This thesis is focused on the study of the interaction of light with amino acid residues and cofactors, resulting in detectable biological activity. Photoactive proteins are good models for the study of proteins in general, due to the easily detectable (even by unequipped eye) change of their absorption, during different stages of the light-induced reactions. Thus, photoactive proteins provide handles for the understanding of the elementary reactions that are the triggers of biological activity.

Light-induced reactions in proteins start by the absorption of a photon by the small chromophore (cofactor) molecule embedded in the protein matrix. This molecule is able to transform the photon energy in extremely short time period, and to utilize it as a driving force for chemical transformations. In this thesis we have explored different light-induced reactions by time-resolved laser spectroscopy. To access the different intermediates during the reactions, we performed ultrafast pump-probe spectroscopy in the visible spectral region. In this way, we could identify the decay rates corresponding to different intermediates and to access the dynamics of the reaction, based on the evolving spectral changes associated with electronic transitions. To access structural changes that often trigger the biological activity of the proteins, we apply vibrational time-resolved spectroscopy, in combination with simulations of the studied systems. We also employed three-pulse different absorption spectroscopy, to rule out overlapping spectral contributions of the intermediates, and to refine the reaction models. We applied combinations of these techniques to three different proteins: proteorhodopsin (chapters 2 and 3), cytochrome P450 (chapter 4) and photoactive yellow protein (chapters 5 and 6).

In chapter 2 the protein of interest was proteorhodopsin, a proton pump protein from the rhodopsin family. Light absorption by its retinal chromophore initiates a photocycle, driven by trans-cis isomerization, leading to a proton translocation through the cytoplasmic membrane. We compared the reaction dynamics for pR at alkaline and acidic pH. We found that the pH (at least in the range that we studied) does not influence the reaction mechanism, only the efficiency of the isomerization is lowered for the sample at acidic pH. Ultrafast vibrational spectroscopy reveals a very fast conformational change of the protein backbone, on the time scale of less than 10 ps, induced by the isomerization of the chromophore.

Chapter 3 is a study of the ground state dynamics of photoisomerization in proteorhodopsin, accessed by three-pulse pump-dump-probe spectroscopy. The application of a pump pulse initiates the photocycle, and with an appropriately tuned dump pulse applied at a time delay after the dump, the molecules in the initial stages of the photochemical process can be de-excited and sent back to the ground state. In this way we resolved an
intermediate on the electronic ground state that represents chromophores, unsuccessful in isomerization. Inclusion of this intermediate in the kinetic scheme led to more consistent spectra of the retinal-excited state, and to a more accurate estimation of the quantum yield of isomerization ($\Phi = 0.4$ at pH 6).

In chapter 4 we studied the photodissociation and rebinding of carbon monoxide from the heme cofactor in cytochrome P450. We monitored the reaction through the spectral changes of the deligated heme in the visible spectral region, and of the photodissociated CO in the mid-IR. We found 100% yield of the photodissociation reaction, and rebinding on a nanosecond time scale. This indicates that before recombination, the protein is docked within the protein. We identified by a cavity search algorithm docking sites within the protein matrix that can accommodate carbon monoxide molecules.

Chapters 5 and 6 are dedicated to photoisomerization in photoactive yellow protein. Absorption of a photon of its chromophore, p-coumaric acid, leads to trans-cis isomerization around the C=C double bond and breakage of the hydrogen bond between the chromophore C=O and the backbone N-H of Cys69. When the chromophore fails to isomerize, it decays to the ground state through an intermediate with a distorted cis conformation (ground state intermediate). To determine how the protein controls the outcome of the light-induced reaction, we studied the isomerization in a series of mutants of PYP, having proline 68 replaced with a series of hydrophobic amino acids. We applied ultrafast spectroscopy probing in the visible and in the mid-IR (chapter 4,5), molecular dynamics simulations of the mutants in the ground state (chapter 4), and pump-dump-probe spectroscopy (chapter 5). We found, that due to its rigid structure, proline 68 is involved in the optimization of the reaction yield. Mutating it with other residues disturbs the hydrogen bond network around the chromophore, and is opening free space for water molecules within the chromophore-binding pocket.

The results presented in this thesis have increased our understanding of the functioning of proteorhodopsin, photoactive yellow protein and cytochrome P450. We showed that the application of complementary techniques provides insight into light-induced changes at the level of individual atoms and chemical groups. These changes are transferred into the living cell and initiate biologically relevant response. This understanding of protein function can find application in biotechnology, medicine and biology.