S8.O2

Insights into redox-driven ion transport from the crystal structure of the Na^+-translocating NADH:ubiquinone oxidoreductase from *Vibrio cholerae*

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Vibrio cholerae maintains a Na^+-gradient across the cytoplasmic membrane. The generated sodium motive force is essential for substrate uptake, motility, pathogenicity, or efflux of antibiotics. This gradient is generated by a NADH:ubiquinone oxidoreductase (NQR) that is related to the RNF complex of archaea and bacteria. NQR is an integral membrane protein complex consisting of six different subunits, NqrA–NqrF. In order to get insights into the redox driven Na^+-transport mechanism we have isolated and crystallized the NQR of *V. cholerae*. The crystals of the entire membrane complex diffract to 3.5 Å. Moreover, we determined independently the structures of the major soluble domains of subunits NqrA, NqrC, and nqrF at 1.9 Å, 1.6 Å and 1.7 Å, respectively, completing large parts of the structure of the respiratory complex at high resolution. Altogether, the structural information gives a detailed picture of the NQR and allows also a close view on the core subunits of homologous Rnf complex. The structural information available allows now the analysis of ion translocation pathways and of the coupling between redox and translocation reactions. From the structure we conclude on a Na^+-translocation pathway and how ion translocation might be coupled to electron transfer in the complex.

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S8.P1

Functional characterization of a dodecaheme c-type cytochrome involved in microbial electron transfer to energy production in MFCs

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In recent years, the interest in alternative and renewable energy sources has been raising rapidly due to the increase in the world population and concerns about the environmental consequences of using fossil fuels to support energy production [1]. Microbial Fuel Cells (MFCs) provide an attractive clean method since they allow direct conversion of biochemical energy into electricity, using microorganisms as biocatalysts [2]. *Geobacter sulfurreducens* is widely studied in the context of bioelectrochemical systems. This organism possesses more than one hundred multiheme cytochromes that contribute to transfer electrons from the cell metabolism, across the periplasm and the outer membrane, to outside of the cell [3]. GSU1996 is representative of a new class of dodecaheme cytochromes c [4–6]. It was suggested that it may work as a molecular nanowire by transferring electrons within the periplasm to the outer membrane proteins [7]. GSU1996 is composed of four tri-heme domains connected by flexible peptide linkers [4]. The aim of this study is to characterize the redox properties of each individual domain, and pairs of domains, determining the reduction potentials of individual hemes as well as their redox and redox-Bohr cooperativities. This characterization will reveal how electron transfer occurs in this protein and provides a good model for understanding electron transfer in microbial nanowires that transfer electrons across large distances.

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S8.P2

Application of solid state physics techniques for electron transport measurements in bacterial pili

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Contemporary solid state physics poses methods and techniques for studying a great variety of objects, including different biological structures. But still there is a great question of interpretation and processing of experimental data with further linking with theoretical models. This particular research is focused on adaptation of surface probe microscopy and transport techniques in application to the problem of long-range electron transport in bacterial pili. Principal problems are: organization of contact in-between the surface of substrate, object itself and probe; enlargement of probe sustainability against irreversible destructions, and decrease of sample modification (destruction, doping etc) during the experiment. Another issue is the data analysis and development of theoretical model, basing on theory of electrons in disordered systems. We've designed conductive sharp cantilever tips for atomic-force microscope from carbon nanotubes doped and covered by gold. They demonstrated high resolution as well as high, comparing to standard cantilevers with deposited metals, sustainability against damage during the scanning. Gentle method of current–voltage characteristic measurement without vacuum and low-temperatures was created and tested. We are eager to develop a technique of contact printing by Focused Ion Beam avoiding modification of biological sample. By that very moment as a result of a careful repeat of experiments no conductivity was found in pili of *Shewanella oneidensis* MR-1. The conditions were reproduced from paper [1]. Also speculations declared in papers [2–3] cause doubts due to: improper data ranges (narrow temperature diapason, too wide voltage range, that causes probe damage), incompatibility of methods and properties (strong sample modification during contact printing, either by FIB or by nanolithography) and inadequate use of theoretical model and data interpretation [4].

References

[1] Kar Man Leung, *Shewanella oneidensis* MR-1 bacterial nanowires exhibit p-type, tunable electronic behavior, NanoLetters. 13 (6) (2013) 2407–2411.
[2] Malvankar, Nikhil S., Tunable metallic-like conductivity in microbial nanowire networks, Nature Nanotechnology Letters, 6 (2011) 573–579.
[3] Gemma Reguera, Extracellular electron transfer via microbial nanowires, Nature, 435 (2005) 1098–1101.
[4] Nicholas F. Polizzi, Physical constraints on charge transport through bacterial, Faraday Discussions, 155 (2012) 43–61.

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