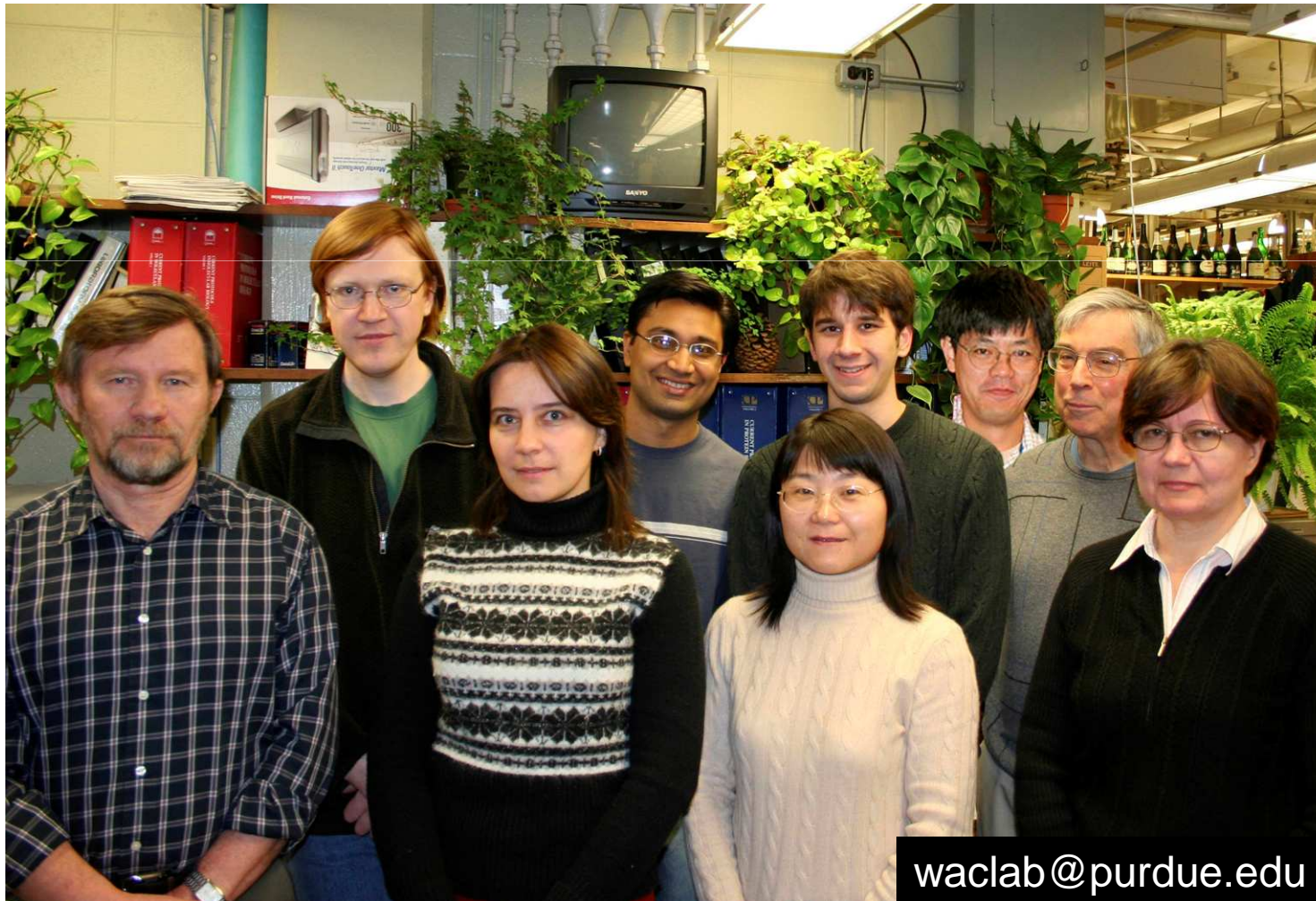
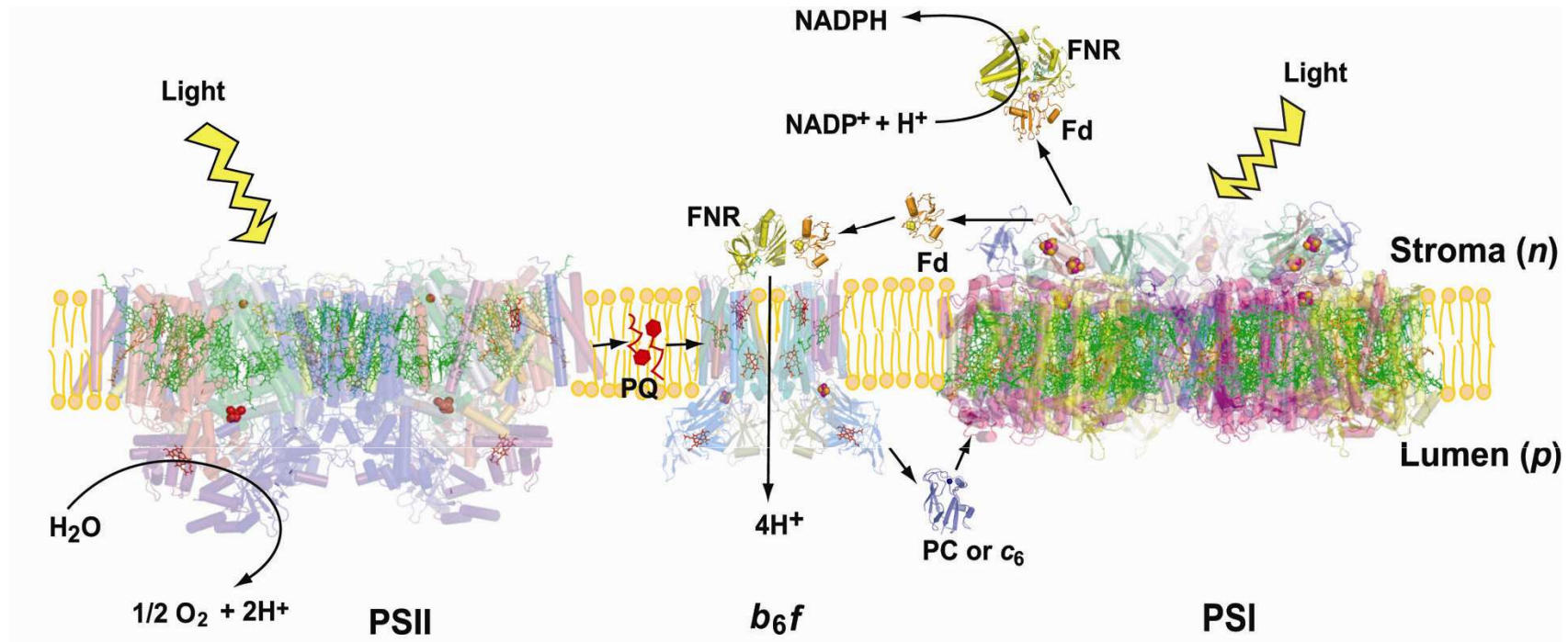


A Limited Bioinformatics Analysis Results in an Improved Cyanobacterial Source for Structure-Function Studies of the Cytochrome b_6f Complex

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Hetero-oligomeric Membrane Protein Structures in Oxygenic Photosynthesis



Crystal structures of the cytochrome *b*₆*f* 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.

