Cryo-EM structure of the *Blastochloris viridis* RC-LH1 complex at 2.9 Å

Pu Qian[1], C. Alistair Siebert[2], Peiyi Wang[3], Daniel P. Canniffe[1], C. Neil Hunter[1]

[1]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK.

[2]Electron Bio-imaging Centre, Diamond Light Source, UK.

[3]Astbury Center for Structural Molecular Biology, University of Leeds, Leeds, UK.

The reaction centre light-harvesting 1 (RC-LH1) complex is the core functional component of bacterial photosynthesis. A 2.9 Å resolution cryo-EM structure of the bacteriochlorophyll *b*-based RC-LH1 from *Blastochloris viridis* reveals the structural basis for absorption of infrared light, and the molecular mechanism of quinone migration across the LH1 complex. The novel triple ring LH1 complex comprises a circular array of 17 β-polypeptides sandwiched between 17 α- and 16 γ-polypeptides. Tight packing of the γ-apoproteins between βs collectively interlocks and stabilizes the LH1 structure, which, together with the short Mg-Mg distances of BChl *b* pairs, contributes to the large red-shift of bacteriochlorophyll *b* absorption. The 'missing' 17th γ polypeptide creates a pore in the LH1 ring, and an adjacent binding pocket provides a folding template for a novel quinone, Q_P, which adopts a compact, export-ready conformation prior to passage through the pore and eventual diffusion to the cytochrome bc_1 complex.

Photosynthesis provides the energy for almost all life on Earth. In the early stages of photosynthesis, light-harvesting complexes absorb solar energy, which migrates to a membrane-bound reaction center (RC), where a charge separation initiates the eventual formation of a reduced electron acceptor[1-3]. The basic functional unit in purple phototrophic bacteria is the RC-light harvesting complex 1 (RC-LH1) complex, in which the RC is surrounded by a ring-like oligomeric assembly of LH1 α and β heterodimers that bind

bacteriochlorophyll (BChl) and carotenoid (Crt) pigments. Three types of RC-LH1 complex[4-6] show a variety of architectures: 16 LH1 α/β pairs completely encircle the RC in *Thermochromatium* (*Tch.) tepidum*[5] and *Rhodospirillum rubrum*[7]; in *Rhodopseudomonas* (*Rps.*) *palustris* the RC is encircled by an open LH1 ring consisting of 15 α/β pairs and a W polypeptide[4]. Finally, *Rhodobacter (Rba.) sphaeroides* has a dimeric core complex[8], in which each monomer has 14 α/β pairs associated with one RC; two monomers associate through two PufX polypeptides to form an S-shaped LH1 ring[6,9].

A high level of structural detail is required to account for the ability of RC-LH1 complexes to absorb within a specific spectral range of solar energy and to drive the formation of a quinol, which must traverse the confines of the LH1 ring encircling the RC. We identified the RC-LH1 complex from *Blastochloris (Blc.) viridis* as a suitable target for a high-resolution structural study because it possesses unique architectural and spectroscopic features. Notably, the *Blc. viridis* RC yielded the first reported structure of a membrane protein complex[10], but for the whole RC-LH1 complex electron microscopy (EM) has provided only low-resolution structures[11,12]. This complex houses BChl *b* rather than BChl *a*, which confers infra-red absorption at 1015 nm, one of the most red-shifted photosynthetic complexes yet described and one proposed as the basis for re-engineered photosynthesis[13]. There is currently no structural basis for this remarkable and enigmatic *in vivo* absorption, which represents one of the largest red shifts observed in a photosynthetic pigment-protein complex, 220 nm from the 795 nm absorption maximum of BChl *b* in methanol. This property could be related to the composition of the *Blc. viridis* LH1 complex, comprising α, β and a third polypeptide, γ, but the position and function of the γ subunit within the LH1 complex remains poorly understood. The *Blc. viridis* LH1 contains rare 1,2-dihydro- derivatives of neurosporene and lycopene as major Crts[14-16]. The RC-LH1 complex forms extensive arrays in the lamellar membranes of *Blc. viridis*[17-20] proposed to consist of closed 16-membered LH1 rings

completely encircling each RC[20]. However, such an arrangement of LH1 subunits, completely enclosing the RC, represents a potential obstacle for quinol export from the RC to the external quinone pool in the membrane, and eventual reduction of the cytochrome bc_1 complex. Here, we report a 3D cryo-EM structure at 2.9 Å of this BChl *b*-based photosynthetic complex from *Blc. viridis*. New insights are gained into the architecture and function of the RC-LH1 complex; the structure shows how γ-apoproteins influence the large red-shift observed in the BChl *b* Q_y absorption band, the position of the internal quinone channel, and identifies a third quinone binding site that prepares quinol for export through the pore in the LH1 ring.

Overall structure of the RC-LH1 from Blc. viridis

The RC-LH1 complex was purified from *Blc. viridis.* Extended Data Fig. 1 shows the absorption spectra of native photosynthetic membranes and purified core complexes. The maximum absorption band at 1015 nm is ascribed to the Q_y band of the BChl *b* in the LH1 complex. This band is slightly blue-shifted to 1008 nm after detergent solubilisation and purification. Following vitrification of monodisperse complexes 6,472 cryo-EM movies were recorded, from which 267,726 particles were picked manually for reference-free two-dimensional classification. Further processing yielded a final resolution of 2.9 Å, enabling compilation of a colour-coded electron density map (Fig. 1 **a-c**) that reveals the detailed structural architecture of this RC-LH1 complex and the relative locations of all pigments, cofactors and subunits. The dimensions of the RC-LH1 are shown in Fig. 1 **c** and **d**. The height of the core complex from top of the periplasmic cytochrome to the bottom of the H subunit on the cytoplasmic side is 128.9 Å (Fig. 1 **a**, **d**), and the diameters of this slightly elliptical structure in projection are 120.2 and 124.5 Å (Fig. 1 **c**); the complex has a molecular weight of 414 kDa. The RC in the cryo-EM map is similar to that determined by X-ray crystallography (e.g., PDB 1PRC)[21]. Structural differences, indicated by residue-residue (RR) distance

