The reaction-center light-harvesting complex 1 (RC-LH1) is the core photosynthetic component in purple phototrophic bacteria. We present two cryo–electron microscopy structures of RC-LH1 complexes from *Rhodopseudomonas palustris*. A 2.65-Å resolution structure of the RC-LH1-W complex consists of an open 14-subunit LH1 ring surrounding the RC interrupted by protein-W, whereas the complex without protein-W at 2.80-Å resolution comprises an RC completely encircled by a closed, 16-subunit LH1 ring. Comparison of these structures provides insights into quinone dynamics within RC-LH1 complexes, including a previously unidentified conformational change upon quinone binding at the RC Q site, and the locations of accessory quinone binding sites that aid their delivery to the RC. The structurally unique protein-W prevents LH1 ring closure, creating a channel for accelerated quinone/quinol exchange.
open (RC-LH114-W) or closed (RC-LH116) ring surrounding the neighbors to form an array of excitonically coupled pigments in a face-to-face arrangement; each resolved from Gly4 to Tyr52 in RC-LH116 and Ser5 to Tyr52 in was observed for the C-terminal 17 residues. The-polypeptides were -polypeptides were resolved from Met1 to Ala46 in both complexes, although no density for the 3 or 4 N-terminal or -polypeptides were also observed. This analysis shows that the -polypeptides consist of residues fMet1 to Asp42/Ala46/Ala47/Ala50, and -polypeptides consist of residues Ser2 to Ala53, in excellent agreement with the mass spectrometry of mixed RC-LH1 complexes prepared from the wild-type strain showed that the missing regions are the result of heterogeneous cleavage of these peptides (figs. S1 and S2). N-terminal formylation (f) of f-Met1 was also observed. Coordination by -His29 and -His36 positions the BChls in a face-to-face arrangement; each  heterodimer assembles with its neighbors to form an array of excitonically coupled pigments in an open (RC-LH114-W) or closed (RC-LH116) ring surrounding the RC (Fig. 1, C and D). The 880-nm absorption of RC-LH116 was redshifted by 3 nm relative to the 877 nm band of RC-LH114-W (Fig. 2A). However, the circular dichroism spectra were almost identical to the RC-LH116 complex (G) and equivalent region of the RC-LH116 complex (H). Protein subunits are colored in yellow for LH1-α, light blue for LH1-β, red for protein-W, cyan for RC-H, orange for RC-L, and magenta for RC-M. Cofactors are shown in stick representation with BChl and BPh molecules in yellow, UQ10 molecules in yellow. (G and H) Zoomed views of the protein-W gap in the RC-LH116-W complex (G) and equivalent region of the RC-LH116 complex (H). Cofactors are shown in space-filling representation with sequestered quinones displayed in blue. The protein-W gap is highlighted with a blue dashed line in (G), and small pores for quinone/quinol diffusion across the LH116 ring are highlighted with black dashed lines in (H).

LH1 structure

Figure 1 (A and B) shows an RC enclosed by open or closed arrays of LH1 αβ heterodimers, each of which binds two BChls and one carotenoid (Fig. 1, C and D). Previous studies have shown that the LH1 complexes of Rps. species contain mixed populations of carotenoids in the spirilloxanthin biosynthesis pathway (17). However, spirilloxanthin is the dominant carotenoid and gave a satisfactory fit to the spirilloxanthin biosynthesis pathway (17). However, spirilloxanthin is the dominant carotenoid and gave a satisfactory fit to the spirilloxanthin biosynthesis pathway (17).

It is also possible that the open LH1 ring in RC-LH114-W is subject to thermal motions not apparent in the cryo-state used for structural analysis and that, at room temperature, conformations of the αβ14 ring exist with longer interpigment distances from BChls at position 1 to the RC.

10× His tag at its C terminus, permitting efficient separation of the protein-W containing complexes from the majority lacking protein-W (16) by immobilized metal affinity chromatography (IMAC).

As shown in Fig. 1, both complexes contain a three-subunit RC (RC-L, RC-M, and RC-H) surrounded by an LH1 antenna. The 2.80-Å structure of the complex that lacks protein-W shows 16 αβ heterodimers forming a closed LH1 ring completely encircling the RC, hereafter referred to as the RC-LH116 complex. The 2.65-Å structure of the complex containing protein-W has a 14-heterodimer LH1 interrupted by protein-W, hereafter RC-LH114-W.
amino acids and pigments of αβ-14. Some polar residues also contribute to the interaction, including a hydrogen bond between W-Thr
and β-Trp on the luminal face of the complex (Fig. 3, F and G). On the cytoplasmic face, Gln is in close proximity to the ketogroup of the αβ-14 carotenoid. In addition, a molecule of n-dodecyl β-d-maltoside (β-DDM) detergent was resolved with its hydrophobic tail extending into the interface between protein-W and αβ-14, where a lipid tail may be located in vivo. We also note that the resolved C-terminal regions of protein-W and RC-H are in close proximity but not within range for specific interactions to form (Fig. 1, A and E). However, it is possible that there are interactions in the unresolved C-terminal amino acids of these two proteins that may provide the mechanism for recruitment of protein-W during assembly of the RC-LH114-W complex.

