The bacterium *Rhodospirillum rubrum* contains a simple photosynthetic system, in which the reaction center (RC) receives energy from the light-harvesting (LH1) complex. We have used high-resolution atomic force microscopy (AFM) to image two-dimensional crystals of the RC-LH1 complex of *R. rubrum*. The AFM topographs show that the RC-LH1 complex is ~94 Å in height, the RC-H subunit protrudes from the cytoplasmic face of the membrane by 40 Å, and it sits 21 Å above the highest point of the surrounding LH1 ring. In contrast, the RC on the periplasmic side is at a lower level than LH1, which protrudes from the membrane by 12 Å. The RC-LH1 complex can adopt an irregular shape in regions of uneven packing forces in the crystal; this reflects a likely flexibility in the natural membrane, which might be functionally important by allowing the export of quinol formed as a result of PCR photochemistry. Nanodissection of the RC by the AFM tip removes the RC-H subunit and reveals the underlying RC-L and -M subunits. LH1 complexes completely lacking the RC were also found, providing ideal conditions for imaging both rings of LH1 polypeptides for the first time by AFM. In addition, we demonstrate the ellipticity of the LH1 ring at the cytoplasmic and periplasmic sides of the membrane, in both the presence and absence of the RC. These AFM measurements have been reconciled with previous electron microscopy and NMR data to produce a model of the RC-LH1 complex.

Photosynthetic organisms harvest light energy and convert it to a chemically useful form, using light-harvesting (LH) and reaction center (RC) complexes. This coupling between LH and RC complexes involves close physical proximity because of the distance term governing energy transfer between the complexes (1). In the purple photosynthetic bacteria, the reaction center receives energy from the LH1 complex (reviewed in Ref. 2). This particular energy transfer step, which takes ~35–45 ps (3), is important, because it is the rate-limiting step in the process of trapping light energy in photosynthetic bacteria (4).

In view of the value of the RC-LH1 complex as a model for coupled light-harvesting energy transfer and photochemistry, structural information on the association between LH1 and RC complexes is required. *Rhodospirillum rubrum*, which represents one of the simplest possible photosynthetic systems, is valuable in this respect. A recent cryo-electron microscopy (EM) study of the RC-LH1 complex of *R. rubrum*, which built upon earlier work on the LH1-only (no RC) complex (5), shows that the RC complex is surrounded by a ring of 16α and 16β LH1 subunits, which would correspond to 32 bacteriochlorophylls (BChls) (6). This structure provides a rationale for the rate-limiting energy transfer step, because the ~45 Å distance from LH1 BChls to the special pair BChls of the RC is considerable (5, 6). This should be viewed in the context of known energy transfer steps and distances in these bacteria: for example, the internal transfer of energy in ~0.5–1 ps between the B800 and B850 BChls within the peripheral LH2 complex (7), which have a center-to-center distance of ~18 Å (8).

Another intriguing observation that emerged from the cryo-EM study of the RC-LH1 complex of *R. rubrum* was that the LH1 ring could adopt both circular and elliptical conformations (6), thus confirming a previously suspected flexibility in this complex (5). The flexibility of the LH1 ring was suggested to have a functional significance (6), because of the need for the RC complex to export reduced quinone, the product of its photochemistry. Breathing motions of the ring could facilitate this export and might be essential because the RC-LH1 complex of *R. rubrum* does not contain the PufX polypeptide, which is required for quinone transfer in *Rb. sphaeroides* and *Rb. capsulatus* (9–11).

