Excitation energy transfer kinetics of trimeric, monomeric and subunit-depleted Photosystem I from Synechocystis PCC 6803

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Abstract

Photosystem I is the most efficient photosynthetic enzyme with structure and composition highly conserved among all oxygenic phototrophs. Cyanobacterial Photosystem I is typically associated into trimers for reasons that are still debated. Almost universally, Photosystem I contains a number of long-wavelength-absorbing ‘red’ chlorophylls (Chls), that have a sizeable effect on the excitation energy transfer and trapping. Here we present spectroscopic comparison of trimeric Photosystem I from Synechocystis PCC 6803 with a monomeric complex from the ΔpsaL mutant and a ‘minimal’ monomeric complex ΔFIJL, containing only subunits A, B, C, D, E, K and M. The quantum yield of photochemistry at room temperature was the same in all complexes, demonstrating the functional robustness of this photosystem. The monomeric complexes had a reduced far-red absorption and emission equivalent to the loss of 1.5–2 red Chls emitting at 710–715 nm, whereas the longest-wavelength emission at 722 nm was not affected. The picosecond fluorescence kinetics at 77 K showed spectrally and kinetically distinct red Chls in all complexes and equilibration times of up to 50 ps. We found that the red Chls are not irreversible traps at 77 K but can still transfer excitations to the reaction centre, especially in the trimeric complexes. Structure-based Förster energy transfer calculations support the assignment of the lowest-energy
state to the Chl pair B37/B38 and the trimer-specific red Chl emission to Chls A32/B7 located at the monomer-monomer interface. These intermediate-energy red Chls facilitate energy migration from the lowest-energy states to the reaction centre.

**Introduction**

Photosystem I (PSI) is a membrane-bound pigment-protein complex essential for all oxygen-evolving photosynthetic organisms. It has a highly conserved structure and composition [1-3]. PSI consists of 11–14 protein subunits coordinating 94–96 chlorophylls (Chls), about 24 carotenoids (Cars) and other cofactors [4-6]. In cyanobacteria, PSI is usually found in a trimeric or monomeric form, though recently, tetrameric forms have been reported in heterocyst-forming species [7]. The oligomerization state may change towards the monomeric form under different growth conditions, such as light, temperature and available nutrients [8-10]. In PSI of the model cyanobacterium *Synechocystis* PCC6803, 86 Chls are associated with the two largest central subunits of the heterodimeric core, PsaA and PsaB, while 9 Chls are bound to the smaller subunits F, J, K, L, and M [6, 11]. PsaL, a 16-kDa hydrophobic protein subunit of the PSI core, was found to be essential for the formation of trimers [12]. The rest of the small subunits, however, could each be deleted without observing any severe growth defects in cells [12, 13]. A minimal PSI complex from *Synechocystis*, consisting only of A, B, C, D, E, K and M subunits, has been recently obtained and crystalized; however, no high-resolution structure could be resolved, casting some doubt on the intactness of the complex [6].

PSI of almost all organisms contains long-wavelength Chl forms, dubbed ‘red’ Chls, absorbing light at wavelengths longer than the absorption of the reaction centre (RC) Chls P700 [14, 15], and thus broadening the absorption spectral range. The red Chls have unusual spectroscopic properties, such as large bandwidth, Stokes shift and electron-phonon coupling, that result from the mixing of excitonic and charge-transfer (CT) states [16, 17]. The number of red Chls and their spectral properties vary among species and oligomeric forms of PSI. Deconvolution analyses of the absorption spectra of PSI of *Synechocystis* PCC 6803 [18] and spectral hole-burning studies [19, 20] have indicated that ~5 red Chls are present in trimers and ~3 in monomers. It has been recognized that the red forms originate from strong Coulombic
interactions between the Chls and several candidate pigments (or pigment groups) have been proposed [4, 21-26], but these assignments remain largely hypothetical.

Although the red Chls account for only a small fraction of the total absorption cross-section [27], they have sizeable impact on the dynamics of energy transfer (ET) and trapping, as the ET from the red Chls to the RC is energetically uphill and thermally activated [28, 29]. At cryogenic temperatures, the thermal energy is not sufficient for the uphill ET and the long-wavelength pigments act as (pseudo)traps increasing the excited-state lifetime in this region and dominating the fluorescence emission [28, 30]. Energy equilibration between the bulk antenna Chls and the low-energy pigments absorbing below 700 nm is shown to take 2–4 ps in PSI from *Synechocystis* sp. PCC 6803 at room temperature (RT) [18, 31-35] – significantly faster than the effective photochemical trapping time of ~20 ps. Recently, significantly slower lifetimes of ET have been uncovered in the PSI core from higher plants at cryogenic temperature [36]. Very few low-temperature time-resolved fluorescence studies have been reported for cyanobacterial PSI and only for thermophilic species, containing a greater number of red Chls [37, 38].

