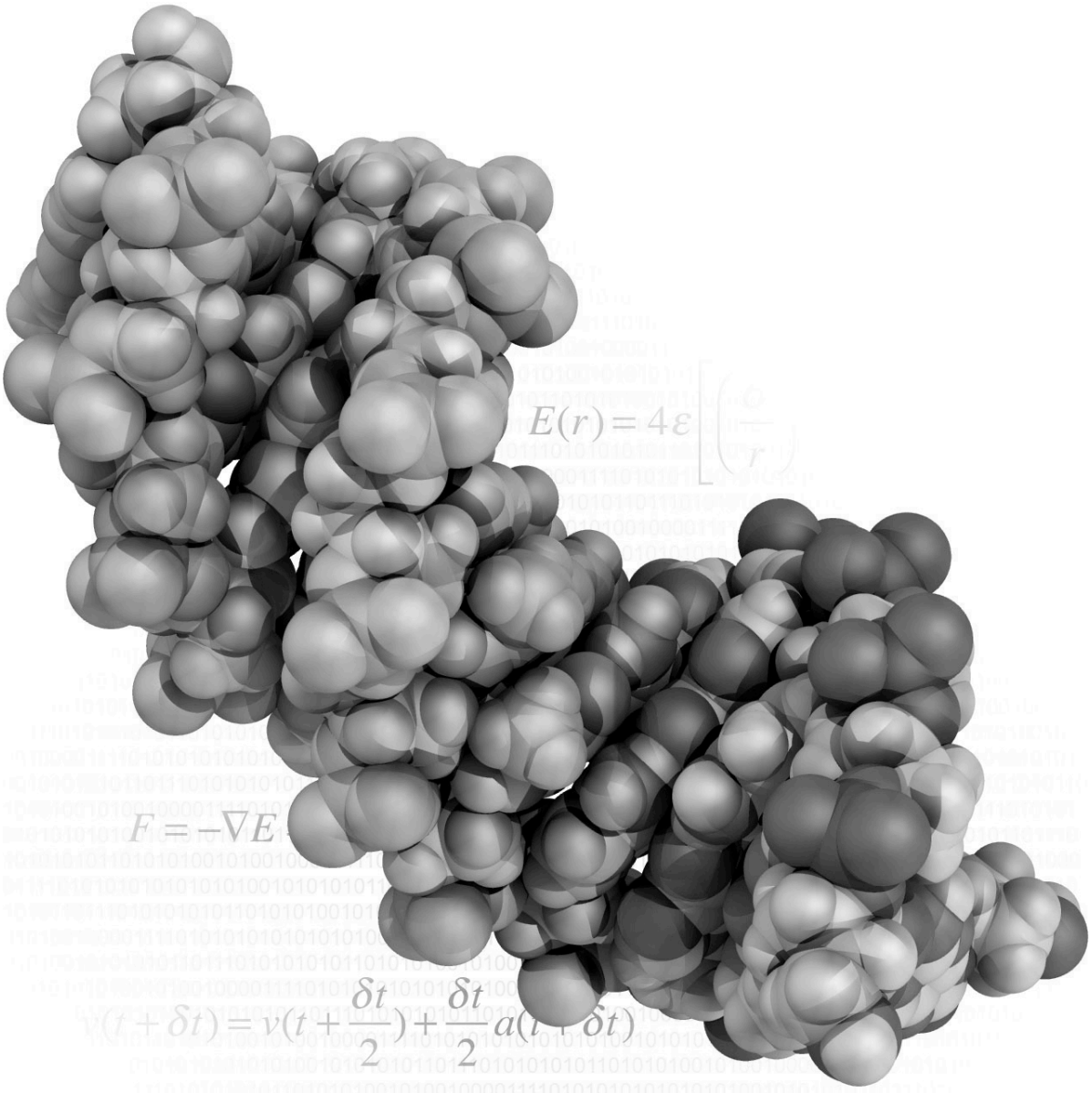
Encontro de Jovens Investigadores de Biologia Computacional Estrutural



Porto, 20 de Dezembro de 2013

Organização

Fátima Lucas
João Martins
Irina Moreira
António Pimenta
João Rodrigues

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, sem o qual não teria sido possível organizar um evento desta qualidade.

Localização

Casa do Infante
Rua da Alfândega 10, Porto

Transportes Públicos

Linhas STCP
500, 900, 901, 906, ZM, ZR
Metro
Estação de São Bento

Parques de Estacionamento
Parque da Alfândega
Parque da Praça do Infante D. Henrique

Programa

O programa do EJIBCE 2013 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:

Alexandre Bonvin, Universiteit Utrecht (NL)
Victor Guallar, Barcelona Supercomputing Center (ES)
Michael Levitt, Stanford University (USA)
Harel Weinstein, Cornell University (USA)

As comunicações orais foram enviadas ao comité científico sem nomes ou afiliações de modo a tornar o processo de selecção o mais idóneo possível, e avaliadas pela sua originalidade e mérito científico. As restantes comunicações serão apresentadas em forma de poster numa sessão a decorrer durante a pausa para almoço. Os posters serão no entanto expostos durante todo o dia.

09:00 **Chegada e registo dos participantes**

10:00 **Rui Travasso**
Center for Computational Physics (CFC) and Institute for Biomedical Imaging and Life Sciences (IBILI), Universidade de Coimbra, Coimbra, Portugal

"*Kinetics of the folding process: Important lessons from coarse-grained models"*

10:40 **Miguel Machuqueiro**
Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

"New in silico methods to model biological membranes with increased realism"

11:05 **Intervalo**

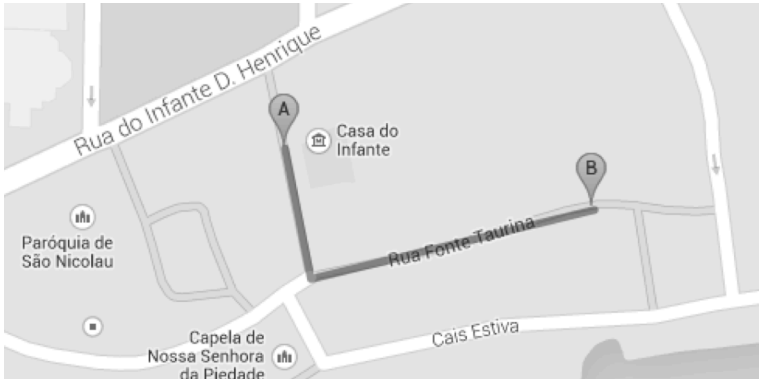
11:30 **João Teixeira**
University of Coimbra, Portugal & CERM, University of Florence, Italy

"Joint use of NMR and bioinformatic tools to unveil the dynamics and the 'tectonic' enzymatic mechanism of MMP-1"

11:55 **Adrià Gil-Mestres**
Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

"How the Intercalation of Phenanthroline Affects the Structure, Energetics and Bond Properties of DNA Base Pairs. Theoretical Study Applied to Adenine-Thymine and Guanine-Cytosine Tetramers"

12:20 **Almoço e sessão de posters**



14:00 **Nuno Cerqueira**
REQUIMTE, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n 4169-007 Porto, Portugal

"The sulfur-shift: a new activation mechanism in mononuclear Mo enzymes."