deviation[22], are low in subunits C, M and L (Extended Data Fig. 2 b,c,d). However, interaction with the LH1 complex constrains a loop region on RC-H (H47-54), producing a larger deviation from the RC-only structure (Extended Data Fig. 2 a, e). A small displacement of RC-C and RC-H subunits is also observed, likely caused by interaction with the LH1 complex, which bends the RC via a hinge point near the interface between RC-C and RC-M/L subunits (Extended Data Fig. 2 a). The LH1 complex encircles the RC, which consists of cytochrome (C), H, M and L subunits, the structures of which are in agreement with previous studies (Fig. 1 **b,c,e**. Extended Data Fig. 2)[23].

The LH1 complex surrounds the RC to form a closed elliptical LH1 ring. The lengths of the major and minor axes of the elliptical rings measured from center to center of the transmembrane helices are 75.2 / 78.7 Å for the α ring, 107.5 / 111.7 Å (β) ring and 109.6 / 114.8 Å (γ). The LH1 ring consists of 17 components, rather than the 16 proposed earlier[21], with 16 heterotrimers of α/β/γ polypeptides and one α/β heterodimer (Fig. 1 **c,f**). Each of α, β and γ has a single transmembrane helix. A short N-terminal helix in α runs parallel to the membrane surface, whereas the C-terminal region contains a loop structure. No helical structures are observed in the C- and N-terminal regions of the β polypeptide. The N-termini of α and β are on the cytoplasmic side of the membrane, but the γ subunit has the opposite topology, with its N-terminus on the periplasmic side (Extended Data Fig. 3). This arrangement of LH1 polypeptides creates a triple ring LH1 complex consisting of an inner circle of 17 α polypeptides, 16 γ polypeptides forming the outer ring and a 17 β polypeptide ring sandwiched in between (Fig. 1 **c**, **f**). Each of the 16 γ polypeptides sits between two βs, with the 'missing' 17th γ leaving a functionally essential gap in the LH1 ring (Fig. 1 **c**, **f**) for quinol exchange.

Two BChl *b* molecules and one Crt, all-*trans* 1,2-dihydro- derivatives of neurosporene (n=9) or lycopene (n=11), are non-covalently bound between each α/β pair. No pigment molecules are bound to the γ polypeptide (Fig. 3). Major cofactors bound within the RC are as previously reported except for a newly found ubiquinone-9, Q_P (Fig. 2). RC cofactors are arranged in the expected local pseudo two-fold rotation symmetry (Fig. 2).

Stabilizing interactions and a proposed assembly sequence for the LH1 ring

The cryo-EM model of the RC-LH1 from *Blc. viridis* reveals a complex interconnecting series of protein-protein, pigment-protein and pigment-pigment associations within the LH1 ring. For the sake of simplicity, the LH1 heterotrimer subunits (1), (2) and (3) are used to demonstrate the stabilising intra- and inter-subunit interactions in the LH1 complex. Inter-subunit H-bonds on the periplasmic side are α(n)-Arg 44 to β(n-1)-Val 55 (3.0 Å); β(n)-Arg 44 to β(n-1)-Ala 48 (3.3 Å) (Fig 3**a**). There is an intra-subunit H-bond between α-Arg 44 on the periplasmic side and the carboxyl group of β-Trp 46 (3.0 Å), which stabilizes the C-terminal loops of both the α- and β-polypeptides (Fig. 3b). The γ(n) polypeptide forms two H-bonds with the α(n) – and β(n) –polypeptides; γ-Asp 14 to β-Trp 41 (3.0 Å) and γ-Arg 36 to carboxyl group of α-Thr 6 (3.1 Å) (Fig. 3**b**). Thus, an LH1 heterotrimer subunit is formed from α(n)/β(n)/γ(n), not α(n+1)/β(n+1)/γ(n). This arrangement suggests an assembly sequence of the LH1 complex of *Blc. viridis*.

It is likely that once an α(1)/β(1) subunit is formed, it interacts with the RC-H to form an anchor point through the H-bond between α(1)Arg 19 and RC-H Ser 256. Then, the γ(1) binds to the α/β subunit to form the first LH1 subunit α(1)/β(1)/γ(1). To do so, γ needs a space to access the α/β subunit by rotating and translating to achieve the correct angle of approach and a suitable orientation. This procedure continues until the 17^{th} α/β subunit is assembled.

At this point, there is no space for a correct direction of approach and orientation that would allow the 17th γ to dock with the 17th α/β, resulting in a "gap" in the LH1 ring.

The RC-LH1 from *Blc. viridis* reveals the basis for the stabilizing effects of Crts, which mainly rely on hydrophobic forces, and for excitation energy transfer from Crts to BChls[15]. Interactions of each Crt with n+1, n, n-1 polypeptides and with bound BChls effectively crosslink one LH1 αβ subunit to the next (Fig. 3c). One end of the Crt is in close proximity to the upstream neighboring LH1 α (n+1) near its C-terminus (Phe 37, 3.1 Å; Leu 33, 3.7 Å; Ala 32 3.4 Å; His 36, 3.9 Å); the other end approaches the downstream neighboring LH1 α(n-1) near its N-terminus (Leu 11, 4.3 Å; Lys 10, 5.1 Å). In particular, this end of the Crt is also in close proximity to the β(n) N-terminus. The middle part of the Crt is close to the phytyl tails of the α- (3.2 Å)and β- (4.0 Å) BChl *b* molecules (Fig. 3c).

