structural preferences enable promiscuous - yet high affinity - binding to a diverse array of molecular targets.

1627-Pos Board B471
Extreme Mechanical Stability In Polyglutamine Chains Identified Using Single Molecule Force-clamp Spectroscopy
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Huntington's disease (HD) is a genetic neurological disorder linked to the insertion of repeats of glutamine (Q) in the protein huntingtin. The increase in the number of Q results in polyglutamine (polyQ) expansions which self-associate to form aggregates. Significantly, there is a strong correlation between the age of onset in HD and the length of polyQ expansions, with postmortem examinations of HD patients identifying large inclusions in the brain. While polyQ aggregation has been the subject of intense studies, very little is known about the structural architecture of individual polyQ chains. An understanding of the molecular properties of polyQ chains is a necessary first step in building a framework to characterize polyQ expansion diseases. Here we demonstrate a single molecule force-clamp technique that directly probes the properties of polyQ. We have constructed polyQ constructs of varying length, namely Q15, Q25, Q50, Q75. Importantly, this length range spans the region where normal polyQ and diseased polyQ expansions have been observed. Each polyQ construct is flanked by the I27 titin module, providing a clear mechanical fingerprint of the molecule being pulled. Remarkably, under the application of force no extension is observed for all lengths of polyQ. We show this is in direct contrast with the random coil protein PEVK of titin which readily extends under force. Our measurements suggest that polyQ form highly stable mechanical structures. We test this hypothesis by disrupting polyQ with insertions of proline residues. Strikingly, upon interruption with prolines the polyQ constructs readily extend under force. These novel experiments provide the first glimpse of the molecular architecture of polyQ expansions, suggesting these structures are mechanically very stable. Such strong structures would be difficult to unravel and degrade in vivo, resulting in polyQ build-up and subsequent aggregation.

1628-Pos Board B472
Small Molecule Binding of Intrinsically Disordered Proteins: Multiple Binders on Multiple Sites
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We have found that structurally diverse small molecules are capable of specific binding to relatively short segments of intrinsically disordered (ID) proteins. We located such sites on the bHLHZip oncogenic transcription factor c-Myc and on the HLH-only inhibitor of transcription Id2. These proteins are disordered in their monomeric state and only upon dimerization with a partner protein does a stable tertiary structure form. The small molecule inhibitors bind to the ID monomer proteins, affecting their structure at a local level only, preserving the overall disorder and preventing dimerization from taking place.

1629-Pos Board B473
Effect of Vesicle Diameter on α-Synuclein Binding
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Parkinson's Disease is characterized by the presence of fibrillar deposits of alpha-Synuclein (αS) in the *substantia nigra*. αS is an intrinsically unstructured protein that becomes α-helical upon binding lipid membranes. Many studies indicate that the toxic form of αS may be pre-fibrillar oligomers formed in solution or upon binding to cell membranes or synaptic vesicles. The effect of curvature on αS binding was studied by using Fluorescence Correlation Spectroscopy (FCS) to monitor the binding affinity of αS for synthetic lipid vesicles with different diameters, comparing the wild-type protein with three pathological mutants: A30P, A53T, and E46K. Our findings indicate that bilayer curvature does affect the affinity of αS for net negatively charged vesicles, which may be related to the native function of the protein.

1630-Pos Board B474
Rejuvenation Of CcdB-poisoned Gyrase By An Intrinsically Disordered Protein Domain
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Toxin-antitoxin modules are small regulatory circuits that ensure survival of bacterial populations under challenging environmental conditions. The ccd toxin-antitoxin module on the F plasmid codes for the toxin CcdB and its antitoxin CcdA. CcdB poisons gyrase, resulting in inhibition of both replication and transcription. The mechanism by which CcdA actively resolves CcdB:gyrase complexes, a process called rejuvenation, has remained elusive. We have shown that the C-terminal domain of CcdA represents a new class of intrinsically disordered proteins with two distinct but mechanistically intertwined regulatory functions: rejuvenation and transcription regulation. CcdA binds consecutive to two partially overlapping sites on CcdB. This creates two affinity windows that differ by six orders of magnitude and constitutes the key element of a regulatory circuit that links the two functions of CcdA. The first, picomolar affinity interaction triggers a conformational change in CcdB that initiates the dissociation of CcdB:gyrase complexes by an allosteric zipper mechanism. The second, low affinity binding event ensures tightly controlled expression of the ccd operon independent of protection against CcdB activation. The mechanistic complexity of this small network illustrates the potential and versatility of intrinsically disordered proteins for a variety of biological tasks.

1631-Pos Board B475
Fuzzy Complexes: Polymorphism And Structural Disorder In Protein-protein Interactions
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The notion that all protein functions are determined via macromolecular interactions is the driving force behind current efforts, which aim to solve the structures of all cellular complexes. Recent findings, however, demonstrate a significant amount of structural disorder or polymorphism in protein complexes, a phenomenon that has been largely overlooked thus far. It is our view that such disorder can be classified into four mechanistic categories covering a continous spectrum of structural states from static to dynamic disorder and from segmental to full disorder. To emphasize its generality and importance, we suggest a generic term, 'fuzziness', for this phenomenon. Given the critical role of protein disorder in protein-protein interactions and in regulatory processes, we envision that fuzziness will become integral to understanding the interactome.

1632-Pos Board B476
Collapse Of Rat And Human Amylin From Nanosecond-resolved Intramolecular Contact Formation
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Amylin is a 37 residue peptide with hormone properties related to nutrient intake regulating glucose levels. It is found in the form of amyloid deposits in the β-cells of type II diabetic patients. Similar to α-syn and Aβ, it is an intrinsically disordered protein. Little is known about amylin's conformational properties in solution and their relation to function and aggregation.
We have used triplet quenching to monitor the dynamics of end-to-end contact formation between the N-terminal disulfide loop of human and rat amylin and a C-terminal tryptophan. The quenching rates for both species increase significantly in aqueous buffer relative to 6M guanidinium chloride (GdmCl), indicating a decrease in the average end-to-end distance. Comparisons with control peptides suggest that backbone-backbone interactions, involving the N-terminal disulfide loop are the principal driving force for collapse in these peptides, rather than sidechain-sidechain hydrophobic interactions. Molecular dynamics simulations on the control sequences indicate that the collapse results from hydrogen-bonding interactions between the central residues of the chain and the disulfide loop, reducing the length of the free chain by ~ 2-fold. This structural feature may contribute to the functional role of the disulfide loop in amylin and in the larger family of calcitonin gene-related peptides. We discuss the newly observed differences between monomeric human and rat IAPP in solution and their possible relation to aggregation.

1633-Pos Board B477
Conformational dynamics of titin PEVK explored with FRET spectroscopy
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Titin's PEVK domain, which is responsible for the molecule's physiological extensibility, is thought to be an instrinsically unstructured protein region. The structural dynamics, induced conformations, and interactions of the PEVK domain are far from being fully understood.

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.

1635-Pos Board B479

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.

1637-Pos Board B481

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.

1638-Pos Board B482

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