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Title

Structures of a phycobilisome in light-harvesting and photoprotected states

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Journal

Nature, 609(7928)

ISSN

0028-0836

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Publication Date

2022-09-22

DOI

10.1038/s41586-022-05156-4

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Structures of the Cyanobacterial Phycobilisome

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28 **Abstract**

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

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53 Cyanobacteria are the most abundant and ecophysiolegically diverse primary producers on Earth.
54 Oxygenic photosynthesis is an ancient cyanobacterial innovation that allowed complex life to emerge¹,
55 and today these organisms are being established as platforms for green biotechnologies².
56 Cyanobacterial phycobilisomes (PBSs)³⁻⁵ are massive pigment-protein complexes that can constitute
57 up to half of the soluble protein content of the cell⁶. The two major PBS substructures, the core and
58 the rod, are composed of phycobiliproteins, and colorless linker proteins (LPs)⁷. Phycobilin pigments
59 are covalently bound to the phycobiliproteins⁸⁻¹⁴, which assemble into disc-like trimers ($\alpha\beta$)₃ or
60 hexamers ($\alpha\beta$)₆. These discs are chained together in the rods and core cylinders by LPs and organized
61 into a complete PBS¹⁵⁻¹⁷. The association of pigments with protein tunes the pigments' energetic
62 properties to establish an energy cascade to the photosynthetic reaction centers that is both extremely
63 fast and highly efficient (ca. 95%)^{18,19}. Four morphological types of PBS are known:
64 hemiellipsoidal^{16,20,21}, block-type¹⁵, hemidisoidal^{10,22-25} and bundle-type²⁶. *Synechocystis* PCC 6803
65 is the primary model system for molecular and spectroscopic studies²⁷ containing the most widespread
66 type of PBS, the hemidisoidal^{8,22,25,28,29}, which is also the evolutionary antecedent of all types of
67 PBS¹⁵. We report three cryo-electron microscopy (cryo-EM) structures of the 6.2 MDa PBS from
68 *Synechocystis* PCC 6803, representing distinct conformers of rod arrangements: the canonical 'up-up'
69 (2.7 Å), and two previously undescribed, 'up-down' (2.8 Å), and 'down-down' (3.5 Å) conformations.
70 We also describe the structure of all the linkers, one of them previously unknown, the arrangement of
71 all bilins, and elucidate the likely energy transfer pathway.

72 **Structural overview**

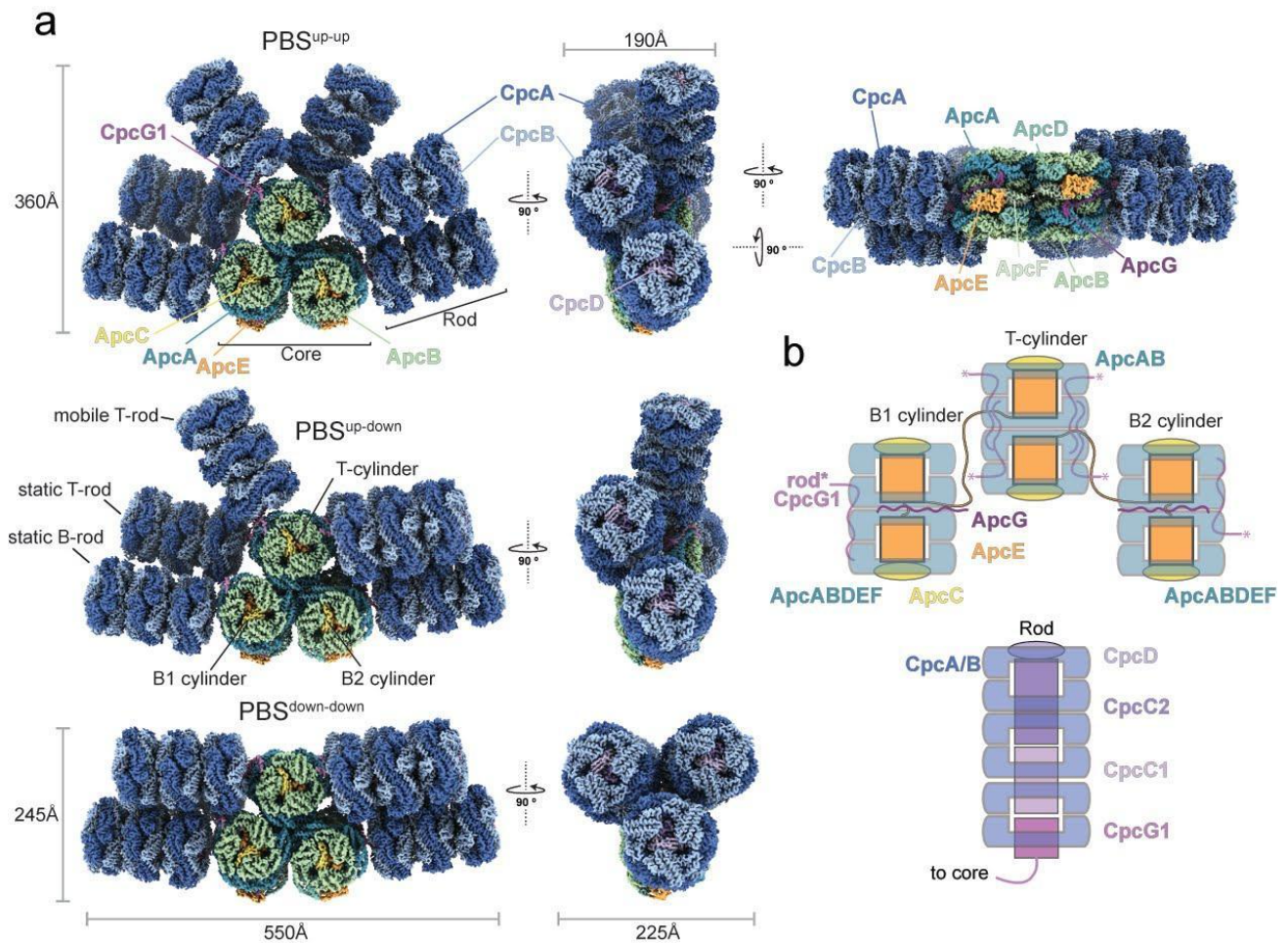
73 Purified and intact PBS complex was biotinylated and subjected to cryo-EM using streptavidin affinity
74 grids³⁰ to reduce preferential particle orientations and to increase stability (Methods, Extended Data
75 Fig. 1, Extended Data Table 1, 2). We determined the structure of three distinct PBS conformations at
76 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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90 **Figure 1: Structural characterization of the phycobilisome from *Synechocystis* PCC 6803.** *a*, Composite cryo-EM density
 91 maps representing the three PBS conformations: Up-up (top), up-down (middle), down-down (bottom). Individual maps
 92 and resolution for the three conformations and the rod can be found in Extended Data Fig. 2. *b*, Schematic overview of
 93 PBS connectivity and subunit arrangement of the core (top) and the rods (bottom).

