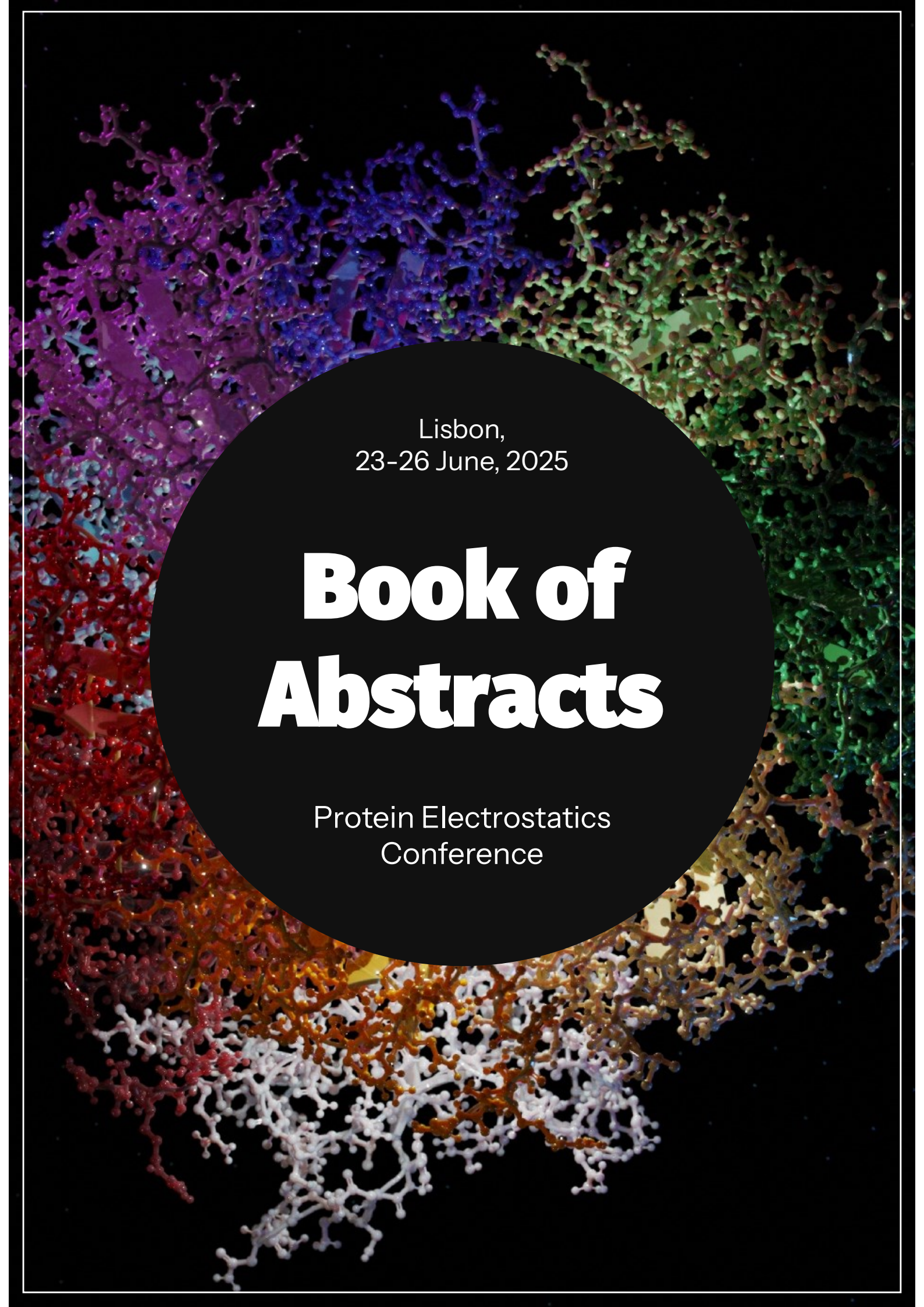

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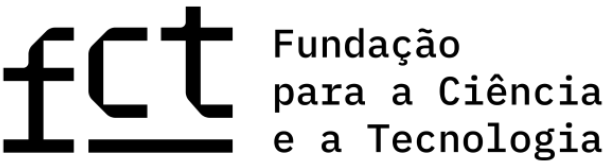
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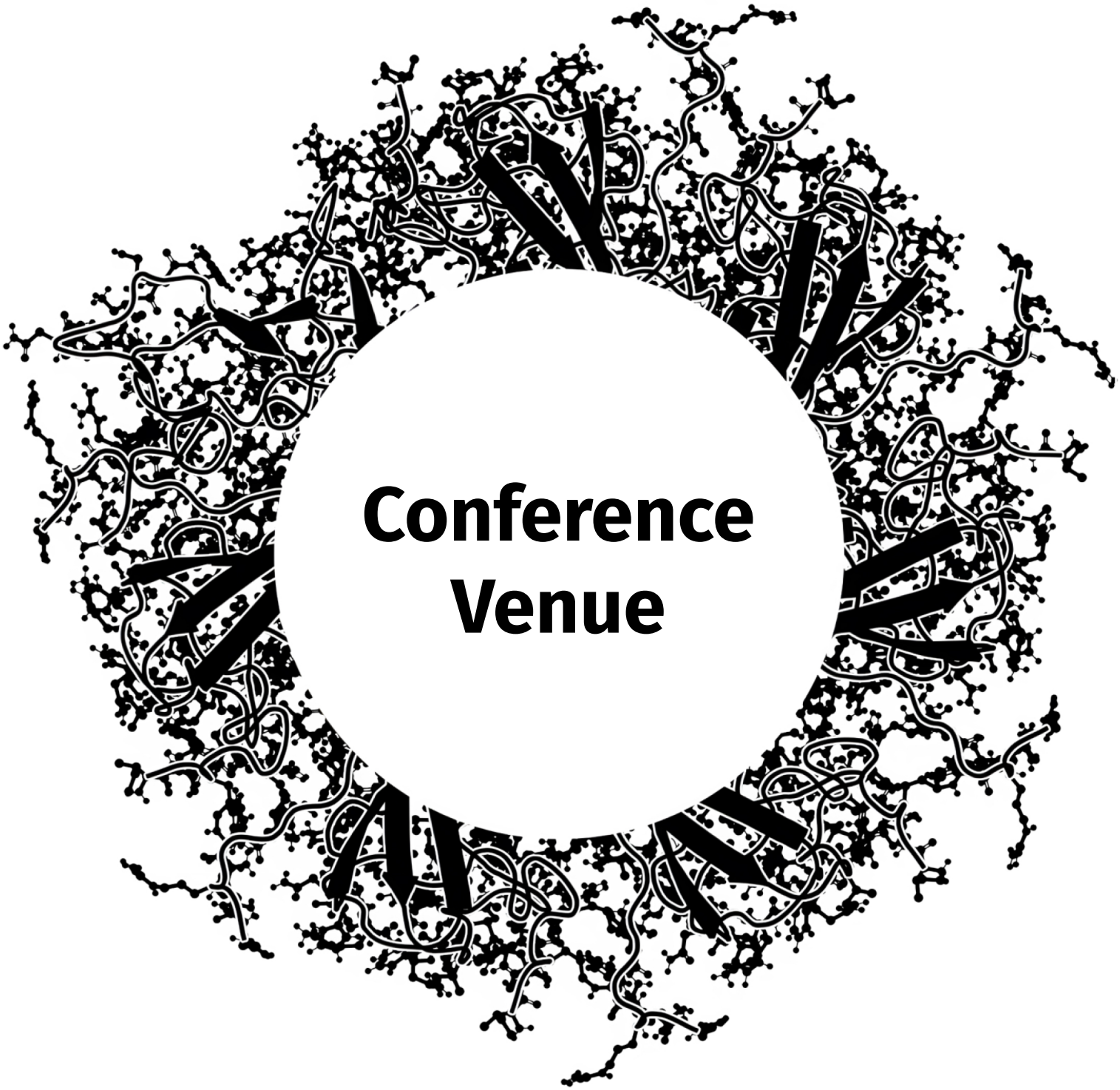
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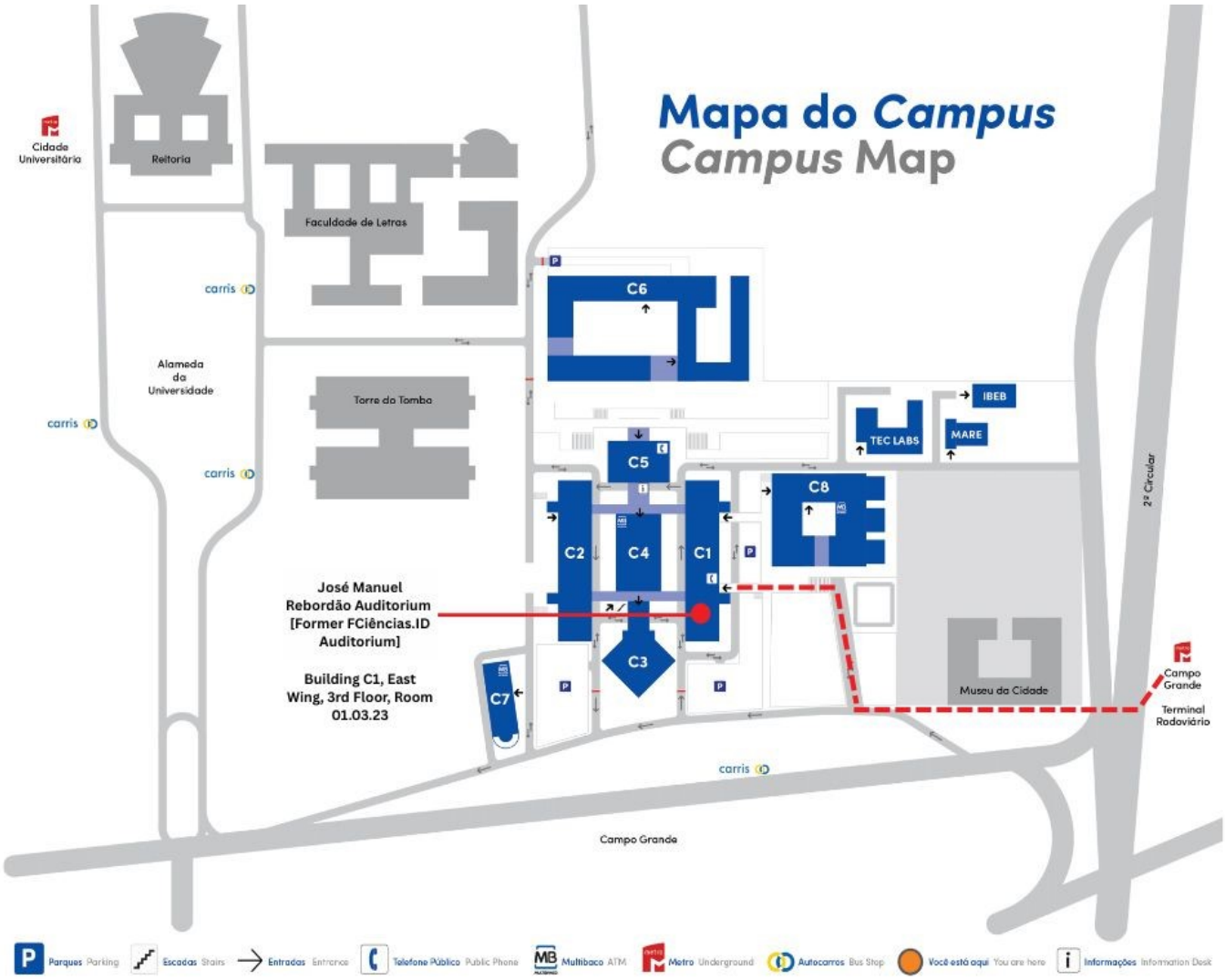
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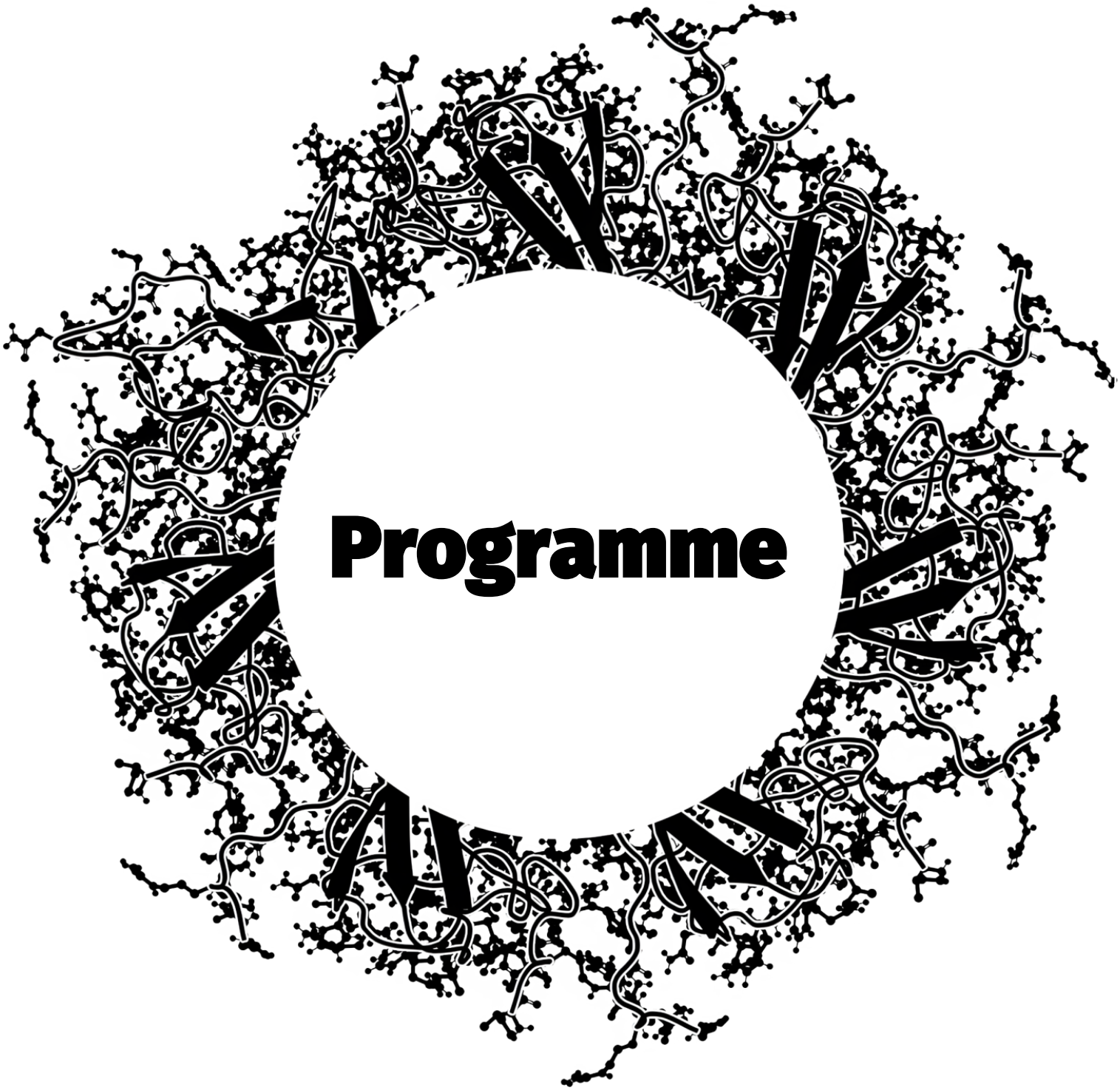

