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# EJIBCE<sup>2019</sup>

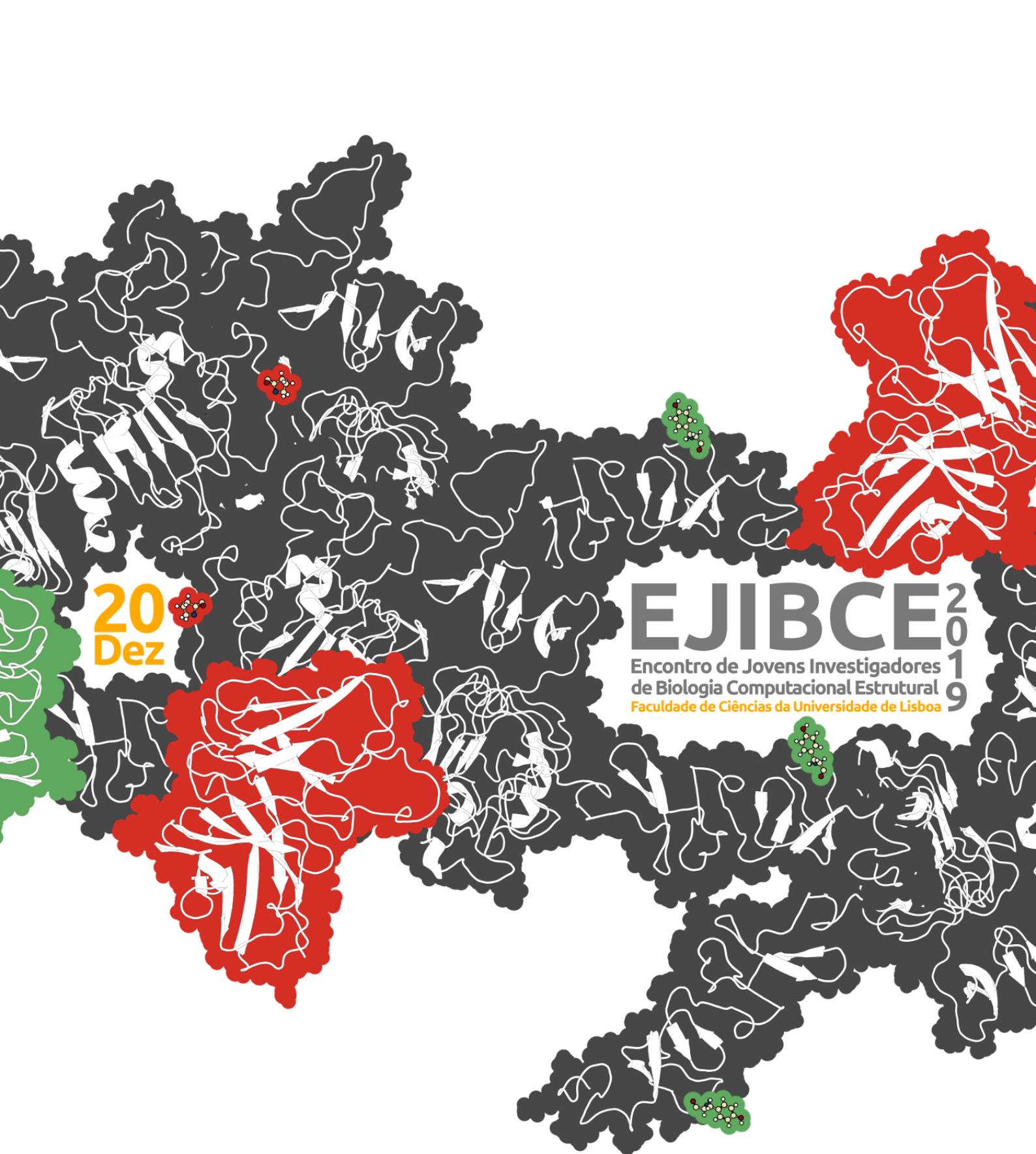
Encontro de Jovens Investigadores  
de Biologia Computacional Estrutural

Faculdade de Ciências da Universidade de Lisboa

## BOOK OF ABSTRACTS



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# PROGRAM

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# EJIBCE<sup>2019</sup>

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08 : 30

## \$ Registration



09 : 30

## \$ Welcome address



09 : 35

\K1 :: Ana Rita Colaço - Mining and Integration of Proteomics Data with Knowledge Graphs



10 : 05

O1 :: Bruno Calçada - In silico methodologies from an agrobusiness point of view



10 : 25

O2 :: Ana Sequeira - Building an automated platform for the classification of peptides/proteins using machine learning



10 : 45

## \$ Coffee break + Poster session



11 : 15

\K2 :: Ana Vila Verde - Molecular mechanisms behind protein halotolerance: a molecular dynamics study with optimised ion-ion force fields



11 : 45

O3 :: João Especial - Hydrophobic confinement modulates thermal stability and assists knotting in the folding of tangled proteins



12 : 05

O4 :: Ricardo Ferreira - Antibiotic uptake across gram-negative outer membranes: enabling targeted synthesis from optimized permeability predictions



12 : 25

## \$ Lunch + Poster session



14 : 30

\K3 :: Ana Sofia Oliveira - Understanding signal propagation in nicotinic acetylcholine receptors



15 : 00

\K4 :: Marta Perez - Addressing challenges in cancer immunotherapy with structural bioinformatics approaches



15 : 30

O5 :: Raquel Gouveia - Stargazin: CACNG2 Mutations in Neuropsychiatric Disorders



15 : 50

## \$ Coffee break + Poster session



16 : 30

\K5 :: Sara Campos - The effect of proton binding on dimer formation and other structural properties of  $\beta$ -lactoglobulin: a constant-pH MD study



17 : 00

O6 :: Carla Teixeira - Unraveling the chemistry beyond the catalytic activity of  $\omega$ -amidase



17 : 20

O7 :: Lucie da Rocha - Study of pH-dependent conformational changes on  $\beta$ -lactoglobulin using molecular modelling



17 : 40

## \$ Closing



18 : 00

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## KEYNOTES

## Mining and Integration of Proteomics Data with Knowledge Graphs

Alberto Santos, Ana R. Colaço, Annelaura B. Nielsen, Johannes Muller, Philipp E. Geyer, Matthias Mann

Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

Development of omics technologies such as genomics, proteomics or metabolomics has aided the analysis of biological systems in a cost-efficient and high-throughput manner. These technologies have produced high-resolution data, which allows an increasingly comprehensive and holistic view of biological processes and complex diseases. However, the huge amount of disparate data generated across the whole spectrum of biology creates great challenges in data aggregation, mining, integration and accessibility.

To overcome these challenges in a translational context, we have designed and implemented a system that integrates multi-omics data and information spread across diverse biomedical databases into a Clinical Knowledge Graph (CKG). This graph-based system uses nodes and edges to represent data points and the relationships between them, providing relevant biological context and producing real-time analytics. Here, we describe the CKG, which already encompasses 16 million entities and more than 112 million relationships between them. This unique platform enables data analysis and provides an excellent ecosystem for machine learning.

# Molecular mechanisms behind protein halotolerance: a molecular dynamics study with optimised ion-ion force fields

Ana Vila Verde

Max Planck Institute of Colloids and Interfaces

Halophilic organisms thrive in habitats with high (up to several mol/dm<sup>3</sup>) NaCl concentration. To counterbalance the high osmotic stress they experience, many halophiles accumulate KCl in their cytoplasm. Whereas proteins of most non-halophilic (i.e., mesophilic) organisms lose activity and/or structure under these conditions, halophilic proteins thrive in them, and sometimes even require them to function properly. Halophilic proteins differ from mesophilic ones in several aspects, one of them being their richness in acidic amino acids. This excess is presumably related to their ability to remain functional at high concentration of KCl, but the molecular mechanisms by which this happens are yet unclear.

Molecular dynamics simulations with atomistic resolution based on simplified descriptions of particle-particle interactions (force fields) are particularly suited to gain this insight, provided that the force fields adequately represent the correct balance of interactions between all species. While an acceptable balance between ion-water and water-water interactions is found in many force fields, ion-counterion interactions are typically poorly represented. Developing internally consistent force fields for ions is hindered in part by incomplete datasets of target experimental properties. To address this issue, my group developed a generic parameterisation approach that combines experimental data and ab initio simulations. The resulting force field is internally consistent, enabling insight into the molecular scale mechanisms behind ion-specific effects. In this presentation I will summarise the parameterisation approach and I will present recent results from all-atom molecular dynamics simulations of multiple halophilic/mesophilic protein pairs, that shed light into the roles played (and not played) by acidic amino acids in halophilic adaptation.

# Understanding signal propagation in nicotinic acetylcholine receptors

A. Sofia F. Oliveira

University of Bristol

Cigarette smoking is considered, nowadays, to be a significant public health problem. Recent estimates indicate that approximately 1/4th of the world's population smokes [1] and that smoking is the second most prevalent cause of death in the world [2]. Currently, the FDA-approved smoking cessation drugs, such as varenicline, are only moderately effective in reducing the symptoms of nicotine withdrawal and may cause undesirable side effects. Consequently, there is a growing need to develop new smoking cessation agents with improved effectiveness and tolerability.

Nicotine is the major biologically psychoactive agent in tobacco, and it binds to the nicotinic acetylcholine receptors (nAChRs) [3]. These receptors mediate synaptic transmission in the nervous system and are therapeutic targets for various neurodegenerative diseases, psychiatric and neurodevelopmental disorders, including nicotine addiction [3]. Over the last decades, nAChRs have been widely explored, and our understanding of their molecular mechanisms has made extensive progress. However, despite a plethora of available structural and biochemical data, it is still not clear how ligand binding induces the conformational changes necessary to modulate the receptor's dynamics. Answering this question requires knowledge of the dynamics of the protein and the identification of the conformational changes that take place upon ligand binding. Molecular dynamics (MD) simulations offer a highly effective method to identify, 'assay' and analyse functionally important motions of proteins and recently we have used a combination of equilibrium and nonequilibrium molecular dynamics simulations to map dynamic and structural changes induced by nicotine in two of the most relevant nAChRs, namely the  $\alpha 4\beta 2$  [4,5] and the  $\alpha 7$  [4,6] subtypes. Our simulations reveal a striking pattern of communication between the agonist-binding pockets and the transmembrane domains and show the sequence of conformational changes associated with the initial steps of signal propagation.

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5. Oliveira et al. Structure, 27, 1171-1183, 2019
6. Oliveira et al. under revision, 2019

## Addressing challenges in cancer immunotherapy with structural bioinformatics approaches

Marta A. S. Perez (1,2), Michal Bassani-Sternberg (3), George Coukos (3), David Gfeller (2,4), Vincent Zoete (1,2)

(1) Computer-Aided Molecular Engineering, Department of Oncology, Ludwig Institute for Cancer Research, University of Lausanne, Lausanne, Switzerland

(2) Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland

(3) Human Integrated Tumor Immunology Discovery Engine, Department of Oncology, Ludwig Institute for Cancer Research, University Hospital of Lausanne, Lausanne, Switzerland

(4) Computational Cancer Biology, Department of Oncology, Ludwig Institute for Cancer Research, University of Lausanne, Lausanne, Switzerland

Recent clinical developments in antitumor immunotherapy involving T-cell related therapeutics have led to a renewed interest for human leukocyte antigen class I (HLA-I) binding peptides, given their potential use as peptide vaccines<sup>1</sup>. Databases of HLA-I binding peptides hold therefore information on therapeutic targets essential for understanding immunity.

In this work, for the first time, we provide a structural view of the HLA-I peptidomics in the source proteins. <sup>2</sup> We use in-depth and accurate HLA-I peptidomics datasets<sup>3</sup>, and analyse properties of the HLA-I peptides in the source proteins with structural bioinformatics approaches. HLA-I binding peptides are studied grouping all alleles together or in allotype-specific contexts. We capitalize on the increasing number of structurally determined proteins to (1) map the 3D structure of HLA-I binding peptides into the source proteins for analysing their secondary structure and solvent accessibility in the protein context, and (2) search for potential differences between these properties in HLA-I binding peptides and in a reference dataset of HLA-I motif-like peptides. This is performed by an in-house developed heuristic search that considers peptides across all the human proteome and converges to a collection of peptides that exhibit exactly the same motif as the HLA-I peptides.

