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Makiko Kosugi (✉ kosugi@bio.chuo-u.ac.jp)

National Institutes of Natural Science <https://orcid.org/0000-0002-7415-1536>

Masato Kawasaki

High Energy Accelerator Research Organization (KEK)

Yutaka Shibata

Tohoku University

Kojiro Hara

Akita Prefectural University

Shinichi Takaichi

Tokyo University of Agriculture <https://orcid.org/0000-0002-1621-9141>

Toshio Moriya

High Energy Accelerator Research Organization <https://orcid.org/0000-0001-7226-5487>

Naruhiko Adachi

High Energy Accelerator Research Organization

Yasuhiro Kamei

National Institute for Basic Biology <https://orcid.org/0000-0001-6382-1365>

Yasuhiro Kashino

University of Hyogo <https://orcid.org/0000-0002-4278-9359>

Sakae Kudoh

National Institute of Polar Research

Hiroyuki Koike

Chuo University

Toshiya Senda

KEK (High Energy Accelerator Research Organization) <https://orcid.org/0000-0002-5153-928X>

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Uphill energy transfer mechanism for photosynthesis in the Antarctic alga

Makiko Kosugi^{1,2,3*}, Masato Kawasaki^{4,5*}, Yutaka Shibata^{6*}, Kojiro Hara⁷, Shinichi Takaichi⁸, Toshio Moriya⁴, Naruhiko Adachi⁴, Yasuhiro Kamei^{9,10}, Yasuhiro Kashino¹¹, Sakae Kudoh^{12,13}, Hiroyuki Koike³, Toshiya Senda^{4,5,14*}

¹Astrobiology Center, National Institutes of Natural Science, 2-21-1 Osawa, Mitaka, Tokyo 181-8588, Japan.

²National Astronomical Observatory of Japan, 2-21-1 Osawa, Mitaka, Tokyo 181-8588, Japan

³Department of Biological Sciences, Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan.

⁴Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK), 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan.

⁵Department of Materials Structure Science, School of High Energy Accelerator Science, The Graduate University of Advanced Studies (Soken-dai), 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan.

⁶Department of Chemistry, Graduate School of Science, Tohoku University, 6-3 Aza Aoba Aramaki, Aoba-ku, Sendai 980-8578, Japan.

⁷Department of Biological Production, Akita Prefectural University, 241-438 Kaidobata-nishi, Shimoshinjo-nakano, Akita, 010-0195, Japan.

⁸Department of Molecular Microbiology, Tokyo University of Agriculture, 1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan.

⁹National Institute for Basic Biology, National Institutes of Natural Sciences, 38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan.

¹⁰Department of Basic Biology, School of Life Sciences, SOKENDAI (The Graduate University for Advanced Studies), 38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585, Japan.

¹¹Graduate School of Science, University of Hyogo, 3-2-1 Kohto, Kamigohri, Ako-gun, Hyogo 678-1297, Japan.

¹²National Institute of Polar Research, Research Organization of Information and Systems, 10-3 Midori-cho, Tachikawa, Tokyo 190-8518, Japan.

¹³Department of Polar Science, School of Multidisciplinary Science, SOKENDAI (The Graduate University for Advanced Studies), 10-3 Midori-cho, Tachikawa, Tokyo 190-8518, Japan.

¹⁴Faculty of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki

1 305-8572, Japan

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3 *These authors contributed equally to this work.

4

5 *Correspondence and requests for materials should be addressed to M. Kosugi
6 (kosugi@bio.chuo-u.ac.jp), Y. Shibata (shibata@m.tohoku.ac.jp), and T. Senda
7 (toshiya.senda@kek.jp).

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Summary

Prasiola crispa, a major green alga in Antarctica, forms layered colonies for survival under the severe terrestrial conditions of Antarctica, which include severe cold, drought, and strong sunlight^{1,2}. As a result of these conditions, the surface cells of *P. crispa* and other Antarctic organisms face high risk of photodamage³. Cells of deeper layer escape from photodamage at the sacrifice of photosynthetic active radiation except infrared. *P. crispa* achieves effective photosynthesis by low energy far-red light for photosystem II excitation with high efficiency similar to that of visible light. Here, we identified a far-red light-harvesting complex of photosystem II in *P. crispa*, Pc-frLHC, and proposed a molecular mechanism of uphill excitation energy transfer based on its cryogenic electron-microscopy structure. While Pc-frLHC is associated with photosystem II, it is evolutionarily related to the light-harvesting complex of photosystem I. Pc-frLHC forms a ring-shaped homo-undecamer in which all chlorophyll *a* molecules are energetically connected and contains chlorophyll *a* trimers. It seems that the trimers are long-wavelength-absorbing chlorophylls for far-red light at 708 nm, and further absorbance extension is accomplished by Davydov-splitting in dimeric chlorophylls. The chlorophyll network should enable a highly efficient entropy-driven uphill excitation energy transfer using far-red light up to 725 nm.

1 Introduction

2 The capture of light energy and its transfer to photosynthetic reaction centers are the
3 primary photosynthetic processes that are performed in light-harvesting proteins with
4 photosynthetic pigments such as chlorophylls and carotenoids. To adapt to the various
5 light conditions under the various micro-environments on earth, pigment species and their
6 binding proteins became highly diversified during their evolution^{4,5}.

7 Eukaryotic photoautotrophs commonly use chlorophyll *a* (Chl *a*)-based light-
8 harvesting complexes (LHCs). Subunits of LHCs typically have three transmembrane
9 helices⁶. LHCs absorb mainly visible light and deliver its energy to photosystem I (PSI)
10 or photosystem II (PSII)⁷⁻⁹. Chl *a*-based PSII needs excitation energy corresponding to
11 680 nm light to split water molecules¹⁰. Since the light-harvesting chlorophyll protein
12 complex of PSII (LHCII) contains Chl *a* and the monomeric Chl *a* shows a red absorption
13 band (Qy band) around 670 nm, monomeric Chl *a* is efficiently used in downhill
14 excitation energy transfer (EET) in LHCII together with a shorter wavelength absorbing
15 Chl, Chl *b*¹¹. On the other hand, PSI requires excitation energy corresponding to 700 nm
16 light¹², and the light-harvesting chlorophyll protein complexes of PSI (LHCI) use up to
17 c.a. 720 nm light for PSI excitation¹³. LHCI possess long-wavelength absorbing Chls
18 (LWC), which consist of aggregates of Chl *a* molecules¹⁴⁻¹⁶. LHCII, however, has little
19 LWC.

20 Effective excitation of the eukaryotic PSII reaction center with far-red light is
21 rather rare but has been reported in some algae¹⁷⁻¹⁹. The EET mechanism of the far-red
22 light-capturing system is noteworthy from the perspective of energy yield. However, its
23 mechanism and structural relevance have not been clarified. Furthermore, oxygenic
24 photosynthesis with far-red light has also attracted the attention of astrobiology
25 researchers. Since an abundance of far-red light characterizes the environments of most
26 observable exoplanets revolving around red-dwarfs (low-mass stars) using next-
27 generation telescopes, confirmation of the molecular mechanism of water-splitting
28 photosynthesis by far-red light is a biological rationale of which oxygen in the atmosphere
29 is a candidate of biosignature on the exoplanets²⁰⁻²³.

