Structures of the Cyanobacterial Phycobilisome

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Abstract

The phycobilisome is an elaborate antenna that is responsible for light-harvesting in cyanobacteria and red-algae. This large macromolecular complex captures incident sunlight and transfers the energy via a network of pigment molecules called bilins to the photosynthetic reaction centers. The phycobilisome of the model organism *Synechocystis* PCC 6803 consists of a core to which six rods are attached but its detailed molecular architecture and regulation in response to environmental conditions is not well understood. Here we present cryo-electron microscopy structures of the 6.2 MDa phycobilisome from *Synechocystis* PCC 6803 resolved at 2.1 Å (rods) to 2.7 Å (core), revealing three distinct conformations, two previously unknown. We found that two of the rods are mobile and can switch conformation within the complex, revealing a layer of regulation not described previously. In addition, we found a novel linker protein in the structure, that may represent a long-sought subunit that tethers the phycobilisome to the thylakoid membrane. Finally, we show how excitation energy is transferred within the phycobilisome and correlate our structures with known spectroscopic properties. Together, our results provide detailed insights into the biophysical underpinnings of cyanobacterial light harvesting and lay the foundation for bioengineering of future phycobilisome variants and artificial light harvesting systems.

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Cyanobacteria are the most abundant and ecophysiologically diverse primary producers on Earth. Oxygenic photosynthesis is an ancient cyanobacterial innovation that allowed complex life to emerge¹, and today these organisms are being established as platforms for green biotechnologies². Cyanobacterial phycobilisomes (PBSs)³⁻⁵ are massive pigment-protein complexes that can constitute up to half of the soluble protein content of the cell⁶. The two major PBS substructures, the core and the rod, are composed of phycobiliproteins, and colorless linker proteins (LPs)⁷. Phycobilin pigments are covalently bound to the phycobiliproteins⁸⁻¹⁴, which assemble into disc-like trimers ($\alpha\beta$)₃ or hexamers ($\alpha\beta$)₆. These discs are chained together in the rods and core cylinders by LPs and organized into a complete PBS¹⁵⁻¹⁷. The association of pigments with protein tunes the pigments' energetic properties to establish an energy cascade to the photosynthetic reaction centers that is both extremely fast and highly efficient (ca. 95%)^{18,19}. Four morphological types of PBS are known: hemiellipsoidal^{16,20,21}, block-type¹⁵, hemidiscoidal^{10,22-25} and bundle-type²⁶. *Synechocystis* PCC 6803 is the primary model system for molecular and spectroscopic studies²⁷ containing the most widespread type of PBS, the hemidiscoidal^{8,22,25,28,29}, which is also the evolutionary antecedent of all types of PBS¹⁵. We report three cryo-electron microscopy (cryo-EM) structures of the 6.2 MDa PBS from *Synechocystis* PCC 6803, representing distinct conformers of rod arrangements: the canonical 'up-up' (2.7 Å), and two previously undescribed, 'up-down' (2.8 Å), and 'down-down' (3.5 Å) conformations. We also describe the structure of all the linkers, one of them previously unknown, the arrangement of all bilins, and elucidate the likely energy transfer pathway.

Structural overview

Purified and intact PBS complex was biotinylated and subjected to cryo-EM using streptavidin affinity grids³⁰ to reduce preferential particle orientations and to increase stability (Methods, Extended Data Fig. 1, Extended Data Table 1, 2). We determined the structure of three distinct PBS conformations at resolutions ranging from 2.7 to 3.5 Å for the entire complexes, enabling us to build all pigments and

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77 protein chains (Fig. 1, Extended Data Fig. 2, 3, and Extended Data Table 3). As previously reported²²,
 78 the rods are flexible in nature and therefore appear smeared in the reconstructions. Therefore, the rods
 79 were processed and reconstructed independently, yielding a resolution of 2.1 Å. The rods cannot be
 80 further sorted into distinct classes, suggesting that all rods within one complex are identical (Extended
 81 Data Fig. 2c).

82 The PBS core consists of three cylinders, a top cylinder (T) stacked on top of two basal cylinders (B1,
 83 B2) (Fig. 1, Extended Data Fig. 4). Each of the two B cylinders have one rod attached while four rods
 84 are attached to the T cylinder. The core consists of 80 polypeptide chains and 72 phycocyanobilins
 85 (PCB), and each rod contains 40 protein subunits and 54 PCBs, adding up to a total of 320 proteins
 86 and 396 bilin molecules that amount to a molecular weight of 6.2 MDa. Extended Data Figure 4 and
 87 Extended Data Table 1 provide an inventory of the subunits, their component protein family (pfam)
 88 domains and functional characteristics.

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Figure 1: Structural characterization of the phycobilisome from *Synechocystis* PCC 6803. a, Composite cryo-EM density maps representing the three PBS conformations: Up-up (top), up-down (middle), down-down (bottom). Individual maps and resolution for the three conformations and the rod can be found in Extended Data Fig. 2. b, Schematic overview of PBS connectivity and subunit arrangement of the core (top) and the rods (bottom).

In contrast to earlier studies, our structures include three different, major rod conformations; two have not been visualized before. They are distinguished by alternate positioning of two of the four T rods (hence referred to as mobile T rods, as opposed to the static T rods). In the ‘up-up’ conformation, both mobile rods are tilted upwards, corresponding to the canonical hemidiscoidal PBS. Two previously unreported conformers were also present: the ‘up-down’ conformation, where one of the mobile rods moved downwards to assume a position parallel to the other rods and the ‘down-down’ conformation, in which all the T cylinder rods are in a down position, reducing the ‘height’ of the complex by more than 100 Å and giving the PBS a more compact profile (Fig. 1a).

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102 Linker architecture and identification of a new linker component, ApcG

103 Both the core and the rods contain linker proteins in their central cavities that provide scaffolding and
 104 organize the arrangement of the Apc and Cpc subunits, thereby ensuring the proper orientation and
 105 spacing of the pigments (Fig. 1b, 2a). Central to the organization are two copies of ApcE (Fig. 2a, b,
 106 Extended Data Fig. 5a) which form the major scaffold for the core by connecting the bottom with the
 107 top cylinders¹⁵⁻¹⁷. The PB loop (residues 87-129) is disordered and could not be modeled, but all other
 108 ApcE domains are well resolved. The three cylinders are ‘capped’ by six copies of ApcC that interact
 109 with ApcE (Fig. 2a, Extended Data Fig. 5b).

