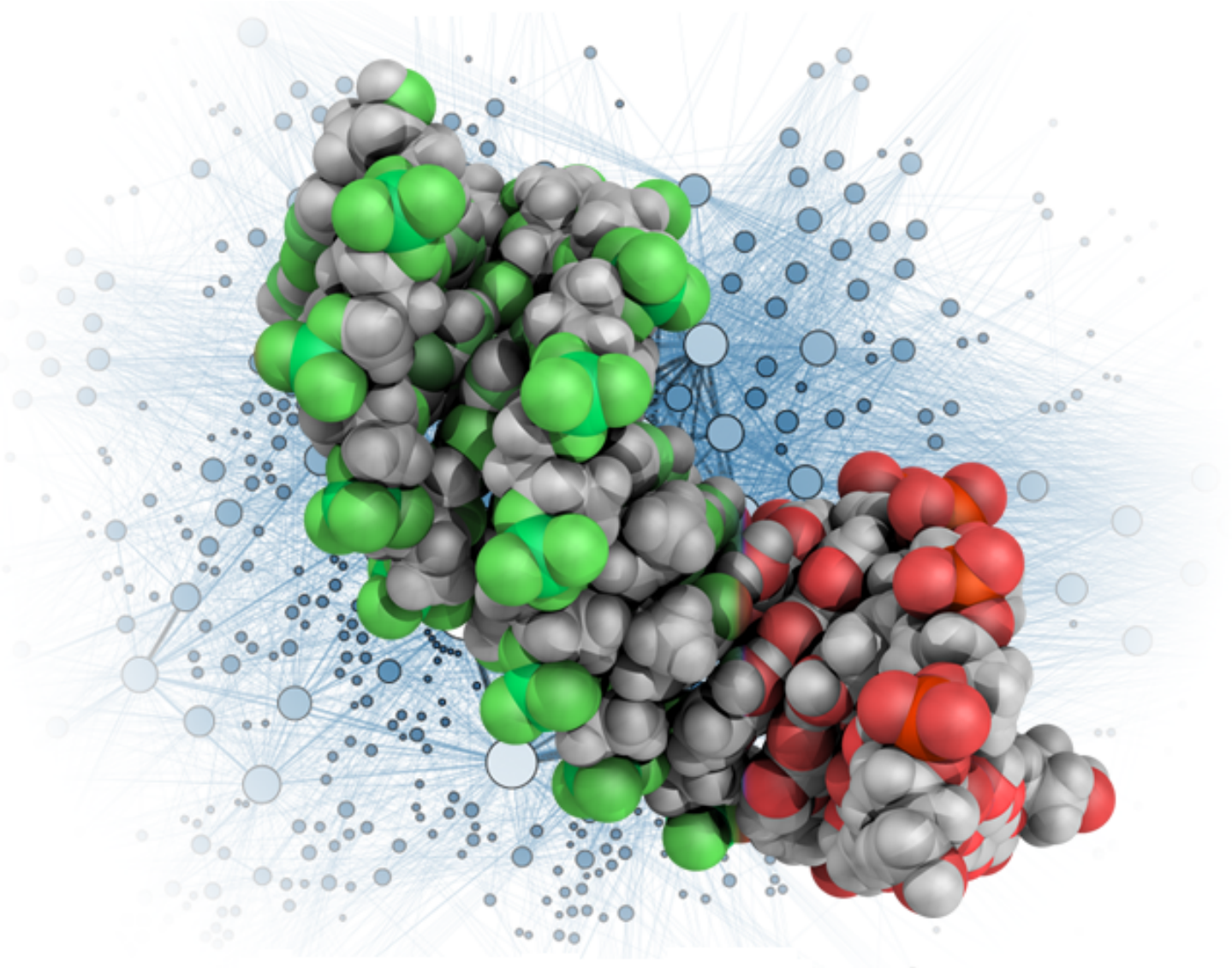
Encontro de Jovens Investigadores de Biologia Computacional Estrutural



Instituto Pedro Nunes

Coimbra, 18 de Dezembro de 2015

Missão e Objectivos

A partilha e discussão de ideias são as sementes para uma comunidade científica forte. Dada a presente situação económica, torna-se cada vez mais difícil manter e estimular um 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? As perguntas surgem naturalmente e as respostas nem sempre são simples de encontrar.

Esta iniciativa pretende dar resposta a algumas destas perguntas. Pretende dar a conhecer o que de melhor se faz na área da Biologia Computacional Estrutural em Portugal, e por outro lado, dar a conhecer o que estudam aqueles investigadores portugueses radicados no estrangeiro. Queremos proporcionar um espaço onde se possam divulgar e discutir projectos e resultados, com vista a estimular colaborações (a nível nacional e internacional) e a alargar os horizontes da Biologia Computacional Estrutural em Português.

Apoios

A organização do EJIBCE gostaria de agradecer o apoio das seguintes entidades e instituições, sem o qual não teria sido possível organizar este evento.

Localização

Instituto Pedro Nunes, Edifício A
Rua Pedro Nunes, Coimbra

Transportes Públicos
Proveniente da estação de comboios de Coimbra-B, apanhe o autocarro #29 até à paragem dos CTT (3 paragens), seguido da linha #24T até ao Parque do Vale das Flores (7 paragens).

