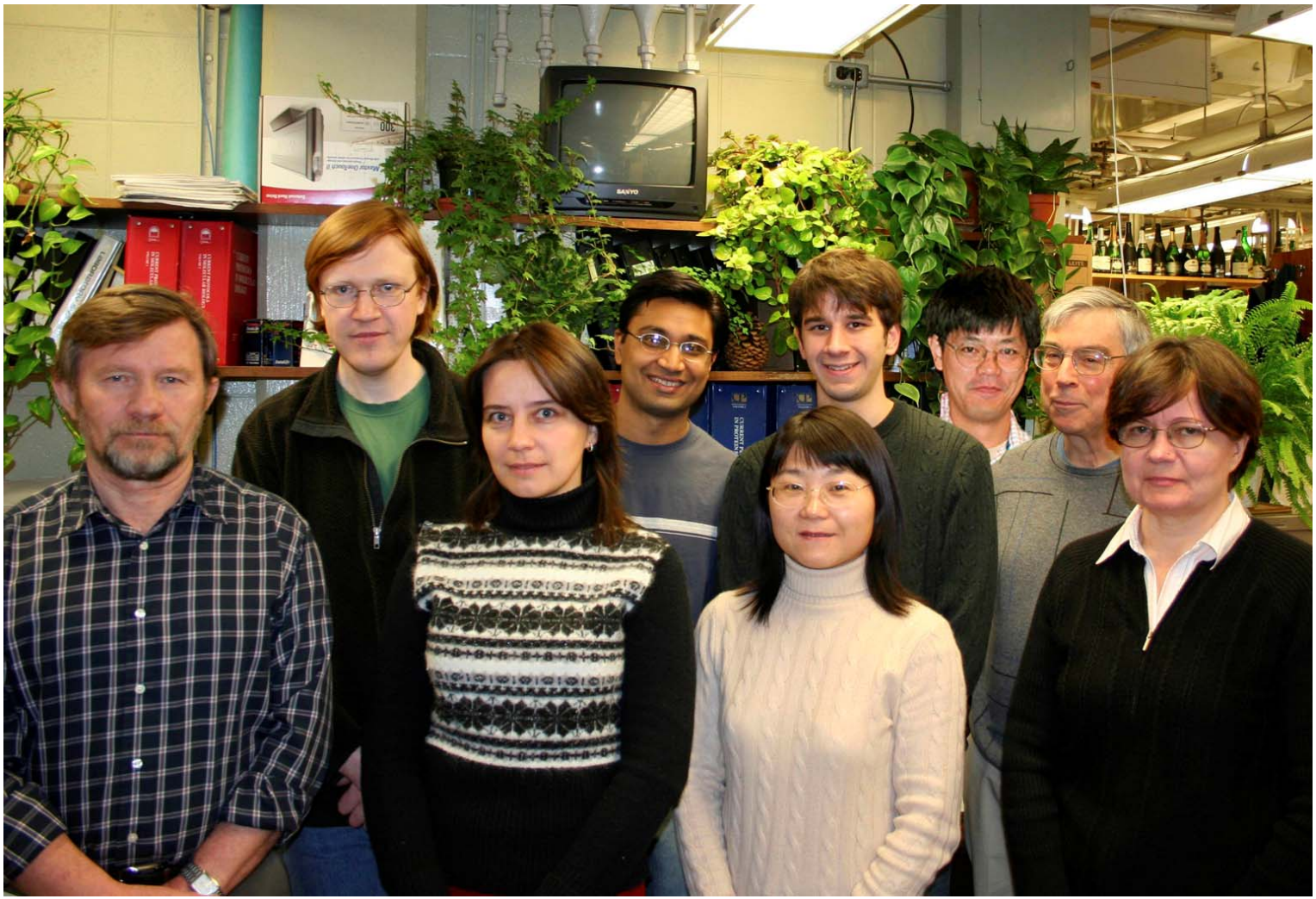
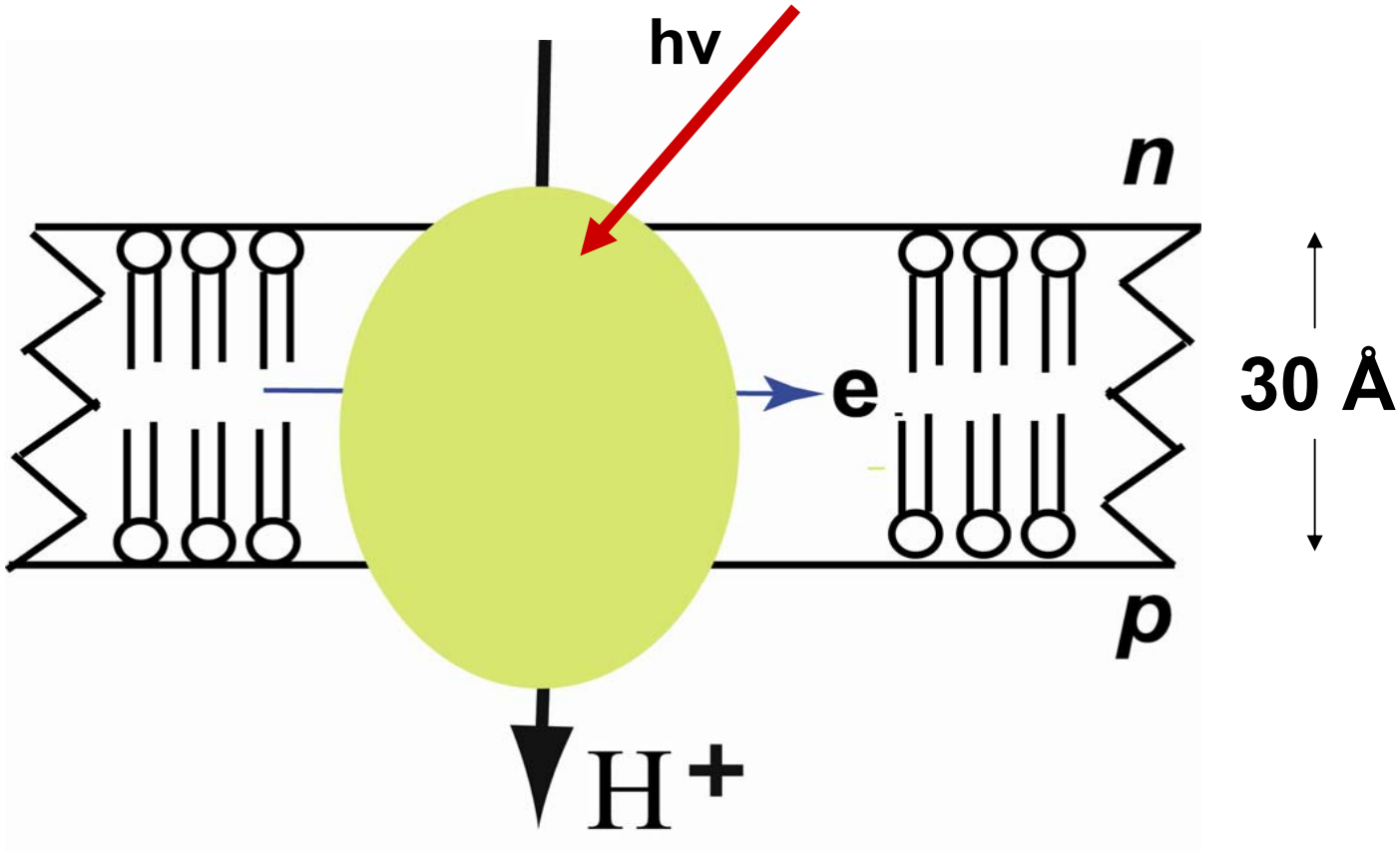
Charge Transfer Across an Energy Transducing Membrane Protein Complex



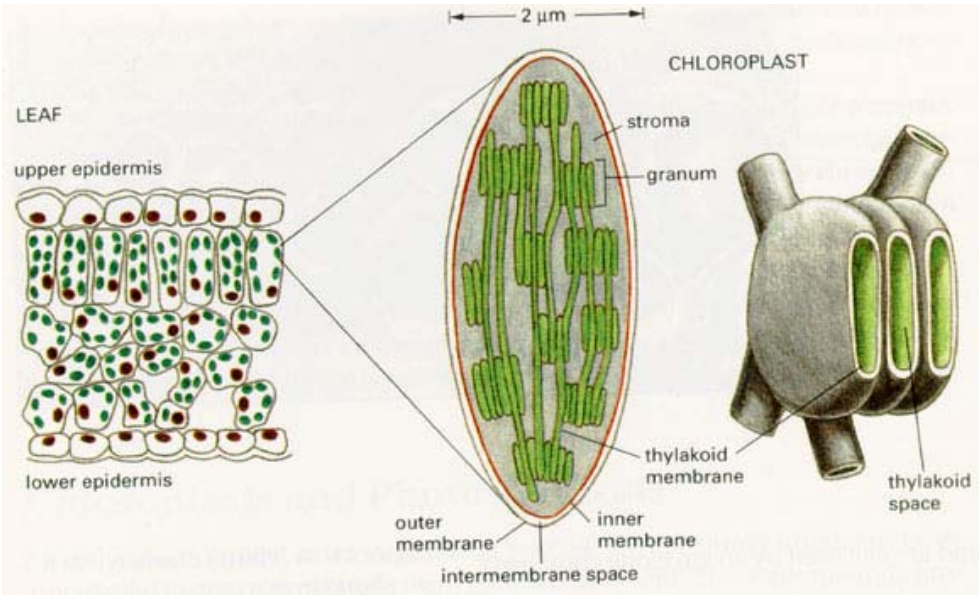
Energy stored in biological membranes in ion (e. g., H+) gradients & membrane potential formed by light or redox–driven ion pumps

