The Role of Exciton Delocalization in the Major Photosynthetic Light-Harvesting Antenna of Plants

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ABSTRACT In the major peripheral plant light-harvesting complex LHCII, excitation energy is transferred between chlorophylls along an energetic cascade before it is transmitted further into the photosynthetic assembly to be converted into chemical energy. The efficiency of these energy transfer processes involves a complicated interplay of pigment-protein structural reorganization and protein dynamic disorder, and the system must stay robust within the fluctuating protein environment. The final, lowest energy site has been proposed to exist within a trimeric excitonically coupled chlorophyll (Chl) cluster, comprising Chls a610-a611-a612. We studied an LHCII monomer with a site-specific mutation resulting in the loss of Chls a611 and a612, and find that this mutant exhibits two predominant overlapping fluorescence bands. From a combination of bulk measurements, single-molecule fluorescence characterization, and modeling, we propose the two fluorescence bands originate from differing conditions of exciton delocalization and localization realized in the mutant. Disruption of the excitonically coupled terminal emitter Chl trimer results in an increased sensitivity of the excited state energy landscape to the disorder induced by the protein conformations. Consequently, the mutant demonstrates a loss of energy transfer efficiency. On the contrary, in the wild-type complex, the strong resonance coupling and correspondingly high degree of excitation delocalization within the Chls a610-a611-a612 cluster dampens the influence of the environment and ensures optimal communication with neighboring pigments. These results indicate that the terminal emitter trimer is thus an essential design principle for maintaining the efficient light-harvesting function of LHCII in the presence of protein disorder.

INTRODUCTION

Photosynthesis begins with solar light absorption by a light-harvesting antenna, followed by energy transfer to a reaction center where charge separation occurs (1–5). The antennae pigment-protein complexes play a dual role by both funneling excitation energy to the reaction center and protecting the organism from the deleterious effects of excess sunlight (6–9). The interchanging light-harvesting and photoregulation functionalities proceed in cooperation with structural reorganization and dynamic disorder in the pigment protein complexes.

In higher plants and algae, the major antenna is the light-harvesting complex LHCII, which is trimeric in its native form. The high-resolution x-ray structure of the LHCII trimer has been obtained at 2.5 to 2.72 Å (10,11). Each monomeric unit contains 8 Chls a, 6 Chls b, and 4 xanthophylls (one neoxanthin, two luteins, and one violaxanthin or zeaxanthin, depending on light conditions). Protein structure and the composition of the binding sites play a significant role in establishing the energy levels of the various Chls (12,13). They also determine relative orientations between the Chls, thus defining mutual resonance interaction between their excited states. This interaction leads to formation of a complex excitonic manifold (4,14,15). Upon photoexcitation, energy is transferred between the excitonic states along an energetic cascade. Over the years, structural (10,11,16), biochemical (17–21), and spectroscopic (22–27) studies have been combined with modeling to map the energy transfer processes in LHCII (28–32).

The structural studies indicate that the chlorophylls are arranged in two layers within the membrane, at the stromal and lumenal sides. Site-specific mutagenesis of pigment binding residues and subsequent protein reconstitution have further mapped the chlorophyll binding sites and substructural pigment spectral signatures (18–20). Novoderezhkin et al. (28,29) utilized a combined Redfield-Förster approach to simultaneously fit both the linear spectra and the transient absorption spectral evolution using the published structural information and an experimentally derived spectral density. The fit of the data also allowed estimation of the site energies for the Chls. Each monomeric subunit contains a group of three strongly excitonically coupled clusters at the stromal side, the Chls a602-a603,
a610-a611-a612, and b608-b609-b601’, where the b601’ pigment is from an adjacent monomeric unit. On the luminal side there are two clusters consisting of Chls a613-a614 and a604-b605-b606-b607. (Nomenclature is from Liu et al. (10).)

The lowest energy state of LHCII has been assigned to the Chls a610-a611-a612 cluster (18,19,28,33). In the PSII supercomplexes this terminal emitter domain is in direct contact with the Chls of either the core complex or of the minor antenna CP29, and is therefore directly involved in the energy transfer to the core (34,35). The same cluster minor antenna CP29, and is therefore directly involved in the energy transfer to the core (34,35). The same cluster minor antenna CP29, and is therefore directly involved in the energy transfer to the core (34,35). The same cluster minor antenna CP29, and is therefore directly involved in the energy transfer to the core (34,35). The same cluster minor antenna CP29, and is therefore directly involved in the energy transfer to the core (34,35). The same cluster

The LHCII-A2 mutant, lacking the ligand for Chl a612, (N/83L, named A2 according to previous nomenclature (42)), does not bind the Chls a611 and a612, thus altering the terminal emitter domain. Previous work on this mutant demonstrated loss of absorbance on the red side of the Qy band and a blue-shift of the room temperature fluorescence (18–20). Circular dichroism and modeling studies further concluded that the arrangement of the remaining chlorophylls and the interactions between them in the monomeric LHCII-A2 are unperturbed relative to the LHCII-WT (43).

