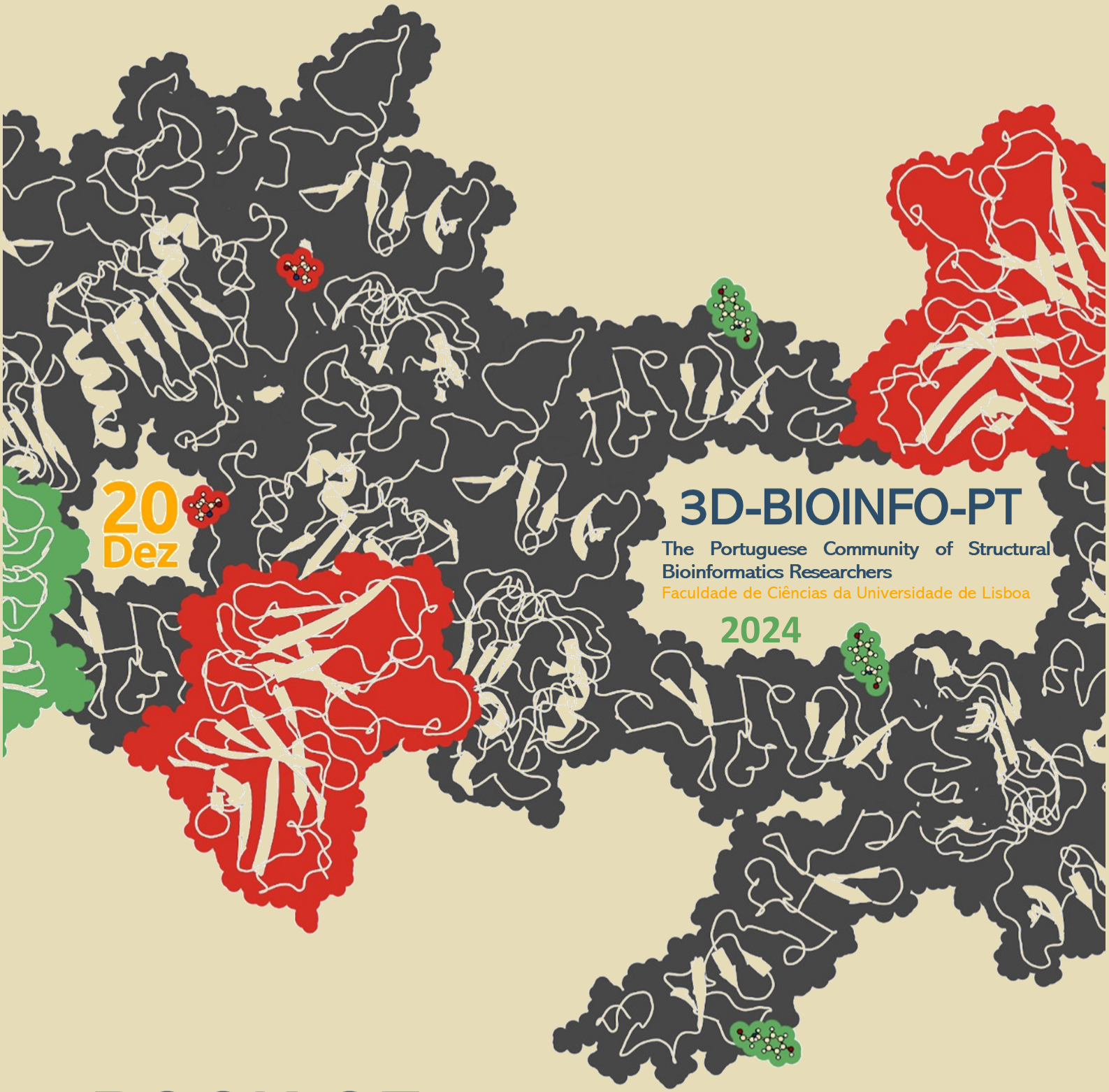

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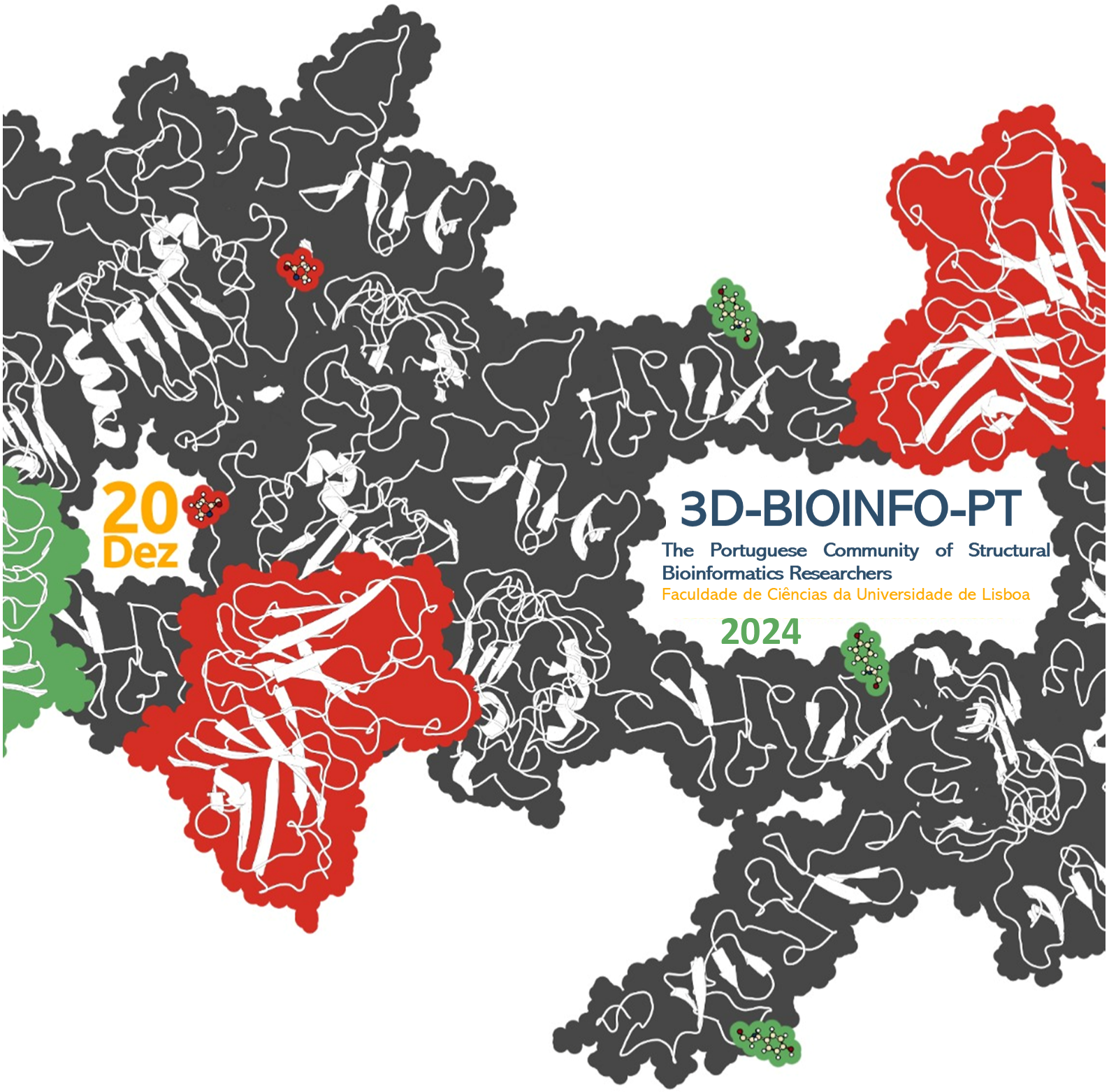
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KEYNOTES

Keynote Presentation 1

Alexandra Carvalho

The INSIGHT enzyme discovery and engineering platform

Alexandra T. P. Carvalho, Daniel F.A.R. Dourado, Jane Mueller, Christine Flemming, Matthew Boyd, Jill Caswell, Stefan Mix, Derek J. Quinn, Thomas S. Moody

Biocatalysis offers a powerful alternative to counteract the waste producing and greenhouse gases emission manufacture processes that still dominate the economy. Biocatalysis further development can lead to a more sustainable world for future generations.Here we will present the discovery and engineering of enzymes of two industrially relevant enzymes classes. Using our INSIGHT platform, we identified from metagenomic sources a smart targeted library of carbonyl reductases. Enzymes were obtained from functional motif annotation using structure-based (physics and machine learning) methods. Top hits were able to convert challenging substrates with conversions up to 80 % at screening scale and were further engineered to be tolerant to different solvent mixtures. The second example is about a milder and greener approach for the synthesis of chiral sulfoxides [1].

