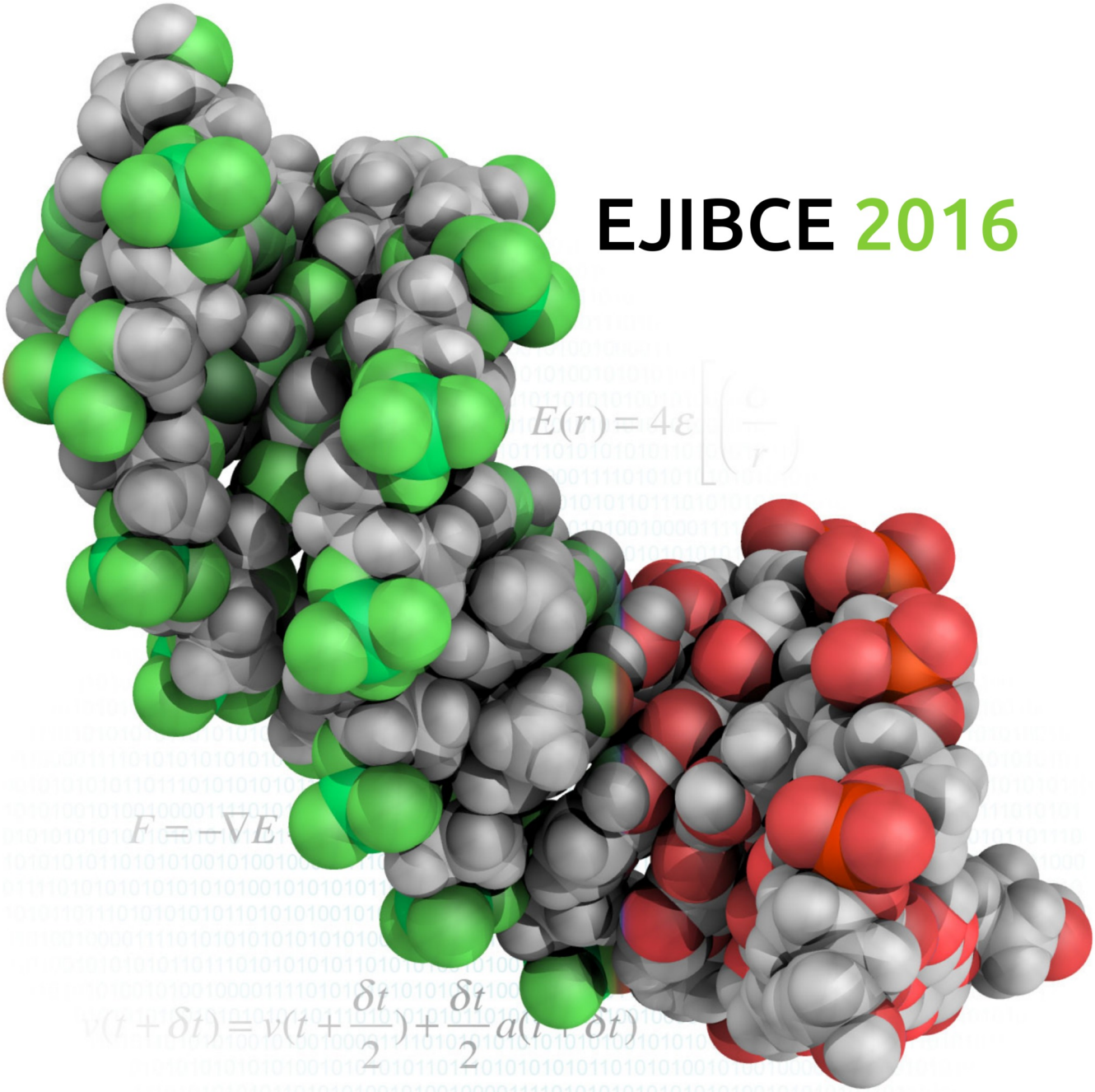

EJIBCE 2016

IV Encontro de Jovens Investigadores de Biologia Computacional Estrutural

ITQB NOVA, Oeiras, 21-12-2016

Patrocínios

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 centrais para o lançamento do EJIBCE em 2013. Em 2016, pretendemos continuar com o mesmo espírito e dar a conhecer o que de melhor se faz na área da Biologia Computacional Estrutural em Portugal e, também, o que estudam os investigadores portugueses radicados no estrangeiro.

http://www.itqb.unl.pt/ejibce2016

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João M. Damas, ITQB NOVA/Acellera

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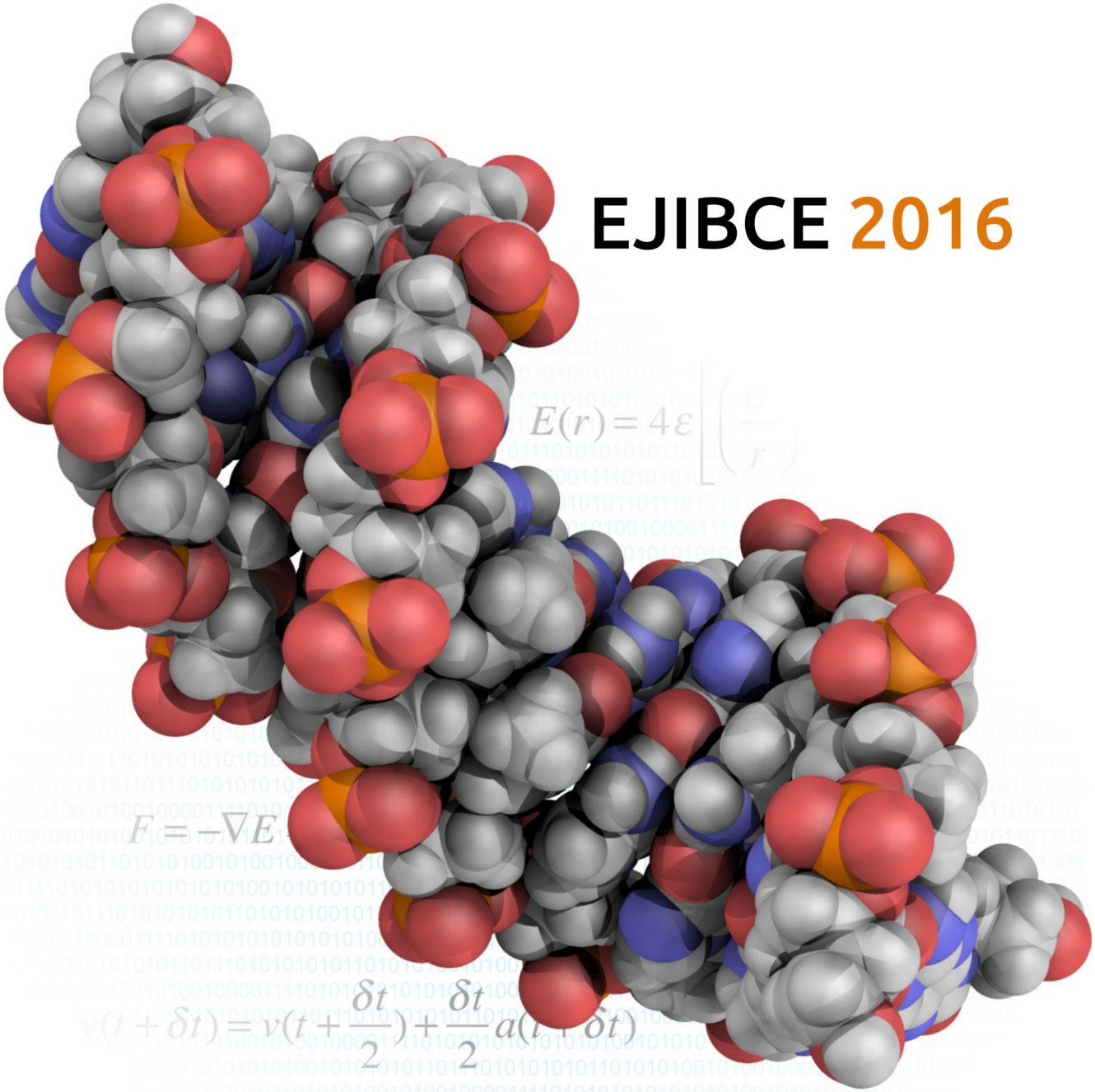

EJIBCE 2016

Program

Wednesday, December 21st

9:00–9:45 Registration

9:45–10:00 Welcome Address

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Acellera, Spain
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11:45–12:00 **Cândida G. Silva**
Universidade de Coimbra, Portugal
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Groningen University, Netherlands
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Round Table
Computational and Structural Biology in Industry

