

Structure of the Cytochrome *b₆f* Complex of Oxygenic Photosynthesis: Tuning the Cavity

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The cytochrome *b₆f* complex provides the electronic connection between the photosystem I and photosystem II reaction centers of oxygenic photosynthesis and generates a trans-membrane electrochemical proton gradient for ATP synthesis. A 3.0-Å crystal structure of the dimeric *b₆f* complex from the thermophilic cyanobacterium, *Mastigocladus laminosus*, reveals a large quinone exchange cavity, stabilized by lipid, in which plastoquinone, a quinone analogue inhibitor, and a novel heme are bound. The core of the *b₆f* complex is similar to the analogous respiratory cytochrome *bc₁* complex, but the domain arrangement outside the core and the complement of prosthetic groups are strikingly different. The motion of the Rieske iron-sulfur protein extrinsic domain, essential for electron transfer, must also be different in the *b₆f* complex.

In oxygenic photosynthesis, three integral membrane protein complexes accomplish electron transport and generate the trans-membrane electrochemical proton gradient used for energy transduction (Fig. 1). Light energy transferred to the photosystem I and II reaction centers (PSI and PSII) activates electron transfer (1). The cytochrome *b₆f* complex mediates electron transfer between the reaction centers by oxidizing lipophilic plastoquinol and reducing plastocyanin or cytochrome *c₆* (2–4). Three-dimensional structures exist for PSI at 2.5 Å (5) and PSII at 3.7 Å (6, 7). The 3.0-Å crystal structure of the cytochrome *b₆f* complex completes the description of the architecture of energy transduction in oxygenic photosynthesis.

Within the *b₆f* complex, one electron is transferred from doubly reduced dihydro-plastoquinone (PQH₂) to a high-potential electron transfer chain, consisting of the Rieske iron-sulfur protein and cytochrome *f* on the electropositive side of the membrane (Fig. 2A). This results in release of two protons to the aqueous lumen phase. Transfer of the second electron from PQH₂ across the complex through two *b*-hemes (8–11), or as anionic plasto-semiquinone (12), and the

resulting proton uptake from the electronegative side generates a proton electrochemical gradient across the membrane.

Electron transfer and proton translocation functions similar to those in the cytochrome *b₆f* complex are performed by the respiratory cytochrome *bc₁* complex using the two *b*-hemes, one high-potential *c*-heme (cytochrome *c₁*, functionally analogous to cytochrome *f*), one [2Fe-2S] cluster, and dihydro-ubiquinol/ubiquinone. Lipophilic quinone species from the bulk membrane bilayer phase move between a site for reduction and proton uptake on the electronegative (*n*) side of the membrane and a site for oxidation and proton release on the electropositive (*p*) side. The *b₆f* and *bc₁* complexes share the basic elements of this process, which is explained by a Q-cycle mechanism (8–11). A structural framework for the Q cycle is provided by the two large central cavities in crystal structures of the dimeric *bc₁* complex from bovine (13–16), avian (14), and yeast (17) sources. Each cavity connects ubiquinone-reactive sites containing hemes *b_p* and *b_n* on the *p* and *n* sides of the complex.

The cytochrome *b₆f* complex from *Mastigocladus laminosus*, a thermophilic cyanobacterium, contains four large subunits (18–32 kDa), including cytochrome *f*, cytochrome *b₆*, the Rieske iron-sulfur protein (ISP), and subunit IV, and four small, hydrophobic subunits, PetG, PetL, PetM, and PetN, leading to a dimer molecular weight of 217 kDa (18). Crystal structures are available for the extrinsic soluble domains of cytochrome *f* (19–21), and the ISP (22). Cytochrome *b₆* and subunit IV are homologous to the N- and C-terminal halves of cytochrome *b* of the *bc₁* complex (23), as is the ISP between the two complexes (22). However, the *c*-type cytochrome *f* of the *b₆f* complex is unrelated to cytochrome *c₁* of the *bc₁* complex (19).

Structure determination. Growth of diffraction-quality crystals of the cytochrome *b₆f* complex required augmentation of the detergent-solubilized preparation with

synthetic lipid (dioleoyl-phosphatidylcholine, DOPC) (24). The structure was solved by isomorphous replacement using Pb and Pt derivatives and multiwavelength anomalous diffraction from native iron atoms (table S1). The initial model was built into a 3.4-Å map of the native complex. Refinement was carried out with a 3.0-Å data set from a co-crystal with the quinone analogue inhibitor tridecyl-stigmatellin (TDS) (Table 1).

Overall structure. All four large and four small subunits of each monomer were positioned in the 3.0-Å electron density map without ambiguity. The monomer includes four hemes, one [2Fe-2S] cluster, one chlorophyll *a*, one β -carotene, one plastoquinone, one added quinone-analogue inhibitor, tridecyl-stigmatellin (TDS), and two added DOPC lipids (Fig. 2B). The monomeric unit contains thirteen trans-membrane helices: four in cytochrome *b₆* (helices A-D), three in subunit IV (helices E-G), and one each in cytochrome *f*, the ISP, and the four small hydrophobic subunits, PetG, L, M, and N (Figs. 2A, C). The extrinsic domains of cytochrome *f* and the ISP are on the *p*-side of the membrane (Fig. 2A), and are ordered in the crystal structure. Loops and chain termini on the *n*-side are less well ordered. The model has no internal gaps for any of the sixteen subunits of the dimer, but a total of 128 terminal residues are missing. Only the large extrinsic *p*-side domain of cytochrome *f* and *n*-side chain termini form crystal lattice contacts. The ISP contributes to dimer stability by domain swapping: its trans-membrane helix obliquely spans the membrane in one monomer and its extrinsic domain is part of the other monomer (Figs. 2A, D).

Viewed along the membrane normal (Fig. 2C), the *b₆f* complex has dimensions of 90 Å x 55 Å within the membrane bilayer and on the *n*-side, and 120 Å x 75 Å on the *p*-side. The complex extends 100 Å along the membrane normal. The trans-membrane domains of the *b₆f* complex obey molecular two-fold symmetry. C α atoms of the trans-membrane helices superimpose with an rmsd of 0.7 Å. The topologies of the subunits of the *b₆f* complex obey the *cis*-positive rule for integral membrane proteins (25). A pronounced asymmetry in the surface potential of the complex is produced by the basic *n*-side and the anionic *p*-side that results from the acidic cytochrome *f* and ISP.