Parques de Estacionamento
Estacionamento grátis no local.

Programa

O programa do EJIBCE 2015 consiste em dez comunicações orais por vários jovens cientistas portugueses e/ou a desenvolver o seu trabalho em Portugal, três das quais por convite. As restantes sete foram seleccionadas por um comité científico composto pelos seguintes membros:

Pedro Beltrão, *European Bioinformatics Institute* (Cambridge, UK)
Alexandre Bonvin, *Universiteit Utrecht* (Utrecht, NL)
Rui Brito, *Universidade de Coimbra* (Coimbra, PT)
Giorgio Colombo, *Consiglio Nazionale delle Ricerche* (Milão, IT)
Xavier Daura, *Institut de Biotecnologia i Biomedicina* (Barcelona, ES)

As comunicações orais foram enviadas ao comité científico e avaliadas pela sua originalidade e mérito científico. As restantes comunicações serão apresentadas em forma de poster numa sessão a decorrer durante todo o dia.

09:00 **Chegada e Registo dos Participantes**

09:45 **Abertura**

10:00 **Fátima Lucas**
Barcelona Supercomputing Center – Centro Nacional de Supercomputación & Anaxomics Biotech, Barcelona, ES

"*Computer-aided protein engineering"*

10:40 **Diogo Vila-Viçosa**
Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Lisboa, PT

"Molecular Modeling and Simulation of pH effects in Lipid Bilayers"

11:05 **Intervalo**

11:30 **Ana Sofia Oliveira**
Instituto de Tecnologia Química e Biológica António Xavier (ITQB) - Universidade Nova de Lisboa, Lisboa, PT

"Coupling between Protonation and Conformation in cytochrome c oxidase: Insights from Constant-pH MD simulations"

11:55 **Ricardo Ferreira**
Research Institute for Medicines (iMed.ULisboa), Lisboa, PT

"in silico Contributions on Drug Adsorption, Membrane Permeation, and P-glycoprotein Efflux Mechanism"

12:20 Espaço dos Patrocinadores: **Susana Tomásio**
CRESSET, Cambridge, UK

"Detailed Electrostatics to Understand Protein Active Sites"

12:40 **Almoço**

14:00 **Bruno Correia**
Laboratory of Protein Design and Immunoengineering, École Polytechnique Fédérale de Lausanne, Lausanne, CH

"Incursions on structural, chemical and computational biology: From the design of 'new' functions to the mapping of 'old' functional sites in proteins"

14:40 **Ana Rita Araújo**
MRC – Clinical Sciences Centre, Imperial College London, UK

"*Temporal insulation of mitosis: one more job for positive feedback"*

15:05 **Intervalo**

15:30 **Hugo dos Santos**
BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, PT

"Untangling the gene regulatory networks underlying motor neuron degeneration: from disease model transcriptomes to cellular systems"

15:55 **Rita Machado**
Universidade Federal de Minas Gerais, Belo Horizonte, BR

"Prediction of Drug Targets in Human Pathogens"

16:20 **Intervalo**

16:45 **Diana Lousa**
Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa, Oeiras, PT

"When simulation and experiment fuse: Analysing the interaction of the influenza fusion peptide with model membranes"

17:10 **Rita Santos**
GlaxoSmithKline (GSK), Cambridge, UK

"Passion and Brigadeiros – Key Ingredients for a Happy Career"

17:50 **Encerramento**

Oradores Convidados

Fátima Lucas

Barcelona Supercomputing Center – Centro Nacional de Supercomputación & Anaxomics Biotech, Barcelona, ES

"Computer-aided protein engineering"

Fátima Lucas é Investigadora sénior no Barcelona Supercomputing Center (BSC) e na empresa Anaxomics Biotech em Barcelona, Espanha. Licenciou-se em Química pela Universidade do Porto, onde efectuou também um mestrado e doutoramento, ambos em Química Computacional, sob orientação da Prof. Maria João Ramos. Após um período de pós-doutoramento de um ano na Universitat della Calabria, em Itália, estabeleceu-se no BSC onde obteve uma bolsa de investigador pós-doutoral da FCT e uma bolsa pós-doutoral Juan de la Cierva do MINECO. Os seus interesses de investigação encontram-se relacionados com a engenharia de proteínas com aplicações industriais. Em particular, no desenvolvimento de métodos computacionais capazes de guiar processos de desenho racional e/ou evolução dirigida de proteínas em laboratório.

Bruno Correia

Laboratory of Protein Design and Immunoengineering, École Polytechnique Fédérale de Lausanne, Lausanne, CH

"Incursions on structural, chemical and computational biology: From the design of 'new' functions to the mapping of 'old' functional sites in proteins"

Bruno Correia licenciou-se em Química na Universidade de Coimbra (2004) e obteve um doutoramento em Biologia Computacional no ITQB-UNL. No seu trabalho de doutoramento foi orientado por David Baker e Bill Schief (2006-2011) onde teve a oportunidade de desenvolver metodologias na área de desenho computacional de proteínas. Muito do seu trabalho foi centrado no desenho de proteínas funcionais, especificamente imunogénios com aplicações no desenvolvimento de novas vacinas. Durante o seu post-doc no grupo de Benjamin Cravatt no Scripps Research Institute - La Jolla (2011-2015), onde desenvolveu metodologias experimentais baseadas em espectrometria de massa para mapear interacções entre moléculas orgânicas e proteínas em proteomas celulares. Em 2015 tornou-se professor assistente em Bioengenharia na Ecole Polytecnhique Federale de Lausanne - Suíca, onde está a estabelecer um laboratório focado em engenharia de proteínas para fins terapêuticos utilizando estratégias experimentais e computacionais.