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Figure 2: Core and rod linkers. **a**, Surface representation of the linker skeleton of the entire PBS. **b**, ApcE with its constituent domains pf00502 and pf00427. **c**, CpcG1 linker conformations from top (T) and bottom (B) cylinders in the up-up conformation. **d**, Linker proteins within a rod. CpcC1/2 contain a pf00427 and pf01383 domain, CpcD only a pf01383 domain. **e**, Interface of CpcC2 with CpcA proteins. Predominant contacts between the linker and CpcA proteins are highlighted in yellow. **f**, CpcC1/2 interact more closely with CpcB than CpcA. **g**, Bilin arrangement within the rod showing spiraling pattern. **h** and **i**, CpcC1 and CpcG1 amino acids interfaces along the long axis of the rod highlighted in yellow.

In the rods, there are four linker proteins (Fig. 2 a, d). CpcG1 is located at the core-proximal side. Its 60 residue C-terminal extension attaches it to the PBS core (Figure 2 c, d, Extended Data Fig. 5c). The homologues CpcC1 and CpcC2 form the bulk of the interior of the rods (Fig. 2d, Extended Data Fig.

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5 d). The interdomain linker of CpcC1 is 15 residues longer than in CpcC2, allowing for a 70° rotation of the pf01383 domain of CpcC1 compared to CpcC2 (Extended Data Fig. 5d). This results in an apparent 10° rotation between each CpcA/B hexamer (Fig. 2g). Even though all linker proteins within the rod employ the same two domains to connect to one another, differences in the binding interface of each domain pair define a unique architecture for the correct assembly of the entire rod (Fig. 2 h, i, Extended Data Fig. 5 e-g).

A previously unknown linker protein (*sll1873*) was discovered in the cryo-EM density and identified by mass spectrometry (Fig. 3a, Extended Data Table 2). We denote it ApcG or L_{C10} in continuation of the established nomenclature. It is found only on the bottom two cylinders extending toward the membrane-facing side. An HMM search reveals that homologues are found in about 80% of cyanobacterial genomes. ApcG contains two conserved sequence motifs including a short helix that binds in a groove on ApcA like CpcG1 (Fig. 3b, Extended Data Fig. 5b). The highly conserved residue R100 of ApcG directly contacts the PCB of ApcA in the bottom cylinder, suggesting that it may finetune its spectroscopic properties. It has previously been suggested that the PBS interacts with lipid head groups³¹; ApcG could mediate this interaction through its positively charged linker region (Fig. 3b). Given its location close to the terminal emitters and the membrane, we propose that ApcG likely plays a key role in PBS localization and energy transfer to PSII.

Terminal emitters

At the membrane facing side of the PBS, the terminal chromophores of ApcD and ApcE are responsible for transferring the absorbed energy to the reaction centers (Fig. 3c, Extended Data Fig. 4). ApcF has been shown to play a crucial role in energy migration to ApcE and also to impact state transitions^{32,33}. The center-to-center distance between the PCBs of ApcE and ApcF is 23 Å, and 35 Å between the PCBs of ApcD and ApcF (Fig. 3d). While these distances fall well within the range of

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other PCB distances, their local environments are likely responsible for the observed shift in fluorescence emission of ApcE and ApcF to 680 nm (as opposed to 660 nm for ApcA). Rigid body docking of our structure into a negative stain map of the related *Anabaena* sp. PBS bound to PSII²⁵ suggests that the closest inter-pigment distance between the PBS and PSII might be as large as ~50 Å (Fig. 3e). The requirement to spectroscopically bridge such a distance might be one of the drivers for the diversification of ApcD, E and F around the terminal chromophores of the PBS and leading to their unique properties.

Figure 3: A newly identified linker ApcG and Terminal emitters. *a*, Position and interaction of ApcG within the PBS. *b*, Sequence conservation logo of ApcG homologs. The numbers refer to residue positions in *Synechocystis* PCC 6803 ApcG. ApcG contains three conserved sequence motifs including a short helix that binds in a central groove of ApcA like CpcG1. *c*, Position and orientation of the subunits ApcD, ApcE, ApcF and ApcG within the core of the PBS. Only the

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156 *phycobiliprotein domain of ApcE is rendered. d, Orientation and distances of the PCBs of ApcD, ApcE and ApcF. e, Rigid*
 157 *body modelling of our PBS core structure onto PSII, using EMDB 2822 as a reference.*

158 Core-rod linker architecture and conformational switching

159 Rod attachment is mediated by CpcG1, which protrudes from the central cavity of each rod and latches
 160 onto the surface of the core cylinders. The PBS core accommodates the CpcG1 linkers through a
 161 network of grooves that span the surfaces of ApcA and ApcB and which can be divided into central
 162 and lateral grooves (Fig. 4a, Extended Data Figure 6a). Unlike the algal PBS complexes, grooves from
 163 separate discs can align to create a curving binding path for the CpcG1 linkers (Figure 4a).

164
 165 **Figure 4: Structural basis for conformational switching.** *a* Side view of the PBS core omitting the rods showing the groove
 166 network that organizes CpcG1 linker attachment. *b*, Same side view of the PBS core emphasizing the CpcG1 position.
 167 Center panel shows an overview while numbered insets show the different hinge conformations. Near the hinge of the

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168 *mobile rod is a PCB molecule that is contacted by R201 of CpcG1. c, Superimposition of PBS^{down-down} (in color) and PBS^{up-}*
 169 *up* (light grey) showing the large conformational differences between the two.

