3D-BIOINFO-PT

Annual Meeting

The Portuguese Community of Structural Bioinformatics Researchers

Oeiras, 21st December 2022

Sponsors of the 2022 3D-BioInfo-PT Activity Cycle

Mission and Goals

In organizing the first 3D-BioInfo-PT Community Meeting we are at once carrying on the tradition of the decade-old EJIBCE meetings (Encontro de Jovens Investigadores em Biologia Computacional Estrutural) and turning a new leaf in the involvement with the community.

We keep the tradition in that this Meeting, just like the EJIBCE before it, is planned as a forum for exchange, networking, and socialization within our vibrant community. Born from the common root of computational techniques, but showcasing a plurality of methods and applications, the Meeting will be technically specific and yet cover broad topics that we hope will foster collaboration between participants working in different sub-fields.

We turn a new leaf in that the rebranding of EJIBCE as 3D-BioInfo-PT is much more than just that. Having been taken under the Biodata.pt umbrella at the end of 2021, we aimed at an expansion of outreach. In 2022, 3D-BioInfo-PT has organized (ongoing) seminar and workshop cycles and established contact with its European-level counterpart towards shared initiatives. These actions already multiply our impact by setting the community also on a teaching mission and by preparing a platform to propel Portuguese research internationally. The future looks bright!

Finally, we hope the organization of this meeting conveys that the EJIBCE-to-3D-BioInfo-PT rebranding was *not* motivated by the "J" in EJIBCE no longer applying to some members of the community. The roster of presenters showcases the next generation of researchers in the field, and attendance was kept free so that precisely the younger researchers can easily participate.

May our community be as young as ever!

In the name of the Organizing Committee,

Manuel N. Melo

Organizing Committee – ITQB NOVA

Ana Carolina Araújo, MSc Student

Carolina C. Buga, PhD Student

Fernando N. Nunes, PhD Student

Gonçalo Vieira, MSc Student

João Correia, MSc Student

Manuel N. Melo, PhD

Mariana Valério, PhD Student

Pedro Moreira, PhD Student

Rita I. Teixeira, MSc Student

Rodrigo Barriga, MSc Student

Susana Parreiras, PhD Student

Scientific Committee

Armindo Salvador, President of the Portuguese Biophysical Society (SPBf), UC

Diana Lousa, ITQB NOVA

Manuel N. Melo, ITQB NOVA

Paulo Souza, CNRS/Université Lyon

Welcome Message From The Organizing Committee

Welcome to the first edition of the 3D-BioInfo-PT Community Meeting!

It is with great honor that we host this event, with the prospect of bringing together the portuguese Computational Structural Biology community and planting the seed of curiosity and fervor in the young researchers who are taking their first steps into this field.

The 3D-BioInfo-PT Community Meetings are a unique and important opportunity for young researchers to get to know the community and learn from its members, as well as for everyone attending to discover the great science that is being done by portuguese researchers across the world. We hope to offer an exciting program, showcasing the works of excellence that the community has invested so tirelessly on for the past year.

In the year of 2022, the event is taking place at Instituto de Tecnologia Química e Biológica António Xavier, from Universidade NOVA de Lisboa (ITQB-NOVA), where the spirit of unity has always been prevalent. We believe that science can only thrive through cooperation and partnership, and the opportunity to host an event like the 3D-BioInfo-PT Community Meeting is a reflection of that.

ITQB-NOVA is centered in Oeiras, a city that continuously invests in science, technology, and innovation. Not only is it one of the leading portuguese cities in education, it is also home for some of the most beautiful beaches in the country. Located near the Tagus river, Oeiras is the perfect choice for a cultural and nature-filled trip.

Still keeping the tradition of the previous EJIBCE (Encontro de Jovens Investigadores em Biologia Computacional Estrutural) meetings, we enter this new stage for the community hoping that our best is still to come.

We look forward to seeing you!

On behalf of the Organizing Committee,

Ana C. Borges-Araújo

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Program

Wednesday, December 21st 2022

8:30 **Registration**

9:30 **Opening Session**

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Cláudio M. Soares, Dean of the ITQB-NOVA
Manuel N. Melo, 3D-BioInfo-PT-Community

9:45 **Carlos S. H. Shiraishi**

"Virtual screening of a library of natural compounds against COX-2 protein"

10:00 **Gabriel F. Martins**

"Polyphenols as Aggregation Inhibitors: Application to α-Synuclein and Related Peptides"

10:15 **Israa Aljnadi**

"In Silico Studies and Chemical Synthesis of Ligands Targeting c-MYC G-Quadruplex"

10:30 **Marta S. P. Batista**

"Structural characterization of the permeation of water and glycerol derivatives through PfAQP for the development of new antimalarial therapies"

10:45 **Rita I. Teixeira**

"The impact of SARS-CoV-2 Omicron variant on the interaction with human ACE2"

11:00 **Coffee Break and Poster Session I**

11:45 **Wallfuture**

11:55 **Keynote Lecturer – Carla Sousa**

"MD simulations applied to Infection research: improving studies of drug permeation across biomembranes"

12:55 **Tomás F. Silva**

"Tackling the realism of transmembrane peptides simulations with a pH-Gradient/CpHMD approach"

13:05 **Pedro C. Rosado**

"Exploring PBP2a protein to fight β-lactam resistance in MRSA"

13:25 **Rodrigo Barriga**

"Simulating substrate binding sites in the *S. aureus* Type II NADH Dehydrogenase"

13:40 **Tatiana F. Vieira**

"What can computational methods do to help find new molecules with antimicrobial properties"

13:45 **Lunch**

SPBf Session

15:50 **Armindo Salvador**

Presentation of the Portuguese Biophysical Society (SPBf)

16:00 **Pedro Beltrão**

"Studying protein interactions and domain evolution using AlphaFold"

16:45 **Coffee Break and Poster Session II**

17:30 **Bruno Calçada**

"Development of machine learning models to predict thyroid peroxidase inhibition"

17:45 **João G. N. Sequeira**

"Extending the stochastic titration CpHMD to the CHARMM36m and AMBER14SB force fields"

18:00 **Urszula Orzeł**

"Oligomerization of metabotropic glutamate receptors"

18:15 **Andreia Fortuna**

"Tackling halogen anisotropy in biomolecular simulations: assessment of force field parameters using hydration free energies"

18:30 **Bárbara Bahls Bruni**

"Design and virtual screening of Indoloisoquinoline derivatives as c-MYC G4 binders"

18:45 **Closing Session**

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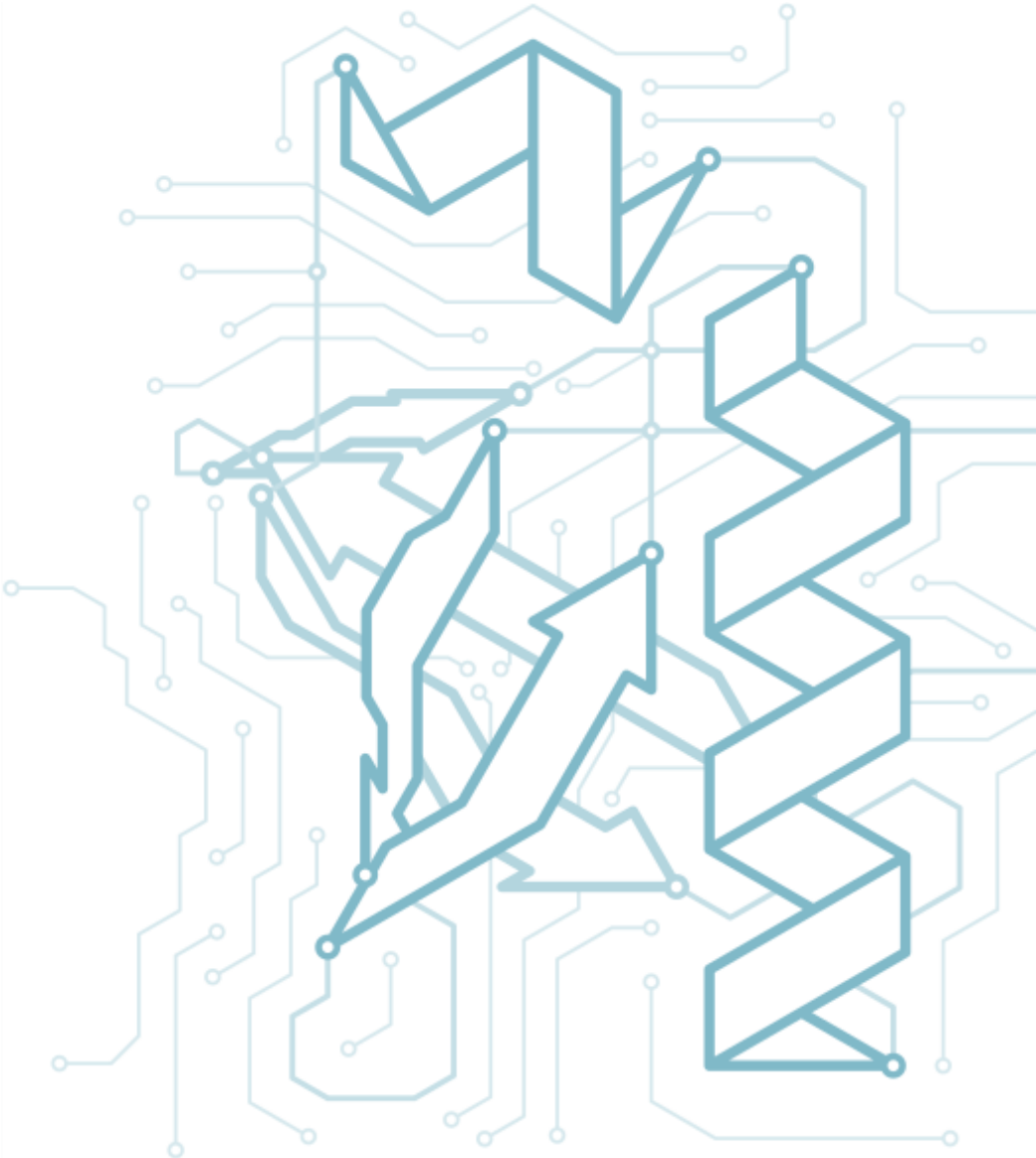
Invited Speakers



Keynote Lecturer – Carla Sousa

After a BSc (2012) and a MSc in Biochemistry (2014), Carla Sousa obtained her PhD degree under the M2B (Medical Biochemistry and Biophysics) International Doctoral Programme (2020). Currently, she is a Post-Doctoral researcher at the Helmholtz Institute for Pharmaceutical Sciences, working in collaboration between the drug bioinformatics and the drug delivery departments. Her current research is mostly focused on the application of computational techniques on the study of the membrane permeation of antibacterial drugs. The aim is to interpret the mechanisms of permeation, obtaining information on the drug's physicochemical properties that are crucial for influx in the bacterial cell.

MD simulations applied to Infection research: improving studies of drug permeation across biomembranes

Carla F. Sousa[1] , Jochen S. Hub[2] , Pedro A. Fernandes[3] , Paula Gameiro[3] ,Claus-Michael Lehr[1], Olga V. Kalinina[1,4]

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[2] Theoretical Physics and Center for Biophysics (ZBP), Saarland University, 66123 Saarbrücken, Germany
[3] LAQV, REQUIMTE, Departamento de Química e Bioquímica, Faculdade deCiências, Universidade do Porto, 4169-007 Porto, Portugal
[4] Center for Bioinformatics, Saarland University, 66123 Saarbrücken, Germany

Molecular dynamics (MD) simulations are a powerful computational tool frequently used to obtain an atomistic view of biological processes, which are difficult to obtain from experimental findings. Specifically in the field of infection research and drug discovery, studying drug permeation across the membrane barrier is crucial, since most of the drugs have intracellular targets. MD simulations can be used to obtain a detailed picture of this process, allowing to follow parameters that usually have strong influence on permeation, such as solute orientation and interactions with the solvent/membrane. The potential of mean force (PMF) for solute permeation can be typically computed using enhanced sampling techniques, such as umbrella sampling (US). Macroscopic constants, such as the partition and permeability constants, can then be calculated and compared with experimental findings. We have used MD simulations to study the effect of copper-chelation of antibiotics on improving permeation across the membrane pathway[1, 2] and also assessed the effect of hydrophobicity on permeability across a bacterial membrane model[3]. These studies shed a light on how MD simulations can give important insights on the

mechanism of drug permeation and help in the development of drugs with improved permeability and, consequently, improved antibacterial activity. Nevertheless, convergence of the PMFs remains a great challenge for bulky drug-like molecules, requiring long simulation times and often resulting in an unacceptable computational cost. Hence, we tested augmenting US with simulated tempering (STeUS) to improve the convergence of these calculations[4]. We found that STeUS accelerates convergence more than five times for bulky molecules, for which standard US converges poorly. This study established STeUS as an efficient and simple method for PMF calculations, thereby strongly reducing the computational cost of routine high-throughput studies of drug permeability.

