mutations on enzyme kinetics and torque production in F_1 from the yeast *S. cerevisiae*.
Mitochondrial Genome Integrity (*mgi*) mutations allow yeast to survive the loss of mitochondrial DNA. A number of these mutations occur in the genes encoding the F_1 portion of the ATP Synthase, and have been shown to uncouple ATP Synthase (*Wang et al. 2007*). The mutations cluster around the collar region of F_1 where the alpha, beta and gamma subunits interact and are thus likely to affect F_1 rotation.
Using a high speed camera and a novel method for laser darkfield microscopy, we captured the rotation of wild-type and *mgi* forms of yeast F_1-ATPase. We show for the first time that at saturating ATP, wild-type yeast F_1 rotates approximately four times faster than the thermophilic F_1. Kinetic and substepping behaviour in wildtype yeast appears to be similar to that observed in bacterial F_1, but some of the *mgi* forms show behaviour that is different to both wildtype and previously reported forms of F_1. We will use the results from these single molecule experiments in conjunction with structural studies to elucidate the mechanisms underlying rotation in wildtype and *mgi* forms of F_1.
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2241-Plat
Mechanisms of Selective Sodium/proton Binding and Coupled Rotation in F1fo ATP Synthases: Insights from Quantitative Computer Simulations
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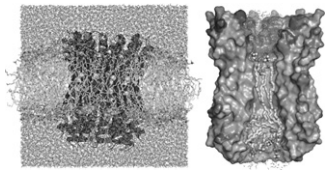
F-ATP synthases are the most prominent ATP source across the living world. These enzymes couple the structural changes required for catalyzing the conversion of ADP and Pi into ATP to the transmembrane flow of protons or sodium ions down their electrochemical gradients. The key, coupling element in these molecular machines is the membrane-embedded Fo rotor, or c-ring. The recent emergence of high-resolution structural data and the close interplay of experimental methods with advanced, quantitative molecular simulations are providing novel and important insights into the mechanisms of these essential proteins. We present an overall summary of our recent progress in this area, particularly pertaining to the mechanism by which ion exchange across the lipid membrane is coupled to the rotation of the c-ring, as well as to the structural basis for the distinct ion-binding selectivity observed for different species. We believe these principles may well apply more generally in the context of ion-coupled membrane transport.
Meier [...] Faraldo-Gómez (2009). J. Mol. Biol. 391:498-507.
Pogoryelov [...] Faraldo-Gómez, Meier. (2009). Nat. Struct. Mol. Biol. (in press).
Krah [...] Meier, Faraldo-Gómez (2009). J. Mol. Biol. (under review).

2242-Plat
Mechanism of Selective Cation Binding to Sodium-Coupled Transporters: Insights from Free Energy Simulations and QM/MM Simulations
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Ion-coupled transport of neurotransmitter molecules by secondary amino-acids transporters plays pivotal role in the regulation of neuronal signaling. One of the major events in the transport cycle is ion-substrate coupling and formation of the high-affinity occluded state with bound ions and substrate. Molecular mechanisms of ion-substrate coupling, specificity for a particular cation and the corresponding ion-substrate stoichiometry in secondary transporters has yet to be understood. We have studied Li+/K+/Tl+/Na+ binding and/or selectivity to several transporters with available crystal structures such as the bacterial aspartate transporter GltPh , leucing transporter LeuT and maltose transporter vSGLT using free energy simulations and QM/MM minimization to evaluate the role of different factors in the observed selectivity and ion binding to the protein. Two different mechanisms were found to co-exist for crystallographically characterized binding sites Na1 and Na2 in LeuT and Glt. Furthermore, site Na1 appeared to be well conserved amongst members of different families. To evaluate the role of Na+ binding in the transporter function, we have performed free energy simulations to determine actual cation selectivity as well as binding affinity for sites Na1 and Na2 in the protein. QM/MM minimization was used to characterize the role of the electronic effects of the stabilization of non-native cations such as Li+ and Tl+ in the Na+-selective sites of different transporters. In the case of Tl+ binding to Glt transporter, neighboring residues from a second solvation shell provide a necessary stabilization to the larger cation due to polarization and charge transfer effects implying a rather large flexibility of the metal binding sites.

