SUMMARY

The content of this thesis is based on single-molecule spectroscopy experiments and aims to bridge the gap from isolated light-harvesting complexes with only a small (10-20) number of interacting chlorophylls to a fully assembled photosystem II with many hundreds of connected pigments. It focuses on the photophysics of individual photosynthetic complexes with respect to the environment and the structural arrangement. The implemented and developed time-resolved measuring techniques span a broad time range from picoseconds to seconds.

Photosynthetic pigment-protein complexes of plants are integral membrane proteins and are therefore usually isolated in detergent micelles to provide a hydrophobic interface and to avoid aggregation. The fluorescence decay of single trimeric LHCII complexes in such a detergent environment is presented and explained in chapter 2. The dominant fluorescence lifetime of about 3.5 ns, associated with the unquenched light-harvesting state of LHCII, is identical to the slowest lifetime component found in ensemble measurements (solubilized sample). The decay further contains an excitation power-dependent fast lifetime component of about 35 ps which is associated with a significant contribution of Singlet-Triplet annihilation. The two-exponential decay is quantitatively explained by a stochastic model for this annihilation process, capturing the statistical switch between a singlet state and one or multiple triplet states.

Photosynthetic complexes can also be isolated by using styrene maleic acid (SMA) as demonstrated in chapter 3. This SMA copolymer solubilizes nanodisk particles that contain a single protein complex in its native lipid environment. The obtained photophysics demonstrates the successful isolation of trimeric complexes in SMA nanodisks and confirms the trimeric structure as a native configuration. The survival time of complexes before they photobleach is increased in SMA compared to detergent which might be explained by a stabilizing effect of the co-purified lipids in these nanodisks. Comparison of the spectroscopic properties of LHCII complexes in detergent and SMA nanodisks yields information on the effect of the native environment on the function of light-harvesting antennae. LHCII in SMA nanodisks also exhibits an unquenched fluorescence lifetime of 3.5 ns which coincides with detergent isolated complexes but is longer than 2 ns typically found in native thylakoid structures. The conformational and energetic flexibility of these complexes in

both detergent and SMA nanodisks can directly be observed by the large dynamic range of partially quenched complexes. This further supports the hypothesis that fluorescence blinking, i.e. the significant changes in fluorescence intensity due to conformational changes, is an intrinsic property of LHCII that may be involved in excess energy dissipation in native light-harvesting.

The dual function of LHC complexes (light-harvesting and photoprotection) has been extensively studied in detergent micelles and ensemble experiments have indicated that the properties of LHC complexes differ in a lipid environment. Combining bulk and single molecule measurements, the fluorescence characteristics of light-harvesting complexes (LHCs) incorporated in liposomes (a spherical vesicle consisting of a lipid bilayer shell around an aqueous core) are investigated in chapter 4 with respect to the number of LHC complexes per liposome. This experiment revealed that the properties of individual LHCs remain similar to that in detergent, in line with the results from LHC in SMA nanodisks. However, clustering of multiple LHCs within one liposome results in fluorescence quenching and the fluorescence lifetime decreases with an increasing density of LHCs. The likely interpretation is that protein interactions of LHC complexes and the extent of crowding modulate the fluorescence lifetime in the lipid membrane. An interesting question that remains is whether and how many LHCs get quenched due to clustering and how much already quenched complexes contribute due to excitation energy transfer. Another intriguing result was the observed monomerization at low protein/lipid ratios that hints at the influence of the membrane curvature on the structural integrity of LHCII trimers or even bigger protein assemblies.

Chapter 5 is a slight detour into the electronic properties of the terminal emitter domain of the monomeric LHCII complex. The reconstituted mutant LHCII-A2, that does not bind Chls *a*611 and *a*612 of the excitonically coupled Chl cluster *a*610-*a*611-*a*612, exhibits two overlapping fluorescence bands which are in turn temperature dependent in relative ratio. The redder peak is assigned to a low energy and relatively low oscillator strength exciton localized on Chl *a*610. The disruption of the Chl trimer results in an increased sensitivity of the excited state energy landscape to the disorder induced by the protein conformations. Exciton delocalization in the wild-type configuration, on the hand, reduces the influence of static disorder and therefore ensures good energy transfer efficiency to neighboring LHCs. We propose this effect as a design principle for maintaining the efficient light-harvesting function of LHCII in the presence of protein disorder.

The established explanation for the molecular mechanism governing NPQ is that conformational changes in antenna complexes open up non-radiative

decay channels via the carotenoids and lead to excitation energy quenching. Fluorescence intermittency observed in all isolated single antenna complexes is likely caused by the same or a very similar mechanism. Another way to at least partially approach the native function of the thylakoid membrane is to measure individual photosystem II particles that contain multiple antenna complexes as well as two reaction centers (see chapter 6) Their average excited state lifetime of about 100 – 150 ps can be explained by multiple quenched LHCs, which is consistent with SMS results on isolated antennae. The observed reversible and light-induced changes of the average lifetime and the corresponding changes in fluorescence intensity on a millisecond time scale directly illustrate how environmental control over a fluctuating antenna can regulate light-harvesting in plant photosynthesis. The described experiment can be utilized to build mathematical models in a bottom-up approach which can then be tested on larger and fully assembled native systems. They also clearly showcase the general property of biological systems to use the same effect for different purposes: LHCs are ingeniously designed nanoparticles that maximize the absorption of sunlight and efficiently transfer the excitation energy further to the RC. The key properties are a long lifetime of electronic excitations on densely packed pigments in a protein environment and their efficient energy transfer without significant losses. At the same time, their conformational flexibility opens up the possibility to induce energy dissipation channels for photoprotection in an environmentally controlled manner.