Because of its high quantum efficiency, relative abundance and structural and functional stability, cyanobacterial PSI is an attractive candidate for the development of bio-hybrid devices for solar energy conversion and biosensor applications [39-41]. Engineering a ‘minimal’ PSI with reduced protein content, requiring less cellular resources to produce and a smaller footprint but retaining full functionality can evidently be advantageous for such developments. Here we report a comparative investigation of PSI trimers and monomers from *Synechocystis* PCC 6803 as well as subunit-depleted ‘minimal’ PSI ΔFIJL with a focus on the pigment spectral forms affected by the oligomerization state and subunit depletion and on the excitation dynamics at room and low temperature. Previously, it has been shown that monomers exhibit differences in absorption, emission, and circular dichroism (CD) spectra compared to trimers [10, 42]. We examined these difference in detail to determine the pigment groups and interactions underlaying them. While trimers and monomers showed similar trapping times at RT [18, 32], specific differences are observed in the kinetics of equilibration with the red Chls at 77 K. We confirm that the subunit-depleted PSI is fully functional with indistinguishable picosecond excitation kinetics.
Materials and methods

Cell culture

Cyanobacterial cells were grown in BG11 medium supplemented with 5 mM HEPES–NaOH (pH 7.5). *Synechocystis* sp. PCC 6803 wildtype (WT), ΔpsaL [10] and the ΔFIJL [6] strains were grown photoautotrophically on a rotary shaker (100 rpm) at 30 °C under continuous white light (~ 35 μmol photons m⁻² s⁻¹). The minimal PSI was obtained from a strain constructed from the ΔFIJL mutant, deleting the PsaJ-PsaF operon, described elsewhere [6]. For the ΔpsaL cells, the medium was supplemented with 40 μg/ml spectinomycin and for PSI minimal with 10 μg/ml kanamycin, and 6 μg/ml chloramphenicol.

Thylakoid membranes preparation

Thylakoid membranes were isolated following the protocol of Zakar et al. [43]. One-week old cells were harvested and washed in buffer A (20 mM MES/NaOH, pH 6.4, 10 mM MgCl₂, 10 mM CaCl₂) at RT by centrifugation for 5 min at 6000 g. The pelleted cells were resuspended in buffer A supplemented with 25% glycerol, 0.5 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine and broken with glass beads (≤ 106-μm) using a Mini bead beater (Precellys Evolution) equipped with dry ice cooling compartment. After breaking, the suspension was centrifuged (4°C, 6 min, 4000 g) to remove the cell debris and remaining glass beads. Then the supernatant was further centrifuged for 30 min at 30000 g, 4°C and the pellet was solubilized in buffer A and used for further experiments.

Photosystem I preparation

PSI samples were isolated from freshly prepared thylakoid membranes following the protocol of Vajravel et al. [42]. The thylakoid membranes were solubilized by incubating with 2% n-dodecyl β-D-maltoside (β-DDM) at 4 °C for 30 min. The suspension was centrifuged for 15 min at 10000 g to remove the unsolubilized material. The supernatant was then loaded on a stepwise (6 steps, 0.2–0.9 M) sucrose gradient containing 20 mM HEPES (pH 7) and 0.05% of β-DM followed by centrifugation at 220,000 g for 17–18 h at 4 °C. The gradient fractions containing PSI trimers (from WT) and monomers (from WT, ΔpsaL and ΔFIJL mutant) were collected by a syringe. The samples were washed in a medium containing 0.03% β-DDM, concentrated using Amicon Ultra filters (Millipore) and stored at −80 °C until use.
Additional experiments were performed on PSI minimal suspension obtained by incubating the crystals [6] in a buffer medium containing 0.03 % β-DDM (while agitating).