30 *Prasiola crispa* is one of the dominant green algae in Antarctica (**Extended Data**
31 **Fig. 1a**)²⁴. *P. crispa* often makes large, layered colonies in terrestrial habitats. While cells
32 beneath of the layered colony can escape from physical damage to their photosynthetic
33 proteins induced by the triple stresses of severe cold, drought, and strong sunlight, the
34 cost of protective strategy from the stresses causes a drastic decrease in net

1 photosynthesis¹⁻³. That is, photosynthetically active radiation (400–700 nm) declines in
2 the deep layers and only far-red light remains (**Extended Data Fig. 1**). Our analysis using
3 cells of *P. crispa* suggested that LWC in *P. crispa* allows efficient PSII activation by far-
4 red light²⁵. The LWC thus has a critical role in increasing photosynthetic productivity
5 inside the layered colony (**Extended Data Fig. 1b**). Since excitation of PSII requires light
6 of approximately 680 nm, the far-red light harvesting system requires the uphill EET for
7 PSII. Our earlier *in vivo* study revealed that *P. crispa* achieves highly efficient uphill EET
8 with far-red light in the similar efficiency as downhill EET with visible light. To clarify
9 the mechanism of the highly efficient uphill EET in *P. crispa*, we purified an LWC-
10 containing complex from *P. crispa* and analyzed it spectroscopically and structurally.

12 **Results**

13 ***Identification of an LWC-binding protein***

14 We purified the LWC-binding protein complex from thylakoid membranes of *P. crispa*
15 by sucrose density gradient centrifugation and anion exchange chromatography by
16 monitoring the far-red absorption band (**Fig. 1a**). The purified LWC-binding protein
17 complex was designated Pc-frLHC (*Prasiola crispa* far-red light-harvesting Chl-binding
18 protein complex). In addition to a typical absorption band at 671 nm (Qy band), Pc-frLHC
19 shows a large far-red absorption band at 706.5 together with fluorescence emission at
20 around 713 nm (F713) at room temperature (**Fig. 1b, Extended Data Fig. 2**). Pc-frLHC
21 is a homo-oligomer that is composed of a subunit of about 29 kDa (**Fig. 1c, right**). While
22 the exact molecular mass of Pc-frLHC was difficult to determine, the band of Pc-frLHC
23 appeared at approximately the same position with the 700 kDa marker protein on the
24 native PAGE (**Fig. 1c, left**). The far-red Qy band can be fitted with two LWC components
25 peaking at 708 and 725 nm, with area ratios of 11:1 (**Fig. 1d**).

26 The amino acid sequence of the subunit of Pc-frLHC was deduced from cDNA
27 sequences of the total mRNA libraries with the help of an internal amino acid sequence
28 analysis of Pc-frLHC (**Extended Data Figs. 3-4**). The LHCII family proteins are known
29 to be the only LHCs contributing to PSII excitation in the green lineage of photosynthetic
30 eukaryotes (green algae and plants). Surprisingly, while Pc-frLHC delivers the excitation
31 energy to PSII, Pc-frLHC is not a member of the LHCII family. The amino acid sequence
32 of the Pc-frLHC subunit was classified into one of the LHCI groups (**Extended Data Fig.**
33 **5**).

Fluorescence measurement revealed two distinct Chl *a* pools

The spectroscopic characteristics of the purified Pc-frLHC were analyzed with the time-resolved fluorescence spectrum at 273 K. The excitation at 740 nm light generates fluorescence at 680 nm (F680) with a rise time of 25 ps (**Fig. 2a**). The longest time constant of the fluorescence decay was 2.2 ns. This result suggests an efficient utilization of far-red light in Pc-frLHC realized by the uphill EET from LWC to bulk Chls. For further analysis, the time-resolved fluorescence at 80 K, 201 K, and 273 K was measured with the excitation at 460 nm (**Fig. 2b**). The fluorescence emission peak of Pc-frLHC showed a temperature-dependent red shift from 710 nm (F710) at room temperature to 730 nm (F730) at 87 K (**Fig. 2c**)²⁵. There are two possible explanations for the temperature-dependent red shift. The first is that the red shift arises due to temperature-dependent structural changes around the pigments that cause a change in the energy level of the Chl *a* pool. However, our analysis supports a second explanation—namely, that there are two intrinsic Chl *a* pools, and lowering the temperature alters the equilibrium between them, causing the red-shifted emission.

The fluorescence decay associated spectra (FDAS) analysis of the time-resolved fluorescence spectra showed two FDAS components at 80 K, one with a time constant of 250 ps peaking at 710 nm and the other with a time constant of 1.9 ns peaking at 730 nm (**Figs 2d-f**). This result supports the co-existence of the two independent pigment pools. The FDAS analysis also revealed a decay of F710 and rise of F730 with the same time constant of 250 ps (**Fig. 2f**), suggesting an energy-transfer from the Chl *a* pool emitting F710 to that emitting F730 with a time constant of 250 ps.

The two pigment pools were further analyzed quantitatively based on the assumption that the red shift of the emission peak upon lowering the temperature is due to the shift of the equilibrium between the intrinsic Chl *a* pools, F710 and F730. For this analysis, the fluorescence spectra at various temperatures were deconvoluted by the fitting of two Gaussian functions with peaks at 713 nm and 730 nm (**Fig 2c**). Based on the analysis using the Arrhenius equation, the ratio of the intrinsic emission intensity of F710 to that of F730 (I_{F713}/I_{F730}) was estimated as approximately 12 (**Fig 2g**). There are two possible models explaining the ratio of I_{F713}/I_{F730} . One attributes the intensity ratio to the different numbers of the Chl *a* molecules which constitute the pigment pools, and the other to the different oscillator strengths of the two emission bands. The second model assumes that the oscillator strength of F730 is 12-fold weakened due to its forbidden-transition nature.

The tertiary structure of Pc-frLHC

To establish a structural basis for the spectroscopic characteristics of Pc-frLHC, the cryo-EM structure of Pc-frLHC was determined at 3.13 Å resolution (**Supplementary Table 1, Extended Data Figs. 6, 7**). Pc-frLHC is an undecamer with eleven-fold symmetry (**Fig. 3a**). Ring-shaped light-harvesting antennas have been reported only from LH1 and LH2 in anaerobic purple bacteria and IsiA in cyanobacteria^{26–28}. Pc-frLHC is the first example of a ring-shaped eukaryotic LHC. While inter-subunit protein-protein interactions are tight at the stromal side, there are only limited inter-subunit interactions at the luminal side. Due to the asymmetric interactions between the subunits, there is a large cavity at the interface of the two subunits. The cavity accommodates nine Chl molecules (**Fig. 4**). PSII in *P. crista* is expected to be loosely bound outside of the Pc-frLHC ring structure, because no PSI/PSII subunits were detected by SDS-PAGE of the Pc-frLHC fraction. Moreover, PSII is too large to fit into the inner space of the ring.