References

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SPBf Keynote Lecturer – Pedro Beltrão

Pedro Beltrão has a Biochemistry degree from University of Coimbra (2002) and a PhD in Biology from the University of Aveiro for research conducted at EMBL-Heidelberg under the supervision of Luis Serrano (2007). He did his postdoctoral research at the University of California San Francisco under the supervision of Prof. Nevan Krogan (2008-2012). He was a group leader at EMBL-EBI from 2013 to 2021 and he is now an Associate Professor at the Department of Biology, ETH Zurich from Jan 2022.

Studying protein interactions and domain evolution using AlphaFold

All cellular functions are governed by complex molecular machines that assemble through protein-protein interactions. Advances in sequence based structural predictions are allowing for unprecedented studies of the evolution of protein structures and the structural characterization of protein interaction networks. I will first describe our work testing the potential and limitations of using AlphaFold2 to predict the structures of complexes from binary complexes to higher order assemblies. AlphaFold2 can predict structures of complexes with 50-80% confidence, depending on stringency, which can be orthogonally confirmed by spatial constraints defined by cross-link data. The prediction of structures for higher order assemblies is limited by lack of knowledge on stoichiometry of subunits and presence of paralogs within complexes. In addition, we are using 200 million predicted structures to study the structural diversity of protein families at the scale of the known protein universe. I will describe the challenges of analyzing data at this scale and an approach to predict protein domain families by clustering of structural similarity scores. I believe these approaches may serve as a rich source of hypotheses for the evolutionary study of protein families.

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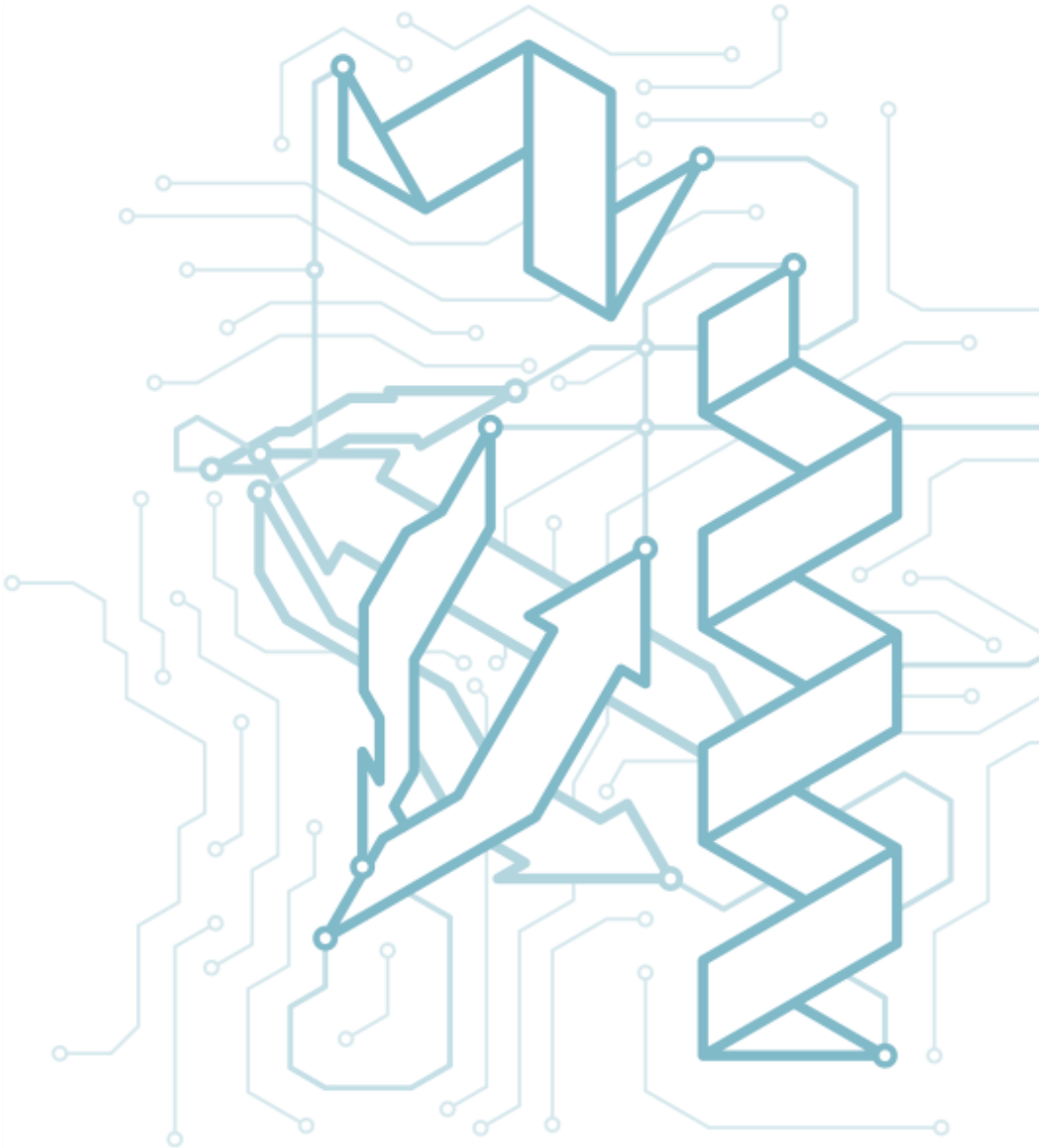
Oral Communications

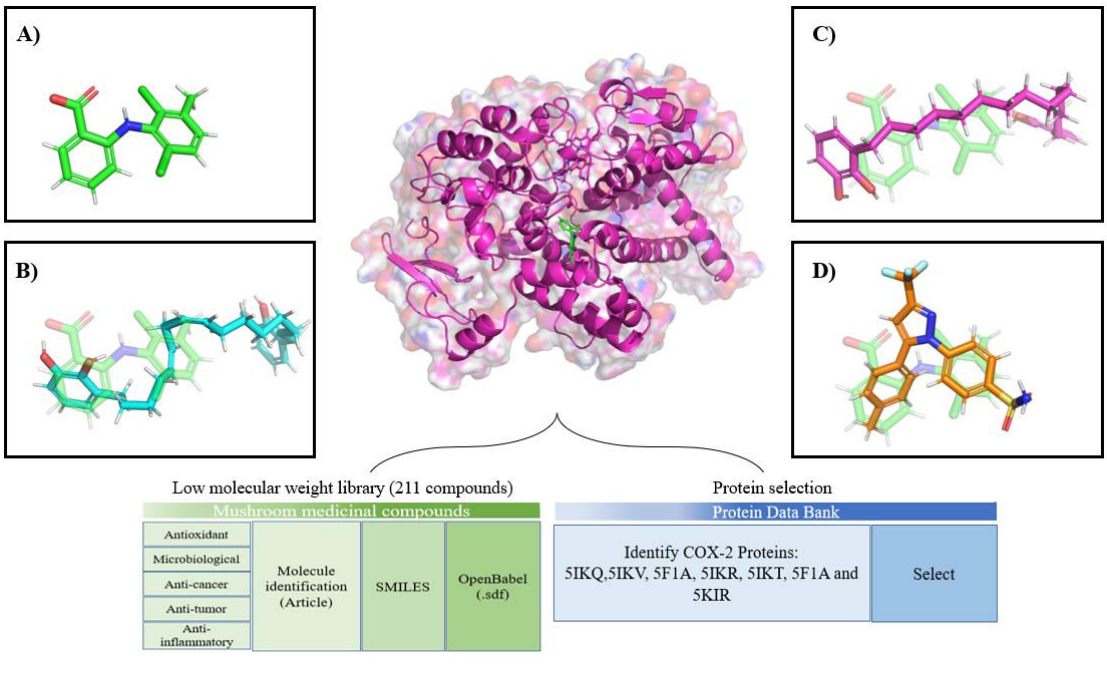


OC1 – Carlos S. H. Shiraishi

Virtual screening of a library of natural compounds against COX-2 protein

Carlos S. H. Shiraishi, José Rufino, Sergio F. Sousa, Miguel A. Prieto, Rui M.V. Abreu*, Lillian Barros

Non-steroidal anti-inflammatory drugs (NSAIDs) used in the treatment of inflammatory diseases act mainly by promoting the inhibition of cyclooxygenase enzymes (COX-1 and COX-2), inducing significant anti-inflammatory, analgesic, and antipyretic activity. However, recent data show prolonged use can lead to cardiovascular side effects. Thus, the present work aims to identify potential COX-2 inhibitors alternatives obtained from natural sources, such as mushrooms, that have already established themselves as a source of natural compounds. In this study, a virtual screening of a library of 211 low molecular weight compounds present in mushrooms was tested. In silico studies against COX-2 protein were conducted with GOLD docking software and the PLPScore function, using a crystallographic ligand for redocking validation. For library preparation, Openbabel was used to obtain the library molecules in .sdf format, and the results were analyzed with Discovery Studio software. The results obtained from Virtual Screening showed that of the 211 compounds present in the library, Gerronemin E and Gerronemin C, both compounds from the mushroom Genorrema, showed the best results with scores of 102.7 and 97.7, respectively (Figure 1B and C). The observation of the ligand overlap in the Re-docking process (Figure 1A) and the results of a commercial inhibitor (Celecoxib) with PLPscore 69.4 were also analyzed (Figure 1D). Thus, these in silico studies predict promising natural inhibitors of COX-2 that need to be experimentally evaluated.



OC2 – Gabriel F. Martins

Polyphenols as Aggregation Inhibitors: Application to α-Synuclein and Related Peptides

Gabriel F Martins, Catarina Nascimento, Nuno Galamba

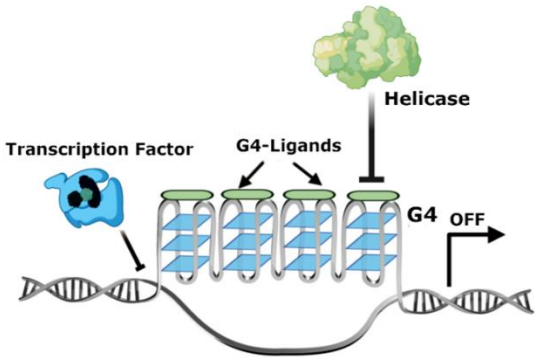
Several polyphenols were shown to inhibit or modulate the aggregation of proteins associated with neurodegenerative diseases, such as Parkinson's disease (PD). However, discrepant action mechanisms have been reported. Furthermore, these compounds are often described as pan-assay interference compounds (PAINS), raising questions concerning their effective potential. In this work, we used molecular dynamics and enhanced sampling methods to study the aggregation of two 11-mer peptides from the non-amyloid-b component (NAC) – an aggregation-prone domain of α-synuclein (α-syn) implicated in PD – in neat water and aqueous solutions of resveratrol (RSV) and gallic acid (GA). We also assessed the structure of α-syn in neat water, and aqueous solution of urea (a protein denaturant), RSV, and GA. Our results indicate that aggregation is not disrupted by either one of the phenolic compounds. Yet, the intrusion of both RSV and GA in the inter-peptide region results in a re-orientation of the peptide's dimer. This favors terminal interactions that translate into barrierless solvent separated configurations, extending over large distances. These findings indicate that, if anything, the studied (poly)phenols delay or modulate peptide aggregation at high concentrations via the stabilization of solvent-separated conformations, as opposed to aggregation inhibition. Structural analysis of the full protein, however, show that the (poly)phenols induce more extended conformations of α-syn, similar to urea, thus possibly also reducing its aggregation propensity.

