

## EBEC 2008 Short Report Supplement

### S1.50 Active ATP Synthase with fused subunits of the proton channel

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We have investigated engineered variants of the *E. coli* ATP synthase where the *a* and *c* subunits of the proton channel were fused together in a head-to-tail fashion, connected by various spacer peptides. If the wild type subunit *c* was present, ATP synthase with *a-c* fusion proteins replacing the wild type subunit *a* had normal H<sup>+</sup>-pumping activity measured by ACMA fluorescence quenching and supported normal oxidative phosphorylation tested by growth on succinate. The *a-c* fusions copurified with the F<sub>0</sub>-complex. In contrast, ATP synthase variants that contained *a-c* - fusion protein, but neither wild type *a*, nor wild type *c* subunits were non-functional. Moreover, the *a-c* fusion proteins in these mutants were not incorporated in the membrane. These results indicate that preassembled *c*<sub>10</sub> ring is required for the incorporation of subunit *a* into the membrane. Since a fusion between the stator and the rotor subunits would preclude rotation of the ATP synthase turbine, the *c* subunit copy that was fused to subunit *a* must be located outside the rotor on the periphery of the F<sub>0</sub>-complex. Easy accommodation of additional transmembrane helices at the *a-c* interface without loss of function is consistent with a structural model that places most transmembrane helices of subunits *a* and *b* at the periphery of the F<sub>0</sub>-complex, limiting rotor-stator contact area to the subunit *a* helices IV-V.

### S1.51 Thermoalkaliphilic F<sub>1</sub>F<sub>0</sub>-ATP synthase: A highly adapted enzyme to synthesize ATP at low protonmotive force

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Alkaliphilic bacteria thrive in environments devoid of protons and are faced with the apparent thermodynamic problem of energizing bioenergetic processes at low protonmotive force. Growth studies with the thermoalkaliphilic bacterium *Bacillus* sp. strain TA2.A1 demonstrated that this strain cannot grow on strictly non-fermentable carbon sources (eg. succinate) below pH 8.5. However, strain TA2.A1 was able to grow from pH 7.5 to 10.0 on fermentable carbon sources (eg. sucrose), suggesting that one or more components of the oxidative phosphorylation machinery are specialized to function only at high pH. To address this hypothesis, we have studied the F<sub>1</sub>F<sub>0</sub>-ATP synthase in strain TA2.A1 (TA2F<sub>1</sub>F<sub>0</sub>) and identified specific adaptations (ie. latent ATPase activity, conserved *a* subunit residues) that are essential for ATP synthesis at high pH, but not low pH. Furthermore, we propose that the TA2F<sub>1</sub>F<sub>0</sub> works against a large inverted pH gradient through the operation of a large oligomeric *c* ring to convert a low electrochemical potential into a significant phosphorylation potential. To address this hypothesis, we have purified the oligomeric *c* ring from strain TA2.A1 and determined the number of *c* subunits to be 13 compared with 10 subunits found in *Bacillus* sp.

growing at neutral pH. The operation of a *c* ring with 13 subunits renders the thermodynamic problem of ATP synthesis at alkaline pH less severe. These data demonstrate that TA2F<sub>1</sub>F<sub>0</sub> is adapted to function at high pH versus neutral pH, thus providing a potential explanation for the lack of growth at pH values below 8.5 on non-fermentable carbon sources.

### S1.52 Structural determination of the proton pathway in the plant plasma membrane H<sup>+</sup>-ATPase

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P-type plasma membrane H<sup>+</sup>-ATPases transport protons against a high electrochemical gradient. In aqueous environments, protons exhibit a very high mobility due to the existence of hydrogen bonded networks of water molecules such as proton wires. Proton wires are linear hydrogen bonded chains of water molecules, where the protons are able to jump from one water molecule to another, and in proteins protonable amino acid residues can also participate in the proton wires. Thus, compared to other cations, protons in solution exhibit markedly different diffusion characteristics as well as distinct interactions with protein molecules. Biochemical and structural data suggests the proton transport pathway to consist of a proton entry/exit pathway and a central proton acceptor/donor (Asp684). In this model, catalytic domain movements are tightly coupled to proton loading of Asp684 by protons entering from the proton entrance pathway, and tightly coupled to proton unloading of Asp684 and release along the proton pathway. As protons and water molecules cannot be traced by X-ray crystallography, the current structural data only offers indications with regard to the proton transport pathway within the H<sup>+</sup>-ATPases. To unravel the proton transport pathway of the H<sup>+</sup>-ATPases, a mutagenic study has been performed on a range of amino acid residues in the membrane domain of the plant membrane H<sup>+</sup>-ATPase AHA2. Mutant enzymes, which were not able to complement the function of the yeast H<sup>+</sup>-ATPase (Pma1p) in a *pma1* yeast mutant, were purified by affinity purification and characterized biochemically. Interestingly, our initial data indicate the direct participation in the proton transport reaction of several conserved amino acid residues projected along the proton transport pathway. Our results are in accordance with a model in which protons are transported to and from the central proton acceptor/donor Asp684 along specific hydrogen bonded networks with participation of conserved acidic and polar amino acid residues and/or specifically bound water molecules.