Programme

Monday 23rd

8h00 - 9h00	Registration	
9h00 - 9h30	Diane Barber	**How Intracellular pH Dynamics Regulates Cell Behaviors: Identifying Endogenous pH Sensors**
9h30 - 10h00	Marilyn Gunner	**MCCE microstates: What can be learned from Monte Carlo microstates**
10h00 - 10h30	Coffee Break	
10h30 - 11h00	Tomás Fernandes da Silva	**Finding the Missing Protons: Constant-pH Simulations from Oligomers to Ribozymes**
11h00 - 11h30	Ana-Nicoleta Bordar	**Protonation- and lipid-coupled hydrogen-bond networks of a proton channel**
11h30 - 12h00	Elias J. Fernandez	**On the Interactions with Distal 5'-flanking DNA and the Control of Transcription by Nuclear Hormone Receptors**
12h00 - 14h00	Lunch	
14h00 - 14h30	Maria Kurnikova	**Active Learning Driven Hit Mining and Optimization Based on Molecular Dynamics Simulated Free Energies**
14h30 - 15h00	Muhamed Amin	**Computational Estimation of Protein Polarizability Using Empirical and Machine Learning Methods: Applications in Alignment, Dielectric Prediction, and Drug Design**
15h00 - 15h30	Poster Pitch Session	
15h30 - 17h00	Coffee Break & Poster Session	

Tuesday 24th

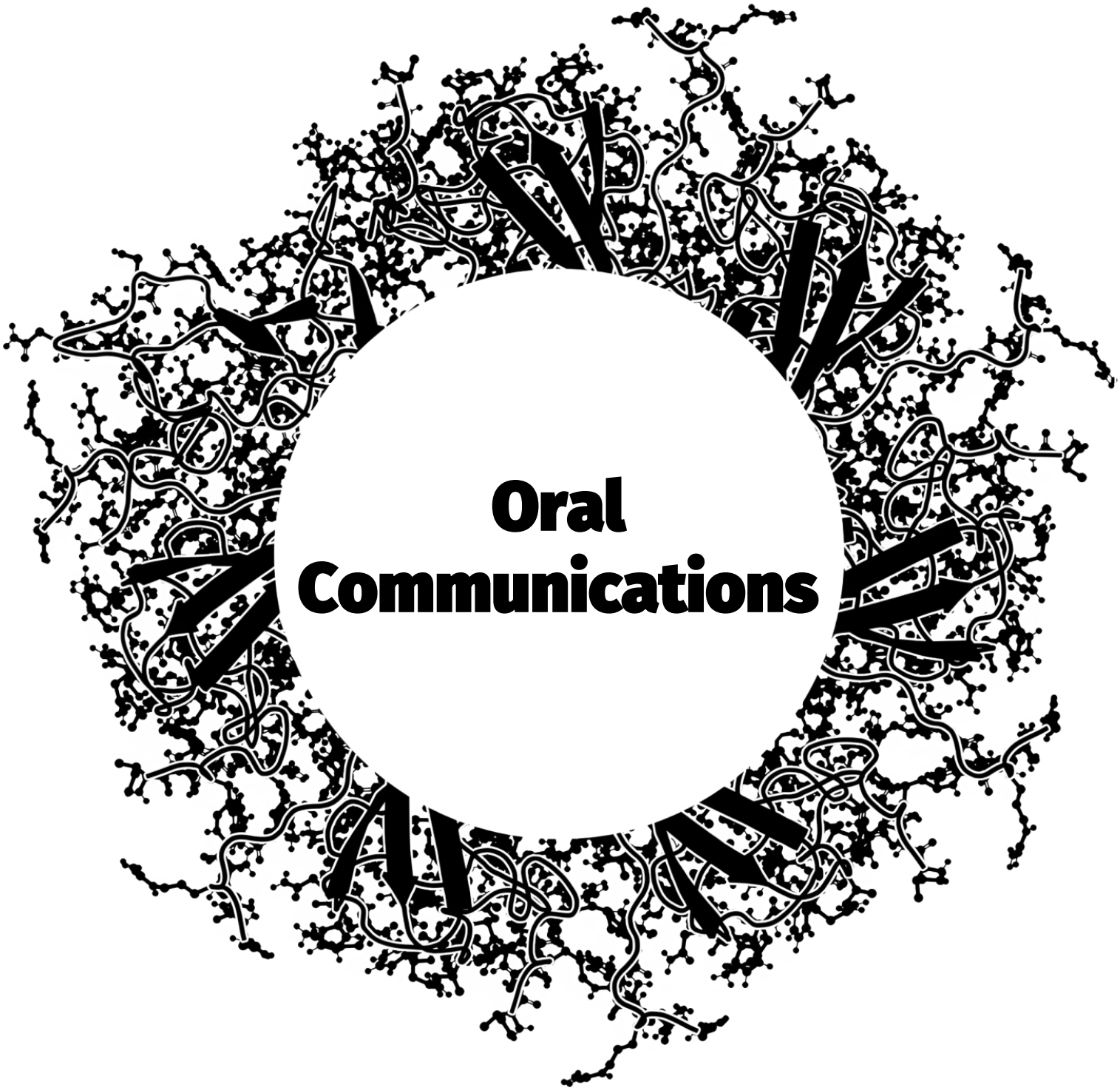
9h00 - 9h30	Charles L Brooks III	**Constant pH MD: An assessment of the state-of-the-art**
9h30 - 10h00	Katharine White	**Protons as a second messenger: pH-dependent allostery in signaling proteins**
10h00 - 10h30	Coffee Break	
10h30 - 11h00	Matthias Ulmann	**Describing Charge Transfer in Proteins: A Microstate Model for Simple Proteins and Complex Machineries**
11h00 - 11h30	Nuno Galamba	**Protein-peptide Recognition and Peptide Induced Structural Transformations of α-synuclein**
11h30 - 12h00	Miguel Machuqueiro	**Constant-pH MD Simulations: Advances, Challenges, and… more Challenges**
12h00 - 14h00	Lunch	
14h00 - 14h30	Michael Feig	**Predictions of pKa shifts and other molecular properties via graph neural networks**
14h30 - 15h00	Ana Sofia Oliveira	**Uncovering pH effects in the glycosylated SARS-CoV-2 spike using nonequilibrium simulations**
15h00 - 15h30	Alexandre S. de Araujo	**Computational Investigation of Dengue Virus Entry Mechanisms for Therapeutic Intervention**
15h30 - 17h00	Free Time or Group Activity	
20h00	Social Dinner	

Wednesday 25th

9h00 - 9h30	Frans Mulder	**Probing protein electrostatics through hydrogen exchange NMR**
9h30 - 10h00	Alexey Onufriev	**Simulations in Implicit Solvent with Explicit Ions**
10h00 - 10h30	Coffee Break & Poster Session	
10h30 - 11h00	Ana Damjanovic	**PKAD-R: curated, redesigned and expanded database of experimental pKa values in proteins**
11h00 - 11h30	Sidney J. de Carvalho	**Protein Adsorption Into Charged Confining Pores**
11h30 - 12h00	António M. Baptista	**Computing the pH-Dependent Thermodynamics of the Allostery between Dimerization and Palmitate Binding in β-Lactoglobulin**
12h00 - 14h00	Lunch	
14h00 - 14h30	Jana Shen	**Protein Electrostatics are Fine-Tuned Through Evolution**
14h30 - 15h00	Ralf Blossey	**On Poisson-Boltzmann equation zoology – and an experimentally validated PB model for proteins**
15h00 - 15h30	Walter Rocchia	**NextGenPB: a novel PB solver bridging numerical accuracy and computational efficiency**
15h30 - 17h00	Free Time or Group Activity or NGPB Tutorial	

