Summary

Photosynthesis is performed by organisms as different as bacteria, algae and vascular plants. They inhabit vast spectrum of environments: aquatic, terrestrial, ranging from cold, ice-covered polar regions to hot and dry deserts. Photosynthesis carries on throughout seasonal changes, day-night cycles, and rapid light variations on hourly, minutes and even seconds time scale. Many regulatory mechanisms are therefore present to make photosynthesis robust and flexible. In this thesis I studied one of them, the so-called state transitions.

Upon state transitions, the photosynthetic antenna complexes LHCII, switch between photosystem I (PSI) (state 2, S2) and photosystem II (PSII) (state 1, S1). As a result, the excitation of PSI and PSII is balanced. Other regulatory functions of state transitions have also been proposed by researchers.

On a molecular level, state transitions result in the following forms of the LHCII antenna: the LHCII complexes bound to PSII, the LHCII complexes bound to PSI and (probably) the uncoupled LHCII complexes. Various research groups have applied different techniques to assess a fraction of each of these LHCII states and the estimates vary between the studies.

In the work described in this thesis, we quantified the different fractions of LHCII mostly based on the measurements of the time-resolved fluorescence and the analysis of the data by means of global and target analysis. This approach enables characterization of the excitation energy transfer between spectroscopically different photosynthetic complexes and, as a result, the functional connectivity between them can be assessed. The analysed samples contained whole cells of *Chlamydomonas reinhardtii* (C.r.), wild-type (WT) (chapters 2, 3, 4 and 5) and photosynthetic mutants (chapters 3 and 4) frozen to 77 K.

In our first experiments described in chapter 2 we estimated the fraction of mobile LHCII complexes depending on the conditions under which WT cells were incubated. We compared two situations: under conditions involving chemical locking of state 1 (with kinase inhibitor, staurosporine) and of state 2 (with photosphatase inhibitor, sodium fluoride) versus no chemical locking of the states. In state 1, around 95% of the energy absorbed by the LHCII antenna was transferred to PSII and only a few percent to PSI. Transition to state 2 in the presence of phosphatase inhibitor (S2c) caused 88% of all LHCII complexes to move away from PSII. In comparison, when no chemical compound was added (S2nc), the fraction of these so-called mobile LHCII was significantly smaller, only 46%. This observation led us to
conclude that chemical locking had a significant influence on the state transition-related energy partitioning between PSI and PSII.

Another observation was that not all LHCII moving away from PSII subsequently transferred energy to PSI in state 2. Our results from target analysis, supported by the 6 K fluorescence spectra, showed a significant fraction of uncoupled LHCII complexes in S2c (29% of all LHCII) and in S2nc (25% of all LHCII), with fluorescence maximum at 685 nm, forming what we have named the X-685 component.

In our further attempt to quantify the state transition-related changes on PSI and PSII, we reached for photosynthetic mutants of *C. r.*, lacking either the PSI core (F15, M18) or the PSII core (FUD7) (chapter 3). The findings obtained in these strains led us to conclude that whether it is the lack of the PSI core or the PSII core, incubation under state transition-inducing conditions will still lead to the LHCII-mediated changes of the excitation energy transfer paths. For example, in the absence of the PSI core, upon transition to S2 some LHCII complexes might disconnect from PSII and if they remain uncoupled, they are in a quenched state, indicated by no increase in the amplitude of the longest living component. We also observed LHCII antenna that transferred excitation energy to Lhca. The relatively long lifetime of this transfer suggests a loose connection.

The lack of the PSII core in another mutant also did not inhibit LHCII rearrangements in S1 or in S2. The amount of LHCII transferring excitation energy towards PSI increased in S2 in comparison with S1, as did the amount of the uncoupled quenched LHCII complexes.

The datasets acquired on the mutant strains presented us with an opportunity to develop a new, more detailed model for target analysis (chapter 4). By applying this model to the new measurements on WT cells, we estimated the fraction of mobile LHCII upon state transitions at 36%. The model did not discern previously observed X-685 component, indicating its absence in datasets from chapter 3 and 5. It is possible that the X-685 component in chapter 2 data resulted from a presence of glycerol added to the samples (measurements in cryostat), as all samples analysed in later chapters were frozen to 77 K instantly without this cryoprotectant (measurements in cold finger).

When the new model was applied to the chemically-locked states from chapter 2, the X-685 component appeared as a residual around 685 nm. Somewhat surprisingly however, the fraction of mobile LHCII estimated with this model was 40%, drastically less than what the previous model indicated (88%) and quite similar to the estimate for the new datasets, where no chemical locking was applied (36%).
Besides the state transitions-related results, two interesting findings came out upon target analysis presented in chapter 4. First, in the PSI core deficient mutants the red-most form, peaking around 716 nm, is related to the PSI core rather than the red-most antenna complexes. Second, in WT and PSI core deficient mutants a quenching was revealed on the PSII-684 compartment. To assess how much this selective quenching has had an influence on the previous conclusions presented in this thesis, we studied it in more details in the final chapter 5.

At first, we suspected that the quenching revealed in chapter 4 was caused by annihilation. However, the relatively low energy per pulse used in the measurements and especially the time after which the quenching remained present in the sample, indicated a different origin. Literature research revealed several works where similar quenching was observed upon illumination of photosynthetic samples at 77 K. Using the model we had developed in chapter 4, we were able to quantify the quenching rate constant and its change during continuing illumination. Under the applied experimental conditions, this quenching speeded up by 1 ns⁻¹. The quenching was identified on the PSII-684, however the more red compartments, PSII-690 and PSII-695, functionally connected to PSII-684 were also affected. With regard to state transitions, the potential dependence between the studied quenching and antenna size did not find confirmation in our data. With this study we showed however that even with the quenching affecting differently PSII vs PSI fluorescence, our earlier interpretation of state transition results had been valid.

Summarizing, based on the results presented in this thesis we can conclude that in state 1 between 95-100% of excitation energy absorbed by LHCII is transferred to PSII. This percentage holds independently of the way we induced state transitions (with vs without chemical lockers), froze the samples (with vs without cryoprotectant), measured the samples (quencher accumulation) and analyzed the data (chapter 2 model vs chapter 4 model). With the model developed in chapter 4 based on 8 diverse datasets, incl. mutant strains, the fraction of mobile LHCII has been estimated between 33-40% consistently for all WT datasets analyzed with this model.