OC3 – Israa Aljnadi

In Silico Studies and Chemical Synthesis of Ligands Targeting c-MYC G-Quadruplex

Israa Aljnadi, Bruno L. Victor, Alexandra Paulo

G-quadruplexes (G4) are four-stranded nucleic acid secondary structures formed by guanine-rich sequences of DNA or RNA. G4 are involved in relevant biological functions of mammalian cells but, more importantly, they are over-represented in cancer cells. Studies have found G4 in telomeres and promoter regions of several oncogenes, including c-MYC, play an important role in cellular regulatory processes as well as in cancer development and progression[1]. G4s are potential anticancer targets to inhibit DNA transcription by blocking the binding of transcription factors. Helicase DHX36 is a helicase that is recruited by cells to bind and unwind G4s in the promoter region of c-MYC. Aiming at targeting this G4:helicase interaction, we used the recently published crystallographic structure of the DHX36 helicase complexed with the c-MYC G4, to develop potential c-MYC G4-DHX36 interaction inhibitors as a novel class of anticancer drugs [1,2]. To achieve such goal, we built a virtual library of 1104 indoloisoquinoline (IDiQ) derivatives and used two different docking softwares, together with a consensus scoring approach, to identify derivatives with a higher probability to bind and inhibit the c-MYC G4-DHX36 interaction. Based on obtained results, we selected a group of the highly ranked compounds and proceeded to their chemical synthesis. In this communication, we will present the results of our computational and chemical studies towards the promising subset of IDiQ derivatives.



G4 ligands stabilize the G4 structure at the promoter region and prevent G4 unwinding by helicase leading to downregulation of oncogene.

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OC4 – Marta S. P. Batista

Structural characterization of the permeation of water and glycerol derivatives through PfAQP for the development of new antimalarial therapies

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

Malaria is one of the largest public health problems. Although most variants are successfully treated with the existing antimalarial drugs, this disease is still responsible for a large number of global deaths. Severe malaria in humans is mostly caused by infection with Plasmodium (P.) falciparum whose complications include severe anemia, end-organ damage, pulmonary complications, and hypoglycemia [1]. The development of hybrid antimalarial agents has been pursued as a promising strategy to tackle resistant parasite strains, eliminating the actively-infecting P. falciparum organisms in human red blood cells, and also the replicative and dormant liver forms of the parasite [2]. The aquaporin of P. falciparum (PfAQP) is a water and glycerol membrane protein channel, allowing the dislocation of these molecules from the host to the parasite. The fast reproduction of P. falciparum in the host red blood cells requires massive biogenesis, in which glycerol is incorporated into the lipids of newly synthesized parasite membranes [3]. Therefore, PfAQP is a promising target for the development of new antimalarial therapies. In this communication, we will present the steps towards a multi-target strategy that couples keystone antimalarial drugs and PfAQP inhibitors. We will show results from Molecular Dynamics, Umbrella Sampling, and Potential of Mean Force calculations aiming at identifying structural determinants regulating the permeation of PfAQP's natural subtracts (water and glycerol), and erythritol and xylitol across the protein channels. These results allowed the identification of the energetic barriers and the structural determinants in this protein's pores that regulate the permeation of the evaluated molecules. Hopefully, the gathered information will boost the future development of a new antimalarial hybrid drug.

Acknowledgments: We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020, UIDP/04046/2020, 2021.09731.CPCA and BioISI Junior Program.

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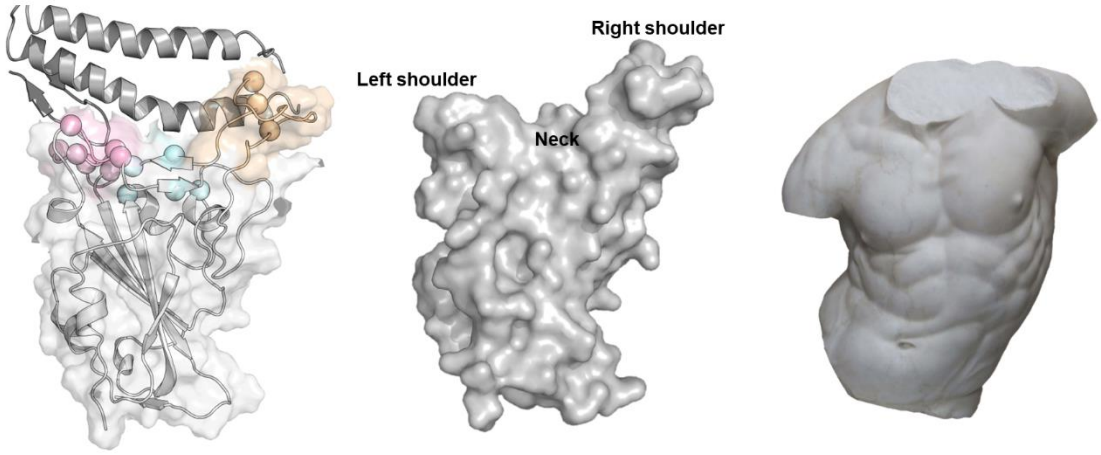
OC5 – Rita I. Teixeira

The impact of SARS-CoV-2 Omicron variant on the interaction with human ACE2

Rita I. Teixeira, Mariana Valério, Luís Borges-Araújo, Manuel N. Melo, João Vicente, Diana Lousa, Cláudio M. Soares

The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 SARS-CoV-2), has led to over 6.6 million deaths worldwide, as of 27th November 2022. The SARS-CoV-2 mechanism of transmission and infection involves the binding of the virus to the angiotensin-converting enzyme 2 (ACE2) host receptor through the Spike (S) protein receptor-binding domain (RBD). The RBD is a privileged target of our immune system and antiviral therapies. Thus, understanding the molecular details of the binding mode is of pharmaceutical interest. Throughout the last few years, multiple vaccines and new therapeutics against SARS-CoV-2 have been developed. However, the emergence of variants of concern (VOC) poses a great challenge due to the loss of natural and vaccine immunity. The rise of the Omicron VOC raised considerable global concern due to the amount of S protein substitutions, fifteen of which are located in the RBD. Later on, the Omicron variant has been divided into seven lineages, of which BA.1, BA.2, and BA.5 became the most concerning ones due to their increased transmissibility. Here, we investigated the impact of the Omicron subvariants on the binding to the human ACE2 (hACE2), by performing microsecond atomistic molecular dynamics (MD) simulations of the Omicron RBDs bound to hACE2. Our analysis of the interface and structural dynamics of the Omicron RBD substitutions provided a detailed characterization of the binding mode, using wt as a control. This allowed us to understand the direct role of key residues on the binding interface with the receptor and identify specific substitutions that may affect binding affinity via the establishment of new interprotein interactions.



RBD interface area definition based on RIP-MD results and RBD anatomy.

OC6 – Tomás F. Silva

Tackling the realism of transmembrane peptides simulations with a pH-Gradient/CpHMD approach

Tomás F. Silva, Diogo Vila-Viçosa and Miguel Machuqueiro

The pHLIP family is composed of pH-dependent membrane-inserting peptides used as tumor biomarkers and drug delivery systems, by taking advantage of their ability to fold into membranes at acidic microenvironments. The thermodynamic stability of the α-helical inserted state of the wt peptide (pKtransition ≈ 6.0) is dictated by crucial deprotonation events of a key aspartate (Asp14 - pKa ≈ 6.0) [1]. Although widely used, the wt peptide lacks tumor specificity and fast insertion/exiting kinetics. Several variants have been designed to address these issues, of which Var3 showed interesting results [2]. The Var3 peptide has better insertion/exit kinetics with a similar key titrating residue (Asp13). Interestingly, it performed poorly as a biomarker (pKtransition ≈ 5.0) in liposome experiments, while outperforming the wt in cell conditions [2]. Among the various differences between cells and liposomes, the pH gradient is a crucial cell property now implemented within the CpHMD methodology [3,4]. The pH gradient setup assigns a constant internal pH value (~7.2) while allowing the external pH to change, thus mimicking the pHLIP-cell microenvironment. Our systematic study has shown that, in the wt cell-like model, Asp14 interacts less with a nearby arginine (Arg11) than in the liposomal setup, therefore promoting pKa shifts to higher values (pKa ≈ 7.1) and corroborating the performance loss in cell conditions [4]. Meanwhile, the Var3 peptide performed similarly in both setups, as a result of a trade-off between the strong interactions in the Asp13 electrostatic network [4]. In conclusion, implementing a pH gradient setup provided more accurate and realistic pKa calculations, aiding in the interpretation of experimental phenomena, while bridging the gap between in silico models and cells.

We acknowledge FCT funding through projects UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017, and SFRH/BD/140886/2018.

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OC7 – Pedro C. Rosado

Exploring PBP2a protein to fight β-lactam resistance in MRSA

Pedro C. Rosado, M. Matilde Marques, Gonçalo C. Justino

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial infections, with a high mortality rate associated with multi-resistance to β-lactams. MRSA resistance comes from the acquisition of a PBP2a coding mecA gene. This protein has low affinity towards β-lactams, meaning that peptidoglycan formation is not blocked, and bacteria survive [1, 2]. Reduced susceptibility to β-lactams is related to PBP2a changes in the active site and to the presence of a loop protecting the active site. Conformational change of this loop is regulated by an allosteric site [2]. Thus, there is a pressing need for innovative antibiotics. In this work, a structure-based computational molecular docking screening approach was employed with Autodock Vina, using the X-ray structures of both closed and open PBP2a conformations (PDB ID 1vqq and 3zg0, respectively). Various lactam scaffolds, fluorenone, flavone and quinazolinone derivatives were tested as possible inhibitors for both sites. Known specific inhibitors were also tested. Molecular dynamics simulations using GROMACS were deployed to understand whether binding of natural substrate and hit compounds can induce protein conformational changes. Known inhibitor L-695256 with best results to the target protein presented affinities of -6.2 kcal.mol 1 (allosteric site in the native PBP2a) and -9.4 kcal.mol-1 (active site of open PBP2a protein). Promising hit compounds tested in this work presented significant improvements in affinity for both catalytic sites, for instance, -8.1 kcal.mol-1 (allosteric site) and -12.1 kcal.mol-1 (active site). Hit compounds recapitulated protein-ligand interactions of known inhibitors. Moreover, binding of promising hit compound to the allosteric site induces protein conformational changes, exposing the catalytic site. These results indicate that tested compounds are promising hits targeting PBP2a protein. Currently, more molecular dynamics simulations are being deployed to understand whether the binding of hit compounds to the allosteric site can induce protein conformational change, contributing to a more accessible active site.

Acknowledgments: This work was funded through Research Grant PTDC/QUI-QAN/32242/2017 (MMM). PCR is an FCT-funded PhD student (UI/BD/152269/2021). Part of this work is funded through Rede Nacional de Computação Avançada (FCT/CPCA/2021/01). Centro de Química Estrutural is a Research Unit funded by Fundação para a Ciência e Tecnologia through projects UIDB/00100/2020 and UIDP/00100/2020. Institute of Molecular Sciences is an Associate Laboratory funded by FCT through project LA/P/0056/2020.

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OC8 – Rodrigo Barriga

Simulating substrate binding sites in the *S. aureus* Type II NADH Dehydrogenase

Rodrigo Barriga, Manuela M. Pereira, Manuel N. Melo

Type II NADH Oxidoreductase (NDH-2) from *Staphylococcus aureus* was established as a therapeutic target against the virulency of this bacterium and an alternative to treat Complex I-derived diseases. To accurately model interactions of NDH-2 with its substrates such as menaquinones and NADH, Coarse-Grain (CG) simulations were employed. We used the Martini 3 CG force field, for which relevant molecules were parameterised. Martini follows a building-block approach; our parameterisation thus yielded a set of 35 molecules including other quinones (parameterised following a bottom-up approach) and nucleotides (which followed a top-down approach). Model validation compared atomistic and CG Connolly surfaces, their solvent accessible surface area (SASA) differences and the calculation of octanol-water partition coefficients (logPs). Overall, SASA differences were below the generally accepted limit of 5%. LogP analysis showed phosphorylated compounds, and alcohols to a lesser extent, are likely too hydrophilic in Martini 3. We employed mitigation strategies. Aqueous simulations showed the expected in vivo interactions and selectivity of NDH-2 towards menaquinones. These quinones were also seen to prefer more extensive binding sites of all quinones. Furthermore, we established that D302 would bind NADH by its adenine and enable its bending to interact with FAD, NDH-2's cofactor. For larger simulations, a model of the *S. aureus* membrane was built. Its fluidity is kept by the existence of methyl branches in its constituting lipids. We mimicked this fluidising effect with a degree of tail bending and were able to recover a gel-to-fluid transition of 293 K. We also observed that NDH-2 was able to pull menaquinones out of the membrane more than their usual fluctuations, highlighting how it is able to catalyse electron transfer monotopically. We hope this work contributes to future research to unveil new potential targets to inhibit NDH-2 and as well for the Martini community.