Protein-W replaces one αβ heterodimer, the 15th in Fig. 1F, preventing closure of the ring and tilting the first three αβ heterodimers. The largest tilt of 25° to 29° relative to the membrane normal is observed for the first αβ-1 heterodimer (Fig. 1, A and E), in stark contrast to the 2° to 8° tilt of αβ-1 in RC-LH116 (Fig. 1, B and F). The second and third heterodimers are tilted by 12° to 22° and 5° to 10°, respectively. Tilting of αβ-1 excludes a second αβ pair (which would have corresponded to the 16th αβ in Fig. 1F) due to steric hindrance by the RC, creating a distinct gap in the LH1 ring (Fig. 1, A and E). Along with the loss of four BChls and two carotenoids due to the absence of two αβ heterodimers, no carotenoid binds to the distorted αβ-1 subunit, resulting in an LH114-W ring containing 13 carotenoids and 28 BChls. Local resolution estimates for the two complexes in the region of αβs 1 to 7 were lower than for the rest of the LH1 ring, which could reflect inherent plasticity of LH1 subunits adjacent to the RC Q₈ site (Fig. 4).

The only other characterized core complex with a 1:14 RC:LH stoichiometry is the Rhodobacter (Rba.) sphaeroides RC-LH1-PufX dimer (13). However, protein-W and PufX share no significant homology and have distinct effects on their respective LH1 structures. PufX is a single TMH with an N-terminal cytoplasmic domain that interacts with the cytoplasmic side of the RC-H subunit in a location corresponding to Rps. palustris LH116 αβ-16. PufX creates a channel for quinone/quinol exchange between RC-LH1 and the cytochrome bc₁ complex and is present in all Rba. sphaeroides core complexes (13). Although the monomer–monomer interface in the Rba. sphaeroides RC-LH1-PufX dimer is in the position that protein-W binds in the RC-LH114-W, the gaps induced by PufX and protein-W are in equivalent positions (fig. S7A). The gap in RC-LH114-W also aligns with the hypothesized quinone channel of Roseiflexus castenholzii LH1 (8), which is formed by peptides bearing no relation to protein-W or PufX (fig. S7B). Further, the quinone channel in the Blc. viridis LH1, formed by exclusion of one γ subunit (7), is found at a similar position (fig. S7C). Despite being mediated by different proteins, the emergence of these quinone/quinol channels in common locations within RC-LH1 complexes appears to be an example of convergent evolution and suggests that the gap produced by protein-W likely serves as a quinone channel.

The gap in the LH114-W ring allows formation of a continuous membrane region between the internal space of the RC-LH114-W complex and the bulk membrane (Fig. 1G), rather than connection of these two domains by protein pores as in the RC-LH116 complex, similar to those of the closed Tch. tepidum complex (22) (Fig. 1H). As quinone diffusion through the membrane is expected to be faster than diffusion through narrow protein channels, the open LH114-W ring may permit faster RC turnover than the closed LH116 ring, in which quinone access to the RC may be more diffusion limited. To test whether protein-W influences the turnover of quinone by the RC, we performed cytochrome oxidation assays over a range of concentrations of ubiquinone 2 (UQ₂), an analog of the native UQ₁₀.

![Fig. 2. Spectral and biochemical analyses of RC-LH114-W and RC-LH116 complexes.](http://advances.sciencemag.org)
with a shorter isoprene tail (Fig. 2E). Although the presence of sequestered quinone precludes the accurate determination of an apparent Michaelis constant \( (fit \ at \ 0.2 \pm 0.1 \ \mu M \ and \ 0.5 \pm 0.2 \ \mu M \ for \ RC-LH1_{14-W} \ and \ RC-LH1_{16}, \ respectively) \), the maximum rate of \( RC-LH1_{14-W} \ (4.6 \pm 0.2 \ e^{-RC^{-1} \ s^{-1}} \) was 28 \pm 5\% greater than that of \( RC-LH1_{16} \ (3.6 \pm 0.2 \ e^{-RC^{-1} \ s^{-1}} \). We originally estimated that protein-W is present in \( \sim 10\% \) of core complexes (16); here, occupancies of \( 15 \pm 0.6\%, \ 11 \pm 1\%, \ and \ 0.9 \pm 0.5\% \) were found for low-, medium-, and high-light grown cells, respectively (Fig. 2F). Comparative quantification by mass spectrometry shows that relative abundance of protein-W is not lowered by addition of a histidine-tag relative to the wild-type strain (\( P = 0.59 \)), so these levels are not an artefact of modifying protein-W (fig. S10). Such low occupancies of protein-W in the RC-LH1 complexes could nevertheless allow some RCs to turn over at an accelerated rate, alleviating slower quinone/quinol exchange in \( RC-LH1_{16} \) complexes. We note that the high-light occupancy is at odds with recent transcriptomics data suggesting increased expression of the \( pufW \) gene under strong illumination (fig. S11) (23). The discrepancy between \( pufW \) transcription and incorporation of protein-W into RC-LH1 complexes is perplexing and may reflect complex regulation of this protein.

**Fig. 3. Structure of protein-W and interactions with LH1.** (A) Protein-W viewed facing the interface with LH1 \( \alpha\beta_{14} \) in cartoon representation with side chains as sticks (red) shown within its portion of the electrostatic potential map (transparent gray surface at a contour level of 0.13). (B) Protein-W in surface representation colored by hydrophobicity. Polar and charged regions are shown in cyan, hydrophobic regions are shown in white, and strongly hydrophobic regions are in orange. (C and D) Protein-W in cartoon representation in the same orientation as in (A) (C) and rotated 180° (D). Resolved residues are in a rainbow color scheme according to position in the sequence with the N terminus in blue and C terminus in red. (E) Protein-W in the same view as in (A) with residues at the protein-W:LH1 interface in stick representation with accompanying labels. (F) Protein-W rotated 90° relative to (E) with LH1 \( \alpha\beta_{14} \) in cartoon representation and interface residues in stick representation. Highlighted residues from the \( \beta \) polypeptide are labeled. Cofactors are shown as sticks with coloring matching Fig. 1, and the resolved \( \beta\)-DDM is shown in gray with oxygens in red. (G) The view in (F) rotated 180° with highlighted residues from the \( \alpha \) polypeptide labeled.

