



Excitation energy transfer between Light-harvesting complex II and Photosystem I in reconstituted membranes

Parveen Akhtar^a, Mónika Lingvay^a, Teréz Kiss^b, Róbert Deák^b, Attila Bóta^b, Bettina Ughy^{a,c}, Győző Garab^a, Petar H. Lambrev^{a,*}

^a Hungarian Academy of Sciences, Biological Research Centre, Institute of Plant Biology, Temesvári krt. 62, 6726 Szeged, Hungary

^b Hungarian Academy of Sciences, Research Centre for Natural Sciences, Institute of Materials and Environmental Chemistry, Magyar tudósok körútja 2, 1117 Budapest, Hungary

^c ELI-ALPS, ELI-HU Non-Profit Ltd., Dugonics tér 13, 6720 Szeged, Hungary

ARTICLE INFO

Article history:

Received 25 November 2015

Received in revised form 22 January 2016

Accepted 27 January 2016

Available online 29 January 2016

Keywords:

Artificial membranes

Light harvesting

Proteoliposomes

State transitions

Time-resolved fluorescence

Thylakoid membranes

ABSTRACT

Light-harvesting complex II (LHCII), the major peripheral antenna of Photosystem II in plants, participates in several concerted mechanisms for regulation of the excitation energy and electron fluxes in thylakoid membranes. In part, these include interaction of LHCII with Photosystem I (PSI) enhancing the latter's absorption cross-section – for example in the well-known state 1 – state 2 transitions or as a long-term acclimation to high light. In this work we examined the capability of LHCII to deliver excitations to PSI in reconstituted membranes *in vitro*. Proteoliposomes with native plant thylakoid membrane lipids and different stoichiometric ratios of LHCII:PSI were reconstituted and studied by steady-state and time-resolved fluorescence spectroscopy. Fluorescence emission from LHCII was strongly decreased in PSI–LHCII membranes due to trapping of excitations by PSI. Kinetic modelling of the time-resolved fluorescence data revealed the existence of separate pools of LHCII distinguished by the time scale of energy transfer. A strongly coupled pool, equivalent to one LHCII trimer per PSI, transferred excitations to PSI with near-unity efficiency on a time scale of less than 10 ps but extra LHCII also contributed significantly to the effective antenna size of PSI, which could be increased by up to 47% in membranes containing 3 LHCII trimers per PSI. The results demonstrate a remarkable competence of LHCII to increase the absorption cross-section of PSI, given the opportunity that the two types of complexes interact in the membrane.

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1. Introduction

Photosynthetic organisms, particularly green plants, have evolved specialized multigene light-harvesting antenna systems that dynamically regulate the flow of excitation energy to the photosynthetic reaction centres (RCs). In vascular plants the two photosystems, Photosystem I (PSI) and Photosystem II (PSII) with their separate peripheral antenna proteins – LHCI (Lhca1–4) and LHCII (Lhcb1–6) – are preferentially located in the unstacked and stacked regions, respectively, of the chloroplast thylakoid membranes. Light harvesting is finely regulated and continuously adapted to the physiological conditions to achieve balanced energy input to the photosystems and prevent photodamage under conditions of excess light [1,2]. LHCII plays a major role in balancing and regulating the excitation energy and electron flow [3–5]. In the so-called state 1 – state 2 transitions in plants and green algae, excitation balance between PSI and PSII is maintained by shuttling of LHCII between them (for reviews see [6,7]). The classical view is that a subpopulation of the LHCII proteins, normally associated

with PSII (in state 1) becomes phosphorylated by the STN7 kinase [8,9] and migrates from the PSII-enriched stacked, granal region to the PSI-containing unstacked, stromal region, interacting with PSI and forming PSI–LHCII supercomplexes [10,11]. PSI–LHCII supercomplexes have been successfully isolated and purified using detergents [12–15] or styrene-maleic acid copolymer [16]. LHCII has been shown to transfer energy to PSI in the PSI–LHCII supercomplexes with high efficiency [12,15], so that Galka et al. [12] proposed that mobile LHCII can be considered as an integral part of PSI in state 2. Recent studies have put forward the notion that a significant amount of LHCII is associated with PSI under normal conditions [15–17] in the absence of phosphorylation. According to Wientjes et al. [15] more than half of the PSI complexes could bind one LHCII trimer, depending on the growth-light conditions. More recently, Bell et al. [16] have isolated PSI–LHCII membrane fractions from stacked spinach thylakoids at estimated stoichiometry of three LHCII trimers per PSI, at least some of them being functionally coupled to the RC. On the other hand, Ünlü et al. [18] examined the antenna sizes of PSII and PSI upon state transitions in *Chlamydomonas* and found that, despite the significantly higher number of mobile LHCII in the green alga, only a very small fraction, less than one trimer, actually delivers excitation energy to PSI in state 2. A similar conclusion was

* Corresponding author.

E-mail address: lambrev.petar@brc.mta.hu (P.H. Lambrev).

drawn in ref. [19] from a comparison of the PSI antenna size in state 1 and 2 estimated from electrochromic absorbance changes.

In the present study we investigated the ability of LHCII to serve as antenna of PSI by utilising a bottom-up approach. Isolated LHCII and PSI were reconstituted into lipid membranes, allowing to test the effective antenna size of PSI under controlled conditions at varying known stoichiometries of LHCII:PSI. We investigated the reconstituted PSI–LHCII membranes by steady-state and time-resolved fluorescence spectroscopy with special focus on determining the efficiency and dynamics of excitation energy transfer from LHCII to PSI and the overall quantum yield of PSI photochemistry. We found that a large fraction of the energy absorbed by LHCII was efficiently transferred to PSI in membranes containing up to three LHCII trimers per RC, thereby significantly increasing the effective PSI absorption cross-section. Understanding and quantifying the functional coupling of LHCII and PSI in the artificial membranes may have important implications for designing future solar devices. PSI is very attractive for engineering hybrid biosolar devices due to its high efficiency and inherent stability [20–24]; the ability to optimize and extend its absorption cross-section can be of significant advantage.

2. Materials and methods

2.1. Isolation of LHCII

PSII-enriched membrane fragments (BBY) were isolated from dark-adapted leaves of green-house grown pea (*Pisum sativum*). LHCII was isolated from BBY membranes, solubilized with 0.7% dodecyl- β -maltoside (β -DM), by sucrose density gradient ultracentrifugation as in Caffarri et al. [25]. LHCII trimer bands collected from the gradients were washed with 10 mM Tricine buffer (pH 7.8) and concentrated with 30 kDa cutoff Amicon filters (Millipore), then frozen in liquid nitrogen and stored at -80°C until use.

2.2. Isolation of PSI

PSI-enriched stromal membrane vesicles were isolated from pea leaves by fractionation with digitonin, following the protocol of Peters et al. [26]. The preparations, with chlorophyll (Chl) a/b ratio of 9–10, were further purified by solubilisation with 1% β -DM and ultracentrifugation on a 0.1–1 M sucrose density gradient in a swing-out rotor at 200,000 g for 20 h. The green bands containing PSI–LHCI supercomplexes were collected, concentrated with Centricon devices (Millipore) and frozen in liquid nitrogen. Immunoblot analysis showed that after β -DM solubilisation and purification the PSI samples were virtually free of LHCII proteins (Supplementary Fig. S1).