14:25 **Daniel Dourado**
Department of Cell and Molecular Biology, Computational and Systems Biology*, Uppsala university, Biomedical Center Box 596, 751 24, Uppsala, Sweden*

"*Local backbone flexibility is key to accurate prediction of the protein-protein binding energy"*

14:50 **Intervalo**

15:15 **Pedro Beltrão**
EMBL-EBI, Cambridge, UK

"Function and Evolution of Protein Post-translational Modifications"

15:55 **Bruno Correia**
The Scripps Research Institute - La Jolla - USA

"Development of the computational structural biology toolbox towards the design of novel vaccines"

16:20 **Intervalo**

16:45 **Manuel Melle Franco**
Centro de Ciências e Tecnologias de Computação, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

"Fundamental interactions and the cellular toxicity of nanomaterials"

17:10 **Carlos Simões**
BSIM^2, Cantanhede, Portugal

"From gloves to keys, from hits to leads, from academia to industry, from hope to hype. Or is it the other way around?"

17:50 **Encerramento**

Oradores Convidados

Rui Travasso

Center for Computational Physics (CFC) and Institute for Biomedical Imaging and Life Sciences (IBILI), Universidade de Coimbra, Coimbra, Portugal

"Kinetics of the folding process: Important lessons from coarse-grained models"

Through coarse-grained models we are able learn about the kinetic properties of protein folding by isolating the role of specific characteristics of proteins on the folding process. Specifically, we use coarse-grained models to study the folding of proteins of different topological complexities, drawing conclusions with respect to the relation between protein structural complexity and folding rate. We also identify the residues that have the most important role in the folding process, indicating how they can be found experimentally and how they are determined by the structure of the protein. Finally, we discuss the role of non-native interactions in the folding process.

Pedro Beltrão

EMBL-EBI, Cambridge, UK

"Function and Evolution of Protein Post-translational Modifications"

Cells have evolved intricate systems to sense changes in internal and external conditions. These changes are processed by the cell triggering an appropriate response most commonly via post-translational modifications (PTMs) of proteins. Recent advances in mass-spectrometry have allowed for the large-scale characterization of post-translational modifications with thousands of novel PTMs identified per experiment. However, little is known about the function of these modifications and how these post-translational regulatory networks change during evolution. To study this we curated over 200.000 published PTMs for 14 species. Our analysis suggests that PTMs diverge at a very fast rate and there are a significant number of non-functional modifications. Using structural information we predicted PTMs that were more likely to be functional by regulating protein-protein interfaces, domain activity or conformational variability. We observe also that the positional conservation of phosphosites significantly under-predicts the conservation of function suggesting that neutral evolution may play a role in shaping phosphorylation networks.

Carlos Simões

BSIM^2, Cantanhede, Portugal

"From gloves to keys, from hits to leads, from academia to industry, from hope to hype. Or is it the other way around?"

1986 has been claimed as the year "rational drug design" was born. Indeed, many (if not most) of the expressions that accompany us today in the relatively new field of computational chemistry were introduced at some point in the 80s. To state a few: protein modelling, conformational searching, molecular docking, free-energy calculations, quantum mechanics, molecular mechanics, molecular dynamics and statistical thermodynamics, QSAR, QSPR, chemometrics, library design, de novo design, bioavailability predictions, chemical databases and searching, virtual screening, high throughput docking, pharmacophore models and searching, data analysis and visualization. The greatest advances observed in the field therefore seem to be more linked to Moore's law and progress in computing power rather than the techniques themselves. Nevertheless, in a time where the challenges posed by the looming patent cliff and the ballooning costs of discovering new chemical entities are causing instability in Pharma and Biotech industries, the role of computational chemistry and biology in the drug discovery process is gaining more and more relevance. Once again, what some regard as a threat to the modelling community others see as opportunity. Mankind needs medicines, and now that waiting for serendipity is becoming a less profitable business, only through research can we find them. This research can burst into a new level if the bond between industry and academia gets tighter. For instance, imagine the benefit of thousands and thousands of X-ray structures of protein–ligand complexes finding their way out of big Pharma's firewalls and flowing to academia… Wouldn't that be a sight? In my talk I will make use of a few experiences collected along a "rocky-RMSD" trajectory in the merger of computational chemistry and structural biology to highlight some of the weaknesses and strengths of these disciplines. The talk will comprise two main parts. The first part will focus on work carried out in academia to study amyloid formation and disease using simulation methods, and to identify new amyloid inhibitors by exploring virtual screening techniques. In the second part I will tell you how we are making use of an entrepreneurial adventure to map pharmacological space towards the optimization of new drug candidates for the treatment of "doença dos pezinhos" and Alzheimer's disease, while building a new kind of relationship between academia and industry. I will briefly tell you the story of BSIMsquare, a small startup company that is slowly thriving under the big thinking of using computers to drive the discovery of new drugs.

Comunicações Orais

New *in silico* methods to model biological membranes with increased realism

Diogo Vila-Viçosa, Hugo Santos, Vitor H. Teixeira, Miguel Machuqueiro

Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

The study of biological membranes has been for quite some time a challenging endeavour for researchers, who must exploit a variety of methodologies. In recent years, molecular modelling is probably the field of research that has more enthusiastically contributed with information at the atomic level. A detailed description of a lipid bilayer has to take in consideration all-important factors that affect the membrane behaviour and stability. pH is recognizably one of these factors even though it is usually ignored due to its high complexity in terms of modelling. The presence of negatively charged groups in the membrane gives rise to a surface electrostatic potential. The negative charges come mainly from anionic lipids, which are prone to protonation under certain conditions. It is not so uncommon to find this fact neglected in the literature. Many studies, both experimental and theoretical, tend to "simplify" the problem by using zwitterionic phospholipids in their membrane models. In the available modelling studies on anionic and negatively charged lipids, their charges are fixed and the authors claim that the pKa values are too low to allow for any protonation. This approach can be compromised, considering that biological membranes can share protons as "acid-anion" dimers and those anionic lipids thus trap and conduct protons along the headgroup domain of bilayers that contain such anionic lipids. The "acid-anion" mechanism suggests that some of these anionic lipids can retain protons at higher pH values, hence altering their structural properties. Here, we introduce a new method that allows the inclusion of pH in molecular dynamics simulations of lipid bilayers. The results on the application of the method to a lipid bilayer constituted by oleic acid will be presented.