The current state of structural knowledge of the RC-LH1 complex, seen only in projection at present, needs to be advanced by incorporating three-dimensional information to define the structure of this complex in more detail, to examine the ellipticity and flexibility of both the periplasmic and cytoplasmic surfaces, and to define the way in which this complex sits in the membrane bilayer. In the absence of three-dimensional x-ray crystallographic data, we have used atomic force microscopy (AFM) (12) to obtain high-resolution surface topographs of both faces of the RC-LH1 complex from *R. rubrum*. The AFM is a remarkable instrument: it allows the topography of biological samples to be acquired at subnanometer lateral resolution and
vertical resolution of ~1 Å under near physiological conditions, i.e. in buffer solution, at room temperature, and under normal pressure. A further feature of the AFM is the high signal-to-noise ratio provided in the topographs, making the study of single membrane protein complexes at work possible (13). Recently, the value of this technique has been demonstrated for the light-harvesting LH2 complexes of *Rb. sphaeroides* and *Rh. sphaeroides* (14, 15) and on membranes of *Rhodopseudomonas viridis* containing RC-LH1 complexes (16).

The aim of this work is to advance our knowledge of the photosynthetic core complex by building on the work of Scheuring et al. (16) on the periplasmically exposed surface of the complex and by obtaining topographs of both the cytoplasmic (RC-H subunit) and periplasmic (c-type cytochrome) faces. This establishes the height of the RC-H subunit and the functionality crucial special pair bacteriochlorophylls in relation to the membrane bilayer. In addition, it is possible to measure directly the position of the membrane-water interface in relation to RC-LH1 aromatic residues to assess their proposed anchoring role (17, 18). Finally, AFM can be used to determine the heights of the LH1 polypeptides above the membrane bilayer, and the ellipticity of the LH1 ring at the cytoplasmic and periplasmic sides of the membrane, in the presence and absence of the RC.

In the present study, we have used high-resolution AFM to image two-dimensional crystals of the RC-LH1 complex of *R. rubrum*. This represents the most detailed analysis of this complex to date, and it is sufficient to show two rings surrounding the RC, one from LH1α and the other from LH1β. The heights of the RC and LH1 protein subunits in the complex measured from AFM topographs have been reconciled with structural data from EM (6), AFM (this study), and NMR (19) experiments. New features of this complex are revealed, including the dimensions and conformations of both periplasmic and cytoplasmic aspects of the complex and the relationship of the RC-LH1 complex and its chromophores to the membrane bilayer. The flexibility of the RC-LH1 complex is emphasized by its adoption of an angular shape in regions of uneven packing in the crystal; this flexibility is expected to be important for the function of this complex by allowing the export of quinol formed as a result of reaction center photochemistry.

**EXPERIMENTAL PROCEDURES**

**Sample Preparation and Crystallization**—The purification of the RC-LH1 complex from *R. rubrum* has been reported previously (20). Briefly, pure membranes obtained from sucrose gradient centrifugation were solubilized with diheptanoyl phosphatidylcholine at a final concentration of 20 mM and a final OD of the protein sample of 60 at 880 nm. The mixture was stirred at 4°C in the dark for 45 min. Unsolvilized material was removed by centrifugation, and the supernatant was loaded onto a DEAE-Sepharose (Sigma) ion-exchange column equilibrated with elution buffer (10 mM Tris-HCl, 1 mM EDTA, 3 mM diheptanoyl phosphatidylcholine, pH 7.9). A gradient of 0–300 mM NaCl in elution buffer was applied. The main peak, which contains pure membranes obtained from sucrose gradient centrifugation, was pooled (A280/H11022 > 2.0). Concentrated sample was then applied to a gel filtration column and eluted in 10 mM Tris-HCl, 2 mM EDTA, 3 mM diheptanoyl phosphatidylcholine, pH 7.9 at 4°C. Fractions with A280/H250 > 2.4 were collected for crystallization. Well ordered two-dimensional crystals were obtained as described (6) by reconstitution of purified protein with *Escherichia coli* lipids (Avanti Polar Lipids Inc., Alabaster, AL) at a lipid-to-protein ratio of 1.0 using the flow dialysis method (21).

