thermodynamic stability of this domain. Interestingly, the beta-strand in GPIBa) platelet receptor. G1324A and G1324S are twoa connecting to A1 suffered drastic changes in secondary structure upon association to A1. Overall, our simulations are in agreement with previous experiments of Tischer et al (J. Ba) platelet receptor. G1324A and G1324S are twoiol. Chem. 291, 38483859, 2016) and add further support to their proposed mechanism of conformational restriction of the A1 domain as the main factor determining the loss-offunction of G1324A and G1324S.

2484-Pos

An Atomic Level Interactions of Phosphorylated Tau Repeat with Microtubule using Molecular Modeling Approach Vishwambhar V. Bhandare, Ambarish Kunwar. Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India.

Tau is a microtubule-associated protein abundantly expressed in brain and neuronal cells. Microtubules (MT) are madeup of ab tubulin heterodimeric subunits. Seven a-tubulin and nine b-tubulin isotypes are reported in humans till date, of which bIII tubulin isotypes are predominantly expressed in the neuronal cells and brain. The C-terminal repeat regions composed of R1, R2, R3 and R4 binds to stabilizes the axonal MT. In several neurodegenerative diseases, tau detaches from MT to form insoluble aggregates leading to tauopathy. Phosphorylation of tau is important for the regulating its structure and function. However, hyperphosphorylation of tau is causes its detachment from the MT. The molecular mechanism which triggers detachment of hyperphosphorylated tau from the MT remains elusive. Therefore, we studied interactions of phosphorylated tau repeat region R2 (TauR2) with neuronal specific bIII tubulin isotypes.

Our MD simulation results show a single and double phosphorylation in the TauR2 does not affect the MT binding. However, hyperphosphorylation of TauR2 causes its detachment from the MT. The negatively charged C-terminal tail of the bIII tubulin repels negatively charged phosphorylated TauR2, hence causes the detachment. Also, phosphorylated TauR2 shows poor binding affinity towards neuronal specific MT composed of bIII tubulin isotypes.

Our strategy can be potentially used to understand detachment of tau from the MTs composed of different b-tubulin isotypes expressed in specific neurodegenerative disorders. We hope that knowledge of precise molecular interactions between phosphorylated tau repeat and neuronal specific MT will pave the way for developing effective treatments against tau related disorders.

2485-Pos

Investigating Novel Hetero-FRET Biosensors for Environmental Ionic Strength using Experimental and Theoretical Approaches

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The ionic strength in living cells is compartmentalized, heterogeneous, and dynamic. Mapping ionic strength in living cells at high spatial and temporal resolution is therefore essential in understanding its effect on cell pathology, intracellular catalytic activities, and protein-protein interactions. In this contribution, we examine the sensitivity of novel ionic-strength biosensors (namely, RE, KE, and RD) that can be genetically encoded in living cells. These biosensors consist of mCerulean3 (a donor) and mCitrine (an acceptor) tethered together with a linker of two charged alpha helical regions, where their electrostatic interaction is sensitive to the environmental ionic strength. Using time-resolved resolved fluorescence and anisotropy measurements, we quantified the fluorescence resonance energy transfer (FRET) efficiency and donor-acceptor distance in these biosensors as a function of the Hofmeister salts concentration. Control studies were carried out on the enzymatically cleaved counterpart of these biosensors as well as E6G2 with neutral alpha helical regions. Our results indicate that as the ionic strength increases, the energy transfer efficiency decreases with an increase in the donor-acceptor distance of these biosensors. There is minor, but consistent, sensitivity of these biosensors to the type of salt solutions within the biologically relevant range of ionic strength. We developed a theoretical framework to model the observed ionic-strength dependent trends of the energy transfer efficiency in terms of the Debye ionic screening of the electrostatic interactions between the two charged alpha helical regions. Our characterization of these biosensors in controlled environments is an important step towards mapping the ionic strength in living cells using fluorescence lifetime imaging microscopy.

2486-Pos

Development of New Methods for Enhanced Conformational Sampling of GPCRs

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G protein coupled receptors (GPCRs) are integral membrane proteins that enable a cell to respond to extracellular stimuli like light, small molecules, peptides, and proteins. These receptors have evolved as highly flexible proteins that possess multiple functionally important conformational states, which enable these receptors to activate pleiotropic signaling events inside the cells. Recent progress in membrane protein structural biology techniques and cryo-electron microscopy is providing structural evidence of two or more distinct receptor conformations for these receptors. Computational structural modeling approaches can complement and supplement these studies by predicting all functionally important states of a receptor, however, they run into the classical protein conformational sampling problem. We are developing a computational biophysical method called Enhanced Conformational Markov-state Sampling in Membrane BiLayer Environment (EnCoMSeMBLE) to solve this sampling problem for GPCRs. We are utilizing Markov-State-Models to combine the Boltzmann sampling of receptor conformations from molecular dynamics (MD) with the brute-force sampling of receptor conformations from our previously developed ActiveGEnSeMBLE method. The method was applied to the A_{2A} receptor starting from the active state by only using traditional MD simulations. This is not surprising because the pre-activce states is a downhill direction on the energy landscape. However, applying both MD and ActivateGEnSeMBLE methods was able to predict pre-active states from the inactive states which, classical MD cannot achieve. Our results indicated that our methods provide an unbiased method to sample the conformational landscape. This can protentional sample unexplored regions of the conformational landscape and find novel conformations that can elucidate the mechanistic properties that GPCRs possess.