### S2/13 Recent advances on the structure and environment of the oxygen evolving centre of PSII

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In oxygenic photosynthesis light energy is used to split water into oxygen and equivalents of hydrogen, a reaction which occurs at the

oxygen evolving centre (OEC) located within the membrane protein complex photosystem two (PSII). The OEC contains a cluster of four Mn ions and Ca<sup>2+</sup> surrounded by a specific protein environment. In addition to the metal ions, Cl<sup>-</sup> is required for the reaction to occur efficiently. Other monovalent anions can substitute for Cl<sup>-</sup> and we have used Br<sup>-</sup> for crystallographic studies. Anomalous dispersive signals in the diffraction data obtained with crystals of the treated PSII (infiltrated and biosynthetic) have allowed us to identify two Br<sup>-</sup> binding sites located about 7 Å from the Mn ion and therefore not direct ligands to metal cluster. Our results are in agreement with recent EXAFS data, but in contrast to some theoretical studies. In addition, using Xe as a hydrophobic oxygen analogue, we have attempted to verify predicted oxygen channels from the OEC by crystallographic analyses.

### S2.14 The properties of electronic junction between photosystem I and solid state substrates

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Light induces a stable electrical potential of 1 V in 250 ns across 9 nm of photosystem I (PS I) with quantum efficiency of 1 and absorbed energy conversion efficiency of 47% (solar energy conversion of 23%). The nano-size dimension and the quantum efficiency of almost 1 make the reaction center a promising unit for applications in molecular nano-electronics. An oriented monolayer of PS I on solid metal surface was fabricated. For this end unique cysteine mutants were induced in cyanobacteria *Synechocystis* sp. PCC 6803 for attachment of PS I through formation of sulfide bonds with the surface. The formation of the monolayer was monitored by AFM and the function by monitoring a single molecule photo-potential of approximately -0.6 V was determined by KPFM. Placed between a metal electrode and a transparent conducting glass PS I micro-photovoltaic cell generated, in preliminary results, photocurrent of 0.7 mA/cm<sup>2</sup> at 0.2 V. Oriented multilayers were fabricated by sequential binding and platinization of PS I. Functional electronic junctions were also fabricated by attachment of PS I to the surface of the semiconductor GaAs and carbon nanotubs. The results clearly indicate that the robust PS I from cyanobacteria can be used as a photoactive component in a solid state template.

### S3.35 A sliding mechanism for oligopeptide binding

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Peptides of an extraordinary wide range of lengths (4 to 35 residues) are transported by the ATP-binding cassette transporter OppABCDF from *Lactococcus lactis*. The oligopeptide-binding protein OppA determines substrate specificity of the transporter by capturing the peptides and delivering them to the membrane-embedded translocator domain. Here we report crystal structures of OppA with bound peptides, both in the closed conformation, with the binding cavity completely buried, and in the open conformation, with the binding site exposed. The structures of the closed conformation

revealed (a) an exceptionally voluminous binding cavity (~5000 Å<sup>3</sup>) explaining how very long peptides can be accommodated; (b) the absence of charged residues to fix the positions of the N- and C-termini of the bound peptide, explaining how peptides of very different lengths can be bound; and (c) the presence of hydrogen bonding between the protein and the peptide backbone only, not with the peptide sidechains, explaining the observed indifference towards the exact sequences. The structures of the open conformation revealed that the peptides could shift register in the partially formed, exposed binding site. We propose a new model for substrate capturing, in which a peptide may bind in different positions to of the open conformation. Only when the protein closes the complete binding pocket is formed, in which is peptides are buried. Differently positioned peptides stabilize the closed conformation to different extents, a process in which side chains are likely to play a role.

### S7.12 Seleno compounds prevent the brain energy impairment induced by methylmercury poisoning in adult mice

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Methylmercury (MeHg), an organic form of mercury, has been shown to cause severe and irreversible neurobehavioral and neuropsychological disorders in both humans and animals. The objective of the present work was to investigate the potential protective effect of chronic subcutaneous administration of sodium selenite - Na<sub>2</sub>SeO<sub>3</sub> - and diphenyl diselenide - (PhSe)<sub>2</sub> - on the respiratory chain function in adult Swiss albino male mice exposed to MeHg poisoning. Adult male mice were treated with MeHg dose of 40 mg · L<sup>-1</sup> diluted in drinking water during 21 days and the seleno compounds were daily subcutaneously injected (5 µmol · kg<sup>-1</sup>). Control animals received drinking water *ad libitum*. The activities of complexes I, II, II-III and IV were assessed in mitochondrial preparations from mice cerebral cortex 24h after the last subcutaneous administration. Enzyme activities were measured spectrophotometrically. It was initially demonstrated that MeHg administration provoked a significant inhibition of the electron transport chain in mitochondrial preparations by diminishing the activities of complexes I, II, II-III and IV. Next, we observed that (PhSe)<sub>2</sub> chronic co-administration significantly prevented the reducing MeHg effect on the activities of complexes I to IV of the respiratory chain in cortical mitochondrial preparations. On the other hand, the inorganic seleno compound showed a differential effect; while it prevented the inhibition of the activity of complexes II-III and IV, it potentiated or not modified the inhibitory effect of MeHg on complexes I and II, respectively [Complex I:  $F_{(5,26)}=40.93$ ;  $P < 0.0001$ ; Complex II:  $F_{(5,25)}=13.16$ ;  $P < 0.0001$ ; Complex II-III:  $F_{(5,21)}=5.55$ ;  $P < 0.01$ ; Complex IV:  $F_{(5,21)}=5.55$ ;  $P < 0.01$ ]. Altogether, these data provide evidence for the first time, that the inhibitory effect of MeHg poisoning of the electron transfer chain in mice cerebral cortex might be prevented by using seleno compounds. This strongly suggests that impairment of brain energy metabolism might be involved in the MeHg deleterious mechanisms leading to

