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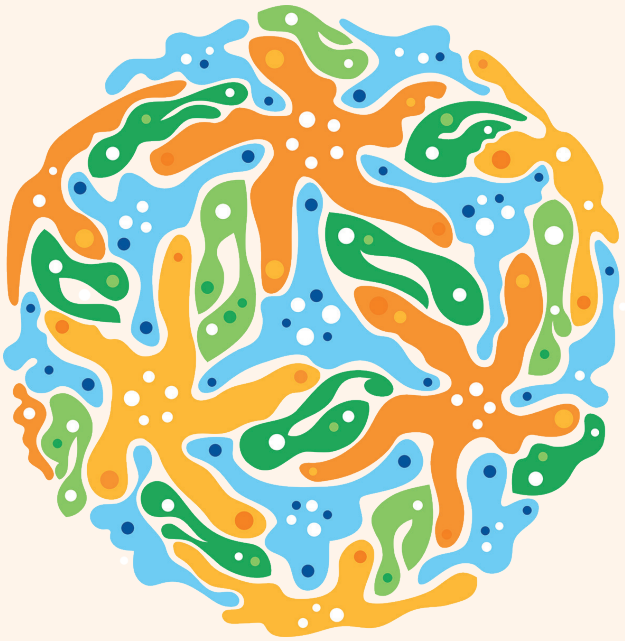
BOOK OF ABSTRACTS



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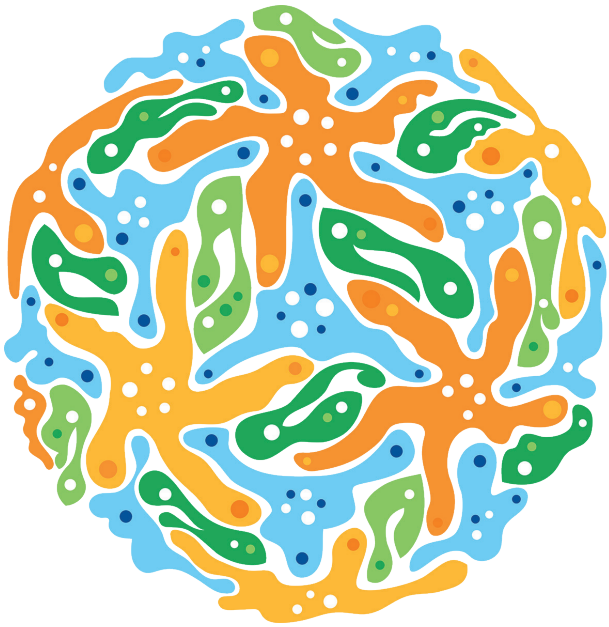

Opening session

Keynote
Learning from molecular simulations

Gerhard Hummer[1,2], Matthias Post[1], Stefan L. Schaefer[1], Ferdinand Horvath[3], Jayashrita Debnath[1], Hendrik Jung[1]

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Molecular dynamics (MD) simulations can give us a detailed and quantitative understanding of complex biomolecular processes. However, many important processes occur on time scales beyond the reach of unbiased MD simulations and thus require the use of advanced simulation schemes. On top, the complexity of the processes makes it challenging to extract mechanistic information from MD simulation trajectories. The problems of sampling rare molecular events and of understanding the underlying reaction mechanism are intertwined: any progress in solving one problem should help solve the other. Building on this connection, we combine enhanced sampling with machine learning techniques to solve both problems at once. We use transition path sampling to learn the commitment probability (in short, the committor) of reaching the product state instead of returning to the reactant state. In turn, we use the learned commitor to boost the efficiency of transition path sampling by a judicious choice of the points from which to initiate new trajectories. The learned committor model is validated on the fly by comparing its predictions for the trajectory endpoints to the observed outcome of actual simulation trajectories. Using these outcomes as input, the committor model is updated if needed. In this presentation, we will present new developments of the aimmd methodology of AI-based molecular mechanism discovery and its applications to autonomous rare-event sampling, state identification, and mechanism learning.

Molecular dynamics session

Invited Lecture
Modeling conformational cycles of ion channels

Erik Lindahl
KTH Royal Institute of Technology

Cryo-electron microscopy has been a revolution for membrane protein structure determination, and in some cases we can even determine multiple structures corresponding to different states. However, even state-of-the-art experimental methods have challenges in resolving the functional properties of states, not to mention that the are not able to resolve the actual transitions between states that achieve their biological function. I will present our integrative structural biology work on determining structures of the pentameric ligand-gated ion channels responsible for chemical signaling between cells, and how we are combining this with molecular simulation tools. I will show how we are able to classify structures with modern computational methods that are able to accurately simulations currents through channels (corresponding to electrophysiology experiments). Coarse-grained representations and free energy calculations have similarly made it possible for us to explain how lipids modulate the function of this channels - despite experimental structures still not resolving bound molecules, and finally I will discuss how Markov state models have finally made it possible for us to model the entire functional cycle between restive, active and desensitized state on millisecond scale.

Molecular dynamics session

Selected Talk
Non-equilibrium simulations with POP-MD

Luca Monticelli[1,2], **Jackson Crowley**[1], **Cecile Hilpert**[1], **Vincent Nieto**[1]
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Abstract Molecular dynamics simulations are generally carried out in equilibrium ensembles, with constant number of particles and constant concentration of each molecular species. However, in living organisms the concentration of different molecules is not constant and molecular distributions do not reach equilibrium, due to active metabolic processes. Here we introduce POP-MD, a simple software that plugs into the well-known GROMACS package and allows MD simulations with non-constant number of particles and non-equilibrium distribution of molecules. We show examples of usage of the software to mimic lipid synthesis during the biogenesis of lipid droplets, cellular organelles responsible for lipid storage and metabolism. POP-MD allows to test different possible mechanisms of biogenesis, and study the effect of localization of lipid synthesis in different regions of the system, as well as asymmetric synthesis. Application of POP- MD to membrane biogenesis and the formation of lipids domains are also envisaged.

Molecular dynamics session

Invited Lecture
Good rates from bad coordinates

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Our ability to calculate rates of biochemical processes using molecular dynamics simulations is severely limited by the fact that the time scales for reactions, or changes in conformational state, scale exponentially with the relevant free-energy barriers. In this work, we improve upon a recently proposed rate estimator that allows us to predict transition times with molecular dynamics simulations biased to rapidly explore one or several collective variables (CVs). This approach relies on the idea that not all bias goes into promoting transitions, and along with the rate, it estimates a concomitant scale factor for the bias termed the CV biasing efficiency γ. First, we demonstrate mathematically that our new formulation allows us to derive the commonly used Infrequent Metadynamics (iMetaD) estimator when using a perfect CV, where γ=1. After testing it on a model potential, we then study the unfolding behavior of a previously well characterized coarse-grained protein, which is sufficiently complex that we can choose many different collective variables to bias, but which is sufficiently simple that we are able to compute the unbiased rate directly. For this system, we demonstrate that predictions from our new Exponential Average Time- Dependent Rate (EATR) estimator converge to the true rate constant more rapidly as a function of bias deposition time than does the previous iMetaD approach, even for bias deposition times that are short. We also show that the γ parameter can serve as a good metric for assessing the quality of the biasing coordinate. We demonstrate that these results hold when applying the methods to an atomistic protein folding example. Overall, our time-dependent rate approach offers a powerful framework for predicting rates from biased simulations.

Molecular dynamics session

Selected Talk

Structure-guided disulfide engineering restricts antibody conformation and flexibility to elicit TNFR agonism in anti-cancer therapeutics

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[2]European Synchrotron Radiation Facility, Grenoble, France

Immunostimulatory antibodies (ISAs) represent a promising strategy for cancer immunotherapy. By activating co-stimulatory molecules expressed on immune cells, such as tumour necrosis factor receptors (TNFRs), ISAs can enhance the immune response towards tumours, resulting in powerful anti-cancer effects. Antibodies comprise two antigen-binding domains linked to an effector domain through a disulfide-containing hinge. There are four isotypes of human (h)IgG, and previous work has shown that the hIgG2 isotype can deliver strong agonistic activity for ISAs, due to its unique hinge disulfide arrangement.
Here we employ an integrative approach to understand how structure and conformational dynamics affect agonistic activity of hIgG2 antibodies. We use cellular assays to ascertain agonistic activity, small angle X-ray scattering (SAXS) to assess flexibility and conformation, X-ray crystallography to determine protein structure and disulfide position, and molecular dynamics simulations with SAXS-guided ensemble reweighting to probe antibody conformational dynamics.
By modifying hinge disulfide patterns using cysteine-to-serine exchange mutations, we show that strong agonistic activity is associated with restricted global antibody flexibility and reduced conformational dynamics due to the presence of a cross-over of disulfides in the hinge. This has been shown for antibodies targeting two co-stimulatory receptors, CD40 and 4-1BB. We then use structure-guided approaches to design new hIgG2 antibody variants with novel disulfide patterns, to further restrict flexibility and enhance biological activity.
Together, these results demonstrate the importance of structure and conformational dynamics in ISAs and provide a strategy for rational design of more powerful antibody therapeutics, and thus more effective anti-cancer treatments.

Molecular dynamics session

Selected Talk

Molecular mechanism of allostery in *E. Coli* dihydrofolate reductase (DHFR)

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Allostery regulates the activity of a protein by changes at a site away from the active site. This regulation typically occurs through ligand binding, but the effect of a distal mutation on the activity of a protein can be seen as latent allostery. Since 1964, several models have been proposed to describe allostery. However, there is still no consensus and the explanation of allostery at the molecular level remains elusive. In this study, we focus on *E. coli* DHFR, in which distal mutations at site G121 have been shown to affect hydride transfer between the cofactor NADPH and the protonated folate H3F+[1]. Using replica- exchange molecular dynamics simulations, we show that the effect of G121V on hydride transfer can be explained by a conformational equilibrium shift due to steric interactions, from conformations in which the NADPH and H3F+ cycles are parallel to conformations in which they are perpendicular. Using an implementation of EVB for Gromacs[2], we show that parallel conformations are more reactive than perpendicular ones. It is consistent with a Monod-WYman-Changeux-type model of allostery[3]. Using out-of-equilibrium MD simulations, we then propose a molecular mechanism for the transition pathway from the parallel conformation to the perpendicular conformation and a kinetic model for this mechanism. It is the first molecular mechanism for allostery in *Ec*DHFR.

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Integrative Modelling session

Invited Lecture
Protein structural ensembles from 3D and 2D cryo-EM data

Max Bonomi
Institut Pasteur, Paris, France

Understanding the molecular mechanisms employed by biological systems to carry out their functions is often essential for rationally targeting associated diseases. In many cases, determining the three- dimensional (3D) structure of these systems provides valuable insights. However, it is frequently the interplay between structural and dynamical properties that determines the behavior of complex systems. While both experimental and computational methods are invaluable tools for studying protein structure and dynamics, limitations in each individual technique can hinder their capabilities [1]. Here, I will present two integrative computational-experimental approaches to combine cryo-electron microscopy (cryo-EM) data (and more) into molecular dynamics (MD) simulations to determine accurate protein structural ensembles [2,3]. I will showcase the capabilities of these methods using different applications to biological systems of outstanding interest. First, I will illustrate how accurate protein structural ensembles can be obtained from 3D cryo-electron microscopy maps with our recently developed EMMIVox approach [4]. Then, I will focus on characterizing the structural and dynamic properties of the CyaA toxin by combining coarse-grained MD simulations with Hydrogen/Deuterium eXchange Mass Spectrometry (HDX-MS), Small Angle X-ray Scattering (SAXS), and 2D single-particle cryo-EM data. Finally, I will show how AlphaFold2 and single-particle cryo-EM images can be synergistically used to characterize the alternative, functional states of a G-Protein Coupled Receptor.

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Integrative Modelling session

Selected Talk
HADDOCK3: a modular integrative modelling platform

Marco Giulini
Utrecht university, Utrecht, Netherlands

HADDOCK has been an important resource for the integrative modeling community and one of the flagship software since the beginning of the Bioexcel project. Our web infrastructure supports nearly 50,000 registered users, who collectively perform approximately 100,000 docking runs per year. Here we introduce HADDOCK3, the new modular version of the program, in which the original, rigid albeit parameterisable pipeline has been first broken down in a catalogue of independent modules and then enriched with powerful analysis tools and third party integrations.
Thanks to this increased flexibility, HADDOCK3 can now handle a multitude of integrative modelling scenarios, providing a valuable, physics-based tool to enrich and complement the predictions made by machine learning algorithms in the post-Alphafold era. We present examples of successful applications of the method, including improved antibody (and nanobody)-antigen modelling, protein-glycan docking, consensus scoring, and iterative model refinement.

Integrative Modelling session

Invited Lecture
Restoring Protein Glycosylation with GlycoShape

Elisa Fadda
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The introduction of machine learning (ML) for protein structure prediction revolutionized structural biology, boosting our ability to source and resolve protein structures, and broadening the potential for therapeutic discovery. One limitation affecting all ML-derived structures is the lack post-translational modifications, which are key to the correct folding, structural stability, and function of the underlying protein. Glycosylation is the most common post-translational modification of proteins, with an estimated 3 to 4% of the human genome dedicated exclusively to encode for glycosylation pathways. Yet, glycans remain largely 'unseen' due to their heterogeneity, complexity and highly dynamic nature. In this talk I will introduce GlycoShape (https://glycoshape.org), a unique resource based on high-performance computing that allows users to rapidly and easily restore glycoproteins from ML (AlphaFold and/or RoseTTAFold), as well as from the Protein Data Bank (www.rcsb.org), to their native, functional state by adding the missing glycan 3D information in seconds. Because of the robustness of its 3D database and of the algorithm, GlycoShape can also predict N-glycosylation site occupancy with a 93% accuracy against all experimentally profiled glycoproteins in the PDB. This remarkable level of agreement with glycoproteomics data provides further evidence that the type of glycosylation and occupancy depend on site accessibility and complementarity of the glycan to the protein surface, revealing a real potential of training upcoming ML algorithms with enormous impact on scientific and therapeutic advances. To this end, I will provide some examples, ranging from pathogen infection to protein folding, to underscore the importance of rebuilding glycosylation to understanding biomolecular structure and function in life sciences.

Integrative Modelling session

Selected Talk

Improving docking and virtual screening accuracy for challenging targets by accounting for protein and ligand flexibility

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Knowledge of atomic-level structures of ligand-protein complexes is key for basic research and structure-based drug design. Computational methods have become a valid complement to experiments, but accuracy of predictions decreases with the extent of the structural changes associated to binding. Accurate description of ligand flexibility is equally crucial, particularly in a virtual screening (VS) context where the structures of many ligands are generated without accounting for structural adaptation in binding.
To address this issue on the proteins side, we recently introduced gEDES – generalized Ensemble Docking with Enhanced-sampling of pocket Shape" [1-3], a computational method based on metadynamics to generate holo-like conformations of proteins by only exploiting their apo structure. Here, we present SHAPER, an algorithm aiming to generate ligand structures snugly fitting into the binding pocket of a generic receptor, adapting to its conformation. Implementation of this dynamic shape-matching algorithm allows to improve the accuracy of VS campaigns compared to docking calculations.
We validated our methodology against other state-of-the-art methods on the DUD-e database [4], a benchmark dataset for docking and virtual screening. In addition, we are setting-up a user-friendly web server that will make it easier for researchers, regardless of their expertise, to set up and run these simulations, as well as to download generated holo-like structures for any available target.

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Integrative Modelling session

Selected Talk

Towards modeling cellular environments from cryo-electron tomography by high-confidence 3D template matching

Sergio Cruz-León[1], Jan Philipp Kreysing[2], Maziar Heidari[1], Tomáš Majtner[2], Patrick C. Hoffmann[2], Sebastian Kehl[3], Maarten W. Tuijtel[2], Stefan L. Schaefer[1], Katharina Geißler[2], Martin Beck[2], Beata Turoňová[2], Gerhard Hummer[1]

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The simulation of biologically realistic systems requires precise knowledge of the composition and spatial arrangement of biomolecules in situ. This information can be obtained from cryo-electron tomography (CryoET), which images the interior of intact cells in 3D. However, feature identification is limited by the low signal-to-noise ratio and anisotropic resolution of the tomographic data. In this talk, I will present our recent advances in high-confidence 3D template matching (hcTM) for CryoET [1] and how we use hcTM to generate simulation-ready molecular models directly from cells [1,2]. hcTM enables the automated and comprehensive detection of a wide variety of macromolecular complexes within crowded eukaryotic cells. We demonstrate high-fidelity and high-confidence localization of nuclear pore complexes, vaults, ribosomes, proteasomes, fatty acid synthases, lipid membranes, microtubules, and individual subunits and substates thereof [1]. The high-confidence molecular assignments have driven both technical advances [3] and biological discoveries [1,2], fostering robust connections between molecular functionality, spatial localization, and cellular context. Thus, hcTM paves the way for modeling and simulating the dynamics of biomolecules in their native environment.

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Integrative Modelling session

Selected Talk
Integrative modeling of glycoproteins, lessons from the pandemic

Mateusz Sikora[1,2], Cyril Hanus[3], Shang-Te Danny Hsu[4], Gerhard Hummer[2]
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[2]Max Planck Institute of Biophysics, Frankfurt am Main, Germany
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Glycans, complex sugars covalently attached to proteins, affect protein stability and function, participate in 'self' recognition, and modulate protein-protein interactions. The glycosylation machinery is frequently hijacked by pathogens, which hide their proteins behind a "glycan shield", making them inaccessible to the immune system and complicating pharmacological interventions. Unlike many biomolecules, glycans do not typically form secondary structures and remain highly mobile, posing a challenge for traditional structural biology techniques. In our research, we combined molecular dynamics simulations with cryo-electron tomography and atomic force microscopy to understand how glycans affect viral fusion proteins, particularly the SARS-CoV-2 spike protein. We discovered a surprising flexibility of the spike protein [1,2] and predicted new antibody binding sites accessible through the dynamic glycan shield [3], which can aid in designing novel vaccines. Additionally, we developed a simplified, open-source method GlycoSHIELD for rapidly predicting glycan shielding with minimal computing power. This method has been applied to refine existing cryo-EM maps of glycoproteins [4].

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Free Energy calculations session

Invited Lecture
From Active Learning to Zinc: adventures with alchemical free energy calculations

Antonia Mey
University of Edinburgh, Edinburgh, United Kingdom

Relative free energy (RFE) calculations are widely used in academia and industry to compute free energies of binding between ligands and proteins as accurately as possible. While alchemical methods do provide good accuracy for many systems some challenges remain. One challenge is how to effectively choose which ligands to screen with RFE calculations when the ligand pool for testing is larger than the computational budget allows. A second challenge involves ligands that interact with metals in the binding site. Both have unique sets of challenges.

I will highlight how we have used active learning (AL), an iterative process that allows for learning of labels of an unlabelled dataset, to identify which compounds to select next for RFE screening from a ligand pool. We benchmarked the influence of different surrogate models (Chemprop and Gaussian processes), AL strategies such as exploration and exploitation cycles, batch sizes, and the effect of noise on the selection efficiency of the data.

To address how we can better model ligands interacting with coordinating metal ions in metalloproteins and devise robust free energy protocols for such systems, I will share our ongoing efforts in this direction. Using different β-lactamases, divalent zinc metallo- β-lactamases, and serine β-lactamases, we can show that conventional RFE methods give good binding affinities with respect to experimental measures of known congeneric series. The same ligands showing activity against metallo-β-lactamases cannot be modelled to the same level of accuracy using naïve ligand and metal force field parameters as well as restraints for the effective coordination of the metal sites.

Free Energy calculations session

Selected Talk
Computational Design Principles for Genetically Encoded Fluorescent Biosensors

Canan Atilgan, Melike Berksoz
Sabanci University, Istanbul, Turkey

Genetically encoded fluorescent biosensors (GEFBs) have emerged as reliable tracers for various metabolites and cellular processes, due to their modular design. These biosensors consist of a fluorescent protein (FP) fused to a sensing protein, which undergoes conformational changes upon ligand binding. This conformational shift alters the chromophore environment, thus modifying the FP's spectral characteristics. However, the key structural elements for effective biosensor performance are typically identified only after the crystal structures of both ligand-bound and ligand-free forms are available. Consequently, the development of new biosensors for specific analytes often involves extensive trial and error processes. In this study, we propose a streamlined workflow for biosensor development which not only accelerates the identification of promising biosensor candidates but also enhances our understanding of the fundamental principles governing their operation. To this end, we examine the structural determinants of high-performance biosensors in both ligand-bound (ON state) and unbound (OFF state) conformations using all-atom classical molecular dynamics (MD) simulations on a range of FPs. These simulations allow us to visualize the dynamic behavior of biosensors at an atomic level, providing detailed insights into the interactions that govern their function. We focus on characterizing the shifts in hydrogen bond occupancies across the entire sensor structure to understand the allosteric modulation of the chromophore environment. Our findings highlight two strong indicators for distinguishing the ON state of high-performance sensors: a continuous network of hydrogen bonds from the ligand binding site to the chromophore environment, and reduced water density around the chromophore. The continuous hydrogen bond network suggests a pathway for the transmission of the conformational change signal, while the reduced water density implies a more stable chromophore environment in the active state. Our integrative strategy involving efficient screening of the PDB for sensor domain candidates, AlphaFold predictions for the fused structures, determinants for correct charge states of residues around the chromophore, and assessment criteria based on dynamics form the groundwork for the design of new biosensors, potentially reducing experimental screening time. This research paves the way for the development of next-generation biosensors with improved performance, broadening their applications in biomedical research, environmental monitoring, and industrial processes.

Free Energy calculations session

Invited Lecture
The past, present, and future of free energy calculations in drug discovery

John Chodera
Memorial Sloan Kettering Cancer Centre

While free energy calculations have played an important role in drug discovery for the past several decades, there is still significant opportunity for computational approaches to deliver significantly more predictive models, play a more central role in driving decision-making in the current discovery paradigm, and even for complete paradigm shifts in how drugs are discovered. In this talk, we review some of the history of free energy calculations in drug discovery, the present state of the art and challenges these methods currently face, and opportunities for the future that will enable the integration of machine learning approaches (and hybrid forms thereof) to transform drug discovery from a research science to an engineering discipline.

Free Energy calculations session

Selected Talk
Applying a force field-centric approach to therapeutic protein design

Mohammad ElGamacy
Universitätsklinikum Tübingen, Tübingen, Germany

Physics-based techniques provide for the most generalizable and rationalizable approaches to protein design. However, given the dimensionality of the protein design problem, several heuristics have been commonly deployed to energy functions, in favor of speeding up design simulations. These knowledge-based approximations could greatly limit the accuracy, and thus experimental success rates of the designed proteins. Here we present a new protein design framework, that aims to solely rely on an input force field for the stages of rotamer library generation and design. Furthermore, in contrast to existing methods which iteratively evaluate non-bonded interactions, our framework calculates the interaction energies across two groups of atoms in a single tensor operation. This greatly improves the throughput and granularity at which rotamers are evaluated in a protein design simulation. Here, we present applying this design framework to create bivalent cytokine receptor binders. Experimental characterization showed these proteins to be highly thermostable and active in vitro. Finally, we implement our tensorized design and analysis tools into an integrated toolkit as a publicly-accessible web server with a simplified interface (https://damietta.de/), which benefits the wider protein research community.

Free Energy calculations session

Selected Talk

Effects of lipid composition and protein–lipid interactions on the free-energy landscape of membrane fusion

Katharina C Scherer, Jochen S Hub
Saarland University, Saarbruecken, Germany

Membrane fusion includes the overcoming of free energy barriers separating the intermediate fusion steps from each other. Therefore, context-specific fusion proteins anchored in the fusing membranes via transmembrane domains (TMDs) facilitate the process. These proteins often need to interact with asymmetric and complex lipid environments of the fusing membranes. Investigating the free-energy landscape of fusion serves as important tool to quantify influences of this complexity along the fusion pathway.
Here, we study how lipid composition and TMDs control the energetics of the first step of membrane fusions, that is, the formation of a stalk. We used a newly designed reaction coordinate for simulating thermodynamically reversible pathways of stalk formation at coarse-grained resolution. The simulations reveal that the inner leaflet of a typical plasma membrane is far more fusogenic than the outer leaflet. To rationalize these findings by the distinct lipid compositions, we computed ~200 free energies of stalk formation in membranes with different lipid head groups, tail lengths, tail unsaturations, and sterol content. We observed a drastic influence of the lipid composition on stalk formation and gained a comprehensive fusogenicity map of many biologically relevant lipid classes (Poojari et al., Nat Commun 2021). Additionally, we saw that the presence of TMDs from SNARE complex as well as from viral fusion proteins in the fusing membranes decrease the stalk free energy in a concentration-dependent manner. We can explain this free energy decrease with an increased disorder in the lipid packing caused by the inclusion of TMDs (Scherer et al., in preparation).

