Here, we characterized the structure, dynamics and ion atmosphere in a bacteriophage MS2 virus using all-atom molecular dynamics simulations. Starting from available experimental information, we built a computational model of a mature MS2 virion containing a 3569 nucleotide RNA genome enclosed inside a mature protein capsid that in turn consists of a single maturation protein embedded within an icosahedral lattice of the protein capsid. Our two 2.5-microsecond replica simulations of the complete virion differing by the initial configurations of the RNA genome revealed the stabilizing effect of the RNA—protein interactions on the structure of the mature capsid and on the conformation of the RNA genome. The virus particle as a whole was observed to undergo a breathing-like motion, whereas parts of the RNA genome not bound specifically to the protein capsid explored a range of structural transformations while retaining their secondary structure. A set of simulations performed upon removal of the RNA genome characterized the effect of the RNA on the internal pressure in the virus and on its ion atmosphere. Taken together, our work introduces a mature viral particle as a system of high dynamic complexity where repulsive RNA-RNA interactions compete with specific RNA-protein binding, with the ion atmosphere serving as intermolecular glue holding the assembly together.

2159-Plat

Identification and characterisation of G-quadruplexes from viral genomes

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G-quadruplexes (G4s) are non-canonical structures identified in all domains of life. In humans, they have been shown to influence a number of biological processes. Our lab has recently identified G4s in viruses containing RNA or DNA as the genetic material using bioinformatics tools and characterized them using biophysical methods such as circular dichroism, and small angle X-ray scattering. For example, we recently established that the Zika virus that contains RNA as its genetic material has a conserved G4 present in its 3′ terminal region. A mutation from G to A nucleotide severely disrupts G4 formation, and the ability of Zika virus G4 to interact with known G4 interacting partners such as DHX36 and G4-binding antibodies. We also demonstrated that Zika virus G4 can be recognized and unwinded by human helicase DDX17. Subsequently, we discovered that the Monkeypox virus which consists of a DNA genome contains two conserved G4s, which are not present in any other pox viruses. After establishing G4 formation using short wild-type and mutant constructs of Monkeypox DNA using biophysical methods, we also demonstrated that a quadruplex binding small-molecule, TMPyP4, that was previously reported to reduce viral replication interacts with Monkeypox G4s with nanomolar affinity. Overall, our work provides novel targets against which antivirals can be developed.

2160-Plat

Predicting 3D RNA structure from solely the nucleotide sequence using Euclidean distance neural networks

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Fast and accurate 3D RNA structure prediction remains a major challenge in structural biology, primarily due to the size and flexibility of RNA molecules, as well as lack of diverse experimentally determined structures of RNA molecules. Unlike DNA structure, RNA structure is far less constrained by base pair hydrogen bonding, resulting in an explosion of potential stable states. Here, we propose a convolutional neural network which predicts all pairwise distances between residues in an RNA, using a recently described smooth parametrization of Euclidean distance matrices. We achieve high accuracy predictions on RNAs up to 100 nucleotides in length in fractions of a second, a factor of 10^7 faster than existing molecular dynamics-based methods. We also convert our coarse-grained machine learning output into an all-atom model using discrete molecular dynamics with constraints. Our proposed computational pipeline accurately predicts all-atom RNA models solely from the nucleotide sequence.

2161-Plat

Charge density effect in counter ion condensation on RNA

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Screening the negatively charged nucleic acid backbone by counter ions is essential for its folding and function. Experimental and computational studies demonstrate the influence of charge density on stability and cation distributions for RNA/DNA duplexes [1-3]. However, a detailed understanding of its thermodynamics aspects remains elusive. We address this question by studying the effect of charge density on the energy landscape of the counterion condensation process in the context of G-Quadruplex RNA. Metadynamics simulations that allow navigating the free energy surface (FES) of the fraction of screened charge variable provided a thermodynamics picture of the condensation process. We study the monovalent ion salt series with decreasing charge density, Li^+, Na^+, K^+, Cs^+, and Rb^+. We found that the counterion condensation process has a single minimum irrespective of the charge density. Surprisingly, the minima are located at around 70-75% charge screening for all monovalent ions under study, consistent with mean field theories. However, unlike mean-field theories, the screening is influenced by charge density. The degree of screening varied in the order of $Li^+ > Na^+ \approx Cs^+ \approx Rb^+ > K^+$. The most notable observation is that K^+ shows a non-monotonic trend with its charge density. We investigate the structural and energetic factors that give rise to the differences in charge screening. Our analysis demonstrates that hydration and preferential binding dictate the charge density-induced screening effect. Together, our study offers detailed insight into the charge compensating cations and highlights the unique properties of K^+, leading to its lower screening.