We report in this article further investigation of fluorescence characteristics of the reconstituted LHCII-A2 monomer. We find that this mutant exhibits two overlapping fluorescence bands, which are in turn temperature dependent in relative ratio. The higher energy (bluer) fluorescence peak corresponds to the previously described room temperature (RT) fluorescence emission. A second emission feature appears at the lower energy (redder) edge of the emission band in low temperature bulk fluorescence experiments and is reported in this study, to our knowledge, for the first time. This feature is furthermore consistent with the disorder dependent appearance of red-shifted fluorescence peaks in SMS experiments (44). We model the steady-state fluorescence properties of an LHCII monomer lacking Chls a611 and a612 using established methods (28,45) and find good agreement with our experimental results. Based on this agreement, we propose that the two fluorescence bands originate from differing protein conformations, which are interchangeable at higher temperatures. These conformations furthermore result in varying excitonic energy level populations. The higher energy emission originates primarily from Chls a602-a603. The previously unobserved fluorescence component is assigned to a low energy and relatively low oscillator strength exciton localized on Chl a610.

MATERIALS AND METHODS

Samples

LHCII-WT and LHCII-A2 apoproteins were prepared using the Lhcb1.3 gene (AT1G29930) as described in Caffarri et al. (46). The mutant sequence was obtained by site directed mutagenesis replacing the Chl 612 ligand, asparagine 183, with leucine, a residue that cannot coordinate the central magnesium of the Chl. WT and A2 apoproteins were produced by expressing them in Rosetta2DE3. The pigment-protein complexes were reconstituted and purified as described in detail in Natali et al. (47) using purified pigments extracted from plants with a Chl a/b ratio of 3. The pigment composition of the obtained proteins was analyzed by HPLC and fitting of the acetone extract (48).

Characterization

Steady-state absorption was done on a Perkin-Elmer Lambda40 spectrophotometer and steady-state fluorescence was recorded on a Fluorolog-3 spectrophotometer and the excitation and emission slits widths were 2 nm (HRIBA Jobin-Yvon, Edison, NJ). Temperature dependent measurements were done using an Oxford cryostat (DN1704) and temperature controller. Samples were in 25 mM Hepes buffer (0.06% β-DM, 1 mM MgCl, pH 7.8) with an OD of 0.1 at the Qy absorption band.

Single-molecule spectroscopy

The fluorescence emission of single LHCII monomers was investigated via a home-built confocal microscope as described earlier (44,49). The complexes were excited at 632.8 nm by a CW He-Ne laser (JDS Uniphase, Eindhoven, The Netherlands) with an excitation power of 1 μW corresponding to a diffraction-limited focal irradiance of ~500 W/cm². This corresponds to an average absorption rate of one photon per μs per complex. The probability of having two excitations in one complex during one pulse is below 0.01% according to the Poisson distribution of absorbed photons. Therefore, singlet-singlet annihilation effects can be neglected. The high Chl triplet yield of ~30%, however, leads to the formation of carotenoid triplets with a lifetime of 9 μs and results in a strong singlet-triplet annihilation regime. Assuming a fraction of 70% of the excitations being annihilated with an annihilation time of 35 ps, <5% of the detected photon counts in a fluorescence emission spectrum originate from the annihilation regime.

Near-circular polarized light was obtained by means of a Berek polarization compensator (5540 New Focus, Santa Clara, CA). Thirty consecutive fluorescence spectra, each with an integration time of 1 s, were obtained by dispersing the fluorescence light via a grating (Optometrics LLC, HR830/800nm, Ayer, MA) onto a CCD camera (Roper Scientific, Spec10:100BR, Trenton, NJ). The particles were diluted down to pM concentrations in a 25 mM Hepes buffer (0.03% β-DM, 1 mM MgCl, pH 7.8) and attached to PLL (poly-L-Lysine, Sigma Aldrich) coated microscope cover slips. The immobilized particles were flushed with an oxygen scavenger system containing 750 μg/ml Pyranose Oxidase, 100 μg/ml Catalase, and 7.5 mg/ml Glucose (all from Sigma). The removal of oxygen prevented the formation of singlet oxygen and therefore significantly improved the photostability of complexes. Furthermore, the temperature within the closed sample chamber was controlled and set to 278K, which further prolonged the average survival time of fluorescent particles. The maximum spectral acquisition time was 30 s per complex. All particles with the
exception of clearly denatured particles (blue-shifted emission of <670 nm) were fitted with a single-skewed Gaussian in Matlab (The MathWorks, Natick, MA) as described previously (44) and further analyzed in OriginPro. In LHCII-WT 90% of particles could be used for analysis, contrary to 75% in LHCII-A2, which is an indication of the decreased stability of the mutant.

