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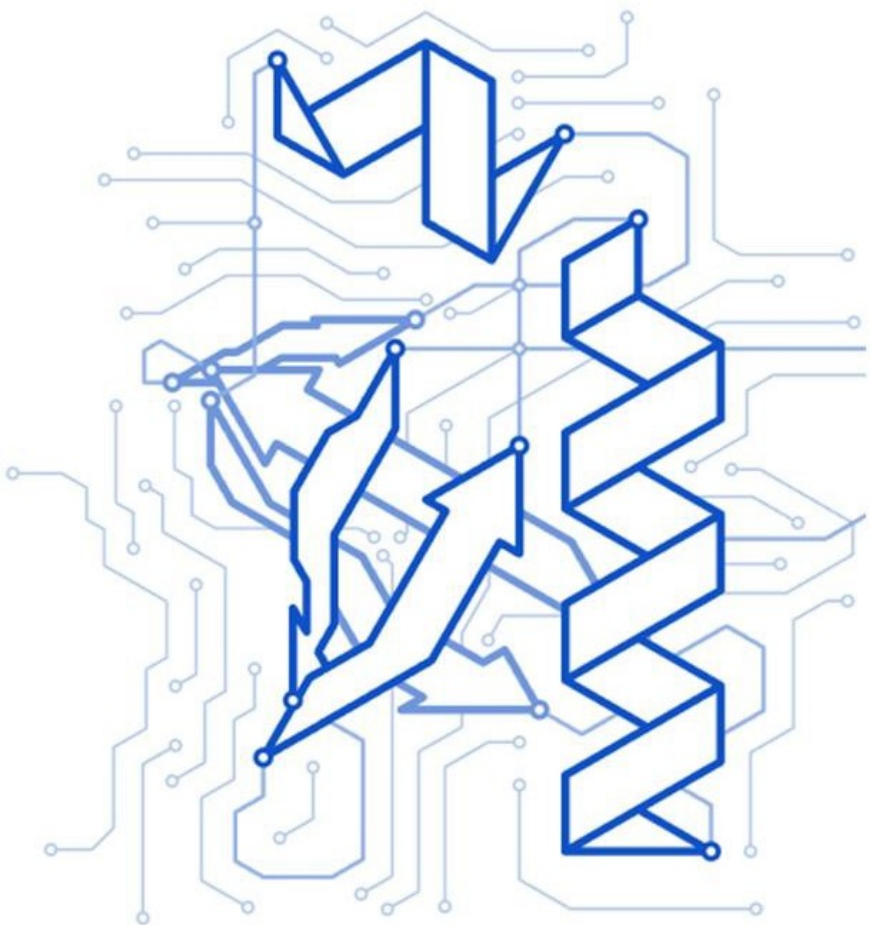
# 4th Annual Meeting

## December 16th 2025



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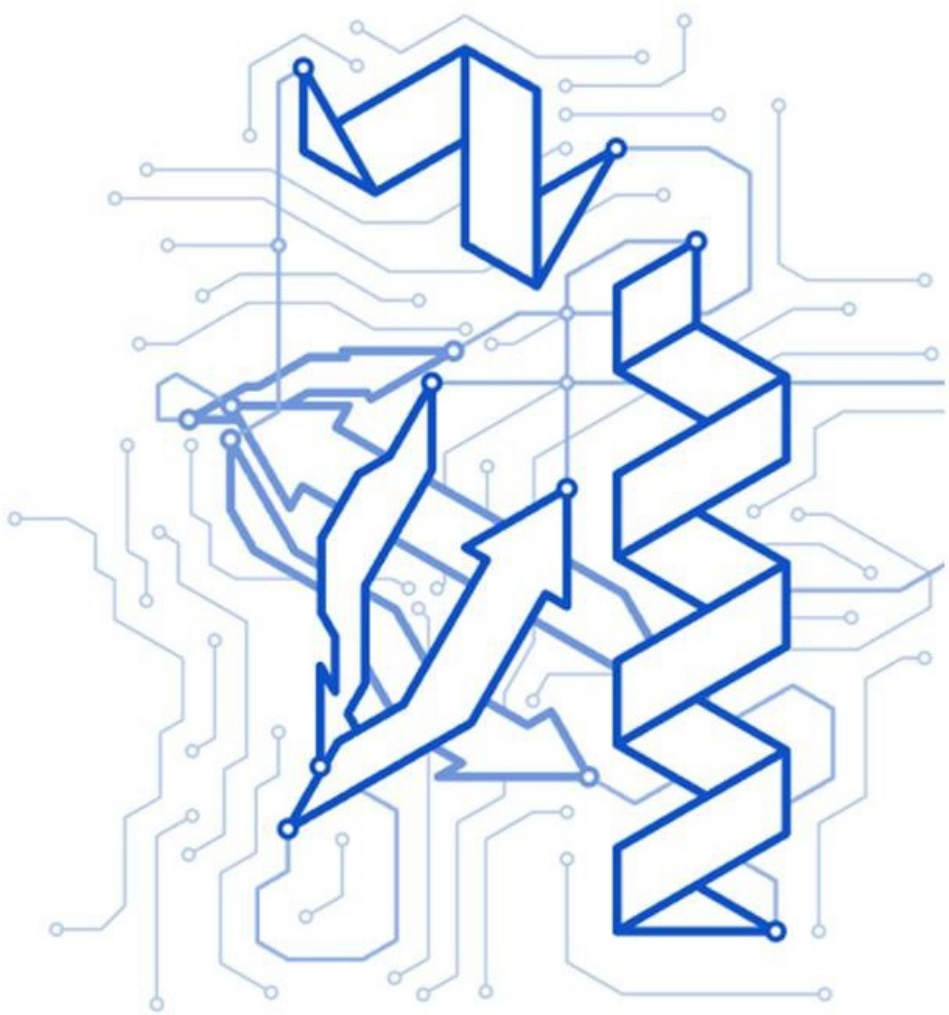

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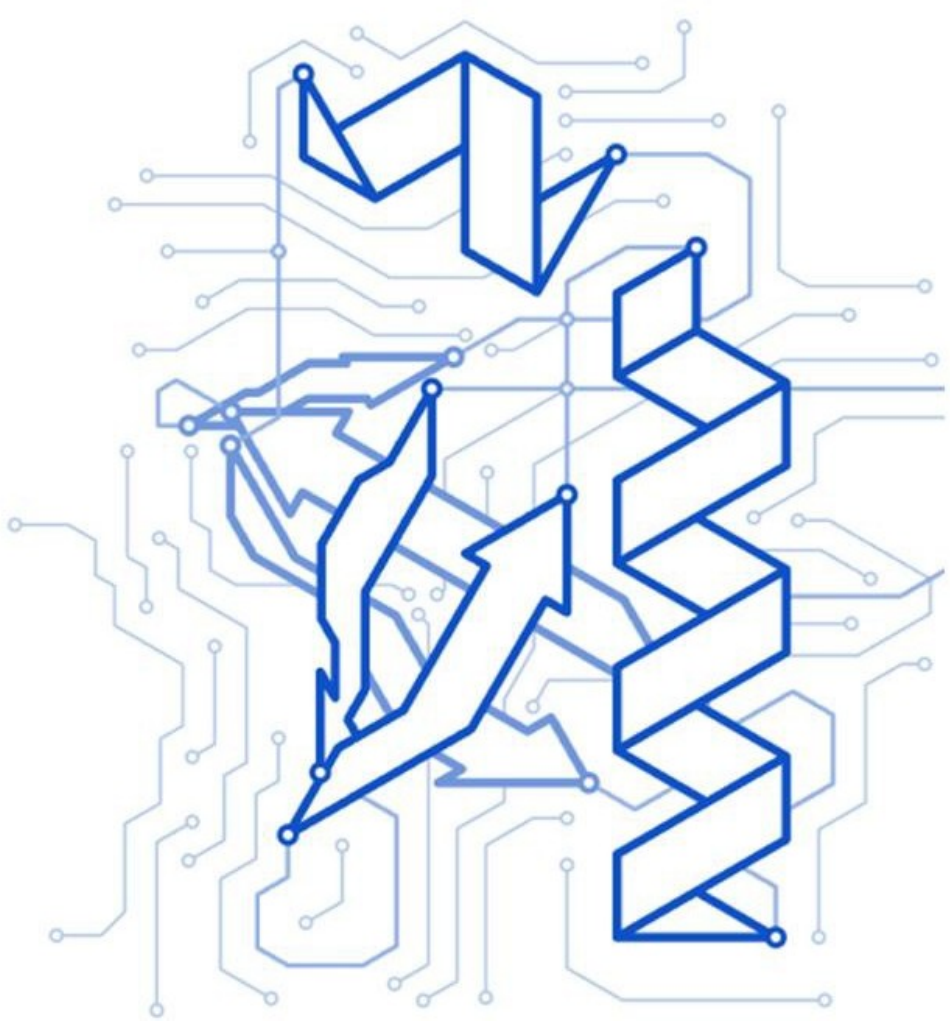
# Tuesday, December 16th

