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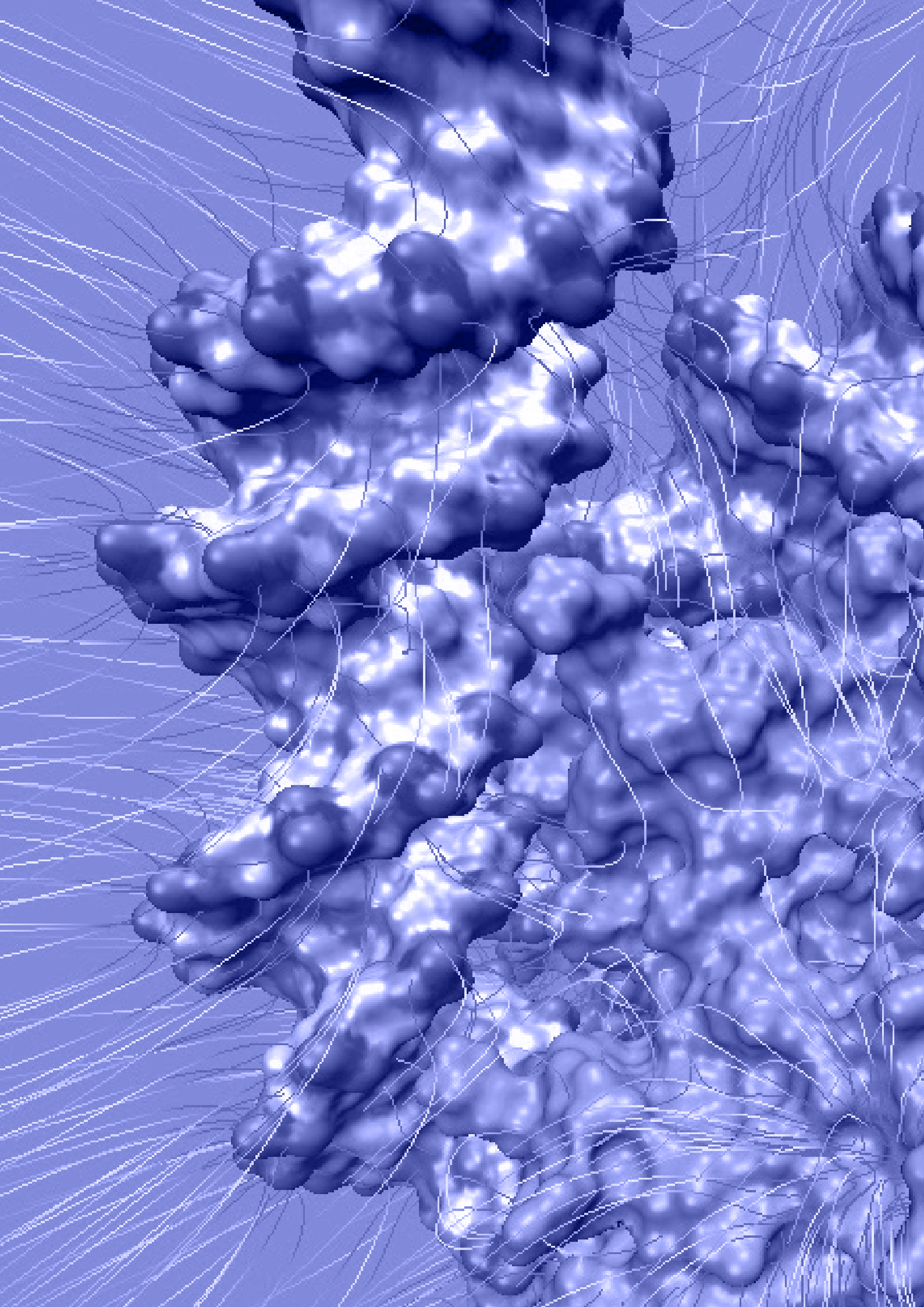

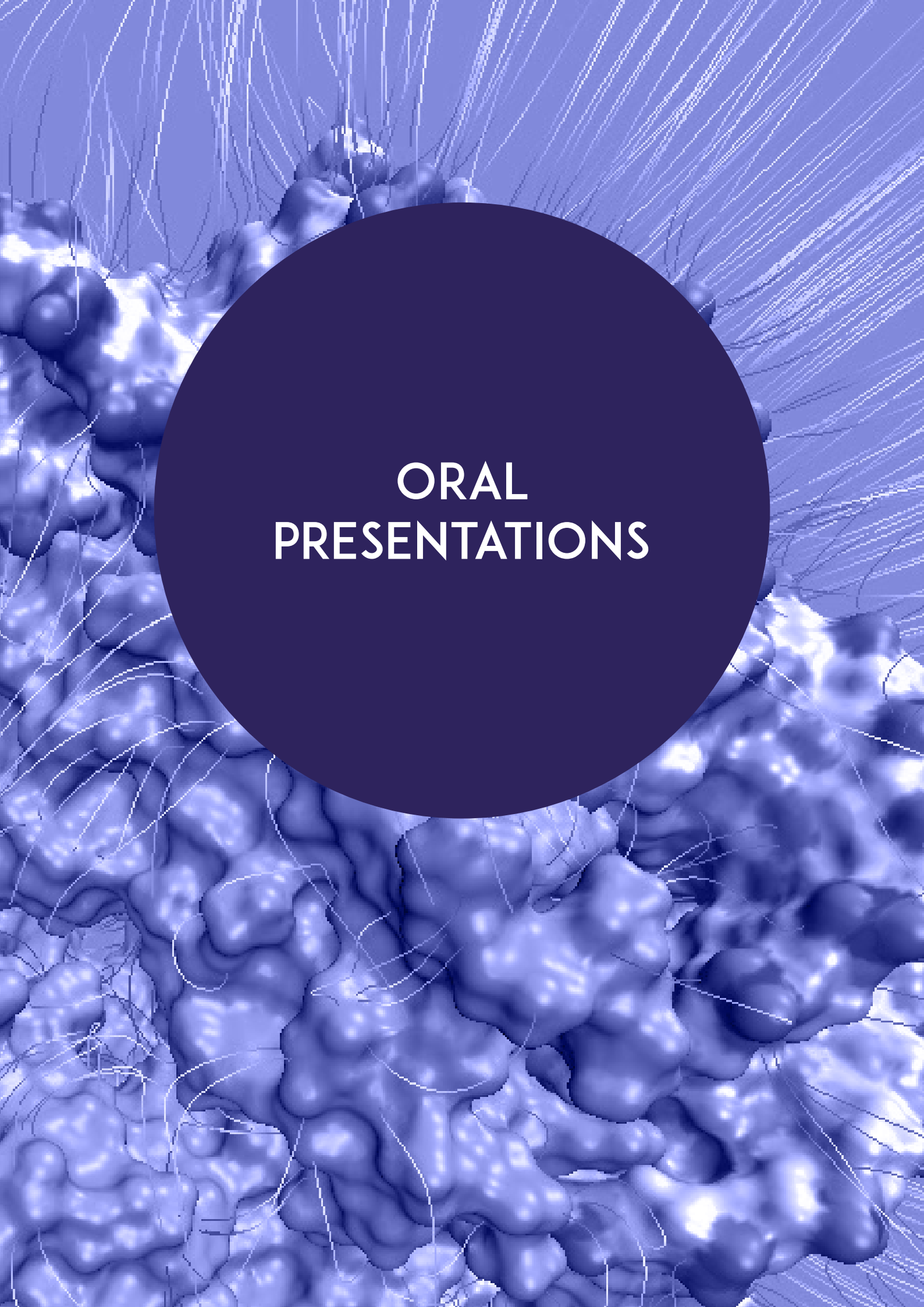

ORAL PRESENTATIONS

Predicting the oxidation states of metals in metalloenzymes using machine learning

Muhamed Amin

Laboratory of Computational Biology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

Serial Femtosecond Crystallography at the X-ray Free Electron Laser (XFEL) sources enabled the imaging of the catalytic intermediates of the oxygen evolution reaction of Photosystem II (PSII). However, due to the incoherent transition of the S-states, the resolved structures are a convolution from different catalytic states. Here, we train Decision Tree Classifier and K-means clustering models on Mn compounds obtained from the Cambridge Crystallographic Database to predict the S-state of the X-ray, XFEL, and CryoEM structures by predicting the Mn's oxidation states in the oxygen-evolving complex. The model agrees mostly with the XFEL structures in the dark S1 state. However, significant discrepancies are observed for the excited XFEL states (S2, S3, and S0) and the dark states of the X-ray and CryoEM structures. Furthermore, there is a mismatch between the predicted S-states within the two monomers of the same dimer, mainly in the excited states. We validated our model against other metalloenzymes, the valence bond model and the Mn spin densities calculated using density functional theory for two of the mismatched predictions of PSII. The model suggests designing a more optimized sample delivery and illumiation systems are crucial to precisely resolve the geometry of the advanced S-states to overcome the noncoherent S-state transition. In addition, significant radiation damage is observed in X-ray and CryoEM structures, particularly at the dangler Mn center (Mn4). Our model represents a valuable tool for investigating the electronic structure of the catalytic metal cluster of PSII to understand the water splitting mechanism.

Notes

How does pH affect the distribution of ions around proteins?

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

Instituto de Tecnologia Quìmica e Biológica António Xavier, Universidade Nova de Lisboa

Ions associated with biomolecules in solution may be bound at specific sites or more loosely associated at their surface (i.e., territorially bound). The study of ion association with nuclei acids has long been the major focus, but ion-protein interactions, often with a marked pH dependency, are prompting a growing interest. This work presents a detailed analysis of the association of ions around _-lactoglobulin using a constant-pH MD (CpHMD) method within the pH range 3-8, comparing it with more traditional Poisson-Boltzmann (PB) models and existing experimental data. The MD simulation box was required to have (approximate) charge neutrality and ionic strength equal to the bulk solution, resulting in an absolute value of ion excess equal to half the protein charge, in agreement with experimental observations on other proteins. Moreover, the MD-estimated protein total charge including territorially bound ions is in excellent agreement with electrophoretic measurements. Calculated ion concentration spatial maps show that CpHMD simulations are overall in good agreement with the nonlinear form of the PB (NLPB) model but not with its linear form, whose use implies a theoretical inconsistency. Nonetheless, some discrepancies between CpHMD and NLPB were observed both at short distances (largely due to the lack of protein dynamics in NLPB) and at long distances (where the methods converge to somewhat different ion concentrations).

Notes

Probing Mastoparan-like Antimicrobial Peptides and Anionic Model Membrane Affinity Through Structural and Electrostatics Analysis

Ingrid Bernardes Santana Martins[1], Rafael Giordano Viegas[1,2], Murilo Nogueira Sanches[1], João Ruggiero Neto[1], Alexandre Suman de Araujo[1,3], Vitor Barbanti Pereira Leite[1].

