

# Multi-Frequency EPR Spectroscopy and Site-Directed Spin Labeling Reveal the Structure and Conformational Dynamics of Membrane Bound Protein Complexes

H.-J. Steinhoff

Fachbereich Physik, Universität Osnabrück, 49069 Osnabrück, Germany

X-, Q- and W-band electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling (SDSL) and molecular dynamics (MD) simulations has emerged as a powerful method to study the structure and conformational dynamics of membrane proteins. Analyses of the dynamics and accessibility of the spin label side chains as well as the determination of inter-spin distances and of the polarity in the vicinity of the spin label binding sites provide information for restraint modeling of protein structures and conformational changes. Multi-frequency EPR spectroscopy has been applied to determine details of the structure and conformational dynamics of colicin A, of bacteriorhodopsin, and of the halobacterial phototaxis receptor sensory rhodopsin (pSRII) in complex with the transducer pHtrII. This member of the archeobacterial rhodopsin family can be considered as a general model system for transmembrane signal transduction. Light activation of pSRII induces a signal which is transferred across the plasma membrane by a receptor-specific transducer protein (pHtrII) that binds tightly to the photoreceptor. Inter-spin distances determined from pairs of interacting nitroxide spin labels introduced into the pSRII-pHtrII complex lead to a unique structural model of the dimeric complex. Distinct structural differences between the solubilized and reconstituted membrane proteins are observed. Time resolved detection of inter-spin distance changes after light activation reveals conformational changes of pSRII and uncovers the mechanism of the signal transfer from pSRII to the associated transducer pHtrII. A second part of the talk focuses on the structure and functional dynamics of the Na<sup>+</sup>/proline transporter PutP of *Escherichia coli*. Four-pulse double electron-electron resonance (DEER) techniques have been applied to study proximity relationships within doubly spin labeled variants of PutP reconstituted in proteoliposomes. Inter-spin distance changes observed between loops 2 and 6 upon Na<sup>+</sup> binding suggest ligand induced structural alterations of PutP which might be essential for the transport mechanism.