Developments in molecular simulations session

Invited Lecture
High-resolution molecular dynamics simulations for biophysics with Tinker-HP

Jean-Philip Piquemal
Sorbonne Université, Paris, France

I will discuss our strategy for high-resolution molecular dynamics towards biophysical applications. As I detail the various protein targets that are currently under study, I will show how the newly developed multi-GPUs version of the Tinker-HP software [1, 2] can accelerate high-resolution molecular dynamics simulations. Indeed, thanks to adaptive sampling and new generation many-body polarizable force fields such as AMOEBA, long (µs) molecular dynamics simulations at enhanced accuracy become possible.[3] As I detail the currently available other enhanced sampling capabilities of the software, I will give some perspectives about the use of new hybrid physically-driven machine learning approaches [4] for condensed phase molecular dynamics.

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Developments in molecular simulations session

Selected Talk

Optimizing Electrostatic Interactions: Electronic Polarization in the prosECCo75 Biomolecular Force Field

Hector Martinez-Seara[1], Ricky Nencini[1,2], Carmelo Tempra[1], Denys Biriukov[1,3,4], Miguel Riopedre-Fernandez[1], Victor Cruces Chamorro[1], Jakub Polák[5], Philip E. Mason[1], Daniel Ondo[5], Jan Heyda[5], O. H. Samuli Ollila[2,6], Pavel Jungwirth[1], Matti Javanainen[1,2]

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The prosECCo75 force field introduces a novel method for enhancing the accuracy of molecular dynamics (MD) simulations by incorporating electronic polarization through charge scaling. This technique addresses a significant limitation of traditional nonpolarizable force fields: their inability to precisely model the intricate electrostatic interactions among highly polar or charged water, ions, proteins, lipids, and polysaccharides, which are crucial for biological functions. By refining partial charges within the CHARMM36 framework, prosECCo75 effectively mitigates overbinding issues and better aligns with experimental observations across diverse biomolecular systems, without altering the detailed molecular structures defined by the original force field. Extensive testing against experimental benchmarks involving ionic solutions, lipid membranes, amino acids/proteins, and saccharides demonstrates prosECCo75's substantial improvements in simulating ion binding to membranes and proteins and when dealing with interactions between charged biomolecules. Achieving such improvements using charge scaling adds zero computational cost to traditional force fields. Consequently, prosECCo75 emerges as a computationally efficient option, offering enhanced accuracy and broader applicability for studying the complex dynamics of biomolecular interactions, essential for advancing our understanding of biological mechanisms.

Developments in molecular simulations session

Invited Lecture
Perspective on Simulating Whole Cells with Martini

Siewert Marrink
Univ. of Groningen, Groningen, Netherlands

In this talk, I will provide the state of the art on the use of the latest version of the coarse-grained Martini mode to simulate complex systems, including the possibility to capture chemical reactions, and provide a perspective on our current efforts to reach the whole cell level.

Developments in molecular simulations session

Selected Talk

Charge Scaling in Potassium Channel Simulations: Implications for Conductance, Ion Occupancy, Voltage Response, and Selectivity

Chenggong Hui[1], Reinier de Vries[1], Wojciech Kopec[1,2], Bert de Groot[1]
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Potassium channels permeate K+ at high rates (~100 pS). They achieve high efficiency and selectivity by a conserved selectivity filter with four adjacent potassium-binding sites. Molecular Dynamics (MD) simulations can provide a detailed, atomistic mechanism for this sophisticated ion permeation. However, there are clear inconsistencies between computational and experimental predictions. Firstly, the ion occupancy of the selectivity filter in simulations is lower than expected (~2.5 in MD compared to ~4 in X-ray crystallography). Secondly, the conductance is typically an order of magnitude lower than what is measured in single-channel electrophysiology experiments. This discrepancy is likely because the force fields does not account for explicit polarization. One proposed force field modification is ECC (Electronic Continuum Correction), which scales down formal charges to introduce polarization in a mean-field way. When ECC is applied to Charmm36m, the simulated conductance significantly increases 7-fold, whereas applying ECC to Amber14 does not consistently increase the conductance. We further investigate this difference between ECC-Charmm36m versus ECC-Amber14sb by looking into the equilibrium distribution of the selectivity filter state. We propose and test new parameters for Amber14sb that also predict a conductance similar to that of experiments and predict the I- V curve qualitatively close to the experiment for different potassium channels. Our modifications for both force fields reconcile Molecular Dynamics (MD) simulations and experimental data, in terms of ion occupancy in the selectivity filter, conductance, current- voltage response, and K+/Na+ selectivity. Overall, these refinements represent possible directions for the development of force fields for simulations of ion channels.

Developments in molecular simulations session

Selected Talk

Mechanoporation in Diffuse Axonal Injury: A Multi-Scale study combining finite element and molecular simulation

Maryam Majdolhosseini, Svein Kleiven, Alessandra Villa
KTH royal institute of technology, Stockholm, Sweden

Diffuse axonal injury (DAI) is a form of traumatic brain injury impacting brain white matter structures from the macroscopic to the molecular level. Although its exact mechanism remains elusive, one hypothesis suggests mechanoporation of axonal membranes as a potential trigger, with mechanoporation defined as the formation of at least one pore in the membrane. Here, we use a multi-scale approach to explore this hypothesis.
First, we developed a coarse-grained model for the axonal membrane, composed of 25 different lipids asymmetrically distributed across the leaflets. The lipid composition was based on experimental data from the human brain. We also considered various protein concentrations to mimic different regions of the axon, such as the node-of-Ranvier (which is considered to have the highest protein concentration). We performed molecular dynamics simulations both at equilibrium and under uniaxial deformation using the GROMACS simulation package and Martini forcefield. Different strain rates were considered to simulate impact conditions.
Then, employing a bottom-up approach, the molecular models were coupled with a finite element model representing the axon at the cellular level, where each element of the cortex of the axon model corresponds to the molecular model. The cellular model of the axon was also investigated under uniaxial deformation. Molecular simulation results indicate that pore formation in the node-of-Ranvier occurs at a lower rupture strain (~ 41% local strain) than other parts of the axolemma. This rupture strain corresponds to axonal strains exceeding 10% at the cellular level, when coupling the results with finite element models, which aligns well with experimental findings. The results suggest that mechanoporation of the membrane might trigger axonal injury.
This enhanced understanding of the injury mechanism of axonal injuries has the potential to develop strategies aimed at mitigating and preventing traumatic brain injuries.

Developments in molecular simulations session

Selected Talk
Nanoparticle induced fusion of lipid membranes

Sofia Blasco[1,2], Lukáš Sukeník[1,3], Robert Vácha[1,2,3]
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Membrane fusion plays a crucial role in various biological processes, including cellular transport and infection mechanisms by membrane-enveloped viruses. Additionally, artificially induced membrane fusion is vital for intracellular drug delivery using lipid vesicles known as liposomes. Nanoparticles can act as fusogens and facilitate membrane fusion. However, the exact mechanisms behind nanoparticle-induced fusion and the optimal properties of these nanoparticles are still largely unknown. In this study, we used molecular dynamics simulations to explore how spheroidal nanoparticles of different sizes, prolateness, and ligand interaction strengths influence fusion between two vesicles. We identified the range of nanoparticle parameters that most effectively promote fusion and demonstrated how changes in each parameter impact the fusion process. Our results offer valuable insights into fusion mechanisms and into the design of fusogenic nanoparticles for biotechnological and biomedical applications.

AI & Drug Design applications session

Invited Lecture

Navigating protein landscapes with a machine-learned transferable coarse-grained model

Cecilia Clementi
Freie Universität, Berlin, Germany

The most popular and universally predictive protein simulation models employ all-atom molecular dynamics (MD), but they come at extreme computational cost. The development of a universal, computationally efficient coarse-grained (CG) model with similar prediction performance has been a long-standing challenge. By combining recent deep learning methods with a large and diverse training set of all-atom protein simulations, we have developed a bottom-up CG force field with chemical transferability, which can be used for extrapolative molecular dynamics on new sequences not used during model parametrization. We have demonstrated that the model successfully predicts folded structures, intermediates, metastable folded and unfolded basins, and the fluctuations of intrinsically disordered proteins while it is several orders of magnitude faster than an all-atom model. This showcases the feasibility of a universal and computationally efficient machine-learned CG model for proteins.

AI & Drug Design applications session

Selected Talk

Human de novo DNA-methyltransferase selectivity is regulated by a base-specific hydrogen bonding network

Ayşe Berçin Barlas[1,2], **Ezgi Karaca**[1,2]
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In mammals, de novo DNA methylation is essential to embryonic development, reprogramming, and gene regulation. The de novo DNA methylation is directed by DNMT3A and DNMT3B enzymes and mainly exerted on CpG islands. Over their enzymatic domains, DNMT3A/B proteins share over 90% of sequence similarity. Even so, DNMT3A predominantly methylates the first cytosine in the CGC and CGT motifs, while DNMT3B prefers the CGG and CGA sequences. To elucidate the mechanistic basis of these selective methylation profiles, we performed extensive molecular dynamics simulations of DNMT3A/B enzymes bound to all possible CGX[C/G/T/A] motifs. Afterwards, we calculated the differential base-specific hydrogen bonding profiles of the systems. As an outcome, we observed that DNMT3A/B sequence selectivity is regulated by an arginine-lysine substitution: the long and branched side chain of DNMT3A-Arg836 stably reads guanine in the complementary strand of the CGC motif, where DNMT3B-Lys777 forms selective hydrogen bonds with two consecutive guanines in the target strand in CGG motif. Interestingly, DNMT3B-Lys777 and DNMT3B- Asn779 assist the sequence selectivity in a cooperative manner. We also observed that when bound to their cognate sequences, the DNMT3A-DNA hydrogen bonding profile is significantly altered by single nucleotide substitution in other CpG motifs, with DNMT3B being less affected by it. We correlated this with the higher CpG specificity of DNMT3A than DNMT3B. Taken together, these findings not only provide the missing molecular links in the DNMT3A/B mechanism of action, but also reveal that the findings obtained by experimental analyzes are provided by molecular dynamics simulations.

AI & Drug Design applications session

Invited Lecture
Learning force fields and reactivity to enhance classical biomolecular simulations

Frauke Gräter
MPI for Polymer Research, Mainz, Germany

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 to choose 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 related machine-learned force fields in accuracy, and can straightforwardly be trained on any chemical species of interest. Our ML-based KIMMDY/GRAPPA simulations are applicable to complex – biological as well as synthetic – molecules and materials, from polymer radical chemistry to protein hydrolysis, and can be extended to other chemistries of interest.

[1] Zapp et al, Nat Comm, 2020
[2] Rennekamp et al, JCTC, 2020
[3] Riedmiller et al, Chem Science, 2024

AI & Drug Design applications session

Selected Talk

Effects of Alzheimer's Disease Drug Candidates on Disordered Aβ42 Dissected by Comparative Markov State Analysis (CoVAMPnet)

Sérgio M. Marques[1,2], Petr Kouba[1,3,4], Anthony Legrand[1,2], Jiri Sedlar[3], Joan Planas- Iglesias[1,2], Jiri Damborsky[1,2], Stanislav Mazurenko[1,2], Josef Sivic[3], David Bednar[1,2]

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Background: Alzheimer's disease (AD) is characterized by the deposition of misfolded tau and amyloid-beta (Aβ). Tramiprosate (TMP) is a phase 3 clinical trial therapeutic, and together with its metabolite 3-sulfopropanoic acid (SPA), is believed to prevent the formation of toxic Aβ oligomers [1]. It is of paramount importance to understand how TMP/SPA modulate the conformations of Aβ. However, studying drug effects on intrinsically disordered biomolecules like Aβ42 is complex due to their vast conformational space.

Methodology: To mitigate this, we recently developed a comparative Markov state analysis (CoVAMPnet) framework [2], which quantifies changes in the conformational distribution and dynamics of proteins from enhanced-sampling dynamics, Markov state models (MSMs), and unsupervised machine learning. More specifically, our approach aligns and compares conformational states from learned MSMs constructed for the different systems and applies a discriminative analysis of aggregated neural network gradients to provide interpretability and biophysical context to those MSMs.

Results: We applied CoVAMPnet to adaptive-sampling dynamic ensembles of Aβ42 alone and in the presence of TMP or SPA. We found that both TMP and SPA preserved more structured conformations of Aβ42 by interacting non-specifically with charged residues. The metabolite SPA affected Aβ42 more extensively than TMP, protecting *α*-helices and suppressing the formation of aggregation-prone β-strands. Experimental biophysical analyses were used to validate the computational findings.

Conclusion: CoVAMPnet proved useful in quantify and interpreting the effects of small molecules on Aβ42. CoVAMPnet is broadly applicable to study the effects of drug candidates on intrinsically disordered biomolecules.

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AI & Drug Design applications session

Selected Talk

A complementarity-driven approach to de novo design of protein binders

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[2]*University Hospital Tübingen, Tübingen, Germany*

Protein-protein interactions form the basis of diverse processes in homeostasis and disease. Consequently, protein binders that promote or antagonize these interactions serve as potent tools for both research and therapeutic purposes. While protein design methods are rapidly advancing, the design of epitope-specific binders remains a formidable challenge, due to the dimensionality of the simultaneous search for an optimal binder scaffold, pose, and sequence. Here, we present a generalizable computational strategy to design novel protein binders, obviating steps of extensive empirical optimization or *in vitro* screening. Our dock-and-design pipeline first retrieves complementary scaffolds from a protein structure database to a given query epitope. This step takes advantage of an ultra- fast docking software HECTOR that evaluates shape complementarity of steric fields in a reduced mathematical representation, wherein a 3D surface patch is represented by a 2D invertible fingerprint. In contrast to conventional trial-and-error docking methods, HECTOR estimates shape compatibility between unaligned surface patches from different proteins through an analytical solution. After scaffold selection and interface design, all sampled mutants are ranked adopting molecular dynamics simulations. As proof-of-concept, we used this pipeline to design inhibitors of a vascular endothelial growth factor (VEGF), a key modulator of tumor progression. Having tested a small set of designs (16 candidates), we identified proteins that bound VEGF with nanomolar affinity, inhibited proliferation and survival of VEGF-dependent cells, and finally had a VEGF-suppressing effect *in vivo*. Moreover, we used the same computational strategy to design binders for Interleukin-7 receptor-*α* (IL-7R*α*). Experimental validation of these binders is ongoing.

Molecular simulations and applications session

Invited Lecture
RNA as a target for drug discovery

Leonardo De Maria
Astrazeneca, Gothenburg, Sweden

Emerging evidence, particularly through the advent of long-read RNA sequencing, shows that many classes of non-coding RNA serve regulatory roles in cells and tissues [1-3]. Hand in hand with the discovery of these new functions has come the realization of the role of RNA in disease. For example, diseases are now associated with defects in splicing [2], and with microRNAs, the DNA-encoded master gene expression regulators [3].
The approval of risdiplam (Evrysdi) [4] for the treatment of spinal muscular atrophy (SMA) is a first in more than one aspect. For SMA patients, it is the first orally available drug. For drug developers, it is the first approved small molecule targeting RNA directly and selectively, a scientific challenge still perceived as formidable. The reason for this being that RNA often operates through the formation of complexes with RNA-binding proteins, with, much like protein-protein interactions, span large surface areas often lacking traditional druggable properties like, for example, hydrophobic pockets.
In this talk I will highlight the challenges for computational methods to address RNA as the macromolecule of interest as the applicability of ‚protein centred' structure-based drug design to RNA is not straightforward and in most of the cases, the is the need to either adapted of completely redesign them. I will also outline how a prototype computational workflow to identify druggable pockets in RNA will look like.

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Molecular simulations and applications session

Selected Talk
Modelling peptide-based RNA delivery systems

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[2]*Bioinformatics, A*STAR, Singapore, Singapore*

RNA is a multipotent polymer of great biological and engineering interest. microRNA mimics have revolutionized the manipulation of gene expression and are being explored as next- generation therapeutics. Despite their high versatility and specificity, their weak pharmacokinetic profile poses serious limitations, that could be bypassed by using biocompatible delivery vectors, such as cell-penetrating peptides (CPPs). Recently, a potent microRNA antagonist (antagomiR) has been successfully delivered using a pH-responsive amphipathic CPP [1].
Molecular-level simulations have proven to be very powerful in optimizing small molecule binding to protein targets, and now routinely feature in commercial drug discovery processes. Here, we present our results from all-atom simulations on sequence-to-structure modelling of antagomiR-CPP complexes. By combining multi-μs medium-to-large-scale simulations with experimental sequence-activity information, we attempt to shed a light on structural details of these complex delivery systems and highlight important interaction features for efficient RNA delivery.
A composite workflow, combining knowledge- and physics-based methods, was developed to fold and sample relevant states of antagomiR, revealing open- and closed-loop conformations. Separate single- and multi-peptide simulations explored charge- conformation relationships of pH-responsive CPPs, revealing key conformations and oligomeric complexes corresponding to the biochemical environments of interest. Large- scale antagomiR-CPP association/dissociation simulations in explicit solvent, at experimentally-relevant molecular concentrations, pHs, and different RNA:CPP ratios, revealed important interactions and residues contributing to RNA coating and RNA/CPP-CPP complex behaviour. Finally, the effect of RNA and peptide crowding on cluster formation was also investigated.
Our study contributes to the elucidation of nanoparticle self-assembling process and the pH- controlled RNA-peptide interaction mechanism, and may assist in the rational discovery of novel CPP designs for tailored antagomiR delivery.

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Molecular simulations and applications session

Selected Talk
A Synergistic Approach to Elucidation of Enzyme Mechanisms & Dynamics

Mikaela Farrugia, Paul Helquist, Olaf Wiest
University of Notre Dame, Notre Dame, USA

Atomistic descriptions of enzyme mechanisms that consider the dynamics of the reaction could help us understand protein function and dysfunction in unprecedented detail but are unattainable with current techniques. This necessitates novel methods which leverage computational-experimental synergy. We describe coupling of time- resolved x-ray crystallographic studies with computational mechanistic investigations through exploitative sampling of the TS-associated complex with molecular dynamics (MD) simulations, structural population partitioning, and predictive model generation. We demonstrate these advantages on the *Pseudomonas mevalonii* HMG CoA Reductase (PmHMGR) system, using computationally generated structures as initial guesses to deconvolute time-resolved x-ray crystallographic data such to better treat the activated complexes in time-resolved experiments. An improvement in fit and diversity of structures identified from experiment then validates the computational data, paving the way for predictive model generation which sews together a 'molecular movie' of the reaction. Application of the described method to slow dynamical events–such as the *Pm*HMGR 2nd hydride transfer–is limited by the ability of force fields to reproduce atomistic behavior at a transition state. This need is met via the generation and application of Transition State Force Fields (TSFFs) with Quantum-guided Molecular Mechanics (Q2MM). Parameterizing TSFFs with many atoms is a high-dimensional problem and often produces suboptimal TSFFs stuck at a local minimum of the penalty function. A hybrid of a constrained particle swarm algorithm with a basic genetic algorithm will automate the optimization of TSFFs with Q2MM. The FUERZA method developed by Seminario is used to estimate initial parameter values for the TSFF before parameterization. These changes will make Q2MM accessible to non-experts simulating a wide range of systems, especially large biomolecules. Initial results of PmHMGR simulation analyses highlighted the need for a customized sampling approach, thus generation of more computational data is underway with an improved biomodel using a new exploitative sampling scheme while Q2MM improvements are tested and benchmarked.

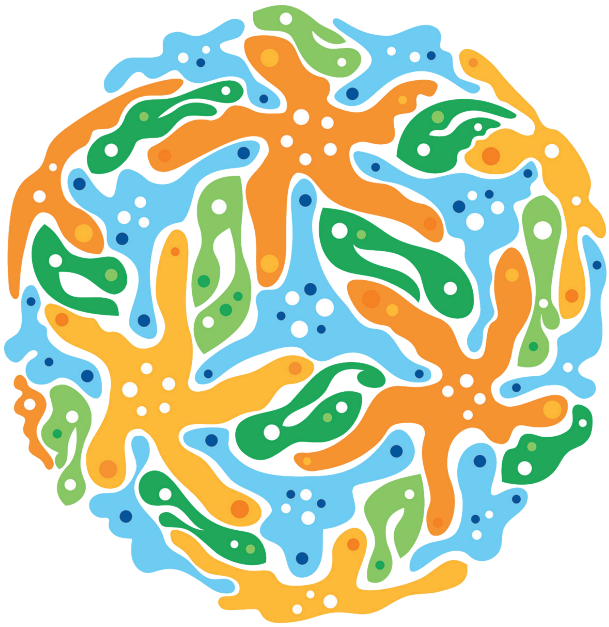
Molecular simulations and applications session

Keynote
Perspectives of a Protein Explorer: An Odyssey Through the Molecular Observatory

Rommie Amaro
University of California, San Diego

Molecular dynamics simulations can provide unparalleled insights into protein structure, dynamics, and function, revealing biological mechanisms that are often inaccessible through experimental techniques. In this evening lecture, I will share a narrative of discovery and exploration through the lens of multiscale computational microscopy. Drawing from personal experience, I will reflect on the current strengths and challenges in the field, including advancements in methods, the evolving landscape of data, and the social dimensions that shape scientific progress. This journey will provide a nuanced perspective on the opportunities and limitations facing today's protein explorers.

Poster Abstracts: 1. MOLECULAR DYNAMICS



1. Molecular dynamics

P 01 Helix stabilizing cyclic peptides with mixed stereochemistry as anti-cancer drugs

Julen Aduriz-Arrizabalaga[1,2], Xabier Lopez[1,2], David De Sancho[1,2]
[1]Donostia International Physics Center, Donostia, Spain. [2]Euskal Herriko Unibertsitatea, Donostia, Spain

In recent years, the use of peptides as drugs has gained notoriety[1]. Recent discoveries showed that macrocyclization, adding a ring of twelve or more atoms, has proved a successful strategy to stabilize a specific secondary structure of a peptide[2]. However, understanding the origin of affinity gains remains challenging. In this work, we focus on peptides including an N-terminal macrocycle designed using the RAPID technique to bind competitively to the anti-apoptotic protein Mcl-1[3], a prime therapeutic target for cancer[4-5]. To explain the enhanced binding affinity we use classical molecular simulations with optimized force-fields and enhanced sampling methods. Additionally, computational chemistry calculations help us rationalize why the presence of the D-phenylalanine in the N-terminal position is key to promote helicity in the cyclic variants, which is crucial for the binding process.

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1. Molecular dynamics

P 02 Deciphering promiscuity of HUWE1 for protein degradation using Molecular Dynamic Simulations

Ritika Aggarwal[1], **Dan Grabarczyk**[2], **Lukas stelzl**[1]
[1]*Johannes Gutenberg University, Mainz, Germany*
[2]*Research Institute of Molecular Pathology, Vienna, Austria*

HUWE-1 is a highly promiscuous enzyme which target a variety of protein substrates of high importance in regulatory mechanisms of cell for ubiquitination. However, the strength by which the substrate binds to HUWE-1 is unknown.
Intrinsically disordered regions (IDRs) in proteins are sequences within the protein that lack a stable three-dimensional structure under physiological conditions. IDR-1 is mesh of negatively charged residues in the center of the HUWE1, and they recognize the substrate protein sequences based on overall surface basicity.
Atomistic molecular dynamics recapitulates experimental trends in how HUWE-1 IDR-1 binds substrates. We did molecular dynamic simulations to explore the mechanisms and the specificity of interactions between HUWE-1 IDR1 and the substrate to understand these interactions better and determined the molecular basis of this substrate selection mechanism using simulations.
We mixed in silico one IDR1 sequence with seven copies of the minimal substrate peptide and performed MD simulation. We observed rapid clustering of all the peptide copies with the IDR, and then stable contact throughout the rest of the simulation. For the control peptide, we instead observed very transient interactions, with peptides rapidly associating and dissociating and no long-term clustering.
While the control simulation showed no specifically enriched contact points, for the wild-type peptide we observed many favored interaction modes. These interactions were diverse in nature, and while electrostatic interactions were most prominent, there were also some polar and hydrophobic interactions. This could potentially explain how HUWE1 could have some preference for disordered hydrophobic proteins.