**Atomic Force Microscopy**—A stock solution of two-dimensional RC-LH1 crystals (0.5 mg/ml protein in 10 mM Tris-HCl, 1 mM EDTA, pH 7.9) was diluted 30-fold in 20 mM Tris-HCl, 150 mM KC1, 25 mM MgCl2, pH 7.8 (imaging buffer) and adsorbed for 20–30 min on freshly cleaved muscovite mica. After adsorption, the sample was gently washed with imaging buffer to remove membranes that were not attached firmly to the substrate. AFM experiments were performed using a Nanoscope Multimode microscope (Digital Instruments, Veeco Metrology Group, Santa Barbara, CA) equipped with an infrared laser head, fluid cell, and oxide-sharpened silicon nitride cantilevers of 100 and 200 μm length and nominal spring constants of 0.08 and 0.06 N/m from Olympus Optical Co. (Tokyo, Japan) and Digital Instruments, respectively. Topographs were acquired in contact mode at minimal loading forces (<100 pN). Trace and retrace signals were recorded simultaneously at line frequencies ranging between 4.1 and 5.1 Hz. Perspective views of raw data were prepared using the SXM program (University of Liverpool, Liverpool, United Kingdom).

**Modeling**—The procedure described here was carried out using the visualization program Dino. The *Rh. sphaeroides* LH1α and LH1β polypeptides (19) were modeled as rigid bodies into a dimer using the following constraints: (i) the side chain nitrogen from the conserved σ-Trp11 is located within hydrogen-bonding distance to the C2 acetyl-oxygen from BCHla (22); (ii) αHis9 serves as the ligand for the Mg-atom in BCHla (23); (iii) the relative orientation of transmembrane helices fits the EM projection (6); and (iv) avoidance of clashes between both domains, even under 16-fold symmetrization. The resulting dimer (not shown) was fitted to the EM projection with the help of the symmetry visualization feature available in Dino, which allows interactive fitting with instant update of the corresponding symmetry elements based on elliptical symmetry, in this case, 16-fold with an axial ratio of 1.04. The *Rh. sphaeroides* RC (Protein Data Bank ID code 1MSX; PubMed ID 12167672) was subsequently inserted into the resulting LH1 ring, and the relative height was adjusted to fit the AFM data.

**RESULTS**

Purified RC-LH1 complexes from the purple non-sulfur bacterium *R. rubrum* were crystallized into two dimensions in the presence of *E. coli* lipids. This yielded exclusively the orthorhombic crystal form, rather than the mixture of crystals with square and orthorhombic symmetry as obtained previously with the lipid dioleoyl phosphatidylcholine (6). A comparison of negatively stained and glucose embedded two-dimensional crystals reconstituted in *E. coli* or dioleoyl phosphatidylcholine lipids by EM revealed no apparent differences. The crystal parameters of the orthorhombic form were almost identical in both lipids, within experimental uncertainty (data not shown).

To visualize the supramolecular organization of the RC-LH1 complex, two-dimensional crystals of the latter were adsorbed to freshly cleaved mica and imaged by AFM in buffer solution. On the overview topograph shown in Fig. 1a, three different surface types are evident: RC-LH1 two-dimensional crystals...
with their orthorhombic lattice lines (a, 1), lipid (a, 2), and mica (a, 3). Compared with the flat and featureless surfaces of lipid and mica, protein two-dimensional crystals were highly corrugated (see section analysis in Fig. 1b). Heights of $111 \pm 5$ Å ($n = 146$) and $45 \pm 3$ Å ($n = 50$) were measured above the mica surface for the RC-LH1 crystal and the lipid bilayer, respectively. These heights together with the section analysis in Fig. 1b indicate that the first domain (Fig. 1a, 1) consists of a single layer of RC-LH1 complexes, and that the crystal lattice is surrounded by a border of lipid (Fig. 1a, 2), as seen previously in two-dimensional crystals of the cyanobacterial Photosystem I complex (24). The height of this lipid border, $45 \pm 3$ Å, is in agreement with previous AFM measurements of the thickness of *E. coli* lipid bilayers (25).