2.3. PSI–LHCII membranes

Liposomes were prepared from mixtures of plant thylakoid lipids (50.0% (w/v) monogalactosyldiacylglycerol (MGDG), 31.0% digalactosyldiacylglycerol (DGDG), 10.7% phosphatidylglycerol (PG) and 8.3% sulfoquinovosyldiacylglycerol (SQDG)) as described in [27] with an additional 10-step freeze–thaw cycle prior to extrusion.

For preparing PSI–LHCII membranes, purified solubilized trimeric LHCII complexes and PSI–LHCI supercomplexes were mixed at molar Chl ratios of LHCII/PSI equal to 0.26, 0.52 and 0.78 – equivalent to approximately one, two and three LHCII trimers per PSI, respectively. The protein mixture was then added dropwise to a suspension of liposomes at molar lipid:protein ratio of 450:1, calculated based on PSI content only. After mild sonication in a bath sonicator the mixture was incubated at room temperature for 30 min. The detergent was then removed by cycled incubation with absorbent beads (Bio-Beads SM2, Bio-Rad). The sample was finally centrifuged for 30 min at 25,000 g to remove unincorporated protein aggregates. LHCII-only and PSI-only proteoliposomes were reconstituted at molar lipid:protein

ratios of 100:1 or 300:1 for LHCII, and 450:1 for PSI. The orientation of the pigment–protein complexes in the reconstituted membranes was tested by measuring the linear dichroism of the samples aligned by gel compression [28].

2.4. Chlorophyll determination

The Chl content of all samples was determined spectrophotometrically from 80% acetone extract using molar absorption coefficients from Porra et al. [29].

2.5. Dynamic light scattering

The average size and size distribution of the sample components were measured by a W130 dynamic light scattering apparatus (AvidNano, UK). After appropriate dilution, 80 μl of the samples was loaded into microcuvettes and thermostated to 25°C for the measurement. Data analysis was performed with the “i-Size” software supplied with the instrument.

2.6. Freeze-fracture electron microscopy

The freeze-fracturing and electron microscopy of reconstituted proteoliposomes was done as described in [27]. Briefly, the sample suspension, deposited onto a gold sample holder, was flash-frozen in partially solidified Freon. Fracturing was performed at -100°C in a freeze-fracture device (BAF 400D, Balzers AG, Liechtenstein) and the surface was etched at -110°C . Replicas were prepared by platinum-carbon shadowing, placed on copper grids and examined in a Morgagni 268D (FEI, The Netherlands) transmission electron microscope.

2.7. Absorption and circular dichroism spectroscopy

Absorption and CD spectra were recorded between 350 and 750 nm with 3 nm spectral resolution using an Evolution 500 dual-beam spectrophotometer (Thermo Scientific) and a J-815 spectropolarimeter (Jasco). Measurements were carried out at room temperature. The samples were diluted in 10 mM NaCl and 10 mM Tris/HCl (pH 7.8) buffer to absorbance of 1.0 at the red maximum. Solubilized complexes were measured in buffer containing 0.03% β -DM. Optical path length was 1 cm.

2.8. Low-temperature fluorescence spectra

For measurements at 77 K diluted samples were deposited on filter paper discs to ca. $0.5\ \mu\text{g cm}^{-2}$ Chl and immersed in liquid nitrogen. Steady-state fluorescence spectra were recorded from the discs with a Fluorolog spectrofluorometer (Jobin Yvon Horiba). Emission spectra were recorded with excitation wavelength of 436 nm and 3 nm detection bandwidth. Excitation spectra were recorded at 730 nm detection wavelength.

2.9. Time-resolved fluorescence

Room-temperature fluorescence decays were recorded by time-correlated single-photon counting using a FluoTime 200 spectrometer (PicoQuant, Germany) equipped with a microchannel plate detector (Hamamatsu, Japan) and a PicoHarp 300 TCSPC system (PicoQuant). Excitation pulses at 633 nm with 6 ps temporal width, 0.1 pJ pulse energy and 20 MHz repetition rate were generated by a WhiteLase Micro supercontinuum laser (Fianium, UK). Fluorescence emission was detected through a monochromator at wavelengths between 670 and 750 nm and binned in 4 ps time channels. The sample was continuously circulated through a flow cell with 1.5 mm path length. The optical density at the excitation wavelength was 0.03. The total instrument

response (IRF) width was 40 ps, measured using 5% Ludox as scattering solution.

Fluorescence decays collected at different detection wavelengths were analysed by a global lifetime fitting routine using a kinetic model and convolution with the measured IRF. Kinetic models were either sums of exponential decays (global analysis) or compartment models (target analysis). The fitting algorithm, written in MATLAB, minimized the squared sum of residuals weighted by the Poisson distribution. The method of variable projection was employed, wherein rate constants (lifetimes) were global nonlinear fit parameters and amplitudes (spectra) were wavelength-dependent linear least-squares fit parameters [30].

3. Results

3.1. Composition and structural organization of the proteoliposomes

The Chl content of PSI, LHCII and PSI–LHCII proteoliposomes was checked to control that the stoichiometry after reconstitution matched the preset one for each sample. The Chl *a/b* ratios from one set of experiments are reported in Table 1. The number of LHCII trimers per PSI was back-calculated from the measured Chl *a/b* ratio, assuming 42 Chls per LHCII [31], 156 Chls per PSI [32] and respective Chl *a/b* ratios of 1.4 and 13 (measured). The calculated LHCII:PSI stoichiometries were equal to the predetermined ones, within 10% margin, attesting that both PSI and LHCII were efficiently incorporated into the lipid membrane.

The size distribution of the proteoliposomes was analysed by dynamic light scattering. The mean hydrodynamic diameter for liposomes without protein was 167 nm (Table 1), whereas in proteoliposomes typically about 90% of the mass was found around particle diameters in the range of 40–80 nm and a secondary peak at 180–270 nm. The smaller diameters probably originate from protein scattering centres, whereas the total vesicle sizes are represented by the latter figures, which incidentally coincide with typical diameters observed in freeze-fracture electron microscopy images (Fig. 1). The freeze-fracture images also show the protein distribution on the membrane surface. The surface of LHCII proteoliposomes appears relatively smooth (Fig. 1A) and individual complexes cannot be discerned, whereas PSI membranes show sharp protrusions that easily identify the positions of the PSI complexes (Fig. 1B). While occasional clusters of PSI and PSI-free regions can be observed in the images, typically the proteins seem to be evenly distributed in the densely packed membranes.

3.2. Absorption and CD spectra

Reconstituted PSI–LHCII membranes were characterized by absorption and CD spectroscopy and compared with membranes containing only PSI or LHCII (Fig. 2). The spectra of PSI (blue curves in Fig. 2A and C) and LHCII in lipid membranes (red curves in Fig. 2B and D) are comparable to the well-known spectra of the respective solubilized complexes [15,33], apart from a slight blue shift of the Q_y maximum of LHCII membranes (674 nm compared to 675 nm in solubilized LHCII).

Table 1
Composition and hydrodynamic diameters of PSI–LHCII membranes.

Sample type	Chl <i>a/b</i>	LHCII/PSI	Diameter 1 (nm)	Diameter 2 (nm)
PSI	12.85 ± 0.16	–	83 ± 14	265 ± 65
LHCII	1.36 ± 0.03	–	51 ± 5	240 ± 32
PSI–LHCII (1:1) ^a	5.99 ± 0.19	1.0	52 ± 8	215 ± 23
PSI–LHCII (1:2)	4.09 ± 0.11	2.1	43 ± 5	181 ± 20
PSI–LHCII (1:3)	3.50 ± 0.03	2.9	41 ± 2	237 ± 32
Empty liposomes	–	–	167 ± 21	

^a The ratio in parenthesis (1:1) refers to the pre-calculated molar ratio LHCII trimers:PSI.

