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energized status of the inner membrane caused dramatic structural alterations in the channel region. In an energized membrane, TMS2 formed a continuous α-helix that was inaccessible to the aqueous intermembrane space. Upon depolarization, the helical periodicity of TMS2 was disrupted and the channel became exposed to the IMS. Real time kinetic measurements confirmed that changes in TMS2 conformation coincided with depolarization. This analysis is extended to the soluble receptor domain of Tim23, where we show protonmotive force-coupled structural changes and key protein interactions that are mediated by specific lipids within the inner membrane. These results reveal how the energized state of the membrane drives functionally relevant structural dynamics in membrane proteins that are coupled to processes such as channel gating.

**1869-Pos Board B599**

**Evolutionary Perspective on the Coupling Mechanism of Complex I and Related Enzymes**

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Complex I is an energy transducing enzyme present in the three domains of life. This enzyme catalyzes the oxidation of NADH and the reduction of quinone coupled to charge translocation across the membrane. In this way, it contributes to the establishment of the transmembrane difference of electrochemical potential which is used for ATP synthesis, solute transport and motility. The research on this enzyme has gained a new enthusiasm, especially after the resolution of the crystallographic structures of bacterial and mitochondrial complexes. Most attention is now dedicated to the investigation of the energy coupling mechanism.

In this work, we made a thorough investigation of complex I and group 4 [NiFe] hydrogenases and established a third member of this family of proteins: the energy-converting hydrogenase related complex. We observed that four subunits (NuoB, D, H and antiporter-like) are common to the 3 types of complexes and we have denominated these subunits as the universal adaptor. We further explored the properties of the adaptor by investigating the structural characteristics of the antiporter-like subunit. We observed that the adaptor contains an antiporter-like subunit with a long amphipathic α-helix. The long helix is a common denominator that has been conserved through evolution. This should reflect a key role of such helix in the coupling mechanism of this family of enzymes.

We are currently investigating the structural motifs involved in $Na^+/H^+$ antiporter activity in complex I and related complexes. These findings are a step forward in the investigation of the coupling mechanism of complex I.

**1870-Pos Board B600**

**Water Gated Transitions in Proton Pumping of Respiratory Complex I**

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Respiratory Complex I or NADH:ubiquinone oxidoreductase is a redox-driven proton-pump. Powered by quinone reduction, Complex I drives the energetically uphill translocation of protons across the mitochondrial inner membrane and bacterial cytoplasmic membrane. The established electrochemical proton gradient provides the driving force for active transport and synthesis of ATP and is thus crucial for biological energy conversion. Complex I comprises a membrane domain that includes three antiporter-like subunits, involved in the proton-pumping process, and a soluble domain, responsible for reduction of quinones by electron transfer from NADH. Remarkably, site-directed mutagenesis experiments show that mutations of titratable residues in the antiporter-like subunits, ~200 Å away from site of quinone reduction, inhibit both proton pumping and quinone reduction. To explain this long-range proton-coupled electron transfer mechanism, both indirect and direct coupling models have been suggested. However, despite the recent elucidation of the complete intact structure of Complex I, the molecular principles of the coupling principles remain elusive. We present here results of large-scale classical and hybrid quantum-classical (QM/MM) molecular dynamics (MD) simulations of Complex I, embedded in biologically realistic environments. Our simulations indicate that water molecules provide important elements in the proton-pumping process. Our findings may form a basis for understanding long-range energy transduction in Complex I, and mechanistic similarities to other redox-driven proton-pumps such as cytochrome c oxidase and bacteriorhodopsin.

