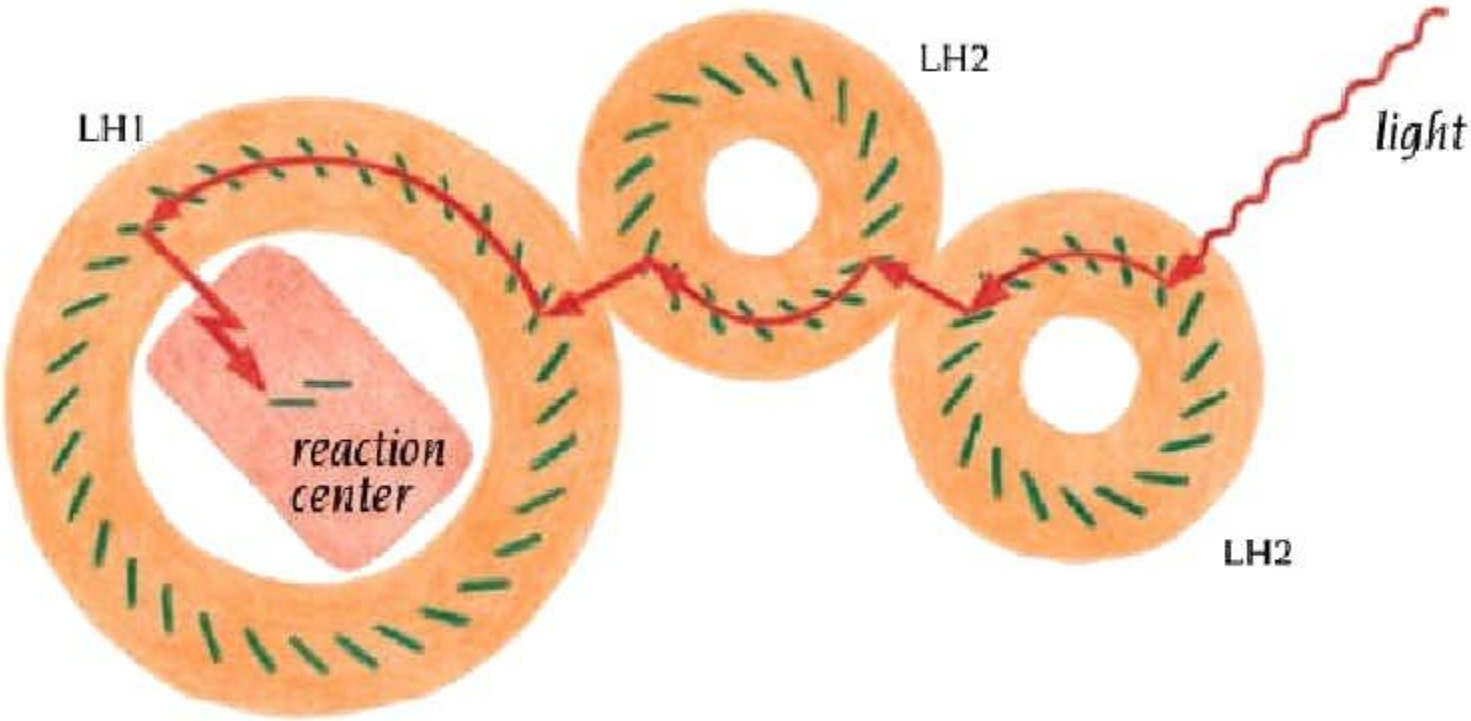
Background

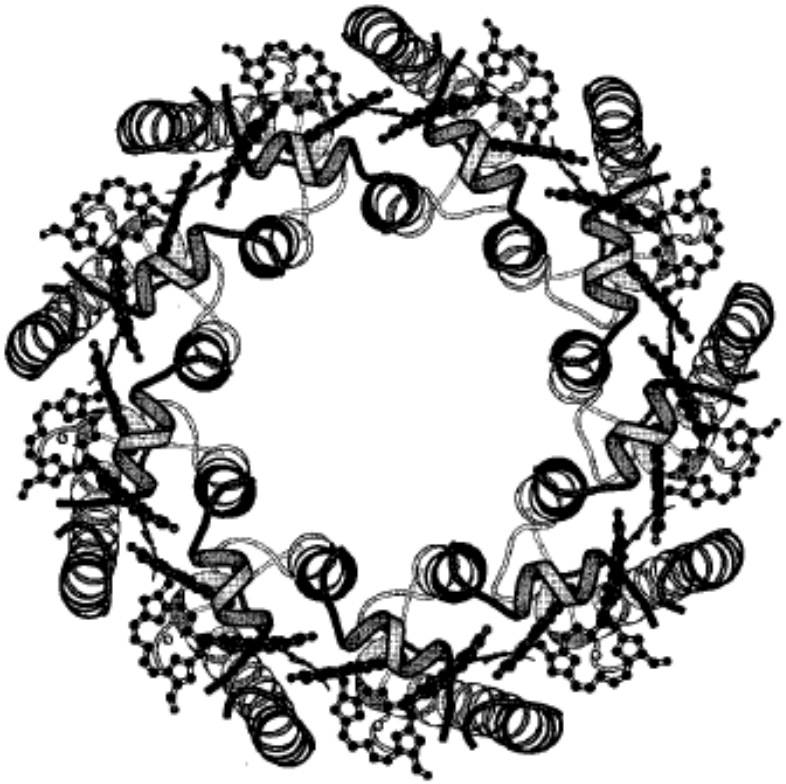
- Light harvesting complexes exist to facilitate and maximize the absorption capacity of the reaction centers (RC) as well as PSI and PSII
- Purple bacteria utilize these functions by having an LH1 complex serving as a primary complex and a peripheral LH2 and LH3 complex
  - As such, LH1 and RC are in stoichiometric equivalence and LH2 and LH3 are expressed as needed for the circumstances
  - LH1, LH2 and LH3 absorb photons shuttle excitons to the RC

It’s really this simple….



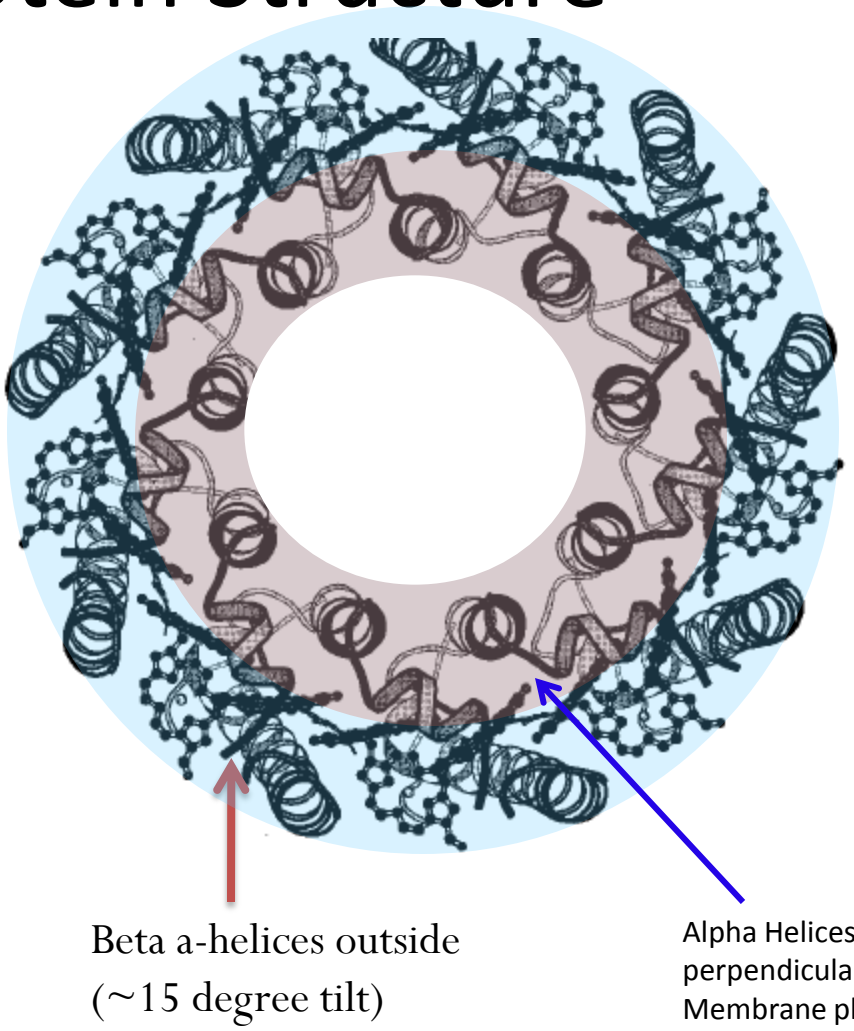
Apoprotein Structure in the LH2 Complex from Rhodopseudomonas acidophila Strain 10050: Modular Assembly and Protein Pigment Interactions