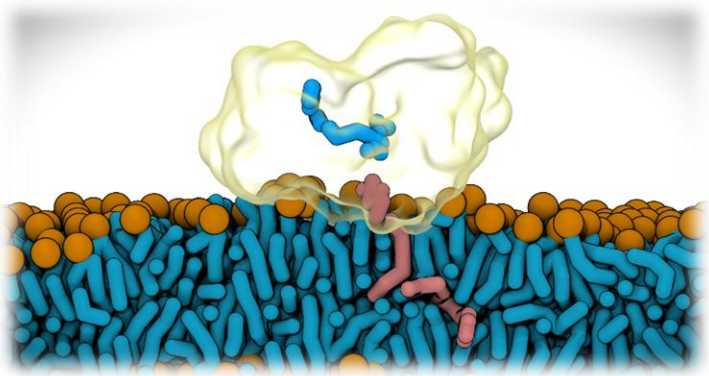

Figure caption - Representation of NDH-2 (Type II NADH Oxidoreductase) of *S. aureus* (in yellow) and its cofactor (FAD, in blue) on top of its membrane (turquoise) pulling out a menaquinone (pink). Phosphate beads are represented in brown.

OC9 – Tatiana F. Vieira

What can computational methods do to help find new molecules with antimicrobial properties

Tatiana F. Vieira, Sérgio F. Sousa

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen, that causes acute and/or chronic infections especially in immune-compromised and hospitalized patients. These infections are very difficult to eradicate because the bacteria can be organized in structured microbial communities forming biofilms, a structured microbial community of surface-attached cells embedded in a self-produced matrix of extracellular polymeric substances (EPS). Biofilms are responsible for 80% of all bacterial infections each year, in the US alone1,2 and finding new strategies to control its formation and development is of the utmost importance. Here we report the optimization of a methodology using docking and virtual screening to identify new drugs against a specific quorum-sensing system in P. aeruginosa responsible for the transcription of virulence genes and biofilm development, the PQS system. Four large databases of compounds (such as IBS InterbioScreen, Mu.Ta.Lig Chemotheca, Chimiothèque Nationale and ZINC), were used for the VS stage after careful optimization and validation. Then, molecular dynamics simulations and free energy calculations were performed in the top 5 results of each database to further validate the docking results and have a better understanding of the residues involved in the protein-ligand interactions. Two compounds from the ZINC database of approved drugs were selected for the experimental study of the inhibition of the QS system, with promising preliminary results.

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This work was supported by national funds from Fundação para a Ciência e a Tecnologia [grant numbers UIDP/04378/2020 and UIDB/04378/2020, SFRH/BD/137844/2018, 2020.01423.CEECIND]. Some of the calculations were produced with the support of INCD funded by FCT and FEDER under project 01/SAICT/2016 number 022153 and projects CPCA/A00/7140/2020 and CPCA/A00/7145/2020.

OC10 – Bruno Calçada

Development of machine learning models to predict thyroid peroxidase inhibition

Bruno Calçada, Andrea Sartini, Adrian Fowkes, Bruno L. Victor, Paulo Costa

The increased incidence of endocrine-related human diseases due to recurrent daily exposure to endocrine-disrupting chemicals (EDCs) has led the European Commission to issue a Guidance focused on the identification of these compounds in the context of biocidal and Plant Protection Products [1]. Therefore, the development of new in silico methodologies capable of identifying EDCs is of utmost importance to support decision-making for chemical risk assessment, but also as a means of ensuring the 3R principles (Replacement, Reduction, and Refinement) regarding the use of animals in product testing. In this communication, the development of machine learning (ML) models focused on thyroid pathways, more specifically on the inhibition of thyroid peroxidase (TPO), will be presented. This target is paramount in the regulation of multiple physiological processes as it is involved in the generation of the T3 and T4 thyroid hormones and importantly validated alternative tests are lacking. Training data was taken from a high-throughput in vitro assay developed for the EDSP by the US EPA [2]. A curation workflow was implemented to appropriately handle the chemical and bioactivity information and compared to previously reported workflows [3]. Dataset curation rules resulted in the training of more accurate models compared to models trained on the raw data. ML models were trained on the enhanced data to identify the best algorithm and settings with the best classifier for TPO inhibition presenting an MCC = 0.76 against a hold-out test set. In the future, workflows and models such as these will help in the accurate identification of endocrine disruptors.

Funding: Ascenza Agro and FCT (Projects UIDB/04046/2020-UIDP/04046/2020, and 2021.09731.CPCA)

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OC11 – João G. N. Sequeira

Extending the stochastic titration CpHMD to the CHARMM36m and AMBER14SB force fields

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

Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to describe pH and its effects on the conformational space of biological systems [1]. The stochastic titration CpHMD method [2] has shown excellent performance over the years [1–3]. Until recently, our implementation of this method only supported the GROMOS 54A7, taking advantage of its compatibility with the Reaction-Field formalism to treat long-range electrostatic interactions [3]. However, we have recently extended this CpHMD method to include CHARMM36m [4], an all-atom force field that is particularly suited for proteins, nucleic acids, and lipids simulations. In this new setup, the long-range electrostatics are dealt mainly by PME, which requires total system charge neutralization, a problem that needs addressing by all CpHMD implementations [3]. To test the new implementation, we did a benchmark study using several proteins, namely, lysozyme, Staphylococcal nuclease, and human/E. coli thioredoxins [4]. The RMSE values (pKa prediction vs experimental) obtained were very encouraging, in particular for lysozyme (0.90 and 0.96) and human thioredoxin (0.82 and 1.00). We have also identified a few challenging residues that highlight scenarios where the method still needs improvement, independently of the force field. The CHARMM36m implementation was more computationally efficient when compared with the GROMOS 54A7, taking advantage of a shorter nonbonded interaction cutoff and a less frequent neighboring list update. We are currently working on the extension of this method to support the AMBER 14SB force field. 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 CpHMD-compatible. Here, we will present our preliminary results using this protocol.

System	Total residues (N)	Titrable residues (N)	FF	CpHMD (ns/day)
HEWL	129	21	G^{54A7}	15.7 ± 0.1
			C^{36m}	17.5 ± 0.1
SNase	149	56	G^{54A7}	10.4 ± 0.0
			C^{36m}	10.7 ± 0.1

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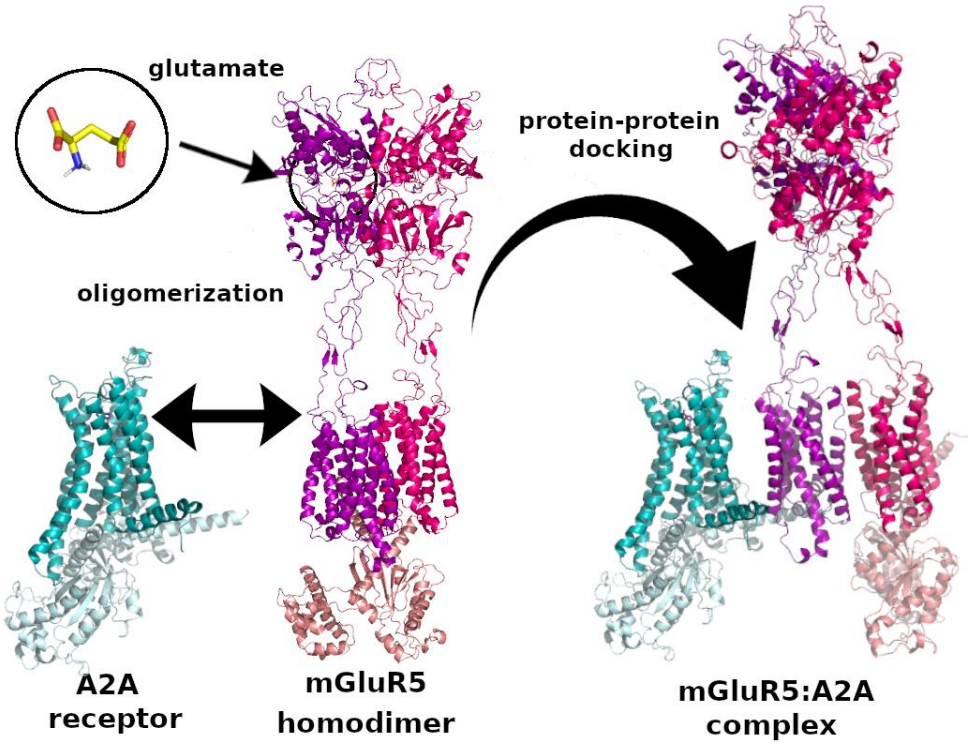
OC12 – Urszula Orzeł

Oligomerization of metabotropic glutamate receptors

Urszula Orzeł, Beatriz Bueshbell, Carlos Baretto, Sławomir Flipek, Irina S Moreira

Metabotropic glutamate receptors (mGluR1-8) belong to class C of G-protein coupled receptors (GPCR) family and have characteristic extracellular domain. They form homodimers, obligatory for their activity [1], as well as oligomers with other GPCRs. The mGluR5 was shown to oligomerize with various class A GPCRs such as dopamine D1 receptor [2], dopamine D2, adenosine A2A receptor [3], and mu opioid receptor (MOR) [4]. Probably mGluR5 forms even higher oligomers, e.g., heterotetramer with the receptors: adenosine A2A, dopamine D2 and cannabinoid CB1 [5]. Due to its interactions with other proteins as well as involvement in synaptic plasticity [6], the mGluR5 was reported as a promising therapeutic target for numerous diseases and disorders, such as drug addiction [7], Alzheimer's Disease [8] and Parkinson's Disease [9]. So far, there are no experimental structures of mGluR5 oligomers available. Therefore, we are developing a multi-component protein-protein docking protocol and analysis pipeline dedicated to GPCR receptors to study the structure of mGluR5 complex with adenosine A2A receptor.



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OC13 – Andreia Fortuna

Tackling halogen anisotropy in biomolecular simulations: assessment of force field parameters using hydration free energies

Andreia Fortuna, Paulo J. Costa

Halogenated compounds are present in drug discovery due to their propensity to improve drug-like properties. Moreover, these compounds can interact with biological targets (e.g. proteins [1] and membranes [2]) via halogen bonds (XB), owing to the anisotropic electrostatic potential of halogens. Force fields typically assign a negative charge to halogens, preventing the establishment of XBs. To overcome this, the introduction of off-center point charges (EP) is commonly used. Various EPs are described in the literature and despite their efficiency in reproducing protein-ligand geometries and sampling of XBs [1], their performance in predicting experimental properties such as hydration free energies (G_hyd) remains unknown. This latter step is crucial in FF validation. Recently, we studied the impact of halogen radii in G_hyd using PBSA calculations [3,4]. Taking experimental G_hyd values as a reference, we provided optimized halogen radii for PBSA calculations using several EP implementations in the scope of AMBER/GAFF and CGenFF/CHARMM. Although PBSA, which treats the solvent as a continuum, substantially decreases the computational cost, proper validation requires the usage of explicit solvent models. Thus, we also evaluated the performance of the literature EP implementations in the prediction of G_hyd using alchemical free energy calculations. In this communication, the results obtained with the different EPs and their comparison with the experimental values, both for implicit and explicit solvent models, will be presented. By assessing the performance of each model, this endeavor will provide the community with properly validated parameters for halogenated species which are relevant in computer-aided drug design and biomolecular simulations.

Acknowledgments: FCT for grant SFRH/BD/146447/2019 (AF), CEECIND/00381/2021 (PJC), and projects UIDB/04046/2020–UIDP/04046/2020 (BioISI) and UID/DTP/04138/2019 (iMed.U-Lisboa).

