

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<sup>+</sup>-translocating NADH:Quinone oxidoreductase from *Vibrio harveyi* confers its sensitivity to low concentrations of Ag<sup>+</sup> ions

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The Na<sup>+</sup>-translocating NADH:ubiquinone oxidoreductase (Na<sup>+</sup>-NQR) is a redox-driven sodium pump that generates a transmembrane electrochemical Na<sup>+</sup> 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<sup>+</sup>-NQR (NqrA-E) do not have any noticeable homology to other proteins with known functions. At present, only a few inhibitors of Na<sup>+</sup>-NQR are known. The antibiotic korormicin specifically inhibits Na<sup>+</sup>-NQR at the level of its interaction with ubiquinone. The effect of HQNO on Na<sup>+</sup>-NQR is similar, but the affinity of this inhibitor to the enzyme is significantly weaker. Na<sup>+</sup>-NQR from *Vibrio sp.* is also sensitive to low concentrations of silver ions, some other heavy metals (Cd<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, and Cu<sup>2+</sup>), and to SH-reagents. These inhibitors influence the initial reactions of the catalytic cycle of Na<sup>+</sup>-NQR and seem to prevent its interaction with NADH. As there was a correlation between inhibition of different Na<sup>+</sup>-NQRs by Ag<sup>+</sup> and NEM, it was proposed that Na<sup>+</sup>-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<sup>+</sup>-translocating NADH:quinone oxidoreductase (Na<sup>+</sup>-NQR) was changed to alanine. 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<sub>3</sub>-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<sup>+</sup>-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<sup>+</sup>-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<sup>+</sup> or Na<sup>+</sup>) 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<sup>+</sup>-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<sup>+</sup> 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<sup>+</sup> to PomB [1]. The conserved aspartate residue in the transmembrane helix of PomB and its H<sup>+</sup>-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<sub>2</sub>) 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<sub>2</sub> 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 affects the biohydrogen production (Khatipov *et al.*, 1998; Ooshima *et al.*, 1998). In this work, growth properties and H<sub>2</sub> production by purple non-sulfur bacterium *Rhodobacter sphaeroides* strain A-10 (isolated from Arzni mineral