Subunits 1-16 of the LH1 complex (Fig. 1**f**) consist of one each of α, β, and γ, two BChl *b* and one all-*trans* Crt., The γ polypeptide has no histidine residue and does not bind BChl *b*. Fig 3b illustrates this point, using subunit 3; α-His 36 forms a ligand with the central Mg of α-BChl *b* (2.5 Å) and β-His 37 ligands β-BChl *b* (2.2 Å) (Fig. 3b, c). The C3 acetyl groups of the α-BChl *b* and β-BChl *b* form an H-bond with α-Trp 47 (2.9 Å) and β-Trp 46 (2.9 Å) respectively to orientate the bacteriochlorin rings of BChl *b*. This orientation is further stabilized by an H-bond between β-Tyr 29 and the ester group of β-BChl *b* on C13^2 (3.0 Å). The OH group of β-Tyr 29 could form a H-bond with the ester group on the phytyl tail of the α- or β-BChl *b.*

RC-LH1 interactions

The resolution of the cryo-EM structure of the RC-LH1 from *Blc. viridis* is sufficient to allow detailed analysis of the protein-protein and protein-pigment interactions within the complex. The pigment-protein interactions within the RC have previously been described in

155 detail[24], and now the relationship between the RC and its encircling LH1 can be defined. Fig.
156 4a shows the overall organization of the RC-LH1 complex, separated into three zones. Zone 1
157 (AOC) includes a close contact between the LH1 and the RC, an H-bond between LH1-α1 Arg
158 19 and RC-H Ser 256 (2.8 Å), which is likely the site for initiating encirclement by LH1 in a
159 manner analogous to that for the RC-LH1-PufX complex of *Rba. sphaeroides*[6]. This α/β/γ
160 triad subunit is assigned as LH1 subunit 1 (see Fig. 1f). Proximity between transmembrane
161 helix RC-L_A and LH1-α2, with a centre-centre helix distance of ~10 Å, could facilitate the
162 encirclement process. A third interaction in this region involves LH1- α3 and LH1- α4 on the
163 cytoplasmic side, which constrains a loop on RC-H (Leu 47-Pro 54). In zone 2 (COB) there is a
164 single point of contact between the RC and LH1, between the RC-M_A helix and LH1 -α9. The
165 gap between the RC and LH1 in this region is mainly filled by the single transmembrane helix
166 of RC-H and lipid molecules, (Extended Fig. 4a). Zone 3 (BOA) is where quinol/quinone
167 exchange occurs at the RC Q_B site, and where newly released quinols, and quinones arriving
168 from outside, create a dynamic quinone pool[6]; Thus, the structure of the gap between the
169 RC and LH1 in this region shows disordered densities arising from lipids and quinones[5]
170 (Extended Fig. 4a). Fig. 4b summarizes all intra- and inter-subunit protein-protein and
171 protein-pigment interactions in the LH1 complex, and highlights the extent of the
172 interactions that stabilize the LH1 complex.
173
174 ***Structural basis for the large red-shift of the BChl b Q_y band***
175 The RC-LH1 complex of *Blc. viridis* accesses the infra-red region of the spectrum by red-
176 shifting its bound BChl *b* pigment to an extraordinary degree; its 1015 nm absorption
177 maximum represents the lowest energy light utilized by a photosynthetic bacterium.
178 Previous studies have identified several influences on the red-shift of the BChl *a*/*b* Q_y
179 absorption maximum in bacterial light-harvesting complex[25,26]. The cryo-EM structure of the

Blc. viridis RC-LH1 complex shows that at least five factors contribute to the large bathochromic shift of the BChl *b* Q_y band.

1. *Chemical structure*. The extra C=C double bond in BChl *b* relative to BChl *a* extends conjugation in the bacteriochlorin ring and red-shifts the Q_y band. The 795 nm absorption maximum of BChl *b* in methanol, is 24 nm further to the red than BChl *a*, which directly affects the 'site energy' within coupled BChl *b* aggregates in the RC-LH1 complex.

2. *Pigment-protein interactions*. As already noted (Fig. 3c) the Crts interlink LH1 αβ subunits (Fig. 3c) and the C3 acetyl groups of α- and β-BChls *b* H-bond to LH1 Trps (Fig. 3b), adopting an in-plane conformation similar those of the B800-850 LH2 complex of *Rps. acidophila*[27,28]. A combination of mutagenesis and Raman spectroscopy showed that H-bonds red-shift the absorption of the *Rba. sphaeroides* LH1 complex[29,30].

3. *Number of coupled BChl a/b molecules*. 17 pairs of coupled BChl *b* molecules in the RC-LH1 complex of *Blc. viridis* represent the largest circular aggregate of pigments reported for light-harvesting complexes from photosynthetic bacteria[31]. Increasing the oligomeric size of LH1 subunits from 2 to 6-7 is accompanied by red shifts of 6-7 nm in absorption and fluorescence emission of the BChl *a* Q_y band, for the LH1 complex of *Rba. sphaeroides*, although larger oligomers produced no further redshifts[32].

4. *Structure of BChl a/b aggregates*. The Mg-Mg distances within BChl pairs reflect the degree of overlap, and therefore the electronic coupling and Q_y red-shifting of BChl *a/b* in light-harvesting complexes. Extended Data Fig. 5 shows the linear correlation of Q_y band maximum versus inter- and intra-subunit Mg-Mg distances in five different light-harvesting complexes, which is stronger for the intra-subunit distances. The intra-subunit (8.8 Å) or inter-subunit (8.5 Å) Mg-Mg distances of BChl *b* in the *Blc. viridis* are the shortest reported for a bacterial light-harvesting complex.

5. *Structural rigidity enforced by the γ-apoproteins.* Sixteen γ-apoproteins pack tightly between βs, and also collectively interlock the LH1 structure through 32 H-bonds to α and β

polypeptides, constraining free movement of the LH1 ring and stabilizing the BChl *b* pairs in the complex and thereby contributing to the red-shift of the BChl *b* Q_y band[33]. There are parallels with the large red shift of BChl *a* to 915 nm within the RC-LH1 complex from *Tch. tepidum* (Extended Data Fig. 6 a). In this case bound Ca^{2+} ions constrain conformational flexibility[34] and limit disorder in site energies. Inhomogeneous narrowing is accompanied by mixing of charge transfer and lowest exciton states, proposed to be the basis for the red shift in this complex[25].