Moderators: **Irina Moreira**
CNC.IBILI

João M. Damas
ITQB NOVA/Acellera

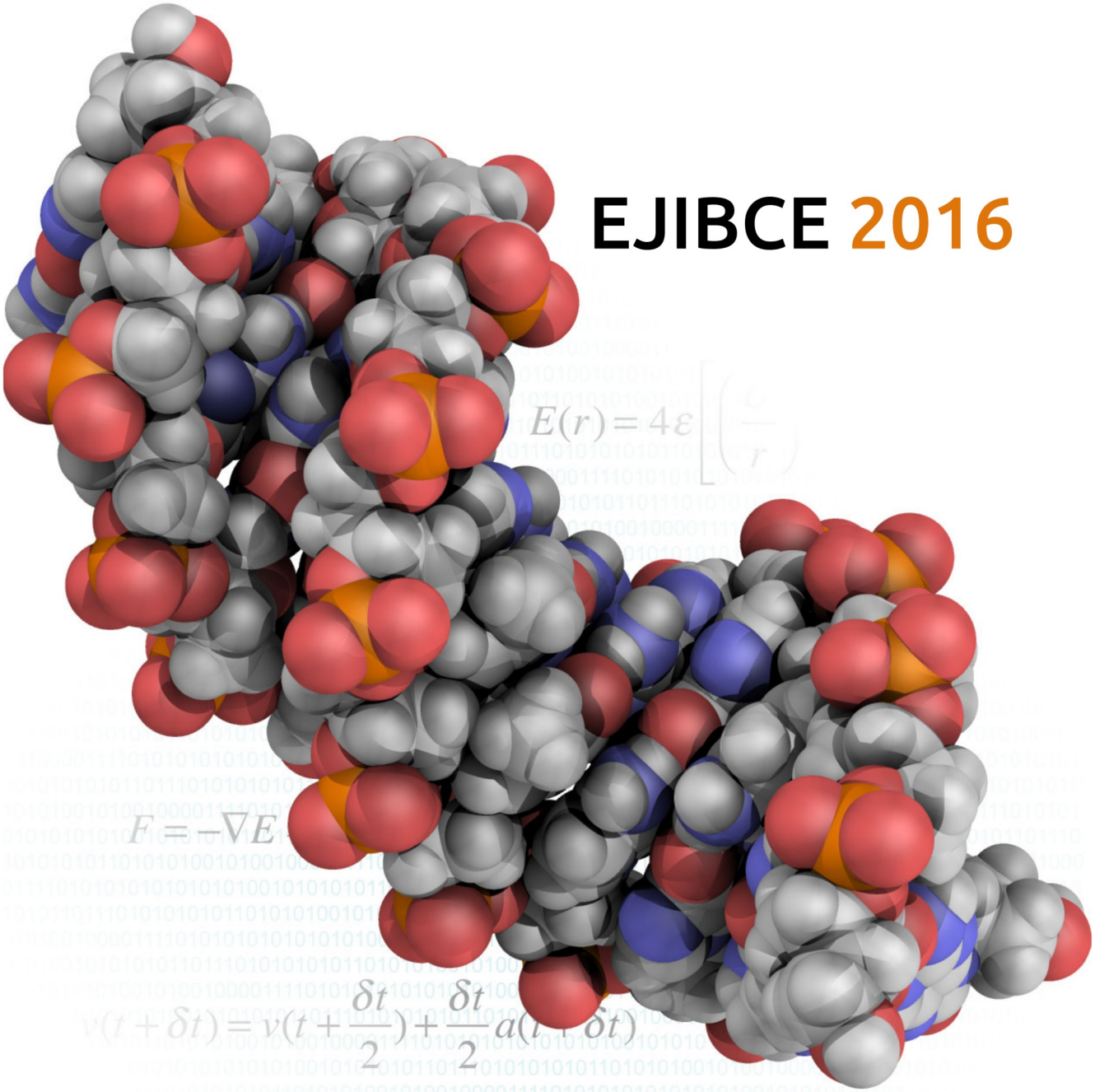
Speakers: **Carlos Simões**
BSIM^2

Isabel Rocha
Biotempo/SilicoLife

José Pereira Leal
Ophiomics

Tiago Bandeiras
IBET

Vera Gonçalves
Patentree



EJIBCE 2016

Invited Speakers

Protonation equilibria in water, membranes and protein active sites

Miguel Machuqueiro, Diogo Vila Viçosa, Pedro Reis, Tomás Silva and Bruno Victor

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pH is a crucial physicochemical property that affects most biomolecules. Changes in protonation equilibrium of susceptible sites will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability and catalysis.1 The pKa values of common titrable sites in peptides or other simple organic molecules can be significantly influenced by changes in solvent mixture, by direct interaction with a protein bindig pocket or due to insertion in a lipid bilayer.2-3

In this work, we present our latest results on the pKa calculations of peptides at the water/membrane interface and known acetylcholinesterase (AChE) inhibitors bound to their receptor. We take advantage of the recent extensions to the CpHMD-L methodology4 and apply it to these different systems, namely, the model Ala-based pentapeptides that have already been well characterized in water by Pace and co-workers,5 the pHLIP peptide, a 36 amino acid peptide derived from bacteriorhodopsin that is able to insert the membrane at acidic pH,6 and to donepezil and galantamine, two commercially available drugs that are inhibitors of AChE.

We acknowledge financial support from FCT through projects PTDC/QEQCOM/5904/2014, UID/MULTI/00612/2013.

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Putting the 'Multi' in Multiscale Modeling

Manuel N. Melo[1,2], Cleo Smeets[3], Dineke S. Verbeek[3], Joost Holthuis[4], Julija Zavadlav[5], Matej Praprotnik[5], Siewert J. Marrink[2]

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The term multiscale, applied to molecular modeling, has become fashionable in recent years — not the least due to partly being the focus of the 2013 Nobel Prize in Chemistry. But what does it really stand for? I will present a series of different examples in my simulation work that could all nowadays be considered multiscale. These examples revolve around protein/peptide interaction with lipid bilayers, modeled — as you can expect — at multiple resolution scales.

Protein-Protein and Human-Human Interactions

João P. Rodrigues

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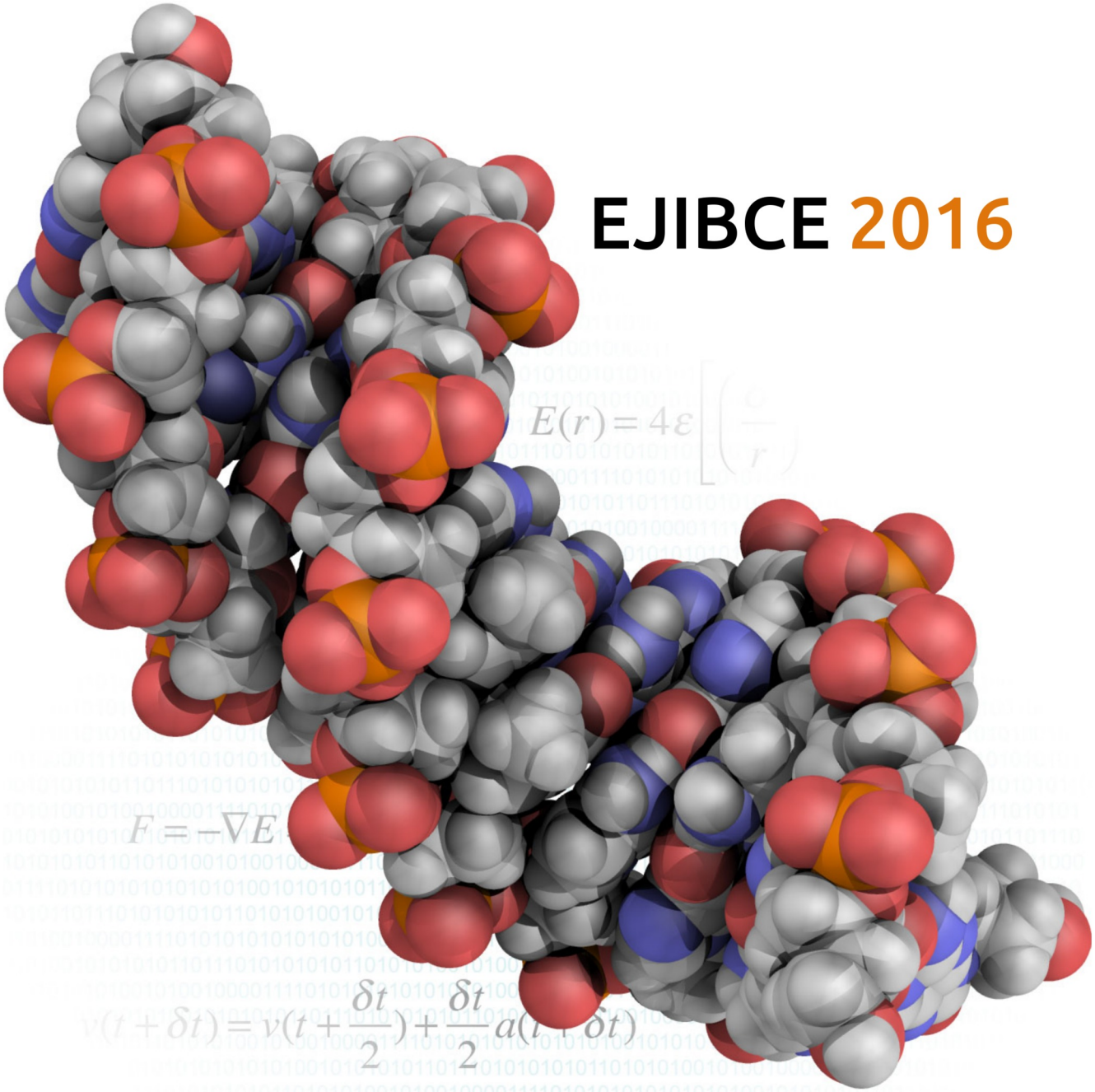
The last decade brought several new and improved players onto the structural biology field, which has been traditionally dominated by x-ray crystallography. The addition of methods such as structural mass spectrometry and resonance energy transfer to the structural biologist toolbox also led to a paradigm shift in the computational modeling community. To be useful, current molecular modeling methods need to handle a variety of experimental information across multiple structural resolutions. In this talk, I will describe methods I helped develop during my graduate studies that tackle modeling of interactions between proteins, DNA, RNA, and small molecules, using a variety of source of experimental data. I will focus on the challenges that current methodologies face when dealing with large molecular assemblies, such as the ribosome, and present solutions to some of these. Finally, I will touch on the importance of healthy scientific communities, to foster collaborations and improve science and education, and on how everyone can contribute to building such communities.



EJIBCE 2016

Selected Talks

A Machine learning based protein-protein hot-spot prediction method - SpotOn

Panos Koukos[1], Rita Melo[2], José G. Almeida[2], António G. Preto[2], Jorg Schaarschmidt[1], Mikael Trellet[1], Zeynep H. Gümüş[3], Joaquim Costa[4], Alexandre M.J.J. Bonvin[1], Irina S. Moreira[1,2]

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Hot-Spot (HS) detection has become an essential area of research in determining important residues in protein-protein interactions (PPIs)[1]. The energetic features of HS are troublesome to experimentally determine, as they involve replacing some residues by alanine — through Alanine Scanning Mutagenesis[2] — or other residues, which proves to be time expensive. As such, computational approaches to this sought out problem have become a valuable resource[3]. SpotOn uses 890 Eevolutionary-based features, along with 38 features related to the contact area (salt-bridges, hydrophobic interactions, solvent-accessible surface areas, among others), in order to determine HS and NS in PPIs. Several Machine-Learning Algorithms (MLAs) have been tested in order to reach the SpotOn model for HS prediction, with a database consisting of 545 mutations across 53 different complexes from ASEdb[4], BID[5], PINT[6] and SKEMPI[7]. The most successful algorithm was RRF-Global, which is essentially a regularized random forest, which works as a regular random forest — randomly selecting features and using them across an ensemble of decision trees — with the advantage that it penalizes similar features, creating a subset of representative features. The SpotOn webserver[8] has managed to reach an AUROC of 0.75, which is significantly better than other webservers and software's available in the literature, with an accuracy of 0.78, sensitivity of 0.84 and specificity of 0.76.

