Summary

In this thesis the red-most absorbing chlorophylls of cyanobacterial Photosystem I (PS I) have been investigated using single-molecule spectroscopy. The excitation energy of these red-shifted chlorophyll spectral forms is lower than that of the primary donor P700 where the charge separation starts. At low temperatures, the red-most chlorophylls act as a trap for the excitation energy due to a lack of vibrational energy for an uphill energy transfer to P700. Under these conditions their fluorescence quantum yield is adequate for single-molecule detection. A confocal spectrometer for low temperatures with sufficient efficiency to detect single-molecule fluorescence was built in the course of this work for this purpose. PS I samples from the species *Thermosynechococcus elongatus* (*T. elongatus*), *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 were probed. In earlier studies based on ensemble techniques, the red-most chlorophylls were assigned to different pools according to their absorption maximum. For PS I from *T. elongatus* pools were found at 708 nm and 719 nm, denoted C708 and C719, while for PS I from *Synechocystis* pools were identified at 708 nm (706 nm) and 714 nm, denoted C708 (C706) and C714. In this thesis a fluorescence polarization analysis on single PS I monomers from *T. elongatus* shows that three pigment pools contribute to the red-shifted emission. From this analysis it can be concluded that at least one of these states is able to emit either in the spectral region of the C708 or C719 pool, varying from complex to complex. The PS I from all three species exhibit intense zero-phonon lines (ZPLs) in the spectral regions of their red-most chlorophylls. Thus, for the first time ZPLs of PS I from *Synechocystis* could be observed, in contrast to an earlier proposition arguing against their existence. While the ZPLs of PS I from *T. elongatus* form two spectrally separated bands corresponding to the spectral ranges of the C708 and C719 pool, the ZPLs of PS I from *Synechocystis* occur without a gap in between in the range of the C706/C708 and the C714 pools. The PS I from *Synechococcus* sp. PCC 7002 mainly shows ZPLs in a band with center wavelength at 698 nm, thus denoted as F698. A characteristic feature of all observed ZPLs is intense spectral diffusion. This is ascribed to structural fluctuations in the vicinity of the corresponding pigment pools which lead to changes of the local interaction strengths and concomitantly to fluctuations of the site energies. For PS I from *T. elongatus* the correlation between fluctuation rates and associated site energy changes shows separated regions. This finding supports the concept of a protein energy landscape organized in hierarchical tiers in which the average height of the energy barriers decreases from top to bottom. However, for PS I from *Synechocystis* in the correlation between fluctuation rates and associated site energy changes no clearly separated regions were found. The substi-

tution of hydrogen for deuterium in the solvent led to significant reduction of spectral fluctuations in the fluorescence of PS I from all three species. This suggests that proton displacement is one of the main processes responsible for fine-tuning of site energies in PS I. An intriguing feature of the deuterium effect on PS I from *Synechocystis* consists in the creation of bi- and tristabilities. These can be explained by the decreased likelihood for deuteron tunneling in contrast to proton tunneling. The occurrence of spectral jumps of ZPLs above 10 cm^{-1} in absence of external excitation was proved to be highly unlikely at 1.4 K. Consequently, at cryogenic temperatures proton tunneling in the vicinity of the red pools is very unlikely without excitation. Interestingly, after substitution of hydrogen for deuterium in the solvent in the correlation between fluctuation rates and associated site energy changes a similar separation into regions was found in PS I from *Synechocystis* as in native PS I from *T. elongatus*. An analysis coping with the effect of spectral diffusion was applied to suppress temporal averaging in the fluorescence signal. This permits to extract spectral profiles of the red-most states close to their homogeneous line shapes. The line shapes of various red-most states were simulated, based on an algorithm that calculates the pigment-protein coupling in linear approximation assuming a Lorentzian spectral density. Surprisingly, even for far red-shifted states of the PS I from all of the three above-mentioned species small Huang-Rhys factors were found, implying only a weak coupling of the electronic transition to the bath of vibrational modes. This finding sheds light on properties of the red-most states by indicating that no distinct electron-phonon coupling is necessary for the strong redshift of these states. The increase of the excitonic splitting by a large contribution of the Dexter mechanism is proposed as an alternative for the strong redshift. To investigate the energy transfer pathway between different states the correlation behavior between their fluorescence bands was analyzed. The repeated observation of anticorrelations for PS I from *T. elongatus* as well as from *Synechocystis* indicate that either direct energy transfer between different pools of red-most chlorophylls occurs or different red-most states are fed independently by one parent excited state. Also a combination of both is possible. By a polarization analysis on single PS I monomers from *T. elongatus* the angles between the transition dipole moments of emitters from C708 and C719 were determined to be near 90°. Hence, in case of direct energy transfer between different red-most states one expects that on linear polarized excitation at ~ 712 nm the polarization of the fluorescence would change. Exactly this behavior occured in an earlier anisotropy study on bulk PS I. An efficient direct energy transfer between states belonging to the pools C708 and C719 is thought to be likely.