A template for preparing quinols for export across the LH1 ring

The RC-LH1 of *Blc. viridis* houses RC Q_A and Q_B, and a novel third quinone, Q_P (See Fig. 5 **a, b, c**). The binding sites of Q_A and Q_B are similar to those reported previously, although their tail structures are significantly different from those in isolated RCs[21,35] (Extended Data Fig. 6 b,c). The third uniquinone-9 molecule, Q_P, sits near the gap in the LH1 ring, some distance (48.9 Å) away from the Q_B binding site. The head of the Q_P molecule is stabilized by π - π interactions with RC-L Phe 40, the aromatic ring of which is roughly parallel to the plane of the quinone head ring at a distance of 3.6 Å. Q_P is also in close proximity, 3.0 Å away, from LH1- α1 Tyr 27 the aromatic ring of which is roughly perpendicular to the Q_P head plane. Unlike RC Q_A and Q_B, the tail of Q_P is not free to move, and instead it is conformationally constrained by a series of contacts, with LH1- α 1 Phe 37 (4.7 Å), RC-L Gln 87(2.4 Å), RC-L Trp 142 (3.5 Å) and RC-L Val 91(4.4 Å) (Extended Data Fig. 6d). This Q_P binding pocket provides a folding template so it assumes a compact conformation and a suitable orientation prior to entering the pore in the LH1 at the position of the absent 17th γ-apoprotein. (See Fig. 5 **c, d**). The RC-LH1 complex of *Blc. viridis* reveals a new strategy for fostering quinone movement across an LH1 ring. Of the 17 subunits 16 are α/β/γ heterotrimers, and only one is an α/β heterodimer. The 16 γ polypeptides, located outside the β ring, pack between β-apoproteins leaving one gap in the LH1 ring between subunits 1 and 17, and dictating the position of the

only pore for quinone/quinol migration. The Q_P binding pocket is located next to the pore, and the Q_P molecule appears to be folded and oriented in the binding pocket in a manner that encourages passage through the LH1 ring (Fig. 5 **d**). A pore measuring ~5 x 7 Å between α17 and α1 can be seen clearly (Fig. 5 **e**), and is created by Arg 18--Phe 25 in α17 (RRVLTALF) and Leu 15--Leu 24 in α1 (LDPRRVLTAL) (Fig. 5 **e**). It should be noted that the electron densities of β(17)-BChl *b*, α(1)-BChl *b* and α(1)/β(1) Crt are weaker than their counterparts in the rest of the LH1 complex; this is particularly evident for those regions of the pigments that are close to the Q-pore, for example the phytyl tails and one end of the Crt as shown in Fig. 5 **f**. This weaker density reflects the relative flexibility of this region; thus the size of this pore could fluctuate transiently, facilitating the movement of the Q/QH_2 molecules through the channel.

1 Blankenship, R. E. *Molecular Mechanisms of Photosynthesis* 2nd edn, (John Wiley & Sons, 2014).

2 Sundström, V., Pullerits, T. & van Grondelle, R. Photosynthetic light-harvesting: Reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *J. Phys. Chem. B* **103**, 2327-2346 (1999).

3 Cogdell, R. J., Gall, A. & Köhler, J. The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to in vivo membranes. *Q. Rev. Biophys.* **39**, 227-324 (2006).

4 Roszak, A. W. *et al.* Crystal structure of the RC-LH1 core complex from *Rhodopseudomonas palustris. Science* **302**, 1969-1972 (2003).

5 Niwa, S. *et al.* Structure of the LH1-RC complex from *Thermochromatium tepidum* at 3.0 Å. *Nature* **508**, 228-232, (2014).

6 Qian, P. *et al.* Three-dimensional structure of the *Rhodobacter sphaeroides* RC-LH1-PufX complex: dimerization and quinone channels promoted by PufX. *Biochemistry* **52**, 7575-7585, (2013).

7 Jamieson, S. J. *et al.* Projection structure of the photosynthetic reaction centre-antenna complex of *Rhodospirillum rubrum* at 8.5 Å resolution. *EMBO J.* **21**, 3927-3935 (2002).

8 Jungas, C., Ranck, J. L., Rigaud, J. L., Joliot, P. & Verméglio, A. Supramolecular organization of the photosynthetic apparatus of *Rhodobacter sphaeroides. EMBO J.* **18**, 534-542 (1999).

9 Qian, P., Hunter, C. N. & Bullough, P. A. The 8.5Å projection structure of the core RC-LH1-PufX dimer of *Rhodobacter sphaeroides*. *J. Mol. Biol.* **349**, 948-960, (2005).

10 Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. X-ray structure analysis of a membrane protein complex: electron density map at 3Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*. *J. Mol. Biol.* **180**, 385-398 (1984).

11 Miller, K. R. & Jacob, J. S. The *Rhodopseudomonas viridis* photosynthetic membrane arrangement in situ. *Arch. Microbiol.* **142**, 333-339 (1985).

12 Ikeda-Yamasaki, I., Odahara, T., Mitsuoka, K., Fujiyoshi, Y. & Murata, K. Projection map of the reaction center-light harvesting 1 complex from *Rhodopseudomonas viridis* at 10 Å resolution. *FEBS Lett.* **425**, 505-508 (1998).

13 Blankenship, R. E. *et al.* Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. *Science* **332**, 805-809, (2011).

14 Magdaong, N. C. M., Niedzwiedzki, D. M., Goodson, C. & Blankenship, R. E. Carotenoid-to-bacteriochlorophyll energy transfer in the LH1-RC core complex of a bacteriochlorophyll *b* containing purple photosynthetic bacterium *Blastochloris viridis*. *J. Phys. Chem. B* **120**, 5159-5171 (2016).

