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
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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).

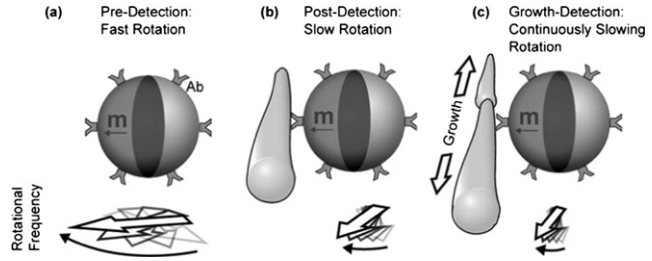

Figure: Schematic of the asynchronous (nonlinear) rotation rates of an antibody-coated magnetic microsphere with (a) no bacteria, (b) a single bacterium and (c) growth of the attached bacterium.

31-Plat
Remote Steering of C. Elegans Using Nanoparticle Heating
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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].
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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
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[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

amino acids that participate in cytokinesis. A random fragment library of Mid1 cDNA was generated using tagged random primer PCR (tPCR). The fragment library was cloned upstream of a monomeric enhanced GFP (mEGFP) and expressed in *E.coli*. The GFP fluorescence of the bacterial colonies expressing the fusion constructs were compared to a threshold level of fluorescence to test if the Mid1 fragment fused to mEGFP is folded. Colonies expressing folded fragments of Mid1 are brighter than control colonies or colonies expressing an unfolded protein fragment fused upstream of mEGFP. Approximately 27000 colonies were screened to identify five soluble folded domains and one insoluble region, accounting for the entire length of Mid1 protein. This large scale approach will be useful in rapidly mapping domain boundaries of proteins in absence of any prior knowledge about the domain organization of the proteins.

34-Plat
A Protein Interaction Network generated from Streptococcus Pneumoniae
Doron Gerber, Sebastian J. Maerkl, Stephen R. Quake.
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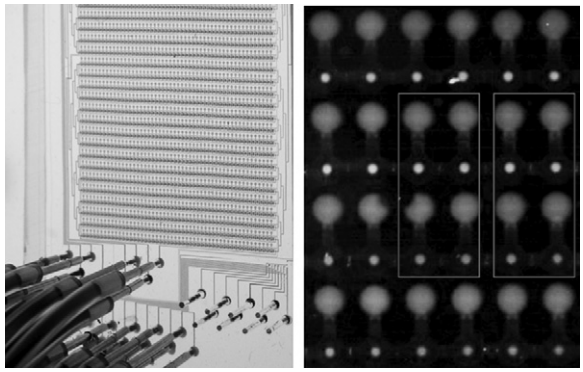
Mapping protein interaction network topologies represents a fundamental step towards a proteome-wide understanding of biological processes. The current high-throughput methods used for protein interactions are yeast two hybrid and affinity purification coupled with mass spectrometry, but both systems require cumbersome cloning steps, are challenging to automate, and have limited ability to detect weak or transient interactions. To overcome these disadvantages we developed a microfluidic in vitro protein expression and interaction platform based on a highly parallel and sensitive microfluidic affinity assay. We used this system to perform 14,792 on-chip experiments which exhaustively measure the protein-protein interaction network of 43 Streptococcus pneumoniae proteins. The resulting network of 157 interactions is denser than one would expect based on the existing data from E. coli and H. pylori. The network shows evidence of being scale free, with the most highly connected nodes derived from chaperones. Analysis of the network reveals previously undescribed physical interactions members of some biochemical pathways.



Left panel - Microfluidic device loaded with food-dyes. Flow lines (blue), ''Sandwich, Neck valves and Button valves'' (red, yellow and green) used for performing PING. Right panel - example of 2 protein-protein interactions (orange).

35-Plat
Diversity-Based Design of Synthetic Gene Networks with Desired Functions
Xiao Wang, Tom Ellis, James J. Collins.
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Constructing predictable gene networks with desired functions remains hampered by the lack of well-characterized components and the fact that assembled networks often require extensive, iterative retrofitting for optimization. Here we present an approach where network components are synthesized with random sequences incorporated into their design, giving rapid parallel production of component libraries with inherent diversity. When coupled with in silico modeling, libraries present a choice of characterized parts for gene network design, and those optimal for the desired function can be selected for network assembly, without the need for post-hoc tweaking. We validated our approach in yeast (S.cerevisiae) by synthesizing a regulatory promoter library and using it to construct negative feedforward loop networks with different, desired input-output characteristics. We then implemented the method to produce a synthetic gene network that acts as a timer, tunable by component choice. We utilize this network to control the timing of the yeast flocculation phenotype, which is crucial to brewing, illustrating a practical application of our approach.