Posters: Protein Stability, Folding, and Chaperones II

2487-Pos

Mechanism of the Disulfide-Coupled Folding of a de novo Designed Prouroguanylin Protein

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The folding of uroguanylin into its native conformation is assisted by the propeptide region, which functions as role as an intra-molecular chaperone. Our previous findings regarding the disulfide-coupled folding of prouroguanylin suggested that a mis-bridged disulfide isomer is stabilized by the formation of slightly larger a-helix structure at the processing site between the propeptide and the mature region at the early stage of the refolding reaction. This processing is under kinetic control and the molecule is then converted to the native conformation via the formation of a b-sheet between the pro-peptide and the mature region along with disulfide shuffling under thermodynamic control.To further investigate and utilize the intra-molecular chaperone function of the propeptide region, we designed a de novo peptide (NDD hybrid peptide) which is hard to fold into its bioactive conformation (G-type: Cys1 and Cys3, and Cys2 and Cys4) by itself, and also designed a de novo protein (pro-NDD hybrid protein), which is able to fold into only G-type, via the fusion of the propeptide region of uroguanylin. Our previous results for refolding reactions of the hybrid protein and peptides suggested that the topological isomer with the same disulfide pairings as G-type plays an important role as an intermediate in the actual folding.In order to further study the folding mechanism of the de novo designed protein, we focused on

the hydrogen bond between the side chain of the Asn residue in the mature region and the carbonyl group at the a-helical structure of the processing site of prouroguanylin. Therefore, a series of mutant of pro-NDD hybrid proteins that were devoid of hydrogen bonds were recombinantly expressed in E. coli cells, and the refolding reactions examined. The results will be discussed in this paper.

2488-Pos

Single-Molecule AFM Imaging of Thermally Denatured Firefly Luciferase

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Firefly Luciferase (FLuc) has been widely used as a substrate to study the mechanisms by which heat shock proteins, specifically the Hsp70 family of chaperones in E.coli (DnaK, DnaJ, and GrpE), assist refolding denatured proteins to their native conformation. However, the denaturation and aggregation of FLuc has rarely been investigated at the single molecule level, despite how critical it is to understand the different states at which FLuc is after different denaturation procedures prior to the addition of the Hsp70 family. Here, we visualize FLuc following different thermal denaturation protocols with varying temperature (42-50 C), FLuc concentration (10 nM - 2 mM), and duration of denaturation (2-20 min) using AFM imaging in air and liquid. We monitor the bioluminescence of each sample to measure the percentage of denatured protein prior to our AFM imaging. We recorded a large number of FLuc images in both its native state and various thermally denatured states, and carried out the following quantitative analysis. We correlated the volume, area, and height of denatured protein from our AFM images with the bioluminescence activity to identify different states of denatured FLuc. This study will help guide interpretation of future Hsp70 studies by furthering the understanding of the denatured FLuc substrate which is at the center of many Hsp70 studies.

2489-Pos

Investigation of Mechanically Labile Type III Secretion Protein Effectors

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Gram-negative pathogenic bacteria have evolved complex machinery to secrete protein effectors, which facilitate establishing a replicative niche and infection. One such complex, the Type III Secretion System (T3SS), spans both bacterial membranes and engages host plasma membrane. Like a syringe, the T3SS can thus inject these protein effectors through its needle directly into the host cell. Due to the narrowness of the needle, protein effectors must be unfolded prior to secretion and then refold once in the host cell. This constraint poses a unique structural challenge for effectors, in which they need to be mechanically unstable to unfold efficiently but also thermodynamically stable, so that they return to their folded state inside the host cell. Previous work in the Sousa lab using single molecule force spectroscopy (SMFS) discovered some of these effectors mechanically unfold under very little force despite ordered folds. However, further characterization and comparisons to their non-secreted homologs is still required. This project aims to thoroughly characterize the mechanical instability of protein effectors compared to their non-secreted homologues, engineer said homologs with comparable mechanical lability, and visualize their secretion dynamics with a live cell assay.