The subunit of Pc-frLHC shares the basic fold of the typical LHC subunit with three transmembrane helices, which have been conventionally designated as helices-B, C, and A from the N-terminus (**Fig. 3b**). However, the subunit of Pc-frLHC has three unique structural features. First, the N-terminal loop region is significantly longer than its homologs (**Extended Data Fig. 4**). Second, the BC loop is relatively short and lacks a well-conserved helix-E. Third, the subunit of Pc-frLHC has a fourth transmembrane helix, helix-F, after helix-D, as observed in LHCI-type homologs of Pc-frLHC (**Fig. 3b, Extended Data Fig. 4**)^{29–32}.

Pigment arrangement in Pc-frLHC

We found eleven Chls and two carotenoids in the subunit of Pc-frLHC (**Fig. 3c, Extended Data Table 1**). While high-performance liquid chromatography (HPLC) pigment analysis using C18-column suggested that at least one Chl in the subunit is Chl *b* (**Extended Data Fig. 8**), it was impossible to distinguish Chl *a* and *b* in the current cryo-EM map. We therefore modeled the eleven Chls as Chl *a*. The numbering of Chls in Pc-frLHC was defined following the nomenclature of spinach LHCII³³. All Chls but Chl708 in the subunit occupy similar positions to those of the corresponding Chls in spinach LHCII monomers. Chls form two layers; one is close to the stromal side (Chls601, 602, 603, 609, 610, 611, and 612) and the other is close to the luminal side (Chls604, 613, and 614) (**Fig. 3c**). Chl708 is located between the two layers.

Due to the inter-subunit interactions, seven consecutive Chls, Chl601-602-603-609-611'-612'-610' on the stromal layer, are aligned from inside to outside of the undecameric ring at the stromal side, with Mg-Mg distances less than 15 Å (**Fig. 4a**). The calculated exciton couplings (**Extended data Table 2**) suggest that two sets of *seven consecutive Chls* are energetically connected to each other through the Chl610-Chl602/603 and Chl601'-Chl603/Chl609 interactions (**Fig. 4a, Extended data Table 2**). All Chls on the stromal layer form an energetically consecutive network throughout the undecameric ring. In addition, Chl708 energetically connects Chl603-609, Chl611', and the luminal Chl613'-614' dimer and the stromal Chls.

Trimeric and dimeric structure of Chls and their spectroscopic assignments

Interestingly, Chl708, Chl609, and Chl603 form a trimer (**Fig. 4a**). Chl708 and Chl603 are located side-by-side, and Chl609 makes stacking (π - π) interactions with Chl708 and Chl603 (**Fig. 4a**). The trimer can be assigned to the main LWC component at 708 nm (LWC_{708}); the ratio of the numbers of the trimeric Chls to the total Chls in the subunit is 3:11 (= 27%), which is consistent with the absorbance area ratio of 22% in the Qy band (**Fig. 1d**).

In addition to the Chl trimer, there is a Chl dimer, Chl613-614, which might be assigned as LWC_{725} . The arrangement of the Chl613-614 dimer seems to allow Davydov splitting with the lower, forbidden-nature excited state³⁴ (**Supplementary Table 2; see the Methods section**). The longest-wavelength absorbance at 725 nm and F730 seem to be assigned to the forbidden transition. The ratio of the absorbance band areas LWC_{708}/LWC_{725} (22/2 ~ 11) (**Fig. 1d**) seems to correlate with the I_{F713}/I_{F730} value (~12) calculated from the temperature-dependent fluorescence spectra (**Fig. 2g**).

Chls610, 611, and 612 are likely other functionally critical Chls, which probably serve collectively as the energy exit site. Since these Chls are located near the outside of the Pc-frLHC ring (**Fig. 4a**), they can interact with PSII on the outer surface of the Pc-frLHC ring structure. Since Chls610, 611, and 612 are located just beside LWC_{708} of next subunit, the excited energy from LWC_{708} can be readily transferred to PSII.

Discussion

In this study, we demonstrated that Pc-frLHC in *P. crisper* can excite PSII with far-red light using uphill energy transfer from LWC to bulk Chls. Pc-frLHC has two LWCs, the main far-red light absorbing LWC, LWC_{708} , and a further red-shifted LWC, LWC_{725} .

Based on the spectroscopic and structural studies, we assigned LWC₇₀₈ and LWC₇₂₅ to the trimeric Chls603-609-708 and dimeric Chls613-614, respectively. The notable ring-shaped structure of Pc-frLHC supports the formation of a large Chl network. This network seems to contribute to highly efficient energy transfer to PSII even using the uphill EET. Due to the uphill EET, only part of the absorbed energy can be used for PSII excitation theoretically. The thermal activation and entropy effect drive the uphill EET from LWC₇₀₈ to bulk Chls. The Boltzmann distribution tells us that there is an approximately 5% probability of finding an excited state in bulk Chls when the energy gap is 28 nm (from 708 nm to 680 nm) at 278 K. In addition, the entropy effect by the abundance ratio of the bulk Chls to LWC₇₀₈ in Pc-frLHC (4:1) increases the probability fourfold, resulting in 20% probability in total. While the probability of the uphill EET of the entire Pc-frLHC was nearly the same as that of the monomer of the Pc-frLHC due to LWC₇₀₈ and the bulk Chls having the same abundance ratios, the probability of energy transfer to PSII would increase significantly in the ring-shaped structure. The ring-shaped structure forms an energetically connected Chl network. Thus, eleven PSII-binding sites can play an equivalent role in the energy transfer to PSII, resulting in sufficient probability for the PSII excitation *per LHC* with the far-red light (**Fig. 4b**). Theoretical calculation based on the current spectroscopic data and the cryo-EM structure suggests that the probability of EET from Pc-frLHC to a bound acceptor complex reaches almost 80% even if only one acceptor complex is bound. When five acceptor complexes are bound, P_{exit} reaches 95% (**Supplementary Note**). In addition, we consider that LWC₇₂₅ plays a role in another uphill EET. The action spectrum analysis of *P. crista*'s cells demonstrated that the highly efficient PSII excitation with far-red light was observed up to 750 nm²⁵, suggesting that the reddest LWC₇₂₅ can contribute to the energy transport to PSII by two-step uphill EET through LWC₇₀₈ and the bulk Chls in Pc-frLHC.

In summary, Pc-frLHC with LWC₇₂₅ and LWC₇₀₈ can excite PSII by uphill EET using far-red light. The ratios of LWC₇₂₅, LWC₇₀₈, and bulk Chls are suitable for the highly efficient uphill EET. Moreover, the ring-shaped arrangement of the eleven subunits enables the formation of an energetically connected Chl network with eleven excited-energy exit sites, increasing the probability of energy transfer to PSII. Pc-frLHC is a well-designed energy transfer molecular machine using far-red light. Pc-frLHC contributes to the predominant growth of *P. crista* in the Antarctic terrestrial habitat through the realization of efficient photosynthesis with a far-red light.

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Figure legends

Figure 1 Purification of Pc-frLHC. **a:** The purification scheme of Pc-frLHC and other photosynthetic proteins from *P. crista*'s thylakoid membranes. **b:** Absorbance spectra (solid line) and fluorescence spectrum (dotted line) of the thylakoids (red) and Pc-frLHC (black) measured at room temperature. **c:** The hrCN-PAGE (left) and SDS-PAGE (right) analyses of thylakoids (lane 1), PSII-LHCII (lane 2), LHCII (lane 3), PSI-LHCI (lane 4) and Pc-frLHC (lane 5). **d:** The fitting analysis of the absorbance spectra of purified Pc-frLHC at room temperature. The peak wavelengths of each component were estimated by the second and fourth derivative of the absorbance spectrum, and fitting analysis with Gaussian functions was performed by Magic plot 2.7.2 (Magicplot Systems). a.u.: arbitrary unit.