**RESULTS**

**Pigment analysis**

The pigment composition of the different samples was measured and is indicated in Table 1. In reconstituted complexes, the Chl a/b ratio depends on the pigment mixture utilized during sample preparation. Therefore, the changes in LHCII-A2 are calculated relative to the LHCII-WT that is reconstituted from the same pigment mixture. Considering that the mutation does not lead to loss of carotenoids, our results suggest the loss of at least two Chls a in LHCII-A2, relative to LHCII-WT, in agreement with previous work (17,18). One of these is assigned to Chl a612, as in the mutant the ligand for this Chl (asparagine) was substituted with an amino acid (leucine) that cannot coordinate the central Mg of the Chl. The second Chl lost is Chl a611, which in the WT interacts strongly with Chl a612 and does not have a protein ligand. For more details on these assignments, see Mozzo et al. and Georgakopolou et al. (17,43). The composition is consistent across different reconstitutions.

**Steady-state absorption**

The steady-state absorption of LHCII-WT and LHCII-A2 is shown in Fig. 1 and Fig. S1 in the Supporting Material for RT and 77 K conditions, respectively. The calculated difference spectra are also shown. The LHCII-A2 sample differs from the WT most notably in the 470 to 500 nm region and the red edge of the Qy band. The loss of absorption around 680 nm in LHCII-A2 relative to the WT is in accordance with the lack of the Chls a611 and a612 and in agreement with previous results (18). These Chls have site energies red-shifted with respect to the rest of the pigments (28,32,33) and consequently the excitonic states in which they participate absorb in the red edge of the LHCII-WT absorption spectrum (18,20). There is a relative increase at 487 nm in the LHCII-A2 absorption, which is also consistent with previous work. This increase has been attributed to a combination of hypochromism and bathochromic shift in the absorption of the carotenoid Lut 620, because of the loss of excitonic coupling between this pigment and the Chls a611 and a612 (17). Although this region corresponds to the absorption of all of the carotenoids in the complex, the mutation was shown to have a very local effect in the overall pigment arrangement (43). Therefore, the assignment to a Lut 620 absorption shift remains the most likely explanation. The LHCII-A2 relative absorption reduction at

**TABLE 1 Pigment composition of samples as determined by acetone extract fitting**

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Chl a/Chl b</th>
<th>Chl/Car</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHCII-WT</td>
<td>1.69 ± 0.05</td>
<td>4.73 ± 0.05</td>
</tr>
<tr>
<td>LHCII-A2</td>
<td>1.31 ± 0.06</td>
<td>3.82 ± 0.08</td>
</tr>
</tbody>
</table>

**Modeling**

The modeling is based on the Hamiltonian and spectral densities used in previously reported work (28,29) with the parameters adjusted to fit simultaneously the spectra of LHCII-WT and LHCII-A2. The fluorescence spectra are calculated from populations Pi of the steady state calculated by the kinetic equations as follows:

\[
\frac{\partial P_i}{\partial t} = \sum_j k_{ij} P_j - \Gamma_i P_i + (P_0 - P_i) \int d\omega W(\omega) \chi_i(\omega),
\]

where \(k_{ij}\) are the population transfer rates, \(\Gamma_i\) are the population relaxation rates, \(\chi_i(\omega)\) is the absorption spectrum of the \(i\)-th exciton, and \(W(\omega)\) is the spectrum of incident light (50).

These equations can be rigorously derived from the Liouville-von Neumann equation for the reduced density matrix using second-order perturbation theory for the interaction with light, the secular approximation, and the fact that populations vary much slower than optical coherences (time-independent population transfer rates). Under these assumptions these equations have general validity and allow calculation of excitation dynamics.

