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Quantum Mechanical Calculations of Xanthophyll–Chlorophyll Electronic Coupling in the Light-Harvesting Antenna of Photosystem II of Higher Plants

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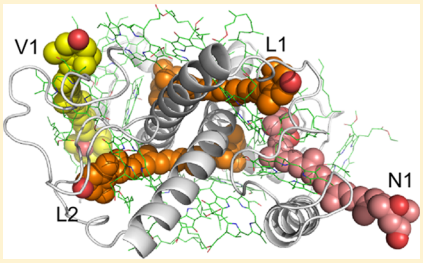
Supporting Information

ABSTRACT: Light-harvesting by the xanthophylls in the antenna of photosystem II (PSII) is a very efficient process (with 80% of the absorbed energy being transfer to chlorophyll). However, the efficiencies of the individual xanthophylls vary considerably, with violaxanthin in LHCII contributing very little to light-harvesting. To investigate the origin of the variation we used Time Dependent Density Functional Theory to model the Coulombic interactions between the xanthophyll $1^1B_u^+$ states and the chlorophyll Soret band states in the LHCII and CP29 antenna complexes. The results show that the central L1 and L2 binding sites in both complexes favored close cofacial associations between the bound xanthophylls and chlorophyll *a*, implying efficient energy transfer, consistent with previously reported experimental evidence. Additionally, we found that the peripheral V1 binding site in LHCII did not favor close xanthophyll-chlorophyll associations, confirming observations that violaxanthin in LHCII is not an effective light-harvester. Finally, violaxanthin bound into the L2 site of the CP29 complex was found to be very strongly coupled to its neighboring chlorophylls.



INTRODUCTION

Photosynthetic light-harvesting by plants is a remarkably efficient process, ensuring a high rate of energy input into the photosynthetic membrane despite frequent periods of low illumination.[1] This efficiency is due to the functional architecture of the photosynthetic antenna, a large modular assembly of various membrane-bound pigment–protein complexes.[2,3] These light-harvesting complexes (LHCs) coordinate the chlorophyll and carotenoid pigment cofactors responsible for the light absorption and energy transfer. The highly specific geometry of these complexes ensures that pigment density is sufficiently high to enable efficient intermolecular energy transfer, while avoiding (under normal circumstances) the concentration quenching that would occur for similar pigment densities in solution.[4]

The most abundant light-harvesting complex is the *major* complex, LHCII, which, along with the *minor* complexes, CP24, CP26, and CP29, forms the antenna of photosystem II (PSII). Far from being a static structure, the PSII antenna possesses remarkable flexibility. During periods of intense illumination, a highly efficient antenna can impact negatively on the plant. The dangers posed by strong illumination arise from the fact that the maximum operating rate of the PSII reaction *center* (RC) is much slower than maximum possible rates of photon absorption and energy transfer in the PSII antenna. As a result, intense illumination leads to saturation of the RCs, leading to a build-up of excitation energy in the antenna and oxidative damage to PSII. This damage is known as photoinhibition,[5] can take many hours to repair,[6] and is highly detrimental to the well-being of the organism. However, photoinhibition is mitigated by a hierarchy of processes collectively known as *photoprotection*. For rapid (minutes) fluctuations in light intensity, photoprotection occurs via a regulation of energy transfer and transduction in the PSII antenna by the formation of excess energy-quenching sites within the PSII antenna. This rapid regulation manifests itself as a decline in chlorophyll fluorescence that occurs following a sudden increase in illumination, a phenomenon known as non-photochemical quenching (NPQ)[7−11] as distinct from the photochemical quenching due to excitation trapping by the RCs. The molecular details of the NPQ process, the precise nature of the excitation quencher, its location within the PSII antenna, and the mechanics of their formation, are subject to intense disagreement, and the reader is directed to our recent review on the topic for a full discussion of the history and current state-of-the-art of the debate.[12]

Central to the structure and function of the PSII antenna are the pigment cofactors, in particular the oxygenated carotenoids known as xanthophylls. As part of the antenna the xanthophylls fulfill a number of important roles. They have a well-established

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function as accessory light-harvesting pigments,[13] absorbing in the green region of the spectrum where chlorophyll *a* and *b* absorption is weak. They are essential to the formation of the tertiary[14] and quaternary[15] structure of the antenna itself. They provide essential protection against photodamage associated with highly oxidizing singlet oxygen, $^1O_2$*, by quenching triplet excited states of chlorophyll, ^{3}Chl*, or by scavenging singlet oxygen directly.[16] Finally, it has been proposed that xanthophylls play an essential role in the NPQ mechanism[12,17−20] although alternative models relegate them to an indirect role.[21]

It is the light-harvesting function, and to a limited extent the NPQ function, of the xanthophylls that concern this work. To understand the light-harvesting properties of the xanthophylls, one must understand its excited state electronic structure, particularly the photophysically important first, S_1, and second, S_2, singlet excited states.[22−24] The xanthophylls present in the PSII antenna are lutein, violaxanthin, and neoxanthin (see Figure 1).

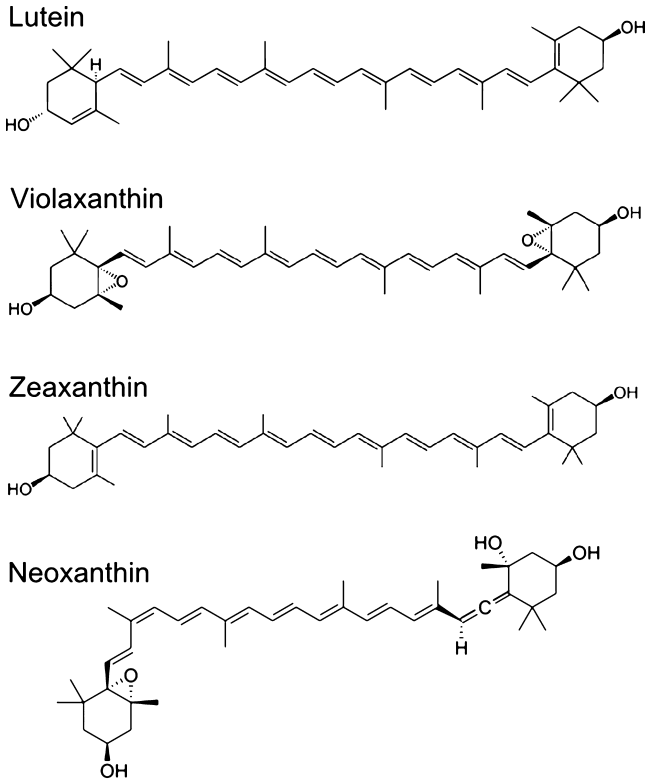

Figure 1. Structural formulas for the xanthophylls found within the PSII antenna.

