $D_2O$  (full diamonds). Inset: Mean square displacements versus  $T$  of H-MBP- $D_2O$ ,  $\langle u^2 \rangle_{MBP-IN5}$ , as calculated from measurements on IN5 (black full diamonds) reported by Paciaroni et al. [9], and on IN16 (gray full triangles) reported by Wood et al. [10]. The latter were rescaled by six to compare with the former.

## References

1. S. Capaccioli and K. L. Ngai, J. Chem. Phys. **135**, 104504 (2011).
2. S. Capaccioli, K.L. Ngai, N. Shinyashiki, J. Phys. Chem B **111**, 8197 (2007).
3. N. Shinyashiki, et al., J. Phys. Chem. B, **112**, 15470 (2008).
4. N. Shinyashiki, et al., J. Phys. Chem. B., **113**, 14448 (2009).
5. K. L. Ngai, S. Capaccioli, M. Shahin Thayyil, N. Shinyashiki, J. Therm. Anal. Calorim. **99**, 123 (2010).
6. K. L. Ngai, S. Capaccioli, S. Ancherbak, N. Shinyashiki, Phil. Mag., **91**, 1809 (2011).
7. S. Capaccioli, K.L. Ngai, S. Ancherbak, A. Paciaroni, J. Phys. Chem. B **116**, 1745 (2012).
8. K. L. Ngai, S. Capaccioli, and A. Paciaroni, K. L. Ngai, S. Capaccioli, arXiv 1106.5924.
9. A. Paciaroni et al. Phil. Mag. **88**, 4071-4077 (2008).
10. K. Wood, A. Frolich, A. Paciaroni, M. Moulin, M. Hartlein, G. Zaccai, D. J. Tobias, M. Weik, J. Am. Chem. Soc. **130**, 4586-4587 (2008).

## WATER STRUCTURE AND ITS RELEVANCE IN PROTEIN STABILITY

*J. Raúl Grigera*

<sup>a</sup>Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB), CONICET-UNLP, 59-789, B1900 La Plata, Argentina. [raul@grigera.com.ar](mailto:raul@grigera.com.ar)

<sup>b</sup>Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de la Plata (UNLP), La Plata, Argentina.

Although the relevance of water properties on the stability of proteins have been long ago recognized, only on the light of recent findings it was possible to have a proper interpretation of its detailed mechanism. Functionality of proteins requires a relative narrow range of temperature and pressure to keep its native folding. This range should be according with the temperature environment of each species.

The interplay between enthalpic and entropic contribution allows tuning the required conditions -going from cryophilic to thermophilic conditions and from 1bar in the earth surface, to high pressures at the deep sea- to keep the protein functional. On one hand hydrogen bonds, electrostatic forces, and disulfide bonds and, on the other, hydrophobic interaction, provide the enthalpic and entropic contribution respectively. A satisfactory explanation of the protein unfolding due to temperature increasing was obtained. In contrast, protein unfolding due to pressure increases has not provided yet a wide consensus.

The equilibrium equation:

$$\frac{\partial(\ln K)}{\partial p} = -\Delta V / RT^2 \quad (1)$$

indicates that pressure facilitates a processes in which the system volume is decreased. Therefore, since in a protein the denaturation decreases the number of internal voids - decreasing the volume - it comes clear that pressure favours denaturation, which have been observed long time ago [1]. This thermodynamics description does not give any explanation regarding the internal mechanism behind the process. What are the interactions that are weakening by pressure producing the unfolding?

Experiments [2] and simulations [3] of systems with pure hydrophobic interaction studied at different temperatures and pressures, show behaviour qualitative and quantitatively similar to the protein denaturation. Being

present only hydrophobic interactions, the properties of such interaction must be carefully considered.

Hydrophobic interaction only can take place in the presence of on a liquid showing tetrahedral structure. For water this condition is present in a certain region of the phase diagram, *i.e.* in the presence of low density liquid phase (LDL). A crossover between LDL and HDL has been described in the literature not only theoretically [4,5] but also in a proceeding that allows to draw the Widom line from experimental data [6].

Heavy water is also capable to induce hydrophobic interaction. However, due to the slight but relevant differences between H-bond and D-bonds, this is an excellent model to test the same peculiarities of the hydrophobic interaction as well as cold denaturation. Precisely, some new unpublished results that we have recently obtained in this direction will be addressed in this keynote.

### **Acknowledgements**

I wish to thanks Dr O. Chara, Dr. C. G. Ferrara, and H. A. Alvarez for their fruitful collaboration. I am Emeritus Professor of the University of La Plata (UNLP), la Plata, Argentina and Superior Researcher of the National Scientific and Technical Research Council (CONICET).

### **References**

1. P. W. Bridgman, J. Biol. Chem. 19, 511(1914).
2. R.L. Baldwin PNAS,83, 8069 (1986).
3. G. Ferrara, O. Chara and J. R. Grigera (unpublished)
4. F. Mallamace, C. Corsaro, P.Baglioni, E. Fratini, and S-H.Chen, J. Phys. Con. Matt. 24, 064103 (2012).
5. P. Kumar, S.V. Buldyrev, and H.E. Stanley, PNAS 106, 22130 (2009)
6. O. Chara , A. N. McCarthy, and J. R. Grigera, Phys. Lett. A. 375, 572–576 (2011).



## THE HYDRATION OF SOLUTES WITH EXTENDED PLANAR SURFACES

*John W. Brady<sup>a</sup>, Udo Schnupf<sup>a</sup>, Letizia Tavagnacco<sup>b</sup>, Philip Mason<sup>a</sup>,  
Marie-Louise Saboung<sup>c</sup> and Attilio Cesàro<sup>b</sup>*

<sup>a</sup>Department of Food Science, Cornell University – Stocking Hall,  
14853 Ithaca, NY, USA. [jwb7@cornell.edu](mailto:jwb7@cornell.edu)

<sup>b</sup>Department of Life Sciences, University of Trieste – via L. Giorgieri 1,  
34127 Trieste, ITALY.

<sup>c</sup>Centre de Recherche sur la Matière Divisée – 1 bis rue de la Férollerie  
45071 Orléans, FRANCE.

Many interesting biological solutes possess not only polar and hydrogen bonding functionalities, but also weakly-hydrating, or hydrophobic, surfaces. Modern theories of the hydration of such surfaces predict that their solvent interactions will change from a wetting type interaction to a dewetting regime as a function of the solute size, with a gradual transition in behavior taking place around lengths of ~ 1 nm. Aggregations of non-polar species over this size range will undergo a transition from being dominated by entropy to being dominated by enthalpy. These transitions can be understood in part in terms of the geometries required of the solvating water molecules. Examples from recent work will be discussed, along with the implications for protein stability and denaturation and ligand binding specificity.

## COOPERATIVE REARRANGING REGIONS IN WATER AT BIOLOGICAL AND INORGANIC INTERFACES

*Giancarlo Franzese*

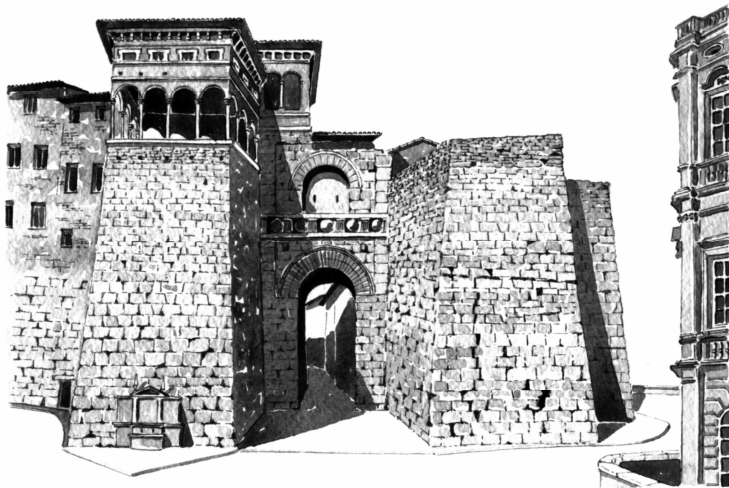
Departament de Física Fonamental, Facultat de Física, Universitat de Barcelona,  
Diagonal 645, 08028, Barcelona, Spain. [gfranzese@ub.edu](mailto:gfranzese@ub.edu)

We study, by simulations and analytic approach, the behaviour of water at the interface with proteins or in hydrophobic nanoconfinement. For water molecules adsorbed on the protein surface, we calculate the temperature dependence of the relaxation time of the dynamics of the hydrogen bond (HB) network, finding two dynamic crossovers, (i) at approximately 252 K and (ii) at approximately 181 K. We show how the two crossovers relate to the presence of two specific heat maxima. The first is caused by fluctuations in the HB formation, and the second, at a lower temperature, is due to the cooperative reordering of the HB network [1,2]. For water between hydrophobic walls, at nanoscopic separation, we study how the diffusion constant parallel to the walls depends on the microscopic structure of water. At low temperature, water diffusion can be associated with the number of defects in the hydrogen bond network [3]. However, the number of defects solely does not account for the peculiar diffusion of water, with maxima and minima along isotherms. We calculate a relation that quantitatively reproduces the behavior of diffusivity, focusing on the high-temperature regime. We clarify how the interplay between breaking of hydrogen bonds and cooperative rearranging regions of 1-nm size gives rise to the diffusion extrema in nanoconfined water and offers a possible explanation for the ultrafast transport of water in nanochannels with size smaller than 1 nm [4]. We finally find a dramatic decrease of compressibility, thermal expansion coefficient, and specific heat for water confined in disordered nanochannels [5].

### **References**

1. M. G. Mazza, K. Stokely, S. E. Pagnotta, F. Bruni, H. E. Stanley, and G. Franzese, Proc. Natl. Acad. Sci. USA 108, 19873 (2011).
2. T. A. Kesselring, G. Franzese, S. V. Buldyrev, H. J. Herrmann & H. E. Stanley, Scientific Reports (Nature Publishing Group) 2, 474 (2012).
2. F. de los Santos, and G. Franzese, J. Phys. Chem. B 115, 14311 (2011).
3. F. de los Santos, and G. Franzese, Phys. Rev. E 85, 010602(R) (2012).
4. E. G. Strelakova, M. G. Mazza, H. E. Stanley, G. Franzese, Phys. Rev. Lett. 106, 145701 (2011).

## ORAL PRESENTATIONS



*Stefano Almi Cardiacoli*

Arco Etrusco – Perugia (Watercolour)  
*Courtesy of the Artist Stefano Alunni Cardinali*  
[WWW.STUDIOARTEAPPIA.IT](http://WWW.STUDIOARTEAPPIA.IT)

## TIME-RESOLVED LASER SPECTROSCOPY ON INTERFACIAL AND NANOCONFINED WATER

*R. Torre<sup>a,b</sup>, A. Taschin<sup>a</sup>, P. Bartolini<sup>a</sup> and R. Righini<sup>a,c</sup>*

<sup>a</sup>European Lab. for Non-Linear Spectroscopy (LENS), Univ. di Firenze, via N. Carrara 1, I-50019 Sesto Fiorentino, Firenze, Italy, [torre@lens.unifi.it](mailto:torre@lens.unifi.it)

<sup>b</sup>Dipartimento di Fisica ed Astronomia, Univ. di Firenze

<sup>c</sup>Dipartimento di Chimica, Univ. di Firenze

The relevance of interfacial and nanoconfined water is spanning from biological studies to material science. Despite the continuous research effort, the structure and dynamic features of interfacial water remain to be understood. Silica nano-pores are recognized to be the prototype hydrophilic matrixes for nanoconfined water studies. This is because they enable a “well-controlled and clean” environment. Nevertheless, the investigations of the interfacial water properties in these systems are still presenting many puzzling phenomena, that did not find a complete explanation and are raising a large debate.

In this scenario, we studied the water dynamics by time-resolved laser techniques [1-3], that enable to achieve new valuable information on interfacial water. In particular, we will report on the investigation the vibrational and structural dynamics of nanoconfined water by ultra-fast optical Kerr [2] and transient grating techniques [3].

### **References**

1. R. Torre, Time-resolved spectroscopy of complex liquids, Springer, New York, (2008).
2. R. Torre, P. Bartolini, R. Righini, Nature 428, 296 (2004); P. Bartolini, A. Taschin, R. Eramo, R. Righini and R. Torre, Jour. of Phys.: Conf. Ser. 177, 012009 (2009);
3. A. Taschin, P. Bartolini, R. Eramo and R. Torre, Phys. Rev. E 74, 031502 (2006); R. Cucini, A. Taschin, P. Bartolini and R. Torre, J. Mech. Phys. Solids 58, 1302 (2010). A. Taschin, R. Cucini, P. Bartolini and R. Torre, Europhys. Lett. 92, 26005 (2010). A. Taschin, R. Cucini, P. Bartolini and R. Torre, Philos. Mag. 91, 1796 (2011)

## COLLECTIVE MODES AND AMORPHOUS DYNAMICAL CHARACTER OF HYDRATION WATER IN BIOMOLECULES

*A. Orecchini<sup>a,b</sup>, A. Paciaroni<sup>a</sup>, F. Sebastiani<sup>a</sup>, M. Jasin<sup>c</sup>, C. Petrillo<sup>a</sup>, J. Zaccal<sup>b</sup> and F. Sacchetti<sup>a</sup>*

<sup>a</sup>Dipartimento di Fisica and CNR-IOM, University of Perugia, Via Pascoli, I-06123 Perugia, Italy. [andrea.orecchini@pg.infn.it](mailto:andrea.orecchini@pg.infn.it)

<sup>b</sup>Institut Laue-Langevin, 6 rue Jules Horowitz, F-38042 Grenoble Cedex 9, France.

<sup>c</sup>Max Planck Institut für Biochemie, Am Klopferspitz 18, D-82152 Martinsried, Germany.

Water dynamics plays a fundamental role for the fulfillment of biological functions in living organisms. As concerns single-particle diffusive and relaxational dynamics, decades of studies on hydrated protein powders have revealed the peculiar properties of hydration water with respect to pure water, due to close coupling interactions with the macromolecule [1]. On the other hand, investigations about collective dynamical properties are extremely rare.

In such a framework, we have recently undertaken a series of extensive and comparative studies about coherent collective dynamics in the hydration water of a number of biological systems, such as DNA, the model protein Ribonuclease A, and intracellular water of living *Escherichia coli* cells.

State-of-art neutron instrumentation allowed us to reveal the actual propagation of coherent density fluctuations in such a peculiar state of water. The corresponding vibrational frequencies are only slightly affected by the coupling with the biomolecular environment. Nevertheless, the effects of the water-biomolecule interaction appear as a marked increase of the modes damping factors, which we ascribe to a perturbation of the water hydrogen-bond network [2]. Such results are interpreted as the signature of a “glassy” dynamical character of hydration water, in agreement with indications from previous measurements of the density of vibrational states [3].

### References

1. P. Ball, Chem. Rev., 108, 74 (2008) and references therein.
2. A. Orecchini, A. Paciaroni et al., J. Am. Chem. Soc., 131, 4664 (2009).
3. A. Paciaroni, A. Orecchini et al., Phys. Rev. Lett., 101, 148104 (2008).

# WATER AND PROTEIN DYNAMICS IN HYDRATED GLOBULAR PROTEINS

*P. Pissis<sup>a</sup>, A. Panagopoulou<sup>a</sup>, A. Kyritsis<sup>a</sup> and N. Shinyashiki<sup>b</sup>*

<sup>a</sup>National Technical University of Athens, Zografou Campus, 15780, Athens, Greece  
[ppissis@central.ntua.gr](mailto:ppissis@central.ntua.gr)

<sup>b</sup>Department of Physics, Tokai University, Hiratsuka, Kanagawa, 259-1292, Japan

## Introduction

The hydration properties of proteins and water and protein dynamics in protein–water mixtures have been studied in the past several decades by a variety of experimental techniques. The results indicated the significant influence of protein–water interactions on the structure, the dynamics and the biological function of proteins. A minimum amount of water is necessary for enzymatic activity of a protein and dielectric results indicated a correlation between the onset of enzymatic activity and of a percolation type displacement process of protons on single macromolecules [1]. Thermal and dynamic studies of hydrated proteins revealed the presence of a thermal glass transition in the temperature range from about 160 to 200 K, depending on the protein, the hydration level and the experimental technique employed. The understanding of both, the origin of the thermal glass transition in proteins and biopolymers in general, and the dynamics of hydration water at biological interfaces, in physical terms, is believed to be essential for applications in Biology and Medicine.

## Experimental-Results and Discussion

In this work we employ a combination of experimental techniques in order to investigate water and protein dynamics in the case of water mixtures of globular proteins, over wide ranges of temperature (123-273 K) and composition. Two globular proteins of different molecular weights, lysozyme and bovine albumin serum (BSA), are used in order to include the molecule size as a parameter. The hydrated samples are in the form of hydrated compressed pellets or in the form of aqueous solutions. The water fraction range extends from practically dry samples (2 wt%) to 82 wt% (grams of water per grams of hydrated protein). The experimental techniques used are differential scanning calorimetry (DSC) and two dielectric techniques, dielectric relaxation spectroscopy (DRS) and thermally stimulated depolarization currents (TSDC). In addition, equilibrium water sorption measurements are performed at room temperature.

BSA exhibited a larger swelling degree as compared to lysozyme at high hydration levels. The thermal phenomena (crystallization and melting) have been studied by DSC and the amount of uncrystallized water (ucw) in the

mixtures has been calculated by the enthalpy of melting [2,3]. The water fraction region where water crystallization occurs only during heating (cold crystallization region) is in the range of 18%-21% for lysozyme, while it obtains higher values in the range of 23%-30% in the case of BSA. The glass transition of the hydrated system was observed by DSC, more clearly in the case of hydrated BSA [2,3]. Dielectric measurements reveal the  $\alpha$  relaxation process associated with the glass transition of the hydrated protein (dynamic glass transition) for both systems. For water fractions where no crystallization of water occurs during cooling our results show a strong plasticization of  $T_g$  [2-4]. The evaluation of the results by all experimental techniques used, leads to an important conclusion relative to the origin of the glass transition of hydrated proteins. It seems that the origin of the segmental dynamics in hydrated proteins is the cooperative motion of uncrystallized water clusters with protein molecules, while this is absent at low hydration levels, where water molecules are adsorbed mainly by primary hydration sites (hydrophilic polar groups) [3,4]. The  $T_g$  and the relaxation time  $\tau$  of the alpha relaxation saturate for water fractions higher than about 30% (in the case of BSA), which corresponds to the region where the protein hydration shell is being completed and water crystallization occurs during cooling. Regarding the dynamics of uncrystallized water, we follow the secondary relaxation of ucw (DRS, TSDC) superimposed on a local dielectric relaxation of small polar groups at the protein surface [4]. Our results indicate the existence of interrelations between the formation of a conductive percolating water cluster and the saturation of the reorientation process of uncrystallized water molecules at the time scale of the water  $v$  process [4]. The dynamic study of hydrated proteins over extremely wide ranges of water fraction proves to provide important findings which contribute to theoretical approaches, but can also be used as an experimental database, with respect to the  $T_g$  and the different states of water.

### Acknowledgements

P. Pissis would like to acknowledge the Scientific Programme for Basic Research, PEBE 2010, of the National Technical University of Athens, for funding.

### References

1. J. A. Rupley, G. Careri, Adv. Protein Chem. 41, 37–172 (1991).
2. A. Panagopoulou, A. Kyritsis, A. M. Aravantinou, D. Nanopoulos, R. Sabater i Serra, J. L. Gómez Ribelles, N. Shinyashiki, P. Pissis, Food Biophysics 6, 199-209 (2011).
3. A. Panagopoulou, A. Kyritsis, R. Sabater i Serra, J. L. Gómez Ribelles, N. Shinyashiki and P. Pissis, J. Biochim. Biophys. Acta 1814, 1984-1996 (2011).
4. A. Panagopoulou, A. Kyritsis, N. Shinyashiki, P. Pissis, J. Phys. Chem. B 116, 4593-4602 (2012).



## BROADBAND TIME-FREQUENCY RESOLVED SPECTROSCOPY: THE MARTELLO PROJECT

*Riccardo Cucini<sup>a</sup>, Andrea Battiston<sup>a,b</sup>, Filippo Bencivenga<sup>a</sup>,  
Alessandro Gessini<sup>a</sup>, Francesco D'Amico<sup>a</sup>, Daniele Fioretto<sup>c</sup> and  
Claudio Masciovecchio<sup>a</sup>*

<sup>a</sup>Sincrotrone Trieste, Strada Statale 14 – Km 163,5, 34149 Basovizza (TS), Italy.

[riccardo.cucini@elettra.trieste.it](mailto:riccardo.cucini@elettra.trieste.it)

<sup>b</sup>University of Trieste, Piazzale Europa 1, 34127 Trieste, Italy

<sup>c</sup>Università di Perugia, Fisica, via Pascoli, 06123, Perugia, Italy

The investigation of liquids properties is a very fundamental topic in many different fields, ranging from biology, chemistry and physics to life science and medicine. In particular, the dynamical properties of liquids have a strong importance in the comprehension of the biological system's behaviour. Due to the very broad time/frequency scale which characterizes this dynamics, a large quantity of different experimental techniques has been developed during the years. So, in order to obtain a complete analysis of the liquid dynamics, different experiments with different techniques have to be improved. This aspect requires to the researcher to move in different laboratories and to make different proposal for each experiment.

We propose a multi technique facilities devoted to the Measure of Acoustic, Raman and Thermal Excitation in Liquid solution and Organic molecule (MARTELLO project), in both time and frequency domain, where a single proposal can be done for making measures on the same sample with all the available techniques. In this way, a very broad time/frequency windows will be covered, permitting a very complete exploration of liquid systems. The following experiments shall be performed:

- Transient Grating (TG) with pulsed probe [1]
- Transient Grating with continuum probe
- Inelastic Visible, UV and VUV scattering [2,3,4]
- Visible, UV and VUV Raman spectroscopy [5]
- EUV Transient Grating (TIMER, in progress) [6]

In detail, the different experiments will cover a time windows from 100 fs to ns (pulsed and continuum TG), with an exchanged wave-vector from  $3 \mu\text{m}^{-1}$  to  $150 \mu\text{m}^{-1}$  (with an extension to  $1 \text{nm}^{-1}$  with EUV TG), depending from the

used technique and configuration. Considering the exploitable dynamical range, this facility will be a unique tool in liquids science.

### **References**

1. R. Cucini et al Opt. Lett 36 1032-1034 (2011)
2. G. Carlotti et al IEEE Trans. Ultrasonics, Ferroelectrics Frequency Control 38 56 (1991)
3. F. Bencivenga et al Rev. Sci. Instrum, submitted
4. C. Masciovecchio et al PRL 92 255507 (2004)
5. F. D'Amico et al J. of Raman Spect., submitted
6. R. Cucini et al NIMA 635 S69-S74 (2011)

# HYDRATION SHELLS OF MACROMOLECULES STUDIED BY HIGH-RESOLVED RESONANT RAMAN SPECTROSCOPY

*Francesco D'Amico*

Sincrotrone Trieste S.c.p.A – Trieste – Italy. [francesco.damico@elettra.trieste.it](mailto:francesco.damico@elettra.trieste.it)

The hydration of organic molecules is the subject of intense debate in the scientific community. Many experiments confirm the presence of a certain rigidity of the water surrounding an organic molecule: the main causes of this stiffness are the hydrogen bonds present at the water-molecule interface. Moreover it has been demonstrated how the water molecules surrounding the hydrophobic groups, such as the methyl one, have a slower dynamics compared to the bulk-water ones. Since both these effects cause the hydration shell it may become difficult to study them separately if both are simultaneously present on the system taken into consideration. Recently, we demonstrated how an appropriate line-shape analysis carried out Raman spectra of small molecules allows to distinguishing between the behavior of hydrogen bonds around hydrophilic and the hydrophobic groups respectively. This was accomplished by a detailed analysis of the Raman features coming from specific group of atoms. Such an approach has been also used to study the interaction between water and macromolecules as peptides, fatty acids and polymers.

## RELAXATION IN AQUEOUS MIXTURES: LOCAL SOLUTE DYNAMICS VS. WATER SPECIFIC MOTIONS

*S. Ancherbak<sup>a</sup>, S. Capaccioli<sup>a</sup> and D. Prevosto<sup>b</sup>*

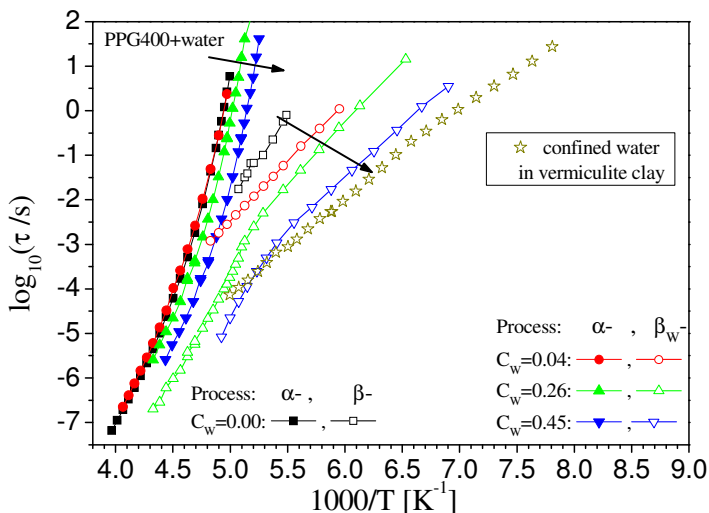
<sup>a</sup>Dipartimento di Fisica, Università di Pisa, Italy, [capacci@df.unipi.it](mailto:capacci@df.unipi.it)

<sup>b</sup>CNR-IPCF, Dipartimento di Fisica, Università di Pisa, Italy

The experimental findings reported in the literature on the dynamics of supercooled aqueous systems are remarkably similar, irrespective of the structure and chemical composition: at least two relaxation processes were always reported, both detected by the highly sensitive calorimetric techniques or by broadband dielectric spectroscopy [1,2]. A slower process, originated by the structural relaxation of the solute molecules hydrogen bonded to water, whose relaxation time has the Vogel-Fulcher  $T$ -dependence, typical of cooperative structural  $\alpha$ -relaxation in glass-formers, attains values between  $10^2$  -  $10^3$  s at the glass transition temperature  $T_g$ , where a jump in heat capacity is observed by differential scanning calorimetry. Moreover, a faster process ( $\beta_w$ -) is present at higher frequency in the dielectric spectra, originating specifically from the independent motions of water molecules in the presence of the solute, analogously to the Johari-Goldstein (JG) secondary  $\beta$ -relaxation, universal feature of glass-formers [3,4].

In this study, by investigating dielectric spectra of water mixtures with glycols, sugars and of hydrated proteins, at different water concentrations and in a wide range of temperature and pressure, we will show how  $\beta_w$ -process is influenced by the hydrogen bonding network surrounding the water molecules. In particular (see Fig.1 as an example), the dependence of  $\beta_w$ -relaxation times on water composition singles out two different regimes: at very low water molar concentration, water molecules are mainly hydrogen bonded to hydrophilic groups of the solute, which usually has lower mobility than water molecule, and  $\beta_w$ -times are very close to those of the secondary  $\beta$ -relaxation of the pure solute. On the other hand, at high water molar concentration the environment of water molecules is mainly composed of the more mobile water molecules themselves:  $\beta_w$ -relaxation shows qualitatively very similar features for many aqueous systems, almost universal, irrespective of the chemical and structural differences, and matching the dynamic behavior of confined water [1,5,6]. For instance, its activation energy in the glassy state is about 52 kJ/mol, which is not much larger than the energy of breaking two hydrogen bonds to enable local relaxation of water. Our findings are potentially important for a better understanding of the mutual influence exerted by hydration water and hydrophilic groups of bio-

macromolecules or proteins in determining dynamics and biological function [7-10].



**Figure 1.** Relaxation times vs. reciprocal T of PPG400-water mixtures at different water weight fraction  $C_w$ . Close and open symbols indicate  $\alpha$ - and  $\beta$ - process, respectively. Stars are data for water confined in vermiculite clay [11]. Arrows show the evolution with increasing  $C_w$ .

### References

1. S. Capaccioli, K.L. Ngai, N. Shinyashiki, J. Phys. Chem B **111**, 8197 (2007).
2. K. L. Ngai, S. Capaccioli, M. Shahin Thayyil, N. Shinyashiki, J. Therm. Anal. Calorim. **99**,123 (2010).
3. G.P. Johari, M. Goldstein, J. Chem. Phys. **53**, 2372 (1970).
4. K. Kessairi, S. Capaccioli, D. Prevosto, M. Lucchesi, S. Sharifi, PA Rolla, J. Phys. Chem. B **112**, 4470 (2008).
5. N. Shinyashiki, S. Sudo, S. Yagihara, A. Spanoudaki, A. Kyritsis, P. Pissis, J. Phys.: Condens. Matter **19**, 205113 (2007).
6. K. L. Ngai, S. Capaccioli, S. Ancherbak, N. Shinyashiki, Phil. Mag., **91**, 1809 (2011).
7. N. Shinyashiki, et al., J. Phys. Chem. B, **112**, 15470 (2008).
8. N. Shinyashiki, et al., J. Phys. Chem. B.,**113**,14448 (2009).
9. H. Frauenfelder et al., Proc. Natl.Acad. Sci. USA **106**, 5129 (2009).
10. S. Khodadadi et al., Biochimica et Biophysica Acta-Proteins and Proteomics 1804, 15 (2010).
11. R. Bergman, J. Swenson, Nature **403**, 283 (2000)

## CLUSTER PHASES OF DECORATED MICELLAR SOLUTIONS WITH MACROCYCLIC LIGANDS

*S. Caponi<sup>a,b</sup>, M. Carlà<sup>c</sup>, G. Cazzollì<sup>a</sup>, A. Defant<sup>a</sup>, C. M. C. Gambi<sup>c</sup>, L. Lanzi<sup>c</sup>; S. Marchetti<sup>c</sup>, M. Mattarelli<sup>a</sup>, M. Montagna<sup>a</sup>, B. Rossi<sup>a</sup>, F. Rossi<sup>a</sup>, F. Sciortino<sup>d</sup> and S. Sennato<sup>d</sup>*

<sup>a</sup>Dipartimento di Fisica, Università di Trento, via Sommarive 14, I-38123, Povo, Trento, Italy, [caponi@science.unitn.it](mailto:caponi@science.unitn.it)

<sup>b</sup>Istituto di Biofisica, Consiglio Nazionale delle Ricerche, via alla Cascata 56/C, I-38123 Trento, Italy

<sup>c</sup>Dipartimento di Fisica, Università di Firenze and CNISM, via G. Sansone, I-50019, Sesto Fiorentino, Firenze, Italy.

<sup>d</sup>CNR-INFM SOFT and Università di Roma "La Sapienza", I-00185 Roma, Italy

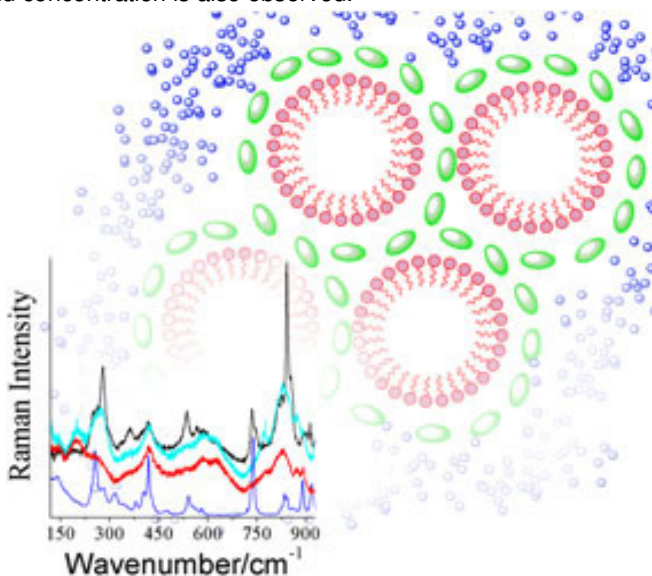
The study of aggregation processes is a topic of broad interest in the physical research because the understanding of the interactions involved in the aggregation mechanisms is fundamental in many industrial processes, in medicine, and for modeling biological systems. In colloidal charged particles suspension, the aggregation can be induced by the addition of ligands: particles or polymers that alter the charge distribution on the particle surface. These aggregates are governed by a delicate balance between short-range attractive and long-range repulsive interactions resulting in the appearance of stable, kinetically arrested cluster phases, where single particles stuck and form relatively large complexes. Ionic surfactants such as Sodium Dodecyl Sulfate (SDS) belong to the amphiphile family: they possess a long hydrophobic hydrocarbon chain and a polar hydrophilic headgroup. In a polar solvent and over the critical micellar concentration these molecules join to form micelles. The micellar solutions, in turn, if doped with various ligands tend to aggregate. The hydrophobicity of the adhesive sites (ligand molecules), gives rise to adhesive forces that induce the aggregation. We name the micelles aggregates Cluster Phases of Micelles (CPM).

Solid SDS, micelles of SDS in water and micelles of SDS doped with two types of macrocyclic ligands, Kryptofix 2.2.2 (K222) and crown ether 18-Crown-6 (18C6), at different concentration are studied by Raman scattering that represents a new approach to such systems [1], by Dynamic Light Scattering (DLS) and Small-Angle X-rays Scattering (SAXS) to disclose the micellar structure interactions, and the aggregation properties [2].

Considering that SDS micelles in water do not aggregate at low concentration, the present study demonstrates that macrocyclic ligands induce the SDS micelles aggregation depending on the ligand/surfactant ratio. The sizes and the percentages of the micelles and of the CPM have been studied in temperature by DLS, and at room temperature by SAXS and Raman. DLS results clearly show that the aggregation processes of the

decorated micelles are reproducible at different time intervals: weeks, months and years. Furthermore, at constant temperature, more ligand is in the sample, larger CPM form. Interestingly, it is found that K222 ligand is more efficient to induce micellar aggregation than the 18C6. The dimension of the CPM is proportional to the ligand concentration in the samples at constant temperature.

The experimental Raman spectrum obtained on crystalline powders of SDS is compared with the *ab initio* computed spectrum in order to assign the vibrational bands. After discriminating sensitive peaks by comparing the crystalline powders of the single components and their water solutions, the aggregation process and the action of the ligands are analyzed following the evolution of the intensity and wavenumber of characteristic Raman peaks. Raman spectroscopy proved to be sensitive to the aggregation dynamics and to the effects induced by the hydration layer on the molecules in solutions. A saturation effect in the aggregation process with the increase of the ligand concentration is also observed.



### References

1. G. Cazzolli et al J. of Raman Spectroscopy (2012).
2. S. Marchetti et al. Submitted to J. of Physical Chemistry.

## DETAILING PROTEIN SOLVATION SHELL IN BINARY MIXTURES

*Maria Grazia Ortore<sup>a</sup>, Francesco Spinozzi<sup>a</sup>, Giuseppe Onori<sup>b</sup> and Paolo Marian<sup>a</sup>*

<sup>a</sup>Department of Life and Environmental Sciences and CNISM, University Polytechnic of Marche, Via breccie bianche, I-60131, Ancona, Italy

[m.g.ortore@univpm.it](mailto:m.g.ortore@univpm.it)

<sup>b</sup>Department of Physics, University of Perugia, Via A. Pascoli, I-60123 Italy

The understanding of the role played by hydration in activating protein intrinsic dynamics, as well as the link between preferential hydration, structural rearranging and biological functionality, requires a direct characterization of protein-solvent interface. In particular, from decades there is a big effort to investigate how non-aqueous solvents affect intermolecular forces, since certain co-solutes are stored in large concentrations in organisms living in conditions of stresses and play a key role in modulating protein stability.

We used Small Angle Neutron Scattering (SANS) technique [1], afterwards matched with Small Angle X-ray Scattering (SAXS) [2] and Differential Scanning Calorimetry (DSC) [3,4], to investigate solvent composition near the protein surface. Our attention focused on a model protein, lysozyme, dissolved in water-glycerol, in water-urea and in water-ethanol mixtures. Glycerol does not change secondary and tertiary structure of lysozyme and it is considered a stabilizer agent. Urea and ethanol are well-known protein denaturants, and despite their ubiquitous use, it is not clear the molecular mechanism leading to protein unfolding. We present a set of SANS experiments that have been performed in several experimental conditions concerning protein concentration, cosolvent concentration and deuteration grade.

Every set of experiments was analysed by a global fit method which estimates a thermodynamic constant  $K$  that describes the exchange equilibrium of water and cosolvent between protein surface and bulk. The thermodynamic constants  $K$  obtained by the three set of experimental data clearly indicate that glycerol is preferentially excluded from lysozyme solvation shell [1,3], while urea [5] as well as ethanol [4] are more concentrated in the solvation shell in respect to the bulk. The common fitted parameters, the  $K$  and the molecular volume of water in contact with protein surface, allow to estimate the preferential binding coefficient and the excess solvation number, as a function of co-solvent molar fraction. These results



are in agreement with preferential interaction coefficients estimated with chemical/physical techniques for water-glycerol and –urea mixtures, and provide for the first time the quantitative description of the protein solvation shell in a water-ethanol mixture. The result is the setting of a new method to investigate in detail proteins hydration features in mixed solvents.

### **References**

1. R. Sinibaldi, M. G. Ortore, F. Spinozzi, F. Carsughi, S. Cinelli, G. Onori and P. Mariani, *J. Chem. Phys.* 85, 235101 (2007).
2. R. Sinibaldi, M.G. Ortore, F. Spinozzi, S. S. Funari, J. Teixeira and P. Mariani, *Eur. Biophys. J.* 37, 673-681 (2008).
3. F. Spinozzi, M. G. Ortore, R. Sinibaldi, P. Mariani, A. Esposito, S. Cinelli and G. Onori, *J. Chem. Phys.* 129, 035101 (2008).
4. M. G. Ortore, P. Mariani, F. Carsughi, S. Cinelli, G. Onori, J. Teixeira, and F. Spinozzi, *J. Chem. Phys.* 135, 245103-245112 (2011).
5. M.G. Ortore, R. Sinibaldi, F. Spinozzi, F. Carsughi, A. Bonincontro and P. Mariani, *J. Phys. Chem. B* 112, 12881-12887 (2008).

## TRANSPORT IN TEMPERATURE GRADIENTS: AQUEOUS SOLUTIONS

*Simone Wiegand, Zilin Wang and Philipp Naumann*

Research center Jülich, ICS-3 – Soft condensed Matter, Leo-Brandt Str.,  
52428 Jülich, Germany. [s.wiegand@fz-juelich.de](mailto:s.wiegand@fz-juelich.de)

The separation of components in binary and multicomponent mixtures in a temperature gradient is one of the unsolved problems in physical chemistry. Nevertheless the effect is used in polymer and colloid analysis in the so-called thermal field flow fractionation. Very recently it has also been utilized to study the bio molecular binding reactions [1]. In order to gain a deeper understanding of the underlying mechanism we study systematically bio- and soft matter in a temperature gradient.

We investigated various low molecular weight structures such as saccharides [2,3], nucleotides [4], fd-viruses [5] and microemulsions by a holographic grating method called infrared thermal diffusion forced Rayleigh scattering (IR-TDFRS). We discuss the concentration and temperature dependence of the thermodiffusion or thermophoresis, but also the influence of charges and hydrogen bonds. Also the open questions such as the radial dependence of the thermal diffusion coefficient and its relation with the interfacial tension are considered. We will also give an intuitive physical picture which explains the universal temperature dependence of aqueous solutions [6].

