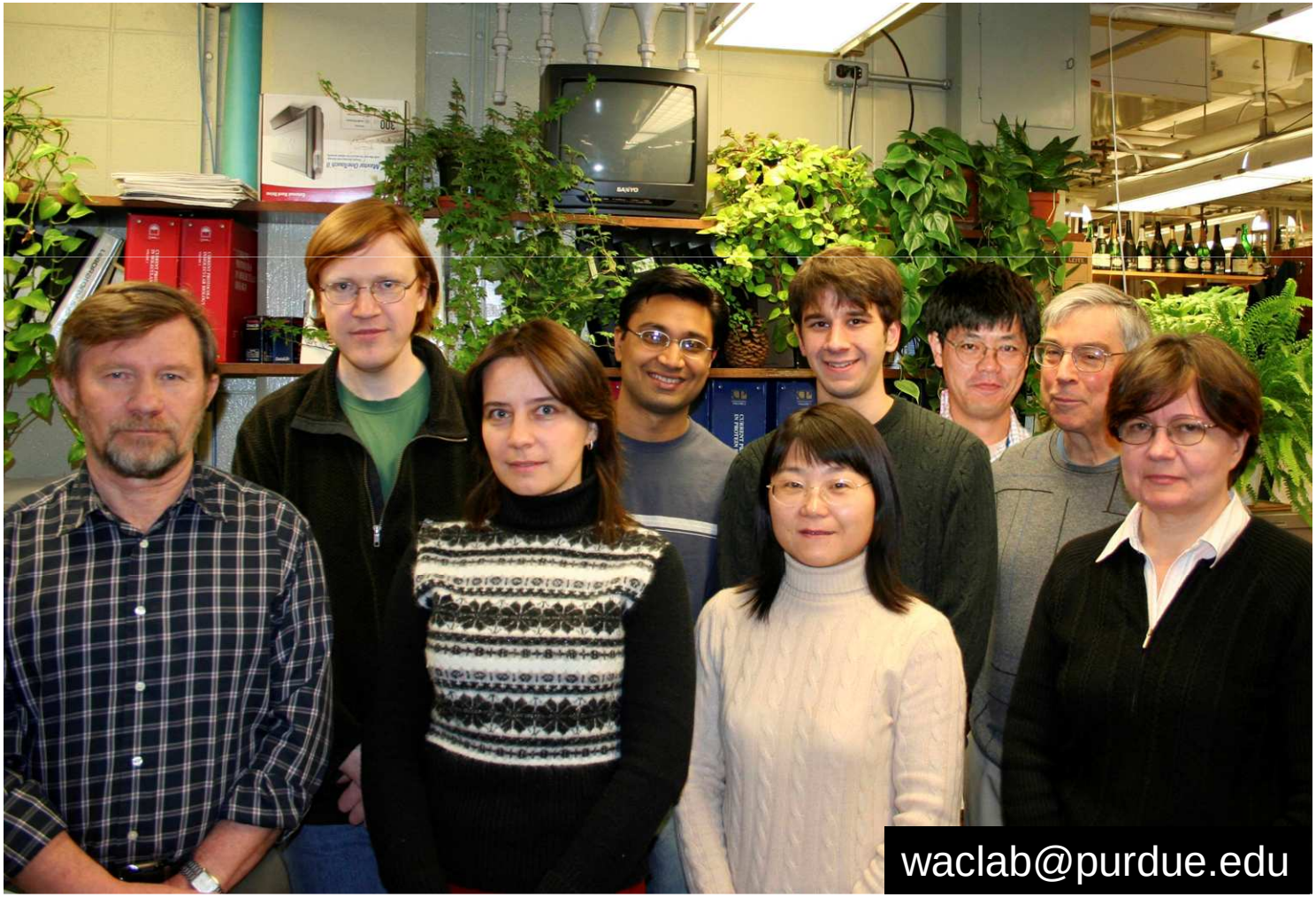
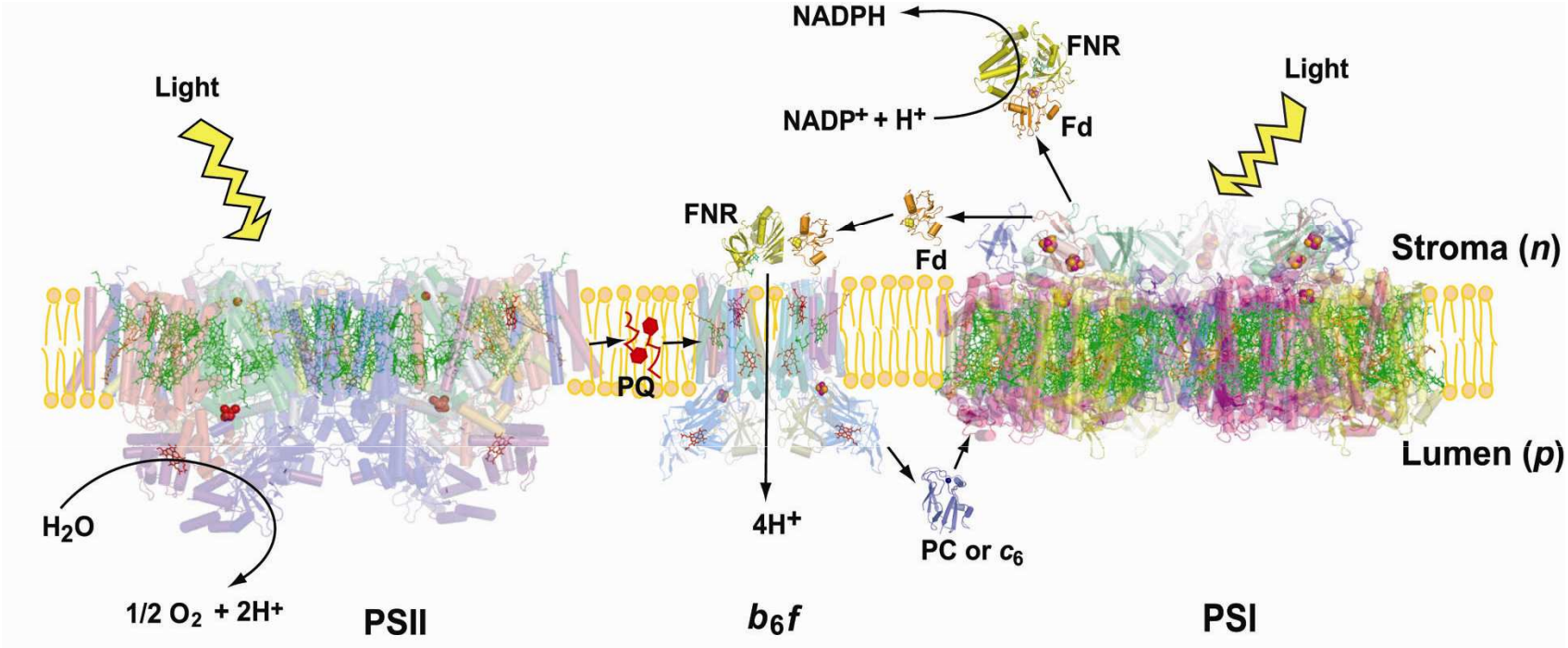
A Limited Bioinformatics Analysis Results in an Improved Cyanobacterial Source for Structure-Function Studies of the Cytochrome b_6f Complex