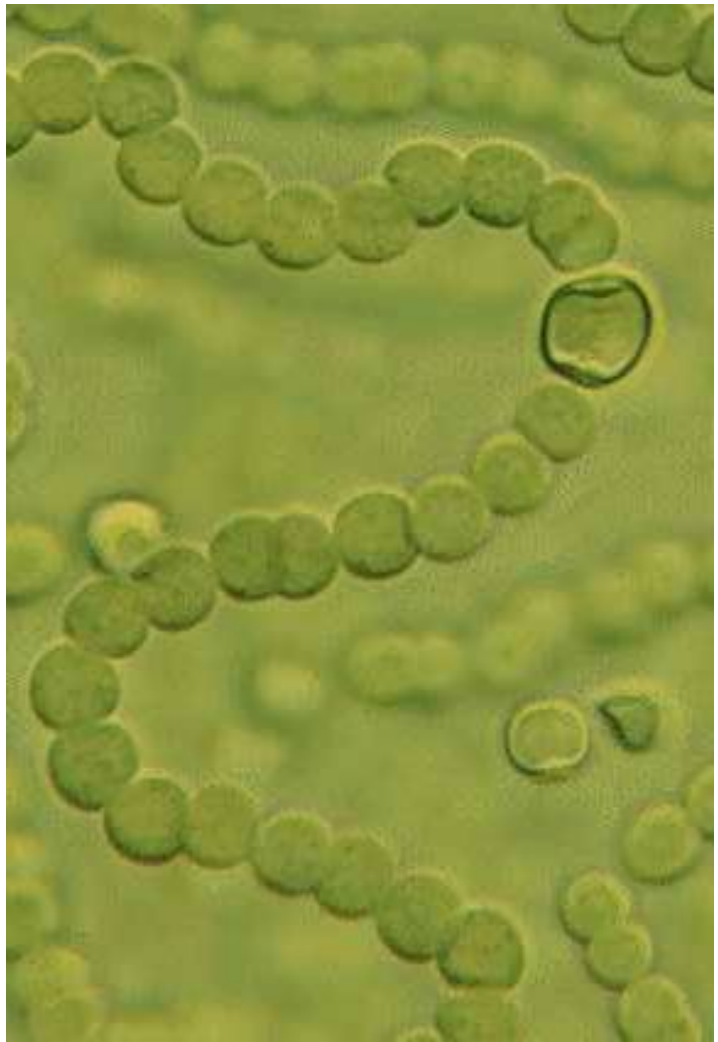


Membrane specific capacitance ≅ 1 μF/cm^2

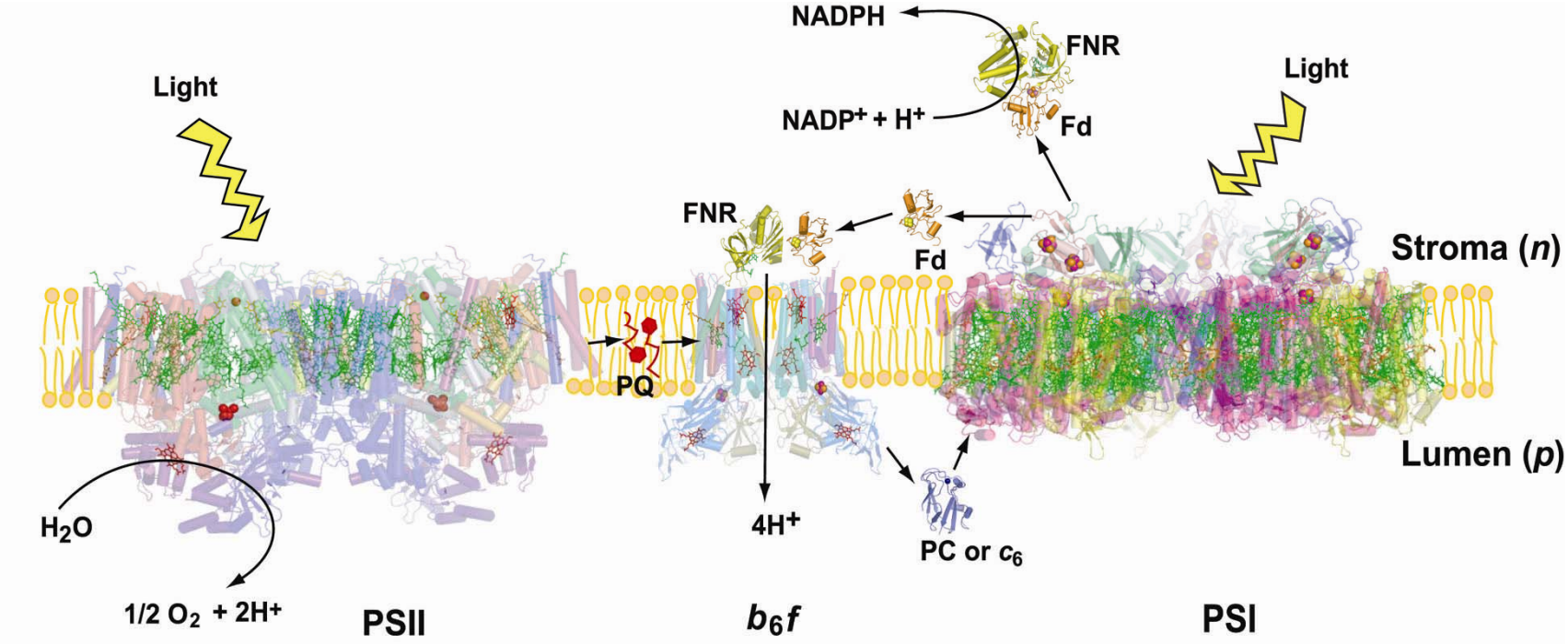
How does this capacitor work in detail, i. e., with respect to biological and biochemical detail?

Two sources of oxygenic photosynthetic membranes: (left) the chloroplast thylakoid membrane; (right) cells of the filamentous thermophilic cyanobacterium, *M. laminosus*





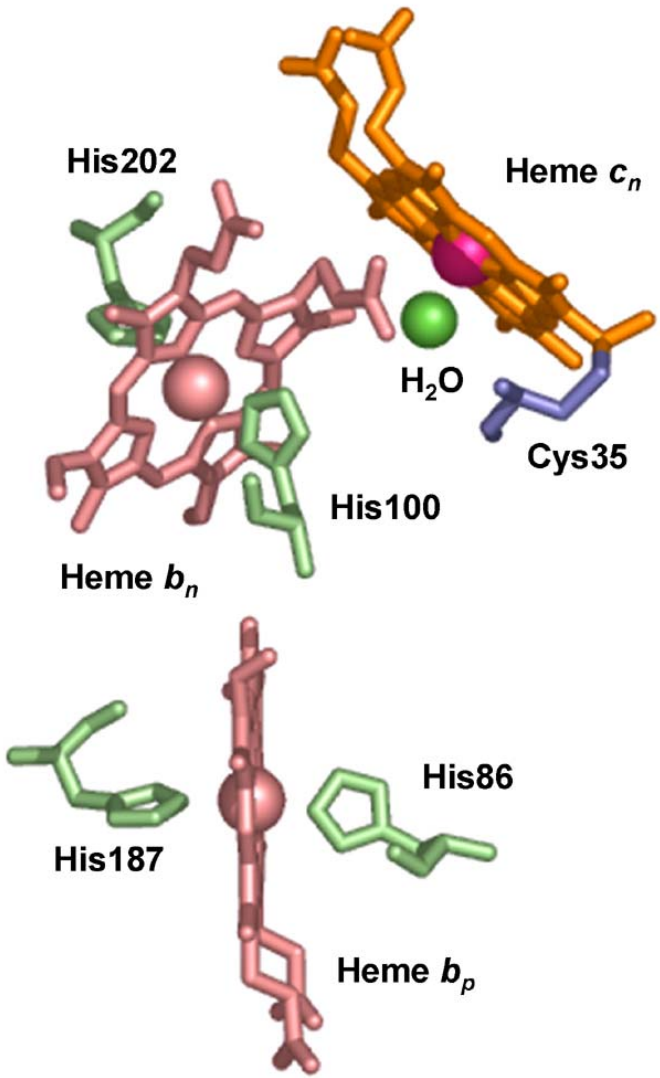
The Electron Transport Chain of Oxygenic Photosynthesis: Electron Transfer from H_2O to $NADP^+$



