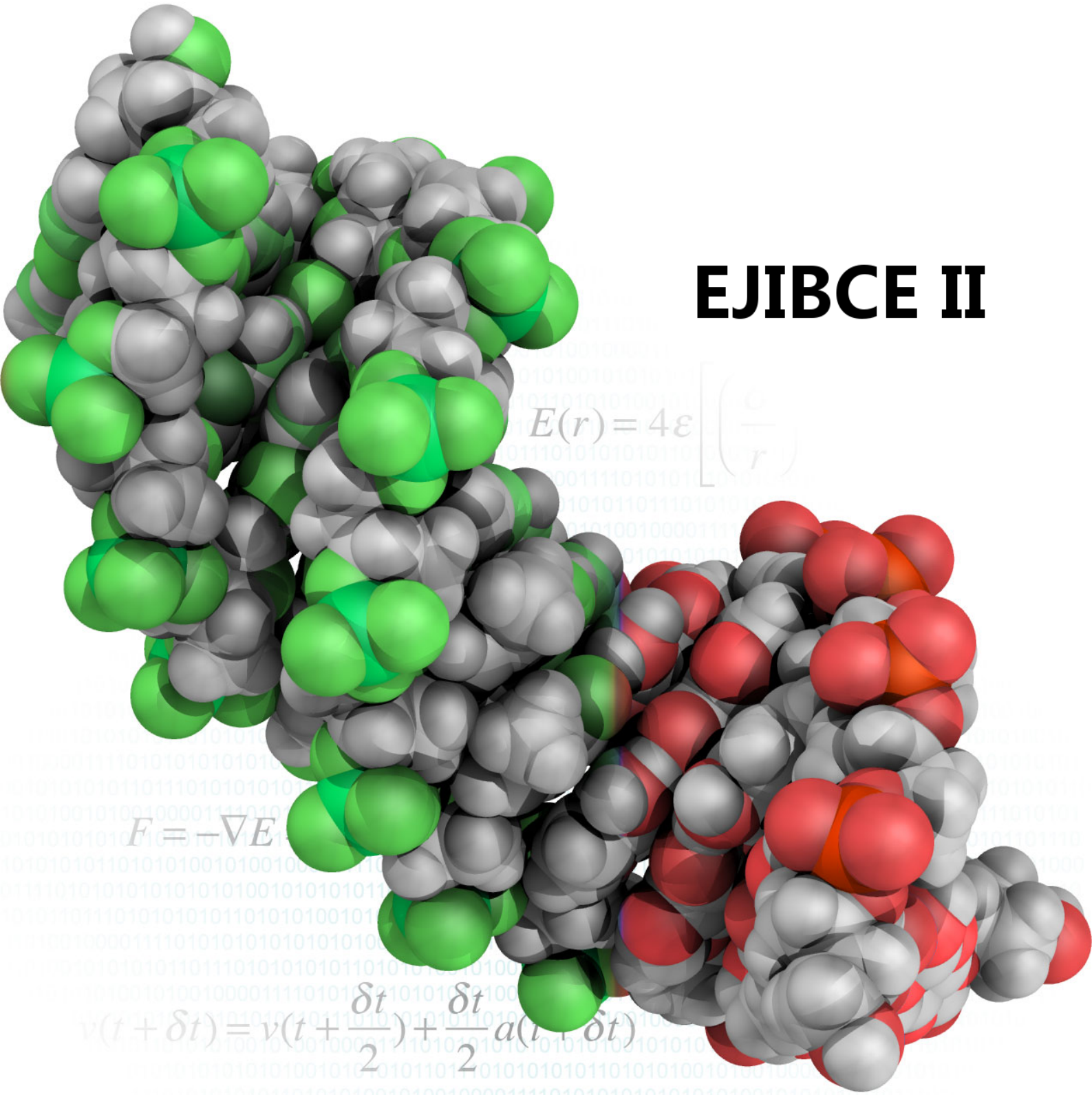


EJIBCE II

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

Missão e Objectivos

A partilha e discussão de ideias são as sementes para uma comunidade científica forte. A presente situação económica, tem dificultado o espírito de abertura e colaboração entre os vários grupos de investigação em Portugal. Ademais, com a acentuada "fuga de cérebros", muitos jovens cientistas portugueses vêem-se forçados a emigrar, perdendo por vezes contacto com o panorama científico nacional.

Este contacto com Portugal torna-se importante no momento de voltar ao país após um doutoramento, um pós-doutoramento, ou qualquer outro período prolongado no estrangeiro. Por outro lado, há quem queira continuar no estrangeiro mas simultaneamente cultivar uma relação de proximidade com a ciência em Portugal. Mas, que grupos existem na área da Biologia Computacional Estrutural em Portugal? E que investigação é levada a cabo nesses grupos? Onde posso contribuir com o meu conhecimento e recursos? Estas questões foram o mote para o lançamento desta iniciativa em Dez. 2013 e que reuniu 57 investigadores na cidade do Porto.

Este ano, pretendemos continuar o mesmo espírito, dando a conhecer o que de melhor se faz na área da Biologia Computacional Estrutural em Portugal e pelos portugueses espalhados por esse mundo fora. Esperam-se discussões animadas, trocas de ideias interessantes e novas colaborações que projectem a Biologia Computacional Estrutural portuguesa.

Comité Científico

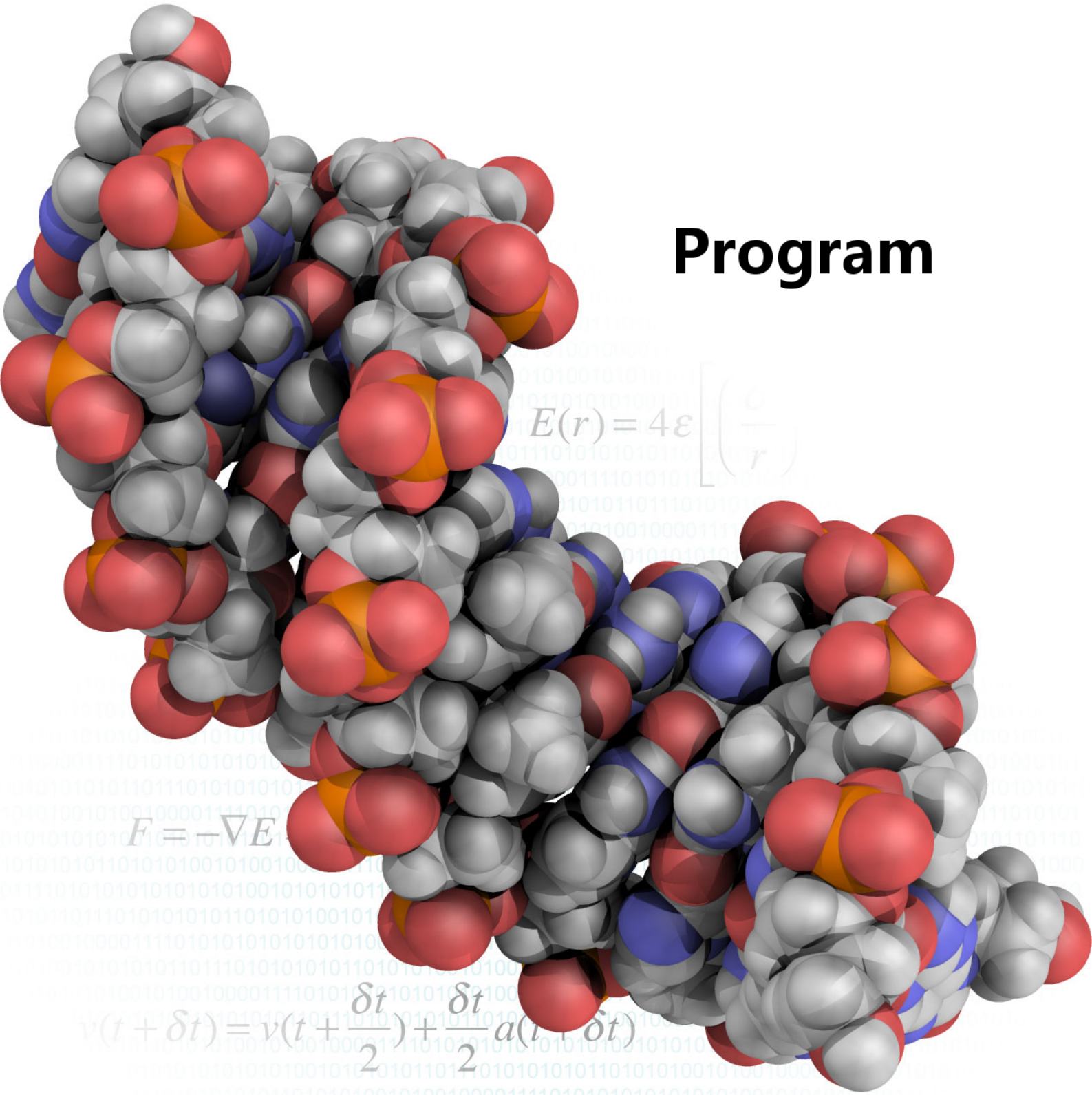
G. Matthias Ullmann, University of Bayreuth, GE
Marcia O. Fenley, Florida State University, Florida, US
Qiang Cui, University of Wisconsin, Madison, US