The absorption spectra of PSI–LHCII membranes, compared to PSI-only membranes (Fig. 2A) feature additional absorption by the Chl *b* molecules in LHCII, as seen in PSI–LHCII supercomplexes from native membranes [12]. For quantitative comparison, we subtracted the PSI absorption spectrum from the spectra of the PSI–LHCII membranes, taking into account the estimated PSI content of each sample. The resulting absorption difference spectra very well matched the spectrum of LHCII-only membranes, not only in shape but also in amplitude, as exemplified by Fig. 2B. The absorption spectra confirm that LHCII and PSI were present in the membranes in the expected stoichiometric ratios. Moreover, linear dichroism spectra were recorded to verify that the proteins were properly inserted into the membrane bilayer (Supplementary Fig. S2).

CD spectra were measured to check for possible changes in protein conformation or exciton interactions within and between complexes in the mixed PSI–LHCII membranes (Fig. 2C and D). The PSI spectra are characterized by strong excitonic bands in the Chl *a* Q_y region – at 685 nm and 671 nm – and a positive carotenoid band at 510 nm [34, 35]. In the CD spectrum of PSI–LHCII membranes, the prominent LHCII peaks at 650 nm and 492 nm are distinguished. The spectra of the mixed membranes can very well be described by linear combinations of the measured CD spectra of LHCII and PSI, with small (<10%) residuals at 482 nm and 668 nm. Conversely, subtracting the PSI CD spectrum from the CD spectra of the LHCII–PSI membranes resulted in CD difference spectra that perfectly matched the CD spectrum of LHCII-only membranes except for small differences at 482 nm and 668 nm (Fig. 2D). The differences might indicate exciton interactions between LHCII and PSI Chls or conformational changes driven by the PSI–LHCII interaction but owing to the highly variable nature of the LHCII CD spectra [27], that cannot be ascertained. Since LHCII exhibits markedly different CD spectra in detergent micelles, lipid membranes, or aggregates [27], the CD spectra can serve as a marker for the presence of complexes in the sample that are inserted in the membrane. The CD difference spectra showed no signature of LHCII aggregation in any of the PSI–LHCII samples.

3.3. Fluorescence emission and excitation spectra at 77 K

Steady-state fluorescence emission and excitation spectra at 77 K were recorded from reconstituted PSI, LHCII and PSI–LHCII proteoliposomes (Fig. 3). PSI and LHCII exhibit rather different excitation and emission spectra (Fig. 3A, B) and are easily distinguishable. The excitation spectrum of LHCII shows higher amplitude in the 460–490 nm region, due to its higher Chl *b* content compared to PSI. The emission spectrum of PSI (Fig. 3B, blue curve) is determined by the red Chl forms acting as traps for all excitations at 77 K and resulting in a broad band at 730 nm [12,36], while the emission at 680 nm is very low. The emission from LHCII liposomes peaks at 680 nm; at 730 nm the intensity was 50% compared to PSI, on an absolute scale. LHCII liposomes also showed a shoulder at 700 nm, as previously observed [37,38] somewhat resembling the spectra of LHCII aggregates [33,39]. The 680 nm band is attributed to the fluorescence emission from the Q_y energy level of molecules of Chl *a* bound to LHCII.

The excitation and emission spectra of PSI–LHCII membranes showed features of both PSI and LHCII – intermediate amplitudes in the Chl *b* region of the excitation spectra (Fig. 3C) and peaks in the emission spectra at both 680 nm – from LHCII – and at 730 nm – primarily from PSI (Fig. 3D). The emission at 680 nm gradually increased with increasing the LHCII:PSI ratio in the membranes, which shows that at least part of the excitation energy in LHCII was not trapped by PSI. On the other hand, the increased 480 nm amplitude in the excitation spectra is evidence that part of the excitations in LHCII were transferred to PSI. These data can be compared with theoretical spectra, computed for PSI–LHCII mixtures at the same PSI:LHCII ratios but in the absence of EET (Supplementary Fig. S3). The measured spectra show weaker emission from LHCII (at 680 nm) and stronger contribution of Chl *b* to the excitation of 730 nm fluorescence, compared to the theoretical ones.

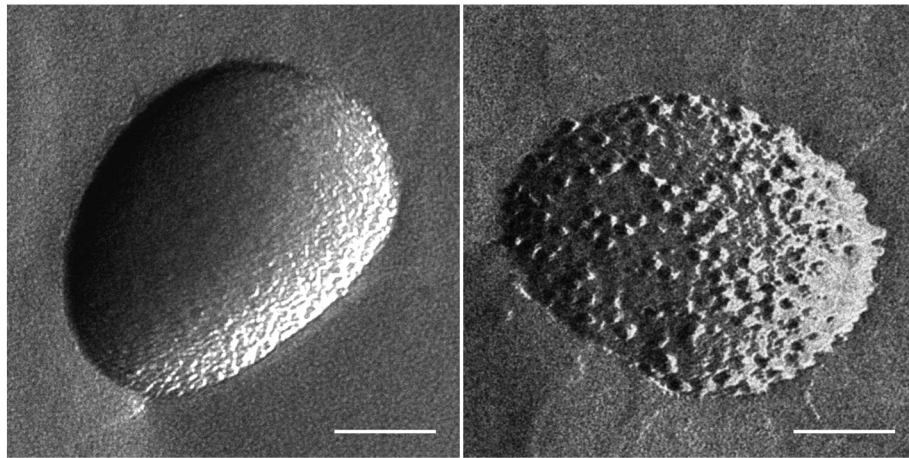


Fig. 1. Representative freeze-fracture electron microscopy images of reconstituted proteoliposomes. A – with LHCII only; B – PSI-LHCII (2 trimers per PSI). Scale bar – 100 nm.

If LHCII fluorescence was indeed quenched by PSI, then the emission should be recovered by disrupting the protein interactions in the membrane with a detergent. After solubilization of the reconstituted PSI-LHCII membranes with 0.03% β -DM, the Chl *b* amplitude of the excitation spectra slightly decreased (Fig. 3E) and the intensity at 680 nm in the

fluorescence emission spectra increased 6-fold (Fig. 3F). In comparison, adding β -DM to LHCII-only membranes led to a two-fold increase in the fluorescence intensity (data not shown). The emission spectrum of the solubilized equimolar PSI:LHCII mixture was comparable to the spectrum of solubilized native PSI-LHCII fraction, reported by Galka et al. [12].