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Antimicrobial Peptides (AMPs) have emerged as promising alternatives to conventional antibiotics due to their capacity to disrupt the lipid packing of bacterial cell membranes and hinder the development of bacterial resistance. Understanding their role in the lipid packing disruption and their structural properties upon interacting with bacterial membranes are highly desirable. Computational methods can provide valuable insights into the mechanism of action of AMPs and describe in atomistic level the structural and electrostatic changes that occur when they adsorb onto an anionic lipid bilayer. In this study, we employed Molecular Dynamics (MD) simulations, Constant pH Molecular Dynamics (CpHMD) simulations and the Energy Landscape Visualization Method (ELViM) to characterize and compare the ionization state of the titratable residues and the conformational ensembles of the mastoparan-like Polybia-MP1 and its analogous H-MP1, in which histidines replace lysines. At first, two types of MD were carried out: (i) the peptides in its free state in an aqueous solution, containing water and ions, and (ii) the peptides spontaneously adsorbing onto an anionic lipid bilayer, used as a bacteria membrane mimetic. CpHMD was used to calculate pKa of each titratable residue of the peptides as well as its net charge at different environments. ELViM was used to project a single-effective conformational landscape for both peptides, enabling a comparative analysis. Furthermore, the single-space analysis was employed to describe structural changes during the adsorption process in the same framework. CpHMD showed average pKa shifts of two to three units, resulting in higher net charge for the analog than for MP1, strongly modulating the peptide adsorption. ELViM analysis indicates that the free MP1 populates a free energy minima structurally close to they adsorbed conformations, while the free H-MP1 is mostly unfolded and has to overcome a larger energy barrier to fold upon adsorption. These discrepancies in the conformational ensembles of these peptides may affect their affinity to the membrane and the adsorption kinetics, corroborating experimental findings on their lytic activity.

Notes

Bondanza Mattia

OpenMMPol: QM/Polarizable MM Calculations Made Easy

Mattia Bondanza, Tommaso Nottoli, Lorenzo Cupellini, Filippo Lipparini, Benedetta Mennucci

Dipartimento di Chimica e Chimica Industriale, Università di Pisa

Polarizable QM/MM methods based on induced point dipole (IPD) force fields (such as AMOEBA) have been developed in our group during the last two decades andnow represent a mature technology. They have been applied to several challenging problems in the fields of light harvesting systems, photoresponsive proteins, and in describing light-induced chemical processes in solution. However, until today, the usage of those methods has been strongly hindered by the lack of publicly available software that provides all the needed machinery to interface QM codes with this particular kind of MM embedding.

In order to fill this gap, we developed OpenMMPol, a library providing all the functionalities needed to interface virtually any QM method to polarizable embedding based on IPD. The usage of the library is illustrated by interfacing it to the popular open source QM software PySCF, thus enabling one to perform DFT/AMOEBA MD simulations, optimizations, and single point TD-DFT calculations.

Notes

Boxer Steven

Enhanced active-site electric field accelerates enzyme catalysis

Steven G. Boxer, Chu Zheng, Zhe Ji

Stanford University

Local internal electric fields in complex organized systems like proteins can be measured using the vibrational Stark effect (VSE). These fields can be very large and can affect chemical reactivity. I will briefly explain the underlying physical concept and strategy we have developed to apply the VSE to a wide range of systems. In all cases to date, mutations made the active site electric field and catalytic rate smaller than the native wild-type enzyme, begging the question whether larger fields and correspondingly larger rates can be created either by design or by evolution? Using the hydride transfer enzyme liver alcohol dehydrogenase (LADH), we have found that mutations and metal replacements at the active site can produce both larger fields and faster rates, extending and strengthening the concept of electrostatic catalysis. Combinations of changes that enhance the field lead to predictions of further enhancements and faster rates. Likewise, changes that enhance the field and rate can be used to rescue changes that slow the rate. In all cases, the predictions agree with the rate-vs-field relationship. This suggests that an important missing link in the quest for better catalyst design, whether biological or non-biological, may be the electric field.

Notes

Computing electrostatics interactions of biomolecular and bio-materials complexes with a robust and user-friendly PB framework

Christopher D. Cooper[1], Horacio V. Guzman[2], Marcia O. Fenley[3]

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The Poisson-Boltzmann equation has been solved using various numerical methods, such as finite differences, finite elements, and boundary elements, and implemented in numerous software packages. This equation plays a crucial role in understanding the electrostatic properties of large macromolecules, such as viruses, where traditional molecular dynamics simulations may be prohibitively expensive. Among these methods, the boundary element method stands out for its accurate representation of the molecular surface, explicit enforcement of the interface condition, precise treatment of charges, and exact handling of vanishing conditions at infinity. Additionally, it demonstrates effectiveness in large-scale systems. Despite these advantages, the boundary element method is not widely adopted by application scientists. In this study, we investigate the application of the boundary element method in molecular electrostatics, emphasizing its accuracy, robustness, applicability, and user-friendliness.

Furthermore, we introduce Poisson-Boltzmann & Jupyter (PBJ), a Python library that serves as a boundary integral solver. PBJ not only allows users to interact with it from a Jupyter notebook but also enables easy integration with other Python-compatible software. Moreover, PBJ's code design is well-suited for mapping to physical problems, providing a flexible platform for exploring new ideas and developing models. These principles align closely with the ongoing community-wide efforts aimed at creating robust and interoperable research software for user-friendly computational modeling frameworks.

Notes

Damjanovic Ana

Protein pKa values through machine learning and selectivity of sodium channels

Ada Y. Chen, Juyong Lee, Bernard Brooks and Ana Damjanovic

Johns Hopkins University
National Institutes of Health
Kangwon National University

I will discuss four tree-based machine learning models for protein pKa prediction. The four models were trained on three experimental PDB and pKa datasets, two of which included a notable portion of internal residues. We provide pKa predictions for proteins in human proteome from the AlphaFold Protein Structure Database and observed that 1% of Asp/Glu/Lys residues have highly shifted pKa values close to the physiological pH.

I will also discuss our latest research on selectivity of bacterial sodium channels. We calculate that the type of ion present in the selectivity filter (Na+ vs K+) modulates the pKa of the selectivity filter glutamates. The resulting change in protonation state of the selectivity filter affects conductance and selectivity.

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Protein pKa Prediction by Tree-Based Machine Learning AY Chen, J Lee, A Damjanovic, BR Brooks, Journal of chemical theory and computation 18 [4], 2673-2686
Ion channel selectivity through ion modulated changes of selectivity filter pKa values, AY Chen, BR Brooks, A Damjanovic, PNAS, accepted

Notes

Ferreira Sara

Mechanical Stability of Knotted Ubiquitin C-terminal Hydrolase-L1 (UCH-L1): Insights from Molecular Dynamics Simulations

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Ubiquitin C-terminal hydrolases (UCHs) are cysteine proteases that are involved in countering the ubiquitination process by hydrolyzing the ubiquitin adduct in proteins. One of the four UCHs found in the human genome, UCH-L1, holds significant importance as it is highly expressed in the brain, representing about 1 to 5% of total neuronal protein [1], and has also been associated with neurodegenerative diseases such as Parkinson's and Alzheimer's, due to its presence in Lewy bodies [2]. UCH-L1, which is a single-domain protein with 223 amino acid residues, possesses a remarkably intricate 3D knotted structure, characterized by a '5 2' or 'Gordian' knot, which is formed by five crossings of the polypeptide backbone. Preliminary results indicate that UCH-L1 exhibits a dynamic folding pathway, with unknotted intermediate states. During the unfolding process, the _-helices of the protein are unfolded, while the _-strands comprising the central hydrophobic core remain intact [3]. The role of the knot in maintaining this remarkable structural stability is an open question we seek to address.
In this study, we aim to investigate the conformational space and mechanical stability of knotted UCH-L1 using extensive molecular dynamics (MD) simulations. Additionally, we have developed a computational protocol to explore truncated versions of UCH-L1 with N- and C-terminus deletions, enabling us to understand the impact of the unknotting process on the protein's overall mechanical stability and its binding to ubiquitin. Both wild-type UCH-L1 and truncated species were simulated in their apo and ubiquitin-complexed forms. The results of this study will provide valuable insights into the structural and functional properties of UCH-L1 and contribute to our understanding of its role in neurodegenerative diseases.

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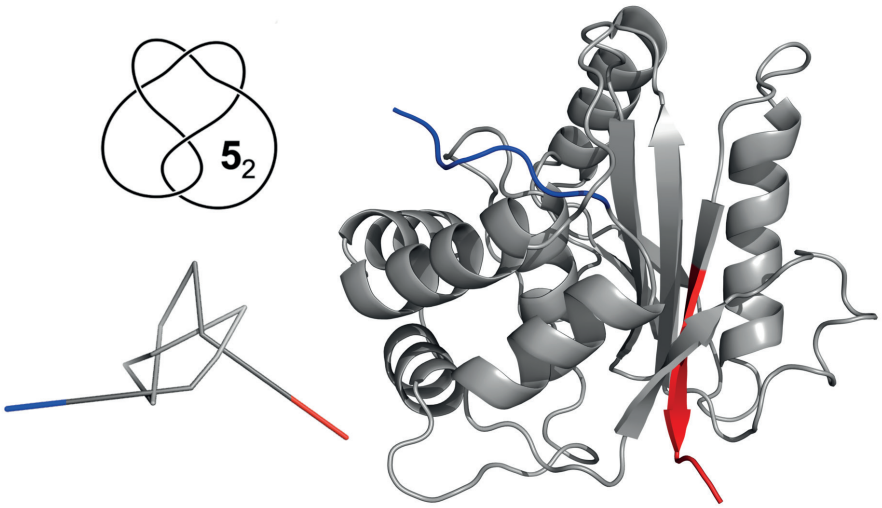

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Notes

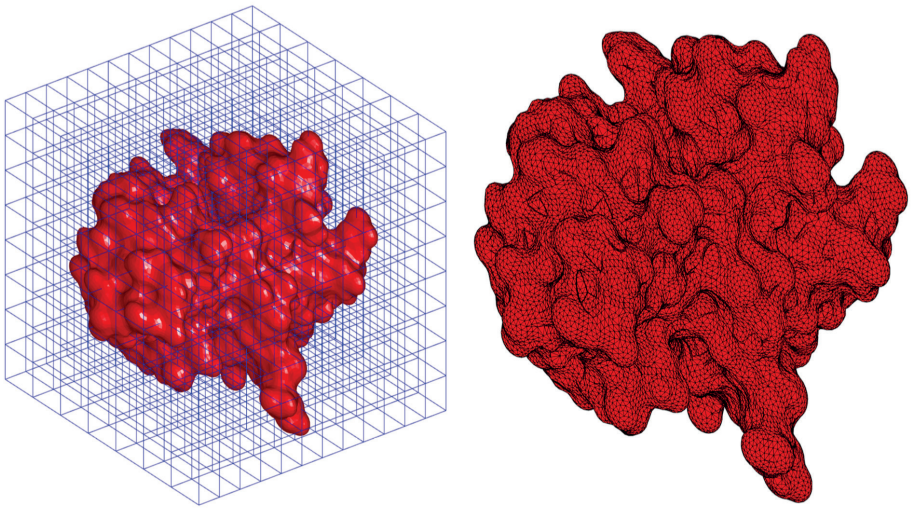
Geng Weihua

Title: Some computational tools for protein electrostatics and structures

Weihua Geng, Robert Krasny, Guowei Wei, Jiahui Chen, Johannes Tausch

Southern Methodist University
University of Michigan
Michigan State University
University of Arkansas
Southern Methodist University

