Supplementary Information:

P450 electron transfer mechanics: unravelling the evolutionary role of transient redox protein complexes.

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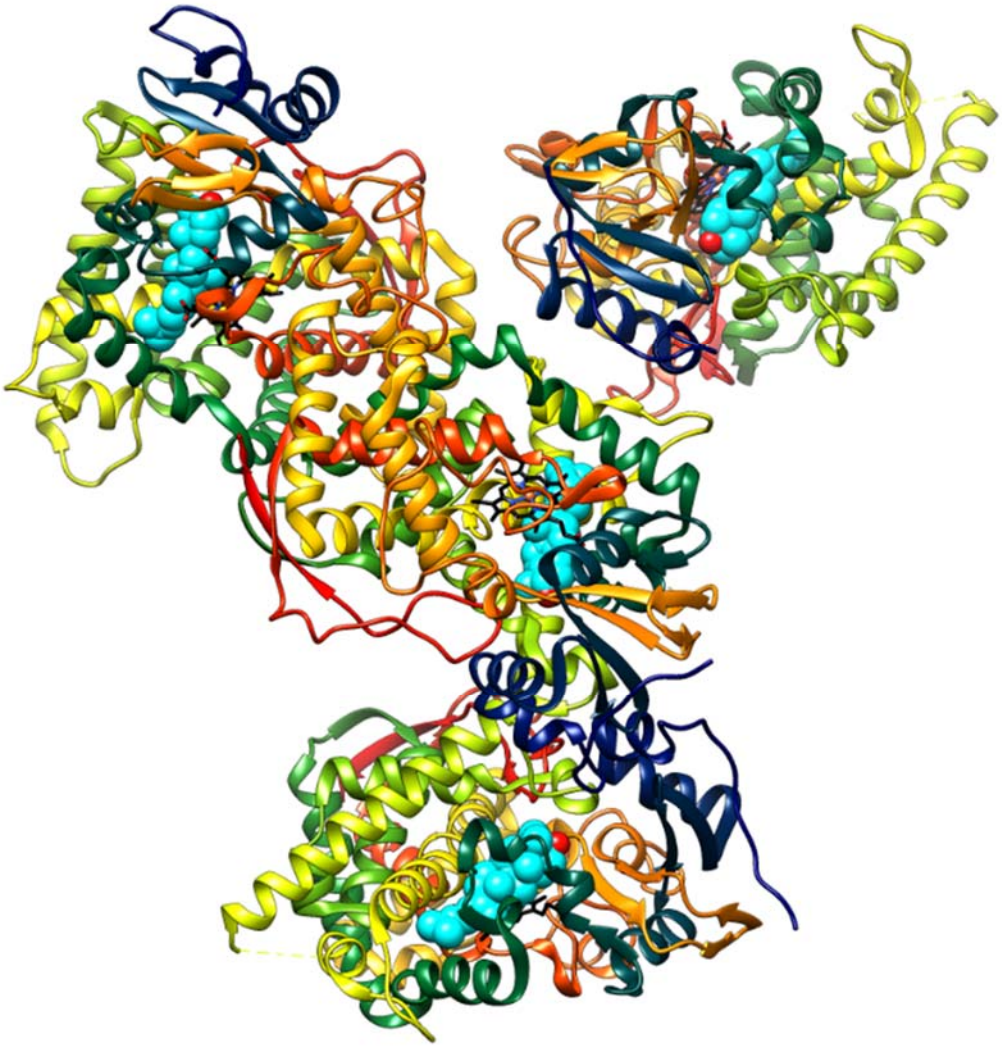
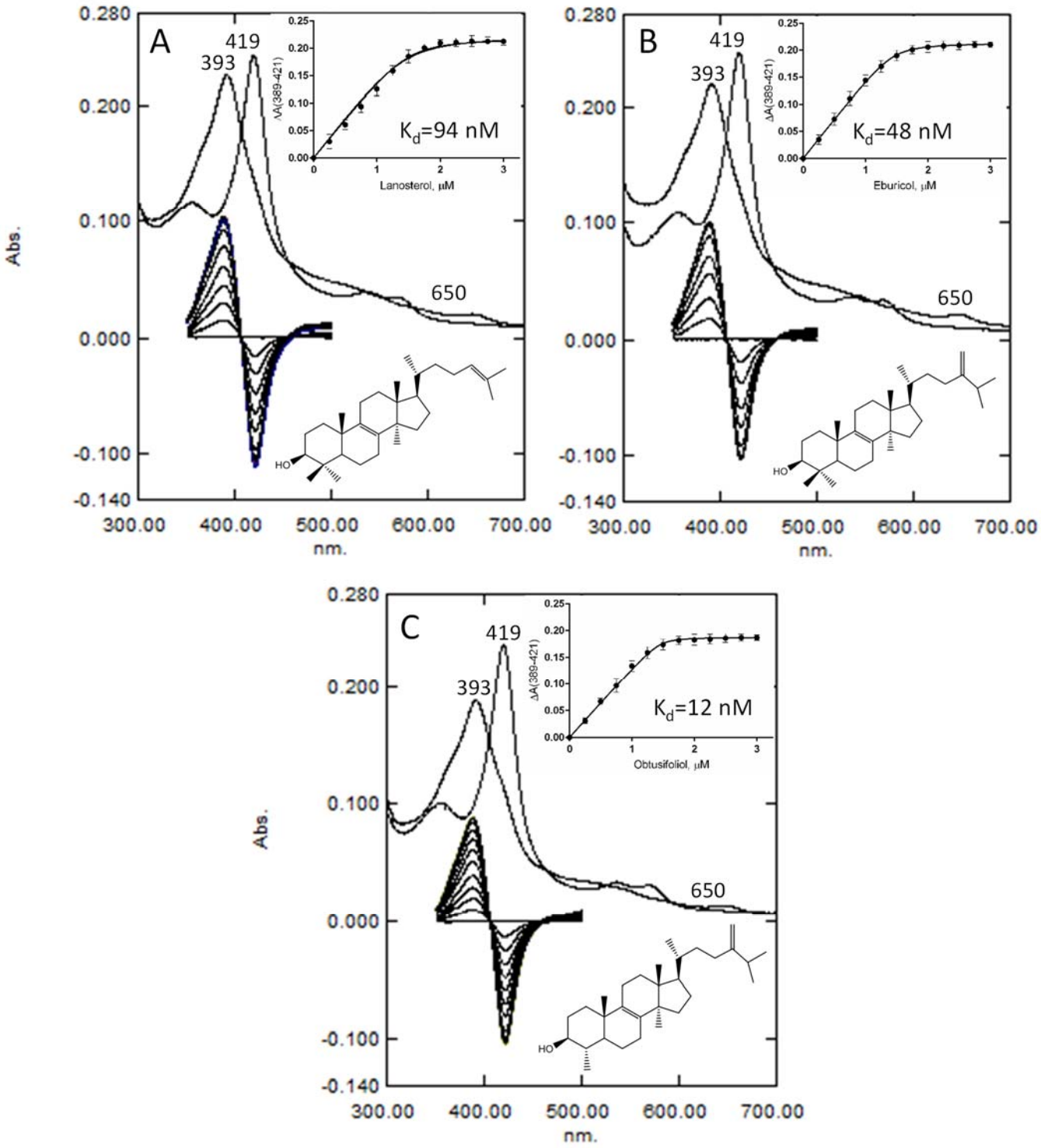
Supplementary Table S1. Data collection and refinement statistics.

