PLATFORM X: Intrinsically Disordered Proteins II

1010-Plat
Modulation of Intramolecular Diffusion in Intrinsically Disordered Protein α-Synuclein under Aggregating Conditions
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It is generally accepted that aggregation takes place from partially unstructured states of initially globular proteins or unstructured states of intrinsically disordered peptides or proteins. However, we know very little about the elementary steps of protein aggregation. The intramolecular diffusion due to formation of contact between two points in a polypeptide chain may be the earliest step of the folding and also aggregation. It is not yet known how intramolecular diffusion of a polypeptide chain is affected under aggregating conditions. In particular it is unknown whether the rate of intramolecular diffusion under physiological conditions is decreased/increased or completely vanished under aggregating conditions. We have addressed this issue by studying the intramolecular contacts formation in an intrinsically disordered protein, α-synuclein under both physiological and aggregating conditions. At low temperatures, the rate of intramolecular diffusion is quite high, comparable to unstructured model peptides. However this rate decreases with increasing temperature, suggesting that the protein reconfigures more slowly under aggregating conditions.

1011-Plat
Single-Molecule FRET Reveals Shifting Folding Landscapes for Parkin-son’s-Related Alpha-Synuclein
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Parkinson’s disease (PD) is the second most common neurodegenerative disease, with the number one risk factor being age. Despite its prevalence and extensive research efforts at many levels to understand the molecular disease mechanism and identify effective drug targets (of which there are currently none), much remains to be understood about PD, including the role of the heavily implicated protein α-synuclein. This small and intrinsically disordered protein (IDP) is the primary component of Lewy bodies (intraneuronal inclusions pathologically associated with PD) and has been linked genetically to PD through mutation and overexpression-linked toxicity. Structural investigation by conventional bulk methods has proven to be quite challenging due to the inherent flexibility of the molecule and subsequent heterogeneity of the population. To reveal critical information about the “misfolding” (or perhaps simply alternative folding) behavior of α-synu-clein, especially as it relates to aggregation, single-molecule Förster Reso-nance Energy Transfer (smFRET) techniques were applied to wild-type and disease-related variants of α-synuclein. The monomeric protein was then observed in misfolding-related conditions, revealing distinct folding characteristics of aggregation-related conformations, which may eventually lead to new insights into α-synuclein’s primary role in human biology by comparison.

1012-Plat
Alpha-Synuclein: Horseshoe Conformation on Intact Large Unilamellar Vesicles
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The intrinsically disordered protein α-Synuclein (αS) is the main component of Lewy-bodies in Parkinson’s disease. The physio-nological and pathological function of αS could involve its interaction with the synaptic membrane. Upon membrane binding, αS adopts an alpha-helical structure, which was found to be either horseshoe or extended (Jao et al. Proc. Natl. Acad. Sci. USA 2008, 105 (50), 19666, Georgieva et al. JACS 2008, 130 (39), 12856, Bortolus et al. JACS 2008, 130 6690), see Fig, top part. We show that both conformations coexist on POPG [1-Palmitoyl-2-oleoyl-sn-Glycero-3-Phosphocholine (1-2O/1)] large unilamellar vesicles (LUVs) using double electron-electron resonance (DEER) EPR on spin labeled variants of αS. In four doubly spin labeled mutants the horseshoe conformation is found, revealing that this conformation is not exclusive to small vesicles and micelles. For αS27/56 the ratio of horseshoe:extended conformation can be directly determined as 1:4 (Fig, lower part). We speculate that the coexistence derives from the energetic proximity of these conformations and that it is a subtle func-tion of the environment.

1013-Plat
Alpha-Synuclein Promotes Structural Reorganization of Raft-Like Membranes
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α-Synuclein is an intrinsically-disordered protein in vitro that undergoes spontaneous coil-helix transitions in the presence of large membranes causing structural disruption [1-2]. One potential membrane target for this interaction is a raft-like lipid mixture that mimics the exocytotic active zone of the neuron. Using solid-state 2D 13C magic-angle spinning (MAS) NMR and electron microscopy (EM), we studied site-resolved molecular and macroscopic structural changes that occur in the presence of wild-type α-synuclein or its N-ter-minal (1-25) consensus sequence. The raft system is a highly-ordered, heterogeneous system composed of liquid-ordered and liquid-disordered phases. This was determined through interpretation of chemical shifts and residual magnetic dipolar couplings (RDCs). We also used a mean-tone structural model that defined average cross-sectional areas and volumetric hydrocarbon thicknesses for the lipid domains. α-Synuclein caused striking changes of the RDCs and chemical shifts of the membrane components. These perturbations suggested that α-synuclein associates interfacially with the membrane, specifically through hydrogen-bonding with EYSM and cholesterol, yielding drastic changes in the membrane hydrocarbon region. Molecular cross-sectional areas for each lipid are significantly increased, and there is a striking 6 Å decrease in membrane thickness. These site-specific changes in molecular structure reflect a protein-assisted mixing of membrane components, and disruption of raft-like domains. Corroborating our NMR structural analysis, the EM images revealed a multilamellar distri-

1014-Plat
Insights Into the Structure and Mechanics of a Mostly Disordered Protein: Lamin A and Progerin Tail Domains
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Hutchinson-Gilford progeria syndrome (HGPS) is a premature aging disease caused by the expression and accumulation of a mutant lamin A protein, progerin. We report experimental and computational results that characterize the biophysical stability of lamin A and progerin tail domains which are mostly dis-ordered. We study protein unfolding with increasing temperature using trypto-

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