Figure 2 Spectroscopic analysis of Pc-frLHC. **a,b:** (a) Fluorescence time profiles of Pc-frLHC excited at 740 nm and monitored at 680 nm (green) observed at 273 K and (b) those excited at 460 nm and monitored at 680 nm (green), 710 nm (orange), and 740 nm (red) and observed at 80 K. Blue curves show the fitting curves to the sum of three exponential components convolved with the instrumental response function shown by red circles in (a). **c:** Temperature dependence of the fluorescence spectrum of Pc-frLHC excited at 460 nm. The blue curves are the fitting curves to the sum of two Gaussian functions (the filled green and orange curves). **d–f:** Fluorescence-decay-associated spectra of Pc-frLHC excited at 460 nm and observed at 273 K (d), 201 K (e), and 80 K (f). **g:** Analysis of the ratio $\text{Area}_{F713}/\text{Area}_{F730}$ using the Arrhenius equation. The blue line is the fitting according to equation S1 (see Methods) with the energy gap fixed to 318 cm^{-1} . a.u.: arbitrary unit.

Figure 3 Overall cryo-EM structure of Pc-frLHC. **a:** Top (upper panel) and side views (lower panel) are shown. Each subunit is shown in a different color. **b:** Top and side views of the subunit of Pc-frLHC in rainbow colors from the N-terminus in blue to C-terminus in red. **c:** Chlorophyll *a* arrangement in Pc-frLHC (the left panel). Each chlorophyll is colored in the same color as the corresponding subunit in (a). The right panel shows the arrangement of chlorophyll *a* in the subunit. Chlorophylls on the stromal and lumenal sides are shown in green and cyan. Chl708 is shown in purple.

Figure 4 The chlorophyll network in Pc-frLHC. **a:** Connections among chlorophyll *a*

1 in Pc-frLHC. Energetically connected stromal chlorophylls (including Chl708) are linked
2 by dotted lines (**Extended data Table 2**). The dotted lines are colored based on the values
3 of excitonic couplings (EC): $EC > 60$ in red, $60 \geq EC > 30$ in orange, and $30 \geq EC > 10$
4 in gray. Chlorophylls on the stromal and lumenal sides are shown in green and cyan,
5 respectively. Chl708 is shown in purple. While Chls613, 614 are energetically connected
6 to Chls708, 612 and 601, they are partly shown in this figure for clarity. **b:** Schematic
7 drawing of excited energy flows in Pc-frLHC. Pc-frLHC absorbs far red light using
8 LWCs and distributes its energy to PSII by uphill excitation energy transfer.

Methods

Protein purification and characterization

Thylakoid membranes were prepared from thalli of *P. crista* harvested from Antarctica as described in our recent report²⁵. For purification of Pc-frLHC, the thylakoids were diluted with Buffer-A (25 mM MES, 1M betaine, 10 mM MgCl₂, 5 mM EDTA, 12.5% glycerol) and solubilized at 0.5 mg Chl ml⁻¹ with 1% dodecyl- β -D-maltoside (β -DDM) on ice for 20 min. The solubilized sample was centrifuged with an OptimaTM TLX and TLA-110 rotor (Beckman Coulter, Brea, CA) at 20,000 $\times$ g for 20 min. The supernatant was fractionated by sucrose density gradient (SGD) centrifugation at 267,000 $\times$ g, at 4°C for 16 h. The middle green band containing PSI-LHCI and Pc-frLHC was collected and diluted ten times with Buffer-B (25 mM MES, 1M betaine, 0.03% β -DDM). The upper green band and lowest light green part were collected as LHCII and PSII-LHCII fractions, respectively. The diluted fraction was adsorbed on a diethylaminoethyl cellulose column (Whatman DE52), and the PSI-LHCI fraction was washed out with 150 mM NaCl containing Buffer-B. The Pc-frLHC fraction was eluted with 250 mM NaCl containing Buffer-B, diluted five times with Buffer-B and precipitated by centrifugation at 417,000 $\times$ g at 4°C for 2 h. For fluorescence measurements, pigment analysis, and cryo-EM analysis, the concentrated fraction was fractionated again by SDG centrifugation and the lowest dark green band was collected. Sucrose was removed by dilution with Buffer-B and ultracentrifugation cycles.

The purified Pc-frLHC fraction was characterized by absorbance and fluorescence spectra measurements, high resolution clear native (hrCN)-polyacrylamide gel electrophoresis (PAGE), SDS-PAGE, and HPLC. hrCN-PAGE and SDS-PAGE were performed according to the previously described protocols^{35,36}. The absorption and fluorescence spectra at room temperature were measured with an MPS-2450 (Shimadzu, Kyoto, Japan) and RF-6000 fluorescence spectrometer (Shimadzu, Kyoto, Japan), respectively, as described in our recent report²⁵. Pigment analysis was performed by HPLC as described by Takaichi et al.³⁷ The purified Pc-frLHC fraction was injected directly into the HPLC system and the absorbance at 440 nm was monitored.

Amino acid sequence analysis

The Pc-frLHC protein was separated with SDS-PAGE, and the gel was stained with CBB solution (0.1% CBB-R, 10% acetic acid and 50% methanol) and destained with 25%

methanol containing 10% acetic acid. The band at 29 kDa was cut out and put in a microtube for lysyl endopeptidase treatment. The peptidase treatment was performed according to an earlier described protocol³⁸. The fragmented peptides were separated by SDS-PAGE and electrophoretically blotted onto a PVDF membrane. The N-terminal amino acid sequences of four peptides were determined with a Procise 492cLC (Applied Biosystems, Carlsbad, CA). The peptide sequence data were deposited in the UniProt Knowledgebase under the accession number C0HLU5. We searched for the most probable cDNA of Pc-frLHC by using the determined internal peptide sequences from the total mRNA libraries of *P. crisper* (BioProject ID: PRJNA329112)³⁹. The cDNA sequence of Pc-frLHC gene was submitted to Third Party data (TPA) of the DDBJ/EMBL/GenBank databases and was assigned the accession numbers TPA: BR001753.

Cryo-EM sample preparation and data collection

For cryo-grid preparation, 3 μ l of 6 mg protein ml^{-1} Pc-frLHC (25 mM MES (pH 6.5), 0.015% β -DDM, and 0.5 M betain) was applied onto a holey carbon grid (Quantifoil, Cu, R1.2/1.3, 300 mesh), which was rendered hydrophilic by a 30 s glow-discharge in air (11 mA current) with a PIB-10 ion bombarder (Vacuum Device, Ibaraki, Japan). The grid was blotted for 5 s with a blot force of 25 at 18°C and 100% humidity. Then the grid was flash-frozen in liquid ethane using Vitrobot Mark IV (Thermo Fisher Scientific). For automated data collection, 1,555 micrographs were acquired with a Talos Arctica (Thermo Fisher Scientific) microscope operating at 200 kV in the nanoprobe mode using EPU software. The movie micrographs were collected by a 4k $\times$ 4k Falcon 3EC direct electron detector (electron counting mode) at a nominal magnification of 92,000 (1.13 Å/pixel). Fifty movie frames were recorded at an exposure of 1.00 $\text{e}^{-}/\text{\AA}^2$ frame, corresponding to a total exposure of 50 $\text{e}^{-}/\text{\AA}^2$. The defocus steps used were 1.0, 1.5, 2.0, 2.5, and 3.0 μm .