15 Thornber, J. P., Cogdell, R. J., Seftor, R. E. B. & Webster, G. D. Further studies on the composition and spectral properties of the photochemical-reaction centers of bacteriochlorophyll *b*-containing bacteria. *Biochim. Biophys. Acta* **593**, 60-75 (1980).

16 Malhotra, H. C., Britton, G. & Goodwin, T. W. Occurrence of 1,2-dihydro-carotenoids in *Rhodopseudomonas viridis*. *J Chem Soc Chem Comm*, 127 (1970).

17 Konorty, M. *et al.* Photosynthetic system in *Blastochloris viridis* revisited. *Biochemistry* **48**, 4753-4761 (2009).

18 Welte, W. & Kreutz, W. Structure of thylakoids in cells of *Rhodopseudomonas viridis* as influenced by growth conditions. *Arch. Microbiol.* **141**, 325-329 (1985).

19 Engelhardt, H., Baumeister, W. & Saxton, W. O. Electron-microscopy of photosynthetic membranes containing bacteriochlorophyll *b. Arch. Microbiol.* **135**, 169-175 (1983).

20 Scheuring, S. *et al.* Nanodissection and high-resolution imaging of the *Rhodopseudomonas viridis* photosynthetic core complex in native membranes by AFM. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 1690-1693, (2003).

21 Deisenhofer, J., Epp, O., Sinning, I. & Michel, H. Crystallographic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction center from *Rhodopseudomonas viridis*. *J. Mol. Biol.* **246**, 429-457 (1995).

22 Chen, J. E., Huang, C. C. & Ferrin, T. E. RRDistMaps: a UCSF Chimera tool for viewing and comparing protein distance maps. *Bioinformatics* **31**, 1484-1486 (2015).

23 Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3Å resolution. *Nature* **318**, 618-624 (1985).

24 Deisenhofer, J. & Michel, H. The photosynthetic reaction centre from the purple bacterium *Rhodospeudomonas viridis. EMBO J* **8**, 2149-2170 (1989).

25 Ma, F., Yu, L. J., Wang-Otomo, Z. Y. & van Grondelle, R. The origin of the unusual Q(y) red shift in LH1-RC complexes from purple bacteria *Thermochromatium tepidum* as revealed by Stark absorption spectroscopy. *Biochim. Biophys. Acta* **1847**, 1479-1486 (2015).

26 Cogdell, R. J., Howard, T. D. & Isaacs, N. W. Structural factors which control the position of the Qy absorption band of bacteriochlorophyll *a* in purple bacterial antenna complexes. *Photosynth. Res.* **74**, 135-141 (2002).

27 McDermott, G. *et al.* Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature* **374**, 517-521 (1995).

28 McLuskey, K., Prince, S. M., Cogdell, R. J. & Isaacs, N. W. The crystallographic structure of the B800-820 LH3 light-harvesting complex from the purple bacteria *Rhodopseudomonas acidophila* strain 7050. *Biochemistry* **40**, 8783-8789 (2001).

29 Olsen, J. D., Sockalingum, G. D., Robert, B. & Hunter, C. N. Modification of a hydrogen bond to a bacteriochlorophyll *a* molecule in the light harvesting 1 antenna of *Rhodobacter sphaeroides. Proc. Natl. Acad. Sci. U.S.A.* **91**, 7124-7128 (1994).

30 Sturgis, J. N., Olsen, J. D., Robert, B. & Hunter, C. N. Functions of conserved tryptophan residues of the core light-harvesting complex of *Rhodobacter sphaeroides. Biochemistry* **36**, 2772-2778 (1997).

31 Cogdell, R. J. & Roszak, A. W. Structural biology: the purple heart of photosynthesis. *Nature* **508**, 196-197 (2014).

32 Westerhuis, W. H. J., Sturgis, J. N., Ratcliffe, E. C., Hunter, C. N. & Niederman, R. A. Isolation, size estimates, and spectral heterogeneity of an oligomeric series of light-harvesting 1 complexes from *Rhodobacter sphaeroides*. *Biochemistry* **41**, 8698-8707 (2002).

33 Yu, L. J., Kawakami, T., Kimura, Y. & Wang-Otomet, Z. Y. Structural basis for the unusual Q(y) red-shift and enhanced thermostability of the LH1 complex from *Thermochromatium tepidum. Biochemistry* **55**, 6495-6504 (2016).

34 Jakob-Grun, S., Radeck, J. & Braun, P. Ca^{2+}-binding reduces conformational flexibility of RC-LH1 core complex from thermophile *Thermochromatium tepidum*. *Photosynth. Res.* **111**, 139-147 (2012).

35 Roszak, A. W. *et al.* New insights into the structure of the reaction centre from *Blastochloris viridis*: evolution in the laboratory. *Biochem. J.* **442**, 27-37 (2012).

Figure Legends

Figure 1. Cryo-EM structure of the RC-LH1 core complex from *Blc. viridis*. Views of the colour-coded RC-LH1 density map. LH1- α (yellow), LH1-β (dark blue), LH1- γ (red), BChl *b* (light sea green), Crt (orange red), RC-C (green), RC-H (cyan), RC-L (orange) and RC-M (magenta). Detergent and other disordered molecules are in grey. **a**, View in the plane of the membrane; two dashed lines indicate the likely position of the membrane bilayer. **b,** 45 degree rotation of **a**. **c,** Perpendicular view from the periplasmic side. Densities outside the membrane region were truncated for clarity. **d**,**e**,**f,** Ribbon models corresponding to **a**,**b**,**c** but without the truncations in **f**. The LH1 subunits are numbered in **f**. Subunits 1 and 17 are outlined with dashed lines.

