An Investigation of the Atomic Structure of Cataract-Forming Mutant Gamma-D-Crystallin Aggregates Formed under Distinct Environmental Conditions

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including curved and straight filaments. Based on these analyzes we predict at least 10-fold enhanced stability of the monomer-monomer association over dimer-dimer association. We tested this prediction by measuring the dimer dissociation constant (Kd) and the critical concentration for polymerization (CC). We also measured the effects of changing the solution composition on dimer Kd (reflecting monomer-monomer association), and on CC (reflecting dimer-dimer association). We examined 4 changes to the solution: addition of ligands 1) GTP or GDP at 0.5 mM, addition of co-solvents 2) urea or 3) Trimethylamine N-oxide (TMAO), each at 0.3 M, and addition of 4) NaCl 0.5 M. Our experimental results demonstrate the enhanced stability and lower sensitivity to solution composition changes of tubulin monomer-monomer association compared to dimer-dimer association.

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Structural Studies of Plant CESA Support Eighteen CESAs in the Plant CSC

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The cellulose synthesis in plants is carried by a large multi-subunit transmembrane protein complex known as cellulose synthesis complex (CSC). The structural information on plant cellulose synthase (CESA) proteins that constitute CSC has remained challenging over years due to inherent complexities in the CSC leading to speculations and debates on the composition of CSC and the number of cellulose chains in a microfibril. Here, we report our findings on the structural properties of catalytic domain of Arabidopsis thaliana CESA1 (ATCESA1CatD) analyzed using small-angle scattering and computational modeling techniques. Our main findings include low resolution structures of ATCESA1CatD in monomeric and trimeric complex forms that provide the first experimental evidence supporting the self-assembly of CESAs into stable trimeric complexes. This is of immense importance in the context of CSC formation by plant CESAs and addresses a long standing question in plant biology - how many CESAs in the plant cellulose synthesis complex? Further, the scattering data in combination with computational modeling provided insight into the potential arrangement the monomers in the catalytic trimer and the relative arrangement of P-CR and CSR regions that are unique in the plants. Comparison of the size of the trimer complex with the dimensions of CSCs from TEM images provides compelling evidence that each lobe of a CSC contains three CESAs rather than the long-standing model of six CESAs within each lobe of a rosette CSC. To our knowledge, these studies are the first experimental evidence that CESA trimers form the lobes of rosette CSCs providing strong support for the hexamer of trimers model that synthesizes an 18-chain cellulose microfibril as the fundamental product of cellulose synthesis in plants.

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Structural Insights into Tc Toxin Action

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Tripartite Tc toxin complexes of bacterial pathogens perforate the host membrane by forming channels that translocate toxic enzymes into the host, including humans. The underlying mechanism is complex but poorly understood. I will present the first high-resolution structure of a complete 1.7 MDa Tc toxin complex composed of TcA, TcB and TcC. TcB and TcC form a large cocoon, in which the toxic domain resides and is autoproteolytically cleaved. Binding of TcB/TcC to the pore-forming and receptor-binding TcA opens the cocoon and results in a continuous protein translocation channel, in which the toxic domain is secreted. Our results allow us for the first time to understand key steps of infections involving Tc toxins at molecular level and shed new light on the interaction of bacterial pathogens, such as the plague pathogen Yersinia pestis, with their hosts.

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An Investigation of the Atomic Structure of Cataract-Forming Mutant Gamma-D-Crystallin Aggregates Formed under Distinct Environmental Conditions

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Human gamma-D-crystallin (HGD) is an extremely soluble (>400mg/mL in vivo) and stable protein that under certain conditions succumbs to protein aggregation, which is associated with cataract formation. Cataracts are the leading cause of blindness globally. Both environmental and genetic factors enable cataract formation by disrupting the solubility of HGD. Several chemical modifications are thought to be linked to loss of solubility and precipitation of HGD. The P23T mutant of HGD is genetically linked to three distinct congenital cataracts. It is crucial to develop a mechanistic understanding of how the reduced solubility of P23T HGD is caused by chemical and/or structural changes. We performed structural and chemical analyses on aggregated or aggregation-prone HGD to gain a molecular understanding of the aggregation mechanism, as well as the nature and location of chemical modifications.
We find P23T HGD is aggregation prone, but forms qualitatively different aggregates as a function of the environmental conditions. Among other factors, we examined the impact of pH and UV irradiation on aggregation and evaluated the resulting aggregates by Electron Microscopy (EM), X-ray powder diffraction, Mass Spectrometry (MS), and multidimensional magic-angle-spinning (MAS) solid-state Nuclear Magnetic Resonance (NMR). Distinct conformations of HGD were observed in the aggregated state by X-ray powder diffraction and EM, and chemical changes were observed by MS. Through comparison to the NMR chemical shifts of the protein in the soluble and aggregated states, we identified structural changes in a residue and site-specific fashion. Our data reveal that aggregation conditions not only affect the macroscopic appearance of the aggregates, but also have a dramatic impact on the aggregates' internal atomic structure. Therefore, distinct molecular mechanisms for crystallin aggregation exist.

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The Giant Cytoskeletal Protein Obscurin acts as a Variable Force Resistor

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Obscurin is a giant cytoskeletal protein that is expressed in a wide variety of cells. This protein is highly modular and functions, in part, by connecting distal regions of the cell through individual obscurin domains binding to specific protein targets. Force resistance is intrinsic in this type of protein organization; as the obscurin targets move relative to each other, the obscurin linker by necessity compresses and expands. Work on the similar protein titin suggests that these long modular proteins intrinsically resist this type of motion through an enthalpic-entropic spring mechanism. Here, we study whether obscurin also behaves through this mechanism. Through the use of NMR, SAXS, and SMD, we find that different segments of obscurin behave differently to stretch. Some obscurin segments (for instance Ig34-39) are moderately rigid relative to each other, and this results in a steeper distance/work slope when the these domains are stretched. In contrast, other domains (Ig58-59) show no interaction with their neighbors. These domains stretch more like a traditional pure entropic spring.

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Force Sensing by the Vascular Protein Von Willebrand Factor is Tuned by a Strong Intermonomer Interaction

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The large plasma glycoprotein von Willebrand factor (VWF) senses hydrodynamic forces in the blood stream and responds to elevated forces with abrupt elongation, thereby increasing its adhesiveness to platelets and collagen. Remarkably, forces on VWF are elevated at sites of vascular injury, where VWF's hemostatic potential is important to mediate platelet aggregation and to recruit platelets to the subendothelial layer. Adversely, elevated forces in stenosed vessels lead to an increased risk of VWF-mediated thrombosis. To dissect the remarkable force sensing ability of VWF, we have performed atomic force microscopy (AFM)-based single-molecule force measurements on dimers, the smallest repeating subunits of VWF multimers. We have identified a strong intermonomer interaction that involves the D4 domain and critically depends on the presence of divalent ions. Dissociation of this strong interaction occurred at forces above ~50 pN and provided ~80 nm of additional length to the elongation of dimers. Corroborated by the static conformation of VWF, visualized by AFM imaging, we estimate that under physiological conditions approximately half of VWF's constituent dimers are firmly closed via the strong intermonomer interaction. As firmly closed dimers drastically shorten VWF's effective length contributing to force sensing, they can be expected to markedly tune VWF's sensitivity to hydrodynamic flow in the blood and to thereby significantly affect VWF's function in hemostasis and thrombosis.