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TEMPORAL CONTROL OF MITOSIS – one more job for positive feedback

Ana Rita Araujo[1], Lendert Gelens[2], Rahuman Sheriff[1,3], Silvia Santos[1]

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The cell cycle is characterized by a sequence of events by which a cell gives rise to two identical daughter cells. Even though the molecular machinery that drives cell division cycles is the same in all tissues, quantification of the length of cell cycle phases in single cells by live-cell imaging shows high variability in the dynamics of cell cycle phases amongst different cell types. An exception to this is mitosis, whose duration remains short and surprisingly constant. In addition, contrary to what happens in other cell cycle phases, there is no correlation between cell cycle length and mitotic duration. In other words, it does not matter if a cell runs through the cell cycle at a fast or slow pace, once it reaches mitosis, it completes mitosis in a short and synchronous manner. It has been shown that positive feedback regulation is crucial to keep mitotic events synchronized (Holt et al 2008, Santos et al 2012) and therefore we hypothesized that positive feedback might be the molecular mechanism that keeps the time of mitosis constant across different cell lines with variable cell cycle lengths. Combining live cell imaging and computational modeling we show that when positive feedback is perturbed the switch-like activation of cyclin dependent kinase 1 (Cdk1) is compromised, leading to a more sluggish mitotic entry and more variable progression through mitosis. Importantly, breaking positive feedback coupled duration of mitosis and cell cycle length. This work shows that positive feedback, a recurrent motif in cell cycle control, may also be important to keep mitosis short, synchronous and temporally insulated from earlier cell cycle events. We anticipate positive feedback might be a simple regulatory strategy to create modularity in other biological systems.

Water dynamics and proton translocation in cytochrome cbb3 oxidase

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Cytochrome c oxidases are large membrane protein complexes found in bacteria and the mitochondria of eukaryotes. They catalyse the final step of aerobic respiration, namely the reduction of oxygen to water, in a biochemical process called oxidative phosphorylation. Oxidases couple the redox energy generated during catalysis to the "uphill" proton pumping across the membrane, thus contributing to the establishment of an electrochemical gradient that is used for ATP synthesis. Our work focuses on the distinctive cbb3 (or C-type) oxidases, which are mostly present in Bacteria and exhibit a number of unique features such as high catalytic activities at low oxygen concentrations, and nitric oxide reduction activity under anaerobic conditions. It has been shown that such characteristics are essential for the colonisation of anoxic tissues by some human pathogens (e.g. Campylobacter jejuni and Helicobacter pylori). At the moment, the functioning mechanism of C-type oxidases is still poorly understood.

In this work we report the results of large-scale, fully-atomistic MD simulations of cbb3 oxidase complimented by continuum electrostatic calculations. We provide a detailed analysis of the water dynamics and proton translocation pathways for both the "chemical" and "pumped" protons and identify key residues controlling the formation of the protonic connections. We also studied the effect of mutations experimentally shown to affect the enzymatic activity. Our work contributes to a better understanding of cbb3 mechanism and provide basis for future experimental and computational studies.

Using Computational Design of Peptides for the Development of Metalloprotease Models

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Computational protein design is a promising approach in the development of new enzymes with high technological potential. We are interested in using such tools for the design of metallopeptides with protease-like activity, given the wide range of industrial applications of the later and our interest in exploring the functional diversity played by metal ions in small protein scaffolds.

We addressed this challenge by first considering which are the factors governing native metalloprotease function. Employment of coarse-grained models (Anisotropic Network Model and β-Gaussian Elastic Network Model) for the study of metalloprotease internal dynamics was evaluated against a set of experimental (X-ray) structures and trajectories from molecular dynamics (MD) simulations. Employment of dynamics-based (ALADYN) and structure-based (DaliLite) comparison methods of MEROPS MA clan members allowed us to make a characterization of its structural and dynamical variability. Results suggest that the requirement for proper functional interactions with the substrate is a selective constraint that acts on distinct manners during the evolution of metalloprotease structure and dynamics [1].

Based on the structural and dynamical features of metalloproteases, we characterized the conserved geometric relations of the MA(M) subclan ((His)3-Zn(II)+Glucat) in order to develop theoretical models of metalloproteases. We then benchmarked the usage of the Rosetta software package (match and enzyme design applications) for the redesign and de novo design of catalytic metallopeptides based on the MA(M) theoretical model. First, we redesigned a native zinc finger peptide in two rounds and the synthesized sequences presented native-like folds and catalytic activity towards a general substrate in the range of other redesigned zinc finger peptides. Then, we explored if other small scaffolds could be similarly designed. The best candidate

(villin headpiece subdomain) from a set of 42 peptides/small proteins with known structure was redesigned to craft the Zn active site and evaluated using MD simulations [2]. The resulting design also has a native-like fold upon zinc binding but presents modest catalytic activity. Therefore, we show that computational enzyme design can be used to generate new catalytically active peptide scaffolds by integrating both computational and experimental methodologies.

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REPRODUCIBLE SYSTEM BUILDING AND SIMULATION OF MEMBRANE PROTEINS WITH HTMD

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HTMD is a programmable scientific platform intended to simplify and increase reproducibility of simulation-based research. In this work we demonstrate the functionalities of HTMD that enable the preparation of a molecular dynamics simulation starting from a PDB structure, parametrize it through well-known forcefields, apply standardized protocols, and launch the corresponding simulations on a variety of computational resources. We demonstrate the automation potential and flexibility of the system building features of HTMD, applying it to hundreds of proteins in the OPM (Orientation of Proteins in Membranes) database, automatically building and performing all-atom simulations of most eukaryotic membrane proteins resolved to date.

Trimming the sails: on how we are fixing docking-based scoring using machine learning

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Molecular docking may be reasonably successful at reproducing X-ray ligand poses within the binding site of a protein (often referred to as pose-fidelity). However, docking scoring functions are typically unsuccessful at correctly ranking ligands according to their binding affinity [1]. Strategies to overcome this problem have included the use of consensus scoring methods, but more recently it has been suggested that machine learning-based scoring functions may yield better outcomes [2].

In this communication, we will reveal how the use of support vector machines (SVM) can dramatically improve the discrimination between active and inactive (decoy) molecules, using a set of challenging pharmaceutical targets deposited in the Directory of Useful Decoys [3]. AutoDock Vina [4] was used to dock all actives and decoys into the target binding sites of the selected enzymes. Re-scoring of all docked complexes was carried out using RF-score. The energy parameters of Vina's scoring function — and more than 30 RF-score terms describing protein-ligand interactions — were used to train classification models with SVM-light [5].

Our results show that Vina offers acceptable pose prediction accuracy for most targets. However, its scoring function performs poorly when compared to our SVM classification models. The superior overall virtual screening (VS) performance of the trained classification models confirms the potential of the use of machine learning methods to eschew the limitations of scoring functions at capturing the non-additive relationship between individual energy terms involved in ligand binding. The inclusion of additional terms produced by RF-score appears to be beneficial to improving scoring in particularly "hard" targets.

Altogether, our results illustrate the potential of our SVM-based protocols for fast, receptor-based VS using freely-available docking and scoring software.

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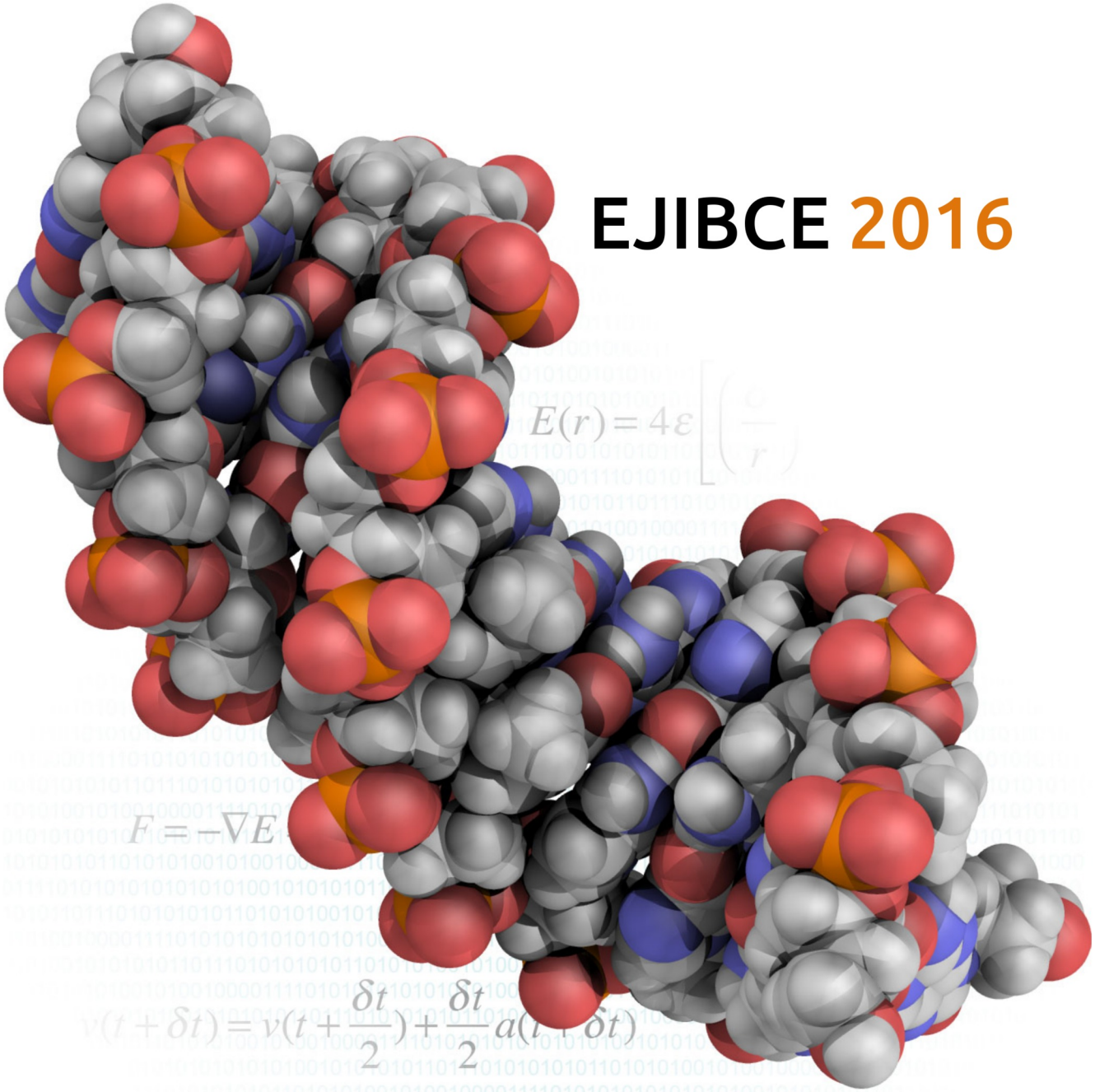

EJIBCE 2016

Posters

Unveiling the role of the mutation F508del in cystic fibrosis

Bárbara Abreu, Emanuel Lopes, A. Sofia F. Oliveira, Cláudio M. Soares

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Cystic fibrosis is a genetic disease that causes the accumulation of mucus in epithelia, mainly affecting the airways of the lungs. These secretions not only cause obstruction, but also inflammation and infections by Pseudomonas and Staphylococcus organisms. (1)(2) The mutated gene in this disease encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is a chloride channel. (1) Although there are over 1500 mutations related to cystic fibrosis, the most common is the deletion of phenylalanine 508 (F508del). F508del causes the misfolding of the CFTR polypeptide leading to its degradation in the ER. Nevertheless, a small amount of defective channels is still able to reach the cell membrane, but display an impaired function leading to minimal chloride transport. (3)

The CFTR channel belongs to the class of ABC transporters. These proteins have been extensively studied using computational methods in our lab (5-7) in order to clarify their mechanism and mode of action.