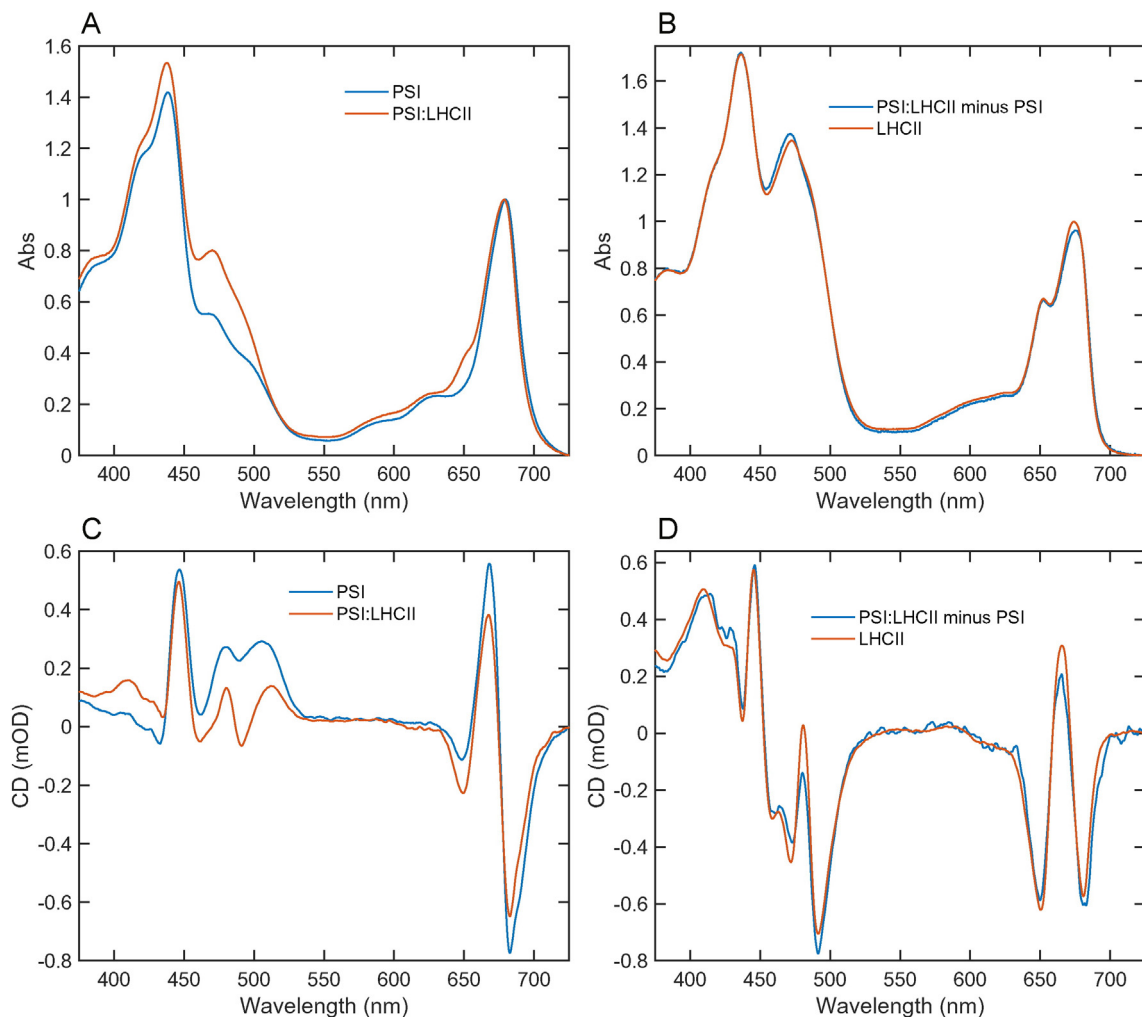


Fig. 2. Absorption and CD spectra of PSI-LHCII membranes. A, C – absorption and CD spectra of proteoliposomes reconstituted with PSI or with PSI-LHCII (2 trimers per PSI); B, D – absorption and CD difference spectra PSI-LHCII minus PSI compared with the spectra of LHCII-only proteoliposomes.

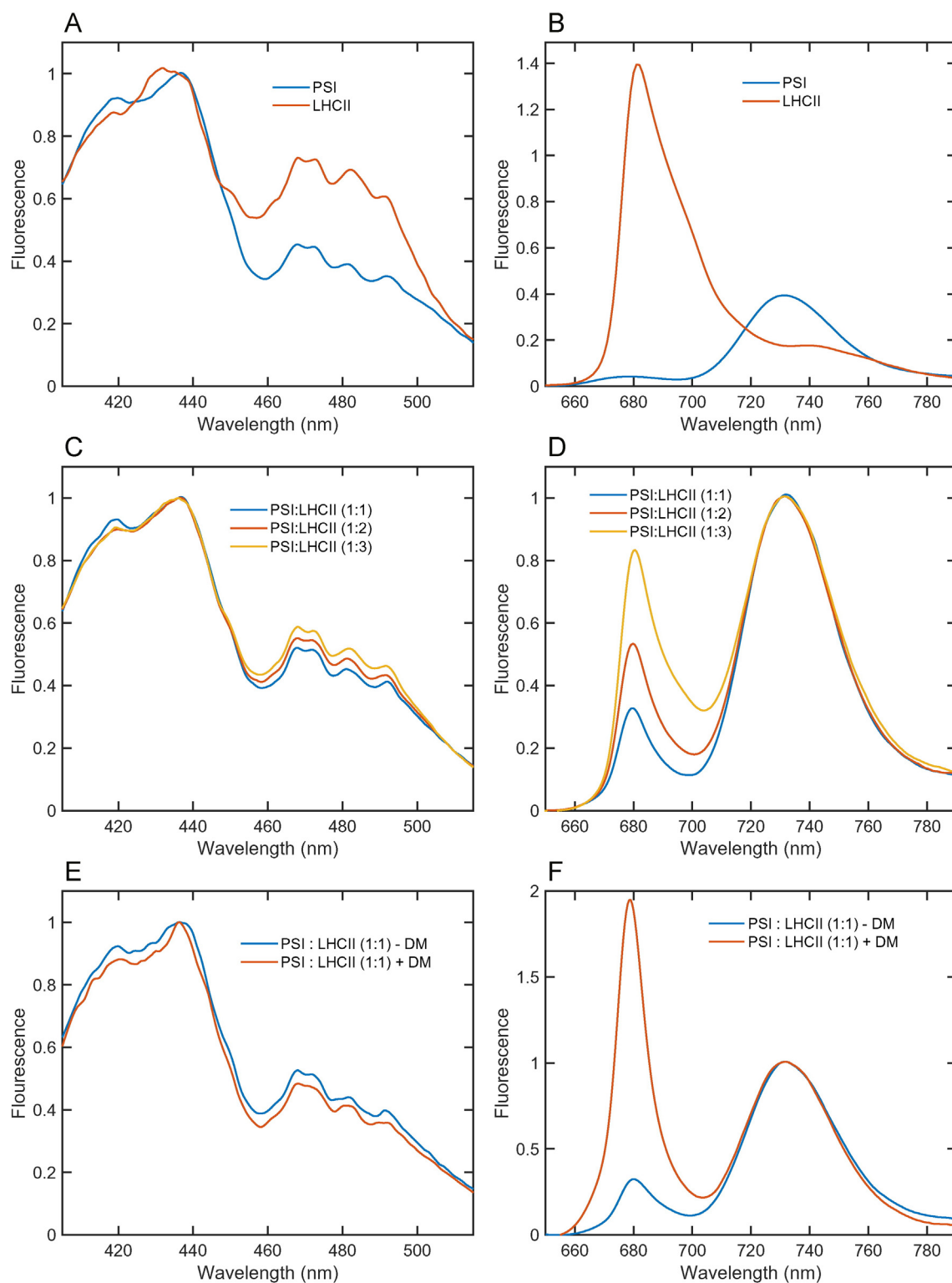


Fig. 3. Fluorescence spectra of PSI-LHCII membranes at 77 K. A – Excitation spectra of the 730 nm emission from PSI- and LHCII-only proteoliposomes; B – emission spectra (436 nm excitation wavelength) of PSI- and LHCII-only proteoliposomes; C, D – Excitation and emission spectra of PSI-LHCII proteoliposomes (1, 2 and 3 trimers per PSI). E, F – Excitation and emission spectra of PSI-LHCII membranes (1 trimer per PSI) before and after solubilisation with 0.03% β -DM;

3.4. Time-resolved fluorescence of solubilized PSI-LHCII

To get more insight into the efficiency and dynamics of energy transfer in the reconstituted PSI:LHCII membranes we recorded the picosecond fluorescence decay kinetics at room temperature. In addition to the reconstituted membranes, we measured the fluorescence

kinetics of isolated LHCII and PSI in detergent micelles as well as proteoliposomes under the same experimental conditions and used these as reference for the analysis of the reconstituted mixtures. The measured fluorescence decays were modelled with a sum of exponential decay components convoluted with the instrument response function (global analysis) and by kinetic compartmental models (target analysis).