[1] Wu, Jingyue, Anselmi, Silvia, Carvalho, Alexandra T. P., Caswell, Jill, Quinn, Derek J, Moody, Thomas S., Castagnolo, Daniele. Expanding the toolbox of Baeyer–Villiger and flavin monooxygenase biocatalysts for the enantiodivergent green synthesis of sulfoxides. Green Chemistry, 2024, 26(15), 1463-9262.

Keynote Presentation 2

Tomás Silva

Titration of RNA systems: introducing CpH-metadynamics

Tomas F.D. Silva[1], Giovanni Bussi[1]

1 Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italia

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 canonical and non-canonical base pairs. Some non-canonical base pair interactions require (de)protonation events to stabilize or perturb 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. pK_a). However, pH-sensitive methods have rarely been explored in the field of nucleic acids, despite growing biological evidence concerning pH regulation of certain motifs' H-bond networks. In this work, we present an extension of the stochastic CpHMD method to RNA from the standard XOL3 AMBER force field and demonstrate the accuracy of our method to reproduce pK_a's of RNA oligomers. Poly-U trimers and pentamers with a single central titrable site were characterized for method validation. A well-tempered (wt) metadynamics approach was integrated into the st-CpHMD methodology (CpH-MetaD) to tackle the systems' high degrees of freedom. The CpH-MetaD technique significantly expanded the sampled conformational space, allowing for more robust and accurate estimates of the oligomers' pK_a shifts to the single nucleoside pK_a's: 0 and 0.4 (A3mer and A5mer); 0 0.1 and 0.7 (C3mer and C5mer). The predicted pK_a values - A3mer: 3.55 (0.1); A5mer 4.0 (0.2); C3mer: 4.7 (0.1); C5mer: 5.0 (0.2) - and relative shifts are in good agreement with experimental data. Nucleobase stacking and electrostatic interactions with phosphate groups clarify the intramolecular phenomena that dictate the experimentally observed pK_a shifts. This work highlights the robustness and accuracy of CpHMD/CpH-MetaD applied to RNA oligomers and the pK_a sensitivity to phosphate group content in the RNA backbone.

Keynote Presentation 3

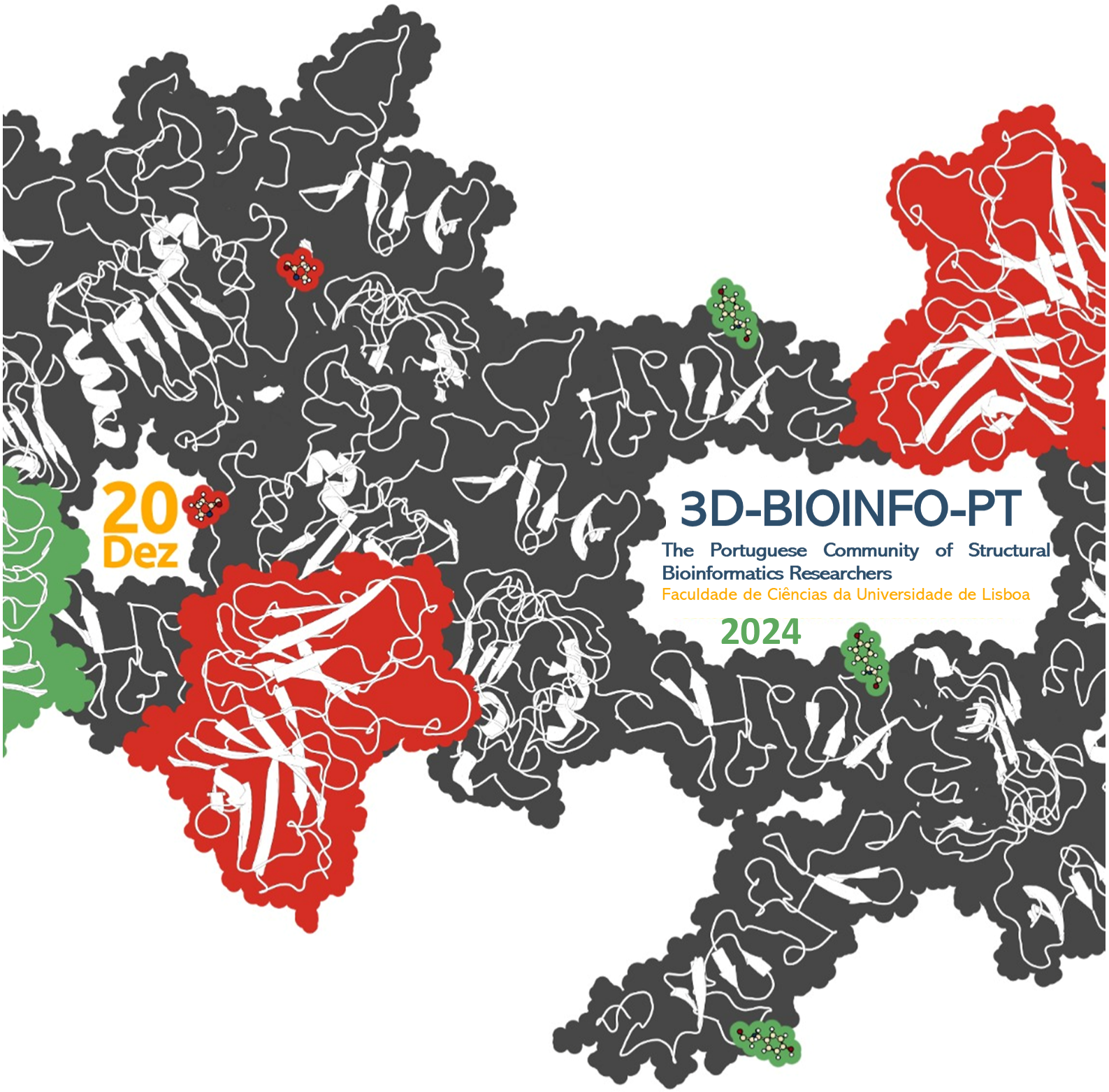
Catarina Santos

Can we use AI to design new drugs?

Catarina A. Carvalheda dos Santos[1]

1 Isomorphic Labs, London UK

Biology at its most fundamental level is an organic information processing system. But life is a phenomenally complex and emergent process, the emergent property of countless interactions. This is quite hard to describe mathematically, but the right type of regime for AI. Classical drug discovery requires trial-and-error and expensive validation, with coarse-grained biology models failing to capture adequate detail and fine-grained physics models with accuracy at small scale and high cost. We think that as maths is the right code for physics, AI might turn out to be the right method for understanding biology. If this conjecture proves true, we should be able to apply the rapid pace of machine learning progress to the modelling of the principles of biology – driving innovation and speed. AI has solved the protein folding problem, just with the amino acid sequence it can generate accurate 3D models of proteins. AlphaFold is the first proof point and marks the dawning of a new era of 'digital biology'. In this talk we will discuss Isomorphic Labs vision of transforming drug discovery and reflecting on the hypothesis that there is a fundamental synergy between biology and information science and how it can be applied to design new drugs.



SELECTED TALKS

Oral Presentation 1

Computer-aided design and validation of novel affinity ligands towards the SARS-CoV-2 spike protein

Carlos Costa, Arménio Barbosa, Margarida Dias e Cecília Roque

The COVID-19 pandemic urged the fast development of tools to prevent, diagnose or treat SARS-CoV-2 infection, at the core of which lies the use of affinity reagents that target its viral proteins. Even now that the World Health Organisation has declared it no longer a public health emergency of international concern on 5 May 2023, since then there have been over 9.5M new cases and 82,000 deaths worldwide [1]. In our lab, we are developing affinity ligands based on the Petasis-Ugi scaffold towards the SARS-CoV-2 spike protein, which are easy, quick and inexpensive to synthesise, offering tuneable affinity for any desired target [2]. The process of designing these ligands involved use of computational tools to model our binders based on structures of known strong ligands and simulate the interactions between them and our protein of interest. An initial combinatorial library of 120 ligands was elaborated and screened with AutoDock 4.2.6, AutoDock Vina 1.2.0 and MOE 2022.02 against the spike protein receptor-binding domain using blind docking. Ligands with the lowest binding energies were selected for further screenings. In parallel, experimental validation of these ligands led to the selection of one lead ligand for the spike protein. Molecular dynamics and free energy calculations were performed to better understand the interactions that our lead ligand establishes with the SARS-CoV-2 spike protein, confirming not only the binding site, but also the most important interactions, namely salt bridges established through a ligand carboxyl group, along with some hydrophobic interactions and hydrogen bonds.

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Acknowledgements: Recombinant spike protein was supplied by the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria; and by iBET – Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal. This work is funded by the European Union's Horizon 2020 programme, under grant agreement no. 899732 (PURE Project); national funds from FCT – Fundação para a Ciência e a Tecnologia, I.P.; within the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO – Research Unit on Applied Molecular Biosciences, the project LA/P/0140/2020 of i4HB – Associate Laboratory Institute for Health and Bioeconomy, the projects 2023.10436.CPCA.A1 and 2023.10437.CPCA.A2, and the PhD scholarship of Carlos Costa (2020.07566.BD).