**Pigment analysis**

The pigment composition of all samples (thylakoid membranes, purified complexes) was determined by fitting the acetone extract spectra in the visible range from 350 to 750 nm with the spectra of the individual pigments. Absorption spectra of pure Chl a (Sigma-Aldrich) and Cars extracted from the thylakoid membranes were recorded in 80% acetone. Then these spectra were used as a reference to obtain a fit of the spectra of the acetone extracts of PSI samples. Molar absorption coefficients were taken from Lichtenthaler [44]. For analysis of the Chls and Cars composition by high-performance liquid chromatography, the pigments were extracted in pure acetone and the quantification was performed using a Shimadzu Prominence HPLC system as described in Zsiros et al. [45]. The pigments were identified according to their retention time and absorption spectrum and quantified by integrated chromatographic peak area recorded at the wavelength of maximum absorbance for each kind of pigments using the corresponding molar decadic absorption coefficient [46].

**Absorption and circular dichroism spectroscopy**

Absorption and circular dichroism spectra in the range of 350–750 nm were recorded at RT with a Thermo Evolution 500 dual-beam spectrophotometer and a Jasco J-815 spectropolarimeter, respectively. The samples were diluted in 20 mM Tricine buffer (pH 7.5) with 0.03% β-DDM to an absorbance of one at the red maximum. The measurements were performed in a standard glass cell of 1-cm optical path length with 1 nm (absorption) or 2 nm (CD) spectral bandwidth.

**Time-resolved fluorescence**

Fluorescence decays were recorded by time-correlated single-photon counting (TCSPC) using an instrument described in Akhtar et al. [47] at emission wavelengths 664–744 nm with 8 nm steps, at RT, and 670–750 nm with 5 nm steps at 77 K. The source of excitation was a Fianium WhiteLase Micro (NKT Photonics, UK) supercontinuum laser, providing white-light pulses at a repetition rate of 20 MHz centred at 440 nm. The total instrument response (IRF) width was 40 ps, measured using 1% Ludox as scattering solution. The samples were diluted to an absorbance of 0.05 (1 mm optical path length) at the excitation wavelength in buffer medium containing 50%
(v/v) glycerol, 20 mM tricine and 0.03 % β-DDM. The suspension was cooled to 77 K in an optical cryostat (Optistat DN, Oxford Instruments, UK). For measurements with closed (oxidized) RCs, 1 mM potassium ferricyanide was added and the sample was preilluminated with white light for 5 minutes before freezing in liquid nitrogen. Control experiments were performed with dark-adapted samples frozen in the presence of 40 μm phenazine methosulfate and 40 mM sodium ascorbate and attenuating the excitation intensity 20-fold.

**Structure-based modelling of the excitation dynamics**

Semi-quantitative calculations of the dynamics of ET in PSI were performed using Förster theory as described in Byrdin et al. [23] with the assumptions and limitations therein and with modifications as follows. Distances, relative orientations and electronic couplings between Chls are calculated using atomic coordinates in the trimeric PSI structure of *Synechocystis* PCC 6803 [6]. Excitonic interactions are neglected, except for the RC Chls. All bulk Chls are assigned gaussian absorption lineshapes with central wavelengths normally distributed within the Qy absorption band and uniform widths (σ = 80 cm⁻¹) along with two vibronic/Qx sidebands shifted by 1100 and 2300 cm⁻¹. The fluorescence spectra mirror the absorption spectra with a Stokes shift of 140 cm⁻¹. Separate spectral parameters are set for the RC and linker Chls as well as for the designated red Chls. The absorption/emission spectra are calculated for 1000 random realizations and Förster ET rates are estimated for each Chl pair based on the distance, orientation and spectral overlap. Additional decay rates of 0.5 ns⁻¹ and 1 ps⁻¹ are imposed for all antenna Chls and for P700, respectively. The population kinetics is calculated by inverting the transfer rate matrix. Fluorescence decays at different wavelengths are calculated from the time-dependent population and the fluorescence emission spectra. Simulated fluorescence data are generated by convolving the disorder-averaged decays with a model IRF and adding Poisson noise.

**Results**

**Absorption and CD spectra**

The absorption spectra of trimeric and monomeric PSI complexes isolated from *Synechocystis* WT, ΔpsaL and ΔFIJL are compared in Figure 1. Both types of monomeric PSI show significantly lower absorption at wavelengths longer than 690 nm in line with numerous studies that have reported loss of red-shifted Chl forms upon monomerization. The absorption difference
spectra show a positive peak around 692–696 nm and a negative peak at 676–677 nm. The magnitude of the differences approximately corresponds to the missing antenna Chls (3 in ΔpsaL and 7 in ΔFIJL). The difference absorption band at 620 nm is due to a small phycocyanin contamination in the monomeric preparations. The ratio of the integrated absorption spectrum beyond 700 nm to the total area under the main Qy absorption band (650–720 nm) corresponds to approximately 3.5 ‘red Chls’ in both monomeric PSI types and five in the trimeric PSI (Supplementary Table S1), in agreement with previous studies [18].