1. Molecular dynamics

P 03 Molecular dynamics simulations of a protein-micelle complex to model contrast-variation SAXS experiments

Noora Aho, Jochen Hub
Theoretical Physics and Center for Biophysics, Saarland University, Saarbrücken, Germany

Small angle X-ray scattering (SAXS) is an experimental technique used to resolve the shape, interactions and large-scale conformational transitions of biomolecules in solution. Interpretation of experimental SAXS data requires an accurate calculation of SAXS curves from structural models, for which one powerful method is explicit-solvent molecular dynamics (MD). In contrast to other modelling techniques for SAXS, MD is able to provide atomistic accuracy, a realistic hydration shell around the biomolecule as well as correct thermal fluctuations in the structural model.
In contrast-variation (CV-) SAXS, the scattering data is recorded at multiple solvent electron densities, achieved with the use of contrast agents, such as salts or sugars. This enables exploring the internal electron density profile of the biomolecule and visualising its distinct parts. In this work, our aim is to expand the application of MD simulations from conventional SAXS to interpret CV-SAXS experiments. We model the FhuA membrane transporter protein solubilised in a LDAO micelle in explicit solvent in the presence of contrast agent molecules, calculate the corresponding SAXS curves from MD trajectories and compare with experimental data. Our simulations provide atomistic and dynamic details to the studied protein-micelle complex, and serve as an example of the possibilities of explicit-solvent MD in interpretation of advanced SAXS experiments.

1. Molecular dynamics

P 04 The molecular dynamics data bank: bridging gaps in bio-molecular simulation interoperability

Adam Bellaiche, Preeti Choudhary, Sameer Velankar
EMBL-EBI, Hinxton, United Kingdom

The Molecular Dynamics Data Bank (MDDB), the global repository for biosimulation data aims to build a research infrastructure tailored for communities engaged from various life science fields and unify these under a common platform using F.A.I.R (findability, accessibility, interoperability, and reusability) data principles. MDDB is a collaborative initiative that unites some of Europe's leading institutions to revolutionise the handling of molecular dynamics simulation data. By creating this platform, we aim to facilitate data sharing within the scientific community, recognizing the necessity of a joint effort to achieve this goal.

Interoperability ensures the seamless collaboration and consistent exchange of information between various systems, devices, applications, and products. By adhering to common data formats, standards, and data exchange protocols, MDDB aims to drive significant advancements in the interoperability of MD data and sharing it effectively, facilitating their integration and interpretation. In this poster, MDDB's approach to achieving interoperability is presented across various dimensions of interoperability:
Technical: providing programmatic access to MD data via a REST API with data interchange primarily using web compatible formats.
Syntactic: MDDB will supports standardised formats for different data types, such as HDF5/H5MD for trajectories and topologies, JSON/YAML or PDBe-KB data exchange format for metadata or other formats like PDBx/mmCIF, which we are exploring to ensure compatibility with the structural domain.
Semantic: by leveraging scalable and extensible formats like PDBx/mmCIF, PDBe-KB data exchange or by using a JSON schema, MDDB will ensures meaningful and automated interpretation of data or metadata.
Cross-domain and cross-organizational interoperability: MDDB aims to join research infrastructures such as ELIXIR INSTRUCT-ERIC and BioExcel to build a community driven project.
MD data integration: with major biological databases such as PDBe, PDBe-KB, 3D-beacons, and UniProt, showing the utility of such data in life sciences.

1. Molecular dynamics

P 05 Prediction of unknown binding sites in proteins by biased molecular dynamics simulations

Jan Beránek, Guglielmo Tedeschi, Vojtěch Spiwok
University of Chemistry and Technology, Prague, Czech Republic

Cryptic pockets are specific sites in the structure of a protein. They can have opened or closed conformation, and the open form can be targeted by drug molecules. However, development of drugs targeting such pockets is challenging due to the fact that the open conformation is visible only in presence of the correct ligand, while in crystal structures used for example for virtual screening the pocket has the closed conformation.
We have developed a new method to screen protein structures to find potential sites where such drug-targettable cryptic pockets could be found. Our method is based on a series of molecular dynamics simulations biased to force conformational changes resembling the opening of potential pockets, while measuring the amount of force necessary to enforce the change. We present the results of this method on two systems, tryptophan cage miniprotein and β-lactamase, a typical protein with a well known cryptic pocket. Our method successfully identified the placement of the existing cryptic pocket in the structure.

1. Molecular dynamics

P 06 Molecular Dynamics Simulation of High-Mobility Group Box Protein 2: Insights into the Conformational Dynamics of Auto-Inhibition

Hao-Ting Chang[1], **Jhih-Wei Chu**[2]
[1]*Institute of Bioinformatics and Systems Biology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan*
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High-mobility group box proteins (HMGBs) are essential nucleic acid-binding proteins involved in gene expression, DNA repair, and immune regulation. HMGB2, a key member of this family, contains two DNA-binding domains (A and B boxes) and a C-terminal intrinsically disordered region (IDR) that modulates DNA-binding through auto-inhibition. While the conformational flexibility of HMGB2 is crucial for its biological functions, particularly in DNA-related processes, understanding its structural behavior in the absence of DNA is critical for unveiling its inherent dynamics and potential as a therapeutic target.
In this study, we employed all-atom molecular dynamics (MD) simulations to explore the conformational dynamics of HMGB2 without DNA. Using homology modeling based on the HMGB1-DNA complex (PDB ID: 6CIJ), we conducted a 1 μs simulation to investigate the structural flexibility of HMGB2. Our analysis includes root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bond occupancy, and the level of bending of alpha helices.
The RMSD analysis revealed a marked difference between HMG-Box A and HMG-Box B, with HMG-Box A exhibiting larger fluctuations throughout the trajectory, particularly in alpha helices α1 and α3. RMSF further confirmed that α1 and α3 in HMG-Box A exhibit more flexibility compared to their counterparts in HMG-Box B, suggesting that these helices play a critical role in HMGB2's structural rearrangements.
Hydrogen bond occupancy calculations for α1 and α3 revealed a correlation between decreased hydrogen bond strength and increased structural flexibility. In α1, the loss of hydrogen bonds, particularly at residues H27-K30, contributed to significant conformational changes. Similarly, in α3, the disruption of hydrogen bonds at residues K65-K68 was associated with helix bending, further linking hydrogen bond dynamics to structural flexibility. Bending analysis of helices for α3 showed a significant decrease in the angle between residues S58-E74, corresponding to the observed fluctuations in RMSD and hydrogen bond occupancy. This suggests that α3 bending plays a crucial role in the overall flexibility of HMG-Box A.
In summary, our calculations provide foundational insights into the conformational dynamics of HMGB2 in auto-inhibition, potentially informing future studies on its DNA-binding affinity and flexibility. In particular, the development of therapeutic strategies call for the conformational states of HMGB2 and the interactions with DNA. Additional simulations are ongoing to broaden the scope of sampling of HMGB2 dynamics and to further understand the biological functions.

1. Molecular dynamics

P 07 Computational Design and Optimization of Aptamer-Based Biosensor for Enhanced West Nile Virus Detection

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Single-stranded DNA and RNA aptamers have shown great potential for the development of ultrasensitive biosensors capable of detecting pathogenic viruses such as the West Nile Virus (WNV). However, immobilization on surfaces may reduce their binding affinity. In this study, we designed and optimized, using *in silico* techniques, a diagnostic device based on aptamers anchored to gold surfaces functionalized with Self-Assembled Monolayers (SAM) for WNV detection.
The aptamer Tr-WNV-37 is the only one for which the binding energy with the WNV E protein is experimentally known, making it an ideal candidate for our study. Following *docking* studies, we performed multi-replica MD simulations of the Tr-WNV-37 aptamer under four conditions: in free solution for conformational analysis; anchored via six thymidines (T_6) to a gold-SAM nanosurface; in complex with the E protein in free solution; and in complex with the E protein anchored via six thymidines (T_6) to the gold-SAM nanosurface. To enhance the sampling, a multi-replica approach was employed, accumulating a total of 2.5 microseconds of simulation time. The T6 linker was used to distance the aptamer from the surface and did not cause significant perturbations in aptamer recognition. Simulations of the complete biosensor system showed that the aptamer remained oriented parallel to the surface, thereby avoiding undesirable direct interactions. Although a slight stiffening of the aptamer was observed, it did not compromise its recognition capability. Binding free energy analyses using MMPBSA/MMGBSA methods demonstrated that the DNA aptamer maintained a strong affinity for the protein both in solution and when anchored to the surface, comparable to its affinity in aqueous solution. Moreover, the anisotropic distribution of Na+ cations, which influences the structure and dynamics of the aptamer, was investigated to better understand their impact on the aptamer's dynamics and behavior.
These findings indicate that the aptamer Tr-WNV-37 is a promising candidate for specific and sensitive electrochemical biosensors for the early diagnosis of WNV and other infectious diseases. This study underscores the potential of aptamers as highly selective recognition elements, crucial for the design of reliable diagnostic sensors of significant importance for public health.t

1. Molecular dynamics

P 08 Facilitating Non-Equilibrium Molecular Dynamics Simulations with POP-MD

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Molecular dynamics simulations generally sample equilibrium ensembles. However, most biological processes occur out of equilibrium, and the non-equilibrium nature of the process may affect both the thermodynamics and the kinetics of the process itself.
We recently developed a software package, coined POP-MD, that works in combination with the popular GROMACS MD simulation package, to facilitate certain types of non-equilibrium simulations. POP-MD allows for the addition, removal, and chemical transformation of molecules as the simulation progresses, by performing a series of cycles wherein the number and/or the nature of particles is altered. Addition of molecules can be used to emulate synthesis, and chemical changes emulate metabolic processes. Under certain conditions, the free energy change during the insertion/transformation process can be calculated.
Here we highlight the various functions of the POP-MD code via four showcases. As a first example, we use atomistic simulations to slowly drive the nucleation of lipid droplets by gradually increasing the number of neutral lipid molecules. The second showcase is about the growth and budding of lipid droplets, triggered by increasing asymmetry between bilayer leaflets in coarse-grained simulations. We then probe the geometry of a unilamellar vesicle during asymmetric membrane biogenesis, achieved by adding lipids to the outer leaflet and inner leaflet at a given, constant ratio. Finally, we model the leaflet asymmetry of red blood cells to observe how cholesterol distributes between leaflets, to balance asymmetric stresses. Each showcase highlights different features of the software, which is highly versatile and can be applied to a variety of systems and forcefields.

1. Molecular dynamics

P 09 MDaRes: An R tool for the Analysis of Protein Residue Dynamics from Molecular Simulations

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Analyzing molecular dynamics (MD) data of proteins is essential for understanding their dynamic behavior, predicting interactions, guiding drug design, and uncovering the mechanisms underlying their functions. MDaRes, a tool developed in R, is designed to analyze MD data using structural alphabets. It performs three primary functions that make it highly valuable in protein dynamics research. First, it enables the detailed analysis of local structural changes, offering insights into specific regions of a protein. Second, it identifies and analyzes allosteric residue coupling, crucial for understanding how changes in one part of a protein affect other regions. Third, it identifies key regions responsible for overall protein movement and function, facilitating the design of specific protein variants for research purposes.
MDaRes is equipped to extract information on both local and global protein motions, providing detailed residue-level data. It facilitates the examination of residue-residue correlations, helping to detect potential allosteric pathways. The tool is particularly well-suited for comparing different protein states, such as bound versus unbound forms or wild-type versus mutated structures, making it a valuable resource for in-depth enzyme function analysis.
The tool is based on the M32K25 structural alphabet [PMID: 20170534], which defines 25 canonical protein fragment states, each composed of four residues focusing on the Cα atoms. This alphabet is vital for accurately encoding the conformational transitions observed in MD simulations. MDaRes matches protein conformations to the closest structural fragment using Root Mean Square Deviation (RMSD) to compress a protein's structure into a string representation. Residue annotations are integrated with the Atlantis database, which gathers data from resources like PDBe, Pfam, InterPro, UniProt, IntAct, PDB, and AlphaFold, ensuring a robust and up-to-date foundation for analysis.
The validation of MDaRes involved molecular dynamics simulations of 600 nanoseconds on two case systems previously studied by the developers: the enzyme galactose-1-phosphate uridyltransferase (wtGALT) and its variant p.Gln188Arg, using GROMACS. Through analysis of fragment frequency, Shannon Entropy, and mutual information, MDaRes showed alignment with existing knowledge.
MDaRes is open-source, user-friendly, and capable of handling large datasets typical of MD simulations. With its statistical analysis tools, it provides a comprehensive evaluation of protein dynamics, making it a versatile tool for both research and variant design. Its accessibility through R further enhances its utility for studying complex protein behaviors.

1. Molecular dynamics

P 10 *De novo* design of peptides that form transmembrane *α*-helical barrel pores with potent antimicrobial activity and optimization for coiled-coil nanopore formation

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Peptides that can self-assemble into transmembrane barrel pore structures have important applications as antimicrobial peptides and for single-molecule sensing. Their *de novo* design and optimization require an understanding of the sequence-structure relationships. We have developed a simulation-guided approach for the rational design and optimization of short peptides that form *α*-helical barrel pores. By evaluating nearly 200 peptides using molecular dynamics simulations, we gained insight into the role of amino acids at each position in the sequence for pore stabilization and formulated design guidelines and sequence patterns for pore-forming peptides. Fluorescent dye leakage and atomic force microscopy experiments validated the pore-forming activity. The designed cationic peptides showed potent antimicrobial activity against ESKAPE pathogens, while exhibiting low toxicity to human cells. We also optimized the designed barrel pores for helicity, symmetry and stability using *α*-helical coiled-coil heptad repeat patterns for tunable nanopore sensing applications.

1. Molecular dynamics

P 11 Molecular Mechanism of Calcium Block in the MthK Potassium Channel

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Potassium channels form one of the most ubiquitous classes of proteins and perform various critical biological functions. MthK is an archaeal calcium-gated potassium channel that has seen wide use as a model system. This channel opens upon binding of Ca^{2+} to the intracellular RCK-domain. Here we focus on a secondary effect of calcium on the channel; increasing calcium concentration lowers the outward current, thereby enhancing the inward-rectification of this channel. This calcium block is not observed in most other potassium channels nor several MthK mutants. These experiments identify key residues for calcium block, but do not provide a mechanism that explains this observation.
We use atomistic Molecular Dynamics (MD) simulations to investigate calcium-MthK interactions. We identify two main calcium binding sites in the pore domain of the channel. Binding of Ca^{2+} to the first site completely blocks the potassium permeation pathway and directly affects the potassium occupancy in the selectivity filter. The second site is formed by a ring of negatively charged residues near the pore entrance. Mutation at this site not only abolishes calcium binding at this site, but also near the selectivity filter, thereby almost completely eliminating calcium block. Simulations of mutant channels qualitatively agree with available electrophysiological data and provide a complete molecular mechanism for calcium block in MthK.

1. Molecular dynamics

P 12 Exploring the dynamics, allostery, and unresolved structure of the PEBP1/LC3 complex in cell death mechanisms

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PE-binding protein 1 (PEBP1) is a multitask and versatile protein in the human body that takes part in or even initiates various cell death programs like ferroptosis, necroptosis, or autophagy [1]. An incorrect course of the degradation pathway mechanisms can lead to various illnesses; therefore, comprehending the PEBP1 protein is crucial regarding its possible binding partners. Since the details of the various degradation pathway mechanisms are still unclear, further studies are conducted to understand the cell death mechanisms in hopes of understanding and potentially inhibiting or activating them as needed (e.g., in therapies or drug design) [2]. The study was conducted to obtain the spatial structure of the PEBP1/LC3 complex, part of the autophagy pathway, essential in maintaining cell homeostasis in the human body. Understanding the mechanism of this interaction is essential for further research regarding possible activation or inhibition of said pathway and, therefore, expanding our knowledge of the versatility of the PEBP1 protein. Molecular dynamics simulations, docking, and the Perturbation Response-Scanning method were used to understand the interactions between proteins and their potential binding sites. The studies analyzed the behavior of the PEBP1/LC3 complex, the most commonly interactive amino acid residues, and stability. The obtained results indicate the successful determination of the spatial structure of the complex.

Acknowledgments:
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1. Molecular dynamics

P 13 Speeding up sampling in molecular dynamics simulations with fast-TIP3P water

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Routine atomistic molecular dynamics (MD) simulations are limited to the microsecond timescale due to the allowed time step for the time integration of the trajectories set by the fastest molecular motions. Time integration becomes unstable for larger time steps, primarily because deep particle collisions result in large forces that, in turn, cause further deep collisions. The default time step of 2 fs in classical MD of biomolecules is well below the stability limit, so that deep collisions and the resulting catastrophic crashes are virtually impossible to occur in any simulation of reasonable length and size.

Here, we present a "fast water model" that fully retains all energetic and thermodynamic properties but substantially increases the sampling efficiency in biomolecular simulations. We combine the approaches of (1) quantifying the stability of MD time integration and (2) mass repartitioning with (3) the fact that in dilute (aqueous) solution, larger-scale molecular motions and thus sampling efficiency are determined by the viscosity of the solvent medium. We repartition and rescale the masses of water to reduce the viscosity without causing time integration instabilities. The resulting "fast water" is then used with a time step as before, to keep the time integration of the vibrational motions of, say, protein solutes stable. However, the reduced water viscosity and faster diffusion result in proportionally faster sampling of the larger-scale motions in the conformation space of both solute and solvent. We illustrate this approach by developing the TIP3P-F model based on the popular TIP3P model, but the approach can easily be applied to other water models and even different solvents.

1. Molecular dynamics

P 14 P2Y12 receptor goes towards activation: insights by Principal Component Analysis

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The G-protein coupled receptor P2Y12, prominently expressed on the cellular membrane of platelets, serves as the primary target for existing antiaggregant medications. Despite the established efficacy of these pharmaceuticals in clinical practice, critical concerns persist, notably concerning fatal bleeding events and variable effectiveness across patient populations. Furthermore, the molecular mechanisms underlying the actions of these drugs remain elusive. To improve our knowledge and for future drug design, this study employs a computational approach to investigate the molecular interactions between the full agonist 2MeSADP and the P2Y12 receptor at a molecular level, to elucidate P2Y12R activation mechanism. Principal Component Analysis was used to pinpoint the Essential Dynamics of P2Y12 and the Gibbs free energy landscape was plotted. This procedure allowed to identify the most visited energy minima and to uncover the P2Y12R mechanism towards activation in response to the full agonist 2MeSADP, through the identification of precise molecular switches, which cause the rearrangement of the helical bundle, in particular helices V and VI. In particular, by inspecting the movement along different eigenvectors, the essential dynamics shows the movement of helix V from a bent to a vertical position, and the slight outward movement of helix VI. These two decomposed movements are crucial as they facilitate the creation of a crevice for G-protein accommodation. Finally, by comparing these findings with experimental structural data, this computational study seems to be aligned with the currently available literature about the P2Y12R, paving the way to the study of the endogenous agonist, ADP.

1. Molecular dynamics

P 15 High-Pressure Response of the Coupled Dynamics of Lipids and Membrane Proteins

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Cell membranes consist of a complex assembly of lipids and proteins that are essential to normal cell function through their role as physical barrier, chemical filter or signal converter [1]. The understanding of the interplay between the lipid properties, the collective physical properties of the membrane and the protein conformational landscape is key to explain the membrane protein function. Experimentally, high hydrostatic pressure can be used as a tool to modulate the lipids dynamics as well as a way to smoothly modulate the protein dynamics without denaturation [2]. By combining high resolution liquid-state NMR and molecular dynamics (MD) simulation, we are characterizing the coupled dynamics of lipids and proteins in response to high pressures at the molecular level [3]. We first demonstrate that state-of-the-art lipid force fields enable us to quantitatively reproduce the membrane phase transition at increasing pressure. As observed experimentally, the presence of a membrane protein such as OmpX shifts the phase transition to higher pressures. The MD simulations are then instrumental in providing a molecular picture of the protein's effect on the membrane lipids, in particular by providing a spatial resolution (that is, how lipids in contact with the protein are affected differently from those that are further away) that is not accessible in the experiments.

Keywords: Biological membranes, Hydrostatic pressure, Membrane proteins, Atomistic molecular dynamic simulations.

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1. Molecular dynamics

P 16 Investigating Nanodiscs as a Membrane Protein Environment

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Nanodiscs serve as an invaluable model system for investigating the structure and properties of membrane proteins. However, a key challenge lies in interpreting the data they provide. While nanodiscs offer a functional environment for proteins, it remains unclear to what extent this environment resembles the natural conditions in which membrane proteins operate within native membranes, or if it reflects any such conditions at all. The primary objective of this work is to use atomic-level computer simulations to elucidate both the structural and dynamic behavior of membrane proteins in nanodiscs, and to determine how this environment differs from that of native GUV-like membrane structures, assuming the membrane composition remains unchanged. Our findings confirm previous observations on protein-free nanodiscs, while providing new insights into the influence of membrane proteins within this environment. Ultimately, this study aims to understand how the structure, conformation, and dynamics of membrane proteins are altered when they are embedded in nanodiscs, as opposed to their native membrane environments. A systematic deep learning analysis of the simulation data reveals a nuanced picture, shedding light on both the advantages and limitations of nanodiscs as membrane mimetics.

1. Molecular dynamics

P 17 Are membraneless organelles truly membrane-less?

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The ability to control the spatio-temporal organization of biomolecules is paramount to the functioning of all living systems. Canonical molecular biology posits that lipids are the key molecules which enable this control via the formation of meso-scale, membrane-bound compartments that dynamically organize biomolecules and their reactions. Recent advancements, however, have shifted this paradigm by developing our understanding of biomolecular condensates - distinct, membrane-less cellular compartments that arise via the liquid-liquid phase separation (LLPS) of intrinsically disordered proteins. Existing near their thermodynamic critical points, condensates exhibit rapid formation and dissolution in response to physicochemical perturbations, in turn providing the cell with intricate spatio-temporal control, beyond what is offered by lipidic structures. The importance of such control is underscored by the ubiquitous presence of condensates in cells, and the functional diversity that they display.
The challenge, lies in understanding how these non-stoichiometric structures achieve such an array of functions. Condensates - initially treated by the field as monophasic entities - are now recognised to exhibit diverse mesoscale architectures, all of which serve clear roles in the functionality of their respective condensates. In this study, we conduct a computational and theoretical investigation into a class of structures scarcely studied by the biomolecular condensate community - membranes. Specifically, motivated by recent works in the field, we directly confront the assumption that biomolecular condensates inherently lack membranes.
To achieve this, we employ a genetic algorithm that is integrated with molecular dynamics simulations in order to conduct a bottom-up, *de-novo* exploration of the intrinsically disordered protein sequence space that governs the formation of membrane structures. Notably, we discover three distinct chain topologies capable of giving rise to membrane structures. Furthermore, we demonstrate their physical properties, and elucidate the molecular grammar that governs their formation. Our investigation into the physical parameters of these protein-based membranes shows that the optimal solutions found using our genetic algorithm form structures with comparable bending moduli to lipid membranes.
Overall, these findings challenge preconceived notions of condensate biology and furthermore, suggest the discovery of a biocompatible material with diverse applications in drug delivery, synthetic biology, and nanotechnology.

