2278-Plat
Quantitative Analysis of Single-Molecule Force Spectroscopy Data on Chromatin Fibers
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Single-molecule techniques allow for piconewton manipulation and nanometer accuracy measurements of single chromatin fibers. However, the complexity of the data, the heterogeneity of the composition of individual fibers, and the relatively large fluctuations in extension of the fibers complicates a structural interpretation of such force-extension curves. Here we introduce a statistical physics model that quantitatively describes the extension of individual fibers in response to forces up to 30 pN: When increasing the force, fully-folded nucleosomes unwrap to complexes with a single turn of DNA, the singly-wrapped nucleosome rotates to increase the fiber extension, and at forces above 8 pN the remaining DNA unwraps in a non-equilibrium fashion. Comparison to force-extension curves of single nucleosomes and between chromatin fibers of 20bp and 40bp linker DNA reveals the physical properties of chromatin unfolding. In particular, chromatin fibers with 20bp linker DNA exhibit features that can be attributed to nucleosome-nucleosome interactions. Overall, the quantitative analysis of chromatin unfolding presented here allows for a structural interpretation of all physical processes that define chromatin folding.

2279-Plat
Kinetics and Energetics of Biomolecular Folding and Binding
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The ability of biomolecules to fold and to bind to other molecules is fundamental to virtually every living process. Advanced experimental techniques can now reveal how single biomolecules fold or bind against mechanical force, with the force serving as both the regulator and the probe of folding and binding transitions. Here, we present analytical expressions [1] suitable for fitting the major experimental output - the distribution of folding or binding forces - from such experiments to enable their analysis and interpretation. The developed theory accounts for the compliance of the unfolded state, as well as for the effect of the tether used to co-localize the ligand and receptor in single-molecule binding experiments. A fit to the derived analytical expressions yields the key determinants of the folding and binding processes: the intrinsic on-rate and the location and height of the activation barrier. The analytical expressions are applicable to a broad range of biomolecular transitions, from the folding of nucleic acids and proteins to the binding of ligand-receptor complexes.
[1] Pierse C.A. and Dudko O.K., Biophysical Journal - Letter (2013), in press.

Platform: Membrane Dynamics

2280-Plat
Mechanism of Nutrient Deprivation Induced Triacylglyceride Accumulation in Alga Indicated by Fluorescence Hyperspectral Imaging
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Microalgae have been identified as a promising renewable feedstock for production of lipids for feeds and fuels. For a wide variety of algal species, depletion of major nutrients from the growth medium results in dramatically increased biosynthesis of triacylglycerides, in some cases exceeding 70% of the dry weight of the biomass. In recent work, we explored this process in a number of promising algal production strains (Chlorophyceae and Eustigmophyceae) using hyperspectral confocal fluorescence imaging with multivariate spectral unmixing. By coupling intensity modulation of the excitation laser and attenuation of the dominant chlorophyll fluorescence emission, we identified a distinct spectral component composed of chlorophyll fluorescence and resonance Raman emission for lipid-solubilized carotenoids allowing label-free projections of lipid bodies in addition to plastids. Furthermore, cells exposed to the lipophilic dye, Nile Red, exhibited distinct spectral components corresponding to the polar and neutral lipid fractions. Combining these capabilities for analysis of nutrient deprivation time courses revealed increases in the lipid fraction of the cellular volume of ~500%, as well as a correlated decrease in the plastid fraction of the total cellular volume. Additionally, diffraction limited tubules connecting the lipids bodies to the plastids and the presence of unbound chlorophyll in the lipid bodies provided evidence for direct shuttling of lipids from the chloroplast to the lipid bodies, in opposition to the expected ER to lipid body transfer dictated by the Kennedy pathway. The results suggest that nutrient deprivation-based lipid accumulation in these species is dominated by plastid membrane recycling, independent of acyl-CoA. Finally, an additional spectral component exhibiting high spectral overlap with Nile Red and varying inversely with the chlorophyll emission was detected during senescence, potentially resulting in false positives for lipid enrichment in low spectral resolution assays.

2281-Plat
Probing the Cell Growth-Dependent Spatial Distribution and Dynamics of Proteins Inserted in the Bacterial Outer Membrane
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The Gram-negative cell envelope incorporates two lipid bilayers: an inner membrane (IM) with a protein and phospholipid composition characteristic of a typical cell membrane and an atypical, asymmetric outer membrane (OM). Protein insertion and time-dependent re-distribution in the IM is fairly well understood, but these processes remain poorly characterised in the OM. The pattern of insertion for an endogenous OM porin over-produced in Escherichia coli and its rearrangement during cell growth has recently been visualised. In this published work, distinct fluorescent puncta coincident with newly inserted proteins appeared randomly in the OM as discrete bursts, and they did not re-distribute appreciably during cell growth. We build on this previous work by addressing whether protein production levels characteristic of normal exponential cell growth alter the protein insertion process in E. coli JM83. To specifically label low-abundance outer membrane receptors (BtuB and Cir), and avoid the problems of over-expression, we have utilised organic dye-labelled colicin molecules (colicins E9 and Ia) engineered to retain high-affinity receptor binding (Kd $\approx$ pM) and prevent OM translocation. This allowed us to follow insertion of BtuB and Cir in live cells and their re-distribution during cell growth and binary fission using both fluorescence recovery after photo-bleaching (FRAP) confocal microscopy and single-molecule total internal reflection fluorescence (TIRF) microscopy. Single particle tracking and FRAP microscopy of colicin-receptor complexes revealed that BtuB and Cir are sequestered to randomly positioned patches in the OM, which persist during cell growth, and through multiple rounds of binary fission. Work is underway to correlate the random sites of receptor protein insertion with the periplasmic/OM molecular machinery necessary to mediate this process.

2282-Plat
Strain Rate-Dependent Membrane Reservoir- Key to Chondrocyte Death by Impact
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Impact/fast loading of articular cartilage causes chondrocyte deaths. Cell membranes can sustain only 3-4% strain, but are protected from direct stretch by their membrane ruffles, also known as membrane reservoir. Based on a numerical model, we suggested that excessive tensile strain rates in chondrocyte membranes in impacted cartilage may render the membrane reservoir inaccessible, thus resulting in membrane rupture and cell death. However, experimental support for this proposal is lacking. The current study was aimed at measuring the accessible membrane reservoir size for different membrane strain rates through membrane tethering techniques. Force spectroscopy was conducted on isolated bovine chondrocytes (n=87) using atomic force microscopy. A micron-sized cantilever was used to extract membrane tethers from cell surfaces at constant pulling rates. Membrane tethers could be identified as force plateaus in the resulting force-displacement curves. Six pulling rates (1-80μm/s) were tested. The normalized membrane tether surface areas, which represent the accessible membrane reservoirs, decreased exponentially with increasing pulling rates (figure). Our findings support the hypothesis that impact-induced chondrocyte deaths are due to membrane ruptures resulting from high tensile membrane strain rates.