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2162-Plat

Chemical probing profiling of 2'-deoxyguanosine sensing RNA reveals magnesium ion-dependent conformational switching

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Maintaining the homeostasis of cellular metabolites is essential for all living organisms. Noncoding mRNA in procaryotes strategically regulates the expression of downstream genes which are linked to the expression of proteins involved in specific metabolite salvage and biosynthesis, in response to binding to that specific metabolite. Such noncoding mRNAs are known as the Riboswitches. A class of riboswitches that recognizes purine analogs were identified. In this present contribution, we report the influence of magnesium and temperature on the conformation of the aptamer domain (the ligand binding domain) of 2'-deoxyguanosine riboswitch from *Mesoplasma florum*. Our comprehensive SHAPE (Selective 2′ Hydroxyl Acylation analyzed by Primer Extension) probing experiments have shown that the aptamer domain exhibited a strong dependence on the Mg^{2+} ion concentration for the efficient ligand binding and the concomitant structural change. Mg^{2+} ions reduce the conformational flexibility mainly by organizing P2 and P3 helices and thereby facilitate ligand binding by shifting the conformational ensemble of the ligand-free aptamer towards a pre-organized, ligand-binding competent conformation. The subtle conformational switching in the presence and absence of 2'-deoxyguanosine is observed at the **(A)** regulatory helix (P1) and **(B)** the interhelical junctions (which serve as the ligand binding pocket) of the aptamer. We have observed the formation of a regulatory helix, the folding of the binding pocket, and the subsequent encapsulation of the ligand is accomplished only in the simultaneous presence of Mg^{2+} and 2'-deoxyguanosine. We have also identified the existence of two conformations in equilibrium in a ligand-free state. Finally, we have shown that for the 2'-dG sensing riboswitch from *Mesoplasma forum*, the conformation of the full-length transcripts populates the functional OFF-state regardless of the presence or absence of ligand, consistent with a kinetic riboswitch mechanism.

Platform: Protein Dynamics and Allostery II

2163-Plat

Increased ionic strength triggers multiple conformations in both apo and holo forms of bacterial ferric binding protein

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This study combines molecular dynamics (MD) simulations with solution X-ray scattering measurements to investigate the range of conformations that can be achieved by a pH/ionic strength (IS) sensitive protein and to quantify its distinct populations. To explore how the conformational multiplicity of

proteins might be modified in the environmental niches of biological media, we focus on the periplasmic protein FbpA from *H. influenzae* which is a part of the mechanism developed by bacteria to capture iron from higher organisms. We examine iron-binding/release mechanisms of FbpA in varying conditions simulating its unique biological environment. The collected SAXS data complemented by SEC analyses and binding assays point to multiple conformations at physiological IS while they are well-explained by single x-ray structures in a 15 mM buffer. Moreover, by fitting the SAXS data with unique conformations sampled by a series of MD simulations carried out under conditions mimicking the buffers, we quantify with high accuracy the populations of the occupied substates. Furthermore, we find the D52A mutant that we predicted by coarse-grained computational approaches to allosterically control the iron binding site in FbpA responds to the environmental changes in our experiments with varied conformational selection scenarios. We show an application of the environment dependence of the conformers by designing a genetically encoded iron biosensor as a chimera of FbpA and green fluorescent protein using the D52 region as the insertion point. This work exemplifies how unifying a range of experimental and computational techniques provides platforms for achieving the next generation of applications that put protein dynamics rather than the static structure at the centerstage. Support by Turkish Atomic Energy Authority, TUBITAK grant no 121Z329 and a scholarship to GL through 2214-A program. Measurements conducted at EMBL-Hamburg.

2164-Plat

Predicting the locations of cryptic pockets from single protein structures using the PocketMiner graph neural network

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Cryptic pockets expand the scope of drug discovery by enabling targeting of proteins currently considered undruggable because they lack pockets in their ground state structures. However, identifying cryptic pockets is labor-intensive and slow. The ability to accurately and rapidly predict if and where cryptic pockets are likely to form from a protein structure would greatly accelerate the search for druggable pockets. Here, we present PocketMiner, a graph neural network trained to predict where pockets are likely to open in molecular dynamics simulations. Applying PocketMiner to single structures from a newly-curated dataset of 39 experimentally confirmed cryptic pockets demonstrates that it accurately identifies cryptic pockets (ROC-AUC: 0.87) >1,000-fold faster than existing methods. We apply PocketMiner across the human proteome and show that predicted pockets open in simulations, suggesting that over half of proteins thought to lack pockets based on available structures are likely to contain cryptic pockets, vastly expanding the druggable proteome.