Joint use of NMR and bioinformatic tools to unveil the dynamics and the 'tectonic' enzymatic mechanism of MMP-1

João M. C. Teixeira[1,2], Claudio Luchinat[1], Carlos F. G. C. Geraldes[2]

1. CERM, University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino (FI) Italy.
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The conformational space explored in solution by human fibroblast collagenase (MMP-1), a two-domain protein, is crucial for the hydrolysis of triple helical type I collagen – an enzymatic role of key importance in cancer metastasis. The recent advancements in protein dynamics investigation through paramagnetic restraints were applied to the active full-length MMP-1, the protein was functionalized with a paramagnetic CLaNP-5 probe bearing four lanthanide ions (Lu^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+}), and NMR (PCS/RDC) restraints were measured. The maximum occurrence (MaxOcc) protocol from the WeNMR Web Server was applied to calculate the conformational space explored by free MMP-1 using NMR restraints integrated with SAXS data. The MMP-1 conformations with large MaxOcc values (up to 47%) are restricted to a relatively small conformational region that favours the enzymatic role and differs from that of the X-ray crystallographic structures. The same NMR experiments were carried out this time in the presence of a triple helical collagen analogue (THP) substrate, revealing a single enzyme conformation in the protein-substrate bound state. We input the paramagnetic NMR restraints in a new version of HADDOCK along with classical NMR, SAXS and X-Ray crystallographic data acquired previously on the same MMP-1/THP interaction. We obtained a very reliable model that satisfies all the input data: the 200 final HADDOCK structures belong to a single cluster (RMSD to the lowest energy str. < 1Å). In this work, we were able for the first time to experimentally isolate and observe, in solution, the full-length MMP-1 (42 kDa) bound to a collagen mimetic peptide (10 kDa) and structurally describe the instant prior to the peptide cleavage when the triple helix is already unwound.

How the Intercalation of Phenanthroline Affects the Structure, Energetics and Bond Properties of DNA Base Pairs. Theoretical Study Applied to Adenine-Thymine and Guanine-Cytosine Tetramers

Adrià Gil-Mestres and Maria José Calhorda

Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

The effects of phenanthroline (phen) intercalation in the structure, energetics and bonding of Adenine-Thymine and Guanine–Cytosine tetramers (A-T/T-A and G-C/C-G) was studied through Density Functional Theory (DFT) using some functionals developed by *Truhlar et al.* and comparing to some DFT-D functionals. As expected, our results show that intercalation produces important changes not only in the hydrogen bonds of base pairs because stacking (S) and hydrogen bonding (HB) are deeply connected, but also in other characteristic geometric parameters of the base pairs. Intercalation energies are higher when intercalation takes place from Major Groove (MG) in G-C/C-G systems but no appreciable differences are found for A-T/T-A systems. On the other hand, for G-C/C-G systems HB interactions are more important than S interactions, whereas for A-T/T-A systems, HB and S become competitive. Interactions and bond properties are analysed in terms of dipole moments, polarizability, density, charge transfer, electrostatic potential maps and frontier orbitals.

The sulfur-shift: a new activation mechanism in mononuclear Mo enzymes

Nuno Cerqueira

REQUIMTE, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n 4169-007 Porto, Portugal

In this communication it will be reported an interesting mechanistic phenomenon that we have called the sulfur-shift. This mechanism is characterized by a displacement of a sulfur atom in the metal site that allows the enzyme to exchange between two states: one inactive form, in which the access to the metal ion is blocked by the formation of a pseudo-dithiolene ligand and an active form that opens a free-coordination position at the metal site that can be occupied by the substrate[1]. This specific rearrangement provides an efficient mechanism to lower the activation barriers for ligand exit or entrance processes and at the same time to protect the metal site from other molecules that can potential destroy or inactive it, including the solvent. This mechanism was first proposed based on theoretical calculations but it has been recently validated by experimental means and has many similarities to the well-known carboxylate-shift mechanism. All these data seems to reinforce the idea that the enzymes in which the metals are involved in the catalytic process own a self-protecting mechanism that allows them to maintain a constant or nearly constant coordination number of the metal throughout an entire catalytic cycle and at the same allows them to protect the metal from other molecule capable of destroying it[2,3].

1. Cerqueira, N. M. F. S. A. ; Fernandes, P.A.; Moura, J. J. G.; Ramos, M.J., Inorg. Chem., **2013**, 52 (19), 10766–10772.
2. Mota, C. S.; Rivas, M. G.; Brondino, C. D.; Moura, I.; Moura, J. J. G.; Gonzalez, P. J.; Cerqueira, N. M. F. S. A. *J Biol Inorg Chem* **2011**, *16*, 1255–1268.
3. Cerqueira, N. M. F. S. A.; Gonzalez, P. J.; Brondino, C. D.; Romao, M. J.; Romao, C. C.; Moura, I.; Moura, J. J. G.Journal of computational chemistry **2009**, *30*, 2466–2484.

Local Backbone flexibility is key to accurate prediction of the protein-protein binding energy

Daniel F.A.R. Dourado, Samuel Coulbourn Flores*

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Experimental expression, purification, and affinity evaluation of protein complexes is an expensive and time-consuming means of evaluating the effect of mutations, making a fast and accurate *in silico* method highly desirable. When the structure of the wild type complex is known, it is possible to evaluate economically the effect of point mutations with Knowledge Based potentials, but accuracy has so far been relatively low due to an inadequate treatment of backbone flexibility. Using an emergent multiscale method, ZEMu (Zone Equilibration of Mutants) flexibilizes a small region around the site of mutation while spending minimal computer resources on regions distant to that site. ZEMu in this way finds an energetically favorable configuration of the backbone and side chains near the mutation site at very low computational cost. This new method was validated with an experimental dataset composed of 1200 mutants (each with 1-15 simultaneous substitutions), 66 different protein complexes, ranging the binding energy ($\Delta\Delta G$'s) from -6.25 to 10.10 kcal/mol. The results suggest applicability to a wide variety of biomedical problems, particularly in the design of pharmaceutically useful proteins.