| | |
|---|---|
| 08:30 - 09:30 | Registration |
| 09:30 - 09:45 | (O1) Fábio Martins — Molecular insights into HIV-1 Intasome-Human Nucleosome Interactions |
| 09:45 - 10:00 | (O2) João Boazinha — Hybrid AI / Physics-based modelling of Human Voltage-gated Sodium Channels for the Development of Isoform Specific Drug Design |
| 10:00 - 11:00 | (K1) Miguel Machuqueiro — Computational Methods to Capture pH Effects in Biomolecules |
| 11:00 - 11:30 | Coffee Break + Poster Session |
| 11:30 - 11:45 | (O3) Ana Amorim — GPCR target discovery via multiclass activity mapping with GPCR-A17 MAAP |
| 11:45 - 12:00 | (O4) Dmitrij Gritsok — Benchmarking Deep Learning Models for Viral Protein Assembly: A Comparative Case Study of the Monkeypox Envelope Protein A29L |
| 12:00 - 12:15 | (O5) Constança Ilunga — Design and characterization of MHETase mutants for improved activity and solubility in PET degradation |
| 12:15 - 12:30 | Poster session |
| 12:30 - 14:00 | Lunch |
| 14:00 - 15:00 | (K2) Pedro Simões — On the Use of Molecular Simulation in Product Engineering |
| 15:00 - 15:15 | (O6) André Gomes — From Chemistry to Code: Automating QM Workflows for Solubility Prediction |
| 15:15 - 15:30 | (O7) Ana Figueiredo — Computational Model of Phosphatidylinositol Protonation in Membranes |
| 15:30 - 16:00 | Coffee Break + Poster Session |
| 16:00 - 17:00 | (K3) Arménio Barbosa — Computational Strategies in applied Molecular Recognition for Affinity Purification, Biomaterials and Drug Discovery |
| 17:00 - 17:15 | (O8) João Vitorino — Studying Protonation-Driven Dynamics in Class A GPCR Activation Mechanism Through Enhanced Sampling |
| 17:15 - 17:30 | (O9) Sara Ferreira — Tied Up in Function: The Role of the Knot in UCH-L1 Activity |
| 17:30 | Closing Session |

# Keynote Speakers



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# K1 – Machuqueiro, Miguel

## Computational Methods to Capture pH Effects in Biomolecules

BioISI: Biosystems and Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
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Assistant Professor in Computational Biochemistry at the Faculty of Sciences, University of Lisbon (Ciências@ULisboa), member of the executive board of BioISI and leader of the Computational Biophysics Lab. His expertise spans biomolecular simulation, constant-pH MD, membrane biophysics and electrostatics and participates in multiple cross-border collaborations and initiatives that promote the internationalization of Portuguese science. In 2023 and 2025, he received the Ciências@ULisboa Scientific Merit Award in Chemical Sciences & Technologies and in 2025, he was awarded the UL/CGD Scientífic Award in Biology, Biologic Eng., Biochemistry, and Biotechnology.

**Abstract:**
Constant-pH molecular dynamics (CpHMD) simulations are powerful computational tools that couple the conformational space of biomolecules to pH, by allowing the protonation states of ionizable sites to change dynamically in response to the environment [1]. This is a significant improvement over traditional MD simulations, which use fixed protonation states, as it provides a more realistic representation of pH effects on molecular structure, function, and binding. In inhomogeneous media, this representation becomes more challenging to capture accurately using these state-of-the-art computational methodologies [2]. Recent advances have focused on enhancing the accuracy and efficiency of these methods, thereby expanding their application to larger and more complex systems, such as protein-drug complexes and membrane proteins. We will present our most recent methodological developments, including the coupling of CpHMD with enhanced sampling schemes [3] to investigate the effects of pH on various biomolecules. However, many challenges remain, including high computational cost, the need for more accurate force fields, and the need to ensure adequate conformational sampling. This presentation will focus on those challenges... the success stories are already in the papers!

**Acknowlegdements:**
We acknowledge financial support from FCT through the project UID/04046/2025.

**References:**
1. Gomes, A. M. M., Costa, P. J., Machuqueiro, M. (2023) BBA Advances, 4, 100099.
2. Teixeira, V. H., Vila-Viçosa, D., Reis, P. B. P. S., Machuqueiro, M. (2016) J. Chem. Theory Comput., 12, 930-934.
3. Oliveira, N. F. B., Machuqueiro, M. (2022) J. Chem. Inf. Model., 62, 2550.

## K2 – Simões, Pedro N.

### On the Use of Molecular Simulation in Product Engineering

Pedro Nuno Simões (PNS) obtained his Ph.D. in Chemical Engineering from the University of Coimbra (UC) in 2001 and in recent years his research has focused on the application of advanced molecular modeling and simulation techniques, supported by high-performance computing (HPC), as a strategic means to advance fundamental knowledge in Chemical Product Engineering. He successfully secured funding for the establishment of an HPC facility at the Research Center of the Department of Chemical Engineering (CERES) at UC and has participated in several interdisciplinary R&D projects across diverse areas of Chemical and Biological Engineering and Materials Science. Within the Department of Chemical Engineering, PNS has held several management positions, including Vice-Director of the Department and Vice-Director of the former DEQ Research Center (CIEPQPF) and has served as Coordinator of the Doctoral Program in Chemical Engineering at UC since 2019.

**Abstract:**
The landscape of Chemical Engineering has shifted dramatically in recent decades. Product Design introduces new classes of problems that lie beyond the scope of traditional Chemical Engineering. In this context, molecular modelling and simulation have gained particular prominence in efforts to unify Molecular Science and Engineering within an integrated, holistic framework, where the boundaries between the physical sciences and engineering increasingly blur. This lecture highlights several representative examples of this emerging practice.