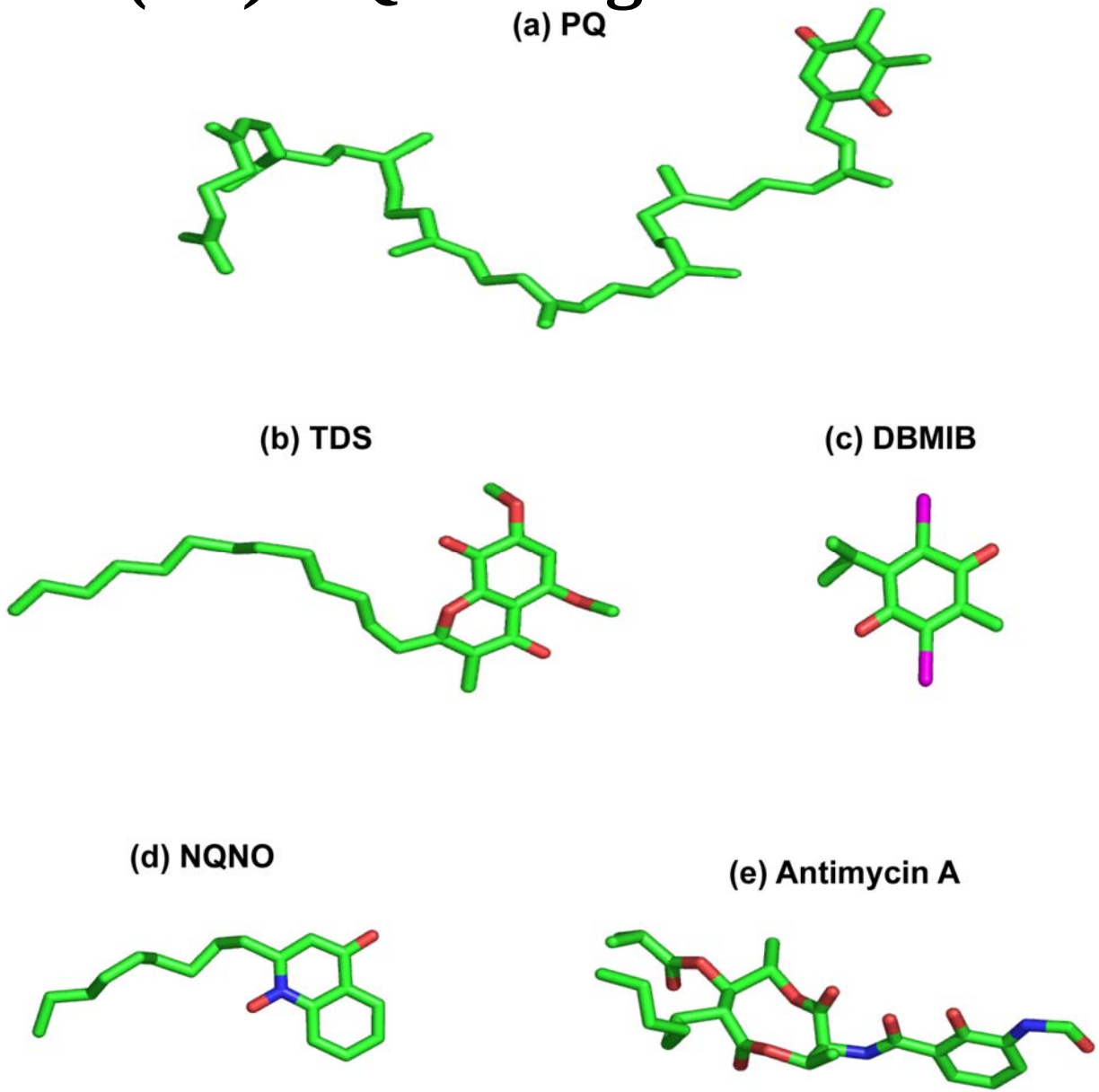
Glossary & Notation

- ***p, n*: electrochemically positive and negative sides of the membrane**
- **Electron transfer groups: hemes; ISP, iron-sulfur protein; FNR, ferredoxin:$NADP^+$ reductase**
- **Electron and proton transfer group, quinone (e.g., plastoquinone).**
- **Quinone analogue inhibitors:**
  - **(i) TDS, tridecyl-stigmatellin**
  - **(ii) NQNO, 2-n-nonyl-4-hydroxyquinoline N-oxide**
  - **(iii) DBMIB: 2, 5-dibromo-3-methyl-6-isopropyl-benzoquinone**

Hemes in the cytochrome b_6f complex



(a) Physiological plastoquinone (PQ) and *(b-e)* PQ-analogue inhibitors

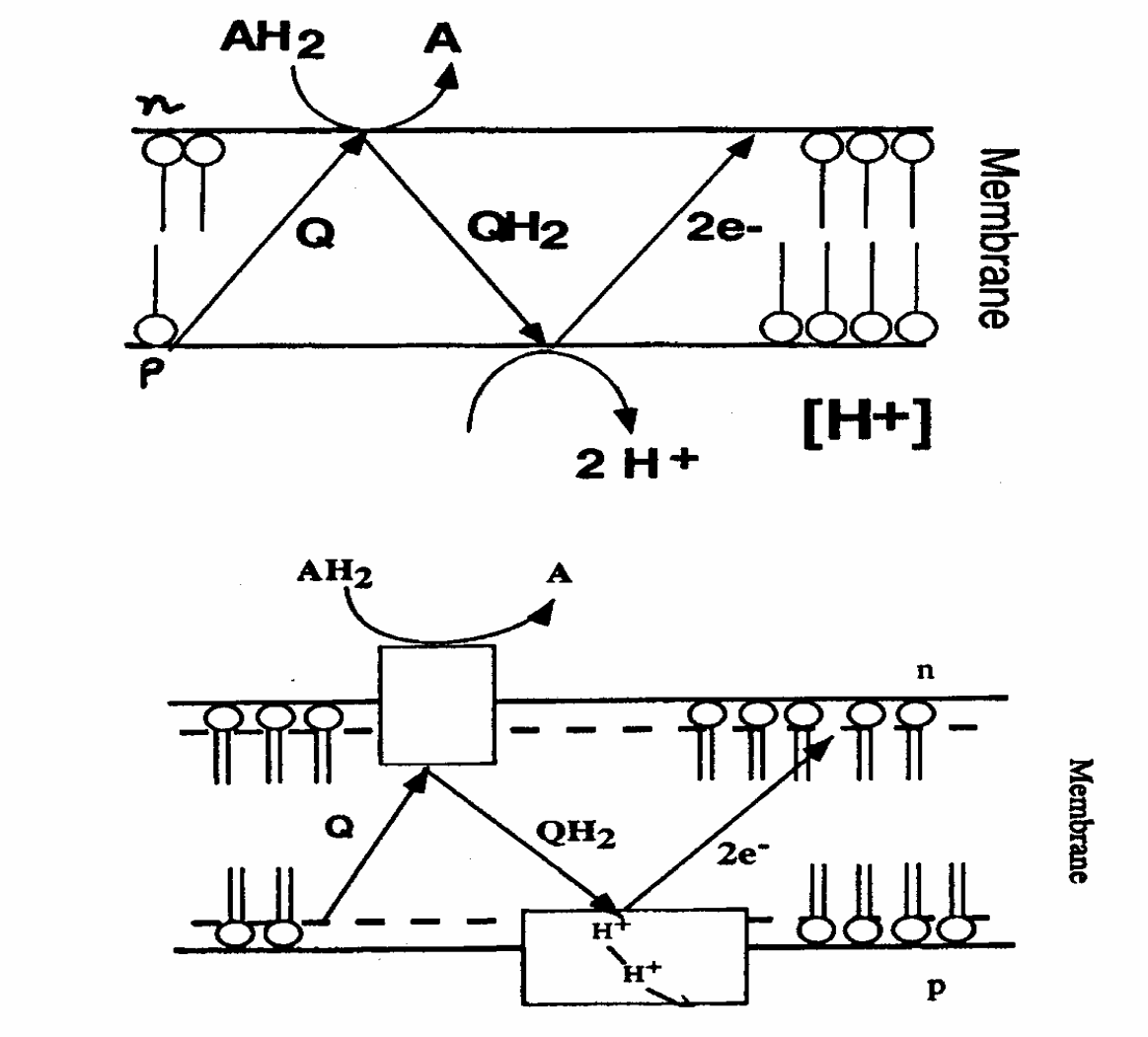


The Cytochrome b_6f Complex: Topics and Questions

Major Question: How does the *bf* complex function in energy transduction; quinone-dependent electron and proton transfer?

1. **Problems in crystallization; lipid requirement.**
2. **Properties of hetero-oligomeric integral membrane protein complex.**
3. **Quinone exchange cavity.**
4. **Novel prosthetic groups: chlorophyll *a* and β-carotene (in a "dark"complex), and a unique heme, c_n.**
5. **Binding sites of *p*- and *n*-side quinone (Q) analogue inhibitors; heme c_n inferred to be PQ-binding site.**
6. **Labyrinthine quinone transfer pathway: exchange cavity, *p*-and *n*-side binding sites.**

Quinone (Q) trans-membrane H^+ carrier (P. Mitchell, 1965; Nobel Laureate, 1978); (bottom) with membrane proteins (in boxes). Thus, electrons, protons, and quinones cross the membrane.



e^--H^+ transfer function of b_6f complex: PQH_2 oxidized on *p*-side, and reduced on *n* –side.