Development of the computational structural biology toolbox towards the design of novel vaccines

Correia BE[1,6], Bates JT[2], Loomis R[4], Jardine JG[1], Rupert P[3], Connel MJ[4], Vittal V[1], Kalyuzhniy O[1,5], MacPhserson S[1,5], Schroeter A[1], Baneyx G[1], Stevens E[1], Li Y[5], Klevit RE[1], Wyatt R[5], Baker D[1], Strong RK[3], Crowe JE Jr[2], Johnson PR[4], Schief WR[1,5]

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2. Department of Pediatrics, Vanderbilt University, Nashville, TN, USA
3. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
4. The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA
5. Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA
6. Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA; PhD Program in Computational Biology, Instituto Gulbenkian Ciência, Oeiras, Portugal

The concept of epitope-focused immunogens for the elicitation of broadly neutralizing antibodies is an attractive strategy for vaccine design. In the past this strategy has failed to deliver notable breakthroughs for vaccine development. Here, we describe a computational methodology to design novel functional proteins – Rosetta Fold From Loops (FFL) – which we applied to the design of epitope-focused immunogens. FFL was devised to insert structurally defined functional sites into protein scaffolds. The scaffolds are folded and designed to stabilize the functional conformation of the inserted site. To test our methodology we sought to design three-helix bundles harboring a respiratory syncytial virus (RSV) epitope, previously co-crystallized with the neutralizing antibody Motavizumab. The designs were thermodynamically stable and showed extremely high affinities to Motavizumab (k_{off}/k_{on}=6.32 pM). Structural characterization of the design showed good agreement with the computational models, the backbone rmsds for the overall structure were 1.7 Å and 0.5 Å for the epitope region. The designed epitope-scaffolds were used to immunize macaques, approximately 75% of the cohort developed RSV neutralizing activity. To better understand the features of the antibodies elicited by the epitope-scaffolds, we isolated several rhesus monoclonal antibodies (RhmAbs). Two RhmAbs bound to the immunogen with low pM affinity and were potent neutralizers of RSV. Interestingly, these RhmAbs were approximately 10-fold more potent than the FDA-approved prophylactic antibody Palivizumab. Our results supply the first proof-of-principle for epitope-focused vaccine design. We anticipate that FFL will be useful for a variety of challenges in the computational design of functional proteins.

Fundamental interactions and the cellular toxicity of nanomaterials

Manuel Melle-Franco[1*], Marco Dallavalle[2], Matteo Calvaresi[2], Siegfried Höfinger[2], Francesco Zerbetto[2]

1. Centro de Ciências e Tecnologias de Computação, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
2. Dipartimento di Chimica "G. Ciamician", Università di Bologna, Italy

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Carbon nanomaterials have been proposed as nano-vehicles to deliver genetic or therapeutic material into the interior of cells because of their capacity to transpose cell membranes. A detailed picture of the molecular mode of action of such a delivery is, however, difficult to obtain because of the concealing effects of the cell membrane. We will present different computational models of the interaction of the cell membrane and representative carbon nanomaterials namely: fullerenes and carbon nanotubes[1] and graphene flakes and discuss how this interaction might be the ultimate cause for the toxicity of these materials.

1. Höfinger S., Melle-Franco M., Gallo T., Cantelli A., Calvaresi M., Gomes J.A.N.F. and Zerbetto F., *A Computational Analysis of the Insertion of Carbon Nanotubes into Cellular Membranes*, Biomaterials, 32, 7079-7085, **2011**

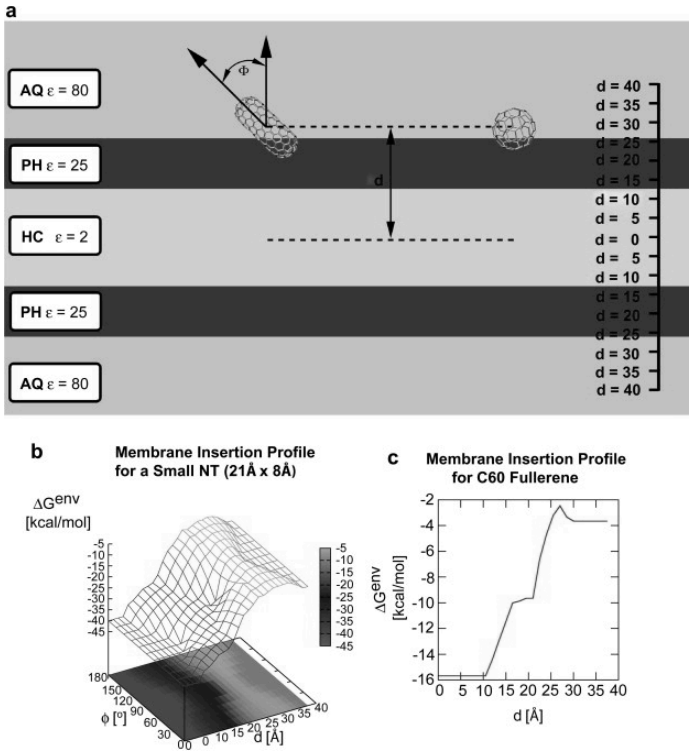

Figure 1. Energy profile of the insertion of a small carbon nanotube and a fullerene with an implicit membrane model[1].

Posters em exposição

P1. Powering Research. Together.

Nuno L. Ferreira

European Grid Infrastructure (EGI), Science Park 140, 1098 XG Amsterdam, The Netherlands

e-infrastructures are geographically distributed computing resources and data storage facilities, and the high-performance networks that link them. They allow scientists to share information securely, analyze data efficiently and collaborate with colleagues worldwide. The European Commission recognizes that the "fifth freedom", the circulation of researchers, knowledge and technology across Europe, is vital to its plan. Computing infrastructures such as the European Grid Infrastructure (EGI) are making this fifth freedom a reality. They are an essential part of modern scientific research and a driver for economic growth. EGI is the result of pioneering work that has, through the federation of national resource providers, built a collaborative production infrastructure of uniform services, which supports multi-disciplinary science. A federation of over 350 resource centers spread across 56 countries in Europe, the Asia-Pacific region, Canada and Latin America, supporting over 22k researchers with access to more than 370k CPU cores and 170 PB of disk capacity. In the Life Sciences, EGI has established collaborations with several external partners within the extended Distributed Computing Infrastructures community: "The Life-Sciences Grid Community", WeNMR, MAPPER, BioMedBridges and ScalaLife, to mention a few. If you are interest to discover our solutions portfolio, and get a high-level overview of key concepts such as grid and cloud technologies, high-throughput computing, science gateways and workflow systems, you are invited to talk with us in person at this event, or find us at http://www.egi.eu/. At the end, you will be empowered with insights on how big science can be performed in the e-infrastructure.

P2. Computer-Aided Drug Discovery toward Chronic Obstructive Pulmonary Disease

Susana D. Lucas, Laurinda R. P. Areias, Eduardo F. P. Ruivo, Lídia M. Gonçalves, Rui Moreira and Rita C. Guedes

iMed.UL, Research Institute for Medicines and Pharmaceutical Sciences, Faculdade de Farmácia da Universidade de Lisboa.

The World Health Organization estimates that 64 million people around the world find it distressingly difficult to breathe due to a combination of emphysema and chronic asthmatic bronchitis — a deadly duo known as chronic obstructive pulmonary disease (COPD) that is currently the fourth leading cause of death[1]. Human Neutrophil Elastase (HNE) is a serine protease which plays a major role through COPD inflammatory process wherein an excess of HNE is produced hydrolyzing elastin, the structural protein which gives the lungs their elasticity[2]. Our group is actively involved with a unified approach to boost the discovery of drug candidates for treatment of COPD relying on the use of advanced computer-aided drug refinement tools such as molecular docking, virtual screening, pharmacophore modelling and de novo design to streamline the lead generation to and optimization process of novel nM inhibitors of HNE, confirming the added value of using chemoinformatic protocols toward COPD drug design[3,4].