**1871-Pos Board B601**

**Dielectric Heterogeneity in the Cytochrome B6F Complex**

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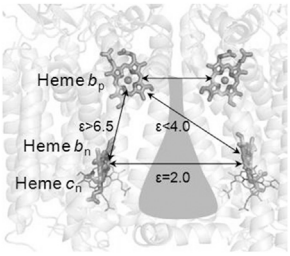
Electron transfer in the dimeric cytochrome $b_6f$ complex, which includes four *b*-type hemes organized as two pairs in symmetric monomers, was studied by simultaneous measurement of the kinetics of heme reduction by dithionite and an associated amplitude increase of Soret band split circular dichroism (CD) spectra diagnostic of heme-heme exciton interactions, for which similar kinetics were determined. Based on inter-heme distances and orientations from crystal structures of the complex, the increase in the split CD signal is dominated by interaction between the two intra-monomer *b*-hemes, located on the electrochemically negative and positive sides of the complex, whose midpoint oxidation-reduction potentials, $E_m$, determined by titrations of isolated complex, differ by 75-100 mV. Kinetics are fit best by preferential reduction of the intra-monomer heme pair. Equilibration of transferred electrons would, however, predict preferential reduction of the two higher potential hemes, one in each monomer. Heterogeneity of the dielectric constant is implied, a consequence of structure inhomogeneity, and/or dielectric reorganization in response to electron transfer. The largest dielectric constant exists between the intra-monomer *b*-hemes, resulting in a lower energy state of the reduced intra-monomer heme pair relative to any other heme pair.




**1872-Pos Board B602**

**Removal of Endogenous Phospholipids of Rhodobacter Sphaeroides Cytochrome C Oxidase affects the Flexibility of the Enzyme**

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The crystal structure of cytochrome *c* oxidase (COX) from *Rhodobacter sphaeroides* shows two phospholipids intercalated inside subunit III and four at the interface between subunits I, III and IV. These phospholipids are necessary for electron transfer, but their exact function in the structure of COX is still unclear.

Phospholipids were removed from COX by incubation with molar stoichiometric amounts of phospholipase $A_2$ for 3 hours at 4 °C in 20 mM MOPS pH 7.2, 20 mM $CaCl_2$ and 0.2 % dodecyl maltoside. The enzyme was then washed on a cytochrome *c*-affinity column; phosphorous, iron and copper content was determined by inductively coupled plasma-mass spectrometry. Wild-type enzyme contained an average of 5 moles phosphorous per mole enzyme, while the delipidated enzyme contained less than one. Electron transfer activity in the treated enzyme was decreased 30% and it exhibited suicide inactivation. Inhibition of electron transfer activity and suicide inactivation were reversed by the addition of 1 mg/ml asolectin. The time dependence of α-chymotrypsin digestion of the enzyme showed that subunit I was digested at a faster rate in the delipidated COX, suggesting a more open conformation in the lipid-depleted COX. To further assess COX conformational flexibility upon delipidation, both COX forms were labeled in subunit III with a sulfhydryl group-directed fluorophore, N-iodoacetylaminoethyl-1-aminonaphthalene-5-sulfonate (IAEDANS). Fluorescence anisotropy measurements showed a 50% increase in the rotational rate of AEDANS-labeled delipidated COX. This increase in flexibility of subunit III affects the flexibility of the adjacent subunit I as shown by the higher chymotrypsin digestion rate of subunit I in the delipidated enzyme. Taken together, these data provide an explanation of the low turnover rates and suicide inactivation, both of which occur in COX in the absence of phospholipids.

**1873-Pos Board B603**

**Energy Transfer in a Molecular Motor in Kramers' Regime**

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We present a theoretical treatment of energy transfer in a molecular motor described in terms of Brownian motion on a multidimensional free-energy landscape. We implement the classical analog of the tight-binding model of quantum mechanics to transform the continuous diffusion equation to a discrete master equation that is analytically tractable [1]. This treatment applies for multidimensional non-separable periodic potentials enabling energy transfer between degrees of freedom to be described explicitly in both the strong and

weak coupling regimes. Our approach provides an opportunity to connect the continuous multidimensional Brownian-motion based theory with experiments, phenomenological models, and established results from non-equilibrium thermodynamics.