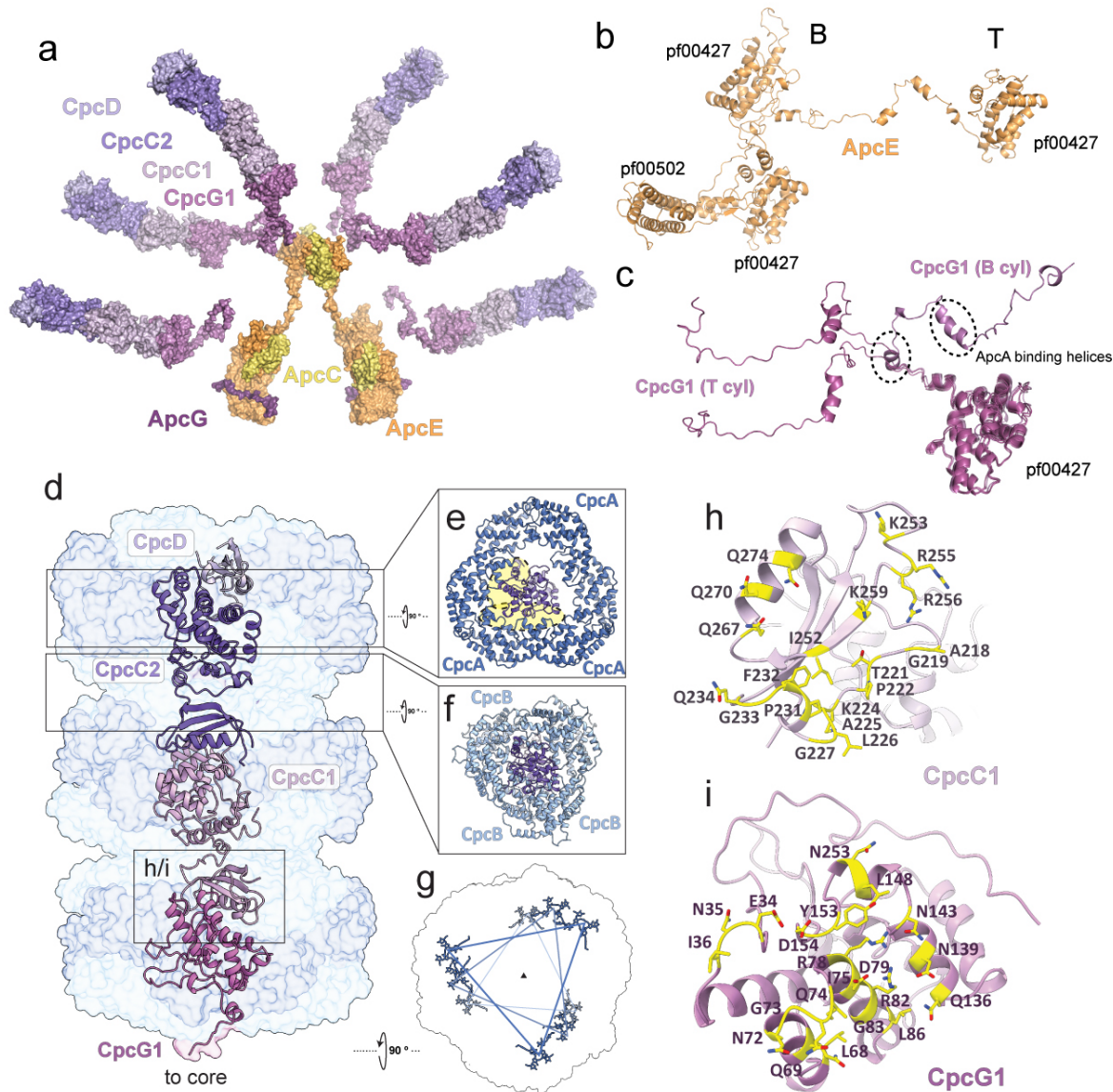
94 In contrast to earlier studies, our structures include three different, major rod conformations; two have
 95 not been visualized before. They are distinguished by alternate positioning of two of the four T rods
 96 (hence referred to as mobile T rods, as opposed to the static T rods). In the ‘up-up’ conformation, both
 97 mobile rods are tilted upwards, corresponding to the canonical hemidiscoidal PBS. Two previously
 98 unreported conformers were also present: the ‘up-down’ conformation, where one of the mobile rods
 99 moved downwards to assume a position parallel to the other rods and the ‘down-down’ conformation,
 100 in which all the T cylinder rods are in a down position, reducing the ‘height’ of the complex by more
 101 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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110

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

117 In the rods, there are four linker proteins (Fig. 2 a, d). CpcG1 is located at the core-proximal side. Its
 118 60 residue C-terminal extension attaches it to the PBS core (Figure 2 c, d, Extended Data Fig. 5c). The
 119 homologues CpcC1 and CpcC2 form the bulk of the interior of the rods (Fig. 2d, Extended Data Fig.

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120 5 d). The interdomain linker of CpcC1 is 15 residues longer than in CpcC2, allowing for a 70° rotation
121 of the pf01383 domain of CpcC1 compared to CpcC2 (Extended Data Fig. 5d). This results in an
122 apparent 10° rotation between each CpcA/B hexamer (Fig. 2g). Even though all linker proteins within
123 the rod employ the same two domains to connect to one another, differences in the binding interface
124 of each domain pair define a unique architecture for the correct assembly of the entire rod (Fig. 2 h, i,
125 Extended Data Fig. 5 e-g).

126

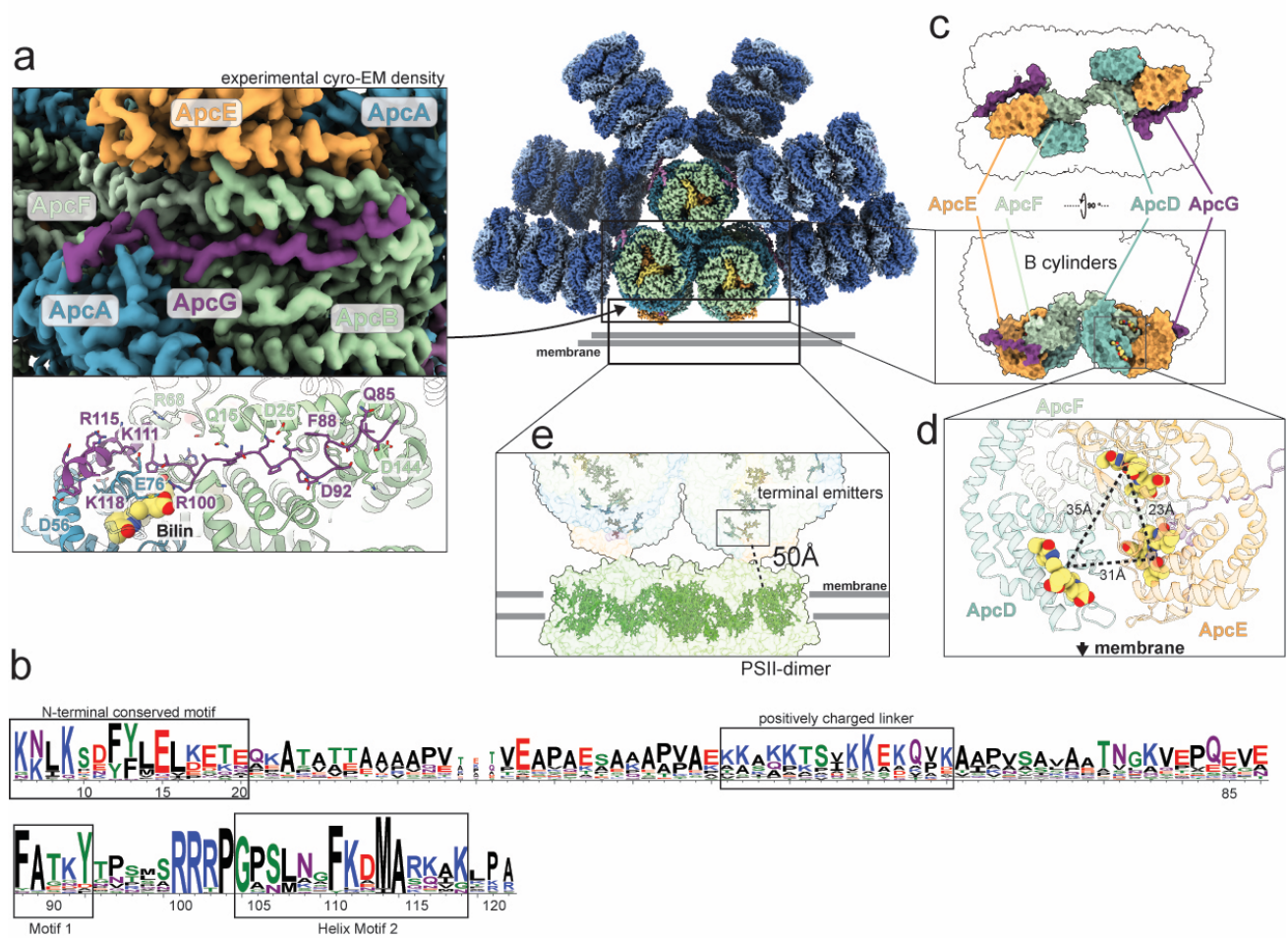
127 A previously unknown linker protein (*sll1873*) was discovered in the cryo-EM density and identified
128 by mass spectrometry (Fig. 3a, Extended Data Table 2). We denote it ApcG or L_{C10} in continuation of
129 the established nomenclature. It is found only on the bottom two cylinders extending toward the
130 membrane-facing side. An HMM search reveals that homologues are found in about 80% of
131 cyanobacterial genomes. ApcG contains two conserved sequence motifs including a short helix that
132 binds in a groove on ApcA like CpcG1 (Fig. 3b, Extended Data Fig. 5b). The highly conserved residue
133 R100 of ApcG directly contacts the PCB of ApcA in the bottom cylinder, suggesting that it may
134 finetune its spectroscopic properties. It has previously been suggested that the PBS interacts with lipid
135 head groups³¹; ApcG could mediate this interaction through its positively charged linker region (Fig.
136 3b). Given its location close to the terminal emitters and the membrane, we propose that ApcG likely
137 plays a key role in PBS localization and energy transfer to PSII.

