Frontiers in Water Biophysics www.waterbiophysics.eu

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BOOK OF ABSTRACTS AND CONFERENCE PROGRAMME

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

WELCOME

A very warm welcome is addressed to all the participants and contributors to this sixth “Frontiers in Water Biophysics” conference, once again located in the spectacular location of Erice.

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

With *Just one word: plastic* and *The future is Plastics*, 55 years ago *The Graduate* movie foretold a great industry and new products. *The Future is 'Plastics'* was uttered in that watershed movie. By confusing the material name (i.e., polylolefines) with the property name, Mr. McGuire did not realize that he said a most unconventional concept at that time. To all of us in the science world the concept clearly does not be applied only to the “plastic commodities” or to the “bioplastics” (e.g., microbial polyesters of late eighties) but extensively also to the life biopolymers, nucleic acids, proteins, polysaccharides, and all other “adaptable” systems. All of them experienced what the biophysicist Careri in a lecture in Naples (1964) called “the breath of life”, referring to the amplitude of statistical displacements of globular proteins out of crystalline state. Nowadays, we face with an extensive use of many other such terms.

This year the Frontiers in Water Biophysics conference marks a step ahead since the beginning in 2010. The adventure begun with the naïve observation that in all constituents of living cells, food, and pharma matters the general presence of water is always acknowledged, either as plasticizer of the biopolymer components, as a solvent and as a reactant. Indeed, several thousands of fundamental pages have been written about water at fundamental research level and its ubiquitous and holistic role in all known aspects of our life. Still no major changes in the molecular archetype have been introduced, yet our knowledge continues to add fascinating experimental and computational results.

Thus, this conference is just another tile in drawing some new perspectives at water properties. If the basic function of every education, in the most conventional meaning, is worth to increase the survival probability of a group of people, it is clear that this goal in the science community continuously receives confirmation. We strongly believe that our scientific endeavor in setting down the Frontiers in Water Biophysics conferences will generate new contacts and collaborations, new ideas, and new relevant results. This objective supported and will support us in pursuing ahead with the strongest consideration of achieving a fantastic socio-scientific goal.

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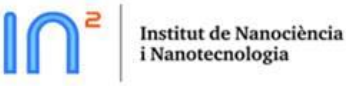

Programme

	FRI 19	SAT 20	SUN 21	MON 22	TUE 23	WED 24
8:45-9:00	ARRIVAL and REGISTRATION	OPENING				DEPARTURE
CHAIR						
9:00-10:20		Naoki Sugimoto	Roland Netz	Jean-Louis Mergny	Frank Jülicher	
10:20-11:00		Daniel Renciuk	Alfonso De Simone	Cristiano De Michele	Sara Del Galdo	
11:00-11:30		COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	
CHAIR						
11:30-11:45		Valeria Libera	Riccardo Morbidini	Ilaria Mosca	Gian Marco Tuveri	
11:45-12:00		Ettore Napolitano	Luigi Caminiti	Luca Bertini	Edward D. Donkor	
12:00-12:30		Francesca Ripanti	Sara Catalini	Nunzia Iaccarino	Jorge H. Melillo	
12:30-13:10		Alessandro Paciaroni	Giuseppe Battaglia	Valeria Conti Nibali	Giuseppe Graziano	
13:10-15:00		LUNCH	LUNCH	LUNCH	LUNCH	
CHAIR						
15:00-16:10		Antonio Randazzo	Francesco Sciortino	Janez Plavec	Ellen Adams	
16:10-16:50		Andrea Lapini	Carmen Galan	Lorena Ruiz Perez	Giancarlo Franzese	
16:50-17:05		EXCURSION to SALINE & DINNER in MARSALA CITY	Sara Venturi	POSTER + COFFEE BREAK	CLOSING REMARKS + COFFEE BREAK	
17.05-17.30			POSTER + COFFEE BREAK			
CHAIR					Touring & shopping	
17:30-17:45			Martina M. Calvino	Ariane Fournier		
17:45-18:25			Sergey Buldyrev	Paola Gallo		
18:25-19:00			Discussion	Discussion		
20:00-	DINNER		DINNER	DINNER	SOCIAL DINNER	

SATURDAY 20

OPENING
09:00-10:20 TU1
N. Sugimoto "*TO B OR NOT TO B in nucleic acids research and water"*
10:20-11:00 IL1
D. Renciuk *"Drunk nucleic acids: ethanol reveals conformational FLEXIBILITY OF DNA and RNA"*
11:30- 11:45 PhD1
V. Libera *"Mutual influence of solvent vibrations and telomere G-quadruplex rearrangements upon thermal unfolding"*
11:45-12:00 PhD2
E. Napolitano *"Discovery of HMGB1 inhibitors based on G-quadruplex-forming aptamers"*
12:00-12:30 OP1
F. Ripanti *"Oxidizing effects on guanine: from nucleotides to G-quadruplex structures"*
12:30-13:10 IL2
A. Paciaroni *"Diffusive dynamics of bacterial proteome as a proxy of cell death"*

15:00-16:10 PL1
A. Randazzo *"An introduction to nuclear magnetic resonance (NMR) spectroscopy and its application to the study of nucleic acid structures and their interaction with water"*
16:10-16:50 IL3
A. Lapini *"Water dynamics in structure breaking ions and in lipidic mesophase confined systems"*
SOCIAL EVENT

SUNDAY 21

09:00-10:20 TU2
R. Netz *"Simulation approaches to hydration effects in biological systems"*
10:20-11:00 IL4
A. De Simone *"Membrane interactions and phase separations of alpha-synuclein"*
11:30-11:45 PhD3
R. Morbidini *"Self and collective dynamics in water-ethanol mixture: spectroscopy with polarized neutrons"*
11:45-12:00 PhD4
L. Caminiti *"Dynamics of lysozyme hydration water investigated by ultrafast time-resolved spectroscopy"*
12:00-12:30 OP2
S. Catalini *"Self-assembled peptides architectures to develop new smart biomaterials"*
12:30-13:10 IL5
G. Battaglia *"Phenotypic association theory and experiment"*

15:00-16:10 PL2
F. Sciortino "*DNA nanostars: a model system for biopolymer gels and condensates"*
16:10-16:50 IL6
M.C. Galan *"Controlling G4 DNA topology with small molecules: towards the development of novel therapeutics"*
16:50-17:05 PhD5
S. Venturi *"Amyloid and non-amyloid aggregation of β-lactoglobulin in self-crowded regime"*
POSTER SESSION
17:30-17:45 PhD6
M.M. Calvino *"Water diffusion in nanoclays: an NMR study"*
17:45-18:25 IL7
S.V. Buldyrev *"Formation of dissipative structures in microscopic models of mixtures with species interconversion"*

MONDAY 22

09:00-10:20 TU3
J.L. Mergny *"Quadruplexes are everywhere!"*
10:20-11:00 IL8
C. De Michele *"Stacking interactions and flexibility of human telomeric multimers"*
11:30-11:45 PhD7
I. Mosca *"Dynamic cluster formation, viscosity and diffusion in monoclonal antibody solutions"*
11:45-12:00 PhD8
L. Bertini *"Polymorphism and ligand binding modulate fast dynamics of human telomeric G-quadruplexes"*
12:00-12:30 OP3
N. Iaccarino *"Use of chemometrics for extracting hidden information from spectroscopic data"*
12:30-13:10 IL9
V. Conti Nibali "Refining the description of the collective dynamics of proteins in the terahertz frequency window"

15:00-16:10 PL3
J. Plavec *"NMR insights into effects of water and cations in folding of G-rich DNA fragments"*
16:10-16:10 IL10
L. Ruiz-Perez *"Imaging Protein Self-Assembly in Water using liquid-phase transmission electron microscopy"*
POSTER SESSION
17:30-17:45 PhD9
A. Fournier *"Influence of photoactivated water on the physico-chemical properties of cosmetic ingredients"*
17:45-18:25 IL11
P. Gallo *"Slow dynamics and local structure of water at bio-interfaces"*

TUESDAY 23

09:00-10:20 TU4
F. Jülicher *"Physics of protein condensates"*
10:20-11:00 IL11
S. Del Galdo *"Combined atomistic and coarse-grained approach for the study of thermoresponsive adsorbent species at the nanoscale: the case of polyoxazolines"*
11:30-11:45 PhD10
G.M. Tuveri *"Computational study of the low-density lipoprotein receptor-related protein 1 structure and dynamics"*
11:45-12:00 PhD11
E.D. Donkor *"Unsupervised machine-learning of water structure: from hydrophobic interfaces to critical behavior"*
12:00-12:30 OP4
J.H. Melillo *"Isotope effect on the dynamics of water in aqueous solutions at supercooled temperatures"*
12:30-13:10 IL13
G. Graziano "R*emarks on the hydration entropy of polar, nonpolar and charged species"*

15:00-16:10 PL4
E. Adams *"Solution properties of biomolecular condensates"*
16:10-16:50 IL14
G. Franzese "Hydration and crowding effect in biomolecular condensates: the case study of sod1 into stress granules*"*
16:50-17:25
CLOSING REMARKS

TUTORIAL

TU1

"TO B OR NOT TO B" IN NUCLEIC ACIDS RESEARCH AND WATER

Naoki Sugimoto

FIBER (Frontier Institute for Biomolecular Engineering Research)
and
FIRST (Graduate School of Frontiers of Innovative Research in Science and Technology), Konan University, Kobe, Japan

The stability of nucleic acids structures cannot be determined from only the sequence composition, as this property critically depends on the surrounding environment of the solution. The intracellular condition is greatly different from that of the diluted buffer typically used for standard experiments and is not constant in each local area of the cell. Thus, stability predictions should reflect the situation under intracellular conditions.

In this presentation, I will provide an overview of the basic concepts, methods, and applications of predicting the stabilities of nucleic acid structures. We explain the theory of the most successful prediction method based on a nearest-neighbor (NN) model. To improve the versality of prediction, corrections for various solution conditions considered hydration have been investigated. I also describe advances in the prediction of non-canonical structures. Finally, studies of intracellular analysis and prediction are discussed for the application of NN parameters.

References (from our group)

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SIMULATION APPROACHES TO HYDRATION EFFECTS IN BIOLOGICAL SYSTEMS

Roland Netz

Physics Department, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany

The layer of water molecules on biological surfaces, around biopolymers and ions, the so-called hydration layer, is crucial for the properties of biological systems. Insight is gained from all-atomistic simulations in conjunction with appropriate continuum interpretation and modeling. We discuss a few different scenarios:

1) The hydration repulsion between polar surfaces can be studied using a simulation technique that allows to determine the interaction pressure at constant water chemical potential. The hydration repulsion is shown to be caused by a mixture of water polarization effects and the desorption of interfacial water. For the interaction between two dissimilar surfaces our simulations demonstrate that between the limiting well-studied regimes representing hydrophobic attraction and hydration repulsion an intermediate novel regime corresponding to hydrophilic attraction exists.

2) On surfaces, the friction coefficient of bound peptides is very low on hydrophobic substrates, which is traced back to the presence of a vacuum layer between substrate and waterforming a lubricating cushion on which a polymer can glide. Conversely, friction forces on hydrophilic substrates are large. A modified Amonton´s law describes the dynamics of hydrogen-bonded matter on the nanoscale.

3) The dielectric properties of hydration water at surfaces are different from bulk water and exhibit pronounced anisotropy effects, causing the well-known reduction of the surface capacitance. The dielectric constant of confined water is generally reduced, leading to an increase of electrostatic interactions in water slabs and water pockets.

4) Although conceptually simple, the air-water interface displays complex static and dynamics properties, which become relevant when describing interfacial ion effects. Different definitions of the electrostatic potential, each relevant for distinct experimental scenarios, lead to widely varying surface potential magnitudes and even different signs. Based on quantum-chemical density-functional molecular dynamics simulations, a few different surface potentials are evaluated and compared. The spatially averaged surface potential, accessible to electron holography, is dominated by the trace of the water molecular quadrupole moment and amounts to more than + 4 Volt inside the water phase, very different from results obtained with force-field water models. The surface potential inside a cavity is much smaller, less than 200 mV in magnitude. This is the electrochemical surface potential relevant for ion transfer reactions and ion surface adsorption.

QUADRUPLEXES ARE EVERYWHERE!

Jean-Louis Mergny

Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS UMR7645 – INSERM U1182, Institut Polytechnique de Paris, 91120 Palaiseau, France

G-quadruplexes ("G4") are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies [1]. G4 can be formed intramolecularly by G-rich DNA or RNA sequences. We are developping tools to understand their folding and polymorphism, both in vitro and in cells 2, as well as the influence of external parameters such as pH, ionic conditions or crowding. In parallel, we proposed a new algorithm for the prediction of G4 propensity 3. We are now applying this prediction tool to many genomes, including cancer or virus genomes 4-8, and very recently on "old genomes": we have analysed the sequences of hepatitis B viruses (HBV) for the presence of G-quadruplex-forming sequences 9. Our work used genomes from ancient and modern HBV stains and represents the first paleogenomic analysis of the propensity for G4 formation in any genome. We are also interested in the role of quadruplexes in parasites such as Plasmodium falciparum 10 and, more recently on parasitic helminths, which are highly prevalent and infect approximately two billion people worldwide 11. Finally, if time allows, I will speak about our latest results on i-motif folding 12.