Thursday 26th

9h00 - 9h30	Adrian Roitberg	**Machine Learning potentials for Molecular Dynamics. The headache of treating electrostatics**
9h30 - 10h00	Jim Warwicker	**Charge interactions and pH-dependence just keep rolling along**
10h00 - 10h30	Coffee Break & Poster Session	
10h30 - 11h00	Lauren Webb	**Electrostatics and Dynamics in Complex Lipid Bilayer Membranes**
11h00 - 11h30	Carles Curutchet	**Characterization of the conformational ensemble of calmodulin from atomistic simulations of Förster resonance energy transfer**
11h30 - 12h00	George Makhatadze	**Modulation of Electrostatic Interactions at the Proteome Level as a Mechanism of Cryptic Adaptation to High Hydrostatic Pressure**
12h00 - 14h00	Lunch	
14h00 - 14h30	Junji Iwahara	**Direct measurement of electrostatic potentials by NMR spectroscopy: Applications to protein post-translational modifications**
14h30 - 15h00	Valerie Welborn	**Protein electrostatics with the AMOEBA polarizable force field**
15h00 - 15h30	Poster Prize Award	
15h30 - 17h00	Closing Remarks	


Oral
Communications

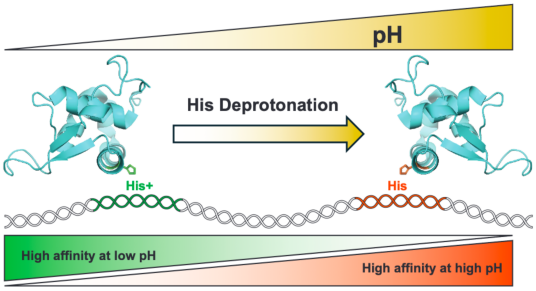
Oral Presentation 1

How Intracellular pH Dynamics Regulates Cell Behaviors: Identifying Endogenous pH Sensors

Diane L. Barber
University of California San Francisco

To understand how intracellular pH (pHi) regulates myriad cell behaviors, we bridge protein electrostatics and cell biology to identify pH sensing proteins with functions regulated within the narrow pHi range of 7.2-7.6. I will describe two projects on pH sensors. In the first project, we found that transcription factors with a histidine in their DNA binding domain that forms a hydrogen bond with nucleotides can have pH-regulated activity, which is relevant to more than 85 transcription factors in more than five different families. Focusing on FOX family transcription factors, we used three unbiased approaches, SELEX-seq, ChIP-seq, and RNA-seq, to identify distinct DNA binding motif preferences at pH 7.2 compared with pH 7.6. We confirmed pH-dependent binding to distinct motif preferences in vitro by using fluorescence anisotropy and in cells by using luciferase reporters, including resolving the critical role of DNA-binding histidine residues. These findings identify pH-regulated transcription factor-DNA binding selectivity with relevance to how pHi dynamics can regulate gene expression for myriad cell behaviors. In the second project guided by disease-associated charge changing mutations, we identified several cancer promoting arginine to histidine mutations, including Arg273His for the tumor suppressor p53, that confer a gain in pH sensing not seen by wild-type proteins. For wild type p53, positively charged Arg273, which is insensitive to pHi dynamics, binds the negatively charged DNA phosphate backbone. For p53-R273H, however, DNA binding and activity are pH sensitive and substantially decreased with a deprotonated His273 at the higher pHi of cancer cells. In collaboration with Emil Alexov at Clemson University we identified small molecules as potential cancer therapeutics that bind deprotonated p53-His273 to impart a positive charge and increase DNA binding and tumor suppressor function. This second project highlights the feasibility of drugging histidine residues to change protein electrostatics for a predicted cell behavior.

Acknowledgements: Work supported by NIH R01 CA197855, NIH F31 GM142284, NSF 2203629, and UCSF Sandler Program for Breakthrough Biomedical Research and Cancer League awards.



Oral Presentation 2

MCCE microstates: What can be learned from Monte Carlo microstates

Marilyn Gunner, Md. Raihan Uddin, Koreena Sookhai, Junjun Mao, Cat Chanel, Gehan Ranepura, Robert Burnap (A), Patricia Saura (B), and Ville Kaila (B)

Physics Department City College of New York, CUNY,

(A) University of Oklahoma,

(B) Stockholm University

MCCE uses Monte Carlo (MC) sampling of residue and water orientation and residue protonation states given a classical electrostatics and molecular mechanics force field. A microstate is one position and charge for each group in the protein. The millions of individual saved microstates form a Boltzmann ensemble that can be analyzed. This moves beyond the standard MC analysis that only gives the probability of uncorrelated, individual degrees of freedom. Here we mine the MC microstates to find correlation between protonation states of residues and of hydrogen bonds in networks. Examples will be given from the comparison of the Complex I proton pump from T. thermophilus, E. coli, T. elongatus (NDH) and mouse. Proton pumps such as Complex I move protons uphill from the side of the membrane with low proton concentration (N-side) to the side at high concentration (P-side). The energy of downhill redox chemistry fuels this process. Protons move via hydrogen bonded chains of waters and Grotthuss competent amino acids. In different reaction intermediates the connections to the N- and P-side differ. In addition, the proton affinity of residues within the protein change through the reaction cycle to load protons and then release them. These residues form a proton loading site (PLS). The E channel, closest to the peripheral arm is unique to Complex I will be described. The proton transfer paths are complex, with multiple competing routes for protons to travel. Residues along these proton transfer paths can play a role in PLS. Comparing the protonation of snapshots from MD trajectories show clusters in the channel whose overall protonation states differs by one proton being bound. However, the Monte Carlo ensemble shows that it is a group of residues stretching over 20Å, whose protonation states are coupled together to load and unload. The comparison of the four evolutionarily separated proteins shows conservation of many but not all PLS residues. Thus, the microstate analysis shows how residues play different roles to maintain function in related proteins.

Oral Presentation 3

Finding the Missing Protons: Constant-pH Simulations from Oligomers to Ribozymes

Tomás F. D. Silva and Giovanni Bussi
SISSA - Scuola Superiore Internazionale di Studi Avanzati

The RNA ecosystem exhibits a remarkable variety of biomolecules with versatile functions due to highly flexible structures, complex structural motifs, and intricate hydrogen-bond networks. These networks are defined by both canonical and non-canonical base pairs, many of which require protonation events to stabilize their structural interactions. Molecular dynamics (MD) methods typically require defining a priori the protonation state of chemical moieties. Typically, nucleobases are neutral as their aqueous pK_a values lie well outside the biologically relevant pH range.Yet, several RNA-based systems exhibit significantly upshifted pKa allowing titration, reshaping hydrogen-bonding patterns and local structural motifs. Constant-pH MD (CpHMD) frameworks couple conformational sampling with proton titration of nucleobases. We previously validated this method on tri- and penta-oligonucleotides by calibrating computed pK_a shifts against experiments [1]. Here, we extend our validation to two larger systems. First, the 30-nucleotide lead-dependent ribozyme (LDZ), one of the smallest catalytic ribozymes, features a protonated adenine–cytosine wobble base-pair near the cleavage site. Second, the 386-nucleotide Tetrahymena ribozyme (intron) adopts a complex tertiary fold exhibiting multiple non-canonical and mediated-Mg^{2+} ion interactions, as revealed by cryo-EM [2,3]. In the LDZ system, we performed CpHMD simulations over the 3.0–8.0 pH range. We accurately reproduced the experimental pK_a's of the known titratable sites [4] and evaluated the pH-dependency of the relevant wobble base pair. For the intron system, an initial 5-ns CpHMD run at pH 8.0 identified 30 potential titratable sites. Then we conducted 50-ns simulations using this subset to reduce computational cost. We measured their average protonations and compared the hydrogen-bond contacts against the cryo-EM structure. Our results reveal potential protonated sites overlooked or misassigned in MD simulations and cryo-EM structures. Overall, our study highlights CpHMD potential to reproduce known pK_a's in small ribozymes, and uncover alternative protonation patterns in large, complex RNA systems.

References

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[2] Z. Su, K. Zhang, K. Kappel, S. Li, M. Palo, G. Pintilie, R. Rangan, B. Luo, Y. Wei, R. Das, W. Chiu, Nature, 596, 603-607 (2021)
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Oral Presentation 4

Protonation- and lipid- coupled hydrogen-bond networks of a proton channel

Honey Jain 1,2 , Michalis Lazaratos 2 , Peter Pohl 3 , and Ana-Nicoleta Bondar 1,4,*

1 University of Bucharest, Faculty of Physics, Atomiștilor 405, Măgurele 077125, Romania,

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3 Institute of Biophysics, Johannes Kepler University, Gruberstraße 40, 4020 Linz, Austria,

*4 Forschungszentrum Jülich, Institute of Computational Biomedicine, IAS-5/INM-9, Wilhelm-Johnen Straße, 5428 Jülich, Germany, *Correspondence to nbondar@fizica.unibuc.ro*

The human voltage-gated proton channel Hv1 uses an internal aspartic residue as essential element of its pH sensing mechanism. To identify the fluctuating hydrogen-bond paths that could serve as proton-transfer wires, we have carried out atomic-level simulations and graph analyses of the channel with distinct protonation states and in membranes with distinct thickness and hydrogen-bonding properties. We find that connections from the internal aspartic residue to either side of the membrane depend on the protonation state of the channel, suggesting that the fluctuating hydrogen-bond network of the channel likely rearranges during proton transfer. We have further identified lipid anchoring sites of the channel, and lipid-protein couplings that shape the conformational dynamics of the channel.

Oral Presentation 5

On The Interactions With Distal 5'-Flanking Dna And The Control Of Transcription By Nuclear Hormone Receptors

David P. Lohry, Taylor A. Stevens, Tongye Shen & Elias J. Fernandez.
University of Tennessee

Optimal gene transcription is achieved through precise interactions between transcription factors and their DNA binding sites. We provide evidence that conserved distally located 5'-flanking sequences interact directly with the intrinsically disordered N-terminal region of the thyroid hormone receptor-alpha (TRalpha) to control transcriptional activity1. Simulated modeling and dynamics with multiple ChIP-seq-derived sequences consistently reveal specific Lysine/Arginine-DNA minor groove interactions. The impact of these interactions is to distort DNA structural conformations, and these are also revealed with atomic force microscopy. The significance of the 5'-flanking DNA is further emphasized with reporter-gene assays and comparisons with canonical response elements. Overall, the study reveals the inadequacy of current definitions of the DNA hormone response element (HRE) and suggests that future descriptions of the HRE include the conserved distal DNA sequences. The broad impact of this study is further underscored by the common occurrence of Lys/Arg-rich motifs within the intrinsically disordered regions of nuclear receptors.

