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Ice Growth Control with Ice-Binding Proteins
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Ice-binding proteins (IBPs) depress the freezing point of body fluids below the melting point, resulting in a thermal hysteresis (TH) that prevents freezing of the organism. The potential of these proteins in the medical sector, in cryopreservation, in the frozen food industry, and in agriculture is enormous. We are investigating the mechanism by which IBPs interact with ice surfaces and inhibit ice growth and recrystallization. We have developed novel methods for these studies, including fluorescence microscopy techniques combined with temperature-controlled microfluidic devices. These techniques have enabled the replacement of the IBP solution surrounding an IBP-bound ice crystal by buffer, without losing the bound IBP or the TH activity. Our results show the irreversibility of the protein:ice interactions and the indirect dependence of TH activity on the protein concentration in solution. We found that the dynamics of the interactions with ice vary dramatically between different types of IBPs. From our results and other recent developments a new understanding of the mechanisms by which various IBPs act is emerging. This understanding is critical for the successful use of IBPs in cryobiological applications.
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Website: http://www.agri.huji.ac.il/~braslavs/
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A Two-Color Non-Muscle SERCA FRET Sensor for Diabetes Drug Discovery Using Fluorescence Lifetime Detection
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We have developed intramolecular FRET sensors capable of detecting cytoplasmic headpiece movements of human SERCA (sarco/endo-plasmic reticulum calcium ATPase) in live-cell assays, including a non-muscle SERCA2b isoform to be used in drug discovery for treatment of diabetes. Two fluorescent proteins, clover (green) and mRuby2 (red) were directly fused to selected locations on human SERCA1a (skeletal muscle), 2a (cardiac muscle), and 2b (non muscle), based on a previously reported SERCA2a construct (Gruber et al., J. Biol. Screening, 2014), and expressed stably in HEK cells. We have used these cells in a novel fluorescence lifetime plate reader (FLT-PR) to screen small-molecule libraries, to discover modulators of SERCA structure and function. The present study focuses on SERCA2b, with the goal of obtaining small molecules that activate SERCA in non-muscle cells. Since recent reports indicate that SERCA overexpression in non-muscle cells can alleviate Type II diabetes, we seek small-molecule SERCA activators for the same purpose. The small-molecule modulators identified in the high-throughput FRET screen were examined for their ability to affect SERCA's function, through assays of ATPase and calcium pumping activities. In order to obtain functional data more directly related to Type II diabetes, we tested the compound's alleviation of endoplasmic reticulum stress in 3T3-L1 adipocytes, using an XF24 Extracellular Flux Analyzer to measure mitochondrial function after inducing ER stress with the inflammatory cytokine TNF-α. While this study is designed to find activators of SERCA2b for treatment of diabetes, constructs based on other SERCA isoforms show promise in targeted therapeutics for muscular dystrophy (SERCA1a) and heart failure (SERCA2a).

1739-Plat
A Novel Molecular Ruler between Fluorescent Proteins
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Förster Resonance Energy Transfer (FRET) is a phenomenon that allows the direct measurement of molecular events through macroscopic observations. The simplicity of FRET translates to its robust performance in complex environments such as living cells, making it immensely powerful in molecular biology. Here, we demonstrate an analogous molecular event between fluorescent proteins, which relies on a different mechanism but shares many parallels with FRET. We demonstrate an exquisite distance sensitivity that allows us to distinguish the lengthening of a protein linker by 1 helical turn in live cells. Further, coupling this discovery with known molecular switches forms the basis of a novel class of biosensors. We therefore report the superresolution imaging of kinase activity and protein-protein interaction for the first time. We utilize one such biosensor to scrutinize the spatial activity architecture of cAMP-dependent protein kinase (PKA). Our results directly confirm the compartmentalization of PKA signaling, and illuminate their characteristics in adherent and migrating cells. In summary, we report a novel molecular ruler that parallels FRET in many aspects and will be useful in a similarly wide range of applications.

Platform: Protein Fold Stability

1740-Plat
The Folding of SasG: A Long and Remarkably Strong Monomeric Protein Responsible for Biofilm Formation is a Highly Cooperative System
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SasG has a long repeat region made up of identical repeating E and G5 domains. Although the domains themselves are relatively unstable (indeed E domains on their own are unfolded), the cooperative folding of the domains results in formation of molecules that are long and remarkably mechanically resistant. We have used small angle X-ray scattering and mechanical unfolding methods, combined with simulations, to show that SasG constructs of physiological length are indeed monomeric, highly extended and mechanically strong. Obligate folding cooperativity of the intrinsically disordered E domain couples spatially separate G5 domains both thermodynamically and structurally, creating a superstructure that supersedes the domain architecture. Our findings provide a simple solution for the efficient assembly of mechano-resistant elongated structures of tunable length from a single polypeptide chain and have significant potential for the development of novel biomaterials.

1741-Plat
Putting on the Squeeze: Solution NMR Investigations of Protein Structure and Hydration under High Pressure
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It is well known that high hydrostatic pressures can induce the unfolding of proteins. The physical underpinnings of this phenomenon have been investigated extensively but remain controversial. Changes in solvation energetics due to applied hydrostatic pressure have been a commonly proposed mechanism for unfolding, but recent studies have provided strong evidence that elimination of void volumes in the native folded state is a principal determinant. Here we use the cavity-containing L99A mutant of T4 lysozyme to examine the pressure unfolding of a multi-domain protein using solution NMR. The cavity-containing C-terminal domain completely unfolds at moderate pressures while the N-terminal domain remains largely structured to high pressures. This pressure response is completely suppressed by benzene binding to the hydrophobic cavity. These results contrast to the pseudo wild type protein, which has a residual cavity volume very similar to that of the L99A-benzene complex but shows extensive subglobal reorganizations with pressure. Encapsulation of the L99A mutant in the aqueous nanoscale core of a reverse micelle suppresses the pressure-induced unfolding transition due to the volume restriction and promotes high-pressure filling of the cavity with water. This result indicates that hydration of the hydrophobic cavity is more energetically unfavorable than global unfolding. Overall these observations point to a range of cooperativity and energetics in the pressure response of proteins and illuminate the fact