ATR-FTIR-Spectroscopy. The CcO with the his-tag attached to subunit II (SU II) was immobilized in a strict orientation on a two-layer gold film deposited on the ATR crystal of the IR spectrometer using the his-tag technology. A lipid bilayer was subsequently reconstituted by in-situ dialysis around the protein to yield a protein-tethered bilayer lipid membrane.
This system enabled us to observe the sequential electron transfer (eT) within the multi-redox-site membrane protein induced by electronic wiring to the gold surface using time-resolved (tr)-SEIRAS. Conformational transitions concerning a large number of single amino acids and also of secondary structures as a consequence of eT could be seen in a wide range of frequencies from 0.7 Hz to 2 kHz. A high resolution of the spectra was achieved by a combination of Two-Dimensional Infrared (2D IR) Spectroscopy and phase-sensitive detection. Kinetic constants were obtained by applying periodic potential pulses and recording spectral changes as a function of time. Methods were developed to separate these kinetic constants from the contribution due to charging currents.

125-Pos
Thermodynamic Properties and Nmr Data Indicate An Inverse Calcium-Myristoyl Switch of Gcap2
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Guanylyl cyclase-activating protein-2 (GCAP-2) is a neuronal calcium sensor protein (NCS) present in vertebrate photoreceptor cells. Depending on the Ca^{2+} concentration, GCAP-2, and its homologue GCAP-1, inhibit or activate their target protein, the rod outer segment guanylate cyclase (ROS-GC). This plays an important role in shaping the photoreceptor light response. Like all members of the NCS, GCAP-2 is myristoylated at the N-terminus. This fatty acid modification is not essential for the basic function of GCAP-2, but required for full activation of the ROS-GC. Up to now, the biological role of this modification has not been fully understood. In order to gain insight into the Ca^{2+}-dependent conformational changes of GCAP-2, we measured the thermodynamic stability of the protein in dependence of Ca^{2+} binding and myristoylation by monitoring thermally and chemically induced folding / unfolding transitions of myristoylated and non-myristoylated GCAP-2. Stabilities observed for myristoylated and non-myristoylated GCAP-2 in absence of Ca^{2+} were indistinguishable. Addition of Ca^{2+} exerted a strong stabilising effect. This effect was more pronounced for the myristoylated GCAP-2 than for the non-myristoylated, indicating a structural role of the myristoyl moiety in Ca^{2+}-bound but not in the Ca^{2+}-free state. Furthermore, from deuterium solid state experiments we have evidence that the myristoyl moiety is highly flexible in the Ca^{2+}-free state when bound to liposomes. In contrast to the Ca^{2+}-myristoyl switch for the prototype NCS Recoverin, which exposes its myristoyl moiety in the Ca^{2+}-bound state, but buries it when Ca^{2+} is missing, the myristoyl moiety ofGCAP-2 appears to be fully solvent-exposed in the Ca^{2+}-free state. As we could show, myristoylation does not significantly enhance membrane binding of GCAP-2. These results are in agreement with a possible direct interaction of the myristoyl moiety with the target protein, the ROS-GC.

126-Pos
How Electron Transfer Is Linked to Conformational Transitions of Peptide Groups of the Cytochrome C Oxidase, a Study By 2d-Ir Spectro-Electrochemistry
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Synchronous 2D-IR spectrum Strong correlation between amide I of α-helices (1654 cm^{-1}) and β-sheets (1600, 1623, 1638, 1682 cm^{-1}) negative cross-correlation peak at 1620 vs. 1654 cm^{-1}
Fast scan cyclic voltammetry is used to follow the sequential electron transfer (eT) through the CcO immobilized in a biomimetic membrane architecture. CcO is immobilized via his-tag technology with the first electron acceptor, Cu_A directed toward the electrode, in a packing density optimized for fast eT to Cu_A. Kinetic constants of the sequential eT including protonations through the rest of the redox centers, heme a, a_3 and Cu_B are obtained by simulations of cyclic voltammograms measured at a wide range of scan rates using the software package MacSpice. Conformational transitions of peptide groups as a consequence of electrochemically-induced redox processes are investigated by static and time resolved 2D-surface-enhanced infrared absorption spectroscopy (tr-SEIRAS). Correlation of kinetic constants obtained from electrochemistry and tr-SEIRAS allows one to discriminate between conformational transitions regarding amino acids of the K and D proton input and exit channels and those regarding the protein backbone of α-helices and β-sheets.

127-Pos
Fitting To Lifetime Distributions in Photoacosutic Calorimetry
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Photoacoustic calorimetry (PAC) is emerging as a powerful technique for extracting volume and enthalpy changes associated with photo-triggered reactions in both chemistry and biology. Current PAC methods allow for the deconvolution of PAC waves using a deconvolution by reconvolution strategy. In this process, the calorimetric reference wave serves as an impulse function to which either a single exponential or multiple exponential functions are convoluted producing a simulated sample wave. The amplitude and rate constants of the associated exponentials are varied using selected parameter estimation algorithms and the reconvolution process repeated until an appropriate chi-square is achieved. Here we investigate a new PAC deconvolution process which allows for the sample acoustic waves to be fit using a variety of reaction models including stretched exponentials and lifetime distribution functions. Our new algorithm is tested against simulated data sets to provide fit validation.

128-Pos
Comparison of Persistence Length Calculations of Model Collagen in Two and Three Dimensions To Afm Measurements
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Molecular-dynamics simulations are performed on a model collagen molecule in SPC water, with and without 100 mM NaCl. To calculate the persistence length, we find the center of mass of each amino acid. We then group the amino acids into triplets, representing each by the (unweighted) average of the three centers of mass. These center-of-mass positions are used as end points for directors. The time-averaged cosine between directors is found (by determining the scalar product of the directors) as a function of contour length between them. Additionally, two-dimensional projections of the three-dimensional images are constructed, in analogy to the experimental deposition of collagen onto a surface. Techniques for measuring and calculating persistence length from AFM images are used on the two-dimensional projection images, and results are compared to the model prediction and to actual experimental results.

129-Pos
Combining Single Molecule Optical Trapping and Small Angle X-Ray Scattering Measurements to Compute the Persistence Length of a Protein Alpha-Helix
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A relatively unknown protein structure motif forms stable isolated single alpha-helices, termed ER/K alpha-helices, in a wide variety of proteins and has been shown to be essential for the function of some molecular motors. The stability of the ER/K alpha -helix arises from the charge-charge interactions between its glutamic acid (E) and Arginine (R) or Lysine (K) side chains. The flexibility of the ER/K alpha-helix determines whether it behaves as a force-transducer, rigid spacer or flexible linker in proteins. The ER/K alpha-helix spans long distances with relatively few amino acid residues, has known salt and temperature sensitivity, and can be expressed in E. coli, making it an important tool in engineering proteins, provided its mechanical properties are clearly established. We have quantified the flexibility of the ER/K alpha-helix in terms of persistence length, namely the length scale over which it is rigid. We use single-molecule optical trapping and small angle x-ray scattering (SAXS), combined with Montecarlo simulations to demonstrate that the ER/K alpha-helix behaves as a worm-like-chain with persistence length of ~ 15 nm. This persistence length is dependent on the relative content of R and K residues in the ER/K alpha-helix. Knowledge of the persistence length enables us to define its function as a rigid spacer in a translation initiation factor, as a force-transducer in the mechanoenzyme myosin VI, and as a flexible spacer in the Kelch motif containing protein.