ISQBP
THE INTERNATIONAL SOCIETY OF QUANTUM BIOLOGY & PHARMACOLOGY

ACADEMY OF ATHENS

BRFAA
BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

ISQBP President's Meeting 2024

19 | 23 May 2024

Athens GREECE

www.isqbp2024.org

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List of Participants **116**

WELCOME

Dear Colleagues and Friends,

It is a pleasure to welcome you to the ISQBP President's Meeting, 19-23 May 2024 taking place at the historical building of the Academy of Athens, Greece, as well as at the Biomedical Research Foundation of the Academy of Athens (BRFAA), which is the most recent addition to the Life Sciences research organizations in Greece.

The International Society of Quantum Biology and Pharmacology is a Society founded in 1970 to provide a forum for chemists, pharmacologists, and biologists to discuss and extend the impact of computational methodologies in the fields of biology, chemistry, medicinal chemistry, chemical biology, drug discovery, pharmacology, bioinformatics, genomics, and proteomics.

The ISQBP President's Meeting is the flagship event of ISQBP being organized biennially for the past 50 years, providing a forum to promote interdisciplinary collaboration and to honor significant scientific contributions in these fields. In this spirit, the ISQBP awards biennially the Computational Biology award, which is awarded this year to Professor Johan Åqvist, Uppsala University, Sweden, as well as an endowed Lectureship honoring Gilda Loew, which is presented by Professor Kenneth Merz, Jr., Michigan State University, USA. Moreover, three awards for the best contributed talk and poster presentations will be given.

Finally, I would like to extend my heartfelt thanks to the ISQBP Board and Founding Members, who have helped in all aspects to organize the Meeting, to the President of BRFAA for his support, to my lab members for their time, to our sponsors for the financial assistance, and to all of you for making this conference a success!

I wish you an exciting ISQBP President's Meeting 2024 and an enjoyable time in the historical and beautiful city of Athens.



Dr. Zoe Cournia
ISQBP President 2022-2024
Senior Investigator, Biomedical Research Foundation Academy of Athens

INVITED SPEAKERS



Johan Åqvist
Uppsala University
SWEDEN



Charlie Brooks
University of Michigan
USA



Matthias Buck
Case Western Reserve University
USA



Andrea Cavalli
CECAM
SWITZERLAND



Vlad Cojocaru
Universitatea Babeș-Bolyai
ROMANIA



Marco de Vivo
Italian Institute of Technology
ITALY



José Duca
Novartis Institutes of Biomedical Research
USA



Serdar Durdağı
Bahçeşehir University
TURKEY



Evi Gkeka
Sanofi
FRANCE



Frauke Gräter
Heidelberg Institute for Theoretical Studies
GERMANY



Ellinor Haglund
University of Hawaii Manoa
USA



Sarah Harris
University of Leeds
UK



Fahmi Himo
Stockholm University
SWEDEN



Wonpil Im
Lehigh University
USA



William Jorgensen
Yale University
USA



Themis Lazaridis
City College of New York
USA



Klaus Liedl
University of Innsbruck
AUSTRIA



Alexander MacKerell
University of Maryland
USA



Alessandra Magistrato
Trieste SISSA
ITALY



Nancy Makri
University of Illinois Urbana - Champaign
USA



Thomas Mavromoustakos
University of Athens
GREECE



Kenneth Merz Jr.
Michigan State University
USA



Emmanuel Mikros
University of Athens
GREECE



Sophie Saquin-Mora
CNRS
FRANCE



Modesto Orozco
University of Barcelona
SPAIN



Jean-Philip Piquemal
University of Sorbonne
FRANCE



Carol Post
Purdue University
USA



Nigel Richards
Cardiff University
UK



Adrian E. Roitberg
University of Florida
USA

PROGRAM

SUNDAY 19 May 2024

ACADEMY OF ATHENS, CENTRAL BUILDING, EAST CONFERENCE ROOM

16:00-18:00	**REGISTRATION**
18:00	**WELCOME REMARKS** • **Zoe Cournia** (ISQBP President) • **Lucas Papademos** (President of the Board, Biomedical Research Foundation, Academy of Athens, Greece)
18:15-18:20	**INTRODUCTION TO THE COMPUTATIONAL BIOLOGY AWARD LAUREATE**
18:20-19:20	**COMPUTATIONAL BIOLOGY AWARD LECTURE** **Johan Åqvist,** Uppsala University, Sweden Computer Simulations of the Temperature Dependence of Enzyme Reactions
19:30-22:00	**WELCOME RECEPTION**

MONDAY 20 MAY 2024

ACADEMY OF ATHENS, CENTRAL BUILDING, EAST CONFERENCE ROOM

09:00-12:40	**FREE ENERGY** Chair: **Marco de Vivo**
09:00-09:30	**William Jorgensen,** Yale University, USA Evolution of Computer-Aided Drug Discovery
09:30-10:00	**Charlie Brooks,** University of Michigan, USA High-throughput approaches to free energy calculations in ligand discovery and protein design
10:00-10:30	**Alex Mackerell,** University of Maryland, USA Site Identification by Ligand Competitive Saturation (SILCS) as a Platform Technology for Computational Investigations of Free Energies of Intermolecular Interactions
10:30-11:15	**COFFEE BREAK**
11:15-11:35	**Jonas Kaidl,** Schrodinger, Germany Is The Functional Response of a Receptor Determined by the Thermodynamics of Ligand Binding?
11:35-12:05	**Klaus Liedl,** University of Innsbruck, Austria Quantifying Thermodynamic Properties in Biological Systems by Grid Inhomogeneous Solvation Theory
12:05-12:25	**Nour Aldin Kahlous,** Uppsala University, Sweden Design of drug efficacy guided by free energy simulations of G protein-coupled receptors
12:25-12:40	**Nishita Mandal,** Adam Mickiewicz University, Poland Beyond the Threshold: Delving into Rare Gate Opening Events in Enzyme Tunnels through Enhanced Molecular Dynamics Simulations
12:40-14:00	**FREE TIME | LUNCH ON YOUR OWN**
14:00-18:20	**BIOMOLECULAR DYNAMICS I** Chair: **Roland Stote**
14:00-14:30	**Carol Post,** Purdue University, USA Syk tyrosine kinase association with immunoreceptors: MD simulation unravels the physical basis for a new phospho-sensor regulating this protein-protein interaction
14:30-15:00	**Ellinor Haglund,** University of Hawaii Manoa, USA Exploring Pierced Lasso Topologies: Novel Insights into Protein Folding, Function, and Therapeutic Potential
15:00-15:20	**Jan Brezovsky,** Adam Mickiewicz University, Poland Exposing unseen passages for ligand transport through proteins and their functional consequences
15:20-15:50	**Frauke Gräter,** Heidelberg Institute for Theoretical Studies, Germany Learning force fields and reactivity to enhance classical biomolecular simulations
15:50-16:30	**COFFEE BREAK**
16:30-17:00	**Wonpil Im,** Lehigh University, USA What can CHARMM-GUI do for you?
17:00-17:20	**Jennifer Sapia,** University of Fribourg, Switzerland Computational insights into the mechanism of triglycerides formation: a study of DGAT1 using molecular dynamics
17:20-17:40	**Paulo Siani,** Università degli Studi di Milano Bicocca, Italy Mechanistic Insights from Molecular Dynamics Simulations in Nanomedicine Research
17:40-18:00	**Thibault Tubiana,** CNRS, France Molecular modelling, homo-oligomerisation and membrane interactions of hepatitis E virus pORF1 replication polyprotein
18:00-18:20	**Fabrizio Pucci,** Université Libre de Bruxelles, Belgium Leveraging structural and (co)evolutionary data for protein fitness prediction

TUESDAY 21 May 2024

ACADEMY OF ATHENS, CENTRAL BUILDING, EAST CONFERENCE ROOM

09:00-12:30	**QUANTUM MECHANICS AND QUANTUM COMPUTING** Chair: **Julian Tirado-Rives**
09:00-09:30	**Jean-Philip Piquemal,** University of Sorbonne, France Hybrid Quantum-classical algorithms for Accurate Quantum Chemistry
09:30-10:00	**Nancy Makri,** University of Illinois, Urbana-Champaign, USA Real-Time Path Integral Simulation of Energy Transfer in Light Harvesting Complexes
10:00-10:20	**Océane Mangel,** Université Grenoble Alpes Study of the Streptomyces tyrosinase reaction mechanism: a dynamic QM/MM approach
10:20-11:00	**COFFEE BREAK**
11:00-11:30	**Fahmi Himo,** Stockholm University, Sweden Mechanism of Vanadium Chloroperoxidase from Quantum Chemical Calculations
11:30-11:50	**Sergio Sousa,** University of Porto, Portugal Probing the Role Played by Different Amino Acid Residues in the Catalytic Mechanism of Plastic PET degrading by QM/MM Methods
11:50-12:10	**Jon Uranga,** University of the Basque Country, Spain A Lysine-Cysteine Redox Switch: The NOS Bridge
12:10-12:30	**Elise White**, University of Bristol, UK Multi-scale simulations provide insights for engineering product outcome of polyketide synthases
12:30-14:00	**RELOCATION FROM THE ACADEMY OF ATHENS TO BRFAA WITH BUSES | LUNCH AT BRFAA**
14:00-16:00	**BIOMOLECULAR DYNAMICS II** Chair: **Annick Dejaegere**
14:00-14:30	**Nigel Richards,** Cardiff University, UK Hidden Conformational Dynamics Controlling Ammonia Transport in Human Asparagine Synthetase
14:30-15:00	**Themis Lazaridis**, City College of New York, USA Classical simulations with proton transfer: insights into proton channels
15:00-15:30	**Sophie Sacquin-Mora,** CNRS, France Investigating the dynamics of protein-protein interfaces in well structured and fuzzy complexes
15:30-16:00	**Matthias Buck,** Case Western Reserve University, USA Learning to use AlphaFoldMultimer and variants - amazing to not so good for transmembrane proteins
16:00-16:30	**COFFEE BREAK**
16:30-17:15	**LOEW LECTURESHIP** Chair: **Zoe Cournia**
	Kennie Merz, Michigan State University, USA Transition Metal Modeling in Aqueous Solution
17:15-20:00	**POSTER SESSION**

WEDNESDAY 22 May 2024

BIOMEDICAL RESEARCH FOUNDATION, ACADEMY OF ATHENS

09:00-12:40	**COMPUTER-AIDED DRUG DESIGN** Chair: **Evi Gkeka**
09:00-09:30	**José Duca,** Novartis Institutes of Biomedical Research, USA Modeling the undruggable; from the computer to the clinic
09:30-10:00	**Marco De Vivo,** Italian Institute of Technology, Italy Targeting the conserved active site of splicing machines
10:00-10:30	**Thomas Mavromoustakos,** University of Athens, Greece Computational Analysis and Drug Discovery
10:30-11:00	**COFFEE BREAK**
11:00-11:20	**Minos Matsoukas,** University of West Attica, Greece Computational approaches in targeting GPCRs and cancer related proteins with small molecules
11:20-11:50	**Serdar Durdağı,** Bahçeşehir University, Turkey Development of New Virtual Screening Methods Incorporating Artificial Intelligence Approaches to Accelerate the Transition from Preclinical to Clinical Stages in Drug Development
11:50-12:20	**Manos Mikros,** University of Athens, Greece In silico tools for the discovery and optimization of new bioactive compounds
12:20-12:40	**Özge Kürkcüoglu,** Instabul Technical University, Turkey In Silico Identification of Allosteric Sites and Modulators on GPCRs Using Residue Network Model and Site Identification by Ligand Competitive Saturation
12:40-14:00	**LUNCH BREAK | POSTER SESSION**
14:00-17:30	**NUCLEIC ACIDS MODELING** Chair: **Thomas Cheatham**
14:00-14:30	**Modesto Orozco,** University of Barcelona, Spain Frontiers in molecular dynamics of nucleic acids
14:30-15:00	**Evi Gkeka,** Sanofi, France Identifying small-molecules binding sites in RNA conformational ensembles with SHAMAN
15:00-15:20	**Nathalie Basdevant,** Université d'Evry Paris Saclay, France DNA translocation through alpha-hemolysin nanopore using the MARTINI coarse-grained force field
15:20-15:40	**Maxime Kermarrec,** Ecole Normale Supérieure de Lyon, France Mapping the Radical Cation Guanine in the nucleosomal DNA using QM/MM approaches
15:40-16:00	**COFFEE BREAK**
16:00-16:30	**Alessandra Magistrato,** Trieste SISSA, Italy Molecular Mechanisms of RNA Metabolism
16:30-17:00	**Vlad Cojocaru,** Universitatea Babeș-Bolyai, Romania Transcription factors meet chromatin under the computational nanoscope
17:00-17:30	**Sarah Harris,** University of Leeds, UK Multi-Scale Simulations of DNA Supercoiling

WEDNESDAY 22 May 2024

BIOMEDICAL RESEARCH FOUNDATION, ACADEMY OF ATHENS

17:30-18:05	**FLASH TALKS** Chair: **Klaus Liedl**
	Isidora Diakogiannaki, University of Naples Federico II, Italy Modeling A40s Aptamer for Advancing Glioblastoma Therapy
	Petr Jurečka, Palacky University Olomouc, Czech Republic Developing Force Field for Nucleic Acids Simulations - Both Canonical and Non-canonical
	Gianfranco Martino, University of Bologna, Italy MD simulatons identify precise and dynamic protein cooperation for the spliceosomal splicing
	Fethiye Aylin Sungur, Istanbul Technical University, Turkey A Multiscale Modelling Study to Elucidate the Citrullination Reaction of the Protein Arginine Deiminase 2 and Identifying New Hit Compounds
	Roxana Geanina Vasarhelyi, Babes-Bolyai University, Romania Exploring RNA-protein interactions: from binding modes to sequence specificity through molecular docking and simulations
	Alessia Visigalli, Italian Institute of Technology, Italy Molecular Dynamics Simulations to study translocation in Human DNA Polymerase
	Merve Yuce, Istanbul Technical University, Turkey Identification of Potent E. coli Ribosome Inhibitors Using a Computationally Efficient Workflow
18:05-19:30	**ISQBP Business Meeting**
20:30	**CONFERENCE DINNER**

THURSDAY 23 May 2024

BIOMEDICAL RESEARCH FOUNDATION, ACADEMY OF ATHENS

09:00-11:00	**BIOMOLECULAR DYNAMICS AND DRUG DISCOVERY** Chair: **Minos Matsoukas**
09:00-09:30	**Andrea Cavalli,** CECAM, Switzerland Physics-based approaches to computational drug discovery
09:30-10:00	**Vangelis Daskalakis,** University of Patras, Greece CRISPR-Cas proteins: Engineering of "silver-bullets" in the arsenal of gene editing
10:00-10:20	**Nishita Mandal,** Adam Mickiewicz University, Poland Probing Ligand Transport Pathways in Proteins with Buried Active Sites: Insights from Coarse-Grained Molecular Dynamics Simulations
10:20-10:40	**Giulia Paiardi,** Heidelberg University, Germany The role of heparin in spike SARS-COV-2 infection: From a model for heparan sulfates to a starting structure for antivirals
10:40-11:00	**Alexios Chatzigoulas,** Biomedical Research Foundation, Academy of Athens, Greece Predicting protein-membrane interfaces of peripheral membrane proteins using AI
11:00-11:30	**COFFEE BREAK**
11:30-12:40	**FORCE FIELD DEVELOPMENT** Chair: **Zoe Cournia**
11:30-12:00	**Adrian Roitberg,** University of Florida, USA Machine Learning Potentials for Molecular Modeling. ANAKIN, The Next Generation
12:00-12:20	**Franz Waibl,** ETH Zürich, Switzerland Validating small-molecule force fields for simulation of macrocycles
12:20-12:40	**Panagiotis Koukos,** Biomedical Research Foundation, Academy of Athens, Greece Martini 3 force field parameters for protein lipidation post-translational modifications
12:40-12:50	**BEST TALK AND POSTER AWARDS**
12:50	**CLOSING REMARKS AND FAREWELL - END OF CONFERENCE**

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ABSTRACTS OF AWARD LECTURES

COMPUTATIONAL BIOLOGY AWARD LECTURE

Computer Simulations of the Temperature Dependence of Enzyme Reactions

Johan Åqvist

Dept. of Cell & Molecular Biology, Uppsala University
Biomedical Center, 751 24 Uppsala, Sweden

The speed of chemical reactions in water and in enzymes varies with temperature, depending on how the free energy of activation is partitioned into enthalpy and entropy. In enzymes, this partitioning is also optimized as a consequence of the organism's adaptation to the environment. Cold-adapted enzymes from psychrophilic species show the general characteristics of being more heat labile and having a different balance between enthalpic and entropic contributions to free energy barrier of the catalyzed reaction, compared to mesophilic orthologs. It is precisely this altered enthalpy-entropy balance that results in a higher catalytic activity at low temperatures. We will show how the temperature dependence of enzymic reaction rates can be obtained from brute force computer simulations. Such calculations shed new light on how enzyme structures have evolved in differently adapted species and allow for computational design of the temperature dependence of catalysis.

LOEW LECTURESHIP

Transition Metal Modeling in Aqueous Solution

Kenneth M. Merz, Jr.

Lerner Research Institute, Cleveland Clinic, 9620 Carnegie Avenue, N Building, Cleveland, Ohio 44106;
Departments of Chemistry and Molecular and Cellular Biology, Michigan State University,
578 S. Shaw Lane, East Lansing, Michigan 48824, United States

Though transition metal ions play an essential role in many cellular processes, they are extremely toxic at high concentrations. Biological metal ion homeostasis maintains the intracellular concentrations of these metal ions through a number of metal sensor proteins that regulate the expression of genes that encode specific transition metal efflux/influx and sequestration proteins. In particular, multiple transition ion efflux and influx pumps have been characterized, but in order to model this class of proteins the interaction of these ions with typical coordinating side chains (*e.g.*, His and Glu/Asp) are essential. To tackle this class of problem we have had to develop novel computational tools and approaches to transition metal ions in aqueous solution. Specifically, we will briefly discuss the history of classical modeling of TM ions and will highlight the 12-6-4 modification to the classic Lennard-Jones (12-6) potential that allows us to accurately model TM ions in condensed phases. Using the 12-6-4 model we will discuss TM ion solvation and the classic coordination chemistry processes involving imidazole and acetic acid as surrogates for the protein side chains of His and Glu/Asp, respectively. Finally, using these novel parameter sets we will describe the direct observation of the elevator transport mechanism of the Zrt/Irt-like protein (ZIP) Zn(II) transporter. With the 12-6-4 model in hand we are now able to tackle a myriad of coordination chemistry problems in chemistry and biology.

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ABSTRACTS OF INVITED LECTURES

Evolution of Computer-Aided Drug Discovery

William L. Jorgensen, Julian Tirado-Rives

Department of Chemistry, Yale University, New Haven, CT 06520-8107 USA

Over the last thirty years, computer modeling has evolved into an essential, widespread activity in drug discovery. The contributions of our group have been in multiple areas including virtual screening, prediction of ADME properties, and FEP-guided lead optimization. The advances were all accompanied by development of new software including the BOMB program for *de novo* design, QikProp for AI-based estimation of pharmacological properties and metabolites, and MCPRO for Monte Carlo simulations of protein-ligand complexes. To pursue prospective studies, our efforts were expanded to include synthesis, biological assaying, and crystallography. Emphasis has been placed on optimization of the hits from virtual screening to yield potent, drug-like inhibitors. MC/FEP simulations are used to identify the most promising choices for substituents on rings, heterocycles, and linking groups. The illustrated applications center on inhibitor design for HIV-1 reverse transcriptase and SARS-CoV-2 main protease. Micromolar leads have been rapidly advanced to low nanomolar inhibitors. Key computational issues are also addressed.

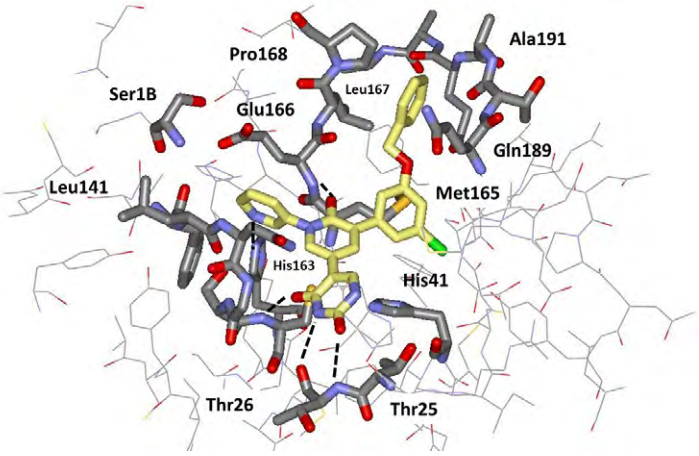


Figure 1. Rendering from a 1.8-Å crystal structure for a complex with the main protease of SARS-CoV-2. Carbon atoms of the inhibitor are in yellow.

[1] Computer-Aided Discovery of Anti-HIV Agents. Jorgensen, W. L. *Bioorg. Med. Chem*. **2016**, *24*, 4768-4788.

[2] Potent non-covalent inhibitors of the main protease of SARS-CoV-2 from molecular sculpting of the drug parampanel guided by free-energy perturbation calculations. Zhang, C.-H.; Stone, E. A.; Deshmukh, M.; Ippolito, J. A.; Ghahremanpour, M. M.; Tirado-Rives, J.; Spasov, K. A.; Zhang, S.; Takeo, Y.; Kudalkar, S. N.; Liang, Z.; Isaacs, F.; Lindenbach, B.; Miller, S. J.; Anderson, K. S.; Jorgensen, W. L. *ACS Central Sci.* **2021**, *7*, 467-475.

[3] Computation of Absolute Binding Free Energies for Non-covalent Inhibitors with SARS-CoV-2 Main Protease. Ghahremanpour, M.; Saar, A.; Tirado-Rives, J.; Jorgensen, W. L. *J. Chem. Info. Model.* **2023**, *63*, 5309-5218.

High-throughput approaches to free energy calculations in ligand discovery and protein design

Charles L. Brooks III

Warner-Lambert/Parke-Davis Chair in Chemistry, Cyrus Levinthal Distinguished University Professor of Chemistry and Biophysics
Departments of Chemistry and Biophysics, University of Michigan

The rapid exploration of functionally relevant chemical spaces associated with the design and refinement of small molecules as potential therapeutic agents and in the design of protein sequences in functional biologics are key to the identification of new or improved treatments for disease. Multi-site λ-Dynamics (MSλD) provides a rigorous statistical mechanically framework for computationally-based free energy calculations that address this goal. MSλD enables the exploration of combinatorically complex chemical spaces at a level of precision that mirrors conventional pairwise free energy approaches but utilizing a fraction of the computer wall time. In this talk, we will illustrate the basic formalism and computational infrastructure of MSλD through examples to several ligand optimization problems comprising tens to hundreds of molecules being explored simultaneously, as well as recent examples in which a sequence space of 32K protein sequences is explored in a single MSλD free energy simulation. These case studies will demonstrate the enhanced ability to explore vast chemical spaces with rigorous free energy methods. Beyond the current developments within the MSλD methodology, we will describe the ability to integrate these calculations into workflows that bring together commonly used Python tools, such as RDKit, OpenBabel, BioPython, etc., with our recent development of pyCHARMM, a Python architecture that enables all of the CHARMM functionality, including our recently developed Basic Lambda Dynamics Engine (BLaDE) GPU accelerated MSλD and molecular dynamics engine to be executed through Python modules and APIs.

Site Identification by Ligand Competitive Saturation (SILCS) as a Platform Technology for Computational Investigations of Free Energies of Intermolecular Interactions

Alexander D. MacKerell, Jr., Anmol Kumar, Erik Nordquist, Wenbo Yu, Mingtian Zhao

Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 20 Penn Street, Baltimore, MD, USA

SILCS involves mapping the functional group affinity pattern of macromolecular systems based on a precomputed ensemble of the distribution of solutes and water obtained from combined oscillating excess chemical potential Grand-Canonical Monte Carlo/Molecular Dynamics simulations. The solutes are low molecule-weight compounds that represent different functional groups ranging from aromatics and aliphatics to polar neutral compounds to charged species. From the precomputed ensemble "FragMaps" are produced that represent the functional group free energy affinity pattern relative to aqueous solution in the full 3D space of the macromolecular system. Contributions to the free energies include macromolecular flexibility, desolvation, and functional group-macromolecular interactions. Once precomputed the SILCS FragMaps may be used for a variety of free energy related computations in a highly computationally accessible manner. These include binding affinities of drug-like molecules, in silico screening or lead compound optimization targeting proteins or RNA, fragment-based drug design, determination of the full distributions of ligands in the 3D space of macromolecules for use in allosteric binding site identification, protein-protein interactions, glycan-protein interactions, and the rational design of excipients for biologics-based drugs. In addition, the SILCS methodology may be applied to membranes allowing for investigations of the partitioning of molecules in interfacial systems and prediction of permeabilities in conjunction with artificial intelligence-based models. Recent advances include the use of the method to estimate unbinding kinetics of drug-like molecules, accounting for the presence of explicit water in ligand-protein docking, and its application in large, heterogeneous systems. An introduction to the SILCS technology and selected applications will be presented.