<u>*p*-side quinol oxidation</u>:

$PQH_2 + FeS\ (ox) \rightarrow PQ\bullet^- + FeS\ (red) + 2H^+$

$PQ^{\bullet -} + b_p\ (ox) \rightarrow PQ + b_p\ (red)$

<u>Trans-membrane electron transfer</u>:

heme b_p (red) + heme b_n (ox) $\rightarrow$ b_p (ox) + b_n (red)

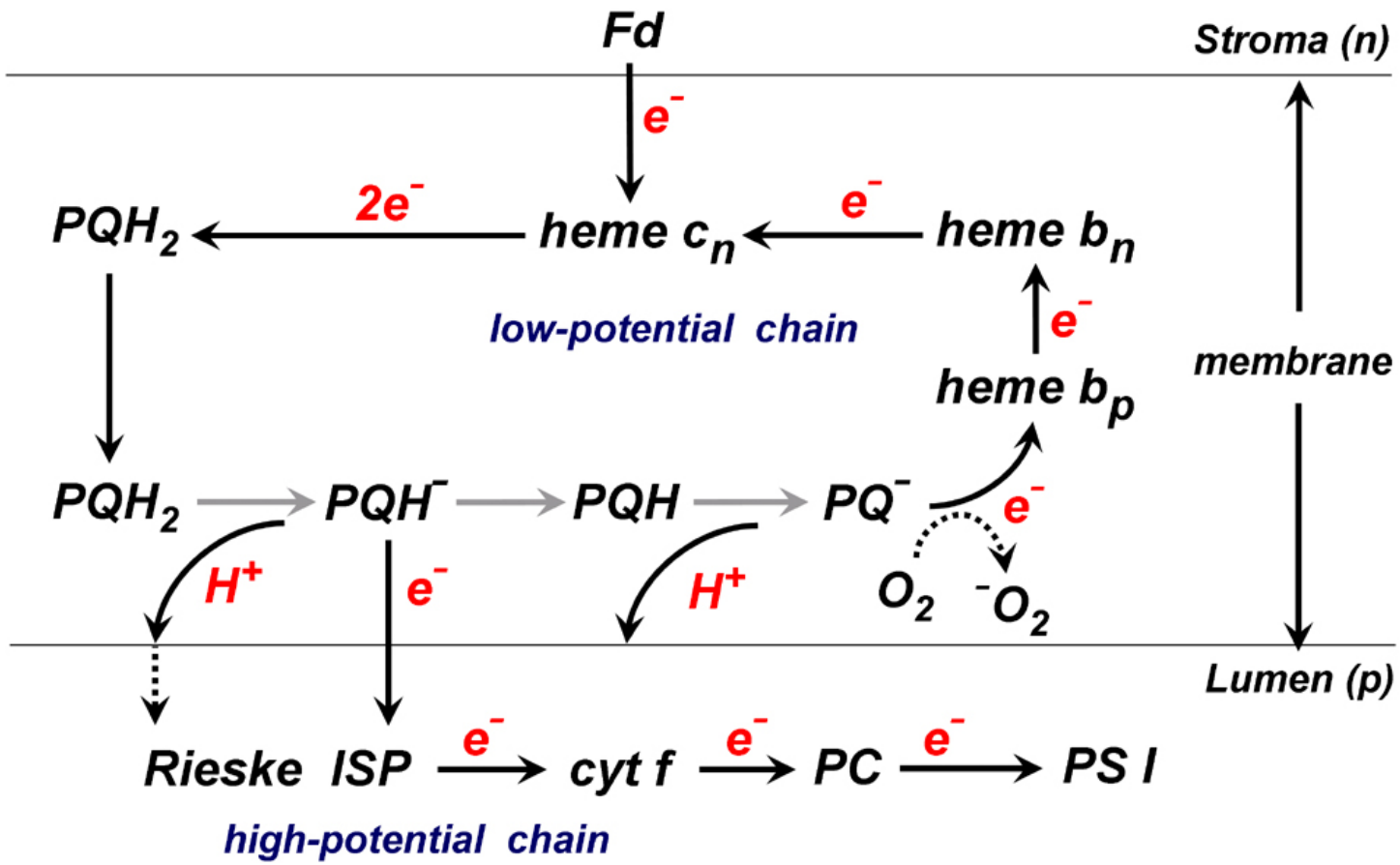
<u>n-side quinone reduction as in *bc*1 complex</u>

(i) heme b_n (red) + $PQ \rightarrow b_n$ (ox) + $PQ^{\bullet -}$

(ii) heme b_n (red) + $PQ^{\bullet -}$ + 2 $H^+ \rightarrow$ 2 b_n (ox) + PQH_2

Thus, e-, H^+, and PQ/PQH_2 must cross the complex.

Electron transfer pathway involving quinone (PQ/PQH_2) cycle in cytochrome *bf* complex



Purification: Masses (electrospray MS) of the 8 subunits of the *bf* complex from *M. laminosus*

Subunit	Measured Mass (Da)	
(I) "Large" Subunits		
Cyt *f*	32,270	
Cyt b_6	24,710	(calc., 24,268)
Rieske ISP	19,295	
Sub IV	17,529	
(FNR in spinach)	35,314	(weakly bound)
(II) "Small" Subunits		
PetG	4057	
PetM	3841	
PetL	3530	
PetN	3304	

Dimer MW = 217 kDa

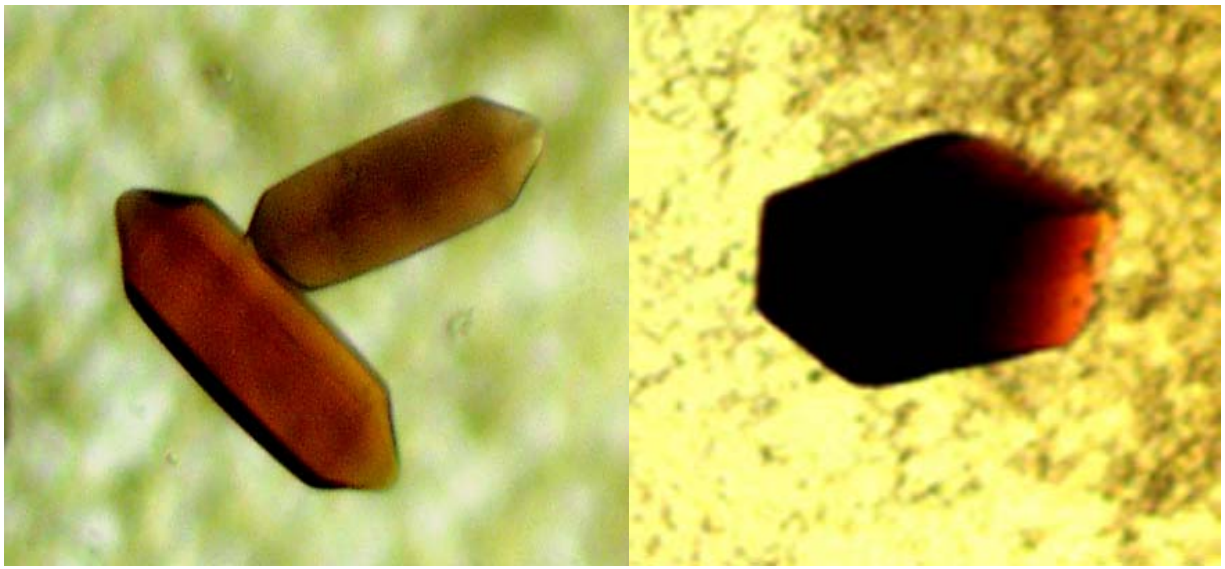
[Whitelegge et al., *Molec. Cell Proteomics* (2002) 1: 816-826]

Hetero-oligomeric proteins; problems of crystallization.

Table 1. Hetero-oligomeric integral membrane protein structures with ≤3Å resolution

Protein name	PDB-ID	Highest resolution (Å)	References
Particulate methane monooxgenase	1YEW	2.8	Lieberman *et al.* (2005) (3)
Photosynthetic reaction center from *T. tepidum*	1EYS	2.2	Nogi *et al.*, 2000 (4)
Light harvesting complex from *Rho. acidophila*	1NKZ	2.0	Papiz *et al.*(2005) (5)
Photosystem I from *T.elongatus*	1JBO	2.5	Jordan et al. (2001) (6)
Photosystem II from *T. elongatus*	2AXT	3.0	Loll *et al.* (2005) (7)
Cytochrome b_6f complex from *M. laminosus* and *C. reinhardtii*	1VF5 1Q90	3.0; 2.95 3.1	Kurisu *et al.* (2003) (8); 2.95 Å, unpublished Stroebel *et al.* (2003) (9)
Calcium ATPase from rabbit sarcoplasmic reticulum	1WPG	2.3	Toyoshima & Nomura (2002) (10)
Rotor of V-type Na^+-ATPase from *Enterococcus hirae*	2BL2	2.1	Murata *et al.* (2005) (11)
Rotor of F-type ATPase from *Ilyobacter tartaricus*	1YCE	2.4	Meier *et al.* (2005) (12)
Fumarate reductase from *Wolinella succinogenes*	1QLA	2.2	Lancaster *et al.* (1999) (13)
Formate dehydrogenase from *E. coli*	1KOG	1.6	Jormakka *et al.* (2002) (14)
Succinate:UQ oxidoreductase from porcine heart mitochondria	1ZOY	2.4	Sun *et al.* (2005) (15)
NarGHI nitrate reductase A from *E. coli*	1Q16	1.9	Bertero *et al.* (2003) (16)
Mitochondrial ADP/ATP carrier from bovine heart	1OKC	2.2	Pebay-Peyroula *et al.* (2003) (17)
Cytochrome *c* oxidase-aa_3 from *P. denitrificans* and bovine heart	1AR1 1OCC	2.8	Iwata *et al.* (1995) (18) Tsukihara *et al.* (1996) (19)
Cytochrome oxidase ba_3 from *T. thermophilus*	1EHK	2.4	Soulimane *et al.* (2000) (20)
Cytochrome bc_1 complex from yeast and bovine heart	1EZV 2FYU 2A06	2.3 2.3 2.1	Hunte *et al.* (2000) (21) Esser *et al.* (2006) (22) Huang *et al.* (2005) (22)