Organizadores

Irina S. Moreira - FCUP, Portugal
Miguel Machuqueiro - FCUL, Portugal

Organização Local

Ana C. Mourato - FCUL, Portugal
Diogo Vila-Viçosa - FCUL, Portugal
Pedro B. Reis - FCUL, Portugal
Rafael Nunes - FCUL, Portugal
Vitor H. Teixeira - FCUL, Portugal



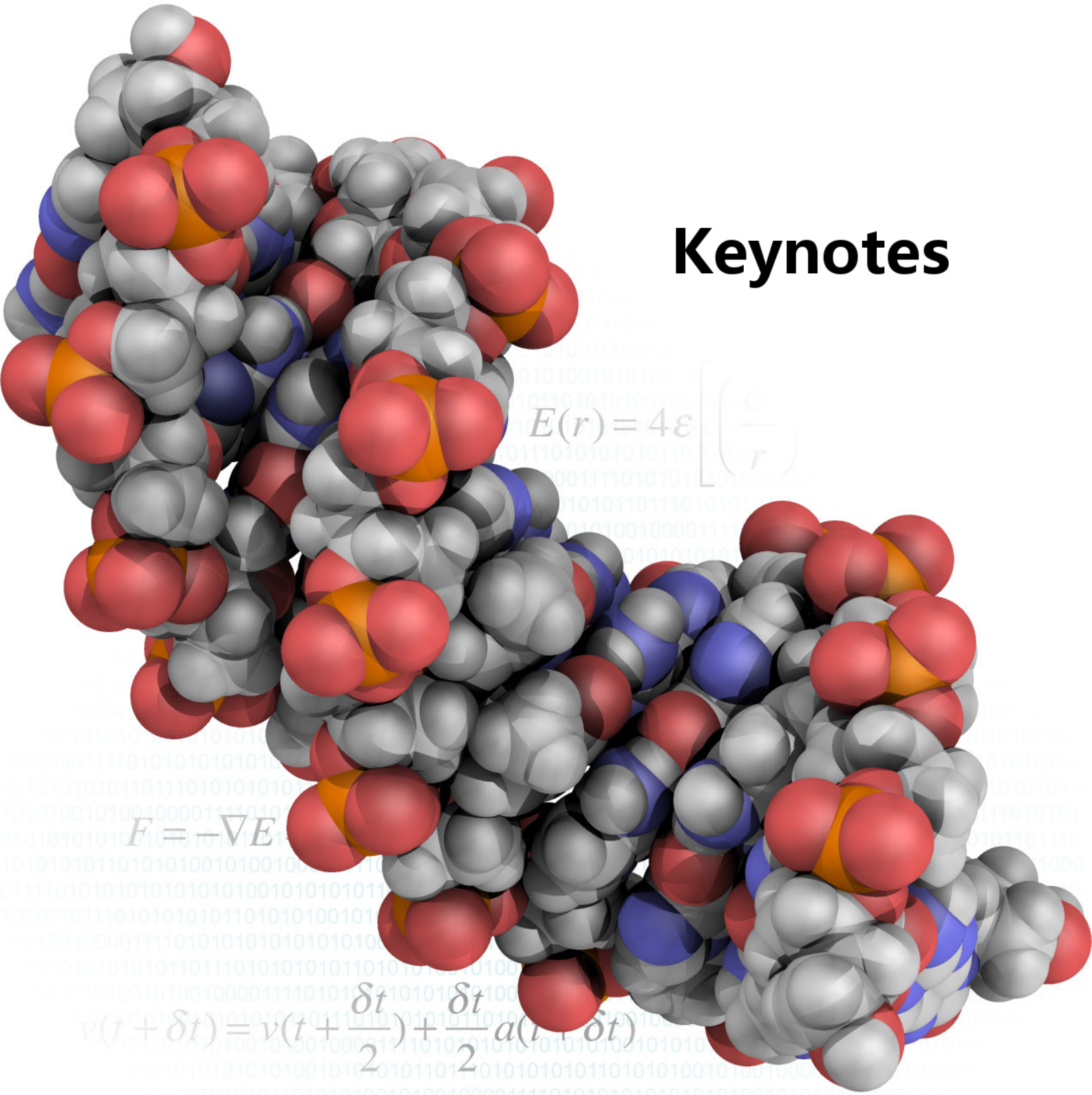
Program

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

Program

Thursday 18th		Friday 19th	
		09:30	**Patrícia F.N. Faísca**
		10:10	Nuno Sousa Cerqueira
		10:30	**Coffee break**
		11:00	**Alexandra Carvalho**
		11:40	Manuel N. Melo
		12:00	João M. Damas
		12:20	Nuno Galamba
		12:40	**Lunch**
13:00	**Registration**	14:00	**Afonso Duarte**
14:30	**Bruno L. Victor**	14:40	**Zeynep H. Gumus**
15:10	**Paulo J. Costa**	15:20	**Closing**
15:50	Cátia A. Bonito		
16:10	Diogo Vila-Viçosa		
16:30	**Coffee break**		
17:00	Poster Session		
19:00	**End poster Session**		



Keynotes

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

Combining NMR spectroscopy and Computational Structural Biology

When the whole is more than the sum of its parts

Afonso M. S. Duarte

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The use of Nuclear Magnetic Resonance (NMR) spectroscopy is wide spread in the field of Structural Biology of biomolecules. The combination of NMR with Computational Structural Biology methodologies is currently imperative to the understanding of protein dynamics and protein-protein interactions at atomic level.

In the last years I have been involved in the study of the dynamics of molecular machines that could provide us information on the cellular molecular processes. In this talk I will give examples of workflows and strategies that combine NMR spectroscopy and Computational Structural Biology to study the interaction mechanism of molecular chaperones with intrinsically disordered proteins and membrane proteins. Moreover, I will also introduce new Cloud and Grid Computing platforms available at European level that can bring new advantages to the community.

Computational challenges in the study of enzymes and DNA

Alexandra Teresa Pires Carvalho, Shina Caroline Lynn Kamerlin

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Without enzymes life would not be possible because the timescales of important biological reactions would be inadmissible long. However, as important to life as enzymes are, and independently of how much we already studied them, we still do not have a complete answer to how they work.

Computational methods have provided much insight about enzyme's mechanisms. I will present different current alternatives ranging from molecular orbital methods, more exactly QM/QM methods to valence bond methods, via the empirical valence bond method.

