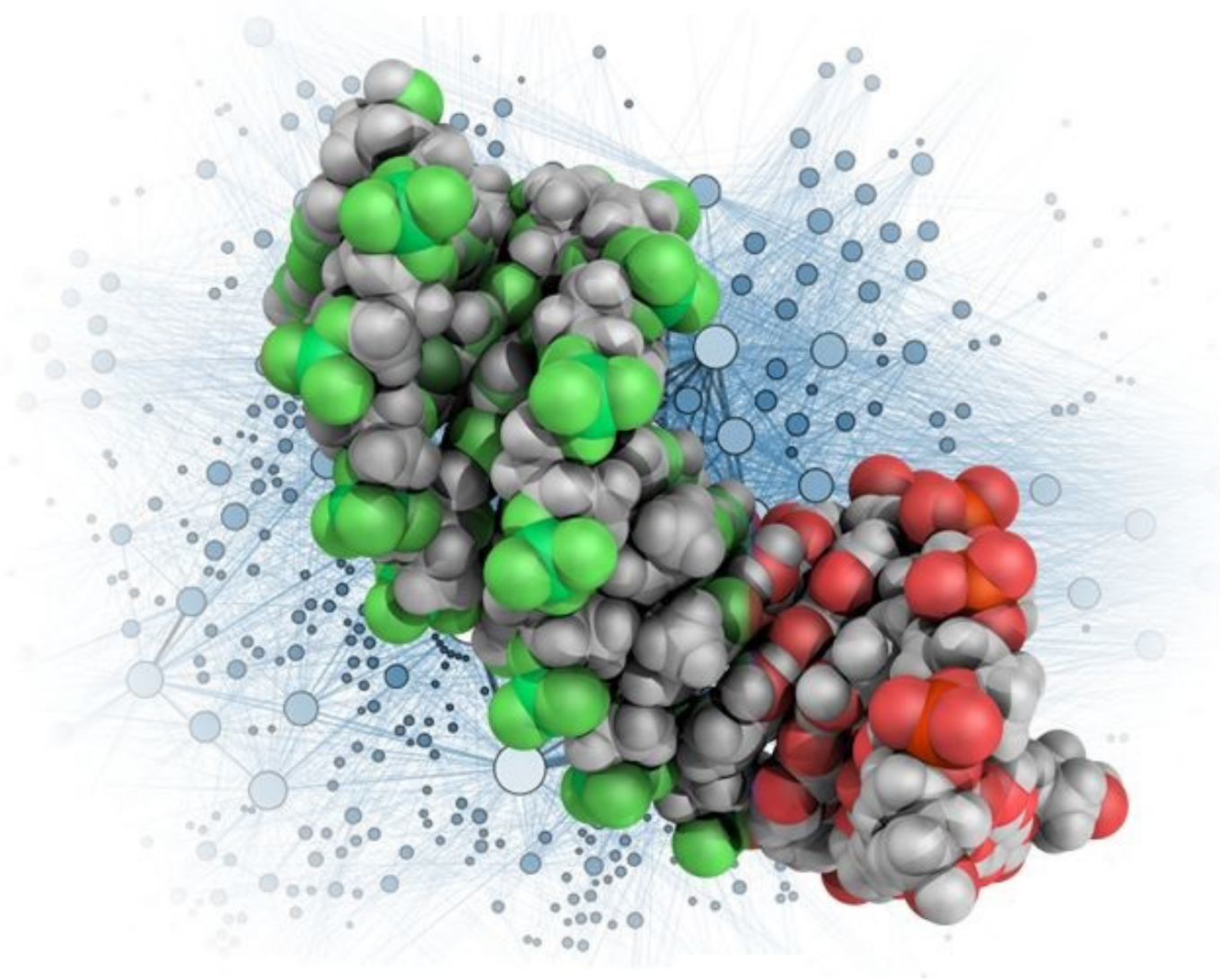
VIII EJIBCE

Coimbra, 20 de dezembro 2021



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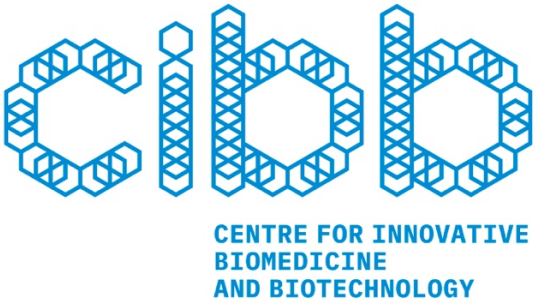
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Mission and Goals

The Meeting of Young Researchers in Structural Computational Biology (EJIBCE) series aims to bring together the Portuguese scientific community in this field in a free-of-charge meeting to provide a forum for discussion and sharing with no strings attached. This eighth edition builds on the success of previous editions, in Porto, Lisbon, Coimbra and Oeiras, which brought together more than 60 participants in a total of more than 10 oral communications per edition.

Sharing and discussing ideas are the seeds for a robust scientific community. Given the current pandemic situation, promoting, and encouraging an open spirit and collaboration between Computational Biology research groups in Portugal is increasingly necessary. Among other factors, this panorama showed the contribution but also a growing need for researchers capable of making the most of computational resources to generate quick, efficient, and rational responses to real, urgent, and unavoidable problems.

This connection with Portugal becomes essential when returning to the country after a PhD, a post-doctorate, or any other prolonged period abroad. On the other hand, some researchers want to continue their work abroad but, at the same time, cultivate a close relationship with science in Portugal. But which groups are working on Structural Computational Biology in Portugal? And what research is carried out in these groups? Questions come naturally, and answers are not always easy to find.

This meeting aims to answer some of these questions. It intends to make known the best that is done in this Structural Computational Biology area in Portugal and, on the other hand, to reveal what Portuguese researchers based abroad are studying. In this way, we want to provide a space where projects and results can be shared and discussed, with a stimulating collaboration view (at national and international level) and broadening the horizons of Structural Computational Biology in Portugal.

Scientific Committee

Alessandra Villa, KTH, Stockholm, Sweden
João Rodrigues, Schrödinger
Ezgi Karaca, Izmir Biomedicine and Genome Center, Balçova/Izmir, Turkey

Organizing Committee

Irina Moreira, CNC

Joana Mourão, CNC

António Preto, CNC

Salete Baptista, C2TN

Catarina Marques, CNC

Contact

ejibce@gmail.com

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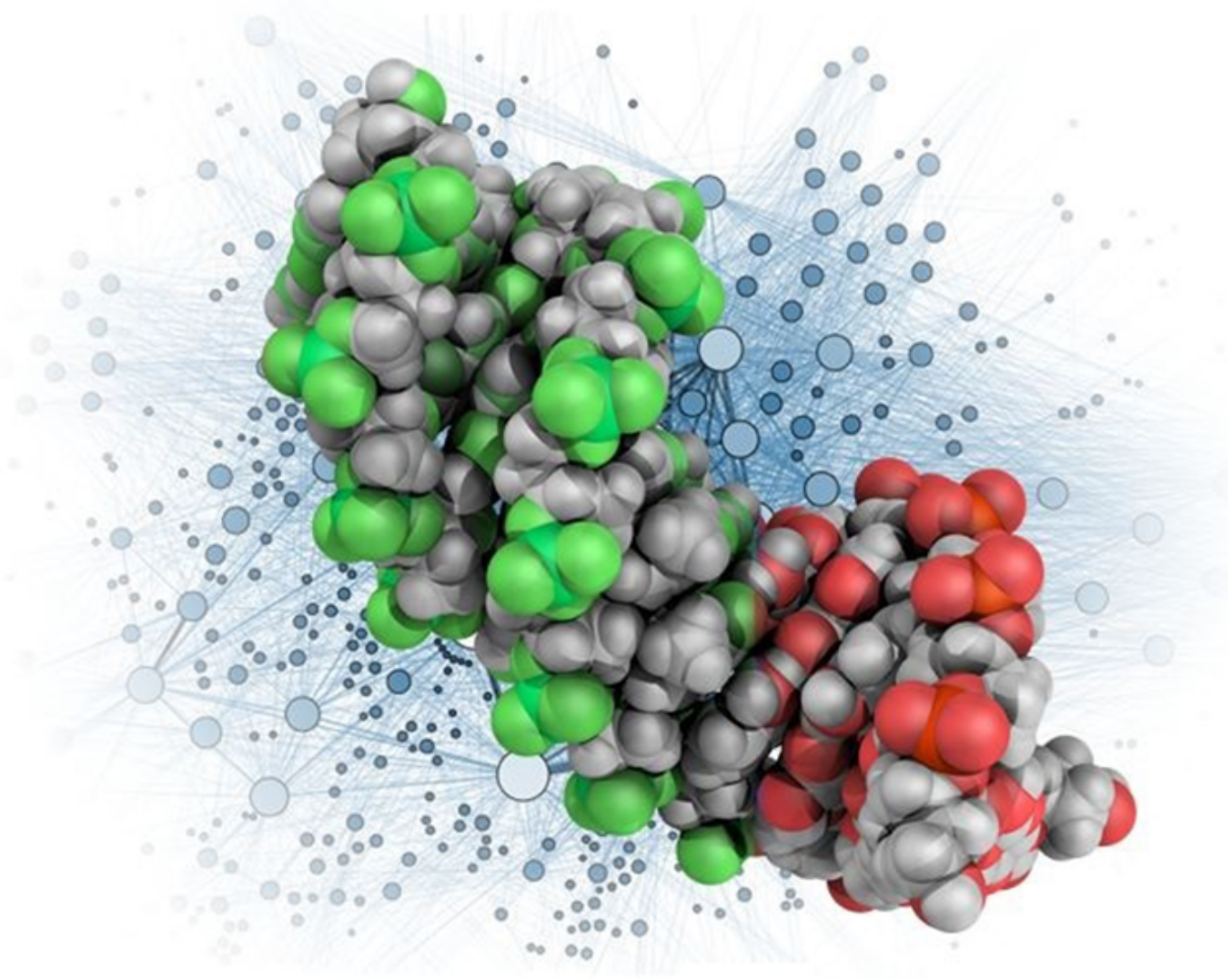

Monday, December 20

08:00 – REGISTRATION

09:00 – Cláudia Cavadas

Opening Session by the Vice-Rector of the University of Coimbra

09:15 – Irina Moreira

3D-BioInfo-PT-Community Inauguration

09:30 – Diana Lousa

"Viral membrane fusion: characterizing and inactivating a crucial step of the infection by devastating pathogens"

10:15 – Joana Pereira

"What is hidden in the darkness? Seeking new families in natural unknown proteins"

10:40 – COFFEE BREAK AND POSTER SESSION I

11:10 – Ona Šivickytė

"Probing new energy descriptors for halogen bonds"

11:35 – Nuno Oliveira

"A novel US-CpHMD protocol to study the protonation-dependent mechanism of the ATP/ADP carrier"

12:00 – LUNCH AND POSTER SESSION II

14:30 – Bruno Victor

"Identification of membrane Pan-Assay INterference CompoundS (PAINS) using an atomistic Molecular Dynamics protocol"

15:15 – Beatriz Bueschbell

"DRD2 homo-dimers - a world of possibilities"

15:40 – Pedro Suzano

"An US-CpHMD protocol to calculate pH-dependent membrane permeability coefficients of antitumor drugs"

16:05 – COFFEE BREAK AND POSTER SESSION III

16:35 – Luís Borges-Araújo

"SARS-CoV-2 variants impact RBD conformational dynamics and ACE2 accessibility"

17:00 – Tatiana Vieira

"Developed of Inverted Virtual Screening Approaches for the Identification of Protein Targets Associated to the Biological Activity of Specific Molecules"

17:25 – Mariana Valério

"Parainfluenza fusion peptide promotes membrane fusion by assembling into oligomeric pore-like structures"

17:50 – CLOSING SESSION

18:00 – First general meeting of PIs of the 3D-BioInfo-PT-Community

Invited Speakers

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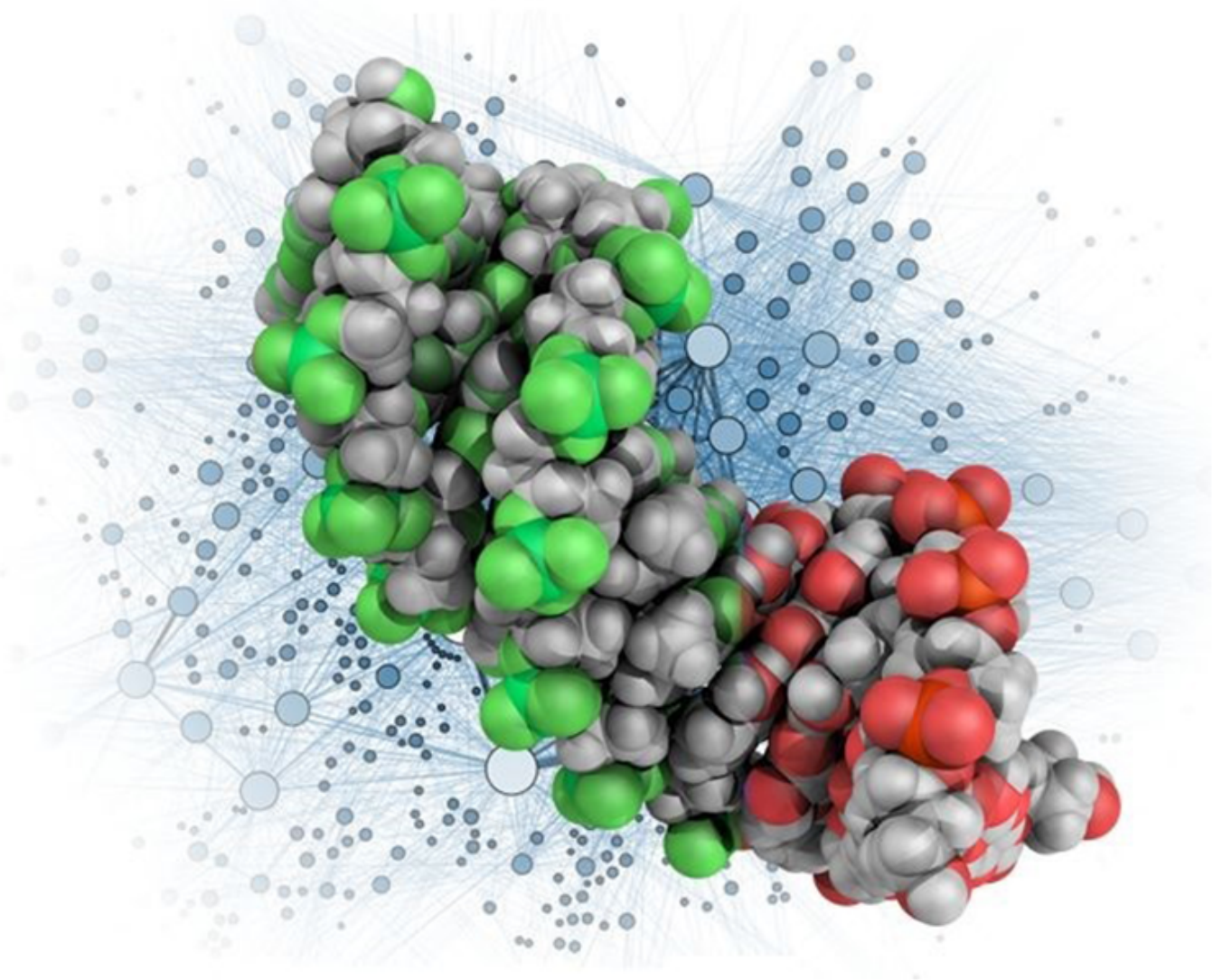

K1 – Lousa, Diana

Viral membrane fusion: characterizing and inactivating a crucial step of the infection by devastating pathogens

ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier

The fusion peptide (FP) is determinant for the infection process of enveloped virus, which include major pathogens such as HIV, influenza and SARS-CoV-2. This peptide, located within the fusion protein, inserts into the host membrane and promotes fusion between the viral and host membrane, which is essential for the infection process. FPs are very promising therapeutic targets and, therefore, there is a tremendous interest in identifying these peptides and characterizing their properties.

FPs from different viral families are quite distinct at the sequence and structural level, although they are all hydrophobic and tend to have high Ala and Gly contents. In some cases (e.g., influenza, HIV and parainfluenza) the FP corresponds to the N-ter end of the fusion protein, whereas in other cases (e.g. dengue) it is internalized. This raises an important question: How can peptides with such distinct features execute the same function? In the last years, we have addressed this question using a combination of nonstandard simulation techniques, machine learning methods and biophysical methodologies performed by collaborators. Using influenza and parainfluenza viruses as models, we show that these peptides use similar molecular mechanisms to promote fusion. We also shed light on important questions, such as the effect of pH and the impact of mutations. Currently we are investigating the effect of the putative fusion peptides from SARS-CoV-2, to determine which peptide(s) are used by this virus to promote fusion. Overall, these studies provide a global perspective of the molecular mechanisms of viral fusion peptides, which are privileged therapeutic targets to fight viral infections.

K2 – Victor, Bruno

Identification of membrane Pan-Assay INterference CompoundS (PAINS) using an atomistic Molecular Dynamics protocol

BioISI - Biosystems & Integrative Sciences Institute

Membrane Pan-Assay INterference compoundS (PAINS) are a class of molecules that interact non-specifically with lipid bilayers and alter their physicochemical properties. An early identification of these compounds avoids chasing false leads and the needless waste of time and resources in drug discovery campaigns. In this work, we optimized an *in-silico* protocol based on umbrella sampling (US)/MD simulations to discriminate between compounds with different membrane PAINS behavior. We showed that the method is quite sensitive to membrane thickness fluctuations, which was mitigated by changing the US-reference position to the P-atoms of the closest interacting monolayer. The computational efficiency was improved further by decreasing the number of umbrellas and adjusting their strength and position in our US scheme. The ISDM-calculated membrane permeability coefficients confirmed that resveratrol and curcumin have distinct membrane PAINS characteristics and indicate a misclassification of nothofagin in a previous work. Overall, we will present a promising *in silico* protocol which can be adopted as a future reference method to identify membrane PAINS.