Our results, based on 9-mers matched to protein 3D structures, clearly show enriched sampling for HLA-I presentation of helical fragments in the source proteins. This enrichment is significant, as compared to 9-mer HLA-I motif-like peptides. The observation that HLA-I peptides have increased helix structure elements in the source is novel and of potential interest for researchers working in the field of antigen presentation and proteolysis. This knowledge refines the understanding of the rules governing antigen presentation and could be added to the parameters of the current peptide-MHC class I binding predictors to increase their antigen predictive ability. We provide several hypotheses regarding the origin of this enrichment.

<sup>1</sup> T cell-based therapeutics including PD-1 and CTLA4 immune checkpoint inhibitors – remarkable studies awarded with the Nobel prize in Medicine 2018.

<sup>2</sup> Perez, M.A.S., Bassani-Sternberg, M., Coukos, G., Gfeller, D. and Zoete, V., Analysis of secondary structure biases in naturally presented HLA-I ligands, *Frontiers in Immunology*, 2019.

<sup>3</sup> Bassani-Sternberg, M., et al., Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry. *Nat Commun*, 2016. 7: p. 13404.

# The effect of proton binding on dimer formation and other structural properties of $\beta$ -lactoglobulin: a constant-pH MD study

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

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

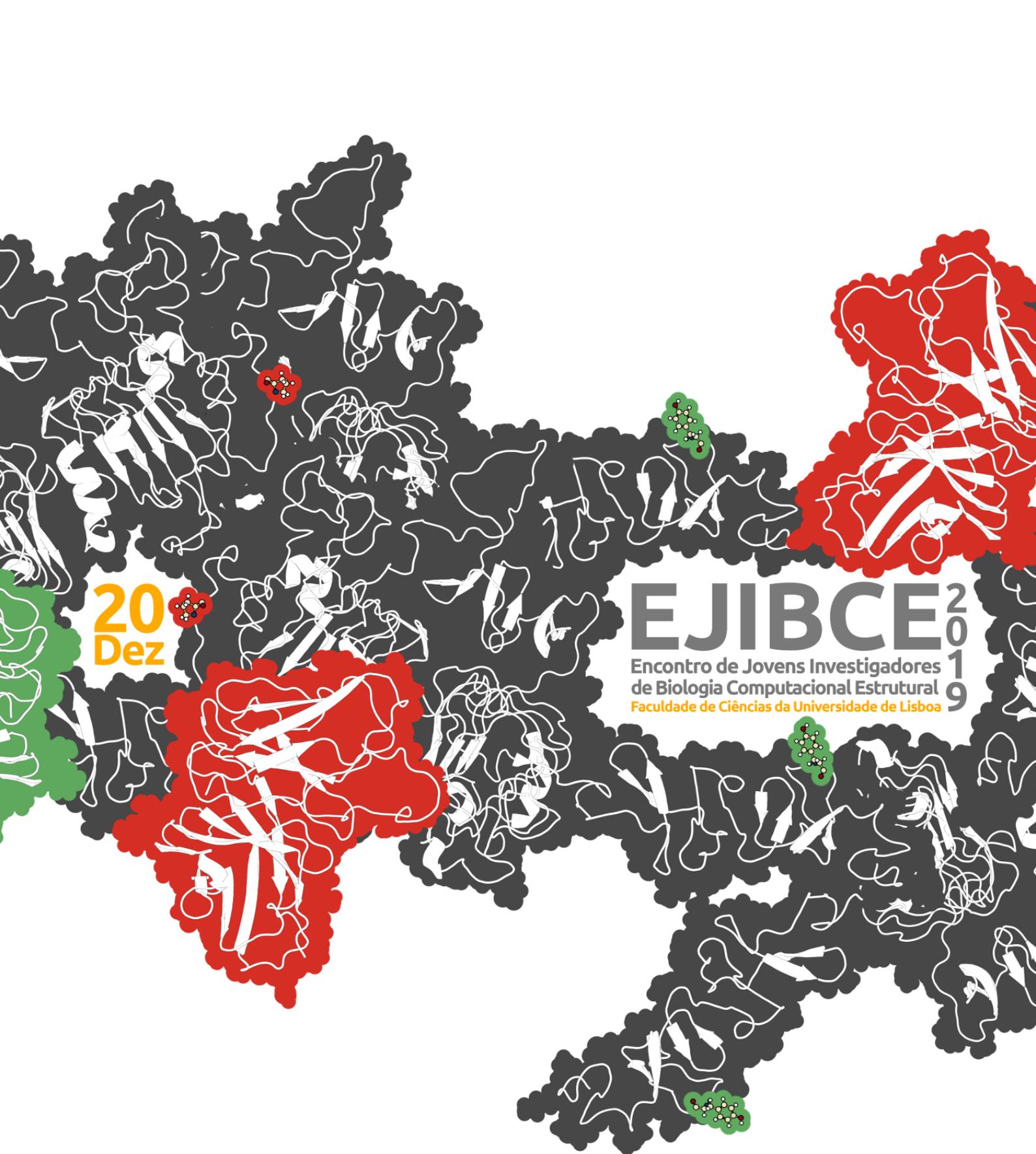
Electrostatic effects, such as pH, are pivotal for cell and protein functioning but, despite their importance, are still coarsely treated by standard biomolecular simulation methods, which ignore the coupling between protonation and conformational change. While molecular mechanics/dynamics methods treat structural equilibrium using fixed protonation states, continuum electrostatics calculations using Monte Carlo sampling treat the multi-site protonation equilibrium using a fixed protein structure. Taking advantage of the complementarity of these methods, the stochastic titration constant-pH MD method [Baptista, 2002], developed at our lab in ITQB, introduced the effect of pH in MD simulations, properly treating the interplay between protonation and conformation.

Our group has been applying this method to a diversity of problems and chemical systems (e.g., protein misfolding, peptidic dendrimers or membrane proteins) and our most recent target is bovine  $\beta$ -lactoglobulin (BLG), which displays interesting pH-dependent traits. This  $\beta$ -barrel protein of 162 amino acids is the most abundant protein in the whey of bovine milk and very widely studied [Singh, 2014]. It can form dimers, oligomers or higher aggregates and the equilibria among these species and the monomeric form is strongly affected by pH [Mercadante, 2012]. BLG also presents a loop conformational transition around pH 7 that exposes the ligand-binding cavity at higher pH values and covers its entrance at lower pH, in a process apparently coupled with the protonation of a glutamate at the entrance of the cavity [Singh, 2014].

This presentation will focus on the electrostatic determinants of dimerization and conformational change in BLG, and how they were studied using constant-pH MD.

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## SELECTED TALKS

## In silico methodologies from an agrobusiness point of view

A. F. Domingues, B. E. S. Calçada and C. S. R. Sousa  
ASCENZA Agro S.A.

The enormous evolution of science and technologies in the last few decades allowed the development and improvement of alternative methodologies to face the animal-based strategies that were implemented until now. One of the major challenges these days, for both the scientific community and industries, is the complete replacement of in vivo studies by in vitro and in silico approaches. This strategy is in line with the implementation of 3Rs principle - Refinement, Reduction and Replacement.

In this scope, Quantitative Structure Activity-Relationships, commonly known as (Q)SARs, can be used to computationally predict physicochemical, biological and environmental fate properties of compounds simply based on their chemical structure. Although these predictions are becoming more accurate with the continuous development of more relevant, reliable and adequate (Q)SAR models, some challenges still persist.

In this communication, the current state of the art on how in silico methodologies are applied to fulfil the regulatory requirements of plant protection products (PPPs) in an industrial context is going to be addressed. Three major toxicological topics will be focused in terms of classification and associated methodologies: skin sensitisation, endocrine disruptors and acute oral toxicity. The upcoming challenges of each topic are going to be addressed, enabling to discuss the potential contribution from computational methods to accomplish the regulatory needs.

Keywords: regulatory, computational sciences, alternative methodologies, challenges

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# **Building an automated platform for the classification of peptides/proteins using machine learning**

Ana Marta Sequeira, Sara Pereira, Diana Lousa, Miguel Rocha  
University of Minho, ITQB

One of the most challenging problems in bioinformatics is to computationally characterize sequences, structures and functions of proteins. Sequence-derived structural and physicochemical properties of proteins have been used in the development of machine learning models in protein related problems. However, tools and platforms to calculate features and perform Machine learning (ML) with proteins are scarce and have their limitations in terms of effectiveness, user-friendliness and applicability.

Here, a generic modular automated ML-based platform for the classification of proteins based on their physicochemical properties is proposed. The tool, developed as a Python package, facilitates the major tasks of ML and includes modules to read and alter sequences, calculate protein features, pre-process datasets, execute feature reduction and selection, perform clustering, train and optimize ML models and make predictions. This platform was validated by testing its ability to classify anticancer and antimicrobial peptides and further used to explore viral fusion peptides.

Membrane-interacting peptides play a crucial role in several biological processes. Fusion peptides are a subclass found in enveloped viruses, that are particularly relevant for membrane fusion. Determining what are the properties that characterize fusion peptides and distinguishing them from other proteins is a very relevant scientific question with important technological implications.

Using three different datasets composed by well annotated sequences, different feature extraction techniques and feature selection methods, ML models were trained, tested and used to predict the location of a known fusion peptide in a protein sequence from the Dengue virus. Feature importance was also analysed. The models obtained will be useful in future research, also providing a biological insight into the distinctive physicochemical characteristics of fusion peptides.

This work presents a freely available tool to perform ML-based protein classification and the first global analysis and prediction of viral fusion peptides using ML, reinforcing the usability and importance of ML in protein classification problems.

Keywords: Machine Learning; Peptide Classification; Viral Fusion Peptides

## **Hydrophobic confinement modulates thermal stability and assists knotting in the folding of tangled proteins**

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(2) BioISI - Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Ed. C8, Lisboa, Portugal.

(3) Departamento de Química Física, Facultad de Ciencias Químicas, Universidad Complutense, Madrid, Spain.

There is growing support for the idea that the *in vivo* folding process of knotted proteins is assisted by chaperonins, but the mechanism of chaperonin-assisted folding remains elusive.

Here, we conduct extensive Monte Carlo simulations of lattice and off-lattice models to explore the effects of confinement and hydrophobic intermolecular interactions with the chaperonin cage in the folding and knotting processes.

We find that moderate to high protein-cavity interactions (which are likely to be established in the beginning of the chaperonin working cycle) cause an energetic destabilization of the protein that overcomes the entropic stabilization driven by excluded volume, and leads to a decrease of the melting temperature relative to bulk conditions. Moreover, mild-to-moderate hydrophobic interactions with the cavity (which would be established later in the cycle) lead to a significant enhancement of knotting probability in relation to bulk conditions while simultaneously moderating the effect of steric confinement in the enhancement of thermal stability.

Our results thus indicate that the chaperonin may be able to assist knotting without simultaneously thermally stabilizing potential misfolded states to a point that would hamper productive folding thus compromising its functional role.

# Antibiotic uptake across gram-negative outer membranes: enabling targeted synthesis from optimized permeability predictions

Ricardo J. Ferreira (1), Peter M. Kasson (2)

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**Aim:** As the outer membrane (OM) of gram-negative bacteria is the major permeability barrier for many drug-like molecules, we have developed a computational method for accurate prospective prediction of OM uptake of drug-like molecules in combination with a new medium-throughput experimental assay using outer-membrane vesicles (OMV).

