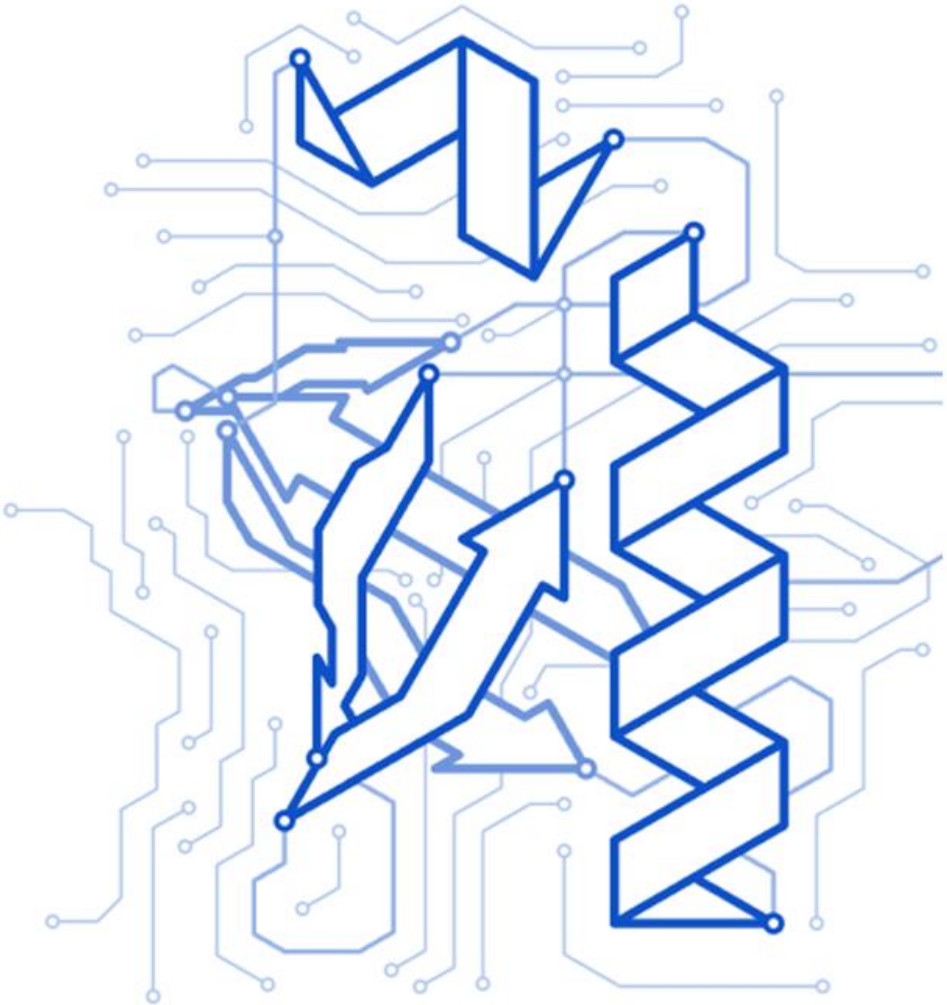
3D-BIOINFO-PT

THE PORTUGUESE COMMUNITY OF
STRUCTURAL BIOINFORMATICS RESEARCHERS

2nd Annual Meeting

December 18th 2023



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Mission and Goals

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.

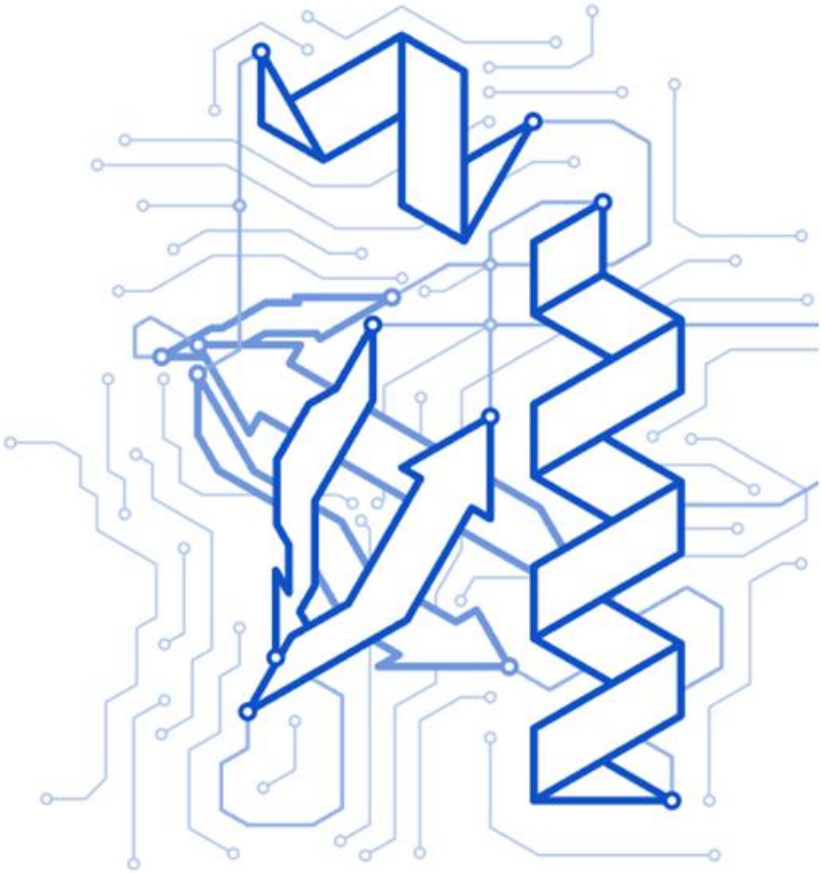
This scientific meeting aims to answer some of these questions. It intends to make known the best that is done in this Structural Computational Biology area in Portugal and, on the other hand, to reveal what Portuguese researchers based abroad are studying. In this way, we want to provide a space where 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.

Organizing Committee

Irina Moreira
Beatriz Caniceiro
Carlos Barreto
Catarina Marques-Pereira
Luana Afonso
Nícia Ferreira
Raquel Gouveia
Urszula Orzeł

Contents

PROGRAM



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Monday, December 18

08:30 – REGISTRATION

09:30 – Irina Moreira
Opening session

09:40 – Armindo Salvador
Presentation of the Portuguese Biophysical Society (SPBf)

9:45 – Oral Communication: Inês Pires
"Are protein pKa predictions affected by the choice of experimental structure?"

10:00 – Oral Communication: Andreia Fortuna
"Binding free energies using MM-PBSA calculations: an assessment using halogenated ligands."

10:15 – Oral Communication: Fábio Martins
"Lysinated multiwalled carbon nanotubes with carbohydrate ligands as a doxorubicin nanocarrier: AMD analysis."

10:30 – Keynote: Rita Melo
"Data driven and computational tools in radiopharmaceutical research."

11:10 – COFFEE BREAK AND POSTER SESSION I

11:45 – Keynote: Ricardo J. Ferreira
"Computer-aided drug discovery (CADD) in CROs and open-source software: new approaches to everyday problems."

12:30 – Oral Communication: Ana Beatriz Caniceiro

"Bridging Quantitative Database Insights with Machine Learning for CPPs."

12:45 – Oral Communication: João Sequeira

"Understanding P-gp inhibition by RuCp compounds using a Molecular Docking protocol."

13:00-15:00 – LUNCH BREAK

15:00 – Oral Communication: Nuno Oliveira

"Mechanism of the conformational switching in diphtheria toxin translocation (T-) domain triggered by protonation."

15:15 – Oral Communication: Sofia Sousa

"Design of novel KDM4C inhibitors for the treatment of triple negative breast cancer."

15:30 – Oral Communication: Tomás Silva

"CpH-MetaD: coupling wt-metadynamics and CpHMD in the study of RNA oligomers."

15:45 – Keynote: Hugo Penedones

"Scientific Computing meets AI."

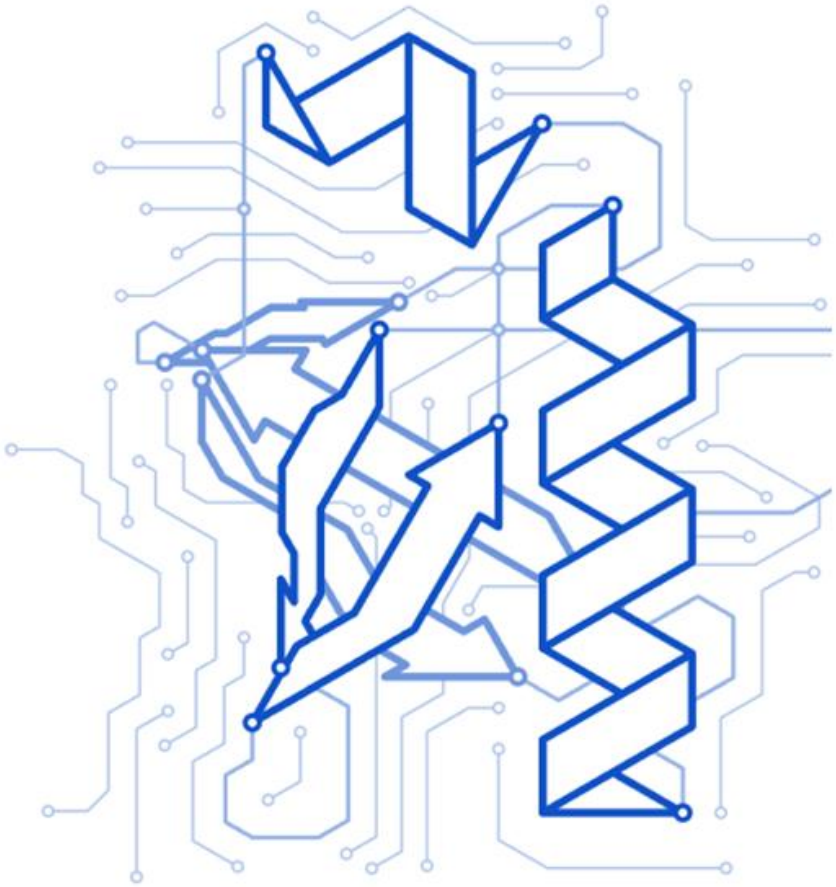
16:30 – COFFEE BREAK AND POSTER SESSION II

17:00 – Round Table: Hugo Penedones | Luís Valente | Nuno Cerqueira | Ricardo J. Ferreira | Irina Moreira

Charting trajectories: Navigating career paths in structural computational biology - a round table discussion on bridging academia and industry.