**Fig. 4. Density maps colored by local resolution as determined with the Relion local resolution tool.** The maps for RC-LH1_{14-W} (A and B) and RC-LH1_{16} (C and D) are shown from the same top/side view in Fig. 1 (A and B) (A and C) and from the lumenal surface of the complex (B and D). The color key is shown on the right.

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Structurally defined lipids and UQs
In RC-LH114-W, 6 cardiolipin (CDL), 7 phosphatidylcholine (POPC), 1 phosphatidylglycerol (POPG), and 29 β-DDM molecules were assigned, and 6 CDL, 24 POPC, 2 POPG, and 12 β-DDM were modeled in RC-LH116 (Fig. 5A and B). In both structures, the CDLs were almost exclusively located on the cytoplasmic side of the complex, while POPC, POPG, and β-DDM were mostly on the luminal side. Two lipid and detergent molecules are sequestered within the αβ-1 to αβ-6 region of the RC-LH114-W complex (Fig. 5A), with five in the equivalent area of RC-LH116 (Fig. 5B). Many more lipids, mostly CDL, are found on the opposite side of the complex, packed between the RC and αβ-7 to αβ-13 (Fig. 5, A and B). Additional structurally resolved lipids and detergents are located on the outside of the LH1 ring with the well-resolved acyl chains extending between LH1 subunits, tentatively assigned as β-DDM in RC-LH114-W and a mixture of β-DDM and POPC in RC-LH116. The similarity in the positions of sequestered lipids and detergents in our structures suggests that these are physiologically relevant binding sites (fig. S12A). There is also good agreement with the positions of equivalent molecules in the *Tch. tepidum* and *Trv. strain 970* RC-LH1s (fig. S12, B to E) (9, 12), and hydrogen-bonding residues to the lipid head groups appear reasonably well conserved in sequence alignments (fig. S13), indicating that, in addition to a conserved CDL bound to the RC (24), these sites may be conserved in RC-LH1 complexes.

In RC-LH116, both the RC Qₐ and Q₈ UQs, which participate in electron transfer during charge separation, were resolved within their binding sites. However, in RC-LH114-W, the Q₉ quinone was not resolved, which will be discussed in detail below. In addition to Qₐ and Q₈ quinones, two sequestered UQ molecules (located in the space between the RC and the LH1 ring) were tentatively assigned in the RC-LH114-W structure, based on their well-resolved head groups (Q1 and Q2 in Fig. 5C). Two isoprene units were assigned for Q1, and the density map resolves the full 10-isoprene tail for Q2. In the RC-LH116 structure, three sequestered UQ₁₀ molecules were resolved (Q1 to Q3, Fig. 5D), all of which had clear density for the entire tail (Fig. 5, D to G). There is excellent agreement in the positions of the quinone head groups for Q1 and Q2 in both structures (fig. S12F), which interact exclusively with the RC. Q1 is located at the entrance to the W-gap of RC-LH114-W (Figs. 1G and 5, C, D, and E), and Q2 is located close to the Q₈ binding site (Fig. 5, C, D, and F). The conserved L-Trp¹⁴³ and L-Trp²⁶⁹ residues are in close proximity to Q1 and Q2 and provide potential π-stacking interactions (Fig. 5, E and F, and fig. S12). L-Gln⁸⁸, 3.0 Å from the distal oxygen of Q1, provides a strong hydrogen bond (Fig. 5E); this residue is conserved in all but the most distantly related RCs (fig. S13). L-Ser⁹¹, which is conservatively substituted for Thr in most other RCs (fig. S13), is 3.8 Å from a methyl oxygen of Q1 and may provide a weak hydrogen bond (Fig. 5E). Q3 does not appear to make specific interactions but is located in a hydrophobic region between the RC-M subunit and LH1-α subunits 5 to 6 (Fig. 5, D and G). Sequestered quinones at or near to Q1, Q2, and Q3 have also been resolved in the *Tch. tepidum*, *Trv. strain 970*, and *Blc. viridis* structures (9, 10, 12), pointing to conserved accessory quinone binding sites within RC-LH1 complexes (fig. S12G). The five resolved UQs in RC-LH116 are in good agreement with the 5.8 ± 0.7 per complex determined by high-performance liquid chromatography (HPLC), while the three in RC-LH114-W is lower than the measured 6.2 ± 0.3 (fig. S14), suggesting the presence of further unresolved UQ molecules in the structure.