Neoxanthin is always present in its 9-cis conformation, and, following exposure to high light, violaxanthin is preferentially de-epoxidised to zeaxanthin. This "xanthophyll variety" is an interesting feature of the PSII antenna and, as shown by Ruban and Johnson,[25] is a central element in the modulation of antenna structure and function. By studying a variety of xanthophyll mutants of *Arabidopsis thaliana*, they showed that PSII quantum efficiency was correlated to the overall hydrophobicity of the xanthophyll complement of the antenna and was optimum for the wild-type (WT) composition. Similarly, NPQ showed the same sensitivity to xanthophyll polarity/hydrophobicity and was also optimum for the WT composition.

The photophysical properties of the xanthophylls are derived from the π-conjugated polyene chain that constitutes the molecular "backbone". The electronic states of the xanthophylls therefore have approximately the same symmetries as those of the linear polyenes. The xanthophyll singlet ground state is labeled $1^1A_g^-$ as it possesses even (A_g) inversion symmetry and odd (−) particle-hole symmetry. The S_1 state possesses the same symmetry as the ground state and is therefore labeled $2^1A_g^-$.[24] Due to the selection rules for electric dipole transitions the $1^1A_g^- \rightarrow 2^1A_g^-$ transition is dipole-forbidden.[26] The S_2 state has opposite spatial symmetry to the ground state and is therefore labeled $1^1B_u^+$ and is dipole-allowed. In fact, the $1^1B_u^+$ state is strongly dipole-allowed, with a dipole strength of ∼200 D^2.[27] However, despite this strong dipole connection to the ground state, the xanthophylls exhibit negligible fluorescence, due to the fact that, following photoexcitation of the $1^1B_u^+$ state, the system undergoes a subpicosecond interconversion (IC) to the $2^1A_g^-$ state,[28] which undergoes an IC to the ground state in a time scale of ∼11 ps.[29] When these pigments are embedded within dense molecular aggregates such as LHCs, these rapid IC processes face competition from energy transfer to chlorophyll. In 2000 van Amerongen and co-workers performed a femtosecond transient absorption study of xanthophyll to chlorophyll energy transfer in LHCII and CP29.[27] They showed that the average lifetime of the xanthophyll $1^1B_u^+$ state was only ∼80 fs in CP29 and ∼100 fs in LHCII. These short lifetimes were a combination of native IC to the $2^1A_g^-$ state, with a weighting of 35−40%, and competing, 60−65%, energy transfer to neighboring chlorophylls. Of the 35−40% of excitations that undergo IC to the $2^1A_g^-$ states, approximately half undergo further IC to the ground state (quenching) with the other half being transferred to the Q_y-band states of the chlorophylls. Therefore, xanthophyll → chlorophyll energy transfer in the PSII antenna occurs with an efficiency of ∼80%. However, measuring the extent to which each xanthophyll contributes to the overall light-harvesting efficiency is difficult. Van Amerongen and co-workers showed that in CP29, the acceptors of xanthophyll excitation energy are exclusively Chl *a*, while this is only true for lutein and violaxanthin in LHCII. In their earlier work, Peterman et al. (1997)[30] also showed that a xanthophyll with an absorption maximum at 486 nm, attributed to neoxanthin, does not play a significant role in either light-harvesting or triplet quenching. More recently, Bassi and co-workers showed, using spectroscopic analysis, that violaxanthin in LHCII does not transfer any energy to neighboring chlorophylls and therefore does not participate in light-harvesting.[31] Determining how (if at all in the case of violaxanthin) the individual xanthophylls contribute to their bulk light-harvesting efficiency is the motivation for the work presented in this paper.

Essential mechanistic insight into the physics of the energy transfer pathways in the PSII antenna could be obtained by careful theoretical modeling of the exciton transfer dynamics of the pigment−protein complexes. This is made possible by the availability of high-resolution structures for LHCII[32,33] and, more recently, CP29.[34] The modified Redfield modeling of Novoderezhkin et al.[35] and the structure-based models of Müh et al.[36,37] reproduce the linear absorption and linear dichroism spectra of LHCII very accurately by considering only the chlorophylls within the complex. The neglect of the xanthophylls is primarily the result of the difficulty in modeling

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their low-lying excited states. This difficulty is due to the strongly correlated nature of the ground and first singlet exited state.[38] These strong electron correlations mean that methods such as linear response time dependent density functional theory (TD-DFT),[39,40] which model many-particle excitations as linear combinations of singly excited determinants, fail to produce an S_1 state with the correct symmetry or optical properties. In fact it has been shown that doubly excited determinants make a significant contribution to the $2^1A_g^-$ state.[18,38,41,42] As such, a higher level of theory is needed for an *ab initio* description of the electronic spectra of linear polyenes. This is currently an important topic in theoretical chemistry, and approaches such as second-order algebraic diagrammatic construction (ADC(2)), developed by Dreuw and co-workers, have been shown to correctly account for the double excitation character of the $2^1A_g^-$ state.[43] More recently, Götze and Thiel have applied multireference configuration interaction calculations on DFT reference state (DFT/MRCI[44]) to calculating the low-lying excited states of violaxanthin and zeaxanthin, generating very close agreement with experimental spectra.[45] At a less demanding level of theory, our previous work employed a full configuration interaction calculation on an active space of molecular orbital eigenstates of the semiempirical MNDO Hamiltonian (MNDO-CAS-CI) to successfully compute the electronic spectrum of lutein.[18,41] This method correctly predicts $2^1A_g^-$ and $1^1B_u^+$ states with the correct symmetry and optical properties due to the inclusion of doubly excited (and higher) determinants. This then allowed for an atomistic model of the central role of lutein in the NPQ mechanism.

