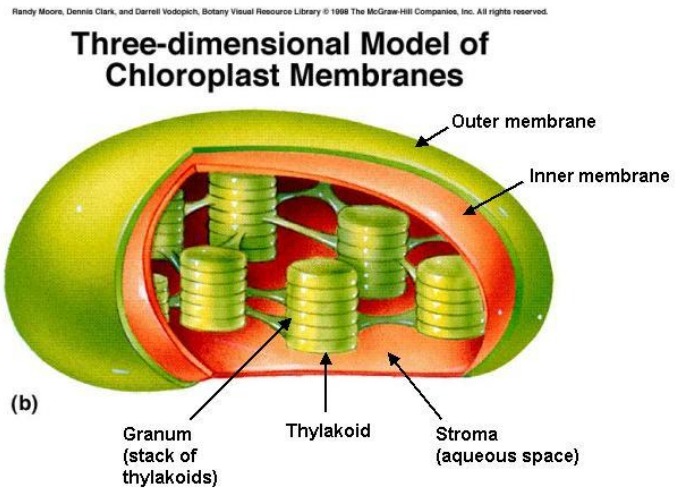
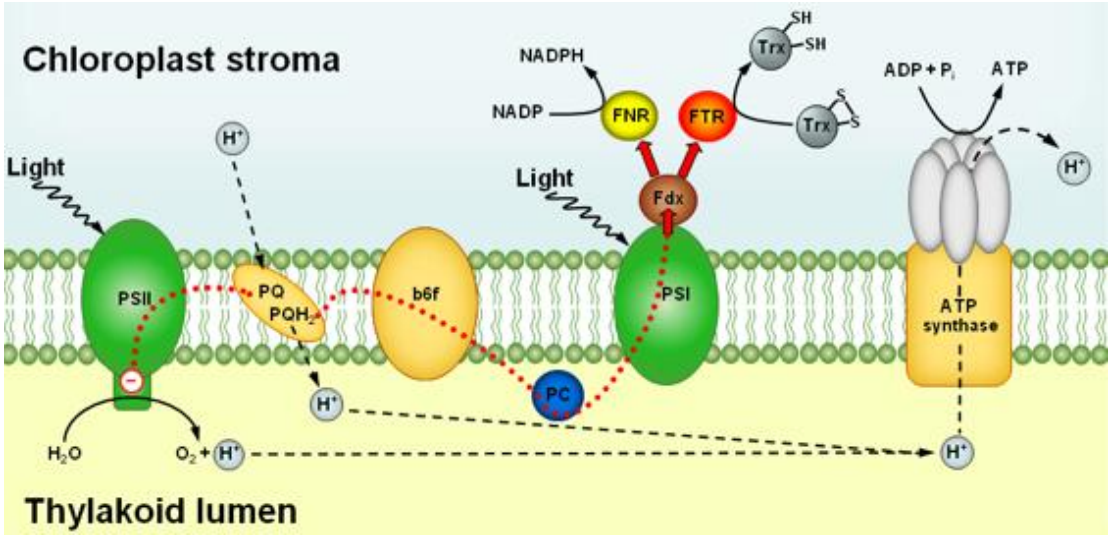
Background

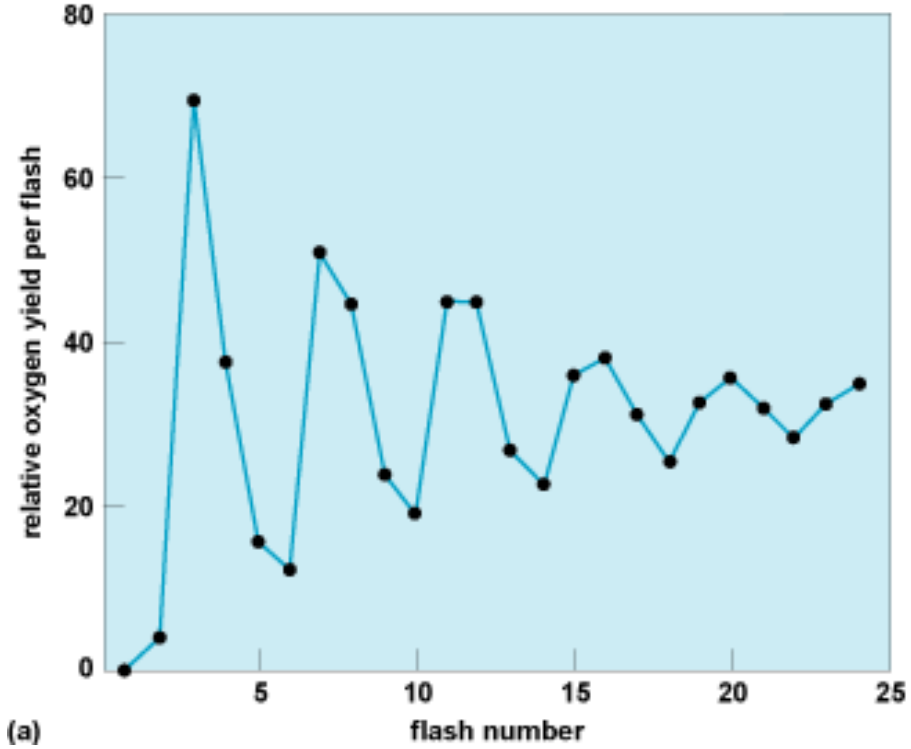
- Photosystems are membrane protein complexes which utilize light to reduce terminal acceptors and make energy
  - Water is oxidized to oxygen. It's electrons are transported through the membrane while H+ are pumped.
  - ATP is generated by the H+ gradient
  - NADPH goes into dark reactions to reduce CO2 and make G3P (the Calvin cycle), a building block of organic molecules
- Type I photosystems use ferredoxin iron-sulfur complexes to accept electrons while type II photosystems use quinones

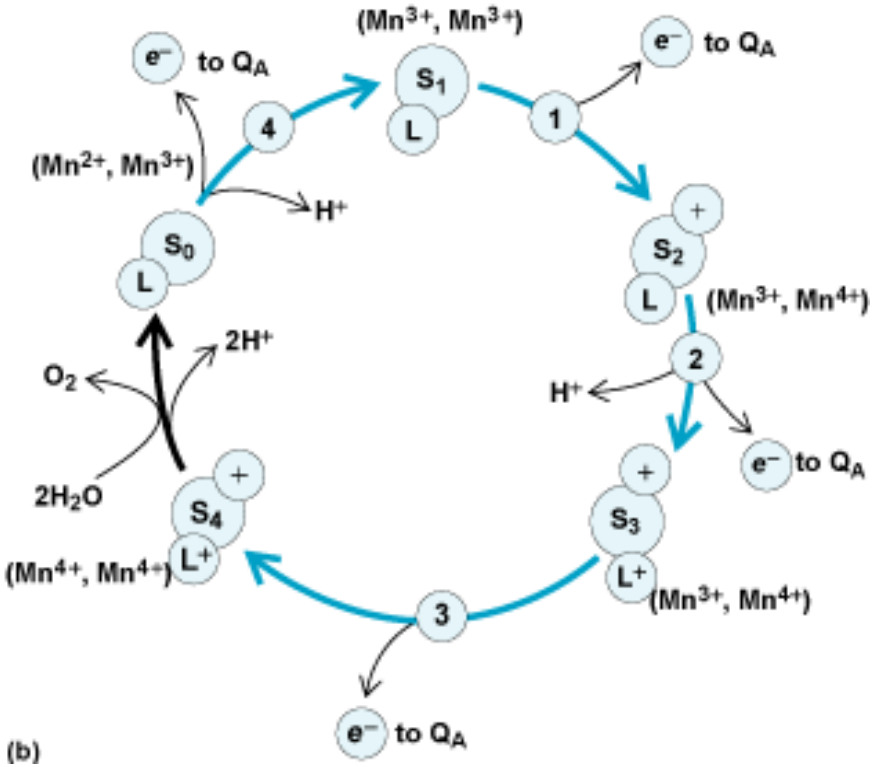




Background: PSII

- PSII is struck by incident light. An electron in P680 (chlorophyll) is excited.
- A series of electron transfer reactions occurs leading to reduction of Q_B
- Q_A, a plastoquinone, transports the electron to Q_B thereby reducing it and taking up protons.
- The oxidized P680 is reduced through the oxidation of water by the Mn cluster


80
60
40
20
0
relative oxygen yield per flash
5
10
15
20
25
flash number
(a)


(Mn3+, Mn3+)
e− to QA
S1
L
1
e− to QA
(Mn2+, Mn3+)
4
H+
S0
L
S2
+
L
(Mn3+, Mn4+)
2
H+
e− to QA
O2
2H+
2H2O
S4
+
L+
(Mn4+, Mn4+)
S3
+
L+
(Mn3+, Mn4+)
3
e− to QA
(b)