The two monomers form a protein-free central cavity on each side of the trans-membrane interface (Fig. 2D). Each cavity is 30 Å high, 15 Å deep, and 25 Å wide at its base near the *n*-side aqueous interface. Cavity walls are formed by helices C, D, and F of one monomer and helices A and E and the ISP trans-membrane helix of the other. The floor of the cavity is formed by the N-terminal 25 residues of cytochrome *b₆* and, presumably, by lipid head groups that fill the cavity in situ. The narrow 13 Å x 13 Å roof of the cavity is formed by cd1 and cd2 *p*-side short peripheral helices connecting helices C and D of cytochrome *b₆*, and the C-terminus of the ISP

trans-membrane helix. A small portal (11 Å x 12 Å) in the wall of each cavity is formed by helices C, cd1, and F, and leads to a *p*-side ante-chamber, or “Q_p pocket,” in each monomer. The Q_p pocket is bounded by the [2Fe-2S] cluster, heme *b_p* and the “ef loop” connecting helices E and F of subunit IV.

Each monomer of the *b₆f* complex contains four heme cofactors and one [2Fe-2S] cluster (Figs. 2A). The two hemes of cytochrome *b₆* (Fig. 3A), the *c*-type heme of cytochrome *f*, and the [2Fe-2S] cluster of the ISP are well studied redox cofactors of the *b₆f* complex and are common to the *b₆f* and *bc₁* complexes. Hemes *b_p* and *b_n* are *bis*-histidine coordinated by imidazole side chains separated by 13 and 14 residues in the B and D helices, effectively cross-linking these two helices (23).

New heme *x*. A surprise of the crystal structure is an additional, unique heme (Figs. 3B, S2) closely linked to heme *b_n*. The flat shape of the electron density (Fig. 2B) at 3.0-Å resolution and a strong peak (4.8 σ) in the iron anomalous-difference map implied a heme with a central Fe atom at a position between heme *b_n* and the central cavity. (The new heme was first identified in the structure of the *b₆f* complex of *Chlamydomonas reinhardtii* (D. Stroebel *et al.*, personal communication)). The new heme appears not to belong to a known heme family and, therefore we propose the temporary nomenclature “heme *x*”. Heme *x* may be the *n*-side heme described spectroscopically in *Chlorella sorokiana* (26, 27). Heme *x* is covalently linked to the protein by a single thioether bond to invariant Cys³⁵ in helix A of cytochrome *b₆*. Cys³⁵ is not part of a signature sequence characteristic of *c*-type cytochromes, in which the heme has thioether bonds to two Cys residues and an orthogonal His ligand. The Fe of heme *x* is not coordinated by protein side chains, but by a small molecule assigned as water. The water is hydrogen bonded to a propionate side chain of heme *b_n*, and to the backbone amide of invariant Gly³⁸ in cytochrome *b₆* (Fig. 3B). The sixth coordination position is unoccupied. Heme *x* is inferred to be a universal feature of the *b₆f* complex because its binding site is well conserved among cytochrome *b₆f* sequences. Invariant Tyr³³, Cys³⁵, Gly³⁸, Phe²⁰³ and Ile²⁰⁶ of cytochrome *b₆*, and Phe⁴⁰ and Ile⁴⁴ of subunit IV contact heme *x*. Invariant Val²⁶, Pro²⁷, Pro²⁸, His²⁹, Asn³¹, Arg²⁰⁷ and Gln²⁰⁹ of cytochrome *b₆* line the heme binding site.

The nearly perpendicular planes of heme *x* and heme *b_n* are in contact through the propionate-H₂O linkage, which implies efficient electron transfer between the two hemes and an electron transfer function for heme *x* that includes heme *b_n*. Heme *x* occludes heme *b_n* from the central quinone exchange cavity. One face of heme *x* packs against an edge of heme *b_n* and the A and D helices. The other face of heme *x* contacts the central cavity and helix E of subunit IV (Fig. 3B). Heme *x* is shielded from the aqueous phase by N- (residues 25–30)

and C-terminal (residues 207–211) segments of cytochrome b_6 .

The weak fifth coordination ligand and unoccupied sixth coordination position of heme x are likely to confer a high-spin character to ferric heme x . A high spin EPR signal in the b_6f complex has been reported (28). A recent pyridine hemochromagen difference spectrum of *M. lamosus* cytochrome b_6f revealed an extra heme whose spectrum is red-shifted by 2 nm relative to that of cytochrome f (H. Zhang, unpublished data).

The lipid phase. Chlorophyll α and β -carotene chromophores have been found in b_6f complexes from plant, algal, and cyanobacterial sources in an approximate 1:1:1 ratio with the protein (29–31). The chlorophyll is bound between helices F and G of subunit IV (Figs. 2A,C; 3A). In the 3.0-Å electron density map, no chlorophyll Mg ligands can be identified from protein or solvent. The chlorophyll a and heme b_n planes are parallel and approximately 16 Å apart (Fig. 4, left). The 20-carbon chlorophyll phytyl tail threads through the p -side redox chamber into the central cavity (Figs. 2C, 3A).

A molecule of 9-*cis* β -carotene is inserted near the center of the trans-membrane region between the helices of PetL and PetM, in contact with the B, E, and PetG helices (Figs. 2B, C). It is oriented obliquely to the membrane plane and chlorophyll α . It is too far from chlorophyll α (at least 14 Å) for effective quenching of the chlorophyll excited triplet state, the presumed function of bound β -carotene (32). The function of the chlorophyll a is unknown, but it may fill structure gaps, similarly to bound lipids in membrane proteins (33).

An endogenous plastoquinone molecule is bound at the n -side of each central cavity adjacent to heme x , identifying this as the Q_n site. The head group near the cavity floor is in contact with heme x and the isoprenoid chain extends upward into the cavity (Fig. 2D, 3A). Two molecules of DOPC, the synthetic lipid that is essential for crystallization, are bound in each cavity (not shown) and appear to provide structural support for the cavity. One lipid is bound with its head group at the cavity roof and the aliphatic chains extending down into the complex. The head group of the second lipid is adjacent to plastoquinone at the cavity floor. Although the phosphatidylcholine head group is not found in *M. lamosus*, the added lipid does not alter the in vitro activity of the complex or the efficiency of Q-analogue inhibitors (H. Zhang, unpublished data).