The L subunit of PSI binds two Cars and the F, I and J subunits bind five more [5, 6]. Accordingly, the monomeric PSI complexes, especially ΔFIJL, have decreased absorption around 500 nm. The HPLC analysis showed 15–30% lower content of carotenoids, echinenone and zeaxanthin in the ΔFIJL PSI (Supplementary Fig. S1, Table S2). For a more precise quantification we performed spectral decomposition of the absorption spectra of acetone pigment extracts from the three PSI types using purified Chl a and total Cars extracted from the thylakoid membranes (Supplementary Fig. S2). From the fits we estimate 24, 22 and 17 Cars in PSI from WT, ΔpsaL and ΔFIJL, respectively. The values exactly match the expected number of Cars, confirming that no additional pigments or subunits were lost during the isolation of PSI.

Figure 1. Absorption spectra of isolated PSI complexes from Synechocystis WT, ΔpsaL and ΔFIJL. (a) Average spectra from four independent preparations, normalized to the area between 650 and 720 nm. (b) absorption difference spectra.

The CD spectra of the monomeric PSI types were recorded to test for possible changes in the pigment exciton interactions (Figure 2). In the red region, the amplitude of the negative excitonic CD band (688 nm) diminished by 25% in both monomeric PSI types with no distinguishable difference among the two. In the blue-green region, the CD spectrum of PSI WT shows an
intense broad band with a maximum at 505 nm, originating mainly from β-carotenes. It was previously observed that this band is sensitive to the oligomerization of PSI [10, 42] and can be used as a fingerprint of trimeric PSI in vivo as well as in isolated complexes [48]. The band intensity was 58% smaller in ΔpsaL complexes relative to WT PSI, whereas the absorption in this region is hardly affected (as ΔpsaL PSI contains just two Cars less than WT). The exact same result was obtained from monomers isolated from WT cells as well (Supplementary Fig. S3). Even more striking is the 85% loss of CD505 in the minimal PSI, where the total Car content is reduced by 29% (Supplementary Fig. S2). Virtually identical CD spectra were recorded from the thylakoid membranes of WT, ΔpsaL and ΔFIJL (Supplementary Fig. S4) – ruling out the possibility that the differences are due to the PSI isolation procedure.

Figure 2. CD spectra of isolated PSI complexes from Synechocystis WT, ΔpsaL and ΔFIJL. (a) average spectra normalized to unity absorption at the red maximum. (b) CD difference spectra.

**Fluorescence kinetics at RT**

The RT fluorescence decay curves over a 2-ns time period were subjected to global analysis with a three-exponential model. The main fluorescence decay lifetime (with 98% amplitude) of trimeric PSI isolated from WT Synechocystis was 25±1 ps (Table 1) – this is the effective trapping time of antenna excitations [31, 34, 49]. No differences in the trapping time were detected in the monomeric PSI types. The other two long-lived components are from contaminations from uncoupled Chls and photosystem II (PSII). Hence, the decay-associated spectrum (DAES) of the 25-ps component (Figure 3) is equivalent to the (stationary) emission spectrum of the pure PSI. The spectra of the monomeric PSI clearly show lower amplitudes at 700–720 nm compared to the trimeric one. Virtually identical PSI spectra (and lifetimes) were recorded from monomeric PSI isolated from WT cells (Supplementary Fig. S5) as well as from
thylakoid membranes and whole cells – confirming that the differences are relevant in vivo (Supplementary Fig. S6).

Table 1. Fluorescence decay lifetimes and amplitudes of PSI complexes at RT

|        | \(\tau_1\) (ps) | \(a_1\) (%) | \(\tau_2\) (ps) | \(a_2\) (%) | \(\tau_3\) (ns) | \(a_3\) (%) |
|--------|-----------------|-------------|-----------------|-------------|----------------|-------------|
| WT     | 25 ± 1.1        | 98.4        | 246 ± 20        | 0.4         | 4.1 ± 0.5      | 1.2         |
| \(\Delta psaL\) | 24 ± 0.4        | 93.7        | 248 ± 20        | 3.1         | 3.1 ± 0.1      | 3.2         |
| \(\Delta FIJL\)  | 24 ± 0.4        | 94.4        | 241 ± 12        | 2.5         | 3.2 ± 0.2      | 3.1         |