Cryo-EM data processing

The cryo-EM data processing is summarized in **Extended data Fig. 7**. First, the movie frames were aligned, dose-weighted, and averaged using an algorithm implemented RELION³⁴⁰ on 5 $\times$ 5 tiled frames with a B-factor of 200. The non-weighted movie sums were used for Contrast Transfer Function (CTF) estimation with the Gctf program⁴¹,

while the dose-weighted sums were used for all subsequent steps of image processing. Particles were picked fully automatically using SPHIRE crYOLO^{42,43} with a generalized model using a selection threshold of 0.02. The subsequent processes, namely, 2D classification, *ab initio* reconstruction, 3D classification, 3D refinement, CTF refinement, and Bayesian polishing, were performed by using RELION3⁴⁰.

A stack of 696,095 particle images was extracted from the 1,555 dose-weighted sum micrographs while rescaling to a box of 144 pixels in size at 3.39 Å/pixel and was subjected to 2D classification (200 expected classes). 195,767 particles corresponding to the best eleven classes, which displayed secondary-structural elements and multiple views of Pc-frLHC, were selected for *ab initio* map reconstruction. Two classes were clearly top views of a ring structure composed of eleven subunits with rotational symmetry (**Extended data Fig. 7c**). Therefore, C11 symmetry was imposed on the generated *ab initio* map and used as an initial reference map for the 3D classification. 654,477 particles from 32 classes after 2D classification were selected with more relaxed criteria for the subsequent 3D classification (4 expected classes). The 3D volume and 185,680 particles of the 3D class with the highest resolution were used as the inputs of the subsequent 3D refinements with C11 symmetry. The refined volume and particle images were rescaled to a box of 432 pixels in size at 1.13 Å/pixel. The particle images which became duplicated as the result of alignments were excluded. 160,157 selected particles were 3D auto-refined (C11 symmetry, with a mask diameter of 280 Å) twice (the 1st run was without, and the 2nd run was with a soft-edged 3D mask). One cycle of Bayesian polishing and CTF refinement was done, followed by 3D refinement with a soft-edged 3D mask (C11 symmetry, with a mask diameter of 360 Å) after each Bayesian polishing and CTF refinement step.

Then, no-alignment 3D classification was conducted (C11 symmetry, two expected classes, T = 4) with a soft-edged 3D mask, and 99,510 particles were selected by choosing the best 3D class. The last 3D refinement (C11 symmetry, with mask diameter 460 Å) with a soft-edged 3D mask generated the result at 3.13 Å resolution. The gold standard FSC resolution with a 0.143 criterion⁴⁴ was used as the global resolution estimation. The local resolution was estimated using an algorithm implemented on RELION3. UCSF Chimera⁴⁵ was used for the visualization.

Model building, refinement, and validation

The initial model was built using Map_to_Model⁴⁶ in PHENIX software. The model was

manually corrected by Coot⁴⁷, followed by Real-space Refinement⁴⁸ in PHENIX. The model was refined by multiple cycles of manual modifications in Coot and Real-space Refinement in PHENIX. NCS restraints were used for the automatic refinement. Validation of the refined model was carried out using MolProbity⁴⁹ in PHENIX. UCSF Chimera and PyMOL (Schrödinger, LLC) were used for visualization.

Fluorescence measurement

The time-resolved fluorescence spectra were measured by the streak scope (C1067; Hamamatsu Photonics Inc., Hamamatsu, Japan). The magic angle configuration was used. For the measurements with the excitations at 740 nm, a femtosecond Ti:S laser (MAITAI; Spectra-Physics, Santa Clara, CA) was used as the excitation source. A short-pass filter with a cut-off wavelength of 700 nm (FESH0700; Thorlabs, Newton, NJ) was set before the detector to block the excitation laser. For the measurements with the excitation at 460 nm, we used the second-harmonics pulse of the Ti:S laser generated by a barium borate (BBO) crystal and a long-pass colored glass filter with a cut-off of 480 nm. The instrumental response function was determined by measuring a standard sample (aqueous solution of Malachite Green) which was known to have a very short fluorescence lifetime⁵⁰. For the steady-state fluorescence spectral measurements, a conventional fluorometer (F4500; Hitachi) was used.

The sample solution was set in the copper sample holder of the home-built Dewar. For the measurements at 273 K, 201 K, and 80 K, the Dewar was filled with ice in a liquid water bath, dry ice in a liquid ethanol bath, and liquid nitrogen as the cooling medium, respectively. The Pc-frLHC solution in the buffer was mixed with a two-fold volume of glycerol to maintain transparency of the solution, except for the measurement of the time-resolved fluorescence at 273 K.

Estimation of the excitonic coupling

We calculated the excitonic coupling between molecules A and B using the following equation:

$$V_{A,B} = \frac{1}{\epsilon} \sum_{I=1}^N \sum_{J=1}^N \frac{q_I^A q_J^B}{r_{IJ}}$$

Here, $q_I^{A/B}$ is the atomic transition charges of the I -th atom in the molecule A/B, and r_{IJ} is the distance between the I -th atom in A and J -th atom in B. ϵ (here set to 2.0) is the

dielectric constant of the protein matrix which shields the electrostatic interaction. We used the values of the atomic transition charges reported previously⁵¹, which were obtained by quantum chemical calculations (Hartree-Fock and configuration interaction with single excitations) of the ground and excited states of Chl *a* in a vacuum. The results of the calculations are shown in Extended data Table 2.

Analysis using the Arrhenius equation

Figure 2c shows a slight but significant broadening of the emission band in the intermediate temperature region from 144 K to 183 K, suggesting the overlap of two emission bands. The blue curves in Fig. 2c are the fitting curves to the sum of two Gaussian functions with peaks at 713 nm and 730 nm. The fitting was done with a constraint that the peak position of each band takes the same value for the data at every temperature. Figure 2g shows the Arrhenius plot⁵² of the ratio of the band areas of the 713 nm band with respect to that of the 730 nm. The blue straight line is the fitting according to the equation

$$\frac{Area_{F713}}{Area_{F730}} = \frac{I_{F713}}{I_{F730}} \exp \left[-\frac{\Delta E}{k_B T} \right]. \quad \dots \text{Equation S1}$$

Here, k_B is Boltzmann's constant. $I_{F713/F730}$ and ΔE are the intrinsic emission intensity of the Chl *a* pool emitting the F713/F730 fluorescence band and the energy gap between the excited states of the two pigment pools, respectively. The fitting line is obtained with the value of ΔE fixed to 318 cm⁻¹ (=458 K), which is calculated from the energy difference of the two peak wavelengths (**Fig. 2c**). Finally, we could estimate the value of $I_{F713/F730}$ as approximately 12, suggesting that the F713 emission is intrinsically about 12-fold brighter than the F730 one.