### **References**

1. Baaske, P., C.J. Wienken, P. Reineck, S. Duhr, and D. Braun, *Angew. Chem. Int. Edit.*, 49 (2010) 2238-2241..
2. Blanco, P., H. Kriegs, B. Arlt, and S. Wiegand, *J. Phys. Chem. B* 314(2010) 10740-10747.
3. Blanco, P. and S. Wiegand, *J. Phys. Chem. B*, 114(2010) 2807-2813.
4. Wang, Z., H. Kriegs, and S. Wiegand, *J. Phys. Chem. B*, 2012.(<http://dx.doi.org/10.1021/jp3032644>, accepted)
5. Blanco, P., H. Kriegs, M.P. Lettinga, P. Holmqvist, and S. Wiegand, *Biomacromolecules*, (2011) 1602-1609.
6. Iacopini, S., R. Rusconi, and R. Piazza, *Eur. Phys. J. E*, 19(2006) 59-67.

## ANISOTROPIC ENHANCED WATER DIFFUSION IN SCLEROGLUCAN/BORAX SWELLED TABLETS STUDIED BY PGSE NMR

*Raffaele Lamanna<sup>a</sup>, Chiara Di Meo<sup>b</sup>, Tommasina Coviello<sup>b</sup>, Pietro Matricardi<sup>b</sup>, Franco Alhaique<sup>b</sup> and Donatella Capitani<sup>c</sup>*

<sup>a</sup>ENEA, Trisaia Research Center, ss 106 Jonica Km 419.5, 75026 Rotondella, MT, Italy. [raffaele.lamanna@enea.it](mailto:raffaele.lamanna@enea.it)

<sup>b</sup>Department of Drug Chemistry and Technologies, University of Rome "Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy

<sup>c</sup>Magnetic Resonance Laboratory Annalaura Segre, Institute of Chemical Methodologies, CNR Research Area of Rome, via Salaria Km 29.300, 00016 Monterotondo Stazione, Rome, Italy

### Introduction

Scleroglucan (ScIgl) is a branched homopolysaccharide secreted by fungi of the genus *Sclerotium* consisting of a main chain of (1–3)-linked  $\beta$ -D-glucopyranosyl units bearing, every third unit, a single  $\beta$ -D-glucopyranosyl unit (1–6)-linked. In the presence of borax ScIgl is able to form a self-sustaining gel, which has been tested as a matrix for the release of model drugs with different steric hindrance. ScIgl tablets, obtained by unidirectional compression of freeze dried hydrogels, show a peculiar swelling behaviour: in fact, the swelling process occurs almost exclusively in the axial direction [1].

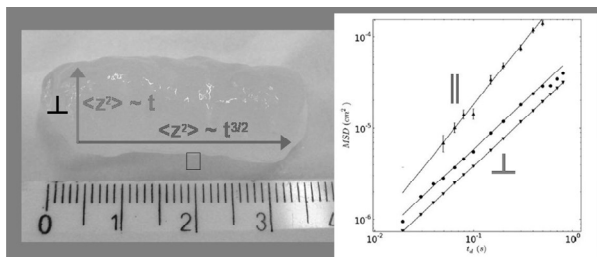
Molecular dynamic (MD) simulations, along with atomic force microscopy (AFM) analyses, evidenced the ability of borax to keep an ordered configuration of parallel-aligned triplexes, which leads to the formation of nanochannel-like structures [2].

Water diffuses inside this peculiar network structure, which modifies its diffusion properties. In fact, the nature and the geometry of the medium strongly influence the diffusion properties of water molecules which, in turn, play some role in the release properties of the gel.

### Results

ScIgl and ScIgl/borax systems were studied by pulsed gradient spin-echo (PGSE) NMR technique. The effects of the compression of the original polysaccharidic dry matter on the diffusion properties of water were studied in connection with the direction of the applied stress. Actually, anomalous and anisotropic enhanced diffusion behaviour of water molecules was observed in swollen ScIgl/borax tablets. This superdiffusive process was

evidenced by a power-law dependence of the mean square displacement (MSD) with diffusion time (Fig. 1). Enhanced diffusion is interpreted in the framework of a solvent mediated diffusion model [3].



**Figure 1.** Mean Square Displacement (MSD) of water in Scgl/borax tablets vs. the diffusion time. Circles and up triangles represent, respectively, the slow and fast diffusion components along the compression direction. The down triangles represent the diffusion along the direction perpendicular to the compression.

### References

1. T. Coviello, M. Grassi, R. Lapasin, A. Marino, F. Alhaique *Biomaterials* 24, 2789-2798 (2003).
2. G. Bocchinfuso, A. Palleschi, C. Mazzuca, T. Coviello, F. Alhaique, G. Marletta *J. Phys. Chem. B* 112, 6473-6483 (2008).
3. C. Di Meo, T. Coviello, P. Matricardi, F. Alhaique, D. Capitani and R. Lamanna *Soft Matter* 7, 6068-6075 (2011).

## ACCOUNTING FOR WATER MOLECULES IN HCV DRUG DISCOVERY

*Maria Letizia Barreca, Nunzio Iraci, Rosy Gaetani,  
Giuseppe Manfroni and Violetta Cecchetti*

Dipartimento di Chimica e Tecnologia del Farmaco, University of Perugia – Via del  
Liceo 1, 06126 Perugia, Italy. [lbarreca@unipg.it](mailto:lbarreca@unipg.it)

### Introduction

Chronic HCV infection is one of the leading causes of liver cirrhosis and hepatocellular carcinoma, as well as the first indication for liver transplantation in industrialized countries. The prevalence of HCV-related disease is increasing, and no vaccine is yet available. The current standard of care for HCV infection is a triple combination therapy (*i.e.*, interferon, ribavirin and NS3 protease inhibitor) which shows long-term toxic effects, is associated with high costs, results in an increased pill burden on the patient, suffers from many drug interactions, and has a low barrier to resistance if used as monotherapy. Thus, novel, more efficacious and tolerable therapies are urgently needed, and a greater understanding of the viral life cycle has led to an increase in the number of possible targets for antiviral intervention.

In this scenario, the identification of novel inhibitors targeting the NS5B RNA-dependent RNA polymerase, a key enzyme in the hepatitis C virus replication, represents an exciting area of research for the work of many industry and university groups [1,2]. Despite these efforts, the field has suffered from high attrition, with several compounds being pulled from the clinic due to lack of efficacy or unforeseen toxicity, and to date no candidates targeting NS5B have reached the market. Thus, the discovery of new chemical entities able to inhibit the NS5B is of great interest, and could help the troubled research of anti-HCV drugs.

As a contribution to the field of HCV drug discovery, this study concerns computational studies aimed at evaluating the impact of tightly bound water molecules in ligand-protein docking simulations at the allosteric NS5B polymerase palm site I (PSI). In fact, one of the most important current challenges in computer-aided drug discovery is gaining insight into the role of the water molecules in the binding site. Water molecules can be involved in protein ligand recognition either by mediating hydrogen bonds between the protein and the ligand or by being displaced by the ligand; both of these mechanisms have been shown to be of substantial importance to drug discovery. In some cases, even if not involved in mediating any ligand-

protein interaction, water molecules have been shown to influence the shape and the flexibility of a ligand-binding site in a protein.

The information gathered in this study could support the hit identification and hit-to-lead optimization of new non-nucleoside inhibitors (NNIs) acting at the NS5B PSI.

## Experimental

Forty-five crystal structures of NS5B polymerase in complex with PSI-NNIs are available on the Protein Data Bank. Highly conserved water molecules at this allosteric site were thus identified by structural investigation coupled with molecular interaction fields analysis. Several sets of ligand-protein cross-docking simulations were then built according to the number of water molecules in the NS5B proteins. The docking poses were then subjected to post-docking refinement and rescoring, and statistical analysis were performed for each simulation to summarize the percentage of correctly predicted ligand conformations (i.e., RMSD < 2 Å).

## Results

The main goal of this study was to understand how the accuracy of ligand-protein docking may be affected by the presence of water molecules in the ligand PSI of the NS5B protein

The results obtained highlight once more the primary role of water molecules in docking studies, since improvements in the quality of the poses were found when including explicit key waters. This information suggests that in a virtual screening experiment, where the type of ligand/water interaction cannot be known *a priori*, it would be important to consider as target an ensemble of NS5B structures with different sets of water molecules included. On the contrary, in a structure-based drug design approach, where the researcher might decide the kind of PSI-NNIs to be explored, the knowledge of the structural water molecules would permit to restrict the targets to use in docking experiments as well as the use of computationally expensive and most rigorous techniques.

In summary, this work suggests the best performing combinations of HCV NS5B protein/water molecules for rational identification and optimization of new PSI-NNIs. These structural models are the ones currently used in our HCV drug discovery projects, and preliminary biological results on new selective anti-HCV inhibitors will be reported.

## References

1. M.J. Sofia *et al.* J. Med. Chem. 55, 2481-2531 (2012)
2. M.L. Barreca *et al.* Future Med. Chem. 3, 1027-1055 (2011)

# WHICH ROLE OF WATER IN DETERMINING THE RESIDENCE TIME OF DRUGS?

*Matteo Ceccarelli*

Dipartimento di Fisica and SLACS/Istituto Officina dei Materiali, University of Cagliari – 09042, Monserrato, Italy. [matteo.ceccarelli@dsf.unica.it](mailto:matteo.ceccarelli@dsf.unica.it)

## **Introduction**

Water is a ubiquitous molecule in life sciences. For this reason it has attracted the attention of several researchers that have tried to assign a specific role to it in many processes. The interest of our group is focused on the interaction between drugs and proteins and the question is how the water can play a role in modulating drug/protein functionalities. Using Molecular Simulations we calculated the kinetics of water molecules in the binding pocket of two membrane proteins. Besides the differences among the two proteins, the first involved in transmitting signals across the membrane and the second in transporting molecules, we found some similarities that suggest a possible role of water in modulating the binding of drugs.

## **Methods**

Molecular Dynamics simulations with empirical force field are the method of choice to obtain microscopic details on the role of specific interactions with enough statistics. We calculated the kinetics of water molecules inside the binding pocket of two membrane proteins, the delta opioid receptor, a GPCR protein that transmits information across the membrane, and the water-filled channel OmpF, considered the main route for the entry of antibiotics inside *Escherichia coli*, a common bacterium of the Gram Negative family.

## **Results**

In the two examples we considered, the binding is not due to the desolvation of the binding pocket. Instead we have seen that drugs are solvated and they interact with protein walls either through hydrogen bonds or through a bridge created by water molecules. We calculated the kinetics of these water molecules inside the binding pocket and in the case of the GPCR protein [1] we observed that water molecules with a slow kinetics (exchange longer than 100 ps) are not present when we docked a drug with a low activity. On the other hand a few slow water molecules are present using a drug with a high activity. The role of slow water would be to increase the residence time of

drug inside the protein and make the drug larger, with a higher probability to transmit the information through a structural change.

In the case of the OmpF channel, antibiotics have to bind the central region, rich of charged amino acids and small in size, in order to permeate [2]. The central region is rich of slow waters and upon binding antibiotics have to replace, completely or in part, them, with an energetic cost to pay [3]. The role of water on the permeation is still under debate [4].

### **References**

1. F. Collu, M. Ceccarelli and P. Ruggerone, submitted (2012)
2. A. Kumar, E. Hajjar, P. Ruggerone and M. Ceccarelli, *J. Phys. Cond. Matt.* 22, 454125 (2010)
3. A. Kumar, E. Hajjar, P. Rugegrone and M. Ceccarelli, *J. Phys. Chem. B* 114, 9608-9616 (2010)
4. R.A. Pearlstein, Q.Y. Hu, J. Zhou, D. Yowe, J. Levell, B. Dale, V. K. Kaushik, D. Daniels, S. Hanrahan, W. Sherman, R. Abel, *Proteins* 78, 2571-2586 (2010)



## TABLET DISINTEGRATION - ROLE OF PRESSURE AND MOISTURE

*Paul Wan Sia Heng, Parind Mahendrakumar Desai and Celine Valeria Liew*

GEA-NUS Pharmaceutical Processing Research Laboratory, Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore. [phapaulh@nus.edu.sg](mailto:phapaulh@nus.edu.sg)

### Introduction

Disintegrants are incorporated into tablets to aid in the break up of the compacts when in contact with an aqueous environment. Swelling, wicking (or capillary action) followed by disruption of particle-particle bonds and strain recovery are the more commonly accepted modes of disintegrant action. However, some of the proposed theories do not adequately explain the observed disintegration behaviors and a good understanding of all disintegrant actions is still deficient. The aim of this study was to investigate the role of pressure and moisture in tablet disintegration using high speed video imaging.

### Experimental

Disintegrant particles were compacted using a universal testing machine. Free disintegrant particles were also entrapped in a stainless steel mesh. Disintegration phenomena of compacts and effect of wetting on free disintegrant particles upon contact with water were then captured by high speed video imaging. MATLAB was used to process the acquired images and analyze changes in compact area as well as instantaneous motions of particles on contact with water. Breakdown behaviors of compacts prepared with different disintegrants were evaluated at different compression pressures to assess the amount and rate of recovery of the compaction strain [1]. Breakdown of compacted disintegrants in different solvents was also studied.

### Results

The relative change in area of compact was less in comparison with that of free disintegrant particles, because compression pressure limited the potential increase in area of all examined disintegrants except crospovidone. Compacted crospovidone particles also showed significantly higher mobility than free crospovidone particles. Disintegrability of crospovidone compacts

increased with compression pressure, suggesting the importance of strain recovery for crospovidone disintegrant action [1]. Differences in the rate of swelling were observed for free and deformed disintegrant particles. Disintegrant compacts had highest disintegration capacity in aqueous medium.

### **Conclusion**

Digital image processing using MATLAB was proven to be a feasible method to gain an in-depth understanding of the characteristics of disintegrants. Disintegrants that acted primarily by swelling and wicking (for example, starch, microcrystalline cellulose) showed decrease in disintegrability with increase in compression pressure. On the other hand, expansive pressure contributed by disintegrant particles upon strain recovery to regain their initial state upon wetting, was responsible for crospovidone disintegrant action.

### **References**

1. P. M. Desai, C. V. Liew and P. W. S. Heng, J Pharm Sci. 101, 2155–2164 (2012)

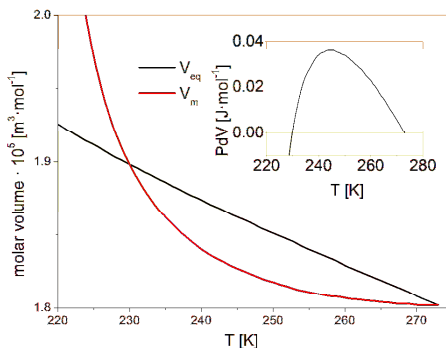
## WATER IS NOT SO ANOMALOUS

*Francesco Aliotta, Rosina Celeste Ponterio and Franz Saija*

Istituto per i Processi Chimico-Fisici, CNR, V.le F. Stagno d'Alcontres 37, 98158  
Messina Italy. [aliotta@me.cnr.it](mailto:aliotta@me.cnr.it)

The properties of liquid water represented the intriguing topic of many experimental and theoretical investigations over several decades, due to the apparent huge number of anomalies detected. The anomalies become more pronounced in approaching the homogeneous nucleation temperature,  $T_h$ , where many thermodynamic quantities exhibit an apparent diverging behaviour [1]. The discovery of different forms of amorphous water [2], observed under extreme temperature and pressure conditions and assumed as metastable phases, triggered the idea for a metastable stability extended down to the region between  $T_h$  and the glass transition temperature,  $T_g$ . At least three plausible conjectures [4-6] have been proposed for the metastable phase diagram of deeply supercooled water. To select the more plausible scenario is an experimental impossible task, since it requires to access temperature so close to  $T_h$  that homogeneous nucleation takes place on shorter times than those required for the experiment. This justifies to name the region  $T_g \leq T \leq T_h$  the *no man's land*. Several attempts have been made to bypass the difficulty by investigating samples under transient heating/cooling or in confinement environment, since confinement prevent freezing. However, such a choice leaves unsolved the question if so obtained data can be relevant for bulk water. In addition, it was recently shown [7] that even if data from bulk water and confined water can appear undistinguishable in the temperature range accessible for bulk water, there is no safe way for assuming that the matching between data persists at lower temperatures.

To exit from such a frustrating situation we propose a change of perspective, moving from a trivial experimental observation: any liquid cooled down its melting temperature, and left reaching the metastable equilibrium, after a finite time will decay abruptly towards the stable equilibrium. The stable equilibrium consists in a mixture of solid and liquid at the melting temperature. We made the assumption that the decay towards equilibrium is a process so fast that it can be considered an adiabatic process. This assumption has been proved by a calorimetric experiment. The assumption of an adiabatic process immediately allows to calculate the volume fraction of ice which forms in reaching the stable equilibrium. A temperature has been detected at which the process towards equilibrium takes place without any volume change (see Fig. 1).



**Figure 1.** Temperature dependence of the molar volume of supercooled water (red line) and of the average molar volume of the corresponding stable state (black line). Inset: temperature dependence of the work required for reaching stable equilibrium.

This means that at this temperature the work required for the formation of ice nuclei and their growth is minimized (see inset in the Fig. 1). This temperature is identified as  $T_h$ . Such a result is independent of the peculiar properties of water and holds for any liquid. It is shown that approaching  $T_h$  negligible temperature fluctuations must produce huge entropy fluctuations. For this reason  $T_h$  must be considered, for any liquid, as the low temperature boundary of the metastability. In this perspective many of the water anomalies disappear, water behaving as any other liquid. The assumption of amorphous water as a metastable phase must be definitely ruled out, together with the description of the *no man's land* as a region of metastability for water (and for any other liquid, of course).

### References

1. R. J. Speedy, C. A. Angell, J. Chem. Phys. 75, 851-858 (1976).
2. O. Mishima, L.D. Calvert, E. Whalley, Nature 314, 76-78 (1985).
3. O. Mishima, J. Phys. Chem. 100, 5910-5912 (1994).
4. R.J. Speedy, J. Phys. Chem. 86, 982-991 (1982).
5. O. Mishima, H.E. Stanley, Nature 392, 164-168 (1998).
6. A. Sastry, P.G. Debenedetti, F. Sciortino, H.E. Stanley, Phys. Rev. E 53, 6144-6154 (1996).
7. F. Aliotta, M. Pochylski, R.C. Ponterio, F. Saija, G. Salvato, C. Vasi, Phys. Rev. Lett., submitted (2012).

## MOLECULAR PROBE DYNAMICS REVEALS SUPPRESSION OF ICE-LIKE REGIONS IN STRONGLY CONFINED SUPERCOOLED WATER

*D. Leporini<sup>a,b</sup>, D. Banerjee<sup>c</sup>, S. N. Bhat<sup>c</sup> and S. V. Bhat<sup>c</sup>*

<sup>a</sup>Dipartimento di Fisica "Enrico Fermi", Università di Pisa, Largo B. Pontecorvo 3, I-56127 Pisa, Italy. [leporini@df.unipi.it](mailto:leporini@df.unipi.it)

<sup>b</sup>IPCF-CNR, UoS Pisa, Pisa, Italy

<sup>c</sup>Department of Physics, Indian Institute of Science, Bangalore 560 012, India

The structure of the hydrogen bond network is a key element for understanding water's thermodynamic and kinetic anomalies. While ambient water is strongly believed to be a uniform, continuous hydrogen-bonded liquid, there is growing consensus that supercooled water is better described in terms of distinct domains with either low-density ice-like structure or high-density disordered one. We evidenced two distinct rotational mobilities of probe molecules in interstitial supercooled water of polycrystalline ice [1].

Here we show that, by increasing the confinement of interstitial water, the mobility of probe molecules, surprisingly, increases [2]. We argue that loose confinement allows the presence of ice-like regions in supercooled water, whereas a tighter confinement yields the suppression of this ordered fraction and leads to higher fluidity. Compelling evidence of the presence of ice-like regions is provided by the probe orientational entropy barrier which is set, through hydrogen bonding, by the configuration of the surrounding water molecules and yields a direct measure of the configurational entropy of the same.

We find that, under loose confinement of supercooled water, the entropy barrier surmounted by the slower probe fraction in the range 130 – 180 K exceeds that of equilibrium water by the melting entropy of ice (within 2.6%), whereas no increase of the barrier is observed under stronger confinement [2]. We argue that the local structure of water surrounding the slower probe fraction in the range 130 – 180 K is well equilibrated and close to ice.

The present results put constraints to recent numerical studies of the lower limit of metastability of supercooled water [3].

### References

1. D. Banerjee, S.N. Bhat, S.V. Bhat and D. Leporini, Proc Natl Acad Sci USA 106, 11448-11453 (2009).
2. D. Banerjee, S.N. Bhat, S.V. Bhat and D. Leporini, PLoS One, in press.
3. E.B. Moore, V. Molinero, Nature 479, 506-509 (2011).



## IDENTIFICATION OF LOW FREQUENCY SHEAR-ELASTICITY IN LIQUID WATER

*Laurence Noirez and Patrick Baroni*

Laboratoire Léon Brillouin (CEA-CNRS)  
91191 Gif-sur-Yvette, France. [laurence.noirez@cea.fr](mailto:laurence.noirez@cea.fr)

On the basis of a Maxwell gas model (1867), it has long been suspected that liquids exhibit only shear elasticity at sufficiently high solicitation frequencies (at GHz). Recent experimental improvements have enabled the identification of shear elasticity in liquids at low frequency (0.1 to 100 Hz). This protocol has been elaborated at the Lab. Léon Brillouin since 2005. Firstly applied on polymer melts, then on molecular glass formers (Glycerol, PPG, o-Terphenyl); a several decades higher mechanical response independent of the frequency, has been obtained revealing a so far neglected shear elasticity (i.e. solid-like component) in the liquid state of various viscous materials [1,4]. We focus here on the response obtained on the H-bond liquid: the water studied for the first time with this method.

The low frequency elasticity is coherent with a series of results emerging from different disciplines as microrheology [5], NMR [6], X-ray photon correlation spectroscopy [7], voltage effects[8] evidencing relaxation modes much slower than those described in conventional theoretical models. These results evidence that time-scales larger than the  $\alpha$ -relaxation times participate to the liquid dynamics.

The consideration of this non-negligible macroscopic component is also of first importance to define relevant parameters for a better understanding of the fluid properties, and in particular of the life at low Reynolds number (microswimmers).

### **References**

1. L. Noirez L, H. Mendil-Jakani, P. Baroni, Polymer Int. **58** 962 (2009).
2. L. Noirez L, P. Baroni, 2010, **972** 16 (2010).
3. L. Noirez, H. Mendil-Jakani, P. Baroni, Philosophical Magazine **91** 1977 (2011).
4. L. Noirez, P. Baroni, H. Cao, J. of Mol. Liq. in press 2012.
5. J. Goyon, A. Colin, G. Ovarlez, A. Ajdari, L. Bocquet, Nature, **454** 84 (2008).

6. U. Tracht, M. Wilhelm, A. Heuer, H. Feng, K. Schmidt-Rohr, H.W. Spiess, Phys. Rev. Lett. **81** 2727 (1998).
7. Y. Chushkin, C. Caronna, A. Madsen, A., EPL, **83** 36001 (2008).
8. E.C. Fuchs, P. Baroni, B. Bitschnau, L. Noirez, J. of Physics D, **43** 105502 (2010).

## DISCLOSING THE ROLE OF HYDROPHOBIC INTERACTIONS THROUGH AGGREGATION OF NON-POLAR SOLUTES IN WATER: A MOLECULAR DYNAMICS STUDY

*Oswaldo Chara<sup>a,b</sup>, H. Ariel Álvarez<sup>b</sup>, C. Gastón Ferrara<sup>b</sup> and J. Raul Grigera<sup>b</sup>*

<sup>a</sup>Center for Information Services and High Performance Computing (ZIH), Technische Universität Dresden, Dresden, Germany. [osvaldo.chara@tu-dresden.de](mailto:osvaldo.chara@tu-dresden.de)

<sup>b</sup>Instituto de Física de Líquidos y Sistemas Biológicos (IFLYSIB), CONICET-UNLP, 59-789, B1900 La Plata, Argentina

Although it is well known that water dynamics is crucial for protein folding, its precise contribution for the stability of the protein is still unknown. What is well known is that the structure and dynamics of water constitute the basis of the so-call hydrophobic interactions. These interactions are not only enthalpically-driven by the hydrophobic residues of the protein but also entropically-driven by the water molecules in the neighbourhood of the protein. The complexity derived from the important number of interactions between protein residues and the water molecules induces additional difficulty to disclose the contribution of these interactions in the stability of a protein. This is why we studied hydrophobic interactions in a simple system composed only by a non-polar solute, Lennard-Jones (LJ) particles, and water SPC/E [1] under different pressure and temperature conditions by means of molecular dynamics simulations.

Previously we demonstrated that water is structurally altered by a hydrophobic surface even in a distances greater than the thickness of the plasma membrane (80 Å) at different temperatures [2]. It is commonly accepted that liquid water structure can be understood in terms of two closely interweaved components: a tetrahedral and hexagonal structures, characterized by a low density and high density arrangement. Based on the calculus of radial distribution function and a definition of a new order parameter we determined a crossover point in structural dominance [3]. At room temperature this point in the range of 1-2 kbar, pressure at which most of the 'anomalous' properties of water vanish.

We here show that 1) temperature triggers the aggregation of non-polar solutes in water within a certain range and 2) pressure induces disaggregation of the clusters. An equilibrium curve defining the coexistence between the non-aggregated (soluble) and aggregated (insoluble) binary system of non-polar solutes and water was extracted. The critical pressure obtained from this curve is 986 bar. The results are in agreement with



previous experimental reports describing hydrocarbon in water mixtures transitions as well as protein unfolding.

We concluded that protein unfolding induced by pressure augmentation could be explained by the reduction in the number of hydrophobic interactions determined by the water hydrogen-bonds network distortion.

### **References**

1. H. J. C. Berendsen, J. R. Grigera and T. P. Straatsma, *J. Phys. Chem.* 91, 6269–6271 (1987).
2. O. Chara, A. N. McCarthy, C. G. Ferrara, E. R. Caffarena and J. R. Grigera, *Physica A*, 388, 4551-4559 (2009).
3. O. Chara, A. N. McCarthy and J. R. Grigera, *Phys. Lett. A*. 375, 572–576 (2011).

## ON THE COUPLING BETWEEN THE DYNAMICS OF PROTEINS AND THAT OF THEIR HYDRATION WATER

*Valeria Conti Nibali<sup>a,b</sup>, Giovanna D'Angelo<sup>b</sup>, Ulderico Wanderlingh<sup>b</sup> and Mounir Tarek<sup>c</sup>*

<sup>a</sup>Ruhr-University Bochum, Institute for Physical Chemistry II, Bochum, Germany.  
[valeria.continibali@rub.de](mailto:valeria.continibali@rub.de)

<sup>b</sup>Dipartimento di Fisica, Università degli Studi di Messina, Messina, Italy

<sup>c</sup>UMR Structure et Réactivité des Systèmes Moléculaires Complexes, Nancy-University, CNRS, France

There is a great interest in elucidating the dynamical features of protein hydration water in the picoseconds time scale and the terahertz frequency window, owing to the fact that numerous processes in water, e.g. hydrogen bond rearrangements and rotational relaxation, occur on this scale. Characterization of incoherent (single-particle) motions have shown that protein hydration water behaves differently from bulk water, due to the topological and energetic disorder induced by the interaction with the fluctuating protein surface. In contrast to what has been observed for single particle dynamics, it was reported that coherent collective dynamics of protein hydration water bears close similarity to that of liquid water i.e. the presence of the protein has negligible effects on the collective motions of the solvent.

In this contribution we present a MD simulation study that has allowed to shed light on very new characteristics of the short wavelength collective dynamics of protein hydration water, highlighting fundamental differences between the latter and that of bulk water. In particular, the data reveals that the spectra of protein hydration water clearly show dynamical features whose origin can be related to specific interactions with the protein collective modes that can be interpreted in terms of anti-crossing events.

Moreover, we show that the collective dynamics of hydration water has a spatially heterogeneous behaviour, being strongly coupled to protein dynamics within few Å from the protein surface and recovering some of its bulk-like properties at an increasing distance from the protein surface.

## ION SPECIFIC EFFECTS IN POLYELECTROLYTE SOLUTIONS: AN EXAMPLE OF IONENE SALTS IN WATER

*Barbara Hribar-Lee<sup>a</sup>, Matjaž Bončina<sup>a</sup>, Sašo Čebašek<sup>a</sup>, Miha Lukšič<sup>a</sup>,  
Natalie Maliková<sup>b</sup>, Mojca Seručnik<sup>a</sup> and Vojko Vlachy<sup>a</sup>*

<sup>a</sup>Faculty of Chemistry and Chemical Technology, University of Ljubljana – Aškerčeva 5  
1000 Ljubljana, Slovenia. [barbara.hribar@fkkt.uni-lj.si](mailto:barbara.hribar@fkkt.uni-lj.si)

<sup>b</sup>Laboratoire Leon Brillouin, CEA Saclay –  
91191 Gif-sur-Yvette cedex, France.

Ionenes are aliphatic cationic polyelectrolytes with quaternized nitrogen atoms in the backbone of the chain. The variety of their application, as well as their use as a model substance to study the ion specific effects in polyelectrolyte solutions, has its origin in their uniqueness: through a properly conducted syntheses the polyion structure and charge density (hydrophobicity), as well as the nature of counterions, can be systematically varied [1]. The physical and chemical properties of aqueous polyelectrolyte solutions strongly depend on the linear charge density of the polyion (or its hydrophobicity), as well as on the chemical nature of the counterions (ion specific effects) [2]. The latter play a major role in analytical, technological, and pharmaceutical procedures, such as ion-exchange chromatography, salting-in and salting-out of proteins (Hofmeister series), and cause specificity in docking interactions.

To understand the mutual influence of the polyion charge density and the nature of the counterions on the thermodynamic and transport properties of these materials, systematic experimental studies of thermodynamic [3-5] and dynamic [6-8] properties of aqueous ionene solutions have been performed. Osmotic coefficients, partial molar volumes, enthalpies of dilution, as well as of mixing, heat capacity, electric conductivity, dielectric relaxation, and transference numbers in these solutions were measured. We showed that the heat effects upon dilution and mixing are linearly related to the enthalpy of counterion hydration. Recently, the conformation of ionene bromides and fluorides was probed by small angle neutron scattering experiment [9]. All these studies allow us to draw some general conclusions about the combined effects of charge density and nature of counterions in polyelectrolyte solutions.

### References

1. A. Rembaum, Applied Polymer Symp. 22, 299-317 (1973).
2. W. Kunz, Specific Ion Effects, Singapor: World Scientific, 2010.

3. S. Čebašek, M. Lukšič, C. Pohar, V. Vlachy, *J. Chem. Eng. Data* 56, 1282-1292 (2011).
4. M. Lukšič, M. Bončina, V. Vlachy, and M. Druchok, *Phys. Chem. Chem. Phys.* 14, 2024-2031 (2012).
5. M. Seručnik, M. Bončina, M. Lukšič, and V. Vlachy, *Phys. Chem. Chem. Phys.* 14, 6805-6811 (2012).
6. M. Lukšič, R. Buchner, B. Hribar-Lee, and V. Vlachy, *Macromolecules* 42, 4337-4342 (2009).
7. M. Lukšič, B. Hribar-Lee, and V. Vlachy, *J. Phys. Chem. B* 114, 10401-10408 (2010).
8. M. Druchok, B. Hribar-Lee, H. Krienke, and V. Vlachy, *Chem. Phys. Lett* 450, 281–285 (2008).
9. N. Malikova, S. Čebašek, V. Glenisson, D. Bhowmik, G. Carrot, and V. Vlachy, submitted to *Phys. Chem. Chem. Phys.*

## LOW TEMPERATURE BEHAVIOUR OF HYDRATED NAFION MEMBRANES

*M. Plazane<sup>a</sup>, P. Bartolini<sup>b</sup>, R. Torre<sup>b</sup>, C. Petrillo<sup>c</sup> and F. Sacchetti<sup>c</sup>*

<sup>a</sup>Laboratoire Interdisciplinaire de Physique, Université Joseph Fourier and CNRS, BP 87, 38402 Saint Martin d'Herès, France. [marie.plazanet@ujf-grenoble.fr](mailto:marie.plazanet@ujf-grenoble.fr)

<sup>b</sup>LENS, Polo scientifico, Via Nello Carrara, 1, 50019 Sesto Fiorentino, Italy

<sup>c</sup>Dipartimento di Fisica, Università di Perugia, 06123 Perugia, Italy

Nafion® is a famous amphiphilic polymer made of fluorated chains carrying hydrophilic sulfonate anions. It is widely used in various applications, for which the most famous is the use as proton conducting membranes in fuel cells. Its useful properties, related to the water structure and dynamics, are the result of a complex polymer organization in which the hydrophilic side chains are organized to form a network of channels and cavities that fills water upon hydration. The structure of the material and the dynamics of water are still a matter of debate and studies.

Using Transient Grating Spectroscopy, we monitored the propagation of the longitudinal acoustic sound wave in the hydrated membranes as a function of temperature. As previously observed by X-ray diffraction [1], we confirmed the progressive formation of ice outside of the membrane as the temperature decreases down to 220 K. Moreover, we highlighted the reversibility of the phenomena, i.e. the re-adsorption of water inside the membrane upon heating [2]. Combining the measurements with neutron diffraction, we emphasized that the crystals formed outside of the membranes are at least of micrometer size, and we were able to monitor the quantity of ice as a function of temperature. The power law followed by this parameter indicates that the phenomena is not related to the pore distribution, but rather to the chemical potential of water inside the membrane that varies with hydration and temperature. A simple model of solution, for which the freezing point depends on the solute concentration, is in very good agreement with the data [3].

Implication of this model in other systems of biological interest containing confined water, like lipid membranes or hydrated proteins, will eventually be discussed.

### References

1. M. Pineri, G. Gebel, R.J. Davies, O. Diat, J. Power Sources 2007, 172, 587-596
2. M. Plazanet, P. Bartolini, R. Torre, C Petrillo and F. Sacchetti, J. Phys. Chem. B, 2009, 113, 10121-10127.
3. M. Plazanet et al, to be published.

## ON THE SOLVATION OF IONS IN HIGH-DENSITY WATER

*Valentina Migliorati<sup>a</sup>, Giordano Mancini<sup>b</sup>, Simone Tatol<sup>a</sup>, Andrea Zitolo<sup>a</sup>, Adriano Filippini<sup>c</sup>, Simone De Panfilis<sup>d</sup>, Andrea Di Cicco<sup>e</sup> and Paola D'Angelo<sup>a</sup>*

<sup>a</sup>Dipartimento di Chimica, Università di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy [dangelo@caspur.it](mailto:dangelo@caspur.it)

<sup>b</sup>CASPUR, Inter-University Consortium for Supercomputing in Research, via dei Tizii 6b, 00185 Roma, Italy

<sup>c</sup>Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Vetoio, 67100 L'Aquila, Italy

<sup>d</sup>CLNS IIT@Sapienza - c/o Dipartimento di Fisica, Università di Roma "La Sapienza", 00185 Roma, Italy

<sup>e</sup>CNISM, Dip. di Fisica, Università di Camerino, Via Mad. delle Carceri, 62032 Camerino (MC), Italy

The hydration properties of metal ions are modified by application of large external pressures (see [1] and ref. therein). In this study, the modification of the hydration properties of the  $\text{Zn}^{2+}$  ion induced by a pressure increase from ambient condition up to 6.4 GPa has been investigated using a combined approach based on extended X-ray absorption fine structure (EXAFS) spectroscopy and Molecular Dynamics (MD) simulations. With increasing pressure the first hydration shell of the  $\text{Zn}^{2+}$  ion has been found to retain an octahedral symmetry with a shortening of the Zn-O distance up to 0.09 Å and an increased width associated with thermal motion, as compared to the ambient condition hydration complex. A very interesting picture of the dynamic behaviour of the first hydration shell has emerged from the analysis of the simulations: up to 2.5 GPa no exchange events between first and second shell water molecules have been observed, while above this pressure value several exchange events take place in the solution following an associative interchange mechanism. This result can be explained by the very high compression and packing of the solvent which force second shell water molecules to enter the  $\text{Zn}^{2+}$  first hydration shell. MD simulations indicate a strong pressure effect also on the structure of the second coordination shell which is compressed and becomes more disordered and unstructured with increasing pressure. The water mobility and the ion diffusion coefficient have been found to increase in the high density conditions, as a consequence of the rupture of the hydrogen bond network caused by pressure.

### References

1. A. Filippini, S. De Panfilis, C. Oliva, M. A. Ricci, P. D'Angelo, and D. T. Bowron, *Phys. Rev. Lett.* 2003, 91, 165505.

## ABOUT EMULSION WATER STRUCTURE

*Fabio Brunì<sup>a</sup>, Silvia Imberti<sup>b</sup>, Maria Antonietta Ricci<sup>a</sup> and Rosaria Mancinelli<sup>a</sup>*

<sup>a</sup>Dipartimento di Fisica "Amaldi", Università di Roma Tre, via della Vasca Navale 84, 00146 Roma, Italia. [mancinelli@fis.uniroma3.it](mailto:mancinelli@fis.uniroma3.it)

<sup>b</sup>STFC, ISIS Facility, Rutherford Appleton Laboratory, Chilton, Didcot, OX11 0QX, U.K.

### Introduction

Emulsions are very common among food and pharmaceutical products. These compounds belong to the class of *colloids*, which have intermediate properties between molecularly dispersed solutions and bulky systems, being characterized by one phase dispersed into another. Typically, one of these phases is made of water and the other one consists of a hydrophobic species, like oil. One of the main topics of discussion for industrial goals on this matter is the *stability* of emulsions: this can be achieved by adding low concentrations of surface active agents to the main phases. Although these are minority components of emulsion, according to Bancroft's rule [1], emulsion type depends more on the nature of emulsifier than on the relative proportion of water to oil. In particular, thermodynamic stability is thought to be guaranteed if emulsifier concentration is below a critical micelle concentration and the storage temperature is lower than 20 K of the phase inversion temperature. A lower limit is usually not mentioned, even if storage in freezers is often applied.

Here we tackle the case of an emulsion made of water (67% in weight), methylcyclohexane (30%) and Span 65, known also as E492 (3%). We have studied the microscopic structure of water in this emulsion and its dependence on temperature, from standard conditions to the ice nucleation point, in order to clarify what exactly happens to water in this heterogeneous system.

### Experimental

To our knowledge, this is the first Neutron Diffraction study on an emulsion, exploiting isotopic substitution H/D [2] of both water hydrogens and methylcyclohexane hydrogens. This technique allows to have direct access to emulsion water structure, via hydrogen-hydrogen structure factors. We performed a set of experiments at the SANDALS diffractometer, installed at ISIS-TS2 (Rutherford Appleton Laboratory, Chilton, UK). We measured the Differential Cross Section of the emulsion at ambient conditions and in the supercooled phase (260 K). We have detected ice nucleation, at 250 K. Interestingly, the wide Q range explored by SANDALS can give information

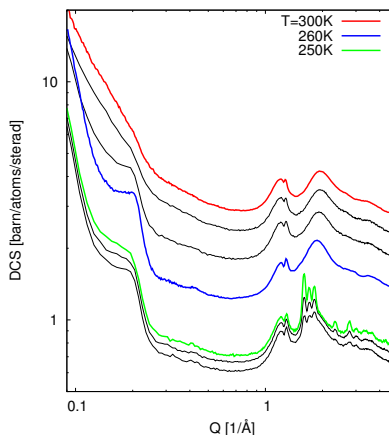
not only at intermolecular level, but also on morphologic changes which occur at the nanoscopic level.

The experiments have been supported by enhanced classical Monte Carlo simulations, in which pair potentials are perturbatively refined by the comparison with the experimental data (the so called Empirical Potential Structure Refinement method [3]).

Modelling an emulsion is not straightforward at the atomistic level, due to the difficulty of giving an atomistic description of the emulsifier. Nevertheless, after many trials, we have identified some stable results and a reliable interpretation of the modifications of the DCS at the nanoscopic level.