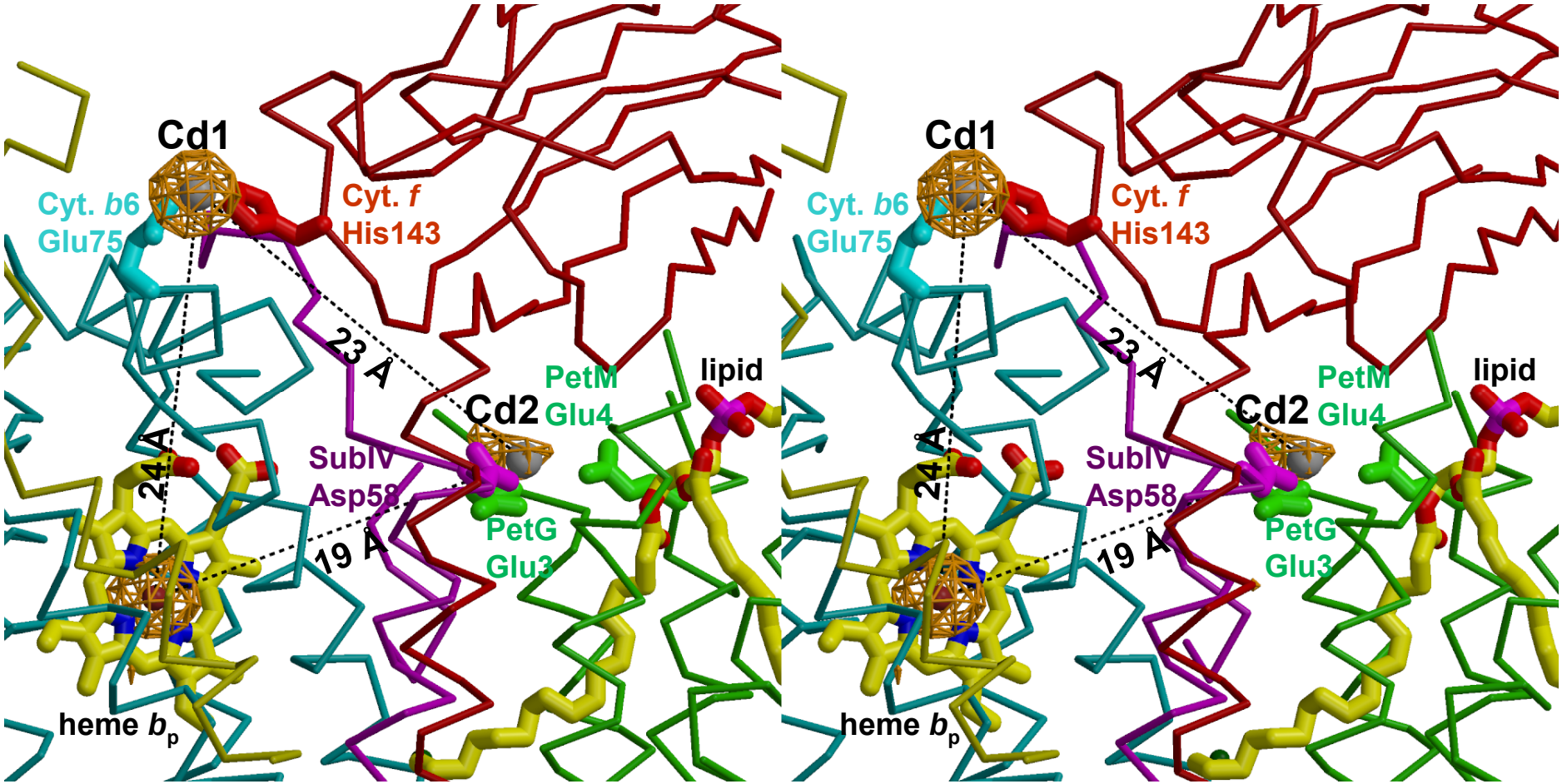
Crystals of b_6f complex are brownish-red (because of additional pigments)



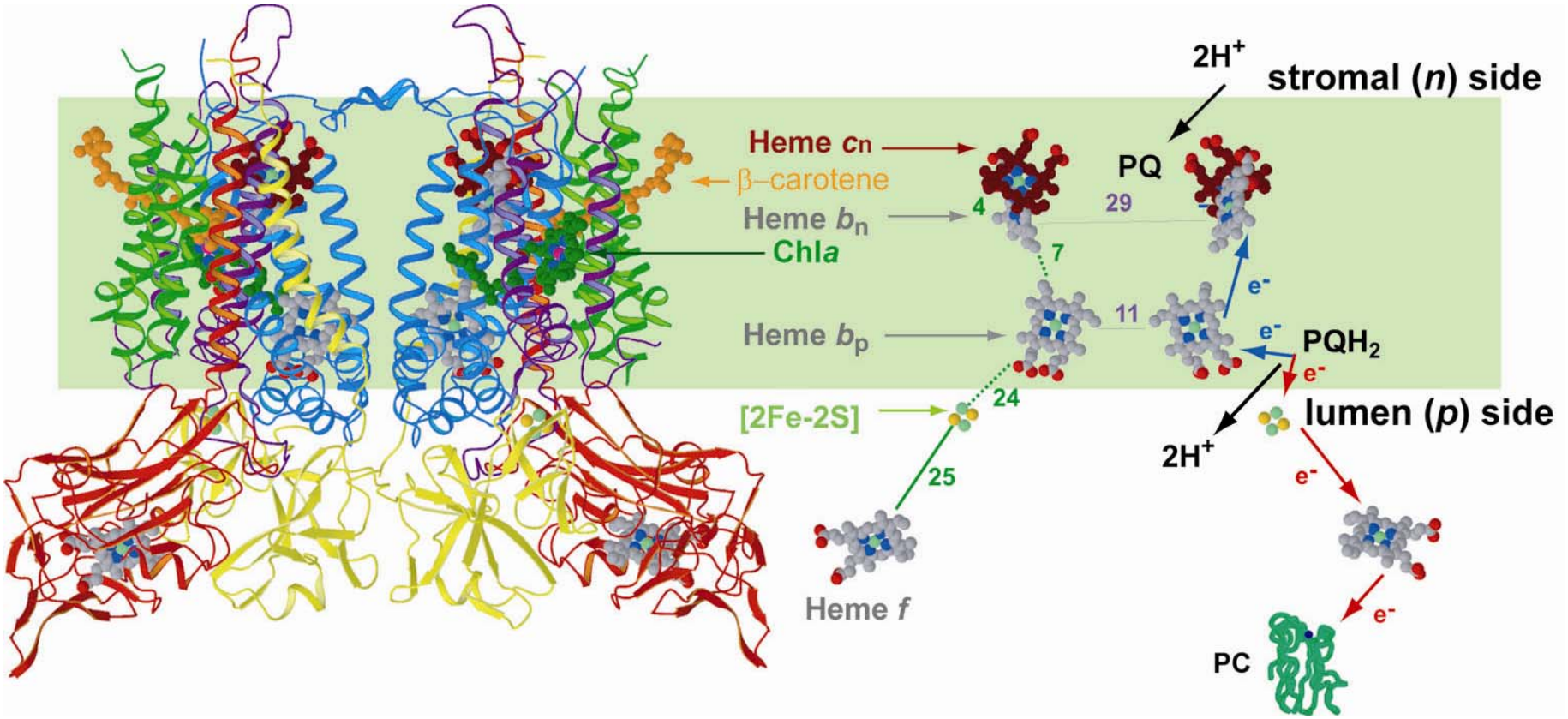
(a) Native: (3.00 Å); R = 0.222; R_f = 0.268); pdb: 2E74
(b) TDS (3.40 Å); R = 0.201; R_f= 0.258; pdb id: 2E76
(c) DBMIB, 3.8 Å [pdb id: 2D2C]
(d) NQNO (3.55 Å); R = 0.224; R_f = 0.273; pdb: 2E75

Crystallization required addition of lipid

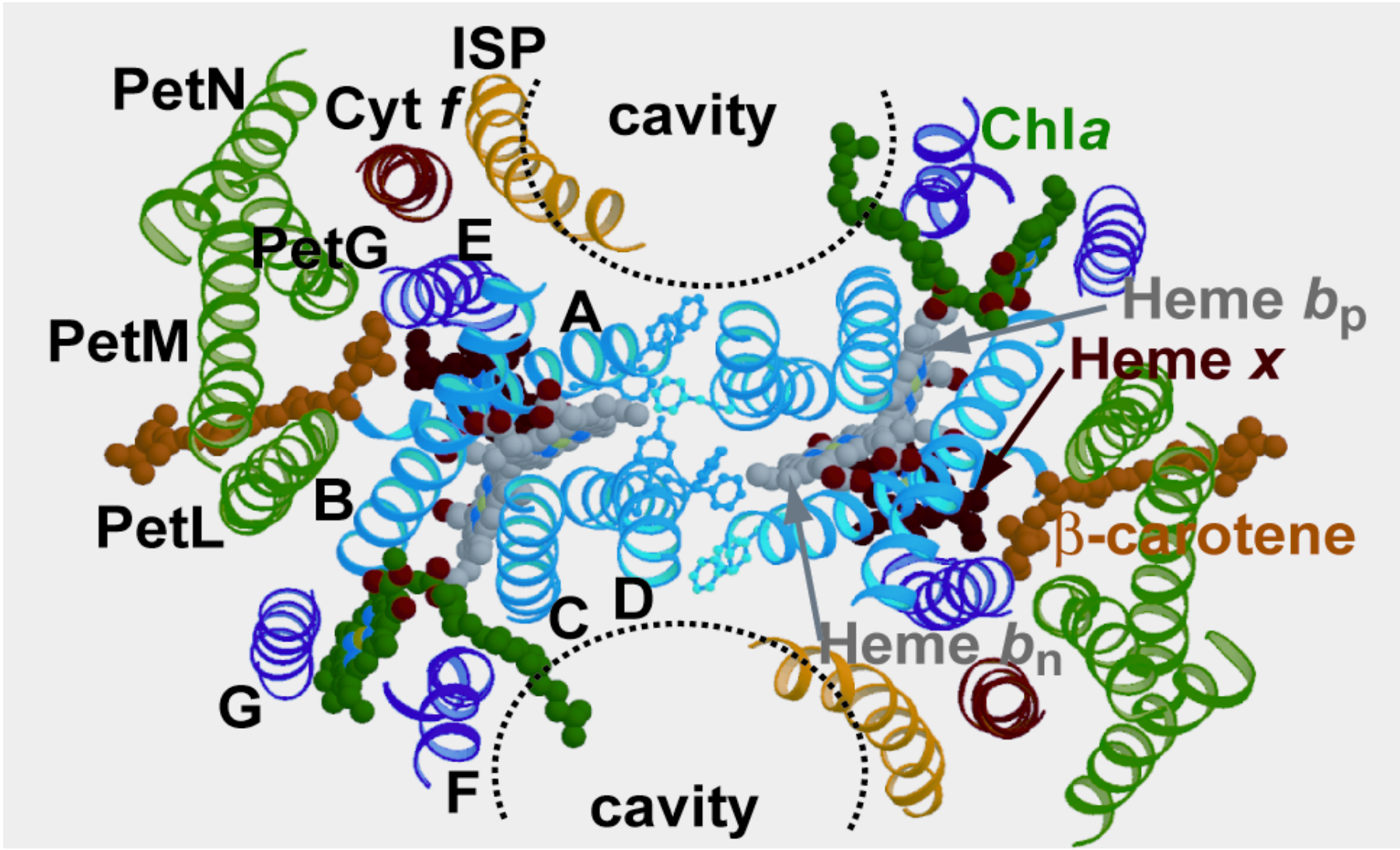
Resolution of native complex greatly improved in presence of Cd2+: *p*-side binding sites