Rita Santos

GlaxoSmithKline (GSK), Cambridge, UK

"Passion and Brigadeiros – Key Ingredients for a Happy Career"

Rita Santos licenciou-se em Bioquímica na Universidade de Lisboa (2007), e obteve um mestrado na área de investigação e desenvolvimento de fármacos, com uma especialização em Química Computational e Toxicologia, na Vrije Universiteit Amesterdam (2009). Durante o mestrado, investigou o papel das moléculas de água em simulações de docking molecular do citocromo Humano P450 2D6, com o intuito de melhorar a predição de metabolismo de fármacos. Seguiu-se o doutoramento em Bioinformática na Universidade de Cambridge (2014), no grupo do Professor John Overington, onde investigou as diferenças entre grupos etários a nível molecular, que podem explicar diferentes reacções a fármacos (ex. criança vs. adulto). Desde Setembro de 2014 que está a trabalhar na GlaxoSmithKline como bióloga computational, e está envolvida em diversos projectos sobre identificação e validação de alvos terapêuticos.

Comunicações Orais

Molecular Modeling and Simulation of pH Effects in Lipid Bilayers

Diogo Vila-Viçosa[1], V. H. Teixeira[1], P. B. P. S. Reis[1], H. A. F. Santos[2], A. M. Baptista[3], M. Machuqueiro[1]

1. Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa (diogo.vicosa@fc.ul.pt)
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3. Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

Biological membranes are complex and diverse but share a common feature: they are all supported by a lipid bilayer. This bilayer is often negative and sensitive to pH and ionic strength. Computational/theoretical methodologies have been used to understand how these two properties influence the bilayer structure. However, the available methods have two significant bottlenecks. First, none of the available Poisson-Boltzmann (PB) solvers are able to deal with both periodicity and pKa calculations. Hence, we used a PB solver (DelPhi) in a new approach to perform pKa calculations taking into account the system periodicity. Secondly, to simulate highly charged membranes, a proper treatment of the ionic strength is crucial and a full neutralization of the system is probably too rough an approximation. Therefore, we developed a PB-based method to determine the number of ions that should be added to the simulations.

With these two problems solved, we developed a new constant-pH molecular dynamics method that is able to correctly model charged lipid bilayers (CpHMD-L). To the best of our knowledge, this is the only available method that can deal simultaneously with pH considering the protonation/conformation coupling, periodic boundary conditions in protonation free energy calculations, and a careful treatment of ionic strength. This represents a significant improvement in simulations of model biological membranes. It is now possible to take a step forward in the direction of biological membranes since we are able to simulate lipid mixtures presenting formal charges.

The Authors acknowledge financial support from Fundação para a Ciência e Tecnologia, Portugal, through project grants PTDC/QUI-BIQ/113721/2009, UID/MULTI/00612/2013, and fellowship SFRH/BD/81017/2011.

Coupling between Protonation and Conformation in cytochrome c oxidase: Insights from Constant-pH MD simulations

Ana Sofia F. Oliveira[1], Sara R. R. Campos[1], António M. Baptista[1], Cláudio M. Soares[1]

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Cytochrome c oxidases (CCOX) are members of the heme-copper oxidase superfamily and are the terminal enzymes of the respiratory chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular O2 to water with the creation of a transmembrane electrochemical proton gradient.

Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping but, until now, these mechanisms are still elusive. In particular, it is still not clear which are the functionally relevant conformational changes, nor how these rearrangements affect proton pumping. The main objective of this work is to identify the redox-induced structural changes and to study the dependence of protein's structure with the protonation state of certain residues. For that, we have performed extensive constant-pH MD simulations [1] of CCOX from Rhodobacter sphaeroides in two states (oxidized and reduced) inserted into a lipid bilayer. From our simulations, several residues with unusual titration behaviors highly dependent on the redox state of the metallic centers were identified.

[1] M. Machuqueiro, A.M. Baptista, Constant-pH molecular dynamics with ionic strength effects: protonation-conformation coupling in decalysine, J Phys Chem B, 110 (2006) 2927-2933.

in silico Contributions on Drug Adsorption, Membrane Permeation, and P-glycoprotein Efflux Mechanism

Ricardo J Ferreira[1], Maria-José U. Ferreira[1], Daniel J. V. A. dos Santos[2]

1. Research Institute for Medicines (iMed.ULisboa), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
2. REQUIMTE, Department of Chemistry and Biochemistry, Rua do Campo Alegre, 4169-007 Porto, Portugal

Multidrug resistance (MDR) to anticancer drugs has become a serious health concern due to increasing chemotherapy failures worldwide. Although P-glycoprotein (P-gp) is one of the main contributors for MDR, the biophysical aspects of drug efflux by P-gp still remain largely unknown. Following our characterization of three drug-binding sites within P-gp\'s internal drug-binding pocket (DBP), a series of molecular dynamics studies were performed using a refined P-gp structure to further clarify how drug efflux occurs and how drug adsorption to either the protein or the membrane affects P-gp activity.