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Outline

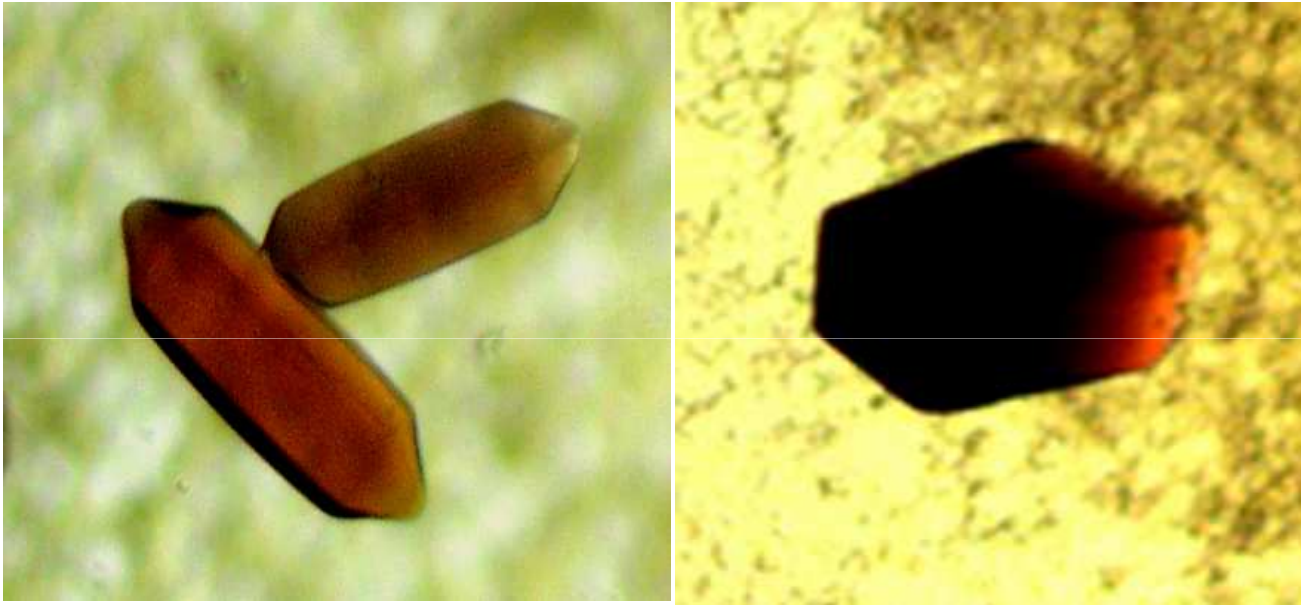
- Crystallization of b6f complex
- Problems (proteolysis, delipidation); unique role of the cyanobacterium, *Mastiocladus laminosus*; but, no genetics
- Results:
 - 3.0 Å structure of 220 kDa native complex; symmetric dimer built around Q/QH2 exchange cavity with 8 subunits, 13 TM helices, 7 prosthetic groups per monomer (4 hemes, 1 Fe₂S₂ 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 structures obtained of b₆f 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 b₆f complex; non-photosynthetic firmicutes
 - How to solve proteolysis problem, obtain a genetically tractable strain?

Masses (electrospray MS) of the 8 subunits of the b_6f complex from *M. lamosus*

<u>Subunit</u>	<u>Measured Mass (Da)</u>
(I) “Large” Subunits	
Cyt <i>f</i>	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

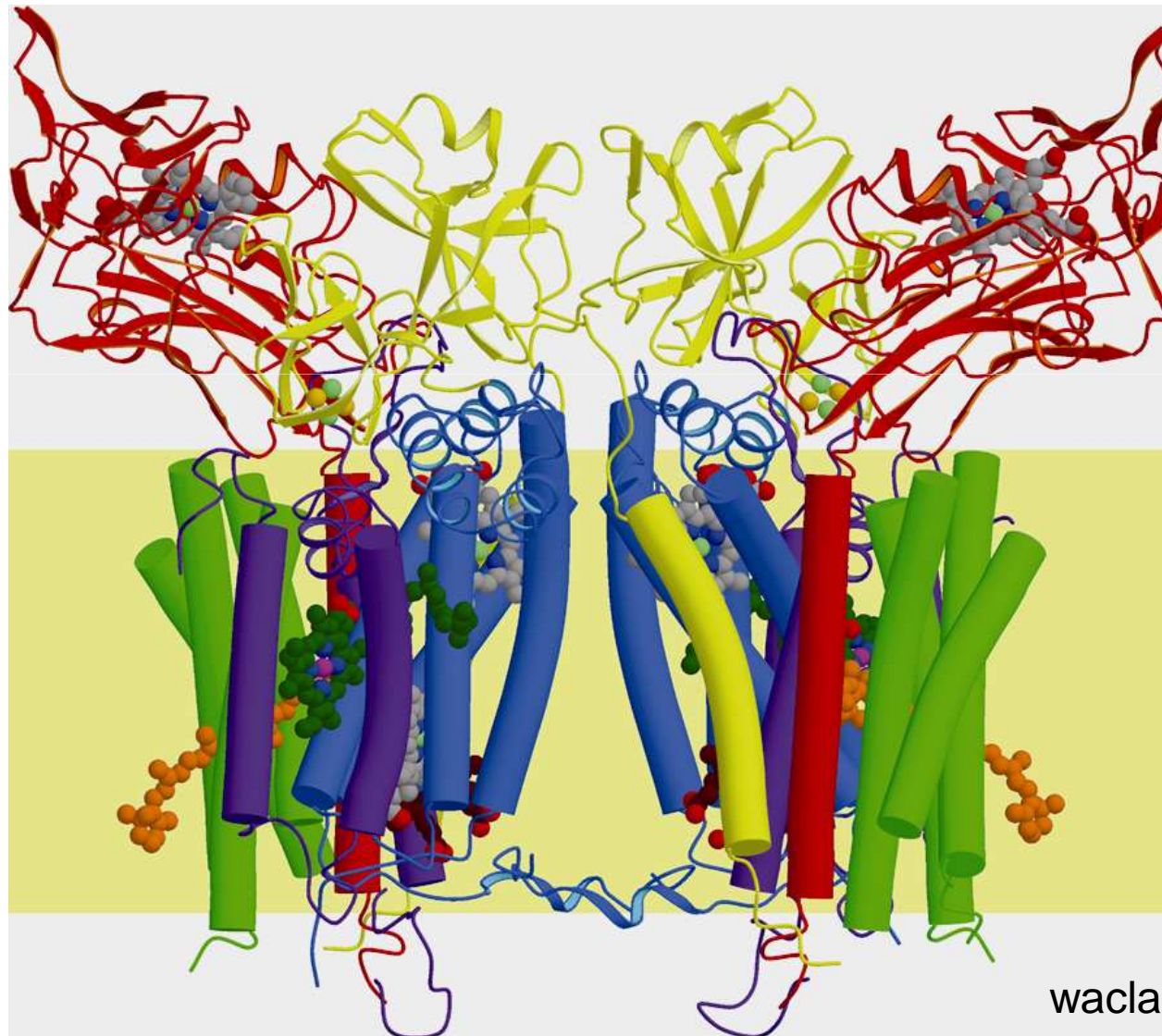
Whitelegge et al., *Molec. Cell Proteomics* (2002) 1: 816-826