Acknowledgments: Fundação para a Ciência e Tecnologia, Pest-OE/SAL/UI4013/2011, SFRH/BPD/64265/2009 (SDL).

1. (a) http://www.who.int/respiratory/copd/en. (b) Nature 2012, 489, S1.
2. Lucas, S. D. et al. Med. Res. Rev., 2013, 33 Suppl 1, E73-101.
3. Lucas, S. D. et al Med. Chem. Commun., 2012, 3, 1299.
4. Lucas, S. D. et al. J. Med. Chem., 2013, accepted.

P3. Applying SASA Descriptors for the Prediction of Interfacial Hot-Spots

J. M. Martins, R. M. Ramos, A. C. Pimenta, I. S. Moreira

REQUIMTE/Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto

The understanding of how proteins interact within its own subunits and with other proteins is of extreme importance towards the goal of developing a greater knowledge about themselves, their inhibitors and their behavior. In this study we develop and calculate novel Solvent Accessible Solvent Area (SASA) descriptors based on residue standardization, allowing us to analyze with further accuracy the changes on the interfacial area of the subunits upon protein complexation. To further understand the applicability of the descriptors, three computationally different data-sets, explicit water molecular dynamics, implicit water MD and static PDB structure, were tested and the performance of the descriptors analyzed for each of the data-sets. We found that no discernible improvement of the descriptors' performance was achieved by using more computationally expensive methods based on molecular dynamics. However, a new method for interfacial hot-spot prediction was developed, SASA-based Hot-Spot Detection (SBHD) applying Support Vector Machines over combinations of the developed SASA-based descriptors. This developed method achieved good precision, recall and F1 scores towards the accurate detection of experimental protein-interface's hot-spots.

1. Martins JM, Ramos RM, Pimenta AC, Moreira IS, Solvent-accessible surface area: how well can be applied to hot-spot detection?, Proteins: Structure, Function, and Bioinformatics, 2013, doi:10.1002/prot.24413
2. Gao Y, Wang R, Lai L. Structure-based method for analyzing protein-protein interfaces., Journal of Molecular Modeling 2004, 10;44-54.
3. Liu Q, Li J. Propensity vectors of low-ASA residue pairs in the distinction of protein interactions., Proteins: Structure, Function, and Bioinformatics 2010, 78;589-602.

P4. What is the "correct" number of ions that should be added to a MM/MD simulation of a charged bilayer?

D. Vila-Viçosa[1], H.A.F. Santos[1], V.H. Teixeira[1], A.M. Baptista[2], M. Machuqueiro[1]

1. Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa 2. Instituto de Tecnologia Química e Biológica,Universidade Nova de Lisboa

The structure of a negatively charged lipid bilayer is strongly influenced by the ionic strength of the solution. Approximations such as Gouy-Chapman model or Poisson-Boltzmann had been used to describe the ion profile along the membrane normal. However, it is not trivial to model a charged membrane using molecular mechanics. The common practice is to completely neutralize the system and use some method to treat long range interactions. This approach can lead to very high ion concentrations in the simulation box and the ion-membrane interactions become unrealistic. In this work, we present a Poisson-Boltzmann based method to estimate the number of ions that should be added to a simulation box containing an anionic lipid bilayer. We calculate the electrostatic potential in the simulation box and use it to obtain an estimation of the number of ions (negative and positive) that must be added to the model. To test the described method, we used model membranes of oleic acid and DMPA/PC mixtures. Our first results showed that, according to the Poisson-Boltzmann estimation, the system must be far from the neutrality to be described accurately. Moreover, comparing our approach with others, we observed a better agreement with experimental data, such as area per lipid and phase transition.

We acknowledge the financial support from FCT (PTDC/QUI-BIQ/113721/2009 and PEst-OE/QUI/UI0612/2013).

P5. Evolution of complex phenotypes: structural and dynamic analysis of TEM enzymes with hybrid mutations

Pimenta, A. C.[1,2,3], Martins, J.M.[1], Fernandes, R.[2,3], Moreira, I. S.[1]

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With the worldwide increase in antibiotic resistances, the potential for the development of new and more complex resistance mechanisms also increased due to the high mutation rate of antibiotic targets in pathogens. A well-studied mechanism of antibiotic resistance is the antibiotic degrading of specific enzymes, such as β-lactamases. TEM-1[1] was the first enzyme of this family to be described and along the years it lead to new families of β-lactamases and to distinct phenotypes within its own family. Inhibitor resistant (IRT)[1,2] and extended spectrum β-lactamases (ESBL)[1] appear as a result of point mutations that are characteristic within each phenotype. Enzymes with mutations typical of both phenotypes start to be isolated but one of each phenotypes appeared to be prevalent until enzymes with more complex phenotypes (CMT)[1] were isolated. In this work we provide insight on the effect of these hybrid mutations on both structure and activity of TEM enzymes by analyzing two enzymes that evolved with mutations of IRT and ESBL phenotypes – TEM-180 and TEM-201[3]. A structural and energetic profile of these enzymes was obtained through molecular modelling, molecular dynamics simulations and free energy calculations. Due to our previous work with TEM-1 complexes we realize that each structure needs a thorough analysis after complexation due to dynamic structure of this type of enzymes[4]. For example, although IRT enzymes previously described the lack of a conserved water molecule or the motion of the catalytic residue as the motive for their phenotype, we observed that conserved water molecules in the active site and catalytic residues such as Ser70 do not suffer great displacement. Rather another catalytic residue, Ser130, is displaced avoiding the formation of the connection Ser70-Oγ-ligand-Ser130-Oγ, which would lead to inhibition of the enzyme. This structural property is only observed in complexes with the inhibitor that therefore stay active against β-lactam antibiotics. Due to the structural and dynamic changes of helix H_{11}, complexes with larger antibiotics (with extended spectrum) were obtained and present reasonable stability during MD simulations.

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P6. The need of explicit lipids to understand pH and deacylation effects on surfactant protein C: a constant-pH MD study

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The pulmonary surfactant protein C (SP-C) is a short highly hydrophobic polypeptide with two covalently linked fatty acyl chains, which adopts a mainly helical structure while associated with the membrane. This protein was shown to play a crucial role in the formation and stabilization of the pulmonary surfactant reservoirs during the compression and expansion cycles of the respiratory cycle. However, while in solution, it misfolds into a β-rich structure, being the α→β transitions promoted by deacylation and exposure to neutral and alkaline pH conditions. To understand the properties relevant for SP-C loss of structure, we have simulated its acylated and deacylated isoforms at different pH conditions in two sets of constant-pH MD runs: (1) using a membrane-mimetic solvent, similar to the ones that have been used to study the protein misfolding and aggregation, and (2) using a dipalmitoylphosphatidylcholine (DPPC) bilayer. While the first set of simulations showed a strong dependence of the protein stability on the pH variation, in agreement with experimental observations, the second set of simulations showed remarkable helix stability with no formation of b motifs regardless of the pH conditions. These contrasting results call into question the suitability of this type of solvent mixtures to mimic a membrane environment, pointing out the essential role of a lipid environment to understand the misfolding process under physiological conditions. In addition, the results obtained in a lipid environment give some insights into the structural determinants for SP-C function and its molecular mechanism of action under physiological conditions.