Oral Communications

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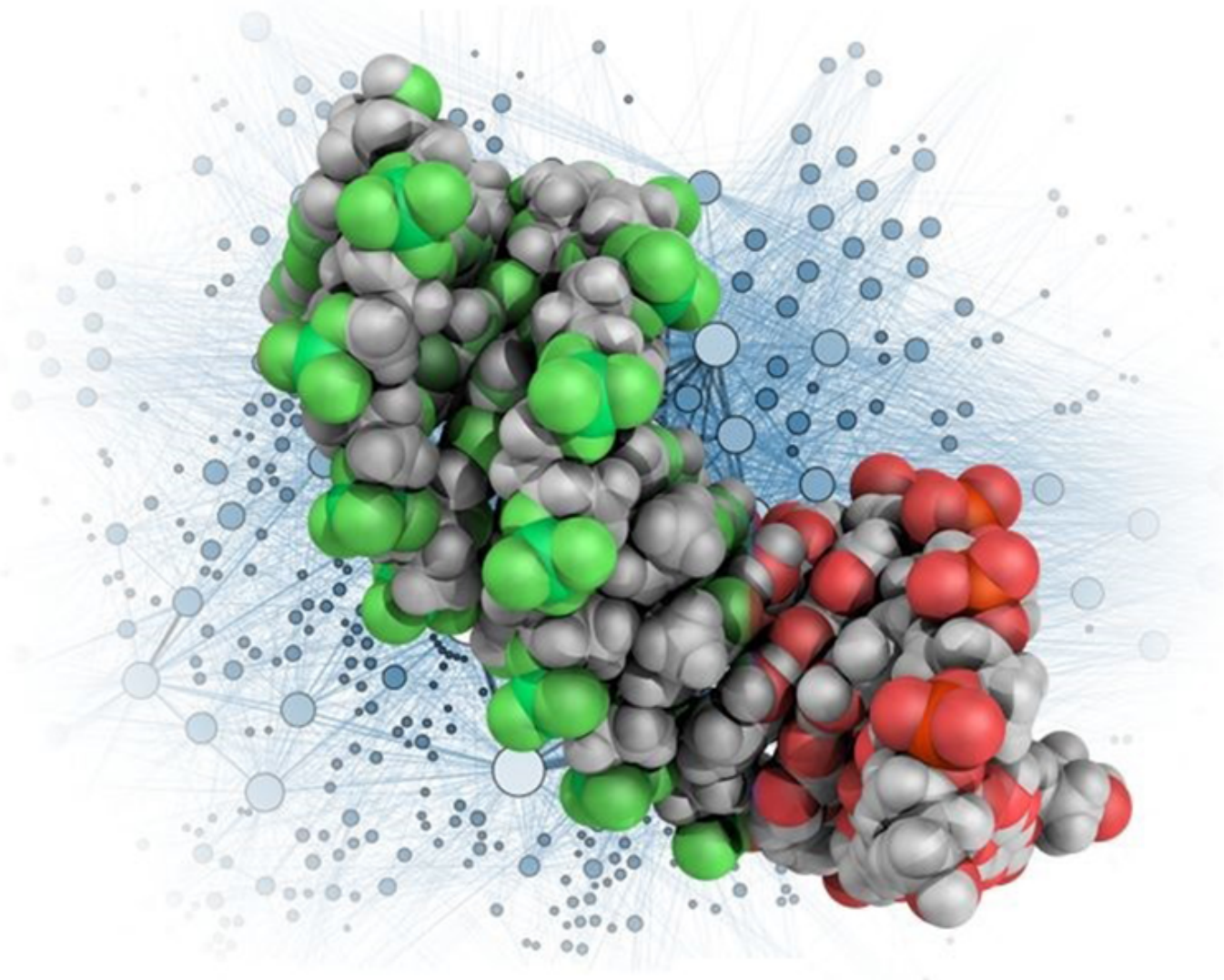


OC1 – Pereira, Joana

What is hidden in the darkness? Seeking new families in natural unknown proteins

Joana Pereira, Torsten Schwede

Biozentrum, University of Basel, Basel, Switzerland

The collection of all natural protein sequences is known as the “protein universe”, where protein families form galaxies and protein superfamilies form clusters of galaxies, surrounded by empty, dark areas seemingly unexplored by Nature. Large-scale genomic projects are promoting an exponential increase in the number of sequences deposited in protein repositories every year. However, the number of “hypothetical proteins” is increasing proportionally, which can be due to low sensitivity of annotation methods but also the presence of sequences belonging to novel biological systems populating dark areas of the protein universe.

We analysed the sequences in UniRef50 and classified them as dark based on the current annotation level of their close homologs. This allowed us to revise and study the proportion and taxonomic distribution of sequence clusters that are poorly annotated for domains and sequence features. We found that almost 40% of all UniRef50 clusters can be considered dark, encompassing sequences across the tree of life. Looking at the 21 proteomes available through the AlphaFold2 database, pathogens and extremophiles are those with the highest frequency of such proteins with no clear homology to well-known families. Preliminary analysis using deep learning protein language models suggest that these proteins complement the landscape of the protein universe occupied by those with at least one well annotated homolog (i.e., bright proteins). While most seem to form bridges between well studied areas, some sequences were found to form unique, well-delimited clusters, suggesting they may correspond to families unrelated to any other previously described.

OC2 – Šivickytė, Ona

Probing new energy descriptors for halogen bonds

Ona Šivickytė[1], Paulo Costa[1]

[1] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisboa, Portugal

Halogen bonds (HaBs) are a type of σ-hole interaction between a halogen, acting as a Lewis acid, and a typical Lewis base [1]. HaBs have been increasingly applied in medicinal chemistry, crystal engineering, and other areas, however, in many cases these applications rely on serendipitous discoveries rather than rational design. This might be due to the lack of fast, accessible, and reliable computational tools that predict HaB interaction energies and other properties a priori [2]. HaBs are typically described as electrostatically driven [3], however, some studies suggest that charge transfer, polarization, and dispersion are also significant [4]. Given that the electrostatic component, quantitatively estimated by the electrostatic potential maximum on the halogen, Vs,max, does not always correlate with interaction energies, in this work we focused on probing two new energy descriptors for HaBs: the orbital overlap distance, D(r) [5], and the intrinsic bond strength index, IBSI [6]. D(r) is a tool to quantify the electron delocalization range and its main purpose is to distinguish soft/diffuse from hard/compact orbitals in molecules, thus taking into account orbital effects. Given that polarization and charge transfer play a non-negligible role in many HaBs, we expected D(r) to complement the commonly used electrostatic descriptor (Vs,max), especially in bonding cases that cannot be rationalized by electrostatics alone. The second descriptor, IBSI [6], evaluates the interaction strength for any pair of interacting atoms and gives each pair an unambiguous and comparable score. The main appeal of this approach is an option to obtain these scores without QM calculations, using only molecular geometries, which would render extremely useful in cases where QM calculations are not feasible such as large biomolecular systems. In this case, we explored how accurately IBSI scores can predict HaB energies and whether using a promolecular electron density is a reasonable alternative to wave-function based methods. Overall, we suggest two completely different descriptors for predicting HaB interaction energy. The results provide useful insights into halogen bonding trends observed in several families of HaBs, and also, we were able to propose an effective and cheap way to evaluate HaB strength thus being a promising method for the fast estimation of HaBs in protein-ligand systems.

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

[2] M. Kaasik, T. Kanger, Front. Chem. 2020, 8, 599064.

[3] T. Clark, Faraday Discuss. 2017, 203, 9.

[4] J. Thirman, E. Engelage, S. M. Huber, M. Head-Gordon, Phys. Chem. Chem. Phys., 2018, 20, 905.

[5] A. Mehmood, S. I. Jones, P. Tao, B. G. Janesko, J. Chem. Inf. Model, 2018, 58, 1836.

[6] J. Klein, H. Khartabil, J.-C. Boisson, J. Contreras-García, J.-P. Piquemal, E. Hénon,J. Phys. Chem. A 2020, 124, 9, 1850.

OC3 – Oliveira, Nuno

A novel US-CpHMD protocol to study the protonation-dependent mechanism of the ATP/ADP carrier

Nuno F. B. Oliveira and Miguel Machuqueiro

BioISI, FCUL

Electrostatic interactions are key participants in biomolecular processes, being the main driving force of molecular interactions [1]. However, describing these forces accurately is quite challenging using both experimental and computational methods. We propose a new computational protocol combining Umbrella Sampling with Constant-pH Molecular Dynamics [2] to overcome the time-scale limitations of conventional MD simulations and to allow protonation changes of all molecules in our simulations. Such protocol was employed to study the transport of two highly negatively charged molecules (ATP and ADP) through the ATP/ADP carrier (AAC), where electrostatic interactions have previously been shown to be very important [3]. Until now, these complete transport processes have not been studied thoroughly and with the correct description of pH. Therefore, our US-CpHMD simulations can bring an unprecedented realism to these complex processes by capturing both conformational and protonation changes occurring during transport.

In our work, the potential of mean force (PMF) profiles of our US-CpHMD simulations at pH 7 show a clear selectivity in the import of ADP, compared to ATP, while in the export, no selectivity was observed. We also observed that, in the import process, AAC was able to sequester both substrates at longer distances and transiently protonate them while crossing the cavity. These features were not observed in the export process and may be an important advantage to counteract the unfavorable mitochondrial membrane potential. Finally, we observed a substrate-induced disruption of the matrix salt-bridge network, which can promote the conformational transition (from the C- to the M-state) required to complete the import process. This work unraveled several important structural features where the complex electrostatic interactions were pivotal to interpret the protein function and illustrated the potentiality of applying the US-CpHMD protocol to other transport processes involving membrane proteins.

[1] Zhang Z, Witham S, Alexov E. On the role of electrostatics in protein–protein interactions. Phys Biol. 2011;8: 035001.

[2] Santos HAF, Vila-Viçosa D, Teixeira VH, Baptista AM, Machuqueiro M. Constant-pH MD Simulations of DMPA/DMPC Lipid Bilayers. J Chem Theory Comput. 2015;11: 5973–5979.

[3] Bidon-Chanal A, Krammer E-M, Blot D, Pebay-Peyroula E, Chipot C, Ravaud S, et al. How Do Membrane Transporters Sense pH? The Case of the Mitochondrial ADP–ATP Carrier. J Phys Chem Lett. 2013;4: 3787–3791.

OC4 – Bueschbell, Beatriz

DRD2 homo-dimers - a world of possibilities

Beatriz Bueschbell, Carlos A.V. Barreto, Rita Melo, Anke C. Schiedel, Irina S. Moreira

CIBB - Center for Innovative Biomedicine and Biotechnology & IIIs –Institute for Interdisciplinary Research, University of Coimbra, Portugal

The concept of dimerization among G protein-coupled receptors has been widely accepted now and opens a completely new point of view on signal transduction [1]. In Addition, GPCR-dimers have been associated with a number of pathological conditions in the last years [1,2]. Dimerization phenomena among dopamine receptors (DRs) have been documented for all five receptors [3]. In particular, dopamine receptor D2 (DRD2) homodimers have been intensively studied as they have been correlated with neurological diseases such as schizophrenia and chronic social defeat stress [4,5]. Although several dimers have been acknowledged, the structural basis behind dimerization has only been partially identified for a very few examples. Regarding the DRD2 homodimers there exist different hypotheses about its interacting interface. The possibility that the interface composition is dependent also on a sharing conformation (active or inactive) between monomers has also to be considered.

In this study we followed a dimer modeling protocol developed by Kaczor et al. (2016) [6]. Starting with homology modeling of the DRD2 monomers in three different conformational states: active with bromocriptine bound (using 6VMS as template [7]), inactive (using 6CM4 as template [8]) and β-Arrestin-bound (Arr) with bromocriptine bound (using 6U1N as template [9]), which were then constructed as dimers in all possible combinations. After validation through PRODIGY algorithms [10,11], the final models were then subjected to long molecular dynamics simulation in replicates of three. Various analyzes were conducted such as solvent accessible surface area, free energy calculations (Molecular Mechanics Poisson-Boltzmann Surface Area, MMPBSA), cross-correlation analysis, types of interactions formed among interfacing residues, localization of ions and residence time of waters in the interface. We identified key aspects that differentiate the various DRD2 homodimer interfaces as well as key residues important for the overall dimerization process.

[1] Somvanshi, R.K. and Kumar, U. (2012) Pathophysiology of GPCR Homo- and Heterodimerization: Special Emphasis on Somatostatin Receptors. Pharmaceuticals 2012, 5, 417-446. Doi: 10.3390/ph5050417.

[2] Quitterer, U. and AbdAlla, S. (2019) Discovery of Pathologic GPCR Aggregation. Front. Med. 6:9. Doi: 10.3389/fmed.2019.00009.

[3] Schiedel, A.C.; Köse M.; Barreto C.A.V.; Bueschbell B.; Morra G.; Sensoy, O.; Moreira I.S. (2018) Prediction and Targeting of Interaction Interfaces in G-protein Coupled Receptor Oligomers. Curr Top Med Chem;18(8):714-746. doi: 10.2174/1568026618666180604082610.

[4] Wang, M.; Pei, L.; Fletcher, P.J.; Kaput, S.; Seeman, P.; Liu, F. (2010) Schizophrenia, amphetamine-induced sensitized state and acute amphetamine exposure all show a common alteration: increased dopamine D2 receptor dimerization. Mol Brain 3, 25. Doi: 10.1186/1756-6606-3-25.

[5] Bagalkot, T.R.; Jin, H.M.; Prabhu, V.V.; Muna, S.S.; Cui, Y., Yadav, B.K., Chae, H.-J.; Chung, Y.-C. (2015) Chronic social defeat stress increases dopamine D2 receptor dimerization in the prefrontal cortex of adult mice. Neuroscience, Vol. 311, 17. Doi: 10.1016/j.neuroscience.2015.10.024.

[6] Kaczor, A.A.; Jörg, M.; Capuano, B. (2016) The dopamine D2 receptor dimer and its interaction with homobivalent antagonists: homology modeling, docking and molecular dynamics. J Mol Model 22, 203. Doi: 10.1007/s00894-016-3065-2.

[7] Yin, J.; Chen, K.M.; Clark, M.J.; Hijazi, M.; Kumari, P, Bai, X.C.; Sunahara, R.K.; Barth, P.; Rosenbaum, D.M. (2020) Structure of a D2 dopamine receptor-G-protein complex in a lipid membrane. Nature. Aug; 584 (7819):125-129. Doi: 10.1038/s41586-020-2379-5.

[8] Wang, S.; Che, T.; Levit, A.; Shoichet, B.K.; Wacker, D.; Roth, B.L. (2018) Structure of the D2 dopamine receptor bound to the atypical antipsychotic drug risperidone. Nature. Mar 8 ;555 (7695):269-273. doi: 10.1038/nature25758.

[9] Staus, D.P.; Hu, H.; Robertson, M.J.; Kleinhenz, A.L.W.; Wingler, L.M.; Capel, W.D.; Latorraca, N.R.; Lefkowitz, R.J.; Skiniotis, G. (2020) Structure of the M2 muscarinic receptor-β-arrestin complex in a lipid nanodisc. Nature. Mar;579 (7798):297-302. doi: 10.1038/s41586-020-1954-0.

[10] Vangone, A. and Bonvin, A.M. (2015) Contacts-based prediction of binding affinity in protein-protein complexes. Elife; 4:e07454. Doi:10.7554/eLife.07454.

[11] Elez, K.; Bonvin, A.M.J.J.; Vangone, A. (2018) Distinguishing crystallographic from biological interfaces in protein complexes: role of intermolecular contacts and energetics for classification. BMC Bioinformatics 19, 438. Doi: 10.1186/s12859-018-2414-9.

OC5 – Suzano, Pedro

An US-CpHMD protocol to calculate pH-dependent membrane permeability coefficients of antitumor drugs

Pedro Suzano, Tomás Silva, Miguel Machuqueiro

BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Many antitumor drugs cross the lipid membrane by passive diffusion to enter tumor cells. In the case of Lewis Base drugs, which are charged in water (pKa values are generally between 8 and 9), a transient deprotonation is required to cross the lipid bilayer [1,2]. Since the Tumor MicroEnvironment (TME) is slightly more acidic than normal cells, it has been proposed that this increased acidity can significantly decrease the antitumor efficiency of these Lewis Bases by impairing the transient deprotonation process [1].

To quantify the impact of the TME in the membrane permeability of some chemotherapeutics, we propose a new protocol based on Constant-pH Molecular Dynamics [2] coupled with an Umbrella Sampling scheme (US-CpHMD) and applied it to two well-known drugs, sunitinib and nintedanib, interacting with a POPC lipid bilayer. The membrane permeability coefficients were calculated using the inhomogenous-solubility diffusion model (ISDM) [3]. The calculations were performed at different pH values, namely 7.5, to mimic a healthy cell, 6.0 to model the TME acidity, and 4.5 to capture the strong acidity of the lysosomes lumen. The latter can provide some insights on the lysosomal sequestration phenomenon, which has been proposed as a drug resistance mechanism [1]. We have calculated the impact of acidity in the bioavailability of both Sunitinib and Nintedanib, which helped us design a new compound as a proof of concept, that is able to circumvent these limitations.

[1] Assaraf YG, Brozovic A, Gonçalves AC, Jurkovicova D, Linē A, Machuqueiro M, et al. The multi-factorial nature of clinical multidrug resistance in cancer. Drug Resist Updat. 2019;46: 100645.