In the present work, we derived a new model for the NBD1-NBD2 association of human CFTR based on existing data using comparative modelling techniques (8), and performed molecular dynamics simulations of the CFTR protein in both mutant and wild-type forms with the goal of studying the conformational consequences of ATP hydrolysis in the mutant and wild-type forms.

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Hair Keratin Molecular Dynamics Studies

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The keratin is a key element of the hair, nails and skin in vertebrates. Understand the keratin features such as its assembling in the mentioned structures, its interaction with some compounds or mechanical properties is of great interest in the fight against some diseases or in the development and optimization of cosmetic products.

Although molecular dynamics simulations provides unique information at molecular level there are only a few studies using this technique on the study of keratin. This is likely the result of the non-existence of full length keratin crystallographic model. In the few works published about keratin using molecular dynamics simulations the authors had to design and build the computational keratin model, to make the simulations of interest.

This work addresses some molecular dynamics studies about hair keratin, from the physicochemical properties of the molecular models to the correlation of the simulations results with experimental data.

Our work on this field, with recently developed computational models of hair fibers, is also discussed. We built molecular dynamics models able to reproduce in simulations some phenomena observed in experimental assays, providing important information at molecular level about the mechanisms that lead to the experimental results.

An Atomistic Study of the Catalytic Mechanism of Dibenzothiophene Monooxygenase DszC with QM/MM Calculations

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The main organic sulfur compounds found in crude oil are dibenzothiophene (DBT) and its alkylated derivatives, which are resistant to the more commonly employed chemical approach in oil industry, hydrodesulfurization. However, they are susceptible to biodesulfurization, a green alternative technique for sulfur removal in fossil fuels.[1] In particular, the bacterium Rhodococcus erythropolis strain IGTS8 metabolizes DBT and its derivatives to 2-hydroxybiphenyl and sulfite through the 4S pathway, which involves four enzymes (DszA, DszB, DszC and DszD).[2,3] Despite that this approach has major advantages, the rate of biodesulfurization is slow compared to hydrodesulfurization. Hence, one strategy to improve the overall rate is to increase the enzymatic activity of DszC, which exhibits a low catalytic efficiency.[4] DszC is a key enzyme in the 4S pathway, making use of a FMNH2 cofactor and O2 to catalyze the oxidation of DBT successively to DBT sulfoxide (DBTO) and DBT sulfone (DBTO2). Despite that X-ray crystallographic studies have provided essential mechanistic insights on the structure of DszC, the reaction mechanism remains unknown.[5,6] We present the catalytic mechanism of DszC investigated computationally with the ONIOM quantum mechanics/molecular mechanics (QM/MM) methodology (B3LYP/6-311+G(2d,2p):AMBER//B3LYP/6-31G(d):AMBER). We propose that the reaction mechanism follows three major steps: (1) the formation of the C4a-hydroperoxyflavin (C4aOOH) intermediate; (2) the oxidation of DBT to DBTO, upon a nucleophilic attack on the distal oxygen of the C4aOOH; and (3) the hydrogen transfer from the NH of the isoalloxazine ring to the Ser163-hydroxyl, releasing a water molecule from the C4a of the C4a-hydroxyflavin intermediate. These calculations further extend the mechanistic knowledge on the chemistry of DszC, and could be instructive for the rational design of DszC mutants with improved catalytic activity that will considerably enhance the role of biodesulfurization in the oil industry.

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Improving Enzyme Design with simple structural descriptors

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Enzyme design is an outstanding innovation in science. Designing new biocatalysts is nowadays accessible without the requirement of tracing back protein evolution trails from a phylogenetic ancestor. This was the most common procedure in improving biocatalysts. [1] The presented approach pushes further our limited knowledge about catalytic mechanisms of enzymes in Nature. It is proposed as a complement of the well-established rational design and directed evolution protocols commonly used in enzyme design. [1]

Within the CHImerASE multilateral ERA-IB consortium, a platform for the design of chimeric enzymes applied to specific biotransformation steps has been under development, since 2014. It is an innovative approach aiming to circumvent the drawbacks of conventional industrial synthetic steps, namely the low value by-products formation. Advanced in silico protein design methods have been applied iteratively: the catalophore method [2] for suitable scaffolds search, the crafting of a given theozyme in each of the selected protein scaffolds, [4] and its integration with a molecular dynamics based evaluation and activity prediction. The usage of computational techniques gives the opportunity to extract crucial data which were implemented in the ranking of designed enzymes. This multi-dimensional analysis led to the optimization of the enzyme design protocol. [3]

We strongly believe that such an innovative approach represents a new trend in the field with a substantial impact at industrial, economic and environmental levels for the next generation of biocatalysts in the decades to come.

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Predictability of the protein partitioning in aqueous two-phase systems using semi-empirical model

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An increasing request for industrial proteins due to their versatile applicability, lead to technological improvements in upstream bioprocessing, resulted in higher yields, and concentrations within a highly complex mixture. For that reason, proteins' separation is very often difficult and economically demanding, so there is a vast interest in the development of unconventional technologies to improve downstream processing, particularly purification. Aqueous two phase systems (ATPSs) are biphasic systems utilized for large scale downstream processing of protein separation. In what concerns their advantages, the ATPSs have been applied in numerous fields such as biomolecules separations, recovery of biopharmaceuticals etc. [1]. However, the major drawback of ATPS is the poor understanding of mechanism involved into various and complex interactions among ATPS compounds, which requires very complex optimization of these systems for practical applications. The free Gibbs energy relationship based model [3], which assumes that electrostatic and nonpolar effects are the main force explaining the protein partitioning in ATPS, was proposed in this work. The partitioning behavior of a series of 14 globular proteins, in 3 different polymer/polymer ATPSs was studied, and model viability was tested.

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UNVEILING THE HUMAN P-GLYCOPROTEIN EFFLUX MECHANISM:INSIGHTS ON DRUG BINDING AND SIGNAL TRANSMISSION PATHWAYS

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Multidrug resistance (MDR) to anticancer drugs is, currently, a major contributor in cancer treatment failures. One of the most significant mechanisms in MDR phenotypes results from the overexpression of P-glycoprotein (P-gp, ABCB1). Therefore, a deeper understanding on P-gp substrate recognition and efflux-related signal transmission mechanism is crucial to develop more potent and selective compounds able to reverse MDR [1].

From a recently published murine P-gp structure, a stable human P-gp model was obtained by our group. Following, four systems with P-gp structures (embedded in a bilayer, water solvated and charge neutralized) and containing the most common mutations experimentally linked with changes in efflux or substrate recognition (G185V, G830V, F978A and ΔF335) were built and equilibrated by means of molecular dynamics (MD) simulations.

When compared with the human homology model, each P-gp variant revealed slight differences in the helices repacking of the transmembrane domains (TMDs), leading to further studies to assess how drug binding may affect the interaction of residues between TMDs and the nucleotide binding (NBD) domains, thought to mediate signal transmission and efflux-related conformational changes.

The G185V and G830V mutations are located at the substrate binding sites H and R respectively and the F978A and ΔF335 at the modulator binding site M. Molecules experimentally known to interact in each of the three sites were docked at the corresponding site and the best top-ranked docking pose were used as starting points for several short 20 ns MD runs (three replicates for each molecule).

For each docked molecule, the free energies of binding were calculated by g_mmpbsa with polar solvation energies corrected through an implicit membrane approach [2] and the herein obtained results could be correlated with

the experimentally determined changes in drug efflux for each specific mutation. Moreover, while in some cases the increase of drug affinity inside the pocket is the major determinant, in other cases variations in residue contacts at the NBD-TMD interface were the main reason for changes in drug efflux.

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The thermodynamics of the influenza fusion peptide orientation in membrane bilayers: a computational study

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One of the key players involved in the entry of the influenza virus in the host cells is the hemagglutinin protein. After the viral uptake by endocytosis and consequent lowering of the pH, this protein suffers major conformational changes. This enables the fusion of the viral envelope and host cell membrane, allowing the entry of the genetic material that will resume the infection process[1]. One particular region of the hemagglutinin protein, called the fusion peptide (FP), has a major role in the membrane fusion process. However, the molecular determinants behind the action of the FP in this process are yet to be elucidated, and experimental studies have not been able to determine the conformation of the FP inside the membrane. Recently, our group has published results that bring new insights on this subject [2]. In the present work, we expand our knowledge on the conformational and structural properties of the FP in a model membrane using enhanced sampling computational methods, such as well-tempered bias-exchange metadynamics simulations.

The results of this work will contribute with a better understanding of the mechanisms of membrane fusion that occur during influenza virus infection.

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Searching for novel antivirals targeting the non-structural protein 1 (NS1) of the Influenza virus

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Influenza A viruses are significant human respiratory pathogens that cause both seasonal, endemic infections and periodic and unpredictable pandemics of flu — altogether resulting in high morbidity and mortality. Influenza viruses fall under three types, A, B and C, of which type A is the most virulent for humans and causes the most severe symptoms. The Influenza A genome contains eight pieces of segmented negative-sense RNAs and encodes 11 proteins, including the non-structural protein 1 (NS1).

The increased lethality observed in influenza A strains has been partly attributed to the NS1 protein. NS1 plays an important role in virus replication and blockade of the host immune response, and it is therefore regarded as a potential therapeutic target. This multifunctional protein holds two distinctive structural domains, a RNA-binding Domain (RBD, with a molecular weight of 10.6 kDa) and an Effector Domain (ED, with a molecular weight of 18.5 kDa), which are separated by a linker region (LR). NS1 is implicated in virus replication and pathogenesis through the interaction of RBD with dsRNA, the interaction of ED with the p85β regulatory subunit of phosphoinositide 3-kinase (PI3K), and also via cleavage and polyadenylation of specificity factor 30 (CPSF30).