138 **Terminal emitters**

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

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



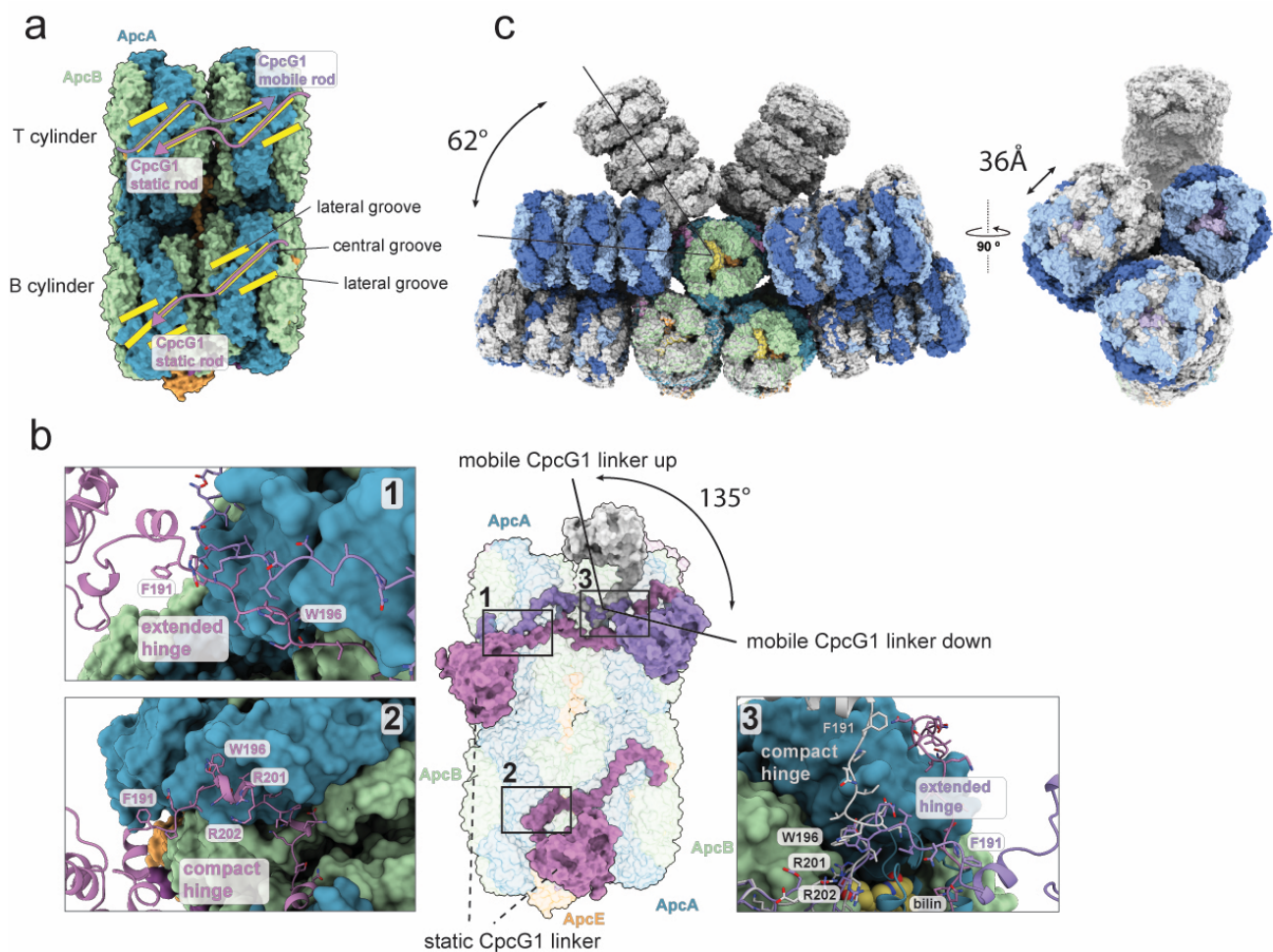
151
 152 **Figure 3: A newly identified linker ApcG and Terminal emitters.** *a*, Position and interaction of ApcG within the PBS. *b*,
 153 Sequence conservation logo of ApcG homologs. The numbers refer to residue positions in *Synechocystis* PCC 6803 ApcG.
 154 ApcG contains three conserved sequence motifs including a short helix that binds in a central groove of ApcA like CpcG1.
 155 *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($\alpha\beta$)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).

