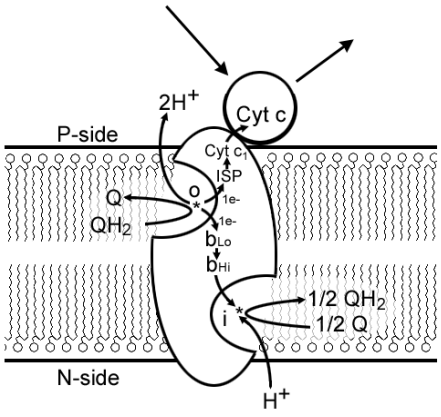
Supplemental Materials



Scheme S1. The protonmotive Q-cycle mechanism by which the cyt bc1 complex is believed to couple electron transfer to proton translocation. The horizontal band shaded with wavy lines represents the lipid bilayer, and the ellipse extending across the bilayer represents the bc_1 complex.

Oxidation of quinol or reduction of quinone results in release or uptake of protons. By arranging sequential oxidation and reduction steps to occur on opposite sides of the membrane, electron transport can be coupled to translocation of protons. If the ***bc₁*** complex simply oxidized quinone at the P-side of the membrane, one "scalar" proton would be released per electron passing through the complex to cytochrome ***c***. In the Q-cycle mechanism, quinol is oxidized at the P side of the membrane (in the Qo site, labeled "o"), but only one of the two electrons released is passed on to cytochrome ***c***. Thus two protons are released on the P side per electron passing through. The second electron is recycled back to the quinol pool by a reduction taking place in protonic equilibrium with the N-side aqueous phase (active site Q_i, labeled "i"), resulting in uptake of one proton per electron. This cycling of electrons from quinol back to quinol does not contribute to the driving force, but results in one proton being translocated from the N phase (normally low protonic potential) to the P phase (normally high protonic potential) and thus requires energy when the membrane is energized with the normal polarity. The energy is provided by the other electron, which passes on to cytochrome ***c*** and eventually to molecular oxygen in cytochrome oxidase. The overall stoichiometry is thus one proton translocated and one scalar proton released per electron, which is consistent with the experimentally determined stoichiometry of proton and charge translocation.

Notice that a single turnover of the Q_O site provides only one electron to the Qi site, while two electrons are required to reduce quinone to quinol. Although some early models proposed dismutation or input of an electron directly from a dehydrogenase to complete the reduction, it appears now that this site undergoes two non-equivalent single-electron reactions: first reducing quinone to a semiquinone and then, on the next turnover of the Qo site, reducing semiquinone to quinol. Thus the Qi site must bind semiquinone, quinone, and quinol at different stages of the catalytic cycle; and an inhibitor acting at the site might be expected to mimic any one of these three forms of the substrate.