Analysis of the time-resolved fluorescence results of solubilized PSI–LHCI supercomplexes is summarized in Fig. 4 and described below.

3.4.1. Global analysis

The fluorescence decay kinetics of PSI over 2 ns time range can be described with three lifetimes – 17 ps, 71 ps, and 163 ps – generally in line with published results from different groups [40–43]. A fourth,

3 ns lifetime of a minor amplitude (<2%) was needed for a good fit, which can be attributed to a small number of free Chls. The decay-associated emission spectra (DAES) are plotted in Fig. 4A. The majority of excitations decay with a 17 ps lifetime, as was previously reported [41–43]. The DAES for this component peaks around 685 nm and is attributed to emission from both core and antenna Chls. The second-largest component – 71 ps – shows a prominent broad peak around 720 nm, originating from low-energy states – the well-known “red” Chls – in PSI. It has the largest contribution to the fluorescence intensity at 720 nm (71%). The third component peaks at 720–730 nm, has a relatively small amplitude (12%) and 27% contribution to the intensity at 720 nm. The average fluorescence lifetime at 688 nm is 44 ps (excluding the emission from free Chls).

3.4.2. Target analysis

To extract more information about the physical origin of the observed fluorescence kinetics and DAES, we performed target compartmental analysis [44,45]. A kinetic model, with compartments corresponding to different physical parts of the system and species-associated emission spectra (SAES) for each compartment, was fitted to the experimental kinetics. The minimal model that could satisfactorily describe the data contained three connected compartments for PSI – one for the bulk of Chls and two for low-energy emitting “red” Chls (See Supplementary Information for details). The fitted SAES for this model are plotted in Fig. 4B and the kinetic scheme with fitted rate constants is shown in Fig. 4C. The lifetimes, DAES and, hence, reconstructed fluorescence decays (Supplementary Fig. S4) were identical to those obtained by global analysis. According to the model, the 17 ps decay component is associated primarily with trapping (charge separation) and energy transfer to the “red” Chls. The two longer-lived components are associated mainly with excitation equilibration between the bulk and the two “red” Chl compartments – peaking at 720 nm and 735 nm. The time scales of equilibration (inverse sum of the forward and backward rate constant) are 22 ps and 125 ps, respectively. The size (absorption cross-section) of the “red” compartments, estimated from the forward-to-backward rate constant ratios and the enthalpy difference between “red” and “bulk” Chls (Supplementary Information), is ~4 Chls for the “Red 1” compartment and ~1 Chl for “Red 2”.

3.5. Time-resolved fluorescence of reconstituted membranes

3.5.1. Global analysis

Representative DAES of reconstituted membranes containing LHCII, PSI or both LHCII and PSI (at a molar ratio of 3:1), are shown in Fig. 5. The decay of LHCII membranes was dominated by two lifetime components in the 1–3 ns range with nearly identical spectra peaking at 680 nm (Fig. 5A). The shortest lifetime was 400 ps. When membranes were reconstituted at lower lipid:protein ratios, slightly shorter lifetimes were found so that the average lifetime dropped from 2 to 2.3 ns at lipid:protein ratio of 300:1 to 1.3–1.6 ns at 100:1 (data not shown), similar to previous studies [46]. The fluorescence kinetics of PSI in liposomes (Fig. 5B) were practically identical to solubilized PSI (cf. Fig. 4A), with very similar lifetimes and no noticeable differences in the shape or relative amplitudes of the DAES.

Five lifetime components were necessary to model the fluorescence decays of PSI–LHCII membranes over 8 ns time range. The lifetimes and relative amplitudes at 680 nm for different PSI–LHCII stoichiometries are reported in Table 2. Several notable features are recognizable in the DAES (Fig. 5C, also see Supplementary Fig. S5). The fastest component (21–25 ps) in all samples is similar in shape to the 19 ps PSI component, but the second-fastest (74–87 ps) DAES appears to have contribution from both “red” antenna Chls and from LHCII. Three longer-lived components are also present with DAES shape similar to LHCII. The summed relative contribution of these components is larger at higher LHCII:PSI ratios. Also the amplitude of the 211–262 ps

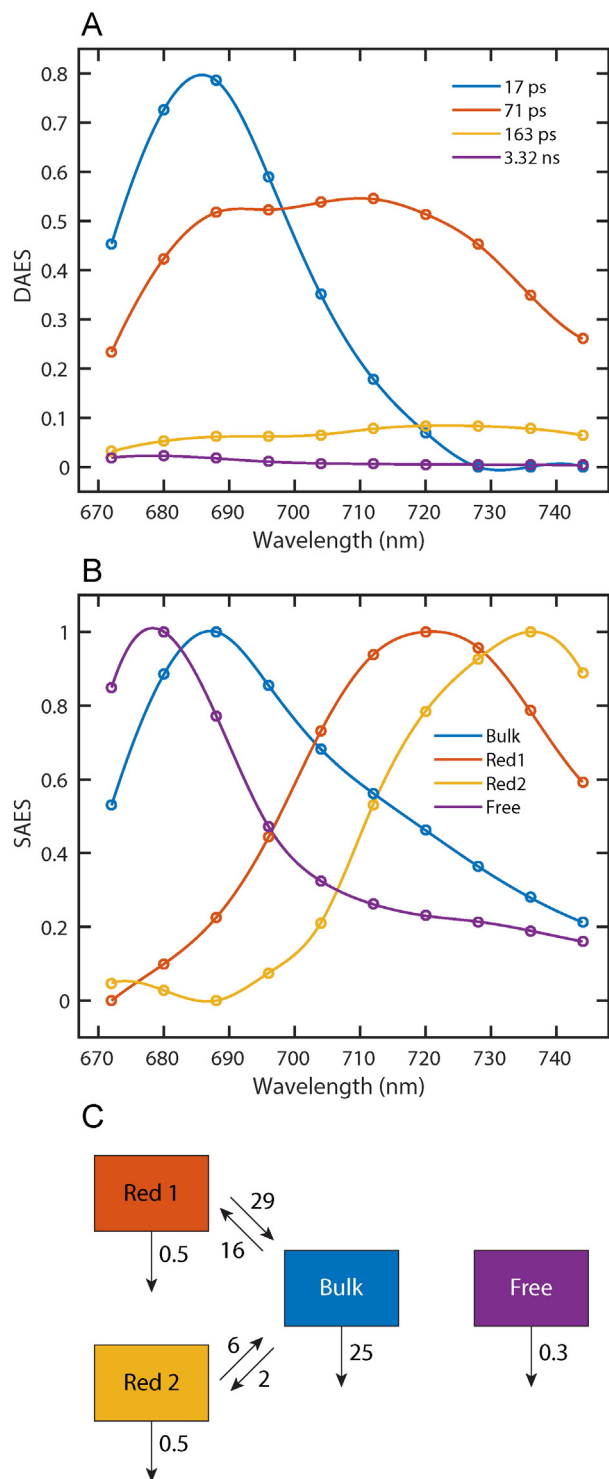


Fig. 4. Fluorescence kinetics of solubilized PSI. A – Decay-associated emission spectra obtained from global exponential lifetime analysis of the fluorescence kinetics; B – Normalized species-associated spectra obtained from target analysis; C – Kinetic scheme used in the target analysis and fitted rate constants (ns^{-1}).