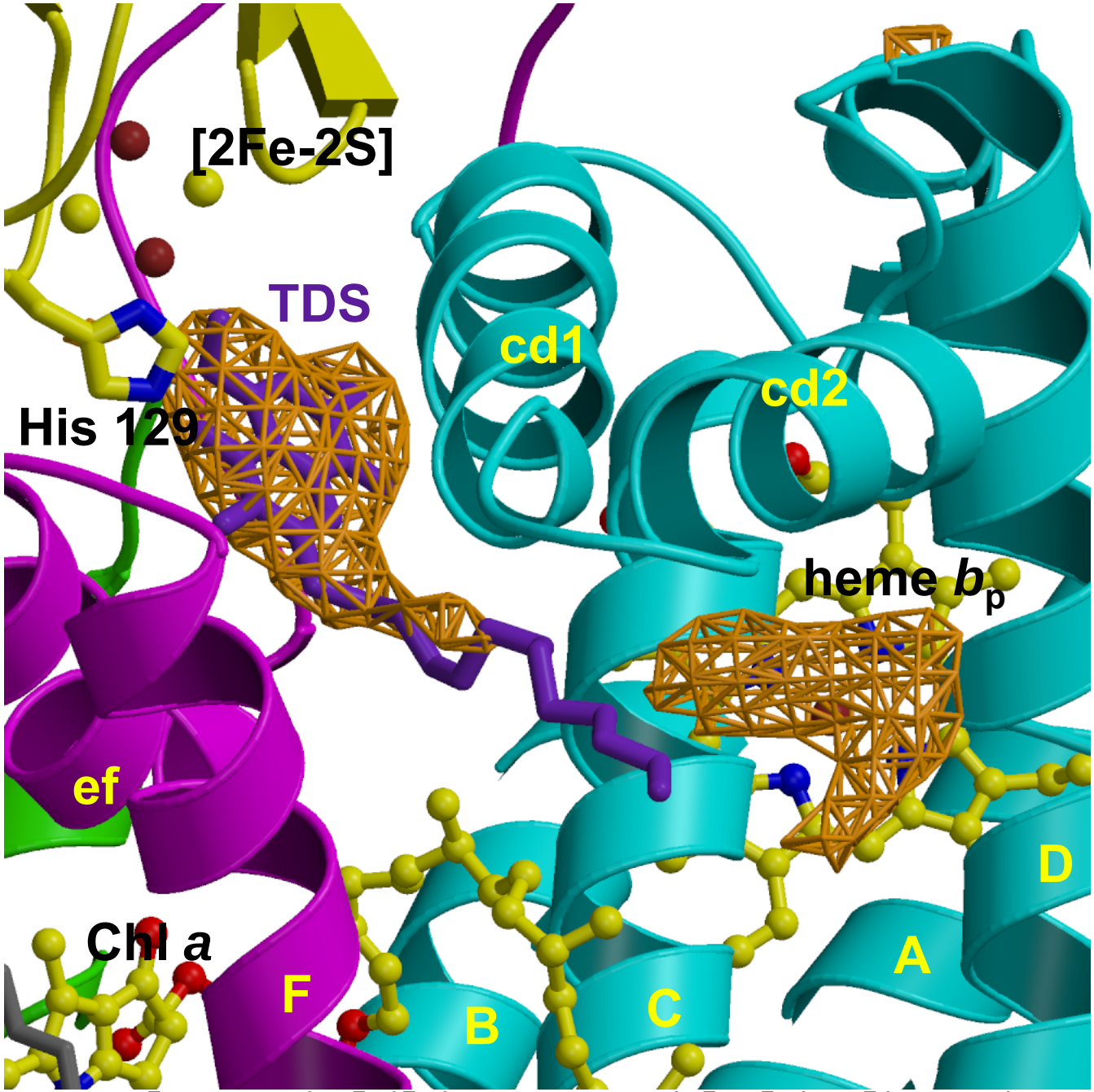


Dimeric b_6f complex: 26 TM helices; 8 subunits per monomer; 7 redox or pigment groups (4 hemes, 1 [2Fe-2S] cluster, 1 Chl *a*, 1 β-carotene); 30 x 25 x 15 Å inter-monomer quinone exchange cavity connects Q_pH_2 oxidation site in 1 monomer with Q_n reduction site in the other.



p-side view of intra-membrane domain along membrane normal showing 26 TM helices, and 3 heme, 1Chl *a*, 1 β-Car prosthetic groups



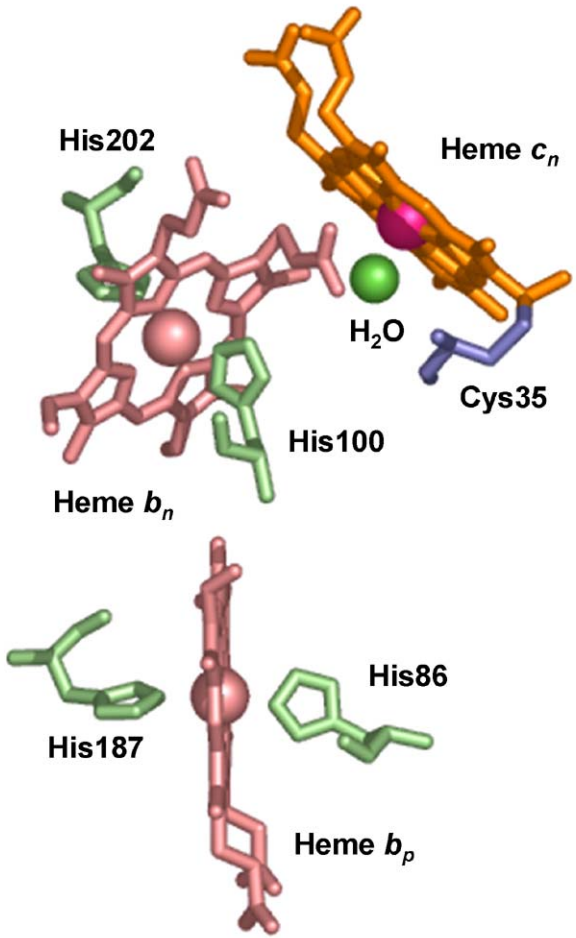


Quinone analogue inhibitors: p-side binding site of TDS

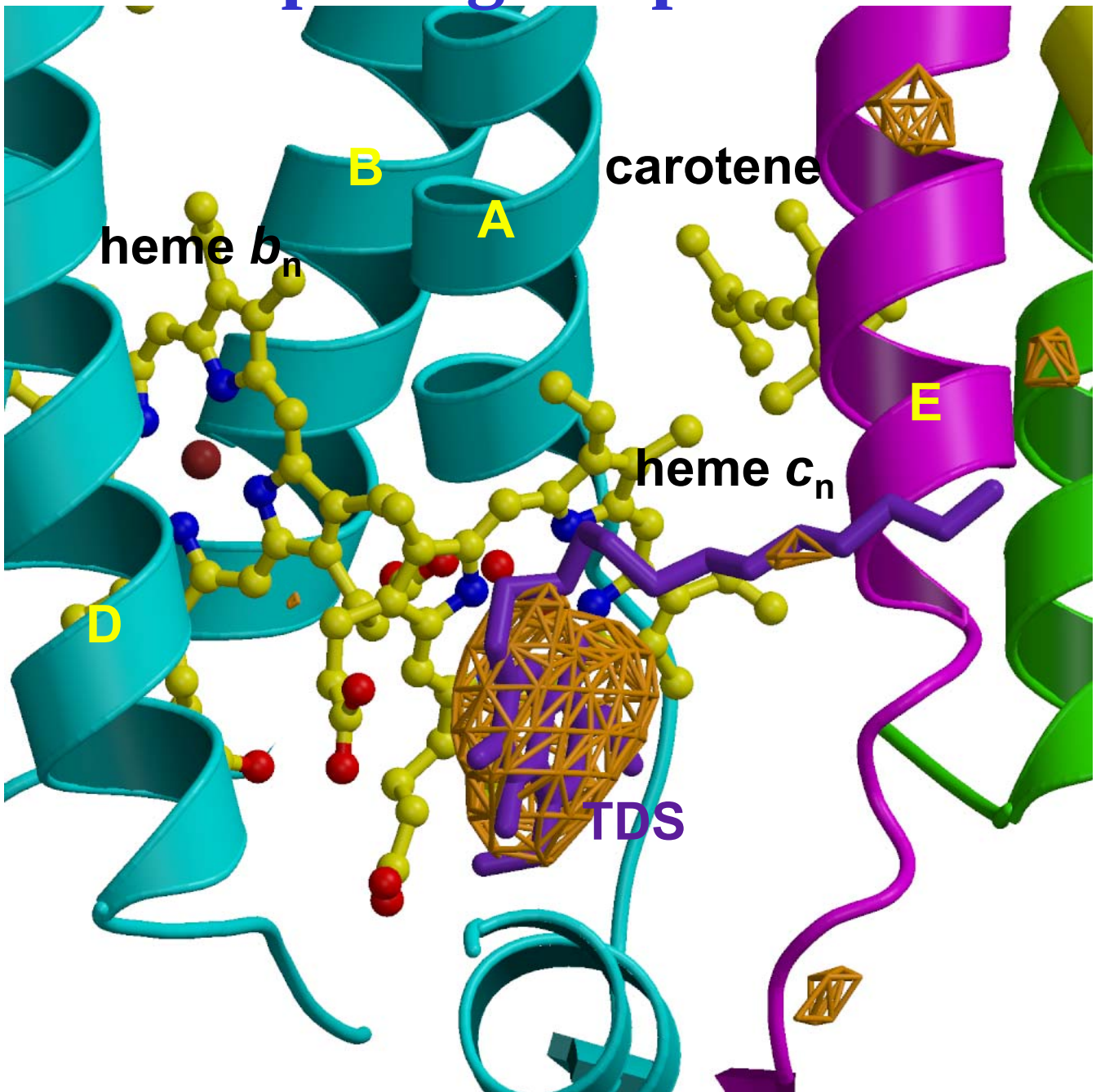
A novel *n*-side heme (c_n) in the b_6f complex

