SFB 1078

Protonation Dynamics in Protein Function

Colloquium

Mon, **Jan. 16**, 2023

15:15 – 17:30

Freie Universität Berlin
Physics Department
Hörsaal B
(Arnimallee 14, 14195 Berlin-Dahlem)

Prof. Petra Hellwig – *University of Strasbourg, Strasbourg, FR*

The reaction of membrane proteins from bacterial respiratory chains studied by IR spectroscopies and electrochemical approaches

Although the architectures of several membrane proteins in respiration as well as the basic chemical reactions have been described, the interactions on molecular level, the diversity and efficiency of the reaction mechanisms in bacterial systems, are under discussion. Electrochemical and infrared spectroscopic experiments are developed to study the coupled electron and proton reactions, the reactivity towards small molecules and, importantly, correlate it with the microenvironment of the cofactors. Studies on different membrane proteins will be presented.

1. Cytochrome bd oxidases. These enzymes catalyze the reduction of oxygen in the respiratory chains of bacteria, including several pathogens and they play a crucial role in protection against oxidative stress, in virulence, adaptability and antibiotics resistance. Electrocatalytic studies of the cytochrome *bd* oxidases from different organisms gave evidence for a different reactivity towards oxygen..These differences in electron transfer can be correlated with different glutamic acids with a pK higher than 9 as evidenced by reaction induced FTIR spectroscopy. The reactivity towards signaling molecules like NO will also be discussed and mutants presented that impair NO release.
2. NADH ubiquinone reductase, or respiratory complex I is the largest enzyme from the respiratory chain. The conformational changes induced by substrate binding and electron transfer are studied by means of an infrared label based on (-SCN) .
3. Finally the insitu identication of the pK value of individual aminoacids different transport proteins by means of surface enhanced infrared spectroscopies will be presented.

Prof. Helmut Grubmüller – *Max Planck Institute for Multidisciplinary Sciences, Göttingen, DE*

Microtubules' bends, cryo-cool ribosomes, and wet proteins

In this talk we will survey some of our current work on large biomolecular systems and new methods for atomistic simulations. Microtubules provide both mechanical support and, via the kinetochore, mechanical forces to the cell. To this aim, the filaments can undergo growth/polymerisation and shrinking/depolymerisation phases, driven by GTP hydrolysis. Through non-equilibrium atomistic simulations of entire plus-end microtubule tips we show that the average nucleotide state of the plus-end MT tip determines the heights of energy barriers between tip conformations, such that the post-hydrolysis MT tip is exposed to higher activation energy barriers, which translates into its inability to elongate.

Much about the ultra-structure of microtubules --- as well as of many other biomolecules and biomolecular complexes has been revealed by the recent resolution revolution in cryo electron microscopy. How much of the ambient temperature ensemble of biomolecules is preserved during shock freezing prior to image acquisition is, however, an unsolved question. In shock cooling atomistic simulations of fully solvated ribosomes at realistic time scales we observed, depending on cooling rates, a marked decrease of structural heterogeneity, which we were able to quantify. The observation that a kinetic two-state model improves the prediction of the decrease in heterogeneity compared to the cooling-rate independent thermodynamic model suggests that kinetic effects do contribute markedly. Small barriers between the states (<10 kJ/mol) are overcome during cooling and do not contribute to the heterogeneity of the structural ensemble obtained by cryo-EM, whereas conformational states separated by barriers above 10 kJ/mol are expected to be trapped during plunge-freezing. Our results will allow one to quantify the heterogeneity of biologically relevant room-temperature ensembles from cryo-EM structures.

In these processes, as well as quite generally in protein folding and the thermodynamics of biomolecular stability, the solvent shell plays a pivotal role as, e.g., the effect of cold denaturation clearly demonstrates. We will present a new method to compute solvent enthalpies and entropies with spatial resolution and thus to quantify the underlying thermodynamic enthalpy/entropy tug-of-war. For the example protein crambin, we quantified the local effects on the solvent free-energy difference at each amino acid and identified strong dependencies of the local enthalpy and entropy on the protein curvature. Remarkably, more than half of the solvent entropy contribution arises from induced water correlations**.**

Coffee and tea will be available during the break from 16:15-16:30. www.sfb1078.de

Freie Universität Berlin · TU Berlin · Humboldt-Universität zu Berlin · Charité Universitätsmedizin Berlin · FMP Leibniz-Institut für Molekulare Pharmakologie · Justus-Liebig-Universität Gießen