[2] Stark M, Silva TFD, Levin G, Machuqueiro M, Assaraf YG. The Lysosomotropic Activity of Hydrophobic Weak Base Drugs is Mediated via Their Intercalation into the Lysosomal Membrane. Cells. 2020;9. doi:10.3390/cells9051082

[3] de Faria CF, Moreira T, Lopes P, Costa H, Krewall JR, Barton CM, et al. Designing new antitubercular isoniazid derivatives with improved reactivity and membrane trafficking abilities. Biomed Pharmacother. 2021;144: 112362.

OC6 – Borges-Araújo, Luís

SARS-CoV-2 variants impact RBD conformational dynamics and ACE2 accessibility

Mariana Valério[1,§], Luís Borges-Araújo[1,2,3,§], Manuel N. Melo[1], Diana Lousa[1], Cláudio M. Soares[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal.
[2] iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[3] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[§] M.V. and L.B.A. contributed equally to this work.

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has killed over 5 million people and is causing a devastating social and economic impact all over the world. The rise of new variants of concern (VOCs) represents a difficult challenge due to the loss vaccine and natural immunity, and increased transmissibility. All circulating VOCs contain mutations in the spike glycoprotein, which mediates fusion between the viral and host cell membranes, via its receptor binding domain (RBD) that binds to angiotensin-converting enzyme 2 (ACE2). In an attempt to understand the effect of RBD mutations in circulating VOCs, a lot of attention has been given to the RBD-ACE2 interaction. However, this type of analysis is limited, since it ignores more indirect effects, such as the conformational dynamics of the RBD itself. Observing that some VOCs mutations occur in residues that are not in direct contact with ACE2, we hypothesized that they could affect RBD conformational dynamics. To test this, we performed long atomistic (AA) molecular dynamics (MD) simulations to investigate the structural dynamics of wt RBD, and that of three circulating VOCs (alpha, beta, and delta). Our results show that in solution, wt RBD presents two distinct conformations: an “open” conformation where it is free to bind ACE2; and a “closed” conformation, where the RBM ridge blocks the binding surface. The alpha and beta variants significantly impact the open/closed equilibrium, shifting it towards the open conformation by roughly 20%. This shift likely increases ACE2 binding affinity. Simulations of the currently predominant delta variant RBD were extreme in this regard, in that a closed conformation was never observed. Instead, the system alternated between the before mentioned open conformation and an alternative “reversed” one, with a significantly changed orientation of the RBM ridge flanking the RBD. This alternate conformation could potentially provide a fitness advantage not only due to increased availability for ACE2 binding, but also by aiding antibody escape through epitope occlusion.

These results support the hypothesis that VOCs, and particularly the delta variant, impact RBD conformational dynamics in a direction that simultaneously promotes efficient binding to ACE2 and antibody escape.

OC7 – Vieira, Tatiana

Developed of Inverted Virtual Screening Approaches for the Identification of Protein Targets Associated to the Biological Activity of Specific Molecules

Tatiana F. Vieira[1], Sérgio F. Sousa[2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy / Faculty of Medicine, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, BioSIM - Departamento de Biomedicina, Faculty of Pharmacy / Faculty of Medicine, University of Porto, 4050-313 Porto, Portugal

In silico techniques such as Virtual Screening have become crucial in the drug discovery process to identify lead molecules, predicting the binding pose and affinity toward a specific target. However, recent data showed that many compounds do not act as "magic bullets" toward one target only (Ehrlich's assumption), but can, in fact, bind to more targets. This is particularly evidenced by the clinical side effects and cross-reactivity observed during clinical trials. Consequently, identifying possible targets for a particular ligand is an appealing approach to prevent side effects but also, to identify targets and mechanisms of action for already known compounds. One approach that can be used to identify potential targets is docking based Inverted Virtual Screening (IVS). In this methodology, a molecular docking process is employed to screen a protein database for a query ligand.

In this work, we report the creation of a docking based IVS protocol that involves the following steps: 1) Creation of Target Databases Associated with a Biological Activity; 2) Identification/Prediction of Associated Binding Pockets; 3) Validation of Target Specific Docking Protocols for the Different Targets; 4) Inverted Virtual Screening for Identification of the Most-likely targets; 5) MD simulations of the top predictions for validation and refinement; 6) MM-GBSA for characterization and prediction of the relative binding free energies. The resulting IVS protocol is robust, can be adjusted and applied to a variety of compounds and sets of targets.

OC8 – Valério, Mariana

Parainfluenza fusion peptide promotes membrane fusion by assembling into oligomeric pore-like structures

Mariana F. Valério[§1], Diogo A. Mendonça[§2], João Morais[2], Carolina C. Buga[1,2], Carlos H. Cruz[1], Miguel A.R.B. Castanho[2], Manuel N. Melo[1], Cláudio M. Soares[†1], Ana S. Veiga[†2], Diana Lousa[†1]

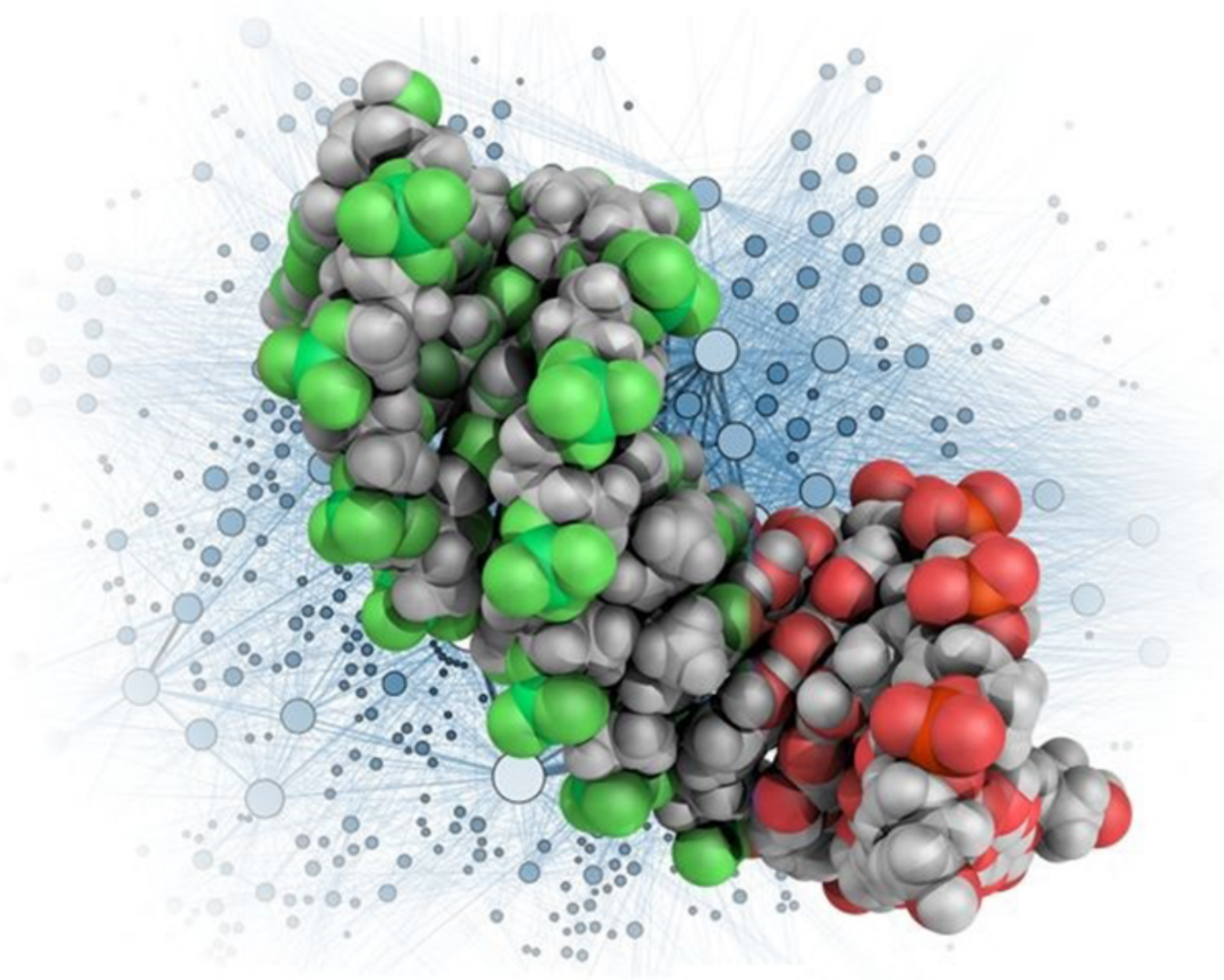
[1] ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa
[2] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa
[§] Both authors contributed equally to this work
[†] Corresponding authors

Paramyxoviruses are enveloped viruses harbouring a negative-sense RNA genome that must enter the host's cells to replicate. In the case of the parainfluenza virus, the cell entry process starts with the identification and attachment to target receptors, followed by proteolytic cleavage of the fusion glycoprotein (F) protein, exposing the fusion peptide (FP) region. The FP is responsible for binding to the target membrane, and it is believed to play a crucial role in the fusion process, but the mechanism by which the parainfluenza FP (PIFP) promotes membrane fusion is still unclear. To elucidate this matter, we performed biophysical experimentation of the PIFP in membranes, together with coarse grain (CG) and atomistic (AA) molecular dynamics (MD) simulations. The simulation results led to the pinpointing of the most important PIFP amino acid residues for membrane fusion and the finding that this peptide, at high concentrations, induces the formation of a water-permeable pore-like structure and promotes lipid head intrusion and lipid tail protrusion, which can facilitate membrane fusion. These results were experimentally validated through biophysical experiments that showed that the peptide's effect in the membrane, including the ability to promote fusion and membrane leakage, depends on the peptide/lipid ratio. Our work provides a step further to understand the membrane fusion process induced by the PIFP, which might foster development in the field of viral entry inhibition.

Posters

VIII EJIBCE

Coimbra, 20 de dezembro 2021



P1 – Aljnadi, Israa

Computational Screening and Design of G-quadruplex Ligands Targeting c-MYC in Breast Cancer

Aljnadi, I[1,2] ; Victor, B L[2] ; Paulo, A[1].

[1] Medicinal Organic Chemistry Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[2] BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Campo Grande, C8 building, 1749-016 Lisboa, Portugal

G-quadruplexes (G4) are four-stranded nucleic acid secondary structures formed by guanine-rich sequences of DNA or RNA. It has been shown that G4 are involved in relevant biological functions of normal mammalian cells, but more importantly, in cancer cells. Several studies reported that G4 is prevalent in telomeres and promotor regions of several oncogenes like c-MYC, which has a key role in several cellular regulatory processes, cancer development and progression [1].

G4 formed in the promoter region of c-MYC may constitute an anticancer drug target by inhibiting the DNA transcription via the block of DNA polymerase and binding of transcription factors. Interestingly, G4 in the c-MYC promoter is reported to be unwounded by the helicase DHX36, a protein of the eukaryotic DEAH/RHA family that recognizes specifically G4s and promotes the regulation of DNA transcription [1,2]. Therefore, we will take advantage of the identified biological relevance of G4, together with the recent published crystallographic structure of DHX36 helicase with the c-MYC G4, to develop an *in-silico* approach to identify inhibitors of this DNAG4-helicase interaction, with the objective to promote an anti-proliferative activity and downregulation of this oncogene expression [2,3]. In this communication, we report a molecular docking workflow that considers different scoring functions coupled to a consensus analysis approach to identify the most promising indoloisoquinoline (IDQ) derivatives capable to bind to c-MYC G4. From an initial library of 1104 ligands, we were able to identify a small group of fragment substituents with a high prevalence in the compounds with higher binding affinities to the c-MYC G4. These results will guide the chemical synthesis of a small subset of IDQ derivatives, and consequent in vitro validation. The obtained results will then afterwards be used in subsequent *in silico* structure/activity studies to assure for the optimization of the most promising c-MYC G4-helicase interaction inhibitors.

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References:

[1] Paulo, A.; Castillo, C. C.; Neidle, S. Compr. Med. Chem. III 2017, 5–8, 308–340.

[2] Chen, M. C.; Tippana, R.; Demeshkina, N. A.; Murat, P.; Balasubramanian, S.; Myong, S.; Ferré-D'amaré, A. R. Nat. 2018, 558 (7710), 465–469.

[3] Duarte, A. R.; Cadoni, E.; Ressurreição, A. S.; Moreira, R.; Paulo, A. ChemMedChem 2018, 13 (9), 869–893.

P2 – Alves, Rodrigo & Lotfi, Maryam

Parameterization of nucleotide cofactors and metabolites for the Martini 3 Coarse-Grain force field

Maryam Lotfi, Rodrigo Barriga and Manuel M. Melo

ITQB NOVA

The biophysics of nucleotides as ubiquitous intracellular molecules is of interest in a wide range of fields. Their crucial importance comes from the fact they are not only the building blocks of nucleic acids, but also cofactors of many enzymes in key biochemical reactions. Understanding the mechanisms of nucleotide-proteins interactions falls within the scope of CG molecular dynamics, which reduce computational complexity to reach size- and time-scales beyond the 10 1 nm and 10 2 μs. To have accurate CG simulation results, we need specific parameters for the required interacting compounds. Sousa et al. [1] developed CG parameters for key metabolites/cofactors (FAD, FMN, riboflavin, NAD, NADP, ATP, ADP, AMP and TPP) in different oxidation and protonation states as well as for smaller molecules derived from them (nicotinamide, adenosine, adenine, ribose, thiamine, lumiflavin, among others) using Martini2. With the recent evolution of Martini2 to Martini3, all these molecules had to be re-parameterized to perform more refined CG simulations to understand those several mechanisms in detail.

In this work, we have successfully established CG parameters for some of these compounds in the framework of Martini3. Afterwards, we simulated a S. aureus' membrane with its most abundant lipids (DAG: Diacylglycerol, PG: Phosphatidylglycerol, CL: Cardiolipin, and L-PG: lysyl-PG). This involved also the parameterization of the iso-/anteiso- terminal methyl branching of the lipidic tails and of the L-PG headgroup. We then focused on simulating the binding of MNQ to NDH-2 (EC 1.6.99.3). This is a possible druggable interaction which might be leveraged to fight S. aureus infection, preventing the propagation of nosocomial disease and overall tip the scales in the war against bacterial resistance.

References:

[1] Sousa, F. M., Lima, L. M. P., Arnarez, C., Pereira, M. M. & Melo, M. N. Coarse-Grained Parameterization of Nucleotide Cofactors and Metabolites: Protonation Constants, Partition Coefficients, and Model Topologies. J. Chem. Inf. Model. 61, 335–346 (2021).

Glossary:

CG – Coarse-Grain

FAD – Flavin Adenine Dinucleotide

FMN – Flavin Mononucleotide

NAD – Nicotinamide Adenine Dinucleotide

NADP – Nicotinamide Adenine Dinucleotide Phosphate

ATP – Adenosine Triphosphate

ADP – Adenosine Diphosphate

AMP – Adenosine Monophosphate

TPP – Thiamine Pyrophosphate

L-PG – Lysyl Phosphatidylglycerol

PG – Phosphatidylglycerol

DAG – Diacylglycerol

CL – Cardiolipin

MNQ – Menaquinone

NDH-2 - Type II NADH:Quinone Oxidoreductase/NADH Dehydrogenase

P3 – Avila, Henrique

Evaluation of Docking Tools on the Search for Adenosine A2A Receptor Antagonists

Henrique V. Avila, Maryam Abbasi, Carlos J. V. Simões, Joel P. Arrais

Univ Coimbra, Centre for Informatics and Systems of the University of Coimbra, Department of Informatics Engineering

Molecular docking is a means of applying molecular simulation methods to provide a quick and inexpensive starting point in drugs and vaccines design. Although, this is a powerful and useful tool, quality assessment is imperative to achieve valid results and be able to appoint the best-suited test molecules in each test set. This process is usually carried over by comparing the tools' performances on docking copies of the native ligands of various crystallographic structures of the same specific protein against the original structure. These practices are namely called Re- and Cross-Docking. Further evaluation can be provided by docking molecules whose chemical affinity for said protein is already known and judging if the tool can rank them accordingly.

In this study, we assess Autodock4, Autodock Vina, Autodock FR, Ledock, rDock, and Plants docking tools using these strategies and their capability of giving accurate results for the adenosine A2a receptor (A2aR). The Docking tools were gauged by performing L-RMSD of the results of Cross-Docking on 10 A2aR structures and then on ranking by binding energy compounds with known inhibition constant (Ki). Autodock 4, Vina, and FR have shown the best acuity on the results, with FR being the most precise when flexible residues are determined, and Vina was the fastest. Moreover, this study has shown that larger sampling correlates positively with better predictions.

P4 – Batista, Marta

First steps towards the identification of a new hybrid antimalarial therapeutic agent targeting PfAQP

Marta S. P. Batista, Paulo J. Costa, and Bruno L. Victor

BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisboa, Portugal

Malaria became a public global health priority and matter of discourse during the transition between millennia. Although most malaria variants are currently successfully treated with the existing antimalarial drugs, in 2019, this disease was still responsible for approximately 409 000 deaths globally [1]. Severe malaria in humans is mostly caused by infection with Plasmodium falciparum whose complications include severe anemia and end-organ damage, pulmonary complications, and hypoglycemia or acute kidney injury [2]. The development of hybrid antimalarial agents has been actively pursued as a promising strategy to overcome the problem of resistant parasite strains since it provides treatment for all P. falciparum that infects human red blood cells and at the same time eliminates the replicative and dormant liver forms of the parasite [3]. In this communication, we will present the first steps towards the development of a multi-target strategy based on the use of keystone antimalarial drugs coupled to inhibitors of the P. falciparum aquaporin (PfAQP). This protein acts as a constitutive water and glycerol channel [4], with a key function in the reproduction of the Plasmodium, making it a promising target for the development of antimalarial therapeutics [5]. An initial structural characterization of PfAQP along with a qualitative assessment on the dynamics and function of the protein will be presented, together with an evaluation of the effect different membrane sizes on simulation times and quality of the model. These results are of utmost importance for the next steps of the project, where the inhibitory efficiency of different glycerol derivatives will be evaluated.