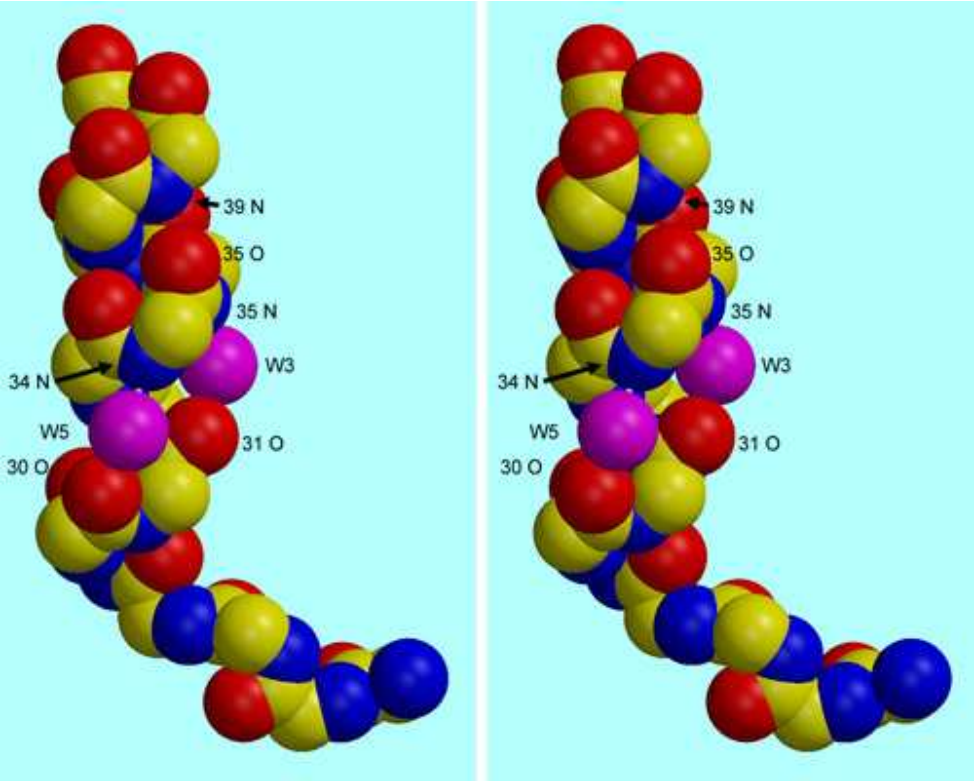


Figure S1. Helix-Intercolated Waters. Stereodiagram of the beginning of helix A of cyt b showing how the two "intercolated" waters fit into the secondary structure of the helix. Purple spheres labeled W3 and W5 are the waters, the other atoms are backbone atoms of cyt b (residues 25 to 39) with Molscript default colors (C, N, O yellow, blue, and red). Side-chain atoms have been removed for clarity. Notice the normal □-helical interaction between atoms 35 O and 39 N, and compare the relation of 31 O with 35 N or 30 O with 34 N.

Omit maps for critical features. Figures in the text show density maps made with phases calculated from the entire structure, as these phases are the most accurate if it is assumed the model is correct. Coefficients of 2Fo-Fc were used to minimize model bias, however there is still some model bias in such maps. As a more stringent test of the validity of critical parts of the model, Figures S2-S7 show features of the structure 1PPJ together with density from an omit map, in which those features were omitted to avoid model bias. Except in Figure S2, residues with atoms withing 3 Å of the specified residues were also omitted. Before map calculation, the structure with omitted residues was subjected to simulated anealing from 1000K in steps of 25 K/cycle, followed by 100 steps of conjugate gradient minimization. No harmonic restraint was applied to the region around the omitted area during the refinement. The map coefficients were sigma-A weighted (2mFo-DFc).

