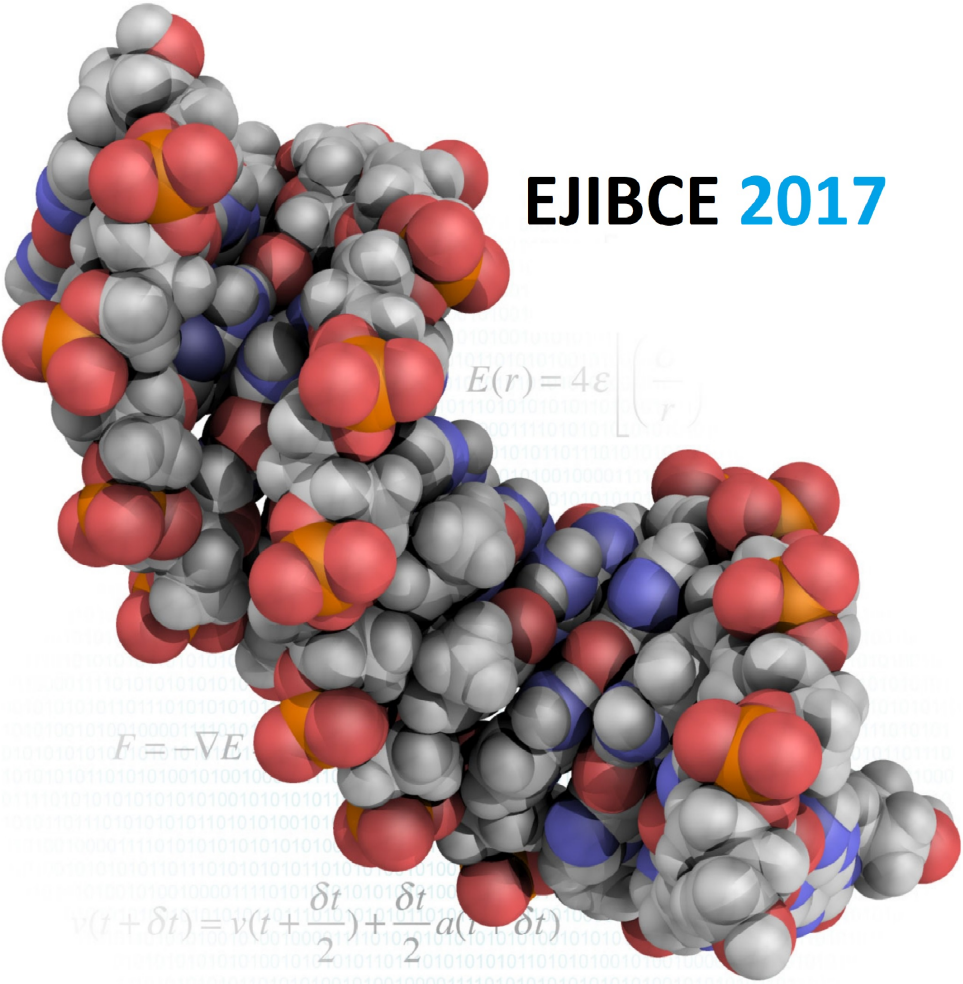
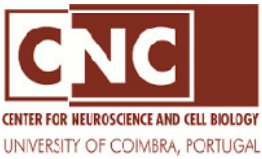


EJIBCE 2017

V Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Departamento de Física, Coimbra, 22-12-2017

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 2017, 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://ejibce.github.io/`

Comité Científico:

Ozge Sensoy, Medipol (Istambul)

Giulia Morria, ICRM (Milan)

Marcel Swart, IQCC (Girona)

Organizadores:

Irina Moreira, CNC

Cândida Silva, CQC

Carlos Simões, BMSIM^2

António Preto, CNC

Jośe Almeida, EBI

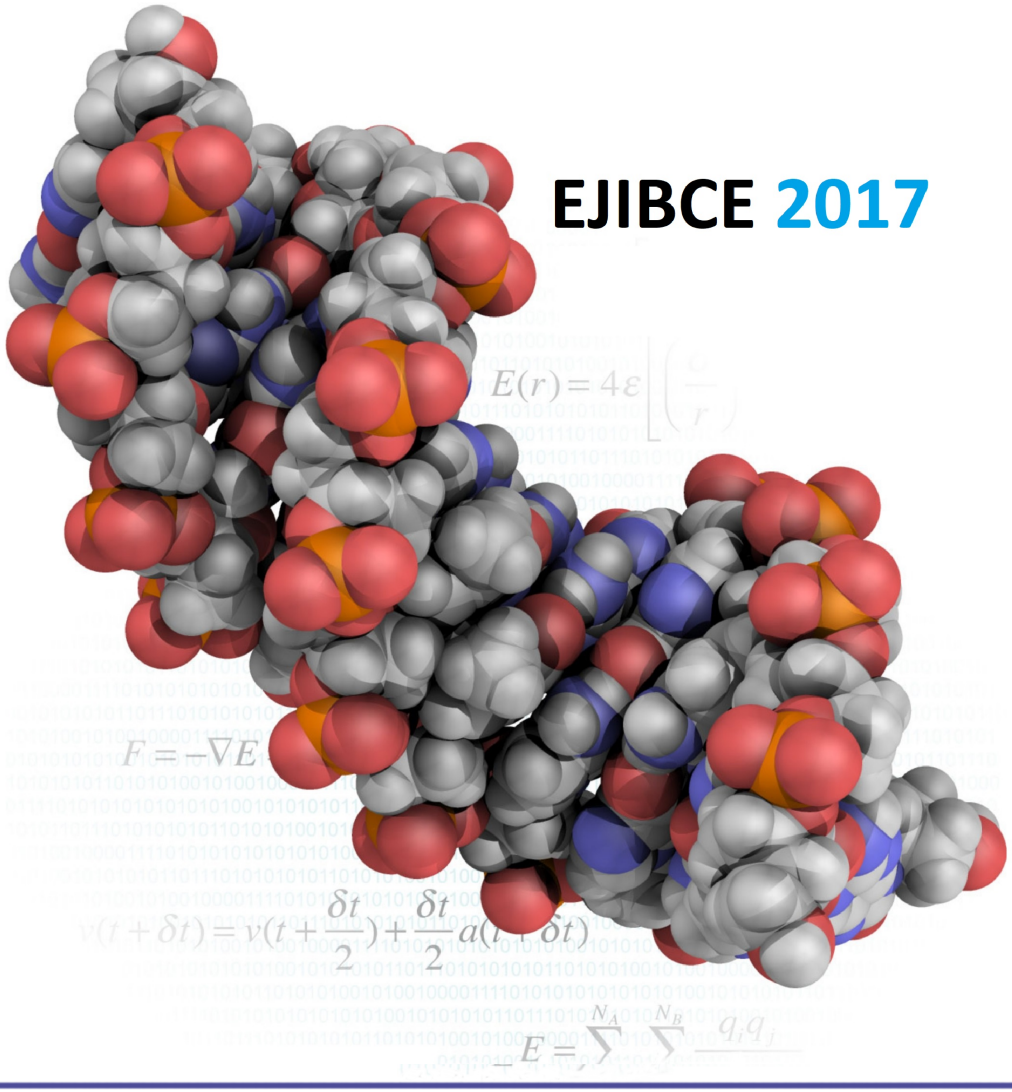
Carlos Barreto, CNC

Alexandra Carvalho, CNC

Rita Melo, CNC

Pedro Filipe, UC

Contents



EJIBCE 2017

Program

Friday, December 22nd

9:00–9:30 Registration

90:30–10:10 **João Ribeiro**
Beckman Institute, University of Illinois
QwikMD - easy and fast molecular dynamics simulations with VMD and NAMD 7

10:10–10:30 **Henrique Fernandes**
University of Oporto, Portugal
Development of computational tools to enhance the study of catalytic mechanisms 15

10:30–11:00 Coffee Break

11:00–11:40 **João M. Damas**
Acellera, Spain
Optimizing Proteins and Ligands for Computerized Drug Discovery 9

11:40–12:00 **Nuno Alves**
Universidade de Lisboa, Portugal
Predicting HIV-1 resistance to protease inhibitors: A new structure-based algorithm exploring binding-site Molecular Interactions Field dissimilarities 17

12:00–12:20 **Andreia Cunha**
University of Coimbra, Portugal
Fragment-based discovery of Influenza A NS1 inhibitors: in silico screening and validation by NMR 19

12:20–12:40 **Beatriz Bueschbell**
University of Bonn, Germany
Structural-based drug design: Dopamine D2 Receptor 21

12:40–14:30 Lunch

14:30–15:10 **António Ribeiro**
European Bioinformatics Institute, UK
Using the Mechanism and Catalytic Site Atlas (M-CSA) to understand enzyme function and evolution 10

15:10–15:30 **Rafael Nunes**
University of Lisbon, Portugal
T4 lysozyme/halobenzene: a test system for modeling biomolecular halogen bonds 24

15:30–15:50 **Tomás Silva**
University of Lisbon, Portugal
In silico studies on the pH induced membrane insertion of pHLIP peptides 26