17:45 – CLOSING SESSION

KEYNOTE SPEAKERS



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K1 – Melo, Rita

Data driven and computational tools in radiopharmaceutical research

Rita Melo (RM) has got a degree in Chemical Engineering at New University of Lisbon in 2003 and completed her PhD in Chemistry at University of Lisbon in 2012. Thereafter, RM has been in ITQB/UNL as postdoctoral researcher and after six months at ITQB/UNL, she was invited to join again IST and she was successfully awarded a research contract by FCT. Since then, she has been working at radiopharmaceutical sciences group with the purpose of develop research activities on the design and preclinical evaluation of radioactive probes for molecular imaging by PET or SPECT and targeted radionuclide therapy, in tune with society priorities in the Life Sciences and Health domain. The line of activity she has driven is related to the development of recombinant virus-like particles in order to increase specificity for tumor receptors, in particular HER2-positive breast cancer cells, since 2014.
RM has started a recent research line at C2TN/IST/Portugal, currently managing and supervising 3 PhD student and 2 MSc students. The research line is growing rapidly in the last 3 years, with increased visibility at host institute. This research work led to the publication of 63 publications among which 40 peer-reviewed scientific articles; 15 proceedings at national/internacional conferences and 8 book chapters. RM's publications obtained over 469 citations (WoS) with an h-index of 13 (WoS). RM contributes to the peer-review process of journals, such as Chemosphere and International Journal of Molecular Sciences., She supervised 11 MSc theses, 3 graduation theses and 3 Erasmus fellowships. Due to her expertise in computational biophysics, RM has been invited to be part of HPC task force team from IST. Currently, she is team member of outreach group from C2TN and has contributed with dissemination activities as social media development.

K2 – Ferreira, Ricardo J.

Computer-aided drug discovery (CADD) in CROs and open-source software: new approaches to everyday problems

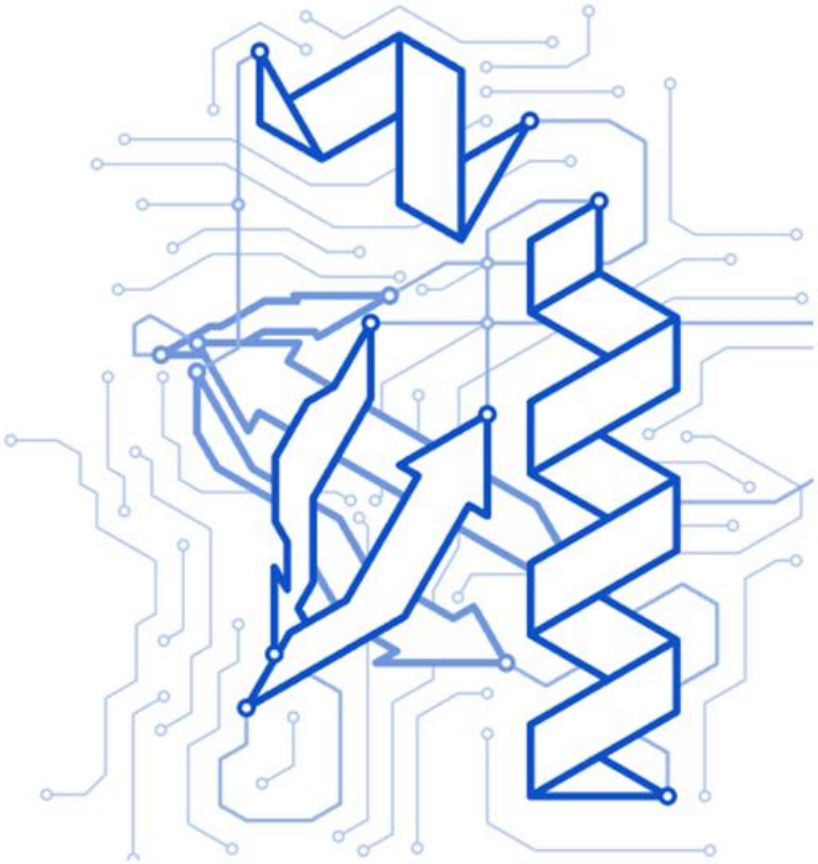
Doctor Ricardo J. Ferreira has obtained his B.Sc degree in Pharmaceutical Sciences in 2004 by the Faculty of Pharmacy, University of Lisbon. Following a short 5-year period working in as pharmacist, he successfully concluded his M.Sc in Pharmaceutical and Therapeutic Chemistry in 2011 and a Ph.D in Pharmacy in 2017. His computational studies on ABC transporters, most particularly P-glycoprotein, were pivotal for a greater understanding on substrate recognition while providing crucial mechanistic insights on the efflux cycle. His 2013 paper provided the first rationale concerning multiple binding sites within the P-gp architecture. Several studies on structure-activity relationships concerning P-gp modulation by natural products isolated from Euphorbia species were also published, as well as phytochemical studies that rendered, among others, a novel highly-potent spiro tetracyclic terpenoid and a diterpene with an unprecedented carbon skeleton containing a bicycle [2.2.1]heptane system as novel P-gp efflux modulators. From 2017 to 2020 he was a Postdoctoral Researcher at the Uppsala University, where he developed a novel computational method to predict antibiotic permeabilities through outer membrane porins of Gram-negative bacteria. He as currently published more than 30 articles, including 2 book chapters. Since 2020 he is the Head of Computational Chemistry at Red Glead Discovery AB, a Swedish company based in Lund, part of the dynamic Medicon Valley region and close to Copenhagen. He is the responsible for all R&D IT while leading a small team of computational chemists to provide support for all client projects, medicinal and peptide chemists.

K3 – Penedones, Hugo

Scientific Computing meets AI

Hugo Penedones is the co-founder and CTO of Inductiva Research Labs, a startup working at the intersection of AI and simulation of physical systems. Prior to Inductiva, Hugo worked as a Machine Learning researcher and engineer at Google DeepMind, where he was part of the initial AlphaFold team -- tackling the problem of protein structure prediction using Deep Learning methods. He also worked at Microsoft, EPFL, and the European Space Agency. He holds a degree in Informatics and Computing Engineering from the Faculty of Engineering of University of Porto.

ORAL COMMUNICATIONS



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OC1 – Pires, Inês

Are protein pKa predictions affected by the choice of experimental structure?

Inês D.S. Pires, Miguel Machuqueiro

Faculdade de Ciências, Universidade de Lisboa
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Abstract:
pH is an important factor to consider when studying proteins. Most, if not all, proteins contain titratable residues in which protonation can play an important activity and/or structural role. Aside from constant-pH MD, most methodologies do not take into consideration residue protonation changes and need the assignment of the most abundant protonation states at a given pH value.
There are multiple pKa predictors available nowadays, which allow for easy protonation state assignment, however, they all assume that the experimental structure used for the calculation is representative of the protein's conformational ensemble in water. This may not be the case for multiple factors, mainly due to the experimental conditions employed to determine these structures in the first place. These conditions are often significantly different from those used in computational protocols, which can lead to mismatches in the position and interaction of side chains, leading to deviations in the obtained pKa values. We studied how different initial structures impacted the pKa predictions of the same proteins. Multiple structures were selected, with varying resolutions, obtained under different crystallization conditions (pH and co-adjuvants), and solved with different methods (X-ray and NMR). The protein pKa values were calculated using the PypKa tool/server[1], as will be shown in the "pKa calculations of proteins using the PypKa tool" workshop, followed by a systematic analysis of the results. In this work, we will report our preliminary data on the benchmark described and assess how each of these properties is impacting the predictions. The results can provide pointers for making informed decisions when picking an initial structure for Molecular Docking, MD, and even CpH-MD simulations.

References:
[1] Reis PBPS, Vila-Viçosa D, Rocchia W, Machuqueiro M. PypKa: A Flexible Python Module for Poisson-Boltzmann-Based pKa Calculations. J Chem Inf Model. 2020;60: 4442–4448.

Acknowledgments:
The authors acknowledge financial support from Fundação para a Ciência e a Tecnologia through grants 2023.01155.BD and CEECIND/02300/2017 and projects UIDB/04046/2020 and UIDP/04046/2020.

OC2 – Fortuna, Andreia

Binding free energies using MM-PBSA calculations: an assessment using halogenated ligands

Andreia Fortuna[1,2] and Paulo J. Costa[1]

[1] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa and Chemistry and Biochemistry Department
[2] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon

Abstract:
Although halogenation of drug molecules is a common strategy to improve ADME (absorption, distribution, metabolism, and excretion) properties and membrane permeability, the description of halogens by empirical force fields (FF) is not sufficient to emulate their anisotropic electrostatic potential (ESP), since usually a negative charge is attributed to these atoms, impairing the establishment of halogen bonds (XBs) that arise from the positive region in the electrostatic potential of these elements (σ-hole). A very simple and efficient approach to overcome this problem is the introduction of an off-center point charge (EP). In past studies,[1,2] our group tackled the determination of hydration free energies of halogenated molecules using Poisson–Boltzmann Surface Area (PBSA) calculations and several EP implementations. In this method, PB radii are assigned to each atom, and for halogens, no optimized radii exist, with standard values often being shorter than the distance that the EP is placed. We therefore provided optimized halogen atomic PB radii (r_opt) that significantly decreased the mean absolute errors against the experimental values[1,2]; however, the impact of these new radii on the determination of ΔG_bind, which is paramount in drug design, remains unknown.
In this work, we studied the casein kinase-2 (CK2) complexes with halogenated ligands that are known to establish XBs with the target. This system has been assessed previously with PM6-DH2X[3] and MM-GBSA[4] calculations, the latter using the AMBER force field with the EP methodology but with non-optimized radii. Herein the impact of using the halogen r_opt as well as different EP implementations, PB setups, and sampling approaches in the determination of ΔG_bind using MM-PBSA calculations will be shown and discussed.

