21 DEZ

POSTER

COMUNICAÇÃO ORAL

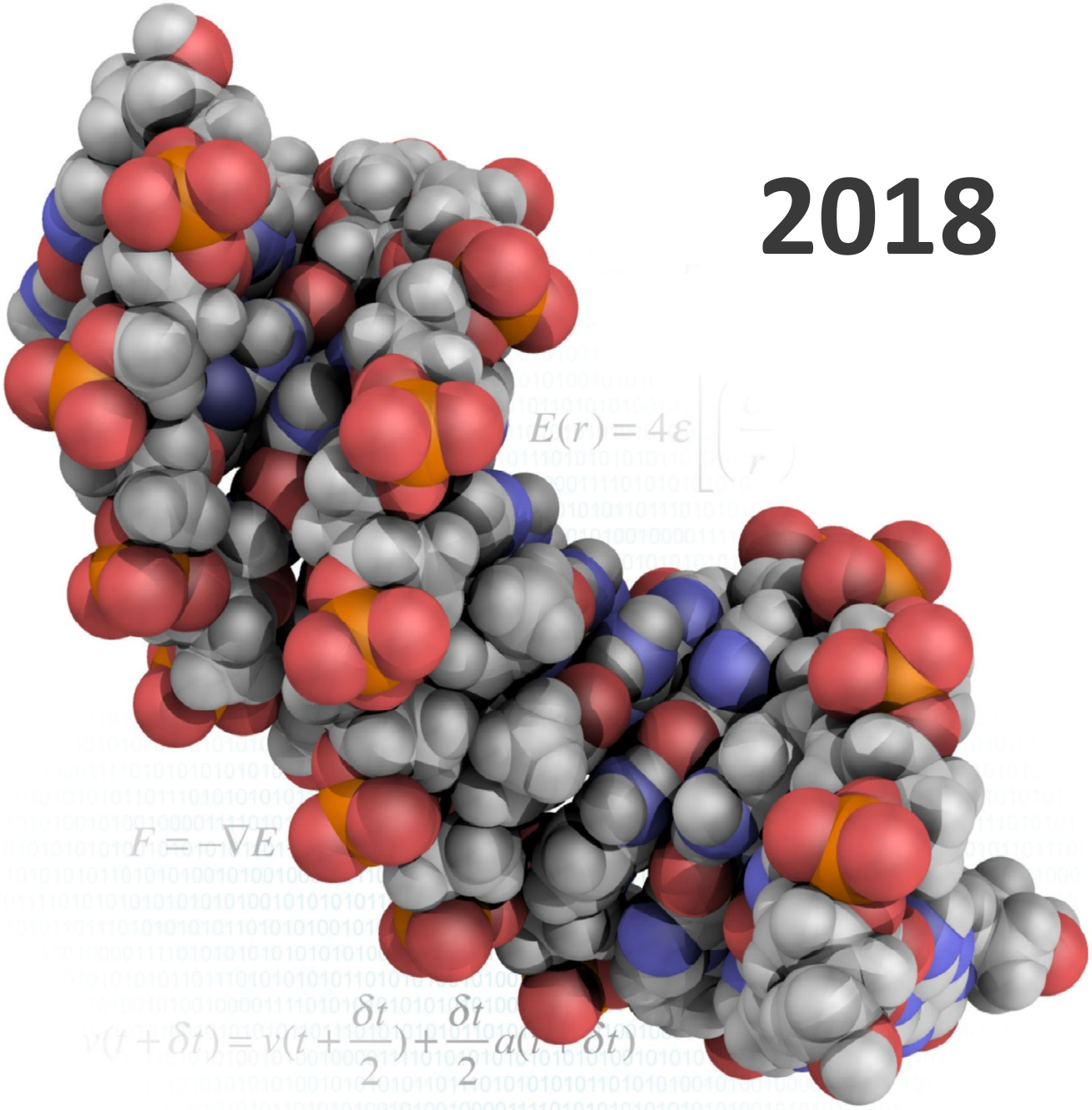
sponsored by molecules

EJIBCE

2018

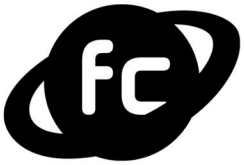
Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



2018

> ${Patrocínios}



> ${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? 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 investigadores portugueses radicados no estrangeiro. Desta forma, 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.

> ${Prémios}

Em parceira com a editora MDPI e a revista científica *Molecules*, serão atribuídos prémios aos melhores posters e comunicações orais do EJIBCE 2018.

`http://ejibce.github.io/`

> ${Comité Científico}

Carmay Lim, National Tsing Hua University (Taiwan)

Vittorio Limongelli, University of Lugano (Switzerland)

Giordano Mancini, Scuola Normale Superiore di Pisa (Italy)

> ${Organização}

Sérgio F. Sousa, BioSIM, REQUIMTE/FMUP

Nuno S. Cerqueira, BioSIM, REQUIMTE/FMUP

Carla Teixeira, BioSIM, REQUIMTE/FMUP

Henrique Fernandes, BioSIM, REQUIMTE/FMUP

Juliana Rocha, BioSIM, REQUIMTE/FMUP

André Pina, BioSIM, REQUIMTE/FMUP

Tatiana Vieira, BioSIM, REQUIMTE/FMUP

Cristina Sousa, BioSIM, DQB/UMinho

Bruno Pinto, BioSIM, REQUIMTE/FMUP

Rita Magalhães, BioSIM, REQUIMTE/FMUP

Irina Moreira, CNC

António Preto, CNC

> ${Contents}

21 DEZ

POSTER

COMUNICAÇÃO ORAL

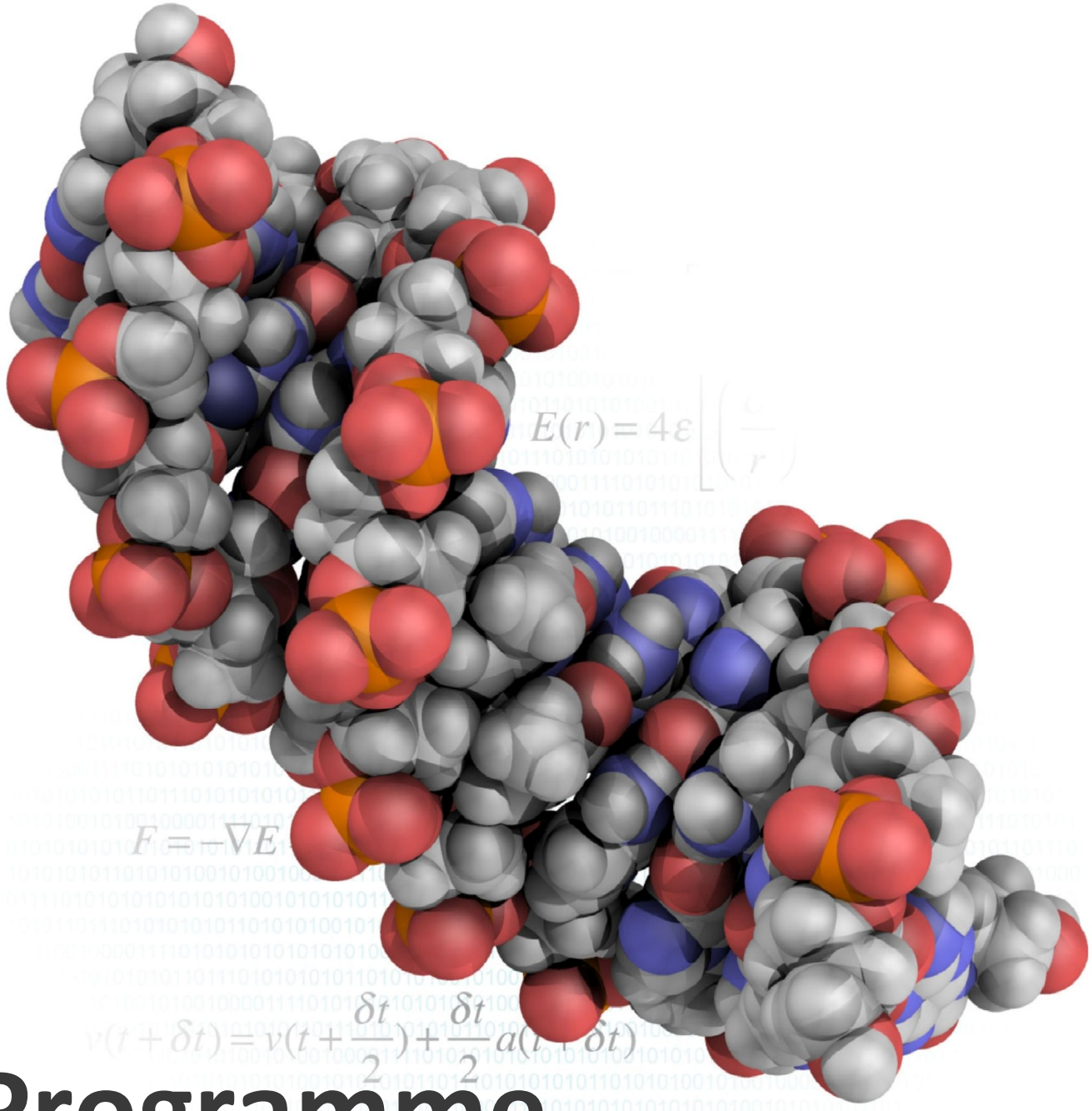
sponsored by molecules

EJIBCE

2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



Programme

EJIBCE 2018

Programme

08:30	Registration		*Foyer* - FMUP
09:30	Opening Ceremony		Room 4 - FMUP
09:35	**K1**: Improving the description of hydrogen bonds in molecular docking using DFT calculations	**Diogo Santos-Martins** The Scripps Research Institute (USA)	
10:05	**OC1**: Molecular Modelling, Analysis of Protein Tunnels and Screening the Binding Trajectories of Inhibitors using the Caver Suite	**Gaspar Pinto** Masaryk University (Czech Republic)	
10:25	**OC2**: An *in silico* predictor of protein dimer interfaces	**António J. Preto** Universidade de Coimbra (Portugal)	
10:45	Poster Session 1 + Coffee Break		*Foyer* - FMUP
11:15	**K2**: Computer-Aided Discovery of JAK2-JH2 Inhibitors	**Ana S. Newton** Yale University (USA)	Room 4 - FMUP
11:45	**OC3**: Computational strategies for the engineering of peptide ligases	**Eduardo F. Oliveira** University of Groningen (Netherlands)	
12:05	**OC4**: Computational campaign to discover novel human 20S proteasome inhibitors	**Pedro M. P. Fernandes** Universidade de Lisboa (Portugal)	**Pedro M. P. Fernandes** Universidade de Lisboa (Portugal)
12:25	Lunch — Poster Session 1, Poster Session 2		*Foyer* - FMUP
14:30	**K3**: A Complete Pipeline for Enabling Efficient and Timely NMR Structural Biology on Challenging Pharmaceutical Targets	**Marta G. Carneiro** ZoBio B.V. (Netherlands)	**Marta G. Carneiro** ZoBio B.V. (Netherlands)
15:00	**K4**: The KH type III domain: From an ancestral peptide to three different protein folds	**Joana Pereira** Max-Planck Institute (Germany)	Room 4 - FMUP
15:30	**OC5**: PypKa: a python module for flexible Poisson-Boltzmann based pKa calculations with proton tautomerism	**Pedro B. P. S. Reis** Universidade de Lisboa (Portugal)	
15:50	Poster Session 2 + Coffee Break		*Foyer* - FMUP
16:30	**K5**: How do buried residues get phosphorylated?	**João Henriques** University of Copenhagen (Denmark)	Room 4 - FMUP
17:00	**OC6**: The impact of using single atomistic long range cutoff schemes with the GROMOS 54A7 force field	**Tomás F. D. Silva** Universidade de Lisboa (Portugal)	
17:20	**OC7**: Accelerating the DszD enzyme for the Biodesulfurization of Crude Oil and Derivatives	**Pedro Ferreira** Universidade do Porto (Portugal)	
17:40	Closing Ceremony + Awards		
18:00			

Keynote Oral Communication

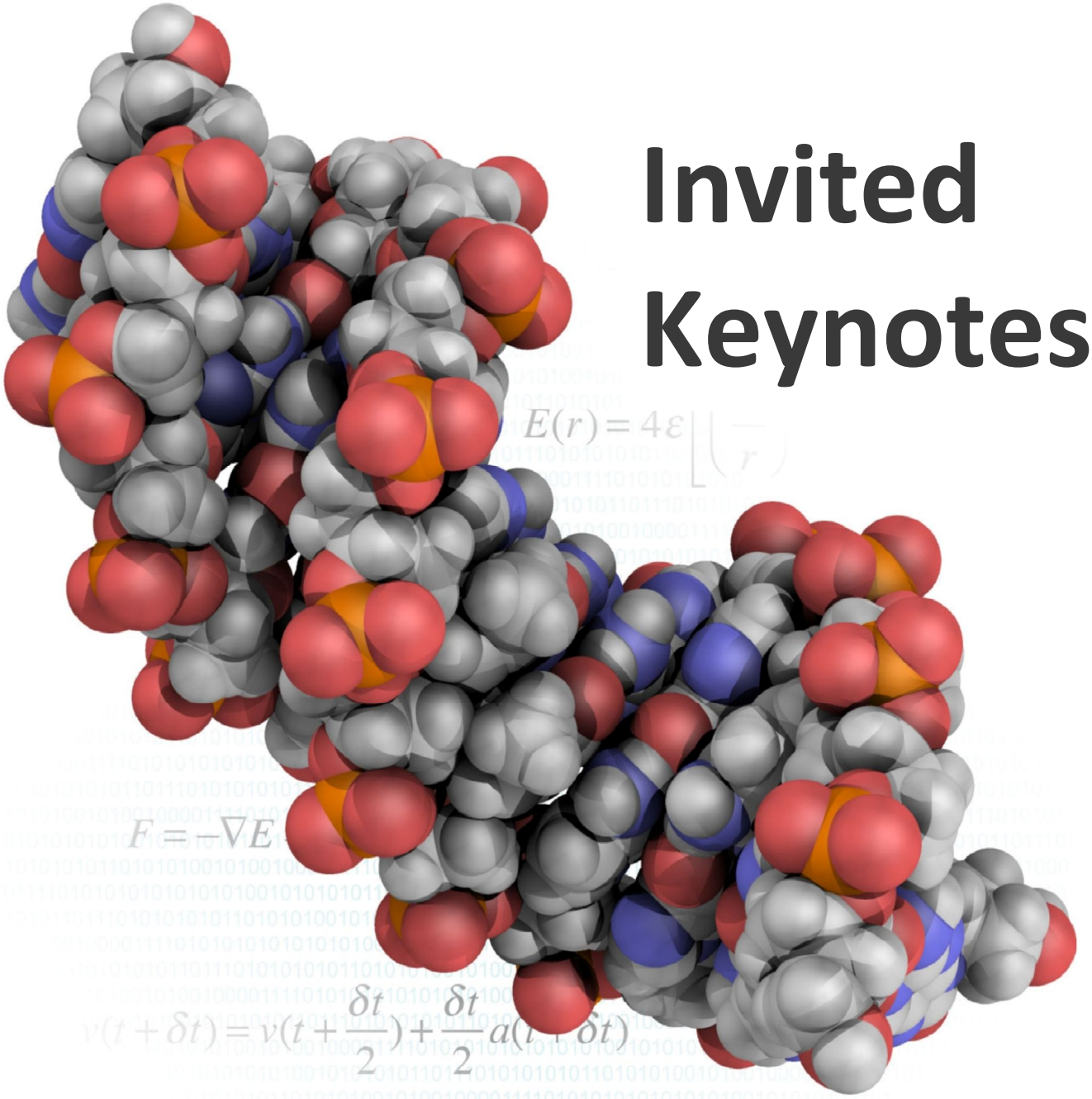
21 DEZ

EJIBCE

2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



Invited Keynotes

K1. Improving the description of hydrogen bonds in molecular docking using DFT calculations

Diogo Santos-Martins

Department of Integrative Structural & Computational Biology, The Scripps Research Institute, La Jolla, CA, USA
email: diogo.stmart@gmail.com

Molecular docking refers to methodologies that predict the binding pose of small molecules given the 3D structure of a larger molecule, usually a protein. Such methodologies are used in drug discovery to help medicinal chemists understand how and why molecules bind to macromolecular receptors.

A key component of molecular docking is the scoring function: it guides the search algorithm towards the best binding pose of a given molecule and may also be used to estimate binding affinities. Therefore, scoring functions should reasonably describe intermolecular interactions.

Our goal is to improve the description of hydrogen bonds (HB) in AutoDock. We used density functional theory (DFT) to calculate the hydrogen-bonding strength of several chemical groups, and also to probe the decay of interaction energy as HB's deviate from their ideal geometry. We then used the DFT data to upgrade the HB potentials in AutoDock, providing better discrimination between strong and weak HB's, and well as more accurate energies for suboptimal geometries.