In this talk, I will introduce several computational tools we recently developed for the study of protein electrostatics and structures. The first is the parallelized treecode and boundary integral Poisson-Boltzmann (TABI-PB) solver. This overlaps Dr. Robert Krasny's talk about TABI 2.0 solver but my focus is on the parallelization features using MPI and GPUs. The second is the Eulerian Solvent Excluded Surface (ESES) software for rendering conjugated Eulerian and Lagrangian surface representations, which enables us to numerically validate and compare the quality of Eulerian PB solvers, such as the MIBPB solver and the Lagrangian PB solvers, such as the TABI-PB solver. If time is permissible, I will introduce our newly developed Cartesian FMM-accelerated Galerkin Boundary Integral Poisson-Boltzmann solver, which shows improvements in accuracy and efficiency at the price of algorithm and coding complexity.



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Notes

Electrochemically Gated Charge Transport in Redox Proteins and Photosynthetic Complexes

Pau Gorostiza

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Institute for Bioengineering of Catalonia (IBEC)
The Barcelona Institute of Science and Technology

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Interprotein electron transport is an essential process in cell respiration and photosynthesis. It takes place between redox proteins and complexes, and it displays an outstanding efficiency and environmental adaptability. Although the biochemistry of electron transport processes is well characterized, nanoscale experimental methods are needed to understand electronic pathways in these redox protein structures, both for fundamental and for technological purposes. Electrochemical scanning tunneling microscopy (ECSTM) is a unique tool to study electronic materials and redox molecules including proteins. It offers single molecule resolution and allows working in aqueous solution, in nearly physiological conditions in the case of proteins, and under full electrochemical control (López-Martìnez et al., 2017). ECSTM also allows performing conductance measurements by current-potential and current-distance tunneling spectroscopy (Artés et al., 2012), notably between cognate redox partner proteins. An overview of these methods will be presented together with recent results of the laboratory in the respiratory (Lagunas et al., 2018; Gomila et al., 2022) and photosynthetic chains (López-Martìnez et al., 2019; López-Ortiz et al., 2022; Zamora et al., 2022). The importance of protein charges in these measurements will be discussed, for example in cytochrome phosphorylation (Gomila et al., 2022) and in the interaction between plastocyanin and photosystem I complexes (Zamora et al., 2022).

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Notes

A multiplicity of protonation states in proteins supports proton transfers.

Marilyn Gunner, Junjun Mao, Gehan Ranepura, Judy Rongmei Wei, Jose Ortiz-Soto, Md. Raihan Uddin, Muhamed Amin[1]

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Proteins are known to exist in many conformations, but the complexity of the equilibrium ensemble of protonation states is under-appreciated. Recent developments in the MCCE program have enabled the analysis of protonation and conformation microstates in Monte Carlo sampling. The individual microstates, which define the protonation states and conformation of each residue and ligand are akin to an MD snapshot. Analysis of these states reveals how protons move within complex clusters of buried protonatable residues. The proton coupled electron transfers in photosynthetic reaction centers, cytochrome c oxidase and complex I provide examples of how the protonation and conformation microstates influence site redox potentials, proton affinities of proton loading sites and the connectivity of extended proton transfer pathways.

Notes

Biomolecular electrostatics by NMR spectroscopy

Junji Iwahara

Department of Biochemistry & Molecular Biology, Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch

Biomolecular electrostatics has been a subject of analysis subsequent to 3D structure determination. This situation is changing. In the past few years, we have developed nuclear magnetic resonance (NMR) methods to directly measure near-surface electrostatic potentials around biomolecules without any use of structure information. Now, local electrostatic potentials can directly be measured for almost every residue of proteins and nucleic acids by these methods. The new electrostatic methods are applicable to various processes, including macromolecular association and liquid-liquid phase separation. Applications to intrinsically disordered proteins and conformationally flexible nucleic acids are particularly useful because structure-based analysis of electrostatics is not straightforward for such molecules. These new NMR methods also facilitate examination of theoretical models for biomolecular electrostatics.

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Notes

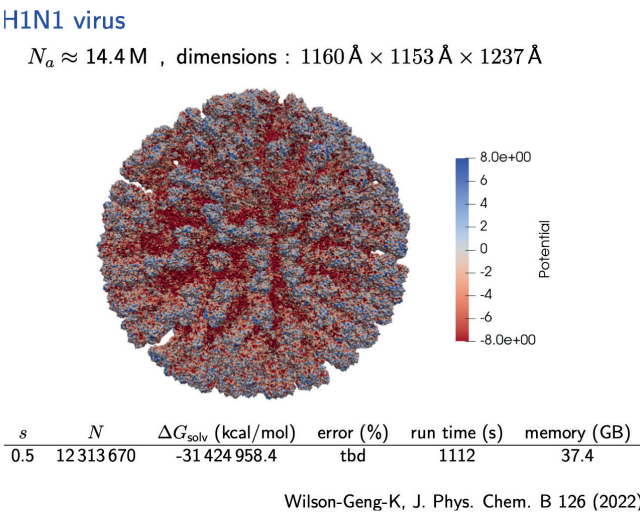
The TABI-PB 2.0 solver and an integral equation method for the 1D steady state Poisson-Nernst-Planck equations

Robert Krasny

University of Michigan, Ann Arbor, USA

TABI-PB 2.0 is an improved version of the treecode-accelerated boundary integral Poisson-Boltzmann solver [1]. The code computes the electrostatic potential on the molecular surface of a solvated biomolecule and further processing yields the electrostatic solvation energy. The new implementation utilizes the NanoShaper surface triangulation code, node-patch boundary integral discretization, a block preconditioner, and a fast multipole method based on barycentric Lagrange interpolation and dual tree traversal [2]. Performance-critical portions of the code were implemented on a GPU. Numerical results for protein 1A63 and two viral capsids (Zika, H1N1) demonstrate the code's accuracy and efficiency.

If time permits I will also report on an integral equation method for the 1D steady-state Poisson-Nernst-Planck equations modeling ion transport through membrane channels [3]. The differential equations are recast as integral equations using Green's 3rd identity yielding a fixed-point problem for the electric potential gradient and ion concentrations. The integrals are discretized by midpoint and trapezoid rules and the resulting algebraic equations are solved by Gummel iteration. Numerical tests for electroneutral and non-electroneutral systems demonstrate the method's 2nd order accuracy and ability to resolve sharp boundary layers. Finally the method is applied to a 1D model of the K+ ion channel.



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Notes

Kurnikova Maria

Structure and Divalent Ion permeation in Tetrameric Ion Channel Proteins in Molecular Dynamics Simulations are Dominated by Electrostatic Interactions

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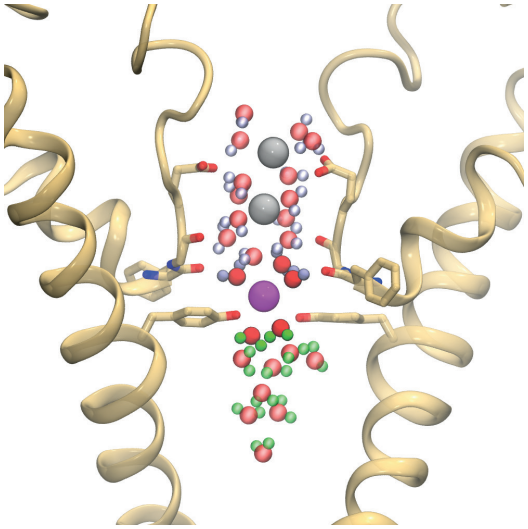
Ion Channel forming proteins present unique challenges for molecular modeling of their structure and function. Due to their integral membrane environment of low dielectric embedded in high dielectric water all polar and charged atom interactions in these systems are enhanced compared to globular proteins. Modeling protein structure via classical molecular dynamics simulations may cause protein deformation due to mis-balance of non-polar and polar interactions that are difficult to quantify. We will present modeling of structure of tetrameric integral membrane ion channel forming proteins (TRPV6, TRPM7 and NMDA) in different conformations that demonstrate stability and lack of thereof based on such balance. Ion permeation through narrow water filled pores formed by these ion channel proteins is also a process strongly influenced by the electrostatic interactions of the ion, water and protein groups confined within a low dielectric membrane environment. While modeling charged species interaction in the protein environment is a known outstanding problem, the difficulty of statistical sampling of configurations in confined environments further exacerbates the difficulty of quantitative and even qualitative predictions of such system behaviors. Selectivity of divalent ions in the glutamate receptors of NMDA type is essential for this receptor physiology, yet it remains unclear how this channel selects between a permeant ion Ca2+ and a blocking ion Mg2+. To compare ion channel selectivity for two similar ions it is typical to compute potentials of mean force (PMF) of a permeating ion. However, a traditional method of computing an ion PMF in an ion channel, which uses umbrella sampling (US) molecular dynamics simulations with an ion position in the channel as a reaction coordinate, turns out to be inadequate for Mg2+. We demonstrate that this occurs due to the slow exchange rate of the first solvation shell ligands of Mg2+, causing significant under-sampling during each umbrella simulation. We thus developed an alternative reaction coordinate (RC) for the PMF of Mg2+ in a narrow channel, which tracks how the Mg2+ ion interacts with each of the six asparagine residues composing the NMDAR selectivity filter. From this alternative RC system, we generate a feasible path for the abstraction of Mg2+ from its binding position in the selectivity filter of NMDAR and hypothesize that the presence of a water reservoir above the selectivity filter provides an energetically stable pathway for the ion to unbind from the asparagine residues. Ca2+ and Mg2+ PMFs are compared to clarify the mechanism of the NMDAR ion selectivity.