Recent semiempirical theoretical models, such as that of Martiskainen et al,[46] have provided a detailed picture of xanthophyll-chlorophyll excitonic interactions in LHCII via treating the xanthophylls as point transition dipole moments located at the center of mass of the relevant molecules. Their approach, which modeled the xanthophyll $1^1B_u^+$ transitions as point dipoles of magnitude 13 D, predicted strong excitonic interactions between lutein and neoxanthin and the Soret band states of chlorophyll *a* and chlorophyll *b*, respectively. This work provides essential insight into the xanthophyll light-harvesting pathways in LHCII and is highly rigorous in its treatment of excitonic delocalization. However, there are limitations to the point dipole-approximation, particularly in such densely packed molecular aggregates, and so it may be complementary to aim for first principle calculation of xanthophyll-chlorophyll couplings in the antenna. Previously we employed TD-DFT with the distance corrected CAM-B3LYP to model the $1^1B_u^+$ states of violaxanthin and zeaxanthin to probe excitonic interactions between these xanthophylls in LHCII aggregates.[47] Recently, Kröner and Götze applied this same method to model chlorophyll−violaxanthin interactions in LHCII.[48] In both cases, the TD-DFT method works due to the weakly correlated nature of the $1^1B_u^+$ state as compared to the $1^1A_g^-$ and $2^1A_g^-$ states. In the calculations of Kröner and Götze, the excited structure of a chlorophyll−violaxanthin heterodimer was embedded within the static chlorophyll/protein binding pocket taken from the LHCII structure. While the chlorophyll−violaxanthin pair was treated at the CAM-B3LYP level, the binding pocket was described semiempirically, and the excitonic couplings were inferred from the calculated level splitting arising from the resonant interaction. The results implied that there may be some coupling between violaxanthin and the Soret band states of chlorophyll, contrary to the observations of Bassi and co-workers.[31] Additionally, they postulate that violaxanthin may accept energy from the chlorophyll Soret band states rather than acting in a light-harvesting capacity. It should be noted here that Neugebauer and co-workers have developed a subsystem TD-DFT method for calculating excitonic couplings between chromophores within arbitrary molecular aggregates, embedded within a protein scaffold.[49,50] Since this formalism exploits the Tam−Dancoff approximation (in which de-excitation terms are neglected in the description of excited states) the intersubsystem matrix elements can be directly interpreted as the interpigment excitonic couplings. This method was applied to modeling the chlorophyll−chlorophyll interactions within LHCII, with particular emphasis on how the loss of specific chlorophylls as a result of mutagenesis affects the coupling network within the complex.[49] This method has recently been shown to be effective in computing couplings involving the $1^1B_u^+$ state of linear polyenes, meaning that this approach could be used to model the light-harvesting role of the xanthophylls in the PSII antenna.[50] In this work we present a much simpler theoretical approach to model the xanthophyll−chlorophyll interactions in specific cases of LHCII and CP29 with the aim of revealing how the xanthophylls are energetically coupled to the chlorophyll pool. Despite the simplicity of the method, it allows for estimates of the contribution made by each xanthophyll to the overall xanthophyll light-harvesting, and we discuss how the binding pocket of each xanthophyll determines their connectivity to the chlorophylls.

METHODOLOGY

The transfer of energy between molecules, *m* and *n*, in a molecular aggregate is governed by the transfer integral,[51]

$$W_{mn} = J_{mn} - K_{mn} \tag{1}$$

where J_{mn} and K_{mn} are contributions from the Coulomb and exchange interactions, respectively.[52,53] Since K_{mn} requires significant atomic orbital (AO) overlap between the two molecules, it decays exponentially with intermolecular distance. We shall therefore assume that

$$W_{mn} \cong J_{mn} \tag{2}$$

Essentially J_{mn} is a measure of the Coulomb interaction between two localized electronic transitions,

$$J_{mn} = \sum_{\substack{i\in m \\ j\in n}} V_{ij}\langle \mathrm{EX}_n|\hat{N}_i|\mathrm{GS}_n\rangle\langle \mathrm{GS}_m|\hat{N}_j|\mathrm{EX}_m\rangle \tag{3}$$

where *i* and *j* label the AOs associated molecules *m* and *n*, $|\mathrm{GS}\rangle$ and $|\mathrm{EX}\rangle$ denote the ground and excited state of a molecule, and $\hat{N}$ is the number operator associated with a particular AO. V_{ij} determines the magnitude of the interaction between AO transition densities. In this work we evaluate J_{mn} using the methodology we previously employed to model chlorophyll−lutein energy transfer in LHCII.[18] Unlike the more detailed approach of Neugebauer, we compute excitonic couplings based on calculations of the excited state structure of isolated chlorophylls and xanthophylls. Essentially, the AO transition densities of the single chromophores are projected onto the atomic centers, and J_{mn} for a pair of chromophores is modeled as the Coulomb interaction between two sets of *transition monopoles/charges*. Equation 3 thus becomes

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$$J_{mn} \cong \sum_{\substack{i\in m \\ j\in n}} \frac{e^2}{4\pi\varepsilon|r_i - r_j|}\langle \mathrm{EX}_n|\hat{N}_i|\mathrm{GS}_n\rangle\langle \mathrm{GS}_m|\hat{N}_j|\mathrm{EX}_m\rangle \quad (4)$$

where e is the electronic charge, ε is the dielectric constant of the medium in which the molecules are embedded, and r_i is the coordinate of the ith atomic center.

