Summary

In this thesis, I present results that have been obtained from measurements with femtosecond time resolution on several photosynthetic and one photoactive protein in both the visible to near infrared and the mid-infrared region of the electromagnetic spectrum.

The pathways of excitons traveling through the Light harvesting complex II of higher plants was investigated by pump probe spectroscopy, with the aim to link spectroscopic data to the actual structure of the protein.

In chapter 2, the findings regarding this protein are presented. We show, by using different excitation wavelengths, that the transfer between chlorophyll $b$ and chlorophyll $a$ molecules in the complex is ultrafast: A time constant of 0.2 ps was found for this process. Furthermore, the presence of a 'bottleneck state' in the system, as predicted by Redfield modeling, could experimentally be shown. The 1685 cm$^{-1}$ band rising in 3-6 ps and decaying in 30 ps we can ascribe to the hydrogen-bound chlorophyll a604 pigment.

Chapter 3 describes the primary steps of charge separation in the Photosystem 1. While the chlorophyll dimer $P_{700}$ has long been identified as the primary electron donor, the components involved in the primary charge separation process in PSI remain undetermined. To shine some light on this issue, we have studied the charge separation dynamics in Photosystem I trimers from Synechococcus elongatus by
femtosecond vispump/mid-infrared-probe spectroscopy. Given the specificity of the infrared region for the redox state and small differences in the molecular structure of pigments, we were able to clearly identify specific marker bands indicating chlorophyll oxidation. We apply to different models, one based on ultrafast charge separation, the other assuming charge transfer character of the red pigments in PSI, to explain our findings. The increase in the amplitude of the cation signals on a subpicosecond time scale indicates the formation of the primary radical pair. Evolution in the cation region with time constants of 7 and 40 ps reveals the formation of the secondary radical pair, involving a secondary electron donor. Modeling of the data enabled us to extract the spectra of the two radical pairs, which yield the IR signatures consistent with $A^+A_0^-$ and $P_{700}^+A_1^-$. This let us conclude that the cofactor chlorophyll A acts as the primary donor in PSI.

Chapter 4 describes experiments performed on PS1, with chemically closed reaction center. We find, that although the RC’s are closed, the first steps of charge separation are the same as the one in PS1 with open RC’s.

Chapter 5 shows results from experiments performed on isolated photosynthetic reaction center-light harvesting I complexes (RC-LHI) of Rhodobacter sphaeroides are reported. We collected difference spectra between 1750 and 1600 cm$^{-1}$ with sub-picosecond time resolution to characterize excited state and radical pair dynamics in these complexes, via the induced absorption changes in the keto carbonyl modes of the bacteriochlorophylls and bacteriopheophytins. Experiments on RC-LHI complexes with and without the polypeptide PufX show that its presence is required to generate the radical pair $P^+Q_A^-$. $P^+Q_A^-$ could be generated in complexes without PufX by addition of the oxidant DMSO, showing that charge separation was blocked after $P^+H^-$ due to the presence of an electron on $Q_A$. Our experiment provides strong support for the hypothesis that the photosynthetic function of PufX is to facilitate the oxidation of $Q_A^-$, in the presence of a partially
reduced quinone pool.

In chapter 6, results from experiments, performed to answer the question if C₄-C₇ single bond rotation plays an important role in the primary photochemistry of Photoactive Yellow Protein, are presented. We therefore synthesized an analogue of this proteins’ 4-hydroxy-cinnamic acid chromophore, in which rotation across the C₄-C₇ single bond has been locked with an ethane bridge, and reconstituted the apo-form of the wild type protein and its R52A derivative with this chromophore analogue. We do find, that in PYP reconstituted with the rotation-locked chromophore: (I) absorption spectra of ground- and intermediate states are blue-shifted, (II) the quantum yield of photochemistry is ~60% reduced, (III) the excited state dynamics of the chromophore are accelerated, and (IV) dynamics of the thermal recovery reaction of the protein are accelerated. Furthermore, the yield of the transient ground-state intermediate in the rotation-locked samples was considerably higher than in the corresponding samples reconstituted with p-coumaric acid. In contrast to theoretical predictions, the initial photocycle dynamics of PYP were observed not to be affected by the charge of the amino acid residue at position 52, which was varied by: (I) varying the pH of the sample between pH = 5 and pH = 10 and (II) site-directed mutagenesis to construct R52A. These results imply that C₄-C₇ single bond rotation in PYP is not an alternative to C₇=C₈ double bond rotation, in case the nearby positive charge of R52 is absent, but rather facilitates with a compensatory movement the physiological cis/trans isomerisation of the blue-light absorbing chromophore.