# K3 – Barbosa, Arménio

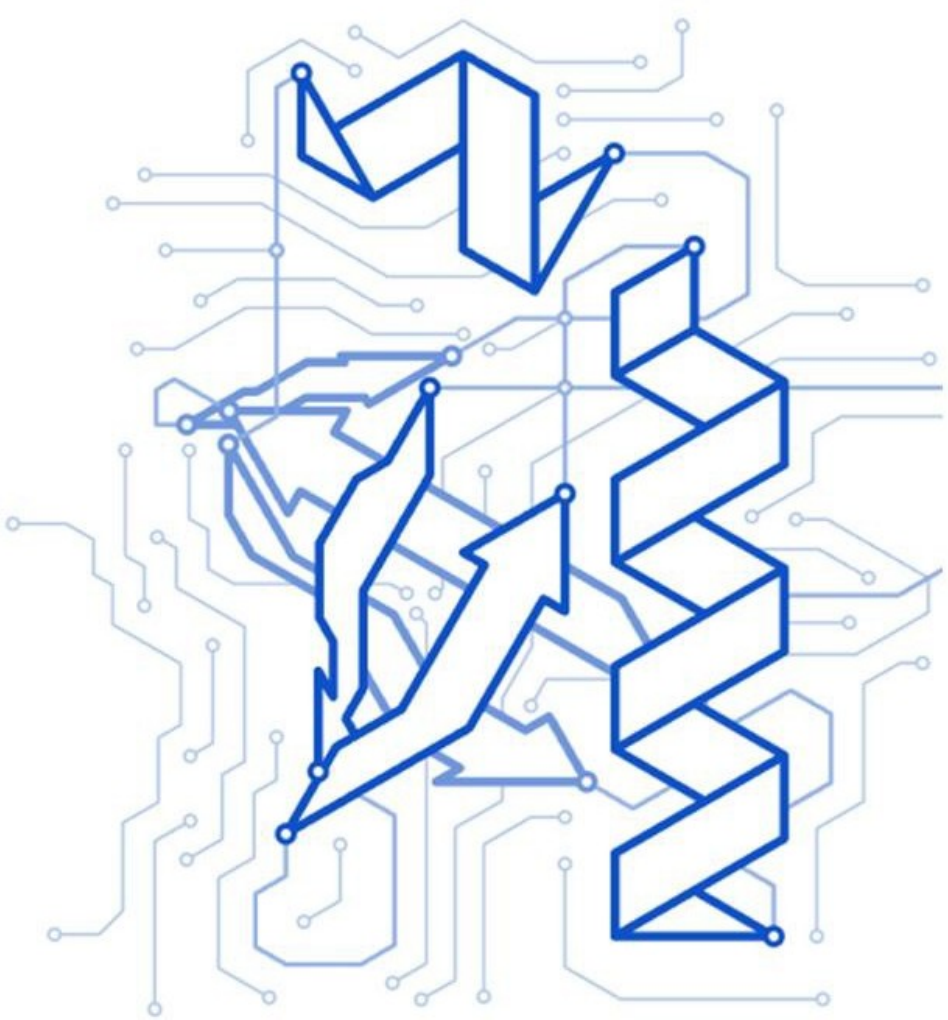
## Computational Strategies in applied Molecular Recognition for Affinity Purification, Biomaterials and Drug Discovery

UCIBIO, i4HB – Chemistry Department, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Quinta da Torre, 2829-516 Almada, Portugal,
email: aj.barbosa@fct.unl.pt

Assistant Professor in Biochemistry and Biophysics at the NOVA School of Science and Technology, NOVA University Lisbon, and a researcher at UCIBIO and i4HB. His expertise involves modelling and simulation of small molecules and biomolecules applied to biotechnology research, using molecular modeling tools to develop ligands for biopharmaceuticals purification, study protein-based materials, dynamic behavior of biomolecules and drug discovery.

**Abstract:**
In the past decades, molecular modeling and simulation of biomolecules became standard techniques in several research and development areas, mainly in pharmaceutical, chemistry, biochemistry and materials science. Simulations of biomolecules and small molecules have become more accessible, user-friendly, and easily realized with non-experts. The flexibility of these techniques allows them to be applied to other areas like biomaterials and niche biotechnological areas such as protein affinity purification and biosensing.

# Oral Communications



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# O1 – Martins, Fábio

## Molecular insights into HIV-1 Intasome-Human Nucleosome Interactions

Fábio G. Martins[1], Sérgio F. Sousa[1,2]

[1] LAQV/REQUIMTE, BioSIM Department of Biomedicine, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, Porto, Portugal
[2] Departamento de Engenharia Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra

**Abstract:**
Integration of the HIV-1 genome into host chromatin is a crucial step in viral infection. This step is carried out by the pre-integration complex, which includes the intasome (viral DNA bound to an integrase) and associated viral components. The intasome targets specific chromosomal "hot spots" for integration. The nucleosome, the core repeating unit of chromatin, consists of 147 base pairs of DNA wrapped around a histone octamer. While prior studies have explored intasome-nucleosome interactions, the molecular mechanisms underlying this interface remain poorly characterized.

In this work, we applied structure-based computational methods, such as molecular docking and molecular dynamics simulations, to investigate how HIV-1 the intasome interacts with the human nucleosome. Initial models were constructed using the HIV-1 intasome and nucleosome crystal structures (PDB: 1KX5 and 9C9M respectively) and docking was performed with the HADDOCK 2.4 platform. Subsequent MD simulations with Amber20 revealed that histone H4 tails and elements of histone H3 contribute significantly for the stabilization of the complex. The analysis also highlighted the relevance, of the 51-56 region of nucleosomal DNA as a key contact site.

Experimental results showed that the HIV-1 intasome is not able to integrate into all nucleosomes of a trinucleosome, unlike other viral intasomes such as the PFV intasome. To understand this, we performed comparative docking using PDB structures 9C9M (HIV-1 intasome) 3S3M (PFV intasome) and 6L4A (human trinucleosome). The results provide insights into the molecular mechanisms of retroviral integration, demonstrating how the PFV intasome is able to integrate in all integration sites, and how a steric hindrance prevents the HIV-1 intasome from doing the same.

Overall, our findings further elucidate the intasome/nucleosome interactions and support further exploration of these interfaces as potential targets for antiviral intervention.

**Acknowledgments:** This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and 2021.07128.BD. We acknowledge the Academy of Finland for awarding this project access to the LUMI supercomputer, owned by the EuroHPC Joint Undertaking, hosted by CSC (Finland) and the LUMI consortium through the Academy of Finland funding programme.

# O2 – Boazinha, João Pedro

## Hybrid AI / Physics-based modelling of Human Voltage-gated Sodium Channels for the Development of Isoform Specific Drug Design

João Boazinha[1], João Carneiro[2], Nuno M.F.S.A. Cerqueira[1] & Sergio F. Sousa[1,3]

[1] LAQV/REQUIMTE – BioSIM – Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research, CIIMAR, University of Porto, Porto, Portugal
[3] Department of Chemical Engineering, FCTUC, University of Coimbra, Portugal

**Abstract:**
Voltage-gated sodium channels (NaVs) are channels that exchange between an open and close states in response to changes in membrane potential, allowing $Na^+$ ions to cross the membranes of excitable cells, such as neurons and muscle. They are essential for starting and transmitting action potentials, enabling neuronal firing, muscle contraction, and electrical signalling throughout the body (Pan et al., 2018; Li, Chen e Li, 2019a).
In human, different NaV isoforms exhibit tissue-specific expression patterns and distinct biophysical properties that determine their physiological roles: NaV1.1, NaV1.2, NaV1.3, and NaV1.6 are critical for central nervous system excitability, NaV1.4 controls skeletal muscle contraction, NaV1.5 initiates cardiac action potentials, and NaV1.7, NaV1.8, and NaV1.9 mediate peripheral pain signalling, making isoform selectivity a key challenge and opportunity in drug development (Pan et al., 2018; Li, Chen e Li, 2019a).
A comprehensive atomic-level description of human NaV channels remains unavailable because experimental X-ray and cryo-EM structures are either scarce, absent for many isoforms, or incomplete.
This study describes the development and application of a hybrid protocol, combining AI-based methods and physics-based approaches to create realistic models of different NaV channels embedded in realistic human cytoplasmic models. The strategy involves: (1) the use of AlphaFold3 to generate the five best full structures in complex with auxiliary structures; (2) the comparison through RMSD values of models created with other AI-based tools, like AlphaFold2, chai-1, boltz-2 and experimental partial structures available on the Protein Data Bank ; (3) manual manipulation of loops using PyMol, to corrected topological inconsistencies; (4) the creating of a realistic heterogenous biomembrane model with Packmol-Memgen with a characteristic lipid compositions of human cell cytoplasm; (5) application of molecular dynamics simulations with Lipid17, Lipid21, and ff14SB force fields to stabilize and refined the full model.

These results offer new insights into the structural differences among NaVs that can be leveraged to design new compounds targeting some of these specific channels while avoiding others that may act as off-targets.