**Methods:** The inhomogeneous solubility-diffusion (ISD) model was used to calculate drug permeabilities through the *E. coli* outer membrane porin F (OmpF), inserted in an equilibrated OM patch. After obtaining an initial permeation path by pulling the molecule through OmpF, 26 umbrella windows (US) were chosen to sample the permeation path. The permeability was further obtained from the inhomogeneous solubility-diffusion model using the calculated local diffusivity coefficient and potential of mean force in each of the US windows. The experimental evaluation of drug permeabilities was performed in crude OMVs from an OmpC knockout strain (*E. coli* MG1655) through vesicle swelling measurements induced by adding 6  $\mu\text{L}$  of the OMV suspension to 6  $\mu\text{L}$  buffer containing 2  $\mu\text{L}$  buffer (control) or 2  $\mu\text{L}$  of the desired antibiotic at 9 mM. At least 5 independent measurements were performed for each molecule and changes in OMV radius were measured by dynamic light scattering (DLS) [1].

**Results:** ISD model predictions show excellent agreement with the experimental data, with a slightly better Pearson correlation coefficient ( $R$ , 0.93 vs. 0.88) and lower root mean square error (RMSE, 0.34 vs. 0.44) for eight  $\beta$ -lactams than a previous analytical model [2]. When compared with the measured OMV permeabilities for 14 small molecules ( $\beta$ -lactams, penems, quinolones, aminoglycosides, sugars and amino-acids), a higher Pearson correlation coefficient ( $R = 0.952$ , RMSE = 4.55) was obtained. All compounds showed a highly significant relationship between swelling relative to glycine and predicted permeability ( $p < 0.001$  for loglinear regression). Finally, the calculated small-molecule permeabilities against whole-cell measurements for 19 compounds were within whole-cell experimental range for 74% of the compounds, in a remarkably good predictive power.

**Discussion:** The results from the ISD model show excellent agreement with experimentally measured permeability coefficients, in which OmpF permeability is a good first-order predictor of overall uptake. The development of an outer-membrane-vesicle swelling assay to specifically measure small molecule permeation across the bacterial outer membrane is also advantageous because it could be used to assess the impact that chemical modifications would have on the permeability of a given ‘hit’ or ‘lead’ compound prior to the synthesis of such modifications. Examples on a possible workflow for antibiotic discovery are provided, based on the undergoing project for developing novel DNA Gyrase B inhibitors.

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- [2] Acosta-Gutiérrez S. et al. ACS Infect Dis. 2018, 4, 1487–1498.

## Stargazin: CACNG2 Mutations in Neuropsychiatric Disorders

Raquel P. Gouveia (1,2), Carlos A. V. Barreto (1,3), A. J. Preto (1,3), Ana L. M. Carvalho (2,4), Irina S. Moreira (1,4)

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The incidence of psychiatric disorders, like intellectual disability, schizophrenia, and depression, has increased worldwide, and as such, the economic burden to society is significant.(1) It has been already shown that aberrant glutamatergic synapses, for example due to mutations in synaptic proteins that modulate synaptic strength, play a key role in the development of these diseases(2,3). A susceptible gene for psychiatric disorders is the CACNG2 gene, which encodes for stargazin, a transmembrane AMPAR regulatory protein.(4,5) This protein is required for the trafficking of AMPAR to the surface and its stabilization at the synapses, the homeostatic synaptic scaling of AMPAR and the modulation of its gating properties.(6-9)

In this work we used molecular dynamics simulations to study how two crucial CACNG2 gene mutations influence stargazin conformation and dynamics. Results showed that, the mainly affected areas were the third transmembrane domain and the first extracellular domain, areas known to be important for the interaction between stargazin and AMPAR.(7)

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# Unraveling the chemistry beyond the catalytic activity of $\omega$ -amidase

Carla S. Silva Teixeira, Sérgio F. Sousa, Nuno. M. F. Sousa A. Cerqueira  
UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto

The enzyme  $\omega$ -amidase or nitrilase 1 (Nit1) (EC 3.5.1.3) is an integral part of both the glutaminase II and asparaginase II pathways. (1) In glutaminase II pathway, a glutamine transaminase catalyzes the transamination of L-glutamine yielding  $\alpha$ -ketoglutarate (KGM; 2-oxoglutarate) which is hydrolyzed by  $\omega$ -amidase to  $\alpha$ -ketoglutarate.

In asparaginase II pathway, an asparagine transaminase catalyzes the transamination of L-asparagine yielding  $\alpha$ -ketosuccinate (KSM; 2-oxosuccinate), which is hydrolyzed by  $\omega$ -amidase to oxaloacetate.

There are evidences of  $\omega$ -Amidase catalytic activity toward KGM and KSM in mammals, plants, bacteria, fungi and some tumors.(2) The mammalian  $\omega$ -amidase is a homodimer composed by two monomers containing a canonical Glu-Lys-Cys catalytic triad in each active site. Mutagenic data showed that the individual mutations of each of these residues result in the inactivation of the enzyme.(3)

The recent reports suggest that  $\omega$ -amidase may present tumor suppressor properties or tumor promoter properties depending on the cancer cell type. These contrary effects have raised several questions and challenges that to date remain unanswered: what are the effects of an induced mutation in the catalytic residues of  $\omega$ -amidase in cancer cells overexpressing this enzyme? Is it possible to develop potent and selective inhibitors for it? In what cancer cells does those selective inhibitors have pro- or anti-cancer effects? (4)

In order to answer all these questions, we built a QM/MM model of a mouse  $\omega$ -amidase (PDB ID: 2W1V) to study the catalytic mechanism of this enzyme. The QM region was treated with density functional theory (DFT) and the MM region was defined by AMBER ff99SB force fields. The relative energies were determined using single-point QM/MM calculations using the domain-based local pair natural orbital coupled cluster DLPNO-CCSD(T) method to calculate further QM contribution. The obtained results showed that the reaction proceeds in six steps, three of which are spontaneous proton transfers.

The transition state structure obtained with this work will allow in the future to design very specific  $\omega$ -amidase inhibitors that may help answer some of the questions regarding the effects of this enzyme in different cancer cells. The knowledge of the specific role played by each residue from the catalytic triad and from the enzyme active site may provide some clues about the potential effect of its individual mutation on the enzyme activity.

## Acknowledgements

The authors thank the financial support from the Foundation for Science and Technology (FCT) through the PhD grant number SFRH/BD/114886/2016, the project PTDC/QUI-QFI/31689/2017, national funds and FEDER cofounding under the partnership agreement with PT2020. This work was also supported by the Applied Molecular Biosciences Unit – UCIBIO which is financed by national funds from FCT/MCTES (UID/ Multi/04378/2019).

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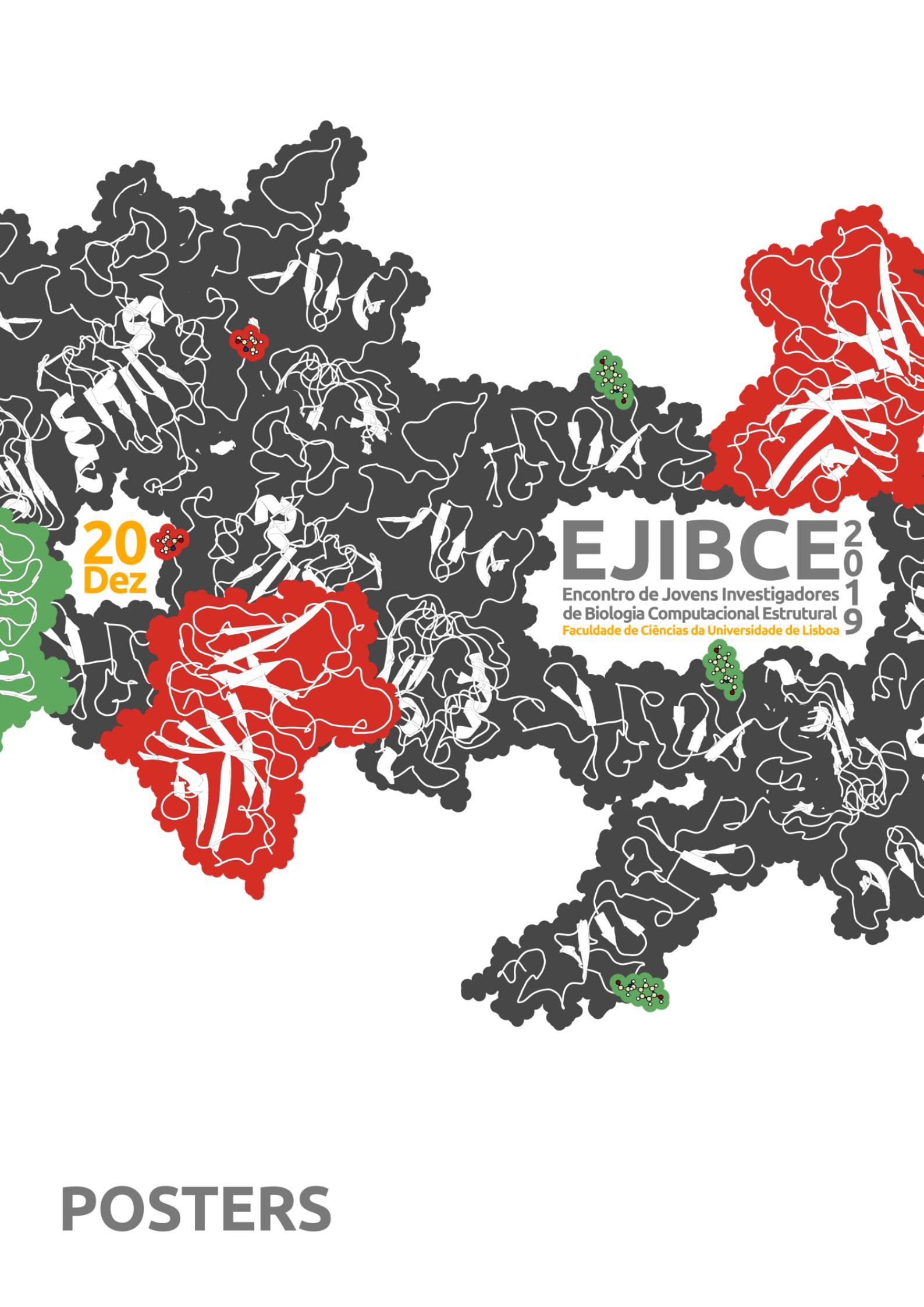
## **Study of pH-dependent conformational changes on $\beta$ -lactoglobulin using molecular modeling**

Lucie da Rocha, Sara R. R. Campos, António M. Baptista  
ITQB NOVA Av. da República 2780-157 Oeiras Portugal

The most abundant protein in the bovine milk whey is the potentially allergenic beta-lactoglobulin (BLG) [1]. Several ligands can bind to BLG through a pH-regulated mechanism, suggesting not only a possible biological role as a transporter, but also a potential pharmacological role as a carrier of bioactive compounds [2]. BLG also presents a monomer-dimer equilibrium strongly affected by pH, which seems to be related to allergenicity [3].

Previous experimental and computational studies have tried to understand these features and the underlying molecular phenomena, using standard methods that focus either on electrostatics or on structure dynamics separately, thus ignoring their interplay [3][4]. In this study we intended to analyse the effect of pH on conformational alterations of the dimer, using two main molecular modeling methods: Plain Constant pH molecular dynamics and its combination with umbrella sampling. This has shown the presence of two different conformational states: a compact state, that resulted from the tight association of the two monomers, observed at pH 3, and a relaxed state, with a less tight interface, observed at pH  $\geq$  5. The umbrella sampling results are also in agreement with experimental dissociation rates [3]. This communication will mostly focus on the different dimer conformations that are characteristic of different pHs.

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The background of the poster features a complex, abstract pattern of protein structures. These structures are represented by black and white ribbon models, some of which are highlighted in red or green. Small molecular models are scattered throughout the background.

**20**  
**Dez**

# EJIBCE<sup>2019</sup>

Encontro de Jovens Investigadores  
de Biologia Computacional Estrutural  
Faculdade de Ciências da Universidade de Lisboa

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## The early phase of the $\beta$ 2m aggregation mechanism: an integrative computational analysis based on the D76N mutant and $\Delta$ N6 variant

Rui João Loureiro, Diogo Vila-Viçosa, Miguel Machuqueiro, Eugene I. Shakhnovich, Patrícia F.N. Faísca  
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Protein beta-2-microglobulin ( $\beta$ 2m) is the causative agent of dialysis related amyloidosis (DRA), which affects patients undergoing long-term ( $>10$  years) hemodialysis. Furthermore, the D76N mutant is associated with a hereditary systemic amyloidosis affecting visceral organs. We carried out a comparative analysis of the early phase of aggregation of two natural variants of  $\beta$ 2m, the D76N mutant and  $\Delta$ N6 variant, to get insights into the early events triggering the  $\beta$ 2m aggregation mechanism.