References

Lohry, D. P., Stevens, T. A., Shen, T. & Fernandez, E. J. Hormone response elements for the thyroid receptor-α include specific distal 5'-flanking DNA. Sci Adv 10, eadr1033 (2024). https://doi.org/10.1126/sciadv.adr1033

Oral Presentation 6

Active Learning Driven Small Molecule Mining and Optimization Based on Molecular Dynamics Simulated Free Energies

Maria G Kurnikova; Olexandr Isayev, Eugeny Gutkin, Phillip Gusev, Samuel Ben Koby, Chamali Narangoda

Chemistry Department, Carnegie Mellon University, Pittsburgh, PA 15217, USA

In silico identification of potent protein inhibitors requires prediction of a ligand binding free energy (BFE). Thermodynamic integration (TI) based on molecular dynamics (MD) simulations is a BFE calculation method capable of predicting accurate BFE, but it is computationally expensive and time-consuming. We have developed an efficient automated workflow for identifying compounds with the lowest BFE among thousands of congeneric ligands which requires only hundreds of TI calculations. Automated Machine Learning (AutoML) orchestrated by Active Learning (AL) in AL-AutoML workflow allows unbiased and efficient search for a small set of best performing molecules. We have applied this workflow to select inhibitors of the SARS-CoV-2 papain-like protease 1, as well as to predict hit and optimized hits for the Leucine-Rich Repeat Kinase 2 (LRRK2) WD40 Repeat (WDR) domain2, a Parkinson's disease target as a part of the Critical Assessment of Computational Hit-Finding experiments (CACHE) Challenge #1. We obtained hit rates that outperform a traditional expert medicinal chemist-guided campaigns, as well as other in silico schemes. Thus, we demonstrate that combination of AL and AutoML with free energy simulations provide at least 20x speedup relative to the naïve brute force approaches. At the same time the limitations of the force-field based MD free energy prediction remain, which include the limited quality of the small molecule parameteriazation of the fixed-charge forcefields, limited applicability of the fixed-charge force-fields per se, as well as deficiencies of the sampling techniques and MD simulation setup often encountered in practice. We have evaluated a number of MD simulation parameters that influence the predicted BFE. Our testing set of small molecules includes charge changing modifications typically deemed especially challenging for BFE simulations. We will demonstrate that the charge changing mutations can be predicted with the same numerical accuracy as similar neutral ligands when the best practices of the simulations are employed.

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Oral Presentation 7

Computational Estimation of Protein Polarizability Using Empirical and Machine Learning Methods: Applications in Alignment, Dielectric Prediction, and Drug Design

Muhamed Amin
National Institutes of Heath

Protein polarizability is a fundamental physical property that describes how a protein's electron density responds to external electric fields. Accurately determining polarizability is essential for understanding how electrostatic landscapes within biomolecules are altered under such perturbations. In this study, we present a suite of methods—ranging from empirical models to machine learning-based approaches—for calculating and predicting the polarizabilities of macromolecules. These methods are validated and applied in diverse contexts, including laser-induced alignment of proteins, estimation of protein dielectric constants, and computer-aided drug design. Our results highlight the utility of polarizability prediction as a versatile tool for advancing both fundamental biophysical understanding and practical applications in molecular modeling.

Oral Presentation 8

Constant pH MD: An assessment of the state-of-the-art

Charles L. Brooks III
University of Michigan

Constant pH molecular dynamics provides a framework for exploring the role that pH plays in modulating structure and function in biological molecules. Over the past two decades there has been significant activity in the development of methods that enable one to explore the role of pH in altering the structure and function of biological molecules by neutralizing or charging amino acids, bases or small molecule effectors in biological systems. This role for pH is ubiquitous throughout cellular function: as molecules enter the cell from the periplasm they typically do so via endocytic processes whereby the pH is lowered; in many cancers the pH profile of the cancerous cell is altered affecting programmed cell death and cellular proliferation. These examples highlight the importance of accounting for pH and pH changes in the computational modeling of biological systems. In this talk I will provide an overview of methods and approaches to incorporate pH and protonation state changes of amino acids and bases into molecular dynamics simulations using the statistical mechanical framework of λ-dynamics. I will discuss the application of these and related methods and provide an assessment of the state-of-the-art achieved by numerous partitioners. I will close by proposing a best-practices workflow that we aim to utilize in enabling the incorporation of pH into molecular dynamics simulations for routine studies of biological systems.

Oral Presentation 9

Protons as a second messenger: pH-dependent allostery in signaling proteins

Papa Kobina Van Dyck, Luke Piszkin, Elijah A. Gorski, Eduarda Tartarella Nascimento, Joshua A. Abebe, Logan M. Hoffmann, Arezo Karimi, Jeffrey W. Peng, Katharine White

Transient changes in intracellular pH (ranging from 6.8 to 7.6) drive various cellular behaviors. Alkanization has been shown to drive cell migration, differentiation, and metabolic adaptation while acidification has recently been shown to be important for maintenance of stem cell pluripotency, mitosis, and entry into the cell cycle. However, the specific pH-sensitive proteins (pH sensors) responsible for these cell behaviors remain largely unknown. To more rapidly identify candidate pH sensitive proteins, we developed an in silico computational pipeline that uses structural data to predict residues with physiological pKas that may function as pH sensing nodes in proteins. We applied this approach to SH2 domain-containing signaling proteins. Our method showed that modular SH2 proteins have predicted pH sensitive networks clustering at the interface between regulatory SH2 domains and catalytic domains. Using biophysical, biochemical, and cell biological approaches, we validated the role of pH in tuning the wild-type basal signaling activity of two SH2 domain containing proteins, Src and Src homology region 2 domain-containing phosphatase-2 (SHP2). At low pHi, these wild-type proteins have high activity with decreasing activity as pHi increases. Additionally, we validated the predictive power of our computational pipeline by mutating the identified residues to abrogate pH dependent activity. Constant pH molecular dynamics simulations performed on both SHP2 and Src support allosteric regulation mediated by pH-dependent binding of inhibitory SH2 domains to the respective catalytic domains. We also show that the pHi-dependent regulation of these kinases functions in concert with traditional regulation by phosphorylation. Moreover, our data suggest that cancer mutations cluster in these conserved networks to abrogate pH-sensitive regulation of activity and contribute to the hyper-activation of cell signaling in cancer. Together, our computational, biophysical, and cellular analyses reveal a conserved role for modular SH2 proteins as mediators of pH-dependent signaling.

Oral Presentation 10

Describing Charge Transfer in Proteins: A Microstate Model for Simple Proteins and Complex Machineries

G. Matthias Ullmann
Computational Biochemistry, University of Bayreuth

Charge transfer through biological macromolecules is essential for many biological processes such as for instance photosynthesis and respiration. In these processes, protons or electrons are transferred between titratable residues or redox-active cofactors, respectively. Often their transfer is tightly coupled. Computational methods based on continuum electrostatics can be used in theoretical biochemistry to analyze the function of even very complex biochemical systems. With these methods, the pH and the redox potential of the solution can be considered in the calculations. Combining continuum electrostatic calculations with a statistical thermodynamic analysis, it is possible to calculate equilibrium parameters such as protonation or oxidation probabilities. Moreover, it is also possible to simulate reaction kinetics by using such parameters. This formalism is applied to the kinetics of electron transfer in the tetraheme-subunit and the special pair of the reaction center of Blastochloris viridis.

Oral Presentation 11

Protein-Peptide Recognition and Peptide Induced Structural Transformations of α-synuclein.

Nuno Galamba

BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

Various amino acid sequences have been suggested to play key roles in the aggregation of α-synuclein (α-syn), implicated in Parkinson's disease and other synucleinopathies. Here, using molecular dynamics simulations, we assessed the specificity of peptides in trans, in both their linear and cyclic forms, toward homologous sequences in the N-terminal and NAC domains of α-syn. We discuss the influence of these peptides on the structure of α-syn in both membrane-bound-like and disordered states. The results are compared with the monomer at high temperatures, at which the protein adopts a more compact structure, and exhibits increased intramolecular -sheet content, associated with an increase of the hydrophobic effect.

Oral Presentation 12

Constant-pH MD Simulations: Advances, Challenges, and... more Challenges

Miguel Machuqueiro

BioISI: Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

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

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Acknowledgements: We acknowledge financial support from FCT through projects UIDB/04046/2020 and UIDP/04046/2020.

Oral Presentation 13

Predictions of pKa shifts and other molecular properties via graph neural networks

Michael Feig
Michigan State University

Machine learning has become an alternative approach for predicting physicochemical properties from molecular structures. One application is the prediction of pKa shifts for amino acids where the main challenge is the relatively small amount of non-redundant experimental data that is available for training. To overcome this challenge, graph neural networks are initially trained on predicting computational data to learn suitable embeddings from structural data which can then be used in a transfer learning framework to retrain output layers of the network to predict pKa shifts with limited experimental training data.

Oral Presentation 14

Uncovering pH effects in the glycosylated SARS-CoV-2 spike using nonequilibrium simulations

Sofia Oliveira,1 Rommie Amaro,2 Andrew Davidson,3 Jonathan Reid,1 Imre Berger,1,4 Christiane Schaffitzel,4 Adrian Mulholland1

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The life cycle of the SARS-CoV-2 virus is intricately regulated by pH, which plays a pivotal role in facilitating viral entry into human cells. Central to this process is the spike protein, the virus's primary molecular pH sensor. The spike is a heavily glycosylated protein that mediates the fusion between the viral and host cell membranes. In acidic environments, such as those found within endosomes, the spike undergoes conformational changes that trigger membrane fusion, thereby initiating infection. In this study, we investigate how pH changes influence the structure and dynamics of the SARS-CoV-2 spike. For this, we leverage the emerging dynamical nonequilibrium molecular dynamics (D-NEMD) simulations approach to characterise the dynamical response of the fully glycosylated ancestral spike, as well as two variants of concern, to pH changes. D-NEMD integrates simulations under stationary and nonequilibrium conditions, enabling us to extract the temporal evolution of the response of a protein to external perturbations (in this case, changes in pH). By combining extensive equilibrium simulations with hundreds of nonequilibrium trajectories, we uncover how the ancestral, Delta and Omicron spike respond to a pH decrease, mimicking the acidic environment of endosomes, and to a pH increase, representative of conditions such as those found inside aerosol particles. These simulations reveal distinct patterns of motion and pH-induced changes between the ancestral protein and its variants, particularly in key functional regions, such as the receptor-binding domains and the fusion-peptide surrounding regions. Our findings provide unprecedented insights into how pH modulates the functional dynamics of the spike, driving the closed-to-open transition of the receptor binding domains and underscoring the critical role of glycans in this process.

Oral Presentation 15

Computational Investigation of Dengue Virus Entry Mechanisms for Therapeutic Intervention

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Dengue virus (DENV) infection is a significant global health concern, with limited specific therapeutic options available. This study utilizes computational simulations to explore DENV entry mechanisms, aiming to identify novel targets for antiviral drug development. Our research focuses on two key aspects of the entry process: stabilization of the E protein dimer at acidic pH and disruption of the fusion peptide interaction with endosomal membranes. First, we performed in silico analyses to identify key residues involved in the pH-dependent dissociation of the E protein dimer, seeking potential "hot spots"for promoting dimer stabilization in acidic pH. For this we used Constant pH Molecular Dynamics (CpHMD) simulations. Second, we investigated the interaction of the E protein domain II (DII) with models of late endosomal lipid bilayers. Adaptive Biasing Force (ABF) simulations were employed to map the free energy landscape of DII integrating into a lipid bilayer, aiming to identify energetically favorable binding sites on the DII surface that could disrupt the DII/bilayer interaction and inhibit viral entry. Initial results revealed key histidine and aspartic acid residues at the dimer interface that are pH-sensitive. Simulations in POPC/DOPA models of late endosomal membranes characterized the energetic impact of the fusion peptide (FP) on the lipid membrane. These findings provide a foundation for structure-based design of novel antiviral compounds targeting DENV entry and support ongoing collaborations for further method development and validation. We are applying the obtained results to guide the development of novel antiviral therapeutic strategies.

