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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Experimental systems based on bacteria of the genus *Rhodobacter* (*R*), particularly *R. sphaeroides* and *R. capsulatus*, have produced many insights into the mechanism of light energy transduction in photosynthesis, including the structural basis of light harvesting and photochemical charge separation. Structural and spectroscopic studies have been greatly assisted by the availability of bacterial strains with altered photosystems, such as mutants lacking one or both types of light harvesting complex for example, or with an altered complement of carotenoids (so-called green or blue-green mutants), together with the ability of the bacterium to assemble the photosynthetic apparatus when growing in the dark under conditions of low aeration. The present work involves a systematic study of the structural and functional consequences of (1) expression of structural genes *in trans* in deletion mutants, (2) variation in growth conditions, (3) deletion of one or more light harvesting complexes, (4) changes in the carotenoid composition of the photosystem and (5) removal of the PufX protein. Particular attention is given to the effects of such changes on the composition of the so-called "core complex" formed between the reaction centre and the LH1 antenna protein, and the ability of the bacterium to grow under photosynthetic conditions.

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### S2/4 The 2-methoxy group of ubiquinone is essential for function of the acceptor quinones in reaction centers from *R. sphaeroides*

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The orientation of a methoxy substituent is known to substantially influence the electron affinity and vibrational spectroscopy of benzoquinones, and has been suggested to be important in determining the function of ubiquinone as a redox cofactor in bioenergetics. Ubiquinone functions as both the primary ($Q_A$) and secondary ($Q_B$) quinone in the reaction centers of many purple photosynthetic bacteria, and is almost unique in its ability to establish the necessary redox free energy gap for 1-electon transfer between them. The role of the methoxy substitution in this requirement was examined using monomethoxy analogues of ubiquinone-4, which were reconstituted into quinone-depleted reaction centers from the purple photosynthetic bacterium, *Rhodobacter sphaeroides*. The analogues used were 2-methoxy-3,5-dimethyl-6-isoprenyl-1,4-benzoquinone (2-MeO-Q) and 3-methoxy-2,5-dimethyl-6-isoprenyl-1,4-benzoquinone (3-MeO-Q) and only 2-MeO-Q was able to simultaneously act as $Q_A$ and $Q_B$. The necessary redox potential tuning was shown to occur in the $Q_B$ site. In the absence of active $Q_B$, the IR spectrum of the monomethoxy quinones was examined *in vitro* and in the $Q_A$ site, and a novel distinction between the two methoxy groups was tentatively identified, consistent with the unique role of the 2-methoxy group in distinguishing $Q_A$ and $Q_B$ functionality.

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### S2/5 The photosystem I reaction centre of oxygenic photosynthesis

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Photosystem I of oxygenic photosynthesis is a large multi-protein complex binding 100 chlorophylls. At its core are two related polypeptides which each bind symetrically-related electron transfer chains. We present evidence from studies utilising spectroscopic approaches in combination with site-directed mutagenesis that demonstrate that light-initiated electron transfer occurs on both branches of electron transfer.

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### S2/6 The unusual configuration of the quinone reduction site of the cytochrome $b_6f$ complex

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Complex cytochrome $b_6f$ couples electron transfer with proton translocation through the thylakoid membrane in oxygenic photosynthesis. Unlike its counterpart in mitochondria and proteobacteria, it exhibits three additional cofactors whose functions are not understood: a chlorophyll a, whose phytyl chain lies at the entrance of site $Q_o$, a β-carotene and an additional haem, called $c_i$. X-ray crystal structures in the presence of inhibitors suggest that the putative substrate binding pocket of the $Q_i$ site is near the iron atom of haem $c_i$, providing an unusual site for a quinone involved in an oxidoreduction reaction, where interaction near the edge of the protoporphyrin ring is sufficient for electron transfer. 2-*n*-nonyl-4-hydroxyquinoline N-oxide (NQNO) binds into the $Q_i$ pocket and is able to coordinate the iron of haem $c_i$. Functional implications need to take into account the configuration of the $Q_i$ site: haem $c_i$ has no axial ligand contributed by the protein, one side is occupied by a water molecule or hydroxide in interaction with haem $b_H$, while the other face of haem $c_i$ is protected by a phenylalanine, which can be displaced to allow ligand coordination. This raises questions on a mechanism that is thought to be very similar to the Q-cycle of the $bc_1$ complex.

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## (S2) Photosystems symposium abstracts (poster and raised abstracts)

### S2.7 The bacteriochlorophyll $B_A$ is missing in H(L153)Y *Rhodobacter sphaeroides* RC

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The reaction center of *R. sphaeroides* is a membrane-bound pigment–protein complex where the photosynthetic charge separation