**Reaction center**
The pseudosymmetric L and M polypeptides each comprise five TMHs and form a heterodimer that binds one BChl dimer, two BChl monomers, two bacteriopheophytin (BPh) monomers, one non-heme iron, and one or two UQ₁₀ molecules. A carotenoid is bound in the M-subunit, assigned as *cis*-3,4-dehydrodorphodin by the presence of a hydrogen bond to the terminal keto group and its known accumulation in *Rps. species* (25). The membrane-extrinsic domain of RC-H is anchored to the membrane by a single TMH. The overall RC structure is similar to three-subunit RCs of related species, such as *Rba. sphaeroides* (PDB ID: 3I4D). The macrocycles of BChl and BPh, the carotenoid backbone, and the non-heme iron superimpose within the resolution limits of these structures, as do the head groups of UQ₁₀ at the Qₐ site and the Q₈ quinone of RC-LH116 (fig. S15).

The availability of two RC structures that differ in the occupancy of the Q₈ site provides a new opportunity to examine the concerted conformational changes that accompany binding of the Q₈ quinone. In the RC-LH116 complex, the Q₈ quinone is located in the fully bound “proximal” position (26), but RC-LH114-W was resolved without a Q₈ quinone. The absence of a Q₈ quinone in RC-LH114-W was
unexpected as the complex is active, more so than the RC-LH116 complex that does have a structurally resolved Q_{B} quinone. Although both LH1 rings sequester approximately six quinones, five are resolved structurally in the closed RC-LH116 ring, whereas only three are structurally defined within the open RC-LH1_{14}-W ring. This increased structural disorder likely reflects faster turnover at the RC-LH1_{14}-W Q_{B} site, more rapid quinone dynamics within the complex, and an increased probability of traversing the LH1 ring. We suggest that the absence of a structurally resolved UQ at the RC Q_{B} site of RC-LH1_{14}-W is likely a consequence of a more disordered and more active complex and that the Q_{B} site in RC-LH1_{14}-W has been frozen instantaneously in a conformation that reflects this activity, in a particular phase of UQ turnover where the entrance to the Q_{B} site has closed.

The absence of Q_{B} is accompanied by rotation of L-Phe\textsuperscript{217} into a position incompatible with UQ_{10} binding as it would cause a steric clash with the first isoprene unit of the tail (Fig. 6A). Further, major conformational changes are apparent, notably the shifting of helix de (a short helix in the loop between TMHs D and E) on which L-Phe\textsuperscript{217} resides into the Q_{B} binding pocket and a rotation of L-Tyr\textsuperscript{223} (Fig. 6A), breaking the hydrogen bond to the backbone of M-Asp\textsuperscript{55} and closing the entrance of the Q_{B} binding site (Fig. 6B). Helix de pivots at its base with the Ca of L-Ser\textsuperscript{209} shifted 0.33 Å, while L-Val\textsuperscript{221} Ca is shifted 3.52 Å with no observable alterations in TMHs D and E, which are superimposable in the two structures (Fig. 6A). To our knowledge, this is the first structure of a closed Q_{B} site in a native RC, and comparison with the holo (Q_{B}-bound) structure shows that conformational changes are required to admit quinone before its reduction. L-Phe\textsuperscript{217} rotates to form a π-stacking interaction with the quinone head group, and helix de shifts outward, allowing the backbone of L-Gly\textsuperscript{222} and side chain of L-Tyr\textsuperscript{223} to form a hydrogen bond network that stabilizes the holo structure (Fig. 6, A and C).

It is unexpected that the conformational changes observed in this study have not previously been reported, despite the availability of several structures of RCs with LH1 removed that lack Q_{B}. These include Q_{B}-depleted structures from Blc. viridis (PDB ID: 3PRC) (27), Tch. tepidum (PDB ID: 1EYS) (28), and Rba. sphaeroides (PDB ID: 1OGV) (29), all of which are near-identical to their respective holo Q_{B} structures. Close inspection of 3PRC reveals that an LDAO (Lauryldimethylamine oxide) detergent molecule is bound at the entrance to the Q_{B} site, which may prevent the rearrangement to the closed conformation. While no LDAO was resolved in the equivalent position in 1EYS or 1OGV, these RCs were prepared using the same detergent and may therefore be subject to the same effect. The crystal structure of the Rba. sphaeroides RC cocryocrystallized with cytochrome c\textsubscript{5} (PDB ID: 1LB9) also appears to have a closed Q_{B} site. However, in this case, the N-terminal region of the RC-M polypeptide, which interacts with the Q_{B} binding site via an H-bond to the helix de Tyr residue, adopts a non-native conformation, and Q_{B} conformational changes were not explored further (30). Reassuringly, we see no such deformations to the M-polypeptide in the RC-LH1_{14}-W structure, which is almost identical to the RC-LH1_{16} RC in its N-terminal region. It should also be noted that the apo RCs in the PDB were solved following detergent-based eradication of the LH1 antenna, which removes the internal quinone pool and lipids that reside in the gap between the RC and the inner face of the surrounding LH1 ring (31, 32). The RC remains functional, because all of its cofactors are retained with the exception of the dissociable Q_{B} quinone, which is more labile and often lost during preparation (33). Furthermore, the removal of LH1 and native annular lipids from the RC is known to have consequent effects on functionality, such as lowered lifetime of the charge separated P^{+}Q_{B} state (34, 35). We therefore speculate that the presence of a native LH1 ring surrounding the RC, preserving the local environment adjacent to Q_{B}, may have facilitated the observation of the “closed” Q_{B} site.