170 The conformations of the CpcG1 linkers correlate within the three rod (Fig. 4b). When comparing the
 171 linkers of the top and bottom static rods it is apparent that the linker can adopt one of two different
 172 conformations: extended or compact. In the compact state, a conserved six amino acid region (W196-
 173 R201), which we define as the ‘hinge’ forms a small coil or helix that changes the local geometry of
 174 the linker relative to the extended state (Fig. 2c, 4b). Of the two static rods, the hinge of the upper rod
 175 adopts the extended conformation, while the hinge of the lower rod is in the compact conformation
 176 (Fig 4b). As a consequence of this conformational difference, the globular domain of the lower CpcG1
 177 is rotated with respect to the globular domain of the upper CpcG1. This rotation is locked in place only
 178 for the static rods, the mobile rod however can toggle between these two states which are determined
 179 by the conformation of the hinge. Superposing the position of CpcG1 of the mobile rod in its ‘down’
 180 conformation with its own ‘up’ conformation reveals that the movement of the mobile rod between
 181 ‘down’ and ‘up’ corresponds to the conformational change of the linker between compact and
 182 extended. As a result, the globular CpcG1 domain along with the entire rod rotates by 135° around its
 183 long axis when swinging from ‘down’ to ‘up’ (Fig. 4b). Most importantly, this switch is accommodated
 184 by a 62° upwards swing of the entire rod, which is the most striking difference between the ‘down’
 185 and ‘up’ conformations (Fig. 4c). A similar conformational change from the mobile rod to the static
 186 rods is precluded because it would result in a clash of the static rods. The bottom static rod would
 187 swing upwards while the upper static rod would swing downwards. This mutual blockade alone is
 188 sufficient to explain the static nature of these two rods.

189 The mobile rod is held in place in its ‘down’ position through interactions with its static rod neighbor.
 190 Two Cpc(αβ)₆ hexamers proximal to the PBS core interact through a salt bridge that is formed between
 191 R150 of one of the CpcB’s in the mobile rod and E131 of one of the CpcA’s in the static rod (Extended
 192 Data Fig. 6b).

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193 The superposition of ‘down’ and ‘up’ conformations also reveals that the upper static rod experiences
194 a 36 Å lateral displacement when the mobile rod is down but relaxes into its central position once the
195 mobile rod swings upwards (Fig. 4c). Once in the central position, the static rod obstructs the
196 downward movement of the mobile rod and stabilizes this conformation. Hence, in every case the
197 mobile rod needs to overcome an energy barrier to move either direction.

198 **Energy transfer pathways in the different PBS conformations**

199 To better understand the impact of the different conformations we computationally modeled the energy
200 transfer within the phycobilisome based on our structural data. We assume energy transfer in the limit
201 of Förster theory using a dipole-dipole approximation for inter-pigment couplings. The map of energy
202 transfer rates for the PBS in the up-up conformation is shown in Fig. 5, demonstrating a robust system,
203 in which excitation of essentially any pigment directs the excitation quickly to the terminal emitters.
204 It also shows that the bottleneck is the rod-to-core energy transfer, as suggested by global fitting
205 models of time-resolved fluorescence data³⁴. Energy transfer between the pigments in the core, both
206 within and between the cylinders, is more efficient than rod-to-core transfer, contradicting the global
207 fitting models that predict slow inter-cylinder transfer³⁵, but the overall flow of energy through the
208 whole PBS obtained from our model matches the experimental data well (Extended Data Fig. 7a, b).

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Figure 5: Energy flow through the PBS. *a*, Pigment distribution within PBS^{up-up} (top) and $PBS^{down-down}$ (bottom). *b*, Maps of individual inter-pigment energy transfer rates of PBS^{up-up} (top) and $PBS^{down-down}$ (bottom). The color bars correspond to rates faster than 1 ps (red), in the 1-10 ps range (blue) and in 10-20 ps range (grey). Inter-pigment rates slower than 20 ps are omitted. *c*, Energy flow through down-down (solid) and up-up (dashed) PBS configurations. The excitation is placed either on the mobile rod R1 (top), middle rod R2 (middle) or bottom rod R3 (bottom). The curves monitor the evolution of excited state population of pigments located in rod, core (APC), and terminal emitters (TEM).

The individual rate constants shown in the map are used to calculate how long the excitation resides in each structural (e.g., rod or core) or spectroscopic unit (Methods, Extended Data Fig. 7a, b). Using this approach, we also investigated the energy transfer through both the up-up and the down-down PBS conformations by placing the excitation to the most distant disc of the respective rods (Fig. 5c). For both conformations, the excitation reaches the APC core in less than 100 ps, in agreement with experimental data (see Extended Data Fig. 7a, b). Moving the mobile rod to the down position (solid lines in Fig. 5c) facilitates inter-rod energy transfer, as the more packed conformation increases the probability of energy transfer between bilins from different rods. Excitation of the mobile rod in down-down conformation leads to about 10 % population of the lowest rod, while for the up-up conformation the population of this rod is essentially zero (Fig. 5c). The same effect occurs for excitation of the bottom rod; essentially no inter-rod energy transfer is observed for the up-up conformation, while a

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non-negligible population is found at the other rods in the down-down PBS (Fig. 5c). Thus, the more compact down-down conformation allows for more pathways to reach the core, slightly increasing the overall trapping efficiency. On the other hand, the fact that significant movement of the mobile rod keeps the overall flow of energy through the system virtually unchanged points to robustness of PBS with respect to light-harvesting capacity. The robustness of PBS is further underscored by comparison with the red algal PBS, which shows that the overall dynamics are comparable^{15,16,36}. We note that two PBS configurations differing by their fluorescence spectra have been identified by single molecule spectroscopy³⁷. It is thus tempting to associate the up-up and down-down conformations with those two species. Here, however, the structures of up-up and down-down conformations do not yet reveal any influence on the structure of the PBS core that is needed to change the fluorescence of terminal emitters. Thus, the relation of the two species identified by different methods remains to be defined.

Importance of conformational switching for light harvesting

The quality of our structural data and the various proportions of the three PBS conformations suggests that we have captured previously unknown conformations of the cyanobacterial antenna. Why have the different rod conformations not been described before? In retrospect, it becomes clear that during earlier attempts to visualize the cyanobacterial PBS different conformation were probably confounded with general flexibility²² or likely disregarded as damaged particles³⁸. Other methods to determine the structural arrangement of the rods like crosslink-MS are not suited to distinguish between inter and intra-rod contacts and therefore are likewise blind to the conformations we identified^{29,39}. Because the static rods already display two different hinge conformations, it is unlikely that the rod movement is an artifact from sample preparation or imaging. The observed movement is directional and quantifiable, hallmarks of true biologically relevant conformational changes. It is tempting to assume that the mechanistic cause for the rod movement is coupled to the transfer of excitation energy through

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the PBS. Pigment molecules can be found near several residues critical for rod movement (Fig. 3d, Extended Fig. 6b), but further studies are now needed to explore whether any of the pigments within the PBS can indeed trigger a conformational change and dictate rod position. The newly described PBS architectures suggests a regulation of light harvesting at the level of supramolecular organization in the thylakoid membrane that is somehow regulated by rod movement.