neurotoxicity and that, particularly, (PhSe)<sub>2</sub> could be considered as a neuroprotective agent in MeHg-induced brain poisoning.

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### S8.29 Modular Control and Regulation Analysis (MoCA) of muscle and heart contraction

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Top-down or Modular approaches to Metabolic Control Analysis (MCA) were used by us and others to overcome the complexity of intra-cellular regulations. By combining MCA with non-invasive <sup>31</sup>P NMR, we recently developed a new approach (MoCA) to study *in situ* control and regulation of cardiac energetics during contraction of a beating perfused heart. While control analysis describes how enzyme concentrations/activities affect steady-state fluxes and metabolite concentrations, regulation analysis has the potential to provide a quantitative description of how regulation by an external effector or internal - pathological - modifications leads the heart to change activity. The first applications allowed us to measure and compare the relative importance of the different routes within intact beating heart by which the effects of Ca<sup>2+</sup> and adrenaline are transmitted, and to demonstrate *in situ* the presence of a parallel activation of both mitochondria and myofibrils, which ensure the almost perfect homeostasis of PCr/ATP in heart. MoCA was supported by Proportional Activation Approach (PAA) in simple analysis and visualization of our results. Our current efforts aim at developing MoCA approach on perfused heart and *in vivo* skeletal muscle, and at the application to the effects of pathologies and drugs modifying cardiac energetics.

### S11.41 Protection of mitochondria and cells against respiratory chain-induced oxidative stress by isochromans

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The aim of the study was to examine the ability of isochromans (ISO), a group of natural occurring antioxidants, to protect isolated mitochondria and cells (PC12) against oxidative stress. For this purpose, we determined the effect of ISO derivatives on (1) the ROS release from the inner mitochondrial membrane (IMM) and (2) the protection of the ROS-sensitive, matrix-enzyme aconitase (AC) using antimycin A-treated mitochondria isolated from rat brain (RBM) and liver (RLM). Impairment of the respiratory chain (RC) by inhibitors of the electron transport is well known to initiate the release of large ROS level from RC. In addition, we examined the suppression of the cytosolic ROS level by ISO in comparison to other anti-oxidants using PC12 cells under condition of depleted cellular glutathione content. Our data show that ISO suppress the ROS-release from RBM into the incubation medium (measured as

resorufin fluorescence), similar to resveratrol. Surprisingly, melatonin, trolox and N-acetylcysteine were without ROS scavenging activity under this condition, whereas ISO or resveratrol do only slightly suppress ROS release from the matrix-side of IMM (measured as MitoSOXred fluorescence) and AC was weakly protected against released ROS. ISO are, on the other hand, able to suppress cytosolic ROS levels, when exposed to the glutathione-depleting agent CDNB. We conclude that ISO permeate the plasma membrane being thereby enabled to protect cytosolic constituents against oxidative stress.

### S12.51 Identification and characterization of novel inhibitors of the mPTP and their efficacy in cardiac infarction

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Mitochondrial dysfunction, resulting from opening of the mitochondrial Permeability Transition Pore (mPTP), has been implicated in the etiology and progression of several diseases. The aim of this work was to identify and characterize novel inhibitors of the mPTP and to assess their efficacy in animal models of cardiac infarction. Compound A was able to protect mitochondria from PTP opening induced by high [calcium], oxidizing agents and chemical cross-linkers and increase the mitochondrial CRC two fold over that of CsA. The compound was able to prevent mPTP opening induced by Arachidonic acid and calcium ionophores in intact HeLa cells. Langendorff-perfused mice hearts were subjected to 45 mins ischemia followed by 15 mins reperfusion. LDH release in untreated animals was 39% of total whilst preperfusion with compound A (1 μM) resulted in only 5% LDH release (1 μM CsA: 19% LDH). Further, in an *in vivo* model of cardiac infarction, oral administration of compound A (15 mg/kg) 1 hr prior to left ventricular ligation, reduced infarct size by 31 % with respect to controls and was similar to that observed with CsA (10 mg/kg i.v.). We have identified novel *bona fide* inhibitors of the mPTP that are efficacious in an animal model of cardiac infarction.