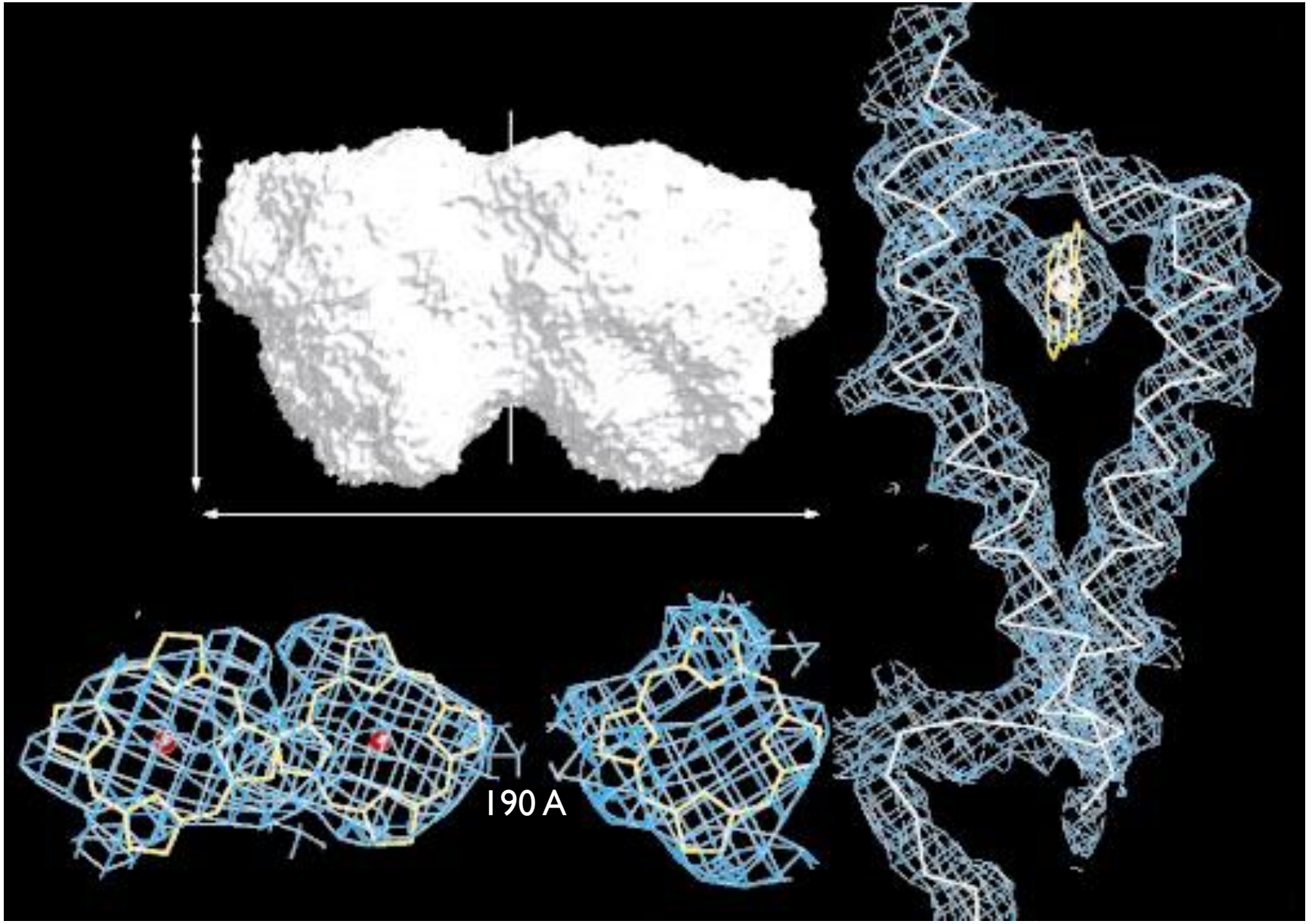
Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8 A resolution

Athina Zouni, Horst-Tobias Witt, Jan Kern, Petra Fromme,,Norbert Krauss, Wolfram Saenger, Peter Orth

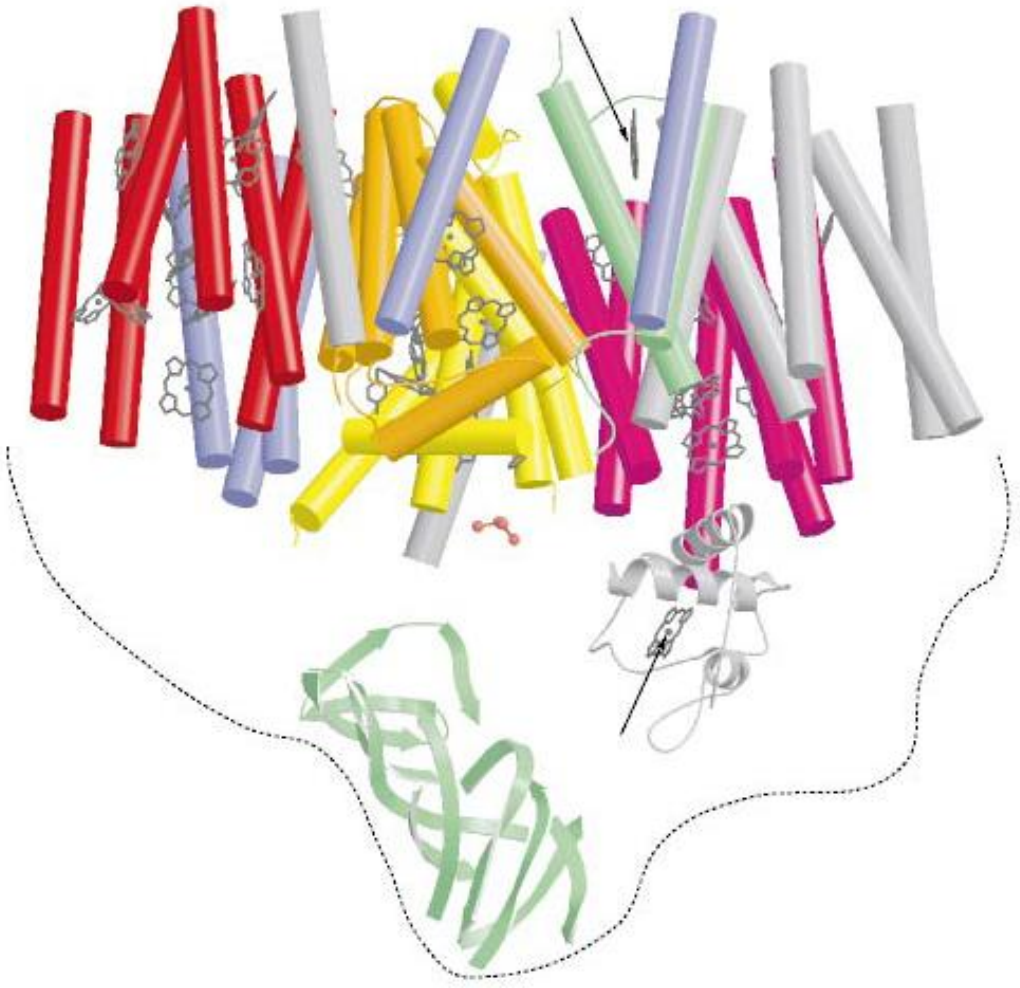
Table 1 Crystallographic statistics

Data collection								
Data set*	Native	Cd	Hg I	Hg II	Hg III	Au	Pt	Native
Wavelength (Å)	0.934†	0.934†	0.934‡	0.9072‡	0.8439§	0.9072‡	0.8439§	1.894‖
Resolution (Å)	20–4.2	20–3.8	20–4.7	20–4.5	20–5.8	20–5.7	20–4.2	20–4.8
Unique reflections	63,639	84,964	45,429	30,210	21,643	22,458	52,611	48,420
Redundancy	3.3	3.2	3.0	1.9	2.6	2.8	3.0	2.3
Completeness (%)¶	98.1 (98.5)	95.4 (84.5)	95.5 (87.5)	55.0 (52.1)	80.3 (82.7)	86.7 (83.9)	81.1 (81.0)	85.0 (70.7)
Completeness of Friedel pairs (%)¶	—	—	76.6 (65.8)	59.8 (53.6)	28.0 (18.7)	—	—	55.9 (44.8)
$\langle I/\sigma \rangle$¶	12.8 (3.5)#	17.1 (3.4)	14.8 (3.2)	17.5 (2.5)	11.3 (3.5)	12.5 (5.3)	13.3 (3.8)	10.5 (5.0)
R_{merge} (%)¶	6.8 (28.8)	6.8 (41.8)	4.1 (28.8)	5.4 (30.0)	4.5 (42.1)	6.2 (26.3)	5.0 (35.6)	9.2 (20.0)
Phasing statistics								
Number of heavy atom sites	—	5	13	16	11	2	6	—
R_{cullis} (centric)	—	0.92 (0.96)	0.85 (0.85)	0.83 (0.91)	0.70 (0.83)	0.95 (0.99)	0.96 (0.99)	—
Phasing power#	—	0.78/0.49	1.07/0.73	1.21/0.91	1.12/0.75	0.62/0.44	0.49/0.51	—
Acentric/centric	—	(0.72/0.52)¶	(1.3/0.76)	(1.02/0.62)	(1.08/0.68)	(0.70/0.51)	(0.42/0.44)	