Oral Presentation 2

Using nonequilibrium simulations to understand drug resistance and allostery in proteins

Sofia Oliveira

Proteins are neither static nor work in isolation in physiological conditions. In fact, it is the opposite; proteins are continuously moving and switching between many different conformations. Changes in the environment can shift the balance between this multitude of conformations, ultimately determining the protein's macroscopic behaviour. The binding of ions and small ligands and peptides, changes in voltage, pH, and temperature and light absorption by light-harvesting complexes are good examples of external perturbations that can induce changes in protein structure and dynamics, thus affecting their function. Several computational approaches have been developed over the years to study functional dynamics in proteins and understand how structural and dynamic changes shape function. Here, we will focus on the emerging dynamical non-equilibrium molecular dynamics (D-NEMD) simulations approach [1-3]. This method combines simulations in equilibrium and non-equilibrium conditions and, by doing so, allows us to extract the evolution of the response of a protein when exposed to an external perturbation. This approach is currently undergoing a renaissance and having increasing impact on the study of biological systems. Here, we will briefly discuss the essential features of the D-NEMD approach and how it can be used in a more general setting to study proteins. We also provide examples of different biomolecular systems and biological questions that can be addressed using this method. The examples, covering different proteins ranging from soluble enzymes involved in antimicrobial resistance to integral membrane receptors and the SARS-CoV2 spike, demonstrate the versatility and general applicability of the D-NEMD approach and how it can provide a comprehensive and unbiased mapping of the structural responses and communication networks in proteins.

[1] Balega, B et al. (2024). Mol. Phys., e2428350, 1-17
[2] Oliveira, A S F et al. (2021). Eur. Phys. J. B, 94 (144), 1-12
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Oral Presentation 3

Computational design of monobody binders to target viral therapeutical epitopes

P Moreira[1,2], Shuhao X[3], Ye W[3], J Schimdt[3], M Parada[1], CJB Conceição[1], M Rocha[2], MN Melo[1], I Abreu[1], JB Vicente[1], BE Correia[3], CM Soares[1], D Lousa[1]

1 ITQB NOVA, Oeiras, Portugal.
2 Centro de Engenharia Biológica, Escola de Engenharia da Universidade do Minho, Braga, Portugal.
3 School of Life Sciences, EPFL, Lausanne, Switzerland.

Monobodies (Mobs) represent a class of synthetic antibody-like proteins derived from Human fibronectin type III [1]. Mobs offer several advantages over monoclonal antibodies, including their monomeric assembly, absence of glycosylation and lack of disulfide bonds. These features make Mobs suitable for expression in bacteria, providing an economic benefit over antibodies. This type of protein has been engineered in laboratory settings to have a high affinity to oncologic and viral targets, suggesting their potential as future therapeutic agents. However, computational pipelines to design Mobs as binders remain scarce. This work aims to develop a computational platform for generating Mob binder sequences with strong affinity for specific protein targets. We envisioned a pipeline combining RFdiffusion [2] and AfDesign [3] to design Mob binders to have strong binding affinity to an input protein epitope. The interfaces of the designed binders are further refined using the soluble weights of ProteinMPNN [4] to reduce the risks of having overly hydrophobic proteins. The most promising outcomes were identified by filtering through AlphaFold2-based [5] metrics. This protocol was validated by creating a library of one thousand Mob sequences designed for strong affinity to the ACE2-binding epitope of the SARS-CoV-2 Receptor Binding Domain (RBD). These sequences were experimentally tested for binding affinity with the RBD using Yeast Display. This screening identified several Mobs with significant affinity signals to the target, confirming the potential of the pipeline. This research paves the way for the rapid development of antiviral antibody-like binders targeting other prominent viral epitopes.

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[5] J Jumper, et al. Nature 596.7873 (2021):583-589.

Oral Presentation 4

Force Field Integration in Constant-pH Molecular Dynamics

João G. N. Sequeira, Adrian E. Roitberg and Miguel Machuqueiro

Constant-pH Molecular Dynamics (CpHMD) methods are indispensable tools for studying pH-dependent conformational dynamics in biological systems [1,2,3]. In this work, we introduce AMBER14SB, a force field particularly suited to model disordered proteins and membrane channels [4], into the stochastic titration CpHMD (st-CpHMD) method, making it the first to support all three major force field families: GROMOS, CHARMM, and AMBER. We will detail the modifications to the side-chain partial charges of the pH-sensitive residues, ensuring compatibility with the Poisson–Boltzmann and Monte Carlo steps used in the st-CpHMD. Validation was done using experimental pK_a data of two proteins (hen egg white lysozyme and staphylococcus nuclease). A performance comparison including GROMOS 54A7 and CHARMM 36m is also shown. In this work, we highlight the method's strengths and shortcomings, while also proposing future enhancements to improve the accuracy and efficiency of CpHMD simulations.

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017 and 2022.10517.BD. This work was also funded by the European Union (TWIN2PIPSA, GA 101079147). We also acknowledge the HiPerGator supercomputer.

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2 - J. G. N. Sequeira, et al., J. Phys. Chem. B. 126 (2022), 7870-7882
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Oral Presentation 5

Solving the experimentally unsolvable – An in-silico approach to the characterization of Suberin

Fernando Neiva Nunes, Cristina Silva Pereira, Manuel N. Melo

This project aims to better understand the antimicrobial behavior of suberin particles, composition and structure. Suberin is a biopolyester ubiquitous in land plants, readily available in cork or in industrial waste. Isolated suberin forms bactericidal particles, which can themselves assemble into bactericidal materials. However, suberin complexity (>40 monomers identified) and irregular oligomerization hinders experimental characterization. Employing in-silico evolutionary algorithms allied with coarse-grained molecular dynamics simulations allowed to better understand suberin particles compositional and structural features, that are majorly determined not only by its chemical composition but also by the number of monomers composing each oligomeric unit, such feature is particle surface composition that may prove to be a key factor of the suberin particles microbicidal activity. With the use of simplified suberin models and bacterial inner membrane models, it is possible to take a closer look at the suberin mode of action linking it to the chemical and structural characteristics of cork suberin. Using these tools, I was able to show not only that composing oligomer length and aggregate particle size are important for bactericidal activity, but also that surface charges/polarity balance has an important role. These insights may help creating suberin derivatives with tunable activity.

Oral Presentation 6

Tying and Untying the Knot: Insights into UCHL-1 Knotting Energetics

Sara G. F. Ferreira[1], Patrícia F. N. Faísca[2], Miguel Machuqueiro[1]

1 BioISI, Departamento de Química e Bioquímica, FCUL, 1749-016 Lisboa, Portugal. 2 BioISI, Departamento de Física, FCUL, 1749-016 Lisboa, Portugal.

Knotted proteins, characterized by the presence of an open knot within their native structure [1], remain a compelling area of study due to their complex folding kinetics and unique topologies. While these knots are thought to have a role in enhancing stability and resisting degradation [2], their functional significance remains unclear. UCH-L1, a monomeric cysteine protease with a 5_2 knot, is pivotal in the ubiquitin-dependent proteolytic pathway and has also been linked to neurodegenerative diseases [3]. In its apo form, the catalytic triad of UCH-L1 is misaligned for catalysis, but undergoes a conformational rearrangement upon ubiquitin binding, enhancing enzymatic activity [4,5]. This interplay between structural rearrangement and function suggests that UCH-L1's activity relies on precise structural features. Notably, the knotted N-terminal tail lies close to the binding pocket, leading us to hypothesize that the knotted topology might influence this process by contributing to the stability and conformational dynamics required for enzymatic activation [5]. To test this hypothesis, we developed a computational protocol using steered molecular dynamics to systematically untangle the knot. We identified the "gate" within the crossing loop formed by the N-terminal tail and used its geometric center as a reference point for applying targeted forces. By gradually pulling these reference points apart, we generated intermediate structures representing various stages of the unknotting process. These intermediates were then analyzed using umbrella sampling to calculate the potential of mean force and estimate the energy barriers associated with both knotting and unknotting, thereby describing the structural and energetic constraints imposed by the knot. Mapping the energy requirements for knot formation and maintenance showed that the knotted topology of UCH-L1 is energetically stable, with a high barrier for unknotting. This high energy barrier highlights the knot's resistance to spontaneous unraveling and emphasizes its potential role in the protein's structural stability.