1. Molecular dynamics

P 18 Molecular Dynamics Simulations Coupled On-the-fly to Experimentally Determined Helical Content for Interpretation of Circular Dichroism Data

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Circular dichroism (CD) spectroscopy enables determination of protein secondary structure content, albeit providing only ensemble- and residue-averaged data.
Molecular dynamics (MD) simulations enable atomistic interpretation, but frequently suffer from force-field inaccuracies and incomplete sampling. Integrating MD simulations with CD addresses these limitations, enabling atomistic interpretation, while the CD data improve force-field and sampling issues related to inaccurate secondary structure propensities.
We develop a method to couple MD simulations on-the-fly to CD-derived secondary structure content by applying harmonic restraints that ensure agreement with experimentally derived secondary structure content. Our differentiable forward-model is based on the Define Secondary Structure of Proteins (DSSP) algorithm. Current implementation of the restraints is acting on a single replica, however we aim to account for the ensemble-averaged data by implementing ensemble restraints with commitment to the maximum entropy principle following the parallel-replica approach, as demonstrated in previous work for refining ensembles against small-angle scattering data. Here, we present MD simulations of a small test peptide, (AAQAA)3, with restraints on the α-helical content.
Coupling of MD simulations to CD-derived secondary structure content will be especially useful for structural ensemble refinement of proteins and peptides with intrinsic disorder, for which force-fields often lack accurate secondary structure propensities.

1. Molecular dynamics

P 19 Transcription factors meet chromatin under the computational nanoscope

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Transcription factors are essential, sequence specific, DNA-binding proteins that regulate gene expression and define cellular identity. 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. From extensive molecular dynamics simulations and experiments, we discovered how the master regulators and inducers of stem cell pluripotency Oct4 and Sox2 cooperate with the unstructured tails of the histones to induce opening of nucleosomes. In addition, we revealed how one point mutation in Sox2 increases the Oct4-Sox2 cooperativity on nucleosome-free DNA, having major consequences on reprogramming skin cells into induced pluripotent stem cells. This mutation raised the developmental potential of the generated induced pluripotent stem cells and enabled highly efficient reprogramming of cells across species.

1. Molecular dynamics

P 20 Martini 3 OliGoōmers: A Scalable Approach for Multimers and Fibrils in GROMACS

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The dynamics of large-scale protein complexes is an active subject of study via molecular dynamics simulations. Such complexes often face computational time and cost limitations when represented on the atomistic scale. To overcome these limitations, coarse-grained (CG) models such as Martini 3 force field can be used instead. One of the challenges of CG protein modeling is realistic description of higher-order protein structures, which requires additional modifications of the model. The Martini-Goō model (originally developed by Poma et al.) enhances Martini 3 by adding interactions between non-local backbone CG beads based on the contact map of the protein's native structure.
As of today, this method has been used only for individual protein monomers. Here, we describe how the Martini-Goō model can be applied to even larger, oligomer systems, taking into account both intramolecular (tertiary) and intermolecular (quaternary) interactions. This extended Martini-Goō model was tested on systems with multiple degrees of complexity: aquaporin tetramer, insulin dimer, and amyloid-β fibril. It is not only able to maintain structural stability, but also allows for assembly/disassembly of protein oligomers. The strength of the Goō potentials can be tuned so that the internal fluctuations of proteins match the behavior of atomistic simulation models.
The presented method paves the way toward future modeling of more challenging large-scale processes, such as protein complex self-assembly.

1. Molecular dynamics

P 21 Influence of the Drug Disulfiram on the Dimer Stability of Gasdermin D

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During infection and inflammation, cells can undergo a form of programmed cell death known as pyroptosis. A crucial step in this process is the assembly of gasdermin protein pores in the plasma membrane. These pores facilitate the leakage of ions and water from the cell and allow the release of specific pyroptotic markers that signal neighboring cells about the ongoing cell death. In cases of sepsis, this can lead to a rapid, avalanche-like spread of cell death. Recently, disulfiram, a drug traditionally used to treat alcohol addiction, has been discovered to block the formation of gasdermin pores, thereby offering a promising therapeutic approach to prevent or mitigate sepsis.
Disulfiram is known to covalently modify accessible cysteine residues, but the exact mechanism by which it prevents gasdermin D from forming pores remains unclear. In addition to modifying Cys191, which is typically palmitoylated in cells, disulfiram may also affect Cys56, located at the protein-protein interface of gasdermin D oligomers, potentially impacting pore formation. To investigate whether disulfiram alters the stability of gasdermin D dimers through modification of Cys56, here we conducted all-atom molecular dynamics simulations. These simulations began with equilibration, followed by enforced dissociation of both wild-type and disulfiram-modified dimers. Subsequently, umbrella sampling was employed to determine the differences in the potential of mean force during the dissociation of the wild-type gasdermin D dimer compared to its disulfiram-modified counterpart.

1. Molecular dynamics

P 22 Computational analysis of flavonoid deglycosylation to enhance antibiotic activity: Predicting substrate affinity by different methods

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The synergistic use of antimicrobial compounds has been proposed as an alternative way to address the problem of increasing antibiotic resistance. One of the potential compounds is the naturally occurring flavonoids [1]. The main sources of flavonoids are fruits, vegetables and plants, where they are mostly found in the C-3 or C-7 glycosylated form (C- or O-glycosylation). Several biochemical studies have shown that flavonoids can enhance the antibiotic effect of drugs and also have antimicrobial properties. In addition to antibiotic activity, flavonoids have been shown to have anti-inflammatory, antioxidant and anti-diabetic properties. They may also be active against some viral enzymes (influenza virus neuraminidase) [2]. Glycosylation affects many properties of flavonoids - toxicity, bioavailability, stability and bioactivity. O-glycosylation has been reported to reduce anti-inflammatory, antibacterial and antifungal bioactivity and in some cases absorption [2, 3]. Fungal alpha-l-rhamnosidase is a family of enzymes capable of cleaving O-linked carbohydrates from glycosylated flavonoids, thereby enhancing their antibiotic activity. In this study, we focused on the validation of computational methods to predict the ability of fungal enzymes (from A. oryzae and A. niger) to hydrolyse the most abundant flavonoids glycosylated with glucose, rhamnose, galactose, xylose, arabinose, glucuronic acid and apiose - the most abundant carbohydrates. Various docking algorithms (Vina, SMINA, PLANTS, AutoDock4) were used to calculate the binding affinity, complemented by stability validation during MD simulation. In silico prediction will be used for comparison with experimental results to select a better methodology for predicting enzyme activity towards different glycosylated flavonoids.

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1. Molecular dynamics

P 23 Lipid composition regulates membrane association and the activity of the human ubiquitin conjugating enzyme UBE2J2.

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The endoplasmic reticulum-associated protein degradation (ERAD) pathway is essential for cellular protein homeostasis. The ubiquitin conjugating enzyme UBE2J2 is a central component of this pathway. The cytosolic UBE2J2 is anchored to the endoplasmic reticulum membrane by an extended, intrinsically disordered region and plays an important role in lipid homeostasis. It can target lipid biosynthetic enzymes for degradation and thus adjusting metabolic activity to cellular demand. It is known that membrane properties regulate the activity of enzymes that interact with UBE2J2, but we have recently shown that the activity of UBE2J2 itself is dependent on lipid composition. In reconstituted membrane systems, the activity of the E2 ligase is strongly correlated with the fraction of saturated fatty acids. Furthermore, the enzyme was less susceptible to limited proteolysis when bound to membranes composed of unsaturated fatty acids. This led to the hypothesis that the cytosolic domain of UBE2J2 binds to membranes with a higher proportion of unsaturated fatty acids. This would prevent other proteins in the ubiquitination cascade from interacting with the E2 ligase.
To test this hypothesis, we performed simulations of UBE2J2 anchored to the membrane. Due to the time scales involved, the simulations were performed with a rescaled Martini 3 force field. This was motivated by recent work showing that rescaling of the Martini 3 protein-protein interactions improves the description of intrinsically disordered regions and multidomain proteins. We tested different lipid compositions, varying the ratio of saturated to unsaturated lipids. We found that the association of UBE2J2 with the membrane increased with increasing amounts of unsaturated fatty acids. We further show that this is primarily due to a decrease in the dissociation rate constant. While the binding on rate UBE2J2 is nearly independent of membrane lipid composition, the protein binds more tightly to membranes with a higher ratio of unsaturated fatty acids. This association occludes the catalytic region and thus loading of the enzyme. Together with the experimental characterization of the enzyme this provides compelling evidence, that membrane composition modulates the activity of the ubiquitination machinery.

1. Molecular dynamics

P 24 How Binding Site Flexibility Promotes RNA Scanning in TbRGG2 RRM: A Molecular Dynamics Simulation Study

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RNA Recognition Motifs (RRMs) are a key class of proteins that primarily bind single-stranded RNAs. In this study, we use unbiased molecular dynamics simulations to obtain insights into the intricate binding dynamics between uridine-rich RNAs and TbRGG2 RRM. Complementing structural experiments that unveil a primary binding mode with a single uridine bound, our simulations uncover two supplementary binding modes where adjacent nucleotides encroach upon the binding pocket. This leads to a unique molecular mechanism through which TbRGG2 RRM is capable of rapidly transitioning the U-rich sequence. In contrast, presence of non-native cytidines induces stalling and destabilization of the complex. By leveraging extensive equilibrium dynamics and large variety of binding states, TbRGG2 RRM effectively expedites diffusion along the RNA substrate while ensuring robust selectivity for U-rich sequences despite featuring a solitary binding pocket. Using recently developed Stafix potential, we substantiate our description of the complex dynamics by simulating fully spontaneous association process of U-rich sequences to the TbRGG2 RRM. Our study highlights critical role of dynamics and auxiliary binding states in interface dynamics employed by RNA-binding proteins, which is not readily apparent in traditional structural studies, but could represent a general type of binding strategy employed by many RNA-binding proteins.

1. Molecular dynamics

P 25 Antimicrobial Action of Essential Oils and CuO Nanoparticles Against Pathogenic Proteins: Elucidation of the Inhibitory Mechanism through Molecular Dynamics and Free Energy Calculations

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In this study, we present our efforts to computationally design self-disinfecting bio-nano coatings based on optimal combinations of specific structures possessing antimicrobial properties. These structures incorporate copper-based nanoparticles (NPs) and bioactive compounds, such as essential oils (EOs) coming from non-edible plants. EOs are natural, safe, easily biodegradable, and particularly effective substances against bacteria, fungi and viruses. Due to the multi-component nature of EOs, their antimicrobial action is not attributable to a specific mechanism, but it is rather a synergistic effect on multiple targets in the cells. For this reason, EOs may prevent antimicrobial (AMR) resistance and therefore fight multi-drug resistant bacteria and viruses.
This work elucidates the binding mechanism of selected EOs and Cu-based NPs against protein targets that directly relate to pathogenic actions, in order to further identify optimal structural and energetic patterns that result in enhanced bioactivity. In particular, a combination of advanced biomolecular modeling approaches was applied to study the binding modes and interactions at the molecular level between a large set of EOs/CuO NPs and selected pathogens, namely, *Staphylococcus aureus*, *SARS-CoV-2*, and *Escherichia coli*. The selection of the aforementioned pathogens was based on their current clinical significance. Molecular docking, extensive all-atom molecular dynamics (MD) simulations, and energetic analyses based on the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) methodology identified the primary structural and thermodynamic parameters that drive binding, along with the individual energy contributions, which favor complex formation, i.e., van der Waals, hydrophobic, electrostatic, and entropy effects between proteins of the microorganisms and essential oils/Cu NPs.
Based on the above scheme, a rational selection of promising EOs and CuO NPs from a comprehensive set of candidates was obtained to provide insight into the nature of host-guest interactions. This information may be particularly helpful to design new formulations with enhanced antimicrobial activity, and to establish a correlation between favorable interactions and physiological responses to the pathogens, for further exploitation. Moreover, the elucidation of the interactions between CuO NPs and the virus proteins facilitates the discovery of NP-containing structures to be used as promising antimicrobial agents.

1. Molecular dynamics

P 26 An integrated protocol for relating Hydrogen-Deuterium exchange data to protein conformational ensembles

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Proteins do not maintain a single static 3D structure, but instead, exist in a dynamic equilibrium, constantly fluctuating between various conformations. While experimental structural determination – for example by X-ray crystallography – will capture one or a few stable structures, there is ample experimental and computational evidence on the existence of transient structures that likely play critical roles in protein function.
An important experimental method for characterizing protein structural dynamics is hydrogen-deuterium exchange, which was historically performed by NMR and now has been revived by mass-spectrometry methods that allow the study of much larger proteins.
For interpreting these data in terms of conformational ensembles, molecular dynamics (MD) simulations are extremely valuable, yet many significant conformational changes occur on timescales that are not routinely accessible, if at all, with conventional MD techniques.
In this study, we present a protocol based on well-tempered metadynamics (WTmetaD) to explore transient fluctuations of protein structure that are relevant for interpreting Hydrogen-Deuterium exchange data.
The protocol was tested on the protein ubiquitin, which has been extensively characterized through both computational and experimental methods. We highlight important points of attention concerning the choice of collective variables for efficient exploration of the conformational landscape. We also discuss important parameters relevant to the calculation of Hydrogen-Deuterium exchange rates from computational trajectories.
Our data show that the WTmetaD trajectories successfully sample the functional states of ubiquitin identified in independent NMR studies and that the overall conformational distribution aligns well with HDX data. The protocol is general and versatile, offering a robust framework for studying protein dynamics across various systems.

1. Molecular dynamics

P 27 Simulations of immunity receptors and more: from micelles to membranes and liposomes

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Toll-like receptors (TLRs) have a primordial role in the activation of the innate immunity through the recognition of pathogen-associated molecular patterns, and have sparked great interest in the Immunotherapy Era. Deep structural understanding of TLRs signaling and mechanism is leading to the discovery of novel molecules with desirable therapeutic properties as antiinflammatory agents or vaccine adjuvants.[1,2] We have addressed the computational characterization, by docking and all-atom MD simulations, of the molecular recognition processes of agonist and antagonist modulators of TLR2, and TLR4,[1,2] and the structural assembly of activated full-length TLRs models embedded into a realistic plasma membrane.[3,4] Also, studies of pathogen bacterial membranes by liposome models can contribute to understand infection mechanisms and antibacterial resistance, capturing, by coarse grained MD simulations, the role of Lipid A on bacterial liposome morphology and physicochemical properties.[5]
On the other hand, the first MD study of the dynamics of a catalytic organometallic system, in micellar media, is presented.[6]

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1. Molecular dynamics

P 28 Evolutionary Molecular Dynamics: Physics-based inverse design of functional peptides

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The design of functional molecules typically entails a combination of lead optimization and rational design. Although these methods are effective, they offer only a narrow exploration of the available chemical space as they rely on existing knowledge of both structure and function. Physics-based inverse design offers an alternative perspective that reverses the conventional approach to design. This method enables a full exploration of the potential chemical space while circumventing the need to rely on pre-existing assumptions about the desired functional mechanisms. It is anticipated that solutions identified through this approach will diverge significantly from existing natural solutions, offering novel insights into the mechanisms underlying functionality.
We demonstrate Evolutionary Molecular Dynamics (Evo-MD) as an implementation of the physics-based inverse design concept. In this approach, coarse-grained molecular dynamics (CG-MD) simulations are integrated into an evolutionary algorithm workflow, enabling the automated set-up, simulation, and evaluation of candidate solutions according to a desired measure of functionality. This process gradually drives the evolution of random peptide sequences towards specific sequences with the desired functionality. The data generated through this evolutionary process is ideal for the training of deep learning models, facilitating straightforward prediction of peptide/protein functionality within seconds.
We present several examples where Evo-MD has provided new insights in the development of functional peptides. These include the design of curvature-sensing peptides, cholesterol-attracting transmembrane helices, and lipid raft-targeting peptides. Additionally, we illustrate how Evo-MD facilitates the straightforward sharing and application of the explored functionality via our webserver, PMIpred, which provides an accessible interface to a transformer deep learning model capable of predicting curvature sensing functionality in peptides and proteins. Furthermore, we explore potential new avenues in which physics-based inverse design could play a role.

1. Molecular dynamics

P 29 Effective Inclusion of Electronic Polarization in Molecular Dynamics of the Human Insulin Receptor–Insulin Complex

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Specific protein–protein binding via large and flexible interfaces is behind many crucial processes in biology. Charge-charge interactions between acidic and basic side chains or N- and C-termini are key contributors to stability and specificity. Atomistic details can be investigated computationally using classical molecular dynamics (MD) simulations. However, a physically correct description of charge-charge interactions is hampered by the omission of electronic polarization in most force fields.
Human insulin receptor (IR) in complex with insulin is a molecular system with a key role in understanding and treating diabetes mellitus. As such, half-a-dozen cryo-EM structures have been obtained showing its conformational plasticity. Yet, the atomistic details of the affinity determinants have not up to now been ascertained.
In this study, we test the utility of electronic continuum correction (ECC) to introduce the missing electronic polarization in classical MD of IR–insulin complex (PDB: 6HN5 with loops modeled). This is accomplished by scaling of charges, which is computationally efficient. We adopt standard (CHARMM36m and ff19SB) and scaled-charge (CHARMM36m-based) protein force fields, combined with TIP3P and SPC/E water models.
Our MD simulations reveal significant differences in terms of hydrogen bond occupancy between IR and insulin. The combinations of the standard CHARMM36m and scaled-charge force fields with SPC/E water, as well as the standard CHARMM36m with TIP3P water, exhibit high occurrences of artificial hydrogen bonds, i.e. those that are not observed in a more recent cryo-EM structure (PDB: 6SOF). In contrast, the scaled-charge force field and ff19SB combined with SPC/E water reproduce well the prominent hydrogen bonds.
In conclusion, our findings quantitatively capture the key residue pairs dominating the hydrogen bonds, suggesting their significant role in the binding affinity between IR and insulin. Most importantly, MD with ECC correction using the TIP3P water model provides the most realistic descriptions of hydrogen bond occurrences between IR and insulin, aligning well with the experimental cryo-EM data. To sum up, MD with ECC correction appears to offer valuable dynamical information without computational overhead. We intend to extend this study to insulin analogues binding to IR.

1. Molecular dynamics

P 30 Oligomerization of G-protein coupled receptors across different classes: structural insights from computational methods

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G-protein coupled receptors (GPCRs), membrane-embedded proteins responsible for cellular communication and signal transduction, are major players in physiological processes in health and disease. They are targets for over 30% of the currently approved drugs (Insel et al. 2019). GPCRs can function as monomers (single receptors), dimers (two interacting receptors), or higher-order oligomers (complexes of more than two receptors). The formation of complexes between receptors, both homooligomers (consisting of the same type of receptor) and heterooligomers (formed by different receptors), significantly affects their functions. Class C GPCRs require dimerisation to perform their biological activities (Kunishima et al. 2000). Additionally, oligomerization can influence the pharmacological properties of ligands and alter downstream signalling pathways, making GPCR oligomers promising drug targets. Despite the importance of this phenomenon, the understanding of oligomerization at the molecular level is still very scarce. Computational methods, such as protein-protein docking and multiscale molecular dynamics simulations, offer invaluable tools to unveil the structural details underlying dimerisation and oligomerization of GPCRs.
In this study, we explored the oligomerization between the class A adenosine 2A receptor (A_2AR) and the class C metabotropic glutamate receptor type 5 ($mGlu_5R$). Experimental studies have confirmed the colocalisation and functional interactions between $mGlu_5R$ and A_2AR in the hippocampus (Tebano et al. 2005, Rebola et al. 2008, Sarantis et al. 2015). $mGlu_5R$ is involved in various pathologies, including mood disorders, addiction, motor control issues, and neurodegenerative diseases, such as Parkinson's and Alzheimer's. The significance of $mGlu_5R$ lies in its modulatory role in N-methyl-D-aspartate (NMDA) receptor activity, implicating its involvement in synaptic plasticity and memory formation. A_2AR has been proposed as a potential pathological mediator, contributing to cognitive impairments associated with aging and Alzheimer's disease (Temido-Ferreira et al. 2018) through its interaction with $mGlu_5R$ and abnormal activation of the $mGlu_5R$/NMDAR pathway, which disrupts calcium ion balance in the hippocampus.
Using a computational pipeline comprised of multicomponent protein-protein docking protocols, molecular dynamics simulations, and atomistic analysis (Kaczor et al., 2015; Bueschbell et al., 2023), we investigated the intricate architecture of the $mGlu_5R$:A_2R oligomer. Our findings provide a detailed molecular mechanism for the interactions between receptors in both inactive and active states, explaining the regulatory role of A_2AR on $mGlu_5R$ activity at a molecular level.

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1. Molecular dynamics

P 31 Effect of two activators on the gating of a K2P channel

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TREK1 is a K^+ mammalian channel that causes leak currents, which control the negative resting potential in the cell membrane, making it a potential therapeutic target for the treatment of neurological disorders. The gating of this channel converges in the narrowest part of the pore: the selectivity filter (SF). Several hypotheses exist regarding the modulation of TREK1 gating, namely: the dynamics of the loops linking the SF to the pore helices and the formation/rupture of hydrogen bond (HB) networks adjacent to the SF. Recently, two small molecules (Q6F and Q5F) were reported as activators of TREK1 by increasing its open probability through stabilization of the SF. Here, we ask how these ligands affect the previously proposed gating modulation mechanisms of TREK1, compared to the apo channel. We found that loop dynamics at the upper region of the SF exhibit only a weak correlation with permeation events and non-permeation periods, whereas the HB network behind the SF appears to correlate more strongly. Non-permeation periods arise from two distinct mechanisms: a distortion similar to C-type inactivation, as previously described, and carbonyl flipping at an SF binding site. We find that, in addition to preventing distortions in the SF, the ligands increase permeation probability by modulating the dynamics of this carbonyl flipping, which is influenced by a threonine residue at the bottom of the SF. These results open the door for the rational design of ligands that optimize these gating mechanisms, as well as the possibility of modulating related channels similarly.

1. Molecular dynamics

P 32 Can Amphipathic Helices Sense Negative Membrane Curvature?

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Precise regulation of biological membrane curvature is essential for key cellular processes such as endocytosis, exocytosis, vesicle trafficking, and cell signaling. This regulation is mediated by specific proteins and peptides capable of both sensing and modulating membrane curvature. Among these, amphipathic helices (AHs)—peptides characterized by distinct polar and nonpolar sides—are recognized for their ability to detect and influence membrane curvature. Traditionally, AHs are thought to primarily recognize positive curvature due to lipid packing defects in convex regions. However, emerging evidence suggests that AHs may also detect negative curvature, indicating a more extensive role in curvature sensing than previously understood.
In this study, we employ a multiscale approach using Martini coarse-grained simulations and all-atom molecular dynamics to investigate the capacity of AHs to sense negative membrane curvature. Coarse-grained simulations allow for efficient exploration of large-scale membrane dynamics, while atomistic simulations provide detailed molecular insights. We focus on amphipathic peptides composed of leucine and serine residues, examining how variations in peptide hydrophobicity impact curvature sensing. We found that increasing the hydrophobicity of these peptides enhances their insertion depth into the membrane and promotes localization in regions with negative curvature, suggesting a direct link between hydrophobic interactions and curvature preference.
These findings challenge the traditional view that AHs are limited to sensing positive curvature and highlight the connection between peptide insertion depth and curvature preference. By broadening our understanding of how AHs interact with membrane curvatures, this study contributes to the development of novel compounds capable of detecting and responding to changes in membrane curvature, with potential applications in targeted drug delivery and synthetic biology.

1. Molecular dynamics

P 33 Molecular dynamics simulation of keratin-derived antimicrobial peptides (KAMPs) in solution and of their interaction with bacterial membranes

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Antimicrobial resistance (AMR) is one of the biggest threats of modern medicine, as pathogens become more resistant to available antibiotics. Overuse and misuse of antibiotics in humans and animals seem to be the main drivers of AMR, thus rendering necessary the search for alternative antimicrobial agents. A promising such class of agents is antimicrobial peptides (AMPs) characterized by selective cytotoxicity against microorganisms and slower development of AMR compared to conventional antibiotics. The structure, size and charge of AMPs directly influence their mechanism of action against cell membranes or intracellular targets.
AMPs derived from keratin (KAMPs) have been considered as a new, promising class of antimicrobial and antifungal agents as experimental studies have shown that they can be effective against a range of bacteria, including both Gram-positive and Gram-negative species. Unlike most antimicrobial peptide candidates, their biological activity is independent of the presence of an α-helix. It has also been established that some KAMPs form pores in the bacterial membrane.
In the present work, we employ molecular simulations to examine the structure of seven KAMPs derived from human cytokeratin 6A (KAMP-10/10G2A/18C/18N/19/19scrumbled/36) and one from poultry feather keratin (Pw-Antibac123), both in dilute and in semi-dilute aqueous solutions, and their interactions against model bacterial membranes. The predictions of detailed molecular dynamics (MD) simulations confirm the experimentally determined structure of KAMPs in dilute solutions, namely a dominant non-canonical secondary structure with a very low helical content (< 10%). The helical content is somewhat promoted by peptide aggregation in semi-dilute conditions, with the formed clusters being stabilized by β-bridges. Enhanced aggregation propensity was observed for KAMP-36 and Pw-Antibac123, while the substitution of glycine by alanine in KAMP-10 was found to hinder the tendency of KAMPs for self-association into a single big cluster.
As no spontaneous pore formation in both Gram-positive and Gram-negative membranes was observed when these were brought into contact with KAMP-10 and KAMP-18C even after about 2 μs of simulation time, systems with pre-assembled pores or with KAMPs embedded into the bilayer membrane were designed and studied in terms of their stability over time or in terms of KAMPs' ability to diffuse through the membrane, self-assemble into larger structures and create defects. Pore stability, in particular, was assessed by computing the time evolution of the volume of the initially formed pore in the presence of different KAMP structures using a geometric algorithm. Results from these calculations will be presented and discussed in due detail.