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 [Cd²⁺] (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]**

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



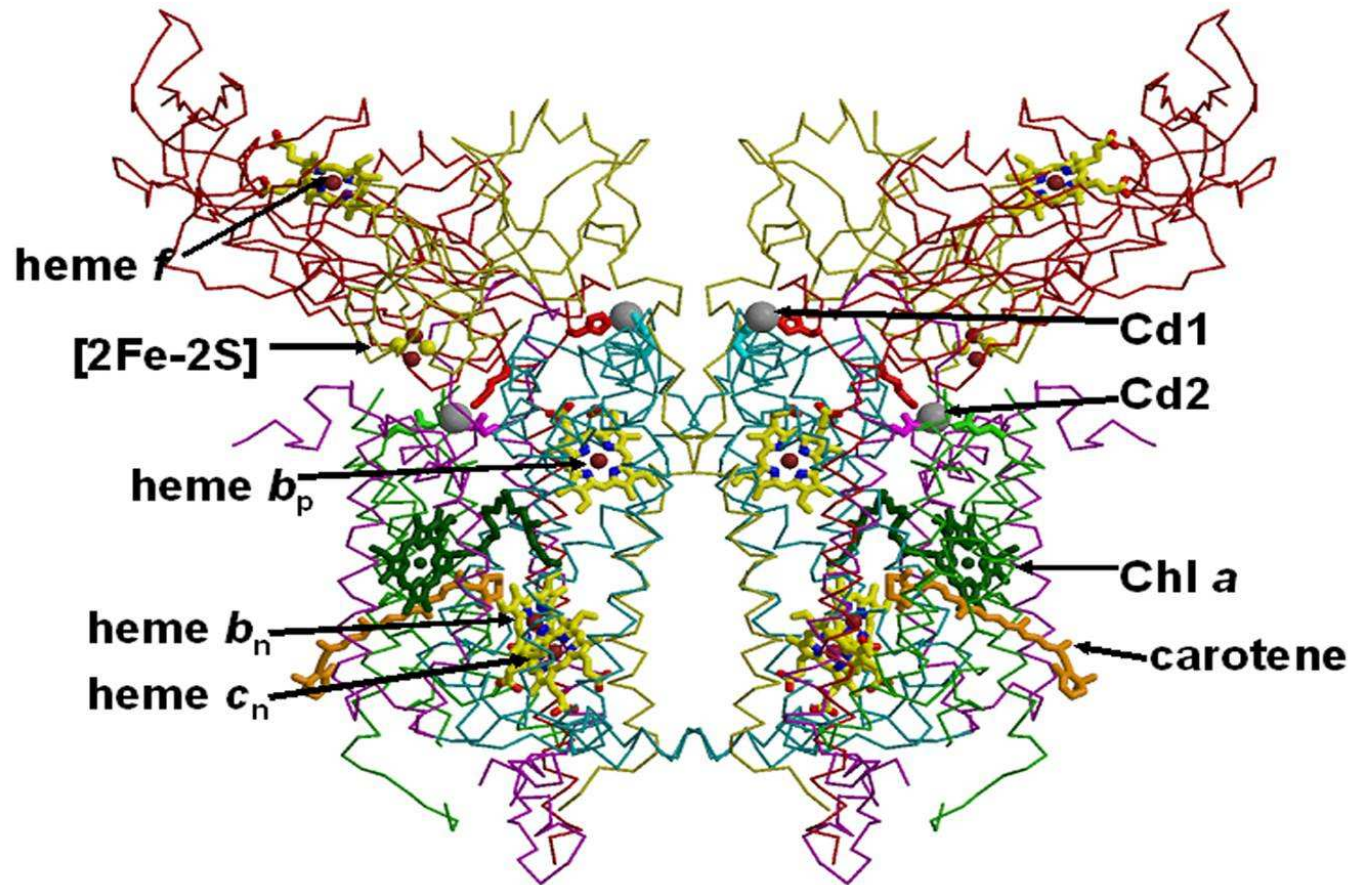
Lumen (p)

Stroma (n)

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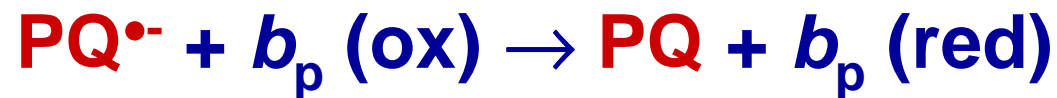
3.0 Å native structure of dimeric b_6f complex from *M. laminosus*, obtained in the presence of Cd^{2+} ;

Minimal Function of the dimer: “Quinone exchange cavity”
How does the quinone navigate across the cavity?