As examples I will present RNA polymerase II, enzymes of the alkaline phosphatase superfamily and succinate quinone oxidoreductase.

Finally, I will also talk about DNA epigenetic modifications and how, depending on the DNA sequence and position of the modification, we obtain subtle changes in structure that may influence recognition.

Carvalho, A.T.P., Barrozo, A., Doron, D., Kilshtain, A.V., Major, D.T., Kamerlin, S.C.L. Challenges in Computational Studies of Enzyme Structure, Function and Dynamics. J. Mol. Mod. Graph, 2014.

Carvalho, A. T. P., Gouveia, L., Kanna. C., Wärmlander, S., Platts, J., Kamerlin, S.C.L. Understanding the Structural and Dynamic Consequences of DNA Epigenetic Modifications: Computational Insights into Cytosine Methylation and Hydroxymethylation. Epigenetics (accepted).

Application of Alchemical Free Energy Calculations (AFEC) to the optimization of Transthyretin stabilizers

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In the past 60 years, the pharmaceutical industry has delivered over 1,200 new drugs that have played an important role in improving public health and extending life expectancy. However, the drug R&D model that has fueled innovation is currently facing critical challenges: the costs per new drug reaching the market are skyrocketing, breakthrough research is ebbing and a cliff of expiring patents is threatening the industry's lifeblood. The rapid progress witnessed in computer hardware, along with the development of simulation methods, enabled their integration in all stages of drug discovery and development. Within the context of medicinal chemistry and lead optimization, Alchemical Free Energy Calculations (AFEC) are increasingly recognized as a powerful tool. Since AFEC are accurate at predicting binding free energy changes upon transformation of one compound into another within a target protein site, they can be used to rule out/in chemical modifications that would result in weaker/stronger analogues. Transthyretin (TTR) is a tetrameric protein found in serum and in cerebrospinal fluid as a carrier of thyroid hormones. Increased dissociation of mutated TTR, and subsequent unfolding of the resulting monomers, results in formation of amyloid arrangements associated with diseases such as Familial Amyloid Polyneuropathy and Familial Amyloid Cardiomyopathy. In this presentation, I will illustrate the application of AFEC in the optimization of a lead series comprised of compounds holding chaperone activity towards the tetrameric form of TTR, while contrasting it with other simulation methods. I will show how Hamiltonian Replica-Exchange Molecular Dynamics (H-REMD) simulations are able to accurately estimate the impact of different alchemical transformations on the affinity of the resulting analogues for TTR. Validation of such predictions upon chemical synthesis and experimental evaluation of the in vitro activity of several analogues has helped pinpointing the limitations of the methods, as well as delineating strategies to overcoming them in future projects.

From ‘simple’ anion recognition phenomena to ‘complex’ biological systems

P. J. Costa

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One of the missions of EJIBCE is to share and discuss ideas bringing to light the research being performed by several young scientists in the field of Computational and Structural Biology. In this talk, instead of focusing on a single research topic, an overview of the work developed at the University of Aveiro will be provided. Starting from our interest in the supramolecular recognition phenomena [1], the development of anion receptors taking advantage of both hydrogen and halogen bonding will be discussed [2]. These anion receptors are extremely important since anion transport across phospholipid bilayers is essential to many cellular processes such as nerve conduction and maintenance of homeostasis and the dysfunction of anion channels is linked with the occurrence of a multiplicity of serious pathologies including the prominent cystic fibrosis. With this knowledge, we have been extensively using Molecular Dynamics simulations to investigate, at the atomistic level, the mechanisms of anion transport by small synthetic carriers, ultimately attempting to create more powerful drug-like molecules [3,4]. Very recently, neuropathological disorders have been considered in our research, namely, the optimization of BACE1 tripartite inhibitors [4] and the study of the amyloid precursor protein intracellular domain by molecular dynamics. Both these studies are relevant for Alzheimer’s disease research.

Acknowledgements: This work is financed by QREN-FEDER, through the Programa Operacional Factores de Competitividade – COMPETE and National Funds through FCT – Fundação para a Ciência e a Tecnologia (project EXPL/QEQ-COM/0821/2013). P. J. C. also acknowledges project CENTRO-07-ST24-FEDER-002034.

[1] S.M. Santos, P.J.Costa, M.D. Lankshear, P.D. Beer, V. Félix, J. Phys. Chem. B 2010, 114, 11173

[2] A. Caballero, F. Zapata, N.G. White, P.J. Costa, V. Félix, Angew. Chem. Int. Ed. 2012, 51, 1876

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[4] P. Linning, U. Haussmann, I. Beyer, S. Weidlich, H. Schieb, J. Wiltfang, H.-W. Klafki, H.-J. Knölker, Org. Biomol. Chem., 2012, 10, 8216

Microscopic insights into the amyloidogenic behavior of beta-2-microglobulin from molecular simulations

Patrícia F. N. Faísca[a], Sílvia G. Estácio[a], Heinrich Krobath[a], Diogo Vila-Viçosa[b], Miguel Machuqueiro[b], Eugene Shakhnovich[c]

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The identification of intermediate states for folding and aggregation is an important challenge not only from a fundamental standpoint but also for the design on novel therapeutic strategies targeted at the so-called conformational disorders. A well-known example is dialysis related amyloidosis (DRA) affecting individuals with kidney impairment undergoing dialysis. In DRA protein beta-2-microglobulin (b2m) aggregates into amyloid fibrils eventually leading to erosion and destruction of oesteoarticular tissues. In this talk I will present and discuss recent computational studies in which an intermediate state for folding and aggregation was identified for b2m. In particular, I will show how structure-based Molecular Dynamics (MD) folding simulations combined with MD simulations at constant pH and Monte Carlo ensemble docking simulations can be successfully combined to explore the conformational space of b2m leading to the identification of intermediate states that dimerize in a pH-dependent manner. I will show that the mechanistically structured-resolved picture of intermolecular association we obtain provides a rationalization for experimental results and leads to testable experimental predictions.

