2911-MiniSymp
Unique Structure Aspects of the Cytochrome b_6f Complex
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The 3.0 Å crystal structure of the cytochrome b_6f complex from the transformable cyanobacterium *Nostoc* sp. PCC 7120 [1) is very similar to that previously determined from the thermophilic *M. laminosus* [2, 3) and the green alga, *C. reinhardtii* [4]. Two unique structure features in the complex are: (i) heme c_n, not found in the bc_1 complex [2, 4]; (ii) the existence of two rotamer states of heme b_p, with the heme in the *Nostoc* structure rotated by 180° about the normal to the membrane plane relative to its orientation in *M. laminosus*. The rmsd between the Cα atom positions of the eight subunits of *M. laminosus* and *Nostoc* is 0.83 Å; rmsd for the *p*-side prosthetic groups, cyt *f* heme and the [2Fe-2S] cluster, are 0.66 and 0.51 Å, and hemes b_p and b_n, 5.74 and 0.28 Å [5].
Heme c_n bound covalently to the n-side of the cytochrome *b* polypeptide has an open coordination site [2, 4] that could bind plastoquinone, as shown [3], or oxygen. EPR spectroscopic analysis [6] shows that ferric heme c_n does not bind a variety of common heme ligands [7]. However, the reduced state binds NO, forming a novel heme-Fe(II)-NO species and could bind O_2. PQ analogues bind, $\leq$ 1 molecule per dimer, to the native complex at high concentrations, implying that they can replace a single PQ molecule in the binding cavity near heme c_n.
[1] Baniulis *et al.*, submitted, 2008.
[2] Kurisu *et al.*, Science, 302, 1009-, 2003.
[3] Yamashita *et al.*, J. Mol. Biol., 370, 39-, 2007.
[4] Stroebel *et al.*, Nature, 426, 413, 2003.
[5] Yamashita et al., in preparation, 2008.
[6] Zatsman *et al.*, JACS, 128: 14246, 2006.
[7] Hendrich *et al.*, in preparation, 2008. [NIH-GM-032383 (WAC), NIH-GM-077387 (MPH)].

2912-MiniSymp
Controlled Motion of the Iron-Sulfur-protein Head Domain in the bc1 Complex - Insights from Famoxadone binding to *Rh. Sph.* bc1
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The membrane protein ubiquionol cytochrome *c* oxido-reductase (cytochrome bc_1, bc_1) combines the oxidation of ubiquinol to ubiquinone with proton pumping in an ingenious way and with high efficiency, building a membrane potential for ATP synthesis. Arguably, this may be the reason why bc_1 is found in the vast majority of life forms ranging from simple bacteria, via yeast to mammals and plants. The reaction bc_1 performs is crucial, featuring both ubiquinol oxidation and ubiquinone reduction. Not surprisingly, the bc_1 complex has been the target of effective pesticides widely used in crop protection and as antibiotics against parasites. Crystallographic studies of inhibitors bound to bc_1 provide both clues for pesticide design as well as mechanistic insights into its function. Here, we report on the crystal structure of *Rh. sph.* bc_1 with famoxadone bound at the Qo site at 3.1 resolution. We compare and contrast it with the same complex from bovine mitochondrial bc_1 and with related complexes formed with stigmatellin as well as other related inhibitors. It appears that famoxadone, while assuming a slightly different conformation in *Rh. sph.* bc_1, arrests the head domain of ISP in the same way as in mitochondrial bc_1. No direct hydrogen bond forms between the inhibitor and Fe2S2 ligands as expected. However, the head domain of ISP lifts off its cyt *b* binding crater compared to the stigmatellin bound form. Furthermore, additional crystal structures of several mutants of *Rh. sph.* bc_1 provide further insight into local changes that affect the cd1/cd2 helix and the ef loop, which is implicated in the control of the motion of ISP head domain.