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Notes

Proton paths in models of the Hv1 proton channel

Laue Tom

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Multivalent antibodies (Ab) and multivalent antigens (Ag) form large, insoluble networks when mixed at their equivalence concentrations (i.e. the concentrations of the antibody and antigen combining sites are equal). This property is at the heart of immune-precipitation, precipitin and Ochterlony assays. The equivalence concentrations for serum Ab:Ag interactions often fall in the physiological concentration range. Protein precipitation in serum leads to serious, even fatal, health problem. Experimental data show that Ab:Ag interactions that form very large complexes in vitro form much smaller complexes in serum. This observation is contrary to molecular crowding theory, where increased concentrations of a neutral crowding agent invariably drives complex formation. The possibility will be discussed that repulsive charge-charge interactions between Ab:Ag complexes and a like-charged background protein (albumin) may serve to block Ab:Ag precipitation.l

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Notes

Proton paths in models of the Hv1 proton channel

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The voltage gated proton channel (Hv1) plays an essential role in numerous biological processes, but a detailed molecular understanding of its function is lacking. The lack of reliable structures for the open and resting states is a major handicap. Several models have been built based on homologous voltage sensors and the structure of a chimera between the mouse homolog and a phosphatase voltage sensor, but their validity is uncertain. In addition, differing views exist on the mode of proton translocation, the role of specific residues, and the mechanism of pH effects on voltage gating. Here we use classical proton hopping simulations under a voltage biasing force to evaluate some of the proposed structural models and explore the mechanism of proton conduction. Some models proposed for the closed state allow proton permeation more easily than models for the open state. An open state model with a D112-R211 salt bridge (R3D) allows proton transport more easily than those with a D112-R208 salt bridge (R2D). However, its permeation rate seems too high considering experimental conductances. In all cases the proton permeates through a water wire bypassing the salt-bridged D112 rather than being shuttled by D112. Attempts to protonate the D112 are rejected due to the high energy gap. At the voltage that H+ permeates easily, Na+ does not, especially in the R2D models. Control simulations with a voltage sensor did not show proton permeation. Hydrogen bond connectivity graphs show a constriction at D112 but cannot discriminate open from closed states.

Notes

Modeling electrostatics and polarization effects in biomolecules: Methodology advancements and applications

Ray Luo

University of California, Irvine

Molecular simulation has become an important tool in modern computational chemistry and biochemistry. Nevertheless, accuracy and efficiency of the approach still need further improvement to achieve the goal of robust and predictive simulation, particularly for large and complex biomolecular systems. The accuracy issue arises from the intrinsic limitations of classical models that have to be used to approximate the quantum molecular processes. The efficiency issue is a direct consequence of the high dimensionality of biomolecular systems: sophisticated molecular machines are complexes of thousands to millions of atoms. What further complicates the picture is the need to realistically model the interactions between biomolecules and their surrounding water molecules. In this talk, I will review our recent developments of Amber electrostatics and polarization modeling methods and their applications to interesting biomedical systems from cancer biology.

Notes

Machuqueiro Miguel

Using computational methods to evaluate the role of tumor microenvironment acidity in multi-drug resistance

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Targeted cancer therapeutics remain a central goal of cancer research. The tumor microenvironment (TME) is an important component of tumor development that influences several key processes such as tumor cell phenotype, proliferation, immune evasion, and drug resistance [1]. An important feature of the TME is the increased acidity of the extracellular milieu (pH 6.0-6.8), which creates a pH gradient between the extracellular and intracellular environments, potentially creating a barrier for hydrophobic Lewis base drugs to enter the cells. The high pKa values of these compounds (7.5-9.5) including for example some tyrosine kinase inhibitors, like sunitinib and nintedanib, require them to first undergo deprotonation before passively diffusing through the plasma membrane into the cells, which may become more difficult in acidic microenvironments, like the TME.

This study aims at investigating the pH-dependent membrane insertion mechanism and quantify the impact of the TME on the membrane permeability of two well-known chemotherapeutics: sunitinib and nintedanib. We propose a new protocol based on Constant-pH Molecular Dynamics [2] coupled with an Umbrella Sampling scheme (US-CpHMD) [3] and applied it to the Lewis-base drugs interacting with a POPC lipid bilayer. The membrane permeability coefficients were calculated using the inhomogeneous-solubility diffusion model [4]. The calculations were performed at pH 7.5 to mimic a healthy cell, 6.0 to model the TME acidity, and 4.5 to capture the strong acidity of the lumen of the lysosome. The latter can provide some insights into the lysosomal sequestration phenomenon, which has been proposed as a drug resistance mechanism [1]. We have calculated the impact of acidity on the bioavailability of both sunitinib and nintedanib, which will help us design a new compound as a proof of concept that can circumvent these limitations.

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

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Notes

The domain decomposition X library for continuum solvation models

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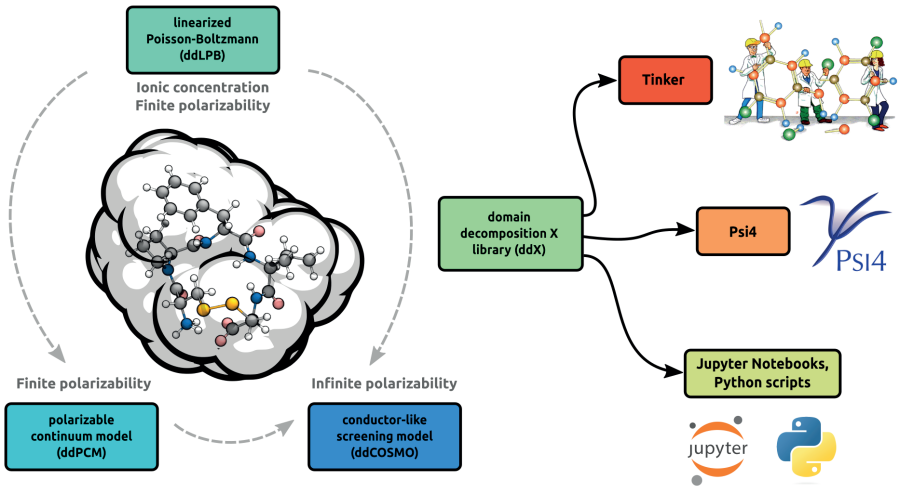
Continuum solvation models can be a simple yet effective strategy to describe the solvation of biomolecules[1]. These models have the advantage of being particularly simple to set up, as they require only a few parameters, and at the same time, they implicitly take into account the statistical sampling over the degrees of freedom of the solvent. In continuum solvation models, the electrostatic effect of the solvent can be modelled by describing the solvent as a homogeneous medium characterized by its dielectric permittivity and the ionic concentration. Depending on the values of the dielectric permittivity and ionic concentration, we distinguish different models, characterized by different equations. The most general case, with a finite dielectric permittivity and a nonzero ionic concentration, is the Poisson-Boltzmann solvation model, which is usually solved in its linearized version (LPB). If the ionic concentration is zero, the model is called polarizable continuum model (PCM), and finally, if the dielectric permittivity goes to infinity, the solvent screening effect is analogous to the one of a conductor and the model is called conductor like screening model (COSMO). These models introduce N-body interactions between the atoms of the system, and require solving a classical electrostatic problem to be able to fully characterize the effect of the environment on the solvated biomolecule. In general, the equations involved are quite simple and well known in the mathematical literature, but at the same time, the domain on which they are defined are rather complicated, thus making the problems difficult. An innovative idea to solve this kind of electrostatic problems is to use the domain decomposition technique, thus transforming them into a collection of coupled problems which are simpler because they are defined on simpler domains. In our research group, we implemented domain decomposition methods for COSMO[2], PCM[3] and LPB[4] using a common framework and a custom FMM library. The methods, the FMM library and various helper functions are collected in the domain decomposition X library (ddX)[5]. ddX is an open source library written in Fortran, with API in Fortran, in C and in Python. The library is available on GitHub, and the Python package can be installed through PyPI and Conda. The library is also coupled to the Tinker molecular mechanics package and to the Psi4 quantum chemistry package. In this talk, we start by presenting a quick overview of the theory and the main features of the library. The Fortran API is discussed by showing the Tinker-ddX interface, the Python API is discussed by showing how to quickly evaluate solvation energies in a Jupyter Notebook. Finally, to highlight the efficiency of ddCOSMO, we present an application in which the Tinker-ddX code was used to compute the solvation energies of viral capsids composed of up to several million atoms[6].



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Notes

Improving the sampling of CpHMD simulations by coupling with Replica-Exchange/Umbrella Sampling techniques

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Obtaining atomic level information on complex biological processes has always been an hard and expensive task for experimental methods. In silico techniques, such as Constant pH molecular dynamics (CpHMD)[1,2], have correctly described the pH effects on biological molecules and have provided crucial data on the dynamics and electrostatics of these systems.
With the increase in size and complexity of our simulation systems, sampling has become a serious problem. To overcome this limitation, we have successfully coupled CpHMD with an umbrella sampling scheme (US-CpHMD)[3]. This CpHMD extension allowed us to study a few interesting systems, but required the use of a high number of umbrellas and replicates to achieve good convergence. Therefore, to avoid such high computational cost, we propose here the implementation of a Replica Exchange scheme in the US-CpHMD method, where each umbrella is allowed to exchange the reference position with their neighbors. Using the membrane insertion of an Aspartate pentapeptide (A-A-D-A-A) as a test system, we aim at quantifying the sampling improvement of using an REUS-CpHMD method vs. US-CpHMD or simple unrestrained CpHMD simulations.

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Notes

Biologically relevant small variations of intra-cellular pH can have significant effect on stability of protein-DNA complexes

Alexey V. Onufriev

Virginia Tech

Stability of a protein-ligand complex may be sensitive to pH of its environment. We have explored stability of a set of protein-nucleic acid complexes using fundamental thermodynamic linkage relationship. The nucleosome, as well as an essentially random selection of 20 protein complexes with DNA or RNA, are included in the analysis.

An increase in intra-cellular/intra-nuclear pH destabilizes most complexes, including the nucleosome. We propose to quantify the effect by ΔΔG0.3 — the change in the binding free energy due to pH increase of 0.3 units, corresponding to doubling of the H+ activity; variations of pH of this amplitude can occur in living cells. We suggest, based on relevant experimental findings, a threshold of biological significance of 0.5kT: a change in the binding affinity above the threshold may have biological consequences. We find that for 70% of the examined complexes, ΔΔG0.3 is larger than the significance threshold. Thus, small but relevant variations of intra-nuclear pH of 0.3 may have biological consequences for many protein-nucleic acid complexes.

The binding affinity between the histone octamer and its DNA, which directly affects the DNA accessibility in the nucleosome, is predicted to be highly sensitive to intra-nuclear pH. Accessibility of the nucleosomal DNA is predicted to positively correlate with pH variations during the cell cycle; an increase in intra-cellular pH seen in cancer cells is predicted to lead to a more accessible nucleosomal DNA; a drop in pH associated with apoptosis is predicted to make nucleosomal DNA less accessible. We speculate that processes that depend on accessibility to the DNA in the nucleosomes, such as transcription or DNA replication, might become up-regulated due to relatively small, but nevertheless realistic increases of intra-nuclear pH.