## Results

At ambient conditions, water within the emulsion seems to be more ordered than its bulk counterpart, due to the existence of hydrophobic forces at interfacial region. Here, where a depletion layer has been detected, the relative arrangement of water molecules appears more perturbed, than in the water layers far from the interface: the perturbation is similar to that brought by pressure. Upon cooling down, water keeps the same average intermolecular structure, although the atomic positions



become more defined and the interfacial layers become more similar to the innermost ones. The origin of this thermodynamic inertia is just the interaction with other species and the weak geometrical confinement. Moreover, predictably, water density decreases whereas the volume occupied by methylcyclohexane decreases. This phenomenon occurs along with a striking morphologic change at nanoscopic level (see Figure): owing to the strengthening of internal water cohesion, Span 65 molecules seem to aggregate around hydrate compounds of methylcyclohexane. In this new phase, which is the cold analogous of micelle, water is the dispersing medium. In conclusion, emulsions cannot be dealt as rigid systems as the balance between phases can be easily altered by thermic stresses.

## References

1. W. D. Bancroft, *J. Phys. Chem.* 17, 501 – 519 (1913).
2. J. L. Finney, A. K. Soper, *Chem. Soc. Rev.*, 1, 1-10 (1994)
3. A. K. Soper, *Chem. Phys.* 202, 295 - 306 (1996); A. K. Soper, *Mol. Phys.* 99, 1503 – 1516 (2001)



## MODELING CHANGES IN FOOD POLYMER PROPERTIES USING HYGRO-THERMAL TIME

*Gaylon Campbell, Brady Carter and Martin Buehler*

Research and Development, Decagon Devices, Inc.  
2365 Hopkins Ct., Pullman WA 99163 USA. [gaylon@decagon.com](mailto:gaylon@decagon.com)

Thermal time refers to a time scale in which the rate of a temperature dependent process is constant (Monteith, 1977; Campbell and Norman, 1998). It has been used to model the rate of plant development where temperature strongly influences the developmental process. Food polymers also change, with time, at rates that depend on their temperature. The thermal time concept can be useful for describing these processes as well. Changes in food polymers, however, depend on both temperature and water activity, so a modified thermal time is needed, called hygro-thermal time. Hygro-thermal time allows the prediction of the response of a polymer to changing temperature and water activity conditions. If, in a laboratory setting, we determine the hygro-thermal time required for a process to complete, then any combination of temperature and water activity giving that same quantity of hygro-thermal time will complete the process. The temperature and water activity determine the rate of the process. Its rate, integrated over time, is a measure of progress toward completion of the process. This is similar to the concept in physics that distance traveled is the integral of speed over time.

### Modeling Rate of Change

Polydextrose was equilibrated at four different water activities, and at temperatures well below the transition temperature. Samples were then monitored without changing water activity, but instantaneously increasing the temperature. The samples were monitored using dielectric spectroscopy. The change in the matrix was detected as the disappearance of a specific peak in the dielectric spectrum. The time required for the peak to disappear was determined. The rate of change was computed as the reciprocal of the time. The rate of change is described by the equation

$$R = \exp[0.48(T + 170.0a_w - 101.57)] \quad (1)$$

which was fit to the data by non-linear least squares.  $R$  is the process rate ( $\text{hr}^{-1}$ ).

### Modeling matrix changes when water activity and temperature vary

With the equation for the rate of change, we can compute the total change that occurs for any combination of water activity and temperature as (Campbell and Norman, 1998)

$$C = \int R(T, a_w) dt \quad (2)$$

where  $T$  and/or  $a_w$  may vary with time.

For any combination of water activity and temperature eq. 1 gives the appropriate rate. In practice we normally know temperature and water activity at discrete points in time, so we would normally compute  $C$  as

$$C = \sum R(T_i, a_{wi}) \Delta t \quad (3)$$

Where, again, eq. 1 is used to compute the rate. When  $C$  reaches 1 the process is complete

### Generalization

This treatment has dealt with just one example, polydextrose. The approach, however, is very general, and should apply to most irreversible temperature and water activity mediated processes. This example relates to modification of the polydextrose matrix, but other processes that should follow this same pattern are lipid oxidation, browning and loss of enzyme activity. The experimental procedure for investigating these processes likely would be similar to the one followed in this study. A sample would be equilibrated at a set water activity, with the temperature well below the transition temperature. The experiment would start by elevating the temperature while maintaining the water activity at a constant value. The samples would be monitored by some suitable method to determine the onset, end or half life of the condition of interest. The analysis is done by plotting either temperature vs. logarithm of time or rate vs. temperature. An equation like eq. 1 is obtained, which, in its general form

$$R = \exp\{\alpha[T - T_b(a_w)]\} \quad (4)$$

where  $T_b(a_w)$  is a polynomial function of water activity.

### References

1. Campbell, G. S. and J. M. Norman. An Introduction to Environmental Biophysics, 2<sup>nd</sup> Ed. New York: Springer Verlag 1998.
2. J. L. Monteith. Climate and the efficiency of crop production in Britain. Phil. Trans. R. Soc. Lond. B. 281:277-294 (1977).

## DIFFERENT MATERIAL BEHAVIORS DURING THE PROTEIN POWDER AGEING

*D. Champion<sup>a</sup> and P. Lillford<sup>b</sup>*

<sup>a</sup>Food process and physical-chemistry, UMR PAM, Agrosup Dijon & University of Burgundy – 1, Esplanade Erasme, 21000 Dijon, France. [d.champion@agrosupdijon.fr](mailto:d.champion@agrosupdijon.fr)

<sup>b</sup>University of Birmingham UK, and CSIRO, Fellow

Most of the ingredients used in commercial complex food products are provided in powder form to reduce the cost of transportation, lengthen storage and reduce the risk of microbial growth. However, this induces the material to go through the glass transition: into a physical state where the powders are sensitive to slow ageing. At temperatures below the glass transition ( $T_g$ ), amorphous glassy materials are thermodynamically non-equilibrium systems, which tend to reach a lower energy state over extended period of time. Their physical properties, such as specific volume, enthalpy and entropy decrease towards lower values. This change in the thermodynamic properties of the glassy material is described as physical ageing or enthalpy relaxation. When heated after ageing, some amount of extra energy is relaxed in the form of enthalpy and this can readily be observed by differential scanning calorimetry (DSC). Thus, any glassy material stored at a temperature below its glass transition temperature experiences a structural relaxation that occurs as a function of time and temperature and moisture content. However, the amount of water in any food matrix differs as a function of water activity depending whether it is determined by water sorption or desorption. In fact, this hysteresis phenomenon may be the consequence of material evolution in the glassy state wherein molecular mobility is still enough to induce molecular reorganizations. The objective of this study is to investigate the influence of the ageing on different protein powders with a systematic study as a function time, of the powder enthalpy relaxation stored in different relative humidity conditions.

The results are discussed in terms of possible modulation of the water protein interactions with time depending on the previous storage conditions of powders and this evolution may induce loss of powder functionality.

# STATES AND MOTIONS OF WATER IN WHEAT BREAD UPON FREEZING AND FROZEN STORAGE

*Guo Chen and Jan Swenson*

Department of Applied Physics, Chalmers University of Technology, SE-412 96  
Göteborg, Sweden. [guoc@chalmers.se](mailto:guoc@chalmers.se), [jan.swenson@chalmers.se](mailto:jan.swenson@chalmers.se)

## Abstract

Formation of ice crystals causes quality deterioration of frozen bread, imposing a great challenge to the baking industry in fully harvesting the benefits of freezing bake-off technology for preserving bakery products. This unfavorable phase transition is mechanistically correlated to the activities of freezable water in the complex crumb and crust materials. In this study we investigated the activities of water and ice in wheat bread upon freezing and frozen storage using differential scanning calorimetry (DSC). Various frozen states were produced through freezing the fresh breadcrumb at different cooling rates (0.5-30 °C/min) to -30 °C. DSC heating traces from these states exhibited characteristically dual endotherms in the ice-melting region, which were attributed to two sources of ice crystals formed in the porous crumb [1]. The impact of frozen storage was further evaluated for the bread stored at -18 °C for a long term of ~4 months. The frozen bread incurred a considerable loss of the crumb water that migrated out and formed ice crystals on the bread surface. Within the frozen crumb water also underwent significant redistributions, resulting in an elevated crumb heterogeneity of freezable water. Such redistributions of freezable water were accompanied by a progressive recrystallization of the crumb-borne ice crystals, which were measured to grow into bulk sizes by the use of a modified calorimetric procedure. It is concluded that the activities of water, shaped by the complex crumb porosity, govern the formation and distribution of ice crystals and thus determine the storage performance of frozen bread.

## References

1. G. Chen, H. Jansson, K. F. Lustrup, and J. Swenson, *J. Cereal Sci.* 55, 279-284 (2012).

# CHALLENGES IN MULTI-HURDLES FOOD PRESERVATION AND ITS STABILITY

*Mohammad Shafiur Rahman*

Department of Food Science and Nutrition, Sultan Qaboos University, P. O. Box 34, Al-Khod 123, Oman. [shafiur@squ.edu.om](mailto:shafiur@squ.edu.om)

## Introduction

In predicting or modelling stability, two approaches could be used in identifying the boundary of the growth/no-growth, and then predicting the rate of growth or reaction within the growth or reacting regions. Fundamental based theoretical concepts of F-value (hurdle: sterilization), water activity (hurdle: water content) and glass transition (hurdle: glassy state) are the most successful in determining food stability during food processing and storage. These concepts consider only three most important hurdles of food preservation separately, while more than 60 hurdles may involve in food preservation. It is a challenge to food scientists and engineers to have unified concept or approach for determining food stability considering multi-hurdles. This paper will explain the concept of macro-micro region in the frame work of multi-hurdles and possible generic rules for individual hurdle alone.

## Concept of F-value, pH and salt

The commercial sterilization criterion states that the minimum thermal process should reduce initial micro-organism concentration by  $10^{12}$  [2]. This is well known as 12D concept or “botulinum cook”. The probability argument says, in 12D treatment there will be one spore in  $10^{12}$  cans [1,3]. It is generally accepted that the limiting pH of 4.6 provides a good margin of safety against the hazards of botulism in acidified foods, and such products are given only a mild heat treatment [4]. Salt should be 34.6% in order to thermodynamic limits to microbial life [5]. For this reason, most of the cases salt is used in combination of other hurdles [6].

## Concept of Water Activity and Glass Transition

In general the rules of water activity concept are: (i) food products are most stable at their ‘BET-monolayer’ content or “BET-monolayer water activity” and unstable above or below BET-monolayer; (ii) there are a critical water activity limit for a specific micro-organism or a class of micro-organism for their growth or toxin production, and biochemical reactions [7-9]. The rules of

the glass transition concept are: (i) the food is most stable at and below its glass transition (i.e.  $T_g$  or  $T_g'$ ), and (ii) the higher the  $T-T_g$  or  $T/T_g$  (i.e. above glass transition), the higher the deterioration or reaction rates [9-10, 12].

### State Diagram and Macro-Micro Region

The state diagram based of freezing curve and glass transition provided four macro-regions for determining food stability [10,11]. Using state diagram, Rahman [9] hypothesized 13 micro-regions having the highest to the lowest stability based on the location from the glass and BET-monolayer lines. For example, region-1 (relatively non-reacting zone, below the BET-monolayer line and glass line) is the most stable and region-13 (highly reacting zone, far from BET-monolayer line and glass line) is the least stable. The stability decreased as the zone number increased [11, 12].

### Future Challenges

Currently empirical approach is being used to identify the growth boundaries when multi-hurdles are used considering probabilistic model of Growth/No-Growth concept [13]. The future challenge is to progress the generic theory or guide lines of food stability combining multi-hurdles.

### References

1. R. Simpson, H. Nunez and S. Almonacid, S. Handbook of Food Process Design, First Edition. Ahmed, J. and Rahman, M. S. eds. Oxford: Willey-Blackwell, 2012, pp. 362-379.
2. C. R. Stumbo, Thermobacteriology in Food Processing, Second Edition. New York: Academic Press, 1973.
3. R. P. Singh, D. R. Heldman, D. R. Introduction to Food Engineering. Fourth Edition. Burlington: Academic Press, 2009.
4. M. H. Brown, I. R. Booth, I.R., Food Preservatives, Russell, N.J., Gould, G.W. Glasgow, 1991, pp. 22-43.
5. A. Oren, Environmental Microbiology 13, 1908-1923 (2011).
6. S. L. Komarik, D. K. Tressler, and L. Long, Food Products Formulary, Vol. 1. Komarik, S. L., ed., Westport: The AVI Publishing, 1974.
7. W. J. Scott, W. J. Australian J. Biol. Sci. 6, 549-564 (1953).
8. T. P. Labuza, S. R. Tannenbaum, M. Karel, Food Technol. 24 (5), 543-544 (1970).
9. M. S. Rahman, Int. J. Food Properties. 12(4), 726-740 (2009).
10. H. Levine, L. Slade, Carbohydrate Polymer. 6, 213-244 (1986).
11. M. S. Rahman, Trends Food Sci. Technol. 17, 129-141 (2006).
12. M. S. Rahman, J. Food Eng. 99(4), 402-416 (2010).
13. I. S. Boziaris, P. N. Skandamis, M. Anastasiadi, G. J. E. Nychas, J. Appl. Microbiol. 102, 796-8005 (2007).

## SOLVATION DYNAMICS OF BIV TAR RNA WITH AND WITHOUT TAT PEPTIDE

*Teena Goel<sup>a</sup>, Santosh Kumar<sup>a</sup> and Souvik Maiti<sup>a,b</sup>*

<sup>a</sup>Proteomics and Structural Biology Unit, Institute of Genomics and Integrative Biology, CSIR, Mall Road, New Delhi 110 007, India. [teenagoel24@gmail.com](mailto:teenagoel24@gmail.com)

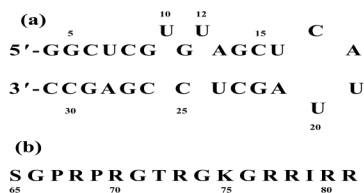
<sup>b</sup>National Chemical Laboratory, CSIR, Dr. Homi Bhabha Road, Pune 411008, India

### Introduction

Bovine immunodeficiency virus (BIV) is a lentivirus which causes lymphocytosis, progressive weakness, and central nervous system disorders in infected cattle. The interaction of Tat peptide with the Trans-activation Responsive (TAR) region of BIV viral transcripts is essential for the replication of the virus and any interference with these interactions hampers their pathogenicity. Hydration of biomolecules such as DNA, RNA, and proteins play important role in their structure, conformation, and function. The classical study on RNA hydration by Egli et. al. [1] described the regular network of water molecules surrounding the RNA duplex based on the X-ray synchrotron diffraction data as the systematic and well organized in both major and minor grooves. There are several studies on RNA hydration using NMR, osmotic stress measurements and molecular dynamics simulations [1-2]. In the present study, we use time resolved fluorescence technique to understand the dynamics of hydration layer surrounding the BIV TAR RNA in absence and presence of peptide binding. To our knowledge this is the first quantitative measurement of solvation dynamics of RNA structure.

### Experimental

The structure and sequence of (a) 28 base BIV TAR RNA and (b) 17-mer Tat peptide are shown in Fig.1. Fluorescence-labelled RNA contains 2-aminopurine (2-AP) incorporated at 11<sup>th</sup> position of the BIV TAR RNA from the 5' end of the RNA strand.



**Figure 1.** (a) Sequence and secondary structure of BIV TAR RNA, numbers correspond to the nucleotide position in BIV mRNA. (b) Sequence of BIV Tat peptide, numbering corresponds to the positions in intact protein.

Picosecond fluorescence decay measurements for BIV RNA in free and bound form were carried out using time-correlated single photon counting (TCSPC) technique. The instrument response function obtained to be 850 ps was detected at 310 nm excitation using a dilute solution of Ludox scatterer. Time resolved emission spectra (TRES) were generated from the fluorescence decay curves collected at different wavelengths spanning the entire emission band of the probe. The spectral-shift correlation function  $C(t)$  was obtained from TRES using the method as described by Maroncelli and Fleming [3].

## Results

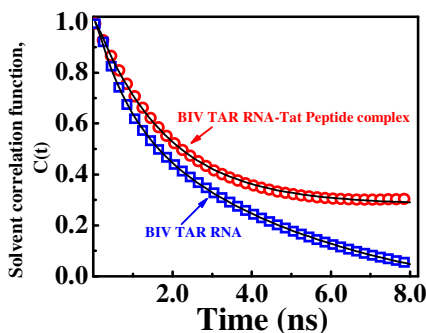
The Tat peptide is known to bind to the bulge region of the hairpin structure of TAR RNA. The quantum yields (Q.Y.) of the free BIV-2AP calculated using free riboside r2AP as the standard is obtained as 0.10, while binding to peptide leads to increase in its quantum yield upto 0.37. The amplitude weighted mean lifetimes ( $\tau_{\text{mean}}$ ) of BIV-2AP increases from 6.85 ns to 8.61 ns upon binding to the Tat peptide due to decrease in collisions of the probe with the solvent molecules or due to decrease in the stacking interactions with the neighboring bases. Using the values of mean lifetime and the quantum yield, the values of  $f_{\text{stacked}}$  for BIV-2AP and BIV-2AP-Tat complex are obtained as 0.67 and 0.15 respectively. The drastic decrease in the value of  $f_{\text{stacked}}$  clearly indicates the significant destacking of 2-AP upon peptide binding. The time dependent fluorescence Stokes' shift obtained from TRES for BIV-2AP and BIV-2AP-Tat complex is used to construct the normalized solvent correlation function,  $C(t)$ , defined as

$$C(t) = \frac{v(t) - v(\infty)}{v(0) - v(\infty)}$$

where  $v(0)$ ,  $v(t)$  and  $v(\infty)$  are the emission maxima (in  $\text{cm}^{-1}$ ) at time zero,  $t$ , and  $\infty$ , respectively.



Figure 2 shows the solvent correlation function of BIV-2AP and BIV-2AP-Tat complex that describes the microenvironment of solvent around 2-AP probe located in the TAR RNA and TAR-Tat complex, respectively.



**Figure 2.** Normalized spectral shift correlation function,  $C(t)$  for the probe 2-AP located in the BIV RNA with (box) and without TAT peptide (circle).

The solvation of BIV-2AP shows single exponential decay with the correlation time of 1.8 ns that arises possibly due to the hydration layer composed of water molecules hydrogen bonded to the RNA bases or due to the diffusion of water molecules between bound hydration layer and the bulk water. In presence of Tat peptide, the solvent correlation function of BIV-2AP shows two exponential fitting with the values of 0.81 ns and 5.4 ns. Due to destacking, the probe experiences faster diffusion between bound water layer and trapped water layer thus may give rise to faster solvent correlation time of 0.81 ns. The slower solvation correlation time (5.4 ns) arises due to relatively restricted motion of the water molecules hydrogen bonded to the RNA in presence of the peptide. Overall the solvent behavior becomes slower for TAR RNA in presence of Tat, which corroborates with the observed unfavorable entropy change during the peptide binding.

### References

1. M. Egli, S. Portmann, N. Usman, *Biochemistry* 35, 8489-8494 (1996).
2. E. Rozners, J. Moulder, *Nucleic Acids Res.* 32, 248-254 (2004).
3. M. Maroncelli, G.R. Fleming, *J. Chem. Phys.* 86, 6221-6239 (1987).

# ANALYSIS OF BULK AND HYDRATION WATER DURING THERMAL LYSOZYME DENATURATION

*G. Bellavia, A. Hédoux, L. Paccou, S. Achir and Y. Guinet*

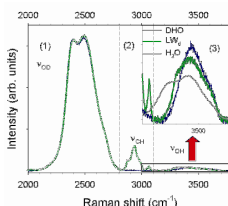
<sup>a</sup>UMET - UFR de Physique - BAT P5 UMR CNRS 8207, Université Lille 1, 59655 Villeneuve d'Ascq, France. [giuseppe.bellavia@univ-lille1.fr](mailto:giuseppe.bellavia@univ-lille1.fr)

## Introduction

Water has a fundamental role in protein stability and the solvent-accessible surface area (SASA) is a parameter intimately related to the native or denatured states of the protein. An experimental method to analyze the organization of bulk and hydration water, based on the detection of H/D isotopic exchange between the protein and hydration water by Raman spectroscopy is described for the model protein lysozyme.

## Experimental

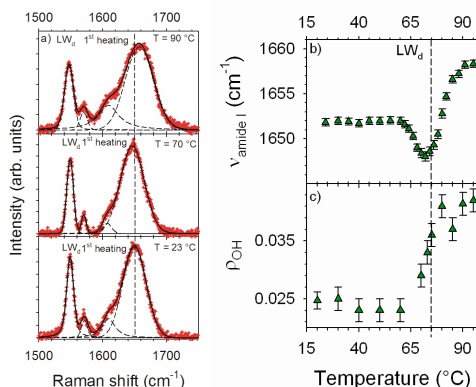
The Raman spectrum of lysozyme dissolved in D<sub>2</sub>O (LW<sub>d</sub>, 10 wt%) was taken in the 1500-3800 cm<sup>-1</sup> range and decomposed in two parts: (i) the Amide I region (1500-1800 cm<sup>-1</sup>); (ii) the 2000-3800 cm<sup>-1</sup> spectrum plotted in Fig.1, dominated by the O–D stretching bands. Isotopic exchanges between the protein and hydration water are responsible for the weak intensity detected in region (3) and corresponding to the dilute DHO spectrum, and then to the uncoupled  $\nu_{OH}$  spectrum [1] (Fig.1inset). A shoulder is detected on the low-frequency side (see Fig.1 inset) of the LW<sub>d</sub> spectrum, not observed in the dilute DHO solution, and therefore corresponding to OH groups of lysozyme not exposed to the solvent.



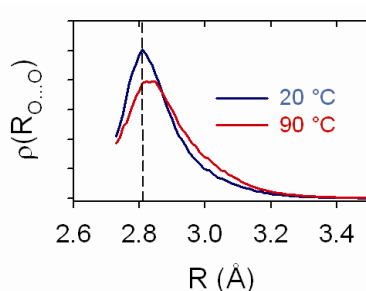
**Figure 1.** Description of the  $\tilde{O}D(H)$  stretching hydrated lysozyme. The spectrum of LW<sub>d</sub> can be divided in 3 regions corresponding to the following intramolecular stretching vibrations: (1)  $\tilde{O}D$  in bulk D<sub>2</sub>O, (2)  $\tilde{C}H$  in lysozyme and (3)  $\tilde{O}H$  in HDO molecules resulting from isotopic exchange. The LW<sub>d</sub> spectrum is compared to DHO and H<sub>2</sub>O in (3) in the inset.

## Results and Discussion

The solution was heated from 20 up to 95 °C. Fig.2 shows Amide I region vs. temperature, together with fitting results. A clear frequency downshift of Amide I band is observed, which was interpreted as reflecting enhanced NH/ND isotopic exchange, associated with the penetration of the solvent in the protein interior [2]. Above 70 °C, a frequency upshift is observed, usually interpreted as  $\alpha$ -helix unfolding process. If we adopt the consideration that the protons exchanges from lysozyme to D<sub>2</sub>O quickly diffuse in bulk heavy water molecules, the intensity ratio between OH and OD stretching bands represents the proportion of water molecules involved in the isotopic exchange. Then the ratio  $\rho_{OH} = I_{OH}/I_{OD} = 0.0246 \pm 0.0015$  is obtained at  $T_{room}$ . This indicates that a lysozyme molecule is surrounded by a bound layer of  $198 \pm 10$  water molecules in the native state.  $\rho_{OH}$  can be then considered as directly representative parameter of the SASA and  $\rho_{OH}(T)$  (see Fig.2c) was interpreted as reflecting the changes in the SASA. It is firstly observed that  $\rho_{OH}$  increases between 60 and 75 °C, as the frequency of amide I band decreases. This confirms the solvent penetration within the tertiary structure. At  $\sim 73$  °C, the number of water molecules bound to the protein in the molten globule state was estimated to  $260 \pm 13$ , indicating the increase of the SASA by a factor 1.3. Secondly, upon further heating above 70 °C, an additional increase of  $\rho_{OH}$  is observed, when the protein denatures and the SASA estimation is 1.75 times greater.



**Figure 2.** Temperature dependence of amide 1 band; a) Raman spectra b)  $\omega(T)$  compared to c)  $\rho_{OH}(T)$  curves.



**Figure 3.** Distribution of O...O distances between D<sub>2</sub>O molecules in bulk water in LW<sub>d</sub> solution at 20 and 90 °C.

Using the uncoupled OH stretching spectrum and the correlation function between O...O distances and the frequencies of OH stretching [3], we can obtain the distribution of O...O distances between water molecules in bulk, plotted in Fig.3 at 20 and 90 °C. Same experiments were performed in presence of trehalose (T). D<sub>2</sub>O+T solution was previously freeze-dried, in order to substitute OH groups by OD. We obtain crucial information on the influence of trehalose on the bulk water, the hydration water and the SASA during the lysozyme denaturation, in relation with the unfolding process monitored by the temperature behavior of the amide I band.

### References

1. T. T. Wall and D. F. Hornig, J. Chem. Phys. 43, 2079-2087 (1965).
2. A. Hédoux, Y. Guinet, L. Paccou, J. Phys. Chem. B 115, 6740-6748 (2011)
3. J. R. Scherer, M.K. Go, S. Kint, J. Phys. Chem. 78, 1304-1313 (1974)

# AQUEOUS SOLUTIONS OF LYSOZYME AND DISACCHARIDES: FOCUS ON CRYOPROTECTION

*Paola Gallo*

Dipartimento di Fisica, Università Roma Tre – Via della Vasca Navale 84,  
I-00146 Roma, Italy. [gallop@fis.uniroma3.it](mailto:gallop@fis.uniroma3.it)

## Introduction

Over the past twenty years, aqueous solutions of carbohydrates have increasingly attracted the attention of scientists. A series of studies have pointed out the extraordinary biopreservation ability of sugars, particularly of trehalose. In fact, this common disaccharide of glucose has been found to be especially effective in preserving membranes structure and in preventing proteins from denaturation at very low water content. It has also been demonstrated that trehalose stabilizes living cells subjected to freezing stresses. These evidences have paved the way to the several and diversified industrial applications of trehalose, which is currently used in pharmaceutical formulations as an eccipient, in food industry to preserve the flavor of dried products, in many cosmetic lines and importantly in cryogenic technology.

In spite of this great number of uses, the microscopic mechanism responsible for the ability of trehalose, and sugar in general, as bioprotectant is still unclear and several hypothesis are currently under debate.

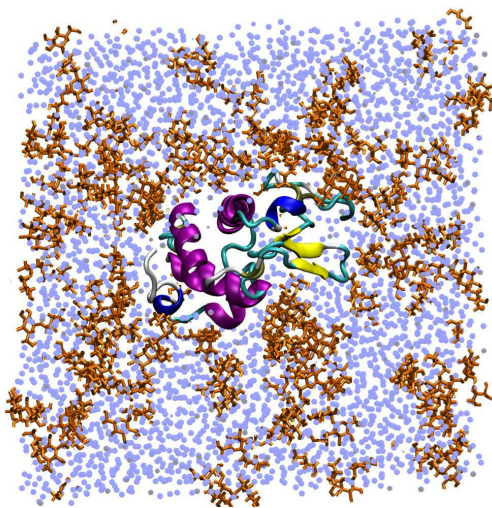
## Results

Molecular dynamics simulations of a solution of water and trehalose, water and maltose and water, trehalose and lysozyme are studied upon supercooling.

Dynamics of water in presence of trehalose and maltose shows two well distinct relaxations. In particular, the fraction of water molecules interacting with the sugar has a completely different dynamical behavior with respect to that of bulk-like water molecules. The analysis of the dynamic of hydration water molecules allows to comprehend the differences between trehalose and maltose aqueous solutions and the microscopic mechanisms that causes a greater capability of trehalose in preserving biomolecules upon cooling [1].

The analysis of the dynamics of water and trehalose around the center of mass of the lysozyme, see the system in Fig.1, indicates the formation of a layer of trehalose containing slow water molecules around the protein. The amount of water between the protein and the trehalose layer progressively

increases when the temperatures is lowered. These results point to a protective action exerted by packing slow water between the protein and a layer of trehalose molecules, accompanied by a global slowing down of water dynamics due to the presence of trehalose [2].



**Figure 1.** Lysozyme in an aqueous solution of water and trehalose.

### **References**

1. A. Magno and P. Gallo, *J. Phys. Chem. Lett.* 115, 1461 (2011)
2. D. Corradini, E. G. Strelakova, H. E. Stanley and P. Gallo, in preparation (2012).

# HOW STRONGLY DOES TREHALOSE INTERACT WITH LYSOZYME AT LOW WATER CONTENTS? INSIGHTS FROM MOLECULAR DYNAMICS SIMULATION AND INELASTIC NEUTRON SCATTERING

*Adrien Lerbret<sup>a</sup>, Frédéric Affouard<sup>b</sup>, Alain Hédoux<sup>b</sup>, Stephanie Krenzlin<sup>c</sup>, Jürgen Siepmann<sup>c</sup>, Marie-Claire Bellissent-Funel<sup>d</sup> and Marc Descamps<sup>b</sup>*

<sup>a</sup>AgroSup Dijon, 1 Esplanade Erasme, 21000 Dijon, France.

[adrien.lerbret@u-bourgogne.fr](mailto:adrien.lerbret@u-bourgogne.fr)

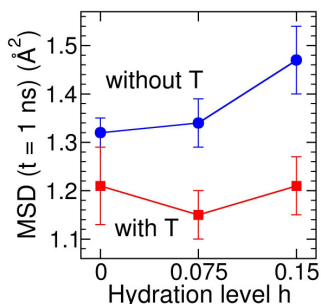
<sup>b</sup>Unité Matériaux Et Transformations, UMR CNRS 8207-Université Lille 1, 59655 Villeneuve d'Ascq Cedex, France.

<sup>c</sup>INSERM U1008, Controlled Drug Delivery Systems and Biomaterials, 3 rue du Professeur Laguesse, 59006 Lille, France.

<sup>d</sup>Laboratoire Léon Brillouin, UMR 12 CEA-CNRS, CEA-Saclay, 91191 Gif-sur-Yvette, France.

Recent advances in molecular biology and recombinant technology have made available a growing number of therapeutic proteins for the treatment of cancers, diabetes or brain diseases. These proteins are usually very labile in solution and are therefore conserved in glassy matrixes composed of stabilizing excipients and a residual amount of water, which both control their long-term stability, and thus their potential use in medical treatments [1]. To shed some light on the protein-matrix interactions in such systems, we performed molecular dynamics (MD) simulations on amorphous matrixes of (i) the model globular protein lysozyme (L), (ii) the bioprotectant trehalose (T), and (iii) the 1:1 (in weight) lysozyme/trehalose mixture (LT), at hydration levels  $h$  of 0.0, 0.075 and 0.15 (in g of water/g of protein or sugar) [2]. We also supplemented these simulations with complementary inelastic neutron scattering (INS) experiments on the L, T and LT lyophilized (freeze-dried) samples [2]. The computed densities and free volume distributions indicate that trehalose improves the molecular packing of the LT glass with respect to the L one. Accordingly, the low-frequency vibrational density of states (VDOS) and the mean-square displacements (MSDs) of lysozyme (Figure 1) reveal that it is less flexible – and thus less likely to unfold – in presence of trehalose. Furthermore, at low contents ( $h=0.075$ ), water systematically stiffens the vibrational motions of lysozyme and trehalose, whereas it increases their MSDs on the nanosecond (ns) time scale. This stems from the hydrogen bonds (HBs) that lysozyme and trehalose form with water, which, interestingly, are stronger than the ones they form with each other,

but which, nonetheless, relax faster on the ns time scale, given the larger mobility of water. Moreover, lysozyme interacts preferentially with water in the hydrated LT mixtures, in agreement with the preferential hydration hypothesis [3], and trehalose appears to slow down significantly the relaxation of lysozyme-water HBs, in line with its well-known retardation effect on the dynamics of water [4]. Overall, our results suggest that the stabilizing efficiency of trehalose in solid matrixes arises from its ability to (i) increase the number of HBs formed by proteins in the dry state and (ii) make stable on long (> ns) time scales the HBs formed by water with proteins.



**Figure 1.** Mean square displacement (MSD) at the nanosecond time scale of the hydrogens of lysozyme in the absence (L matrix) or in the presence of trehalose (LT matrix) as a function of the hydration level  $h$  (in g of water/g of protein or sugar) determined from MD simulations.

### References

1. W. Wang, *Int. J. Pharm.* 203, 1-60 (2000).
2. A. Lerbret, F. Affouard, A. Hédoux, S. Krenzlin, J. Siepman, M.-C. Bellissent-Funel, M. Descamps, to be submitted to *J. Phys. Chem. B* (2012).
3. S. N. Timasheff, *Biochemistry* 41, 13473-13482 (2002).
4. M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D. M. Leitner, M. Havenith, *J. Am. Chem. Soc.* 130, 5773-5779 (2008).



## DO WE UNDERSTAND THE DEHYDRATION PROCESS?

*R. Heyd<sup>a</sup>, G. Lelong<sup>b</sup>, J. Fichot<sup>a</sup>, C. Josserand<sup>c</sup>, I. Chourpa<sup>d</sup>, E. Gombart<sup>e</sup>, J-F Tranchant<sup>e</sup>, G. Charalambopoulou<sup>f</sup>, T. Steriotis<sup>f</sup>, A. Brandt<sup>g</sup>, A. Rampino<sup>i</sup>, J. W. Brady<sup>h</sup>, A. Cesàro<sup>j</sup> and M-L Saboungi<sup>a</sup>*

<sup>a</sup>CRMD, CNRS & Université d'Orléans, 1b rue de la Férollerie, 45071 Orléans La Source, France. [rodolphe.heyd@cnrs-orleans.fr](mailto:rodolphe.heyd@cnrs-orleans.fr)

<sup>b</sup>Institut de Minéralogie et Physique des Milieux Condensés, Université Pierre et Marie Curie, CNRS-UMR 7590, Paris, France.

<sup>c</sup>Institut D'Alembert, CNRS & UPMC, Université Paris VI, UMR 7190, Paris, France.

<sup>d</sup>Université François Rabelais, EA4244 Physico-Chimie des Matériaux et des Biomolécules, Group Nanovecteurs Magnetiques pour la Chimiothérapie, 31 avenue Monge, Tours, F-37200, France.

<sup>e</sup>LVMH Recherche, 185 avenue de Verdun 45800 Saint Jean de Braye, France.

<sup>f</sup>National Centre for Scientific Research "Demokritos", Athens, Greece.

<sup>g</sup>Helmholtz-Zentrum Berlin für Materialien und Energie, Lise-Meitner Campus, Berlin, Germany.

<sup>h</sup>Department of Food Sciences, Cornell University, Ithaca, New York 14853, USA.

<sup>i</sup>Laboratory for Physical and Macromolecular Chemistry, Dept. of Life Sciences, University of Trieste, Via Giorgieri 1, 34127 Trieste, Italy.

Dehydration involves interesting fundamental principles of physics and is important for many technologies ranging from food, arts, medicine and cosmetics. Drying kinetics depend on several mechanisms such as diffusion, convection, Marangoni effects and contact line motion. These processes are also implicated in the protection of living organisms under conditions of extreme dehydration

We are studying the drying kinetics of complex materials covering several scales from microscopic to macroscopic. For example, polymeric formulations for cosmetics are an example of the former and plant and food systems of the latter. Although the kinetics involve different mechanisms, they share crucial key parameters such as the chemical activity of water and its diffusivity through the residual matrix. In both cases we observe that the initial chemical composition has a strong impact on the morphology of the final dry product.

Drying of such complex systems has been explored by a combination of experimental techniques including *in-situ* gravimetric measurement in controlled atmosphere, optical microscopy and Raman confocal microspectroscopy, micro-rheology by laser light scattering, small-angle neutron scattering, atomic force microscopy and water activity measurements. Numerical modeling complements the experimental approach and allows a

further understanding of the multiple steps involved in the dehydration process.

This talk will illustrate two recent examples of our work: the complex drying dynamics of polymeric mixtures [1], and the dehydration of aqueous silica gels with and without the presence of sugar molecules [2].

### **References**

1. J. Fichot, R. Heyd, C. Josserand, I. Hourpa, E. Gombart, J.-F. Tranchant, and M.-L. Saboungi, submitted to Phys. Rev. Letters (2012).
2. G. Lelong, R. Heyd, G. Charalambopoulou, Th. Steriotis, A. Brandt, K. Beck, M. Vayer, D. L. Price, J.W. Brady and M.-L. Saboungi, J. Phys. Chem. C , 116(17):9481–9486, (May 2012).

## **ANOMALOUS PROTON DYNAMICS OF WATER IN NEURAL TISSUE AS SEEN BY QUASI-ELASTIC NEUTRON SCATTERING. IMPACT ON MEDICAL IMAGING TECHNIQUES.**

*F. Natali<sup>a,b</sup>, Y. Gerelli<sup>b</sup>, C. Stelletta<sup>c</sup>, J. Peters<sup>b,d,e</sup> and J. Ollivier<sup>b</sup>*

<sup>a</sup>CNR-IOM, OGG, 6 rue Jules Horowitz, 38042 Grenoble Cedex 9, France. [natali@ill.fr](mailto:natali@ill.fr)

<sup>b</sup>Institut Laue-Langevin, 6 rue Jules Horowitz, 38042 Grenoble Cedex 9, France

<sup>c</sup>Dep. of An. Med., Prod. & Health, Univ. of Padova, Viale dell'Università 16, 35020 Agripolis, Legnaro (Padova), Italy

<sup>d</sup>Univ. Joseph Fourier, 38041 Grenoble, France

<sup>e</sup>Inst. Biol. Str. J. P. Ebel, 41 rue J. Horowitz, 38027 Grenoble, France

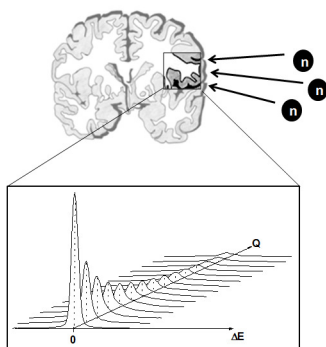
Brain tissues are heterogeneous systems containing numerous compartments: glia cells, neurons, neuronal fibers, myelin sheaths and extracellular space. The major tissue constituent is water (>70%) which, besides its key role in all life processes, can also be used as a tool to probe neuronal tissue morphology at microscopic scale, through continuous interactions with cell membranes during their random motion [1]. On the other hand, the heterogeneity of the cell structure in its macromolecular composition, well visible at the micron scale, influences the estimation of the water diffusion, acting as an obstruction. This effect is even more pronounced in tissue samples where different types of cells coexist.

We propose here the investigation of water diffusion in brain, for the first time spotlighted at atomic scale, i.e. at the scale experiencing macromolecular separation, using neutron spectroscopy. The results are relevant for improving the modelling of the physics driving intra- and extracellular water diffusion in brain, with evident benefit for modern medical diagnostic technologies based on water diffusion processes, such as the diffusion (DMRI) and diffusion kurtosis magnetic resonance imaging (KDMRI) techniques, nowadays widely used to reveal and characterize a number of brain pathologies on the micrometric scale (ischemia, tumors and, recently, inherited prion disease) [2].

From the elementary building blocks of the cell (proteins, membranes etc) to bacteria and in vivo cells, neutrons have proven to be unique in pinpointing proton dynamics at atomic scale in biological compounds regardless of the macromolecular complexity involved.