**References:**

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**Acknowledgements:**

This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and FCT (2023.04909.BDANA)

# O3 – Amorim, Ana Miguel

## GPCR target discovery via multiclass activity mapping with GPCR-A17 MAAP

Ana B. Caniceiro[1,2,3,4,5], Ana M. B. Amorim[1,2,3,4,5], Nícia Rosário-Ferreira[4,5], Irina S. Moreira[1,3,4,5]

[1] CNC - Center for Neuroscience and Cell Biology, Center for Innovative Biomedicine and Biotechnology, University of Coimbra,
Coimbra, Portugal.
[2] PhD in Bioscience, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.
[3] Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.
[4] CoLAB4Ageing - Sítio Paço das Escolas, Reitoria da Universidade de Coimbra, 3000-530 Coimbra, Portugal.
[5] PURR.AI

**Abstract:**
Predicting ligand behaviour in the GPCR-A17 subfamily is difficult, as the same molecule can act as an agonist, antagonist, or allosteric modulator depending on the receptor context. To address this complexity, we developed GPCR-A17 MAAP, an ensemble model that integrates detailed ligand physicochemical descriptors with protein sequence embeddings to infer functional behaviour across A17 receptors. Using 6,919 curated ligand–receptor interactions, the combined XGBoost, Random Forest, and LightGBM framework achieved strong performance, yielding an F1 score of 0.9179 on the testing set and 0.7151 when evaluated on an independent ligand set. To investigate whether data quality influences predictive accuracy, we constructed a Ki-filtered dataset of 4,274 interactions supported by experimentally determined binding affinities. Training a parallel ensemble on this refined subset further boosted performance, producing F1 scores of 0.9330 on the testing set and 0.8267 for independent ligands. Feature-importance analyses showed that both ligand descriptors and receptor embeddings contribute critically to model decisions, underscoring the necessity of incorporating receptor-specific information when classifying ligand function [1].

**References:**
[1] A. B. Caniceiro, A. M. B. Amorim, N. Rosário-Ferreira and I. S. Moreira, J. Cheminform., 17, 102, (2025).

# O4 – Gritsok, Dmitrij

## Benchmarking Deep Learning Models for Viral Protein Assembly: A Comparative Case Study of the Monkeypox Envelope Protein A29L

Dmitrij Gritsok, Célia Gomes Amorim, Martin Hedström, Maria C.B.S.M. Montenegro, Sérgio F. Sousa

LAQV@REQUIMTE, University of Porto

**Abstract:**
The Monkeypox virus envelope protein A29L is a critical mediator of viral fusion, yet its 3D structure remains experimentally unsolved due to its complex oligomeric nature. In this study, we employed a bottom-up approach, integrating physicochemical sequence analysis with a comparative assessment of deep learning-based structure prediction methods.

Prior to modeling, we characterized the structural determinants of A29L. Multiple sequence alignments with the Poxviruses and homologous Vaccinia virus protein A27L (94.5% identity) and heptad repeat analysis identified a clear coiled-coil signature. Crucially, examination of the A27L template (PDB: 3VOP) suggested a non-canonical topology comprising two parallel chains and one antiparallel chain.

To resolve this assembly, we evaluated AlphaFold2, AlphaFold3, OpenFold2, OpenFold3, RoseTTAFold, Chai-1, Protenix, and Boltz-2. Standard "black-box" predictions failed to consistently reproduce the stable oligomer suggested by our sequence analysis, yielding low confidence scores (ipTM ~0.52) and incorrect parallel bundles. By introducing geometric constraints derived from our physicochemical characterization, we compared the ability of Protenix, Chai-1, and Boltz-2 to incorporate these data. While Protenix struggled to respect these restraints and Chai-1 didn't reach good confidence, Boltz-2 robustly generated a high-confidence trimeric model (ipTM 0.82) matching the specific parallel/antiparallel topology. This study demonstrates the necessity of guiding AI pipelines with chemical intuition for complex viral targets.

**Acknowledgements:**
This research was funded by PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia; Ministério da Educação, Ciência e Inovação) through the project UID/50006 –

Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos. Dmitrij Gritsok is grateful to FCT (Fundação para a Ciência e Tecnologia) for his PhD research grant with ref. PRT/BD/154971/2023 established within EUGLOH–U.Porto protocol.

# O5 – Constança Ilunga

## Design and characterization of MHETase mutants for improved activity and solubility in PET degradation

Constança Ilunga[1], Alexandra Balola[1], Sofia Ferreira[1], Cláudio Soares[1], Isabel Rocha[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Oeiras, Portugal

**Abstract:**
Polyethylene terephthalate (PET) is a petroleum-based plastic valued for its durability and chemical inertness. However, it has become a major environmental challenge due to its inefficient recycling and improper disposal. The bacterium Ideonella sakaiensis can biodegrade PET with two key enzymes: PETase, which breaks down PET into smaller intermediates like mono-(2-hydroxyethyl) terephthalate (MHET), and MHETase, which hydrolyzes MHET into the monomers terephthalic acid (TPA) and ethylene glycol (EG). Although PETase has been extensively studied and engineered, MHETase remains comparatively underexplored. This work addresses this gap by engineering mutants to improve both its catalytical efficiency and solubility.
Using our in-house platform GDEE (Gene Discovery and Enzyme Engineering), thousands of MHETase mutants were generated by systematically modifying amino acid residues in its active site. Mutants were ranked based on Autodock Vina metrics, and the 14 promising candidates were selected for laboratory validation. Enzymatic activity assays revealed four mutants with improved performance, highlighting the value of computational design. Molecular dynamics simulations indicated that catalytic efficiency depends on factors beyond substrate binding, underscoring the influence of enzyme flexibility on catalysis.
To address solubility, the machine-learning platforms AggreProt and MutCompute were applied to identify mutations outside the active site. Split-GFP assays demonstrated that all designed mutants displayed higher solubility than the wild type.
Additionally, size-exclusion chromatography combined with AlphaFold3 modeling revealed that MHETase exists as both monomer and dimer in solution, challenging previous assumptions of that it functions exclusively as a monomer. These findings provide the first combined computational and experimental evidence for MHETase dimerization and offer new structural insights into the enzyme.
This study demonstrates an integrated in silico–in vitro framework for MHETase optimization, contributing to enhance PET biodegradation and contributing to a more sustainable plastic waste management.

# O6 – Gomes, André

## From Chemistry to Code: Automating QM Workflows for Solubility Prediction

BioISI - Instituto de Biossistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa; Hovione FarmaCiência S.A.

**Abstract:**
The pharmaceutical industry is evolving rapidly, driven by the need for efficiency, cost reduction, and faster drug development. A major challenge in early drug formulation is predicting key physicochemical properties such as solubility and stability. Quantum mechanics (QM)-based computational methods provide valuable molecular insights, reducing reliance on costly experimental testing. Despite these advantages, QM adoption in the pharmaceutical industry remains limited due to expertise requirements, computational cost, and the need for accurate molecular conformations. Poorly chosen conformers can impact solubility predictions, affecting drug development reliability. Ensuring relevant conformers is essential for maximizing predictive accuracy. [1]
To address this, we evaluated how different conformer generation approaches influence QM-based solubility and Gibbs free energy of solvation (ΔGsolv) predictions. We compared multiple strategies to balance accuracy and computational cost while automating workflows for efficiency. By integrating these approaches, we streamlined QM calculations, minimizing manual intervention and bridging the gap between computational tools and industry applications. We compiled a dataset of 189 commercially available molecules with experimentally determined solubility across 36 solvents. A subset also included experimental ΔGsolv data. To assess QM-based calculations and the impact of conformer generation, we developed automated workflows incorporating four distinct approaches: (1) RDKit conformer generation with Merck molecular force field (MMFF) optimization; (2) RDKit + MMFF followed by DFT/BP86/def2-TZVPD optimization; (3) a basin-hopping-inspired method with GFN2-xTB [2] and DFT optimization; and (4) the openCOSMO-RS conformer generation workflow [3], as the refence methodology, featuring three consecutive DFT optimization steps. Each workflow used the SMILES code as input to determine the lowest energy conformation. The lowest energy conformers underwent Single Point calculations (DFT/BP86/def2-TZVPD) to extract molecular features for thermodynamic

predictions, including solubility via COSMO-RS. The sigma profile was directly extracted from QM calculations to compute activity coefficients and compare predicted solubility with experimental data. [4,5]

Our preliminary results demonstrate promising solubility prediction performance with comparable accuracy across workflows, despite the substantial methodological differences and computational cost. Notably, simpler methods like RDKit-based workflow (1) achieved similar accuracy while offering significant computational efficiency. This suggests that, despite initial conformations being a source of error, fast and accessible methods can provide reliable predictions in certain cases.