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PHYSICS OF PROTEIN CONDENSATES

Frank Jülicher

Max Planck Institute for the Physics of Complex Systems (MPI-PKS), Dresden, Germany
Center for Systems Biology Dresden, Dresden, Germany
Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany

Many membraneless organelles in cells are biochemical compartments that form via the condensation of specific proteins together with RNA. Such condensation can be recapitulated in vitro where protein droplets can form by phase separation from buffer. Protein condensates are complex fluids with variable material properties. I will provide an introduction to biological condensates and highlight general aspects of the physics of protein phase separation and its regulation by chemical components. In order to explore how condensates can organize chemical processes in cells, we study the interplay of chemical reactions and phase separation. This work reveals that chemically active droplets can exhibit a rich phenomenology and unconventional behaviors. Finally, protein condensates exhibit interesting rheological behaviors. Microrheology of protein droplets shows that the material properties of protein condensates are those of a visco-elastic fluid that is well described by a Maxwell model. However, material properties can depend on age of the material. This suggests that condensates that harden over time exhibit glassy behaviors. We call these materials Maxwell glasses.

PLENARY LECTURES

PL1

AN INTRODUCTION TO NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY AND ITS APPLICATION TO THE STUDY OF NUCLEIC ACID STRUCTURES AND THEIR INTERACTION WITH WATER

Antonio Randazzo

Netherlands Dept. of Pharmacy, University of Naples ''Federico II'', Via D. Montesano 49, 80131 Napoli, Italy

Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as a powerful tool for studying the structure, dynamics, and interactions of nucleic acids. Nucleic acids, including DNA and RNA, play crucial roles in genetic information storage, transmission, and regulation within living systems. Understanding their structure and behavior is essential for unraveling the mechanisms underlying vital cellular processes such as replication, transcription, and translation.
In this communication, an overview of the applications of NMR spectroscopy in the study of nucleic acids will be reported. Particularly, the first part of the presentation will highlight the fundamental principles of NMR, including nuclear spin and the interaction between magnetic fields and atomic nuclei. It will also report on the concept of chemical shift, scalar and dipolar couplings.

The second part will focus on the use of NMR to study the three-dimensional structures of nucleic acids at atomic-level, providing information about base pairing, helical geometries, and higher-order structures. Example of assignment strategy and description of the main 2D NMR experiments will be discussed. Furthermore, the second part will also focus on water molecules present in the sample and how these can provide interesting structural information, allowing the characterization of the conformational flexibility and fluctuations in solution of nucleic acids e suggesting the mechanisms of nucleic acid folding, recognition, and binding to various ligands and proteins.

DNA NANOSTARS: A MODEL SYSTEM FOR BIOPOLYMER GELS AND CONDENSATES

Francesco Sciortino

Department of Physics, Sapienza University of Rome, P.le A. Moro, 5, 00185 Rome, Italy

DNA oligomers can nowadays be used to produce a large variety of nanometric constructs, via a cascade of self-assembly processes, each one guided by the length of complementary sequences of distinct DNA strands. In the lecture I will show that it is possible to build bulk quantities of DNA-made nanoparticles that closely match idealized colloids, transferring modern in-paper and in-silico intuitions into experimental realizations.

I will show how unconventional phase-behaviors, recently explored theoretically and numerically, can indeed be reproduced in the lab. Specifically, I will discuss how to exploit limited valence interactions to suppress phase separation, enhancing the stability of the equilibrium gel phase. The ability to control in details the interaction between the different nano-stars makes these systems ideal models for investigating liquid-liquid phase separation, a topic of biological relevance.

The ability to control in details the interaction can also be exploited for material properties. I will show how to exploit competing interactions to generate a material that is fluid both at high and at low temperatures and a solid-like disordered open network structure in between and how to exploit bond-swap dynamics to create an all-DNA vitrimer.

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NMR INSIGHTS INTO EFFECTS OF WATER AND CATIONS IN FOLDING OF G-RICH DNA FRAGMENTS

Janez Plavec

Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia
University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia
EN-FIST Centre of Excellence, Trg OF 13, Ljubljana, Slovenia

Water molecules play an essential role in stabilizing the structure of DNA and other biomolecules. The polar nature of water molecules allows them to interact with the charged phosphate groups on the outside of the DNA helix through electrostatic interactions. In addition, water molecules can form hydrogen bonds with the nitrogenous bases of DNA, which helps stabilize the double helix structure and prevents DNA strands from separating. Understanding the localization of water molecules is critical to understanding DNA function in replication and transcription. Quadruplex DNA is a secondary structure formed by G-rich DNA sequences. These sequences can form four-stranded structures that are stabilized by hydrogen bonds between guanine bases. The unique structure of G-quadruplex DNA makes it an attractive target for drug discovery. Small molecule ligands targeting G-quadruplexes have been developed as potential cancer therapeutics. Different ligands prefer different folds and very few complexes have been solved at high resolution. Human telomeric G-quadruplex structures are attractive targets, but target polymorphism complicates their development. Phen-DC3, bisquinolinium-derivatized phenanthroline dicarboxamide, one of the best-known G-quadruplex ligands characterized by high binding affinity and selectivity, causes dTAGGG(TTAGGG)$_3$] to completely change fold in KCl solution, with the ligand intercalating between two quartet units.

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for determining the three-dimensional structure of DNA. In water, hydrogen atoms are surrounded by other atoms in the molecule and by neighboring water molecules, which can affect the frequency of the NMR signal. Cations such as K^+ and Na^+ are important for the stability and function of DNA, and their interaction with water molecules is critical for their role in solvating DNA. In an aqueous environment, cations are hydrated by water molecules, which form a hydration shell around the cation. This hydration shell can affect the ability of the cation to interact with DNA and can also affect the structure and dynamics of the DNA itself.

Our laboratory uses NMR spectroscopy in combination with complementary methods to reveal structural details of four-stranded DNA architectures in terms of sequence details, presence of cosolutes and inorganic salts, pH, interaction with (heterocyclic) ligands, and folding pathways. We have recently described a new family of tetrahelical structures that are distinctly different from G-quadruplexes, although they contain the G-quadruplex folding motif (i.e., d[(GGGNn)$_3$GGG]). These sequences with Nn=AGCGA exhibit topologies characterized by a tetrahelical core of AGCGA repeats connected by edge-like loops of different lengths stabilized by G-G base pairs in N1-carbonyl symmetric geometry. Remarkably, AGCGA-quadruplexes are not very sensitive to the type of cations present in the nearby solution and exhibit specific water localization in their interior.

SOLUTION PROPERTIES OF BIOMOLECULAR CONDENSATES

Ellen Adams

Cluster of Excellence Physics of Life, Technische Universität Dresden, 01307 Dresden, Germany
Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, 01328 Dresden, Germany

In recent years the importance of the aqueous solvent in influencing protein structure, function, and dynamics has been recognized. Coupling of water molecules to the protein surface results in an interfacial region in which water molecules within this region have distinctly different properties than bulk water. However, the structure and dynamics within this interfacial region are still not easy to access experimentally. Terahertz (THz) spectroscopy has been shown to be a powerful tool to investigate solvent dynamics in bulk solutions. Radiation in the THz regime is directly sensitive to the low frequency collective intermolecular hydrogen-bonding vibrations of water (0.3-6 THz or 10-200 cm^{-1}), and thus to any changes in the hydrogen-bonding network. Changes in these sub-picosecond collective motions, such as protein-water interactions, result in changes in the measured THz absorption. Individual hydrations shells of proteins have been shown to contribute largely to structure-function relationships and ultimately modulate the binding properties of proteins. Here the role of solvation dynamics in the liquid-liquid phase separation (LLPS) of the intrinsically disordered protein fused in sarcoma (FUS) is probed. Characterization of the hydrogen bonding network reveals that water solvating hydrophobic groups is stripped away in the membrane-less FUS biomolecular condensates. Additionally, water left inside of the biomolecular condensates is highly constrained, indicative of a population of bound hydration water. These results uncover the vital role of hydration water in LLPS: the entropically favorable release of unfavorable hydration water serves as a driving force for LLPS.

INVITED LECTURES

DRUNK NUCLEIC ACIDS: ETHANOL REVEALS CONFORMATIONAL FLEXIBILITY OF DNA AND RNA

Daniel Renciuk

Department of Biophysics of Nucleic Acids, Institute of Biophysics of the Czech Academy of Sciences, Brno, CZ-61200, Czech Republic

Nucleic acids are structurally polymorphic molecules. They might fold into various topologies based on their primary sequence and environmental conditions. One of the important factors is the hydration, indirectly related to so-called molecular crowding. Structural transitions from regular right-handed double helical B-DNA into a more condensed A-DNA or left-handed Z-DNA, induced by dehydrating agents such as alcohols were described several decades ago. In our subsequent work we have been interested whether and how the alcohols influence other alternative nucleic acids structures such as guanine quadruplexes (G4) and cytosine i-motifs (iM). We revealed ethanol as a powerful G4 inducer and stabilizer even in conditions with low potassium ions concentration, though the G4 topology might be significantly different in the presence of ethanol, compared to K^+ water solutions. In contrast to reported positive effects of molecular crowding on iM formation and stability, our data indicate that ethanol-induced dehydration neither facilitates iM formation nor stabilizes existing iM. Our data might contribute to understanding conformational dynamics of nucleic acids in systems with inherently crowded or dehydrating environment such as cytosol or virus particles.

DIFFUSIVE DYNAMICS OF BACTERIAL PROTEOME AS A PROXY OF CELL DEATH

Alessandro Paciaroni[1], Daniele Di Bari[1,2], Stepan Timr[3], Marianne Guiral[4], Marie−Thérèse Giudici-Orticoni[4], Tilo Seydel[5], Christian Beck[5], Caterina Petrillo[1], Philippe Derreumaux[6], Simone Melchionna[7], Fabio Sterpone[6], Judith Peters[2]

[1]Dipartimento di Fisica e Geologia, Università degli Studi di Perugia
[2]Laboratoire Interdisciplinaire de Physique, Université Grenoble Alpes, CNRS
[3]J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences
[4]Laboratoire de Bioénergétique et Ingénierie des Protéines, IMM, CNRS, Aix−Marseille Université
[5]Institut Laue−Langevin
[6]ISC−CNR, Dipartimento di Fisica, Università Sapienza
[7]Laboratoire de Biochimie Théorique, CNRS, Université de Paris Cité

Temperature variations have a big impact on the bacterial metabolism and death, yet an exhaustive molecular picture of these processes is still missing. For instance, whether the thermal death is determined by the deterioration of the whole or a specific part of the proteome is hotly debated. In our work, by monitoring the proteome dynamics of E. coli we show that only a minor fraction of the proteome unfolds at the cell death. First, we prove that the dynamical state of the E. coli proteome is an excellent proxy for the temperature dependent bacterial metabolism and death. The proteome diffusive dynamics peaks at about the bacterial optimal growth temperature, then a dramatic dynamical slowdown is observed which starts just below the cell's death temperature. Next, we show that this slowdown is caused by the unfolding of just a small fraction of proteins which establish an entangling inter-protein network—dominated by hydrophobic interactions— across the cytoplasm. Finally, we prove that the deduced progress of the proteome unfolding and its diffusive dynamics are both key to correctly reproduce the E. coli growth rate.

WATER DYNAMICS IN STRUCTURE BREAKING IONS AND IN LIPIDIC MESOPHASE CONFINED SYSTEMS

Sara Catalini[1,2], Chiara Calvagna[1], Yang Yao[3], Paolo Bartolini[1], Andrea Taschin[1,4], Samuele Fanetti[1,5], Mariangela di Donato[1,5], Paolo Foggi[1], Raffaele Mezzenga[3], Renato Torre[1], RobertoBini[1], Roberto Righini[1], Andrea Lapini[1,6]

[1]LENS, Laboratorio Europeo di Spettroscopia Non-Lineare, via Nello Carrara 1, 50019 Sesto F.no, Italy
[2]Dipartimento di Fisica e Geologia, Università di Perugia. Via Alessandro Pascoli, 06123 Perugia, Italy
[3]ETH-Zurich, Schmelzbergstrasse 9 8092 Zürich, Switzerland
[4]ENEA, Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile, Centro Ricerche Frascati, via Enrico Fermi 45, 00044 Frascati, Roma, Italy
[5]ICCOM-CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy
[6]Dipartimento di Scienze Chimiche della vita e della sostenibilità ambientale (SCVSA), Università di Parma, Italy

The presence of ions induces perturbations in the water network, these structural and dynamic modifications can extent over space scales overcoming the local solvation shell: aqueous solutions of sodium perchlorate ($NaClO_4$) are characterized by extended phenomena of structure breaking of the solvent network. Ultrafast Optical Kerr Effect (OKE) and time resolved infrared absorption are suitable experimental techniques: OKE is mostly sensitive to the collective properties of the sample, while transient IR provides access to local properties of the solvent. The experiments and simulations have been performed at room temperature, varying the concentrations (0–6 M) and varying the applied pressure (10^{-4}–1.3 GPa). Overall, our results underline that pressure and concentration have convergent effects on the water dynamics, the common feature being the modification of the short-range liquid structure that results from the merging of the second shell into the first one [1,2].
Confinement effects on water dynamics has been very well characterized in the paste decades through static as well as time resolved vibrational spectroscopy. The main target of such studies has been the investigation of water within AOT reverse micelles [3] or Nafion membranes [4]. Lipidic lyotropic liquid crystals (LLCs), also known as lipidic mesophases, forming by the self-assembly of lipids in water, offer an alternative confining media, and an ideal biocompatible platform for protein crystallization drug delivery and enzymatic reactions. Contrary to the AOT and other water-in-oil systems, LLC provides continuous/bicontinuous water channels which enables the diffusion and transportation of substrates and products. A variety of liquid crystal structures can be obtained depending on the amphi-philic properties of the lipid, the degree of hydration, and the temperature. Here we present our recent characterization of Water in Phythantriol-based LLC-water system.