It was hypothesized that molecules have access to the internal DBP through entrance gates but no experimental proof was given thus far. The molecule transfer from the membrane\'s hydrophobic environment into the water-filled DBP revealed similar permeation profiles for both substrate (colchicine) and modulator (tariquidar), with a ΔΔGentry between both molecules of 12 kJ.mol-1. These results show that drug permeation to the lipid, entry to the DBP and release to the extracellular medium (as shown by Oliveira et al. for the Sav1866 transporter) are characterized as energetically favorable processes.

Drug-membrane interactions are also predicted to affect ATPase function by inducing long-range mechanical alterations. We assessed the effect of drug adsorption to both protein-water and lipid-water interfaces and important differences in drug-protein interactions, protein dynamics and membrane biophysical properties were observed between non-substrates, substrates and modulators. Therefore, we hypothesize that drug adsorption to the protein- or lipid-water interface account for a complex network of events that affects the transporters' ability to transport drugs.

Temporal insulation of mitosis: one more job for positive feedback

Ana Rita Araújo[1], Rahuman Sheriff[1], Lendert Gelens[2], Sílvia D. Santos[1]

1. MRC-Clinical Sciences Centre, Imperial College London, London, UK
2. Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, USA

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, preliminary quantification of the length of cell cycle phases in single cells by live-cell imaging showed high variability in the dynamics of cell cycle phases amongst different cell types. An exception was seen in mitosis, where different cells seem to maintain a short and constant length of time to complete this phase. Surprisingly, there is no correlation between cell cycle length and mitosis 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 will complete mitosis in a short and synchronous manner. It was shown that positive feedback regulation is crucial to keep mitotic events synchronized (Holt et al 2008, Santos et al 2012) and so, we hypothesize 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 modelling we showed that when positive feedback is perturbed the switch-like activation of Cdk1 is compromised, leading to a more variable duration of mitosis and a correlation between the length of the cell cycle and length of mitosis. 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.

Untangling the Gene Regulatory Networks Underlying Motor Neuron Degeneration: From Disease Model Transcriptomes to Cellular Systems

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1. BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa - Lisboa, PT
2. Instituto de Medicina Molecular (IMM), Faculdade de Medicina, Universidade de Lisboa – Lisboa, PT
3. Formation and Regulation of Neuronal Connectivity Research Unit, Okinawa Institute of Science and Technology Graduate University - Okinawa, JP
4. Department of Cell Biology, Harvard Medical School - Boston, USA

Spinal Muscular Atrophy (SMA), the leading cause of genetically linked infant death, is a neurodegenerative disorder characterized by low levels of the ubiquitous Survival of Motor Neuron (SMN) protein, a key player for the assembly of spliceossomal small nuclear ribonucleoproteins (snRNPs). Strikingly, low levels of SMN mainly affect motor neurons (MN), disrupting neuromuscular junctions (NMJ) thus leading to MN degeneration. Despite robust knowledge of SMA's genetics, the exact molecular mechanisms underlying SMA\'s phenotype remain largely elusive, preventing the development of rational therapeutics. Multiple, non-mutually exclusive hypothesis have been proposed, ranging from the increased sensitivity of MN to altered splicing to the involvement of SMN in non-splicing related, MN-specific functions.

In order to assess this question, we performed RNA-Seq profiling of the central nervous system (CNS) transcriptome of a Drosophila melanogaster SMA disease model, in parallel with a similar analysis focused on human motor neuron cultures derived from patient induced pluripotent stem cells (iPSCs).
Upon SMN down-regulation we observe inter-species conserved changes in exon usage in a subset of genes crucial for neuronal development, viability and NMJ function. This suggests that SMN-dependent changes in the splicing machinery do not have widespread effects, affecting specific genes possibly due to the existence of certain features in their primary and secondary structure. Interestingly a large proportion of identified genes with altered splicing are known genetic modifiers of the SMN loss-of-function phenotype in SMA fly models, thereby supporting the search for innovative therapeutic approaches to SMA.

Prediction of Drug Targets in Human Pathogens

Rita Silvério-Machado[1], Miguel Rocha[2], Carlos J. V. Simões[3], Rui M.M. Brito[3,4], Bráulio R.G.M. Couto[5], Marcos A. Dos Santos[6]

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The identification of new and "druggable" targets in bacteria is a critical endeavour in pharmaceutical research of novel antibiotics to fight infectious agents. The rapid emergence of resistant bacteria makes today's antibiotics more and more ineffective, consequently increasing the need for new pharmacological targets and novel classes of antibacterial drugs.

A new model that combines the singular value decomposition technique with biological filters comprised of a set of protein properties associated with bacterial drug targets and similarity to protein-coding essential genes of E. coli has been developed to predict potential drug targets in the Enterobacteriaceae family [1]. This model identified 99 potential target proteins amongst the studied bacterial family, exhibiting eight different functions that suggest that the disruption of the activities of these proteins is critical for cells.

