SUMMARY

In photosynthetic light harvesting, the energy of sunlight is captured by colourful pigments and carried in the form of their electronic excitation. The excitation energy is transported through the photosystem, to the reaction center, where it powers charge separation. The pigments are held in place by their binding to a protein scaffold, which determines the structure (and therefore functionality) of the light-harvesting complexes (LHCs). Both the pigment and protein molecules are moving on many timescales, from high-frequency intrapigment vibrations to slow protein switching. The work in this thesis focuses on the influence of the molecular motion across timescales (the bath) on the excitation (the system). To this end, it develops and utilizes a combination of spectroscopic and theoretical tools.

The first two chapters are introductory, welcoming the reader in the colourful world of light harvesting dynamics. Chapter 1 starts with the structure and composition of the photosynthetic pigment-protein complexes, introducing the partially delocalized excitons and the resulting spatio-energetic landscape. It continues with the theoretical description of the excitonic states, their dynamics and interaction with light, using the perspective of an open quantum system description. Finally, it presents optical spectroscopy as the method of choice for their study, focusing on single-molecule spectroscopy. It describes in detail the newly-developed technique of two-pulse single-molecule spectroscopy of light-harvesting complexes, with a practical description of the setup and measurement, in the hope that it will become useful for those wishing to implement and further develop this technique. The following chapter 2 is based on a published review article; it describes the role of single LHCs within the context of the whole photosynthetic membrane of higher plants. It offers a single-molecule spectroscopy perspective on the function of LHCs, from light collection to energy dissipation. It also introduces the timescales of photosynthetic processes and describes how they can be measured by single-molecule spectroscopy (SMS).

The following chapters are based on original research papers, investigating the influence of fast and slow molecular motion on the photosynthetic light harvesting. We start with the fastest motion: in chapter 3 the role of high-frequency, intramolecular vibrational modes is put forward. One such vibrational mode, strongly-coupled to the electronic degrees of freedom (DOF), is explicitly quan-
tized and included in the system. As a result, it becomes coupled to the rest of the bath modes. The system interacts effectively with two baths, one for the electronic DOF (visible in transition lineshapes, especially of the zero-phonon line), and one for the quantized vibrational mode (accessible through the rate of vibrational relaxation). A vibronic Redfield-type theory is derived for an aggregate of such pigments, and the excitation energy transfer (EET) in a dimer of pigments is followed when changing the energy gap. We find that when the vibrational mode is resonant with the energy gap between the pigments, the vibronic wavepacket leaks through partially delocalized vibronic states through the potential energy surface (PES) intersection, and the EET is faster. As a spectroscopic signature of this regime we identify the presence of long-lived coherent oscillations of mixed electron-vibrational origin.

In chapter 4 we continue with a slow switching of the LHCII protein conformation, observed by blinking of individual LHCII monomers in SMS. We tested the possibility of extending the standard equations of motion of a master equation type to slower timescales. We found that with surprisingly low degree of approximation this is possible for interaction with weak light: one gets equations for the system eigenstate dynamics, driven by the absorbed light. Using these equations it is possible to control the inner dynamics of a Frenkel-exciton model by external parameters. In our case, the external parameter was a coupling of chl a612 to a Lut 1 Car molecule, which facilitated excitation dissipation, causing the observed dark (off) states. The coupling fluctuations were controlled by a random walk of the LHCII protein on its PES, with a possibility of switching between two conformations.

In the next chapter 5 we turn our attention to the interplay of fast and slow molecular motion. To this end, we have developed a new technique, two-pulse SMS of LHCs. Using a confocal microscope gives us the single-complex resolution, while the pulsed excitation with controlled delay provides the time resolution. Changing the pulse delay and observing the modulation of fluorescence of single LH2 antennae of purple bacteria, we are able to extract the ultrafast energy relaxation times in the individual complexes. We found that the effective energy relaxation time is on the order of 100 fs and changes with excitation wavelength. The relaxation time is fluctuating on the course of seconds, that is, in the domain of protein motion, and the ensemble seems ergodic in the sense that one complex experiences the whole distribution of relaxation times, from 50 fs to 250 fs.

In the last chapter 6, several pieces of the LH2 energy relaxation puzzle are put together to pin down the mechanism of how does the slow protein motion actually modify the ultrafast energy transfer. We measured steady state ab-
sorption and fluorescence, broadband and narrowband pump and probe (TA), and extended our two-pulse SMS measurement to a range of excitation wavelengths. We describe all the measurements by the same Frenkel-exciton model. Due to the high degree of LH$_2$ symmetry, we have effectively only four fitting parameters. Thus constrained, we succeed in describing quantitatively all the experiments. The ensemble data are averaged over a static disorder in the pigment energies, while the single-LH$_2$ data are generated by drawing single realizations of the pigment energies from the distribution; the ‘static’ disorder becomes dynamic on the slow timescale of protein motion. By constructing the effective three-state description, used for the two-pulse SMS fitting, from the excitonic model, we describe our method by the same model as the traditional spectroscopies, making an important step towards its broader application as an established technique. From our unified model description we conclude that the LH$_2$ is constructed in such a way that even in the inevitable presence of the environmental fluctuations, the excitation remains delocalized over several pigments, ensuring fast energy transfer. By observing the interplay of the fast and slow molecular motion, we are able to conclude that LH$_2$ is constructed as a robust light-harvesting antenna.

In the work described in this thesis, we have developed theoretical (chapters 3, 4, 6) and experimental (chapters 5, 6) techniques to study the photosynthetic light harvesting dynamics across many timescales. This allowed us to make several observations regarding the function of light-harvesting complexes, mentioned above. Our hope, however, lies also in the potential of the developed techniques to shed light on the fascinating interplay of timescales in many other (non)photosynthetic systems.