1. Molecular dynamics

P 34 Observing RAS through the computational microscope – lessons learned from molecular dynamics simulations

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Mutated small GTPase RAS is a key driver of oncogenesis across human cancers, and nearly 20% of all tumors harbor a RAS alteration. Targeting this oncoprotein with small molecule inhibitors has proven difficult. To this end, the KRAS switch-II pocket (SII-P) has offered one of the most invaluable tools to date. Its value has been demonstrated with several KRAS(G12C)-targeting covalent inhibitors, already resulting in two FDA-approved drugs, accompanied with earlier-stage compounds targeting the other position 12 mutants. To date, dozens of SII-P binder co-crystal structures are available, which have considerably improved our understanding of this pocket. The SII-P, however, is very enigmatic in nature as it is enclosed by the highly dynamic switch-II loop region. Therefore, the "frozen" structural data can only offer a limited view of this pocket.
In this contribution I will provide several insights into what our classical microsecond timescale molecular dynamics (MD) simulations suggest about the pocket behavior with SII-P bound ligands, including two clinical candidates with 200 µs simulation data. Furthermore, our simulations with the approved drugs, sotorasib and adagrasib, highlight important mechanistic aspects related to RAS isoform selectivity and resistance arising from secondary mutations. Finally, I will discuss the outlook of transferring the SII-P targeting strategy to other RAS GTPases beyond KRAS, by disclosing our key findings from our 1 millisecond MD simulations of MRAS, RRAS and RRAS2 GTPases.

1. Molecular dynamics

P 35 Rationalizing the tridimensional structure of Hyaluronan and Heparin based on their monosaccharide sequence and sulfation pattern

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Glycosaminoglycans (GAGs) are complex polysaccharides found in the extracellular matrix. They are vital regulating cellular processes, such as cell-cell communication and molecular recognition. A significant feature of all GAGs is their high negative charge, which facilitates interactions with positively charged regions of proteins. Most GAGs present local structural modifications in the form of sulfations, which can vary in number or location, forming distinct characteristic patterns called "sulfation patterns".
The tridimensional structure of biomolecules is essential for their activity, as shape and charge distribution are important factors influencing intermolecular interactions. However, understanding the secondary structure of GAGs is challenging due to their big size, flexibility and intrinsic sequence inhomogeneity.
Here, we explore the impact of sulfation patterns on the local structure of GAGs using Molecular Dynamics (MD). Our systems contain mixtures of fifty-monosaccharide long chains of Hyaluronan (HA) and Heparin (Hep). HA, the only GAG that never presents sulfation, acts as a relevant reference. On the other hand, Hep is a heterogeneous polysaccharide with variable sulfation patterns and monosaccharide types. Our Hep systems include two common sulfation patterns: NANS, with moderate sulfate and Iduronic acid (IdoA) content, and NS, with high sulfate and IdoA content.
We observe that the highly sulfated NS chains present a significantly reduced end-to-end distance due to the formation of kinks. The more compact structure is favored despite the increased electrostatic repulsion caused by the kinking. This results from the internal flexibility of IdoA, and the fact that its sulfation at position 2 locks its pucker conformation in the 1C4 chair.
We use a range of metrics to track the development of secondary structures (referred to as Hep–helix), highlighting characteristic chain shortenings, local kinking, constrained dihedral angle distributions, and above all, the locking of IdoA residues in the 1C4 conformer.
Furthermore, we present an objective metric to quantify the Hep–helix, and we employ an additional set of shorter GAG systems with custom sequences and sulfation patterns to rationalize the factors that promote its appearance, with sulfation at position 2 of IdoA being the most prominent.
This opens a door for biological regulation through control of Hep secondary structure, allowing the concentration of charges in a reduced space of the GAG to regulate the interactions with positively charged regions of proteins.

1. Molecular dynamics

P 36 Investigating Collectivity in Self-Assembled Protein Filaments Involved in Homologous Recombination

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Protein filaments are crucial for cellular functions, acting as scaffolds in the cytoskeleton and aiding in force and torque transmission. This study examines nucleoprotein filaments involved in homologous recombination (HR), specifically those formed by RecA protein polymerization on DNA. Unlike cytoskeletal filaments, HR filaments interact primarily with DNA, with the stress in the bound DNA promoting strand exchange during HR.
HR, a vital DNA repair mechanism, is driven by RecA recombinase in nucleofilaments that polymerize on single-stranded DNA (ssDNA) and integrate double-stranded DNA (dsDNA), enabling homology search and pairing exchange. Tension among the three DNA strands in the filament facilitates strand exchange. Research by Dr. Mara Prentiss's group at Harvard University shows that the length of inserted DNA affects reverse strand exchange kinetics and filament stability.
We investigated the impact of DNA strain during strand exchange on subsequent HR steps using molecular dynamics simulations. Our findings reveal that the mechanical properties of filaments are sensitive to the length of inserted DNA, especially with a displaced strand present. We observed global deformations such as axis bending, protein-protein interface perturbations, and localized switching of the displaced strand between binding sites. Structural analysis indicates non-uniformities in bound DNA strands, with fluctuating inter-phosphate distances and reduced stress in B-form DNA regions. Multiple DNA turns cause local filament curvature and deformation, impacting contact networks, strand positions, and filament structures, which may affect HR events.
In conclusion, our study demonstrates how RecA nucleoprotein filaments adapt to inserted DNA length, leading to non-homogeneous structures. These insights into filament mechanics enhance our understanding of HR mechanisms and may inform strategies for DNA repair, genetic disease, and cancer therapies.

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1. Molecular dynamics

P 37 Exploring interactions in between Pep-1 peptide and Hyaluronan with molecular simulations

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The extracellular matrix (ECM) plays a critical role in cancer progression, with hyaluronan (HA) significantly influencing metastasis and tumor aggressiveness. Pep-1, a unique HA-binding peptide, lacks typical HA-binding motifs, making it an intriguing candidate for anticancer therapytargeting hyaluronan. We used all-atom molecular dynamics (AAMD) simulations to explore Pep-1's interaction with HA, comparing it to a scrambled variant (scrPep-1) and control peptide from the work, where Pep-1 was initially introduced.
We tested two force fields, CHARMM36m and prosECCo75, the latter being specifically chosen for its electronic continuum correction (ECC), which is crucial for accurately modeling Pep-1's high hydrophobicity, especially with the fact that literature states high importance of hydrophobic residues in Pep-1's sequence for HA binding. Our simulations show that Pep-1 exhibits a higher propensity to bind HA than the control peptide, although the differences between Pep-1 and scrPep-1 were modest. Despite that general binding propensities were similar, Pep-1 consistently bridges two HA strands for longer duration than scrPep-1, possibly explaining the reason why *in vitro* studies have higher binding activity observed for Pep-1.
Neither peptide adopted stable secondary structures upon binding to HA, despite scrPep-1 being known to adopt more β folding propensity in solvent and additionally no evidence of Pep-1 aggregation or stable multimer formation was found, despite its hydrophobicity.
These results were validated through comparison with circular dichroism (CD) and ^{1}H NMR data, ensuring accuracy in our parameterization.
These findings address how well one can represent biological effects by simplistic biophysical numerical models and how to compare results from in *in vivo* and *in vitro* experiments with *in silico* computations.

1. Molecular dynamics

P 38 The capacity of GM1 to modulate the properties of biomembranes

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Gangliosides play a significant role in cell signaling. They form one class of glycosphingolipids, occurring in the outermost leaflet of the plasma membrane. GM1 glycosphingolipids are known to exhibit significant variation in the length and saturation of their ceramide chains, and based on preliminary experimental data, this has a clear impact on their behavior in membranes and further also a significant impact on their ability to modulate the properties of membranes and their membrane proteins. GM1s are also related to, e.g., lipid traffic and intracellular distribution, but in ways that are not well understood so far. Translocation (flip-flop) is not a likely mechanism supporting this, because GM1-glycosphingolipids have large hydrophilic head groups, so it is more obvious that GM1s are distributed through the cell via, for example, vesicular traffic.
Using atomic-level molecular dynamics simulations, we investigated how different types of GM1 modulate the dynamics of cell membranes. Above all, we investigated the effect of the length of the ceramide chain and the position of the double bond in it by studying several members of the GM1 family, such as saturated ceramide chains (e.g., C14:0) and subtly different unsaturated chains (e.g., C22:1Δ13, C24:1Δ15).
On this basis, we investigated the biophysical properties of membranes and their dependence on GM1, including membrane order and thickness, hydrogen bonding capacity, nanodomain formation, and the role of GM1 in these environments. Preliminary computational results together with experimental results (collaboration reported elsewhere) are promising, demonstrating the intriguing effect of gangliosides on membrane dynamics.

1. Molecular dynamics

P 39 Exploring Photodynamic Cycle of EL222 Transcription Factor: A Molecular Dynamics Approach

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The EL222 protein contains a light-oxygen-voltage (LOV) domain and undergoes substantial conformational changes upon illumination, playing a crucial role in various photobiological processes. When exposed to light, the conformational changes in the LOV domain are transmitted to the HTH domain in EL222, altering its ability to bind DNA. This process modulates the transcription of target genes in response to blue light. Additionally, the FMN chromophore in EL222, essential for light sensing, undergoes structural, redox and protonation state changes upon photon absorption, transitioning from a dark state to covalently-bound semiquinone (FMN-Cov, lit1) and hydroquinone (FMN-Red, lit2) forms. This study explores the atomic-level mechanisms behind the conformational transitions of the EL222 protein from its dark to lit states using molecular dynamics (MD) simulations and Metadynamics enhanced sampling techniques via the PLUMED plugin (using Gromacs version 2023.2). Light-induced alterations in the hydrogen bond network within the FMN chromophore were significant, particularly involving the rotation of Gln138 residue sidechain in the flavin binding pocket. This conformational transition is coupled with significant fluctuations of residues (based on RMSF analysis) in the A'α to Bβ, Eα to Fα, Iβ-Jα loop, and Jα-helix regions of the protein upon light activation. Additionally, the FMN-Cov (lit1) state showed a faster LOV-HTH domain separation time compared to the FMN-Ox (dark) and FMN-Red (lit2) states. The shorter separation time in this state suggests that covalent bond formation accelerates the separation of the LOV and HTH domains, highlighting the critical role of covalent bonding in EL222's structural dynamics.

1. Molecular dynamics

P 40 In silico characterization of a rare disease that affects the dynamics and function of linker histone H1

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The eukaryotic genome is wrapped in nucleosomes to form chromatin. Nucleosomes contain 147 bp of DNA wrapped ~1.65 times around a histone octamer comprising two copies each of the core histones H2A, H2B, H3, and H4. In higher eukaryotes, a fifth histone, known as the linker histone H1, binds the nucleosome and the linker DNA between nucleosomes to facilitate the folding of the chromatin fiber into a higher-order three-dimensional structure.
In late 2019, germline frameshift mutations involving one of the H1 subtypes, H1.4, were linked to a syndrome called Rahman syndrome (RS). Affected individuals show intellectual disability and skeletal and cardiac anomalies. We hypothesize that chromatin domains with RS-H1.4 could exhibit a less condensed and differently organized structure, disrupting chromatin architecture and function. To understand how RS mutant H1.4 affects nucleosome dynamics, we performed atomistic molecular simulations of the wild-type (WT) and RS-H1.4 bound nucleosome. We focused on the most affected RS-H1.4 from "patient #1." Indeed, we found that H1.4 from patient #1 not only failed to fold the linker DNA strands but also destabilized them compared to the WT and a "tailless" mutant lacking the CTD.
In conclusion, this study provides insights into RS and critical findings about 3D chromatin architecture. These findings are expected to shed light on the mechanism of RS and serve as an example of a "workflow" for rare disease studies.

1. Molecular dynamics

P 41 Design Guidelines for Antimicrobial Peptides that Kill Antibiotic-Resistant Bacteria via Transmembrane Pores

Robert Vacha
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Pore-forming peptides can be utilized in various medical and biotechnological applications, including antimicrobial peptides that can kill antibiotic-resistant bacteria. However, designing peptides that self-assemble into transmembrane barrel-like nanopore structures is challenging due to the complexity of several competing interactions involving peptides, lipids, water, and ions. We develop a computational approach for the de novo design of *α*-helical peptides that self-assemble into stable and large transmembrane barrel pores with a central nano-sized functional channel. We address the lack of existing design guidelines for the de novo pore-forming peptides and propose 52 sequence patterns, each of which can be tailored for different applications using the identified role of its residues. Atomic force microscopy, channel electrical recording, leakage of small fluorescent molecule and transport of macromolecule experiments confirm that the designed peptides form stable, large, and functional barrel-shaped nanopores in model membranes. The custom-designed peptides act as potent antimicrobial agents able to kill even antibiotic-resistant ESKAPE bacteria at nanomolar concentrations, while exhibiting low toxicity to human cells. Peptides and their assembled nanopore structures can be similarly fine-tuned for other medical and biotechnological application.

1. Molecular dynamics

P 42 Lys716 in the transmembrane domain of yeast mitofusin Fzo1 modulates anchoring and fusion

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Outer mitochondrial membrane (OMM) fusion is an important process for the cell and organism survival, as its dysfunction is linked to neurodegenerative diseases and cancer. The OMM fusion is mediated by members of the dynamin-related protein (DRP) family, named mitofusins. The exact mechanism by which the mitofusins contribute to these diseases, as well as the exact molecular fusion mechanism mediated by mitofusin, remains elusive. We have performed extensive multiscale molecular dynamics simulations using both coarse-grained (Martini 2 and 3) and all-atom approaches to predict the dimerization of two transmembrane domain (TM) helices of the yeast mitofusin Fzo1. We identify specific residues, such as Lys716, that can modulate dimer stability. Comparison with a previous computational model reveals remarkable differences in helix crossing angles and interfacial contacts. Overall, however, the TM1-TM2 interface appears to be stable in the Martini and CHARMM force fields.
Replica-exchange simulations further tune a detailed atomistic model, as confirmed by a remarkable agreement with an independent prediction of the Fzo1-Ugo1 complex by AlphaFold2.
Functional implications, including a possible role of Lys716 that could affect membrane interactions during fusion, are suggested and consistent with experiments monitoring mitochondrial respiration of selected Fzo1 mutants.

1. Molecular dynamics

P 43 Disruption of monoamine oxidase enzymes by sars-cov-2 spike protein: implications for neurodegenerative diseases

Lucija Vrban, Robert Vianello
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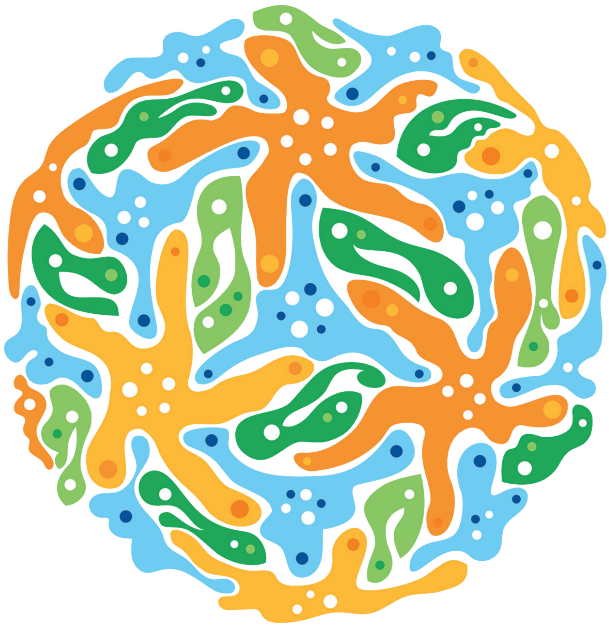
COVID-19, caused by SARS-CoV-2, is primarily associated with respiratory symptoms, but emerging evidence suggests significant neurological impacts, possibly due to the virus's interaction with monoamine oxidase (MAO) enzymes.Our research explores the binding of the SARS-CoV-2 spike protein to MAO enzymes, hypothesizing that this interaction disbalances the delicate monoaminergic system, potentially leading to neurodegenerative processes.
We utilized an extensive computational approach, incorporating flexible docking algorithms to predict binding orientations and affinities, molecular dynamics (MD) simulations to refine and stabilize these complexes, and quantum mechanics/molecular mechanics (QM/MM) simulations to understand catalytic and inhibitory processes. This approach allowed us to investigate reactions involving the natural substrates dopamine and phenylethylamine, as well as the inhibitors selegiline and clorgyline.
Our results identified high-affinity binding sites for the spike protein on MAO A and MAO B. MD simulations confirmed the stability of these interactions and indicated significant conformational changes in the enzymes and subsequent free binding energy changes. QM/MM calculations showed that the spike protein alters MAO catalytic activity, affecting neurotransmitter metabolism and potentially contributing to neurodegenerative disease pathways.
These findings suggest a mechanistic pathway through which COVID-19 could impact the monoaminergic system, contributing to neurological symptoms in patients. Our study underscores the need for further experimental validation and the development of therapeutic interventions to mitigate these effects. Understanding these interactions opens new avenues for treating the neurodegenerative impacts associated with COVID-19.

Final category: 2. FREE ENERGY CALCULATIONS



2. Free Energy calculations

P 44 On the challenge of predicting the conformational ensembles of unordered biomolecules

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Computational methods based on physical and data-driven modelling have been pivotal for the determination of biomolecular structures, which are crucial for a rationalization of their biochemical and biological functions. A still very open research goal is the elucidation of conformational ensembles of biomolecules that are mostly unordered or least present a large degree of flexibility, and the association of their functions with unambiguously defined structural features. Enhanced-sampling molecular dynamics (MD) methods offer a viable way of computationally predicting such conformational ensembles, provided that a series of challenges are successfully addressed and solved, including: (i) the accurate and efficient calculation of interatomic interaction energies and forces allowing MD simulations on the microsecond time scale; (ii) the choice of the collective coordinates spanning explorable phase spaces in which the ensembles can be rationally analysed; (iii) the visual representation of these multidimensional spaces in reduced dimensions, typically in 2D diagrams. The validation of the predicted ensembles via comparisons between calculated and measured properties poses further challenges related with the estimation of free-energy observables and of the biomolecular responses to excitations at the basis of common spectroscopic methods. These are hard to infer both experimentally and computationally.
In this talk, I will present examples of own studies aiming at solving these challenges, coupling MD simulations of disordered proteins, nucleic acids and glycans with experimental measurements of their optical and vibrational spectra, their adsorption energies to inorganic surfaces and their responses to externally applied forces. Future directions aiming at elucidating so-far unexplored relationships between conformational microstates and circular-dichroism spectral features will also be presented.

2. Free Energy calculations

P 45 Unraveling Heme flipping in biological transport phenomena with an effective data-driven collective variable

Mina Ebrahimi, Ahmad Reza Mehdipour
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In this project, we try to model the free energy profile of the spontaneous passive and protein-mediated heme transport across the lipid membrane, employing a data-driven collective variables (CVs) approach. This approach offers insights into the mechanism of heme binding and translocation of an ABC transporter in Mycobacterium tuberculosis. Experimental observations indicate heme spontaneous flipping is a slow process with a high energy barrier. Obtaining a free energy profile that accurately maps into the heme flip is challenging and beyond the scope of conventional CVs regularly used in small molecule permeation, even in biased advanced sampling methods. The newly formulated CVs overcome the challenge by describing heme transport with the consideration of all relevant degrees of freedom of the ligand concerning the binding site. The associated coefficients accounted for in the CV are initially well-tuned based on a training thermal equilibrated data set using a deep learning algorithm, an autoencoder. Finally, the optimal CVs with the precise characterization of the metastable states are improved by the iterative training of coefficients based on biased samplings. The adjustable data-driven CVs can predict the high energy barrier associated with the translocation of charged heme across an impenetrable biological membrane and discriminate the transport mechanisms of heme, either neutral or positively charged.

2. Free Energy calculations

P 46 Targeting RNA with Small Molecules - Absolute Binding Free Energy Calculations on a Riboswitch-like RNA-ligand complex from the Hepatitis C Virus Internal Ribosome Entry Site

Krystel EL HAGE[1], Narjes Ansari[1], Chengwen Liu[2,1], Florent Hedin[1], Pengyu Ren[2,1], Jay Ponder[3,1], Jean-Philip Piquemal[1,4], Louis Lagardere[1,4]

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In this work we describe and leverage Qubit's advanced computational technics to compute the affinity of a class of small molecules for the HCV-IRES IIA subdomain. We have put in place a first tailored state of the art approach for absolute binding free energies (ABFE) of Riboswitch-like RNA using the newly developed lambda-ABF scheme combined with positional, orientational and conformational restraints. Calculations are done using AMOEBA, an advanced multipolar polarizable force field which accounts for quantum many-body effects, as well as polarization effects, and the massively parallel molecular dynamics package Tinker-HP. Results show a solid reproduction of the binding mode, a prefect ranking with Ligands 12 and 13 as most potent and Ligand 2h as a non-binder, and a very good correlation with experiment (R2 = 0.93 - RMSE = 0.33 kcal/mol - MAE = 0.26 kcal/mol).

2. Free Energy calculations

P 47 Comparing Free Energy Profiles of PFKL and PFKP Isoform Interfaces: A Multiscale MD Simulation Study

Mehrnoosh Hazrati, Štěpán Timr
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Phosphofructokinase-1 (PFK1), a pivotal enzyme in the glycolytic pathway, catalyzes the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate. In mammalian cells, this reaction is carried out by three enzyme isoforms: PFKL (liver), PFKM (muscle), and PFKP (platelet), exhibiting distinct regulatory behaviors. The assembly of PFK1 tetramers into large-scale filaments has been suggested to contribute to PFK1 regulation and play a key role in the formation of a glycolytic metabolon. The ability to form filaments is isoform-specific: while they are formed by PFKL, the PFKP isoform lacks this capability due to differences at the interaction interfaces.
To provide mechanistic insights into PFK1 filament formation and to investigate the ability of all-atom (AA) and coarse-grained (CG) force fields to capture isoform-specific differences, molecular dynamics (MD) simulations were performed on PFKL and its N702T mutant, which represents the PFKP interface. The simulations were conducted at both the AA and CG levels using the GROMACS 2022.5 software. We employed Replica Exchange Umbrella Sampling (REUS) to generate free energy profiles for fragment-fragment interactions, utilizing force fields including AMBER ff14SB, ff19SB, Martini 3, and Martini 3 + OLIVES. The Weighted Histogram Analysis Method (WHAM) was employed to reconstruct the free energy profiles in all simulations.
We found that all the above force fields captured the qualitative difference between PFKL and the N702T mutant binding free energies. In all cases, PFKL fragments exhibited stronger interactions compared to the mutant, aligning well with experimental data. The ff19SB force field revealed larger differences between PFKL and the N702T mutant than ff14SB. While correctly capturing the difference between the two constructs, the Martini 3 force field significantly underestimated the stability of the interface. On the other hand, the Martini 3 + OLIVES model overestimated both the absolute binding strength and the relative differences between the constructs compared to the AA results, indicating that further optimization of the OLIVES parameters is necessary for accurate protein-protein interaction studies. Overall, this study forms an important starting point for a multi-scale description of PFK1 assemblies, providing insights into the structural features that distinguish PFKL from PFKP, and deepening our understanding of isoform-specific regulation in glycolysis.