K2. Computer-Aided Discovery of JAK2-JH2 Inhibitors

Ana S. Newton

Department of Chemistry, Yale University, New Haven, CT USA 06520-8107
email: ana.newton@yale.edu

Drug discovery is being pursued for JAK2-JH2 kinase involved in malignant blood diseases that can develop into leukemia. The approach combines state-of-the-art technology for molecular design, synthetic organic chemistry, biological assaying, and crystallographic determination of structures of the designed molecules bound to their protein target. Lead identification features *de novo* design with the ligand growing program *BOMB* or docking of commercial compound libraries. Emphasis is placed on optimization of the resultant leads to yield potent, drug-like inhibitors. Monte Carlo/free-energy perturbation (FEP) simulations are often executed. Micromolar leads have been rapidly advanced to nanomolar inhibitors, and numerous crystal structures for protein-inhibitor complexes have been obtained. Development and use of fluorescence polarization assays provide direct binding data.

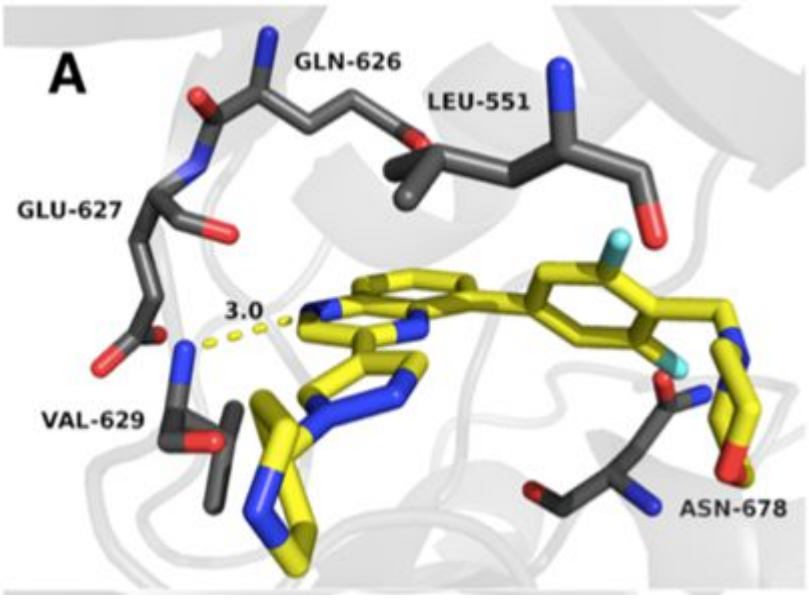


Figure 1. Rendering from a 2 Å x-ray crystal structure (5UT4) of NVP-BSK805 to JAK2-JH2 WT. The compound yields a K_d of 0.8 mM in a fluorescence polarization assay using a fluorescein-labelled tracer.

K3. A Complete Pipeline for Enabling Efficient and Timely NMR Structural Biology on Challenging Pharmaceutical Targets

Marta G. Carneiro, Stephan Theisgen, Eiso AB, Kavya Chepuri, Johan Hollander, Dipen Shah, Elseline van der Spek, Willem-Jan Waterreus, Gregg Siegal*

ZoBio B.V., Leiden, Netherlands *Corresponding author

email: martagcarneiro@gmail.com

By now the power of structure-based drug design (SBDD) is so widely recognized that it has become essentially the de facto approach for target based, small molecule campaigns. NMR can provide structural information at different levels of resolution, with a trade-off between the amount of information/ambiguity versus throughput, and is indispensable for cases where crystallization fails, or where crystal contacts create artefactual binding sites. However, NMR structural biology efforts on pharmaceutical targets are often hindered by a variety of challenges including: poor yields of recombinant protein, limited solubility or instability of the protein and long experimental and analysis time needed for NMR resonance assignment and structural information. ZoBio has been implementing and developing comprehensive strategies to enable efficient and timely NMR structural biology and has routinely obtained protein-ligand co-structures by combining sparse NOE data with data-driven docking. The impact of these strategies on enabling NMR structural biology on difficult targets will be highlighted with various examples.

K4. The KH type III domain: From an ancestral peptide to three different protein folds

Joana Pereira, Anja Rau, Murray Coles, Mohammad ElGamacy, Kosuke Fujishima, Jörg Martin, Andrei N. Lupas

Department of Protein Evolution, Max-Planck-Institute for Developmental Biology, Tübingen, Germany
email: joana.pereira@tuebingen.mpg.de

Since the time of the Last Universal Common Ancestor (LUCA), folded proteins have been the fundamental catalysts of life. However, it is still unclear how they first emerged. Our hypothesis is that they resulted from the increased complexity of peptides in the "RNA-peptide world" that preceded LUCA[1,2]. Evidence for these peptides can be derived even today from regions of local sequence similarity within globally dissimilar folds. One example is the KH-motif, a 45-residue motif found at the core of Universal RNA/ssDNA-binding hnRNP K homology (KH) domains[1,3]. KH domains are known to adopt two topologically different folds, but no single genetic event could be conceived to explain their evolutionary connection[3,4]. By combining sequence classification with deep homology searches, protein structure comparison, and experimental structure determination, we found a third fold that contains the KH motif at its core. This KH type III[5] domain corresponds to the Small Domain (SD) of bacterial Ribonucleases G/E, which is involved in protein-protein interactions, but carries a KH-motif compatible with RNA/ssDNA-binding in some bacterial species. Like the type I and type II KH folds, the type III fold can also not be related to any of the others by a single genetic event, supporting the KH-motif as an ancestral peptide predating folded proteins.

We thank Jens Baßler, Vikram Alva, Laura Weidmann and Marcus Hartmann for helpful and stimulating discussions.

(1) Alva, V.; Söding, J.; Lupas, A. N. A Vocabulary of Ancient Peptides at the Origin of Folded Proteins. *Elife* **2015**, *4*, e09410.

(2) Lupas, A. N.; Alva, V. Ribosomal Proteins as Documents of the Transition from Unstructured (Poly)Peptides to Folded Proteins. *J. Struct. Biol.* **2017**, *198* (2), 74–81.

(3) Grishin, N. V. KH Domain: One Motif, Two Folds. *Nucleic Acids Res.* **2001**, *29* (3), 638–643.

(4) Lupas, A. N.; Ponting, C. P.; Russell, R. B. On the Evolution of Protein Folds: Are Similar Motifs in Different Protein Folds the Result of Convergence, Insertion, or Relics of an Ancient Peptide World? *J. Struct. Biol.* **2001**, *134* (2–3), 191–203.

(5) Pereira, J.; Lupas, A. N. The Ancestral KH Peptide at the Root of a Domain Family with Three Different Folds. *Bioinformatics* **2018**.

K5. How do buried residues get phosphorylated?

João Henriques

Structural Biology and NMR Laboratory & Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark
email: joao.henriques@bio.ku.dk

Protein phosphorylation is the most common post-translation modification of proteins and regulates many biological processes. As a biologically important example we have studied the complex formed by cyclins and cyclin-dependent kinases (CDKs), which play an essential role in the control of the eukaryotic cell cycle. Regulated inhibition of these complexes has been identified as an intrinsic component of cell-cycle control. p27 is 27-kDa protein that binds to and prevents the activation of different G1 and S phase cyclin-CDK complexes, and a number of studies have characterized p27 as an independent prognostic factor in various human cancers, including breast, colon, and prostate adenocarcinomas.

Three sequential phosphorylation events on specific sites of p27, regulate the activity of different cyclin-CDK complexes and ultimately control cell cycle proliferation or arrest. Notably, the first two post-translational modifications, which are required for the initial activation of these complexes, occur on solvent inaccessible (i.e., buried) tyrosine residues. If these residues are inaccessible to kinases, how do they get phosphorylated then?

We hypothesize that a dynamic equilibrium between the dominant buried state and a transiently open, kinase-accessible state is present in the p27-cyclin A-CDK2 complex, and aim to test this hypothesis through the use of unbiased molecular dynamics and metadynamics simulations. From these simulations we aim to obtain a more detailed understanding of the conformational ensemble of this complex and the binding and release mechanism of p27, as well as to be able to calculate the free energy difference between the bound and unbound states. The latter should prove important in understanding whether the functionally important, but transiently populated state, where Tyr88 of p27 is solvent accessible, is likely to occur spontaneously or not.

More generally, bioinformatics analyses have shown that ~15% of all phosphorylated residues are buried in the non-phosphorylated state, suggesting that transient exposure might be a general

mechanism involved in protein regulation. Thus, our work could open up for a novel and detailed understanding of the structural and dynamical changes involved in a much larger set of proteins.

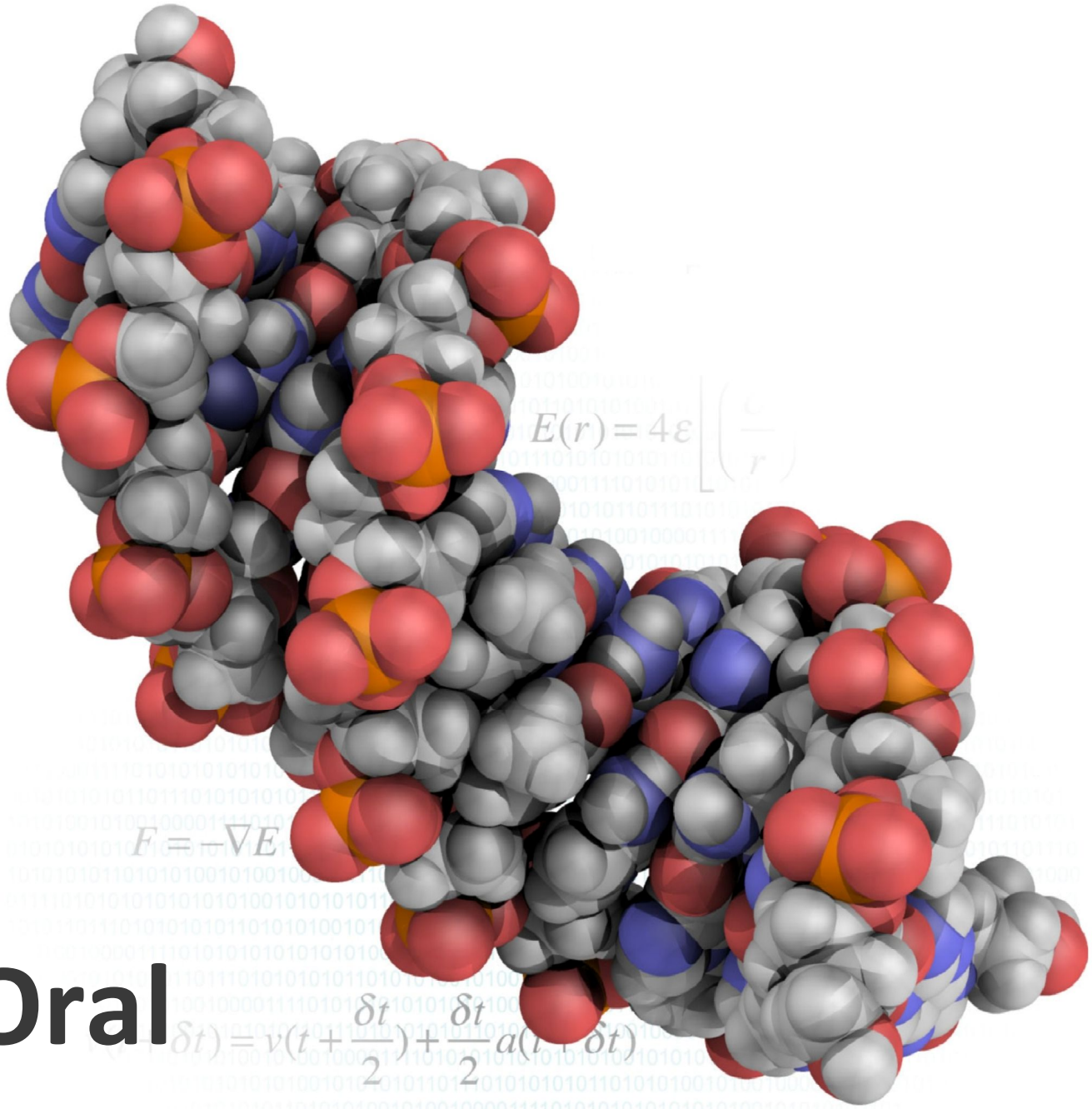
21 DEZ

EJIBCE

2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



Oral Communications

OC1. Molecular Modelling, Analysis of Protein Tunnels and Screening the Binding Trajectories of Inhibitors using the Caver Suite

Pinto, G.[1,2], Kokkonen, P.[1,2], Filipovic, J.[3], Vavra, O.[1], Jurcik, A.[4], Kozlikova, B.[4], Bednar, D.[1,2], Damborsky, J.[1,2]

[1] Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic
[2] International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
[3] Institute of Computer Science, Masaryk University, Brno, Czech Republic
[4] Human Computer Interaction Laboratory, Faculty of Informatics, Masaryk University, Brno, Czech Republic

Protein tunnels and gates are attractive targets for drug design [1]. The drug molecules blocking the access of natural substrate or release of products are very efficient modulators of biological activity. Tunnels are important for the transport of ligands, solvent and ions, and can be found in many enzymes and membrane-bound proteins. Caver Analyst, [2] which is part of the Caver suite, [3] can be used to identify tunnels in both static structures as well as molecular dynamics trajectories. Moreover, we have developed CaverDock for the study of the transport of ligands through the protein tunnels. CaverDock is fast, robust and an accurate tool which allows the screening of binding and unbinding processes for pharmacologically interesting compounds. It is based on a modified AutoDock Vina algorithm [4]. We have used FDA-approved drugs for two targets: (i) cytochrome P450 17A1 and (ii) leukotriene A4 hydrolase/aminopeptidase. Oncological drugs (133 molecules) were taken from the NIH website and anti-inflammatory drugs (56 molecules) were taken from the drugbank website. The tunnels studied in the cytochrome P450 17A1 had lengths of 15.1 Å, 24.9 Å and 28.2 Å, while the tunnels studied on the leukotriene A4 hydrolase/aminopeptidase had lengths of 20.4 Å and 25.4 Å. We were able to correctly reproduce the positions of ligands observed by X-ray crystallography with RMSD of 3.0 Å, 5.3 Å and 1.9 Å in cytochrome P450 17A1; and 5.4 Å and 2.8 Å in A4 hydrolase/aminopeptidase. The screening took 3260 s and 1760 s per molecule on average, using 4 processors, and successfully finished for >90% studied cases. We conclude that CaverDock is sufficiently fast, robust and accurate to allow screening of binding and unbinding processes for pharmacologically important targets containing molecular tunnels or channels. The software Caver Analyst 2.0 and CaverDock 1.0 are available free of charge at the website https://www.caver.cz.

References:

[1] Marques, S.M., et al. 2016: Enzyme Tunnels and Gates as Relevant Targets in Drug Design. Medicinal Research Reviews 37: 1095-1139.

[2] Jurcik, A., et al. 2018: Caver Analyst 2.0: Analysis and Visualization of Channels and Tunnels in Protein Structures and Molecular Dynamics Trajectories. Bioinformatics doi: 10.1093/bioinformatics/bty386.

[3] Chovancova, E., et al. 2012: CAVER 3.0: A Tool for Analysis of Transport Pathways in Dynamic Protein Structures. PLOS Computational Biology 8: e1002708

OC2. An *in silico* Predictor of Protein Dimer Interfaces

Preto, A. J.[1], Matos-Filipe, P.[1], Almeida, J. G.[1], Koukos, P. I.[2], Bonvin, A. M. J. J.[2], Moreira, I. S.[1#]

[1] Centro de Neurociências e Biologia Celular, UC - Biotech Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal
[2] Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, The Netherlands

Membrane Proteins (MPs) are implicated in a variety of medical conditions ranging from neurological diseases to cancer, due to their ubiquity in the cell membranes and the specificity conveyed by their sequence, structure and multimeric forms. In the current landscape, MPs and its oligomeric forms are increasingly and steadily gaining attention as specific targets for disease treatment [1]. As such, the identification of the interfacial residues at MPs is key for the correct understanding of their multimeric structure and function [2].

MENSA, MEmbrane protein dimers Novel Structure Analyser is a novel approach that makes use of evolutionary and structural information to characterize the currently available experimentally determined MP dimer structures. Indeed, by deploying machine-learning algorithms, MENSA is a new platform for drug discovery that allows the prediction of the MP interfacial residues.

References

[1] D. T. Moore, B. W. Berger, and W. F. DeGrado, "Protein-protein interactions in the membrane: sequence, structural, and biological motifs," *Structure*, vol. 16, no. 7, pp. 991–1001, Jul. 2008.