References:
[1] Nunes, R. et al (2019) JChemTheoryComput, 15, 4241
[2] Fortuna, A et al (2021) JChemInf.Model, 61, 3361
[3] Dobes, P. et al (2011) J. Phys. Chem. B, 115, 8581
[4] Ibrahim, M. et al (2012) J. Phys. Chem. B, 116, 3659

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FCT for SFRH/BD/146447/2019 (AF), IDB/04046/2020–UIDP/04046/2020 (BioISI), UID/DTP/04138/2019 (iMed.ULisboa), and 2021.00381.CEECIND (PJCosta). European Union for TWIN2PIPSA, GA 101079147

OC3 – Martins, Fábio

Lysinated multiwalled carbon nanotubes with carbohydrate ligands as a doxorubicin nanocarrier: A MD analysis

Fábio G. Martins, Sérgio F. Sousa

BioSIM

Abstract:

Doxorubicin is an anticancer drug widely used as a chemotherapeutic agent for the treatment of many forms of cancer. However, the clinical usefulness of this drug is challenged because of dose dependent side effects. To overcome these challenges, drugs are often delivered through nanocarriers to enhance their safety and efficacy.

Nanocarriers such as carbon nanotubes (CNTs) can shield drug molecules from degradation, provide a sustained drug release and also perform targeted drug delivery, leading to less side effects. CNTs have several benefits when compared with other nanocarriers, such as larger surface area, high loading capacity, prolonged release of drug, easy perforation and conjugation. However, the major issue of CNTs is the lack of solubility. These nanotubes tend to aggregate and accumulate in the tissues leading to various side effects. To overcome these issues the CNTs are functionalized to a variety of linkers and ligands to improve the solubility and the efficacy while reducing associated side effects.

This work used molecular dynamics simulations, to investigate the pH-dependent loading, arrangement, configuration, and release of Doxorubicin on carboxylated multi-walled CNTs (MWCNTs), and on MWCNTs functionalized with lysine, galactose, mannose and lactose. All systems were studied at both neutral and acidic pH. Using lysine as the linker is a cost-effective alternative for the functionalization of MWCNTs. Additionally, lysine residues are nontoxic, biocompatible and biodegradable. Carbohydrates such as galactose, lactose and mannose are used as ligands. These molecules also are nontoxic, biocompatible, and cost-effective. Furthermore, these ligands have the capacity to interact with cancer cell receptors.

Our results presented a dynamic molecular-level demonstration that the systems with acidic pH had lower adsorption when compared to their neutral pH counterparts. Furthermore, carbohydratemodified lysinated MWCNTs had higher adsorption than the pristine or carboxylated nanotubes. These results indicate that sugar-tethered multiwalled carbon nanotubes are promising tools for the delivery of doxorubicin to cancer cells.

OC4 – Caniceiro, Ana Beatriz

Bridging Quantitative Database Insights with Machine Learning for CPPs

Ana B. Caniceiro[1,2], António J. Preto[1,3], Francisco Duarte[1], Hugo Fernandes[5,6], Lino Ferreira[5,6], Joana Mourão[5] and Irina S. Moreira[4,5*]

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[2] PhD in Biosciences, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal;
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[4] Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal;
[5] CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal;
[6] FMUC - Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
E-mail: abcaniceiro@cnc.uc.pt

Abstract:
Short sequences of amino acids known as "cell-penetrating peptides" (CPPs) have demonstrated a remarkable ability to pass through cell membranes and introduce linked therapeutic cargoes to cells[1]. Although testing several CPPs *in vivo*/*in vitro* can be time-consuming and expensive[2], it is essential to design and test various CPPs to target cells or tissues to guarantee a high delivery efficiency and low toxicity. Computational tools such as machine learning (ML) approaches have received attention as faster and less expensive ways to build CPPs and predict uptake. Nevertheless, only databases offering qualitative information on CPPs are currently available[3]. As a result, most ML models in use today focus on categorization.
In response to these difficulties, we created an updated, publicly accessible database – POSEIDON database - with experimental quantitative absorption values for more than 2,300 entries and the physicochemical characteristics of 1,315 peptides. This extensive database contains the physicochemical characteristics of each CPP, including cell line, cargoes, uptake method and sequence, in addition to the quantitative uptake values. By leveraging this extensive database alongside cell line genomic features, we processed a dataset of over 1,200 entries to develop an ML regression model for predicting CPP uptake. Our results, which obtained a Pearson correlation of 0.87, Spearman correlation of 0.88, and r2 score of 0.76 on an independent test set, demonstrated the precision of the model in predicting peptide cell line uptake. The POSEIDON database represents a breakthrough in CPP research and development, particularly when combined with its strong ML predictor.

References:

[1] Xie, J. et al. Cell-penetrating peptides in diagnosis and treatment of human diseases: From preclinical research to clinical application. Front. Pharmacol. 11, 697 (2020).

[2] Gao, S., Simon, M. J., Hue, C. D., Morrison, B., 3rd & Banta, S. An unusual cell penetrating peptide identified using a plasmid display-based functional selection platform. ACS Chem. Biol. 6, 484–491 (2011).

[3] Agrawal, P. et al. CPPsite 2.0: a repository of experimentally validated cell-penetrating peptides. Nucleic Acids Res. 44, D1098-103 (2016).

Acknowledgements:

This work was supported by the European Regional Development Fund through the COMPETE 2020–Operational Programme for Competitive-ness and Internationalization and Portuguese National Funds via Fundação para a Ciência e a Tecnologia (FCT) [LA/P/0058/2020, UIDB/04539/2020, UIDP/04539/2020, and DSAIPA/DS/0118/2020, http://doi.org/10.54499/DSAIPA/DS/0118/2020). We further acknowledge the Faculty of Sciences and Technology of the University of Coimbra and the Center for Neuroscience and Cell Biology. A.J.P. and A.B.C. were supported by the FCT through PhD scholarships SFRH/BD/144966/2019 and 2022.12479.BD, respectively. J.M. was supported by a Junior Postdoctoral Research Contract (2021.03416.CEECIND - Individual Call to FCT Scientific Employment Stimulus 2021) granted by the FCT/MCTES through national funds.

OC5 – Sequeira, João

Understanding P-gp inhibition by RuCp compounds using a Molecular Docking protocol

João G. N. Sequeira[1], Ricardo G. Teixeira[2], Nuno F. B. Oliveira[1], Andreia Valente[2], Miguel Machuqueiro[1]

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

Abstract:
The search for more effective and selective drugs to overcome cancer multidrug resistance is urgent. As such, we paired a computational approach and a wet-lab protocol to study P-gp's inhibition using a new series of ruthenium-cyclopentadienyl ("RuCp") compounds[1]. In this work, we used molecular docking calculations to identify the R-site P-gp pocket as the preferred one for RuCp binding. A detailed analysis of the amino acid residues interaction network helped select key positions that were validated using site-directed mutagenesis experiments in P-gp.
In this presentation, we will showcase the intricacies of the developed methodology, highlighting the steps that led to the identification of those key residues for P-gp inhibition, showcasing a great example of the complementarity between *in silico* studies and "wet lab" experiments.

References:
[1] Teixeira RG, Salaroglio IC, Oliveira NFB, Sequeira JGN, Fontrodona X, Romero I, et al. Fighting Multidrug Resistance with Ruthenium–Cyclopentadienyl Compounds: Unveiling the Mechanism of P-gp Inhibition. J Med Chem. 2023 [cited 29 Nov 2023]. doi: 10.1021/acs.jmedchem.3c01120

Acknowledgments:
The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/00100/2020, UIDP/00100/2020, LA/P/0056/2020, UIDB/04046/2020 & UIDP/04046/2020 and grants CEECCIND/01974/2017, CEECIND/02300/2017, SFRH/BD/135830/2018, COVID/BD/153190/2023, 2021.06409.BD and 2022.10517.BD.

OC6 – Oliveira, Nuno

Mechanism of the conformational switching in diphtheria toxin translocation (T) domain triggered by protonation

Nuno F. B. Oliveira, Alexey S. Ladokhin, Miguel Machuqueiro

Faculdade de Ciências, Universidade de Lisboa

Abstract:
pH-dependent conformational transitions have been identified as key factors in the cellular entry of toxins and viruses[1,2]. A very promising system for these processes is the diphtheria toxin translocation domain (T-domain), which can translocate its catalytic domain out of an acidified endosome. Currently, the mode of action of this translocation process is still not clear, however, the trigger for T-domain membrane translocation seems to reside on the full protonation of key His residues[3].
In our work, we combined NMR data from our collaborators with constant-pH MD simulations to study the effects of pH on several residues of wt T-domain, the H223Q, H257Q, and E259Q single mutants, and the H223Q/H257Q double mutant. Combining all the pKa and protonation data from these 5 systems, we identified key features of the initial stages of the conformational transition of T-domain translocation. We also shed some light on the effects of the specific mutations in the activity of the translocation domain and revealed the existence of a tight latch between H223 and E259 that could modulate the triggering of the translocation domain.

References:
[1] Neale EA. Moving across membranes. Nat Struct Biol. 2003;10: 2–3.
[2] Mair CM, Meyer T, Schneider K, Huang Q, Veit M, Herrmann A. A histidine residue of the influenza virus hemagglutinin controls the pH dependence of the conformational change mediating membrane fusion. J Virol. 2014;88: 13189–13200.
[3] Rodnin MV, Vasques-Montes V, Kyrychenko A, Oliveira NFB, Kashipathy MM, Battaile KP, et al. Histidine Protonation and Conformational Switching in Diphtheria Toxin Translocation Domain. Toxins . 2023;15: 410.

Acknowledgments:
We acknowledge Fundação para a Ciência e Tecnologia for funding through projects UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017 and 2021.06409.BD.