Folding simulations predict the existence of two intermediates in the D76N folding transition, one termed I1 featuring an unstructured and detached C-terminus and another termed I2, with both termini unstructured and detached. The intermediate I1 is topologically similar to the intermediate populated by  $\Delta$ N6 in which the unstructured terminus is the N-terminus [1]. Monte Carlo docking simulations indicate an essential role of the unstructured termini, as well as of the BC-, DE- and EF-loops in  $\beta$ 2m aggregation [2]. The termini tend to be more relevant in dimerization at acidic conditions while the BC-, DE- and EF-loops are the dominant regions at physiological pH. Moreover, results from simulations also corroborate experimental evidence according to which residues Tyr10 (A-strand), Phe30 and His31 (BC-loop), Trp60 and Phe62 (DE-loop) and Arg97 (C-terminus) play an important role in the aggregation mechanism of  $\beta$ 2m [2]. They further predict the occurrence of novel hot-spot residues, such as Lys-75 (EF-loop) and Trp-95 (C-terminus), i.e. new testable theoretical predictions to guide the research on  $\beta$ 2-microglobulin amyloidogenesis.

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# The disulfide bond modulates the folding landscape of beta-2-microglobulin

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Protein beta-2-microglobulin ( $\beta$ 2m) is the causative agent of dialysis related amyloidosis (DRA), which affects patients undergoing long-term ( $>10$  years) hemodialysis. Together with the wild-type protein, the  $\Delta$ N6 variant and the D76N mutant have been recently used as model systems of  $\beta$ 2m aggregation. In all of them the native structure is stabilized by a disulfide bridge between the cysteine residues at positions 25 (at B strand) and 80 (at F strand), which has been considered fundamental in  $\beta$ 2m fibrillogenesis at neutral pH. Here, we use extensive Discrete Molecular Dynamics simulations of a full atomistic structure-based model to explore the role of the cysteine bond as a modulator of the folding space of  $\beta$ 2m. In particular, by considering two different models for the disulfide bond, one in which the bond is locked (i.e. permanently formed), and one in which the bond is modeled as a native interaction with tunable interaction strength, we explore the thermodynamics of the folding transition, and the formation of intermediate states that may have the potential to trigger the aggregation cascade. Our results show significant differences between the folding thermodynamics and free energy landscapes of the considered model systems. In particular, as the strength of the disulfide bond increases novel intermediate states appear with considerably higher thermodynamic stability.

## **Hydrophobic confinement modulates thermal stability and assists knotting in the folding of tangled proteins**

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There is growing support for the idea that the *in vivo* folding process of knotted proteins is assisted by chaperonins, but the mechanism of chaperonin-assisted folding remains elusive.

Here, we conduct extensive Monte Carlo simulations of lattice and off-lattice models to explore the effects of confinement and hydrophobic intermolecular interactions with the chaperonin cage in the folding and knotting processes.

We find that moderate to high protein-cavity interactions (which are likely to be established in the beginning of the chaperonin working cycle) cause an energetic destabilization of the protein that overcomes the entropic stabilization driven by excluded volume, and leads to a decrease of the melting temperature relative to bulk conditions. Moreover, mild-to-moderate hydrophobic interactions with the cavity (which would be established later in the cycle) lead to a significant enhancement of knotting probability in relation to bulk conditions while simultaneously moderating the effect of steric confinement in the enhancement of thermal stability.

Our results thus indicate that the chaperonin may be able to assist knotting without simultaneously thermally stabilizing potential misfolded states to a point that would hamper productive folding thus compromising its functional role.

# Motif amplification and fold change: explaining the bacterial origin of mitochondria-specific outer membrane $\beta$ -barrels

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The universe of protein folds we know today is the result of at least 3.7 billion years of evolution. Since the time of the Last Universal Common Ancestor (LUCA), proteins have been the fundamental catalysts of life and it is becoming more evident that new protein folds may emerge by the re-use of already optimised subdomain-sized fragments. One of the most successful mechanisms is amplification: the tandem repetition of small peptide motifs. The reason may be that by promoting the periodic recurrence of favourable interactions, it reduces the amount of sequence sampling until a stable fold is found.

The origins of many protein folds can be explained by this mechanism and one important example is the Outer Membrane  $\beta$ -Barrel (OMBB), the most abundant protein fold at the outer membrane of Gram-negative bacteria. OMBBs fold into a closed anti-parallel  $\beta$ -sheet whose strands traverse the outer membrane and form a scaffold for different important biological processes. Many bacterial OMBB families are known with a variable even number of  $\beta$ -strands between 8 and 36. Internal sequence and structure symmetry suggest that they arose independently through the amplification of a homologous pool of ancestral  $\beta\beta$ -hairpins. Given the endosymbiotic origin of eukaryotes, OMBBs are also found in mitochondria and chloroplasts. However, the diversity of eukaryotic OMBBs is not as remarkable as that of bacterial OMBBs and only a small number of families are known. Most of these can be traced to a bacterial family, with the exception of one: the 19-stranded family found only in mitochondria (MOMBBs) and whose origins remain unclear.

In order to shed light into the origins of this atypical fold, we preformed a large-scale computational survey of mitochondrial and bacterial OMBB sequences and structures. Our results suggest that MOMBBs, contrary to other OMBBs, evolved by amplification of a double  $\beta\beta$ -hairpin and were not acquired from the symbiont. Instead, they emerged in the proto-mitochondrion, probably at the time of the Last Eukaryotic Common Ancestor (LECA), driven by the need for a general-purpose polypeptide importer. The amplification of the 4-stranded fragment would have yielded a 20-stranded barrel, a fold never observed in nature. This ancestor 20-stranded barrel may have been quickly converted into a 19-stranded barrel by accumulating a few point mutations in the 1st strand, which rehabilitated it as a N-terminal  $\alpha$ -helical plug.

While providing a scenario for the origins of the MOMBB fold, our results shed light into an important step in the origins of mitochondria and eukaryotes. Furthermore, they highlight the role of motif amplification and local fold change in the de novo emergence of new folds for established protein architectures and the shaping of the protein fold universe we observe today.

## Refining an umbrella-sampling protocol to describe membrane PAINS

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Pan-assay interference compounds (PAINS) are promiscuous molecules with apparent bioactivity that can interfere with the result of biological assays. These compounds are often mistakenly flagged as positive hits [1], acting as burden agents in drug screening processes. There are several categories of PAINS, but an especially problematic and underestimated category are the so-called membrane PAINS [2]. These compounds interact directly and nonspecifically with lipid membranes, promoting changes in their biophysical properties and ultimately affecting the function of mechanosensitive membrane proteins. Despite developed efforts, the identification of these compounds in initial compound screening phases of drug discovery is still very imprecise.

We will describe a new computational protocol to identify and characterize membrane PAINS. This protocol is based on an already validated method [2], featuring atomic detail potential of mean force (PMF) calculations using umbrella sampling (US) techniques. These calculations allow the estimation of the perturbing effect of these compounds on membrane permeability and stability. Our validation set comprises molecules with reported minor, mild and major membrane PAINS activity [2,3]. Since one of the main concerns limiting the accuracy of these calculations is related with long equilibration times and insufficient sampling in each US window, we will also show new advances to the initial protocol based on longer simulation times and the use of enhanced sampling methods.

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## **Molecular details of the pH-dependent interaction of the influenza fusion peptide with a model membrane**

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Influenza pandemics represent serious health threats, in view of the limited treatments available. Research on the molecular mechanisms of infection by the influenza virus (IV) is needed to find new therapeutic targets and inactivating the fusion of the viral and host membranes is considered a promising strategy, but this process is poorly understood at the molecular level.

Given that fusion takes place when the virus is exposed to the low pH of the endosome, we analyzed the effect of pH on the influenza FP structure and membrane-interacting properties, by using an in-house developed constant-pH molecular dynamics method. Experimental biophysical methods were also used to analyse the peptide-membrane interaction at different pH values. The realistic treatment of protonation introduced by the CpH MD simulations allowed us to provide a detailed molecular characterization of the pH effect on the fusion peptide properties and its ability to interact and disturb the host membrane.

## **Characterization of maltose binding and translocation in different states of the MalFGK2E transporter**

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The Escherichia coli MalFGK2E maltose importer is a type I ATP-binding cassette (ABC) importer responsible for the uptake of malto-oligosaccharides [1]. ABC transporters use the energy from ATP hydrolysis for harnessing substrate translocation, undergoing substantial conformational changes [2]. The MalFGK2E importer is composed by several subunits: two ATPase domains MalK, two membrane-spanning domains MalF and MalG and a substrate binding protein MalE. It is one of the most studied ABC transporters and it is a model system for type I importers [3]. However, little is known about the effect of ATP hydrolysis and nucleotide exit on maltose translocation and binding. In this work, three states were simulated using molecular dynamics: a pre-hydrolysis system with ATP bound, a post-hydrolysis system with ADP and phosphate bound, and an apo system with no nucleotides bound. In this work, we study the effect of each nucleotide and their absence in substrate binding and translocation, along with the associated structural changes.

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# The hydrophobicity of fluorinated amino acids varies non-monotonically with degree of fluorination

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The incorporation of fluorinated amino acids in proteins is a well-established means of improving their thermal stability, which is believed to relate to the hydrophobicity of these amino acids. Quantifying this change in local hydrophobicity and the consequent change in protein properties is, however, still challenging. Using molecular dynamics simulations, together with in-house force fields [1,2], we quantify fluorination-induced changes in the hydration free energy ( $\Delta\text{GHyd}$ ) of amino acids. The free energy unexpectedly becomes either more positive or negative regardless of the degree of fluorination. We then derive a phenomenological model that allows for the prediction of changes in  $\Delta\text{GHyd}$  and for the decomposition of these changes into physical observables [2]. We find two major contributions to changes in  $\Delta\text{GHyd}$ : i) direct interactions between water and the side chain, depending monotonically on the degree of fluorination and ii) side chain-backbone interactions, which change the hydrogen-bonding ability between backbone polar groups and water and vary unpredictably with the degree of fluorination [2]. Our model introduces the ability to predict and rationalize, based on short simulations, the impact of fluorination on the local hydrophobicity of proteins. Further, this work emphasizes the need for a synergistic coupling of experiment and simulation to fully understand the extent to, and the mechanism by which, amino acid modifications impact hydrophobicity.

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# **Targeting Neuroinflammation: a combined Virtual Screening protocol towards the discovery of Interleukin-1 Receptor type I (IL-1R1) modulators**

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In recent years, neuroinflammation has been increasingly recognized as a major determinant of several neurodegenerative and central nervous system (CNS) disorders. Hence, the modulation of neuroinflammatory processes holds potential prospects for halting, or at least slowing down, the progression of such disorders.<sup>1</sup> One of the most important group of cytokines implicated in neuroinflammation is the interleukin 1 (IL 1) family, a well-characterized cluster that plays a critical role in acute inflammatory responses.<sup>2</sup> Importantly, several reports have demonstrated that blocking the IL-1 signalling pathway via interleukin-1 receptor type 1 (IL-1R1) leads to reduced neuroinflammation and may prevent related disorders.<sup>3</sup> Notably, while there is substantial body of research on IL-1R1, there have been no small molecule modulators reported to date.