2. Free Energy calculations

P 48 Binding of arginine peptides to non-planar lipid bilayers

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Short cationic peptides with the ability to passively translocate across the cell membrane and carry their cargo with them have many potential applications, such as targeted drug delivery. Despite intensive research over the past 30 years and numerous theories proposed, the exact mechanism of passive cell penetration remains unknown. Molecular dynamics (MD) simulations offer atomistic resolution of peptide-membrane interactions and have thus become a useful complementary method to experimental studies of cell-penetrating peptides. Fluorescence microscopy experiments on living human cells reveal that R9 peptides penetrate cells effectively, while shorter R4 and K9 peptides do not. The difference between R9 and K9 arises from the unique guanidinium group in arginine, which promotes peptide aggregation and facilitates passive membrane penetration. Additionally, R9 was observed to penetrate at specific membrane sites, likely involving peptide aggregation and local membrane curvature.
To further investigate these interactions, we performed MD simulations of R9 on curved membranes using the EnCurv technique, with K9 as a negative control. Free Energy profiles obtained from metadynamics confirm that R9 strongly interacts with membranes, particularly on concave surfaces where binding increases with curvature. In contrast, on convex surfaces, higher curvature reduces R9 binding. These results suggest a link between peptide aggregation and membrane shape. Future research will focus on whether R9 stabilizes or induces membrane curvature, and on its potential role in multilamellar membrane formation, as suggested by cryoEM data.

2. Free Energy calculations

P 49 Impact of Charged Residue Distribution on Peptide Translocation

Ivo Kabelka[1,2], Radim Brožek[1,2], Peter Pajtinka[1,2], Robert Vácha[1,2]
[1]*CEITEC - Central European Institute of Technology, Brno, Czech Republic*
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Cell membranes are selectively permeable barriers that regulate the exchange of matter between a cell and its surrounding environment. While essential for cell survival, this property poses a significant challenge for drug delivery. One possible solution to this challenge may involve the use of cell-penetrating peptides. These compounds are known for their ability to spontaneously enter cells by translocating through the membrane. Despite the large number of known translocating peptides, there remains an ambiguity regarding the underlying translocation mechanism, and particularly the sequence-activity relationship. In this study, we employed coarse-grained molecular dynamics simulations and discovered that the peptide's ability to translocate isn't solely determined by its overall physico-chemical properties. Instead, we show that the spatial distribution of charged residues within a peptide sequence emerges as a stronger determinant than the overall net charge. These findings have important implications for targeted therapeutic applications, as tuning the peptide net charge can enable selectivity towards distinct cell types. Ultimately, our study provides insights for the design optimization of cell-penetrating peptides.

2. Free Energy calculations

P 50 Absolute binding free energy calculations: a systematic force field comparison by a newly designed computational pipeline

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Absolute binding free energy (ABFE) calculations based on free energy perturbation (FEP) techniques have emerged as a useful tool for guiding drug design. Although accuracy of ABFE calculations relative to experiment critically depends on the quality of the force field, systematic force field comparisons are still rare in the literature. To close this gap, we devised a computational pipeline "BindFlow" for fully auto-mated ABFE calculations. We computed ABFEs using the small-molecule force fields OpenFF-2.0.0, OpenFF-2.0.1, GAFF-2.11, and Espaloma-0.3.1. A total of 1239 affinities were computed, involving seven biological targets including a pentameric membrane channel. By computing uncertainties from three in-dependent replicas for each ABFE calculation, we find that common methods such as MBAR underestimate the statistical uncertainties. Our results reveal marked differences among the force fields, both in terms of mean unsigned error relative to experiment and in terms of binding contributions from Lennard-Jones or Coulomb terms. Remaining challenges and future improvements are discussed.

2. Free Energy calculations

P 51 Applications of Ligand-Based Grand Canonical Nonequilibrium Candidate Monte Carlo to Drug Design

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[2]*Astex Pharmaceuticals, Cambridge, United Kingdom*

For several years, Grand Canonical Monte Carlo (GCMC) based simulations have been used to efficiently sample buried water molecules within protein-ligand systems, accurately reproducing crystallographic water sites, and predicting the effects of water on ligand binding. To further improve sampling and acceptance rates, we have incorporated Nonequilibrium Candidate Monte Carlo (NCMC) such that an insertion or deletion move happens over a short amount of time with the molecule being coupled or decoupled alchemically. When applied to water, this GCNCMC method yields increased acceptance rates as well as a greater understanding of the binding process. Improved sampling of both protein and ligand is also observed as they respond to the nonequilibrium water move.
Here, in the context of fragment and structure-based drug design, we have further developed the protocol to facilitate the thermodynamically rigorous insertion and deletion of small molecules, with a focus on the MiniFrags library of Astex Pharmaceuticals.
We demonstrate that fragment GCNCMC, when combined with mixed solvent molecular dynamics, can rapidly find and map occluded binding pockets that are often inaccessible in traditional simulations. Once a binding site is known, fragment-like molecules often bind in multiple orientations which may be kinetically trapped; we show how these binding modes are naturally sampled in GCNCMC simulations. Finally, we show that GCNCMC is able calculate fragment binding affinities using a novel titration protocol which is free of complicated restraints, symmetry corrections and the need for prior knowledge of the binding site.

2. Free Energy calculations

P 52 Elucidating the mechanism of ligand control of PAR1 receptor activation by molecular dynamics simulations

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Leipzig University, Leipzig, Germany

The protease-activated receptor 1 (PAR1) is a key regulator in homeostasis. Current drug research targets PAR1 to prevent thrombotic events, with an already clinically used antagonist vorapaxar (VPX). However, the distinct signaling outcomes of PAR1 in response to different proteases challenge the application. VPX administration not only blocks proinflammatory thrombotic effects but also interferes with the cytoprotective signaling induced by activated protein C, potentially leading to bleeding complications. The rational design of a biased PAR1 modulator, which would maintain cytoprotective signaling while inhibiting proinflammatory signaling, is hampered by the lack of knowledge on the structural basics of differential PAR1 signaling. Here, the effects of different ligands on the conformational free energy landscape of PAR1 activation were investigated using molecular dynamics (MD) simulations with enhanced sampling. First, an experimentally validated molecular model was generated that reveals the binding mode for the thrombin-generated tethered ligand (tTL) on PAR1. Subsequent MD simulations using the string method with swarms of trajectories uncovered the free energy landscapes of the ligand-free, tTL-bound and VPX-bound PAR1 receptor complexes. Comparison of these landscapes revealed an activation increasing effect for tTL and a hampering effect for VPX consistent with experimental data. Furthermore, it was found, that binding of a sodium ion at the receptor intramembrane core allosterically modulates the PAR1 activation state. These findings shed light on the structural basics of PAR1 signaling and motivate further investigations of more ligands to characterize their differential effects and uncover distinct conformational receptor states.

2. Free Energy calculations

P 53 Hydrothermal scenario of the amino acids formation

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Chemical evolution is a field of chemistry, which is focused on exploration of the plausible scenario for DNA and RNA building blocks and its formation from simple molecules under geothermal conditions on the Early Earth. Particularly, amino acids are essential building blocks of proteins and play a key role in a various biological processes. The question about amino acids formation on the Early Earth is an interesting topic and its modern investigation is based on the pioneer work of *Urey* and *Miller*, published on May 15, 1953 in *Science*. Their series of experiments elegantly show the amino acids formation from simple molecules, using as an energy source heat and electric sparks (mimicking lightning).
However, the absence of a logical geological context in works, mentioned above, opens a broad field for finding a new plausible reaction pathways for amino acids formation, especially for sulphur-containing amino acids. The hydrothermal systems are a unique natural source of sulphur and provide a feasible medium for sulphur-containing amino acids formation, since it constitutes essential molecules for living organisms.

Methodology

Theoretical approaches, based on *ab initio* quantum-chemical calculations and molecular dynamics were used in this work. A new approach, called the *ab initio* nanoreactor, was employed, which allows to construct the reaction network from simple compounds, where the initial set of molecules are treated under spherical border conditions with alternative cycles of collisions and relaxation.

Results

As a result, we obtain a potential reaction pathways for sulphur-containing amino acids from simple molecules, such as methionine, cysteine, homocysteine and taurine. In addition, experiments with *ab initio* nanoreactor provide an insight into amino acids formation, starting from water, formamide and hydrogen sulphide molecules. Based on the obtained results, the potential energy diagrams for the initial reaction pathways were established, which allow to visualize the energetic and thermodynamic characteristics of the reaction network.

Conclusions

As a result of the research, current investigation supplies the original reaction mechanisms and reaction pathways for sulphur-containing amino acids formation from simple molecules under hydrothermal conditions.

Acknowledgements

The Bulgarian National Science Fund within the financial support for project of junior basic researchers and postdocs – 2021 (contracted as КП-06 ПМ59/2) is acknowledged for the financial support for this investigation. The research that led to these results was carried out using the infrastructure purchased under the National Roadmap for RI, financially coordinated by the MES of the Republic of Bulgaria (grant № D01-325/01.12.2023).

2. Free Energy calculations

P 54 Calculation of errorbars only from numbers of transitions: molecular dynamics and parallel tempering

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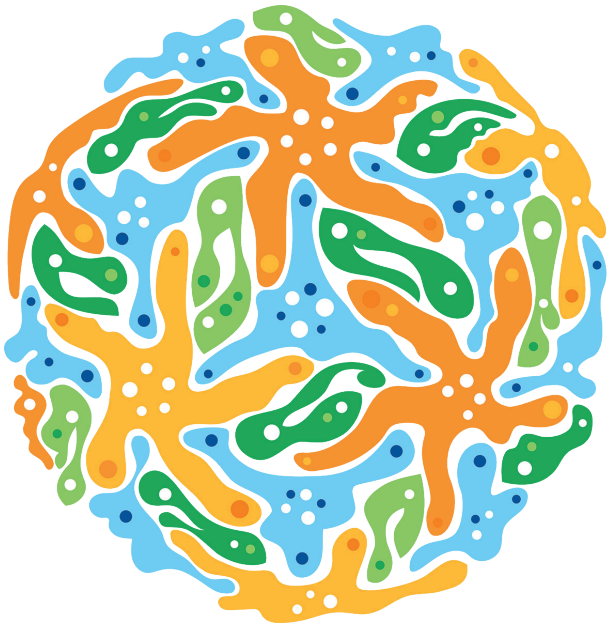
Molecular dynamics simulation and its parallel tempering variant are often used to predict equilibrium constants and free energy differences. Assessment of the accuracy of such predictions is crucial. Here we show that errorbars (standard errors or confidence intervals) can be calculated solely from the number of events, e.g. protein folding and unfolding, protein-ligand binding and unbinding etc. Markovianity of the process is a prerequisite.

Final category: 3. FORCE FIELD DEVELOPMENT



3. Force field development

P 55 Refinement of ionic force fields through electronic continuum correction utilizing a global optimization method

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Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

The Electronic Continuum Correction (ECC) method addresses the lack of electronic polarization in non-polarizable force fields by rescaling charges in a mean-field manner. This approach, implemented in force fields like prosECCo75, has demonstrated improved agreement with experimental ion binding data across diverse systems, including lipid membranes, proteins, and saccharides. However, existing ECC-integrated force fields are based on water models with dielectric constants ranging from 60 to 100, which already partially account for the high-frequency electronic component, leading to overcorrection and raising concerns about the physical consistency of the charge scaling approach.
Recently, a water model compatible with ECC (ECCw) was developed, providing a foundation for constructing force fields from the ground up. In this work, we present the parameterization of several biologically relevant atomic ions—including alkali metals (Li^+, Na^+, K^+), halides (Cl^-, Br^-, I^-), and Ca^{2+}—based on ECCw. The ion models were optimized to match experimental data on hydration structure (from neutron diffraction and isotopic substitution), as well as thermodynamic properties such as density and viscosity.
The best-fitting models exhibit an optimal scaling factor of 0.81 and show good agreement with experimental hydration structures,and achieve acceptable accuracy in viscosity and density. A comparison with the Madrid2019 ion models, which utilize a scaling factor of 0.85, reveals that the ECC ions better capture short-range hydration structures (first hydration shell) and viscosity, but are less accurate in reproducing densities. Radial distribution functions (RDFs) in both r-space and q-space highlight that deviations in long-range ion-water and ion-ion interactions drive these density discrepancies.
From analyses of NaCl, LiCl and $CaCl_2$ solutions, we found that short-range cation-anion interactions influence long-range water structure, suggesting that the limitations of classical force fields prevent them from accurately capturing complex ion-water and ion-ion interactions across both long and short ranges. This study provides key insights into the development of ECC-based force fields and highlights the trade-offs between short-range accuracy and overall thermodynamic consistency.

3. Force field development

P 56 Influencing calcium-membrane binding in classical molecular dynamics simulations by varying dielectric constant of water models

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A known drawback of full charge non-polarizable force fields for biological systems is their inherent overestimation of the binding of charged groups, particularly divalent ions (frequently Ca^{2+}). This issue has been addressed either through corrections to nonbonded parameters (such as the NBFX in CHARMM36) or through charge scaling in the prosECCo75 force field. Charge scaling, also referred to as Electric Continuum Correction (ECC), reduces overbinding by scaling down charges, effectively accounting for the missing electronic component of the dielectric constant of the medium, which is fairly constant for most biological relevant environments including water solutions and membranes.
To test how charge scaling and ECC models depend on the used water parameters, we developed 60 optimized water models. These models correctly recover the dependence of density with temperature and pressure, and viscosity even though they vary in the dielectric constants (ranging from 45 to 77). Based on these models, one can create compatible ion parameters which reproduce well experimental data of ionic solutions. Here we evaluate the water models using the prosECCo75 and CHARMM36 force fields in more complex, biologically relevant systems such as POPC bilayer membrane. We study the binding of calcium chloride to the membrane, a known difficult problem for non-polarizable force fields, in two concentration of the salt (at 75 mM and 150 mM). Previous simulations of calcium chloride in pure water have shown that ion paring of calcium and chloride for optimized $CaCl_2$ models was reasonably reproduced and was practically invariant to the chosen water model. On the other hand, our results for more complex membrane system indicate that the choice of water model is crucial for accurate description of the interaction of calcium to those membranes. We see that lower water dielectric constants lead to more Ca^{2+} aggregation at the surface. At very low dielectric constant the Ca^{2+} ion binds fully to the membrane leading to zero concentration of the Ca^{2+} ions in the bulk.
This is a setback in the ECC framework where the Ca^{2+} membrane interaction was corrected without the need of further fudging of the parameters. Comparably, NBFIX approaches are also not immune to lowering the water dielectric constant. How to correct for this new overbidding needs further investigation and the question arises: what does it mean to be a compatible ECC water model when it comes to its dielectric constant.

3. Force field development

P 57 Combining CHARMM36m and OPC to improve accuracy

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The choice of force field parameters profoundly determines the accuracy of molecular dynamics (MD) simulations. A major challenge in recent force field development is achieving high accuracy for simulations of intrinsically disordered proteins (IDPs). Here, only small changes is the force field can largely impact e.g., compactness, secondary structure propensities, and kinetics, due to their much shallower free energy landscape compared with their globular counterparts. However, many biological systems contain both globular and disordered regions and therefore force fields that accurately describe both components are needed.
The Charmm36m [Huang et al., 2017] force field has been optimised for IDPs, but in some cases yields too small radii of gyration compared to FRET experiments. This problem of overly compact structural ensembles is common among other force fields as well. It arises from a wrong balance between protein-protein, protein-water, and water-water interactions and is usually addressed by modifying protein-water van der Waals interactions.
Here, we attempt to rebalance these interactions by replacing the original water model Tip3p* [Jorgensen et al., 1983] with the Optimal Point Charge (OPC) model [Izadi et al., 2014]. This strategy looked promising due to the recent successes of OPC in accurately reproducing bulk water properties and, e.g., solvation free energies. Indeed, we observed an overall improved accuracy in IDP simulations with the new combination. However, Charmm36m was developed and tested with Tip3p* and any other water model will require thorough validation also for globular proteins.
To this end we tested the new combination for nine globular proteins of different sizes and structure classes by comparing a total of 4,2 ms MD simulations with observations from NMR, X-ray crystallography, SAXS, circular dichroism, infrared spectroscopy, and T-jump relaxation experiments. We also compare these results to the established Charmm36m+Tip3p*, Amber99sb-disp, and Amber99sb-ildn force field combinations.
Our results suggest that Charmm36m+OPC provides similar accuracy for globular proteins and slightly improved accuracy IDPs.

3. Force field development

P 58 Bringing the last decade of AMBER force field improvements to GROMACS

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Experimental scientists rely on a variety of machines in their laboratories, often provided by different vendors, to advance their research efforts. Regardless of the machine vendors, their age of production, and their usage terms and conditions, results between machines can be compared and cross-validated. Molecular dynamics simulation software, which can be considered a type of digital machine for computational scientists, is no different. CHARMM and AMBER are two popular force fields used in the simulation of dynamics in biomolecular systems, used primarily by the academic software packages with the same name, but also ported for usage in free and open-source tools such as GROMACS, OpenMM, and LAMMPS. The energies computed by different tools are comparable, which enables the validation of the ports.
The GROMACS molecular dynamics simulation engine has historically provided support to a number of molecular mechanics force fields, including CHARMM and AMBER. Over time, the force fields used for simulations evolved by refining existing and defining additional special types of bonded interactions, which are then applied as energy corrections to existing interactions to more faithfully match the real-world experiments. The newest generations of force-fields, however, introduce functional forms that may require changes to the internal engine structure and functionality. Namely, the newest generation of AMBER, ff19SB, utilizes residue-specific backbone dihedral energy correction maps (CMAPs). Specifically, in older force fields such as CHARMM27, CMAPs are parametrized using only atom types and ff19SB extends this with additional amino-acid typing.
We describe our software development and physical validation efforts to extend GROMACS with support for amino-acid-specific CMAPs, their transformation with lambda dynamics, and provide AMBER ff19SB to users. As a part of this effort, we aim to provide a reproducible force field conversion and validation procedure that can be reused for modified variants of ff19SB and possibly future versions. As the result of our effort, the computational chemists will be able to perform more accurate (free energy) simulations of the protein backbone for all twenty amino acids using the first new force field released in the last decade that is intended to be added to the official version of GROMACS.

3. Force field development

P 59 Incorporating Electronic Polarization into All-Atom Molecular Dynamics Simulations of Glycans: From Simple Saccharides to Glycosaminoglycans and Lipopolysaccharides

Miguel Riopedre-Fernandez[1], Hector Martinez-Seara[1], Denys Biriukov[2,3,1]
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Glycans are crucial components of cell surfaces, being the primary constituents of glycosaminoglycans (GAGs) in animal cells and lipopolysaccharides (LPS) in Gram-negative bacteria. The biological functionality of GAGs and LPS is largely driven by charge–charge interactions. While molecular dynamics (MD) simulations can offer detailed atomic-level insights into glycan-related processes, the lack of electronic polarization in nonpolarizable MD force fields (FFs) often limits their accuracy.
To address this, we have developed "scaled-charge" MD models for glycans that incorporate electronic polarization using a mean-field approach [1]. Our new models for D-glucuronic and D-galacturonic acids—essential components of larger glycans—effectively overcome limitations of nonpolarizable FFs, such as nonphysical aggregation [1]. Further, by combining MD with NMR, we demonstrated a high affinity of arginine-rich sequences for hyaluronan (the only non-sulfated GAG), revealing enhanced dynamic interactions that are accurately reproduced only by our scaled-charge models [2]. Additionally, we investigated calcium binding to sulfated GAG motifs, identifying solvent-shared ion pairing as the primary interaction mode, consistent with ab initio MD findings and again properly captured by our models [3]. Using scaled-charge models, we also explored LPS membrane stabilization through cation–phosphate interactions.
Our charge-scaling approach not only refines the modeling of charge–charge interactions in glycans but also enables relevant biological applications, such as designing implant coatings with minimized bacterial affinity to prevent adhesion and developing glycan-targeting antimicrobial therapeutics.

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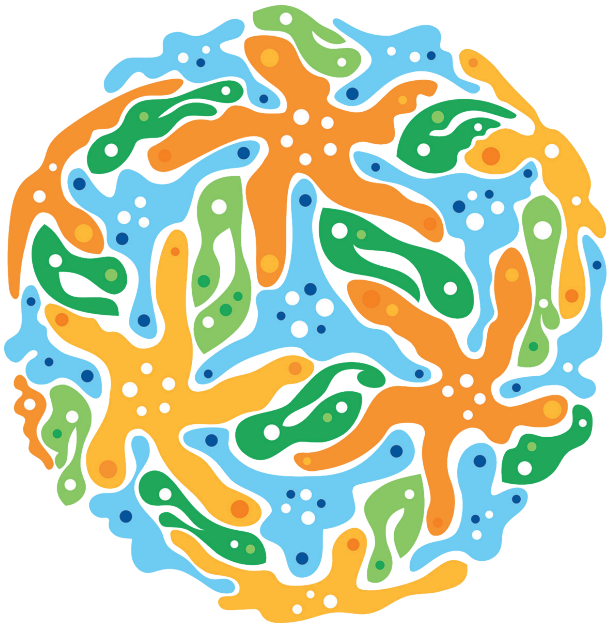
[3] Riopedre-Fernandez et al. bioRxiv 10.1101/2024.05.31.596767

Final category: 4. INTEGRATIVE MODELLING



4. Integrative Modelling

P 60 Computational modelling driven biosynthetic protein materials design

Adam L. Harmat[1,2], Dmitry Tolmachev[1,2], Alberto Scacchi[3,2,4], Markus Linder[3,2], Maria Sammalkorpi[1,2]
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Liquid-liquid phase separation (LLPS) of protein solutions is an established pre-assembly step associated with basic biological cell function and pathological conditions. LLPS is also an intermediate step in the formation of advanced biobased materials, such as mussel feet adhesives, squid beaks or silk-like fibers. The rational design of protein condensates is necessary for understanding LLPS and paving the way for engineered biosynthetic protein materials with exceptional properties.
To this purpose, we investigate engineered silk-like proteins using computational modelling to explore molecular level interactions and their implications on LLPS as observed in experiments [1,2]. Silk-like proteins have two folded domains connected by an intrinsically disordered region (IDR), making them an ideal model system for systematically investigating the interplay of folded and disordered regions in protein assembly.
We present a methodology advancement based on using protein-protein docking combined with molecular dynamics simulations to study interactions between the folded domains. We demonstrate the approach for a well-characterized ubiquitin system after which we map the response of four different folded domains matching our experimentally realized systems [2]. The simulations results show a difference in the type and anisotropicity of the interactions that regulate coacervate propensity and physical properties. A mesoscale coarse-grained model was constructed to uncover the complex interplay of folded domain and IDR interactions.

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4. Integrative Modelling

P 61 Transient Non-local Interactions of the Measles Virus Nucleocapsid Tail Domain

Gabor Nagy
Max Planxk Institute, Göttingen, Germany

The Measles virus nucleocapsid is made of thousands of nucleoprotein (N) repeats, which hold the viral RNA in a helical structure. The last 125 amino acids of each N repeat (N_{TAIL}) are intrinsically disordered and protrude radially outward from the nucleocapsid. N_{TAIL} promotes virus replication by binding to the X domain of the phosphoprotein (P_{XD}), which in turn brings the viral polymerase close to the nucleocapsid to transcribe and replicate the viral RNA. Only 18 amino acids of N_{TAIL} directly bind to P_{XD} via coupled folding and binding. The majority of N_{TAIL}, on either side of this molecular recognition region (MoRE), remains disordered. While it has been shown that these disordered regions weaken the binding affinity, interactions involving these regions, and their possible functional role have not been identified.
We apply photo-induced electron transfer (PET) experiments of full-length N_{TAIL} in solution to probe intra-molecular contact formation times between tryptophan (W) and cysteine (C) residues introduced in different regions of the protein and under different salt and pH conditions. To better understand N_{TAIL} dynamics, we combine PET measurements with analytical models, coarse-grained and all-atom molecular dynamics simulations, and co-evolutionary analysis. Our integrated approach identifies key transient non-local interactions between two regions outside of MoRE that are functionally important. These interactions dominate the dynamics of the entire N_{TAIL} in solution and affect the conformational preference of the MoRE. Supported by available N_{TAIL}-P_{XD} binding data, we propose multiple mechanisms by which key non-local interactions in N_{TAIL} may play a role in the viral replication of Measles. These mechanisms involve the recruitment and tethering of the polymerase complex to the nucleocapsid, as well as the regulation of N_{TAIL}-P_{XD} binding via an ensemble allosteric model, facilitating polymerase progression along the nucleocapsid-bound RNA.