As no radiation damaging effects have to be feared, neutrons are an ideal probe for the study of the dynamical properties of biological samples. Moreover, as protons' (H) incoherent neutron cross section is extremely high when compared to the other atoms (differing by almost 2 orders of magnitude), neutron scattering overcomes the limits of X-ray based

techniques in which light atoms, such as H, are masked by other contributions. This makes neutron scattering a privileged tool for the investigation of H-rich samples, such as biology-related molecules, in which the typical composition includes almost 50 % of H atoms. So far, the domain of biological fields studied with neutrons spans from the single amino acid (the smaller component of the protein primary sequence) [3] to in vivo cells [4-6], suggesting that water dynamics is different on micrometric and atomic scales.



**Figure 1.** Bovine brain tissue exposed to neutron beam and resulting QENS spectra measured at room temperature; the different scattering contributions, convoluted with the instrument resolution, are mainly due to a) rotational motion of  $\text{CH}_2$  groups observed in protein amino-acids and lipids and b) rotational and translational motions experienced by water molecules in intra- and extra-cellular spaces.

### References

1. D. Le Bihan, Bull. Mem. Acad. R. Med. Belg. 163(1-2), 105 (2008).
2. H. Hyare, S. Wroe, D. Siddique, T. Webb, N. C. Fox, J. Stevens, J. Collinge, T. Yousry, J. S. Thornton, Neurology 74(8), 658 (2010).
3. G. Schiró, C. Caronna, F. Natali, A. Cupane, J. Am. Chem. Soc. 132, 1371 (2010).
4. A. Frolich, F. Gabel, M. Jasnin, U. Lehnert, D. Oesterhelt, A. M. Stadler, M. Tehei, M. Weik, K. Wood, G. Zaccai, Faraday Discuss. 141, 117 (2009).
5. M. Jasnin, M. Moulin, M. Haertlein, G. Zaccai, M. Tehei, EMBO Rep. 9, 543 (2008).
6. A. M. Stadler, J.P. Embs, I. Digel, G.M. Artmann, T. Unruh, J. Am. Chem. Soc. 130, 16852 (2008).

# WATER MOBILITY, DENATURATION AND THE GLASS TRANSITION IN PROTEINS

*David Porter and Fritz Vollrath*

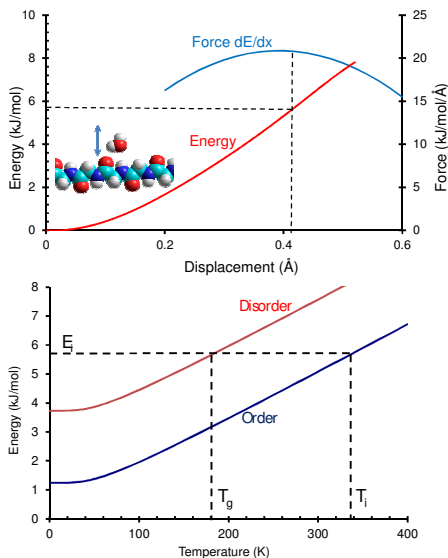
Department of Zoology, University of Oxford, South Parks Road,  
Oxford OX1 3PS, UK. [david.porter@zoo.ox.ac.uk](mailto:david.porter@zoo.ox.ac.uk)

## Introduction

Two key transition temperatures or conditions are associated with biological activity in proteins. The onset of activity in a protein is attributed to the protein-water glass transition,  $T_g$ , at about 200K, and the loss of activity usually occurs through denaturation,  $T_i$ , at about 333K. This work suggests that these two processes are due to a single water-protein instability mechanism, at which point the interacting molecules becomes mobile [1,2]. After predicting the two transitions using 'first principle' quantum mechanics simulations of the water-protein hydrogen bond energy instability, we will show that observations of thermodynamics and kinetics support the hypothesis of a common mechanism. The energetic instability approach provides a consistent mechanism for important biological processes such as protein function, refolding and aggregation as the conformational constraints of strong water-amide bonds are changed with increasing molecular mobility.

## Mobility and Transition Model

At the simplest level, a structural transition occurs at the onset of mobility of molecules relative to each other. This happens when the intermolecular bond stiffness tends to zero or force is a maximum. We calculate this condition for hydrogen bonded interactions between water molecules and the backbone chain amide groups in the protein using density functional theory predictions of energy as a function of bond length, as shown in Figure 1a. The  $T_g$  and  $T_i$  transitions are then differentiated by means of the different zero point energy values for disordered and ordered interactions, which are calculated from their quantised values of bond vibrational energy. The two energy values for the transitions are then translated into transition temperature values by means of the thermal energy needed to attain the transition condition from the two zero point energy values, as shown also in Figure 1b. Predictions are in good general agreement with observed transition temperature values, with a clear physical mechanism.



**Figure 1.** Potential energy well to calculate the instability energy of water-protein interactions and the translation of energy to transition temperatures  $T_g$  and  $T_i$  using the order-disorder energies.

If the transition represents an onset of molecular mobility, the associated degrees of freedom can be quantified as six for each water molecule for both  $T_g$  and  $T_i$ , which agrees with observations of the thermodynamics of both transitions using DSC. In addition, the activation energy for the transitions can be translated into kinetics using an Arrhenius relation using parameters predicted in the energy simulations.

## Discussion

The implications of the model are important, in that it predicts a temperature window for protein function and a mechanism for refolding, since constraints on torsional angles in the protein chain backbone must be released as the protein-water bonds become mobile above  $T_i$ . Similarly, aggregation can proceed once bonded water no longer inhibits stronger interchain hydrogen bonding. We also suggest that protein-water order is essential for electronic function in proteins due to ferroelectric domains in the hydration shell.

## References

1. D. Porter and F. Vollrath, *Biochim. et Biophys. Acta* 1824, 785-791 (2012).
2. D. Porter, F. Vollrath, *Soft Matter* 4, 328-336 (2008).

## TEMPERATURE AND COMPOSITION EFFECTS ON PROTEIN HYDRATION. INSIGHT ON THERMOSTABILITY

*F. Sterpone, R. Obaidur, M. Kalimeri, D. Laage and S. Melchionna*

Laboratoire de Biochimie Theorique, IBPC, 13 rue Pierre et Marie Curie, Paris 75005.  
[fabio.sterpone@ibpc.fr](mailto:fabio.sterpone@ibpc.fr)

Proteins from (hyper)-thermophilic organisms exhibit an extraordinary stability being able to function at very high temperature (up to 100 °C) [1]. The microscopic origin of such a resistance to thermal stress is still unknown. For many years the internal packing of the protein hydrophobic core was seen as the key factor for protein stability. However, recently, the attention has shifted toward surface effects, e.g. electrostatic interactions and coupling with the solvent [2].

Here we present a systematic analysis of protein hydration for a pair of homologue proteins (the g-domain from EF-Tu) from a mesophilic and a hyperthermophilic organism based on computer simulations.

We show that the average water dynamics at the protein surface is not affected by the different composition of the two proteins and we provide a rational basis for this result considering that the main perturbative contribution to water dynamics is an excluded volume effect.

Even if the average dynamics is rather insensitive to different protein composition among the homologues this does not suffice to rule out the role of hydration on protein thermostability. We will then briefly report on other key aspects: the localized behavior of hydration during unfolding, the role of surface hydrogen bond net-working [4], and the role of water on electrostatic local interactions (ion-pair).

### **References**

1. C. Vieille, G.J. Ziekus, *Microbiol. Mol. Biol. Rev.* 65, 1 (2001).
2. F. Sterpone, S. Melchionna, *Chem. Soc. Rev.* 41, 1665-1676 (2012).
3. F. Sterpone, G. Stirnemann, D. Laage, *J. Am. Chem. Soc.* 134, 4116-4119 (2012).
4. F. Sterpone, C. Bertonati, G. Briganti, S. Melchionna, *J. Phys. Chem. B*, 113, 131-137, (2007).





**POSTER PRESENTATIONS**



*Stefano A. Codrial*

Acquedotto – Perugia (Ink)  
*Courtesy of the Artist Stefano Alunni Cardinali*  
[WWW.STUDIOARTEAPPIA.IT](http://WWW.STUDIOARTEAPPIA.IT)

## WATER TRANSPORT DURING FREEZING OF CELLS IN THE PESENCE OF CRYOPROTECTIVE AGENTS

*Maryam Akhoondi<sup>a</sup>, Harriëtte Oldenhof<sup>b</sup>, Harald Sieme<sup>b</sup> and Willem F. Wolkers<sup>a</sup>*

<sup>a</sup>Institute of Multiphase Processes, Leibniz Universitaet Hannover – Callinstrasse 36, 30167 Hannover, Germany. [akhoondi@imp.uni-hannover.de](mailto:akhoondi@imp.uni-hannover.de)

<sup>b</sup>Clinic for Horses - Unit for Reproductive Medicine, University of Veterinary Medicine Hannover – Buenteweg 15, 30559 Hannover, Germany

In order to predict optimal cooling rates for cryopreservation of cells, the cell specific membrane hydraulic permeability and corresponding activation energy for water transport need to be experimentally determined. We have used Fourier transform infrared spectroscopy (FTIR) and cryomicroscopy to study mouse embryonic fibroblast (3T3) cells during freezing and to derive the subzero membrane hydraulic permeability and activation energy for water transport. Coulter counter measurements were used to determine the suprazero membrane hydraulic permeability parameters.

The activation energy for water transport in the ice phase is about three fold greater compared to that at suprazero temperatures [1]. Cellular dehydration as observed by cryomicroscopy precedes dehydration of the bound water surrounding the phospholipid head groups as observed by FTIR. Both DMSO and glycerol increase the membrane hydraulic permeability at subzero temperature and reduce the activation energy for water transport [2]. Cryoprotective agents facilitate dehydration to continue at low subzero temperatures thereby decreasing the incidence of intracellular ice formation. The increased subzero membrane hydraulic permeability likely plays an important role in the cryoprotective action of DMSO and glycerol. In the presence of DMSO water permeability was found to be greater compared to that in the presence of glycerol. Two temperature regimes were identified in an Arrhenius plot of the membrane hydraulic permeability. The non-linear Arrhenius behavior of  $L_p$  has been implemented in the water transport model to simulate cell volume changes during freezing. At a cooling rate of 1 °C/min, ~5% of the initial osmotically active water volume is trapped inside the cells at -30 °C.

### **References**

1. M. Akhoondi, H. Oldenhof, C. Stoll, H. Sieme and W. F. Wolkers, *Biochim. Biophys. Acta* 1808, 642–648 (2011).
2. M. Akhoondi, H. Oldenhof, H. Sieme, W.F. Wolkers, *Mol. Mem. Biol.* DOI:10.3109/09687688.2012.699106 (2012).

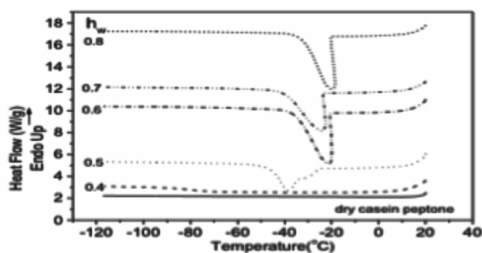
## GLASS TRANSITION AND WATER DYNAMICS IN AQUEOUS SOLUTIONS OF CASEIN PEPTONE

*D. Aravopoulou, A. Panagopoulou, A. Kyritsis and P. Pissis*

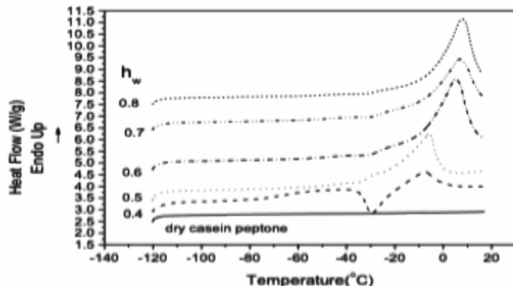
National Technical University of Athens, Zografou Campus, 15780, Athens, Greece.

[dnsaravopoulou@gmail.com](mailto:dnsaravopoulou@gmail.com)

We present a study on the glass transition and water dynamics in the case of aqueous solutions of highly water soluble casein peptone, a digest derivative of the protein casein. Differential Scanning Calorimetry (DSC) was used for the study of glass transition and crystallization effects. Also Dielectric Relaxation Spectroscopy (DRS) was used to analyze dynamics.



**Figure 1:** Heat Flow thermogram, normalized to water fraction, during cooling for a dry casein peptone compressed pellet and several aqueous casein peptone solutions of water fraction  $h_w$  indicated on the plot

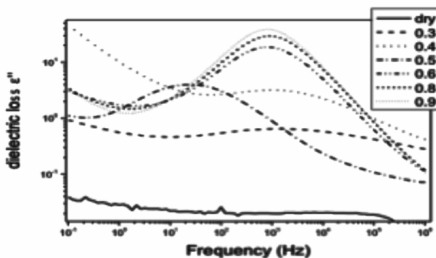


**Figure 2:** Heat Flow thermogram, normalized to water fraction, during heating for a dry casein peptone compressed pellet and several aqueous casein peptone solutions of water fraction  $h_w$  indicated on the plot.

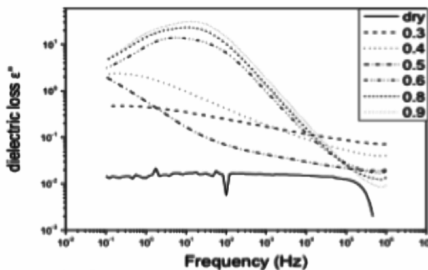
The glass transition of the solutions and the crystallization and melting events of water have been studied by differential scanning calorimetry (DSC). No water crystallization effects during cooling were recorded for the solution of water fraction  $h_w=0.4$  (grams of water per grams of solution), (Figure 1).

The glass transition temperature ( $T_g$ ) has been recorded for all the solutions, but was absent for the dry casein peptone (at least within the temperature range of our measurements), which was measured for comparison. The  $T_g$  has been found at about  $-65^\circ\text{C}$  for  $h_w=0.4$  and at about  $-50^\circ\text{C}$  for higher  $h_w$ . The heat capacity step ( $\Delta C_p$ ) of

the glass transition seems to be enhanced for the solution of water fraction  $h_w=0.4$ , compared to the solutions of higher  $h_w$ , where crystallization of water occurs during cooling (Figure 1). The calorimetric data have been analyzed and the values of  $T_g$  during heating,  $\Delta C_p$ , crystallization temperature,  $T_c$ , melting temperature,  $T_m$ , crystallization enthalpy,  $\Delta H_c$ , have been calculated, together with the fractions of crystallized and uncrystallized water,  $X_{cw}$ ,  $X_{ucw}$ , respectively, according to [1]. After an isothermal scan during heating at  $T=-30^\circ\text{C}$ , which is in the region of the cold crystallization phenomena during heating (figure 1) and below the respective melting temperature, a subsequent cooling and heating scan, reveals the increase of the  $T_g$  from  $-63^\circ\text{C}$  to  $-50^\circ\text{C}$  and the significant reduction of the  $\Delta C_p$ . This fact implies that the amount of uncrystallized water does contribute to the  $T_g$  and the nominal water fraction in the two phase system is now lower. In combination with the fact that the  $T_g$  is not detectable for the dry sample, it becomes clear that the glass transition of the system is originated from the combined motion of uncrystallized water molecules and casein peptone, as it is suggested in several globular proteins [1,2]



**Figure 3:** Comparative dielectric loss  $\epsilon''$  spectra for  $T=-90^\circ\text{C}$  for a dry casein peptone compressed pellet, a hydrated pellet ( $h_w=0.3$ ) and several aqueous casein peptone solutions of water fraction  $h_w$  indicated on the plot.



**Figure 4:** Comparative dielectric loss  $\epsilon''$  spectra for  $T=-130^\circ\text{C}$  for a dry casein peptone compressed pellet, a hydrated pellet ( $h_w=0.3$ ) and several aqueous casein peptone solutions of water fraction  $h_w$  indicated on the plot.

Water dynamics has been studied by broadband dielectric spectroscopy (DRS) technique. Figures 3 and 4 show an example of the recorded dielectric loss,  $\epsilon''$ , versus frequency, at  $T=-90^\circ\text{C}$  and  $T=-130^\circ\text{C}$  respectively. The dry casein peptone sample did not present a pronounced dielectric response (Figures 3,4). The dielectric loss peaks corresponding to the solutions in Figures 3 and 4, are mainly connected to water dynamics. It is interesting that the maximum of the peak for  $h_w=0.4$ , which corresponds to the reorientation of uncrystallized water molecules in the mixture [1], moves to lower frequencies and the peak changes in shape for

$h_w=0.5$ , where crystallization of water occurs during cooling. The dynamics of uncrystallized water becomes “slower” due to the presence of ice. For the solutions of higher  $h_w$  a more intense peak saturates in position and increases in magnitude, with increasing  $h_w$ . In the case of partially crystallized solutions, relaxations due to ice are expected to contribute to the total dielectric loss [3].

From the data received we calculated the Arrhenius plot. For  $h_w=0.3,0.4$  we observe a secondary relaxation of water which stays stable in position and presents a similar activation Energy  $E_a$  to the  $\nu$  relaxation of water [4]. For  $h_w=0.5$  the ucw relaxation becomes slower. We saw in DSC (Figure 1) that 0.5 sample presents crystallization of water during cooling. In DSC also we saw that the ucw fraction initially decreases until a minimum value at  $h_w=0.6$  and then increases again. At this water fraction region a reorganization of water occurs in the system and a decrease of activation energy  $E_a$  is observed for higher  $h_w$  [3].

### References

1. A. Panagopoulou, A. Kyritsis, R. Sabater I Serra, J.L. Gómez Ribelles, N. Shinyashiki, P. Pissis, BBA, 1814 (2011) 1984-1996.
2. A. Panagopoulou, A. Kyritsis, A.-M. Aravantinou, D. Nanopoulos, R. S. i Serra, J. L. Gómez Ribelles, N. Shinyashiki, P. Pissis. , 6, (2011) 199-209.
3. N. Shinyashiki, W. Yamamoto, A. Yokoyama, T. Yoshinari, S. Yagihara, K.L. Ngai, S. Capaccioli, J. Phys. Chem. B., 113 (2009) 14448-14456.
4. A. Panagopoulou, A. Kyritsis, N. Shinyashiki, P. Pissis, J. Phys. Chem. B, 116 (2012) 4593- 4602

### Acknowledgements

D. Aravopoulou and P. Pissis would like to acknowledge the Scientific Programme for Basic Research, PEBE 2010, of the National Technical University of Athens, for funding.

## HIGH-RESOLUTION TANDEM FABRY-PEROT INTERFEROMETER FOR ULTRA-VIOLET BRILLOUIN SCATTERING MEASUREMENTS

*A. Battistoni<sup>a</sup>, F. Bencivenga<sup>b</sup>, D. Fioretto<sup>c</sup> and C. Masciovecchio<sup>b</sup>*

<sup>a</sup>Physics department, University of Trieste, via A. Valerio n.2,1 34127 Trieste, Italy.  
[andreabat@gmail.com](mailto:andreabat@gmail.com)

<sup>b</sup>Sincrotrone Trieste S.C.p.A., Strada Statale 14 - km 163,5 in AREA Science Park, 34149 Basovizza, Trieste, Italy

<sup>c</sup>Physics department, University of Perugia, via Alessandro Pascoli, I-06123 Perugia, Italy

We present the features of a new high-resolution tandem Fabry-Perot interferometer for Ultra-Violet (UV) Brillouin scattering measurements. The use of this new table top setup, equipped with a tunable scattering angle, enables us to investigate the momentum-energy (Q-E) region between  $10^2$  and  $10^{-1} \text{ nm}^{-1}$ , and between  $10^{-3}$  and  $10^{-2} \text{ meV}$ , previously accessible only by large scale facilities. Dissipation of longitudinal acoustic modes as a function of Q and E is reported, giving information on relaxation of density fluctuations in prototypical liquids, like water, glycerol and water-peptide solutions.

## WATER EFFUSION AS A PROBE FOR DRUG RELEASE FROM HYDROGELS

*B. Bellich, M. Borgogna, A. Rampino, M. Cok and A. Cesàro*

*Dept. Life Sciences, University of Trieste, Via Giorgieri - 34127 Trieste, Italy.  
[bbellich@units.it](mailto:bbellich@units.it)*

### Introduction

The interest in hydrogels relies on their valuable properties for applications in pharmaceutical and biomedical field. Due to their safety and biodegradability, polysaccharide represents interesting biomaterials. Among polyelectrolytes, alginate is one of the most employed. Diffusional properties of alginate gel have been extensively studied [1]. The network structure of hydrogels is essentially characterized by the polymer volume fraction in the swollen state, molecular weight of the polymer chain between two neighbouring crosslinking points and the corresponding mesh size [2]. In particular, mesh size depends on degree of crosslinking and chemical structure of the monomers. Typical mesh size for biomedical hydrogels ranges from 5 to 100 nm, therefore diffusion of small molecules is totally unaffected. On the contrary, diffusion of peptides, proteins or large macromolecules are strongly influenced by the mesh size of the matrix [3]. Many factors influence the properties of the gel and the drug release behaviour, including physicochemical properties of the drug and polymer(s), formulation composition, processing conditions and environmental characteristics [2,4].

The aim of the work was to assess the release process of macromolecular drugs and in parallel to investigate the behaviour of water diffusion by means of differential scanning calorimetry. A model gel system was employed. The ambitious intent is to scale the two phenomena with respect to composition in order to correlate one another.

### Experimental

Calcium-alginate beads were prepared by dropwise addition of the alginate solution (2% w/v) into the gelling solution (calcium chloride 0.050 M) with a syringe. Release of proteins (e.g. lysozyme or insulin) was carried out in different medium either buffered or not, employing a simple and fast colorimetric assay. Thermograms of water evaporation were obtained by using a heat flux calorimeter (mod. DSC6-Perkin Elmer Instruments). Fitting of the evaporation curve was performed as described in Bellich *et al* [5]. Release data were also fitted.



## Results

The model gel used is represented by alginate beads. Release data collected in several different medium concerns the effect of the surrounding aqueous environment. The effect of the ionic strength was investigated. Not only the physico-chemical properties of the gel matrix and the external environment, but also the characteristics of the proteins have to be taken into account. After selecting one particular medium, in which diffusion is the main mechanism occurring, the effect of additional components of the gel matrix was studied. The release profiles of proteins from alginate gel were fitted according to the Weibull equation. Thus, a sort of a ternary system came up in which each component of the system is characterized by several different properties. In parallel, thermograms of water evaporation from different systems were recorded and analysed. A mathematical fitting of the first part of the curve was performed, with the aim of come up with a functional parameter. More specifically, the parameter used is the slope of the initial part of the curve. One of the main concerns of this hypothetical correlation resides on the geometry of the gel system, which in turn could be tricky and in some way misleading. Thus, water evaporation from model sugar systems, in order to assess the effect of the geometry, is currently under investigation.

## Conclusions

Formulation of biopolymeric hydrogels for the specific encapsulation of labile proteic drugs is still a topic of particular interest in pharmaceutical field. The study of the release behaviour of proteins from gel matrix is here presented. Many factors are involved in the process and a special focus was given to the external aqueous environment. The calorimetric approach represents an intriguing tool to further characterize the gel matrix and to provide additional information on the complex gel carrier.

## References

1. A. Martinsen, I. Storrø, G. Skjåk.Bræk, *Biotechnol Bioeng* 39, 186-194 (1992).
2. N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, *Eur. J. Pharm. Biopharm* 50, 27-46 (2000).
3. M. Hamidi, A. Azadi, P. Rafiei, *Adv. Drug Deliv. Rev.* 60, 1638-1649 (2008).
4. X. Mu, M.J. Toba, J.N. Staniforth, *Drug Dev Ind Pharm* 29, 19-29 (2003).
5. B. Bellich, M. Borgogna, M. Cok, A. Cesaro, *J. Therm. Anal. Calorim.* 103, 81-88 (2011).

# COLLECTIVE DYNAMICS IN THE THZ RANGE BY INELASTIC SCATTERING SPECTROSCOPIES

*F. Bencivenga<sup>a</sup> and A. Cunsolo<sup>b</sup>*

<sup>a</sup>Sincrotrone Trieste S.C.p.A. – SS 14 km 163,5 in AREA Science Park, 34149 Basovizza (Italy). [filippo.bencivenga@elettra.trieste.it](mailto:filippo.bencivenga@elettra.trieste.it)

<sup>b</sup>Photon Sciences Division, Brookhaven National Laboratory, P.O. Box 5000 Upton, New York 11973, USA.

## Introduction

Most of peculiarities of water can be ascribed to the highly ordered local structure, that continuously rearranges itself on ps-nm time-length scales. The spectrum of density fluctuations correlation function,  $S(Q,\omega)$ , probed in a momentum-frequency ( $Q-\omega$ ) range of the Thz-nm<sup>-1</sup> is indeed characterized by distinctive features, such as the presence of an anomalous dispersion of longitudinal acoustic (LA) modes as well as by the onset of transverse-symmetry mode (TS).

## Experimental

We report on Inelastic X-ray Scattering (IXS) data, interpreted within the memory function approach, aimed at determining how the anomalous dispersion of LA modes evolves on going from the liquid to the supercritical phase [1]. Water data are then compared with IXS results on other systems [2] and with a recently developed theoretical framework [3]. We also discuss the behaviour of the TS mode, by comparing IXS data with those of a very high resolution Inelastic Neutron Scattering (INS) experiment [4]. Finally, a new instrument (presently in construction at the FERMI@Elettra free electron laser source), which is able to probe THz dynamics in the time domain is briefly presented [5].

## Results

We observed that the anomalous dispersion of LA modes in water can be related to a structural relaxation and it tends to disappear on approaching supercritical conditions [1]. Concurrently, the characteristic timescale of the structural relaxation departs from the Arrhenius temperature dependence, observed in the liquid phase. The comparison with the results obtained in other systems let us to suppose that in the liquid phase the relaxation can be associated to making and breaking of intermolecular bonds, while in the supercritical phase is mainly due to intermolecular collisions [2]. Finally, the

combined use of IXS and very high resolution INS allowed us to underline how the  $S(Q,\omega)$  lineshape bears evidence of TS modes, which can be related with the structural relaxation phenomenology [4]. We also pointed out how, on increasing  $Q$ , the TS mode undergoes a transition from a non-propagating character (as that predicted by classical hydrodynamics) to a propagating one.

### **References**

1. F. Bencivenga et al., Phys. Rev. E 75, 051202 (2007).
2. F. Bencivenga et al., Phys. Rev. Lett. 98, 085501 (2007).
3. F. Bencivenga et al., J. Chem. Phys. 136, 114508 (2012).
4. A. Cunsolo et al., Phys. Rev. B 85, 174305 (2012).
5. F. Bencivenga et al., Nucl Instrum Methods phys res A 606, 785 (2009).

## **FREEZE-DRYING AND SPRAY-DRYING OF LIPID NANOPARTICLE SUSPENSIONS**

*P. Blasi<sup>a</sup>, A. Schoubben<sup>a</sup>, S. Giovagnoli<sup>a</sup> and M. Ricci<sup>a</sup>*

<sup>a</sup>Dipartimento di Chimica e Tecnologia del Farmaco, University of Perugia – Via del Liceo 1, 06123 Perugia, Italy.  
[kaolino@unipg.it](mailto:kaolino@unipg.it)

### **Objectives**

The aim of the work was to prepare and characterize lipid nanoparticles (NPs) and to investigate the possibility of obtaining a redispersible powder, preferable due to its superior stability. To this aim, both lyophilisation and spray-drying were investigated.

### **Experimental**

Polysorbate 80 stabilized cetylpalmitate (CP) NPs were prepared using the hot high pressure homogenization technique [1]. Particle size was determined with photon correlation spectroscopy, using a Nicomp 380 autocorrelator equipped with a Coherent Innova 70-3 argon ion laser. The suspension was then dried using both freeze-drying (Benchtop 2K, Freeze Dry VirTis) and spray-drying (nano spray-dryer B90, Büchi). Prior to drying, lipid NP suspensions with a volumetric fraction of 0.121 were appropriately diluted (1:5, 1:10, and 1:20) and ethanol, trehalose, and polyethylene glycol (PEG) have been added to evaluate their usefulness in avoiding irreversible aggregation during water removal. After drying, the obtained powder was dispersed in ultrapure water under stirring and submitted to particle size analysis to evaluate aggregation. Size increase after drying was expressed as  $\Delta_{size}$  calculated using the following equation.

$$\Delta_{size} = \frac{mhd \text{ after drying} - \text{Initial mhd}}{\text{Initial mhd}} \cdot 100$$

### **Results**

Lipid NPs were successfully produced and characterized by dimensions suitable for parenteral administration. In fact, the mean hydrodynamic diameter (MHD) was around 180 nm. After freeze-drying, an increase of MHD has been observed in all cases and in absence of additives a MHD > 2  $\mu$ m was recorded even at the highest dilutions. Among additives, trehalose was the most successful in reducing aggregation. Using trehalose and 1:20 dilution, a  $\Delta_{size}$  of 18% (MHD = 199 nm) was obtained. Satisfactory results

were also obtained for the other dilutions ( $\Delta_{size} = 28$  or  $39\%$ ), while the addition of ethanol worsen the situation. With PEG, MHD comprised between 269 and 290 nm were obtained using water/ethanol dilution ( $61 < \Delta_{size} < 73$ ).

In the case of spray-drying, an instrument of new generation, in which the powder recovery mechanism is completely different from the conventional instruments, was employed [2]. Without additive, the small fraction of powder that was recovered was characterized by large particle size. As in the case of freeze-drying, the use of additives appeared to be essential to limit lipid NP aggregation. Also with spray-drying, the best results were obtained with trehalose as additive and when dilution was performed with an ethanol/water mixture with respect to water. Using the solvent mixture, the inlet temperature in the instrument can be set to lower values, limiting the effect of temperature on lipid NP aggregation. With trehalose,  $\Delta_{size}$  of 20, 34 and 106% were registered for 1:20, 1:10 and 1:5 dilutions, respectively. The  $\Delta_{size}$  was almost doubled when water was used to dilute the suspensions. Unfortunately, the  $\Delta_{size}$  with PEG was always higher than 150% except for the 1:20 water dilution where lipid NPs were characterized by a MHD of 330 nm ( $\Delta_{size}$  of 98%).

## Conclusions

Both freeze-drying and spray-drying can be used to obtain an easy redispersible powder starting from lipid colloidal suspension. Besides, working conditions (e.g., additives, dilution) have to be accurately chosen to limit particle growing and/or sintering. In the present study, high dilutions and trehalose showed the overall best performance in lipid NP drying.

## References

1. P. Blasi, S. Giovagnoli, A. Schoubben, C. Puglia, F. Bonina, C. Rossi, M. Ricci, *Int. J. Pharm.* 419, 287-295 (2011).
2. K. Schmid, C. Arpagaus, W. Friess, *Pharm. Dev. Technol.* 16, 287-294 (2011).

## STABILITY OF LIPID NANOPARTICLES IN PHYSIOLOGICAL RELEVANT MEDIA

*P. Blas<sup>a</sup>, A. Schoubben<sup>a</sup>, V. Romano<sup>a</sup> and M. Ricci<sup>a</sup>*

<sup>a</sup>Dipartimento di Chimica e Tecnologia del Farmaco, University of Perugia – Via del Liceo 1, 06123 Perugia, Italy.

[kaolino@unipg.it](mailto:kaolino@unipg.it)

### Objective

The aim of this work was to investigate the stability of lipid nanoparticles (NPs) upon dilution in different media and temperatures. This kind of investigation is overlooked in literature even though very important. In fact, in order to assess the real potential of colloidal carriers in drug delivery, aggregation phenomena should be known and, if possible, avoided.

### Experimental

Polysorbate 80 (P80) and poloxamer 338 (P338) stabilized cetylpalmitate (CP) NPs were prepared using the hot high pressure homogenization technique [1]. Particle sizes were determined with photon correlation spectroscopy, using a Nicomp 380 autocorrelator equipped with a Coherent Innova 70-3 argon ion laser. Lipid NPs dimensions were expressed as mean hydrodynamic diameter (MHD) and Gaussian distribution width (GDW). The suspensions were diluted 50, 100, 250, and 500 times with cell culture medium F12 without FCS or alternatively physiological solution and stored at 4 or 37°C. CP NPs were monitored for 7 days in cell culture media and 21 days in physiological solution.

### Results

Lipid NPs were successfully produced and characterized by dimensions suitable for parenteral administration. P80 and P338 stabilized NPs showed a MHD around 180 nm and a GDW of 50 and 70 nm, respectively. Upon dilution with liquid culture broth, both suspensions were stable for 7 days at 4 and 37°C. Dilution did not affect the MHD nor the GDW, except for P80 stabilized NPs diluted 500 times and maintained at 37°C. In this case, aggregation was evident already after 24 hours. Upon dilution with physiological solution, P338 CP NPs were stable for 21 days at all dilutions and temperatures. In the case of P80 lipid NPs, the MHD and GDW values did not increase for 21 days at both temperatures only when the suspension was not diluted. After 21 days at 4°C, the MHD was 30 nm larger

for all dilutions. The situation worsened at 37°C. In the worst case (dilution 500 folds), both MHD and GDW were higher already after 24 hours and, after 21 days, MHD and GDW were 80 nm and 140 nm higher, respectively.

### **Conclusions**

NPs stabilized with P80 and P338 were stable for 7 days when diluted up to 500 times at both 4 and 37°C. After 21 days, similar results were obtained for P338 stabilized NPs diluted with physiological solution. On the contrary, P80 stabilized NPs, when diluted with physiological solution, showed some aggregation after 24 hours at 37°C and after 21 days at 4°C.

### **References**

1. P. Blasi, S. Giovagnoli, A. Schoubben, C. Puglia, F. Bonina, C. Rossi, M. Ricci, *Int. J. Pharm.* 419, 287-295 (2011).

## MULTIPLE PROTEIN GLASS TRANSITIONS AT LOW WATER CONTENT

*Jacob Bouman<sup>a</sup>, Susan Barker<sup>d</sup>, Paul Venema<sup>c</sup> and Renko de Vries<sup>b</sup>*

<sup>a</sup>School of Pharmacy, University of East Anglia – Norwich Research Park,  
Norwich, United Kingdom. [jacob.bouman@wur.nl](mailto:jacob.bouman@wur.nl)

<sup>b</sup>Laboratory of Physical Chemistry and Colloid Science, Wageningen University –  
Dreijenplein 6, 6703 HB Wageningen, The Netherlands

<sup>c</sup>Chair group Physics and Physical Chemistry of Foods, Wageningen University –  
Bomenweg 2, 6703 HD Wageningen The Netherlands

<sup>d</sup>School of Pharmacy, University of East Anglia – Norwich Research Park, Norwich,  
United Kingdom.

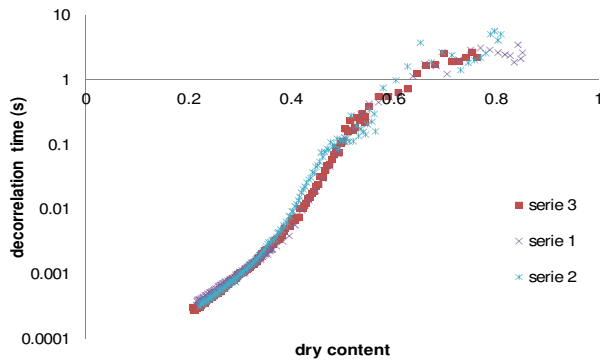
### Introduction

Concentrated protein films are widely used in foods and pharmaceuticals as a barrier material. When concentrated protein solutions enter the glassy state their physical transport properties change sharply, making these systems potential candidates as e.g. coating or encapsulation materials [1]. While their macroscopic physical properties have been widely examined, the underlying microscopic physical mechanism it is not well understood. This project focusses on the properties of  $\beta$ -lactoglobulin (BLG) solution near the glassy state. The dynamic behaviour of high-density protein systems are examined on multiple length scales using several techniques.

### Techniques and findings

Diffusion Wave Spectroscopy (DWS) is used as a form of micro-rheology, which enables continuously monitoring the drying process of a BLG film. The technique itself can be regarded as an extension of DLS, to the limit of strong multiple scattering [2]. Using this technique, no sudden increase in the decorrelation time ( $\sim$ proportional to viscosity) was observed between 20-80% protein content (see Figure 1). This suggests no sharp glass transition is present at the typical length scale probed (600 nm). The next step is to compare these results with glass transition temperatures at different protein contents as determined by differential scanning calorimetry and local thermal analysis. In addition, the GARField approach for NMR imaging introduced by Glover et al.[3] is examined as an alternative technique. It enables imaging with a high resolution of about 5  $\mu\text{m}$  and is promising towards monitoring the water distribution during the drying process of BLG films.





**Figure 1.** Diffusion wave spectroscopy on a drying  $\beta$ -lactoglobulin solution

### **References**

1. J. J. Kester and O. R. Fennema, Edible films and coatings: a review; Food technology, Food technology, 1987, pp. 47 -59.
2. G. Maret, Diffusion-wave spectroscopy; Current Opinion in Colloid & Interface Science, 1997, pp. 251-257.
3. P.M. Glover; P.S. Aptaker; J.R. Bowler; E. Ciampi; P.J. McDonald, Journal of Magnetic Resonance, 1999, pp. 90-97.

## EFFECT OF TEMPERATURE AND WATER ON POLY(LACTIDE-CO-GLYCOLIDE) INVESTIGATED BY FT-IR SPECTROSCOPY

*S. Casagrande<sup>a</sup>, M. Paolantonio<sup>b</sup>, P. Blas<sup>a</sup>, M. Ricci<sup>a</sup> and P. Sass<sup>b</sup>*

<sup>a</sup>Dipartimento di Chimica e Tecnologia del Farmaco, University of Perugia, 06123 Perugia, Italy. [fuliggine00@libero.it](mailto:fuliggine00@libero.it)

<sup>b</sup>Dipartimento di Chimica, University of Perugia, 06123 Perugia, Italy.

### Objectives

Thanks to their biocompatibility and biodegradability, poly-lactide (PLA) and poly-(lactide-co-glicolide) (PLGA) are approved by the FDA for human clinical applications and different controlled release formulations, based on these polymers, are actually marketed [1]. Water, acting as a plasticiser, might drastically change polymer features, compromising the performance of the drug delivery system [2].

### Experimental

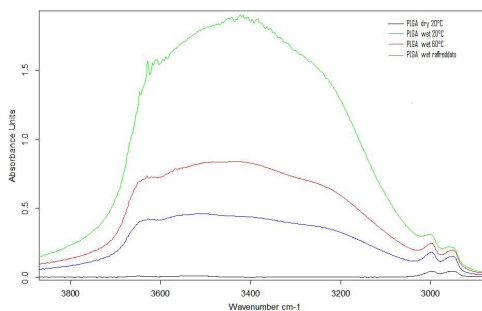
PLGA RG502H, RG503H, and RG504H have been casted on CaF<sub>2</sub>, and analyzed by FTIR in dry and completely wet state. Spectra were collected in the range of 20-70 °C.

### Results

For the three dry polymers several vibrational signals located in the 1000-2000 cm<sup>-1</sup> frequency range, show temperature-dependent intensity changes. In particular, the absorbance of the bands ascribed to C=O and O-C-O stretching, CH<sub>3</sub> and CH bending, and CH<sub>3</sub> rocking modes [3], plotted as a function of temperature, evidences an inflection point corresponding to the T<sub>g</sub> region determined by DSC measurements. Differences between the first and the second heating ramp, due to the cancellation of polymer thermo-mechanical history, are also observed.

The C=O and O-C-O stretching bands of the completely hydrated RG503H sample shift to lower frequencies compared to the signal of dry polymeric films at 20 °C. Moreover, these bands slightly upshift upon temperature rising, downshifting back after subsequent cooling at 20 °C. These findings can be rationalized considering the formation of O-H...O hydrogen bonds between water and PLGA groups and suggest the presence of non-freezable (bound) water in the completely hydrated polymer [4].