OC7 – Sousa, Sofia

Design of novel KDM4C inhibitors for the treatment of triple negative breast cancer

Sofia M. Sousa[1-3], Fernanda Proença[2], Marta F. Costa[1], Sérgio F. Sousa[3]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, Braga, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
[2]Department of Chemistry, University of Minho, Campus of Gualtar, Braga, Portugal
[3]LAQV/REQUIMTE, BioSIM Department of Biomedicine, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, Porto, Portugal
E-mail: id10589@alunos.uminho.pt

Abstract:
Breast cancer is the most diagnosed cancer worldwide and the leading cause of cancer death in women. It is a clinically and genetically heterogeneous pathology with four distinct subtypes: luminal A, luminal B, HER2-positive and triple-negative. Triple-negative breast cancer (TNBC) accounts for about 15-20% of all breast cancers and is the most aggressive. TNBC is characterised by fast proliferation, high recurrence and mortality rates. Patients are mainly treated with conventional chemotherapy, associated to poor efficacy and severe side-effects, highlighting the need for new therapies. Targeted therapies based on epigenetic targets have been identified as promising treatment options for cancer, including TNBC. Lysine-specific demethylase 4C (KDM4C) catalyses the removal of lysine 9 and 36 di- and trimethyl marks from histone 3 lysine residues via an Fe2+-dependent dioxygenase mechanism, regulating chromatin structure and gene expression. When overexpressed, several tumorigenic processes that contribute to the aggressive phenotype of TNBC are promoted. The combined use of computational biochemistry, organic synthesis and molecular biology methods has proven to be a powerful strategy for the rational design of new therapies. This work describes the first computational step of a multidisciplinary work, aiming the development of selective and potent KMD4C inhibitors.
For the virtual screening, we started with the selection of 6 experimental structures of KDM4C from the Protein Data Bank. The ability of 4 scoring functions to reproduce the experimental poses was evaluated by protein-ligand docking with the GOLD software. In the ChEMBL database, 30 KDM4C inhibitors with IC50 values below 100 nM were identified. For each, 50 decoys were generated, resulting in a training set of 30 actives and 2000 decoys. The performance of different docking protocols in discriminating active molecules from decoys was evaluated by calculating the enrichment factor at 1% (EF1%) and the area under the curve (AUC). The best results were obtained with the 5FJH and 5FJK structures, and with the ChemPLP and ASP scoring functions. The 5FJK/ChemPLP combination presented an AUC of 70.2% and an EF1% of 6.8, while the 5FJH/ASP and 5FJH/ChemPLP combinations resulted in an AUC of 83.5% and 74.7%, respectively, and an EF1% of 6.8 for both. We selected the protocol/structure combinations with the highest discriminative ability in active/decoy recognition and accuracy in predicting the structures of the complexes.

With this work, molecules with potential activity for KDM4C can be discriminated from inactive molecules using the established protocol/structure combinations. Several libraries of commercial and in-house molecules (ca. 100.000) were screened using the three protocols, allowing the selection of a set of molecules with the best *in silico* performance. These identified molecules will be experimentally validated in the near future, using the appropriate biological models.

Acknowledgments:
This work was supported by national funds from Fundação para a Ciência e a Tecnologia UIDP/50006/2020 and 2022.11395.BD.

OC8 – Silva, Tomás

CpH-MetaD: coupling wt-metadynamics and CpHMD in the study of RNA oligomers

Tomás F.D. Silva, and Giovanni Bussi

SISSA

Abstract:
RNA molecules have a wide range of biological functions due to their highly flexible structures. Their flexibility stems from complex H-bonding networks defined by both canonical and non-canonical Watson-Crick (i.e. Wobble and Hoogsteen) base pairs. Some non-canonical base pair interactions may require (de)protonation events to either stabilize (i.e. Hoogsteen G-C+) or perturb (i.e. conformational switches/rybozimes) their H-bond networks.
Constant pH molecular dynamics (CpHMD) methods provide a reliable tool to describe the conformational space of dynamic structures and to obtain robust calculations of pH-dependent properties (i.e. pKa). However, pH-sensitive methods have rarely been explored in the field of nucleic acids, despite growing biological evidence concerning pH regulation of nucleic acid H-bond networks.
Here, we present an extension of the stochastic constant pH molecular dynamics (CpHMD) method[1] to ribonucleic acids (RNA) from the standard XOL3 AMBER force field[2]. In this work, we demonstrate the accuracy of the method to reproduce all RNA nucleoside pKa's and a set of relevant conformational properties.
Poly-U trimers and pentamers with a single central titrable A site (A or C) were also characterized for further method validation[3]. In order to overcome the high degrees of freedom of these oligomers, we have integrated a well-tempered (wt) metadynamics approach[4] into the CpHMD methodology (CpH-MetaD). The wt-metadynamics promotes the crossing of free energy barriers by adaptively scaling the deposited gaussians along the chosen collective variables (CVs), leading to a smoother convergence. The CpH-MetaD technique significantly expanded the sampled conformational space, allowing for more robust and accurate estimates of the pKa shifts with respect to the single nucleoside: 0 and 0.4 (A3mer and A5mer, respectively); 0~0.1 and 0.7 (C3mer and C5mer, respectively).
The predicted pKa values - A3mer: 3.55 (0.05); A5mer 4.0 (0.2); C3mer: 4.2 (0.05); C5mer: 4.9 (0.3) - and relative shifts are mostly in good agreement with experimental data[4]. Further analysis of nucleobase stacking and electrostatic interactions of phosphate groups clarified the balance between nucleobase shielding and strong negative electrostatic effects on the titrable site, which dictate the experimentally observed pKa shifts. This work highlights the robustness and accuracy of CpH-MetaD applied to RNA nucleotide monomers and oligomers, and the modularity of the nucleobases' proton binding affinity with increasing phosphate group content in the RNA backbone.

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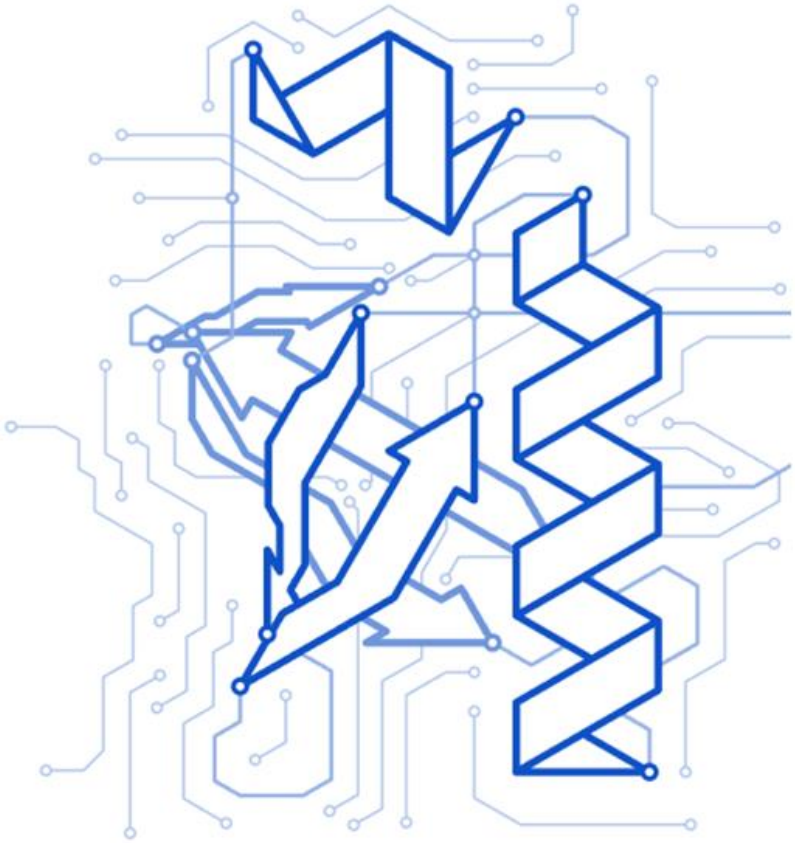

3D-BIOINFO-PT

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P1 – Abbasi, Maryam

Enhanced Prediction of Anticancer Drug Efficacy Using Deep Neural Networks

Instituto de Investigação Aplicada (i2A), Instituto Politécnico de Coimbra (IPC)

Abstract:
Due to the persistent rise in cancer rates, the global impact of this disease as a leading cause of death has intensified, underscoring the imperative for improved detection and treatment. In the age of personalized medicine, the focal objective is to integrate individual variability to tailor therapy and prevention strategies more precisely to each individual. Nevertheless, the challenge of accurately predicting tumor sensitivity to anticancer treatments persists. This study introduces two novel deep neural network models designed to forecast the impact of anticancer drugs on tumors, specifically through the determination of half-maximal inhibitory concentration (IC50). These models integrate biological and chemical data to capture pertinent features of genetic profiles and drug compounds, respectively. To predict drug responses in cancer cell lines, various deep learning methods, including Recurrent Neural Networks (RNNs) and Convolutional Neural Networks (CNNs), were employed. Initially, two autoencoders were pre-trained using high-dimensional gene expression and mutation data from tumors. Subsequently, this acquired genetic background was transferred to the prediction models, which yielded the IC50 value, a metric indicating the potency of a substance in inhibiting a cancer cell line. The results demonstrate the efficacy of the extracted deep representations in predicting IC50, surpassing the performance of previous state-of-the-art models.

P2 – Afonso, Luana Ferreira

Combining network biology and artificial intelligence to improve cancer drugs synergy predictions

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Abstract:
Cancer treatment often faces the challenge of drug resistance, a phenomenon that leads to relapse in the majority of patients. Addressing this issue, drug combination therapies have shown superiority over monotherapy, intending to reduce dosage and toxicity. High-throughput screening (HTS) technologies offer vast datasets for analyses. However, the impracticality of experimentally screening all drug combinations underscores the importance of computational methods. Despite the potential of deep learning models, a critical issue is their lack of interpretability. This study investigates the integration of domain knowledge with biological networks to enhance drug synergy prediction and understanding. Project goals include constructing an integrated knowledge graph and refining cancer drugs synergy predictions. This approach seeks to bridge the gap between computational efficiency and interpretability in advancing anticancer drug combination strategies.

Keywords: anticancer drugs; synergy; artificial intelligence; networks

Acknowledgements:
This work was supported by the European Regional Development Fund through the COMPETE 2020–Operational Programme for Competitiveness and Internationalization and Portuguese National Funds via Fundação para a Ciência e a Tecnologia (FCT) [LA/P/0058/2020, UIDB/04539/2020, UIDP/04539/2020, and DSAIPA/DS/0118/2020, http://doi.org/10.54499/DSAIPA/DS/0118/2020). We further acknowledge the Faculty of Sciences and Technology of the University of Coimbra and the Center for Neuroscience and Cell Biology. Luana Ferreira Afonso was supported by the FCT through PhD scholarship 2022.12975.BD.

