

Dynamic LHCI antenna and inter-photosystem spillover enable flexible light harvesting in *Euglena gracilis*

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SUMMARY

Photosynthetic light-harvesting antenna systems of aquatic algae show remarkable evolutionary diversity. *Euglena gracilis*, a unicellular flagellate that acquired its chloroplast through secondary endosymbiosis with a green alga, exemplifies evolutionary innovation in light harvesting. *E. gracilis* possesses a distinctive photosynthetic apparatus with loosely stacked thylakoid membranes and atypical light-harvesting complexes (LHCs). Previous studies proposed shared antenna systems serving both photosystems I and II (PSI and PSII) and inter-photosystem excitation energy transfer (spillover) as a regulatory mechanism. Here, we investigate the mechanisms and dynamics of light harvesting in isolated photosynthetic protein complexes and whole cells of *E. gracilis* by absorption, circular dichroism, fluorescence, and time-resolved fluorescence spectroscopy. Isolated PSII and PSI–LHCI were found to have spectroscopic characteristics distinct from their green-lineage counterparts. PSI–LHCI showed exceptionally long excitation lifetimes of 100–200 ps, resulting from energy transfer through a large mosaic LHCI antenna. Time-resolved fluorescence of intact cells and thylakoid membranes revealed a characteristic 50 ps energy transfer component at room temperature from PSII-associated antenna to PSI-associated red chlorophyll forms, providing direct evidence for prevalent spillover *in vivo*. The mobile LHC complex, previously thought to contain both LHCI and LHCII, comprises exclusively multiple LHCI types and likely regulates excitation energy redistribution via spillover.

Keywords: photosynthesis, light harvesting, energy transfer, light-harvesting complex, photosystem, euglenophytes, microalgae, far-red light, energy spillover, *Euglena gracilis*.

INTRODUCTION

The core machinery of oxygenic photosynthesis, including the photochemical reaction centers (RCs) and electron transfer chains of photosystems I and II (PSI and PSII), is highly conserved (Oliver et al., 2023). In contrast, the light-harvesting antenna systems show much greater diversity (Li et al., 2025), reflecting adaptation to varying light environments (Suga & Shen, 2020). Most eukaryotic phototrophs employ LHC family proteins (Neilson & Durnford, 2010)—membrane-intrinsic pigment–protein complexes binding chlorophyll (Chl) and carotenoids (Cars). Although the overall LHC structure is conserved, variations in pigment composition, oligomeric organization, and photosystem association enable considerable evolutionary flexibility, especially in algal lineages (Bai et al., 2021).

Aquatic algae have evolved diverse strategies to thrive in complex light environments, including dynamic

excitation energy redistribution and chromatic adaptation using specialized pigments and LHCs. Green algae possess more flexible, less strictly partitioned antenna systems than land plants (Iwai et al., 2010; Takahashi et al., 2006). Phototrophic euglenids—and *Euglena gracilis* in particular—exemplify extreme evolutionary innovation in light harvesting. This unicellular flagellated alga acquired its chloroplast through secondary endosymbiosis with a green alga, resulting in a photosynthetic apparatus with pronounced mosaic character shaped by endosymbiotic and horizontal gene transfer. Consequently, *Euglena* combines green-lineage ancestry with lineage-specific structural, biochemical, and regulatory features. While its chloroplast genome has been sequenced, the nuclear genome remains only partially resolved owing to its large size and complex organization (Hallick et al., 1993).

Early studies suggested that *Euglena* lacks the canonical separation of PSI-associated LHCI and PSII-associated

LHCII. Winter and Brandt (1986) provided physiological evidence for a shared, functionally flexible antenna system serving both photosystems—the concept was reinforced by genetic and structural studies (Miranda-Astudillo et al., 2025). Comparative genomic analyses revealed that *E. gracilis* lacks a full complement of green-lineage LHCI proteins and instead possesses an expanded set of LHCII-related proteins, including multiple Lhcbm groups (Kozioł & Durnford, 2008). Many antenna proteins are encoded as large polyprotein precursors post-translationally cleaved into individual subunits—this suggests extensive functional reassignment following secondary endosymbiosis. The pigment composition further distinguishes *Euglena* from green algae and land plants: instead of lutein and violaxanthin, it employs a Car system dominated by diadinoxanthin, diatoxanthin, neoxanthin, and β -carotene (Doege et al., 2000).

Recent cryoelectron microscopy analyses of the PSI–LHCI supercomplex revealed a striking departure from the typical green-lineage architecture: a minimal PSI core of eight subunits (PsaA–F, PsaJ, and PsaM) surrounded by up to 16 asymmetrically arranged antenna proteins derived from Lhcbm/Lhcb-related clades rather than the Lhca group (Bai et al., 2026; Kato et al., 2025). This expanded PSI antenna binds diadinoxanthin and retains long-wavelength Chls, giving rise to pronounced far-red fluorescence emission (Brown, 1980)—a conserved feature despite the organism's green algal ancestry and atypical pigment composition.

Light harvesting regulation and photoprotection involve metabolic acclimatory mechanisms, including synthesis and accumulation of photoprotective pigments—carotenoids and flavonoids (Kato et al., 2017; Zheng et al., 2025). Euglenophytes can also switch to negative phototaxis under high light, actively evading excess radiation (Ogawa et al., 2016). Spectroscopic evidence of pigment-dependent control of excitation energy transfer (EET) and reversible non-photochemical quenching (NPQ) has been reported (Doege et al., 2000; Nagao et al., 2021). However, despite the presence of diatoxanthin, *Euglena* does not employ the xanthophyll cycle associated with NPQ (Casper-Lindley & Björkman, 1998). Euglenophytes also lack the pH-sensing NPQ effectors found in plants and green algae (PsbS and LHCSR, respectively), thus relying on different mechanisms. Doege et al. (2000) hypothesized spillover—EET from PSII to PSI—as a mechanism of dissipation of excess light and photoprotection.

Another intriguing feature is an oligomeric LHC assembly reported to contain both LHCI and LHCII with extended far-red absorption (Doege et al., 2000). It was proposed to participate in NPQ and dynamically associate with either PSI or PSII depending on light conditions, or interconnect the two photosystems mediating spillover (Miranda-Astudillo et al., 2025; Tschiersch et al., 2002). This

behavior could explain the shared, reconfigurable antenna system proposed by early studies and represents a regulatory strategy distinct from plant-type state transitions.

In this study, we analyze the spectral and kinetic properties of *E. gracilis* pigment–protein complexes *in vitro* and *in vivo*, and experimentally test the occurrence of PSII–PSI spillover. We isolate LHCII, PSII core complexes, and PSI–LHCI complexes and characterize them by absorption, fluorescence, circular dichroism (CD), and time-resolved fluorescence spectroscopy. The results reveal unique properties of both photosystems distinct from green-lineage counterparts. We find that LHCII is not an essential constituent of the far-red-absorbing oligomeric LHC by purifying an LHCI-only assembly carrying the far-red states. Analysis of isolated complexes enables unambiguous interpretation of time-resolved fluorescence data from intact cells and thylakoid membranes, demonstrating that spillover is indeed prevalent *in vivo*.

RESULTS

Fluorescence spectra and kinetics of intact cells and thylakoid membranes

Fluorescence spectra of cells and thylakoid membranes

Fluorescence emission spectra from intact *E. gracilis* cells at room temperature (RT) and 77 K are shown in Figure 1. RT spectra reflect thermally equilibrated Chl *a* pools in PSI and PSII with their light-harvesting antenna complexes. We compared the spectra recorded with two excitation wavelengths—440 nm (predominantly Chl *a*) and 480 nm (predominantly Chl *b*). Both wavelengths excite Cars as well. The spectra peak at 688 nm with a broad shoulder around 715 nm. The main fluorescence maximum represents Chl *a* from both photosystems, while extended far-red emission represents long-wavelength Chl *a* forms (“red” Chls) characteristic for PSI. The far-red shoulder is enhanced upon 440 versus 480 nm excitation. Since Chl *b* binds primarily to PSII, this difference confirms that far-red emission originates mainly from PSI and shows that the two photosystems are at least partially energetically separated.

At low temperatures (77 K), EET is mainly unidirectional—from higher to lower energy states, revealing terminal emitters in different pigment systems. The 77 K fluorescence spectra of intact cells peak at 735 nm with almost no emission below 690 nm. This contrasts strongly with higher plants and many algae, where PSII fluorescence at 685–695 nm is prominent at low temperature. The spectra are consistent with previous *Euglena* observations (Doege et al., 2000; Kanna et al., 2021). The lack of PSII emission can be interpreted two ways: either PSII has far-red emitting energy sinks or PSII transfers absorbed energy to PSI (spillover).