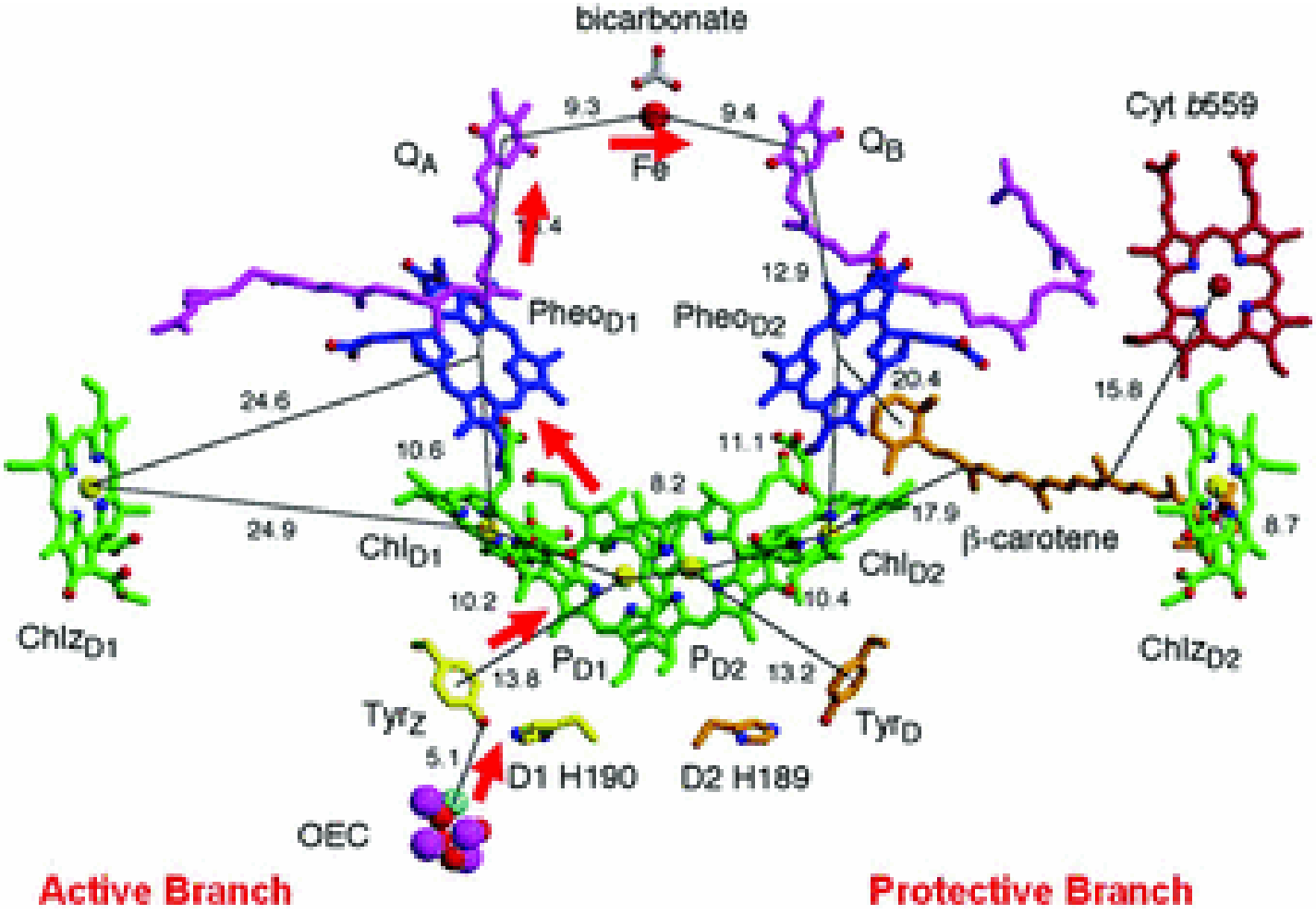
*Heavy atoms derivatives: Cd, cadmium sulphate; Hg I, ethylmercuriphosphate; Hg II, chloromercuriacetate; Hg III, *p*-chloromercuriphenylsulphonic acid; Au, potassium dicyanoaurate (I); Pt, potassium tetracyanoplatinate(II).
† ID14-EH1 ESRF.
‡ X11 DESY.
§ BW7B DESY.
‖ ID13 ESRF.
¶ The numbers in parentheses indicate the values in the highest resolution shell (taken from SCALEPACK[26]).
# Phasing power was calculated between 20 and 4.2 Å.

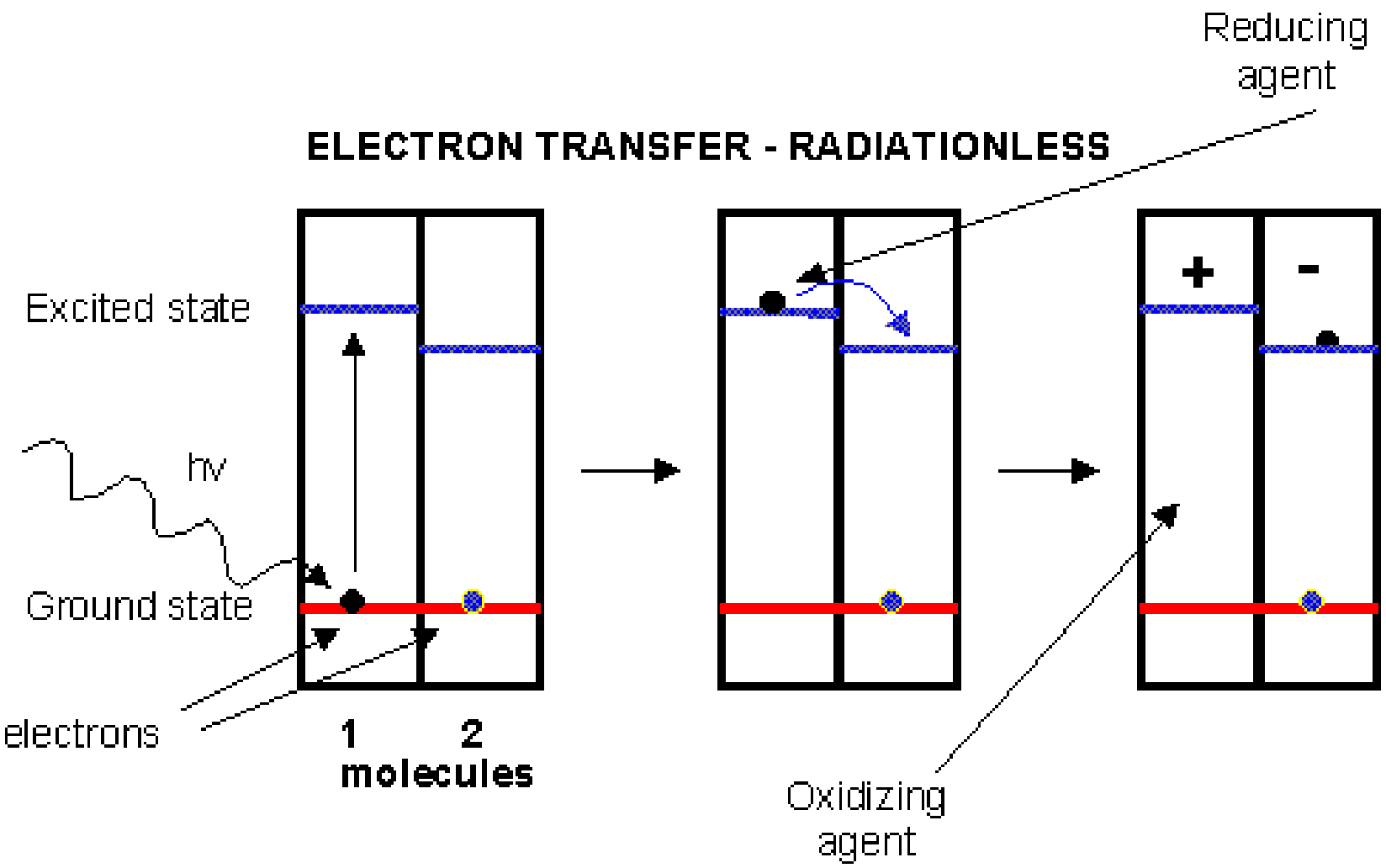

190 A

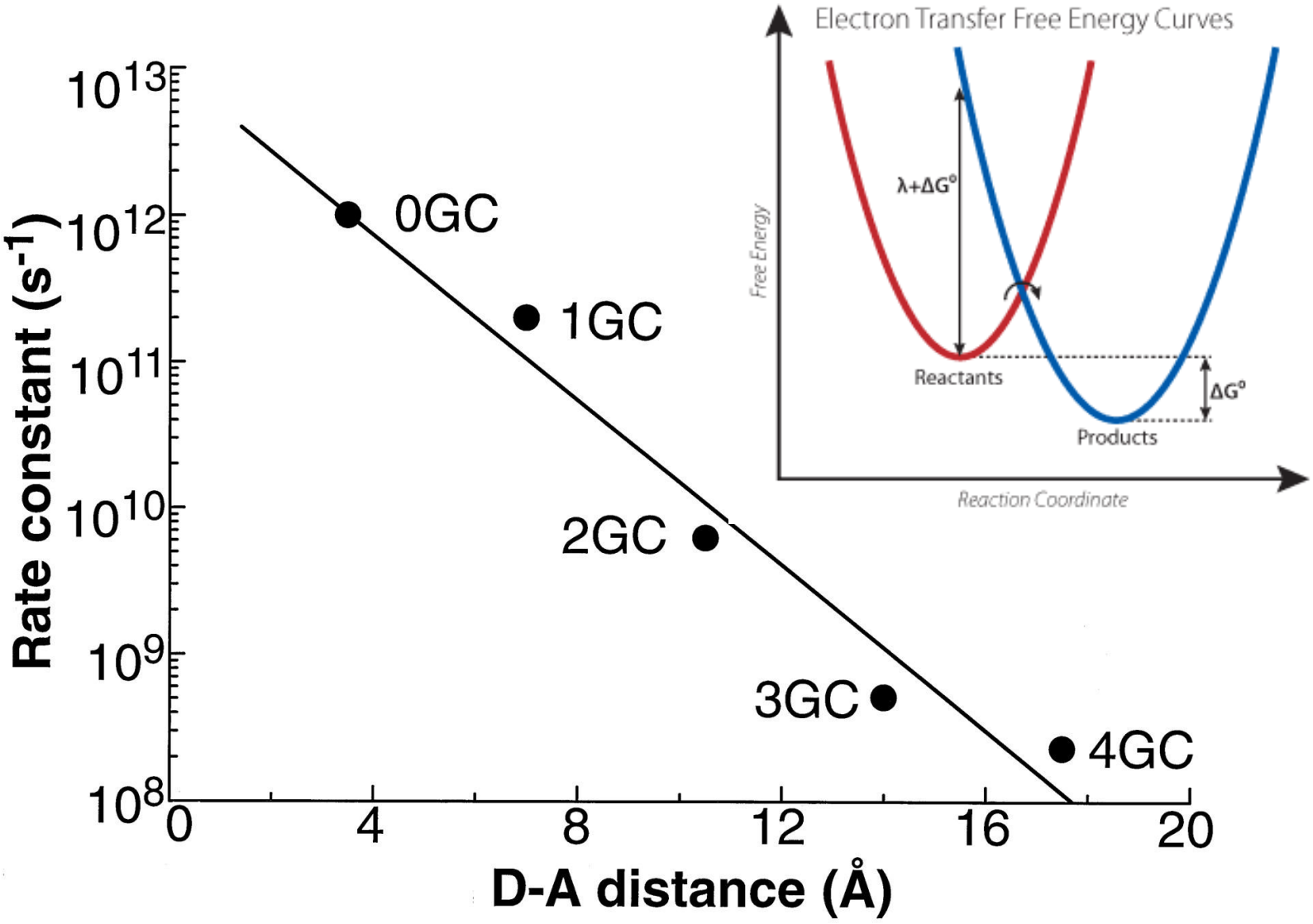
- CP43 and CP47 contain 12 and 14 chlorophyll a, located between the dimers of the trimer
- Located at the stromal and luminal sides: why?