The RT emission spectrum of PSI represents a thermal quasiequilibrium between all emitting Chls – bulk and red – and can reveal the different red spectral forms and their abundance. Note that the steady-state emission spectrum measured from trimeric and monomeric PSI [32], is not suitable for such quantification because of the very high relative fluorescence yield of the free Chls and PSII. We decomposed the 25-ps DAES into two ‘bulk’ Chl pools and three red pools emitting at 707, 715 and 720 nm (see Supplementary Table S3 and accompanying text on the details of the calculation). A near-perfect fit of the trimeric PSI spectrum (Figure 3) was obtained with 89 bulk Chls plus 4 emitting at 707 nm and one at 715–720 nm. The best fit for both monomeric PSI types was achieved by decreasing the 707-nm pool by ~2 Chls. Thus, the quantification of the red states from the DAES is in agreement with the analysis of the absorption spectra, while additionally revealing the emitting wavelength of the red Chls lost in monomers.

![Figure 3](image-url)  

Figure 3. Decay-associated emission spectra obtained from global analysis of the fluorescence decays of PSI recorded at RT from WT (black), \(\Delta psaL\) (red) and \(\Delta FIJL\) (yellow) corresponding to the decay components with lifetimes of 24–25 ps. The solid lines represent simulated spectra as described in the text. Long-lived components with very small amplitudes are not plotted.
Fluorescence kinetics at 77 K

Time-resolved fluorescence measurements were performed at 77 K to resolve the dynamics of excitation relaxation and trapping by the different pools of red Chls. Six lifetimes were necessary to fit the fluorescence decays at 670–750 nm over a 4-ns time period. As excitations are gradually transferred from shorter- to longer-wavelength pigments at cryogenic temperature, the DAES shift to the red (Figure 4). The two fastest components – 13–15 ps and 37–44 ps – show transfer from 690 nm to 700–710 nm and from 700 nm to 715–725 nm. The long-lived components with lifetimes 130–160 ps, 410–460 ps and 1.1–1.3 ns have all-positive DAES with maxima around 710, 716, and 722 nm, respectively (red-shifted by 1–2 nm in trimeric PSI). Although the lifetimes and DAES are very similar in all PSI types, some specific differences can be noted for the monomeric compared to trimeric PSI: 1) all but the first decay lifetimes are 10–25% longer; 2) the amplitudes of the DAES peaking at 710 and 715 nm are smaller –the former especially is reduced by 50%; 3) correspondingly, the negative amplitudes of the ET components at 710–716 nm are also smaller. No meaningful differences in the fluorescence kinetics or spectra were detected between the two monomeric PSI types, ΔpsaL and ΔFIJL. Virtually the same DAES were resolved in thylakoid membranes (Figure 4, d-f), apart from changes in the short-lived components below 700 nm, which evidently show PSII emission. In thylakoid membranes with monomeric PSI, the amplitude of the DAES peaking at 710 nm is reduced by half.
Figure 4. Decay-associated fluorescence emission spectra of isolated PSI complexes (a-c) and thylakoid membrane fragments (d-f) obtained from global analysis of fluorescence decays measured by TCSPC at 77 K. (a,c) WT; (b,e) ΔpsaL; (c,f) ΔFIJL.

The changes in the fluorescence kinetics described above affect the overall excitation trapping time. The average fluorescence lifetime plotted as a function of the wavelength shows significant changes between trimeric and monomeric PSI (Figure 5). The average lifetime at 730 nm was 40% longer in ΔpsaL and ΔFIJL PSI compared to WT.

Figure 5. Wavelength dependence of the amplitude-weighted average fluorescence lifetime $\tau_{av} = \sum_i a_i \tau_i / \sum_i a_i$ for the PSI complexes at 77 K.
To test how the oxidation state of the RC may affect the fluorescence decay kinetics, measurements were also performed with P$_{700}$ pre-oxidized by preillumination in the presence of 1 mM potassium ferricyanide. Under these conditions the average fluorescence lifetime in the far-red region was longer, mainly due to the increased relative amplitude of the 1 ns decay component. Conversely, in samples that were frozen in darkness with electron donors (ascorbate and phenazine methosulfate) to increase the proportion of open RCs, the average lifetimes at 730 nm were shorter (Table 2). These results indicate that excitations at the longest wavelength Chls can be transferred to P$_{700}$ and used for photochemistry at 77 K.

