molecules into single viral proheads in real time. We can measure DNA binding and initiation of translocation, DNA translocation dynamics, force generated by the motor, and can infer the forces resisting DNA confinement. We have developed approaches to study three different viruses: Bacteriophages phi29, lambda, and T4. These viruses have different capsid sizes and shapes, genome lengths, and structural and biochemical differences in their packaging motors, resulting in differing DNA packaging dynamics. All three motors translocate DNA processively and generate high forces exceeding 50 piconewtons, but the motor velocities vary 10-fold. In the lambda system we have found evidence for an effect of procapsid expansion on the packaging dynamics and evidence for force-induced capsid rupture in the absence of a putative stabilizing protein. We are currently investigating motor structure-function relationships by analyzing effects of point mutations. Amongst the mutants we have identified are one that exhibits a motor velocity roughly one-tenth that of the wild type, and one that exhibits increased pausing and slipping. These studies shed light on the various functional domains of viral packaging motors.

81-MiniSymp
Response of Viral Shells under Nano-Indentation
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Viral capsids are self assembled nano-containers with remarkable material properties. They combine extreme simplicity of construction with both, toughness and resilience protecting the viral genome, and with complex functionality that the virus needs for targeting and infecting new host cells. We have experimentally, with atomic force microscopy, and numerically, with finite element analysis, studied viral shells under external mechanical stress. While gently probing bacteriophage Φ29 shells with small forces, we could measure linear response properties and estimate a Young's modulus for the shell proteins. In images we observed patterns following symmetry elements. When we irreversibly destroyed the shells in a controlled fashion with higher applied forces, we found that the capsids fractured along well-defined lines revealing trimers as stable building blocks. Similar experiments on capsids of the cowpea chlorotic mottle virus (CCMV) at pH 4.8 revealed an initial reversible linear regime up to indentations of ~ 20% of the diameter followed by irreversible deformation. At a pH 6.0, the response of the shell changes dramatically and becomes soft. Modeling predicts that the nature of structural failure is determined by a simple and universal physical characteristic, namely, the Föppl-von Kármán (FvK) number, a dimensionless control parameter that emerges from the continuum theory of thin shells.

Platform G: Imaging & Optical Microscopy

82-Plat
Stereo Photoactivated Localization Microscopy for Super-Resolution 3D Bioimaging
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Serial localization of single photoactivated fluorescent molecules allows imaging of cells and tissues with theoretically unlimited high resolution. Recently significant progresses have been made towards the extension of this technique to three dimensions. However, to obtain axial localization of single molecular emitters, most of current techniques rely on extracting information from the out-of-focus region. Moreover, the axial resolution is generally lower than the lateral resolution.
Here, we demonstrate a 3D super-resolution imaging technique by stereo photoactivated localization microscopy (Stereo PALM), in which a mirror is placed at 45 degree with respect to the microscope stage, at the sample region. The mirror creates a side-view image of the activated molecules and thereby the axial localization turns into the lateral localization in the mirrored image. In this fashion, a 3D high resolution image can be reconstructed with an equal resolution in lateral and axial directions. This technique is very simple to implement and can be readily combined with other imaging techniques. Stereo PALM imaging of micron-size beads coated with photoswitchable fluorophores is demonstrated, and its application to super-resolution 3D imaging of mitochondria and other biological samples is discussed.

83-Plat
Flurogen Activating Peptides for Single Molecule Localization based Superresolution
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Fluorgen Activating Peptides (FAPs) operate using an expressible dye binding peptide and a concentration of dye molecules that, upon binding to the receptor, have increased fluorescence excitation cross-sections by factors of hundreds to thousands [1] (see abstract by Qi Yan etal. for single-molecule characterization.) Depending on dye/receptor combination, affinities range from nanomolar to micromolar, corresponding to bound lifetimes up to 10s. The same receptor can repeatedly bind and activate new dye molecules, resulting in resistance to photobleaching when suitable concentration of unbleached dye remains and the FAP module hasn't been photodamaged. Binding rates can be controlled by dye concentration whereas fluorescent to dark state transitions can occur from unbinding and photobleaching. Adjusting dye concentration and excitation intensity allows tuning to maximize dyes localized per second per area. The following combined properties make the FAP system ideal for localization-based superresolution: 1)Expressible binding regions allow live cell studies; 2)Dye replenishment allows unlimited receptor position measurements, therefore arbitrary localization accuracy; 3)Only one excitation wavelength required; 4)Dye specific receptors allow multi-color superresolution.
We demonstrate FAP superresolution by imaging live and fixed cells expressing beta-2 adrenergic receptor labeled with an extra-cellular FAP. Cell treatments show protein clustering details not apparent in diffraction limited images. Superresolution images are generated by placing Gaussian blobs at the found location of each activated dye molecule. Dye locations are found using a recently developed, iterative method that performs a maximum likelihood parameter estimation of the background count rate, dye location and dye emission rate. The blob widths are calculated from the Cramer-Rao Lower Bound (CRLB) corresponding to combined estimation of background, position and emission rate. Localization and CRLB are performed on GPU hardware using NVIDIA's CUDA architecture, achieving up to 10^5 combined fits and CRLB calculations per second.
1. Szent-Gyorgyi et al, Nature Biotechnology, 2008. 26(2):p235-p240.

84-Plat
Three-dimensional Super-resolution Fluorescence Microscopy and Its Application to Clathrin Mediated Endocytosis
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The recent invention of super-resolution fluorescence microscopy allows nanoscopic investigation of cellular structures. Among these techniques, the Stochastic Optical Reconstruction Microscopy (STORM) is based on precise single molecule localization of photoswitchable fluorescent probes. By stochastically activating, imaging and deactivating subsets of fluorophores, it makes their images optically resolvable and determines their positions with nanometer precision. A super-resolution image is then reconstructed using these localizations. We now extend this approach to three-dimensional (3D) microscopy by determining the 3D coordinates of activated probes through astigmatism imaging: a cylindrical lens is inserted into the imaging optical path such that the image of individual molecules appear elliptical with the ellipticity depending on its z-position. Using this approach, we have achieved an optical resolution of 20-30 nm in the x-y direction and 50-60 nm in the z direction, representing an order of magnitude improvement over conventional fluorescence microscopy in all three dimensions. We have resolved the nanoscopic morphology of cellular structures that was previously deemed impossible by light microscopy.
As a specific application, we use STORM to study the mechanism of clathrin mediated endocytosis in an in vitro reconstituted system. Proteins of interest in this system are directly labeled with photoswitchable fluorescent probes. This procedure is facilitated by covalently linking the two components of the probe, an activator dye and a photoswitchable reporter fluorophore, to form a single chemical unit prior to protein labeling. Using multicolor 3D STORM, we have characterized the spatial organization of plasma membrane, clathrin, actin and tubule forming proteins dynamin at the site of clathrin mediated endocytosis. These results reveal the molecular architecture of the nascent clathrin-coated pits at the nanometer scale and help to establish the role of actin and dynamin in membrane invagination, scission and vesicle formation.