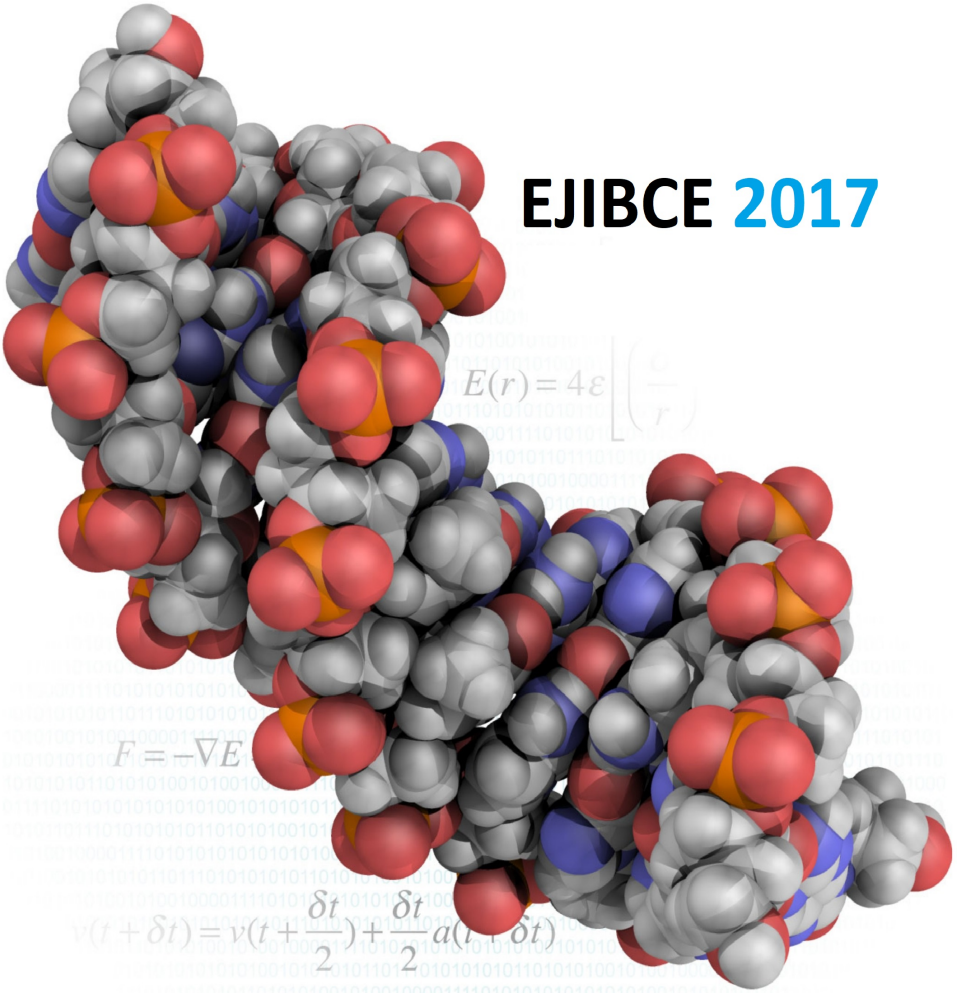
15:50–16:20 Coffee Break

16:20–16:40 **José Almeida**
European Bioinformatics Institute, UK
Using big-data to understand the protein interface landscape 28

16:40–17:20 **João Martins**
University of Copenhagen, Denmark
Back to the Library: Bridging the Gap Between Experiments and Theoretical Ensembles 11

17:20–18:20 Poster Session

18:20 Closing Remarks



EJIBCE 2017

Invited Speakers

QwikMD: easy and fast molecular dynamics simulations with VMD and NAMD

João V. Ribeiro, Rafael C. Bernardi1, Till Rudack1,2, Klaus Schulten1,3+, Emad Tajkhorshid1,4

+ In memoriam.; 1) Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, 61801; 2) Department of Biophysics, Ruhr-Universitat Bochum, Germany; 3) Department of Physics, University of Illinois at Urbana-Champaign, Urbana, 61801; 4) Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Richard Feynman's remarks in the early 1960's summarize what is today widely accepted, namely, that molecular processes can be described by the dynamics of biomolecules. Molecular dynamics (MD) simulation, in this regard, is the major methodology employed in structural biology to explore the dynamical behavior of macromolecules. Aided by MD, researchers were able, for instance, to resolve atomic structures of multi-protein complexes from cryo-EM densities; unveil the atomistic details of enzymatic mechanisms and characterize the binding of small molecules to proteins. To achieve all this, the capabilities of MD packages are constantly evolving, providing a multitude of complex MD simulation and analysis techniques. Although applicable to a great variety of research problems, a broad usage of MD is often hindered by a steep initial learning curve imposed by nearly every MD software. To make MD accessible to the general community, we developed an intuitive tool named QwikMD(1), which assists the users in the preparation, execution and analysis of MD simulations. While assisting the user, QwikMD ensures reproducibility of the results by writing all parameters and steps into two log files, one in a script-like format and another in a m̈ethods sectionf̈ormat. The user-friendly graphical interface of QwiKMD allows the preparation of MD simulations in just a few minutes, in a point-and-click fashion, offering the user multiple MD protocols, such as unbiased MD simulations, Steered MD, MD Flexible Fitting (MDFF), and, most recently, hybrid QM/MM simulations. The latter exploits the recently developed VMD and NAMD interface

to common quantum mechanics software packages. QwikMD facilitates performing MD simulations for nearly any user, novice or expert. QwikMD also serves as a learning tool, providing the theoretical background of the different MD protocols and options in many ïnfo buttons.

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Optimizing Proteins and Ligands for Computerized Drug Discovery

João M. Damas1, Alberto Cuzzolin1, Raimondas Galvelis1, Stefan Doerr1,2, Gerard Martinez-Rosell2, Matt Harvey1, Gianni De Fabritiis1,2

1) Acellera, PRBB, C/Dr. Aiguader 88, 08003 Barcelona, Spain; 2) Computational Biophysics Laboratory (GRIB-IMIM), Universitat Pompeu Fabra (PRBB), C/Dr. Aiguader 88, 08003 Barcelona, Spain

The reliability of physics-based in-silico studies of protein-ligand complexes highly depends on the quality of available structures and force-field parameters. Both these subjects have been largely addressed by both experimental and computational scientists from industry and academia. Yet, tasks like obtaining an initial structure with the correct protonation states and hydrogen-bond network or accurate force-field parameters for a given ligand can still be out of reach for the non-experts in those particular fields. Here we showcase two software tools that aim at bridging this gap: proteinPrepare and parameterize. We show how these softwares can be easily used by the community and how we are integrating these tools within a wider computational pipeline for drug discovery.

Using the Mechanism and Catalytic Site Atlas (M-CSA) to understand enzyme function and evolution

António J.M. Ribeiro, Gemma L. Holliday, Nicholas Furnham, Janet M. Thornton

European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK

M-CSA (Mechanism And Catalytic Site Atlas) is a database of enzyme mechanisms that can be accessed at www.ebi.ac.uk/thornton-srv/m-csa. Our objectives with M-CSA are to provide an open data resource for the community to browse known enzyme reaction mechanisms and catalytic sites, and to use the dataset to understand enzyme function and evolution. Practical applications that could benefit from this data include the design of new enzymes and inhibitors. M-CSA annotation includes the curly arrow description of the stepwise chemistry, the role of each catalytic residue and any cofactors, and the primary literature that supports such data. We also provide annotation for the associated protein sequences, structures, and their homologues. M-CSA results from the merging of two previous databases, MACiE (Mechanism, Annotation and Classification in Enzymes), a database of enzyme mechanisms, and CSA (Catalytic Site Atlas), a database of catalytic sites of enzymes. In comparison with the parent databases, M-CSA supports the inclusion of several mechanism proposals, better tools for curators, and the creation and edition of new entries through the website. For people consulting the website, we improved the search and browsing tools, as well as the presentation of database statistics. In addition to the changes in the database and website, we are also carrying out a complete revision of existing data. At the moment, M-CSA contains 961 entries, 423 of these with detailed mechanism information, and 538 with information on the catalytic site residues only. In total, these cover 81% (195/241) of third level EC numbers with a PDB structure, and 30% (840/2793) of fourth level EC numbers with a PDB structure, out of 6028 in total.

Back to the Library: Bridging the Gap Between Experiments and Theoretical Ensembles

António J.M. Ribeiro

The Image Group/SBinLab, University of Copenhagen, Denmark

The long standing relationship between structure and function of proteins has evolved over time due to improvements in both theoretical and experimental scientific capabilities. This evolution led to the current understanding of protein function as arising from an ensemble of structures sampled from low- and high-populated states of its free energy landscape. In order to address this relationship, sophisticated methods bridging the gap between experimental measurements and theoretical predictions give us the tools to further our understanding of proteins' structures as well as their functions. Using previously establish methods[1], I introduce a method capable of rapidly predicting Double Electron-Electron Resonance (DEER) distance distributions from an arbitrary structural ensemble[2, 3]. I further introduce two other methods for prediction of Paramagnetic Relaxation Enhancement (PRE) effects[4] and Forster resonance energy transfer (FRET) efficiencies[5]. These allow rapid comparison between experiment and theory while also be- ing useful as a tool for experiment design and interpretation.