S.M. Prince, M.Z. Papiz, A.A. Freer, G. Mcdermott, A.M. Hawthorethwaite-Lawless, R.J. Cogdell and N.W. Isaacs



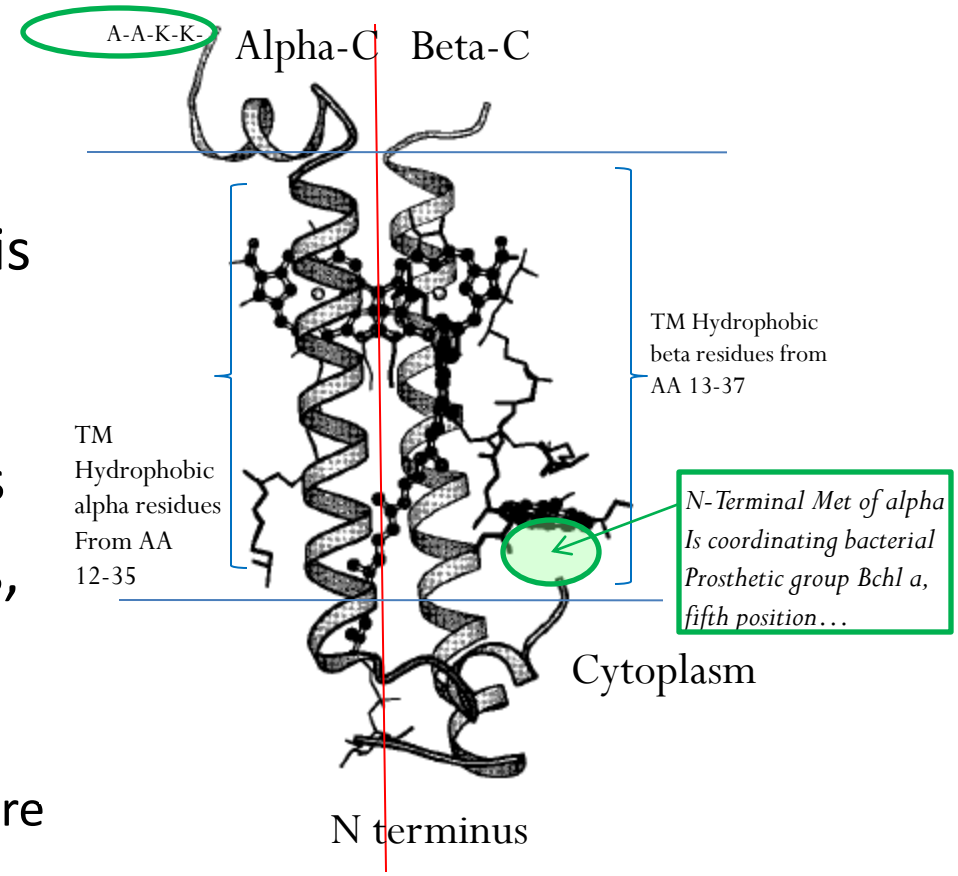
Apoprotein Structure

- LH2 is a nonomeric symmetrical assembly of alpha-beta apoproteins, with alpha helical secondary structure, noncovalently binding prosthetic groups
- Oligomerize into concentric circles: alpha on the inside, perpendicular to the membrane normal, and beta on the outside, tilted roughly 15 degrees to the alpha plane



LH2 Molecule

- The alpha-beta subunits of LH2 is composed of two TM alpha helices, noncovalently bound, each with unique characteristics
- Beta apoprotein has 41 residues, all shown, while apha has 53 residues, 49 of which are shown
  - Remaining alpha residues shown are on C-terminus and are –K-K-A-A

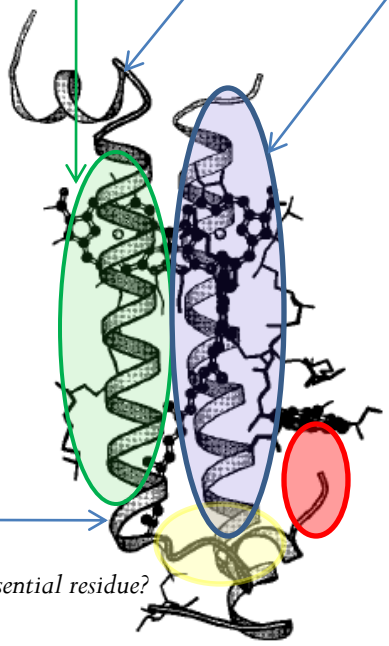


LH2's 2' structure reflected in amino acids

- One can look at the amino acid sequnces to deduce at least some level of structural detail
- What amino acids would you expect in the kinks of the beta helix?
- What kinds of amino acids should coordinate pigments?

Table 1. Secondary structure of apoproteins

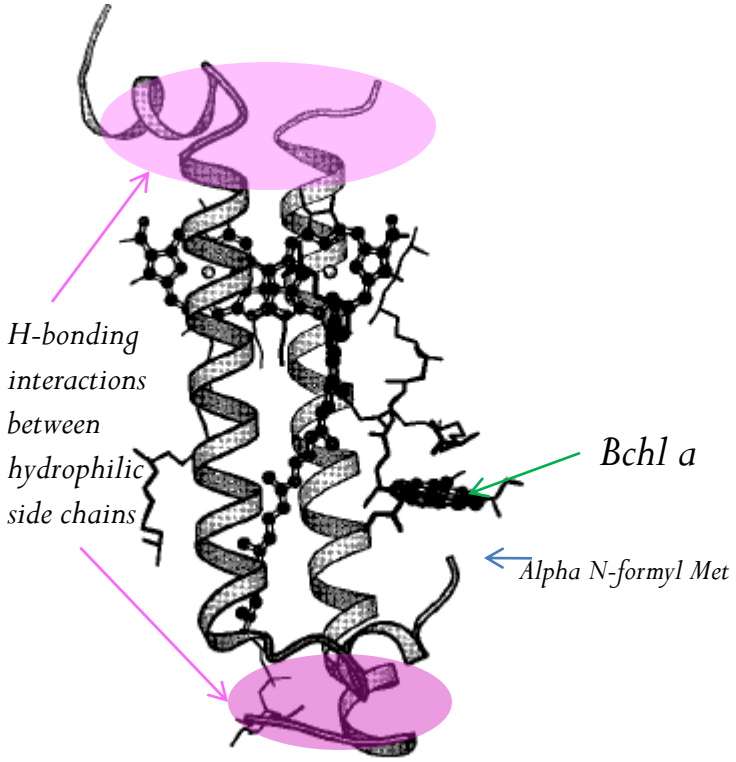
Residues	Secondary structure assignment	Sequence
α1-α3	–	MNQ
α4-α8	3_{10} Helix	GKIWT
α9-α11	–	VVN
α12-α36	α Helix	PAIGIPA LLGSVLV IAILVHL AILS
α37-α39	–	HTT
α40-α46	α Helix	WFPAYWQ
α47-α49	–	GGV
α50-α53	[Disordered]	KKAA
β1-β4	–	ATLT
β5-β36	α Helix	AEQSEEL HKYVIDG TRVFLGL ALVAHFL AFSA
β37-β41	–	TPWLH



This is roughly where alpha-12 Starts. Note the proline, is this an essential residue?

