quantified by surface plasmon resonance. NDPK-D was also able to cross-link anionic phospholipid-containing liposomes as seen in light scattering assays, suggesting that the hexameric kinase could promote intermembrane contacts. Mutation of the central arginine (R90) in a surface exposed basic RRK motif unique to NDPK-D strongly reduced these membrane interactions. In a model using HeLa cells naturally almost devoid of NDPK-D, wt protein and R90D mutant were stably expressed, but only wt protein was found attached to membranes. Respiration was significantly stimulated by the NDPK substrate TDP only in mitochondria containing wt NDPK-D, but not in those expressing R90D mutant that is catalytically equally active. This indicates local ADP regeneration in the mitochondrial intermembrane space and a tight functional coupling of NDPK-D with oxidative phosphorylation that depends on the membrane-bound state of the kinase. A model is proposed for a mitochondrial NDPK microcompartment.

39-Plat

Using Two-photon Excited Fluorescence Intensity- and Lifetime-based NADH Imaging to Investigate Cochlea Metabolism

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Metabolism and mitochondrial dysfunction are thought to be involved in many different hearing disorders including noise induced hearing loss and presbycusis. We have employed two-photon fluorescence imaging of intrinsic mitochondrial reduced nicotinamide adenine dinucleotide (NADH) to study the metabolic status of the different cell types in excised yet intact mouse organ of Corti preparations. Recent published studies employ fluorescence lifetime imaging (FLIM) to determine the ratio of the free to enzyme-bound fluorophores populations that occur during changes in metabolism. We have compared traditional intensity based methods to FLIM in order to evaluate the two different methods in both cultured cells and the excised organ of Corti. Treatment with both metabolic uncouplers and inhibitors caused systematic shifts in both the lifetime and populations of the free and bound pools of NADH, resulting in significant differences in the calculated concentration of NADH when compared to using intensity alone to calculate the same value. Mapping of the locations of the individual lifetimes, shows that the lifetime of NADH varies in different cellular locations as well as in different cell types. Possible implications for the study of hearing loss will be discussed.

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40-Plat

Mitochondrial Energy Metabolism and Ca^{2+} Handling in Pancreatic Beta-cells. A System Analysis Approach

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Pancreatic islet beta-cells respond to rising blood glucose by increasing oxidative metabolism, leading to both an increased mitochondrial membrane potential (Ψm) and ATP/ADP ratio in cytoplasm. This leads to a closure of K_{ATP} channels, depolarization of the plasma membrane, influx of calcium and the eventual secretion of insulin. Such a signaling mechanism suggests that mitochondrial metabolism and ATP/ADP ratio regulation in beta-cells may be specially coupled in comparison with other cell types. We performed mathematical modeling to quantitatively assess how cytoplasmic ATP/ADP ratio can be controlled by mitochondria. The cytoplasmic part of the model includes glucokinase, glycolysis, pyruvate reduction, NADH and ATP production and consumption. The mitochondrial part of the model includes production of NADH, which is regulated by pyruvate dehydrogenase. NADH is used in the electron transport chain to establish a proton motive force, driving the F_1F_0-ATPase. Mitochondrial matrix Ca^{2+} is determined by the Ca^{2+} uniporter and Na^+/Ca^{2+} exchanger. The model is described by ordinary differential equations for cytoplasmic and mitochondrial parameters. The model simulates the response of the ATP/ADP ratio to changes in substrate delivery, to inhibition of the mitochondrial Ca^{2+} exchanger and other effects. We found that mitochondrial Ψm should be in a range lower than 150 mV (where F_1F_0-ATPase is sensitive to Ψm) to provide a sensitivity of the ATP/ADP ratio to glucose in beta-cells. On other hand, Ψm can work in the range above 150 mV to provide a maximal F_1F_0-ATPase productivity in other cell types (for example in myocytes). Kinetic analysis of the model reveals that these differences can be simulated by a decreased respiratory activity and higher leak capacity in beta-cell mitochondria in comparison with muscle cell mitochondria that were found recently by Affourtit and Brand (2006).