- Incident light hits P680. It's electron is excited
- Electron must be replaced. Uses electrons from nearby Mn cluster oxidizing water
- Y residues of PSII facilitate electron abstraction from Mn

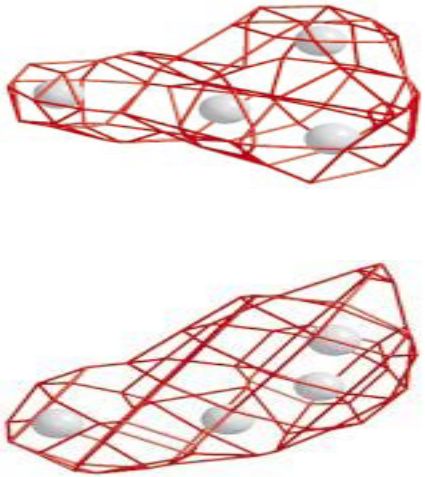


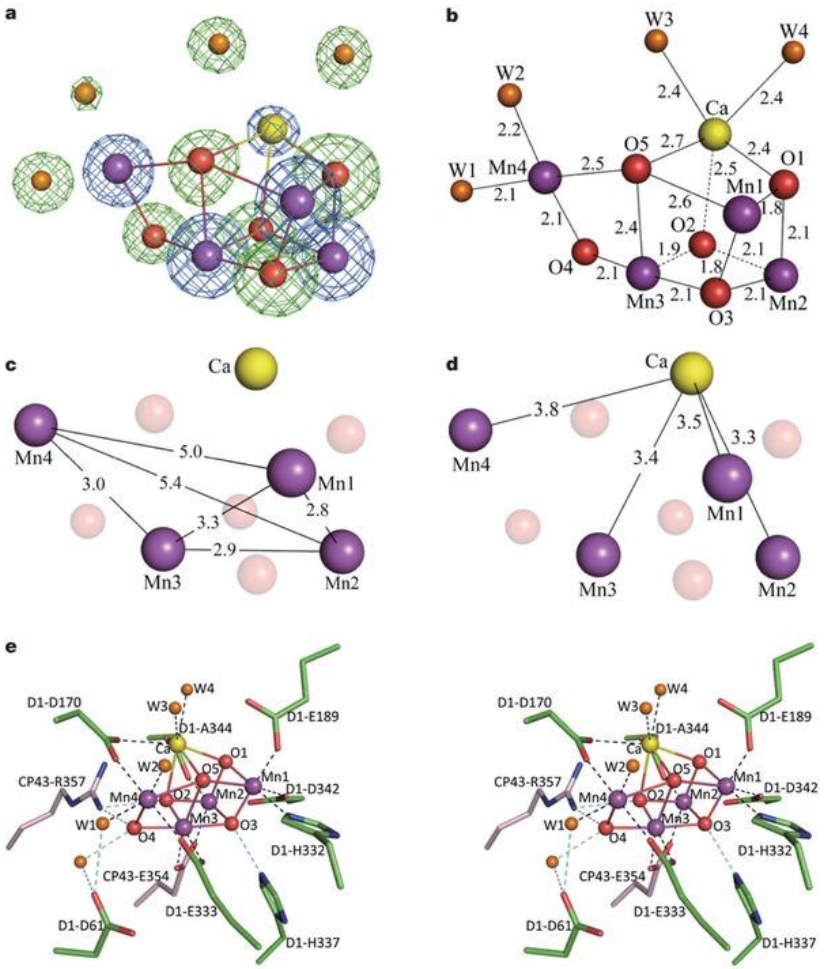

ELECTRON TRANSFER - RADIATIONLESS
Reducing agent
Excited state
hν
Ground state
electrons
1
2
molecules
+
-
Oxidizing agent

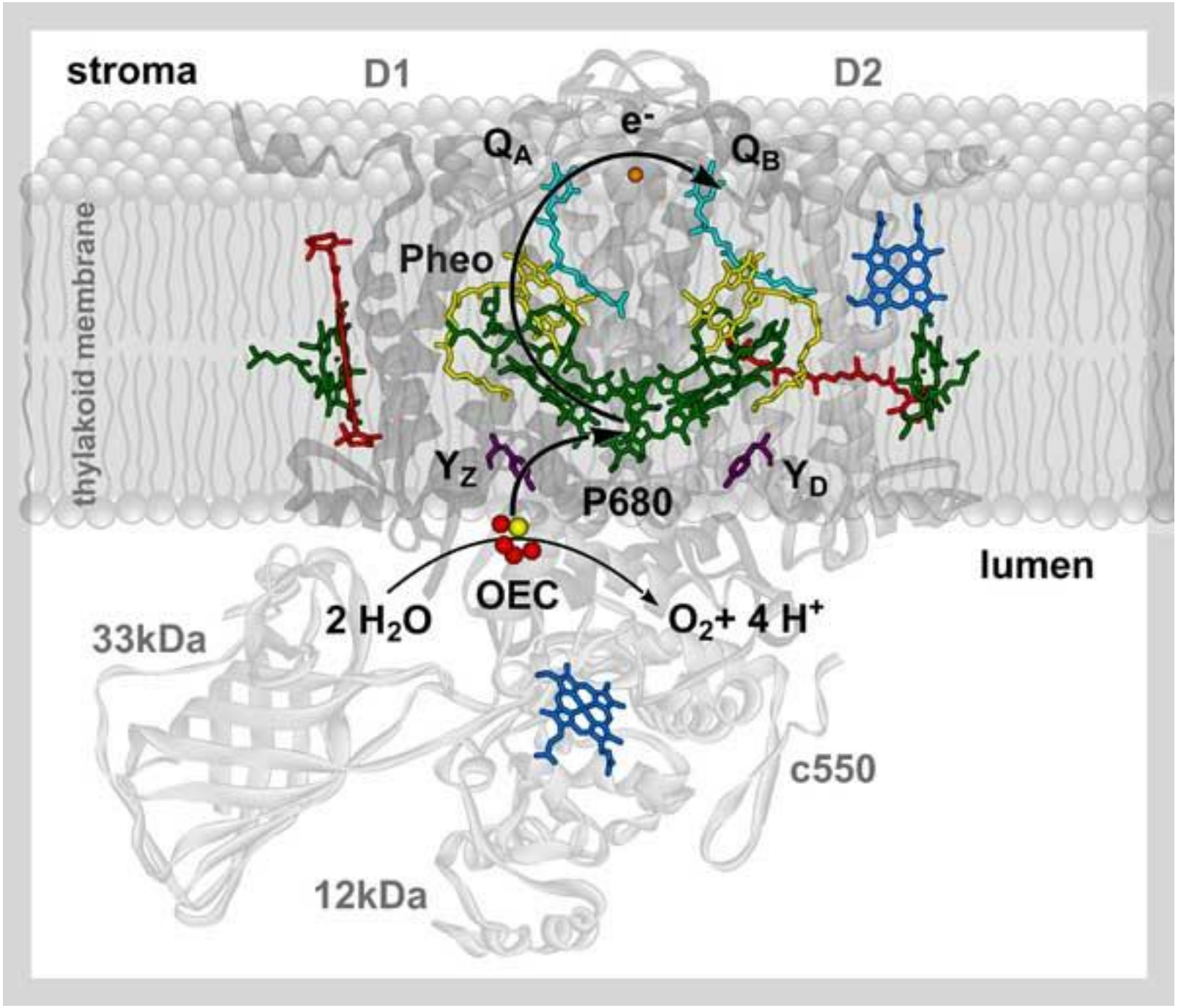

Rate constant (s-1)
10^13
10^12
10^11
10^10
10^9
10^8
0
4
8
12
16
20
D-A distance (Å)
0GC
1GC
2GC
3GC
4GC
Electron Transfer Free Energy Curves
Free Energy
λ+ΔG°
Reactants
ΔG°
Products
Reaction Coordinate