References

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Notes

The Multi-Faceted Roles of Electrostatics in Biomolecular Simulation: What We Want to Aim For?

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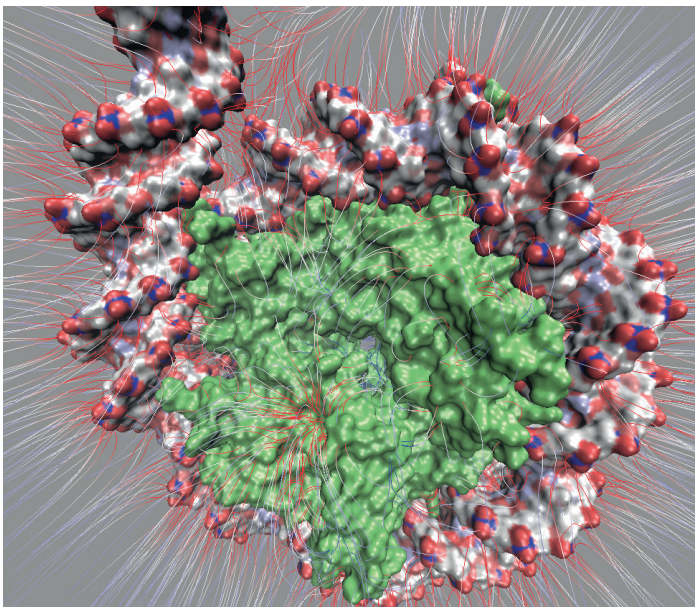
Microscopic electrostatic phenomena are widely studied in biology due to the great importance they have in describing molecular behavior in aqueous environments. The interest of the scientific community in this topic is actually still very high.[1] Recent advances in biomolecular simulations showed that it is possible to use electrostatics in many flavors, and to get interesting insights on relevant biomolecular processes[2-3]. Here, I will show some of them and what we are working on in order to push forward the research in this interesting field.[4-5]



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Notes

Computing the energetics of palmitate-BLG complex using constant-pH molecular dynamics

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Beta-lactoglobulin (BLG) is the most abundant protein in bovine milk whey. Several ligands can bind to BLG through a pH-regulated mechanism associated with a conformational change of a loop that acts as a gate to the binding site. This reversible conformational change occurs near pH 7, above which the gate opens, allowing ligands to bind or be released [1]. Among the various BLG ligands, the fatty acid palmitate holds a significant importance due to being the most abundant natural ligand found in bovine milk. Several measurements of the binding constants of palmitate-BLG complex have been performed, although a large variety of values arises due to different experimental conditions and BLG variants. In this work, we performed constant-pH molecular dynamics simulations at a pH range 3-8 and computed the binding free energies for both the monomeric and dimeric forms of BLG, using a linkage relation [2][3]. The dimerization free energy for the holo form was also computed and compared with the previously obtained value for the apo form [4]. The titrable residues that most contribute to the binding free energy were also identified.

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Notes

An in-silico study on the pH-dependent structure of cationic peptide dendrimers and their potential as vectors for siRNA

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Peptide dendrimers are symmetrical, tree-like molecules with a well-defined and uniform topology, composed of amino acid residues. They interact with several biological targets, such as nucleic acids and biological membranes, exhibiting activities as antimicrobial agents and being singular vectors for nucleic acids [1]. More recently, the use of such structures as vector molecules for siRNA molecules has been explored [2], which resulted in the identification of the dendrimers MH13, MH18, and MH47. These consist exclusively of lysine and leucine residues and contain as hydrophobic cores two palmitoyl chains or a leucine tetrapeptide. The hydrophobic cores increase their capability to interact with the cellular membrane and facilitate internalization via endocytosis. This is synergized by their different protonation states at physiological pH and low pH, which is vital for the interaction with the negative nucleic acid molecules, while also providing a way to escape the endosomal entrapment. Moreover, some mutations in MH18 where L-amino acids are replaced by their D-counterparts were detrimental to binding and activity with only the homochiral D-dendrimer reaching the same activity as the L-dendrimer. Despite a large number of experimental results, our understanding of the overall molecular mechanisms and the factors that govern the acquisition or loss of specific properties in these structures remains limited [3].
In this work, we will present our findings regarding the application of our state-of-the-art CpHMD methodology to the pH-dependent conformational space of MH13, MH18, and MH47 and other variants composed of different combinations of L and D-amino acids. We will present the pH titration behavior and structural characterizations, including the radius of gyration and the permuted root mean square deviation (RMSD). This study was done both in solution and with a lipid membrane model, which allowed us to assess the impact of the membrane on the conformational space and protonation behavior of the dendrimers. Altogether, these results will help experimentalists interpret their data and better understand the molecular mechanisms behind their properties to internalize cells and design new and improved sequences.

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Notes

Extending Stochastic Titration CpHMD to AMBER14SB Force Field for Enhanced Drug Discovery

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

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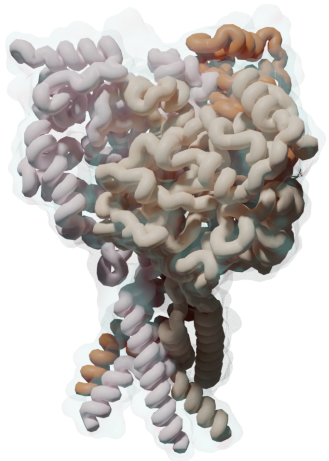

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Notes

Machine Learning Models for Proteome-wide Covalent Drug Design

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Currently, only a small fraction of human proteins have pharmaceutical modulators; there is a need to significantly expand the druggable proteome space using nontraditional approaches such as targeted covalent drug design. In this talk I will discuss our lab's progress in developing molecular simulation and machine learning (ML) tools towards prediction of cysteine-directed covalent ligandable sites in the entire human proteome. I will present the ML models trained on the X-ray structural data and external validated against the proteomic data using cell lines.

Notes

Computational enzyme design

Thomas Simonson

Ecole Polytechnique

Methionine gamma-lyase (MGL) breaks down methionine, with the help of its cofactor pyridoxal-5'-phosphate (PLP), or vitamin B6. Methionine depletion is damaging for cancer cells but not normal cells, so that MGL is of interest as a therapeutic protein. We have used MD and free energy simulations to elucidate the structure and function of its active site, including PLP protonation states, to help guide engineering efforts. High throughput enzyme engineering can be done using computational protein design, using a physics-based energy function and adaptive landscape flattening Monte Carlo simulations. Validation is presented for a different enzyme, methionyl-tRNA synthetase, where predictions were confirmed by blind experimental tests. The method was then used to engineer activity for a nonnatural substrate, beta-methionine, in view of genetic code expansion.

Notes

A dipole model explains secondary pore formation in the c-ring of F1Fo ATPsynthase

Abhishek Singharoy

Assistant Professor, School of Molecular Science, Biodesign Institute, Arizona State University

It has long been a matter of contention whether the c-ring of mammalian ATP synthase operates as a proton channel. While a number of imaging and conductivity experiments have alluded to this possibility, implicating c-ring as part of the enigmatic membrane permeability transition complex, past molecular simulations from yeast c-ring has brought forth apparently contradictory arguments. Armored with the latest structural evidence from the Sazanov team (at IST Austria) and a number of new mutational assays and patch clamp experiments by the Mnatsakayan team (Penn State & Yale), we employ a combination of continuum electrostatic computations, voltage-driven all atom molecular dynamics, Brownian dynamics and adaptive biasing force calculations on exacale computers to determine the impact of c-ring's protonation equilibrium on its expansion, pore formation and channel activation. These simulations bring forth an experimentally verified voltage-gated dipole reorientation mechanism for c-ring activation that we have simulated both an isolated Fo unit, and for the very first time, within an entire ATP synthase. Our model sheds fundamental insights on how electrostatics has guided evolution of c-ring geometries to be pH vs voltage dependent.

Notes

Characterization of a stable and specific peptide inhibitor for insulin-degrading enzyme as for diabetes treatment

Yossi Tsfadia and Dan Frenkel

Tel Aviv University

Insulin-degrading enzyme (IDE) is a zinc-metalloprotease that cleaves Insulin and many other substrates like glucagon, amylin and TGFa, yet insulin has the highest affinity to IDE. Inhibition of IDE has a potential to be an effective anti-diabetic therapy since it affects insulin degradation. However, it has not yet evolved into a medical intervention, mainly because most developed inhibitors target the zinc in IDE's catalytic site, potentially causing toxicity to other essential metalloproteases. Since IDE is a cellular receptor for the varicella-zoster virus (VZV), we constructed a VZV-based peptide inhibitor. Using Molecular Dynamics simulations, we computationally characterized the peptide interaction site with IDE, showing that the peptide specifically binds inside IDE's central cavity, however not in close proximity to the zinc ion. We demonstrated effectiveness of our designed peptide in in vitro studies and in vivo mouse models by ameliorating insulin-related defects in type1 and 2 diabetes. We believe that this inhibitor can be a valuable addition to the current arsenal of anti-diabetic therapies through its ability to reduce insulin degradation.

Notes

From Monomers to Polymers: Unraveling the Early Stages of Protein Aggregation

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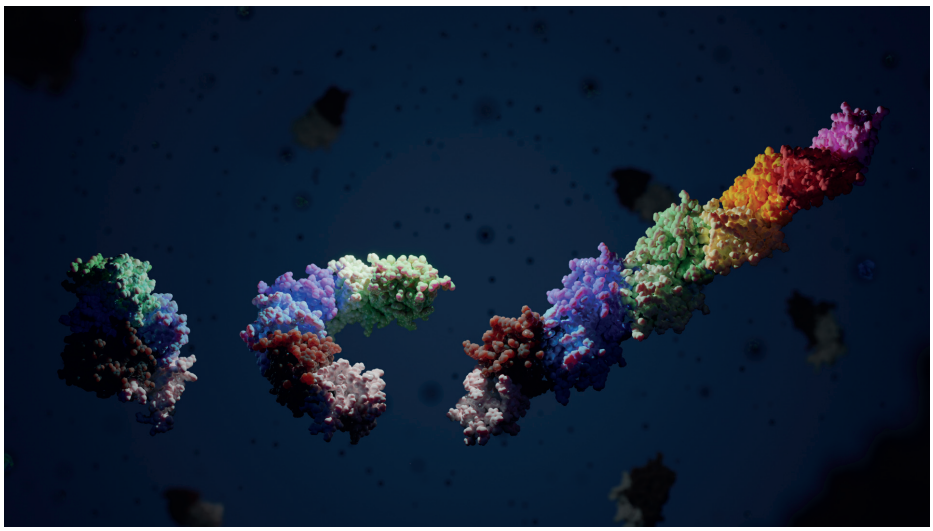
Protein aggregation is a defining trait of many conformational diseases, such as Parkinson's and Alzheimer's disease [1]. These processes can start with an increased propensity of a monomeric species towards self-association, forming dimers, and subsequently promoting the formation of potential polymer-like chains [2,3]. In Dialysis Related Amyloidosis (DRA), aggregation of protein beta-2 microglobulin (B2M), occurs in patients undergoing long-term hemodialysis. This work focuses on the aggregation-prone monomeric state I2 populated by the D76N mutant of B2M [4], s. Monte Carlo Ensemble Docking (MCED) and Molecular Dynamics (MD) simulations were employed to generate and assess the stability and binding affinity of various I2 dimer configurations [3]. An in-house implementation of the Molecular Mechanics Poisson Boltzmann Surface Area Method (PyBindE, available at: https://github.com/mms-fcul/PyBindE) was utilized to calculate binding energies and determine key binding forces. Stable binding interfaces with crucial residues were identified. Clustering protocols and extended MD simulations confirmed the stability and growth potential of select binding modes. Additionally, we explore the plausibility of polymerization, using a novel simple polymer growth model based on a minimal representation of binding interfaces, that is capable of predicting several types of unlimited and limited growth modes. Our comprehensive framework provides valuable insights into the growth potential and stability of B2M binding interfaces, shedding light on the mechanisms underlying protein aggregation in conformational diseases. Moreover, the universalityof the methodologies employed in this study offers a versatile toolset for probing protein dynamics in a broader scientific context [5].

We acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through Grant CEECIND/02300/2017 and projects PTDC/FIS OUT/28210/2017, UIDB/04046/2020 & UIDP/04046/2020



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Notes

Electrostatic and electrodynamic fields in lipid bilayer membranes

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Lipid bilayer membranes are complex, dynamic, and functional structures composed of a wide diversity of lipids, proteins, small molecules, and water organized in heterogeneous domains through noncovalent interactions. The structure and motion of these molecules generate large electric fields within the interior of the membrane that are critical to membrane structure and function. Here, we describe how vibrational spectroscopy of unnatural nitrile chromophores places throughout the membrane structure is used to measure electrostatic fields in peptides intercalated in free-standing lipid bilayer membranes of increasing chemical complexity. In combination with electrodynamics simulations, these experiments highlight how common small molecules such as cholesterol dramatically affect membrane structure and dynamics through large changes to membrane electric fields.

Notes

Zuzic Lorena

A pH-dependent cluster of charges in a conserved cryptic pocket on flaviviral envelopes

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Flaviviruses are enveloped viruses which include human pathogens that are predominantly transmitted by mosquitoes and ticks. Some, such as dengue virus, exhibit the phenomenon of antibody-dependent enhancement (ADE) of disease, making vaccine-based routes of fighting infections problematic. The pH-dependent conformational change of the envelope (E) protein required for fusion between the viral and endosomal membranes is an attractive point of inhibition by antivirals as it has the potential to diminish the effects of ADE. We examined six flaviviruses by employing large-scale molecular dynamics (MD) simulations of raft systems that represent a substantial portion of the flaviviral envelope. We utilised a benzene-mapping approach that led to a discovery of shared hotspots and conserved cryptic sites. A cryptic pocket previously shown to bind a detergent molecule exhibited strain-specific characteristics. An alternative conserved cryptic site at the E protein domain interfaces showed a consistent dynamic behaviour across flaviviruses and contained a conserved cluster of ionisable residues. Constant-pH simulations revealed cluster and domain-interface disruption under low pH conditions. Based on this, we propose a cluster-dependent mechanism that addresses inconsistencies in the histidine-switch hypothesis and highlights the role of cluster protonation in orchestrating the domain dissociation pivotal for the formation of the fusogenic trimer.

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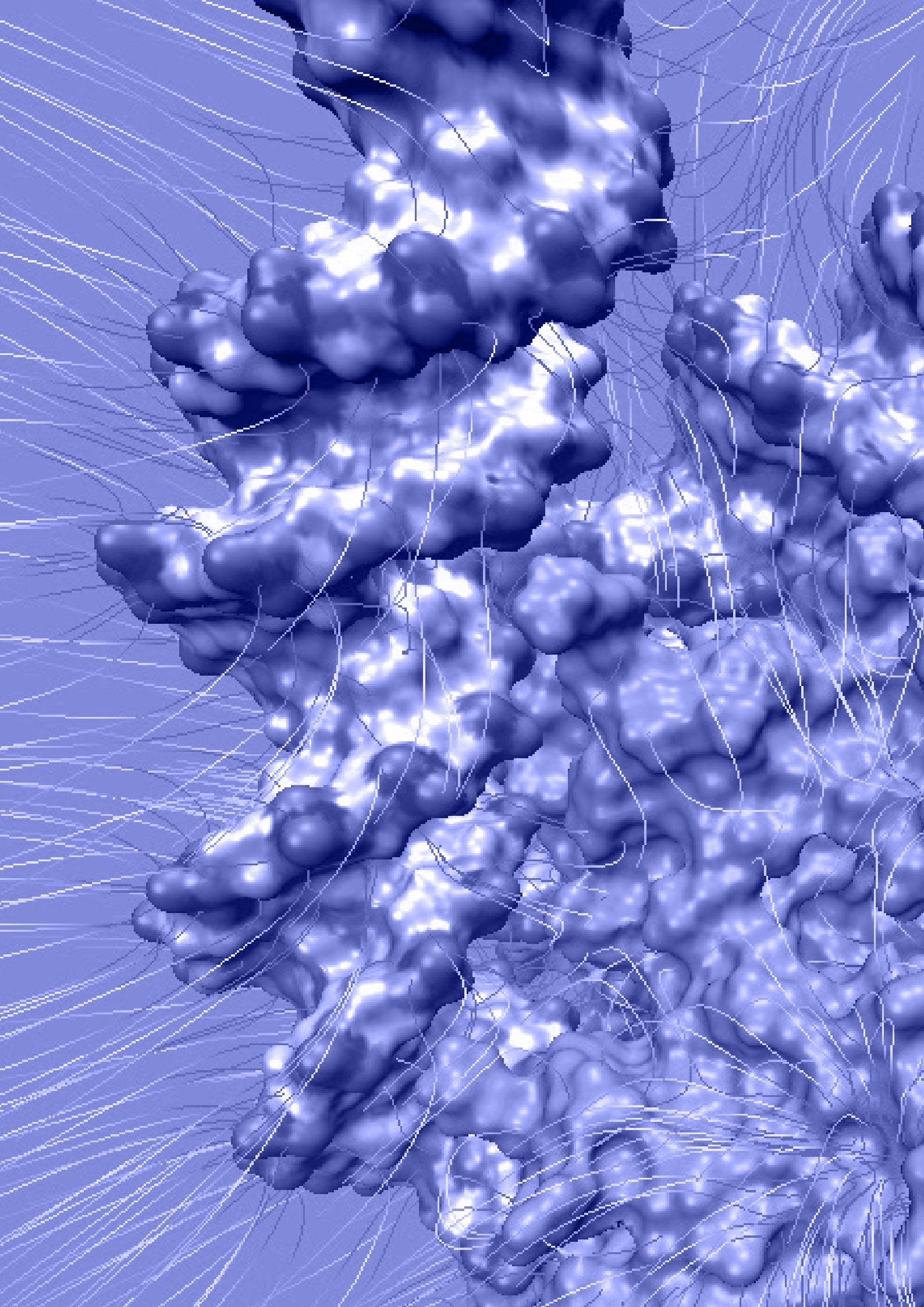
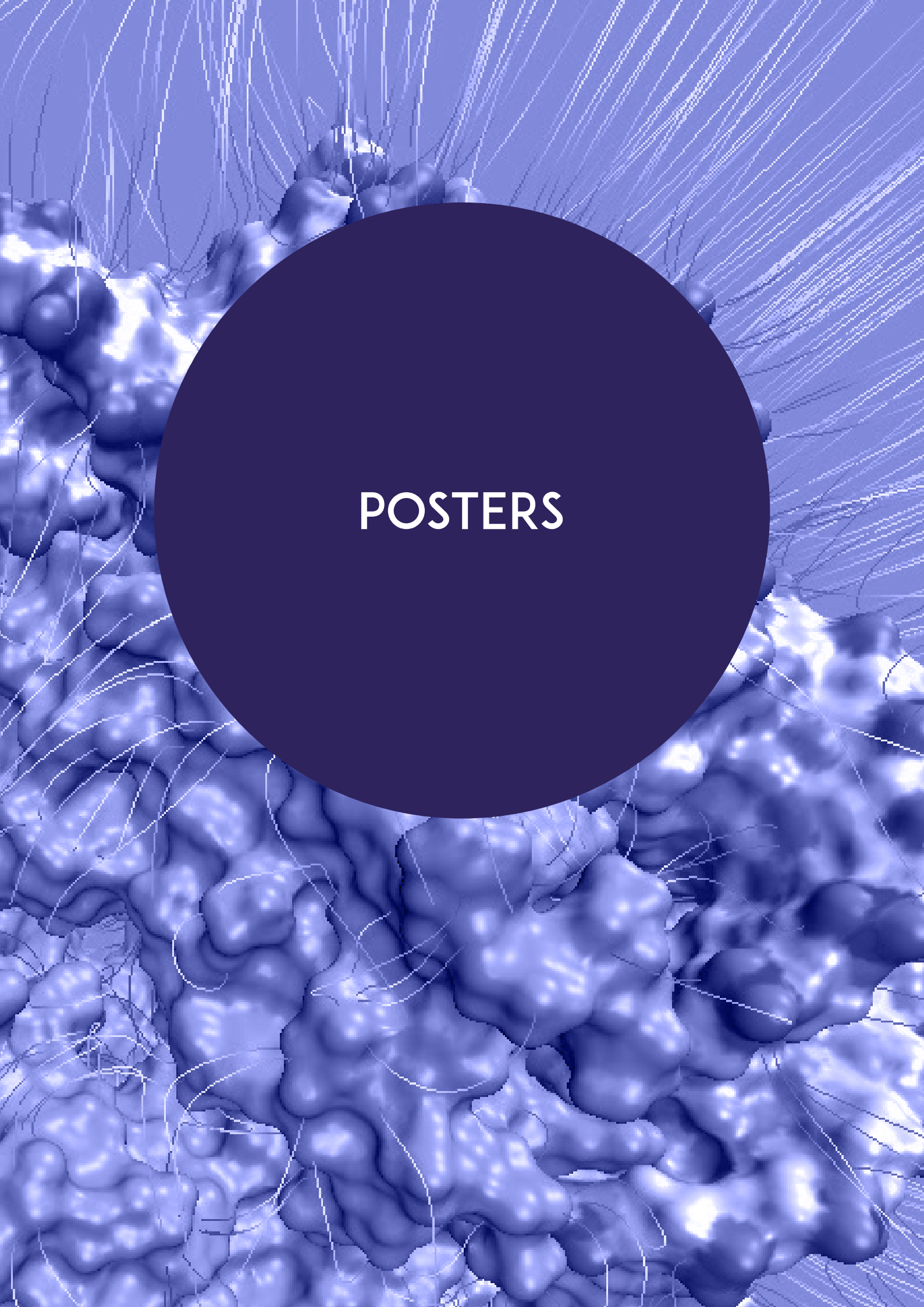
Lorena Zuzic, Jan K. Marzinek, Ganesh S. Anand, Jim Warwicker, Peter J. Bond (2023) A pH-dependent cluster of charges in a conserved cryptic pocket on flaviviral envelopes. eLife 12:e82447. DOI: https://doi.org/10.7554/eLife.82447.