In this work we model the interactions between the xanthophyll S_2 transitions and several chlorophyll transitions in the Protein Data Bank structures of LHCII (1RWT)[32] and CP29 (3PL9).[34] We neglect the xanthophyll $2^1A_g^-$ states for several reasons. First, as showed by van Amerongen and co-workers, the dominant channel for xanthophyll → chlorophyll energy transfer is via the S_2 state. Moreover, they showed that transfer from the $2^1A_g^-$ state to chlorophyll is only significant for lutein.[27] Lastly, our previous MNDO-CAS-CI work on the interactions between the $2^1A_g^-$ state of lutein and the chlorophyll Q_y band in LHCII[18] found that these interactions were only significant for very small intermolecular distances and even then were an order of magnitude smaller than the typical chlorophyll-chlorophyll couplings. The coupling between the strongly dipole-allowed xanthophyll S_2 state and the Q_y, Q_x and Soret band states of chlorophyll is expected to be much stronger and is therefore assumed to be a clear indicator of the *energetic connectivity* between the two sets of pigments.

The first step is to obtaine quantum mechanically optimized structures for the pigment cofactors of LHCII and CP29: chlorophyll *a*, chlorophyll *b*, lutein, violaxanthin, and neoxanthin. In the LHCII monomer, two luteins are bound at the central L1 and L2 sites and were labeled by Liu et al.[32] as lut620 and l621, respectively. Neoxanthin, labeled neo623, is bound at N1, and violaxanthin, vio622, is peripherally bound at the V1 site[32] (see Figure 2). In CP29, L1 is occupied by lut620 and N1 by neo623. The L2 site is, however, occupied by vio622, and the complex does not bind anything at an analogous V1 site[34] (see Figure 2).

In this work we do not consider zeaxanthin, as there are no equivalent structures for the de-epoxidized complexes. The optimizations were performed without any geometric constraints, therefore resulting in idealized planar geometries.

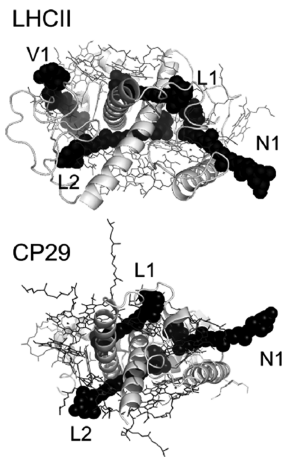

Figure 2. The xanthophylls in the LHCII monomer and CP29. In LHCII, lutein is bound at the central L1 and L2 sites, neoxanthin is bound at the N1, and violaxanthin is bound at the peripheral V1 site. In CP29, as in LHCII, lutein and neoxanthin are bound at the L1 and N1 sites, respectively. The L2 site is occupied by violaxanthin, and there is no equivalent of the V1 site.

Additionally, the phytyl tails of the chlorophylls were removed and replaced with a methyl group in the manner described by Müh and co-workers.[36] This drastically reduces computational expense and, because the phytyl tail does not contribute to the electronic transitions of the tetra pyrole ring, does not affect the calculated electronic spectra. The xanthophyll geometries were obtained via a ground state density functional theory (DFT)[54,55] calculation employing the long-range corrected CAM-B3LYP hybrid exchange-correlation functional[56] and the 6-31G** Pople basis set.[57] CAM-B3LYP has been shown to be highly effective in modeling the geometries and S_2 transition of the linear polyene-based xanthophylls.[47,48] For comparative purposes, the geometries of the methyl-chlorophylls were computed using the CAM-B3LYP functional and its uncorrected equivalent, B3LYP.[58] The basis set used was also 6-31G**. The electronic spectrum of each molecule was computed using TD-DFT. For the xanthophylls, we employed a three state CAM-B3LYP/6-31G** calculation. For the chlorophylls, the Q_y and Q_y transitions and the different transitions that collectively make up the Soret band were computed via B3LYP/6-31G** and CAM-B3LYP/6-31G** calculations. Both the optimizations and the excited state calculations were performed using the Gaussian 09 quantum chemistry package.[59] For both the chlorophylls and the xanthophylls, it was found that de-excitation terms (virtual → occupied) do not contribute significantly ($C > 0.1$) to the electronic states being studied. This is a reflection of the accuracy of the Tam−Dancoff approximation in TD-DFT calculations on these pigments. The AO transition densities for the electronic transitions of the isolated molecules are then calculated according to the textbook method outlined in our previous work and then projected onto the nuclear coordinates of the pigments, yielding the transition monopole description of the transition.[18] The transition charges (calculated for the planar chlorophylls and xanthophylls) were then projected on to the crystal structures of LHCII and CP29. Since the crystal structures do not contain hydrogen coordinates, these were added, and their positions relaxed relative to the frozen heavy atoms via a constrained B3LYP/6-31G* optimization. Generally, the transition monopoles associated with an electronic transition are rescaled so that the transition dipole moment matches some vacuum extrapolated value.[60] The solvent environment is then represented as a continuous dielectric medium with $\varepsilon_{opt} = 2$. This was the approach used by Müh et al. in their highly accurate TrESP (transition charges from an electrostatic potential) modeling of chlorophyll−chlorophyll energy transfer[36,37] and in our previous model of chlorophyll−lutein interactions in LHCII.[18] However, we have no vacuum extrapolated values for the dipoles strengths of xanthophyll S_2 transitions or the higher ($\geq S_2$) transitions of the chlorophylls. Therefore, the couplings we calculate here are only meaningful in a relative sense. As described below, all couplings are expressed relative to the strongest coupling identified in the two complexes. The details of the continuum dielectric environment are thus completely neglected.