D. Baniulis, E. Yamashita, J. Whitelegge, H. Zhang, and W.A. Cramer



Hetero-oligomeric Membrane Protein Structures in Oxygenic Photosynthesis



Crystal structures of the cytochrome b_6f complex

Kurisu, G. *et al*. (2003) *Science*, 302: 1009-1014

Stroebel, D. *et al*. (2003) Nature, 426: 413-418 from *C. reinhardtii*

Yan, J. *et al*. (2006) PNAS, 103: 69-74

Yamashita, E., *et al*. (2007) J. Mol. Biol, 370: 59-72.

waclab@purdue.edu

Outline

- **Crystallization of b6f complex**
- **Problems (proteolysis, delipidation); unique role of the cyanobacterium, *Mastiocladus laminosus;* but, no genetics**
- **Results:**

  3.0 Å structure of 220 kDa native complex; symmetric dimer built around Q/QH2 exchange cavity with 8 subunits, 13 TM helices, 7 prosthetic groups per monomer (4 hemes, 1 Fe_2S_2 cluster, 1 Chl *a*, 1 β-carotene).

  Unique heme c_n, no aa side chains as axial ligands; unique g = 12 EPR spectral band.

  3 stuctures obtained of b_6f co-complexed with quinone analogue inhibitors: DBMIB, NQNO, stigmatellin.

  Stg is both n- and p-side inhibitor; Stg and NQNO are axial ligands of heme c_n, defining n-side PQ reduction site.
- **Evolution of b_6f complex; non-photosynthetic firmicutes**
- **How to solve proteolysis problem, obtain a genetically tractable strain?**

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Masses (electrospray MS) of the 8 subunits of the b_6f 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	
PetN	4057
PetM	3841
PetG	3530
PetL	3304

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

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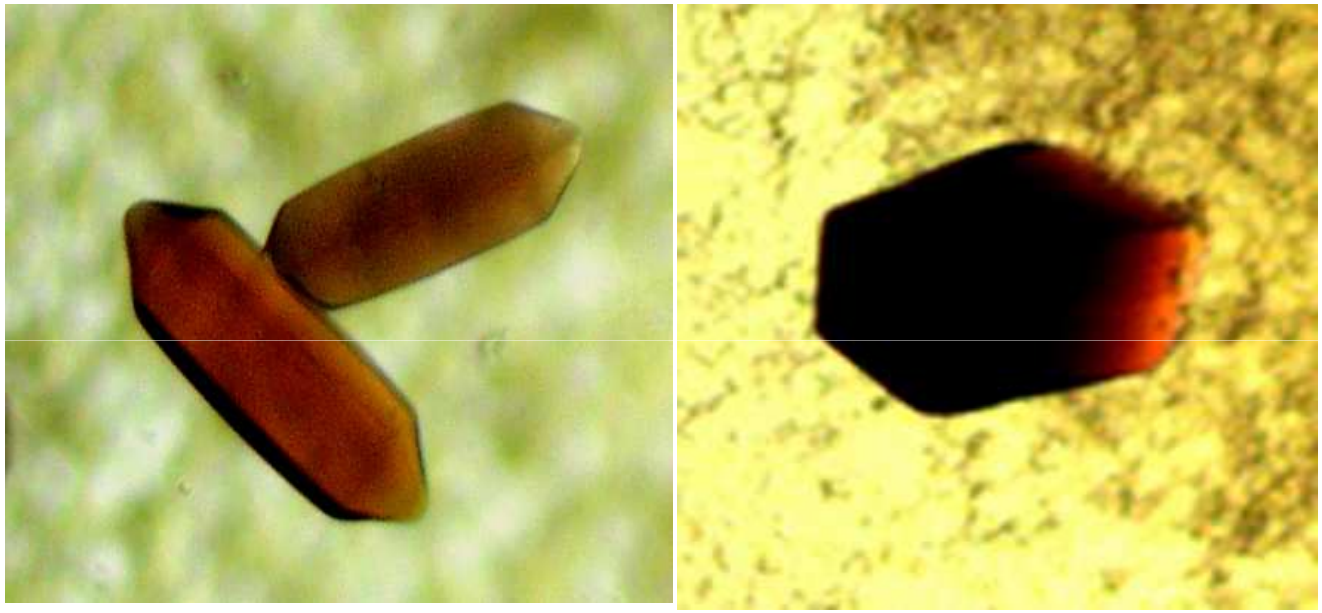
Increased crystallization rate was achieved by lipid addition: approx. 10 lipids (DOPC or PG) per cytochrome *f*. Crystals such as those shown below grew overnight.