Direct information on water structuring within the polymer matrix are obtained by analysing the O-H stretching region ( $3100\text{-}3700\text{ cm}^{-1}$ ). The high intensity detected for the hydrated polymer drastically reduces with increasing temperature due to the loss of freezable (unbound) water [4]. Thus, the OH distribution detected after cooling back the sample at  $20\text{ }^{\circ}\text{C}$ , can be ascribed to water molecules which remain trapped into the polymer matrix (Figure 1). Interestingly, this OH band is very broad and partially structured, showing components assigned to OH oscillators involved either in very weak ( $3600\text{ cm}^{-1}$ ) or very strong cooperative ( $3200\text{ cm}^{-1}$ ) hydrogen bonds [5]. Similar features have been observed for hydration water “confined” in a hydrophobic solvent [5].



**Figure 1.** The OH stretching band of dry and wet polymer at different temperatures.

## Conclusions

The reproducibility of the data obtained with different PLGA, namely Resomer<sup>®</sup> RG504H, RG503H, and RG502H, confirms the ability of FTIR to detect the glass-to-rubber transition on one side, and investigate the state of water in water/PLGA mixtures on the other.

## References

1. S. S. D'Souza and P. P. DeLuca, *Pharm. Res.* 23, 460-474 (2006).
2. O. J. Karlsson, J. M. Stubbs, L. E. Karlsson and D. C. Sundberg, *Polymer* 42, 4915-4923 (2001).
3. P. Pan, B. Zhu, T. Dong, K. Yazawa, T. Shimizu, M. Tansho and Y. Inoue, *J. Chem. Phys.* 129, 184902/1-10 (2008).
4. P. Blasi, S. S. D'Souza, F. Selmin and P. P. DeLuca, *J. Control. Release* 108, 1-9 (2005).
5. F. Palombo, T. Tassaing, M. Paolantoni, P. Sassi and A. Morresi, *J. Phys. Chem. B* 114, 9085-9093 (2010).

## MOLECULAR DYNAMICS OF HYDRATION WATER IN SOLUTES OF DIFFERENT COMPLEXITY BY EXTENDED DEPOLARIZED LIGHT SCATTERING (EDLS) EXPERIMENTS

L. Comez<sup>a</sup>, D. Fioretto<sup>b</sup>, L. Lupi<sup>b</sup>, A. Morresi<sup>c</sup>,  
M. Paolantoni<sup>c</sup> and P. Sassi<sup>c</sup>

<sup>a</sup>IOM-CNR, c/o Dipartimento di Fisica, Università di Perugia, via A. Pascoli 1, I-06123 Perugia, Italy. [lucia.comez@fisica.unipg.it](mailto:lucia.comez@fisica.unipg.it)

<sup>b</sup>Dipartimento di Fisica, Università degli studi di Perugia, via A. Pascoli 1, I-06123 Perugia, Italy

<sup>c</sup>Dipartimento di Chimica, Università di Perugia, via Elce di Sotto 8, I-06123 Perugia, Italy.

We investigated the influence of hydrophilic and amphiphilic solutes of different chemical nature, molecular size and complexity on the fast rearrangement dynamics of water. In particular, diluted aqueous solutions of sugars, amino acids, peptides and the model protein lysozyme were considered. We made coupled use of interferometric and dispersive devices for collecting depolarized light scattering (EDLS) spectra in a wide frequency range, going from fraction of gigahertz to tens of terahertz [1-5]. This method allowed us to separate the solute from the solvent dynamics and bulk from hydration water, providing both characteristic times and relative fractions.

Hydration water was found to slow down when compared to bulk water and a relative retardation factor was estimated. An increasing of the dynamical perturbation was observed on going from simple sugars to amino acids, polypeptides and proteins. Moreover, while for sugar molecules this dynamical effect essentially involves the first hydration layer, a larger perturbation extending up to 2-3 layers was found for both simple peptides and lysozyme.

Overall, sugars showed a reduced influence on the picosecond restructuring of water when compared to simple peptides and proteins. Concerning these latter, similar dynamical slowing down effect and long-range perturbation degree were revealed, suggesting that increasing the molecular size and complexity does not change the mechanism responsible for the observed phenomena and that the chemical nature of the molecular surface plays a fundamental role in determining hydration features.

### References

1. M. Paolantoni, L. Comez, M. E. Gallina, P. Sassi, F. Scarponi, D. Fioretto and A. Morresi J. Phys. Chem. B 113, 7874-7878 (2009).

2. M. E. Gallina, L. Comez, A. Morresi, M. Paolantoni, S. Perticaroli, P. Sassi and D. Fioretto, *J. Chem. Phys.* 132, 214508 (2010).
3. S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, L. Lupi, D. Fioretto, A. Paciaroni and A. Morresi, *J. Phys. Chem. B* 114, 8262-8269 (2010).
4. S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, A. Morresi and D. Fioretto, *JACS* 133, 12063-12068 (2011).
5. L. Lupi, L. Comez, M. Paolantoni, D. Fioretto and B. M. Ladanyi, *J. Phys. Chem. B* 116, 7499-7508 (2012).

## SOLVENT DYNAMICS DURING ENZYMATIC CATALYSIS: A MOLECULAR DYNAMICS SIMULATIONS STUDY

*Valeria Conti Nibali<sup>a</sup>, Matthias Heyden<sup>b</sup>, Moran Grossman<sup>c</sup>, Benjamin Born<sup>c</sup>, Dmitry Tworowski<sup>c</sup>, Irit Sagi<sup>c</sup> and Martina Havenith<sup>a</sup>*

<sup>a</sup>Ruhr-University Bochum, Institute for Physical Chemistry II, Bochum, Germany.  
[valeria.continibali@rub.de](mailto:valeria.continibali@rub.de)

<sup>b</sup>University of California, Department of Chemistry, Natural Sciences, Irvine, USA

<sup>c</sup>The Weizmann Institute of Science, Department of Structural Biology, Rehovot, Israel.

Solvent dynamics can play a major role in enzyme activity, but obtaining an accurate, quantitative picture of solvent activity during catalysis is quite challenging.

Recently, Grossman et al. have investigated changes in water and protein dynamics as a zinc metalloprotease enzyme (MT1-MMP) binds a peptide substrate and have shown that a retardation of hydration water dynamics is connected to the formation of the Michaelis complex during the enzymatic reaction [1].

Here, we present a molecular dynamics simulation study to characterize the solvation dynamics at the active site during a more physiologic scenario, namely the interaction between MT1-MMP and collagen-like molecules.

MT1-MMP belongs to a broad family of zinc-dependent endopeptidases that mediate peptide hydrolysis reactions of extracellular matrix proteins under both physiological and pathological conditions. Given the role of MMPs in numerous diseases associated with unregulated collagenolysis, such as atherosclerosis, rheumatoid arthritis and cancer, it is highly desirable to study the specific molecular mechanism of MMPs and to point out the role of the solvent during collagen hydrolysis at atomic resolution.

The results obtained by MD simulations have been combined with time resolved kinetic terahertz absorption and experiments and X-ray absorption analyses on the same enzyme-substrate systems in aqueous environment to gain insight to the complete catalytic reaction ensemble.

### **References**

1. M. Grossman et al., Nature Structural & Molecular Biology 18, 1102–1108 (2011).

## PHOTON CORRELATION STUDY OF THE DYNAMICS OF CYCLODEXTRIN-BASED NANOSPONGES IN WATER

*S. Corezzi<sup>a</sup>, D. Bonfigli<sup>a</sup>, L. Comez<sup>a,b</sup> and F. Trotta<sup>c</sup>*

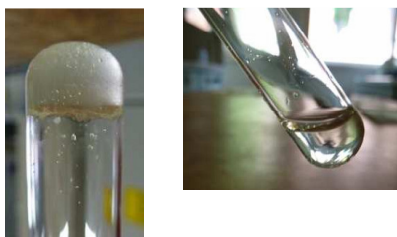
<sup>a</sup>Dipartimento di Fisica, Università di Perugia – Via A. Pascoli, I-06123 Perugia, Italy. [silvia.corezzi@fisica.unipg.it](mailto:silvia.corezzi@fisica.unipg.it)

<sup>b</sup>IOM-CNR, c/o Università di Perugia, Perugia, Italy

<sup>c</sup>Dipartimento di Chimica IFM, Università di Torino, Torino, Italy

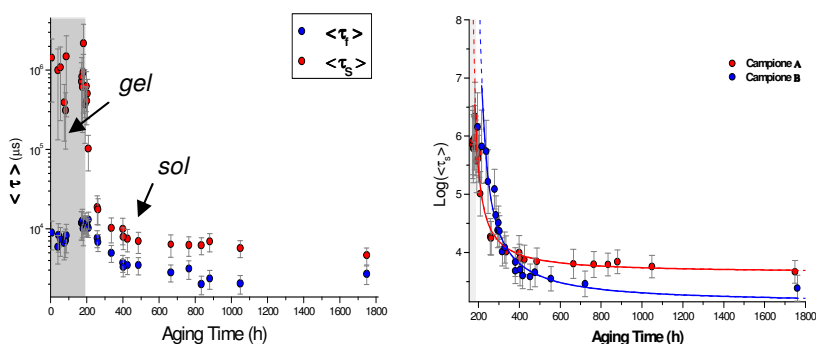
Cyclodextrins (CDs) appear to be intriguing materials for the development of new drug carriers because of their unique physico-chemical properties, mainly related to the ability of CD molecules to form inclusion complexes. More recently, polymer-network structures involving CDs have attracted interest as even more efficient host systems, with specific functional properties intimately connected to the cross-linking density of the polymer structure. Cyclodextrin nanospunges (CDNSs) are a new class of amorphous network-polymers obtained by reacting CD with a suitable polyfunctional agent such as pyromellitic anhydride (PMA) [1]. The reaction products turn out to be nanoporous systems showing interesting properties of swelling and absorption/inclusion of chemicals. Although their promising use for biotechnological applications, a detailed structural and dynamical characterization of these polymers is still lacking in the literature [2].

Here we use photon correlation spectroscopy (PCS) to study the dynamic evolution of CDNS-based hydrogels, obtained at different CDNS/water ratios. The technique appears to be an effective tool to characterize the huge evolution of the relaxation processes of the swollen network, which evolves in time due to the ester bond hydrolysis inside the CDNS grains, determining a progressive change of the sample from a gel to a liquid phase (Figure 1).



**Figure 1.** CDNS-based sample in the gel (left) and liquid state (right).

The de-gelation process induced by covalent bond breakage inside the CDNS structure exhibits, although temporally reversed, significant similarities with the hydro-gelation process induced by physical bond formation. Two relaxation processes can be clearly observed: a faster exponential process, which progressively increases with time, and a slower non-exponential process, which directly probes the gel-sol transition and progressively decreases (Figure 2). Measurements performed at different values of exchanged wavevector allowed to characterize the evolution with time of the effective hydrodynamic radius associated to these relaxation processes and to suggest a possible physical interpretation.



**Figure 2.** (Left): Time evolution of the relaxation time of the slow and fast processes in a CDNS-based hydrogel. (Right): Time evolution of the slow relaxation time in two CDNS-based hydrogels with different CDNS/water ratio.

### References

1. F. Trotta and W. Tumiatti, Patent number WO 03/085002, 2003.
2. B. Rossi, S. Caponi, F. Castiglione, S. Corezzi, A. Fontana, M. Giarola, G. Mariotto, A. Mele, C. Petrillo, F. Trotta, and G. Viliani, *J. Phys. Chem. B* 116, 5323-5327 (2012).



## INTERPLAY OF WATER MOBILITY AND SUGAR CRYSTALLIZATION IN GLASSY MIXTURES

*Elena Elisei<sup>a</sup>, Attilio Cesàro<sup>a</sup>, Fabio Tedoldi<sup>b</sup> and Alessandro Maiocchi<sup>b</sup>*

<sup>a</sup>Department of Life Sciences, University of Trieste – Via Licio Giorgieri 1, 34127 Trieste(TS), Italy. [elena.elisei@phd.units.it](mailto:elena.elisei@phd.units.it)

<sup>b</sup>Bracco Research Centre, Bracco Imaging SpA, – Via ribes 5, 10010 Colletterto Giacosa(TO), Italy

The glassy and amorphous states of sugars are of fundamental importance for the bio-preservation, both in nature and in laboratory [1]; in fact many food materials are present in the glassy state due to the food processing [2]. The dependence of the glass transition temperature  $T_g$  with composition, and water in particular, has been widely studied in literature and subjected to several phenomenological interpretations.

The study of the relaxation enthalpy and the structural arrest (the temperature at which the mobility of the glass almost vanishes,  $T_K$  Kauzmann temperature) is essential not only in food science, but also in other fields, such as the biomedical field, to investigate how the internal nanostructuring of the glass matrix could affect the magnetic transport properties (DNP, Dynamic Nuclear Polarization) of imaging agents used in Nuclear Magnetic Resonance.

This paper reports the comparison of the structural stability below the glass transition of three glassy systems based on carbohydrates: Sucrose, Trehalose and Glucose.

Several techniques and procedures for amorphization of these sugars, including their binary and ternary mixtures, were investigated and the importance of the residual water inside the sugar matrix as one of the destabilizing factors of the glassy samples was highlighted. The other concurrent factor of interest is the partial recrystallization which occurs at temperature higher than the  $T_g$ , often parallel to water evaporation.

Particularly, the phenomenon of Physical Aging as a function of the aging time was studied by different calorimetric procedures, i.e., by changing the scan rate of the cooling process or by aging the sample at constant temperature below the glass transition temperature. This investigation was carried out to collect data on the changes in the relaxation enthalpy and the fictive temperature, and then to derive the fragility parameter of the glass, a signature of the molecular mobility of the sugar glass matrix as a function of composition and residual moisture.

New experimental results concern the anomalous behavior of Trehalose alone which shows two different fragility parameters depending on the procedure by which amorphization was achieved [3,4]. Indeed, the two procedures refer to the fact that two crystalline polymorphs can be amorphized. Comparison is made with the well known behavior of the homologous sugar, Sucrose, which shows a fragility parameter in line with that expected for this system [5].

Greatest attention was paid to the third sugar, Glucose, to avoid the risk of degradation that occurs just above the melting point, in addition to possible anomerization effects. The data so-far collected, however seem to indicate that Glucose behavior is more close to that of Sucrose than to that of Trehalose.

An attempt to correlate the percentage of residual water and the presence of micro-heterogeneity in the glass matrices would be enlightening. Given the modern understanding of a heterogeneous matrix of the glassy state, several structural approach have been proposed, such as Phosphorescence [6] and Positron Annihilation Lifetime Spectroscopies (PLS [7,8] and PALS [9-11]) or Fourier Transform Infrared Attenuated Total Reflectance (FTIR ATR) Microspectroscopy [12]. Comparison of the calorimetric data with those obtained in other laboratories with these techniques is not always straightforward because of the different procedures, set-up and samples used. Experiments are planned toward collecting all the data on uniformly prepared samples.

### **References**

1. C. Walters, *Biophys. J.* 86, 1253-1258 (2004).
2. Y. Liu, B. Bhandari and W. J. Zhou, *Agric. Food Chem. Rev.* 54(16), 5701-5717 (2006).
3. A. Cesàro, O. De Giacomo and F. Sussich, *Food Chem.* 106, 1318-1328 (2008).
4. F. Sussich and A. Cesàro, *Carbohydr. Res.* 343, 2667-2674 (2008).
5. R. Urbani, F. Sussich, S. Prejac et al, *Thermochim. Acta* 304/305, 359-367 (1997).
6. S. Shirke, Y. You and R. D. Ludescher, *Biophys. Chem.* 123, 122-133 (2006).
7. R. Tiwari and R. D. Ludescher, *Food Chem.* 132, 1814-1821 (2012).
8. L. C. Pravinata, Y. You and R. D. Ludescher, *Biophys. J.* 88, 3551-3561 (2005).
9. D. Racko, R. Chelli, G. Cardini et al, *Eur. Phys. J. D* 32, 289-297 (2005).
10. D. Kilburn, J. Claude and R. Mezzenga, *J. Phys. Chem. B* 108, 12436-12441 (2004).
11. D. Kilburn, J. Claude and T. Schweizer, *Biomacromolecules* 6, 864-879 (2005).
12. O. De Giacomo, A. Cesàro and L. Quaroni, *Food Biophys* 3, 77-86 (2008).

## A NOVEL ANTI-FOG PULLULAN COATING FOR FOOD PACKAGING APPLICATIONS

*S. Farris, L. Introzzi, L. Piergiovanni and A. Schiraldi*

DeFENSE Department of Food, Environmental and Nutritional Sciences, University of Milan – via Celoria 2, 20133 Milan, Italy. [stefano.farris@unimi.it](mailto:stefano.farris@unimi.it)

Current food packaging materials are conceived as a multifunctional tool enabling containment, protection and preservation of foods. One of the additional properties required is the anti-fog property, which concerns the capability of the packaging material to avoid the forming small droplets of water on the internal side of the packaging film. Fog has a detrimental effect on the transparency of the material, mainly due to the shape of the droplets [1], which is reflected by the balance between the three interfacial energies (solid-liquid  $\gamma_{SL}$ , liquid-vapor  $\gamma_{LV}$ , and solid-vapor  $\gamma_{SV}$ ) of the three-phase system, as described by Young's equation [2]. The higher the contact angle, the higher the incident angle of the light normal to the substrate at the water/air interface, hence the more intense will be the scattering of the visible light. Not only the shape, but also the size of the droplets is another important factor to consider with regards to the anti-fog property of a material [1]: the smaller the size, the larger the number of droplets and the more pronounce the foggy effect. As both the shape and the size of the water droplets depend on the physicochemical characteristics of the substrate [3], modifying the original physicochemical properties of the plastic surface can be the key to controlling water droplet formation [4].

The approach of applying a bio-based coating is here described, and a new anti-fog coating made of pullulan is proposed [5]. The anti-fog properties are discussed in terms of wettability, surface chemistry / morphology, and by quantitative assessment of the optical properties (haze and transparency) before and after fog formation. The work also presents the results of anti-fog tests simulating the typical storage conditions of fresh foods. In these tests, the anti-fog efficiency of the pullulan coating was compared with that of two commercial anti-fog films, whereas an untreated low-density polyethylene (LDPE) film was used as a reference. The obtained results revealed that the pullulan coating behaved as a 'wetting enhancer', mainly due to the low water contact angle ( $\sim 24^\circ$ ), which in turn can be ascribed to the inherent hydrophilic nature of this polysaccharide, as also suggested by the X-ray photoelectron spectroscopy experiments. Unlike the case of untreated LDPE and commercial anti-fog samples, no discrete water formations (i.e., droplets or stains) were observed on the anti-fog pullulan coating on refrigeration during testing. Rather, an invisible, continuous and thin layer of water occurred on the biopolymer surface, which was the reason for the unaltered haze and increased transparency, with the layer of water possibly behaving

as an anti-reflection layer. As confirmed by atomic force microscopy analysis, the even deposition of the coating on the plastic substrate compared to the patchy surfacing of the anti-fog additives in the commercial films is another important factor dictating the best performance of the anti-fog pullulan coating.

### **References**

1. J. A. Howarter and J. P. Youngblood. *Macromol. Rapid Commun.* 29, 455–466 (2008).
2. T. Young, *Philosophical Transactions of the Royal Society of London* 1805, Vol. 95, pp 65–87.
3. S. Farris, L. Introzzi, P. Biagioni, T. Holz, A. Schiraldi and L. Piergiovanni. *Langmuir* 27, 7563–7574 (2011).
4. F. C. Cebeci, Z. Wu, L. Zhai, R. E. Cohen and M. F. Rubner. *Langmuir* 22, 2856–2862 (2006).
5. L. Introzzi, J. M. Fuentes-Alventosa, C. A. Cozzolino, S. Trabattoni, S. Tavazzi, C. L. Bianchi, A. Schiraldi, L. Piergiovanni and S. Farris. *ACS Appl. Mater. Interfaces* (accepted). DOI:10.1021/am300784n

## WETTING OF BIOPOLYMER COATINGS: CONTACT ANGLE KINETICS AND IMAGE ANALYSIS INVESTIGATION

*S. Farris, L. Introzzi, L. Piergiovanni and A. Schiraldi*

DeFENSE Department of Food, Environmental and Nutritional Sciences, University of Milan – via Celoria 2, 20133 Milan, Italy. [stefano.farris@unimi.it](mailto:stefano.farris@unimi.it)

The use of coatings made of biopolymers on packaging materials, especially plastics, has significantly increased over the past few years [1,2]. However, the modification of the surface properties of packaging materials after biocoating deposition has not been fully investigated so far [3]. In particular, few papers in literature deal with the behavior of bio-coated surfaces with respect to water, especially concerning the physicochemical phenomena involved at the solid/liquid interface [4].

In this work [5], the surface wetting of five biopolymers, used as coating materials for a plastic film, was monitored over a span of 8 min by means of the optical contact angle technique. Because most of the total variation was observed to occur during the first 60 s, we decided to focus on this curtailed temporal window. Initial contact angle values ( $\theta$ ,  $^{\circ}$ ) ranged from  $91^{\circ}$  for chitosan to  $30^{\circ}$  for pullulan. However, the water drop profile began to change immediately following drop deposition for all biocoatings, confirming that the concept of water contact angle equilibrium is not applicable to most biopolymers. First, a three-parameter decay equation [ $\theta(t) = \theta(0) \exp(kt^n)$ ] was fit to the experimental contact angle data to describe the kinetics of the contact angle change for each biocoating. Interestingly, the  $k$  constant correlated well with the contact angle evolution rate and the  $n$  exponent seemed to be somehow linked to the physicochemical phenomena underlying the overall kinetics process. Second, to achieve a reliable description of droplet evolution, the contact angle (CA) analysis was coupled with image analysis (IA) through a combined geometric/trigonometric approach. Absorption and spreading were the key factors governing the overall mechanism of surface wetting during the 60 s analysis, although the individual quantification of both phenomena demonstrated that spreading provided the largest contribution for all biopolymers, with the only exception of gelatin, which showed two quasi-equivalent and counterbalancing effects. The possible correlation between these two phenomena and the topography of the biopolymer surfaces are then discussed on the basis of atomic force microscopy analyses.

**References**

1. J. W. Rhim, J. H. Lee and S. I. Hong. Food Sci. Technol.-Leb. 39, 806–813 (2006).
2. S. Farris, L. Introzzi and L. Piergiovanni. Packag. Technol. Sci. 22, 69–83 (2009).
3. T. Karbowiak; F. Debeaufort and A. Voilley. Crit. Rev. Food Sci. 46, 391–407 (2006).
4. S. Kokoszka, F. Debeaufort, A. Hambleton, A. Lenart and A. Voilley. Innovat. Food Sci. Emerg. Tech. 11, 503–510 (2010).
5. S. Farris, L. Introzzi, P. Biagioni, T. Holz, A. Schiraldi and L. Piergiovanni. Langmuir 27, 7563–7574 (2011).

## WATER EFFECTS ON SACCHARIDE MATRICES STUDIED THROUGH MD AND FTIR

*Sergio Giuffrida, Grazia Cottone and Lorenzo Cordone*

Dipartimento di Fisica, Università degli Studi di Palermo,  
Via Archirafi 36, I-90123 Palermo, Italy. [sergio.giuffrida@unipa.it](mailto:sergio.giuffrida@unipa.it)

### Introduction

Saccharides, and in particular trehalose, are known for their high efficiency in protecting biostructures against adverse environmental conditions [1], although the preservation mechanism is still debated. Experiments and simulations 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. Trehalose effects have been related to its hydrogen-bonding (HB) properties, which have been studied by Infrared Spectroscopy (FTIR) and Molecular Dynamics simulations (MD) [2,3,4].

FTIR, through the study of the water association band and of the CO stretching band, pointed out the existence of a reciprocal protein-matrix coupling modulated by water content. In these systems different classes of water molecules could be distinguished, whose ratio is altered by chaotropic and kosmotropic solutes [3,5].

MD simulations pointed out that water molecules shared between protein and sugar could modulate the *strength* of the constraints imposed by the surrounding matrix on the protein, thus affecting its internal dynamics (atomic fluctuations on ps/ns time scale) [4].

A deeper understanding of the role of water molecules in the dynamics of these system could shed light on trehalose peculiarity.

### Experimental

Here we report on MD study of model systems constituted by a MbCO molecule embedded in a highly viscous (amorphous) trehalose-water matrix (830 trehalose molecules and 1909 water molecules, 89% w/w). NAMD code was used with CHARMM22 parameters. The system was equilibrated in the NPT ensemble with the Nosé-Hoover Langevin piston pressure control and then left to evolve in the NVT ensemble up to 5 ns. Runs were performed at various temperatures in the range 50-400K. For each temperature parallel simulations with either free or fixed water molecules (unable to either translate or translate *and* rotate) were performed in order to elucidate the

contribute of water dynamics (disentangled from trehalose dynamics) to the total dynamics of the system, and its influence on the protein.

To further investigate the water role, we performed analogous simulations with model systems at the same water/trehalose ratio containing various salts with kosmotropic/chaotropic effect, able to modulate the hydrogen bond strength of the matrix. These latter results are compared with FTIR measurement performed on analogous systems, both containing and not-containing added salts.

## Results

The analysis of the mean square fluctuations of the protein C-alpha and of the trehalose glycosidic linkage O atoms, evidenced the damping of anharmonic contributions to the protein and matrix dynamics, even in the high temperature range, in the fixed water systems. In particular it has been possible to set apart the effect of the protein presence on the matrix as a function of the distance from protein surface, as well as the selective water effect on the protein dynamics by fixing only the water in a given distance range from protein surface. This allows to discriminate which fraction of water has the maximum effect on the protein dynamics and up to which distance these effects are important. Further information on the effects of the HB strength modulations in systems with free water has been obtained by comparing the dynamics of the previous systems with those of salt-containing ones.

## References

1. J.H. Crowe, *Adv. Exp. Med. Biol.* 594, 143–158 (2007).
2. L. Cordone, G. Cottone, S. Giuffrida, *J. Phys.: Condens. Matter* 19, 205110 and ref. therein (2007).
3. S. Giuffrida, G. Cottone, L. Cordone, *Biophys. J.* 91, 968-980 (2006).
4. G. Cottone, *J. Phys. Chem. B* 111, 3563–3569 (2007).
5. S. Giuffrida, G. Cottone, E. Vitrano, L. Cordone, *J. Non-Cryst. Solids* 357, 677–682 (2011).



## HIGH DENSITY PROTEIN SOLUTIONS: THEORY & EXPERIMENT

*John Ioannou<sup>a</sup>, Joris Stegen<sup>b,c</sup>, Athene Donald<sup>a</sup>,  
Paul van der Schoot<sup>b,c</sup> and Hans Tromp<sup>d,e</sup>*

<sup>a</sup>Sector of Biological & Soft Systems, Department of Physics, University of Cambridge, Cavendish Laboratory, 19, J J Thomson Avenue, Cambridge CB3 0HE, United Kingdom. [jci22@cam.ac.uk](mailto:jci22@cam.ac.uk)

<sup>b</sup>Theory of Polymer and Soft Matter, Department of Physics, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands. [i.stegen@tue.nl](mailto:i.stegen@tue.nl)

<sup>c</sup>Institute for Theoretical Physics, Utrecht University.

<sup>d</sup>NIZO The Food Researchers, B.V.Kernhemseweg 2, 6718 ZB Ede P.O. Box 20, 6710 BA Ede, The Netherlands.

<sup>e</sup>Department of Physical and Colloid Chemistry, Utrecht University

### Introduction

High-density protein systems are of increasing interest to food industry. Whey proteins in particular, a constituent of milk, have a significant importance in this context, not least as they are known to be prone to aggregation under relatively mild denaturing conditions. Consequently, an understanding of the aggregation mechanism of whey proteins is vital if considering heat or salt treatment or if utilising whey proteins as a gelling agent. Furthermore creating 'concentrates' of protein-based foods via the removal of water is standard practice. Indeed, dehydrated foods could be rehydrated at later time, thereby reducing food volume and weight in between production and consumption. This allows for a significant reduction in food freight and storage costs. Unfortunately, the behaviour of dense protein systems is poorly understood. At high concentrations, a lack of hydration water may cause the (partial) unfolding of proteins, potentially affecting the mesoscopic and macroscopic properties of the dispersion by the subsequent expulsion of water. Such a (partial) unfolding of the protein molecules can ultimately have an adverse effect on food texture, which has obvious implications for the food industry.

The aim of our work is to attempt to elucidate the temporal behaviour of high-density protein solutions. More specifically, we investigate the properties of dense whey protein solutions. Current work focuses on producing high-density protein systems of varying protein concentrations at a fixed pH from which the effects on mechanical behaviour, viscosity, and conformational changes to the microstructure of these dense protein systems can be characterised. In future work we explore varying pH conditions and

salt inclusions. Theoretical work focuses on modeling the conditions under which phase separation, gelation and crystallization in a dense protein solution will occur, and what the time- and length-scales are involved with the various phase transitions. Finally, we hope to combine both theory and experiment in a comprehensive study of the behaviour of dense protein solutions.

### **Theoretical Work**

A simple two-state model for proteins is used to model a protein solution. Proteins are either in their native state or in a denatured state. These two states are separated by a free energy difference: unfolding of proteins is an activated process. After unfolding, hydrophobic parts of the protein are exposed to solvent. This results in an effective attractive interaction between denatured proteins due to hydrophobic effects. The corresponding phase behaviour reflects the competition between the energy scale associated with the native - denatured transition, and the energy scale associated with aggregation of denatured proteins. This results in a competition between a dilute phase of native proteins and a dense phase of denatured proteins. The phase diagram shows rich and non-trivial phase behaviour, including a multi-critical point. Phase transitions are slowed down by a lag time associated with the conversion time between native and denatured state.

### **Experimental Work**

Type A  $\beta$ -lactoglobulin, freeze-dried whey protein powder (as produced by NIZO food research), was dissolved in a buffer at pH = 3 (0.2 M  $\text{Na}_2\text{HPO}_4$ /ml and 0.1 M Citric Acid/ml) at initial dilute concentrations of 50 mg/ml protein-concentration. From these dilute solutions, concentrates of high-density protein solutions up to 780 mg/ml were made via centrifugation filtration. Oscillatory shear bulk rheology and passive video particle tracking (VPT) micro-rheology techniques were used to determine the mechanical properties of these concentrates and assess the possible existence of heterogeneity in the structure. The Infrared absorption spectrum of a drying droplet of the solution (protein concentration of 307 mg/ml) over a 90 minute duration was determined via Fourier Transform Infrared Spectrometry (FTIR). All the bulk and microrheology results indicated that these solutions behave simply as viscous fluids and exhibit typical Newtonian behaviour. Bulk rheological analysis indicated that there was no gelation occurring (not even a weak gel) in the liquids. All solutions exhibited viscous fluid behaviour with FTIR derived drying-spectra showing the possibility of a phase separation/gelation at 15% protein-volume fraction.

## EFFECTS OF POTASSIUM IONS ON THE VISCOSITIES IN THE POTASSIUM CHLORIDE-GLUCOSE-WATER TERNARY SYSTEM

*Kazuhiro Kajiwara, Yumi Yanaihara, Hiroko Ando and Kiyoshi Kawai*

School of Bioscience and Biotechnology, Tokyo University of Technology – 1404-1 Katakura Hachioji, Tokyo 192-0982 Japan. [kajiwara@bs.teu.ac.jp](mailto:kajiwara@bs.teu.ac.jp)

### **Introduction**

Many electrolytes, including sodium and potassium chloride, exist in a mammal's living body. Despite the chemical similarities of the sodium and potassium ion, their cellular distributions are different and asymmetric; the extracellular concentration of sodium ions exceeds their intracellular concentration, the reverse being the case for potassium ions, suggesting that the ions have differing functions in the cell. The saccharides are also important tissue components that influence tissue fluid behavior. To clarify the roles of sodium ions and potassium ions in living bodies, in our previous research we examined the physicochemical characteristics of each ion in the alkali halide-saccharide-water ternary system by measuring its solubility[1] and freezing-thawing behavior[2]. The viscosity has been employed previously as physicochemical index, often related to potential solution structural changes. In this study of KCl-glucose-water ternary system, the influence of potassium ions on solution viscosities was studied as a potential structural probe.

### **Experimental**

The density of the KCl-glucose-water ternary system was measured with a Gay-Lussac-type pycnometer. The flow time of the ternary system was measured with a Ubbelohde viscometer. All measurements were carried out in a temperature-controlled water bath ( $25.0 \pm 0.01$  °C). The viscosity of the solutions was calculated by using the density and the flow-time results.

### **Results**

The viscosity curve is not simple. It decreases at lower KCl concentration then increases gradually with increases KCl concentration. The tendency is emphasized with increases glucose concentration. A increase or decrease in the dependence of viscosity on KCl concentration is seen in the binary KCl-water system as a function of temperature.

***References***

1. K. Kajiwara and F. Franks, J. Solution Chemistry, 33, 157-167 (2004).
2. K. Kajiwara, A. Motegi and N. Murase, CryoLetters, 22, 311-320 (2001).

## EFFECT OF WATER ON THE SOFTENING TEMPERATURE OF COOKIE PRODUCTS

*Kiyoshi Kawai<sup>a,b</sup>, Midori Toh<sup>b</sup>, and Yoshio Hagura<sup>a,b</sup>*

<sup>a</sup>Department of Biofunctional Science and Technology, Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan.

[kawai@hiroshima-u.ac.jp](mailto:kawai@hiroshima-u.ac.jp)

<sup>b</sup>Faculty of Applied Biological Science, Hiroshima University, Hiroshima, Japan.

### Introduction

Cookie is a major bakery product composed of wheat flour, sugar, butter, and egg. The quality of cookie has been often characterized by mechanical properties such as shortness and hardness. When cookie is stored in an ambient condition, softening is caused by water adsorption, and then the shortness and hardness are lost almost completely. It is suggested that softening of cookie can be related to glass transition of the constituents [1-3]. The previous studies, however, were not large enough to permit the control and prediction for the softening of cookie. Thus, effects of sugar-type and water content on the softening temperature ( $T_s$ ) of cookie were investigated using a thermal rheological analysis (TRA). In addition, relationship between the  $T_s$  of cookie and the glass transition temperature ( $T_g$ ) of sugar contained in the cookie was discussed for the establishment of softening prediction.

### Experimental

Three types of mixed sugars (100% sugar, sugar containing 40% trehalose, and sugar containing 40% sorbitol per dry basis) were prepared. The sugar, butter, whole egg, and wheat flour were mixed homogeneously, and the mixture (dough) was held at 4 °C for 2 h. The dough was baked at 180 °C for 12 min, and then cookie was obtained. The cookie was grinded homogeneously, and then equilibrated under various relative humidity conditions. Water content of the sample was evaluated gravimetrically.

$T_s$  of cookie samples was investigated using a rheometer (CR-150; Sun Scientific Co.,Ltd., Tokyo, Japan). The sample (100-105 mg) was compressed at 12 MPa in a holder ( $\phi = 7$  mm), and set to a heat sink cup attached on the sample stage of the rheometer. Then, the sample was compressed at 2 MPa at 10 °C, and subsequently heated at 3.5 °C/min up to 80 °C.

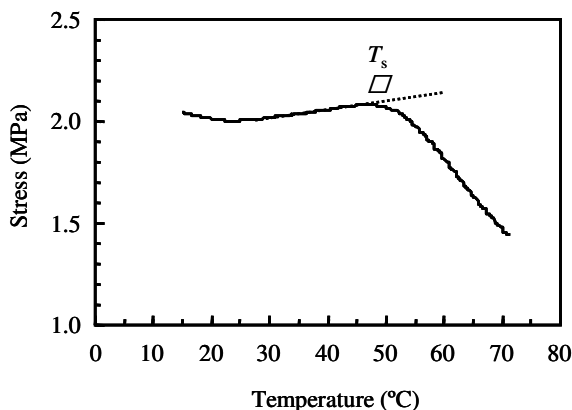
$T_g$  of sugar was investigated using a DSC (DSC120; Seiko Instruments Inc., Tokyo, Japan). Each mixed sugar was dissolved by distilled water and 10% (w/w) aqueous solution was prepared in a glassy vial. The solution was

placed in a pre-cooled freeze-drier, and vacuumed at a pressure below 70 Pa with increasing temperature from -40 °C to 10 °C over a period of 36 h. The freeze-dried amorphous samples (4-12 mg) were placed in a DSC pan. In order to remove the residual water of sample, the sample in the pan was held at 60 °C for 18 h in a vacuum desiccator (below 8 kPa). Then, the fully-dried sample was hermetically sealed, and heat-scanning was performed at 5 °C/min in the temperature range between 0 °C and 100 °C.

## Results and discussion

A typical TRA result for a cookie sample is shown in Fig. 1. The cookie sample showed a drastic decrease of stress at a certain temperature, and  $T_s$  value could be evaluated from the onset point. In order to understand effect of water content on the  $T_s$  of the cookie samples, the  $T_s$  was plotted against the water content. It was found that the  $T_s$  decreased linearly with increase in the water content. From the linear function, the water content of which the cookie showed softening at 25 °C could be calculated. This will be useful for the prediction of softening condition of cookie.

DSC thermograms for anhydrous mixed sugar samples showed an endothermic shift reflecting glass transition, and  $T_g$  was evaluated from the onset point. When a relationship between the anhydrous  $T_s$  of cookie calculated by above-mentioned linear function and the  $T_g$  of anhydrous sugar was investigated, there was a liner relationship between them. This indicates that the softening of cookie can be characterized by the glass transition of sugar. This will be useful for the control of cookie texture.



**Figure 1.** A typical TRA result for a cookie sample.

**References**

1. Y. H. Roos, Phase transitions in foods, London: Academic Press, 1995, pp. 109-155.
2. L. Slade and H. Levine, J. Food Eng. 22, 143-188 (1994).
3. S. Chevallier, G. Della Valle, P. Colonna, B. Broyart and G. Trystram, J. Cereal Sci. 35, 1-10 (2002).

## COMPARATIVE STUDY OF AQUEOUS AND ETANOLIC EXTRACTS OF THE DRUG PRODUCT PREPARED ON THE BASIS OF PLANT SAMPLE

*Armen Kirakosyan<sup>a</sup>, Astghik Sukiasyan<sup>b</sup> and Armen Humbarzumyan<sup>a</sup>*

<sup>a</sup>Department of Chemical technology and Environmental engineering of SEUA,  
105 Teryan Str., 0009 Yerevan, Armenia.

[kirakosyan\\_armen@yahoo.com](mailto:kirakosyan_armen@yahoo.com)

<sup>b</sup>Department of Biotechnology and Environmental of SEUA,  
105 Teryan Str., 0009 Yerevan, Armenia.

### Introduction

The antioxidant power some of a drug is not only its capacity to protect against oxidant species, but also to maintain constant the physiological production of oxygen reactive species and to improve antioxidant defence systems. Lipid oxidation products can alter the balance of these mechanisms in both beneficial and damaging way. The major emphasis was to study the kinetics of the inhibitors in protecting against oxidant stress in erythrocytes prepared from whole blood. To inhibit oxidant stress, we will use Artemisia Absinthium L. extracts, which was widely employed in Armenian traditional medicine as a remedy for digestive diseases.

### Experimental

In a first was investigated of some physico-chemical properties of our experimental samples. For study of antioxidant properties of Artemisia Absinthium L extract all using organically solutions (methanol, ethanol, chloroform etc.) was cleaned by method purified of double outstrip. Extraction of plant was carried out with using extractor of the Soxlet. It was obtained water and ethanol extracts of the degree of extraction cleanliness our examples was detection by spectroscopy method. Our experimental extracts of Artemisia Absinthium L. for studies their antioxidant effect on lipid peroxidation were tested. We also studied the influence ethanol extracts on hemolysis kinetics in different situation.

All data were expressed as the mean  $\pm$  standard error of six experiments using Student's test and special computer program MatLab.