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OC14 – Bárbara Bahls Bruni

Design and virtual screening of Indoloisoquinoline derivatives as c-MYC G4 binders

Bárbara Bahls Bruni, Alexandra Paulo and Bruno L. Victor

Mutations, amplifications and translocations in specific regions of the genome, such as in c-MYC, a gene encoding for a “master” transcription factor, are associated with cancer development and progression. c-MYC protein has for long be considered a drug target for cancer therapy, but so far all the efforts have been unsuccessful [1]. c-MYC transcription can be regulated by several mechanisms and proteins, including DNA secondary structures like G-quadruplexes (G4) located in its promoter region [1]. These dynamic but relatively stable secondary DNA structures have been currently tested as therapeutic targets, since it was found that they inhibit the transcription of oncogenes such as c-MYC [2]. On the other hand, helicases such as DHX36, control the formation of G4s, by binding and unfolding them [3]. Currently, several small molecules are being developed to target G4 structures, however with still low therapeutic efficacy [4]. With this project, we propose a new approach to the identification of new anticancer drugs, focused on targeting the interaction of c-MYC G4 and its negative regulator, the helicase DHX36. To achieve this goal, we built and evaluated a custom-made virtual library of 1080 ‘drug-like’ indoloisoquinoline derivatives for binding to c-MYC G4 region recognized by DHX36. Molecular docking studies showed that asymmetric N11-N5-di-substitutions with methylpyridines and acetylpyridines of the 5-amino-indoloisoquinoline core exhibited better binding energies to c-MYC G4. Taking these results into account, we designed two synthetic routes to achieve the desired compounds. The first one explored the nucleophilic reactivity of the 5-amino group, but without success, while the second route explored the reactivity of the indole nitrogen, allowing the synthesis of 5 indoloisoquinoline derivatives with moderate (21-39%) to good (57-86%) yield of isolated pure compounds. Using a FRET melting assay we concluded that all synthesized mono-substituted indoloisoquinolines are poor binders/stabilizers of G4, which agrees with the molecular docking studies. These results indicate that different synthetic approaches are required to synthesize the most promising derivatives identified from the docking calculations.

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3D-BIOINFO-PT

Annual Meeting

Poster Communications

PC1 – Ana B. Carniceiro

3D interactome of GPCR sub-family A17: understanding the binding specificity of GPCRs complexes with G proteins and Arrestins

Ana B. Caniceiro, Beatriz Bueschbell, Irina S. Moreira

G protein-coupled receptors (GPCRs) mediate several signalling pathways through a general mechanism that involves their activation and, consequently, interaction with binding partners, such as G protein and arrestin [1]. The structural determinants that mediate the coupling between GPCR and the binding partners remain unknown. In this project, we developed a protocol to assemble the GPCR-Complexes, to reach the main interactome of these proteins. The project was focused on a particular relevant sub-family of receptors, A17, that are highly associated with neurological diseases. The GPCR structures in active state, as monomer and complex with the binding partners, were obtained by homology modelling and their interfaces were refined with HADDOCK [2]. A detail description of protein interfaces of all members of the GPCRs sub-family A17 with the corresponding binding partners was performed [3,4]. Understanding structural and dynamic determinants of complexes may reveal the underlying molecular mechanisms that regulate several physiological and pharmacological outcomes associated with the GPCR sub-family A17.

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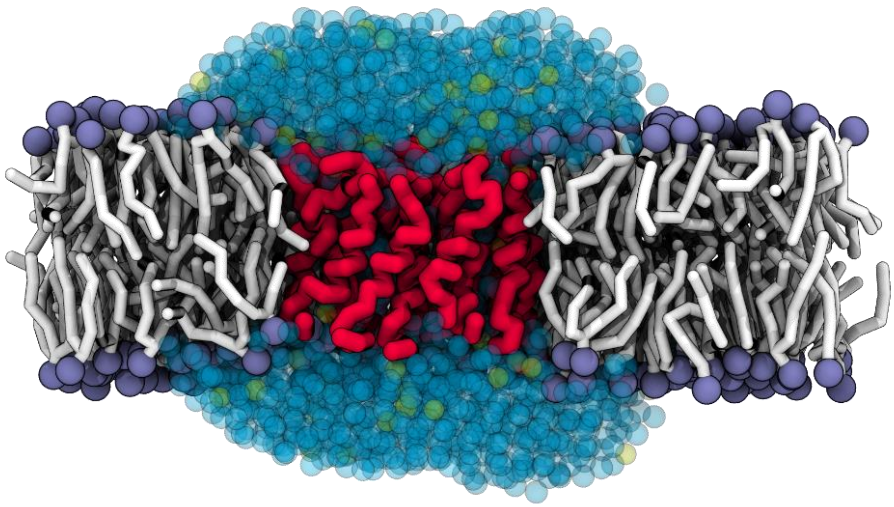
PC2 – Ana C. Borges-Araújo

A tale of peptides and their pores: Study of alamethicin pore formation in lipid membranes using the Martini 3 coarse-grained force field

Ana C. Borges-Araújo, Manuel N. Melo

Alamethicin is an antimicrobial peptide produced by the fungus Trichoderma viride, famously known for its activity against Gram-positive bacteria and fungi. It aggregates to form ion-conducting channels according to the barrel-stave model - in which the peptides' hydrophilic group is aligned towards the centre of the pore. Given the growing number of antibiotic resistant bacterial strains, the action and behaviour of antimicrobial peptides, like alamethicin, have been thoroughly studied to target this concern. Previously, it was not possible to study alamethicin's pore behaviour in Martini 2 coarse-grained simulations due to the Martini 2 water beads not being able to sufficiently penetrate into the membrane as well as the establishment of continuous interpeptide contacts between alamethicin residues. However, with the improved protein-protein interactions and the new and specific water bead type of Martini 3, these hurdles can now be easily overcome. In this work, we studied alamethicin's pore behaviour in Martini 3 coarse-grained simulations. We were able to see alamethicin peptides aggregating into clusters and successfully forming several working channels, with water and ions going through them. In comparison with experimental results, the pore conductance behaviour in coarse-grain is still not faithfully represented, which is something that still needs to be futurely addressed.



PC3 – Carolina C. Buga

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

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

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronaviruses 2 (SARS--CoV--2), emerged in late 2019 and quickly spread worldwide. SARS-CoV-2 is an enveloped virus and its entry into host cells is mediated by the spike glycoprotein (S--protein) [1]. The S--protein is composed of two subunits (S1 and S2) that contain essential domains for the viral entry mechanism, such as the fusion peptide (FP) which inserts into and disturbs the host cell membrane promoting the fusion between viral and host membranes. Despite its relevance for viral entry, there is still no consensus among scientists for its location on the S--protein and amino acid sequence, although two major candidate regions have been proposed [2, 3]. To shed light on this matter, we combined computational and experimental methods to characterize and compare the effect of the two putative SARS--CoV--2 FPs. We performed a systematic analysis of the SARS--CoV--2 putative FPs, using Molecular Dynamics simulations, to dissect how these peptides interact with the membrane. In parallel, we evaluated the putative FPs behavior in membrane model systems applying biophysical techniques. Since both FPs revealed modest fusogenic activity, we hypothesized that a longer FP or a cooperation among the individual FPs might be required to achieve fusion between viral and host membranes. Given the pivotal role of the FP to viral entry, our work provides relevant insights on the SARS--CoV--2 entry mechanism.

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PC4 – Catarina Marques-Pereira

A Deep Learning Approach for Protein-Ligand Binding Motifs Prediction using Sequence Derived Information

Catarina Marques-Pereira,* Ana Teresa Gaspar*, António José Preto, Irina Sousa Moreira

* Co-authors: authors contributed equally to this work

Drug discovery for new protein targets and drugs is a challenging task, and it takes on average 1.8 billion US dollars and 10 years for a single drug to arrive at the market. As such, pharmaceutical companies are interested in accelerating the drug discovery process recurring to in silico approaches. Since docking simulations require protein and ligand structures that are not always available, Drug-Target Interactions (DTIs) have been successfully predicted through sequence-based methods. In this study, we developed a Deep Learning (DL)-based approach to predict protein-ligand binding motifs. Our algorithm was optimized and evaluated with several metrics (i.e., Accuracy, Area Under Receiver Operating Characteristic Curve, Area Under Precision-Recall Curve, F1-Score, Matthews Correlation Coefficient, Precision and Recall,) facilitating comparison with the literature. The results attained in the validation dataset point to the high performance of our algorithm. It was particularly promising the correct identification of the positive interacting residues in an extremely unbalanced dataset.

Keywords:

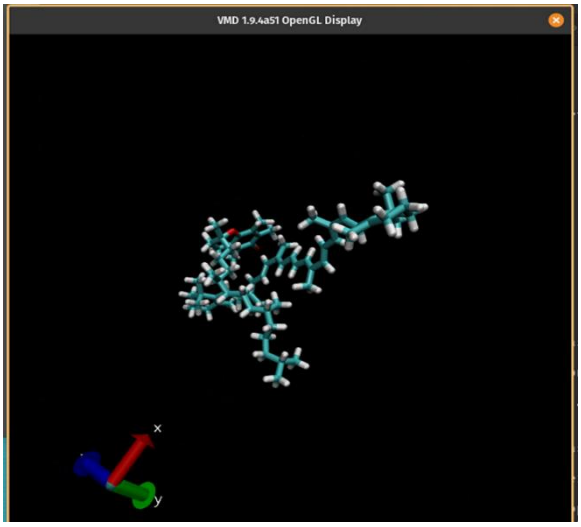
Drug Discovery; Drug-Target Interactions; Machine Learning; Supervised Learning; Neural Networks

PC5 – Cláudia Novais

Molecular dynamics studies targeting coloring and preserving molecules: proposal for the development of bio-based hybrid molecules

Cláudia Novais, Sérgio Sousa, Rui Abreu, Celestino Santos-Buelga, Lillian Barros, Carla Pereira

Although substantial progress has been made in food additives, the controversy in which some of them are still involved has encouraged research into the next generations of safer and healthier foods. These additives can come from natural sources and confer health benefits beyond coloring or preserving, among others. Issues of stability, sustainability, and cost-effectiveness are often related limiting factors that justify the need for innovative solutions.[1] Non-covalent complexation is a natural process and an important mechanism responsible for the stabilization and enhancement of blue, violet, and red colors in flowers, vegetables, and fruits. In this context, copigmentation with antioxidant/antimicrobial molecules can be explored, and the use of new cheminformatics tools and models can be used to support the development of unique dual-function hybrid compounds by predicting and verifying experimental results in order to develop new bio-based molecules as the next generation of food additives.[2] For that purpose, molecular dynamics (MD) simulations were performed considering the crossover of two molecules used as colorants (β-carotene and betanin), against two antioxidants (ascorbic acid and α-tocopherol), evaluating whether they may have binding affinity by calculating their ΔG. System minimizations were performed using the Sander program from the AMBER18 software package; for the equilibrium and production phases, PMEMD was used, and subsequent analysis performed using CPPTRAJ, both from AMBER18 software package. For both colorants, the cross with the antioxidant that showed a higher mean of ΔG was that of α-tocopherol, and it stood out for the cross with β-carotene (-10.08). As for ascorbic acid, the values of ΔG were around zero. Given these results, it would be interesting to explore a possible hybridization between the β-carotene molecule and α-tocopherol.



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PC6 - André M. M. Gomes

Modeling the effect of pH and halogen bonds in the passive membrane permeation of drugs

André M. M. Gomes, Miguel Machuqueiro, Paulo J. Costa

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 less studied halogen bonds might also impact membrane permeability owing to the existence of halogen-membrane recognition phenomena mediated by those interactions [2]. In this communication, we report the first steps of a study of the permeation mechanism of a drug (Cobimetinib) for which both pH and halogen bonding are important. Indeed, Cobimetinib is an anti-cancer drug used to treat patients with melanoma, comprising a halogen atom (iodine) and a titrable group (Lewis base) in its structure. We started with the parameterization of the molecule by employing quantum-chemical calculations to generate a reference ESP and subsequently generated RESP charges taking into account the anisotropic features of the halogen by using an extra point (EP). In the following step, we employed constant-pH molecular dynamics (CpHMD) simulations in solution to calibrate the compound's pKa values (with and without EP). In the future, we will perform CpHMD simulations in a lipid bilayer (POPC) using an Umbrella Sampling scheme to calculate the membrane permeability coefficient values. By performing these calculations at normal cell pH (~7.4) and tumor cell acidic conditions (pH~6.2), we will evaluate the impact of tumor acidosis in the compound membrane passive diffusion while also evaluating the role of halogen bonds in the overall process.