Figure 2. Pigment arrangement in the *Blc. viridis* RC-LH1 core complex. a, Pigment molecules viewed from the periplasmic side by tilting 45 degrees in the plane of the

membrane. **b**, RC pigment molecules, viewed from the membrane plane. A local pseudo C2 symmetry axis is shown as a dashed line.

Figure 3. Intra- and inter-subunit protein-protein and protein-pigment interactions. **a**, LH1 subunits 1-3 (see Fig. 1f) illustrate inter-subunit interactions. Colours as in Figure 1 except BChl *b* molecules in medium blue and all-trans 1,2-dihydroneurosporene in orange. H-bonds are indicated by dashed lines. **b**, A single LH1αβγ subunit, with the polypeptides shown in loop representation for clarity. The red arrow indicates a putative direction of approach for γ to the α/β pair during assembly of the complex. **c**, Projection view to show interactions made by a Crt with nearby pigments and polypeptides.

Figure 4. Interaction between the RC and LH1 complex, and within the LH1 complex. a, Periplasmic side of the RC-LH1 core complex; colour coding as in Figure 1. RC-H Ser 256 and LH1-α1 Arg 19 are highlighted using space-fill. The RC-H loop Leu47 to Pro 54 is highlighted in orange red. **b**, Summary of intra- and inter-subunit interactions in the LH1 complex. Only transmembrane helices of LH1 polypeptides are shown for clarity. All arrows indicate H-bonding interactions.

Figure 5. A quinone/quinol channel in the RC-LH1 core complex. a, RC-LH1 from the periplasmic side, with 80% transparency applied to the RC, and LH1 subunits 9-17. A green arrow indicates the gap between subunits 1-17 . **b**, RC-LH1 rotated 90 degrees from **a**, with Q_B and Q_P viewed by removing LH1 subunits 1-8. **c**, Close-up of the Q_P binding pocket. **d**, Ribbon representation of the Q_P region. Green arrow as in a. **e**, Close-up view of the Q-channel (dashed circle) from outside the LH1 ring. **f**, Electron densities of pigments adjacent to the LH1 pore.

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgments 78

C.N.H. and P.Q. acknowledge support from the Biotechnology and Biological Sciences Research Council (BBSRC) UK, award number BB/M000265/1, the European Research Council Advanced Award 338895, and the Sheffield University Imagine programme. C.N.H. was also partially supported by the Photosynthetic Antenna Research Center (PARC), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences (Award number DE-SC0001035). D.P.C. acknowledges funding from the European Commission (Marie Skłodowska-Curie Global Fellowship 660652).

Author contributions

P.Q. and C.N.H. conceived the study. P.Q. and C.N.H. designed the experiments. P.Q., C.A.S., D.P.C., and P.W. performed the experiments. P.Q. analysed the results and generated structural models. P.Q. and C.N.H. wrote the paper.

Author Information

The authors declare no competing financial interest. Correspondence and requests for materials should be addressed to P.Q. (p.qian@sheffield.ac.uk) or C.N.H. (c.n.hunter@sheffield.ac.uk).

METHODS

Protein purification. Wild type *Blc. viridis* (DSM-133) was obtained from DSMZ. Photosynthetic cultures of the *Blc. viridis* were grown in sodium succinate medium 27 (N medium) under illumination (100 µmol of photons $m^{-2}.s^{-1}$) at 30 °C in 20 L screw-capped vessels, completely filled with N_2-sparged medium, as described by Lang and Oesterhelt[36]. Cells were harvested when the culture reached an optical density (OD) of 1.6 at 680 nm by centrifugation at 3290 g for 30 minutes. Washed cells were broken by passing them through French Press three times at 18,000 psi. The crude cell lysate was applied to a two-step sucrose gradient (15% and 40% (w/w) in an ultracentrifugation tube). Photosynthetic membrane was collected at the interface of 15% and 40% sucrose after 5 hours of centrifugation at 100,000 g. Membranes were pelleted and resuspended into working buffer (20 mM HEPES, pH 7.8). The OD of the membrane was adjusted to ~100 at 1015 nm. For solubilization of the core complexes, the OD at 1015 nm of the photosynthetic membrane was adjusted to 60, and 3% (w/w) β-DDM was added. This mixture was then stirred in the dark at 4 °C for 30 minutes. Unsolubilized material was removed by centrifugation for 1 hour at 211,000 g.

The clarified supernatant was loaded onto an ion exchange column pre-equilibrated with working buffer solution containing 0.03% β-DDM. The core complexes eluted at ~ 250 mM NaCl and were collected and concentrated. These were further purified using a Superdex 200 gel filtration column. The fractions with an absorption ratio of A_{1008}/A_{280} higher than 1.22 were pooled together and used for cryo-EM data collection.

Cryo-EM data collection. The protein concentration was adjusted to OD 40 at 1008 nm. 3.0 μl protein solution was applied to a glow-discharged holey carbon grid (Quantifoil grid R1.2/1.3, 300 mesh Cu). The grid was plunged into liquid ethane cooled by liquid nitrogen using a Leica EM GP vitrobot. Parameters were set as following: blotting time 4 seconds, humidity 99%, sample chamber temperature 5 °C. The frozen grid was stored in liquid nitrogen before use. A second grid was prepared using a Quantifoil grid R3.5/1.0 covered by a thin carbon film (EM resolution, Inc.), with protein diluted 10 fold. Vitrification conditions were the same as for the first grid. Data were recorded at eBIC on a Titan Krios electron microscope with a Gatan 968 GIF Quantum with a K2 summit detector operating at 300 kV accelerating voltage, at nominal magnification of 130k in counting mode. Movies were collected in super-resolution mode and Fourier cropped to give a resulting calibrated pixel size of 1.06 Å at the specimen level. An energy selecting slit of 20 eV was used. An exposure rate of 5 electrons/pixel/second was set and a fresh super-resolution gain reference was performed at this dose rate prior to data acquisition. A total dose of 45 electrons per $Å^2$ was used for movies of 20 frames. In total, 6,472 movies were collected with defocus values from 1.0 to 3.0 μm. Two typical cryo-EM images, which are averaged from motion corrected movie frames, are shown in Extended Data Fig. 7a, b.