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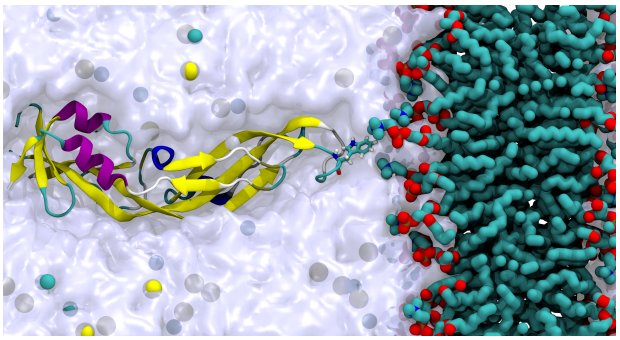

Oral Presentation 16

Probing protein electrostatics through hydrogen exchange NMR

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As charge interactions in intrinsically disordered proteins (IDPs) are sufficiently weak, it is adequate to apply a hybrid mean-field approach to estimate the electrostatic potential at various side chain positions to explain side chain titration behavior as a function of pH [1,2,3]. In similar vein, the potential present at the polypeptide backbone amide groups can be approximated. We demonstrate that this potential can significantly influence hydrogen exchange (HX) kinetics of IDPs because this process is base catalyzed at neutral pH. Using the protein alpha-synuclein as an example, we show that slow HX observed for the acidic C-terminal tail can be completely explained by electrostatic rather than structural effects, using the concept of the thermodynamic electrochemical potential [4]. Co-solute paramagnetic relaxation experiments [4,5] further support these conclusions.

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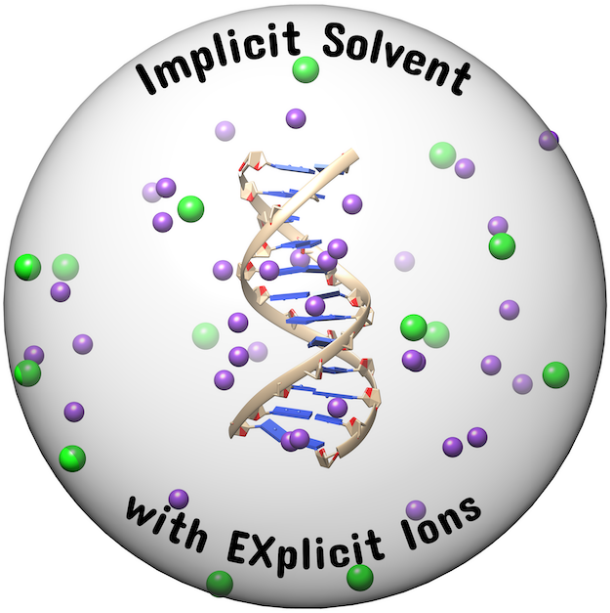
Oral Presentation 17

Simulations in Implicit Solvent with EXplicit Ions

Alexey Onufriev
Virginia Tech

Ion atmosphere surrounding biomolecules is crucial for their dynamics, structure and interactions. We have developed an approach for the explicit treatment of ions within the implicit solvent framework, suitable for atomistic simulations. The proposed implicit solvent/explicit ions model, GBION, is based on a significantly modified generalized Born (GB) model. The proposed model, now available in AMBER, is capable of simulating biomolecules in a large volume of solvent with explicit ions, with little additional computational overhead compared with the fully implicit GB treatment of ions. Ions simulated using GBION explore conformational space at least 2 orders of magnitude faster than in the explicit solvent. These advantages have allowed us to observe, and explore, a novel "stacking" mode of DNA condensation in the presence of trivalent counterions. As the next step, we have demonstrated how the efficiency of GBION can lead to insights into dynamics of the fundamental structural unit of chromatin packaging – the nucleosome – paving the way for using GBION for large complexes where ions are important.



Oral Presentation 18

PKAD-R: curated, redesigned and expanded database of experimental pKa values in proteins

Ana Damjanovic

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Understanding pKa values in ionizable protein residues is critical for understanding fundamental protein properties, such as structure, function and interactions. I will discuss PKAD-R, the newly curated, redesigned and expanded database of experimental pKa values. The database builds upon its predecessors, PKAD and PKAD-2, with significant updates and improvements through: (1) careful data curation to remove incorrect entries and consolidate redundant entries by offering alternative structures and pKa values for each unique residue (2) database redesign, to enhance its usability by adding additional information such as protein and species names, detailed notes, as well as sequence identity (3) database expansion through identification of 214 new (128 non-redundant) pKa entries from the literature. I will also discuss the critical need for more experimental pKa values in our community.

Oral Presentation 19

Protein Adsorption Into Charged Confining Pores

Ricardo B. de Souza Junior, Daniel Lucas Z. Caetano, Icaro P. Caruso and Sidney J. de Carvalho
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The confinement of proteins on porous materials is essential for several applications since protein stability and biological activity are enhanced. In the case of charged confining surfaces, like silica, the protein charge distribution is one of the main factors for this applicability since it determines their orientation regarding the surface and the exposure of active sites. In this study the adsorption properties of lysozyme and cytochrome c into a negatively charged silica pore are investigated using a coarse-grained model and constant–pH Monte Carlo simulations. The effects of pH and ionic strength on the protein orientation and spatial distribution of its residues regarding the pore surface are evaluated. We observe that pH variation promotes an orientational transition in the adsorbed lysozyme, unlike the cytochrome c behavior, which keeps the same region in contact with the pore surface for the whole pH range in which the protein is adsorbed. These findings are related to changes in the charge-patch distribution caused by the adsorption due to the charge variation of some key residues, which stabilizes the adsorption in the observed protein orientation. This study highlights the significant interplay between charge regulation and charge-patch mechanisms on the protein adsorption.

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Oral Presentation 20

Computing the pH-Dependent Thermodynamics of the Allostery between Dimerization and Palmitate Binding in β-Lactoglobulin

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The study of pH-dependent allosteric processes presents a significant challenge, both experimentally and computationally. In this work, we apply the constant-pH molecular dynamics method to explore an interesting case of allostery involving protein–ligand binding and dimerization. As a model system, we use β-lactoglobulin (BLG), a small protein from bovine milk known to dimerize and bind palmitic acid in a hydrophobic pocket - both processes sensitive to pH. This study focuses on the holo form of BLG, and, when combined with our previous study of the apo form (da Rocha et al. J. Chem. Theory Comput. 2022 18, 1982), completes the thermodynamic cycle of the allosteric process. The corresponding pH-dependent free energy profiles are obtained through the use of a thermodynamic linkage relation, avoiding the need of performing heavy computational calculations. Dimerization is found to be more favorable near the isoionic point, as observed in the apo form. Palmitate binding is found to be more favorable around pH 6–7, a biologically relevant pH range at which the gate covering the binding site is known to open. A pH-dependent measure of allosteric coupling is computed, showing that ligand binding and dimerization exhibit an antagonist relationship within the studied pH range of 3–8, with binding destabilizing dimerization and vice versa.

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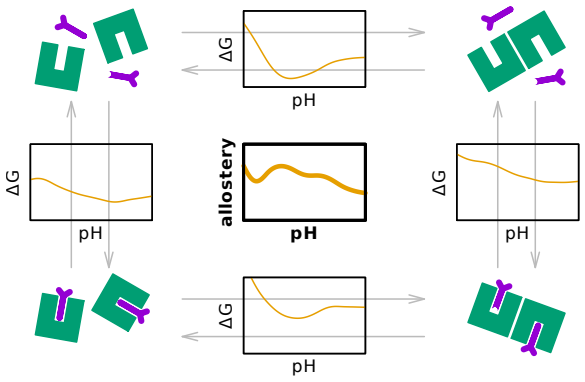

Oral Presentation 21

Protein Electrostatics are Fine-Tuned Through Evolution

Mingzhe Shen, Guy W. Dayhoff II, and Jana Shen*
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Protein ionization states provide electrostatic forces to modulate protein structure, stability, solubility, and function. Until now, predicting ionization states and understanding protein electrostatics have relied on structural information. Here we demonstrate that primary sequence alone enables remarkably accurate pKa predictions through KaML-ESM, a model that leverages evolutionary representations and pretraining with a synthetic pKa dataset. The KaML-ESM model achieves RMSEs approaching the experimental precision limit of 0.5 pH units for Asp, Glu, His, and Lys residues, while reducing Cys prediction errors to 1.1 units. We provide KaML, a sequence-based end-to-end ML platform that enables researchers to map protein electrostatic landscapes, facilitating applications ranging from drug design and protein engineering to biomolecular simulations.

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Oral Presentation 22

On Poisson-Boltzmann equation zoology – and an experimentally validated PB model for proteins

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The Poisson-Boltzmann equation is an indispensable tool for the calculation of the electrostatic potential of proteins and further physical quantities derived from its knowledge. The limitations of the equation are well-known, and several modifications of the basic (linear) Poisson-Boltzmann equation have been proposed over the years. Two such modifications have enjoyed particular interest that both concern the treatment of the protein environment, i.e. the solvation of a protein. Within a phenomenological, often also termed nonlocal, approach, solvent properties are built into the PB equation via the space-dependent dielectric properties of the solvent. This approach leads in its most simple version to a system of coupled, in general nonlinear equations for two scalar fields, a structural field and the electrostatic potential. A still simpler, but highly nonlinear theory is the Poisson-Boltzmann Dipolar Langevin equation, in which the dielectric properties of the solvent molecules is built in by explicitly considering their dipolar nature. The validation of these and other "animals" from the "Poisson-Boltzmann equation zoo" is hampered by the usually only indirectly available knowledge of "true" (i.e., experimentally measured) electrostatic potentials. Recent experimental advances, notably in AFM and NMR-techniques, are changing this picture – a direct validation of PB models for protein electrostatics now comes into reach. Against the background of these experimental insights, in this talk I briefly describe the state of the art of modern PB models and propose an effective PB model applicable to proteins electrostatics.

Oral Presentation 23

NextGenPB: a novel PB solver bridging numerical accuracy and computational efficiency

V. Di Florio, P. Ansalone, S. V. Siryk, S. Decherchi, C. De Falco, W. Rocchia
Istituto Italiano di Tecnologia, Genoa, Italy

The Poisson-Boltzmann equation (PBE) is likely the most reliable and widely used tool to estimate the electrostatic contribution to the energy of biomolecular systems immersed in electrolytic solutions. Due to the complexity of the geometries, advanced numerical solvers are needed to perform this task in an accurate and efficient way. Many of them have been developed in the past and some are proficiently used still nowadays. However, the availability of increasingly bigger systems and larger datasets calls for advanced and particularly efficient solutions, capable on one side to exploit at best the modern computational architectures, and on the other, to perform accurate and fast calculations also on less powerful personal computers. We present here NextGenPB (NGPB), a computational tool developed to improve the accuracy and efficiency of electrostatic energy calculations in complex biomolecular environments. NGPB integrates analytical surface corrections into a finite element method framework, enabling precise electrostatic potential and energy estimates without the need for costly mesh refinement. Additionally, grid de-refinement at boundaries of the computational domain further reduces the computational overhead related to the enforcement of boundary conditions while preserving accuracy. We benchmark NGPB against analytical solutions and demonstrate its scalability and robustness across a variety of molecular systems. We also show its performance with respect to the size of the studied systems. Specific advantages given by the increased accuracy in the estimate of the potential at the molecular surface will be explored. Overall, these advances make NGPB a powerful and practical tool for routine electrostatics calculations, with direct applications in computational biochemistry, molecular design, and drug discovery.