Lastly, we make a qualitative estimate of the relative efficiency of energy transfer between the xanthophyll S_2 states and the chlorophyll Soret band states. We neglect excitonic delocalization and assume that the total rate of energy transfer from the ith xanthophyll,

$$k_i^{\mathrm{total}} \propto \sum_{j\in \mathrm{Chlorophyll}} |J_{ij}|^2 \quad (5)$$

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By taking into account the empirically obtained efficiency for light-harvesting through the xanthophyll $1^1B_u^+ \rightarrow$ chlorophyll Soret pathway ($\sim$ 65%[27]), it is possible to make qualitative estimates of the absolute efficiencies of this light-harvesting channel.

RESULTS

Xanthophyll $1^1B_u^+$ State. The excitation energies and dipole lengths of the vertical xanthophyll $1^1A_g^- \rightarrow 1^1B_u^+$ transitions are listed in Table 1.

Table 1. The Calculated Excitation Energies and Dipole Lengths of the Vertical $1^1A_g^- \rightarrow 1^1B_u^+$ Transitions of Lutein, Violaxanthin, and Neoxanthin for the Planar CAM-B3LYP/6-31G Geometry**

	$E(1^1B_u^+)$ (eV)	$E(1^1B_u^+)$ (nm)	dipole length (D)
lutein	2.9	427.5	19.2
violaxanthin	2.9	427.5	22.0
neoxanthin	3.0	413.3	21.7

The vacuum TD-DFT calculations yield a strongly dipole-allowed S_2 state with a single (HOMO $\rightarrow$ LUMO) + (HOMO-1 $\rightarrow$ LUMO+1) character and pseudo B_u^+ symmetry. The excitation energy is $\sim$3 eV, an overestimate of the order of 0.5 eV when compared to the typical solvated energy.[27] The dipole lengths are typically 20−22 D, which is larger than the experimental vale of $\sim$15 D. However, this method clearly produces a qualitatively correct prediction of the xanthophyll $1^1B_u^+$ state.

Chlorophyll Electronic Spectra. Chlorophylls *a* and *b* were more complex due to the need to describe the multitransition Soret band. It was found that a total of six electronic transitions were needed to describe the chlorophyll *a* spectrum, and a total of five were needed for chlorophyll *b*. The electronic spectra as calculated by the B3LYP/6-31G** and CAM-B3LYP/6-31G** methods are shown in Figure 3. The vacuum calculations gave spectra that were typically blue-shifted by $\sim$0.4 eV. The calculated electronic spectra were therefore rescaled so that the first singlet excited states (S_1) of chlorophylls *a* and *b* coincide, in both energy and amplitude, with the Q_y band of their respective absorption spectra as measured by Frigaard et al.[61] Obviously, comparisons of real spectra and the line spectra obtained for excited state calculations using the ground state geometry only are of limited validity. In particular, if we consider only the excitation energy of the relevant vertical transitions, there is little to promote one method over the other. However, as shown in Figure 3, there is a large disparity between the oscillator strengths of the Q_y and Soret band states predicted by the CAM-B3LYP method.

When both the B3LYP and CAM-B3LYP spectra are rescaled, B3LYP seems to give better qualitative agreement. For chlorophyll *a*, S_1 corresponds to the Q_y band, the weakly dipole-allowed S_2 corresponds to the Q_x band, and the S_5 and S_6 apparently belong to the Soret band. The S_3 and S_4 states are very weakly dipole-allowed and therefore make no significant contribution to the spectrum. For chlorophyll *b*, the S_1 and S_2 states also correspond to the Q_y and Q_x bands and the S_4 and S_5 constitute the Soret band. The S_3 state is very weakly dipole-allowed.

Xanthophyll−Chlorophyll Couplings. The magnitudes of the couplings, $|J|$, between the xanthophyll S_2 states and all chlorophyll states were computed for both complexes. For LHCII, all 42 chlorophylls in the trimer were calculated in order to account for any significant couplings that occur across the interfaces between monomers. These couplings are listed in their entirety in the Supporting Information.[62] However, we are interested primarily in couplings that are could lead to energy transfer or a significant excitonic perturbation of the uncoupled states. Therefore we restrict this discussion to interactions between the xanthophyll S_2 states and the chlorophyll Soret transitions. Additionally, inspection of the Supporting Information shows that the most significant couplings involve the chlorophyll Soret band states. Figure 4 shows the sum of the magnitude of the couplings between the xanthophyll S_2 states and the two Soret states for chlorophylls in LHCII and CP29.

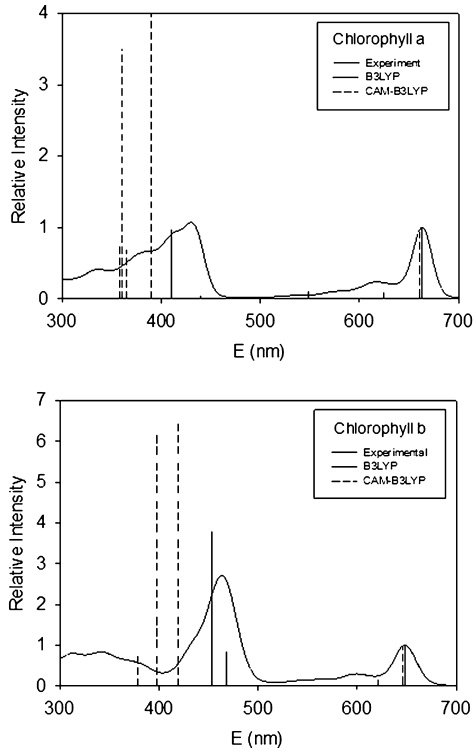

Figure 3. The electronic spectra of chlorophylls *a* and *b* as calculated by time-dependent B3LYP/6-31G** and CAM-B3LYP/6-31G**. The electronic spectra have been rescaled so that the S_1 transitions coincide with the Q_y band of the absorption spectra of Frigaard et al. (1996).[61]