We recorded 77 K emission spectra of isolated thylakoid membranes to test whether the low PSII emission in

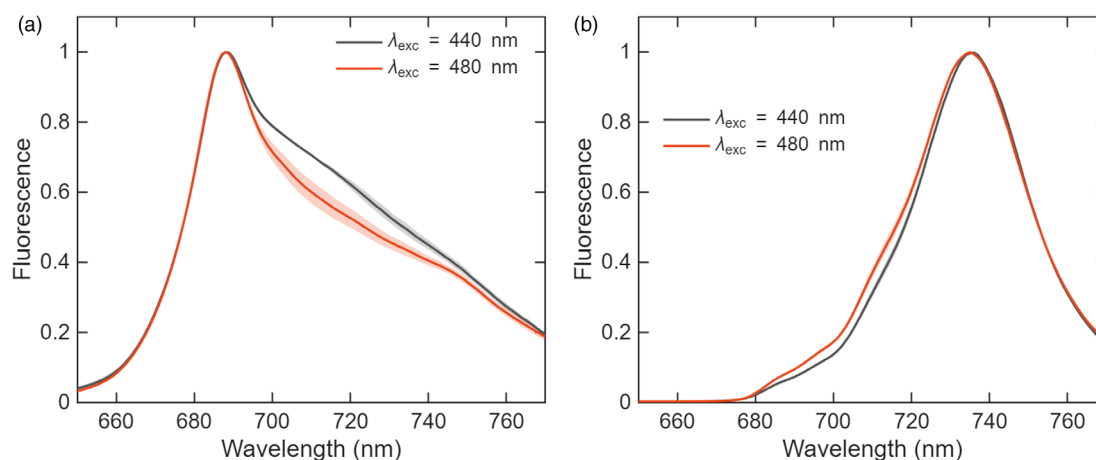


Figure 1. Fluorescence emission spectra of intact cells measured at RT (a) and 77 K (b) upon excitation at 440 nm and 480 nm. Spectra were normalized at 688 nm (RT) and 734 nm (77 K). Spectra represent the average of three independent replicates for RT and 10 replicates for 77 K measurements.

cells results from strong reabsorption. Thylakoid membranes were resuspended in low-salt buffer (avoiding artificial membrane stacking) and in buffer containing 0.03% β -DDM to break the macromolecular organization and inter-complex interactions. Membranes in low-salt buffer exhibit a broad emission band centered at 733 nm with a shoulder at 711 nm (Figure S1). The higher relative intensity at 711 nm compared to intact cells suggests that some LHCI complexes functionally disconnect from PSI upon thylakoid membrane isolation. Little difference appeared between spectra recorded at 440 and 480 nm excitation—the emission at 680–690 nm was negligible in both cases. In contrast, membranes in buffer with detergent had an emission maximum at 684 nm. The 684 nm band intensity increased further with 480 nm excitation relative to the 733 nm band—confirming the emission was mainly from PSII.

The supramolecular organization of PSII and PSI in granal chloroplasts produces three-dimensional pigment order with a specific strong CD signature, called “psi-type” CD (Garab & van Amerongen, 2009; Lambrev & Akhtar, 2019). CD spectra of isolated chloroplasts show a strong psi-type CD signal in the far-red region, with a prominent positive maximum at 695 nm and a negative maximum at 668 nm (Figure S2). The psi-type CD magnitude and spectral shape closely match those previously reported for intact *Euglena* cells (Kanna et al., 2021), indicating macroorganization is retained in isolated chloroplasts. Notably, this large CD signal appears despite the absence of grana stacking, demonstrating that long-range chiral organization does not require granal membrane architecture. Adding 0.03% β -DDM completely abolishes the psi-type CD, revealing the summed excitonic CD of pigment–protein complexes.

Time-resolved fluorescence spectroscopy at RT

Fluorescence decay kinetics of intact cells were measured with picosecond resolution using two excitation wavelengths—440 and 480 nm, preferentially exciting Chl *a* (PSI) and Chl *b* (PSII), respectively. Conditions maximizing either open PSII RCs (low-intensity excitation, fast sample circulation) or closed PSII RCs (high-intensity excitation, slow circulation with DCMU) were employed. Fluorescence decays recorded at emission wavelengths 664–744 nm under each experimental condition were subjected to global lifetime analysis with IRF deconvolution to obtain decay lifetimes and decay-associated emission spectra (DAES). Four kinetic components fit the experimental decays over a 4-ns time window. DAES for different experimental conditions are plotted in Figure 2.

The fastest component (~50 ps) exhibited DAES characteristic of EET. The positive maximum shows decaying Chl population emitting around 680 nm and the negative maximum shows corresponding rise of long-wavelength states emitting around 710 nm. Thus, the DAES strongly suggest that EET between these two Chl pools occurs with ~50 ps lifetime. Red Chls are typically associated with PSI, identifying acceptor states as belonging to the PSI antenna (LHCI). Notably, rise amplitudes are larger upon Chl *b* excitation (preferential PSII), compared to Chl *a* (PSI) excitation.

The remaining DAES had all-positive amplitudes, representing decay of different pigment groups. The second component (~220 ps) peaked at 705 nm in all but one experimental condition, with a long-wavelength tail to 730 nm. This component can confidently be assigned to PSI–LHCI despite the unusually long lifetime (for PSI), for two reasons: (1) red-shifted DAES suggests red Chl decay; (2) its amplitude is ~60% greater upon preferential PSI excitation (440 nm) versus PSII excitation (480 nm). However,

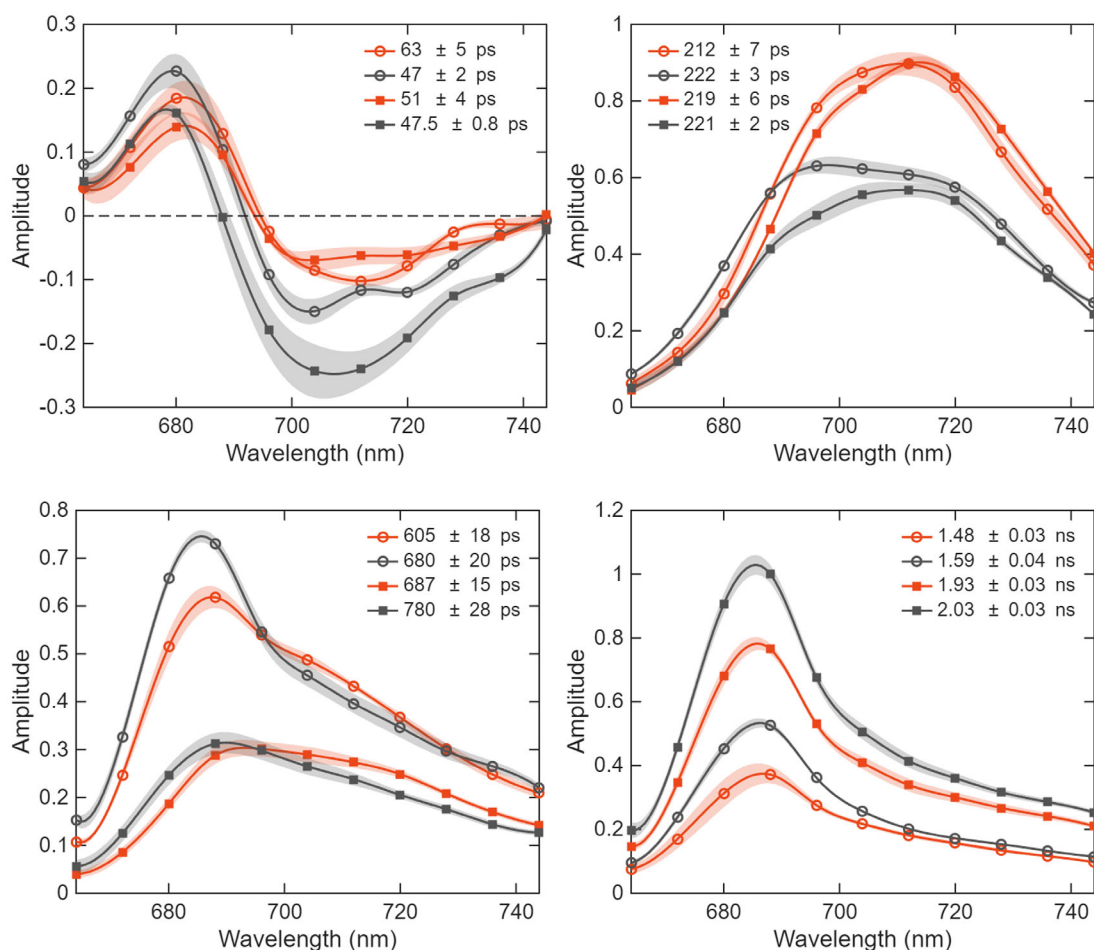


Figure 2. Decay-associated emission spectra of intact cells obtained from global analysis of fluorescence decays measured by TCSPC at RT. The decay kinetics were measured at two excitation wavelengths—440 nm (red curves) and 480 nm (black curves) under low excitation intensity, high sample flow rate to open the PSII RCs (open circles) and high excitation intensity with added DCMU to close the PSII RCs (black squares). The spectra are averages of five independent measurements and normalized to the summed wavelength-integrated amplitudes; the shaded area represents standard error. The corresponding lifetimes are indicated.