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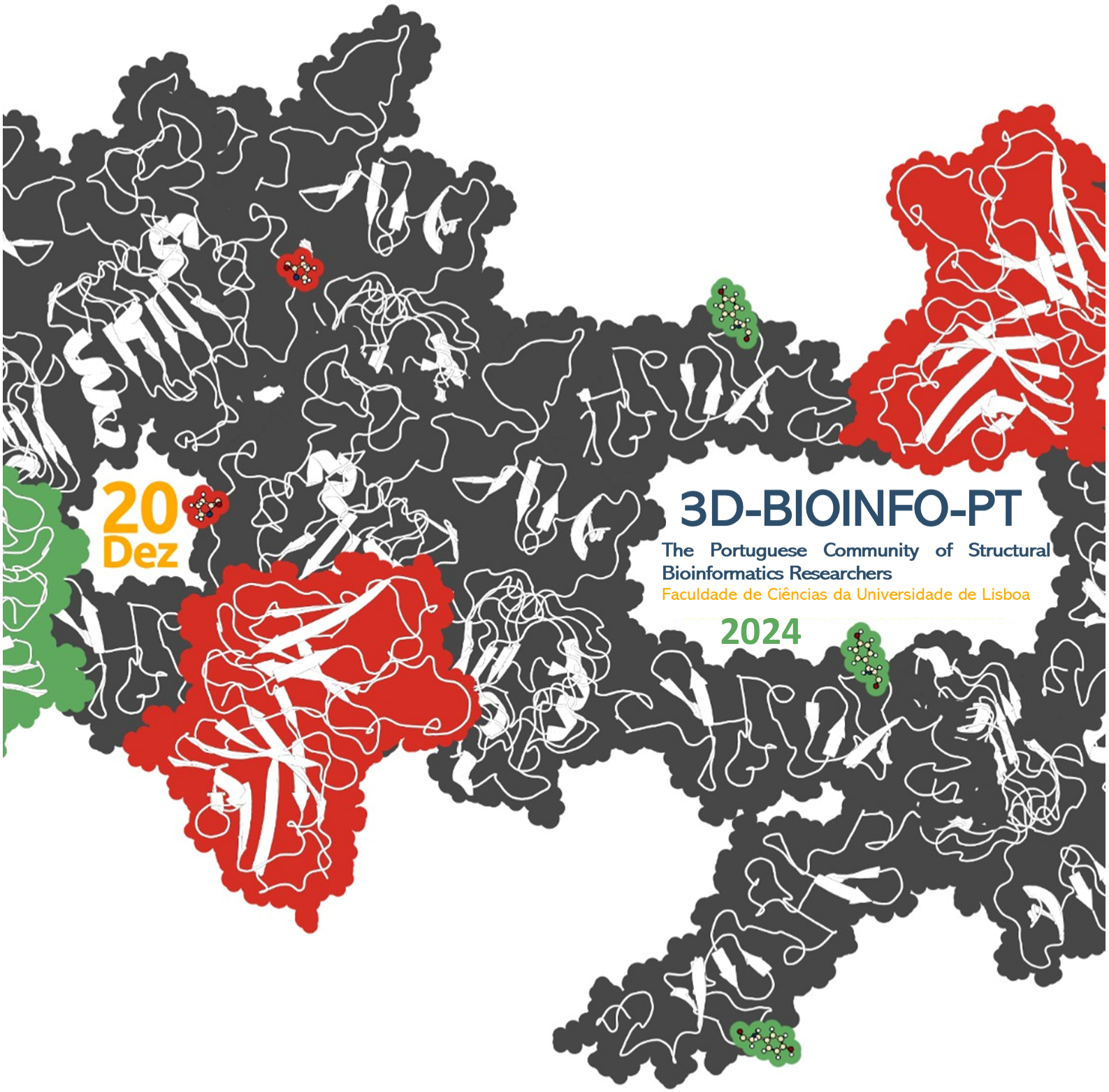
Oral Presentation 7

From structural variability to mechanical energetics of a molecular motor assembly

Victor Hugo Mello, GIMM, Oeiras Jiri Wald, CSSB, Hamburg Thomas Marlovits, CSSB, Hamburg Pablo Sartori, GIMM, Oeiras

Molecular motors harness chemical energy from molecules, such as ATP, to perform mechanical work. Through this process, energy is transiently stored in deformations of the protein structure. However, the assessment of these deformations at an atomic scale is often based on visual comparisons of resolved structures. Here we apply the formalism of elasticity theory to quantify deformation in protein atomic models. In particular, we use this approach for understanding how molecular motors perform mechanochemical energy transduction. We choose RuvB as a model system, a hexameric AAA+ crucial for branch migration in bacterial homologous recombination. Importantly, multiple cryo-EM conformations are available for different substeps of the catalytic cycle. We compute the spatial distribution of elastic energy across the protein structure throughout the mechanochemical cycle. For each residue, we obtain a 30-states energy trajectory, spanning a range of four orders of magnitude and revealing an intricate spatiotemporal energy redistribution. Analysing this data, we identify outlier residues on the energy distribution, which constitute regions of interest that are mechanically active in different sets of conformations. These regions link structure and energy transduction in protein-protein and protein-nucleotide interactions. Finally, we build a stochastic model consisting of ATPases coupled in a ring which is constrained by the energetics inferred from the structural data. Our kinetic model is consistent with single-molecule biophysical experiments and highlights the significance of subunit cooperativity for sequential dynamics. In summary, our methodology provides a physical interpretation of structural data, bridging detailed atomic-scale information and molecule-scale modelling. This systematic and general approach helps us to uncover structural mechanisms driving biological function from an interdisciplinary perspective.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POSTERS

Poster Presentation 1

Improving transient pore formation in Martini 3 lipid membranes

Ana C. Borges-Araújo, Manuel N. Melo

The lipid membrane is a crucial structure as it plays many roles in the cell, such as regulation of metabolite transport. Ion flux is typically controlled by membrane transporters but they can also leak through transient lipid pores. The control over the metabolite concentration in the cell, as well as over the electrochemical gradient that is generated through their transport, is essential for the maintenance of cell life, as it plays a major role in the mechanisms of certain cell processes, like signal transduction and ATP synthesis. Thus, pores are crucial structures to gain insight into — since they allow for these exchanges between the intra and extracellular environments. Coarse-grained (CG) molecular dynamics (MD) simulations allow us to study systems at time and size scales otherwise inaccessible, at the expense of some atomic detail. In the Martini 3 CG force field, transient lipid pores are not well modeled — with higher energies of formation than those obtained by atomistic (AA) methods. We studied Martini 3 simulations of transient pore formation in lipid bilayers and compared them to AA behavior, to try to identify the limitations of lipid pore simulations with the CG forcefield and subsequently try to address them. We found a potential target — the lipid tail splay — that may affect formation behavior, and tested lipid prototypes with new tail topologies to analyze whether the free-energy profile improved. Whilst we did not obtain better profiles, we believe this target can still be causing incorrect modeling as we were not able to mimic the AA tail splay distribution with accuracy. Other potential targets and solutions will also be further explored in the near future.

Poster Presentation 2

DFT studies applied to the synthesis of new G4-ligands

Bahls, B. (1,2), Costa, P. J. (2) and Paulo, A. (1)

(1)Faculty of Pharmacy, Research Institute for Medicines (iMed.Ulisboa), Universidade de Lisboa, Av. Prof Gama Pinto, 1649-003 Lisbon, Portugal;
(2)Faculty of Sciences, Biosystems and Integrative Sciences Institute (BioISI), Universidade de Lisboa, Campo Grande 016, 1749-016, Lisbon, Portugal;

Guanine-rich nucleic acids can form quadruplex structures (G4) which have important biological roles in regulating processes such as gene replication, transcription, translation, and epigenetic events, where they often function as protein recognition sites. G4s have been explored as drug targets, primarily for treating cancer, but also for parasitic, bacterial, or viral infections and, recently, neurological disorders [1]. While four G4-targeting molecules have reached clinical trials, two were withdrawn due to toxicity and low efficiency [2]. All G4-ligands that reached clinical trials have four-fused aromatic rings, being potent G4 stabilizers but not selective to specific G4s. These findings are in line with another study suggesting that the desired selectivity/specificity may be achievable by sacrificing binding affinity [3]. The pyrrolo[4,3,2-de]quinolinone (PQ) scaffold is present in natural alkaloids that show different biological activities such as cytotoxicity against cancer cell lines and inhibition of topoisomerase I [4]. We aim to derivatize the PQ core, first by Suzuki Coupling, followed by the derivatization of the amine and carboxylate groups. The PQ core was obtained using the procedure of Yang et. al., with a global yield of 18% [5]. To improve the yield of Suzuki Coupling, the reaction mechanism was studied using DFT calculations. Initially, we studied the solvent effect (methanol, dioxane, and dimethoxyethane), showing that the polarity of the solvent interfere with the calculated barriers. Reactions were reproduced on a small scale and analyzed by HPLC-ESI-MS to validate the in silico studies. Preliminary results showed that slightly increasing the solvent polarity boosts reactivity. However, too much increase can lead to other issues, e.g. product and initial material degradation and problems with solubility. We will continue to study the effect of the base and halogen position through DFT calculations to understand their effect on the final yield.

