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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
Adam Muschielok, Joanna Andrecka, Barbara Treutlein, Jens Michaelis.
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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].
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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

formation, and monitoring protein folding and membrane insertion processes. This dynamic information can be a valuable complement to the more detailed structural information produced by crystallography and NMR spectroscopy.
Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy, which uses the intense light of a synchrotron for the measurements, has a number of advantages for membrane protein studies over conventional CD spectroscopy: The higher penetration of the light means proteins can be examined in detergents and lipid environments, as well as in high salts and buffer conditions used for crystallisation, so comparisons can be made as to the physiological relevance of structures. In addition it permits the use of high lipid-to-protein ratios which are more similar to native membranes. The higher signal-to-noise levels in SRCD enable the use of smaller amounts of protein and the detection of smaller conformational changes, as well as the detection of faster dynamic processes over a wider wavelength range. The lower wavelength data measurable improve the accuracy of secondary structure determinations and provide additional information on supersecondary motifs and folds. Plus, using oriented SRCD it is possible to determine the dispositions of different structural elements with respect to the membrane.
I will use voltage-gated sodium channels as a case study demonstrating the types of information that can be gleaned from CD and SRCD studies, including ligand and drug binding, thermal stability, and comparisons of wildtype, modified and mutant proteins.
This work was supported by grants from the U.K. BBSRC.

1091-Wkshp
Using Atomic Force Microscopy for Membrane Structural Analyses
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No Abstract.

Workshop 3: Biophysics of Renewable Energy and Cellular Power Plants

1092-Wkshp
The Development of Cellulosic Fuels
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Because plants can be deployed inexpensively on a large scale to capture and store solar energy in biomass, one way of moving toward the development of carbon neutral fuels is to use plant biomass for production of fuels. The efficient production of biofuels by routes other than gasification will require innovation in three main areas: sustainable production of feedstocks that do not compete with food production, depolymerization of feedstocks, and conversion of feedstocks to fuels. At present, it is expected that gasoline and diesel replacements will ultimately be derived from cellulosic biomass. In this respect there is renewed interest in identifying plants that have optimal biomass accumulation and understanding the production issues associated with large-scale cultivation and sustainable harvesting of such species. Additionally, the importance of enhancing soil carbon and nutrient retention while minimizing inputs will require an integrated approach to the development of cellulosic energy crops. The challenges on the processing side include the development of improved catalysts for polysaccharide and lignin depolymerization and conversion to fuels as well as the development of microbial strains that can convert a wide range of sugars to next generation fuels under harsh conditions.

1093-Wkshp
Ultrastructural Plant Cell Wall 3D Organization and Microbial Deconstruction
Manfred Auer.
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No Abstract.

1094-Wkshp
Biophysics in Cellulose Biosynthesis and Biodegradation
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Cellulose is considered to be one of the most abundant biopolymers on earth. Although the chemical composition of cellulose, β-1,4 linked linear glucose polymer (glucan), is relatively simple compared to other plant cell wall polysaccharides, the physical structure of cellulose is complex. It is generally believed that the β-1,4-glucans are synthesized by 36-unit synthase rosettes, each of which forms a 36-chain cellulose elementary fibril (CEF). Our preliminary results based on nanoscale imaging of samples from living maize cell walls suggest that several CEFs may then coalesce to a bundle, termed a macrofibril, which eventually splits at the end to form parallel microfibrils with concurrent deposition of other cell wall components (i.e. hemicellulose and pectin) secreted from Golgi apparatus. Cellulose microfibrils may twist during dehydration process of natural senescence. High resolution surface measurements suggest that native plant cellulose is a well-organized bundle of β-1,4-glucans. Celluloses can be crystalline, para-crystalline, and even amorphous, depending on their tissue source in native plants, or the way that cellulose is isolated. The structural integrity of cellulose is believed to be one of the major causes of resistance to chemical and enzymatic hydrolysis. Imaging of chemically pretreated corn stover has revealed that enzyme digestibility is positively correlated to the degree of disorder of plant cell wall microfibrils. The ordered nature of the cell wall microfibril probably represents the last and most crucial biological barrier to enzyme hydrolysis. Chemical pretreatment may cause both disordering of native cellulose structure and increased surface accessibility; different pretreatment approaches may be effective in bringing out either of these two changes, or both. Our current work is also focused on investigating the specific interaction between individual enzymes and biomass substrates using single molecule spectroscopy.

1095-Wkshp
The Genus Prevotella, A Resource of Enzymes for Hemicellulose Degradation
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Hemicellulose and cellulose constitute two major targets in plants for cellulosic ethanol production. Whereas cellulose is a highly homogenous polymer of glucose joined in beta-1,4-glycosidic linkages, hemicellulose is mostly a heterogenous polymer of xylose and arabinose. Thus, the common arrangement of sugars in hemicelluloses, such as xylans from bioenergy feedstocks, is a beta-1,4-linked xylose backbone with side chains of arabinofuranosyl, acetyl, and 4-O-methyl glucuronyl groups. Complete hydrolysis of hemicellulose, therefore, requires a complex set of enzymes. Nature has selected for microorganisms that derive their carbon and energy sources from hemicellulose by an enzymatic action that deconstructs the polymer into its component sugars. Such microorganisms include the genus *Prevotella*. We are, therefore, using genomics, bioinformatics, biochemical, and structural analyses to unravel the strategies used by *Prevotella* spp to break down hemicellulose. Our ultimate goal is to rationally assemble enzyme cocktails from these microorganisms for use in the bioenergy industry. We have demonstrated that *Prevotella bryantii* grows rapidly on hemicellulose. Furthermore, the sequencing of its genome has allowed identification of genes likely to encode products for deconstruction of hemicellulosic substrates. Several of these genes have been expressed as recombinant proteins in *E. coli*. Experiments that aimed at examining the synergistic activities of the *P. bryantii* enzymes have led us to reconstitute an enzyme complex that degrades wheat arabinoxylan into its component sugars. This enzyme mixture is a promising product in our effort to deconstruct hemicellulose. We are currently using transcriptomic analyses to improve the enzyme cocktail, and structural analysis is also being applied to rationally synthesize new carbohydrate active enzymes with enhanced activities.

1096-Wkshp
Engineering Feedstocks for Biofuel Production
Pamela Ronald.
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Researchers in JBEI's Feedstocks Division are developing plants whose lignocellulosic biomass can be more economically and efficiently deconstructed into fermentable sugars for the production of biofuels. Achieving this requires a far better scientific understanding of plant cell wall structure, as well as identifying all the genes and enzymes involved in making lignocellulose. JBEI Feedstocks Division researchers focus their studies on rice, a genetic model for switchgrass and *Miscanthus*, two perennial grasses with great potential as energy crops; and on *Arabidopsis*, a small flowering plant related to mustard, which is a model for poplar, a tree that's also touted as a future source of biofuels. Rice and *Arabidopsis* go from seed to maturity in a matter of weeks, as compared to the year or more required for the biofuel plants they model.