Platform C: Oxidative Phosphorylation & Mitochondrial Metabolism

36-Plat
Molecular Basis of Substrate Selectivity in the ADP/ATP Carrier
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The ADP/ATP carrier (AAC) is a membrane transporter that mediates the exchange of ADP and ATP across the mitochondrial inner membrane. During an exchange cycle, AAC switches between two conformational states, the cytoplasm-open state (c-state), and the matrix-open state (m-state). Our recent molecular dynamics simulations revealed spontaneous binding of ADP to the c-state AAC, and identified the unknown binding site for ADP as a pocket deeply positioned inside the lumen that forms through significant conformational changes of several basic residues in response to substrate binding. We also showed that ADP binding likely triggers AAC transition to the m-state by breaking a salt bridge network. The identified binding site has allowed us to explore substrate selectivity in AAC by simulating the ''binding'' of various ligands, e.g., AMP and Mg-ADP, to AAC. AMP does bind but is not transported by AAC, and Mg is known to have an inhibitory effect on AAC. However, the molecular details involved in these processes are largely unknown. Our results suggest that the presence of a minimum of two phosphate groups in their Mg-free form is absolutely necessary for proper binding and for initiating the structural changes required for activation of AAC. AMP and Mg-ADP cannot establish sufficient contact with the salt bridge ring at the bottom of AAC lumen, either due to lack of the beta-phosphate in AMP, or interference of $Mg2+$ with the phosphate groups in Mg-ADP. These results provide additional evidence for the ADP binding site characterized in our earlier study, and suggest a mechanism for substrate selectivity in AAC.

37-Plat
VDAC Regulation by Cytosolic Proteins
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Voltage-dependent anion channel VDAC, positioned on the interface between mitochondria and the cytosol, is at the control point of mitochondria life and death. This large channel plays the role of a ''switch'' that defines in which direction mitochondria will go: to normal respiration or to suppression of mitochondria metabolism that leads to apoptosis and cell death. As the most abundant protein in the mitochondrial outer membrane (MOM), VDAC is known to be responsible for ATP/ADP exchange and for the fluxes of other metabolites across MOM. It controls them by switching between the open and ''closed'' states that are virtually impermeable to ATP and ADP. This control has dual importance: in maintaining normal mitochondria respiration and in triggering apoptosis when cytochrome c and other apoptogenic factors are released from the intermembrane space into the cytosol. Emerging evidence indicates that VDAC closure promotes apoptotic signals without direct involvement of VDAC in the permeability transition pore or hypothetical Bax-containing cytochrome c permeable pores. Closure of VDAC induced by such dissimilar cytosolic proteins as pro-apoptotic tBid and dimeric tubulin is compared to show that the involved mechanisms are rather distinct. While tBid mostly modulates VDAC voltage gating, tubulin blocks the channel with the efficiency of blockage controlled by voltage. Tubulin strikingly increases voltage sensitivity of VDAC reconstituted into planar phospholipid membrane and could induce VDAC closure at < 10 mV transmembrane potentials. Experiments with isolated mitochondria confirm a tubulin-induced VDAC closure. Our findings suggest a novel mechanism of regulation of mitochondrial energetics, governed by VDAC and tubulin at the mitochondria-cytosol interface. Overall, we demonstrate that VDAC gating is not just an observation made under artificial conditions of channel reconstitution but is a major mechanism of MOM permeability control.

38-Plat
A Microcompartment Of Mitochondrial Nucleoside Diphosphate Kinase: Cardiolipin Interaction And Coupling Of Nucleotide Transfer With Respiration
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[1]Inserm, U884, Univ Joseph Fourier, Grenoble 1, Grenoble, France, [2]Inserm, UMR S893, CdR Saint-Antoine, UMPC Univ Paris 6, Paris, France.
Molecular functions of mitochondrial nucleoside diphosphate kinase (NDPK-D) were studied using different biophysical and biochemical techniques. Subfractionation of rat liver and HEK 293 cell mitochondria revealed that NDPK-D is essentially bound to the inner membrane. The kinase interacted electrostatically with anionic phospholipids, showing highest affinity for cardiolipin as