In Kramers' regime of deep potential wells, we derive a range of formal expressions for molecular motors. We determine physical properties including the drift and diffusion, the rate and efficiency of energy transfer, and the entropy generated, thereby unifying known results within a single theoretical framework [2]. We also consider thermal fluctuations for the motor and show that energy transfer between degrees of freedom creates statistical correlations between fluctuations in those degrees of freedom [3]. The fluctuation statistics provide an opportunity to distinguish the different operating regimes of the motor.

[1] K. J. Challis and M. W. Jack, Phys. Rev. E 87, 052102 (2013).
[2] K. J. Challis and M. W. Jack, arXiv:1208.5818, to appear in Phys. Rev. E.
[3] K. J. Challis and M. W. Jack, arXiv:1308.6354.

**1874-Pos Board B604**

**Mitochondrial Thermodynamic Efficiency and P/O Ratios are Controlled by the F1F0 ATP Synthase C-Subunit Stoichiometry**

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Recently the $F_0$ portion of the bovine mitochondrial $F_1F_0$-ATP synthase was shown to contain eight 'c' subunits ($n = 8$). This surprised many in the field, as previously, the only other mitochondrial $F_0$ solved (for yeast) had $n = 10$ 'c' subunits. The metabolic implications of 'c' subunit copy number are explored in this paper: Typical aerobically respiring *E. coli* ($n = 10$) and mitochondria ($n = 8$) are both found to have very high $F_1F_0$ thermodynamic efficiencies of $\approx$ 90%, whereas efficiency is lower at $\approx$ 65% for chloroplasts ($n = 14$). Reasons for this difference are discussed. Maximum mitochondrial P/O ratios in animal mitochondria ($n = 8$) are calculated to be 2.73 ATP/NADH and 1.64 ATP/$FADH_2$, yielding 34.5 ATP/glucose. The experimentally measured values of 2.52, 1.53, and 32.3, respectively, are only about 7% lower. Finally, the thermodynamic efficiency of oxidative phosphorylation is *not* lower than that of substrate level phosphorylation, as previously believed. The overall thermodynamic efficiencies of oxidative phosphorylation, glycolysis, and citric acid cycle are $\approx$ 80% in all three processes.

**1875-Pos Board B605**

**Cytoplasmic Loops of Subunits C and A in E. Coli F1Fo ATP Synthase Interact to Gate $H^+$ Transport to the Cytoplasm**

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Rotary catalysis in F1Fo ATP synthase is powered by proton transport through the membrane-embedded Fo sector, which functions like a proton-driven turbine. Proton binding and release occur in the middle of the membrane at Asp61 on the second transmembrane helix (TMH) of subunit c, which folds in a hairpin-like structure with two transmembrane helices (TMHs). Previously, the aqueous accessibility of Cys substitutions in the TM regions of subunits c and a were probed by testing the inhibitory effects of $Ag^+$ or Cd+2 on function, and defined two half-channels leading to the proton binding site at cAsp61. The half channel from the periplasm lies in the center of a four-helix bundle in subunit a. We show here that the gating of protons from the periplasmic half channel to the Asp61 binding site requires repositioning of helices at the aAsn214/aGln252 site of interaction between TMH4 and TMH5. In addition we show that $Ag^+$ and $Cd2^+$ sensitive Cys substitutions on the proton transporting pathway to the cytoplasm extend into the polar loop of subunit c. Further, $Ag^+$ and $Cd2^+$ sensitive Cys substitutions that are directly involved in proton transport through Fo are also found in two cytoplasmic loops of subunit a. We show here that Cys substitutions in the hairpin loop of subunit c and the two loops of subunit a, each of which are directly implicated in proton transport, can be cross-linked to each other. We suggest that the three loops pack as a single domain that serves to gate proton release to the cytoplasm.