Notes





POSTERS

Efficient and Scalable implementation of a Linearized Poisson-Boltzmann Solver on Hierarchically refined Cartesian Meshes

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Most existing solvers for the PBE adopt either i) tensor product cartesian grids, or ii) adaptive, simplicial, conforming meshes. Both options have distinctive advantages and limitations. In particular, the former can be implemented with high memory efficiency, and it produces matrices with some very desirable structural properties, but demands a very large number of degrees of freedom to achieve the required spatial resolution. The latter, on the other hand, allows for achieving good geometrical resolution with a limited number of additional degrees of freedom. However, it requires much more complex data structures for storing the mesh, which results in a larger memory footprint and more complex partitioning algorithms for parallelization. In this work, we present a prototype of an efficient and scalable implementation of a PBE solver, based on hierarchically refined cartesian meshes, that tries to take the benefits of both approaches above while limiting the corresponding issues. We show through numerical experiments that the resulting solver allows improving the accuracy-per-degree-of-freedom with respect to tensor product cartesian grid. Moreover, it can still be represented and stored in simple data structures that allow for the use of highly effective parallel partitioning, traversal, and balancing algorithms. Therefore, we obtain an overall better parallel scaling performance.

Notes

Circular dichroism study of the pH effect on the induction of α-helix in the intrinsically disordered region of the viral phosphoprotein responsible for binding to the M2-1 transcriptional antitermination factor of hRSV

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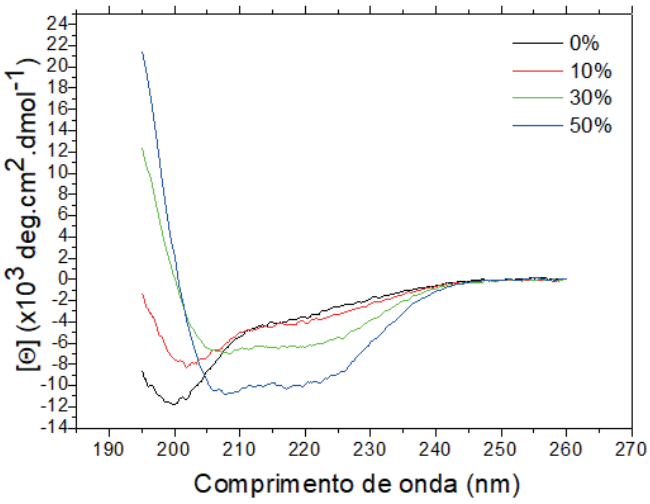
The human Respiratory Syncytial Virus (hRSV) is one of the main causative agents of acute respiratory infections in newborns. During the replication cycle of this virus, a crucial interaction occurs between the M2-1 antitermination factor and the P phosphoprotein, specifically between the core domain of M2-1 (cdM2-1) and the intrinsically disordered region (IDR) of residues 90 -110 from P (P90-110). The present work aimed to characterize the effect of pH on the induction of the α-helix of the IDR P90-110 responsible for the interaction with the hRSV M2-1 protein. For this purpose, the formation of the α-helix was induced by titrations of dgM2-1 at pH 7 and 2,2,2-trifluoroethanol (TFE) at different pH conditions (from 3 to 10), and this formation was investigated by the technique of Circular Dichroism (CD) spectroscopy. The results show that the TFE titration provided an increase in the negativity of the molar ellipticity at 222 nm, indicating the formation of an α-helix in the P90-110 peptide structure characterized by a cooperative transition. At acidic pHs, especially at pH 4 (isoelectric point), the conformational transition from random structure to α-helix induced by TFE showed greater cooperativity than at basic pHs. Considering a two-state equilibrium to model the peptide-water-TFE interactions, the value of the thermodynamic parameter m, which describes the effectiveness of the TFE solvent in interconverting between the helix and random coil structure states in terms of solvent exposure, presents clear evidence of increase with the acidification of the buffer solution, indicating a greater propensity for the formation of a secondary structure in an α-helix in the conformation of the P90-110 peptide. The difference spectra between the CD-UV signal of the P90-110/dgM2-1 complex and free dgM2-1 report a gain in secondary structure in α-helix of P90-110 in the interaction with dgM2-1. In the interaction with dgM2-1, the value of m corroborates with the thermodynamic parameters determined for acidic pH, mainly at pH 4, in the induction of the promoted α-helix conformation by TFE. Based on the results of peptide titrations with TFE and dgM2-1, it can be suggested that the interaction interface between P90-110 and dgM2-1 is a microenvironment with more acidic characteristics that favor the protonation of negatively charged residues.

Keywords: intrinsically disordered region, phosphoprotein, M2-1 protein, hRSV, circular dichroism



Notes

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

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Membrane permeability plays a crucial role in many biological processes, directly affecting the ADMET properties of drugs, thus being paramount in rational drug design. Several factors influence the permeability of small molecules, including size, charge, and lipophilicity. The pH [1] and the existence of chemical groups prone to establish specific noncovalent interactions are also core parameters that impact this biological event. Among noncovalent interactions the impact of the ubiquitous hydrogen bond is usually addressed, however, the less studied halogen bond might also impact membrane permeability owing to the existence of halogen-membrane recognition phenomena mediated by those interactions [2].

In this communication, we report the first steps of a study of the permeation mechanism of cobimetinib for which both pH and halogen bonding are important. This compound is an anti-cancer drug used to treat patients with melanoma and comprises a halogen atom (iodine) and a titrable group (Lewis base) in its structure. Our workflow started with the parameterization of the molecule by employing quantum-chemical calculations to generate the RESP charges taking into account the anisotropic features of the halogen by using an extra point (EP) of charge. In the following step, we employed constant-pH molecular dynamics (CpHMD) simulations in solution to calibrate the compound's pKa values (with and without EP). This allowed us to perform further CpHMD simulations in a lipid bilayer (POPC), namely, classic unrestrained MD and Umbrella Sampling (US) simulations to obtain relevant sampling across the entire membrane aiming at calculating the membrane permeability coefficient values. By performing these calculations at normal cell pH (~7.4) and tumor cell acidic conditions (pH~6.2), we will evaluate the impact of tumor acidosis in the compound membrane passive diffusion while also evaluating the role of halogen bonds in the overall process.

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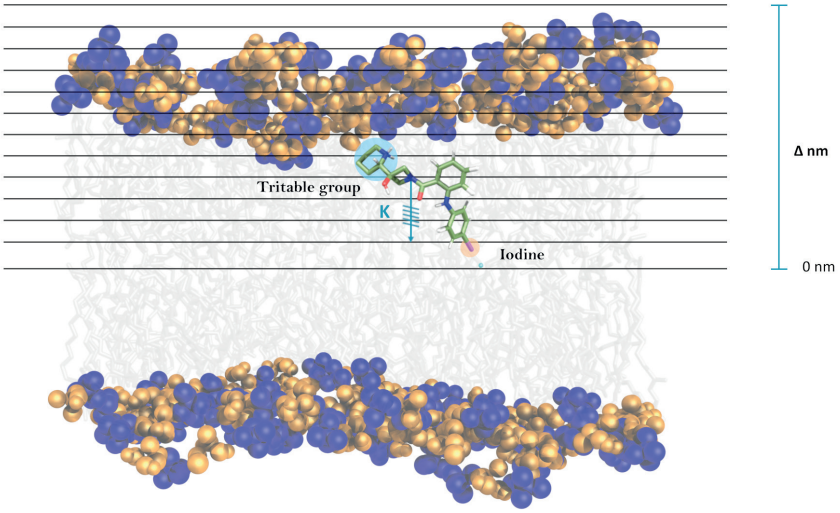

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Notes

Guerra Rita

Development of a computational protocol to evaluate tyrosine kinase inhibitor derivatives with improved membrane permeabilities

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Despite tumor multi-drug resistance (MDR) being multi-factorial in nature [1], it has been proposed that the acidity in the lumen of lysosomes (pH~4.5-5) and tumor microenvironment (pH~6.2-6.8) play a significant role hindering the anti-cancer activities of hydrophobic weak base drugs (Lewis bases; pKa~7.5-9.5), by efficiently entrapping/excluding them, via protonation events [2]. Some of these compounds, the tyrosine kinase inhibitors (TKI), exhibit high and complementary clinical relevance by being vital mediators of signal transduction and cancer cell proliferation, angiogenesis, and apoptosis [3]. We have developed a computational strategy to chemically modify this class of TKI molecules and exchange the cationic amino groups with anionic ones. The rationale is that the anionic group should also have good solubility in the aqueous media and, in contrast to the weak base, have its membrane permeability increased with acidity. These acidic derivatives should selectively target cancer cells over normal tissues and effectively evade lysosomal sequestration, circumventing several crucial factors related to MDR.

In this study, we are optimizing a molecular docking protocol based on different search methods and scoring functions to study systematically the impact of replacing cationic groups found on TKIs with negative chemical building blocks on the binding to RTKs. This chemical modification strategy will allow us to simultaneously improve the druggability of such compounds, and evaluate the impact on the affinity to their therapeutical targets. We use a consensus docking approach to combine the score and/or rank of various freely available docking suits, including Autodock 4.2, Autodock-GPU, Autodock Vina 1.2, and Dock 6.10, to achieve the best results.

The authors acknowledge Fundação para a Ciência e Tecnologia (FCT) for funding through projects UIDB/04046/2020 and UIDP/04046/2020 and grants CEECIND/02300/2017 and 2021.06409.BD.

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Notes

Constant pH calculation on Agp1

Oanh Tu Hoang, Maria Andrea Mroginski

Technical University berlin

Phytochromes are red-light photoreceptors in plants, funghi and bacteria. When radiated with (far) red-light they undergo a photocycle. This reaction is responsible for growth control, seed germination and movement. Although their functions are known, the mechanism of the signal transduction remains unknown. Phytochromes utilize a chromophore, a linear methine-bridged tetrapyrrole, to absorb light. After absorption a series of structural changes in the protein take place, as well as proton transfer processes involving the chromophore and titrable residues in the protein. In order to get a better understanding of the photoconversion reaction it is essential to determine the pKa value of the chromophore and the titrable amino acids. Thus, we use the constant pH molecular dynamic method to calculate the pKa values. Additionally, this method is also taking the conformational changes into account.