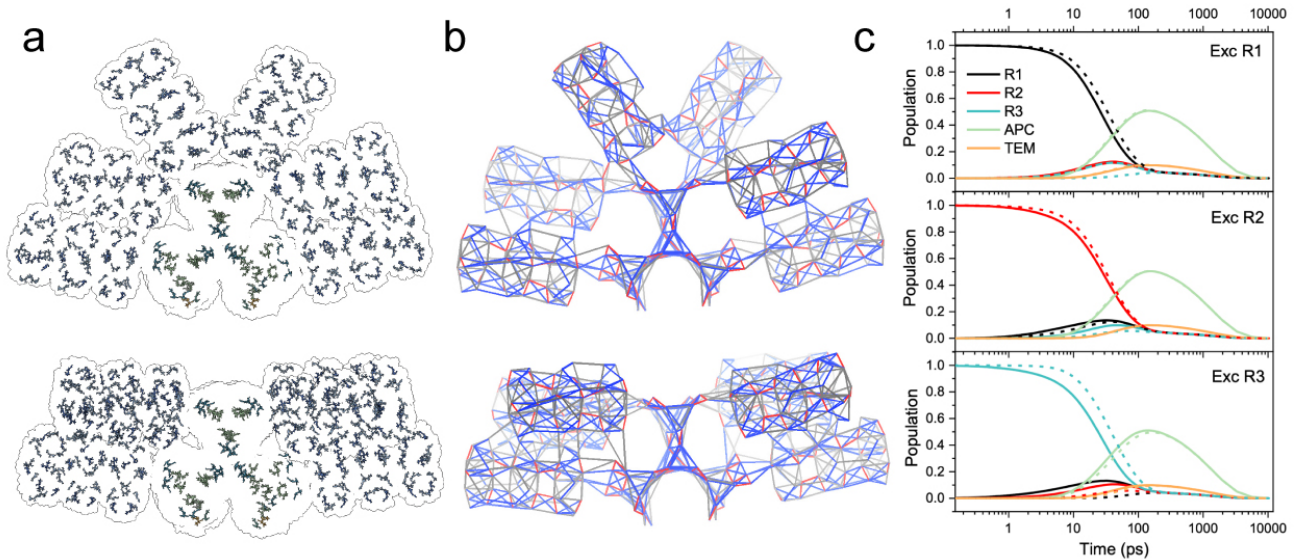
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193 The superposition of ‘down’ and ‘up’ conformations also reveals that the upper static rod experiences
194 a 36 Å lateral displacement when the mobile rod is down but relaxes into its central position once the
195 mobile rod swings upwards (Fig. 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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210 **Figure 5: Energy flow through the PBS.** *a*, Pigment distribution within PBS^{up-up} (top) and $PBS^{down-down}$ (bottom). *b*, Maps
211 of individual inter-pigment energy transfer rates of PBS^{up-up} (top) and $PBS^{down-down}$ (bottom). The color bars correspond to
212 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
213 ps are omitted. *c*, Energy flow through down-down (solid) and up-up (dashed) PBS configurations. The excitation is placed
214 either on the mobile rod R1 (top), middle rod R2 (middle) or bottom rod R3 (bottom). The curves monitor the evolution of
215 excited state population of pigments located in rod, core (APC), and terminal emitters (TEM).

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

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227 non-negligible population is found at the other rods in the down-down PBS (Fig. 5c). Thus, the more
228 compact down-down conformation allows for more pathways to reach the core, slightly increasing the
229 overall trapping efficiency. On the other hand, the fact that significant movement of the mobile rod
230 keeps the overall flow of energy through the system virtually unchanged points to robustness of PBS
231 with respect to light-harvesting capacity. The robustness of PBS is further underscored by comparison
232 with the red algal PBS, which shows that the overall dynamics are comparable^{15,16,36}.

233 We note that two PBS configurations differing by their fluorescence spectra have been identified by
234 single molecule spectroscopy³⁷. It is thus tempting to associate the up-up and down-down
235 conformations with those two species. Here, however, the structures of up-up and down-down
236 conformations do not yet reveal any influence on the structure of the PBS core that is needed to change
237 the fluorescence of terminal emitters. Thus, the relation of the two species identified by different
238 methods remains to be defined.

239 **Importance of conformational switching for light harvesting**

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

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

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

264
265 Under certain conditions, PBS arrays might need to be dismantled to rebalance PSII versus PSI
266 excitation pressure. Given that the down-down and down-up PBS conformations cannot be fitted into
267 arrays, (Extended Data Fig. 7d, e), a conformational change in one PBS unit could therefore help to
268 break up such arrays by acting as a terminator or interrupter of PBS array formation.

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

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

288

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

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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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324 **Absorption and fluorescence spectrum measurement**

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

329 **PBS biotinylation**

330 The ChromaLink™ Biotin Labeling reagent, purchased from Solulink (see
331 <https://vectorlabs.com/chromalink-biotin-antibody-labeling-kit.html>), was used as it incorporates a
332 chromophore as part of the linker. 120 µl of the PBS in 0.75 M potassium-phosphate pH 7.5 was buffer
333 exchanged with the 1X Modification buffer (10X Modification buffer- 100 mM sodium phosphate and
334 150 mM sodium chloride pH 8.0-diluted in 0.75 M potassium-phosphate pH 8.0). Then, the sample
335 amount was quantified using an absorbance spectrum and using the values at 280, 354 and 620 nm to
336 calculate the amount of biotin to add to the reaction. The samples were then incubated for 120 min at
337 room temperature. The sample was buffer exchanged back into 100 µl of 0.75 M potassium-phosphate
338 pH 7.5 using a Zeba Spin Desalt 7 kDa MWCO column, Thermo Scientific). UV–vis spectra were
339 measured from 230–800 nm for biotinylated protein samples using a Tecan Safire microplate reader.
340 Using the absorbance values at 280, 354 and 620 nm, and the molar extinction coefficients, the average
341 number of biotin molecules covalently attached per PBS were calculated to be about 1. Fluorescence
342 emission spectra were used to confirm functional energy transfer in the isolated PBS before and after
343 biotinylation (Extended Data Fig. 1d).

344 **Cryo-EM sample preparation from purified PBS**

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

356 **Cryo-EM data collection PBS**

357 For initial assessment of the sample, grids were loaded onto a Talos Arctica microscope (Thermo
358 Fisher Scientific) equipped with a Gatan K3 direct electron detector and operating at 200 kV. 1954
359 movies were collected at a super-resolution pixel size of 0.558 Å, a defocus range of -0.6 μ m to -1.8
360 μ m and a total exposure of 50 e⁻/Å² using SerialEM⁴⁵. Images were processed as described below.
361 After evaluation of the first dataset, a second dataset was collected on a Titan Krios G3i microscope
362 operating at an acceleration voltage of 300 kV, equipped with a Gatan K3 direct electron detector
363 operating in CDS mode⁴⁶ and a GIF Quantum energy filter with a 20-eV slit width. 12,051 movies
364 were acquired with SerialEM in super-resolution counting mode with a super-resolution pixel size of
365 0.525 Å using an image shift collection scheme with active beam tilt correction, a defocus range from
366 -0.5 μ m to -1.6 μ m and a total electron exposure of 50 e⁻/Å².

367 **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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393 ~2,100,000 good particles were obtained that refined to a resolution 2.1 Å after CTF refinement,
394 reaching Nyquist frequency. Further classification of the particle set to distinguish between different
395 rod protomers within one PBS complex was unsuccessful and always yielding the same conformation.
396 To visualize the contact area between two neighboring rods in the PBS^{up-down} conformation, particle
397 subtraction followed by local refinement was carried out on the PBS^{up-down} reconstruction. The map
398 yielded an improved resolution of 2.6 Å.

399 All processing was carried out using C1 symmetry and resolutions were estimated with the FSC =
400 0.143 criterion⁴⁹. Reconstructions were sharpened using DeepEMhancer⁵⁰ with the default tight mask
401 preset.