Herein, we present the results of an integrated structure-based virtual screening protocol targeting the extracellular domain of IL 1R1, combining molecular dynamics (MD) simulation studies, 3D-pharmacophore modelling, and molecular docking – towards the discovery of small-molecule modulators of IL 1R1 and related molecular networks. Binding site prediction followed a grid-based pocket detection methodology prioritizing druggable cavities. This has been accompanied by the study of receptor dynamics via MD runs totalling 600 ns, with particular focus on the stability and conformational flexibility of the putative ligand-binding site. Several receptor-based pharmacophore hypotheses were then generated, which allowed retrieval of 13.814 virtual hit compounds from a CNS-tailored virtual screening deck. As a post-screening filter, said screening hits were docked into IL 1R1, with the best candidates ranked via quantitative analysis of protein-ligand interactions, docking scores and pharmacophore fitness levels. Thus far, 21 promising compounds have been selected on a basis of prior bioactivity data and chemical diversity, and acquired from their respective chemical vendors for in vitro evaluation.

The ongoing experimental validation, together with the innovative in silico framework presented here, represents a pioneering attempt to discover IL-1R1 small molecule modulators, and will hopefully shed light on their use as potential neuroinflammation modulators.

# Deep Reinforcement Learning Framework for Drug Design with Optimized Drug-Like Properties

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In de novo drug design, computational strategies have been used to generate new molecules with bespoke properties that have a good affinity towards the desired biological purpose.

In this work, we explore the use of Reinforcement Learning (RL) strategies for improving the design of de novo drug-like compounds. This is a policy-based approach that captures the syntax of molecular representation in terms of Simplified Molecular Input Line Entry (SMILES) and generates potential new compounds with desirable properties. The proposed RL strategy consists of two interdependent neural networks, one that acts as a generator of new compounds and the other acting as an evaluator. Firstly, the generative model is built using Recurrent Neural Networks (RNN). We use a Long Short-Term Memory (LSTM) architecture with two layers and then it is trained to generate valid molecules. Afterwards, a Quantitative Structure-Activity Relationship model (QSAR) was established, also with two LSTM layers that estimates the desired property of each generated structure. Subsequently, the policy gradient method is applied to make the model produce fine-tuned molecules.

The performed experiments demonstrate the efficiency of the proposed strategy in a single task regime where each endpoint of interest is independently optimized. For instance, regarding the coefficient partition - which is the measure of the lipophilicity of a drug and an indication of its ability to cross the cell membrane - we managed to increase in 5% the percentage of generated drugs inside the interest region. This means that 92% of the generated drugs have a coefficient partition admissible, according to Lipinski's rule. Nevertheless, this approach can be expanded simultaneously to allow multi-objective optimization of several drug-like properties, which is the need for drug discovery where the molecule should be optimized with respect to the potency, selectivity and pharmacokinetic properties to avoid unwanted side effects and minimize toxicity. Our future studies will address this issue.

Acknowledgements: This research has been funded by the Portuguese Research Agency FCT, through D4 - Deep Drug Discovery and Deployment (CENTRO-01-0145-FEDER-029266).

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# An MD-based protocol to assess the stability of $\beta$ 2M dimers obtained from MC-ED

Filipe E. P. Rodrigues, Nuno F. B. Oliveira, Diogo Vila-Viçosa, Patrícia F. N. Faísca, Miguel Machuqueiro  
BioISI

$\beta$ 2 Microglobulin ( $\beta$ 2M) is a protein that aggregates and forms amyloid plaques under several conditions, including low pH. Amyloidosis caused by  $\beta$ 2M is extremely common in people undergoing long-term hemodialysis due to the slightly acidic pH of the synovial fluid in patients.[1] This disease can also have a genetic origin, where the single point mutation D76N has been shown to increase the aggregation propensity of  $\beta$ 2M.[2] Two folding intermediate states of the  $\beta$ 2M D76N variant were identified, one having the C-terminus unstructured (I1) and another having both termini unstructured (I2).[3]

$\beta$ 2M dimers with the D76N mutation in the conformation I2 were previously obtained from the Monte Carlo ensemble docking (MC-ED) protocol,[4] generating a vast ensemble of dimer interfaces. A cost function was used to select the most stable interfaces and identify the key residues in the dimerization process. An ensemble of 221 dimers was selected based on the lowest energies generated by the MC-ED scoring function.[4]

We used molecular dynamics (MD) simulations to evaluate the stability of the obtained dimers. A simple solvent-accessible surface area (SASA) protocol was used to assess the stability of the original dimers interfaces along with the MD simulations. For the most stable dimers, the ones whose interface is not disrupted, we applied an equilibrium analysis based on molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) calculations to obtain the binding free energies and establish a correlation between the conformational stability and binding energies, both from MM/PBSA and MC-ED scoring function. These results are pivotal to help evaluate the MC-ED protocol, especially since the experimental information at the molecular level on  $\beta$ 2M dimerization is scarce.

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# Nucleoside and nucleotide analogues as potential kinase inhibitors: a molecular docking study

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Kinases are enzymes that transfer phosphate groups from phosphate donating molecules, such as ATP, to specific substrates. This process, known as phosphorylation, plays a crucial role in biological processes such as cell signaling, protein regulation, and metabolism. Moreover, cyclin-dependent kinases (CDKs) are key regulators of the cell cycle which have shown an abnormal expression/regulation in several human neoplasms. Another interesting kinase involved in several important biological processes, such as Type II diabetes, Alzheimer's disease, and bipolar disorder, is the glycogen synthase kinase 3 (GSK3). Both these targets have been studied as potential therapeutic targets for cancer [1] and neuronal disorders [2]. Nucleosides and nucleotides play a role in fundamental biological processes such as a synthesis of DNA and RNA, cell division, and metabolism. Therefore, the development of nucleoside and nucleotide synthetic analogues has been explored in drug design leading to several drugs used for the treatment of cancer and viral diseases [3]. Their potential to mimic ATP could lead to the inhibition of ATP-dependent enzymes such as kinases, which would be an interesting approach to explore.

In this work, we used molecular docking to explore the CDK- and GSK-binding affinity of three different family SETs of nucleos(t)ide analogues extracted from the PubChem database or custom-built by us. In particular, we explored the possibility of replacing the phosphate group by a more stable analogue such as phosphonate [4] and the capacity of the guanidine moiety to act as a mimetic of guanine.

Acknowledgments: FCT/MCTES (IF/00069/2014, IF/00069/2014/CP1216/CT0006, IF/01488/2013 and IF/01488/2013/CP1159/CT0006, UID/MULTI/00612/2019 and UID/MULTI/04046/2019). FCT/MCTES, Lisboa 2020, Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

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# Siamese Neural Networks for One-Shot Drug Discovery

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The application of deep neural networks in drug-discovery is mainly due to their enormous potential in significantly increasing the predictive power when classifying drugs as well as their fundamental properties and interactions. One of the major drawbacks of this kind of approach is that a large number of training examples per class is needed, therefore it is not possible to classify instances whose classes were not considered in the training phase or in data where the number of classes is high and oscillates dynamically.

In drug-discovery, the reduced amount of biological data available is a common culprit for the failure of novel compounds as potential drugs with the desired therapeutic activity. The main objective of this work is to optimize the discovery of drug analogues, with increased therapeutic activity, for the same pharmacological target based on a reduced set of candidate drugs. We apply a Siamese neural network architecture for one-shot classification, based on Convolutional Neural Networks (CNNs), that learns from a similarity score between two input molecules according to a given similarity function. The main advantage of this approach is the low amount of data and computational resources required while only one instance is needed per class for training. The preliminary results of this study showed that a one-shot learning strategy allowed us to achieve strong results given the low data available in one-shot classification tasks for drug discovery.

Acknowledgments: This research has been funded by the Portuguese Research Agency FCT, through D4 - Deep Drug Discovery and Deployment (CENTRO-01-0145-FEDER-029266).

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## **Targeting acetylcholinesterase with halogenated ligands: finding halogen bonding hotspot**

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Acetylcholinesterase (AChE) is one of the most relevant therapeutic targets for the symptomatic treatment of Alzheimer's disease. Hence, the development of new molecules capable of inhibiting AChE activity is an effective therapeutic strategy. The incorporation of halogens (X) in drug molecules is a common way to enhance drug ADME (absorption, distribution, metabolism, and excretion) properties, often leading to increased potency. However, halogenation can also improve drug-target binding affinity due to the existence of halogen bonds (HaBs) established with the receptor. Halogen bonds (R-X···B) are noncovalent interactions between a positive region on the electrostatic potential of X, called sigma-hole, and a nucleophile, such as a lone pair of a Lewis base (B).

Since there were virtually no reports on the use of HaBs to target AChE, in this work, we searched for amino acids frequently targeted by halogen bonding (called hotspots) in the AChE binding site. For that purpose, all the compounds containing a moiety capable of halogen bonding (Ph-X, Ph = phenyl, and X=Cl, Br, I) were retrieved from the ChEMBL database and docked into the AChE binding site using AutoDock Vina XB whose scoring function takes into account the sigma-hole. Multiple X-ray structures and molecular dynamics snapshots from the target were used to account for conformational variability. By applying a geometrical criteria, we selected all halogens atoms engaged in HaBs, thus being able to identify halogen bonding hotspots on the binding pocket. These preliminary results will be the starting point for obtaining new halogenated scaffolds to target AChE, hopefully helping in the design of new and more effective AChE inhibitors.

Acknowledgements: FCT/MCTES (IF/00069/2014, IF/00069/2014/CP1216/CT0006, SFRH/BD/116614/2016, UID/MULTI/00612/2019 and UID/MULTI/04046/2019). FCT/MCTES, Lisboa 2020, Portugal 2020, FEDER/FN, and European Union (LISBOA-01-0145-FEDER-028455, PTDC/QUI-QFI/28455/2017).

## **Novel tools and approaches to predict biomolecular partitioning in aqueous two phase system**

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The significance of aqueous two-phase systems (ATPSs) in bioprocessing with commercial application has been reported for the separation of a large number of biopharmaceutical products. ATPS is a liquid–liquid separation technique that has shown significant potential for the purification of biological compounds. For both industrial- and laboratory-scale purposes it is essential to know the partitioning behavior of biomolecules and which parameters lead to an optimal separation [1]. A better understanding of the factors which govern partitioning contributes for the prediction of the partitioning behaviour in ATPSs.

The present work aims to achieve an additional insight into the protein partitioning behaviour in (ATPSs), together with a study on the viability of a semi-empirical model based on continuum electrostatics to predict the protein partition characteristics. Electrostatic energy contributions were estimated using a Poisson-Boltzmann computational method [2]. A linear correlation of calculated nonpolar energies with the solvent accessible surface area was observed which was essential for development and applications of semi-empirical model.

The model is very sensitive to polymer phase concentrations and the protein characteristics (e.g. solubility, aggregation). Therefore, an optimization of polymer phase concentration is very important to improve its feasibility on prediction of protein partitioning in ATPS.

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# Potential C–X···π halogen bonds in halogenated sugar mimetics: a DFT study

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Protein-carbohydrate interactions have a prominent role in several biological processes.[1] The understanding of the underlying molecular mechanisms is therefore paramount for applications in carbohydrate-based drug design. Carbohydrate recognition by specialized proteins often occurs via C–H···π noncovalent interactions involving two or three C–H groups from the sugar and the aromatic side-chains of aminoacids (i.e. Phe, Tyr, Trp).[2] While fluorination of carbohydrates is quite common in the design of carbohydrate mimetics aiming at improving their binding affinity, the use of heavier halogens (Cl, Br, I) is not common, remaining practically unexplored. Nonetheless, in that case, halogen bonds[3] can potentially be formed with protein acceptor residues, particularly, C–X···π halogen bonds which could replace the C–H···π bonds originally observed in protein-carbohydrate complexes.

In this work, we used DFT calculations aiming at studying the potential role of C–X···π halogen bonds in protein-carbohydrate recognition. By using a b-d-fucose template, we evaluated the C–X···π halogen-bond capability of several brominated b-d-fucose mimetics towards benzene, phenol, and indole as protein side-chain models. These studies provide the first insights on how this specific type of noncovalent interaction can be explored in protein-carbohydrate systems.

