conformational transformation when it does not interact with the NTD. The RfaH-CTD in the all-α topology is involved in regulating transcription whereas in the all-β topology it is involved in stimulating translation by recruiting a ribosome to an mRNA. Calculations of free-energy landscape and transfer entropy elucidate the details of the RfaH-CTD transformation process. The importance of interfacial interactions between the two domains of RfaH is highlighted by the compromised structural integrity of the helical form of the CTD in the absence NTD.

**1881-Pos Board B18**
**Small-Angle X-Ray Scattering and Biochemical Studies of an Intramolecular Tandem Coiled Coil**
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Coiled coil has served as an excellent model system for studying protein folding and developing protein-based biomaterials. Most designed coiled coils function as oligomers, namely intermolecular coiled coils. However, less is known about structural and biochemical behavior of intramolecular coiled coils where coiled coil motifs are covalently linked in one polypeptide. Here we prepare a protein which harbors three coiled coil motives with short linkers, termed tandem coiled coil (TCC) and characterize its structural and biochemical behavior in solution. TCC consists of three coiled coil motives whose sequences are derived from Coil-Ser and its domain swapped dimer (DSD). Modifications include positioning E (Glu) residue at ''e'' and K (Lys) at ''g'' throughout heptad repeats to enhance ionic interaction among its constituent coiled coil motives. The linkers are four-residue-long with sequence G[S/T]GG to ensure flexibility. Molecular modeling of TCC suggested a compact triple helical bundle structure with the second and the third coiled coil motives forming a canonical coiled coil. TCC exists as a mixture of monomeric and dimeric species in solution. Small-angle X-ray scattering (SAXS) revealed ellipsoidal molecular envelopes for both dimeric and monomeric TCC in solution. The theoretically modeled structures of TCC docked well into the envelopes of both species. Higher ionic strength shifted the equilibrium into monomer with apparently more compact structure. Secondary structure of TCC at various ionic strengths remains unchanged probed by circular dichroism. Taken together, our results suggest that our designed TCC is predominantly monomeric structure through the enhanced ionic interactions, and it is affected by the concentration of ionic species in the buffer.

**1882-Pos Board B19**
**Characterization of Amynthas Gracilis Hemoglobin (HbAg) and its Subunits by AUC and MALDI-TOF-MS**
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The giant extracellular hemoglobin (HbAg) of the annelid *Amynthas gracilis* has a molecular mass (MM) of 3400kDa. In the current work, the characterization of MM values of HbAg and its subunits is presented. Electrophoresis, MALDI-TOF-MS and AUC show that the MM values of HbAg subunits are very close, but not identical to those of *Glossoscolex paulistus* (HbGp) and *Rhinodrilus alatus* (HbRa) hemoglobins. Analytical ultracentrifugation (AUC) sedimentation velocity experiments were performed to obtain M for HbAg in oxy- form. value of 59.3 ± 0.2 S was obtained for native HbAg. From the ratio between sedimentation and diffusion coefficients values for M of approximately 3400 ± 100 kDa for oxy-HbAg was obtained. MALDI-TOF-MS data gave MM for HbAg subunits. Monomer *d* is found to exist in, at least, four isoforms with MM 16,244 ± 3Da, 16,459 ± 5Da, 16,667 ± 5Da and 16,855 ± 3Da, as noticed for HbGp, and not observed for HbRa. Furthermore, the trimer subunit presents two isoforms (*(abc)$_1$ and (abc)$_2$*) with *MM* 51,415 ± 20Da and 51,610 ± 14Da, respectively. This might indicate that the monomers *a*, *b* and *c* do have isoforms, as found for HbGp and not for HbRa. The monomeric chains *a*, obtained from the trimer *abc* reduction, present three isoforms with *MM* 17,015Da, 17,061Da and 17,138Da, differing from HbGp that presents four isoforms. A less intense species is observed at 67,717, and is due to the tetramer *abcd* contribution. Finally, AUC and MALDI-TOF-MS data are very close as compared to that obtained for HbGp and HbRa. Our results show total consistency between M obtained by AUC and recent partial characterization by mass spectrometry. Finantial support: FAPESP and CNPq Brazilian agencies.

**1883-Pos Board B20**
**Structure and Function of Clostridial Yter**
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Pectin found in fruit and vegetable waste is a potential renewable feedstock for production of butanol from Clostridium acetobutylicum. Pectin is an abundant complex carbohydrate found in the cell wall of plants, but its fermentation results in low butanol yields. Understanding the process of degradation and metabolism of the carbohydrate matrix is key to improving butanol yields from pectin. Transcriptomic analysis of C. acetobutylicum during growth on pectin identified several genes with potential degradation activity. One of these genes, CA_C0359, encodes a putative unsaturated rhamnogalacturonyl hydrolase (URH). The crystal structure of the recombinant CA_C0359 protein was solved to a 1.6 Å resolution. We present here an overview of the crystal structure of the CA_C0359 protein, and theoretical results docking various disaccharides into this structure to assess its capabilities for sugar degradation in the context of functionally similar proteins.