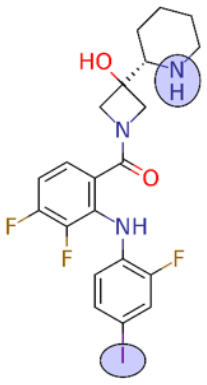

Fig. 1. Chemical structure of Cobimetinib highlighting the presence of both halogens and titrable groups.

We acknowledge FCT for projects UIDB/04046/2020-UIDP/04046/2020 (BioISI) and grants CEECIND/02300/2017 (M.M) and CEECIND/00381/2021 (PJC).

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PC7 – Bárbara Almeida

Structural characterization and identification of novel inhibitors of respiratory proteins from human bacterial pathogens

Bárbara Almeida, Manuela M. Pereira, Bruno L. Victor

Antibiotic resistance is becoming more prevalent as bacteria develop resistance to different drugs. Recently, the World Healthcare Organization created a priority list for the pathogens for research and development of new antibiotics. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are included in this list due to their persistence against a wide range of antimicrobials. *S. aureus* is a common cause of bacteraemia and infective endocarditis, and is found as a commensal bacterium in about 30% of the population.[1] The same is observed for *P. aeruginosa*, which frequently is involved in cystic fibrosis, burn wounds, hospital-acquired pneumonia, or urinary tract infections.[2] Both exhibit a great ability to adapt to diverse environmental conditions due to the relatively complex composition of their respiratory chains. The type II NADH:quinone oxidoreductase (NDH-2) membrane protein is involved in their respiratory chains and metabolic regeneration of NAD^+. [3] Therefore, the structural characterization and identification of novel inhibitors of these proteins may pave the way to the development of new therapeutic strategies to address multidrug resistance. Aiming at identifying a subset of promising compounds that inhibit NDH-2's activity, we will initially characterize this protein's structure from both organisms using multiple computational approaches. Since the Protein Data Bank currently only contains the structure of the *S. aureus* protein, we used homology modeling to create an optimized 3D model of *P. aeruginosa*'s NDH-2 based on the similarity of both proteins. To structurally characterize the dynamics of these two proteins, we performed molecular dynamics simulations in the absence and presence of a POPC model membrane. The gathered information will afterward be important to conduct a computational molecular docking screening campaign using purchasable compound databases to identify promising inhibitors of these enzymes. Most promising compounds will then be experimentally evaluated for their NDH-2 inhibitory effect as potential future multidrug resistance therapies.

Acknowledgments: We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020, UIDP/04046/2020, and PTDC/BIA-BQM/2599/2021.

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PC8 – Beatriz Farinha

Structural characterization and functional role of TGR5 in adipose tissue – liver axis in the context of non-alcoholic fatty liver disease

Beatriz Farinha, André L. Simão, Rui Castro, Bruno L. Victor

Obesity has become a worldwide epidemic, resulting in an increased prevalence of comorbidities such as non-alcoholic fatty liver disease (NAFLD). In turn, NAFLD represents the leading cause of chronic liver disease worldwide, and currently available therapeutic strategies to tackle this disease are very limited. NAFLD involves complex interactions between different regulatory pathways and different organs, including adipose tissue (AT) [1]. Hence, a better understanding of these molecular mechanisms governing may help in identifying new therapeutic targets that can accelerate drug development. In this regard, activation of the metabolic receptor TGR5 in AT has been reported to increase energy expenditure and prevent obesity and insulin resistance [2]. In parallel, it has been shown that AT comprises an important source of circulating exosomal miRNAs, capable of regulating mRNA translation in other tissues, such as the liver [3]. Altogether, exosomal miRNAs, whose expression might be modulated by TGR5 activation in AT, may represent a still unidentified link between AT and the liver. We aim to better understand the signaling pathways connecting metabolic impaired AT and hepatic dysfunction to identify novel biomarkers and therapeutic strategies for NAFLD. Herein, we will present our initial computational studies, focused on the structural characterization of TGR5 and on our virtual screening campaigns intending to identify protein agonists. The most promising compounds will afterward be experimentally evaluated to unveil the role of TGR5 in adipose exosomes (ad-exos) pathways and ad-exos function in stressed liver cells.

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PC9 – Fábio G. Martins

Computer-Guided Development of New Drugs Against COVID-19

Fábio G. Martins, Henrique S. Fernandes, Rita P. Magalhães, Tatiana F. Vieira, Sérgio F. Sousa

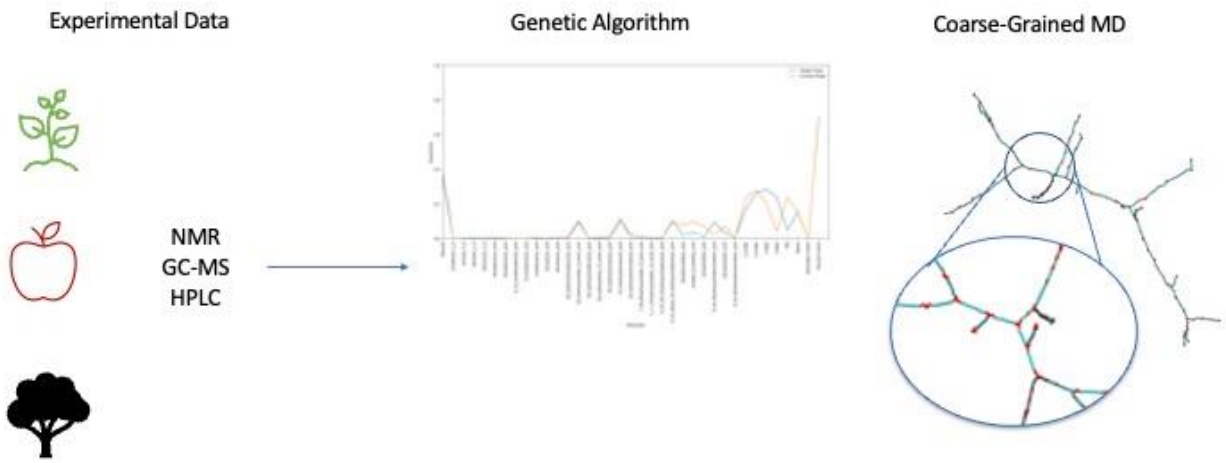
SARS-CoV-2 is the infectious agent behind the COVID-19 pandemic. This positive-strand RNA virus causes severe respiratory syndrome in humans. Although multiple vaccines have been developed since the start of the pandemic, there are no specialised, effective drugs against this virus. The infection relies on the interaction between the viral spike S protein and the angiotensin-converting enzyme 2 (ACE2). This enzyme is expressed in the in human airway epithelia, lung parenchyma, vascular endothelia, kidney cells, and small intestine cells. The S/ACE2 interaction is the central entry pathway for SARS-CoV-2. Therefore, one of the ways to block the infection is to disrupt this interaction. In this work, in silico models were used to study the S/ACE2 complex and discover drugs that may target the S/ACE2 interface. The interaction between spike and ACE2 was studied by performing molecular dynamics simulations with a length of 400 ns using the AMBER 19 software. The interfacial binding pocket was then defined using FPocket 2.0. Subsequently, a virtual screening protocol was employed. Using Autodock Vina and GOLD molecular docking software, 139,146 compounds were screened. These compounds belonged to different chemical libraries: the Chimiothèque Nationale, MuTaLig Virtual Chemotheca, and the Inhibitors of Protein-Protein Interactions Database. The virtual screening experiment resulted in 10 compounds that were selected for experimental validation. These experimental studies tested the inhibition of infectivity, dose dependency, S/ACE2 binding inhibition, viral replication, and drug cytotoxicity. From these experimental studies, two novel compounds were found which are capable of hindering S/ACE2 interaction and therefore inhibiting SARS-CoV-2 replication.

PC10 – Fernando Neiva Nunes

Solving the experimentally unsolvable: Unveiling cork Suberin chemical and structural characteristics

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

Suberin is a glycerol polyester ubiquitous in land plants, that serves as a barrier between the outside and the plant – Shields from UV radiation, limits the progression of pathogens – and acts as an effector of major plant-microbe interactions. The polymer chemistry is plant-, species- and tissue-specific, this plethora of chemical compositions enables the construction of fine connections between composition and bioactivity. The Applied and Environmental Mycology Lab, developed a green approach to extract suberin from different natural sources, using an ionic-liquid to selectively cleave acylglycerol esters, releasing suberin nanoparticles with near-native cross-linking and antimicrobial activity. The average chemistry of these nanoparticles is solved using NMR and GC-MS. However finer details of individual suberin oligomers remain unclear, like weight and chemical composition. So, I developed a Genetic Algorithm that creates in-silico probabilistic methods of individual suberin oligomers. This aims at answering latent questions as to what the most probable weight distribution of the extracted suberin nanoparticles is. This methodology is to be used in tandem with Coarse-grained molecular dynamics simulations, using the novel Martini3 forcefield, to assess the antimicrobial mode of action already seen experimentally. With this it I expect to create relationships between different suberins (with different chemistries) and microbicidal activity. This knowledge also can also be translatable into other biopolyesters like cutin, also of biotechnological interest.



PC11 – Filipe E. P. Rodrigues

pH-dependent cationic peptide dendrimers as vectors for siRNA: an in silico study

Filipe E. P. Rodrigues, Tamis Darbre and Miguel Machuqueiro

Peptide dendrimers are tree-like symmetric molecules, with a well-defined and homogeneous topology, constituted by amino acid residues. They have been reported to interact with several biological targets leading to good activity as antimicrobial agents and superior vectors for nucleic acids [1]. Recently, the use of such structures as vector molecules for siRNA molecules has been explored [2], which resulted in the MH13 and MH18 sequences. These are solely constituted by lysines and leucines, and contain as hydrophobic cores two palmitoyl chains or a leucine tetrapeptide, respectively. The hydrophobic core increases their capability to interact with the cellular membrane and internalize the cell via endocytosis. This is synergized by their cationic nature at low pH, which is vital for the interaction with the negative nucleic acid molecules, while also providing a way to escape the endosomal entrapment. Furthermore, some mutations in MH18 from L- to D-amino acids, result in improved binding to siRNA, as well as improved resistance to proteolytic degradation [2]. However, these mutations are accompanied by lower silencing performance of the carried siRNA [2]. Little is known about their overall molecular mechanisms and the reasoning why some combinations lose or acquire specific properties [3]. In this work, we will present our findings regarding the application of our state-of-the-art CpHMD methodology to the pH-dependent conformational space of MH13 and MH18 and its variants composed of different combinations of L and D-amino acids. We will present the pH titration behavior and perform several structural characterizations, including the radius of gyration, the permuted root mean square deviation (RMSD). We will also present our preliminary data on the interaction of some of these dendrimers with a lipid model membrane. Altogether, these results will help experimentalists interpret their data, as well as design new and improved sequences.

We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017 and 2021.05909.BD.

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PC12 – Inês D. S. Pires

Computational biophysics evaluation of promising smart metallodrug delivery systems

Inês D. S. Pires, Tânia S. Morais, Miguel Machuqueiro

Cancer, especially breast cancer, has been rising to the top of most prevalent and deadly diseases. The triple-negative (TN) subtype of breast cancer (BC) is associated with high aggressiveness and poor prognosis[1]. Other subtypes currently employ targeted therapies, taking advantage of receptors like hormone (estrogen and progesterone) and human epidermal growth factor receptor 2. The TN subtype lacks their expression, thus, its treatment is still heavily reliant on chemotherapy, especially with cisplatin-like drugs, which are known for lack of selectivity and tendency to develop multidrug resistance. TM34 is a ruthenium-based compound that has been suggested to be a more efficient and selective therapy than cisplatin[2]. TM34 derivatives have been under study in the last years, in an attempt to increase their selectivity but preserve their activity, by adding a pH sensitive linker and a peptide sequence that is recognized by receptor proteins from the FGFR family (overexpressed in TNBC cancers)[3]. Once in the presence of the altered pH of the tumour micro-environment, the linker hydrolyses and releases the active species. This work aimed to study the impact of different substituent groups in the active specie's biophysical profile. This included studying the interaction of several TM34 derivative compounds with a membrane model (POPC) and calculating their membrane crossing energy profiles that can be used to estimate the membrane permeability coefficients. We used Molecular Dynamics simulations coupled with an Umbrella-sampling scheme to obtain the potential of mean force profiles, which allowed the calculation of the membrane permeability using the inhomogeneous solubility-diffusion model[4].