Conflict of Interest: ADM is cofounder and CSO of SilcsBio LLC

Quantifying Thermodynamic Properties in Biological Systems by Grid Inhomogeneous Solvation Theory

Klaus Liedl

University of Innsbruck, Austria

Advancements in Grid Inhomogeneous Solvation Theory (GIST) aiming at quantifying thermodynamic properties in biological systems are presented. The method directly calculates hydrophobicity from enthalpic and entropic contributions, enabling the identification of regions with specific enthalpic and entropic properties. Additionally, an extended version of GIST facilitates the calculation of partition coefficients between water and chloroform, showing high correlation with experimental data and aiding in drug development.

Further applications include assessing cell permeability of macrocycles, where GIST demonstrates a strong correlation with experimental data and emphasizes the importance of conformational sampling. The extension of GIST to various solvent molecules allows for reliable calculation of localized thermodynamic properties, significantly broadening its applicability. Moreover, an extension to estimate solvation properties involving salt–water mixtures enhances its usefulness in fields ranging from biopharmaceuticals to environmental research. We also provide a tutorial on GIST analysis, offering practical guidance and addressing common pitfalls. Furthermore, a GPU-accelerated version of GIST is introduced, providing efficient estimation of solvation free energy for evaluating protein surface hydrophobicity.

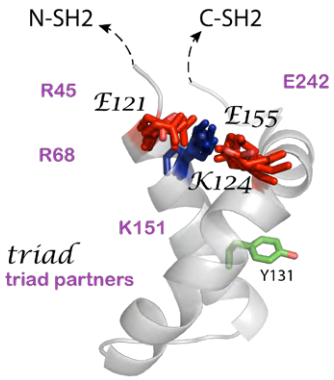
Finally, a physics-based approach using GIST to describe antibody hydrophobicity based on hydration free energy is presented, emphasizing the importance of dynamic conformational ensembles for accurate predictions. These advancements collectively contribute to a more comprehensive understanding of thermodynamic properties in biological systems, with implications for drug development and biomolecular interactions.

Syk tyrosine kinase association with immunoreceptors: MD simulation unravels the physical basis for a new phospho-sensor regulating this protein-protein interaction

Carol Beth Post, Duy P. Hua, Chao Feng, Jacob J. Kinnun

Departments of Medicinal Chemistry and Biological Sciences, Purdue University, West Lafayette, IN, U.S.A.

Spleen tyrosine kinase (Syk) serves a key role in immunity through its ability to couple a variety of membrane immunoreceptors to alternative intracellular signaling pathways that initiate an immune response. Receptor activation leads to the recruitment of Syk to a cytoplasmic region of the receptors called ITAM. Syk associates with high-affinity (nanomolar K_D) to the receptor ITAM. A unique phosphorylation mechanism established from NMR relaxation measurements and isothermal titration calorimetry regulates this proteinprotein interaction; phosphorylation of Syk on a tyrosine distant to the binding site causes substantial reduction in affinity by an increased entropy penalty from conformational disorder, without altering SH2-ITAM binding contacts, and binding enthalpy is unchanged [1-3]. To begin to understand how phosphorylation triggers disorder without affecting binding interactions, we analyzed μs equilibrium molecular dynamics simulations of unphosphorylated and phosphorylated Syk [4]. We discovered the Syk phosphosensor response to be a transition in the interdomain structure (iDS) of the Syk regulatory module from a narrow ensemble with highly correlated motions to a broader ensemble comprising multiple forms of domain structure with greatly diminished correlated motion [5].



A triad of charged residues centrally located in the Syk regulatory module serves as the basis for interdomain electrostatic networks that stabilize the different forms of iDS of unphosphorylated and phosphorylated Syk. The phospho-sensor response illuminated by MD is fully consistent with the domain decoupling measured experimentally, and appears to be exquisitely tuned to achieve a transition from a wellordered iDS to a less ordered iDS, but not one that is fully disorder.

1. Zhang, Y., et al., *Tyr130 phosphorylation triggers Syk release from antigen receptor by long-distance conformational uncoupling.* Proc Natl Acad Sci U S A, 2008. **105**(33): p. 11760-5.
2. Feng, C. and C.B. Post, *Insights into the allosteric regulation of Syk association with receptor ITAM, a multi-state equilibrium.* Physical Chemistry Chemical Physics, 2016. **18**(8): p. 5807-5818.
3. Feng, C., A. Roy, and C.B. Post, *Entropic allostery dominates the phosphorylation-dependent regulation of Syk tyrosine kinase release from immunoreceptor tyrosine-based activation motifs.* Protein Science, 2018. **27**(10): p. 1780-1796.
4. Roy, A., D.P. Hua, and C.B. Post, *Analysis of Multidomain Protein Dynamics.* Journal of Chemical Theory and Computation, 2016. **12**(1): p. 274-280.
5. Hua, D.P., J.J. Kinnun, and C.B. Post, *An electrostatic network with strong connectivity is a phospho sensor for Syk tandem SH2.* submitted, 2024.

Exploring Pierced Lasso Topologies: Novel Insights into Protein Folding, Function, and Therapeutic Potential

Ellinor Haglund

University of Hawaii Manoa, USA

The relationship between structural motifs in proteins has been well-established to influence biology. However, less is known about the biological implications of protein topology. Leptin is the founding member of the Pierced Lasso Topology (PLT), a newly discovered protein family sharing the unique features of a "knot-like" topology. A PLT is formed when the protein backbone pierces through a covalent-loop formed by a single disulfide bond. PLTs are found in all kingdoms of life, with 14-different biological functions, found in different cell compartments. Despite the large number found in nature, where more than 18% of disulfide-linked proteins are folded into a PLT, a connection between topology and biological function has not yet been determined.

Molecular understanding of the folding and function of proteins with complex topologies will aid in the development of therapeutics in the treatment of protein misfolding disorders. Our model protein leptin, a hormone primarily secreted by adipose tissue, plays a crucial role in regulating energy homeostasis and metabolism. The folding of leptin into its native structure is essential for its biological activity, as misfolding can lead to dysfunction and disease. Our results show that the threaded state of leptin folds via a unique flat energy landscape, with few native contacts formed in the transition state ensemble, whereas the linearized protein folds through a diffuse nucleus.

Moreover, the threaded state leads to aggregation from the native state, while the linearized protein aggregates from its unfolded state. We attribute this propensity for aggregation to the development of leptin resistance, a condition linked to obesity and metabolic disorders. Understanding the folding, misfolding, and aggregation of leptin is crucial for elucidating its biological role and developing therapeutic strategies for diseases related to leptin dysfunction.

Learning force fields and reactivity to enhance classical biomolecular simulations

Frauke Gräter

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Proteins are inherently reactive. They undergo post-translational modifications, can be targeted by covalent ligands, and degraded by reactive radicals. I will present our newly developed hybrid kinetic Monte Carlo / Molecular Dynamics (KIMMDY) scheme that incorporates chemical reactions into classical molecular simulations in a highly efficient and yet accurate manner. KIMMDY makes use of reaction barriers predicted by graph neural networks and chooses reactions to occur among the jiggling and wiggling of the protein.

Alongside KIMMDY, we have developed a new framework to parametrize a classical force field. GRAPPA leverages graph attention and a transformer model, outperforms previous conventional and machine-learned force fields in accuracy, properly folds small proteins in water, and can straightforwardly be trained on any chemical species of interest such as radicals.

Using KIMMDY and GRAPPA, we simulate the mechanochemistry of collagen, protein hydrolysis, and polymer chain scission and recombination. Our scale-bridging ML-enhanced simulation technique is applicable to complex – biological as well as synthetic – molecules and materials and can be extended to other chemistries.

[1] Zapp et al, Nat Comm, 2020

[2] Rennekamp et al, JCTC, 2020

[3] Riedmiller et al, Chem Science, 2024

What can CHARMM-GUI do for you?

Wonpil Im

Departments of Biological Sciences and Chemistry, Lehigh University, Bethlehem, PA, USA

Since its original development in 2006, CHARMM-GUI (https://www.charmm-gui.org) has proven to be an ideal web-based platform to interactively build complex molecular systems and prepare their simulation inputs with well-established and reproducible simulation protocols for state-of-the-art molecular simulations using widely used simulation packages. The CHARMM-GUI development project has been widely adopted for various purposes and now contains a number of different modules designed to set up a broad range of molecular simulation systems. Our philosophy in CHARMM-GUI development is less about providing the nuts and bolts of molecular modeling, but instead focused on helping users to achieve a task, such as building a membrane system or solvating a protein, by providing a streamlined interface. This design principle helps us to think of the workflow critically when designing the interface, which leads CHARMM-GUI to be accessible to users with little experience in modeling tools and remains useful to experts, especially for batch generation of systems. The CHARMM-GUI development project is still ongoing. CHARMM-GUI will continue to help expert and non-expert researchers from a broader range of the modeling and simulation community to build the complex molecular systems of their interest and prepare the input files for any general and advanced modeling and simulation through the large and unique scope of CHARMM-GUI functionality, allowing the research community to carry out innovative and novel molecular modeling and simulation research. In this talk, I will present the past, present, and future of the CHARMM-GUI development project, and some applications for specific modules will be also discussed.

Hybrid Quantum-classical algorithms for Accurate Quantum Chemistry

Jean-Philip Piquemal

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Hybrid quantum-classical Adaptive Variational Quantum Eigensolvers (VQE) hold the potential to outperform classical computing for simulating quantum many-body systems. However, their practical implementation on current quantum processing units (QPUs) faces challenges in measuring a polynomially scaling number of observables during the operator selection so as to optimise a high-dimensional and noisy cost function. In this talk, I will present an extension of the ADAPT-VQE strategy denoted Overlap-ADAPT-VQE. The algorithm iteratively generates a compact quantum approximation of a classical target wave function through a quasi-greedy procedure that maximises, at each iteration, the overlap of the current iterate with the target. This is achieved by adding onthe-fly, the most relevant unitary operator from a finite size pool of admissible operators, with a selection criterion based on the gradient of the overlap of the ansatz with the target. I will also detail how the approach can be used for state preparation purpose. Finally, I will present new results obtained with our in-house Hyperion-1 GPUaccelerated quantum emulator and explain how one can use it to perform fully adaptive-VQE simulations using large basis sets.

[1] Overlap-ADAPT-VQE: Practical Quantum Chemistry on Quantum Computers via Overlap-Guided Compact Ansätze. C. Feniou, M. Hassan, D. Traoré, E. Giner, Y. Maday, J.-P. Piquemal, Commun. Phys., **2023,** 6, 192 (Open Access), DOI: 10.1038/s42005-023-01312-y

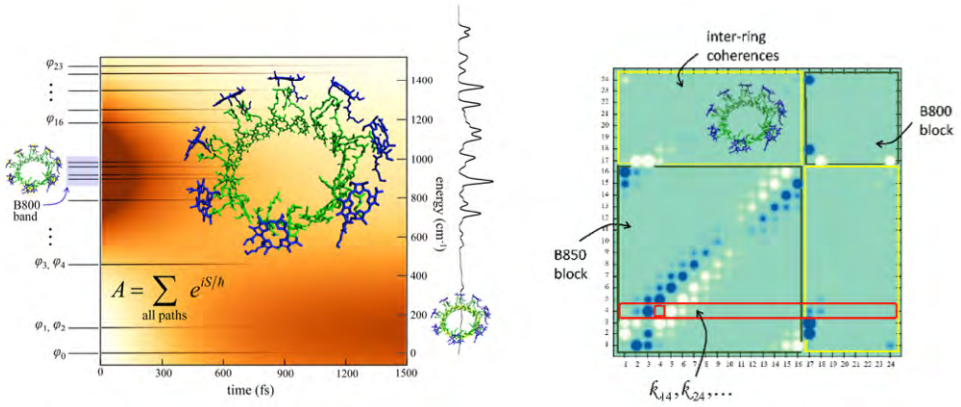
[2] Sparse Quantum State Preparation for Strongly Correlated Systems. C. Feniou, B. Claudon, J. Zylberman, O. Adjoua, E. Giner, J.-P. Piquemal, J. Phys. Chem. Lett., **2024,** online (COVER), DOI: 10.1021/acs.jpclett.3c03159

Real-Time Path Integral Simulation of Energy Transfer in Light Harvesting Complexes

Nancy Makri

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Fully quantum mechanical simulations of excitation energy transfer within the peripheral light harvesting complex (LH2) of *Rhodopseudomonas molischianum* at room temperature have been performed using the numerically exact small matrix decomposition of the quasi-adiabatic propagator path integral (SMatPI). The exciton-vibration Hamiltonian comprises the 16 singly excited bacteriochlorophyll (BChl) states of the B850 (inner) ring and the 8 excited states of the B800 (outer) ring with all available electronic couplings. The electronic states of each chromophore couple to 50 intramolecular vibrational modes with spectroscopically determined Huang-Rhys factors and to a weakly dissipative bath that models the biomolecular environment. The energy trasnfer process is investigated following photoexcitation of individual pigments or electronic eigenstates. The time constant obtained from the simulation results is in very good agreement with experimental values. We find that the flow of energy to the B850 states is enabled by molecular vibrations, and that nuclear quantum effects increase the amount of tranferred energy. Further, the relaxation cascade from B800 eigenstates is characterized by two distinct time scales and is nonmonotonic owing to significant energy shifts of vibrationally dressed states. Coherence maps clarify the intra- and inter-ring pathways of energy transfer and the role of quantum coherence.



[1] N. Makri, Small matrix disentanglement of the path integral: overcoming the exponential tensor scaling with memory length, *J. Chem. Phys.* **2020,** *152*, 041104.

[2] S. Kundu and N. Makri, Real-time path integral simulation of exciton-vibration dynamics in light-harvesting bacteriochlorophyll aggregates, *J. Phys. Chem. Lett.* **2020,** *11*, 8783-8789.

[3] S. Kundu, R. Dani and N. Makri, Tight inner ring architecture and quantum motion of nuclei enable efficient energy transfer in bacterial light harvesting, *Science Advances* **2022,** *8* (43), eadd0023.

[4] S. Kundu, R. Dani and N. Makri, B800-to-B850 relaxation of excitation energy in bacterial light harvesting: All-state, all-mode path integral simulations, *J. Chem. Phys.* **2022,** *157*, 115101.

[5] R. Dani, S. Kundu and N. Makri, Coherence Maps and Flow of Excitation Energy in the Bacterial Light Harvesting Complex 2, *J. Phys. Chem. Lett*. **2023**, *14*, 3835-3843.

Mechanism of Vanadium Chloroperoxidase from Quantum Chemical Calculations

Fahmi Himo

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Vanadium-dependent haloperoxidases (VHPO) catalyze the oxidation of halides to hypohalous acids using hydrogen peroxide:

$$X^- + H_2O_2 + H^+ \rightarrow XOH + H_2O$$

X= Cl, Br, I

Many aspects of the reactivity and selectivity of these enzymes remain unknown. Herein, we use density functional theory calculations employing a large cluster model of the active site (ca 350 atoms) to elucidate the reaction mechanism of chloroperoxidase (VCPO) from *Curvularia inaequalis*. A detailed mechanism is proposed on the basis of the calculations and new insights into the formation of the key vanadium-peroxide intermediate (first half-reaction) and the oxidation of the chloride ion (second half-reaction) are developed.

Very interestingly, in the absence of the halogen substrate, VCPO can catalyze sulfide oxidation to sulfoxide. We have investigated the mechanism and the potential stereochemistry features of this promiscuous reactivity as well. The insights developed by the calculations will be valuable for the design of variants of the enzyme capable of asymmetric sulfoxidation.

Hidden Conformational Dynamics Controlling Ammonia Transport in Human Asparagine Synthetase

Nigel G. J. Richards[1], Adriana Coricello[2], Yuro Takagi[3], Wen Zhu[4], Vincent Chaptal[5], Alanya J. Nardone[4], Carmen Gratteri[6], Antonio Lupia[7,8], Matthijn Vos[9], Stefano Alcaro[6,8]

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[3]Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[4]Chemistry & Biochemistry, Florida State University, Tallahassee, FL 32306, USA
[5]Molecular Microbiology and Structural Biochemistry, CNRS UMR 5086, University of Lyon, 69367 Lyon, France
[6]Dipartimento di Scienze della Salute, Università "Magna Græcia" di Catanzaro, 88100 Catanzaro, Italy
[7]Dipartimento di Scienze della vita e dell'ambiente, Università degli Studi di Cagliari, 09042 Cagliari, Italy
[8]Net4Science Academic Spin-Off, Università "Magna Græcia" di Catanzaro, 88100 Catanzaro, Italy
[9]Centre de Resources et Recherches Technologiques, Institut Pasteur, 75015 Paris, France

How motions in enzymes might be linked to catalytic function is of considerable general interest. Recent advances in X-ray crystallography and cryogenic electron microscopy offer the promise of elucidating functionally relevant motions in proteins that are not easily amenable to study by other biophysical methods. In this lecture I will outline the use of 3D variability analysis (3DVA) [1] on cryo-EM maps for wild type (WT) human asparagine synthetase (ASNS) and the R142I ASNS variant to identify conformational changes in the Arg-142 side chain, which mediates the formation of a catalytically relevant intramolecular tunnel. Our 3DVA results for WT ASNS are consistent with independent molecular dynamics (MD) simulations on a model generated from the X-ray structure of human ASNS [2]. I will also discuss MD simulations of computational models for the ASNS/β-aspartyl-AMP/$MgPP_i$ and R142I/β-aspartyl-AMP/$MgPP_i$ ternary complexes, which suggest that the structural integrity of the tunnel is impaired in the R142I variant only when β-aspartyl-AMP is present in the synthetase active site; a conclusion that is supported by steady-state kinetic experiments. This study illustrates the power of cryo-EM to identify localized motions and to dissect the conformational landscape of large proteins. It also shows how combining MD simulations with 3DVA is a powerful approach to understanding how conformational dynamics might regulate function in multi-domain enzymes possessing multiple active sites.

[1] P.V. Afonine, A. Gobet, L. Moissonnier, J. Martin, B.K. Poon, V. Chaptal, *BBA Biomembranes*, **2023**, *1865*, 184133.$

[2] W. Zhu, A. Radadiya, C. Bisson, S. Wenzel, B.E. Nordin, F. Martínez-Márquez, T. Imasaki, S.E. Sedelnikova, A. Coricello, P. Baumann, A.H. Berry, T.K. Nomanbhoy, J.W. Kozarich, Y. Jin, D.W. Rice, Y. Takagi, N.G.J. Richards, *Commun. Biol*, **2019**, *2*, 345.$.

Classical simulations with proton transfer: insights into proton channels

Themis Lazaridis

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An important limitation of mainstream classical molecular dynamics simulations is their inability to make or break chemical bonds, as in processes involving proton transfer. We recently proposed a simple combination of molecular dynamics with periodic stochastic proton hops. The proton hop acceptance criterion is heuristic, and adjusted to reproduce experimental proton diffusion coefficients and transfer rates. The method has been implemented in the program CHARMM and has been applied to investigate questions related to the structure and function of two proton channels: the influenza M2 [1] and the human voltage gated proton channel Hv1 [2]. In the former system we investigated the origin of asymmetric proton conduction, i.e. the lack of outward proton conduction when the proton gradient is inverted. We identified three factors that may account for asymmetric conduction: hydronium orientation at the barrier, pore shape, and electrostatic trapping. In the Hv1 system we significant differences in the facility of proton permeation among the various proposed structural models for the open and closed state. The hydronium was seen to bypass, rather than break, the Asp-Arg salt bridge.

[1] T. Lazaridis, *Biophysical J*, **2023**, *122*, 90-98

[2] T. Lazaridis, *J Phys Chem B*, **2023**, *127*, 7937-7945

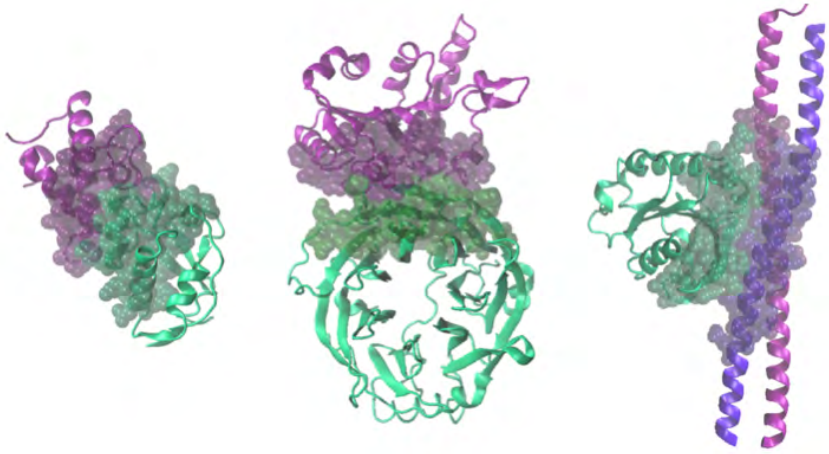
Investigating the dynamics of protein-protein interfaces in well structured and fuzzy complexes

Sophie Sacquin-Mora[a,b], **Chantal Prévost**[a,b]

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[b] Institut de Biologie Physico-Chimique-Fondation Edmond de Rotschild, PSL Research University, Paris, France

The modeling of protein assemblies at the atomic level remains a central issue in structural biology, as protein interactions play a key role in numerous cellular processes. Over the recent years our group started focusing on the dynamic properties of the protein/protein interface using classical modeling tools such as Molecular Dynamics simulations (1).

For globular proteins, predicting the structure of the assembly is traditionally addressed using docking tools, where the quality of the models is based on their similarity to a single reference experimental structure. However, using a static reference does not take into account the dynamic quality of the protein interface. Using all-atom classical MD simulations, we investigate the stability of the reference interface, and of models of various accuracies, for three complexes that previously served as targets in the CAPRI competition. To assess the quality of the models from a dynamic perspective, we set up new criteria which take into account the stability of the reference experimental protein interface (2). We show that, when the protein interfaces are allowed to evolve along time, the original ranking based on the static CAPRI criteria no longer holds.



For IDPs and proteins comprising disordered fragment the issue of dynamics is even more crucial as the protein-protein interface is likely to undergo important conformational changes along time. Here we present the case of the fuzzy complex formed by tubulin and the tau IDP. In particular we highlight the part play by the disordered tubulin C-terminal tails in stabilizing the assembly (3).

[1] E. Karaca, C. Prévost and S. Sacquin-Mora, *Molecules*, **2022**, 27, 1841

[2] C. Prévost and S. Sacquin-Mora, *Proteins*, **2021**, 89, 1315-1323

[3] J. Marien, C. Prévost, and S Sacquin-Mora, *Biochemistry*, **2023**, 62, 2492–2502

Learning to use AlphaFoldMultimer and variants - amazing to not so good for transmembrane proteins

Matthias Buck, Amita Sahoo and Nisha Bhattarai

Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States of America

In this presentation I will provide an overview of work in my lab. on all atom and coarse grained molecular dynamics simulations on transmembrane proteins and our use of AlphaFoldMultimer and related structure prediction programs. Giving a perspective on the field of Artificial Intelligence/Machine Learning based tools with respect to their impact on the understanding of protein biophysics, the report includes studies of transmembrane helix dimer systems [1], the Eph- [2] and Plexin [3] receptors as well as Plexin-small GTPase interactions. Overall it is apparent that there are instances of excellent predictions, but in a significant number of cases, AlphaFold predictions need to be enhanced/filtered with what is biologically and physically reasonable.

[1] Sahoo, A.R., Souza, PCT., Meng,, Y., & Buck, M. (2023) "Transmembrane region dimer structures of Type 1 receptors readily sample alternate configurations: MD simulations using the Martini 3 coarse grained model compared to AlphaFold2 Multimer" *Structure* 31(6):735-745.e2

[2] Sahoo A.R., & Buck M. (2021) "Structural and Functional Insights into the Transmembrane Domain Association of Eph Receptors." Int J Mol Sci. 22:8593

[3] Buck, M. (2021) "Membrane Proteins: The family of Plexin Transmembrane Receptors" 3rd Ed. of Elsevier Encyclopedia of Biological Chemistry 3, 594-610

Modeling the undruggable; from the computer to the clinic

José Duca, PhD

Novartis Biomedical Research, Cambridge MA (USA)

This presentation serves as a collection of unique research vignettes where traditional methodologies could not provide tangible direction or solutions. Where standard practices fell short, the power of computation sprung to action, providing innovative perspectives to drive research forward. Utilizing computationally inspired hypotheses turned out to be invaluable in these scenarios, paving the way for subsequent hit generation and optimization. These computational strategies, coupled with a rigorous and robust approach to experimentation, served as the cornerstone of several successful transitions into the clinical stage.

