1148-Pos Board B99
Phosphorylation Modulates Conformational Bias of a Disordered Peptide
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Disordered proteins, proteins lacking a stable three-dimensional structure, are often enriched in a broadly conserved sequence composition and patterning that imbues them with residual conformational bias toward the polyproline-II (PII) conformation. Since post-translational modification often effects biological function, we ask how phosphorylation impacts PII bias and overall geometry of a short, phosphorylatable peptide. We developed a method to use time-correlated single-photon counting data to measure Forster Resonance Energy Transfer (FRET), then subsequently calculate polymer properties of the peptide. We explore the impact of phosphorylation on effective persistence length and end-to-end distance, and compare the results of our experiments to behaviors modeled by a hard-sphere collision simulation.

1149-Pos Board B100
Single-Molecule Force Spectroscopy on Unfolded and Intrinsically Disordered Proteins
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Transcription factors and cell-signaling proteins contain a hyper abundance of domains or segments that are intrinsically disordered (ID) under native conditions. In many cases, intrinsically disordered proteins (IDPs) or ID segments undergo coupled folding and binding and this transition is associated to its biological role. Many techniques for dissecting folding are not readily amenable to IDPs because they are aggregation prone and insoluble. Single-molecule methods provide an avenue to mitigate these issues. We have performed single-molecule force spectroscopy experiments on a Trimethylamine N-oxide (TMAO) refolded IDP and found that this IDP unfolds cooperatively under mechanical force. The data are rich in information and reveal that what appears to be a two-state transition in bulk is actually multi-state at the single-molecule level. We found that the analysis methods typically employed in such experiments may not be applicable because of the high concentrations of TMAO. To address these issues, we have designed unfolded mutants of T4 lysozyme using a random mutagenesis phenotypic screen. We are actively performing both bulk and single-molecule experiments to characterize unfolded mutants of T4 Lysozyme and TMAO refolded IDPs.

1150-Pos Board B101
Label-Free Detection of Protein Secondary Structure Content in Biological Specimen by Fourier-Transform Infrared Spectroscopy
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Fourier-transform infrared (FT-IR) spectroscopy, a non-destructive, label-free, and high sensitive and specific analytical method is a vibrational technique that gives information on the chemical composition of a sample of biological specimens. It is also a powerful approach to detect changes in the protein composition and structure of intact living cells. To determine a cellular function or change of biological materials (cells, tissues, microorganisms, etc.), we have studied the amide I band of protein by using both FT-IR spectra based on attenuated total reflection (ATR) and their second derivative spectra. we have performed the breast cancer cells and the pathogenic microorganisms exposed to anticancer and antibiotic drugs, respectively. Based on these studies, we found that the potential to use this technique for the detection and quantification of the protein secondary structure components of biological specimens under drug application

1151-Pos Board B102
Transitional Disorder: Calcineurin as an Example
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How intrinsically disordered proteins and intrinsically disordered regions evade degradation by the cellular machinery evolved to recognize unfolded and misfolded chains remains a vexing question. One potential means by which this can occur is that the disorder is transitional in nature. Calcineurin (CaN) is a highly-conserved, heterodimeric Ser/Thr phosphatase that plays vital roles in memory development and retention, cardiac growth, and immune system activation. Alterations in the regulation of CaN contributes to disorders such as Alzheimer's disease, Down syndrome, autoimmune disorders and cardiac hypertrophy. At low calcium levels the 95 residue regulatory domain in CaN appears to be folded. As levels rise, the CaN B chain binds calcium and undergoes a conformational change that releases the regulatory domain into a disordered state. The subsequent binding of CaM to CaN results in the regulatory domain folding. Folding of the regulatory domain in turn causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. The transitional disordered state of the regulatory domain is essential in the process of activation.

1152-Pos Board B103
A Substantial Improvement in Predictions of Intrinsically Disordered Protein
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The most accurate predictors of Intrinsically Disordered Protein (IDP) structure, as determined by their performance on the DP_NEW data set in 2013 [Mizianty et al] are, for the true positive rate (TPR), true negative rate (TNR), and area under the ROC curve (AUC) respectively: MFDp2: 75.9%, 95.3%, 0.940; and CSpritzL: 83.5%, 85.9%, 0.909. The sum of type I and type II errors for these predictions is, for MFDp2 29%, and for CSpritzL 30%. The Random Forests predictor reported here, named RFDPred, applied to the same test data, yields a TPR, FNR, and AUC of 88.7%, 94.3%(0.4%), and 0.959 respectively, where the sum of type I and II errors is 17%. A similar improvement is seen when RFDPred is tested against the Spritz test data. In a 31 fold cross validation that includes all of Disprot, with no optimizations between tests, TPR, FNR, and AUC are 78.3%, 94.3%(0.4%), and 0.94(0.04), respectively. The hypothesis: residues missing from the PDB evidence are not disordered, was tested with RFDPred. Disprot residues assigned to disordered (D) only on the strength of their being missing from their PDB structures were not counted as disordered in an otherwise same cross validation, TPR, FNR, and AUC improved to 81.1, 96.7, and 0.96(0.01) respectively, and the sum of type I and II errors decreased by 19%. Missing residues (M) are weakly predicted to be ordered and disordered with equal frequency. These results indicate that missing residues should not be assigned as intrinsic disorder without supporting evidence. The improvement seen here is attributed to a much larger set of variables, more than 500, where a PCA yields 130 components, and a much larger training set, including all unique (E-value $>$ 1) PDB data at and below 3 angstroms resolution.

1153-Pos Board B104
Identification of Novel Intrinsically Disordered Proteins in Eukaryotes
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During the past decade, one of the most significant advances in the field of structural biology has been the paradigm-shifting recognition of proteins which are functional under physiological conditions without having a well-defined, rigid 3D structure. Contrary to Anfinsen's dogma indicating that a single unique native structure of a protein is the prerequisite of its function, intrinsically disordered proteins (IDPs) lack stable tertiary structure and exist as ensembles of time-fluctuating structural conformations, while still possessing the ability to carry out functions across a wide spectrum of cellular processes. Due to advantages arising from structural disorder, such as adaptability in partner binding, specific dynamics and enlarged binding surfaces, IDPs are often implicated in complex functions such as cell signaling and gene expression regulation. Bioinformatic predictions suggest that IDPs comprise a significant percentage of numerous eukaryotic proteomes which necessitates high-throughput studies focusing on experimental and computational characterization of such widely-predicted IDPs. In plants, IDPs play important roles in response to abiotic stress, which directly affects plant growth and yield.
We have demonstrated through bioinformatic analyses that the genome of industrially important Beta vulgaris L. plant contains a large portion of IDPs. We confirmed the presence and roles in abiotic stress response for several known but also several previously undocumented IDPs using a combination of experimental methods. In order to gain further functional insights, our studies involve molecular dynamics simulations of the newly identified IDPs for ab initio prediction of pre-formed secondary structural elements in short binding regions termed Pre-Structured Motifs. The existence of functionally important transient secondary structure in some IDPs has previously been shown by others using NMR, advancing recent developments of suitable computational approaches for its prediction and thus enabling a new approach to understanding IDP function.

1154-Pos Board B105
Charge Patterning, Salt Screening and Denaturant Expansion in the CGRP Neuropeptide
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Calcitonin gene related peptide (CGRP) is a neuropeptide of the calcitonin peptide family, which acts as a vasodilator and is involved in the transmission of pain signals in the nervous system upon binding to the correspondent receptor. It also triggers migraine attacks, and is a major therapeutic target for the