(CIDR) mechanism [5, 6]. Using synchrotron small-angle X-ray scattering (SAXS), we show that n-dodecyl-β-D-maltoside solubilized bovine rhodopsin is reconstituted spontaneously to form 2-D proteomembrane arrays, which in turn are coupled along the trans-membrane direction to form a 3-D multilamellar structure. The lamellar periodicity is ~5.7 nm, which matches closely the trans-membrane dimension of rhodopsin. Using time-resolved UV-visible spectroscopy, we are currently examining the photoactivity of embedded bovine rhodopsin, and its dependency on the surface charge states and membrane moduli of the artificial membrane. [1] M. F. Brown (1997) Curr. Top. Membr. 44, 285-356. [2] A.V. Botelho et al. (2006) Biophys. J. 91, 4464-4477. [3] A.V. Struts et al. (2014) Meth. Mol. Biol. (in press). [4] V. Subramaniam et al. (2005) JACS 127, 5320-5321. [5] L.J. Kuang et al. (2014) ACS Nano 8, 537-545. [6] D.B. Hua et al. (2011) JACS 133, 2354-2357.

2530-Plat

Coarse-Grained Modeling of Minute-Timescale Co-Translational Membrane Protein Integration via the Sec-Translocon

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Integration into the cell membrane in the correctly folded structure is essential for membrane protein function. Membrane protein integration takes place at the Sec-translocon and typically occurs during translation of the protein sequence. Although extensive structural data is available, study of the dynamics of Sec-facilitated membrane protein integration is difficult due to roles of long-timescale dynamics and ribosomal translation. We present a coarse grain simulation approach that is capable of reaching the experimentally relevant (i.e., minute) timescales, while retaining the level of detail required for reproducing available experimental data. The CG model enables direct simulation of the dynamics of co-translational integration at the Sec-translocon and analysis of the resulting trajectories provides mechanistic explanations for observed experimental data. We will demonstrate use of this CG model to investigate thermodynamic and kinetic factors that govern membrane protein topology, integration efficiency of trans-membrane domains, and hydrophilic loop translocation.

Platform: Protein Folding and Chaperones

2531-Plat

Proteomics-Level Identification of Degradation-Resistant Proteins Provide Insight about their Potential Roles in Organismal Adaptation to Stress

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Some proteins are hyperstable, as demonstrated by their resistance to proteolysis and detergents, and their long half-life. Some of these proteins are characterized by having a high energy barrier toward unfolding that virtually traps them in their native state. This property of proteins, known as ''kinetic stability'' (KS), appears to be a strategy used by ''mother nature'' to protect certain proteins against aggregation, and premature degradation under stress conditions. In addition to saving organismal resources by avoiding frequent degradation-biosynthesis of proteins, KSPs could function under extreme stress to maintain vital functions needed for survival. On the basis of our observed correlation between KS and a protein's resistance to the detergent SDS, we developed a diagonal two-dimensional (D2D) SDS-PAGE method for the proteome-level identification of KSPs. We applied D2D SDS-PAGE to the lysate of Thermus thermophilus and E. coli followed by proteomics analysis, and identified over 100 KSPs. We also analyzed the cell lysates of various prokaryotic and eukaryotic organisms. Our results suggest that KS was likely a critically important property of proteins for the adaptation and survival of microbial organisms under stress conditions. In contrast, the minimal abundance of KSPs in eukaryotic organisms implies an evolutionary compromise of KS in favor of more complex cellular defense, function, and regulation. Thus, D2D SDS-PAGE is a simple and powerful method that may be applied to any complex mixture of proteins to explore the biological and pathological significance of protein kinetic stability.

2532-Plat

Surprising Abundance of Misfolding during Refolding of Multidomain Proteins

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The covalently linked domains constituting a multidomain protein typically share a similar fold and sequence, are generally stable in isolation, and are largely capable of independent folding. However, the elegant interplay of forces leading an amino acid sequence to its native state is more complex in proteins with multiple domains because of inter-domain interactions, which complicate the folding energy landscape.
In previous experiments, employing immunoglobulin-like (Ig-like) domains of the human multidomain protein titin, we showed that tandem repeats of domains with high sequence identity can form a stable misfolded state upon refolding in physiological solution conditions. Conversely, tandem constructs of natural neighbouring domains, which display a low sequence identity, did not misfold. This supported the hypothesis that sequence identity between neighbouring domains in multidomain proteins is reduced as a result of evolutionary pressure to avoid misfolding.
With a combination of single-molecule Förster resonance energy transfer, microfluidic mixing, stopped-flow kinetics and molecular dynamics simulations, we now demonstrate that Ig-like domains can transiently populate a surprisingly broad range of misfolded conformations on the sub-second timescale. Using tandem repeats of Ig-like domains, we can resolve both strand-swapped misfolds dominated by native-like interactions and, remarkably, a non-native-like, largely disordered type of misfolded state which so far was never observed experimentally, characterized by promiscuous interactions. Even more surprisingly, both types of misfolding are detected also for the naturally occurring tandem repeat, showing how finely the propensities of folding and misfolding have been balanced by co-evolution of adjacent domains to avoid stable misfolded states formation. On longer timescales, however, all or most of the protein molecules are able to reach the native state, demonstrating that the overall free energy surface is still sufficiently optimized for the protein to efficiently reach its correctly folded state.

2533-Plat

Two-Dimensional Fluorescence Lifetime Correlation Spectroscopy on the Folding Mechanism of B Domain of Protein A

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Molecular-level description of the folding process of proteins is of fundamental importance for understanding how proteins acquire their unique conformations to show various biological functions. Elucidation of the folding mechanism requires quantitative analysis with a high time-resolution. To this aim, we recently developed two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS)1,2. This method enables us to examine the microsecond conformational dynamics of proteins at the single-molecule level, through the analysis of the fluorescence lifetime correlation. In this study, we applied 2D FLCS to study the folding mechanism of B domain of protein A (BdpA). This small protein was believed to show a two-state folding process in which the native state unfolds without exhibiting any intermediate states. However, a recent single-molecule study suggested that the folding mechanism of BdpA is not so simple3. To quantitatively analyze the complex folding process of BdpA, we performed 2D FLCS for two FRET mutants of BdpA. The results showed that the conformations of both the native and unfolded states are highly heterogeneous and that the conformational dynamics within each conformational ensemble occurs on a time scale shorter than ten microseconds. Furthermore, our results indicated that the conformational distribution in the native and unfolded ensembles gradually change with the change of the denaturant concentration.
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2534-Plat

Conformational Dynamics of Molecular Chaperones Investigated by Single Molecule Multicolor Förster Resonance Energy Transfer

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Molecular chaperones of the heat shock protein family 70 (Hsp70) are ubiquitously expressed and involved in a variety of cellular processes. While participating in protein folding and protein complex remodeling, Hsp70 chaperones found in cellular organelles additionally assist in protein import, protection from misfolding, and transfer to the proteosomal machinery.