Data processing. All images that were empty, contained few particles, or were ice contaminated were discarded. Dose fractionated images were subjected to beam-induced motion correction using MotionCorr[37]. Images derived from the sum of all frames were used for further data processing by the use of RELION 2.0[38-40]. CTF parameters were determined using gctf[41]. In total, 267,726 particles were picked manually. These particles were subjected to reference-free two-dimensional classification. Those particles that categorized into poorly defined classes were rejected. This cleaning procedure by the use of 2D classification was repeated three times, resulting in rejection of 9.45 % of total particles. The resulting 2D classes were subjected to an initial 3D model calculation using EMAN2[42] for maximum-likelihood-based 3D classification. One of the four stable 3D classes accounting for 62.3% total particles was selected for high resolution refinement and 3D reconstruction without subtraction of detergent micelle from the raw micrographs. This resulted in a map at a global resolution of 3.3 Å. The density map was corrected for the modulation transfer function (MTF) of the Gatan K2 summit camera and further sharpened by the post-processing subroutine in the RELION 2.0 using an estimated temperature factor and a mask was created using RELION 2.0 with a lowpass of 15 Å and a soft-edge of 7 Å. The Fourier Shell Correlation (FSC) curve corrected for masking is shown in Extended Data Figure 7c. The estimate of final resolution of 2.9 Å for the RC-LH1 map was based on a FSC cut off of 0.143. ResMap[43] was used for a calculation of the local resolution map (Extended Data Fig. 4b,c).

Modeling and refinement. Initially, the crystal structure of the *Blc. viridis* (PDB 1PRC) was fitted to the cryo-EM map as a rigid body using the *fit in map* routine of Chimera[44]. COOT[45] was then used for manual adjustment and real-space refinement for both polypeptides and cofactors. All amino acid sequences of polypeptides in the RC are listed in Extended Data Fig. 8. Ubiquinone-9 molecules (Q_B and Q_P) were also fitted to the density map independently using COOT.

For LH1, the electron density of the LH1 subunit 3 was selected for modeling first. Based on structural similarity compared with the LH1 of *Tch. tepidum*[5] and LH2 of *Rps. molischianum*[46], the location of His residues, which ligate BChl *b* molecules in the

α/β polypeptides (Extended Data Fig. 9), were located in the density map. The fitted RC was used as a reference to determine the orientation of the α/β polypeptides. Their amino acid sequences, taken from previous work[47], were fitted into electron density map using COOT. Two BChl *b* molecules and one all-*trans* Crt are added into the model based on their densities. Analysis of pigment composition shows that the major Crt in the core complex is all-*trans* 1,2-dihydroneurosporene[15]; this Crt therefore was modeled into the density map. Having no His residues, the γ-polypeptide does not bind BChl *b* molecules. No 3D structural information of the γ subunit was available, but the 2.9 Å resolution allows assignment of the larger amino acid side-chains such as Trp and Tyr. By matching three Trps and one Tyr residue in the γ polypeptide, its orientation was ascertained and all other residues were traced based on the density map using COOT. Comparison with the sequence of the γ-polypeptide[47] leaves 12 N-terminal residues unaccounted for. The structure of the LH1 α/β/γ subunit was then used as a rigid body to fit into the density map for other LH1 subunits. For the LH1 subunit 17, only α/β and pigments were used. All of the LH1 subunits then underwent real-space refinement using COOT. The final model was subjected to global refinement and minimization using REFMAC5[48]. The final refinement statistics are summarized in Extended Data Table 1. The quality of fit for the structural model within the electron density map was validated using EMRinger[49].

Data availability. The cryo-EM density map has been deposited in the World Wide Protein Data Bank (wwPDB) under accession code EMD-3951 and the coordinates have been deposited in the Protein Data Bank (PDB) under accession number 6ET5.

36 Lang, F. S. & Oesterhelt, D. Microaerophilic growth and induction of the photosynthetic reaction center in *Rhodopseudomonas viridis*. *J. Bacteriol.* **171**, 2827-2834 (1989).

37 Zheng, S. Q. *et al.* MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat. Methods* **14**, 331-332 (2017).

38 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J. Struct. Biol.* **180**, 519-530 (2012).

39 Scheres, S. H. W. Semi-automated selection of cryo-EM particles in RELION-1.3. *J. Struct. Biol.* **189**, 114-122 (2015).

40 Scheres, S. H. W. Processing of structurally heterogeneous cryo-EM data in RELION. *Methods Enzymol.* **579**, 125-157 (2016).

41 Zhang, K. Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1-12 (2016).

42 Tang, G. *et al.* EMAN2: an extensible image processing suite for electron microscopy. *J. Struct. Biol.* **157**, 38-46 (2007).

43 Kucukelbir, A., Sigworth, F. J. & Tagare, H. D. Quantifying the local resolution of cryo-EM density maps. *Nat. Methods* **11**, 63-67 (2014).

44 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605-1612 (2004).

45 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr. D* **60**, 2126-2132 (2004).

46 Koepke, J. *et al.* The crystal structure of the light-harvesting complex II (B800-B850) from *Rhodospirillum molischianum*. *Structure* **4**, 581-597 (1996).

47 Brunisholz, R. A., Jay, F., Suter, F. & Zuber, H. The light-harvesting polypeptides of *Rhodopseudomonas viridis* - the complete amino acid sequences of B1015-alpha, B1015-beta and B1015-gamma. *Biol. Chem. H-S* **366**, 87-98 (1985).

48 Murshudov, G. N. *et al.* REFMAC5 for the refinement of macromolecular crystal structures *Acta Crystallogr. D* **67**, 355-367 (2011).