PSII emission is expected to decay with ~220 ps lifetime, similar to the timescale of electron transport from Pheo to Q_A . Thus, when PSII RCs are open, the 220 ps lifetime represents both PSI and PSII decay, explaining higher amplitudes on the blue side. This is especially clear for PSII-specific excitation with open RCs—the DAES peak around 690 nm.

The third component (600–700 ps) shows predominantly PSII decay, evidenced by the 685 nm maximum and the enhanced amplitudes upon preferential PSII excitation. However, DAES shape also depends on excitation wavelength—showing a shoulder at 710–720 nm upon PSI-specific excitation. This indicates part of the PSI emission decays with 600–700 ps lifetime, which is unusual as such long lifetimes have not been reported for PSI-LHCI.

The longest lived component (1.5–2 ns) originates from PSII with closed RC—its amplitude is enhanced by

DCMU addition. A 2 ns lifetime is typically observed for PSII in the presence of DCMU (Szczepaniak et al., 2009).

We measured the fluorescence kinetics of isolated thylakoid membranes (Figure S3). The main qualitative features of the *in vivo* fluorescence kinetics are retained, including a 40–50 ps EET lifetime component and similar lifetimes and DAES as in whole cells. The difference between kinetics with open and closed PSII RCs is present in thylakoid membranes because the exogenous electron acceptors maintain RC open state. When thylakoid membranes were solubilized with 0.03% β -DDM, the fluorescence kinetics changed (Figure S4)—the 50 ps EET was no longer present and the DAES had distinct shapes ascribable to PSI (150 ps), PSII (1.2 ns), and detached LHCII (4 ns).

Fluorescence decays of cells and thylakoids show that PSI has unusual kinetics in *E. gracilis*, with relatively slow

50 ps EET to red Chls and 220 ps trapping, possibly extending to 600 ps in some complexes *in vivo*. We postulate that the 50 ps lifetime represents EET between PSII and PSI, that is, spillover (see Discussion section).

Time-resolved fluorescence spectroscopy at 77 K

The fluorescence spectra of cells recorded at 77 K show complex dynamics between several Chl pools. Six lifetimes were necessary to fit the kinetics, spanning from 23 ps to 3 ns. DAES, plotted in Figure 3 for 440 and 480 nm excitation, showed a gradual shift from shorter to longer wavelength emitting states as expected. The first four DAES have positive–negative amplitudes, representing EET. With 440 nm excitation, DAES show the subsequent rise of emission at 715 nm (27 ps lifetime), 725 nm (79 ps), 735 nm (253 ps), and 740 nm (774 ps). Therefore, the red Chl population (presumably in PSI) spans several timescales up to hundreds of ps at 77 K. Similar to RT measurements, negative amplitudes (representing EET to red Chls) are enhanced upon PSII excitation—especially rise amplitudes in the intermediate range 710–720 nm. If these EET components originated solely from PSI, their relative amplitudes would be expected to decrease with preferential PSII excitation, not increase. This is evidence for PSII–PSI spillover at 77 K. The last two components, with lifetimes around 1.7 and 2.9 ns, have only positive amplitudes and show the decay of red Chl forms with emission maxima at 725 and 735 nm, respectively. Essentially the same results were obtained by measuring fluorescence kinetics of isolated thylakoid membranes at 77 K (Figure S5).

Isolated pigment–protein complexes

To gain insight into the functional organization of the *E. gracilis* photosynthetic apparatus and confidently

interpret excitation kinetics *in vivo*, we isolated the main pigment–protein complexes from thylakoid membranes and analyzed them biochemically and spectroscopically.

Biochemical characterization

After sucrose density gradient centrifugation of solubilized thylakoid membranes, six fractions were collected (Figure 4): B1—LHCII monomers and free pigments; B2—LHCII trimers; B3—LHCI oligomers (LHCI'); B4—PSII core complex; B5—PSI–LHCI (small); B6—PSI–LHCI (large). It is worth mentioning that B6 contained more Chl than B5 (~2:1), so it likely represents the main functional form *in vivo*. Fractions B2, B3, and B4 were further purified by size-exclusion chromatography (SEC).

Figure 4(b) shows SDS–PAGE analysis of antenna complexes from bands B2 (LHCII) and B3 (LHCI') before and after SEC. B2 displays a characteristic LHCII polypeptide pattern (~25–30 kDa), consistent with trimeric LHCII. In contrast, B3 before chromatography contains a heterogeneous mixture of LHCII and LHCI-type proteins, indicating incomplete separation and the presence of free antenna components, consistent with other spectroscopy (Figure S6). After SEC, however, B3 resolves into a more homogeneous banding pattern, consistent with the purified LHCI complex, demonstrating effective removal of LHCII and other loosely associated proteins.

To obtain more information on the protein composition of LHCI', we analyzed gel cutouts of B3 by mass spectrometry. Using BLAST searches against available *E. gracilis* sequences in the UniProt database, we identified several polypeptides with high confidence (Table 1). All can be identified as proteins found in the PSI structure, either Lhca or Lhcbm type. However, one group of proteins has prominent abundance—Lhca5/6/7/8, which together account for 84% of the total. These four sequences are

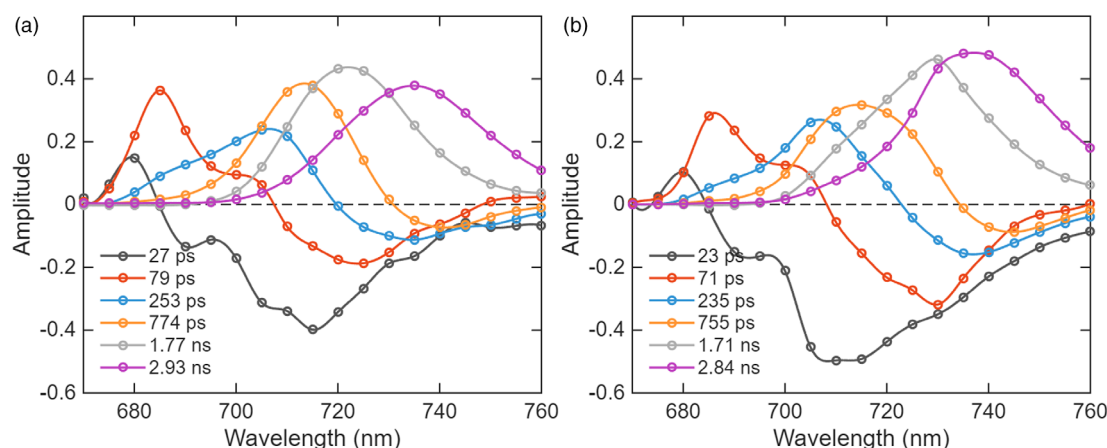


Figure 3. Decay-associated emission spectra of intact cells obtained from global analysis of fluorescence decays measured by TCSPC at 77 K. (a) 440 nm excitation and (b) 480 nm excitation.

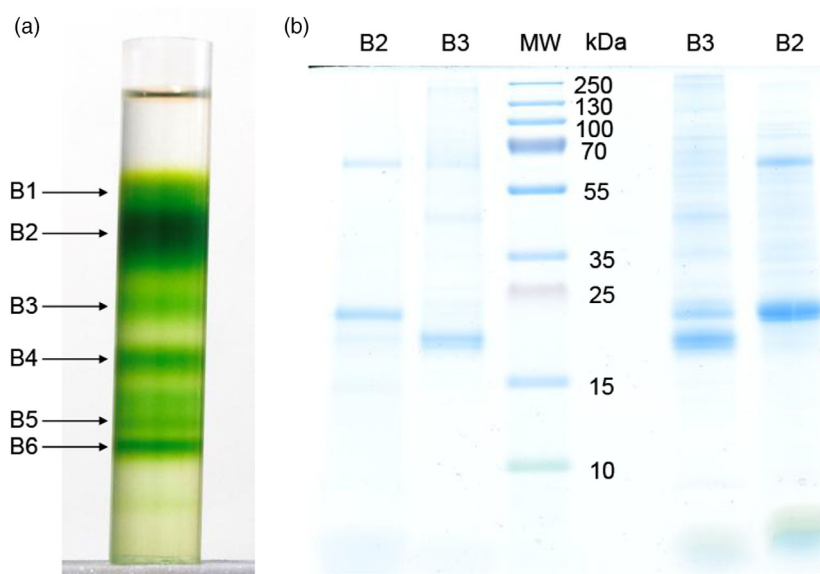


Figure 4. Isolation and purification of pigment-protein complexes. (a) Bands B1–B6 after sucrose density gradient ultracentrifugation of thylakoid membranes solubilized with 0.7% β -DDM. (b) SDS-PAGE of bands B2 (LHCII) and B3 (LHCI') before (right-hand lanes) and after (left-hand lanes) size exclusion chromatography.