Fig. 2a shows a high resolution AFM topograph of an RC-LH1 two-dimensional crystal, which clearly indicates that it is composed of alternating rows of complexes exposing high and prominent protrusions (bright rows), and the lower, opposite face of the complex (dark rows). The high protrusions correspond to the RC-H subunit, which is surrounded by an elliptical LH1 ring. It is known that the RC-H subunit is located on the cytoplasmic surface of the membrane in *vivo* (26); the oppositely oriented complexes, therefore, expose the periplasmic face of the RC-LH1 complex. Within the array of molecules seen in Fig. 2a, it is apparent that, in some cases, the RC-H subunit is missing, revealing a shape arising from the underlying LC-L and -M subunits inside the LH1 ring (see complexes marked 1 and 2). When images were recorded with minimal force applied to the AFM tip, *i.e.* $\leq 100$ pN, it was evident that this occurred only in a minority of the complexes. As originally demonstrated by Fotiadi et al. (24) and more recently by others (16), extrinsic or weakly bound subunits of a complex can be displaced by increasing the force applied to the AFM tip. When such nanodissections were performed on RC-LH1 crystals, the RC-H subunit could be removed routinely (data not shown). This manipulation, however, is not desirable because it may disrupt the membranes and it also increases drastically the probability of contaminating the AFM tip, thus hindering the acquisition of clear images. One important aspect of LH1 that emerges from the complexes in Fig. 2a is the intrinsic ability of LH1 to adopt either a round (broken circle, 1) or a larger, elliptical shape (broken ellipse, 2), while conforming to both the shape of the RC and the close packing of neighboring complexes. The asterisks and the empty arrowhead indicate LH1 rings without RC, seen from the cytoplasmic and periplasmic side, respectively.

Fig. 2b displays two typical RC-LH1 complexes. Careful inspection of these molecules reveals that the central, prominent elevation arising from the RC-H subunit is slightly skewed either to the left or to the right (see arrows), indicating two preferred orientations of the H subunit in the LH1 ring, which is consistent with Jamieson et al. (6). Proximity of the RC-H subunit to either side of the LH1 ring, depending on the orientation of H, obscures measurement of LH1. This point is underlined by Fig. 2c, which shows in more detail a comparison of an intact RC-LH1 complex (particle at right) with one lacking the H-subunit (particle at left). Removal of the RC-H subunit did not only reveal the RC L and M subunits (arrowhead), but, most interestingly, the second, inner ring of the LH1 complex is visualized for the first time by AFM (arrow). LH1 complexes completely lacking the RC were found, al-
though infrequently. Both sides of LH1 were imaged. Fig. 2a shows the cytoplasmic (asterisks) and periplasmic (empty arrowhead) faces of the LH1-only complex. As the comparison with the intact RC-LH1 complex on the left of Fig. 2d clearly shows, the absence of all of the RC subunits (particle at right) provides ideal conditions for imaging the LH1 complex, and two distinct rings, one for LH1α, and the other for LH1β, are seen. Another striking finding is the presence of elliptical LH1-only rings, whether or not the RC is present.

The crystalline lattice contains defects (Fig. 2a, bottom right), which imposes uneven packing constraints on the LH1 complex. Thus, in addition to the round or elliptical shapes in the crystal lattice, the RC-LH1 complex can also assume irregular, angular shapes (Fig. 2a, bottom right and Fig. 2c). The adopted shape of the central RC-LH1 complex is dictated by the surrounding complexes (denoted 1–4 in Fig. 2e), which exert uneven packing forces. This result not only reflects the intrinsic flexibility of the LH1 ring in the presence of the RC but also explains why empty LH1 complexes also distort into ellipses when packed in a two-dimensional crystal with orthogonal symmetry. Carefully inspecting the RC-LH1 ring shapes in Fig. 2, we perceive that their contours in the crystal are not perfect ellipses; individual shapes strongly depend on the orientation of the RC in the surrounding complexes, again reflecting the intrinsic flexibility of the LH1 ring. Consequently, a large diversity of possible contours emerges.