P7. Exploring O2 diffusion in aa3-type Cytochrome C Oxidases: MD simulations uncover an alternative channel towards the binuclear site.

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Cytochrome c oxidases (CCOX) are members of the heme-copper oxidase superfamily and they are the terminal enzymes of the respiratory chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular dioxygen to water with the creation of a transmembrane electrochemical proton gradient. Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping and little emphasis has been given to the pathways used by dioxygen to reach the binuclear site. The main objective of this work is to identify possible alternative dioxygen pathways in the reduced CCOX from Rhodobacter sphaeroides[1] using extensive Molecular Dynamics (MD) simulations. Our simulations allowed the identification of two possible dioxygen channels, whose entrances are both located in the membrane spanning region. The first channel is a Y-shaped hydrophobic cavity with a constriction point near F282I and W172I, and it corresponds to the oxygen pathway previously identified in the X-ray structure[2]. The second channel follows the hydroxylfarnesyl tail of haem a3 and ends near the Y288I (which is covalently linked to the H284I imidazole group).

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P8. A fruitful integration of Molecular Dynamics with experimentation: Development of synthetic transmembrane anion transporters

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The anion transport across phospholipid bilayers is crucial to many cellular processes and its malfunction is linked with the occurrence of serious pathologies, including cystic fibrosis, caused by a defective transmembrane transport of chloride[1]. In this context, the development of synthetic molecules capable of promoting anion transport as suitable candidates for replacement therapies is of great importance. We use Molecular Dynamics (MD) simulations as a powerful tool to investigate the mechanisms of anion transport by small synthetic carriers, in collaboration with experimental groups, in an effort to understand the factors that govern that transport with the ultimate goal of creating more efficient drug-like molecules. In this communication, we will present an overview of the recent developments of this synergetic collaboration with examples of MD simulations on chloride transporter systems ranging from bis-indolylureas[2], ortho-phenylenediamine-based bisureas[3], tris-thiourea tripodal-based molecules[4], and calix[4] arene plataforms[5].

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P9. Peptide dendrimers conformational determinants: a computational study

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Peptide dendrimers are a new class of dendrimers formed by alternating functional amino acids with branching diamino acids such as lysine, thus taking advantage of dendritic architecture, while incorporating known biocompatible peptides[1]. Despite significant advances, the field of peptide dendrimers is coming to a cross-road where new systems for specific applications are needed but the information to construct truly tailor-made molecules is missing. Herein we present a comprehensive structural characterization of peptide dendrimers using molecular simulation methods[2]. Multiple long molecular dynamics (MD) simulations were used to extensively sample the conformational preferences of several third-generation peptide dendrimers, including some known to bind aquacobalamine. We used several conformational analysis procedures (clustering, energy landscapes and multivariate analysis) to analyze conformational changes that can be correlated with particular structural trends. The results clearly show that a trade-off between electrostatic effects and formation of hydrogen bonds controls structure acquisition in these systems. Moreover, by selectively changing the dendrimers charge we are able to manipulate the exhibited compactness. These conclusions bring new insight into the conformational behaviour of these systems and may provide better routes for their functional design.

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P10. Unveiling the Kinetics of O2 Diffusion in a Multicopper Oxidase through ILS and Markov Models

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Dioxygen (O2) was crucial for the evolution of higher organisms, mainly as the final electron acceptor of the respiratory chain. Among many other enzymes that use O2 as substrate are the multicopper oxidases (MCOs), whose biotechnological interest has increased in these past decades due to their "green" process of oxidizing small organic molecules while reducing O2 to water. Deeply buried inside MCOs lies the catalytic center where O2 gets reduced, and while X-ray crystallography structures have shown at least two solvent channels leading to the center, the pathways and kinetics for O2 to reach the center are still unknown. Biomolecular simulation tools have been used to tackle these issues on other O2-catalyzing enzymes and, in this work, we have performed standard molecular dynamics simulations of CotA laccase with explicit O2, as well as the implicit ligand sampling (ILS) technique. As a result, we obtained a high-accuracy description of O2 affinities inside a MCO in the form of a tridimensional energy landscape. Analytic tools were developed to determine the minima and the lowest-energy pathways connecting the solvent to the catalytic center of that MCO. From this analysis, a kinetic Markov model was constructed and, together with the transition path theory, the kinetically relevant pathways to the internalized center have been unveiled, together with important residues in the process. Altogether, this study provides new insights onto the MCO catalysis as well as a valuable combination of tools to tackle similar problems.

P11. Virtual screening in the discovery of 5α-reductase inhibitors potential

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The 5α-reductase is a microsomal protein that converts testosterone into dihydrotestosterone (DHT). There are two isoenzymes of 5α-reductase (5α-R1 and 5α-R2), which vary in different tissues with age. When disturbances occur in the function of this enzyme, disorders such as pseudohermaphroditism, baldness, benign hyperplasia and prostate cancer may arise. Currently, there are only two marketed drugs, finasteride and dutasteride, which have long term side effects, stressing the need for the development of better inhibitors. In the present study, we used a dataset of compounds with known activity on 5α-reductase obtained from ChEMBL database, and employed machine learning methods (Random Forests and Support Vector Machines) to build classifiers for high-throughput virtual screening campaigns to help in prioritizing molecules for further analysis. The performance of the classification models was evaluated based on sensitivity, specificity, precision and F-score. Additionally, the compounds were further analysis is search for common substructures using a Maximum Common Substructure (MCS) algorithm. Our results show that for both isoenzymes, the classification models produced by the two algorithms (Random Forests and Support Vector Machines) present similar performance. However, the classifiers for 5α-R2 perform better than the classifiers obtained for 5α-R1. The identification of potentially enriched substructures in the known inhibitors of 5α-R1 and 5α-R2 revealed that 4-azasteroid and 6-azasteroid are key components when designing inhibitors for these isoenzymes.