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Acknowledgments: Work supported by UIDB/04046/2020 (DOI: 10.54499/UIDB/04046/2020) and UIDP/04046/2020 (DOI: 10.54499/UIDP/04046/2020) Centre grants from FCT, Portugal (to BioISI), UIDB/04138/2020-UIDP/04138/2020 (iMed) and 2022.06099.PTDC. PhD grant 2023.01798.BD (BB), and 2021.00381.CEECIND (PJCosta). European Union for TWIN2PIPSA, GA 101079147

Poster Presentation 3

SMILESRender: User-friendly website for rendering molecules

Carlos S. H. Shiraishi, Gabriel Grechuk, Miguel A. Prieto, Sandrina A. Heleno and Rui M. V. Abreu

In today's technological landscape, user-friendly design is crucial to the development of bioinformatics tools, with accessibility and intuitiveness standing out. SMILESRender aims to make molecule rendering easier to implement, improving the user experience. With a logical and clear interface, his design reduces the learning curve and extends the reach of the software. This results in greater user satisfaction, increased adoption rates, and the expansion of bioinformatics. SMILESRender (https://smiles-render.onrender.com) is a user-friendly web application designed to render molecule representations from SMILES (Simplified Molecular Input Line Entry System) chemical format to a two-dimensional representation image. Built on Python and utilizing the RDKit library as its main engine, SMILESRender supports multiple image formats, including PNG, JPEG, and TIFF. The platform offers multiple endpoints for rendering, including individual processing of SMILES strings and batch processing from CSV files. Its RESTful API framework enables efficient rendering, ensuring compatibility with complex SMILES strings through Base64 encoding. The frontend, developed in HTML, CSS, and JavaScript, offers an intuitive interface, facilitating both direct input and batch processing of CSV files. The deployment utilizes Docker and Docker Compose for scalability and ease of use in different environments. The system is currently hosted on Render.com, demonstrating its cloud adaptability. SMILESRender provides researchers and chemists with an affordable, high-performance tool for molecular visualization, promoting improved analysis and communication of chemical data.

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Poster Presentation 4

Leveraging Computational Approaches for Improved Toxicity Prediction in Drug Development

Ana M. B. Amorim[1,2,3,4], Nícia Rosário-Ferreiram[1,2], Irina S. Moreiram[1,2,3]

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2 CNC - Center for Neuroscience and Cell Biology, Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
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The success rate of drugs in clinical trials is notably low, with about 90% of candidates not making it th-rough the entire process. Toxicity is a major contributor to this failure rate, significantly impacting overall success and escalating development costs, especially when detected late in clinical trials or after market release. Notably, unexpected toxicity issues identified during preclinical stages account for approximately 30% of these failures, posing a major challenge. Evaluating toxicity and safety is critical in drug development, however traditional methods are time-consuming and costly. Artificial Intelligence (AI) greatly improves the speed and accuracy of drug toxicity prediction, playing a significant role in pharmaceutical innovation. The implementation of AI in drug development promises to significantly shorten the timeline and reduce the costs of bringing safe drugs to market. Given that toxic reactions can cause severe and irreversible damage to critical organs, significant advancements in these fields can open new pathways for improving healthcare outcomes and patient safety1. Keywords Safety, Predictive Toxicology, Artificial Intelligence, Drug Discovery, Therapeutical Drugs.

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Poster Presentation 5

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

Luana Ferreira Afonso, Nícia Rosário-Ferreira, Cátia Pesquita and Irina Sousa Moreira

Drug combinations can enhance therapeutic efficacy and overcome drug resistance. However, systematic testing of all possible drug combinations experimentally is infeasible owing to the vast combinatorial space. Computational prediction of synergistic drug combinations is therefore highly desirable for prioritising experiments, but accurate prediction and ensuring interpretability remain challenging. Synpred 2.0 is an evolving machine learning framework that integrates drug and cell line features to predict synergistic drug combinations. Large-scale drug combination screening data and multi-omics profiles of cancer cell lines were curated and preprocessed. This pipeline supports various prediction tasks and rigorous evaluation schemes. To enhance predictive performance and interpretability, future work will explore the integration of biological knowledge in the form of graph-structured information. Iterative refinement is necessary to facilitate the development of precision medicine strategies for cancer treatment.

Poster Presentation 6

Glycan Shielding Simulation of Respiratory Syncytial Virus Fusion Protein: Implications for Biopharmaceutical Design

André Bagão, Raquel Domingues, Rita I. Teixeira, Cláudio M. Soares, João B. Vicente, Diana Lousa

Respiratory Syncytial Virus (RSV) is a leading cause of acute respiratory infections, which is more severe in neonates and older adults. The virus's Fusion (F) protein is a glycoprotein that has a crucial role in viral infection and is one of the main targets for neutralizing antibodies. RSV F is a class I fusion protein that mediates the fusion of viral and host cell membranes, allowing viral entry. The prefusion conformation of RSV F is metastable and changes conformation during the fusion process. This study investigates the glycan shielding of known epitopes on the prefusion F protein to identify potential targets for protein design and discover of new potential epitopes. Molecular dynamics (MD) simulations of the prefusion trimeric F protein structure with glycans were performed for the first time, providing novel insights into the protein's surface accessibility. The results revealed that while glycans shield a significant portion of the protein surface, they offer less protection to two specific epitopes only present in the prefusion state: site Ø and site V. These findings may have important implications for RSV vaccine and therapeutic development. The inferior glycan shielding of sites Ø and V suggests that these epitopes remain more accessible to antibodies, making them promising targets for protein design strategies. Furthermore, this study's approach of including glycans in MD simulations of the RSV F protein provides a more accurate representation of the protein's dynamics in its native environment.

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Poster Presentation 7

The effect of ATP binding in ABCG2 conformation

Pedro Suzano, Bruno Gonçalves, Maria Ferreira, Daniel dos Santos

The ABC (ATP-Binding Cassette) proteins is a superfamily of membrane transporters that bind ATP to perform their function. Upregulation of these transporters is a defense strategy against administered drugs, leading to drug resistance, and poor chemotherapy response and prognostic factor.1 Recent advances in experimental techniques, such as Cryo-EM, improved the resolution of structural details, although the complete transport mechanism of ABC transporters is still unclear.2 Herein, we report on the dynamics of the inward-facing conformation of the ABCG2 protein in its apo and ATP-bound states, and the effects of ATP binding. Homology modeling was used to complete a structure of the ABCG2 protein, starting from a cryo-EM structure available on the Protein Data Bank (pdb: 6ETI). Missing sequences were built using our previous BCRP model3 as template, along with the PredictProtein secondary structure prediction server4. The protein was inserted into its native environment and simulated for data collection for 500 ns under physiological conditions (total of 760 ns). We analyzed and compared the conformational changes occurring in the transmembrane and nucleotide binding domains of the ABCG2 apo and ATP-bound forms, providing interesting insights. Our results and the overall motion of the protein appear to support a hypothesis proposed by Locher et al5 for the ABCG2 efflux mechanism. Although our research helped elucidate the ABCG2 structural differences between the Apo and ATP-bound forms, further research is required to completely characterize these mechanisms. A more complete understanding of these transporters is essential for anticancer research, allowing to design more specific and efficient anticancer drugs.

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Poster Presentation 8

Computational design and production of ACE2-based inhibitors against SARS-CoV-2

Rita I. Teixeira, Bárbara Fernandes, Carlos Cruz, Susana Parreiras, Carolina C. Buga, Christine Cruz-Oliveira, Ana S. Veiga, Miguel A. R. B. Castanho, João B. Vicente, Diana Lousa, Cláudio M. Soares

Targeting viral entry represents a promising therapeutic strategy to combat the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this study, triple helical bundle antiviral proteins (ARMYs) were computationally designed and optimized to target the Spike protein receptor-binding domain (RBD), preventing its interaction with the angiotensin-converting enzyme 2 (ACE2) receptor. The design strategy incorporated the two adjacent α-helices from ACE2, which mediate most interactions with the RBD, into backbone scaffolds stabilized by an additional helix and a short loop. For the interface design, the amino acid sequences were designed to optimize target binding, folding and stability. This resulted in five candidate proteins. Further structural validation was performed using structural prediction methods. Experimental assays were performed and confirmed that four designed proteins (ARMY 1 to 4) effectively bound to the RBD with nanomolar affinities, similar to the ACE2 affinities to the viral target and successfully blocked SARS-CoV-2 infection. Thus, these design proteins showed great potential as viral therapeutics. Further molecular dynamics simulations of the designs were performed in the unbound and the bound state to the RBD to access their stability and the key interactions between these and the RBD that may have affected the binding affinity, respectively. As expected, ARMY 5 demonstrated a distinct dynamic behavior from the others. The knowledge taken from this work led to a new workflow for anti-SARS-CoV-2 design and production.