In order to discover compounds endowed with ability to inhibit NS1 activity, we have started a new research program combining computational and experimental approaches. First, and to allow the experimental validation of new inhibitors, we have independently cloned and characterized the RBD and ED domains of NS1. Here, we report results on the characterization of the cloned domains, based on Circular Dichroism (CD), Differential Scanning Calorimetry (DSC), Size-Exclusion Chromatography (SEC) and Nuclear Magnetic Resonance (NMR). Additionally, we have initiated computational work

for the identification of druggable hot spots in RBD-NS1, i.e. putative pockets on the protein surface capable of binding high-affinity drug-like molecules. We proceeded by creating pharmacophore models of the identified hot spots, which are now being used for virtual fragment and ligand screening.

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FRAGMENT BASED LEAD DISCOVERY BY MOLECULAR DYNAMICS SIMULATIONS

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Using our software for molecular dynamics run and trajectories analysis, we performed a proof-of-concept study with common force fields to develop inhibitors of Factor Xa. A selection of small molecule fragments from chemical libraries served as starting point. This effort required substantial methodological advances in our software to be completed. I will present the methods and the results obtained, a comparison with experimental results as well as some general conclusions for future work.

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Prediction ncRNA-mRNA interactions network from Herbaspirillum seropedicae SmR1

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The β-proteobacterium Herbaspirillum seropedicae SmR1 is a non-pathogenic, endophytic and diazotrophic bacterium that colonizes the intercellular spaces of several plants, including economically important crops such as sugarcane, maize, wheat and rice. The association of this microorganism promotes the better plant growth and its ability was attributed to nitrogen fixation and the biosynthesis of phytohormones. H. seropedicae SmR1 express nifHDK operon and other nif genes in microaerobic inside the plant. The genome contains noncoding RNAs (ncRNAs) with regulatory function (size between 50 and 500 nt) wich modulates diverse physiological responses and acts through RNA-RNA pairing bases and RNA-protein interactions. ncRNAs may regulate a dozen or more targets acting at different levels of gene expression. Analysis in eleven RNA-Seq experimental conditions confirmed the differential expression of ncRNAs and mRNAs in H. seropedicae SmR1. Only 15 out of 173 identified ncRNAs feature match in Rfam database. The objective of this study is to identify new ncRNAs with regulatory function in the H. seropedicae SmR1, review their possible targets in silico and propose a new computational approach to construct ncRNA-mRNA interaction networks. We identified the ncRNA trans-encoded Betaproteobacteria_toxic_sRNA, crcB riboswitch, sucA riboswitch and ykkC-yxkD riboswitch. The ncRNA-mRNA interaction networks were inferred using the algorithms: Algorithm for the Reconstruction of Accurate Cellular Network (ARACNE), Maximum Relevance Minimum Redundancy (MRNET), Context Likelihood (CLR), Spearman Correlation and Sequential Floating Forward Selection (SFFS). Similar interactions present in at least three of them were selected for creating a consensus network. The network inferred with CLR has a

greater number of equal relationship with MRNET and then with CLR, Correlation and SFFS. The Hs_noco_790 sRNA with size of 464 nt showed putative interactions with 28 genes participants nif cluster including the nifA gene, the major transcriptional regulator of biological nitrogen fixation in H. seropedicae Smr1. This approach will help us to identify possible targets several ncRNA of H. seropedicae SmR1 that don't have yet defined function.

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Rational design of a (S)-selective-transaminase for asymmetric synthesis of (1S)-1-(1,1'-biphenyl-2-yl)ethanamine

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Amine transaminases offer an environmentally sustainable synthesis route for the production of pure chiral amines. However, their catalytic efficiency towards bulky ketone substrates is greatly limited by steric hindrance and therefore presents a great challenge for industrial synthetic applications. Hereby we report an example of rational transaminase enzyme design to help alleviate these challenges. Starting from the *Vibrio fluvialis* amine transaminase that has no detectable catalytic activity towards the bulky aromatic ketone 2-acetylbiphenyl, we employed a rational design strategy combining *in silico* and *in vitro* studies to engineer the transaminase enzyme with a minimal number of mutations, achieving an high catalytic activity and high enantioselectivity. We found that by introducing two mutations W57G/R415A detectable enzyme activity was achieved. The rationally designed variant, W57F/R88H/V153S/K163F/I259M/R415A/V422A, showed an improvement in reaction rate by > 1716-fold towards the bulky ketone under study, producing the corresponding enantiomeric pure (S)-amine (ee value of > 99%).

ITRANS database - sorting structural motifs in cellular transporters

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Cells interact with their surroundings via molecular transporters located in the cellular membrane. These transporters are fundamental for the uptake of nutrients and removal of toxic compounds and its malfunction has been correlated with different diseases.

Over the last five decades great advances have been taken in understanding the mechanism behind ions and solutes transport. A key tool that triggered the understanding at atomic level of this mechanism is the determination of high resolution structures of membrane proteins.

Currently, over 2000 high resolution structures of molecular transporters are deposited in the PDB databank and available to researchers. As this number increases, the presence of structure motifs and patterns that define the mechanism, dynamics and regulation of ion or solute transporters starts to emerge. In order to organize and fetch this information several tools are available. Nevertheless, the systematization of the structural information based on tertiary and quaternary structural motifs and its relation with ion transport is still time consuming and not optimized.

In this work we present the ITRANS database. ITRANS is a relational database focusing on ion or solute transporters for which high resolution structures were experimentally obtained. The database contains information on: classification, structural repeats topology and function of cellular transporters, providing the community a novel tool to study the mechanism of these proteins.

The database spans information of different organisms and different families of ion and solute transporters, allowing the user to organize molecular transporters using different structural motifs and biophysical properties.

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Structural and Functional insights into a dodecameric machine

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RuvBL1 and RuvBL2 are AAA+ ATPases essential for many cellular activities such as chromatin remodelling, transcriptional regulation and DNA damage. RuvBL1 and RuvBL2 form hexamers or dodecamers and are also overexpressed in different types of cancer interacting with major oncogenic factors, such as b-catenin and c-Myc regulating their function. Each monomer contains 3 domains, I and III involved in ATP binding and hydrolysis, and domain II which is unique among AAA+ proteins and absent in the bacteria homologue. Related to their diverse cellular functions, they are part of several complexes either together or alone, and our work focuses on the R2TP complex where the RuvBLs interact with key players required for box C/D snoRNP complex assembly which in turn is involved in Ribosome biogenesis. It was later found to participate in other cellular roles including apoptosis, PIKK signalling, and RNA polymerase II assembly.

Our main players in the complex, RuvBL1 and RuvBL2, are homologous to each other but can exert antagonistic functions. Our objective is to understand the triggering of these opposing roles by unravelling the structural determinants of RuvBL1/RuvBL2 in the context of their function in R2TP complex providing new insights on how RuvBL1 and RuvBL2 may be regulated towards diverse cellular activities. We have determined the crystallographic structure of ΔII_RuvBL1:2 heterohexamer, as well as the full length homohexamer RuvBL1, and we will use this experience to assess relevant protein:protein interactions of RuvBLs with key players of the R2TP complex.

Computational studies addressed to Histidine decarboxylase

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Mammalian histidine decarboxylase (mHDC) is an enzyme that requires pyridoxal-5'-phosphate (PLP) as a cofactor [1-3]. mHDC belongs to the group II of PLP-dependent decarboxylases, and catalyses the L-histidine decarboxylation from which results histamine. Histamine is important in several physiological events such as immune response, gastric system modulation, and also as a neurotransmitter. Unfortunately, several diseases are related to the deregulation of histamine production, i.e., atopic dermatitis, allergies, and cancer.

The main goal of this project was the characterization of the catalytic mechanism, with atomistic detail, of mHDC through computational means.

This study used the recent X-ray structure of mHDC (PDB: 4E1O) [4] and an ONIOM QM/MM methodology. The MM part was always considered under molecular mechanics approach, whereas the QM part was calculated using DFT method B3LYP/6-31G(d) for geometry optimizations and M06/6-311++G(3df,2pd) for single point-energy calculations.

This work confirmed a two-step type of catalytic mechanism. The first step is the rate-limiting one (free activation energy = 17.6 kcal/mol; free reaction energy = 13.7 kcal/mol), and involves the decarboxylation of the substrate and, the formation of a stable carbanion (quinonoid intermediate). In the second step, the quinonoid intermediated is protonated by a tyrosine (Tyr-334B) residue from which results histamine (the final product). In contrast with the first step, this one is almost spontaneous(free activation energy = 1.9 kcal/mol) and, highly exergonic step (free reaction energy = −33.8 kcal/mol).

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Molecular Dynamics studies on the ABCG transporter family

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ATP binding cassete (ABC) transporters are a large family of membrane transporters. In eukariotic cells, 48 members have been identified so far, acting mainly as export pumps in order to lower the intracellular concentration of endogenous metabolites or xenobiotics (1,2).

The recent publication of the ABCG5/G8 structure (3) revealed a new transmembrane organization thought to be characteristic of the ABCG family. Thus, and despite the low sequence identity between ABCG5/G8 and ABCG2, this new crystallographic structure may become a template for studying the ABCG56/G8 heterodimer or, by homology modeling, the ABCG2 homodimer. Therefore, the behavior of both ABCG5/G8 and ABCG2 was studied by means of molecular dynamics (MD) simulations.

Regarding the ABCG5/G8, as the crystal structure was incomplete, de novo modeling of the missing sequences was performed and added to the transporter. The transporter was then inserted in a DMPC:CHOL membrane, water soaked and neutralized. Following, the system was energy minimized and its temperature (303 K) and pressure (1 bar) equilibrated. After membrane equilibration, all de novo modeled segments were equilibrated by removing their spatial restraints, thus allowing a better adjustment of these domains to the transporter. Finally, a production MD run was performed during 200 ns.

For the ABCG2 transporter, a homology model was generated from ABCG5. As some ABCG2 α-helical domains were missing or are longer than those in ABCG5, they were reconstructed as fully helical domains. This complete homology model showed a good agreement with a previously published three-dimensional structure of ABCG2 by cryo-electron microscopy (4) . Finally, the previously described MD protocol for ABCG5/G8 was applied and a 200 ns production run was obtained.