The advent of the digital age witnesses a substantial surge in the capabilities of machine learning and artificial intelligence methods, both of which have enriched our scientific exploration. This presentation will delve into some of the opportunities presented by these advanced digital tools: how to deploy computational and experimental approaches, powered by AI and ML technologies, on a scale that is unprecedented.

Targeting the conserved active site of splicing machines

Marco de Vivo

Laboratory of Molecular Modelling & Drug Discovery
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In my contribution, I will illustrate our current efforts in targeting RNA, using small organic molecules. Specifically, I will explain our work on targeting the conserved active site of splicing machines. This work is based on our mechanistic investigations of the self-splicing group II introns, which are bacterial and organellar ancestors of the nuclear spliceosome. I will show how we integrate atomistic molecular simulations and alchemical free energy calculations with enzymatic and crystallographic studies to design potent splicing modulators. Furthermore, I will delve into the mechanism by which the evolutionarily conserved RNA core of splicing machinery exhibits specificity in recognizing small molecules. This capability paves the way for designing innovative splicing modulators potentially applicable to a broad spectrum of human diseases.

[1] Targeting the conserved active site of splicing machines with specific and selective small molecule modulators

I. Silvestri, J. Manigrasso, A. Andreani, N. Brindani, M. De Vivo, M. Marcia https://doi.org/10.1101/2023.06.21.545906 - available in BioRxiv – **2024.**

Development of New Virtual Screening Methods Incorporating Artificial Intelligence Approaches to Accelerate the Transition from Preclinical to Clinical Stages in Drug Development

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[a]Computational Biology and Molecular Simulations Laboratory, Department of Biophysics, School of Medicine, Bahçeşehir University, Istanbul, Türkiye;
[b]Lab for Innovative Drugs (Lab4IND), Computational Drug Design Center (HITMER), Bahçeşehir University, Istanbul, Türkiye;
[c]Molecular Therapy Lab, Department of Pharmaceutical Chemistry, School of Pharmacy, Bahçeşehir University, Istanbul, Türkiye;
[d] Department of Biostatistics and Medical Informatics, School of Medicine, Bahçeşehir University, Istanbul, Türkiye

Virtual screening is one of the indispensable computational tools in drug development and medicinal chemistry and offers the potential to accelerate the identification of promising drug candidates. However, this approach faces significant challenges and limitations.[1-3] The accuracy and quality of virtual screening results are highly dependent on the models used and can produce misleading results when generated with incorrect parameters or insufficient data. Furthermore, virtual screening is constrained by its inability to access comprehensive information regarding target proteins and chemical compounds, potentially overlooking crucial interactions and drug candidates. Despite its utility, current virtual screening methods often fail to fully capture the complexity of molecular interactions, particularly with regards to orally administered molecules encountering barriers such as cell membrane crossing and metabolism. In our laboratory, over the last decade, we have sought innovative solutions by identifying the shortcomings of existing virtual screening approaches through methodological developments and application-oriented studies. By integrating machine learning techniques into ligand- and structure-based CADD approaches, our goal is to increase the effectiveness, accuracy, and efficiency of virtual screening methods in drug design, discovery, and development research. The talk will cover the studies on the discovery of new enzyme inhibitors and protein-protein interaction inhibitors that we carry out in our laboratory for this purpose.[3-5]

[1] N. B. Iyison, K. Kahveci, M.B. Duzgun, A.E. Atis, A. Yilmaz, A. Shahraki, B. Coskun, S. Durdagi, *Nature Comm.* **2024** (in press)

[2] S. Durdagi, T. Avsar, M.D. Orhan, M. Serhatli, B.K. Balcioglu, H.U. Ozturk, A. Kayabolen, Y. Cetin, S. Aydinlik, T. Bagci-Onder, S. Tekin, H. Demirci, M. Guzel, A. Akdemir, S. Calis, L. Oktay, I. Tolu, Y.E. Butun, E. Erdemoglu, A. Olkan, N. Tokay, S. Isik, A. Ozcan, E. Acar, S. Buyukkilic, Y. Yumak, *Molecular Therapy*, **2022**, *30(2)*, 963-974.

[3] S. Calis, B. Dogan, S. Durdagi, A. Celebi, O. Yapicier, T. Kilic, E.T. Turanli, T. Avsar. *Cell Death Discov.* **2022**, 8(1), 433. doi: 10.1038/s41420-022-01225-9.

[4] E. Sayyah, L. Oktay, H. Tunc, S. Durdagi, *ChemMedChem*, **2024**, in press: DOI: 10.1002/cmdc.202300644

[5] S. Ikram, E. Sayyah, S. Durdagi, *ChemBioChem*, 2024, in press, DOI: 10.1002/cbic.202400008

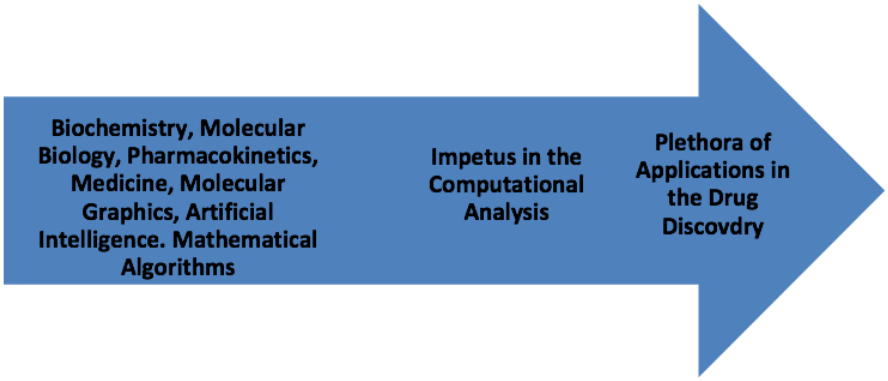
Computational Analysis and Drug Discovery

T. Mavromoustakos

National Kapodistrian University of Athens, Chemistry Department, Laboratory of Organic Chemistry

Computational Analysis has become a valuable tool in in Drug Discovery. This is attributed to the recent advancements of various fields such as Biochemistry, Molecular Biology, Pharmacokinetics, Pharmacodynamics, Physics and Medicine. The advancements in the Molecular Graphics, artificial intelligence and versatility of mathematical algorithms provided a further impetus in the applied computational analysis related to the drug discovery.

Recent examples from our research activities will illustrate the aid of Computational Analysis in the Drug Discovery. Such examples include the rational design of discovery of new leads for the treatment of the risk factor hypertension and the major neurodegenerative diseases of Alzheimer, Parkinson and multiple sclerosis.



In silico tools for the discovery and optimization of new bioactive compounds

Emmanuel Mikros

Department of Pharmacy, University of Athens, Panepistimiopolis Zografou, Greece

Drug discovery is a laborious and lengthy process spread over a sequence of phases. In the last decades the preclinical stages of drug discovery pipeline have been considerably shortened by successful implementation of innovative physicochemical methods for screening of compound libraries as well as chemoinformatics and bioinformatics methods for primary hit identification, hit-to-lead optimization and rational drug design. Of utmost importance in the discovery of new and promising drug candidates are the various computational algorithms used for predicting the chemical affinity of small molecules to therapeutically relevant biological targets in silico. Solvation mapping algorithms can be a valuable tool suggesting possible lead modifications. By identifying hydration sites in the vicinity of the ligand hints solvation mapping can provide hints for the optimization of the protein–ligand interactions increasing enthalpic gain and decreasing unfavorable entropy.

The aim of this presentation is to illustrate examples of the implementation of computational tools in conjunction with screening using biophysical methods for the identification and optimization of bioactive compounds. In a first example we address issues related to robustness of virtual screening in a systematic manner by comparing in silico calculations performed under a consensus ranking scheme with the in vitro experimental evaluation of a small chemical library against four well validated kinase targets. In a second example, we shall demonstrate how consensus ranking can be used in close relation with computational mapping of the binding site solvation to hit-to lead optimization of pharmacological targets like the bromodomain PB1 and estrogen receptor.

Frontiers in molecular dynamics of nucleic acids

Modesto Orozco

Institute for Research in Biomedicine, Baldiri i Reixac 10, Barcelona 08028, Spain

After decades of force-field improvements, molecular dynamics (MD) simulations of nucleic acids have reached maturity and have predictive power, even in the case of properties that were not considered at all during the parametrization of the force-fields. This success is opening the universe of systems to be subjected to simulation, with a long-term dream: to simulate real nucleic acids, i.e., chromatin and long RNAs with complex folds. I will summarize during my talk what are the current challenges and the recent methodological advances designed to treat ultra-large systems of biological revelevance.

Identifying small-molecules binding sites in RNA conformational ensembles with SHAMAN

Paraskevi Gkeka, Francesco-Paolo Panei, Massimiliano Bonomi

The rational targeting of RNA with small molecules is hampered by our still limited understanding of RNA structural and dynamic properties. Most in silico tools for binding site identification rely on static structures and therefore cannot face the challenges posed by the dynamic nature of RNA molecules. Here, we present SHAMAN, a computational technique to identify potential small-molecule binding sites in RNA structural ensembles. SHAMAN enables exploring the conformational landscape of RNA with atomistic molecular dynamics and at the same time identifying RNA pockets in an efficient way with the aid of probes and enhanced-sampling techniques. In our benchmark composed of large, structured riboswitches as well as small, flexible viral RNAs, SHAMAN successfully identified all the experimentally resolved pockets and ranked them among the most favorite probe hotspots. Overall, SHAMAN sets a solid foundation for future drug design efforts targeting RNA with small molecules, effectively addressing the long-standing challenges in the field.

Molecular Mechanisms of RNA Metabolism

Alessandra Magistrato

National Research Council-Institute of Material Foundry (CNR-IOM) at Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste (Italy)

Complex protein/RNA macromolecular engines, catalyze formation of mature messenger and ribosomal RNAs from their precursors, giving rise to functional RNAs.

In this talk I will illustrate how atomic-level simulations have contributed to elucidate: (i) the function of specific protein components of the spliceosome assembly [1,2]; (ii) the chemical details of premature-messenger and premature-ribosomal RNA splicing [3-4], and (iii) the mechanism of spliceosome helicases [7], and (iv) can contribute to refine the cryo-EM structures of complex RNA-based machineries.

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[4] J Aupič, J Borišek, SM Fica, WP Galej, A Magistrato Monovalent metal ion binding promotes the first transesterification reaction in the spliceosome Nature Communications 2023 14 (1), 8482

[5] Movilla, S.; Roca, M.; Moliner, V.; Magistrato, A Molecular Basis of RNA-Driven ATP Hydrolysis in DExH-Box Helicases. *J. Am. Chem. Soc.* 2023, https://doi.org/10.1021/jacs.2c11980

Transcription factors meet chromatin under the computational nanoscope

Vlad Cojocaru

STAR-UBB Institute, Babeş-Bolyai University, Cluj-Napoca, Romania
Computational Structural Biology Group, Utrecht University, Utrecht, the Netherlands

Transcription factors are essential, sequence specific, DNA-binding proteins that regulate gene expression and define cellular identity. How the binding of these proteins to small sequences of DNA provokes highly specific cascades of events leading to the activation or repression of transcription is still not understood. In the cell nucleus, the DNA is packaged into chromatin, a highly dynamic structure formed of repeating structural units known as nucleosomes. In a nucleosome 145-147 DNA base pairs are wrapped around an octamer of histone proteins in which each of the four histones is present twice. A special class of transcription factors known as pioneer factors are capable of binding DNA in closed, inactive states of chromatin, recognizing their sequence specific binding sites on the nucleosome and inducing local opening of chromatin. How pioneer factors induce the opening of nucleosomes and how this opening translates into chromatin opening and then in gene activation is to a large extent still unknown.



I will present our recent efforts dedicated to understand how Oct4 and Sox2, two master regulators of stem cell pluripotency with different DNA binding features induce large nucleosome opening. Moreover, I will show how rengineering the DNA binding cooperativity of these two pioneer factors by a single point mutation has dramatic implications for biology and potentially therapeutic approaches. To uncover these mechanisms we used a feed-back loop between extensive molecular dynamics simulations and experimental validations. I will also discuss the main challenges the simulations are facing in describing accurately the conformational transitions occurring in such large, heterogeneous systems.

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Multi-Scale Simulations of DNA Supercoiling

Sarah Anne Harris[1], Tom Bishop[2], Charlie Dorman[3], Carsten Kroger[3], Steve Levene[4], Aalap Mogre[3], Penn Rainford[5], Massa Shoura[6], Susan Stepney[5], Thana Sutthibutpong[7], Victor Velasco Berrelleza[1]

[1]School of Physics and Astronomy, University of Sheffield, UK
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[6]Phinomics, San Carlos California, USA
[7]Department of Physics, King Mongkut's University of Technology Thornburi, Thailand

Molecular dynamics simulations have shown how DNA responds to supercoiling at the atomistic level. But how does the mechanics that we see influence DNA recognition and gene expression? I will describe the insights we have obtained from atomistic MD, and how we are now using these as the foundation for highly simplified physics based models of transcription in bacterial plasmids, which we are testing experimentally.

Physics-based approaches to computational drug discovery

Andrea Cavalli

CECAM, EPFL, Lausanne, Switzerland

Computational drug discovery has become an increasingly important tool in the search for new drugs to treat a wide range of diseases. One of the critical challenges in this field is simulating the complex behavior of biomolecules at the atomic level, which can be computationally very expensive and time-consuming. The thermodynamics and kinetics of drug-target binding play a crucial role in understanding and optimizing the interactions between drugs and their target molecules. To address this challenge, molecular dynamics (MD) and related approaches combined with machine learning and artificial intelligence play a crucial role in accelerating the exploration of the conformational space of molecules. These methods also enable researchers to compute the free energy differences between different states of a molecule and estimate thermodynamic and kinetics properties such as binding free energies and residence time. This talk overviews various enhanced sampling methods combined with machine learning in computational drug discovery and their advantages and limitations. The talk focuses on using these methods for free energy and kinetics estimations, reporting on the significant limitations toward accurate estimations for large datasets of compounds. The talk then describes recent applications in drug discovery and discusses the challenges and limitations of these methodologies when applied to increasingly complex biological systems. In conclusion, the talk illustrates how MD, enhanced sampling methods, and machine learning can improve the efficiency and effectiveness of computational drug discovery and accelerate the development of new therapeutics to treat significant unmet medical needs in the era of precision medicine.

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Machine Learning Potentials for Molecular Modeling. ANAKIN, The Next Generation

Adrian E. Roitberg

Department of Chemistry and Quantum Theory Project, University of Florida
Gainesville, Florida, USA

I will present the new generation of machine learning potentials that follow our initial ANI-Family initially parametrized to reproduce gas-phase DFT energies and forces for small molecules.

In these new training sets and networks, we have extended the reach of the potentials to:

+ Implicit Solvents

+ Transition states

+ Chemical reactions

+ Stable Dynamics (implemented in Amber and LAMMPS)

+ Reactive Dynamics (Highly Scalable over GPUS)

+ Electrostatics

+ ML/MM

+ NMR Chemical Shifts in Proteins

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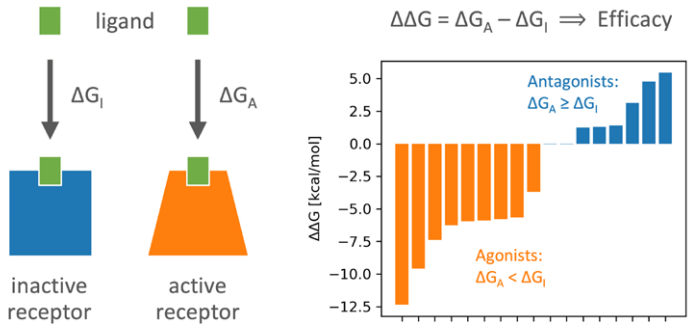
ABSTRACTS OF CONTRIBUTED LECTURES

Is The Functional Response of a Receptor Determined by the Thermodynamics of Ligand Binding?

Martin Vögele[1], Bin W. Zhang[1], Jonas Kaindl[2], Lingle Wang[1]

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[2]Schrödinger GmbH, Glücksteinallee 25, 68163 Mannheim, Germany

While strong binding to its target protein is crucial for a molecule's drug efficacy, it is often insufficient alone. To produce a particular functional response, drugs need to either block the proteins' functions or modulate their activities by changing the conformational equilibrium. The binding free energy of a compound to its target is routinely calculated but the time scales for protein conformational changes are prohibitively long to be efficiently modeled via physics-based simulations. Thermodynamic principles suggest that binding free energies of the ligands with different receptor conformations may infer their efficacy. However, this hypothesis has not been thoroughly validated and its practical viability has remained under-explored. We introduce a robust protocol and an exhaustive study demonstrating that binding thermodynamics provides a strong predictor for the efficacy of a ligand. Using the Absolute-Binding Free Energy Perturbation (ABFEP) method, we assess ligands bound to both active and inactive forms of eight G protein–coupled receptors (GPCRs) and a nuclear receptor, then compare the resulting values for the binding free energy. Our findings suggest the occasional need for specialized restraints to model the respective conformational ensembles accurately, and we propose an efficient approach to set them up. Remarkably, our method categorizes ligands as agonists or antagonists with unparalleled accuracy across the various investigated receptors, all of which are important drug targets. By examining receptor ensembles and ligand poses, we identify necessary conditions and limitations for real-world application. Overall, our insights have significant implications for the drug discovery process as they allow detailed predictions of a ligand's effects on its target.



M. Vögele, B.W. Zhang, J. Kaindl, L. Wang, J. Chem. Theory Comput., 2023, 19 (22), 8414-8422.

Beyond the Threshold: Delving into Rare Gate Opening Events in Enzyme Tunnels through Enhanced Molecular Dynamics Simulations

Nishita Mandal[a,b], **Bartłomiej Surpeta**[a,b], **Jan Brezovsky**[a,b]

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Biochemical reactions unfold within the intricate confines of enzyme active sites, safeguarded by many essential catalytic residues. These crucial components, typically nestled deep within the protein core, and connected to the bulk solvent through molecular transport pathways called tunnels [1]. Moreover, the engineering of tunnel residues can yield mutants endowed with enhanced functional properties, and tunnels also represent very interesting drug targets [1]. Since most of these tunnels are transient in their nature, the preferred method to study them is molecular dynamics (MD) simulations. However, the infrequent occurrence of tunnel openings imposes a significant hurdle, demanding exhaustive and time-intensive sampling for accurate identification and analysis.

In our investigation, we have explored to what extent Gaussian accelerated MD simulations (GaMD) models [2] are suitable for the analyses of the rare opening of gates in transient tunnels. We investigated in three model systems, wild-type haloalkane dehalogenase (DEHAL) enzyme along with its two mutants. Notably, These simulations unveiled that GaMD simulations managed to identify all experimentally confirmed tunnels. Additionally, we observed that, indeed, GaMD simulations tend more frequent detection of tunnels, which were observed only exceedingly rarely in the Classical MD (cMD) simulations, but still on the biologically relevant time scales. In-death analysis of rare tunnels using GaMD, allowed us to understand the mechanism of opening of gates (figure 1) in mutants. Overall, the presented research highlights GaMD models as a promising approach to investigate tunnels in proteins, without compromising the geometrical properties of these ligand transport pathways. **As such application of GaMD opens up new possibilities for the identification of complex networks of transient tunnels in large protein systems efficiently.**

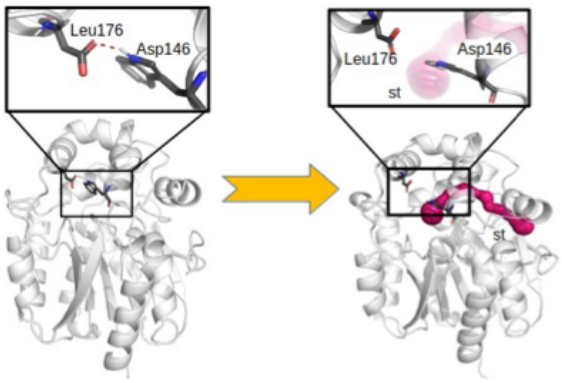


figure1. Opening of gate in rare st tunnel (shown in magenta) in DEHAL mutants

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Design of drug efficacy guided by free energy simulations of G protein-coupled receptors

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[3]Science for Life Laboratory, Department of Applied Physics, KTH Royal Institute of Technology, SE-12121, Solna, Sweden
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[&]Authors contributed equally

G protein-coupled receptors (GPCR) play important roles in physiological processes and are modulated by drugs that either activate or block signaling. Rational design of the pharmacological efficacy profiles of GPCR ligands could enable the development of more efficient drugs, but is challenging even if high-resolution receptor structures are available. We performed molecular dynamics simulations of the ß2 adrenergic receptor (ß2R) in active and inactive conformations to assess if binding free energy calculations can predict differences in ligand efficacy for closely related compounds. Previously identified ligands were successfully classified into groups with comparable efficacy profiles based on the calculated shift in ligand affinity upon activation. A series of ligands were then predicted and synthesized, leading to the discovery of partial agonists with nanomolar potencies and novel scaffolds. Our results demonstrate that free energy simulations enable the design of ligand efficacy and the same approach can be applied to other GPCR drug targets.

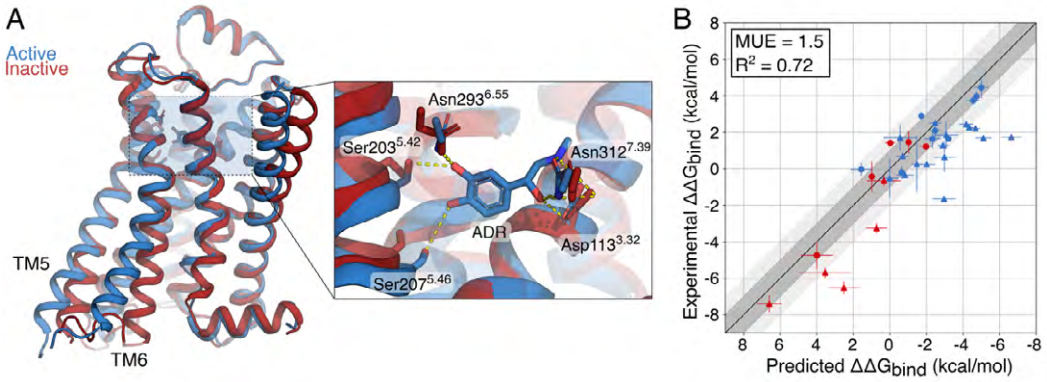


Figure1. Molecular basis of β2R activation and relative binding free energy calculations in active and inactive receptor conformations. A) Structures of the active and inactive conformations of the β2R. Adrenaline and key binding site residues. B) Correlation between calculated and experimental relative binding-free energies.

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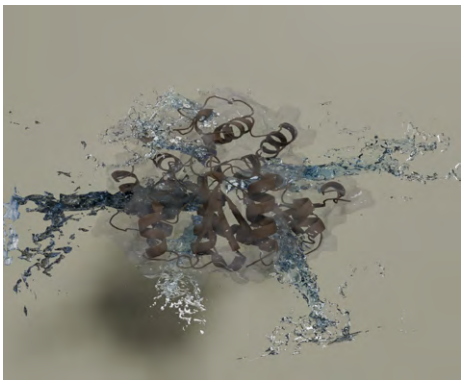
Exposing unseen passages for ligand transport through proteins and their functional consequences

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Active sites of approximately 50 % of enzymes are deeply buried in their structure and connected to the bulk solvent through molecular transport pathways called tunnels. Tunnel anatomy, physicochemical properties, and dynamics often determine substrates and products' access and release rates. Engineering of tunnel-forming residues delivered markedly improved biocatalysts, while inhibitors targeting these tunnels became viable drugs [1&2]. Despite the evident importance of transport processes in enzymes, their fundamental understanding is difficult due to the complicated topologies of tunnel networks and the fact that tunnels are usually equipped with one or more molecular gates, making their opening a rare event and their actual utilization by small molecules even rarer [2].

Accurate analyses of tunnel conformations and explicit ligand migrations from very long and usually highly parallel simulations are required to uncover the details of these elusive transport mechanisms. To alleviate this often rather daunting task, we have developed the TransportTools library to perform effective quantitative analyses of empty space in protein interiors from long as well as highly parallel simulations and couple them with explicit assignment of ligand utilizing those voids without compromising a level of details involved [3].



