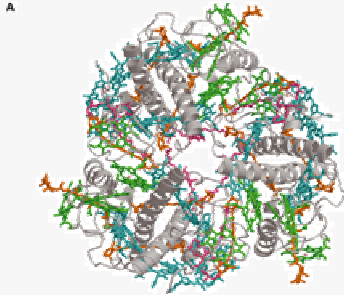
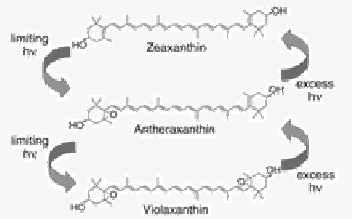


**Non-photochemical quenching in LHC-II**

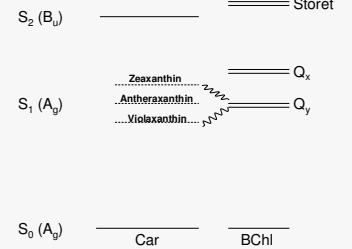
The recently obtained high resolution structures of LCH-II complexes [1,2] provide detailed insight into the molecular organization of the major light harvesting protein in plants and should be complemented with a dynamical picture for a fundamental understanding of photosynthesis on a molecular scale. The down-regulation of photosynthetic light harvesting in excess light is mainly achieved via the NPQ-mechanism. The central process is the formation of zeaxanthin (Zea) from violoxanthin (Vio) by deepoxidation in the so called xanthophyll cycle.



**Xanthophyll cycle**

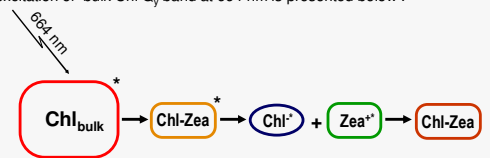


**Energy level diagram of carotenoids and chlorophylls**



**Outlook**

In order to unambiguously prove the proposed mechanism, we want to investigate isolated LHC-II complexes which are enriched in Zea (Zea-LCH-II) and compare the energy transfer dynamics with Vio containing LHC-II by ultrafast spectroscopy. Upon excitation of the lowest excited singlet state of Chl ( $Q_y$ ), we plan to record the kinetics in the carotenoid radical cation band in the NIR region (900 - 1100nm). These experiments represent a critical test of the quenching mechanism proposed in [1], which assumes that excitation energy from bulk LHC-II Chl is transferred (very likely via Chl 8) to Zea, where it is trapped and nonradiative dissipation remains as the only decay channel. The qE quenching scheme, which present generation of Zea, upon selective excitation of bulk Chl  $Q_y$  band at 664 nm is presented below .



P7 (Kühlbrandt): biochemical experiments and the structural determinations  
P29 (Dreuw): quantum chemical calculations of NPQ

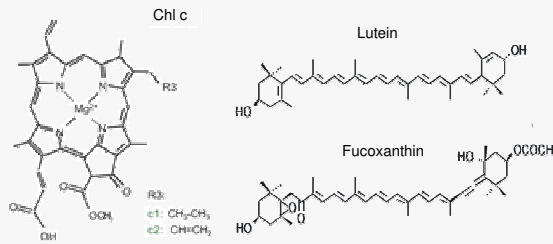
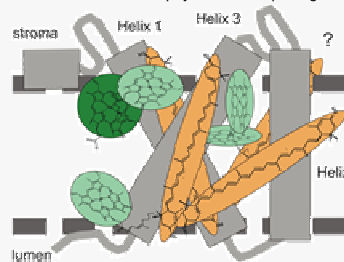
**Photosynthetic excitation energy transfer in FCPs:**

Fucoanthin-chlorophyll protein (FCP), the peripheral light harvesting complex in diatoms exhibits a high sequence homology with the LHC-II complex of green plants, but differs substantially in its pigment composition [3], employing carotenoids rather than chlorophylls for the capturing of sunlight.

This light harvesting antenna binds Chl a, fucoxanthin and Chl c molecules in a 4:4:1 ratio. Upon excitation of the carotenoid to its  $S_2$  state, a significant path of the excitation energy is transferred very fast to Chl a. The energy transfer dynamics that takes place in FCP will be investigated, knowing that the combination of fucoxanthin with a multi-chlorophyll system results into efficient light harvesting.

**Future plans**

The ultrafast excitation energy transfer to Chl a following the photoexcitation of fucoxanthin ( $S_0 \rightarrow S_2$  transition) shall be studied by vis/near-IR and vis/mid-IR pump-probe experiments. We hope to establish a time scale for the various Car-Chl and Chl-Chl energy transfer reactions in a mode sensitive way.



P28 (Büchel): biochemical work and the structural analysis  
P24 (Hellwig): spectroscopic studies in the far-IR  
P29 (Dreuw): QM calculation of a model for singlet and triplet energy transfer

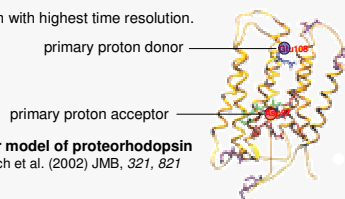
**Primary reactions of proteorhodopsin**

The structural dynamics of retinal proteins, especially details of the initial photoisomerization of the chromophore is an area of intense efforts. At present, several different models are discussed. The ambiguities are partially due to a lack of experimental time resolution. We therefore plan to study the photodynamics of wild type and mutant proteorhodopsin as well as channelopsin with highest time resolution.

