Symposium 4: Systems Biology

72-Symp
Designing Biological Systems
Pamela Silver.
Harvard Medical School, Boston, MA, USA.
Biology presents us with an array of design principles. From studies of both simple and more complex systems, we understand at least some of the fundamentals of how Nature works. We are interested in using the foundations of biology gleaned from Systems Biology to engineer cells in a logical way to perform certain functions. In doing so, we learn more about the fundamentals of biological design as well as engineer useful devices with a myriad of applications. For example, we are interested in building cells that can perform specific tasks, such as counting mitotic divisions, measuring life span and remembering past events. Moreover, we design and construct proteins and cells with predictable biological properties that not only teach us about biology but also serve as potential therapeutics, cell-based sensors and factories for generating bioenergy.

73-Symp
Design principles of biological circuits
Uri Alon.
Weizmann Institute of Science, Rehovot, Israel.

74-Symp
Nature, nurture, or just blind chance: Stochastic gene expression and its consequences
Alexander van Oudenaarden.
Massachusetts Institute of Technology, Cambridge, MA, USA.

75-Symp
Information is critical for cellular life
Ravi Iyengar.
Mount Sinai School of Medicine, New York, NY, USA.
Functional Organization of Cells
The ability to receive, process and respond to information is critical for cellular life. This ability arises from the cell signaling network that processes information from external and internal sources. There are at least three sources of information: chemical signals, cell shape and mechanical forces. Integrating and processing information from all of these sources to coordinately control multiple cellular machines is essential for both homeostasis and regulated change in cell state. A key feature of signaling networks is the topology of regulatory motifs. Determining regulatory motif topology and the resultant information processing capability requires an integrated computational approach that blends graph theory-based analyses to identify and characterize regulatory motifs with differential equation-based models to determine functional capabilities of these motifs and how information processing changes with time and specific locations within the cell. Graph theory analyses indicate that large networks have a head-to-head topology that results in a depletion of long loops and such systems appear to be more dynamically stable. Interactions between motifs such as feedfoward loops and bifans indicate that multi-motif organization may be critical for biological processes. Differential equation models show that a set of three stacked and nested feedforward loops that is spatially specified is required for the β-adrenergic receptor triggering of the differentiated state in podocytes. These predictions have been experimentally verified. Thus the size of regulatory motifs and how the motifs are juxtaposed with respect to each other give rise to functional organization. There is partial overlap between the structural organization that arises from the presence and location of intracellular organelles and functional organization. Together the functional and structural organization of the cell determines how information is integrated, and how this integrated information is used to co-ordinate and regulate biological processes.

Minisymposium 1: Virus Mechanics: Material Properties and Assembly

76-MiniSymp
Biophysical Studies of Virus Particles and their Maturation: Insights into Elegantly Programmed Nano-machines
Jack Johnson.
Scripps Research Inst, La Jolla, CA, USA.
Complex virus particles such as HIV, Herpes Viruses and dsDNA bacteriophages are programmed nano machines that assemble in a fragile shell that matures through a series of intermediates to form an infectious, robust particle. We have analyzed mature bacteriophage and intermediates in maturation with X-ray crystallography and a variety of biophysical methods, defining the biochemical nature of the transitions and their driving forces. Through experimentally defined chemistry and physics, these particles shape an energy landscape resulting in an exothermic transition and continued maturation that relies on a Brownian ratchet. The presentation will describe the synthesis of structural and biophysical data that lead to an understanding of emergent biological behavior.

77-MiniSymp
Modeling the Size and Structure of RNA: Viral vs. Non-viral Sequences
Avinoam Ben-Shaul[1], Aron M. Yoffe[2], Peter Prinsen[2], William M. Gelbart[2].
[1]Hebrew University Jerusalem, Jerusalem, Israel, [2]Department of Chemistry and Biochemistry, University of California, Los Angeles, CA, USA.
Based on a simple linear polymer model we derive a scaling relationship between the ''maximum-ladder-distance'' characterizing the secondary structure of folded, single stranded, RNA and the radius of gyration characterizing its spatial dimensions. Secondary structures are calculated for a large number of viral RNA sequences as well as non-viral sequences of comparable length and nucleotide composition. The results show that viral RNAs fold into significantly smaller 3D structures than non-viral sequences, consistent with an evolutionary pressure to package the viral sequences inside small rigid protein capsids. We also present a theory explaining why the two ends of a folded RNA are generally found close to each other, independent of nucleotide sequence and length.

78-MiniSymp
Structural Mechanics of Viral Shells: Stretching the Limits of Continuum Models
William S. Klug.
UCLA, Los Angeles, CA, USA.
The last several years have seen a number of successful applications of continuum elasticity theory to the study of virus mechanics. Continuum modeling has been particularly effective in connection with atomic force microscopy nanoindentation experiment for understanding and predicting capsid material properties, and may hold promise for illuminating the physics of capsid assembly as well. I will consider the question of the limitations of continuum modeling of capsids, and discuss some examples of how conventional continuum theory is being extended or "stretched" to study features linked to the inherently discrete character of these molecular assemblies.

79-MiniSymp
The Influenza Virus Mechanical Properties Are Dominated By Its Lipid Envelope
Frederic Eghiaian[1], Iwan Alexander Schaap[2], Amédéé Des Georges[3], John James Skehel[1], Claudia Veigel[1].
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The influenza (flu) virus causes yearly epidemics, and has claimed the life of tens of millions of people in the last century. Flu viruses need to travel from one host to a new one, where they inject their RNA genome by a membrane fusion mechanism. Before fusion the flu virus RNA genome protected by an envelope made of a matrix protein (M1) layer surrounded by a lipid membrane. The exact role of M1 protein and its mode of interaction with the viral membrane are unknown.
We have set out to investigate the mechanical design of influenza virus: we imaged and characterized mechanical properties of influenza virions by atomic force microscopy (AFM). We compared the response of the viral particle with the behavior of simplified model systems to understand the role of the various parts of the viral structure in its mechanical properties.
Influenza virions proved to be very soft compared to the other ''protein-enveloped'' viruses that have been characterized by AFM so far. The stiffness of viral particles was comparable to that of similar-sized small unilamellar lipid vesicles and virosomes. Our results suggest that the M1 protein does not mechanically reinforce the flu virus envelope and that M1 may not directly interact with the inner side of the viral membrane. Hypothesis on the conditions under which influenza virus will persist during transmission will be discussed.

80-MiniSymp
Single-Molecule Studies of Viral DNA Packaging with Optical Tweezers: Molecular Motor Function and DNA Confinement
Douglas E. Smith, James M. Tsay.
University of California, San Diego, La Jolla, CA, USA.
A key step in the assembly of many viruses is the packaging of double-stranded DNA into a procapsid shell by the action of an ATP-powered molecular motor. We use optical tweezers to measure the packaging of single DNA

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
Irena L. Ivanovska[1], Pedro J. de Pablo[2], Roberto Miranda[2], Melissa M. Gibbons[3], Jean-Philipe Michel[3], Gijs J.L. Wuite[1], Fred C. MacKintosh[1], Jose L. Carrascosa[2], William S. Klug[3], Robijn F. Bruinsma[3], Charles M. Knobler[3], **Christoph F. Schmidt[4]**.
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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
Jianyong Tang, Alipasha Vaziri, Charles V. Shank.
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA.
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
Keith A. Lidke[1], Qi Yan[2], Sierd C. Smith[3], Nikolai Joseph[1], Marcel Bruchez[2].
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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.