Symposium: Advances in Computer Simulations in Structural Biology and Biophysics
&
Workshop: Data Science and Molecular Dynamic Simulation for Drug Discovery

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Abstract Book

Govardhan Reddy

Anionic Ligand Sensing by a Bacterial Riboswitch

Riboswitches sense various ions in bacteria and activate gene expression to synthesize proteins that help maintain ion homeostasis. The crystal structure of the aptamer domain (AD) of the fluoride riboswitch shows that F- ion is encapsulated by three Mg2+ ions bound to the ligand-binding domain (LBD) located at the core of AD. The assembly mechanism of this intricate structure is unknown. To this end, we performed computer simulations using coarse-grained and all-atom RNA models to bridge multiple timescales involved in riboswitch folding and ion binding. We show that F- encapsulation by the Mg2+ ions bound to the riboswitch involves multiple sequential steps. Broadly, two Mg2+ ions initially interact with the phosphate groups of the LBD using water-mediated outer shell coordination and transition to a direct inner shell interaction through dehydration to strengthen their interaction with the LBD. We propose that the efficient binding mode of the third Mg2+ and F- is that they form a water-mediated ion pair and bind to the LBD simultaneously to minimize the electrostatic repulsion between the three Mg2+ bound to the LBD. The tertiary stacking interactions among the LBD nucleobases alone are insufficient to stabilize the alignment of the phosphate groups to facilitate Mg2+ binding. We show that the stability of the whole assembly is an intricate balance of the interactions between the five phosphate groups, three Mg2+, and the encapsulated F- ion aided by the Mg2+ solvated water.

Nisanth N. Nair

Exploring Rugged Energy Landscapes of Enzymatic Reactions

Exploration and computation of free energy surfaces of chemical reactions are pivotal to computationally predicting reaction mechanisms, pathways, and kinetics. Computing free energy surfaces of complex reactions, especially in soft matter systems, requires advanced molecular dynamics (MD) simulation techniques. Although tremendous progress has been made in recent years in this direction, several limitations are yet to be addressed to make the computations efficient and more accurate. Over the past few years, my group has proposed several new methods to address the outstanding challenges. In my talk, I will present some of our recent contributions in this area. In particular, I will demonstrate how the new methods and codes we developed can aid in developing new covalent inhibitors for beta-lactamase enzymes to compact antibiotic resistance.

Durba Sengupta

Benchmarking a dual-scale hybrid simulation framework for small globular proteins combining the CHARMM36 and Martini2/Martini3 models

Multi-scale models in which varying resolutions are considered in a single molecular dynamics simulation setup are gaining importance in integrative modeling. However, combining atomistic and coarse-grain resolutions, especially for coarse-grain force fields derived from top-down approaches, have not been well explored. In this talk, we will discuss our implementation and validation of a dual-resolution simulation approach to model globular proteins in atomistic detail (represented by the CHARMM36 model) with the surrounding solvent in Martini2/Martini3 coarse-grain detail. The hybrid scheme considered is an extension of a model implemented earlier for mainly lipid and water molecules. We have considered a set of small globular proteins and have extensively compared to atomistic benchmark simulations as well as a host of experimental observables. We show that the protein structural dynamics sampled in the hybrid scheme is robust, and the intra-protein contact maps are reproduced, despite increased fluctuations of the loop regions. A good match is observed with experimental small angle X-ray scattering (SAXS) and NMR observables, such as chemical shifts and 3J-coupling, with the best match obtained for the chemical shifts. However, deviations are observed in the water dynamics and protein-water interactions which we attribute to the limitation of solvent screening in the coarse-grain force field. The computational speed-up achieved is about 2-3 times compared to an all-atom system. Overall, the hybrid model is able to retain the main features of the underlying atomistic conformational landscape with a two-fold speed-up in computational cost.

Shubhra Ghosh Dastidar

Allosteric Changes in Proteins: Structure, Dynamics and Thermodynamics

The phenomena of allosteric transitions between the inactive and active conformational states of proteins are of high importance, not just because such mechanisms dictate macromolecule's functions, but also because their characterizations open up unique opportunities to design drugs with higher precisions. The allosteric changes could also be induced by administering small molecules to activate a macromolecule to function when required or to inactivate it, as desired by the molecular therapeutic strategists. While *in-vitro* experiments can sense and register the existence of allosteric transitions in a molecular system, but the allostery being a dynamics process, the actual mechanisms are non-trivial to be visualized just from the static models of molecular structures. We have been working in this field of such induced conformational transitions using all-atom molecular modelling and dynamics simulations to characterize the dynamic details of such mechanism in several model systems of systems, e.g. α,β-Tubulin [1-4], Bcl2 [5], kinases [6-7], all of which are of tremendous importance in the field of drug design to combat cancer. Not only the structural and thermodynamic insights which will be shown in this presentation, but also our latest initiatives to make use of the machine learning [7] methods to complement the molecular dynamics simulations will be briefly discussed.