Table 2. Fluorescence decay lifetimes and average lifetime at 730 nm of PSI with preoxidized and mixed reduced/oxidized RC

|                | $\tau_3$ (ps) | $a_3$ (%) | $\tau_4$ (ps) | $a_4$ (%) | $\tau_5$ (ns) | $a_5$ (%) | $\tau_{730\text{nm}}$ (ns) |
|----------------|---------------|-----------|---------------|-----------|---------------|-----------|----------------------------|
| WT (red/ox)    | 112           | 33        | 377           | 38        | 0.95          | 28        | 0.45                       |
| WT (oxidized)  | 139           | 29        | 402           | 34        | 1.10          | 37        | 0.58                       |
| ΔFIJL (red/ox) | 110           | 19        | 409           | 28        | 1.01          | 45        | 0.61                       |
| ΔFIJL (oxidized)| 151           | 18        | 430           | 30        | 1.30          | 50        | 0.82                       |

**Discussion**

**Induced CD of carotenoids**

Of the 24 carotenoids per monomer in the trimeric PSI [6], only 20 were resolved in the monomeric PsaL$^{\text{His}}$ PSI [11] – two are missing from the L subunit and two in subunit A may be unresolved in the lower-resolution structure. Five more Cars are coordinated by the F, I, and J subunits and therefore a total of at least seven Cars should be absent in the minimal PSI. In principle, several other Cars located between these and the ‘core’ subunits, could also be lost – for instance, hydroxyechinenone I4021 – but this does not seem to be the case if we rely on the quantification of pigment acetone extracts. It is remarkable then, that the loss of seven Cars results in >80% reduction of the CD intensity at 505 nm. Clearly, only a few Cars are responsible for the CD.

Cars in solution rarely exhibit optical activity but they can acquire CD when bound to proteins [50, 51]. There are two types of induced CD – one is when the achiral molecules adopt different conformations such that atom displacement breaks the molecular symmetry, and another is when
the chromophores are engaged in dipole-dipole interactions creating excitonic CD. The CD difference spectra of PSI (Figure 2) suggest the former type, i.e. the Car signals are induced Cotton effects resulting from the protein environment forcing the Car molecules into a chiral conformation, rather than intermolecular excitonic interactions. Some Cars, including xanthophylls, are chiral and can have Cotton effect depending on the configuration of the polyene chain and the end cyclohexene rings. Upon crude inspection of the 24 Cars in the PSI structure, several stand out as having highly distorted polyene chains and twisted rings (Supplementary Fig. S7). Interestingly, the majority of them are xanthophylls (B4011, F4016, I4020, M4021), such as zeaxanthin and echinenone, but also several carotenes and 9-cis carotenes (A4019, B4018). Despite a very minor loss of Cars in ΔpsaL PSI (only 2–3 Cars), it has a greatly reduced CD spectrum. The CD difference spectrum shows that the missing Car is red-shifted (512 nm maximum), which may indicate a contribution from xanthophylls like echinenone that have more conjugated π-bonds compared to carotene [52]. The I subunit, shown to be lost in other psaL-deficient monomers [6], binds echinenone or other xanthophylls at position I4020 – this Car might contribute to the CD band at 511 nm. The F/J subunits bind only three Cars, including the 9-cis zeaxanthin F4016, that contribute 30% of the total CD. A more comprehensive analysis comparing PSI from different cyanobacterial strains augmented with structure-based calculations [53] could validate the assignment of the Car CD.

Red Chls in trimeric PSI
Trimeric PSI from different cyanobacterial species has been shown to contain additional red absorption that is not observed in monomers [18, 20]. Upon comparison of the absorption as well as emission spectra of trimeric and monomeric PSI from *Synechocystis*, we determined that trimers contain an equivalent of 1.5–2 additional red Chls (based on equal oscillator strength). These red Chls emit with a maximum at 707–708 nm at RT (Figure 3) and 710–715 nm at 77 K (Figure 4) – in contrast, the lowest-energy states peak at 722–724 nm. Since a high-resolution crystal structure of PSI from a thermophilic cyanobacterium has been available, several groups have attempted to identify the Chls responsible for the red absorption/emission, assuming that they need to be strongly excitonically coupled [4, 21, 23, 26]. One strongly coupled pair that has been promoted as a likely candidate for the lowest-energy red Chls is A32/B7, located at the monomer-monomer interface, close to the I/L subunits. As our time-resolved fluorescence shows, the longest-wavelength emission is hardly affected in monomeric PSI. Hence, if Chls A32/B7
give rise to the trimer-specific red absorption, they cannot harbour the lowest-energy state, as previously suggested [21, 23]. We propose that A32/B7 forms a state emitting around 710 nm in the trimer but is blue-shifted in the monomeric complex due its altered environment (that can modify the pigment excitonic coupling as well as the charge-transfer character of the excited state). The lowest-energy states emitting at 722–724 nm remain to be located on one or more Chls outside the monomerization region, e.g. A38/A39, B31/B32, B37/B38 [23-25, 54].