Tertiary structure

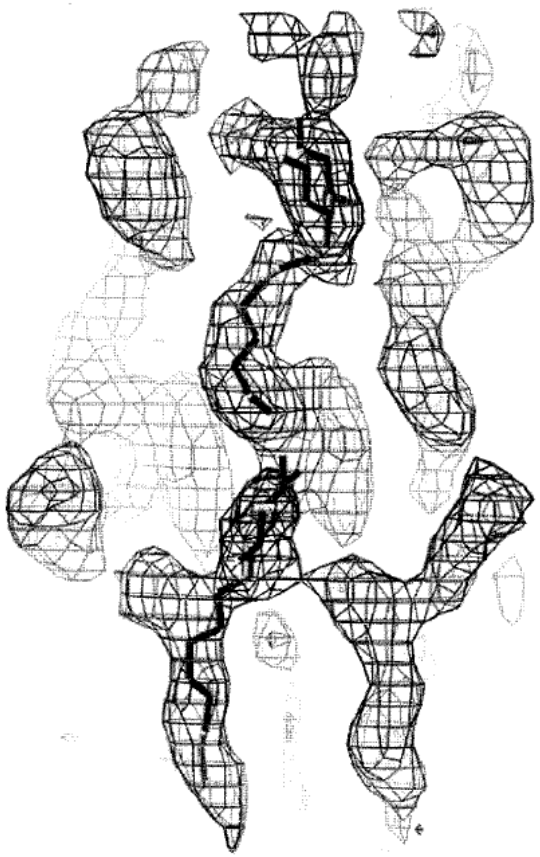
- Interestingly, the N and C termini of the helices are exposed to the aqueous environment and must H-bond the hydrophilic side residues of the interior
- With regards to the alpha helix, it's turns coordinate oligomerization and pigment binding

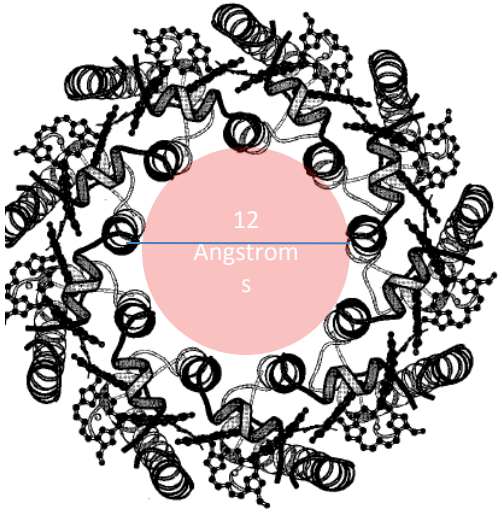


Membrane Interaction

- The LH2 complex forms a ring, and as such gives way to a 12 A cylindrical enclosure
- Lipids are inside, but a single alpha helix could have fit… Why not?
  - Hydrophobic interactions between alpha helices and core
- The outside is made of largely hydrophobic beta apoproteins, which lack protruding side chain anchors
  - Lots of cofactors are present, which dominate over lipid-protein interaction

LH2's Hole…



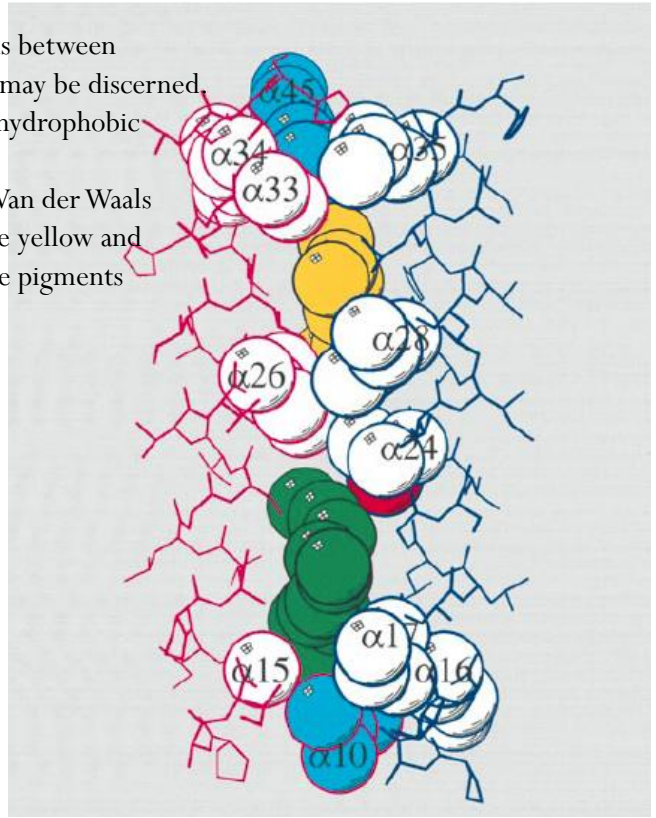


Protein-Protein Interactions

- Pigment distribution important for protein-protein as well as protein-pigments interactions
- H-bonding interactions observed at cytosolic and periplasmic faces between proteins and pigments
- Alpha-Y-Bchl a does double duty with oligomer contact and Bchl a coordination
- Beta transmembrane helices don't touch: pigments form lining bewteen them
- Alpha helices do form contacts of hydrophobic nature

Here, interactions between two alpha chains may be discerned. They are mostly hydrophobic

Spheres indicate Van der Waals Interactions while yellow and Green spheres are pigments



Pigment Coordination

- Bchl a molecules posses two main interactions with protein
- Coordination of Mg++ ion, and an H-bond to C-3 acytl group on ring a
- Protein donor residue for Mg++: B850 is His residue and for B800 is alpha Met-1
- For C-3 acetyl: B850 is alpha-W45/Y44 and B800 is beta-R20

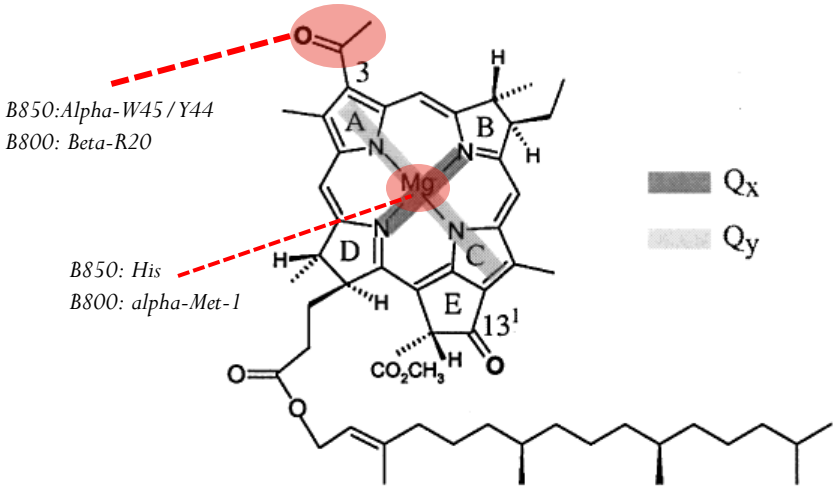


Table 3. Apoprotein contacts to the αB850 bacteriochlorin

αB850 atom	Residue	Atom	Distance (Å)
Mg	His α31	NE2	2.34
$O3^1$	Trp α45	NE1	2.97
C5	Phe α41	CZ	3.73
$C3^2$	Val (−)α30	CG1	4.36
$C7^1$	Tyr α44	CE2	4.00
$C8^1$	Trp α40	CH2	3.73
$C8^8$	Ile α34	CD1	3.98
$C12^1$	Ala β33	CB	4.14
C13	Ala β29	CB	3.43
$O13^1$	His β30	CE1	3.19
	Ala β26	CA	3.43
$C13^4$	Leu β25	CB	3.56
$O17^3$	Ala α27	CB	3.35
OP	Phe β22	CE1	3.34

Pigment Contacts cntd.

Table 6. Apoprotein contacts to the rhodopin glucoside chromophore

Rhodopin glucoside atom	Residue	Atom	Distance (Å)
C4	Ile (−)α6	CA	3.81
	Lys (−)α5	O	3.78
	Val (−)α9	CG2	3.70
CM3	Tyr β14	CD2	3.43
C6	Leu β11	CD2	4.10
CM4	Gln (−)α3	OE1	3.54
C12	Val β15	O	3.87
C13	Leu α20	CD2	3.82
CM3	Gly β18	O	3.66
C14	Thr β19	OG1	3.37
C15	Phe β22	CB	4.26
C18	Val α23	CG2	3.64
C25	Ile α26	CG2	3.73
CM8	Ala (+)α27	CB	4.06
	Ile (+)α28	CG1	4.02
C27	His (+)α31	NE2	4.11

Table 7. Closest approach of bacteriochlorins and carotenoids

Pigment atom	Pigment atom	Distance (Å)
αB850 $C13^2$	βB850 $O13^1$	3.36
αB850 $O17^3$	(−)Rhodopin CM8	3.59
αB850 $C2^1$	(−)βB850 C20	3.57
βB850 OP	Rhodopin C19	3.42
B800 $O17^3$	Rhodopin CM6	3.42