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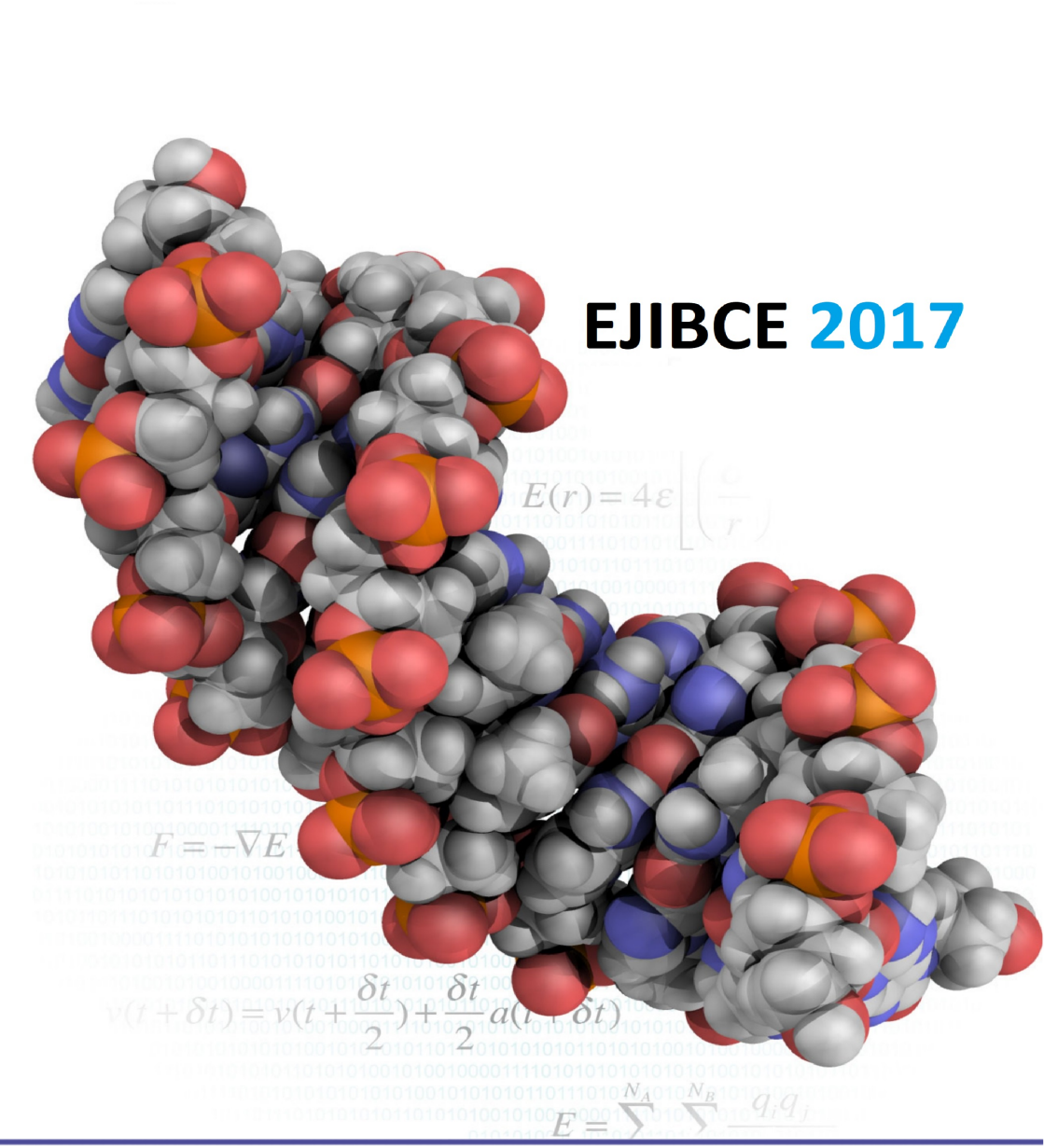

Selected Talks

Development of computational tools to enhance the study of catalytic mechanisms

Henrique S. Fernandes, Maria João Ramos, Nuno M. F. S. A. Cerqueira

UCIBIO@REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

Computational methods have been widely used to characterize the catalytic mechanisms of several chemical systems namely enzymes. However, enzymes are studied using big chemical systems containing several thousands of atoms generating huge amounts of data that are hard to manipulate and analyze efficiently. Therefore, we developed molUP that is a user-friendly plugin for VMD to handle QM and ONIOM calculations performed using Gaussian software. MolUP allows loading output files from Gaussian calculations and performs analysis concerning the structure of the chemical system as well as their energies and vibrational frequencies. Furthermore, molUP provides a graphical interface to manipulate the length of atomic bonds and the amplitude of angles and dihedral angles. Users can also easily choose which atoms belong to each ONIOM layer and the atoms that are free to move during a geometry optimization. At the end, molUP is capable of saving the new structure as a new Gaussian input file, ready to run a new calculation. Since molUP is a VMD extension, users can also benefit from the many features and resources available on VMD. In order to demonstrate the potential of MolUP, we will also present the results that have been carried out in our research group regarding the catalytic mechanism of Serine HydroxyMethylTransferase (SHMT), using a QM/MM approach. SHMT is a pyridoxal-5'-phosphate (PLP)-dependent enzyme [1-3] that catalyzes the α -elimination of L-serine, where a methyl group is transferred from the substrate to a second cofactor, tetrahydrofolate (THF). The reaction occurs in six sequential steps from which the first one is the rate-limiting step with an activation barrier of 18.3 kcal/mol that closely fits the experimental kcat of

0.98+/- 0.06 s-1 [4] (Δ G‡18.2 kcal/mol). This first step involves the nucleophilic attack of nitrogen from THF to the α-carbon of the substrate, promoting the α-elimination of the CH2OH group of the substrate. The subsequent steps involve an intramolecular cyclization within the THF cofactor where the elimination product of the first step is incorporated, generating the 5,10-methyl-THF. At the end, the quinonoid intermediate (substrate + PLP) is protonated, producing glycine.

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Predicting HIV-1 resistance to protease inhibitors: A new structure-based algorithm exploring binding-site Molecular Interactions Field dissimilarities

Nuno G. Alves1 , João P. Luís1, Carlos J. V. Simões1,2, João Pereira-Vaz3, Daniela C. Vaz1,4, Vítor Duque5, Rui M. M. Brito1,2

1) CQC, Chemistry Department, Faculty of Science and Technology, University of Coimbra, 3004-535 Coimbra, Portugal; 2) BSIM^2 , Instituto Pedro Nunes, 3030-199 Coimbra, Portugal; 3) Laboratory of Molecular Biology, Clinical Pathology Unit, Centro Hospitalar e Universitário de Coimbra, 3000 Coimbra, Portugal; 4) Health Research Unit, School of Health Sciences, 2411-901 Leiria, Portugal; 5) Infectious Diseases Unit, Centro Hospitalar e Universitário de Coimbra, 3000 Coimbra, Portugal

Over the last 30 years, HIV has grown to a pandemic status with more than 36 million people infected worldwide. Current therapies provide a significant improvement in the quality of patients' lives, specifically the Highly Active Anti-Retroviral Therapy (HAART). Yet, viral resistance development towards anti-HIV medication stands as the main obstacle to an effective therapy, having also a substantial economic impact on healthcare systems worldwide. Such viral resistance is primarily related to mutations occurring mainly on the active site of viral key enzymes, capable of decreasing the pocket's capability to establish the necessary non-covalent interactions with the drugs. Even so, mutations outside the enzyme's active site can also lead to resistances, by causing changes on its structure and/or chemical environment. Among the two HIV virus types, HIV-1 stands as the most studied and prominent, with HIV 1 protease being one of the main viral targets for therapy. Given the ease of quickly and affordably sequence HIV from infected individuals, considerable progress - in the sense of predicting resistance towards drugs - could be made by developing tools to link specific genetic mutations with the resulting structural and chemical alterations in the active site of the target enzymes. In recognition of a serious medical need identified by a team of virologists working at the

University of Coimbra teaching Hospital and with the intent of helping rationalize and personalize the choice of anti-HIV therapies, we set out to develop a new computational algorithm to predict resistance to protease inhibitors in HIV 1 via detection of binding-site Molecular Interactions Field (MIF) dissimilarities. Briefly, the algorithm works by 1) automatically generating high-quality 3D protein model structures from HIV 1 protease sequences; 2) capturing subtle, mutation-induced, chemical perturbations within the binding sites of resistant and non-resistant HIV 1 protease structures using a MIF-based approach; and 3) quantifying binding site dissimilarities based on MIF analysis, and translating these into a resistance score. In terms of its predictive power, preliminary testing of the algorithm using several different HIV protease sequences showed promising levels of sensitivity and specificity. Despite both sequence- and structure-based computational approaches to the prediction of HIV drug resistance have been proposed in the past, our present work stands out from other known algorithms as a first implementation of a fast structure-based algorithm capable of discriminating between HIV sequences that may be susceptible or resistant to commercially available protease inhibitors. Since the problem of mutation-induced resistance cuts across virtually all pathogenic virus, we believe that our approach may be extended to a wide range of viral targets besides HIV-1.

1. CQC, Chemistry Department, Faculty of Science and Technology, University of Coimbra, 3004-535 Coimbra, Portugal

2. BSIM^2, Instituto Pedro Nunes, 3030-199 Coimbra, Portugal

3. Laboratory of Molecular Biology, Clinical Pathology Unit, Centro Hospitalar e Universitário de Coimbra, 3000 Coimbra, Portugal

4. Health Research Unit, School of Health Sciences, 2411-901 Leiria, Portugal

5. Infectious Diseases Unit, Centro Hospitalar e Universitário de Coimbra, 3000 Coimbra, Portugal

Fragment-based discovery of Influenza A NS1 inhibitors: in silico screening and validation by NMR

Andreia E.S. Cunha1, Zaida Almeida1, Pedro Cruz1, Pedro Castanheira2, Carlos J. V. Simões1,3, Rui M. M. Brito1,3

1) Chemistry Department and Coimbra Chemistry Centre, Faculty of Science and Technology, University of Coimbra, 3004-535 Coimbra, Portugal; 2) Biocant - Biotechnology Innovation Center, Parque Tecnológico de Cantanhede, Núcleo 4, Lote 8, 3060-197 Cantanhede, Portugal; 3) BSIM^2 , Instituto Pedro Nunes, 3030-199 Coimbra, Portugal

