Abstracts

– Biological Electron and Proton Transfer –

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Ion translocation by respiratory Complex I: The role of NuoL subunit

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Complex I is the least understood enzyme of the respiratory chain and its deficiencies have been implicated in several neurodegenerative diseases. It couples the oxidation of NADH and reduction of quinone to charge translocation across the membrane, contributing for the establishment of the membrane potential. The membrane arm contains Na^+/H^+ antiporter-like subunits (NuoL, M and N), which are homologous to Na^+/H^+ antiporters (Mrp), suggesting that these subunits may participate in charge translocation. Understanding the coupling of the enzymatic reaction to charge translocation is still a major question in complex I research. Crystallographic structural data showed the presence of a ∼110 Å long amphipathic helix part of the C-terminal of NuoL subunit, which may function as a coupling element.
In this work we investigate the role of NuoL. We used an *E. coli* mutated strain in NuoL, obtained from the 'Keio collection' and monitored the ion transport. Proton translocation was studied by quenching of ACMA fluorescence and ^{23}Na-NMR spectroscopy was used to investigate sodium transport. We observed that the presence of NuoL subunit is not essential for entire proton translocation by complex I, but is determinant for sodium transport.

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Interaction mechanisms between plant GALDH and cytochrome c

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The flavoprotein L-galactono-γ-lactone dehydrogenase (GALDH) catalyzes the terminal step of vitamin C biosynthesis in plant mitochondria. Here we investigated the interaction between *Arabidopsis thaliana* GALDH and its natural electron acceptor cytochrome *c* (Cc). Using laser spectroscopy we observed that $GALDH_{SQ}$ oxidation by Cc follows a kinetic mechanism involving protein association, to form a transient bimolecular complex prior to electron transfer. The kinetic analysis of both $GALDH_{SQ}$ and $GALDH_{HQ}$ oxidation suggests that GALDH reduction by its carbohydrate substrate limits the overall rate of Cc reduction. ITC analysis showed that GALDH weakly interacts with both oxidized and reduced Cc. Chemical shift perturbations for ^{1}H and ^{15}N nuclei of Cc mapped the interacting surface of Cc to a single surface surrounding the heme edge. In summary, the results point to a relatively low-affinity GALDH/Cc interaction, similar for all partner redox states, directed by electrostatic complementary forces and involving protein-protein dynamic motions.

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Inter- and intra-monomeric communication in the cytochrome bc1 complex as revealed by MD simulations

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The cytochrome bc_1 complex (bc_1) plays an important role in cell bioenergetics acting as a dimeric redox-driven proton translocase. Mitchell's Q-cycle in general describes mechanism of bc1 activity: ubiquinol is oxidized in the site Q_P of the cytochrome *b* subunit, whereby the two released electrons are transferred to the heme of cyt. c_1 (via the mobile "head" domain of the Rieske protein) and to a ubiquinone molecule in the Q_N site of cyt. *b*.
As it was discussed before [1] the ubiquinol reduction in the Q_N site should be coupled with the mobility of the Rieske "head" domain, while thermodynamics considerations [2] imply the coupling between the two "head" domains. However, the details of both the coupling events in bc_1 remain elusive. We performed equilibrium and metadynamics MD simulations of the bc_1 with different ligands to establish inter- and intra-monomeric coupling in bc_1 dimer while application of various computational techniques allowed us to track possible pathways for allosteric signal propagation.
References
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Molecular details of electron transfer in fumarate reduction by flavocytochrome c3

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Numerous redox enzymes catalyze two-electron processes, even though the connection from the protein surface to the active site is made through chains of redox cofactors that only exchange one electron at a time. The soluble fumarate reductase from the periplasmic space of *Shewanella* is one of these proteins. The three-dimensional structure showed that this protein contains a chain of four hemes, which interacts with the FAD catalytic centre that performs the obligatory two electron-two proton reduction of fumarate to succinate. In order to investigate the role played by the redox chain in the catalytic activity of this enzyme, transient kinetic studies of flavocytochrome reduction in the absence and in the presence of substrate were performed. The kinetic contribution of each heme for electron uptake and conduction to the catalytic centre was determined. This enabled the observation that the catalytically most competent states of the enzyme are those least prevalent in a quasi-stationary condition of turnover. Comparison of the results from fumarate reductases from two different organisms enabled the identification of the role of the redox properties of individual hemes in the modulation of the catalytic activity of the enzymes.