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Platform: Protein Dynamics and Allostery I

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Allosteric Communication within the Cytoplasmic Region of the Histidine Kinase CpxA, Revealed by Molecular Dynamics Simulations of the Wild-Type and M228V Proteins

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The histidine kinases belong to the family of two-component systems, which serves in bacteria to couple environmental stimuli to adaptive responses. Most of the histidine kinases are homodimers, in which the HAMP and DHp domains assemble into an elongated helical region flanked by two CA domains. Recently, X-ray crystallographic structures of the cytoplasmic region of the Escherichia coli histidinekinase CpxA were determined [1] and a phosphotransferase-defective mutant, M228V, located in HAMP, was identified. We used molecular dynamics simulations to compare the behavior of the WT and M228V protein dimers.The modification of M228 removed the inter-monomer interaction between Methionines, which is the only Methionine-Methionine interaction inside the HAMP hydrophobic core. This removal locally induced the appearance of larger voids within HAMP, destabilizing the hydrophobic packing. More globally, the modification of M288 induced a destabilisation of the ATP binding in the CA domain, as well as the disruption of hydrogen bonds connecting residue sidechains between HAMP and CA. These variations correlate with an overall modification of the covariance of the protein internal strain [2], showing that the protein modification interferes with a path of long-range communication between the HAMP and the CA domains. This model gives an explanation for the phosphotransferase-defective effect of the M288V modification.

1. Mechaly AE, Sassoon N, Betton JM, Alzari PM. Segmental helical motions and dynamical Asymmetry modulate histidine kinase autophosphorylation. PLoS Biol 2014;12:e1001776.
2. Selwa E, Davi M, Chenal A, Sotomayor-Pérez AC, Ladant D, Malliavin TE. Allosteric Activation of Bordetella pertussis Adenylyl Cyclase by Calmodulin: MOLECULAR DYNAMICS AND MUTAGENESIS STUDIES. J Biol Chem 2014;289:21131-21141.

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Studying the Conformational Equilibrium of the N-Terminal Domain of Dsbd by NMR and Computer Simulation

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The N-terminal domain of the membrane protein DsbD (nDsbD) acts as a ‘redox hub’ in the periplasm of Gram-negative bacteria. Reductant, from cytoplasmic thioredoxin, is channelled via a series of thiol-disulphide exchange reactions involving the transmembrane domain of DsbD, the C-terminal domain (cDsbD) and finally nDsbD. nDsbD supplies reductant to crucial biosynthetic pathways. The active-site cysteines of nDsbD are protected from the oxidising periplasm by a loop. This ‘cap’ loop must open when nDsbD associates with its physiological interaction partners. To understand the mechanism of loop opening we have applied NMR relaxation methods alongside molecular dynamics (MD) simulations, probing the dynamic behaviour of the ‘cap’ loop in the oxidised (with C103-C109 disulphide) and reduced (without disulphide) forms of nDsbD. Spin-relaxation experiments demonstrate the ‘cap’ loop is rigid on a ps-ns timescale. 15N relaxation dispersion experiments revealed a slow conformational change in the ‘cap’ loop of oxidised but not reduced nDsbD. The chemical shift differences measured from fits of the nDsbDox data provide insights into its lowly-populated excited state. MD simulations confirm that the loop protects the active site of nDsbDred. It is more flexible in nDsbDox; the disulphide bond induces frustration in the active site, which leads to spontaneous loop opening in some trajectories. The simulations agree well with experimental S2, RDCs and torsion angle predictions from TALOS-N. The differences in the conformational flexibility of oxidised and reduced nDsbD may account for the observed redox-state dependence of its interaction affinities. Once reductant is transferred from cDsbDred to nDsbDox, the complex dissociates, enhancing turnover in the pathway. NMR experiments have revealed the kinetic basis of the redox-state dependent formation of the cDsbD-nDsbD complex. MD simulations identified dynamic encounter complexes in the association of cDsbD with nDsbD.

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Functional Mechanism of the Photoactive Yellow Protein: A Transient Absorption Spectroscopy Perspective

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Photoactive yellow protein (PYP) is a photoreceptor from extremophile bacteria Halorhodospira Halophila that swims out of blue light. PYP is supposed to be the responsible for the physiological action. Since the 3D structure of PYP resembles to the PAS domain proteins, PYP is proposed as a model system. The knowledge on how PYP responds to blue light- will shed light on the signal transduction mechanism of the PAS domain proteins. The excitation of blue light triggers the photocycle of PYP through an isomerization of para-coumaric acid chromophore with successive events of the protonation of chromophore, partial-unfolding of protein and resuming of initial state within the seconds of time scale. The partial unfolded state is supposed to be the signaling state. Here, the comprehensive results on the kinetics, dynamics and equilibria of these photocycle intermediates- recently discovered from the transient absorption measurements of the wild type and several key mutants- will be presented. PYP will continue to be the significant part of a newly introduced field of Optogenetics.

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Dual Allosteric Inhibitors Exhibit Antagonistic Effects in the Hepatitis C Virus Polymerase

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The Hepatitis C Virus (HCV) affects close to 200 million people globally. This growing epidemic urgently requires the development of more effective therapies. The HCV polymerase (gene product NS5B) has served as a valuable target for therapeutics due to its critical role in replicating the viral genome. Numerous studies have identified inhibitors for this enzyme, including several allosteric non-nucleoside inhibitors (NNIs). A therapeutic approach that has gained prominence in recent years is the combined use of multiple inhibitors in order to enhance their inhibitory effects. Nevertheless, the molecular mechanisms responsible for enhanced inhibition of NS5B when multiple inhibitors are involved remain unclear, particularly with regard to NNIs. In this study, we use molecular dynamics simulations to understand the mechanisms that govern inhibition when two allosteric NNIs simultaneously bind to the HCV polymerase. Our results suggest that NS5B is able to simultaneously bind dual NNIs at non-overlapping sites. Even though the two inhibitors induce distinct structural and dynamic changes, they are also able to jointly modulate specific enzyme conformations and dynamics. We observe that the overall affinity when two inhibitors bind is comparable to the sum of individual affinities, suggesting that these inhibitors act in an additive manner. We discuss the ramifications of these findings for optimizing the efficacies of combination therapies targeting the HCV polymerase.

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Microsecond Motion Modulates Ubiquitin Binding through an Allosteric Backbone/Side Chain Network

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Motion is involved in a large number of protein functions. Relaxation dispersion (RD) NMR experiments sensitively probe microsecond to millisecond motions. We conducted an in-depth RD analysis of the backbone and side chain methyl groups of ubiquitin. This survey showed a large number of atoms (>30) with microsecond fluctuations. These atoms are distributed throughout the structure. Strikingly, nearly all show the same exchange rate, which suggests that ubiquitin undergoes collective motion involving both the backbone and side chains. Furthermore, comparison of different methyl nuclei indicates that the nature of the side chain fluctuations is almost entirely due to changes in rotamer populations. Thus, collective microsecond backbone motion is coupled to redistribution of side chain rotamer populations through a mechanism we term “population shuffling”. We present a single collective mode of motion that yields a reaction coordinate corresponding to the relaxation dispersion data. The resulting model indicates that a localized conformational