Table 1 Proteins in LHCI' identified by mass spectrometry analysis

UniProt ID	Gene name	Abundance (%)
A8HPC6	Lhca5	80.1
A8HPD0	Lhca6	
A8HPD7	Lhca8	
A8HPD3	Lhca7	4.1
Q39725	Lhcbm1	2.4
A8HPE4	Lhca10	1.2
A8HPE8	Lhca11	
A4QPI5	Lhca1	0.3
A4QPI4	Lhca2	
A8HPC1	Lhca4	
A8HPH1	Lhcbm10	0.1

parts of polypeptide precursors to LHCI (Koziol & Durnford, 2008) and can be identified with PSI–LHCI supercomplex subunits bound on the PsaM side of the PSI core (Bai et al., 2026; Kato et al., 2025). Moreover, together they match 10 of the 11 LHCE subunits comprising the M-side

belt in the structure of Bai et al. (2026). Among the rest of the detected proteins, Lhca10/11 and Lhcbm1/10 are also present in the PSI–LHCI supercomplex (Bai et al., 2026). However, their low abundance in B3 suggests that they are contamination rather than a functional subunit of LHCI'. Note that these additional subunits are located on the PsaF-side of PSI and are more stably bound than the M-side subunits (Kato et al., 2025).

HPLC analysis of pigment extracts (Table 2) showed all isolated complexes contained Chl *a*, but only LHCII was enriched in Chl *b*, with a Chl *a/b* ratio of ~3. No Chl *b* was detected in PSII and LHCI' after SEC purification and the Chl *a/b* ratio of the two PSI fractions, B5 (S) and B6 (L), was 41 and 26, respectively. This suggests that PSI (L) contains more LHCS than PSI (S), which is consistent with its larger size. Three main Car types were identified: diadinoxanthin in LHCII, LHCI' and PSI; neoxanthin in LHCII; β -carotene—found in PSII and PSI. In essence, LHCII contains Chl *a* + *b*, diadinoxanthin and a small amount of neoxanthin; LHCI contains Chl *a* + *b* and diadinoxanthin; PSI and PSII core

Table 2 Pigment analysis of the isolated pigment-protein complexes

Sample	Chl <i>b</i> [mol mol ⁻¹ Chl <i>a</i>]	Diadinoxanthin [mol mol ⁻¹ Chl <i>a</i>]	Neoxanthin [mol mol ⁻¹ Chl <i>a</i>]	β -carotene [mol mol ⁻¹ Chl <i>a</i>]	Chl/Car [mol mol ⁻¹]
LHCII	0.31 ± 0.02	0.56 ± 0.04	0.02 ± 0.01	0	2.0 ± 0.2
LHCI'	0.08 ± 0.01	0.34 ± 0.03	0	0	4.0 ± 0.2
LHCI' (SEC)	0.01 ± 0.01	0.23 ± 0.02	0	0	4.4 ± 0.4
PSII	0	0.05 ± 0.00	0	0.15 ± 0.01	4.9 ± 0.1
PSI (S)	0.03 ± 0.01	0.16 ± 0.00	0	0.07 ± 0.01	4.5 ± 0.1
PSI (L)	0.04 ± 0.01	0.18 ± 0.01	0	0.06 ± 0.01	4.3 ± 0.1

complexes contain Chl *a* and β -carotene. The results align with the expected pigment composition according to previously published data (Miranda-Astudillo et al., 2025) and attest the relative purity of the isolated complexes.

Absorption, CD, and fluorescence spectra of isolated complexes

Stationary absorption, CD, RT, and 77 K fluorescence spectra of trimeric LHCII and LHC oligomers (LHCI') are shown in Figure 5. LHCII absorption and CD spectra resemble those from green algae and higher plants but with notable differences: lower Chl *b* absorption (650 nm shoulder) and higher Car absorption, consistent with pigment analysis. The specific pigment composition is reflected in the CD spectrum, especially in the blue region showing a particularly strong negative CD band at 490 nm. Oligomeric LHCI' has dramatically red-shifted absorption with a pronounced band around 700 nm. It shows no Chl *b* and much lower

Car absorption compared to LHCII. The highly non-conservative CD spectrum shows two negative bands corresponding to the Q_y transitions of Chl *a*—one at 684 nm and one at 700 nm, mirroring the two principal absorption bands. The 700 nm CD band indicates that the red-shifted absorption originates from delocalized excitonic states of strongly coupled Chl *a*.

RT and 77 K fluorescence spectra of LHCII show expected emission band at 682 nm and its red-shifted vibronic side wings. In contrast, LHCI' has broad emission maximum at 698 nm at RT and 715 nm at 77 K, showing multiple red-shifted states thermally equilibrated at RT. The remaining 680 nm emission is most probably from contaminations and uncoupled Chls as its intensity varies between samples.

Notably, RT emission and absorption spectra of LHCI' are strongly red-shifted, even compared to higher plants. This means red Chls energies in LHCI' are relatively higher,

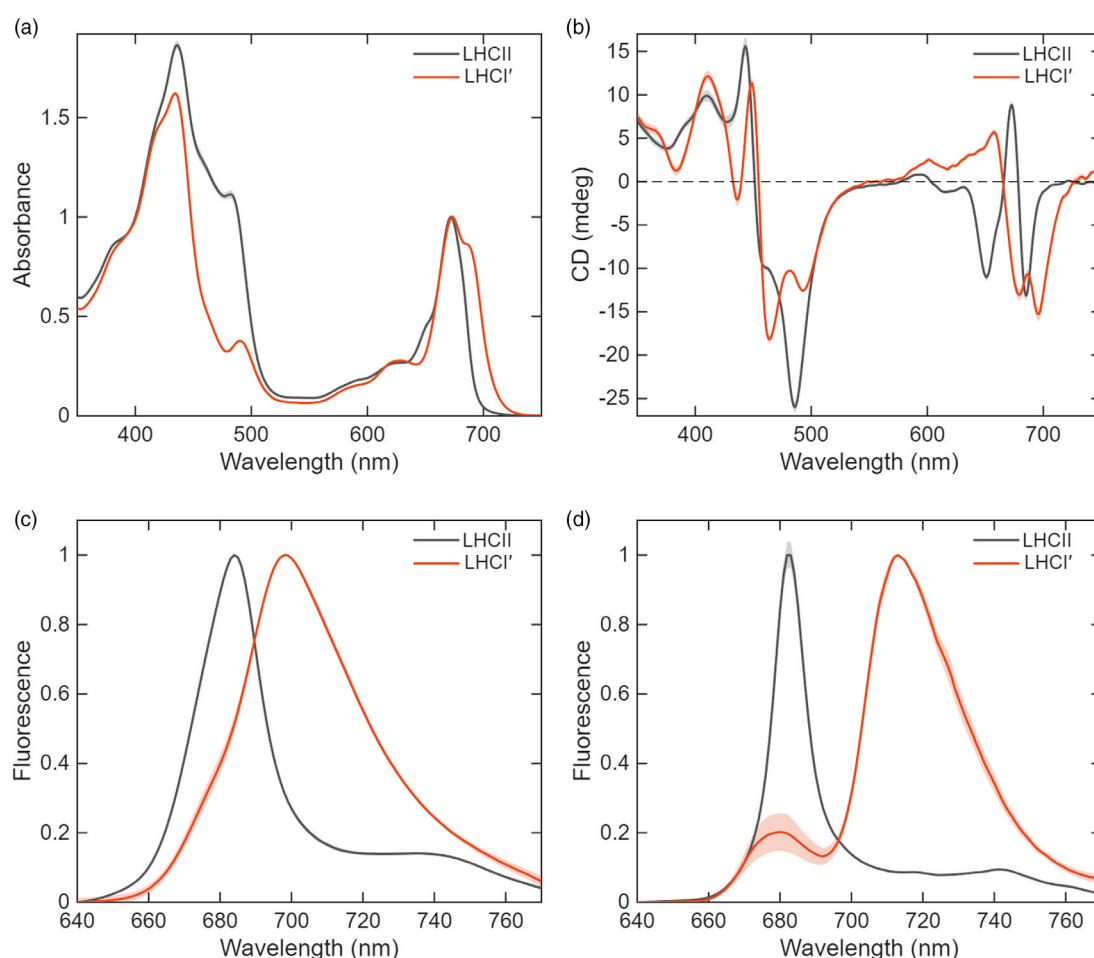


Figure 5. Stationary spectra of LHCII and LHCI'.