The excitonic absorption spectrum is given by the transition dipole strength, which can be calculated from the structure and coupling between pigments, and the calculated lineshape. The lineshape and population transfer rates are given by the bath properties, which are expressed by means of the spectral density. The absorption and fluorescence spectra are then calculated as follows (45,51):

\[
\text{ABS}(\omega) \propto \omega \sum_i |\mu_{i0}|^2 2\text{Re} \int_0^\infty d\tau \ e^{i(\omega-\omega_0)\tau} g_i(\tau) \frac{\Gamma_i}{2}\tau.
\]

\[
\text{FL}(\omega) \propto \omega^3 \sum_i P_i |\mu_{i0}|^2 2\text{Re} \int_0^\infty d\tau e^{i(\omega-\omega_0+2\omega_l)\tau} g_i(\tau) \frac{\Gamma_i}{2}\tau.
\]

Here, \(|\mu_{i0}|^2\) is the transition dipole moment length for the \(i\)-th exciton, \(\omega_0\) is the optical transition frequency of the \(i\)-th exciton, and \(g_i(\tau) = \sum_n |c_{i0}|^2 g_n(\tau)\) is the lineshape function of the \(i\)-th exciton (\(c_{i0}\) are coefficients of the site \(i\) exciton basis transformation, and \(|c_{i0}|^2\) is then the participation of the \(n\)-th site in the \(i\)-th exciton). The lineshape function of the \(n\)-th site is given by \(g_n(\tau) = \int_0^\infty dt \int_0^\infty dt' C_n(t,t');\) where \(C_n(t)\) is the bath correlation function related to the spectral density by Fourier transform (45). We assume the same but uncorrelated bath oscillations on all the different sites here. The reorganization energy of the \(i\)-th exciton is given by \(\lambda_i = \sum_i |c_{i0}|^2 \lambda_o\) where \(\lambda_o = (1/\pi) \int_0^\infty d\omega \text{Re}(C_n(\omega)/\omega)\) is the reorganization energy of the \(n\)-th site and \(C_n(\omega)\) is the bath spectral density. The dependence of the excitation reorganization energy on the participation coefficients \(|c_{i0}|^2\) leads to the difference between reorganization energies assigned to low energy states of the LHCII-WT and LHCII-A2, with increased delocalization leading to smaller reorganization energy.

Based on the crystal structure determined by Liu et al. (10), the excitonic coupling between pigments is calculated in the dipole-dipole approximation. Effective dipole strengths used were 4 D for Chl a and 3.4 D for Chl b (for definition, see Novoderezhkin et al. (32)), and the spectral density was taken from previous work (53). The Chl orientations were taken into account, and the Mg-Mg distances were used for dipole distances. The Chl population lifetime is taken to be 3 ns and the light intensity is, in accordance with the experimental conditions of the ensemble measurements, chosen to be arbitrarily small so as to ensure a nonannihilation regime.
650 nm in the 77K measurements is attributed to the loss of a small amount of Chl b, made clearer because of the band narrowing at low temperature (Fig. S1). The concomitant absorption differences around 472 nm may also be explained by the same reasoning, although since all the pigments have some absorption at this wavelength, it is difficult to assign this change conclusively.

Steady-state fluorescence

Temperature dependent steady-state emission spectra of LHCII-WT and LHCII-A2 samples of equivalent OD are shown in Figs. S2 and 2, respectively, for λex = 600 nm. The fluorescence emission of the reconstituted LHCII-WT monomer exhibits a peak at 681 nm at 290 K and 679 nm at 77 K, in agreement with literature reports of isolated native complexes (24,54). The emission peak narrows with lowering temperature. LHCII-A2 exhibits a fluorescence peak at 677 nm at 290 K. This 4 nm blue-shift relative to LHCII-WT is in agreement with earlier studies of this mutant at this temperature (18,20). The mutant also shows an increase in intensity of the vibronic band centered at 735 nm, relative to the primary emission. With lowering temperature, the emission of LHCII-A2 broadens, particularly on the redder side of the fluorescence band, differing significantly from the behavior of LHCII-WT. Furthermore, whereas the vibronic band of LHCII-WT narrows with lowering temperature, that of LHCII-A2 does not narrow. The fluorescence quantum yield of LHCII-A2 is quenched with respect to LHCII-WT, and the amount of quenching increases from 30% at RT to 50% at 77 K.

The fluorescence emission lineshape of LHCII-A2 at 77 K is dependent on excitation wavelength (Fig. 3). Particularly with excitation λex = 475 nm, two emission peaks are observed at 674 and 684 nm. In contrast, LHCII-WT spectra do not show such a change in lineshape under the same conditions (Fig. S3). These steady-state and temperature-dependent measurements suggest that the mutant exhibits two overlapping fluorescence bands in bulk measurements.