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References:

[1] World malaria report 2020: 20 years of global progress and challenges. geneva: World health organization; 2020.

[2] Phillips, M., Burrows, J., Manyando, C. et al. Malaria. Nat Rev Dis Primers 3, 17050 (2017).

[3] Capela, R., Cabal, G. G., Rosenthal, P. J., Gut, J., Mota, M. M., Moreira, R., Lopes, F., & Prudêncio, M. (2011). Antimicrob Agents Chemother, 55(10), 4698–4706.

[4] Newby, Z., O'Connell III, J., Robles-Colmenares, Y. et al. Nat Struct Mol Biol 15, 619–625 (2008).

[5] Kun, J. F., & de Carvalho, E. G. (2009). Expert Opin Ther Targets, 13(4), 385–394.

P5 – Bruni, Bárbara

Design of Indoloisoquinoline derivatives as potential inhibitors of the interaction between c-MYC:G4 and helicase

Bárbara Bahls Bruni, Alexandra Paulo e Bruno L. Victor

MedOrgChem@iMed, Faculdade de Farmácia,BioISI Faculdade de Ciências, Universidade de Lisboa Universidade de Lisboa

Guanine rich DNA or RNA sequences may form a noncanonical higher-order structure called G-quadruplexes (G4). The structural features of G4 have been described to promote genomic instability in DNA replication, modulate transcription and translation and have been found with high prevalence in promoter regions of many cancer-related genes such as c-MYC [1]. G4s are transient structures that can be unfolded by helicases, a protein family that binds and remodel nucleic acid structures and nucleic acid protein complexes. Some helicases, such as DHX36, prefer binding and unwinding G4 nucleic acid structures [2]. In previous reports, G4 structure stabilization by small organic molecules, has shown promising results as anticancer drug target [1,3]. However, many difficulties related to lipophilicity and specificity towards different G4s have been found. To overcome these obstacles, in this project, we propose to design, synthesize and evaluate indoloisoquinoline (IDQ) derivatives as potential inhibitors of the c-MYC:G4-DHX36 interaction, taking advantage of the recently resolved crystallographic structure of DHX36 helicase in complex with this G4 [2]. The IDQ core was combined with a library of purchasable fragments to create a final library of compound derivatives, which was then used in a molecular docking screening campaign targeting the c-MYC:G4 structure in complex with DHX36 [5]. Different scoring functions from different molecular docking softwares [4-6] were used to derive a final consensus scoring [7], and consequently identify a subset of IDQ fragment substituents shown to be prevalent in the lowest binding affinity docking solutions with c-MYC:G4. These results will now guide the synthesis of the most promising ligands, which selectivity and stabilization will afterwards be validated with several in vitro assays. The obtained results will guide additional structure-activity *in silico* calculations, to allow the optimization of the most promising inhibitors.

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References:

[1] Paulo A. et. al. (2017) Third Edition. Vols. 5–8, Comprehensive Medicinal Chemistry III. Elsevier; 308–340 p.

[2] Chen, M. C., et al. (2018). Nature, 558(7710), 465–483.

[3] Cadoni E, et al. (2021). Pharmaceuticals. 14(7) 669.

[4] Trott, O., Olson, A. J. (2009). Journal of Computational Chemistry, NA-NA.

[5] Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). Journal of Chemical Information and Modeling, 61(8), 3891–3898.

[6] Koebel MR, et al. (2016) J Cheminform. May 18;8:27. 7. Ochoa, R. et al., (2021) Journal of Molecular Graphics and Modelling, Volume 109, 108023.

P6 – Buga, Carolina

Molecular determinants of the SARS-CoV-2 fusion peptide activity

Carolina C. Buga[1,2], Mariana Valério[1], Diogo A. Mendonça[2], João B. Vicente[1], Ana S. Veiga[2], Miguel A. R. B. Castanho[2], Cláudio M. Soares[1], Diana Lousa[1]

[1] ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[2] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, emerged in late 2019 and quickly spread worldwide, resulting in a health and economic crisis. Given its major impact, it is of utmost importance to understand the virus mechanism of infection at a molecular level to develop efficient treatments.

SARS-CoV-2 is an enveloped virus, being encapsulated by a lipid membrane, which needs to be fused to the host membrane to begin the infection process. Fusion between viral and host membrane is promoted by the Spike (S) glycoprotein. The S protein is composed of essential elements for the infection mechanism, namely the receptor binding domain (RBD) known to bind to the host cell angiotensin-converting enzyme 2 during the viral entry pathway. Another important region, known as the fusion peptide (FP), plays an essential role in the fusion mechanism, by inserting into and disturbing the host membrane. There is still not a consensus among scientists in terms of the fusion peptide location on the S protein sequence, with two major candidate regions having been proposed.To shed light into this matter, we combined computational and experimental methods to characterize and compare the effect of the two putative SARS-CoV-2 FPs.

We performed a systematic analysis of the SARS-CoV-2 putative FPs, using Molecular Dynamics (MD) simulations, to dissect how these peptides insert and interact with the membrane. In parallel, we evaluated the putative FPs ability to disturb membrane bilayers applying fluorescence spectroscopy techniques. The overall data support the understanding of how the two peptides interact with the membrane. Since FP has a pivotal role in the virus infection process, this work provides relevant insights and contributes to the fight against COVID-19.

P7 – Caniceiro, Ana

MUTPROT database: a mutation overview of GPCR sub-family A17 receptors

Ana B. Caniceiro[1], Beatriz Bueschbell[1,2], António J. Preto[1,2], Carlos A. V. Barreto[1,2] and Irina S. Moreira[3,4]

[1] CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal (A.B.C.);
[2] PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão, 3030-789 Coimbra, Portugal;
[3] Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal;
[4] CNC - Center for Neuroscience and Cell Biology, Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal;
irina.moreira@cnc.uc.pt (I.S.M.)

More than 800 genes code for G protein-coupled receptors (GPCRs), making them the largest family of membrane proteins [1]. GPCRs mediate several signaling pathways through a general mechanism that involves their activation and subsequent downstream signaling cascades that lead to the release of molecules responsible complex physiological actions [2,3]. These physiological functions can be severely altered by mutations in GPCR genes [4]. The most frequent mutations that alter the function of GPCRs are generally classified according to a decrease in the relevant signaling, that is called loss-of-function, or with an increase, called gain-of-function. Besides many physiological functions GPCRs were also reported to be associated with various pathophysiological states and many diseases [4]. The root-cause of such pathologies involving GPCRs are mostly genetic errors, which alter the normal function of the receptor [4]. A detailed classification of all known non-synonymous mutations in GPCRs would help to understand deregulation and guide the appropriate therapy.

In this project, we constructed a comprehensive and detailed database – MUTPROT Database - which gives an overview about mutations and their effect on receptors of the sub-family A17 (dopamine, serotonin, adrenergic and trace amine receptors), for which the host groups have former knowledge and expertise. Mutations were also sub-classified into groups of residues which are important for receptor signaling: ligand binding pocket, allosteric binding pocket, known activating microdomains, C-terminus or key cysteine residues, GPCR-G-protein interaction, GPCR-Arr-s interaction, and other relevant residues. Here, we provide an analysis of the type of mutations occurring overall and in the different families of the sub-family A17, as well what effects they have on the receptor functionality, and in which part of the receptor they are located. Moreover, we provide an overview into what type of amino acid mutations usually mutate preferably within the sub-family A17.

Lastly, the residues were further analysed considering population and age distribution. Our results reveal a diversity of mutations in the diverse GPCRs sub-family A17 structures, drawing attention to the considerable number of mutations in conserved residues and domains, especially in Adrenergic family. Mutated residues were enriched at the orthosteric binding pocket and β-Arrestin binding. It was also identified that the residues prefer to switch to hydrophobic residues, especially in Dopamine and Trace amine families. Furthermore, heterogeneous distribution of mutations in the different age groups was observed, suggesting that the number of mutations does not seem to increase with age but was rather found to be restricted to specific age groups. This in turn are dependent on the receptor family: while we found that there is an incidence of the number of mutations in the age group greater than 80 years for the Dopamine family, for the Serotonin family it affects the 35 to 40 age group and for the Adrenergic family it affects those under 30 years old. We expect that this interactive database helps to explore mutations, their influence, and their effects, which will be useful as the first step in elucidating the structural and molecular interactions at the atomic level that give rise to different signalling pathways.

References:

[1] Lv X, Liu J, Shi Q, Tan Q, Wu D, Skinner JJ, et al. In vitro expression and analysis of the 826 human G protein-coupled receptors. Protein Cell 2016 75. 2016 Apr;7(5):325–37.

[2] DM R, SG R, BK K. The structure and function of G-protein-coupled receptors. Nature. 2009 May;459(7245):356–63.

[3] Syrovatkina V, Alegre KO, Dey R, Huang X-Y. Regulation, Signaling and Physiological Functions of G-proteins. J Mol Biol. 2016 Sep;428(19):3850.

[4] Schöneberg T, Liebscher I. Mutations in G Protein–Coupled Receptors: Mechanisms, Pathophysiology and Potential Therapeutic Approaches. Pharmacol Rev. 2021 Jan;73(1):89–119.

P8 – Calçada, Bruno

Towards a framework to unify *in silico* methods for endocrine disruptors identification: the inhibition of thyroid peroxidase

Bruno Calçada[a,b], Andrea Sartini[c], Adrian Fowkes[c], Bruno L. Victor[b], Paulo Costa[b]

[a] Ascenza Agro S.A, Alameda dos Oceanos Lote 1.06.1.1 D - 2º, 1990-207 Lisbon,
[b] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences of the University of Lisbon,
[c] Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds LS11 5PS, England

The recurrent daily exposure of human beings to different chemicals along with an increasing trend of endocrine-related human diseases [1] led to a growing interest in understanding how endocrine-disrupting chemicals (EDCs) can affect the human endocrine system. These EDCs can be present in the environment from different sources, e.g., plant protection products (PPPs), pharmaceuticals, or dietary components [2]. Recently, a Guidance to identify this class of compounds in the context of PPPs and biocidal products was issued by the European Chemical Agency (ECHA) and the European Food and Safety Agency (EFSA) [3] upon a request by the European Commission (EC). Considering this new guidance, the development of new *in silico* methodologies capable of identifying EDCs is of utmost importance, not only for drug discovery but also for chemical risk assessment campaigns. In this context, this project aims at maximizing the predictive performance of different *in silico* methodologies by combining ligand-based and structural-based techniques with machine learning algorithms, overcoming the limitation of each approach, and leveraging their individual strengths. In this communication, the initial steps of this project, which focused on thyroid pathways, more specifically on the inhibition of thyroid peroxidase (TPO), will be discussed. TPO catalyses the iodination as well as the coupling of tyrosine residues to thyroglobulin to generate T3 and T4 thyroid hormones, which are of utmost importance in the regulation of multiple physiological processes of the endocrine system. Preliminary results, highlighting the criteria for the curation of a dataset assembled from a high-throughput in vitro assay developed in the Endocrine Disruptor Screening Program by the United States Environmental Agency to predict TPO inhibition via the oxidation of Amplex UltraRed in the AUR-TPO assay [4] will be shown. These results will be compared with previously reported workflows [5,6] applied to the same dataset, and a discussion on the importance of the selected curation steps will be undertaken. These steps are of major importance not only to increase the quality of the dataset but also to reduce the noise than non-relevant compounds could have in the performance of the predictive models that will be generated in the future.

References:

[1] Bergman, A., et al., World Health Organization, 2013

[2] Frye, C.A., et al., J. Neuroendocrinol. 2012, 24, 144

[3] Andersson, N. et al., EFSA Journal 2018, 16, 5311

[4] Friedman, F.K., et al., Tox. Sci., 2016, 151, 160

[5] Lomana, M. G., et al., Chem. Res. Toxicol., 2021, 34, 396

[6] Gadaleta, D., et al., , Front. Pharmacol., 2021, 12, 713037

P9 – Carvalho, Filipa

Predicting Anticancer Drug Response through deep learning

Filipa G. Carvalho, Maryam Abbasi, Bernardete Ribeiro, Joel P. Arrais

Univ Coimbra, Centre for Informatics and Systems of the University of Coimbra, Department of Informatics Engineering

Many commonly prescribed drugs have unexpected side effects based on individual inherited genetic variants. To overcome this problem, the pharmacogenomics field focuses on the association between genetic variants and drug responses. In the era of precision medicine, the main goal is to build a scenario where drug prescription is based on patient profile. However, due to intra- and inter-tumor heterogeneity, drug response prediction for cancer therapy remains a challenge.

In this work, we propose a deep neural network model to predict the effect of anti-cancer drugs in tumors through the half-maximal inhibitory concentration (IC50). The model can typically be seen as two-fold: First, we process three distinctive inputs, namely gene expression profile, mutations data, and molecular fingerprints of the drugs; Then we converge them to predict the impact of the genetic variants on a given drug. Furthermore, we use drug sensitivity data correlated to the genomic data to identify genetic features that are predictive of sensitivity. Given a set of mutation and expression profiles and drugs' fingerprints, the model predicts IC50 values of these drugs.

P10 – Cruz, Carlos

Antibodies Elicited by Rationally-Designed FP-Based Immunogenic Protein have High-Affinity for the SARS-CoV-2 Spike Protein

Carlos Cruz, Ana Carolina Buga, Lindsay Kosack, Marta Alenquer, Jocelyne Demengeot, Maria João Amorim, Claúdio M. Soares, João B. Vicente and Diana Lousa.

Instituto de Tecnologia Química e Biológica (ITQB)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of coronavirus disease 2019 (COVID19) responsible for the most important pandemic in recent decades, with several critical outcomes, including the worst economy crisis in the last decades and the death of over 5 million people worldwide. In this scenario, rational protein design techniques emerged as a rapid strategy to combat this disease. Here, we propose the design of an immunogenic protein based on the Spike fusion peptide (FP) capable of eliciting high-affinity antibodies. The SARS-CoV-2 Spike protein is a transmembrane protein attached on the surface of the virus responsible for mediating interactions with the host cell receptor ACE2 and also for starting a membrane fusion process that results in the virus replication. During the fusion process, Spike assumes a pre-fusion conformation, induced by furin-proteases, that exposes the fusion peptide (817-855) and anchors it into the host cell membrane. Through a Spike refolding mechanism, the membranes are fused and the viral genetic material inserted into the host cell. Hence, preventing this early stage of infection by targeting on the FP can be explored to develop protein-based vaccines. Based on this hypothesis, we designed a hyper-stable immunogenic protein by grafting of 804-824 FP fragment onto scaffold protein and redesigning the amino acids around it to stabilize the chimeric protein. This construct was expressed in Escherichia coli, purified and used to immunize rabbits. Antibodies elicited by rabbit immunization showed high affinity towards the Spike protein, showing that this may be a promising strategy to fight COVID19.

P11 – Cunha, Ricardo

Influence of delivery peptides in the binding affinity of Peptide Nucleic Acids

Ricardo D. González[a,b], Pedro R. Figueiredo[a,b], Beatriz C. Almeida[a,b], Alexandra T. P. Carvalho[a,c,*]

[a] CNC – Center for Neuroscience and Cell Biology, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, 3004-504 Coimbra, Portugal
[b] PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão, 3030-789 Coimbra, Portugal
[c] Almac Sciences, Department of Biocatalysis and Isotope Chemistry, Almac House, 20 Seagoe Industrial Estate, Craigavon BT63 5QD, Northern Ireland, United Kingdom
* atpcarvalho@uc.pt

RNAs have been pointed out as the primary genetic molecule before the outcome of DNA-based organisms [1]. Due to the RNA's key functions, namely inhibition of gene expression, coding of proteins, and protein targeting, RNA has gathered interest as a potential therapeutic tool, especially during the current COVID-19 pandemic [2,3]. Polymers of N-(2-aminoethyl)glycine have been suggested as possible backbone for peptide nucleic acids (PNA), which may have facilitated the transmission of genetic information in the pre-RNA world [1]. PNAs can bind in a sequence-specific manner to disease-causing genes, down-regulating their expression, binding to nucleic acids more effectively than natural nucleic acids given the lack of charged phosphate groups in their backbone, which confers a state of no-electrostatic repulsion [4]. Nevertheless, cell delivery remains an issue, and conjugation with efficient delivery peptides seems to be a promising approach to overcome it, as an alternative to mainstream nanostructure's delivery.

Thus, we have been studying PNAs as potential therapies for infectious diseases, namely COVID-19, by gene expression inhibition. We retrieved sequences for antisense targeting and modelled the PNA-cell penetrating peptide conjugates prior to the analysis of the binding efficiency of PNA to RNA.

References:

[1] S.A. Banack; J.S. Metcalf; L. Jiang; D. Craighead; L.L. Ilag; et al. PLOS ONE, 2012, 7(11), e49043.

[2] M.Y.T Chow; Y. Qiu; J.K.W. Lam, Trends Pharmacological Sciences, 2020, 41(10), 715-729.

[3] DeLong, R. Communications biology, 2021, 4(577).

[4] E. Quijano; R. Bahal; A. Ricciardi; W.M. Saltzman; P.M. Glazer, The Yale journal of biology and medicine, 2017, 90(4), 583.