(a) Native [Cd^{2+}] (3.00 Å); R = 0.222; R_{free} = 0.268 (pdb: 2E74)
(b) TDS (3.40 Å); R = 0.201; R_{free}= 0.258 (pdb: 2E75)
(c) NQNO (3.55 Å); R = 0.224; R_{free} = 0.273 (pdb: 2E76)
(d) DBMIB, 3.8 Å [pdb: 2D2C]

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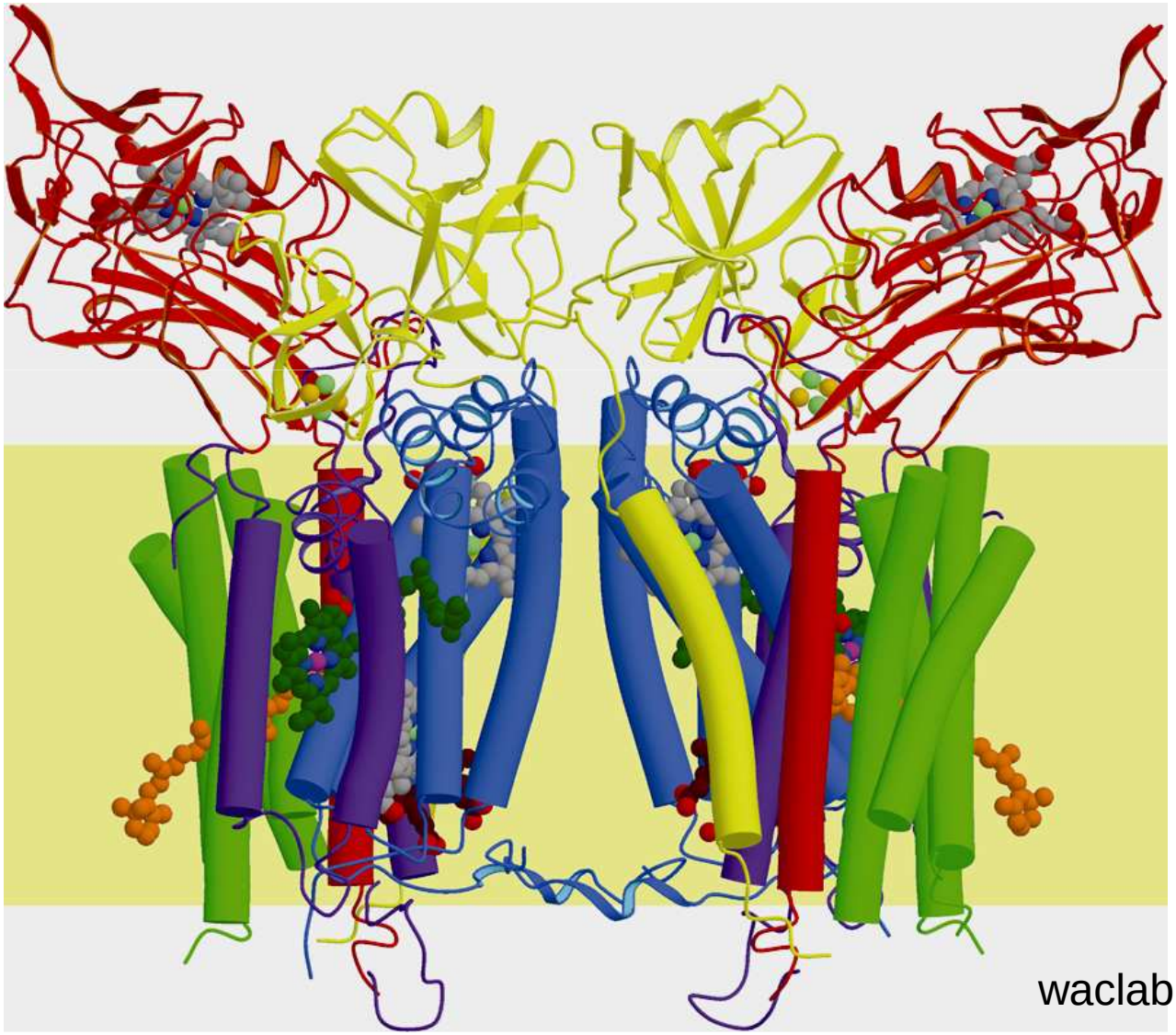
Dimeric b_6f complex: 26 TM helices; 8 subunits per monomer; 7 prosthetic groups (4 hemes, 1 [2Fe-2S] cluster, 1 Chl *a*, 1 β-carotene); central "quinone exchange cavity"; domain swapping of ISP; exposed ISP flexible loop - protease site



Lumen (*p*)

Stroma (*n*)

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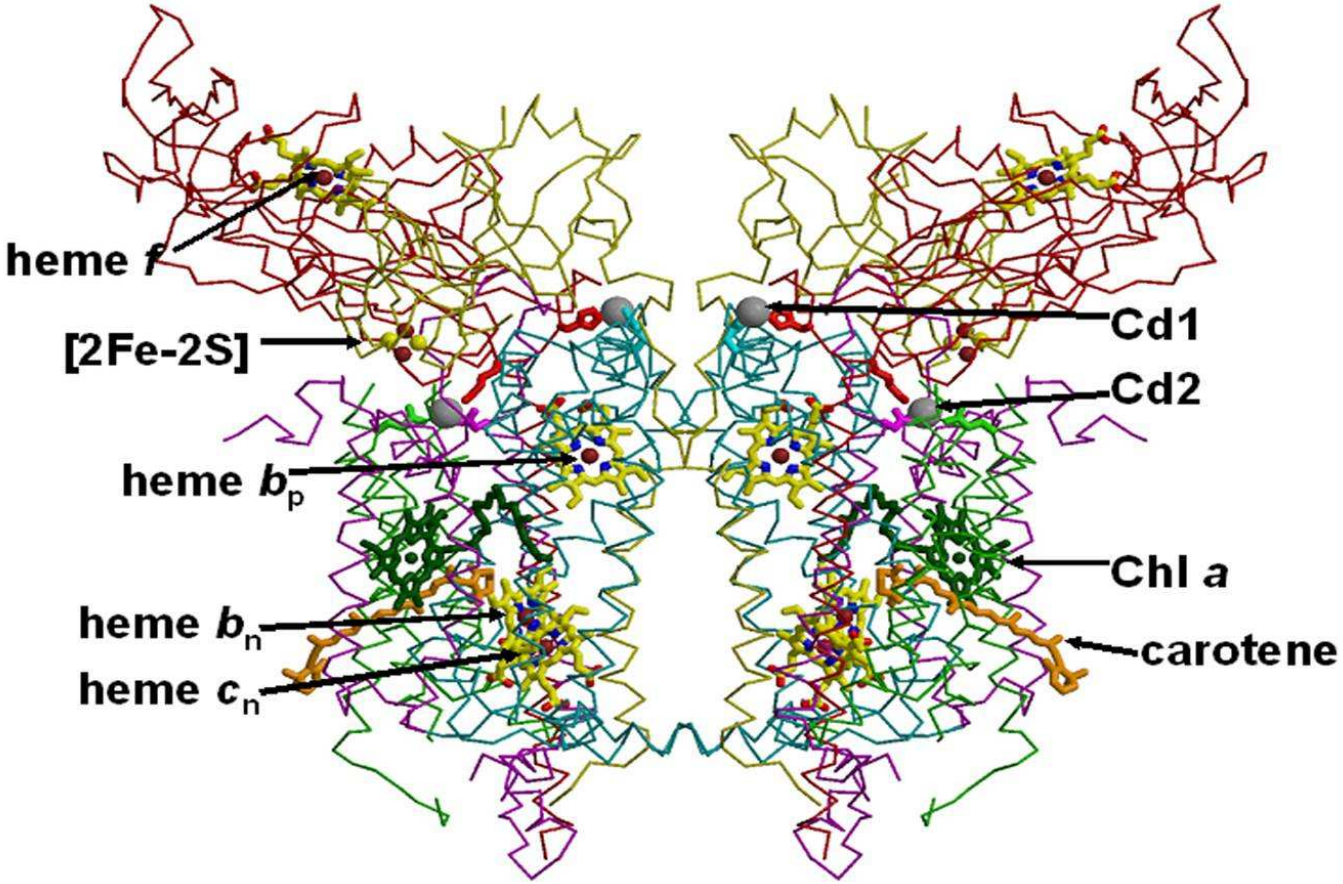
3.0 Å native structure of dimeric b_6f complex from *M. laminosus*, obtained in the presence of Cd^{2+};