[2] C. Yan, F. Wu, R. L. Jernigan, D. Dobbs, and V. Honavar, "Characterization of Protein–Protein Interfaces," *Protein J.*, vol. 27, no. 1, pp. 59–70, Jan. 2008.

OC3. Computational strategies for the engineering of peptide ligases

Oliveira, E.F.[1], Wijma, H.J.[1], Toplak, A.[2], Marrink, S.J.[1], Janssen, D.B.[1]

[1] University of Groningen

[2] EnzyPep B. V.

There are currently 60 approved peptide pharmaceutical drugs, with many more candidates in clinical development. Solid-phase chemical synthesis is the prevailing method for large-scale production of small peptides, however the yield drops sharply as the size of the peptide grows. For peptides larger than 15 amino acids, we have to couple two shorter fragments either by conventional chemical fragment condensation or enzymatic coupling. The latter does not require side-chain protection, prevents racemization, facilitates purification and is not limited by the sequence of the coupling residues. Moreover, the development of coupling enzymes selective for specific sequences has several advantages: allows optimizing the activity for each enzyme, permits to combine multiple ligation steps in one-pot reaction and use of unprotected peptide fragments.

The most common tool to re-engineering enzymes is directed evolution, but it requires construction of a large mutant library, exhaustive analysis by functional screening and it might become prohibitive if expression and characterization are slow or cannot be miniaturized. Consequently, the way forward is to use computational simulations to design smaller and rational libraries.

We are currently developing computational protocols to model engineered enzymes and assess their catalytic activity. The protocol consists of 3 steps: sampling the sequence-conformational space of the enzyme active region, model near attack conformations (NACs) and, finally, assess the stability of NACs by high-throughput molecular dynamics.

To guide the protocol development, we have access to a dataset developed by EnzyPep that consist of a library with more than 300 mutants of peptiligase, with corresponding experimental activity on more than 100 peptide substrates. Herein, we will benchmark our computational predictions against the experimental activity of a library of 35 mutants developed for coupling of thymosin-α1, a peptide of 28 amino acids approved for the treatment of Hepatitis B and C.

OC4. Computational campaign to discover novel human 20S proteasome inhibitors

Fernandes, P.M.P.[1], Guedes, R.A.[1], Victor, B.L.[1], Guedes, R.C.[1]

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

The Ubiquitin Proteasome Pathway (UPP) plays a pivotal role in intracellular protein degradation and turnover in eukaryotic cells. [1] Therefore, modulation of the UPP emerged as a rational therapeutic approach in cancer, neurodegenerative diseases, among others. [2] During the last two decades academia and pharmaceutical industry made huge efforts to develop natural and synthetic proteasome inhibitors (PI). However, despite the enormous potential of PI, their use is still limited to certain types of blood cancer and shows severe side effects, limited activity in solid tumor and innate or acquired drug resistance. [3] This work encompasses a computational drug discover campaign to find new small molecules that inhibit proteasomal activity, with the goal of obtaining new anti-cancer drugs. A set of several compounds were identified in our lab as PIs obtained from virtual screening procedure. Since the proteasome can be found both on the cell cytoplasm and nucleus, inhibitors developed to target it, must be able to cross the membrane barrier. To acquire more information on how they interact with the lipid bilayer restrained (PMF) and unrestrained MD simulations at the water/membrane interface. The results showed that our compounds have similar permeability rates and behavior in the lipid bilayer when compared with known proteasome inhibitors. Furthermore, one of the major challenges with the approved PIs is acquired resistance, possibly from point mutations in the catalytic subunits of the proteasome. We have used MD simulations to focused our analysis on three different point mutations in the β5 catalytic subunit, with recognized importance in PI's resistance: Ala49Thr, Ala50Val and Cys52Phe. Hopefully, our studies will be able to shed the light on the structural key determinants that regulate the observed PI's resistance in the different mutations, and ultimately use the acquired knowledge in the development of new alternative and efficient proteasome inhibitors.

Acknowledgements: We thank the Fundação para a Ciência e a Tecnologia for financial support PTDC/QEQ-MED/7042/2014, UID/DTP/04138/2013 and SAICTPAC/0019/2015.

References

[1] Sommer, T.; Wolf, D. H. Biochim. Biophys. Acta - Mol. Cell Res. 2014, 1843 (1).

[2] Nalepa, G.; Rolfe, M.; Harper, J. W. Nat. Rev. Drug Discov. 2006, 5 (7), 596–613.

[3] Crawford, L. J.; Walker, B.; Irvine, A. E. J. Cell Commun. Signal. 2011, 5 (2), 101–110.

OC5. PypKa: a python module for flexible Poisson-Boltzmann based pKa calculations with proton tautomerism

Reis, P.B.P.S.[1], Vila-Viçosa, D.[1], Rocchia, D.[2], Machuqueiro, M.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[2] CONCEPT Lab, Istituto Italiano di Tecnologia (IIT), Genoa, Italy

pKa values have a significant impact on the structure and function of biomolecules, influencing many physicochemical and ADME properties. Thus, the calculation of pKa values is widely used in different scientific communities, including bioinformatics, structural biology and medicinal chemistry. We have implemented a flexible tool to predict Poisson-Boltzmann-based pKa values of biomolecules. This is a free and open source project that provides a simple, reusable and extensible python API for pKa calculations with a valuable trade-off between fast and accurate predictions. With PypKa one can enable pKa calculations, including optional proton tautomerism, within existing protocols by adding two extra lines of code. PypKa supports CPU parallel computing on anisotropic (membrane) and isotropic (protein) systems, and allows the user to find a balance between accuracy and speed. Due to its open source nature, there is an opportunity to continually evolve a user-friendly, reliable and flexible API that has applicability across a wide range of fields.

OC6. The impact of using single atomistic long range cutoff schemes with the GROMOS 54A7 force field

Silva, T. F. D.[1], Vila-Viçosa, D.[1], Reis, P. B. P. S.[1], Victor, B. L.[1], Diem, M.[2], Oostenbrink, C.[2], and Machuqueiro, M.[1]

[1] Faculdade de Ciências da Universidade de Lisboa

[2] University of Natural Resources and Life Sciences Vienna

Due to the recent increase in computing power, the molecular modeling community has been focused on improving the accuracy and overall quality of biomolecular simulations. These technological improvements centered on the development of new force fields and simulation packages, allowed for more complex and heavier systems to be tackled, while maintaining, or even improving simulations speed. Some force fields, such as GROMOS, have been parameterized and validated using a reaction field (RF), charge groups and a twin-range cutoff scheme (0.8 and 1.4 nm) to treat long range electrostatics [1]. However, in GROMACS software package, the use of group-based cutoff scheme will be deprecated in future versions. As to properly use the newer and faster versions of this simulation package coupled with GROMOS 54A7 and RF, it is crucial to assess the impact on the system sampling when using a single atomistic cutoff (based on the Verlet method) instead of the twin-range group-based scheme.

We reproduced the GROMOS parameterization procedure [1] with both schemes and measured very similar hydration free energy values of small amino acid side chains analogs between both protocols [2]. However, we observed a small, yet significant, difference on the conformational spaces of G1-PAMAM and DMPC when using the atomistic cutoff scheme [2]. The DMPC Al values decrease to a region outside the experimental range, which is not surprising since the force field parameters were optimized for a group-based scheme. Nevertheless, the structural properties for both systems are better converged for the used atomistic cutoff range (1.4-2.0 nm) relative to the group-based cutoff simulation set [2]. The use of a single atomistic cutoff scheme seems a viable approach for MD simulations of biomolecules using G54A7 force field, even if in some cases, new calibration protocols are needed.

References

[1] Oostenbrink, C.; Villa, A., Mark, A. E.; van Gunsteren, W. F. A biomolecular force field based on the free enthalpy of hydration and solvation: The GROMOS force-field parameter sets 53A5 and 53A6. J. Comput. Chem. 2004, 25, 1656–1676.;

[2] Silva, T. F. D.; Vila-Viçosa, D.; Reis, P. B. P. S.; Victor, B. L.; Diem, M.; Oostenbrink, C.; and Machuqueiro, M. The impact of using single atomistic long range cutoff schemes with the GROMOS 54A7 force field. J. Chem. Theory Comput., Just Accepted, DOI: 10.1021/acs.jctc.8b00758

OC7. Accelerating the DszD enzyme for the Biodesulfurization of Crude Oil and Derivatives

Ferreira, P.[1], Sousa, S. F.[1], Fernandes, P. A.[1], Ramos, M. J.[1]

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

It is known that fossil fuel combustion is one of the main environmental problems of the modern era, and the sulfur content of crude oil [1] contributes heavily to this. One of the main sulphurous compounds present in crude oil is dibenzothiophene (DBT). Due to its harmfulness, several governments around the world have been imposing stricter restrictions regarding the sulfur content in fossil fuels. The desulfurization of crude oil is currently carried out using costly chemical processes. One alternative to these costly chemical processes involves the use of specific microorganisms, such as Rhodococcus erythropolis, capable of utilizing DBT as a sole source of sulfur. The process carried out by R. erytrhopolis is called the 4S pathway and is conducted by the action of four enzymes of the dibenzothiophene desulfurization enzymes (dsz) family. DszA, DszB, DszC and DszD. The major limitation of this pathway is the slow catalytic rates of the four enzymes, which limits its application in industry.

The enhancement of the catalytic power of enzymes is a subject of enormous interest both for science and for industry. The latter, in particular, due to the vast applications enzymes can have in industrial processes.

In this work, we sought to enhance the turnover rate of DszD from Rhodococcus erythropolis, a NADH-FMN oxidoreductase responsible to supply FMNH2 to DszA and DszC in the biodesulfurization process of crude oil, the 4S pathway. For that purpose, we replaced the wild type spectator residue of the rate-limiting step of the reduction of FMN to FMNH2 catalysed by DszD, known to have an important role in its energetic profile, with all the natural occurring amino acids, one at a time, using computational methodologies, and repeated the above-mentioned reaction with each mutant. To calculate the different free energy profiles, one for each mutated model, we applied quantum mechanical methods (namely DFT) within an ONIOM scheme. The free energy barriers obtained for the different mutated models varied between 15.1 $kcal.mol^{-1}$ and 29.9 $kcal.mol^{-1}$. Multiple factors contributed to the different ΔGs. The most relevant were electrostatic interactions and the induction of a favourable alignment between

substrate and cofactor. These results confirm the great potential that chirurgic mutations have to increase the catalytic power of DszD in relation to the wt enzyme.

References

[1] N. Kamali, M. Tavallaie, B. Bambai, A. A. Karkhane and M. Miri, Biotechnol Lett, 2010, 32, 921-927.

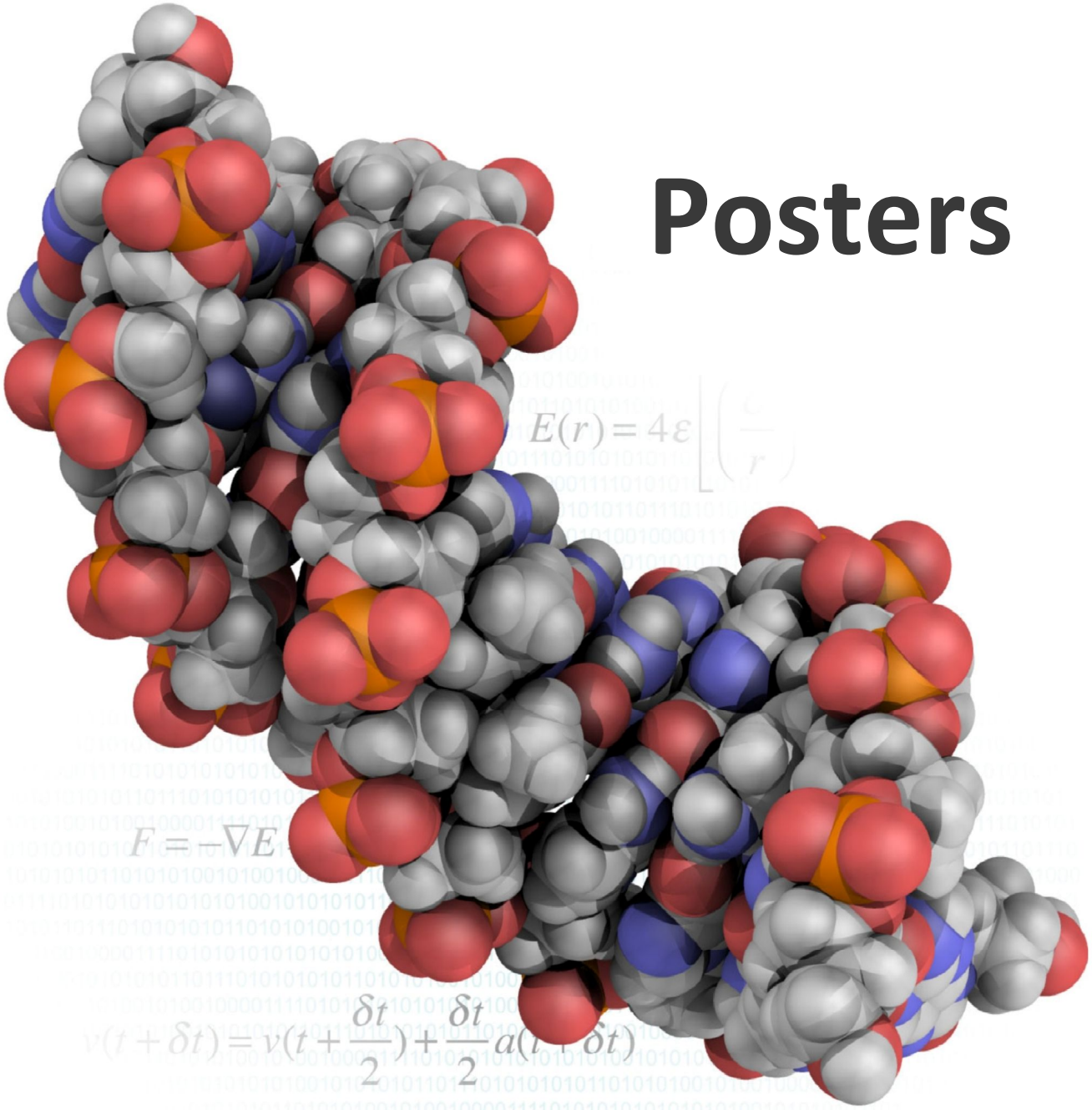
21 DEZ

EJIBCE

2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



Posters

P1. A multi-target approach for hormone-dependent breast cancer: estrogen receptors and aromatase

Almeida, C.[1,2], Oliveira, A.[1], Amaral C.[2], Ramos, M. J.[1], Correia-da-Silva, G.[2], Teixeira, N.[2] and Fernandes, P. A.[1]

[1] Laboratório de Bioquímica Teórica, UCIBIO e Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto
[2] UCIBIO, REQUIMTE, Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto

Introduction: Estrogen receptor positive breast cancer (ER+) is the most common form of cancer cell death in woman worldwide. Aromatase is the enzyme responsible for the conversion of androgens into estrogens, hormones implicated on growth of this type of cancers [1]. ERα and ERβ mediate the action of estrogens on cells. Therefore, in order to promote tumor cell growth, the aromatase and ERα are up-regulated, while ERβ is down-regulated since it is linked with anti-proliferative/pro-apoptotic effects. Considering these functions, aromatase and ERs are attractive therapeutic targets against this type of tumors.

Besides the clinical success of the current first-line treatments, the occurrence of endocrine resistance and undesired side effects [2], highlights the need to discover novel drugs that may improve breast cancer treatment and overcome resistance. Furthermore, development of multi-target drugs is a challenge. Taking this into account, our goal is to increase our knowledge on the chemical evolution of the drugs that interact with these targets and understand their specificity. In order to improve the multi-target affinity, a pharmacophore model will be designed.

Methods: We have made use of the ChEMBL Database to retrieve all the active compounds that inhibit receptors and aromatase functions. Using the ChemAxon software we computed two types of chemical descriptors: extended connectivity fingerprints and the pharmacophore fingerprints of each compound. The compounds were then hierarchical clustered. In parallel, the biological activity of the best compounds was selected to determine a 3D QSAR model using the open 3D-QSAR software.

Conclusions: For the best of our knowledge, this is the first attempt to a multi-target approach that aims to discover new compounds that simultaneously inhibit aromatase activity and modulate the actions of ERs. In this context, we have identified the selectivity and promiscuity of the

targets. Based on the results, we will perform a virtual screening and after evaluate their effects in in vitro assays using appropriate cell lines.

References

[1] Amaral C et al. Anti-tumor efficacy of new 7alpha-substituted androstanes as aromatase inhibitors in hormone-sensitive and resistant breast cancer cells. J Steroid Biochem Mol Biol. 2017;171:218-28.