Q analogue binding: p-side. Two p -side quinone-analogue inhibitors, TDS, and 2,5-dibromo-5-methyl-6-isopropyl-benzoquinone (DBMIB) were separately co-crystal-lized with the b_6f complex. In the 3.0-Å structure of a co-crystal with TDS, the head group of TDS binds at the roof of the central cavity on the p -side of the complex, and its thirteen-carbon tail extends through a portal into the Q_p

pocket (Figs. 2B, D; 3A). This orientation is opposite to that described for stigmatellin in the cytochrome bc_1 complex (17) (Fig. 4, center), where the head group directly contacts the [2Fe-2S] cluster, while its tail extends into the central cavity. In the b_6f complex, the TDS head group is 20 Å from the [2Fe-2S] cluster. TDS inhibition may be due to occlusion of the Q_p portal, a new mode of p -side inhibition by the quinone analogues. Restriction of the portal by the chlorophyll phytyl tail raises the problem of tight passage of plastoquinone through the portal. The binding of plastoquinone on the n -side of the large cavity and the quinone analogue inhibitor TDS on the p -side (Fig. 2D) illustrates the transfer of plastoquinone between the p - and n -side of different monomers.

The other inhibitor, DBMIB, binds between the E and F trans-membrane helices of subunit IV in a region of the Q_p pocket that is highly conserved in b_6f and bc_1 complexes. The DBMIB binding site, identified independently in several co-crystal data sets, is also approximately 20 Å from the [2Fe-2S] cluster (Fig. 4, center). The site in the crystal structure may correspond to a high-affinity site with little inhibitory effect (34).

Comparison with the cytochrome bc_1 complex. The inter-cofactor distances (Fig. 4) and the organization of 8 of the 13 trans-membrane helices (A-D in cytochrome b_6 , E and F in subunit IV, ISP and cytochrome f) are similar in the b_6f and bc_1 complexes. The rmsd for C α atoms is 2.0 Å for helices A-F of the dimer. The G helix of subunit IV has a position intermediate to the G, H helices of cytochrome bc_1 , and helices F and G are further apart in cytochrome b_6f in order to accommodate the chlorophyll a (Figs. 2C, 3A). The four small subunits of cytochrome b_6f form a hydrophobic "picket fence" around the core subunits (Figs. 2A, C) and occupy positions unlike any subunits of cytochrome bc_1 .

Hemes b_p and b_n are oriented identically in the b_6f and bc_1 complexes (1.1 Å rmsd for the four b -heme Fe atoms of the dimer). Relative to cytochrome b in the bc_1 complex, cytochrome b_6 has an additional residue (Thr¹⁸⁸) between the two histidine ligands in the D helix (23). Thr¹⁸⁸ is accommodated by a 25° kink in the helix, centered at Phe¹⁸⁹ (Fig. 3A). Heme x in cytochrome b_6f is located at the same position as the n -side ubiquinone and antimycin A binding site in the bc_1 complex (Fig. 4, center). Thus, the two complexes have different Q_n sites. This also explains why no inhibitors of n -side electron transfer in the cytochrome b_6f complex have been identified that are similar to antimycin A.

Though the central cavity is a prominent feature of both the b_6f and bc_1 complexes, there are differences. Access to the Q_p and Q_n sites within the cavity is quite different in the two complexes. Due to constriction of the portal by the chlorophyll phytyl tail, the Q_p site of cytochrome b_6f is less accessible than the Q_p site of the bc_1 complex. In contrast, the Q_n site is more accessible in cytochrome b_6f because it is not

in an enclosed pocket as in cytochrome bc_1 . The central cavity is more exposed to the quinone pool in the center of the membrane bilayer in the b_6f than in the bc_1 complex, where the entrance is narrowed by small subunits 10 and 11 (15, 35). Instead, the F and ISP trans-membrane helices define the mouth of the cavity in the b_6f complex (Figs. 2C, D).

***p*-side extrinsic domains: cytochrome *f*.** The extrinsic domains of the b_6f and bc_1 complexes are strikingly different. Just as cytochromes *f* and c_1 are unrelated (19), so their hemes map to positions differing by 12 Å relative to the trans-membrane *b* hemes (Fig. 4, center). Cytochrome *f* consists of two extrinsic structural domains. The larger heme-binding domain occupies a position similar to part of the cytochrome c_1 extrinsic domain. The small domain occupies a position analogous to subunit 8 in cytochrome bc_1 . There is no analog in the b_6f complex for subunit 7, a pedestal for subunit 8 (Fig. 4, right). Cytochrome *f* also lacks inter-monomer contacts, which exist in cytochrome c_1 . The contrast of the 48° angle between heme and membrane planes in the present structure, in which cytochrome *f* is reduced, and the 30° angle when cytochrome *f* is oxidized (36), suggests the possibility of movement of the cytochrome. However, the structure has not provided evidence for such motion, as the large heme-binding domain of cytochrome *f* conforms to the molecular two-fold symmetry of the core of the complex. The small domain exhibits a 5° difference in hinge angle between the monomers of the complex, similar to its variability in the structures of isolated cytochrome *f* (19–21).

***p*-side electron transfer.** Large-scale motion of the ISP extrinsic domain is essential for cytochrome bc_1 function (14). The ISP extrinsic domain is flexibly tethered to its trans-membrane helix and, in different crystal structures, lies in extreme positions that vary between proximal to the *p*-side quinol binding niche and proximal to the heme of cytochrome c_1 (Fig. 4, center) (14, 15). The extrema differ by a 60° rotation resulting in a net displacement of the [2Fe-2S] cluster by 16 Å (14). Among the isomorphous crystal structures reported here, no such changes are seen for the ISP in cytochrome b_6f . Different motional trajectories may be employed in the b_6f and bc_1 complexes, because the extrinsic domains of cytochrome *f* and c_1 dictate different interactions of the [2Fe-2S] cluster. In the present structure, the [2Fe-2S] cluster and cytochrome *f* heme Fe atoms are separated by 29 Å (Fig. 4), which is too long for electron transfer to occur at a competent rate. Therefore, the extrinsic domain of the ISP and/or cytochrome *f* must move.