Although the apo (no Q_{B} quinone) and holo structures represent only two snapshots of turnover at the Q_{B} site rather than a sequence of events, there is an indication that binding could be gate to prevent substrate inhibition by rebinding of quinol. A quinol approaching the apo Q_{B} site could interact differently to a quinone, resulting in its rejection by the RC. Conformational changes have long been suggested to play a role in the binding and reduction of quinone. RCs that have been frozen after dark adaptation are impaired in their ability to reduce quinone (36); x-ray crystallography has shown that this impairment is due to the Q_{B} quinone being trapped in a “distal” conformation ~4.5 Å from the active proximal position (26, 37). We propose that this distal binding conformation is a snapshot of an intermediate state between the apo and holo structures that follows the initial interaction with quinone and opening of the Q_{B} site.

Fig. 6. Conformational changes upon quinone binding to the RC Q_{B} site. (A) Overlaid cartoons of holo (chain L, orange/chain M, magenta) and apo (gray) structures with key residues displayed in stick representation. UQ_{10} is shown in stick representation in yellow. Dashed lines show hydrogen bonds formed in the holo structure. (B and C) Surface representations of apo and holo structures, respectively, with L-Phe\textsuperscript{217} highlighted in blue and the side chain oxygen of L-Tyr\textsuperscript{223} in red. Subunit L is orange; and subunits M and H are not colored. (D and E) The apo (D) and holo (E) RC Q_{B} sites (colored as in (A)), respectively, aligned to the Thermosynechococcus vulcanus PSII (green with plastoquinone in blue; PDB ID: 3WU2) (58).
There is structural and functional conservation within the type II RCs found in some phototrophic bacteria and the PSIII complexes of cyanobacteria, algae, and plants (38). The structural alignments shown in Fig. 6 (D and E) emphasize similarities between Qb sites of PSIII RCs and the bacterial RC complex; such comparisons have long served as models to study quinone binding and reduction in these closely related systems. Previous publications have suggested that conformational changes accompany quinone reduction by PSIII (39, 40). Thus, given the evolutionary conservation of RCs, this previously unobserved binding mechanism may also apply to the Qb site of the PSIII RCs in oxygenic phototrophs.

**MATERIALS AND METHODS**

**Growth of *Rps. palustris* strains**

The ΔpuFW (unmarked deletion of *puFW*) and PuFW-His (C-terminally 10× His-tagged protein-W expressed from the native *puFW* locus) strains of *Rps. palustris* CGA009 were described in our previous work (16). These strains and the isogenic wild-type parent were recovered from cryo-stocks [stored at −80°C in LB with 50% (w/v) glycerol] by streaking a small quantity of cells onto PYE (5 g liter⁻¹ each of peptone, yeast extract, and succinate) agar [1.5% (w/v)] plates. Plates were incubated under anaerobic conditions overnight in the dark at room temperature and then illuminated with white light (~50 μmol m⁻² s⁻¹) provided by OSRAM 116-W halogen bulbs in the dark at room temperature and then illuminated with white light (~50 μmol m⁻² s⁻¹) provided by OSRAM 116-W halogen bulbs (RS Components, UK) for 3 to 5 days until single colonies appeared. A single colony was used to inoculate 10-ml M22+ medium (41) supplemented with 0.1% (w/v) casamino acids (from now on referred to as M22). This culture was grown under microoxic conditions in the dark at 34°C with shaking at 180 rpm for 48 hours and then used to inoculate a 70-ml culture grown under the same conditions for 24 hours. A volume of 1 ml of the semi-aerobic culture was used to inoculate 30 ml of M22 media in a screw-capped clear glass 30 ml universal and grown with illumination (~50 μmol m⁻² s⁻¹) for 48 hours with agitation via a sterile magnetic stir bar. The 30-ml culture was then used to inoculate ~9-liter cultures illuminated at ~200 μmol m⁻² s⁻¹ for 72 hours.

**Preparation of solubilized *Rps. palustris* cellular membranes**

After thawing, a few crystals of deoxyribonuclease I (Merck, UK), lysozyme (Merck, UK), and two Roche Complete Protease Inhibitor Cocktail tablets (Merck, UK) were added to the resuspended cells. Cells were broken by 8 to 12 passes in a French pressure cell (Aminco, USA) at 20,000 psi. Following removal of unbroken cells and insoluble debris by centrifugation at 18,500 RCF for 15 min at 4°C, membranes were pelleted from the deeply pigmented lysates by centrifugation at 113,000 RCF for 2 hours at 4°C. The soluble fraction was discarded, and the pigmented membranes were resuspended in 100 to 200 ml of 20 mM tris-HCl (pH 8.0) and homogenized until no visible aggregates remained. Resuspended membranes were solubilized by incubation in 20 mM tris-HCl (pH 8.0) containing 2% (w/v) β-DDM (Anatrace, USA) for 1 hour at 4°C in the dark with gentle stirring, followed by centrifugation at 150,000 RCF for 1 hour at 4°C to remove residual insoluble material.