Minimal Function of the dimer: "Quinone exchange cavity"

How does the quinone navigate across the cavity?



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e^--H^+ Transfer Function: PQH_2 oxidized on *p*-side; PQ reduced on *n* –side; according to conventional "Q cycle" (as in bc_1)

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 quinone reduction**

(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

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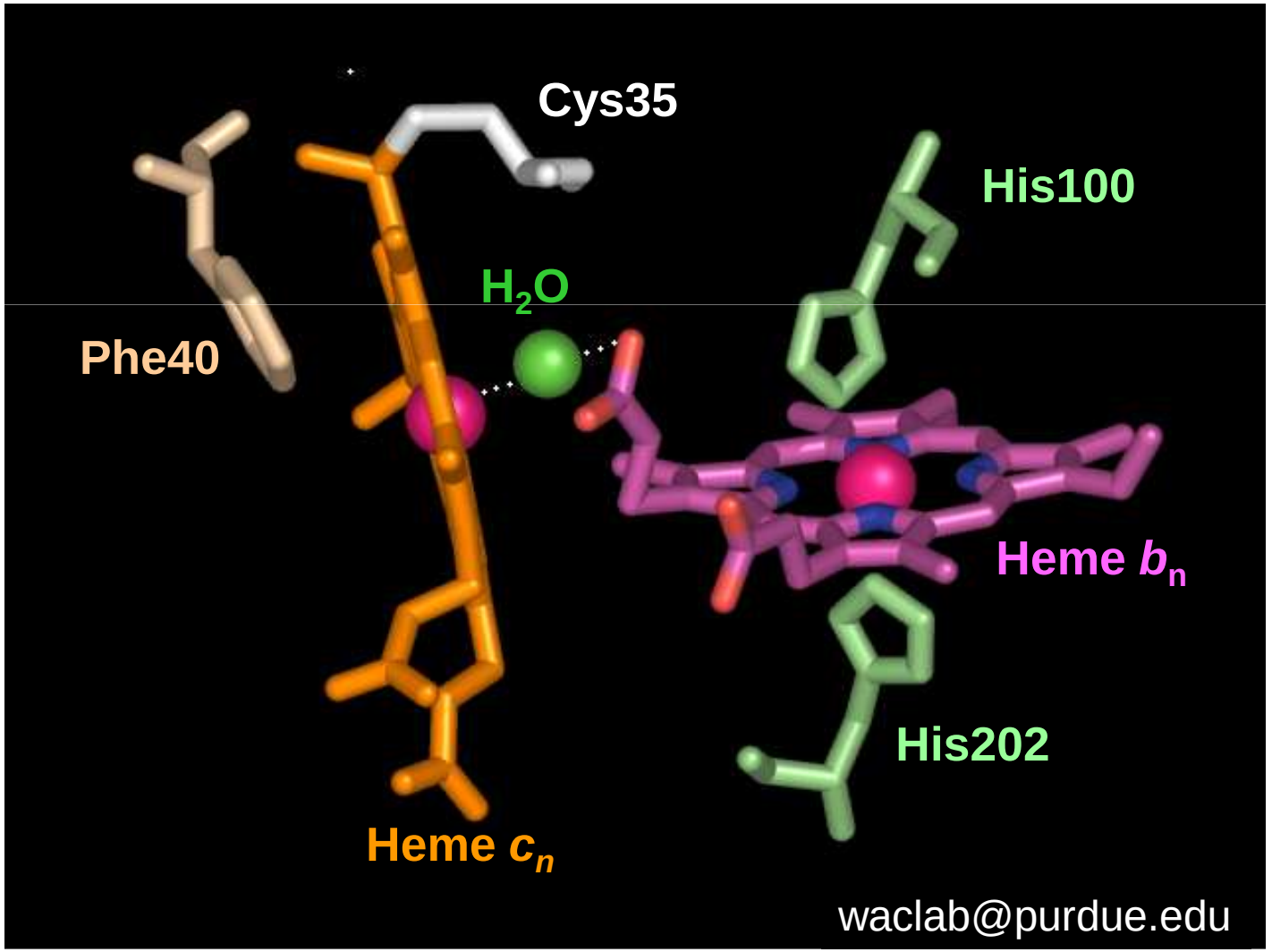
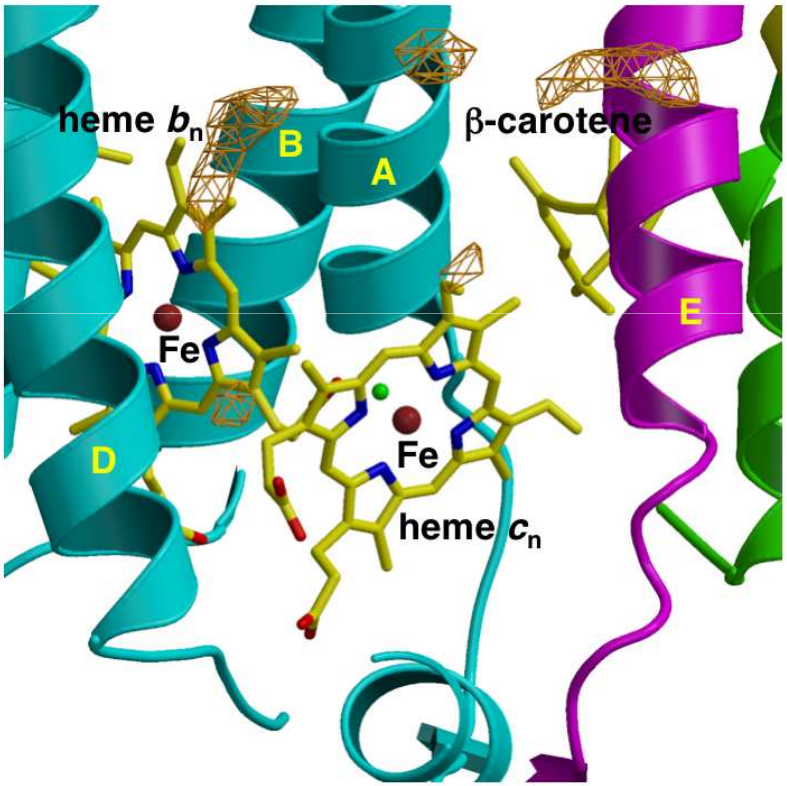