When imaging at minimal force, only a little information is gained from complexes exposing the periplasmic face (central particle in Fig. 2e). This is because this face of the complex forms grooves in the lattice, which are poorly accessible to the AFM tip. Increasing the force applied to the AFM tip to 200–300 pN improves the contrast on the periplasmic side of the complex significantly, unveiling a structure in the approximate shape of an “X” arising from the periplasmic face of the RC (Fig. 2f). This RC surface is at a lower level than the surrounding ring formed by the C-terminal regions of LH1. However, the higher force applied to the AFM stylus involves a loss in resolution on the periplasmic side of the RC-LH1 complex and the introduction of defects in the H-subunit (Fig. 2f, arrows).

The solid circle in Fig. 2a indicates a lipid region where the AFM tip can descend to touch the surface of the lipid bilayer; this is invaluable because it provides a way to measure the heights of the LH1 and RC complexes above the membrane. Thus, on the cytoplasmic side, the height from the lipid bilayer to the highest point of the protruding RC is 40 ± 3 Å (n = 48), and from the highest point on the LH1 ring to the highest point of the RC is 22 ± 2 Å (n = 48). On the periplasmic side, the distance from the surface of the LH1 complex to the membrane bilayer is 12 ± 2 Å (n = 120). Therefore, given the measured thickness of the membrane bilayer of 45 ± 3 Å (n = 50), the overall height of the RC-LH1 complex is −94 Å. All of this height information is incorporated into the representation of the RC-LH1 complex in Fig. 3, which incorporates structural and modeling information for the homologous Rb. sphaeroides complex. This model of the complex shows the RC (structure of the Rb. sphaeroides homolog; Protein Data Bank ID code 1M3X), surrounded by the LH1 complex, which was modeled on the Rb. sphaeroides LH1αβ dimer from Conroy et al. (19) and fitted to the EM projection map of the R. rubrum RC-LH1 complex in Jamieson et al. (6).

DISCUSSION

The Shape of the RC-LH1 Complex—The elliptical shape of the LH1 complex observed in the present work is consistent with the overall shape of the RC (27–29). However, adoption of this shape by the encircling LH1 ring depends on external packing constraints. When these packing forces are evenly applied, as in the tetragonal crystals of Jamieson et al. (6), or absent, as in solution (30), then they can be circular. In contrast, when packing forces are anisotropic, the complex can be squeezed so that it adopts an elliptical shape (6). The extent of ellipticity is also variable. The present AFM data are important...
in measuring these ellipses directly at room temperature. They also dramatically illustrate the point about packing constraints; there is an area of the crystal (Fig. 2a, bottom right and at higher magnification in Fig. 2e) within which lattice imperfections are induced by faulty packing of the RC-LH1 complexes. One poorly packed complex in this region clearly exhibits an angular shape where it makes contact with neighboring complexes.

The EM projection maps in Jamieson et al. (6) clearly established the capacity of the RC-LH1 complex to adopt both circular and elliptical conformations. This flexibility of the LH1 complex was suggested to be important for its function, by allowing the export of quinol formed as a result of reaction center photocatalysis (6). Subsequently, a similar ellipticity was observed in AFM topographs of the membrane-bound Rps. viridis RC-LH1 complex, only to alter to a circular shape upon removal of the RC (16). Our topographs of the R. rubrum complex differ, in that ellipses are observed whether or not the RC is present. Additionally, because we are able to image both sides of the complex, we have demonstrated that this ellipticity is conserved on periplasmic and cytoplasmic sides of the complex, regardless of the presence of the RC. However, this does not imply that there is a “correct” answer to the question of the shape of the LH1 complex, only that this shape depends on local forces exerted by neighboring complexes, which were clearly different in the orthorhombic and tetragonal two-dimensional crystals in Jamieson et al. (6) and which are probably different again in Rps. viridis membranes (16). In fact, the shape of the LH1 complex is subject to subtle distortions; this fact is revealed because AFM provides information on individual molecules, whereas EM, for example, averages out such differences. Furthermore, crystallographic techniques necessarily ignore any complexes that do not form regular lattices. Careful inspection of the complexes in Fig. 2 shows that the LH1 complex is not perfectly elliptical but instead is more “D” shaped. Thus, the flexibility of LH1 is sufficiently pronounced to allow it to conform very closely to the real shape of the RC, which is also not perfectly elliptical in projection. Another aspect of the pliability of the LH1 complex has emerged from a closer examination of the projection structure of the RC-LH1 complex (6); variable α-β and αβ-αβ spacings are found within the LH1 ring (not shown).