Davydov splitting of the Chl613-614 dimer

Extended data Table 2 reveals several Chl pairs with significantly strong excitonic couplings. These Chl pairs are considered to form mixed excited states delocalized over the two molecules. These two mixed excited states are energetically separated with an energy known as the Davydov splitting, which is approximated by the double of the excitonic couplings. This effect is thought to be one of the major causes of the red shift of LWC. Since LWC₇₂₅ was predicted to have a low oscillator strength, its potential candidate is assigned to a Chl pair that has the lower, forbidden-transition-nature excited state. A famous example of such a lower, forbidden-nature excited state is an H-type molecular aggregate, in which the transition dipole moments are aligned in a side-by-side

configuration³⁴. When the excitonic coupling between molecules A and B is positive, the transition dipole moment of the lower excited state can be approximated by

$$\vec{\mu}_- \approx (\vec{\mu}_A - \vec{\mu}_B)/\sqrt{2},$$

while when the coupling is negative it can be approximated by

$$\vec{\mu}_- \approx (\vec{\mu}_A + \vec{\mu}_B)/\sqrt{2}.$$

Here, $\vec{\mu}_{A/B}$ is the transition dipole moment of the molecule A/B. The transition dipole moment of a Chl *a* is known to be aligned nearly parallel to the vector from NB to ND (the nomenclature of the nitrogen atoms according to the PDB format)⁵³. As shown in Supplementary Table 2, we found that only the Chl613-614 pair fulfills the above criterion. A simple calculation assuming the same excitation energy for Chl613 and 614 resulted in a transition dipole moment of the lower excited state of the Chl613-614 pair that was reduced by approximately 33% from that of the monomeric Chl *a*.

Kinetic analysis of the EET

The fluorescence decay curves at various wavelengths were fitted to sum of exponential functions:

$$F_\lambda(t) = \left[\sum_{i=1}^n A_i \exp\left(-\frac{t}{\tau_i}\right) H(t) \right] \otimes I(t).$$

Here, $I(t)$ is the instrumental response functions (Fig. 2a open circles), the symbol $\otimes$ indicates convolution, and $H(t)$ is the Heaviside step function. The fitting was done under a constraint that the time constants take the same values for every monitoring wavelength (global fitting). Three exponential components were found to be sufficient to fit the data. The pre-exponential factors plotted against the monitoring wavelength are called fluorescence decay-associated spectra (FDAS)⁵⁴ and are shown in Figs. 2d,e and f. Positive and negative signs in FDAS mean that the fluorescence at that wavelength has components that decay and rise with the time constant of the FDAS, respectively. Thus, the energy transfer is reflected in the profile of FDAS having positive and negative signs on the shorter and longer wavelength sides, respectively.

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Author contributions

M.Ko. conceived and designed the experiments. M.Ko. and S.K. collected the *Prasiola crispera* sample from Antarctica. M.Ko. purified the samples. M.Ko., S.T., and Y.Kas. performed biochemical analyses. M.Ko., Y.Kam., and K.H. performed primary sequence analysis. M.Ko., M.Ka., N.A. prepared the cryo-EM samples and collected data. M.Ka. and T.M. processed the cryo-EM data. M.Ka. performed model building, refinement, and validation of the cryo-EM structure. M.Ko., and Y.S. performed the spectroscopic experiments and analysis. M.Ko., Y.S., and T.S. analyzed data and interpreted results with contributions from H.K. and wrote the manuscript.

Competing interests: The authors declare no competing financial interests.

Data availability

Cryo-EM map of Pc-frLHC is deposited in the Electron Microscopy Data Bank under accession codes EMD-31486. Structure coordinate related to the cryo-EM map is deposited at the Protein Data Bank with accession code 7F78.

Additional information

Supplementary Information accompanies this paper at <http://#####>

Correspondence and requests for materials should be addressed to M.Ko, Y.S., or T.S.

Extended data figure/ table legends

Extended Data Figure 1| *Prasiola crispa* in Antarctica, light conditions of the *P.*

***crispa* colony, and spectroscopic properties of the cells. a:** Colonies of *P. crispa* spreading in an Antarctic habitat. **b:** Light spectral conditions on the surface and the middle and lower parts of a colony of *P. crispa* simulated using an HAL-100 solar simulator (Asahi Spectra, Tokyo) were measured by a spectrometer, Black comet (StellarNet, Tampa, FL). A drought colony harvested from Antarctica was sliced in the upper, middle, and lower parts. Each part of a colony was sandwiched with quartz plates, respectively, and vertically set under the irradiation of the solar simulator. The light source and the transmitted light of samples were determined by a spectrometer five to ten times under each condition and averaged. **c:** Absorption spectra of thylakoid membranes isolated from the upper, middle, and lower parts of a colony were measured by an MPS-2450 spectrometer (Shimadzu, Kyoto, Japan). All spectra were normalized at 679 nm. **d:** Emission fluorescence spectra of thylakoid membranes were measured at room temperature with excitation at 435 nm by an RF-6000 fluorescence spectrometer (Shimadzu). The slit widths of the excitation and emission sides were 5 nm and 3 nm, respectively. **e:** Excitation spectra of fluorescence emission at 680 nm were measured by an RF-6000. Shorter wavelengths than 400 nm of the excitation light were cut by long-pass filters. The slit widths of the excitation and emission sides were 3 nm and 5 nm, respectively. a.u.: arbitrary unit.

Extended Data Figure 2| Absorbance spectra of isolated proteins. Each spectrum was normalized at the peak wavelength of the red absorption band. a.u.: arbitrary unit.

Extended Data Figure 3| Amino acid sequence analysis. a: Pc-frLHC fraction after the DE53 column was loaded onto an SDS-PAGE gel and the separated proteins were stained by a CBB. The main protein band detected at 29 kDa (*) was cut out and used for lysyl endopeptidase treatment. The fragmented peptides after lysyl endopeptidase treatment were separated by SDS-PAGE and detected by silver staining (b) or electrophoretically blotted onto a PVDF membrane (c). **b:** The right and left lanes were thylakoid membranes and the fragmented peptides of the 29 kDa protein of (a), respectively. **c:** N-terminal

amino acid sequences of fragmented peptides of 14, 10, 6, and 4 kDa were determined by a Procise 492cLC peptide sequencer (Applied Biosystems, Carlsbad, CA). Fragments 14 and 10 had the same N-terminal sequence.