In both cases, the structural integrity of the transporters was thoroughly characterized. The structural features of both the ABCG5/G8 and ABCG2 systems improved (when compared with the initial models), already providing valuable information on the structural dynamics of the transporters from the

ABCG family but will also allow new structure-based studies on drug efflux by both efflux pumps.

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COULD AN OCTAHEDRAL METAL COMPLEX FIT INSIDE A G-QUADRUPLEX? THE JOURNEY OF [Mo(η3-C3H5)Br(CO)2(phen)] METAL COMPLEX THROUGH DNA'S MIDDLE-EARTH

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Several flat ligands, alone or in coordination complexes (CCs), are active against tumor cells and can be used in chemotherapy. This activity is related to their capacity to interact with G-quadruplexes, stabilizing these DNA tertiary structures.[1,2] This stabilization inhibits the activity of telomerase in cells,[3] which is responsible for maintaining the length of telomeres, and is involved in around 85% of all cancers. It causes apoptosis of cancer cells[4,5] and, since telomerase is overexpressed in the majority of tumor cells and in relative few somatic cells,[5] G-quadruplexes are recognized as a potential cancer specific target. In this work we studied the interaction of [Mo(η3-C3H5)Br(CO)2(phen)], which was found to be cytotoxic against tumoral cells,[6] with the G-quadruplex obtained from the 2jwq PDB structure by means of docking, semiempirical methods including dispersion (PM6-DH2) and QM/MM methods. PM6-DH2 method gives good results since it reproduces the crystal 2jwq PDB structure, whereas QM/MM calculations with M11 and UFF yield a distorted structure. Moreover, a surprising result is obtained when the interaction of [Mo(η3C3H5)Br(CO)2(phen)] with the G-quadruplex is studied using PM6-DH2 and the metal complex is found totally inside the G-quadruplex. The next most stable structures interact with the G-quadruplex by means intercalation.

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The importance of unstructured termini in the aggregation cascade of beta-2-microglobulin: insights from molecular simulations of D76N mutant

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The identification of intermediate states for folding and aggregation is important, both from a fundamental standpoint and for the design of new therapies targeted at conformational disorders. Here, we use the single point mutant (D76N) of β2m, the causing agent of a hereditary systemic amyloidosis affecting visceral organs, as a model system to study the aggregation mechanism of β2m using molecular simulations. We present our predictions on the early molecular events triggering the amyloid cascade for the D76N mutant. Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C-terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2. Additionally, Monte Carlo docking simulations suggest that the I2 is considerably more amyloidogenic than I1. These simulations support an essential role of the DE-loop and of the C-terminus in the dimerization of both intermediates. The relevance of the C-terminus is higher at the acidic pHs 5.2 and 6.2. Additionally, the AB-loop becomes an important player in the dimerization of both I1 and I2 at pH 6.2. A key specific finding of I2 dimerization is the relevance of Tyr 10 at the end of strand A at pH 6.2. These predictions rationalize experimental results that support the involvement of the AB-loop and DE-loop, particularly of Glu 16, Lys 19, Phe 56, Trp 60 and Tyr 63, in amyloidogenesis in the wild-type and other model systems of β2m.

The effect of mutations on the structure and activity of the influenza fusion peptide analysed by bias-exchange metadynamics simulations and spectroscopic methods

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The emergence of an influenza pandemic is one of the biggest health threats of our time and, therefore, there is an urgent need to develop vaccines and drugs against a broad spectrum of influenza viruses (IV). A promising strategy to combat IV is to inactivate the fusion process between the viral and host membranes, which is mediated by the surface protein hemagglutinin (HA). During this process, the N-terminal region of HA, known as fusion peptide (FP), inserts into the host membrane. Although it has been shown that the FP plays a crucial role in the fusion process, the molecular effect of the peptide remains unclear

In order the shed light into this problem, we used a combination of state-of-the-art experimental and simulation techniques to analyse the WT influenza FP and four mutants.[1] Fluorescence based methods were used to analyse the partition coefficient of the WT and mutant peptides in model membranes, and their ability to promote lipid-mixing was analysed using a (FRET)-based assay. To rationalize the results obtained in these experiments, we analysed the energy landscape of the peptides by performing bias-exchange metadynamics (BE-META) simulations. This allowed us to characterize the conformational properties of the WT peptide in a model membrane and understand how this structure is affected by the mutations studied. This study also elucidated the factors that explain the reduced activity of the mutants, which contributes to a better understanding of the role of the influenza FP in the fusion process.

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Unraveling the Nitrate Reductase reaction mechanism

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Nitrate Reductases (NR) catalyse the reduction of nitrate to nitrite. Due to the different cell location, structure and function they have been grouped into cytoplasmic (Nas), membrane bound (Nar) and periplasmic (Nap) nitrate reductases, all belonging to the dimethyl sulfoxide reductase (DMSO) family of molybdopterin enzymes.

The monomeric NapA from Desulfovibrio desulfuricans was the first crystal structure of a NR to be published [1]. By then it was established that in the active site of NapA was the molybdopterin guanosine dinucleotide cofactor (MGD) coordinating molybdenum with two cis-dithioline groups, as well as a sulfur from cysteine side chain and apparently a water/hydroxo ligand.

New Nap crystal structures demonstrated that the sixth ligand is in fact another sulfur atom [2,3], contrary to the previous believed water/hydroxo ligand. The sulfur ligand seems to have a partial disulfide bond with the same cysteine thiolate group that coordinates molybenum, blocking nitrate from interacting directly with the molybdenum atom unless some rearrangement occurs during catalyse [2]. Nap reaction mechanism has been rethought since then, and the main options discussed concern substrate binding directly to the molybdenum atom, or indirectly, through the sulfido ligand.

Independent studies evolving computational chemistry and modeling, demonstrate that a first coordination-sphere type mechanism with the rearrangement of the coordinating thiolate group is the most probable mechanism [4-7].

Through crystallization with various important molecules as the substrate, homologous and inhibitors; Isothermal titration calorimetry; and possibly revolutionary techniques such as time-resolved crystallography and X-Ray free electron laser, we intend to study and prove the true Nap reaction mechanism.

This poster will present how computational chemistry and modeling contributed [4-7] to the present knowledge of the Nap reaction mechanism [8], and how the proposed techniques can provide more valuable insight.

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Structural mechanism of HER2-antibodies complexes by molecular dynamics studies

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Human Epidermal Growth Factor Receptor 2 (HER2) is, among the EGFR family, the most relevant target from a biological perspective. Its overexpression on tumor cells, namely in breast cancer, is associated with tumor aggressiveness and an increased probability for recurrent disease. The anti-HER2 monoclonal antibody trastuzumab (Herceptin®) or specific tyrosine kinase inhibitors are used to treat HER2 positive tumors.

In this work, we have employed computational modelling and Molecular Dynamic (MD) simulations to attain a deeper understanding of the interaction of specific anti-HER2 antibodies and HER2. The dynamic behavior of HER2 receptor in complex with three antibodies (F0178, A21 and scFv from Trastuzumab) was investigated by two replicas of 0.5 μs MD simulations for each system as well as for the individual ones. A variety of structural characteristics ranging from pairwise interactions formation to covariance analyses are currently being employed. Our aim is to understand how to modify and control the formation of these macromolecular systems, which would be fundamental to the development of new approaches on anti-HER2 therapies.

Coupling between protonation and conformation in cytochrome c oxidase: Insights from Constant-pH MD simulations

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Cytochrome c oxidases (CcOs) are the terminal enzymes of the respiratory chain in mitochondria and most bacteria. These enzymes reduce dioxygen (O2) to water and, simultaneously, generate a transmembrane electrochemical proton gradient. Despite their importance in the aerobic metabolism and the large amount of structural and biochemical data available for the A1-type CcO family, there is still no consensually accepted description of the molecular mechanisms operating in this protein. A substantial number of questions about the CcO's working mechanism remains to be answered, including how the protonation behavior of some key residues is modulated during a reduction cycle and how is the conformation of the protein affected by protonation.

The main objective of this work was to study the protonation-conformation coupling in CcOs and identify the molecular factors that control the protonation state of some key residues. In order to directly capture the interplay between protonation and conformational effects, we have performed constant-pH MD simulations of an A1-type CcO inserted into a lipid bilayer in two redox states (oxidized and reduced) at physiological pH. From the simulations, we were able to identify several groups with unusual titration behavior that are highly dependent on the protein redox state, including the A-propionate from heme a and the D-propionate from heme a3, two key groups possible involved in proton pumping. The protonation state of these two groups is heavily influenced by subtle conformational changes in the protein (notably of R481I and R482I) and by small changes in the hydrogen bond network.

Bioinformatics and NGS as tools to study regulation of gene expression

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In the last years with the advancement of sequencing techniques several new bioinformatic tools were developed to deal with the analysis of vast amounts of data. This lead to new ways of look and interpret data and consequently required different methods and approaches to be integrated. In this work, we used RNA-Seq data to study RNA degradation focusing on the role of exoribonucleases and small RNAs in the control of gene expression. In Escherichia coli, there are three main exoribonucleases (RNase II, RNase R and PNPase) involved in RNA degradation and there are 68 identified small RNAs with many more predicted. Most of the sRNAs control the degradation of several different mRNA targets making complex regulatory networks that are tightly regulated.

We sequenced the total RNA (RNA-Seq) from wild-type cells and from mutants for each of the exoribonucleases (Δrnb, Δrnr and Δpnp) in exponential and stationary phase. We then compared each of the mutant transcriptomes with the wild-type to determine the global effects of the deletion of exoribonucleases. Several bioinformatic approaches were used to analyse these data sets. We started by using a global approach to identify all the transcripts that were affected by the deletion of the different exoribonucleases followed by a functional categorization approach to determine specific pathways affected. The small RNAs were specifically looked and regulatory networks were constructed to identify potential mRNA targets for several small RNAs with unknown function.

The three exoribonucleases affected transcripts from several distinct functional categories. Interestingly, in exponential phase all the exoribonucleases affected transcripts related to motility and biofilm formation and further experimental validation confirmed that deletion of the exoribonucleases affect the cell motility and their ability to form biofilm. In stationary phase, all

exoribonucleases seem to affect transcripts involved in oxidation-reduction processes and transcripts related to stress responses. Among the transcripts found to be affected by the deletion of the exoribonucleases there are several small RNAs that are known to regulate stress responses.

This work shows that the integration of several bioinformatic approaches to analyse RNA-Seq data can greatly improve our knowledge of regulatory mechanisms in the cell.