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MEMBRANE INTERACTIONS AND PHASE SEPARATIONS OF ALPHA-SYNUCLEIN

Alfonso De Simone

Dipartimento di Farmacia, Università di Napoli Federico II, Via Montesano 49, 80131 Napoli, Italy

The aggregation of α-synuclein (αS), a neuronal protein that is abundant at the pre-synaptic terminals, is associated with a range of highly debilitating neurodegenerative conditions including Parkinson's Disease. Fibrillar aggregates of αS are the major constituents of proteinacious inclusions known as Lewy bodies that form in dopaminergic neurons of patients suffering from these conditions. The function of αS, however, is currently unknown, with evidences suggesting a role in the regulation of the trafficking of synaptic vesicles (SV).
To elucidate the nature of the normal and pathological forms of αS, we have established a research programme based on structural biology and cellular biophysics to reveal the properties of its transient interactions with biological membranes. Another key focus of our recent research is the self-assembly of αS into liquid-like spherical condensates, from which pathologically relevant fibrils are formed in vitro. Our data highlight the role of the solvent in this process indicating that the depletion of bulk water is at the origin of the underlying molecular mechanism of αS aggregation.

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PHENOTYPIC ASSOCIATION THEORY AND EXPERIMENT

Giuseppe Battaglia

Catalan Institution for Research and Advanced Studies, Barcelona, Spain
Institute for Bioengineering of Catalunya, The Barcelona Institute of Science and Technology, Barcelona
Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine, Spain

The central dogma of biological interactions dictates that the strength of a drug's affinity to its receptor is crucial in effective drug design. A higher negative binding energy leads to better targeting of cells or tissues that express the same receptor [1]. However, it is important to note that achieving maximum selectivity at the molecular level may result in high-affinity ligands targeting all cells expressing the receptor in question. It is essential to bear in mind that biological units are more intricate than just single molecules. The combination of biomolecules into single cells leads to an immense increase in the number of possible configurations. This implies that each cell in our body is distinct from the others. To create more selective drugs, it may not be necessary to analyse the complexity of single cells down to the quantum level. However, it is crucial to enhance molecular design to incorporate more holistic effects for more precise biological target differentiation. Over the past decade, we have utilised statistical and soft matter physics tools to match the internal state energetic configurations of biological targets with complementary multivalent units. This approach favours selective associations based on multiple bonds, addressing the challenge at hand. According to the super-selectivity theory (SST) [2], multivalent units interact through the collective effect of single affinities (or avidity) and association changes with receptors or ligand numbers. This results in entropy-driven interactions that are not linear. By combining low-affinity ligands, we can target cells that overexpress the desired receptor only when receptors are high in numbers. This unique nature of the interaction allows for effective targeting. Through experimental evidence [3-6], we have confirmed the existence of SST and shown that the interaction involves a combination of specific ligand/receptor bonds and a mean-field repulsive potential resulting from steric effects. To address the multidimensional nature of this problem, we have adopted a nomenclature similar to that used in quantum physics.I here define the different states that characterise a cell phenotype and the multivalent unit to target using a vector of features, one that defines the cell phenotype as$|\varphi_j\rangle$ representing the specific cell receptors compositions and is the mean-field steric potentials. We can define a multivalent unit vector of features is defined as $\langle\nu_j|$and state that two are complementary if

$$\langle\nu_i|\boldsymbol{\Theta}|\varphi_j\rangle = \delta_{ij} = \begin{cases} 1, & \text{if } i \equiv j \\ 0, & \text{if } i \not\equiv j \end{cases} \quad (1)$$

the *ith* multivalent unit, the *jth* cell phenotypes are complementary via the hierarchical operator $\boldsymbol{\Theta}$. Using such a formalism, I will show that we can adapt molecular engineering tools to design highly selective drugs.

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CONTROLLING G4 DNA TOPOLOGY WITH SMALL MOLECULES: TOWARDS THE DEVELOPMENT OF NOVEL THERAPEUTICS

M. Carmen Galan

School of Chemistry, University of Bristol, Cantock's Close, Bristol, United Kingdom

G-quadruplexes oligonucleotides (G4) are a fascinating class of nucleic acid structures formed from the self-association of guanine-rich sequences. This kind of four-stranded structures have potential applications in biological chemistry and responsive nanotechnology that may be exploited for therapeutic effect. While many examples of ligands that are able to stabilize G4 sequence are reported in the literature, those ligands do not induce reversible and controllable structural perturbations such as the re-folding of the G4 to an alternative topology or the unfolding of the G4 structure through binding modes at physiological pH. In this sense, light offers high spatiotemporal precision for the regulation of oligonucleotide structure [1,2]. During this lecture I will describe recent examples of photoresponsive ligands for G4 DNA regulations developed within our research group. From stiff-stilbene ligands which are capable of unfolding G4 DNA in physiological conditions in a reversible manner [3,4] dithienylethene chromophores with inherently superior photoresponsive properties for the study of G4-binding properties which can be used for the photo-reversible control of ligand binding mode and oligonucleotide folding employing exclusively red and blue visible light [5].

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FORMATION OF DISSIPATIVE STRUCTURES IN MICROSCOPIC MODELS OF MIXTURES WITH SPECIES INTERCONVERSION

Thomas J. Longo[1], Nikolay A. Shumovskyi[2], Betül Uralcan[3], Sergey V. Buldyrev[4], Mikhail A. Anisimov[1], Pablo G. Debenedetti[5],

[1]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD 20742
[2]Department of Physics, Boston University, Boston, MA 02215
[3]Department of Chemical Engineering and Polymer Research Center
[4]Department of Physics, Yeshiva University, New York, NY 10033
[5]Department of Chemical and Biological Engineering, Princeton University, NJ 08544

The separation of substances into different phases is ubiquitous in nature and important scientifically and technologically. This phenomenon may become drastically different if the species involved, whether molecules or supramolecular assemblies, interconvert. In the presence of an external force large enough to overcome energetic differences between the interconvertible species (forced interconversion), the two alternative species will be present in equal amounts, and the striking phenomenon of steady-state, restricted phase separation into mesoscales is observed. Such microphase separation is one of the simplest examples of dissipative structures in condensed matter. In this work, we investigate the formation of such mesoscale steady-state structures through Monte Carlo and molecular dynamics simulations of three physically distinct microscopic models of binary mixtures that exhibit both equilibrium (natural) interconversion and a nonequilibrium source of forced interconversion. We show that this source can be introduced through an internal imbalance of intermolecular forces or an external flux of energy that promotes molecular interconversion, possible manifestations of which could include the internal nonequilibrium environment of living cells with a flux of ATP molecules or photons. The main trends and observations from the simulations are well captured by a nonequilibrium thermodynamic theory of phase transitions affected by interconversion. We show how a nonequilibrium bicontinuous microemulsion or a spatially modulated state in biomolecular condensates may be generated depending on the interplay between diffusion, natural interconversion, and forced interconversion [1].

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STACKING INTERACTIONS AND FLEXIBILITY OF HUMAN TELOMERIC MULTIMERS

Cristiano De Michele

Dipartimento di Fisica, Sapienza Università di Roma, P.le A. Moro 5, 00185 Roma, Italy

G-quadruplexes (G4s) are helical four-stranded structures forming from guanine-rich nucleic acid sequences, which are thought to play a role in cancer development and its malignant transformation. Most current studies focus on G4 monomers, yet under suitable and biologically relevant conditions G4s undergo multimerization. Here, we investigate the stacking interactions and structural features of telomeric G4 multimers by means of a novel low-resolution structural approach that combine small angle X-ray scattering (SAXS) with extremely coarse-grained (ECG) simulations. The degree of multimerization and the strength of the stacking interaction is quantitatively determined in G4 self-assembled multimers. We show that self-assembly induces a significant polydispersity of the G4 multimers, with an exponential distribution of contour lengths, consistent with a step-growth polymerization. On increasing the DNA concentration, the strength of the stacking interaction between G4 monomers increases, as well as the average number of the units in the aggregates. We utilize the same approach to explore the conformational flexibility of a model single-stranded long telomeric sequence. Our findings indicate that its G4 units frequently adopt a beads-on-a-string configuration. We also observe that the interaction between G4 units can be significantly affected by complexation with benchmark ligands. The proposed methodology, which identifies the determinants that govern the formation and structural flexibility of G4 multimers, may be an affordable tool aiding in the selection and design of drugs that target G4s under physiological conditions.

REFINING THE DESCRIPTION OF THE COLLECTIVE DYNAMICS OF PROTEINS IN THE TERAHERTZ FREQUENCY WINDOW

Valeria Conti Nibali[1], Francesco Sacchetti[2], Alessandro Paciaroni[2], Caterina Petrillo[2], Mounir Tarek[3], Giovanna D'Angelo[1,4]

Department of Mathematical and Computational Sciences, Physical Science and Earth Science, University of Messina, Messina, Italy
Department of Physics and Geology, University of Perugia, Perugia, Italy
Université de Lorraine, CNRS, LPCT, F-54000 Nancy, France
Institute for Chemical-Physical Processes, National Research Council of Italy (IPCF-CNR), Messina, Italy

An in-depth knowledge of protein dynamics is essential for a thorough understanding of their functionality. Among the wide range of motions -from the femtoseconds to seconds time scale-that proteins sustain, the term “protein collective dynamics” refers to the intricate patterns of coordinated motions of a large fraction of protein atoms in the sub-picosecond time scale, which are hypothesized to be involved in functional dynamical mechanisms. Using the theoretical framework of hydrodynamics, the collective dynamics of proteins had previously been described in a manner akin to that of simple liquids, i.e. in terms of a single acoustic-like excitation, related to intra-protein (secondary structure) vibrational motions. We investigate herein the intra-protein collective dynamics of a model globular protein by analysing -in terms of an interacting phonon model-its longitudinal and transverse modes, calculated from molecular dynamics simulations. This approach allows us to reveal a complex low-frequency vibrational landscape, populated by multiple acoustic-like and low-frequency optic-like modes, with mixed symmetry and interfering with each other. We propose an interpretation of the observed collective dynamical behavior, by means of a correlation to the structure of the investigated protein and an evaluation of the origin of the excitations. Finally, we discuss the coupling between the collective dynamics of proteins and their hydration water, which is particularly effective in the terahertz frequency range. The present findings, likely to be encountered for all globular proteins, provide a molecular-level perspective for describing energy-transfer mechanisms in proteins and their hydration environment.

IMAGING PROTEIN SELF-ASSEMBLY IN WATER USING LIQUID-PHASE TRANSMISSION ELECTRON MICROSCOPY

Lorena Ruiz-Perez

Institute for Bioengineering of Catalonia, IBEC, C. Baldiri Reixac, 10-12, 08028, Barcelona, Spain
Centre for Liquid Phase TEM Imaging at UCL, Department of Chemistry, University College London, United Kingdom

Liquid Phase Transmission Electron Microscopy (LPTEM) is a groundbreaking technique that allows for the imaging of soft structures in real time within their native liquid media. This method prevents the artefacts that can be caused by traditional drying or cryogenic treatments. LPTEM presents significant potential for the study of the molecular machinery structures of cells, especially proteins, as the liquid nature of samples permits access to previously unattainable protein states. This feature is a unique advantage for investigations in structural biology.

LPTEM has enabled us to observe how intrinsically disordered proteins form liquid-like condensates through liquid-liquid phase separation. We used CPEB4, a neuronal protein that is a member of the cytoplasmic polyadenylation element binding (CPEB) family of proteins, to investigate this process. We were able to image the dynamic process of CPEB4 protein self-assembly, from monomers to dimers, multimers, and eventually into condensed nanodroplets. LPTEM provided us with insight into the nanodroplets' morphology and liquid nature, and we compared these results with liquid-like structures formed by the misfolded protein Amyloid-β in solution.

