nucleocytoplasmic transport. Furthermore, synergistic molecular dynamics simulations permitted visualization of previously unknown steps and binding modes during Nup●NTR interactions at atomic resolution. These results have molecular implications for the diversity of transport routes within nucleocytoplasmic transport and on how nuclear transport can pursue specifically and very fast inside the nuclear pore complex.

27-Subg
The Role of Protein Disorder and Self-Association in the Formation of Cellular Bodies

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Cellular "bodies", i.e. organelles in cells that are not enclosed by membranes, have been characterized as large protein assemblies with liquid-like properties, though the biophysical basis for their formation is currently unclear. Recent work demonstrated that weak, multivalent protein interactions can result in the formation of large higher-order complexes, can undergo liquid demixing phase separation in vitro and may enable the formation of cellular bodies. The inherent size heterogeneity of higher-order complexes renders them difficult to characterize biophysically and structurally. As a result, their size distributions remain largely unquantified, limiting molecular insight into their biological functions. We report novel mechanisms governing the formation of nuclear SPOP bodies and stress granules in which the self-association of folded domains into large homo-oligomers and of long disordered regions play key roles. We have used biophysical, biochemical and cell biological approaches to explore the self-association of proteins in vitro, to quantify the size distribution of the resulting higher-order oligomers, and to relate these to the function of cellular bodies in cells. We further explore how changes in self-association properties of constituent proteins, i.e. the destabilization or rigidification of cellular bodies, can lead to cancer and neurodegenerative diseases. We propose that dynamic, higher-order protein self-association is a general mechanism underlying the formation of cellular bodies. These may serve as hotspots of enzymatic or signaling activity, which can be dynamically turned on or off through the regulated assembly and disassembly of the organelle bodies, respectively.

Subgroup: Biopolymers in vivo

28-Subg
Dynamics of Bacterial Ribosome Assembly in Cells

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The assembly of the bacterial ribosome involves coordinated rRNA transcription, rRNA processing, and binding of over 50 ribosomal proteins in a highly efficient biogenesis program. Using quantitative mass spectrometry to study both the rRNA and ribosomal proteins, we are investigating the dynamics of ribosome assembly in cells with pulse labeling experiments. Using electron microsopy, we can characterize both the distributions and structures of assembly intermediates. The analysis focuses on use of assembly cofactor deletion strains to perturb the assembly and create a bottleneck in the otherwise efficient assembly process. Using this approach, we are starting to understand the basic order of events that must occur during ribosome assembly in rapidly growing bacteria.

29-Subg
Assembling the Pieces of Protein Puzzles

Gerhard Hummer, Pilar Cossio, Amir Bahrami, Alfredo Jost López, Jürgen Köfinger.
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Dynamic supramolecular assemblies are central to many biological functions, from the processing of genetic information to sensing and signaling. Structural and functional studies face enormous challenges associated with large molecular sizes, transient interactions, extensive motions, and partial disorder. To overcome these problems, we combine simulation and theory with experiment. In our coarse-grained simulation approach, we use ensemble refinement techniques that allow us to integrate data from diverse experiments, including X-ray crystallography, small-angle X-ray scattering (SAXS), spin-label dis-

tance measurements (EPR), single-molecule fluorescence energy transfer (FRET), paramagnetic relaxation enhancement (PRE), nuclear magnetic resonance (NMR), and single-particle cryo electron microscopy (cryo-EM). The combination of simulation and experiment allows us to build ensemble representations of the dynamic structures of protein supercomplexes and gain insight into their function.

30-Subg
Probing Spatiotemporal Regulation of Signal Transduction in Living Cells

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The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of signaling molecules, such as protein kinases, phosphatases and second messengers. I will present studies where we combined genetically encoded fluorescent biosensors, targeted biochemical perturbations, mathematic modeling and superresolution imaging to probe the spatiotemporal regulation of signaling molecules such as cAMP, Protein Kinase A, calcineurin and Protein Kinase B.

31-Subg
Super-Resolution Fluorescence Imaging with STORM

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Dissecting the inner workings of a cell requires imaging methods with molecular specificity, molecular-scale resolution, and dynamic imaging capability such that molecular interactions inside the cell can be directly visualized. Fluorescence microscopy is a powerful imaging modality for investigating cells largely owning to its molecular specificity and dynamic imaging capability. However, the spatial resolution of light microscopy, classically limited by the diffraction of light to a few hundred nanometers, is substantially larger than typical molecular length scales in cells. Hence many subcellular structures and dynamics cannot be resolved by conventional fluorescence microscopy. We developed a super-resolution fluorescence microscopy method, stochastic optical reconstruction microscopy (STORM), which circumvent the diffraction limit based on stochastic switching and imaging of single molecules. This approach has allowed multicolor and three-dimensional imaging of living cells with nanometer-scale resolution and enabled discoveries of novel sub-cellular structures. In this talk, I will discuss the recent technological development and biological applications of STORM.

Subgroup: Nanoscale Biophysics

32-Subg
Insights into Nucleic Acids Structural Dynamics with Single Molecule FRET Studies

Victoria Birkedal.
Interdisciplinary Nanoscience center (iNANO), Aarhus University, Aarhus, Denmark.

Single molecule methods allow following individual molecules in action and thus obtaining a direct view of how they work providing insights into the "machinery" of biological systems. Fluorescence resonance energy transfer (FRET) is a versatile technique that allows investigating molecular interactions, folding pathways and structural dynamics of biomolecules on the nanoscale.
DNA can form a wide range of natural and artificial functional structures. We focus here on different complex structures, such as DNA G-quadruplexes and a DNA actuator device. Their conformational diversity and dynamics are revealed through single molecule FRET microscopy and the detailed analysis of these experiments. We will also discuss ways to control the structural dynamics properties of these DNA molecules. Direct observation of the molecules in action allows providing a detailed picture of structural heterogeneity and dynamics.

33-Subg
Ultrastable AFM: Improved Stability, Precision, and Bandwidth for Bio-AFM

Thomas T. Perkins.
JILA, NIST & CU-Boulder, Boulder, CO, USA.

Enhancing the short-term force precision and temporal resolution of atomic force microscopy (AFM) while maintaining excellent long-term stability would