mechanism. Whereas these enzymes produce three ATP molecules per rotation in the F_1 triple stroke motor, the F_o motor transfers ions via turbines of variable size, the c rings. Structurally determined c ring sizes revealed coupling ratios (ions per ATP) between the F_o and F_1 motors geared from 3.3 to 5 depending on different species. We have measured the molecular mass of bacterial c rings by 'Laser induced liquid bead ion desorption' (LILBID). The novel method allows the mass determination of non-covalently assembled membrane protein complexes even in the MDa-range with high accuracy and therefore also allows the exact determination of the c ring stoichiometries and hence the enzyme's coupling ratios. It requires only microgram amounts of protein in detergent solution.

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S1. 45 Single pair FRET with fusion proteins of the F_0F_1-ATP synthases from *Escherichia coli*

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A rotor and a stator subunit of EF_0F_1 have previously been selectively labelled with appropriate organic fluorophores. Time resolved single pair fluorescence resonance energy transfer (spFRET) has revealed a stepwise rotational subunit movement during ATP synthesis (Diez et al., Nat. Struct. Mol. Biol. 2004). To simplify the labelling and reconstitution procedure necessary for double labelling of EF_0F_1 an enhanced green fluorescent protein (EGFP) was genetically fused to the γ-subunit. In order not to disturb the conformational changes during the catalytic steps, a leucine zipper helix was used as linker between the γ-subunit and EGFP. This helix elongated the C-terminus of the γ-subunit and its rotation was transduced to EGFP. The b-subunit contains the mutation b64C, which allows covalent labelling of the fusion protein with an organic acceptor fluorophore. This construct offers the opportunity to analyze the dynamics of the enzyme during ATP synthesis and ATP hydrolysis by spFRET with freely diffusing proteoliposomes. In addition fluorescence anisotropy measurements can be carried out with immobilised proteoliposomes.

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S1.46 Supramolecular organization of mitochondrial ATP synthases: Electron microscopy study

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Cryo-electron microscopy is applied to obtain a medium resolution structure of the dimeric ATP synthase in mitochondria. Although the enzyme functions as a monomer, dimeric ATP synthase supercomplexes were found in yeast, bovine heart, *Arabidopsis* and *Chlamydomonas*. Recently a very stable ATP synthase supercomplex was described in the alga *Polytomella*. The supercomplex includes a number of additional subunits that are speculated to be involved in dimer formation. Structural analysis by single particle analysis of negatively stained molecules revealed that monomers specifically interact via the F_0 parts and an angle between the two F_0 parts is about 70° in *Polytomella*. This arrangement is considered to induce a strong local bending of the membrane. In order to increase a resolution and to obtain a native state of the protein cryo-electron microscopy (EM) method was used. Preliminary EM data on a set of about 70,000 projections allow us to expect at least 20 Å resolution in a 3D model of the dimeric ATP synthase.

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S1. 47 Epsilon subunit, an ATP sensor of ATP synthase

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Our recent studies showed that the ε subunit of F_1-ATPase from the thermophilic *Bacillus* PS3 (TF_1) can bind ATP in a very specific manner. From these results, we have proposed a regulatory mechanism of ATP synthase involving ATP binding to the ε subunit. One of the critical issues is how the ATP binding to the ε subunit may concern with its regulatory role. To address this question, eleven mutants of the ε subunit were prepared, in which one of the basic or acidic residues was substituted with alanine to alter their ATP binding. ATP binding to these mutants was measured by gel-filtration chromatography. Among them, four mutants that showed no ATP binding were selected and subjected to further study. The mutant ε subunits can be reconstituted with the $\alpha_3\beta_3\gamma$ complex of TF_1. The ATPase activity of the resulting $\alpha_3\beta_3\gamma\varepsilon$ complexes was measured and the extent of inhibition by the mutant ε subunits was compared in each case. With one exception, weaker binding of ATP correlated with greater inhibition of ATPase activity. These results clearly indicate that ATP binding to the ε subunit plays a regulatory role and that ATP binding may stabilize the ATPase active form of TF_1 by fixing the ε subunit into the folded conformation.