Network approaches in computational biology and medicine

Zeynep H. Gumus
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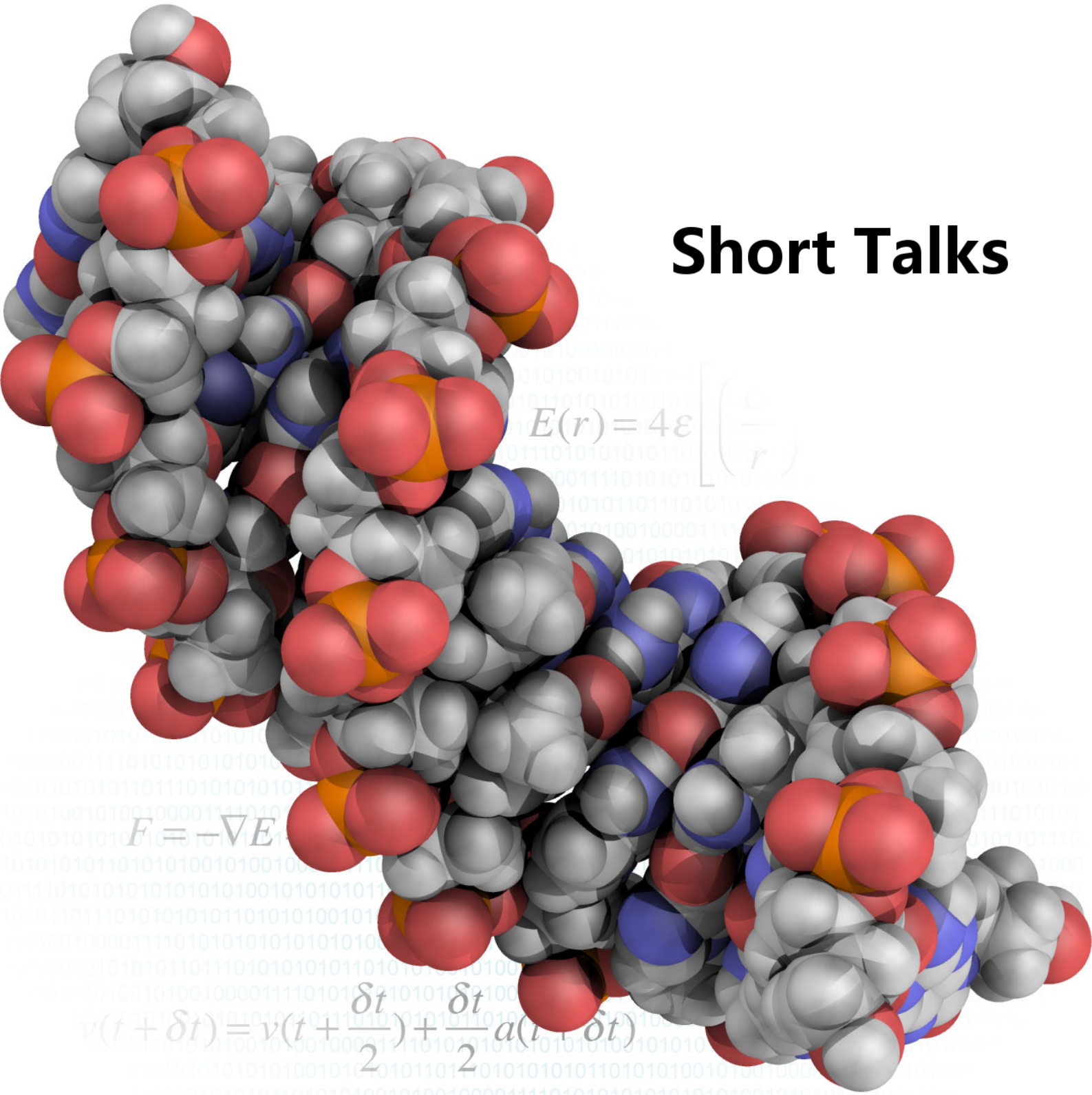
The massive quantities of biomolecular data generated by the recent new high-dimensional –omics technologies hold great promise for characterizing human physiology. The challenge lies in the development of novel approaches to analyze, integrate and interpret these data with high accuracy. Addressing this challenge can enable us to improve our understanding of the underlying biomolecular mechanisms of disease pathology and translate the information into diagnostic, prognostic and therapeutic strategies in patient treatment.

I will present three new computational systems approaches I designed for this purpose. The first aims to identify the key cellular disease mechanisms that either contribute to or drive synergistic response to drug combinations in chemotherapy. Second is a novel immersive 3D network visualization and analysis platform to address the complexities that arise from integrated analysis of large and dense biomolecular networks, and to provide a freely available resource for gaining novel insights from complex HT datasets. The platform also enables novel modalities in 3D visualization of residue correlation networks in protein structures. Third aims to better distinguish mutations in cancer genomes that affect protein function by utilizing protein sequence evolution and structural domain information.



Short Talks

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

A new approach to elucidate the molecular mechanisms of Medium-Chain Acyl-CoA dehydrogenase deficiency (MCADD)

Cátia A. Bonito[abc], Paula Leandro[ab], Fátima V. Ventura[ab], Rita C. Guedes[cd]

[a]*Department of Biochemistry and Human Biology*
[b]*Metabolism and Genetics Group, Research Institute for Medicines, iMed.ULisboa*
[c]*Medicinal Chemistry, Research Institute for Medicines, iMed.ULisboa*
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The MCAD deficiency is the most frequent genetic disorder of the mitochondrial fatty acid β-oxidation (mFAO) pathway. The MCAD enzyme is a homotetramer and catalyzes the first step of mFAO, a dehydrogenation reaction of the fatty acyl substrates. The most common mutation found in MCADD patients is translated in the substitution of lysine 304 residue by a glutamic acid (p.K304E), being associated with protein conformational changes. To better understand the molecular mechanisms underlying MCADD an in silico assessment of structural features of the wild-type and p.K304E mutant were performed through molecular dynamics simulations. The enzyme´s coordinates were obtained from the reverted crystallographic structure of the Glu376Gly/Thr255Glu mutant of human MCAD (hMCAD) into the hMCAD wild-type, in complex with FAD cofactor and octanoyl-CoA (CCOA) as substrate (PDB: 1EGC). The *sus scrofa* (pig) MCAD (pMCAD), has more than 90% of homology with hMCAD and its crystallographic structure, in complex with FAD and 3-thiaoctanoyl-CoA (SCOA) as substrate (PDB: 1UDY), was used to validate the obtained hMCAD wild-type. Three systems were build: in the absence (APO) or presence of cofactor (FAD) and with cofactor and substrate (LIPID). Our results show that the reverted hMCAD wild-type crystallographic structure is a good model to study the structural affected mutants. The p.K304E mutation induces structural modifications in the catalytic pocket, which compromise the FAD binding mode and may impair substrate access to the catalytic residue.

Constant-pH MD simulations of oleic acid in a lipid bilayer

Diogo Vila-Viçosa[a], Vitor H. Teixeira[a], António M. Baptista[b], Miguel Machuqueiro[a]

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Oleic acid is a simple molecule with fatty acid chain and a carboxylic group whose ionization and, consequently, their intermolecular interactions are strongly dependent on the solution pH. The pK_a of these molecules is difficult to obtain and can vary between 7.5 at low concentrations and 9.85 in pure monolayers. In this work, taking advantage of the recent implementations of PBC in PB calculations and ionic strength treatment in simulations of charged lipid bilayers, we studied the ionization dependent behavior of an oleic acid bilayer using a new extension of the stochastic titration constant-pH MD method.

The obtained titration curves are in strong agreement with experimental curves for this molecule. This comparison was performed for ionizations until ~50% since, above this value, a macroscopic transition to micelles occur. As previously observed for a binary mixture of a zwitterionic and an anionic lipid, we were able to reproduce experimental results with simulation boxes usually far from neutrality. This observation further supports the idea that a charged membrane strongly influences the ion distribution in its vicinity and the neutrality is achieved significantly far from the bilayer surface.

The good results obtained with this extension of the stochastic titration constant-pH MD method strongly supports its usefulness to sample the coupling between configuration and protonation in these types of biophysical systems. This method stands now as a powerful tool to study more realistic lipid bilayers where pH can influence both lipids and solutes that might be interacting in the lipidic environment.

Acknowledgements: Fundação para a Ciência e a Tecnologia for supporting grant SFRH/BD/81078/2011 and projects PTDC/QUI-BIQ/113721/2009 and Pest-OE/QUI/UI0612/2013.

A New Pathway for O_2 Diffusion inside Laccases and Multicopper Oxidases

João M. Damas, António M. Baptista, Cláudio M. Soares

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Laccases and multicopper oxidases (MCOs) oxidize a wide range of organic compounds while reducing dioxygen (O_2) to water, enabling numerous biotechnological applications. Deeply buried inside MCOs lies the catalytic center where O_2 gets reduced, and while X-ray crystallography structures suggest a solvent channel as a possible pathway, it is still unknown how O_2 reaches the center. Herein [1], an alternative new pathway is found through the use of a combination of free energy calculations (implicit ligand sampling), landscape analysis, and Markov modeling. The reported pathway is shown to be the one mostly contributing to O_2 reaching the catalytic center. This pathway is considered in light of the whole MCO family, and a possible relation between the O_2 diffusion and the protonation state of a structurally conserved acidic residue right above the center is advanced.