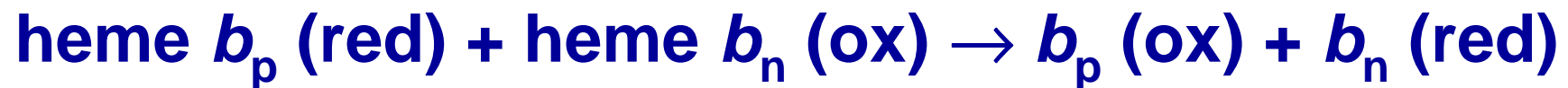


e⁻-H⁺ Transfer Function: PQH₂ oxidized on *p*-side; PQ reduced on *n*-side; according to conventional “Q cycle” (as in *bc*₁)

p-side quinol oxidation:



Trans-membrane electron transfer:

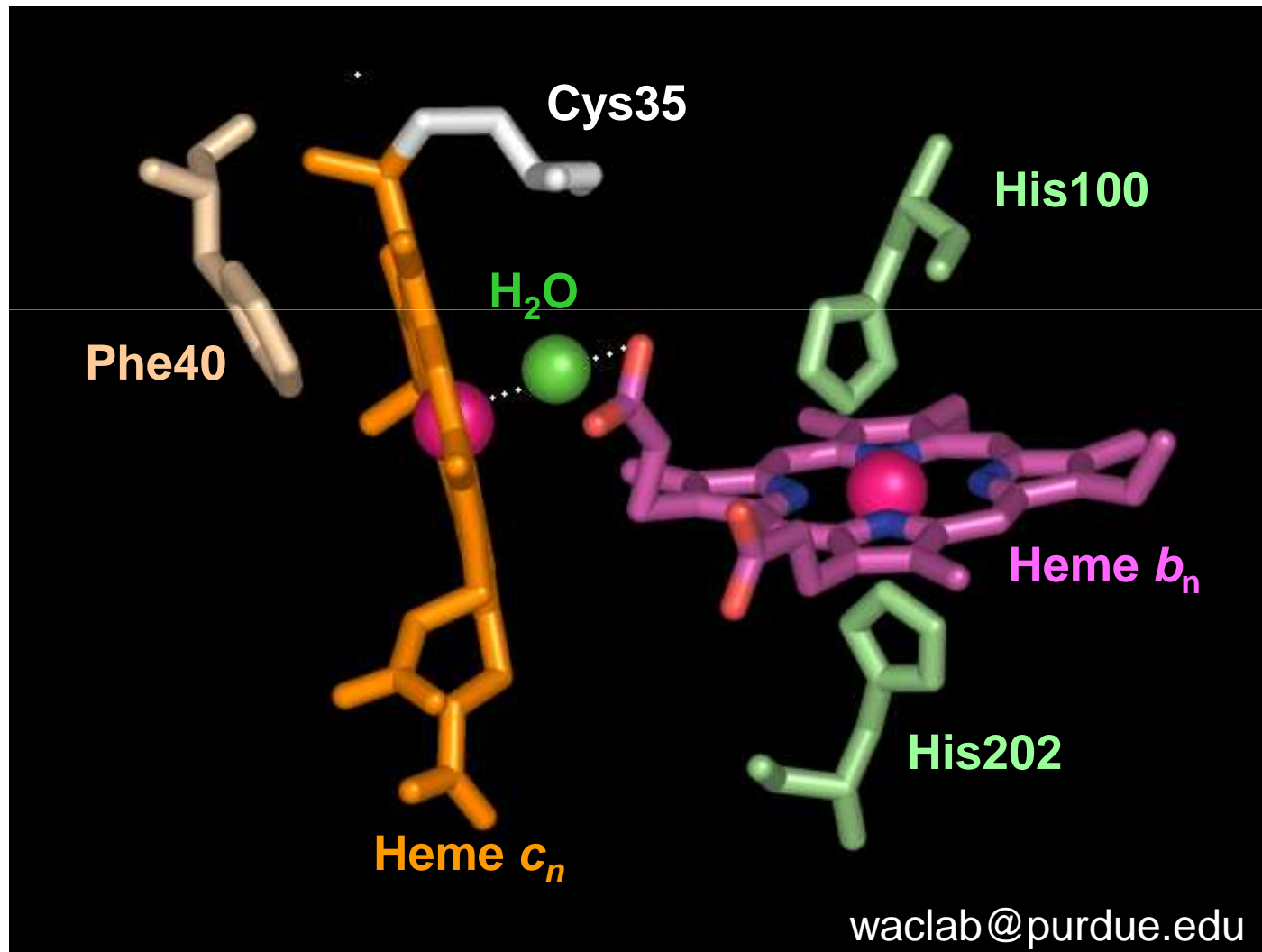


n-side quinone reduction

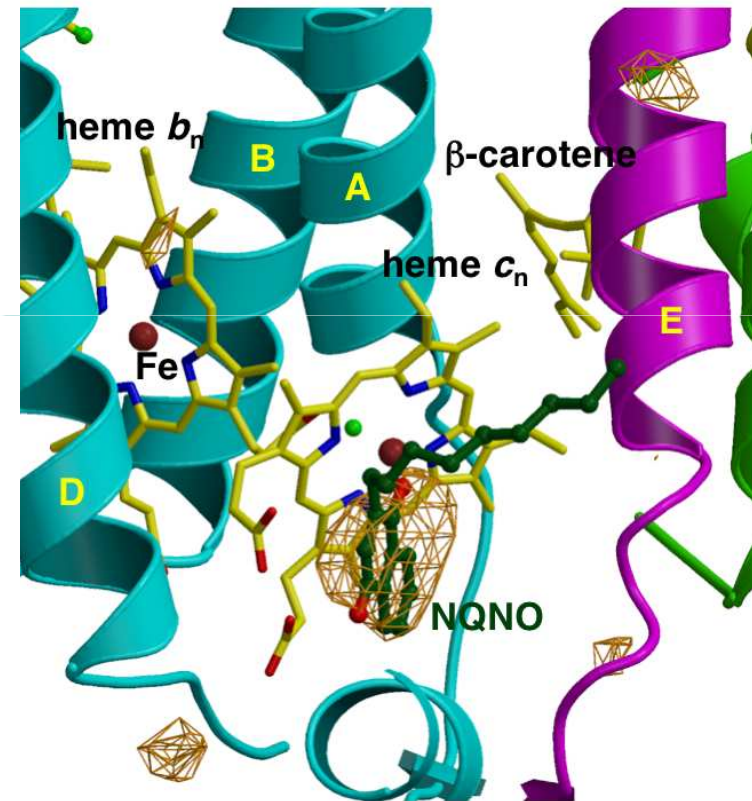