Single molecule spectroscopy

SMS of LHCII-A2 and LHCII-WT was conducted to further elucidate the bulk fluorescence characteristics of LHCII-A2. Continuous illumination of surface-attached single monomers at λex = 633 nm allowed measurement of the fluorescence dynamics over tens of seconds. The sample temperature was maintained at 278 K. LHCII-WT particles exhibit a constant narrow (FWHM ≈ 18 nm) emission centered at 681 nm (Fig. S4). The exact peak position varies because of static disorder altering the steady-state exciton
composition. These results are consistent with SMS of LHCII-WT trimers isolated from spinach (44). Additionally, ~3% of the LHCII-WT particles exhibit switching behavior to redder emitting states at and above 700 nm, also in agreement with previous results on isolated LHCII trimers (44). SMS of LHCII-A2 demonstrated lower fluorescence intensity than LHCII-WT, which is consistent with the bulk measurements. The average spectra of all measured single particles furthermore match the bulk steady-state emission spectra for both the LHCII-WT and LHCII-A2 (Fig. S5). The mutant particles exhibited spectral features of four general profiles (Fig. 4). The first category (a) represents particles that give a narrow (FWHM ≈ 18 nm) emission band centered at 678 nm, in agreement with the peak emission of LHCII-A2 in bulk measurements at RT. The second category (b) consists of particles with a broadened spectrum (FWHM up to 27 nm) and a red-shifted peak wavelength up to 684 nm. Accounting for shifts because of temperature, this center wavelength falls between the two emission peaks resolved at 77 K in bulk LHCII-A2 measurements. The third profile (c) is associated with particles that demonstrate switching between profiles (a) and (b). About half of all the measured particles fall into category (a) and the second half of particles with broadened spectra include a fraction of ~5% of the sampled particles that exhibit the switching feature of category (c). Finally, ~3% of all particles emit at and above 700 nm, analogous to that seen in the WT sample and depicted in profile (d).

The measured SMS spectra were each fit with a single-skewed Gaussian after subtracting the vibronic contribution (Fig. S6). The spectral peak width distribution shows the FWHM of these fits versus fluorescence peak position (Fig. 5). This allows us a statistical overview of our data sample. In LHCII-WT, the peak width remains constant, across the emission wavelength. LHCII-A2, however, exhibits narrower emission peaks for bluer peak positions and a trend toward broader peaks at redder wavelengths. There is also no evidence of a narrow emission band from LHCII-A2 at the longer wavelengths. This observation, in addition to the switching behavior observed in profile (c) (Fig. 4), suggests that both of the overlapping emissive bands observed in the bulk LHCII-A2 measurements are originating from single complexes and are thus not due to separate subpopulations of a heterogeneous sample preparation. These two emission bands are furthermore present at all temperatures.

DISCUSSION

The LHCII-A2 mutant has been previously shown to lose Chls emitting and absorbing near the red edge of the spectrum. These were identified as the Chls a611 and a612, members of the strongly excitonically coupled cluster forming the terminal emitter in LHCII. The pigment composition as well as steady-state absorption at RT and 77 K of the LHCII-A2 preparation used in this work match previous reports (17–20,43), and the observed 4 nm blue-shift of the LHCII-A2 fluorescence peak relative to LHCII-WT at RT is also in agreement with earlier studies (18,20). The experiments and simulations presented in this study are fully consistent with the disruption of excitonic states involving Chls a610, a611, and a612 (28,33). The comparison of
mutated and WT LHCII systems thus provides us with a framework to study the role of excitonic delocalization in establishing efficient excitation energy transfer pathways within and out of LHCII. The primary goal in this work is to elucidate the nature of the LHCII-A2 monomer emission and in so doing establish the functional role that the excitonic trimer Chl \(a_{610-611-612}\) plays within the WT complex.

Our experimental results show that LHCII-A2 exhibits a broader primary fluorescence band than LHCII-WT, an increase in relative intensity of the vibronic band at 735 nm, and a reduced quantum yield of fluorescence. Additionally, at 77 K, the LHCII-A2 emission resolves into a lineshape suggestive of two overlapping fluorescence bands with peaks at 674 and 684 nm. This indicates that the absence of Chls 611 and 612, which has a local effect on the complex structure, has a more drastic effect on the energy distribution in the system.

The samples used in this study were prepared by pigment-protein reconstitution, a well-established biochemical technique for photosynthetic proteins (47,55). The earlier circular dichroism modeling on LHCII-A2 finds that the mutation results in minimal changes to the overall organization of the remaining pigments and consequently of the relevant protein structure (43). Moreover, recent in vivo mutation analysis of minor light-harvesting complexes has shown that reconstitution produces complexes with the same properties as complexes from plants, even in case of mutants (56). However, the reproducibility and homogeneity of samples prepared by this in vitro process should be carefully considered, as it is critical for the reliability of the conclusions we draw from the comparison between LHCII-WT and LHCII-A2. The emission behavior reported in this article was observed in multiple sample preparations, with small differences attributable to slightly differing pigment ratios in the reconstitution. It is thus reasonable to conclude that the observed specific emission features are reproducible and inherent characteristics of the LHCII-A2 monomer.