P12 – Fernandes, Henrique

The Catalytic Mechanism of the SARS-Cov-2 Main Protease

Henrique S. Fernandes, Sérgio F. Sousa, and Nuno M.F.S.A. Cerqueira

Associate Laboratory i4HB - Institute for Health and Bioeconomy, BioSIM, Faculty of Medicine, University of Porto, 4050-313 Porto, Portugal
UCIBIO – Applied Molecular Biosciences Unit, BioSIM, Faculty of Medicine, University of Porto, 4050-313 Porto, Portugal

SARS-CoV-2 Main Protease (Mpro), also known as 3C-like protease, is a key enzyme involved in the replication process of the virus that is causing the COVID-19 pandemic. It is also the most promising antiviral drug target targeting the SARS-CoV-2 virus. In this work [1], the catalytic mechanism of Mpro was studied using the full model of the enzyme and a computational QM/MM methodology [2] with 69/72-atoms included in the QM region treated at DLPNO-CCSD(T)[3][4]/CBS//B3LYP/6-31G(d,p):AMBER level, containing the catalytic important oxyanion-hole residues. The transition state of each step was fully characterized and described together with the related reactants and products. The rate-limiting step of the catalytic process is the hydrolysis of the thioester-enzyme adduct, and the calculated barrier closely agrees with the available kinetic data. Our simulations have disclosed important aspects of the mechanism, namely: (1) the role of the interaction between the P2 residue of the substrate and the catalytic His; (2) the important role of P1' residue of the substrate in the stabilization of the ion pair intermediate; (3) the critical role of Gly143, Ser144, and Cys145 in the stabilization of the substrate's oxo group.

The obtained Gibbs free energy profile, together with the full atomistic detail of the structures involved in catalysis, can be helpful for the rational drug design of transition state analogs as new inhibitors targeting the SARS-CoV-2 virus.

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References:

[1] H. S. Fernandes, S. F. Sousa, N. M. F. S. A. Cerqueira, Mol. Divers. 2021, DOI 10.1007/s11030-021-10259-7.

[2] H. S. Fernandes, M. J. Ramos, N. M. F. S. A. Cerqueira, J. Comput. Chem. 2018, 39, 1344–1353.

[3] Y. Guo, C. Riplinger, U. Becker, D. G. Liakos, Y. Minenkov, L. Cavallo, F. Neese, J. Chem. Phys. 2018, 148, 011101.

[4] F. Neese, F. Wennmohs, U. Becker, C. Riplinger, J. Chem. Phys. 2020, 152, 224108.

P13 – Figueiredo, Pedro

Accelerated Molecular Dynamics to access the Human Carboxylesterase 2 Active Site

Pedro R. Figueiredo[a,b], Ricardo D. González[a,b], Beatriz C. Almeida[a,b], and Alexandra T. P. Carvalho[a,c*]

[a] Center for Neuroscience and Cell Biology (CNC), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, 3004-504 Coimbra, Portugal;
[b] PhD Programme in Experimental Biology and Biomedicine, Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Casa Costa Alemão, 3030-789 Coimbra, Portugal;
[c] Almac Sciences, Department of Biocatalysis and Isotope Chemistry, Almac House, 20 Seagoe Industrial Estate, Craigavon, BT63 5QD, Northern Ireland, UK; *atpcarvalho.com

Human Carboxylesterases (hCEs) belong to the serine hydrolase superfamily (E.C. 3.1.1.1) and are localized within the lumen of the endoplasmic reticulum in several tissues. [1,2]

Two primary hCEs (hCE-1 and hCE-2) have been reported but only the hCE-1 was extensively studied in the past decade. They share a 47% sequence identity and 71% similarity but exhibit different tissue distribution as well as distinct substrate and inhibitor specificities. [3,4] These human enzymes catalyse the hydrolysis of a vast number of structurally diverse ester- or amide-containing moieties to their corresponding products (carboxylic acid and alcohol). [1-5] Their base-catalysed mechanism takes place in a multistep reaction, which is conserved in all serine hydrolases. The process relies on an essential catalytic triad of residues (serine, histidine, and glutamic acid) and an oxyanion hole within the active site. [6] However, to allow molecules to access the active site, the enzymes must present an "open" conformation, especially for bulky substrates. The α1 and α10' helices line the active site and act as a "lid" that permits substrate entry, closing afterwards for the reaction to occur. [7]

In this work, we investigated the structural changes on the helices α1 and α10' of the hCE-2 enzyme. These changes are required to grant xenobiotic and endogenous compounds access to the enzyme's active site. For this purpose, we have employed classical and accelerated Molecular Dynamics calculations. [8]

References:

[1] T. Satoh; M. Hosokawa Annu Review Pharmacol Toxicol 1998, 38, 257-88.

[2] M.R. Redinbo; P.M. Potter; Drug Discov Today 2005, 10, 313-25.

[3] M. Hosokawa; Molecules 2008, 13, 412-31.

[4] H. Schwer; T. Langmann; R. Daig; A. Becker; C. Aslanidis; G. Schmitz Biochem Biophys Res Commun 1997, 233, 117-20.

[5] T. Satoh; P. Taylor; W.F. Bosron; S.P. Sanghani; M.Hosokawa; B.N. La Du; Drug Metab Disp 2002, 30, 488-93.

[6] S. Bencharit; C. Morton; Y. Xue; Nat Struct Mol Biol 2003, 10, 349–56.

[7] V.W. Dolinsky; D. Gilham; M. Alam; Cell Mol Life Sci 2004, 61, 1633–51.

[8] P.R. Figueiredo; R.D. González; A.T.P. Carvalho; Mol Catal 2021, 515, 111938.

P14 – Fortuna, Andreia

Impact of halogen radii in the prediction of hydration free energies using PBSA calculations

Andreia Fortuna[#,+], Paulo J. Costa[#]

[#] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016 Lisboa, Portugal
[+] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal

The estimation of protein-ligand binding energies, which is important for structure-based virtual screening, can be performed over molecular dynamics (MD) trajectories, combining Molecular Mechanics (MM) energies with Poisson-Boltzmann surface area (PBSA) continuum solvation methods. In this context, the PBSA calculations, which estimate hydration free energies, rely on the assignment of atomic radii (PB radii). Several drug and drug-like molecules are halogenated and in force field methods, an off-center point-charge, also called extra point (EP), is often introduced at a given distance from halogen atoms (X) to emulate a positive region of the electrostatic potential of these elements (σ-hole). This simple strategy overcomes the fact that empirical force fields typically consider halogen atoms to carry a negative charge leading to unfavorable interactions halogen bond interactions. However, standard halogen PB radii are often incompatible with typical X-EP distances, placing the EP within the solvent dielectric [1]. To overcome this issue, we previously optimized the halogen PB radii for a single EP implementation taken from the literature in the context of AMBER/GAFF [1]. Given the multitude of EP implementations, herein, we present an extension of the PB radii optimization to other EP approaches in the context of the same (GAFF) and other force fields (e.g., CHARMM) [2]. For that purpose, the performance of PBSA was evaluated for 142 halogenated compounds for which the experimental hydration free energies are known. The optimized values for the halogen PB radii were chosen based on the minimization of the mean absolute error against experimental values. Additionally, the effect of the solute flexibility and nonpolar contributions was also explored.

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References:

[1] Nunes, R.; Vila-Viçosa, D.; Costa, P. J., J. Chem. Theory. Comput. 2019, 15, 4241−4251.

[2] Fortuna, A; Costa, P. J., J. Chem. Inf. Model. 2021, 61, 7, 3361–3375.

P15 – Gabriel, Guilherme

In silico approach on the biochemistry of perception: interaction between DMT and 5-HT2AR and its biased signaling

Guilherme Lopes Gabriel [1,2,3], Raquel Gouveia [1,2,3], Irina S. Moreira [1,2,3]

[1] Center for Neuroscience and Cell Biology; UC - Biotech, Biocant Park, Parque Tecnológico de Cantanhede, Núcleo 04, Lote 3, 3060-197 Cantanhede, Portugal
[2] Center for Neuroscience and Cell Biology; Rua Larga, Faculty of Medicine, Pólo I, 1st floor, University of Coimbra, 3004-504 Coimbra, Portugal
[3] Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

During the past years there was a crescent interest on psychedelics and their possible role as neurotherapeutics [1]. This lies on their potential as psychoplastogens, a class of compounds that promote neural plasticity [2]. When considering contemporary neuropsychiatric disorders such as anxiety, mood or disorders related to abuse of substances, these compounds arise as a possible solution for the maladaptive behavioral changes, that characterize these brain disturbances. We need to understand how these compounds bind to specific receptors and how different mechanisms call for a different molecular intermediate, resulting in specific physiological responses [3].

As we need to atomistically assess these interactions, we are using state-of-the-art computational methods [4]. By homology modelling, docking, molecular dynamics, and interfacial analysis we are looking into a better understand how key drugs binds to 5-hydroxytryptamine receptor 2A. We will also clarify the natural preference for G-protein or β-arrestin biased pathway. Our goal is to correlate the psychedelic response with a specific pathway. This becomes an essential part of research when designing new pharmaceuticals and helps to better understand how psychedelics work on the human mind.

[1] Dunlap, L. E., Azinfar, A., Ly, C., Cameron, L. P., Viswanathan, J., Tombari, R. J., Myers-Turnbull, D., Taylor, J. C., Grodzki, A. C., Lein, P. J., Kokel, D., & Olson, D. E. (2020). Identification of Psychoplastogenic N,N-Dimethylaminoisotryptamine (isoDMT) Analogues through Structure-Activity Relationship Studies. Journal of medicinal chemistry, 63(3), 1142–1155.

[2] Olson D. E. (2018). Psychoplastogens: A Promising Class of Plasticity-Promoting Neurotherapeutics. Journal of experimental neuroscience, 12, 1179069518800508.

[3] Barker S. A. (2018). N, N-Dimethyltryptamine (DMT), an Endogenous Hallucinogen: Past, Present, and Future Research to Determine Its Role and Function. Frontiers in neuroscience, 12, 536.

[4] Kazmi, S. R., Jun, R., Yu, M. S., Jung, C., & Na, D. (2019). In silico approaches and tools for the prediction of drug metabolism and fate: A review. Computers in biology and medicine, 106, 54-64.

P16 – Gouveia, Raquel

The study of AMPAR:Stargazin interface through molecular dynamics

Raquel P. Gouveia[#a], Carlos A. V. Barreto[#a,b,c], Salete J.Baptista[a,d], A.J.Preto[a,b,c], Gladys L. Caldeira[a], Ana Luisa Carvalho[a,e], Rita Melo[a,d] and Irina S. Moreira[*,e]

[a] CNC – Center for Neuroscience and Cell Biology. University of Coimbra, UC Biotech Building, 3060-197 Cantanhede, Portugal.
[b] IIIUC – Institute for Interdisciplinary Research, University of Coimbra, 3030-789 Coimbra, Portugal.
[c] PhD Program in Experimental Biology and Biomedicine (PDBEB), University of Coimbra, Portugal.
[d] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, ao km 139,7; 2695-066 Bobadela, Portugal.
[e] – Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal.
[#] Co-first authors
[*] Corresponding author

Glutamate is the key excitatory neurotransmitter in the Central Nervous System and together with glutamate receptors, such as AMPAR, plays a critical role in synaptic plasticity, which is believed to be involved in the process of learning [1,2]. STarGazin (STG), a transmembrane AMPAR regulatory protein, is required for the transport of AMPAR to the surface and its stabilization at synapses, as well as the homeostatic synaptic scaling of AMPAR and the modulation of its gating properties [3,4].

In this work we used microsecond molecular dynamics simulations to study the structure of AMPAR:STG. We extensively analyzed the dynamics of this interface through interactions (H-bonds and Salt-Bridges), ΔSASA and MMPBSA. Our dynamical study showed specific and stable interactions of the two main STG binding sites, potentiating the understanding of the mechanism of these biological machineries.

References:

[1] Louros, S. R., Hooks, B. M., Litvina, L., Carvalho, A. L. & Chen, C. A role for stargazin in experience-dependent plasticity. Cell Reports 7, 1614–1625 (2014).

[2] Martin, S. J., Grimwood, P. D. & Morris, R. G. M. Synaptic Plasticity and Memory: An Evaluation of the Hypothesis. Annu. Rev. Neurosci vol. 23 (2000).

[3] Tomita, S. et al. Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Nature 435, 1052–1058 (2005).

[4] Ben-yaacov, A. et al. Molecular Mechanism of AMPA Receptor Modulation by TARP / Stargazin. Neuron 93, 1–12 (2017).

P17 – Loureiro, Rui

Non-structural protein 1 (NS1) interaction with TRIM25 as a target for influenza antiviral therapy

Rui João Loureiro, Andreia Cunha, Rui M. M. Brito

Coimbra Chemistry Center, Chemistry Department, Faculty of Sciences and Technology, University of Coimbra, 3004-535 Coimbra, Portugal

Influenza viruses evolve at a high rate, which poses a challenge for antiviral therapies, making the development of new antivirals directed to novel targets an urgent need. One of the most promising new targets for influenza antiviral therapy is the non-structural protein 1 (NS1). NS1 binds to TRIM25, leading to the inhibition of the RIG-I/MAVS signalling pathway and the suppression of IFN production. We therefore explored the interaction between NS1 and TRIM25 as a possible druggable target using molecular dynamics (MD) simulations and druggability predictions.

Analysis of classical 100 ns MD simulations reveals a high stability of the interface in the NS1-TRIM25 complex, with low RMSD and RMSF values. Additionally, druggability predictions suggest a potential druggable site in NS1 at the most stable region of the binding interface with TRIM25.

The data presented provide rationale for targeting the NS1 interaction site with TRIM25 in new antiviral drug discovery efforts. Accordingly, in future work we will conduct virtual screening campaigns directed to this protein-protein interaction site.

P18 – Magalhães, Pedro

Identification of membrane PAINS via an optimized computational protocol

Pedro R. Magalhães[*], Pedro B. P. S. Reis[§], Diogo Vila Viçosa, Miguel M. Machuqueiro[§], Bruno L. Victor[§]

[*] Centro de Neurociências e Biologia Celular, Rua Larga, FMUC, 3004-517 Coimbra
[§] BioISI: Biosystems and Integrative Sciences Institute; Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Membrane Pan-Assay INterference compoundS (PAINS) are a subcategory of molecules that interact with lipid membranes in a nonspecific way and alter their physicochemical properties [1]. A prompt detection of these compounds in the drug discovery process is therefore crucial, as it avoids wasting precious time and resources chasing after false leads. Here we present an optimized umbrella sampling/molecular dynamics-based computational protocol to identify compounds with varying degrees of membrane PAINS behavior. We observed that the method was extremely susceptible to fluctuations in membrane thickness, which we were able to alleviate by changing the US-reference position from the membrane center to the closest interacting monolayer. The computational performance was further improved by adjusting the number, strength, and position of the umbrellas. The membrane permeability coefficients calculated using the inhomogeneous solubility diffusion model were able to accurately assess the membrane PAINS character of both curcumin and resveratrol [2] but indicated a possible misclassification of notophagin in a previous work [3].

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References:

[1] Baell, J.B. et al. (2010) JMC 53(7):2719 doi.org/10.1021/jm901137j

[2] Ingólfsson, H.I. et al. (2014) ACSCB 9(8):1788 doi.org/10.1021/cb500086e

[3] Jesus, A.R. et al. (2017) JMC 60(2), 568-579 doi.org/10.1021/acs.jmedchem.6b01134

P19 – Magalhães, Rita

Optimization of Classification Models for Identification of Ligand-Target Inhibition Pairs in Biofilm Formation in *P. aeruginosa*

Rita P. Magalhães[1,2], Victor de la Oliva[1,2], João Carneiro[3], and Sérgio F. Sousa[1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, BioSIM – Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal
[3] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal

Biofilms are highly resistant communities of bacteria enclosed in a self-produced matrix. Biofilm formation is complex and involves several mechanisms. *P. aeruginosa* is a biofilm forming highly pathogenic and resistant bacteria, responsible for up to 20% of hospital bacterial infections. Several biofilm-formation *P. aeruginosa* related protein targets have been identified, mainly involved in Quorum-Sensing, the cell-to-cell communication in bacteria. Several ligands with biofilm formation inhibitory activity have been identified and experimentally tested. However, for many of these ligands the specific molecular targets are unknown. Adequate knowledge of each ligand-target pair would allow for directed drug-design and development, increasing chances of more potent inhibition.

In this work, a classification algorithm for the pairing of inhibitory ligands and protein targets in *P. aeruginosa* biofilm formation mechanisms was developed. From a curated database of known ligand-target pairs, PaDEL software was used to calculate over 600 descriptors for each ligand. An algorithm to select the most relevant features was developed. Finally, K-nearest neighbours, Support Vector Machines, Random Forest, Naïve Bayes, XG-Boost and Neural Networks classification models were developed, trained, and evaluated, using Python programming language scikit-learn libraries. Several metrics such as F1-score, Jaccard score, Accuracy, Precision and Recall were calculated to evaluate model behaviour. The top performing models were used to screen a set on unknown ligands with anti-biofilm activity and are now ready to be used in cases of unknown ligand-target pairing.

P20 – Marques, Jéssica

Development of new computational workflow for the identification of hAQP5 modulators

Jéssica L. Marques[1,2]; Bruno L. Victor[1]; Graça Soveral[2]

[1] BioISI, Faculty of Sciencies, Universidade de Lisboa
[2] iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa

Aquaporins (AQPs) are membrane channels which facilitate the flow of water and other small molecules, such as glycerol, across biological membranes. They play a crucial role in cell homeostasis and volume regulation, being widely distributed in all organisms. In mammals, there are three subsets of AQPs, divided according to their permeability profiles and sequence homology. Due to its biological importance, deregulation of AQPs activity and/or expression can induce changes in the cell homeostasis, causing health problems and diseases, such as carcinogenesis [1-3]. The relationship between AQPs and cancer has been thoroughly studied and, as a result, it was concluded that AQPs are overexpressed in a wide variety of tumors, especially AQP1, AQP3 and AQP5 isoforms. Moreover, the discovery of efficient and selective modulators of human AQPs (hAQPs) has been considered as a potential strategy for cancer treatment/therapy. However, the inhibitors reported thus far exhibit high toxicity and poor selectivity, making them inappropriate to proceed for drug development [4]. Therefore, the main goal of this work, was to develop and apply a new computational workflow to identify hAQP5 modulators from a Sigma-Aldrich database of compounds. This approach combined the use of Molecular Dynamics, Molecular Docking, and MM-PBSA methodologies, allowed for the identification of compounds with high affinity for hAQP5 [5]. The five most promising hits were experimentally tested for their inhibitory effect on hAQP5 in an optimized yeast cell model expressing this isoform, by permeability assays using the fluorescence stopped-flow technology. Here, we present and discuss our results highlighting the limitations of our approach and propose the future methodological perspective that will be pursued to find better and specific hAQP5 inhibitors.

