In the present work we investigated the structural dynamics of PEVK, using FRET spectroscopy, on synthetic peptides of different contour length (11 and 21 residues) containing donor and acceptor fluorophores Trp and IAEDANS on the N- and C-termini, respectively. Because in this molecular arrangement FRET efficiency allows the calculation of the equilibrium mean end-to-end distance of the peptides, predictions based on statistical polymer models may be tested, and the effect of solution variables on global configuration may be measured. We find that the scaling of end-to-end distance with contour length deviates from the square-root law predicted for a purely statistical polymer chain, suggesting that the PEVK fragments studied acquire non-random conformation. To explore structural dynamics further, we measured the effect of temperature, chemical denaturation, pH and ionic strength on FRET efficiency. Increasing temperature, pH or ionic strength increased FRET efficiency. By contrast, denaturation with guanidine-HCl resulted in decreasing FRET efficiency. We hypothesize that PEVK may acquire a non-random structure in which electrostatic interactions play an important role. Whether local flexibility of the domain may be tuned by electrostatic mechanisms under physiological conditions, remains to be explored further.

1634-Pos Board B478

Cyanylated Cysteine Is a Site-Specific Vibrational Probe of Disorder-to-Order Transitions In Helical Protein Domains

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The time scale of molecular vibrations allows infrared spectroscopy to be a picosecond probe of fluctuations in the local solvent and the structural environment of distinct vibrations. Solvent-exposed, free cysteine side chains are easily modified to thiocyanate through established reaction chemistry. Following site-directed mutagenesis to introduce cysteine, this modification allows the site-specific placement of thiocyanate in disordered domains implicated in binding or other structure-inducing events. Using a natural system (the Ntail protein from measles virus) and model helical peptides, we demonstrate that the frequency and line shape of the CN stretching band of cyanylated cysteine are sensitive to formation of both secondary structure and tertiary/quaternary or lipid contacts. The CN line shape indicates significant attenuation of the dynamics of water surrounding well-formed secondary structures.

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Effects of Phosphorylation on the unbound states of an intrinsically disordered protein: A Computational Approach

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Intrinsically disordered proteins (IDP) can exist as ensembles of disordered conformations under physiological conditions, and such intrinsic disorder often plays important roles in their functions. The kinase-inducible transactivation domain (KID) from cAMP-response element-binding protein (CREB) has distinct ordered structure with its binding partner KIX, but is mostly unstructured in unbound state. The phosphorylation on Ser133 residue of KID increases the binding potency of the peptide toward KIX, but its impacts the disordered states remain unclear. We have carried out atomistic simulations in an implicit solvent to study effects of above-mentioned phosphorylation on the structure of unbound KID peptide. The results reveal that while the phosphorylation does not affect the average residue helicities, but has importance consequences on the flexibility of the peptide as well as the length and population of the transient helical segments. In particular, phosphorylation appears to restrict the accessible conformational space of the loop connecting two helices, and reduces the entropic penalty of folding upon binding. This entropic contribution, estimated to be ~1.5R from 4D joint backbone torsion distributions of Arg130 and Arg131 residues of KID, supplements the salt-bridges between pSer133 of KID and Lys662 and Tyr658 residues of KIX. This effect was not previously recognized due to inaccessibility of the structural details of the disordered ensembles from experiments. Success of these simulations is very encouraging, and demonstrates the feasibility of an implicit solvent-based computational framework for accurate atomistic simulation of IDPs.