(a) Absorption; (b) CD; (c) room temperature fluorescence (440 nm excitation); (d) 77 K fluorescence (440 nm excitation). Absorption and CD spectra are normalized to the red absorbance maximum; fluorescence spectra are normalized to their maximum. The spectra are an average of 5–7; shaded areas show the standard error.

but so are the number of states (contributing Chls) and total oscillator strength. Thus, for increasing absorption cross-section in the far-red, LHCI' is a much more potent absorber than typical LHCI.

Stationary spectra of PSII (B4) and PSI complexes (B6) are compared in Figure 6. Although their absorption spectra are broadly similar, PSI can be readily distinguished by additional red-shifted absorption bands from its associated LHCI antenna. LHCI also contributes to the negative CD signal in the blue region (450–500 nm). The most distinctive PSI spectral signature is far-red Chls emission in the range 700–750 nm. At 77 K, PSI fluorescence peaks at 733 nm with minimal emission below 700 nm, reflecting low-energy Chls in the PSI–LHCI antenna. Notably, the emission maximum is red-shifted ~15 nm relative to the LHCI' oligomer, indicating the most strongly red-shifted Chl forms are absent from LHCI'. The two PSI-containing fractions, B5 and B6, display nearly identical spectral characteristics

(Figure S7), confirming highly similar pigment–protein composition.

Somewhat surprisingly, PSII fluorescence spectra also contain some red-shifted forms emitting around 710 nm. While the spectra shown in Figure 6 were recorded from PSII samples purified by SEC, purification had very little effect on far-red emission (Figure S8). Hence, the far-red emitter is likely an integral part of the complex and not an impurity. Nonetheless, the 710 nm band intensity is relatively small even at 77 K, which means that either the red-shifted Chls are poorly energetically connected to the rest of the PSII antenna or present only in a small subpopulation of complexes.

Time-resolved fluorescence spectroscopy of isolated complexes

Fluorescence kinetics of LHCII, LHCI', PSII, and PSI complexes were recorded at RT and 77 K. All except PSI were

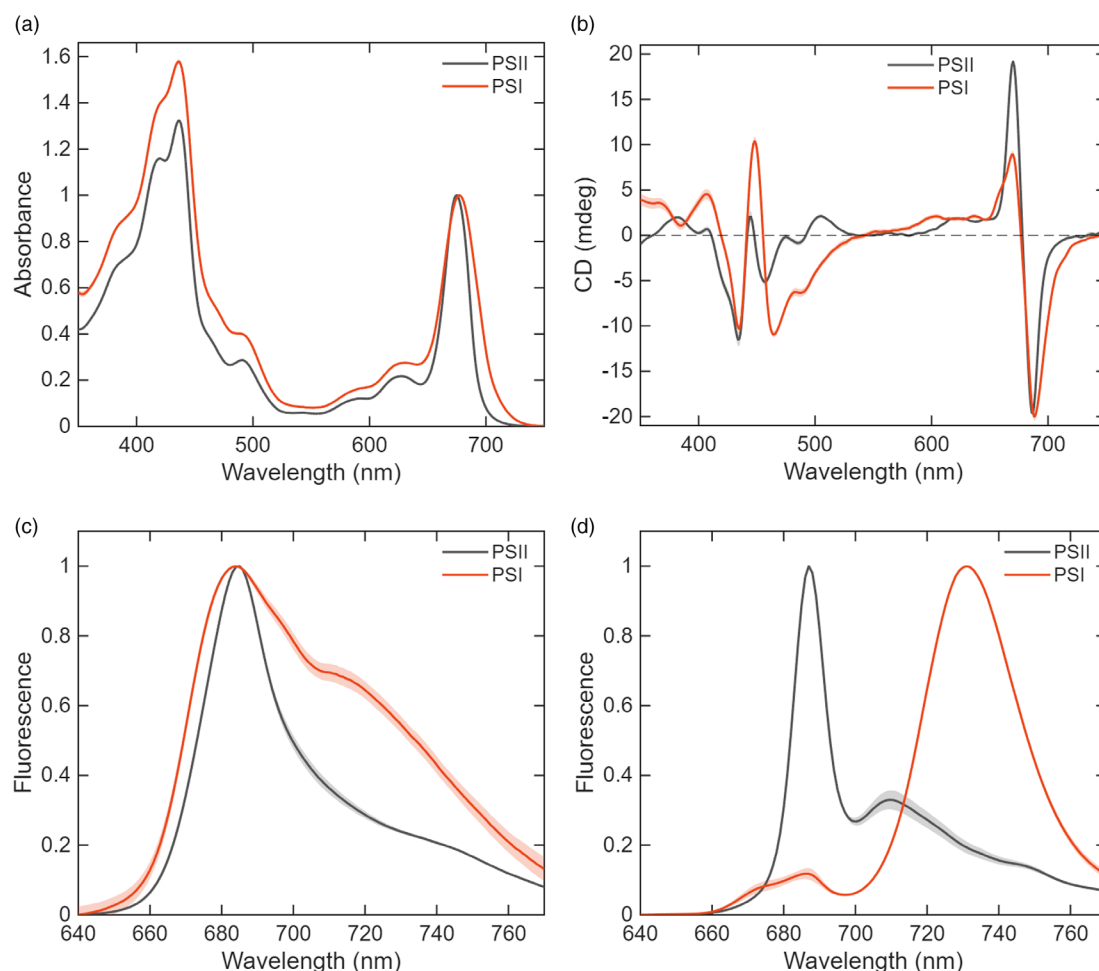


Figure 6. Stationary spectra of PSII and PSI.

(a) Absorption; (b) CD; (c) room temperature fluorescence (440 nm excitation); (d) 77 K fluorescence (440 nm excitation). Absorption and CD spectra are normalized to the red absorbance maximum; fluorescence spectra are normalized to their maximum. The spectra are an average of 5; shaded areas show the standard error.

tested before and after SEC; purified complexes were used when significant changes occurred. Figure 7 shows RT kinetics represented as DAES. LHCII fluorescence decayed with major lifetime of 4.3 ns; a small-amplitude (<10%) sub-ns component could originate from quenching in a minor complex population. The spectra and kinetics are typical for LHCII (Akhtar et al., 2020; Lambrev et al., 2007). LHCI' oligomer fluorescence decayed with main lifetime of 2.8 ns and had markedly red-shifted spectrum as found in steady-state measurements. The DAES peaks at 696 nm and a broad shoulder at 710–720 nm. A 300 ps component shows quenching in a minor fraction of complexes that varies between samples. A longer lifetime (5 ns), blue-shifted DAES probably represents pigments energetically uncoupled during isolation. This blue-shifted emission has significantly higher amplitude in B3 samples before SEC, confirming it originates from energetically unconnected LHCs (Figure S9). Fluorescence kinetics at 77 K showed

similar decay components plus an EET component with 18 ps lifetime (Figure S10).

The PSII kinetics resembles published measurements of plant and cyanobacterial PSII—described by three lifetimes with similar DEAS peaking around 680 nm. PSII typically shows multiexponential fluorescence decay of equilibrated antenna due to reversible charge separation kinetics and dynamic fluctuations of the EET network (Caffarri et al., 2011; Chmeliov et al., 2014). Lifetimes of 30–50 ps and 200–400 ps are typically observed in PSII with open RCs, assigned to primary (to pheophytin) and secondary (to Q_A) electron transfer, respectively (Holzwarth et al., 2006), whereas ~2 ns lifetime is typical for PSII with closed RCs, where fluorescence is emitted after $P_{680}^{+}Pheo^{-}$ radical pair recombination (Szczepaniak et al., 2009). Still, *E. gracilis* PSII fluorescence has characteristic features—enhanced emission at 710–720 nm consistent with steady-state spectra, and slightly longer decay lifetimes than in