References:

[1] Direito, I., Madeira, A., Brito, M. A., & Soveral, G. (2016). Cellular and Molecular Life Sciences, 73(8), 1623–1640.

[2] Huber, V. J., Tsujita, M., & Nakada, T. (2012). Molecular Aspects of Medicine, 33(5–6), 691–703.

[3] S. Verkman, A., O. Anderson, M., & C. Papadopoulos, M. (2014). Nature Reviews Drug Discovery, 13(4), 259–277.

[4] Abir-Awan, M., Kitchen, P., Salman, M. M., Conner, M. T., Conner, A. C., & Bill, R. M. (2019). International Journal of Molecular Sciences, 20(7).

[5] Horsefield, R., Nordén, K., Fellert, M., Backmark, A., Törnroth-Horsefield, S., Terwisscha Van Scheltinga, A. C., Kvassman, J., Kjellbom, P., Johanson, U., & Neutze, R. (2008). Proceedings of the National Academy of Sciences of the United States of America, 105(36), 13327–13332

P21 – Martins, Fábio

Specialized Multi-Level Computational Protocol for the Identification of Potential New Drugs

Fábio G. Martins[1,2], Tatiana F. Vieira[1,2], Joel P. Moreira[1,2], Tiago Barbosa[1,2], Sérgio F. Sousa[1,2]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, BioSIM - Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, 4200-319 Porto, Portugal

The use of computer-aided drug design in a preliminary stage of drug design has been gaining increased importance over the years, finding application in the study of many biologic problems. These methods make the entire process more cost-efficient and minimize failures in latter stages.

This work reports the development of a specialized multi-level computational approach involving the application of molecular docking, Molecular Dynamics (MD) simulations and MM/PB(GB)SA calculations to find promising compounds against two important biologic targets: CviR, the quorum sensing receptor from *Chromobacterium violaceum* and PknB, a Serine/Threonine protein kinase responsible for phosphorylation-mediated signalling in *Mycobacterium tuberculosis*. *C. violaceum* is a model organism for the study of quorum sensing, a key process during biofilm formation. Biofilm infections have been recognized as a serious threat to our society, being associated with 80% of all bacterial infections in humans. PknB is involved in many key bacterial processes such as cell wall synthesis, cell division and metabolism in M. tuberculosis, one of the most common causes of mortality in the world.

Autodock 4, Autodock Vina, GOLD (with 4 different scoring functions) and LeDock were the molecular docking programs used. The ability to discriminate the active molecules within a large database was optimized by screening a library containing known active molecules and decoys. The optimized protocol was then applied to a ZINC/FDA Approved database, the Mu.Ta.Lig Virtual Chemotheca and the French Chimiothèque Nationale. Finally, Molecular dynamics simulations of the most promising molecules in complex with the target proteins were performed using the Amber20 software to validate the stability of the Target-Ligand association. MM/PBSA and MM/GBSA calculations were done in order to estimate the affinity of each molecule towards the target protein. These studies yielded multiple promising compounds which in the future can be tested and validated experimentally.

P22 – Moreira, Pedro

ViralFP: A web application of viral fusion proteins

Pedro Moreira, Ana Marta Sequeira, Sara Pereira, Rúben Rodrigues, Miguel Rocha, Diana Lousa

ITQB NOVA & Universidade do Minho - Centro de Engenharia Biológica

Viral fusion proteins are essential to allow enveloped viruses (such as Influenza, Dengue, HIV and SARS-CoV-2) to enter their hosts' cells, in a mechanism referred to as membrane fusion. This makes these proteins (with special relevance to their fusion peptides, the component of the protein that can insert into the host's membrane by itself) interesting potential therapeutic targets for preventing or treating for some well-known diseases [1,2,5,8,10,11,14,16]. However, there is no centralized data repository containing all the relevant information regarding viral fusion proteins.

With that in mind, the main purpose of this work is to develop a CRUD (Create, Read, Update and Delete) web application that will allow researchers to find all the necessary data regarding enveloped viruses and their viral fusion proteins (this data was gathered from biological repositories like NCBI Protein [7] and NCBI Taxonomy [6], UniProt [15] and PDB [3]), through an easy-to-use web interface. The web application will also contain other bioinformatics functionalities, such as sequence alignment (through BLAST [9], Clustal [12] and Weblogo [4]) to allow researchers to retrieve key pieces of information regarding a fusion protein, as well as machine learning models capable of predicting the location of fusion peptides inside the viral fusion protein sequence [13].

The implementation of the server used Django as its back-end, retrieving the data from a MySQL database, and Angular as its front-end.

The main result of the work is, therefore, a working web application, with a web interface available online through the URL https://viralfp.bio.di.uminho.pt/.

The web application allows users to explore the gathered data related to viral fusion proteins in a user-friendly way. This tool contains all the proposed functionalities and machine learning models. As expected in an application's development, there are several aspects that require future work to improve the usefulness of this tool to the scientific community.

References:

[1] Apellániz, B., Huarte, N., Largo, E., & Nieva, J. L. (2014). The three lives of viral fusion peptides. Chemistry and Physics of Lipids, 181, 40–55. https://doi.org/10.1016/j.chemphyslip.2014.03.003

[2] Baquero, E., Albertini, A. A., Vachette, P., Lepault, J., Bressanelli, S., & Gaudin, Y. (2013). Intermediate conformations during viral fusion glycoprotein structural transition. Current Opinion in Virology, 3(2), 143–150. https://doi.org/10.1016/j.coviro.2013.03.006

[3] Burley, S. K., Berman, H. M., Kleywegt, G. J., Markley, J. L., Nakamura, H., & Velankar, S. (2017). Protein Data Bank (PDB): the single global macromolecular structure archive. Protein Crystallography, 627-641.

[4] Crooks, G., Hon, G., Chandonia, J., & Brenner, S. (2004). WebLogo: A Sequence Logo Generator. Genome Res, 14, 1188–1190. https://doi.org/10.1101/gr.849004.1

[5] Epand, R. M. (2003). Fusion peptides and the mechanism of viral fusion. Biochimica et Biophysica Acta - Biomembranes, 1614(1), 116–121. https://doi.org/10.1016/S0005-2736(03)00169-X

[6] Federhen, S. (2012). The NCBI taxonomy database. Nucleic acids research, 40(D1), D136-D143.

[7] Geer, L. Y., Marchler-Bauer, A., Geer, R. C., Han, L., He, J., He, S., ... & Bryant, S. H. (2010). The NCBI biosystems database. Nucleic acids research, 38(suppl_1), D492-D496.

[8] Harrison, S. C. (2015). Viral membrane fusion. Virology, 479–480, 498–507. https://doi.org/10.1016/j.virol.2015.03.043

[9] Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: a better web interface. Nucleic acids research, 36(suppl_2), W5-W9.

[10] Marsh, M. (2005). Current topics in microbiology and immunology. In Immunological Communications (Vol. 6). https://doi.org/10.3109/08820137709055812

[11] Marsh, M., & Helenius, A. (2006). Virus entry: Open sesame. Cell, 124(4), 729–740. https://doi.org/10.1016/j.cell.2006.02.007

[12] Sievers, F., & Higgins, D. G. (2014). Clustal omega. Current protocols in bioinformatics, 48(1), 3-13.

[13] Sequeira, A. M., Lousa, D., & Rocha, M. (2020, June). ProPythia: A Python Automated Platform for the Classification of Proteins Using Machine Learning. In International Conference on Practical Applications of Computational Biology & Bioinformatics (pp. 32-41). Springer, Cham.

[14] Teissier, É., & Pécheur, E. I. (2007). Lipids as modulators of membrane fusion mediated by viral fusion proteins. European Biophysics Journal, 36(8), 887–899. https://doi.org/10.1007/s00249-007-0201-z

[15] UniProt Consortium. (2015). UniProt: a hub for protein information. Nucleic acids research, 43(D1), D204-D212.

[16] White, J. M., Delos, S. E., Brecher, M., & Schornberg, K. (2008). Structures and mechanisms of viral membrane fusion proteins: Multiple variations on a common theme. Critical Reviews in Biochemistry and Molecular Biology, 43(3), 189–219. https://doi.org/10.1080/10409230802058320

P23 – Oliveira, Alexandre

Modelling Gd-DOTA for molecular dynamics simulations in water and in presence of lipid bilayers

Alexandre C. Oliveira[1*], Hugo A. L. Filipe[1], Armindo Salvador[2], Rui Sun[3], Gregory A. Voth[4], Carlos F. G. C. Geraldes[1,5], Maria João Moreno[1,2], Luís M. S. Loura[1,2,6]

[1] Coimbra Chemistry Centre (CQC), Department of Chemistry, University of Coimbra, 3004 535 Coimbra, Portugal
[2] CNC—Center for Neuroscience and Cell Biology, University of Coimbra, P-3004-517 Coimbra, Portugal
[3] Department of Chemistry, The University of Hawai'i, Monoa, Honolulu, Hawaii 96822, USA
[4] Department of Chemistry, James Franck Institute, and Institute for Biophysical Dynamics, The University of Chicago, Chicago, Illinois 60637, USA
[5] Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-393 Coimbra, Portugal
[6] Faculty of Pharmacy, University of Coimbra, P-3000-548 Coimbra, Portugal

The correct parametrization of lanthanide complexes is of utmost importance for their characterization using computational tools such as molecular dynamics simulations, allowing the optimization of the properties for a wide range of applications, including medical imaging. Here we present a systematic study to establish the best strategies for the correct parametrization of lanthanide complexes using the Gd-DOTA as reference, which is used as contrast agent in MRI. We chose the bonded model to parameterize the lanthanide complexes, this being especially important when considering the study of the complex as whole (e.g., for the study of the dynamics of its interaction with proteins or membranes). We followed two strategies: a heuristic approach based on a previously published work1, and another with the MCPB.py tool2. Adjustment of the Lennard-Jones parameters of the metal was required. The final topologies were able to reproduce ion to oxygen distance (IOD), vibrational frequencies and other structural properties. For the first time, we report the correct assessment of the mean residence time (τm) of the coordinated inner sphere water by recording the dissociative events over up to 10 μs all-atom simulations. The analysis is extended to exchange kinetics of the second coordination sphere, allowing the quantitative characterization of its contribution to the overall water relaxivity. With the correct description of the Gd-DOTA, we studied its interaction with a POPC membrane by means of unbiased simulations to understand its membrane location and interactions. Biased simulations have also been performed to obtain the Potential of Mean Force (PMF) across the bilayer normal and calculate the partition to and the rate of permeation through biomembranes.

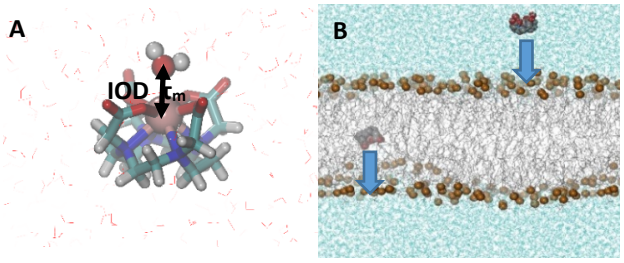

Figure. A) Snapshot illustrating the observables IOD and τm used for parameter optimization B) Snapshot illustrating the acquisition of a PMF of Gd-DOTA interacting with a POPC membrane.

References:

[1] Henriques, E. S., Geraldes, C. F. G. C. & Ramos, M. J. Mol. Phys. 101, 2319–2333 (2003).

[2] Li, P. & Merz, K. M. J. Chem. Inf. Model. 56, 599–604 (2016).

P24 – Parreiras, Susana

Design and production of antiviral proteins targeting SARS-CoV-2

Susana Parreiras, Carlos H. Cruz, Mariana Valério, Pedro M. F. Sousa, Cláudio M. Soares, João B. Vicente and Diana Lousa

ITQB NOVA

SARS-CoV-2 is the virus responsible for the current COVID-19 pandemic, which has caused >250 million infections and >5 million deaths, as of November 2021. Despite the vaccination efforts, it remains urgent to develop strategies to control the infection and treat patients [1,2]. This is a positive-sense RNA virus that belongs to the Coronaviridae family. Its outer structure is spherical, it is encapsulated by a viral membrane and, in order to infect the host cell, it needs to fuse its membrane with the host cell membrane [3].

One of the proteins that is attached to the viral membrane of the virus is the spike (S) protein, which is composed of two subunits: S1, containing a receptor binding domain (RBD) responsible for binding to the host cell receptor, and S2, that facilitates membrane fusion between the viral and host cell membranes [4]. Thus, this protein is primarily responsible for the ability of the virus to enter the host cells, making it one of the most promising therapeutic targets of coronaviruses.

The goal of this work was to design and produce antiviral proteins that can inactivate the S protein and block infection. These proteins are engineered to bind to the RBD region and block its interaction with the host receptor, the angiotensin converting enzyme-2 (ACE2) protein [5,6].

In a first step, several antiviral proteins were computationally designed with the Rosetta program [7,8], based on the interactions between ACE2 and the receptor-binding domain (RBD) of protein S. Next, molecular dynamics (MD) simulations of three candidates, free in solution and in complex with the RBD, were performed in order to test their interaction with the RBD. This was followed by experimental validation that began with the expression and purification of the three candidates. After obtaining pure fractions, the secondary structure and thermal stability of these proteins were tested by far-UV circular dichroism spectropolarimetry and differential scanning fluorimetry, respectively. In order to assess the affinity of each candidate for RBD, surface plasmon resonance was employed. Finally, neutralization assays were performed to study the neutralization ability of the proteins. The experimental results show that one of the designed proteins is a promising therapeutic lead that will be further improved in the future.

P25 – Pereira, Tiago

Deep generative model for de-novo molecular design using disease gene expression data

Tiago Oliveira Pereira, Maryam Abbasi, Joel Arrais

Univ Coimbra, Centre for Informatics and Systems of the University of Coimbra, Department of Informatics Engineering

Drug development is a complex process that is extremely costly as it requires domain expertise and time. However, having an efficient and reliable drug development pipeline is essential to address the pressing needs for novel therapeutic solutions. Despite these problems, the steady decrease of approved drugs over the past few decades demonstrates the ineffectiveness of conventional drug discovery methods.

Several computational strategies based on deep learning have been applied to the drug discovery process. A paradigmatic example is the de novo drug design. Its main objective is to identify drug candidate molecules, with optimized properties, to streamline the expensive drug discovery testing steps. Nonetheless, finding novel molecules with desired properties regarding their effectiveness, toxicity, and bioavailability is a difficult task. As a result, strategies based on artificial intelligence and deep learning have proved to be insufficient to solve this problem. The principal challenges faced are the lack of reliable biological data quantifying the drug-target affinity, the multi-objective nature of the problem that is not properly considered, and the view of the drug-target interaction as being solely a chemical aspect.

In this work, we propose a novel method to explore the chemical space based on Deep Reinforcement Learning, i.e., a combination of the capabilities of Deep Learning with the ability of Reinforcement Learning to learn complicated tasks through trial and error. It was applied breast cancer gene expression data to guide the molecular optimization process. The general architecture is composed of different sub-models including a molecular generator and two variational autoencoders – to encode molecules and gene expression profiles. These three models were interconnected to generate promising molecules that can induce a desired gene expression profile, avoiding the application of biological activity prediction models to guide the optimization process. In summary, the aim was to implement an efficient but interpretable molecule generation framework to deliver promising lead molecules.

P26 – Pina, André

A computational study on the catalytic mechanism of Pdx2: a glutaminase containing the Cys-His-Glu triad

André F. Pina[1,2], Sérgio F. Sousa[1,2], Nuno M. F. S. A. Cerqueira[1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, BioSIM – Department of Biomedicine, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

Pdx2, the glutaminase subunit of the pyridoxal 5'-phosphate (PLP) synthase, is a key enzyme in the synthesis of PLP. It employs a non-canonical Cys-His-Glu triad to catalyze the deamination of glutamine to glutamate and ammonia – the source of the nitrogen of PLP. For this reason, Pdx2 is considered a novel and promising drug target against diseases such as Malaria and Tuberculosis, whose pathogens rely on this enzyme to obtain PLP. Therefore, the catalytic mechanism of Pdx2 was studied with atomic detail using the computational ONIOM QM/MM methodology with an 80/81 atoms QM region, which includes all catalytic relevant residues, treated at the DLPNO-CCSD(T)/CBS//B3LYP/6-31G(d,p):ff14SB level. The results demonstrate that the catalytic mechanism of Pdx2 occurs in six steps divided into four main stages: (i) activation of Cys87, (ii) deamination of glutamine with formation of the glutamyl-thioester intermediate, (iii) hydrolysis of the formed intermediate, and (iv) enzymatic turnover. The rate-limiting step of the complete catalytic mechanism is the hydrolysis of the glutamyl-thioester intermediate (18.2 kcal.mol-1), which closely agrees with the available kinetic data (19.1–19.5 kcal mol-1). The catalytic mechanism of Pdx2 differs from other known amidases in three main points: i) it requires the activation of the nucleophile Cys87 to a thiolate; ii) the hydrolysis occurs in a single step without formation of a second tetrahedral intermediate, and iii) Glu198 does not have a direct role in the catalytic process.