In the crystal structure, the [2Fe-2S] cluster points away from cytochrome *f*, into the Q_p site (Fig. 5A). Therefore, motion of at least the ISP is necessitated by the inaccessibility of the [2Fe-2S] cluster and its distance from the cytochrome *f* heme. Motion is consistent with the different ISP orientations

in the two monomers. When transformed about the molecular twofold, the superimposed ISP extrinsic domains differ by a 12° rotation and approximately 2 Å translation parallel to the membrane plane. Motion is also consistent with the flexibility in the multi-glycine hinge region of the ISP in cytochrome b_6f . Mutagenesis of the hinge region implied that less movement of ISP is required in cytochrome b_6f than in the bc_1 complex (37). A 14-Å displacement of the [2Fe-2S] cluster can be achieved by rotation of the ISP by 25° towards cytochrome *f*, which shortens the distance between the [2Fe-2S] cluster and the His²⁶ heme ligand to 14 Å. This would bring the [2Fe-2S] cluster into apposition with the backbone carbonyl of Leu²⁷ of cytochrome *f* (Figs. 5A, B). Electron transfer from this position to the imidazole of the His²⁶ heme Fe ligand would be very efficient as it would involve transfer through a pathway of eight covalent bonds (Fig. 5B) (38), and a distance of less than 8 Å between the carbonyl of Leu²⁷ and the His²⁶ imidazole ring (39).

Heme *x* and cyclic electron transfer. Heme *x* does not appear to be required for Q-cycle function (Fig. 2A) because the other elements of the Q cycle (hemes b_p and b_n) are identically oriented in the b_6f and bc_1 complexes, have identical inter-heme distances, and similar hydrophobic environments between hemes. However, the oxygenic photosynthetic electron transport chain also carries out ferredoxin-dependent cyclic electron transport, whose purpose is to support levels of ATP relative to NADPH needed for carbon fixation (Fig. 1). In cyanobacteria, the cyclic pathway is dominant at physiological CO₂ concentrations (0.03%) or low illumination levels (40). Heme *x*, which can readily contact plastoquinone in the central cavity (Fig. 3A), may be the hitherto elusive ferredoxin-plastoquinone reductase inferred to be essential for this activity. The positive stromal-side surface potential of cytochrome b_6f would facilitate docking of anionic ferredoxin to the stromal (*n*) side of the complex near heme *x*.

The 3.0 Å structure of the cytochrome b_6f complex completes the structural description of the photosynthetic electron transport chain, extends the high resolution structural description of the family of cytochrome *bc* complexes, identifies a new heme cofactor, and describes the structural changes that have occurred in and around the central quinone exchange cavity in response to the demands of photosynthesis.

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Supporting Online Material

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SOM Text

Figs. S1 and S2

Table S1

References and Notes

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Fig. 1. The integral membrane protein complexes responsible for electron transport and proton translocation in oxygenic photosynthesis. The structures are from thermophilic cyanobacterial sources, *S. elongatus* for the reaction centers of photosystems I (purple, PDB: 1JB0) and II (cyan, PDB: 1IZL), and *M. laminosus* for the cytochrome *b₆f* complex (orange), described in the present work. Lumen (*p*) and stromal (*n*) side soluble electron transfer proteins are plastocyanin (green) or cytochrome *c*₆, ferredoxin (dark brown), and ferredoxin:NADP⁺ reductase (yellow).

Fig. 2. The eight-subunit dimeric cytochrome *b₆f* complex. (**A, left**) Electron and proton transfer pathway through the *b₆f* complex and distances between redox cofactors. (**A, right**) Side view showing bound cofactors and protein subunits. Cytochrome *b*₆ (blue), subunit IV (purple), cytochrome *f* (red), ISP (yellow), PetG,L,M,N (green); the membrane bilayer (yellow band). (**B**) Electron density (rmsd level of the 2Fo-Fc map) of prosthetic groups, heme *x*, tridecyl-stigmatellin (TDS), plastoquinone, chlorophyll *a*, β-carotene, and DOPC. (**C**) View normal to the membrane plane of the 26 trans-membrane helices in the dimer; color code as in (A). Dimer interface is enriched in aromatic residues, Phe⁵², Phe⁵⁶

and Phe¹⁸⁹ in the A and D helices of cytochrome *b*₆. Central cavities formed at the dimer interface are indicated by dotted lines. **(D)** Molecular surface of the complex showing the central quinone exchange cavity including plastoquinone (magenta) and TDS (orange).

Fig. 3. Stereo views of intra-membrane core and bound molecules. **(A)** Side view. The two *b*-hemes (gray) are bis-histidine coordinated on the *n*- and *p*-sides of the B and D helices (blue). Chlorophyll *a* (green) is sandwiched between the F and G helices of subunit IV; the 20-carbon phytyl chain (green) extends normal to the figure into the *p*-side of the quinone-exchange cavity. Heme *x* (red-brown), ligated by water and the heme *b*_n propionate, lines the quinone exchange cavity, in contact with plastoquinone (magenta) near the *n*-side of the cavity. TDS (yellow) is near the *p*-side. **(B)** Linkage and coordination of heme *x*. Colors as in panel (A); Cys³⁵ (yellow) on the *n*-side of the A helix makes the single covalent thioether bond with heme *x*. The fifth ligand is a water that is hydrogen bonded (dashed line) to a heme *b*_n propionate. Phe⁴⁰, on the *n*-side of the E-helix, is parallel to heme *x*, and near (6-9 Å) plastoquinone (PQ, magenta) in the cavity.