Over the last 30 years, HIV has grown to a pandemic status with more than 36 million people infected worldwide. Current therapies provide a significant improvement in the quality of patients' lives, specifically the Highly Active Anti-Retroviral Therapy (HAART). Yet, viral resistance development towards anti-HIV medication stands as the main obstacle to an effective therapy, having also a substantial economic impact on healthcare systems worldwide. Such viral resistance is primarily related to mutations occurring mainly on the active site of viral key enzymes, capable of decreasing the pocket's capability to establish the necessary non-covalent interactions with the drugs. Even so, mutations outside the enzyme's active site can also lead to resistances, by causing changes on its structure and/or chemical environment. Among the two HIV virus types, HIV-1 stands as the most studied and prominent, with HIV 1 protease being one of the main viral targets for therapy. Given the ease of quickly and affordably sequence HIV from infected individuals, considerable progress - in the sense of predicting resistance towards drugs - could be made by developing tools to link specific genetic mutations with the resulting structural and chemical alterations in the active site of the target enzymes. In recognition of a serious medical need identified by a team of virologists working at the University of Coimbra teaching Hospital and with the intent of helping rationalize and personalize the choice of anti-HIV therapies, we set out to develop a new computational algorithm to predict resistance to

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2. Biocant - Biotechnology Innovation Center, Parque Tecnológico de Cantanhede, Núcleo 4, Lote 8, 3060-197 Cantanhede, Portugal

3. BSIM^2, Instituto Pedro Nunes, 3030-199 Coimbra, Portugal

Structural-based drug design: Dopamine D2 Receptor

Beatriz Bueschbell1,2, Carlos Barreto2, António Preto2, Anke C. Schiedel1 and Irina S. Moreira2,3

1) Pharmaceutical Chemistry I, PharmaCenter Bonn, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany & Structural; 2) Computational and Chemical Biology group, Center for Neuroscience and Cell Biology Rua Larga, FMUC, Polo I, 1st floor, Universidade de Coimbra, 3004-517 Coimbra, Portugal; 3) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, the Netherlands

Targeting dopaminergic neurotransmission is a growing field of investigation since imbalances and alterations on dopamine (DA) signalling can be related to several neuropsychiatric and neurodegenerative disorders such as Tourette's Syndrome, schizophrenia, Parkinson's disease and Huntington's disease (1), (2), (3). DA signalling occurs through five subtypes of dopamine receptors (DR), which can be subdivided into two families: the D1-like with D1DR and D5DR and the D2-like with D2DR, D3DR and D4DR (3). The DR belong to the G protein-coupled receptors (GPCRs) superfamily and have therefore the typical canonical seven-transmembrane structure and G protein dependant and -independent signalling (4). Several drugs target DR and their molecular mechanism of ligand-binding and selectivity remain unclear (5). One of the challenges is the identification of the amino acid residues responsible for ligand binding. With the resolved X-ray crystallography structures of the D3DR and D4DR (5), (6) more structural features, especially among the D2-like family, are revealed, which can facilitate ab initio computer modelling of the D2DR. In this study we used homology modelling with the D3DR as template and molecular docking of a large set of known D2DR ligands to investigate the orthosteric (OBP) and the secondary binding pocket (SBP) of the D2DR in the inactive state in order to group ligands by structural features and interacting residues. Results showed that all ligands, despite their size, form a salt-bridge with the crucial aspartic acid (3.32ASP) and form hydrogen

bonds with the aromatic microdomain (6.51PHE, 6.52PHE, 6.48TRP and 6.55HIS) in the OBP (7), (8). Furthermore, catecholamine hydroxyl-groups interacted with the serine microdomain (5.42SER, 5.43SER and 5.46SER) (9), while chloride-substituted ligands interacted with a cysteine (3.36CYS) in the OBP. Bulky ligands, e.g. apomorphine-like ligands interacted with non-conserved residues of the extracellular-loop 2 (10). More prolonged ligands such as atypical antipsychotics had access to the SBP, where they interacted with a conserved tyrosine (7.43TYR). In conclusion the study showed, that for the D2DR 3.32ASP and 6.48TRP are crucial for ligand binding, since both residues face each other towards the binding crevice and stabilize the position of the ligands in the binding pocket. Besides, both residues are conserved among all DR (5). Regarding structural and functional motifs for GPCR activation according to Moreira, S. (2014) (11), we were able to show that the aromatic microdomain is important not only for receptor activation, but also for ligand coupling. This comprehensive ligand-docking is a promising base for future studies on D2DR.

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T4 lysozyme/halobenzene: a test system for modeling biomolecular halogen bonds

Rafael Nunes, Diogo Vila-Viçosa, Miguel Machuqueiro, Paulo J. Costa

Centro de Química e Bioquímica, DQB, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

Halogen bonds (XBs) are non-covalent R-X***B interactions where heavy halogens (X = Cl, Br, I) act as electrophilic species interacting with Lewis bases (B). This highly directional type of interaction is mostly explained by the existence of a positive region on the molecular electrostatic potential located at the tip of the halogen (called s-hole), arising from polarization of the R-X covalent bond. Following the recognition of the significance of XBs in biomolecular structures [1], their application in rational drug design, amongst other areas, has been increasingly explored. In this context, the development of computational tools accurately modelling XB is of paramount importance. This is particularly challenging in the case of force field (FF)-based methods, where XBs are typically modelled by introducing a positive extra-point (EP) of charge to mimic the s-hole [2]. Though different schemes for EP parameterization have been proposed for AMBER or other FFs, their application to lengthy molecular dynamics (MD) simulations is still uncommon. In this work, we assessed the performance of distinct EP models and their transferability to the popular united-atom GROMOS FF, using bacteriophage T4 Lysozyme as a prototype system. The L99A mutant of this enzyme contains a large non-polar cavity that binds iodobenzene and related ligands, via XBs [3]. MD simulations were carried out and the network of intermolecular interactions, particularly XBs targeting different acceptors in the protein, was analysed. The results showed the dramatic impact of varying the X - EP distance and the associated sets of charges on the description of XBs. This, together with the implications for computer-aided drug design will be discussed.

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In silico studies on the pH induced membrane insertion of pHLIP peptides

Tomás Silva, Diogo Vila-Viçosa, Yana K. Reshetnyak, Oleg A. Andreev, Miguel Machuqueiro
Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

The pH (low) insertion peptide (pHLIP) belongs to a family of peptides originated from a segment of the transmembranar C helix of bacteriorhodopsin. The peptide has three major states: state I - soluble and unstructured; state II - adsorbed at the membrane surface and unstructured; state III - inserted in the bilayer as an a-helix at low pH values. One of the major applications of pHLIP befalls on its ability to insert into membrane cells with an acidic vicinity, such as tumoral cells, thus working as an efficient tumor-specific biomarker [1]. However, wt-pHLIP has a significant limitation, since it accumulates in the kidneys in considerable amounts due to their naturally acidic extracellular pH. This limitation led to a need for increased pHLIP specificity by delimiting the pH range of insertion, further strengthening its application as a biomarker and possible drug-delivery system for inflammatory tissues. The stochastic titration constant-pH molecular dynamics (CpHMD) method has been successfully used to sample protonation behaviour of titrable amino acids inserted in a lipid bilayer, presenting, however, an insufficient amount of data to extensively describe pKa profiles [2]. The newly developed pH-replica exchange (pHRE) method, allows the exchange of pH values between replicas within a certain probability. This approach enhances the transitions between energy minima, improving the sampling of non-favorable protonation states, which leads to a better description of the pKa profiles. This new method was applied to simulations of wt-pHLIP and L16H variants. The pHRE simulations led to more detailed, accurate and consistent pKa profiles and allowed the identification of Asp14 as the key residue whose protonation state triggers the insertion process. The calculated insertion pKa value of this residue is in good agreement with the experimental insertion pK value for the wt sequence. Moreover, the simulations of L16H

showed that this variant exhibits a second insertion pKa, at lower pH, indicating that, below this value, the peptide would exit the membrane. These results were corroborated by new experimental data performed by our collaborators, Prof. Oleg Andreev in Rhode Island, USA.

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Using big-data to understand the protein interface landscape

José G. Almeida1*, Alexandre.M.J.J. Bonvin 2, Irina S. Moreira1,2
*1) CNC - Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Rua Larga, 3004-517 Coimbra, Portugal; 2) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, the Netherlands, *) Current affiliation: Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; European Bioinformatics Institute EMBL - EBI, Wellcome Genome Campus, Hinxton CB10 1SD, UK*

Protein-protein interactions (PPIs) are the foundation of basic organism functions and understanding them is key in determining the importance of different proteins in a wide array of complex networks and processes. The variety underlying PPIs is immense and some residues are more essential in interface stabilization than others. Such is the case of hot-spots (HS), residues whose mutation to alanine is detrimental for the stability of the PPI, as opposed to null-spots (NS), which constitute the remaining interfacial residues. Considering the complex landscape in protein interfaces, some patterns and characteristics arise when a high amount of data is considered, by minimizing the effect of less prevalent interactions and characteristics. In this work, the SpotOn pipeline - developed by our group - custom scripts and conservation servers were used to determine structural features of interfacial residues and to classify them as HS and NS in the PPI4DOCK database, comprising over 1400 non-redundant complexes. This study allowed us to further understand the structural differences between HS and NS and will be available in a web-server in the near future.