**1884-Pos Board B21**
**NMR Structural Characterization for Proteases of Dengue and West Nile Viruses and its Insight into Drug Discovery**
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*Flaviviruses* are a major cause of infectious disease in human, which include the Dengue Virus (DENV), West Nile virus (WNV). The genomic RNA encodes a polyprotein precursor which is processed proteolytically upon translation to 10 proteins, including three structural proteins (capsid [C], premembrane [prM] and envelope [Env]), and seven nonstructural (NS) proteins (NS1, NS2A/B, NS3, NS4A/B, and NS5). The NS3 is of great interest in drug discovery because of its N-terminal domain has the protease activity required for viral replication. The activity of NS3 is regulated by membrane protein NS2B. Previous studies were using a construct that contains 40 amino acids from NS2B fused with NS3 protease domain though an artificial $G_4SG_4$ linker. We have developed a series of peptidic inhibitors targeting WNV and DENV proteases. We analyzed their interactions with the protease using both chemical shift perturbation and docking studies. For the DENV protease, there is still no potent inhibitor available so far. Using NMR spectroscopy, we discovered that the conventional DENV protease construct in which NS2B cofactor region linked with NS3 protease through a flexible liner is not suitable for drug discovery due to the protein dynamics. Using a co-expression system, we obtained a protease complex that contains the NS3 protease domain and 50-residue segment of the NS2B. Our results show that this protease complex exists as a close conformation and active under physiological conditions, which might be a better construct for drug discovery targeting DENV. We also expressed and purified a natural form of DENV protease which was shown to be active in detergent micelles. Our studies will be useful for development of DENV protease inhibitors.

**1885-Pos Board B22**
**Monitoring Protein Structure on the Surface of Gold Nanoparticles using NMR Spectroscopy**
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Because of their unique spectroscopic properties and biocompatibility, protein-functionalized gold nanoparticles (AuNPs) have many potential diagnostic and therapeutic applications. Surface-bound proteins can be used both as molecular sensors and drug delivery vectors, and the plasmonic properties of gold enable the detection of very small changes in surface chemistry. Unfortunately, the design of general-purpose, functionalized AuNPs is severely complicated by our limited understanding of protein structure on nanoparticle surfaces. Some enzymes remain active on AuNPs while others are inactive, and it is currently impossible to predict which behavior will be observed. To address this problem, we have recently developed several new NMR-based approaches for monitoring protein structure on AuNP surfaces. We find that the adsorption capacity of 15 nm AuNPs can be predicted using the native structure, suggesting that proteins remain globular on the AuNP surface. Additionally, we demonstrate that proteins can be displaced from AuNPs by organothiols, supporting a case for reversible binding. Finally, we have used hydrogen/deuterium exchange (HDX) to monitor structural perturbations of two model proteins,

GB3 and Ubiquitin, when bound to AuNPs. We find no significant changes in slow HDX rates (5-300 min), suggesting that AuNP-induced structural changes are small for these two proteins. Together, these results support a model where most of a protein's native contacts are preserved upon adsorption, although larger changes may occur over long timescales.

**1886-Pos Board B23**

**3D Reconstruction of the S885A Mutant of the Human Mitochondrial Lon Protease**

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The Lon protein is a protease belonging to the superfamily of ATPases Associated with diverse cellular Activities (AAA+). Its main function is the control of protein quality and the maintenance of proteostasis by degrading misfolded and damaged proteins, which occur in response to numerous stress conditions. Lon protease has been also shown to participate in regulation of levels of transcription factors that control pathogenesis, development and stress response. Furthermore, it seems to play an important role in aging, and it is supposed to be involved in mtDNA replication, translation, or repair. We focus our interest on the structure of human mitochondrial Lon (hLon) protease whose altered expression levels are linked to some severe diseases, such as epilepsy, myopathy, or lateral sclerosis.

At the moment, it is assumed that Lon subunits assemble into oligomeric structures whose conformations are supposed to differ at ATP, ADP, and protein substrate binding. However, neither the full 3D structure of the Lon holoenzyme nor the mechanism of Lon's action is known. Several sub-structures of bacterial and human Lon have been resolved by X-ray scattering, and one 3D structure of an E. Coli Lon dodecamer active at physiological protein concentrations was resolved with electron microscopy.

Here, we present two conformations of an ADP-bound hLon S885A mutant obtained as a result of cryo-EM data analysis. The S885A mutant has a point mutation on the proteolytic domain, which completely disables its proteolytic function but does not affect its ATP-binding properties. The 3D reconstructions reveal that human Lon is a hexamer whose proteolytic and ATPase domains are arranged into a helix. The opening and pitch of the helix depend on the N-terminal domain interactions. These structures provide an insight toward the understanding of the protein mechanism of action.