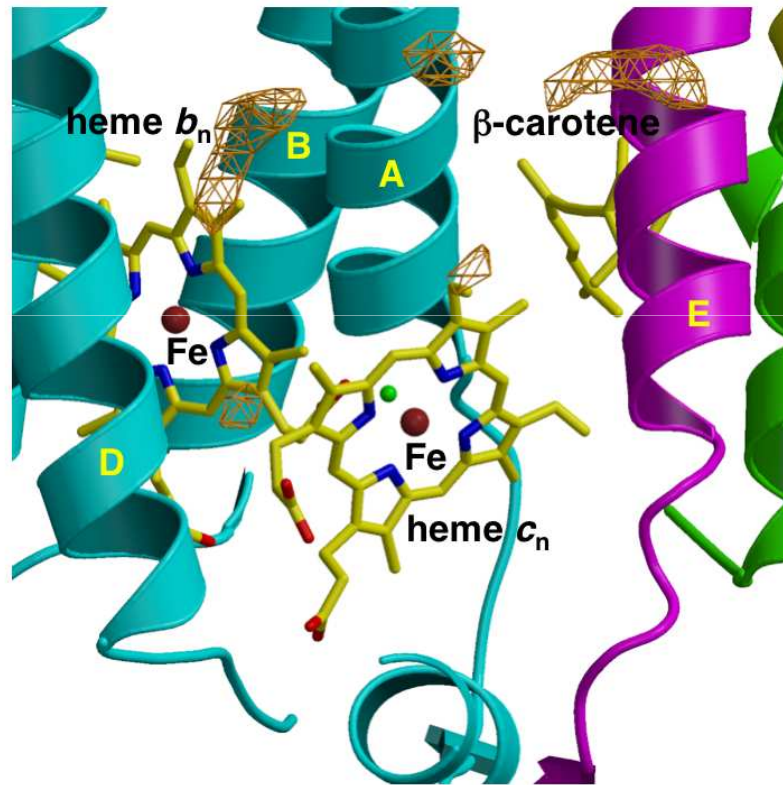


Thus, e⁻, H⁺, and PQ/PQH₂ must cross the complex

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.

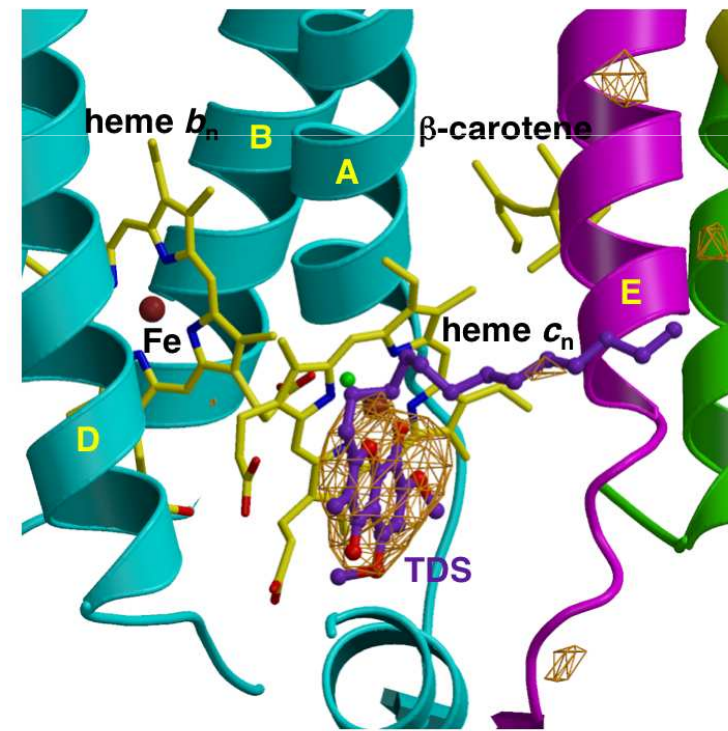
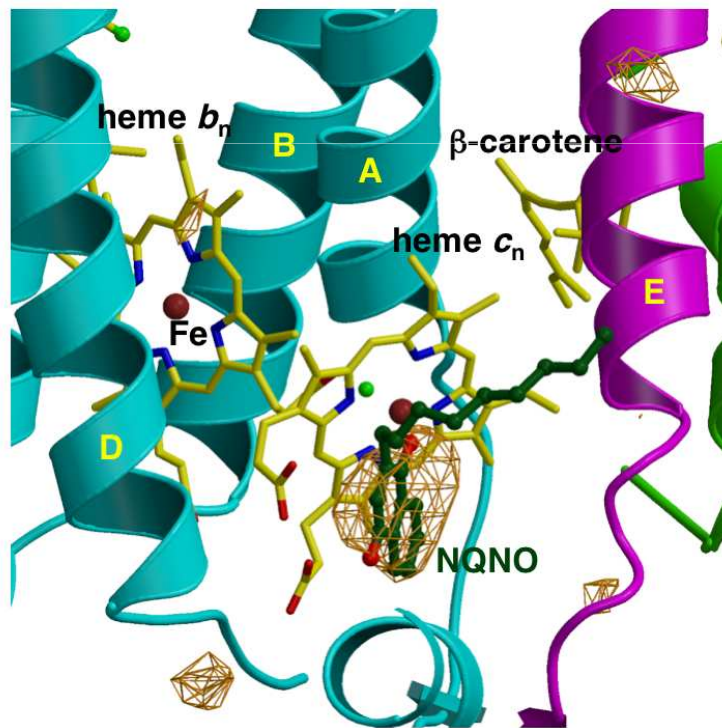


Function of heme c_n : quinone analogue inhibitor, NQNO, binds at free axial position of heme c_n