The model in Fig. 3 presents the LH1 complex as a perfect ellipse for convenience, although we have already noted that this is one of many shape variations for this complex. The panels in Fig. 3 a–c depict space-filling representations of the model of the RC-LH1 complex to show how it relates to the AFM topographs in Fig. 2. Thus, the complete complex in Fig. 3a (cf. Fig. 2b) is accompanied by the complex lacking the RC-H subunit (Fig. 3b; see Fig. 2c for the AFM topograph), and the LH1 ring with no RC enclosed (Fig. 3c; see also Fig. 2d).

The Heights of Extrinsic Regions of the RC-LH1 Complex in Relation to the Membrane Bilayer—The relative heights of the RC and LH1 complexes and the positions in the membrane were obtained from the AFM data. These heights can only be obtained directly by AFM, and the precision of this measurement allows several structural and functional aspects of the RC-LH1 complex to be examined. The AFM data, obtained for the first time for both periplasmic and cytoplasmic faces of the RC-LH1 complex, show that the highest part of the AFM topograph can be unequivocally assigned to the RC-H subunit, which protrudes from the membrane by 40 ± 3 Å and which is apparently easily removed by the AFM tip during scanning. Once fixed, this parameter allows the positions of aromatic residues of the RC to be examined, because the vertical distance between these residues and the extremity of the RC-H subunit is known from the Rb. sphaeroides RC structure, which is highly homologous to the RC of R. rubrum (31). The vertical positions of these residues are of interest because they have been proposed to lie at the interface between hydrophobic and head group regions of a membrane bilayer, acting as membrane “anchors”. Specifically, it has been proposed that in the bacterial RC, Trp residues will be found at the membrane interface (32), and our AFM data allow this proposition to be examined. The results of this analysis were inconclusive and demonstrated an interfacial location for only a few Trp and other aromatic residues. In contrast, this proposed anchoring role for aromatic residues is more clearly seen for LH1 (see below).

The LH1 complex of R. rubrum completely encloses the RC, and on the cytoplasmic side, it protrudes from the membrane surface by 19 ± 2 Å (n = 72). There have been predictions of substantial hydrophilic N-terminal domains for both the α and β polypeptides of LH1, based upon sequence alignments (33), and AFM measurements of LH2 complexes have shown protrusions of up to 15 Å (14, 15). The absence of the RC-H subunit from some complexes, and even of the whole RC complex from others, afforded the opportunity of comparing the heights of the LH1 complex under these different conditions, but no height differences were detected. The AFM topographs in Fig. 2 are of sufficient quality to allow two rings within LH1 to be distinguished in the absence of the RC-H subunit, particularly so in the absence of the whole RC complex. No differences in height, with respect to the membrane, were noted for the inner and outer rings of LH1. On the periplasmic side, LH1 protrudes ~12 Å from the membrane surface, and it also protrudes slightly from the periplasmic surface of the RC. This cannot be quantified, because relatively high forces are applied to the AFM tip to obtain images such as that displayed in Fig. 2f, but qualitatively speaking, it means that the LH1 complex forms a slightly raised rim around the RC. Our measurements can be compared with those of Scheuring et al. (16), who examined the periplasmic side of membranes from Rps. viridis, and found that the LH1 complex protruded from the membrane by ~10 Å.