402 **Atomic model building and refinement**

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

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

412 Gel bands were digested in-gel according to⁵² with modifications. Briefly, gel bands were dehydrated
413 using 100 % acetonitrile and incubated with 10 mM dithiothreitol in 100 mM ammonium bicarbonate,
414 pH~8, at 56 °C for 45 min, dehydrated again and incubated in the dark with 50 mM iodoacetamide in
415 100 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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465 where \mathbf{K} represents the transition matrix whose off-diagonal elements (K_{ij}) represent the pairwise rates
466 of energy transfer, k_{ij} , between donor (i) and acceptor (j) pigments. The diagonal elements are defined
467 as:

$$468 \quad K_{ii} = - \left(k_{ii}^0 + \sum_{i,i \neq j} k_{ij} \right) \quad (4)$$

469
470 where k_{ii}^0 corresponds to the intrinsic excited state decay rate of the i -th pigment; we used $k_{ii}^0 = (1500$
471 $\text{ps})^{-1}$ for all bilins, except for b-84, which was assigned the lifetime of 900 ps, based on⁵⁷, however,
472 the exact value of this parameter has little effect on the simulation results. Solving (3) for initial
473 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
474 flow through the PBS following excitation into k -th pigment. A sum of $y_i(t)$ over i then describes the
475 total decay of the excitation in the PBS. The validity of the model was tested by comparing the results
476 with experimental data reported earlier. Extended Data Fig. 7 shows that correspondence between the
477 simulation and experimental data is reasonable, considering that the model was not in any way adjusted
478 to fit the experimental data and is based solely on the structural information and previously reported
479 pigment spectra.

480 **Bioinformatics**

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

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488 that contained a complete PBS protein set (as described in ref.⁴¹). Sequences of the 50 proteomes with
489 a protein composition closest to *Synechocystis* PCC 6803 were used for sequence alignments.
490 Sequences were aligned ClustalW^{58,59}, trimmed with trimAl⁶⁰ and the protein sequence conservation
491 was visualized with Weblogo 3.74⁶¹.
492 Structures were analyzed with ChimeraX⁶² and Pymol (The PyMOL Molecular Graphics System,
493 version 1.7 Schrödinger, LLC).

494 **Acknowledgements**

495 CAK and MADM would like to dedicate this manuscript to Dr. Nicole Tandeau de Marsac. The authors
496 want to thank Dr. Bryan Ferlez for taking TEM images. We thank Dr. Douglas Whitten from the
497 Proteomic Facility at Michigan State University, A. Chintangal and P. Tobias for computational
498 support, Dr. Daniel Toso and Jonathan Remis at the Cal-Cryo facility for support with cryo-EM data
499 collection, Dr. Robert Glaeser and Dr. Bong-Gyoon Han for advice concerning streptavidin grid
500 preparation, and Dr. Lisa Eshun-Wilson for help with data processing. Research in the Kerfeld lab was
501 supported by the Office of Science of the U.S. Department of Energy DE-FG02-91ER20021. This
502 project has received funding from the European Union's Horizon 2020 research and innovation
503 programme under the Marie Skłodowska-Curie grant agreement No. 795070. DB and TP thank the
504 Czech Science Foundation, grant No. 19-28323X. DB also acknowledges institutional support
505 RVO:60077344. Molecular graphics and analyses were performed with UCSF ChimeraX with support
506 from NIH R01-GM129325. E.N. is a Howard Hughes Medical Investigator.

507 **Author contributions**

508 MADM and PVS designed and performed experiments, interpreted results. CK designed and
509 supervised the project. HK helped with the sample preparation, interpreted results. MS refined the
510 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
LR33	<i>cpcC1</i>	CpcC1	Internal rod linker	pfam00427/ pfam01383	--	6	32.5
LR30	<i>cpcC2</i>	CpcC2	Internal rod linker	pfam00427/ pfam01383	--	6	30.8
LR10	<i>cpcD</i>	CpcD	Internal rod linker	pfam01383	--	6	9.3
LRC	<i>cpcG1</i>	CpcG1	Rod-to-core linker	pfam00427	--	6	28.9
LRC	<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
LR33	<i>cpcC1</i>	236	61	33
LR30	<i>cpcC2</i>	219	60	31
LR10	<i>cpcD</i>	148	88	9
LRC	<i>cpcG1</i>	215	83	27
LRC	<i>cpcG2</i>	21	43	29
α -APC	<i>apcA</i>	224	93	17
β -APC	<i>apcB</i>	322	94	17
Lc	<i>apcC</i>	86	63	8
LCM	<i>apcE</i>	671	72	100
α -B	<i>apcD</i>	61	83	18
α -B18	<i>apcF</i>	66	71	19
Lc10	<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
LR33	<i>cpcC1</i>	236	61	33
LR30	<i>cpcC2</i>	219	60	31
LR10	<i>cpcD</i>	148	88	9
LRC	<i>cpcG1</i>	215	83	27
LRC	<i>cpcG2</i>	21	43	29
FNR	<i>petH</i>	177	78	46

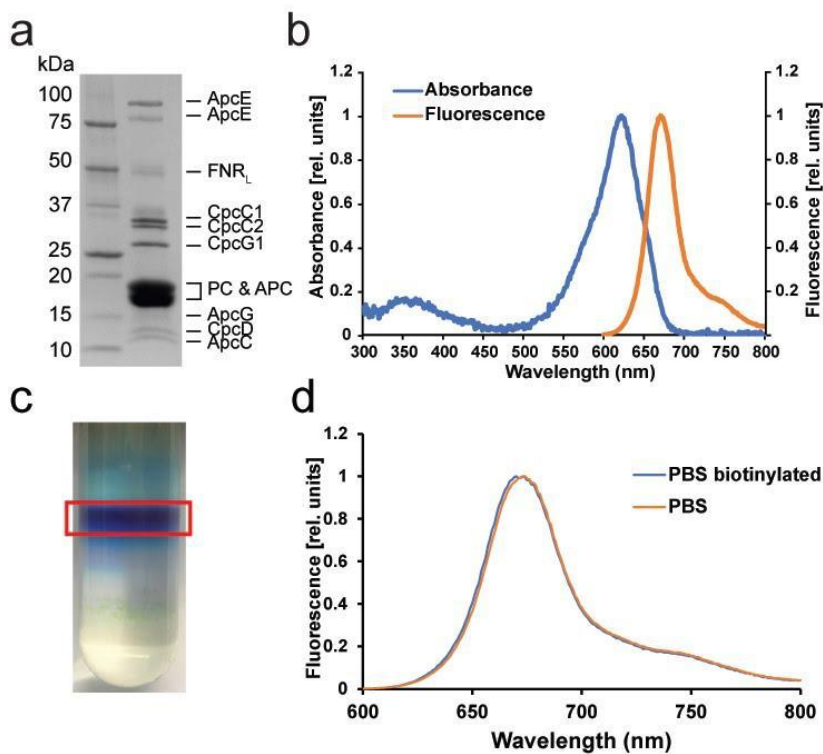
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531 **Extended Data Table 3: Cryo-EM data collection, refinement and validation statistics**

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		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
<i>B</i> 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