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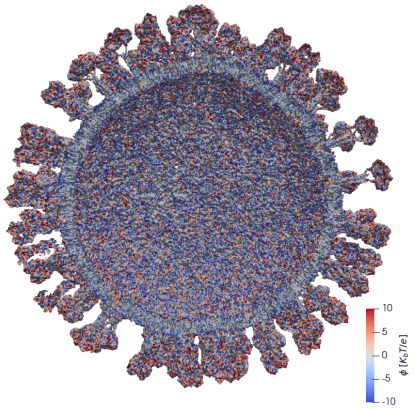
Acknowledgements: We acknowledge the financial support from the European Union - NextGenerationEU and the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP): Research program CN00000013 “National Centre for HPC, Big Data and Quantum Computing”, funded by the D.D. n.1031 del 17.06.2022 and Mission 4, Component 2, Investment 1.4 - Avviso “Centri Nazionali” - D.D. n. 3138, 16 December 2021; and PNRR MUR Project PE0000013 "Future Artificial Intelligence Research (FAIR)", CUP J53C22003010006.



Oral Presentation 24

Machine Learning potentials for Molecular Dynamics. The headache of treating electrostatics

Adrian Roitberg

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I will present some ideas and results of training and using Machine Learning Potentials for computing energies and forces. Those potentials are usually "near-sighted", meaning that long range interactions, such as dispersion and electrostatics must be done as add-ons. This is non-trivial and many options are available. I will discuss pros and cons of the different ideas. If one wants to do ML/MM like one would do QM/MM, then even more care needs to be taken.

Oral Presentation 25

Charge interactions and pH-dependence just keep rolling along

Shalaw Sallah, Sifan Zhang, Jim Warwicker
University of Manchester, UK

In common with many other groups we have been beguiled by charge interactions and molecular function in biology, in particular pH-dependence. It is an exciting time, with ML methods promising to extend their reach in structure and function. We're not that, well not much. What will be presented is an attempt to predict pH-dependence, firstly in the somatic mutations that are associated with tumour development and secondly, more generally, in the human proteome. The complexity of both application areas has been a challenge. For example, what is functional pH-dependence, and how much of this has been recorded in the literature? In terms of the methodology, where we transit from benchmarking with known systems to predicting pH-dependence (where it is not known experimentally), what is our degree of confidence? A separate area that will be mentioned is investigation of proteome sequences and solubility.

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Oral Presentation 26

Electrostatics and Dynamics in Complex Lipid Bilayer Membranes

Lauren J. Webb

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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.

Oral Presentation 27

Characterization of the conformational ensemble of calmodulin from atomistic simulations of Förster resonance energy transfer

C. Curutchet

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Förster resonance energy transfer (FRET) is widely used as a molecular ruler to interrogate the structure of biomolecules. Förster theory, introduced over 70 years ago, allows linking transfer rates to simple structural and spectroscopic properties of the energy-transferring molecules. In biosystems, however, significant deviations from Förster behavior often arise due to breakdown of the ideal dipole approximation, dielectric screening effects due to the biological environment, or departure from the weak-coupling regime.[1] Here, we examine Förster limits in the combined application of MD simulations and FRET experiments to investigate the conformational preferences of disordered proteins. Indeed, simulations have emerged as an ideal complement to FRET due to its ability to provide structural models that can be compared with experiments in disordered systems.[1] We use a rigorous theoretical framework based on extensive unbiased all-atom MD simulations and FRET calculations based on electrostatic potential-fitted transition charges coupled to an atomistic polarizable classical environment.[2] Such TrESP-MMPol approach is validated from rigorous polarizable embedding TD-DFT QM/MMPol calculations,[3] and applied to investigate the Ca2+-dependent conformational preferences of calmodulin, a protein that plays a major role in the transmission of calcium signals.[4]

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Oral Presentation 28

Modulation of Electrostatic Interactions at the Proteome Level as a Mechanism of Cryptic Adaptation to High Hydrostatic Pressure.

George I. Makhatadze

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The role of various interactions in determining the pressure adaptation of the proteome in piezophilic organisms remains to be established. The adaptation is not limited to one or two proteins but has a more general evolution of the characteristics of the entire proteome, the so-called cryptic evolution. Using the synergy between bioinformatics, computer simulations, and some experimental evidence, we probed the physico-chemical mechanisms of cryptic evolution of the proteome of psychrophilic strains of the model organism, Colwellia, to adapt to life at various pressures, from the surface of the Arctic ice to the depth of the Mariana Trench. From the bioinformatics analysis of proteomes of several strains of Colwellia, we have identified the modulation of interactions between charged residues as a possible driver of evolutionary adaptation to high hydrostatic pressure. Computational modeling suggests that these interactions have different roles in modulating the function-stability relationship for different protein families. For several classes of proteins, the modulation of interactions between charges evolved to lead to an increase in stability with pressure, while for others, just the opposite is observed. The latter trend appears to benefit enzyme activity by countering structural rigidification due to the high pressure.

Oral Presentation 29

Direct measurement of electrostatic potentials by NMR spectroscopy: Applications to protein post-translational modifications

Junji Iwahara
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In recent years, it has become possible to directly measure the near-surface electrostatic potentials of biomolecules using NMR spectroscopy. This is achieved through solvent paramagnetic relaxation enhancement (PRE) caused by charged paramagnetic cosolute molecules for which the spatial distributions around the biomolecules are biased due to electrostatic interactions. This NMR method allows for the simultaneous measurement of electrostatic potentials for many residues without relying on structural information. As a result, it is extremely useful for examining theoretical electrostatic models and investigating the electrostatic properties of highly flexible macromolecules, such as intrinsically disordered proteins and multi-domain proteins connected by flexible linkers. We and others have applied this powerful NMR technique to various proteins, nucleic acids, and their complexes. Moreover, direct measurements of electrostatic potentials by NMR spectroscopy are valuable in studying the effects of post-translational modifications. This presentation will briefly overview recent progress in NMR-based measurements of electrostatic potentials and show applications to investigate how post-translational modifications impact the molecular properties of proteins from an electrostatic perspective.

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Acknowledgements: This work was supported by Grant R35-GM130326 from the National Institutes of Health and Grant H-2104-20220331 from the Welch Foundation.

Oral Presentation 30

Protein electrostatics with the AMOEBA polarizable force field

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In aqueous solutions, macromolecules act as local networks of molecular dipoles. Changes in conformation, due to thermal fluctuations and bonded/non-bonded interactions will affect the orientation and magnitude of these dipoles, which generate an electric field that governs macromolecular function. In this talk, I will present how electric field calculations can be used to predict chemical reactivity in classical molecular dynamics simulations, including protonation state in enzymes. Although seldom used outside enzymatic catalysis, the lan- guage of electric fields can be generalized to other biological macromolecules. In this talk, I will also present how electric field calculations help to revisit our molecular picture of ion transport through ion channels.

Poster Communications

Poster Presentation 1

Sequence-Specific Binding of Linear and Cyclic Peptides to Aggregation-Prone Regions of α-Synuclein

Gabriel F. Martins, Cristiano Rocha and Nuno Galamba

BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences of the University of Lisbon, C8, Campo Grande, 1749-016 Lisbon, Portugal

Specific amino acid regions within α-synuclein (α-syn), a protein implicated in Parkinson's disease and other synucleinopathies, have been identified as key contributors to its aggregation. One therapeutic strategy involves designing molecules that can selectively bind to these regions in the monomeric form of α-syn, thereby potentially preventing primary nucleation by either disrupting protein–protein interactions or stabilizing the monomer in its soluble or membrane-associated states. In this study, molecular dynamics simulations were employed to evaluate the binding preferences of both linear and cyclic peptides made of homologous sequences of the protein – P1, NACore and NACterm – towards the corresponding segments in the N-terminal and NAC domains of α-syn (regions previously confirmed to influence aggregation). The findings indicate that these peptides, though differing in size and nature, generally show sequence-specific interactions with their target regions. Furthermore, several of these peptides promote stabilization of α-helical structures within the NAC domain for when α-syn adopts a membrane-like conformation, while others favor more extended configurations in its intrinsically disordered state. However, some peptides may disrupt intramolecular contacts that could otherwise serve protective roles against aggregation. These results were also compared to simulations of the monomer at higher temperatures, which revealed a more compact structure enriched in intramolecular β-sheets, likely driven by enhanced hydrophobic interactions. Keywords: Molecular dynamics; Cyclic Peptides; Linear Peptides; Charmm36m; P1; NACore; NACterm; α-synuclein.

Poster Presentation 2

Estimation of pKa values in proteins

Jesse W. Jones1, Nereu Montserrat i Busquets1,2, Ana P. Gamiz-Hernandez3, Ville R.I. Kaila3, Maria A. Mroginski1

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3Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

Many key bioenergetic processes involving electron and proton reactions take place in membrane bound protein complexes, generating a proton motive force. Yet the ionizable groups which facilitate these reactions are often buried in hydrophobic pockets in the membrane. These processes are mainly described through pKa values, which continue to be poorly understood and difficult to obtain despite structural, biochemical and computational advances. Hence, es- timating pKa values of these residues without the need for weeks of work in a laboratory, is important to describe the dynamics of the system, providing in- formation on possible proton pathways. In this work we preview Karlsberg3, a software which solves the Poisson Boltzmann Equation for proteins with APBS and calculates pKa values. Karlsberg3 is, in contrast to its predecessor Karls- berg2+, parallelized, and running in modern software environments.

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Poster Presentation 3

What do AI Tools for Protein Structure Prediction Know About Electrostatics?

George I. Makhatadze

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A set of amino acid substitutions affecting electrostatic interactions in a small globular protein has led to significant structural changes, including increased helical content and a transition from monomer to oligomer. These experimentally detected structural changes were not predicted by widely used AI-based structure prediction tools such as AlphaFold, EsmFold, and OmegaFold. To assess the ability of these tools to account for electrostatic interactions, we conducted a computational screen of over 100,000 electrostatic variants across three different proteins. Our analysis revealed that none of these AI tools effectively capture electrostatic interactions. Interestingly, the accuracy varied among them, with AlphaFold unexpectedly demonstrating the weakest performance.

Poster Presentation 4

Electrostatic calculation of the redox behavior of 2[4Fe-4S]-ferredoxins

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4Fe-4S clusters are redox cofactors found in various important proteins and enzymes. To fully understand these proteins, it is essential to accurately predict and model the redox properties of these clusters. The presented work focuses on 2[4Fe-4S]. Calculations with the Linearized Poisson-Boltzmann electrostatics model are used in combination with a Monte Carlo Titration algorithm implemented in GMCT to determine the redox behaviour of these clusters, utilizing re-refined crystal structures and in-silico mutants. A central part of the work shows that in case of long-fold 2[4Fe-4S] ferredoxins, modeling internal water molecules and different hydrogen rotamers of residues near to the cluster significantly improves the agreement between calculated and experimental redox potential across 20 structural models (including in-silico mutants).

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Poster Presentation 5

Patchy charge distribution affects the pH in protein solutions during dialysis.

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When using dialysis or ultra/diafiltration to purify protein solutions, a dialysis buffer in the permeate is employed to set the pH in the protein solution. Failure to achieve the target pH may cause undesired precipitation of the valuable product. However, the pH in the permeate differs from that in the retentate, which contains the charged proteins. [1] Experimental optimization of the process conditions is time-consuming and expensive, while accurate theoretical predictions still pose a major challenge. Current models of dialysis account for the Donnan equilibrium, acid–base properties, and ion–protein interactions, but they neglect the patchy distribution of ionizable groups on the proteins and its impact on the solution properties. Here, we present a simple computational model of a colloidal particle with weakly acidic sites on the surface, organized in patches. [2] This minimalistic model allows systematic variation of the relevant parameters, while simultaneously demonstrating the essential physics governing the acid–base equilibria in protein solutions. Using molecular simulations in the Grand-Reaction ensemble, we demonstrate that interactions between ionizable sites significantly affect the nanoparticle charge and thereby contribute to pH difference between the permeate and retentate. We show that the significance of this contribution increases if the ionizable sites are located on a smaller patch. Protein solutions are governed by the same physics as our simple model. In this context, our results show that models which aim to quantitatively predict the pH in protein solutions during dialysis need to account for the patchy distribution of ionizable sites on the protein surface. Keywords: colloidal nanoparticles, patchy distribution, Donnan effect, protein clustering/aggregation, pH.