Extended Data Figure 4| Sequence alignment of Pc-frLHC from *P. crispa* and the closely related LHCs. The deduced amino acid sequence of Pc-frLHC was classified into one of the LHCI groups along with Lhca 2 of *Chlamydomonas reinhardtii* (Cr_Lhca2), Lhca-J of a marine green alga, *Bryopsis corticulans* (Bc_LhcaJ), and Lhca 5 of a halophilic green alga, *Dunaliella salina* (Ds_Lhca5) with amino-acid sequence identities of 32%, 32%, and 29%, respectively. Recent analyses revealed that Cr_Lhca2, Bc_LhcaJ, and Ds_Lhca5 are orthologs and loosely bound to the side of green algal PSI in a state of a heterodimer together with Cr_Lhca9, Bc_Lhcl, and Ds_Lhca6, respectively^{29,30,31,32}. Based on these results, we concluded that Pc-frLhc is phylogenetically a member of LHCI, while it transfers excitation energy to PSII. Transmembrane helices are shaded by different colors, the signal peptides are shaded, chlorophyll-binding sites (followed by nomenclature of Liu et al.) are shown as red characters, and the sequences detected by N-terminal sequences are shown by arrows. The signal peptides and the transmembrane helices were predicted from the 3D structures registered in the PDB (Cr_Lhca2; 6JO5, 6IJO, Bc_LhcaJ; 6IGZ, Ds_Lhca5; 6SL5) and from the results of secondary structural prediction using the Jpred 4 (doi: 10.1093/nar/gkv332) and the TargetP-2.0 (<http://www.cbs.dtu.dk/services/TargetP/>). The Chl708 binding site in *P. crispa* is in blue.

Extended Data Figure 5| Phylogenetic tree of light-harvesting complexes (LHCs) of PSI and PSII in green algae and plants. Evolutionary analyses of LHCs were conducted with the neighbor-joining method in MEGA7. We only used amino acid sequences of LHCs in which 3D structural analysis were achieved. The optimal tree with the sum of branch lengths = 17.75479796 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Poisson correction method. The rate variation among sites was modeled with a gamma distribution (shape parameter = 3).

While most LHC subunits have only three transmembrane helices, a few LHC subunits, such as Cr_Lhca2, Bs_LhcaJ, and Ds_Lhca5, have been known to possess a fourth

transmembrane helix (Four TMH).

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(lower panel). Loroanthin was also identified by its molecular weight (584) using liquid chromatography-mass spectrometry. Three carotenoid-binding sites, L1, L2 (both surrounding central helices A and B), and N1 (near helix C), are conserved among LHCs. Biochemical analysis shows that Pc-frLHC binds only two carotenoids: vio and loro. The vio and loro in the all-trans configuration were assigned in the L1- and L2-binding sites of Pc-frLHC, respectively. The N1 site is not occupied in Pc-frLHC. a.u.: arbitrary unit.

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Figures

Figure 1 Purification of Pc-frLHC. **a:** The purification scheme of Pc-frLHC and other photosynthetic proteins from *P. crista*'s thylakoid membranes. **b:** Absorbance spectra (solid line) and fluorescence spectrum (dotted line) of the thylakoids (red) and Pc-frLHC (black) measured at room temperature. **c:** The hrCN-PAGE (left) and SDS-PAGE (right) analyses of thylakoids (lane 1), PSII-LHCII (lane 2), LHCII (lane 3), PSI-LHCI (lane 4) and Pc-frLHC (lane 5). **d:** The fitting analysis of the absorbance spectra of purified Pc-frLHC at room temperature. The peak wavelengths of each component were estimated by the second and fourth derivative of the absorbance spectrum, and fitting analysis with Gaussian functions was performed by Magic plot 2.7.2 (Magicplot Systems). a.u.: arbitrary unit.

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Figure 2 Spectroscopic analysis of Pc-frLHC. **a,b:** (a) Fluorescence time profiles of Pc-frLHC excited at 740 nm and monitored at 680 nm (green) observed at 273 K and (b) those excited at 460 nm and monitored at 680 nm (green), 710 nm (orange), and 740 nm (red) and observed at 80 K. Blue curves show the fitting curves to the sum of three exponential components convolved with the instrumental response function shown by red circles in (a). **c:** Temperature dependence of the fluorescence spectrum of Pc-frLHC excited at 460 nm. The blue curves are the fitting curves to the sum of two Gaussian functions (the filled green and orange curves). **d-f:** Fluorescence-decay-associated spectra of Pc-frLHC excited at 460 nm and observed at 273 K (d), 201 K (e), and 80 K (f). **g:** Analysis of the ratio $\text{Area}_{713}/\text{Area}_{730}$ using the Arrhenius equation. The blue line is the fitting according to equation S1 (see Methods) with the energy gap fixed to 318 cm⁻¹. a.u.: arbitrary unit.

Figure 3 Overall cryo-EM structure of Pc-frLHC. **a:** Top (upper panel) and side views (lower panel) are shown. Each subunit is shown in a different color. **b:** Top and side views of the subunit of Pc-frLHC in rainbow colors from the N-terminus in blue to C-terminus in red. **c:** Chlorophyll *a* arrangement in Pc-frLHC (the left panel). Each chlorophyll is colored in the same color as the corresponding subunit in (a). The right panel shows the arrangement of chlorophyll *a* in the subunit. Chlorophylls on the stromal and lumenal sides are shown in green and cyan. Chl708 is shown in purple.

Figure 4 The chlorophyll network in Pc-frLHC. a: Connections among chlorophyll *a* in Pc-frLHC. Energetically connected stromal chlorophylls (including Chl708) are linked by dotted lines (**Extended data Table 2**). The dotted lines are colored based on the values of excitonic couplings (*EC*): $EC > 60$ in red, $60 \geq EC > 30$ in orange, and $30 \geq EC > 10$ in gray. Chlorophylls on the stromal and luminal sides are shown in green and cyan, respectively. Chl708 is shown in purple. While Chls613, 614 are energetically connected to Chls708, 612 and 601, they are partly shown in this figure for clarity. **b:** Schematic drawing of excited energy flows in Pc-frLHC. Pc-frLHC absorbs far red light using LWCs and distributes its energy to PSII by uphill excitation energy transfer.

Extended data

Extended Data Figure 1| *Prasiola crispa* in Antarctica, light conditions of the *P. crispa* colony, and spectroscopic properties of the cells. **a:** Colonies of *P. crispa* spreading in an Antarctic habitat. **b:** Light spectral conditions on the surface and the middle and lower parts of a colony of *P. crispa* simulated using an HAL-100 solar simulator (Asahi Spectra, Tokyo) were measured by a spectrometer, Black comet (StellarNet, Tampa, FL). A drought colony harvested from Antarctica was sliced in the upper, middle, and lower parts. Each part of a colony was sandwiched with quartz plates, respectively, and vertically set under the irradiation of the solar simulator. The light source and the transmitted light of samples were determined by a spectrometer five to ten times under each condition and averaged. **c:** Absorption spectra of thylakoid membranes isolated from the upper, middle, and lower parts of a colony were measured by an MPS-2450 spectrometer (Shimadzu, Kyoto, Japan). All spectra were normalized at 679 nm. **d:** Emission fluorescence spectra of thylakoid membranes were measured at room temperature with excitation at 435 nm by an RF-6000 fluorescence spectrometer (Shimadzu). The slit widths of the excitation and emission sides were 5 nm and 3 nm, respectively. **e:** Excitation spectra of fluorescence emission at 680 nm were measured by an RF-6000. Shorter wavelengths than 400 nm of the excitation light were cut by long-pass filters. The slit widths of the excitation and emission sides were 3 nm and 5 nm, respectively. a.u.: arbitrary unit.

Extended Data Figure 2| Absorbance spectra of isolated proteins. Each spectrum was normalized at the peak wavelength of the red absorption band. a.u.: arbitrary unit.