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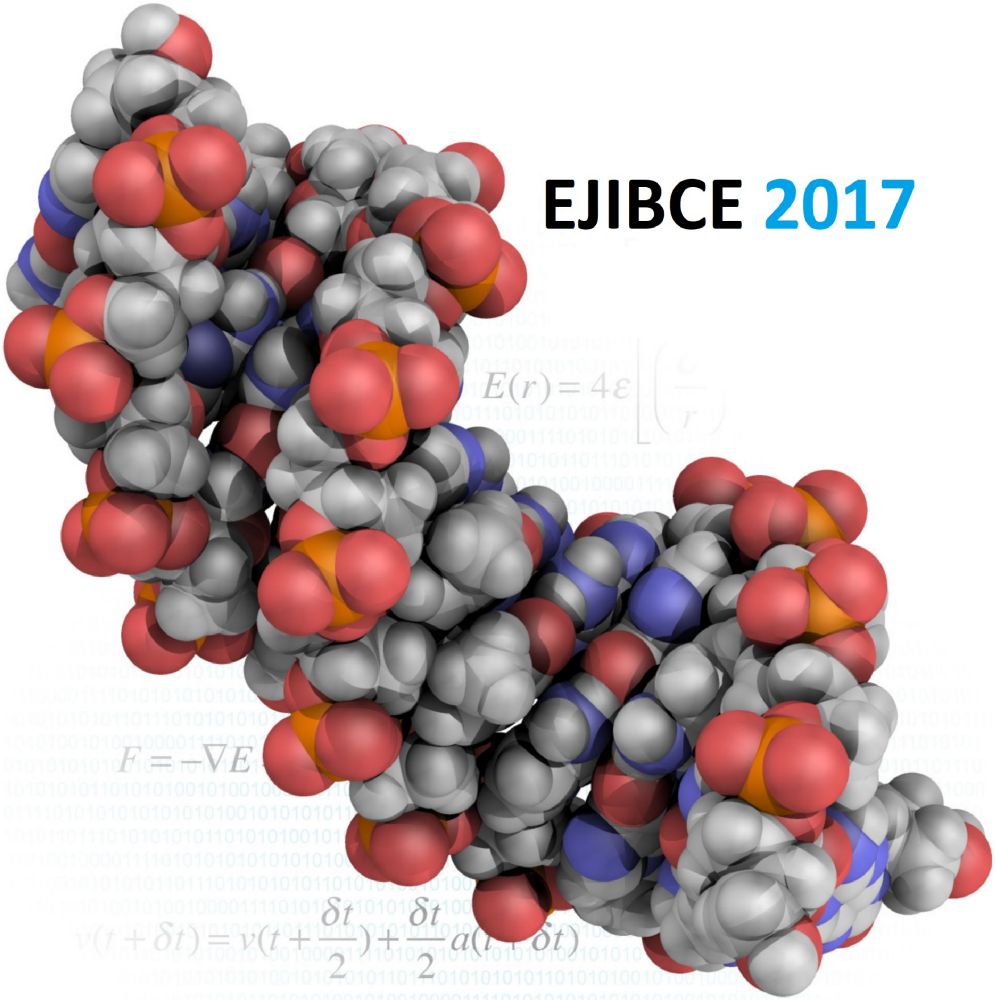

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Posters

Structural and dynamic understanding of the ghrelin receptor high constitutive activity

Carlos Barreto1, Miguel Machuqueiro2, Alexandre M. J. J. Bonvin3, Irina S. Moreira1,3

1) Computational and Chemical Biology group, Center for Neuroscience and Cell Biology Rua Larga, FMUC, Polo I, 1st floor, Universidade de Coimbra, 3004-517 Coimbra, Portugal ; 2) Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; 3) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, the Netherlands

Ghrelin is a peptide secreted in the gastric fundus and acts in hypophysis and hippocampus through a Class A G-protein Coupled Receptor (GPCR), the GHS-R1a. GPCRs are characterized by a seven transmembrane (TM) spanning a-helices, connected to three extracellular (ECL) and three intracellular loops (ICL). Activation of this receptor is involved in many functions from feeding and growth hormone release and more recently discovered to promotion of learning and memory. Among GPCRs, GHS-R1a is characterized by an unusual high constitutive activity, in the absence of specific ligands. Regarding this distinctive characteristic, a mutation, A204E, of this receptor leads to a reduction of its constitutive activity. To reveal more information about this distinctive characteristic, 3D structure models of different stages of activation (the inactive, the pre-activated, the G-protein activated and Arrestin-activated) and a particular mutation (A204E) of the GHS-R1a were created through homology modelling molecular docking and their differences analysed. Fully-atomistic molecular dynamics (MD) simulations are being initially performed with the pre-active model and the mutant to understand dynamic differences. These simulations will give atom-level information about the necessary conformation rearrangements responsible for receptor activity. The understanding of structural and functional features of GHSR1a constitutive activity will open new doors to design specific drugs.

Early Stages of Parkin Activation - a computational study

Catarina A. Carvalheda, Juan A. Bueren-Calabuig, Atul Kumar, Helen Walden, Andrei V. Pisliakov

School of Life Sciences and School of Science and Engineering, University of Dundee, UK

Parkin is an E3-ubiquitin ligase involved in regulation of mitophagy. Upon activation, it mediates the transfer of ubiquitin (Ub) from an ubiquitin-conjugating enzyme (E2) to specific substrate proteins, labelling those for degradation. Recent biochemical and structural data suggested that several factors are involved in parkin activation. However, the available data cannot unambiguously explain the mutual effect of these factors or the detailed sequence of steps on the molecular scale. In this work, we use a combination of computational tools to examine the effect of several factors on parkin activation. The effects of parkin phosphorylation, removal of its inhibitory domain, and allosteric binding of phosphoubiquitin are addressed. Additionally, the effect of several mutations which are thought to promote the displacement/detachment of the inhibitory domain and render parkin active are analysed. Our results suggest that i) phosphorylation alone is unlikely to promote the transition from the inactive to active conformation; ii) UBL removal might facilitate E2 binding, but its complete detachment from Parkin is rather improbable in a physiological context; iii) pUb binding stabilises the active conformation of Parkin likely priming the activation process; iv) mutations involving residues at the UBL/RING1 interface lead to structural rearrangements suggested to be involved in the activation mechanism.

Molecular modeling study of pH effects on β-lactoglobulin

Lucie da Rocha, Sara R. R. Campos, António M. Baptista

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

Milk and its derivatives are an important worldwide food source, particularly for infant nutrition, but their use faces a major health complication: some of their proteins are allergens, especially β-lactoglobulin (BLG), a major component of the bovine milk. The fate of BLG upon human ingestion remains unsettled, being unclear how extensive BLG proteolysis is and how it relates to allergenicity. The fact that its proteolytic resistance and antigenic response remain related even in the case of non-oral administration [1] suggests that they are not causally related but rather reflect an underlying common feature. This feature may be the formation of dimers, which can hinder proteolysis and seems to facilitate the binding of protein allergens to IgE antibodies; indeed, BLG is dimeric when complexed with IgE Fab fragments [2] and shows lower antigenicity when in the monomeric form [3]. As shown in experimental studies, this form is predominant at pH below 3 and above 8 and between these there's the formation of a reversible dimer at a moderate ionic strength [4]. The changes in pH are also associated to the Tanford transition, that is, a change in the conformation in a loop near the binding site, allowing or inhibiting the binding of ligands, regulated by the protonation of Glu89 [5]. Previous studies have shown that the dimerization involves electrostatic interactions, for which a better understanding at a molecular-level is essential. In this study, we intended to analyse the effect of the pH in conformational alterations on the monomer and dimer and its dissociation process. For that, two main molecular modeling methods have been used: Constant pH molecular dynamics (CpHMD) method for the monomer and dimer, which allow us to treat pH as an explicit parameter and couples the MM/MD and Poisson-Boltzmann/Monte Carlo (PB/MC) algorithms; and umbrella sampling method for the dimer using CpHMD,

providing us the potential of mean force (PMF) as a function of the distances between the monomers

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Mapping the conformational and structural regulators involved in the inhibition of the human 20S proteasome inhibitors

Pedro M. P. Fernandes, Bruno L. Victor, Rita C. Guedes

iMed.ULisboa, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

The Ubiquitin Proteasome Pathway (UPP) plays a pivotal role in intracellular protein degradation and turnover in eukaryotic cells. (1) Therefore, modulation of the UPP emerged as a rational therapeutic approach in cancer, neurodegenerative diseases (Alzheimer, Parkinson), inflammatory pathologies (arthritis, psoriasis, asthma, colitis), organ transplant, infective diseases (malaria), among others. (2) During the last two decades academia and pharmaceutical industry made huge efforts to develop natural and synthetic proteasome inhibitors (PI). In 2003 FDA approved the pioneering dipeptidyl boronic acid derivative PI bortezomib for the treatment of refractory multiple myeloma (MM) and subsequently frontline therapy for MM. However, despite the enormous potential of PI, their use is still limited to certain types of blood cancer and shows severe side effects, dose limiting toxicity, peripheral neuropathy, limited activity in solid tumour and innate or acquired drug resistance. (3) In this work, we have used Molecular Dynamics (MD) simulations to perform the first conformational and structural characterization of the human native 20S proteasome structure (4). We focused our analysis on the three catalytic subunits well known for their proteolytic activity (β1, β2 and β5) and we further extended our study to additional MD simulations of three different point mutations in the β5 catalytic subunit, with recognized importance in PI's resistance: Ala49Thr, Ala50Val and Cys52Phe. Hopefully, our studies will be able to shed the light on the structural key determinants that regulate the observed PI's resistance in the different mutations, and ultimately use the acquired knowledge in the development of new alternative and efficient proteasome inhibitors.

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Molecular determinants of the influenza fusion peptideś activity

Diana Lousa1, Antónia R. T. Pinto2, Bruno L. Victor1, Alessandro Laio3, Ana S. Veiga2, Miguel A. R. B. Castanho2, Cláudio M. Soares2

(1) ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal; (2) Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, 1649-028 Lisboa, Portugal; (3) SISSA/ISAS, Statistical and biological physics, Via Beirut 2-4 Trieste, Italy

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 to obtain a comprehensive description of the molecular determinants underlying the IV FP, we are using a combination of simulation and experimental techniques. We have applied a broad range of molecular simulation and spectroscopic techniques to analyse the properties of this peptide and its interaction with model membranes. The combination of these techniques allowed us to characterize the peptide effect on the membrane and the impact of mutations and external conditions, such as pH, on the peptide activity. These results can be useful for the design of novel therapies against this devastating pathogen.