Keywords: Allostery, Kinase, Tubulin, Molecular Dynamics, Machine Learning

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Rajesh Mishra

Osmolyte assisted refolding of bacterial α-amylases

α-amylases are industrially important industrial enzymes which are used in different industries. Herein, α-amylases from *Bacillus amyloliquifaciens* (BAA) and *Bacillus licheniformis (*BLA), representing mesophilic and thermophilic like proteins respectively, have been refolded after

chemical denaturation. We have used a series of polyols, sugars, amino acids and the derivatives on the refolding of these enzymes from chemically denatured state. The effect of polyols with varying number of -OH groups from 2 to 6 (Ethylene glycol, glycerol, erythritol, xylitol and sorbitol) and sugars (trehalose and sucrose) has been studied on the refolding of BLA and BAA. Our study demonstrates that glycerol, sorbitol and trehalose are the efficient osmolyte for BAA refolding, while comparatively less effective for BLA. Among amino acids and glycine derivatives, betaine is the most promising osmolyte, while arginine and glycine exhibit moderately positive effect at their lower concentrations on the refolding of BAA only. Aggregation kinetics monitored by static light scattering indicates suppression of aggregation by amino acids and derivatives. Our study highlights the differential effect of protein-osmolyte interactions during refolding of thermophilic and mesophilic proteins which may have implications in protein formulations, refolding and inhibition of aggregation.

Hari O. S. Yadav

Characterizing the Hydrophobicity of Nanoscale Surfaces

Hydrophobicity controls many biological processes such as protein folding, protein-ligand binding, enzymatic reactions, and membrane formation. Similarly, it has potential applications in self-cleaning and antifouling industries. Although this effect is well recognized and often visible to the naked eye in nature, it is still poorly understood. Intuitively, hydrophobicity is thought of as the tendency of a surface to “repel” water, but it is much more than that. Experimentally, this effect is characterized with contact angle measurement of water droplets on the surfaces, but the approach is limited to the flat surfaces. Here, we present a method to characterize the hydrophobicity of nanoscale surfaces based on quantifying the density fluctuations of water near the surface–water interface. The method is highly efficient molecular simulation technique and applicable to both flat and non-flat complex surfaces. The robustness of the approach is demonstrated by evaluating the surface hydrophobicity of several self-assembled monolayers of widely varying surface chemistries. It is shown that not only surface chemistry and topology, but also chain flexibility can play an important role in controlling the hydrophobicity of surfaces.

Sudhanshu Shanker

Advancing Peptide Docking: Incorporating Non-Natural Amino Acids with AutoDock CrankPep v1.1

Peptide-based drug design heavily relies on non-standard amino acids to optimize binding affinity, stability, and selectivity. However, incorporating these modified residues into molecular docking workflows presents significant challenges. In this talk, I will discuss AutoDock

CrankPep v1.1, an advanced tool for flexible peptide docking that now supports non-natural amino acids. I will cover the development of ADCP, the methodology for incorporating non-standard amino acids into docking studies, the availability of molecular mechanics parameters for further analysis of docked complexes, and the support for custom-designed non-natural amino acids in docking workflows. These new features expand the scope of peptide docking by enabling the inclusion of a wider range of bioactive non-standard amino acids, facilitating the rational design of next-generation peptide therapeutics.