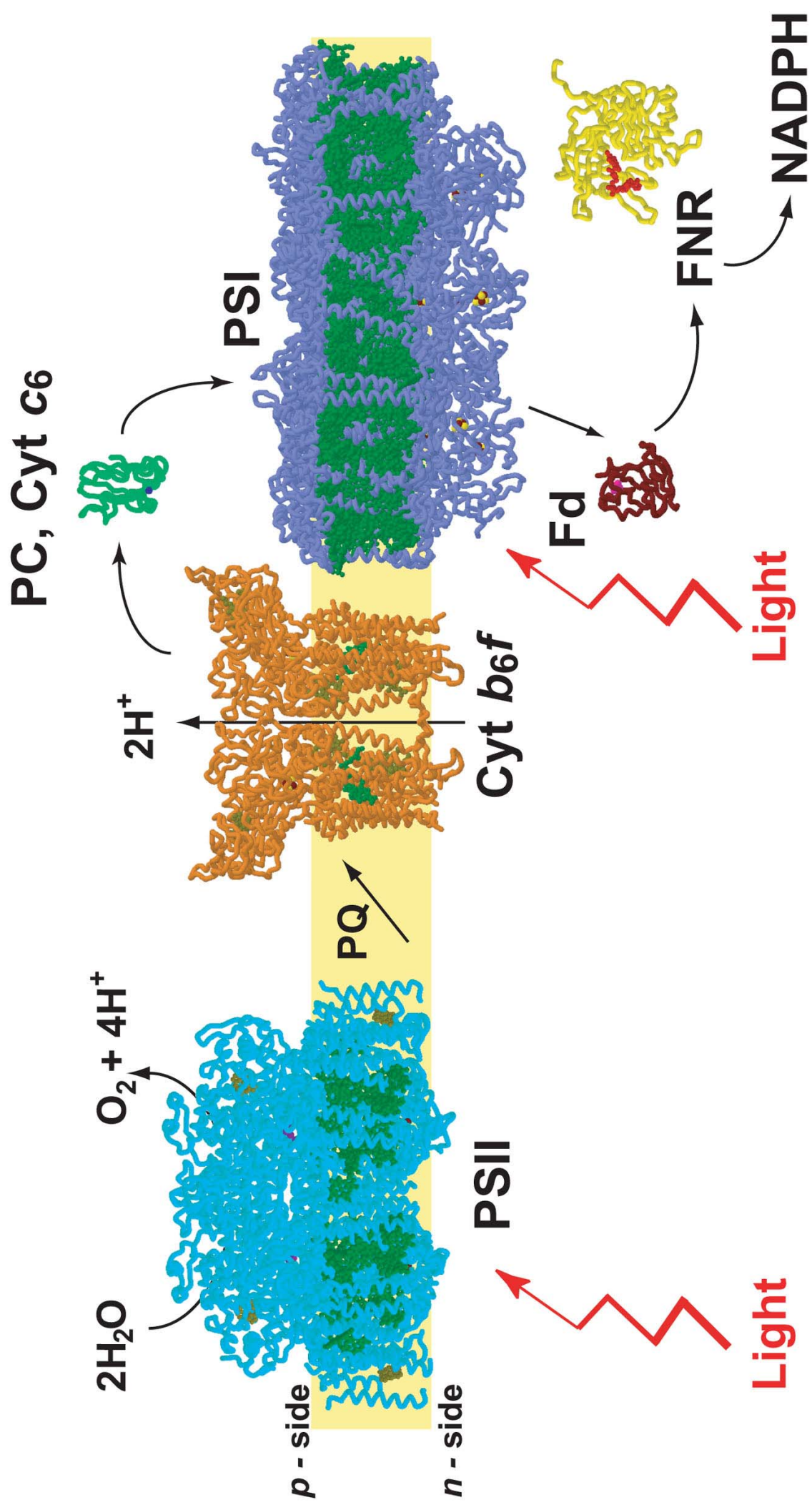
Fig. 4. Comparison of cytochrome *b*₆*f* (**left**) and yeast mitochondrial *bc*₁ (**right**) monomers, showing inhibitor binding sites and different positions of cytochromes *f* and *c*₁ and the [2Fe-2S] clusters. Positions of the [2Fe-2S] clusters and the *c*-type cytochromes of the superimposed complexes are shown in the center. *p*-side inhibitors of the *b*₆*f* complex are TDS (**left, center**), and DBMIB (**center**). The *p*- and *n*-side inhibitors of the *bc*₁ complex are stigmatellin (**center, right**), and antimycin A (**center**). The binding site of DBMIB is near Glu⁷⁸ in the conserved ef loop of subunit IV. Color code as in Fig. 2A.