532

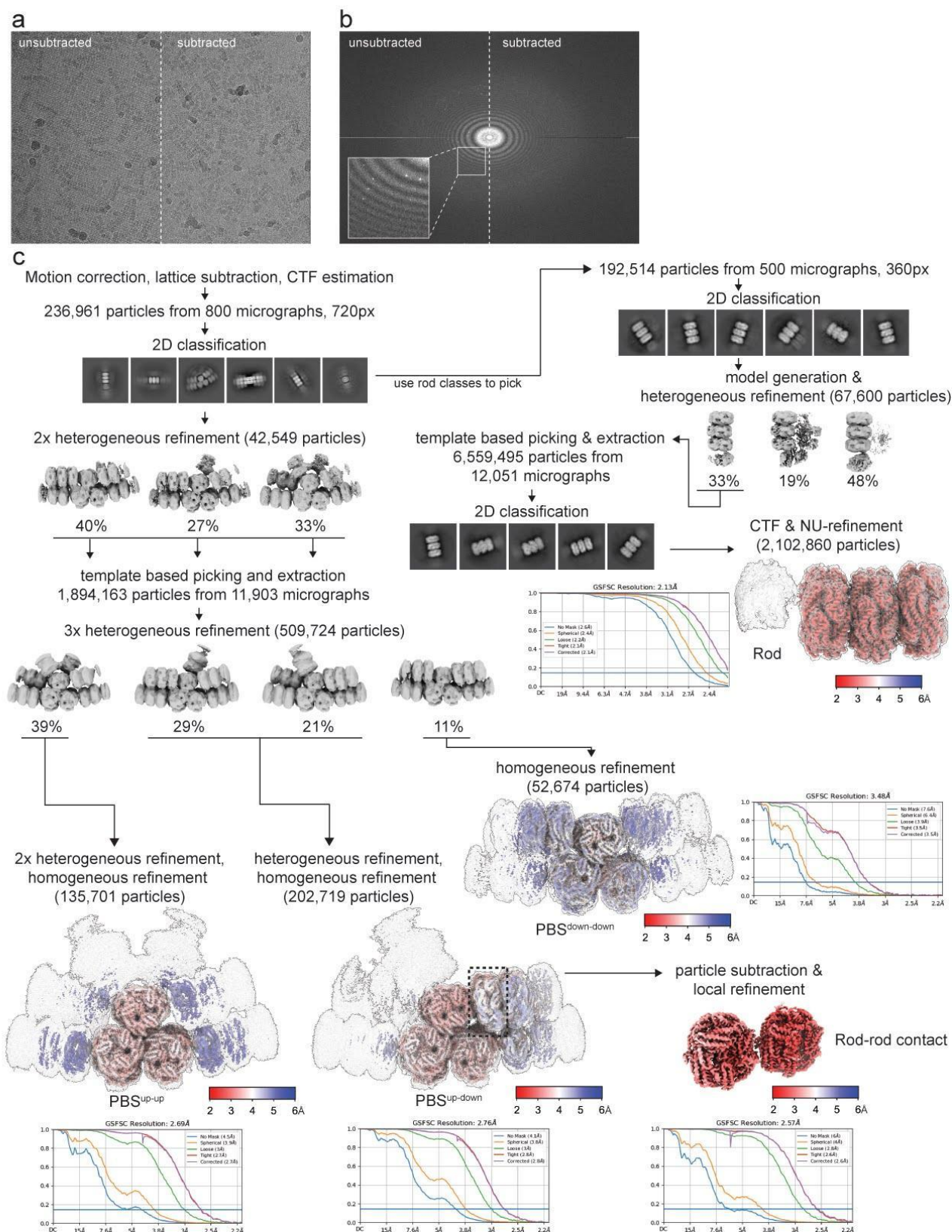
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533

534 **Extended Data Figure 1: Biochemical and spectroscopic PBS characterization.** *a*, Protein composition analysis of the
535 PBS sample by SDS-PAGE. Identification of the components by MS can be found in Extended Data Table 2. *b*, Absorption,
536 and fluorescence spectrum of the isolated PBS in 0.75 M K-phosphate pH 7.5. The PBS shows a maximum absorbance at
537 620 nm corresponding to phycocyanin (PC), and a shoulder at 650 nm corresponding to allophycocyanin (APC). The
538 fluorescence peak maximum is ~670 nm when PC is preferentially excited (excitation at 580 nm), and a shoulder from 740
539 nm to 780 nm. *c*, Sucrose gradient of PBS showing the intense blue band that was used for cryo-EM. *d*, Fluorescence
540 spectra of PBS before and after in vitro biotinylation. Collectively, these data show that the PBS preparation was
541 structurally and functionally intact.

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542

543 *Extended Data Figure 2: Cryo-EM data processing of PBS data. a, Example raw micrograph before and after*

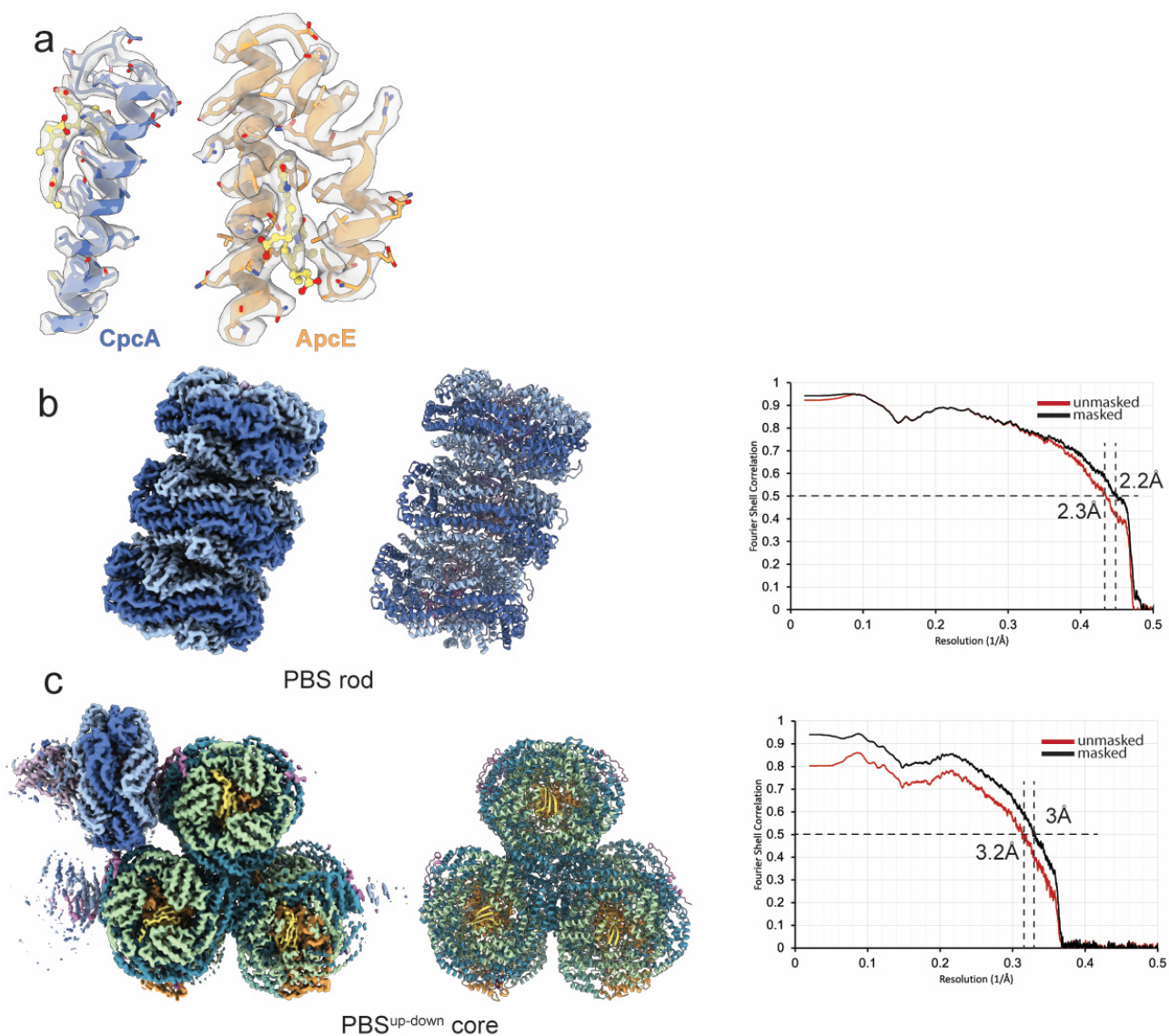
544 *streptavidin lattice subtraction. b, Fourier transform of a showing Bragg diffraction of streptavidin crystal before*