In the case of Amyloid-β, we propose combining all-atom simulations with imaging to complement protein structural studies with dynamic investigations. Aβ is a short peptide that aggregates into larger assemblies, including neurotoxic oligomers, fibrils, and plaques and is highly associated with Alzheimer's disease. The details of the aggregation pathway remain elusive, with much of the current knowledge coming from computational simulations and chemical kinetics investigations. Using LPTEM, we were able to visualize processes such as oligomers attaching to the surface of fibres, a vital step in the secondary nucleation of Aβ and one of the most critical aggregation steps to consider for developing oligomer-targeting drugs. We were also able to visualize the growth of a short fibril over a video, demonstrating the capabilities of LPTEM for imaging molecular aggregating systems over time in solution and in situ. While still in the early stages, our findings promise to provide relevant and novel biological information on Aβ aggregation pathways.

SLOW DYNAMICS AND LOCAL STRUCTURE OF WATER AT BIO-INTERFACES

Paola Gallo

Dipartimento di Matematica e Fisica, Università Roma Tre, Via della Vasca Navale 84, 00146 Roma, Italy

Molecular dynamics simulation results of the behavior of water at bio-interfaces are discussed for two systems: water in the proximity of a protein, water and trehalose in proximity of a protein, the Lysozyme. Translational dynamics of the hydration water of a Lysozyme protein upon cooling is studied through the self van Hove function and the mean square displacement. In the deep supercooled region, it shows two different temperature activated relaxation mechanisms. The low-temperature hopping regime has a time scale of tenths of nanoseconds and a length scale on the order of 2–3 water shells, it represents the glassy dynamics, and it is also present in bulk water. The second hopping regime is active already at high temperatures, on the nanoseconds time scale and over distances of nanometers. This regime is connected to water displacements driven by the protein motion, and it is observed very clearly at high temperatures and for temperatures higher than the protein dynamical transition. Modification of dynamics in presence of trehalose is discussed. A detailed local structural analysis assessing the difference between the hydration water influenced by the protein and bulk water in terms of high density and low density distribution in local structures is also shown and discussed.

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COMBINED ATOMISTIC AND COARSE-GRAINED APPROACH FOR THE STUDY OF THERMORESPONSIVE ADSORBENT SPECIES AT THE NANOSCALE: THE CASE OF POLYOXAZOLINES

Sara Del Galdo, Carlo Andrea De Filippo, Barbara Capone

Science Department, Roma Tre University, Via della Vasca Navale 84, 00146, Roma, Italy

Polyoxazolines (POAs) are a class of thermoresponsive polymers (having a phase transition in response to thermal stimuli) characterised by a lower critical solution temperature (LCST) in water. The thermoresponsiveness makes POAs smart materials that can be exploited in the design and development of thermosensitive nanoscale devices. Recently, POAs-based nanodevices have been used in environmental remediation to remove heavy metal ions from water [1]. Colloidally stable superparamagnetic nanoparticles (SPMNPs) coated with poly(2-alkyl/aryloxazoline)s introduced into a water solution contaminated with heavy metal ions, have been used to adsorb and remove the contaminants [1]. By changing the chemical nature of the groups attached to the POA backbone, different polymeric systems can be designed to both adsorb diverse cargos and undergo controlled solubility transitions in response to thermal triggers. The ability to control and tune both polymer-cargo and polymer-solvent interactions offers the potential to develop a wide range of effective - and potentially selective - smart nanoadsorbers.

Using a combined atomistic and coarse-grained approach, we are able to investigate the undermining features that regulate adsorption and thermoresponsiveness in polyoxazolines at different scales. From one side, the simplest case of thermoresponsive POA (namely polyisopropyloxazoline or PiPOx) in water has recently been studied using atomistic simulations [2]. Our data show that the thermoresponsive behaviour of PiPOx is dominated by intermolecular interactions leading to the experimentally observed liquid-liquid phase separation (LLPS). We also found that the system behaviour can be well modelled by remapping the polymer chains into effective ellipsoids [2]. Such a simplified description allowed us not only to observe that in the LLPS phase the average distance between polymer chains mirrors the experimental lattice spacing measured on crystalline phases, but also to suggest the presence of a liquid crystalline phase in the polymer rich region, at high temperatures [3]. From the other side, scaling theories and coarse-grained simulations are employed to study the physics of adsorption when the atomistic details are embedded into effective potentials. Such a route allowed us to derive simple predictions linking the adsorption potential to general properties of classes of macromolecules and introduce a set of measurable quantities that can be exploited as an indirect measurement for loading [4].

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REMARKS ON THE HYDRATION ENTROPY OF POLAR, NONPOLAR AND CHARGED SPECIES

Giuseppe Graziano

Dipartimento di Scienze e Tecnologie, Università del Sannio, Via Francesco de Sanctis snc, 82100 Benevento, Italy

It is often not well known that, at room temperature and atmospheric pressure, the hydration process leads to an entropy decrease for all types of solutes: nonpolar molecules, polar non-charged molecules and ions [1] (note that it is necessary to use the so-called Ben-Naim standard that refers to the transfer from a fixed position in the ideal gas phase to a fixed position in water, at constant temperature and pressure).

Experimental data indicate that the magnitude of the hydration entropy of neutral solutes (i.e., both polar and nonpolar) increases with the molecular volume according to a trend that proves to be largely insensitive to the polar nature of the solute molecule [2]. Surprisingly, the general trend is well reproduced by the values of the entropy change upon cavity creation in water, as calculated by means of classic scaled particle theory [2,3]. This means that the solvent-excluded volume effect plays the major role in determining the magnitude of the hydration entropy change [2,4,5].

In contrast, for the 20 alkali halides analysed, the magnitude of the hydration entropy change decreases on increasing the size of the constituent ions [2], demonstrating that the strength of charge-water dipole interactions plays the dominant role [6], overwhelming the contribution due to the solvent-excluded volume effect.

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HYDRATION AND CROWDING EFFECT IN BIOMOLECULAR CONDENSATES: THE CASE STUDY OF SOD1 INTO STRESS GRANULES

Luis Enrique Coronas[1], *Emeline Laborie*[2], *Stepan Timr*[2], *Fabio Sterpone*[2], *Giancarlo Franzese*[1]

[1]Departament de Física de la Matèria Condensada & Institute of Nanoscience and Nanotechnology (IN2UB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain
[2]CNRS Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, Université Paris Cité, PSL Research University, 75005 Paris, France

Superoxide Dismutase 1 (SOD1) proteins are involved in the disease progression of amyotrophic lateral sclerosis (ALS) and, under heat stress, are sequestered into Stress Granules (SGs) and Fused in Sarcoma (FUS) biomolecular condensates in vivo and in vitro, respectively. In vivo, the cytoplasm controls the SOD1 partition coefficient (PC) into SGs. In vitro experiments with the main cytoplasm component—albumin—reproduce this effect with FUS condensates even after 60 min of heat stress [1]. Implicit solvent (IS) simulations show no preferential interactions of SOD1 with (globular) BSA or (intrinsically disordered) FUS, despite their conformational difference, concluding that the decrease in PC with BSA compared to FUS is due to the lack of SOD1 preference between the two [1]. Here we show that the free-energy landscape of SOD1 in solution with BSA is entirely different from that of SOD1 in a FUS condensate once the solvent is considered [2]. We conclude that SOD1 PC decreases in FUS condensate in the presence of BSA crowders because of the hydration entropy. Our results show how relevant could be the role of water's entropy in biomolecular condensates.

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ORAL PRESENTATIONS

OP1

OXIDIZING EFFECTS ON GUANINE: FROM NUCLEOTIDES TO G-QUADRUPLEX STRUCTURES

Francesca Ripanti[1], Valeria Libera[1,2], Claudia Fasolato[3], Paolo Postorino[4], Caterina Petrillo[1], Alessandro Paciaroni[1], Lucia Comez[2]

[1]Physics and Geology Department, University of Perugia, Via A. Pascoli, 06123 Perugia, Italy
[2]CNR-IOM c/o University of Perugia, Via A. Pascoli, 06123 Perugia, Italy
[3]CNR-ISC c/o Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[4]Physics Department, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Roma, Italy

Guanine-rich nucleic acid sequences are able to form non canonical DNA and RNA structures called G-quadruplexes (G4s), which are promising programmable metal-mediated assemblies with a wide range of applications, including molecular computing and biosensing. G4 structures are characterized by the stacking of planar arrangements of four guanines linked via Hoogsteen hydrogen bonds, called G-tetrads [1]. Due to their polymorphic nature and role in many life processes, it has been long suggested that targeting G4s with small molecules regulates their biological functions. In this context, a plethora of small molecules has been shown to interact with G4s, leading to conformational changes of the native structure or even its unfolding. Among them, photosensitive ligands are a class of versatile systems able to trigger topological variations using different wavelengths and/or exposure times of the light source. In particular, TMPyP4 porphyrin downregulates gene expression through quadruplex formation or induction in the promoter region [2]. Porphyrins can generate singlet oxygens, thus oxidizing guanines at the exterior faces of the quadruplex scaffold. By considering the human telomeric G4-forming sequence AG3(TTAG3)3 (Tel22), we observed that, if complexed with TMPyP4 and gradually illuminated with blue light, it is subjected to, at least partial, destruction of the tetrads, result that can be useful for photodynamic applications. These structural changes are close to those obtained by introducing oxidized guanines in the G4 sequence [3].
The oxidized state of G4 structures was also deeply investigated in their synthesis precursors, i.e. guanosine triphosphates [4]. The identification, detection, and quantification of oxidized nucleotides at low concentration was carried out by exploiting a novel method based on micro-Raman spectroscopy combined with ab initio calculations. We showed that the Raman signature in the terahertz spectral range contains information on the intermolecular assembly of guanine in tetrads, which allowed to further boost the oxidative damage detection limit. We also provided evidence that similar analyses can be carried out on samples in very small volumes at very low concentrations by exploiting the high sensitivity of surface-enhanced Raman scattering combined with properly designed superhydrophobic substrates [5]. These results pave the way for employing such advanced spectroscopic methods for quantitatively sensing the oxidative damage of nucleotides in the G4 synthesis precursors, which is a crucial step for a comprehensive study of oxidizing effects on G4s.

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OP2

SELF-ASSEMBLED PEPTIDES ARCHITECTURES TO DEVELOP NEW SMART BIOMATERIALS

Sara Catalini[1,2,3], Francesco Bagni[3,4], Stefano Cicchi[4], Mariangela Di Donato[3,5], Alessandro Iagatti[2,3], Andrea Lapini[3,6], Paolo Foggi[2,3,7], Caterina Petrillo[1], Alessandro Di Michele[1], Marco Paolantoni[7], Lucia Comez[8] and Alessandro Paciaroni[1]

[1]Dip.Fisica e Geologia, Università di Perugia, 06123 Via Pascoli, PG, Italy
[2]CNR-INO, Largo Fermi 6, 50125, FI, Italy
[3]European Laboratory for Non-Linear Spectroscopy, Via Nello Carrara 1, 50019, Sesto Fiorentino, FI, Italy
[4]Dip. Chimica, Università di Firenze, via della Lastruccia, 3-13, 50019, Sesto Fiorentino, FI, Italy
[5]CNR-ICCOM, via Madonna del Piano 10, I-50019, Sesto Fiorentino, FI, Italy
[6]Dip. Scienze Chimiche, Vita e Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze, 17/A, 43124 Parma, PR, Italy
[7]Dip. Chimica, Biologia e Biotecnologie, Università di Perugia, Via Elce di sotto 8, 06123, PG, Italy
[8]CNR-IOM, c/o Dip. Fisica e Geologia, Università di Perugia, 06123 Via Pascoli, PG, Italy

Nano- and micro-structures based on the self-assembly of biological molecules have interesting physicochemical properties, which can be exploited to develop new promising smart materials. Different strategies are developed to induce the formation of multi-length scale ordered architectures and control their structure and morphology [1]. In this context, short peptides raise much interest due to their easy availability and capability to form versatile supramolecular architectures [2] that display unique physical and chemical properties [3]. The short peptide diphenylalanine (FF) is one of the most suitable building blocks, due to its ability to form extended ordered architectures with a wide morphological differentiation, conferring it unique functional properties. Indeed, FF is the key structural motif of the Aβ amyloid polypeptide with a strong propensity to self-assemble in aqueous environment [4] [5] [6]. Here we investigate how FF self-assembly properties can be modulated acting on the interaction between the aromatic sides and their microenvironment through the acetonitrile/water (AcN/H_2O) good/bad solvent ratio. Our measurements revealed a hierarchical aggregation where morphologies can be tuned by changing the AcN content. Interestingly, even subtle changes in AcN content (2-10 %) induce a large morphological investigated by scanning electron microscopy (SEM). FTIR spectra (mostly sensitive to peptide conformation) show the same spectral features regardless of the aggregate morphologies, while CD spectra (accounting for both peptide conformation and stacking interaction) reveal differences among the different samples, suggesting that the most relevant interaction determining either isotropic or preferential growth of the aggregates is the stacking of aromatic rings.