α-helical and β-Sheet Membrane- Membrane Protein Dimers: Centralizing Information

Pedro Matos-Filipe1, António J. Preto1, Panagiotis I. Koukos2, Alexandre M. J. J. Bonvin2, Irina S. Moreira1,2

1) Computational and Chemical Biology group, Center for Neuroscience and Cell Biology Rua Larga, FMUC, Polo I, 1st floor, 2) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, the Netherlands

Bioinformatics allows to automatically characterize a large number of proteins from numerous different databases1, thus, uncovering new possible interactions between biomolecules in a huge set of individuals in a conscious and cost-efficient way2. Membrane proteins are indisputably important for the assurance of major processes in the cell, occupying approximately 25% of the whole cell genome3. In this work, some of the major features displayed at Protein Data Bank4 (original species, chains and ligands, oligomer state, multimeric states, stoichiometry, among others) of membrane proteins listed in the Membrane Proteins with Known 3D Structure5 database were registered together using manual and automated methods - some of these methods include the usage of python specific tools (like Selenium6 and BioPython7). We aimed to construct a membrane-membrane dimers database that will serve as input for data-mining algorithms to unveiling new functional and evolutionary knowledge.

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Structural mechanism of HER2-antibodies complexes by molecular dynamics studies

Ruta Melo1,2, José G. Almeida2*, S. Cabo Verde1, Zeynep H. Gumus3, Irina S. Moreira2,4, João D. G. Correia1

*(1) Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal; 2) Computational and Chemical Biology group, Center for Neuroscience and Cell Biology Rua Larga, FMUC, Polo I, 1st floor; 3) Department of Genetics and Genomics and Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; 4) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, the Netherlands, *) Current affiliation: Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; European Bioinformatics Institute EMBL - EBI, Wellcome Genome Campus, Hinxton CB10 1SD, UK*

Human Epidermal Growth Factor Receptor 2 (HER2) is, among EGFR family, one of the most relevant members as it remains overexpressed on tumor cells and provides resistance to well-studied anti-HER2 monoclonal antibody, Trastuzumab (Herceptin), or tyrosine kinase inhibitor. Furthermore, HER2 plays a key role in the HER family due the interaction with other HER receptors via a complex signaling network to regulate cell growth, differentiation and survival. 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 F0178 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, energetic and dynamic characteristics ranging from pairwise interactions formation to covariance analyses were performed to the 2 bundle complexes. Our aim was to understand the all-atom details of these intermolecular couplings, fundamental for the development of new therapies.

Quantum Chemical Modeling of Citrate Synthase reaction mechanism

Eduardo F. Oliveira, Pedro A. Fernandes, Maria J. Ramos, Fahmi Himo

REQUIMTE - UCIBIO - Theoretical and computational biochemistry

Citrate synthase (CS) catalyzes the first step of the citric acid cycle, the condensation of acetyl-coenzyme-A with oxaloacetate (OAA) to yield, after hydrolysis, citrate and coenzyme-A. The remarkable rate enhancement and the ubiquity of this enzyme granted it extensive computational and experimental studies. Nevertheless, several aspects of CS reaction mechanism, such as the stabilization of the citryl-CoA or the hydrolysis reaction pathway, still need to be clarified or disclosed. We use a large quantum-chemical description of CS active site to investigate for the first time the entire reaction mechanism of CS. We propose an original pathway for hydrolysis and active site interactions responsible for CS catalytic enhancement are identified. The amino acids involved and the predicted barriers are in good agreement with previous mutagenesis and kinetic experimental studies.

G-Protein Coupled Receptors Complexes with intracellular partners

António J. Preto1, José G. Almeida1*, Agostinho Lemos2,3, Zeynep Kurkcuoglu4, Rita Melo1,5, Maxim Telle1, André Melo3, Maria N. D. S. Cordeiro3, Giulia Morra6,7, Ozge Sensoy8, Alexandre M. J. J. Bonvin4, Irina S. Moreira1,4

*1. CNC - Center for Neuroscience and Cell Biology, Faculty of Medicine, University of Coimbra, Rua Larga, 3004-517 Coimbra, Portugal; 2. GIGA Cyclotron Research Centre In Vivo Imaging, University of Liége, 4000 Liége, Belgium; 3. LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal;4. Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, The Netherlands; 5. Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal; 6. Weill-Cornell Medical College, Department of Physiology and Biophysics, 1300 York Ave, New York, NY 10065; 7. ICRM-CNR Istituto di Chimica del Riconoscimento Molecolare, Consiglio Nazionale delle Ricerche, Via Mario Bianco 9, 20131 Milano, Italia; 8. Istanbul Medipol University, The School of Engineering and Natural Sciences, 34810, Istanbul,Turkey, *) Current affiliation: Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK; European Bioinformatics Institute EMBL - EBI, Wellcome Genome Campus, Hinxton CB10 1SD, UK*

G-Protein Coupled Receptors Complexes with intracellular partners G-Protein Coupled Receptors (GPCRs) are membrane proteins involved in a wide variety of processes that pertain to many biological roles dependent on intercellular communication 1. Due to their ubiquity, GPCRs are also associated with many diseases, some of the most commonly referred in the literature are Alzheimer's Disease (AD), Parkinson's Disease (PD) and many other neurological diseases 2. The interaction of GPCRs and G-proteins is basal to a large array of matters,

as it is in many times the backbone of intracellular signalling propagation, triggered by GPCRs' activation by a ligand 3. Arrestins, traditionally thought to provoke GPCRs' detachment from G-proteins and later internalization, are now being researched as possible contributors for intracellular signalling pathways 4. Dopamine Receptors, in particular, play a huge role in many brain areas and functions, reportedly interacting with both G-proteins 5 and arrestins 6. In this work, 33 complex models were constructed with MODELLER 7 undergoing further refinement with HADDOCK 8, making use of two known active templates 9. These models were analysed with a wide variety of tools, many of them developed for the purpose, rendering results on the differences of interactions between the arrestins (ARR2 and ARR3) and G-proteins (Gi, Gs, Gt, Go, Gq and Gz) with the Dopamine Receptors (D1-5R). Particularly, intracellular substructures ICL1-3 and HX8, as well as, in some cases, the residues of the TMs closer to these structures, were confirmed to be highly determinant for complex formation with the different partners. More importantly, was possible to notice differences of interactions between the complexes, more evident between arrestins and G-proteins. Specific residues on the GPCRs' interface can be pinpointed as being present in the interaction with all the complexes and motifs of residues arise for different complexes' groups, such as Gs and Gi groups. Furthermore, D4R exhibits a deviating behaviour, in relation to the other dopamine receptors.

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"In silico" estimation of encapsulation-induced pKa shifts in drugs

Diogo M. C. Reis, Miguel Machuqueiro, Diogo Vila-Viçosa

Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Molecular machines have recently been associated with the development of molecular carriers to enhance drug properties, such as solubility or bioavailability. One possible approach is the drug encapsulation by a host molecule, such as cucurbituril (CB) rings, modifying the environment of the guest molecule. CB rings are able to encapsulate guest molecules providing a hydrophobic cavity and several carbonyl groups that stabilize cationic hosts that interact with this region. This will result in significant pKa shifts for drugs with titrable (cationic) groups that can be exploited in order to improve drug bioavailability, whether by enhancing their solubility, stabilizing their active form or by protecting them against external agents. This approach can be used for medical targeting, such as cancer therapy, by designing carriers that deliver guest molecules at specific conditions, knowing the target properties. Computational tools are a powerful way to help the rational design of CB-guest complexes. In particular, the stochastic titrations constant-pH MD (CpHMD) method allows a molecular dynamics simulation to have the pH value as an external parameter and, consequently, obtain full titration curves and pKa values. The main goal here is to develop a strategy to model benzimidazole (BZ) pKa shifts, our p̈roof-of-conceptm̈olecule, and then extrapolate this process to other host-guest complexes. BZ has a well-known shift of 3.5 pKa units when encapsulated by a CB ring and, with the refinement and fine tuning of this process, it is possible to elucidate the molecular details of these host-guest interactions.

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Improving pKa calculations of membrane inserting amino acids using replica exchange CpHMD simulations

Pedro B. P. S. Reis, Diogo Vila Viçosa, Miguel Machuqueiro

Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

pH is one of the most important solution parameters. It plays a major role in most biochemical processes by, among other, inducing protein conformational changes and influencing protein-lipid interactions. These systems have been modeled using constant-pH molecular dynamics (CpHMD) methods since they are able to correctly capture the conformational/protonation coupling. In a previous CpHMD study,1 we have shown that, upon membrane insertion, the titrable amino acids are prone to adopt a neutral state. In that work, CpHMD experienced difficulties sampling ionized conformations in inserted regions, since in the time scale of our simulations most residues retained their neutral state upon insertion/desolvation. Enhanced sampling techniques are a widely used solution to deal with kinetic traps in molecular dynamics simulations. Since our sampling problems are related with protonation, we have implemented a pH-based replica exchange (pHRE)2. In this method, each simulation replica is assigned a unique pH value and attempts to exchange the simulated pH value of simulations pairs are periodically performed. The acceptance criterion is influenced by the exchanging simulations pH values and protonation states of the titrable sites. In this work, a more accurate description of the membrane influence on the pKa profiles of titrable amino acids is provided by using the pHRE methodology, a newly developed method to calculate insertion, and more rigorous criteria to define the acceptable protonation sampling. Since in pHRE, due to replica mixing, all pH values sample similar insertion regions, a larger amount of inserted conformations in the ionized state are obtained. Our efficient pHRE results outperformed previous CpHMD ones, granting more sampling in less simulation time. In the future, pHRE will replace CpHMD as our go-to method to study pH-dependent phenomena.