Table 4. Apoprotein contacts to the βB850 bacteriochlorin

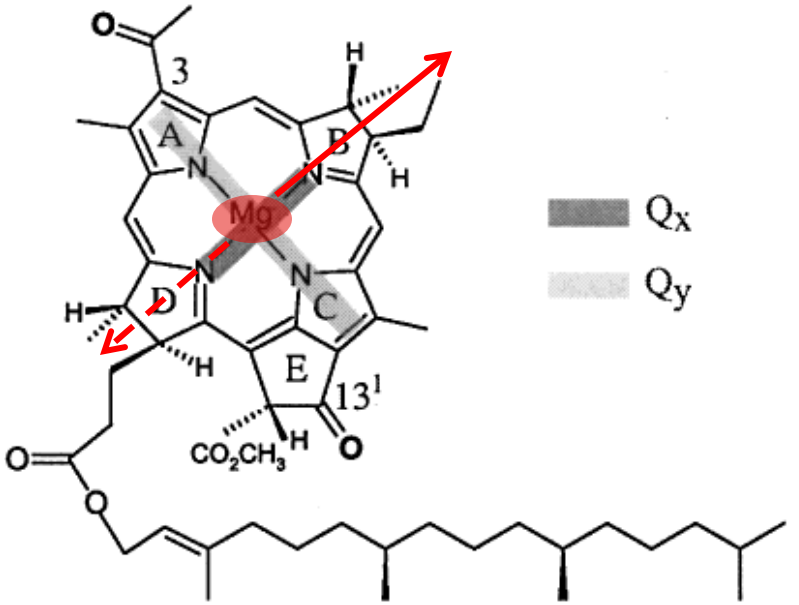
βB850 atom	Residue	Atom	Distance (Å)
Mg	His β30	NE2	2.34
$O3^1$	Tyr (+)α44	OH	2.64
C10	Ile α34	CD1	3.63
C7	Trp β39	CE2	3.43
$C7^1$	Trp (+)α45	CD1	3.54
$C8^2$	Trp α40	CH2	3.98
	Thr β37	CG2	4.09
	Ala β33	CB	3.88
C12	Val α30	CG1	3.64
$C12^1$	His α31	CE1	3.41
$O13^1$	Ala α27	CB	3.51
$C13^4$	Val α23	O	3.72
	Ile α26	CG2	4.12
$O17^3$	Leu β23	CA	3.86
	Ala β26	CB	3.28
OP	Phe β22	CD2	3.48

Table 5. Apoprotein contacts to the B800 bacteriochlorin

B800 atom	Residue	Atom	Distance (Å)
Mg	fMet α1	OF	2.46
$O3^1$	Arg β20	NH2	2.91
C1	Ile β16	CD1	3.68
C10	Gln α3	NE2	3.83
$C2^1$	Thr β19	CG2	3.63
$C7^1$	Asn α2	OD1	3.47
$C12^1$	Gly (+)β18	CA	3.65
	Val (+)β21	CB	4.08
$O13^4$	Leu (+)α20	CD1	3.91

Coordination Effects

- Because Bchl a has two transition dipoles, Qx and Qy, two absorption spectra result
- The conjugated pie system is susceptible to distortion, and ultimately changes in wavelength absorption which accompany this
- Thus, the local protein environment determines the absorptive properties of the pigments
  - Generally arise from axial ligand and peripheral contact coordination



Mg++ is penta-coordinated. Which side this coordination Is on depends on the protein environment. If on one side of the plane, then the B800 species results. The other side produces B850

Coordination

- The alpha-B800 species is slightly domed while the alpha-B850 species is roughly planar

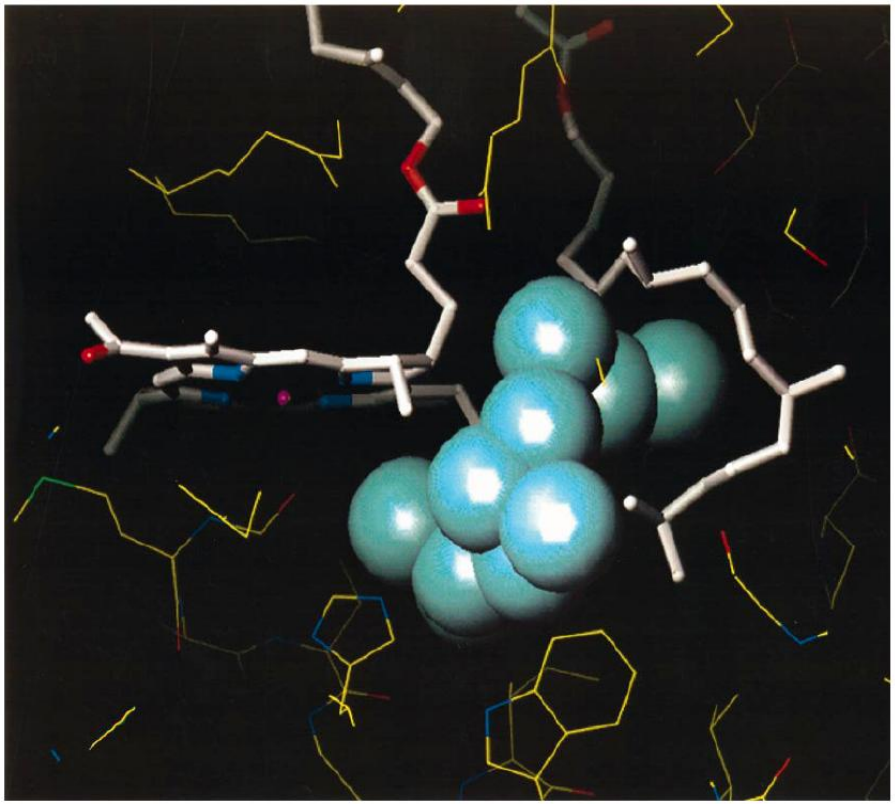


Figure 6. The grey van der Waals spheres show the likely repositioning of the αB850 phytyl chain. The B800 Bchl *a* and the phytyl chain of the αB850 are highlighted as thick bonds, the remaining protein is represented in thinner bonds (O; Jones *et al.*, 1991).

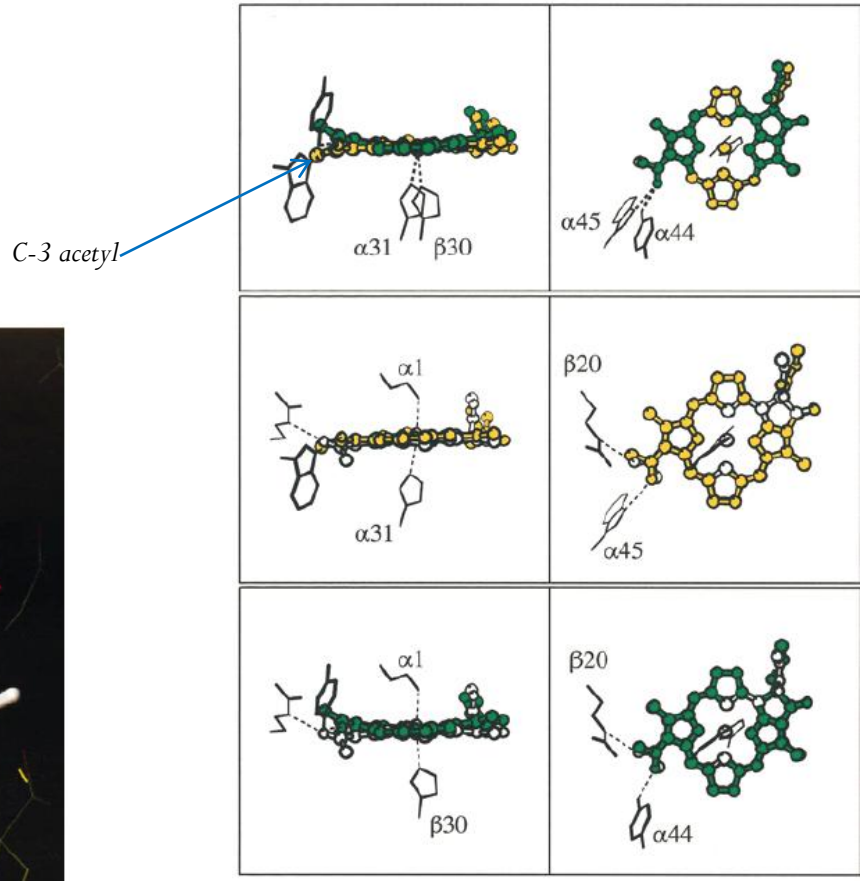


Figure 4. Overlay of PC bacteriochlorophylls and their contacts: Plan and elevation views. Overlays were generated by superimposing the core nitrogen atoms plus a carbon atom to define chirality (rms deviations were of the order 0.03 Å, program LSQKAB (CCP4, 1994)). αB850, yellow; βB850, green; B800, white; for clarity, peripheral groups on rings B and D have been removed, along with the phytyl chains (MOLSCRIPT; Kraulis, 1991).

