Computational Assessment of Trimethoprim Resistance in Dihydrofolate Reductase

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has important ramifications for the development of new antibacterial compounds. In streptococcus pneumoniae, pilis are formed by three Sortase C (SrtC) enzymes. In contrast to the housekeeping SrtA class of proteins, these SrtC enzymes possess an N-terminal extension that is thought to constitute a flexible lid that modulates access to the active site during the pilus construction process. In this work, we have used enhanced sampling and free energy molecular dynamics simulations to study the conformational dynamics and probe the lid opening mechanism of three wild-type SrtC enzymes (SrtC-1, SrtC-2, and SrtC-3), as well as for several experimentally studied SrtC-1 mutants. The salt bridge between the conserved aspartate residue in the lid region and arginine in the active site leads to a robust anchoring of the lid that is stable during long conventional molecular dynamics simulations of all the SrtC proteins. In contrast, the results suggest that the aforementioned mutations lead to a dynamic lid, in accordance with experimental findings. We then carried out umbrella sampling calculations for SrtC-1 and its mutants which showed that the wild-type had the highest barrier for lid opening. This indicates that opening of the active site likely require interactions with the sorting signal (SS) and possibly other regions of the pilin building block. Calculations on SrtC in complex with either the SS or SS containing pilin subunit were then implemented. The data shows that the conformation of the lid is altered in the presence of substrate as proposed above. We also explore the structural and energetics underpinnings of the exquisite substrate specificity observed experimentally in the SrtC class of enzymes.

247-Pos Board B27
A Novel Signal Transduction Mechanism in LOV Domain Proteins
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In light-oxygen-voltage (LOV) domain photoreceptors, blue light irradiation leads to a hetero bond formation between the C4a carbon of the flavin cofactor isoolaxazine ring and a conserved cysteine residue. Adduct formation results in N5 nitrogen protonation and changes in the hydrogen bond network surrounding the cofactor, instigating protein conformational changes and downstream signaling. Interestingly, when the circadian-clock LOV-protein Vivid from Neurospora crassa is devoid of the adduct-forming cysteine, the neutral semiquinone forms upon photoreduction of the cofactor. This variant is surprisingly still capable of dimerization and in vivo signaling. Moreover, analogous LOV domains of natural cysteine-less proteins were discovered to exhibit conformational changes after either chemical reduction or photoreduction. As flavin N5 protonation is a shared event in both the formations of the adduct and the neutral semiquinone, our findings indicate that 1) the neutral semiquinone is a biologically functional state in LOV-proteins and 2) flavin N5 protonation is sufficient for triggering signal transduction.

248-Pos Board B28
NMR Structural/Functional Characterization of an Oncogenic Mutant of cAMP-Dependent Protein Kinase A: PRKACA-DNAJB1
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Cyclic AMP (cAMP)-dependent Protein kinase A (cAMP-PKA) is involved in a multitude of biological processes including cell growth and division, cell differentiation, as well as metabolism and immune responsiveness, as such misregulation of PKA has been implicated in tumorigenesis. A non-receptor Src-family protein tyrosine kinases (SFKs) play a critical role in cell growth, differentiation, and various metabolism by controlling cell signaling. The regulation of cell signaling by SFKs is mediated by the conformational activation/inactivation of the tyrosine kinases. We investigated the conformational change of c-Src, one of the member of SFKs, from the inactive form (PDB id: 2SRC) to the active form (PDB id: 1Y57) employing targeted molecular dynamics (TMD) simulation. In this study, we propose the dynamical scenario for the activation process of the c-Src tyrosine kinase. Also, we discuss the role of key residue (W260) and hydrophobic pocket formed by (L325, V328, L308, and A311) and correlation between the domains (SH2, SH3 and catalytic domain) in the process of the activation of c-Src complex.

Protein-Small Molecule Interactions I

250-Pos Board B30
Biophysical Studies on the Interaction of Thionine Gold Nanoconjugate to Serum Albumin
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Nanoparticles have attracted remarkable recent interest for drug delivery application and control of protein structure and activity. The interaction of gold nanoparticles (GNP), thionine (TH), two complexes of GNP-TH, namely S1 and S2 with the transport protein Human Serum Albumin (HSA) was studied. GNP was prepared by citrate reduction method, while S1 and S2 were synthesized by mixing GNP and TH at different ratios - at room temperature and at 80°C, respectively. GNP, S1 and S2 were characterized by strong plasmon resonance absorption in 500-600 nm region. The adsorption of TH on GNP surfaces was characterized by FTIR spectroscopy. In order to understand the particle size domains of the synthesized GNP, S1 and S2, dynamic light scattering technique was used. Absorbance and fluorescence quenching experiments revealed the formation of strong complexes of S2 and HSA, and comparatively weaker complex between S1 and HSA. Spectroscopic analysis suggested the binding affinity of S1-HSA to be of the order of 10^4 M^-1 and that of GNP-HSA, TH-HSA, S2-HSA to be of the order 10^5 M^-1. Synchronous fluorescence confirmed alteration in the microenvironment of the Trp residues of HSA while practically no shift in the maximum emission wavelength reflected little transformation around Tyr residues. Circular dichroism studies revealed that binding, in all cases, altered the protein conformation by reducing the alpha-helical content. The binding also caused perturbation of the tertiary structure leading to unfolding of the protein and induced optical activity in the bound molecules, but to varying extents. The present study through multifaceted biophysical experiments is an effort to use GNP’s as delivery vehicles for multiple therapeutic purposes.