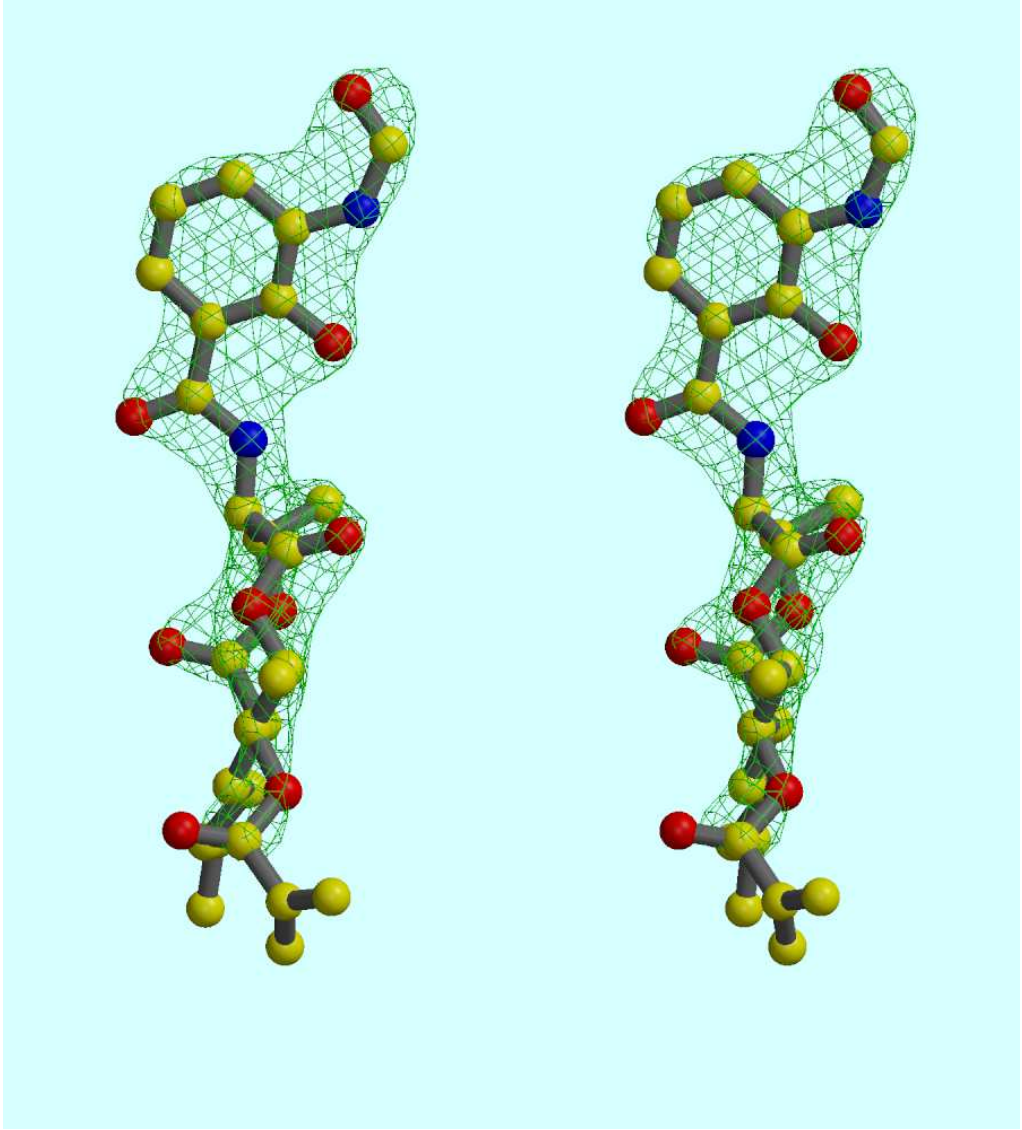


Figure S2. Stereo views of omit map density for antimycin in 1PPJ.
As Figure 1b except the map is a Sigma-A weighted simulated-anealing omit map with coefficients . Contour level 1.4 σ

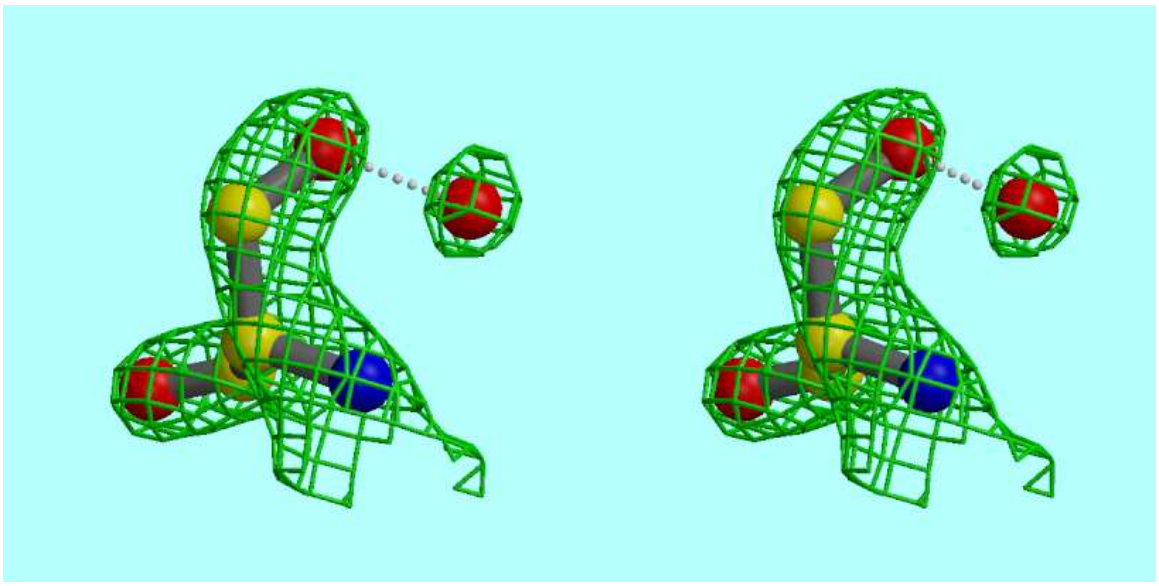


Figure S3. Omit map for C:Ser35. Map as in S2 except that C and P:Ser35 were omitted, together with residues within a 3Å sphere. Contour level 2.0 σ. The water molecule W is also included.