Acknowledgments: We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/00100/2020 (CQE), UIDB/04046/2020 & UIDP/04046/2020 (BioISI), and PTDC/QUI-QIN/0146/2020. T.S. Morais and M. Machuqueiro thank the CEECIND 2017 Initiative for projects CEECIND/00630/2017 and CEECIND/02300/2017, respectively (acknowledging FCT, as well as POPH and FSE-European Social Fund).

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PC13 – Inês Gomes

Biomolecular Stability on a Betaine-Glycerol Deep Eutectic Solvent

Inês Gomes, Alexandre Paiva, Nuno Galamba

Deep eutectic solvents (DESs) are an emerging class of green solvents formed by a hydrogen bond (HB) donor and an HB acceptor characterized by a depression of the melting point relative to their components (1). DES are biodegradable, cheap, and less toxic than ionic liquids (2). Furthermore, water can be used to further tailor their physicochemical properties (3). DESs have been explored in multiple potential applications including biocatalysis and nucleic acid preservation and stabilization (4, 5). Recent reports have shown that enzymatic activity can be enhanced by using DESs as the reaction media (6). The molecular origin of these effects remains, however, poorly understood. A key question concerns the chemical nature of the solvation spheres of biomolecules and the minimum amount of water required to allow biomolecules to maintain their structure and function. Herein, the structure of a prototypical globular protein, Ubiquitin, was probed in water and in a betaine:glycerol DES at different water contents through molecular dynamics simulations. A force field for the DES, derived from the General AMBER Force Field was optimized to accurately reproduce the experimental density and shear viscosity. Further, preliminary results of the structural transformations of the protein as a function of the solvent dewetting are reported.

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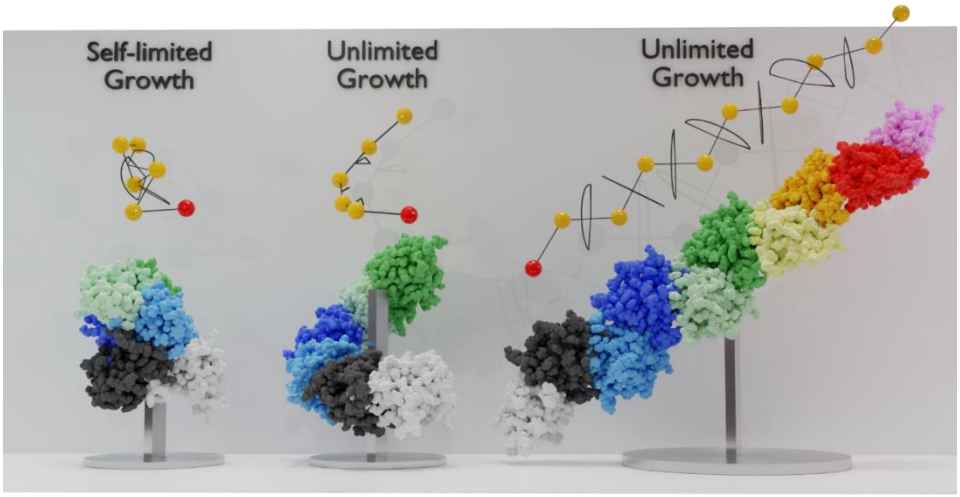
PC14 – João N. M. Vitorino

Predicting aggregation-triggering B2M oligomers with the help MM/PBSA calculations

João N. M. Vitorino, Nuno F. B. Oliveira, Filipe E. P. Rodrigues, Patrícia F. N. Faísca and Miguel Machuqueiro

A variety of conformational disorders, including Parkinson's and Alzheimer's disease, are characterized by protein aggregation and amyloid production [1], processes that begin with the simple self-association of two monomers into a dimer. Computational methodologies have been invaluable in understanding protein aggregation pathways, including the identification and characterization of some of the most cytotoxic, early-formed, oligomeric species [2,3], which can be used as potential therapeutic targets to hinder the aggregation associated with those diseases. Following an extensive Monte Carlo Ensemble Docking (MCED) protocol that generated an ensemble of possible dimers resulting from Beta-2-microglobulin (B2M) self-association, and the analysis of aggregation hotspots [4], we recently reported the prediction of stable binding modes from simulated dimers of the B2M D76N, an aggregation-prone, clinically relevant mutant [5], associated with a fatal form of Dialysis Related Amyloidosis (DRA) [6]. This prediction relied on MM-PBSA free binding energy calculations, using PyBindE (https://github.com/mms-fcul/PyBindE), which also allowed us to identify the key hydrophobic character of the interactions in the stable binding modes [3]. We have since expanded on this work by studying the conformational dynamics of B2M dimers in long MD simulations, and B2M polymerization growth modes with the development of geometric expansion models used to predict the unlimited or self-limiting potential of each of these growth modes. Here, we present the results of our recent studies of B2M aggregation and polymerization, as well as ongoing work.



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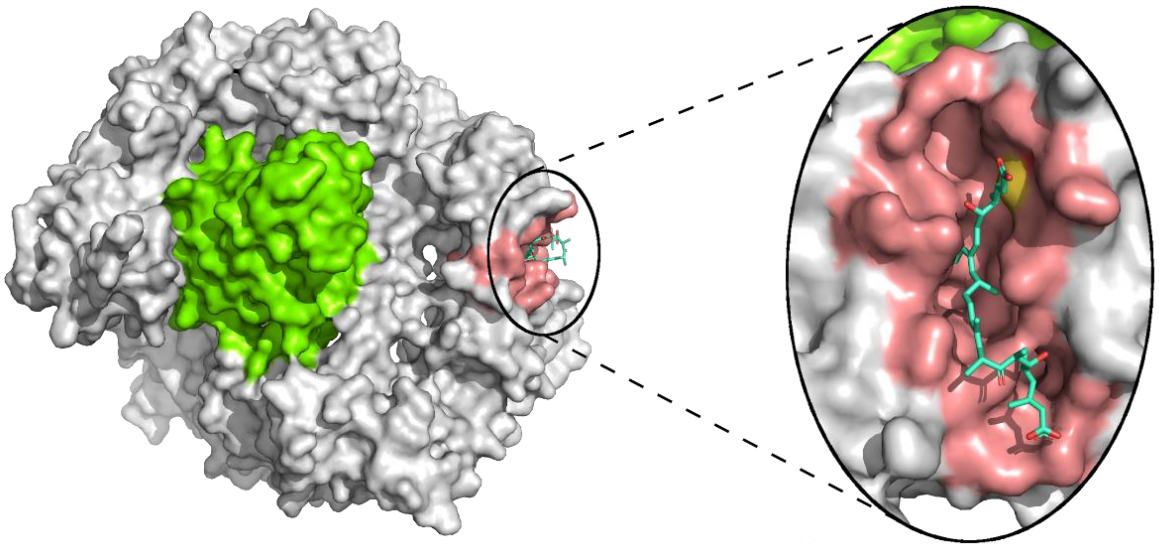
PC15 – João G. N. Sequeira

Searching for CRM1 inhibitors using a HTVS protocol

João G. N. Sequeira, Wolfgang Link, Miguel Machuqueiro

Protein function depends on its subcellular localization, as it determines the access to binding partners and enzymes that catalyze post-translational modifications. The best-studied export protein is the Chromosome Region Maintenance 1 (CRM1), a transversal protein across all eukaryotic cells, whose inhibition has been used for the treatment of cancer [1] and possibly against viruses [2]. CRM1 inhibition usually involves the binding of a compound to the NES-binding groove to prevent cargo binding. However, all known CRM1 inhibitors establish a covalent bond with Cys528, leading to irreversibility, high toxicities, and impairing its in vivo application. The first inhibitor that showed non-covalent binding capacity was NCI-1 [3]. However, this inhibitor also binds covalently to Cys528 in the CRM1 wild-type form. In this work, we implemented a HTVS protocol using a compound database provided by a collaborator (Dr. Romano Silvestri, Rome, IT) to identify non-covalent inhibitors of CRM1 after a conformational study of the NES-binding groove. This led to the selection of 30 lead compounds, which were tested experimentally by Dr. Wolfgang Link (Madrid, ES) and helped identify a very promising inhibitor [4]. Here, we present our preliminary data on the development of a new computational protocol to identify non-covalent inhibitors of CRM1. We will use NCI-1 and the newly found promising compound in a reverse docking approach to search for representative structures of an apo NES-binding groove which will be used in new HTVS protocols.



Acknowledgements: We 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.

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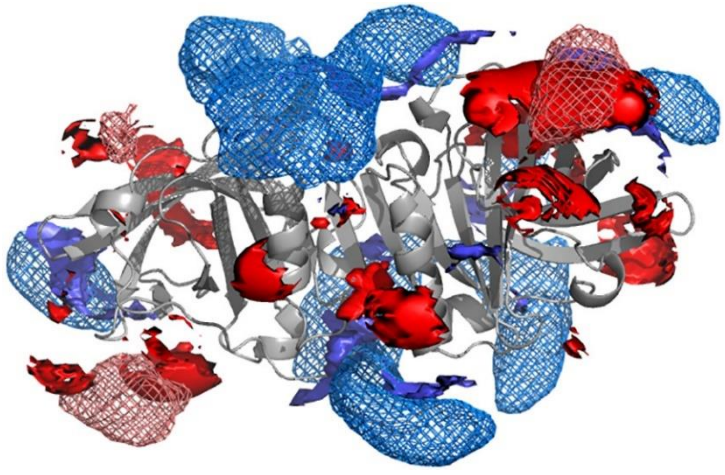
PC16 – Lucie Rocha

How is the distribution of ions around proteins affected by pH?

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

Ions are involved in multiple biological processes and may exist bound to biomolecules or may be associated with their surface. Although the presence of ions in nucleic acids has traditionally gained more interest, ion–protein interactions, often with a marked dependency on pH, are beginning to gather attention. Here we present a detailed analysis on the binding and distribution of ions around β-lactoglobulin using a constant-pH MD (CpHMD) method, at a pH range 3–8, and compare it with the more traditional Poisson–Boltzmann (PB) model and the existing experimental data. The requirements of approximate charge neutrality and ionic strength equal to bulk, imposed on the MD box, imply that the absolute value of the ion excess should be half the protein charge, which is in agreement with experimental observation on other proteins and lends support to this protocol. In addition, the protein total charge (including bound ions) estimated with MD is in excellent agreement with electrophoretic measurements. Overall, the CpHMD simulations show good agreement with the nonlinear form of the PB (NLPB) model but not with its linear form, which involves a theoretical inconsistency in the calculation of the concentration maps. In several analyses, the observed pH-dependent trends for the counterions and co-ions are those generally expected, and the ion concentration maps correctly converge to the bulk ionic strength as one moves away from the protein. Despite the overall similarity, the CpHMD and NLPB approaches show some discrepancies when analyzed in more detail, which may be related to an apparent overestimation of counterion excess and underestimation of co-ion exclusion by the NLPB model, particularly at short distances from the protein.



PC17 – Nuno F. B. Oliveira

Computational estimation of protein/drug pH-dependent binding affinities

Nuno F. B. Oliveira, Mohannad Yousef, Miguel Machuqueiro

Binding events are the basis of life, being the key of all interactions that occur in cells. These events are usually strongly influenced by pH, however, computational approaches that tackle binding processes, often overlook this effect due to its complexity [1,2]. To showcase this problem, we used acetylcholinesterase (AChE) as our protein system, since it has been targeted for inhibition in the treatment of the Alzheimer's disease [3]. Therefore, there are several specific inhibitors of AChE, such as donepezil, for which the crystal structures of the bound complex has been solved [4]. In this work, we used CpHMD simulations[5] of AChE/donepezil to identify which amino acid residues, located in the active site, are pH-sensitive at physiological pH values. The most relevant sites were used in a systematic molecular docking study where all protonation combinations were explored and the specific binding energies were calculated. Finally, we combined the CpHMD data to correctly weight the docking results of each protonation state, allowing us to estimate the binding affinities of the AChE/donepezil system in a range of pH from 6 to 8. This work highlights the importance of capturing the correct protonation states of each system to have a good estimation of the binding energy at the desired pH.

We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects 2021.09635.CPCA, UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017 and 2021.06409.BD.