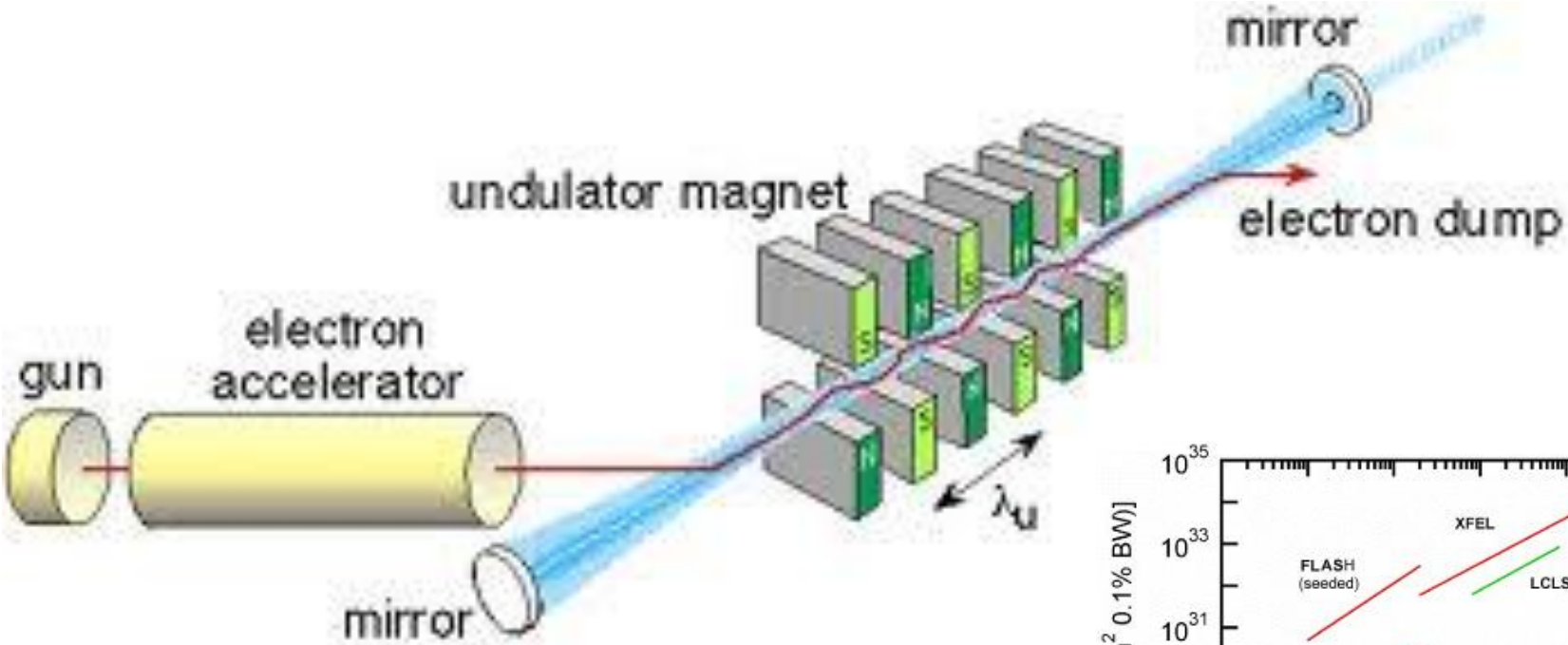
Electron Transfer Reactions

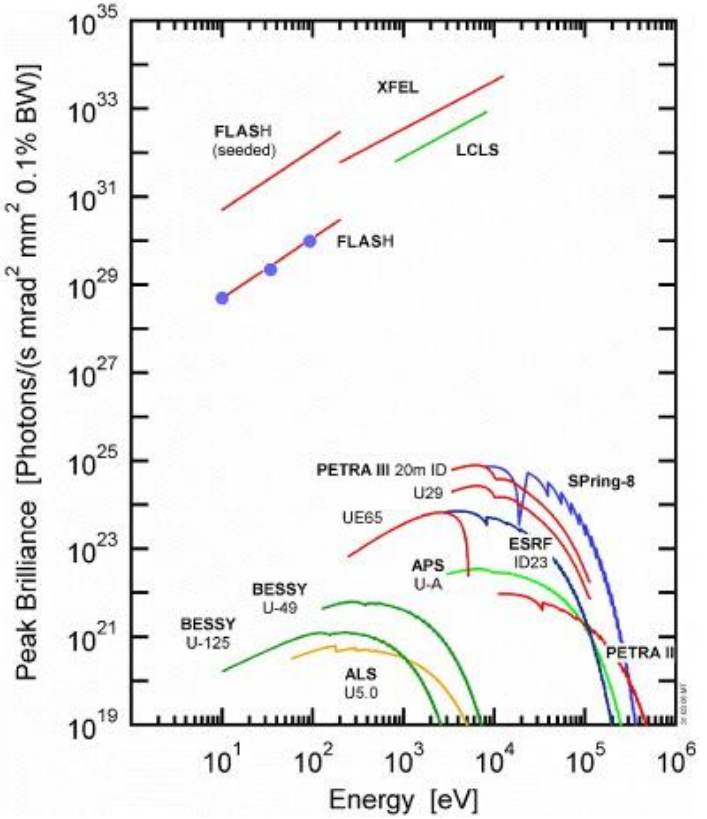
- TyrZ (Y161) bridges P680 and the Mn cluster
- The Mn cluster bulges in three directions
  - 6.8x4.9x3.3 A

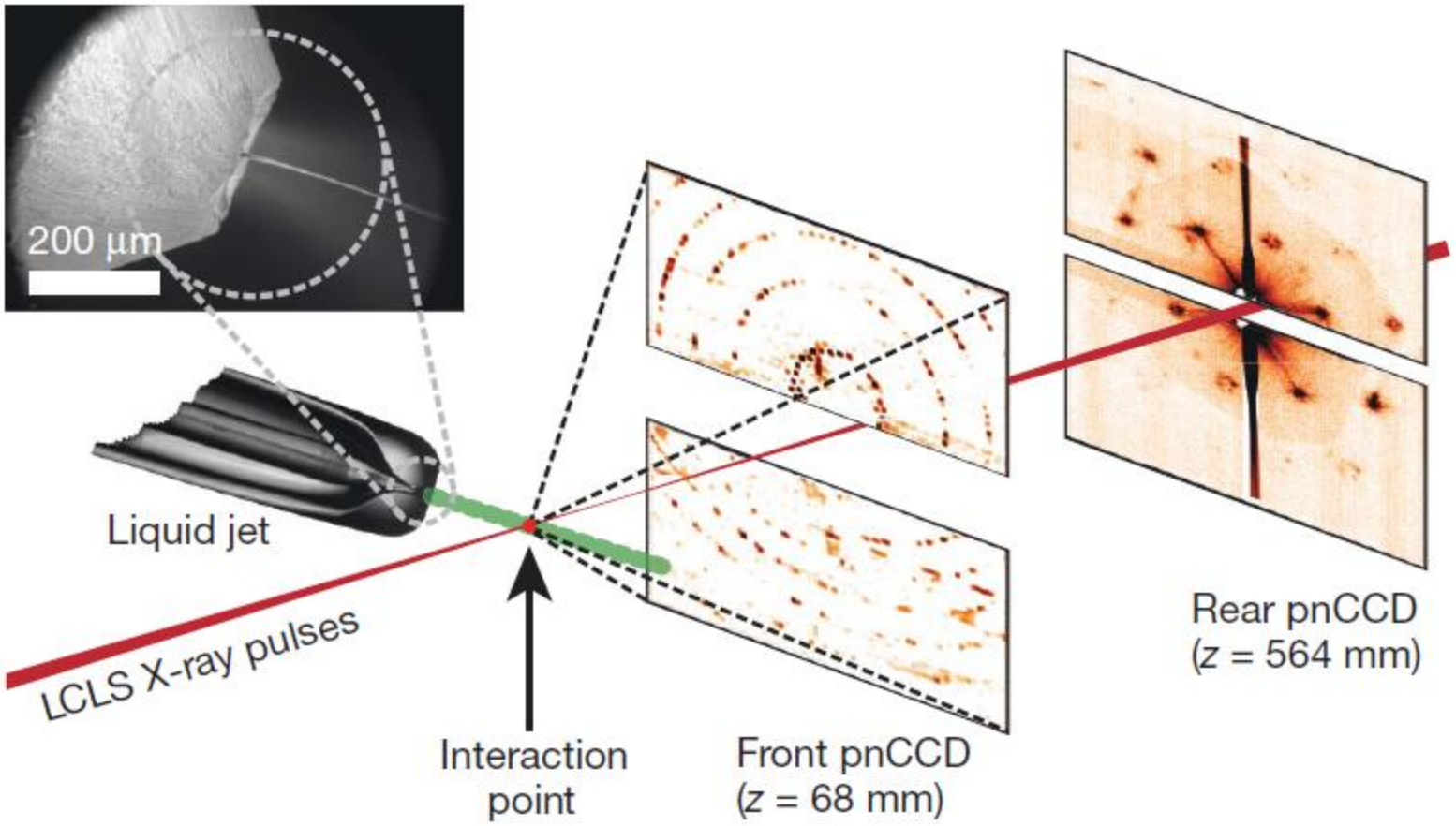





stroma
D1
D2
e-
Q_A
Q_B
Pheo
thylakoid membrane
Y_Z
P680
Y_D
lumen
OEC
2 H_2O
O_2+ 4 H^+
33kDa
c550
12kDa


mirror
undulator magnet
electron dump
electron
accelerator
gun
mirror
λ_u


Peak Brilliance [Photons/(s mrad^2 mm^2 0.1% BW)]
Energy [eV]
XFEL
FLASH
(seeded)
LCLS
FLASH
PETRA III 20m ID
U29
SPring-8
UE65
ESRF
ID23
APS
U-A
BESSY
U-49
BESSY
U-125
ALS
U5.0
PETRA II


200 µm
Liquid jet
LCLS X-ray pulses
Interaction point
Front pnCCD (z = 68 mm)
Rear pnCCD (z = 564 mm)

Three-Dimensional structure of cyanobacterial photosystem I at 2.5 A resolution

Patrick Jordan, Petra Fromme, Horst Tobias Witt, Olaf Klukas, Wolfram Saenger & Norbert Krauss

