3. Pushkarev D, Neff NF, Quake SR: Single-molecule sequencing of an individual human genome. Nature Biotechnology 2009, 27:847–850.
4. Lipson D, Raz T, Kieu A, Jones DR, Giladi E, Thayer E, Thompson JF, Letovsky S, Milos P, Causey M: Quantification of the yeast transcriptome by single-molecule sequencing. Nature Biotechnology 2009, 27:652–658.
5. Ozsolak F, Platt AR, Jones DR, Reifenberger JG, Sass LE, McInerney P, Thompson JF, Bowers J, Jarosz M, Milos PM: Direct RNA sequencing. Nature 2009, advance online publication.

1084-Wkshp
Nano-Devices for Probing Single Molecules
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By confining molecules to 2-dimensional sheets or zero-dimensional wells, with nanometer-scale dimensions, one can observe individual fluorescently labeled molecules for long times, at high concentrations, and without surface attachment. We present two simple nano-devices that achieve these goals. We use these devices to obtain previously inaccessible information on molecular size, composition, and dynamics.
2-dimensional confinement is achieved near the point of contact between a convex lens and a planar coverslip. The lens-coverslip spacing varies smoothly from zero to many microns as the radial distance from the point of contact is increased. Commercial fused silica optics have surface roughness of approximately 1 nm, so one can select a vertical confinement with nanometer accuracy simply by imaging at a given radius from the point of contact. The lens-coverslip system allows: a) fluorescence imaging of immobilized single molecules in the presence of a micromolar concentration of diffusing fluorophores; b) long-time observations of freely diffusing single molecules in dilute solution, which further allows determination of diffusion coefficients, brightness, and spectral dynamics molecule-by-molecule; and c) direct mechanical measurement of the size distribution in a population of fluorescently labeled molecules.
Zero-dimensional confinement is achieved in nanometer-scale wells in a fused silica coverslip. A solution of fluorophores is washed over the wells, and then the bulk solution is replaced with a fluorinated oil. At most one molecule, or molecular complex, is immobilized in a nanoscale water droplet in each well. As with the lens-coverslip system, the dimple machine allows long-time observations of individual molecules, without surface attachment and in the presence of a high fluorescence background. By counting the number of photobleaching steps in each of several thousand chambers, we determine the *distribution* of stoichiometries in multimeric complexes.

1085-Wkshp
Physics and Engineering of Biological Molecular Motors
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No Abstract.

1086-Wkshp
Selectivity Mechanism of the Nuclear Pore Complex Characterized by Single Cargo Tracking
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[1]Univ California, Berkeley, Berkeley, CA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [3]National Cancer Institute, Bethesda, MD, USA, [4]Illumina Inc, Hayward, CA, USA.
The Nuclear Pore Complex (NPC) is the selective filter that facilitates all exchange between the cytoplasm and the nucleus in eukaryotic cells, allowing small molecules to passively diffuse through, while larger cargos require specific transport receptors to translocate. How NPCs achieve their exquisite selectivity remains unclear. We have developed a single molecule assay based on small (18 nm diameter) protein-functionalized Quantum Dots (QDs) for studying (with a mean spatial precision of 6 nm and a temporal resolution of 25 ms) the motion of single cargos as they approach, translocate, and exit the NPC. Optical tracking of single QD cargos reveals the individual steps involved in the import reaction. There is a size-selective cargo barrier in the cytoplasmic moiety of the central channel. The majority of QDs are rejected early rather than spending long times partitioned in the channel. Translocation is not governed by simple receptor-NPC binding interactions; rather, the central channel behaves in accordance with the 'selective phase' model. Finally, in the absence of Ran, cargos still explore the entire volume of the NPC, but have a dramatically reduced probability of exit into the nucleus, suggesting that NPC entry and exit steps are not equivalent and that the pore is functionally asymmetric to importing cargos. The overall selectivity of the NPC appears to arise from the cumulative action of a cascade of filters, only the last of which is irreversible.

1087-Wkshp
The Nano-Positioning System - A Fret-Based Tool for Macro-Molecular Structural Analysis
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Single-Pair Fluorescence Resonance Energy Transfer (FRET) experiments reveal structural and dynamic information about macro-molecules by monitoring the change in FRET efficiency between fluorescent dyes attached to a macromolecule. The Nano-Positioning System (NPS) developed recently [1] uses data from several of such experiments to infer the position of a dye attached to protein sites unresolved by x-ray crystallography.
While triangulation, the basic underlying principle, is not new and has already been reported in this or a similar context [2,3], the NPS applies probabilistic data analysis to the problem. That allows us to calculate the distribution of possible dye positions in a simple and objective way without relying on ad-hoc procedures, while at the same time we account for various error sources that usually accompany FRET measurements, for instance dye orientation effects. We have applied the NPS to determine the position of the nascent RNA [1] as well as to map the pathway of the non-template and upstream DNA in yeast RNA polymerase II elongation complexes [4].
[1] A. Muschielok, J. Andrecka, A. Jawhari, F. Brückner, P. Cramer & J. Michaelis, Nat. Meth. 5, 965–971 (2008).
[2] M. Margittai, J. Widengren, E. Schweinberger, G.F. Schroöder, S. Felekyan, E. Haustein, M. König, D. Fasshauer, H. Grubmüller, R. Jahn, and C. A. M. Seidel, PNAS 100, 15516–15521 (2003).
[3] J.L. Knight, V. Mekler, J. Mukhopadhyay, R.H. Ebright, and R.M. Levy, Biophys. J. 88, 925–938 (2005).
[4] J. Andrecka, B. Treutlein, M.A. Izquierdo Arcusa, A. Muschielok, R. Lewis; A.C.M. Cheung, P. Cramer, and J. Michaelis, NAR doi:10.1093/nar/gkp601 (2009).

Workshop 2: Complementary Methods for Studying Membrane Protein Structure

1088-Wkshp
The Role of Detergents and Lipids in Membrane Protein Crystallography
Robert Stroud.
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No Abstract.

1089-Wkshp
NMR Structural Studies of Membrane Proteins in Lipid Micelles and Lipid Bilayers
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Integral membrane proteins regulate major cellular processes in health and disease, including transport, signaling, secretion, adhesion, pathogenesis, and apoptosis, and therefore, represent important targets for structural and functional characterization. Membrane protein structures and functions are regulated by their physical interactions with the surrounding lipids, and NMR is unique in its ability to provide high-resolution information in lipid environments that closely resemble the cellular membranes. Solid-state NMR experiments with proteins in oriented bilayers, and solution NMR experiments with proteins in weakly oriented micelles, provide high-resolution orientation-dependent restraints, which can be combined for protein structure determination and refinement. As previously observed for helical membrane proteins, the NMR spectra of outer membrane barrels in lipid bilayers exhibit characteristic patterns that reflect both protein structure and intra-membrane orientation. Results are presented for mammalian and bacterial α-helical and β-stranded membrane proteins. The NMR structures characterized in lipids provide insights to their distinct functions.
(This research was supported by the National Institutes of Health.)

1090-Wkshp
Using Circular Dichroism (CD) and Synchrotron Radiation Circular Dichroism (SRCD) Spectroscopy to Study Membrane Proteins
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Circular dichroism (CD) spectroscopy can provide valuable information on membrane protein structures, including determination of secondary structures of intact proteins and domains, detection of conformational changes associated with binding ligands and different functional states, examination of environmental effects and intermolecular interactions associated with complex