Ananya Debnath

Plant Thylakoid Polymorphism and Dynamical Heterogeneity

Chlorophyll (CLA) binding light harvesting complex II (LHCII) and thylakoid membranes are essential for photosynthesis in plants. To investigate how CLA and LHCII influence the structure and dynamics of thylakoid membranes, coarse-grained molecular dynamics (CG MD) simulations using MARTINI-2 force fields are conducted at 293 K, varying the CLA and lipid concentrations. The simulations reveal that above a critical CLA concentration, the thylakoid membranes undergo a transition from a lamellar to a non-lamellar phase and LHCII resist the polymorphism. CLA molecules tend to aggregate into structures of varying orders and attract nonbilayer forming lipids with fewer unsaturated bonds, leading to a non-lamellar phase characterized by fused membrane regions. These fused areas cause structural immobilization of CLA and lipids and introduce dynamical heterogeneity, as evidenced by non-Gaussian parameters and van Hove correlation functions. This transition from lamellar to non-lamellar phases is associated with a significant decrease in the correlation length of immobile CLA and lipids due to the fused membrane topology. Understanding these CLA-induced structural transitions can be extended and applied to LHCII induced thylakoid phase transitions for studying nonphotochemical quenching mechanisms and offers potential for developing artificial photosynthetic materials and applications in photodynamic therapy.

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Ragothaman M. Yennamalli

Deciphering Thermostability and Structural Dynamics in Carbohydrate Active Enzymes

Understanding the structural and dynamic determinants of enzyme function is fundamental for enzymes that function under extreme conditions. Using an integrative approach combining sequence-structure analysis, molecular dynamics simulations, and machine learning, we explore the biophysical principles governing thermostability and substrate specificity in two carbohydrate-active enzyme families: Endoglucanases and Lytic Polysaccharide Monooxygenases (LPMOs). Our comparative analysis of thermophilic and mesophilic endoglucanases reveals that thermostability arises from fold-dependent amino acid compositions, altered intramolecular interactions, and global dynamic behavior. Also, a single mutation in *Trichoderma reesei* endoglucanase induced thermostability through allosteric rigidity, demonstrating that long-range interactions can significantly impact enzyme stability. Structural dynamics studies on endoglucanases using elastic network models further highlight distinct slow-mode motions that influence catalytic residues. In LPMOs, coarse-grained and all-atom molecular simulations reveal family-specific flexibility in substrate-binding regions, with dynamic loop regions playing a critical role in function. Frustration analysis indicates that local energetic variations modulate enzymatic activity, particularly in substrate recognition and binding. Additionally, machine learning-based classification of LPMOs using sequence and structural features improved sequence-based functional annotation, outperforming traditional methods. By looking at sequence and structural dynamics, we highlight basic biophysical understanding of enzyme stability, flexibility, and function. We demonstrate how computational methods can uncover fundamental biophysical principles governing protein behavior of endoglucanases and LPMOs.

Shailja Singh

Target based repurposing of anti-diarrheal drug as irresistible antimalarial

Malaria elimination faces challenges from drug resistance, stemming from mutations within the parasite's genetic makeup. Genetic adaptations in key erythrocyte proteins offer malaria protection in endemic regions. Emulating nature's approach, and implementing methodologies to render indispensable host proteins inactive, holds the potential to reshape antimalarial therapy. This study

delves into the functional implication of the single-span membrane protein Kell ectodomain, which shares consensus sequence with the zinc endopeptidase family, possesses extracellular enzyme activity crucial for parasite invasion into host erythrocytes. Through generating Kell-null erythrocytes from an erythroid progenitor, BEL-A, we demonstrate the indispensable nature of Kell activity in *P. falciparum* invasion. Additionally, thiorphan, a metallo-endopeptidase inhibitor, which specifically inhibits Kell activity, inhibited Plasmodium infection at nanomolar concentrations. Interestingly, individuals in malaria-endemic regions exhibit low Kell expression and activity, indicating a plausible Plasmodium-induced evolutionary pressure. Both thiorphan and its prodrug racecadotril, demonstrated potent antimalarial activity *in vivo*, highlighting Kell's protease role in invasion and proposing thiorphan as a promising host-oriented antimalarial therapeutic.

Sanjib Senapati

Room temperature DNA storage to enhanced Drug bioavailability: Magic molecules Ionic Liquids in action

DNA stability is a prerequisite in many of its applications, ranging from DNA-based vaccine, data storage to gene therapy. However, the existing strategies to enhance the DNA stability are ineffective and limited in scope. Ionic liquids (ILs), molten salts of organic cations and organic/inorganic anions, are showing tremendous prospects in myriads of applications. In this talk, I will show that ILs could be ideal media for long-term preservation of genomic and short-strand DNA at room conditions. Our experimental measurements show that the ILs enhance DNA melting temperature significantly, while unaltering its functional B-conformation. Molecular dynamics simulations and quantum mechanical calculation results suggest that the intramolecular Watson-Crick H-bonding in DNA remains unaffected and, in addition, the ILs induce stronger H-bonding network in solution. In the 2nd half of the talk, I will show that ILs conjugated with representative APIs (API-ILs) enhanced the solubility of the parent APIs by 10^2-10^3 folds without compromising the cell permeability. Measured dissolution rate of the API-ILs indicate the much quicker availability of the APIs in solution compared to the parent APIs. Our finding signifies the rapid onset of action of our IL-based formulations and their requirements in significantly lower volumes that can be delivered in liquid form. Lastly, a glimpse of the use of the ILs in producing soft and adhesive hydrogels useful for surface coating and wound healing will be presented.