**1887-Pos Board B24**

**Structure and Dynamics of the EIIC Sugar Uptake System**

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The phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) is a sugar uptake system unique to bacteria. It is a multicomponent system consisting of several cytosolic proteins and a dimeric transmembrane protein (EIIC in most PTS systems), which transports extracellular sugar across the membrane. Although EIIC is a uniporter, it is able to drive concentrative transport of its ligand because the sugar is phosphorylated by a cytosolic protein, EIIB, while still bound to the transporter. Phosphorylation prevents the sugar from escaping the cell and primes it for consumption by the cell. Little is known regarding the mechanism of sugar translocation and phosphorylation. Currently, the only available crystal structure of an EIIC is that of the N,N′-diacetylchitobiose transporter, bcChbC (1). We have proposed a mechanism for sugar translocation and phosphorylation, and we will report our progress in characterizing the mechanism.

Reference

1. Cao Y, Jin X, Levin EJ, et al. Crystal structure of a phosphorylation-coupled saccharide transporter. Nature. 2011;473(7345):50-4.

**1888-Pos Board B25**

**Predicting the Effects of Clinically Observed Kinase Mutations using Molecular Modeling and Machine Learning Algorithms**

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Many cellular processes are impacted by signaling through receptor and non-receptor kinase proteins. These include such diverse cellular actions as proliferation, differentiation, and motility, as well as tissue level phenomena such as angiogenesis and development. This important role in the cell is reflected also in the relative overrepresentation of kinases among known cancer mutations to proteins. In order to better understand the functional effects of these mutations, we have developed computational methods that seek to predict the effect of point mutations on kinase activation. By predicting whether a given mutation causes a kinase to be more active, we can gain insight into the overall impact of the mutation on cell phenotype and give insight to clinicians on patient cohorting for efficacious treatment with targeted kinase inhibitors. We have developed two separate but complementary methods to predict kinase activation status. The first uses molecular dynamics (MD) simulations and scoring criteria to predict if a mutation preferentially stabilizes the protein's active state. As a complimentary approach to MD, we have developed machine learning techniques that utilize the method known as support vector machines to predict whether mutations in a large number of kinases (>450) are activating. This method has proven to be almost as effective at predicting activation mutations as the mechanistic picture gained from MD simulations. We think these methods are both broadly applicable and have the potential to greatly impact both our understanding of mechanisms of kinase activation as well as to guide best practices in the clinical setting of targeted therapy in cancer treatment.

**1889-Pos Board B26**

**Activation Mechanism of a Signaling Protein at Atomic Resolution**

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The interconversion between the inactive and active state is the heart of signaling. This process has traditionally been described by the two corresponding structures, sometimes complemented with kinetic data. However the question of how these folded states interconvert is largely unknown due to the inability to experimentally observe the transition pathways.

Here we present a recent investigation of the full free energy landscape of the receiver domain of the response regulator NtrC (NtrC ) by combining several computational methods including the string method, Markov state models of massive unbiased MD simulations, and long MD simulations on ANTON, with new NMR structural data.

The results unveil several unexpected features underlying efficient signaling: The active and inactive states have to be considered purely in kinetic terms. The functional need of attaining a stable and well-defined conformer, crucial to the active form of the protein, is absent in the inactive state. The inactive state comprises a structurally heterogeneous collection of sub-states that interconvert on timescales shorter than the transition to the active state. The transitions between the two functional states occur through multiple pathways characterized by transition states with dramatically different structural features. In addition to this entropic lowering of the transition barrier, a number of polar side-chains engage in unspecific transient interactions during the barrier crossing and thus make the activation mechanism flexible, efficient and robust. These novel findings challenge the structural paradigm of signaling and may represent general features for functional conformational transitions within the folded state.

**1890-Pos Board B27**

**Crystal Structures of Trehalose Synthase from Deinococcus Radiodurans Reveal a Closed Conformation for Intramolecular Isomerization Catalysis and Mutant Induction of an Active-Site Aperture**

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Trehalose has been used in food, cosmetic, and biotechnological industries due to its exceptional stability. Trehalose synthase (TS) catalyzes a simple conversion of inexpensive maltose into trehalose and hence has a great potential. TS consists of a catalytic (β/α)8 barrel, a subdomain B, a C-terminal β domain and two TS-unique subdomains (S7 and S8). The apo TS structures from Mycobacterium smegmatis and M. tuberculosis showed an unusual inactive conformation, in which the S7 loop blocks the substrate-binding pocket. Here we report structural and mutational studies of TS from Deinococcus radioduran (DrTS). The complex structures of DrTS with the inhibitor Tris share high homology with the substrate-bound sucrose hydrolase, amylosucrase, and sucrose isomerase, particularly virtually identical active-site architectures. A maltose was modelled into the active site and subsequent mutational analysis suggested that Tyr213, Glu320, and Glu324 are essential for the TS activity. In addition, the interaction networks between subdomains B and S7 seal the active-site entrance. Disruption of such networks through replacement of Arg148 and Asn253 with alanine resulted in a decreased isomerase activity but an increased hydrolase activity. The R148A and N253A structures showed a small pore created for water entry.