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S1.48 Yeast cells depleted in subunit *h* fail to assemble subunit *6* within the ATP synthase and exhibit altered mitochondrial cristae morphology

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Within the yeast mitochondrial ATP synthase, subunit *h* is a nuclear-encoded protein belonging to the so-called "peripheral stalk". To examine the role of subunit *h* in ATP synthase function and assembly, we used a regulatable, doxycycline-repressible, subunit *h* gene, to overcome the strong instability of the mtDNA observed in deletion mutants. Yeast cells expressing less than 3% of subunit *h*, but still containing intact mitochondrial genomes, grew poorly on respiratory substrates because of a major impairment of ATP synthase-borne ATP synthesis, whereas the respiratory chain was not affected. The lack of ATP synthesis in the subunit *h*-depleted (d*h*) mitochondria was attributed to defects in the assembly/stability of the ATP synthase. A main feature of d*h* mitochondria was a very low content (<6%) in the mitochondrially encoded subunit *6*, a

component of the enzyme proton channel, that was in large part due to a slowering in translation. Interestingly, depletion of subunit *h* resulted in dramatic changes in mitochondrial cristae morphology, which further supports the existence of a link between the ATP synthase and the folding/biogenesis of the inner mitochondrial membrane.

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S1.49 Kinetics of the F-ATPase of *E. coli* before and after blocking the C-terminal end of γ

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The F-ATPase of *E. coli* consists of the F_1 ($\alpha\beta\gamma\delta\varepsilon$) and the F_o (abc) part. The subunits γ, c and ε form the rotor of the protein. Former studies showed that a swivel joint in γ was created after fixing the rotor to the stator. That allows a rotation of γ despite a closed crosslink. In these studies we asked for the location of the swivel joint along the length of γ. For this purpose four new F_1-mutants, that carried a Cysteine at the C-terminal end of γ and a Cysteine at α, were created. Under oxidizing conditions a crosslink between stator- and rotor-subunits could be closed. Using a microvideographic test for single-molecule-rotation the different mutants were tested in respect of their hydrolysing behaviour under reducing and oxidizing conditions. Three mutants showed normal rotational behaviour. The rotational velocity dropped by appr. 25%. One mutant (γG282C) showed rotation despite an intact crosslink (verified by SDS-PAGE with appr. 90% closed crosslink) but the rotational velocity dropped by appr. 56%. The untwisting of the α-helical structure behind γI279 allows rotation around the dihedral angels of the peptide-backbone. This rotation could meet sterical hindrance causing greater activation energy. The untwisting of the α-helical portion of γ is one option for a rotary joint in F_1.

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(S2) Photosystems symposium lecture abstracts

S2/1 Membrane protein structure determination using crystallography and lipidic mesophases
Recent advances and successes

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A primary impasse on the route that leads to membrane protein structure through to activity and function is found at the crystal production stage. Diffraction quality crystals, with which structure is determined, are particularly difficult to prepare currently when a membrane source is used. The reason for this is our limited ability to manipulate proteins with hydrophobic/amphipathic surfaces that are usually enveloped with membrane lipid. More often than not, the protein gets trapped as an intractable aggregate in its watery course from membrane to crystal. As a result, access to the structure and thus function of tens of thousands of membrane proteins is limited. In contrast, a veritable cornucopia of soluble proteins has offered up their structure with insight into function, reflecting the relative ease with which they are crystallized. There exists therefore an enormous need for new ways of producing crystals of membrane proteins. One such promising approach makes use of lipidic liquid crystalline phases (mesophases). In my presentation, I will describe the method, our progress in robotizing and miniaturizing it for high-throughput applications, and our understanding of how it works at a molecular level. Major advances in applying the method for membrane protein structure determination will be presented.

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S2/2 Structure and function of photosynthetic membrane proteins

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Oxygenic photosynthesis is the main biological process on earth that converts the light energy from the sun into chemical energy. The primary step in this energy conversion, the light induced charge separation, is catalyzed by two distinct, membrane intrinsic protein complexes, photosystems I and II. Cyanobacterial Photosystem I consists of 12 protein subunits, to which 127 cofactors are non-covalently bound. It catalyzes the light-driven electron transfer from plastocyanin to ferredoxin. In the talk, the structure of the PSI-Ferredoxin will be presented. The second part of the talk focuses on the chloroplast ATP Synthase. Crystals of an oligomeric form of subunit c were grown from the spinach chloroplast enzyme diffracting as far as 2.5 Å. Though we are currently collecting experimental phases using nobel gases, the high symmetry of the oligomer allows for the determination of valuable structural insights from the native data. The native Patterson reveals the presence of 14-fold symmetry in the oligomer, confirming previous AFM studies. A huge surprise is the finding, that these crystals possess a strong yellow color. The pigment analysis shows that the c-ring chloroplast ATP-synthase contains chlorophylls and carotenoids.

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S2/3 Structural plasticity of the *Rhodobacter* photosystem

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