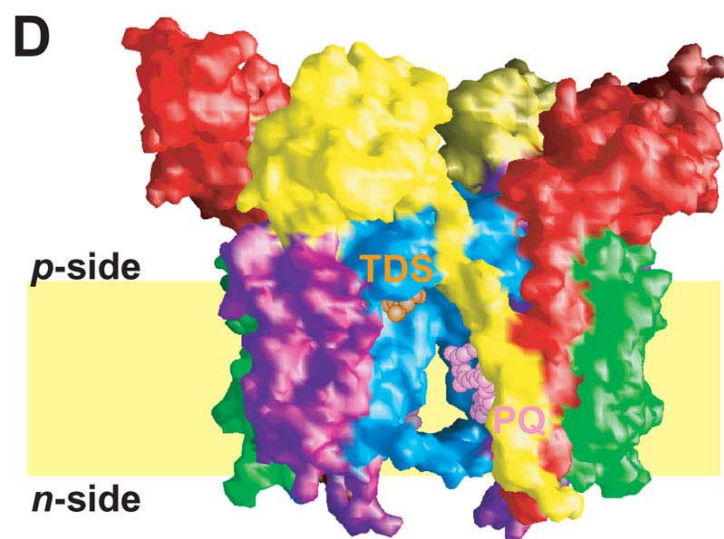
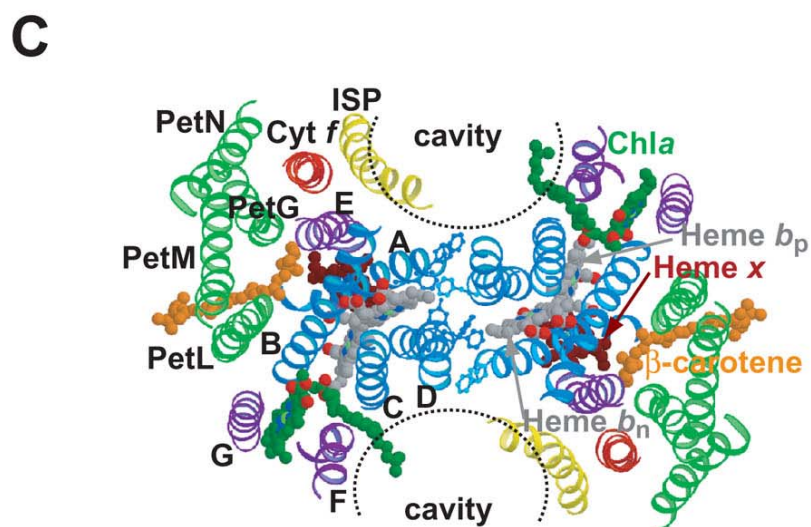
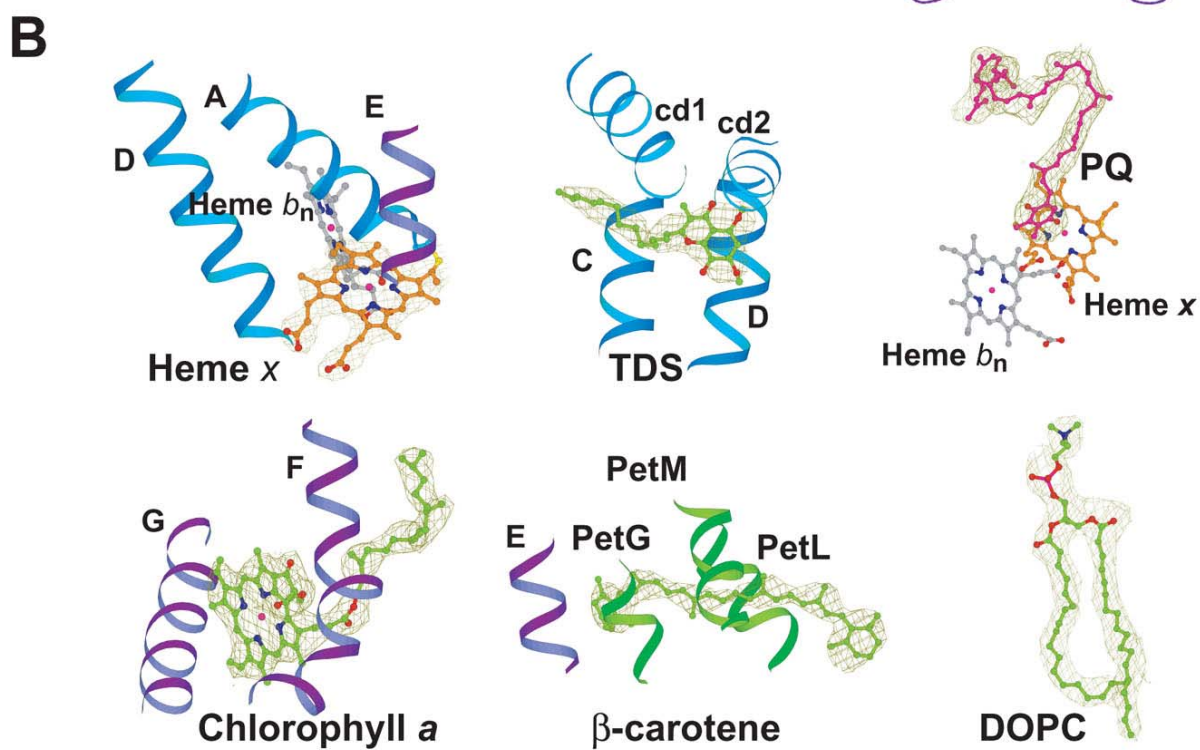
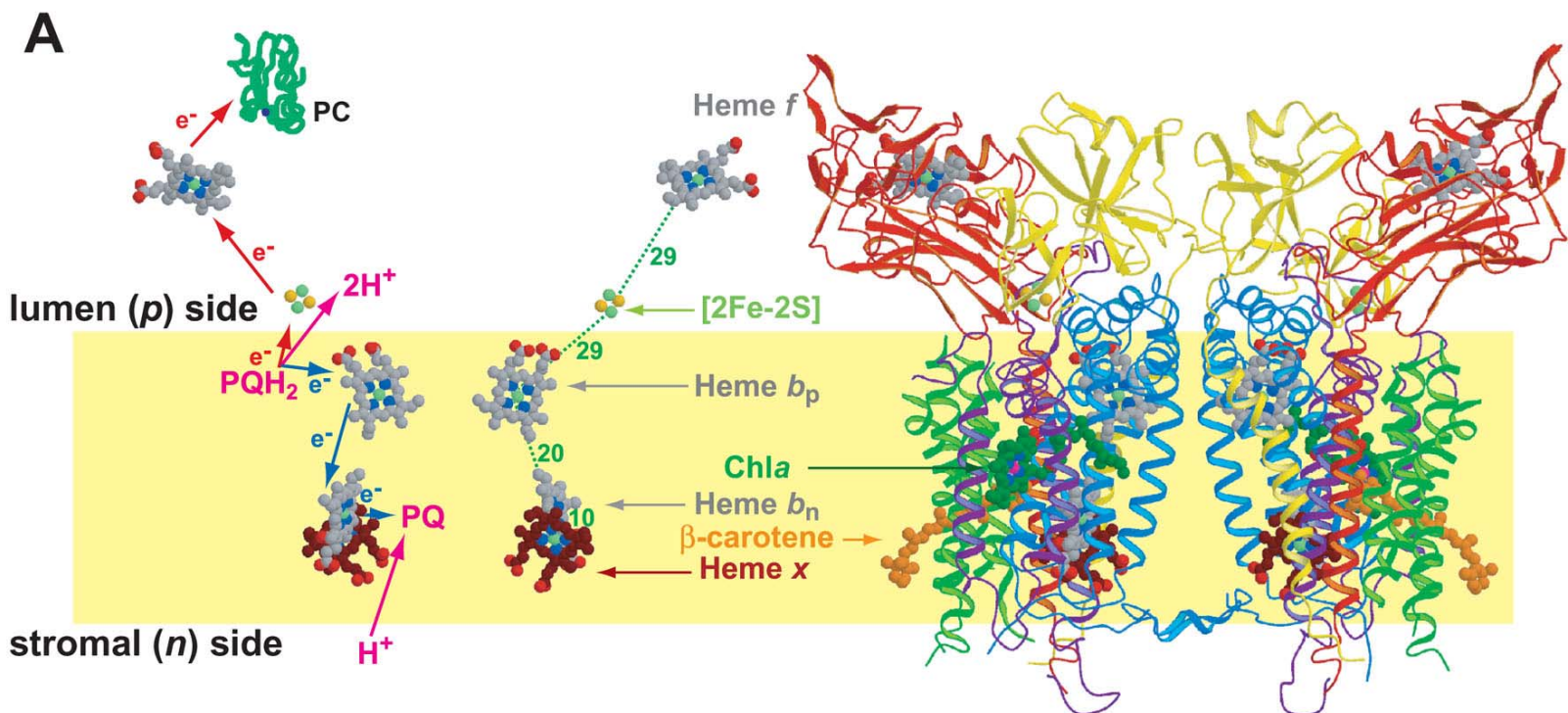
Fig. 5. Hypothesis for pathway of electron transfer between ISP and cytochrome *f*. **(A)** Side views showing the cytochrome *f* and ISP extrinsic domains in the crystal structure (**left**) and the model in which the soluble domain of ISP is rotated by 25° towards cytochrome *f* (**right**). His²⁶ and Leu²⁷ (orange) are shown in a ball-and-stick model. Distances between Leu²⁷ (cytochrome *f*) and His¹²⁹ (ISP) are shown as dotted lines, 21 Å in the crystal structure and 5 Å in the model after 25° rotation. The rotational trajectory of the [2Fe-2S] cluster in the proposed model is shown as a dotted arrow. Color code as in Fig. 2A. **(B)** Schematic drawing around the [2Fe-2S] cluster and cytochrome *f* heme showing the distances in the experimental and model structures.