41-Plat

Modeling Regulation of Mitochondrial Free Ca^{2+} by ATP/ADP-Dependent Ca^{2+} Buffering

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Introduction: Mitochondrial free $[Ca^{2+}]$ ($[Ca^{2+}]_m$) is regulated by cation fluxes through the Ca^{2+} uniporter (CU), Na^+/Ca^{2+} exchanger (NCE), Na^+/H^+ exchanger (NHE), and Ca^{2+}/H^+ exchanger (CHE) as well as via Ca^{2+} buffering by the mitochondrial proteins. However, the regulation of $[Ca^{2+}]_m$ via ATP/ADP-dependent dynamic Ca^{2+} buffering mechanism inside the mitochondrial matrix during transient state-3 respiration is not well known. **Methods:** To gain a quantitative understanding of this Ca^{2+} buffering phenomenon, we developed a computational model of mitochondrial bioenergetics and Ca^{2+} handling by integrating our recent biophysical models of the CU, NCE, NHE, and CHE into our well-validated model of mitochondrial oxidative phosphorylation, TCA cycle, and electrophysiology. The model also accounts for binding and buffering of cations with metabolites, including ATP, ADP and Pi. Experiments were performed to spectrofluorometrically measure $[Ca^{2+}]_m$, pH_m, membrane potential ($\Delta\Psi_m$), and NADH redox state in guinea pig heart mitochondria suspended in Na^+ and Ca^{2+} free buffer medium (ensured with ~50 μM of EGTA) with 0.5 mM pyruvic acid (HPyr). Dynamics were inferred with various addition of $CaCl_2$ (0, 10, 25 μM of $CaCl_2$; 16, 88, 130 nM of free $[Ca^{2+}]$ followed by 250 μM of ADP in the presence or absence of carboxyatractyloside (ANT blocker) and oligomycin (F_1F_0-ATPase blocker). **Results and Discussion:** Model analysis of the data on (i) initial decrease of $[Ca^{2+}]_m$ with addition of Na^+-independent substrate HPyr, and (ii) transient increases of $[Ca^{2+}]_m$ with addition of ADP suggests ATP/ADP-dependent dynamic Ca^{2+} buffering inside the cardiac mitochondrial matrix. This model will be helpful to understand mechanisms by which $[Ca^{2+}]_m$ both regulates, and is modulated by, mitochondrial energy metabolism.

42-Plat

The External Stalk of the FoF1-ATPase: 3D-Structure of the b-Dimer

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The structure of the external stalk of the FoF1-ATP synthase and its function during catalysis remain one of the important questions in bioenergetics.
Proteomics, structure prediction, molecular modeling and ESR spectroscopy using site-directed spin labeling were employed to elucidate the structure and interfacial packing of the E. coli b-subunit homodimeric and Synechocystis bb' heterodimeric stalks of ATP synthases.
b-Subunits of different origin demonstrate little sequence similarity. Structure prediction algorithms, however, showed that all of the compared sequences contain extensive heptad repeats, suggesting that these proteins may favorably pack as left-handed coiled coils (LHCC).
Molecular modeling of homo- and heterodimeric b produced low energy LHCC. Extensive mutagenesis followed by site-directed spin labeling and subsequent ESR investigations in soluble homo- and heterodimeric b-constructs allowed the determination of inter- and intra-subunit distances.
Inter-spin distances obtained by ESR agreed very well with distances derived from LHCC molecular models of b- and bb'-dimers and therefore strongly support our proposition that dimeric external stalks of ATP synthases indeed form left-handed coiled coils.
The extreme C-terminal part of the b-dimer is not predicted to form a coiled coil structure. We are presently investigating this part of the second stalk both when in solution and when in complex with soluble F1-ATPase. The influence of subunit δ is of particular interest due to its proposed direct interaction with the C-terminus of subunit b.
Initial site-directed spin labeling and ESR experiments using complete E. coli FoF1-ATP synthase indicate that the inter-subunit packing of the b-dimer changes during catalytic turnover, which may be a mechanism for elastic coupling of the different rotating parts of the enzyme.