P3 – Amorim, Ana Miguel

Drug Toxicity Prediction using Artificial Intelligence

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Abstract:
Drug discovery is a complex process involving sequential stages, such as target and lead discovery, pre-clinical evaluation, clinical trials, and subsequent registries. Ensuring safety and assessing the toxicity of compounds throughout these phases is crucial. However, identifying compounds with toxicity issues incurs significant resource, time, and financial costs, leading to their elimination from development.
In response to this challenge, computational methods for toxicity prediction have emerged as a promising solution. With the proliferation of big data and advancements in Artificial Intelligence (AI), various algorithms, particularly Machine Learning (ML) and Deep Learning (DL) techniques, have been optimized and applied to the field of drug toxicity to enhance efficiency. Exploiting the recent surge in algorithmic development and computational power is essential for constructing a more robust and powerful model for predicting drug compound toxicity.

Keywords:
Drug discovery; Toxicity prediction; Artificial intelligence; Machine Learning; Deep Learning.

Acknowledgements:
This work was supported by the European Regional Development Fund through the COMPETE 2020–Operational Programme for Competitiveness and Internationalization and Portuguese National Funds via Fundação para a Ciência e a Tecnologia (FCT) [LA/P/0058/2020, UIDB/04539/2020, UIDP/04539/2020, and DSAIPA/DS/0118/2020, http://doi.org/10.54499/DSAIPA/DS/0118/2020). We further acknowledge the Faculty of Sciences and Technology of the University of Coimbra and the Center for Neuroscience and Cell Biology.

P4 – Annunciato, Isabelly

An *In silico* prospection for new therapeutic strategies: the impact of nitrosamines in PAF regulation

Annunciato I., Nicodemo, I., Rafael, M., Roggero A., Cruz C.R. , Belchor, M., Santos Junior A.B., Antonio, G.F., Oliveira, M.A., Sousa S.F.,Toyama M.H.

BioSim/FMUP

Abstract:
Nitrosamines, identified as emerging contaminants, harbor both carcinogenic and oxidant traits, thus commanding significant attention in the realm of biochemical research. These compounds can alter the cellular membrane dynamics, engendering an escalation in the concentration of biologically active lipids, notably arachidonic acid, and the platelet-activating factor (PAF)[1]. The latter is a potent pro-inflammatory agent, intricately linked with the initiation of liver diseases. At the forefront of inflammation control is the enzyme lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase (PAF- AH), revered for its anti-inflammatory attributes. This enzyme adeptly moderates the plasma concentrations of PAF, a critical lipid mediator instrumental in the evolution and exacerbation of inflammatory phenomena[2].
Thus, our research endeavors to elucidate the potential of specific nitrosamines—N- Nitrosodiethylamine (NDMA) and N-Nitrosodimethylamine (NDEA)—in provoking hepatocyte dysfunction, influencing energy metabolism, and potentiating hepatotoxicity through their influence on the platelet aggregation factor.
Utilizing sophisticated Molecular Docking techniques and Molecular Dynamics, our investigation scrutinizes the capabilities of nitrosamines as potential non-selective inhibitors of PAF-AH enzymes. In this framework, NDMA and NDEA were assessed as binding agents, utilizing the lipoprotein associated PLA2 from Homo sapiens as a representative molecular model. To bolster the credibility of our data, we incorporated a comparative analysis utilizing a recognized covalent inhibitor of PAF-AH —paraoxon (DEP) —as a benchmark, thereby elevating the reliability and discernment of the acquired data.
Remarkably, these compounds did not demonstrate interactions with the enzyme when compared to paraoxon interaction. Future work will seek to identify the interaction between these compounds and other enzymes belonging to the membrane remodeling cycle.

P5 – Bruni, Bárbara

Pyrrolo[4,3,2-de]quinolinones as DNA-G4 targeting molecules

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Abstract:
Guanine-rich nucleic acids can form quadruplex structures (G4) which have important biological roles in genome regulation. Cumulative evidence supports the involvement of G4s in regulating gene replication, transcription, translation, and epigenetic events, where G4s function, in many cases, as protein recognition sites. Therefore, G4s have been explored as drug targets, particularly for the treatment of cancer, but also for parasitic, bacterial, or viral infections. Moreover, G4s have also been recently proposed as drug targets in neurological disorders[1]. Three G4-targeting molecules have reached clinical trials, but the design of clinically useful G4 ligands still faces some challenges. In a recent review of the literature on G4-ligands capable of binding to the c-MYC oncogene promoter region and inducing cancer cell death by downregulating this gene expression[2], we noticed that all these small molecules have differently organized aromatic cores, which are able to interact with G-quartets and stabilize the G4. Moreover, all molecules reaching clinical trials have four-fused aromatic rings, being potent G4 stabilizers, but not selective for c-MYC G4. Conversely, some c-MYC G4-ligands with less fused aromatic rings and consequently more flexible, were shown to have a preference for binding to this G4, without losing anticancer activity[2]. Other studies on the potential of indoloquinolines as G4-ligands have also shown that the position of side chains is important to discriminate between topologically different G4s, but the increased number of protonable groups in the molecule, although increasing potency, is detrimental for selectivity to G4 structures[3,4]. These conclusions are in agreement with another study suggesting that the desired selectivity/specificity may be achievable by sacrificing binding affinity[5]. The above insights led us to propose that an aromatic core of 3 fused rings, such as the pyrrolo(4,3,2-de)quinolinone core, decorated with different side chains containing H-bond acceptor/donor groups could present the right balance between binding strength and selectivity. The different reactivity and associated reaction mechanisms will be tentatively explained by DFT calculations while the ligand-G4 interactions will be subsequently evaluated by *in vitro* studies. MD simulations will then be performed to understand the binding and further guide the synthesis of the libraries.

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P6 – Batista, Marta

Evaluation of the most appropriate bilayer membrane size for the simulation of AQPs

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Abstract:
Aquaporins (AQPs) are responsible for permeating solutes accross membranes, and can be divided into two subgroups depending on their permeability profile and sequence homology: strictly selective for water (classical aquaporins) and permeable to water and small solutes, such as glycerol (aquaglyceroporins). The identification of AQP function modulators has proven to be difficult due to the low target druggability and suitability of the commonly used assays and computational approaches employed to address this problem. The crystallographic structures of AQPs often present inadequate conformations due to the crystal packing, which impairs the binding of modulator candidates. Despite attempts to use computational approaches to mitigate the mentioned problems[1], recently a fundamental issue appeared: are we using the most adequate models and parameters to simulate these membrane protein channels? In this work, we initially focused on studying the impact of different membrane sizes on AQPs stability and function. For this, we set up long Molecular Dynamics (MD) simulations with the recently solved structure of the aquaglyceroporin hAQP7 (6QZI)[2] in a POPC lipid bilayer of different sizes (200, 300, 400, and 500 lipids) with the Amber ff14SB forcefield. This protocol helped us identify the best lipid embedding environment that fully stabilizes the conformational space of the protein, and which will be useful in the future for the identification of structural features that regulate the function of hAQP7.

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P7 – Ferreira, Sara

Computational insights into the structure of an important grapevine serine protease (VviSBT4.19) involved in the defense against *P. viticola*

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Abstract:
In 2022, Portugal ranked as the fifth largest wine producer in the European Union[1], with the grapevine industry constituting a pivotal component of the national economy. Unfortunately, grapevine pathogens, such as *Plasmopara viticola*, the causative agent of the downy mildew disease, pose a considerable threat to grape and wine production. Addressing this challenge without resorting to extensive pesticide use is imperative due to associated environmental and health risks. To achieve this, understanding how tolerant grapevine species recognize and mount a successful defense response against *P. viticola* is crucial. Previous research has highlighted the role of serine proteases, particularly grapevine subtilase VviSBT4.19, in establishing the incompatible grapevine-*P. viticola* interaction[2]. This protease exhibits high expression in resistant genotypes and post-pathogen infection. However, a comprehensive understanding of protease activity modulation by *P. viticola* has been delayed by the absence of a robust structural model. Here, we constructed a computational model for VviSBT4.19 using an approach based on homology modeling (Modeller)[3]. Molecular Dynamics (MD) and Constant-pH MD simulations were then conducted to assess the overall stability of the model structure. Our analyses focused on the active site and the relative positioning of the catalytic triad residues to evaluate their topology and functionality. Additionally, we also investigated the interactome of VviSBT4.19 and its role in plant immunity. This work aims to fill the existing gap in understanding the molecular details of grapevine defense against *P. viticola*, potentially paving the way for the development of innovative strategies to enhance disease resistance in grapevine cultivars.

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P8 – Gomes, André

The interplay of pH, hydrogen bonds, and halogen bonds in modulating Cobimetinib membrane permeability

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Abstract:
Membrane permeability plays a crucial role in many biological processes, directly affecting the ADMET properties of drugs, thus being paramount in rational drug design. Several factors influence the permeability of small molecules, including size, charge, and lipophilicity. The pH[1] and the existence of chemical groups prone to establish specific noncovalent interactions are also core parameters that impact this biological event. Among noncovalent interactions the impact of the ubiquitous hydrogen bond is usually addressed, however, the less studied halogen bond might also impact membrane permeability owing to the existence of halogen-membrane recognition phenomena mediated by those interactions[2]. In this communication, we report a study of the permeation mechanism of Cobimetinib for which pH, hydrogen, and halogen bonding are important. This compound is an anti-cancer drug used to treat patients with melanoma and comprises a halogen atom (iodine), a hydroxyl, and a titrable group (Lewis base) in its structure. We used QM calculations to generate the RESP charges taking into account the anisotropic features of the halogen through the implementation of an extra-point (EP) model. CpHMD and US-CpHMD simulations in a POPC membrane model were performed to compute the permeability coefficients for both Cobimetinib models by applying the Inhomogeneous Solubility Diffusion Model (ISDM). In this work, we studied two different biological conditions, considering the normal cell physiological pH (~7.4) and tumor microenvironment acidic conditions (pH~6.2), aiming to evaluate the impact of tumor acidosis in the compound membrane passive diffusion while also considering the role of halogen bonds in the overall process.

