2318-Pos

Separating the thermodynamic impact of non-specific interfacial interactions from confinement effects on protein stability

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The intracellular milieu is incredibly complex, containing myriad degrees of spatial restriction and highly variable interfacial chemistry. It is well established that this environmental complexity impacts diffusive properties, stability, and functional rates of proteins. The relative thermodynamic contributions of confinement versus non-specific interfacial interactions (quinary interactions), however, remain poorly understood. We are using reverse micelles (RMs) as a tool to differentiate between these effects. RMs are spontaneously organizing nanopools of water stabilized by surfactants in bulk non-polar solvent. By using optimized surfactant mixtures, the native states of proteins can be maintained in the RM water core. We have executed folding studies on a wide variety of proteins using varied surfactant mixtures with uniform degrees of confinement. We investigate both equilibrium folding thermodynamics and folding kinetics using various spectroscopic approaches. Our findings begin to unveil the complex relationship between a protein's thermodynamic stability and its local solvating environment, helping to dissect the differential impacts of confinement and quinary interactions.

2319-Pos

Mapping protein energy landscapes with X-ray footprinting mass spectrometry

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Understanding how the linear sequence of a protein can encode the diversity of protein structures and functions has been a central goal of biology for the last half-century. Recent advances now allow one to predict the three-dimensional structure for a given protein sequence with incredible accuracy; the sequence of a protein, however, encodes much more than just this native structure - it encodes the entire energy landscape - an ensemble of conformations whose populations (energetics) and dynamics are finely tuned. Therefore, proteins should not be thought of as single static structures but as statistical ensembles. To truly predict protein function from sequence, we must also understand protein energy landscapes. Unfortunately, current methods for measuring protein stability suffer from several limitations, requiring milligram quantities of purified protein in isolation at micromolar concentrations. We have developed an approach based on x-ray radical footprinting mass spectrometry (XF-MS), in combination with chemical denaturation, that overcomes these challenges and can accurately measure protein stabilities using $\sim 10^3$ fold less protein, including following the stabilities of individual proteins in complex mixtures. Importantly, we can follow the energetics of individual regions in a protein allowing us to study complex multidomain proteins refractory to standard tools for measuring protein stability. Together, this approach enables us to dramatically increase the number of proteins for which we can quantitatively measure energy landscapes.

2320-Pos

The purely thermodynamic anti-prionic mode of action for the treatment of neurodegenerative diseases - realized by target protein specific all-D-peptides

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Neurodegenerative protein-misfolding diseases, like Alzheimer's (AD) and Parkinson's disease (PD), are driven by prion-like self-replicating and propagating protein assemblies of Aβ, α-synuclein, and many more. Therefore, we have developed the so-called anti-prionic mode of action (MoA). It is realized by all-D-enantiomeric peptide ligands that bind the monomeric protein of interest with high affinity, thereby stabilizing the physiological intrinsically disordered monomer structure. We call this purely thermodynamic driven mode of action ''anti-prionic'', because it is eliminating already existing prion-like aggregates by disassembling them into non-toxic and functional monomers. I will summarize the current, and yet unpublished, progress in realizing the anti-prionic MoA for the target proteins α-synuclein and Aβ. Briefly, the most promising all-D-enantiomeric ligand for α-synuclein, SVD-1a, disassembles preformed α-synuclein fibrils (PFF) as shown by AFM, DLS and SEC analysis. SPR and NMR demonstrate picomolar affinity of SVD-1a to α-synuclein monomers, while keeping them in their physiological intrinsically disordered conformation. The all-D-enantiomeric ligand for Aβ, RD2, disassembles Aβ oligomers obtained from brain tissue of former AD patients. A clinical phase Ib, double-bind, placebo-controlled study with 20 ATN positive patients treated once daily orally with RD2 or placebo for 4 weeks with an additional 4 weeks follow up period demonstrates good safety and tolerability, and yields interesting results on efficacy. This is valuable information for the scheduled phase II study. I will also acknowledge the many contributors of both developments that are too many to be included here in the abstract. Conclusion: The unique anti-prionic mode of action for the treatment of AD, PD and other protein misfolding diseases is promising.

2321-Pos

NanoLuc tandem repeats show misfolding during thermal denaturation which is reversible by *E. coli* chaperones (DnaK/DnaJ/GrpE)

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Monomeric NanoLuc (Nluc) is a thermally robust bioluminescent protein with its thermal denaturation temperature at 58°C. In order to further examine this thermal behavior, we developed three protein constructs (monomeric, dyad, and triad Nluc). Interestingly, thermal denaturation experiments at 58°C showed sudden decrease in thermal stability when Nluc was linked to itself, dyad and triad Nluc constructs, while monomeric Nluc remained mostly folded under same conditions. Also, all three constructs maintained their activity (bioluminescence) when brought back to room temperature with no spontaneous recovery observed. However, when we examined the E. coli DnaK/DnaJ/GrpE chaperone system effect on this loss of thermal stability in refolding assays, we observed recovery in bioluminescence signal up to 70% for the poly-Nluc constructs. This observation strongly supports poly-Nluc is a strong chaperone substrate. Another interesting observation was that the refolding of these poly-Nluc constructs was possible at similar percentages even in the absence of GrpE showing a deviation from the canonical chaperone mechanism described up to now in literature. However, high GrpE concentrations were inhibitory to the protein refolding. Similarly, addition of disaggregase ClpB chaperone, showed no further assistance in refolding of the proteins and in some cases proved to be inhibiting the refolding. Lastly, we performed MD simulations of thermally unfolded and refolded monomeric and poly-Nluc constructs. These show that monomeric Nluc is able to correctly refold, while dyad Nluc misfolds with 78% and triad Nluc misfolds with 63%. Also, additional MD simulations suggest the reason of dyad and triad Nluc constructs misfolding is due to domain swapping preventing the correct refolding of these two constructs during thermal renaturation.

2322-Pos

Exploring Hsp90 as a possible pseudo-substrate receptor for E3 ligases

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Hsp90 is a chaperone protein responsible for aiding in the proper folding of its client proteins, particularly in response to cellular stresses such as heat shock. It has been demonstrated in human cell lines that inhibition of Hsp90's ATPase activity, which is required for its chaperone activity, leads to proteolytic degradation of c-Raf, which is an oncogenic kinase in the MAPK/ERK pathway and a known client protein of Hsp90. In eukaryotes, the primary mechanism of protein degradation is through the ubiquitin-proteasome system, in which E3 ligases post-translationally modify substrate proteins with ubiquitin, which marks the protein for degradation by the proteasome. It has been shown that siRNA silencing of the E3 ligases HECTD3 and Cullin5 in Hsp90-inhibited cells leads to recovery of c-Raf, suggesting that HECTD3 and Cullin5 are responsible for initiating the degradation of c-Raf. Furthermore, it has been shown that increasing concentrations of the Hsp90 inhibitor AUY922 causes HECTD3 to pull down both c-Raf and Hsp90, implying that HECTD3, and perhaps also Cullin5, directly interact with Hsp90 in order to immediately ubiquitinate misfolded Hsp90 clients and mark them for proteasomal degradation. In order to investigate the validity of this model, we are expressing these proteins in vitro and will report which proteins directly interact with which, with the goal of reconstituting the full E3 ligase complex responsible for c-Raf ubiquitination.