Table 1. Crystallographic data.

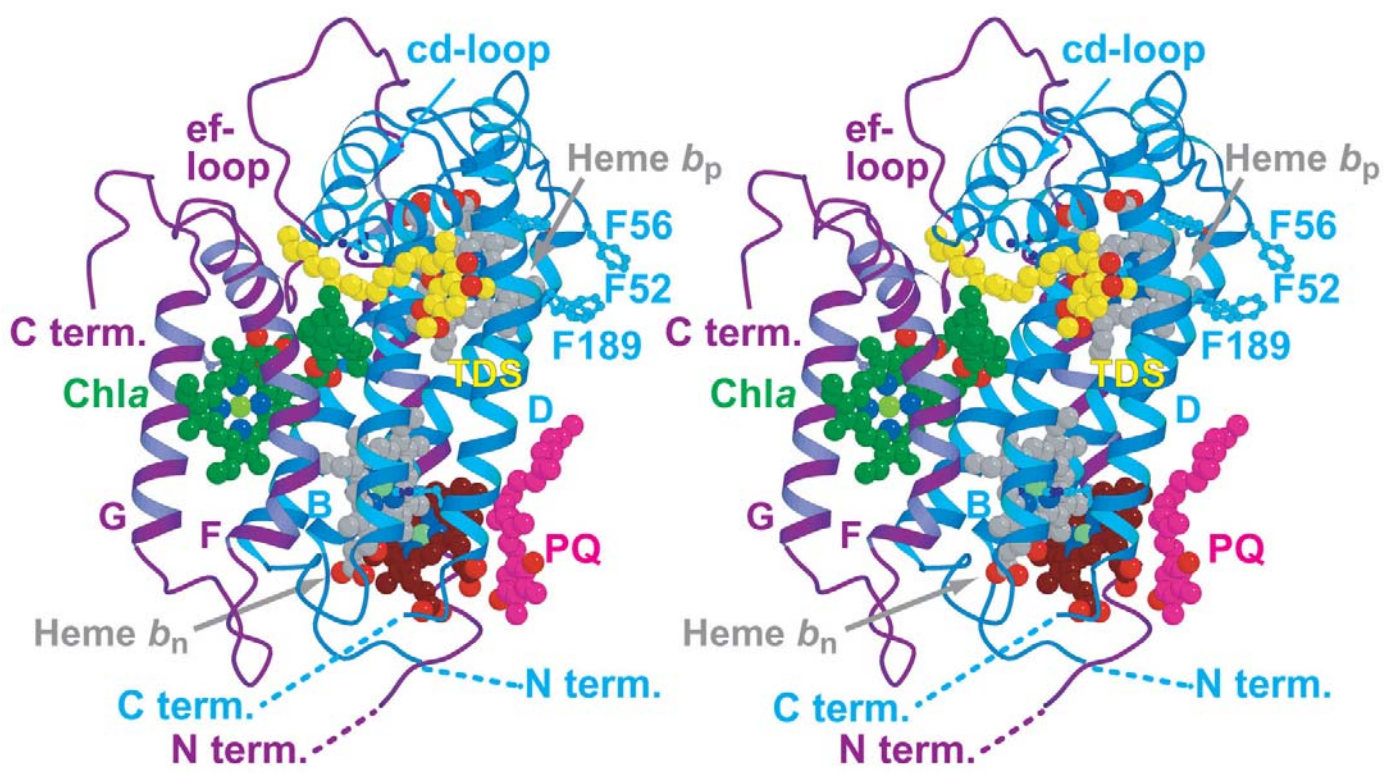
Data set	TDS	DBMIB	Native
X-ray source	<u>APS SBC-19ID</u>	<u>SPring-8 BL44XU</u>	
Wavelength (Å)	1.0332	0.9000	0.9000
Cell constants ($P6_1$)			
a, b (Å)	157.5	156.4	156.6
c (Å)	360.4	363.3	361.8
Resolution* (Å)	3.0 (3.11–3.0)	3.4 (3.52–3.4)	3.4 (3.52–3.4)
Unique reflections (#)	100,622	65,488	67,633
Redundancy	7.0	3.1	7.8
Completeness* (%)	99.8 (100.0)	95.0 (87.7)	98.4 (96.4)
$\dagger R_{\text{merge}}^*$	0.068 (0.540)	0.068 (0.449)	0.089 (0.372)
$\langle I/\sigma_I \rangle^*$	17.9 (2.2)	13.7 (1.8)	18.0 (3.6)
$\ddagger R_{\text{cryst}}$	0.259	0.326	0.256
$\ddagger R_{\text{free}}$	0.346	0.361	0.336
rmsd bonds (Å)	0.011	0.015	0.013
rmsd angles (°)	1.9	1.9	1.9
mean B-values (Å ²)	88	121	96

*Values in parentheses apply to the highest-resolution shell. $\dagger R_{\text{merge}} = \sum_{\mathbf{h}} \sum_i |I_i(\mathbf{h}) - \langle I(\mathbf{h}) \rangle| / \sum_{\mathbf{h}} \sum_i I_i(\mathbf{h})$ where I_i is the i^{th} measurement of reflection $\mathbf{h}$ and $\langle I(\mathbf{h}) \rangle$ is a weighted mean of all measurements of $\mathbf{h}$. $\ddagger R = \sum_{\mathbf{h}} |F_{\text{obs}}(\mathbf{h}) - F_{\text{calc}}(\mathbf{h})| / \sum_{\mathbf{h}} |F_{\text{obs}}(\mathbf{h})|$. R_{cryst} and R_{free} were calculated from the working and test reflection sets, respectively. The test set comprised 3% of the total reflections not used in refinement.