## Results

The changing of ethanol solution per cent from 30 to 96 % we have increasing the time of extraction around 60%, in a case if concentration of plant material is same. We obtained changes by the measuring of redox potential and value of pH for extracts with different degree of ethanol. It is means, that our extract can be use as natural inhibitors in during oxidative damage in organism.

The obtained results showing depended antioxidant activity (AOA) from per cent of ethanol extracts. It is necessary for determination of total control phone in our experiments. According its ethanol extract inhibits the concentrations of Diene Conjugate (DC) and Malone Dialdehyde (MDA) and we have the antioxidant effects of plants for DC and MDA. However, when concentration of erythrocytes suspensions was increased, for example in case of determination of MDA, we obtained more high per cent of inhibition. The kinetics of these processes is different. The cover for controls and for control+96% ethanol systems is same. But in case adding in control systems 70%, 50% and 30% ethanol solution we obtained another by same cover. May be in last situation we must to take into account that quality of water, which adding for obtaining 70%, 50% and 30% ethanol solution.

Hemolysis induced by HCl solution was significantly by addition of *Artemisia Absinthium L.* extracts. The rate of hemolysis of biological target (human erythrocyte suspension) was reduced after influence plant extract. As control systems we used ethanol extract with different per cent. According those results for human health can be dangerously 96% ethanol extract. It is means 96% ethanol extracted more biological active compounds, which in case complex influence will have negative effect.

So, we try to develop novel approaches to correlate the level of lipid peroxidation and membrane damage for erythrocyte system. Our results shown that choose a method of extraction (time, temperature, using solution, etc.) can be determinate that pharmaceutical effect, which necessary for concrete case of organism damage. The important link in the biomedicine is an analytical processing of experimental results. It is a component of informational basis at prognoses of different diseases. We suggested using as novel statistical insights a computer program MatLab for modeling diseases connected with infringement in lipid peroxidation. One of the stronger sites of that computer program is repeated using of special program, getting up as M-files. Then by help MatLab-function's plot we has obtained the graphical view of study biological processes at its development. The approximations of that kinetic curve allow us to make interpolation by polynomial-function with necessary power. Then we obtain a coefficient and roots of polynomials exactness and other statistical insights. The using of MatLab by analytical processing of experimental results to allow us with once program file to get the results, which is a basic at the same time evaluation a lot of kinetic parameters for studying processing in whole.

Also, the method of peroxide haemolysis we suggest for using as specific test for detection of the presence different disease in an organism carried out by the activation of the lipid peroxidation processes. Our works out of mathematical computer program simulation for the determination of factors of stability membrane allow creating model kinetics of oxidation. These experiments was supported by ANSEF grant 07-NS-biochem-1440.

## X-RAY INELASTIC SCATTERING STUDY OF HUMAN CELL THz DYNAMICS USING A URANIUM BASED COLORANT

*M. Longo<sup>a</sup>, A. Orecchini<sup>a</sup>, A. Paciaroni<sup>a</sup>, C. Petrillo<sup>a</sup>, F. Sacchetti<sup>a</sup>, I. Pirisinu<sup>b</sup>, M. Calvitti<sup>b</sup> and R. Romani<sup>b</sup>*

<sup>a</sup>Dipartimento di Fisica, Università di Perugia, Via Alessandro Pascoli I-06123 Perugia, Italy. [marialucia.longo@fisica.unipg.it](mailto:marialucia.longo@fisica.unipg.it)

<sup>b</sup>Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università di Perugia, Via del Giochetto 06122 Perugia, Italy

### Introduction

In the framework of modern biophysics, the internal motions of key biomolecules in the picosecond domain play an essential role to turn on their biological activity [1,2,3]. Of course, the dynamical features of biomolecules strongly depend on the environment where they are embedded in. So far, mainly hydrated powder and solution states have been studied to simulate the coupling between the biomolecules and the aqueous solvent [4]. In order to deepen our understanding of the dynamical behaviour of biological macromolecules in their physiological environment, an inelastic x-ray scattering experiment on samples of U937 human cells has been performed. The main goal was to single out the dynamics of nuclear DNA in its natural condition. With this aim we stained the cells with a Uranyl salt, which has both high atomic number and high affinity with phosphoryl groups. We also compared the collective dynamics of the whole cell with that of nucleic acid.

### Results and Discussions

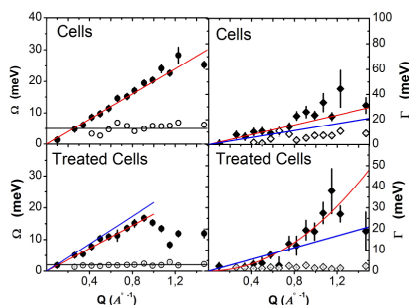
The analysis of the coherent scattering data, for the sample treated without and with staining by uranyl-acetate, has pointed out the existence of two collective excitations: a high-frequency propagating mode, which reveals a dispersive character, and a low-frequency mode of almost constant energy. From a first look at Figure 1, which provides the dispersion curves, a strong resemblance between the dynamical features of the whole cells and that of pure liquid water is shown.

Indeed, the propagation speed of 3000 m/s is absolutely comparable to that revealed in IXS and INS experiments on bulk water [5,6]. This value is also surprisingly higher than the sound velocity of 1500 m/s measured by an in-house ultrasound apparatus in the same sample. This finding suggests that

also in this sample a phenomenon reminiscent of the so-called fast sound is observed.

Due to the high concentrations of macromolecules in cellular environments, this unexpected likeness may be ascribed to a strong dynamical coupling between the biomolecules and the solvent, as if the collective dynamical behaviour of the cell as a whole was mainly ruled by water.

The dynamical features of the dyed nuclear DNA are instead quite different. The high-frequency mode shows a dispersive character with an initial linear trend in the low-Q region, which corresponds to a lower propagation speed of 2700 m/s. Moreover, the damping factors displays a quadratic dependence, a behaviour similar to that found in biological water and in several glassy systems.



**Figure 1.** Left panels: dispersion curves of the high-frequency (full circles) and low-frequency (empty circles) mode in the cells treated with and without staining by uranyl-acetate. The red continuous lines represent the linear fit yielding the propagation speed of the high-frequency mode. The black continuous lines describe the trend of the low-frequency mode, localized at 5 meV and 2 meV in the case of the entire cells and treated cells respectively. Right panels: damping factors of the high-frequency (full diamonds) and low-frequency (empty diamonds) mode in the cells treated with and without staining by uranyl-acetate. The red continuous lines represent the fit to the experimental data, which are compared with the linear behaviour observed in bulk water (blue continuous lines).

### References

1. P. W. Fenimore, H. Frauenfelder, B. H. McMahon and R. D. Young, Proc. Natl. Acad. Sci. USA ,101, 14408 (2004).
2. J. N. Onuchic, C. Kobayashi, O. Miyashita, P. Jennings and K.K. Baldrige, Phil. Trans. Roy. Soc. B-Biological Sciences 361, 1439 (2006).
3. A. Xie, A.F.G. Van der Meer and R.H. Austin, Phys. Rev. Lett. 88, 018102 (2002).

4. A. Orecchini, A. Paciaroni, A. De Francesco, C. Petrillo, and F. Sacchetti, *J. Am. Chem. Soc.* 131,4664 (2009).
5. F. Sacchetti, B. Dorner, J-B Suck, *Phys. Rev. E* 69,061203 (2004).
6. G. Ruocco, F. Sette, *J. Phys. Condens. Matter* 11, R259

## MODE OF ACTION OF DIFFERENT CRYOPROTECTIVE AGENTS USED FOR CRYOPRESERVATION OF STALLION SPERM

*Harriette Oldenhof<sup>a</sup>, Marina Gojowsky<sup>a</sup>, Shangping Wang<sup>b</sup>, Willem F. Wolkers<sup>b</sup> and Harald Sieme<sup>a</sup>*

<sup>a</sup>Clinic for Horses - Unit for Reproductive Medicine, University of Veterinary Medicine Hannover – Buenteweg 15, 30559 Hannover, Germany.

[harriette.oldenhof@tiho-hannover.de](mailto:harriette.oldenhof@tiho-hannover.de)

<sup>b</sup>Institute of Multiphase Processes, Leibniz Universitaet Hannover – Callinstrasse 36, 30167 Hannover, Germany.

Cryopreservation of sperm can be done using a combination of cryoprotective agents including glycerol, ethylene glycol, sucrose, hydroxyl ethylene starch (HES), egg yolk, and skim milk. Glycerol and ethylene glycol are small membrane permeable molecules. Sucrose (disaccharide) and HES (polysaccharide) are membrane impermeable and only provide extracellular protection. The aim of this study was to determine the mode of protective action of the various components that can be used for cryopreservation of stallion sperm. Differential scanning calorimetry was used to determine glass forming properties of the different cryoprotective agents. Liposomes with trapped carboxyfluorescein were used to study the ability of the protectants to stabilize membranes and prevent leakage during freezing and thawing. Post-freeze motility was determined using computer assisted sperm analysis. In addition, membrane integrity and osmotic tolerance limits of sperm were assessed by microscopic and flow cytometric analysis. Post-freeze plasma membrane integrity typically increases with increasing concentration of cryoprotectant if sperm is kept in cryopreservation medium after thawing. Post-thaw motility percentages using glycerol, sucrose or HES were found to be similar when used at their optimal concentrations. When cryopreserved sperm is diluted in isotonic buffer after thawing, however, sperm viability decreases. The decrease in viability upon return to isosmotic conditions is greater using non-permeable osmotically active protectants such as sucrose compared to glycerol or osmotically inactive HES. Skim milk-based protection buffer supplemented with egg yolk and glycerol exhibits a glass transition temperature at approximately -55 °C. Addition of sucrose or HES increases the glass transition temperature. Studies with liposomes showed that glycerol and ethylene glycol are able to protect against membrane leakage during freezing and thawing, whereas sucrose and HES do not. Taken together, cryoprotectants such as glycerol and ethylene glycol reduce the damaging effects of osmotic stress and stabilize membranes during freezing and thawing, whereas sucrose and HES increase the glass transition temperature.

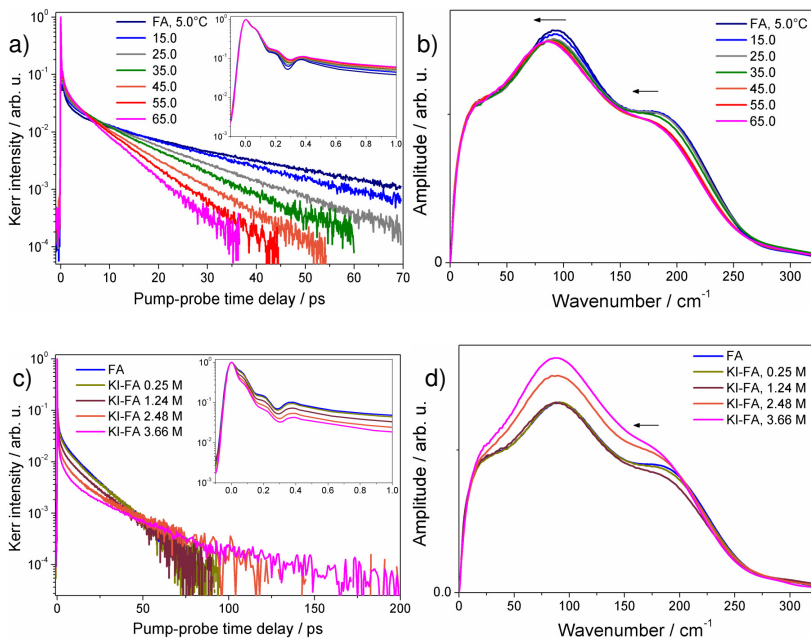
## IONIC EFFECTS ON H-BONDING LIQUID DYNAMICS STUDIED BY ULTRAFAST OKE SPECTROSCOPY

*Francesca Palombo and Stephen R. Meech*

School of Chemistry, University of East Anglia, Norwich Research Park,  
Norwich NR4 7TJ, United Kingdom. [F.Palombo@uea.ac.uk](mailto:F.Palombo@uea.ac.uk)

Ion solvation is relevant to a number of processes and mechanisms in technological, biological and pharmaceutical fields. Molecular dynamics of aqueous alkali halide solutions have previously been investigated using the time-resolved ultrafast optical Kerr effect (OKE) with a polarization-resolved approach to study the effects of ion solvation on water structure and dynamics [1,2].

Here, we focus on transient OKE and the corresponding low-frequency (THz) Raman spectra of formamide solutions of alkali metal halide salts. Through these studies we observe the effects of ion solvation on the dynamics of a non-aqueous high-permittivity H-bonding solvent. The picosecond orientational and ultrafast intermolecular dynamics of liquid formamide as a function of concentration of NaI and KI are compared with the temperature effect on the pure solvent dynamics. The effects of a range of other salts at fixed concentration are also recorded. Transient OKE and THz Raman spectra of the solutions revealed differences in the solvent dynamics caused by ion solvation. Increasing concentrations of NaI and KI have the effect of slowing down the diffusive reorientation and reducing the librational frequencies of formamide. Further a number of cation-specific effects on the THz Raman spectrum are reported. These effects are discussed in terms of an ion perturbation of the H-bonding structure in the solution. This approach provides a valuable means of investigating the dynamics, structure and interactions in complex, interacting systems [3-5].



**Figure 1.** Evolution in (a,c) Kerr transient and (b,d) THz Raman spectrum of (a,b) pure liquid formamide as a function of temperature, and (c,d) KI formamide solution as a function of concentration, up to near saturation, at 25°C. Arrows indicate the direction of the observed shifts with increasing temperature/concentration.

### References

1. I. A. Heisler, K. Mazur and S. R. Meech, *J. Phys. Chem. B*, 2011, **115**, 1863-1873.
2. I. A. Heisler and S. R. Meech, *Science*, 2010, **327**, 857-860.
3. F. Palombo and S. R. Meech, submitted to *J. Phys. Chem. B*.
4. F. Palombo, C. Barontini, M. Paolantoni, P. Sassi, A. Morresi and M. G. Giorgini, to be submitted to *Phys. Chem. Chem. Phys.*
5. F. Palombo, M. Paolantoni, P. Sassi, A. Morresi and M. G. Giorgini, *Phys. Chem. Chem. Phys.*, 2011, **13**, 16197-16207.



## MOLECULAR DYNAMICS OF WATER IN HYALURONIC ACID HYDROGELS

*A. Panagopoulou<sup>a</sup>, J. Vazquez Molina<sup>b</sup>, A. Kyritsis<sup>a</sup>, M. Monleón Pradas<sup>b,c</sup>, A. Vallés Lluch<sup>b</sup>, G. Gallego Ferrer<sup>b,c</sup> and P. Pissis<sup>a</sup>*

<sup>a</sup>National Technical University of Athens, Zografou Campus, 15780, Athens, Greece  
[panagann@central.ntua.gr](mailto:panagann@central.ntua.gr)

<sup>b</sup>Center for Biomaterials and Tissue Engineering, Universitat Politècnica de València,  
Camino de Vera s/n, 46022 Valencia, Spain

<sup>c</sup>CIBER en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

### Introduction

Hyaluronan is a carbohydrate, more specifically a mucopolysaccharide, occurring naturally in most living organisms. It is a linear polyanion, with a poly repeating disaccharide structure [(1→3)-β-D-GlcNAc-(1→4) β-D-GlcA-], which is energetically very stable. It can be found in various molecular forms like the acid form, hyaluronic acid (HA), and the salts, such as sodium hyaluronate, which form at physiological pH.

HA is one of the most hydrophilic molecules in nature and its role in living organisms is mainly that of a moisturizer and a lubricant. In solution the HA biopolymer forms a helical configuration which can be attributed to hydrogen bonding between the hydroxyl groups along the chain. Due to the significance of this molecule for biological procedures, a variety of commercially available preparations of HA derivatives and cross-linked HA materials have been developed for drug delivery and an extraordinary broad range of biological applications [1].

### Materials and Methods

Here we present a thermal and dielectric study in the case of mixtures of water and crosslinked HA (crosslinker: divinyl sulfone (DVS) at a molar ratio 0.8). We follow an experimental approach which has been previously used in the case of globular protein-water mixtures in wide ranges of temperature (123-273 K) and water composition [2,3]. Here the samples are in the form of hydrated solid specimens, hydrated either by vapor adsorption, or by swelling. The water fractions achieved are in the range of 70 wt% to 0 wt% (practically dry samples). The experimental techniques used are differential scanning calorimetry (DSC) and two dielectric techniques, dielectric relaxation spectroscopy (DRS) and thermally stimulated depolarization currents (TSDC). In addition, water equilibrium sorption-desorption (ESI) measurements are performed at room temperature.

## Results

ESI measurements showed a maximum water uptake of  $h=1.33$  (corresponding to 57 wt%) at relative humidity  $a_w=0.95$ , while a hysteresis was observed at desorption. The thermal transitions of water (crystallization and melting) were studied by DSC. Samples of water fraction up to 17 wt% showed no water crystallization nor during cooling or heating. At higher water fraction values, crystallization of water was present during cooling, showing multiple complex peaks. Cold crystallization of water was observed during heating. In addition, segmental dynamics (glass transition) was detected by DSC, depending on the water fraction. Dynamics of uncrystallized water, ice and segmental dynamics were followed by DRS.

The importance of the results is twofold. First, the dielectric study of HA dynamics in literature is rare and focused more in HA salt solutions at physiological temperatures [4], so these results are somehow novel. Second, the combination of the above mentioned experimental techniques have led to very important conclusions relevant to the origin of the polysaccharide glass transition and the dynamics of water at the polysaccharide interface, analogous to those in the case of BSA-water mixtures [2,3]. The parallel studies of protein-water systems and a structurally simpler molecule like HA, regarding the evolution of dynamics with water composition, may help in clarifying the role of water in biological function.

## Acknowledgements

The authors would like to acknowledge Sara Poveda Reyes and María Hernández Palacios for the preparation of the hyaluronic acid sheets.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

## References

1. J. Necas, L. Bartosikova, P. Brauner, J. Kolar, *Veterinari Medicina* 53 (8): 397-411 (2008).
2. A. Panagopoulou, A. Kyritsis, R. Sabateri Serra, J. L. Gómez Ribelles, N. Shinyashiki and P. Pissis, *J. Biochim. Biophys. Acta* 1814, 1984-1996 (2011).
3. A. Panagopoulou, A. Kyritsis, N. Shinyashiki, P. Pissis, *J. Phys. Chem. B* 116, 4593-4602 (2012).
4. T. Vuletić, S. Dolanski Babić, T. Ivek, D. Grgičin, S. Tomić, R. Podgornik, *Phys. Rev. E* 82, 011922-011932 (2010).

## HYDRATION DYNAMICS OF BIOLOGICAL WATER: MOLECULAR MODELING OF DEPOLARIZED LIGHT SCATTERING EXPERIMENTS

*M. Paolantonj<sup>a</sup>, L. Lupi<sup>b</sup>, L. Comez<sup>c</sup>, D. Fioretto<sup>b</sup> and B. M. Ladanyi<sup>d</sup>*

<sup>a</sup>Dipartimento di Chimica, Università di Perugia, via Elce di Sotto 8, I-06123 Perugia, Italy. [marcopa@unipg.it](mailto:marcopa@unipg.it)

<sup>b</sup>Dipartimento di Fisica, Università degli studi di Perugia, via A. Pascoli 1, I-06123 Perugia, Italy

<sup>c</sup>IOM-CNR, c/o Dipartimento di Fisica, Università di Perugia, via A. Pascoli 1, I-06123 Perugia, Italy.

<sup>d</sup>Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523-1872, United States

Information on the hydration dynamics of biorelevant molecules at picosecond time scales can be obtained both in the frequency and in the time domain, by depolarized light scattering (DLS) and time-resolved optical Kerr effect (OKE) experiments, respectively [1,2]. In simple terms, both techniques depend on the relaxation of the collective polarizability anisotropy of the system and probe a water restructuring process, connected with the fast reorganization of the hydrogen bonding network [1].

Recently DLS experiments extended in a wide frequency range (EDLS) have been employed in our laboratories here in Perugia to study the hydration dynamics in the shell of several biomolecules, such as sugars, peptides and proteins [3-6]. Owing to its relevance in biophysical and biochemical processes this interfacial water is often referred to as "biological water" [7].

For all the aqueous solutions investigated, two relaxation processes related to hydration and bulk water have been detected, providing an estimate of the dynamical retardation induced by the solute on the surrounding water and of the number of solvent molecules dynamically affected (i. e. dynamical hydration number) [3-6]. Nevertheless, considering the collective character of the physical properties probed by these techniques and the complexity of the processes under exam, molecular dynamics (MD) simulations have been also employed to deeply understand experimental results [8].

In the present study MD simulations were used to calculate time correlation functions (TCFs) of the anisotropic component of the collective polarizability of aqueous solutions of glucose and trehalose at different concentrations. Corresponding spectral profiles were found to be in agreement with experimental ones. Computed TCFs were separated into solute, solvent and cross terms between the two, highlighting the presence of two distinct

relaxation processes in water, related to hydrating and bulk components. A concentration-independent retardation factor of *ca.* 5 is estimated for hydration water compared to the bulk, while hydration numbers are found to decrease at higher sugar concentrations. These results agree with experimental findings suggesting that DLS and OKE represent suitable approaches to explore the hydration dynamics of biological water [8].

### **References**

1. M. Paolantoni, P. Sassi, A. Morresi and S. Santini, *J. Chem. Phys.* 127, 024504 (2007).
2. K. Mazur, I. A. Heisler and S. R. Meech, *J. Phys. Chem. A* 116, 2678-2685 (2012).
3. M. Paolantoni, L. Comez, M. E. Gallina, P. Sassi, F. Scarponi, D. Fioretto and A. Morresi *J. Phys. Chem. B* 113, 7874-7878 (2009).
4. M. E. Gallina, L. Comez, A. Morresi, M. Paolantoni, S. Perticaroli, P. Sassi and D. Fioretto, *J. Chem. Phys.* 132, 214508 (2010).
5. S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, L. Lupi, D. Fioretto, A. Paciaroni and A. Morresi, *J. Phys. Chem. B* 114, 8262-8269 (2010).
6. S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, A. Morresi and D. Fioretto, *JACS* 133, 12063-12068 (2011).
7. S. K. Pal, J. Peon, A. H. Zewail, *PNAS* 99, 1763-1768 (2002).
8. L. Lupi, L. Comez, M. Paolantoni, D. Fioretto and B. M. Ladanyi, *J. Phys. Chem. B* 116, 7499-7508 (2012).

## BIOLOGICAL HYDRATION WATER DYNAMICS PROBED BY EXTENDED LIGHT SCATTERING AND BROADBAND DIELECTRIC SPECTROSCOPIES

*S. Perticaroli, M. Nakanishi and A. P. Sokolov*

Department of Chemistry, The University of Tennessee, Knoxville, TN 37996–1600, USA; Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6453, USA. [spertica@utk.edu](mailto:spertica@utk.edu)

We have studied the dynamics of water in aqueous solutions of selected sugars and peptides at room temperature by Extended Depolarized Light Scattering (EDLS) and Broadband Dielectric (BDS) Spectroscopies.

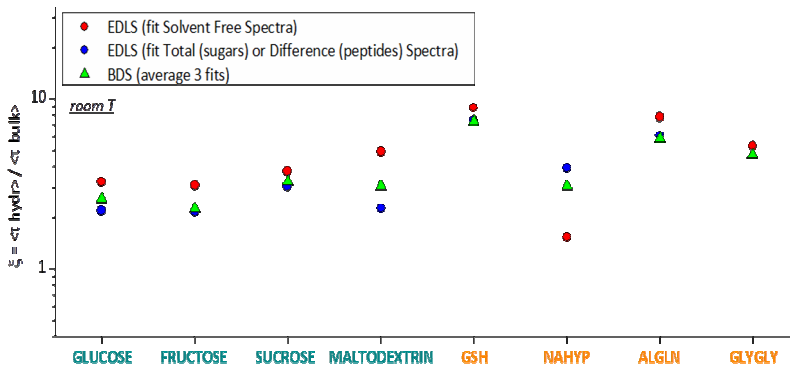
This is the first combined EDLS – BDS study on hydrophilic and amphiphilic solutes where both the slowing down of the hydration water dynamics and the spatial extent of the solvent perturbation revealed by both techniques have been directly compared.

We show here that it is possible to obtain consistent parameters from each technique for sugars, and note systematic differences in the estimation of the hydration number for peptide solutions.

The figure below shows the water retardation factors,  $\xi$ , in very good agreement for both experimental techniques. Our analysis reveals that water molecules located in the hydration shells of carbohydrates are slowed down  $\sim 4$  times relative to the bulk solvent, while water molecules surrounding peptides are more strongly retarded ( $\xi$  up to  $\sim 8$ ).

This peculiarity in the hydration water relaxation of peptides solutions had been ascribed to frustration of the structural dynamics of the hydrogen-bond network which is induced by the presence of hydrophobic and hydrophilic functional groups of solutes molecules [1-4].

EDLS and BDS results also illustrate that the solute-induced perturbations in carbohydrates-water systems are short-ranged: in 10 wt% sugar solutions, only  $\sim 15$ -25 % water molecules surrounding sugars are affected by the solute. Interestingly, there appear to be systematic differences in the estimation of hydration number for peptides samples. Values estimated from EDLS are always higher than those obtained from the analysis of the dielectric spectra, indicating a perturbation extending beyond the first hydration shell. These measurements highlight the strength of estimates of retardation factor from both techniques and the challenges stemming from comparisons of complimentary, but fundamentally different, experimental techniques.



**Figure 1.** Retardation factor  $\xi$  of hydration water dynamics obtained by EDLS (circles) and BDS (triangles) measurements on carbohydrates and peptides solutions ( $\Phi_{\text{solute}}=10\%$  wt, room Temperature).

### References

1. Murarka, R. K.; Head-Gordon T. *J. Phys. Chem. B* **2008**, *112*, 179-186.
2. Murarka, R. K.; Head-Gordon T. *J. Chem. Phys.* **2007**, *126*, 215101/1-9.
3. Sasisanker, P.; Weingärtner *Chem. Phys. Chem.* **2008**, *9*, 2802-2808.
4. Perticaroli, S.; Comez, L.; Paolantoni, M.; Sassi, P.; Morresi, A.; Fioretto, D. *J. Am. Chem. Soc.* **2011**, *133*, 12063–12068.

# THERMAL CHARACTERISTICS OF FREEZE-DRIED CAMEL MILK AND ITS MAJOR COMPONENTS

*Mohammad Shafiur Rahman, Halaa Al-Hakmani, Ahmed Al-Alawi, and Insaaf Al-Marhubi*

Department of Food Science and Nutrition, Sultan Qaboos University, P. O. Box 34, Al-Khod 123, Oman. [shafiur@squ.edu.om](mailto:shafiur@squ.edu.om)

## Introduction

Thermal characteristics, such as glass transition, melting and freezing point of foods are important in determining its stability during processing and storage [1]. It is important to know the melting point of fat since milk storage above melting could initiate undesirable agglomeration and caking [2]. Most of the cases in the literature, thermal characteristics of whole foods or food ingredients were measured. However negligible work was presented by measuring thermal characteristics of foods and its extracted or separated components as well. Negligible research works were reported on the thermal characteristics of camel milk powder, especially for its major components. The objectives of this study were to determine the thermal characteristics of whole and skimmed camel milk; and its isolated components (i.e. fat, cream, casein, whey protein and lactose) using Differential Scanning Calorimetry (DSC). These thermal characteristics data could be used in determining the storage stability of dried camel milk.

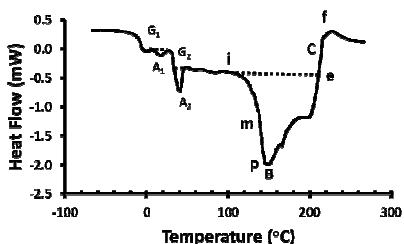
## Materials and Methods

Whole camel milk and its extracted components (skimmed milk, cream, fat, casein, whey, and lactose) were freeze dried in a laboratory freeze-drier at 20°C and 100 Pa. All freeze-dried samples were equilibrated in air-sealed jar maintained at relative humidity 11.3%. Differential Scanning Calorimetry (DSC Q10, TA Instruments, New Castle, Delaware, USA) were used to measure the glass transition and melting. The procedures for thermal analysis were similar as discussed by Rahman et al. [3].

## Results

The thermogram of whole milk showed three endothermic peaks (two for fat-melting and one for non-fat solids-melting) and three shifts (Figure 1). Two shifts at low temperature were related to the glass transitions. The shift at higher temperature after melting of non-fat solids could be related to the structure ordering in milk after solids-melting. Different major components of

the camel milk (fat, cream, casein, whey protein, and lactose) were separated and then measured its thermal characteristics.



**Figure 1.** DSC thermogram of freeze-dried whole milk powder equilibrated at 11.3% relative humidity (G<sub>1</sub>: first glass transition, G<sub>2</sub>: second glass transition, A<sub>1</sub>: first fat-melting, A<sub>2</sub>: second fat-melting, B: solids-melting, i: onset, m: maximum slope, p: peak, e: end, C: structure-forming, and f: end of structure-forming)

The first melting peak of fat started at -5°C and second peak started at 38°C and ended at 52°C, respectively. Casein showed one endothermic peak due to solids-melting and two shifts in the thermogram line indicating two glass transitions. Similarly whey protein precipitated by ammonium sulfate and ethanol also showed two glass transitions and one melting endotherm. In the cases of all types of protein, the second glass transition was observed just before solids-melting. In the case of first scan of lactose, only two endothermic melting peaks were observed without any trace of glass transition. However, the second scan with annealing showed two glass transitions and two endothermic peaks, onset of the first and second glass transitions were at 56 and 114°C, respectively. The onsets of first and second melting endotherms were observed at 145 and 213°C, respectively.

### Acknowledgement

This study was funded by SQU-UAEU collaborative project CL/SQU/UAEU/08/01.

### References

1. M. S. Rahman, Trends Food Sci. Technol.17, 129-141 (2006).
2. B. Adhikari, T. Howes B. R. Bhandari, V. Truong, Int. J. Food Properties. 4(1), 1-33 (2001).
3. M. S. Rahman, G. Al-Saidi, N. Guizani, A. Abdullah, Thermochimica Acta. 509, 111-119 (2010)



## EFFECT OF WATER CONFINEMENT IN CYCLODEXTRIN-BASED POLYMERS: A JOINT INFRARED AND RAMAN SPECTROSCOPIC STUDY

*Vincenza Crupi<sup>a</sup>, Domenico Majolino<sup>a</sup>, Andrea Mele<sup>b</sup>, Barbara Rossi<sup>c</sup>,  
Francesco Trotta<sup>d</sup> and Valentina Venuti<sup>a</sup>*

<sup>a</sup>Department of Physics, University of Messina and CNISM UdR – Viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy

<sup>b</sup>Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano – Via Mancinelli 7, 20131 Milano, Italy.

<sup>c</sup>Department of Computer Science, University of Verona – Strada le Grazie 15, 37134 Verona, Italy. [rossi@science.unitn.it](mailto:rossi@science.unitn.it)

<sup>d</sup>Department of Chemistry, University of Torino, Via Pietro Giuria 7 – 10125 Torino, Italy.

### Introduction

Cyclodextrins (CD) are cyclic oligomers of amilose with consolidated and well assessed characteristics of host molecules for supramolecular inclusion complexes [1]. These host-guest complexes find several applications, mainly in the pharmaceutical field, such as water solubility enhancement, controlled release and stabilization of active pharmaceutical ingredients.

More recently, great effort has been devoted to the designing and production of cyclodextrins-based polymers able to improve the performance of CD as host molecules [2]. In this framework, the easy preparation of cross-linked polymers by reaction of CD with suitable activated derivatives of carboxylic acids has been proposed [3]. The corresponding materials are referred to as cyclodextrin nanospunges (CDNS). The main feature of these polymeric matrices is the simultaneous presence of lyphophilic cavities and hydrophilic channels which can be used to encapsulate, carry and/or release a great variety of substances. CDNS are insoluble in water and in most common organic solvents, but some classes of CDNS may undergo swelling in the presence of aqueous solutions, giving rise to a characteristic gel-like behaviour.

The thorough physical-chemical and structural characterization of these materials is a challenging task and several spectroscopic methods are being used [4-6]. In particular, the swelling process of CDNS was investigated by the analysis of the O–H and C–H groups vibrational modes, whereas high resolution magic angle spinning nuclear magnetic resonance (HRMAS-NMR) measurements on hydrated samples gave clues of “free” and “bound” water molecules in the polymeric gel with different diffusion coefficients [7].

## Results and discussion

Here, we present some results on the investigation of swelling properties in PMA-nanosponges, a class of CDNA obtained by polymerization of CD with pyromellitic dianhydride (PMA) as cross-linking agent. In particular the effect of water confinement into the nanopores of PMA-nanosponges will be studied by using Raman and Fourier transform Infrared (in Attenuated Total Reflectance geometry, FTIR-ATR) spectroscopies as a function of temperature, level of hydration and cross-linking degree of the polymers. Moreover, the differences in the vibrational dynamics of PMA-nanosponges swollen with H<sub>2</sub>O and D<sub>2</sub>O will be exploited, in order to better understand the role played on the dynamic peculiarities of water arrangements both by the bare geometrical confinement and the specific interaction with chemical groups of the polymers.

We will focus the attention on the experimental FTIR-ATR and Raman spectra of the intramolecular O–H stretching vibration, whose spectral contributions are assigned to different water molecules arrangements.

## References

1. M.L. Bender and M. Komiyama, Cyclodextrin chemistry. Springer, New York 1978
2. F. Van de Manacker, T. Vermonden, C.F. Van Nostrum and W.E. Hennink, *Biomacromolecules* 10, 3157–3175 (2009).
3. F. Trotta, W. Tumiatti, Cross-linked polymers based on cyclodextrin for removing polluting agents. WO 03/085002 (2007).
4. F. Castiglione, V. Crupi, D. Majolino, A. Mele, W. Panzeri, B. Rossi, F. Trotta and V. Venuti, *J. Incl. Phenom. Macrocycl. Chem.*, DOI: 10.1007/s10847-012-0106-z (in press).
5. B. Rossi, S. Caponi, F. Castiglione, S. Corezzi, A. Fontana, M. Giarola, G. Mariotto, A. Mele, C. Petrillo, F. Trotta and G. Vilianni, *J. Phys. Chem. B*, 116(17), 5323-5327 (2012).
6. F. Castiglione, V. Crupi, D. Majolino, A. Mele, B. Rossi, F. Trotta and V. Venuti, *J. Phys. Chem. B*, DOI: 10.1021/jp303006a (in press).
7. A. Mele, F. Castiglione, L. Malpezzi, F. Ganazzoli, G. Raffaini, F. Trotta, B. Rossi and A. Fontana, *J. Incl. Phenom. Macrocycl. Chem.*, 69(3-4), 403-409 (2011).

## RAMAN AND FLUORESCENCE INVESTIGATIONS OF PHASE TRANSITION IN SWOLLEN CYCLODEXTRIN NANOSPONGES: FROM GEL TO WATER SOLUTION

*Barbara Rossi<sup>a,b</sup>, Valentina Allodi<sup>a</sup>, Francesca Castiglione<sup>c</sup>, Graziano Guella<sup>b</sup>, Gino Mariotto<sup>a</sup>, Andrea Mele<sup>c</sup> and Francesco Trotta<sup>d</sup>*

<sup>a</sup>Department of Computer Science, University of Verona – Strada le Grazie 15, 37134 Verona, Italy. [rossi@science.unitn.it](mailto:rossi@science.unitn.it)

<sup>b</sup>Department of Physics, University of Trento – Via Sommarive 14, 38123 Povo, Trento, Italy

<sup>c</sup>Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano – Via Mancinelli 7, 20131 Milano, Italy.

<sup>d</sup>Department of Chemistry, University of Torino, Via Pietro Giuria 7 – 10125 Torino, Italy.

Cyclodextrin nanosponges (CDNS) are a new class of cross-linked nanoporous polymers obtained by reaction of cyclodextrins (cyclic oligomers made of 6, 7 or 8 glucopyranose units) with a suitable polyfunctional cross-linking agents [1]. Cyclodextrins (CD) polymerize in a three-dimensional network, whose main feature is the presence of both lyphophilic cavities and hydrophilic channels which can be used to encapsulate, carry and/or release a great variety of substances. Moreover, the use of different cross-linking agents provides a suitable modulation of physico-chemical properties of the final material. For these reasons CDNS have been exploited in a variety of fields, including bio-catalysis, agriculture, environmental control and pharmaceutical applications [2,3].

Recently, we focused our attention on a specific class of CDNS obtained by polymerization of  $\beta$ -CD with pyromellitic dianhydride (PMA) as cross-linking agent with different  $\beta$ -CD/PMA molar ratios [4-6]. These polymers are insoluble in water and in most common organic solvents, but they may undergo swelling in the presence of aqueous solutions, giving rise to a characteristic gel phase. Moreover, the swelling properties of these materials, i.e. the level of hydration, the viscosity of the gel and its stability with respect to ageing, depends on their degree of cross-linking, which is in turn correlated to nanopores dimensions.

The thorough physico-chemical and structural characterization of nanosponges is a challenging task and several spectroscopic methods are being used for investigating the cross-linking properties of these polymers in dry state [4-6]. In particular, the inspection of the vibrational dynamics of CDNS in different frequency regimes allowed to recognize some important descriptors of the cross-linking degree of the polymers, as a function of the molar ratio between the monomers CD and PMA used during the reaction of

polymerization. Moreover, the analysis of fluorescence spectra of the same samples revealed interesting correlations between the stiffness of the polymers and the wavelength of their emission band.

Here, special attention will be devoted to the ageing process of swollen PMA-nanosponges which evolve in time, passing from a gel phase to a liquid solution. The Raman and fluorescence spectra recorded on swollen CDNS with H<sub>2</sub>O and D<sub>2</sub>O at different ageing times will be analysed and the observed spectral changes will be correlated with the changes observed in the viscosity of the system.

Comparison of the results obtained for the D<sub>2</sub>O solvent with those for the H<sub>2</sub>O solvent will elucidate the deuteration influence on the kinetics of the swelling process.

### **References**

1. F. Trotta, W. Tumiatti, Cross-linked polymers based on cyclodextrin for removing polluting agents. WO 03/085002 (2007).
2. B. Boscolo, F. Trotta and E. Ghibaudi, J. Mol. Catal. B: Enzym. 62, 155 (2010).
3. B. B. Mamba, R. W. Krause, T. J. Malefetse and E. N. Nxumalo, Environ. Chem. Lett. 5, 79 (2007).
4. A. Mele, F. Castiglione, L. Malpezzi, F. Ganazzoli, G. Raffaini, F. Trotta, B. Rossi and A. Fontana, J. Incl. Phenom. Macrocycl. Chem., 69(3-4), 403-409 (2011).
5. B. Rossi, S. Caponi, F. Castiglione, S. Corezzi, A. Fontana, M. Giarola, G. Mariotto, A. Mele, C. Petrillo, F. Trotta and G. Viliiani, J. Phys. Chem. B, 116(17), 5323-5327 (2012).
6. F. Castiglione, V. Crupi, D. Majolino, A. Mele, B. Rossi, F. Trotta and V. Venuti, J. Phys. Chem. B, DOI: 10.1021/jp303006a (in press).

## ACTIVITY OF HORSERADISH PEROXIDASE AND BOVINE LACTOPEROXIDASE AS AFFECTED BY PHYSICAL PROPERTIES OF THE SOLUTION

*Giampiero Sacchetti, Lilia Neri, Dino Mastrocola and Paola Pittia*

Department of Food Science, University of Teramo – Via Lerici 1,  
64023 Mosciano S. Angelo (TE), Italy. [gsacchetti@unite.it](mailto:gsacchetti@unite.it)

### Introduction

Enzymatic activity in solution is largely influenced by environmental factors such as the physical properties of the system.