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## **Development of a structural database to inhibit biofilm formation**

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Most of the microbial life present in nature, is found in surface bound communities called biofilms. [1] They are also ubiquitous in both normal and pathogenic human processes. [2] Still, biofilm associated bacteria are less sensitive to antibiotics and that often leads to resistance. [3] Therefore, it is fundamental to have a deeper knowledge of the proteins and enzymes involved in biofilm formation and development. Understanding the mechanisms of action of several key proteins opens the way for the rational development of new compounds with anti-biofilm activity.

This database [4] is a fully searchable curated platform that gathers atomic level information on the proteins directly involved in biofilm formation and development. It can be an excellent starting point for those not only who wish to study the proteins responsible for biofilm formation, but also by structural and computational biologists, who want to study these targets at a molecular level. This database contains X-ray crystallographic data for 253 proteins. It is organized by name of the protein, category, mechanism, gram-type, and resolution. The user may also find structural and chemical information on the natural substrates and inhibitors as well as a characterization of each binding pocket, and main interactions formed. Each target is also associated with other databases such as CHEMBL and BindingDB, UniProt, BRENDA, ExPASy and KeGG.

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This work was supported by national funds from Fundação para a Ciência e a Tecnologia [grant numbers SFRH/BD/137844/2018, IF/00052/2014, UID/Multi/04378/2019].

## **Structural characterization of Aquaporin-1 and evaluation of a PMF based protocol to determine water permeability rates**

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Aquaporins (AQPs) are a class of membrane water channels whose function is to facilitate the passive transport of water across membranes of cells in response to osmotic gradients (1). These membrane proteins are essential for water homeostasis and cell volume control, and members of this protein family have been implicated in numerous physiological processes such as trans epithelial fluid transport, CNS function, cell migration and proliferation.

AQPs have been shown to be mechanosensitive (2), since they are able to sense subtle changes in the membranes properties and be affected in their function if changes in these properties are observed (3). Within the framework of an on-going project being developed on our research group, we have performed a structural characterization of AQP-subtype 1 and evaluated a Potential of Mean Force (PMF) protocol to accurately determine water permeability rates through the protein's pores. The sensitivity of the evaluated method is of utmost importance, since it constitutes an initial reference for subsequent studies, where we will be evaluating the effect of different membrane PAINS on this protein's function. This type of compounds are known to promote changes in the properties of membranes, indirectly affecting the function of transmembrane mechanosensitive proteins such as Aquaporins. All the obtained results will have an experimental counterpart, whose results will be used to validate the computational predictions.

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## Poisson-Boltzmann based pKa estimations with a user-friendly python API

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The structure and function of biomolecules are highly dependent on the ionization state of titrable sites. In an average globular protein, 25% of all residues are ionizable in water [1] and their pKa values have a direct influence on its physicochemical properties, such as polarity or lipophilicity. In silico cost-effective pKa calculation tools have numerous applications, ranging from optimization of chemical leads, to building QSAR models or understanding structural/functional properties of biological systems.

Here, we present an open source python API for pKa calculations with a valuable trade-off between fast and accurate predictions, that can be used to extend existing protocols by adding two extra lines of code and a few minutes of computation time. This module streamlines pKa calculations by providing validated radii [2], charge distributions [3], and default PB parameters [4], and by pipelining the intrinsic pKa, site-to-site interactions calculations and Monte Carlo sampling. Although default values are provided, user-defined models are allowed and easily implementable.

PypKa supports CPU parallel computing on anisotropic (membrane) and isotropic (protein) systems. As an example, we show how to easily calculate pKa values of a membrane interacting peptide using a linear response approximation (LRA) approach.

We acknowledge financial support from FCT through grant SFRH/BD/136226/2018 and SFRH/BPD/110491/2015 and projects PTDC/QEQCOM/5904/2014, UID/MULTI/00612/2019 and UID/MULTI/04046/2019.

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# pH-dependent membrane crossing mechanism of lipophilic anti-tumoral drugs

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Tumor targeting and treatment constitutes one of the greatest challenges for the scientific community due to the highly complex environment present in different tumors [1]. This tumor microenvironment (TME) is an important component for tumor development by influencing several key processes such as cell proliferation and phenotype and, more specifically, therapy resistance [1]. A key feature of the TME is the increased acidity of the extracellular vicinity (pH values from 6.0 to 6.8) due to enhanced anaerobic glycolysis coupled with higher levels of proton extrusion via upregulated proton pumps. This process creates a pH gradient between the extra and intracellular environments effectively creating an entrapment mechanism for hydrophobic Lewis base drugs with high pKa values (7-10), such as sunitinib or nintedanib, impairing effective tumoral treatment. A similar entrapment mechanism is observed in lysosomes due to its strong acidity (pH 5.0). This acidity-induced entrapment mechanism occurs since it becomes more difficult for the drug to deprotonate, which would enable the neutral molecule to cross passively through the membrane. The present study aims at investigating the pH-dependent membrane insertion mechanism of some of these molecules. pKa profiles along the membrane insertion pathway can help interpret the available experimental data on how some of these compounds struggle to insert into tumor cells or why other compounds are completely entrapped in lysosomes. We performed pH replica-exchange (pHRE)[2] simulations of nintedanib and sunitinib, interacting with a 128 DMPC lipids membrane bilayer. We calculated pKa profiles for each system, which mainly captures the desolvation effect along the membrane normal [3]. Simultaneously, we obtained each molecule's average charge with its relative abundance, for each pH value, therefore providing insight into the membrane permeability process coupled to (de)protonation events.

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# Improving the druggability of Aquaporin-1 for future drug discovery campaigns

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Aquaporins (AQPs) are a family of 13 small integral membrane proteins, whose primary function is to facilitate the passive transport of water across the plasma membrane of the cell, in response to osmotic gradients created by the active transport of solutes. These small membrane-spanning proteins assemble as homotetramers in which each monomer is composed by an extracellular and cytoplasmic vestibule connected by a central amphipathic pore region in a barrel like arrangement. [2]

These proteins are widely expressed throughout the animal and plant kingdom, being localized in the plasma membrane and in the cytoplasmic compartments, particularly in cell types that are involved in fluid transport. AQPs have been proven to play key roles in tumor biology, including histological tumor grade, proliferation, migration, angiogenesis, or tumor-associated edema, namely due to its over-expression when compared to normal tissues. Therefore, AQPs can work as potential diagnostic and therapeutic targets in anticancer treatment, since their inhibition in endothelial and tumor cells might limit tumor growth and spread. [3] Unfortunately, the hit rate for the identification of small-molecule AQP modulators appears to be very low when compared to other membrane proteins, and the few available pharmacological modulators lack specificity or show high toxicity. [1] One of the possible explanations for this low druggability is the small size of the functional AQP monomer and its small pore diameter. Nonetheless, the continuous growing of structure/function knowledge on AQPs, particularly the atomic-level geometry of specific hydrophobic and hydrophilic residues in the pore region, makes AQPs a promising therapeutic target to be used in future computational drug discovery campaigns. [2] However, in order to achieve successful results, new and innovative approaches and methods must also be developed. Consequently, we are currently developing a new computational workflow based on several distinct methods that combine innovative ligand and structure-based approaches to identify new AQP modulators. In this work, we will present the first steps of the developed protocol, focusing our studies on AQP – subtype 1.

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## Marine Natural Products as new anti Acute Myeloid Leukemia drugs

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Innovative drug therapies are crucial to improve Acute Myeloid Leukemia (AML) patients poor prognostic. The epigenetic reader protein ENL-YEATS 3-D structure was recently unveiled [1]. This protein is required for the maintenance of AML and its depletion results in anti-leukemic effects in vitro and in vivo [1,2]. A structure-based drug discovery protocol was employed to search for new inhibitors for this novel anti-AML target. A marine compound library, based on free-access databases [3], was assembled and curated for Virtual Screening. Residues from the binding site of ENL were then selected to perform docking-based virtual screenings with MOE induced-fit docking protocol [4]. A set of lead compounds with higher docking scores than the native acetylated lysine were selected. Cross-dockings with other important targets for AML, DOT1L and PTEF-b were also performed. The most promising common compound, Mandelalide-C, was submitted to MD Simulations with each previously mentioned protein to test its behavior as a putative multi-target lead compound. Mandelalide-C and other most promising ligands will be experimentally tested for their binding affinity.

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## **pKa shifts calculations of encapsulated drugs through a CpHMD approach**

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Molecular machines have recently been associated with the development of molecular carriers to enhance drug properties, such as solubility or bioavailability. One possible approach is through drug encapsulation by a host molecule, such as cucurbituril (CB) rings, which modifies the environment of the guest molecule. CB rings are able to encapsulate guest molecules providing a hydrophobic cavity and several carbonyl groups that stabilize cationic guests that interact with this region. This results in significant pKa shifts for drugs with titrable (cationic) groups that can be exploited in order to improve drug bioavailability, whether by enhancing their solubility, stabilizing their active form or by protecting them against external agents.

Computational methods are a powerful way to rationalize the design of CB-guest complexes. In particular, the stochastic titration constant-pH MD (CpHMD) method allows a molecular dynamics simulation to have the pH value as an external parameter and, consequently, obtain full titration curves and pKa values. Our first step is to develop a strategy to model benzimidazole (BZ) pKa shifts, which has a well-known shift of 3.5 pKa units when encapsulated by a CB ring. The obtained parameters were tested in three different drugs for validation purposes: carbendazim, 2-aminoanthracene and cyclohexylmethylamine. This will be helpful to elucidate the molecular details of these host-guest interactions and to extend this procedure for many other host-guest complexes. Ultimately, we aim to develop a method that predicts the best host which optimizes the drug delivery properties of any chosen drug, enabling the design of multiple complexes with different pKa shifts. This strategy can be beneficial for novel drug design and medical applications such as cancer therapy, by designing carriers that deliver guest molecules at specific conditions, knowing the specific target properties.

We acknowledge financial support from FCT through project UID/MULTI/00612/2019.

## Decoding Partner Specificity on Dopamine Receptor Family

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The determinants of partners (G-Protein and Arrestins) selective binding to G-protein-coupled receptors (GPCRs) are not entirely known. In order to bring new insights into this subject we developed an extensive characterization of the Dopamine Receptor (DXR) family. The crystal structures of the  $\beta 2$ -Adrenergic Receptor bound to Gs and the Rhodopsin bound to Gi and Arrestin (ARR) were used to generate reliable structures of complexes of DXR family (D1R, D2R, D3R, D4R and D5R) bound to their partners (Arrestins: ARR2, ARR3; G-protein: Gq, Gz, Gt2, Gi1, Gi2, Gi3, Gs(sh), Gs(lo), Go, Gob).

An ensemble of computational methods was applied to give a detailed description of the complexes structure and their interface. A dynamic analysis using Normal Mode Analysis was also performed to test structure fluctuations and flexibility. A total of 40 models were constructed and analysed, representing an unprecedented big data analysis of GPCR-intracellular partner interface determinants.

# Comparing Different Scoring Functions for Docking and Virtual Screening against bacterial Quorum-Sensing Receptors

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Biofilms are a community of bacteria that show higher resistance to antibiotics and host immune response, resulting in severe issues for public and human health [1]. Several mechanisms are responsible for biofilm formation, such as Quorum-Sensing (QS). QS is the intercellular chemical communication in bacteria. It allows bacterial communities to collaboratively alter gene expression in a population density dependant manner. Two proteins (a synthase and a receptor) and a diffusible signal molecule participate in QS. Inhibiting the receptor-ligand binding process could decrease the pathogenic effects caused by this mechanism, reducing biofilm formation and development [2].

Molecular Docking [3] is a computational method used in structure-based drug design to accurately predict the binding pose between two molecules. The technique allows for the ranking of the generated poses based on the strength of association between the two molecules. When used in the context of Virtual Screening [4], it is possible to screen large libraries of compounds against a target protein.

In this work, we have compared the performance of different Molecular Docking scoring functions in predicting accurate binding poses and distinguishing active and non-actives molecules through ranking against LasR, a quorum-sensing receptor from *P. aeruginosa*. AutoDock, LeDock, GOLD, FFLD and AutoDock Vina were employed in this study. Decoys of the known active ligands were generated and used to test virtual screening protocols. Several early recognition evaluation metrics such as enrichment factors and receiver operating characteristic curves were calculated to evaluate the performance. The results allow us to conclude on which scoring functions are more efficient in identifying active molecules against the target.

