microbalance-dissipation (QCM-D) instrumentation, we investigate mechanical properties of intact clathrin-coated vesicles (CCVs), reassembled pure clathrin cage baskets, and single triskelia. CCVs are purified subcellular trafficking organelles of about 100 nm diameter, consisting of an outer clathrin lattice shell over a receptor- and adaptor- loaded lipid vesicle. The clathrin cages formed by self-assembly of clathrin triskelia are structurally similar to the outer shell of CCVs. From AFM and complementary QCM-D measurements, we now provide an integrative mechanical model of the clathrin protein shell and of the coupling between the clathrin coat and the membrane vesicle, in biologically relevant fluid environments. Whereas the Young's modulus of the triskelia arm is as high as a few MPa, the effective shear moduli of clathrin triskelia, clathrin baskets, and CCVs are one to two orders of magnitude smaller and vary with the buffer pH and ionic strength, as well as their proximity to a given substrate support. Such modulation of the nanomechanical properties of the assembling/disassembling protein shells may be an essential aspect of cellular function.

1087-Plat

Proline Periodicity Modulates the Self-Assembly Properties of Elastin-Like Polypeptides

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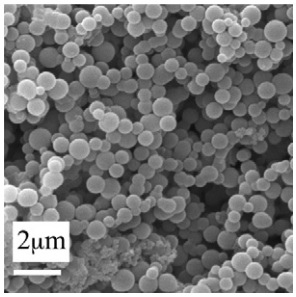
Elastin is a self-assembling protein of the extracellular matrix that provides tissues with elastic extensibility and recoil. The monomeric precursor, tropoelastin, is highly hydrophobic yet remains substantially disordered and flexible in solution, due in large part to a high combined threshold of proline and glycine residues within hydrophobic sequences. In fact, proline-poor elastin-like sequences are known to form amyloid-like fibrils, rich in β-structure, from solution. On this basis, it is clear that hydrophobic elastin sequences are in general optimized to avoid an amyloid fate. However, a small number of hydrophobic domains near the C-terminus of tropoelastin are substantially depleted of prolines. Here we investigated the specific contribution of proline number and spacing to the structure and self-assembly propensities of elastin-like polypeptides. Increasing the spacing between proline residues significantly decreased the ability of polypeptides to reversibly self-associate. Characterization of the assembly process revealed the presence of smaller colloidal droplets with enhanced propensity to cluster into dense networks, enriched in β-structure. These data strongly support a model where proline-poor regions of the elastin monomer provide a unique contribution to assembly, and suggest a role for localized β-sheet in mediating self-assembly interactions.



1088-Plat

Thrombin Flux and Wall Shear Rate Regulate Fibrin Fiber Deposition State during Polymerization Under Flow

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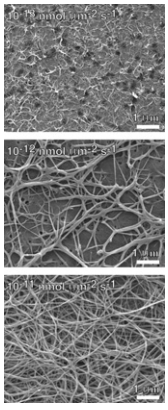
Thrombin is released as a soluble enzyme from the surface of platelets and tissue factor bearing cells to trigger fibrin polymerization during thrombosis. While isotropic fibrin polymerization under static conditions involves protofibril extension and lateral aggregation leading to a gel, factors regulating fiber growth are poorly quantified under hemodynamic flow due to the difficulty of setting thrombin fluxes. A membrane microfluidic device allowed combined control of both thrombin wall flux (10^{-13} to 10^{-11} nmol/um^2 s) and the wall shear rate (10 to 100 s^{-1}) of a flowing fibrinogen solution. At a thrombin flux of 10^{-12} nmol/um^2 s, both fibrin deposition and fiber thickness decreased as the wall shear rate increased from 10 to 100 s^{-1}. Direct measurement and transport-reaction simulations at 12 different thrombin flux-wall shear rate conditions demonstrated that two dimensionless numbers, the Peclet number (Pe) and the Damkohler number (Da), defined a state diagram to predict fibrin morphology. For Da<10, we only observed thin films at all Pe. For 10<Da<900 and Pe<100, we observed three-dimensional gels. These results indicate that increases in wall shear rate first quench lateral aggregation and then protofibril extension.



1089-Plat

Fluorescent S-Layer Fusionproteins; Reassembling Behaviour and Spectral Properties

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S-layer proteins are crystalline bacterial cell surface layer proteins. When they are isolated or genetically modified with functional moieties they are still able to reassemble spontaneously into their distinctive monomolecular lattice symmetry as originally found on the cell surface. In solution they form differently sized self-assembly products while they build crystalline nanostructured biocoatings o nsolid supports. In our case we fused the pH-dependent green fluorescent protein (EGFP) in a 1:1 stoichiometry with a truncated form of the S-layer protein SgsE from Geobacillus stearothermophilus NRS2004/3a. We investigated the formation of the 2D nanostructured monomolecular crystalline self-assembly products with the functional moiety in defined position and orientation, representing a new patterning material for biomolecular construction kits. With transmission electron microscopy (TEM) we could demonstrate that isolated SgsE-EGFP subunits build mono- and double-layered self-assembly products, forming differently sized flat sheets and cylinders with p2 lattice symmetry (a=11.9+/-0.6nm, b=14.7+/-0.6nm, g=81.2+/-1.1nm). Atomic force microscopy (AFM) was used to resolve the crystalline domain nanostructure of the fluorescent S-layer proteins on functionalised solid supports. We demonstrated the application as building blocks by coating silica particles with the fluorescent S-layer protein, fabricating in this way a pH-dependent nanostructured surface-coating. The pKa value of the pH-dependent fluorescent S-layer coating was calculated after measuring the EGFP fluorescence emission in different pH environements using flow cytometry and fluorescence microscopy. The colloidal behaviour was investigated with Zeta-potential measurements. In conclusion, these fluorescent S-layer fusion protein assemblies can be used for investigating systematically changes in pH and surface potential at the nanoscale.

AWARDS CEREMONY AND NATIONAL LECTURE

1090-Natl

Chaperonin-Mediated Protein Folding

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Chaperonins are remarkable ring assemblies that provide kinetic assistance to protein folding to the native state. They function through the action of the central cavities of their rings. The best studied such system is bacterial GroEL and its cooperating co-chaperonin GroES, which mediate productive folding of a large number of proteins in the presence of ATP. I will recount the early functional studies of chaperonins in mitochondria, carried out collaboratively with Ulrich Hartl, and describe our subsequent X-ray, EM, and biochemical studies of GroEL leading to an understanding of the structure and reaction cycle, studies carried out with Paul Sigler and Helen Saibil. The structure and mechanism studies revealed that an open ring of GroEL exposes hydrophobic surfaces that capture non-native proteins and prevent them from aggregation. In the presence of ATP, which binds cooperatively in the seven equatorial sites of an open ring, the cochaperonin ring GroES associates, producing large rigid body movements of the apical and intermediate domains of the bound, so-called *cis* ring, releasing substrate protein into a now-encapsulated central cavity that has hydrophilic walls. Folding occurs in this space in what is the longest-lived state of the reaction cycle. ATP hydrolysis gates rapid entry of ATP into the opposite ring, which both allosterically ejects the *cis* ligands and sets up a new folding active ring. I will present recent cryoEM studies of the allosteric movements driven by ATP binding, which both facilitate substrate protein binding and enable initial contact of GroES with GroEL, carried out with Helen Saibil, as well as discuss *cis* folding studies, carried out in part with Kurt Wüthrich.