Excitation dynamics in PSI

The deletion of the four subunits F, I, J, L from PSI results in discernible changes in its spectroscopic characteristics but the overall excitation trapping rate is unaffected – it remains around 25 ps at RT in PSI with open RC (where we have used external electron donors to quickly re-reduce P_{700}). This means that despite the changes in the energetics of the antenna, the quantum yield of photochemistry of the minimal PSI is the same as in the WT trimeric complex. These results illustrate the functional robustness of this remarkable enzyme. Nevertheless, we observed differences in the low-temperature fluorescence kinetics of monomeric complexes that parallel the reduction in far-red emission.

Energy equilibration between the bulk antenna and the red Chls in PSI is thought to occur considerably faster than the photochemical trapping, at least at RT, resulting in trapping of a quasiequilibrated state [27, 55]. Equilibration times in the order of 3–4 ps have been detected by time-resolved absorption and emission spectroscopy [18, 33, 34]. Our RT time-resolved fluorescence data are in line with this – although we are not able to resolve lifetimes shorter than ~10 ps, it is evident that the bulk-red equilibrium is reached well before trapping, which occurs with an effective lifetime of 25 ps. At 77 K, however, the red Chls are populated on different timescales spanning up to 50 ps. ET slows down at lower temperatures, on the one hand because of the reduced spectral overlap and, on the other, because of the restricted uphill transfer. Significant slowdown of ET at 77 K has been shown in different photosynthetic complexes [see 56 and refs. therein, 57] and also modelled [58, 59]. Another possible explanation for the slow spectral changes is that protein dynamics lowers the energy of the red states shifting the emission to longer wavelengths. CT states are associated with large reorganization energies [19, 20] and protein motions are generally more temperature-dependent than ET and frozen at cryogenic temperatures [60]. If this is indeed the case, then any assignment of spectral forms to red Chls
becomes more difficult as multiple spectral forms originate from CT states involving the same Chl molecules.

Quenching by open and closed RCs

Global analysis of the time-resolved fluorescence data at 77 K resolved three far-red components with lifetimes from ~200 ps to 1.3 ns (Figure 4), resembling previous results on PSI from T. elongatus [38] as well as the PSI core from higher plants [36]. It has been previously postulated that excitations on red Chls can be quenched by the RC (P700) either in its neutral or oxidized (P700+) state [14, 23, 38]. The broad near-infrared absorption of P700+ allows it to efficiently quench excitations on the redmost Chls in Arthrospira (Spirulina) platensis and T. elongatus [37, 38, 61]. In the present work, however, we found that P700+ is a less efficient quencher for the red Chls. The different behaviour is probably because of the shorter-wavelength emission of the red Chls in Synechocystis, and, consequently, larger spectral overlap with the absorption of P700 and smaller overlap with P700+.

A notable result is that the quenching of red states at 77 K is 30–40% faster in trimers (Figure 5) than in monomers, showing that the oligomeric state of PSI has a measurable effect on the excitation dynamics, at least at low temperature. The differences appear to hold irrespective of the oxidation state of the RC (Table 2). One possible reason for the accelerated fluorescence decay could be connectivity between monomers. Byrdin et al. [37] pointed that the 5 K fluorescence yield of PSI with 50% closed RCs is lower than the expected average yield of PSI with fully open and fully closed RCs, attributing the deviation to inter-monomer transfer. On the other hand, the additional red states in the trimers with intermediate energies between the bulk Chls and the lowest-energy forms, can mediate detrapping of excitations on the latter. As our theoretical calculations indicate, this has a more significant effect on the dynamics.