Table 8. Refinement of LH2

	Constrained (1 × PC)	Restrained (3 × PC)
Resolution limits (Å)	12.0–2.5	12.0–2.5
No. independent non-Hydrogen atoms[a]	988	3078
No. reflections $F > 2\sigma(F)$	27,855	27,855
R_{cryst} (%)	22.73	20.98
R_{free} (%)	25.32	24.65
Rms deviation from target geometry	0.019 Å; 2.129°	0.019 Å; 2.043°
Mean *B*-factor (Å^2)	34.52	38.97
Rms deviation on PC superimposition (Å)	–	0.060 (PC1-PC2), 0.053 (PC1-PC3)
Coordinate precision (Å)[b]	0.07	0.10
Estimated rms accuracy (Å)[c]	0.40	0.38

Unit cell: $a = b = 120.3$, $c = 296.2$ Å (hexagonal index). Spacegroup *R*32.

Anisotropic scaling correction (Å^2) applied to F_{obs}: $U_{11} = -0.0550$, $U_{22} = -0.0550$, $U_{33} = 0.1099$, $u_{12} = -0.1718$, $U_{13} = 0.0000$, $U_{23} = 0.0000$.

[a] In the initial report (McDermott *et al.*, 1995) hydrogen atoms used by X-PLOR were included in the number of independent atoms reported.

[b] Calculated with the expression for coordinate precision of atoms with low *B*-factors by D. W. J. Cruickshank (Dodson *et al.*, 1996). The geometrical and non-crystallographic symmetry restraints were included as observed parameters in the calculation. The high non-protein volume of the LH2 unit cell (73%) leads to an artificially low value for this figure.

[c] From the CCP4 (1994) program SIGMAA (Read, 1986).

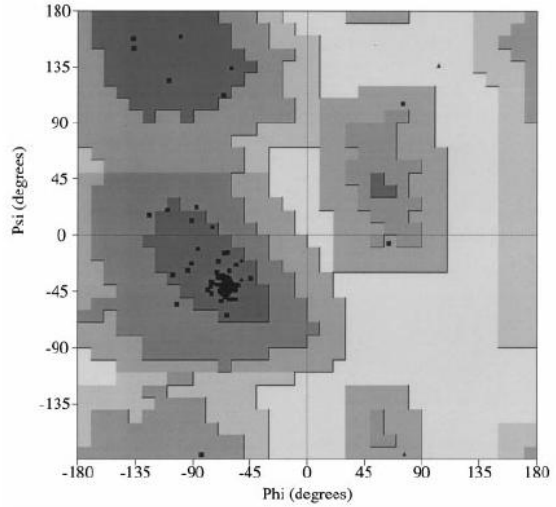
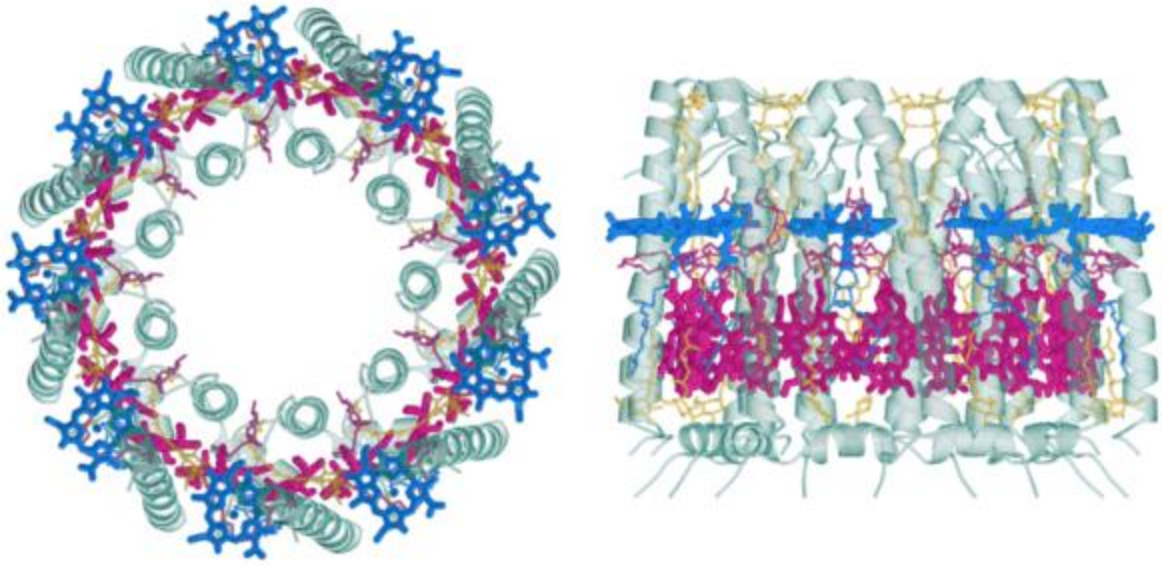


Figure 5. A Ramachandran (Ramachandran & Sasisekharan 1968) plot of the PC apoproteins (PROCHECK; Laskowski *et al.*, 1993).

The Crystallographic Structure of the B800-820 LH3 Light-Harvesting Complex from the Purple Bacteria Rhodopseudomonas Acidophila

K. McLuskey, S.M. Prince, R.J. Cogdell, and N.W. Isaacs

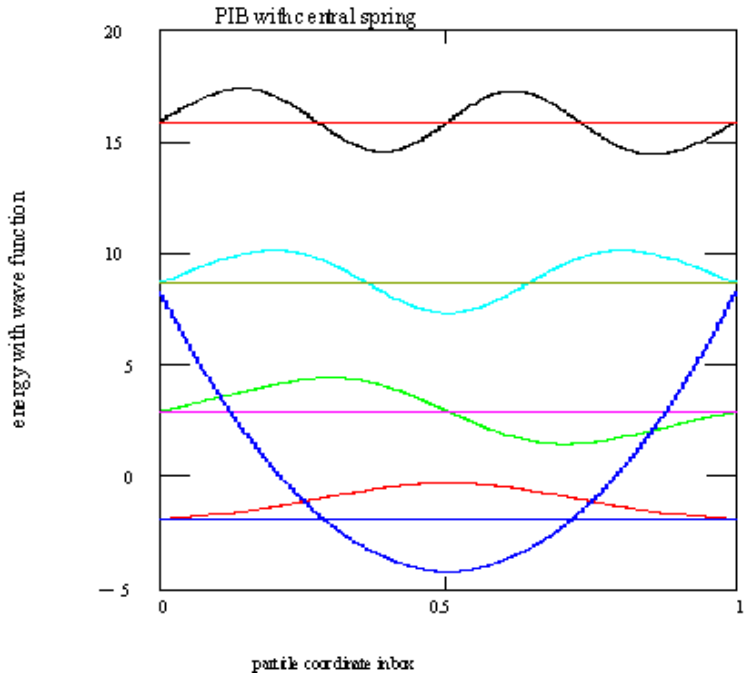


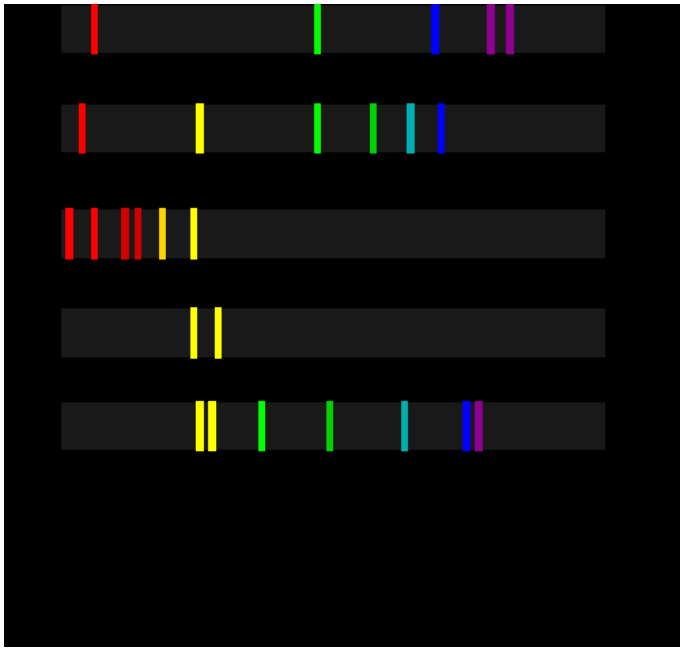
Background

- LH3 is a lot like LH2 in structure, with a few minor differences (a few AA's)
- It is from a different gene than LH2…Why? It seems alternative splicing could do this job quite well, as well as other post transcriptional modifications…
- It's expressed in stressful conditions (low illumination/temperature) when different wavelength light needs to be absorbed and maximized
  - It follows these different wavelengths of light can be attributed to different structures. Thus, the crystal structure can help explain functional differences
  - The differences arise in primary structure, while secondary structure is more or less the same
    - Thought to be differences in H-bonding patterns on C-3 acetyl position of Bchl a