Ajeet Kumar Yadav

Exploring Hydration Thermodynamics through Analytical Models

Water has anomalous behaviour both in its pure state volumetric and thermodynamic properties as well as solvation properties. Therefore, understanding solvation in water is vital for a range of biological processes, including protein folding, enzyme activity, drugbinding interactions, membrane fusion, and cellular signaling. It also plays a crucial role in water purification, global geochemical cycles, and the Earth's hydrological systems. The most used technique to estimate the hydration properties, explicit simulations, is very powerful but is computationally expensive. To boost the computation speed, we have developed an analytical model for (quasi) spherical hydrophobic solutes. The model is based on the cage-water model for water, where water can take any of the three predefined states (hydrogen bonded, Lennard-Jones, and Open states) and their partition function is obtained analytically. In the solvation modeling, the insertion of solute affects neighboring water in two steps: (a) loss of hydrogen bonds (is calutaded using a geometric approach) and (b) energetic approach for sulute-water interaction (modelled based on surface heat capacity). We have got good quantitative agreement with experimantal data for hydration free energy, enthalpy, entropy for a series of inert gases and quasi-spherical solutes. Further, we employed our method for a set of 643 small organic molecules' hydration free energy data and our results are within the experimental uncertainties.

Sangeeta

Unravelling the Role of Local DNA Deformation on the Target Search Dynamics of DNA Binding Proteins

Accurate transcription of genetic information is crucial, involving precise recognition of the binding motifs by DNA-binding proteins. While some proteins rely on short-range hydrophobic and hydrogen bonding interactions at binding sites, others employ a DNA shape readout mechanism for specific recognition. In this mechanism, variations in DNA shape at the binding motif resulted from either inherent flexibility or binding of proteins at adjacent sites are sensed and capitalized by the searching proteins to locate them specifically. Through extensive computer simulations, we investigate both scenarios to uncover the underlying mechanism and origin of specificity in the DNA shape readout mechanism. Our findings reveal that deformation in shape at the binding motif creates an entropy funnel, allowing information about altered shapes to manifest as fluctuations in minor groove widths. This signal enhances the efficiency of nonspecific search of nearby proteins by directing their movement toward the binding site, primarily driven by a gain in entropy. We propose this as a generic mechanism for DNA shape readout, where specificity arises from the alignment between the molecular frustration of the searching protein and the ruggedness of the entropic funnel

governed by molecular features of the protein and the arrangement of the DNA bases at the binding site, respectively.

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Tarak Karmakar

Thermodynamics and Kinetics of Biomolecular Recognition

Biomolecular recognition processes, like the binding of small molecules to proteins and nucleic acids, protein-protein and protein-nucleic acid associations, occur over milliseconds to seconds timescales, posing a challenge for brute-force molecular dynamics simulations in capturing their thermodynamics and kinetics. Enhanced sampling (ES) simulations, particularly collective-variable (CV)-based methods such as umbrella sampling and metadynamics, alleviate this limitation by enabling efficient sampling. A crucial aspect of CV-based ES methods is selecting appropriate CVs that distinguish between the system's metastable states, such as bound and unbound states in biomolecular recognition. A bias, based on these CVs, is

introduced into the system's Hamiltonian to enhance CV fluctuations, improving sampling efficiency. However, identifying CVs for biomolecular systems is complex, often requiring a large number of variables to describe the system's states, making the use of such large dimensional CVs in ES methods impractical. To tackle this, deep learning methods are employed to non-linearly combine a large number of descriptors, typically pairwise contacts between host and guest atoms, to create effective CVs. These CVs are then used for ES simulations to study peptide-RNA binding,

small RNA-protein binding, and small molecule- DNA binding and calculate the free energy landscapes for the host-guest binding process. Moreover, infrequent metadynamics simulations enable the calculation of binding kinetics, with rate constants showing excellent agreement with spectroscopic measurements.