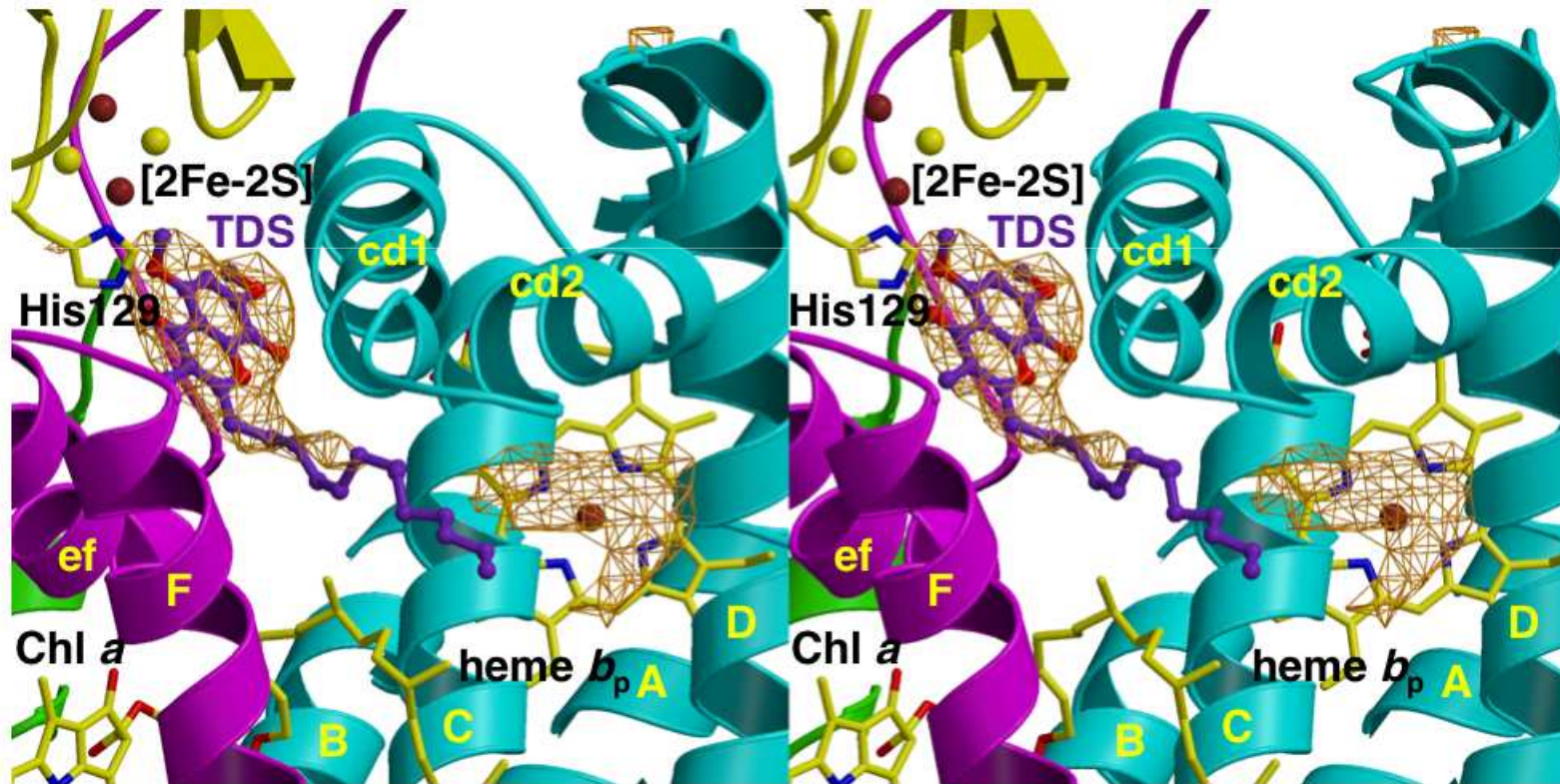


In fact, 2 quinone analogue inhibitors, NQNO and tridecyl-stigmatellin (TDS), are ligands of heme c_n .

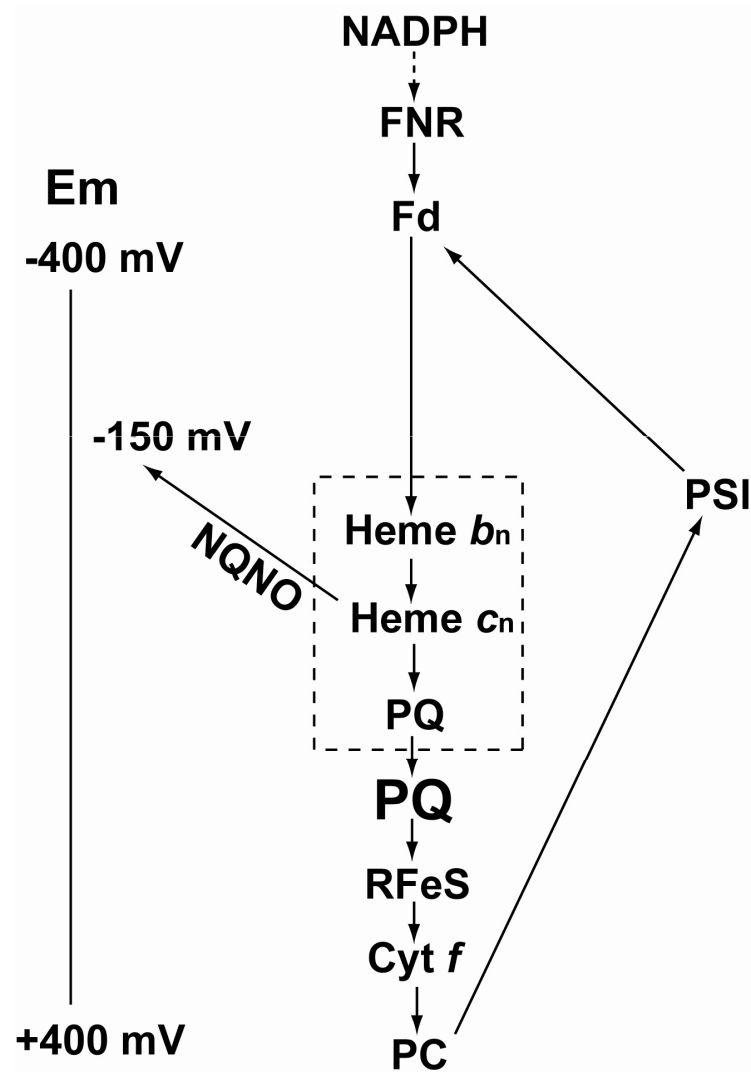
Implication: physiological ligand of heme c_n is bound plastoquinone



Quinone analogue inhibitor, TDS, binds near His-129 ligand of Rieske iron-sulfur protein; as in *bc*₁



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 E_m of heme c_n (Alric *et al.*, 2005).