Out of these candidates, one was selected for target confirmation. To find target modulators, receptor-based pharmacophore hypotheses were built and used in the screening of a virtual library of compounds. Post-screening filters were based on physicochemical and topological similarity to known Gram-negative antibiotics and applied to the retrieved compounds. Screening hits passing all filters were docked into the protein's catalytic groove and 15 of the most promising compounds were purchased from their chemical vendors to be experimentally tested in vitro.

To the best of our knowledge, this is the first attempt to rationalize the search of compounds to probe the relevance of this candidate as a new pharmacological target.

[1] Silverio-Machado, R., Couto, B.R. and Dos Santos, M.A. (2015) Retrieval of Enterobacteriaceae drug targets using singular value decomposition. Bioinformatics, 31, 1267-1273.

When simulation and experiment fuse: Analysing the interaction of the influenza fusion peptide with model membranes

Diana Lousa, Antónia R. T. Pinto, Ana Salomé Veiga, Alessandro Laio, Miguel A. R. B. Castanho and Cláudio M. Soares

1. ITQB-UNL, IMM-FMUL, SISSA

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. 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 studies. 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.

Espaço dos Patrocinadores

Detailed Electrostatics to Understand Protein Active Sites

Susana Tomásio

CRESSET, Cambridge, UK

The Cresset XED force field has shown to be excellent at computing the electrostatic properties of small molecules. One of the goals at Cresset has been to apply the unique features of XED to proteins. We present results from two research projects in this area: describing the electrostatic environment in a protein active site and using that to assist in ligand design, and using XED combined with 3D-RISM theory to get an accurate picture of the energetics of bound waters in a protein active site.

Posters em Exposição

P1. Molecular events triggering the aggregation cascade in beta-2-microglobulin amyloidosis

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The identification of intermediate states for folding and aggregation is an important challenge, not only from a fundamental standpoint, but also for the design of novel therapeutic strategies targeted at the so-called conformational disorders. A well-known example is dialysis related amyloidosis (DRA), a fatal condition that affects individuals with kidney impairment undergoing dialysis. In DRA, protein beta-2-microglobulin (β2-m) aggregates into amyloid fibrils in the osteoarticular system eventually leading to tissue erosion and destruction. Recently, a single point mutant of β2-m (D76N) was identified as the causing agent of a new systemic amyloidosis affecting visceral organs. We are currently using D76N as a model system to study the aggregation mechanism of β2-m with molecular simulations. This poster presents results of ongoing work on a comparative study of the early molecular events triggering the amyloid cascade in two β2-m variants (D76N and ΔN6). In particular, discrete molecular dynamics simulations of the folding transition highlight the existence of an aggregation-prone folding intermediate state whose topology is conserved across the two variants and a new intermediate that is unique to D76N, while Monte Carlo docking simulations provide a structurally resolved picture of the dimerization phase highlighting key molecular events that are common to both model systems.

P2. Applying molecular structural similarity for hERG inhibition prediction

Joana M. Barros[1], João Monteiro[1], André A. O. Falcão[1]

1. LaSIGE, Faculdade de Ciências, Universidade de Lisboa, Lisboa, PT

The hERG protein is crucial in maintaining the heart's normal function, however, due to its promiscuity to a variety of molecules it is estimated that about 40-70% of all new drug-like molecules affect hERG. Given this problem, we propose a quantitative structure-activity relationship approach (QSAR) to determine hERG inhibition. To achieve this, we focused on three aspects: (1) collecting and curating a diverse data set of 2719 molecules, which were retrieved from the literature and from the ChEMBL database; (2) applying and testing several feature reduction methods over different molecular descriptors and (3) the application of molecular structural similarity, calculated through the Noncontiguous Atom Matching Structural Similarity (NAMS) method, to improve the models. After the selection of the feature reduction method, Random Forests and Support Vector Machines were applied to build the prediction models. To choose the best models, we applied a strict validation procedure with 5-fold cross-validation, which resulted in a selection of three different models, two of them reliant on the availability of structurally similar molecules in the database. These models were validated using an independent set and yield a percentage of explained variance up to 62.5 and mean squared errors between 0.523 and 0.320. Given the results obtained, and online prediction tool named hERGIP, which is available at http://hergip.lasige.di.fc.ul.pt/, was developed using the models achieved.

P3. Theoretical structural studies for the development and synthesis of stereoselective aza-sugars with improved activity towards Golgi alpha-mannosidase II

Bárbara Abreu[1,2], Nuno Micaêlo[1,2]

1. Protein Modelling Lab, ITQB-UNL Estação Agronómica Nacional 2780-157, Oeiras, PT
2. Molecular Modelling and Simulation Lab, Chemistry Centre, University of Minho, 4710-730, Braga, PT

Most tumors show altered glycosylation patterns. Some of them are associated to cancer progression events, such as metastasis, tissue invasion, growth and non-recognition by the immune system [1] [2]. Golgi alpha-mannosidase II (GMII) plays a key role in the N-glycosylation pathway, trimming two mannose residues. The inhibition of GMII leads to a decrease in cancer-associated oligossacarides, providing a potential target for chemotherapy. Swainsonine is

the most potent inhibitor of GMII known. However, it is known to have side effects resulting of Lysossomal α-mannosidases (LM) inhibition, which is involved in glycoprotein degradation [3].