Bulk measurements provide only averaged information about the energy transfer properties of the studied system. SMS results give further insight into the emissive behavior of individual complexes forming the studied ensemble. The sampling population of type \(d\) in LHCII-A2 (Fig. 4) matches the red-shifted emission behavior observed in both the LHCII-WT and in isolated LHCII trimers (44). This supports earlier conclusions that this far-red emission is not originating from the terminal emitter trimer. Regarding the emission of LHCII-A2, the sampling population of type \(c\) demonstrates switching between the narrow blue emission of population type \(a\) and the broad red emission of population type \(b\), which indicates that varying fluorescence profiles occur within single particles (Fig. 4). The experimental peak width versus emission wavelength distribution shows no evidence for narrow emission bands from the red edge of the LHCII-A2 fluorescence (Fig. 5). The SMS results thus demonstrate that the overlapping fluorescence bands observed in the bulk measurements of LHCII-A2 are not because of a heterogeneous sample preparation. Varying emission profiles of single light-harvesting complexes have been observed previously in bacterial systems, and were therein attributed to fluctuations in the electron-phonon coupling because of differing protein conformation and concomitantly exciton localization (57). We analogously assign the varying LHCII-A2 emission profiles to a change in the excitonic manifold of the mutant, resulting in altered energy transfer and distribution relative to LHCII-WT. Considering again the temperature dependent bulk measurements, the population of these two bands is furthermore dependent on excitation wavelength at 77 K (Fig. 3). This result, combined with the SMS experiments, suggests either that the system is not fully equilibrated at 77 K, and/or that there is a significant change in absorption cross section of the realizations associated with the two emissive bands.

To explore this further, we have modeled the steady-state spectral properties of LHCII-A2. Previous work reported a model of the excitonic manifold, including site energies and energy transfer pathways, in LHCII trimers and monomers based on a quantitative fit of linear and transient absorption spectra using structural information from crystallography studies (28,29). We have applied this model to this study. The initial set of site energies was from the work of Novoderezhkin et al. (29). The temperature dependence was incorporated only through the spectral density, i.e., through the lineshapes and energy transfer rates. LHCII-A2 is considered to have the same structure as LHCII-WT, but lacking the Chls 611 and 612, as supported by the previously discussed experimental evidence (43). As we wanted the simplest model that explained our results, we chose not to alter the LHCII-A2 site energies relative to LHCII-WT. This allows for a simultaneous fit of the absorption and fluorescence spectra of both LHCII-WT and LHCII-A2 at RT and 77 K with the same set of parameters. All simulations are averaged over 110 cm\(^{-1}\) FWHM Gaussian disorder on all the site energies, and the fluorescence spectra are calculated for 650 nm illumination. Since the reconstituted samples are in the monomeric state, the Chl \(b601^+\) is not associated with the \(b608\) as in the trimer model. Further details on the theory and input parameters are presented in the Materials and Methods section.

Both the LHCII-WT and LHCII-A2 spectra were fitted and, from the diagonalization of the Hamiltonian with the resulting site energies (Table S1), excitonic contributions to the spectra were calculated at 290 and 77 K. These are shown in Fig. 6 for LHCII-A2 and Fig. S7 for LHCII-WT. The steady-state spectral lineshapes of LHCII-A2 and LHCII-WT are both reasonably well reproduced. The origins of the absorption and emission lineshapes can be
elucidated by considering the energies of the excitonic states and the participation of the Chls in these states, shown for the lowest six energy levels in Table S2. The differences between the excitonic energy levels of the two samples manifest predominantly in the lowest six levels. This is reasonable as these correspond as well to the excitonic levels with altered participation of Chl $a_{610}$, the only remaining pigment of the disrupted terminal emitter trimer in LHCII-A2. Fig. 7 illustrates the differences between the lowest energy states of the LHCII-A2 and LHCII-WT excitonic manifolds. In LHCII-WT the primary contribution to the fluorescence, at both RT and 77 K, is from the lowest excitonic state of Chl $a_{610-a611-a612}$ terminal emitter trimer, which furthermore has a high oscillator strength because of oscillator strength borrowing facilitated by the large exciton delocalization (an effect known as superradiance). In LHCII-A2 we find that the lowest two excitonic levels contribute strongly to the fluorescence. The bluer of these two, denoted A2-02 (see Table S2 for the exciton notation) is composed mostly of Chl $a_{602-a603}$, and also has high oscillator strength, agreeing with its analog in LHCII-WT, exciton WT-03. The redder emission (A2-01) originates primarily from Chl $a_{610}$, which is now less coupled to other Chls and consequently demonstrates a much reduced oscillator strength because of the loss of superradiance. From the modeling, the fluorescence quantum yield of LHCII-A2 is reduced 23% at room temperature and 36% at 77 K relative to LHCII-WT, supporting the experimentally observed fluorescence quenching of the mutant. Furthermore, the A2-01 emission is redder than that observed for WT-01. In LHCII-A2, A2-01 is localized on Chl $a_{610}$, resulting in an increased reorganization energy with respect to the delocalized WT-01 exciton, and subsequently a greater Stokes shift. The modeling also suggests that the broader 735 nm band results from overlapping vibronic contributions from A2-01 and A2-02. Additionally, the localized $a_{610}$ state exhibits increased electron-phonon coupling as well as larger inhomogeneous broadening. This effect is the straightforward consequence of the change in delocalization as demonstrated by the equations for the reorganization energy of a delocalized excitonic state in the Materials and Methods section. These factors also contribute to the higher relative intensity and broadened lineshape of the 735 nm vibronic band in LHCII-A2 as compared with the analogous ratio in the LHCII-WT spectrum.