This novel toolbox allowed us to study water migration paths in 250 simulations of five hydrolases in total 25 µs long. We have traced almost 250,000 water transport events via more than 14,000,000 conformations of distinct transport tunnels present in these enzymes. Based on this dataset, we could look into some fundamental questions. *What enzyme tunnels allow migrations of water molecules – what are the minimal enabling requirements, and what are the implications to the functional analyses of proteins?* Curiously, we find out those tunnels are more prevalent than previously expected, suggesting the presence of a considerable knowledge gap in our understanding of structure-dynamics-function relationships in enzymes with buried active sites [4]. To *what extent is water transport via enzyme tunnels affected by a water model used?* By systematic comparative analyses employing TransportTools, we inferred some notable differences in various transport properties among water models [5]. Additionally, this toolbox and derived knowledge can be applied to shed more light onto molecular principles underlying the effect of distal mutations in human epoxide hydrolase [4] and ABCG transporters [6] as well as to drive protein engineering of N-terminal hydrolases towards enhanced activity and selectivity with alternative substrates by re-designing two molecular gates controlling access to their binding site [7&8].

[1] S.M. Marques, L. Daniel, T. Buryska, Z. Prokop, J. Brezovsky, J. Damborsky, *Med. Res. Rev.* **2017**, *37*, 1095-1139.
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This research was supported by National Science Centre, Poland (2017/26/E/NZ1/00548) and the calculations were performed at the Poznan Supercomputing and Networking Center.

Computational insights into the mechanism of triglycerides formation: a study of DGAT1 using molecular dynamics

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Lipids represent an efficient source of energy into the cell. They are stored in special bodies, known as lipid droplets (LDs). LDs are intracellular compartments that play a central role in lipid energy homeostasis and protect the cells from lipotixicity. LDs present a particular cellular composition: a neutral core consisting mostly of triglycerides (TGs) and sterol esters (SEs), covered by a phospholipid monolayer, enriched with a variety of LD-specific proteins. The TGs biosynthesis is catalyzed by DGAT1 (acyl-coenzyme A: diacylglycerol O- acyltransferase 1) and occurs mainly at the endoplasmic reticulum (ER). To date, a detailed understanding of how TG molecules are synthesized in the ER is still elusive, but the recent determination of the 3D structure of DGAT1 dimer, experimentally solved using cryo-EM microscopy[1,2], paves the way for a structural understanding of its mechanism. To investigate the structure and dynamics of DGAT1, we performed molecular dynamics simulations at atomistic and coarse-grain detail, using the 3D structure of the DGAT1-dimer in a model lipid bilayer, enriched of its natural substrates: oleoyl-CoA and di- oleoyl-glycerol (DOG). Our findings provide new insights into the mechanisms underlying LD formation in the ER and the role of DGAT1 in this process.

[1] Sui et al, Nature, 2020

[2] Wang et al, Nature, 2020

Mechanistic Insights from Molecular Dynamics Simulations in Nanomedicine Research

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Molecular dynamics simulation techniques have been in the spotlight of recent nanomedicine research, becoming an indispensable tool for unveiling complex molecular mechanisms that are sometimes unreachable by experimental methods. Here, we demonstrate how MD simulations can complement existing experimental knowledge or provide new mechanistic insights into relevant aspects of nanoscale devices designed for nanomedicine. Through some case studies - from how thermodynamic variables (e.g., pH and ionic strength) affect the protein corona formation onto organic-functionalized nanoparticles[1] to the impact of lipid composition in the permeation process of anti-tumoral drugs in membranes[2] - this work compilation illustrates how classical MD simulations can be helpful bridging the simulated microscopic behaviors to their corresponding macroscopic manifestation.

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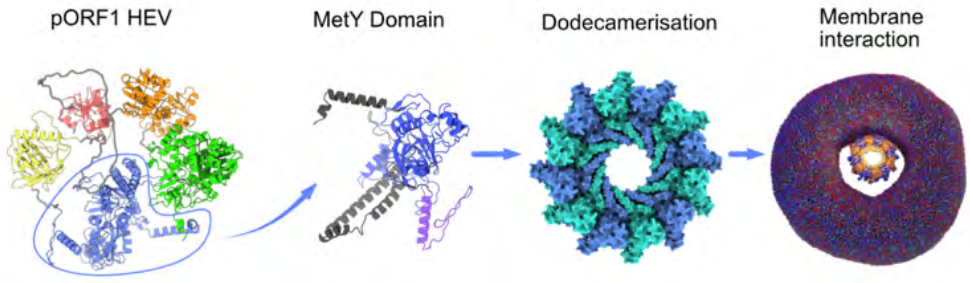
Molecular modelling, homo-oligomerisation and membrane interactions of hepatitis E virus pORF1 replication polyprotein

Thibault Tubiana[1], Sonia Fieulaine[1], Stéphane Bressanelli[1]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

Hepatitis E virus (HEV) is one of the main causative agents of acute hepatitis and jaundice, affecting both developed and developing countries. At least 20 million people are infected each year worldwide. HEV usually causes a minor and asymptomatic infection that resolves on its own but around 70,000 people die per year after HEV infection. HEV is particularly problematic in immunocompromised patients such as tranplant recipients, where infection progresses in most cases to a chronic and highly liver-damaging disease, for which antivirals are needed. However, despite its severity, its prevalence and its impact in health systems, HEV is poorly characterized and many questions remain unanswered about its functioning, especially at the structural level.

In recent years, the subject of structural biology has witnessed major revolutions. The most recent of these is the capacity of the software AlphaFold to properly predict protein 3D structures from their sequences alone. We used AlphaFold modeling to study I) the full-length pORF1 and ii) oligomers of a pORF1 subdomain (MetY)[1]. It turns out that MetY is highly similar to nsP1 of Chikungunya virus, for which the structure was published of a dodecamer putatively topping the membrane spherule harbouring the replication complex [2]. We modelled a similar MetY dodecamer and used coarse-grained[3] molecular dynamic simulations (MD) to investigate its interaction with membranes. To mimic a membrane spherule system, we used a torus (or *donut-like*) membrane system (generated with BUMPy[4] and TS2CG[5]we present a multiscale algorithm that backmaps a continuum membrane model represented as a dynamically triangulated surface (DTS) to reprensent the membrane curvature at a spherule exit.



We will discuss the conclusions we can draw from these models, specifically I) the breakdown of pORF1 into a limited number of domains and possible alternate conformations, ii) the possible organization, oligomerization, and membrane association of the pORF1 MetY.

[1] S. Fieulaine, T. Tubiana, et S. Bressanelli, « De novo modelling of HEV replication polyprotein: Five-domain breakdown and involvement of flexibility in functional regulation », *Virology*, vol. 578, p. 128-140, janv. 2023, doi: 10.1016/j.virol.2022.12.002.

[2] R. Jones, G. Bragagnolo, R. Arranz, et J. Reguera, « Capping pores of alphavirus nsP1 gate membranous viral replication factories », *Nature*, vol. 589, nº 7843, p. 615-619, janv. 2021, doi: 10.1038/s41586-020-3036-8.

[3] L. Monticelli, S. K. Kandasamy, X. Periole, R. G. Larson, D. P. Tieleman, et S.-J. Marrink, « The MARTINI Coarse-Grained Force Field: Extension to Proteins », *J. Chem. Theory Comput.*, vol. 4, nº 5, p. 819-834, 2008.

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Leveraging structural and (co)evolutionary data for protein fitness prediction

Matsvei Tsishyn, Gabriel Cia, Pauline Hermans, Jean Kwasigroch, Marianne Rooman, Fabrizio Pucci

Computational Biology and Bioinformatics, Université Libre de Bruxelles, Brussels, Belgium

Accurately predicting how mutations impact the fitness and stability of a protein is of major interest for both the interpretation of genetic variants and protein design. To address this challenge, we introduce FiTMuSiC [1], a simple linear model that combines structural, evolutionary and coevolutionary features to predict the fitness of protein variants. Although FiTMuSiC only has a few parameters, it outperforms more complex deep-learning models when benchmarked on deep-mutagenesis scanning data. Moreover, our simple approach allows us to biologically interpret the phenomenon underlying the predicted fitness. Indeed, FiTMuSiC returns two scores that separately describe the functional and structural effects of the variant, thus providing mechanistic insight into why genetic variants lead to fitness loss or gain.

We showcase the application of FiTMuSiC on hydroxymethylbilane synthase (HMBS), an enzyme involved in the heme biosynthesis pathway and whose mutations have been associated with acute intermittent porphyria, which is a rare metabolic disease with life-threatening neurovisceral attacks. HMBS was one of the targets in the last round of the Critical Assessment of Genome Interpretation blind challenge (CAGI) [2], in which our method was independently assessed to predict HMBS variant fitness and ranked as one of the best [3]. To further demonstrate FiTMuSiC's robustness, we also compared its predictions on human glucokinase (GCK) variant fitness data, as well as on in vitro activity and clinical data on both HMBS and GCK.

Finally, we explore potential strategies to further improve the predictive capabilities of our method while avoiding dataset biases that are known to be one of the major problems in the construction of machine learning models. For example, we investigate how to more efficiently extract evolutionary information from multiple sequence alignments of homologous proteins, and also show that simple models perform as well as more complex methods on independent test sets.

[1] M. Tsishyn, G. Cia, P. Hermans, J. Kwasigroch, M. Rooman, F. Pucci, FiTMuSiC: Leveraging structural and (co)evolutionary data for protein fitness prediction, *in press Human genomics* (2024)

[2] CAGI consortium, „CAGI, the Critical Assessment of Genome Interpretation, establishes progress and prospects for computational genetic variant interpretation methods." *Genome Biology* 25.1 (2024): 1-46.

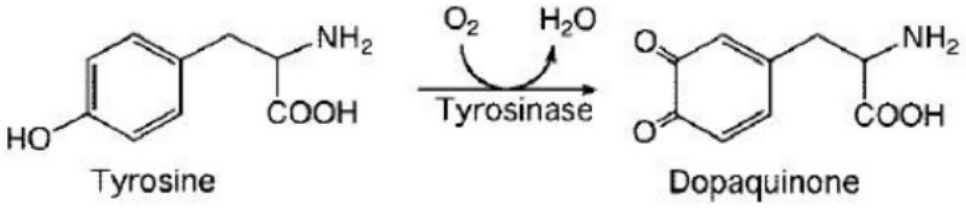
[3] J. Zhang et al., "Assessing predictions on fitness effects of missense variants in HMBS in CAGI6 ", submitted (2024)

Study of the *Streptomyces* tyrosinase reaction mechanism: a dynamic QM/MM approach

Océane Mangel, Catherine Belle, Hélène Jamet

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During the biosynthesis of melanin, the conversion of tyrosine into dopaquinone is catalysed by tyrosinase, a di-copper enzyme, that contains a molecular dioxygen bound as a peroxide in a μ-η2:η2 mode in one of its active form. As a dysfunction in the production of melanin can lead to health issues[1], tyrosinase reaction mechanism is actively studied. To do that, the Streptomyces tyrosinase is often used and has recently been characterised by crystallographic approach[2,3].



To get a better understanding of this system, QM/MM metadynamic approach and thermodynamic integrations were used on this system using the CP2K software. Metadynamic approach allows us to take int account dynamic effects on this reactivity and thermodynamic integration to obtain an accurate estimation of the energy barriers. As it was experimentally described, we modelled both the deprotonation of the tyrosine and the movement of one of the coppers. An opening of the peroxo group was witnessed after the deprotonation, in agreement with a QM/MM static study previously published by the group of Solomon *et al*, in which this opening was described as well[4]. Then, the formation of a bond between one of the oxygens of the peroxo group and a carbon of the tyrosinase was also described.

To describe the antiferromagnetic coupling between the two copper atoms in the active site of the tyrosinase, we used a broken symmetry approach. However, this coupling along with the coupling with the two oxygens is evolving through the reaction. Thus, the electronic structures of some intermediates were described using QM/MM static calculations with the ASH interface.

Altogether, these results combining static and dynamic studies provide unprecedented molecular-level insights into the rate-limiting step in melanin biosynthesis.

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Probing the Role Played by Different Amino Acid Residues in the Catalytic Mechanism of Plastic PET degrading by QM/MM Methods

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Plastic accumulation is one of the main environmental issues of our time. In 2016, two enzymes capable of degrading polyethylene terephthalate (PET), one of the most common plastic polymers, were discovered. PETase and MHETase from *Ideonella sakaiensis* (*Is*PETase and *Is*MHETase, respectively) work sequentially to degrade PET to its constituent monomers. PETase catalyzes the cleavage of PET repetitive units ((mono-(2-hydroxyethyl)terephthalic acid (MHET)), whereas MHETase hydrolyses MHET into terephthalic acid (TPA) and ethylene glycol (EG). In this work, the catalytic mechanism of *Is*PETase was studied by QM/MM with DLPNO-CCSD(T)/CBS:AMBER14SB//B3LYP/6-31G(d,p):AMBER14SB [1].

The reaction was found to progress in four distinct steps, divided into two major events: formation of the first transition intermediate and hydrolysis of the adduct. The transition state and respective reactant and product of each step were fully characterized and described. The rate-limiting step was found to be step 3, with an activation barrier of 12.5 kcal mol^{-1}. Furthermore, in this study, we have shown the critical role of a triad of residues composed by Ser207, Ile208, and Ala209 in stabilizing the catalytic Asp206 residue. This finding confirms the importance of using a larger QM region since our results disclose some important differences when compared with previous computational studies of the same mechanism. These results provide valuable insights into the catalytic mechanism of *Is*PETase that can contribute to the rational development of more efficient engineered enzymes.

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A Lysine-Cysteine Redox Switch: The NOS Bridge

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Recently, a new naturally occurring covalent linkage in peptides was characterised, involving a cysteine and a lysine bridged through a single oxygen atom. The latter was coined as *NOS bond*, reflecting the individual atoms directly involved in this rather uncommon bond which finds little parallel in lab chemistry.[1] This bond acts as an allosteric redox switch, which is crucial for the activity of the enzyme. It is found to form under oxidising conditions and reversible upon addition of reducing agents. Further studies have shown that this bond is found over a large variety of systems and organisms, potentially playing an important role in regulation, cellular defense and replication.[2] Herein, Quantum Mechanics (QM) based key structural data for the identification of the NOS is shown, which allows to differentiate it from the reduced Cys and Lys residues. Moreover, double NOS bonds have recently been identified as a regulatory unit in the main protease of SARS-COV-2 (Mpro),[3] playing an important role in the catalytic activity of the enzyme and its oligomerization. Finally, with the aim of characterizing the reaction mechanism that leads to the formation of the *NOS bond*, we present a reaction network with more than 30 intermediates which provides one of the most encompassing pictures for cysteine oxidation pathways to date.[4]

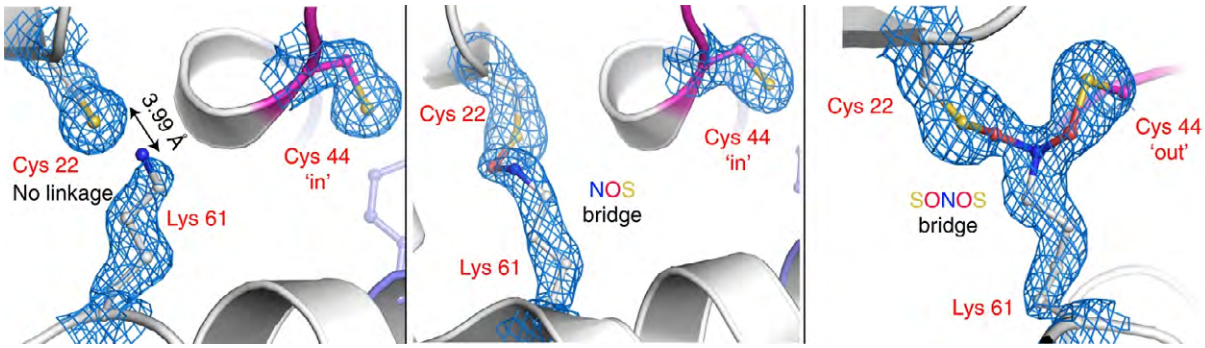


Figure 1. NOS and SONOS bridges in Mpro from SARS-CoV-2. The corresponding 2mFo–DFc electron density maps are shown in blue at a contour level of 1σ. Left: structure of Mpro in the reduced state (PDB: 7JR3) showing the redox switch at the protein surface formed by residues Cys 22, Cys 44 and Lys 61 (highlighted in red). Center: Structure of Mpro in a mono-oxidized state with an NOS bridge formed between Cys 22 and Lys 61 (PDB: 6XMK). Right: Structure of Mpro in a dioxidized state with a SONOS bridge formed between Cys 22, Lys 61 and Cys 44 (PDB: 7JR4).

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Multi-scale simulations provide insights for engineering product outcome of polyketide synthases

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Polyketides are a diverse group of natural compounds; many are biomedically active. For example, the antibiotic actinorhodin (*act*) is biosynthesised by a type-II polyketide synthase (PKS) multi-enzyme complex. A ketosynthase-chain-length-factor (KS-CLF) catalyses initial chain elongation reactions to iteratively build a polyketide backbone. This involves a repeating cycle of binding of an acyl carrier protein (ACP) with a phosphopantetheine (Ppant) arm attached to a malonyl-group, a condensation reaction to join the malonate units together, and unbinding of ACP. This occurs until the polyketide reaches the desired chain length, controlled by the substrate channel. Subsequent enzymes then tailor the polyketide to generate the final molecule. Due to the reactive nature of polyketide substrates and the transient nature of protein-protein interactions, these systems are difficult to study experimentally, and obtaining structural insight into the states relevant for reaction is a challenge. As such, the detailed mechanism of type-II PKS polyketide chain elongation is debated.

Here, we build models of *act*ACP-*act*KS-CLF complexes for multiple stages of the chain elongation reaction cycle using protein-protein docking and iteratively refining Ppant and polyketide substrate positioning using molecular mechanical (MM) molecular dynamics (MD) simulations. We then use these optimised models in extensive combined quantum mechanical / MM (QM/MM) simulations. Semi-empirical QM methods were selected through benchmarking against small (QM-only) models at DFT level. Combined QM/MM CI-NEB (climbing image nudge elastic band) and umbrella sampling reaction simulations successfully revealed the mechanism of the first step in chain elongation and highlight an energy difference in penultimate and final rounds of elongation. Detailed mechanisms of the second and third steps of the reaction help generate a complete picture of the reaction. MM MD simulations further indicate that ACP-Ppant is released when the expected polyketide chain length is reached.

This mechanistic insight into the chain elongation reaction, coupled with other work regarding chain length control, provides a platform for PKS engineering to develop novel polyketides with potential novel biomedical activities.

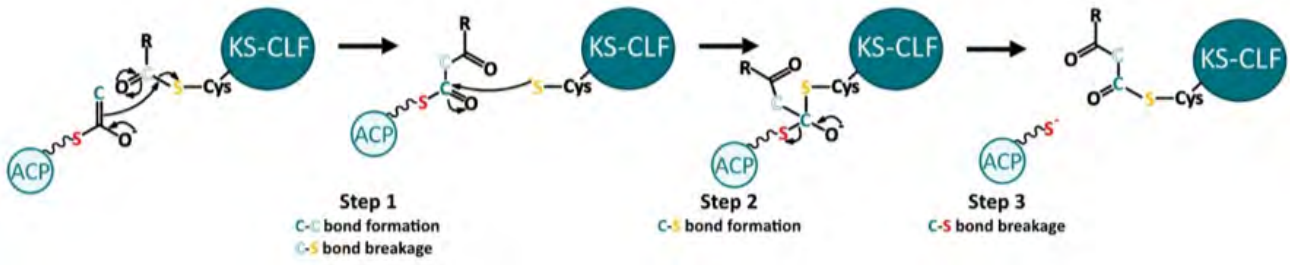


Figure 1: Type-II PKS KS-CLF-catalysed chain elongation reaction steps. Step 1 is the transfer of the growing polyketide from the KS-CLF active-site cysteine to the malonyl-loaded-Ppant to elongate it by one ketide unit. Step 2 is the formation of the tetrahedral intermediate, before step 3 which is the complete transfer back to the active-site cysteine to yield an 'empty' ACP-Ppant which can unbind.

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Computational approaches in targeting GPCRs and cancer related proteins with small molecules

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Protein structural information can be used for virtual screening approaches to identify novel small molecules as potential hits which may act as antagonists, agonists, or allosteric modulators. In this context we have targeted different biological systems, single transmembrane proteins for inhibition, GPCR dimers for protein-protein interaction disruption at the cellular level, and other proteins of pharmaceutical interest. Pharmacophore modeling and docking are well-established methods for screening large databases for drug design and can be a feasible approach in targeting the signaling outcomes of the aforementioned types of systems. Using the structures of these druggable protein targets, we have developed docking and multi-pharmacophore modeling approaches to perform virtual screening of large compound databases and identify small molecule binders. Such methodologies have been implemented on family B GPCRs and specifically GHRHR, the CB_1R-$5HT_{2A}$ heterodimer, as well as the MALT1 paracaspase, which have resulted in bioactive molecules that function either as orthosteric binders, dimer disruptors or allosteric modulators.

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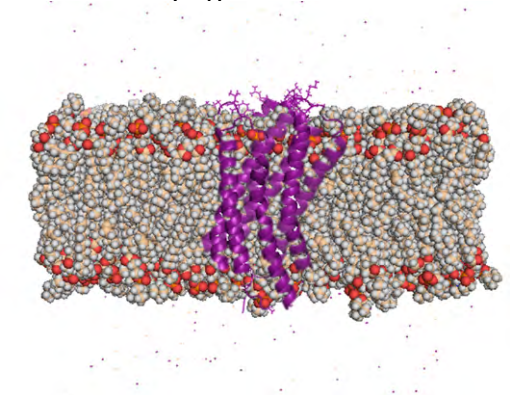
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In Silico Identification of Allosteric Sites and Modulators on GPCRs Using Residue Network Model and Site Identification by Ligand Competitive Saturation

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G protein-coupled receptors (GPCRs) located at the cell membrane play key roles in the internal signalling system of the cell. As they are implicated in numerous diseases, the pharmaceutical industry has developed many commercially successful medications specifically targeting GPCRs, constituting ~17% of all approved drugs [1]. Considering the side effects of conserved orthosteric site-binding drugs, the current focus is on developing allosteric modulators with a high selectivity for the target protein. In this study, we combined the residue interaction network (RIN) model [2] and site identification by ligand competitive saturation (SILCS) technology [3] to explore putative allosteric sites for the class A GPCRs. RIN determined the hub residues with a high capacity to participate in the allosteric mechanism of the protein based on its contact topology. SILCS-Hotspots carried out a fragment-based *in silico* search to determine the druggability of the solvent-accessible hub residues. We statistically tested this approach on structural data of 8 distinct class A GPCRs and showed that the known orthosteric or allosteric sites were predicted by up to 78.6% sensitivity, 93.4% specificity, 51.9% precision, and 92.6% accuracy based on the experimental and theoretical binding site data. Calculations also suggested numerous putative allosteric sites for the receptors. Among these, CXCR4, a chemokine receptor critical for HIV-1 infection and cancer, was investigated and 20 hit compounds were determined for its monomeric and homodimeric forms using SILCS-Monte Carlo docking. *In vitro* assays involving the ligand CXCL12 and mAB 12G5 showed that all hit compounds demonstrated either positive or negative allosteric activities underlying the success of our computational approach.



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DNA translocation through alpha-hemolysin nanopore using the MARTINI coarse-grained force field

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Nanopore experiments are a powerful method for studying charged biomolecules at the single-molecule level. A biological or artificial nanopore is inserted in a lipid or solid membrane, surrounded by an ionic solution, and an electric potential difference is applied to drive a charged biopolymer through the pore. When a macromolecule passes through, it partially blocks the pore, inducing a decrease in the ionic current that depends on the nature of the molecule and the pore properties. The alpha-hemolysin toxin channel is a protein nanopore broadly used for these experiments since it enables the passage of single-stranded DNA, making ultra-fast sequencing possible. Numerous DNA translocation and unzipping experiments have been conducted by experimentalists in our lab [1]. Unfortunately, the molecular details of these processes are not directly accessible by experiments.

Molecular dynamics simulations are thus a precious tool for understanding the physical processes underlying these experiments. Coarse-grained (CG) models are an excellent alternative to classical all-atom models, as they can provide longer and faster simulations for large systems, closer to the characteristic experimental times. In collaboration with experimentalists of our lab, we successfully achieved CG molecular dynamics simulations of the ionic transport through alpha-hemolysin, inserted into a lipid bilayer surrounded by solvent and ions, using the MARTINI CG force field and polarizable solvent, in the presence of several different electric fields to mimic the electric potential difference [2]. We validated specific features of this pore, in agreement with experimental data [3].

We now employ CG constant-force steered molecular dynamics to investigate the mechanisms of ssDNA translocation through alpha-hemolysin. We performed simulations of several systems (~500,000 atoms, 140,000 CG beads) for at least one microsecond, with ssDNA of different lengths and orientations at the nanopore entrance. Our simulation results reveal a very wide distribution of translocation times per base, in agreement with experimental measurements. This wide variability in translocation times underlines the importance of significant statistics, which the CG approach allows. The impact of the ssDNA molecule orientation and phosphate charges on the translocation dynamics is also explored and compared with experimental results.