- A CO-reactive heme on the *n*-side of the membrane that equilibrates with heme b_n was identified spectrophotometrically through Soret band absorbance changes (Lavergne, 1983; Joliot and Joliot, 1988), and given the notation, "G."
- A heme "c_n" was found near heme b_n in ~ 3.0 Å X-ray structures of the b_6f complex from the green alga, *C. reinhardtii* (Stroebel *et al*., 2003), and the thermophilic cyanobacterium, *M. laminosus* (Kurisu *et al*., 2003).
- EPR studies have shown heme c_n to be associated with high 'g' values(Zhang *et al*., 2004; Zatsman *et al*., 2006).
- What is the function of heme c_n?

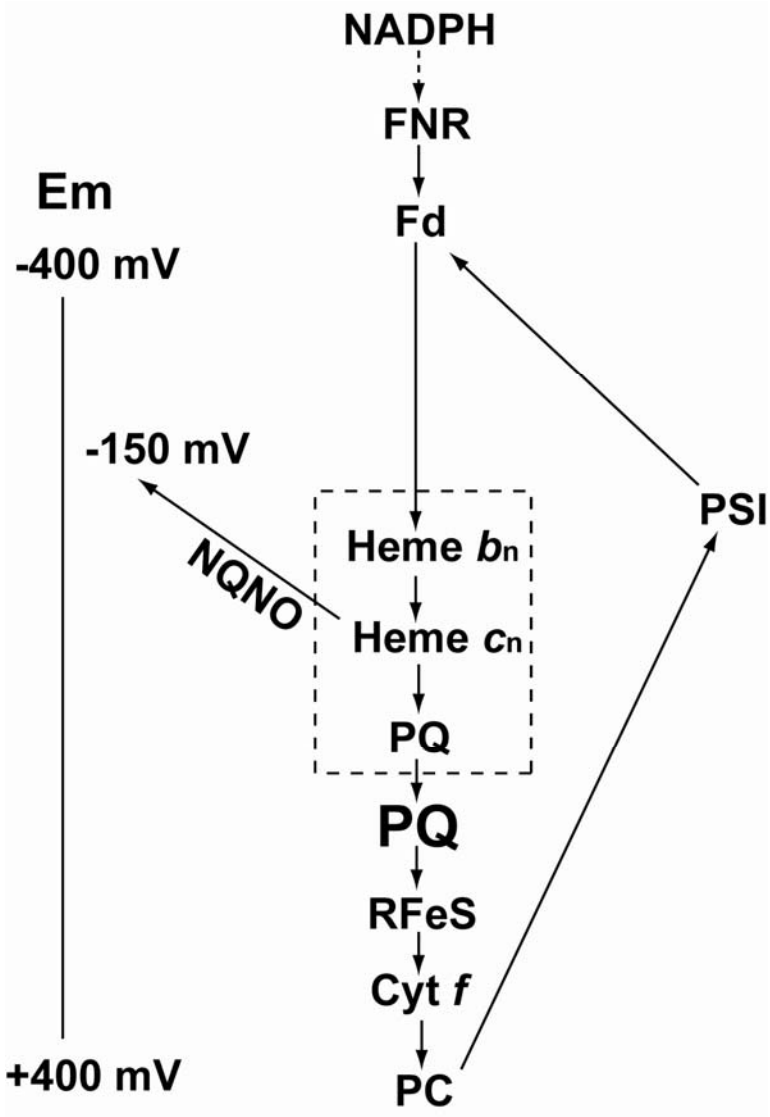
Novel redox prosthetic group: heme c_n (n-side) covalently bound to cyt b_6 Cys35, close (4 Å) to heme b_n, no amino acid side chain as axial ligand; H_2O connects heme b_n propionate and heme c_n Fe.



TDS binds to open ligand position of heme *c*n



n-side binding of quinone analogue inhibitors implies that PQ bound to heme *c*n is the entry and interface to the PQ pool, from which PQH2 serves as the donor to the [2Fe-2S] cluster



Modified Q cycle in cytochrome b_6f complex including heme c_n

p-side quinol oxidation

$PQH_2 + FeS\ (ox) \rightarrow PQ^{\bullet -} + FeS\ (red) + 2H^+$

$PQ^{\bullet -} + b_p\ (ox) \rightarrow PQ + b_p\ (red)$

trans-membrane electron transfer

heme b_p (red) + heme b_n (ox) $\rightarrow$ b_p (ox) + b_n (red)

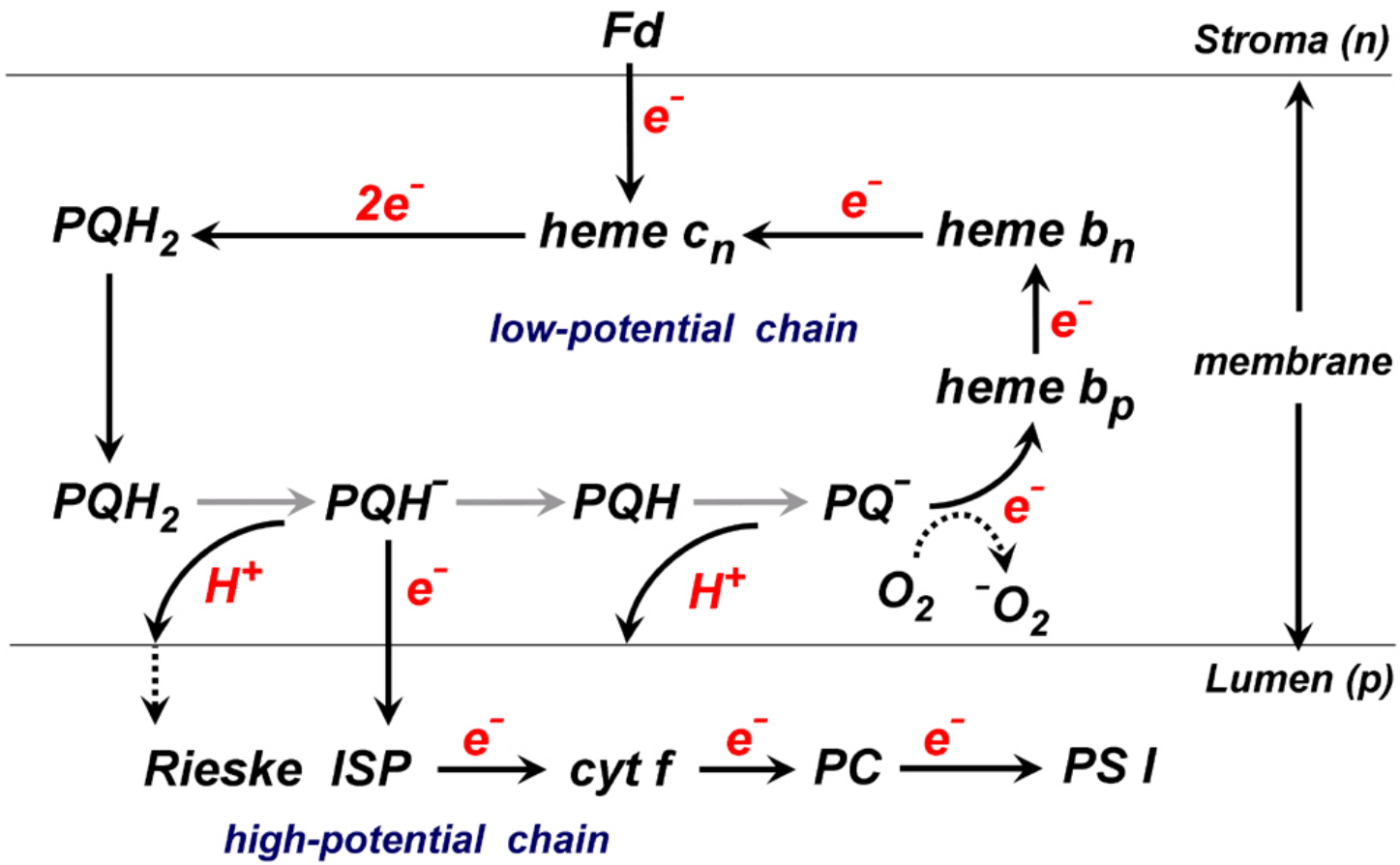
n-side 2 electron reduction of plastoquinone (PQ)