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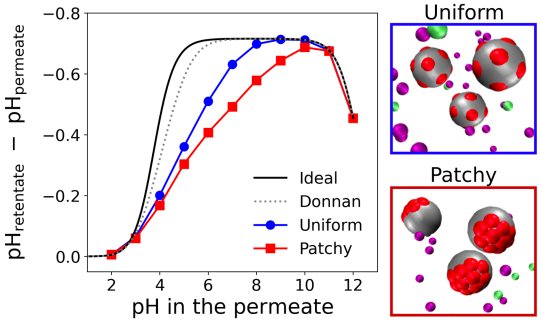

Poster Presentation 6

Using molecular dynamics simulations to study the monomer-dimer equilibrium of the pH sensing protein PsbS

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Under intense sunlight, plants prevent photooxidation by dissipating excess absorbed energy as heat, a protective mechanism known as non-photochemical quenching (NPQ) [1]. This process is triggered by PsbS, a membrane protein responsive to pH changes in the thylakoid lumen [2]. Although the precise mechanism of PsbS activation remains unclear, experimental evidence suggests that certain glutamate (Glu) residues on the lumen side may act as pH sensors, and that both the monomer-dimer equilibrium and the dimer conformation are pH-dependent. A recent computational study on the PsbS monomer [3] supports these observations, identifying unusually high pKa values for several lumen-exposed Glu residues, suggesting a key role in pH sensitivity under physiological conditions. The study also reported pH-induced folding of a loop likely involved in dimerization and revealed correlations between protonation states across the membrane, potentially enabling lumen-stroma communication. The present work extends this investigation to the PsbS dimer, using constant-pH molecular dynamics (CpHMD) simulations, as in the previous study, to determine pKa values, conformational dynamics, and correlations between protonation and structural changes. By combining monomer [3] and dimer data, the pH-dependence of dimerization and the role of key residues will be assessed using a thermodynamic linkage relation [4]. This study employs the GROMACS molecular dynamics package, alongside in-house tools: meadTools, PETIT, and ST-CpHMD. The outcomes are expected to enhance our understanding of the PsbS activation mechanism and contribute to the development of strategies for improving plant photoprotection and crop performance [5].

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Poster Presentation 7

INVESTIGATING POLYBIA-MP1 ACTION IN TUMOR MEMBRANES MODELS THROUGH MOLECULAR DYNAMICS SIMULATIONS

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Antimicrobial peptides (AMPs) are short, predominantly cationic molecules of the immune system with a broad spectrum of activity. They act through electrostatic and hydrophobic interactions leading to adsorption and perturbation of cell membranes, thereby increasing permeability and potentially causing lysis. In addition to their antimicrobial effects, AMPs also exhibit antiviral, antifungal, antiparasitic, and antitumor potential. Polybia-MP1 (MP1), extracted from the wasp Polybia-paulista, possesses a broad antimicrobial spectrum with low toxicity. The selectivity of MP1 for tumor cells is fundamentally attributed to the externalization of the anionic phospholipid phosphatidylserine (PS) on the outer leaflet of these cells, in contrast to healthy cells where PS remains confined to the inner leaflet. This exposure enables electrostatic interactions that significantly enhance MP1's binding affinity for PS-containing membranes. Additionally, phosphatidylethanolamine (PE), which is often externalized alongside PS in cancer cells, increases membrane susceptibility to MP1-induced disruption by facilitating the formation of larger transmembrane pores. Crucially, PS and PE act synergistically: while PS optimizes initial binding, its combination with PE amplifies membrane permeabilization and rupture. In this study, we employed molecular dynamics (MD) simulations to investigate the interaction and mechanism of action of MP1 on model membranes mimicking the composition of tumor cells (PC:PS:PE 70:20:10). We initiated simulations with a single MP1 peptide, placed 10 Å from the membrane surface in a random coil conformation. We observed partial adsorption and minor disturbances in the bilayer, including changes in thickness, curvature, and order parameter, suggesting initial permeability induction. Subsequently, starting from the partially adsorbed single-peptide system, a second MP1 peptide was introduced into the water phase. These simulations highlight the potential cooperative effects between peptides and provide molecular-level insights into how the selective interaction of Polybia-MP1 with PS and PE drives its lytic activity against cancer cell membranes.

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Poster Presentation 8

Biomolecular Electrostatics applications of the NextGenPB solver

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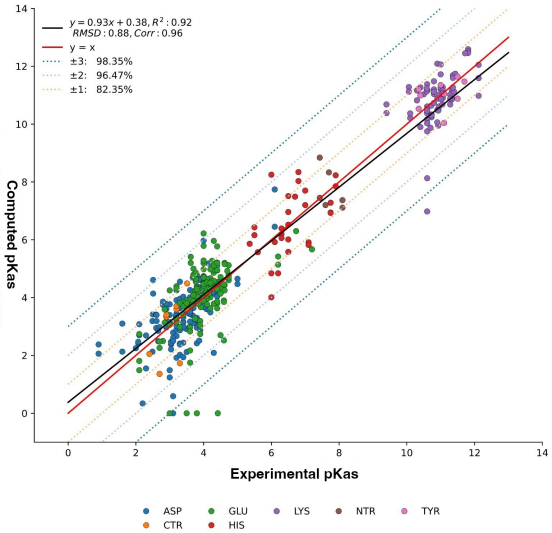
Accurate estimation of electrostatic quantities, such as electrostatic potential and solvation free energy, is fundamental for understanding biomolecular stability, interactions, and function. The Poisson–Boltzmann equation (PBE) is widely used to model these electrostatic effects in biomolecular systems immersed in electrolyte solutions. In this work, we present practical applications of NextGenPB (NGPB), a newly developed PBE solver tailored for biomolecular electrostatics. NGPB incorporates analytical surface corrections within a finite element method (FEM) framework, enabling the precise estimation of electrostatic potential and energies without the need for costly mesh refinement. We demonstrate the versatility of NGPB by computing various electrostatic quantities in biomolecular systems solvated in ionic solutions, including electrostatic potential distributions on molecular surfaces and solvation free energies, key parameters for characterizing biomolecular systems. Furthermore, we integrate NGPB into the MCCE protocol for pKa prediction, achieving excellent agreement with experimental data. We also apply NGPB to binding energy calculations and are incorporating it into the MM-PBSA framework for extensive protein-protein binding energy estimation.

Acknowledgements: We acknowledge the financial support from the European Union - NextGenerationEU and the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP): Research program CN00000013 “National Centre for HPC, Big Data and Quantum Computing”, funded by the D.D. n.1031 del 17.06.2022 and Mission 4, Component 2, Investment 1.4 - Avviso “Centri Nazionali” - D.D. n. 3138, 16 December 2021; and PNRR MUR Project PE0000013 "Future Artificial Intelligence Research (FAIR)", CUP J53C22003010006.



Poster Presentation 9

Using QM parameterizations and CpHMD simulations to study the proton pumping mechanism in Cytochrome c Oxidase

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The terminal enzyme of the electron transport chain transfers electrons from Cytochrome c to its catalytic site along a series of metal cofactors, where they will reduce molecular oxygen to water. Complex IV, also denominated Cytochrome c Oxidase (CcO), pumps protons from the N-side, with some being used as "chemical protons", leaving as water, while others are pumped to the P-side. This transport against the proton concentration gradient is integral for the maintenance of the electrochemical gradient, which facilitates, among other phenomena, the production of ATP by Complex V. The "gating mechanism" attempts to explain the directionality of protonic transport. As postulated, key residues near the catalytic center of CcO have their proton affinity (pKa value) modulated by the surrounding electrostatic environment, which in turn varies as the oxidation state of the metal cofactors changes with electronic transport. Despite being a hallmark system of bioenergetics, there is still no consensus on the CcO catalytic cycle, and the gating mechanism still lacks definitive evidence, such as the identification of key "gate" residues. This work investigates the gating mechanism by following the pKa shifts of residues in the vicinity of the catalytic center. To achieve this, we developed an enhanced computational model for simulation using the Constant pH Molecular Dynamics method (CpHMD). CcO is a complex system with metal cofactors that are not part of standard force fields, requiring parameterization using Quantum Mechanics (QM) methods to obtain adequate partial charge sets. We will be showcasing the results of this quantum parametrization, as well as preliminary results from CcO CpHMD simulations.

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Poster Presentation 10

The Role of Electrostatics in Host:Guest Binding Affinities: The Case of Cyclodextrin Complexes

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Pharmacokinetics plays a significant role in determining the pharmacological effect of drugs, thus, exploring delivery systems that can enhance these properties is crucial for drug development. Within this scope, cyclodextrins (CDs) are a group of cyclic oligosaccharides characterized by a hydrophilic outer surface and a lipophilic central cavity that can form inclusion complexes with various drugs, often enhancing their aqueous solubility and making them suitable vehicles for drug delivery. In this communication, we highlight how Molecular Docking calculations with a tweaked scoring function and Molecular Dynamics (MD) simulations can be used to study the interactions between drugs and CDs. Three cyclodextrin systems (HPαCD, HPβCD, and HPγCD) will be discussed in combination with 36 different drugs. Several analyses have been performed, including root mean squared deviation (RMSD) and the deviation of the drug from the center of geometry of the CD, which enables the determination of energy landscapes and provides essential insight into the optimal binding pose. Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) calculations were also performed. The Molecular Docking and MM/PBSA calculation results were compared with experimental data, revealing a moderate correlation between the vacuum (MM) components of the energy and a poor correlation with the solvation (PBSA) energy, indicating severe limitations in this approach. The MM part was further decomposed into van der Waals and Coulombic contributions, and the number of interactions between the drug and CD was evaluated and correlated with both. It was possible to conclude that most systems tend to show fewer unbinding events with HPβCD and HPγCD, compared to the smaller HPαCD. Despite the prevalence of van der Waals interactions, the Coulomb component appears to have a greater impact on binding, primarily through hydrogen bonding.

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Poster Presentation 11

Integrating Constant-pH Molecular Dynamics to Multiple Force Fields

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Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to accurately model pH effects on the conformational dynamics of biological systems [1,2,3]. In this work [4], we introduce AMBER14SB, a force field particularly suited for studying disordered proteins and membrane channels [5], into the stochastic titration CpHMD (st-CpHMD) method, making it the first to support all three major force field families: GROMOS, CHARMM, and AMBER. We will detail the modifications to the side-chain partial charges of the pH-sensitive amino acids to make them compatible with the Poisson–Boltzmann and Monte Carlo steps used in the st-CpHMD. This strategy was validated and the method's overall performance using AMBER14SB was benchmarked with the experimental data of two proteins (hen egg white lysozyme and staphylococcus nuclease). A comparison of GROMOS 54A7 and CHARMM 36m performance is also shown. In this work, we highlight the method's strengths and shortcomings, while also proposing future enhancements to improve the accuracy and efficiency of CpHMD simulations.