In LHCII, it was found that significant couplings only occurred for pigments within the same monomer, and therefore longer-range couplings are not shown. Since the couplings are only meaningful in a comparative sense, they are measured relative to the strongest coupling, vio622−Chl*a*603 in CP29. The second biggest coupling in CP29 is lut620−Chl*a*612 (J = 0.77). Equivalently in LHCII, the strongest couplings are lut620−Chl*a*612 (J = 0.81) and lut621−Chl*a*603 (J = 0.91). The couplings between the neo623 $1^1B_u^+$ state and the chlorophyll Soret band states were smaller, with the strongest couplings being neo623-Chl*b*606 for LHCII (J = 0.61) neo623-Chl*a*609 in CP29 (J = 0.30), although the neo623−Chl*a*604 couplings in LHCII (J = 0.27) and CP29 (J = 0.26) and the neo623−Chlb606 coupling (J = 0.28) are also significant. Lastly, the couplings between vio622 and chlorophyll in LHCII is typically smaller than all others, with the strongest coupling being vio622−Chl*b*601 (J = 0.51).

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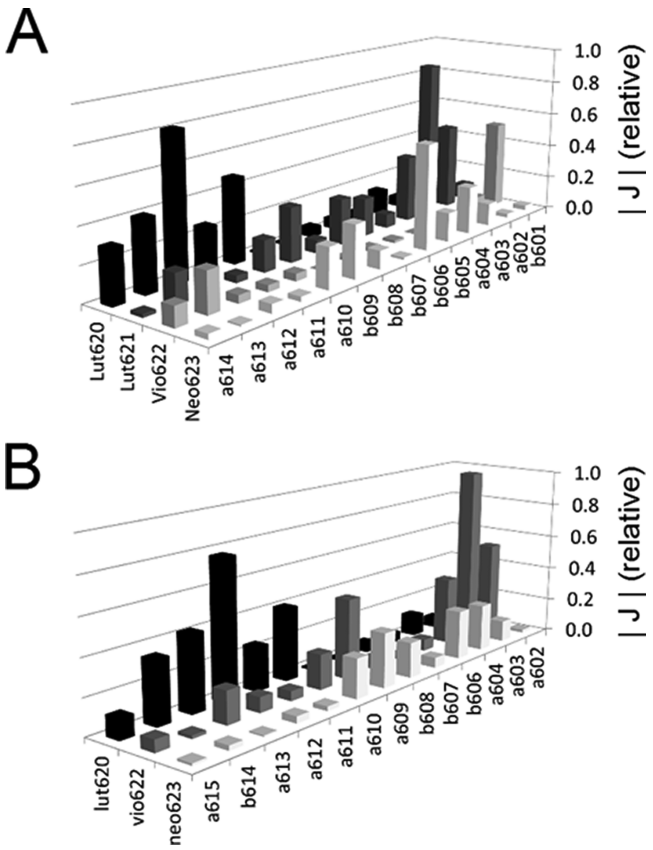

Figure 4. The relative magnitudes of the couplings between the xanthophyll S_2 transitions and the chlorophyll Soret band states in LHCII (**A**) and CP29 (**B**).

Efficiency of Xanthophyll−Chlorophyll Energy Transfer. Figure 5 shows the estimated relative total rates of energy transfer from xanthophylls to the chlorophyll pool of LHCII and CP29.

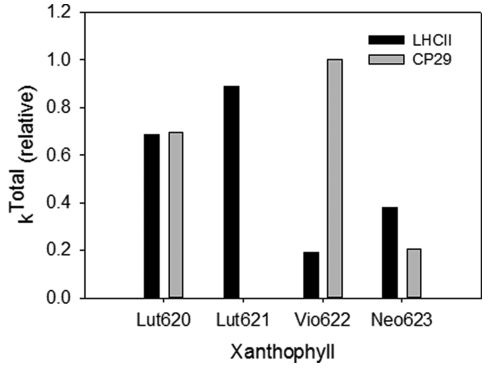

Figure 5. The relative rates of total xanthophyll $1^1B_u^+ \rightarrow$ chlorophyll Soret energy transfer in LHCII and CP29.

This graph shows that lut620 → chlorophyll energy transfer is essentially the same in both LHCII and CP29. Light-harvesting by vio622 in CP29 is highly efficient, due to the strong vio622-Chl*a*603 coupling, and is comparable to the efficiency of lut621 bound to the same L2 site in LHCII. Light harvesting by neoxanthin in both complexes appears to be less efficient than that of the central L1 and L2 xanthophylls, but is particularly low in CP29. Vio622 in LHCII has the lowest energy transfer efficiency of all of the xanthophylls belonging to either complex, being <20% of that of Vio622 in CP29. If we assume that the total efficiency of xanthophyll light-harvesting via the xanthophyll $1^1B_u^+ \rightarrow$ chlorophyll channel is ∼65%,[27] then it is possible to (very tentatively) estimate the light-harvesting efficiencies of each xanthophyll, in LHCII and CP29, through this pathway (see Table 2).

Table 2. Estimated Light-Harvesting Efficiencies of Each Xanthophyll, in LHCII and CP29, through the Xanthophyll $1^1B_u^+ \rightarrow$ Chlorophyll Soret Pathway

	$1^1B_u^+ \rightarrow$ Soret efficiency	
	LHCII	CP29
lutein	48%	24%
neoxanthin	11%	7%
violaxanthin	6%	34%

From Table 2 we see that, in LHCII, lutein has a combined efficiency of ∼48%, neoxanthin ∼11%, and violaxanthin only ∼6%. In CP29, the lone lutein has an efficiency of ∼24%, neoxanthin only ∼7%, and violaxanthin ∼34%. This would seem to be in agreement with the observations of Bassi and co-workers,[31] implying that violaxanthin, which constitutes the major part of the whole xanthophyll cycle, plays a minimal role in light-harvesting in LHCII. However, it would appear that violaxanthin in CP29 contributes significantly to light-harvesting.