Viscosity is referred to as an index of the mobility of a system since it is related to the translational diffusion by the Stokes relationship. Kramers' theory [1] predicts that the rate for the dynamics should have an inverse relationship with viscosity. Even if a large body of work indicates that Kramers' theory does not always predict protein dynamics properly [2], large amplitude (global) protein motions and, in particular, those involving exterior portions of the protein, appear to follow Kramers' relation at viscosities above  $\approx 1$  cP [3,4] whilst diffusive motions involving smaller portions of the protein are less likely to follow Kramers' relation at high viscosities [2]. Deviations from Kramers' relation for exterior protein motions has been attributed to solvent composition and viscosity in the immediate vicinity of the protein.

Independently of viscosity, other physical variables could affect the enzyme kinetics in solutions. In particular, in highly viscous concentrated solutions, water activity, which is a thermodynamical variable extensively used to describe the chemical potential of water in binary and complex systems, could play a significant role [5].

The aim of this study was to investigate and compare the effect of viscosity and water activity ( $a_w$ ) on the activity of horseradish peroxidase (HRP) and bovine lactoperoxidase (LPO) in buffered solutions. HRP (44,173.9 Da) is an enzyme with an highly accessible active site whilst LPO is a larger enzyme (78,431 Da) with a deeply buried active site with a restrictive substrate access channel which should limit the influence of physical properties of the solution (such as viscosity and water activity) on enzymatic catalysis.

## Experimental

Concentrated binary and ternary buffered solutions with were prepared by using 0.1 M potassium phosphate buffer, maltose, trehalose and maltodextrins ( $\overline{M}_w$  : 30.000).

HRP and LPO activity was tested in buffered solutions using the method of Keesey [6]. Viscosity was measured using a falling ball viscometer while water activity was measured using a dew point hygrometer. All measurements were carried out at 25 °C. Differential scanning calorimetry was used for the T'g determination. Thermograms were obtained after annealing at T'g + 10 °C.

## Results

In viscous solutions characterized by different composition, the inhibition of the HRP activity was dependent both on the thermodynamical properties of the solution, as described by water activity, and on the mobility of the system, as described by the inverse of viscosity and T-T'g. Viscosity was the most important factor in the inhibition of enzymatic activity in solutions characterized by the same T'g value, but when T'g was changed, due to changes in the solutes composition, the latter became the key factor in the regulation of the enzyme activity.

HRP activity was much more influenced by changes in the water activity than LPO activity which, in turn, was much more influenced by changes in viscosity. The results of this study suggest that for large enzymes the translational diffusivity which, according to the Stokes-Einstein law, is inversely related to viscosity and molecular weight, is a far more critical factor than the accessibility of the active site. On the other hand, the water status of the solution seems to be a far more critical factor for the activity of enzymes with an active site highly accessible and expose to the external environment.

## References

1. H. A. Kramers, *Physica* 7, 284-304 (1940).
2. M. T. Cicerone and C. L. Soles, *Biophys. J.* 86, 3836-3845 (2004).
3. A. C. Ansari, C. M. Jones, E. R. Henry, J. Hofrichter and W. A. Eaton, *Science* 256, 1796-1798 (1992).
4. S. Hagen, J. Hofrichter and W. A. Eaton, *Science* 269, 959-962 (1995).
5. L. Neri, P. Pittia, G. Bertolo, D. Torreggiani and G. Sacchetti, *J. Food Eng.* 101: 289-295 (2010).
6. J. Keesey, *Biochemica Information*, Indianapolis: Boehringer Mannheim Biochemicals, 1987, pp.58.

## DISSOCIATION OF WATER UNDER ELECTRIC FIELD

*F. Saija<sup>a</sup>, G. Cassone<sup>b</sup>, P. V. Giaquinta<sup>b</sup> and A. M. Saitta<sup>c</sup>*

<sup>a</sup>CNR-IPCF, Messina, Italy [saija@me.cnr.it](mailto:saija@me.cnr.it)

<sup>b</sup>Università degli Studi di Messina, Dipartimento di Fisica, Messina, Italy

<sup>c</sup>IMPMC, CNRS-UMR 7590, Université P. and M. Curie, Paris, France

The behavior of liquid water under an electric field is a crucial phenomenon in science and engineering. However, its detailed description at a microscopic level is difficult to achieve experimentally. Here we report on the first *ab initio* molecular dynamics study on water under electric field. Fields beyond a threshold of 0.35 V/Angstrom are able to dissociate molecules, and sustain an ionic current that occurs via a series of correlated proton jumps. This result paves the way to a whole new branch of quantum-accurate microscopic theoretical calculations on the effect of electric fields on aqueous solutions, and thus to massive applications of *ab initio* molecular dynamics in neurobiology, electrochemistry, and the nascent hydrogen economy.

## DYNAMICS OF LIQUID AND SUPERCOOLED WATER AND OF THE LiCl-6H<sub>2</sub>O SOLUTION INVESTIGATED BY BRILLOUIN LIGHT SCATTERING AND INELASTIC SCATTERING OF SYNCHROTRON RADIATION

S. C. Santucci<sup>a,b,c</sup>, C. Masciovecchio<sup>c</sup>, L. Comez<sup>b</sup>,  
J-F Legrand<sup>d</sup>, D. Fioretto<sup>b</sup>

<sup>a</sup>European Synchrotron Radiation Facility, 6 Rue Jules Horowitz,  
38000, Grenoble, France. [silvia.santucci@gmail.com](mailto:silvia.santucci@gmail.com)

<sup>b</sup>Dipartimento di Fisica, Università degli Studi di Perugia,  
Via Pascoli 1, 06100 Perugia, Italy.

<sup>c</sup>Sincrotrone Elettra, Strada Statale 14, Km 163,5  
in AREA Science Park, 34149 Basovizza, Trieste, Italy.

<sup>d</sup>Institut Charles Sadron, 23 rue du Loess - BP 84047, 67034  
Strasbourg Cedex 2, France.

The diagram of the metastable phases of water is a long-time puzzle, because of the presence of the *no-man's land*, located between the supercooled and the glassy phase, where non-crystalline bulk water has never been observed and where singularities of transport and thermodynamic properties are inferred at 228 K and ambient pressure. Due to the experimental inaccessibility of *the no-man's land*, a commonly agreed explanation of the eccentric properties of water is not yet reached. Dynamical and thermodynamical interpretations have been proposed. The presence of a second critical point is very debated [1].

We measured the relaxational dynamics of liquid and supercooled water down to 253 K, by means of inelastic ultraviolet and Brillouin light scattering [2, 3]. The measured structural relaxation time follows a power law, which diverges at 220 K [2], in agreement with the predictions of molecular dynamic simulations [4]. We availed of the use of ionic solutions to circumvent the freezing of water. We measured the dynamics of the LiCl-6H<sub>2</sub>O solution at ambient pressure down into the glassy phase (T<sub>g</sub> = 135 K) and over 5 frequency decades, by inelastic scattering of synchrotron radiation and light [5]. At this particular concentration, the system remains in a liquid metastable phase at those temperatures corresponding to the *no-man's land* of water.

At high temperature, the dynamics of the solution is characterized by a single relaxation process. The high frequency speed of sound is comparable to that of water. The relaxation splits into a structural relaxation and a



secondary relaxation at about 220 K, where the structural relaxation time of the pure solvent apparently diverges.

According to recent experiments and calculations, this scenario could be compatible with the hypothetical liquid-liquid transition in pure water, which would occur at lower temperatures and higher pressures than those studied ( $T'_{cr} \approx 220$  K,  $P'_{cr} \approx 100$  MPa). The presence of ions would hence have the effect to translate the phase diagram in the pressure-temperature plane. Indeed, the comparison of water to aqueous solutions is currently being proposed by several groups as a route to the pure solvent, e.g. [6]. A deeper investigation of the nature and the role of the hydrogen bond in the pure solvent and the solutions is also encouraged by several authors. Recent efforts have been done by molecular dynamics simulations [7], X-ray absorption and resonant and non-resonant X-ray emission spectroscopies [8, 9]. The pursuit of a picture of water which conciliates coherently the dynamical and structural aspects, will be a fascinating challenge.

### **References**

1. P. G. Debenedetti, *J. Phys.: Condens. Matter* 15, R1669–R1726 (2003).
2. C. Masciovecchio, S. C. Santucci, A. Gessini, S. Di Fonzo, G. Ruocco, F. Sette, *Phys. Rev. Lett.* 92, 255507 (2004).
3. S. C. Santucci, D. Fioretto, L. Comez, A. Gessini, C. Masciovecchio, *Phys. Rev. Lett.* 97, 225701 (2006).
4. F. Sciortino, L. Fabbian, S-H Chen, P. Tartaglia, *Phys. Rev. E* 56, 5397 (1997).
5. S. C. Santucci, L. Comez, F. Scarponi, G. Monaco, R. Verbeni, J-F Legrand, C. Masciovecchio, A. Gessini, D. Fioretto, *J. Chem. Phys.* 131, 154507 (2009).
6. D. Corradini, M. Rovere and P. Gallo, *J. Chem. Phys.* 132, 134508 (2010).
7. Y. Tu, S. V. Buldyrev, Z. Liu, H. Fang and H. E. Stanley, *Europhys. Lett.* 97, 56005 (2012).
8. J.-H. Guo, Y. Luo, A. Augustsson, J.-E. Rubensson, C. S  the, H.   gren, H. Siegbahn, and J. Nordgren, *Phys. Rev. Lett.* 89, 137402 (2002) ; J. Forsberg, J. Gr  sjo, B. Brena, J. Nordgren, L-C. Duda, and J-E Rubensson, *Phys. Rev. B* 79, 132203 (2009).
9. A. Nilsson and L. G. M. Pettersson, *Chem. Phys.* 389, 1 (2011).

## OPTICAL MANIPULATIONS OF BIOLOGICAL SINGLE SAMPLES IN AQUEOUS ENVIRONMENTS FOR SYNCROTRON RADIATION INVESTIGATIONS

S. C. Santucci<sup>a,b</sup>, D. Cojoc<sup>b</sup>, H. Amenitsch<sup>c</sup>,  
M. Burghammer<sup>a</sup> and C. Riekel<sup>a</sup>

<sup>a</sup>European Synchrotron Radiation Facility, 6 Rue Jules Horowitz,  
38000, Grenoble, France. [silvia.santucci@gmail.com](mailto:silvia.santucci@gmail.com)

<sup>b</sup>Laboratorio TASC, CNR-IOM Istituto Officina dei Materiali, Area Science Park  
Basovizza, I-34149, Trieste, Italy.

<sup>c</sup>Institute of Biophysics and Nanosystems Research, Austrian Academy  
of Sciences, A-8042 Graz, Austria.

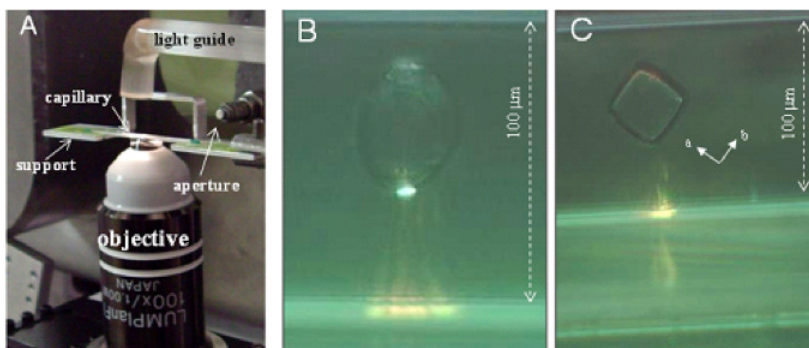
It is well known in biological research that the investigations of single samples such as cells and biomolecular complexes, may provide a more accurate understanding of the relation between the macroscopic functionalities and the microscopic properties, with respect to the averaged properties measured over a large population [1]. Single particle studies open wide domains of knowledge and applications, for example in cancer diagnose, drug delivery, single particle chemistry, serial protein crystallography. Optical tweezers [2], based on the trapping capability of focused laser beams, provide forces in the femto- to nanoNewton range, ideal for the manipulations of soft materials in aqueous environments, such as cells, colloidal crystals, liposomes and viruses.

On the other hand, the availability of intense microbeams and, more recently, nanobeams at 3rd generation synchrotron radiation sources allows probing local structures in hierarchically organized materials by small-angle and wide-angle X-ray scattering techniques (SAXS/WAXS). The small beam dimension also allows the reduction of the investigated object dimension, making it possible the study of membrane protein crystals of a few  $\mu\text{m}$  dimensions, which are difficult to crystallize. Complementary imaging techniques such as X-ray microscopy and coherent X-ray diffraction imaging contribute to an understanding of the structural properties of such systems.

We have developed a dedicated optical tweezers setup at the ID13 microfocus beamline of the ESRF, with a compact, portable, and versatile geometry for sample manipulations in synchrotron radiation applications [3]. Objects of a few micrometers up to a few tens of micrometers size can be trapped for extended periods of time. The selection and positioning of single objects out of a batch of many can be performed semi-automatically by software routines. The performance of the setup has been tested by wide-

angle and small-angle X-ray scattering experiments on single optically trapped starch granules, using a synchrotron radiation microbeam.

In addition, we have demonstrated for the first time the feasibility of microdiffraction on optically trapped protein crystals. Starch granules and insulin crystals were repeatedly raster-scanned at about 50 ms exposure/raster-point up to the complete loss of the structural order. Radiation damage in starch granules results in the appearance of low-angle scattering due to the breakdown of the polysaccharide matrix. For insulin crystals, order along the densely packed [110] direction is preferentially maintained until complete loss of long-range order.



**Figure 1.A** Sample environment of the OT setup on the ID13 beamline sample stage. An aqueous suspension of samples is contained in a 100 microns inner side square-section glass capillary. A water immersion microscope objective in upright position focuses the traps. The objective is also used for the real time bottom-view imaging of the investigated sample. The microfocus X-ray beam is delivered through the aperture visible in the figure. SAXS/WAXS mesh scans have been performed. **1.B** Optically trapped 40 microns diameter starch granule and **1.C** 35 microns diameter insulin crystal.

### References

1. A. E. Knight, *Single Molecule Biology*. (Elsevier, Oxford), 2008 ; D. Di Carlo and L. P. Lee, *Anal. Chem.* 78, 4925–4930 (2008).
2. A. Ashkin, *Phys. Rev. Lett.* 24, 156 (1970) ; A. Ashkin, *Optical trapping and manipulation of neutral particles using lasers*; World Scientific Publishing Co. Pte. Ltd. London, 2006.
3. S. C. Santucci , D. Cojoc, H. Amenitsch, B. Marmiroli , B. Sartori, M. Burghammer, S. Schoeder, E. Di Cola, M. Reynolds and C. Riekel, *Anal. Chem.* 83, 4863 (2011).

## PRE-MELTING EFFECTS ON GLOBULAR PROTEINS AND WATER PROPERTIES

*P. Sassi<sup>a</sup>, L. Comez<sup>b,c</sup>, S. Perticaroli<sup>a</sup>, M. Paolantoni<sup>a</sup>, D. Fioretto<sup>b</sup>,  
A. Morresi<sup>a</sup>, A. Giugliarelli<sup>a</sup> and G. Onori*

<sup>a</sup>Dipartimento di Chimica, Università di Perugia, Via Elce di sotto 8, 06123 Perugia, Italy. [sassipa@unipg.it](mailto:sassipa@unipg.it)

<sup>b</sup>Dipartimento di Fisica, Università di Perugia, Via Pascoli, 06123 Perugia, Italy  
<sup>c</sup>CRS-SOFT INFN-CNR, c/o Dipartimento di Fisica, Università La Sapienza, P.le A. Moro 4, I-00185, Roma, Italy

Low frequency Raman spectra (LFRS) of salt free aqueous solutions of hen egg white lysozyme (HEWL), were collected in HV geometry in the 25-85°C range. A solvent free (SF) spectrum was obtained after subtraction of elastic and the solvent contributions, and assigned to a genuine vibrational contribution of hydrated lysozyme. A straight similarity is obtained between this profile and the vibrational density of states (VDOS) of lysozyme in water solution as recently obtained by a room temperature MD simulation [1].

The goal of present work is manifold. On one side, we confirm the volume expansion observed for lysozyme in the pre-melting region by light scattering and dielectric spectroscopies [2,3]; to this extent, we analyse the results of density and low-frequency Raman measurements. Moreover, due to this comparison, we evidence the relation between this spectroscopic observable and the protein volume, and the sensitivity of the scattering technique to reveal even subtle conformational changes. Finally, we emphasize the role of water in the protein activity, not only to assist but particularly to trigger the local structural changes.

### **References**

1. A. Leuret, F. Affouard, P. Bordat, A. Hedoux, Y. Guinet and M. Descamps, *J. Chem. Phys.* 131, 245103 (2009)
2. A. Bonincontro, E. Bultrini, V. Calandrini and G. Onori, *PCCP* 3, 3811-3813, (2001)
3. A. Esposito, L. Comez, S. Cinelli, F. Scarponi and G. Onori, *J. Phys. Chem. B*, 113, 16420-16424, (2009)

# ABOUT OF MODULATION EFFECT ON WATER EXCHANGE IN SEED IN METABOLIC ACTIVE AND INACTIVE STATES

*A. Sukiasyan<sup>a</sup>, Y. Mikaelyan<sup>b</sup> and S. Ayrapetyan<sup>b</sup>*

<sup>a</sup>Department of Biotechnology and Environmental, State Engineering University of Armenia, 105 Teryan Str., 0009 Yerevan, Armenia.

[sukiasyan.astghik@gmail.com](mailto:sukiasyan.astghik@gmail.com)

<sup>b</sup>UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St., 0040 Yerevan, Armenia.

## Introduction

It is known that a cell hydration is one of the fundamental parameter for determining cell function. In present work, a comparative study of non-ionizing (NIR) (static magnetic fields (SMF), extremely low frequencies electromagnetic field (ELF EMF), ELF EMF-modulated millimetre waves, and ionizing radiation (IR) effects on winter wheat seeds water absorption and dry mass changes was performed.

## Experimental

The winter wheat seed hydration during 2 and 72 hours incubation was treated in a special saline solution, which had previously been treated by SMF, ELF EMF, ELF EMF-modulated millimetre waves and ionizing radiation, respectively for a fixed period of time. For determination of seed dry mass, they were dried in thermostat at 1040C during 24 hours. It is suggested that in these conditions, the free water in seed is evaporated and any factor-induced water structure changes. So, any changes of water thermodynamic properties predict the changes of free and bound water ratio in cells, the determination of seed dry mass after incubation could give information on changes of this ratio.

## Results

In during our experiments the water absorption was determined by osmotic gradients between seeds bathing medium in during of time of incubation. As during 2 hours, metabolism of seeds is in inactive state, these data can be explained by thermodynamic reversible processes, that is, the water fluxes and solubility can be considered as pure physical processes. In case of 72 hours incubation (i.e. the germination processes are in active state) these impact had reversed character. The results were shown that NIR and IR

effectively modulate the water molecule dissociation. It is also known that carboxyl solubility decreasing depresses water thermodynamical activity, followed by the radiation-induced decrease of water absorption and dry mass solubility. At the same time, NIR- and IR-induced water molecule dissociation in the presence of oxygen brings to the formation of reactive oxygen species (ROS), which are able to modulate cell metabolism effectively, especially peroxide, having longer lifetime among ROS.

This work was supported by grant of the State Committee of Science of the Ministry of Education and Science of the Republic of Armenia and the Belarusian Republican Foundation for Basic Research - 2011 (OCG RA - BRFFR – 2011-014 “Increased radio resistance of crops by modifying the structure of the irrigation water”)

## GLASS TRANSITION AND RELAXATION BEHAVIOR OF WATER AND AQUEOUS SOLUTIONS

Jan Swenson

Department of Applied Physics, Chalmers University of Technology  
SE-412 96 Göteborg, Sweden.  
[jan.swenson@chalmers.se](mailto:jan.swenson@chalmers.se)

This presentation focuses on two topics; the glass transition temperature of water and an ultraslow (i.e. slower than the viscosity related  $\alpha$ -relaxation) relaxation process in water and aqueous solutions. In the case of the ultraslow Debye-like relaxation it is well known that such a process exists in monoalcohols [1], although its origin is still not clear. For monoalcohols this slow Debye-like relaxation is 2-3 orders of magnitude slower than the viscosity related  $\alpha$ -relaxation, and it is also much more intense [1]. Here we show that a similar Debye-like process exists also in other hydrogen bonded liquids, such as water and aqueous solutions, although its intensity is substantially weaker than in the monoalcohols, and also a few more orders of magnitude slower than the  $\alpha$ -relaxation [2,3]. Whether this weak and ultraslow process in a wide range of hydrogen bonded liquids has the same physical origin as the well-known Debye-like relaxation in monoalcohols remains to be elucidated.

Regarding the glass transition of water many different scenarios have been discussed in recent years, although the most accepted glass transition temperature of bulk water is still the 136 K, as proposed by Johari *et al.* [4]. Here we try to understand the glass transition related dynamical properties of supercooled bulk water from experimental results on interfacial water. We propose [5] that the commonly accepted glass transition temperature of bulk water at 136 K is not a true glass transition, but rather due to the freezing in of a local secondary  $\beta$ -relaxation. The true glass transition should instead be located close to the critical temperature  $T_s \approx 228$  K when the build-up of an ice-like tetrahedral network structure is completed. The proposed interpretation is simple and able to explain many of the peculiar properties of supercooled water. For instance, it explains (a) the weakness of the calorimetric feature at 136 K, (b) why bulk water crystallizes immediately slightly above 228 K, (c) the unusual density behavior below 277 K, and (d) the similarities to ice at low temperatures. Furthermore, the model is closest to the findings for confined water and aqueous solutions, and no speculative liquid-liquid transition or fragile-to-strong transition is needed to explain the properties of supercooled and glassy water.

**References**

1. L. M. Wang and R. Richert, J. Chem. Phys. 121, 11170 (2004).
2. R. Bergman, H. Jansson, and J. Swenson, J. Chem. Phys. 132, 044504 (2010).
3. H. Jansson, R. Berman, and J. Swenson, Phys. Rev. Lett. 104, 017802 (2010).
4. G. P. Johari, A. Hallbrucker and E. Mayer, Nature (London) 330, 552 (1987).
5. J. Swenson and J. Teixeira, J. Chem. Phys. 132, 014508 (2010).



## WATER HYDRATION IN CAFFEINE-SUGAR SOLUTION

*Letizia Tavagnacco<sup>a</sup>, Olof Engström<sup>b</sup>, Udo Schnupf<sup>c</sup>,  
Philip E. Mason<sup>c</sup>, Marie-Louise Saboungi<sup>d</sup>, Michael Himmel<sup>e</sup>,  
Göran Widmalm<sup>b</sup>, John W. Brady<sup>c</sup> and Attilio Cesàro<sup>a</sup>*

<sup>a</sup>Department of Life Sciences, University of Trieste – via L. Giorgieri 1,  
34127 Trieste, ITALY. [letizia.tavagnacco@phd.units.it](mailto:letizia.tavagnacco@phd.units.it)

<sup>b</sup>Department of Organic Chemistry, Stockholm University  
S-10691 Stockholm, SWEDEN

<sup>c</sup>Department of Food Science, Cornell University – Stocking Hall,  
14853 Ithaca, NY, USA.

<sup>d</sup>Centre de Recherche sur la Matière Divisée – 1 bis rue de la Férellerie  
45071 Orléans, FRANCE.

<sup>e</sup>National Renewable Energy Laboratory – 1617 Cole Boulevard  
80401-3393 Golden, CO, USA.

The achievement of a better knowledge on how water molecules interact with biomolecules and how this affects the association of biomolecules in aqueous solution is of primary interest to understand the fundamental role of active substances in all life-related fields.

In this work the behavior of small biological solutes in aqueous solution was studied through molecular dynamics simulations and NMR experiments.

MD simulations of caffeine in aqueous solution were first performed to collect information on the water-caffeine interaction and on the water structuring due to the presence of the caffeine molecule. These studies offer the possibility of testing various models for aqueous solvation [1]. The caffeine molecule was modeled using a newly developed CHARMM-type force field [2]. The simulations show that caffeine in aqueous solution imposes a complex organization on its adjacent solvent molecules and the details of this structuring are a complicated function of the molecular architecture of the solute. Indeed, caffeine self-aggregation was observed in aqueous solution as expected from thermodynamic measurements [3].

Further MD simulations have been conducted on several independent system consisting of sugars in caffeine aqueous solution. The sugar under consideration were sucrose,  $\alpha$ -glucopyranose and  $\beta$ -glucopyranose. All the sugar exhibited an affinity of the caffeine molecule. The nature of the interaction was a stacking between the hydrophobic faces of the caffeine molecule and the hydrophobic faces generated by the sugar proton atoms. This mode of interaction did not interfere with the hydrogen bonding of the sugar molecules to water. At variance of cyclic sugars, linear sugar

molecules (like sorbitol) are characterized by a larger conformational degree. As a consequence, they do not show oriented (stacked) interactions although the affinity between caffeine and sugar is still relatively high. Intermolecular NOE has been previously used to detect the forming of complexes between sugars and small aromatic compounds [3] and indeed small positive NOEs were observed from both D-glucopyranose and sucrose to caffeine in D<sub>2</sub>O. <sup>1</sup>H NMR titration experiments were carried out on similar systems. The magnitude of the chemical shift change of the sugar ring proton atoms in the <sup>1</sup>H NMR titration experiments validate the stacking interaction detected through MD experiments.

### **References**

1. D. Chandler, *Nature*, 2005, 437, 640-647.
2. Tavagnacco, L.; Schnupf, U.; Mason, P. E.; Saboungi, M.-L., Cesàro, A., Brady, J. B., *J. Phys. Chem. B*, 2011, 115(37), 10957-10966.
3. Cesàro, A.; Russo, E.; Crescenzi, V. *J. Phys. Chem.* 1976, 80, 335–339.
4. Vandenbussche, S.; Díaz, D.; Fernández-Alonso, M. C.; Pan, W.; Vincent, S. P.; Cuevas, G.; Cañada, F. J.; Jiménez-Barbero, J.; Bartik, K. *Chem. Eur. J.* 2008, 14, 7570-7578.

## NON-DESTRUCTIVE MEASUREMENT OF MOISTURE, SOLUBLE SOLIDS CONTENT AND WATER SELF-DIFFUSION COEFFICIENT OF RIPE AND UNRIPE OSMO-DEHYDRATED KIWIFRUIT SLICES BY NIR SPECTROSCOPY

U. Tylewicz<sup>a</sup>, *P. Santagapita*<sup>b,c</sup>, *V. Panarese*<sup>c</sup>, *P. Rocculi*<sup>a,c</sup> and *M. Dalla Rosa*<sup>ac</sup>

<sup>a</sup>Interdepartmental Centre for Agri-Food Industrial Research, Alma Mater Studiorum, University of Bologna, Piazza Goidanich 60, Cesena (FC), Italy.

[urszula.tylewicz@unibo.it](mailto:urszula.tylewicz@unibo.it)

<sup>b</sup>Faculty of Exact and Natural Sciences, Industry Department – Member of the National Council of Scientific and Technical Research (CONICET), University of Buenos Aires, Buenos Aires, Argentina

<sup>c</sup>Department of Food Science, Alma Mater Studiorum, University of Bologna, Piazza Goidanich 60, Cesena (FC), Italy

Kiwifruit is one of the most suitable fruit for osmotic dehydration process (OD), because offers the possibility to treat unripe fruits to obtain final products with acceptable firmness and organoleptic characteristics. The effect of OD on the quality and the water state of kiwifruit has been widely studied [1], [2], [3]. The aim of present work was to evaluate the feasibility of a non-destructive and rapid method based on NIR spectroscopy to determine the changes on moisture, soluble solids content and water self-diffusion coefficient promoted by OD on kiwifruit at different ripening stages. Two kiwifruit (*A. deliciosa* cv Hayward) groups with refractometric index values of  $9.5 \pm 1.1$  (LB) and  $14.1 \pm 0.9$  (HB) °Bx were selected. The OD process was carried out by dipping the samples (10 mm thick slices) in 61.5% (w/v) sucrose solution at 25 °C, with continuous stirring for 0, 30, 60, 180 and 300 min. The moisture content was determined gravimetrically and soluble solids content (SSC) by measuring the refractive index with a digital refractometer. The proton transverse relaxation time ( $T_2$ ) and its peak intensity were determined at 24 °C through the CPMG sequence using a 20 MHz spectrometer. Moreover, water self diffusion coefficient ( $D_w$ ) measurements through pulsed field gradient spin-echo (PGSE) sequence were performed [3]. Non-destructive moisture, SSC and  $D_w$  of kiwifruit slices were determined by means of FT-NIR spectrometer using a fibre optic probe. Diffuse reflectance spectra were acquired in the spectral range between 4,000 and 12,000  $\text{cm}^{-1}$ , by averaging 32 scans at a resolution of 4  $\text{cm}^{-1}$ .

OD leads to a great water loss (WL) from kiwifruit and the simultaneous counter-diffusion of solutes from the hypertonic solution into the kiwifruit tissues. The highest WL rates occurred during the first treatment hour, because the dehydration driving force was the greatest. The samples with LB presented the higher WL. As expected, the kiwifruit  $D_w$  coefficient values measured in the raw kiwifruit were lower than the free water one, as the structures and solutes of raw kiwifruit reduce water mobility, and decreased even more during OD, due to the water loss and sugar gain. For both the maturity groups, a principal component analysis (PCA) was carried out in order to set up a model to analyze the spectral data. Four PCs were obtained, which explained more than the 95% of the variance. Partial least square (PLS) regressions were performed between the NIR spectra and the analyzed physico-chemical parameters. The best predictive models showed coefficient of determination ( $R^2$ ) values of 0.897, 0.882 and 0.763 (cross validations) for moisture content, SSC and water self-diffusion coefficient, respectively.

A multy-analytical approach is often needed to deeply investigate the behaviour of vegetable tissues during minimal processing. Then, by knowing the extent of changes on mass transfer parameters and water behaviour during OD, which in turn modified the structural and textural characteristics of the final product, it is possible to exploit both raw unripe and ripe kiwifruits from a technological point of view. In this scenario, NIR spectroscopy appears as an interesting tool to take on-line decisions about the optimum OD time, according to a specific target of final product.

### **References**

1. U. Tylewicz, V. Panarese, L. Laghi, P. Rocculi, M. Nowacka, G. Placucci, M. Dalla Rosa, *Food Biophysics*, 6, 2, 327 (2011).
2. Panarese V., Tylewicz U., Santagapita P. et al., *Innovative Food Science & Emerging Technologies*, 15, 66–71 (2012).
3. Santagapita P., Laghi L., Panarese V. et al. *Food and Bioprocess Technology*, Doi 10.1007/s11947-012-0818-5 (2012)

### **Acknowledgments**

This research was supported by POR-FESR 2007-2013 Emilia-Romagna Region.

# WATER AND PROTEIN DYNAMICS IN LYSOZYME-WATER MIXTURES OVER WIDE RANGES OF COMPOSITION

*K. Vartzeli-Nikaki, A. Panagopoulou, A. Kyritsis and P. Pissis*

National Technical University of Athens, Zografou Campus, 15780, Athens, Greece.

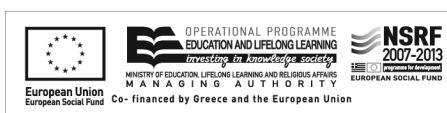
[popvar@central.ntua.gr](mailto:popvar@central.ntua.gr)

## Introduction

The hydration properties of protein dynamics in protein-water mixtures have been studied in the past several decades by a variety of experimental techniques. Dielectric techniques are very effective for studies on the dynamics of protein water systems, since, owing to the strong electric dipole moment of the water molecule, molecular motions of water can be detected as dielectric relaxation processes. While thermal studies (Calorimetry) [1-3] enables analysis of the calorimetric glass transition and thermal phenomena (crystallization and melting), dielectric (DRS) studies reveal the overall molecular motions (uncrystallized water, ice, protein segmental dynamics) [2-4] in a broad frequency range ( $10^{-4}$  -  $10^6$  Hz) and contribute to determination of the origin of relaxation phenomena.

## Experimental

In this work we employ differential scanning calorimetry (DSC) and two dielectric techniques, broadband dielectric relaxation spectroscopy (DRS) and thermally stimulated depolarization currents (TSDC), in order to investigate water and protein dynamics in protein-water mixtures over wide ranges of composition. In addition, water equilibrium sorption measurements (ESI) are performed at room temperature. The protein used is a globular protein, hen egg lysozyme, in the form of white powder. The mixtures are in the form of dilute solutions, concentrated solutions and hydrated solid pellets, in order to cover a broad composition range, from samples of about 82 wt% in water to practically dry solid pellets, in order to follow the evolution of dynamics with increasing hydration level.



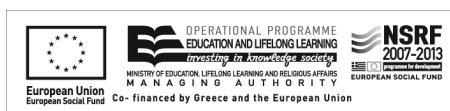
## Results

Three relaxation regions are observed by the dielectric TSDC and DRS techniques, in good agreement to each other. In the order of increasing temperature/decreasing frequency, these are (a) a broad local relaxation of polar groups on the protein surface plasticized by water, most likely with contribution of the secondary  $\beta$  relaxation of water molecules themselves in the uncrystallized water phase, (b) two relaxations of water in the crystallized water phase (ice crystals), and (c) the  $\alpha$  relaxation associated with the glass transition of the hydrated protein.

In particular, TSDC, due to its high peak resolving power and its low equivalent frequency in the range of  $10^{-2}$ - $10^{-4}$  Hz, but also due to fact that conductivity contributions may be less significant (steps of applying the stimulus and of recording the response, i.e. polarization and depolarization step, are separated from each other) compared to DRS measurements, proves to be an exceptional tool for recording the evolution of the protein glass transition temperature,  $T_g$ , with increasing level of hydration. The  $T_g$  is found to decrease significantly with increasing hydration and then to stabilize at about  $-90^\circ\text{C}$  for hydration levels where water crystallization occurs during cooling. The above mentioned results are in good accordance with calorimetry measurements of lysozyme - water mixtures [1,2]. This behavior, probes us to discuss the results according to the phase diagram, using the Rault model [2], where the plasticization of the  $T_g$  of polymer-solvent systems is explained using thermodynamic terms, taking into account no strong interactions between the two components. The strong plasticization of the  $T_g$  along with the stabilization of the latter, for water fractions where crystallization of water occurs during cooling, is a novel result with potential impact to cryoprotection and pharmaceuticals.

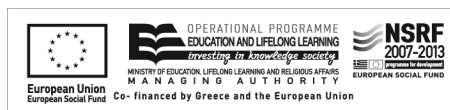
## Acknowledgements

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.



**References**

1. Y. Miyazaki, T. Matsuo, and H. Suga, J. Phys. Chem. B 104, 8044-8052 (2000).
2. A. Panagopoulou, A. Kyritsis, A. M. Aravantinou, D. Nanopoulos, R. Sabater i Serra, J. L. Gómez Ribelles, N. Shinyashiki, P. Pissis, Food Biophysics 6, 199-209 (2011).
3. A. Panagopoulou, A. Kyritsis, R. Sabater i Serra, J. L. Gómez Ribelles, N. Shinyashiki and P. Pissis, J. Biochim. Biophys. Acta 1814, 1984-1996 (2011).
4. A. Panagopoulou, A. Kyritsis, N. Shinyashiki, P. Pissis, J. Phys. Chem. B 116, 4593-4602 (2012).



# MOLECULAR DYNAMICS OF WATER IN HYALURONIC ACID/SILICA HYDROGEL NANOCOMPOSITES

*J. Vazquez Molina<sup>a</sup>, A. Panagopoulou<sup>b</sup>, A. Kyritsis<sup>b</sup>, M. Monleón Pradas<sup>a,c</sup>, A. Vallés Lluch<sup>a</sup>, G. Gallego Ferrer<sup>a,c</sup> and P. Pissis<sup>b</sup>*

<sup>a</sup>Center for Biomaterials and Tissue Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.

[joavzmo@etsii.upv.es](mailto:joavzmo@etsii.upv.es)

<sup>b</sup>National Technical University of Athens, Zografou Campus, 15780, Athens, Greece

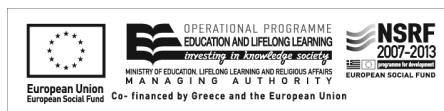
<sup>c</sup>CIBER en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

## Introduction

Hyaluronic acid is a natural carbohydrate used for several biomedical applications due to its high hydrophilicity and biocompatibility. While pure HA is soluble in water, crosslinked HA forms a hydrogel that can be employed for scaffolds for tissue engineering. In order to improve the mechanical properties of the material and the scaffolds, HA/SiO<sub>2</sub> nanocomposites are being investigated, in an approach similar to other hydrogels [1,4]. Nevertheless, polymer-filler-water structure and interactions are not fully understood for silica nanocomposites in general, and are unknown for the particular HA/SiO<sub>2</sub> system.

## Materials and Methods

Here we present a dielectric study of hydrated HA/SiO<sub>2</sub> nanocomposites. For the synthesis, silica nanoparticles (1, 2, 5 and 10% of total mass) were properly dispersed in a solution with HA, which later was crosslinked with divinyl sulfone (DVS) at a molar ratio 0.8. The hydrogels were hydrated up to equilibrium in water vapour environments with different relative humidities, achieving water mass fractions in the range of 60 wt% to 0 wt% (practically dry samples).





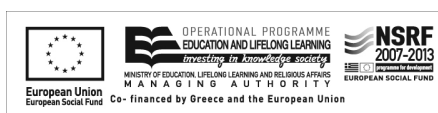
Experimental techniques employed include Thermally Stimulated Depolarization Currents (TSDC), Dielectric Relaxation Spectroscopy (DRS) and water equilibrium sorption-desorption measurements (ESI).

## Results and Conclusions

DRS of HA with 10% of silica shows that, for the samples with lower water content, two relaxations appear, one of them clearly plasticized by water. For the sample with the highest water content (more than 50% in the wet basis), at least four relaxations appear, which can be discussed in terms of water dynamics. TSDC measurements show a relaxation at around  $-135^{\circ}\text{C}$ , in accordance with DRS.

ESI experiments show that the nanocomposites with 10% of silica absorb significantly less water than pure HA ( $h=1$  versus  $h=1,33$  at relative humidity  $a_w=0.95$ ). A study of the intermediate compositions might reveal a turning point and thus a percolation threshold.

The importance of the results is threefold. First, there is no dielectric study of HA/SiO<sub>2</sub> nanocomposites in the literature, so these results are novel. Second, the study can help to decide whether HA/SiO<sub>2</sub> nanocomposites are interesting materials for tissue regeneration purposes, at which silica content, and motivate the development of scaffolds for tissue engineering. Third, the combination of the above mentioned experimental techniques have led to very important conclusions relevant to the origin of the polysaccharide glass transition and the dynamics of water at the polysaccharide interface, analogous to those in the case of BSA-water mixtures [2,3], as well as the effect of silica on polymer dynamics [4]. The studies of HA and the evolution of dynamics with water and silica contents may help in clarifying the interactions between hydrogel matrix, filler nanoparticles and water molecules, three elements that play a crucial role in the properties of the final material.