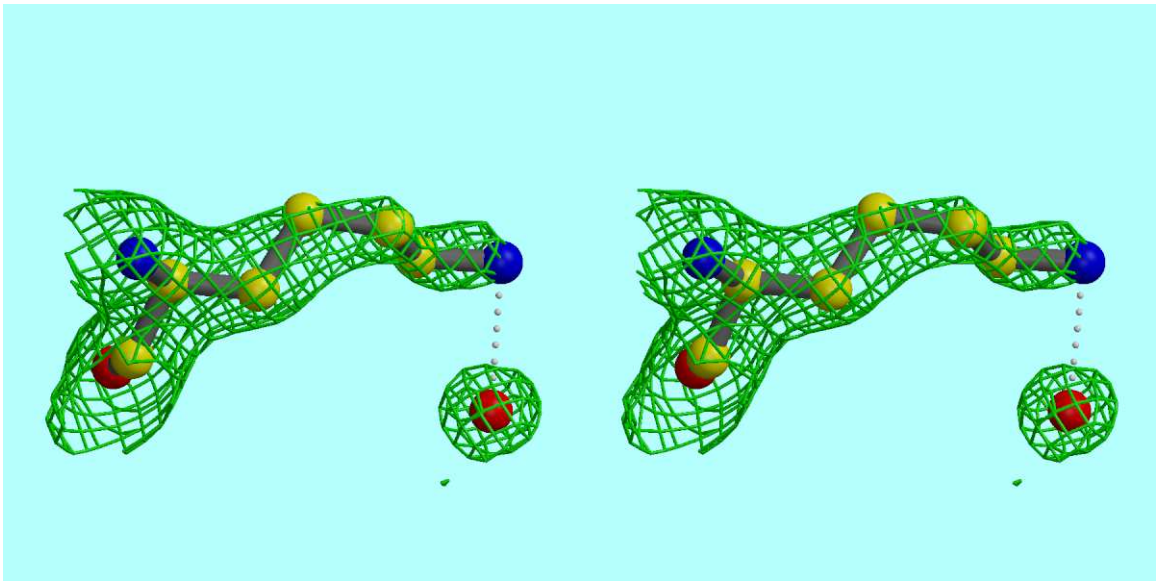


Figure S4. Omit map for P:Lys227. Map as in S3 except that C and P:Lys227 was omitted, together with residues within a ?Å sphere. Contour level 1.8 σ

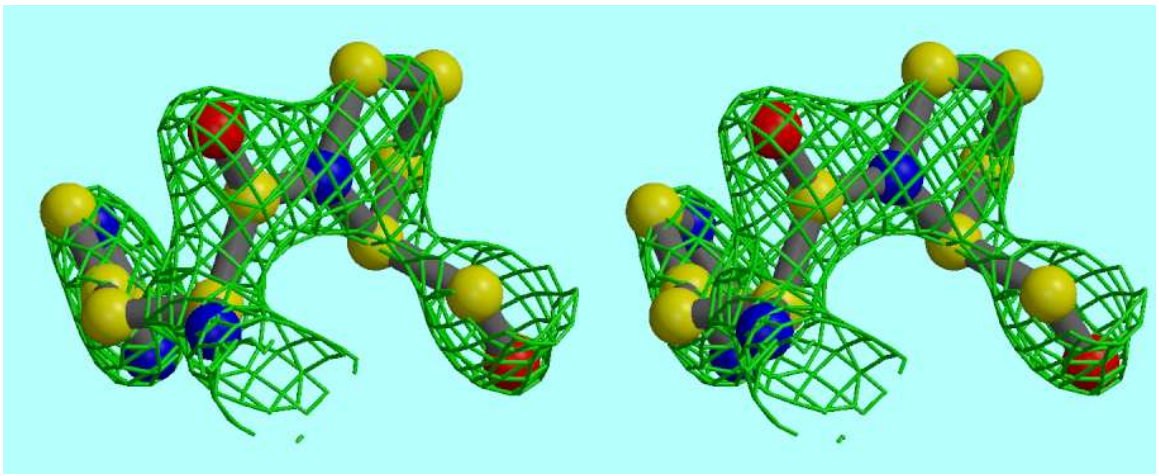


Figure S5. Omit map for C:His221-Pro222. Map calculated as in S3 except that C: and P:His221-Pro222 were omitted, Contour level 1.8 σ