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OP3

USE OF CHEMOMETRICS FOR EXTRACTING HIDDEN INFORMATION FROM SPECTROSCOPIC DATA

Nunzia Iaccarino

Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy

Chemometrics is a multidisciplinary field that uses mathematical and statistical methods to extract hidden information from any kind of data. The aim of chemometrics is to develop models that can accurately predict or classify samples based on their chemical or physical properties. This approach has been widely applied in various fields of chemistry, such as environmental monitoring, drug discovery, and food analysis.

The use of chemometrics allows for the identification of patterns and correlations that may not be easily observable by traditional methods. Indeed, chemometric models are capable of handling large and complex datasets, making it possible to analyze multiple variables simultaneously by reducing the dimensionality of the data and simplifying the interpretation of the results.

One of the most common applications of chemometrics is in spectroscopic analysis. In particular, spectroscopic data can be analyzed using chemometric techniques such as principal component analysis (PCA) and multivariate curve resolution (MCR) to extract information about peculiar features of the samples being analyzed. During this communication, examples of applications of such techniques to various kind of spectral data originating from non-canonical DNA samples will be provided.

ISOTOPE EFFECT ON THE DYNAMICS OF WATER IN AQUEOUS SOLUTIONS AT SUPERCOOLED TEMPERATURES

Jorge H. Melillo[1], Silvina Cerveny[1,2]

[1]Donostia International Physics Center (DIPC), Paseo Manuel de Lardizabal 4, 20018 San Sebastián, Spain
[2]Centro de Física de Materiales (CSIC-UPV/EHU)-Material Physics Centre (MPC), Paseo Manuel de Lardizábal 5, 20018 San Sebastián, Spain

The interaction of water with biological materials is one of the most exciting topics in science: life is impossible without water. Fundamental biological processes occur in aqueous environments; water characteristics, such as its hydrogen bond network, are responsible for biomolecules' structure, stability, dynamics, and function [1]. On the other hand, water isotopes have different characteristics, and consequently, aqueous solution dynamics also differ when different water isotopes are considered [2]. The differences observed in the dynamical properties can be explained by classical theory, such as mass or inertia moments of the water molecules, and by nuclear quantum effects (NQEs) [3], which affect the nature of water hydrogen bonds. In particular, and due to the small mass of the molecules, water is significantly affected by NQEs, affecting the dynamics of biomolecules.

In this work, we employed aqueous solutions with a water content of c_w = 35 wt% of tri-propylene glycol (3PG), poly (vinyl methyl ether) (PVME), and 1-lysine (1-Lys) using three water isotopes as a solvent: $H_2{}^{16}O$, $D_2{}^{16}O$, and $H_2{}^{18}O$. We applied broadband dielectric spectroscopy (BDS) combined with calorimetric measurements to analyze the effects of the mass, the moment of inertia, and the impact of NQEs in the dynamics of the aqueous solutions at supercooled temperatures. When analyzing the dynamics of an aqueous solution, two scenarios can be distinguished. For some solutes, such as 3PG and PVME, two significant relaxations are detected: the fast-water relaxation and the relaxation of the solute affected by water (α-relaxation); For other types of solutes, such as 1-Lys, an additional process is found: a water relaxation affected by the solute (slow-water relaxation) [4].

We found no difference in the dynamics using $H_2{}^{16}O$ and $H_2{}^{18}O$ as a solvent; however, when the solvent is $D_2{}^{16}O$, the α-relaxation shifts to higher temperatures, in good agreement with the calorimetric glass transition ($T_{g,DSC}$). On the other hand, water-relaxation processes are more affected under H→D substitution. In particular, below $T_{g,DSC}$, the effects are more substantial. These results indicate that NQEs play a fundamental role in the water dynamics in aqueous solutions and are more prominent at low temperatures. However, we will also show that NQEs are detected in the water even at room temperature.

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PhD1

MUTUAL INFLUENCE OF SOLVENT VIBRATIONS AND TELOMERE G-QUADRUPLEX REARRANGEMENTS UPON THERMAL UNFOLDING

Valeria Libera[1,2], Federico Bianchi[1], Barbara Rossi[3], Francesco D'Amico[3], Claudio Masciovecchio[3], Caterina Petrillo[1], Francesco Sacchetti[1], Alessandro Paciaroni[1], Lucia Comez[2]

[1]Dipartimento di Fisica e Geologia, Università degli Studi di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy
[2]CNR-IOM, c/o Perugia, Università degli Studi di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy
[3]Elettra Sincrotrone Trieste, S.S. 14 Km 163.5, 34012 Trieste, Italy

G-quadruplexes (G4s) are non-canonical four-stranded DNA helical structures formed through the stacking of four in-plane Hoogsteen-paired guanine bases [1,2]. Their location in the human genome is often related to cancer biology and genome activities like transcription, replication, genome stability, and epigenetic regulation [3, 4].

In general, not much is known about the hydration layer around G4. Most of the work has been performed in crowded conditions, where it seems that their pronounced polymorphism is strictly regulated by their hydration [5–7]. Water molecules can go in both the medium and the narrow grooves; however, recent research suggested that the latter can accommodate extended filiform networks of water molecules, called spines [8]. Antiparallel and hybrid topologies are indeed more prone to host stable water spine structures. In diluted conditions, many factors affect the G4 structure because each state is separated by small energy barriers, therefore it is intriguing to investigate the relation between solvation and G4 conformations. We exploited UV Resonance Raman scattering to simultaneously explore the vibrational behaviour of a human telomeric G4 (Tel22) and its aqueous solvent as the biomolecule undergoes thermal unfolding [9]. We found that the OH stretching band, related to the local hydrogen-bonded network of a water molecule, was in strict relation with the vibrational features of the G4 structure as a function of temperature. In particular, the modifications to the tetrahedral ordering of the water network were strongly coupled to the Tel22 rearrangements, showing changes in temperature that mirrored the DNA multi-step melting process. The comparison between circular dichroism and Raman results supported this view.

The present findings provide novel insights into the impact of the molecular environment on G4 conformation. Improving current knowledge on the solvent structural properties will also contribute to a better understanding of the role played by water arrangement in the complexation of G4s with ligands.

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PhD2

DISCOVERY OF HMGB1 INHIBITORS BASED ON G-QUADRUPLEX-FORMING APTAMERS

Ettore Napolitano[1], Andrea Criscuolo[1], Claudia Riccardi[1], Carla L. Esposito[2], Domenica Musumeci[1], Daniela Montesarchio[1]

[1]Department of Chemical Sciences, University of Naples Federico II, via Cintia 21, 80126 Napoli (Italy)
[2]Institute of Experimental Endocrinology and Oncology "Gaetano Salvatore" (IEOS), National Research Council (CNR), via Tommaso De Amicis 95, 80131 Napoli (Italy)

High-Mobility Group Box 1 (HMGB1) is an abundant, highly conserved, non-histone nuclear protein present in all eukaryotic cells [1,2]. In inflammatory conditions, HMGB1 is actively secreted from immune cells in the extracellular matrix, where it behaves as a proinflammatory cytokine [3]. Once released, it can mediate various cellular responses, including cell migration/proliferation and release of other proinflammatory cytokines [4]. Moreover, HMGB1 contributes to various chronic inflammatory and autoimmune diseases as well as of cancer [5]. Given the crucial roles of HMGB1 in these pathologies, identification of inhibitors of this protein is of considerable interest [6]. We here identified 14 G-quadruplex (G4) forming aptamers as potential HMGB1 inhibitors, using a SELEX-based procedure [7] from a properly designed G-rich oligonucleotide library. These aptamers were fully characterized using several biophysical techniques to determine their preferred conformation as well as their thermal and enzymatic stability. We also evaluated the interaction between the aptamers and HMGB1, as well as their ability to inhibit HMGB1-induced migration in cancer cells [8] so to identify the best candidate for more advanced biological assays aimed at repressing the protein pathological functions.

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PhD3

SELF AND COLLECTIVE DYNAMICS IN WATER-ETHANOL MIXTURE: SPECTROSCOPY WITH POLARIZED NEUTRONS

Riccardo Morbidini[1,2], Tilo Seydel[1], Robert M. Edkins[3], Gøran Nielsen[4], Katharina Edkins[2]

[1]Institut Laue-Langevin, 71 Avenue des Martyrs, F-38042 Grenoble, France
[2]School of Pharmacy and Optometry, University of Manchester, Oxford Road, Manchester M13 9PT, UK
[3]WestCHEM Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, UK
[4]ISIS Neutron and Muon Source, Rutherford Appleton Laboratory, Didcot, OX11 0QX, UK

Globular proteins in aqueous environment fold into their native and biologically active conformation with a compact shape to minimize the interactions among water molecules and protein hydrophobic chains [1].
Due to their outstanding role in food and pharmaceutical industry, as well as for biological and chemical research, water and its binary mixtures with monohydroxy alcohols have been the subject of sustained focus [1]. Questions regarding the intriguing macroscopic properties [2], such as negative excess entropy, non-monotonous dependencies of volume, refractive index and viscosity on the mixing ratio have been interpreted in terms of cluster formation at molecular level [3]. To investigate the dynamic of such elusive meso-structures, whose lifetime is in the ps range, different experimental techniques (NMR and dielectric spectroscopy) have been employed but only with neutron spectroscopy is possible to simultaneously access spatial and time correlations in the Å-ps domain. So far, with quasi-elastic neutron scattering (QENS) we probed self-diffusion of hydrogen rich water-ethanol [4] without gauging any information on the collective dynamics of the hydrogen bond network. We performed sub-meV resolution QENS with polarization analysis on LET (ISIS, UK) in combination with selective deuteration to study the structural dynamics in D_2O/C_2H_5OD mixtures at different concentrations, around the maximum of the macroscopic viscosity (0.2 ethanol mole fraction). The self-diffusion from the purely incoherent signal of the ethanol non-exchangeable hydrogens in the water network follows the same trend of the previously probed ensemble average self-diffusion of H_2O/C_2H_5OH but with lower values. From the analysis of the purely coherent signal we observe the collective density fluctuations of D_2O perturbed by ethanol, building on what has been recently measured on pure water [5]. Our findings indicate that relaxation times of the hydrogen bond network exhibit a slowing down as the ethanol fraction increases reflecting a so-called “wait and switch” process for the breaking and formation of HB bonds [6]. Thanks to polarised neutrons we are able to observe for the first time this effect as a function of momentum transfer q, unraveling a more significant slowing down in the mesoscale, where the presence of clusters has been predicted for lower alcohols [3].
This work, which will be soon submitted, is part of the InnovaXN PhD thesis project about “Diffusion in supramolecular gels for drug delivery” in collaboration with the industry partner AstraZeneca.

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DYNAMICS OF LYSOZYME HYDRATION WATER INVESTIGATED BY ULTRAFAST TIME-RESOLVED SPECTROSCOPY

Luigi Caminiti[1,2], Maria Taddei[1,2], Sara Catalini[1,2,3], Paolo Bartolini[2], Andrea Taschin[4], Renato Torre[1,2]

[1]Dipartimento di Fisica e Astronomia, Università di Firenze, Via Giovanni Sansone 1, 50019 Sesto Fiorentino, Italy
[2]European Laboratory for Non-Linear Spectroscopy, Università di Firenze, Via Nello Carrara 1, 50019 Sesto Fiorentino, Italy
[3]Dipartimento di Fisica e Geologia, Università di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy
[4]ENEA Centro di Ricerche Frascati, Via Enrico Fermi 45, 00044 Frascati, Italy

Water is the fundamental element for the development of life as the main biological processes take place in aqueous environments. In fact, water molecules can strongly influence the structure and functions of biological molecules, such as proteins and nucleic acids [1]. Numerous physical techniques have been utilized to investigate the dynamics of water molecules at the protein-water interface across different time scales. Despite some consensus on the mechanisms and dynamics of water-protein interactions, there remain several critical points to consider [1-6]. In this study, we present the results of a time-resolved optical Kerr effect spectroscopic analysis on the structural and vibrational dynamics of hydration water in lysozyme-water samples on a very fast time scale. By varying the protein concentration, we observe the effects of concentration increase on the intermolecular dynamics of hydration water. Our experiments validate the existence of two structural dynamics of hydration water, which have previously been linked to hydrogen bond exchange relaxation and water molecule reorganization by structural protein fluctuations, respectively [7]. Additionally, we measured the vibrational dynamics of the water hydration layer up to the sub-picosecond time range. The concentration-dependent dynamics of the hydration water indicate a probable clustering phenomenon taking place in high concentration solutions. Overall, our findings provide further insights into the complex dynamics of hydration water and the impact of protein concentration on these dynamics.