We propose two scenarios for placing our structures in that context. PBS arrays efficiently pack light harvesting pigments on the surface of the thylakoid membrane and thus increase the overall photon absorption cross-section of a cell. Additionally, they create redundancy of light harvesting and photochemical energy conversion at the reaction centers and can thus alleviate energetic losses due to damaged PSII. Using the published structure of an array as a model⁴⁰, we found that only the ‘up-up’ state of the PBS could be fitted into the array (Extended Data Fig. 7c). Modeling of the energetics of our structure in arrays indicates that lateral energy transfer between PBSs within an array is possible, mainly between adjacent PBS cores.

Under certain conditions, PBS arrays might need to be dismantled to rebalance PSII versus PSI excitation pressure. Given that the down-down and down-up PBS conformations cannot be fitted into arrays, (Extended Data Fig. 7d, e), a conformational change in one PBS unit could therefore help to break up such arrays by acting as a terminator or interrupter of PBS array formation.

The second scenario in which PBS conformation may regulate the balance between light harvesting and photoprotection is coupled with the activity of the photoreceptor OCP. OCP quenches the excitation energy by converting it to vibrational energy. Activated OCP binds to the lateral side of the PBS core in the space between the rods⁴¹. However, the binding site is completely blocked when the mobile rod is in the ‘down’ position. Therefore, the mobile rod controls access of OCP to the PBS core and determines whether the PBS can be quenched.

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Having more than one mechanism could be a means for the cell to fine tune its light harvesting efficiency to adjust to altered metabolic and cellular requirements. Moreover, these scenarios are not mutually exclusive and could work concurrently, but on different time scales, to adjust light-harvesting in response to environmental changes. The OCP binding site is still accessible in PBS arrays⁴¹, and thus PBS arrays are readily available for OCP induced excitation energy quenching upon rapid changes in the environment. Supramolecular rearrangements of the photosynthetic machinery can then take place to rebalance light harvesting, which might involve rod movement and array dispersion. Several studies have revealed that excitation energy harvested at the PBS can be transferred to PSI³⁹. Given that PSI does not require excitation energy quenching at the light-harvesting system, it can be hypothesized that the down-down position obscuring the OCP binding site might be involved mainly in transferring its harvested energy to PSI. In fact, in such a scenario, OCP induced quenching would be counterproductive.

Here, we present the first high-resolution structures of cyanobacterial phycobilisomes, revealing an unexpected variety of conformational states that help to collect and deliver light energy to photosynthetic reaction centers. Further analysis of these structures will inform future experiments that will help us to understand and harness the power of these large light harvesting machines.

301 **Methods**

302 **Preparation of the *Synechocystis* PCC 6803 Phycobilisome**

303 *Synechocystis* PCC 6803 were grown photoautotrophically in a BG11 medium. Cells were kept in a
 304 rotary shaker (100 rpm) at 30°C, under 3% CO₂ enrichment, illuminated by white fluorescent lamps
 305 with a total intensity of about 30 μmol photons m⁻² s⁻¹. The protocol used for PBS isolation was
 306 based on^{42,43}. The cells were collected at 8,000 rpm, 10 min at room temperature. The pellet was
 307 resuspended in 10 mL of PBS isolation buffer (0.75 M potassium phosphate buffer pH 7.5, 1 mM
 308 EDTA, 0.5 mM PMSF) per 2 g of fresh weight of cells. Then, the cells were washed twice by
 309 centrifugation at 30,000 g during 15 min. The cells were broken through French Press at 1,000 psi
 310 three times. The supernatant was incubated in the presence of 2% (v/v) Triton X-100 under dim
 311 stirring, at 23°C, 15-20 min. The cell debris and the aggregates were removed by ultracentrifugation
 312 at 20,000 rpm (Ti-70 rotor) for 20 min at room temperature. The dark blue supernatant was directly
 313 loaded onto a discontinuous sucrose gradient (0.5, 0.75, 1, and 1.5 M sucrose layers in 0.75 M
 314 potassium phosphate buffer, pH 7.5), and spun at 150,000 g, 23°C, for 16 h using a SW28 rotor. The
 315 intact PBS was recovered from the 0.75 – 1 M interface of the sucrose gradient. The PBS were buffer
 316 exchanged into 0.75 M potassium phosphate buffer pH 7.5 using Amicon (30 kDa). The samples were
 317 kept at room temperature for further use.

318 **Protein separation**

319 The isolated PBS samples were concentrated by precipitation with 20% (v/v) trichloroacetic acid prior
 320 to loading on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 5-20%
 321 gradient gel. The gels were run for 25 min at 240 V and were stained by Coomassie Brilliant Blue.
 322 The protein composition of the SDS-PAGE confirms the presence of all PBS subunits (Extended Data
 323 Tables 1, 2).

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Absorption and fluorescence spectrum measurement

PBS ultraviolet–visible (UV-vis) absorption spectra were collected with a Cary 60 spectrophotometer (Agilent). The fluorescence emission spectra of the PBS were recorded at room temperature from 600 to 800 nm in a fluorimeter (TECAN Spark 20M multimode microplate reader) with an excitation wavelength of 580 nm.