## Acknowledgements

This work has been supported by the Fundação para a Ciência e a Tecnologia (FCT) (UID/Multi/04378/2019)

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# Theoretical insight from molecular dynamics simulations into the structural and functional understanding of of human ABCG2

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ABCG2 (also known as BCRP) is an ATP-binding-cassette (ABC) protein transporter that plays a pivotal role in tissue protection. However, in cancer cells, the over-expression of this protein along with two other ABC transporters (P-glycoprotein and MRP1), has been closely associated with the development of multidrug-resistance (MDR). An efficient modulation of the ABCG2 function could be a powerful therapeutic strategy to overcome MDR by improving the pharmacokinetics and efficacy of chemotherapeutic agents. Despite many efforts, the development of clinically useful modulators of ABCG2 has been hampered by the lack of knowledge about the structural and functional understanding of this efflux pump. Indeed, the first Cryo-EM structure of human ABCG2 (PDB ID: 5NJ3) was published only in 2017 [1]. It is important to note that preceding the publication of these structures, our research group developed a homology model of ABCG2 that proved to be comparable with the cryo-EM structure. This model brought new insights into the structural dynamics of ABCG transporters sub-family and how drug binding and recognition occurs in the ABCG2 protein [2]. However, new and independent assays needed to be conducted.

The present study aims at clarifying key points of the mechanism of ABCG2— substrate recognition and ATP-driven transport. For this purpose, a refined model of ABCG2 based on the cryo-EM structure was built. This full-length ABCG2 protein modeled was inserted in a membrane bilayer at physiological conditions (ATP, ions) and refined through molecular dynamics simulations. The global motion patterns displayed by apo ABCG2, in comparison with the ones displayed by ABCG2 in the presence of specific modulators or substrates were determined and extensively analyzed. We were particularly interested in understanding how conformational changes in ABCG2 structure leads to substrate efflux, also unveiling possibilities for setting modulation strategies. In the absent of ATP, the equilibration of the apo ABCG2 induced a distinct conformational change resulting in the closure of the access to the cavity 1 from cytoplasm. Distinct efflux-like motions were observed when the substrate Estrone-3-sulphate was positioned inside cavity 1, while milder changes in the motion patterns were observed upon inhibitor binding. These preliminary observations shed some light on the proper interpretation of available structural information on this ABC protein as well as on its functional understanding.

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## Molecular modeling studies of halogen bonding in drug-membrane recognition

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Halogen bonds (XBs) are noncovalent interactions where halogenated species interact with electronegative acceptors through a region of positive electrostatic potential, named  $\sigma$ -hole [1]. These interactions play a relevant role across many disciplines and have been increasingly exploited as tools to modulate biomolecular recognition phenomena, mainly protein-ligand binding events, in the context of chemical biology or drug discovery applications.

The development of biomolecular simulation techniques that accurately model these interactions is particularly challenging since traditional force field-based methods, e.g. molecular dynamics (MD) simulations, rely on a single-point-charge description of the system and therefore typically fail to account for the charge anisotropy in halogenated ligands [2]. This limitation can be circumvented by introducing a positively charged extra-point (EP) to emulate the  $\sigma$ -hole. We have shown that, in addition to reproducing experimental geometries and energetics, this type of methodology can yield an accurate sampling of halogen-bonded conformations in MD simulations of a model protein-ligand complex [3].

Recently, we have been applying this type of methodology to probe the role of XB interactions involving other relevant biological systems, namely phospholipid membranes. Indeed, membrane permeability is a key modulator of pharmacological activity, while halogenated compounds represent c.a. 25% of marketed drugs [4]. Therefore, the eventual role of halogen bonding targeting multiple oxygen acceptor sites within phospholipid molecules merits further investigation. This was addressed by performing MD simulations with the EP approach to model halogenated ligands interacting with model lipid bilayers. The results provided important insights into the (halo)drug-membrane recognition mechanisms which will be disclosed in this communication [5].

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## **Pyridoxine/pyridoxamine 5'-phosphate oxidase: a computational study**

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Pyridoxine/pyridoxamine 5'-phosphate oxidase (PNPOx) is a FMN-dependent homodimeric enzyme responsible for the recycling of pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP), into pyridoxal 5'-phosphate (PLP) [1]. PLP, the active form of the vitamin B6, is an essential cofactor in the synthesis of several neurotransmitters involved in both neuronal excitation and inhibition. In fact, low PLP concentrations in cerebrospinal fluid have been correlated with the occurrence of severe neurological disorders, such as neonatal epileptic encephalopathy [2]. Therefore, the fine regulation of PLP levels is of the utmost importance for the correct function of the brain, a role comprised by the PNPOx.

To unveil the catalytic mechanism of PNPOx, computational studies were employed using quantum mechanics/molecular mechanics (QM/MM) methodologies [3]. The results show that the mechanism for both substrates is mostly different. While the catalytic process for the formation of PLP occurs in a single step with PNP, several steps are needed with PMP. It is also shown that the first step for both substrates is an hydride transfer from carbon C4 of PNP/PMP to FMN, as postulated in the literature. Ongoing work is being done in order to understand if the steps involved in the deamination of PMP occur in the enzyme or in solution.

Together, these results provide knowledge and insight about the catalytic mechanism of PNPOx, helping us to understand the importance of some key residues in the active site that can have implications in some PLP-deficiency disorders.

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# Tumour targeting Photodynamic Therapy: A Strategy to Increase Selectivity

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Photodynamic therapy (PDT) is based on light irradiation of a photosensitiser (PS), in the presence of oxygen, to produce reactive oxygen species (ROS) that will destroy nearby cells [1,2]. It is an established anticancer treatment presenting advantages over traditional therapies, such as being minimally invasive, having low mutagenic potential, low systemic toxicity, and being a light delivered treatment [3]. The low systemic toxicity results from photoactivation of the PS only in the irradiated area, and not necessarily of the PS selectivity for the tumour tissue. The known side effects of PDT are caused by this lack of selectivity: tissue photosensitivity and eventual destruction of healthy tissue in the surrounding area of the tumour [2]. To tackle this problem of low selectivity in PDT, strategies have been developed for: 1) passive targeting by PS modification, use of delivery vehicles, and serum proteins associations; or 2) active targeting by conjugation of the PS (alone or loaded) with endogenous ligands, monoclonal antibodies, or growth factors [2].

Herein, we propose the development of a tumour targeted strategy by identifying an overexpressed receptor in cancer cells and screen for small molecules through computational approaches, such as, similarity searches, and docking, among others, in order to identify a relevant ligand to conjugate with a novel chlorin PS to increase the selectivity of its PDT protocol. Several strategies to increase PS selectivity have been developed and produced positive results, but a targeted PS has yet to reach the clinic [2,4,5].

Acknowledgements: Ana I. Mata thanks the MedChemTrain PhD programme (PD/00147/2013) in Medicinal Chemistry – Foundation for Science and Technology (FCT), Ministry of Science, Technology, and Higher Education (MCTES) – for the grant of a PhD fellowship (PD/BD/135289/2017).

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# Using a computational approach to enhance enzymes as industrial biocatalysts

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Nowadays, directed evolution has been used more and more to modify and/or improve the catalytic mechanism of enzymes [1-2]. This approach has been employed to aid the transfer of the catalytic power of enzymes from the cell to the industrial field, where they are used as biocatalysts in the synthesis of commercially valuable compounds.

Computational studies devoted to the study of the catalytic mechanism of enzymes have been crucial to increase the set of studied reactions and also to speed up the investigation of mutations that could improve catalytic efficiency. According to this, the reversible retro-aldol mechanism of Serine Hydroxymethyltransferase (SHMT) [3] enzyme was studied using ONIOM QM/MM approach.

Different mutations of the Glu51 residue were assessed, seeking for an improvement in the catalytic efficient associated with the production of  $\alpha,\alpha$ -dialkylamino acids. Models for wild-type and mutated forms of the enzyme were submitted to molecular dynamics simulations followed by ONIOM QM/MM calculations according to the DLPNOCCSD(T)/CBS//B3LYP/631G(d,p):AMBER scheme. The VMD, molUP [4], AMBER, and Gaussian09 software were used to perform the calculations and analyze the results.

The results offer important clues about the catalytic mechanism of SHMT and can be used as a blueprint to test other mutations. Additionally, this data contributes to the development of more efficient bioengineered enzyme for the synthesis of  $\alpha,\alpha$ -dialkylamino acids. These commercially valuable compounds are precursors in the synthesis of some drugs, such as, as lactacystin and myriocin.

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Acknowledgments: The authors thank the financial support from the Foundation for Science and Technology (FCT) through the PhD grant number SFRH/BD/115396/2016, the project PTDC/QUI-QFI/31689/2017, national funds and FEDER cofounding under the partnership agreement with PT2020. This work was also supported by the Applied Molecular Biosciences Unit – UCIBIO which is financed by national funds from FCT/MCTES (UID / Multi/04378/2019).

## **Improving the Threonine Aldolase activity to produce beta-hydroxy-alpha-amino acids by computational means**

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Enzymes offer a variety of advantages as biocatalysts in the pharmaceutical industry, such as, regio and stereo selectivity, work at mild conditions and, are environmentally friendly. However, their limited catalytic rates are still a hindrance for a broader industrial usage.

The beta-hydroxy-alpha-amino acids (HAA) is a core building block of several drugs, such as L-threo-3,4-dihydroxyphenylserin (L-threo-DOPS) and an interesting anti-Parkinson's disease drug. Threonine Aldolase (TA) is a powerful PLP-enzyme that enhance the catalysis of asymmetric carbon-carbon bond formation to produce a wide range of HAAs [1]. However, the reaction rates continue to be poorly optimized which compromise its use in the industry.

Computer modelling, in particular quantum mechanics/molecular mechanics (QM/MM) schemes, have proven to be exceptional tools to unveil the catalytic mechanisms of enzymes with atomistic detail and provide the free energy profile of the catalytic reactions. This enables a better understanding of the reaction and aids on the rational prediction of mutations, which can be used to improve the catalytic rate of the reactions.

In this project, we are studying, with atomistic level of detail, the catalytic mechanism of TA to produce L-threo-DOPS, using QM/MM at the B3LYP/6-31G(d):Amber level of theory.

Acknowledgments: The authors thank FCT (Fundação para a Ciência e a Tecnologia) for PhD Grant SFRH/BD/136746/2018 and UID/Multi/04378/2019.

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## RDB-TS: Development of a Database for Storage of Transition State Structures for Organic Reactions

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A Transition State (TS) is a first-order saddle point on a potential energy surface hovered between the chemical structures of reactants and products of a chemical reaction. This is the point where the chemical bonds are broken or formed and thus dictates the rate of a chemical reaction. Since all the chemical reactions must go through a TS, these structures give important clues about all ephemeral stages that govern all chemical or biochemical processes present in life. Once the TS structures are known, one can predict reaction outcomes, determine the necessary reaction conditions to perform desirable reactions, design new drugs and, most importantly, explain chemical transformations for which experimental data is not or cannot be available.

The transition state (TS) structures determined by computational means are currently not available for general use, due to the difficulty to get them and the needed skilled labour. It is estimated that 75% of the time spent by a computational chemist to study a chemical reaction is spent determining a transition state structure. Even when the TS structures are known, access to this information is difficult.

This work describes the creation of a database for storage of TS structures derived from quantum chemical calculations for application in organic chemistry. This information organized and available for computational analyses, providing a key resource for the identification, classification and comparative analysis of TS structures. Additional information will also be stored on the kinetic and thermodynamic parameters of the reactions related to them.

**Acknowledgements:** The authors thank the financial support from the Foundation for Science and Technology (FCT) through the project PTDC/QUI-QFI/31689/2017, national funds and FEDER co-founding under the partnership agreement with PT2020. This work was also supported by the Applied Molecular Biosciences Unit – UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019).