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PhD5

AMYLOID AND NON-AMYLOID AGGREGATION OF β-LACTOGLOBULIN IN SELF-CROWDED REGIME

Sara Venturi[1], Barbara Rossi[2], Mariagrazia Tortora[3], Renato Torre[1,4], Andrea Lapini[1,5], Paolo Foggi[1], Marco Paolantoni[5], Sara Catalini[6]

[1]European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy
[2]Elettra-Sincrotrone Trieste, Italy
[3]AREA SCIENCE PARK, Trieste, Italy
[4]Dipartimento di Fisica ed Astronomia, Sesto Fiorentino, Italy
[5]Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Parma, Italy
[6]Dipartimento di Fisica e Geologia, Perugia, Italy

Globular proteins in aqueous environment fold into their native and biologically active conformation with a compact shape to minimize the interactions among water molecules and protein hydrophobic chains [1]. Varying the system properties such as solvent composition, solution pH, ionic strength and temperature, globular proteins can unfold losing their native structure. The unfolding process promotes the exposition to the solvent of the hydrophobic chains and the possibility to activate the aggregation among monomers [2]. Proteins aggregates can be of different complexity, going from small transient oligomers up to mature amyloid fibrils [3]. Proteins aggregates can further interact forming extended polymeric network leading to the formation of hydrogels, soft materials capable to self-sustaining and retain into their meshes a great amount of water. Protein aggregates and hydrogels are of increasing interest in biomedical field due to their involvement in amyloid pathologies [4] and in material science thanks to their emergent properties [5] [6] and biocompatibility. Exploiting the natural propensity of proteins to self-assemble is a smart strategy to realize biomaterials using a bottom-up approach, baying the synthetic effort. Simply acting on the aggregation conditions, the chemical-physical properties of the material can be tuned. In this work, molecular insights on the β-Lactoglobulin (β-Lg) thermal unfolding and aggregation are obtained by means of structural-sensitive techniques. The commonly used Fourier Transform Infrared (FTIR) spectroscopy gives information on the secondary structure variation of β-Lg during thermal unfolding and on the formation of amyloid aggregates thanks to the marker amyloid signal at 1620 cm^{-1}. The UV Resonance Raman (UVRR) spectroscopy with an excitation wavelength at 226 nm is a tryptophan-sensitive probe and gives information on the environmental changing around this amino acid residue. Depending on the solution pH, two different aggregation pathways have been identified; one is amyloidogenic while the other is of different nature.

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PhD6

WATER DIFFUSION IN NANOCLAYS: AN NMR STUDY

Martina Maria Calvino[1], Daniel Topgaard[2], Giuseppe Cavallaro[1], Giuseppe Lazzara[1]

[1]Dipartimento di Fisica e Chimica "Emilio Segrè", Università degli Studi di Palermo, Viale delle Scienze, 90128, Palermo, Italy
[2]Division of Physical Chemistry, Chemistry Department, Lund University, Lund, 22100, Sweden

Nowadays, clay nanoparticles have attracted significant attention due to their peculiar features and widespread applications as natural materials in different fields. Among them, halloysite nanotubes possess a peculiar tubular morphology, biocompatibility, unique surface properties, and distinctive features.
Water confinement within the cavity of halloysite was examined by Knudsen thermogravimetric technique in a previous work [1] aimed to describe the loading mechanism of different compounds inside the lumen of the tubular clay. As a matter of fact, the confinement of active molecules inside tubular nanoparticles has a potential impact on both fundamental sciences and nanotechnologies. According to existing literature, the confinement of water was investigated in numerous systems [2], such as cellulose or carbon nanotubes, but insights on clay nanotubes are needed.
With this in mind, here we propose a detailed study of self-diffusion and transverse relaxation time T2 of water in clays by means of NMR techniques. Both tubular and platy clays were investigated, in particular halloysite and kaolinite, pointing out the differences between them.
Experiments were performed in a temperature range between 263 and 330 K and by changing the relative humidity conditions, highlighting the presence of different kind of water populations in the investigated systems.

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DYNAMIC CLUSTER FORMATION, VISCOSITY AND DIFFUSION IN MONOCLONAL ANTIBODY SOLUTIONS

Ilaria Mosca[1], Christian Beck[1,2], Kevin Pounot[3], Olga Matsarskaia[2], Christoph Grapentin[4], Lauren Matthews[3], Tilo Seydel[2], Frank Schreiber[1]

[1]Institut für Angewandte Physik, Universität Tübingen, Germany
[2]Institut Max von Laue - Paul Langevin, Grenoble, France
[3]European Synchrotron Radiation Facility, Grenoble, France
[4]Lonza AG, Ltd., Basel, Switzerland

Antibodies, formally known as immunoglobulins, are proteins secreted by lymphocites B and therefore present in mammal blood sera, playing an essential role in the immune response of those organisms against intruding pathogens.
Monoclonal antibodies (mAbs) are particularly relevant for therapeutic applications due to their high specificity and versatility. One of the current pharmaceutical challenges related to these interesting molecules concerns their formulation for subcutaneous (SC) administration, which is currently becoming the preferred route of delivery due to its convenience. For SC administration, highly concentrated antibody formulations are needed to achieve a significant therapeutic effect, which may lead to high solution viscosities and altered injectability. The main interest is therefore keeping the viscosity of mAb formulations under 15-20 mPas [1, 2] without altering the solution stability, in order to make the drug administration less difficult for injection devices and less painful for patients.
Since the understanding of macroscopic viscosity requires an in-depth knowledge on protein diffusion, mutual interactions and potential cluster formation [3, 4], we study the self-diffusion of five mAbs of the IgG1 subtype (produced and characterized at Lonza AG) in aqueous solution as a function of antibody type, concentration and temperature, by quasi-elastic neutron scattering (QENS). QENS allows to determine the hydrodynamic cluster size of the solutions [5] and sheds light on the mAb internal dynamics. Data have been treated using analysis frameworks we developed [6] and interpreted using colloid physics theory, as established in previous works [7].
A subset of these mAbs has been also investigated using small angle neutron and X-ray scattering (SANS and SAXS) to probe mutual interactions between mAb molecules. Complementary information is provided by molecular dynamics (MD) simulations and rheology measurements. As a reference, we use polyclonal antibody (IgG from bovine serum) solutions [8], thus obtaining a comprehensive picture of mAb diffusion.

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PhD8

POLYMORPHISM AND LIGAND BINDING MODULATE FAST DYNAMICS OF HUMAN TELOMERIC G-QUADRUPLEXES

Luca Bertini[1], Valeria Libera[1,2], Francesca Ripanti[1], Francesca Natali[3], Andrea Orecchini[1], Alessandro Nucara[4], Caterina Petrillo[1], Marco Paolantoni[5], Lucia Comez[2], Alessandro Paciaroni[1]

[1]Dipartimento di Fisica e Geologia, Università degli Studi di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy
[2]CNR-IOM, c/o Perugia, Università degli Studi di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy
[3]CNR-IOM and INSIDE@ILL c/o OGG, 71 avenue des Martyrs, 38042 Grenoble Cedex 9, France
[4]Dipartimento di Fisica, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Roma, Italy
[5]Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia, Via Elce di Sotto 6, 06123 Perugia, Italy

G-quadruplexes (G4s) formed by the human telomeric sequence $AG_3(TTAG3)_3$ (Tel22) play a key role in cancer and ageing. G4 structures are known to display a variety of topologies, which are determined by several factors, resulting in structural polymorphism. Neutron Scattering techniques are a valuable tool to investigate how G4 structural polymorphism and ligand binding affect their sub-nanosecond dynamics. Within this context, we combined Fourier Transform Infrared Spectroscopy (FTIR) to monitor the Tel22 conformation and Elastic Incoherent Neutron Scattering (EINS) to assess the corresponding dynamical properties. K+ and Na+ stabilized G4s were found to be in the parallel and mixed parallel-antiparallel topologies, respectively, with the latter resulting to be dynamically more stable. This result is compatible with the presence of ordered hydration-water structures in the antiparallel conformation. Complexation with the model ligand BRACO19 (BR19) resulted in an overall increase of Tel22 mobility. Such a dynamical enhancement, which is uncorrelated to the G4 topology, can be ascribed to a preferential binding of water molecules to Tel22 rather than to BR19.

INFLUENCE OF PHOTOACTIVATED WATER ON THE PHYSICO-CHEMICAL PROPERTIES OF COSMETIC INGREDIENTS

Ariane Fournier[1], Sandrine Delpeux[1], Rémi Guyomarc[2], Louis Hennet[1]

[1]ICMN (Interface, Confinement, Materiaux et Nanostructures), 1 Rue de la Férollerie,45071 Orléans, France
[2]Terre de Couleur, 6 Rue de Châtenay, 37210 Rochecorbon, France

Water has always been used as a solvent in cosmetics as it is effective and non-harmful. By applying an external excitation such as UV lights [1] or cold plasma [2], it is possible to modify the physicochemical properties of water. Some studies have also shown macroscopic modifications such as a change in the surface tension on nano-droplets of water irradiated by electrons [3].
The French company *Terre de Couleur* has developed a device using a particular external excitation, being innovative as different from what can be found in the literature, in order to obtain "photoactivated water". By using activated water in their cosmetic formulations, *Terre de Couleur* has also observed changes in the surface tension as well as an improvement in the efficiency of its formulations (tested by external laboratories) on skin properties. The effect of this "photoactivation" process has been observed on different cosmetic ingredients, such as dyes, essential oils, and surfactants.
In this framework, we focused on trying to understand the multiple physico-chemical mechanisms involved in the water activation process and the interactions between the different activated components of the cosmetic formulation. More precisely, various analysis techniques have been used to investigate the modifications induced in multiple ingredients (dyes, vegetal oil, ...) mixed with water activated under
different conditions (temperature, time of activation, nature of the container). We have also investigated the impact of the mineralisation of water on the photoactivation process.
The results of our work may provide more information about how the "photoactivation" process affects the water molecules dynamic. The observed structural modifications will help us understand the macroscopic observations such as the changes observed with the surface tension. Overall, this "photoactivated" water used within the cosmetic field could be beneficial in terms of decreasing the amount of each active ingredients while enhancing the effectiveness of formulations.

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PhD10

COMPUTATIONAL STUDY OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1 STRUCTURE AND DYNAMICS

Gian Marco Tuveri[1], Silvia Acosta-Gutierrez[1,3], Giancarlo Franzese[2], Lorena Ruiz-Perez[1,3], Giuseppe Battaglia[1,3,4]

[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology,
[2]Department of Condensed Matter Physics & Institute of Nanoscience and Nanotechnology (IN2UB), University of Barcelona
[3]Department of Chemistry, University College London
[4]Catalan Institution for Research and Advanced Studies -ICREA

The brain is the most energy-expensive organ in humans consuming around 20% of the body metabolic resting rate. At the same time, the molecular balance that leads to biochemical reactions in the brain makes it extremely delicate to alterations from the outside environment, that is, the blood circulation. Evolution led our bodies to develop a special wall between the neuronal environment and the blood flux, the so-called Blood-Brain Barrier BBB. This barrier comprises endothelial cells that tightly wrap the capillaries and applies strict control over the molecules that enter and exit the brain. In this control, the membrane proteins called receptors, located in the BBB, play a fundamental role by binding to the molecular agents and activating the inwards/outwards transport mechanism. The present research focuses on the structure and function of a specific receptor, the low-density lipoprotein receptor-related protein 1, LRP1. LRP1 comprises 4544 amino acids, around 1200 of which are involved in three long and flexible structures that contain coordinated calcium ions and are decorated with small sugar chains called glycans. These three structures are believed to have an active role in ligand binding activity [1][2] and to activate a peculiar transport mechanism [3]. No crystal structure of LRP1 is currently available. Hence the receptor structure has been predicted with the AlphaFold2 deep learning tool [4]. Moreover, a membrane protein closely related to it, LRP2, has recently been resolved [5]. The information from this crystal structure has been used as well since it provides a ground truth for our prediction. This investigation approaches the problem from a computational biophysical point of view, using the atomistic molecular dynamics MD implemented in Gromacs. The MD results, even if preliminary, allow us to speculate on the structural characterization of LRP1 and the role of the calcium ions and glycans in the evolution in solution of flexible domains.

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PhD11

UNSUPERVISED MACHINE-LEARNING OF WATER STRUCTURE: FROMHYDROPHOBIC INTERFACES TO CRITICAL BEHAVIOR

Edward Danquah Donkor[1,2], Adu Offei-Danso[1], Alessandro Laio[2], Alex Rodriguez[2], Ali Hassanali[1]

[1]Abdus Salam International Centre for Theoretical Physics, 34151, Trieste, Italy
[2]SISSA – via Bonomea 265, 34136 Trieste, Italy

Hydrophobic interfaces are ubiquitous in nature and play an integral part of chemical and biological systems. Despite its importance, understanding the structure of water in general and also near hydrophobic surfaces remains an open challenge. In this work, state-of-the-art statistical techniques and Unsupervised Clustering methods are used to characterize water structure at hydrophobic interfaces and in the supercooled regime. We illustrate that local atomic descriptors used in Machine Learning (ML) to describe local environments of water are not equivalent to the commonly used chemical-intuition based order parameters, for example, the tetrahedrality. We illustrate the implication of these findings on understanding the nature of local structural fluctuations close to the putative second liquid-liquid critical point under supercooled conditions, highlighting the importance of non-local structures. We also identify the structural fingerprints of water near the interface with air and oil, allowing for an agnostic characterization of water near hydrophobic surfaces. This approach provides a framework for re-visiting textbook notions of the physical chemistry of solvation.