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Poster Presentation 12

Identification of Key Titratable Residues Mediating pH Regulation in hAQP7

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

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Poster Presentation 13

Constant pH Molecular Dynamics (CpHMD) study of analogous peptides from the Mastoparan family

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Antimicrobial peptides (AMPs) are small natural molecules that, in addition to playing a role in innate immunity, have promising applications in medicine, agriculture, and the food industry, among others [1]. In this study, peptide analogs from the Mastoparan family (L1A and L1Am) were designed to explore their insertion in anionic membrane systems. Conventional molecular dynamics (MD) simulations and constant-pH molecular dynamics (CpHMD) simulations were employed to investigate how the peptide's protonation and conformation influence its interactions with the lipid bilayer. The presence or absence of acidic residues significantly affects the peptide's propensity to form alfa-helix upon interacting with the membrane. Experimental analysis with L1A revealed deep insertion into the membrane and significant structural perturbations, supporting its proposed antimicrobial mechanism [2]. This study presents a comprehensive analysis of the dynamics of peptide insertion into the membrane, highlighting its impact on lipid organization and the role of pH in modulating peptide charge and conformation. CpHMD simulations also provide important insights into the key residues that modulate these pH effects, thus advancing our understanding of these systems and improving the rational design of antimicrobial agents.

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Poster Presentation 14

Deciphering the pH-Dependent Mechanisms of Cationic Peptide Dendrimers as Vectors: an in silico approach

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Transfection is a crucial process in therapeutic applications, including gene therapies and vaccines, but many transfection methods face challenges related to cytotoxicity and immunogenicity [1]. Researchers have investigated the use of peptide dendrimers as carriers for siRNA molecules [2]. These branched, tree-like structures, composed of cationic and hydrophobic amino acid residues such as lysine and leucine, form strong interactions with biomolecules, including nucleic acids and biological membranes [2]. Their ability to change protonation states between physiological and low pH is key to binding nucleic acids and facilitating endosomal escape. However, despite extensive experimental research, a comprehensive understanding of the molecular mechanisms underlying the specific properties of these dendrimers remains incomplete [3]. In this study, we report our findings using our advanced CpHMD methodology to explore the pH-dependent conformational behavior of select peptide dendrimers. Simulations were conducted in three environments: an aqueous solution, interaction with a lipid bilayer, and a complex with siRNA, to evaluate how pH influences both conformation and protonation. Our results show that these molecules become highly charged under acidic conditions, and this high charge density is critical for inducing membrane destabilization. These observations align well with experimental data and offer insights into why certain dendrimers struggle with efficient endosomal escape, thereby hindering the transfection process. This understanding of their mechanism of action will be instrumental in designing new peptide dendrimers with enhanced transfection efficiency.

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Poster Presentation 15

Evaluating the effect of side chain torsion angles in G4 targeting molecules

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G-quadruplexes (G4) are secondary DNA/RNA structures formed in guanine-rich sequences. They are involved in several important cellular processes and are present in biologically relevant sequences, such as telomeres and proto-oncogenes. Thus, G4s are promising anti-cancer targets, and both the stabilization and destabilization of these structures with small molecules have been explored as therapeutic strategies [1,2]. The pyrrolo[4,3,2-de]quinolinone (PQ) scaffold is found in several natural alkaloids that exhibit diverse biological activities, including cytotoxicity against cancer cell lines [3]. Due to their aromatic structure, we hypothesize that these compounds can target G4s. In this work, we aim to comprehend the interaction between the PQ derivatives and different G4 structures. More specifically, we aim to determine whether there is a correlation between their binding capabilities and the torsion angles of the aromatic side chains of the PQ derivatives. Three libraries of compounds (26 in total) were screened by molecular docking (AutoDock Vina) using two different PDB structures (5W77 and 3R6R) to identify the most common binding sites. Four binding sites were identified for 5W77 and three for 3R63. Then, for each site, the small library was systematically docked, and both docking score and dihedral angle values were retrieved. The analysis of this data showed that the torsion angles varied considerably and were not directly correlated with the scores. For instance, compounds with the same score can have different torsion angles or vice versa. Additionally, all of the compounds presented good binding energies, being promising G4 binders. These results will guide the design of new derivatives that will be synthesized and their binding strength to G4 evaluated.

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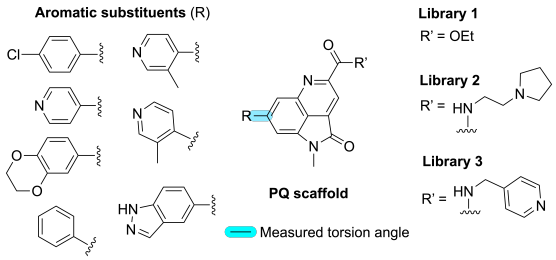

Poster Presentation 16

Knot Your Average Protein: The Role of the Knot in UCH-L1 Activity

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Knotted proteins, with their non-trivial topologies, offer a fascinating window into how protein architecture can influence folding, stability, and function [1]. UCH-L1, a monomeric cysteine protease containing a 5_2 knot, plays an essential role in the ubiquitin-proteasome system and has been implicated in several neurodegenerative diseases [2]. Intriguingly, the proximity of its knotted N-terminal segment to the catalytic site suggests that knotting may influence enzymatic regulation by modulating conformational flexibility and substrate access [3]. To explore this hypothesis, we developed a computational framework combining steered molecular dynamics, umbrella sampling, and molecular dynamics simulations to systematically investigate the energetic and structural consequences of unknotting. By applying controlled forces along selected reaction coordinates, we generated a series of partially unknotted intermediates that captured progressive stages of the unknotting pathway. These intermediates were then analyzed to reconstruct the free energy landscape of knot disruption, allowing for the characterization of associated structural rearrangements. Fully unknotted variants were further simulated in both apo and ubiquitin-bound (holo) states to assess the impact of unknotting on protein stability, substrate binding, and active-site organization. Our findings revealed that, although the knotted topology appears to be slightly energetically favored, it is separated from the unknotted state by a substantial free energy barrier, indicating that knot formation likely occurs early in the folding process. In holo simulations, knot removal shifts the conformational ensemble and alters the population of catalytically active states. Altogether, our observations hint at a model in which catalytic activity may emerge from an interplay between topological constraints and the positioning of the N-terminal, where the knot may help define the spatial arrangement of key elements.

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Poster Presentation 17

Computational Mocel of Phosphatidylinositol protonation: Insights into membrane and Protein interactions.

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Phosphatidylinositols (PIPs) are ubiquitous signaling molecules with distinct biological roles and metabolisms that are tightly controlled. Their structural properties are primarily defined by the characteristics of the polar group, which at physiological pH is highly negatively charged and has the potential to establish strong electrostatic interactions. The global protonation state of PIPs has a significant influence on their binding affinities and specificity for certain protein domains, as well as their interactions with other lipids (including PIPs themselves) and divalent cations. PIPs have been studied at the molecular level using computational methodologies that introduce large approximations on their preferred protonation states. In light of this, our project aims to develop a computational model that accurately describes the protonation dynamics of PIPs. To achieve our goals, we used our in-house constant-pH molecular dynamics (CpHMD) code to simulate the inositol ring, employing the CHARMM force field. The results presented regarding Ins(1,2,3)P3, Ins(1,2,6)P3, and Ins(1,4,5)P3 are in agreement with the experimental data, confirming the model's predictive ability. Equilibration of three membrane systems (PI(3,4,5)P3, PI(4,5)P2, and PI4P) in a phosphatidylcholine (PC) lipid bilayer is currently being performed. PIPs/PC CpHMD simulations will then allow us to quantify the impact of the lipid bilayer on PIPs' pKa values, fully capturing the effect of the environment. This is a crucial step towards understanding how pH affects PIPs' interactions and further comprehending the role of protonation in their binding affinities, which are essential for their diverse functions. In this communication, we will validate our approach by presenting our predictions of the pKa values for Ins(1,2,3)P3, Ins(1,2,6)P3, and Ins(1,4,5)P3, and comparing them with the experimental NMR data.

Poster Presentation 18

CpHMD in a container: the power of the stochastic titration method in everyone's hands

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pH is a physicochemical property that has a significant impact on biological systems, as it affects the protonation and deprotonation of titratable molecules, influencing protein folding, enzyme activity, drug permeability, and binding events, among others. Throughout the years, computational tools have been developed to evaluate pH effects in biological systems, from rigid pKa calculations to higher-level techniques such as lambda dynamics and stochastic Constant pH Molecular dynamics. Currently, the developers of CHARMM, AMBER, and GROMACS, the primary molecular dynamics (MD) software developers, have implemented lambda-dynamics constant-pH MD (CpHMD) in their respective packages. In direct contrast, the stochastic titration (st-)CpHMD, developed by the Baptista and Machuqueiro Labs, is often perceived as having a steep learning curve and being relatively complex to use. In the past two years, we have extended the st-CpHMD methodology also to include the CHARMM 36m and AMBER 14sb force fields. However, the complexity of using this software remains a significant challenge. This year, we focused on improving the st-CpHMD code and making it more user-friendly so that everyone can run st-CpHMD simulations. Building on the advancements in containerization of recent years, we have developed a Singularity container that includes all the necessary machinery to run CpHMD. Inside, we provide the complete CpHMD code, GROMACS, Delphi, and the three supported force fields (GROMOS 54A7, CHARMM 36m, and AMBER 14sb), helping the user avoid the struggle of gathering all the required files. A significant advantage of releasing all software in a container is its independence from the user's system, eliminating the hassle of recompilations due to OS or software upgrades. As a result of this work, we provide a container that can run CpHMD on both CPU and GPU, with high system compatibility, and tools to (i) prepare the system, (ii) extract force fields for custom parameterization, (iii) perform CpHMD simulations, and (iv) analyze simulation results.

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Poster Presentation 19

WHAT IS THE ROLE OF PROTONATION IN CLASS A GPCR (DE)ACTIVATION?

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G protein-coupled receptors (GPCRs) constitute the most prominent family of membrane proteins in the human cell. Being involved in nearly all physiological processes, they are the pharmaceutical targets for approximately 35% of currently approved drugs. Evidence suggests that class-A GPCRs, the largest subfamily, share a conserved mechanism of activation and deactivation characterized by specific changes in their residue interaction networks [1]. Although the residue Asp2.50 has been proposed as a microswitch governing this mechanism, its precise functional contribution remains unclear [2]. To investigate this, we conducted pKa calculations based on 100-ns molecular dynamics (MD) simulations using the CHARMM36m force field. We analyzed five distinct class-A GPCRs in both active and inactive states, as well as in both protonated and deprotonated forms of Asp2.50. Macroscopic pKa estimates were initially obtained using a linear response approximation. We identified a clear pKa shift (1.3 units) between active and inactive conformations for non-constitutive GPCRs, while constitutively active receptors showed minimal and variable shifts (ranging from –0.3 to 0.3) [3]. Constant-pH Molecular Dynamics (CpHMD) simulations were also run for select receptor systems. Notably, in inactive states, the limited hydration within the Asp2.50 binding pocket impeded accurate Poisson–Boltzmann assessments of solvent accessibility, yielding artificially elevated pKa values. Our CpHMD analyses suggested potential inadequacies in capturing sufficient hydration of Asp2.50 using CHARMM36m, leading to deficient protonation state sampling. Our results underscore the pivotal influence of hydration on accurately modeling Asp2.50 protonation states, highlighting the importance of meticulous system preparation for pKa and protonation analyses. This work presents a refined approach for investigating GPCR activation mechanisms, which may inform future research on receptor functionality and the discovery of novel therapeutic agents.

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Poster Presentation 20

Structure-Guided Discovery of Small Molecules to Prevent TrkB-FL Cleavage in Alzheimer's Disease and Epilepsy

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

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