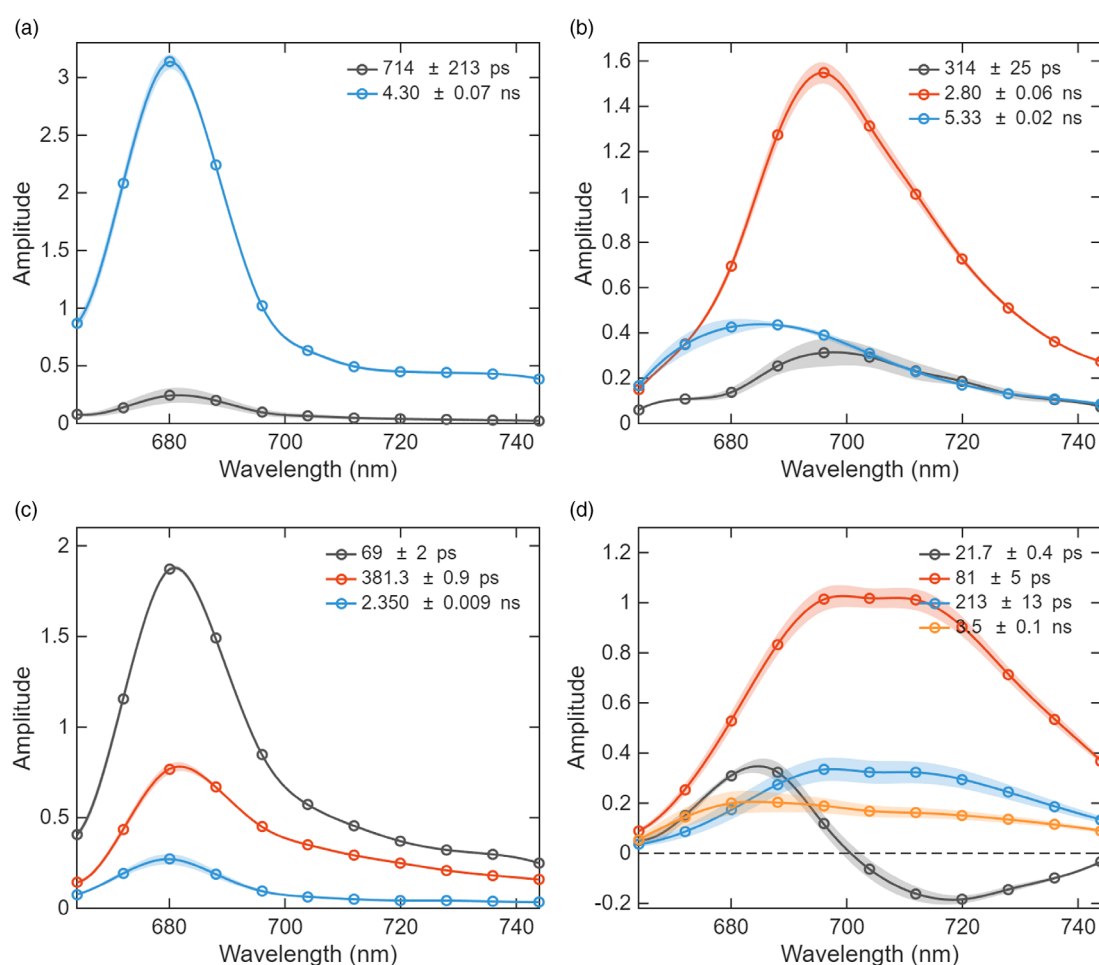


Figure 7. Time-resolved fluorescence spectroscopy of isolated pigment-protein complexes: decay-associated emission spectra obtained by global analysis of the fluorescence decays recorded at RT with 440 nm excitation.

(a) LHCII; (b) LHCI'; (c) PSII; (d) PSI.

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isolated core complexes. Far-red emission is revealed more clearly in time-resolved fluorescence measurements at 77 K (Figure S10). A fluorescence component decaying with 2.4 ns has a well-resolved maximum at 710 nm. The main trapping component, DAES (100–500 ps lifetime) also shows that far-red-emitting states are populated on this timescale, confirming that low-energy states are connected to the bulk antenna pool.

PSI fluorescence decays required a minimum of four lifetimes in a 2-ns time window (Figure 7d). The shortest resolved lifetime, around 20 ps, was unmistakably associated with EET from bulk antenna Chls to red forms. The main decay lifetime was ~80 ps, showing trapping of transiently equilibrated excitations between bulk and red Chls. A third lifetime (200 ps) was necessary to fit the kinetics with a similar DAES. This suggests that spectrally equilibrated antenna excitations decay nonexponentially, with a spectrally integrated amplitude-weighted average lifetime of 110 ps (the 3.5-ns component is excluded from the calculation). These values correspond to the larger PSI fraction (B6); the average lifetime of fraction B5 was slightly shorter (~100 ps, see DAES in Figure S11). The fourth decay component with 3.5 ns lifetime represents a mix of functionally detached LHCI (still retaining the red emission) and free Chls.

Isolated PSI fluorescence confirms effective trapping lifetime is considerably longer than that of PSI–LHCI from most studied organisms (40–70 ps); however, not as long as 220 ps suggested by time-resolved fluorescence of intact cells. Importantly, effective EET to red Chls is faster than the 50 ps lifetime observed *in vivo*. Moreover, even the 20 ps lifetime in PSI is probably only the shortest resolvable lifetime in our experiments but from the relatively low amplitude of the DAES, we infer there might be faster components below experimental time resolution. PSI fluorescence kinetics at 77 K reveal all red Chl forms resolved in intact cells (Figure S10). However, the longest resolved EET lifetime to the red Chls in isolated PSI was 118 ps (Figure S10)—considerably shorter than the EET lifetimes in intact cells that extend to few hundreds of ps.

DISCUSSION

PSI trapping slowed by LHCI

The uncommon evolutionary characteristics of *E. gracilis* extend to the photosynthetic apparatus organization, including the PSI supramolecular architecture. The combination of endosymbiosis with an early-diverging green alga and horizontal gene transfers creates a mosaic of photosynthetic genes predicting distinctive PSI architecture, confirmed by structural studies (Kato et al., 2025; Miranda-Astudillo et al., 2025). The *E. gracilis* genome lacks the canonical LHCI clade but contains proteins evolved from green algal LHCI and other phototrophic taxa (Maruyama et al., 2011).

Additionally, the PSI core lacks small peripheral subunits that bind LHCI in other organisms. Consequently, *E. gracilis* has an innovative PSI–LHCI supercomplex organization. Instead of the well-defined heterodimeric LHCI belts in typical green-lineage PSI, *E. gracilis* employs a cluster of LHCI/Lhcbm-related complexes, yielding distinctive light-harvesting characteristics.

The average excitation lifetime of isolated PSI, calculated as the spectrally integrated amplitude-weighted average lifetime, neglecting the nanosecond component, was 110 ps (using the DAES in Figure 7d). This is substantially longer than typical PSI–LHCI (50–70 ps in green algae and higher plants). *In vivo* measurements (Figure 2; Figure S3) suggest that the excitation lifetime may reach 200 ps (at least in a fraction of complexes). The long lifetime strongly suggests that EET between LHCI Chls and the PSI core contributes majorly to the trapping time. Three factors rationalize this: (1) a larger antenna creates more excitation states, thermodynamically favoring antenna residence and slowing trapping; (2) more red-shifted states drive excitations to escape from core to antenna; (3) long Chl-to-Chl distances create an EET bottleneck. In green algae, the excitation lifetime is influenced mainly by entropic factors (Akhtar et al., 2025); in higher plants, by strongly red-shifted LHCI. All three factors contribute in *E. gracilis*.

The trapping lifetime is heterogeneous—isolated PSI shows a main component of 80 ps and a second ~200 ps component. These lifetimes varied with fitting time range (Figure S12), suggesting they average more complex dynamics. Longer lived components occur in PSI from other species, though typically with low amplitudes (Giera et al., 2014, 2018). The heterogeneity may reflect the complex LHCI antenna architecture creating a cascade of EET over multiple timescales, or actual heterogeneity among PSI complexes in the number of attached LHCI subunits.

Spectroscopically distinct PSII core complex

The spectroscopic characterization of *E. gracilis* PSII revealed peculiar characteristics. Most notable is the near-total absence of the F695 band in 77 K fluorescence emission spectra, typically attributed to the lowest energy Chl states of CP47 (Andrizhiyevskaya et al., 2005). Although F695 intensity depends on species and conditions, it is present almost universally if CP47 is intact (Reinot et al., 2016). Without a high-resolution structure, judging the molecular basis is difficult. The Chl molecule responsible for the red-shifted emission has been debated, but most authors assign it to Chl B16 (Müh & Zouni, 2020), coordinated by His114 of PsbB (CP47). Interactions with the neighboring PsbH subunit may be necessary for the red shift (D'Haene et al., 2015). Both PsbB and PsbH are universally found in PSII of oxygenic organisms (Yang et al., 2022), including *E. gracilis* (Miranda-Astudillo et al., 2025), and His114 is conserved.

More investigation is needed to understand why Chl B16 behaves differently in *E. gracilis*.

Another notable aspect is that *E. gracilis* PSII has a long-wavelength absorption tail beyond 700 nm and corresponding far-red emission—traits typical for PSI but not PSII. One explanation is that the missing F695 emission is red-shifted beyond 700 nm in some conformational states. The far-red emission is unlikely from LHCI/LHCII contamination, as pigment and protein analyses exclude their presence. The low-energy states may explain why PSII trapping kinetics are slower than in cyanobacterial or plant PSII cores.

Far-red-absorbing LHCI oligomers

A unique *E. gracilis* feature is the oligomeric LHCI complex (LHCI') isolated as a separate entity. Earlier studies reported that it contains mixed LHCI and LHCII (Doege et al., 2000; Miranda-Astudillo et al., 2025). Although the sucrose-gradient band contains trimeric LHCII, these appear not genuinely attached—they are not energetically connected to far-red-emitting states and are nearly completely removed by gel filtration. MS analysis revealed that purified LHCI' consists exclusively of LHCI proteins from two gene clades—Lhca5/6/8 and Lhca7 (Kozioł & Durnford, 2008)—both encoding PSI–LHCI supercomplex subunits (Kato et al., 2025). These subunits are located on the PsaM side of the PSI core (opposite PsaF side). The PsaM-side belt has fewer core contacts, suggesting looser LHCI connections than PsaF side subunits (Kato et al., 2025). Thus, LHCI' is an integral or transiently connected part of PSI *in vivo*.

