### 2P.16 3D-reconstruction and overall topology of the dimeric mitochondrial ATP synthase of the colorless alga *Polytomella* sp

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Mitochondrial $F_1F_O$-ATP synthase of chlorophycean algae like *Chlamydomonas reinhardtii* and *Polytomella* sp. is a stable dimeric complex of 1,600,000 Da. It contains the conserved subunits of the rotor and catalytic sectors (α, β, γ, δ, ε, OSCP, a and c) but lacks the classic subunits that constitute the peripheral stator-stalk, regulatory elements, and the polypeptides involved in the dimerization of the complex (b, F6, A6L, $IF_1$, h, and g). Instead, it contains nine polypeptides of unknown evolutionary origin named ASA1 to ASA9. Therefore, the algal enzyme seems to have modified the structural features of its peripheral scaffold, while conserving almost intact the structure of its rotor and catalytic subunits. The isolated enzyme exhibits a very low ATPase activity (0.03 U/mg), that increases upon heat treatment or by incubation in the presence of low concentrations (0.01% w/v) of several non-ionic detergents. The detergent-activated enzyme is fully sensitive to oligomycin. In this work, we readdressed the overall topology of the enzyme with different experimental approaches: dissociation of the enzyme into subcomplexes, detection of close vicinities between subunits based on cross-linking experiments, and inference of subunit stoichiometry based on cysteine residue labelling. Monomer–monomer interactions seem to be mediated by the membrane-bound subunits ASA6 and ASA9, while ASA4, ASA2 and ASA7 appear to be closely-associated forming an important structural element of the peripheral stalk. In addition, three-dimensional structural features of the algal dimeric $F_1F_O$-ATP synthase were obtained using different experimental approaches: small angle X ray scattering with the enzyme in aqueous solution, and electron microscopy image reconstruction from single particle images.

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### 2P.17 How to survive and thrive without mitochondrial DNA: A protozoan's guide to ATP synthase modification

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*Trypanosoma brucei* is a single cellular eukaryotic parasite which normally has a complex host–vector life cycle. This organism undergoes extensive physiological modification in order to survive and proliferate within the very different host/vector organisms. Amongst other adaptations, ATP generation shifts from mitochondrial oxidative and substrate level phosphorylation in the insect vector to glycolysis in the mammalian bloodstream form. In spite of this, mitochondrial function and mitochondrial DNA gene expression are still essential in the bloodstream form. The procyclic forms utilize ATP synthase (complex V) and the proton gradient to generate ATP from ADP. Bloodstream forms, however, possess an incomplete respiratory chain and use ATP synthase in reverse in order to maintain the essential membrane potential of the mitochondrion; metabolizing ATP and pumping protons out of the organelle in the process. In trypanosomes, only one subunit of the $F_O$ moiety of ATP synthase, A6, is encoded in the mitochondrial DNA. Despite the fact that mitochondrial DNA replication and gene expression in *T. brucei* are normally essential processes, so called dyskinetoplastic forms, which lack mitochondrial (=kinetoplast) DNA have been induced in the lab and also occur in nature. Thus, the dyskinetoplastic forms must have developed mechanisms to compensate for the essential mitochondrial gene product(s), reminiscent of petite-negative yeasts [1]. Indeed, we have previously identified a candidate mutation in the nuclearly encoded $F_1$ subunit γ of a laboratory induced dyskinetoplastic strain [2] and similar sequence polymorphisms have been identified in γ subunits of naturally occurring dyskinetoplastic forms [3]. We are currently testing the hypothesis that the identified mutations in the nuclearly encoded γ subunit of ATP synthase can allow the loss of the mitochondrial genome in *T. brucei*. Evidence will be presented indicating that a single point mutation in this subunit is indeed necessary and sufficient to permit survival of bloodstream *T. brucei* in the absence of mitochondrial DNA. Interestingly, this mutation appears to render the ATP synthase complex insensitive to oligomycin, suggesting that it results in uncoupling of ATP hydrolysis from proton transport across the inner mitochondrial membrane.

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### 2P.18 Crystallographic insight into the catalytic mechanism of subunit A of the A-ATP synthase and the P-loop switch in evolution

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In archaea type ATP synthases (A-ATP synthases), subunits A and B alternate in an $A_3$:$B_3$ hexamer, forming the $A_1$ headpiece. The hexamer is attached by a central and two peripheral stalks to a membrane-embedded ion-translocating part known as $A_O$, and a collar-like structure [1]. The central stalk is made of subunits C, D and F. ATP is proposed to be synthesized or hydrolyzed in the interface of A and B subunits and the energy provided for or released during that process is transmitted to the membrane-bound $A_O$ domain, consisting of subunits *a* and *c*. The energy coupling between the two active domains occurs via the stalk part [2]. Crystallographic structures of the nucleotide-binding subunits A and B of A-ATP synthases reveal, that they are composed of the N-terminal β barrel, the α-β domain, and the C-terminal α-helical bundle [3–5]. Most recently, transition position of ADP and ATP could be described in crystallographic structures of subunit B, providing information on the ATP traversing pathway to the final binding pocket [6]. However, the mechanism of nucleotide-binding and ATP synthesis in subunit A of A-ATP synthases still remains a puzzle. Here we describe the crystal structure of subunit A from *P. horikoshii* OT3 A-ATP synthase in the absence and presence of AMP-PNP as well as ADP at 2.47 Å and 2.4 Å resolutions, with defined features of the nucleotide-binding sites and