Summary

Carotenoids are important molecules in nature. In addition to endowing color to many plant and animal species, they are involved in cellular process such as light harvesting and photoprotection. The work of this thesis was to study the vibrational properties of specific carotenoid molecules using resonance Raman spectroscopy; carotenoids were investigated in solvents and in purified protein complexes to deduce information ranging from their conformational structure to insight into the way the function in vivo. Specifically, we have looked at different carotenoids in light harvesting complexes from dinoflagellate, cyanobacteria, and plants, work that was done in collaboration with groups around Europe and Asia. Chapter one is a general introduction to carotenoids, resonance Raman spectroscopy, photosynthesis, and my projects.

Chapter 2 is work that began with the peridinin–chlorophyll a-protein (PCP). We realized that a preliminary study of peridinin in solvents would be necessary to understand the carotenoid in vivo. Thus, this publication came from this preliminary fundamental work on peridinin in solvents. We discovered a Fermi resonance in the carbonyl region of the RRS spectra. Our experimental results were corroborated with calculated spectra and a model done with our collaborators.

Chapter 3 is an extension of the work done in Chapter 2. This study looks at peridinin photophysics and investigates its vibrational ground state properties. Again, calculations were done to validate the experimental findings. In the step-scan FTIR spectrum of PCP new peaks have been identified belonging to a triplet state induced by light. The nature of this triplet state has been largely debated, mainly on the basis of time-resolved FTIR studies, thus this paper brings us several steps closer to a full understanding of peridinin photophysics.

Chapter 4 investigates the vibrational properties of carotenoid molecules that contain substitutions of chain methyl groups. The $\tilde{\nu}1$ stretching mode in the resonance Raman spectrum of a carotenoid arises from C=C stretching modes and is normally found around 1520 cm$^{-1}$. Density functional theory (DFT) was used to theoretically analyze this peak. From the calculations we have found that while the effective conjugation length of carotenoids increases in linear polyenes upon s-cis isomerization towards the end of the chain, it lengthens in conformers of
carotenoids containing β-rings. It was also found that methyl groups attached to the conjugated chain of carotenoids induce a splitting of the $\pi_1$ band.

Chapter 5 examines the orange carotenoid protein (OCP). This 35 kDa soluble protein has been implied in photoprotection in certain cyanobacteria. It contains an echinenone molecule spanning its N and C terminal domains that gives the protein its distinct orange color. Upon subjugation to high light intensities, the purified protein appears red. Our work was to investigate the vibrational differences of the echinenone molecule in the two OCP forms. We have discovered a type of intermediate form of echinenone in OCP that is relatively red-shifted, and have found that the crystal structure that exists of OCP is probably not the most blue-shifted form of the protein.

Chapter 6 has not been published yet. It is an investigation on the triplet-triplet energy transfer in artificially made carotenoporphyrin dyads. These molecules are used as models for the carotenoid and chlorophyll molecules that exist in light harvesting complexes found in higher plants. We have studied the triplet-triplet energy transfer between these two molecules and found that in the para linked dyad the transfer rate is much slower than in the ortho linked dyad. Using RRS we have investigated the carbon double bond stretching modes of the dyads. The triplet state peak is different in the two dyads; a surprising find since the absorption spectra are almost identical. Calculations on these molecules suggest that there is an effect of the triplet state porphyrin on the ground state of the carotenoid in the ortho dyad.

Chapter 7 is nearly ready for submission. It is a study of wild-type and point-mutated light harvesting complex II (LHCII) proteins. Spectra from RRS showed that the complex was trimerized, made apparent by the lutein 2 peak. The chlorophyll b 605 binding site was also investigated. Notably, it is difficult to obtain LHCII trimer in a reconstitution; often monomers are observed. Further experiments are being completed before submission of this work, such as low temperature absorption and HPLC.