P12. Modelling The Oxidation Site Of Plasmodium Falciparum Bc1 Complex

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The electron transport chain (ETC) in malaria parasites was first recognized as an attractive drug target since the development and clinical use of atovaquone in 1992. Atovaquone selectively inhibits electron transport by binding to the Qo binding site (oxidation site) of the parasite mitochondrial bc1 complex. More specifically, this compound induces the collapse of the mitochondrial membrane potential which results in parasite death. Therefore, being crucial for the survival of P. falciparum (Pf), the cytochrome bc1 complex is currently a validated target for antimalarial drug development. In the absence of a crystallographic structure for the bc1 complex of Pf, much of the key structural and mechanistic information has been obtained from analogous bc1 systems. In particular, the bc1 complex of Saccharomyces cerevisiae was already chosen to model this pocket and to understand the mechanism of action of potential inhibitors of the Pf bc1 complex. Nevertheless, a reliable three-dimensional structure of the Pf enzymatic complex is essential for successful drug design. As a result, having in mind the increasing interest in obtaining potential antimalarial drugs that can act in this target, we developed a homology model of cytochrome bc1 Qo binding site based on yeast crystallographic structure. Here, we present the methodology followed to obtain the homology model and all the validation procedure employed to verify the reliability of the model generated.

P13. Transmembrane transport of chloride by Squaramides: an in silico study

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The anion transmembrane transport is essential to cell functioning and its regulation depends on transmembrane channels. The malfunction of ion channels leads to channelopathies. In particularly, the impairment of chloride ion channels is associated with cystic fibrosis. These diseases have been inspired the supramolecular chemists for the development of new chloride synthetic transporters with potential use in channel replacement therapies. In this context, we report here an in silico study performed in membrane models to evaluate the ability of a series of squaramides to assist the chloride transmembrane transport across a POPC vesicle. Indeed, previous experimental studies have shown that these compounds were able to transport chloride out of POPC vesicles much faster than their analogous thioureas and ureas[1]. This study was carried out by quantum calculations followed by Molecular Dynamics (MD) simulations in a POPC membrane model. The transporters were described with GAFF[2] coupled with specific parameters developed for squaramide moiety. The phospholipids were described with parameters from LIPID11[3]. The passive diffusion of chloride complexes was investigated placing each of them in two different starting positions. When the complex is placed in water slab the chloride is released before the receptor reaches the water/lipid interface. By contrast, when the receptor starts in the membrane core, the anion release occurs concomitantly with the approach of the receptor to the interface.

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P14. The study of complex biological processes - from the electronic to the atomic level.

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For years structural biologists have used an ensemble of experimental techniques to unveil the molecular structure/function of large biomolecules. In recent years and thanks to constant advances in both software and hardware, computational methods have taken a place in this field. We can now investigate protein folding, reaction pathways, binding and many other processes. Here we will show how a combination of different techniques can, at the computational level, aid in the long path connecting structural information to function.

P15. Toward the discovery of inhibitors of babesipain-1, a Babesia bigemina cysteine protease: in vitro evaluation, homology modeling and molecular docking studies

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Babesia bigemina is a protozoan parasite that causes babesiosis, a disease with a world-wide distribution in mammals, principally affecting cattle and man. The unveiling of the genome of B. bigemina is a project in active progress that has already revealed a number of new targets with potential interest for the design of anti-babesiosis drugs. In this context, babesipain-1 has been identified as a proteolytically active enzyme whose three-dimensional structure has not been resolved yet, but which is known to be inhibited by cysteine proteases inhibitors such as E64, ALLN, leupeptin, and vinyl sulfones. In this work, we introduce (1) a homology model of babesipain-1; (2) a comparison between babesipain-1 and falcipain-2, a cysteine protease of the malaria parasite Plasmodium falciparum; (3) in vitro data for babesipain-1 inhibition by HEDICINs and HECINs, previously reported as modest inhibitors of falcipain-2; and (4) the docked binding conformations of HEDICINs and HECINs in the model of babesipain-1. HEDICINs presented similar preferred binding conformations for both babesipain-1 and falcipain-2. However, in vitro bioassay shows that HEDICINs and HECINs are better inhibitors of babesipain-1 than of falcipain-2, which could be explained by observed differences between the active pockets of these proteins in silico. Results presented herein provide a valuable contribution to future computer-aided molecular design of new babesipain-1 inhibitors.

P16. Coarse-Grained Modelling Of β -Casein - Effect of pH and Electrolyte.

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β -casein is one of the most abundant milk proteins and belongs to the Ca^{2+}-sensitive phosphoprotein family. It is rich in prolines and contains five phosphorylated serines. Its lack of stable tertiary structure when studied as an isolated polypeptide chain under physiological conditions makes it a part of the intrinsically disordered protein (IDP) family[1]. Moreover, this protein has an amphiphilic character. Its adsorption to hydrophobic surfaces has been the subject of many studies. At neutral pH, the β-casein hydrophobic region adsorbs to the hydrophobic surface, anchoring the protein, while the hydrophilic N-terminus protrudes into the solution and forms a brush- like structure. Studies have also shown that the adsorbed amount increases as the pH is lowered towards the isoelectric point[2]. For hydrophilic surfaces, there is some contradiction with some studies showing that adsorption can be both strengthened[3] and weakened[4] by increasing the ionic strength. A recent theoretical study[5] suggests that this protein changes its properties according to the chemical environment, adsorbing to any kind of surface, not only due to direct electrostatic and hydrophobic interactions, but also due to conformational arrangements at the surface and charge regulation. In an effort to better understand β-casein adsorption mechanisms and pinpoint its key elements, we have developed a coarse-grained model for this protein and we are currently performing Monte Carlo simulations on the adsorption of a single β-casein to different types of surfaces, under different conditions, such as pH, surface charge density, salt concentration and valency.

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P17. 3D models of protein complexes from evolutionary information

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The evolutionary information contained in sequenced genomes could potentially be used in tandem with computational docking methods to structurally characterize complexes that have traditionally been challenging to study experimentally, such as membrane-embedded systems. We combined a recently developed method for extracting co-evolving pairs of amino acids in protein sequences (EVFold) with a state-of-the-art information-driven docking protocol (HADDOCK). We first developed an extension of EVFold to reveal pairs of co-evolving residues across protein sequences. Then, we developed a filtering algorithm that analyzes the candidate pairs and removes those not meeting certain structural criteria (e.g. solvent accessibility). The best pairs are then implemented as distance restraints in HADDOCK, which then produces and scores a number of structural models of the interaction. This workflow was applied to a dataset of 22 protein-protein complexes that included integral membrane proteins, molecular transporters and multi-domain proteins. Only 2 cases fail to produce models of the interaction that resemble the native complex. The remaining 20 cases produce very accurate structural models (interface RMSD <4Å). Furthermore, these near-native models are always selected by our scoring function. We propose a method that uses sequence co-variation analysis to derive extremely accurate spatial restraints between proteins of a complex. Such combination of genomic information with physics-based modelling approaches can be used to greatly increase the structural coverage of interactomes.

