them to fulfill their adaptable physiological role, is a space-filling expanded hydrogel held together by tunable interfilament associations. These interfilament associations are predominantly electrostatic and established by the polyanhydrol nature of the sidearms, the unstructured C-termini of the three constituent subunits: NF-Low (NF-L), NF-Medium (NF-M), and NF-High (NF-H) [1]. Using synchrotron x-ray scattering and polarized optical microscopy, we examined the strength of these regulatory electrostatic associations and their effect on filament re-orientation organization. As a function of decreasing ionic strength, for binary and ternary NF systems, three distinct salt-induced hydrogel phases are found: nematic liquid-crystal (NG), isotropic (IG), and anisotropic NF blue phase (BG). At low ionic strength (< 5mM) with weak screening, sidearm overlap is maximal producing BG hydrogels with very high elastic modulus comparable to cross-linked gels with a phenomenonal ability to retain shape and water over long times. Upon melting into the IG phase, with reduced sidearm overlap, filaments re-organize across at large angles to minimize electrostatic repulsion. At still higher salt concentrations, NF gels exhibit an abrupt transition from the IG with mesh size ≈ 1000Å to an Onsager-type oriented nematic liquid-crystalline gel with interfilament spacing d ≈ 500Å (NF-LM) and 700Å (NF-LH) [1]. Remarkable to the NF system is the fully reversible interchange of NF hydrogel properties, most notably the elastic modulus transition from chemical-gel mimicking BG to physical-gel-like (IG and NG), prompted merely by variation in ionic strength. Supported by DOE-BES DOE-DE-FG02-00ER46314 and NSF DMR 1101900.


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Shear-Optimized Platelet-Like-Particles from High Ploidy MKS: From Segregation to Composition and Activation
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Cone and plate rheometry is used to impart physiologically relevant shear stress on the human megakaryocyte (MK) cell line, MEG01. These experiments reveal that platelet-like-particle (PLP) generation is sensitive to both biomechanical and pharmacological factors namely blebbistatin inhibition of NMM-II. We demonstrate that shear stress reduces phospho-deactivation of NMM-II heavy chain at Ser1943 to approximately 30% of the unsheared level, indicating a restoration of NMM-II activity necessary for proper platelet function. Stimulation of rheometer generated PLP cultures with collagen-I showed aggregation and phosphatidyserine exposure (with Annexin-V binding in the presence of Ca2+). These data demonstrates that PLPs generated in this system retain some degree of functionality such that MKs exposed to shear stress and blebbistatin result in approximately 6.5 fold more PLPs than untreated MK cultures. To assess partitioning and segregation of proteins from MKs in sheared membranes, we used fluorescence-imaged micro-deformation (FIDM) to monitor CD41 and NMM-I in a micropipette aspiration. Antibody labeling of MK surface CD41 shows a homogeneous intensity along the aspirated projection of membrane, but the whole cell bodies shows approximately 3 fold higher intensity, suggesting an excess of CD41 in the MK. Pre-treatment with blebbistatin increases fragmentation frequency, and these fragments show a similar trend with CD41 expression. Nucleofection was used to introduce either GFP tagged WT NMM-I or phosphomimetic, myosin deactivating, GFP tagged NMM-I, to assess whether pSer impacts partitioning of this cytoskeletal protein that is abundant in platelets. Both WT and S1943D NMM-I are seen in the aspirated cell projection. WT NMM-I accumulates at the leading edge of the aspirated projection and at sites of membrane fragmentation, whereas S1943D remains uniformly dispersed. These findings underscore the central role of NMM-II heavy chain phosphorylation, and thus activity, in pro-platelet formation and platelet fragmentation.