PBS biotinylation

The ChromaLink™ Biotin Labeling reagent, purchased from Solulink (see <https://vectorlabs.com/chromalink-biotin-antibody-labeling-kit.html>), was used as it incorporates a chromophore as part of the linker. 120 µl of the PBS in 0.75 M potassium-phosphate pH 7.5 was buffer exchanged with the 1X Modification buffer (10X Modification buffer- 100 mM sodium phosphate and 150 mM sodium chloride pH 8.0-diluted in 0.75 M potassium-phosphate pH 8.0). Then, the sample amount was quantified using an absorbance spectrum and using the values at 280, 354 and 620 nm to calculate the amount of biotin to add to the reaction. The samples were then incubated for 120 min at room temperature. The sample was buffer exchanged back into 100 µl of 0.75 M potassium-phosphate pH 7.5 using a Zeba Spin Desalt 7 kDa MWCO column, Thermo Scientific). UV–vis spectra were measured from 230–800 nm for biotinylated protein samples using a Tecan Safire microplate reader. Using the absorbance values at 280, 354 and 620 nm, and the molar extinction coefficients, the average number of biotin molecules covalently attached per PBS were calculated to be about 1. Fluorescence emission spectra were used to confirm functional energy transfer in the isolated PBS before and after biotinylation (Extended Data Fig. 1d).

Cryo-EM sample preparation from purified PBS

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For sample preparation we used Quantifoil Au 300 mesh 2/1 grids covered with a home-made streptavidin monolayer, which were manufactured as described previously³⁰. To apply the sample, grids were first rehydrated in buffer A (375 mM potassium phosphate, pH 7.5) and then blotted dry with filter paper. 4 µl of PBS sample at 5.3 mg/ml in buffer B (750 mM potassium phosphate, pH 7.5) were added to the grid and then incubated on the bench for 60 seconds. Grids were washed on two 10 µl drops of buffer C (375 mM potassium phosphate, pH 7.5, 3% w/v trehalose, 0.01% v/v NP40, 0.05% w/v beta-octylglucoside) before being carefully wicked with Whatman filter paper. 1 µl of buffer C was added quickly, and the grid lifted into a FEI Mark IV Vitrobot. The grid was then manually blotted for 2-3s at 18°C and 100% humidity before plunging into a liquid ethane-propane (3:1) mix⁴⁴. During the process, the PBS was exposed to ambient light for a total of approximately 2-3 min between pipetting and vitrification.

Cryo-EM data collection PBS

For initial assessment of the sample, grids were loaded onto a Talos Arctica microscope (Thermo Fisher Scientific) equipped with a Gatan K3 direct electron detector and operating at 200 kV. 1954 movies were collected at a super-resolution pixel size of 0.558 Å, a defocus range of -0.6 µm to -1.8 µm and a total exposure of 50 e⁻/Å² using SerialEM⁴⁵. Images were processed as described below. After evaluation of the first dataset, a second dataset was collected on a Titan Krios G3i microscope operating at an acceleration voltage of 300 kV, equipped with a Gatan K3 direct electron detector operating in CDS mode⁴⁶ and a GIF Quantum energy filter with a 20-eV slit width. 12,051 movies were acquired with SerialEM in super-resolution counting mode with a super-resolution pixel size of 0.525 Å using an image shift collection scheme with active beam tilt correction, a defocus range from -0.5 µm to -1.6 µm and a total electron exposure of 50 e⁻/Å².

Image processing

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368 All movies were aligned, gain corrected and binned by 2 using MotionCorr2 as implemented in
369 RELION³⁴⁷. The background streptavidin lattice in the motion corrected micrographs was subtracted
370 using in-house scripts³⁰. These subtracted micrographs were then imported into Cryosparc⁴⁸ for patch
371 CTF estimation and further image processing. For dataset 1 from the Talos Arctica microscope,
372 particles were picked using the blob picker, extracted with a box size of 720 pixels, and subjected to
373 one round of 2D classification. Good classes representing different views of the PBS were selected
374 and used as templates for a second round of autopicking, yielding ~156,000 particles. After another
375 round of 2D classification, ~43,000 particles in good classes were used to generate an ab-initio model
376 of the PBS. A subsequent round of heterogenous refinement using this reference yielded two distinct
377 classes representing the PBS^{up-down} (66 %) and PBS^{up-up} (34 %) conformations. These classes were
378 refined to 4.1 Å and 4.5 Å, respectively.

379 For dataset 2 from the Titan Krios microscope, particles were picked from 800 micrographs using
380 templates generated from the PBS^{up-up} reconstructions from dataset 1. After 2D classification and
381 heterogenous refinement three distinct classes corresponding to PBS^{up-down}, PBS^{up-up} and PBS^{down-down}
382 conformations became apparent. Using these classes as references, 11,903 micrographs were picked
383 to yield ~1,900,000 particles. After several rounds of heterogenous refinement, approximately 510,000
384 good particles were sorted into four classes representing the three conformations (PBS^{up-down} 50.6%,
385 PBS^{up-up} 38.9%, and PBS^{down-down} 10.5%). Refinement of the three conformations yielded overall
386 resolutions of 2.7 Å (PBS^{up-up}), 2.8 Å (PBS^{up-down}) and 3.5 Å (PBS^{down-down}).

387 During initial rounds of 2D classification of the datasets, we observed that several classes
388 corresponded to PBS-rods that aligned independently of the core section of the PBS. To obtain
389 reconstructions of the rods, these classes were used as templates for autopicking on a subset of 500
390 micrographs of dataset 2 and extracted with a box size of 360 pixels. 2D classification followed by ab-
391 initio model generation and heterogenous refinement yielded a particle set that reached a resolution of
392 2.9 Å during non-uniform refinement. Using the same templates for picking from the entire dataset 2,

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~2,100,000 good particles were obtained that refined to a resolution 2.1 Å after CTF refinement, reaching Nyquist frequency. Further classification of the particle set to distinguish between different rod protomers within one PBS complex was unsuccessful and always yielding the same conformation. To visualize the contact area between two neighboring rods in the PBS^{up-down} conformation, particle subtraction followed by local refinement was carried out on the PBS^{up-down} reconstruction. The map yielded an improved resolution of 2.6 Å.

All processing was carried out using C1 symmetry and resolutions were estimated with the FSC = 0.143 criterion⁴⁹. Reconstructions were sharpened using DeepEMhancer⁵⁰ with the default tight mask preset.

Atomic model building and refinement

Using our PBS-OCPR^R atomic models as a basis⁴¹, atomic models of the PBS core were refined using a cropped PBS^{up-down} map with the real space refinement program in PHENIX 1.19.2 (ref. ⁵¹). Similarly, models of the core of other PBS conformations were refined but found to be virtually identical. Models of the PBS-OCPR^R rod were used as a basis for refinement of the PBS rod. To arrive at models for all three holo-PBS complexes, maps and models for the individual rods were rigid-body docked into the density of each holo-PBS conformer, which resulted in an unambiguous orientation of each rod. Because the holo-PBS maps are not resolved enough in the distal rod regions to allow for model refinement, models of the full complexes are for visualization only.