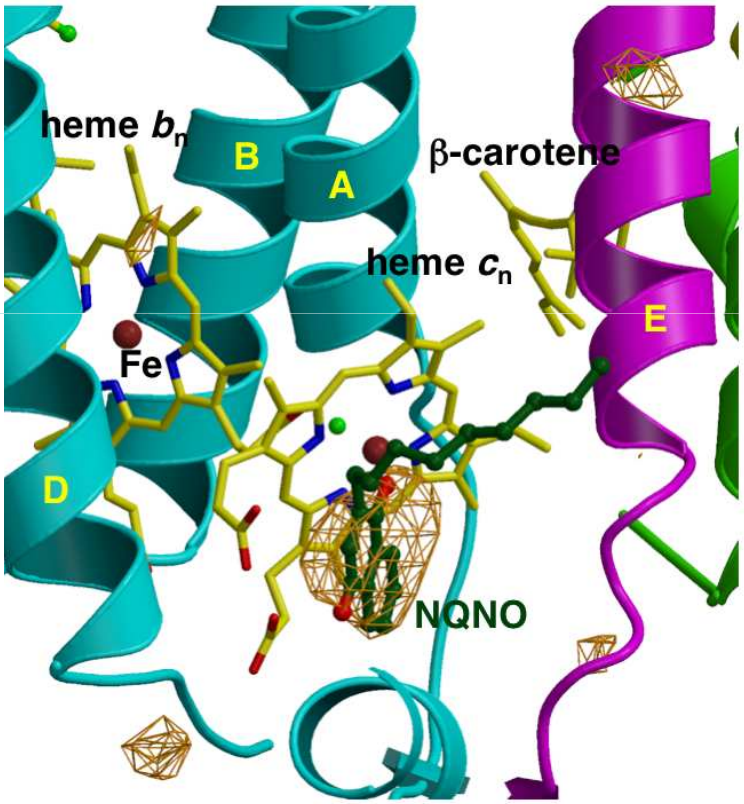
Novel redox prosthetic group: heme c_n (n-side) covalently bound to cyt b_6 Cys35, close (4 Å) to heme b_n; no amino acid side chain as axial ligand; H_2O connects heme b_n propionate and heme c_n Fe.



Function of heme c_n: quinone analogue inhibitor, NQNO, binds at free axial position of heme c_n





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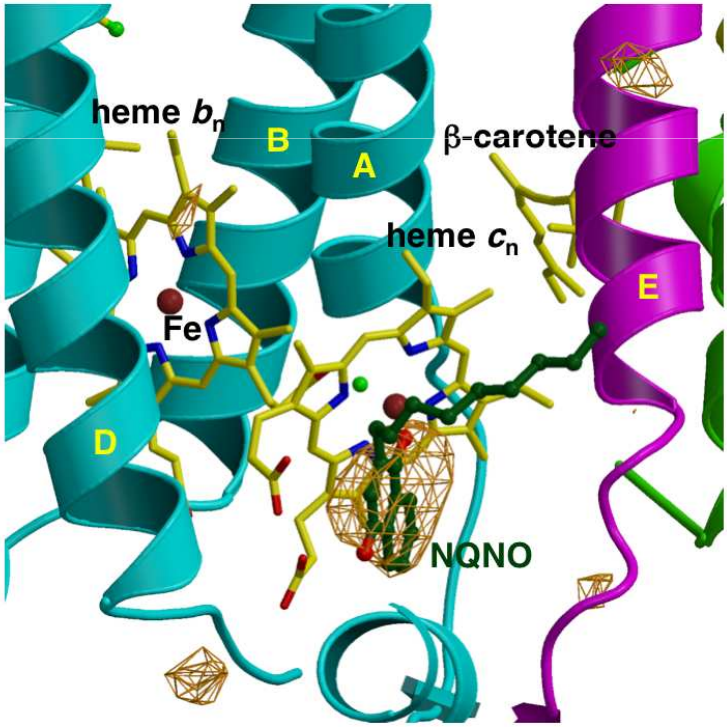
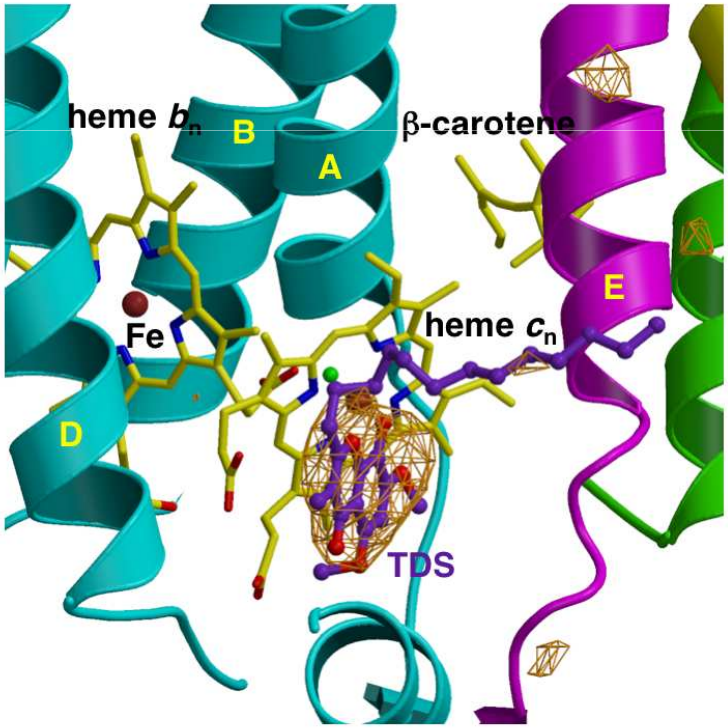
In fact, 2 quinone analogue inhibitors, NQNO and tridecyl-stigmatellin (TDS), are ligands of heme c_n.