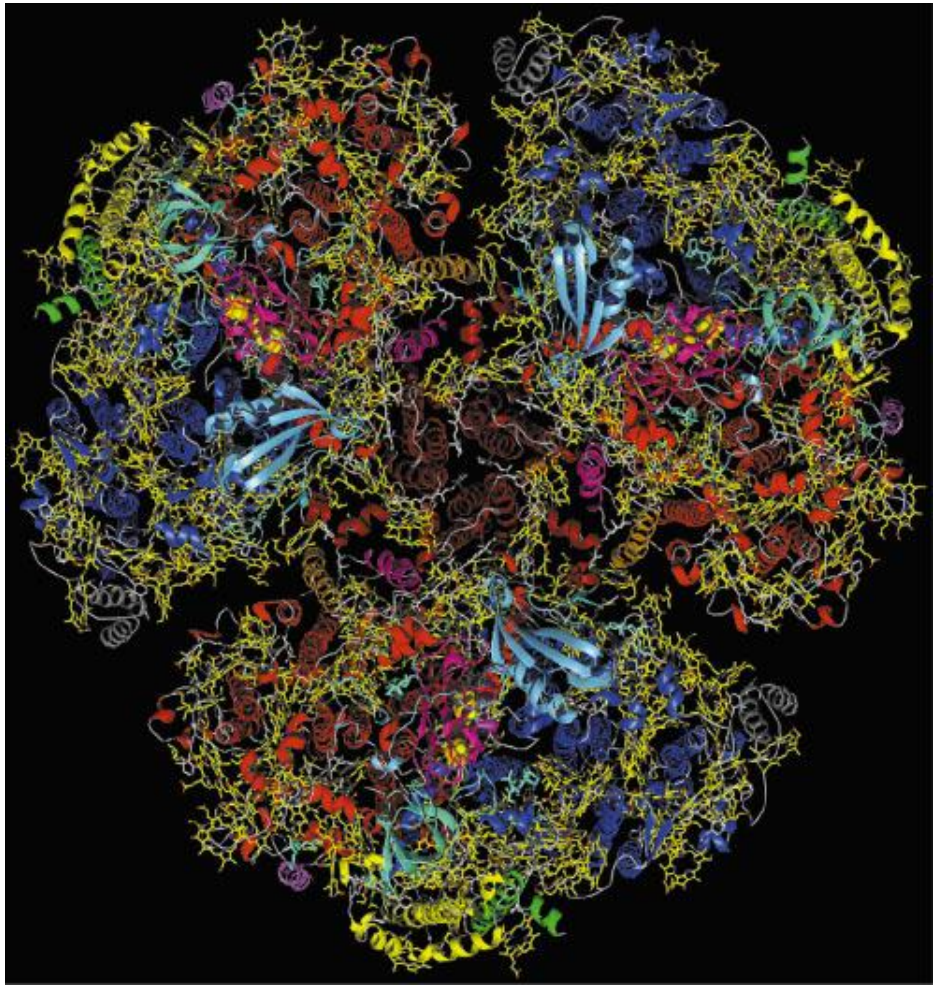
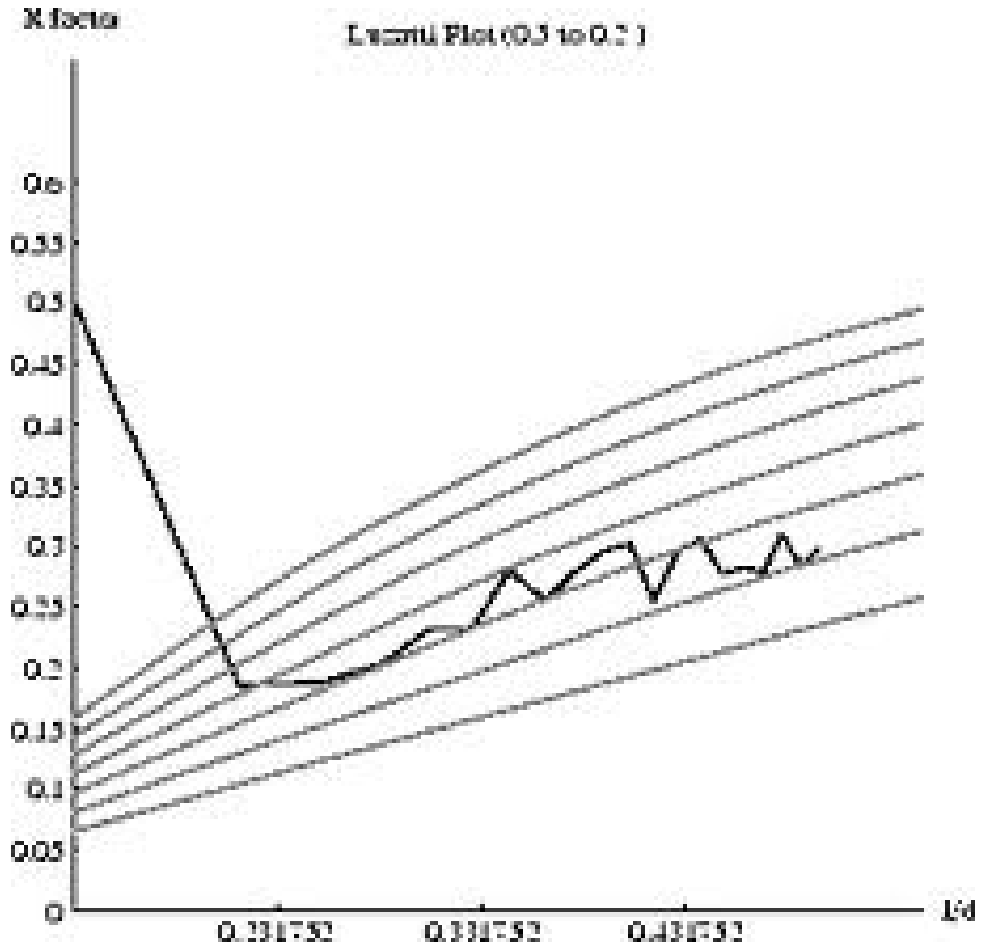


Table 1 Data collection, phasing and refinement statistics

Data set	Native (1)	Native (2)	EMTS	PIP
Wavelength (Å)	0.99	1.44	0.99	0.99
Resolution (Å)	30–2.5	30–2.9	50–3.0	50–3.0
R_{merge}*	0.064 (0.247)	0.079 (0.207)	0.076 (0.224)	0.065 (0.254)
Completeness (%)	97.1 (88.9)	90.4 (76.3)	98.8 (95.6)	93.3 (68.3)
$\langle I/\rangle$	10.6 (3.4)	10.8 (4.6)	20.5 (8.9)	21.1 (4.2)
Phasing statistics				
No. of heavy atom sites	–	–	4	8
R_{cullis} (centric)†			0.86	0.81
Phasing power (iso/ano)†		–/0.51	1.23/1.03	1.62/1.07
Resolution (Å)	30–2.5	30–3.0	30–3.0	30–3.0
FOM‡	0.57			
Refinement statistics				
Reflections (working set)	233,377		Number of non hydrogen atoms	
Reflections (test set)	4,743		Protein	17,404
R_{work}/R_{free} (%)	19.8/21.7		Cofactors	6,602
R.m.s.d. bond lengths (Å)	0.0126		Water	201
R.m.s.d. bond angles (°)	1.475		Metal	1
Coordinate error (Å)‡				
Luzzati	0.32			
SigmaA	0.36			

Numbers in parentheses correspond to values in the highest resolution shell.
*$R_{merge} = \Sigma_h \Sigma_i |I_i(h) - \langle I(h)\rangle| / \Sigma_h \Sigma_i I_i(h)$, where $I_i(h)$ are individual intensities of any reflection and $\langle I(h)\rangle$ is the mean intensity.
† FOM (figure of merit), R_{cullis} (centric) and phasing power determined by the program SHARP (see Supplementary Information).
‡ From Luzzati plot and SigmaA analyses, as determined with CNS (see Supplementary Information).

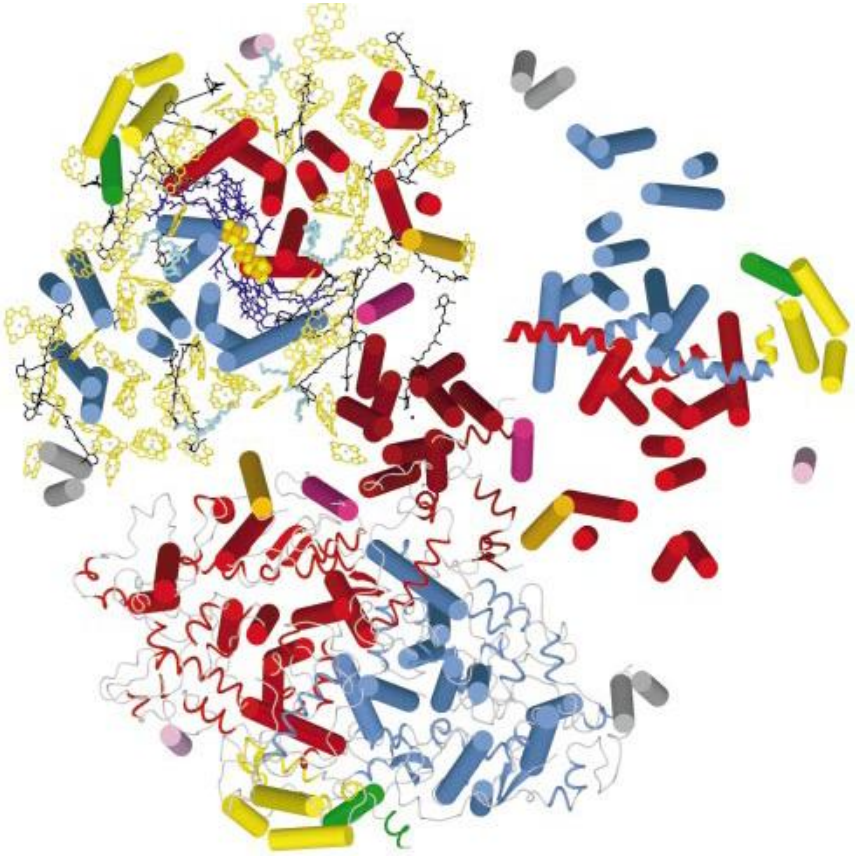

X facto
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.331752
0.331752
0.431752

Intro to structure

- PS1 exists as a trimer of three monomers, 11 subunits each
  - Each subunit coordinates 100 cofactors
  - The general structure is a photoreaction center surrounded by an antenna system whose job is to funnel excitons to RC core

