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P8 Membrane Channels, Pumps and Transporters

P8.1

A potassium "transporter" regulated by the ATP/ADP ratio

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The KtrAB potassium "transporter" plays an important role in adaptation to osmotic shock in bacteria. This membrane protein complex is composed by two polypeptides: KtrB is the membrane protein involved in potassium and sodium permeation and KtrA is the cytosolic protein involved in regulation of transporter activity. KtrA binds ATP and/or ADP. We have determined the structure of the KtrAB complex in the ATP bound state and have been performing biochemical and structural studies to unravel the mechanism of regulation of this complex by the ATP/ADP ratio.

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P8.2

Functional and structural dynamics of NhaA, a prototype for Na^+ and H^+ antiporters, which are responsible for Na^+ and H^+ homeostasis in cells

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The crystal structure of the down regulated NhaA crystallized at acidic pH 4 [1] has provided the first structural and functional insights into the antiport mechanism and pH regulation of an Na^+/H^+ antiporter (reviewed in [2]). NhaA is organized into two functional regions: (i) a cluster of amino acids responsible for pH regulation, and (ii) a catalytic region at the middle of the TM IV/XI assembly, containing unique antiparallel unfolded regions that cross each other, forming a delicate electrostatic balance in the middle of the membrane. This unique structure contributes to the cation-binding site and facilitates the rapid conformational changes expected for NhaA. Although extended chains interrupting helices have since emerged as a common feature for ion-binding in transporters, the NhaA fold, shared by ASBTNM [3] and NapA [4] is unique among the three structural folds that comprise the secondary transporters e.g., MFS, LeuT and NhaA [5]. Computational and electrophysiological methods (reviewed in [2]) have been used to develop intriguing models for the mechanism of NhaA. However, the dynamics of the conformational changes and how energy is transduced in this "nano-machine" are still unknown. Ultimately, interdisciplinary integrative results will shed light on the mechanism of activity and pH regulation of NhaA, a prototype of the CPA2 family of transporters.

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P8.3

Mitochondrial machinery for import and assembly of proteins

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Mitochondria contain more than 1000 different proteins, most of which are synthesized as precursor proteins on cytosolic ribosomes. Mitochondrial outer and inner membranes possess translocases that import the precursor proteins. The translocase of the outer mitochondrial membrane (TOM) initially recognizes and transports the large majority of precursor proteins across the outer membrane. Subsequently, at least four different pathways sort the precursor proteins to their intramitochondrial destinations [1]. Presequence-carrying preproteins are translocated by the presequence translocase of the inner membrane (TIM23) and the associated import motor PAM [2]. Metabolite carriers are transferred through the intermembrane space by the small TIM chaperones and are inserted into the inner membrane by the carrier translocase (TIM22) [3]. The mitochondrial intermembrane space import and assembly machinery (MIA) mediates the import of cysteine-rich proteins in a redox-regulated manner [4]. Beta-barrel proteins use small TIM chaperones and the sorting and assembly machinery (SAM) of the outer membrane. Additionally, some alpha-helical outer membrane proteins bypass the TOM channel and are inserted into the outer membrane by the MIM machinery. The mitochondrial contact site and cristae organizing system (MICOS), located at crista junctions between inner boundary membrane and cristae, plays a dual role. MICOS is required for maintaining the characteristic inner membrane morphology and interacts with TOM and SAM of the outer membrane, thus promoting protein biogenesis.

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P9 General Bioenergetics

P9.1

Toward the biogenesis of manmade oxidoreductases working in cells

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The idea of integrating manmade oxidoreductases and allied proteins into cells and organelles to replace or augment component parts of metabolic and bioenergetic machinery has some way to go to before any benefit to mankind can be expected. Yet potential impact is considerable, for instance in the conversion of solar energy into selected clean chemical fuels or in the remediation of respiratory diseases of genetic or aging origins.

We find that combining first-principles of electron transfer engineering common to natural oxidoreductase proteins with first-principles of α-helical protein design offers a practical path toward reproducing oxidoreductase and related functions in structurally transparent man-made proteins we call maquettes. Transparency has recently increased with crystal structures of maquettes accommodating hemes and other metal tetrapyrroles and dimetal ion clusters at 1.45 Å resolution. Maquettes not only continue to meet demands in their structural versatility and functional diversity, but also prove to be readily assembled, highly stable and biocompatible.

With minor sequence alterations, maquettes can be turned to promote diverse natural functions, including light harvesting and charge-separation emulating the core reactions of photosynthesis, and as well as dioxygen binding and electron transfer including resolved superoxide/peroxide/hydroxyl radical generation typical of respiration and oxidative metabolism (see Sheehan et al. poster "Resolution of ROS"). They also promote second-order inter-protein electron-transfer with natural cytochrome *c* displaying rate constants matching those typical of diffusive steps in natural photosynthesis and respiration. Moreover, we have recently designed and expressed in high yields in *Escherichia coli* our own cytochrome *c* maquette, the first demonstration of cofactor biogenesis (of dithiolated heme C) in our manmade proteins.

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P9.2

From chemiosmotic redox loops to proton motive molecular machines

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The origins of Peter Mitchell's chemiosmotic theory are to be found in the elegant concept of vectorial hydrogen and electron transfer reactions (redox loops) of respiratory and photosynthetic redox complexes, orientated with respect to the mitochondrial, bacterial, or chloroplast membrane in such a way as to produce net translocation of hydrogen ions [1]. The generated proton motive force is utilised by the F_oF_1 ATP synthase in the same membrane to produce ATP. Mitchell suggested that ATP synthesis would be directly coupled to flux of H^+ ions via the active site of ATP synthase [2], but we now know from the work of Boyer, Junge, Walker, Yoshida, and others [3] that the protonic coupling is indirect and mediated by rotatory conformational changes. Analogously, the proton motive mechanisms of the respiratory complexes I and IV also cannot be explained by Mitchell's redox loop principle, but they work as true redox-linked proton pumps [4,5]. However, complex III, bacterial nitrate and nitrite reductases, cytochrome *bd*, and photosystems I and II are indeed redox loops. Moreover, apart from being a true proton pump complex IV also has features of a redox loop, being a hybrid between the two coupling principles. This hybrid function might tell us something about how cell respiration evolved, especially when different heme–copper oxygen reductases [6] are compared. Some currently controversial properties of the proton pump of complex IV will also be discussed.

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