Overall, this QM-based automated approach offers a robust framework for predicting key molecular properties, refining predictive models, and accelerating drug discovery, particularly when experimental data is limited. These workflows hold potential for broader applications in predicting thermodynamic properties using QM calculations, and optimizing pharmaceutical industry processes.

**Acknowledgements:**

The authors thank Fundação para a Ciência e a Tecnologia (FCT) for doctoral grant 2024.05026.BDANA (AMMG), projects UID/04046/2025 (BioISI), 2023.10475.CPCA (PJC, DOI: 10.54499/2023.10475.CPCA.A2), and 2024.16247.PEX (PJC).

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# O7 – Figueiredo, Ana Teresa

## Computational Model of Phosphatidylinositol Protonation in Membranes

Ana Teresa Figueiredo, João Vitorino, Miguel Machuqueiro

BioISI

**Abstract:**
Phosphatidylinositols (PIPs) are ubiquitous signaling molecules with distinct biological roles and tightly controlled metabolism. They distinguish themselves from other lipids due to their inositol ring head group, which can be reversibly phosphorylated up to three times. PIPs' structural properties are primarily defined by the characteristics of this polar head group. At physiological pH, the inositol ring head group is highly negatively charged, enabling strong electrostatic interactions. The global protonation state of PIPs significantly influences their binding affinities and specificity for certain protein domains, as well as their interactions with other lipids (including PIPs themselves) and divalent cations.
Molecular-level studies of PIPs have been performed computationally to examine their protonation dynamics and interactions with proteins. However, these methodologies fix PIPs' protonation to a single state, introducing significant approximations to their results. Therefore, in this work, we developed a computational model that accurately describes the protonation dynamics of PIPs by employing our in-house constant-pH molecular dynamics (CpHMD) code and the CHARMM force field. Three membrane systems (PI4P, PI(4,5)P2, and PI(3,4,5)P3) in a POPC lipid bilayer were tested under two conditions: infinite dilution (ID) and 9% molar fraction of PIPs. CpHMD simulations were performed over a pH range that allowed us to capture PIPs' total protonation curves and calculate the corresponding macroscopic pKa values. This is a crucial step towards understanding how pH affects PIPs' interactions and further comprehending the role of protonation in their binding affinities, which are essential for their diverse functions.

# O8 – Vitorino, João

## Studying Protonation-Driven Dynamics in Class A GPCR Activation Mechanism Through Enhanced Sampling

João Vitorino, Carlos Barreto, Irina Moreira, Miguel Machuqueiro

BioISI - FCUL

**Abstract:**
G protein-coupled receptors (GPCRs) are the largest family of membrane proteins in the cell, and they are involved in nearly all physiological processes. They constitute the pharmaceutical targets for >35% of all drugs currently on the market. Research indicates that class-A GPCRs (the largest subfamily) exhibit a conserved (de)activation mechanism defined by changes in their residue interaction networks [1]. Although residue Asp2.50 has been previously proposed as a microswitch for this mechanism, its precise functional contribution remains unclear [2].
In our studies, we conducted pKa calculations based on 100-ns molecular dynamics (MD) simulations using the CHARMM36m force field (in 5 different class A receptors) that led to the identification of a clear pKa shift (~1.3 units) between active and inactive conformations for non-constitutive GPCRs. In contrast, constitutively active receptors showed minimal and variable shifts (ranging from –0.3 to 0.3) [3].
Constant-pH Molecular Dynamics (CpHMD) simulations were also run for select receptor systems. Notably, in inactive states, the limited hydration within the Asp2.50 binding pocket impeded accurate Poisson–Boltzmann assessments of solvent accessibility, yielding artificially elevated pKa values. Our CpHMD analyses suggested potential inadequacies in capturing sufficient hydration of Asp2.50 with CHARMM36m, leading to deficient protonation-state sampling. Preliminary results with GROMOS 54a7 parameters indicate that this force field, or at least different atomic parameters, might be better suited to capture the nuances of this mechanism, showcasing clear protonation preferences between active and inactive conformations.
Moreover, using PLUMED metadynamics CpHMD simulations with GROMOS 54a7, we aim to study the complete Active-to-Inactive state transition and associated residue protonation changes. The elucidation of this mechanism to this level of detail is unprecedented and may therefore fuel future research on receptor functionality and the discovery of novel therapeutic agents.

**Acknowledgements:**
Work supported by Fundação para a Ciência e Tecnologia, for grant 2022.11124.BD and

projects UID/04046/2025 (Biosystems and Integrative Sciences Institute Centre). Also funded by the European Commission (TWIN2PIPSA, GA 101079147).

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[2] Ranganathan et al., 2014, doi:10.1021/bi5008723
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# O9 – Ferreira, Sara Gabriela Ferraz

## Tied Up in Function: The Role of the Knot in UCH-L1 Activity

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**Abstract:**
Knotted proteins present a compelling paradigm for understanding how topological complexity can shape protein folding, stability, and function. Among these proteins, UCH-L1, a monomeric cysteine protease featuring a 5_2 knot, plays a key role in the ubiquitin-proteasome system and its association with neurodegenerative disorders. Notably, the knotted region resides near the catalytic core, raising the possibility that the knot may modulate enzymatic regulation by influencing conformational dynamics and substrate access.
To investigate this hypothesis, we developed a computational approach combining steered molecular dynamics, umbrella sampling, and classical MD simulations to probe the energetic and structural implications of unknotting. By systematically applying directional forces along defined reaction coordinates, we generated a continuum of partially unknotted intermediates, capturing distinct stages of the unknotting pathway. These intermediates enabled the reconstruction of the free energy landscape associated with knot disruption and allowed for the characterization of associated structural rearrangements. Fully unknotted forms of UCH-L1 were further explored in both apo and ubiquitin-bound states to evaluate the effects of this topological change on structural integrity, substrate interaction, and active site configuration.
Our results suggest that while the knotted topology is slightly more energetically favorable, it is separated from the unknotted state by a significant free energy barrier, consistent with knot formation occurring early in the folding process. In the presence of ubiquitin, removal of the knot reshapes the conformational ensemble and perturbs the distribution of catalytically active states. Together, these findings support a model in which catalytic function arises from an interplay between topological constraints and the spatial positioning of the N-terminal, implicating knotting as a potential regulator of enzymatic activity.