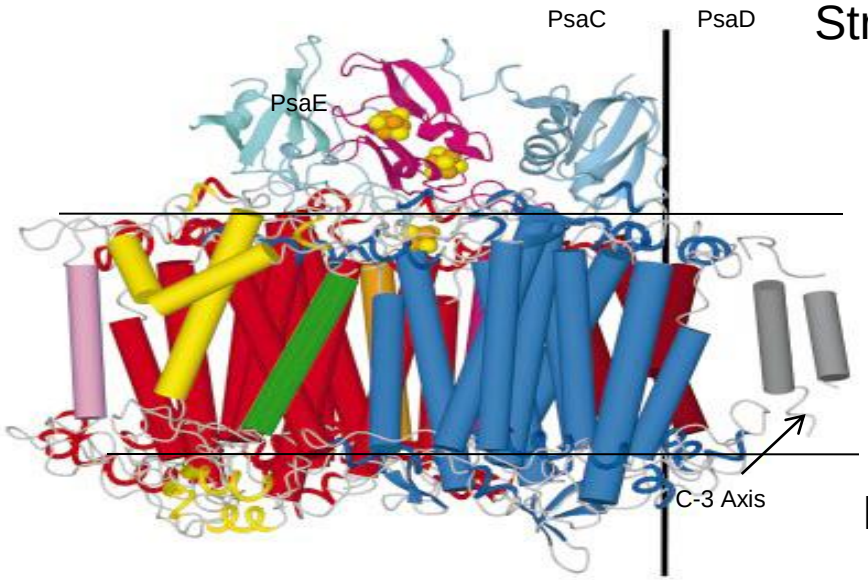
Overall Architecture

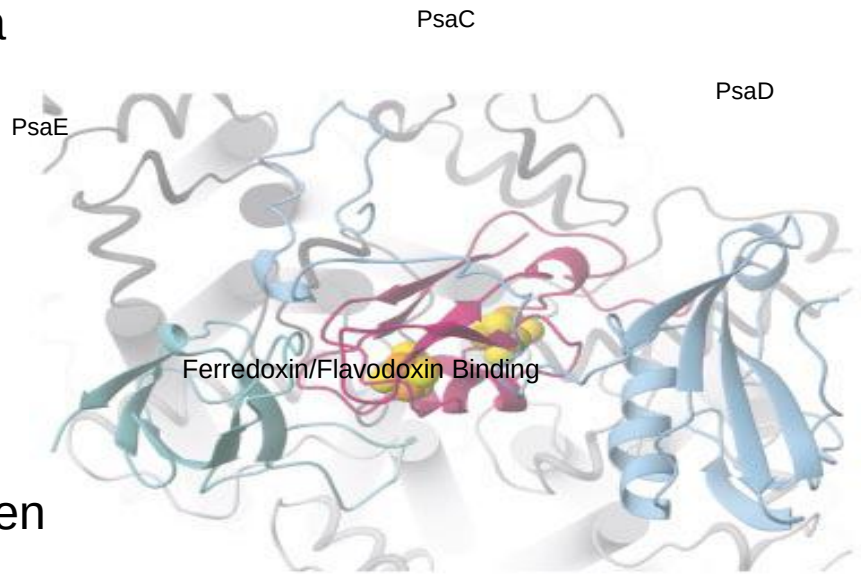
- Cloverleaf structure with C3 axis perpendicular to membrane plane, PsaL in the center forming many monomer-monomer interactions
- 9 TM subunits: Psa-A,B,F,I,J,K,L,M,X and three stromal: Psa-C,D,E
- Cofactors organized into two branches on either side of psuedo-C2 axis, most chlorophylls around PsaA AND PsaB, the business end.



Overall Architecture cntd.

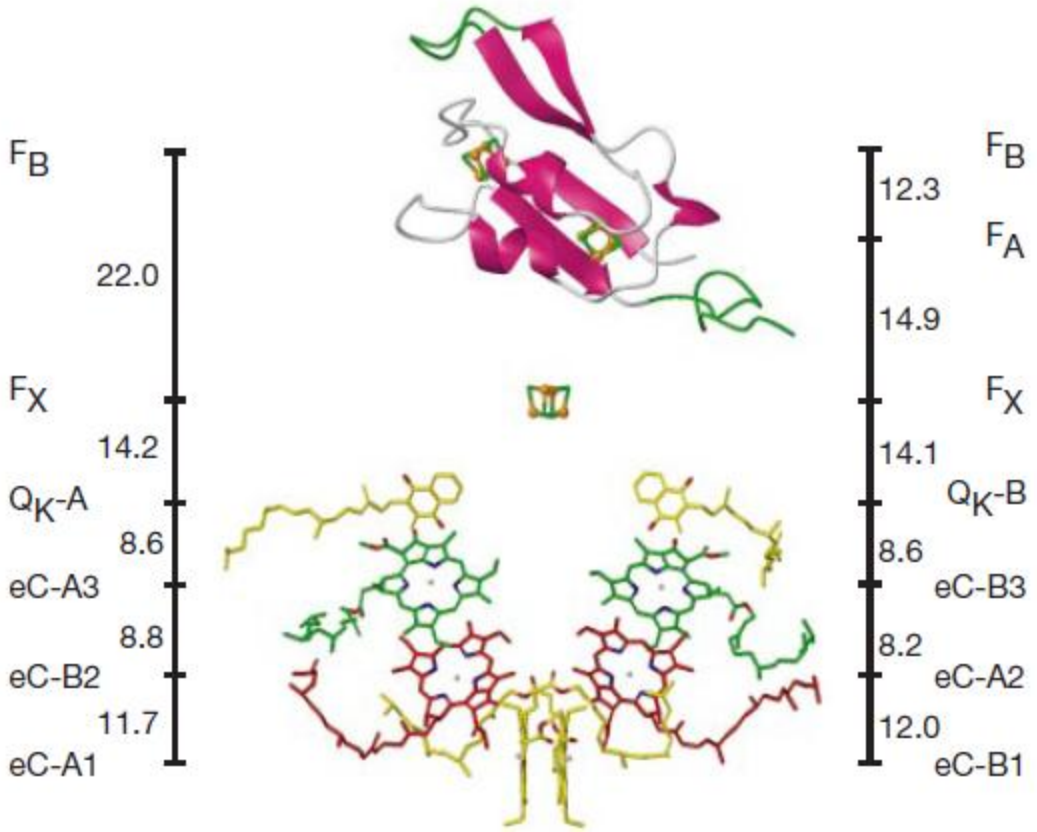
- As mentioned, Psa-E,D,C not TM proteins. Rather, they suggest a docking site from Cyt-c6 or plastocyanin on the lumenal side of the protein
  - Donating e- to PS1
- Structure also suggests stromal side docking for ferredoxin and flavodoxin
  - Accepting e- from PS1 utilizing Psa-E,D,C