Physics

- To change the wavelength of light absorbed, the quanta of the atom(s), or electronic structure, must change in energy
- What is being changed in the structure ultimately results from intermolecular bonding, as pigment-protein interactions are noncovalent
- It follows that these differing noncovalent interactions result in the change of the atomic quanta, allowing different wavelength light to be absorbed





Absorptive properties

- Bchl a is asymmetric and has two transition dipoles on a pie conjugated plane
- Qy is lower energy (higher wavelength) and is influenced by the local environment
- Because Bchl a is used in multiple harvesting complexes, and in each one has a range of spectra, It follows that it's coordination determines its absorptive properties
- LH3 absorbs at 800 and 820 nm

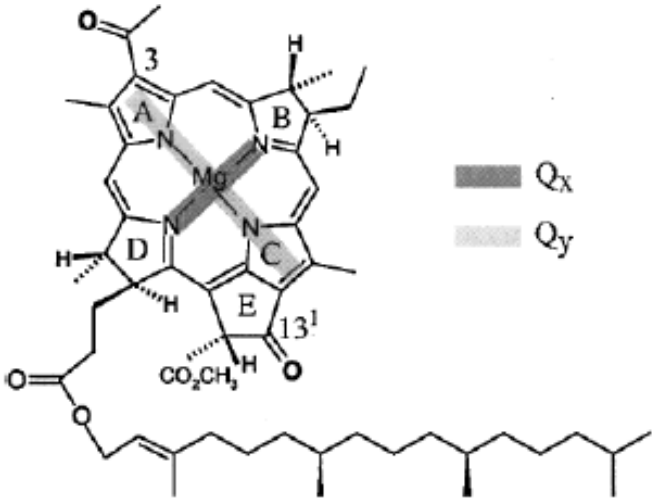


FIGURE 1: Bacteriochlorophyll *a* showing the directions of the Q_x and Q_y dipoles of the bacteriochlorin ring and the numbering of groups referred to in the text.

Structural Differences in LH3

- As mentioned, the differences in LH3 (when compared to LH2) arise in primary structural differences
- The most notable differences are in Bchl a C-3 acetyl group binding proteins on alpha-W44/Y45, which have been changed to F and L respectively
  - Mutagenesis confirmed this, as an 850->820 shift was seen after F/L replacement

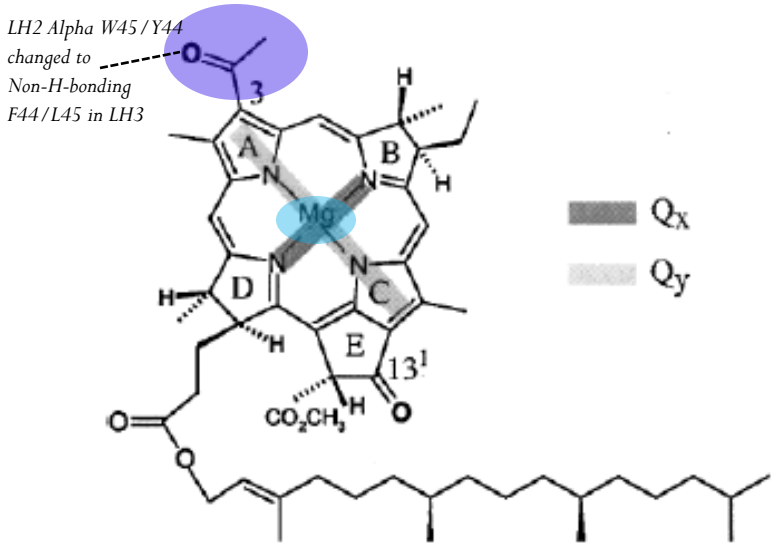


FIGURE 1: Bacteriochlorophyll *a* showing the directions of the Q_x and Q_y dipoles of the bacteriochlorin ring and the numbering of groups referred to in the text.

Structure determination of LH3

- During crystallization, hard to separate LH2 and LH3
  - Beta-Glu-7 and Beta-K-7 are involved in crystal contacts and lack NCS contraint. This results in abnormally high B-factor
  - Overall B factor less than we would prefer

Table 1: Data Collection and Structure Determination[a]

Native 1	
spacegroup	*R*32
(hexagonal) unit cell at 100 K	$a = 116.76$, $c = 294.61$ Å
resolution interval	12.5−3.30 Å
R_{merge} multiplicity and completeness	6.9 (12.1)%, 3.3 (2.7), 86.8 (77.4)%
molecular replacement resolution interval	12.0−4.0 Å
solution correlation and R^*_{cryst}	55.2 (39.3)%, 42.4 (48.8)%
Native 2	
(hexagonal) unit cell at 100 K	$a = 117.26$, $c = 295.92$ Å
resolution interval	42.0−3.0 Å
R_{merge} multiplicity and completeness	8.5 (37.0)%, 3.7 (2.9), 97.0 (96.4)%

[a] Items in parentheses denote values for the outer resolution shell, 3.39−3.31 Å, and 3.04−2.95 Å for natives 1 and 2, respectively. Except * where the bracketed terms are the figures for the first incorrect solution.

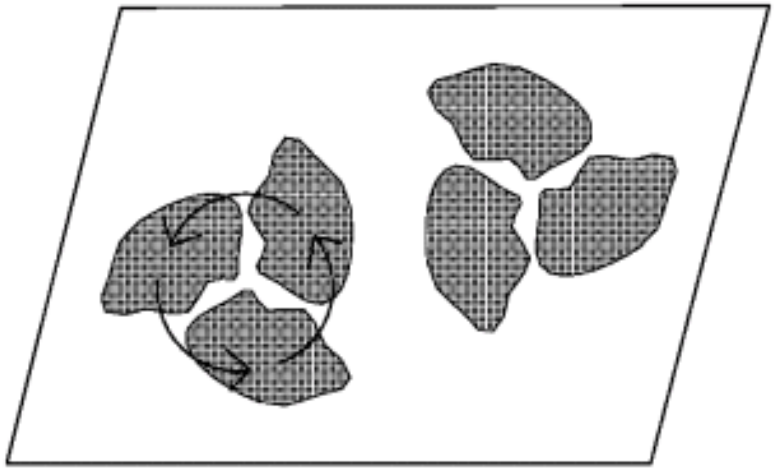
Table 2: Refinement[a] of LH3 at 3.0 Å

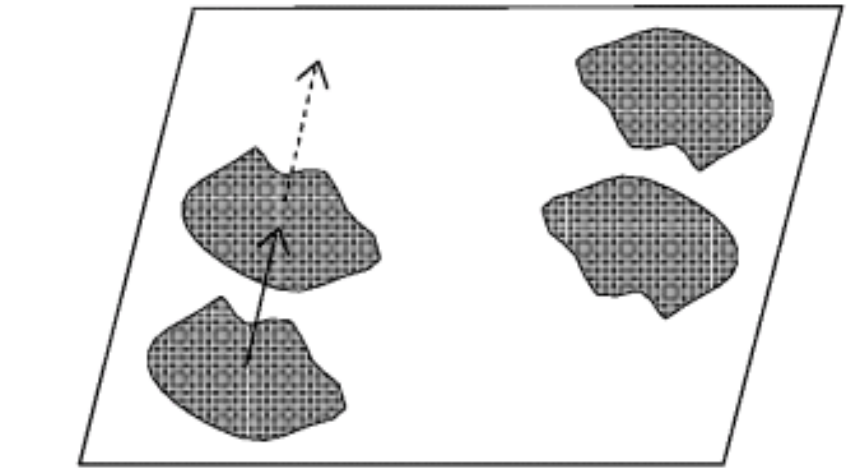
resolution interval	42.0−3.0 Å
no. parameters	3772
no. reflections ($F > 2\sigma(F)$)	15500
rms deviations from target geometry[b]	0.013, 0.021 Å
R_{cryst}, R_{free} (4.8% reflections)[c,d]	24.3, 25.5%
average B-factor	47.02 Å^2
Sigmaa (*45*) estimated coordinate error	0.39 Å