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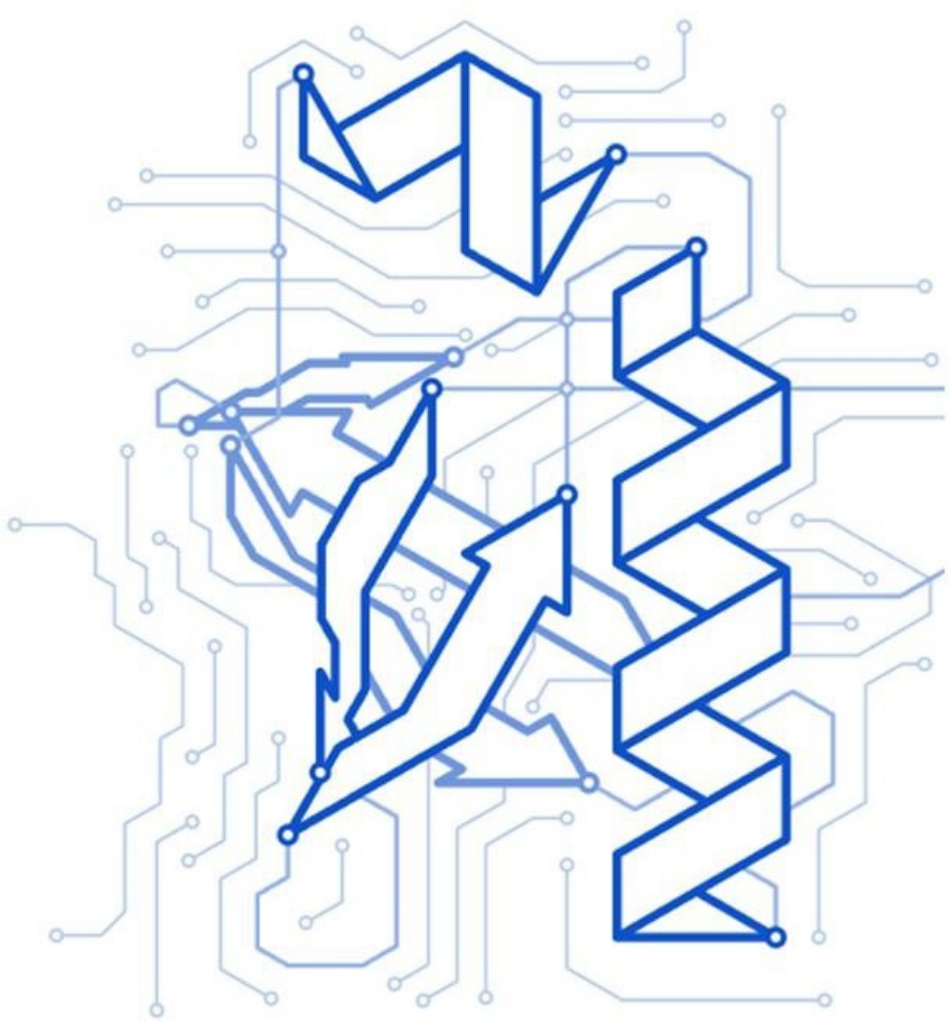
# Posters



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# P1 – Roggero, Airam

## Structural and Dynamical Determinants of Kaempferol Interaction with PLA2 and LPCAT

LAQV/REQUIMTE - BioSIM

**Abstract:**

Kaempferol, a naturally occurring flavonoid, is widely recognised for its antioxidant and anti-inflammatory potential. Increasing evidence, however, points to a strong context-dependent behaviour. Here, we employed an integrated computational and experimental strategy to investigate the Janus-like dual activity of kaempferol toward key enzymes of the Lands cycle, focusing on phospholipase $A_2$ ($PLA_2$) and LPCAT3. Molecular docking indicated that kaempferol binds reversibly to the catalytic region of both enzymes with moderate affinity. Molecular dynamics simulations revealed stable, non-disruptive complexes under physiological conditions, maintaining structural compactness and persistent hydrogen-bonding networks. Under oxidative and pro-inflammatory simulation parameters, however, kaempferol induced higher structural fluctuations and partial destabilisation of the lipid-interacting interface, suggesting a shift toward a pro-oxidant profile. Experimental data supported these predictions. In vitro by HPLC and enzyme inhibition assay and in vivo analyses showed hepatoprotective and lipid-modulating effects under basal conditions. Conversely, under elevated $PLA_2$ activity, kaempferol administration increased GGT, cholesterol, and renal markers, indicative of redox imbalance and enhanced membrane turnover. Inflammatory challenge models further demonstrated exacerbation of lipid dysregulation and oxidative stress. Overall, our results show that kaempferol functions as a bifunctional modulator of the Lands cycle: antioxidant and protective under homeostasis, but potentially pro-oxidant when phospholipid remodelling is overactivated. These findings highlight the value of combining in silico predictions with biochemical validation to better characterise natural compounds and guide their safe therapeutic application.

# P2 – Caniceiro, Ana Beatriz

## GPCR-A17 MAAP: A predictive model to identify modulator, agonist, antagonist roles in GPCR-A17 interactions

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**Abstract**:
G protein-coupled receptors (GPCRs) are central to cellular signalling and represent critical targets for developing safe and effective therapeutics. Within the GPCR-A17 subfamily, which is linked to diverse disorders, ligand interactions can influence both efficacy and toxicity. To address these challenges, we developed GPCR-A17 MAAP, an ensemble machine learning framework combining XGBoost, Random Forest, and LightGBM to classify ligands as agonists, antagonists, or modulators. Trained on over 3,000 ligands and 6,900 protein-ligand interactions from the Guide to Pharmacology, Therapeutic Target Database, and ChEMBL, the model achieved high predictive performance (F1 scores of 0.92 and 0.72; AUCs of 0.98 and 0.86 on testing and independent validation sets). A Ki-filtered subset further enhanced accuracy (F1 up to 0.93 and 0.83). By supporting the early identification of functional roles, GPCR-A17 MAAP offers a valuable tool to guide experimental validation and reduce downstream toxicological failures in drug discovery [1].

**Keywords:**
Agonist, Antagonist, Modulator, Binding Affinity (Ki), Drug Discovery, Ensemble Learning, GPCR subfamily A17.

**References:**

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# P3 – Pereira, Ana Catarina

## Molecular docking reveals CDK1 as a therapeutic target of novel purine derivatives in triple negative breast cancer

Ana Catarina Pereira[1,2], Soraia P. Fernandes[2], Bruna Leite[2], Rafael Vieira[3], Cátia Santos-Pereira[1,5], Débora Ferreira[1,5], Sérgio F. Sousa[3,4], Alice Dias2 and Lígia Rodrigues[1,5]

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**Abstract**:
Triple negative breast cancer (TNBC) accounts for about 15% of the more than two million breast cancer cases diagnosed annually. Defined by the lack of estrogen (ER), progesterone (PR), and epidermal growth factor receptor 2 (HER2) expression, TNBC is highly aggressive, exhibits rapid proliferation, and shows poor response to conventional and hormonal therapies, highlighting the urgent need for new therapeutic strategies. Given the emerging anticancer potential of purine scaffolds, newly synthesized 6-cycloalkylaminoadenine derivatives were investigated for their therapeutic potential. These purine-based molecules were evaluated for antiproliferative activity in TNBC cells. Among them, one compound (3.2a) displayed notable growth-inhibitory effects and reduced motility and invasiveness of the TNBC MDA-MB-231 cell line. Further analyses indicated that this compound promotes cytoskeletal alterations and triggers apoptotic pathways. Computational docking was performed against a panel of signaling proteins associated with cell cycle regulation and oncogenic survival pathways. A validated molecular docking pipeline was established through redocking assays using GOLD with PLP and GoldScore scoring functions. Compound 3.2a showed a favorable docking score against CDK1, a key regulator of cell cycle progression, forming hydrogen bonds and hydrophobic interactions characteristic of high-affinity ATP-competitive inhibitors. Taken together, these results highlight the 6-cycloalkylaminoadenine scaffold as a promising lead structure for the rational design of selective CDK1 inhibitors for TNBC therapy.

# P4 – Vitorino, Diana

## Protonation-Dependent Stability of DJ-1 Dimers and Its Implications for Parkinson's Disease

Diana I. M. Vitorino, Miguel Machuqueiro

BioISI, FCUL

**Abstract**:

DJ-1 is a multifunctional, neuroprotective protein that acts as both an oxidative stress sensor and a protein deglycase. Its stability and function are highly dependent on its oligomeric state, naturally occurring as a dimer. The dimerization process has been proposed to be strongly modulated by the protonation states of two carboxylic acids, Glu15 and Asp24, which form a unique hydrogen bond at the dimer interface. Mutations in DJ-1, as well as oxidation-state modifications, such as those at Cys106, can compromise its structural integrity, impair dimerization, and ultimately lead to aggregation, which is intimately associated with neurodegenerative diseases, such as Parkinson's Disease (PD) [1].