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Extended Data Figure 3| Amino acid sequence analysis. **a:** Pc-frLHC fraction after the DE53 column was loaded onto an SDS-PAGE gel and the separated proteins were stained by a CBB. The main protein band detected at 29 kDa (*) was cut out and used for lysyl endopeptidase treatment. The fragmented peptides after lysyl endopeptidase treatment were separated by SDS-PAGE and detected by silver staining (**b**) or electrophoretically blotted onto a PVDF membrane (**c**). **b:** The right and left lanes were thylakoid membranes and the fragmented peptides of the 29 kDa protein of (**a**), respectively. **c:** N-terminal amino acid sequences of fragmented peptides of 14, 10, 6, and 4 kDa were determined by a Procise 492cLC peptide sequencer (Applied Biosystems, Carlsbad, CA). Fragments 14 and 10 had the same N-terminal sequence.

Extended Data Figure 4| Sequence alignment of Pc-frLHC from *P. crista* and the closely related LHCA2s. The deduced amino acid sequence of Pc-frLHC was classified into one of the LHCA2 groups along with Lhca 2 of *Chlamydomonas reinhardtii* (Cr_Lhca2), Lhca-J of a marine green alga, *Bryopsis corticulans* (Bc_LhcaJ), and Lhca 5 of a halophilic green alga, *Dunaliella salina* (Ds_Lhca5) with amino-acid sequence identities of 32%, 32%, and 29%, respectively. Recent analyses revealed that Cr_Lhca2, Bc_LhcaJ, and Ds_Lhca5 are orthologs and loosely bound to the side of green algal PSI in a state of a heterodimer together with Cr_Lhca9, Bc_LhcaI, and Ds_Lhca6, respectively^{29,30,31,32}. Based on these results, we concluded that Pc-frLhc is phylogenetically a member of LHCA2, while it transfers excitation energy to PSII. Trans-membrane helices are shaded by different colors, the signal peptides are shaded, chlorophyll-binding sites (followed by nomenclature of Liu et al.) are shown as red characters, and the sequences detected by N-terminal sequences are shown by arrows. The signal peptides and the transmembrane helices were predicted from the 3D structures registered in the PDB (Cr_Lhca2; 6JO5, 6IJO, Bc_LhcaJ; 6IGZ, Ds_Lhca5; 6SL5) and from the results of secondary structural prediction using the Jpred 4 (doi: 10.1093/nar/gkv332) and the TargetP-2.0 (<http://www.cbs.dtu.dk/services/TargetP/>). The Chl708 binding site in *P. crista* is in blue.

Extended Data Figure 5| Phylogenetic tree of light-harvesting complexes (LHCs) of PSI and PSII in green algae and plants.

Evolutionary analyses of LHCs were conducted with the neighbor-joining method in MEGA7. We only used amino acid sequences of LHCs in which 3D structural analysis were achieved. The optimal tree with the sum of branch lengths = 17.75479796 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Poisson correction method. The rate variation among sites was modeled with a gamma distribution (shape parameter = 3).

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Abbreviations: Cm: *Cyanidioschyzon merolae* (red alga); Cre: *Chlamydomonas reinhardtii*; Bc: *Bryopsis corticulans*; Ds: *Dunaliella salina*; Pc: *Prasiola crista* (green algae); At: *Arabidopsis thaliana* (plant). Blast accession numbers: Cm_Lhcr1 (XP_005538084), Cm_Lhcr2 (XP_005537362), Cm_Lhcr3 (5ZGB_3), Cre_LHCA1 (6IJJ_1), Cre_LHCA2 (XP_001691031), Cre_LHCA3 (PNW76422), Cre_LHCA4 (6IJJ_4), Cre_LHCA5 (6IJJ_5), Cre_LHCA6 (6IJJ_6), Cre_LHCA7 (AAO16495), Cre_LHCA8 (6IJJ_8), Cre_LHCA9 (XP_001692548), Cre_CP29 (XP_001697193), Cre_PSBS2 (XP_001689923.1), Cre_CP26 (XP_001695927), Cre_LHCII (XP_001700243.1), Bc_Lhca-j (6IGZ_0), Bc_Lhca-a (6IGZ_1), Bc_Lhca-c (6IGZ_2), Bc_Lhca-d (6IGZ_3), Bc_Lhca-b (6IGZ_4), Bc_Lhca-g (6IGZ_6), Bc_Lhca-h (6IGZ_7), Bc_Lhca-i (6IGZ_9), At_Lhca1 (NP_191049.1), At_Lhca2 (NP_191706.2), At_Lhca3 (NP_001185280.1), At_Lhca4 (NP_190331.3), At_Lhca5 (NP_175137.1), At_Lhca6 (NP_173349.1), At_CP26 (NP_192772.1), At_CP29 (NP_195773.1), At_CP24 (NP_173034.1), At_LHCII (NP_174286.1), At_PSBS (NP_001319163.1), Ds_Lhca1 (6RHZ_1), Ds_Lhca2 (6RHZ_2), Ds_Lhca3 (6SL5_3), Ds_Lhca4 (6QPH_4), Ds_Lhca5 (6SL5_5), Ds_Lhca6 (6SL5_6).

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Chl	Ligand	Mg-ligand distance (Å)
601	unidentified	
602	Glu124	2.77
603	Asn127	2.56
604	none	
609	Glu178	2.23
610	Glu219	3.58
611	Glu63	2.62
612	Asn222	3.36
613	Gln236	2.67
614	His251	3.48
708	His171	2.59

Extended data Table 2| Excitonic couplings (cm^{-1}) between Chl *a* molecules bound to two subunits. Strong interactions are highlighted with hatching

	601	602	603	604	609	610	611	612	613	614	708
601	-	75	1	-2	4	-5	-5	-6	-13	-21	-2
602	75	-	34	8	-39	-11	-5	-8	-2	2	9
603	1	34	-	-4	154	15	-1	8	4	0	-67
604	-2	8	-4	-	-6	-3	-4	-2	3	-1	1
609	4	-39	154	-6	-	3	6	0	-4	-1	86
610	-5	-11	15	-3	3	-	-36	-86	8	4	-6
611	-5	-5	-1	-4	6	-36	-	-73	-7	-7	-2
612	-6	-8	8	-2	0	-86	-73	-	25	14	-1
613	-13	-2	4	3	-4	8	-7	25	-	66	3
614	-21	2	0	-1	-1	4	-7	14	66	-	0
708	-2	9	-67	1	86	-6	-2	-1	3	0	-
601'	2	1	15	1	-11	3	0	1	-2	-1	-15
602'	1	-3	5	-2	-2	2	1	0	-1	0	-9
603'	0	1	-1	1	1	0	0	0	0	0	0
604'	0	0	-1	1	1	0	0	0	0	0	2
609'	0	-1	2	-1	-2	1	0	0	0	0	-1
610'	0	2	-4	2	7	-2	-1	-1	0	0	3
611'	-2	9	-33	6	65	-6	-2	-1	1	1	64
612'	0	4	-9	5	22	-1	-1	0	0	0	14
613'	-1	2	-9	3	9	-2	-1	-1	2	1	-7
614'	-2	-3	-14	-1	15	-2	0	-2	3	0	-92
708'	0	0	-1	0	0	0	0	0	0	0	1

Supplementary Files

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