force is abolished by blebbistatin treatment which inhibits myosin II, suggesting that this >54 pN integrin force is generated by actomyosin. However, blebbistatin treatment does not abolish the rupture of TGT with T_{tol} of 33 pN and lower, consistent with the previously reported ~40 pN integrin force generated by cell membrane during cell adhesion. FAK activation and cell spreading beyond initial adhesion also require ~ 40 pN integrin force. dHL-60 cells were poorly polarized and migration rate was significantly reduced on 54 pN TGT but not on unrupturable TGT or fibronectin coated surfaces. Collectively, our results demonstrated that cell membrane generates ~40 pN molecular force on integrins and this force activates FAK and mediates cell initial adhesion and spreading, while actomyosin generates a >54 pN force to single integrins and this force mediates neutrophil-like cell polarization and migration. The discovery and decoupling of these two regimes of integrin forces was enabled by TGT which measures molecular forces and also regulates cellular functions by restricting integrin molecular forces under a designed level, providing a global molecular force control for cell mechanics study.

2498-Plat
Dynamic Traction Forces of Human Neutrophil Adhesion
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The focus of our presentation will be the mechanics of human neutrophil adhesion and the associated role of the cell cytoskeleton during this process. The principle tool we employ to measure adhesive forces is microfabricated-Post-Array-Detectors (mPADs). We achieve high spatial and temporal resolution of neutrophil adhesion to arrays of sub-micron diameter vertical pillars functionalized with the adhesive ligand fibronectin (FN). Additionally, we will present adherent cell profiles (vertical contours) of neutrophils on pillars via confocal microscopy. The adhesion event is stimulated by haptokinetic interaction with FN alone, consistent with our previous work on continuous fields of FN (Henry et al. 2014. *Integr Biol*. 6:348). Our preliminary data shows the adhesion event is initially a fast (38 ± 8 s, mean ± sd, n = 7 cells) protrusive front that spreads radially outwards with an average per-pillar force of 32 ± 17 pN (mean ± sd, n = 7 cells). Over the next 2-5 min the cell transitions from a protrusive phenotype to a contractile phenotype with the peripheral pillars being deflected inwards towards the cell center and having an average per-pillar force of −43 ± 26 pN (mean ± sd, n = 7 cells). Pillar behavior can be dichotomized according to the position of the pillar relative to the cell's geometric centroid. Peripheral pillars (those residing at the edge of the cell footprint) are highly contractile and persistent in their contractility, core pillars are less so. Pretreatment of quiescent neutrophils with a variety of small molecule inhibitors of the cell cytoskeleton alter the long-time (i.e. minutes) contractile phase of adhesion but have little effect on the short-time (i.e. seconds) protrusive signature. A notable exception is pretreatment with Jasplakinolide which rigidifies the quiescent cortical actin shell and abrogates adhesion completely.

Platform: Intrinsically Disordered Proteins (IDP) and Aggregates

2499-Plat
Sequence Specific Radiolytic Footprinting Study of Monomer, Oligomeric and Fibrillar Amyloid Beta (1-42)
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Increasing evidence suggests that soluble aggregates of amyloid-β (Aβ) are the pathogenic species in Alzheimer's disease (AD). However, detailed structural information on these species remains scarce due to low levels of endogenous Aβ oligomers and uncertainties surrounding current in vitro model systems. Herein, we describe a hydroxyl radical footprinting (HRF) study of Aβ42 fibrils and a stable and homogeneous preparation of Aβ42 oligomers. Specific side chain solvent accessibilities of individual residues in the aggregated and fibril forms of Aβ42 are measured with respect to the same residues of Aβ42 in a fully exposed reference state. These data provide residue specific side chain solvent accessibility protection factors and are used in complement with biophysical characterizations and ss-NMR analyses of these systems. Results are discussed in the context of proposed NMR models of Aβ oligomers and fibrils with implications towards further development of therapeutic and diagnostic strategies.

2500-Plat
Novel Methodologies for the Computational Study of Protein Aggregation
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Aggregation is a major challenge in the development of antibody-based therapeutics. Therapeutic antibodies are produced and stored in high concentrations and generated under varying and unfavourable conditions for the stability of monomeric proteins. The aggregation of these proteins in solution can lead to serious consequences for patients in the form of the initiation of immune reactions, which have the potential to be fatal, and in the loss of clinical potency. Further to this, the type of aggregates formed by antibodies, and the processes that lead to their propagation, are relatively poorly understood. By investigating these molecules as a model system we may find out more about other, more complex systems known to involve aggregation - including amyloids.
Here we present a novel application of coarse-grained molecular dynamics, in a high throughput scheme, coupled with statistical techniques to investigate aggregation and self association of proteins. The use of a high throughput methodology, developed to be modular, user friendly, and efficient, allows the collection of much larger datasets of specific structures than previous work. These can then be analysed using statistical techniques to gain information on specific regions of interactions, and relative rates of interaction.

2501-Plat
Multiscale Simulations Provide Mechanistic Insights into the Effects of Sequence Contexts on Early-Stage Polyglutamine-Mediated Aggregation
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Huntington's disease is associated with an expanded polyglutamine (polyQ) tract in huntingtin. The relevant aggregation prone toxic species encompasses an N-terminal 17-residue amphipathic stretch (N17), the polyQ tract, and a C-terminal 38-residue proline-rich stretch (C38). Experiments have shown that C38 decreases the overall driving force to form aggregates, whereas N17 decreases the overall solubility and accelerates fibril formation by destabilizing non-fibrillar species. We present results from multiscale simulations to explain how N17 and C38 accomplish their distinct modulatory behaviors. Our inverse Boltzmann procedure yields a systematic coarse-graining method whereby we bootstrap against atomistic simulations of monomers and reversible associations for pairs of molecules. We demonstrate that the species distributions and kinetics of forming early-stage aggregates are governed by the relative favorability of intermolecular interactions between flanking sequence regions and the polyQ tract. Specifically, the two flanking sequences reduce the favorable polyQ interaction surface and hence reduce the frequency of intermolecular interactions between and entanglements of the polyQ tract, thus slowing aggregation kinetics regardless of the molecular architecture at the monomer level. The latter affects the morphologies and number of aggregates that form during early aggregation events. Overall, we show that N17 and C38 diminish the formation of large, polydisperse early-stage aggregates that are intrinsic to polyQ. Our results also show how polyQ expansions can override the effects of flanking sequences. Taken together with cellular data, we propose that flanking sequences help diminish the formation of toxic species and this functionality is overwhelmed by mutational expansion of polyQ tracts. We use the conceptual framework of Janus colloids to propose a connection between our results and other aggregation prone systems because they can be partitioned into cores that drive aggregation and contexts that control the aggregation process.

2502-Plat
New Insight into Amyloid-β Fibril Growth and its Inhibition: Kinetic Network Analysis of Multi-Scale Molecular Dynamics Simulations
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Formation of amyloid fibrils by amyloid-β peptides (Aβ) in patient brains is a hallmark of Alzheimer's disease. A major step of Aβ fibril formation is elongation of fibrils by unstructured Aβ peptides, involving Aβ binding and structural transitions. The atomic detail of the structural transitions remains poorly