LC-MS/MS analysis of the PBS SDS-PAGE fractions

Gel bands were digested in-gel according to⁵² with modifications. Briefly, gel bands were dehydrated using 100 % acetonitrile and incubated with 10 mM dithiothreitol in 100 mM ammonium bicarbonate, pH~8, at 56 °C for 45 min, dehydrated again and incubated in the dark with 50 mM iodoacetamide in 100 mM ammonium bicarbonate for 20 min. Gel bands were then washed with ammonium bicarbonate

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416 and dehydrated again. Sequencing grade modified trypsin was prepared to 0.01 µg/µl in 50 mM
417 ammonium bicarbonate and ~100 µl of this was added to each gel band so that the gel was completely
418 submerged. Bands were then incubated at 37 °C overnight. Peptides were extracted from the gel by
419 water bath sonication in a solution of 60% Acetonitrile (ACN) /1% Trifluoroacetic acid (TFA) and
420 vacuum dried to ~2 µl.

421 Dried samples were re-suspended to 20 µl in 2% ACN/0.1% TFA and an injection of 5 µl was
422 automatically made using a Thermo (www.thermo.com) EASYnLC 1000 onto a Thermo Acclaim
423 PepMap RSLC 0.1mmx20mm C18 trapping column and washed for ~5 min with buffer A. Bound
424 peptides were then eluted over 35 min onto a Thermo Acclaim PepMap RSLC 0.075mm x 250mm
425 resolving column with a gradient of 5%B to 40%B in 24 min, ramping to 90%B at 25 min and held at
426 90%B for the duration of the run (Buffer A = 99.9% water/0.1% formic acid, Buffer B = 80%
427 acetonitrile/0.1% formic acid/19.9% water) at a constant flow rate of 300 nl/min. Column temperature
428 was maintained at a constant temperature of 50 °C using an integrated column oven (PRSO-V1,
429 Sonation GmbH, Biberach, Germany).

430 Eluted peptides were sprayed into a ThermoScientific Q-Exactive mass spectrometer
431 (www.thermo.com) using a FlexSpray spray ion source. Survey scans were taken in the Orbitrap
432 (70,000 resolution, determined at m/z 200) and the top 15 ions in each survey scan are then subjected
433 to automatic higher energy collision induced dissociation (HCD) with fragment spectra acquired at
434 17,500 resolution. The resulting MS/MS spectra are converted to peak lists using Mascot Distiller,
435 v2.7.1 (www.matrixscience.com), and searched against a database containing all *Synechocystis* PCC
436 6803 protein sequences and appended with common laboratory contaminants (downloaded 2020-11-
437 17 from www.uniprot.org and www.thegpm.org, respectively) using the Mascot searching algorithm,
438 v2.7. The Mascot output was then analyzed using Scaffold, v5.0 (www.proteomesoftware.com) to
439 probabilistically validate protein identifications. Assignments validated using the Scaffold 1%FDR
440 confidence filter are considered true.

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441 They performed LC-MS/MS on the protein bands to confirm the identity of all the PBS components
442 (Extended Data Table 2).

443 **Modelling the energy flow within PBS complex.**

444 Excitation energy transfer within PBS was studied in the limit of the Förster theory, using dipole-
445 dipole approximation for the inter-pigment coupling. The energy transfer rates k (in ps⁻¹) were
446 computed using the equation⁵³:

$$447 \quad k_{ij} = 1.18 V^2 J \quad (1)$$

448 where J is the spectral overlap between donor emission and acceptor absorption, and the coupling V
449 (in cm⁻¹) is given by

$$450 \quad V = 5.04 \frac{\mu_i \mu_j \kappa}{R^3} \quad (2)$$

451 where R is the center-to-center dipole separation in nm, κ is the orientation factor, and μ_i and μ_j are the
452 transition dipole moments (in Debye) of the donor and acceptor, respectively. We have used effective
453 transition dipole moments, including the correction for the effect of dielectric properties of the protein
454 (see ref.⁵³ for discussion of this point). The direction of the pigment transition dipoles of bilin
455 chromophores were placed along the axis of the conjugated parts of the molecules. The geometrical
456 axis was obtained using the singular-value decomposition on the atom coordinates. For bilins, the
457 transition dipole moment of 13 D were used for the bilins in rod (CPC), and 15 D for bilins in the core
458 (APC) pigments⁵⁴. The spectral overlap J is calculated from the area-normalized spectra of donor and
459 acceptor reported in literature^{34,55,56}. Using the full matrix of the excitation transfer rates computed
460 using (1), it is possible to write down a master equation describing the time evolution of probability of
461 exciton residence on i -th chromophore, with a vector-valued $y(t) = y_i(t)$ (for i running from 1 to the
462 number of pigments):

$$463 \quad \frac{dy(t)}{dt} = K \cdot y(t) \quad (3)$$

464

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where $\mathbf{K}$ represents the transition matrix whose off-diagonal elements (K_{ij}) represent the pairwise rates of energy transfer, k_{ij} , between donor (i) and acceptor (j) pigments. The diagonal elements are defined as:

$$K_{ii} = -\left(k_{ii}^0 + \sum_{i,i \neq j} k_{ij}\right) \quad (4)$$

where k_{ii}^0 corresponds to the intrinsic excited state decay rate of the i -th pigment; we used $k_{ii}^0 = (1500 \text{ ps})^{-1}$ for all bilins, except for b-84, which was assigned the lifetime of 900 ps, based on⁵⁷, however, the exact value of this parameter has little effect on the simulation results. Solving (3) for initial conditions given as $y_i(0) = 1$ for $i = k$, and $y_i(0) = 0$ for $i \neq k$, yields the model for the excitation flow through the PBS following excitation into k -th pigment. A sum of $y_i(t)$ over i then describes the total decay of the excitation in the PBS. The validity of the model was tested by comparing the results with experimental data reported earlier. Extended Data Fig. 7 shows that correspondence between the simulation and experimental data is reasonable, considering that the model was not in any way adjusted to fit the experimental data and is based solely on the structural information and previously reported pigment spectra.