[1] – Damas, J.M. et al. (2014) J. Chem. Theory Comput., 10: 3525–3531, doi: 10.1021/ct500196e

Adaptive multiscale simulations with the Martini coarse-grain model. Successes and pitfalls

Manuel N. Melo, Alex de Vries, Ana M. Cunha, Siewert-Jan Marrink

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The bridging together of coarse-grain (CG) and atomistic (AA) MD models is a promising approach to simulating systems with the high detail of AA models while still being able to reach the large time- and space-scales provided by CG. AdResS is a region-based multiscale scheme that has been successfully used for the multiscale simulation of different systems. In my talk I will go through the applications and limitations of the scheme, focusing mostly on the use of the GROMACS simulation package with the Martini model as the CG representation. I'll further describe the ongoing research, both at the application and the implementation level, and discuss the challenges involved in expanding the use of AdResS.

The Dynamics of Water in Phospholipid Reverse Micelles and Bilayers

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The dynamics of water in confined environments such as the interior of reverse micelles (RM) is significantly different from that of bulk water. These differences are thought to be associated with both excluded volume effects and water interactions with specific hydrophilic and hydrophobic groups, and manifest, for instance, on a slower rotational anisotropy relaxation and on a blue or red shift of the OH(D) stretch vibrational frequency. Here we present a study on the dynamics of water in dioleoylphosphatidylcholine (DOPC) reverse micelles and on a DOPC lipid bilayer, with recourse to molecular dynamics simulations. Our results show that although the dynamics of water at the headgroup / water interface exhibits a size dependence similar to that found for distinct reverse micelles, bulk water-like dynamics is weakly size dependent, with water molecules at 5 Å and 6-7 Å from the surface portraying a vibrational and orientational dynamics, respectively, similar to bulk water's dynamics. Further, we show that phosphate water HBs and choline-water interactions are at the origin of an increasing red shift of the OH stretch absorption spectrum with the decrease of water content, consistent with experimental and theoretical studies of phospholipid (DLPC) multi-bilayers. However, the more interior, and less hydrated, carbonyl groups induce instead a blue shift on the OH stretch frequency of water, while the much less hydrated phosphate ester oxygens induce either a red or blue shift depending on the RM size. Frequency time correlation functions show that spectral diffusion decreases for the smaller RM although at short times (<1 ps) a faster decay takes place for low water content RM, apparently induced by large electrostatic fluctuations.

The Activation Mechanism of PLP Dependent Enzymes

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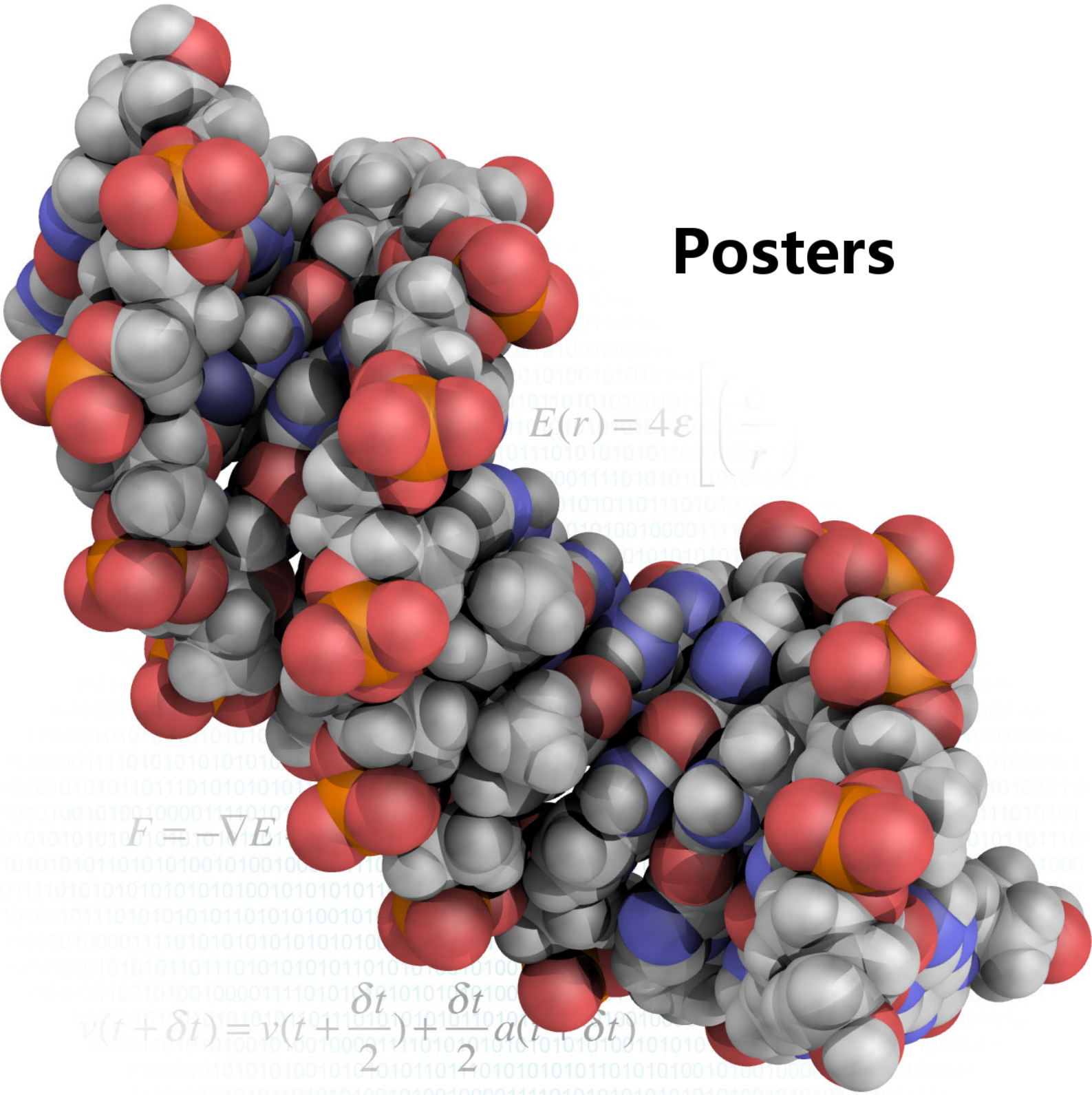
The pyridoxal-5′-phosphate-dependent enzymes (PLP enzymes) catalyze a myriad of biochemical reactions, being actively involved in the biosynthesis of amino acids and amino acid-derived metabolites as well as in the biosynthetic pathways of amino sugars and in the synthesis or catabolism of neurotransmitters. Although the scope of PLP-catalyzed reactions appears to be bewilderingly diverse, all enzymes employ a similar mechanism of activation and involve two stages: the binding of the cofactor to the active site of the enzyme and, the transimination reaction. Only after this step, the mechanistic pathway for each PLP-catalyzed reaction diverges.

In this communication, QM and QM/QM calculations regarding the activation mechanism of the PLP dependent enzymes will be presented (1,2,3). These results provide for the first time an atomistic portrait of these reactions and allow to assign a novel role to several active site residues that were never proposed.



Posters

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

Theoretical Study on the Intercalation of Phenanthroline in DNA: When Dispersion Forces are Important but the Electrostatic Contribution Becomes Crucial

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The effects of phenanthroline (phen) intercalation in the structure, energetics and bonding of Adenine-Thymine and Guanine–Cytosine tetramers (A-T/T-A and G-C/C-G) were studied through Density Functional Theory (DFT) using functionals that were recently improved considering the effect of dispersion forces. Our results given by the Energy Decomposition Analysis show that dispersion contribution, ΔE_{disp}, is the most important contribution to the interaction energy, ΔE_{int}. However, it is not enough to compensate the Pauli repulsion term, ΔE_{Pauli}, and the role of the orbital contribution, ΔE_{orb}, and specially the role of the electrostatic contribution, ΔE_{elstat}, become crucial for the stabilization of the structures in the intercalation process. Moreover, formation energies are higher when intercalation takes place from Major Groove (MG) in G-C/C-G systems but no appreciable differences are found for A-T/T-A systems. For G-C/C-G systems hydrogen bonding (HB) interactions are more important than stacking (S) interactions, whereas for A-T/T-A systems, HB and S become competitive. On the other hand, intercalation produces important changes not only in the hydrogen bonds of base pairs because S and HB are deeply connected, but also in other characteristic geometric parameters of the base pairs. Interactions and bond properties are analyzed in terms of dipole moments, polarizability, molecular electrostatic potential maps, electronic density, charge transfer, and frontier orbitals.