[2] Augusto TV et al. Acquired resistance to aromatase inhibitors: where we stand! Endocr Relat Cancer. 2018;25(5):R283-R301.

P2. Understanding Structural and Dynamic Differences of GHSR-Gq and GHSR-Arr Complexes

Barreto, C.[1], Melo, R.[1,2], Magalhães, P. R.[3], Renault, P.[1], Machuqueiro, M.[3], Moreira, I. S.[1]

[1] Centro de Neurociências e Biologia Celular, UC - Biotech Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal

[2] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal

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

G-protein-coupled receptors (GPCRs) are considered as highly relevant drug targets and are the focus of more than ~27% of all drugs in the market [1]. Therefore, it is not surprising that they are the subject of major mechanistic efforts to understand their function. GPCRs are characterized by a seven transmembrane (TM) spanning alpha-helices, connected to three extracellular (ECL) and three intracellular loops (ICL) [2]. When activated, GPCRs can interact with different intracellular partners such as G-proteins and Arrestins [2]. Understanding the dynamical behaviour of GPCR-partner complexes can lead to the design of new selective drugs that induce a specific pathway in the receptor.

Herein, we will employ Molecular Dynamics (MD) simulations to attain a deeper understanding of the molecular interaction between G-protein (Gq) and Arrestin-2 with ghrelin receptor (GHSR), a member of family A of GPCRs superfamily [3]. Special attention was given to possible conformational rearrangements in the receptor structure between all different activation states.

References

[1] A. S. Hauser, M. M. Attwood, M. Rask-Andersen, H. B. Schiöth, and D. E. Gloriam, "Trends in GPCR drug discovery: new agents, targets and indications," *Nat. Rev. Drug Discov.*, vol. 16, no. 12, pp. 829–842, Oct. 2017.

[2] I. S. Moreira, "Structural features of the G-protein/GPCR interactions," *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1840, no. 1, pp. 16–33, 2014.

[3] T. Evron *et al.*, "G protein and β-arrestin signaling bias at the ghrelin receptor," *J. Biol. Chem.*, vol. 289, no. 48, pp. 33442–33455, Nov. 2014.

P3. The influence of conformational diversity on enzyme catalysis

Calixto, A. R.[1], Ramos, M. J.[1] and Fernandes, P. A.[1]

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

The role of conformational changes on enzyme catalysis has been a matter of discussion on recent studies, being the active site pre-organization pointed as one of the main reasons for the large catalytic power of enzymes, by many authors. Some specific conformations make the active site perfectly organized to allow the chemical reaction to happen with the lowest activation barriers.

In the present work this aspect were explored for the catalytic mechanism of two different enzymes (alpha-amylase and HIV-1 protease) with an adiabatic mapping method, starting from different initial structures, collected from a molecular dynamics simulation. Their catalytic mechanism were studied using the ONIOM QM/MM methodology, with activation and reaction energies calculated at the M06-2X/6-311++G(2d,2p):ff99SB level of theory, for different conformations of each enzyme:substrate complex.

The results showed that some enzyme conformational changes influence, not only the energetic profile of the reaction, but also its chemical progress. For alpha-amylase we found that reactive states require a hydrogen bond between a buried water molecule and a glutamate residue, which acts as the general acid on the catalyzed reaction mechanism. This hydrogen bond increases the acidity of this residue, facilitating its role as an acid. The conformational changes on this enzyme led to activation energy oscillations from 9.3 kcal/mol to 28.3 kcal/mol [1]. For HIV-1 protease two different chemical mechanisms, leading to the same intermediate, were found. Small structural differences of the active site hydrogen bonding network determine the progress of the reaction by on mechanism or the other. These small variations lead also to fluctuations in the activation barriers.

These studies help to understand how enzymatic reactivity is influenced by conformational diversity and they are important to gain a finer understanding of the results obtained from molecular ensembles.

References

[1] Santos-Martins D., Calixto A. R., Fernandes P. A., Ramos M.J., A buried water molecule influences reactivity in alpha-amylase on a sub-nanosecond timescale, ACS Catalysis, 2018, 8(5), 4055–4063

P4. Coarse grain-molecular dynamics simulations of cyclic peptide nanotubes on biological membranes for different negative charge content

Calvelo, M.[2], Claro, B.[1], Bastos, M.[1], Garcia-Fandiño, R.[1,2]

[1] CIQ-UP Dep. Chemistry & Biochemistry, FCUP, Porto, Portugal
[2] CIQUS Singular Research Centre in Chemical Biology and Molecular Materials, USC, Santiago de Compostela, Spain

The use of intelligent materials that form active species when in contact with bacterial membranes has been studied in recent years, with various applications. Cyclic Peptides (CP) of planar conformation are one promising application of these smart materials. [1] The orientation of their amide group perpendicular to the ring plane facilitates the formation of Self-assembling Cyclic Peptide Nanotubes (SCPNs) when in contact with membranes. With appropriate amino acid composition, they are currently seen as very promising antimicrobial candidates, due to their robust secondary structure and proven activity [2, 3], combined with a high resistance to protease degradation.

Here we used an in-silico/in-vitro approach to try to elucidate the mechanism of action of antimicrobial SCPNs, having as goal the design of new and improved antibacterial agents with a reduced toxicity and a minimized propensity for inducing resistance. Coarse-grained molecular dynamics (CG-MD) simulations have been employed to obtain information of the self-assembling of the SCPNs on several mammalian and bacteria model membranes with different DMPC:DMPE:DMPG composition, and thus about their disruptive action mechanism.

Acknowledgements: Thanks are due to Fundação para a Ciência e a Tecnologia (FCT), Portugal, for the financial support to Project UID/QUI/0081/2013, POCI-01-0145-FEDER-030579, Starting Grant IF/01133/2015 (RGF) and PhD grant PD/BD/135095/2017 (BC), and to FEDER (COMPETE 2020) for the financial support to SAM Project Norte-01-0145-FEDER-000028. RGF thanks Spanish Government MINECO for the Ramon y Cajal (RYC- RYC-2016-20335) contract and MC thanks to Xunta de Galicia for a predoctoral fellowship.

References

[1] Rodríguez-Vázquez, N; Ozores, H. L.; Guerra, A.; González-Freire, E.; Fuertes, A.; Panciera, M.; Priegue, J. M.; Outeiral, J.; Montenegro, J.; García-Fandiño, R.; Amorín, R.; Granja, J. R. Current Topics in Medicinal Chemistry 2014, 14, 2647-2661.

[2] Fernández, L.; Hancock, R. E. W. Clin Microbiol Rev, 2012, 25, 661-681.

[3] Fernandez-Lopez, S.; Kim, H. S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R., Nature 2001, 412 (6845), 452-455.

P5. From All Atoms to Coarse Grain: Simulating the Molecular Imprinting Process of a Silica Polymer

Concu, R.[1], Cordeiro, M. N.[1]

[1] REQUIMTE/ Faculdade de Ciências da Universidade do Porto

Molecular imprinted polymers (MIP) are used in very different fields such as solid-phase extraction, enantiomer separations, drug delivery, drug discovery, and so on. Due to this, different techniques have been investigated in the past few years. In this contest, sol-gel polycondensation technique is an interesting alternative since MIP produced with this technique has been proved to present several advantages such as physical robustness, long shelf life, simple preparation, great selectivity, etc. The most widely used precursors for preparing sol–gel materials have been silicon alkoxides, such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS). In a recent paper for the first time, we simulated a complex sol-gel system aimed at preparing the (S)-naproxen-imprinted xerogel with an explicit representation of all the ionic species at pH 91. With that simulation we were able to undercover the molecular mechanism behind the imprinting process. However, the simulation ran for only 100ns and we were unable to simulate other important process such as the polymer formation. One possible solution is to move on to a coarse-grain (CG) simulation based on the Martini force field2. The model uses a four-to-one mapping, i.e. on average four heavy atoms and associated hydrogens are represented by a single interaction center. One of the main advantages of this approach is that larger systems may be simulated for longer time. Due to this, the main aim of this study is the simulation of the molecular imprinting process using the Martini force field, in order to simulate all the relevant aspects occurring during the imprinting and polycondensation process.

References

Concu, R.; Perez, M.; Cordeiro, M. N.; Azenha, M., Molecular dynamics simulations of complex mixtures aimed at the preparation of naproxen-imprinted xerogels. J Chem Inf Model 2014, 54 (12), 3330-43.

Marrink, S. J.; Risselada, H. J.; Yefimov, S.; Tieleman, D. P.; de Vries, A. H., The MARTINI Force Field: Coarse Grained Model for Biomolecular Simulations. The Journal of Physical Chemistry B 2007, 111 (27), 7812-7824.

P6. Estimation of solvation free energies by continuum methods: How to tackle halogenated species?

Nunes, R.[1] and Costa, P. J.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Incorporating halogens (X) into drug candidates occupies an important role in drug discovery. While traditionally this strategy mainly aimed at improving drug-like properties, the pharmaceutical potential of halogenated compounds has been increasingly explored for their ability to modulate protein–ligand binding by establishing halogen bonds (XBs) [1]. These are non-covalent interactions (R-X...B) explained by the existence of a positive region on the electrostatic potential (ESP) of X, called sigma-hole, which interacts with Lewis bases (B). The development of computational methods that accurately model the charge anisotropy of halogenated compounds is of great importance, in view of their use in computer-aided drug design. Particularly challenging is the case of molecular mechanics (MM)-based methods since these rely on point charges, therefore failing to describe XBs. One approach to describe the ESP anisotropy involves the addition of an positive extra-point (EP) of charge mimicking the sigma–hole [2]. We have successfully applied this methodology to study protein–ligand complexes by means of molecular dynamics (MD) simulations [3]. Regarding the prediction of protein–ligand binding free energies, the use of MM energies combined with Poisson-Boltzmann surface area (MM-PBSA) continuum solvation is a popular methodology. While EP addition has been shown to improve the MM/MD description of halogen-containing systems, its effect on the binding free energy estimated by MM-PBSA is yet to be assessed. PBSA relies on the calculation of the solvation free energy of the ligand, among other terms, for which empirical parameters, such as PB radius, are required. We conducted a study on the effect of varying the halogen PB radii on the performance of MM-PBSA-based solvation free energy calculations for a library of halogenated ligands. The results highlight the impact of the choice of X's radii on the accuracy of the calculated values.

Acknowledgements: Investigador FCT (IF/00069/2014, IF/00069/2014/CP1216/CT0006), PHD grant SFRH/BD/116614/2016, IUD projects UID/QUI/UI0612/2013,

UID/MULTI/04046/2013. Lisboa 2020, Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

References

[1] Lu, Y. et al. J. Med. Chem. 2009, 52, 2854.

[2] Wolters, L. P. et al. WIREs Comput. Mol. Sci. 2014, 4, 523.

[3] Nunes, R. et al. J. Chem. Theory Comput. 2018, 14, 5383.

P7. The pH-dependent membrane stability and insertion mechanism of GALA peptide

Dias, J. R.[1], Silva, T.[1], Machuqueiro, M.[1]

[1] Faculdade de Ciências da Universidade de Lisboa

The cellular membrane is an essential component of all cells. Distributed on the cellular membrane are many different proteins, either integrated or in interaction, with a variety of functions, namely assisting in membrane transport. GALA is a helical peptide that is prone to protonation, at acidic pH, followed by insertion in the membrane [1]. In its folded form, GALA has an amphiphilic structure, and shows a concentration-dependent tendency to aggregate in multimeric structures inside the membrane, such that the polar glutamate residues face toward each other and the apolar residues face toward the membrane's lipid tails [1].

In this preliminary study, we used Constant-pH molecular dynamics (CpHMD) simulations [2] to study the conformation and protonation behaviour of GALA as a monomer and as a dimer (parallel or anti-parallel configurations) in the membrane. From these results, we obtained the effects of the initial step of GALA aggregation, particularly, in the secondary structure stabilization and the shifts in the pKa values of the different glutamate residues [2]. In the future, these results will serve as the basis for expanding this study to larger aggregated complexes with 3, 5 or 10 monomer subunits.

References

[1] Subbarao, N. K.; Parente, R. A.; Szoka, Jr., F. C.; Nadasdi, L.; Pongracz, K.; The pH-dependent bilayer destabilization by an amphipathic peptide. Biochemistry, 1987, 26, 2964-2912.

[2] Teixeira, V. H.; Vila-Viçosa, D.; Reis, P. B. P. S.; Machuqueiro, M.; pKa Values of Titrable Amino Acids at the Water/Membrane Interface. J. Chem. Theory Comput., 2016, 12, 930-934.

P8. Is the 5,10-methylenetetrahydrofolate cofactor synthesized through a non-enzymatic or enzymatic mechanism?

Fernandes, H. S.[1], Sousa, S. F.[1], and Cerqueira, N. M. F. S. A.[1]

[1]UCIBIO@REQUIMTE, BioSIM (www.biosim.pt), Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319, Porto, Portugal

The 5,10-methylenetetrahydrofolate (5,10-mTHF) is a cofactor essential for the synthesis of purines and thymidine, which are crucial for the cell viability [1]. The α-elimination of L-serine, catalyzed by the serine hydroxymethyltransferase (SHMT), is the primary source of 5,10-mTHF in the cell. However, the catalytic mechanism behind the synthesis of 5,10-mTHF was unknown, and two divergent theories were proposed for the mechanism. Some authors suggested that the final steps of the 5,10-mTHF synthesis occur in the cytoplasm whereas other authors showed some evidence that the reaction must occur inside the SHMT [2].

In this study, we addressed the entire catalytic mechanism of the PLP-dependent enzyme SHMT using a QM/MM approach and the mechanism of 5,10-mTHF synthesis in aqueous solution. The calculations were prepared and analyzed using molUP [3] for VMD and run on Gaussian09 and ORCA.

This work [4] resulted in the entire e detailed catalytic mechanism of SHMT. The results showed that both hypotheses for the synthesis of 5,10-mTHF shared the two first steps where the -OH group is transferred from the serine to the THF. These reactions occur inside the SHMT and have a ΔG‡ of 18.0 and 2.0 kcal/mol. Th en, the reaction can proceed inside the enzyme through 5 sequential steps or in the cytoplasm where only 3 steps are needed. The calculations showed that the mechanism is kinetic and thermodynamically favorable by 0.8 and 24.3 kcal/mol, respectively, when it takes place inside the SHMT. Although the reaction is not impossible in solution, it is very improbable that the THF intermediate might be released to the cytoplasm to overcome a set of reactions that are less favorable when compared to the ones that would occur in the SHMT.

Acknowledgments: FCT (SFRH/BD/115396/2016, IF/01310/2013, IF/00052/2014 and PTDC/QUI-QFI/31689/2017)

References

[1] Froese, D. S.; et al., Structural basis for the regulation of human 5,10-methylenetetrahydrofolate reductase by phosphorylation and S-adenosylmethionine inhibition. Nature comm 2018, 9 (1), 2261-2261.

[2] Schirch, V.; et al, Serine hydroxymethyltransferase revisited. Curr Opin Chem Biol 2005, 9 (5), 482-7.

[3] Fernandes, H. S.; et al., molUP: A VMD plugin to handle QM and ONIOM calculations using the Gaussian software. J Comput Chem 2018, 39 (19), 1344-1353.

[4] Fernandes, H. S.; et al., Catalytic Mechanism of the Serine Hydroxymethyltransferase: A Computational ONIOM QM/MM Study. ACS Catalysis 2018, 10096-10110.

P9. Text Mining for Recognition of Cancer Biomarkers

Gaspar, A. C.[1,*], Pires, M. N.[1,*], Preto, A. J.[1], Moreira, I. S.[1]

[1] Centro de Neurociências e Biologia Celular, UC – Biotech, Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal

[*] Joint first co-authors

Nowadays, cancer is one of the deadliest diseases in the world. Due to its incidence on a major part of the worlds' population and the difficulty to attain an overall cure, oncology research occupies a very large percentage of the medical, pharmaceutic and biological scientists worldwide. Various studies are looking for new biomarkers for more appropriate and accurate patient diagnosis and therapeutics. Herein, our main purpose is the identification and grouping of cancer biomarkers and the understanding of molecular and/or cellular responses or any other type of trace of cancer. We are building an algorithm, using unsupervised machine learning techniques, capable of identifying biomarkers in scientific articles databases. More specifically, we are currently deploying text mining techniques to establish a new *in silico* pipeline.

References

Hui Li, Chunmei Liu, "Biomarker Identification Using Text Mining", Computational and Mathematical Methods in Medicine Volume 2012, Article ID 135780, 4 pages

Vinaitheerthan Renganathan, "Text Mining in Biomedical Domain with Emphasis on Document Clustering", Healthc Inform Res. 2017 Jul;23(3):141-146. English.

