ia leading to the proposal that other enzymes involved in oxidative phosphorylation may also be essential (e.g. electron transport chain components). Tibotec BVBA, Johnson and Johnson Phamaceutical Research and Development described the first mycobacterial F_1F_O-ATP synthase inhibitor (TMC207) and showed that TMC207 has fast *in vivo* activity in animal models and TB patients. In this seminar I will discuss new energetic targets and their importance in the adaptation of mycobacteria to persistence i.e. low energy conditions and hypoxia.

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Posters

14P.1 Electrogenicity of succinate: Menaquinone oxidoreductase from *Bacillus subtilis* depends on the direction of electron transfer

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Respiration of *Bacilli* with succinate is known to require energization of the membrane. In order to test whether $\Delta\mu H^+$ can serve as a driving force for the reduction of membrane-bound menaquinone (MQ) by succinate, we have, firstly, investigated a *B. subtilis* strain with cytochrome *bd* as the only terminal oxidase which generates $\Delta\mu H^+$ with low efficiency (1 H^+/e^-). If succinate: menaquinone oxidoreductase (SQR) needs to spend 1 H^+/e^- to reduce MQ, the overall succinate oxidase activity will be electroneutral and conserving no energy. In the minimal growth medium with succinate as a sole energy source, the mutant cells were unable to divide and the subcellular membrane vesicles did not oxidize succinate. These observations are consistent with the energy-consuming mode of the succinate:menaquinone oxidoreductase operation. Second, tightly coupled inside-out membrane vesicles were isolated from *B. subtilis* strain overproducing SQR in which respiration with succinate approached the rate observed with the whole cells. The respiration is coupled to $\Delta\mu H^+$ generation and is completely inhibited by the uncouplers. This finding supports energy-dependence of SQR activity which could be explained either by $\Delta\mu H^+$ consumption in the SQR reaction or by $\Delta\mu H^+$-dependent enzyme conversion to the active form. Third, in the same vesicles, the menaquinol:fumarate reductase (QFR) reaction appears to be electroneutral. Anaerobic reduction of fumarate by NADH proceeds linearly for at least 2 h. The reaction is neither inhibited nor stimulated by the uncouplers, and is not coupled to acidification inside the vesicles. As the fumarate reduction is not inhibited by the uncouplers, conversion of SQR to the active state by $\Delta\mu H^+$ is unlikely. Presumably, SQR in *B. subtilis* has two modes of operation: the reaction in succinate → MQ direction is associated with $\Delta\mu H^+$ consumption, whereas the reverse reaction is electroneutral. Such a behavior resembles the situation with the liposome-reconstituted QFR from *Wolinella succinogenes*, despite the opposite direction of the physiological activity of the two enzymes.

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14P.2 The sodium translocation pathway in Na^+-NQR from *Vibrio cholerae*

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The physiological role of the Na^+-pumping NADH:quinone oxidoreductase (Na^+-NQR) is to convert energy from one form to another. The enzyme uses the energy of a redox reaction, oxidation of NADH and reduction of quinone, to drive the transport of sodium from the inside to the outside of the cell, against its concentration gradient. Turnover of Na^+-NQR produces both, a transmembrane Na^+-gradient and an electrical potential ($\Delta\Psi$), negative inside the cell. Here we report, the characterization of the sodium pathway in Na^+-NQR using a combination of site-directed mutagenesis and kinetics. We characterized several conserved acid residues located in the transmembrane helices of Na^+-NQR that appear to be involved in sodium uptake and release. Seventeen such residues were found, all of them in subunits B, D and E. We then constructed mutants in which these residues were individually replaced by aliphatic groups (alanine or leucine), which should interact poorly with Na^+. Many of these mutations resulted in slowing of the redox reaction (70% to 4% residual quinone reductase activity) and a corresponding decrease in Na^+ translocation. Our results suggest the participation of seven of these residues in the translocation process of sodium. Mutations at NqrB-D397, NqrD-D133 and NqrE-E95 produced a decrease of approximately ten times or more in the apparent affinity of the enzyme for sodium (Kmapp), during turnover, which suggests that these residues may form part of a sodium-binding site. Mutation at other residues, including NqrB-E28, NqrB-E144, NqrB-E346 and NqrD-D88 had a large effect on the quinone reductase activity of the enzyme and its sodium sensitivity, but less effect on the apparent sodium affinity, consistent with a possible role in sodium conductance pathways. Taken together these results, we propose a possible binding site and pathway for sodium in Na^+-NQR.

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14P.3 Heterodimeric bc_1 complex from *Paracoccus denitrificans*: A validation of the half-of-the-sites mechanism

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The bc_1 complex from *Paracoccus denitrificans* is the product of the *fbc* operon, coding for ISP, cytochrome *b* and cytochrome c_1 respectively. In the latter subunit, a very particular feature was identified, an acidic domain showing high homology to the hinge protein in the bovine enzyme or the QCR6p in yeast [1–3]. The domain has been deleted and the resulting complex used to test the half-of-the-sites mechanism [4–7] by constructing a heterodimeric bc_1 in which one of the two monomers carries an inactivating mutation at the Q_O site [8] and therefore, only half of the complex is able to oxidize quinol. The use of the deletion mutant was necessary since the wild type complex resulted to be a stable association of two

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