We also compared the modeling results with that of the SMS experiments. The timescale of the switching observed in LHCII-A2 is seconds, which means that the protein-pigment conformation responsible for switching process can be regarded static for the duration of the absorption-emission cycle (few ns). The modeling of the SMS
The three lowest exciton levels, all within the Chls energy gap between the lowest exciton levels. In LHCII-WT a trimer in the WT thus ensures good connectivity to neighboring pigments, as well as stabilized and optimized energy levels because of the reduced influence of static disorder, as evidenced by the simulations of single realizations of disorder. The increased sensitivity to the dynamic protein disorder in LHCII-A2 highlights another important design principle in the native complex. In the mutant, the site energy of the decoupled Chl a610 varies because of the disorder, resulting in unfavorable situations wherein excitations populate mainly Chl a602. Our modeling results show that this happens roughly one-third of the time. In the WT, the lowest exciton, and thus final population, is almost always on the Chl a610-a611-a612 trimer. Additionally, in the native complex, the terminal emitter must be able to back transfer excitation energy in the event that a suitable neighboring antenna is not available to accept transfer out of the LHCII. This requires that the Chls a610-a611-a612 cluster is well connected to the Chls a602-a603 cluster. In LHCII-A2, however, for some realizations of the disorder, excitations that populate Chl a610 will become trapped because of the above-mentioned increased energy gap between the lowest exciton levels in the mutant. The stabilized energetic structure of the terminal emitter trimer in the WT is thus essential for the necessary energy migration within and between the monomeric subunits of an LHCII trimer.

In this work we have demonstrated that disrupting the terminal emitter trimer in LHCII-A2 results in emissive features consistent with a loss of energy transfer robustness and the average efficiency because of an increased sensitivity of the excited state energy landscape to the disorder induced by the protein conformations. In light of these results, the existence of the terminal emitter trimer with strong resonance coupling and a correspondingly high degree of excitation delocalization is found to be an essential design principle of LHCII functional structure.
**AUTHOR CONTRIBUTIONS**

R.C. and R.v.G. designed the research. C.R., J.M.G., and M.N. performed the experiments. C.R., J.M.G., and T.P.J.K. analyzed the data. V.N., T.M. and P.M. designed the modeling, and P.M. carried out the modeling calculations.

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**REFERENCES**


Supporting Material

**Figure S1.** 77K steady state absorption spectra of LHCII-WT, LHCII-A2 and the calculated difference.

**Figure S2.** Temperature dependent steady-state emission of WT, $\lambda_{ex} = 600$ nm.

**Figure S3.** 77 K emission spectra of LHCII-WT at various excitation wavelengths exhibit the same lineshape. Dashed line indicates peak at 679 nm.
**Figure S4.** Fluorescence spectral traces of single WT complexes at 278 K with continuous illumination at $\lambda_{ex} = 633$ nm exhibits two primary profiles: a,b) narrow emission at 681 nm and c,d) blinking behavior to red emitting states. Top profiles correspond to selected 1 second time bins.

**Figure S5.** Comparison of ensemble fluorescence emission spectra and the average from 300 individually measured single particle spectra of a) LHCII-WT and b) LHCII-A2. The blue edge of the ensemble spectrum measured on the confocal single molecule setup is cut off due to the dichroic beam splitter and the fluorescence filter.
Figure S6. Examples of single particle spectra (1 s acquisition time) fit to a skewed Gaussian. FWHM of the Gaussians were used to build Figure 5 in the main text.