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*

Spinach	----MSKVD	WFERLE-IQ	AIADDITSKY	VFHVN----	IFYCLOGITL	TCFLWQVATG	FAMTFYYRPT	VTDAFASVQY
Chlamydomonas_reinhardtii	----MSKVD	WFERLE-IQ	AIADDITSKY	VFHVN----	IFYCLOGITF	TCFLWQVATG	FAMTFYYRPT	VAEAFASVQY
Synechococcus_elongatus	----MNKVD	WFERLE-IQ	AIADDITSKY	VFHVN----	IFYCLOGITL	TCFLIQFATG	FAMTFYYKPT	VAEAFASVQY
Synechocystis_sp._PCC_6803	(6)TESKVFQ	WFNDRLVQ	AISDDIASKY	VFHVN----	IFYCLOGGLTL	TCFLIQFATG	FAMTFYYKPT	VTEAFASVQY
Synechococcus_sp._PCC_7002	(6)TDSKLVK	WFNERLE-IQ	AISDDISSKY	VFHVN----	IFYCLOGITL	TCFLIQFATG	FAMTFYYKPT	VAEAFASVQY
Nostoc_sp.	----MANVD	WFERLE-IQ	AIAEDVTSKY	VFHVN----	IFYCLOGITL	TCFLIQFATG	FAMTFYYKPT	VAEAFSSVEY
M._laminosus	----MANVD	WFQERLE-IQ	ALADDVTSKY	VFHVN----	IFYCLOGITL	TCFLIQFATG	FAMTFYYKPT	VTEAFASVQY
Helicobacterium_gestii	-----MK	WLEERFPFGIG	HVAKDVADHP	VSHTLN---	IFFCLOGGLTL	LCFIVQCLEG	IFLAFYYKPT	PEAFTSVQM
Bacillus_subtilis	---HLNKIYD	WVDERLD-IT	PMWRDIADHE	VDEHVN(6)	FVYCFQGLTF	FVTVIQVLSG	MFLTHYYVD	IKNAMESVYY
Bacillus_cereus	---HLNKIYD	WVDERLD-IT	PIWRDIADHE	VDEHVN(6)	FVYCFQGLTF	FVTVIQILSG	MFLTHYYVD	IKNAMESVYY

Spinach	IMTEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	KRPELTHVTO	VVLGVLTSASF	QVTVYSLPND	QIGYWAVKIV
Chlamydomonas_reinhardtii	IMTEVNPQWL	IRSYRWSAS	MMVLMVLYV	FRVYLTGGFK	RPELTHVTO	VIMAVCTVSF	QVTVYSLPND	QVGYWAVKIV
Synechococcus_elongatus	IMNEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	KRPELTHVTO	VVLAVITVSF	QVTVYSLPND	QVGYWAVKIV
Synechocystis_sp._PCC_6803	IMNEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	KRPELTHVVO	VMLAVITVTF	QVTVYSLPND	QVGYWAVKIV
Synechococcus_sp._PCC_7002	IMNEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	RPELTHVTO	VIMATITVSF	QVTVYSLPND	QVGYWAVKIV
Nostoc_sp.	IMNEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	KRPELTHVVO	VILAVITVSF	QVTVYSLPND	QVGYWAVKIV
M._laminosus	IMNEVNPQWL	IRSYRWSAS	MMVLMILV	FRVYLTGGFK	KRPELTHVVO	VILAVITVSF	QVTVYSLPND	QVGYWAVKIV
Helicobacterium_gestii	ITNEVNPQSV	IRSMHWSCQ	LMILLVFLM	LRVYVYGAFK	RPELTHVVO	CFLLVLSLAL	AFTVYLLPND	QLSYWASVIG
B.subtilis	IQNEVAPQOI	VIGMHWGAS	LVIYMMFLIT	LRVFFQDAYK	KRPELNHIVO	VLIFFVMLGL	QFTVYLLPND	MKALFATEWG
B.cereus	IQNEVAYQOI	VIGMHWGAS	LVIYMMFLIT	LRVFFQDAYK	KRPELNHIVO	VLIFFVMLGL	QFTVYLLPND	MKALFATEWG