2165-Plat

Quasi Markov state models elucidates the role of bacterial RNA polymerase loading gate and trigger loop dynamics in antibiotics inhibition

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The rise of antibiotic resistance urges the development of new antibiotics. Bacterial RNA Polymerase (RNAP) transcribes DNA to RNA and is an effective target for antibiotics. RNAP has a crab-claw-like shape with two pincers, clamp and β-lobe domains, forming a loading gate. During the initiation of transcription,the opening of the loading gate is necessary to load the template DNA. Myx is an antibiotic that binds to the switch 2 region, under the clamp, to prevent clamp closing/opening and inhibit transcription initiation. To study the inhibition mechanism of Myx in atomic resolution, we built quasi-Markov State Models (qMSM) based on extensive molecular dynamics (MD) simulations. qMSM can obtain the slow timescale dynamics of complex conformational change using shorter MD simulations compared to traditional MSM. qMSM identifies four states of clamp opening: closed, two partially closed, and open states. We have shown that the opening of the Myx binding site only occurs in a partially closed state, which allows binding of Myx. This suggests that Myx selectively binds to a partially closed state and follows the conformational selection mechanism. Notably, we find that β-lobe opening is sufficient for loading of DNA during transcription initiation. This highlights the critical role of β-lobe to initiate transcription and β-lobe's potential as a target for future antibiotics development. We also utilize qMSMto study the folding of the trigger loop (TL) of RNAP. During RNA elongation, TL closes/folds to stabilize the NTP substrate in the active site for catalysis. CBR is antibiotics that inhibit the folding of TL. Studying the molecular mechanism of TL folding will be useful to understand the inhibition mechanism of antibiotics, CBR, and future drug design targeting TL.

2166-Plat

From closed to open: Omicron mutations increase interdomain interactions and reduce epitope exposure

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Omicron BA.1 is a highly infectious variant of SARS-CoV-2 that carries more than thirty mutations on the spike protein in comparison to the Wuhan wild type (WT). Some of the Omicron mutations, located on the receptor binding domain (RBD), are exposed to the surrounding solvent and are known to help evade immunity. However, the impact of buried mutations on the RBD conformations and on the mechanics of the spike opening is less evident. Here, we use all-atom molecular dynamics (MD) simulations with metadynamics to characterize the thermodynamic RBD-opening ensemble, identifying significant differences between WT and Omicron. Specifically, the Omicron mutations S371L, S373P, and S375F make more RBD interdomain contacts during the spike's opening. Moreover, Omicron takes longer to reach the transition state than WT. It stabilizes up-state conformations with fewer RBD epitopes exposed to the solvent, potentially favoring immune or antibody evasion.

2167-Plat

Perturbation-HDX reveals glycosylation sites as weakest links in dengue capsid quaternary structure

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Dengue virus (DENV) is a mosquito-borne, icosahedral flavivirus that represents a significant threat to human health worldwide. Vaccine development for dengue has long eluded scientists due to serotype and strain diversity associated with altered viral capsid dynamics. Despite being largely conserved, dengue strains show distinctive reversible fluctuations referred to as 'breathing'. DENV capsids have T=3 icosahedral symmetry, consisting of 180 copies of out envelope (E) protein. E-protein dimer contacts confer stability of the native virus particle. To localize and rank the strengths of E-protein interfaces on the surface, we carried out urea and temperature perturbation of DENV particles analyzed using amide hydrogen-deuterium exchange mass spectrometry. Our results highlight the weakest links on the virion surface. These sites are spanning the glycosylation sites of dengue. We have isolated and characterized the viable deglycosylated mutant which shows a different temperature stability profile. These sensitive sites are prime targets for drug development and predict sites that may change due to the selective pressure that impact viral evolution.

2168-Plat

Charactering ionizable residue networks at SH2 interfaces in pH-sensitive proteins

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Intracellular pH (pHi) dynamics (7.0 to 7.8) regulate myriad cell processes but the molecular mechanisms driving these behaviors are largely unknown. While some pH-sensitive proteins use titratable histidine residues to sense physiological changes in pHi, others have unknown or histidine-independent mechanisms. Charged amino acids (Asp, Glu, and Lys) can have pKas that are up- or down-shifted into the physiological range depending on protein environment, but the role for these residues in regulating pH-dependent protein function is understudied. Here, we use computational and biochemical approaches to investigate pH sensors that use networks of cooperative ionizable residues with a cumulative physiological pKa. We performed computational analyses of two pH sensitive signaling proteins with unknown mechanisms: (phosphatidylinositol-3-kinase (PI3K) and SHP-2 tyrosine-protein phosphatase). In both proteins, we identified networks of ionizable residues with upshifted pKas at the inhibitory SH2 domain interface. We next used biochemical mutational analyses to probe the accuracy of our computational approaches by measuring pH sensitive activity of wild-type and mutant proteins *in vitro*. Importantly, because SH2 domains are structurally conserved, this pH-sensing mechanism could be conserved in a subset of kinases and phosphatases with inhibitory SH2 domains. We tested this hypothesis by identifying a network of ionizable residues in other signaling proteins that contain inhibitory SH2 domains. We analyzed JAK2 kinase, which has not been shown to have pH sensitive