Protein Dynamics II

1636-Pos Board B480

Computational Study of Signal Propagation in The Complex of *Thermus Thermophilus* Leucyl-tRNA Synthetase (leuRS) and Its Cognate tRNA

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Aminoacyl-tRNA synthetases (aaRS's) play a critical role in decoding genetic information located on genome DNA, through catalyzing attachment of their cognate amino acid to 3′-end of the specific tRNA. The fidelity of translation is assured by their strict discrimination of the specific amino acids from non-cognate ones. For Ile, Val, and Leu, which are similar in the sizes and hydrophobicity, their specific aaRS's generate mis-aminoacylated tRNA, such as Val-$tRNA^{Leu}$; those enzymes accomplish ''editing'' through which mis-products are hydrolyzed. However, reaction mechanisms have not yet been clarified; the reasons are as follows: (i) No crystal structures of the enzymes in complex with the mis-aminoacylated tRNA have not yet been determined. (ii) Nucleophile for the reaction has not been identified.
In this study, to perform molecular docking of LeuRS, $tRNA^{Leu}$, and a nonspecific amino acid such as Val, we adopted a novel molecular docking algorithm developed by our group; characteristic features of our scheme are to enable us to predict conformational changes of protein induced by interaction with substrate and waters. Accordingly, this scheme is referred to as the Fully Solvated Dynamical Docking (FSDD). Thereby, we have successfully identified structural water molecules forming stable hydrogen bond networks in the active site of the enzyme. It has been found that one of such waters is located at the appropriate position as nucleophile in the modeled structure. Furthermore, using MD simulations of the LeuRS●Val-$tRNA^{Leu}$ complex, we have identified dynamical motions correlated between two distinct tRNA-binding domains of the enzyme, which are apart by ~100 Å. We have further found that those dynamical properties are induced by the interdomainal communication, for which the signal is propagated through the $tRNA^{Leu}$ molecule connecting the two domains in the complex.

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Transient Nonlinear Infrared Spectroscopy of Ubiquitin Unfolding Dynamics

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We have structurally resolved the nanosecond to millisecond unfolding of ubiquitin with transient amide I two dimensional infrared (2D IR) and dispersed vibrational echo (DVE) spectroscopy following variable temperature jumps. 2D IR and DVE, a measurement related to the 2D IR spectrum projected onto the ω_3 axis, are nonlinear techniques capable of measuring secondary structure content with picosecond time resolution. The equilibrium 2D IR spectrum reveals features resulting from delocalized β-sheet vibrations with dipoles oriented parallel ($\nu_{\parallel}$) and perpendicular ($\nu_{\perp}$) to the strands. Transient 2D IR spectra show a blue shift of the $\nu_{\perp}$ vibration and disappearance of a cross peak between $\nu_{\perp}$ and $\nu_{\parallel}$ over μs to ms time scales. Diagonal peak intensities and homogeneous linewidths also indicate the melting away of sheet structure and the concomitant increased mobility of β-strand amide groups. These changes reflect the sequential unfolding of the β-sheet beginning with the labile strands III-V and followed by strands I-II. This pathway is confirmed through transient DVE of ubiquitin mutants, in which local mutations affect the timescales assigned to specific structures. The free energy landscape is evaluated through comparison of experiment and 2D IR spectra calculated from molecular dynamics simulations of ubiquitin unfolding using a structure-based model. The separation of timescales, stretched exponential relaxation, and probe-dependent response are consistent with the observation of μs downhill unfolding of a sub-ensemble that is prepared at the transition state followed by ms activated unfolding kinetics. The downhill unfolding is characterized through temperature jumps initiated and ending at variable temperatures. The increased downhill unfolding amplitudes and slowing timescales that accompany increases in temperature indicate that multiple unfolding pathways become accessible at higher temperatures.

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Class A β-lactamase backbone dynamics - At the crossroads of molecular dynamics and NMR spectroscopy

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Protein dynamics reveal crucial information about structure-function relationships. We complement the information obtained through NMR spectroscopy relaxation experiments and model-free analysis for class A beta-lactamases TEM-1 and PSE-4 with results from bioinformatics techniques, chiefly molecular dynamics (MD). Molecular dynamics allows the simulation of a protein's dynamics. The timescales probed using this technique differ from those accessible by NMR spectroscopy, giving a more complete picture of the backbone dynamics. Moreover, comparison of order parameters where the timescales of motions are accessible to both methods serves to validate our in silico approach. Also, MD hints at the atomic details associated with a residue's