Co-evolution role on binding hot-spot prediction

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Co-evolution algorithms and features have recently taken a relevant spot on the scene of protein structure determination as well as on protein-protein interaction determination. These methods can generally extract information on the conservation of residues between pairings, or groupings of residues, allowing for the identification of active sites, Hot-Spots (HS), or, more widely, functional information on the protein. In this work, we aimed the assessment and implementation of a prediction algorithm for binding HS that combines the already published features[1] with known co-evolution metrics. In particular, relevant algorithms such as EVFold[2], CoeViz[3], InterEV[4] were used and subjected to Machine-Learning techniques. The accuracy attained with these new features was compared with previous methods.

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Does the Ionic Strength Treatment Influence the Protonation/Conformational Space of Charged Biomolecular Systems?

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We have recently shown that the most common approach to deal with charged membrane systems, namely the full system neutralization, leads to excessively ordered lipid bilayers in a 30% DMPA/DMPC system. However, when a significantly smaller number of ions, estimated from Poisson-Boltzmann calculations at a defined ionic strength value, is used, we were able to reproduce the correct isothermal pH dependent lipid phase transition. An important conclusion of these findings is that, in charged membrane systems, full neutralization only takes place at several nanometers away from the lipid interface. Therefore, we are now raising the question of whether this issue of estimating and using the correct number of counter-ions in MD simulations is also determinant to correctly model charged globular systems. In these systems, like proteins, dendrimers, etc., it is possible to become significantly charged, depending on pH, and the amount of counter-ions added could influence its conformation space and protonation profile. In this work, we used our Constant-pH MD method to study the conformational space and titration profile of polyamidoamine (PAMAM) dendrimers (2nd generation) with two different ionic strength treatments: an implicit approach (with no explicit counter-ions) using the generalized reaction field; and PME with explicit ions to approximate system neutrality. The main question now is whether the most common approach used by the scientific community (PME/neutralization) is able to correctly describe highly charged globular systems. We already know the answer and will share it with you in our poster.

Unveiling the molecular determinants responsible for NAD(P)(H) cofactor specificity using enzyme structural information

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In this post-genomic era, gene homology annotations have become the foundation of systems biology. However, errors spread easily when functional annotation is not performed carefully due to overly unconstrained homology metrics [1,2].

As metabolic model reconstructions become a relevant tool for performing fundamental studies and bioprocess design, the impact of accurate enzymatic function assignments becomes evident [3].

The uncertainty of the usage of NADP(H) or NAD(H) as co-factors [4], even in well performed annotations, has a major impact in metabolic engineering applications, severely affecting Genome-scale metabolic model reconstruction due to the potential insertion of misleading reactions.

In this work, we unveiled the molecular determinants for cofactor specificity, using enzyme structural information. In order to do so, we created a representative dataset of all enzymes present in the PDB with NAD(P)(H) as cofactors and measured the occurrence of every aminoacid residue at a distance of 3.5 Angstrom[5] of all cofactor atoms. This allowed us to create a matrix with the total number of aminoacid residues at interacting distances from all cofactor atoms, for all structures.

After analyzing the matrix, we identified the residues that had a significantly higher number of contacts with the cofactors, and in which atoms this occurred.

With the intent of applying these findings in the unveiling of cofactor specificity for enzymes that are not structurally characterized experimentally we successfully replicated these findings using machine learning algorithms.

In future work we will apply the results and machine learning models obtained in order to assign cofactor specificity to enzymes with uncertain cofactor specificity.

These results may represent an important development in systems biology by allowing the reduction of annotation errors and the implementation

of erroneous or redundant reactions in GEM models, improving the overall performance of metabolic simulations.

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Potential targets against HIV infections — Viral surface glycoproteins

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HIV infection is a major threat to public health [1] and it has been marked by a long and elusive search for an effective HIV vaccine. There are two types of viruses, HIV-1 and HIV-2. While HIV-1 is responsible for a pandemic worldwide infection, HIV-2 causes localized epidemics mainly in West Africa, India, Brazil and in Europe (mainly in Portugal and France). There are several differences between them visible in the form how the infection evolves upon contamination with the respectively virus which can suggest a more effective immune response against HIV-2. Approved drugs target protease, reverse transcriptase, integrase, transmembrane envelope glycoprotein (gp) and the CCR5 co-receptor of HIV. Until now, no "cure" is available and drug efficacy is reduced with time and that has encouraged alternatives. HIV establishes a permanent link between the cell and the host and it integrates the genomic DNA like a latent provirus. New targets under investigation include the viral envelope gp of viral entry into cells. Computational tools allow us to rationalize the investigation and screen more targets to develop potential vaccine to the virus. [2,3]

Our goal relies on the study and modulation of the most important envelope surface glycoproteins. We are currently developing and optimizing an homology model of gp125 (HIV-2) due to the lack of complete crystallography structure. The recent crystallographic structure misses the variable areas, kwon as V1, V2, V3, V4 and V5, with a major role in terms of interactions with the receptors and co-receptors to invade the cells and spread the infection. [4] Using MOE and GROMACS software, structure (homology model), structural interactions and dynamic behaviour is being explored. Molecular dynamics simulations are being performed with Gromacs program package. [5] Preliminary results will be presented and discussed.

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Improved parameters in MM-PBSA calculations applied to computational alanine scanning mutagenesis

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The knowledge of how proteins make stable complexes enables the development of inhibitors to preclude protein-protein (P:P) binding. The identification of the specific interfacial residues that contribute the most for protein binding, denominated as hot-spots, is thus critical. Here we refine a computational alanine scanning mutagenesis protocol, based on a residue-dependent dielectric constant version of the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method. To this purpose, we have used a large data set of structurally diverse P:P complexes to re-define the residue-dependent dielectric constants used in the calculation of binding free energies. The accuracy of the method was validated through comparison with experimental data, considering the per-residue P:P binding free energy (ΔΔGbinding) differences upon alanine mutation. Different protocols were tested, including a geometry optimization protocol and three molecular dynamics (MD) protocols: 1) one using explicit water molecules, 2) another with an implicit solvation model, and 3) a third where we have carried out an accelerated MD with explicit water molecules. Using a set of protein dielectric constants (within the range of 1 to 20) we showed that the dielectric constants of 7 for non-polar and polar residues and 11 for charged residues (and histidine) provide optimal ΔΔGbinding predictions. An overall mean unsigned error (MUE) of 1.4 kcal.mol^{-1} relative to experiment was achieved in 210 mutations only with geometry optimization, which was further reduced with MD simulations (MUE of 1.1 kcal.mol^{-1} for the explicit solvent MD). This recalibrated method allows for a better computational identification of hot-spots, avoiding expensive and time-consuming experiments or thermodynamic integration/ free energy perturbation/ uBAR calculations, and will hopefully help new drug discovery campaigns in their quest of searching spots of interest for binding small drug-like molecules at P:P interfaces.

Computational studies addressed to the catalytic mechanism of the alpha subunit of the bifunctional enzyme Tryptophan Synthase

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Tryptophan Synthase (TS) is a bifunctional enzyme that catalyzes the last two steps in the synthesis of tryptophan (trp). Each reaction is catalyzed in different active sites that are located in separate α and β subunits. The active site of the α-subunit catalyzes the formation of indole and gliceraldeyde-3-phosphate (G3P) from indole 3- glycerolphosphate (IGP). Indole is then transported trough a 25Å physical tunnel to the active site of the β-subunit where it is added to a molecule of acrylate, derived from serine, to produce trp, in a PLP dependent reaction [1].

In this work we studied the reaction that takes place in the α-subunit active site using computational means and QM/MM hybrid methodologies [2]. The results have shown that the reaction occurs in a stepwise general acid-base mechanism. The 1st step requires the participation of a water molecule that protonates C3 of the indole ring and receives a proton from αGlu49 yielding the indolenine tautomer. In the 2nd step the αGlu49 carboxylate deprotonates the water molecule that subsequently deprotonates the glycerolyl hydroxyl of IGP, triggering the C–C bond cleavage to give indole and G3P.

The results go in line with previous experimental studies who demonstrated that a TS mutant in which αGlu49 is substituted by aspartic acid is totally devoid of α activity [3], while a mutant form with glutamic acid at position 60 instead of an aspartate has partial activity [4].

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pKa calculations in the heme pocket of KatG during its catalytic cycle

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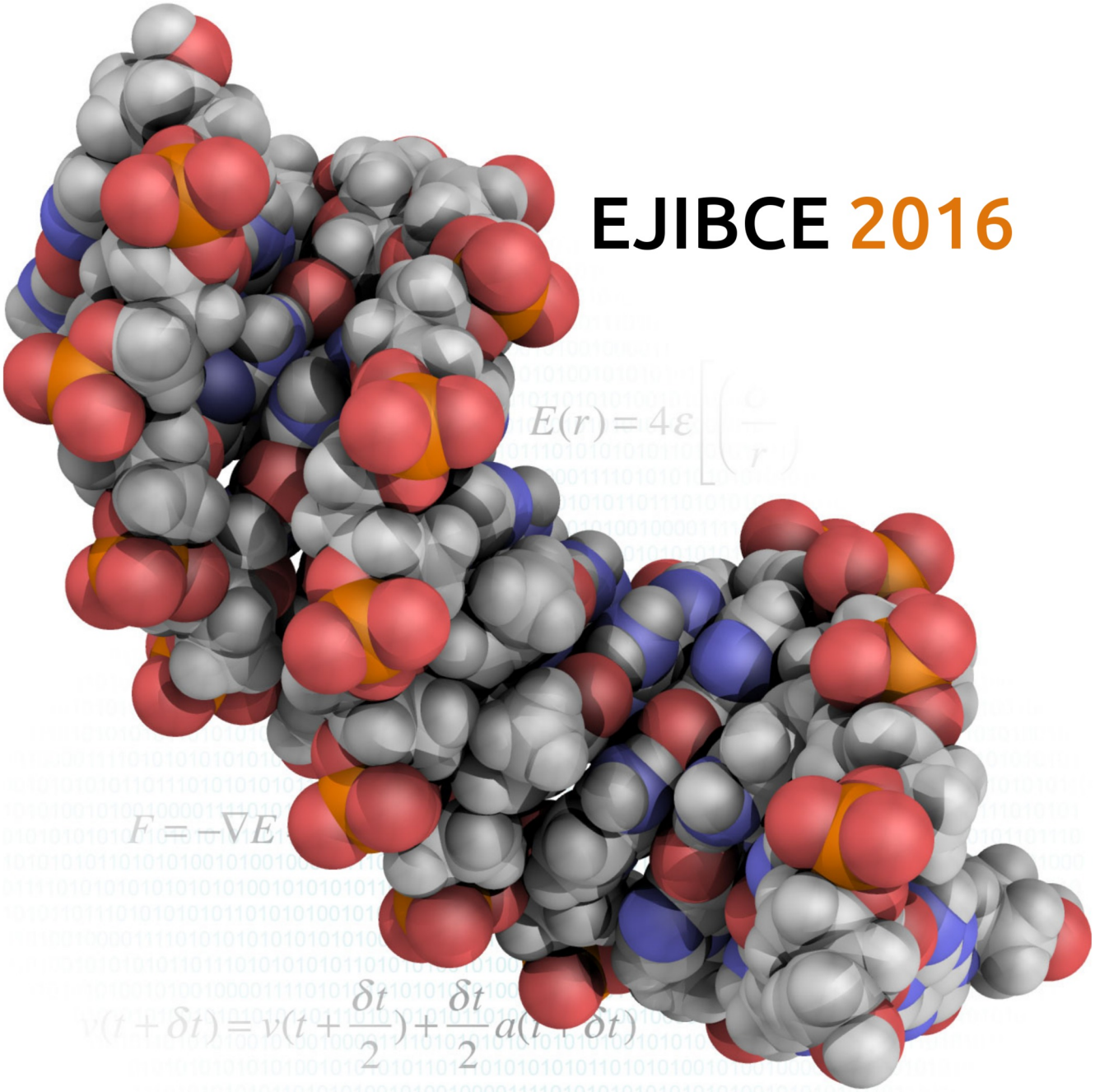
KatG proteins are a class of bifunctional heme-proteins that exhibit both catalase and peroxidase activities. These proteins assemble as functional homodimers, with each monomer being composed by two different domains. In the N-terminal domain one can find a heme-b prosthethic group where electron catalysis occurs, and an adduct composed by three covalently bonded residues (Trp 107, Tyr 229, and Met 255) known to be involved in catalase but not in peroxidase activity of the protein. These overlapping reaction mechanisms, although not yet fully understood, are key regulated not only by the adduct itself but also by its association with Arg 426 and a reversible perhydroxy modification on Trp 111 indole.