In the present work, new swainsonine derivatives were conceived and evaluated in a close collaboration with synthetic chemists. Their activity and selectivity of was evaluated using docking against a flexible model comprising crystallographic structures and a set of structures obtained by molecular dynamics simulations. Thermodynamic integration (TI) calculations were made in order to check structure-activity relations in a more accurate way.

It was found that most swainsonine derivatives showed higher or similar affinity and similar binding modes. A set of molecules possessing longer sidechains bound to an extra pocket, exclusive of GMII, displaying potential selectivity. TI calculations allowed the assessment of the energetic contribution of extra groups in binding affinity. The most promising compounds are currently being synthetized.

Acknowledgements: The authors acknowledge to GRIUM cluster of University of Minho and to FCT for financial support and a research grant (PTDC/QEQ-MED/1671/2012).

[1] Dennis, J. W.; Laferte, S., Cancer research 45 , 6034-40 (1985); [2] van den Elsen, J. M.; Kuntz, D. A.; Rose, D. R., The EMBO journal 2

P4. Adding explicit sigma-holes in force fields for biomolecular simulations: the do's and don'ts

Diogo Vila-Viçosa[1], Miguel Machuqueiro[1], P.J. Costa[1]

1. Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, PT

Halogen atoms are commonly used in drug design to fill hydrophobic cavities in protein binding sites, improve blood–brain barrier crossing or facilitate membrane permeability. Additionally, halogens are capable of establishing a directional, non-covalent interaction, known as halogen bond, consisting on an R-X···B interaction, where X = Cl, Br, I and B = Lewis base. These bonds are explained by the existence of an electron deficient region at the tip of X, called sigma-hole, which interacts with Lewis bases.

Halogen bonds play an important role in many protein-drug interactions and several structures deposited in the RCSB Protein Data Bank show this type of interaction, thus showing its potential for rational drug design. Notwithstanding its importance, the implementation of halogen bonding in biomolecular force fields is rare and often rely on the use of a massless points of charge at a fixed distance to emulate the sigma-hole, depending solely on the nature of the halogen. [1] Here we show, for a prototype ligand containing iodine, that ignoring the chemical substituents near the halogen in the definition of the massless points of charge, leads to an incorrect description of the charge at that point. Ultimately, given the predominantly electrostatic nature of the halogen bonds, this also leads to an incorrect description of the halogen bond length and strength.

[1] (a) M. A. A. Ibrahim, J. Comput. Chem. 2011, 32, 2564–2574. (b) Bronowska et al, Chem. Commun., 2013, 49, 981-983.

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P5. Structural mechanism of HER2-antibodies complexes by molecular dynamics studies

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Various types of nanoparticles have been developed as multifunctional, target-specific platforms for cancer Theranostics. Among them, virus-like particles (VLPs) hold great potential owing for to their superior intrinsic biological properties. Indeed, effective tumor targeting can be achieved by modification of viral tropism through

conjugation to ligands that bind to specific receptors expressed on target cells. Overexpression of the Human Epidermal Growth Factor Receptor 2 (HER2) plays a key role in the development and progression of certain aggressive types of breast cancer. Thus, the development of innovative approaches on anti-HER2 therapies is a field of intense research where the potential role of modified VLPs is still under researched.

In order to attain a deeper understanding of the interaction of specific anti-HER2 antibodies and HER2, computational methods modelling and Molecular Dynamic (MD) simulations were employed. 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 being employed. Our aim is to understand how to modify and control the formation of these macromolecular systems.

P6. Insights on molecule recognition and substrate specificity by P-glycoprotein

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Despite the advances in medical and in pharmacological treatment, some types of cancer acquire a Multidrug Resistant (MDR) phenotype, allowing them to overcome chemotherapeutical regimens. One of the main causes for MDR is related to the increased expression of ABC transporters at the surface of cancer cells. To that matter, P-glycoprotein (P-gp) is still the most representative member in MDR, being involved in the multidrug-resistance phenomenon by exporting a huge range of xenobiotics and lowering their intracellular concentration. Thus, ABC transporters and particularly P-gp are still considered a potential molecular target to overcome MDR by modulating drug efflux in cancer and tumor cell lines.

Nevertheless, the molecular mechanism by which P-gp recognizes its substrates is still poorly understood. Yet, it is known that some mutations in the drug-binding pocket are related with changes in substrate specificity and recognition by the transporter. From a recently obtained homology model for human P-gp based on the published murine crystallographic structures, three mutations (G185V, F978A and ΔF335) were introduced to assess the molecular mechanisms underpinning substrate specificity.

Our preliminary results suggest that rotations in the transmembranar helices may be one of the causes that leads to alterations in the substrate specificity, in agreement with experimental results available in literature. Moreover, the angle of rotation appears to be intimately correlated with the location of the mutation, promoting shifts in the orientation of the residue side-chains and/or helix repacking within the drug binding site, that ultimately affects substrate specificity.