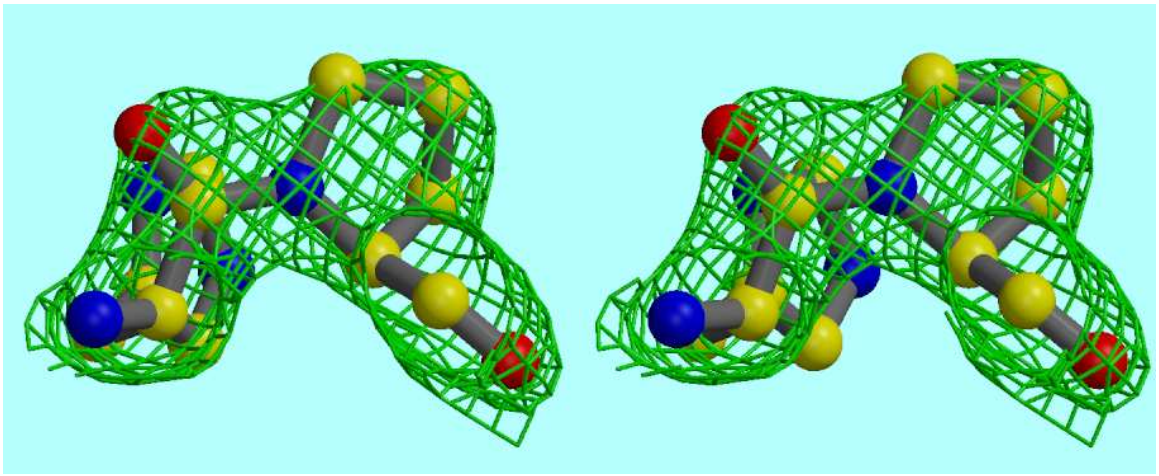


Figure S6. Omit map for C:His345-Pro346. Map calculated as in S3 except that C: and P:His345-Pro346 were omitted. Contour level 0.7 σ

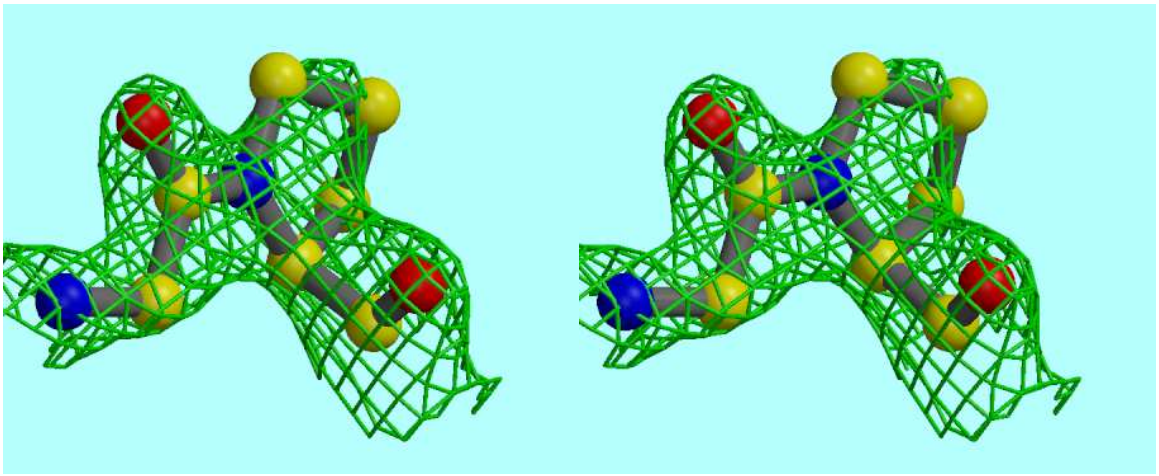


Figure S7. Omit map for D:Gly73-Pro74. Map calculated as in S3 except that D: and Q:Gly73-Pro74 were omitted. Contour level 1.6 σ

Table S1. Definition of Rotamers reffered to in the text."Freq" is the frequency with which the rotamer occurs in in the population used to define the rotamers.
The Lysine rotamer 26 seen in C:Lys227 is from the more extensive collection of ref. 77.

residue	rotamer	Freq.	chi1	chi2	chi3	chi4
Ser	**1**	**45%**	**63**			
	2	**30%**	**-62**			
His	**3**	**16%**	**-169**	**80**		
	5	**8%**	**-59**	**169**		
Lys	**26**	**3%**	**-66**	**180**	**67**	**180**