LHCI' exhibits strikingly different spectra than typical LHCII and LHCI—strongly red-shifted absorption with a maximum at 696 nm at RT (Figure 5a). Since LHCI' emission falls between 710 and 720 nm at 77 K (Figure 5d), while PSI's longest wavelength states emit around 730 nm (Figure 6d), the reddest Chls are absent from Lhca5/6/7/8. Unless the red-most emission is exclusively a PSI-connected LHCI property, it should originate from LHCI subunits on the PsaF side (Kato et al., 2025).

The strong far-red absorption of LHCI' is highly unusual—about 40% of Q_y band oscillator strength lies beyond 682 nm, requiring substantial red-shifted Chls. Our results affirm the excitonic nature and multiplicity of long-wavelength states, but more investigation is needed to uncover their molecular identity. The red shift may be an oligomer property not present in single units; however, the far-red absorption and emission differ fundamentally from far-red states formed upon LHCII oligomerization (Miloslavina et al., 2008; Ruban et al., 2007) or FCP (Gelzinis et al., 2024; Miloslavina et al., 2009), which are associated with fluorescence quenching (Chmeliov et al., 2016; Ostroumov et al., 2020). In contrast, LHCI' emits far-red fluorescence (maximum ~696 nm at RT) with high

quantum yield and 3 ns lifetime, indicating it is a fully functional light-harvesting antenna.

Energy exchange between PSI and PSII

The thylakoid membranes in *E. gracilis* chloroplasts lack the distinct appressed and non-appressed sheets characteristic of vascular plant grana (Albertsson, 2001; Andersson & Anderson, 1980; Dekker & Boekema, 2005), instead existing as largely non-appressed lamellae unable to support strict lateral separation of the two photosystems. Additionally, the peripheral light-harvesting complexes of both photosystems are similar, being mostly LHCII-derived and possibly partially shared (Doege et al., 2000; Winter & Brandt, 1986). Thus, the two photosystems or their antenna systems have significant physical contacts enabling excitation energy migration between them (spillover).

Fluorescence and time-resolved fluorescence spectroscopy of intact cells and thylakoid membranes provide strong evidence for considerable spillover in *E. gracilis*. First, PSII emission at 77 K is very low in cells and thylakoids. The most conspicuous spillover sign is EET between Chls emitting at 680 nm (donors) and 710–720 nm (acceptors) over ~50 ps at RT. This is most easily explained as energy exchange between PSII antenna (emitting at 680 nm) and PSI (710–720 nm). The alternative hypothesis—that the EET pathway exists within PSII or PSI—is inconsistent with isolated complex measurements, where this component was absent. Furthermore, the EET component vanished when thylakoid membranes were solubilized with detergent, disrupting inter-complex membrane interactions (Figure S4). At 77 K, multiple EET lifetimes to red Chls were observed (Nagao et al., 2021), but not in isolated PSI or PSII.

We can exclude the blue–red EET component originating from PSII core or PSII–LHCII, as both have little far-red emission. If the EET originated from PSI, its amplitude should increase with preferential PSI excitation. Conversely, the EET amplitudes at RT and 77 K were significantly enhanced with preferential Chl *b* excitation, primarily found in LHCII. Therefore, energy migrates from PSII–LHCII to PSI. Additionally, the RT fluorescence decay lifetimes of red Chls are considerably longer *in vivo* than in isolated PSI, suggesting *in vivo* red Chls equilibrate with a larger Chl pool, consistent with energy exchange between PSII–LHCII and PSI.

If PSII and PSI coexist in the same membrane domains and are energetically connected, this might impair PSII efficiency since PSI traps energy faster (Anderson et al., 2012). However, in *E. gracilis*, the PSI trapping lifetime is relatively long (≥ 110 ps), allowing excitations to equilibrate within a shared antenna and supply both RCs, though excitation flow remains biased toward PSI. Whether spillover bestows physiological advantage or is merely an unavoidable consequence of lacking specific control mechanisms remains unclear. PSI already employs

a large antenna that slows trapping, so further antenna size increase is unlikely the driving force. Alternatively, spillover may have photoprotective effects on PSII when other rapid NPQ mechanisms are unavailable (Doege et al., 2000). Spillover has been proposed as photoprotective in other species under specific conditions, for example, desiccated lichens (Slavov et al., 2013), overwintering conifers (Bag et al., 2020), red algae (Yokono et al., 2011), or cyanobacteria (Terashima et al., 2025). While spillover is prevalent in *E. gracilis*, concrete evidence for its use as a controlled photoprotective mechanism is lacking. With an established method to probe spillover *in vivo* (RT time-resolved fluorescence), this question can be addressed experimentally in future studies.

CONCLUSIONS AND PERSPECTIVES

The biochemical and spectroscopic characterization of isolated *E. gracilis* complexes reveals extensive insights into their composition, organization, and photophysical function. PSI–LHCI supercomplexes exhibit unusually prolonged excitation lifetimes (100–200 ps) arising from EET through a large, structurally mosaic LHCI antenna delivering excitations to a minimal core lacking specialized anchoring subunits. This demonstrates that functional light-harvesting antennas can assemble through alternative binding mechanisms independent of co-evolved protein–protein interfaces—a principle with potential applications for designing optimized photosynthetic systems.

We demonstrate that a mobile, red-shifted LHC assembly (LHCI'), previously considered to comprise both LHCI and LHCII, is composed exclusively of LHCI proteins and likely functions in dynamic regulation of PSI antenna size. The molecular basis for pronounced far-red absorption remains incompletely understood, though our data clearly implicate multiple excitonically coupled Chls. Further investigations are necessary to elucidate the precise mechanisms underlying the complex's unusual spectral signature. The *E. gracilis* PSII core displays atypical properties—most notably, diminished or absent F695 emission from CP47—warranting mechanistic investigation.

Re-analysis of whole-cell and thylakoid membrane fluorescence kinetics, using data from isolated complexes as reference, provides compelling evidence for substantial PSII-to-PSI spillover *in vivo*. The existence of spillover in principle supports earlier hypotheses that it functions as a regulatory mechanism for distributing and dissipating excess light energy. The relatively long PSI excitation lifetime allows spillover to occur without considerable loss of PSII efficiency. At the same time, it inherently reduces the energy dissipation through spillover, limiting its regulatory role. Hence, spillover should be viewed primarily as an energy redistribution rather than energy dissipation pathway. It is tempting to assign the loosely attached LHCI' antenna a role in facilitating energy redistribution via spillover.

MATERIALS AND METHODS

Cell culture

Cells were cultivated in TAP medium on a rotary shaker with gentle shaking at 130 rpm and 23°C under constant illumination (80 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$). The experiments were performed on 4-day-old cells, with age defined from the start of the main culture following dilution of a 4-day-old preculture (grown in 100 ml TAP) into fresh medium to an initial concentration of 0.5 ml^{-1} .

Isolation of thylakoid membranes and pigment–protein complexes

For isolation of thylakoid membranes, cells were harvested and washed at RT by centrifugation at 4000 $\times g$ for 4 min in buffer containing 50 mM Tricine (pH 7.5), 5 mM MgCl_2 , 5 mM Na-EDTA, 0.3 M sorbitol, and 1% (w/v) BSA. The resulting cell pellet was disrupted with glass beads ($\leq 106 \mu\text{m}$) using a Precellys Evolution homogenizer (Bertin Technologies, France) with dry ice cooling. The homogenate was centrifuged at 300 $\times g$ for 2–3 min to remove unbroken cells, debris, and glass beads. Supernatant was subsequently centrifuged at 6500 $\times g$ for 5 min at 4°C to pellet thylakoid membranes. The pellet was washed once in buffer containing 50 mM Tricine (pH 7.5), 0.3 M sorbitol, and 5 mM MgCl_2 , and then resuspended in the same buffer to obtain a concentrated thylakoid membrane stock.

Thylakoid membranes were solubilized by incubation with 0.7% (w/v) n-dodecyl- β -D-maltoside (β -DDM) on ice. Insoluble material was removed by centrifugation at 20 000 $\times g$ for 15 min. The supernatant was loaded onto a step sucrose density gradient (six steps, 0.2–0.9 M) containing 20 mM Tricine (pH 7.5) and 0.05% (w/v) β -DDM, followed by ultracentrifugation at 220 000 $\times g$ for 20–22 h at 4°C. Gradient fractions containing PSI, PSII, or LHCs were collected using a Hamilton syringe. Collected fractions were washed in buffer containing 0.03% β -DDM, concentrated using Amicon Ultra filters (Millipore), and stored at -80°C until further use. Fractions containing LHCI' and PSII were further purified by size-exclusion chromatography using a Superose 6 Increase 10/300 GL column (Cytiva, USA).