In this study, we use constant-pH molecular dynamics (CpHMD) simulations to understand the roles of protonation and carboxylic acid H-bonds in regulating DJ-1 dimer stability. We will also investigate the effects of a set of Parkinson's disease-associated mutations and Cys106 oxidation to determine their impact on dimerization and overall protein aggregation propensity. CpHMD is particularly important for this study because it enables titratable residues to change protonation state during the simulation, allowing simultaneous sampling of conformational and protonation states that influence DJ-1 dimer stability [2]. This provides valuable insights into dimerization and the mechanisms underlying neurodegeneration in PD.

We have built and equilibrated wild-type monomeric and dimeric models of human DJ-1 and simulated them at three pH values (5.2, 6.2, and 7.2), each with five replicas, to obtain titration curves and evaluate protonation under physiological and stress-related conditions. These simulations are complete, and preliminary data will be presented in this communication.

**Acknowledgments:**
We acknowledge Fundação para a Ciência e Tecnologia for funding through the project UID/04046/2025.

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# P5 – Kiriwan, Duangnapa

## Identification of Electron Transfer Binding Sites on OmcS Nanowires in Geobacter sulfurreducens

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**Abstract**:

Geobacter sulfurreducens is a well-known bacterium capable of generating electric current1-3. Like other organisms that lack oxygen or soluble electron acceptors, it transfers electrons to external acceptors during respiration via conductive filaments (nanowires), a process known as extracellular electron transfer (EET). The bacterium relies on nanowires composed of the c-type cytochrome OmcS to dispose of respiratory electrons by transferring them to solid external acceptors such as Fe(III) oxides4. Electron transport begins at the inner membrane and proceeds across the periplasm and outer membrane before reaching the extracellular environment4,5. Recent studies have shown that periplasmic cytochromes PpcABCDE can transfer electrons directly to OmcS nanowires through transient interaction at specific regions of OmcS located near the periplasmic side of the outer membrane5. However, the precise interaction surfaces and electron injection points remain unclear. In this study, we aim to identify electron transfer binding sites on OmcS filaments to better understand their structure and function. Using two monomers from the OmcS structure (PDB ID: 6EF8) as the initial model, we performed molecular dynamics simulations, cavity detection and characterization, pocket prediction, and solvent-accessible surface area calculations to identify potential interaction sites. We identify structurally and electrostatically compatible sites on OmcS that may facilitate electron transfer from partner cytochromes.

**Keywords:**

Extracellular Electron Transfer (EET), OmcS Nanowires, Periplasmic c-type Cytochromes

(PpcA–E)

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# P6 – Duarte, Francisco

## Studying the Behavior of sigma-holes of Sulfur-Containing Compounds by Probing Electrostatic Potential Maps

BioISI-FCUL

**Abstract:**

The importance of noncovalent interactions in molecular recognition is well established, with hydrogen bonding ranking as the most studied one. In recent years, sigma-hole interactions, particularly halogen bonding, have gained significant attention for their role in modulating binding properties. However, chalcogen bonding (ChB), a closely related yet distinct interaction, remains underexplored despite its relevance in diverse chemical and biological contexts. A major limitation hindering their systematic study and application lies in the absence of a universal parameterization framework to study these interactions using computational methods based on classical force fields. . Building on prior work modeling halogen bonds via force field methods, this work shifts the focus toward chalcogen bonds, aiming to develop robust computational tools for their accurate representation. Herein, we show a preliminary analysis focused on the analysis of the topology of electrostatic potetential maps of a library of sulfur-containing compounds with around 300 different sulfur atoms in total. All compounds went through QM calculations, in particular geometry optimization and vibrational frequency calculations at the B3LYP/6-311G* level of theory. The electrostatic potential surface was then calculated, allowing the obtain the extrema, particularly the maxima. Further QM calculations were performed on a select group of compounds to determine if all these maxima found near the sulfur atoms (sigma-holes) are able to form a chalcogen bond with a Lewis base (NH3 or HCN). Our results show that despite other types of sulfur atoms presenting sigma-holes, only those with a coordination number two, excluding thiols, seem to be able to interact with the Lewis basis in such an interaction. In the near future, extra-points of charge will be implemented in a force field to describe these regions and solvation energy calculations will be performed and fitted against experimental data.

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# P7 – Pires, Inês D. S.

## Building a computational model of Rhodobacter Sphaeroides Cytochrome c Oxidase to study the proton pumping mechanism

Pires, Inês D. S., Baptista, António M., Machuqueiro, Miguel

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**Abstract:**

Cytochrome c Oxidase (CcO) is the terminal enzyme of the electron transport chain, whose activity reduces oxygen to water, with the uptake of protons from the mitochondrial matrix. There is an additional pumping of protons to the intermembrane space, contributing to the maintenance of the proton electrochemical gradient, responsible for regulating ATP production, among other phenomena. The directionality of the pumping has been likened to a gate, where key residues, sensitive to electrostatic environment variations, prevent the return of protons to the N-side through modulations of their proton affinity (pKa value). Still, important details regarding the catalytic cycle and gating residues are still missing.

We built a computational model of Rhodobacter Sphaeroides CcO to investigate these key residues which pKa shifts. Constant pH molecular dynamics (CpHMD) simulations allow for an adequate description of proton affinity dynamics, however it is not trivial to apply to a system as complex as this. Parameters for both the metal cofactors and bound residues are not included in standard protein force fields, and those already published are not compatible with the alternative catalytic cycle we compiled from the literature. Hence, we obtained partial charges for these groups using Quantum Mechanics (QM) calculations coupled with RESP. We will be showcasing results from the QM analysis, validating the catalytic cycle proposed, and from molecular dynamics simulations, validating the stability of this charge set.

**Acknowledgements:**

The authors would like to acknowledge FCT and BioISI for funding (2023.01155.BD and UID/04046/2025).

# P8 – Matias, João Gomes

## Computational Approach towards selection of novel DprE1 inhibitors

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**Abstract:**

Mycobacterium Tuberculosis (MTB) is a prominent infectious agent that causes more than one million deaths worldwide every year. Recently, a novel group of compounds, dinitrobenzamides (DNBs), has shown antibacterial effects towards MTB due to their interaction with a critical enzyme, DprE1 located in the periplasm of the bacterium. In our work, we explored the potential of molecular docking for the development of a predictive model of activity of DNBs against DprE1 using a library of more than 600 compounds. The post-processing of the docking poses also allows for the extraction of regions of high density for the presence of certain chemical groups, which could provide clues for compound selectivity.

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# P9 – Gonçalves, João Nuno Dias

## Target Prediction and Optimization of New Fluorescent Nucleoside Analogues

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**Abstract:**

Fluorescent nucleoside analogues (FNAs) are a class of molecules that share structural similarities with natural nucleosides while emitting fluorescence. However, the spectral properties of most FNAs synthesized so far remain in or near the UV region, limiting their applications to spectral analysis and making them non-viable options for imaging applications.[1] The resemblance of FNAs with endogenous nucleosides suggests a strong possibility of interaction with the cell membrane transport and signalling systems, as well as nucleoside-associated intracellular machinery. This motivated our group to engineer new FNAs with improved emissive properties that are suitable for imaging and high-throughput applications, such as fluorescence microscopy and flow cytometry, respectively. The new class of improved FNAs was developed and preliminary biological assessments proved their potential as putative fluorescent probes.[2,3] Since the targets with which they interact remain elusive, we aim to investigate the potential targets of ours FNAs through a reverse virtual screening protocol and improve target binding via targeted-oriented chemical modifications to the fluorescent nucleobase scaffold. Particular emphasis will be given to nucleoside transporters and

purinergic receptors to explore a potential dual pharmacological modulation, as well as building towards an alternative fluorimetric method for studying nucleoside transporters as opposed to the currently used radioisotopic techniques.