**Purification of RC-LH1₄-W complexes**

Solubilized membranes from the ΔpuFW strain were applied to a 50-ml DEAE Sepharose ion-exchange column pre-equilibrated with three column volumes (CVs) of binding buffer [20 mM tris-HCl (pH 8.0) containing 0.03% (w/v) β-DDM]. The column was washed with two CVs of binding buffer and then with two CVs of binding buffer containing 50 mM NaCl. RC-LH1₄-W complexes were eluted over a linear gradient of 150 to 300 mM NaCl (in binding buffer) over 1.75 CVs, with residual bound complexes eluted over 0.5 CVs with binding buffer containing 300 mM NaCl. Absorption spectra were collected between 250 and 1000 nm, and fractions with a ratio of absorbance at 880 to 280 nm (A880/A280 > 1) were retained, diluted twofold in binding buffer, and purified on the DEAE column again using the same procedure. Fractions with A880/A280 ratios above 1.7 and A880/A805 ratios above 3.0 were diluted and subjected to a third round of ion-exchange, retaining fractions with A880/A280 ratios above 2.2 and A880/A805 ratios above 5.0. The partially purified complexes were concentrated to ~2 ml in Amicon 100,000 molecular weight cutoff (MWCO) centrifugal filters (Merck, UK), loaded onto a Superdex 200 16/600 size exclusion column (GE Healthcare, USA) pre-equilibrated with 1.5 CVs of binding buffer containing 200 mM NaCl, and then eluted over 1.5 CVs in the same buffer. Absorption spectra of the size exclusion fractions were collected, and those with A880/A280 ratios over 2.4 and A880/A805 over 5.8 were concentrated to an A880 of 100 and immediately used for cryo–transmission EM (cryo-TEM) grid preparation or stored at −80°C until required.

**Cryo-TEM grid preparation**

Cryo-TEM grids were prepared using a Leica EM GP plunge freezer. Complexes were diluted in IMAC buffer to an A880 of 50, and 5 μl was loaded onto freshly glow-discharged QUANTIFOIL 1.2/1.3.
carbon-coated copper grids (Agar Scientific, UK). The grids were incubated for 30 s at 20°C and 60% relative humidity before blotting for 3 s and plunge-freezing in liquid ethane at −176°C.

Cryo-TEM data collection
Data for the RC-LH1 16 complex were recorded at eBIC (Electron Bio-imaging Centre) (Diamond Light Source, UK) on a Titan Krios microscope operating at 300-kV accelerating voltage at a nominal magnification of 130,000 × with an energy-selecting slit of 20 eV. Data were collected in counting mode recording images with a Gatan 968 GIF Quantum with a K2 summit detector. The calibrated pixel size was 1.048 Å, and the dose rate was 3.83 e− Å−2 s−1. Movies were collected over 11 s and dose-fractionated into 40 fractions. The microscope was refocused using a carbon-coated area before collecting three movies per hole. Overall, 3130 movies were collected with defocus values between −1 and −3 μm.

Data for the RC-LH1 16 complex were recorded at the Astbury Biostructure Laboratory (University of Leeds, UK) using an identical microscope. Data were collected in counting mode at a magnification of 130 k with a calibrated pixel size of 1.065 Å at a dose of 4.6 e− Å−2 s−1. Movies were recorded over 12 s and dose-fractionated into 48 fractions. Overall, 3359 movies were collected with defocus values between −1 and −3 μm.

Cryo-TEM data processing
All data processing was performed within the Relion 3.0 pipeline (42). Beam-induced motion correction was performed with dose weighting using Motioncorr 2 (43), followed by determination of CTF (contrast transfer function) parameters with CTFFIND 4.1 (44). Typical micrographs after these initial processing stages are shown in fig. S16. Autopicking templates were generated by manual picking of ~1000 particles with a box size of 250 pixels and reference-free two-dimensional (2D) classification, rejecting those classes that conform to sample contamination or had no discernible features. Autopicking was then performed on all micrographs resulting in 849,359 particles for the RC-LH1 14-W and 476,547 particles for the RC-LH1 16 complex. All picked particles were subjected to two rounds of reference-free 2D classification, and particles conforming to carbon areas, sample contamination, particles with no discernible features or strongly overlapping particles were rejected following each run, resulting in 772,033 (90.9%) and 359,678 (75.5%) particles being used for 3D classification for RC-LH1 14-W and RC-LH1 16, respectively. Initial 3D reference models were generated using the stochastic gradient descent method. Selected particles were subjected to 3D classification into four classes with the initial model as a reference. The particles within the largest class were subjected to 3D refinement using the model from this class as a reference followed by masking of solvent areas using an initial low-pass filter at 15 Å, adding a soft edge of 6 pixels, and postprocessing correcting for the modulation transfer function of the Gatan K2 summit detector. For the RC-LH1 14-W dataset, this initial model was modified by removing strong density at the mask edge, which was disconnected from the core-complex density in UCSF Chimera. The resulting models (at 3.91 and 4.16 Å resolution for RC-LH1 14-W and RC-LH1 16, respectively) were used as a reference for a second round of 3D classification using particles grouping into initial 3D classes that did not contain strong overlap with neighbors or lack discernible structural features. Following the second round of 3D classification, the highest resolution classes were selected [one class of 377,703 particles (44.5%) for RC-LH1 14-W and two classes with a combined total of 260,752 particles (54.7%) for RC-LH1 16, which were identical when aligned after only initially differing by a small rotation]. Selected particles were reextracted in a 400-pixel box and refined by 3D refinement. A solvent mask was generated with an initial low-pass filter of 15 Å, map extension by 3 pixels, and a soft mask of 3 pixels. The resultant maps were further refined using per-particle CTF refinement, per-particle motion correction, and a second round of per-particle CTF refinement, with 3D refinement, solvent masking and postprocessing after each step. Using an FSC (Fourier shell correlation) cutoff at 0.143, the final models for RC-LH1 14-W and RC-LH1 16 were at resolutions of 2.65 and 2.80 Å, respectively. The FSC curves of the final models are shown in fig. S17.