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Mapping the Radical Cation Guanine in the nucleosomal DNA using QM/MM approaches

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An overexposure of DNA to an oxidative stress may result to various diseases related to mutations, especially cancers[1]. Therefore, understanding the behavior of the DNA oxidative damages is of medical and pharmaceutical interest. Guanine has the lowest ionization potential among the 4 DNA nucleobases[2] thus it is the most likely to be oxidized. However, the guanine redox properties can be impacted by the biomolecular environment around the nucleobases, which modulates the charge transfer ability of the DNA double strand. Then, the specific environment of the nucleosome, the fundamental unit of the chromatin, must be considered to understand DNA oxidative damages in cell. The nucleosome is composed of double-stranded DNA wrapped around four histone dimers rich in positively charged residues, which leads to a wide landscape of DNA-protein interaction (Figure 1).

Here, we investigate the factors that can modulate the ionization potential of guanines in the nucleosome with a focus on their interactions with histone flexible N-terminal tails by means of a multi-scale approach. We combine classical molecular dynamics simulation at the µs scale and QM/MM calculations based on the FO-DFTB/MM[3,4] approach. This method allows us to determine the ionization potential of a large number of nucleobases and the electronic coupling between them.

According to our results, the proximity of positive charges from histone tails seems to be the most predominant cause of variation of the ionization potential. Sequence and orientation towards the nucleosome core have little/limited impact on this redox property. The electronic coupling values mostly depend on the geometry of the considered guanine pair, but the strongest ones are compatible with sub-nanosecond charge transfers (tens of meV). Our understanding of the combinatorial impacts of the structure and dynamics of nucleosome on the DNA charge transfer parameter is currently limited by our sampling but also by our use of conventional approach. Consequently, we want to develop machine learning algorithms to improve our analysis and predictive power of nucleosomal guanine redox properties.

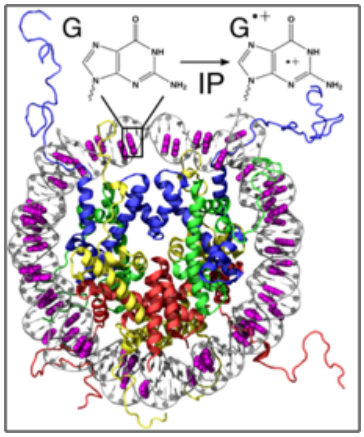


Figure 1: Nucleosome core particle (NCP) (PDB 1KX5) with DNA in grey ribbons and histone in cartoon representation: H3 in blue, H4 in green, H2A in yellow and H2B in red. Guanine nucleobases are represented in purple; their formula and their oxidation to $G^{\bullet+}$ is schematically represented.

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CRISPR-Cas proteins: Engineering of "silver-bullets" in the arsenal of gene editing

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CRISPR has become a programmable and powerful state-of-the-art genome editing tool that has already revolutionized the life sciences [1]. The CRISPR-associated (Cas) nucleases can cleave double-stranded (ds) DNA guided by a Cas-bound small single RNA (sgRNA). Markov state models (MSMs) and machine learning (ML) algorithms can extrapolate the long-term behavior of large biomolecules from atomistic modelling (molecular dynamics trajectories). These methods serve as key tools in biomolecular dynamics and engineering, providing the dynamics of biological molecules, including protein conformational changes, structure-function relationships, and their response to perturbations (mutations), at all-atom resolution. Using large-scale enhanced sampling replica-exchange simulations and a combination of MSM-ML, insight is provided into the dynamics of two Cas proteins (Cas9, Cas12f1) [2,3]. In addition, mutations are proposed to improve both their efficiency and their delivery into target cells for gene editing applications. The configurational space of Cas9 is provided with a focus on the engineering of efficient and high fidelity Cas9 variants to minimize off-target effects. Cas12f1 is a small nuclease of the bacterial immunity CRISPR system with an ideal size for cellular delivery, in contrast to the larger Cas9 effectors. However, Cas12f1 works best at low salt concentrations. Key Cas12f1 domains involved in DNA target recognition and activation of the catalytic RuvC domain are negatively affected by physiological salt concentrations in human cells, and mutations are proposed for engineering salt-tolerant Cas12f1 variants.

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Probing Ligand Transport Pathways in Proteins with Buried Active Sites: Insights from Coarse-Grained Molecular Dynamics Simulations

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In the realm of biochemical processes, enzymes play a vital role by facilitating reactions within their active sites. These sites are secured by crucial catalytic components, often nestled deep within the protein structure and linked to the surrounding environment via molecular pathways known as tunnels [1]. These tunnels have emerged as significant target for protein engineering and drug development. However, due to the dynamic nature of proteins and the transient existence of many tunnels, their examination necessitates sophisticated techniques. Molecular dynamics (MD) simulations are the method of choice for analysing tunnels. However, given tunnel opening is often very rare, the their identification and analysis require extensive and time-demanding simulations, a common limitation of classical MD approach. Coarse-grained (CG-MD) simulations are, in general, capable of overcoming sampling limitations by reducing the complexity of atomic structures through CG beads, which allows for longer simulation time step (up to 20 fs) [2,3]. Therefore, we are investigating the efficacy–utility dilemma of CG models for tunnel analyses due to their reduced resolution.

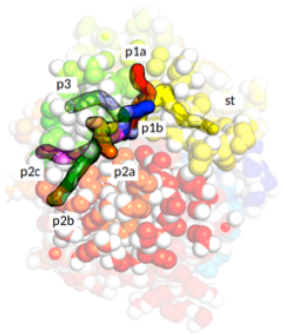


Figure1.Coarse grained representation of enzyme with transport tunnels (p1a, p1b, p2a, p2b, p2c, p3,and st).

We performed replicated cMD & CG simulations (5x 5 µs for each model) for model system haloalkane dehalogenase (DEHAL) enzyme using AMBER22 package following recommended standards in the field and using SIRAH 2.0 CG [2] and MARTINI 3.0 CG models [3]. Tunnels were analyzed with the CAVER3 package (Figure 1). We further assessed the generalization of these methods on a unique dataset of ~40 enzymes belongs to 5 EC classes.

In case of MARTINI, we have used Gō-MARTINI and ELNEDIN methods for carrying out the simulations with 20 fs time step. For MD simulations using SIRAH, we used in-house restraint protocol to prevent the loss of enzyme secondary structure. Regarding their application for tunnel analysis, all CG methods managed to identify all experimentally confirmed tunnels in agreement with cMD. The geometry of CG tunnels was similar to cMD despite the reduced resolution, but we observed a tendency for tunnels being somewhat narrower and longer. Additionally, we have systematically identified several tunnels not present in cMD, suggesting improved sampling of tunnels in significantly reduced run-times. In conclusion, CG models shows promise for investigation of transport tunnels in proteins while **significantly reducing computational demands compared to classical methods, opening new doors for the identification of tunnels in large protein systems and massive datasets**.

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The role of heparin in spike SARS-COV-2 infection: From a model for heparan sulfates to a starting structure for antivirals

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The continued proliferation of new SARS-CoV-2 variants highlights the need for a deeper understanding of infection mechanisms and the development of broad-acting therapeutics to reduce viral burden. SARS-CoV-2 infection is driven by the interaction of the viral spike glycoprotein with the host cell angiotensin-converting enzyme 2 (ACE2) receptor and heparan sulfate (HS) proteoglycans, which are co-receptors for SARS-CoV-2 infection [1,2]. Experimental data indicate that the HS-analog heparin acts as an antiviral against SARS-CoV-2 by binding the spike glycoprotein [2]. We previously performed molecular dynamics (MD) simulations, revealing that long basic grooves on the spike accommodate heparin which, in turn, exerts its antiviral effects by direct and allosteric mechanisms [3]. Here, we applied conventional and enhanced sampling MD simulations with two aims:

(i) The role of HS as co-receptors for spike infection is not yet fully understood since it evades experimental characterization. We have investigated this mechanism by performing conventional MD followed by random acceleration MD (RAMD) simulations for systems of spike glycoprotein homotrimer bound to ACE2 and zero or three HS-analog heparin chains. We find that N-glycans and HS/heparin energetically stabilize the protein-protein interaction while influencing ACE2 dynamics. Direct crosstalk between HS/heparin and ACE2 N-glycans extends the residence time of the complex. Allosteric communication pathways from ACE2 and modulated by HS/heparin regulate the spike-TMPRSS2 site rearrangement.

(ii) The anticoagulant effect of heparin hinders its application as an antiviral to early-stage COVID-19 patients. We aimed to design heparin derivatives with antiviral but without anticoagulant activity by application of MD simulations corroborated by biophysical, biochemical, and cellular assays. Our results suggest that N-O-sulphated and O-sulphated heparin derivatives exhibit higher antiviral activity than heparin, due to greater hindrance of HS and ACE2 binding to spike. These results support the therapeutic potential of these heparin analogs.

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Predicting protein-membrane interfaces of peripheral membrane proteins using AI

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Abnormal protein-membrane attachment is involved in deregulated cellular pathways and in disease. Therefore, the possibility to modulate protein-membrane interactions represents a new promising therapeutic strategy for peripheral membrane proteins that have been considered so far undruggable [1]. A major obstacle in this drug design strategy is that the membrane binding domains of peripheral membrane proteins are usually unknown. The development of fast and efficient algorithms predicting the protein-membrane interface would shed light on the accessibility of membrane-protein interfaces by drug-like molecules. Herein, we describe an ensemble machine learning methodology for predicting membrane-penetrating amino acids. Evaluation of the ensemble classifier produced an F_1 score = 0.92 and an MCC = 0.84 for predicting correctly membrane-penetrating amino acids on unknown proteins of a validation set [2]. The code is available at https://github.com/zoecournia/DREAMM.

We also present a drug design pipeline for drugging protein-membrane interfaces using the DREAMM web-server[3]. DREAMM works in the back-end with a fast and robust ensemble machine learning algorithm for identifying protein-membrane interfaces of peripheral membrane proteins. Additionally, DREAMM also identifies binding pockets in the vicinity of the predicted membrane-penetrating residues in protein conformational ensembles provided by the user or generated by DREAMM. DREAMM has been made accessible via a web-server at https://dreamm.ni4os.eu.

Recently, we tested the capability of protein Language Models (pLMs) to predict protein-membrane interacting amino acids [4]. Initially, we used UniProt sequences from the PePrMInt dataset [5], which includes proteins from nine families that bind transiently to the membrane. pLM embeddings were employed as features to train multi-layer perceptron MLP models, which showed that the predictive ability is limited only to the protein superfamilies represented in the training set. Subsequently, only the protein sequences that are structurally resolved and reported in the PDB were included in the training dataset and the LGBM classifier was trained. The robustness and performance of these models increased (F_1 score = 0.8 with an MCC = 0.76), and additionally indicated a decreased dependence on superfamily-specific datasets. The code is available at https://github.com/zoecournia/pLM-PMI.

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Validating small-molecule force fields for simulation of macrocycles

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Because physical and biological properties of a compound depend on its 3-dimensional structure, molecular dynamics (MD) is increasingly important for pharmaceutical development. However, the accuracy of MD simulations also depends crucially on the force field. Therefore, high-quality force fields are required for many applications, and continuous research efforts have been directed towards their optimization.

Macrocycles as a class of drug-like molecules have received increased attention because they often exhibit strong binding affinity combined with favourable biophysical properties such as solubility or membrane permeability. While the ring-closure often improves biophysical properties, it might pose special challenges for force-fields in terms of the balance between rigidity and flexibility, combined with a strong influence of interactions with the solvent.

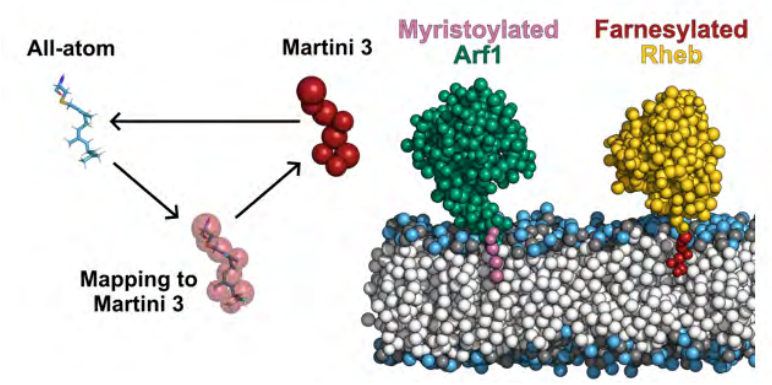
Here, we benchmark the performance of several small-molecule force fields, in combination with REST2 simulation, at reproducing experimental distance bounds from NOESY experiments. Our work highlights the recent advances in force field development, while also showing cases where further improvements are needed. We hope that these findings will help guide future MD-studies and showcase the importance of reliable and widely transferable force-fields.

Martini 3 force field parameters for protein lipidation post-translational modifications

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Protein lipidations are vital co/post-translational modifications that tether lipid tails to specific protein amino acids, allowing them to anchor to biological membranes, switch their subcellular localization, and modulate association with other proteins.[1] Such lipidations are thus crucial for multiple biological processes including signal transduction, protein trafficking and membrane localization, and are implicated in various diseases as well. Examples of lipid-anchored proteins include the Ras family of proteins that undergo farnesylation; actin and gelsolin that are myristoylated; phospholipase D, which is palmitoylated; glycosylphosphatidylinositol-anchored proteins and others.[2]



Here, we develop parameters for cysteine-targeting farnesylation, geranylgeranylation and palmitoylation, as well as glycine-targeting myristoylation for the latest version of the Martini 3 coarse-grained force field.[3] The parameters are developed using the CHARMM36m all-atom force field parameters as reference.[4] The behavior of the coarse-grained models is consistent with that of the all-atom force field for all lipidations and reproduces key dynamical and structural features of lipid-anchored peptides such as solvent-accessible surface area, bilayer penetration depth, and representative conformations of the anchors. The parameters are also validated in simulations of the lipid-anchored peripheral membrane proteins Rheb and Arf1, after comparison with independent all-atom simulations. The parameters, along with mapping schemes for the popular martinize2 tool, are available for download at https://doi.org/10.5281/zenodo.7849262.

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ABSTRACTS OF FLASH TALKS

Modeling A40s Aptamer for Advancing Glioblastoma Therapy

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Glioblastoma (GBM) is the most aggressive primary brain tumor in adults. Despite the benefits associated with radiotherapy and chemotherapy for GBM treatment, GBM stem cells (GSCs) cause chemoresistance and tumor recurrence.[1] A40s is a 2-fluoropyrimidine 30-nt RNA aptamer, identified by the research group using systematic evolution of ligands by exponential enrichment (SELEX) procedure, and the first reported aptamer to directly identify GSCs in fixed human GBM tissue. A40s presents interesting biological value as it selectively binds GSCs by direct recognition of the ephrin type-A receptor 2 (EphA2) and inhibits cell growth, stemness, and migration of GSCs, while it can also serve as selective vehicle for therapeutic drugs.[2,3] Optimization of A40s aptamer will improve its potential applicability as a therapeutic tool to block GSC population and GBM recurrence. Here, we model A40s and investigate its binding with EphA2 through computational and experimental studies. Initially, using 2D prediction tools and experimental data, we predict A40s' structure. Subsequently, we simulate its 3D conformation with µs-long all-atom Molecular Dynamics (MD) simulations in solution, and we study its binding with EphA2. Our findings will highlight key EphA2/A40s interaction points, offering insights for enhancing A40s' binding affinity and in vivo stability.

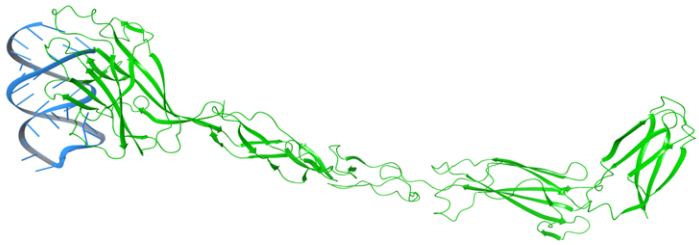


Figure 1. EphA2/A40s complex.

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Developing Force Field for Nucleic Acids Simulations - Both Canonical and Non-canonical

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In developing nucleic acids (NA) force fields, equal attention should be paid to canonical duplexes and non-canonical backbone conformations, which play important roles, for example in protein-NA interactions and in unfolded NA ensembles. An overview of recent developments in AMBER force fields for nucleic acids will be given, focusing on the dihedral angle modifications introduced in our group in Olomouc. Our novel dihedral parameterization methodology[1], which includes conformation-dependent solvation effects, has provided consistent and general improvements for a variety of canonical and non-canonical NA structures. Refinements of the glycosidic dihedral, χ_{OL3}[2] and χ_{OL4}[3], and other backbone dihedrals, ε/ζ_{OL1}[4], β_{OL1}[5], and α/γ[6] merged into the OL21[6] force field, the successor of OL15[5] (OL stands for the city of Olomouc, Czech Republic). OL force fields showed remarkable improvements in modeling of DNA and RNA canonical duplexes, as well as non-canonical RNA motifs, Z-DNA or guanine quadruplexes. However, current NA force fields are still far from perfect and many problems remain to be solved.

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MD simulations identify precise and dynamic protein cooperation for the spliceosomal splicing

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The spliceosome is a dynamic macromolecular complex composed of both proteins and RNA. It catalyzes the pre-mRNA splicing reaction, ultimately regulating gene expression[1]. Notably, the spliceosome catalytic cycle encompasses several intermediate states, with diverse proteins and RNA cofactors being recruited and released to ensure proper catalytic function. These states enable correct folding of the active site and catalytic activation.

Recent advances in cryogenic electron microscopy (Cryo-EM) have provided high-resolution structures of the spliceosome[2,3], revealing insights into the interplay between protein recruitment and catalytic site activation across the various intermediate states for splicing. However, the role of numerous proteins surrounding the active site at different splicing intermediates remains still unclear. Here, we employ a comprehensive structural analysis of existing splicing structures and molecular dynamics (MD) simulations of large model systems (>2 Million atoms) of wild-type spliceosome and selected mutants to elucidate molecular mechanisms by which proteins surrounding the active site contribute to splicing, at each step of the catalytic cycle.

Our preliminary results show that certain positively charged residues cooperate to preserve the active site folding. Certain residues help maintaining the overall active site architecture, occupying a binding site reminiscent of the potassium ion K1 observed in group II introns[4], ancestors of the spliceosome, crucial for catalysis. We show that such positively charged residues are precisely and timely placed in the surrounding of the spliceosome active site to regulate its catalytic function. Notably, our results agree well with mechanistic hypotheses proposed previously for splicing at the group II introns[4], suggesting a unified splicing mechanism for all splicing machines.

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A Multiscale Modelling Study to Elucidate the Citrullination Reaction of the Protein Arginine Deiminase 2 and Identifying New Hit Compounds

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Peptidylarginine deiminase 2 (PAD2) is a member of guanidinium group modifying superfamily of enzymes. It catalyses the conversion of protein arginine residues to citrulline in the presence of Ca^{2+} ions[1]. PAD2 has a significant effect on various aspects of tumour cells, including growth, metastasis, invasion and apoptosis, making PAD2 an attractive target for anti-cancer drug development[2]. Multi-scale modelling studies will help us understand the molecular mechanisms underlying PAD2 activation and help design new potent inhibitors to prevent citrullination reactions. In order to understand different aspects of the inhibition and reaction mechanism, a computational study with three different approaches was designed. The protonation states of active site residues play an important role on the reaction mechanism. To investigate the effect of protonation state changes on the active site and ligand binding, five different PAD2-BAEE systems were prepared (Figure 1). We run ten independent, 1 µs-long MD simulations using random initial velocities for each system to understand the dynamics of the homodimer complex and, in particular, its active sites[3]. The second part of the study involved density functional theory calculations at the M06-2X/6-31+G(d,p) level, starting from the determined near attack conformation where the overall results enabled us to understand the mechanism and elucidate reaction energetics for the citrullination reaction. Finally, a docking study was carried out with a library of natural products to the structures obtained from the MD simulation of the PSI system, which was found to have the most favourable protonation states for the substrate, and possible hit compounds for the inhibition of the citrullination reaction were identified.

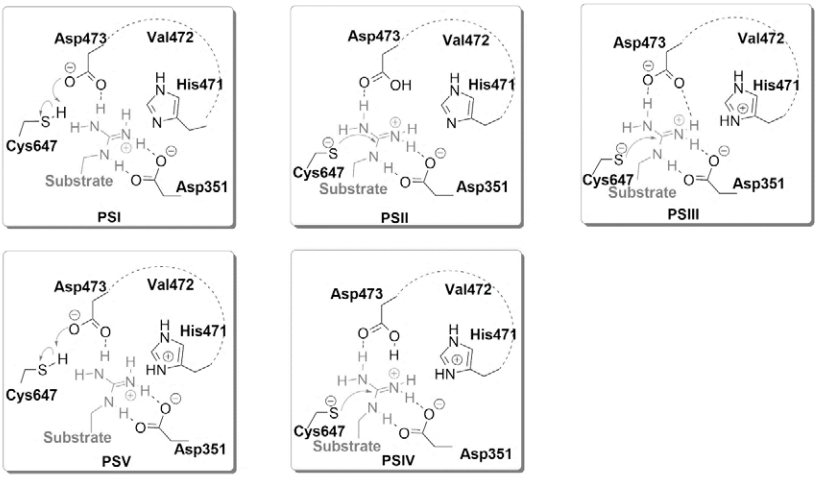


Figure 1. Protonation states of active site residues in the designed systems[3]

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Exploring RNA-protein interactions: from binding modes to sequence specificity through molecular docking and simulations

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Protein-RNA interactions mediated by RNA recognition motifs (RRMs) play pivotal roles in regulating gene expression, RNA processing, and cellular stress responses. These interactions are also implicated in various diseases such as cancer, neurodegenerative disorders, and viral infections. Despite their significance, predicting these interactions remains challenging, presenting an obstacle in understanding cellular processes. In this study, we investigated the intricate mechanisms underlying protein-RNA interactions using computational methodologies such as modelling, docking, and molecular simulations. Initially, we assessed the performance of Haddock through docking protocols using experimentally determined data, obtaining high docking scores for all complexes. Subsequently, we refined the docking process by restricting the information to a minimal set of residues, resulting in outcomes comparable to the initial experiments, indicating the efficacy of our docking protocol for these interactions. During the analysis of these structures and their experimental data, we developed a pipeline integrating multiple tools to map sequence specificity, determining protein affinity for mutations in the RNA sequence based on docking scores. These findings offer deeper insights into the mechanism of protein-RNA binding and demonstrate the versatility of our approach for extracting new information from experimental data. Furthermore, our docking methodologies show promise for advancing drug discovery efforts and exploring molecular patterns in greater detail.

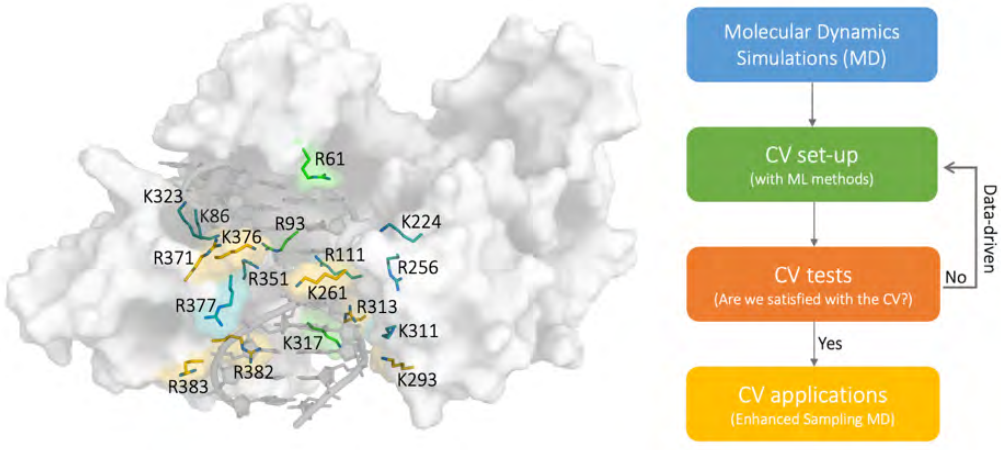
Molecular Dynamics Simulations to study translocation in Human DNA Polymerase η

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DNA and RNA polymerases (Pols) are essential enzymes involved in fundamental cellular processes, such as gene expression, regulation, transcription, and repair [1][2]. Given their critical role, Pols have emerged as promising targets for therapeutic interventions in various diseases, including infections, neurodegenerative disorders, and cancer [3]. Translocation, a fundamental process in DNA replication and transcription, involves the movement of polymerase enzymes along the DNA strand after the nucleotide addition [2]. This dynamic step is essential for accurate DNA synthesis. Despite significant improvements in understanding Pols' catalytic mechanisms, the translocation step is poorly understood, yet [4][5].

