

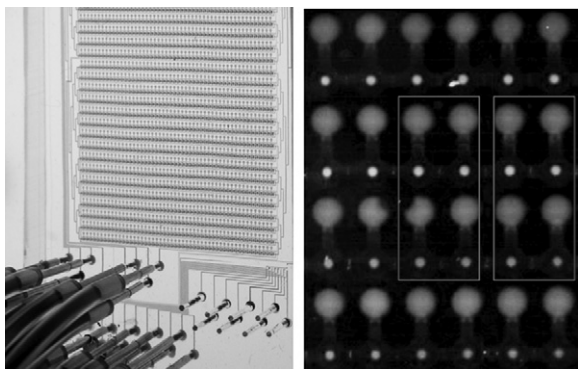
amino acids that participate in cytokinesis. A random fragment library of Mid1 cDNA was generated using tagged random primer PCR (tPCR). The fragment library was cloned upstream of a monomeric enhanced GFP (mEGFP) and expressed in *E. coli*. The GFP fluorescence of the bacterial colonies expressing the 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*

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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

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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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Molecular functions of mitochondrial nucleoside diphosphate kinase (NDPK-D) were studied using different biophysical and biochemical techniques. Sub-fractionation 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