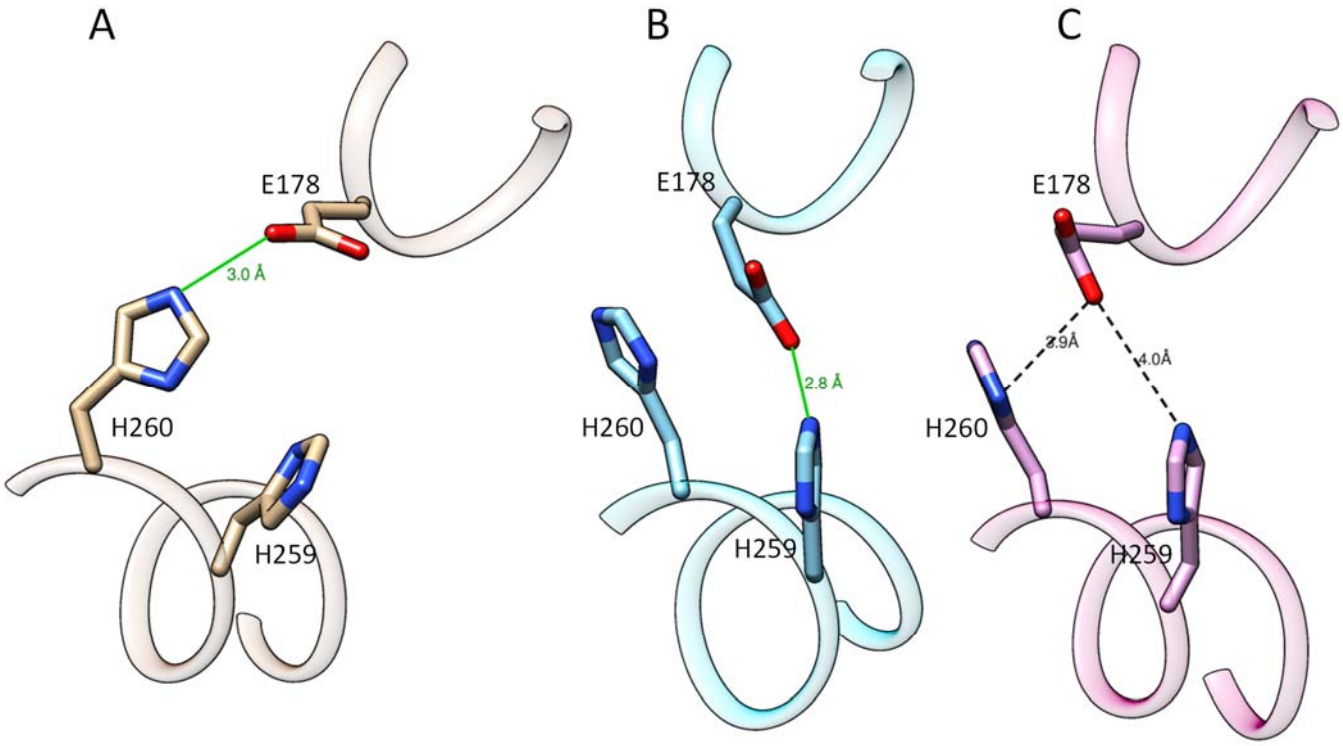
PDB ID	7SNM
Data collection	
Wavelength, Å	1.000
Space group	P_12_11
Cell dimensions	
a, b, c, Å	73.2 206.20 81.4
α, β, γ, °	90.0 114.1 90.0
Molecules per asynetric unit	4
Resolution (upper shell), Å	74.45-2.55 (2.62-2.55)
Solvent content, %	47.7
R_{merge} (upper shell)	0.065 (0.637)
CC (1/2) (upper shell)	0.998 (0.960)
I/σ(I) (upper shell)	13.4 (2.2)
Completeness (upper shell), %	99.0 (99.9)
Redundancy (upper shell)	7.1 (7.4)
Refinement	
No. of reflections	67008
R_{work}/R_{free}	0.204/0.246
R.m.s deviations	
Bond lengths, Å	0.002
Bond angles, °	1.3
Ramachandran plot	
Favorable/allowed, %	94.0/99.8
Outliers, %	0.2
Average B factor, Å^2	54.9
Model	
No. of atoms	14767
No. of residues per molecule	
Protein (B factor, Å^2, A/B/C/D)	443 (45.5/48.6/66.6/77.7)
Heme (B factor, Å^2)	1 (13.8/16.2/35.5/31.0)
Water (B factor, Å^2)	341 (47.1)
Lanosterol [Lan] (B factor, Å^2)	1 (13.5/20.0/31.0/34.3)