In this context, molecular dynamics (MD) simulations can be a powerful tool to investigate the translocation step in human DNA polymerase η (Polη). Through microsecond-long atomistic simulations and machine learning (ML) -guided enhanced sampling MD[6][7], we investigate the role of competing interactions at the Pol•DNA interface, which involve positively charged protein residues and phosphate atoms on the DNA substrate, and their dynamic interactions. Our preliminary results support that the cooperative and collective dynamics of such interactions are critical to an efficient translocation process in Polη.



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Identification of Potent E. coli Ribosome Inhibitors Using a Computationally Efficient Workflow

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Ribosome is a macromolecular machine responsible for protein synthesis in all living cells. The bacterial ribosome comprises the small subunit 30S and the large subunit 50S, both containing antibiotic binding sites at functional regions. The decoding center (DC) on the 30S subunit controls the proper codon-anticodon pairing whereas the peptidyl transferase center (PTC) on the 50S subunit catalyzes the peptide bond formation1. There exist several types of antibacterial agents binding the ribosome to hinder translation; however, there is an urgent need to develop novel therapeutics to combat antibiotic resistance in bacteria2. In this study, a total of 12,449 compounds including FDA-approved drugs, experimental and investigational compounds, were subjected to virtual screening against DC and the PTC on E. coli ribosome using Glide (Schrodinger Release 2021-4) and AutoDock vina3 programs. First, SP-, XP-, vina-docking scores, and Prime MMGBSA of the native inhibitors bound to the DC (Hygromycin B) and PTC (Clindamycin) regions were calculated and then used as reference values to decide on the hit compounds. The known aminoglycoside antibiotics such as Netilmicin, Paromomycin, Kanamycin, and Dihydrostreptomycin exhibited the highest binding affinity towards the rRNA target sites, thereby confirming our approach. A hierarchical filtering method comprising an antibiotic binding fingerprint was applied; 7 hit compounds were selected for DC and 53 for PTC. These were further subjected to three independent molecular dynamics simulations of 100 ns long using Desmond4 to monitor the ligand stability, and the extent of interactions as well as to calculate the binding free energies. To keep the computational efficiency for the full-atom simulations, we focused on a spherical region with a 40 Å radius centered at the ligand-binding site on the ribosome structure, comprising rRNA and ribosomal proteins. Several aminoglycoside derivatives, anticancer, antiseptic, and antimalarial drugs as well as peptide drugs were suggested as promising drug candidates, based on their extent of interactions with nucleotides critical in bacterial ribosome function. In addition, the computational approach involving detailed filtering procedures developed in this study results in successful implementation of the protein-centered molecular docking programs to rRNA and offers an efficient method to explore plausible inhibitors against supramolecular assemblages such as the ribosome.

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ABSTRACTS OF POSTERS

P01

Deciphering the mechanism of avibactam against KPC β-Lactamases through QM/MM computational assays

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Diazabicyclooctanes (DBOs), such as avibactam, represent a crucial class of covalent β-lactamase inhibitors with clinical significance. Together with β-lactam antibiotics, they provide powerful combination therapies to treat complicated infections, as exemplified by avibactam-ceftazidime. However, the global rise of novel KPC β-lactamases threatens the effectiveness of DBO inhibitors in potentiating β-lactam antibiotics. It remains elusive how the KPC-variants alter the reaction rates of DBOs as a possible path towards resistance.

Using multiscale QM/MM simulations, we aim to explain the molecular basis behind the experimentally observed differences between DBOs and different enzyme variants. Using the clinically relevant β-lactamase KPC-2 with avibactam, MM MD simulations were first used to establish protonation states of key active site residues. Then, the potential energy profile of avibactam acylation was modelled using QM/MM with climbing image-nudged elastic band (CI-NEB) calculations. The rate-limiting step of acylation was revealed and corroborated by DFT/MM CI-NEB calculations (at the ωB97x/def2-TZVP//ff14SB level). To enable fast reaction simulations, 2D potential energy surfaces using the semi-empirical PDDG-PM3/ff14SB showed excellent agreement to the CI-NEB benchmarking studies and this method was then used successfully to determine the minimum free energy pathway using two-dimensional umbrella sampling (2D US).

2D US was used as an "avibactam acylation assay" to investigate different KPC variants of clinical interest bearing single amino acid substitutions. Good agreement between experimental and predicted activity amongst different enzymes was obtained. For example, our protocol was able to clearly differentiate between avibactam activity of KPC-2 vs. KPC-3 (His274Tyr mutation). Work is currently underway to establish the origins for the variation of free energy barriers amongst multiple KPC variants. Our computational assays will help to dissect the reaction mechanism of DBOs on an atomistic level and rationalize experimental differences. This can help anticipate variants with reduced DBO susceptibility and may aid design of new, efficient inhibitors or antibiotics to treat life-threating infections.

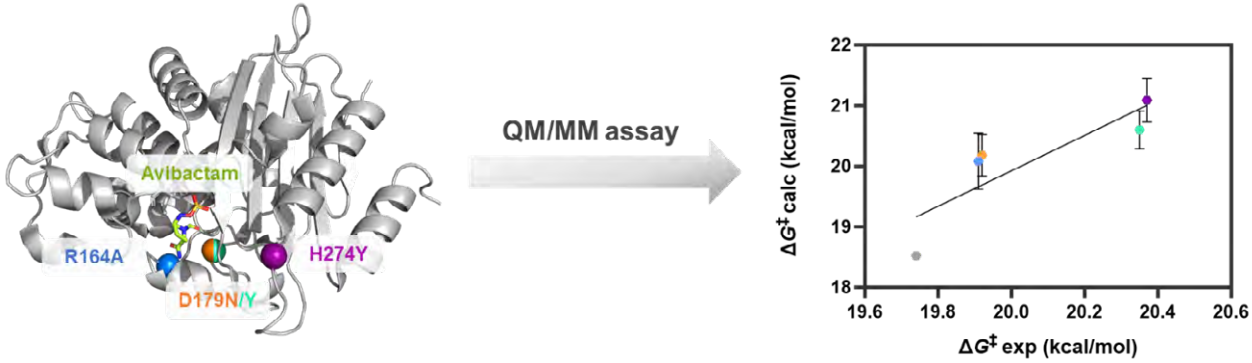


Figure 1. *Left: KPC-2 β-lactamase (grey) in complex with covalently bound avibactam (green). Single amino acid substitutions of KPC-2 shown as spheres, Right: Experimental activation energies versus calculated free energy barriers for rate-limiting step of acylation. Error bars represent the standard deviation from three replicas.*

P02

High-throughput analyses of internal voids in biomolecules and ligand transport through them with TransportTools library

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Living systems contain thousands of small organic molecules that need to arrive at their sites of action to exert their function. The transport of these molecules around the cell and beyond is governed primarily by channels and tunnels formed within the internal voids of biomolecules [1]. Hence, the investigation of these pathways is critical to drug discovery [2] and protein engineering efforts [3]. Unfortunately, these pathways are often equipped with dynamic gates, making them transient and challenging to study [4]. The advent of high-throughput molecular dynamics simulations has enabled the study of such pathways, and their rare utilization for ligand transport events. It is not uncommon to collect datasets of thousands simulations, imposing a substantial burden on researchers in establishing the identity of the pathways observed across all simulations, determining utilization of pathways by ligands, and developing means of specific quantitative analyses.

To alleviate these difficulties, we developed the TransportTools Python library [5] that provides access to (i) efficient analyses of transport pathways across extensive simulations; (ii) integrated data regarding transport pathways and their utilization by ligands; and (iii) straightforward means to compare transport processes under different settings, contrasting transport in an original and perturbed systems. The utility of the tool is showcased by delivered insights into biological problems connected with ligand transport obtained with this tools on model proteins from dehalogenase family: (i) discovering rare yet functional water tunnels, (ii) understanding the effect of mutations on transport pathways, and (iii) studying substrate selectivity for pathways leading to an enzyme active site. Finally, benefitting from the ability of Transport Tools to analyse massive simulation datasets effectively without compromising the accuracy and level of details of its outputs, we could reconcile some of the confounding discrepancies in insights derived by geometric analyses of transport pathways and direct tracking of ligand migration trajectories between enzyme active sites and the bulk solvent [6&7].

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P03

Predicting protein-membrane interfaces of peripheral membrane proteins using ensemble machine learning

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Abnormal protein-membrane attachment is involved in deregulated cellular pathways and in disease. Therefore, the possibility to modulate protein-membrane interactions represents a new promising therapeutic strategy for peripheral membrane proteins that have been considered so far undruggable [1]. A major obstacle in this drug design strategy is that the membrane binding domains of peripheral membrane proteins are usually unknown. The development of fast and efficient algorithms predicting the protein-membrane interface would shed light into the accessibility of membrane-protein interfaces by drug-like molecules. Herein, we describe an ensemble machine learning methodology and algorithm for predicting membrane-penetrating amino acids. We utilize available experimental data in the literature for training 21 machine learning classifiers and a voting classifier [2]. Evaluation of the ensemble classifier accuracy produced a macro-averaged F_1 score = 0.92 and an MCC = 0.84 for predicting correctly membrane-penetrating amino acids on unknown proteins of a validation set. Predictions were further verified in independent test sets. The Python code is available at https://github.com/zoecournia/DREAMM.

The allosteric modulation of peripheral membrane proteins by targeting protein-membrane interactions with drug-like molecules represents a new promising therapeutic strategy. We also present a drug design pipeline for drugging protein-membrane interfaces using the DREAMM (Drugging pRotein mEmbrAne Machine learning Method) web-server [3]. DREAMM works in the back-end with a fast and robust ensemble machine learning algorithm for identifying protein-membrane interfaces of peripheral membrane proteins. Additionally, DREAMM also identifies binding pockets in the vicinity of the predicted membrane-penetrating residues in protein conformational ensembles provided by the user or generated by DREAMM. DREAMM has been made accessible via a web-server at https://dreamm.ni4os.eu.

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P04

Using deep learning and large protein language models to predict protein-membrane interfaces of peripheral membrane proteins

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Characterizing interactions at the protein-membrane interface is crucial as abnormal peripheral protein-membrane attachment is involved in the onset of many diseases [1]. However, a limiting factor in studying and understanding protein-membrane interactions is that the membrane-binding domains of peripheral membrane proteins are typically unknown. By applying Artificial Intelligence techniques in the context of Natural Language Processing (NLP), the accuracy and prediction time for protein-membrane interface analysis can be significantly improved compared to existing methods. Here, we assess whether NLP and protein language models (pLMs) can be used to predict membrane-interacting amino acids for peripheral membrane proteins. We utilize available experimental data and generate protein embeddings from two pLMs (ProtTrans [2] and ESM [3]) to train classifier models. Overall, the results demonstrate the first proof of concept study and the promising potential of using deep learning and pLMs to predict protein-membrane interactions for pe-ripheral membrane proteins faster, with similar accuracy and without the need for three-dimensional structural data, compared to existing tools [4].

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P05

Predicting RNA Structure by using Evolutionary Information captured by Unsupervised Language Models

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Non-coding RNAs (ncRNAs) possess functional significance that extends beyond transporting genetic information. They are involved in various regulatory mechanisms, including gene expression control, protein synthesis and molecular signalling. These functions are mediated through the structure adopted by ncRNAs. Understanding the trends and mechanisms behind RNA structure, holds great potential for uncovering more insights about these promising molecules with implications in disease mechanisms, diagnostics, and therapeutics. Inspired by the success of using evolutionary information in protein structure prediction, our research draws on the ground-breaking ESMFold[1] framework, that uses an unsupervised language model pre-trained on vast amounts of protein sequences from diverse organisms to efficiently capture conserved structural insights[2]. In a similar manner, we explore the suitability of DNABERT[3] and RNABERT[4], the only available pre-trained nucleic acid language models, for RNA structure prediction. Specifically, we employ traditional Machine Learning and Deep Learning models in order to refine the machine-learnt evolutionary knowledge of the language model and predict the secondary and tertiary RNA structures, represented as pairwise contacts. This approach has the potential to overcome the limited structural data availability problem by utilizing the features learnt from processing the abundant sequence data that the post-omics era yields, as a natural language. However, here, we show that machine learning models cannot recognize RNA structural patterns or retrieve any structural information from the two current nucleic acid language models. Overall, this approach offers the potential to uncover novel ncRNA structure insights provided that suitable language models are developed.

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P06

Mechanism of Ligand-dependent G-protein-coupled Receptor Activation Reveled by Free-energy Landscapes

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G-protein-coupled receptors (GPCRs) respond to a variety of extracellular stimuli to mediate diverse cellular signals. Despite the high similarity of their orthosteric binding sites and endogenous ligand chemistry, GPCRs are selectively activated by specific ligands/drugs. Thus, understanding and predicting the underlying mechanisms for ligand-induced signaling responses is critical for the development of efficient drugs, which remains a major challenge. Here we used enhanced sampling molecular dynamic (MD) simulations to disclose the activation mechanism for D2 dopamine receptor (D2R) in the presence of agonists with similar chemical structures but various efficacies. Free energy landscapes projected along functional microswitches located from ligand-binding to G-protein binding sites revealed agonist-induced conformational transitions between the inactive and the active states. Further analyses of the correlation between ligand efficacy and the outward movement of transmembrane helix 6 (TM6) identify that D2R exists in a ligand efficacy-dependent equilibrium between an inactive, partially active, and fully active conformation. Together, these findings provide mechanistic insights into the dynamic activation of GPCRs and useful information for designing specific GPCR ligands with desired activity profile.

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P07

A virtual screening approach to target allosteric binding sites on PI3Kα and PTEN

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PI3Kα is an ATP-dependent membrane kinase within the PI3K/Akt signaling pathway that phosphorylates $PI(4,5)P_2$ to $PI(3,4,5)P_3$. PI3Kα has emerged as a promising therapeutic target due to its importance in cancer.[1] However, a key challenge in targeting PI3Kα is the high similarity of PI3K isoforms in their active site, which often leads to the development of non-selective PI3K drug candidates that may yield undesirable side effects. Allosteric modulators typically bind to less conserved sites compared to the active site of an enzyme, and thus, they may confer greater specificity in kinase regulation. Recently, a crystal structure of PI3Kα with an allosteric ligand, STX-478, within the kinase domain was discovered, paving new paths for drug design and drug discovery.[2] In the present study, molecular docking of 8.3 million drug-like compounds from the ZINC 20 database was performed in the allosteric binding pocket of STX-478. The top 10,000 compounds with the best docking scores were selected. These compounds were subsequently subjected to filtering for undesirable van der Waals contacts and toxic moieties as well as clustering for chemical diversity using the ChemBioServer.[3] Compounds that passed these post-processing steps underwent rescoring with MM/GBSA.. Experimental validation of the selected compounds for inhibition of PI3Kα using in vitro cell-free assays is currently in progress.

PTEN acts as an antagonist to PI3Kα, dephosphorylating $PI(3,4,5)P_3$ to $PI(3,4,5)P_2$.[4] Positive or negative modulators of PTEN may find use in cancer and neurodevelopmental diseases or neurodegenerative and nerve injury conditions, respectively. Currently used first-generation PTEN inhibitors suffer from specificity, selectivity and adverse effects. In this study, we screened drug-like compounds of ZINC 20 database, both in the active site and in predicted allosteric sites of PTEN generated by Allosite Pro.[5] Following the same workflow as in PI3Kα, several compounds were selected for each pocket and their effect on PTEN activity will be experimentally validated.

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P08

Exploring the interactions: Novel iron chelators and 5-LOX binding effects

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Lipoxygenase enzymes are implicated in various diseases, such as cancer, Alzheimer, Parkinson, arthritis, COVID and asthma [1], [2]. The only commercially available inhibitor, zileuton, that targets human 5-LOX is associated with numerous side effects, highting the necessity for the development of novel inhibitors. In our previous study, we discovered through *in silico* and *in vitro* assays that aspartame could serve as a significant inhibitor of lipoxygenase [3]. However, important concerns exist regarding the safety of aspartame's metabolic products that mainly arise from its overconsumption. In this study we focused on designing novel compounds, based on aspartame's structural skeleton, to target human 5-LOX. *In silico* studies, including molecular docking, molecular dynamics and QM/MM MD simulations have been implemented in order to discover the binding mode of 11 compounds to 5-LOX. The computational predictions pinpointed 2 compounds as prominent 5-LOX inhibitors, acting by chelating the catalytic iron in 5-LOX's active site (Figure 1). To experimentally assess the binding potency of these compounds to 5-LOX, Saturation Transfer Difference NMR experiments were conducted. Subsequently, *in vitro* biological assays were performed to evaluate the anti-inflammatory effects of the compounds, determining their IC_{50} values.

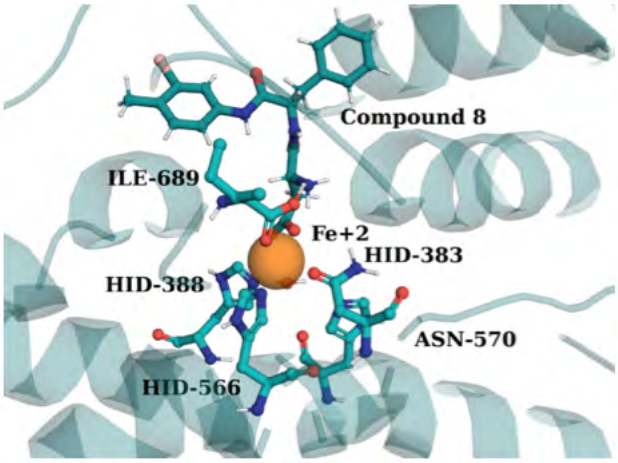


Figure 1. 3D active site of human 5-LOX with the most prominent inhibitor derived from QM(DFT)/MM MD simulations.

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P09

How do AI based-approaches could improve computational enzymology?

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Computational biology is a well-established and widespread discipline that enables the deciphering of complex biological properties using computational tools. Among these, Molecular Dynamics (MD) is used for simulating the motion of atoms by calculating their respective energies and forces over a specific time frame *in silico*. When applied correctly and in conjunction with accelerated sampling techniques, such as Umbrella Sampling and Metadynamics, it allows to capture relevant and reliable dynamical features. This includes elucidating the behavior of ligands interacting with enzymes—a cornerstone of computational enzymology.

Despite its advantages, the optimal balance between accuracy and computational time often relies on force field approaches, which, however, constrain the observation of reactive events within the dynamics. Despite its advantages, the optimal balance between accuracy and computational time often relies on force field approaches, which, however, constrain the observation of reactive events within the dynamics. While techniques like QM/MM offer an alternative, the substantial cost associated with the computational time required to accurately recover free energy barriers (for instance, through Free Energy Perturbation) drastically limits its applicability to large-scale systems. In contrast, Machine Learning potentials (MLPs), trained with Density Functional Theory data, emerge as the most effective surrogate for overcoming this issue by enabling to perform MDs with accuracy comparable to ab initio methods at a significantly reduced cost. Nonetheless, constructing MLPs for complex environments remains a tremendous challenge, one that has focused the efforts of numerous researchers during the last decade.

This study aims to demonstrate how AI can substantially enhance the results obtained with computational tools, particularly in the context of enzyme dynamics. Focusing on acetylcholinesterase, we first reveal through a multiscale approach how the reorganization of a highly polarized water molecule within its buried active site significantly contributes to regulating its reactivity. We further present AI-based approaches that not only improve the accuracy of our simulations but also induce solvent-assisted reactivities in MD, highlighting the potential of AI to overcome traditional limitations.

By integrating these advancements, we provide concrete answers to the questions posed in this abstract and suggest new directions for future research. Specifically, our findings pave the way for exploring the application of AI in elucidating complex biological reactions, thereby opening novel avenues for computational biology and enzyme science.

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P10

A computational approach to reveal the mechanism of nucleosome unravelling by pioneer transcription factors

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Human genetic information is stored inside the cell's nucleus in the form of 23 pairs of chromosomes. Each chromosome consists of the chromatin fiber, which is composed of genomic DNA wrapped around a protein construct (histones). This association between genomic DNA and histones is called a nucleosome. Pioneer transcription factors are a special class of proteins that help in the process of unpacking the genetic information from the nucleosome. The theme is to investigate the mechanism of unwrapping genomic DNA from the nucleosome using computational methods.

First, we will develop a molecular docking pipeline using HADDOCK3 to reliably dock pioneer factors on free genomic nucleosomes. With this pipeline, we aim to predict preferred binding locations of different pioneer factors on genomic nucleoosmes and understand how the interaction interfaces depend on the local DNA conformation and the positioning of the histone tails. Then, we will choose representative docked models of pioneer factor – nucleosome complexes and perform molecular dynamics simulations to predict the impact of the binding on nucleosome flexibility and how the histone tails modulate this process.

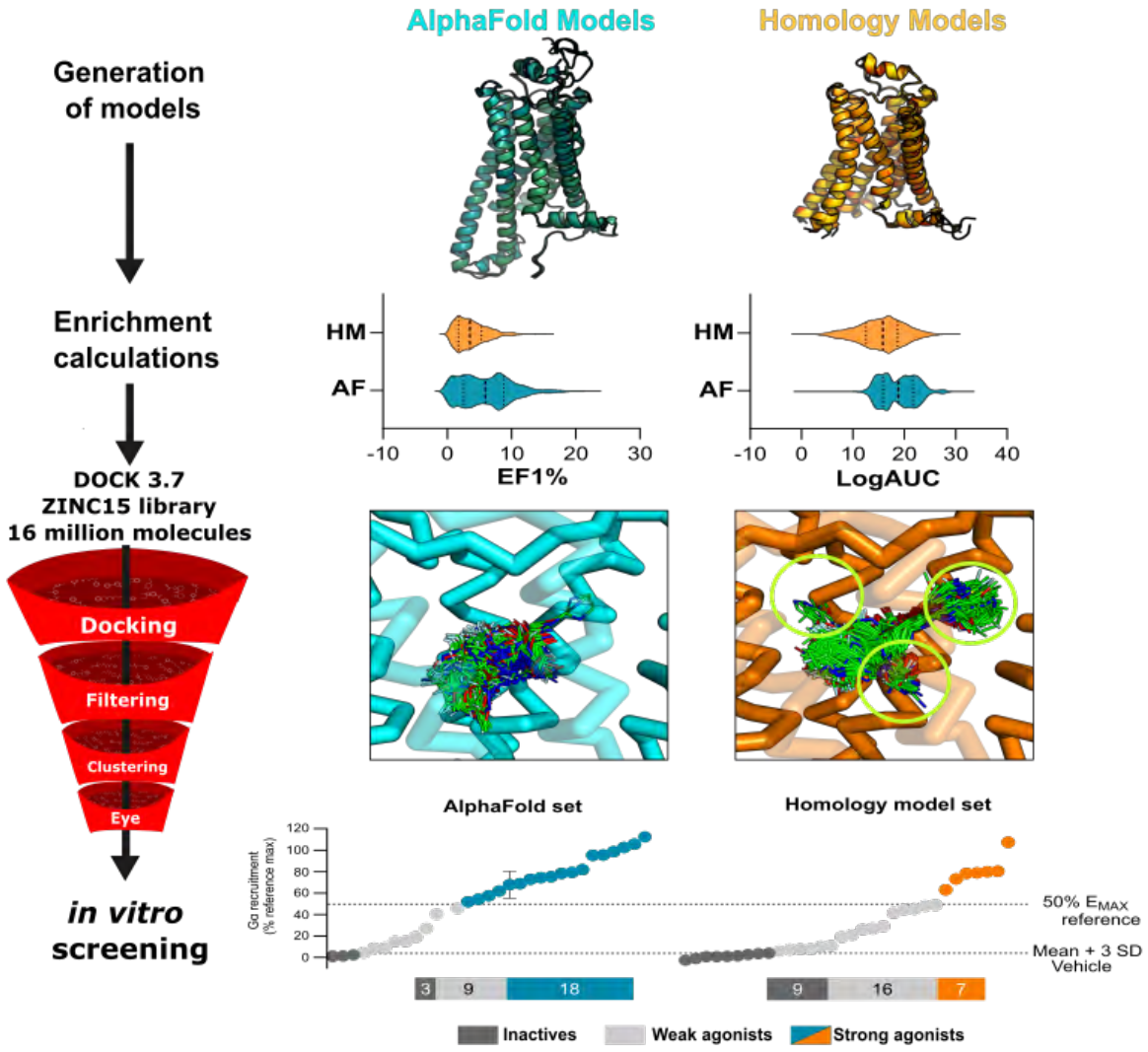
P011

AlphaFold accelerated discovery of psychotropic agonists targeting a GPCR

Alejandro Díaz-Holguín, Marcus Saarinen, Duc Duy Vo, Andrea Sturchio, Niclas Branzell, Israel Cabeza de Vaca, Huabin Hu, Mark J Millan, Yunting Yan, Jens Carlsson*, Per Svenningsson*

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AlphaFold has significantly advanced computational drug discovery, particularly in predicting protein structures with exceptional accuracy. This study evaluates AlphaFold against traditional homology modelling in structure-based virtual screening, focusing on GPCRs—a key drug discovery target. AlphaFold showed superior performance, with a 60% hit rate for identifying new GPCR ligands, surpassing the 22% hit rate of homology models. An ensemble of AlphaFold models led to the discovery of potent GPCR agonists, demonstrating an enhancement in predictive capabilities for drug design. Compound DO8173052, optimized to DO8175010 using AlphaFold, showed promising in vivo results. These findings highlight AlphaFold's role in revolutionizing drug discovery, especially in identifying and optimizing GPCR agonists, indicating a major advancement in computational chemistry's contribution to therapeutics.