Bioinformatics

Homologues of ApcG were found by searching with a profile HMM (constructed from sll1873 homologs obtained by BLAST search) against 420 non-redundant UniProt proteomes that contained a complete PBS protein set (as described in ref.⁴¹). Sequences of the 50 proteomes with a protein composition closest to *Synechocystis* PCC 6803 were used for sequence alignments. Sequences were aligned ClustalW^{58,59}, trimmed with trimAl⁶⁰ and the protein sequence conservation was visualized with Weblogo 3.74⁶¹. Homologues of ApcG were found by searching with a profile HMM (constructed from sll1873 homologs obtained by BLAST search) against 420 non-redundant UniProt proteomes

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Author contributions

MADM and PVS designed and performed experiments, interpreted results. CK designed and supervised the project. HK helped with the sample preparation, interpreted results. MS refined the structures and interpreted results. DB and TP performed the model for the energy transfer. BJG

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511 performed initial characterization of the specimen for cryo-EM. MADM, PVS and CK wrote the
512 manuscript with help from all authors.

513 **Competing interest**

514 The authors declare no competing interests.

515 **Data availability**

516 The atomic coordinates have been deposited in the Protein Data Bank with the accession codes 7SC8
517 for the rod and 7SC7 for the PBS^{up-down} core.

518 The EM maps have been deposited in the Electron Microscopy Data Bank with the accession codes
519 25029 for the rod, 25028 for the PBS^{up-down} core, 25069 for the full map of the PBS^{up-down} conformation
520 and the rod-rod contact, 25070 for the full map of the PBS^{down-down} conformation and 25071 for the
521 full map of the PBS^{up-up} conformation.

522 All other data are available from the corresponding authors upon reasonable request.

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523 Extended Data

524 **Extended Data Table 1: *Synechocystis* PCC 6803 Phycobilisome Protein subunits.** (*) Nomenclature used along the text.

525 (**) New linker discovered and incorporated in this study.

Protein component	Gene symbol	Protein name (*)	Function	pfam	#bilins per monomer	#proteins per PBS	MW (kDa)
α -PC	<i>cpcA</i>	CpcA	Rod antenna protein	pfam00502	1 PCB	108	17.6
β -PC	<i>cpcB</i>	CpcB	Rod antenna protein	pfam00502	2 PCB	108	18.1
L _{R33}	<i>cpcC1</i>	CpcC1	Internal rod linker	pfam00427/ pfam01383	--	6	32.5
L _{R30}	<i>cpcC2</i>	CpcC2	Internal rod linker	pfam00427/ pfam01383	--	6	30.8
L _{R10}	<i>cpcD</i>	CpcD	Internal rod linker	pfam01383	--	6	9.3
L _{RC}	<i>cpcG1</i>	CpcG1	Rod-to-core linker	pfam00427	--	6	28.9
L _{RC}	<i>cpcG2</i>	CpcG2	Rod-to-core linker	pfam00427	--	--	28.5
α -APC	<i>apcA</i>	ApcA	Major core antenna protein	pfam00502	1 PCB	32	17.4
β -APC	<i>apcB</i>	ApcB	Major core antenna protein	pfam00502	1 PCB	34	17.2

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L _C	<i>apcC</i>	ApcC	Internal core protein	pfam01383	--	6	7.7
L _{CM}	<i>apcE</i>	ApcE	Core-to- membrane linker	pfam00427/ pfam00502	1 PCB	2	100.3
α -B	<i>apcD</i>	ApcD	Minor core antenna protein	pfam00502	1 PCB	2	17.9
α -B18	<i>apcF</i>	ApcF	Minor core antenna protein	pfam00502	1 PCB	2	18.9
L _{C10} **	<i>apcG</i>	ApcG	Linker	Newly discovered no assigned pfam	--	2	12.9

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527 **Extended Data Table 2: MS analysis of the *Synechocystis* PCC 6803 Phycobilisome Protein subunits.** The criteria were
528 2 peptides, False Discovery Rate (FDR) = 1%, percentage of coverage (% coverage) > 30%, and the number of total
529 peptide count of each protein (# total peptide count). MW (kDa) = Molecular Weight assigned by the software used in the
530 analysis, Scaffold 5.0 software.

Protein component	Gene symbol	# total peptide count	% coverage	MW (kDa)
α -PC	<i>cpcA</i>	420	94	18
β -PC	<i>cpcB</i>	414	93	18
L _{R33}	<i>cpcC1</i>	236	61	33
L _{R30}	<i>cpcC2</i>	219	60	31
L _{R10}	<i>cpcD</i>	148	88	9
L _{RC}	<i>cpcG1</i>	215	83	27
L _{RC}	<i>cpcG2</i>	21	43	29
α -APC	<i>apcA</i>	224	93	17
β -APC	<i>apcB</i>	322	94	17
L _C	<i>apcC</i>	86	63	8
L _{CM}	<i>apcE</i>	671	72	100
α -B	<i>apcD</i>	61	83	18
α -B18	<i>apcF</i>	66	71	19
L _{c10}	<i>apcG</i>	27	78	13
FNR	<i>petH</i>	177	78	46
α -PC	<i>cpcA</i>	420	94	18
β -PC	<i>cpcB</i>	414	93	18
L _{R33}	<i>cpcC1</i>	236	61	33
L _{R30}	<i>cpcC2</i>	219	60	31
L _{R10}	<i>cpcD</i>	148	88	9
L _{RC}	<i>cpcG1</i>	215	83	27
L _{RC}	<i>cpcG2</i>	21	43	29
FNR	<i>petH</i>	177	78	46

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531 **Extended Data Table 3: Cryo-EM data collection, refinement and validation statistics**