Supplementary Figure S1. The asymmetric unit of *M. capsulatus* CYP51. Four polypeptide chains of the P450 molecule are shown in a rainbow-colored ribbon representation, from the N-terminus (blue) to the C-terminus (red). The heme is seen in a stick representation, the carbon atoms are black. The atoms of lanosterol are presented as spheres, the carbons are cyan. The RMSD of Cα between the four molecules is 0.35 Å.



Supplementary Figure S2. **Spectral changes observed during titration of *M. capsulatus* CYP51-fx with (A) lanosterol (B) eburicol, and (C) obtusifoliol**. Absolute (top) and difference (bottom) spectra. The P450 concentration was ~2 μM. Inset: The titration curves fitted to the quadratic (Morrison) equation.

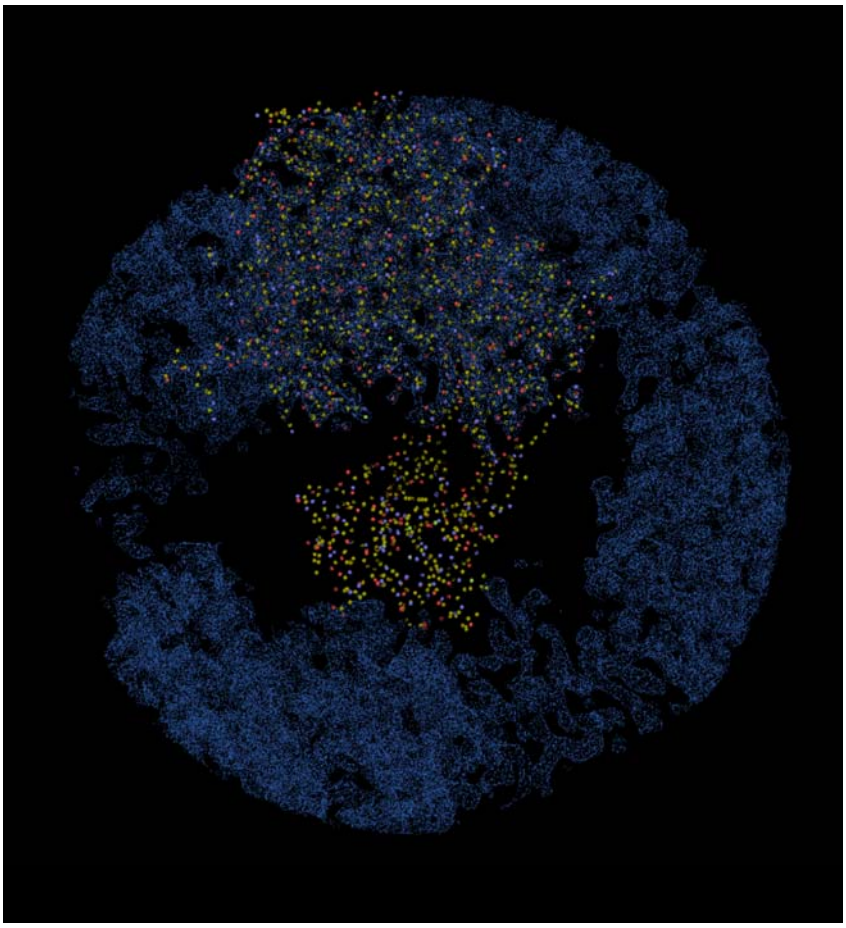


Supplementary Figure S3. **Rearrangements in the proton relay network in *M. capsulatus* CYP51.** Ligand-free (salt bridge E178-H260, 3.0 Å) (**A**), lanosterol-bound (salt bridge E178-H259, 2.84 Å) (**B**) structures of *M. capsulatus* CYP51, and a snapshot from MD simulations of the CYP51-ferredoxin complex, |Fe-Fe1|=16.6 Å. (E178 is freed, proton delivery route activated) (**C**).