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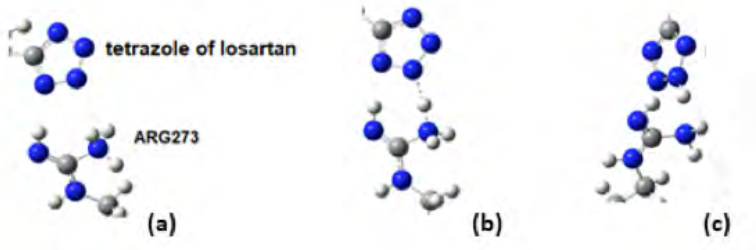
P012

DFT calculations of the interactions of sartans with ACE2 active center. Study of proton transfer in ACE2

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The binding affinities of eight drugs, i.e., benzimidazole-N-biphenyltetrazole, benzimidazole bis-N,N'-biphenyltetrazole, 4-butyl-N,N-bis{[2-(2H-tetrazol-5-yl)biphenyl-4-yl] methyl (BV6), losartan, losartan carboxylic acid (EXP 3174), candesartan, nirmatrelvir, and telmisartan, with the active site of ACE2 were calculated via DFT methodology to find out which of them are the best candidates to prevent the infection of SARS-COV-2. Various reactivity descriptors of above drugs were calculated. We found that, telmisartan and benzimidazole bis-N,N' biphenyltetrazole favor the stronger binding with the active center of ACE2 and they easily accept H+ from the guanidinium group of ARG273. The relative ordering of the eight studied drugs with respect the binging affinity and others descriptors starting from the most promising one is telmisartan > benzimidazole bis-N,N' biphenyltetrazole > candesartan > benzimidazole-N-biphenyltetrazole > losartan carboxylic acid > BV6 > losartan > nirmatrelvir. Additionally, proton transfer among the amino acids (tyrosine, phenylalanine, and histidine) was examined, revealing the necessity of all three amino acids for proton transfer, suggesting a chain-like behavior in proton transfer. In conclusion, the computational findings, coupled with the binding affinity of these molecules to the ACE2 active site, suggest their potential as ACE2 enzyme inhibitors and as prospective compounds for COVID-19 therapy.



Scheme: H-transfer from the guanidinium group of ARG273 to losartan (a) Interactions of losartan tetrazole with ARG273 segment, (b) Transfer of hydrogen from ARG273 to nitrogen of losartan tetrazole (c) The hydrogen from ARG273 is transferred to tetrazole of losartan.

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P013

Using Truncated Structures in Molecular Dynamics Simulations to Investigate Protein-Ligand Interactions: A Case Study with Triose Phosphate Isomerase

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Molecular docking and molecular dynamics (MD) simulations are widely used computational methods in drug discovery to estimate the binding affinity of ligands. MD simulations can be computationally intensive and time-consuming when considering an intact supramolecular complex as a target. Different approaches exist to focus on the region of interest such as cutting out a spherical region[1] and employing a stochastic boundary-based approach[2] to perform MD simulations. In these approaches, the spherical region is divided into multiple zones, where the inner zone is unrestrained and different harmonic restraints are applied to the outer zones. Here, we studied Triose phosphate isomerase (TIM), a key alpha-beta barrel enzyme in glycolysis, to investigate the effect of using different harmonic restraints for the distinct zones centering the catalytic site and the flexible loop 6, on the secondary structure of the protein as well as on ligand-catalytic site interactions. Validation docking using the XP docking method of Schrödinger Glide[3] was conducted for the closed loop 6 TIM structure (PDB ID: 1TPH) with ligand phosphoglycolohydroxamate that is an intermediate analog of dihydroxyacetone phosphate (DHAP). Subsequently, DHAP was docked to the open loop 6 TIM structure (PDB ID: 8TIM), where it maintained a correct orientation at the catalytic site. A spherical region of 30 Å around the flexible loop 6 of TIM was extracted from the DHAP-docked open loop 6 structure that was subjected to 100 ns long MD simulations using Desmond[4] with OPLS2005 forcefiled[5]. The truncated structure was studied for three cases, where the number of zones (3 or 4) and associated harmonic restraints (10, 25, 35, and 50 kcal/mol) changed. The secondary structure of the truncated region and the ligand-binding site interactions were monitored to understand the effect of restraints on the dynamics of the protein involving a highly flexible loop over the catalytic site. In this line, the unrestrained 300 ns long simulations of the intact DHAP-bound TIM structure were used as the reference case. Molecular Mechanics/Generalized Born Surface Area calculations were performed to estimate binding free energies. For the intact structure, the binding free energy was determined as -34.48 ± 2.70 kcal/mol. For the truncated structures, the binding free energies were -37.22 ± 1.54 kcal/mol (Case 1), -39.86 ± 0.04 kcal/mol (Case 2), and -37.16 ± 2.85 kcal/mol (Case 3). Results showed that dividing the truncated structure into 4 zones and using a smooth transition between restraints as 0 kcal/mol (zone 1), 10 kcal/mol (zone 2), 25 kcal/mol (zone 3), and 50 kcal/mol (zone 4) (Case 3) maintained appropriate dynamics of the catalytic site during the MD simulations. These also showed that the usage of different restraints should be carefully adjusted for the region of interest to prevent a stiff structure and low flexibility. This approach highly promises to reduce computational time and cost in estimating binding free energies, especially for large protein complexes involving flexible regions, paving the way for more efficient drug discovery methods.

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P014

Water-Cyclohexane Partition Coefficients via Grid Inhomogeneous Solvation Theory

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Partition coefficients, which quantify the solute distribution between water and apolar solvents, play a crucial role in modeling transfer processes across various biological environments. While many existing computational tools focus on estimating water-octanol partition coefficients, fewer are available for other apolar solvents like alkanes or cyclohexane. Additionally, building highly accurate parametrized computational models for partition coefficients other than water-octanol is difficult due to a lack of available training data.

However, such other apolar solvents, more distinct from water than octanol in their physicochemical properties, have been suggested as improved model partition coefficient systems for membrane permeability[1].

In this work, we propose a physics-based approach using the Grid Inhomogeneous Solvation Theory (GIST)[2,3,4] to tackle these challenges. By leveraging solvent densities calculated from molecular dynamics simulations, GIST enables the computation of accurate free energy of solvation. Thereby, this method facilitates a spatial analysis of the thermodynamic components governing the solvation process, offering valuable insights at the molecular level. From the free energy of solvation of a single solute in various solvents, the partition coefficient between these solvents can then be estimated[5].

We compute water-cyclohexane partition coefficients for various small molecules, demonstrating promising agreement with experimental data. Additionally, we point out current challenges associated with this methodology, shedding light on areas for improvement and future development. The present study underscores the potential of GIST in accurately estimating partition coefficients beyond conventional solvent systems, paving the way for enhanced understanding of compound behaviour in complex biological environments.

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P015

Conformational Changes in ABCG2 Triggered by ATP

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The ABC (ATP-Binding Cassette) protein superfamily of membrane transporters is a large family of transmembrane transporters that bind ATP as a part of their function. Upregulation of ABC transporters emerges as one defense strategy against the administered drug, leading to clinical drug resistance, poor response to chemotherapy, and poor prognostic factor [1]. Advancements in experimental techniques, such as Cryo-EM, have largely contributed towards elucidating structural details. However, the full mechanism through which ABC transporters carry substrates is still unclear and a source of debate [2]. Herein, we report on the dynamics of the inward-facing conformation of the Breast Cancer Resistance Protein (BCRP/ABCG2) protein in its apo and ATP-bound states, and the effects of ATP binding.

Homology modeling was used to complete a structure of the ABCG2 protein, starting from a cryo-EM result available on the Protein Data Bank (pdb: 6ETI). Missing sequences were built using our previous BCRP model [3] as template along with the PredictProtein secondary structure prediction server [4]. The protein was then inserted in its native environment and under physiological conditions.

From the results obtained in our molecular dynamics runs, we analyzed and compared the conformational changes occurring in the transmembrane and nucleotide-binding domains of the ABCG2 apo and ATP-bound forms, providing interesting insights. Our results appear to support a hypothesis proposed by Locher et al [5] for the ABCG2 efflux mechanism.

Although our research helped elucidate the ABCG2 structural differences between the Apo and ATP-bound forms, further research is required to completely characterize these mechanisms. Nevertheless, a complete understanding of these transporters is central in pharmacodynamics/pharmacokinetics, not only in cancer research.

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P016

Investigating the C-terminal activation in WT and oncogenic PI3Kα using enhanced sampling simulations

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PI3Kα is the most frequently mutated kinase in human cancers with mutations often occurring at the C-terminus. The C-terminus has a dual function in regulating the kinase, playing an auto-inhibitory role for kinase activity and also mediating protein binding to the cell membrane. When PI3Kα attaches to the cell membrane, it phosphorylates its substrate PIP2 and converts it to PIP3, initiating a signaling cascade for cell proliferation.[1] Oncogenic mutations in the C-terminus of PI3Kα lead to overactivation of the kinase,[2,3] however, the molecular mechanisms by which these C-terminal oncogenic mutations cause PI3Kα overactivation remain unclear. H1047R, G1049R, and M1043L mutants increase ATPase activity compared to the WT, and H1047R, G1049R, and a frameshift mutation (N1068KLKR) increase membrane binding compared to the WT.[2,3] Moreover, after comparing available crystal structures of the WT and H1047R mutant, different conformations of the C-terminus are observed with the H1047R mutant structure displaying an open C-terminal conformation associated with PI3Kα activation.[4]

To understand how C-terminal mutations of PI3Kα alter kinase activity, we perform unbiased and biased Molecular Dynamics simulations of the above-mentioned mutants and report the free energy needed for the C-terminal "closed to open" transition and associated conformational changes. H1047R and G1049R mutants have the lowest free energy for the transition compared to the WT, M1043L and N1068KLKR mutants. In the open state of M1043L mutant, a different direction of the C-terminus is observed, which corroborates with experimental work showing that M1043L has a lower membrane-binding ability compared to the other mutants.[3] To validate our findings, we compare the results from Molecular Dynamics simulations with the existing HDX-MS experimental data,[3,5] and find that the results align. The differences in the free energy needed for the closed to open transition and the observed conformational changes across the different mutants provide valuable insights into the molecular mechanisms underlying the C-terminal activation in oncogenic and WT PI3Kα.

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P017

A GIST end-state approach to estimate binding free energies

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Binding free energies play a important role in guiding drug development. Despite their significance, the computationally efficient in-silico estimation of these energies in solution remains challenging. To address this, we introduce a novel Grid Inhomogeneous Solvation Theory (GIST) end-state approach. Thereby, we utilize a thermodynamic circle to independently compute interaction energies between the binding partners in vacuum and solvation energies of each binding partner as well as of the complex. Combining these terms yields an approximation of the binding free energies. We optimize our approach using a host-guest system from the SAMPL8 challenge. The highly negatively charged Gibb deep-cavity cavitand tetra-endo-methyl Octa-acid (TEMOA) serves as the host molecule, while 3-hydroxy-2-naphthoic acid, p-bromophenol, cyclopentylacetic acid, piperonylic acid, and p-toluic acid are used as single negatively charged guest molecules. Throughout method development, we underline the significance of accurately accounting for all electrostatic interactions in free binding energy computations. Multiple strategies were explored, evaluated, and discussed to achieve satisfying results. In conclusion, our findings underscore the promising potential of the GIST end-state approach in efficiently estimating binding free energies, offering a pathway to enhance drug development with improved cost-effectiveness.

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P018

Unraveling Dynamics-Driven Allostery in Tyrosine Kinase 2 through Community Analysis of Large-Scale Molecular Dynamics Simulations: Implications in Diseases

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Protein kinases (PKs) play a crucial role in various key processes in human cells by orchestrating cellular signaling. They are highly dynamic molecules and their regulation mechanism relies on the conformational plasticity and concerted motions within their catalytic domains (KD) as they pass through their catalytic cycle.

TYK2, a non-receptor tyrosine kinase and member of the Janus kinases (JAK) family, is involved in various signaling pathways with major role in several diseases, including cancer. While the KDs of JAKs are highly conserved, the isolated TYK2-KD exhibits unique specificities. Previous studies have highlighted the significance of specific amino acid changes in altering TYK2 functionality. In a previous work [1], using molecular dynamics (MD) simulations of a catalytically impaired TYK2-KD variant (P1104A) in its APO form, we found that this disease associated amino acid change acts at the dynamics level.

Given that structural dynamics is key to the allosteric activation of PKs, in a recent study [2], we employed long-scale (microsecond-scale, 3 μs) MD simulations and investigated an active TYK2-KD form that corresponds to a dynamic and crucial step of the catalytic cycle in other PKs (in the presence of adenosine 5'-triphosphate and one magnesium ion).

Here we present highlights from the community-network analysis of the 3 μs MD trajectory. In brief, our results elucidated, for the first time, the dynamic profile and dynamics-driven allosteric communications within the TYK2-KD during activation and identified several TYK2 amino acids, including P1104, with a pivotal role in serving intra-KD allosteric signaling. Our findings are validated by the observation that the majority of the amino acids of the identified dynamic allosteric network are associated with cancer-related missense/splice-site mutations of the Tyk2 gene. In total, our findings provide insights into TYK2's unique substrate recognition and specificity, underscoring the potential for targeted therapeutic interventions via a rational design of allosteric TYK2-specific inhibitors.

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P019

Unveiling Membrane Dynamics of a K-RAS4B variant: Insights from Coarse-Grained Molecular Dynamics Simulations

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KRAS-4B, the predominant isoform of KRAS, is a lipid-anchored GTPase protein crucial for regulating cellular proliferation and differentiation through its GTPase activity. Dysregulation, primarily driven by mutation at codon 12, results in constitutive KRAS-4B activation implicated in hyper-proliferative disorders and cancer. Notably, the G12D mutation stands out as the most frequent alteration at codon 12 and is associated with various cancers, including pancreatic carcinoma, colorectal cancer, and lung malignancies[1].

Structurally, KRAS-4B consists of a catalytic GTP/GDP binding domain (G-domain) and a hypervariable C-terminal region (HVR), which anchors to cellular membranes. Conformational changes within the catalytic domain are crucial for the activity of KRAS-4B. Recent atomistic Molecular Dynamics (MD) simulations studies have revealed two predominant membrane orientations of KRAS-4B G12D variant on model lipid bilayers: orientation state 1 (OS1), which facilitates effector interactions by the G-domain, and orientation state 2 (OS2), where the G-domain interacts with the membrane[2-5]. Due to the intensive computational demands of atomistic MD simulations, exploring the full free energy landscape of KRAS-4B membrane interactions would be more easily explored using coarse-grained MD simulations that simplify biomolecular representations and enable the study of longer simulation length- and time-scales. This study aims to validate whether the newly-released Martini 3 force field[6] can reproduce the atomistic MD simulation results of the two dominant conformations (OS1 and OS2) that have been previously identified in all-atom MD simulations of the G12D variant[2].

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P020

Studying the dimerization of K-Ras4B^{G12D} through atomistic Molecular Dynamics simulations

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K-RAS mutations, particularly the G12D activating variant, play a central role in the pathogenesis of aggressive cancers.[1] Current therapeutic options for these *K-RAS*-driven malignancies are limited, underlining the urgent need for innovative approaches to disrupt K-Ras activity. The active GTP-bound K-Ras4B^{G12D} variant may form dimers in the plasma membrane, which could act as scaffolds that facilitate the recruitment of Raf to the membrane and initiate Raf dimerization.[2] These events lead to sustained activation of the MAPK signaling pathway contributing to uncontrolled cell growth and tumor formation. [3]and its treatment and outcomes have been dramatically revolutionised by targeted therapies. As the most frequently mutated oncogene, Kirsten rat sarcoma viral oncogene homologue (KRAS However, the dimerization of K-Ras4B^{G12D} remains largely uncharacterized. We have recently analyzed the dimerization free energy landscape of K-Ras4B^{G12D} using enhanced sampling Coarse-Grained Molecular Dynamics (CG-MD) simulations and extracted several low energy dimers. This research project evaluates the structure and dynamics of selected K-Ras4B^{G12D} dimers arising from the CG-MD simulations using all-atom MD simulations. Atomistic configurations of K-Ras4B^{G12D} dimers in the presence and absence of the CRD and RBD domains of C-Raf on a model membrane were obtained after backmapping the CG structures. MD simulations were then performed; our analyses confirmed the stable association of the K-Ras4B^{G12D} dimers with the lipid bilayer. However, during system equilibration, instability issues were identified that led to GTP unbinding and protein-protein dissociation. Different strategies were explored, including the reconstruction of the GTP interaction network from a dimer crystal structure of K-Ras4B^{G12D}, the use of gradual restraints, and the extension of equilibration time. These observations may indicate inaccuracies in atom positions resulting from the backmapping procedure causing the loss of key intermolecular interactions, instability of the CG dimer structures, or a need for optimization in the employed atomistic simulation protocol.

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P021

MD simulations identify precise and dynamic protein cooperation for the spliceosomal splicing

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The spliceosome is a dynamic macromolecular complex composed of both proteins and RNA. It catalyzes the pre-mRNA splicing reaction, ultimately regulating gene expression[1]. Notably, the spliceosome catalytic cycle encompasses several intermediate states, with diverse proteins and RNA cofactors being recruited and released to ensure proper catalytic function. These states enable correct folding of the active site and catalytic activation.

Recent advances in cryogenic electron microscopy (Cryo-EM) have provided high-resolution structures of the spliceosome[2,3], revealing insights into the interplay between protein recruitment and catalytic site activation across the various intermediate states for splicing. However, the role of numerous proteins surrounding the active site at different splicing intermediates remains still unclear. Here, we employ a comprehensive structural analysis of existing splicing structures and molecular dynamics (MD) simulations of large model systems (>2 Million atoms) of wild-type spliceosome and selected mutants to elucidate molecular mechanisms by which proteins surrounding the active site contribute to splicing, at each step of the catalytic cycle.

Our preliminary results show that certain positively charged residues cooperate to preserve the active site folding. Certain residues help maintaining the overall active site architecture, occupying a binding site reminiscent of the potassium ion K1 observed in group II introns[4], ancestors of the spliceosome, crucial for catalysis. We show that such positively charged residues are precisely and timely placed in the surrounding of the spliceosome active site to regulate its catalytic function. Notably, our results agree well with mechanistic hypotheses proposed previously for splicing at the group II introns[4], suggesting a unified splicing mechanism for all splicing machines.

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P022

In silico analysis of quinolone derivative indicates the transcription factor NF-κB as a potential target

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Quinolones are a broadly known class of antibiotics that inhibit bacterial nucleic acid synthesis by disrupting the enzymes topoisomerase IV and DNA gyrase, and by causing breakage of bacterial chromosomes. This interference is responsible for their antimicrobial functions. Quinolones also possess anti-inflammatory functions. The focus of this study is novel quinolone molecules that were tested as potential modulators of the transcription factor NF-κB, a known proinflammatory pathway in innate immune system. *In vitro* screening of quinolone derivatives using an NF-κB-induced luciferase reporter cells line revealed that one compound had an inhibitory effect, causing a reduction of the NF-kB-induced luciferase signal. Docking studies using the Autodock software showed favorable binding energy (-6,5kcal/mol). Molecular dynamics simulations using the Desmond software verified the stability of the protein-ligand complex. These results indicate that the compound may exert its anti-inflammatory effects by directly binding to the p65/p50 NF-κB dimer. This binding potentially inhibits its DNA-binding activity and therefore its transcriptional activity. Currently, many NF-κB inhibitors are used in the clinic, but none of them targets the NF-κB proteins directly.

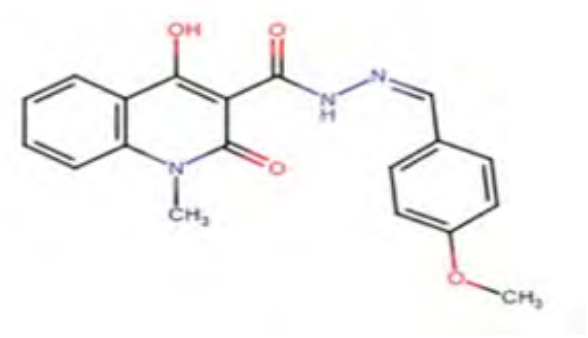


Figure 1: Quinolone Derivative

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P023

Impact of force field polarization on the collective motions of proteins

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Correlated motions of proteins are thought to underpin various physiological mechanisms, such as substrate binding, signal transduction and allostery.[1] These motions, arising from low frequency collective movements of residues, have mostly been studied using MD simulations. In our work, we compared effects of two different force fields used for MD simulations on correlated motions – the non-polarizable CHARMM 36m FF and the polarizable Drude FF.

The study was conducted on two proteins, ubiquitin - a small protein with a well-described dynamic, and the nuclear receptor protein PPAR gamma. The ligand binding domain of PPAR gamma was of particular interest since its function is to regulate transcription through ligand and coregulator protein binding. It has been previously shown that a dynamical network of correlated motions ensures the transmission of information related to ligand binding.[2]

In this study, we present the results of the MD simulations which include results of residue fluctuations, correlation maps and visual inspection of highly correlated and anti-correlated regions, which we use to explain the functional dynamics of these proteins.[3]

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P024

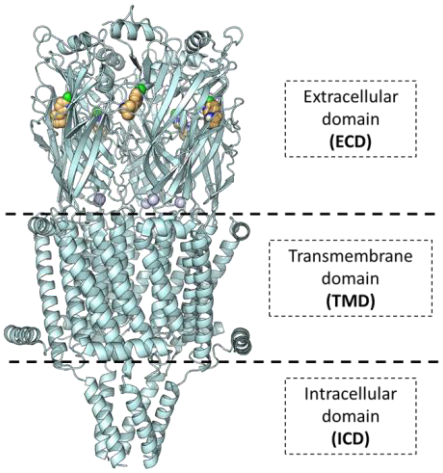
The mechanism of desensitization of the α7 nicotinic acetylcholine receptor by Molecular Dynamics simulations and Markov State Modeling

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Nicotinic acetylcholine receptors (nAChR) mediate intercellular communication in the brain by opening an ion channel in response to increased levels of neurotransmitter. The homomeric nAChR α7 has been recognized as a relevant pharmacological target in humans for the treatment of severe nervous-system disorders including Alzheimer's disease, Parkinson's disease, schizophrenia, and inflammation [1]. This pharmacological relevance of α7 has spurred the development of numerous compounds that enhance channel activation. However, its rapid desensitization limits the utility of traditional agonists that mimic neurotransmitter activity.

Markov State Models (MSM) are stochastic models that describe the dynamics of complex molecular systems by combining information collected from a large set of independent molecular dynamics (MD) simulations [2]. This powerful technique aims at capturing the structural (the biologically relevant states), thermodynamic (their population at equilibrium) and kinetic (the transition pathways and barriers) properties of the system beyond the time scales accessible by unbiased computer simulations.



In the last year, we have been working on the construction of a MSM of α7-nAChR desensitization. By capitalizing on a generous CPU allocation from GENCI [3], we have finalized a model that illuminates the mechanism of receptor desensitization with atomic resolution. Our analysis supports the conclusion that desensitized α7 receptors are in reversible equilibrium between open and closed channel states. Albeit surprising, this finding has profound implications on the function and regulation of nAChRs, it provides an understanding of type-I versus type-II allosteric modulation and rationalizes the paradoxical pharmacology of the L246T anomalous mutant. Our results demonstrate that MSM provides a convenient theoretical framework for the interpretation of synaptic receptors modulation by allosteric ligand binding or allosteric mutations.

The emerging mechanism of desensitization opens to new pharmacological strategies against neurological disorders.

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P025

Machine Learning for allosteric site prediction

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Allosteric regulation is a fundamental biological mechanism that can control critical cellular processes via allosteric modulator binding to protein distal functional sites[1]. The advantages of allosteric modulators over orthosteric ones have sparked the development of numerous computational approaches, such as the identification of allosteric binding sites, to facilitate allosteric drug discovery[2]. An allosteric binding site should correlate its motions with functional sites of the protein, and such correlations can be investigated using computational allosteric communication analysis methods. Building on the success of Machine Learning (ML) in solving complex problems in biology and chemistry, several ML tools for the prediction of allosteric sites that use descriptors derived from these allosteric communication models have been developed. In this poster, the utility and shortcomings of current computational methods for allosteric communication analysis and ML models for allosteric site prediction are discussed. In addition, recently emerged protein Language Models (pLMs)[3] are proposed as a new resource to exploit the simultaneous prediction of allosteric communication pathways and allosteric sites, which may improve the efficiency and/or accuracy of existing methods.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956314.