Implication: physiological ligand of heme c_n is bound plastoquinone





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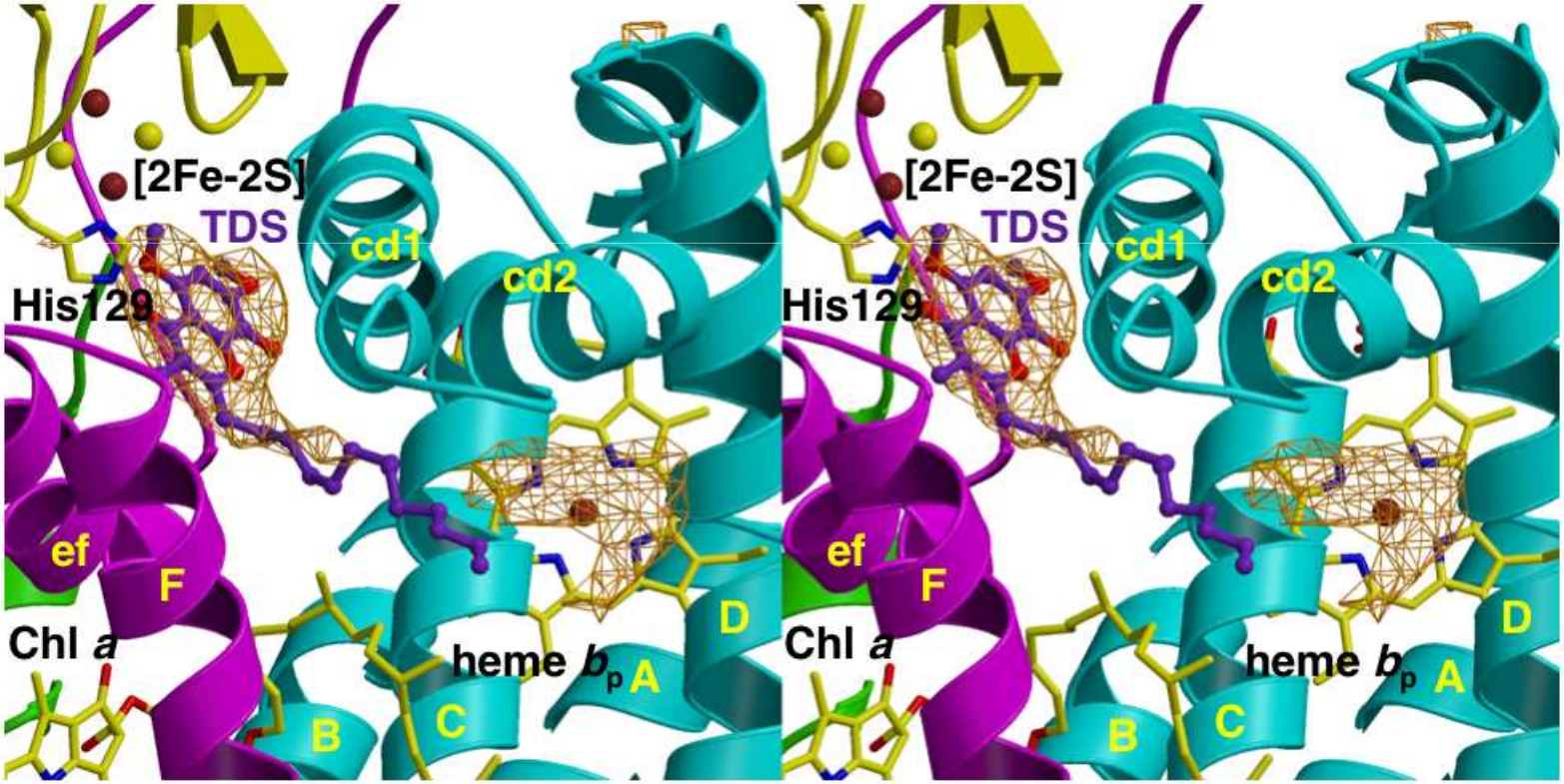
Quinone analogue inhibitor, TDS, binds near His-129 ligand of Rieske iron-sulfur protein; as in bc_1



