dimers [9], complicating the preparation of such a heterodimer. The acidic domain lacking complex, on the contrary, is a dimer (results confirmed from Mass Spectrometry experiments and structural observation). Further experiments are currently ongoing. On the kinetic side, the deletion mutant is functional and shows properties comparable to the wild type. Here we show by Stopped-flow kinetics that the catalytic efficiency of the heterodimer is not affected by the mutation, the affinity for quinol is not changed and under pre steady-state conditions only one cytochrome $c_1$ and two cytochrome $b_H$ are reduced per dimer. Moreover, it is not possible to see the antimycin stimulation observed in the WT [7] since the second quinol oxidation site is not functional.

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### 14P.4 The Cys-377 in NqrF subunit of $Na^+$-translocating NADH: Quinone oxidoreductase from *Vibrio harveyi* confers its sensitivity to low concentrations of $Ag^+$ ions

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The $Na^+$-translocating NADH:ubiquinone oxidoreductase ($Na^+$-NQR) is a redox-driven sodium pump that generates a transmembrane electrochemical $Na^+$ potential. This enzyme is shown to operate in respiratory chain of various bacteria, including several pathogenic microorganisms. The enzyme consists of six subunits (NqrA-F) encoded by the six genes of the *nqr* operon. The NqrF subunit is a polypeptide that combines NADH:ferredoxin oxidoreductase and ferredoxin. The five other subunits of $Na^+$-NQR (NqrA-E) do not have any noticeable homology to other proteins with known functions. At present, only a few inhibitors of $Na^+$-NQR are known. The antibiotic korormicin specifically inhibits $Na^+$-NQR at the level of its interaction with ubiquinone. The effect of HQNO on $Na^+$-NQR is similar, but the affinity of this inhibitor to the enzyme is significantly weaker. $Na^+$-NQR from *Vibrio sp.* is also sensitive to low concentrations of silver ions, some other heavy metals ($Cd^{2+}$, $Pb^{2+}$, $Zn^{2+}$, and $Cu^{2+}$), and to SH-reagents. These inhibitors influence the initial reactions of the catalytic cycle of $Na^+$-NQR and seem to prevent its interaction with NADH. As there was a correlation between inhibition of different $Na^+$-NQRs by $Ag^+$ and NEM, it was proposed that $Na^+$-NQR inactivation by heavy metals ions is caused by modification of some of its cysteine residue in the NqrF subunit. By means of site-directed mutagenesis conserved cysteine (Cys-377) residue in NqrF subunit of $Na^+$-translocating NADH:quinone oxidoreductase ($Na^+$-NQR) was changed to alanin. This mutation has been demonstrated to cause loss of sensitivity of the enzyme activity to SH-modifying inhibitors, such as silver ions and other heavy metals. Nevertheless enzyme possessed NADH:$K_3$-oxidoreductase and sodium-dependent and HQNO-sensitive NADH: quinone oxidoreductase activities and their ratio was the same as for wild-type enzyme. But this change of the Cys-377 residue results in approximately 14-fold decrease of $Na^+$-NQR turnovers as a consequence of the rate of the electron entrance reduction. Also it was estimated that mutant protein is much more stable than wild-type control protein in course of its aerobic incubation with NADH and that it has the same thermal stability as wild-type protein. The subunit composition is also the same as for wild-type protein.

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### 14P.5 Functional role of a conserved aspartic acid residue in the motor of the $Na^+$-driven flagellum from *Vibrio cholerae*

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The flagellar motor consists of a rotor and a stator and couples the flux of cations ($H^+$ or $Na^+$) to the generation of the torque necessary to drive rotation of the flagellar filament. The inner membrane proteins PomA and PomB are stator components of the $Na^+$-driven flagellar motor from *Vibrio cholerae*. Affinity-tagged variants of PomA and PomB were co-expressed in trans in the non-motile *V. cholerae* pomAB deletion strain to study the role of the conserved D23 in the transmembrane helix of PomB. At pH 8.0 and under saturating $Na^+$ concentrations, the D23E variant restored motility to 84% of that observed with wild type PomB, whereas the D23N variant resulted in a non-motile phenotype, indicating that a carboxylic group at position 23 in PomB is of functional importance for flagellar rotation. Motility tests at decreasing pH under otherwise identical conditions revealed a pronounced decline of flagellar function with a motor complex containing the PomB-D23E variant. This indicates that the protonation state of the glutamate residue at position 23 determines the performance of the flagellar motor, most likely by altering the affinity of $Na^+$ to PomB [1]. The conserved aspartate residue in the transmembrane helix of PomB and its $H^+$-dependent homologs is proposed to act as a ligand for the coupling cation in the flagellar motor.