**1876-Pos Board B606**

**Experimental Determination of the Ion Selectivity of an ATP-Synthase Membrane Rotor by Isothermal Titration Calorimetry**

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ATP synthases are rotating nanomachines that couple ATP synthesis or hydrolysis to the transmembrane flow of protons or sodium ions down or against their electrochemical gradient. The key coupling element is a membrane-embedded subcomplex, the c-ring. We have recently proposed a principle that explains the ion selectivity of the c-ring, and validated this through functional studies of several ATP synthases. Specifically, we have proposed that a conserved Glu/Asp confers a universal $H^+$ selectivity to all c-ring binding sites, and that additional amino-acids, which vary among species, have evolved to modulate this selectivity. In particular, we have shown that polar groups can suppress the $H^+$ selectivity of the c-ring by a factor of $1\text{-}10^3$. Thus, the enzyme becomes coupled to $Na^+$, due to the large excess of $Na^+$ over $H^+$ under physiological conditions. Here, we further assess this theory by directly measuring the selectivity of a representative c-ring through Isothermal Titration Calorimetry. Specifically, we characterized the c-ring from the ATP synthase of *Ilyobacter tartaricus*. From titrations at different pH values, we established that $K_d(Na^+) \sim 0.3$ mM while $K_d(H^+) \sim 0.3$ μM, confirming the notion that this prototypical $Na^+$-coupled c-ring is in fact $H^+$ selective, although to a much smaller degree than those actually $H^+$-driven. Comparing our results with those obtained for *Enterococcus hirae* demonstrates that, as predicted by our theory, the *I. tartaricus* c-ring is 100 times less $Na^+$ selective. The weaker affinity for $Na^+$ of the *I.tartaricus* ring is also coherent with the 10-fold difference in $K_m(Na^+)$ values between these enzymes, at high pH. Taken together, these experiments demonstrate that the c-ring is the main determinant of the physiological ion specificity of rotary ATPases, and provide a conclusive validation of our theory.

**1877-Pos Board B607**

**On the Functional Differentiation of F- and V-Type Rotary Atpases Atomic Mechanism of a Hybrid F/V Membrane Rotor**

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Rotary ATPases/ATP synthases have a common architecture and overall mechanism, whereby the activity of the soluble catalytic domain is coupled to the rotation of a membrane-embedded sub-complex, or c-ring, which in turn is coupled to the translocation of $H^+$ or $Na^+$ across the membrane. Physiologically, however, V-ATPases function only as ion pumps, while F-type enzymes function as ATP synthases. It has been proposed that this differentiation stems from the greater spacing between consecutive ion-binding sites along the circumference of V-type c-rings, relative to the F class, resulting from the different topology of the constituent c-subunits. Here, we use molecular simulation methods and bioinformatic tools to assess the atomic mechanism of the $Na^+$-coupled ATP synthase from Acetobacterium woodii, whose unusual c-ring features one V-type c-subunit inserted along nine of the F-type, and therefore lacks one ion-binding site. Our results demonstrate that rotation of the A. woodii c-ring in either direction is not fundamentally hampered by the inserted V-type c-subunit, and explain in atomic detail why this enzyme can be driven by a transmembrane $Na^+$-gradient and thus function as an ATP synthase. In sum, we conclude that the physiological differentiation between V- and F-type enzymes is likely to arise primarily from the availability of suitable substrates and ionic gradients in the specific environment of these enzymes, rather than from a precise structural feature.

## Gene Regulatory Systems: Prokaryotic and Eukaryotic

**1878-Pos Board B608**

**Multicolor Timelapse Luminescence Microscopy: Optimizing Luciferases to Track Fast Gene Dynamics in Single Yeast Cells**

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Timelapse fluorescence microscopy is an important tool for measuring in vivo gene dynamics during cell differentiation, cell cycle, and circadian clocks. However, fluorescent proteins are limited by high cellular auto-fluorescence, photo-toxicity, and slow chromophore maturation times. This problem becomes acute when the goal is to measure multiple, weakly transcribed genes and faithfully track fast dynamics. A promising alternative is luciferase, which is an enzyme that produces photons from a chemical substrate and does not require a maturation step. The drawback is that photon flux is much lower than fluorescent proteins. To date, timelapse luminescence microscopy has