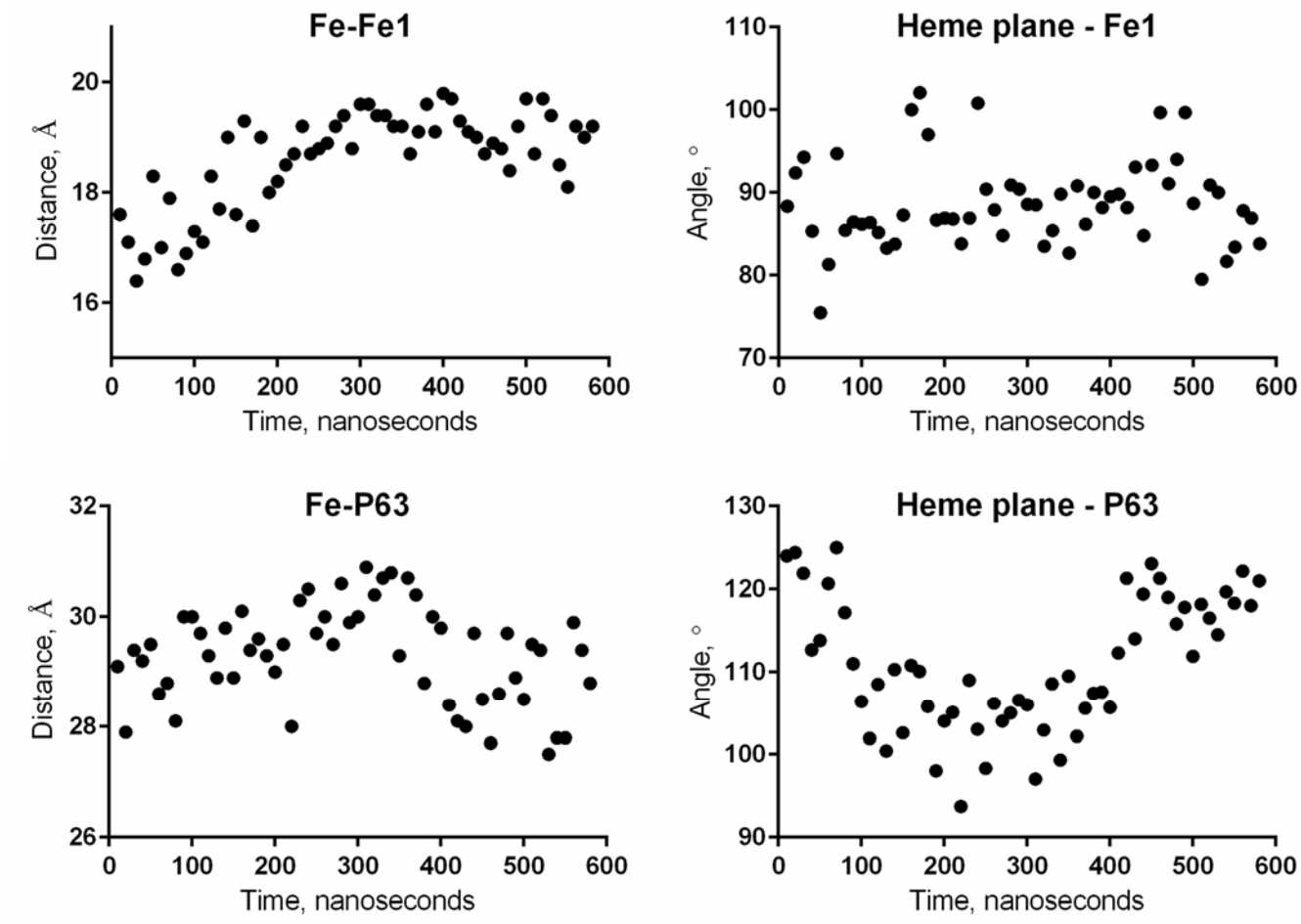
Supplementary Table S2. Active site cavity lining residues in the substrate-bound bacterial (*M. capsulatus*, 7SNM), protozoan (*T.cruzi*, 6FM0), and mammalian (human, 6UEZ) CYP51 structures

Structural element	*M. capsulatus*	Human D231A/H314A	*T. cruzi* I105F
	Substrate (Lanosterol)	Substrate (Lanosterol)	Substrate (Obtusifoliol)
B' helix	**Y79** I81 M82 **F86**	**Y131** L134 T135 **F139**	**Y103*** F105 M106 **F110**
B'' η-turn	**V90** V91 F92	**V143** A144 Y145	**V114** A115 Y116
C helix	K99 **Q102** L103 M105 **L106** M107	F152 **Q155** K156 M158 **L159**	M123 **Q126** L127 F129 **L130**
F'' helix	 I181 Y186	 F234	M204 S207 L208 I209 P210 V213
G' helix			L219
I helix	**G249** **M250** L251 A253 T254 F256 **A257** **H260** **T261**	**G303** **M304** G307 L308 L310 **A311** **A314**(H314)** **T315**	**G283** **M284** I285 A287 A288 F290 **A291** **H294** **T295**
K/b1-4 loop	**P321** L322 I323 *L324***	**P376** I377 M378 *L379****	**P355** L356 L357 *M358****
β1-4	L325 **M326**	M380 **M381**	V359 **M360**
β4 hairpin	**M433** V434	**M487** I488	**M460** V461
Total #, volume, Å^3	32 913	29 865	36 1203