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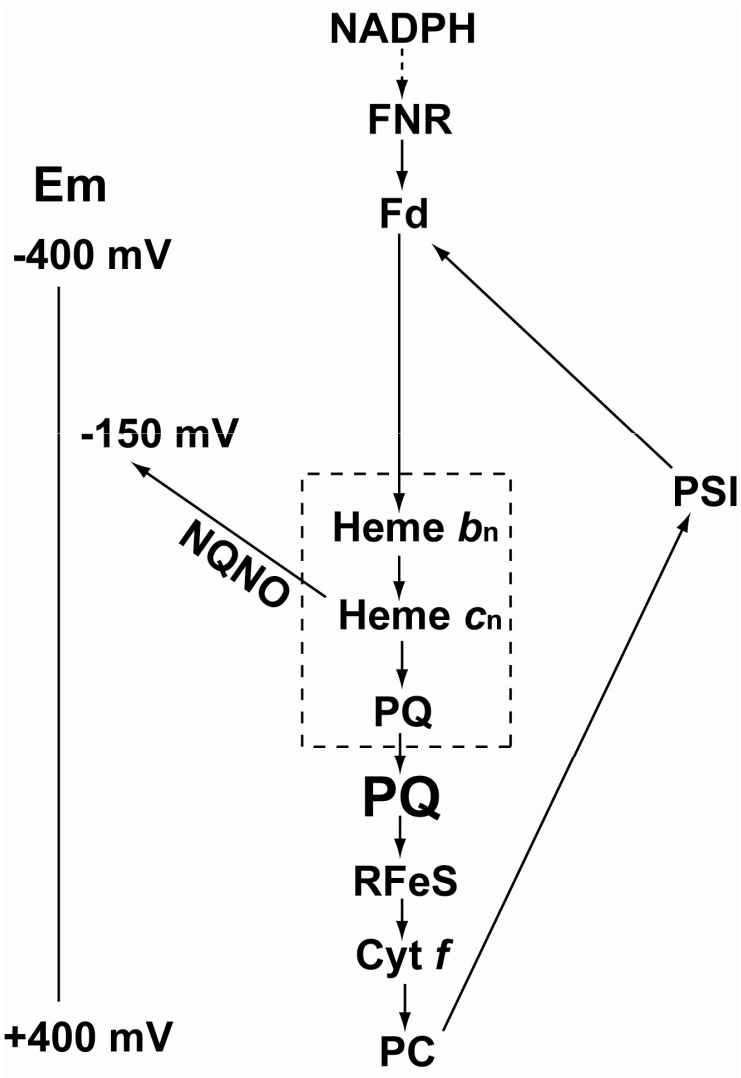
Electron transfer pathway; PQ bound to heme c_n provides *n*-side entry to PQ pool; NQNO inhibits by causing a -200 mV shift in Em of heme c_n (Alric *et al*., 2005).



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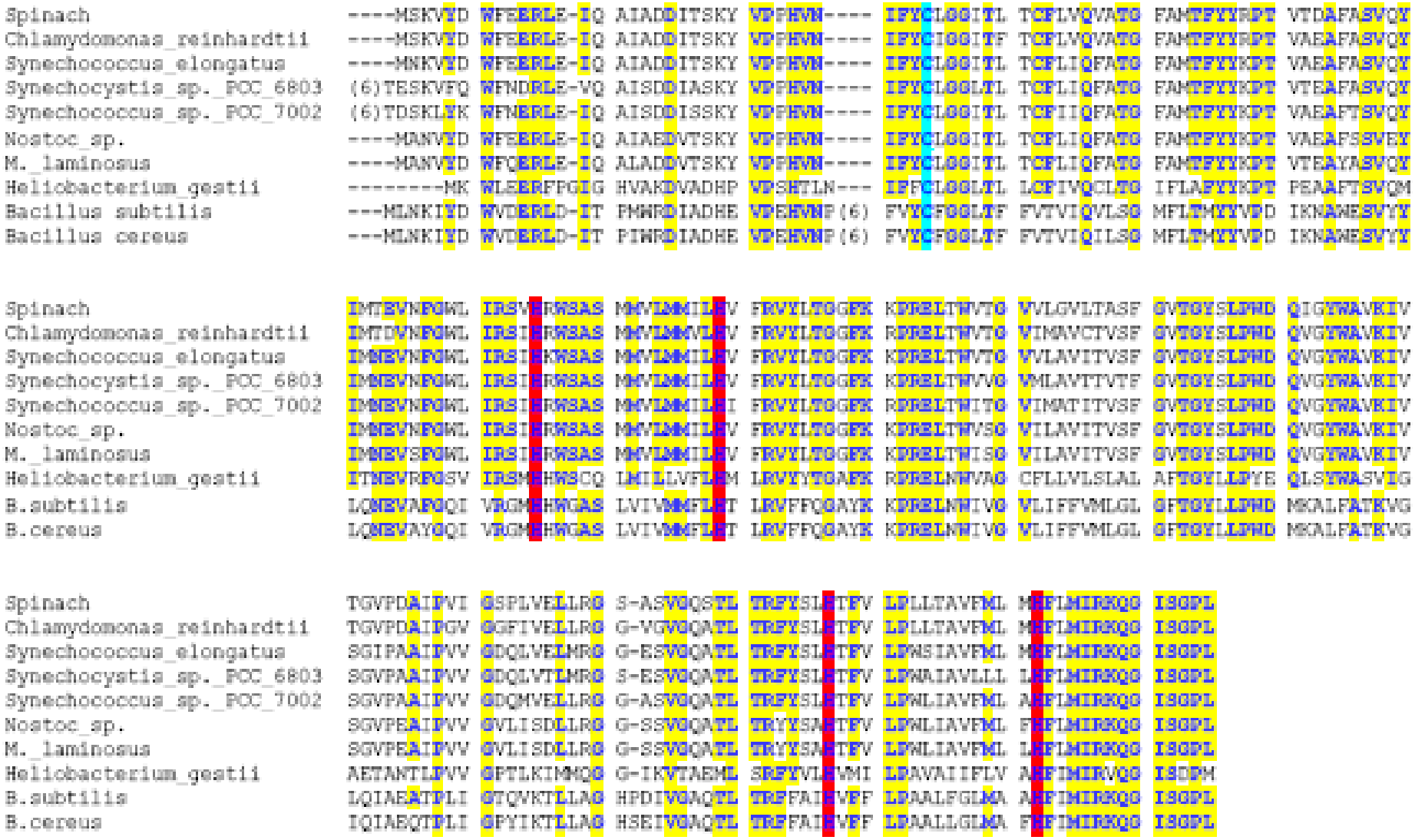
Heme c_n not in cyt bc_1; implies role in PSI cyclic pathway; however, *n. b.*, it is present in non-photosynthetic firmicutes, e. g., *B. subtilis*



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Advantage and deficiencies of *M. laminosus* for studies on b_6f complex and perhaps other photosynthetic membrane proteins

- **Advantage: from this filamentous cyanobacterium, can isolate active complex that can be crystallized (with added lipid).**
- **Deficiencies:**

  (1) No genetics, His-tag

  (2) Problem of proteolysis,

  but much less of a problem than it is in the unicellular *Synechocystis*, *Synechococcus, or Thermosynechococcus elongatus*

  Let's look for a cyanobacterium that is more closely related to *M. laminosus* and which has a sequenced genome.