251-Pos Board B31
Computational Assessment of Trimethoprim Resistance in Dihydrofolate Reductase
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We characterize structural and dynamical changes induced on dihydrofolate reductase (DHFR). We investigate the structural features of the mutant protein that allow it to survive natural selection. Single/double/triple mutants detected via the systematic experimental approach carried out in the morbidostat1 under the selection pressure induced by the antibiotic trimethoprim (TMP), a competitive inhibitor of dihydrofolate, are studied. We investigate the
Interactions between a Classical Allosteric Protein and a Strong Effector Revisited

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According to the Two-State Model of Monod-Wyman-Changeux, the allosteric regulation in human tetrameric hemoglobin (Hb) is achieved upon binding of heterotropic effectors, such as 2,3-bisphosphoglycerate (BPG), to the allosteric site located between the beta subunits of unliganded, low oxygen affinity T-structure Hb. As a result, the new complex exhibits an additional decrease in the affinity for oxygen when compared with Hb in the absence of BPG. On the other hand, liganded Hb in the high oxygen affinity R-structure does not bind BPG, and thus its oxygen affinity remains unaffected.

We have studied the interactions between inositol hexakisphosphate (IHP), an allosteric effector stronger than DPG, and Hb, both in the liganded and unliganded forms, by isothermal titration calorimetry (ITC) and oxygen binding measurements in the equimolar range at pH 7.0 and 15°C. For the liganded "R"-structure, we chose the cyammetHb derivative (Hb^{+}-CN), and for the unliganded "T"-structure, the nickel-porphyrin Hb (NiHb). ITC experiments showed that IHP binds to both tetrameric derivatives in equimolar amount and with relatively high affinity.

Tertiary/quaternary structural perturbations introduced systemically into Hb+CN by removal of specific amino acid residues suggested that the IHP binding site is identical to that exhibited by NiHb, i.e., between the beta subunits. Under these conditions, we have not found any evidence that suggests IHP bind between the alpha subunits, or in the central cavity, as recent reports have suggested. At pH 8.2, we observed that while unliganded Hb with equimolar amounts of IHP remained practically invariable, the addition of above 100-fold molar excess indeed produced a pronounced decrease in the affinity for oxygen, suggesting the existence of an additional form of interaction. The function of liganded Hb, on the other hand, remained unchanged in the presence of IHP in excess.

Human Serum Albumin-[Ru(Phen)3]^{2+} Complex Formation Studied by Optical Spectroscopy

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Assessment of the tissue oxygenation is an important factor allowing to analyze various processes depending on the presence of oxygen. However, the oxygen sensitive probes can interact with proteins or lipids (depending on the hydrophobic character of the probe) what can interfere with the precise determination of the oxygen concentration. Dichlororut(1,10-phenanthroline)-ruthenium(II) hydrate ([Ru(Phen)3]^{2+}) is a water soluble non-phototoxic compound serving as a sensor of molecular oxygen. This type of heterocyclic ligands can also be used for antitumor, antibacterial and antiviral purposes. Thus, its interaction with carrier protein such as serum albumin is of high importance since this complex formation has a substantial influence on the in vivo distribution of the sensor. In this work, we have investigated the mechanism of [Ru(Phen)3]^{2+} interaction with human serum albumin (HSA), the most abundant protein in human plasma, by using different optical spectroscopy techniques: UV-visible absorption, fluorescence, Raman spectroscopy and surface-enhanced Raman spectroscopy. The obtained experimental data suggest only weak interaction of [Ru(Phen)3]^{2+} with HSA (K_{d}= 64 μM). Further, Raman spectroscopy and surface-enhanced Raman spectroscopy allowed us to register so-called fingerprint vibrational spectra of both molecules as well as to detect small changes in the polypeptide backbone conformations induced by the [Ru(Phen)3]^{2+}-molecule binding.

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Nanoscale Measurements of Biochemical Interactions at the Surface of Optically Trapped Particles

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Molecular interactions at biological surfaces are central to numerous physiological functions, such as cell-cell communication, adhesion, or the immune response to pathogens. Most analyses of these interactions require the use of purified proteins, which are studied in suspension or after immobilization on artificial carrier objects.

To characterize instead biomolecular dynamics directly at the surface of small biological particles, we have combined optical tweezers with a multi-channel microfluidics chamber. This instrument allows us to expose a laser-trapped particle to a series of microenvironments that are created without walls by laminar flow. We monitor biochemical reactions between the particle surface and supplied ligands by measuring minuscule changes in the particle’s response to a well-controlled fluidic drag force. Among others, this response is highly sensitive to the particle size.

Once a particle is trapped in a typical experiment, the chamber is moved perpendicularly to the main flow direction in a sinusoidal wave to apply a periodic drag force. Using custom-developed software, the resulting particle motion is tracked with a resolution of ~5 nm at a rate of up to 3,200 frames per second. A linear-systems analysis allows us to monitor the particle radius in real time, which is directly correlated to the surface adsorption state.

In first measurements, we used this setup to examine the interaction between protein-A and human IgG. We found that at saturation, bound IgG added an apparent thickness of 12.6±1.4(σ) nm to protein-A coated beads, in