Poster Presentation 9

Exploring the Impact of the Stargazin V143L Mutation on the Dynamics of the AMPA Receptor:Stargazin Complex with Different Conductance Levels

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AMPA Receptors (AMPAR), the fastest ionotropic glutamate receptors, are activated by the agonist glutamate and play a critical role in synaptic plasticity believed to be involved in the learning process. AMPAR can achieve different conductance levels (O1-O4) depending on number of agonist molecules bound to the ligand-binding domains of the protein. Stargazin (STG), a transmembrane AMPAR regulatory protein (TARP), plays a crucial role in facilitating the transport of AMPAR to the cell surface, stabilising their localisation at synapses, and influencing their gating properties. In this study, we investigated the effect of the STG V143L mutation, previously linked to intellectual disability, on the interaction between stargazin and AMPAR using eight distinct AMPAR:STG complexes, associated with different conductance levels. Through extensive analysis of the complex interface structures and dynamics, we revealed that the STG V143L mutation had a more pronounced destabilising effect on complexes with lower conductance levels compared to the conductive states of the receptor, suggesting a potential association with impaired synaptic transmission in individuals with this mutation.

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Poster Presentation 10

An In Silico Protocol to Evaluate and Optimize Cyclodextrins for Drug Delivery

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Pharmacokinetics plays a significant role in the pharmacological effect of drugs and exploring delivery systems that can improve such properties is paramount for drug development. In this scope, cyclodextrins (CDs) are a group of cyclic oligosaccharides containing a hydrophilic outer surface and a lipophilic central cavity that can form inclusion complexes with several drugs, often improving their aqueous solubility, thus, being suitable vehicles for drug delivery 1,2. In this communication, we highlight how a combination of computational methods, such as Quantum Mechanical Calculations (QM), Molecular Docking, and Molecular Dynamics (MD) simulations, can be used to build a pipeline for the estimation of CD:drug binding energies. The final goal is to use features extracted from the above-mentioned computational protocols along with Machine Learning and Artificial Intelligence methods to develop a fast and efficient model capable of predicting the binding affinities of a compound to a series of cyclodextrin hosts. Three cyclodextrin systems (HPαCD, HPβCD and HPγCD) are being studied in combination with 39 different drugs. Since Molecular Docking calculations of these systems with both rigid and partially flexible receptors usually do not correlate well with the experimental binding constants, Molecular Dynamics Simulations of all drug-CD systems were performed. The analysis of the MD trajectories showed a remarkable ability of CDs to form stable inclusion complexes with several drugs. Most of these complexes correlate well with the host:guest configurations predicted by the molecular docking tool (Autodock Vina). Therefore, the difficulty of this protocol to predict experimental binding affinities is most likely due to the unsuitability of its scoring function for these non-protein systems. The work completed until now allows for a good understanding of the systems at hand and will allow us to move forward to the estimation of properties related with drug:CD binding and the full development of the ML model.

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Poster Presentation 11

Targeting Respiratory Syncytial Virus (RSV): Structural Bioinformatics insights into the stability of a therapeutic nanobody

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Respiratory syncytial virus (RSV) is a major cause of respiratory infections, particularly in children under five and older adults. In 2019, RSV caused 33 million infections and 101,400 deaths in children. Treatment of patients primarily focuses on symptom relief, with approaches varying by age and severity. The fusion protein (F), essential for RSV's ability to infect host cells, is a key target for therapeutic and preventive measures. In 2023, two vaccines targeting the F protein were approved for older adults: Arexvy and Abrysvo. Later in May of 2024, mRESVIA, an mRNA vaccine with similar efficacy to Abrysvo, was also approved. For infants, two monoclonal antibody therapies targeting the fusion (F) protein are currently approved for RSV prevention (palivizumab and nirsevimab). Nanobodies (VHHs), with their unique antigen-binding properties and ease of production in microbial expression systems, are emerging as promising tools for combating RSV. For example, nanobody ALX-0171 has been evaluated in a phase II clinical trial in children that were hospitalized with RSV lower respiratory tract infection [1]. Another VHH, named F-VHH-4, potently neutralizes RSV A and B strains by targeting a prefusion F-specific quaternary epitope [2]. However, the biopharmaceutical potential of F-VHH-4 remains to be explored. In this work, we focus on studying the structural stability of F-VHH-4 through molecular dynamics (MD) simulations. We analyzed its stability through RMSD, the flexibility using RMSF and the secondary structure using DSSP. Our simulations revealed that F-VHH-4 is a highly stable structure. Our studies will be important for the improvement of nanobody's biopharmaceutical potential.

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Poster Presentation 12

A systematic computational study of the membrane-induced $\mathrm{p}K_\mathrm{a}$ shifts in amino acids

Nuno F. B. Oliveira, Pedro B. P. S. Reis, Miguel Machuqueiro

Protonation equilibrium is an often forgotten key physicochemical property of titrable molecules that is highly influenced by the surrounding environment. In our cells, titrable molecules face a vast array of environmental effects that change their proton binding affinities ($\mathrm{p}K_\mathrm{a}$ values), from conformational changes in proteins to insertions in highly apolar regions, such as lipid bilayers. Being able to describe the effects of inserting titrable molecules from aqueous phase into a lipid bilayer, can provide helpful insights into several important pharmaceutical systems such as membrane-inserting peptides or even charged drugs permeating in a membrane. Although an accurate description of these effects can be hard to achieve experimentally, computational methods such as the stochastic titration Constant pH Molecular Dynamics (CpHMD) can provide crucial insights into this complex interplay between environment changes and protonation equilibrium, allowing for a better understanding of experimental results. In this work, we complemented our previous description of the $\mathrm{p}K_\mathrm{a}$ profiles of the Ala pentapeptides residues (AA-X-AA) inserting into a DMPC lipid bilayer [1] by employing advanced enhanced sampling techniques such as pHRE [2], and US-CpHMD [3]. We compared the efficiency of each technique to describe the $\mathrm{p}K_\mathrm{a}$ profile of all 6 titrable residues and 2 termini when inserting in a POPC membrane. We observed an improvement in the protonation sampling between regular CpHMD and pHRE, however, both methods still struggle to obtain ionized conformations at more bilayer-inserted positions. The use of biasing potentials (US-CpHMD) to fix the titrable group at more inserted positions led to a slight improvement of both the conformational and protonation sampling. From our simulations, we observe a distinctive shift in the residues' $\mathrm{p}K_\mathrm{a}$ values upon membrane insertion that is due to a stabilization of their neutral states, leading to a clear $\mathrm{p}K_\mathrm{a}$ increase in the anionic residues and the inverse for the cationic ones.

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Poster Presentation 13

Utilizing Artificial Intelligence for GPCR Activation State Prediction: A Computational Perspective

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G protein-coupled receptors (GPCRs) are essential proteins involved in cellular communication, and are key targets for drug development. This work explores how artificial intelligence (AI) can help predict the activation states of GPCRs, providing insights into their complex behaviour. By combining AI with structural data, molecular dynamics simulations, and ligand-based approaches, we can better understand how these proteins function and respond to various stimuli. This knowledge is critical for identifying new drug candidates and for improving therapeutic strategies. Our findings highlight the potential of AI to simplify GPCR research and accelerate the discovery of effective treatments (1).

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Poster Presentation 14

Parameterization of bacterial peptidoglycan for state-of-the-art molecular dynamics coarse-grain force fields

João Catarino, Manuel N. Melo

The cell wall is one of the most defining characteristics of bacteria. It plays a crucial role in cell integrity while regulating the passive transport of many nutrients and proteins. The main culprit behind the cell wall properties is the peptidoglycan polymer. It is composed of a backbone of two alternating sugars (NAG and NAM) and a pentapeptide linked to every NAM sugar. This pentapeptide is capable of cross-linking with other ones to create large meshes. Being able to computationally simulate peptidoglycan meshes at the molecular level would possibilitate several research venues, such as drug targeting and screening towards development of novel medicine. However, simulating these meshes is unfeasible using standard all-atom (AA) simulations due to their considerable size. Coarse-grain (CG) molecular dynamics trade molecular detail for lighter calculation and larger time steps, reaching time and size scales compatible with simulation of the peptidoglycan mesh. In this work, we develop a peptidoglycan model for one of the most widely used coarse-grain frameworks for biomolecular simulations, the Martini 3 force field. Martini 3 parameterization entails comparing behaviour from tentative CG models with that of an AA counterpart. Using this information, CG parameters are then iteratively tweaked to best reproduce target behavior. Here, the glycan chain of peptidoglycan was first parameterized using separate simulations of single disaccharide units ran with existing AA models. These parameters were then extended to successfully represent a full glycan chain. Peptide stems were then attached to each NAM residue and its parameters were determined based on the standard Martini 3 guidelines. Additionally, the parameterization process used an exclusion and pair scheme to reproduce the rate and directionality preference with which disaccharide units flipped between two relevant torsional states. This is one of the first Martini 3 parameterization effort that takes these aspects into account as well.

