Project C2: Mapping the electric field in protein-membrane interfaces

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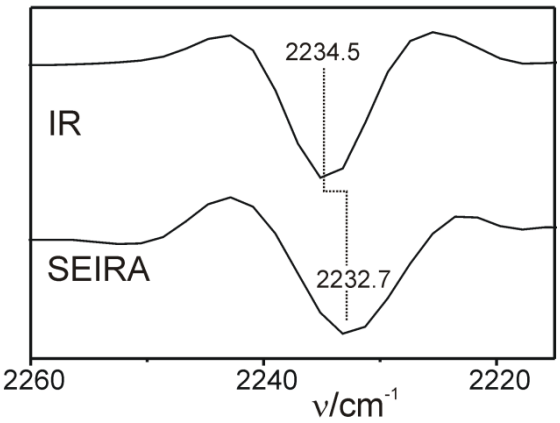
US partner: **Franzen (NCSU)**

Outline. The interfacial potential distribution across membranes is associated with strong local electric fields that may affect structure and reaction dynamics of proteins attached to the lipid bilayer. Whereas numerous examples of the electric-field control of biological processes at interfaces have been reported, the quantification of the electric field is yet a challenge. On the basis of the methodology developed as preliminary work, we will now aim at determining the electric field strength at different positions of protein-membrane adducts as a prerequisite for a comprehensive understanding of interfacial processes of proteins.

Research within the German group. The studies will focus on the heme protein cytochrome c (Cyt-c) immobilised on model systems that are designed to mimic biological membranes, i.e. phospholipid vesicles and electrodes coated with self-assembled monolayers (SAM) of amphiphiles or SAM/lipid hybrid bilayers. To determine the local electric field, we will exploit the vibrational Stark effect (VSE) of reporter groups introduced at specific sites on the protein surface as well as in the model membranes. Such reporter groups are nitrile functions that may be site-specifically introduced at cysteine residues of engineered protein variants by binding of mercaptobenzonitrile (MBN) or incorporated into the model membranes via co-immobilisation of octadecyltetracyanoquinodimethane (TCQM) or chemical modifications of lipids. To probe the VSE in such protein/membrane devices, IR, surface enhanced IR absorption (SEIRA), resonance Raman (RR), and surface enhanced RR (SERR) spectroscopy will be employed (see figure). The analysis of the VSE requires complementary theoretical methods including quantum chemical calculations of the Stark tuning rate as well as molecular dynamics simulations combined with electrostatic calculations (Franzen, NCSU). The results will be correlated with available experimental data on the thermodynamics and dynamics of Cyt-c on membrane models that are either already available. The studies will also include nitrile-labelled dehaloperoxidase which are in the focus of the Franzen group.



The nitrile stretching mode of the K8C Cyt-c variant carrying a CN-label at the cysteine side chain. The IR and SEIRA second-derivative spectra refer to the protein in solution and bound to a SAM-coated electrode.

Longer-term perspective. Later in the continuation period, the studies will be expanded to multicomponent liposomes that represent more realistic models for biological membranes [collaboration with C3 (Dimova)]. Here methodological developments are required to adapt Raman and IR microscopic approaches in order to probe electric-field heterogeneities in domain-containing liposomes.

Complementary work in the US partner group. Franzen will provide theoretical analyses on the VSE of nitrile groups and IR spectroscopic studies on dehaloperoxidase which will further analysed by SERR and SEIRA spectroscopy in our group.

Status of the project. In collaboration with the projects of C3 (Dimova) and C4 (Lipowsky) we will extended the studies to multicomponent lipid membranes. In addition, we will make available vibrational spectroscopic techniques to all experimental projects in the IRTG where these techniques are relevant.