O_2 Diffusion in Cytochrome c Oxidase: Insights from MD simulations

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Cytochrome c oxidases (CCOX) are members of the heme-copper oxidase superfamily and they are the terminal enzymes of the respiratory chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular dioxygen to water with the creation of a transmembrane electrochemical proton gradient.

Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping and little emphasis has been given to the pathways used by dioxygen to reach the binuclear site. The main objective of this work is to identify possible alternative dioxygen pathways in the reduced CCOX from Rhodobacter sphaeroides using extensive Molecular Dynamics simulations. Our simulations allowed the identification of three possible dioxygen channels, all starting in the membrane hydrophobic region and connecting the surface of the protein to the BNC. One of these channels corresponds to the pathway inferred from the X-ray data available, whereas the other two are alternative routes for O_2 to reach the BNC. Both alternative channels start in the membrane spanning region and terminate close to Y288I (which is covalently linked to the H284I imidazole group).

Exploring PELE web server for protein-protein interactions: the binding of the intrinsically unstructured AICD as a test case

Cátia S. R. Sousa, Paulo J. Costa

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In recent years the Protein Energy Landscape Exploration (PELE) method [1] which combines Monte Carlo sampling with protein structure prediction tools has been successfully used in the exploration of protein-ligand interactions. On the other hand, protein-protein interactions are also important. Proteins lacking a stable secondary structure in the unbound state often fold upon binding to a receptor, e.g., the C-terminal intracellular domain of the amyloid precursor protein (AICD), intrinsically unstructured in solution,[2] folds when bound to a partner protein like Fe65.[3] Given the simplicity of PELE, we sought to explore its application in predicting the interaction of AICD with several partners. In our preliminary simulations, total energies and binding energies were analyzed as a function of the RMSD relative to the X-ray structure. Lower total energies and favourable binding energies yield a lower RMSD, indicating that PELE is able to sample the binding site even starting from a random structure. Although the results are promising, the predicted binding poses are not yet accurate. The methodology is currently being tuned trough the integration with Rosetta FlexPepDock,[4] a high-resolution peptide refinement protocol. In this approach, PELE is used for exploration of the binding site while FlexPepDock refines the selected structures.

Acknowledgements. This work is financed by QREN-FEDER, through the Programa Operacional Factores de Competitividade – COMPETE and National Funds through FCT – Fundação para a Ciência e a Tecnologia (project EXPL/QEQ-COM/0821/2013).

[1]Madadkar-Sobhani et al, Nucleic Acids Research, 2013, 41, W322
[2]Ramelot et al, Biochemistry, 2000, 39, 2714-2725
[3]Radzimanowski et al, EMBO reports, 2008, 9, 1134
[4]Raveh et al, Proteins, 2010, 78, 2029

What is the role of the influenza fusion peptide in membrane fusion? A computational study

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Influenza virus (IV) is responsible for worldwide outbreaks of flu, causing hundreds of thousands of deaths every year, which rise to millions in pandemic years. The finding of effective drugs against IV is, therefore, a matter of the utmost importance and urgency. To infect host cells IV fuses its membrane with the host membrane. The fusion process is promoted by the glycoprotein hemagglutinin (HA), which is located on the surface of the virus. HA has a highly conserved N-terminal domain, comprising ~20 residues, which inserts and destabilizes the host membrane during fusion - fusion peptide (FP). To elucidate the molecular determinants that lead to the destabilization of biological membranes by the FP, we used a molecular dynamics approach. These simulations enabled us to analyze the structural properties of the peptide inside the membrane and characterize the interactions between the peptide and the lipids. This knowledge contributes to a better understanding of the role of the FP in the fusion process, which can be useful for the development of anti-viral drugs against influenza.

Protein partitioning in aqueous two phase system

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Advances in biotechnology have made possible the production of a large number of new proteins important to the food, pharmaceutical, medical and chemical industry. There is growing request for these proteins due to their versatile applicability [1]. Technological improvements in upstream bioprocessing of biomolecules, brought a demands of a new technological platforms for downstream processing, particularly purification. Aqueous two phase system (ATPS) is a liquid-liquid extraction technique that exhibits several advantages including: biocompatibility, economically attractive, scalable, and low processing time. As additional tool to enable better process predictability and optimization, modeling of separation at the molecular and process level can be used [2]. The application of ATPS for protein separation and application of the semi-empirical model, based on continuum electrostatic, to predict protein partition coefficient, was uncovered by present work [3].

In this study partitioning of a fourteen different globular proteins, with defined Protein Data Base (PDB) structure, was examined in 4 different polymer/polymer ATPSs: PEG-Dextran, Ficoll-Dextran and two PEG-Ficoll systems with different polymer phase composition. The model performance was analysed regarding to the influence of different PDB structure and protein charges.

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Structural Enhancement of Water near a Hydrophobic Amino Acid

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The way water organizes next to hydrophobic surfaces and how this organization impacts on hydrophobic interactions, believed to be at the origin of many biological processes, is key to understand hydrophobicity. Hydrophobic effects manifest differently for small and large hydrophobic surfaces and this is closely associated with a distinctive organization of water. Here we study the structure of water next to a small hydrophobic amino acid, valine, through Born-Oppenheimer and classical molecular dynamics. We show that while water loses some hydrogen bonds (HBs) next to valine's side chain, the structure of a subset of water molecules, those with four nearest water neighbors, resembles that of liquid water at low temperatures, rearranging in more tetrahedral pentamers, with a larger number of HBs and shorter inter-molecular oxygen-oxygen and oxygen-hydrogen distances, relative to bulk water. In spite of several important differences, this structural organization is captured by density functional theory (DFT) and polarizable and non-polarizable water models, with DFT water exhibiting the largest tetrahedrality enhancement both at low temperatures and near valine's hydrophobic side chain. These results indicate that while those water molecules nearest to hydrophobic residues in proteins cannot adopt a tetrahedral geometry, losing both donor and acceptor HBs, those further away, adopt a more tetrahedral geometry, resembling liquid water at low temperatures. The possibility of this local structural enhancement to be observed through neutron diffraction experiments is discussed.

Accurate Prediction of Kinetics and Thermodynamics of a Peptide Model using μs-long MD Simulations

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cyc-RKAAAD is a short cyclic peptide known to adopt a stable single turn α-helix in water. Due to its simplicity and the availability of thermodynamic and kinetic experimental data, cyc-RKAAAD poses as an ideal model for evaluating the aptness of current molecular dynamics (MD) simulation setups to sample conformations that reproduce experimentally observed properties. Herein [1], we extensively sample the conformational space of cyc-RKAAAD using μs-long MD simulations and, using Cartesian-coordinate PCA (cPCA), we construct its energy landscape, thus obtaining a detailed description of the helical and non-helical subensembles. The cPCA state discrimination, together with a Markov model built from it, allowed us to estimate the free energy of unfolding (−0.57 kJ/mol) and the relaxation time (~0.435 μs) at 298.15 K, which are in excellent agreement with the experimentally reported values (−0.22 kJ/mol and 0.42 μs) [2]. Additionally, we compared this landscape with the ones obtained by REMD and bias-exchange metadynamics and discuss the sampling and computational gains achieved. Overall, modern simulations methods are shown suitable to explore the conformational behavior of peptide systems with a high level of realism.

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How do peptidic tree-like molecules fold?

