natural cavity, and variants with substitutions to polar residues to affect the state of hydration of cavities to study its role in pressure unfolding. For 27 variants studied we obtained (a) crystal structures, (b) thermodynamic stabilities using chemical denaturation, and (c) ΔV of unfolding measured by pressure denaturation monitored with Trp fluorescence. ΔV of unfolding were also measured using NMR spectroscopy for select variants. The cavities generally did not affect the structure. Although large enough to hold several waters, water molecules were only detected in the cavities when lined with polar groups. The measured ΔV of variants was always larger than for the wild-type. A near-linear correlation between the ΔV measured experimentally and the one calculated from structures illustrates the importance of cavities in pressure sensitivity. A correlation between measured ΔV and thermodynamic stability (ΔG) suggests that 1 kcal/mol is lost per 12 mL/mol of increased void volume. This study demonstrates irrefutably the significant contributions cavities make towards the pressure sensitivity of proteins and their effects on internal hydration and structural fluctuations of proteins.

241-Pos Board B21

Crystal Structures of Streptococcus Pyogenes Cas2 Protein at Various pH Conditions

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Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (cas) proteins provide RNA-mediated adaptive immunity against foreign invading nucleic acids such as phages and plasmids in archaea and bacteria. Cas2 protein is one of the two core Cas proteins are present in all types of CRISPR-Cas systems and is required for new spacer integration into CRISPR loci. Cas2 homologues from several CRISPR-Cas subtypes were characterized as metal-dependent nucleases with different substrate preferences, and it was proposed that a pH-dependent conformational change mediates metal binding and catalysis. Here, we report the X-ray crystal structures of Streptococcus pyogenes Cas2 protein at three different pHs (5.6, 6.5, and 7.5), and the results of its nuclease activity assay at varying pHs (6.0-9.0). While S. pyogenes Cas2 exhibited strongly pH-dependent catalytic activity, there was no significant conformational difference among the three crystal structures. However, structural comparisons with other Cas2 homologues suggest structural variability and the flexible nature of its putative hinge regions, supporting the supposition that conformational change is important for catalysis.

242-Pos Board B22

Crystal Structures of Streptococcus Pyogenes and Xanthomonas Oryzae Cas5d Proteins

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Clustered regularly interspaced short palindromic repeats (CRISPRs) are repetitive genetic elements found in archaeal and bacterial genomes that are involved in RNA-mediated adaptive immunity against foreign invading nucleic acids such as phages and plasmids. CRISPR-associated (cas) genes are also found adjacent to CRISPR loci and encode Cas proteins with a variety of nucleic acid-related functions. Cas5d proteins are subtype I-C specific Cas5 proteins that constitute one of the most plevalent Cas protein families in CRISPR-Cas systems, and have been predicted to have RNA-recognition motif (RRM) domains. Here, we report crystal structures of Cas5d proteins from Streptococcus pyogenes and Xanthomonas oryzae, which represent two Cas5d subgroups. S. pyogenes Cas5d contains extra C-terminal residues while X.oryzae Cas5d has an extended helical region protruding from the N-terminal RRM domain, which has not been observed in other Cas5d structures previously. Despite structural differences, the two Cas5d proteins share common functional properties such as specific endoribonuclease activity for pre-crRNA and non-specific double-stranded DNA binding. These findings suggest that Cas5d may have multiple roles in CRISPR-mediated immunity system and provide an explanation for the conserved mode of the pre-CRISPR RNA processing.

243-Pos Board B23

Understanding Structural and Dynamic Effects Induced by Key Components of the HCV Polymerase Replication Complex

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Hepatitis C virus (HCV) is a wide spread health concern for which there is no vaccine available. HCV contains a single-stranded RNA genome and replicates with the aid of an RNA-dependent RNA polymerase known as non-structural protein 5B (NS5B). NS5B adopts at least two different conformations to replicate viral RNA. The closed conformation is thought to be necessary for initiation of replication while the open conformation is required for elongation of the newly synthesized RNA strand. Transitions between these two conformations play a crucial role in NS5B function. Our goal is to understand how the distribution of conformations sampled by the enzyme is altered during different stages of replication. In previous studies we observed that the free enzyme is able to occupy conformational states that are expected to be relevant for the different stages of RNA replication. Our current studies examine the impact of specific components of the replication complex in shifting the ratio of conformational states adopted by the enzyme. To accomplish this goal we performed Molecular Dynamics simulations of the enzyme bound to various combinations of nucleotides and RNA template. We anticipate that the binding of these components will change the relative population of the different conformations in a way that facilitates enzyme function.

244-Pos Board B24

Modeling Macromolecular Bodies using 3D Medial Axis Transforms and Normal Mode Analysis

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Many large protein or protein/DNA machines are difficult to simulate on time scales long enough for complete turnover cycles. One way to overcome this general problem is to treat the protein at a (much) lower level of detail. In the approach described here, the protein complex is modeled as a collection of interacting flexible bodies, with low levels of detail over most of the protein surface (and interior), and high levels of detail only where necessary. The bulk shapes of all protein components are described with a low-resolution, purely repulsive potential, together with local high-resolution sites of interaction. The low-resolution potential function is built using the three-dimensional analog of the Medial Axis Transform (MAT) to generate an efficient set of basis functions that can represent the protein's repulsive isopotential surface.

In addition to the general theory, a model for small DNA polymerases (specifically, the HIV Reverse Transcriptase) interacting with a coarse-grained DNA will be presented. As above, the polymerase model treats the protein as a single continuum body decorated with interaction sites. The system energy has several components: a) the repulsive body potential based on the MAT; b) a set of low order protein normal modes (from all-atom MD runs) whose amplitudes act as coordinates for the deformation energy function; c) an elastic energy function that includes both harmonic and non-harmonic terms, and accounts for stress/strain energy as the polymerase undergoes conformatioinal changes; d) a set of interaction sites (roughly corresponding to key individual amino acids) that give local (usually attractive) interactions with DNA, with nucleotides, and between sites on the polymerase body.

245-Pos Board B25

Simulating Protein and Nucleic Acid Dynamics on the Microsecond to Millisecond Timescale

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Molecular dynamics simulation is an invaluable tool for studying biomolecular structure, dynamics and function. Currently this approach suffers from high computational cost, restricting direct simulation of dynamics to the microsecond timescale. Implicit treatment of solvent can offers orders of magnitude increases in sampling, due to both reduced viscosity and high computational efficiency on consumer graphics processors (GPUs). In the past, these fast implicit solvent models typically had significant weaknesses, and thus the advantages in speed were offset by poor accuracy. We present the application of our newly developed implicit solvent model and physics-based force field, with application to unraveling puzzles in protein and nucleic acid folding. Specifically, we show that within weeks of simulation in implicit solvent, we were able to fold a benchmark series of protein with a variety of sizes and topologies. Comparable calculations in explicit solvent would take years and are largely intractable. Our solvent model is also extended to highly charged (and more challenging) systems such as DNA and RNA.

246-Pos Board B26

Environmental and Mutation Effects on the Folding and DNA-Binding of the Primary DNA Recognition Subdomain of Sleeping Beauty Transposase

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The reaction of DNA transposition begins when the transposase enzyme binds to the transposon DNA. Sleeping Beauty (SB) is a member of the mariner