Poster Presentation 15

Setting up a reliable model to study the structure and function of aquaporins using MD simulations

Marta S. P. Batista, Miguel Machuqueiro, Bruno L. Victor

Aquaporins (AQPs) are responsible for permeating solutes across membranes. They can be divided into two subgroups: classical aquaporins, strictly selective for water, and aquaglyceroporins, which are permeable to water and glycerol. Identifying AQP function modulators is crucial, as they hold significant potential for treating diseases. However, this has remained a challenge due to the low target druggability and the unsuitability of the commonly used computational approaches. The crystallographic structures of AQPs often exhibit unrepresentative conformations due to the crystal packing, which hinders the accurate binding of the best modulator candidates. Despite attempts to use computational protocols to address these issues [1], a fundamental question remains: are we using adequate models, parameters, and force fields to simulate these membrane proteins effectively? In this work, we explored the impact of different membrane sizes and force fields on the stability and function of AQPs. For this, we set up Molecular Dynamics (MD) simulations with the solved structure of the aquaglyceroporin hAQP7 (6QZI) [2] in a POPC lipid bilayer of different sizes (170, 200, 300, 400, and 500 lipids) with the Amber ff14SB and the CHARMM 36m forcefields. This protocol helped us understand the impact of simulation parameters on the structure and function of the protein. These results will be valuable for correlating specific structural features with hAQP7's function. Moreover, they will aid in the identification and development of specific and efficient functional modulators that could be explored as therapeutic approaches for various diseases.

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Poster Presentation 16

Assessing the Affinity of Cyclic Peptides Towards the Amyloid-β Peptide

Cristiano Rocha; Nuno Galamba; Gabriel Martins

Several amino acid sequences are believed to be especially important to the aggregation of the amyloid beta peptide (Aβ), implicated in Alzheimer's disease. A potential strategy in the design of aggregation inhibitors foresees using peptides with a primary structure homologous to these sequences of the Aβ peptide. Here, we studied, through molecular dynamics, the affinity of cyclic peptides formed by different amino acid sequences of the Aβ 42 peptide and their specificity towards their homologous sequences in the monomer. Our results show that some peptides have a good affinity to specific domains, although not necessarily their precursor sequences, suggesting that relatively small cyclic peptides may have long residence times next to these sequences, possibly influencing the Aβ 42 peptide aggregation propensity.

Poster Presentation 17

Ultra-Large Library to Discover Novel Synthetic Affinity Ligands for Antibody Purification

Jéssica Rodrigues, Ana Cecília Roque, Arménio Barbosa

Affinity chromatography is essential for biopharmaceutical purification, a market projected to reach 300 billion USD by 2025. However, the production of antibodies remains costly due to the affinity chromatography step. Synthetic affinity ligands like A2P, 22/8, and DAAG are gaining attention, but Protein A, an expensive biological ligand, remains the standard. Its drawbacks include high resin cost, ligand leakage, and potential antibody denaturation during acidic elution. Additionally, the process involves significant water use and chemical waste, raising environmental concerns for large-scale production. This work explores novel synthetic affinity ligands for antibody purification to reduce costs while maintaining efficacy. By screening virtual compound libraries, the number of experimental candidates is reduced while ensuring chemical diversity. Using the Petasis-Ugi scaffold and fragments from different chemical suppliers, a virtual combinatorial library of 68 million structures was generated. The library underwent curation and filtering, followed by molecular docking virtual screening to assess ligand binding to a target monoclonal antibody. Top ligands were further analyzed using Molecular Dynamics simulations for deeper insights into molecular interactions and ligand stability. Binding free energy calculations were performed on the most promising candidates. Two synthetic affinity ligands were identified for antibody purification. Additionally, the ultra-large Petasis-Ugi library developed in this study shows potential for applications in other biopharmaceutical targets. These results represent a significant step toward cost- effective antibody purification and affinity ligand discovery, with experimental validation planned in the laboratory.

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Poster Presentation 18

Understanding the Binding of Cyclic Peptides to Amyloidogenic Amino Acid Sequences of α-Synuclein through Molecular Dynamics Simulations

Gabriel Martins and Nuno Galamba

Neurodegenerative diseases are associated with the formation of amyloid structures of intrinsically disordered proteins (IDPs), such as α-synuclein (α-syn) in the case of Parkinson's disease and other synucleinopathies. Understanding the driving forces and mechanisms involved in the formation of toxic oligomers is, therefore, pivotal to the development of drugs for these pathologies. Several different compounds have been studied as anti-amyloidogenic agents. Amongst them, cyclic peptides (CPs) show some key advantages over other candidates, such as enhanced resistance to proteolysis compared to linear peptides and a higher surface area than small molecules, important to block protein-protein interactions. Herein, we study through molecular dynamics the binding affinity and specificity of several cyclic peptides designed to bind to aggregation-prone domains of α-syn. Furthermore, their influence on the structure of α-syn is assessed. This work highlights the potential of specific protein-peptide interactions to inhibit protein-protein interactions, setting the stage for further investigations on aggregation inhibition, starting with α-syn dimer models for the most promising CPs candidates.

Keywords: Molecular dynamics; α-synuclein; IDPs ; Cyclic Peptides

Poster Presentation 19

Artificial Intelligence-based Design of Antibody-like Engineered Protein Scaffolds

Rafael F. Salgueiro, Pedro Moreira, Cláudio M. Soares, Diana Lousa

Viral pandemics have profoundly impacted human societies, underscoring the urgent need for enhanced pandemic preparedness and effective antiviral solutions. Currently available antiviral solutions present some limitations, highlighting the need to explore and invest in innovative alternatives. Monobodies, a class of engineered proteins derived from the 10th domain of the human Fibronectin type III (FN3) and with unique characteristics, have emerged as promising antiviral candidates. Given the lack of diversity between the monobodies already described in the literature, this work aims to create a computational framework streamlining monobody development, positing that language models can generate a large, diverse monobody library suitable for further protein design approaches. The research began with the 10th human FN3 domain. Sequence search was preformed to find existing sequences with high identity; the obtained hits served as input for the MSA Transformer, the selected language model for this work. Generated sequences were clustered, and the structures of the cluster representatives were predicted. Predicted structures were docked with the SARS-CoV-2 Receptor Binding Domain as proof of concept. Sequences in the interface were optimized and interface metrics were calculated to filter promising results. Findings showed that language models can be used to generate a large, diverse monobody library, integrable into a broader antiviral design framework. Also, MSA Transformer significantly enhanced the selection process for target-specific antiviral discovery, compared with the dataset obtained directly from the sequence search step . Finally, we found that the most effective approach involved starting from the predicted docked complex structure, and improve it by optimizing the entire monobody sequence. These advances could significantly enhance pandemic preparedness by streamlining target-specific antiviral development.

Poster Presentation 20

An Integrated Platform for Enhancing Enzyme Catalysis in Metabolic Pathways

João Correia, Sofia Ferreira, Isabel Rocha, Diana Lousa, Caio S. Souza, Cláudio M. Soares

The performance of microorganisms in natural and synthetic metabolic pathways is often limited by issues such as low enzyme activity, insufficient substrate or co-factor levels, and toxicity of the final product. These challenges can be mitigated through protein engineering, which modifies enzyme catalytic properties to favor specific reactions and enhance pathway efficiency (Du et al., 2011). To tackle these limitations, we created an automated computational platform for gene discovery and enzyme engineering. The platform targets enzymes involved in rate-limiting steps, aiming to improve their efficiency or enable them to catalyze new reactions beyond their native function. The workflow consists of three main modules: a random mutation generator to produce enzyme variants, an atomistic homology modeling tool for structural predictions, and a binding energy evaluator to screen for high-performing candidates. This approach allows the generation and prioritization of mutant enzymes for subsequent experimental validation. Validation across multiple case studies demonstrated the platform's effectiveness in identifying both natural and engineered enzyme variants capable of catalyzing specific reactions with improved efficiency (Hanko et al., 2023). The results underscore the promise of computational enzyme engineering as a strategy to address metabolic pathway bottlenecks, offering a powerful tool for advancing microbial engineering and synthetic biology.