EMD PDB	PBS ^{up-up}	PBS ^{up-down}	PBS ^{up-up} 25071	PBS ^{up-down} 25069	PBS ^{up-down} core 25028 7SC7	PBS ^{down-down} 25070	Rod 25029 7SC8
Dataset	1		2				
Data collection and processing							
Magnification	36000 x		81000 x				
Voltage (kV)	200		300				
Electron exposure (e-/Å ²)	50		50				
Defocus range (µm)	0.6-1.8		0.5-1.6				
Pixel size (Å)	1.115		1.05				
Symmetry imposed	C1		C1				
Initial particle images (no.)	156,000		1.9M				6.6M
Final particle images (no.)	14,434	28,109	135,701	202,719	52,674	2.1M	
Map resolution (Å)	4.5	4.2	2.7	2.8	3.5	2.1	
FSC threshold 0.143	(C2 imposed)						
Map resolution range (Å)	N/A	N/A	2.5-10	2.6-10	3.2-10	2.1-2.5	
Refinement	N/A						
Initial model used (PDB code)			7SC9				7SCA
Model resolution (Å)			3				2.2
FSC threshold 0.5							
Model composition							
Non-hydrogen atoms			107930				54106
Protein residues			13756				6835
Ligands			72				54
B factors (Å ²)							
Protein			130.4				88.8
Ligand			125.5				88.6
R.m.s. deviations							
Bond lengths (Å)			0.003 (0)				0.002 (0)
Bond angles (°)			0.610 (207)				0.793 (13)
Validation							
MolProbity score			1.79				1.53
Clashscore			15.2				10.2
Poor rotamers (%)			0.01				0.00
Ramachandran plot							
Favored (%)			97.48				98.9
Allowed (%)			2.50				1.1
Disallowed (%)			0.01				0.00

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Extended Data Figure 1: Biochemical and spectroscopic PBS characterization. **a**, Protein composition analysis of the PBS sample by SDS-PAGE. Identification of the components by MS can be found in Extended Data Table 2. **b**, Absorption, and fluorescence spectrum of the isolated PBS in 0.75 M K-phosphate pH 7.5. The PBS shows a maximum absorbance at 620 nm corresponding to phycocyanin (PC), and a shoulder at 650 nm corresponding to allophycocyanin (APC). The fluorescence peak maximum is ~670 nm when PC is preferentially excited (excitation at 580 nm), and a shoulder from 740 nm to 780 nm. **c**, Sucrose gradient of PBS showing the intense blue band that was used for cryo-EM. **d**, Fluorescence spectra of PBS before and after in vitro biotinylation. Collectively, these data show that the PBS preparation was structurally and functionally intact.

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Extended Data Figure 2: Cryo-EM data processing of PBS data. *a*, Example raw micrograph before and after streptavidin lattice subtraction. *b*, Fourier transform of *a* showing Bragg diffraction of streptavidin crystal before subtraction. *c*, Processing pipeline of dataset 2, yielding the structures presented in this paper. Red to blue color shadings

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546 represent local resolution estimates at FSC=0.5 ranging from 2 to 6 Å. The three PBS conformations and the structure of
547 the rod are shown at two different thresholds to visualize both overall shape and higher resolution details.

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549 **Extended Data Figure 3: Map and Model quality of *PBS^{up-down}*.** **a**, EM density examples of CpcA, ApcE and associated
550 bilins showing map quality of the *PBS^{up-down}*. **b** and **c**, Cropped *PBS^{up-down}* map, model and corresponding Fourier shell
551 correlation (FSC) curves. Resolution of masked and unmasked models are indicated at FSC = 0.5.

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Extended Data Figure 4: Assembly of the PBS. The core cylinders consist of four stacked discs; each disc contains three α - and three β -type Apc proteins that form the signature $(\alpha\beta)_6$ hexamers. Besides the canonical ApcA and ApcB proteins, B1 and B2 cylinders contain one copy each of ApcD and ApcF, as well as the phycobiliprotein domain of ApcE. These are symmetrically arranged in the two bottom cylinders, oriented towards the membrane. ApcG, ApcD and ApcE protrude out of the core, indicating their likely role in contacting the photosystems. Each rod contains three stacked discs, each consisting of one CpcA/B hexamer. The overall architecture of the PBS core is in agreement with modelling predictions²⁹.

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Extended Data Figure 5: Molecular details of the PBS. **a**, Alignment of ApcE with red algae homologs (PDB:5Y6P *Griffithsia pacifica*, PDB:6KGX *Porphyridium purpureum*). RMSD is 1.9 Å, differences are circled. **b**, Structural models of ApcC, ApcG and CpcD. CpcD and ApcC consist of a single pf01383 domain. **c**, Alignment of CpcG1 with red algae

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563 homologs. Circle in CpcG1 shows C-terminal domain. CpcG1 is related to algal LRC1 linker with RMSD of 0.7 Å in the
 564 pf00427 domain. The C-terminal domain of CpcG1 has homology to LRC1a with RMSD of 0.6 Å. **d**, Comparison of CpcC1
 565 and CpcC2. **e-g**, interaction between CpcD, CpcC2, CpcC1 and CpcG1. Interacting residues are highlighted. **h**, Alignment
 566 of ApcD and ApcF with red algae homologs. ApcD is related to the red algal homolog with RMSD of 0.6 Å while ApcF
 567 aligns with an RMSD of 0.6 Å with its homolog. The only major differences exist in loop regions.

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570 **Extended Data Figure 6: PBS rod conformation.** *a*, Side view of AcpAB disc showing the groove network that organizes
571 CpcG1 linker attachment to the PBS core. Selected residues are labeled. *b*, Salt bridge formed between two neighboring
572 rods when the mobile rod adopts the 'down' conformation. EM density is transparent, dark grey and shows the
573 conformation of R150 when the mobile rod is 'up'. A bilin molecule in very close proximity of R150 of CpcB is also shown.

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Extended Data Figure 7: Decay associated spectra (DAS) and PBS arrays. DAS spectra obtained either **a**, from simulation or **b**, from fitting the data obtained from time-resolved fluorescence (right, adapted from ref.³⁴) monitoring the excitation energy flow in the PBS. In simulations, PBS was excited into the far end of the rod. The ~100 ps component (green) in the experimental data was interpreted as due to excitation annihilation³⁵, hence it is not present in the simulation. **c**, PBS arrays were modeled based on cryo-electron tomography data from ref.⁴⁰. **a**, only the up-up conformation is compatible with PBS arrays. **d**, The up-down conformation would result in array termination. **e**, A PBS switching to the down-down conformation could result in array dispersion.

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