P18. Hot-Spot Detection at Protein-DNA Interfaces

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Proteins and protein-based complexes are the basis of many key systems in nature and have been the focus of a high number of studies. For the comprehension of protein interfaces one approach has been commonly used: Alanine Scanning Mutagenesis. This technique is able to determine the most important residues for complex formation, the Hot-Spots. However, it is usually only applied to the study ofprotein-protein interfaces. With that in mind we verified its transferability to protein-DNA systems. In this work we performed MD simulations of protein-DNA complexes and evaluated the influence of several parameters on the determination of the binding free energy terms of a set of mutations. Among the evaluated parameters were the solvent representation, Linear and Nonlinear Poisson-Boltzmann equation, Generalized Born model, simulation time, number of MD trajectories, force field used and energetic terms involved. Overall, and for a set of 78 mutations, we developed a new computational alanine scanning mutagenesis approach, with an average error of 1.55 kcal/mol. This method can be used to explore the behaviour of protein-DNA complexes and to obtain a clear picture of the physical-chemical characteristics of theses interfaces.

P19. Constant-pH MD simulation of PA/PC lipid bilayer models

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It is accepted that electrostatic interactions have a major effect on the structure and dynamics of membranes[1]. Nevertheless, the problem regarding the number of counter-ions solvating the membrane is often overlooked. This is especially important when dealing with more realistic membrane models composed of anionic binary mixtures. In certain organelles, phosphatidic acid (PA) is the major anionic lipid. Being a phosphomonoester, PA has three different charge states (0, –1 and –2) that are interchanging at physiological pH. This lipid can act as a pH biosensor by dramatically changing its charge upon small intracellular pH variations[2]. We performed several CpHMD simulations of 25% PA/PC binary mixture at different pH values and used the Poisson-Boltzmann formalism to estimate the amount of Na+ and Cl– counter-ions at 0.1 M ionic strength. From the results, we observed a pH-induced isothermal gel-to-fluid phase transition, in very good agreement with experimental data.

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P20. Molecular dynamics simulations of antimicrobial deoxy glycoside-based surfactants: micellization and structural insights

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Carbohydrate-based surfactants are widely used in the extraction and crystallization of membrane properties and have a broad range of other applications. *Rauter et al.* have developed a new class of alkyl deoxy glycosides that display potent antibacterial activities and particular selectivity against Bacillus spp.[1]. However, their mechanism of action needs to be further explored. Surface-active properties data is indicative that surfactant self-association into micelles is a prerequisite for biological activity, which is significantly achieved above the critical micelle concentration. Therefore, it is thought that surfactants aggregate and interact with phospholipid bilayers in a detergent-like manner. Molecular dynamics (MD) methodologies have been previously employed to characterize surfactant systems, including structurally similar glycoside micelles in aqueous solution[2]. Thus, we were encouraged to study the formation of micelles and their interaction with model lipid bilayers in attempt to rationalize the physical and biological properties of these compounds with additional molecular detail. In this communication, we will disclose preliminary results concerning atomistic MD simulations of the micellization process and structural stability characterization at different concentrations of the most active compound reported to date, dodecyl 2,6-dideoxy-α-L-arabino-hexopyranoside. Subsequent work is in progress towards the study of both the behaviour of these micelles at a model lipid bilayer interface and the comparative analysis of the behaviour of structural analogous systems.

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P21. Free Energy Studies addressing P-glycoprotein Multidrug Resistance Phenomena

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Multidrug resistance is becoming a serious impairment in cancer chemotherapy. Following our characterization of three putative drug-binding sites within P-glycoprotein's internal drug-binding pocket (DBP), a series of free energy studies are undergoing to address interactions between drugs, P-gp and the lipid bilayer. Free energy profiles for molecules transfer from the DBP into the lipid bilayer revealed a low energetic cost for permeation from the membrane's hydrophobic environment to the water-filled DBP. In addition, a remarkable influence of P-gp's cytoplasmic domains on the drug's free energy profiles are also being unveiled, suggesting that P-gp may have the ability to increase local drug concentration in order to promote higher membrane concentrations that may increase efflux rates.

P22. Assessing the impact of water pollution on mussel populations using genomics

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This work intends to uncover the biological mechanisms behind the differential resistance of some species to various types of dangerous chemicals. Considering the crucial role of cytochrome P450 and glutathione S-transferases in detoxification, we propose that particular functional combinations of enzymes from both families are major contributors for the success of some organisms that are challenged by the exposure to dangerous chemicals. This will improve the design of experiments meant to investigate the molecular mechanisms that enable some species to deal with otherwise lethal chemicals. Furthermore, we will be able to detect evolutionary processes going on in species that are exposed to man-made chemicals.

P23. Tris-Thiourea Tripodal-based Molecules as Chloride Transmembrane Transporters: Insights from Molecular Dynamics Simulations

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The interaction of six tripodal synthetic chloride transmembrane transporters with a POPC bilayer was investigated by means of molecular dynamics simulations using the general Amber force field (GAFF)[1,2] for the transporters and the LIPID11 force field for the phospholipids[3]. These transporters are structurally simple molecules, based on the tris(2-aminoethyl)amine scaffold, containing three thiourea binding units coupled with three n butyl (1), phenyl (2), fluorophenyl (3), pentafluorophenyl (4), trifluoromethylphenyl (5), or bis(trifluoromethyl)phenyl (6) substituents[4,5]. The passive diffusion of 1-6⊃Cl- was evaluated with the complexes initially positioned either on the water phase or inside the bilayer. In the first scenario the chloride is released in the water solution before the transporters achieve the water-lipid interface and permeate the membrane. On the latter one, only when the chloride complex reaches the interface is the anion released to the water phase, with the transporter losing the initial tripodal shape. Independently of the transporter used in the membrane system, the bilayer structure is preserved and the synthetic molecules interact with the POPC molecules at the headgroups level, via N-H···O hydrogen bonds. Overall, the structural and dynamic results support that this series of small carriers can mediate the chloride transmembrane transport.

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P24. Computational studies on the P-glycoprotein efflux modulation

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I will conduct a review regarding the advances achieved by my group in the last 2 years, specifically, in understanding multidrug resistance linked to the P-glycoprotein (P-gp) efflux pump. A unified view over the mechanistic mode of action of modulators and substrates provided by the combination of pharmacophore, molecular dynamics and docking techniques will be presented, focusing on the following recent developments: a) We have identified the most common features for modulation through a pharmacophore[1,2]. b) The relevance of a linker between the two P-gp halves (absent from the crystallographic structure and playing a role in substrate recognition) was assessed through several molecular dynamics experiments[3,4]. c) Substrates and modulators studied inside the drug-binding pocket revealed striking mechanistic differences. Global motion patterns were also characterized that correlate with conformational changes in the initial step of the efflux mechanism[4]. d) A docking study revealed three putative drug-binding sites. These sites match the modulators (M-site) and substrate-binding sites (H-site and R-site), partially described in literature[5]. e) A new classification scheme (based on binding and physicochemical properties, and "cross-linking" ability displayed by modulators) able to discriminate substrates from modulators will be presented, integrating a vast number of theoretical and experimental data[5].

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