Theoretical modelling of the excitation dynamics

To aid the interpretation of the results, we created a kinetic model based on the crystal structure of PSI from Synechocystis [6], calculating the Förster ET between Chls. The Chl–Chl distances, orientations, and Coulomb couplings are very similar as in Byrdin et al. [23]. To keep the model as simple as possible, we assigned three strongly coupled Chl pairs as the sites of the red states – A32/B7, B31/B32, and B37/B38, carrying a combined oscillator strength equivalent to five Chls. The spectral parameters used for the calculation are shown in Supplementary Table S4. In
accordance with the experimental data, the first pair, residing at the monomer-monomer interface, is not the longest-wavelength form but emits at 713 nm in the trimeric PSI. The same three sites have been recently assigned as red Chls in *Synechocystis* PCC 6803 absorbing at 706, 707 and 714 nm [24, 62]. We tentatively set the same absorption wavelengths (see Supplementary table S4), which resulted in an excellent fit of the experimental 77 K absorption and emission spectra (Supplementary Figure S8). We then calculated the Förster ET rates between all Chls in the trimeric and the monomeric complexes. The only change imposed in the monomers was setting the absorption of the pair A32/B7 to 683 nm instead of 706 nm. The simulated DAES (Figure 6) qualitatively resemble the experimental results of both trimers and monomers, mimicking the noteworthy features and differences between them. While it is possible (and probably the case) that additional Chls contribute to the far-red absorption and emission in the real system, the model serves well to illustrate the dynamics of the red Chls and the mechanisms of excitation quenching.

Figure 6. Simulation of the fluorescence kinetics of PSI trimers (a) and monomers (b) using a structure-based model: decay-associated emission spectra obtained by global lifetime analysis of the simulated fluorescence decays.

The model shows ET to red Chls occurring on different timescales and two main decay components in the far-red region, with DAES peaking at 715 and 725 nm. Notably, the final decay lifetimes of the monomers are significantly longer. The model can also reproduce the dependence of the red Chl dynamics on the oxidation state of the RC (Supplementary Table S5). The time-dependent population of the three red Chl states confirms that B37/B38 is the longest-lived, whereas A32/B7 rapidly decays (Supplementary Figure S9). This is not surprising as the former is the lowest-energy form and A32/B7 is closer and more strongly coupled to P<sub>700</sub>. In monomers, the decay of the B31/B32 and B37/B38 pairs is slower than in trimers. The main
Reason for the slowdown is the lack of the red state at A32/B7, which can be verified by comparing the population dynamics in monomers with and without a red state on A32/B7 (Supplementary Figure S10). In other words, the intermediate-energy red state on A32/B7 acts as a bridge between the Chls emitting at longer wavelengths (especially B37/B38) and P_{700}, increasing the overall probability for excitations on the red Chls to be transferred to the RC and used for photochemistry. Obviously, this depends on the mutual arrangement of the red Chls and the RC. The pair A32/B7 located on the donor side of the complex, close to P_{700}, is well positioned to mediate energy transfer from the longest-wavelength form B37/B38 on the stromal side. Figure 7 illustrates the assigned red Chl pairs and the main routes of effective uphill ET between them according to the theoretical calculations. Note that the scheme is only qualitative as the actual ET rates will depend on the exact spectra and transition dipole orientations of the red Chls as well as on whether the RC is open or closed.

Figure 7. Red Chls in the PSI complex of *Synechocystis* PCC 6803 assigned in the theoretical model based on the crystal structure of trimeric PSI [6] – bulk Chls are in grey, P_{700} in blue, red Chls in yellow/red. The PsaL subunit is also shown for reference (cylinders). The A32/B7 pair (yellow) harbours a long-wavelength state only in the trimeric complex. The numbers indicate the absorption/emission wavelengths of the red states. The arrows represent different pathways of effective uphill ET from the red Chls to the RC with thicker arrows indicating faster ET.
Conclusions

In summary, we have resolved the timescales of ET to different red spectral forms in trimeric and monomeric PSI showing that equilibration with the red states spans timescales up to tens of ps competing with photochemical trapping at low temperatures. Excitations are not irreversibly trapped by the red states but are slowly (hundreds of ps) quenched by the RC. Monomeric PSI complexes lacking the PsaL subunit have fewer red Chls emitting at 710–715 nm. The loss of these intermediate-energy Chls results in a slower transfer of excitations from the lowest-energy red Chls (emitting at 720–725 nm) to the RC. Our theoretical calculations support the assignment of the Chl pair A32/B7 as the site of the trimer-specific state and B37/B38 as the site of the lowest-energy state. In PSI trimers, the A32/B7 pair facilitates ET from the lowest-energy state to the RC, thus increasing the overall efficiency of photochemical trapping. Our data also show that, despite a 20% lower protein content, ΔFIJL PSI has virtually identical kinetics of light harvesting and quantum efficiency of charge separation as wild-type PSI under physiological conditions, highlighting the remarkable functional robustness of the PSI core.

Data availability statement

The data (absorption spectra, circular dichroism spectra, fluorescence kinetics and DAES) presented in this manuscript are available for download at https://doi.org/10.6084/m9.figshare.13477101.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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