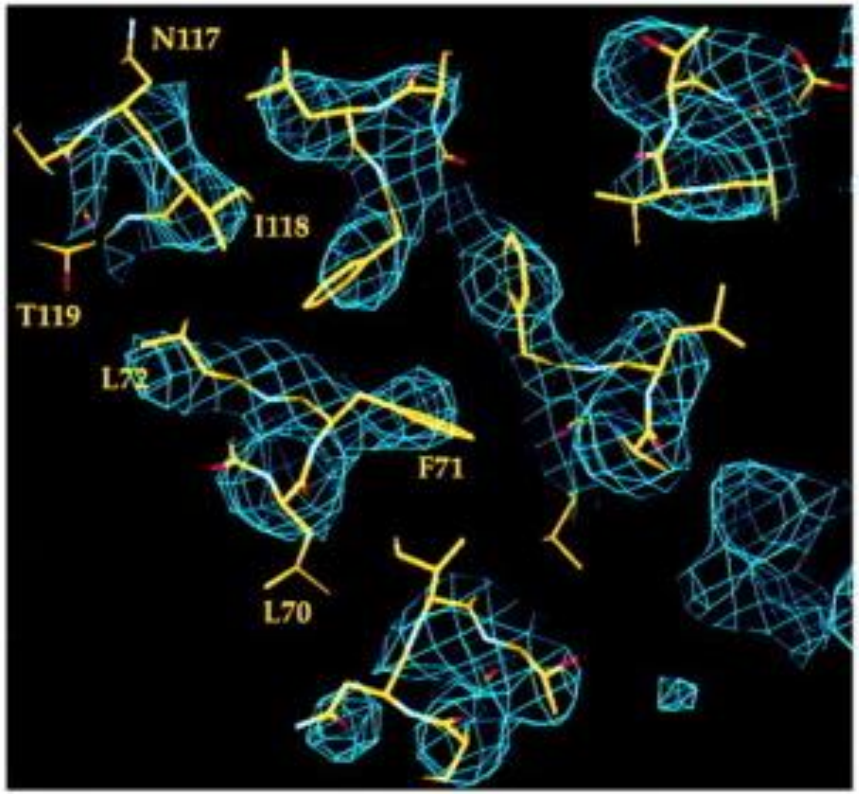
[a] NCS constrained. [b] Bonded and nonbonded restrained distances. [c] Bulk solvent correction parameters S_{B1} and S_{B2} were refined specifying the correction $f' = f - S_{B1}\exp[-1/2 \cdot S_{B2} \cdot q^2]$ where $q = 2\pi \sin(\theta)/\lambda$. [d] Anisotropic scaling (not applied) over all reflections gives R_{cryst} = 23.7%: $k \exp[b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + 2(b_{12}hk + b_{13}hl + b_{23}kl)]$ where $k = 0.9224$ and $b_{11} = b_{22} = -8.6 \times 10^{-5}$, $b_{33} = -3 \times 10^{-6}$, $b_{12} = -4.3 \times 10^{-5}$, and $b_{13} = b_{23} = 0.0$.

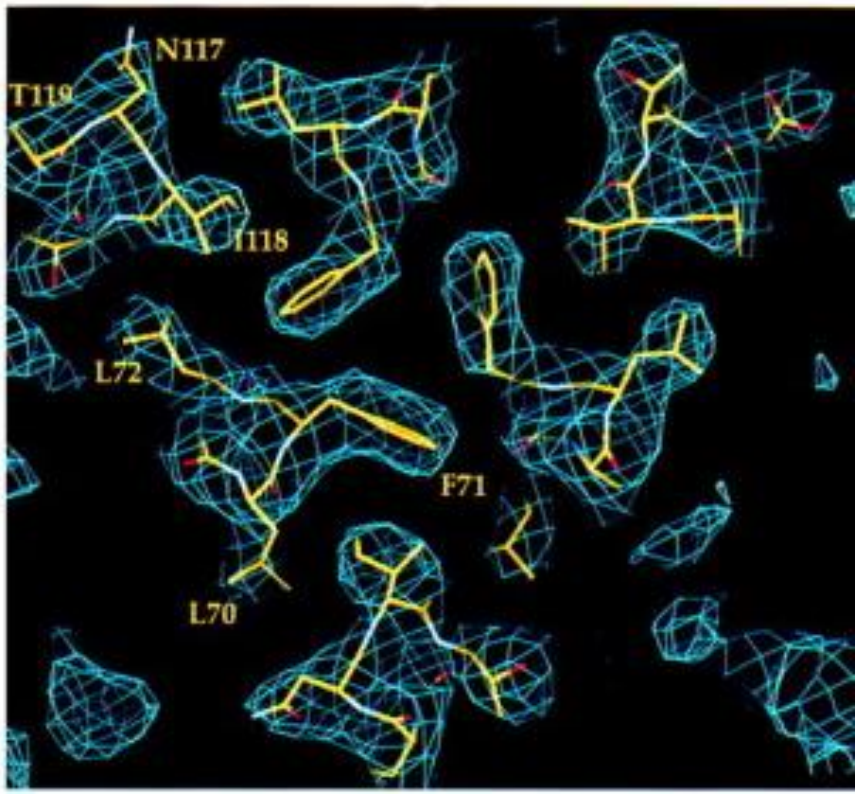
‘closed’ (proper) ncs

‘open’ (improper) ncs









Results: comparison of LH2 and LH3

- LH3 also nonameric, but differs in pigment coordination
- LH2 and LH3 nearly identical at secondary structural level
- Pigments also close but deviate in phytyl chain

Superimposition of LH2 and LH3

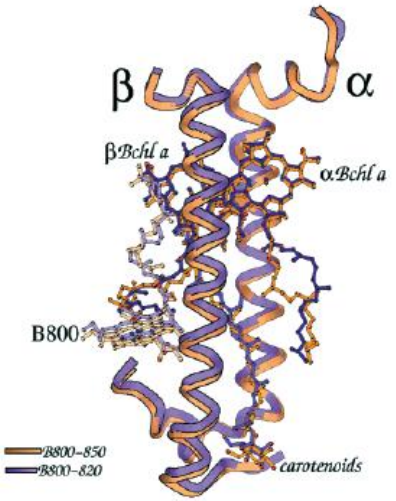


FIGURE 3: A comparison between PCs of LH3 (B800-820, purple) and LH2 (B800-850, tan) (Molscript (46) diagram).

Changes in H-bonding patterns can be seen here
With respect to C-3 acetyl group, among others…

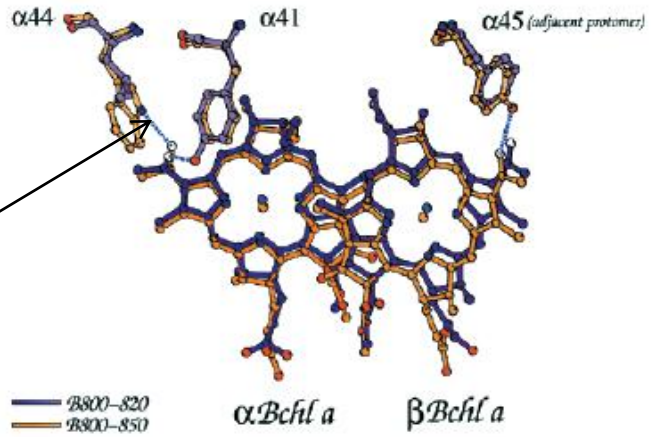


FIGURE 5: Overlays of LH3 B820 (purple) and LH2 B850 (tan) molecules and their H-bond contacts (Molscript (46) diagram).