2490-Pos

Denaturing Effect of Guanidine Hydrohloride on Amyloid Fibrils

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According to modern concepts, monomeric proteins under native conditions have a unique three-dimensional structure determined and optimized evolutionarily. This structure encoded by amino acid sequence that ensures the correct folding of protein molecules. Amyloid fibrils formed from the same protein under different conditions, on the contrary, can vary in their structure. Given the significant differences in the morphology of amyloid fibrils and monomeric proteins, it can be assumed that conformational stability of these states of the proteins also varies significantly. To revealing these differences denaturing effects can be used. In this work the depolymerization of amyloid fibrils formed from lysozyme and Abeta-peptide induced by denaturating agent guanidine hydrochloride was studied. Using the wide range of physicochemical approaches (including specially elaborated) we showed that amyloids are far from as stable as they are considered (even less stable than monomeric proteins). These results are a step towards identifying effects that can lead to degradation of amyloids and their clearance in vivo without adverse effects on the functionally active state of the proteins forming them.

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2491-Pos

Structural Dynamics of Mammalian Prion Protein Correlates with Degree of Susceptibility to Prion Diseases

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Prion diseases, featured by the autocatalytic misfolding of the cellular form of the prion protein, PrP^{C}, to the infectious form, PrP^{Sc}, are fatal neurodegenerative disorders in mammals, including humans. Compelling evidence correlates gene polymorphisms with susceptibility to developing prion diseases. Intriguingly, the prion gene and protein overall fold are highly conserved. Prion protein from bank voles, for example, is considered to be a universal receptor for a wide range of prion strains. On the opposite, prion protein from rabbits show low susceptibility. We hypothesize that the local structural dynamics encoded in PrP^{C} is distinct in the prion protein of each species and may be modulated by the extracellular space. Using structural bioinformatics techniques, our study examines the conformational dynamics of PrP^{C} from a number of species and the effect of the cell surface environment to which PrP^{C} is anchored. Our results indicate that while the overall shape of the prion protein from each species is similar, distinctions in the network of residue interactions result in distinct conformational coupling between secondary structure elements. We interpret our results in light of the zoonotic potential of prion diseases.

2492-Pos

Characterizing the Interplay between Dynamics and Regulation in the Trypsinogen/Trypsin Protease System

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Trypsin (Tn) is a digestive serine protease found in eukaryotes and prokaryotes. Tn is manufactured by the vertebrate pancreas as its proenzyme trypsinogen (Tgn) which includes an N-terminal six amino acid zymogen region. The zymogen form, Tgn, is disabled against cleavage of substrate until Tgn reaches the small intestine. Within the small intestine, enterokinase cleaves the inhibiting peptide from folded Tgn to release active Tn protease. Fluorescence experiments with bovine Tn demonstrate remarkably slow global unfolding, with a kinetic barrier to unfolding as large as kinetically stabilized bacterial homologues. Whereas bacterial homologues have evolved to resist proteolysis, thereby prolonging extracellular activity, mammalian Tgn/Tn is tightly regulated through a series of auto-cleavage events. We propose that Tgn/Tn experiences subglobal dynamics, and that sampling of these semi-unfolded states under physiological conditions causes this rapid autolysis. To explore this, we will perform native-state hydrogen exchange mass spectrometry of a purified inactive mutant. Exploring the Tgn/Tn subglobal dynamics which expose the native-state to autolysis will grant understanding of the link between Tn dynamics and the regulation of its function through its specific sequence of autocleavage events. By comprehensively characterizing the sampling of subglobally and globally unfolded states of Tgn/Tn, more can be inferred about the occurrence of non-native state protein aggregation and susceptibility to proteolytic attack. Studying Tgn/Tn from the inactive Tgn/Tn mutant allows direct assessment of the role of subglobal protein dynamics and the regulation of these dynamics in Tn folding

disorders without the complication of autoproteolysis. The Tgn/Tn system will allow investigation of diseases characterized by abnormal protein aggregation and vulnerability to proteolytic attack, in addition to diseases directly related to Tn function such as pancreatitis.

2493-Pos

Evaluation the Protein Stability by Molecular Dynamics Simulation Tomoshi Kameda[1], Kaito Kobayashi[1], Shin Irumagawa[2], Ryoichi Arai[2], Yutaka Saito[1], Takeshi Miyata[3], Mitsuo Umetsu[4].

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Protein stability has been a central issue of biophysics, and improvement of protein stability is important for biotechnology and pharmaceuticals. We propose a novel method to evaluate the protein stability using molecular dynamics simulation. We applied the method to 5 proteins with various structure (alpha only, beta only and both alpha and beta), and the result showed good correlation between experimental melting temperature with predicted one. Our study also shows that it is possible in silico screening of high stable mutants using this method.

2494-Pos

Using Circular Permutation to Probe the Role of Chain Connectivity in the Co-Translational Folding Process of Halotag Natalie R. Dall, Susan Marqusee.

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