(i) b_n (r) / c_n (ox)/PQ (o) $\rightarrow$ b_n (o)/c_n (r)/PQ(o)

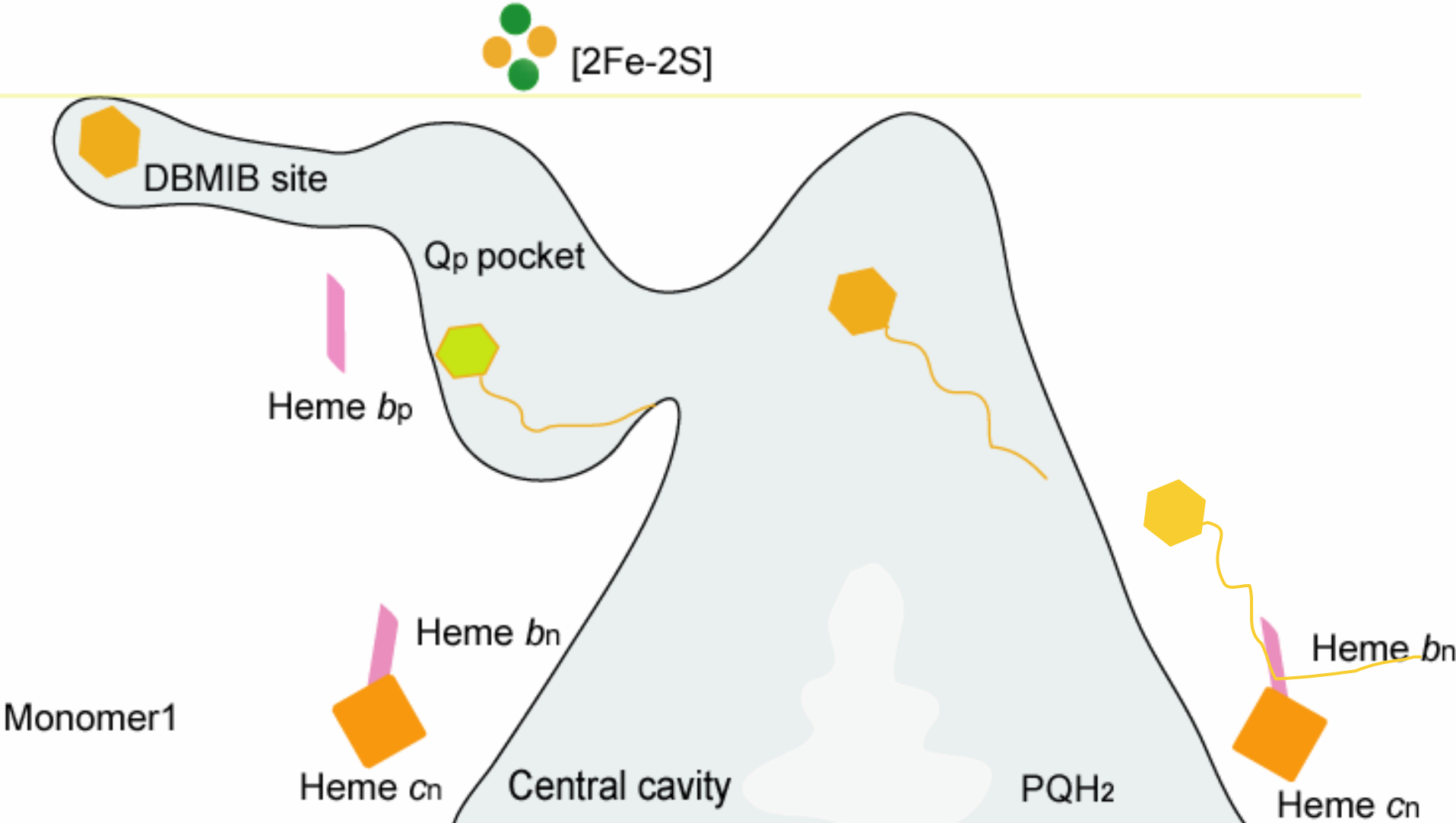
(ii) Fd (r) + b_n (o)/c_n (r)/PQ(o) $\rightarrow$
Fd(o) + b_n (r)/c_n (r)/PQ(o)

(iii) b_n (r)/c_n (r)/PQ(o) + 2 H^+ $\rightarrow$
b_n(r)/c_n(r)/PQ(o) + PQH_2

Electron transfer pathway involving quinone (PQ/PQH_2) cycle in cytochrome *bf* complex



Quinone transfer through *b6f* complex: labyrinthine pathway through lipophilic cavity/exit portal; distal (19 Å from His129) DBMIB binding site



Summary

- **3.00 Å native structure of hetero-oligomeric 8 subunit, 220 kDa dimeric b_6f complex with 8 different prosthetic groups. Central core conserved in evolution.**
- **Movement of Q/QH_2 across complex is not simple "flip-flop," but a "labyrinthine" quided diffusion through caverns and portals.**
- **3 novel prosthetic groups: Chl *a*, β-carotene; novel high spin heme.**
- ***n*-side: unique high spin heme c_n; proximal to b_n; PQ axial ligand displaced by quinone analogue NQNO; b_n-c_n-PQ electron wire, donor to PQ pool. The "Q cycle" mechanism functions differently in mitochondria and chloroplasts.**

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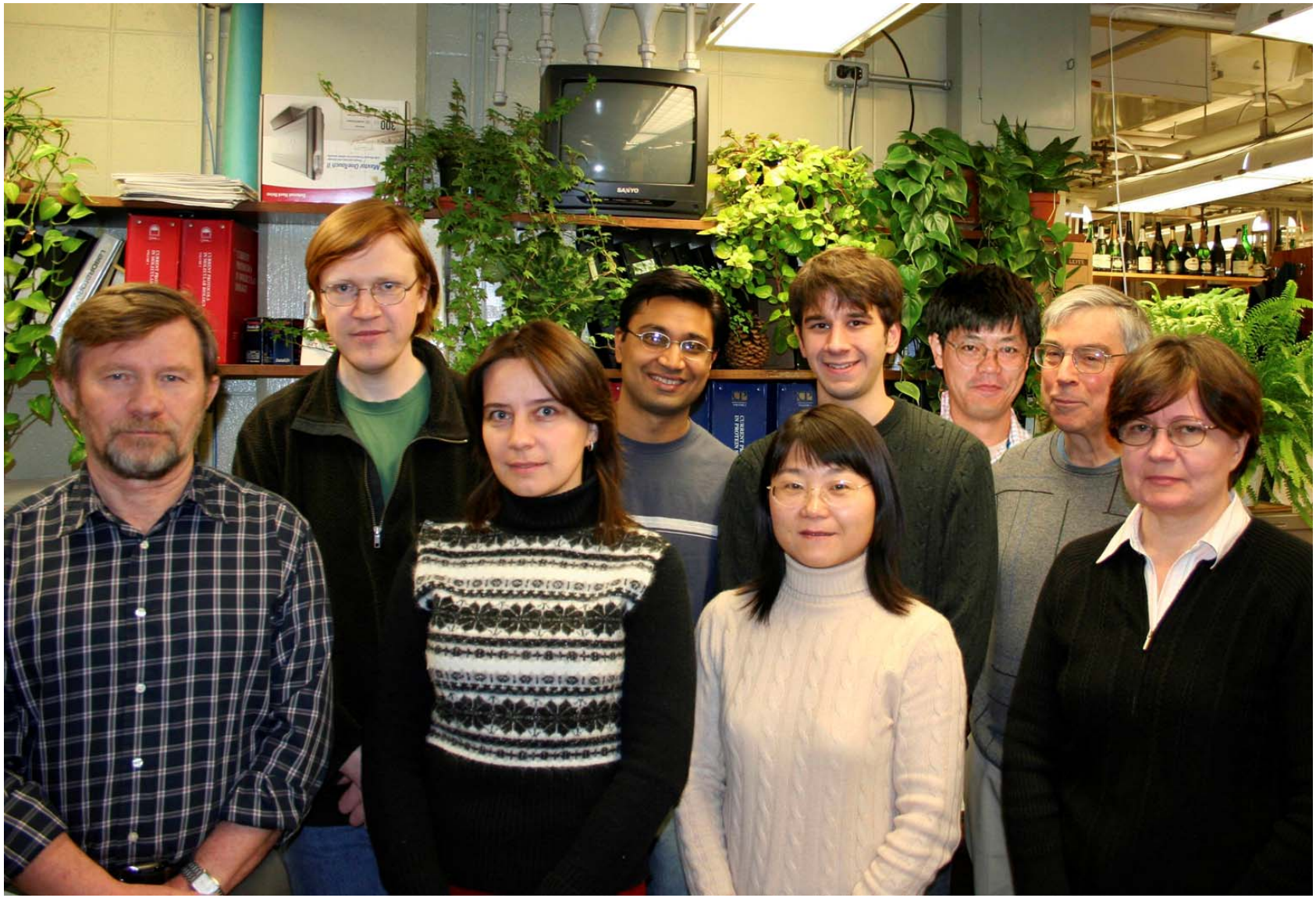
We thank you for your attention!
(except for the two guys sleeping in the back)



Questions & Answers

Questions 2

Questions 3