P10. The importance of unstructured termini in the aggregation cascade of beta-2-microglobulin: insights from molecular simulations of D76N mutant

Loureiro, R.J.S.[1], Vila-Viçosa, D.[2], Machuqueiro, M.[2], Shakhnovich, E.I[3], Faísca, P.F.N.[1,4]

[1] BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa

[2] Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa

[3] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts

[4] Departamento de Física, Faculdade de Ciências da Universidade de Lisboa

The identification of folding and aggregation intermediate states is important, both from a fundamental standpoint and for the design of new therapies for conformational disorders. Here, we use the single point mutant (D76N) of β2m, the causing agent of a hereditary systemic amyloidosis affecting visceral organs, as a model system to study the aggregation mechanism of β2m using molecular simulations. We present our predictions on the early molecular events triggering the amyloid cascade for the D76N mutant. Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C-terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2. Additionally, Monte Carlo docking simulations suggest that both intermediates have high aggregation-propensity, particularly at acidic pH. These simulations support an essential role of the DE and EF-loops and the termini in the dimerization of both intermediates. The relevance of the C-terminus is higher at the acidic pH 5.2 while the N-terminus become important at pH 6.2. At physiological pH, the DE and EF-loops are the crucial regions for dimerization. These predictions rationalize experimental results that support the involvement of Lys-19, Phe-56, Trp-60 and Tyr-63 in amyloidogenesis in the wild-type and other model systems of β2m.

Acknowledgments: RJSL thanks FCT for financial support through doctoral fellowship PD/BD/108259/2015. PFNF and RJSL thanks FCT for financial support through grant UID/MULTI/04046/2013 from FCT/MCTES/PIDDAC (to BioISI).

P11. Influence of codon 35 amino acid insertion in HIV-1 protease: insights from molecular dynamics

Luís, J. P[1#], Mata, A. I.[1#], Alves, N. G.[1#], Simões, C. J. V.[1,2], Pereira-Vaz, J.[1,3], Vaz, D. C.[1,4], Duque, V. and Brito, R. M. M.[1,2]

[#] Equal author contribution,

[1] CQC, Chemistry Department, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal

[2] BSIM Therapeutics, Instituto Pedro Nunes, Coimbra, Portugal

[3] Laboratory of Molecular Biology, Clinical Pathology Unit, CHUC, Coimbra, Portugal

[4] Health Research Unit, School of Health Sciences, Leiria, Portugal e Infectious Diseases Unit, CHUC, Coimbra, Portugal

One of the main challenges facing the development of effective anti-HIV-1 medicines relates to the high mutation rate of essential enzymes, such as HIV-1 Protease (HIV1Pr) [1]. Pereira-Vaz et al. [2] first reported a threonine insertion at position 35 (E35E_T), in the HIV1Pr coding region, among treatment-naïve subtype C infected individuals. Undetectable viral loads were attained after antiretroviral therapy in such individuals, with no associated major mutations, implying null contribution of E35E_T to viral resistance. Interestingly, a new study suggests a potential additive effect of position 35 insertions when in presence of major mutations – ultimately leading to resistance to HIV1Pr inhibitors in higher extent [3].

In order to study the role of the E35E_T insertion in the structure and ligand-binding propensity of HIV1Pr, homology models were generated from subtype B and subtype C base sequences, using available X-ray structures corresponding to highest identity sequences as template. Fifty (50)-nanoseconds Molecular Dynamics (MD) simulations were then performed for unbound and bound (HIV1PR:darunavir complex) structures of the wild-type form and a singlepoint major mutation variant of HIV1PR – in all cases in presence and absence of E35E_T.

Combining simple measurements like the root mean square (RMS) deviations and fluctuations, applied to the whole protein and to its two functional flap regions, with principal component analysis (PCA) of the multiple MD trajectories, we herein contrast the behaviour of all systems in attempt to dissect the putative role of E35E_T in the resistance towards HIV1PR inhibitors.

References

[1] Baxter D. J. et al., An Update on HIV-1 Protease Inhibitor Resistance. J AIDS Clin Res. 2016, 7, 581.

[2] Pereira-Vaz J. et al., Detection of the protease codon 35 amino acid insertion in sequences from treatment-naïve HIV-1 subtype C infected individuals in the Central Region of Portugal. J Clin Virol. 2009, 46, 169.

[3] Kozísek M. et al., Ninety-nine is not enough: molecular characterization of inhibitor-resistant human immunodeficiency virus type 1 protease mutants with insertions in the flap region. J Virol. 2008, 82, 5869.

P12. pH effects on PG/PC and PS/PC lipid binary mixtures: a CpHMD study

Magalhães, P. R.[1], Vila-Viçosa, D.[1], Silva, T.[1], Machuqueiro, M.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Membranes are vital components of biological systems, fulfilling a myriad of roles in the cell [1]. Despite their highly diverse composition, they are primarily comprised of zwitterionic and anionic lipids which, in some cases, makes them sensitive to changes in pH. In computational methods, the inherent complexity of these systems is often simplified via the use of model membranes normally composed of a single lipid type or, in some cases, of binary or ternary mixtures. While these approximations are generally adequate, there are particular instances where the pH, and consequently, the titration of lipid headgroups, plays a key role in membrane stability and function, meaning that the development of more realistic membrane models is extremely important.

Previously, we reported in simulations of a 25% PA/PC (phosphatidic acid/phosphatidylcholine) mixture a pH-dependent phase transition from gel to fluid [2]. In this work, we assembled binary mixtures of either phosphatidylglycerol (PG) or phosphatidylserine (PS) in phosphatidylcholine (PC) with different molar fractions (10%, 25%, 50% or 75% of PG or PS) and studied the effect of pH using the latest implementation of our constant-pH MD method with lipid titration (CpHMD-L) [2].

Acknowledgments: We acknowledge financial support from FCT through grant SFRH/BPD/110491/2015 and projects PTDC/QEQCOM/5904/2014, UID/MULTI/00612/2013 and UID/MULTI/04046/2013.

References

[1] Edidin, M., NRMCB 2003, 4:414.

[2] Santos, H.A.F., Vila-Viçosa, D., Teixeira, V.H., Baptista, A.M., Machuqueiro, M. JCTC 2015, 11:5973.

P13. Creation of a Structural Database for Inhibition of Biofilm Formation

Magalhães, R. P.[1,2], Vieira, T. F.[1], Fernandes, H. S.[1], Cerqueira, N. M. F. S. A.[1], Melo, A.[2], Simões, M.[3], Sousa, S. F.[1]

[1] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[2] LAQV/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[3] LEPABE, Faculdade de Engenharia, Departamento de Engenharia Química, Universidade do Porto, Porto, Portugal

Biofilms can be prevalent in natural, industrial and hospital settings and are associated to ca. 80% of all human infections [1]. The increased antimicrobial resistance and mutation rate of bacteria in biofilms contributes to the development of antibiotic resistance, greatly limiting the therapeutic options to a variety of infections, posing a critical problem to the biomedical sector. Preventing biofilm formation could dramatically reduce the effects of infectious diseases [2].
Quorum-sensing (QS) is the cell-to-cell communication in bacteria and contributes to the formation of organized structural communities of bacteria in biofilms [3]. Several different microbial-derived signalling molecule types and receptors have been recently identified, offering a very appealing opportunity for rational design of new drugs.
This work reports the creation of a database containing all the available experimental X-ray structures for all the synthases and receptors known to be involved in quorum sensing and includes also structural and biological information on all the known compounds with demonstrated inhibitory activity against each of these protein targets.
This database will provide useful atomic-level information for researchers working on this field with direct application in drug design and development efforts through docking, virtual screening, molecular dynamics and QSAR techniques.

References

[1] Worthington, R. J.; Richards, J. J.; Melander, C., Small molecule control of bacterial biofilms. Organic & biomolecular chemistry 2012, 10 (37), 7457-74.

[2] Subhadra, B.; Kim, D. H.; Woo, K.; Surendran, S.; Choi, C. H., Control of Biofilm Formation in Healthcare: Recent Advances Exploiting Quorum-Sensing Interference Strategies and Multidrug Efflux Pump Inhibitors. Materials (Basel, Switzerland) 2018, 11 (9).

[3] Banerjee, G.; Ray, A. K., Quorum-sensing network-associated gene regulation in Gram-positive bacteria. Acta microbiologica et immunologica Hungarica 2017, 64 (4), 439-453.

P14. MENSAdb: A Major Structural Statistical Analysis of Membrane Protein Dimers

Matos-Filipe, P.[1], Preto, A. J.[1], Almeida, A. J.[1], Koukos, P. I.[2], Bonvin, A. M. J. J.[2], Moreira, I. S.[1#]

[1] Centro de Neurociências e Biologia Celular, UC – Biotech, Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal
[2] Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Utrecht, 3584CH, The Netherlands

Membrane Proteins (MPs) account for around 30% of organism's genome [1,2] and 60% of current drug targets are reported to target this type of biological systems [3]. Structure-based molecular design is one of the first methods to be used in medicine design [4], and as such gathering as much as possible all available information about MPs is a crucial step when developing new and more suited therapies from a holistic point of view. Previous work in this field proved the relationship between the presence of Hot-Spots (HS) and the suitability of small molecules to target binding pockets in MPs [5]. However, the role of Protein-Protein Interactions (PPIs) within MP dimers, has also a huge potential as a target to specific therapeutic drugs.
As such, in this work, we listed an overall statistical analysis of all kind of interactions (Close, Hydrophobic and Hydrogen Contacts, Salt Bridges, π interactions), various surface features and evolutionary conservation from a curated dataset retrieved from the known interacting MPs at mpstruc – Membrane Proteins of Known 3D Structure [6]. This allowed the tracing of a pattern of common characteristics of all possible interacting regions within MPs and its residues.

References

[1] Almén, M. S., Nordström, K. J. V, Fredriksson, R., et al. (2009) Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin., BMC Biol., 7, 50.
[2] Gromiha, M. M. and Ou, Y.-Y. (2014) Bioinformatics approaches for functional annotation of membrane proteins, Brief. Bioinform., 15, 155–168.
[3] Yin, H. and Flynn, A. D. Drugging Membrane Protein Interactions.
[4] Singh, S., Kumar Malik, B. and Kumar Sharma, D. Current Trends Molecular drug targets and structure based drug design: A holistic approach; 2006; Vol. 1.

[5] Bakail, M. and Ochsenbein, F. (2016) Targeting proteineprotein interactions, a wide open field for drug design Inhiber les interactions prot eineeprot eine, un large champ ouvert pour le d eveloppement de nouveaux m edicaments, Comptes rendus - Chim., 19, 19–27.
[6] White, S. H. mpstruct - Memebrane Proteins of Known 3D Structure http://blanco.biomol.uci.edu/mpstruc/ (accessed Nov 20, 2018).

P15. Dynamical Rearrangement of Human Epidermal Growth Factor Receptor 2 upon Antibody Binding: Effects on the Dimerization

Melo R.[1,2,*], Almeida J. G.[3], Melo A.[4], Cordeiro M. N. D. S.[4], Gumus, Z. H.[5], Cabo-Verde S.[1], Moreira I. S.[2], Correia J. D. G.[1]

[1] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal

[2] Centro de Neurociências e Biologia Celular, UC- Biotech Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal

[3] EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridge, UK

[4] REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

[5] Department of Genetics and Genomics and Icahn Institute for Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

[*] ritamelo@ctn.tecnico.ulisboa.pt

Human Epidermal Growth Factor Receptor 2 (HER2) is overexpressed in some types of tumours. In this work, we employed computational modelling and Molecular Dynamics (MD) simulations to attain a deeper understanding of the interaction between anti-HER2 antibodies, an antigen-binding (Fab) fragment (F0178) and a single chain variable fragment (scFv) from trastuzumab, and HER2. We performed MD simulations for each system as well as for the unbounded HER2. A variety of structural, energetic and dynamic characteristics was calculated for an ensemble of MD structures. An all-atom characterization of these intermolecular couplings and the breakthrough of its mechanism is fundamental for further development of innovative therapies. Our results clarified F0178 and scFv interactions with HER2. Interestingly, we observed that not only the interfacial residues in HER2: scFv are relevant, but the residues at ECDII-III, the HER2 dimerization arm, also have an important role on Protein-Protein Interactions (PPI). This fact supports and advances the described trastazumab effect on HER2 dimerization blocking through synergistic inhibition and/or steric hindrance. Overall, our approach offers a new strategy for fine-tuning target activity through allosteric ligands.

P16. Drug-Target Interaction Prediction: End-to-End Deep Learning Approach

Monteiro, N.R.C[1], Arrais J.P.[1], Ribeiro B.[1]

[1] Department of Informatics Engineering (DEI), Center for Informatics and Systems of the University of Coimbra (CISUC), University of Coimbra, Coimbra, Portugal

The discovery of potential Drug-Target Interactions (DTIs) is a determining step in the drug discovery and repositioning process. Establishing effective computational methods, instead of the traditional in vivo or in vitro methods, is decisive to find new leads in a considerable short of amount of time. Deep Learning have shown to outperform state-of-art methods in multiple classification problems.

In this work we evaluate an experimental setup that exploits the particular ability of Convolutional Neural Networks (CNNs) to obtain 1D representations from protein sequences (amino acid sequence) and SMILEs (Simplified Molecular Input Line Entry System, which represent the chemical structure as a string). This can be interpreted as features that express local dependencies or patterns that can then be used in a Fully Connected Neural Network (FC), acting as a binary classifier.

The results achieved show that the use of CNNs to obtain representations of the data, instead of using the traditional descriptors of proteins sequences and chemical structures, lead to a more effective performance.

Acknowledgements: This research has been funded by the Portuguese Research Agency FCT, through D4 - Deep Drug Discovery and Deployment (CENTRO-01-0145-FEDER-029266).

References

Öztürk, H., Özgür, A. & Ozkirimli, E. DeepDTA: deep drug–target binding affinity prediction. Bioinformatics 34, i821-i829 (2018). doi:10.1093/bioinformatics/bty593

Coelho, E. D., Arrais, J. & Oliveira, J. Computational Discovery of Putative Leads for Drug Repositioning through Drug-Target Interaction Prediction. J. Comput. Biol. 12, e1005219 (2016). doi:10.1371/journal.pcbi.1005219

Yamanishi, Y., Kotera, M., Kanehisa, M. & Goto, S. Drug-target interaction prediction from chemical, genomic and pharmacological data in an integrated framework. Bioinformatics 26, i246-i254 (2010). doi:10.1093/bioinformatics/btq176

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3: 673-683. doi: 10.1038/nrd1468

Dudley, J. T., Deshpande, T. & Butte, A. J. Exploiting drug–disease relationships for computational drug repositioning. Bioinformatics 12, 303-311 (2011). doi:10.1093/bib/bbr013

P17. In silico design of halogenated carbohydrate mimetics as potential halogen-bonding ligands

Nunes, R.[1,2,3], Xavier, N. M.[1,3], and Costa, P. J.[1,2]

[1] Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[2] BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa
[3] Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

The molecular recognition of carbohydrates by proteins is characterized by the presence of classical hydrogen bonds which stabilize binding together with contributions from other intermolecular interactions conferring high specificity. [1] The design of glycomimetic ligands as modulators of protein-carbohydrate binding events is a common approach in the context of chemical glycobiology [2] and carbohydrate-based drug discovery [3].

While a diversity of functional groups has been successfully introduced in carbohydrate structures, [2] the use of halogens has been largely neglected, except for fluorine. However, heavier halogens (X = Cl, Br, or I) can establish highly directional, R–X···B interactions with Lewis bases (B), known as halogen bonds (HaB). These interactions have been mostly explained by the presence of an electropositive site at the outermost region of X species, named sigma-hole [4]. HaB-mediated molecular recognition phenomena are widespread across biological systems and have been used as tools in medicinal chemistry, [5] amongst other fields.

In the search for novel glycomimetics with the potential to modulate carbohydrate-protein recognition via HaB interactions, we performed a quantum mechanical study on the HaB donor propensity of model halogenated carbohydrate derivatives by computing the respective molecular electrostatic potential surface maxima. This procedure allowed us to map the chemical space of halogenated sugars in terms of their potential to act as HaB interaction partners with HaB acceptor species commonly found in biomolecules and the results encourage further in silico optimization towards new halogen-bonding glycomimetic ligands.

Acknowledgements: Investigador FCT (IF/00069/2014, IF/00069/2014/CP1216/CT0006), PhD grant SFRH/BD/116614/2016, IUD projects UID/QUI/UI0612/2013,

UID/MULTI/04046/2013. Lisboa 2020, Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

References

[1] Lacetera, A. et al. in Chemical Biology, No. 3, Computational Tools for Chemical Biology (Ed.: S. Martín-Santamaría), Royal Society of Chemistry, Croydon, UK, 2018, pp.145-164.

[2] Sliba, R. C., Pohl, N. L. B. Curr. Opin. Chem. Biol. 2016, 34, 127.

[3] Ernst, B., Magnani, J. L. Nat. Rev. Drug. Discov. 2009, 8, 661.