2243-Plat
Computational Approaches to Understanding the Mechanism of Transport in the Na+/galactose Co-Transporter vSGLT
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A number of recent high resolution structures suggest that a larger family of cation coupled substrate transporters share a common core architecture. At the molecular level, it is not known how this architecture enables them to harness the energy stored in ionic gradients to move small molecules across the membrane. We have studied the details of substrate and ion entry and exit to the cytoplasm of the galactose symporter vSGLT. We used equilibrium molecular dynamics (MD) simulations to determine the role of key residues in stabilizing galactose and sodium in their respective binding sites. The simulations show that the transporter is stable when simulated as a monomer having only small deviations from the x-ray structure. We also used steered MD simulations to pull galactose and sodium from their site into the cytoplasm to obtain the free energy for unbinding.

2244-Plat
A Mutation Associated with DCM Increases Phospholamban Oligomerization and Decreases SERCA-Binding, but Does Not Change Phopholamban Tertiary Strucuture or Phosphorylation by PKA
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To better understand the pathological mechanism of a human dilated cardiomyopathy phospholamban (PLB) mutation (R9C), we investigated the effects of this mutation on PLB structure and regulatory interactions. Notably, we observed efficient phosphorylation of R9C-PLB by PKA *in vitro*, and nuclear magnetic resonance (NMR) spectroscopy showed no change in R9C-PLB structure compared to WT. To test R9C-PLB binding interactions in live cells, PLB was expressed as cyan and yellow fluorescent protein (CFP/YFP) fusions in AAV-293 cells, and PLB oligomerization and SERCA-binding were quantified by fluorescence resonance energy transfer (FRET). 100 micromolar H_2O_2 applied to the cells induced a rapid quench of CFP-R9C-PLB fluorescence and a concomitant increase in YFP-R9C-PLB fluorescence, indicating an increase in intraoligomeric FRET after oxidation. FRET enhancement after peroxide addition was not observed for CFP/YFP-WT-PLB. To test whether the FRET increase was due to increased oligomerization or a quaternary conformation change, we measured intraoligomeric FRET in a population of cells expressing a wide range of R9C-PLB protein concentrations. FRET dependence on concentration yielded oligomer intrinsic FRET efficiency ($FRET_{max}$) and relative dissociation constant (K_D). Compared to WT, R9C-PLB had a decreased K_D and increased $FRET_{max}$, indicating an increased oligomerization affinity and more compact oligomer structure, respectively. The enhanced oligomerization of R9C-PLB was matched by a decrease in SERCA-binding compared to WT. Overall the data suggest a new mechanism by which the R9C mutation may exert a pathological effect: decreased SERCA regulation and increased oligomerization, as consequences of increased sensitivity of R9C-PLB to oxidation.

2245-Plat
The Na,K-ATPase Beta1 and Beta2 Subunits Associate with Different Quality Control Pathways in the ER
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The catalytic Na,K-ATPase α-subunit is not able to exit the ER or catalyze ion transport unless assembled with the β-subunit. However, requirements for the ER exit of the Na,K-ATPase β-subunit that plays an additional, ion-transport-independent, role in intercellular adhesion are not clear. The Na,K-ATPase β_1- or β_2-subunits and their N-glycosylation-deficient mutants were expressed in renal MDCK cells. Confocal microscopy, immunohistochemistry, and immunoprecipitation were employed to evaluate the role of N-glycans of the β-subunit isoforms in the quality control of the Na,K-ATPase in the ER. Mutagenic removal of as few as two of the eight N-glycosylation sites from the β_2-subunit precludes its assembly with the α_1 subunit and results in full retention of the unassembled β_2-subunit in the ER. However, removal of all three N-glycosylation sites from the β_1-subunit only slightly affects its