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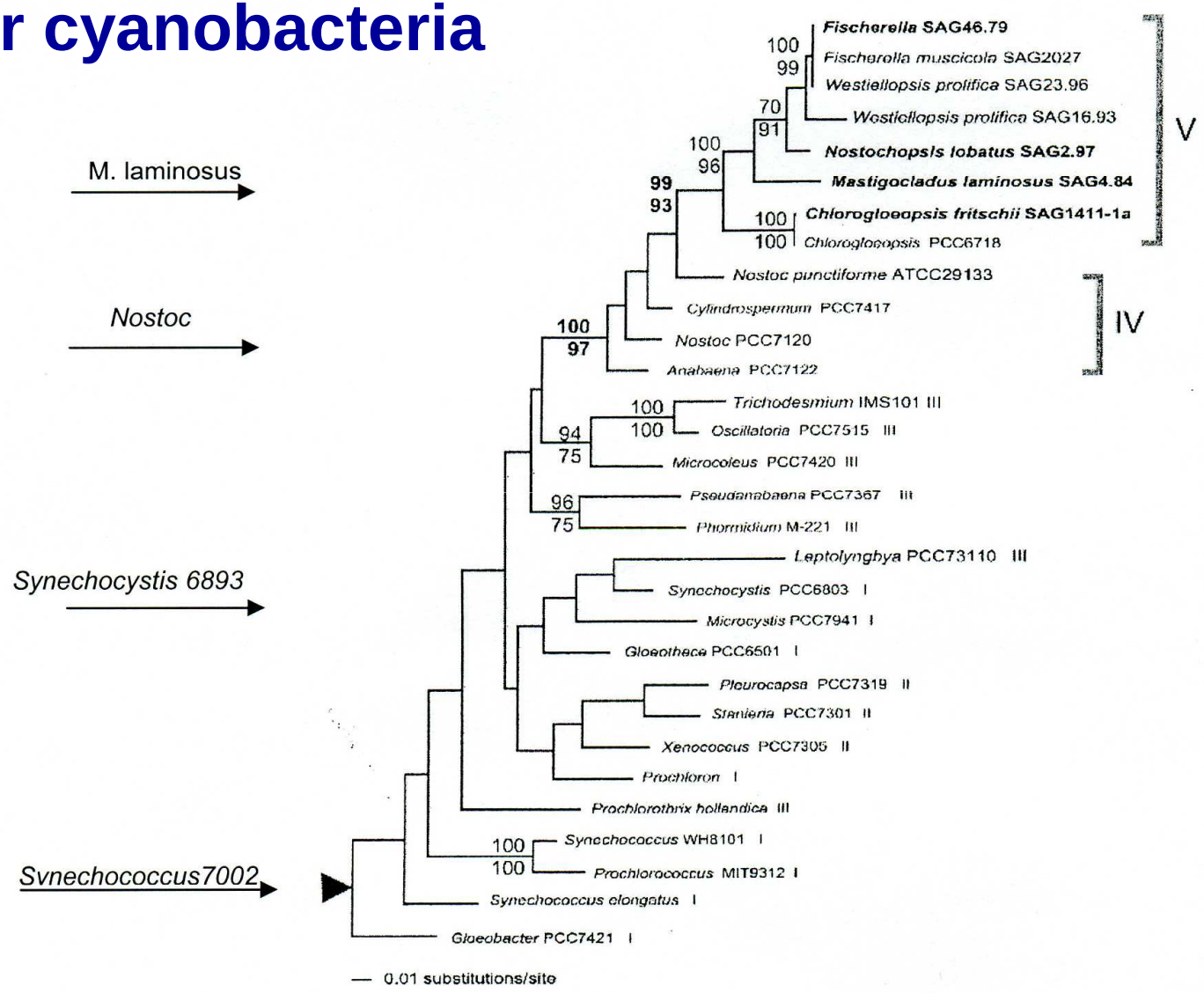
16s RNA partial phylogenetic ladder for cyanobacteria



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Bipyramidal crystals of *Anabaena* b_6f complex diffracted to < 3.0 Å in first diffraction trials

His-tagged b_6f complex cloned by R. Mella-Herrera/J. Golden (Texas A and M)

waclab@purdue.edu

Summary

- **Problems (not unique) in crystallization of b_6f complex.**
- **Using filamentous *M. laminosus*, 3.0 Å native structure in presence of Cd^{2+}; complex is hetero-oligomeric 8 subunit, 220 kDa dimeric b_6f complex with 8 different prosthetic groups. Central core conserved in evolution.**
- **3 novel prosthetic groups: Chl *a*, β-carotene; novel high spin heme on *n*-side proximal to heme b_n.**
- **PQ axial ligand displaced by quinone analogues, Stg, NQNO; implies b_n-c_n- PQ electron wire is n-side donor to PQ pool.**
- **b_6f complex not just another bc_1 complex; also illustrated by evolution of b_6f from firmicutes, e. g., *B. subtilis*.**
- **Movement of Q/QH_2 across complex is not simple "flip-flop," but a "labyrinthine" quided diffusion through caverns and portals.**
- **Used 16s RNS cyanobacterial "tree" to select *Anabaena* sp. PCC 7120 as a cyanobacterium with less proteolysis and a sequenced genome.**
- **Purified *Anabaena* b6f is dimeric, active, crystallizes readily, diffracts well, and is being His-tagged.**
- **All unicellular cyanobacteria from which active dimeric b6f complex cannot be isolated have proteases not in *Anabaena*, *Thermosynechococcus elongatus* having the fewest.**

waclab@purdue.edu