The model displayed in Fig. 3d relates the heights of the LH1 and RC complexes in relation to the membrane normal, according to the AFM data. We note two belts of aromatic residues for the LH1 complex, which coincide with the position of the hydrophobic/hydrophilic head group membrane interface. These aromatic regions are more clearly seen for the LH1 complex than for the RC, possibly because the RC is not required to anchor itself in the membrane bilayer because it is surrounded by LH1. Perhaps the LH1 complex provides a stabilizing “lifebelt” around the RC. One exception to this possibility might be in the earliest stages of assembly of the photosynthetic unit before the LH1 ring has fully formed around the RC (34). The surface color coding of the LH1 ring (Fig. 3d) shows that polar, extrinsic regions of this complex, indicated by the positive belt on both the cytoplasmic and periplasmic sides, are found in the lipid head group region of the membrane bilayer. Specifically, the positively charged residues are in a position to interact with the lipid phosphate groups.

The Heights of the Protein and Bacteriochlorophyll Components of the RC-LH1 Complex in Relation to the Membrane Bilayer—The features of the RC-LH1 complex revealed by this AFM study also provide information on the positions of BCHl cofactors in relation to the membrane. The distance along a vertical plane from the cytoplasmic extremity of the RC-H subunit to the special pair BCHls of the RC is known. Thus, it can be demonstrated directly that the special pair BCHls are located in the hydrophobic core of the membrane (see Fig. 3e). In contrast, the Fe atom on the cytoplasmic side of the complex lies just outside the head group region of the membrane. Re-
cently, the RC was crystallized by using the cubic lipidic-phase method (35), and the positions of the membrane bilayer deduced in this work are in approximate agreement with our direct measurements using AFM. As a consequence of using AFM to measure the positions of the RC and LH1 complexes along the membrane normal, the ring of LH1 BChls falls into alignment with the special pair BChls of the RC (Fig. 3e), in such a way that excitation energy could transfer along a horizontal plane from LH1 to the RC special pair BChls, as originally suggested (36).