43-Plat

Torque Generation Mechanism of ATP Synthase and Other Rotary Motors

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Ion driven rotary motors, including F0-ATP synthase (F0) and the bacterial flagellar motor, convert energy from ion translocation into torque and rotary

motion. Here we propose a mechanism whereby electric fields emanating from channels in the stator(s) create forces acting on ion binding sites in the rotor, driving it to rotate. The model predicts a scaling law relating torque to ion motive force and number of stators, consistent with experiment. The rotor of F0 drives the gamma-subunit to rotate within the ATP-producing complex (F1), working against an opposing torque that rises and falls periodically with angular position. Drawing an analogy with the washboard potential of the superconducting Josephson junction, we compute ATP production rate vs. ion motive force, finding excellent quantitative agreement with experiment and accounting for the crossover from ATP production to consumption. Plausible mechanisms for reversing the direction of bacterial flagellar rotation become self-evident in the model. (Supported by NIH R21CA133153, TcSUH, Welch (E-1221), and THECB-ARP.)

Platform D: RNA Folding & Ribosome

44-Plat
Laser Assisted Single-molecule Refolding
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In vivo, many RNA molecules can adopt multiple conformations depending on their biological context. For example, an RNA molecule initially in a stable hairpin conformation may later interact with a second RNA molecule, thus triggering a dimerization reaction. This is the case of the HIV Dimerization Initiation Sequence (DIS) and the DsrA RNA in bacteria. It is quite common that the initial interaction between the two RNAs takes place via complementary unpaired regions, forming a so-called kissing complex. However, the exact kinetic mechanism by which the two RNA molecules reach the dimerized state is still not well understood.
To investigate the refolding energy surface of RNA molecules, we have developed new technology based on the combination of single molecule spectroscopy with laser induced temperature jump kinetics, called Laser Assisted Single-molecule Refolding (LASR). LASR enables us to induce folding reactions of otherwise kinetically trapped RNAs at the single molecule level, and to characterize their folding landscape. Single molecule time trajectories show that we can drive the dimerization reaction between two trapped kissing RNA hairpins with LASR and use this data to calculate folding enthalpies and entropies. Our LASR experiments indicate that the RNA kissing complex is a stable intermediate state that facilitates the dimerization reaction.
LASR provides an exciting new approach to study molecular memory effects and kinetically trapped RNAs in general. LASR is readily applicable to study DNA and protein folding as well.

45-Plat
Single Molecule Analysis of Group I Ribozyme Folding Reveals Pronounced Ruggedness Throughout Its Folding Landscape
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It is well established that biological activity of macromolecules is intimately related to their structures. Significant efforts are directed at characterizing macromolecular structures and structure-function relationships. It is less appreciated that because of their complexity macromolecules are likely to fold not into unique structures, but into ensembles of different conformations distinct in their biological activity. Strong evidence for such complex behavior has been recently obtained in several macromolecular systems, mostly by single molecule methods. Understanding biological activity of macromolecules requires detailed characterization of the species that co-exist in the ''folded'' states, in other words - mapping of their folding energy landscape.
We used single molecule FRET to map folding energy landscape of a catalytic RNA - ribozyme derived from a group I intron. Labeling several different positions within the ribozyme allowed us to probe different parts of the folding landscape: from essentially unfolded to fully folded ribozyme. These experiments revealed broad heterogeneity of folding behaviors of individual molecules. Strikingly, even in conditions in which the ribozyme is fully folded and active, different conformations retain distinct activity. Distinct folding behaviors are ''remembered'' by individual molecules upon long incubation in conditions in which the ribozyme lacks most of its tertiary structure. This finding suggests that the origins of heterogeneity might appear at the level of secondary structure. Pinning down the structural basis for ruggedness of RNA folding landscapes and understanding its role in biology represents an exiting future challenge.