# Computational approach to study the interaction between the milk protein lactoferrin and V-ATPase

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Lactoferrin (Lf) is an iron-binding protein abundant in milk and colostrum that exhibits several biological activities, from which its anticancer activity stands out. Although many reports attest Lf activity against different types of cancer cells, information about the underlying mechanisms and targets is scarce.

We previously demonstrated that bLf is selectively cytotoxic to highly metastatic cancer cells but not to lowly metastatic or non-tumorigenic cells by targeting and inhibiting the plasmalemmal V-ATPase (1, 2). V-ATPase is a proton pump that actively transports protons across cellular membranes, which is localized at the plasma membrane of highly metastatic cancer cells. Although our data show that bLf inhibits V-ATPase activity, we now aim to find which residues of both bLf and V-ATPase are involved in their interaction.

For that purpose, we present a computational approach based on the use of the molecular docking program HADDOCK (High Ambiguity Driven protein-protein DOCKing) to predict and rank the protein-protein complexes (3). Molecular docking is a computational method used to predict the preferred binding pose between two molecules. The docking protocol will be validated using known Lf interactors. The best scored complexes will then be pre-embedded into a pre-equilibrated POPC (1-palmitoyl-2-oleoyl phosphatidylcholine) membrane model and the resulting complexes will be subjected to molecular dynamic (MD) simulations using the AMBER software. With this approach, we expect to identify the hot spot residues on the interface of the proteins critical for their interaction, for future validation by experimental means.

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Acknowledgements: The authors thank the financial support from the Foundation for Science and Technology (FCT) through the PhD grant PD/BD/128032/2016 under the scope of DP\_AEM. This work was also supported by the Applied Molecular Biosciences Unit - UCIBIO, which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019).

## **Computational approach to the structural elucidation of an Electrogenerated Hydrophilic Carbon Nanomaterial**

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In the last decade, the interest in generating and fully characterizing Carbon-based nanomaterials grew exponentially due to their excellent properties and wide potential to be applied in several fields. An Electrogenerated Hydrophilic Carbon (EHC) nanomaterial was recently developed by our group [1] and its physical and chemical properties were investigated by numerous techniques as TEM, HRTEM, AFM, Raman, FTIR, XPS and, UV-Vis. EHC nanomaterial comprises amorphous-like carbonaceous clusters displaying sp<sub>2</sub> carbon suggesting that the aromatic structure is predominant. Various oxygen-containing functional groups were detected by XPS and FTIR with an O/C atomic ratio of 0.42. In order to elucidate the EHC structure, computational means were used to model, different molecules and simulated the resulting IR, Raman and, UV-Vis spectra with different computational methods using Gaussian09 and ORCA. These results were then compared with the available experimental data. Our preliminary results enabled us to discard several possibilities, giving also important clues about the functional groups and number of benzene rings present in the EHC nanomaterial. More computational studies are required to fully elucidate the structure of this promising nanomaterial. These calculations are currently being carried out.

**ACKNOWLEDGMENTS:** The authors thank the financial support from the Foundation for Science and Technology (FCT) through the PhD grant number SFRH/BD/138425/2018, the projects UID/QUI/00616/2013, UID/NAN/50024/2013 and UID/Multi/04378/2019. FEDER (Partnership Agreement PT2020) ERDF (NORTE-01-0145-FEDER-000024).

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## Alignment-Free Method to Predict Enzyme Classes and Subclasses

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The Enzyme Classification (EC) number is a numerical classification scheme for enzymes, established using the chemical reactions they catalyze. This classification is based on the recommendation of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Six enzyme classes were recognised in the first Enzyme Classification and Nomenclature List, reported by the International Union of Biochemistry in 1961. However, a new enzyme group was recently added as the six existing EC classes could not describe enzymes involved in the movement of ions or molecules across membranes. Such enzymes are now classified in the new EC class of translocases (EC 7). Several computational methods have been developed in order to predict the EC number. However, due to this new change, all such methods are now outdated and need updating. In this work, we developed a new multi-task quantitative structure–activity relationship (QSAR) method aimed at predicting all 7 EC classes and subclasses. In so doing, we developed an alignment-free model based on artificial neural networks that proved to be very successful.

## **Computational studies on the CFTR ion channel**

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ATP-binding cassette (ABC) proteins are ubiquitous super-family of membrane transporters present in all phyla with 7 sub-families (A-G) playing central roles in drug disposition, metabolism and pharmacokinetics. They are flexible transporters that act with the membrane environment to efflux undesirable metabolites or xenobiotic drugs from inside the cell providing this way a highly relevant protective role. At least 11 transporters of the ABC family have well characterized roles in human diseases while others are expressed in many tissues and appear to be particularly related with multidrug resistance (MDR) in cancer.

Cystic fibrosis is the most common life-shortening rare disease (median age at death 28yrs), affecting 34,000 individuals in EU ( 85,000 worldwide). These numbers rise daily due to extensive newborn screening and increased diagnosis across the world. CF results from mutations in the gene encoding CFTR, an epithelial anion channel, also an ABC transporter family member. The F508del mutation, occurring in 85% CF patients, causes CFTR protein to misfold and its recognition by the endoplasmic reticulum quality control (ERQC) which prevents mutant CFTR from reaching the cell surface, thus causing a traffic defect. Despite symptomatic therapies, quality of life/life expectancy are still limited. Thus, rescue of CFTR by small molecules that correct the basic defect is the much ambitioned alternative, but so far with limited success. Indeed, 2 drugs have been approved that: 1) one (potentiator VX-770) restores the function in mutant CFTR, but only applies to a small number ( 4%) of all CF patients; 2) another (corrector VX-809) rescues F508del-CFTR traffic to cell surface and together with a potentiator restores its function, but at low efficacy. Here, we will present our recent findings resulting from studying the dynamic behavior of the CFTR ion channel using computational approaches in order to understand how selected mutations impact the gating mechanism, with the ultimate goal of identifying new leads/drugs which may improve the lives of CF patients carrying these mutations.

# A molecular dynamics approach to Non-Structural Protein 1 from influenza virus to understand protein-protein interactions through interface evolution

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Influenza (flu) is a contagious viral disease, which attacks the respiratory tract spreading through the population in seasonal infections. In fact, every year around 10% of world population is infected and an estimated 650.000 people die from Influenza according to the World Health Organization (WHO)[1]. Since the vaccination's efficacy is limited, and due to high rates of mutation and recurrent genetic assortment, new preventive and therapeutic approaches and better understanding of the virus-host interactions are urgently needed[2].

NS1, one of the 11 proteins encoded by the virus, became a potential target as it promotes enhancement of viral replication, while negatively affects the host's innate immune response[3]. NS1 protein has indeed a plethora of functions by interacting with different host partners[4]. Structurally, NS1 is a 26 kDa multifunctional protein with around 230 residues and formed by 2 domains, a linker and a disordered C-terminal tail. The linker that connects the N-terminal RNA-Binding Domain (RBD) and the Effector Domain (ED) is a short, flexible region without a defined/fixed number of residues[5]. In host cells, NS1 is likely to be a homodimer that can shift between different quaternary conformational statuses possibly depending on its partners, location in cell, and linker size[6]. This biological system presents itself as a highly evolutionary-conserved protein, but there is lack of information about its structure and behavior [7]. We performed six replicas of 1  $\mu$ s Molecular Dynamics (MD) simulation of each NS1 system in order to fully characterize the conformational space visited by both domains and how the length of the flexible linker affects the interface of both monomers in order to infer differences in the protein-protein interactions that NS1 can have. In particular, we carried out MDs of 2 different strains of full-length NS1 proteins: i) H6N6 strain with a 15 amino-acids linker; ii) H6N6 strain with a 10 amino-acids linker after a 5 residue deletion intended to mimic the behavior of a H5N1 strain. Overall, our approach offers a new strategy for a better understanding of the NS1 homodimer dynamical behavior in human cells.

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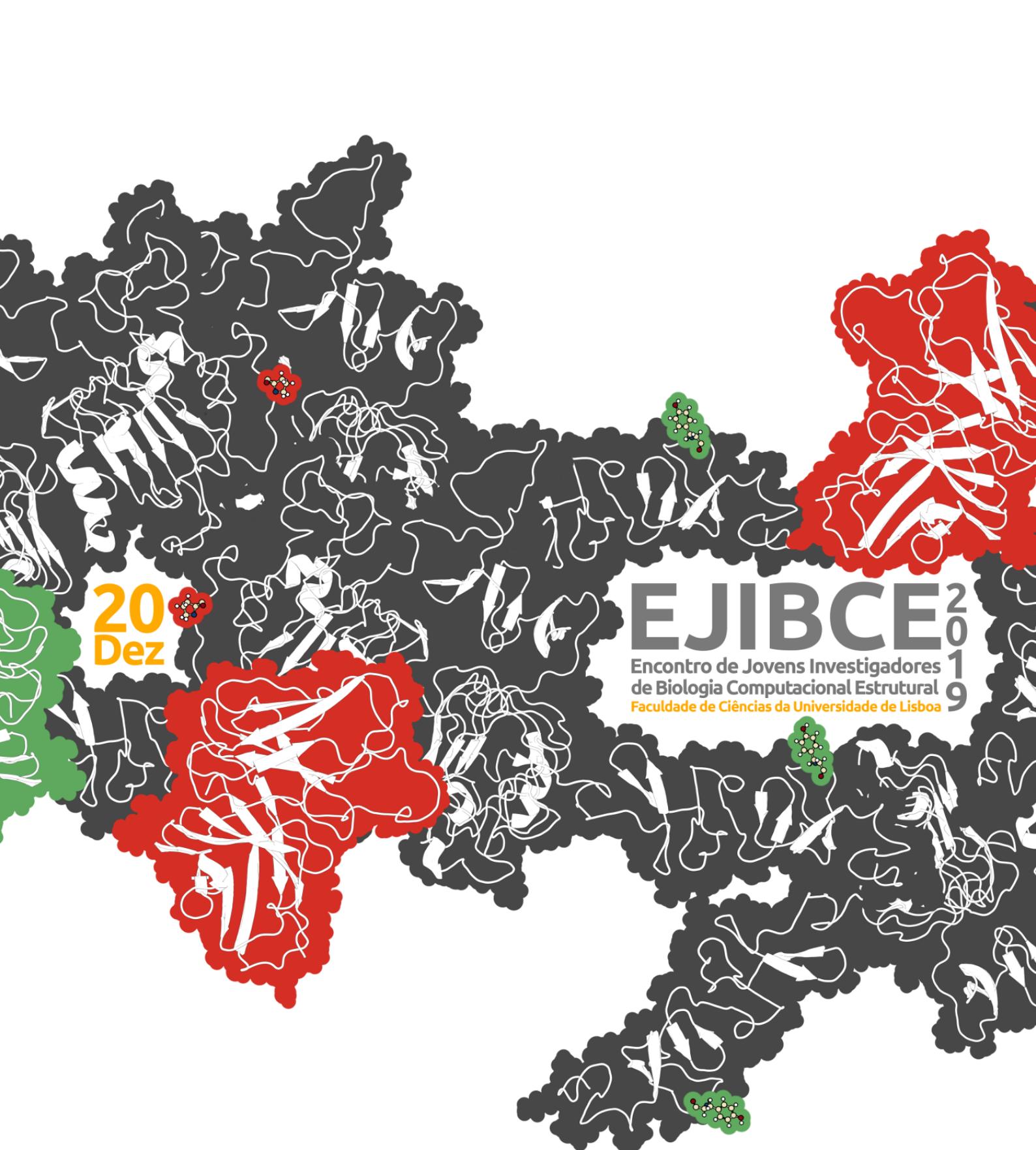
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## A Deep Learning Approach for Ligand-Target Binding Affinity Prediction

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Here we present an algorithm that exploits big data to deliver accurate ligand-target interaction predictions. It makes use of simple features extracted from the protein and SMILE sequences and deploys them in neural network frameworks that assess the interaction in multi-classification and regression approaches.



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# EJIBCE<sup>2019</sup>

Encontro de Jovens Investigadores  
de Biologia Computacional Estrutural

Faculdade de Ciências da Universidade de Lisboa

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