[4] Clark, T. et al. J. Mol. Model. 2007, 13, 291.

[5] Wilcken, R. et al. J. Med. Chem. 2013, 56, 1363.

P18. New phosphorylated amino acid parametrization to correctly reproduce their acid/base equilibria, including in protein binding events

Oliveira, N. F. B. M.[1], Pires, I. D. S.[1], Machuqueiro, M.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa

Phosphorylation is one of the most important post-translational modifications in living cells and the phosphate groups are present in many of its biomolecules. Despite the fact that the phosphate group is ubiquitous, their role and the environment to which it is exposed can change dramatically. Therefore, in computational methods, the phosphate parameters are probably the among the least transferable, e.g. a phosphate group in phospholipids is significantly different from a phosphoryl tyrosine, in terms of electrostatics. In current forcefields, phosphate groups have been parameterized in the context of lipids and in connection with nucleotides, while parameters for phosphorylated amino acids are still scarce. Most attempts made at these parameters, were based on RESP calculations on the ESP generated from QM calculations [1]. The phosphate group is pH-active, with the second a pKa value around 6, which is problematic to be studied using conventional MD simulations. With constant-pH MD (CpHMD) simulations [2], we can capture the coupling between protonation and conformation for the phosphate group in their different environments.

We have devised several strategies to obtain the GROMOS 54A7 charge parameters for phosphoryl tyrosine (pTyr), serine (pSet), and threonine (pThr), namely: RESP calculations performed on the ESP generated either by Hartree-Fock or DFT calculations; directly adapting the charge set from Wojciechowski et al. [1]; and by manually curating the best charge set obtained taking in consideration the experimental data available. With all charge sets it was possible to run CpHMD simulations on simple pentapeptides to calibrate the pKa value against experimental data. We tested the parameters with simple systems like the Gly-Gly-X-Ala tetrapeptides and with more complex systems, like the phosphorylated dodecapeptide, free or complexed with a phospholipase [3]. We observed that for the simple systems, all charge sets, after calibration, are able to predict their unshifted pKa values. However, all recipe-based charge parameterization processes, failed (sometimes even qualitatively) in predicting the pKa shift for the complex, which could only be corrected by manually curating the charges.

References

[1] Wojciechowski, M., Grycuk, T., Antosiewicz, J.M., Lesyng, B., Biophys. J., 2003, 84, 750-6.

[2] Machuqueiro, M., Baptista, A. M., J. Phys Chem. B, 2006, 110, 2927-2933.

[3] Singer, A.U., Forman-Kay, J.D., Prot. Sci., 1997, 6, 1910-9.

P19. Exploring the Catalytic Mechanism of the Malonyl-Acetyl Transferase Domain of Human Fatty Acid Synthase

Paiva, P.[1], Sousa, S.F.[2], Ramos, M.J.[1], Fernandes, P.A.[1]

[1] UCIBIO@REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal
[2] UCIBIO@REQUIMTE, BioSIM – Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal

Human fatty acid synthase (hFAS) is a multidomain enzyme that catalyzes all steps of fatty acid synthesis, which is overexpressed in many cancer cells [1]. Studies have shown that FAS inhibitors exhibit antitumor activity without relevant effects over normal cells [2]. Thus, the molecular description of active sites in hFAS is an important goal for the development of novel anticancer therapies. The malonyl-acetyl transferase (MAT) domain is responsible for loading acetyl and malonyl moieties to the acylcarrier protein (ACP) domain, a carrier for fatty acid reaction intermediates [3]. In this work, we have employed computational QM/MM methods at the DLPNO-CCSD(T)/CBS:AMBER level of theory to study the MAT catalytic mechanism. The results indicate that the first catalytic stage occurs in two steps: (1) nucleophilic attack on the thioester carbonyl group of the substrate through a concerted pathway centered on a Ser-His dyad and (2) tetrahedral intermediate breakdown and release of the free coenzyme A. The Gibbs activation energies for the first and second steps are 13.0 and 6.4 kcal·mol^{-1} and 10.9 and 8.0 kcal·mol^{-1}, whether the substrate transferred to the MAT domain is acetyl-CoA or malonyl-CoA, respectively. The mutation of the Arg606 residue by an alanine severely impairs the malonyl transacylase reaction, while leading to an improvement of the transferase activity for acetyl-CoA, which is in agreement with previous experimental evidences [4]. The backbone amines of Met499 and Leu582 form an oxyanion hole that accommodates the negative charge of the substrate carbonyl, lowering the first step activation barriers for both substrates. The results from this work encourage future studies that aim for the full comprehension of the MAT catalytic reaction and that explore the therapeutic potential of hFAS.

References:

[1] Smith, S.; Tsai, S. C. The Type I Fatty Acid and Polyketide Synthases: A Tale of Two Megasynthases. Nat. Prod. Rep. 2007, 24, 1041–72.

[2] Pandey, P. R.; Liu, W.; Xing, F.; Fukuda, K.; Watabe, K. AntiCancer Drugs Targeting Fatty Acid Synthase (Fas). Recent Pat. AntiCancer Drug Discovery 2012, 7, 185–197.

[3] Maier, T.; Leibundgut, M.; Ban, N. The Crystal Structure of a Mammalian Fatty Acid Synthase. Science 2008, 321, 1315–22.

[4] Riplinger, C.; Neese, F. An Efficient and near Linear Scaling Pair Natural Orbital Based Local Coupled Cluster Method. J. Chem. Phys. 2013, 138, 034106.

P20. Comparing Molecular Dynamics Force Fields in Bacteria Membrane Models

Peón, A.[1], Claro, B.[1], Bastos, M.[1], Piñeiro, Á.[2], García-Fandiño, R.[1,3]

[1] CIQ-UP – Centro de Investigação em Química, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal

[2] Facultad de Física, University of Santiago de Compostela (USC), Santiago de Compostela, Spain

[3] Singular Research Centre in Chemical Biology and Molecular Materials, (CIQUS), Organic Chemistry Department, University of Santiago de Compostela (USC), Santiago de Compostela, Spain

The cell membrane is the first physical barrier against various pathogens found in both prokaryotic and eukaryotic cells, which is composed mainly of a series of lipid mixtures. In contrast to mammalians cytoplasmic membranes that have a neutral external membrane, bacteria membranes are negatively charged, a fact that can be used to obtain antimicrobial selectivity. The development of molecular-level models that incorporate the heterogeneity of the nonprotein constituents is one of the most exciting advances in the scope of Molecular Dynamics (MD) simulations of bacterial membranes in the last years, and now frequently a mixture of phospholipids is used in simulation studies [1, 2]. The quality of the force field is a crucial issue of the reliability of such simulations. Whereas much effort has been dedicated to parametrize and optimize the force fields for biomembrane modelling, most of the comparisons have been done for homogeneous bilayers composed of a single phospholipid type, which may not work optimally or even fail when used in description of complex heterogeneous systems.

Preliminary results comparing different force fields (Slipids, CHARMM36, GROMOS 54A7 and Lipid17) with several homogeneous and heterogeneous bacterial membrane models will be presented. Models consisting of five hundred lipids were studied, as gram-positive bacteria mimetic model, POPE/POPG (1:3) as gram-negative mimetic model, POPE/POPG (3:1) and POPE/POPG (7:3) as well as homogeneous bilayer models to simulate neutral membrane systems, anionic membrane systems or the outer layer of the membranes will be used respectively POPE, POPG and POPC. For comparison: area per lipid, thickness, number of hydrogen bonds, lateral diffusion, order parameters, lateral density, radial distribution function and number of clusters were used. These outcomes would be useful to understand the behaviour of lipids at

atomistic-level at lipid-bilayer/water interfaces and provide a point of reference for making the appropriate decision on the force field in bacterial membrane models MD simulations.

References

[1] Boags, A.; Hsu, P.-C.; Samsudin, F.; Bond, P. J.; Khalid, S., J. Phys. Progress in Molecular Dynamics Simulations of Gram-Negative Bacterial Cell Envelopes. Chem. Lett. 2017, 8 (11) 2513.

[2] Parkin, J.; Chavent, M.; Khalid, S., Molecular Simulations of Gram-Negative Bacterial Membranes: A Vignette of Some Recent Successes. Biophysical Journal 2015, 109 (3) 461.

P21. Application of QM/MM Methods in the Study of PNPOx

Pina, A.[1], Sousa, S. F.[1], Cerqueira, N. M. F. S. A.[1]

[1] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal

Pyridoxal 5'-phosphate (PLP), the active form of the vitamin B6, is an essential cofactor required by more than 160 families of enzymes. Its role as an electron sink makes it imperative for the catalysis of a myriad of chemical reactions. Contrarily to microorganisms and plants, humans and other mammals are not able to synthesize PLP de novo, resorting to a "salvage pathway" that helps to maintain PLP homeostasis [1]. The correct functioning of this salvage pathway is crucial for the cell, as demonstrated by the correlation between low levels of PLP and the occurrence of severe neurological disorders [2]. It was found that the major culprit is pyridoxine/pyridoxamine 5'-phosphate oxidase (PNPOx), an FMN-dependent homodimeric enzyme responsible for the recycling of pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP) into PLP [3]. Therefore, in order to better understand its role in these disorders, it is of the utmost importance to unveil the catalytic mechanism of PNPOx. To do so we used computational means, namely QM/MM hybrid methodologies [4], to evaluate different mechanistic proposals related to PNPOx reactivity. Models were prepared and evaluated enabling important aspects related to the catalytic modelling of this enzyme to be validated. The results obtained in the present work provide important details about the catalytic mechanism of PNPOx, helping us to understand the importance of some key residues in the active site that can have implications in some PLP-deficiency disorders. More studies are required to fully understand the catalytic mechanism of this important enzyme.

Acknowledgments: We acknowledge FCT for financial support to the PhD grant SFRH/BD/136594/2018, the Starting grants IF/01310/2013 and IF/00052/2014, and to the Project PTDC/QUI-QFI/31689/2017.

References

[1] Di Salvo, M L, et al. Vitamin B6 Salvage Enzymes: Mechanism, Structure and Regulation. BBA-Proteins Proteomics 2011, 1814(11), 1597–1608

[2] Mills, P B, et al. Neonatal Epileptic Encephalopathy Caused by Mutations in the PNPO Gene Encoding Pyridox(Am)Ine 5′-Phosphate Oxidase. Hum Mol Genet 2005, 14(8), 1077–1086

[3] Di Salvo, M L, et al. Structure and Mechanism of Escherichia Coli Pyridoxine 5′-Phosphate Oxidase. BBA-Proteins Proteomics 2003, 1647(1–2), 76–82

[4] Chung, L. W, et al. The ONIOM Method and Its Applications. Chem Rev 2015, 115(12), 5678–5796

P22. Machine Learning to Predict Binding Affinity of Ligand-Target Interactions

Pinho, X.[1], Preto, A. J.[1], Moreira, I. S.[1]

[1] Centro de Neurociências e Biologia Celular, UC - Biotech Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal

Due to the high cost and labor required for drug discovery and robust characterization of interactions between ligands and targets, various Machine Learning (ML) models have been proposed as cost-effective means to advance this process in terms of predicting the interactions for subsequent verification [1]. Most of the model predictions that have been proposed to predict interactions have focused on binary classification [2]. Herein, we tested a computational approach to predict the binding affinity in a continuous display. In this study we deployed various ML techniques to a heterogeneous set of drugs from public databases, after establishing an *in silico* pipeline for feature extraction of both ligand and target.

References

[1] Öztürk, H., Özgür, A. & Ozkirimli, E. DeepDTA: Deep drug-target binding affinity prediction. in *Bioinformatics* (2018). doi:10.1093/bioinformatics/bty593

[2] Kundu, I., Paul, G. & Banerjee, R. A machine learning approach towards the prediction of protein-ligand binding affinity based on fundamental molecular properties. *RSC Adv.* (2018). doi:10.1039/c8ra00003d

P23. Unravelling the molecular details of the pH-dependent gating mechanism in the outer membrane protein G

Pires, I. D. S.[1] and Machuqueiro, M.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
email: inesdspires@gmail.com

Outer membrane protein G (OmpG) is a monomeric protein of E. coli outer membrane that mediates a pH-dependent non-specific oligosaccharide transport [1]. Two X-ray structures have been determined at different pH values: a closed conformation at low pH that inhibits metabolite transport; and an open one at neutral pH that allows it [1,2]. The key structural difference behind the mechanism lies in the position of the external loops, that determine the open or closed conformation of the channel. Given the important function of this protein, a detailed description of the conformation changes that result from varying the pH has enormous biological relevance, possibly contributing in making it a better antibiotic target and more flexible biosensor [3]. Here, we will perform constant pH molecular dynamics (CpHMD) simulations of a membrane-embedded OmpG in order to characterize the conformational/protonation space of both end states (open and closed). pH-induced conformational transitions and metabolite transmembrane diffusion through the pore are examples of other objectives in the current project.

References

[1] Yildiz, Ö., Vinothkumar, K.R., Goswami, P., Kühlbrandt, W. EMBO J., 2006, 25, 3702

[2] Zhuang, T., Chisholm, C., Chen, M., Tamm, L.K. J.Am.Chem.Soc., 2013, 135, 15101

[3] Chen, M., Khalid, S., Sansom, M. S., Bayley, H. Proc. Natl. Acad. Sci. U.S.A., 2008, 105, 6272

P24. Estimating pKa shifts of encapsulated drugs through a CpHMD approach

Reis, D.[1], Machuqueiro, M.[1], Vila-Viçosa, D.[1]

[1] Faculdade de Ciências da Universidade de Lisboa

Molecular machines have recently been associated with the development of molecular carriers to enhance drug properties, such as solubility or bioavailability. One possible approach is through drug encapsulation by a host molecule, such as cucurbituril (CB) rings, which modifies the environment of the guest molecule. CB rings are able to encapsulate guest molecules providing a hydrophobic cavity and several carbonyl groups that stabilize cationic hosts that interact with this region. This results in significant pKa shifts for drugs with titrable (cationic) groups that can be exploited in order to improve drug bioavailability, whether by enhancing their solubility, stabilizing their active form or by protecting them against external agents. The aforementioned approach can be used for medical targeting, such as cancer therapy, by designing carriers that deliver guest molecules at specific conditions, knowing the specific target properties [1].

Computational tools are a powerful way to help the rational design of CB-guest complexes. In particular, the stochastic titration constant-pH MD (CpHMD) method allows a molecular dynamics simulation to have the pH value as an external parameter and, consequently, obtain full titration curves and pKa values. Our main goal is to develop a strategy to model benzimidazole (BZ) pKa shifts, a «proof-of-concept» molecule, and then extrapolate this process to other host-guest complexes. BZ has a well-known shift of ~3.5 pKa units when encapsulated by a CB ring and, with a CpHMD method, it is possible to elucidate the molecular details of these host-guest interactions.

Acknowledgements: We acknowledge financial support from FCT through project UID/MULTI/00612/2013 and grant SFRH/BPD/110491/2015.

References

[1] Ghosh, I.; Nau, W. M., The strategic use of supramolecular pKa shifts to enhance the bioavailability of drugs. Advanced Drug Delivery Reviews. 2012, 64, 764-783.

P25. Implementation of a biocomputing platform to settle a new drug discovery pipeline towards post-synaptic receptors

Ribeiro R.[1], Giorgetti A.[1,2]

[1] Dep. Biotechnology, University of Verona, Italy;
[2] IAS-5 / INM-9: Computational Biomedicine - Institute for Advanced Simulation (IAS) / Institute of Neuroscience and Medicine (INM) Forschungszentrum Jülich D-52425 Jülich, Germany

GOMoDO is a GPCRs online modelling and docking web-server, developed in the Applied Bioinformatics Laboratory of the University of Verona. This biocomputing platform puts together state-of-the-art bioinformatics tools which allow users to effortlessly model GPCR structures and dock ligands to the model, and obtain biologically and pharmacologically relevant data. With a very easy user interface GOMoDo works through a consistent pipeline: protein sequence alignment, homology modelling and model quality assessment, and docking.

Although, GOMoDo was meant to be used by expert and non-expert users for GPCR-targeting drug design, we want to improve and extend it to other post-synaptic receptor families, specially to Cys- loop receptors. This superfamily of pentameric ion channels are widespread across the nervous system, being particularly involved in the learning and memory processes. They are also fundamental targets for clinically relevant drugs, such as neuromuscular blockers, barbiturates, benzodiazepines, and anaesthetics.

Today's cell and molecular biology no longer focus only on single macromolecules, but on the understanding of the chemical and biological functions of organisms. Therefore, we intend to implement in our biocomputing platform the ability to unveil the metabolic pathways and catalogue all the biological complexes and the relationships between them of the post-synaptic receptors.

Such complex biological systems rely on fundamental mathematical equations applied by computational methods. Hence, we will use computational frameworks that combine the strengths of rule-based and programmatic approaches (BioNetGen and Kappa) and Python numerical tools to assemble accurate, extensible, and reusable biological models.