P7. Computational studies addressed to the catalytic mechanism of 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.

In this work we studied the reaction that takes place in the α-subunit active site using computational means and QM/MM hybrid methodologies [1]. 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 (Ea=18,07 Kcal/mol, Er=8,24 kcal/mol) 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 (Ea=14,16 Kcal/mol, Er=-4,87 Kcal/mol).

The results go in line with previous experimental studies that have shown that a TS mutant in which αGlu49 is substituted by Asp is totally devoid of α activity [2], while a mutant form with Glu at position 60 instead of an Asp has partial activity [3].

[1] Oliveira EF, Cerqueira NM, Fernandes PA and Ramos MJ 2011. J Am Chem Soc 133: 15496-15505; [2] Yutani K, Ogasahara K, Tsujita T, et al. (1987) J Biol Chem 262: 13429-13433; [3] Nagata S, Hyde CC and Miles EW 1989. J Biol Chem 264: 6288-6296

P8. Computational studies addressed to the catalytic mechanism of Histidine Decarboxylase

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Mammalian histidine decarboxylase (mHDC) is an enzyme that requires pyridoxal-5'-phosphate (PLP) as a cofactor [1]. mHDC belongs to the group II of PLP-dependent decarboxylases together with L-DOPA and glutamate decarboxylases, and catalyses the L-histidine decarboxylation from which results histamine.

Histamine plays a key role in several biological events such as immune response, gastric system modulation and as a neurotransmitter in the nervous system. Several inhibitors for histamine action have been studied in order to treat some diseases such as atopic dermatitis, allergies, and cancer.

mHDC has been studied for a long time, but only in 2012 Komori's [2] group was able to determine X-ray structure of the enzyme and revealed the active site environment. Until date, only hypothesis about the mechanism of mHDC were available and based on homology models (that propose a different active site configuration).

In this work we are studying the catalytic mechanism of mHDC by computational means using the recent X-ray structure of mHDC and a QM/MM methodology.

The results have shown that mHDC catalyses the reaction in a two-step type of mechanism. The first step involves a decarboxylation that is followed by the formation of a carbanion. In the second step, the carbanion is protonated by a base from which results histamine. Our early results indicate that the first step is the limiting reaction step and the full reaction is endothermic by approximately 25 kcal/mol.

[1] Ngo HP, Cerqueira NMFSA, Kim JK, Hong MK, Fernandes PA, et al. 2014. Acta Crystallogr D Biol Crystallogr 70: 596-606; [2] Komori H, Nitta Y, Ueno H, Higuchi Y. 2012. Acta Crystallogr Sect F Struct Biol Cryst Commun 68: 675-7

P9. Characterization of the β2m-ThT complex using molecular dynamics and alchemical free energy calculations

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The development of new and better molecular probes that can be used for the early diagnosis of amyloid diseases such as Alzheimer's, Parkinson's and type II diabetes are of critical essence. β-2 microglobulin (β2m) is a component protein of the Major Histocompatibility Complex class I. In patients on long term hemodialysis, β2m can aggregate into amyloid fibers that deposit in joint spaces causing a disease known as dialysis-related amyloidosis. To evaluate the structural stability of both the β2m dimer and its complex with the well-known amyloid probe thioflavin-T (ThT), we performed several MD simulations. The results obtained in these simulations were in agreement with several reports found in the literature, where the Q8 and Y10 residues of β2m were shown to be crucial for the interaction with ThT. To better characterize the topological constraints imposed by these residues, we have mutated Q8A, Y10A and Y10F, and performed additional MD simulations. According to our results, the benzene ring of the Tyr residue is crucial to the interaction of ThT with β2m, as with the mutation Y10A, this interaction is strongly affected. Additionally, in order to quantify the energetic contribution of these residues to the stability of the β2m-ThT complex,

we have also performed several AFEC. These calculations show that the most important residue of β2m for the interaction with ThT is Y10, as the mutation of Y10A is the most unfavorable.

P10. Design space methodology maps protein abundance patterns to phenotype of cellular responses to hydrogen peroxide

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The peroxiredoxin II (Prx2) / thioredoxin 1 (Trx1) / thioredoxin reductase (TrxR) system (PTTRS) shows distinct responses to H2O2 in distinct cell types. E.g., exposure of human erythrocytes to micromolar H2O2 causes oxidation of Prx2 and Trx1 to disulfide forms and little Prx2 sulfinylation, whereas a similar treatment to Jurkat T cells causes extensive Prx sulfinylation and limited Trx1 oxidation. Can these distinct responses be explained by the distinct gene expression profiles in these cells based on the known kinetic properties of the PTTRS proteins? What expression patterns originate each type of behavior?

By applying the systems design space methodology [1,2] to a model of the PTTRS that accounts for Prx1/2, Trx1, TrxR1, sulfiredoxin, peroxidases and catalase, we derived a concise approximate condition that predicts the response to H2O2 from the relative abundances of these proteins. Further, the analysis of quantitative proteomic datasets for human cell lines indicates that despite considerable variation in the protein abundances all operate in a well-defined region of the design space. Namely, one that permits near-ideal transduction of H2O2 signals at low concentrations but prompts extensive sulfinylation, bi-stability and hysteresis upon exposure to high H2O2 concentrations. Given that Prx2 sulfinylation is known to arrest the cell cycle, the analysis suggests a potential novel strategy in cancer therapy.