References:

Pina, A., Sousa, S. and Cerqueira, N.M.F.S.A. (2021), The catalytic mechanism of Pdx2 glutamine driven by a Cys-His-Glu triad: a computational study. ChemBioChem. Accepted Author Manuscript. DOI: 10.1002/cbic.202100555

P27 – Pires, Inês

New antitumor Ru-based compound derivatives optimized using *in silico* methods

Inês D. S. Pires[a], Tânia Morais[b], Miguel Machuqueiro[a]

[a] BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, Lisboa, Portugal.
[b] Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

Cancer has become one of leading causes of death around the globe, with female breast cancer as one of the most prevalent. Among the multiple types of breast cancer (BC) identified to date, the triple-negative (TN) subtype (lacking expression of estrogen and progesterone receptors and human epidermal growth factor receptor 2) is associated with higher aggressiveness and poor prognosis [1]. TNBC lacks targeted therapies and presents heterogeneous responses to treatment with traditional cisplatin-like drugs, in part due to the development of multidrug resistance (MDR). TM34 is a Ruthenium-based compound that has been suggested to be a more efficient and selective therapy than cisplatin [2]. More recently, new derivatives of TM34 have been developed with increased selectivity by adding peptide sequences that are recognized by receptor proteins from the FGFR family [3].

The main goal of this work is to study the interaction of several TM34 derivatives with a membrane model (POPC) and to calculate their membrane crossing energy profiles that can be used to estimate the membrane permeability coefficients. We used Molecular Dynamics simulations coupled with an Umbrella-sampling scheme to obtain the potential of mean force profiles, which allowed the calculation of the membrane permeability using the inhomogeneous solubility-diffusion model [4].

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References:

[1] Rakha EA, Ellis IO. Triple-negative/basal-like breast cancer: review. Pathology. 2009;41: 40–47.

[2] Lin K, Zhao Z-Z, Bo H-B, Hao X-J, Wang J-Q. Applications of Ruthenium Complex in Tumor Diagnosis and Therapy. Front Pharmacol. 2018;9: 1323.

[3] Machado JF, Machuqueiro M, Marques F, Robalo MP, Piedade MFM, Garcia MH, et al. Novel “ruthenium cyclopentadienyl”--peptide conjugate complexes against human FGFR (+) breast cancer. Dalton Trans J Inorg Chem. 2020;49: 5974–5987.

[4] Dickson CJ, Hornak V, Pearlstein RA, Duca JS. Structure-Kinetic Relationships of Passive Membrane Permeation from Multiscale Modeling. J Am Chem Soc. 2017;139: 442–452.

P28 – Pires, Manuel

SARS-CoV-2 membrane protein: from genomic data to structural new insights

Manuel N. Pires[1,2**], Catarina Marques-Pereira[1,2**], Nádia N. Pereira[1,2], Ana B. Caniceiro[1,2], Raquel P. Gouveia[1,2], Nícia Rosário-Ferreira[1,3], Irina S. Moreira[4,1,2*]

[1] University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal.
[2] University of Coimbra, Center for Innovative Biomedicine and Biotechnology, Coimbra, Portugal.
[3] Coimbra Chemistry Center, Department of Chemistry, University of Coimbra, Coimbra, Portugal.
[4] University of Coimbra, Department of Life Sciences, Calçada Martim de Freitas, Coimbra, Portugal.
[**] Co-Authors

SARS-CoV-2 genome codes four structural proteins and a wide range of non-structural and accessory proteins. The most common of the structural proteins is the Membrane protein. This is a highly conserved protein with three transmembrane domains that plays a pivotal role in both immune evasion and the formation of the viral envelope, which makes it an interesting study subject for SARS-CoV-2 therapeutics. In this work we propose an *in-silico* approach to obtain new structural insights for the M protein dimer and the possible effect of known mutations. No experimentally resolved structure is available for this protein, due to the difficulties associated with the crystallization process for this protein. As such, we used the predicted structure by AlphaFold for the assessment of membrane orientation and dimer structure. Furthermore, sequences for over 1.2M genomes and proteins were downloaded from Global Initiative on Sharing All Influenza Data. From these, 91 different single mutations were detected on residues predicted to play a part on the dimer interactions. Among those, we identified mutations in Variants of Concern (VOC) and Variants of Interest (VOI). Binding free energy differences were evaluated for dimer interfacial mutations to infer mutant protein stabilities. A few high-prevalent mutated residues were found to be especially relevant in VOC and VOI. This realization may be a game changer to structure driven formulation of new therapeutics for SARS-CoV-2.

P29 – Rocha, Juliana

Unraveling the catalytic mechanism of Threonine aldolase – a critical enzyme in the pharmaceutical industry

Juliana F. Rocha[1,2], Sérgio F. Sousa[1,2] and Nuno M. F. Sousa A. Cerqueira[1,2]

[1] Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
[2] UCIBIO – Applied Molecular Biosciences Unit, BioSIM – Department of Biomedicine, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

The catalytic mechanism of threonine aldolase (TA) was herein studied with atomic detail employing the computational ONIOM hybrid QM/MM methodology. TA is a PLP-dependent enzyme that catalyzes the retro-aldol cleavage of threonine into glycine and acetaldehyde, as well as the reverse reaction. This enzyme is currently seen as optimal approach for the regio selective synthesis of beta-hydroxy-alpha-amino acids (HAAs) which are very difficult to obtain by standard methods.

The results obtained show that the catalytic mechanism of TA occurs in three steps: (i) deprotonation of the hydroxyl group of EA1, (ii) covalent bond cleavage and (iii) hydrolysis. According to the Gibbs free energy profile, the rate-limiting step of the catalytic process is the covalent bond cleavage from which results the formation of acetaldehyde. The calculated energy barrier for this step is 16.7 kcal.mol-1, which agrees very well with the kinetic data available in the literature (17.4 kcal.mol-1).

All these results can now be used for the optimization of HAAs synthesis that serve as building blocks of several commercial drugs, such as antibiotics, immunosuppressants, and the anti-Parkinson's disease drug L-threo-3,4-dihydroxyphenylserine.

Keywords: Threonine aldolase (TA), Pyridoxal 5'-phosphate (PLP), beta-hydroxy-alpha-amino acids (HAAs), catalytic mechanism, QM/MM, ONIOM.

P30 – Rocha, Lucie

The effect of pH and bound palmitate on the dimerization of beta-lactoglobulin: a constant-pH molecular dynamics study

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

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

The most abundant protein in the bovine milk whey is the potentially allergenic beta-lactoglobulin (BLG) [1]. Several ligands can bind to BLG through a pH-regulated mechanism, suggesting not only a possible biological role as a transporter, but also a potential pharmacological role as a carrier of bioactive compounds [2]. BLG also presents a monomer-dimer equilibrium strongly affected by pH [3]. Previous experimental and computational studies have tried to understand these features and the underlying molecular phenomena, using standard methods that focus either on electrostatics or on structure dynamics separately, thus ignoring their interplay [3,4]. In this study we analyzed the effect of pH on the dimerization of BLG using constant-pH molecular dynamics. We studied both the apo and holo forms and the differences between them, including the pH-dependent profiles of the dimerization free energy.

References:

[1] Huby et al. (2000) Toxicol. Sci. 55: 235

[2] Niemi et al. (2007) Structure 15: 1413

[3] Mercadante et al. (2012) Biophysical J. 103:303

[4] Gutierrez-Magdaleno et al. (2013) J. Mol. Recognit. 26: 67

P31 – Rodrigues, Filipe

In silico study of peptidic dendrimers as transfection agents in DNA/RNA vaccines

Filipe E. P. Rodrigues[1], Tamis Darbre[2], Miguel Machuqueiro[1]

[1] BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[2] Department of Chemistry, Biochemistry and Pharmacy, Bern University, 3012 Bern, Switzerland
Email: ferodrigues@ciencias.ulisboa.pt

Peptide dendrimers are compounds related to dendrimers by virtue of their branched and polymeric structure, and to peptides and proteins because only amino acids are present in the dendrimer branches. In the past years peptide dendrimers with two or three amino acids in the branches have been reported to interact with biological molecules and cell membranes leading to good activity as antimicrobial agents, pathogenic biofilm inhibitors and superior vectors for DNA, siRNA and small oligonucleotides [1].

Recently, the use of such structures as vector molecules for mRNA and siRNA vaccines has been explored [2], which resulted in some promising peptidic dendrimers, namely MH13 and MH18, which are solely constituted by lysines and leucines, and contain two palmitoyl chains or a leucine tetrapeptide as hydrophobic cores, respectively. Furthermore, some mutations in MH18 from L- to D-amino acids, results in improved transfection and delivery efficiencies, as well as improved resistance to proteolytic degradation [2]. Despite these promising results in penetrating the target cells, being resistant to degradation, protecting and delivering their cargo (DNA and RNA), and not triggering a significant cytotoxic or immunogenic reaction, it is remarkable how little we know about the molecular mechanisms of their actions [3].

In this work, we will present our preliminary findings regarding the pH-dependent conformational space of MH18 and its variants composed of a different number (and position) of D-amino acids. We will present a specific protocol to build our dendrimers without bias, coupled to a robust initialization/equilibration scheme that prepares the peptidic dendrimers to be used in our state-of-the-art CpHMD simulations. We will present the pH titration behavior and perform several conformational characterizations, including the radius of gyration and the root mean square deviation (RMSD). These results are pivotal to help us choose the next steps of the project, where the interactions with lipid membranes and DNA/RNA are planned, to help experimentalists interpret their data, and to design new and improved peptidic dendrimers.

References:

[1] Reymond J-L, Bergmann M, Darbe T. ChemInform Abstract: Glycopeptide Dendrimers as *Pseudomonas aeruginosa* Biofilm Inhibitors. ChemInform. 2013. p. no–no. doi:10.1002/chin.201335206

[2] Heitz M, Javor S, Darbre T, Reymond J-L. Stereoselective pH Responsive Peptide Dendrimers for siRNA Transfection. Bioconjug Chem. 2019;30: 2165–2182.

[3] Filipe LCS, Campos SRR, Machuqueiro M, Darbre T, Baptista AM. Structuring Peptide Dendrimers through pH Modulation and Substrate Binding. J Phys Chem B. 2016;120: 10138–10152.

P32 – Santos-Pereira, Cátia

Bridging the gap between lactoferrin and V-ATPase through a multi-stage computational approach

Cátia Santos-Pereira[1,2,3], Juliana F. Rocha[3], Henrique S. Fernandes[3], Lígia R. Rodrigues[2], Manuela Côrte-Real[1], Sérgio F. Sousa[3]

[1] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Braga, Portugal
[2] Centre of Biological Engineering (CEB), Department of Biological Engineering, University of Minho, Braga, Portugal
[3] UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Porto, Portugal

Lactoferrin (Lf), a bioactive milk protein, exhibits strong anticancer and antifungal activities [1,2]. The search for Lf targets and mechanisms of action is of utmost importance to enhance its effective applications. A common feature among Lf-treated cancer and fungal cells is the inhibition of a proton pump essential for pH homeostasis called V-ATPase. Lf-driven V-ATPase inhibition leads to cytosolic acidification, ultimately causing cell death of cancer and fungal cells [2–4]. Given that a detailed elucidation of how Lf and V-ATPase interact is still missing, in this work we aimed to fill this gap by employing a multi-level computational approach. Molecular dynamics (MD) simulations of both proteins were performed to obtain a robust sampling of their conformational landscape, followed by clustering and protein-protein docking. Subsequently, MD simulations of the docked complexes and free binding energy calculations were carried out to evaluate the dynamic binding process and built the final ranking. This computational pipeline unraveled a putative mechanism by which Lf inhibits V-ATPase and identified key binding residues that will certainly aid in the rational design of follow-up experimental studies, bridging in this way computational and experimental biochemistry.

References:

[1] Acosta-Zaldívar M, Andrés MT, Rego A, Pereira CS, Fierro JF, Côrte-Real M. Human lactoferrin triggers a mitochondrial- and caspase-dependent regulated cell death in Saccharomyces cerevisiae. Apoptosis. 2016;21: 163–173. doi:10.1007/s10495-015-1199-9

[2] Pereira CS, Guedes JP, Gonçalves M, Loureiro L, Castro L, Gerós H, Rodrigues LR, Côrte-Real M. Lactoferrin selectively triggers apoptosis in highly metastatic breast cancer cells through inhibition of plasmalemmal V-H+-ATPase. Oncotarget. 2016;7: 62144–62158. doi:10.18632/oncotarget.11394

[3] Guedes JP, Pereira CS, Rodrigues LR, Côrte-Real M. Bovine milk lactoferrin selectively kills highly metastatic prostate cancer PC-3 and osteosarcoma MG-63 cells in vitro. Front Oncol. 2018;8: 1–12. doi:10.3389/fonc.2018.00200

[4] Pereira CS, Andrés MT, Chaves SR, Fierro JF, Gerós H, Manon S, Rodrigues LR, Côrte-Real M. Lactoferrin perturbs lipid rafts and requires integrity of Pma1p-lipid rafts association to exert its antifungal activity against Saccharomyces cerevisiae. Int J Biol Macromol. 2021;171: 343–357. doi:10.1016/j.ijbiomac.2020.12.224

P33 – Sequeira, João

Extension of the stochastic CpHMD method to the CHARMM36m force field

João G. N. Sequeira, Filipe E. P. Rodrigues, Telmo G. D. Silva, Pedro B. P. S. Reis, Miguel Machuqueiro

BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Constant-pH Molecular Dynamics (CpHMD) methods are nowadays essential to describe pH and the pH effects on the conformational space of biological systems [1]. The stochastic CpHMD method [2] has shown excellent performance over the years [1–3]. Until recently, our implementation of this method only supported the GROMOS 54A7, a force field compatible with the Generalized Reaction-Field (GRF) formalism to treat long-range electrostatic interactions, hence allowing for non-neutral systems [3]. Despite GROMOS popularity, one of the most used force fields is CHARMM36m, which is all-atom and particularly suited for protein, nucleic acids, and lipids simulations [4]. However, it uses mainly PME to treat the long-range electrostatics, which requires a system charge neutralization, a major limitation in its CpHMD implementation [3].

In this work, we present an extension to the stochastic CpHMD to include the CHARMM36m force field. In this preliminary benchmark study, we simulated two well-known proteins - lysozyme and thioredoxin - for which there is a significant amount of experimental data available [5]. These systems were thoroughly studied (pH range 1-12) and the final pKa values were compared between force fields and with the experimental data [5]. Please visit our Poster to see the performance of both force fields, the details on how to circumvent the PME neutralization step, and the code efficiency (ns/day).

References:

[1] Vila-Viçosa D, Reis PBPS, Baptista AM, Oostenbrink C, Machuqueiro M. A pH Replica Exchange Scheme in the Stochastic Titration Constant-pH MD Method. J Chem Theory Comput. 2019;15: 3108–3116. doi:10.1021/acs.jctc.9b00030

[2] Baptista AM, Teixeira VH, Soares CM. Constant-pH molecular dynamics using stochastic titration. J Chem Phys. 2002;117: 4184–4200. doi:10.1063/1.1497164

[3] Silva TFD, Vila-Viçosa D, Reis PBPS, Victor BL, Diem M, Oostenbrink C, et al. The Impact of Using Single Atomistic Long-Range Cutoff Schemes with the GROMOS 54A7 Force Field. J Chem Theory Comput. 2018;14: 5823–5833. doi:10.1021/acs.jctc.8b00758

[4] Huang J, Rauscher S, Nawrocki G, Ran T, Feig M, de Groot BL, et al. CHARMM36m: an improved force field for folded and intrinsically disordered proteins. Nat Methods. 2017;14: 71–73. doi:10.1038/nmeth.4067

[5] Pahari S, Sun L, Alexov E. PKAD: a database of experimentally measured pKa values of ionizable groups in proteins. Database. 2019;2019

P34 – Sequeira, João

HTVS protocol to identify non-covalent inhibitors of CRM1

João G. N. Sequeira[1], Wolfgang Link[2], Miguel Machuqueiro[1]

[1] BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[2] Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain

The protein function depends on its subcellular localization, as it determines the access to binding partners and enzymes that catalyze post-translational modifications. The best-studied export protein is the Chromosome Region Maintenance 1 (CRM1, also known as XPO1 or exportin 1), which is a transversal protein across all eukaryotic cells. Inhibition of CRM1 has long been idealized for the treatment of cancer and several viruses and it consists of binding a compound to the NES-binding groove to prevent the association of CRM1 with its cargo. However, all known inhibitors of CRM1 establish a covalent bond with Cys528, leading to high toxicities and impairing it's in vivo application.

Until recently, all known inhibitors bound covalently to the NES-binding groove. However, in a recent paper, Lei et al. presented the first inhibitor that was able to bind non-covalently to the NES-binding groove - the non-covalent CRM1 inhibitor 1 (NCI-1) [1]. Unfortunately, and despite the name suggesting otherwise, this inhibitor also binds covalently to Cys528 in the wild-type form. Nevertheless, NCI-1 ability to bind to CRM1 non-covalently serves as a proof-of-concept that such inhibitors are viable and can be developed.

With the intent of discovering non-covalent inhibitors of CRM1, a high-throughput virtual screen (HTVS) protocol was developed and implemented using a database provided by our collaborator, Prof. Romano Silvestri (Head of Medicinal Chemistry, Sapienza Univ., Italy). This HTVS was done using both the NES-binding groove from the crystallographic structure available in the PDB (ID: 6TVO) and two new conformations sampled from MD simulations, which we expect to be better descriptors of the apo structure. The top rank compounds were selected and are now being tested experimentally by our collaborator, Professor Wolfgang Link (University of Madrid, Spain).