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# P10 – Sequeira, João

## Optimizing Lennard–Jones Radii to Improve Constant-pH Molecular Dynamics

João G. N. Sequeira, João N. M. Vitorino and Miguel Machuqueiro

BioISI - FCUL

**Abstract:**

Accurate protonation-state sampling in Constant-pH Molecular Dynamics (CpHMD) depends critically on the electrostatic model used. In the stochastic titration CpHMD method, Poisson–Boltzmann calculations are used. However, the Lennard-Jones radii currently employed were derived for older force fields and may not optimally represent modern biomolecular parameter sets. This project aims to systematically evaluate alternative LJ radii for GROMOS54A7, CHARMM36m, and AMBER14SB by combining pKmod calibration with extensive CpHMD simulations of benchmark proteins with well-characterized experimental pKa values.

Although the work is ongoing, this poster will present the computational design, early pilot simulations, and the rationale for a unified radius optimization strategy. By improving the electrostatic accuracy underlying CpHMD, we aim to strengthen the methodological foundation for pH-aware biomolecular simulations in the community.

**Acknowledgments:**

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# P11 – Batista, Marta S. P.

## Identification of Key Titratable Residues Mediating pH Regulation in hAQP7

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**Abstract:**

Aquaporins (AQPs) facilitate the permeation of solutes across membranes. They can be divided into two subgroups: classical aquaporins, which are strictly selective for water, and aquaglyceroporins, which can permeate water and glycerol. Human aquaglyceroporin 7 (hAQP7) plays a crucial role in adipose tissue metabolism by mediating glycerol efflux for gluconeogenesis in the liver [1]. Notably, AQP7 is pH-sensitive, exhibiting high glycerol permeability at physiological pH (7.4), which decreases by approximately 50% under acidic conditions [2]. However, the molecular mechanisms underlying this pH-regulation remain unclear. To address this, we performed Constant-pH Molecular Dynamics (CpHMD) simulations [3] to investigate the behavior of titratable key residues in AQP7 at pH 5.0, 6.2, and 7.4. Our simulations revealed histidine and glutamate residues within the AQP7 channel pores and vestibules, whose protonation states change significantly, suggesting their relevance to channel dynamics and substrate transport efficiency. Additionally, our results indicate that pH-induced structural rearrangements modulate the hydrophobicity and steric properties of the permeation pathway, thereby modulating glycerol flux. These findings provide molecular-level insights into the pH-dependent regulation of AQP7 and highlight potential mechanisms by which acidity may influence glycerol transport in adipose tissue. A deeper understanding of these regulatory mechanisms could support the development of targeted strategies for modulating AQP7 function in metabolic disorders.

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# P12 – Suzano, Pedro Miguel Sousa

## Preventing TrkB-FL Loss: A Small Molecule Approach to Target Alzheimer's & Epilepsy

Colab Accelbio

**Abstract:**

Neurological disorders like Alzheimer's disease (AD) and epilepsy (EP) represent significant societal and economic burdens globally. Current treatments often fail to halt disease progression, underscoring the urgent need for novel therapeutic strategies [1]. Emerging approaches aim to modulate neurotrophic signaling pathways, particularly those involving brain-derived neurotrophic factor (BDNF), a critical protein for neuronal survival, differentiation, and synaptic plasticity [2]. Under normal physiological conditions, BDNF binds to the full-length TrkB receptor (TrkB-FL), activating downstream signalling cascades essential for neurogenesis and brain function [2,3]. However, recent studies have shown that in both AD and EP, calpain-mediated cleavage of TrkB-FL disrupts this signaling by generating the non-functional fragments identified as TrkB-ICD and TrkB-T' [4]. The accumulation of TrkB-ICD within neurons has been associated with neurodegenerative processes and transcriptomic dysregulation [5]. While direct calpain inhibition is not viable due to its widespread physiological roles, a novel peptide, TAT-KK-TrkB, has been shown to act as a competitive substrate, preventing TrkB-FL cleavage [6]. Nevertheless, the therapeutic application of peptides is limited by challenges such as poor stability, low bioavailability, and limited blood-brain barrier permeability [5,7]. Our goal in this project is to identify small-molecule (SM) alternatives that replicate the protective mechanism of TAT-KK-TrkB against TrkB cleavage. To achieve this, we propose a multi-step workflow in which we will, first, characterize the interaction between TAT-KK-TrkB and calpain to pinpoint the key structural determinants regulating binding. Based on these insights, we will perform a structure-guided virtual screening campaign using a curated database of small molecules to identify promising therapeutic candidates. Top hits will then be experimentally validated with in vitro assays, with western blot analysis serving as the primary readout to assess TrkB-FL integrity. Ultimately, this approach aims to identify SM-based therapeutics that preserve TrkB-FL and restore BDNF signaling,

paving the way for novel treatment strategies for AD and EP.

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# P13 – Vitória, Sara

## Constant-pH Molecular Dynamics Study of the Monomer–Dimer Equilibrium of the pH-Sensing Protein PsbS

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**Abstract:**

Under intense sunlight, plants prevent photooxidation by dissipating excess absorbed energy as heat, a protective mechanism known as non-photochemical quenching (NPQ). This process is triggered by PsbS, a membrane protein responsive to pH changes in the thylakoid lumen. Although the precise mechanism of PsbS activation remains unclear, experimental evidence suggests that certain glutamate (Glu) residues on the lumen side may act as pH sensors, and that both the monomer-dimer equilibrium and the dimer conformation are pH-dependent. A recent computational study on the PsbS monomer supports these observations, identifying unusually high pKa values for several lumen-exposed Glu residues, suggesting a key role in pH sensitivity under physiological conditions. The study also reported pH-induced folding of a loop likely involved in dimerization and revealed correlations between protonation states across the membrane, potentially enabling lumen-stroma communication. The present work extends this investigation to the PsbS dimer, using constant-pH molecular dynamics (CpHMD) simulations, as in the previous study, to determine pKa values, conformational dynamics, and correlations between protonation and structural changes. By combining monomer and dimer data, the pH-dependence of dimerization and the role of key residues will be assessed using a thermodynamic linkage relation. This study employs the GROMACS molecular dynamics package, alongside in-house tools: meadTools, PETIT, and ST-CpHMD. The outcomes are expected to enhance our understanding of the PsbS activation mechanism and contribute to the development of strategies for improving plant photoprotection and crop performance.

# P14 – Melo, Tatiana

## Seaweeds as Sustainable Protein Sources: *In Silico* Identification of Bioactive Peptides Targeting sACE for Hypertension and Fibrosis Management

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**Abstract:**
The continuous growth of the human population is intensifying global food security concerns, highlighting the need for sustainable and nutritionally rich protein sources. Marine macroalgae (seaweeds) have emerged as promising alternatives to conventional plant and animal proteins due to their sustainability, high biomass productivity, and richness in bioactive compounds. Among these, peptides with health-promoting properties have gained particular interest, especially those capable of inhibiting the angiotensin-converting enzyme (ACE), a key therapeutic target for hypertension and fibrosis. In this study, an *in silico* workflow was applied to simulate the gastrointestinal digestion of 300 seaweed-derived protein sequences and to assess the inhibitory potential of the resulting peptides against the N- and C-domains of ACE. Domain selectivity was evaluated through a molecular docking protocol. This approach enabled the identification of eight peptides with predicted bioactivity, originating from *Porphyra purpurea*, *Corallina officinalis*, *Nonlabens ulvanivorans*, *Formosa agariphila*, and *Phocaeicola plebeius*. The peptides TDPSAEDF, ATQAR, TTDGEEQTL, and PGPIGDVY showed higher affinity for the C-domain, suggesting potential antihypertensive effects. Conversely, QVQVSF, DAASVEY, CGVEVTES, and GDGVAEAW displayed preferential binding to the N-domain, indicating possible antifibrotic activity. Allergenicity assessment further revealed that TDPSAEDF, DAASVEY, and PGPIGDVY pose no predicted risk to the general population. Overall, this study highlights the potential of seaweed-derived peptides as selective ACE domain inhibitors with antihypertensive and antifibrotic properties. Incorporating seaweeds into dietary patterns may support the development of sustainable and health-promoting food systems. Nevertheless, experimental validation is required to confirm these bioactivities in vivo and to refine the safety, bioavailability, and effective dosage of the identified peptides.

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