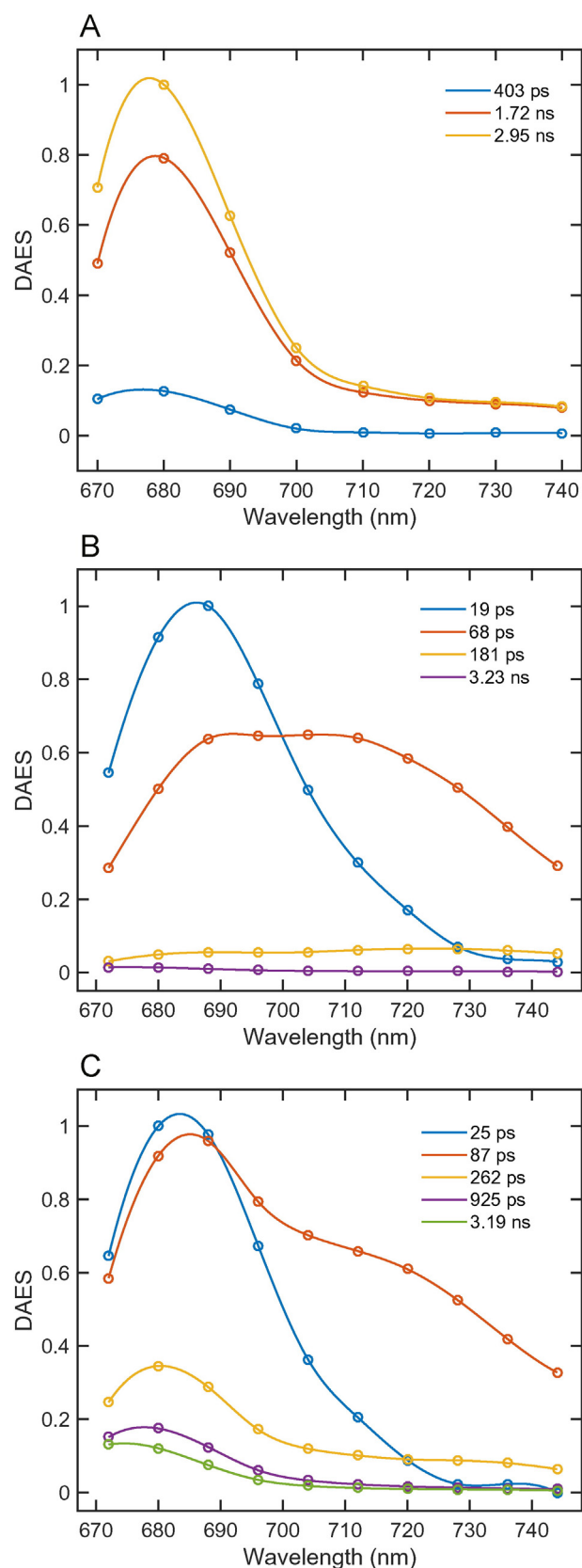


Fig. 5. Decay-associated fluorescence emission spectra of LHCII and PSI reconstituted in membranes. A – LHCII; B – PSI; C – PSI-LHCII (1:3).

component increases with LHCII content relative to the nanosecond components. Thus, the main LHCII decay component in PSI-LHCII membranes is about one order of magnitude faster than the main decay

Table 2

Fluorescence lifetimes and relative amplitudes at 680 nm of reconstituted membranes.

LHCII		PSI		PSI:LHCII (1:1)		PSI:LHCII (1:2)		PSI:LHCII (1:3)	
τ (ps)	a	τ (ps)	a	τ (ps)	a	τ (ps)	a	τ (ps)	a
–	–	19	62	21	51	24	45	25	39
–	–	68	34	73	34	80	37	87	36
403	7	181	3	214	5	232	7	262	13
1720	41	–	–	1478	3	1155	4	925	7
2950	52	3230	1	3904	8	3625	8	3193	5

component in LHCII-only membranes – a strong evidence for excitation energy transfer from LHCII to PSI.

The lifetime of the major decay component of PSI appeared to increase progressively with the proportion of added LHCII, up to 30% compared to isolated PSI, while its relative amplitude diminished (Table 2). The second decay lifetime component also increased by up to 25%. A similar relative increase in the lifetimes was observed by Wientjes et al. [15].

3.5.2. Target analysis

The fluorescence kinetics of PSI-LHCII membranes were analysed by fitting kinetic schemes based on the isolated PSI model and additional compartments representing LHCII. The results obtained with a 5-compartment model that best describes the kinetics are shown in Fig. 6 in terms of DAES, SAES and rate constants. The kinetic scheme is similar to the one applied by Le Quiniou et al. [47,48] to model the kinetics of PSI-LHCI and PSI-LHCI-LHCII supercomplexes from *Chlamydomonas*. For clarity, the deactivation rate constants of the antenna compartments (0.5 ns^{-1}) are omitted from the figure. The PSI compartments, “Bulk” and “Red”, have very similar SAES as in isolated PSI (Fig. 4B). In place of a second red antenna, the model includes two connected LHCII compartments with linked SAES (Fig. 6C, yellow curve). The SAES, peaking at 680 nm, undoubtedly identifies these compartments as LHCII. Finally, a free compartment represents unconnected LHCII with minor contribution from free pigments. The connected LHCII transfer energy to PSI on time scales of $<10 \text{ ps}$ (the forward rate constant was constrained to 80 ns^{-1}) and 300 ps. According to this model the 280 ps decay lifetime mainly represents decay of excited states in LHCII complexes energetically connected to PSI. The model reproduces the increased PSI decay lifetimes (due to the larger antenna) as well as the characteristic change in the 80 ps DAES compared to isolated PSI – a direct result of the coupling with LHCII. Because the decay of PSI excitations is faster than the rate of donation from LHCII, there are no observable negative-amplitude DAES.

It must be noted that a simpler 4-compartment model having only one connected LHCII compartment could fit the data (Supplementary Fig. S6). In fact the goodness of fit is equal or better than the 5-compartment model (Supplementary Fig. S7 and S8); however, the PSI rate constants and the increase in absorption cross-section are not accurately quantified. In this model the strongly coupled LHCII pool is implicitly included as an intrinsic part of PSI, hence the fitted effective PSI charge separation rate is slower by 30%.