DISCUSSION

By computing the relative Coulombic couplings, |*J*|, of the xanthophyll $1^1B_u^+$ state and Q_y, Q_x, and Soret band states of chlorophyll, it is possible to qualitatively examine how each xanthophyll contributes to the overall light-harvesting capability of the major, LHCII, and minor, represented by CP29, antennae of PSII in plants. First, we will consider lutein. Our previous work on lutein−chlorophyll interactions in LHCII revealed that a close, cofacial association with Chl*a*612 allowed chlorophyll $Q_y \rightarrow$ lut620 $2^1A_g^-$ energy transfer.[18] This close association is also reflected in the strong coupling between the lut620 $1^1B_u^+$ state and the Chl*a*612 Soret band states (see Figure 6).

In fact this close, cofacial association with chlorophyll *a* appears to be conserved for all luteins in both complexes (see Figure 6). Lut620 in CP29 is also strongly coupled to its Chl*a*612 neighbor and lut621 in LHCII is similarly coupled to Chl*a*603. It appears that it is this close, cofacial association that leads to a significant transfer of energy from lutein to chlorophyll (see Figure 5) as observed by van Amerongen and co-workers.[27] Inspection of Figure 4 shows that the luteins in both complexes are almost exclusively coupled to chlorophyll *a*. This is again consistent with the observations of van Amerongen and co-workers who showed that chlorophyll *a* is the exclusive acceptor of energy from lutein in the PSII antenna.[27] For neoxanthin, Figure 4 shows that neo623 is coupled to the Soret band states of both chlorophyll *a* and *b* in LHCII and CP29. However, it lacks the close cofacial association with its nearest neighbors that a feature of the lutein-chlorophyll *a* interactions (see Figure 7).

This is reflected in the fact that neoxanthin is less strongly coupled to its chlorophyll environment than lutein and is likely a less efficient light-harvesting channel (see Figure 4). This is

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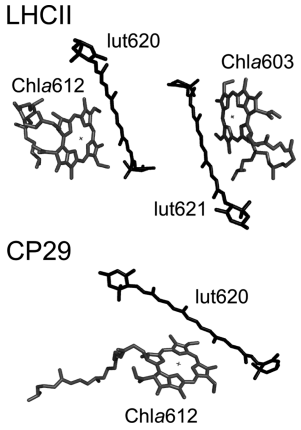

Figure 6. The close, cofacial associations between the luteins and their neighboring chlorophylls in both LHCII and CP29 ensure strong Coulombic couplings between the lutein $1^1B_u^+$ state and the chlorophyll Soret band states. In both LHCII and CP29, lut620 is strongly coupled to Chl*a*612, while lut621 in LHCII is closely associated with Chl*a*603.

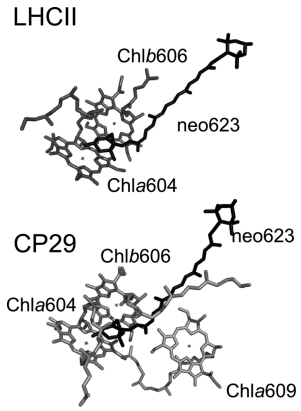

Figure 7. Neo623 is most strongly coupled to Chl*a*604 and Chl*b*606 LHCII. In CP29 there is an additional coupling to Chl*a*609. Note that the lack of the close cofacial geometry observed for lutein in LHCII and CP29 explains why neoxanthin–chlorophyll coupling is typically weaker when compared to lutein.

consistent with the low efficiency of light-harvesting and triplet quenching as observed by van Amerongen and co-workers.[27,30] The violaxanthin–chlorophyll couplings/energy transfer efficiency vary considerably between LHCII and CP29, reflecting their very different binding pockets with the two complexes. In LHCII, vio622 is weakly associated with its chlorophyll neighbors as compared to lutein, while in CP29 it is the most strongly coupled of all xanthophylls (see Figure 4). Figure 8 shows the geometries of the violaxanthin binding pockets in each complex.

For LHCII we see that vio622 is most closely associated with Chl*b*601 with the weak intermolecular coupling resulting from the fact that Chl*b*601 is most closely associated with the end of the violaxanthin polyene chain rather than being cofacially configured with its center, like the close lutein-chlorophyll associations. In CP29, vio622 occupies the central L2 site, and there its association with the CP29 chlorophylls is similar to the lutein–chlorophyll coupling of lut621 in LHCII. Figure 5 shows that the different violaxanthin binding sites lead to very different estimates for violaxanthin → chlorophyll energy transfer. The fact that this efficiency is very low in LHCII, only

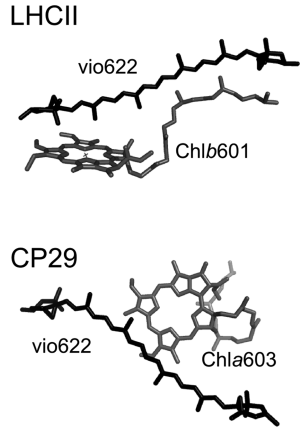

Figure 8. In peripheral V1 binding pocket of LHCII, vio622 is weakly coupled to Chl*b*601, while in the central L2 site of CP29, vio622 is very strongly coupled to Chl*a*603. The L2 site in CP29 is characterized by the same close, cofacial xanthophyll–chlorophyll association as in L2 in LHCII. This geometry is not as apparent in the V1 site of LHCII.

