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Unraveling the link between nonlinear mechanics, microstructure, and molecular packing of fibrin

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Platform: Protein Assemblies

193-Plat
The Cooperative Assembly of IFI16 Filaments on dsDNA Provides Insights into Host Defense Strategy
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Whether host DNA-receptors have any capacity to distinguish self from nonself at the molecular level is one of the foremost questions in the innate immunity of mammals. By using quantitative assays and electron microscopy, we show that cooperatively assembling into filaments on dsDNA may serve an integral mechanism by which human interferon inducible protein 16 (IFI16) engages foreign DNA. IFI16 is essential for defense against a number of different pathogens, and its aberrant activity is also implicated in several autoimmune disorders such as Sjögren's syndrome. IFI16 cooperatively binds dsDNA in a length-dependent manner and clusters into distinct protein filaments even in the presence of excess dsDNA. Consequently, the assembled IFI16●dsDNA oligomers are clearly different from the previously proposed noninteracting entities resembling beads on a string. The isolated DNA-binding domains of IFI16 engage dsDNA without forming filaments and with weak affinity, and it is the non-DNA binding pyrin domain (PYD) of IFI16 that drives the cooperative filament assembly. The surface residues on the PYD that mediate the cooperative DNA-binding are conserved, suggesting that related receptors use a common mechanism. These results suggest that IFI16 clusters into signaling complexes in a switch-like manner, and that it may use the size of naked dsDNA as molecular ruler to distinguish self from nonself.

194-Plat
A Helical Transport Mechanism for Type III Secretion
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Gram-negative pathogenic bacteria, like Salmonella typhimurium and Shigella flexneri, employ the Type III Secretion System (T3SS) to infect human cells. The T3SS is a large protein secretion channel that assembles to span a ~50nm gap between the bacterial and target cell walls. A key component of the S. typhimurium SPI-1 T3SS is the 80 residue needle subunit PrgI which polymerizes to form a 25 Å wide channel through which proteins are transported. During needle assembly, the PrgI subunits pass through the nascent channel before attaching to the tip.
We have studied the mechanism of PrgI transport using near-atomistic molecular dynamics simulations. We found that the channel's inward facing amino acids and its helical symmetry direct PrgI diffusion along a helical pathway (the i+1 crystallographic axis) with 2.4nm axial displacement per 360 degrees rotation. In vivo assays have shown that mutations of channel residues inhibit the subunit secretion required for needle self-assembly.
Our combined studies evidence that the channel surface plays an active role in substrate secretion, rather than being a passive corridor for linear diffusion. Our evidence of rotation-translation coupling suggests the that the T3S needle might rotate during effector secretion.

195-Plat
Unraveling the Link between Nonlinear Mechanics, Microstructure, and Molecular Packing of Fibrin
Nicholas A. Kurniawan[1], Jos Grimbergen[2], Izabela K. Piechocka[3], Karin A. Jansen[1], Fred C. MacKintosh[4], Jaap Koopman[2], Gijsje H. Koenderink[1].
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When we cut ourselves, our body's immediate response is to stop the bleeding by repairing the damage to the wall of the blood vessel_forming a ''blood clot''. This is physically carried out by forming a network of semiflexible fibrin fibers, which bind together red blood cells and platelets, thus effectively plugging the hole that results from the injury. Recent works have shown that fibrin exhibits extraordinary material properties: it can be stretched up to 5 times its original length and it can stiffen more than 100 fold in the process. Unraveling the biophysical mechanisms behind these phenomena not only can help us better understand how our body maintains haemostasis, but also can provide useful design principles for (bio)materials.
In this work, we relate the nanoscale polymerization kinetics to the microscale fiber and network structure, and to the macroscale rheological properties of fibrin. We identified distinct temporal stages in which fibrils aggregate laterally to form floppy fibers, followed by slow compaction of the fibril bundles. Furthermore, we show a direct correlation between the slow formation of high-molecular-weight chain oligomers with a slow decrease in fiber diameter and a concomitant slow increase in clot stiffness. A comparison with theoretical model of bundled semiflexible polymer networks reveals that cross-linking enhances the tightness of coupling between protofibrils within fibers. This compaction leads to the stiffening of fibers, and thus underlies the stiffening of clots. Strikingly, the stiffening effect becomes negligible when the samples are subjected to a large mechanical deformation, suggesting that the mechanics of highly stressed clots is governed by intrinsic fibril stretching. Together, our work provides a detailed biophysical picture explaining how the hierarchical structure of fibrin is interconnected with its formation and mechanics at multiple length-scales.

196-Plat
Dimerization of the PTEN Tumor Suppressor and its Structural Characterization by SAXS
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The PTEN tumor suppressor is a dual-specificity phosphatase whose main target is the phosphatidylinositoltriphosphate (PI(3,4,5)P3) pool in the inner plasma membrane. It acts as the PI3K antagonist in the PI3K/Akt signaling pathway that controls cell growth and apoptosis and is the second most frequently mutated protein in human cancers. Because the propensity for tumor formation depends on PTEN dose in a way that is inconsistent with the ''two-hit hypothesis'', it was postulated that PTEN multimers may form the active species of the phosphatase, and recently strong evidence has been presented for the formation of functional PTEN dimers in the cell (Papa et al., Cell 157, 2014, 595). Here, we use SAXS to investigate the multimerization of PTEN in buffer and show that it indeed forms dimers following elution from a size-exclusion column as a monomer. Electron density envelopes for the PTEN monomer and dimer obtained from SAXS could clearly be distinguished and were assigned by placing the PTEN crystal structure which was earlier determined for a truncated protein. In addition, the monomer envelope was validated by μs-long all-atom MD simulations of full-length PTEN. In these simulations, the auto-inhibitory, flexible C-terminal tail associates closely with the PTEN core domains while hopping between different bound conformations. The equilibrium ensemble of the resulting structures is in excellent agreement with the SAXS data. A structure prediction using the Rosetta docking protocol revealed a putative dimer arrangement that fits the dimer envelope derived from SAXS very well and is consistent with neutron reflectometry results for membrane-bound PTEN.

197-Plat
Molecular Simulations of the Capsid Release and Membrane Binding Processes of Flock House Lytic Peptides
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During the infection process, a virus particle encounters membrane barriers, which must be overcome in order for the viral genome to be delivered to the correct cellular location for viral transcription. While enveloped viruses have fusion peptides to facilitate this process, the mechanisms by which non-enveloped viruses cross these membranes barriers is poorly understood. Many non-enveloped virus contain a membrane lytic component to the viral capsid, which is sequestered on the capsid interior until a signal is received to externalize/activate the lytic component. One such model system is the Flock House virus (FHV), which is an animal virus of the nodaviridae family, which infects insects. FHV has a T=3 icosahedral capsid, and autocatalytic cleavage separates the lytic peptide (γ) from each of the 180 subunits. Lysosome leakage experiments have shown acidic conditions are critical for FHV membrane lysis. Using molecular simulation methods we have been investigating three aspects of this phenomena i) γ externalization form the capsid interior, ii) structural