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[1] Coelho et al. (2009) PLoS Comput Biol 5, e1000319.; [2] Savageau et al. (2009) PNAS 106, 435–6440.

P11. Molecular Dynamics and Pharmacophore Modeling studies aiming the discovery of novel PARP-1 inhibitors

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PARP-1 is the best characterized member of PARP (Poly-(ADP-ribose) polymerase) superfamily and plays a critical role as a DNA damage sensor. It recognizes and binds DNA strand-breaks through the N-terminal region. Following this binding event, its C-terminal catalytic domain becomes activated, causing formation and addition of polyadenosine-ribose to acceptor proteins, thereby modulating their functions [1, 2, 3].

The development of medicinal chemistry approaches to synthesize potent and selective PARP-1 inhibitors has been pursued with therapeutic relevance in a range of diseases including stroke, inflammation, diabetes and cancer. In spite of all the effort to find out promising leads, recent data indicate that these compounds usually lack binding specificity among PARP family members. For this reason, more in depth studies of the determinants of PARP-1 recognition features are needed which ultimately have to be linked to the development of novel and more selective PARP-1 inhibitors [4, 5, 6].

In this work we performed explicit-solvent Molecular Dynamics (MD) simulations with different inhibitors bound to PARP-1 catalytic domain to analyze complex functional dynamics and to characterize the dynamic features of active site-ligand interactions in the enzyme. The combined statistical characterization of ligand-protein interactions derived from MD trajectories was used to generate selective structure-based pharmacophores.
The pharmacophore models were used for virtual screening of large compounds databases. After docking analysis, the best candidates were selected for evaluation by enzyme inhibition assays and showed interesting enzyme inhibiting activities.

[1] Rouleau, M.; Patel A.; Hendzel, M.J.; Kaufmann, S.H.; Poirier GG PARP inhibition: PARP1 and beyond. Nat Rev Cancer 10(4):293-301 (2010); [2] Eustermann. S.; Videler, H.; Yang, J.; Cole, P.T.; Gruszka, D.; Verpreintsev D, Neuhaus D. The DNA-binding domain of human PARP-1 interacts with DNA single-strand breaks as a monomer through its second zinc finger. J Molec Biol, 2011, 407 (1), 149-170.; [3] Benafif, S.; Hall, M. An update on PARP inhibitors for the treatment of cancer. OncoTargets and Therapy, 2015, 8 519-528.; [4] Ferraris, D.V.; Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem, 2010, 53 (12), 4561-4584.; [5] Ekblad, T.; Camaioni, E.; Schüler, H.; Macchiarulo, A. PARP inhibitors: polyphar macology versus selective inhibition. FEBS J. 2013; [6] Steffen, J.; Brody, J.; Armen, R.; Pascal, J. Strutural implications for selective targeting of PARPs. Front Oncol, 2013, 3:301

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P12. pKa values of titrable amino acids at the water/membrane interface

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Many important biological processes happen at the membrane/water interface, for which there is scarce information at the molecular detail. It is commonly accepted that peptides and proteins protonation equilibrium is strongly influenced by its surrounding media. Until now, there are no quantitative and systematic studies reporting the pKa shifts in the common titrable amino acids upon lipid membrane insertion. Here, we applied our recently developed CpHMD-L method to calculate the pKa values of titrable amino acid residues incorporated in Ala-based pentapeptides at the water/membrane interface. We observed that membrane insertion leads to desolvation and a clear stabilization of the neutral forms. Consequently, we quantified the increases/decreases of the pKa values in the anionic/cationic residues along the membrane normal. This work highlights the importance of properly modeling the protonation equilibra of peptides and proteins interacting with membranes using MD simulations.

P13. Sugar-based surfactants as antibacterial agents targeting lipid bilayers: insights from molecular dynamics simulations

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Sugar-based surfactants have been used in several applications, ranging from membrane protein crystallography to food industries, mainly due to their biocompatibility properties and low toxicity. Recently, a family of alkyl deoxy glycosides with relevant antibacterial activities against several pathogens was developed [1]. Experimental data shows that the deoxygenation of the sugar moiety leads to an increased surface activity of these molecules, which seems to modulate their antimicrobial properties. In this work, we used atomistic molecular dynamics simulations to characterize the micellization process of these glycosides in aqueous media and the adsorption of these micelles to a model phospholipid bilayer. We also simulated phospholipid/glycoside binary mixtures to analyze the effect of these glycosides on the structural features of the lipid bilayer upon partitioning. The results herein presented provide valuable information regarding the mechanism of action of these antibacterial molecules and may have implications for the design of new antibiotics with increased potency.

[1] A. Martins, M. S. Santos, C. Dias, P. Serra, V. Cachatra, J. Pais, J. Caio, V. H. Teixeira, M. Machuqueiro, M. S. Silva, A. Pelerito, J. Justino, M. Goulart, F. V. Silva, A. P. Rauter, Eur. J. Org. Chem., 2013, 8, 1448-1459.

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