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P9 – Gomes, Inês

RNA Tetraloop Structural Stabilization in a Natural Deep Eutectic Solvent

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Abstract:
RNA is inherently unstable and prone to degradation. Traditional approaches to mitigate RNA degradation primarily revolve around storing RNA at ultra-low temperatures, below −20 °C. This strategy, however, entails a significant investment of time and resources. Deep eutectic solvents (DESs)[1], more specifically, natural deep eutectic solvents (NADESs), have recently garnered significant attention as promising biomolecular stabilizers. NADESs are formed by mixing two or three components, generally osmolytes, at a particular molar ratio, to produce an eutectic mixture with a melting point lower than any of its individual constituents.
Herein, we investigated the potential stabilization of a small RNA tetraloop, GAGA, in water and a betaine-glycerol-water (Bet:Gly:Wat) (1:2:ζ) NADES with different water contents (ζ = 0, 1, 2, 5, 10), using molecular dynamics simulations. This work aims to explore the potential structural stabilization of an RNA tetraloop in a NADES and the associated molecular mechanisms.

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P10 – Gouveia, Raquel

The study of Aminomethyltransferase Mutations in Nonketotic Hyperglycinemia Disease

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Abstract:
NonKetotic Hyperglycinemia (NKH) is a rare autosomal recessive metabolic disorder characterized by complex multisystemic manifestations. NKH etiology is characterized by mutations in the Glycine Cleavage System (GCS) complex, a complex of P, T, L, and H proteins. As its name suggests, GCS breaks down glycine, an amino acid and neurotransmitter, by cleaving it into smaller molecules. Glycine is both an excitatory and inhibitory neurotransmitter and has an excitatory effects when bound to the NMDA receptor. Elevated glycine levels in the central nervous can cause seizures, hypotonia, and lethargy. The breakdown of excess glycine is necessary for the normal development and function of nerve cells in the brain. In this study, we conducted Molecular Dynamics Simulations and structural and functional analyses to investigate mutations in the T protein and their association with different severities of the disorder, including mild, attenuated, and severe forms. Our study reveal significant perturbations in the stability of key structural elements, such as α8, α9, and β6, and alterations in hydrogen bonding networks pivotal to T-protein catalysis.

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P11 – Guerra, Rita

Is the binding affinity of Lewis base anti-tumor drugs affected by charge?

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Abstract:
Multidrug resistance (MDR) in cancer remains a major cause of chemotherapy failure being characterized by resistance to a wide array of anticancer drugs and multi-factorial development including inversion of pH gradient and decreased drug uptake[1]. Many conventional antineoplastic drugs are Tyrosine Kinase Inhibitors (TKIs), which are often weak bases with basic pKa values (ranging from 7.5 to 9.0) that can undergo deprotonation, and in their neutral form, can passively cross the cell membrane[1-3]. However, with the development of MDR, there is an acidification of the extracellular tumor microenvironment (TME; pH = 6.0–6.8) which translates into a stronger protonation of these drugs and, therefore, a reduction of cellular drug uptake since ionized drugs do not efficiently cross the cell membrane[1-4]. Additionally, a large pH gradient relative to the acidic lumen of lysosomes is generated (pH = 4.5–5) allowing the entrapment of hydrophobic weak base drugs within lysosomes via protonation in the acidic lumen of this compartment[1]. Without circumventing these MDR-related barriers, the TKIs will not reach the target and perform their antitumor activities. To improve the low membrane permeability coefficients of TKIs in the acidic environment, we could just remove the offending cationic group. However, such a strategy can lead to changes in the binding affinities of the new TKIs in their targets. Therefore, in this work, we investigated the influence of different protonation states on the binding affinity of sunitinib and nintedanib to their corresponding TK targets using CpHMD and molecular docking calculations.

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P12 – Marques-Pereira, Catarina

ProLigResDB: A new Protein-Ligand Interaction Database

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Abstract:
Protein–ligand Interactions (PLI) are fundamental for understanding molecular mechanisms in biology and drug design. The Protein Data Bank (PDB) contains valuable experimental data on several PLIs. However, systematically classifying residue-level information on protein chains interacting with specific ligands remains a challenge. To address this problem, ProLigResDB was developed, leveraging PDB experimental data to classify protein chain residues as interacting or non-interacting, based on distance metrics between residues and ligands of interest. Our ProLigResDB database represents a comprehensive repository of experimentally derived information. For each protein chain that interacts with a specific ligand, ProLigResDB provides residue-level classification, distinguishing between interacting (≤ 5 Å) and non-interacting (> 5 Å) residues. To enhance its utility, we incorporated protein residue descriptors along with ligand descriptors extracted from MORDRED. This integration offers a multi-layered perspective, enriching the understanding of PLIs at both residue and ligand levels. ProLigResDB is a pioneering resource that provides unprecedented access to detailed residue-level information derived from the experimental PDB data.

Keywords: ProLigResDB; Protein-Ligand Database; Protein-Ligand Interactions; Protein Chain Residue Interactions; Protein Data Bank.

Acknowledgements:
This work was supported by the European Regional Development Fund through the COMPETE 2020–Operational Programme for Competitive-ness and Internationalization and Portuguese National Funds via Fundação para a Ciência e a Tecnologia (FCT) [LA/P/0058/2020, UIDB/04539/2020, UIDP/04539/2020, and DSAIPA/DS/0118/2020, http://doi.org/10.54499/DSAIPA/DS/0118/2020). We further acknowledge the Faculty of Sciences and Technology of the University of Coimbra and the Center for Neuroscience and Cell Biology. C. Marques-Pereira and A.G. Preto were supported by the FCT through PhD scholarships 2020.07766.BD and SFRH/BD/144966/2019, respectively.

P13 – Martins, Daniel

A Machine Learning-based alternative for the identification of Candidate Genes for Schizophrenia

Daniel Martins; Maryam Abassi; Conceição Egas; Joel P. Arrais

CISUC; CIBB

Abstract:
Introduction: Heritability studies have indicated a substantial contribution of genetic factors to the manifestation of Schizophrenia (SCZ). However, it still lacks definitive hypotheses to explain its etiology. This uncertainty forces SCZ diagnosis to rely on broad frameworks, which, in turn, hinders the collection of robust case-control cohorts. As a paradigmatic example of this issue, one of the largest cohorts on SCZ was drawn from the Swedish Hospital Discharge Register, for which there have been reported relatively high SCZ misdiagnosis rates (ranging from 6% to 19%).
Methodology: This large-scale case-control Whole-Exome Sequencing dataset from the Swedish population was reduced to 18,970 variants with significant associations to the phenotype. A gene-annotation-based Machine Learning model was built and trained on the entire data to enhance the within-group distinctions. The outlying cases, associated with a higher likelihood of misclassification, were excluded from the subsequent analysis. A second iteration of the model was trained to analyze the capability of the refined dataset to identify candidate genes for Schizophrenia.
Results: After excluding samples on a proportion equivalent to the misdiagnosis rate associated with a narrow SCZ definition (19%), the classification model presented an AUC value of approximately 0.81 for the test set. This degree of separability between cases and controls would be in line with Schizophrenia heritability estimates from the existing literature. Further analyses point to a greater weight of contribution of genes on glucose metabolism pathways.
Discussion: Our findings suggest that the reported misdiagnosis rates for Schizophrenia may be reflected on its case-control cohorts. This could compromise the performance of purely genetic studies relying on such datasets. In comparison to the original data, the genetic profiles leading to the distinction of cases and controls in the refined dataset were more consistent across different replications of the training process. This supports the presented strategy to define more informative subsets from large-scale cohorts and opens the door to reusing such data with adapted machine learning approaches for complex disease research.

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P14 – Martins, Gabriel

Wild Type and Mutated α-synucleins' Structure from Coarse-Grained Molecular Dynamics Simulations

Gabriel Martins, Hugo Martiniano, and Nuno Galamba

BioISI Faculdade de Ciências da Universidade de Lisboa

Abstract:
α-synuclein (α-syn), a 140-amino acid intrinsically disordered protein (IDP) mainly expressed in the central nervous system, has been implicated in the etiology of Parkinson's disease (PD). Despite the increasing use of *in silico* methods to study PD and other neurodegenerative diseases, the development of force fields (FFs) that accurately capture the behavior of IDPs remains a challenging endeavor. These proteins are characterized by large ensembles of conformations that most FFs seemingly fail to correctly describe. Furthermore, these proteins can adopt elongated conformations that require the use of prohibitively large simulations boxes in all-atom molecular simulations. In face of these limitations coarse-grained (CG) FFs along with enhanced sampling methods emerge as potentially viable alternatives. Herein, we assessed the structure of the monomer of α-syn, through molecular dynamics simulations, using two different CG FFs, MARTINI3 (M3) and SIRAH2 (S2). Various simulation factors were probed, including, the dependence of the protein's initial structure, protein-solvent interactions, enhanced sampling (replica exchange), and long-range electrostatic interactions. Our results indicate that enhanced sampling methods may not be fundamental to capture CG model α-syn structural conformations, and no dependence of the method used to compute long-range electrostatic interactions is found. However, the results do show a dependence of the initial structure for M3. A discussion around the putative ability of these FFs to describe missense mutations, such as A30P, A53T and E46K, involved the familial forms of PD, is provided.

Keywords: Molecular dynamics; coarse-grained; MARTINI3, SIRAH2; REST2; A-53T; A30-P; E-46K, α-synuclein.

P15 – Nunes, Fernando

Room for improvement in the initial Martini 3 parametrization of peptide interactions

Fernando Neiva Nunes, J. K. Spinti, Manuel N. Melo

Multiscale modeling lab - ITQB NOVA

Abstract:
Coarse-grained simulation rose in popularity during the last decade, and one of the most widespread forcefield is the general purpose forcefield Martini. That currently is in its third iteration, Martini 3, and has been used with great success in various applications. However, this work focus on inconsistencies of the initial Martini 3 forcefield regarding peptide interactions in biologically relevant systems. These inconsistencies can be explained by the incorrect depiction of hydrophobic / hydrophilic balance in this initial parametrization. Firstly, coiled coil dimers were represented using heptad repeats which are drivers of coiled coil, not only by a mere polarity matching but leverages from the spatial fitting of each helix's side chains into inter-residue gaps in the other helices. However, Martini 3 failed to have any kind of propensity to dimerize, even when dimerized preferred to cluster around polar/charged residues. Transmembrane peptides, using WALPs a series of single pass transmembrane peptides which has a well described tilt angle behavior in response to membrane thinning. Although Martini 3 was able to represent the tilting behavior of the WALPS, the smaller WALPs were consistently ejected from the membrane showing a preference for the adsorbed. With this report we show there is still much work left in protein parametrization in Martini3 and these results may be used as steps towards a more robust forcefield.