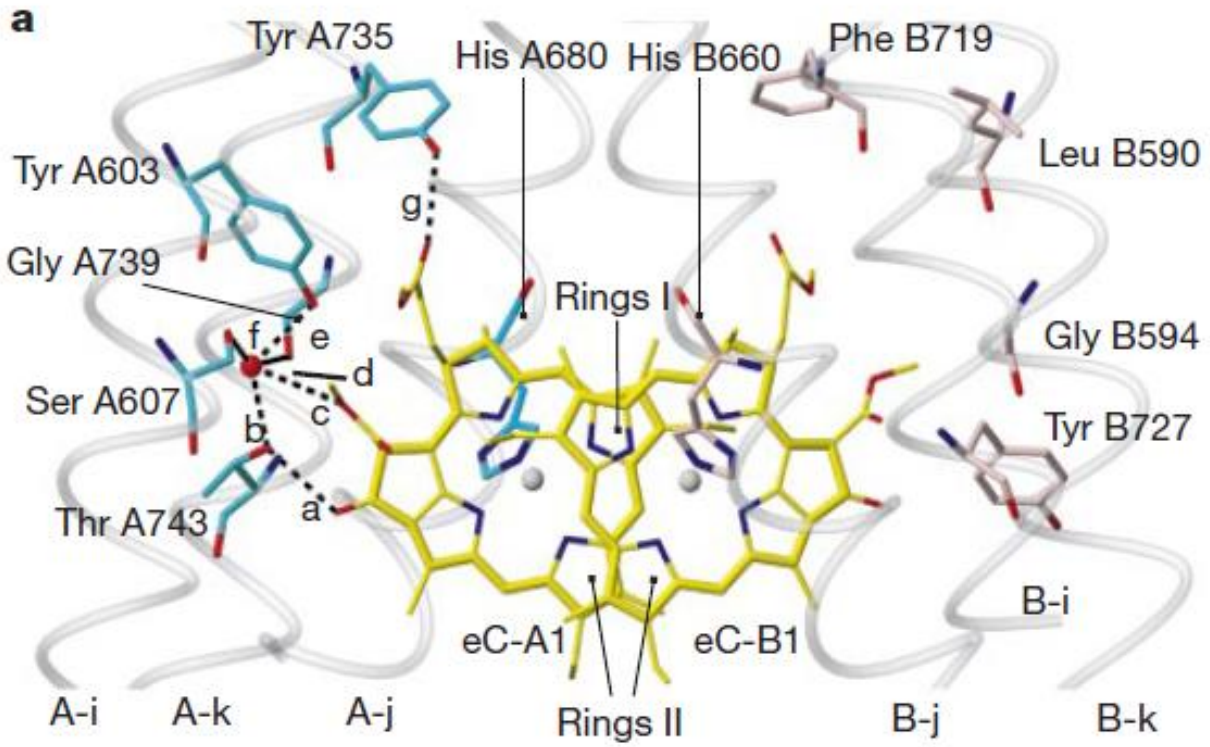


PsaC

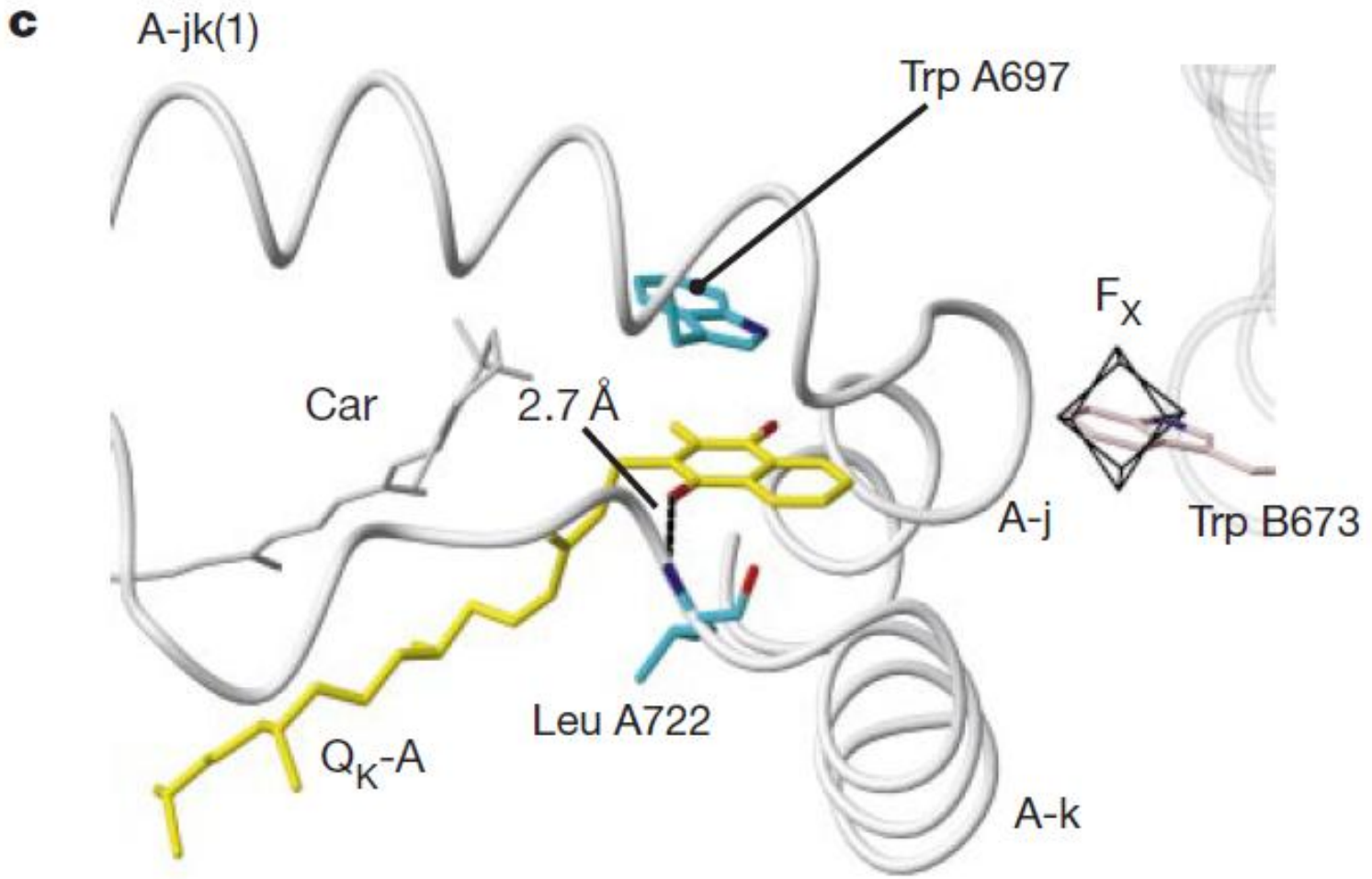
- PsaC is in the middle of PsaE and PsaD
- It harbors two Fe4S4 clusters Fa and Fb
  - Extending from these clusters is a loop thought to be involved in ferredoxin and flavodoxin binding



- Functionally most important structure of protein
- Consists of 2 phylloquinones, 6 chlorophylls, 3 Fe4S4 clusters
- Arranged in two branches: PsaA and PsaB related by Pseudo C-2 axis
  - PsaA branch is eC-A1, -B2, -A3 and PsaB is eC-B1, -A2, -B3
  - Special pie interaction between eC-A1 and eC-B1 with p700 initiates charge separation

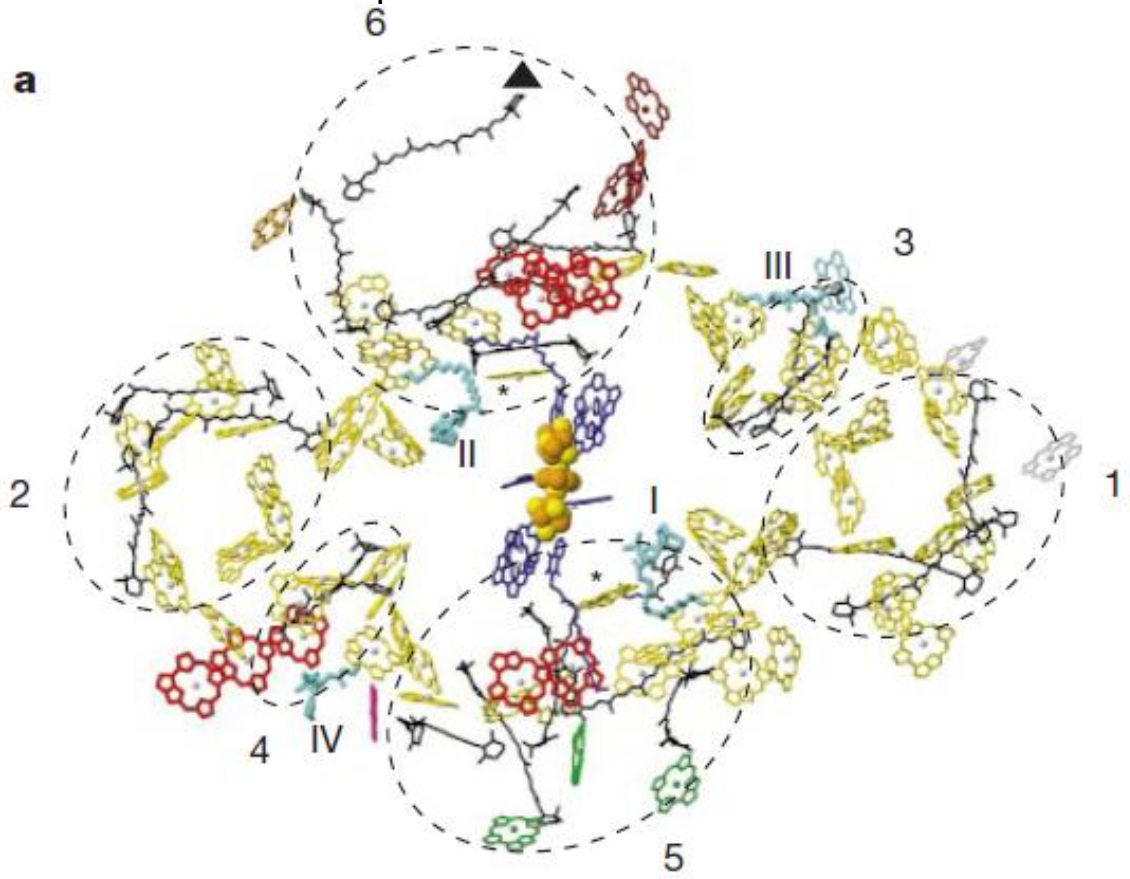


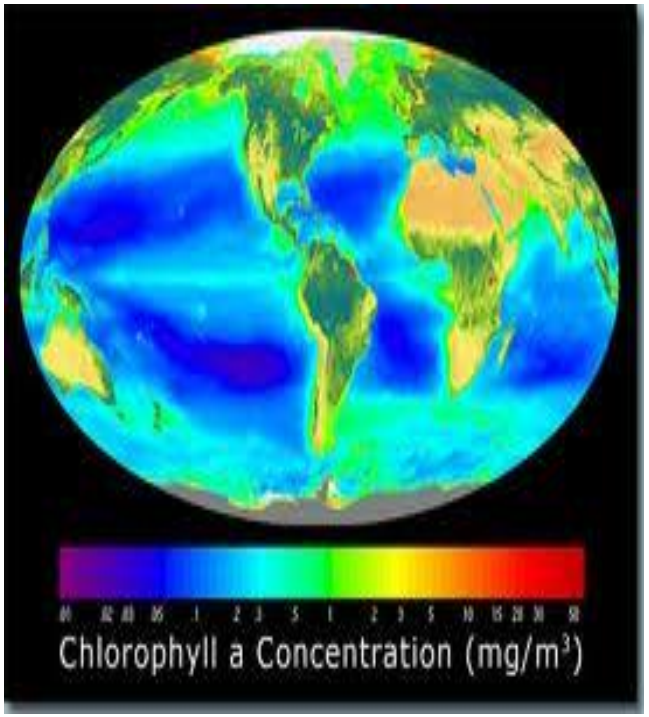
Other Important Cofactors



Antenna System

- 90 Chla molecules, 22 carotenoids
- 79 of Chla bound to PsaA and PsaB, others coordinated to nearby helices
- Show distribution about psudeo C-2 axis
- Most coordinated through His imidazole moiety, but some through other AA side chains
  - Most important are aC-A40 and aC-B39, which connect to eC-A3 and eC-B3 in antenna-RC hook-up





Carotenoids

- Beta-carotene dominant in PS1
- Deeply inserted into membrane
- Bound to PsaA and PsaB by hydrophobic interactions
- Harvest light in 450-570 nm range
- Contact Chlas to facilitate exciton transfer
- Pie Bond interactions

- PSI works in a similar fashion to PSII. It has a photoreaction center which, upon excitation, promotes an electron to a higher energy level. Instead of p680, PSI uses p700 for this
- The excitons then travel across the membrane (charge separation) to reduce quinones to quinol. The exciton proceeds to reduce NADP+, which reduces CO2 in the dark reactions (Calvin Cycle).