4. Discussion

4.1. Kinetics of PSI

The ultrafast kinetics of energy transfer and trapping in PSI has been the subject of many works in the past several years and exhaustive treatment can be found in the literature. Nevertheless, in order to understand the excitation dynamics of the reconstituted PSI-LHCII membranes, we measured and analysed the picosecond fluorescence kinetics of solubilized PSI and PSI-lipid membranes under the same experimental conditions. This allows us to draw parallels with similar studies on eukaryotic PSI. In our results the fluorescence decays were

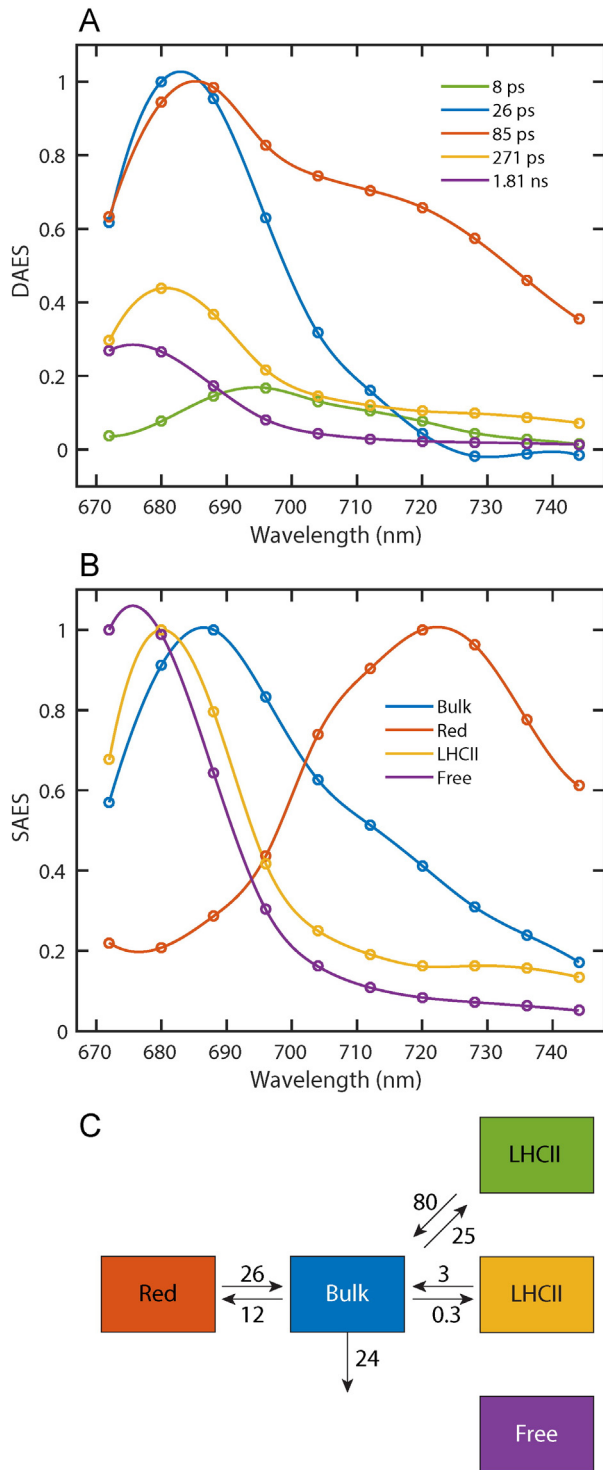


Fig. 6. Target analysis of the fluorescence kinetics of PSI-LHCII (1:3) membranes. A – Decay-associated emission spectra; B – Normalized species-associated spectra; C – Kinetic scheme and fitted rate constants (ns^{-1}). Additionally the model includes decay rate constants of 0.5 ns^{-1} for all antenna compartments. See Supplementary Information (Fig. S6) for a comparison with a simpler, 4-compartment model.

described with two dominant lifetimes, 17 ps and 71 ps. Lifetimes of 16 ps [41], 19–24 ps [40,43,47,49] and 11–14 ps [12,42] in PSI-LHCI preparations from higher plants and green algae have been attributed to a different extent of trapping of excitations in the PSI core as well as equilibration of bulk antenna Chls and the low-energy emitting states. The DAES in the present data show features of both processes and

judging from the kinetic modelling this lifetime has about 70% contribution from trapping and 30% from energy equilibration with “red” Chls. The main decay component associated with the “red” antenna in higher plant PSI is typically found in the 60–80 ps range [40,50], as is the present case, or as two separate components in the range of 30–100 ps [42]. These lifetimes most probably originate from far-red emitting states in both the core and peripheral LHCI antenna [40,50]. A longer-lived decay component (160–180 ps) with a spectrum peaking around 735 nm was also necessary for a complete description of the kinetics in our measurements. There is much discrepancy in the literature regarding the lifetime, spectral shape, and physical assignment of this decay component [43]. Because of this variability it likely originates from structural heterogeneity in the PSI supercomplexes.

Based on the lifetime analysis, we constructed a minimal kinetic model with three compartments. As the pigment/subunit heterogeneity in PSI-LHCI is clearly much more complex, the rate constants in the model represent aggregated effective time scales of energy equilibration and trapping. We could of course describe the kinetics with more complex models as well. For example, using the kinetic scheme of Slavov et al. [41], which includes additional fast charge separation, an equally good fit was achieved with rate constants of secondary charge separation and recombination exactly as reported, but all other parts of the model (spectra and rate constants) remained the same as with the simpler scheme.

The average fluorescence lifetime of PSI (at 688 nm) was 44 ps – in good agreement with published values [40–43] and almost equal to the effective “Bulk” trapping time of 40 ps (the inverse decay rate constant of the “Bulk” compartment). The quantum yield of photochemistry can be calculated from the average lifetime as $\Phi_p = 1 - \tau/\tau'$, where τ is the fluorescence lifetime of the system and τ' is the lifetime in the absence of charge separation. If we assume $\tau' = 2 \text{ ns}$ – the average fluorescence lifetime of isolated LHCII [51], the estimated quantum yield is 97.8%. However, because the time scales of trapping and equilibration with the “red” antenna Chls are comparable, the fluorescence lifetime at a given wavelength is not equal to the overall excited-state lifetime. Using the kinetic model to calculate the average excited-state lifetime we obtained a value of 73 ps. Hence, the presence of “red” Chls slows down the effective trapping time by a factor of 1.8 – from 40 ps to 73 ps. The quantum yield of photochemistry estimated from the kinetic model is 96.4%. Nevertheless, as discussed by Wientjes et al. [43], this drop in quantum efficiency of PSI caused by the red Chls is surpassed by the benefit they offer in terms of enhanced absorption.

4.2. Dynamics and efficiency of energy transfer between LHCII and PSI

The steady-state fluorescence emission at 77 K served as a straightforward check for the presence of energy transfer in the reconstituted PSI-LHCII membranes, because a significant fraction of LHCII fluorescence was quenched and at the same time the relative emission from PSI was increased. From the increase in emission intensity at 680 nm after detergent solubilisation of the membranes it could be estimated that 60–70% of LHCII fluorescence was quenched. The energy transfer efficiency is overall lower compared to the results from similar measurements on PSI-LHCII supercomplexes isolated from native thylakoid membranes [12,14,15], but in the present case the number of LHCII complexes is larger.

The time-resolved fluorescence data provided a more consistently reproducible and reliable basis for estimation of the energetic connectivity. The following numbers are based on the measurement of PSI-LHCII membranes reconstituted at stoichiometric ratio of 3 LHCII trimers per PSI (Fig. 4C), or about 57% Chls in PSI and 43% Chls in LHCII. The average lifetime of this sample at 688 nm was 222 ps, compared to 61 ps for PSI and ~2 ns for LHCII-only membranes (Table 3). A simple rough estimation of the energy transfer efficiency can be made based on the average lifetimes alone. If there was no energetic

Table 3
Average lifetimes and photochemical quantum efficiencies of PSI–LHCII membranes^a.