For spectroscopic measurements, thylakoid membranes were diluted in 20 mM Tricine buffer (pH 7.5), with or without 0.03% β -DDM. Solubilized complexes were resuspended in the same buffer containing 0.03% β -DDM.

Chlorophyll extraction and estimation

Chl and Car content was determined spectrophotometrically using molar absorption coefficients reported by Porra et al. (1989) and Lichtenthaler (1987), respectively. Samples were resuspended in 80% acetone and incubated in the dark for 2–3 min. The suspension was then centrifuged at 10 000 $\times g$ for 4 min to remove starch and protein debris. Absorbance of the supernatants was measured at 470, 646.6, and 663.6 nm, with scattering correction at 750 nm, using Shimadzu 2700i (Shimadzu, Japan) spectrophotometer. At least four replicates were measured, and the 750 nm scattering value was subtracted.

For analysis of the Chls and Cars composition by high-performance liquid chromatography (HPLC), the pigments were extracted in pure acetone, and the quantification was performed using a Shimadzu Prominence HPLC system as described in Nagy et al. (2025). The pigments were identified according to their retention time and absorption spectra and quantified by integrated chromatographic peak area recorded at the wavelength of

maximum absorbance for each kind of pigment. For most Cars, extinction coefficients from Mantoura and Llewellyn (1983) were used.

Gel electrophoresis

For tricine-SDS-PAGE, the purified pigment-protein complexes were mixed with SDS-containing loading buffer (3% SDS, 1.5% β -mercaptoethanol, 7.5% glycerol, 0.0125% Coomassie Blue G-250 in 37.5 mM Tris-HCl pH 7.0) and denatured at 60°C for 10 min. Depending on the number of expected protein bands, 1–3 μ g Chl was loaded on 4% stacking and 10–16% gradient resolving SDS gel (Schägger, 2006) and after separation, stained by standard Coomassie R-250 staining protocol.

Mass spectrometry

From SDS-PAGE, a single gel lane per sample was excised for mass spectrometric analysis. Proteins were reduced with 10 mM dithiothreitol and alkylated with 22 mM iodoacetamide prior to in-gel tryptic digestion. Resulting peptides were extracted, dried, and reconstituted in 0.1% formic acid for LC-MS/MS analysis. Digestion mixtures were acidified and transferred to single-use trapping mini-columns (Evotips, Evosep Biosystems); one-third of each sample was loaded per Evotip. Peptides were analyzed on an Evosep One system (15 samples per day method) online-coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) operating in positive ion mode.

MS1 survey scans were acquired in the Orbitrap at a resolution of 60 000, and data-dependent acquisition was carried out by selecting multiple charged precursor ions for HCD fragmentation. MS/MS spectra were acquired in the ion trap with a resolution of 0.6 Da. Raw data were processed using Proteome Discoverer 3.0 (Thermo Fisher Scientific) and searched against the *E. gracilis* UniProt protein database (520 entries). Trypsin was specified as the proteolytic enzyme, allowing up to two missed cleavages. Carbamidomethylation of cysteine was set as a fixed modification, and oxidation of methionine as a variable modification. Protein identifications were filtered to a false discovery rate (FDR) of 1%.

Absorption and circular dichroism spectroscopy

Absorption spectra were measured using Evolution 500 (Thermo Scientific, USA) and Shimadzu 2700i (Shimadzu, Japan) dual-beam spectrophotometers, while CD spectra were recorded on a J-815 spectropolarimeter (Jasco, Japan). Spectral bandwidths of 1 and 3 nm were used for absorption and CD measurements, respectively. All experiments were performed at RT. Samples were diluted in buffer to an absorbance of 1 at the red maximum within the 600–700 nm range and placed in semimicro quartz cuvettes with a 1-cm path length.

Steady-state fluorescence spectroscopy

Fluorescence emission spectra in the visible region were measured from the same samples at RT and at 77 K using an FP-8500 spectrofluorometer (Jasco, Japan). Samples were diluted in buffer to an absorbance of 0.1 per cm at the red maximum. Emission spectra were recorded over the 620–780 nm range with excitation wavelengths of 440 and 480 nm, using excitation and emission bandwidths of 2.5 nm. Spectra were collected with a 0.5-nm step size and integration times of 1 and 2 s for RT and 77 K measurements, respectively. For low-temperature measurements, samples were cooled using a home-built accessory used with the FP-8500 spectrofluorometer. All spectra were corrected for the spectral

sensitivity of the instrument using a calibrated light source (ESC-842, Jasco) as a reference.

Time-resolved fluorescence spectroscopy at RT and 77 K

Picosecond time-resolved fluorescence measurements were performed with a time-correlated single-photon counting instrument (FluoTime 200/PicoHarp 300 spectrometer, PicoQuant). Excitation was provided by the Fianium WhiteLase Micro (NKT Photonics, UK) supercontinuum laser, generating white-light pulses with a 20 MHz repetition rate. Excitation wavelengths of 440 and 480 nm were used to excite selectively either Chls *a* or *b*. Fluorescence decays were measured at emission wavelengths of 664–744 nm with 8 nm steps, at RT, and 670–780 nm with 5 nm steps at 77 K. Samples, diluted to an absorbance of 0.03 at the excitation wavelength, were placed in a 1-mm flow cell and circulated at a flow rate of 4 ml min⁻¹. In addition, the buffer medium of the thylakoid membranes and PSII (B4) was supplemented with 25- μ M dichlorophenolindophenol and 0.5-mM potassium ferricyanide. Alternatively, measurements with closed PSII RCs were performed by adding 20 μ M DCMU to the medium and lowering the flow rate by ~10-fold. For 77 K measurements, the suspension was placed in a 1-mm demountable cryogenic quartz cell and cooled in an optical cryostat (Optistat DN, Oxford Instruments, UK).

The total instrument response function (IRF) measured using 1% Ludox as scattering solution has full width at half maximum (FWHM) of $\approx$ 40 and $\approx$ 60 ps with 440 and 480 nm excitation, respectively. Data are corrected for the spectral response of the detector using a calibrated light source (ESC-842, Jasco). Global multiexponential lifetime analysis with IRF deconvolution was performed using MATLAB, whereby the decay lifetimes and wavelength-dependent IRF time shifts were determined by non-linear least squares minimization of squared sum of Poisson-weighted residuals. Pre-exponential amplitudes were obtained by linear least squares fit.

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AUTHOR CONTRIBUTIONS

Parveen Akhtar: conceptualization, funding acquisition, resources, investigation, formal analysis, visualization, and writing; László Kovács: high-performance liquid chromatography, review & editing; Ildikó Domonkos: SDS-PAGE analysis, review & editing; Aladár Pettkő Szandtner and Ádám Pap: MS analysis; Soujanya Kuntam: maintaining the algal cultures, review & editing; Petar H. Lambrev: conceptualization, funding acquisition, investigation, software, formal analysis, supervision, and writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data reported in this paper (absorption, fluorescence, circular dichroism, and decay-associated emission spectra) are available for download at <https://doi.org/10.6084/m9.figshare.31444114>.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Fluorescence emission spectra of isolated thylakoid membranes measured at 77 K upon excitation at 440 and 480 nm, normalized at 734 nm.

Figure S2. CD spectra of isolated thylakoid membranes measured in the absence (control) and presence of 0.03% β -DDM.

Figure S3. Decay-associated emission spectra of isolated thylakoids obtained from global analysis of fluorescence decays measured by TCSPC at RT.

Figure S4. Decay-associated emission spectra of thylakoid membranes solubilized with 0.03% β -DDM.

Figure S5. Decay-associated emission spectra of isolated thylakoid membranes obtained from global analysis of fluorescence decays measured by TCSPC at 77 K.

Figure S6. Stationary spectra of LHCl' before and after size-exclusion chromatography purification.

Figure S7. Stationary spectra of PSI small (S) and large (L).

Figure S8. Fluorescence emission spectra of PSII (B4) before and after purification by size-exclusion chromatography.

Figure S9. Decay-associated emission spectra of LHCl' (B3) obtained from sucrose gradient obtained by global analysis of the fluorescence decays recorded at RT with 440 nm excitation.

Figure S10. Time-resolved fluorescence spectroscopy of isolated pigment-protein complexes at 77 K: decay-associated emission spectra obtained by global analysis of the fluorescence decays recorded with 440 nm excitation.

Figure S11. Decay-associated emission spectra of PSI (B5) obtained by global analysis of the fluorescence decays recorded at RT with 440 nm excitation.

Figure S12. Decay-associated emission spectra of PSI (B6) obtained by global analysis of the fluorescence decays recorded at RT with 440 nm excitation in 4-ns range with five components.

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