Model building
All protein sequences were downloaded from UniProtKB: LH1-β (PufB; UniProt ID: Q6N9L5); LH1-α (PufA; UniProt ID: Q6N9L4); RC-L (PufL; UniProt ID: O83005); RC-M (PufM; UniProt ID: A0A4Z7); RC-H (PufA; UniProt ID: A0A4Z9); protein-W (PufW; UniProt ID: Q6N1K3). A homology model of the RC was constructed using SWISS-MODEL (45) with protein sequences for RC-L, RC-M, and RC-H and a crystal structure of the Rba. sphaeroides RC as a template (PDB ID: 5LSE) (46). The resulting model was fit into the map using the “fit in map” tool within UCSF Chimera (47), the protein structure was refined, and cofactors [4× BChl a (monomer library residue name = BCL), 2× Bph a (BPH), one or two UQ10 (U10), one nonheme iron (Fe), and one 3,4-didehydروورودوپين (QAK)] were added using Coot (48). As QAK was not available in the monomer library, it was parameterized using the eLBOW tool in PHENIX (49).

Next, the LH1 subunits were constructed. Initially, the Auto-build tool in PHENIX (49) was used to automatically build part of the LH1 sequence using the map and the LH1-α and LH1-β protein sequences as inputs. The most complete LH1 subunit was selected, extracted, and loaded into Coot where missing sequence was manually added, and the entire structure was manually refined before addition of the two BChls a (BCL) and one spirilloxanthin (CRT) [assigned on the basis of the density and known carotenoid content of LH1 complexes of related Rps. species (17)]. The complete LH1 subunit was duplicated and docked into a neighboring nonmodeled region of LH1 density using the UCSF Chimera “dock in map tool” followed by refinement in Coot; this process was repeated until all LH1 subunits had been modeled. For the RC-LH1 14-W structure, the remaining subunit (protein-W) was modeled by extracting the unassigned density in Coot, segmenting the protein from the remaining nonprotein components of the map in USCF Chimera and building an initial model with the Autobuild tool in PHENIX (49). Any missing sequence was added to the resulting model in Coot (48) followed by manual refinement of the entire subunit. The remaining unassigned density was fit with a combination of lipids (PDB monomer library IDs for CDL = CDL, POPC = 6PL, and POPG = PG1), β-DM detergent (LMT), and UQ10 molecules (U10). The complete initial models were refined using cycles of PHENIX refine (49) and manual refinement in Coot (48) until the model statistics and visual quality of the fits could not be improved further. Last, local map sharpening was applied using LocScale (50), followed by several additional cycles of modeling unassigned density and automatic and manual refinement.

The individual peptides, cofactors, and additional lipids andquinones docked within their corresponding density are shown in figs. S18 to S23. Statistics for the final models are displayed in table S1.
Ultraviolet/Vis/NIR absorption spectroscopy

Ultraviolet/visible/near-infrared absorption spectra were collected on a Cary60 spectrophotometer (Agilent, USA) scanning between 250 and 1000 nm at 1-nm intervals with a 0.1-s integration time, unless otherwise stated.

Circular dichroism spectroscopy

Samples were diluted to an A880 of 1 in a 2-mm path quartz cuvette, and absorption spectra were collected between 400 and 1000 nm. Circular dichroism spectra were collected on a Jasco 810 spectropolarimeter (Jasco, Japan) between 400 and 950 nm at 1-nm intervals at a scan rate of 20 nm min⁻¹.

Determination of molar extinction coefficients

Molar extinction coefficients were determined by diluting core complexes to an A880 of ~50. A 10-μl volume was diluted in 990-μl binding buffer or methanol, and absorption spectra were collected immediately to minimize BChl degradation. Extinction coefficients were determined by calculating the BChl content of each methanol sample using an extinction coefficient of 54.8 mM⁻¹ cm⁻¹ at 771 nm (51). The measured BChl concentration was divided by 32 (RC-LH1₁₄-W) or 36 (RC-LH1₁₆) to determine the core complex concentration, which was subsequently used to determine an extinction coefficient from the absorption spectra of the same samples in buffer collected in parallel. Each sample was measured in triplicate, and the average absorbance for the BChl Qₐ maxima was used for the calculations. The extinction coefficients determined were 3280 ± 140 mM⁻¹ cm⁻¹ at 878 nm for RC-LH1₁₄-W and 3800 ± 30 mM⁻¹ cm⁻¹ at 880 nm for RC-LH1₁₆.

Quantification of UQ₁₀

UQ₁₀ was quantified on the basis of the method in (52). Briefly, reverse-phase HPLC (RP-HPLC) was performed using an Agilent 1200 HPLC system. Approximately 0.02 nmol of RC-LH1₁₆ or RC-LH1₁₄-W was dissolved in 50 μl of 50:50 methanol:chloroform containing 0.02% (w/v) ferric chloride and injected onto a Beckman Coulter UltraspHERE ODS 4.6 mm × 25 cm column preequilibrated in HPLC solvent (80:20 methanol:2-propanol) at 40°C at 1 ml min⁻¹. Isocratic elution was performed in HPLC solvent for 1-hour monitoring absorbance at 275 nm (UQ₁₀), 450 nm (carotenoid), and 780 nm (BChl). The peak in the 275-nm chromatogram at 25.5 min, which did not contain any other detectable compounds, was integrated. The integrated area was used to calculate the molar quantity of extracted UQ₁₀ by reference to a calibration curve calculated from injection of 0 to 5.8 nmol of pure standard (fig. S14). Each sample was analyzed in triplicate with reported errors corresponding to SD of the mean.