Type	$\langle\tau\rangle$, ps	Φ_p
PSI	44	0.98
PSI:LHCII (1:1)	267	0.87
PSI:LHCII (1:2)	269	0.87
PSI:LHCII (1:3)	222	0.89

^a $\langle\tau\rangle$ – average fluorescence lifetime at 688 nm; Φ_p – quantum yield of photochemistry; $\Phi_p = (1 - \langle\tau\rangle) / 2000$.

connectivity, the lifetime of this stoichiometric mixture should be close to 1 ns. Conversely, the “LHCII lifetime” in the PSI–LHCII membranes can be calculated as $(222 - 0.57 \times 61) / 0.43 = 435$ ps. It follows that PSI traps excitations from LHCII with overall efficiency of 78%. This result is an experimental validation of the postulated function of PSI as potentially photoprotective quencher of excess excitations [52] – reduction of the excitation lifetime prevents from potential detrimental reactions in LHCII but also in PSII, if LHCII is connected to both PSI and PSII in the membrane.

Target analysis of the fluorescence decay kinetics produces kinetic models of the PSI–LHCII system, which can quantitatively answer all questions regarding the time scales of energy transfer, partitioning of excitations between the compartments, the effective absorption cross-section of the connected antenna and the efficiency of energy transfer and trapping (charge separation). The analysis reveals the existence of different pools of LHCII with respect to the time scale and efficiency of energy coupling. Three separate pools can be distinguished – “strongly coupled”, transferring energy on a time scale of less than 10 ps, “weakly coupled” – with energy transfer time scale of 300 ps, and “free” LHCII.

The size (absorption cross-section) of the compartments can be estimated in the following way. The initial excitation distribution between PSI and the different LHCII compartments was adjusted to achieve equal amplitudes of the fitted SAES. Assuming that the SAES of bulk PSI Chls and LHCII should have similar intensity (per Chl) and that the absorption cross-section of LHCII and PSI Chls is about the same at the excitation wavelength (633 nm), then the initial excitation is approximately proportional to the number of Chls in each compartment. The excitation distribution for the extended scheme was as follows: PSI – 1, LHCII (strongly connected) – 0.3, LHCII (weakly connected) – 0.2, “Free” LHCII – 0.15. Thus, the total estimated absorption cross-section of LHCII was 65% compared to PSI, equivalent to about 2.4 trimers.

The efficiencies of excitation energy trapping from each LHCII pool (number of photons trapped by PSI charge separation over the number of photons absorbed by LHCII) were estimated by solving the model with initial excitation on each LHCII compartment separately. The resulting efficiencies were 96% for the strongly coupled LHCII and 84% for the weakly coupled LHCII. It must be noted that the lifetimes of “free” LHCII components also slightly decreased with increasing LHCII content, suggesting weak coupling to PSI. If the decay rate constant of the “Free” compartment was fixed at 0.5 ns^{-1} (as for all other compartments), the estimated trapping efficiency was 9%. The overall efficiency of energy transfer from all LHCII pools combined to PSI was 72% (for the given sample). Hence, the addition of LHCII increased the effective absorption cross-section of PSI by 47%. From a similar analysis of membranes with 2 LHCII:PSI we could conclude that 67% of the excitations on the 2 LHCII are trapped by PSI, corresponding to a 33% increase of the effective absorption cross-section of PSI.

It must be noted that although the kinetic schemes used for target analysis depict different pools of LHCII directly coupled to the PSI core, this is not necessarily the actual case. The exact topology of the pigment–protein network cannot be uncovered by the fluorescence data alone. In the presented case of 3 LHCII per RC, the size of the strongly coupled LHCII pool was 20% relative to PSI, i.e. equivalent to one trimer. Interestingly, the estimated time scale and efficiency of transfer (96%)

are about the same as in PSI–LHCII supercomplexes isolated by Wientjes et al. [15]. Hence, this pool could represent LHCII forming similar PSI–LHCII supercomplexes in the artificial membrane. The kinetics could also be modelled with a scheme in which only one LHCII compartment was coupled to PSI and the rest were daisy-chained (data not shown). LHCII is able to self-assemble into energetically well-connected networks [53,54] and reconstituted LHCII proteoliposomes have displayed high degree of energetic connectivity [27] – excitations can hop multiple times between LHCII trimers within their lifetime. In this context the boundary between “strongly coupled” and “weakly coupled” antenna pools, and the respective energy transfer time scales, is arbitrary – at least for membranes with large number of antenna complexes per RC.

How does the increased antenna size affect the quantum yield of photochemistry in PSI? The average fluorescence lifetime and hence the photochemical yield of Photosystem II were shown to depend on the antenna size [55,56], whereas in PSI–LHCII supercomplexes the yield was almost the same as in PSI [12]. Very recently, Le Quiniou et al. [48] have reported the excitation dynamics in a large PSI–LHCII supercomplex isolated from PSII-deficient *Chlamydomonas* mutant under state 2 conditions and containing 9 LHCI and 7 LHCII monomeric subunits. The authors determined that LHCII was energetically coupled to the PSI core with energy equilibration occurring on a time scale of ~60 ps (i.e. intermediate between the fast and slow time scales reported here). The average lifetime of the supercomplexes was ~70 ps, translating to a remarkable 96% quantum efficiency of trapping – only marginally lower in comparison to PSI–LHCI.

The calculated average fluorescence lifetimes of PSI and PSI–LHCII membranes, measured at 688 nm, and the calculated quantum yields of PSI photochemistry are shown in Table 3. All components of the PSI–LHCII decay were included in the calculation, including those attributed to free Chls, so the result encompasses all pigments in the system. The results show that in the presence of LHCII, the overall quantum yield of charge separation decreased to 0.87–0.89, i.e. by only about 10% even as the total Chl content was increased by 70%. The photochemical yield calculated from the kinetic model of PSI–LHCII membranes (Fig. 6C) was 0.87. The energetic coupling of LHCII to PSI and consequently the trapping efficiency of the artificial membranes seem lower compared to the recent reports on isolated PSI–LHCII supercomplexes [12,15,16,48] but in the present case the numbers represent the bulk membrane system, which is evidently highly heterogeneous. It would be interesting to test whether and what type of supercomplexes are formed in the reconstituted membranes.

In conclusion, our results bring further support to other recent studies suggesting that LHCII can be a very efficient antenna for PSI even in the absence of phosphorylation [15,17], given the opportunity that the two interact in the membrane. We demonstrated that in artificial membranes containing up to three LHCII trimers per PSI, 70% of the photons absorbed by LHCII were effectively transferred to PSI. It can be indeed expected then that in the grana margins and in unstacked thylakoid membranes PSII and PSI would share a common pool of LHCII antenna and only the steric exclusion of PSI from the appressed granal regions prevents “spillover”.

Energy transfer from LHCII to PSI in the reconstituted membranes was found to occur on different time scales – from less than 10 ps extending to nanoseconds. We envision that the rapid transfer is associated with LHCII directly interacting with PSI that can be considered as part of the PSI supercomplex. As LHCII complexes self-organize in an energetically well-connected network, the slower transfer times may reflect hopping of excitations over multiple LHCII before eventually being trapped by the RC. Due to the remarkably efficient charge separation in PSI, the overall photochemical quantum yield remained very high (87%). A positive outcome is the demonstrated ability to construct artificial systems with a desired functional antenna size without a significant loss in quantum efficiency – hence maintaining an optimal net productivity per area.

Conflict of interest

We declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

This work was supported by grants from the European Commission (TÁMOP 4.2.2.D-15/1/KONV-2015-0024) and the Hungarian Scientific Research Fund (OTKA-PD 104530 to P.H.L. and OTKA-K 112688 to G.G.). We wish to thank Ms. Divya Sai Kanna for partaking in some of the experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbabo.2016.01.016>.

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