Notes

Hristova Svetlana

Protein electrostatics, intermolecular interaction and anticancer effect of cytochrome C adsorbed on montmorillonite nanosheets

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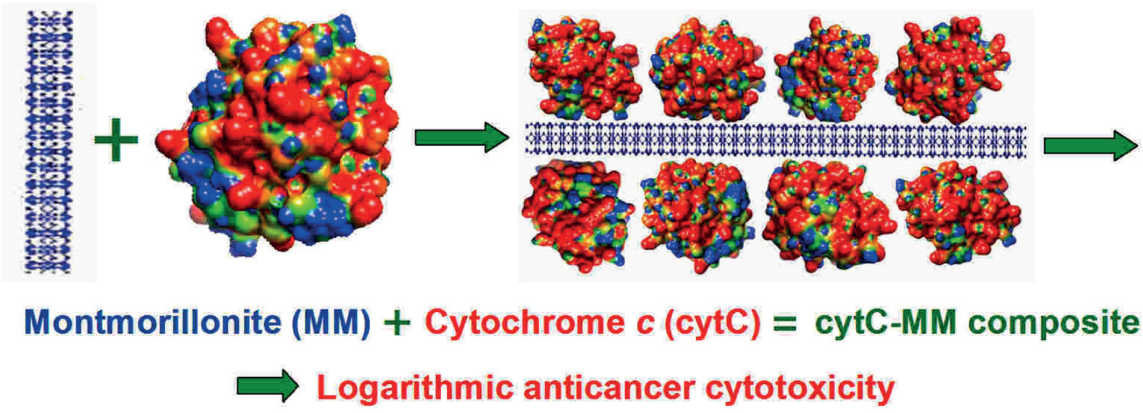
Cytochrome C is a globular protein, part of the respiratory chain of mitochondria and a molecule triggering the intracellular pathway of programmed cell death (apoptosis). By means of protein electrostatics and a combination of physicochemical methods (microelectrophoresis, static light scattering and light scattering in an electric field, isoelectric focusing), the charge properties of the protein, its isoelectric point and 3D electrostatic potential were investigated [1 , 2]. The variation of these properties upon adsorption of cytochrome C on montmorillonite nanoplates was investigated and the formation of protein dimers and trimers on the negatively charged surface of the mineral (cooperative effect) and maximum saturable adsorption was found [3]. The exogenous introduction of cytochrome C by montmorillonite nanoplates (as a drug delivery system) into metastatic cancer cells induced cell death, with a 96 hours' viability from treatment of only 4% [4, 5]. Inorganic particles (free or incorporated into composite hydrogels) are often used as carriers for various drugs, including anticancer chemotherapeutics [6]. The selective action of the protein-mineral complexes is due to the ability of cancer cells to phagocytose colloidal-sized particles, which is not characteristic of the vast majority of healthy cells in the human body.
This research was funded by Bulgarian National Science fund, grand K_-06-H69/4-2022.



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Notes

How protein pKa predictions are affected by the choice of experimental structure?

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pH can have a significant impact in protein structure and function which motivated the development of simple pKa predictors that help assign the most abundant protonation states at a given pH value. Several computational protocols require this user-defined step which is a common source of errors. There are many pKa predictors, but all require an experimental structure of the protein that ideally should be representative of its conformational ensemble in water. However, the conditions under which these structures are determined may be significantly different from those needed for the computational protocol and this mismatch can lead to important errors in the pKa predictions.

In this work, we aim to study how different initial structures impact the pKa prediction of the same proteins. We selected structures with different resolutions, obtained under different crystallization conditions (pH and co-adjuvants), and solved with different methods (X-ray and NMR). We calculated the protein pKa values using the PypKa tool/server [1] and performed a systematic analysis of the results. We will report our preliminary data on this benchmark and assess how each of these properties is impacting the predictions. This can also provide pointers for making informed decisions when picking an initial structure for Molecular Docking, MD, and even CpH-MD simulations.

The authors acknowledge financial support from Fundacao para a Ciencia e a Tecnologia through grant CEECIND/02300/2017 and projects UIDB/04046/2020 and UIDP/04046/2020.

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Notes

In silico structural analysis of the grapevine serine protease VviSBT4.19 involved in defense against P. viticola

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In 2022, Portugal was the fifth largest wine producer in the EU, making grapevine one of the key components of the Portuguese economy. However, grapevine pathogens, such as Plasmopara viticola, the downy mildew disease-causing agent, pose a significant threat to grape and wine production. To mitigate the use of heavy pesticide applications, and their resulting environmental and health risks, it is essential to understand how tolerant grapevine species recognize and are able to mount a successful defense response against P. viticola (incompatible interaction). Previous research has shown that serine proteases, namely grapevine subtilase VviSBT4.19, play a crucial role in the establishment of the incompatible grapevine-P. viticola interaction, due to its high expression both in resistant genotypes and after pathogen infection. However, a comprehensive understanding of protease activity modulation by P. viticola is lacking, most likely due to a lack of a good structural model.

In this work, we built a computational model for this protein based on both AI (ESM Atlas) and homology modeling (Modeller) approaches. We performed Molecular Dynamics (MD) and Constant-pH MD simulations to study the model structure's overall stability. Among other more common structural analyses, we also focused on the relative positioning of the residues that comprise the active site and the catalytic triad, to evaluate its topology and functionality. Efforts are also being pursued to study the interactome of VviSBT4.19 and its role in plant immunity. The findings from this study will help to better understand the molecular details of grapevine defense against P. viticola and may help the development of novel strategies to enhance disease resistance in grapevine cultivars.

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Notes

Suzano Pedro

MembIT - a Powerful Tool for Analyzing Solute Membrane Insertions and Deformations in MD Simulations

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In MD simulations of membrane systems, it is common for the lipid bilayers to deform when interacting with small molecules or large transmembrane proteins. To fully characterize these interactions, the properties of solutes and membrane need to be accurately described.

Two of the main properties of interest when analyzing these systems are the local monolayer thickness and the compound's membrane insertion, which directly result from the membrane-compound interactions. Membrane insertion is generally calculated by comparing the z-position of the compound of interest with the average position of the phosphate head groups of the interacting leaflet. Monolayer thickness is usually determined by halving the difference between the average z-positions of the phosphate head groups in both monolayers. However, membrane deformations can influence the position of the local reference atoms surrounding the compound, causing these properties to no longer be accurately estimated using the average z-position of the phosphate head groups as references. To account for these local deformations, the membIT [1] tool makes a distinction between local (in close proximity) and bulk lipids (further away from the solute), which allows for the calculation of the average position of undisturbed phosphate head groups. Consequently, the local monolayer deformation calculations become straightforward, as the difference between bulk and local lipids head group positions. The solute membrane insertion calculations can also take advantage of this strategy by using the affected phosphate group atoms as reference, while ignoring the unperturbed bulk lipids.

The membIT tool application is showcased here with example systems, namely that of inserting compounds like sunitinib [2] and the pHLIP peptide [3], and a large transmembrane protein of the ABC transporter family, the ATP/ADP carrier [4].

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Notes

The electrostatic differences among mammalian haemoglobin proteins

Isabel Vinterbladh and Mikael Lund

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Experimental studies of the haemoglobin protein from different mammals have been carried out, studying the protein hydrophobicity-hydrophilicity, its primary structure and erythrocyte water content (see P. Bogner et. al. [1]). The study concluded that haemoglobins from mammals living in arid environments contained more charged amino acids, tended to be more hydrophilic and showed a greater osmotic resistance. Meaning that the haemoglobin of the desert animal camel contains more charged amino acids compared to human haemoglobin or that of mammals living in a more fertile environment.

This work will further investigate the electrostatic differences of different mammalian haemoglobin proteins, the isotropic-anisotropic effects and the osmotic properties. Computational methods such as Monte Carlo, one-body and many-body, simulations will be utilized and developed for this cause. A simplified amino acid model of the protein structures has been developed to employ in the simulations, where the haemoglobin PDB-structure is used as input. For the haemoglobins where no PDB-structure exists, the AlphaFold2 ColabFold notebook [2] is used for obtaining three dimensional protein structures. The aim of the project is to evaluate how the charge distribution of the different mammalian haemoglobins affect the protein electrostatics and interactions in crowded environments.

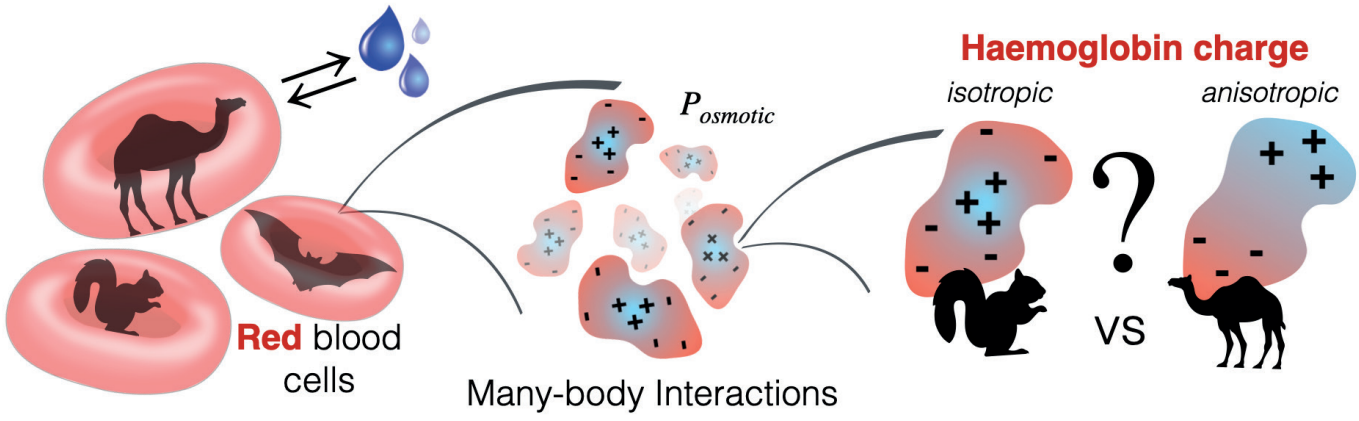

Figure 1: Illustration of project approach and of the charge distribution in haemoglobin for different mammals.

References

[1] Bogner P, Csutora P, Cameron IL, Wheatley DN, Miseta A. Augmented water binding and low cellular water content in erythrocytes of camel and camelids. Biophys J. 1998 Dec;75[6]:3085-91. doi: 10.1016/S0006-3495(98)77749-5\\
[2] Mirdita, M., Schütze, K., Moriwaki, Y. et al. ColabFold: making protein folding accessible to all. Nat Methods 19, 679-682 (2022). https://doi.org/10.1038/s41592-022-01488-1

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