P026

Sequence and force effects on ssDNA translocation through a protein nanopore: Coarse-grained constant-force steered molecular dynamics simulations

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Protein nanopores are highly effective for single-molecule studies, especially in the context of single-stranded DNA (ssDNA) translocation.[2, 4] Previous experiments have successfully distinguished between purine and pyrimidine bases[4] and have revealed insights into the effect of applied electric voltage on ssDNA translocation dynamics.[5, 3] Unfortunately, the microscopic details of ssDNA translocation over experimental timescales,which are not accessible through all-atom molecular dynamics (MD), remain to be further explored. Coarse- grained (CG) MD simulations offer a promising avenue to bridge this gap, allowing for simulations over timescales closer to experimental ones. Our study investigates ssDNA translocation through the α-hemolysin nanopore using constant-force (cf) steered MD with the MARTINI CG force-field, building on our previous work validating thisapproach for ionic [1] and ssDNA transport through this nanopore. The influence of the sequence and the magnitude of the pulling force on translocation dynamics are explored. We simulated the translocation of poly(dA) and poly(dC) molecules with varying lengths through the α-hemolysin nanopore. Different pulling forces were applied to mimic the effect of applied voltage used in the experiments. To investigate the sequence effects, simulations were run for at least one microsecond, while those exploring the pulling force effect were performedfor at least five microseconds, with both sets having two replicas.

Our results demonstrate that CG MD simulations efficiently generate statistically significant data and are successful to capture experimental properties of ssDNA translocation, such as the differences in translocation timeof purine and pyrimidine bases. Despite the simplification of the CG model, our findings are in accordance with previous experimental and all-atom MD simulations results, underscoring the potential of the model for studying further translocation phenomena.

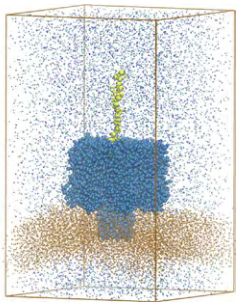


Figure 1: Coarse-grained representation of the system at its initial conformation with 10-nucleotide poly(dA) molecule. The DPPC lipid bilayer is depicted with brown points, ions with cyan and purple points, poly(dA) in yellow beads, and the α- hemolysin nanopore in blue beads. Water is not shown.

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P027

Exploring Protease Regulation: A Comprehensive Database of Cathepsin-GAG *in silico* Interactions

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Cathepsins are a family of proteases critical for protein degradation in all living organisms. Their enzymatic activity may be regulated by GAGs, linear periodic anionic polysaccharides, that are essential for numerous biological processes, including cell proliferation, adhesion and anticoagulation [1]. Despite their significant biological roles, the structural details of cathepsin-GAG complexes remain largely undefined due to the scarcity of experimental structures [2, 3]. To bridge this knowledge gap, we are developing a web-based database specifically focused on systematic calculation of GAG interactions with cysteine cathepsins covering the entire family of 11 cathepsins and 6 classes of GAGs, resulting in over 300 unique complexes. This database utilizes advanced *in silico* methods including all-atom and coarse-grained molecular dynamics simulations, alongside replica exchange molecular dynamics techniques, facilitating a total of over 5000 simulations. This tool is under development to provide comparative framework for computational approaches in biomolecular research that could be useful for rational development of novel strategies in GAG-driven drug design.

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P028

Pharmacodynamic and pharmacokinetic studies of potential bioactive natural products in two Achillea species found in Greece

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The use of medicinal plants and their products as a mean to cure diseases and practice that continues even to this day and has its roots to the early history of mankind. The development of molecular docking alongside the rise of novel computational technologies has managed to considerably the cost of drug development. In the present study the chemical compounds that are found at Achillea Millefolium L. and Achillea Holosericea Sibth. Sm., which grows at the geographic area of Epirus, Greece, are as well as collected and then examined regarding their pharmacodynamic and pharmacokinetic properties, as well as their potential use as bioactive compounds. The compounds that show the desired properties and then examined *in silico* using the AutoDock software, in order to determine their ability to effectively bind to two proteins, MAO-A and MAO-B.

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P029

All-atom molecular dynamics simulations of antimicrobial branched peptide interaction with bacterial membrane

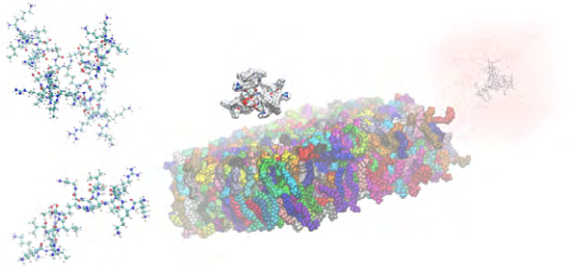
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In the recent years, the emergence of an increasing number of antibiotic-resistant bacteria poses a serious threat to human health. The rapid spread of resistance among bacteria significantly challenges the management of bacterial infections. Antimicrobial peptides (AMPs) are short amino acid sequences with positive net charge which makes them capable to permeating into the anionic bacterial membrane, leading to cell death [1].

These peptides can be combined together via suitable linkers to create more effective compounds, such as so-called branched peptides. This helps overcome some limitations of peptides, such as a short half-life and instability in the cellular environment. Branched peptides are based on a promising peptide structure with enhanced stability and specificity to combat resistant bacterial infections and cancer cells [2]. Synthetic Set-M33 is a branched antimicrobial peptide specifically developed to fight multi-drug resistant bacteria. Tetra-branched and double-branched forms of Set-m33 consist of four and two copies of the KKIRVRLSA sequence, respectively. They are connected via a core made of modified amino acids forms [3]. The strong antibacterial activity of the tetra-branched peptide against multi-resistant gram-negative bacteria has prompted researchers to investigate the peptide's mechanism of actions at the atomic level and to enhance its activity.

All-atom molecular dynamics simulations of different variants of the branched peptide together with bacterial membrane models can offer insights into the most influential molecular determinants, unveiling the differences in their mechanisms of action. Interestingly, differences in behaviour between the double and tetra branched structures as they interact with the membrane can be studied and used to improve their design. To address this, we generated specific force field parameters for the non-standard amino acids used as building blocks for the branched peptides, and we parameterized the entire structures of tetra and double branched peptides. This enabled performing molecular dynamics simulations for single, double, and tetra branched peptides in the absence and in the presence of a *Pseudomonas aeruginosa* outer membrane (OM) model. Conformational analysis of the molecules in different environments pointed to the main differences between each peptide form and identified key points of interaction with the anionic surface of the membrane.



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P030

Multispecific Recognition in Antibodies: A Molecular Dynamics Investigation of SPE-7 IgE Binding to Haptens and Proteins

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SPE-7 is a monoclonal IgE antibody originally raised against a 2,4-dinitrophenyl (DNP) ligand. The antibody is capable of adopting multiple conformations, which greatly increases its binding repertoire. This allows it to bind several different small haptens as well as large proteins. In addition, the antibody is thought to be able of self-binding due to its cytokinergic properties.

This study examines a dataset of experimentally determined crystal structures of the SPE-7 antibody, including unbound conformations, structures bound to different haptens, and a structure bound to a protein, confirming its high multispecificity [1]. Bias-exchange metadynamics as well as conventional molecular dynamics simulations were used to access the paratope dynamics on a micro- to millisecond timescale. We were able to clearly distinguish between the different binding states of the paratope and calculated transition timescales and state probabilities. We also described the residues responsible for discriminating between the different binding capacities and identified the key residue as a tryptophan in a central position of the CDR L3 loop. The CDR L3 and the CDR L1 seem to have the biggest impact on the overall paratope dynamics.

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P031

Conformational diversity of Cytochrome P450 3A4s' tunnels co-determines promiscuity

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Cytochrome P450 3A4 (CYP3A4) is a membrane bound monooxygenase. It metabolizes the largest proportion of all orally ingested drugs. Ligands can enter and exit the enzyme through flexible tunnels[1], which co-determine CYP3A4s' ligand promiscuity. Classical molecular dynamics simulations were used to characterize the tunnel opening and closing, in terms of their diameter, opening frequencies and joint movements. We observe correlated opening and closing events and find that the opening and closing mechanisms serve the tunnels exit or access purposes and suit their positioning in the membrane. Known cytosol facing tunnels are stabilized by salt bridges in their open conformation, while membrane facing, and inserted tunnels are closed by the phenylalanine cluster. These results lead to a further understanding of the entrance and exit pathways and will help to elucidate CYP3A4s ligand promiscuity.

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P032

Cation-π Interactions: a Key Interaction in Lowering the Energy Barrier of Phosphatidylcholine Extraction from Lipid Bilayers by STARD2

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[2]Computational Biology Unit, University of Bergen, Bergen, Norway

STARD2 is a lipid transfer protein that extracts phosphatidylcholine (PC) lipids from the endoplasmic reticulum membrane and carries them to other intracellular membranes. Like other START domains, the STARD2 structure features a helix-grip fold surrounding a large hydrophobic cavity holding a PC lipid. Available X-ray structures show that the choline group of the PC lipid is positioned within an aromatic cage formed by W101, Y114, Y116, and Y155. Cation-π interactions are widespread noncovalent interactions that play important roles in protein folding and stability, ligand recognition, and the recognition of choline-containing lipids by peripheral membrane proteins. The mechanism of PC extraction by STARD2 remains undisclosed. Here, we employ microsecond-long molecular dynamics simulations of the protein in the presence of multicomponent lipid bilayers, supplemented by free energy perturbation (FEP) calculations to shed light on the PC extraction mechanism by STARD2 from a bilayer mimicking the endoplasmic reticulum membrane. We propose a stepwise mechanism driven by cation-π interactions and by which the choline moiety of a PC: *(1)* interacts with W186 and W190 at protein-membrane interface, *(2)* moves to Y84 and Y105 located on the cavity walls, and *(3)* reaches the aromatic cage. From earlier work, we know that interfacial choline-tryptophane cation-π interactions such as those in step *(1)* contribute about 2.5-3 kcal/mol. We used FEP calculations to evaluate the contribution of the two tyrosines in step *(2)* to the binding affinity of the PC lipid to STARD2. The substitution of Y84 and Y105 to alanine resulted in the loss of 1.8 and 2.5 kcal/mol, respectively. Our findings aid in understanding the mechanism of PC extraction by STARD2 and suggest that cation-π interactions play a critical role in lowering the energetic cost of extracting a PC lipid from the membrane.

P033

Exploring RNA-protein interactions: sequence specificity and docking insights across diverse binding motifs

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RNA recognition motifs (RRMs) are essential RNA-binding motifs found in diverse proteins, regulating gene expression, RNA processing, and cellular stress responses. Dysregulation of RNA-binding protein (RBP) interactions is implicated in various diseases, emphasizing the need to understand their mechanisms. This study aims to explore the intricate mechanisms underlying protein-RNA interactions mediated by RRMs, utilizing computational methodologies such as modeling, docking, and molecular simulations. We applied bound-bound docking protocols with HADDOCK3 to evaluate complex RNA-protein interactions, resulting in favorable outcomes. Moreover, we conducted experiments involving mutations on the RNA sequence combined with docking to determine the sequence specificity of experimentally determined complexes. Sequence specificity is critical in determining the precise binding preferences of RBPs, underscoring its importance in understanding their regulatory mechanisms. The implication of RNA-binding protein (RBP) interactions in a range of diseases underscores the critical necessity to elucidate their underlying mechanisms. Understanding these mechanisms is essential for comprehending the molecular basis of diseases and underscores the importance of this project in addressing the challenges posed by RNA-related disorders.

P034

Investigating Targeted Therapies for Cystic Fibrosis through D-NEMD Simulations of the CFTR Protein

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Cystic fibrosis (CF) is a debilitating genetic disorder caused by the malfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, an anion channel that regulates epithelial ion transport. The treatment of CF has been revolutionized by orally administered drugs that directly target defects in CFTR called correctors and potentiators. The potentiator ivacaftor partially restores function to CFTR channels with gating mutations, such as G551D. Ivacaftor binds CFTR at the protein-lipid interface in a cleft formed by transmembrane segments 4, 5, and 8,[1] but the conformational and dynamical changes it induces remain incompletely understood.

Here, we investigate how the ivacaftor-binding site is allosterically coupled to other functionally important regions of CFTR using a new computational approach named dynamical-nonequilibrium molecular dynamics (D-NEMD) simulations.[2] The D-NEMD process combines molecular dynamics (MD) simulations under equilibrium and nonequilibrium conditions to map the evolving structural response of a protein to a perturbation.

We constructed a model of CFTR from the cryo-EM structure of phosphorylated, ATP-bound human CFTR in complex with ivacaftor.[1] Missing loops were filled in using AlphaFold.[3] The regulatory domain that is absent from the cryo-EM dataset was not included due to its considerable size and low-confidence AlphaFold prediction. The model was inserted into a lipid membrane composed of POPC, POPE, and cholesterol. The simulations were consistent with a 60 % lipid contribution to the ivacaftor binding site,[1] with interactions formed during a preliminary 20 ns "pre-production" stage. Consequently, ivacaftor remained in the cryo-EM-deduced binding site throughout all the subsequent unconstrained 500 ns runs (5 replicas for each system), unlike systems set up without prior equilibration of the lipid binding site. This allowed us to refine our understanding of ivacaftor-CFTR dynamics and its response to ivacaftor perturbation.

These equilibrium MD simulations were used to generate a large set of short D-NEMD simulations (305 x 5 ns) where ivacaftor was removed as the perturbation, to identify communication pathways through the observed responses (ensuring the statistical significance of our findings).

Our work reveals that there are allosteric signals related to ivacaftor binding that are propagated from the binding pocket up towards the extracellular vestibule of the CFTR pore and, significantly, downwards via neighbouring transmembrane segments to both ATP-binding sites at the interface of the dimer (the nucleotide-binding domains). For example, there are three routes from the ivacaftor-binding site that reach ATP-binding site 2. One of these goes through transmembrane segment 5 and intracellular loop 2 to reach ATP-binding site 2. By studying disease-causing mutations that map to these allosteric pathways, such as G551D, we can gain a better understanding of how ivacaftor restores CFTR function, potentially guiding the rational design of future therapies for CF.

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P035

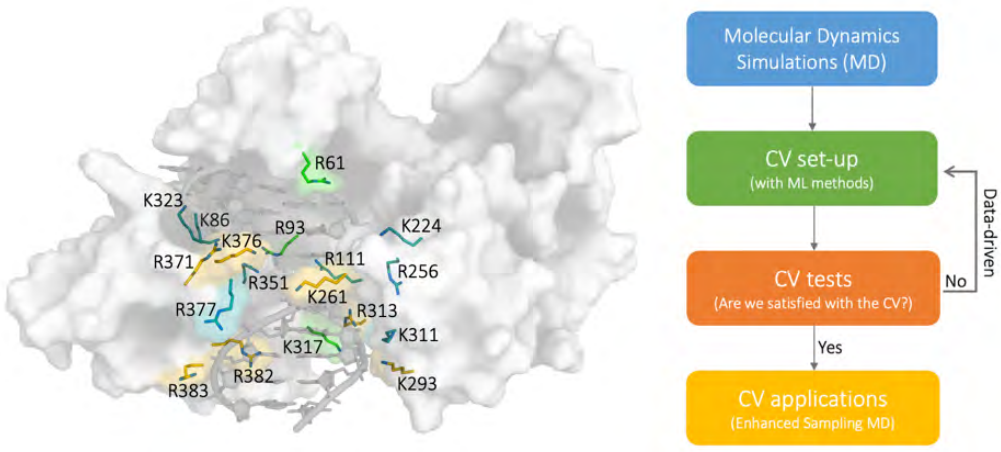
Molecular Dynamics Simulations to study translocation in Human DNA Polymerase η

Alessia Visigalli[1], Enrico Trizio[2], Luigi Bonati[2], Pietro Vidossich[1], Michele Parrinello[2], Marco de Vivo[1]

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DNA and RNA polymerases (Pols) are essential enzymes involved in fundamental cellular processes, such as gene expression, regulation, transcription, and repair [1][2]. Given their critical role, Pols have emerged as promising targets for therapeutic interventions in various diseases, including infections, neurodegenerative disorders, and cancer [3]. Translocation, a fundamental process in DNA replication and transcription, involves the movement of polymerase enzymes along the DNA strand after the nucleotide addition [2]. This dynamic step is essential for accurate DNA synthesis. Despite significant improvements in understanding Pols' catalytic mechanisms, the translocation step is poorly understood, yet [4][5].

In this context, molecular dynamics (MD) simulations can be a powerful tool to investigate the translocation step in human DNA polymerase η (Polη). Through microsecond-long atomistic simulations and machine learning (ML) -guided enhanced sampling MD[6][7], we investigate the role of competing interactions at the Pol•DNA interface, which involve positively charged protein residues and phosphate atoms on the DNA substrate, and their dynamic interactions. Our preliminary results support that the cooperative and collective dynamics of such interactions are critical to an efficient translocation process in Polη.



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P036

Investigating origins of differential carbapenem hydrolysis by OXA-48 variants through multiscale simulation

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β-lactam antibiotics are the most widely used way to treat bacterial infections and have saved numerous human lives. Bacteria can produce β-lactamases (BLs), which are enzymes that first acylate β-lactams, forming an acyl-enzyme complex, and then release the hydrolyzed antibiotic through deacylation, leading to inactivation of the drug.

OXA-48 has a strong hydrolysis activity towards the carbapenem antiobiotic imipenem, while OXA-163 has only low efficiency. OXA-163 has a four residue deletion β5-β6 loop alongside a S212D mutation. A newly discovered OXA-48 variant, OXA-517, has just a two residue deletion in the same loop and partially rescues imipenem hydrolysis activity compared to OXA-163 [1]. The molecular basis of the different imipenem hydrolysis activity and the role of β5-β6 loop for carbapenem hydrolysis remain unclear.

MM molecular dynamics was applied to investigate the acyl-enzyme complex of the three variants with imipenem. Principal component analysis shows that OXA-48 and OXA-517 have a slightly looser active site than OXA-163. Clustering indicates that the biggest structural difference is at the backbone of T213 (Figure 1A). The first cluster is dominant in OXA-48 and OXA-517, with T213 closer to hydroxyethyl group of imipenem. This may lead to different H-bond patterns of the deacylating water (DW) – acting as hydrogen donor or acceptor to hydroxyethyl group (Figure 1B). Previously, we showed that DW acting as hydrogen acceptor can increase the subsequent energy barrier.[2] The deacylation reaction of the most reactive conformation (with DW acting as hydrogen donor) was simulated with QM/MM umbrella sampling. Our results show that a less hydrated carboxylated lysine (KCX73) is beneficial for the reaction (Figure 1C). Regardless of the hydration state of KCX73, the energy barriers of OXA-48 are always the lowest, and the energy barriers of OXA-517 are between OXA-48 and OXA-163, which is consistent with experimental data. This work can not only indicate how different residue mutation and deletion may cause a change in hydrolysis activity, but also help obtain a deeper insight into the dynamics and reaction mechanisms related to antibiotic resistance.

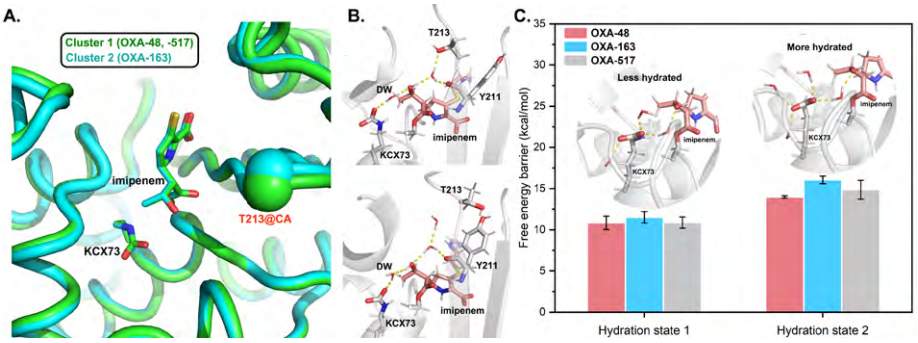


Figure 1. (A) Representative active site structures from clustering. (B) Structures of deacylating water (DW) acting as hydrogen donor (upper) or hydrogen acceptor (lower) to the imipenem hydroxyethyl group. (C) Calculated free energy barriers for imipenem hydrolysis with different hydration states around carboxylated lysine (KCX73).

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P037

Photoexcited Proton-coupled Electron Transfer Reactions in Cryptochrome

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While Quantum Chemical/Molecular Mechanical (QC/MM) calculations have been reported on models of Cryptochromes, much less has been reported about the calculated excited state properties of these photoreceptors and the coupling of proton transfer to electron transfer from a proximal Trp residue to the isoalloxazine ring of flavin adenine dinucleotide (FAD). In addition, alternative electron transfer pathways have gone largely unexplored. Here, we reveal the geometrical and charge redistributions that occur upon photoexcitation of FAD and how these changes facilitate proton transfer from Asp396 to N5 of FAD in the excited state while also showing why this proton transfer is unlikely to occur in the ground state. Prior to complete proton transfer from Asp396, an electron from Trp400 is also transferred to the isoalloxazine ring. The energy of this charge transfer state steadily reduces while progressing along the proton transfer coordinate consistent with a PCET reaction. In addition, the calculations suggest that if Trp400 were to fluctuate outside favorable electron transfer range, the adenine (Ade) moiety of FAD could serve as an electron donor given the stacked orientation between Ade and the isoalloxazine ring. Electron transfer between these two moieties has also been characterized in DNA photolyases that share the same stacked FAD configuration though the two moieties are in the different oxidation states. QC calculations on isolated Trp and Ade radical cations reveal distinct overlapping absorptions suggesting the need to possibly reinterpret experimental data and to consider alternative identities of the radical pair important to magnetic field sensitivity.

P038

Advances in DNA structure simulation: bridging canonical and non-canonical motifs with the OL21 force field

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As the importance of non-canonical DNA motifs in the molecular biology of living organisms becomes increasingly apparent, accurate modeling of these unique conformations is crucial for many applications. The Z-DNA molecule can be considered a touchstone of the universality of empirical force fields, since the non-canonical alpha and gamma backbone conformations native to Z-DNA are also found in protein-DNA complexes, i-motif DNA and other non-canonical DNAs. In 2021, we introduced a modification of the alpha/gamma parameter which, together with our previous torsion modifications, is referred to as OL21. These parameters enhance the stability of native alpha/gamma Z-DNA backbone substates while keeping the canonical DNA description essentially unchanged, and thus represent a significant improvement over previous AMBER force fields. Here, we present an extensive validation of the OL21 parameters for various DNA structures, both canonical and non-canonical.

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P039

Structural elucidation of MANNAN (polymannose) conjugate with the myelin oligodendrocyte glycoprotein 35-55 epitope (MOG_{35-55}) using NMR spectroscopy

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Multiple sclerosis (MS) is an immunologically mediated [1], inflammatory, autoimmune disorder of the Central Nervous System (CNS) and it is characterized by destruction of the myelin sheath leading to paralysis and serious health problems. One of the main proteins of myelin is the myelin oligodendrocyte glycoprotein MOG and is heavily implicated in the progress of MS. MOG protein is associated with the demyelination in animals in Experimental Autoimmune Encephalomyelitis (EAE). Especially the 35-55 epitope of MOG protein is considered to be a main autoantigen in the pathogenesis of MS and is connected with the induction of EAE in mice. Nevertheless, this epitope conjugated with polysaccharide mannan in its oxidized form, was found to protect mice from EAE and could be a promising approach for the immunotherapy of MS [2].

Mannose, mannan polysaccharide, oxidized mannan and the conjugates of immunodominant MOG_{35-55} epitope with mannan in oxidized form were studied by high field Nuclear Magnetic Resonance (NMR) spectroscopy to explore their structural characteristics and possible structural alterations during storage conditions. Conformational Analysis via Density Functional Theory (DFT) was combined with NOESY NMR experiments contributing to the revealing of low energy conformations of molecules (Figure 1).

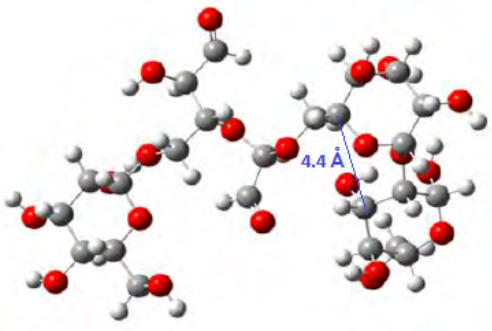


Figure 1. Low energy structure of mannan derived using DFT computations.

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