Lastly, we also intend to provide the user the possibility of running in silico target fishing and virtual screening campaigns to find new targets against the post-synaptic receptors.

P26. A Survey of Enzyme Chemistry using the Mechanism and Catalytic Site Atlas

Ribeiro, A. J. M.[1], Tyzack, J. D.[1], Borkakoti, N.[1], Thornton, J. M.[1]

[1] European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton CB10 1SD, UK

M-CSA (Mechanism and Catalytic Site Atlas) is a database of enzyme mechanisms with two main purposes: to gather all the information about enzyme mechanisms in the same place, so it can be consulted by the community; and to provide a dataset to study enzyme function and evolution.

The database was last updated in the Summer of 2018, when we increased the number of annotated mechanisms from 350 to 684. The database also contains another 280 entries with annotated catalytic sites, but no mechanistic information. The entries in M-CSA are associated with 852 EC numbers and 1001 CATH domains. By using homology search, we link these entries to about 50 000 PDB structures and 5 000 000 UniProt sequences. We are trying to use this data to better understand enzymatic catalysis and evolution.

P27. Combined experimental and computational studies devoted to the synthesis of 1,4-lactones

Rocha, J. F.[1], Freitas, D. S.[2], Noro, J.[2], Teixeira, C. S. S.[1], Sousa, C. E.[2], Sousa, S. F.[1], Alves, M. J.[2], Cerqueira, N. M. F. S. A.[1]

[1] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernani Monteiro, 4200-319 Porto, Portugal
[2] Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Lactones are important biological molecules that offer a new molecular scaffold to develop new and more selective inhibitors targeting glycosidases [1]. The chemical routes that can speed up their synthesis in a stereo- and regio-selective way have become a major demand. A new derivative of 2,4-O-alkylidene-D-erythrose, enclosing a C=C moiety into a 1,5-lactone ring, was found to induce a complete facial selectivity in 1,3-dipolar cycloadditions [2]. This new D-erythrosyl 1,5-lactone was studied as a Michael acceptor with sulfur and nitrogen nucleophiles and from which a complete facial selectivity was demonstrated in all reactions [3]. Sulfides attack exclusively at C-4, but primary amines and hydrazine attack both at C-2 and C-4. The sulfur adducts formed are 1 (D-erythrose derivative):1 (nucleophile), and the nitrogen adducts are 1:2. The theoretical and computational results clearly explain the origin of the stereo-selectivity, and the energetic course of the reactions, starting with nitrogen and sulfide nucleophiles. Considering that the 1,4-lactones obtained in this work offer a new molecular scaffold for organic synthesis, these new results provide a solid theoretical platform that can be used to speed up synthesis of other derivatives in a stereo- and regio-selective way.

References

[1] Rocha, J. F.; Cerqueira, N. M. F. S. A. Glucosidases and galactosidases as drug targets. Curr. Top. Biochem. Res. 2017, 18, 117– 126.

[2] Sousa, C. E.; Ribeiro, A. M.; Gil Fortes, A.; Cerqueira, N. M.; Alves, M. J. Total Facial Discrimination of 1,3-Dipolar Cycloadditions in a d-Erythrose 1,3-Dioxane Template: Computational Studies of a Concerted Mechanism. J. Org. Chem. 2017, 82 (2), 982–991.

[3] Rocha, J. F.; Freitas, D. S.; Noro, J.; Teixeira, C. S. S.; Sousa, C. E.; Alves, M. J.; Cerqueira, N. M. F. S. A. Total Stereoselective Michael Addition of N- and S- Nucleophiles to a d-Erythrosyl 1,5-Lactone

Derivative. Experimental and Theoretical Studies Devoted to the Synthesis of 2,6-Dideoxy-4-functionalized-d-ribono-hexono-1,4-lactone. J. Org. Chem. 2018, 83 (15), 8011-8019 DOI: 10.1021/acs.joc.8b00769.

P28. Metabolic Engineering of Cyanobacteria for Bisabolene Production

Rodrigues, J.[1], Dienst, D.[1], Lindberg, P.[1]

[1] Department of Chemistry - Ångström, Uppsala University, Sweden

The ever-increasing consumption of non-renewable resources in an unsustainable fashion has been a driving force for the expansion of the Biotechnology field over the last decades, especially in the genetic engineering of microorganisms to produce biofuels and other high-value compounds. Most of the approaches rely on heterotrophic microorganisms; however, their need for carbon supplementation is disadvantageous, as it competes with the food sector and increases substantially the production costs. Unlike the bio-production on heterotrophs, the usage of photosynthetic microorganisms, such as cyanobacteria and green algae, allows the solar-powered fixation of carbon dioxide from the atmosphere and its conversion into desired organic chemicals. This second approach is more advantageous in the sense that there is no need for carbon supplementation, making all the production process cheaper, and its application on large scales would allow the establishment of a carbon-neutral based economy.

Among other secondary metabolites, cyanobacteria produce terpenoids, a structurally diverse group of natural products that play different pivotal roles in the cell. These metabolites have high relevance to the industrial sectors as fragrances, colorants and precursors for drugs and biofuels [1,2]. Our research is currently focused on the metabolic engineering of cyanobacteria for terpenoid production, specifically the sesquiterpenoid bisabolene. This 15 carbon-atom terpenoid has been shown to be a suitable biofuel, when chemically hydrogenated into bisabolane, and its production in heterotrophic microorganisms in high titres was already reported [3].

Acknowledgements: This work is supported by the Swedish Energy Agency (grant no. 38334-1), the ÅForsk Foundation (grant no. 16-538) and the NordForsk NCoE program NordAqua (project no. 82845).

References

[1] Pattanaik, B.; Lindberg, P. Terpenoids and Their Biosynthesis in Cyanobacteria. Life 2015, 5 (1), 269–293.

[2] Englund, E.; Shabestary, K.; Hudson, E. P.; Lindberg, P. Systematic Overexpression Study to Find Target Enzymes Enhancing Production of Terpenes in Synechocystis PCC 6803, Using Isoprene as a Model Compound. Metab. Eng. 2018, 49 (1), 164-177.

[3] Peralta-Yahya, P. P.; Ouellet, M.; Chan, R.; Mukhopadhyay, A.; Keasling, J. D.; Lee, T. S. Identification and Microbial Production of a Terpene-Based Advanced Biofuel. Nat. Commun. 2011, 2 (1), 483–488.

P29. A Molecular Dynamics Insight to Non-Structural Protein 1 (NS1) – A Hub Protein Essential for Influenza Infection

Rosário-Ferreira, N.[1,2], Melo, R.[2,3], Moreira, I. S.[2], Brito, R. M. M.[1]

[1] Departamento de Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra, 3004-535 Coimbra, Portugal. nicia.ferreira@student.uc.pt and brito@ci.up.pt

[2] Centro de Neurociências e Biologia Celular, UC - Biotech Parque Tecnológico de Cantanhede, Núcleo 04, Lote B, 3060-197 Cantanhede, Portugal. irina.moreira@cnc.uc.pt

[3] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal. ritamelo@ctn.tecnico.ulisboa.pt

Influenza (flu) is a contagious viral disease, which attacks the respiratory tract spreading through the population in seasonal infections. In fact, every year around 10% of world population is infected and an estimated 650.000 people die from Influenza according to the World Health Organization (WHO) [1]. Since the vaccination's efficacy is limited, due to high rates of mutation and recurrent genetic assortment, new preventive and therapeutic approaches and better understanding of the virus-host interactions are urgently needed [2].

Thus, NS1, one of the 11 proteins encoded by the virus, became a potential target as it promotes enhancement of viral replication, while negatively affects the host's innate immune response [3]. NS1 protein has indeed a plethora of functions by interacting with different host partners [4]. Structurally, NS1 is a 26 kDa multifunctional protein with around 230 residues and formed by 2 domains, a linker and a disordered C-terminal tail. The linker that connects the N-terminal RNA-Binding Domain (RBD) and the Effector Domain (ED) is a short, flexible region without a defined/fixed number of residues [5]. In host cells, NS1 is likely to be a homodimer that can shift between different quaternary conformational statuses depending on its partners and/or location in cell [6].

This biological system presents itself as a highly conserved protein, but there is lack of information about its structure and dynamics [7]. To this end, we are performing several replicas of 100 ns to complete one 1 μs Molecular Dynamics (MD) simulation in order to fully characterize the conformational space visited by both domains and how the presence of the flexible linker affects mobility and binding to the different partners.

References

[1] Iuliano, A. D.; Roguski, K. M.; Chang, H. H.; Muscatello, D. J.; Palekar, R.; Tempia, S.; Cohen, C.; Gran, J. M.; Schanzer, D.; Cowling, B. J. The Lancet 2018, 391(10127), 1285-1300.

[2] Mubareka, S.; Palese, P.; Rino, R.; Giudice, G. D., Eds.; Springer, Basel, 2011.

[3] Das, K.; Aramini, J. M.; Ma, L. C.; Krug, R. M.; Arnold, E. Nat Struct Mol Biol 2010, 17(5), 530-538.

[4] Ayllon, J.; Garcia-Sastre, A. Curr Top Microbiol Immunol 2015, 386, 73-107.

[5] Engel, D. A. Antiviral Res 2013, 99(3), 409-416.

[6] Carrillo, B.; Choi, J. M.; Bornholdt, Z. A.; Sankaran, B.; Rice, A. P.; Prasad, B. V. J Virol 2014, 88(8), 4113-4122.

[7] Kleinpeter, A. B.; Jureka, A. S.; Falahat, S. M.; Green, T. J.; Petit, C. M. J Biol Chem 2018.

P30. The HIV-2 envelope glycoprotein as a differentiated target in structure-function relationships to structural elucidation and characterization

Serra, P.[1], Martins A.[1], Taveira, N. [1,2], Guedes, R.C. [1]

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

The discovery of multiple new hit compounds that can be used as useful starting points towards drug candidates for HIV-1 and HIV-2 therapy is the main goal of this work. Until now, it has been marked by the use of computational techniques to study the viral surface glycoproteins as potential drug targets against HIV-1 and HIV-2 infections. The glycoproteins gp120 and gp125 are critical to the receptor's recognition and internalization of viral material into the cell. The modulation of its activity can lead to the disturbance of this mechanism.

Our work has been focused in HIV-2 structure. In the absence of a crystallographic structure of HIV-2 envelope gp125 comprising variable domains, computer aided modulation is crucial to identify structural features in the variable regions that correlate with HIV-2 tropism and susceptibility to neutralization. HIV-2ROD is an X4 T-cell adapted isolate naturally resistant to antibody neutralization. A 3D structure of HIV-2ROD gp125 was generated by homology modelling, using MOE2016 and MODELLER 9v19. Additionally, to disclose the importance of the main structural features and compare with experimental results, 3D-models of six V3 mutants were also generated using the C2V3C3 domain. These mutations revealed selectively impact in the behaviour of the protein. Additionally, molecular dynamics is being performed, using Gromacs 2006.3, in order to better characterize this protein and disclose its the biological dynamic behaviour. The same procedure was extended to the all protein in order to perform a full model of the protein.

Acknowledgements: Fundação para a Ciência e a Tecnologia for PhD Grant SFRH/BD/100643/2014 of Patrícia Serra and UID/DTP/04138/2013.

References

Kwong, P. D. et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393, 648–659 (1998).

Uchtenhagen, H. et al. Crystal structure of the HIV-2 neutralizing Fab fragment 7C8 with high specificity to the V3 region of gp125. PLoS One 6, e18767 (2011).

Davenport, Y. W., West, A. P. & Bjorkman, P. J. Structure of an HIV-2 gp120 in Complex with CD4. J. Virol. 90, 2112–2118 (2016).

P31. Carbohydrate-aromatic interactions in halogenated sugar mimetics: the role of C-Br...pi bonds

Šivickytė, O.[1], Nunes, R.[1], and Costa, P. J.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

Protein-carbohydrate interactions play an important role in many biological processes, [1] the understanding of the underlying mechanisms being crucial for carbohydrate-based drug design. Besides the ubiquitous hydrogen bond, noncovalent interactions involving aromatic rings are particularly important in sugar binding [2]. These interactions, normally referred to as C-H ...pi bonds, can involve two or three C-H groups from pyranoses and the aromatic side-chains of aminoacids (i.e. Phe, Tyr, Trp). One of the strategies used to improve properties such as carbohydrate-protein binding affinity is to design carbohydrate mimetics [3]. In this scope, fluorination is quite common, however, the use of heavier halogens (Cl, Br, I) is not usually employed. In the latter case, the existence of halogen bonds, which are noncovalent interactions of the type R-X ... B (X = Cl, Br, I; B = Lewis base),[4] is possible. Besides being extremely relevant in the context of biological systems,[5] C-X ...pi halogen bonds share some common features with the C-H ...pi bonds, namely their directionality. In this work, we employed DFT calculations to compare the energetics and geometries of carbohydrate-aromatic interactions in model systems. In particular, we used a variety of b- D- fucose ... phenol, b- D -fucose ... indole, and b- D -fucose ... benzene dimers possessing C-H ... pi bonds [6] and compared them with equivalent brominated fucose derivatives featuring C-Br ... pi halogen bonds. With these studies, we provide the first insights on how this specific type of noncovalent interaction can be explored in protein-carbohydrate systems.

Acknowledgements: Investigador FCT (IF/00069/2014, IF/00069/2014/CP1216/CT0006), PHD grant SFRH/BD/116614/2016, IUD projects UID/QUI/UI0612/2013, UID/MULTI/04046/2013. Lisboa 2020), Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

References

[1] Gabius, H.-J. et al. Trends Biochem. Sci. 2011, 36, 298.

[2] Asensio, J. L. et al. Acc. Chem. Res. 2013, 46, 946.

[3] Sliba, R. C. et al. Curr. Opin. Chem. Biol. 2016, 34, 127.

[4] Costa, P. J. Phys. Sci. Rev. 2017, 2, 20170136.

[5] Auffinger, P. et al. PNAS 2004, 101, 16789.

[6] Tsuzuki, S. et al. J. Phys. Chem. A 2011, 115, 11256.

P32. Experimental and Computational Studies Addressed to 1,3-Dipolar Cycloadditions of D-Erythrose 1,3-Dioxane 1,5-Lactone with Regio- and Stereo-selectivity

Sousa, C. E. A.[1], Ribeiro, A. M. P.[1], Fortes, A. G.[1], Cerqueira, N. M. F. S. A.[2], and Alves, M. J.[1]

[1] Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[2] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal

A new D-erythrose 1,3-dioxane 1,5-lactone derivative was synthetized and found to be a highly stereo-selective template as dipolarophile in 1,3-dipolar cycloadditions. Different regio-selectivities were obtained depending on the nature of the 1,3-dipole: complete, with alkyl azides and diazomethylbenzene, inexistent, with nitrile oxides.

To understand the mechanisms of cycloadditions with the three types of 1,3-dipoles, computational studies were performed, giving full agreement with the experimental data.

The computational results showed that all the studied cycloadditions are concerted processes, involving exoenergonic, and small free activation energies. The stereoselectivity of the reactions is due to a combination of the steric effect endorsed by hydrogen H-8 and the hyper conjugative effect of the incoming 1,3-dipole with the lactone. The regioselectivity observed in alkyl azides and phenyldiazomethane is mostly dependent on the distortion effect during the cycloaddition process. This distortion effect is however higher in the alkyl azide compounds than in phenyldiazomethane. This distortion effect is absent with nitrile oxides. This study provides a specific example where an apparent similar chemistry was found to proceed via different mechanisms, leading to different output results.

Acknowledgements: We thank FCT for project funding PTDC/QEQ-MED/1671/2012; the NMR Portuguese network (PTNMR, Bruker Avance III 400-Univ. Minho), and FCT and FEDER (European Fund for Regional Development)-COMPETE-QREN-EU for financial support to CQ/UM.

References

Sousa, C.E.A.; Ribeiro, A. M. P.; Gil Fortes, A.; N. M. F. S. A. Cerqueira; Alves, M. J., Total Facial Discrimination of 1,3-Dipolar Cycloadditions in a D-Erythrose 1,3-Dioxane Template: Computational Studies of a Concerted Mechanism, J. Org. Chem., 2017, 82 (2), pp 982–991.

P33. Single vs Multi Conformational QM/MM approach for enzymatic catalysis: The case of study of the HBPS desulfinase from the 4S pathway

Sousa J. P. M.[1], Sousa S. F.[1], Ramos M. J.[1], Fernandes P. A.[1]

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

Sulfur oxides emission is one of the major causes for the formation of acid rain and other noxious atmospheric pollutants. Fossil fuels have sulfur containing molecules that, upon combustion, are degraded producing sulfur oxides. Conventional methods become unprofitable in order to achieve the level of desulfurization required by legislation. One alternative is the use of sulfur metabolizing bacteria that possess enzymatic machinery capable of removing sulfur, without degrading the calorific content of the molecule. Rhodococcus erythropolis is capable of performing such task using four different enzymes: DszA, DszB, DszC and DszD. DszD is an HBPS desulfinase which ultimately cleaves the carbon-sulfur bond.