Cytochrome c oxidation assays

Solutions containing RC-LH1 complexes at a maximum Qₐ absorption of 0.1 were prepared with 30 μM reduced horse-heart cytochrome c₅₅₃ (Merck, UK) and 0 to 50 μM UQ₂ (Merck, UK). Three 1-ml samples were prepared at each UQ₂ concentration and incubated at 4°C in the dark overnight to ensure they were fully dark-adapted before measurement. Solutions were loaded into an OLIS RSM1000 modular spectrophotometer equipped with a 300-nm blaze/500-line grating and a 1.24-mm entrance, 0.12-mm intermediate, and 0.6-mm exit slit. The sample and reference photomultiplier tubes had 600-nm long-pass filters placed at their entrances to exclude excitation light. Absorbance was monitored at 550 nm with an integration time of 0.15 s. Excitation light was delivered via a fiber optic cable from an 880-nm M880F2 LED (Light-emitting diode) (Thorlabs Ltd, UK) driven at 90% intensity using a DC2200 controller (Thorlabs Ltd, UK) at 90° to the measurement beam with a mirror opposite to return any light that was not initially absorbed by the sample. Absorbance was monitored for 10 s before illumination for 50 s. The absorbance was then monitored for a further 60 s in darkness to assess the extent of the spontaneous reduction of cytochrome c₅₅₃ by quinol (see fig. S8 for raw data).

Data were processed by fitting of the linear initial rate over 0.5 to 10 s (dependent on UQ₂ concentration) and averaging the rates of all three samples at each UQ₂ concentration. Rates were converted to catalytic efficiencies using RC-LH1 concentrations calculated with their respective extinction coefficients, plotted in Origin pro 2019 (OriginLab, USA), and fit to the Michaelis-Menten model to determine apparent Kₘ and Kₐ₄ values.

Transient absorption spectroscopy

For transient absorption measurements, RC-LH1 samples were diluted to ~2 μM in IMAC buffer containing 50 mM sodium ascorbate (Merck, USA) and 0.4 mM terbutryn (Merck, USA). The ascorbate was used as a sacrificial electron donor, while the terbutryn acts as a Qₐ₄ inhibitor to ensure that the RC primary donor remains in the reduced (i.e., not photooxidized) state throughout the measurements. Approximately 3 ml of sample was added to a 2-mm path length custom-built spinning cell (~0.1 m in diameter, 350 RPM) to ensure that the sample in the laser path has sufficient time to dark-adapt between excitation pulses. Samples were excited at 880 nm using ~100-fs laser pulses at a repetition rate of 1 kHz (20 nJ for NIR or 100 nJ for Vis) provided by an amplified Ti:Sapphire laser system (Spectra Physics, USA). The samples were exposed to excitation light for a duration of ~30 min before data collection, the exposure results in QA inactivation (possibly by singly or doubly reducing QA). Note, however, that this process is reversible, because the RCs slowly return to the QA active state following prolonged dark adaption. Transient spectra were measured using a Helios spectrometer (Ultrafast Systems, USA) with delay times from −10 to 7000 ps. The datasets were dechirped using Surface Xplorer software (Ultrafast Systems, USA), then combined, and normalized. The combined datasets were used to obtain decay-associated difference spectra using the CarpetView software package (Light Conversion Ltd., Lithuania) or fit to single-wavelength spectral evolutions in Origin using functions that consist of multiple exponentials convoluted with the instrument response (OriginLab, USA).

Photosynthetic membranes containing the LH1 complex lacking both the RC and peripheral LH2 antenna were prepared as described previously (53). The membranes were diluted in 20 mM tris (pH 8.0) and loaded into a 2-mm path length quartz cuvette. The sample was excited at 540 nm using 30-nJ laser pulses with delay times from −10 to 7000 ps. Datasets were processed as described for the Rps. palustris samples.

Mass spectrometry

Membranes were pelleted by centrifugation at 150,000 RCF for 2 hours at 4°C and resuspended at an absorbance of 100 at 880 nm in 20 mM tris-HCl (pH 8.0) and 200 mM NaCl. Membranes were solubilized by gentle stirring in 2% (w/v) β-DDM for 1 hour at 4°C in the dark. Samples were diluted to a protein concentration of 2.5 mg ml⁻¹ (Bio-Rad assay) in 100 mM triethylenediammonium bicarbonate (pH 8.0) (TEAB; Merck, UK). Further processing was adapted from...
Immunodetection of protein-W

Cells were grown under low (10 μM m⁻² s⁻¹), medium (30 μM m⁻² s⁻¹), or high (300 μM m⁻² s⁻¹) illumination for 72 hours in 100 ml of NF-M22 medium (M22 medium where the ammonium sulphate is omitted, and sodium succinate is replaced with sodium acetate) (23) in 100-ml screw-cap bottles. Cells were lysed by bead beating in 1:1 volumetric ratio with 0.1 μm glass beads over five 30-s cycles, cooling on ice for 5 min in between. Insoluble material, unbroken cells, and glass beads were removed by centrifugation at 16,000 RCF for 10 min in a benchtop microcentrifuge. Membranes were isolated on 40/15% (w/v) sucrose gradients in 20 mM tris-HCl (pH 8.0) in a Ti 70.1 rotor, 100,000 RCF for 10 hours.
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Structures of *Rhodopseudomonas palustris* RC-LH1 complexes with open or closed quinone channels

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