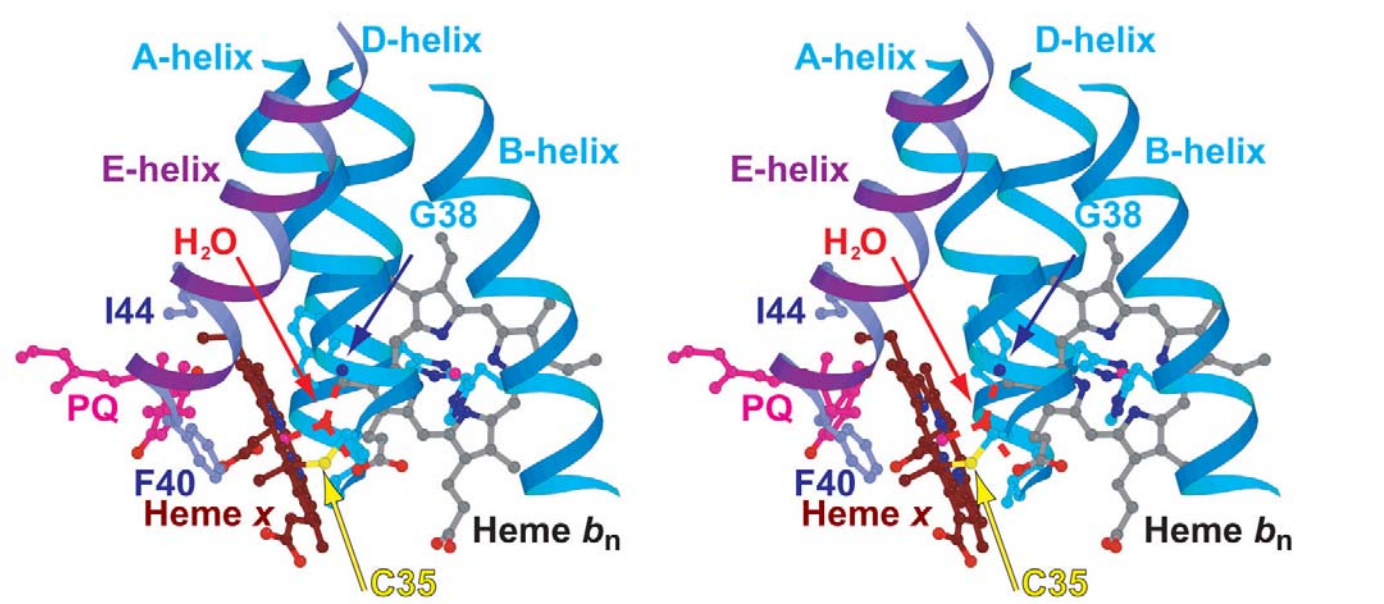


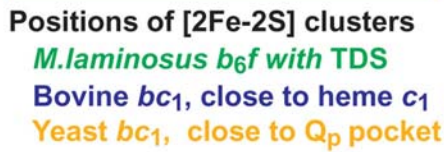


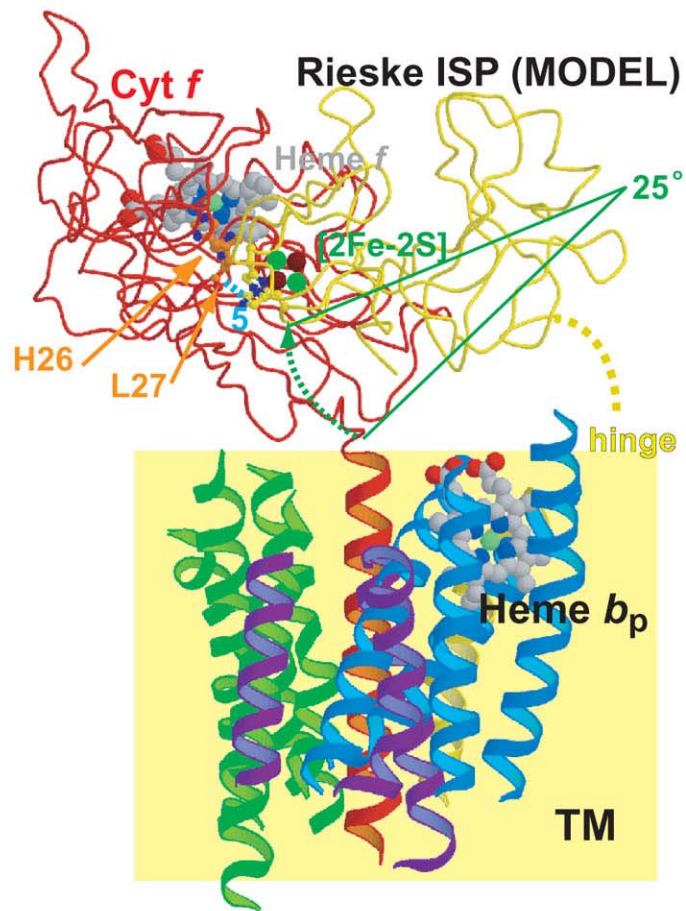
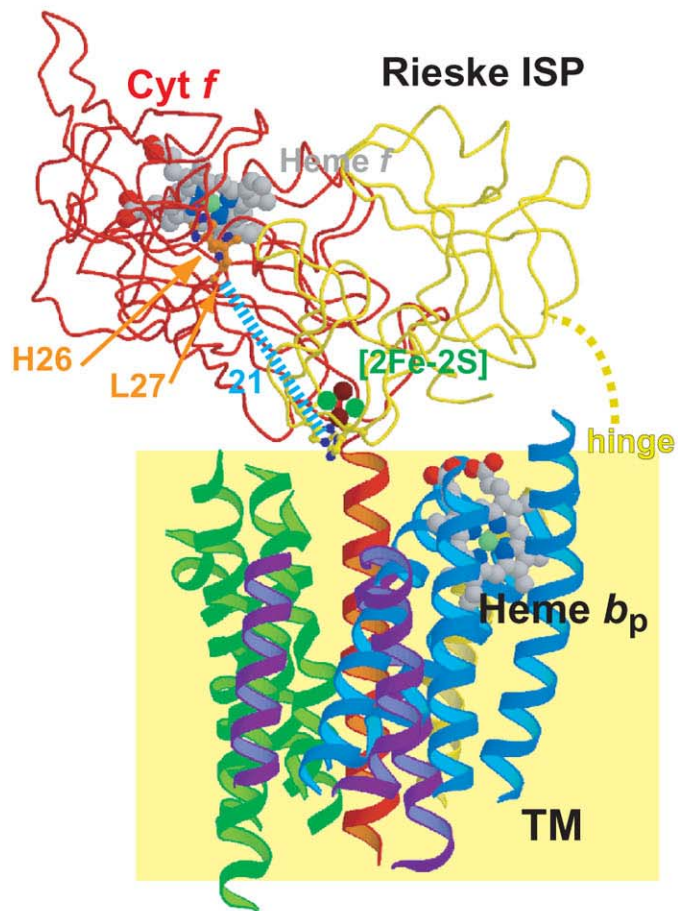
A



B





A**B**