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Peptide dendrimers are tree-like molecules formed by alternating functional amino acids with branching diamino acids such as lysine [1]. Dendrimers of this kind have already rendered several models for different applications, such as vitamin B12 transporters, antimicrobial agents and catalytic systems. Unfortunately these molecules have not yet yield to experimental structural characterization, hampering the possibility of constructing truly tailor-made peptide dendrimers and improve the existing ones. Computational methods seem to be an adequate tool to address these issues.

Herein, we present a comprehensive set of computational studies using molecular dynamics simulation methods, including stochastic titration constant-pH simulations, to explore the conformational behavior of these molecules and the key determinants to such behavior [2,3]. Moreover, we unravel the first hints on the influence of pH in the folding of these molecules, and the role played by dendrimer-substrate interactions during dendrimer-catalyzed ester hydrolysis. We used several conformational analysis procedures (clustering, energy landscapes and multivariate analysis) to analyze conformational changes that can be correlated with particular structural trends.

Our results show that peptide dendrimers exhibit a remarkable structural plasticity which is crucial for their activities. This work provides new insights into the atomic-level structures of these systems and might, in a near future, contribute to the development of novel knowledge-based dendritic systems with enhanced functionalities.

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Searching for new probes for amyloid

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The formation and inhibition of amyloid is nowadays a major subject of research since amyloidogenesis is the hallmark of a series of diseases, such as Alzheimer's, Parkinson's, and type II diabetes. One way to identify and characterize amyloid arrangements is through the use of fluorescent probes and dyes. However, due to the molecular polymorphism of amyloid species it is urgent to develop new and better dyes and probes. Taking these facts into account, we have tried to better characterize the interaction of the protein β-2 microglobulin (β2m) with the well-known amyloid probe thioflavin-T (ThT) using molecular modelling and molecular dynamics (MD) simulations. The crystallographic structure of β2m with PDB code 3MZT has been considered an amyloid-like oligomer consisting of 3 homodimers arranged in a ring where two alternative ThT conformations intercalate between the β-sheets of each dimer of the protein. To evaluate the structural stability of both the β2m dimer and its complex with ThT, we performed several molecular dynamics simulations using GROMACS 4.6.5. The results obtained from these simulations were in agreement with several reports found in the literature, where the Gln8 and Tyr10 residues of β2m were identified to be crucial in the interaction with ThT. To have a better understanding of this interaction, in particular to characterize the topological constraints imposed by those two critical aminoacid residues, we have mutated both Gln8 and Tyr10 to alanine residues, and using molecular dynamics simulations, we have analysed the stability of the β2m-ThT complex. The preliminary results of this work are presented here, and may help in the design and development of new and better probes for amyloid species.

pH gradients in continuum electrostatics calculations of Ccox

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Cytochrome c oxidase (Ccox) is an integral membrane protein complex which couples the oxidation of cytochrome c to the reduction of O2 to H2O. Ccox is also a proton pump, generating an electrochemical gradient, which provides the energy to fuel different cellular processes.[1,2] As a proton pump, Ccox is negatively affected as the pH gradient that it generates across the membrane increases. In order to study the effect of the pH gradient on the titration behavior of several key sites, we have modified our continuum electrostatics method in order to include this gradient, similarly to what was done by Calimet et al.[3].

Ccox was inserted in an explicit DMPC membrane, and all titratable sites were considered to be connected to either the P- or N-side of the membrane, which were exposed to different pH values. The effect of the pH gradient on key residues, particularly those in the catalytic site and in the proton transport channels, was analyzed by calculating their two-dimensional titration curves as function of the pH on each side of the membrane. In particular, we found that Lysine-362, in the K-channel, although located in the N-side of the membrane, was affected by the pH on both sides.

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New insights into the interaction of sugar-based surfactants with lipid bilayers from molecular dynamics simulations

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Sugar-based surfactants are known for their use in membrane protein crystallography and feature many other applications as a result of their biocompatibility properties and low toxicity when compared to traditional cationic detergents. We have developed a new family of alkyl deoxy glycosides with relevant antibacterial activities against Bacillus spp. and other microbes [1]. Experimental data indicates that deoxygenation of the sugar moiety leads to an increase on the surface activity of these molecules, which seems to modulate their antimicrobial properties. Many amphiphilic antibiotics are known to target bacterial membranes, destabilizing the thermotropic properties of lipid bilayers upon insertion. Thus, unraveling the molecular details of the interactions of these glycosides with model membranes is paramount to understand their mechanism of action. In this work, molecular dynamics simulations were applied to characterize the micelles formed by these glycosides in aqueous media and study their behavior at a model phospholipid bilayer interface. We also simulated phospholipid/glycoside binary mixtures to analyze the effect of partitioning increased molar fractions of glycosides into the bilayer on its structural features. The results herein presented provide valuable information with respect to the physical properties of these molecules and their biological relevance, which may have implications on the design of new antibiotics with increased potency.

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Insights on P-Glycoprotein and Membrane Function upon Drug Adsorption

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The biophysical aspects by which multidrug resistance (MDR) relates with ABC transporters function still remain unknown. Notwithstanding the central role that efflux pumps like P-glycoprotein have in MDR onset, experimental studies classified additionally the lipid microenvironment where P-gp is inserted as determinant for the increased efflux capability demonstrated in MDR cell lines [1,2].

Recently, a nonlinear model of drug-membrane interactions stated that, upon drug adsorption, long-range mechanical alterations are predicted to affect the ATPase function [3]. However, drug adsorption also occurs at the nucleotide-binding domains where conformational changes driving efflux takes place. Thus, we assessed the effect of drug adsorption to both protein-water and lipid-water interfaces, by means of molecular dynamics simulations [4].

Our results show that free energies of adsorption are lower for modulators in both interfaces, whereas transported and non-transported substrates share similar adsorption energies. Important differences in drug-protein interactions, protein dynamics and membrane biophysical characteristics were observed between the different classes. Therefore, we hypothesize that drug adsorption to the protein or lipid-water interface account for a complex network of events that affects the transporters' ability to transport drugs.

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Oxo-β-lactam computer-aided optimization toward COPD therapeutics and molecular probing

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Oxo-β-lactams are reactive warheads toward protease inhibition and our group is engaged with the tuning of its protease activity by scaffold re-decoration. The excess of extracellular Human neutrophil elastase (HNE) plays an important role in a variety of inflammatory diseases, namely in Chronic Obstructive Pulmonary Disease (COPD) which is currently the third leading cause of death worldwide.[1,2].

Molecular dynamics and experimental x-Ray studies previously reported that HNE operates by an induced-fit mechanism that involves a surface loop adjacent to the binding pocket, which contracts toward the inhibitor, bringing active site residues into a catalytically productive alignment.[3,4] Hence, in the work presented herein, molecular docking studies were performed to evaluate the more reliable interactions of oxo-β-lactams with different available HNE PDB structures in order to take in account the induced-fit character of HNE activity in the evaluation of more probable recognition counterparts for the synthesized inhibitors. Moreover, to overcome the flexibility contribution for HNE activity, a pharmacophoric model based on potent HNE inhibitors was created as a powerfull tool for compound making decision and it was used to filter a virtual oxo-β-lactam library toward the election of a virtual hit for experimental development.