P16 – Oliveira, Tiago

Genotype-phenotype correlations in PMM2-CDG through molecular dynamics simulations

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Abstract:
Phosphomannomutase 2 (PMM2) deficiency (PMM2-CDG), the most common N-glycosylation disorder, leads to hypoglycosylation of several proteins, resulting in a multisystem involvement[1]. This disease can be caused by a huge variety of mutations that could impact PMM2 function and, consequently, result in a broad phenotypic spectrum, raising the challenge of making genotype-phenotype correlations[2-4].
In order to understand the correlation between different mutations in PMM2 and their impact on the phenotype in PMM2-CDG, several three-dimensional structures of PMM2 were modelled. Subsequently, molecular dynamics simulations were performed to analyse the impact of these mutations at both energetic and molecular levels. This assessment was mainly focused on PMM2 dimerization, folding, substrate-binding and structure stability.
Our results predict the effect on PMM2 function, mainly on the dimerization free energy and protein folding destabilization. In particular, it was possible to determine a blueprint of the energetical contribution of the residues that compose the dimerization interface towards the dimerization free energy. Moreover, molecular analysis revealed that p.Asp223Asn/WT and p.Pro113Leu/p.Pro113Leu mutations induced drastic conformational changes, possibly leading to protein denaturation. Additionally, p.Arg162Trp/p.Arg162Trp mutation resulted in the displacement of the substrate (glucose-1-phosphate) from the binding pocket.

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P17 – Orzeł, Urszula

GS-SMD server: the structural insight into the processing of substrates by γ-secretase

Urszula Orzeł[1,2], Paweł Pasznik[1], Przemysław Miszta[1], Marcin Lorkowski[1],Szymon Niewieczerzał[1], Jakub Jakowiecki[1], Irina S. Moreira[2], Sławomir Filipek[1]

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Abstract:
IGS-SMD server (https://gs-smd.biomodellab.eu/) is a platform that allows to investigate the cleaving and unfolding of the γ-secretase (GS) substrates. GS, a membrane protein complex consisting of 4 subunits, is known to play an important role in Alzheimer's disease (AD). Due to its proteolytic activity, it is responsible for cleaving numerous peptide substrates (currently over 170 known substrates). Among them, investigated the most is Amyloid Precursor Protein (APP), trimming of which leads to the production of pathogenic amyloid β (Aβ) peptide. Despite of the growing knowledge about GS, the mechanism of cleaving APP or any other substrate is not yet fully understood. Our server provides very efficient tool to shed a light on the structural details underlying the GS:peptide interactions. For that, it uses the steered molecular dynamics (SMD) simulations of pulling the substrate in GS active site towards the next expected cleavage site. The simulations are performed in an implicit water-membrane environment, that significantly reduces the computational cost (2–6 h per job), maintaining the reliability of the results. The obtained trajectories are presented in an interactive way, along with the extensive analysis including SMD force, energy and interactions between GS and a substrate. The server is also applicable to study the effects of mutations. We tested several known APP mutations and observed a good alignment with the experimental results, showing that the GS-SMD results are reliable and the server can be used to study the processing of substrates by γ-secretase. The structural knowledge can be further used to search for specific GS modulators that are promissing in the treatment of AD.

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P18 – Rodrigues, Filipe

In silico study of cationic peptide dendrimers as pH-dependent vectors for siRNA

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Faculdade de Ciências, Universidade de Lisboa

Abstract:
Transfection is a pivotal process in both the industry and therapeutic fields, with recent applications to nucleic acid vaccines. However, most transfection processes exhibit problems regarding cytotoxicity and immunogenicity[1]. Recently, researchers have explored the use of peptide dendrimers as vectors for siRNA molecules[2] These branched molecules, when composed of cationic and hydrophobic amino acid residues, can interact strongly with nucleic acids and biological membranes, making them effective carriers, for example, for siRNA[2]. As a result, several dendrimers, including MH18, MH13, and MH47, have been identified. These dendrimers consist exclusively of lysine and leucine residues and contain hydrophobic cores composed of two palmitoyl chains or a leucine tetrapeptide. Their different protonation states at physiological and low pH values play a crucial role in interacting with negatively charged nucleic acids and escaping endosomal entrapment. Despite numerous experimental results, our understanding of the overall molecular mechanisms and the factors governing specific properties of these molecules still needs to be improved[3].
In this study, we present our findings on the application of our advanced CpHMD methodology to investigate the pH-dependent conformational space of MH18, MH13, and MH47. We performed the simulations in the aqueous phase and in interaction with a lipid bilayer to assess how conformation and protonation are affected by pH in different environments. We found that these dendrimers become highly charged upon acidification, and a high charge density is essential to trigger membrane destabilization. These findings are in excellent agreement with the experimental data and help explain why some dendrimers, like MH47 which has four fewer titratable lysines, are unable to undergo efficient endosome evasion and complete the transfection process. This evidence provides further understanding of the mode of action of these peptide dendrimers and will be pivotal for the future design of new sequences with improved transfection capabilities.

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P19 – Sequeira, João

Stochastic titration CpHMD with AMBER14SB: an approach to look for non-opioid analgesics

João G. N. Sequeira[1], Adrian E. Roitberg[2], Miguel Machuqueiro[1]

[1] BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
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Abstract:
Acid-sensing ion channels (ASICs) are voltage-insensitive, proton-gated cation channels, widely expressed across the central and peripheral nervous systems, that are involved in diverse physiological processes ranging from nociception to brain ischemia[1]. ASICs are activated by extracellular acidosis and ligands can act as antagonists or agonists for the channel's affinity for protons[2]. To discover ASIC activity modulators, one must understand the pH effects on the conformational rearrangement of the protein channel that leads to a change in the cation membrane permeability.
Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to describe pH and its effects on the conformational space of biological systems[3]. The stochastic titration constant-pH Molecular Dynamics (CpHMD) method has shown excellent performance over the years[3,4]. Until recently, our implementation of this method only supported the GROMOS 54A7[3] and the CHARMM36m force fields[4]. We are currently working on extending this method to support the AMBER 14SB force field, an all-atom force field particularly suited for studying disordered proteins and membrane channels. However, since the charge parameterization procedure of this force field allows side chain charge propagation to the main chain, we propose a small modification to the official ff14SB atomic partial charges to make them st-CpHMD-compatible. Here, we will present our most recent results using this protocol.

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P20 – Suzano, Pedro

Multidrug resistance reversal through ABC transporter modulation

Pedro M. S. Suzano, Daniel J. V. A. dos Santos

CBIOS / Universidade Lusófona

Abstract:
Cancer has been showing a rapid increase in the number of cases, especially in industrialized countries. Furthermore, despite the constant advancements in the different types of therapeutic options targeting cancer such as chemotherapy, immunotherapy and radiotherapy, the lack of effective treatment options namely due to acquired resistance remains a major health challenge. This resistance can be owed to a multitude of factors, but when it comes to chemotherapy, the multidrug resistance seen in cancer cells is due mainly to the alterations of the tumor microenvironment, reduced drug import and upregulation of ABC transporters leading to increased efflux of anticancer drugs[1].
When subjected to pharmacological pressure, cancer cells benefit greatly from overexpressing exporters of the ABC transporter family. As anticancer drugs are removed from the cell interior, they become unable to reach their target and perform the intended action. As such, mediating the overexpression or activity of these transporters is a viable strategy in designing anticancer drug strategies.
Recently, our group has found novel allosteric binding sites while probing the P-gp protein, where P-gp inhibitors can bind[2]. Later, in the middle of 2023, researchers Chow et al found a similar binding site[3]. In their work, it is not clear if the published molecule is directly modulating P-gp or if a different mechanism (or combination of mechanisms) is at work. The clarification of this protein's modulation would be greatly beneficial in cancer therapy development. In addition to these reports, in the beginning of December, Moesgaard et al published their results where compounds targeting the same binding area showed potential for P-gp inhibition without toxicity[4]. Thus, in our work, we propose the rational design and development of novel inhibitors through the modulation of these newly described hotspots of the ABC transporter family.

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P21 – Vitorino, João

Studying the role of Asp2.50 protonation in GCPRs activation/deactivation mechanism

João N. M. Vitorino, Carlos A. V. Barreto, Irina S. Moreira, Miguel Machuqueiro

BioISI - FCUL

Abstract:
GPCRs are arguably the most important family of drug targets in the human body, being targeted by more than 35% of all drugs currently on the market[1]. Since these systems are difficult to study experimentally, the mechanistic details about their activation and interaction with partners remain somewhat elusive. Despite this, some possible conformational switches have been identified in GPCRs such as a2ar, b2ar, amongst others. Some of these conformational switches include changes in protonation of residue Asp2.50, a known key residue related to the mechanism of activation/deactivation[2,3].
In this work, we provide insights into the protonation states of Asp2.50, by devising a Linear Response Approximation (LRA) protocol that allows the sampling of both the two protonation states of this Asp residue and the conformational ensembles of the GPCRs. We performed MD simulations (Gromacs + CHARMM36m) of 5 GPCR systems, in both their active and inactive conformations and with their Asp2.50 in its protonated and deprotonated forms. The pypka tool was used to calculate the pKhalf values of this key residue in each ensemble conformation, followed by an LRA protocol to estimate the macroscopic pKa values. The final pKa estimations at both activation states for all GPCRs studied, provide a simple way to identify protonation changes upon activation for these systems. In the near future, we plan to use CpHMD simulations on the most promising systems, even in the presence of their binding partners, to obtain a detailed molecular description of GPCR activation.