2913-MiniSymp
Probing membrane proteins: Proton translocation by respiratory Complex I subunits and mrp antiporters
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Respiratory Complex I (NADH:ubiquinone oxidoreductase) plays a key role in bioenergetics, coupling electron transfer to proton translocation across the mitochondrial inner membrane. Malfunction of Complex I contributes to normal aging as well as neurodegenerative diseases. Despite its key role in biology and medicine, the mechanism of energy coupling in Complex I is far from understood. This mechanism uses electron transport through the hydrophilic domain to drive proton translocation through the relatively distant membrane-bound domain. While the hydrophilic domain is structurally determined and the electron pathway is well characterized, very little is known about the membrane-bound domain, and how it performs proton translocation.
Four of the seven membrane-bound subunits are homologous to mrp antiporters, proteins that transport Na^+ and H^+ simultaneously in opposite directions across membranes. This homology makes these subunits prime candidates for harboring proton channels, and thus participating actively in Complex I function. We probe these subunits individually, monitoring their proton-pumping activity and antiporter-inhibitor sensitivity. For this purpose we have developed a new, quantitative method for monitoring proton translocation across membranes[1,2]. The method utilizes a pH-sensitive, membrane-impermeable nanoprobe[3] enclosed inside closed membrane vesicles and monitored by a semi-automatic titration and spectrometer[4], enabling robust, high-precision data from membrane-bound proton pumps (see Thom Leidings poster).
1. Gustavsson, T., Eek, M., Leiding, T., Peterson Årsköld, S., and Hägerhäll, C. (2008) Submitted to Biochimica et Biophysica Acta Bioenergetics.
2. Leiding, T., Górecki, K., Vinogradov, S., Hägerhäll, C., and Peterson Årsköld, S. (2008) Submitted to Analytical Biochemistry.
3. Finikova, O., Galkin, A., Rozhkov, V., Cordero, M., Hagerhall, C., and Vinogradov, S. (2003) J. Am. Chem. Soc. 125, 4882-4893.
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2914-Plat
Nucleosome Sliding by ACF is Processive and Bidirectional
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The packaging of DNA into chromatin presents significant challenges to essential nucleic acid transactions such as transcription, replication, and repair. This challenge is overcome by a variety of chromatin remodeling enzymes, which couple the energy of ATP hydrolysis to the assembly and mobilization of nucleosomes, thereby modulating the accessibility of DNA. It is generally accepted that most chromatin remodeling complexes employ a nucleosome 'sliding' mechanism, wherein a histone octamer is translated along DNA without trans-displacement. However, it has been difficult to directly characterize structural dynamics and kinetic intermediates during the remodeling process. Here we report a single-molecule Förster resonance energy transfer (FRET) based assay to monitor in real time the remodeling of individual nucleosomes by the human ATP-utilizing chromatin assembly and remodeling factor (ACF). We demonstrate that ACF can processively slide histone octamers along the DNA in an ATP dependent manner, exhibiting multiple kinetic pauses during translocation. A predominant pause was observed after ~7 base pairs of DNA translocation, independent of the DNA sequence. Surprisingly, ACF exhibits bidirectional translocation activity and rapid switching of directionality: a single ACF functional unit can dynamically translate the histone octamer back and forth many times along the DNA prior to dissociation, suggesting that the functional unit of ACF is a dimer. These previously unknown remodeling intermediates and dynamics have significant implications on the mechanistic understanding of chromatin remodeling enzymes.

2915-Plat
A Biophysical Model Of Interactions Between Transcription Factors And Chromatin
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Binding of transcription factors (TFs) to DNA is critical for triggering a cascade of events that lead to gene expression. The role of chromatin in this process is not considered by traditional biochemical models of protein-DNA interaction, or is limited to the passive DNA sequestration by the nucleosomes. Taking into account dynamic structure of chromatin is important for understanding transcription regulation in eukaryotes.
Here we present a biophysical model of interactions between TFs and chromatinized DNA. The model takes into account dynamics of nucleosomes as well as other important features of eukaryotic regulatory regions such as the clustering TF binding sites, nucleosome-positioning DNA signals etc. Our model