46-Plat
Identifying Energy Barriers in RNA Folding Through Kinetic Model Enumeration
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Large RNA molecules are known to fold through multiple parallel pathways to achieve their functional conformation. These pathways include stable intermediate structures that we identify using our KinFold algorithm (http://simtk.org/home/kinfold) for the analysis of time-resolved hydroxyl radical footprinting experiments. KinFold enumerates kinetic model topologies to determine the structure, lifetimes, and abundance of intermediates along the folding pathways of RNA. We are therefore able to identify the best fitting kinetic model for each folding reaction studied. We used this approach to study the folding of the L-21 T. thermophila group I intron, a catalytic ribozyme, at 6 temperatures ranging from 25° to 51° C. Our analysis of this data reveals that the rates for a majority of the transitions (between intermediate, folded and unfolded states) obey the Arrhenius equation, allowing us to estimate the relative energies of activation for inter conversion between the states of an RNA. Furthermore, we are able to estimate the lifetimes of the different intermediate structures. These results reveal a simple landscape, where folding of the peripheral elements of the RNA create significant barriers to folding. Our approach is unique in that it allows us to simultaneously estimate the activation energies for all transitions in an RNA folding reaction.

47-Plat
Uncovering The Mechanism Used By Drosophila Snf Protein To Specifically Bind Two RNAs
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The Drosophila SNF protein is a natural chimeric protein that evolved from the metazoan U1A and U2B'' proteins. In the fly, SNF is found in both the U1 and U2 snRNPs, where it is assumed to bind to the U1 snRNA Stemloop II (the site of U1A binding) and U2 snRNA Stemloop IV (the site of U2B'' binding), respectively. Like U1A and U2B'' proteins, SNF has two RNA Recognition Motifs (RRM). By analogy to U1A protein, the N-terminal RRM should bind RNA, while the C-terminal RRM does not. Our experiments show that indeed the SNF C-terminal RRM does not bind RNA; NMR data show it to be soluble and stable. The SNF N-terminal RRM alone is not stable, however, and requires the C-terminal domain to maintain a folded form. NMR experiments and ^{15}N-relaxation data show that the N-terminal RRM undergoes conformational exchange on the chemical shift timescale, while the C-terminal RRM has uniformly high order parameters characteristic of a more rigid protein.
We hypothesize that the increased flexibility of the N-terminal RRM is part of its RNA binding mechanism, since SNF does indeed bind both U1 snRNA Stemloop II and U2 snRNA Stemloop IV. The affinity of SNF for these two RNAs differs by three orders of magnitude, indicating that one is preferentially bound. By comparison, however, the affinities of the human U1A protein for these two RNAs differ by more than 10^6-fold, indicating that SNF has found a way to bind both RNAs. This hypothesis will be tested by mutation of the N-terminal domain, with subsequent determination of protein structure, dynamics, and RNA binding.

48-Plat
Parallel Pathways in 30S Ribosome Assembly
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The bacterial 30S ribosomal subunit self-assembles in vitro to form an 850kDa RNP. Assembly is generally thought to advance when the core 16S rRNA navigates through many low-energy kinetic traps, guided by the 20 small-subunit proteins that recognize and lock in native RNA tertiary structure. Kinetic analyses of protein binding reveal a highly choreographed, ordered assembly process, consistent with large-scale qualitative observations of ordered protein binding and measurements of thermodynamic cooperativity within synthetic fragments of the 30S. However, the precise order of events in this complex process are not well understood.
We have constructed a 2-photon, 3-color detection Fluorescence Correlation Spectroscopy (FCS) microscope to measure the kinetic cooperativity in the assembling 30S ribosome. Up to three ribosomal proteins of interest can be labeled with different fluorophores, and the populations of up to seven different bound species with different combinations of labels can be discriminated by