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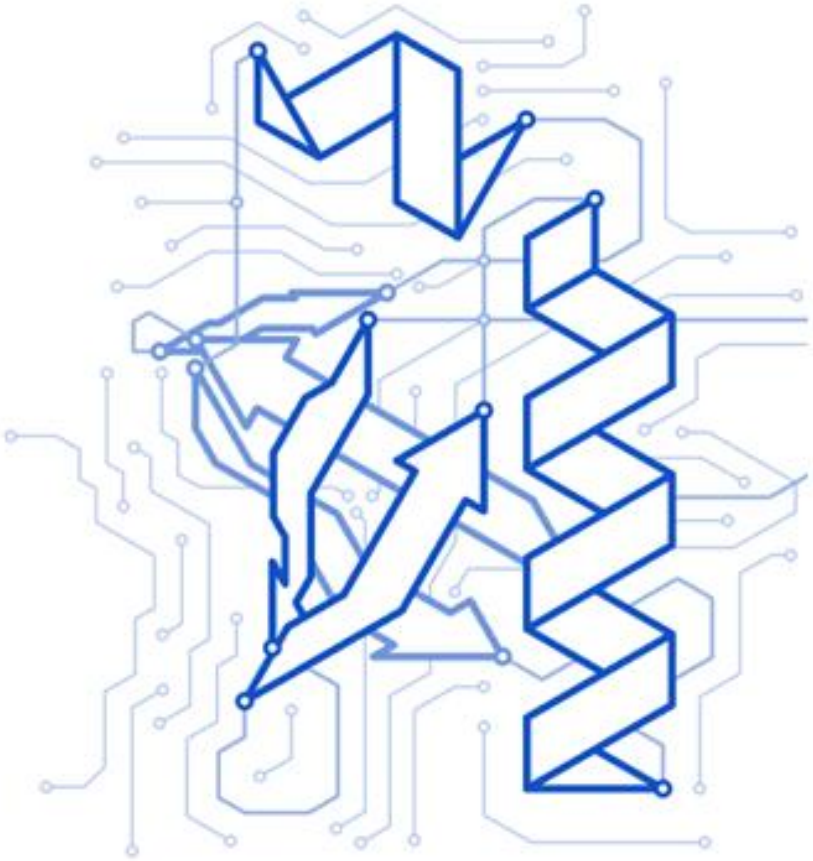
ROUND TABLE



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December 18th 2023

RT1 – Penedones, Hugo

Hugo Penedones is the co-founder and CTO of Inductiva Research Labs, a startup working at the intersection of AI and simulation of physical systems. Prior to Inductiva, Hugo worked as a Machine Learning researcher and engineer at Google DeepMind, where he was part of the initial AlphaFold team -- tackling the problem of protein structure prediction using Deep Learning methods. He also worked at Microsoft, EPFL, and the European Space Agency. He holds a degree in Informatics and Computing Engineering from the Faculty of Engineering of University of Porto.

RT2 – Valente, Luís

Business manager, strategist, and entrepreneur with a drive for using innovative and creative strategies to solve complex problems.
Forbes 30 under 30 for Science and Healthcare, with a background in Computer Science, I have spent several years focusing on innovation and improving strategies in companies from Tech to Healthcare. My passion for entrepreneurship led me to create my first business at 18, founding and operating several ventures specializing in optimized demand prediction, lean operations, and design for growth. A deep belief in the power of Technology as the core driver for democratizing Personalized Medicine had me co-founding iLoF: a fast-growing deep tech company using AI to build a cloud-based library of disease biomarkers and biological profiles.
Currently on a mission to accelerate adequate treatments for millions of patients living with complex, heterogeneous diseases around the globe.

RT3 – Cerqueira, Nuno

Nuno M. F. Sousa A. Cerqueira is a senior computational chemist at BIAL since 2018.
Applies computational methods, together with machine learning and artificial intelligence, to study therapeutic targets and develop new drugs. Uses data analytics to streamline the drug discovery process in the pharmaceutical industry.
Accomplished scientist with a PhD in computational chemistry/biochemistry, >98 papers published and 10 book chapters (h-index 28). Assistant professor and invited professor at University of Trás-os-Montes and Alto Douro, Universidade Católica Portuguesa, Minho University and Faculty of Sciences of Porto University. Full-time researcher at Faculty of Sciences and Associated laboratory REQUIMTE, during 10 years, where he led two 2 FCT projects and worked as a team member in another 5. During this period of time, he founded 2 spin-offs, 1 research group (Biomolecular SIMulations Research Group – www.BIOSIM.pt) and supervised 8 pHD students, 6 MSc students and 3 BsC students. He was also a reviewer in 60 international journals and developed several scientific software applications.
Areas of expertise: Computer-aided drug design; Computer simulations, Bioinformatics and Chemoinformatics; Machine learning, Artificial intelligence and Data science; Programming languages; Data analytics.

RT4 – Ferreira, Ricardo J.

Doctor Ricardo J. Ferreira has obtained his B.Sc degree in Pharmaceutical Sciences in 2004 by the Faculty of Pharmacy, University of Lisbon. Following a short 5-year period working in as pharmacist, he successfully concluded his M.Sc in Pharmaceutical and Therapeutic Chemistry in 2011 and a Ph.D in Pharmacy in 2017. His computational studies on ABC transporters, most particularly P-glycoprotein, were pivotal for a greater understanding on substrate recognition while providing crucial mechanistic insights on the efflux cycle. His 2013 paper provided the first rationale concerning multiple binding sites within the P-gp architecture. Several studies on structure-activity relationships concerning P-gp modulation by natural products isolated from Euphorbia species were also published, as well as phytochemical studies that rendered, among others, a novel highly-potent spiro tetracyclic terpenoid and a diterpene with an unprecedented carbon skeleton containing a bicycle [2.2.1]heptane system as novel P-gp efflux modulators. From 2017 to 2020 he was a Postdoctoral Researcher at the Uppsala University, where he developed a novel computational method to predict antibiotic permeabilities through outer membrane porins of Gram-negative bacteria. He as currently published more than 30 articles, including 2 book chapters. Since 2020 he is the Head of Computational Chemistry at Red Glead Discovery AB, a Swedish company based in Lund, part of the dynamic Medicon Valley region and close to Copenhagen. He is the responsible for all R&D IT while leading a small team of computational chemists to provide support for all client projects, medicinal and peptide chemists.

RT5 – Moreira, Irina

Irina Moreira completed a PhD in Chemistry, after which she went to pursue postdoctoral training in the field of Structural Computational Biology and joined the laboratory of Prof. Weinstein, who was recognised worldwide for his work in GPCRs, and the director of the Institute for Computational Biomedicine at WCM in NYC, USA. Studies during this period resulted in four high-profile publications. She returned to Portugal under the FCT's initiative "Investigator Ciência 2008", aimed at attracting excellent expatriated researchers back to the country.

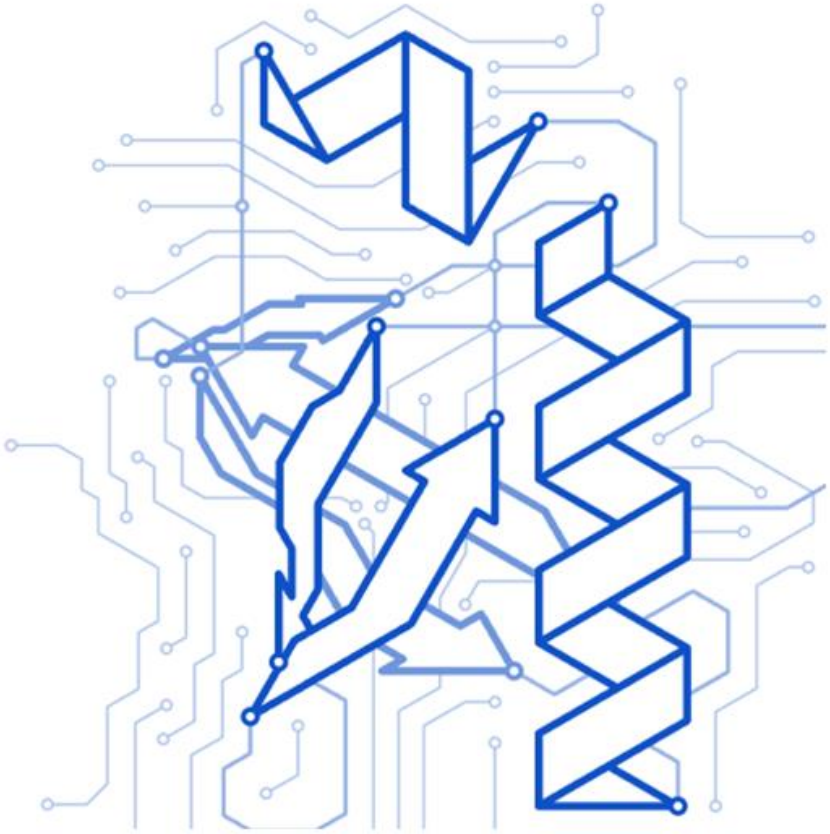
In 2015, she was awarded a highly competitive and prestigious Investigator Starting Grant following an international FCT call, with the goal of establishing a new generation of scientific leaders in Portugal. This grant allowed her to start her own research group, Data-Driven Molecular Design, at the University of Coimbra (UC). In early 2016, she took sabbatical leave to join Prof. Alexandre Bonvin's laboratory (the Netherlands) under an internationally renowned H2020-MSCA-IF grant. This collaboration resulted in the development of a widely used tool for hotspot detection (SpotON) and several new publications.

In 2019, she was awarded a highly competitive tenure-track position as an Assistant Professor at the UC's Life Sciences Department. To accept this, she had to decline another University of Coimbra (UC) tenure-track analogous position and a researcher fix-term contract awarded in an FCT's Scientific Employment Stimulus International call.

Her capacity to seamlessly connect several disciplines can be clearly attested by the different research areas of scientific journals, including Bioinformatics, Chemistry, Biochemistry/Molecular Biology, Computer Science, Pharmacology, and Mathematics. She co-authored over 85 scientific publications with a diverse team of 246 colleagues(s). Of these, 28 publications/book chapters feature international collaborations (most notably from the USA and the Netherlands) because of the fruitful international network that she established, nurtured, and expanded. Peer recognition and her capacity to attract competitive national and international research funding are well demonstrated and support her forward-looking mindset and the potential to conduct ground-breaking work beyond the state-of-the-art (a highly cited researcher).

Irina Moreira works on the intersection of two crucial science domains, Structural Bioinformatics and Computer Science, opening new horizons in the development of innovative integrative computational tools to better characterize and predict the biochemical properties and mechanistic understanding of key biological systems. She has now co-founded a start-up called PURR.AI, which aims to revolutionise the G-protein-coupled receptor field by utilising advanced Artificial Intelligence techniques to analyse massive amounts of existing data on this important family of pharmaceutical proteins. In doing so, the company seeks to identify significant functional and structural relationships that can lead to breakthroughs in the field.

LIST OF PARTICIPANTS



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