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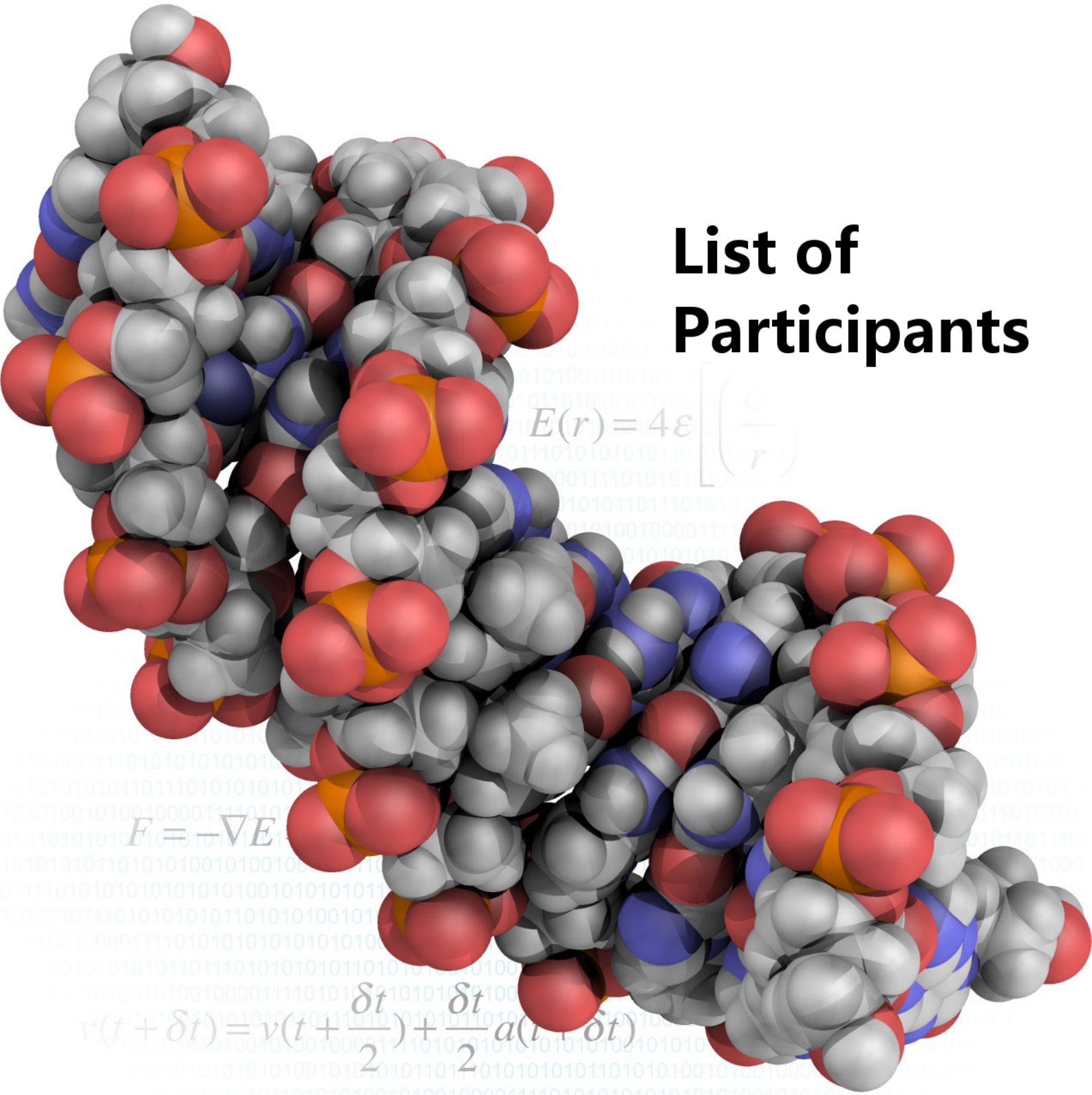

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Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

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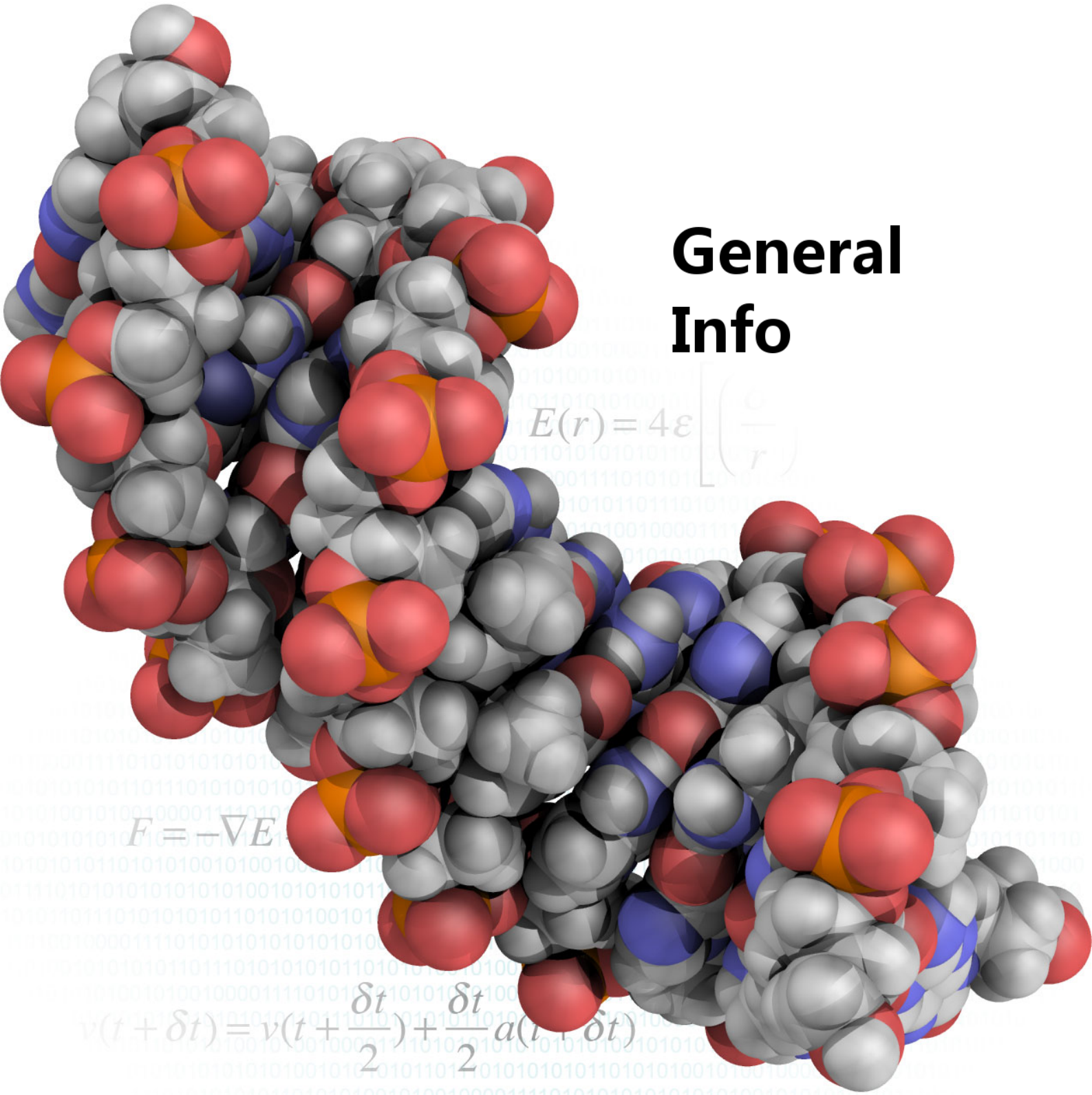

General Info

Segundo Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Lisboa, 18 e 19 de Dezembro de 2014

Local

O campus da Faculdade de Ciência da Universidade de Lisboa está localizado na zona norte do Jardim do Campo Grande. O EJIBCE 2014 terá lugar no edifício C1, no terceio andar (ver mapa).

Morada

Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Como chegar

- Metro: Estação do Campo Grande linhas Amarela ou Verde. Para mais informações: Metro de Lisboa.
- Autocarro: Para uma lista completa de autocarros que passam no Campo Grande: Carris.

Alojamento

Na eventualidade de necessitarem de passar a noite em Lisboa, aqui ficam duas sugestões, uma imediatamente ao lado da FCUL, o Hotel Radisson Lisboa, e outra um pouco mais em conta, o Typical Lisbon Guest House.