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Allostery in a GPCR:mini-G complex studied by molecular dynamics simulations

Pedro Renault, Jacky Marie, Nicolas Floquet

IBMM, Montpellier

G-protein coupled receptors (GPCRs) are membrane proteins implicated in several physiological functions and constitute important drug targets. They can couple to G-proteins to trigger complex cellular responses to various extracellular signals, such as organic molecules and peptides, for example. Experimental and theoretical methods have been used to investigate the dynamical character of these receptors and how their interactions with binding partners shift the dynamical equilibria, that are related to GPCR function. In particular, molecular simulations have contributed to clarify the allosteric communication between the agonist binding site and distal regions of the receptor [1] and the effect exerted by the receptor on the G-protein [2]. Interestingly, recent experiments have demonstrated that the G-protein can also allosterically modulate the receptor and favor a closed conformation of the agonist binding site, even in the absence of a bound agonist [3]. Analysis of available crystal structures of GPCRs suggests possible allosteric mechanisms [3], but a dynamical view of this process is still missing. In this work, we used molecular dynamics simulations to investigate this allosteric modulation, using the adenosine A2 receptor (A2AR) coupled to the mini-Gs protein as a model system [4]. We have observed smaller volumes of the agonist binding site of the receptor in the presence of the mini-Gs protein and we have shown how this partner modifies the network of contacts of the receptor. We have also identified interactions between side chains that are critical for the propagation of this effect from the intracellular domain to the binding pocket. In our simulations, we have observed similar effects when only the C-terminal helix of the mini-Gs protein is bound to the A2AR, suggesting that the local rearrangement of interactions resulting from direct contacts with the bound protein partner causes a rewiring of the receptor's interaction newtork, with global effects.

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Interactive Analysis of Protein Energetics

João Rodrigues

Stanford University

Pairwise residue interactions within and between proteins are fundamental for structure and function. Here, we introduce a chord-based visualization to represent pairwise quantities, such as energy, between protein residues. Taking advantage of recent development in web technologies, we couple 2D and 3D representations of the molecule(s), allowing for seamless interaction between the two. We demonstrate the simplicity and usefulness of this visualization tool with several examples of protein-protein and protein-DNA interactions. The tool is under active development and will be made available free-of-charge and open-source upon publication.

Effect of protonation state on the interaction of Hoechst 33342 with lipid membranes - An experimental and computational study

Patrícia Santos1, Hugo A. L. Filipe1,2, Joaã P. Prates Ramalho3, Luís M. S. Loura1,4, Maria João Moreno1

1) CQC - Coimbra Chemistry Center, University of Coimbra, P-3004-535 Coimbra, Portugal; 2) CNC - Center for Neuroscience and Cell Biology, University of Coimbra, P-3004-517 Coimbra, Portugal; 3) CQE - Évora Chemistry Center, University of Évora, P-7000-671 Évora, Portugal; 4) Faculty of Pharmacy, University of Coimbra, P-3000-548 Coimbra, Portugal

Hoechst 33342 (H33342) is a fluorescent probe that stains the DNA of living cells, permeating though cell membranes.[1] However, the influence of the probe ionization state [2] in this process is poorly characterized. The knowledge of H33342 ionization state in lipid bilayers will help to predict and interpret its passive permeation through cell membranes. In this work we characterized the acid/base properties of the interaction of H33342 with POPC bilayers using an experimental and computational combined approach. H33342 pKa values in aqueous solution of 6.4 and 11.1 were measured by its UV/Visible spectra at different pH. H33342 partition coefficient (Kp) to POPC bilayers at different pH was measured by isothermal titration calorimetry (ITC). An increase of Kp for higher pH values was obtained, indicating stronger interaction with membranes for the less charged or neutral forms of the probe. The enthalpy variation (?H0) for the partition to the bilayer was negative at all pH values, with higher absolute values at low pH. This may indicate that when H33342 is more protonated, it adopts a more external position in the bilayer, being able to make favorable interactions in this membrane region. Detailed characterization of H33342-membrane interactions was also obtained through Molecular Dynamics (MD) simulations. This allowed to support experimental results by the calculation of membrane transverse location and preferential orientation of the H33342 in different protonation states, as well as possibility of hydrogen bonding between the probe and the membrane. We conclude

that at physiological pH H33342 presents a high fraction of the neutral form while associated with POPC bilayers, justifying the fast permeation observed through cell membranes.

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From structural elucidation and molecular dynamics to characterize viral surface glycoproteins in HIV-2

Patrícia Serra1, Andreia Martins1, Nuno Taveira1,2, Rita C. Guedes1

1) iMed.ULisboa, Research Institute for Medicines and Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; 2) Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM), Quinta da Granja, 2829-511 Monte da Caparica, Portugal

The efficacy of some of the available antiretroviral drugs is very limited against HIV-2 and, most importantly, none of the current drugs effectively prevents entry into the cells. HIV envelope glycoproteins mediate binding to the receptor CD4 and to CCR5 and/or CXCR4 co-receptors at the surface of the target cell, enabling fusion with the cell membrane and viral entry [1,2]. Relying on the computational tools to study and to modulate the envelope surface glycoproteins of the HIV-2 involved in the entry mechanism of the virus consists on the mail goal of this work. Aiming to pinpoint specific structural features that could correlate with known genotypic determinants of HIV-2 tropism located in the gp125 V3 loop region, a three-dimensional (3D) structure of C2V3C3 domain of HIV-2rod gp125 was generated by homology modelling, due to the lack of a complete crystallography structure that includes variable domains.[3] To disclose the importance of these structural features and compare with experimental results, a wild type model was generated besides six other models incorporating specific modifications using MOE software package. The binding of the glycoprotein with receptor CD4 lead to conformational changes and determine the co-receptor specificity. Modifications on the aromatic equilibrium of the protein suggest an important feature to determine co-receptor usage. [1] It was seen that modifications at these positions in different variants originate an increment in the CCR5 usage. The analysis of these variants suggests that the presence of aromatic moieties increment CXCR4 usage. The modification of specific residues resulted in an easier neutralization when compared with the wild type as well as alteration of capacity of replication in different targets. The models were

furthermore subjected to MD simulations to account for structural flexibility and structure optimization and infer if the mobility led to modification of the molecular interactions. Energy minimization and molecular dynamic simulations were performed using Gromacs 2016.01 packages. These new insights into the structure-function relationship will help in the design of better models and into the following of the design of new small molecules potential capable of use to inhibit the attachment and binding of HIV with host cells mediated by the envelope surface glycoproteins.

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Computational studies addressed to the catalytic mechanism of the alpha sub-unit of Tryptophan Synthase

Carla S. Silva Teixeira, Nuno M. F. S. A. Cerqueira

UCIBIO@REQUIMTE

Tryptophan Synthase (TSase) is a bi-functional enzyme that catalyzes the last two steps in the synthesis of tryptophan (trp), in different actives site. The active site of the a-subunit catalyzes the formation of indole and gliceraldeyde-3-phosphate (G3P) from indole 3- glycerolphosphate (IGP). Indole is then transported through 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 a-active site of TSase using computational means and QM/MM hybrid methodologies [2]. The results show that the reaction occurs in a stepwise general acid-base mechanism. The first step requires the participation of a water molecule that protonates C3 of the indole ring and receives a proton from aGlu49. In the second step, aGlu49 abstracts a proton from the glycerolyl hydroxyl of IGP through a water molecule, triggering the C-C bond cleavage to give indole and G3P. The rate-limiting step of this reaction is the first one that requires an activation free energy of 17.74 kcal/mol. This result agrees extremely well with the available experimental data that predicts reaction rate of 3.0-3.7 s-1, which corresponds to a free energy barrier of 17.37-17.50 kcal/mol. The results obtained in this work provide important details about TSase that can now be used for the development of new transition state analogues inhibitors targeting TSase - an important drug target used in the treatment and prophylaxis of tuberculosis that is caused by the Mycobacterium tuberculosis pathogenesis.

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Characterization of the membrane permeability of different proteasome inhibitors using molecular dynamics methods

Bruno L. Victor, Pedro Fernandes, Romina A. Guedes, Rita C. Guedes

iMed Ulisboa, Faculdade de Farmácia da Universidade de Lisboa

Protein degradation is a key function developed by organisms to remove damaged and abnormal proteins, preventing their accumulation, and serving at the same time to regulate cellular processes by removing enzymes and regulatory proteins that are no longer needed.1 This regulatory process can be achieved through two independent pathways: proteolysis in lysosome, or a ubiquitin-dependent process targeting unwanted proteins to proteasome. Due to its shattering function, proteasome has constituted an important therapeutic target to the control of different diseases such as malaria, cancer, multiple sclerosis, psoriasis, among others.2 Since this protein can be found both on the cell cytoplasm and nucleus, inhibitors developed to target it, must be able to cross the membrane lipidic barrier. Until now, it is unclear if transport involves simple passive diffusion or occurs via a yet unidentified transport system. In both scenarios, associations with the cell wall and the membrane are to be expected. Modeling the interaction of different inhibitors derivatives with the cell wall is not feasible because of its complicated and variable structure. However, it is possible to model and compare the interactions of different proven proteasome inhibitors with a lipid bilayer. In this work, by using restrained (Potential of Mean Force - PMF) and unrestrained Molecular Dynamics simulations at the water/membrane interface, we have evaluated the membrane permeability rates of different proteasome inhibitors (available on the market and identified in our lab) and their configurational and positional preference in this mixed medium. Our results will allow us to compare the trafficking of the evaluated compounds through the cell membrane and to relate it with the proteasome inhibition efficiency. Keywords: Protein degradation, inhibitors, cellular permeability, membrane

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Enhancing protonation sampling via a pHRE replica exchange scheme

Diogo Vila-Viçosa1, Pedro B. P. S. Reis1, Chris Oostenbrink2, Miguel Machuqueiro1

1) Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal; 2) Institute of Molecular Modeling and Simulation; University of Natural Resources and Life Sciences

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. However, the protonation behavior of pH sensitive biomolecules is difficult to study using experimental techniques and can strongly benefit from using computational approaches. In this context, we have successfully studied several systems using the stochastic constant-pH molecular dynamics (CpHMD) method. In these studies, we were able to obtain titration curves for proteins, membranes, and peptides at the membrane water interface. In the later case, it was observed that, when the titrable groups are deeply inserted in the membrane, the conformational / protonation sampling becomes very limited. In this project, we extended the stochastic CpHMD method to introduce enhanced protonation sampling. We implemented a pH replica exchange scheme and applied it to ethylenediamine, a simple molecule with two strongly coupled macroscopic pKa values, and to hen egg white lysozyme (HEWL), a typical test system for pKa prediction methods. In the future, we will use this method to study challenging pH dependent phenomena in complex biological systems.