POSTER PRESENTATIONS

STRUCTURAL RELAXATION AND THERMODYNAMICS OF VISCOUS AQUEOUS SYSTEMS: A SIMPLIFIED REAPPRAISAL

Alberto Schiraldi

Dept. of Food Environment and Nutrition Sciences (DeFENS), University of Milan, Via Celoria 2, 20133 Milano, Italy

The attainment of true equilibrium conditions is a dynamic process that encompasses a time span. For slow relaxing systems, non-equilibrium steady states can often look like equilibrium states.
The present paper deals with homogeneous and heterogeneous aqueous systems and suggests a modified expression for the chemical potential of water by addition of an extra term that accounts for the extra energy related to the residual strains within a given aqueous solution. This allows estimation of the effects of the viscosity on the freezing point of aqueous polymers and leads to expressions for the viscosity in line with Eyring's and Angell's equations for homogeneous liquids.
As for simple heterogeneous systems, like "reversible" hydrogels, the paper describes the gel/sol transition and related hysteresis as the result of the "dynamic" character of relevant phase diagram, and suggests an estimation of the excess configurational entropy of the components that form the gel framework.
Dispersed aqueous systems, where interphase physical hurdles can reduce the water exchange rate, could host different aqueous phases with different water activities. A further modification of the expression for the chemical potential of water can accounts for such situations.
The proposed approach overcomes sophisticated theoretical visions and mathematical tools reported in recent papers. The formal expressions used substantially coincide with the classical thermodynamic equations for stable systems, save for the extra terms that account for time dependent quantities that tend to vanish for $t \rightarrow \infty$.

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QUANTITATIVE PHASE MICROSCOPY AND DYNAMIC LIGHT SCATTERING ON THE STUDY OF POLYELECTROLYTE DYNAMICS

Ioannis Sampson[1,2], Antje Larsen[2], Anthony A. Hyman[3], Emmanouela Filippidi[1,2,3]

[1]Department of Meterials Science and Technology, University of Crete, Greece
[2]IESL, Foundation for Research and Technology-Hellas, Crete, Greece
[3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

The study of polyelectrolyte dynamics is a complex field of study in polymer physics. Polyelectrolytes exhibit a permanent or partial charge which alters their conformational behaviour in solution, which is relevant also for proteins. Both polyelectrolytes and proteins undergo phenomena such as liquid-liquid phase separation, coacervation and aging. Close resemblance of proteins and polyelectrolytes makes polyelectrolytes the perfect candidate for developing a simpler, replicable bulk system, to understand the driving forces behind biological processes and confirm techniques we want to use for the study of proteins. Therefore, we use a previously documented [1] complex coacervate polyelectrolyte system of carboxy methyl dextran (CM-Dextran) with poly-dialyldiamethyl ammonium chloride (PDDA) in order to characterise the dynamics by light scattering, and in the future by similar techniques. Characterization methods include quantitative phase microscopy (QPM) as a means to obtain concentration from the refractive index increment (dn/dc) [2] and dynamic light scattering (DLS) quantifying the diffusion dynamics in solution [3], [4].

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PP3

IMPACT OF G-QUADRUPLEX BINDERS ON THE G-QUADRUPLEX/HELICASE INTERACTION

Elisa De Piante[1], Federica D'Aria[2], Luisa MR Napolitano[1], Jussara Amato[2], Simone Pirrello[1], Silvia Onesti[1], Concetta Giancola[2]

[1]Structural Biology Laboratory, Elettra-Sincrotrone Trieste S.C.p.A, 34149 Trieste, Italy
[2]Department of Pharmacy, University of Naples Federico II, 80131 Napoli, Italy

DNA G-quadruplexes (G4s) are formed in relevant genomic regions and involved in key cellular processes, including telomere maintenance and modulation of oncogene transcription. G4 unwinding helicases play strategic roles in G4 metabolism and function and are sensibly overexpressed in several cancers. Due to the complex role played by helicases in the onset of cancer, their mere inhibition is not a viable option. Several small organic molecules able to bind and stabilize G4s have shown the ability to reduce tumour cell growth also by affecting G4 recognition by helicases.

Despite numerous reports on the interactions of G4s with helicases, systematic analysis addressing the selectivity and specificity of each helicase towards a variety of G4 topologies are scarce. Here, we focused our attention on the binding and unwinding activities of the DinG FeS helicase towards different G4s, and evaluated the impact of three well-known G4 ligands, namely Pyridostatin (PDS), PhenDC3 and BRACO-19.

Our results indicate for the first time that DinG also binds and unwinds unimolecular G4s. Although DinG shows low discrimination in terms of affinity to G4s with different topologies, it exhibits a clear degree of unwinding specificity towards them. In addition, when the G4 structures were stabilized by ligands (Pyridostatin, PhenDC3 or BRACO-19), the unwinding activity of DinG was decreased and, in most cases, abolished, with a pattern that is not simply explained by a change in binding affinity. Understanding how G4 ligands interfere with the G4/helicase recognition is a key point in upgrading the current generation of G4-interacting drugs.

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THE ROLE OF WATER HYDRATION INTHE STABILIZATION OF THE THROMBIN-BINDING DNA APTAMER G-QUADRUPLEX BY MOLECULAR DYNAMICS

Attilio Cesàro[1], Silvia Di Fonzo[1], John W. Brady[2], Udo Schnupf[3]

[1]Elettra Sincrotrone Trieste, Area Science Park, 34149, Trieste, Italy
[2]Department of Food Science, Cornell University, Ithaca, NY 14853, USA
[3]Department of Chemistry and Biochemistry, Bradley University, Peoria, IL 61625, USA

The hydration layer surrounding complex biomolecules in solution is defined as the sheath of water molecules whose properties differ from those in the bulk due to the biomolecule's proximity. A rigorous definition of this labile layer is experimentally challenging, with numerical simulations being more versatile since they can use simple (although arbitrary) geometric criteria. Following the methods developed for caffeine,1here all water contacts to specific atoms of the Thrombin Binding Aptamer G-Q(a still active and intriguing research field2,3), have been averaged as a function of time for several microsecond MD simulations (up to 5 μs), in the presence of Na^+, or K^+, and Na^+/K^+ mixed cation solutions by using the TIP3P water model and the AMBER Nucleic Acid Force Field DNA-OL15. The starting structure from the PDB X-Ray data(5EW1). In addition to numerical data for hydration water distribution, the analysis of the trajectories pointed to several relevant structural characteristics:

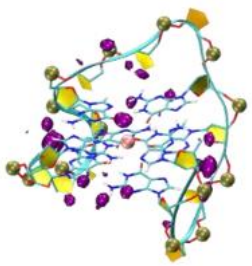
1) Ion-exchange in the G4 channel was observed with long term (up to 4 μs) counterion residence, with the ion approach preferred from the area of the 4-thymine-gating function; significant oscillations of side chains were characteristically monitored during the gating functions.

2) The Hoogsteen planes remain stable throughout the simulations, with structural flexibility of Hoogsteen planes observed.

3) Different extents of puckering of sugars in the complex was detected in planes 1 and 2 (as well as sugar thymine puckering).

4) Up to 20% of the short term changes in hydration levels can be attributed to loop movements, while water density between the Hoogsteen planes is zero regardless of the fluctuations.

5) The volume density maps show several areas of preferred water-solute interactions; the difference in the average hydration levels can be related to the stability of the TBA-15.



The figure shows a cartoon representation of TBA-15 backbone with detailed Hoogsteen planes and the purple regions of high water density. In conclusion, TBA-15 is an excellent model to investigate the dynamics and hydration behavior of G4s. This kind of investigation can be extended to other larger systems including homo-and hetero-tactic molecular interactions where water has been experimentally found to play a fundamental role.

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ON THE G-QUADRUPLEX DNA RECOGNITION BY PEPTIDES DERIVED FROM THE RAP1 PROTEIN

Francesco Merlino, Simona Marzano, Pasquale Russomanno, Federica D'Aria, Antonio Randazzo, Jussara Amato, Bruno Pagano

Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy

G-quadruplexes (G4s) are non-canonical nucleic acid secondary structures involved in several important biological processes such as transcription, replication, translation and telomere maintenance [1]. Traczyk A. *et al*. recently reported the crystal structure of a G4 in complex with the DNA-binding domain of the yeast protein Rap1, which consists of Myb1 and Myb2 homeodomains [2]. Here, aiming to develop peptide-based molecules capable of binding to target G4s, we analyzed the interaction of two peptide fragments with different lengths derived from Myb1, Myb1$^{399-410}$ and Myb1$^{397-415}$, with some G4-forming DNA sequences. Myb1$^{397-415}$ showed a greater ability to bind and stabilize the investigated G4 structures than Myb1$^{399-410}$. Combining the results of peptide-induced thermal stabilization and binding constant values, the G4s from the promoter sequences of *c-KIT* and *HER2* oncogenes (i.e., *c-kit2 G4* and *her2 G4*, respectively) came out as the most promising targets of Myb1$^{397-415}$. The two DNA-peptide systems were further investigated by isothermal titration calorimetry (ITC) analysis, thus obtaining the thermodynamic signature of the interactions. Moreover, an alanine-scanning mutagenesis was performed to evaluate the contribution of each amino acid of the peptide to the interaction with the G4s. We obtained a library of 19 Ala-substituted derivatives differing from Myb1$^{397-415}$ by a single amino acid. Surprisingly, circular dichroism melting experiments showed that 3 of the 19 peptides were able to induce an even greater increase of *c-kit2 G4* thermal stability. These interactions were further validated by ITC analysis, which confirmed the higher affinity of these Ala-substituted peptides for the G4 structure compared to Myb1$^{397-415}$. The biological activity of Myb1$^{397-415}$ and its most promising derivatives is currently under study.

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PP6

AQUEOUS PERCHLORATE SOLUTIONS: A NUMERICAL STUDY

Paolo La Francesca, Paola Gallo

Dipartimento di Matematica e Fisica, Università degli studi Roma Tre,
Via della Vasca Navale 84, 00146 Roma, Italy

The study of water in solutions plays a key role in the research regarding water anomalies both because aqueous solutions are naturally found in large quantities and because the experimental conditions under which many thermodynamic quantities are measured are more complicated to achieve for bulk water. Here we show the calculations of the phase diagrams of sodium perchlorate solutions in supercooled water derived through molecular dynamics numerical simulations. These solutions are relevant due to the recent experimental evidences of liquid water in perchlorate solutions beneath the Martian soil. By modelling water using the TIP4P/2005 potential, we obtain an agreement with the hypothesis of existence of a liquid-liquid phase transition where the liquid-liquid critical point shifts to slightly higher temperatures and lower pressures. By investigating the structure of the systems, we find that even at the highest concentrations considered, water retained its anomalous behaviour.

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PP7

PHASE DIAGRAM OF AQUEOUS SOLUTIONS OF LICL: A STUDY OF CONCENTRATION EFFECTS ON THE ANOMALIES OF WATER

Leonardo Perin, Paola Gallo

Dipartimento di Fisica, Università Roma Tre, Via della Vasca Navale 84, 00146 Roma, Italy

We perform molecular dynamics simulations in order to study thermodynamics and the structure of supercooled aqueous solutions of lithium chloride (LiCl) at concentrations c = 0.678 mol/kg and c = 2.034 mol/kg. We model the solvent using the TIP4P/2005 potential and the ions using the Madrid-2019 force field, a force field particularly suited for studying this solution. Aqueous solutions of electrolytes are of great interest as they are the media where biological processes take place, and they have great importance in the fields of physical chemistry, electrochemistry, and medicine, as well as in the industry. Given the water anomalous properties, it is of interest to study how these are modified in electrolytes solutions. We find that for c = 0.678 mol/kg the behavior of the equation of state, studied in the P-T plane, indicates the presence of a liquid-liquid phase transition, similar to what was previously found for bulk water. We estimate the position of the liquid-liquid critical point to be at T_C = 174 K, P_C = 1775 bar e ρ_c = 1.065 g/cm^3. When the concentration is tripled to c = 2.034 mol/kg no critical point is observed, indicating its possible disappearance at this concentration. We also study the water–water and water–ions structure in the two solutions, and we find that at the concentrations examined the effect of ions on the water-water structure is not strong and all the features found in bulk water are preserved. We also calculate the hydration number of the Li and Cl ions and in line with experiments we find the value of 4 for Li^+ and between 5.5 and 6 for Cl^-, confirming the good performances of the Madrid-2019 force field.