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Mechanics and Structure of Fibrin Networks Polymerized under Oscillatory Shear Perturbations
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The mechanical properties of a blood clot are of crucial importance for its ability to stem the flow of blood at a site of vascular injury. These properties are largely determined by the underlying structural scaffold which forms during blood coagulation, a branched network of the biopolymer fibrin. Alterations in the structure of this network, such as changes in fiber density, fiber thickness or branching probability, strongly affect the mechanics of the network. The relationship between network architecture and bulk-level mechanics is commonly investigated using in vitro fibrin networks polymerized under static conditions. However, in vivo, blood clots form under a highly dynamic mechanical environment: Nascent fibrin fibers are constantly exposed to the pulsatile shear flow of blood and the concurrent oscillatory dilation of the vessel walls. However, the effects of mechanical perturbations during polymerization on clot structure and the resultant mechanical properties remain unknown. Here, we polymerize fibrin networks while applying continuous oscillatory shear perturbations of varying strain amplitude. Despite these mechanical perturbations, fibrin can form rigid clots which exhibit a significantly later onset of the non-linear strain stiffening response, a postponed rupture strain, and a lowered linear modulus compared to clots formed without perturbations. Up to perturbation amplitudes of 45% shear strain, the typical non-linear stiffness of these clots as well as their rupture stresses are of similar magnitude to those formed without perturbation. We show by confocal microscopy that these changes in the mechanical properties result from a formation of two architecturally distinct layers within the clot: one layer shows a highly bundled structure, while the other layer is virtually unaltered. Architectural adjustments may serve as a means for adapting blood clots to the mechanical loading conditions of the environment in which they form.

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Superresolution Investigation of the Dynamics of FtsZ Structures during E. Coli Cell Division
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FtsZ is an E. coli tubulin homolog that forms single-stranded protofilaments that can interact laterally to form superstructures. In vivo, FtsZ localizes to the midcell plane and assembles into a ring-like structure known as the Z-ring, which is necessary for cell division. The Z-ring serves as an scaffold to recruit all other division proteins and may also generate contractile force for cytokinesis. However, details about the arrangement of protofilaments within the Z-ring, which would suggest possible contraction mechanisms, remains unclear. We have used photoactivated localization microscopy (PALM) to characterize the in vivo structural dynamics of the Z-ring in E. coli at a spatial resolution of ~35 nm. Using Photoactivated Localization Microscopy (PALM), we have previously shown that the Z-ring is a loose bundle of overlapping protofilaments that form either a single-ring conformation at midcell or a multiple-ring conformation reminiscent of a tight helix. We are currently investigating the structural changes that the Z-ring undergoes during the cell cycle and the role of GTPase activity in the structural rearrangements using the variant FtsZ46, which has diminished GTPase activity in vitro.

729-Pos Board B498
Metamorphic Pattern Formation and Deformation: In Vivo and In Vitro Mechanisms
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Mitotic spindle formation into a bipolar structure suitable for chromosome segregation requires reorganization of the interphase microtubule cytoskeleton. A multi-protein complex at spindle poles acts as a microtubule organizing center (MTOC). Microtubules are assembled from this site through the addition of α/ß-tubulin heterodimers onto a template complex containing γ-tubulin (γ-TuRC) imbedded in a larger macromolecular ring (γ-TuRC). Our goal is to apply insights on patterning biological polymers in vivo to development of hybrid biosynthetic systems capable of utilizing microtubules in self-assembling metamorphic patterns including parquet deformation behavior. Dynamic patterning has applications in biosensing, materials design and new nanomanufacturing paradigms. Building off of recent structural insights into γ-TuRC and GCP4 with our own detailed genetic analysis, site-directed mutagenesis, cross-species functional studies and biochemical purification and nucleation assays we provide novel insights on MTOC structural requirements to nucleation. Additionally we have identified an associated regulatory mechanism utilized by a subset of Kinesin-14 members for targeting and regulatory interference at poles (TRIP) distinct from microtubule targeting elements found in other Kinesin-14 members such as Drosophila Ncd. In our in vivo analysis we support application of the recent Kollman-Agard structure as a general eukaryotic model however with species-specific protein and domain constraints as well as contact sites for Kinesin-14 regulation of γ-TuRC. Our findings have broad application towards a general understanding of cellular MTOC machinery and reiterates the flexibility of Klps to localize to multiple spindles.