49. Barad, B.A. *et al.* EMRinger: side chain–directed model and map validation for 3D cryo-electron microscopy. *Nature Methods* **12**, 943–946 (2015).

Extended Data Figure Legends

Extended Data Figure 1. Absorption spectra of photosynthetic membranes and the purified RC-LH1 core complex from *Blc. viridis*. Absorption spectra of isolated membranes (dashed line) and the purified RC-LH1 complex (solid line) were recorded at room temperature and normalized at their Q_y bands at 1015 and 1008 nm. The peak at 831 nm together with a shoulder at ~ 970 nm arise from BChl *b* in the RC. Bacteriopheophytin appears as a poorly-resolved peak at ~810 nm. The Q_x bands give rise to a composite peak at 602 nm. The minor peak at ~558 nm arises from the cytochromes, the Soret band of which contributes in the ~410 nm region. Absorption features at 482, 450 and 420 nm belong to Crts and the 399 nm maximum corresponds to the Soret band of BChl *b* in the core complex. No oxidized BChl *b* is observed which, if present, would appear at ~685 nm.

Extended Data Figure 2. Residue-residue distance deviation between cryo-EM and X-ray structures of the RC from *Blc. viridis.* a, Superimposition of the RC from the X-ray structure (1PRC) in grey with the cryo-EM structure in colour. The colour coding is the same as that for Figure 1. A putative hinge point is indicated with a red dot. The bending direction of the cryo-EM structure is indicated with two green arrows. A red arrow points to a flexible RC-H loop. **b,c,d,e**, Residue-residue (RR) distance deviation maps[22] of the individual RC subunits, C, M, L and H, respectively, comparing the structures from cryo-EM and X-ray crystallography (PDB 1PRC)[21]. Each

vertical scale shows the standard deviation (SD) in Ångstroms. The flexible loop of RC-H is indicated with a red perpendicular arrow in **e**.

Extended Data Figure 3. Cryo-EM densities and structural models of polypeptides and pigments in the *Blc. viridis* RC-LH1 complex. The colour code is the same as in Fig. 1. The contour levels of the density maps were adjusted to mirror their molecular weights.

Extended Data Figure 4. Electron densities between and outside the LH1 and RC complexes, and local resolution maps of the RC-LH1 core complex from *Blc. viridis*. **a,** The RC-LH1 complex is as shown in Fig. 1f, but displayed at 70% transparency. Electron densities belonging to detergent, lipid and other disordered molecules are in grey. **b,** Side view of the core complex with the periplasmic side uppermost. **c**, View of the periplasmic side. All membrane-extrinsic parts of the complex were truncated for clarity. The coloured bar chart on the right shows the local structural resolution in Å.

Extended Data Figure 5. Relationship between BChl *a*/*b* Mg-Mg distances and Q_y band absorption in bacterial light harvesting complexes. a, Correlation of Q_y band maximum and inter-subunit BChl *a*/*b* Mg-Mg distances in five bacterial light-harvesting complexes. **b**, as in **a**, but for intra-subunit Mg-Mg distances. Values for the linear correlation coefficient *R*, calculated using least square linear regression (n=5 biologically independent samples in each case; one-sided significance test), are shown in **c.**

Extended Data Figure 6. Structural comparisons of selected cofactors and details of the Q_P binding site. a, The LH1-B1008 BChl *b* pair from *Blc. viridis* (blue) compared with the LH1-B915 BChl *a* pair (green) from the X-ray structure of the *Tch. tepidum*

RC-LH1 complex (PDB 3WMM). **b**, Comparison of the Q_A menaquinone-9 (blue) from the cryo-EM model of the *Blc. viridis* RC-LH1 with the Q_A (green) from the X-ray structure of the *Blc. viridis* RC (PDB 3T6E). **c**, as in **b**, but comparing Q_B. **d**, The Q_P binding site. Only LH1-α1 and part of RC-L are shown for clarity. LH1- α1 is in yellow, RC-L in brown, Q_P in blue and Q_B in rosy brown. Amino acid residues making close contacts around Q_P are numbered and listed accordingly.

Extended Data Figure 7. **Cryo-EM micrographs of the RC-LH1 complex from *Blc. viridis* and calculation of the cryo-EM map resolution. a**, Protein particles embedded in vitrified ice. Examples of RC-LH1 complexes are circled. 6,472 cryo-EM movies were recorded, from which 267,726 particles were picked manually for reference-free two-dimensional classification. During data processing, datasets of ~100,000 and ~167,000 particles were used independently for 3D reconstruction. They generated very similar 3D maps for the RC-LH1 complex, so they were then combined. **b**, The RC-LH1 particles are covered by a thin layer of vitrified ice on a supported carbon film. Each image has a size of 393.2 x 406.8 nm. **c**, Gold standard refinement was used for estimation of the final map resolution. The global resolution of 2.9 Å was calculated using a Fourier shell correlation (FSC) cut-off at 0.143.

Extended Data Figure 8. Amino acid sequence of polypeptides in the RC-LH1 complex from *Blc. Viridis.* Black---genome sequence, Red---protein sequence, Blue---missing in protein sequence.

Extended Data Figure 9. Amino acid sequence alignment of LH1 α- and β-polypeptides in RC-LH1 core complexes from purple photosynthetic bacteria. All sequences have been aligned relative to the His residue that ligates BChls in the LH1

complexes. The α- and β-polypeptides of the *Phs. molischianum* LH2 complex are included for comparison. The sequence alignment was performed using CLUSTAL O(1.2.4).

Extended Data Table 1. *Peter B Rosenthal and Richard Henderson (2003) Optimal determination of particle orientation, absolute hand and contrast loss in single particle electron cryomicroscopy. J. Mol. Biol., 333(4):721-745. ‡These results are calculated from a density map, in which electron density contributed by the surrounding belt of detergent was removed by masking. The results from the unmasked model are presented in parentheses.