4. Integrative Modelling

P 62 Intuitive, three-dimensional agent-based simulation of complex molecular mechanisms: application to the dynein walk cycle

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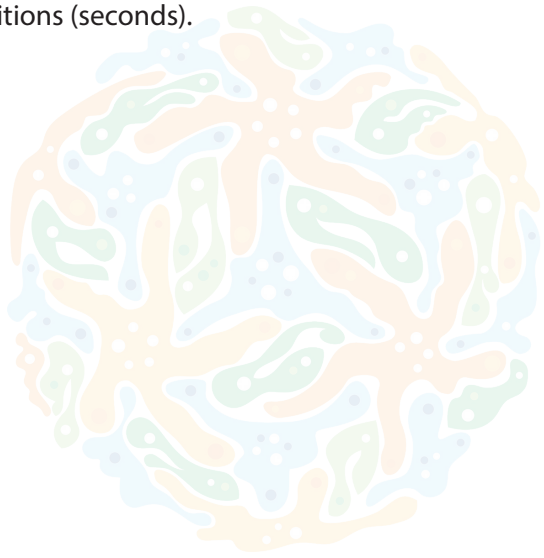
Understanding complex molecular mechanisms requires spanning broad scales in space (nm to dozens of µm) and even greater in time (ps-ns to ms-sec). Different methods can be used to carry out simulations on specific ranges of these scales, but all require tradeoffs to balance spatial resolution and simulation length with computational cost. High resolution, single-particle tracking methods such as those based on Molecular Dynamics (MD) cannot yet routinely reach scales that are functionally relevant for all biological systems; on the other end of the spectrum, methods that focus on bulk cellular properties, for example those based on Ordinary Differential Equations (ODEs), cannot provide detailed information about individual molecules. By translating outputs from one level of representation into inputs for a neighboring one, multiscale modeling aims to connect across scales and understand how changes at different levels influence each other. However, linking representations to bridge the molecular and cellular scales remains challenging because of computational cost and the relative lack of experimental data at this intermediate scale. This calls for novel, efficient mesoscopic modeling methods.
Agent-based modeling (ABM) is an alternative, stochastic and bottom-up approach to simulate a complex system and its emergent properties from the perspective of its individual components, termed "agents". Instead of being directly driven by biophysical forces, each agent behaves according to a set of rules that can be derived from very diverse information. Some of these rules can still rely on biophysical equations, while others may be defined from outputs of simulations carried out at lower scales, or stem from experimental data. Importantly, this approach achieves extended time scales, which allows for direct comparison to experimental results for both validating the models and testing further predictions.
While several ABM tools already exist, we are developing a new workflow to build intuitive, three-dimensional simulations that explicitly include intramolecular conformational changes in addition to Brownian diffusion and molecular interactions.
As a first case study, we apply this approach to simulate dynein walking on microtubules. We are able to incorporate and test the influence of molecular characteristics on the processive walk of dynein - the scale currently accessible to wet-lab experiments - despite the large size of the system (>1MDa) and long timescale of a typical run in physiological conditions (seconds).



4. Integrative Modelling

P 63 Combining Computational and Experimental Approaches for Unraveling the Molecular Mechanisms of Phase Behavior of Intrinsically Disordered Proteins.

Dmitry Tolmachev[1,2], Isabell Tunn[1,2], Adam Harmat[1,2], Nea Möttönen[1,2], Alberto Scacchi[3], Markus Linder[1,2], Maria Sammalkorpi[1,2]

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[3]*University of Turku, Turku, Finland*

Intrinsically disordered proteins lack well-defined ordered structures, leading to a complex configurational landscape. This poses significant challenges for extracting molecular-level structural assembly information and molecular studies, both experimentally and computationally. However, combining experimental and computational methods enables in part overcoming these challenges. We have pursued approaches, where experimental data validates simulation results and reveals a macroscopic response, while the simulations provide molecular-level insight.
Using a combination of microscopy, Fourier-transform infrared (FTIR) spectroscopy, and atomistic molecular dynamics simulations we investigated the temperature response of spidroins. Spidroins, the main proteins of spider silk, are highly promising molecules for a wide range of applications, including fibers and advanced composite materials [1,2]. Controlling the protein phase behavior by manipulating environmental conditions, such as temperature, is crucial for the development of such materials.
Our findings demonstrate that the repetitive hydrophilic/hydrophobic structure of intrinsically disordered regions of spidroins results in water being a poor solvent at a wide temperature range (10-80°C). This leads to phase separation, such as liquid-liquid phase separation or/and protein aggregation. At low temperatures, phase separation is driven by the hydrophilic, flexible glycine regions, while at high temperatures, the hydrophobic collapse of alanine regions. At 70-80°C, hydrophobic interactions, coupled with structural rearrangements, lead to the formation of beta-sheets, which act as crosslinks resulting in gelation at the macroscale.

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4. Integrative Modelling

P 64 Molecular architecture of the plant callose synthase complex

David Ušák[1,2], Dominique Eeckhout[3], Tereza Korec Podmanická[1], Daniela Kocourková[1], Michal Daněk[1], MIchaela Neubergerová[1,2], Michael Kraus[3], Daniel van Damme[3], Roman Pleskot[1]

[1]Institute of Experimental Botany, CAS, Prague, Czech Republic
[2]Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic
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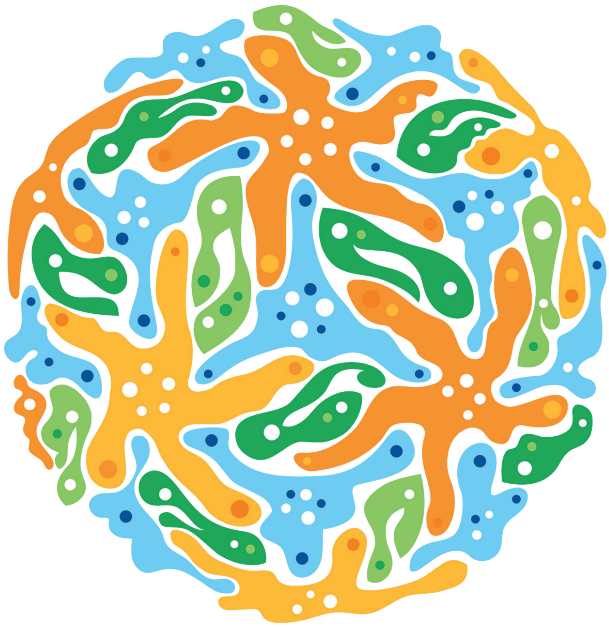
Final category: 5. COARSE GRAINING



5. Coarse graining

P 65 A coarse-grained model for disordered and multi-domain proteins

Fan Cao, Sören von Bülow, Giulio Tesei, Kresten Lindorff-Larsen
Structural Biology and NMR Laboratory & the Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Copenhagen, Denmark, Copenhagen, Denmark

Many proteins contain more than one folded domain, and such modular multi-domain proteins help expand the functional repertoire of proteins. Because of their larger size and often substantial dynamics, it may be difficult to characterize the conformational ensembles of multi-domain proteins by simulations. Here, we present a coarse-grained model for multi-domain proteins that is both fast and provides an accurate description of the global conformational properties in solution. We show that the accuracy of a one-bead-per-residue coarse-grained model depends on how the interaction sites in the folded domains are represented. Specifically, we find excessive domain-domain interactions if the interaction sites are located at the position of the C*α* atoms. We also show that if the interaction sites are located at the centre of mass of the residue, we obtain good agreement between simulations and experiments across a wide range of proteins. We then optimize our previously described CALVADOS model using this centre-of-mass representation, and validate the resulting model using independent data. Finally, we use our revised model to simulate phase separation of both disordered and multi-domain proteins. Our results provide a starting point for understanding interactions between folded and disordered regions in proteins, and how these regions affect the propensity of proteins to self-associate and undergo phase separation.

5. Coarse graining

P 66 In Silico Activation of Arrestin by Means of Small Molecules

Zeynep Cinviz[1], Özge Şensoy[1], Giulia Morra[2]
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[2]*SCITEC CNR, Milano, Italy*

The discovery that Arrestins, besides their roles as terminators of G protein-coupled receptor (GPCR)-mediated signaling, also activate certain signaling pathways has been a breakthrough, and promoted development of biased ligands that enable coupling of the receptor to a specific Arrestin subtype. Towards this end, either i) the orthosteric site or ii) allosteric regions like cytosolic or membrane-exposed regions of receptor are targeted, both of which has its own limitation. Hereby, we propose an alternative strategy, where we propose to stabilize a certain conformation of b-Arrestin, which is required for binding to a specific receptor, by means of small molecules. As our reference system, we chose Arrestin-biased ligand, carvedilol,-bound b_2-adrenergic receptor (b_2AR) in complex with b-Arr2 and aimed to stabilize conformation of Arrestin in that complex by small molecules. To enable observation of rearrangements associated with Arrestin activation, we performed coarse-grained simulations using Martini force-field, and optimized parameters accordingly. Since Arrestin shuttles between cytosol and the membrane, we performed simulations in both environments to examine possible conformations. We clustered conformations adapted by b-Arr2 and identified possible binding pockets, in comparison to b-Arr1 to increase the possibility of finding molecules specific for b-Arr2. We show that b-Arr2 transiently samples active-like conformations both in cytosol and at the membrane, whereas b-Arr1 requires interaction with the membrane. Interestingly, we demonstrate that b-Arr2 samples small domain-rotation angles without any need of C-tail detachment as long as the tail doesn`t interact with the lariat loop as opposed to what has been proposed in the field.

5. Coarse graining

P 67 Modelling mechanical properties of nucleic acid structural motifs

Eva Matoušková[1], Filip Lankaš[1]
[1]*Department of Informatics and Chemistry, University of Chemistry and Technology Prague, Czech Republic*

Structure and deformability of nucleic acids play a key role in their biological function and in nanostructure design. Within the range of small deformations, these features can be deduced from structural fluctuations observed in unrestrained, all-atom molecular dynamics (MD) simulations, using a suitable set of coarse-grained internal coordinates. This approach has long been used to examine mechanical properties of the DNA double helix [1], but its application to other NA structures has been limited. We have recently proposed an MD-based model to predict sequence-dependent shape and stiffness of DNA and RNA duplexes with high accuracy (Dohnalová et al., submitted). We also used extensive MD simulations to infer elastic properties of recurrent non-helical motifs, such as RNA C-loops [2], kink-turns [3], or the double-crossover (DX) motif [4], a key building block of NA nanostructures. Our approach can be extended to other non-helical motifs and paves the way for a comprehensive description of nucleic acid structural mechanics.

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5. Coarse graining

P 68 Untangling the Network Interactions Inside a Model of Transcriptional Condensate

William Morton, Marek Sebesta, Richard Stefl, Robert Vácha
CEITEC MUNI, Brno, Czech Republic

The interaction between RNA polymerase II (Pol II) and transcription factors (TFs) is essential for proper cellular function. TFs act as controllers and aids for Pol II's transcription of RNA. Many TFs can work in unison with Pol II, forming condensates where the interaction between disordered regions precisely controls the size and makeup of these molecular hubs[1.] Our understanding of species' spatial organization and concentration within the condensates is limited by the microscopic resolution of densely packed proteins[2.] Here we focus on RECQL5, a TF known to be present during the elongation phase of transcription when Pol II is hyperphosphorylated[3.] In vitro mixtures of proteins were recreated in silico, giving insight to their interaction network. The disordered region of RECQL5 is responsible for localization in a homotypic condensate. The uMol affinity between phosphorylated serine and RECQL5's SRI domain increases the valency of the heterotypic condensate and may be responsible for recruiting Pol II. This work is the first of its kind to compare simulations of multi-domain proteins to cryo-tomography images *in vitro*.
Funding: This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101180586, in association with funding from GAČR.

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5. Coarse graining

P 69 Development of a coarse-grained model of disordered RNA for protein–RNA phase separation

Ikki Yasuda[1,2], Sören Bülow[2], Giulio Tesei[2], Eiji Yamamoto[1], Kenji Yasuoka[1], Kresten Lindorff-Larsen[2]
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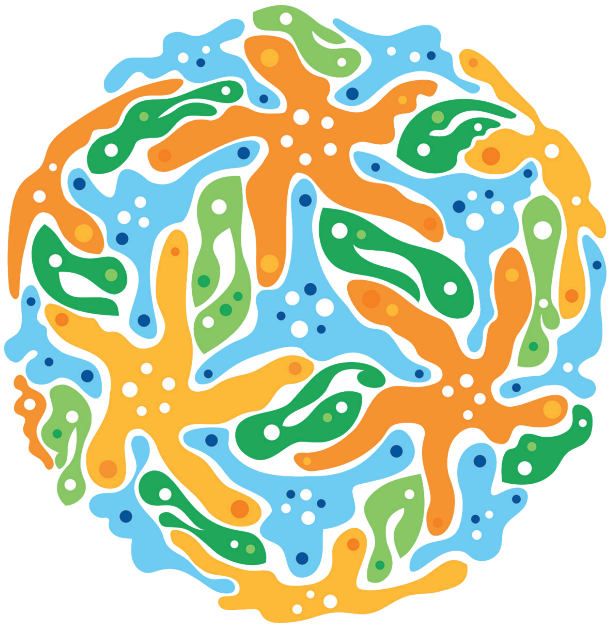
[2]University of Copenhagen, Copenhagen, Denmark

Protein-RNA condensates are involved in various cellular activities. Coarse-grained molecular models of intrinsically disordered proteins have been developed to predict single-chain properties and phase separation. RNA models compatible with these disordered protein models enable the study of complex biomolecular mixtures involving RNA. Here, we present a coarse-grained, two-bead-per-nucleotide RNA model based on a hydropathy scale. Our RNA model was tuned using a combination of bottom-up and top-down approaches to reproduce RNA geometry and intramolecular interactions based on atomistic simulations and in vitro experiments. The model describes relevant aspects of RNA-RNA interactions, as demonstrated by the agreement of calculated and experimental virial coefficients. We have used this model to simulate the reentrant phase behavior of protein-RNA mixtures, focusing on the RGG domain of FUS and polyU40. The phase behavior was quantified using phase diagrams to assess whether our model could predict experimentally observed phase separation. Additionally, we tested selective partitioning when another intrinsically disordered region, the prion-like domain of FUS, was added. Finally, we simulated the formation of mixed condensates consisting of MED1 IDR and RNA chains, and observed reentrant phase behavior. Furthermore, by adding intrinsically disordered regions from transcription factors, we found that selective partitioning into the transcriptional condensates was RNA-dependent. These results demonstrate that our RNA model can capture several important aspects of the phase behavior of protein-RNA condensates.

Final category: 6. AI & DRUG DESIGN APPLICATIONS



6. AI & Drug Design applications

P 70 A Data-Driven Approach to Enhancing Cell-Penetrating Peptide Uptake Predictions

Ana B. Caniceiro[1,2], António J. Preto[1], Francisco Duarte[1], Hugo Fernandes[3,4], Lino Ferreira[3,4], Joana Mourão[3], Irina S. Moreira[3,5]

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Cell-penetrating peptides (CPPs), which are short amino acid sequences capable of crossing cell membranes, are promising tools for delivering therapeutic molecules into cells[1.] However, *in vivo* and *in vitro* experiments of CPPs are often expensive and time-consuming, posing a significant challenge to optimising delivery efficiency and minimising toxicity. Machine learning (ML) approaches are emerging as powerful alternatives for the rapid design and prediction of CPP behaviour. Despite this potential, current databases primarily offer qualitative insights into CPPs, limiting the application of quantitative ML models[2.]

To address this, we developed the POSEIDON database, a freely accessible resource containing over 2,300 entries with quantitative absorption data and detailed physicochemical properties of 1,315 peptides. This rich dataset includes cell line information, cargo type, uptake methods, peptide sequences, and quantitative uptake values. By leveraging this dataset, we processed more than 1,200 entries to build an ML regression model capable of predicting CPP uptake. Our model achieved high performance, with a Pearson correlation of 0.87, Spearman correlation of 0.88, and r2 score of 0.76, on an independent test set, demonstrating its robustness in predicting peptide uptake across different cell lines.

The POSEIDON database, combined with a powerful ML predictor, represents a significant advance in CPP research and offers a promising avenue for the development of more efficient and targeted peptide-based delivery systems[3.]

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Acknowledgements:
This work was supported by the European Regional Development Fund through the COMPETE 2020–Operational Programme for Competitiveness and Internationalization and Portuguese National Funds via Fundação para a Ciência e a Tecnologia (FCT) [LA/P/0058/2020, UIDB/04539/2020, UIDP/04539/2020, and DSAIPA/DS/0118/2020, http://doi.org/10.54499/DSAIPA/DS/0118/2020). We acknowledge the Faculty of Sciences and Technology of the University of Coimbra and the Center for Neuroscience and Cell Biology. A.J.P. and A.B.C. were supported by the FCT through PhD scholarships SFRH/BD/144966/2019 and 2022.12479.BD, respectively. J.M. was supported by a Junior Postdoctoral Research Contract (2021.03416.CEECIND - Individual Call to FCT Scientific Employment Stimulus 2021) granted by the FCT/MCTES through national funding.

6. AI & Drug Design applications

P 71 Machine Learning Applied to Drug Discovery for the Treatment of Colorectal Cancer

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Universidad de los Andes, Bogotá, Colombia

Colon cancer is a significant health issue, largely due to the overexpression of mdm2, which prevents apoptosis in cancer cells.[1] The interaction between p53 and mdm2 proteins is crucial in this process. Despite experimental studies on some molecules, none have passed clinical trials to become effective drugs.[2] Therefore, it is essential to design new drugs to treat colon cancer by targeting this interaction. We present a machine-learning-based approach to discover and repurpose new molecules from known molecules with potential pharmacological activity to treat colon cancer. By incorporating the latest machine-learning scoring function (MLSF) advances, we generated and evaluated five MLSFs (SVM, RF, XGB, DNN, and CNN) to identify small molecules that inhibit the p53-mdm2 interaction. These algorithms were used because it was evidenced that generic MLSFs like Smina [3] have low performance in identifying MDM2-P53 inhibitors, with a precision equivalent to 5% and NEF1% of 0.3. Using MLSF, we expect to identify a machine learning model and its corresponding scoring function with good performance to identify new inhibitors of the MDM2-P53 interaction. This approach will allow the identification of small molecules with high potency to treat colon cancer.

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6. AI & Drug Design applications

P 72 Sampling-friendly MD Dimensionality Reduction with Autoencoders

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The machine learning technique of autoencoder (AE) can be applied to MD trajectories compressing the information on the molecule configuration to a low-dimensional latent space, the bottleneck of the AE. After an AE is trained on a specific MD trajectory, its latent space can be sampled and the molecule configurations recovered with feeding the samples to the decoder part. However, with plain AE the distribution of the latent space is completely random.
Variational Autoencoders, and Adversarial Autoencoders (AAE) with less technical difficulties, can impose an arbitrary probability distribution (prior) on the latent space. However, AAE itself does not relate the probability density of the latent space with the probability of occurrence of the corresponding structures in the MD trajectory. Therefore we assign a „density" value (how many similar structures are around, or, more technically, a reciprocal of the average RMSD of N closest neighbours) to each step of the trajectory. The AAE training is extended, besides following the prior distribution, also to reflect the density. For example, with a normal prior, folded and nearly folded protein configurations, which are typically the most frequent on a trajectory, are mapped close to the centre of the latent space, while unfolded and misfolded configurations are distributed in the farther areas.
The resulting latent space can be sampled according to its prior distribution, and the samples fed through the decoder part of AE to recover the structure. Due to the low dimensionality of the latent space, the decoder outputs are not always feasible structures. We correct them with force field energy optimization, which starts from the nearest structure in the original trajectory, and it restrains dihedrals to the values coming out from the decoder; we end up with a trade-off structure, still feasible with respect to the force field while favoring the decoder output as much as possible.
Altogether, with this approach we are able to use relatively short MD trajectories to generate much longer ones (or bigger ensembles) with realistic geometries while preserving their basic statistical (kinetic) properties.

6. AI & Drug Design applications

P 73 Analysis and Sampling of Molecular Simulations by Adversarial Autoencoders

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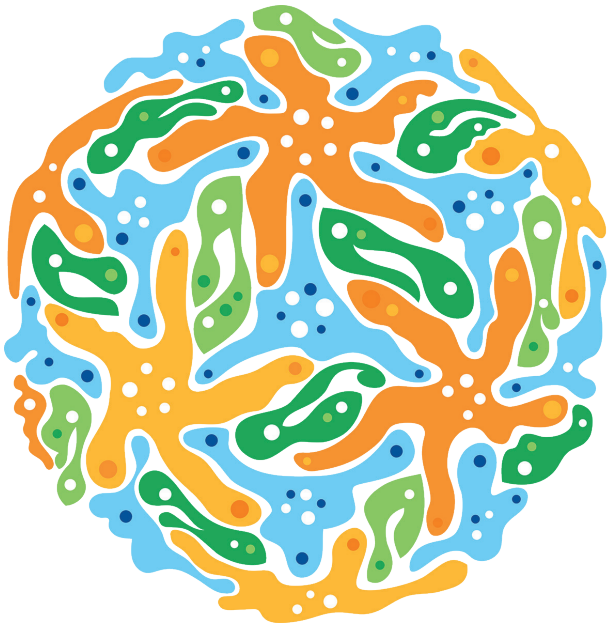
Molecular dynamics is a computational technique which describes how a given molecular system evolves over time. It facilitates the exploration of stability, conformational changes and a plenty of other properties. However, the application of molecular dynamics is affected by its large computational costs. The computation involves steps that must be in order of femtoseconds, to assure numerical stability and accurately describe molecular motions which typically occur in nature on a scale ranging from milliseconds to seconds. Among numerous techniques used to enhance simulations, metadynamics operates by encouraging the system to cross high free energy barriers and explore configurations that might otherwise be inaccessible or challenging to sample. However, to make metadynamics successful, the scientist must select the so-called Collective Variables. Designing good Collective Variables is not a trivial task and it relies on the knowledge of the system and experience of the scientist. Hereby we present an Adversarial Autoencoder as an unsupervised method that relies on the design of optimal Collective Variables. By encoding high non-linear dimensional data, collected from unbiased TrpCage molecular dynamics, into a two-dimensional latent space, the model demonstrates the capability and the efficiency of meaningfully interpreting the distribution of the training data. By considering the latent space as a combination of the most relevant system features, we derived optimal combinations of Collective Variables based on internal coordinates. These CVs were then used to enhance TrpCage molecular dynamics via metadynamics, enabling the observation of folding events on a significantly reduced timescale. The developed method proves effective in analyzing high-dimensional molecular dynamics simulation and in designing efficient CVs. We aim to further apply and validate this approach on more complex molecular systems.

Final category: 7. QM/MM



7. QM/MM

P 74 Substrate Positioning and Mechanism of Non-Heme Fe(II)/ α-Ketoglutarate-Dependent Hydroxylases and Epoxidases: A QM/MM Study

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Mono-nuclear non-heme oxygenase enzymes catalyze oxidative transformation reactions, such as hydroxylation, epoxidation, halogenation, ring expansion, desaturation, epimerization, and ring closure [1]. The reactive species is Fe(IV)-Oxo which is formed from Fe(II) by incorporation of 2-oxoglutarate (2OG) and their reactivity with O_2. The rate determining step is usually the C–H activation of the substrate. Here, we used QM/MM methods to get insights into the mechanism underlying the reactivity of different mono-nuclear non-heme iron enzymes and to probe the role of different residues in the active site. We examined the reactivity and the substrate positioning of alpha-ketoglutarate-dependent taurine dioxygenase (TauD), hyoscyamine 6β- hydoxylase (H6H) and the quaternary complex of AsqJ- Fe3+-2OG-cyclopeptin (AsqJ) enzymes with their natural substrate. For example, while TauD enzymes only perform the hydroxylation of taurine [2], H6H catalyzes a subsequent dehydrogenation step leading to the epoxidation of hyoscyamine [3] and AsqJ catalyzes desaturation step followed by the oxygen atom transfer that leading to epoxidation of Cyclopeptin [4]. Moreover, to validate and accurately predict the reaction energy barriers, we studied two DFT models, and two QM/MM models for each enzyme which include the first and second coordination sphere residues, resulting in a total of six models. According to the activation energy barriers, the selectivity of taurine at the C1-position, hyoscyamine 6β-hydoxylase at the C6-position, and Cyclopeptin at the C3-position are favorable hydroxylation positions for each substrate.