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Poster Presentation 21

Molecular Dynamics Study of H5N1 Hemagglutinin: Insights into Viral Inhibition

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Highly pathogenic avian influenza virus (HPAIV) H5N1 poses a significant threat due to its increasing ability to infect mammals. The latest strain has caused an outbreak in dairy cattle across the United States, that has also spread to poultry and humans working in close contact with infected animals. While no human-to-human transmission has been confirmed, the virus' continued spread and adaptation to new mammalian hosts raise concerns about its potential to acquire this capability, potentially leading to human-to- human transmission[1]. This study focuses on the hemagglutinin (HA) protein, which is crucial for host cell interaction, since it binds to the host cell sialic acid receptors and is also responsible for fusing the viral envelope with the host cell membrane, making it a key target for viral inhibition. To advance our understanding and inform potential inhibitor designs, µs-long molecular dynamics simulations were conducted to assess the structural characteristics and dynamic behavior of the HA globular head, which contains the receptor binding site (RBS)and is, thus, a very relevant target. The simulation was performed on the predicted structure of the A/cattle/Texas/2024 strain and included three replicates to ensure robustness in the findings. The data extracted allows for a detailed examination of conformational changes and stability in regions critical for receptor binding and may provide valuable insights into HA-receptor interactions and specificity. By enhancing our understanding of H5N1 HA dynamics, a foundation is laid for future targeted interventions to contain the pandemic potential of this evolving virus.

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Poster Presentation 22

In silico development of a P. viticola VviSBT4.19 mutant to circumvent its wt autoproteolytic activity

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Grapevine cultivation is a cornerstone of the Portuguese economy, with the country ranking as the fifth-largest wine producer in the European Union in 2022 [1]. However, grapevines face significant threats from pathogens, particularly Plasmopara viticola, the causative agent of downy mildew [2]. This disease can drastically reduce crop yield and quality [3], and while fungicides are commonly used for control, their environmental and health risks highlight the need for sustainable alternatives [4]. Serine proteases, particularly VviSBT4.19, have been identified as key factors in grapevine immunity against P. viticola, with high expression observed in resistant genotypes and during post-infection responses [5]. However, understanding the functional role of this protease has posed a major challenge arising from the absence of a reliable structural model, due to its autoproteolytic activity. To address this hurdle, we constructed a computational model of VviSBT4.19 using homology modeling. Simulations with our advanced Constant-pH molecular dynamics methodology were then performed to assess the stability and structural features of the protease model, focusing on its catalytic center. Additionally, we introduced a targeted mutation to disable the autocatalytic activity of VviSBT4.19 while preserving its structural integrity. Simulations of the mutant protein confirmed that the mutation did not induce significant structural rearrangements nor activated a putative secondary catalytic triad. Furthermore, cross-RMSD analysis revealed that the wild-type and mutant proteins sampled indistinguishable conformational landscapes, further validating the structural integrity of the mutant and reinforcing the reliability of both models. By providing a reliable structural model of VviSBT4.19, this work enhances our understanding of the molecular mechanisms underlying resistance to P. viticola, paving the way for future experimental studies aimed at exploring its functional role in grapevine immunity and its potential applications in engineering disease-resistant cultivars.

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Poster Presentation 23

The pH-dependent ionization of peptide dendrimers is key to modulating its affinity for membrane and siRNA

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Emerging therapeutic applications, such as gene therapies and vaccines based on RNA, require a delivery system to safely and accurately deliver the therapeutic molecules to their targets. There are many methods to achieve transfection, but several have significant limitations related to toxicity and immunogenic responses[1,2]. Researchers have recently investigated ways to address these hurdles, namely the use of novel compounds, such as peptide cationic dendrimers[3]. These branched structures, composed of cationic and hydrophobic amino acid residues, such as lysine and leucine, establish strong interactions with nucleic acids, as well as with biological membranes[3]. Their ability to change their protonation states between physiological and acidic pH values is crucial to bind nucleic acids and enable endosomal escape. However, despite extensive experimental efforts, the molecular mechanisms driving the unique properties of these dendrimers are not yet fully understood[4]. Here, we present our findings using the CpHMD method to investigate the pH-dependent conformational behavior of these peptide dendrimers. Simulations were performed in aqueous media, interacting with a lipid bilayer, and in complex with a siRNA double helix. We found that these molecules become highly charged under acidic conditions and that the derived high charge density is critical to destabilizing the membrane. These observations align well with experimental data and offer insights into why certain dendrimers struggle with efficient endosomal escape. Additional early results also show that the binding of the dendrimers to the siRNA cargo seems to be mainly driven by electrostatics. The comprehension of their mode of action will be pivotal in designing new peptide dendrimers with improved transfection efficiency.

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Poster Presentation 24

Improving the accuracy of a computational model of Cytochrome c Oxidase to study the proton gating mechanism

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Cytochrome c Oxidase (CcO) is the last electron transport chain enzyme that reduces oxygen to water while pumping protons against their concentration gradient. The activity of CcO is an integral part of the maintenance of the transmembrane electrochemical potential in the mitochondria which is used, among other things, to facilitate the production of ATP by ATP-synthase. CcO contains a set of metal cofactors proposed to play a pivotal role in the H+ pump control. There are three cofactors of interest in CcO: a CuA center, containing two copper atoms; a hemea center; and a binuclear center (BNC), composed of a hemea3 and CuB groups. All these cofactors are coordinately bound to specific amino acid residues of the protein, which due to the presence of the metals will have different properties than the standard residues. These metal cofactors, in particular the BNC, are proposed to control proton uptake via a conformational gating mechanism that upholds directionality. The gating is hypothesized to be dependent on fluctuations of the pK_a value, the proton affinity, of nearby residues as a result of electrostatic shifts induced by changes in the oxidation states of the metal groups. However, important details are still missing to fully describe this gating model, like identifying key residues and elaborating on the role of the cofactors as proton loading sites. To shed light on these issues, we built a model of R. sphaeroides CcO to perform Constant pH Molecular Dynamics (CpHMD). We parameterized the metal cofactors with quantum mechanics (QM), to obtain an ESP charge set and customize our force field parameters aiming to improve the accuracy of the description of these complex organometallic cofactors. We will be showcasing the results of our QM parameterization and some CpHMD preliminary results with our CcO:membrane system.

Poster Presentation 25

Insights into the Protonation Dynamics of (Phosphatidyl)inositols

Ana Figueiredo, João Vitorino, Miguel Machuqueiro

Phosphatidylinositols (PIPs) are valuable molecules for cells, offering a vast array of functions, ranging from membrane trafficking to cellular proliferation. Most PIPs, if not all, have distinct biological roles and their metabolisms are tightly controlled. Their structural properties are mainly determined by the characteristics of the polar group, which, at physiological pH, is very negatively charged and with the possibility of establishing strong electrostatic interactions. The global protonation state of PIPs has a large influence on their binding affinities and specificity for certain protein domains, their interaction with other lipids (including PIPs themselves), and with divalent cations. Although their structural properties rely on the characteristics of the polar group, which is affected by pH, PIPs have been studied at the molecular level using computational methodologies that introduce large approximations regarding their preferred protonation states. In light of that, our project aims to use constant-pH molecular dynamics (CpHMD) simulations to investigate the acid-base equilibrium of PIPs in different environments, including their specific interaction with certain proteins. To achieve our goals, we simulated differently phosphorylated inositol rings (Ins1P, Ins(1,4)P2, Ins(4,5)P2 and Ins(1,4,5)P3) and PIPs (PI4P, PI(4,5)P2, PI(3,4)P2, PI(3,5)P2, and PI(3,4,5)P3), employing the CHARMM force field and pH values ranging from 0 to 9. In this communication, we will present all titration curves and respective pK_{a} values obtained from the simulations, together with their validation with experimental NMR data [1,2].

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Poster Presentation 26

(De)Activation Mechanisms in Class A GPCRs: Protonation Insights from MD and CpHMD Simulations

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

G protein-coupled receptors (GPCRs) are key regulators of many cellular processes. In human cells, more than 800 different GPCRs act as a therapeutic target for roughly 35% of all pharmaceutical drugs currently on the market. Evidence suggests that class A GPCRs might share common (de)activation pathways involving (de)protonation of key residues that will eventually lead to the major conformation changes seen upon the transition between the 2 activation states[1]. In particular, the protonation state of Asp2.50 has been suggested to act as a pivotal microswitch within this mechanism[2]. Using pK_a calculations performed with PypK_a, over the trajectories of short CHARMM36m MD simulations (100 ns), we have explored the protonation behaviors of protonated and deprotonated Asp2.50 in active and inactive starting conformations of 5 class A GPCRs. A Linear Response Approximation protocol was employed to estimate macroscopic pK_a values for each conformational state[3]. Recently, we have extended our latest CpHMD protocol to membrane proteins in CHARMM36m, and we have run longer CpHMD simulations (250 ns) for one of the GPCRs systems, giving us more robust insights about the protonations and conformational changes of this system. Here, we present our current data regarding our MD+pK_a calculations protocol and preliminary data from CpHMD. Using these approaches, we aim to clarify the molecular mechanisms underlying GPCR (de)activation regarding their (de)protonation events. These are the first steps in understanding disease-causing mutations and dysfunctions within these proteins, which can offer valuable insights for the development of next-gen therapeutic approaches.

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