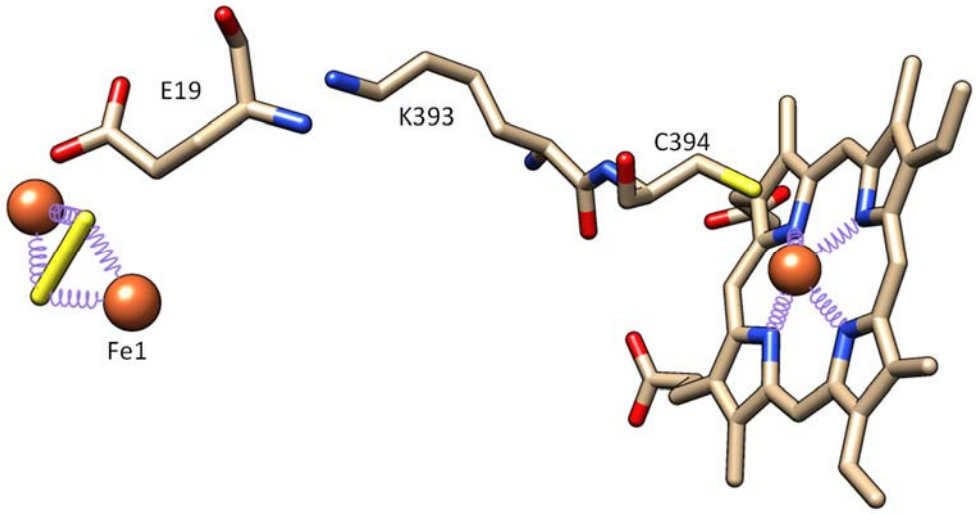
*Residues invariant across the biological kingdoms (**in bold**), **mutant (H314A), ***H-bond with the substrate OH group (*italic*)



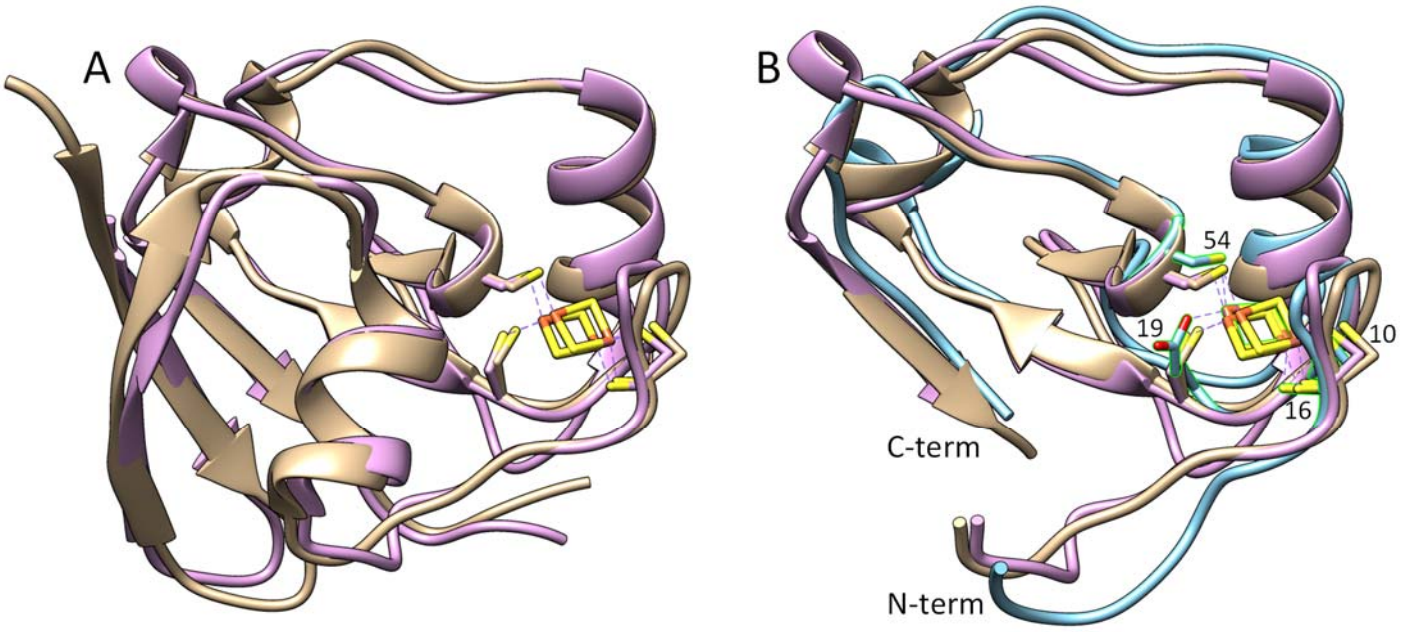
Supplementary Figure S4. **A Coot snapshot of the $2F_0$-F_c density map contoured at 1.5 σ, map radius 50 Å.** The dots correspond to one molecule of P450 (top, proximal face down) and one molecule of ferredoxin (bottom, the docked pose, selected for MD simulations). The electron density is clearly seen for the P450 domain only, while any density close to the proximal P450 surface (where the ferredoxin domain is supposed to interact) is absent.