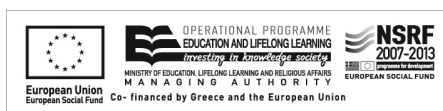
### Acknowledgements

The authors would like to acknowledge Sara Poveda Reyes and María Hernández Palacios for the preparation of the HA sheets and Dr Daniel Beltrán and Dr Pedro Amorós for the preparation of the silica nanoparticles.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

### References

1. J.C. Rodríguez Hernández, M. Monleon Pradas, J.L. Gómez Ribelles, J. Non-Cryst. Solids 354, 1900–1908 (2008).
2. A. Panagopoulou, A. Kyritsis, R. Sabater i Serra, J. L. Gómez Ribelles, N. Shinyashiki and P. Pissis, J. Biochim. Biophys. Acta 1814, 1984-1996 (2011).
3. A. Panagopoulou, A. Kyritsis, N. Shinyashiki, P. Pissis, J. Phys. Chem. B 116, 4593-4602 (2012).
4. A. Kyritsis, A. Spanoudaki, C. Pandis, L. Hartmann, R. Pelster, N. Shinyashiki, J. C. Rodríguez Hernández, J. L. Gómez Ribelles, M. Monleón Pradas, P. Pissis, J Therm Anal Calorim 108, 1067–1078 (2012)



## **FREEZE-DRIED HEART VALVE MATRIX IMPLANTS**

*Shangping Wang<sup>a</sup>, Tobias Goecke<sup>b</sup>, Carsten Meixner<sup>c</sup>, Axel Haverich<sup>b</sup>, Andres Hilfiker<sup>b</sup> and Willem F. Wolkers<sup>a</sup>*

<sup>a</sup>Institute of Multiphase Processes, Leibniz Universitaet Hannover – Callinstrasse 36, 30167 Hannover, Germany. [wang@imp.uni-hannover.de](mailto:wang@imp.uni-hannover.de)

<sup>b</sup>Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover Medical School – Carl-Neuberg Strasse 1, 30625 Hannover, Germany

<sup>c</sup>Corlife GbR – Buenteweg 15, 3000 Feodor-Lynen-Strasse 23, 30625 Hannover, Germany

In this study, structure and biomechanical properties of freeze-dried decellularized porcine pulmonary heart valve scaffolds were investigated. Various strategies for freeze-drying porcine heart valves were studied. After the cellular material was removed, heart valve scaffolds were freeze-dried with or without sucrose and hydroxyl ethylene starch, and the stability and elasticity of the freeze-dried scaffolds were compared to assess the effectiveness of these lyoprotectants in preventing degradation of the scaffold. Heart valves were dissected from porcine hearts and freeze-dried. Rehydrated freeze-dried scaffold has a more porous structure compared to native tissue or decellularized scaffolds. Freeze-drying in the absence of lyoprotectants caused an overall more disintegrated appearance of the histological architecture of the porcine valves, especially between the fibrosa and the ventricularis layers. Freeze-dried scaffolds with lyoprotectants have a looser network with bigger pore sizes. Scaffolds freeze-dried in the absence of lyoprotectants had the largest pore sizes, whereas scaffolds freeze-dried in the presence of protectants showed pores of intermediate sizes between decellularized scaffolds and unprotected freeze-dried samples. Qualitatively, matrices freeze-dried with sucrose looked more intact than matrices freeze-dried using a sucrose/HES mixture. Scaffolds freeze-dried with sucrose alone displayed less porosity compared to those freeze-dried with a sucrose/HES mixture, whereas no significant differences in biomechanical properties were observed. Taken together, freeze-drying could provide a promising tool for storage of decellularized heart valve scaffolds. The in vivo performance and durability of freeze-dried heart valve scaffolds remains to be elucidated.

### **References**

1. S. Wang, T. Goecke, C. Meixner, A. Haverich, A. Hilfiker, W.F. Wolkers, Tissue Engineering doi:10.1089/ten.TEC.2011.0398 (2012).



## INVITED LECTURES

ULTRAFAST OKE SPECTROSCOPY FOR THE THZ RAMAN SPECTRA OF AQUEOUS SOLUTIONS <i>Stephen R. Meech, Kamila Mazur, Ismael A. Heisler and Francesca Palombo</i>	3
THEORY OF DIELECTRIC RESPONSE OF PROTEINS IN SOLUTION FROM MHZ TO THZ <i>Dmitry Matyushov</i>	5
PHARMACEUTICAL PROCESSING: A PHYSICAL PERSPECTIVE ON INDUCED TRANSFORMATIONS AND WATER INVOLVEMENT <i>Marc Descamps</i>	7
WATER IN PROTEIN-LIGAND INTERACTIONS: CHALLENGES AND OPPORTUNITIES FOR DRUG DESIGN <i>Julien Michel</i>	8
MAGNITUDE AND MOLECULAR ORIGIN OF WATER SLOWDOWN NEXT TO A PROTEIN <i>Damien Laage, Fabio Sterpone, Guillaume Stirnemann and Aoife Fogarty</i>	9
CRYSTALLIZATION OF WATER <i>Valeria Molinero</i>	10
PROTEIN MOBILITY AND DYNAMIC COUPLING IN AMORPHOUS GLASSY SUGARS <i>Richard D. Ludescher, Andrew Draganski and Joel M. Friedman</i>	11
EFFECTS OF SUGARS ON FLAVOUR RELEASE IN SOLUTION AND REAL FOOD MATRICES AT DIFFERENT HYDRATION DEGREE <i>Paola Pittia</i>	13
CRYOPRESERVATION OF CORD BLOOD-DERIVED HAEMATOPOIETIC STEM CELLS: AN INTERNATIONAL BIO-BANKS NETWORK <i>Riccardo Saccardi and Letizia Lombardini</i>	15
WATER TRANSPORT PROCESSES DURING FREEZING OF CELLS <i>Willem F. Wolkers</i>	17

## KEYNOTE LECTURES

A UNIFIED VIEW OF THE DYNAMICS OF WATER, AQUEOUS MIXTURES AND HYDRATED PROTEINS DERIVED FROM EXPERIMENTS AND THEORY <i>S. Capaccioli, K. L. Ngai and A. Paciaroni</i>	21
WATER STRUCTURE AND ITS RELEVANCE IN PROTEIN STABILITY <i>J. Raúl Grigera</i>	23
THE HYDRATION OF SOLUTES WITH EXTENDED PLANAR SURFACES John W. Brady, Udo Schnupf, Letizia Tavagnacco, Philip Mason, Marie-Louise Saboungi and Attilio Cesàro	25
COOPERATIVE REARRANGING REGIONS IN WATER AT BIOLOGICAL AND INORGANIC INTERFACES <i>Giancarlo Franzese</i>	26

## ORAL PRESENTATIONS

TIME-RESOLVED LASER SPECTROSCOPY ON INTERFACIAL AND NANOCONFINED WATER <i>R. Torre, A. Taschin, P. Bartolini and R. Righini</i>	29
COLLECTIVE MODES AND AMORPHOUS DYNAMICAL CHARACTER OF HYDRATION WATER IN BIOMOLECULES <i>A. Orecchini, A. Paciaroni, F. Sebastiani, M. Jasnin, C. Petrillo, J. Zaccai and F. Sacchetti</i>	30
WATER AND PROTEIN DYNAMICS IN HYDRATED GLOBULAR PROTEINS <i>P. Pissis, A. Panagopoulou, A. Kyritsis and N. Shinyashiki</i>	31
BROADBAND TIME-FREQUENCY RESOLVED SPECTROSCOPY: THE MARTELLO PROJECT <i>Riccardo Cucini, Andrea Battistoni, Filippo Bencivenga, Alessandro Gessini, Francesco D'Amico, Daniele Fioretto and Claudio Masciovecchio</i>	33
HYDRATION SHELLS OF MACROMOLECULES STUDIED BY HIGH- RESOLVED RESONANT RAMAN SPECTROSCOPY <i>Francesco D'Amico</i>	35
RELAXATION IN AQUEOUS MIXTURES: LOCAL SOLUTE DYNAMICS VS. WATER SPECIFIC MOTIONS <i>S. Ancherbak, S. Capaccioli and D. Prevosto</i>	36
CLUSTER PHASES OF DECORATED MICELLAR SOLUTIONS WITH MACROCYCLIC LIGANDS <i>S. Caponi, M. Carlà, G. Cazzolli, A. Defant, C. M. C. Gambi, L. Lanzi; S. Marchetti, M. Mattarelli, M. Montagna, B. Rossi, F. Rossi, F. Sciortino and S. Sennato</i>	38
DETAILING PROTEIN SOLVATION SHELL IN BINARY MIXTURES <i>Maria Grazia Ortore, Francesco Spinozzi, Giuseppe Onori and Paolo Mariani</i>	40
TRANSPORT IN TEMPERATURE GRADIENTS: AQUEOUS SOLUTIONS <i>Simone Wiegand, Zilin Wang and Philipp Naumann</i>	42
ANISOTROPIC ENHANCED WATER DIFFUSION IN SCLEROGLUCAN/BORAX SWELLED TABLETS STUDIED BY PGSE NMR <i>Raffaele Lamanna, Chiara Di Meo, Tommasina Coviello, Pietro Matricardi, Franco Alhaique and Donatella Capitani</i>	43
ACCOUNTING FOR WATER MOLECULES IN HCV DRUG DISCOVERY <i>Maria Letizia Barreca, Nunzio Iraci, Rosy Gaetani, Giuseppe Manfroni and Violetta Cecchetti</i>	45
WHICH ROLE OF WATER IN DETERMINING THE RESIDENCE TIME OF DRUGS? <i>Matteo Ceccarelli</i>	47

TABLET DISINTEGRATION - ROLE OF PRESSURE AND MOISTURE <i>Paul Wan Sia Heng, Parind Mahendrakumar Desai and Celine Valeria Liew</i>	49
WATER IS NOT SO ANOMALOUS <i>Francesco Aliotta, Rosina Celeste Ponterio and Franz Saija</i>	51
MOLECULAR PROBE DYNAMICS REVEALS SUPPRESSION OF ICE-LIKE REGIONS IN STRONGLY CONFINED SUPERCOOLED WATER <i>D. Leporini, D. Banerjee, S. N. Bhat and S. V. Bhat</i>	53
IDENTIFICATION OF LOW FREQUENCY SHEAR-ELASTICITY IN LIQUID WATER <i>Laurence Noirez and Patrick Baroni</i>	54
DISCLOSING THE ROLE OF HYDROPHOBIC INTERACTIONS THROUGH AGGREGATION OF NON-POLAR SOLUTES IN WATER: A MOLECULAR DYNAMICS STUDY <i>Oswaldo Chara, H. Ariel Álvarez, C. Gastón Ferrara and J. Raul Grigera</i>	56
ON THE COUPLING BETWEEN THE DYNAMICS OF PROTEINS AND THAT OF THEIR HYDRATION WATER <i>Valeria Conti Nibali, Giovanna D'Angelo, Ulderico Wanderlingh and Mounir Tarek</i>	58
ION SPECIFIC EFFECTS IN POLYELECTROLYTE SOLUTIONS: AN EXAMPLE OF IONENE SALTS IN WATER <i>Barbara Hribar-Lee, Matjaž Bončina, Sašo Čebašek, Miha Lukšič, Natalie Malikova, Mojca Seručnik and Vojko Vlachy</i>	59
LOW TEMPERATURE BEHAVIOUR OF HYDRATED NAFION MEMBRANES <i>M. Plazanet, P. Bartolini, R. Torre, C. Petrillo and F. Sacchetti</i>	61
ON THE SOLVATION OF IONS IN HIGH-DENSITY WATER <i>Valentina Migliorati, Giordano Mancini, Simone Tatoli, Andrea Zitolo, Adriano Filipponi, Simone De Panfilis, Andrea Di Cicco and Paola D'Angelo</i>	62
ABOUT EMULSION WATER STRUCTURE <i>Fabio Bruni, Silvia Imberti, Maria Antonietta Ricci and Rosaria Mancinelli</i>	63
MODELING CHANGES IN FOOD POLYMER PROPERTIES USING HYGRO- THERMAL TIME <i>Gaylon Campbell, Brady Carter and Martin Buehler</i>	65
DIFFERENT MATERIAL BEHAVIORS DURING THE PROTEIN POWDER AGEING <i>D. Champion and P. Lillford</i>	67
STATES AND MOTIONS OF WATER IN WHEAT BREAD UPON FREEZING AND FROZEN STORAGE <i>Guo Chen and Jan Swenson</i>	68



CHALLENGES IN MULTI-HURDLES FOOD PRESERVATION AND ITS STABILITY <i>Mohammad Shafiur Rahman</i>	69
SOLVATION DYNAMICS OF BIV TAR RNA WITH AND WITHOUT TAT PEPTIDE <i>Teena Goel, Santosh Kumar and Souvik Maiti</i>	71
ANALYSIS OF BULK AND HYDRATION WATER DURING THERMAL LYSOZYME DENATURATION <i>G. Bellavia, A. Hédoux, L. Paccou, S. Achir and Y. Guinet</i>	74
AQUEOUS SOLUTIONS OF LYSOZYME AND DISACCHARIDES: FOCUS ON CRYOPROTECTION <i>Paola Gallo</i>	77
HOW STRONGLY DOES TREHALOSE INTERACT WITH LYSOZYME AT LOW WATER CONTENTS? INSIGHTS FROM MOLECULAR DYNAMICS SIMULATION AND INELASTIC NEUTRON SCATTERING <i>Adrien Lerbret, Frédéric Affouard, Alain Hédoux, Stephanie Krenzlin, Jürgen Siepmann, Marie-Claire Bellissent-Funel and Marc Descamps</i>	79
DO WE UNDERSTAND THE DEHYDRATION PROCESS? <i>R. Heyd, G. Lelong, J. Fichot, C. Josserand, I. Hourpa, E. Gombart, J-F Tranchant, G. Charalambopoulou, T. Steriotis, A. Brandt, A. Rampino, J. W. Brady, A. Cesàro and M-L Saboungi</i>	81
ANOMALOUS PROTON DYNAMICS OF WATER IN NEURAL TISSUE AS SEEN BY QUASI-ELASTIC NEUTRON SCATTERING. IMPACT ON MEDICAL IMAGING TECHNIQUES. <i>F. Natali, Y. Gerelli, C. Stelletta, J. Peters and J. Ollivier</i>	83
WATER MOBILITY, DENATURATION AND THE GLASS TRANSITION IN PROTEINS <i>David Porter and Fritz Vollrath</i>	85
TEMPERATURE AND COMPOSITION EFFECTS ON PROTEIN HYDRATION. INSIGHT ON THERMOSTABILITY <i>F. Sterpone, R. Obaidur, M. Kalimeri, D. Laage and S. Melchionna</i>	87

## POSTER PRESENTATIONS

WATER TRANSPORT DURING FREEZING OF CELLS IN THE PRESENCE OF CRYOPROTECTIVE AGENTS <i>Maryam Akhooondi, Harriëtte Oldenhof, Harald Sieme and Willem F. Walkers</i>	91
GLASS TRANSITION AND WATER DYNAMICS IN AQUEOUS SOLUTIONS OF CASEIN PEPTONE <i>D. Aravopoulou, A. Panagopoulou, A. Kyritsis and P. Pissis</i>	92
HIGH-RESOLUTION TANDEM FABRY-PEROT INTERFEROMETER FOR ULTRA-VIOLET BRILLOUIN SCATTERING MEASUREMENTS <i>A. Battistoni, F. Bencivenga, D. Fioretto and C. Masciovecchio</i>	95
WATER EFFUSION AS A PROBE FOR DRUG RELEASE FROM HYDROGELS <i>B. Bellich, M. Borgogna, A. Rampino, M. Cok and A. Cesàro</i>	96
COLLECTIVE DYNAMICS IN THE THZ RANGE BY INELASTIC SCATTERING SPECTROSCOPIES <i>F. Bencivenga and A. Cunsolo</i>	98
FREEZE-DRYING AND SPRAY-DRYING OF LIPID NANOPARTICLE SUSPENSIONS <i>P. Blasi, A. Schoubben, S. Giovagnoli and M. Ricci</i>	100
STABILITY OF LIPID NANOPARTICLES IN PHYSIOLOGICAL RELEVANT MEDIA <i>P. Blasi, A. Schoubben, V. Romano and M. Ricci</i>	102
MULTIPLE PROTEIN GLASS TRANSITIONS AT LOW WATER CONTENT <i>Jacob Bouman, Susan Barker, Paul Venema and Renko de Vries</i>	104
EFFECT OF TEMPERATURE AND WATER ON POLY(LACTIDE-CO-GLYCOLIDE) INVESTIGATED BY FT-IR SPECTROSCOPY <i>S. Casagrande, M. Paolantoni, P. Blasi, M. Ricci and P. Sassi</i>	106
MOLECULAR DYNAMICS OF HYDRATION WATER IN SOLUTES OF DIFFERENT COMPLEXITY BY EXTENDED DEPOLARIZED LIGHT SCATTERING (EDLS) EXPERIMENTS <i>L. Comez, D. Fioretto, L. Lupi, A. Morresi, M. Paolantoni and P. Sassi</i>	108
SOLVENT DYNAMICS DURING ENZYMATIC CATALYSIS: A MOLECULAR DYNAMICS SIMULATIONS STUDY <i>Valeria Conti Nibali, Matthias Heyden, Moran Grossman, Benjamin Born, Dmitry Tworowski, Irit Sagi and Martina Havenith</i>	110
PHOTON CORRELATION STUDY OF THE DYNAMICS OF CYCLODEXTRIN-BASED NANOSPONGES IN WATER <i>S. Corezzi, D. Bonfigli, L. Comez and F. Trotta</i>	111

INTERPLAY OF WATER MOBILITY AND SUGAR CRYSTALLIZATION IN GLASSY MIXTURES <i>Elena Elisei, Attilio Cesàro, Fabio Tedoldi and Alessandro Maiocchi</i>	113
A NOVEL ANTI-FOG PULLULAN COATING FOR FOOD PACKAGING APPLICATIONS <i>S. Farris, L. Introzzi, L. Piergiovanni and A. Schiraldi</i>	115
WETTING OF BIOPOLYMER COATINGS: CONTACT ANGLE KINETICS AND IMAGE ANALYSIS INVESTIGATION <i>S. Farris, L. Introzzi, L. Piergiovanni and A. Schiraldi</i>	117
WATER EFFECTS ON SACCHARIDE MATRICES STUDIED THROUGH MD AND FTIR <i>Sergio Giuffrida, Grazia Cottone and Lorenzo Cordone</i>	119
HIGH DENSITY PROTEIN SOLUTIONS: THEORY & EXPERIMENT <i>John Ioannou, Joris Stegen, Athene Donald, Paul van der Schoot and Hans Tromp</i>	121
EFFECTS OF POTASSIUM IONS ON THE VISCOSITIES IN THE POTASSIUM CHLORIDE-GLUCOSE-WATER TERNARY SYSTEM <i>Kazuhito Kajiwara, Yumi Yanaiharu, Hiroko Ando and Kiyoshi Kawai</i>	123
EFFECT OF WATER ON THE SOFTENING TEMPERATURE OF COOKIE PRODUCTS <i>Kiyoshi Kawai, Midori Toh, and Yoshio Hagura</i>	125
COMPARATIVE STUDY OF AQUEOUS AND ETANOLIC EXTRACTS OF THE DRUG PRODUCT PREPARED ON THE BASIS OF PLANT SAMPLE <i>Armen Kirakosyan, Astghik Sukiasyan and Armen Humbarzumyan</i>	128
X-RAY INELASTIC SCATTERING STUDY OF HUMAN CELL THz DYNAMICS USING A URANIUM BASED COLORANT <i>M. Longo, A. Orecchini, A. Paciaroni, C. Petrillo, F. Sacchetti, I. Pirisinu, M. Calvitti and R. Romani</i>	131
MODE OF ACTION OF DIFFERENT CRYOPROTECTIVE AGENTS USED FOR CRYOPRESERVATION OF STALLION SPERM <i>Harriëtte Oldenhof, Marina Gojowsky, Shangping Wang, Willem F. Wolkers and Harald Sieme</i>	134
IONIC EFFECTS ON H-BONDING LIQUID DYNAMICS STUDIED BY ULTRAFAST OKE SPECTROSCOPY <i>Francesca Palombo and Stephen R. Meech</i>	135
MOLECULAR DYNAMICS OF WATER IN HYALURONIC ACID HYDROGELS <i>A. Panagopoulou, J. Vazquez Molina, A. Kyritsis, M. Monleón Pradas, A. Vallés Lluçh, G. Gallego Ferrer and P. Pissis</i>	137

HYDRATION DYNAMICS OF BIOLOGICAL WATER: MOLECULAR MODELING OF DEPOLARIZED LIGHT SCATTERING EXPERIMENTS <i>M. Paolantoni, L. Lupi, L. Comez, D. Fioretto and B. M. Ladanyi</i>	139
BIOLOGICAL HYDRATION WATER DYNAMICS PROBED BY EXTENDED LIGHT SCATTERING AND BROADBAND DIELECTRIC SPECTROSCOPIES <i>S. Perticaroli, M. Nakanishi and A. P. Sokolov</i>	141
THERMAL CHARACTERISTICS OF FREEZE-DRIED CAMEL MILK AND ITS MAJOR COMPONENTS <i>Mohammad Shafiur Rahman, Halaa Al-Hakmani, Ahmed Al-Alawi, and Insaaf Al-Marhubi</i>	143
EFFECT OF WATER CONFINEMENT IN CYCLODEXTRIN-BASED POLYMERS: A JOINT INFRARED AND RAMAN SPECTROSCOPIC STUDY <i>Vincenza Crupi, Domenico Majolino, Andrea Mele, Barbara Rossi, Francesco Trotta and Valentina Venuti</i>	145
RAMAN AND FLUORESCENCE INVESTIGATIONS OF PHASE TRANSITION IN SWOLLEN CYCLODEXTRIN NANOSPONGES: FROM GEL TO WATER SOLUTION <i>Barbara Rossi, Valentina Allodi, Francesca Castiglione, Graziano Guella, Gino Mariotto, Andrea Mele and Francesco Trotta</i>	147
ACTIVITY OF HORSE RADISH PEROXIDASE AND BOVINE LACTOPEROXIDASE AS AFFECTED BY PHYSICAL PROPERTIES OF THE SOLUTION <i>Giampiero Sacchetti, Lilia Neri, Dino Mastrocola and Paola Pittia</i>	149
DISSOCIATION OF WATER UNDER ELECTRIC FIELD <i>F. Saija, G. Cassone, P. V. Giaquinta and A. M. Saitta</i>	151
DYNAMICS OF LIQUID AND SUPERCOOLED WATER AND OF THE LiCl-6H <sub>2</sub> O SOLUTION INVESTIGATED BY BRILLOUIN LIGHT SCATTERING AND INELASTIC SCATTERING OF SYNCHROTRON RADIATION <i>S. C. Santucci, C. Masciovecchio, L. Comez, J-F Legrand<sup>d</sup>, D. Fioretto</i>	152
OPTICAL MANIPULATIONS OF BIOLOGICAL SINGLE SAMPLES IN AQUEOUS ENVIRONMENTS FOR SYNCHROTRON RADIATION INVESTIGATIONS <i>S. C. Santucci, D. Cojoc, H. Amenitsch, M. Burghammer and C. Riekel</i>	154
PRE-MELTING EFFECTS ON GLOBULAR PROTEINS AND WATER PROPERTIES <i>P. Sassi, L. Comez, S. Perticaroli, M. Paolantoni, D. Fioretto, A. Morresi, A. Giugliarelli and G. Onori</i>	156
ABOUT OF MODULATION EFFECT ON WATER EXCHANGE IN SEED IN METABOLIC ACTIVE AND INACTIVE STATES <i>A. Sukiasyan, Y. Mikaelyan and S. Ayrapetyan</i>	157

GLASS TRANSITION AND RELAXATION BEHAVIOR OF WATER AND AQUEOUS SOLUTIONS <i>Jan Swenson</i>	159
WATER HYDRATION IN CAFFEINE-SUGAR SOLUTION <i>Letizia Tavagnacco, Olof Engström, Udo Schnupf, Philip E. Mason, Marie-Louise Saboungi, Michael Himmel, Göran Widmalm<sup>b</sup>, John W. Brady<sup>c</sup> and Attilio Cesàro</i>	161
NON-DESTRUCTIVE MEASUREMENT OF MOISTURE, SOLUBLE SOLIDS CONTENT AND WATER SELF-DIFFUSION COEFFICIENT OF RIPE AND UNRIPE OSMO-DEHYDRATED KIWIFRUIT SLICES BY NIR SPECTROSCOPY <i>U. Tylewicz, P. Santagapita, V. Panarese, P. Rocculi and M. Dalla Rosa</i>	163
WATER AND PROTEIN DYNAMICS IN LYSOZYME-WATER MIXTURES OVER WIDE RANGES OF COMPOSITION <i>K. Vartzeli-Nikaki, A. Panagopoulou, A. Kyritsis and P. Pissis</i>	165
MOLECULAR DYNAMICS OF WATER IN HYALURONIC ACID/SILICA HYDROGEL NANOCOMPOSITES <i>J. Vazquez Molina, A. Panagopoulou, A. Kyritsis, M. Monleón Pradas, A. Vallés Lluch, G. Gallego Ferrer and P. Pissis</i>	168
FREEZE-DRIED HEART VALVE MATRIX IMPLANTS <i>Shangping Wang, Tobias Goecke, Carsten Meixner, Axel Haverich, Andries Hilfiker and Willem F. Wolkers</i>	171

## AUTHOR INDEX

Achir, S.	74	Caponi, S.	38
Affouard, F.	79	Carlà, M.	38
Akhoondi, M.	91	Carter, B.	65
Al-Alawi, A.	143	Casagrande, S.	106
Alhaique, F.	43	Cassone, G.	151
Al-Hakmani, H.	143	Castiglione, F.	147
Aliotta, F.	51	Cazzoli, G.	38
Allodi, V.	147	Čebašek, S.	59
Al-Marhubi, I.	143	Ceccarelli, M.	47
Álvarez, H. A.	56	Cecchetti, V.	45
Amenitsch, H.	154	Cesàro, A.	25, 81, 96, 113, 161
Ancherbak, S.	36	Champion, D.	67
Ando, H.	123	Chara, O.	56
Aravopoulou, D.	92	Charalambopoulou, G.	81
Arapetyan, S.	157	Chen, G.	68
		Chourpa, I.	81
Banerjee, D.	53	Cojoc, D.	154
Barker, S.	104	Cok, M.	96
Baroni, P.	54	Comez, L.	108, 111, 139, 152, 156
Barreca, M. L.	45	Conti Nibali, V.	58, 110
Bartolini, P.	29, 61	Cordone, L.	119
Battistoni, A.	33, 95	Corezzi, S.	111
Bellavia, G.	74	Cottone, G.	119
Bellich, B.	96	Coviello, T.	43
Bellissent-Funel, M.-C.	79	Crupi, V.	145
Bencivenga, F.	33, 95, 98	Cucini, R.	33
Bhat, S. N.	53	Cunsolo, A.	98
Bhat, S. V.	53		
Blasi, P.	100, 102, 106	D'Amico, F.	33, 35
Bončina, M.	59	Dalla Rosa, M.	163
Bonfigli, D.	111	D'Angelo, G.	58
Borgogna, M.	96	D'Angelo, P.	62
Born, B.	110	De Panfilis, S.	62
Bouman, J.	104	de Vries, R.	104
Brady, J. W.	25, 81, 161	Defant, A.	38
Brandt, A.	81	Desai, P. M.	49
Bruni, F.	63	Descamps, M.	7, 79
Buehler, M.	65	Di Cicco, A.	62
Burghammer, M.	154	Di Meo, C.	43
		Donald, A.	121
Calvitti, M.	131	Draganski, A.	11
Campbell, G.	65		
Capaccioli, S.	21, 36	Elisei, E.	113
Capitani, D.	43	Engström, O.	161

Farris, S.	115, 117	Jasnin, M.	30
Ferrara, G. C.	56	Josserand, C.	81
Fichot, J.	81		
Filipponi, A.	62	Kajiwara, K	123
Fioretto, D.	33, 95, 108, 139, 152, 156	Kalimeri, M.	87
Fogarty, A.	9	Kawai, K.	123, 125
Franzese, G.	26	Kirakosyan, A.	128
Friedman, J. M.	11	Krenzlin, S.	79,
		Kumar, S.	71,
		Kyritsis, A.	31, 92, 137, 165, 168
Gaetani, R.	45		
Gallego Ferrer, G.	137, 168	Laage, D.	9, 87
Gallo, P.	77	Ladanyi, B. M.	139
Gambi, C. M. C.	38	Lamanna, R.	43,
Gerelli, Y.	83	Lanzi, L.	38,
Gessini, A.	33	Legrand, J-F	152
Giaquinta, P. V.	151	Lelong, G.	81
Giovagnoli, S.	100	Leporini, D.	53,
Giuffrida, S.	119	Lerbret, A.	79,
Giugliarelli, A.	156	Liew, C. V.	49,
Goecke, T.	171	Lillford, P.	67,
Goel, T.	71	Lombardini, L.	15,
Gojowsky, M.	134	Longo, M.	131
Gombart, E.	81	Ludescher, R. D.	11,
Grigera, J. R.	23, 56	Lukšič, M.	59,
Grossman, M.	110	Lupi, L.	108, 139
Guella, G.	147		
Guinet, Y.	74	Maiocchi, A.	113
		Maiti, S.	71
Hagura, Y.	125	Majolino, D.	145
Havenith, M.	110	Malikova, N.	59
Haverich, A.	171	Mancinelli, R.	63
Hédoux, A.	74, 79	Mancini, G.	62
Heisler, A.	3	Manfroni, G.	45
Heng, P. W. S.	49	Marchetti, S.	38
Heyd, R.	81	Mariani, P.	40,
Heyden, M.	110	Mariotto, G.	147
Hilfiker, A.	171	Masciovecchio, C.	33, 95, 152
Himmel, M.	161	Mason, P.	25, 161
Hribar-Lee, B.	59	Mastrocola, D.	149
Humbardzumyan, A.	128	Matricardi, P.	43
		Mattarelli, M.	38
Imberti, S.	63	Matyushov, D.	5
Introzzi, L.	115, 117	Mazur, K	3
Ioannou, J.	121	Meech, S. R.	3, 135
Iraci, N.	45	Meixner, C.	171

Melchionna, S.	87	Ricci, M. A.	63
Mele, A.	145, 147	Riekkel, C.	154
Michel, J.	8,	Righini, R.	29
Migliorati, V.	62	Rocculi, P.	163
Mikaelyan, Y.	157	Romani, R.	131
Molineró, V.	10	Romano, V.	102
Monleón Pradas, M.	137, 168	Rossi, B.	38, 145, 147
Montagna, M.	38	Rossi, F.	38
Morresi, A.	108, 156		
		Saboungi, M.-L.	25, 81, 161
Nakanishi, M.	141	Saccardi, R.	15
Natali, F.	83	Sacchetti, F.	30, 61, 131
Naumann, P.	42	Sacchetti, G.	149
Neri, L.	149	Sagi, I.	110
Ngai, K. L.	21	Saija, F.	51, 151
Noirez, L.	54	Saitta, A. M.	151
		Santagapita, P.	163
Obaidur, R.	87	Santucci, S. C.	152,154
Oldenhof, H.	91, 134	Sassi, P.	106, 108, 156
Ollivier, J.	83	Schiraldi, A.	115, 117
Onori, G.	40, 156	Schnupf, U.	25, 161
Orecchini, A.	30, 131	Schoubben, A.	100, 102
Ortore, M. G.	40	Sciortino, F.	38
		Sebastiani,	30
Paccou, L.	74	Sennato, S.	38
Paciaroni, A.	21, 30, 131	Seručník, M.	59
Palombo, F.	3, 135	Shinyashiki, N.	31
Panagopoulou, A.	31, 92, 137, 165, 168	Sieme, H.	91, 134
Panarese, V.	163	Siepmann, J.	79,
Paolantoni, M.	106, 108, 139, 156	Sokolov, A. P.	141
Perticaroli, S.	141, 156	Spinozzi, F.	40
Peters, J.	83	Stegen, J.	121
Petrillo, C.	30, 61, 131	Stelletta, C.	83
Piergiovanni, L.	115, 117	Steriotis, T.	81
Pirisinu, I.	131	Sterpone, F.	9, 87
Pissis, P.	31, 92, 137, 165, 168	Stirnemann, G.	9
Pittia, P.	13, 149	Sukiasyan, A.	128, 157
Plazanet, M.	61	Swenson, J.	68, 159
Ponterio, R. S.	51		
Porter, D.	85	Tarek, M.	58
Prevosto, D.	36	Taschin, A.	29
		Tatoli, S.	62
Rahman, M. S.	69, 143	Tavagnacco, L.	25, 161
Rampino, A.	81, 96	Tedoldi, F.	113
Ricci, M.	100, 102, 106	Toh, M.	125



Torre, R.	29, 61
Tranchant, J.-F.	81
Tromp, H.	121
Trotta, F.	111, 145, 147
Tworowski, D.	110
Tylewicz, U.	163
Vallés Lluch, A.	137, 168
van der Schoot, P.	121
Vartzeli-Nikaki, K.	165
Vazquez Molina, J.	137, 168
Venema, P.	104
Venuti, V.	145
Vlachy, V.	59
Vollrath, F.	85
Wanderlingh, U.	58
Wang, S.	134,171
Wang, Z.	42
Widmalm, G.	161
Wiegand, S.	42
Wolkers, W. F.	17, 91, 134, 171
Yanaihara, Y.	123
Zaccai, J.	30
Zitolo, A.	62

## CONTENTS

<b>Programme</b>	<b>IV</b>
<b>Invited Lectures</b>	<b>1</b>
<b>Keynote Lectures</b>	<b>19</b>
<b>Oral Presentations</b>	<b>27</b>
<b>Poster presentations</b>	<b>89</b>
<i>Index</i>	
<b>Invited Lectures</b>	<b>173</b>
<b>Keynote Lectures</b>	<b>174</b>
<b>Oral Presentations</b>	<b>175</b>
<b>Poster Presentations</b>	<b>178</b>
<b>Author Index</b>	<b>182</b>

## addendum

**DEHYDRATION OF MODEL SUGAR SYSTEMS: ROLE OF GEOMETRY AND COMPOSITION**

Antonio Rampino<sup>a</sup>, Rodolphe Heyd<sup>b</sup>, Barbara Bellich<sup>a</sup>, Massimiliano Borgogna<sup>a</sup>, Marie-Louise Saboungi<sup>b</sup> and Attilio Cesàro<sup>a</sup>

<sup>a</sup>Dipartimento di Scienze della Vita, Università di Trieste, Via Giorgieri 1, 34100 Trieste, Italy. [ANTONIO.RAMPINO@phd.units.it](mailto:ANTONIO.RAMPINO@phd.units.it)

<sup>b</sup>Centre de Recherche sur la Matière Divisée, Equipe MCC, CNRS, rue de la Férellerie 1B, 45071 Orléans Cedex 2, France.

The hydrogel properties make them one of the ideal controlled release systems. Since the most common mechanism of release from hydrogels is diffusion, molecules of different sizes and characteristics can freely diffuse into/out of hydrogel matrix, provided that mesh size is large enough. This means not only that solute molecules diffuse out of the matrix, but also that solvent molecules penetrate into the gel thus influencing solute release [1].

Several systems have been selected for the preparation of complex gels, with current interest in biotechnology and biomedicine as well as in various fields of pharmaceutical and food technology, in order to understand the diffusion behaviour out of the matrix. Initially, the work was aimed at the study of the rate (kinetics) and the equilibrium (thermodynamics) of water effusion from complex polysaccharidic matrices [2-4], focusing on alginate gels with spherical form (beads). The main drawback in using spherical beads is the continuous changing of the surface area. In particular, the attention was given to the study of the non-isothermal dehydration process of these gels.

Therefore, in order to elaborate simple and useful equations, the model was simplified as much as possible, changing the geometry of the system by using paper disks as sample holders. They were prepared by using filter-paper disks first soaked in polymer solutions, and then *in-situ* gelled through immersion into suitable gelling solutions. The main feature of these gels is that they are characterized by a well-defined homogeneous internal structure, and with pore sizes, ranging in the order of the nanometers.

Moreover, The system composition was changed, moving from polysaccharide gels to (mono-, di-)saccharides solutions, analysing the process of saccharide-water interaction during dehydration. In this set of experiments, therefore, the focus of the research was pointed to monitor the solute-solvent interaction as a function of composition change following dehydration.

In all cases, water evaporation was analyzed both with isothermal thermogravimetric and non-isothermal calorimetric experiments. Isothermal experiments were carried out with a custom-made humidity and temperature controlled gravimetric apparatus (CNRS laboratories), connected to a PC.

*addendum*

While isothermal data follow the well-known diffusion equation in the initial range, the non-isothermal data are slightly more complex showing the convolution of the diffusion equation with its temperature dependence.

Differences and peculiar dehydration profiles were observed among systems characterized by different geometries. The sample composition influenced the dehydration process: in particular the presence of sugars decreases water evaporation rate. At a first glance, bead geometry seems to highlight differences related to composition. A mathematical fitting has been used both isothermal and non-isothermal evaporation rate and correlate this behaviour with the process of drug release.

Then, based on several complex hydrogel results, a system modeling [5] has been exploiting for rationalizing the non-isothermal evaporation rate and correlate this behaviour with the process of drug release, which is of fundamental relevance in biopharmaceutics [6].

**References**

1. Amsden, B. *Macromol.* 1998, *31*, 8382.
2. Bellich, B., Borgogna, M., Carnio, D., Cesàro, A. J. *Therm. Anal. Calorim.* 2009, *97*, 871.
3. Bellich, B., Borgogna, M., Cok, M., Cesàro, A. *Food Biophysics*, 2011, *6*, 259.
4. Bellich, B., Borgogna, M., Cok, M., Cesàro, A. J. *Therm. Anal. Calorim.* 2011, *103*, 81.
5. Lin, C.-C., Metters, A. T. *Adv. Drug Deliv. Rev.* 2006, *58*, 1379.
6. Hamidi, M., Azadi, A., Rafiei, P. *Adv. Drug Deliv. Rev.* 2008, *60*, 1638.

<b>Monday 24</b>		<b>Tuesday 25</b>		<b>Wednesday 26</b>	
9.00	<b>Opening</b>	9.00	<b>Invited - LAAGE</b>	9.00	<b>Invited - SACCARDI</b>
9.15	<b>Invited - MEECH</b>	9.40	ALIOTTA	9.40	GOEL
9.55	TORRE	9.55	LEPORINI	9.55	BELLAVIA
10.10	ORECCHINI	10.10	NOIREZ	10.10	GALLO
10.25	PISSIS	10.25	CHARA	10.25	LERBRET
10.40	coffee break/poster	10.40	coffee break/poster	10.40	coffee break/poster
11.10	<b>Invited - MATYUSHOV</b>	11.10	<b>Invited - MOLINERO</b>	11.10	<b>Invited - WOLKERS</b>
11.50	CUCINI	11.50	<b>Keynote - GRIGERA</b>	11.50	<b>Keynote - FRANZESE</b>
12.05	D'AMICO	12.10	CONTI NIBALI	12.10	HEYD
12.20	CAPACCIOLI	12.25	HRIBAR-LEE	12.25	NATALI
12.35	CAPONI	12.40	PLAZANET	12.40	PORTER
13.00	Lunch	13.00	Lunch	12.55	STERPONE
14.00	poster session	14.00	poster session		<b>CLOSING</b>
15.00	<b>Keynote - NGAI</b>	15.00	<b>Keynote - BRADY</b>		
15.20	ORTORE	15.20	D'ANGELO P.		
15.35	WIEGAND	15.35	MANCINELLI		
15.50	<b>Invited - DESCAMPS</b>	15.50	<b>Invited - LUDESCHER</b>		
16.30	coffee break/poster	16.30	coffee break/poster		
17.00	<b>Invited - MICHEL</b>	17.00	<b>Invited - PITTIA</b>		
17.40	LAMANNA	17.40	CAMPBELL		
17.55	BARRECA	17.55	CHAMPION		
18.10	CECCARELLI	18.10	CHEN		
18.25	HENG	18.25	RAHMAN		
		20.30	<b>SOCIAL DINNER</b>		