545 *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.*

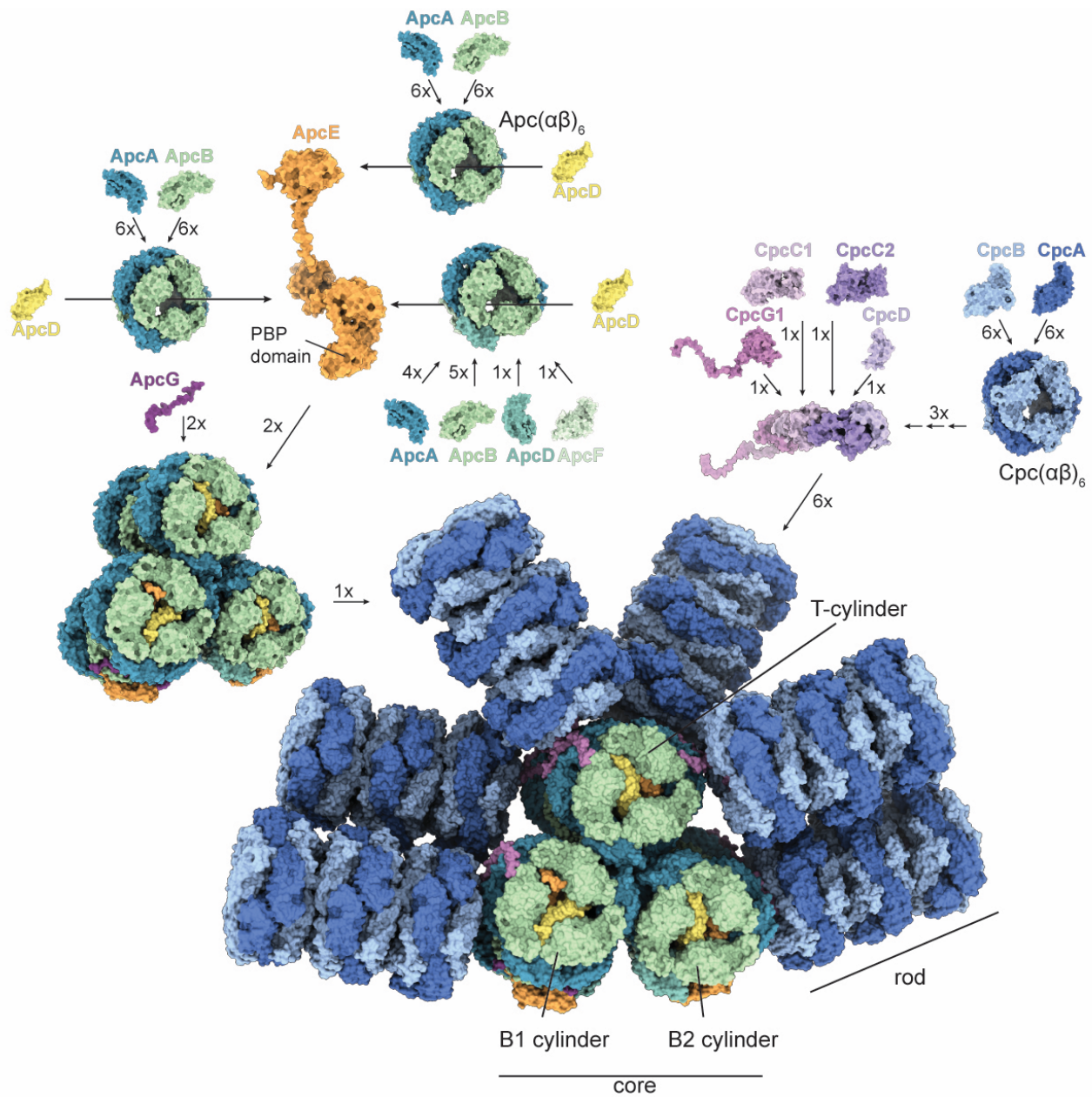
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548

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.

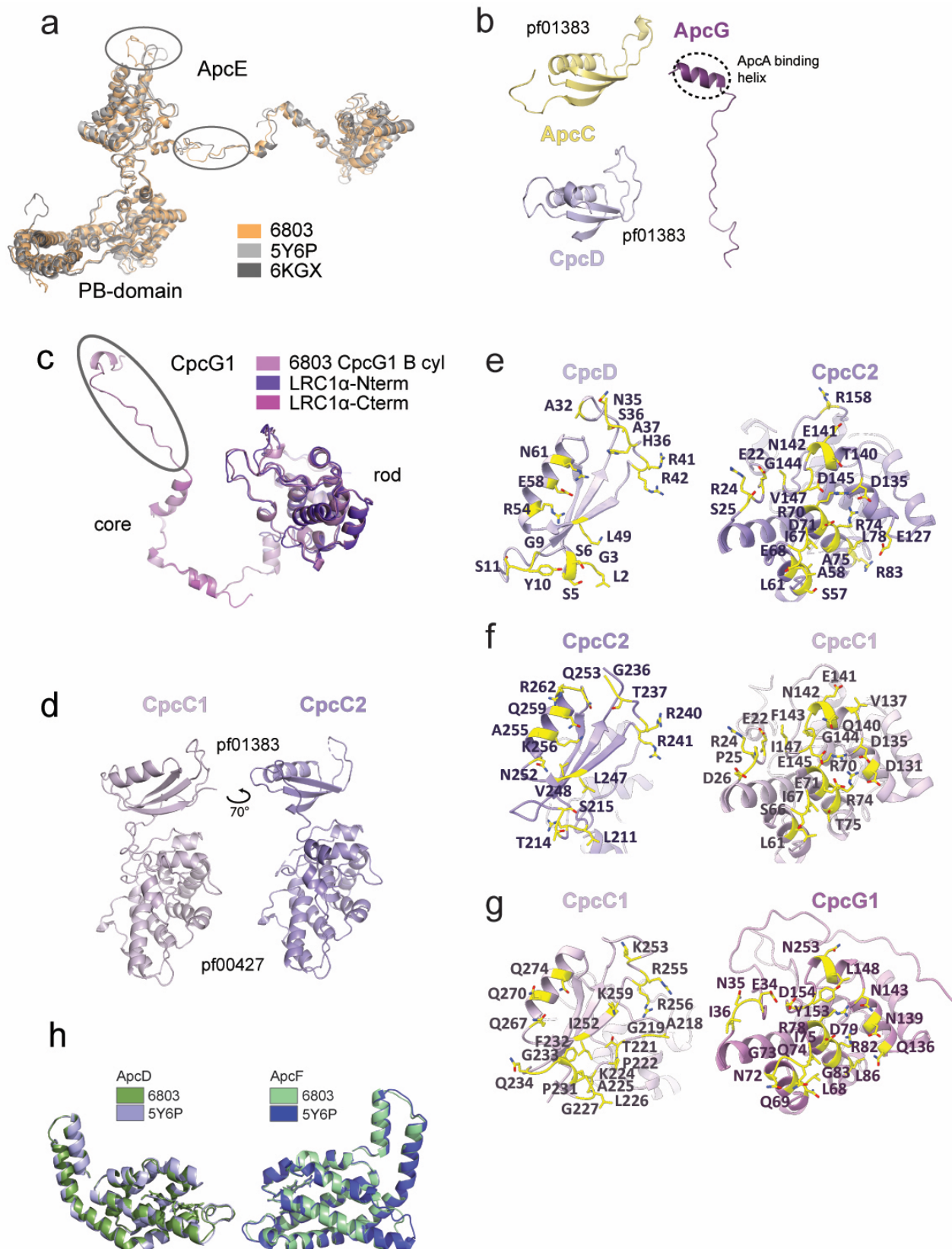
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552