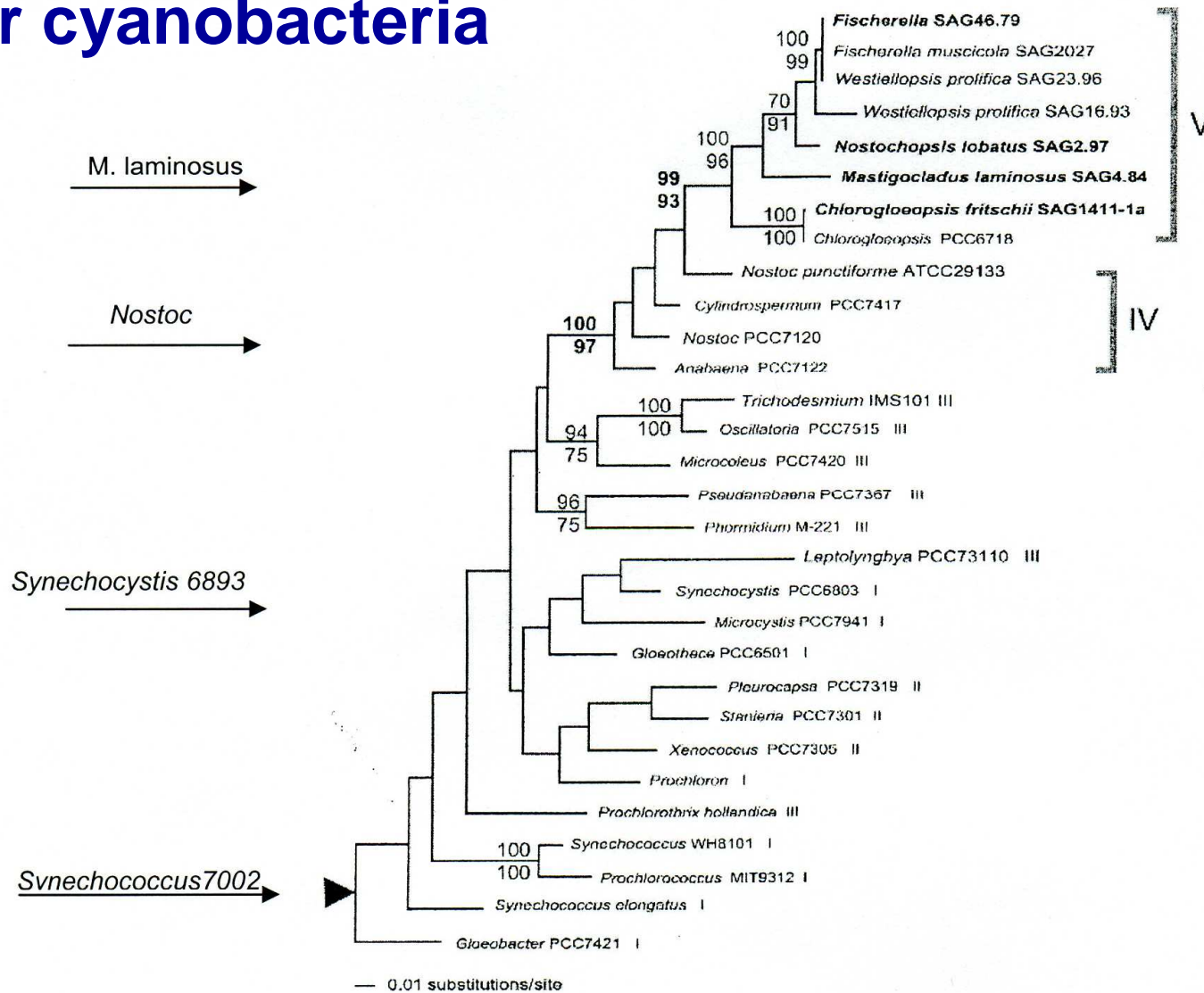
Spinach	TGVPDAIDVI	GSPIVELLRG	S-ASVQSTL	TRFYSLTFV	LFLLTAVFML	MAFIMIRKQQ	ISGPL
Chlamydomonas_reinhardtii	TGVPDAIDGV	GGFIVELLRG	G-VGVQATL	TRFYSLTFV	LFLLTAVFML	MAFIMIRKQQ	ISGPL
Synechococcus_elongatus	SGIPAAIDVV	GDQIVELMRG	G-ESVQATL	TRFYSLTFV	LQNSIAVFML	MAFIMIRKQQ	ISGPL
Synechocystis_sp._PCC_6803	SGVPAAIDVV	GDQIVELMRG	S-ESVQATL	TRFYSLTFV	LQNAIAVLLL	LAFIMIRKQQ	ISGPL
Synechococcus_sp._PCC_7002	SGVPAAIDVV	GDQIVELLRG	G-ASVQATL	TRFYSLTFV	LQNLIAVFML	AFIMIRKQQ	ISGPL
Nostoc_sp.	SGVPEAIDVV	GVLI SDLLRG	G-SSVQATL	TRYYSATFV	LQNLIAVFML	FAFIMIRKQQ	ISGPL
M._laminosus	SGVPEAIDVV	GVLI SDLLRG	G-SSVQATL	TRYYSATFV	LQNLIAVFML	LAFIMIRKQQ	ISGPL
Helicobacterium_gestii	AETANTIDVV	GPTLKIMQQG	G-IKVTAEML	SRFYVLYMI	LDVAVAIFLV	AFIMIRVQQ	ISDPH
B.subtilis	IQIAEATPLI	GTQVKTLIAG	HPDIWQAQTL	TRFFAIVFF	LDAALFGLSA	AFIMIRKQQ	ISGPL
B.cereus	IQIABQTPLI	GPIYKTLIAG	HSEIWQAQTL	TRFFAIVFF	LDAALLGLSA	FAFIMIRKQQ	ISGPL

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

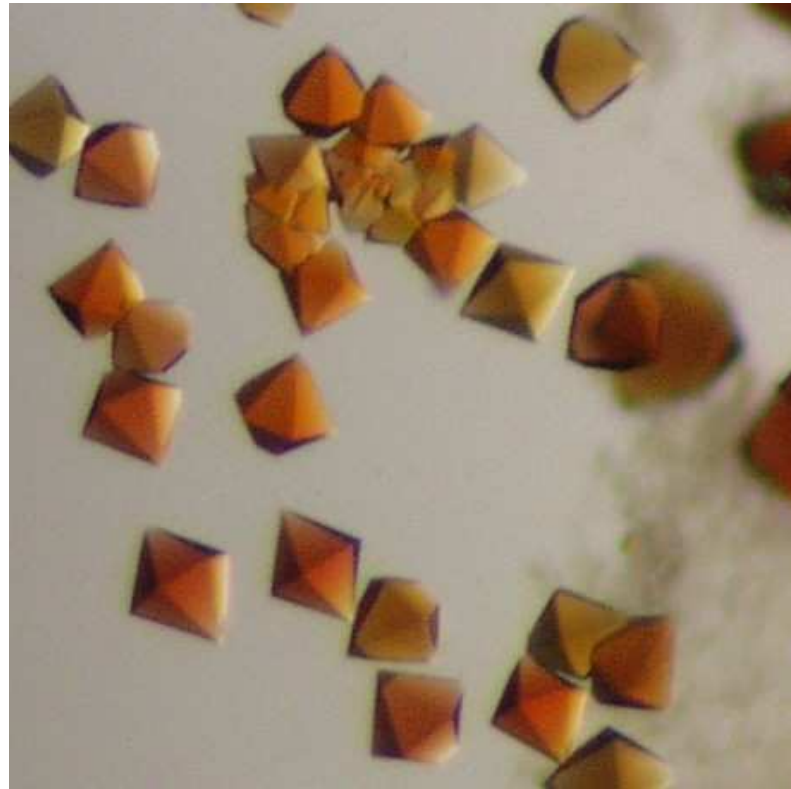
- **Advantage:** from this filamentous cyanobacterium, can isolate active complex that can be crystallized (with added lipid).
- **Deficiencies:**
 - (1) No genetics, His-tag
 - (2) Problem of proteolysis,but much less of a problem than it is in the unicellular *Synechocystis*, *Synechococcus*, or *Thermosynechococcus elongatus*

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

16s RNA partial phylogenetic ladder for cyanobacteria



Bipyramidal crystals of *Anabaena* b_6f complex diffracted to $< 3.0 \text{ \AA}$ in first diffraction trials



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

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/QH₂ 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* b_6f is dimeric, active, crystallizes readily, diffracts well, and is being His-tagged.
- All unicellular cyanobacteria from which active dimeric b_6f complex cannot be isolated have proteases not in *Anabaena*, *Thermosynechococcus elongatus* having the fewest.