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COVALENTLY LINKED DIMERS OF AN ANTI-HMGB1 APTAMER: SYNTHESIS AND CHARACTERIZATION

Andrea Criscuolo, Ettore Napolitano, Claudia Riccardi, Domenica Musumeci, Daniela Montesarchio

Department of Chemical Sciences, University of Naples Federico II,
Via Cintia 26, 80126 Naples, Italy

High-Mobility Group Box 1 (HMGB1) is an abundant, highly conserved, non-histonic nuclear protein present in almost all eukaryotic cells [1,2]. It is also a DNA binding protein, involved in critical biological processes, such as DNA transcription, replication, repair, and recombination [3]. In an inflammatory state, HMGB1 is actively secreted from immune cells in the extracellular matrix, where it behaves as a proinflammatory cytokine [4], contributing to the pathogenesis of various chronic inflammatory and autoimmune diseases as well as cancer [5]. Given the multiple roles of the protein in these pathologies, identification of HMGB1-inhibitors is of considerable interest [6,7]. Considering the ability of this protein to induce bending in double-stranded DNA [8,9], as well as the identification of HMGB1 as a telomeric and non-telomeric G-quadruplex (G4)-interacting protein [10,11], in a recent work we identified a set of G4-forming aptamers from a focused library of G-rich oligonucleotides able to interact with high affinity with the protein and also inhibit the HMGB1-induced cell migration [12]. A more in-depth biophysical and biological characterization of one of the best anti-HMGB1 aptamers revealed that its efficacy was mostly due to its spontaneously formed dimer. Thus, the aim of this work is the design and synthesis of covalent dimers of the best G4-forming anti-HMGB1 aptamer to develop optimized constructs that can better interact with HMGB1 and also better inhibit the protein pathological activities.

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A QUANTITATIVE-CORRECT WATER MODEL FOR LARGE-SCALE SIMULATIONS UNDER LIFE-RELEVANT CONDITIONS

Luis Enrique Coronas, Oriol Vilanova, Giancarlo Franzese

Departament de Física de la Matèria Condensada & Institute of Nanoscience and Nanotechnology (IN2UB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

All-atom simulations of biologically-relevant systems come at a tremendous computational cost. To overcome this problem, coarse-grained models in simulation often use implicit water or replace water with a polar solvent without hydrogen bonds. Consequently, many physical properties are lost in this representation, and the models are not transferable. Here, we develop a transferable coarse-grained model with hydrogen bonds and many-body interactions. Initially introduced by Franzese and Stanley (FS) for water monolayers [1, 2], the model is analytically tractable [3] and suitable for Monte Carlo free-energy calculation [4] for large systems (10^7 molecules) and extreme conditions [5]. In its bulk version, we parametrize the FS model to get an excellent quantitative agreement with the experimental water thermodynamics in a life-relevant range of pressures and temperatures around ambient conditions, a prerequisite for its use as a solvent in biological simulations. The region of the quantitative agreement is as large as 60 degrees at ambient pressure and 50 MPa at ambient temperature [6]. Therefore, correctly accounting for the hydrogen bonds allows us to provide a transferable water model with an unprecedented agreement with the experimental phase diagram for large-scale free-energy calculations.

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PP10

THE METASTABLE LIQUID-LIQUID CRITICAL POINT OF SUPERCOOLED WATER IN A QUANTITATIVE MODEL

Luis Enrique Coronas, Giancarlo Franzese

Departament de Física de la Matèria Condensada & Institute of Nanoscience and Nanotechnology (IN2UB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

Water has thermodynamic, structural, and dynamic anomalies. To explain its unique properties, several thermodynamic scenarios for supercooled water have been proposed. Recent experiments support the hypothesis that water exhibits a liquid-liquid phase transition between low-density liquid and high-density liquid phases, ending in a liquid-liquid critical point (LLCP) at extreme thermodynamic conditions [1]. However, the lack of definitive experimental evidence leaves the debate open. Here, we show that the extrapolation to extreme supercooled conditions of a quantitative model [4], the Franzese-Stanley model for bulk water allowing unprecedented large-size simulations [2, 3], predicts a LLCP at T_C = (186 ± 2) K and P_C = (174±14) MPa in the thermodynamic limit, confirming the estimates of best-fitted atomistic models [4]. Furthermore, our finite-size-scale analysis demonstrates that the LLCP belongs to the 3D Ising universality class once the correct (non-trivial) order parameter is selected.

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PP11

SOLVENT FREE-ENERGY BARRIERS FOR THE VON WILLEBRAND FACTOR UNDER SHEAR

Luis Enrique Coronas[1], Emeline Laborie[2], Philippe Derremaux[2], Fabio Sterpone[2], Giancarlo Franzese[1]

[1]Departament de Física de la Matèria Condensada & Institute of Nanoscience and Nanotechnology (IN2UB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain
[2]CNRS Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, Université Paris Cité, PSL Research University, 75005 Paris, France

Calculations of conformational free energy barriers for large-scale hydrated biological systems are challenging due to the abundant water interactions and the difficulty of generating statistically uncorrelated configurations. Implicit solvent (IS) models, such as the OPEP for amino acids [1], reduce the computational cost by incorporating water contributions as effective interactions. However, by definition, IS models cannot explicitly calculate the solvent contribution to the free energy landscape. Here, we show that hydrating IS-generated configurations with the quantitative water model introduced by Franzese and coworkers [2, 3] allows us to overcome the limitation [4]. As an example, we consider the von Willebrand factor (vWf)—a protein involved in cardiovascular diseases made by globular domains connected by intrinsically disordered regions—that expands under hydrodynamic stress [5]. The explicit inclusion of water allows us to calculate the solvent free-energy barriers separating collapsed, detached, and extended conformations of three domains as a function of the shear rate [4]. This application of the method opens the perspective of accounting for the hydration contributions in a detailed way in large-scale numerical calculations for biological systems.

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PP12

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

Giuliana Fusco

Consiglio Nazionale delle Ricerche, Via Pietro Castellino 111, 80131, Napoli

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

To elucidate the nature of the normal and pathological forms of αS, we have established a research programme based on structural biology and cellular biophysics to reveal the properties of its transient interactions with biological membranes. A major focus of our research is the binding to SV and plasma membrane in the regulation of the SV homeostasis during neurotransmitter release. In the context of αS aggregation, we focus on the pathological membrane interactions of oligomers and fibrils that form on pathway during the self-assembly into mature amyloids as found in post-mortem analysis of patients affected by synucleinopathies.

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PP13

ROLE OF FAST DYNAMICS IN THE COMPLEXATION OF G-QUADRUPLEXES WITH SMALL MOLECULES

Luca Bertini[1], Valeria Libera[1,2], Marco Paolantoni[3], Caterina Petrillo[1], Francesca Ripanti[1], Tilo Seydel[4], Alessandro Paciaroni[1], Lucia Comez[2]

[1]Dipartimento di Fisica e Geologia, Università degli Studi di Perugia, 06123 Perugia, Italy
[2]IOM-CNR c/o Department of Physics and Geology, Università degli Studi di Perugia, 06123 Perugia, Italy
[3]Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia, 06123 Perugia, Italy
[4]Institute Max Von Laue – Paul Langevin, 71 avenue des Martyrs, 38042 Grenoble, France

In this study we combined incoherent quasielastic neutron scattering (QENS), incoherent elastic neutron scattering (EINS) and Fourier-transform infrared spectroscopy (FTIR) to characterize the dynamical properties of G-quadruplexes (G4) formed in the human telomeric sequence $AG_3(TTAG_3)_3$ (Tel22) when it is complexed with BRACO19 and Berberine.
By using FTIR we could determine that the Tel22 sequence is able to form G4 structures in hydrated powders. EINS and QENS, which give access to the nanosecond timescale dynamics, provided somewhat counterintuitive results. Indeed, our measurements allowed us to determine that the overall mobility of Tel22 is increased upon complexation while its stiffness decreases. The entropic contribution to the conformational free energy of fast motions might be crucial in the complexation mechanisms.

PP14

CORRELATION BETWEEN SECONDARY AND QUATERNARY PROPERTIES IN G-QUADRUPLEX COMPLEXED WITH PHOTOSENSITIVE LIGANDS BEFORE AND AFTER IRRADIATION

Valeria Libera[1,2], Elena A. Andreeva[3], Caterina Petrillo[1], Giorgio Schirò[3], Lucia Comez[2], Alessandro Paciaroni[1]

[1]Dipartimento di Fisica e Geologia, Università degli Studi di Perugia, 06123 Perugia, Italy
[2]IOM-CNR c/o Department of Physics and Geology, Università degli Studi di Perugia, 06123 Perugia, Italy
[3]Univ. Grenoble Alpes, CNRS, CEA, Institut de Biologie Structurale, F-38000 Grenoble, France

Nucleic acid sequences rich in guanines can organize into non-canonical DNA G-quadruplexes (G4s) of variable size. The design of small molecules stabilizing the structure of G4s is a rapidly growing area for the development of novel anticancer therapeutic strategies and bottom-up nanotechnologies. Among a multitude of binders, porphyrins are very attractive due to their light activation that can make them valuable conformational regulators of G4s. Here, a structure-based strategy, integrating complementary probes, is employed to study the interaction between TMPyP4 porphyrin and a 22-base human telomeric sequence (Tel22) before and after irradiation with blue light. Porphyrin binding is discovered to promote Tel22 dimerization, while light irradiation of the Tel22-TMPyP4 complex controls dimer fraction. Such a change in quaternary structure is found to be strictly correlated with modifications at the secondary structure level, thus providing an unprecedented link between the degree of dimerization and the underlying conformational changes in G4s. The combined use of information at different structure levels will represent a diagnostic strategy to implement the photocontrol of G4 dimerization and stabilization.

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QUADRUPLEXES ARE EVERYWHERE!
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LIST OF PARTICIPANTS

SURNAME	Name	e-mail address
ADAMS	Ellen	ellen_marie.adams@tu-dresden.de
BATTAGLIA	Giuseppe	g.battaglia@ucl.ac.uk
BERTINI	Luca	luca.bertini1@studenti.unipg.it
BULDYREV	Sergey	buldyrev@verizon.net
CALVINO	Martina Maria	martinamaria.calvino@unipa.it
CAMINITI	Luigi	caminiti@lens.unifi.it
CATALINI	Sara	sara.catalini@unipg.it
CESARO	Attilio	attilio.cesaro@elettra.eu
COMEZ	Lucia	comez@cnr.iom.it
CONTI NIBALI	Valeria	valeria.continibali1@unime.it
COTTONE	Grazia	grazia.cottone@unipa.it
CRISCUOLO	Andrea	andrea.criscuolo2@unina.it
D'ANGELO	Giovanna	giovanna.dangelo@unime.it
DE MICHELE	Cristiano	cristiano.demichele@uniroma1.it
DE SIMONE	Alfonso	a.de-simon@imperial.ac.uk
DEL GALDO	Sara	sara.delgaldo@uniroma3.it
DONKOR	Edward Danquah	edonkor@sissa.it
FOURNIER	Ariane	ariane.fournier@cnrs-orleans.fr
FRANZESE	Giancarlo	gfranzese@ub.edu
FUSCO	Giuliana	giuliana.fusco@cnr.it
GALAN	M. Carmen	m.c.galan@bristol.ac.uk
GALLO	Paola	paola.gallo@uniroma3.it
GIANCOLA	Concetta	giancola@unina.it
GRAZIANO	Giuseppe	graziano@unisannio.it
IACCARINO	Nunzia	nunzia.iaccarino@unina.it
JÜLICHER	Frank	julicher@pks.mpg.de
LA FRANCESCA	Paolo	paolo.lafrancesca@uniroma3.it
LAPINI	Andrea	andrea.lapini@unipr.it
LIBERA	Valeria	valeria.libera@studenti.unipg.it
MELILLO	Jorge H.	jorge.melillo@dipc.org
MERGNY	Jean-Louis	jean-louis.mergny@polytechnique.edu
MONTESARCHIO	Daniela	montesar@unina.it
MORBIDINI	Riccardo	morbidini@ill.fr
MOSCA	Ilaria	mosca@ill.fr

NAPOLITANO	Ettore	ettore.napolitano@unina.it
NETZ	Roland	rnetz@physik.fu-berlin.de
PACIARONI	Alessandro	alessandro.paciaroni@unipg.it
PAGANO	Bruno	bruno.pagano@unina.it
PERIN	Leonardo	leonardo.perin@uniroma3.it
PLAVEC	Janez	janez.plavec@ki.si
RANDAZZO	Antonio	antranda@unina.it
RENCIUK	Daniel	renciuk@ibp.cz
RIPANTI	Francesca	francesca.ripanti@unipg.it
RUIZ-PEREZ	Lorena	l.ruiz@ibecbarcelona.eu
SAMPSON	Ioannis	ioannissampson@materials.uoc.gr
SCHIRALDI	Alberto	alberto.schiraldi@unimi.it
SCIORTINO	Francesco	francesco.sciortino@uniroma1.it
SISSI	Claudia	claudia.sissi@unipd.it
SUGIMOTO	Naoki	sugimoto@konan-u.ac.jp
TUVERI	Gian Marco	gtuveri@ibecbarcelona.eu
VENTURI	Sara	venturi@lens.unifi.it