References:

[1] Lei Y, An Q, Shen X-F, Sui M, Li C, Jia D, et al. Structure-Guided Design of the First Noncovalent Small-Molecule Inhibitor of CRM1. J Med Chem. 2021;64: 6596–6607.

P35 – Silva, Tomás

Improved realism of peptide-membrane simulations using a pH Gradient/CpHMD protocol

Tomás Silva, Miguel Machuqueiro

BioISI: Biosystems and Integrative Sciences Institute

The pH-low insertion peptides (pHLIP) are a family of pH-dependent membrane inserting peptides used as tumor biomarkers and drug delivery systems, by taking advantage of the peptide's ability to attach and fold into membranes in low pH microenvironments. The wt peptide possesses three states: unfolded in solution, adsorbed to the membrane, and inserted in a α-helix (pKtransition ≈ 6.0); and a key aspartate (Asp14 - pKa ≈ 6.0), whose (de)protonation events are essential to dictate the thermodynamics of each state [1]. Although widely used, the wt peptide lacks tumor specificity and fast state transition kinetics between the inserted and adsorbed states. Several variants have been designed to address these issues, of which variant 3 (Var3) showed interesting results [2].

The Var3 peptide has a similar key titrating residue (Asp13), and it has shown better transition kinetics than its predecessor, however, performing poorly as a biomarker (pKtransition ≈ 5.0) in liposome experiments [2]. Yet, in cell conditions, Var3 outperformed the wt. Among the various differences between cells and liposomes, the pH gradient is a crucial cell property that has been now implemented within the pH-replica exchange (pHRE) methodology [3]. The pH gradient is defined by assigning a constant internal pH value (∼7.2), while allowing the external pH to titrate, thus mimicking the pHLIP-cell microenvironment. A systematic study between both variants and liposomal and cell-like setups has shown that, on one hand, in the wt cell-like model, Asp14 interacts less with a nearby arginine (Arg11) than in the liposomal setup, therefore promoting pKa shifts to higher values (pKa ≈ 6.9) and corroborating the loss of performance in cell conditions. On the other hand, the Var3 peptide performed similarly in both setups, as a result of a trade-off between the strong interactions in the Asp13 electrostatic network. In conclusion, the implementation of a pH gradient setup within the pHRE method provided more accurate and realistic pKa calculations, aiding in the interpretation of experimental phenomena and, ultimately, in bridging the gap between *in silico* models and cells.

References:

[1] D. Vila-Viçosa, T. F. Silva, G. Slaybaugh, Y. K. Reshetnyak, O. A. Andreev, and M. Machuqueiro, “Membrane-induced pKa shifts in wt-pHLIP and its L16H variant”, Journal of chemical theory and computation, vol. 14, no. 6, pp. 3289–3297, 2018.

[2] D. Weerakkody, A. Moshnikova, M. S. Thakur, V. Moshnikova, J. Daniels, D. M. Engelman, O. A.Andreev, and Y. K. Reshetnyak, “Family of pH (low) insertion peptides for tumor targeting”,Proc.Natl. Acad. Sci. USA, vol. 110, no. 15, pp. 5834–5839, 2013

[3] T. F. Silva, D. Vila-Viçosa, and M. Machuqueiro, “Improved protocol to tackle the ph effects on membrane-inserting peptides”, J. Chem. Theory Comput., 2021

P36 – Sofia, Raul

USP7 inhibitors generative model

Raul Sofia, Maryam Abbasi, Joel Arrais

Univ Coimbra, Centre for Informatics and Systems of the University of Coimbra, Department of Informatics Engineering, Coimbra, Portugal

Time spent in drug design is a limiting factor in pharmacological research. In efforts to boost the discovery of new drug-like compounds, generative artificial intelligence models revealed themselves as a promising approach to de novo drug design. In this work we used
recurrent neural networks based on long short-term memory cells. The network was fed with valid molecules, in the form of SMILES, so it could capture the basics of molecule design. After accessing its capability of successfully generating compounds, transfer learning was
employed to adapt it to the design of USP7 inhibitors. USP7 is a deubiquitinase which has recently been object of studies for its role in protecting p53 from degradation in ubiquitin related pathways. Overexpression of this enzyme leads to oncogenesis and viral diseases.
The low existing data on USP7 inhibitors lead us to try an alternative approach, with the use of inhibitors of enzymes with similar catalytic sites.

P37 – Teixeira, Carla

Unveiling the catalytic mechanism of the Cys-Glu-Lys catalytic triads using the Nitrilase 2 as a case study

Carla S. Silva Teixeira; Nuno M. F. S. A. Cerqueira; Sérgio F. Sousa

Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Medicine, University of Porto, Porto, Portugal; UCIBIO – Applied Molecular Biosciences Unit, BioSIM – Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal

The Nitrilase 2 (Nit2) is a member of the nitrilase superfamily, which encompasses a group of thiol enzymes that share a common Cys-Glu-Lys catalytic triad in their active site. Those enzymes catalyze the condensation and hydrolysis of non-peptide carbon-nitrogen bonds. Although it is well established that all catalytic triad's residues are essential for catalysis the precise role played by each residue is not fully understood [1].

The Nit2 is an ω-amidase that has the capability to supply rapidly dividing cells (e.g., tumor cells) with anaplerotic carbon, but, surprisingly, it has also been associated with tumor suppressor characteristics, an observation that suggest that it might have separate catalytic and tumor suppressor domains [2].

This work describes with atomic- and electronic-level detail the catalytic mechanism of Nitrilase 2. This was accomplished by applying Quantum Mechanics /Molecular Mechanics (QM/MM), particularly the ONIOM approach [3]. The QM region was treated with density functional theory (DFT), and the MM region was defined by AMBER ff99SB force fields. The final free energies were calculated using single-point QM/MM calculations using the DLPNO-CCSD(T)/CBS method to calculate further the QM contribution [4].

The results obtained enabled a detailed understanding of the catalytic mechanism of Nit2 and of the exact role played by each catalytic triad residue. The structures and relative energies for all the intermediates and transition state structures were obtained. The results show that the catalytic triad's Cys and Glu amino acid residues play an active role during the catalytic process, while Lys plays only a secondary role. The results also show that catalytic mechanism involves four steps: 1- the nucleophilic attack of Cys to the reactant. 2,3-the formation of two tetrahedral enzyme adducts and 4-the hydrolysis of a thioacyl-enzyme intermediate. The rate limiting step of the catalytic process is the formation of the first tetrahedral intermediate [5].

This acquired knowledge can now be used to evaluate the role of the catalytic domain of Nit2 in the progression of different cancer cells.

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References:

[1] H. C. Pace, C. Brenner, Genome Biol. 2001, 2, REVIEWS0001.

[2] S. Jaisson, M. Veiga-da-Cunha, E. Van Schaftingen, Biochimie 2009, 91, 1066-1071.

[3] F. Maseras, K. Morokuma, J. Comput. Chem. 1995, 16, 1170-1179.

[4] G. Bistoni, I. Polyak, M. Sparta, W. Thiel, F. Neese, J. Chem. Theory Comput. 2018, 14, 3524-3531.

[5] C. S. Silva Teixeira, S. F. Sousa, N. M. F. S. A. Cerqueira, Chemphyschem 2021, 22,796-804.

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PyBindE: Development of a Simple Python MM-PBSA Implementation for Estimating Protein-Protein and Protein-Ligand Binding Energies

João N. M. Vitorino, Pedro P. B. P. S. Reis, Miguel Machuqueiro

BIOISI

There are several approaches for calculating binding free energies, with single-trajectory MM-PBSA being particularly useful when the relative energy differences between configurations are most significant. These methods also become a very popular option since they can be applied to a vast variety of systems, including protein-protein, protein-ligand and even protein-membrane binding events. MM-PBSA can generate binding energies over time, with various force-fields, and can be used to investigate the impact of protonation changes in a complex stability.

With this in mind, we have just developed PyBindE, a single-trajectory MM-PBSA Python implementation designed to be easily inserted into existing MD protocols [1]. Although PyBindE is in its early stages of validation it has already been applied to a few different systems of protein-protein and protein-ligand. Here, we provide a detailed description of the PyBindE implementation, how it can be easily installed and inserted into MD simulations pipelines and some of the results from on-going and published projects [2].

References:

[1] PyBindE: Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations in protein-protein and protein-ligand systems. Github; Available: https://github.com/mms-fcul/PyBindE

[2] Oliveira NFB, Rodrigues FEP, Vitorino JNM, Loureiro RJS, Faísca PFN, Machuqueiro M. Predicting stable binding modes from simulated dimers of the D76N mutant of β 2-microglobulin. Comput Struct Biotechnol J. 2021;19: 5160–5169.

List of Participants

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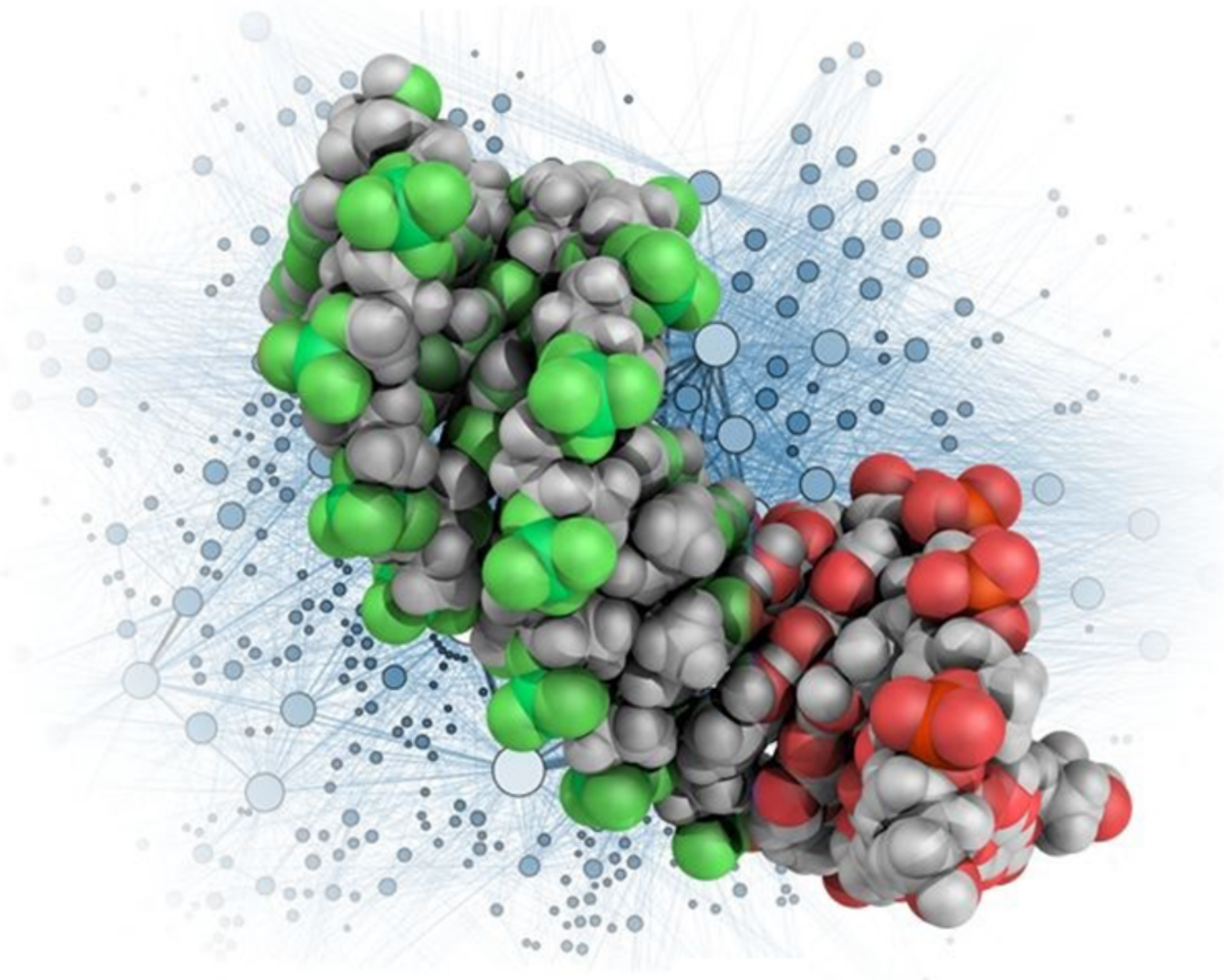

ORGANIZING COMMITTEE	
Irina Moreira	Centro de Neurociências e Biologia Celular, Universidade de Coimbra
Joana Mourão	Centro de Neurociências e Biologia Celular, Universidade de Coimbra
António Preto	Centro de Neurociências e Biologia Celular, Universidade de Coimbra
Salete Baptista	Centro de Ciências e Tecnologias Nucleares
Catarina Marques-Pereira	Centro de Neurociências e Biologia Celular, Universidade de Coimbra
LIST OF PARTICIPANTS	
Alexandre Oliveira	Universidade de Coimbra
Ana Beatriz R. Caniceiro	Universidade de Coimbra
Ana Catarina D. Machado	Universidade de Coimbra
Ana Teresa Gaspar	Universidade de Coimbra
André F. Pina	BioSIM, UCIBIO@REQUIMTE, FMUP
Andreia J. L. Fortuna	Faculdade de Ciências, Universidade de Lisboa
Bárbara B. Bruni	Faculdade de Ciências, Universidade de Lisboa
Bárbara Fernandes	Instituto de Tecnologia Química e Biológica António Xavier
Beatriz Bueschbell	Centre for Innovative Biomedicine and Biotechnology, University of Coimbra
Bruno Calçada	Faculdade de Ciências da Universidade de Lisboa
Bruno Victor	BioISI, FCUL
Carla S. S. Teixeira	UCIBIO@REQUIMTE - BioSIM, Univ Porto - Fac Med
Carlos Barreto	Universidade de Coimbra
Carlos H. Bezerra da Cruz	Instituto de Tecnologia Química e Biológica (ITQB)
Carolina C. Buga	ITQB-UNL
Cátia Santos-Pereira	UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto
Claúdio Soares	ITQB
Daniel A. R. Ramalhão	Universidade Porto
Daniel Rainho	Universidade de Coimbra
Diana Lousa	ITQB NOVA
Fábio G.Martins	BioSIM (UCIBIO@REQUIMTE)
Filipa G. Carvalho	Universidade de Coimbra
Filipe Rodrigues	Faculdade de Ciências da Universidade de Lisboa
Francisco Fernandes	INESC-ID
Guilherme Lopes Gabriel	Universidade de Coimbra
Henrique de V. Avila	Universidade de Coimbra
Henrique S. Fernandes	BioSIM, UCIBIO@REQUIMTE, Faculdade de Medicina da Universidade do Porto
Hugo A. L. Filipe	Instituto Politécnico da Guarda
Inês Pires	Faculdade Ciências Universidade Lisboa

Israa M. A. Aljnadi	Universidade de Lisboa
Jéssica L. Marques	Faculdade de Ciências da Universidade de Lisboa
Joana Pereira	Biozentrum, University of Basel
João Sequeira	Faculdade de Ciências da Universidade de Lisboa
João Vitorino	Faculdade de Ciências da Universidade de Lisboa
Juliana F. Rocha	BioSIM - Departamento de Biomedicina da FMUP
Lucie da Rocha	ITQB NOVA
Luis P. B. Araújo	Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa & Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa
Luiz Felipe Piochi	Universidade de Coimbra
Manuel N. Melo	ITQB NOVA
Manuel Pires	Universidade de Coimbra
Mariana Valério	ITQB NOVA, Universidade Nova de Lisboa
Marta S. P. Batista	Faculdade de Ciências da Universidade de Lisboa
Maryam Abbasi	University of Coimbra
Maryam Lotfi	ITQB NOVA
Miguel Machuqueiro	Faculdade de Ciências da Universidade de Lisboa
Nicia Ferreira	Universidade de Coimbra
Nuno Oliveira	Faculdade de Ciências da Universidade de Lisboa
Nuno Oliveira	Faculdade de Ciências da Universidade de Lisboa
Ona Šivickytė	Universidade de Lisboa
Paulo J. Costa	BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon
Paulo Martel	Universidade do Algarve
Pedro M. F. da Silva	Universidade de Coimbra
Pedro Moreira	ITQB NOVA
Pedro R. Figueiredo	Center for Neuroscience and Cell Biology
Pedro R. Magalhães	Centro de Neurociências e Biologia Celular, Universidade de Coimbra
Pedro Suzano	Faculdade de Ciências da Universidade de Lisboa
Raquel Gouveia	Universidade de Coimbra
Raul J. C. Sofia	Universidade de Coimbra
Ricardo D. G. da Cunha	CNC/CIBB - Universidade de Coimbra
Rita Ângelo	Faculdade de Ciências e Tecnologia da Universidade de Coimbra
Rita M. Fonseca	Universidade de Aveiro
Rita Melo	Centro de Ciências e Tecnologias Nucleares
Rita P. Magalhães	BioSIM@UCIBIO/REQUIMTE, FMUP
Rita Teixeira	Instituto de Tecnologia Química e Biológica António Xavier (ITQB Nova)

Rodrigo M. de S. Barriga Alves	Instituto de Tecnologia Química e Biológica António Xavier (ITQB)
Rui João de S. Loureiro	Centro de Química de Coimbra, Universidade de Coimbra
Sara Ferreira	Faculdade de Ciências da Universidade de Lisboa
Sérgio F. Sousa	UCIBIO/REQUIMTE - Univ Porto
Susana C. M. Parreiras	ITQB NOVA
Tatiana F. Vieira	BioSIM/FMUP
Taynara Pontes Franco	Faculdade de Ciências da Universidade do Porto
Tiago O. Pereira	Universidade de Coimbra
Tomás Silva	Faculdade de Ciências da Universidade de Lisboa
Vasco Santos	Universidade de Coimbra