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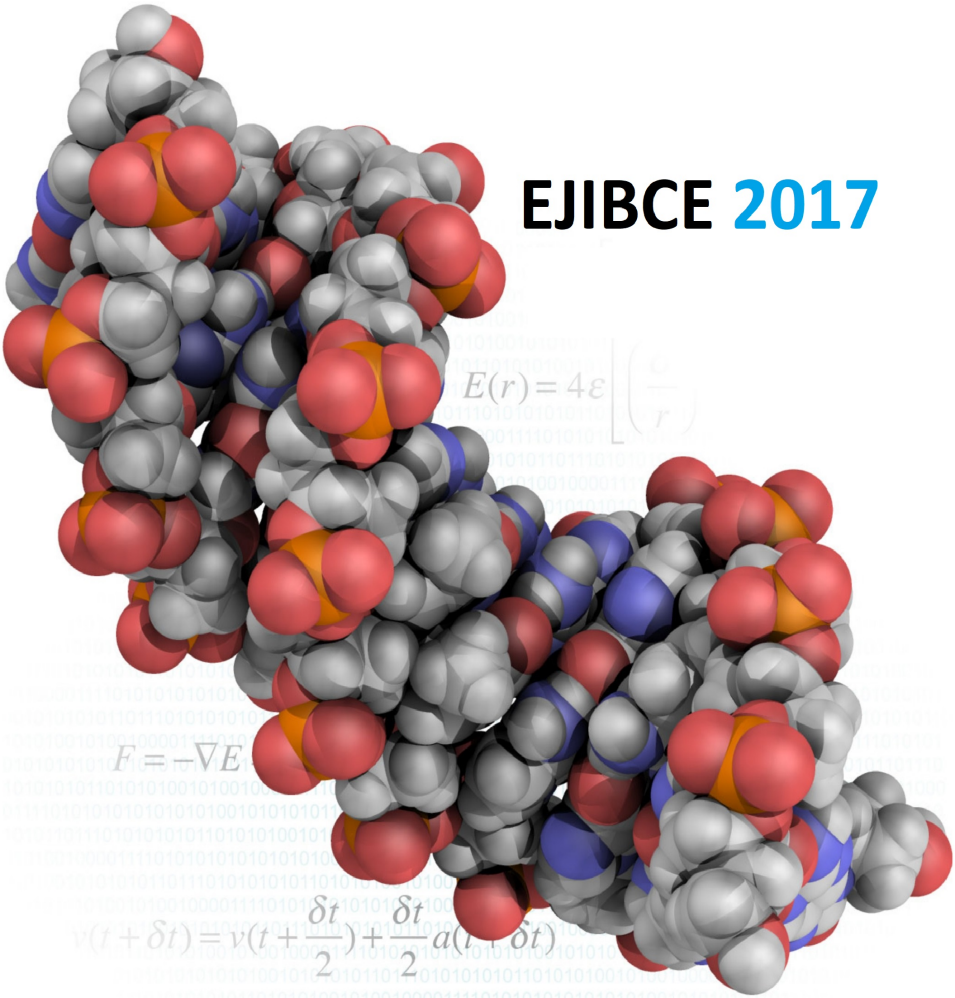

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List of Participants

Abreu, Bárbara
barbara.abreu@itqb.unl.pt
ITQB NOVA (Oeiras, Portugal)

Almeida, José 28
jose.gcp.almeida@gmail.com
EMBL-EBI (Cambridge)

Alves, Nuno 28
nunoagalves@gmail.com
Faculdade de Ciências e Tecnologias da Universidade de Coimbra (Coimbra, Portugal)

Antunes, José
egipto@ceb.uminho.pt
Universidade do Minho (Braga, Portugal)

Araújo, Ana Rita
anritaraujo@gmail.com
Imperial College London (London, UK)

Barreto, Carlos 33
carlosavbarreto@gmail.com
Universidade de Coimbra (Coimbra, Portugal)

Brito, Rui
rbrito@ci.uc.pt
Universidade de Coimbra (Coimbra, Portugal)

Bueschbell, Beatriz 21
s6bebues@uni-bonn.de
University of Bonn (Bonn, Germany)

Campos, Rita
scampos@itqb.unl.pt
ITQB NOVA (Oeiras, Portugal)

Carvalheda, Catarina 34
cacdossantos@dundee.ac.uk
University of Dundee (Dundee, UK)

Carvalho, Alexandra
apirescarvalho@gmail.com
CNC/Coimbra University (Coimbra, Portugal)

Costa, Paulo Jorge
pjcosta@fc.ul.pt
CQB - DQB - FCUL (Lisboa, Portugal)

Cunha, Andreia 19
andreiacunha94@hotmail.com
Universidade de Coimbra (Coimbra, Portugal)

da Rocha, Lucie 19
lucie@itqb.unl.pt
ITQB (Oeiras, Portugal)

Damas, João M. 9
j.damas@acellera.com
Acellera Ltd (Barcelona, Spain)

Dias, José
josericardoodias@gmail.com
FCUL (Lisboa, Portugal)

Fernandes, Henrique 15
henrique.fernandes@fc.up.pt
Faculdade de Ciências da Universidade do Porto (Porto, Portugal)

Fernandes, Pedro 37
pmpfernandes@ff.ulisboa.pt
iMed.ULisboa (Lisboa, Portugal)

Fernandes, Rúben
ruben@ess.ipp.pt
Escola Superior de Saúde; Politécnico do Porto (Porto, Portugal)

Filipe, Hugo
hugolourofilipe@gmail.com
Universidade de Coimbra (Coimbra, Portugal)

Guedes, Rita
rguedes@ff.ulisboa.pt
Faculdade de FarmÃącia (Lisboa, Portugal)

Lousa, Diana 39
dlousa@itqb.unl.pt
ITQB NOVA (Oeiras, Portugal)

Machuqueiro, Miguel
machuque@ciencias.ulisboa.pt
FCUL (Lisboa, Portugal)

Martins, M. João 11
jmartins@di.ku.dk
Copenhagen University (Copenhagen, Denmark)

Mata, Ana Isabel
ana.ig.mata@gmail.com
Universidade de Coimbra (Coimbra, Portugal)

Matos-Filipe, Pedro 40
p.pintofilipe@gmail.com
Universidade de Coimbra (Coimbra, Portugal)

Meireles, António José
ribeiro@ebi.ac.uk
EMBL-EBI (Cambridge, UK)

Melo, Manuel N.
ritamelo@ctn.tecnico.ulisboa.pt
ITQB NOVA (Oeiras, Portugal)

Melo, Rita 42
m.n.melo@itqb.unl.pt
IST (Lisboa, Portugal)

Moreira, Irina
irina.moreira@cnc.uc.pt
CNC (Coimbra, Portugal)

Nunes, Rafael
rsnunes@fc.ul.pt
CQB (Lisboa, Portugal)

Oliveira, Alexandre
ac_oliveira10@hotmail.com
Universidade de Coimbra (Coimbra, Portugal)

Oliveira, Eduardo 43
eduardoftoliveira@gmail.com
Universidade do Porto (Porto, Portugal)

Oliveira, Nuno
nunogrupo23@gmail.com
FCUL (Lisboa, Portugal)

Pires, Inês
inesdspires@gmail.com
FCT-UNL (Lisboa, Portugal)

Pires, Manuel
manuelmoreirapires@hotmail.com
Universidade de Coimbra (Coimbra, Portugal)

Preto, António José 44
martinsgomes.jose@gmail.com
CNC (Coimbra, Portugal)

Ramalhão, Daniel
dani.ramalhao.97@gmail.com
Universidade de Coimbra (Coimbra, Portugal)

Reis, Diogo 47
diogoreis992@gmail.com
FCUL (Lisboa, Portugal)

Reis, Pedro 48
pdreis@fc.ul.pt
FCUL (Lisboa, Portugal)

Renault, Pedro 50
barrosvic.renault@gmail.com
IBMM, Université de Montpellier (Montpellier, France)

Ribeiro, António 10
EBI (Cambridge, UK)

Ribeiro, João 7
jribeiro@illinois.edu
University of Illinois (Urbana-Champaign, Illinois, USA)

Rodrigues, João 52
joaor@stanford.edu
Stanford University (CA, USA)

Serra, Patrícia 55
pfserra@ff.ulisboa.pt
iMed.ULisboa, Faculdade de Farmácia da Universidade de Lisboa (Lisboa, Portugal)

Silva, Tomás 26
tfsilva@fc.ul.pt
FCUL (Lisboa, Portugal)

Simões, Carlos
carlos.simoes@bsimsquare.com
BSIM^2 (Cantanhede, Portugal)

Teixeira, Carla 57
carlateixeira_10@sapo.pt
Departamento de Quimica e Bioquimica da Faculdade de Ciências da Universidade do Porto (Porto, Portugal)

Victor, Bruno 59
blvictor@fc.ul.pt
iMed ULisboa, Faculdade de Farmácia da Universida de Lisboa (Lisboa, Portugal)

Vila-Viçosa, Diogo 61
diogo.vicosa@fc.ul.pt
CQC, FCUL (Lisboa, Portugal)

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