By using different crystallographic structures corresponding to different catalytic stages of the KatG protein and also the presence and absence of molecular oxygen and acetate, we have evaluated changes in the free energies of protonation of the residues found at the catalytic region of the protein. The use of Monte Carlo simulations of protonation states based on Poisson-Boltzmann free energies, allowed us to compute individual and pairwise terms needed to obtain these changes in free energies of protonation and ultimately determine pKa values for the protonable residues found at the catalytic center of KatG. His 112 was found to be quite sensitive to pH and to the electrostatic potential of the environment, and by using it as a probe we were able to quantify these changes along the catalytic cycle of KatG.

We acknowledge financial support from FCT through project UID/MULTI/00612/2013.



EJIBCE 2016

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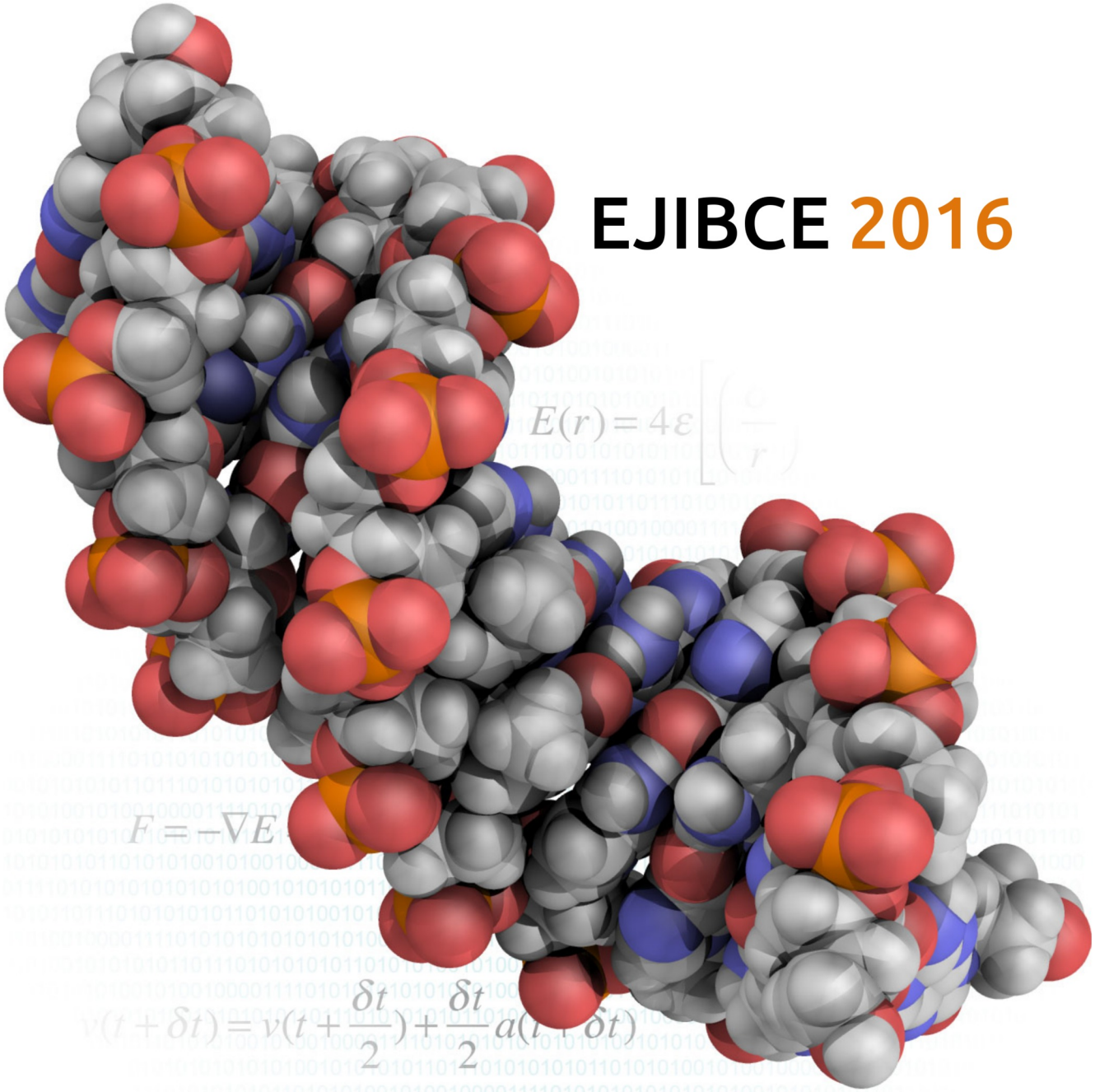

EJIBCE 2016

General Info

EJIBCE 2016

EJIBCE 2016 will take place in **ITQB NOVA** on **21st October**.

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ejibce2016@itqb.unl.pt

ITQB NOVA Location

ITQB NOVA is located in **Oeiras**, approximately 15 Km from Lisbon. There are two direct road connections between Oeiras and Lisbon: one by motorway, Autoestrada de Cascais — A5, and another by coastline road, Av. Marginal. The main building is located within the campus of the National Agricultural Station[1] (EAN). The main entrance of the EAN is on Avenida da República in Nova Oeiras.

Address

ITQB NOVA
Av. da República
2780-157 OEIRAS

Coordinates

latitude: 38° 41' 38" (38.694 N)
longitude: 9° 19' 7" (-9.318 W)

[1] Also known as Quinta do Marquês.

Arriving by Car

To reach Oeiras from Lisbon you have to take Autoestrada de Cascais (**A5**) and leave the road at the Carcavelos access. Then, follow the signs to Nova Oeiras.

If you come from the North of Portugal, you do not need to pass through Lisbon to reach Oeiras. You leave Autoestrada do Norte in Alverca following the signs to CREL. Follow CREL almost until the end (after the toll at Queluz). Then, you can follow through Autoestrada de Cascais (A5). You take a similar path if you come from Autoestrada do Oeste.

ITQB can also be reached by car using the seaside **Av. Marginal**.

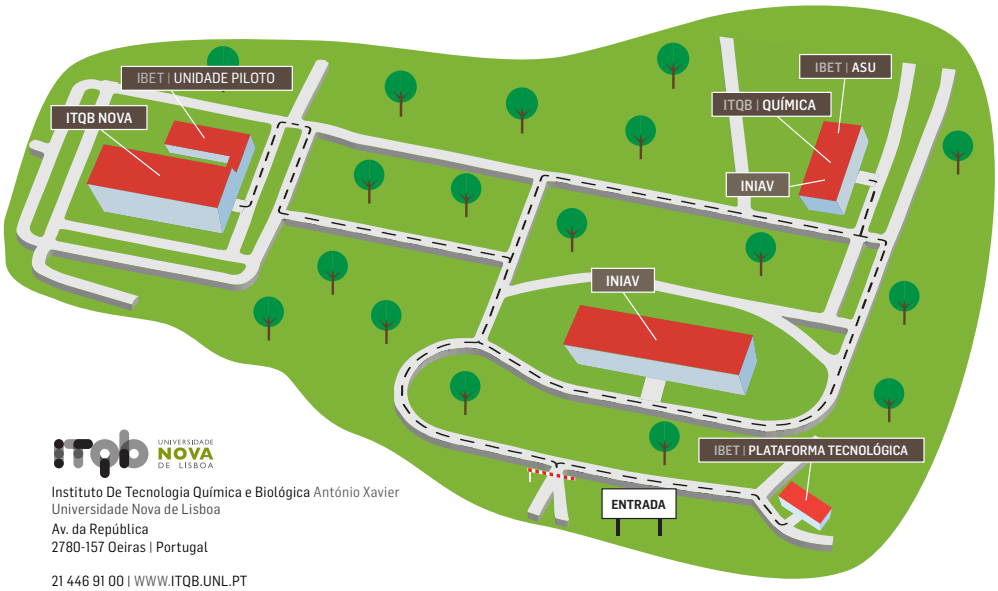
Coming from Lisbon from Av. Marginal take the right turn at Oeiras near the fast food chain. At the first roundabout turn left cross the small bridge and turn right going under the train bridge. Keep in that direction. At the red lights turn left and drive for 300 m. On the first turn go right till the end of the road reaching Av. da República. Then turn left and 100 m after turn right into the main gate of "Estação Agronómica Nacional (EAN)".

Arriving by Train

There are very frequent, fast and reliable train connections from Lisbon to Oeiras (13 km).

The train station in Lisbon is called **Cais do Sodré** and the trip should take less then 25 minutes to Oeiras (ticket costs about €1.85). From Oeiras train station a 12 minutes walk in a straight line or 5 minutes by taxi (about €5) to ITQB.

Inside EAN



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