6% (as compared to CP29), is consistent with the observations of Bassi and co-workers that violaxanthin is not an efficient light-harvester in CP29.[31] Inspection of Figure 4 shows that in CP29 violaxanthin interacts most appreciably with chlorophyll *a*, in agreement with the observations of van Amerongen and co-workers.[27,30]

CONCLUSION

In this work we used single-molecule TD-DFT to generate a simple model of the Coulombic interactions between the xanthophyll $1^1B_u^+$ states and chlorophyll in the major, LHCII, and minor, CP29, PSII antenna. We found that the CAM-B3LYP/6-31G** approach yielded a qualitatively correct description of the xanthophyll $1^1B_u^+$ state, while, following a rescaling procedure, the B3LYP/6-31G** approach gave a reasonable representation of the electronic spectra of chlorophylls *a* and *b*. A transition monopole approach, of the kind previously employed in our model of lutein-mediated NPQ in LHCII,[18] was employed to compute the Coulomb coupling. It was found that the strongest interactions occurred between the xanthophylls $1^1B_u^+$ state and the chlorophyll Soret band states. Since these states are likely to have significant energetic overlap, these interaction are likely to result in energy transfer of excitonic delocalization. Knowledge of the relative interaction strengths then allowed for a qualitative estimate of the relative efficiency of xanthophyll energy transfer in both complexes, yielding clues as to the different contributions made by each pigment to the overall light-harvesting capability of the PSII xanthophylls. We showed that the central L1 and L2 binding sites in both complexes favored close cofacial associations between the bound xanthophylls (lutein in LHCII and lutein and violaxanthin in CP29) and chlorophyll *a*, which led to energy transfer of comparable efficiency. The fact that chlorophyll *a* appears to be the most significant acceptor of lutein excitation energy has previously been observed by van Amerongen and co-workers. Additionally, we found that the peripheral V1 binding site in LHCII did not favor close xanthophyll–chlorophyll associations, which in turn explains why Bassi and co-workers concluded that violaxanthin in LHCII is a poor light-harvesting pigment.[31] Our estimates of xanthophyll light-harvesting efficiencies are very approximate in

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nature, and much further work is needed to produce quantitative estimates based on first priciples calculations. First, the excited state calculations were carried out in vacuum, meaning that the xanthophyll and chlorophyll electronic spectra had to be rescaled. As such, we obtained no conclusive information regarding the relative excitation energies of the in vivo pigments. This may be rectified by calculations that account for the protein environment.[36,37,48] Second, since our calculations were based on a simple single-molecule approach (i.e., each chromophore considered separately), the calculated couplings themselves are only approximate. A more formal subsystem TD-DFT method such as that developed by Neugebauer and co-workers, would likely produce a more accurate picture of the landscape of xanthophyll−chlorophyll interactions within the antenna.[49,50] Additionally, our estimates of the relative energy transfer efficiencies assumed that the degree of energetic overlap between the states were the same. This is a reasonable assumption given the broadness of both the xanthophyll $1^1A_g^- \rightarrow 1^1B_u^+$ transition and the chlorophyll Soret band states, but is still a drastic oversimplification. A quantitative assessment of transfer efficiencies will require a detailed knowledge of the relative positions of states. Perhaps most importantly, our rough estimates of energy transfer efficiencies assumed transfer between localized excited states. Due to the very strongly allowed nature of the xanthophyll $1^1B_u^+$ state and the chlorophyll Soret band states, it is unlikely that energy transfer proceeds via transfer between a localized $1^1B_u^+$ state and pure Soret band states. Martiskainen et al. showed in their semiempirical model that this is indeed not the case for lutein and neoxanthin in LHCII, as the absorbing states in this region are excitonic mixtures of xanthophyll $1^1B_u^+$ and chlorophyll Soret states.[46] Lastly, our treatment of LHCII refers to the dark adapted state only as we have no structure for the deopoxidised complex. How zeaxanthin binds to the V1 pocket and how it is coupled to neighboring chlorophylls may be studied if a de-epoxidised structure were available or via a binding geometry generated via a quantum mechanical/molecular mechanical (QM/MM) calculation. We may expect de-epoxidation, and NPQ as a whole, to give rise to measurable changes in xanthophyll−chlorophyll interactions. It is now widely accepted that the transition between light-harvesting and dissipative states in the antenna is achieved via a subtle conformational change that modulates the profile of pigment−pigment interactions in the complexes.[63−70] Certainly, it is known that the de-epoxidation component of the photoprotective switch is associated with absorption changes in the Soret region, indicating some change in the L1 domain.[64] It is also possible that there is some change in V1 domain, meaning that violaxanthin and zeaxanthin are coupled to the local V1 chlorophylls to different degrees. Further experimental and theoretical work is needed to clarify these points.

The issue of de-epoxidation is less important for CP29, as there is considerable evidence, both experimental[71] and theoretical,[72] that violaxanthin in CP29 is too strongly bound to the L2 site to be available for de-epoxidation. This lack of violaxanthin de-epoxidation in CP29 does raise the question of the functional purpose of the binding of violaxanthin at the L2 site rather than lutein, as in the major antenna. Our calculations indicate that both pigments can serve as efficient light-harvesters, and that it is the geometry of the L2 binding pocket that essentially determines the strong lutein/violaxanthin−-chlorophyll coupling. In light of the earlier work of Ruban and Johnson,[25] we may hypothesize that the intrinsic binding of violaxanthin in CP29 plays a structural role, in which violaxanthin polarity/hydrophobicity is an important factor in tuning the light-harvesting properties of the minor antenna. In conclusion, despite the limitations of the model, it represents a first-principles model of xanthophyll−chlorophyll coupling in the PSII antenna that offers qualitative insight into the light-harvesting role of xanthophylls in the PSII antenna.

■ ASSOCIATED CONTENT

Supporting Information

Several supplementary tables are listed within the Supporting Information. Tables S1−S6 list the Coulombic couplings between the xanthophyll $1^1B_u^+$ state and the chlorophyll *a* S_1-S_6 states in LHCII respectively. Tables S7−S11 list the same couplings for the chlorophyll *b* S_1-S_5 states in LHCII. Tables S12−S17 list the chlorophyll *a* couplings (states S1−S6, respectively) and Tables S18−S22 list the chlorophyll *b* couplings (states S1−S5, respectively) for CP29. All couplings are measured relative to the largest overall coupling in our calculations (Vio622-Chla603 (S_6) in CP29). This information is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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