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

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559

560 **Extended Data Figure 5: Molecular details of the PBS.** *a*, Alignment of ApcE with red algae homologs (PDB:5Y6P

561 *Griffithsia pacifica*, PDB:6KGX *Porphyridium purpureum*). RMSD is 1.9 Å, differences are circled. *b*, Structural models

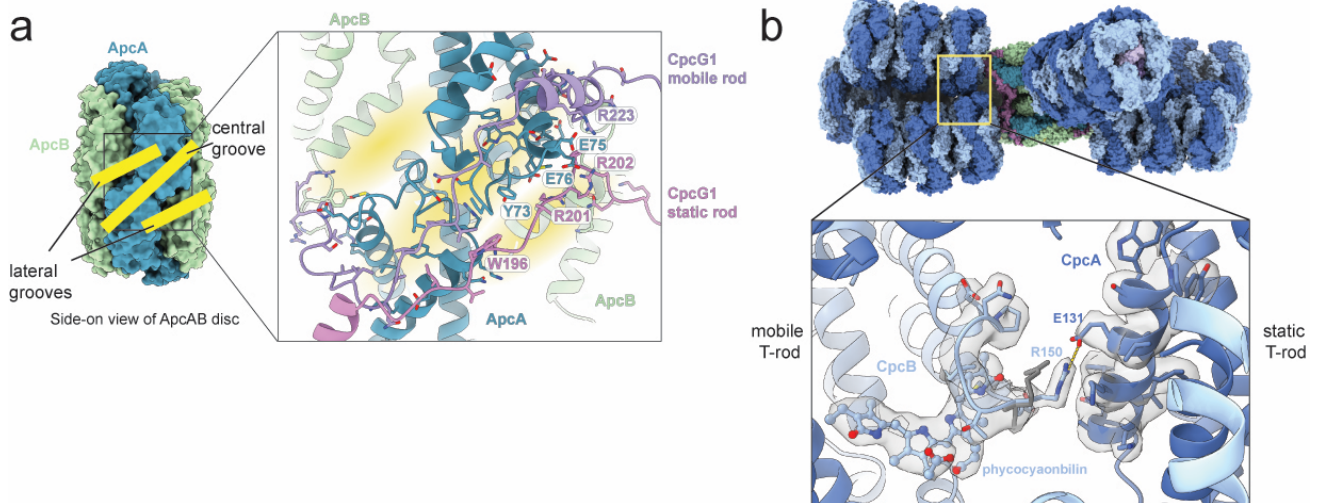
562 of ApcC, ApcG and CpcD. CpcD and ApcC consist of a single pf01383 domain. *c*, Alignment of CpcG1 with red algae

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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.

568

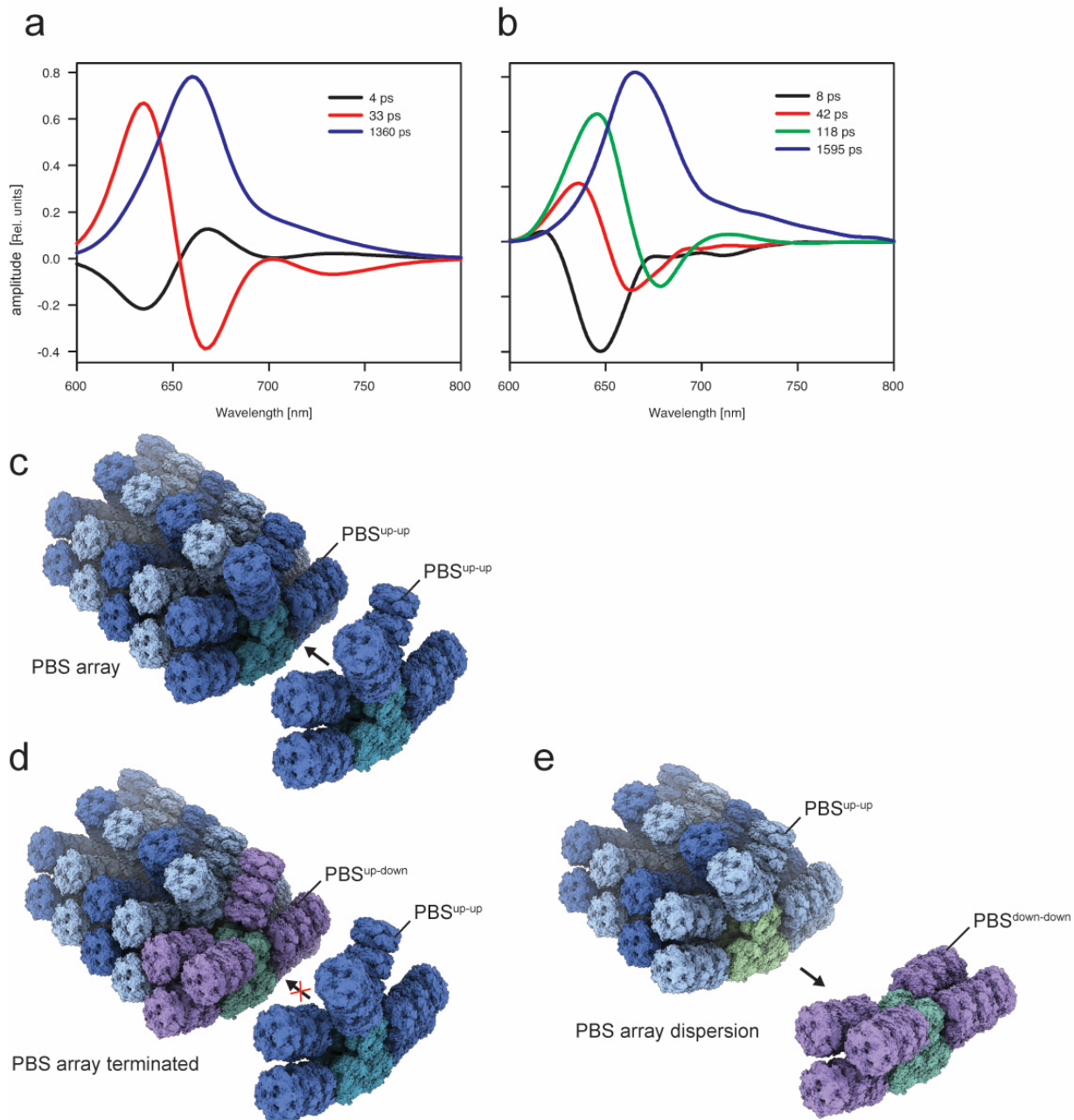
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569

570 **Extended Data Figure 6: PBS rod conformation.** **a**, Side view of ApcAB 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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574

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

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