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PC18 - Ona Šivickytė

Predicting halogen bond strength with simple descriptors: the usage of the Intrinsic Bond Strength Index

Ona Šivickyte, Paulo Costa

Halogen bonds (XBs) are a type of sigma-hole interaction between a halogen, acting as a Lewis acid, and a Lewis base [1]. XBs have become increasingly popular over the past few years with numerous applications in catalysis, material design, anion recognition, and medicinal chemistry. Rational design of X-bonded systems requires computational tools to predict potential interactions and their strength, however, it has been challenging to obtain reliable and accessible XB descriptors for such task, meaning that most of the applications so far have been rationalized post factum [2]. Recently, the Intrinsic Bond Strength Index (IBSI) [3] has been proposed as a new tool to evaluate the interaction strength for any pair of interacting atoms and give each pair an unambiguous and comparable score. Despite this, IBSI has not yet been systematically studied in the context of XBs. Using tabulated promolecular densities and performing QM calculations to obtain a more accurate molecular density, in this work [4] we show that IBSI values linearly correlate with the interaction energy of diverse sets of halogen-bonded complexes. The results suggest that IBSI is a promising method for cheap and fast estimation of XBs in protein-ligand systems.

Acknowledgments: FCT for CEECIND/00381/2021 (PJC), and projects UIDB/04046/2020–UIDP/04046/2020 (BioISI)

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PC19 – Pedro M. S. Suzano

MembIT – a tool to calculate membrane-insertion properties of solutes

Tomás, F. D. Silva, Pedro M. S. Suzano, Inês D. S. Pires, Pedro Reis, Miguel Machuqueiro

The greatest hurdle of MD simulations has always been the limited computational power available. Nevertheless, recent software and hardware developments have allowed the atomistic study of complex systems, like those containing membranes and/or large proteins. It is common for membranes to deform when interacting with both small molecules and large transmembrane proteins. To fully characterize the interactions taking place in these systems, both molecule and membrane properties need to be accurately described. Two of the main properties of interest when analyzing these systems are the local monolayer thickness and the compound's membrane insertion, which directly result from the membrane-compound interactions. Membrane insertion is generally calculated by comparing the z-position of the compound of interest with the average position of the phosphate head groups of the interacting leaflet. Monolayer thickness is usually determined by half the difference between the average z-positions of the phosphate head groups in both monolayers. However, membrane deformations can influence the position of the local reference atoms surrounding the compound, and these properties are no longer accurately estimated using the average z-position of the phosphate head groups as references. To account for these local deformations, the membIT [1] tool makes a distinction between local (in close proximity) and bulk lipids (further away from the solute), which allows for the calculation of the average position of undisturbed phosphate head groups. Consequently, the local monolayer deformation calculations become straightforward, as the difference between bulk and local lipids head group positions. The solute membrane insertion calculations can also take advantage of this strategy by using the affected phosphate group atoms as reference, while ignoring the unperturbed bulk lipids. The membIT tool application is showcased here with example systems, namely that of inserting compounds like sunitinib [2] and the pHLIP peptide [3], and a large transmembrane protein of the ABC transporter family, the ATP/ADP carrier [4].

We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects 2021.09635.CPCA, UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017, SFRH/BD/136226/2018, and SFRH/BD/140886/2018.

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PC20 – Raquel P. Gouveia

The computational study of AMPAR:Stargazin interface in different subconductance 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.[1,2] STarGazin (STG), a transmembrane AMPAR regulatory protein, is required for the transport of AMPAR to the surface and its stabilization at synapses and is also responsible for the homeostatic synaptic scaling of AMPAR and the modulation of its gating properties. [3,4] It was shown that conductance levels (O1-O4) of AMPAR are associated with the number of agonist molecules bound to the ligand-binding domains of the protein.[5] Still, the molecular details are scarce. As such, in this work we used molecular dynamics simulations to study the structure of AMPAR:STG with different amounts of glutamate in the presence of a mutation found in a patient with intellectual disability (V143L). We extensively analyzed the structure and dynamics of this interface by calculating pairwise interactions (H-bonds and Salt-Bridges) and their prevalence, and changes in the solvent accessibility. We also decomposed the binding free energy at the residue level. Our dynamical study showed that structures with fewer glutamates and in a closed state are much more vulnerable to changes in the interface upon V143L mutation.

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PC21 – Rita F. C. C. Guerra

A computational study on the impact of adding anionic groups to tyrosine kinase inhibitors

Rita F. C. C. Guerra, Nuno F. B. Oliveira, Pedro M. S. Suzano, Bruno L. Victor, Miguel Machuqueiro

Despite tumor multi-drug resistance (MDR) being multi-factorial in nature [1], it was proposed that the acidic lumen of lysosomes (pH~4.5–5) plays a major role by efficiently entrapping hydrophobic weak base drugs (Lewis bases; pKa~7.5–9.5), via their protonation, markedly hindering their anti-cancer activities [2]. Some of these weak bases, the tyrosine kinase inhibitors (TKI), exhibit high and complementary clinical relevance by being vital mediators of signal transduction and cancer cell proliferation, angiogenesis, and apoptosis [3]. There are several chemotherapeutic options in the market based on TKIs and there is a myriad of information about their targets, the receptor tyrosine kinases (RTKs) [4]. We have a strategy to chemically modify several TKIs and exchange the cationic amino groups with anionic ones. The rationale is that the anionic group should also provide good solubility in the aqueous media and, in contrast to the weak base, will have its membrane affinity increased with acidity. These acidic derivatives should selectively target cancer cells over normal tissues and effectively evade lysosomal sequestration, circumventing several crucial factors related to MDR. In this work, we will optimize a molecular docking protocol based on different search methods and scoring functions to study systematically the impact of replacing cationic groups found on TKIs with negative chemical building blocks on the binding to RTKs. This chemical modification strategy will allow us to simultaneously improve the druggability of such compounds, and evaluate the impact on the affinity to their therapeutical targets. We will use a consensus docking approach where the score and/or rank of several different freely available docking suits, including Autodock 4.2, Autodock-GPU, Autodock Vina 1.2, and Dock 6.9, will be combined to achieve the best results.

Acknowledgments: We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020, UIDP/04046/2020, 2021.09731.CPCA, and grants CEECIND/02300/2017 and 2021.06409.BD.

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PC22 – Rita Emídio

Molecular docking and synthesis of indoloisoquinoline bioisosters as new anticancer therapeutics

Rita Emídio, Bárbara B. Bruni, Bruno L. Victor, Alexandra Paulo

The role of DNA helicases in repairing damaged DNA and the maintenance of genomic stability is under increased scrutiny as part of efforts to combat cancer. c-Myc is a constitutively transcription factor aberrantly expressed in over 70% of human cancers. [1] Its direct and selective inhibition was shown to trigger rapid tumor regression and enhance anti-cancer therapies, making it an attractive target for anticancer therapy. [2] However, the c-MYC protein lacks a define binding site making the design of specific interacting modulators extremely difficult. Therefore, the special transient secondary structure of DNA entitled G-quadruplex (G4) which is found in the c-MYC promoter region has emerged as a potential drug target to control c-MYC expression. [3] Additionally, helicases such as DHX36 are G4 -binding proteins with the capacity to enzymatically unwind DNA and RNA. By exerting its G4 helicase function, DHX36 regulates transcription, genomic stability, telomere maintenance and translation, making it an additional potential drug target to focus on the quest for alternative anti-cancer therapies. [4] In this communication, we will present the preliminary results of a large project focused on the identification, synthesis, and validation of indoloisoquinoline (IDIQ) derivative compounds targeting c-MYC G4 in complex with DHX36. We will show the results regarding the molecular docking and synthesis of a small set of di-substituted IDIQ bioisosters, which will afterward be experimentally evaluated for their inhibitory effect. The most promising compounds will then be studied by molecular dynamic simulations aiming to understand the SAR. This innovative approach focused on targeting the complex c-MYC:G4 with DHX36 helicase is expected to lead to an increased selectivity and efficacy of G4-stabilization, and consequently allow the identification of new alternative anti-cancer therapies.

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PC23 – Sara G. F. Ferreira

The role of protein knots in the UCH-L1 mechanical stability and function

Sara G. F. Ferreira, Patrícia F. N. Faísca and Miguel Machuqueiro

Ubiquitin C-terminal hydrolases (UCHs) are papain-like cysteine proteases that hydrolyze the ubiquitin adduct, countering the ubiquitination process in proteins. This procedure consists of a posttranslational modification, is therefore involved in regulating both membrane trafficking and protein degradation pathways. Besides its importance in the ubiquitin-dependent proteolytic pathway, UCH-L1, one of the four UCHs present in the human genome, is also highly expressed in the brain. It is estimated to make up 1 to 5% of total neuronal protein [1] and has been characterized as being involved with Parkinson's, Alzheimer's, and other neurodegenerative diseases [2]. UCH-L1 has one of the most complicated 3D knotted structures yet discovered, where five crossings of the polypeptide backbone form a '5 2', or 'Gordian' knot. This protein has been reported to unfold with three populated states, transitioning from its folded state to fully denatured via an intermediate stage where its α-helices are already unfolded, but the β-strands central hydrophobic core remains intact [3]. There is an open question regarding the role of the protein knots in this remarkable structural stability. To address this question, we used long MD simulations to study the conformational space of the knotted UCH-L1. Furthermore, we developed a computational protocol to explore several N- and C-terminus truncated versions of this protein to study the impact of the unknotting process in its mechanical stability and function. We will present the preliminary results using the wild type and the truncated species, both in the apo form and complexed with its substrate, ubiquitin.

We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020 & UIDP/04046/2020 and grants CEECIND/02300/2017 and UI/BD/153055/2022.

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PC24 – Tiago Dias

Characterization of noncovalent interactions in biomolecular simulations for drug discovery improvement: using a robust and unsupervised energy-based criterion

Tiago Dias, Bruno L. Victor, and Paulo J. Costa

Noncovalent interactions, such as hydrophobic interactions, hydrogen and halogen bonds, salt bridges, and aromatic stacking, are essential in structural biochemistry, drug discovery, and biology [1]. The assignment of such interactions in 3D structures (e.g. molecular dynamics snapshots), often relying on automatic tools, is extremely useful in drug design since a comparative analysis of binding patterns for a given target is frequently performed [1]. These tools commonly use geometric criteria to assign interactions such as hydrogen or halogen bonds, often using empirical angle and distance thresholds. Such thresholds are arbitrary and can lead to the erroneous inclusion or exclusion of interactions. In this work, inspired by the popular method Define Secondary Structure of Proteins (DSSP) algorithm, we aim to develop an unsupervised energy-based criterion to assign noncovalent interactions in biomolecular systems. Based on previous work done by the group [2,3], a fast python-based workflow was developed to analyze molecular dynamics trajectories. In this workflow, energy-based criteria relying on the probability density functions were implemented for the identification of non-covalent interactions. We will show that besides bivariate data (distance, angle), a newer and faster descriptor based on the potential energy is an accurate method for bond assignment, which will hopefully improve the accuracy of drug discovery tools, specifically, non-covalent interaction calculations.

Acknowledgments: FCT for projects UIDB/04046/2020–UIDP/04046/2020 (BioISI) and CEECIND/00381/2021 (P. J. Costa).

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PC25 – Vasco Neto

Probing the Anti-sickling Power of Distinct Drugs in Sickle Cell Disease

Vasco Neto, Bruno L. Victor, Nuno Galamba

Sickle cell disease (SCD) is a highly debilitating monogenic blood disorder that affects millions, especially in sub-Saharan African countries. A possibly evolutionary missense mutation in the beta-globin gene that codes for adult hemoglobin (HbA) results in a Glu6-Val6 substitution in the beta-chains. Although not significantly perturbing the protein conformation, this mutation causes a decrease in the solubility and prompts the aggregation of the deoxygenated form of this variant (deoxy-HbS), leading to hemolysis, vaso-occlusive crisis, and regular infections. Despite having been investigated for over a century and its underlying mechanism being well understood, an easily applicable and effective treatment remains elusive. A potential therapeutic strategy involves the design of deoxy-HbS aggregation inhibitors that either hinder or delay the aggregation process. Therefore, it remains pivotal to find novel inhibitors for the treatment of this illness. The focus of this work is the identification and validation of a small molecule or peptide capable of directly inhibiting or delaying the aggregation process, preventing the multiple downstream effects associated with this pathology. To this end, in this communication we will present the preliminary results focused on the use of different computational techniques, such as molecular dynamics and molecular docking simulations to identify promising binding sites and screen for compounds with promising inhibitory effect of the deoxy-HbS aggregation process.

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