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### 14P.6 Molecular hydrogen production during various nitrogen source photo-fermentations by *Rhodobacter sphaeroides*

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Molecular hydrogen ($H_2$) production by photosynthetic bacteria is considered as one of the more perspective ways to generate clean and sustainable energy from various substrates. Purple bacteria such as *Rhodobacter* species have the ability for $H_2$ production with high rates (Kapdan, Kargi, 2006; Gabrielyan, Trchounian, 2009). The selection of the nitrogen source for bacterial growth is of great importance because the nitrogen source strongly effects the biohydrogen production (Khatipov *et al.*, 1998; Ooshima *et al.*, 1998). In this work, growth properties and $H_2$ production by purple non-sulfur bacterium *Rhodobacter sphaeroides* strain A-10 (isolated from Arzni mineral

springs in Armenia), which was grown anaerobically at pH 7.0 under light, from succinate using different amino acids (glutamate, alanine, tyrosine, and glycine) as nitrogen sources have been studied. The maximal cell growth rate and $H_2$ production by *R. sphaeroides* were obtained when glutamate was used as nitrogen source. The $H_2$ production rate, in the presence of alanine and tyrosine, in comparison with glutamate, decreased about 3-fold. In the presence of glycine the bacterial growth, but not $H_2$ production was observed. This process was suppressed at the presence of the *N,N′*-dicyclohexylcarbodiimide (DCCD), the $F_OF_1$-ATPase inhibitor. The addition of DCCD (0.5 mM) in succinate–glutamate, succinate–tyrosine or succinate–alanine medium caused a decrease of $H_2$ production rates (about 50%).

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### 14P.7 EPR-HYSCORE study of quinone binding in respiratory nitrate reductase: Molecular basis for the adaptation to anaerobiosis–aerobiosis transition

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Nitrate reductase A (NarGHI) is a respiratory complex that is preferentially expressed under anaerobic conditions and in the presence of nitrate in *Escherichia coli* cells. This membrane-bound enzyme is then associated with formate dehydrogenase (FdnGHI) to constitute a redox loop enabling the energetic coupling between transmembrane electron and proton transfers. By shuttling electrons between these two complexes, quinones are key elements of this bioenergetic chain. NarGHI is physiologically able to oxidize either menaquinols or ubiquinols associated to anaerobic or aerobic growing conditions, respectively. However, due to the absence of quinone in the crystal structure of NarGHI, the number and location of the quinol binding sites were largely debated. By combining EPR spectroscopy and site-directed mutagenesis, we have recently shown that a semiquinone radical species can be stabilized in close vicinity of the distal heme $b_D$ located in the NarI membrane subunit. This radical was identified as a menasemiquinone (MSQ) intermediate. Surprisingly, it exhibits the highest stabilization constant reported so far in respiratory enzymes [1,2]. To understand the molecular basis of this unusual stabilization, a multifrequency HYSCORE study was directly undertaken on NarGHI-enriched inner membrane vesicles (IMVs) [3]. Analysis of the $^{14}N$ and $^{15}N$ hyperfine couplings reveals that MSQ is specifically H-bound to a nitrogen atom which was assigned to the $N_d$ imidazole nitrogen of the heme $b_D$ axial ligand His66. Moreover, the EPR study of NarGHI-enriched IMVs purified from a menaquinone-deficient *E. coli* strain shows that endogenous ubisemiquinones (USQ) can also be detected. The use of $^{14}N$ HYSCORE enabled to distinguish the USQ radicals bound to various membrane-bound enzymes, and to clearly identify the USQ species bound to NarGHI. Noticeably, MSQ and USQ bind in a single site of the NarGHI complex in a similar mode involving one of the His heme $b_D$ ligand [4]. This work provides the first spectroscopic evidence to address at the molecular level the question of the adaptation of an anaerobic enzyme to oxygenic conditions.

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### 14P.8 Novel electrochemical methods to characterise ubiquinol oxidase activity in native-like model-membrane systems

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Bacteria have highly diverse and highly branched respiratory chains, which consist of a range of enzymes that transfer electrons from many different substrates into a common pool of lipid soluble electron carriers, known collectively as quinones. In the aerobic respiratory pathway of *Escherichia coli* there are two types of ubiquinol oxidases that catalyse the oxidation of ubiquinol to ubiquinone and reduce molecular oxygen to water. The ubiquinol oxidase cytochrome $bo_3$ ($cbo_3$) is structurally related to the mammalian terminal oxidase, cytochrome *c* oxidase, and its catalytic cycle is coupled to the pumping of protons across the membrane. We have developed two native-like membrane systems to investigate different bioenergetic aspects of $cbo_3$ using electrochemical methods. In the first model system, planar orientated membranes are formed onto gold electrodes functionalised with cholesterol derivatives. C$bo_3$ activity in these planar membranes is monitored using cyclic voltammetry with electron transfer to $cbo_3$ mediated by the ubiquinol/ubiquinone (UQ) pool. Using impedance spectroscopy, the diffusion rate of UQ is found to be orders of magnitude slower than accepted values for lateral diffusion. It is therefore hypothesised that these rates represent perpendicular diffusion of UQ 'head-group' across the membrane, corresponding to a 'flip' time between 0.05 and 1 s. The apparent $K_M$ of $cbo_3$ for oxygen was measured at $1.1 \pm 0.4$ μM, in good agreement with literature values for whole cell experiments and for purified $cbo_3$. Increasing the concentration of lipophilic UQ above 5–10 pmol/cm$^2$ in the membrane, either by incorporating UQ in the membranes before assembly of the planar membrane or adding it *in situ* during the voltammetric experiments, leads to a decrease in $cbo_3$ activity. Analysis of the data indicates that $cbo_3$ is inhibited by ubiquinol at high concentrations (substrate inhibition), but not by ubiquinone (product). In the second model-membrane system, vesicles are adsorbed intact on the electrode surface. By incorporating a pH-sensitive fluorescent dye inside the vesicles, the generation of a proton gradient (DpH) by $cbo_3$ is monitored. The rate of pH increase inside the vesicle is measured after $cbo_3$ is electrochemically activated. After correcting for proton leakage into the vesicles, the proton pumping activity (in DpH/s) of $cbo_3$ is shown to be linearly related to DpH.

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