Figure S7. Modeling (solid line) as compared to experimental results (dotted line) of LHCII-WT absorption and emission at 290K and 77K.
Figure S8. Single realizations of the modeled exciton composition of LHCII-A2 corroborate SMS results (Figure 5, main text.)
Table S1. Full Hamiltonian used for simulations. Off-diagonal elements are pigment interaction energies and on-diagonal elements are the pigment site energies. All values are in cm\(^{-1}\).

| Chl  | \(a602\) | \(a603\) | \(a610\) | \(a611\) | \(a612\) | \(b608\) | \(b609\) | \(b601\) | \(a613\) | \(a614\) | \(a604\) | \(b605\) | \(b606\) | \(b607\) |
|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| \(a602\) | 15188   | 35.95   | -11.7   | 9.55    | 16.1    | -6.18   | -19.6   | 52.4    | -5.56   | 0.94    | 6.45    | -0.73   | 5.69    | 7.32    |
| \(a605\) | 15315   | 13.2    | -2.52   | -0.91   | 7.81    | 98.0    | -6.20   | 1.86    | -6.79   | -3.74   | 1.24    | -9.28   | 1.53    |
| \(a610\) | 15138   | -25.7   | 24.6    | 62.2    | 4.37    | -6.08   | 7.33    | -1.65   | -4.28   | 1.61    | -3.38   | -0.13   |
| \(a611\) | 15232   | 137.9   | 4.43    | 4.52    | 26.73   | -6.09   | -4.41   | -3.96   | 1.40    | -2.62   | -2.91   |
| \(a612\) | 15145   | -1.10   | -2.80   | 9.36    | -0.68   | -0.01   | 5.06    | -2.98   | 3.25    | 3.21    |
| \(b601\) | 15678   | 39.0    | 2.93    | -1.98   | 1.37    | -2.88   | 5.36    | 5.22    | 4.82    |
| \(b602\) | 15618   | 4.03    | -2.95   | 2.40    | -7.48   | -0.84   | 0.54    | -12.3   |
| \(b603\) | 15883   | -10.8   | 3.56    | -2.60   | 0.81    | -1.99   | -2.62   |
| \(a613\) | 15336   | -53.8   | 1.96    | -1.46   | 1.39    | 2.05    |
| \(a614\) | 15337   | -3.48   | 0.40    | -2.25   | -3.41   |
| \(a604\) | 15508   | 3.61    | 112.3   | 37.1    |
| \(b605\) | 15660   | 33.0    | -4.51   |
| \(b606\) | 15771   | 61.0    |
| \(b607\) | 15601   |

Table S2. Energies of six lowest excitonic states and disorder averaged participation of the pigments in these states for \(a)\) LHCII-WT and \(b)\) LHCII-A2.

\(\begin{array}{|c|c|c|c|c|c|}
\hline
\text{Energy (cm}^{-1}\text{)} & WT-01 & WT-02 & WT-03 & WT-04 & WT-05 & WT-06 \\
\hline
\text{Oscillator Strength} & 30.36 & 14.34 & 27.87 & 8.86 & 7.10 & 4.37 \\
\hline
\text{a602} & 0.03 & 0.28 & 0.49 & 0.14 & 0.05 & 0.00 \\
\text{a603} & 0.00 & 0.05 & 0.13 & 0.51 & 0.26 & 0.02 \\
\text{a610} & 0.21 & 0.45 & 0.30 & 0.02 & 0.00 & 0.01 \\
\text{a611} & 0.27 & 0.08 & 0.02 & 0.20 & 0.42 & 0.00 \\
\text{a612} & 0.48 & 0.13 & 0.04 & 0.12 & 0.23 & 0.00 \\
\text{b608} & 0.00 & 0.01 & 0.01 & 0.00 & 0.00 & 0.70 \\
\text{b609} & 0.00 & 0.00 & 0.01 & 0.02 & 0.02 & 0.27 \\
\text{b601} & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 \\
\hline
\text{b) A2-01} & A2-02 & A2-03 & A2-04 & A2-05 & A2-06 & \\
\hline
\text{Energy (cm}^{-1}\text{)} & 14564.74 & 14785.76 & 14904.92 & 15256.05 & 15369.08 & 15308.21 \\
\hline
\text{Oscillator Strength} & 11.04 & 32.42 & 13.63 & 4.09 & 12.46 & 9.04 \\
\hline
\text{a602} & 0.31 & 0.52 & 0.17 & 0.00 & 0.00 & 0.01 \\
\text{a603} & 0.06 & 0.12 & 0.76 & 0.02 & 0.03 & 0.00 \\
\text{a610} & 0.63 & 0.34 & 0.02 & 0.01 & 0.00 & 0.00 \\
\text{b608} & 0.01 & 0.01 & 0.00 & 0.65 & 0.32 & 0.01 \\
\text{b609} & 0.00 & 0.01 & 0.02 & 0.31 & 0.58 & 0.05 \\
\text{b601} & 0.00 & 0.00 & 0.00 & 0.07 & 0.92 & 15
\end{array}\)