REFERENCES

1. Förster, T. (1965) in Modern Quantum Chemistry (Sinanoglu, O., ed) Vol. IIIB, pp. 93–137, Academic Press, New York.
2. Sundström, V., Pullerits, T., and van Grondelle, R. (1999) J. Phys. Chem. B 103, 2327–2346.
3. Visscher, K. J., Bergström, H., Sundström, V., Hunter, C. N., and van Grondelle, R. (1989) Photosynth. Res. 22, 211–217.
4. Beckman, L. M., van Mourik, F., Jones, M. R., Visser, H. M., Hunter, C. N., and van Grondelle, R. (1994) Biochemistry 33, 3143–3147.
5. Karrrsch, S., Bullough, P. A., and Ghosh, R. (1995) EMBO J. 14, 631–638.
6. Jamieson, S. J., Wang, P., Qian, P., Kirkland, J. Y., Conroy, M. J., Hunter, C. N., and Bullough, P. A. (2002) EMBO J. 21, 3927–3935.
7. Monshouwer, R., Ortiz de Zarate, O., van Mourik, F., and van Grondelle, R. (1995) Chem. Phys. Lett. 246, 341–346.
8. Freer, A., Prince, S., Sauer, K., Papiz, M., Hawthornthwaite-Lawless, A., McDermott, G., Cogdell, R., and Isaacs, N. W. (1996) Structure 4, 449–462.
9. Barz, W. P., Vermeglio, A., Francia, F., Venturaoli, G., Melandri, B. A., and Oesterhelt, D. (1995) Biochemistry 34, 15248–15258.
10. Barz, W. P., Francia, F., Venturaoli, G., Melandri, B. A., Vermeglio, A., and Oesterhelt, D. (1995) Biochemistry 34, 15235–15247.
11. Liburn, T. G., Haith, C. E., Prince, R., and Beatty, J. T. (1992) Biochim. Biophys. Acta 1100, 160–170.
12. Binnig, G., Quate, C. F., and Gerber, C. (1986) Phys. Rev. Lett. 56, 930–933.
13. Engel, A., and Müller, D. J. (2000) Nat. Struct. Biol. 7, 715–718.
14. Scheuring, S., Reiss-Husson, F., Engel, A., Rigaud, J. L., and Ranck, J. L. (2001) EMBO J. 20, 3029–3035.
15. Scheuring, S., Seguin, J., Marco, S., Levy, D., Breyton, C., Robert, B., and Rigaud, J. L. (2003) J. Mol. Biol. 325, 569–580.
16. Scheuring, S., Seguin, J., Marco, S., Levy, D., Robert, B., and Rigaud, J. L. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1690–1693.
17. Schabert, F. A., Henn, C., and Engel, A. (1995) Science 268, 92–94.
18. de Plaenque, M. R. R., Bonev, B. B., Demmers, J. A. A., Greathouse, D. V., Koeppe, R. E., Separovic, F., Watts, A., and Killian, J. A. (2003) Biochemistry 42, 5341–5348.
19. Conroy, M. J., Westerhuis, W. H., Parkes-Loach, P. S., Loach, P. A., Hunter, C. N., and Williamson, M. P. (2000) J. Mol. Biol. 298, 83–94.
20. Qian, P., Addlessee, H. A., Ruban, A. V., Wang, P., Bullough, P. A., and Hunter, C. N. (2000) J. Biol. Chem. 275, 23678–23685.
21. Jap, B. K., Zulauf, M., Scheybani, T., Heifi, A., Baumeister, W., Asbi, U., and Engel, A. (1992) Ultramicroscopy 46, 45–84.
22. Olsen, J. D., Sorkalingum, G. D., Robert, B., and Hunter, C. N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7124–7128.
23. Olsen, J. D., Sturigis, J. N., Westerhuis, W. H., Fowler, G. J. S., Hunter, C. N., and Robert, B. (1997) Biochemistry 36, 12625–12632.
24. Pasiadis, D., Muller, D. J., Tsitsiotis, G., Hasler, L., Tittmann, P., Mini, T., Jeni, P., Gross, H., and Engel, A. (1998) J. Mol. Biol. 283, 83–94.
25. Pasiadis, D., Hasler, L., Muller, D. J., Stahlberg, H., Kistler, J., and Engel, A. (2000) J. Mol. Biol. 300, 779–789.
26. Hall, R. L., Doorley, P. F., and Niederman, R. A. (1978) Photochem. Photobiol. 28, 273–276.
27. Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., and Rees, D. C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6162–6166.
28. Arnaux, B., Durruex, A., Reiss-Husson, F., Lutz, M., Norris, J., Schiffer, M., and Chang, C. H. (1989) FEBS Lett. 258, 47–50.
29. Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985) Nature 318, 618–624.
30. Gerken, U., Lupo, D., Tietz, C., Wrachtrup, J., and Ghosh, R. (2003) Biochemistry 42, 10354–10360.
31. Yeates, T. O., Komiya, H., Chirino, A., Rees, D. C., Allen, J. P., and Feher, G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7993–7997.
32. Schiffer, M., Chang, C. H., and Stevens, F. J. (1992) Protein Eng. 5, 213–214.
33. Brunisholz, R. A., Wiemken, V., Suter, F., Bachofen, R., and Zuber, H. (1984) Hoppe-Seyler’s Z. Physiol. Chem. 365, 689–701.
34. Pugh, R. J., McGlynn, P., Jones, M. R., and Hunter, C. N. (1998) Biochim. Biophys. Acta 1406, 301–316.
35. Katena, G., Andreasson, U., Landau, E. M., Andreasson, L. E., and Neutze, R. (2003) J. Mol. Biol. 331, 681–692.
36. Hunter, C. N., van Grondelle, R., and Olsen, J. D. (1989) Trends. Biochem. Sci. 14, 72–76.