provide a mechanism on how proteins remotely modulate bound ligands to create preferred kinetic pathways(5,6).
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Platform B: Biotechnology & Bioengineering

28-Plat
Sequencing Paired Reads using True Single Molecule Sequencing (tSMS)™ Technology
Jeff G. Reifenberger, Doron Lipson, Jennifer Colonell, Tal Raz,
Bill Pierceall, John Thompson, Patrice Milos.
Helicos BioSciences Corp., Cambridge, MA, USA.
Single molecule DNA sequencing provides novel methods for interrogating DNA molecules. For example, genomic rearrangements such as insertions, deletions, and inversions that are often associated with cancers or variations within the transcriptome of specific genes can be difficult to detect with conventional sequencing strategies. Paired reads sequencing, where a spacer is inserted between two single molecule sequencing reads, offers a more viable method for detecting genomic rearrangements. We have developed a paired reads strategy using True Single Molecule Sequencing (tSMS)™ in which a large number of individual templates of DNA were analyzed using a proprietary form of sequencing-by-synthesis. To create paired reads DNA strands are attached to a surface and sequenced-by-synthesis for a known number of cycles. A spacer was then added to the DNA strands in a controlled manner and then sequencing by synthesis continued for the same number of cycles. Data on test oligonucleotides of known length and sequence demonstrate the viability of the technique and our ability to control the length of the spacer between the two reads on an individual strand. We have now extended our Paired Reads technique to biological samples, initially with a 12kb PCR product encompassing the CETP gene to demonstrate our ability to sequence the whole gene product and identify mutations which have been inserted into the CETP reference. Finally we have utilized this novel method to examine a human placental transcriptome cDNA library to demonstrate the ability to span exon boundaries.

29-Plat
Self-assembly via Active Transport By Biomolecular Motors
Parag Katira, Isaac Luria, Shruti Seshadri, Henry Hess.
University of Florida, Gainesville, FL, USA.
Modified kinesin gliding motility assays display striking self-assembly phenomena,[1] in particular the formation of non-equilibrium structures. Biotinylated microtubules partially coated with streptavidin form ‘‘wires’’ and ‘‘spools’’ while gliding on kinesins adhered to the surface.[2] The spool formation process was investigated in detail, and we found that the assembled spools exhibit a narrow distribution of spool diameters, and that the average diameter is an order of magnitude smaller than what would be obtained from a thermally driven assembly process. We also observe that pinning of the microtubule leading tip on the surface initiates the spool formation in 80% of the cases. By modeling the mechanism of microtubule tip buckling and the resultant microtubule spool diameter as a function of the microtubule persistence length and the number of motors attached to the microtubule, we can predict the average spool diameter and the observed spool diameter distribution. The model suggests a strong dependence of spool diameters and size distribution on the surface kinesin density. The goal of our research is to obtain a better understanding of the dynamics of this multi-agent process and its implications for self-assembly in general.
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30-Plat
Single Cell Detection and Analysis with Asynchronous Rotation of Driven Magnetic Microspheres
Brandon H. McNaughton, Paivo Kinnunen, Rodney R. Agayan,
Ron G. Smith, Alan J. Hunt, Raoul Kopelman, Roy Clarke.
University of Michigan, Ann Arbor, MI, USA.
The nonlinear rotational response of a magnetic micropshere, suspended in a viscous fluid, occurs when a driving magnetic field, used to rotate the magnetic particle, exceeds a critical frequency. Above this critical frequency, the particle is asynchronous with the external field. Shifts in this nonlinear rotational frequency of the magnetic microsphere offer a dynamic approach for the detection (see Appl. Phys. Lett. 2007 **91**, 224105) and analysis of bacterial cells (i.e. growth and response to chemical agents).

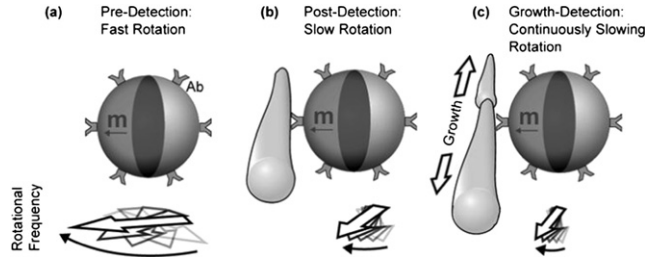

Figure: Schematic of the asynchronous (nonlinear) rotation rates of an antibody-coated magnetic microsphere with (a) no bacteria, (b) a single bacterium and (c) growth of the attached bacterium.

31-Plat
Remote Steering of C. Elegans Using Nanoparticle Heating
Heng Huang, Savas Delikanli, Hao Zeng, Arnd Pralle.
SUNY Buffalo, Amherst, NY, USA.
Remote and parallel stimulation of a subpopulation of neurons will aid the understanding of signal processing in the complex neuronal networks tremendously. We present a method capable of stimulating neurons deep inside the body. It relies on manganese iron oxide nanoparticles targeted to the neuron’s plasma membrane to convert energy from an alternating magnetic field to local heat which opens the temperature sensitive Calcium channel TRPV1. Expressing this channel in neurons will allow us to remotely stimulate and control the neurons. We apply the method to trigger the ASH and ADL neurons in C. elegans which control the chemical avoidance reaction.
In the course of this study, we have characterized nanoscale heating and heat propagation inside cells quantifying heat conduction over nanometer distances, important knowledge to optimize the efficacy of hypothermia treatment for cancer.

32-Plat
Probing Conformational Changes In Rhodopsin With Site-specific Azido Labels
Shixin Ye[1], Thomas Huber[1], Reiner Vogel[2], Thomas P. Sakmar[1].
[1]Rockefeller University, New York, NY, USA, [2]Albert-Ludwigs-University Freiburg, Freiburg, Germany.
Transmembrane signaling via heptahelical G protein-coupled receptors (GPCRs) is essential for a cell’s communication with its environment and represents a major target for drug development. Although recent advances have provided high-resolution crystal structures of several GPCRs, understanding the conformational dynamics of receptor activation in bilayers remains paramount. Fourier-transform infrared (FTIR) difference spectroscopy has proven to be a powerful biophysical technique for structure/function relationships in the prototypical GPCR, rhodopsin. Here we report a new conceptual advance in FTIR difference spectroscopic analysis of heterologously expressed eukaryotic proteins. We demonstrate the site-directed incorporation of an IR-active unnatural amino acid, *p*-azido-L-phenylalanine (azidoF), into rhodopsin using amber condon suppression technology. The intense antisymmetric stretch vibration of the azido group absorbs at around 2100 cm^{-1} in a clear spectral window devoid of other protein bands and is exceptionally sensitive to the polarity of its surroundings. Using FTIR difference spectroscopy on azidoF rhodopsin mutants, we report the changes in the electrostatic environments of selected side chains on both the cytoplasmic and extracellular receptor surfaces during the conformational transition associated with receptor activation.

33-Plat
Towards Mapping Domain Boundaries of Proteins
Shambaditya Saha[1], Thomas D. Pollard[1,2].
[1]Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA, [2]Dept. of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA.
GFP fluorescence from bacterial colonies expressing a polypeptide upstream of GFP depends on the solubility of the polypeptide. Inspired by this idea, we developed a method to test for the folding of any polypeptide longer than about 66 amino acids. This method bypasses the need for purification of the polypeptide to test for folding. We employed this technique to identify the independently folded domains of Mid1, a fission yeast protein of 920