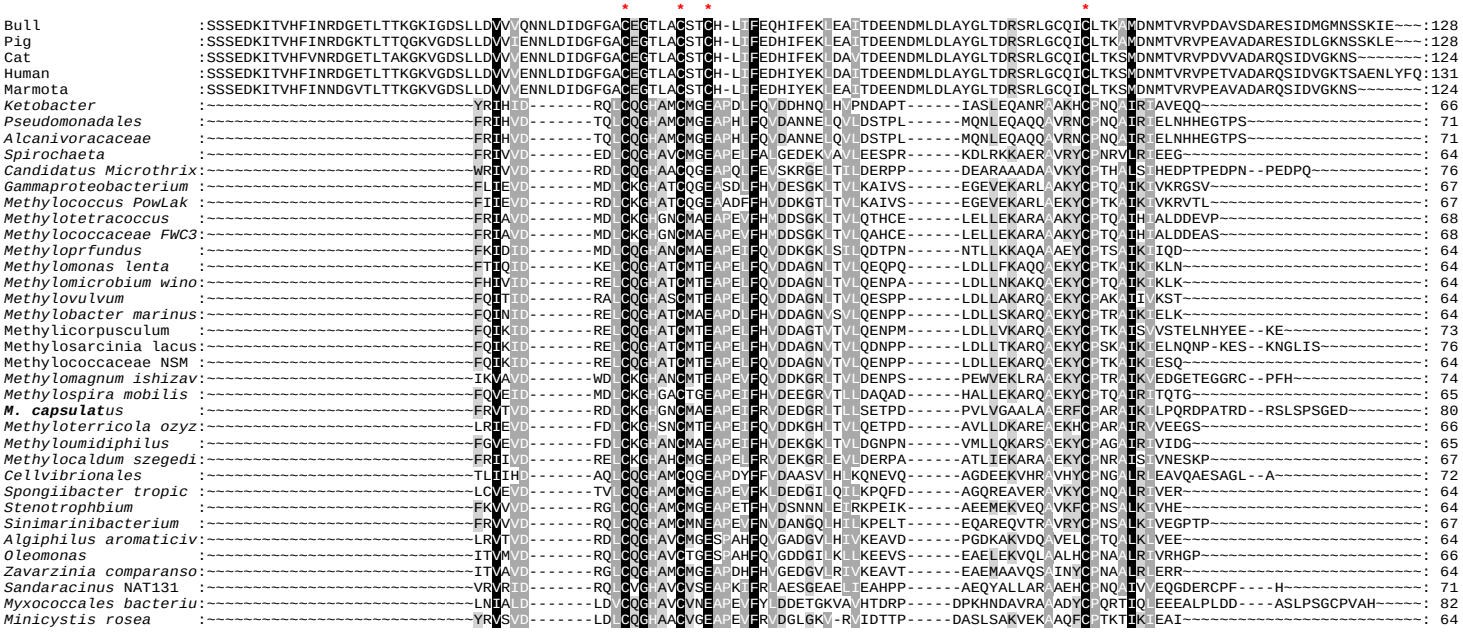
Supplementary Figure S5. Molecular-dynamics trajectories. The pbd files were written each 10 ns.



Supplementary Figure S6. Possible ET path in *M. capsulatus* CYP51-fx.



Supplementary Figure S7. **Ferredoxins that serve as P450 redox partners display highly conserved structural fold**. **A**. Overlaid structures of putidaredoxin from *Pseudomonas putida* [1OQQ, tan] and adrenodoxin from human [2Y5C, plum]. **B**. The 35-103 fragments of the same structures overlaid with the model of *M. capsulatus* ferredoxin fusion (blue). The 2Fe-2S cluster, Cys10, 16, 54, and Glu19 of the model are selected (in green) and the residues are numbered.



Supplementary Figure S8. Alignment of 5 mammalian ferredoxins and 30 bacterial ferredoxin domains that exist as naturally fused C-terminal portion of CYP51 genes. Four residues that form the interactions with the [Fe_2-S_2] cluster are marked with red stars.