The way DszB performs this reaction is still an object of discussion. Different mutagenesis studies have revealed key aminoacid residues for the reaction to occur of which can be named Cys27, His60 and Arg70.

Through the use of MD we obtain different conformations of the active site which are then used to elaborate QM/MM models in order to study of the desulfination reaction of DszB. Our results assure the importance of Cys27, His60 and Arg70 for the reactivity of the enzyme, as well as revealing other important residues such as Gly73, which functions as a stabilizer of Cys27.

P34. Unraveling the Catalytic Mechanism of Tryptophan Synthase, a Drug Target Against *Mycobacterium Tuberculosis*

Teixeira, C. S. S.[1], Sousa, S. F.[1], Cerqueira, N. M. F. S. A.[1]

[1] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal

Tryptophan Synthase (TSase) 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 through a 25 ≈ physical tunnel to the active site of the β-subunit where it is added to a molecule of acrylate, derived from serine, to produce TRP, in a PLP dependent reaction [1].

Since TSase is absent in mammals, it is a promising target for the development of new antibiotics and vaccines against infectious bacteria, such as Mycobacterium tuberculosis.

The complex allosteric regulation of the enzyme has turn it difficult to co-crystalize the enzyme in its closed conformation with both substrates correctly placed in the α and β-active sites. In this work, we modulated three enzyme models for the posterior construction of QM/MM models: Model 1 (α-IGP and β-Ain); Model 2 (β-Aex-Ser); Model 3 (β-Q2). All the models were based on the crystallographic structure with PDB ID: 3PR2 and the ligands were either obtained from other crystallographic structures (PDB ID:1QOQ) or modulated from the analogs. Each of the three models were emerged in a box of waters and subjected to a MD simulation of 30 ns for detailed analysis and sampling of the interactions formed. The RMSd analysis of the last 20 ns of the three MD simulations did not evidence any abnormal fluctuation, and the equilibrated region presents a low RMSd average value of respectively 2.90 ± 0.17 Å for MD1; 2.64 ± 0.12 Å for MD2 and 2.37± 0.10 Å for MD3. We concluded that all the models are stable and can be the basis for further studies.

Afterwards four ONIOM QM/MM hybrid model were built for geometry optimization, validation of the initial enzyme-ligand interaction, and posterior study of the catalytic mechanism of both α and β subunits of the enzyme.

References

[1] Dunn, M. F., Niks, D., Ngo, H., Barends, T. R. & Schlichting, I. Tryptophan synthase: the workings of a channeling nanomachine. Trends in biochemical sciences 33, 254-264, doi:10.1016/j.tibs.2008.04.008 (2008).

P35. Initial studies on the sensibility of Aquaporin-4 to membrane PAINS

Luz, A.F.S.[1], Victor, B.L.[1,2]

[1] iMed - Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal
[2] BioISI – BioSystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal

Pan-assay interference compounds (PAINS) is a class of compounds emerging as hits in many different assays. These promiscuous compounds show an apparent bioactivity and/or interfere with assay statistics across unrelated biological targets and tests. PAINS can emerge from many different sources such as chemical aggregation, redox activity, nonselective reactivity with proteins and membrane destabilization. This last category has gained increased importance due to its broad range of action. Membrane PAINS can act directly and indiscriminately at the membrane level, promoting changes in their physicochemical properties, such as viscosity and permeability, affecting mechanosensitive membrane proteins functions such as Aquaporins (AQPs). Within the framework of an on-going project whose objective is the development of a computational workflow capable to identify membrane PAINS, we have performed Molecular Dynamics simulations to characterize the structure key factors regulating the function of AQPs. This type of proteins is an excellent model system to evaluate the effect of membrane PAINS, due to their mechanosensitive properties and also to its biological importance. AQPs play key biological roles in the regulation of cell volume and internal osmotic pressure. Its dysfunction is behind the onset of several pathologies, associated with imbalanced water homeostasis, tumorigenesis, fat metabolism, liver gluconeogenesis, autoimmunity, etc. In cancer, AQPs have proved to be highly expressed in different tumor types, and a positive correlation between histological tumor grade and the AQP expression was also verified, showing the involvement of this proteins in tumor invasion, metastasis and growth. The results here presented will afterwards be used in the evaluation of the effect membrane PAINS have on the function of AQPS. This will be performed in a multidisciplinary approach combining in silico and in vitro functional evaluation assays.

Acknowledgements: The Authors acknowledge financial support from Fundação para a Ciência e a Tecnologia, Portugal, through project grant with reference PTDC/BIA-BFS/28419/2017 and Funding of R&D Units Strategic Plan with reference UID/MULTI/04046/2013.

P36. Evaluation of Different Scoring Functions for Docking and Virtual Screening against GPCR Drug Targets

Vieira, T. F[1], Magalhães, R. P.[1,2], Cerqueira, N. M. F. S. A.[1], Sousa, S. F.[1]

[1] UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[2] LAQV/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

G-protein-coupled receptors (GPCRs) constitute a large family of structurally similar proteins that respond to diverse physiological and environmental stimulants and that includes many therapeutic targets. In fact, 40% of all modern medicinal drugs are thought to target G-protein-coupled receptors (GPCRs), making this large family of proteins a particular appealing target for drug discovery efforts [1, 2].

Protein-ligand docking is a computational method that tries to predict and rank the structure resulting from the association between a ligand and a target protein [3]. Virtual screening (VS) can use docking to evaluate databases with millions of compounds to identify promising new molecules that could bind to a specific target of pharmacological interest, including GPCRs [4]. This strategy if often used to limit the amount of molecules that has to be tested experimentally and to reduce the cost in the identification of new lead molecules for drug development.

This work reports a detailed comparison of the popular Autodock and Vina software programs in ligand/decoys discrimination against 5 GPCR proteins, (Adenosine 2a receptor, Beta-1 adrenergic receptor, Beta-2 adrenergic receptor, C-X-C chemokine receptor type 4 and Dopamine D3 receptor), for a total of 1480 ligands and 99763 decoys. The results show that AutoDock is more efficient in recovering real ligands among the top 1% solution than VINA, when applying virtual screening to GPCR receptors.

Acknowledgements: The authors thank FCT for funding (IF/00052/2014 and SFRH/BD/137844/2018).

References

[1] Lagerstrom, M.C. and H.B. Schioth, Structural diversity of G protein-coupled receptors and significance for drug discovery. Nature Reviews Drug Discovery, 2008. 7(4): p. 339-357.

[2] Overington, J.P., B. Al-Lazikani, and A.L. Hopkins, Opinion - How many drug targets are there? Nature Reviews Drug Discovery, 2006. 5(12): p. 993-996.

[3] Sousa, S.F., P.A. Fernandes, and M.J. Ramos, Protein-ligand docking: Current status and future challenges. Proteins-Structure Function and Bioinformatics, 2006. 65(1): p. 15-26.

[4] Shoichet, B.K. and B.K. Kobilka, Structure-based drug screening for G-protein-coupled receptors. Trends in Pharmacological Sciences, 2012. 33(5): p. 268-272.

P37. Folding of cyclic peptides stabilized by halogen bonds

Vila-Viçosa, D.[1], Nunes, R., Costa, P. J.[1]

[1] Centro de Química e Bioquímica and BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

The folding process of proteins and peptides is often stabilized by hydrogen bonds. In fact, most secondary structures observed in proteins are stabilized by these non-covalent, and mainly electrostatic, interactions (namely, alpha-helices and beta-sheets). Recently, it was proposed that, when halogen atoms are present in non-natural amino acids, the stabilization of folded structures can also be associated with halogen bonds (HaB) [1]. These are highly directional non-covalent interactions explained by the existence of a positive region on the electrostatic potential (ESP) of heavier halogens, called sigma-hole, which is available to interact with electron-rich species (i.e. Lewis bases). The improvement of computational methods to model the charge anisotropy of halogenated compounds is therefore mandatory to describe HaB interactions. The simplest approach to describe the ESP anisotropy in halogenated species involves the addition of an off-centre positive extra-point of charge mimicking the sigma-hole.

Here, we studied a family of cyclic peptides with 8 common residues and 2 that can interact via hydrogen bonds, HaB or none: NVXAGPVXQG (where X indicate the variable residues and P is a D-Proline) [1]. When the two X residues are serines, the folded state is stabilized by hydrogen bonding. On the other hand, when replaced with non-natural amino acids, (the -OH group of each serine is replaced by -Me and -OMe, respectively), the folded state became unstable, owing to the impossibility to form hydrogen bonds. With the replacement the -Me group with a chlorine atom, the folded state is recovered via a HaB interaction. These three peptides are studied using different force fields, with and without the addition of an extra-point to describe the sigma-hole. The folding equilibrium and the prevalence of stabilizing non-covalent interactions are the main focus of this study.

Acknowledgements: Investigador FCT (IF/00069/2014, IF/00069/2014/CP1216/CT0006), PhD grant SFRH/BD/116614/2016, IUD projects UID/QUI/UI0612/2013, UID/MULTI/04046/2013. Lisboa 2020, Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

References

[1] Danelius, E. et al; 2017, Biochemistry 56:3265

21 DEZ

POSTER

COMUNICAÇÃO ORAL

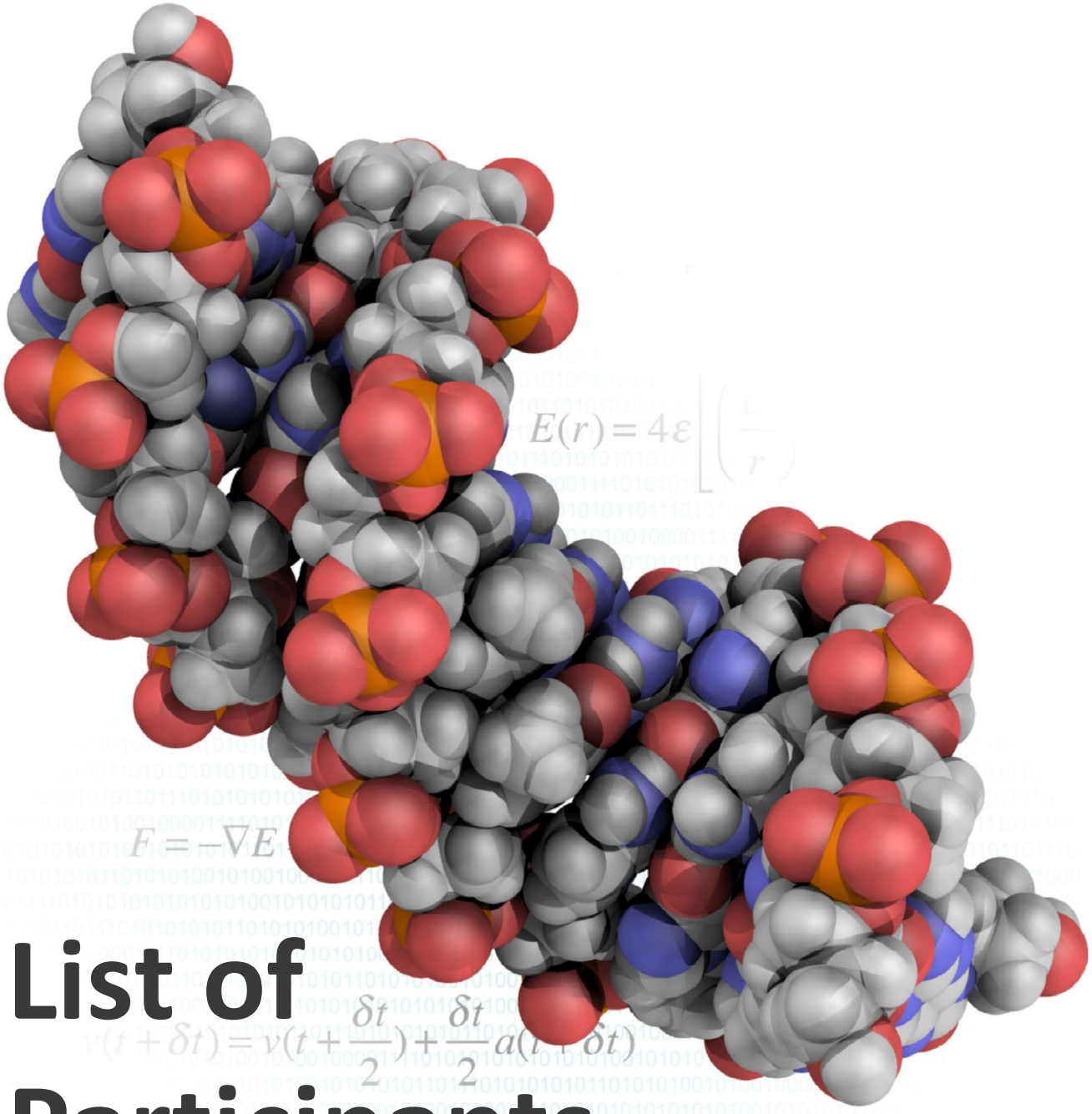
sponsored by molecules

EJIBCE

2018

Encontro de Jovens Investigadores de Biologia Computacional Estrutural

Faculdade de Medicina da Universidade do Porto



List of Participants

Agostinho Lemos	Uni. Liège
Alexandre Pinto	FCUP
Alexandre Seabra	FCUP/ICBAS
Ana I. Mata	Univ. Coimbra
Ana Oliveira	Univ. Porto
Ana Rita Calixto	Univ. Uppsala, SE
Ana Teresa Gaspar	Univ. Coimbra
Anabela Borges	FEUP
André Pina	BioSIM, FMUP
André Santiago	Univ. Minho
Andreia Cunha	Univ. Coimbra
Antonio Peón	Univ. Porto
António Preto	CNC
António Ribeiro	EMBL-EBI
Bárbara Claro	CIQUP-FCUP
Bruno L. Victor	FCUL
Bruno Pinto	FCUP
Carla Teixeira	BioSIM, FMUP
Carlos Barreto	CNC
Carlos J. V. Simões	BSIM Therapeutics
Carlos Pereira	FCUP
Catarina A. Carvalheda	BSIM Therapeutics
Cláudio Nóvoa	Univ. Minho
Cristina Almeida	FCUP/FFUP
Cristina Sousa	UM/UP
Diogo Reis	FCUL
Diogo Vila-Viçosa	FCUL
Eduardo Oliveira	Univ. Groningen, NL
Fabiola Medina	FCUP
Filipa Domingues	FMUP
Filipe Rodrigues	FCUL
Gaspar Pinto	FNUSA-ICRC, CZ
Henrique Fernandes	BioSIM, FMUP
Hugo A. L. Filipe	CQC–UC
Inês Garrido	FCUP
Inês Pires	FCUL
Irina Moreira	CNC
J. Ricardo Dias	FCUL
Joana Malheiro	FEUP
Joana Mourão	FFUP
João Botelho	FFUP
João Luís	Univ. Coimbra
João N. M. Vitorino	FCUL
João Rodrigues	Univ. Uppsala, SE
João Sousa	FCUP
Joel P. Arrais	Univ. Coimbra
Judite Coimbra	FFUC
Juliana F. Rocha	BioSIM, FMUP
Manuel Melle-Franco	CICECO–UA
Manuel Moreira Pires	Univ. Coimbra
Marta Dias	iBiMED–UA
Miguel Machuqueiro	FCUL
Nelson Monteiro	FCTUC
Nícia Ferreira	FCTUC
Nuno Cerqueira	BioSIM, FMUP
Nuno Oliveira	FCUL
Olena Shevchuk	FCUP/ICBAS
Ona Šivickytė	FCUL
Patricia Fechine	FAMENE, BR
Patrícia Serra	FFUL
Paulo J. Costa	FCUL
Pedro Fernandes	Univ. Lisboa
Pedro Ferreira	FCUP
Pedro Figueiredo	Biotech–UC
Pedro M. Filipe	Univ. Coimbra
Pedro Nicola	FFUP
Pedro Paiva	FCUP
Pedro Rafael Magalhes	FCUL
Pedro Reis	FCUL
Rafael Nunes	FCUL
Riccardo Concu	FCUP
Rita P. Magalhães	BioSIM, FMUP
Rita Paiva de Melo	IST
Rui João Loureiro	BioISI
Rui M. M. Brito	Univ. Coimbra
Rui Ribeiro	Univ. Verona, IT
Sérgio F. Sousa	BioSIM, FMUP
Tarsila Castro	Univ. Minho
Tatiana F. Vieira	BioSIM, FMUP
Tomás Silva	FCUL
Xavier Pinho	Univ. Coimbra