elevated in short-term, but not long-term, exposure. Our work discovered that ELF-EMF exposure could elevate de novo generation and propagation of yeast prion and supports the hypothesis that ROS may play some roles in the effect of ELF-EMF on protein misfolding. Both ROS and proteins are widely involved in various biological processes, therefore, our finding may be of importance for uncovering the mechanism underlying the biological effect of ELF-EMF.

1575-Pos

Building a mimetic system for unraveling protein-protein interactions on membranes

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Eukaryoteshave proteinsanchoredto the cells'plasma membrane viathe glycolipid anchor glycosylphosphatidylinositol (GPIs). GPI anchored proteins (GPIAPs) can play a role in the protection, regulation and activation of cells via protein-protein interactions. Studies of GPIs and GPI-APs are hindered by the difficulty of isolating and producing these complex molecules in pure forms. Herein, we apply a semi-synthetic method to synthesize well-defined GPI-APs by combining protein expression and chemo-selective attachment of synthetic GPI to proteins. Specifically, we will express the 19 kDa fragment of the Plasmodium berghei merozoite surface protein 1 (MSP1-19) and ligate it to a synthetic GPI. The GPI-anchored MSP1-19 will be inserted into cell-sized model membranes (giant unilamellar vesicles) of different composition and into supportedlipidbilayerstoinvestigatetheroleofGPIsonMSP1-19anchoredprotein using various fluorescence microscopy-based techniques and surface plasmon resonance. MSP1-19 protein is rich in cysteine. Evaluating its folding is a warranted prerequisite to studying, how MSP1-19 integrates into model membranes and how the structure of the GPI anchor affects this. We utilize a NMR based approach to study the protein folding post expression and ligation.

1576-Pos

To refold or not to refold: first generation undergraduate students study refolding across the tree of life

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Our laboratory has previously used a limited proteolysis mass-spectrometry (LiP-MS) approach to show that a third of the soluble E. coli proteome is unable to efficiently refold to their native structures on physiological timescales following denaturation. In this method, we take advantage of a permissive protease that preferentially cleaves at more flexible regions to examine structural differences between native and refolded lysates. Here, we seek to apply this technique to the proteomes of different mesophilic fungi and bacteria across various phylogenetic clades. These studies were accomplished by first generation college freshmen conducting research in the Fried Lab. By comparing the refoldability of orthologous proteins across different species, we determine that refoldability is a conserved trait among certain - but not all - protein families. These results illuminate which proteins can universally refold and which proteins have potentially diverged to require co-translational folding or chaperones for assembly.

1577-Pos

Mutations protective against prion disease redirect the folding pathway of PrP in single-molecule trajectories

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Propagation of misfolded prion protein (PrP) is associated with a wide range of prion diseases in mammals. Variations in the amino acid sequence of PrP appear to play a key role in determining the disease susceptibility for any species. Several single-point mutations in PrP have been identified as protecting against prion infections, apparently by preventing PrP misfolding and/or conversion. However, the mechanism by which these mutations act to protect against disease is unclear. Here we investigate two such protective mutants: G127V, found in humans and N159D, found in canids. We inserted these mutations into PrP from bank voles, which are the most prion-susceptible species known, and used optical tweezers to study the unfolding and refolding of single mutant bank vole PrP molecules. We found that these mutants differed in several aspects of their unfolding and refolding behavior: G127V folded homogeneously with a narrow force range, whereas N159D showed significant heterogeneity in folding with a wide range of unfolding forces. Notably, both mutations showed no evidence of forming metastable misfolded states, as seen in wild-type bank vole PrP. However, both mutations led to a similar effect on the native folding pathway: analyzing the transition maps showing all the transitions between distinct structural states of the protein in hundreds of unfolding and refolding trajectories, we found that the protective mutations redirected the pathways away from a particular partially folded intermediate into the same new intermediate states that were not present in the folding of the wildtype protein. We propose that this change in the pathway avoids an intermediate that is particularly misfolding-prone, thereby protecting against misfolding disease.

1578-Pos

Engineering robust chaperone substrates for refolding studies using the highly bioluminescent protein NanoLuc

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Chaperones play critical functions during protein de novo folding, refolding after denaturation and during translocation and degradation in all organisms. The Hsp70 chaperone system (DnaK/J/E in E. coli) has been studied over more than 40 years, yet its mechanism remains poorly understood. This is partly, due to the complexity and fragility of its most studied protein substrate, namely Firefly Luciferase (550 amino acids), which even in its native state is rather unstable and displays a complex co-translational-like folding behavior that obscures chaperone contributions to its refolding, following thermal or chemical denaturation. Therefore, in this work we aimed at developing a new, simpler, and more robust DnaK/J/E substrate that could allow deciphering mechanistic details of the chaperones reaction. As a promising starting candidate, we chose NanoLuc, an engineered (171 aa) highly bioluminescent enzyme (100 fold brighter than Firefly Luciferase) that is very stable under most stress conditions, such as chemical (urea) and thermal denaturation. While monomeric NanoLuc denatures minimally at 58C and refolds spontaneously without chaperones, NanoLuc-based tandem repeat proteins that we engineered proved to be excellent Hsp70 substrates. Specifically, two and three tandem repeats of the NanoLuc monomer (NLuc-NLuc and NLucNLuc-NLuc) remain stable for a long time at room temperature but unlike NanoLuc monomer denature significantly at 58 C, and they both require DnaK/J/E and ATP for successful refolding, when they recover more than 70% of their original bioluminescent activity. Interestingly, a similar behavior was also observed for a hybrid polyprotein composed of NanoLuc monomer units that we assembled into a polyprotein, where they were separated by titin I91 domains (I91-Nluc-I91-Nluc-I91-Nluc-I91). The three NanoLuc-based protein constructs that we engineered qualify as superb substrates for studying Hsp70 refolding reactions and their use promises to lead to mechanistic breakthroughs.

1579-Pos

Probing charged aminoacid sidechains in protein-protein interfaces using NMR

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Topoisomerase V from Kandleri plays an important dual role in DNA isomerization and repair. The 12 helix-hairpin-helix ((HhH)2) domains are responsible for DNA binding and for the AP lyase activity of the protein. Previous work in the Barrick lab on this system has shown that repeats can be expressed individually and in pairs, and Gdn-HCl-induced unfolding transitions can be fit using a global Ising fit to determine the intrinsic and interfacial free energies of the entire array. Both the intrinsic and interfacial free energies show a broad range of stabilities. To begin to understand the structural origins of interfacial stability we have focused our attention on the interface between the domain repeats H and I. This interface buries 675 square angstroms of surface area and has an interfacial free energy of about 3 kcal/mol. The crystal structure shows two salt-bridges that are formed across the HI interface. The first is between residue glutamic acid in H and lysine in I; the second is between residue aspartic acid in H and arginine in I. We have used heteronuclear NMR to attempt to identify side-chain interactions that contribute to the stability of the HI interface. ^{15}N, ^{1}H NMR spectra reveal a highly solvent-protected lysine

residue; as a result, we can directly observe this side-chain amide ammonium group in a ^{15}N, ^{1}H -HSQC spectrum. This is unusual, since, lysine ammonium groups are not observed in such spectra due to rapid exchange with water. We have developed some new and highly sensitive NMR techniques using optimal control pulses to assign and characterize charged sidechain residues on the interfaces. These experiments enable us to determine the contribution of these specific residues to the stability of HI interface through chemical shift perturbation studies and hydrogen exchange.