Keywords: non-heme iron, hydroxylation, epoxidation, QM/MM, molecular dynamic simulations, density funtional theory.

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7. QM/MM

P 75 Comparative study of MnmE GTPase catalysis mechanisms

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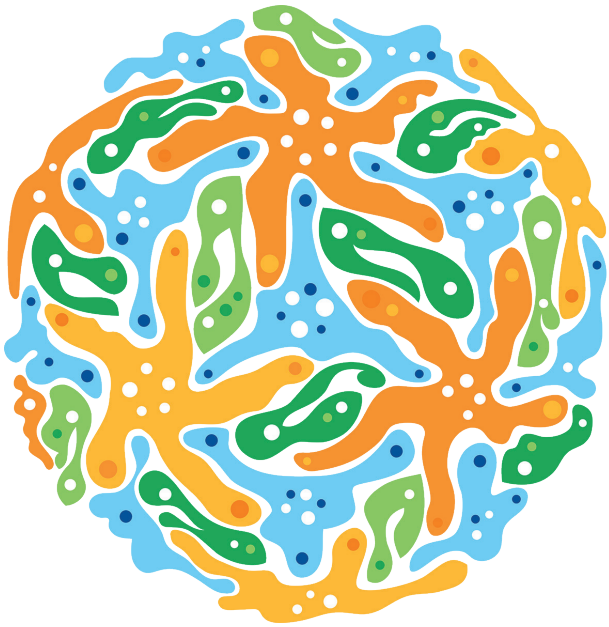
Regulation of NTPase activity is a complex, multi-step process. A crucial aspect for hydrolysis to occur is the necessity of an activating positive charge in the enzyme's active site alongside magnesium cation. In ancient enzymes, this charge was provided by sodium or potassium cations, while in more evolved enzymes, that role is taken by positively charged amino acids like arginine or lysine. Most research focuses on amino acids' roles in modern enzymes' hydrolysis mechanisms, with less attention to cations' roles.
The aim of the study was to investigate the role of cations in the hydrolysis mechanism of MnmE GTPase, an NTPase present in both prokaryotes and eukaryotes, which modifies tRNA and ensures high translational accuracy. The structure and function of MnmE GTPase have been evolutionarily conserved, and its enzymatic activity depends on potassium cations, marking it as an ancient NTPase.
Understanding the role of cations in hydrolysis requires atomic-level knowledge. No existing studies provide such insights into MnmE GTPase's hydrolysis mechanism. However, hypotheses and experiments suggest the involvement of Asp270 and Glu282 amino acid residues.
We used hybrid quantum mechanics/molecular mechanics (QM/MM) modeling to investigate two potential GTP hydrolysis mechanisms of MnmE GTPase, involving Asp270 and Glu282. Comparative analysis of both mechanisms allowed us to propose which one is carried out by the enzyme. Additionally, we examined the role of potassium cation in hydrolysis, revealing evolutionary changes related to the activating charge in the hydrolysis mechanism. The findings contribute to a deeper understanding of enzyme evolution and functionality.

Final category: 8. MOLECULAR SIMULATIONS AND APPLICATIONS



8. Molecular simulations and applications

P 76 Salicylic Acid Binding to Redox-Related Enzymes: Insights from Arabidopsis Catalase 2

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Salicylic acid (SA) is a key phytohormone involved in plant physiology, particularly in response to pathogen attacks. Following such attacks, SA accumulates and induces the expression of major PR (Pathogenesis-related) genes, which play a role in the plant immune response. This process is mainly controlled through the NPR1 (Non-expresser of Pathogenesis Related 1) pathway, a transcriptional regulator that requires changes in the intercellular redox balance to be activated[1].
For SA to act within the cell, it must bind to certain proteins, which then trigger cellular events that alter the redox balance. Catalase was the first Salicylic Acid Binding Protein (SABP) to be identified. Recently, other SABPs were discovered in *Arabidopsis thaliana*[2] through high-throughput screening, revealing that several SABPs are involved in primary metabolism and redox regulation[3].
This project aims to uncover the molecular details of SA binding to redox-related enzymes, focusing first on Arabidopsis thaliana catalase 2 (AtCAT2). Using C-I-TASSER, we modeled the AtCAT2 structure and ran simulations with the AMBER suite in the presence of water. Docking studies with AutoDock Vina identified several potential SA binding pockets, one of which aligns with the inhibition of catalase activity by SA. We are now studying this pocket in detail through *in silico* analyses, examining SA's Potential of Mean Force (PMF) along the binding channel[4] for both wild-type and mutant forms of the protein. Concurrently, we are producing wild-type and mutant proteins in vitro to experimentally test their SA binding and inhibitory effects.

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8. Molecular simulations and applications

P 77 Insertases scramble lipids: Molecular simulations of MTCH2

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Scramblases play a vital role in facilitating the bidirectional translocation of lipids across cellular membranes, a process that is critical for maintaining lipid metabolism, membrane integrity, and cell signaling. In this study, we focus on the mitochondrial outer membrane protein MTCH2, which is recognized for its function as a protein insertase. MTCH2 features a hydrophilic groove reminiscent of those seen in established lipid scramblases, leading us to hypothesize that it may also function as a scramblase.
To investigate this, we employed a combination of coarse-grained and atomistic molecular dynamics simulations with free energy calculations to analyze MTCH2's ability to facilitate lipid movement across the membrane. Our simulations reveal that MTCH2 significantly lowers the free energy barrier for lipid flip-flop along its groove, indicating that it indeed functions as a scramblase. Notably, the lipid scrambling activity of MTCH2 observed in our coarse-grained simulations is comparable to that of the voltage-dependent anion channel (VDAC), a known scramblase in the outer mitochondrial membrane. This similarity suggests that MTCH2 and VDAC might have complementary roles in lipid transport within mitochondria.
Moreover, our results imply that other protein insertases with similar hydrophilic pathways could also exhibit lipid scrambling activity. Our results suggest that insertases and scramblases may share a common functional mechanism. This dual functionality could be crucial for mitochondrial membrane dynamics, including processes such as membrane biogenesis and the synthesis of specialized lipids like cardiolipin. Our findings not only expand the functional repertoire of MTCH2 but also offer new insights into the potential overlap between protein insertion and lipid scrambling mechanisms in mitochondrial biology.

8. Molecular simulations and applications

P 78 Mechanistic insights into P-glycoprotein ligand transport and inhibition revealed by enhanced molecular dynamics simulations

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P-glycoprotein (P-gp) is integral to cellular detoxification and drug efflux, transitioning between inward-facing (IF) open, occluded, and outward-facing (OF) states to facilitate substrate transport. P-gp's role is particularly crucial in cancer therapy, as it contributes to the multidrug resistance (MDR) phenotype. In our study, we employed classical and advanced MD simulations, including kinetically excited targeted MD (ketMD) and adiabatic biasing MD (ABMD), to dissect the structural and functional features of P-gp's conformational states.
Our advanced MD simulations revealed that the unkinking of transmembrane helices TM4 and TM10 is essential for achieving the OF conformation. Simulations of the IF-occluded conformations, characterized by kinked TM4 and TM10 helices, consistently demonstrated altered communication between the transmembrane domains (TMDs) and nucleotide-binding domain 2 (NBD2). This alteration suggests that the TMD-NBD2 interface plays a critical role in inhibiting P-gp's efflux function.
Additionally, we focused on the unstructured linker segment connecting NBD1 to TMD2 and its impact on the transporter's dynamics. With the linker present, we observed entry for cholesterol (CHOL) through the TM4-TM6 portal, identifying key residues involved in CHOL accommodation. This finding suggests a mechanism for ligand entry that parallels cholesterol's characteristics.
Our results provide significant insights into P-gp functioning and propose a novel mechanism for ligand entry, offering potential targets for developing new P-gp inhibitors. These findings contribute to the advancement of strategies against MDR in cancer therapy.

8. Molecular simulations and applications

P 79 Structural and Functional Insights of a Novel Bacterial Copper Radical Oxidase

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Copper radical oxidases (CROs) are a diverse group of enzymes that catalyze the chemo-selective oxidation of alcohols into aldehydes. They have a copper ion in the catalytic center that combined with an adjacent radical formed between a Cys and Tyr couple the oxidation of substrates with the reduction of molecular oxygen to hydrogen peroxide. CROs are classified under the auxiliary activity family 5 (AA5) in the Carbohydrate active enzymes (CaZy) database, and are divided in two subfamilies, AA5_1 and AA5_2 that share a common catalytic domain and mechanism. While the AA5_1 subfamily efficiently oxidizes aldehydes like glyoxal, methylglyoxal, and glycolaldehyde into carboxylic acids, the AA5_2 subfamily exhibits a wide substrate scope, including aromatic, aliphatic, and furan-based alcohols. The latter encompasses enzymes such as galactose oxidases, raffinose oxidases, alcohol oxidases, 5-hydroxymethyl furfural oxidases, and aryl alcohol oxidases (1). Despite their interesting properties, this subfamily remains structurally underexplored, with only three members having their structures determined. These include the archetypal galactose oxidase from *Fusarium graminearum* (FgrGalOx, PDB 1GOG), an alcohol oxidase (*Cgr*AlcOx, PDB 5C92) and an aryl alcohol oxidase (*Cgr*AAO, PDB 6RYV), both from *Colletotrichum graminicola*. This study focuses on advancing the biochemical and structural understanding of a bacterial CRO belonging to the AA5_2 subfamily. The presence of two carbohydrate binding modules (CBM) contrasts with other known AA5_2 enzymes. Through biochemical, structural and *in silico* analyses, we investigated the functional characteristics of this new enzyme. The analysis of x-ray crystal structures of CBM-truncated forms of the enzyme, free and in complex with *g*alactose, combined with molecular dynamics and molecular dockings, revealed structural factors that define substrate specificity within the CROs superfamily. Our findings contribute to a deeper understanding of CROs catalytic mechanisms and their potential applications.

8. Molecular simulations and applications

P 80 Rest assured: programmed translational stalling studied by means of MDsimulations

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One way gene regulation occurs in bacteria is through programmed translational stalling. Specific arrest peptides have evolved to pause the ribosome under certain conditions while being translated. The SecM arrest peptide, e.g., up-regulates secretion of proteins through the cell membrane by inducing stalling. A mechanical pulling force acting on the N-terminus terminates stalling and enables translation to continue.
Recently, novel arrest peptides like ApdP have been discovered which share with SecM a conserved Arg-Ala-Pro-Pro motif. Cryo-EM structures of the stalled E. coli ribosome containing the peptide-Arg-Ala-ProtRNA in the ribosomal P site and a Pro-tRNA in the A site, suggest that peptide bond formation is affected during translation of ApdP and SecM. In all current models of peptide bond formation, the nucleophilic attack of the aminoacyl-tRNA α-amino group to the carbonyl-carbon of the peptidyl-tRNA is facilitated by the extraction of a proton from the attacking α-amino group. Using molecular dynamics (MD) simulations of the E. Coli ribosome in complex with wt ApdP and non-stalling variants, we determined the protonation state of the A-site Pro and found that specific hydrogen bonds between the Arg-Ala-Pro peptide and the A-site Pro prevent the efficient adoption of conformations that allow the proton extraction as well as the subsequent nucleophilic attack required for peptide bond formation. Additionally, we investigated, via unbiased and pulling simulations, how pulling of the N-terminus of SecM relieves stalling, identifying the sequence of events that leads to the disruption of the stalling conformation of the Arg-Ala-Pro-Pro motif.

8. Molecular simulations and applications

P 81 Molecular dynamics-driven selection of vectors for targeted delivery of doxorubicin

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Side effects of chemotherapeutics may be alleviated by employing active targeting drug delivery systems (DDSs). Those based on recognition of extracellular receptors overexpressed on the surface of cancer cells are quite promising [1]. In the current work, promising vector molecules for targeted delivery of the chemotherapeutic doxorubicin are put forward after multi-step atomistic molecular dynamics computations. They are selected among a set of folic acid-based ligands. The simulated models scale up from a single ligand in saline, through the free vectors in the presence of the target folate receptor-a, embedded in a salinized lipid bilayer mimicking a neoplastic cell membrane [2], to an entire DDS consisting of a vector ligand, covalently bound to a drug-binding peptide with four adsorbed molecules of doxorubicin.
The simulations show that five out of the six tested vector ligands are able to bind to the target receptor in unbound state. Four of them also guide the cargo to the receptor but raltitrexed and folate are highlighted as the most prospective vectors. The protein-ligand coupling is a result of subtle balance between electrostatic and van der Waals attraction and hydrogen bonding. The molecular modelling predicts highly specific behaviour of the targeting ligands, strongly influenced by the immediate surrounding of the receptor. Overall, the proposed targeted DDSs have potential for application after further optimization of the cargo.

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8. Molecular simulations and applications

P 82 Crucial role of the correlation between glycan conformations and ring distortion in the catalytic reaction of CAZymes

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Structural biology has traditionally focused on protein folding, complex formation, and ligand binding. However, the structural elucidation of glycans, which are frequent post- translational modifications on protein surfaces, remains largely unexplored. While still little is known about their function, even less is understood about their structural behavior, especially because state-of-the-art experiments can hardly resolve glycan flexibility. This is e.g. to the disadvantage of understanding the synthesis of glycans in the cytoplasm, involving several carbohydrate-active enzymes (CAZymes) whose malfunction is linked to cancer and disease progression.
Therefore, we employ molecular dynamics simulations to investigate the correlation between adopted glycan conformations in CAZyme binding sites and the reduction of kinetic barriers for glycan hydrolysis. Our focus is on alpha Golgi-mannosidase 2, a promising but underexplored drug target. Massive enhanced sampling molecular dynamics simulations of the enzyme/glycan complex reveal:

[1.] a direct correlation between torsion angle settings and monosaccharide ring distortion, often necessary for making hydrolytic reactions energetically feasible.
[2.] a disruption of this correlation induced by key mutations in the catalytic site, leading to experimentally observed loss of CAZyme function.
[3.] the ring distortion to be induced by binding of the glycan to the catalytic site of the protein and not adopted prior to substrate binding.

8. Molecular simulations and applications

P 83 Collaborative Rotamer Network Guides Mutation-Driven Multi-State Switch in EGFR Kinase Domain

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Epidermal growth factor receptor (EGFR) is a multi-state protein receptor kinase that is commonly mutated in various types of cancer. Our analysis of crystal structures and molecular dynamics simulations shows that multiple mutations in the kinase domain can trigger backbone movements in the C-helix and DFG domain causing the adoption of drug resistant multi-states. Backbone movements appear to be transmitted from mutation sites by linked side-chain movements involving a network of protein surface rotamers. Future work will investigate potential allosteric hotspots in this network that might be used for therapeutic solutions to drug resistance.

8. Molecular simulations and applications

P 84 Self-assembly of sucrose esters studied by molecular dynamics simulations

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Currently, there is a demand to replace the alkyl sulfate type of surfactants in different formulations by more sustainable 'green' substances like sucrose esters. To keep the properties of the final formulations stabilized by the new surfactants, it is necessary to understand in detail the behavior of the latter in aqueous solution with respect to phases they form. Thereby, molecular dynamics (MD) simulations may reveal the origin of the experimentally observed phenomena at atomistic level.
In the current work, we model by all-atom classical MD the phase behavior of sucrose esters with different length of the alkyl chain. The simulations are performed at experimental conditions. The self-assembly of the surfactants is monitored in the course of 10^2 ns.
Initial formation of spherical micelles is observed, driven by hydrophobic interactions between the alkyl tails. The micelles assemble subsequently into long threads stabilized by hydrogen bonds between sucrose heads located in the outer shell of the primary micelles. The process is faster for longer chains and at higher temperature and concentration. Interestingly, the sucrose esters threads seem to be strings of aligned spherical micelles. This contrasts the conduct of conventional surfactants which form homogeneous rod-like assemblies as a result of rearrangement of the molecules from the initial spherical micelles.

Acknowledgments
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8. Molecular simulations and applications

P 85 Aggregation, binding and phase separation of disordered proteins from short model peptides

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In the study of the conformational dynamics of biomolecules, short peptides have been instrumental as model systems for more complex processes like protein folding. By studying short peptides we have gained an understanding of fundamental folding events like alpha helix nucleation or hairpin folding and in the determination of the microscopic origin of internal friction. Short peptides are also challenging tests for the calibration of modern force fields, and hence have been extensively used in recent optimization efforts. In my talk I will present recent work from our laboratory where we use short peptides to understand different aspects of the behaviour of intrinsically disordered proteins, including their binding to folded partners [1], aggregation [2,3] and phase separation [4].

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8. Molecular simulations and applications

P 86 Exploration of intermolecular interactions between folate-based vectors and the folate receptor-*α*

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Conventional chemotherapy has been widely applied in combating cancer over the past few decades. Unfortunately, this treatment exhibits many drawbacks such as limited bio-availability of the chemotherapeutic, leading to high dose requirements and partiality to drug resistance, non-specific targeting and adverse side effects [1], stemming from its cytotoxic nature. The desire to improve therapeutic targeting on malignant cells and suppress unwanted side effects leads to the development of systems that possess the ability to deliver specific chemotherapeutics to tumor sites. The design of drug delivery systems (DDSs), implemented within an active targeting method, relies on binding the chemotherapeutic agent to a vector-molecule that steers its delivery and release to specific types of cells by recognizing their cellular components [2]. Vector-ligands, such as folic acid (FA), 5-methyltetrahydrofolate (MTHF), methotrexate (MTX), pteroyl ornithine (PON), pemetrexed (PTX) and raltitrexed (RTX) are potential small molecules that possess high affinity to bind to folate receptor-*α* (FR*α*), the latter being overexpressed on many types of tumor cells.
Our current work aims to investigate and decipher computationally the dominant intermolecular interactions involved in the binding of these biomolecular ligand-receptor complexes. For quantitative assessment of the binding, the key supramolecular interactions within the complexes are determined for representative structures extracted from molecular dynamics simulations. Utilizing the MM-PBSA method, we evaluate the relative stabilities of the ligand-receptor associates and assess the various contributions to the total binding free energy. The outcome of our study reveals that, across all studied structures, electrostatic interactions have the predominant share of the interaction energy between ligands and FR*α*. However, variations in the polar energy contribution of each ligand complexed with FR*α* are responsible for the specificity of the binding interaction of the vector-ligands. The dielectric constant of the surrounding medium exerts minor influence on the ligand-FR*α* coupling.
Acquiring the energy-based characterization and molecular-level description of the binding mechanism of folate and its derivatives to FR*α*, combined with molecular dynamics modelling [2], could enable better control over the essential attributes needed for successful attachment of the vectors to the receptor active site and outline prominent vector-molecules for inclusion into FR*α*-focused DDS for active targeting.

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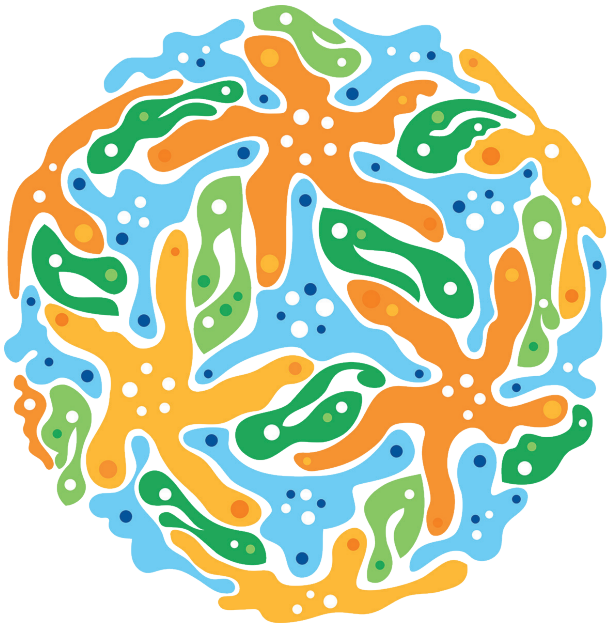
Final category: 9. OTHER



9. Other

P 87 The shape of the endocytic TPLATE complex drives vesicle formation

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Clathrin-mediated endocytosis (CME) is the primary route by which plant cells internalize molecular cargoes from the plasma membrane and outside of the cell. During CME, adaptor proteins recognize molecular cargoes and facilitate their packaging into clathrin-coated vesicles. The process of vesicle formation involves the invagination of a flat membrane patch, which is subsequently pinched off and released into the cytoplasm. While actin plays a critical role in membrane deformation in animals and yeasts, it seems only to be required to transport endocytic vesicles after vesicle scission in plants [1]. The TPLATE complex is an evolutionarily ancient protein complex lost in animals and yeasts. The TPLATE acts as an adaptor protein complex [2, 3], which was also proposed to contribute to the membrane deformation during vesicle formation [4]. However, mechanistic understanding of how the TPLATE complex drives the membrane deformation is, however, lacking.
Here, we used an integrative structural approach to determine the molecular architecture of the TPLATE complex. Our model allowed us to visualize the key structural features of the TPLATE complex that further hallmarked its roles in clathrin-mediated endocytosis (CME). Using coarse-grained molecular dynamic simulations, we identified lipid-binding preferences associated with specific domains at the membrane-binding interface. The simulations also revealed that the crescent shape of the complex generates the membrane curvature. To further test whether the TPLATE complex-generated membrane curvature can lead to membrane tubulation, we performed extensive coarse-grained MD simulations with an asymmetric membrane model. These simulations demonstrated that the combined action of multiple TPLATE complexes induces membrane budding, while a single complex is insufficient for this process. Supported by wet lab experiments, these findings address the longstanding question of how plants can carry out endocytosis without actin-based force generation.

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9. Other

P 88 Beyond Metadata: Leveraging Similarity Search in Molecular Dynamics Data

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The exponential growth of molecular dynamics (MD) simulation data, now being standardized and shared in community-established data repositories, presents a challenge. While comprehensive metadata annotations enhance data discovery, reuse, and provide context, they are insufficient for identifying and comparing complex MD trajectories. The metadata model offers essential information about the simulation, such as system composition, simulation parameters (e.g., pressure, temperature, force field), and biomolecule identification. Although these details are valuable for categorizing and filtering datasets, they lack the granularity needed to capture the dynamic, structural, and kinetic features of molecular trajectories. As MD data continues to rise, advanced methods are needed to search, compare, and analyze these datasets beyond metadata-based approaches.

Similarity search offers a solution to address metadata limitations by enabling comparisons of molecular dynamics trajectories based on actual data rather than annotations. By identifying patterns, stable states, and characteristic motions within simulation data, similarity search can describe relationships across diverse biomolecular systems. This approach can reveal underlying mechanisms, functional similarities, or structural motifs not evident from metadata alone.

To facilitate similarity search in MD trajectories, the data must be transformed into compact, fixed-length vectors (embeddings). This transformation preserves essential information in the data, such as trajectory features, enabling efficient comparisons across biomolecular systems. Such embeddings allow the identification of common conformational states, transition pathways, and kinetic basins, supporting analyses like clustering dynamic behaviors, detection of functional motifs, and elucidating mutation impacts. These capabilities enhance applications in biomolecular function prediction, pathway analysis, and drug discovery, providing the MD community with tools for data-driven exploration.

One example of a purely data-driven search in biomolecular data is AlphaFind [1,2], which employs a learned index with compact embeddings for similarity searching within protein structures. This application demonstrates the feasibility of deep learning in biomolecular comparisons. Inspired by AlphaFind's success, we aim to explore a similar methodology with MD trajectories, where finding suitable embeddings will be crucial for capturing dynamic conformational changes. This involves developing embeddings that effectively represent the temporal and spatial features of MD trajectories, enabling efficient similarity search and deeper insights into molecular mechanisms.

[1] https://academic.oup.com/nar/article/52/W1/W182/7673488
[2] https://alphafind.fi.muni.cz/

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