As in similar experiments with ultrashort pulses we expect to detect oscillatory contributions, reflecting wave packet dynamics on the excited state potential energy surface. This approach to extract vibrational information from electronic spectra shall be complemented by the recently completed fs mid-IR spectroscopy setup (see P27 report). In 2006 we plan to set up a broadband fs-fluorescence experiment. In collaboration with the Munich group we could already show that this technique also complements fs time resolved absorption experiments and allows detailed observation of the excited state dynamics of retinal proteins.

**Electron transfer in cytochrome  $c_{552}$ :**

For the photoinitiation of electron transfer in cytochrome  $c_{552}$  we plan the design and the spectroscopic characterization of ruthenium complexes  $C_{552}$ /ruthenium. The soluble domain of this protein is ideally suited for these studies, since the structure is known for both redox-states [4]. The ruthenium-labelled cytochrome  $c_{552}$  is also an excellent model system for the study of electron transfer induced folding/unfolding dynamics [5].



Does the replacement of Asp97 by Asn confirm the pH dependent measurements (see P27 report)?

P1 (Bamberg): electrophysiological studies

This project requires electrochemical techniques and will thus be carried out in close cooperation with:

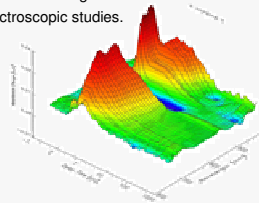
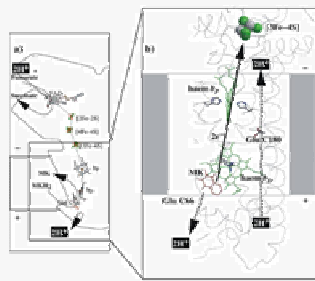
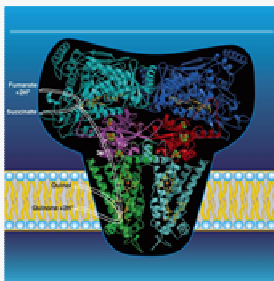
P21 (Mäntele) P24 (Hellwig) P8 (Ludwig).

These experiments have already been proposed for the current funding period, but were delayed due to availability problems of the suitable ruthenium reagent and difficulties with the coupling reaction.

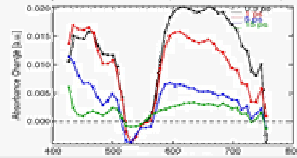
**Photoinduced dynamics in QFR**

The enzyme quinol:fumarate reductase (QFR) from the anaerobic  $\epsilon$ -proteobacterium *Wolinella succinogenes* is part of the anaerobic respiratory system of this organism. It couples the reduction of fumarate to succinate to the oxidation of menaquinol to menaquinone. The three-dimensional structure of the *Wolinella succinogenes* QFR [6] revealed the exact location of the two heme b groups ( $b_p$  and  $b_b$ ) within the hydrophobic subunit C. These two hemes are actively involved in the electron transfer reactions driving succinate oxidation and quinone reduction, their proximity and spectral properties make them ideal candidates for optical spectroscopic studies.

Using ultrafast spectroscopy with selective excitation of the hemes, we would like to investigate the interactions between hemes  $b_p$  and  $b_b$  and the differences in this interaction between the mixed valence and the fully reduced enzyme (DMNH<sub>2</sub> vs. dithionite reduced QFR).

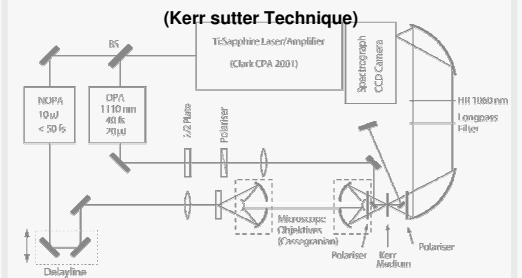


First time resolved data acquired from a reduced QFR sample by exciting the  $\alpha$  band of the hemes at 387 nm and probing the  $\beta$  band transition (530 and 560 nm)



P19 (Lancaster): electron transfer studies of the diheme center in QFR

**Layout of the proposed femtosecond fluorescence**



- Standfuss, J., Terwisscha van Schellinga, A.C., Lamborghini, M. and Kühlbrandt, W. (2005) EMBO J. (in press).
- Dreuw, A., Fleming, G.R. and Head-Gordon, M. (2003) Phys.Chem. Chem. Phys., 5, 3247-3256.
- Büchel, C. (2003) Biochemistry, 42, 13027-13034.
- Harrenga, A., Reincke, B., Rüterjans, H., Ludwig, B. and Michel, H. (2000) J. Mol. Biol., 295, 667-678.
- Wittung-Stafshede, P., Lee, J.C., Winkler, J.R., Gray, H.B. (1999) Proc. Natl. Acad. Sci. U.S.A., 96, 6587-6590.
- Lancaster, C.R.D., Kröger, A., Auer, M. and Michel, H. (1999) Nature, 402, 377-385.