Pigment Comparison

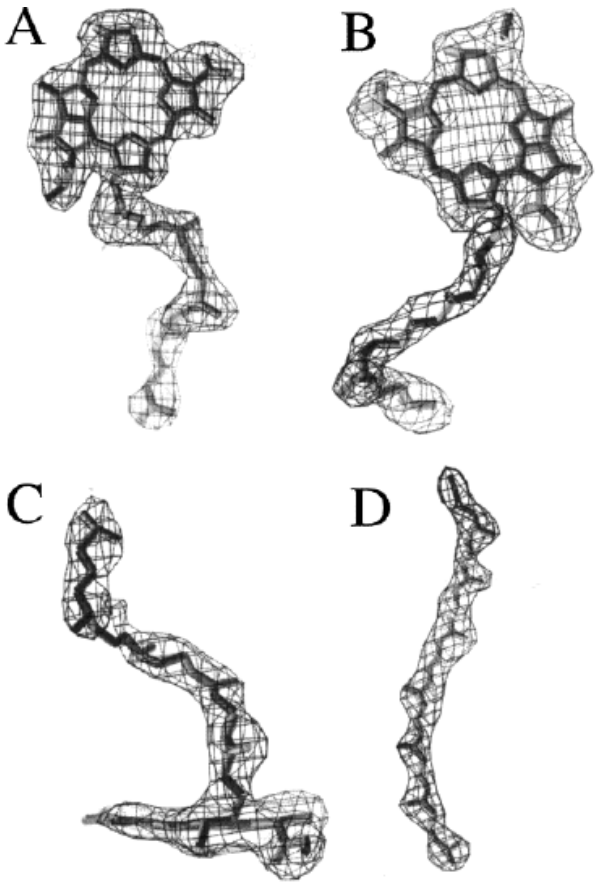


FIGURE 6: F_O-F_C omit maps contoured at 3σ for each of the pigments in LH3 (O (*34*) diagram). (A) αB820, (B) βB850, (C) B800, (D) carotenoid.

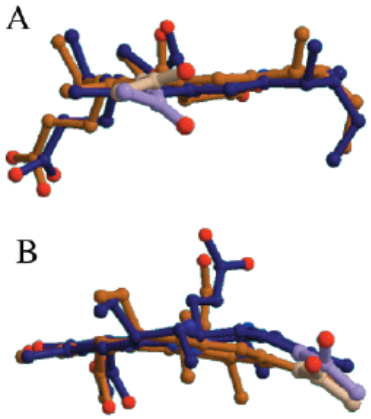


FIGURE 7: Comparison of the conformations of the bacteriochlorin rings in LH3 (purple) and LH2 (tan). (A) αB820/850 molecules, (B) βB820/850 molecules. The C3-acetyl groups are colored light purple in the LH3 molecules and cream in the LH2 molecules (Molscript (*46*) diagram).

Table 3: Contacts to the Pigments of LH3

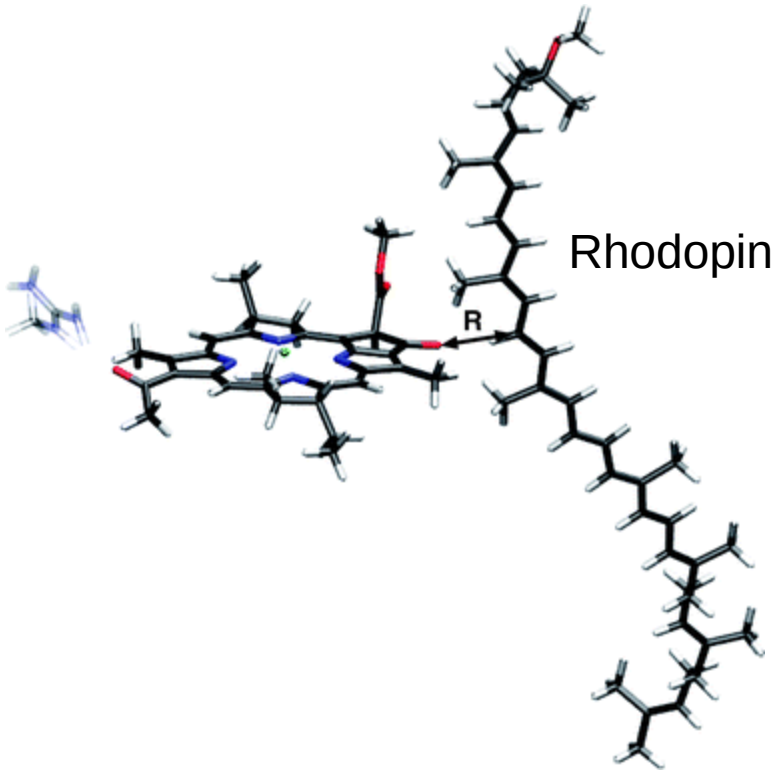
atom	atom[a]	distance Å
B800 Mg	f-Met α OF	2.49[b]
B800 O3[1]	Arg β21 NE	3.08
αB820 Mg	His α31 NE2	2.42[b]
αB820 O3[1]	Tyr α41 OH	2.74
βB820 Mg	His β31 NE2	2.43[b]
αB820 C2	+βB820 C2	3.95
αB820 C12	βB820 C12	3.52
carotenoid C26	+αB820 C20	3.93
carotenoid C11	−B800 O13′	3.47
B800 Mg	+αB820 Mg	17.62
B800 Mg	βB820 Mg	18.35
αB820 Mg	βB820 Mg	9.51
βB820 Mg	+αB820 Mg	8.97

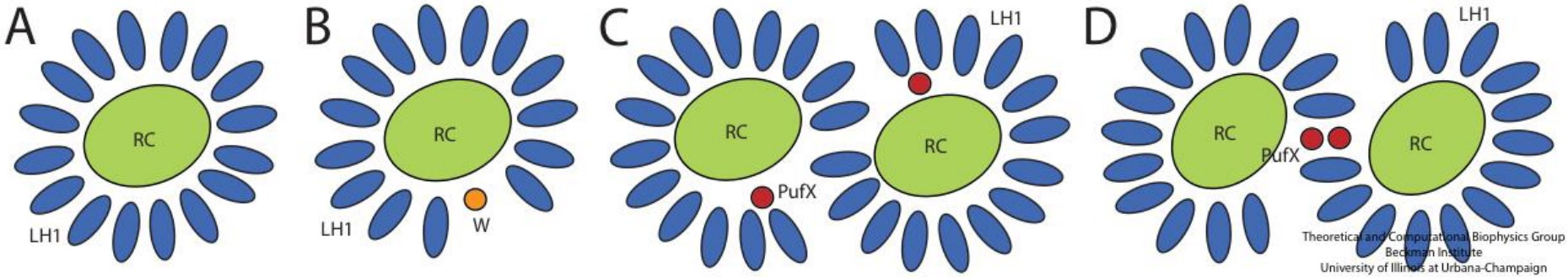
[a] ± signs denote adjacent protomers. [b] These contacts were restrained.

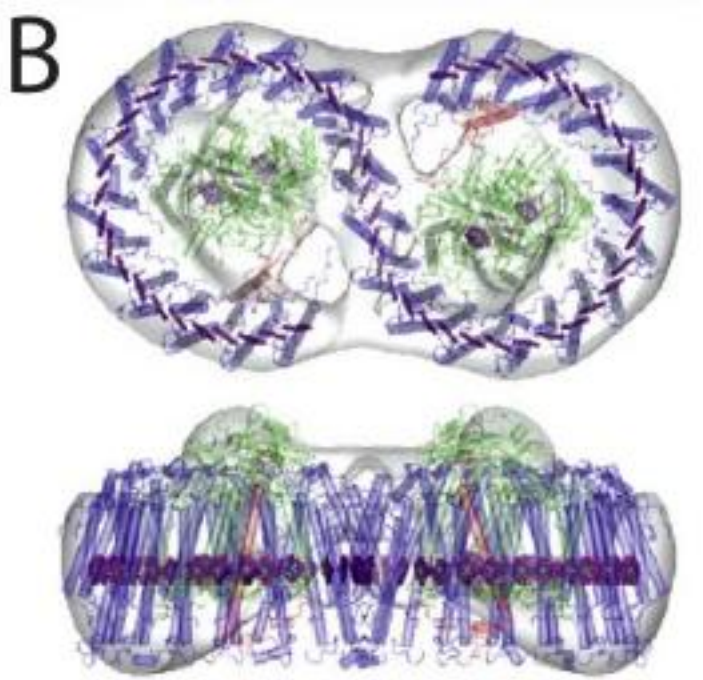
2.46 for LH2 B800

Other Differences

- LH3 contains rhodopinal glucoside, a modified form of rhodopin glucoside which is present in LH2
- The difference in both is the 3rd of 4th methyl group (chemically equivalent) on rhodopin which is replaced by a keto group in rhodopinal




A
RC
LH1
B
RC
LH1
W
C
RC
PufX
LH1
RC
D
RC
PufX
LH1
RC
Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign


B