

Supplementary Information:

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

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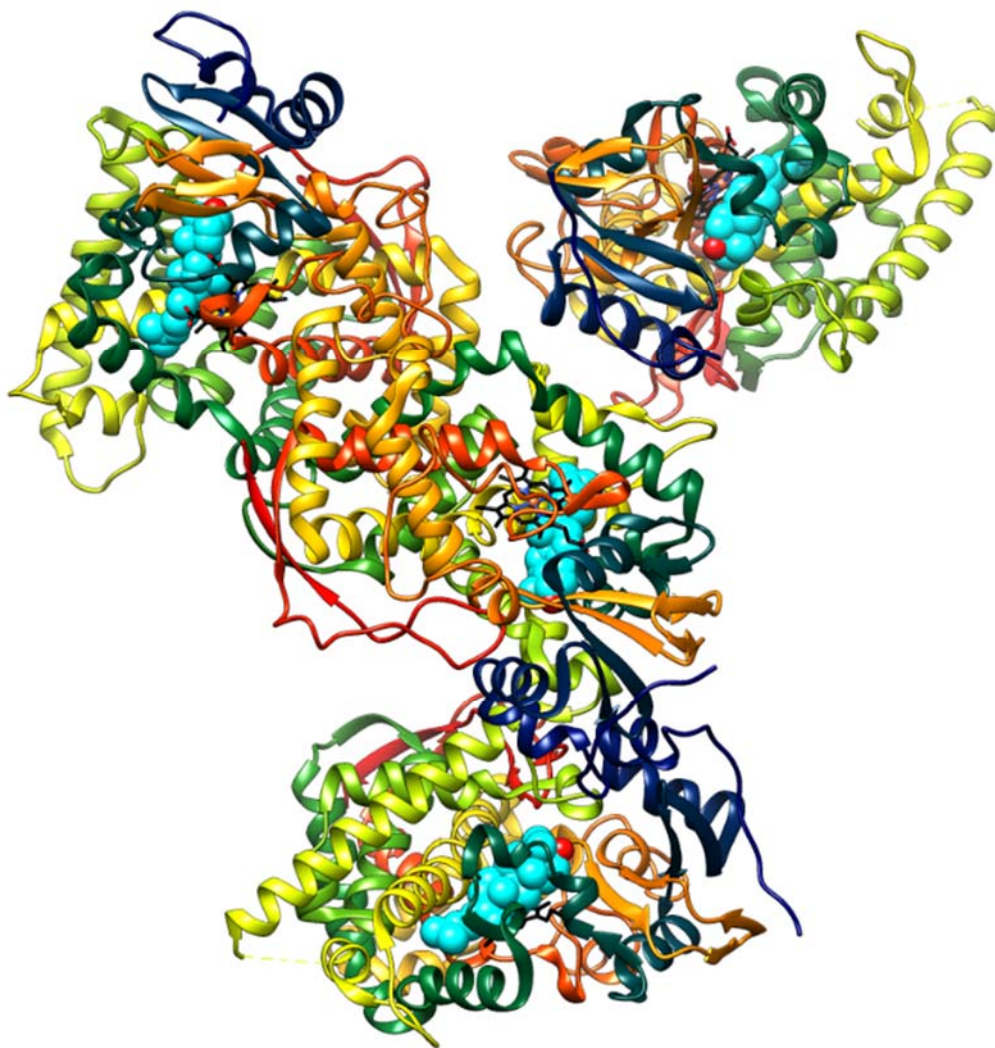
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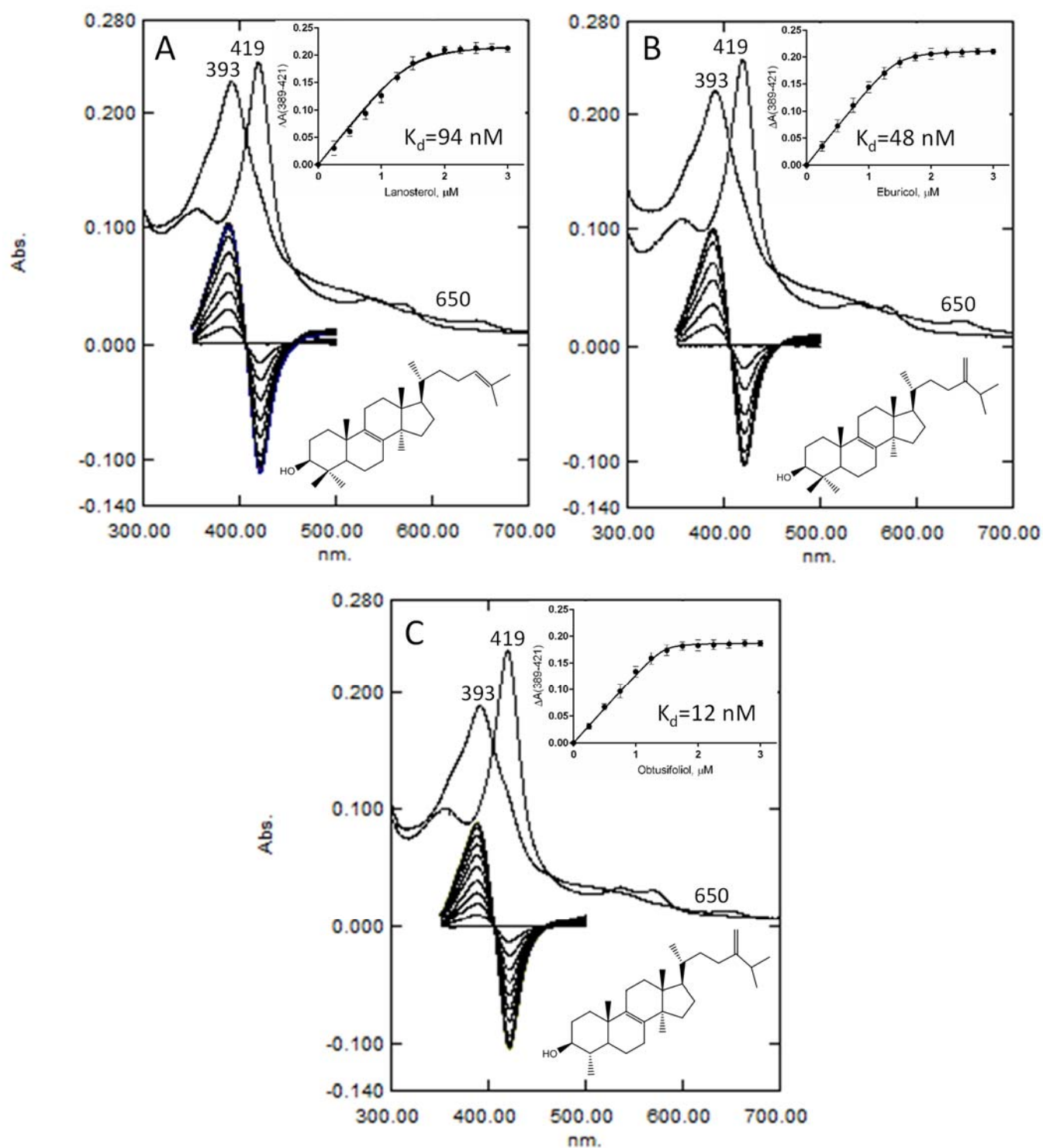
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Supplementary Table S1. Data collection and refinement statistics.

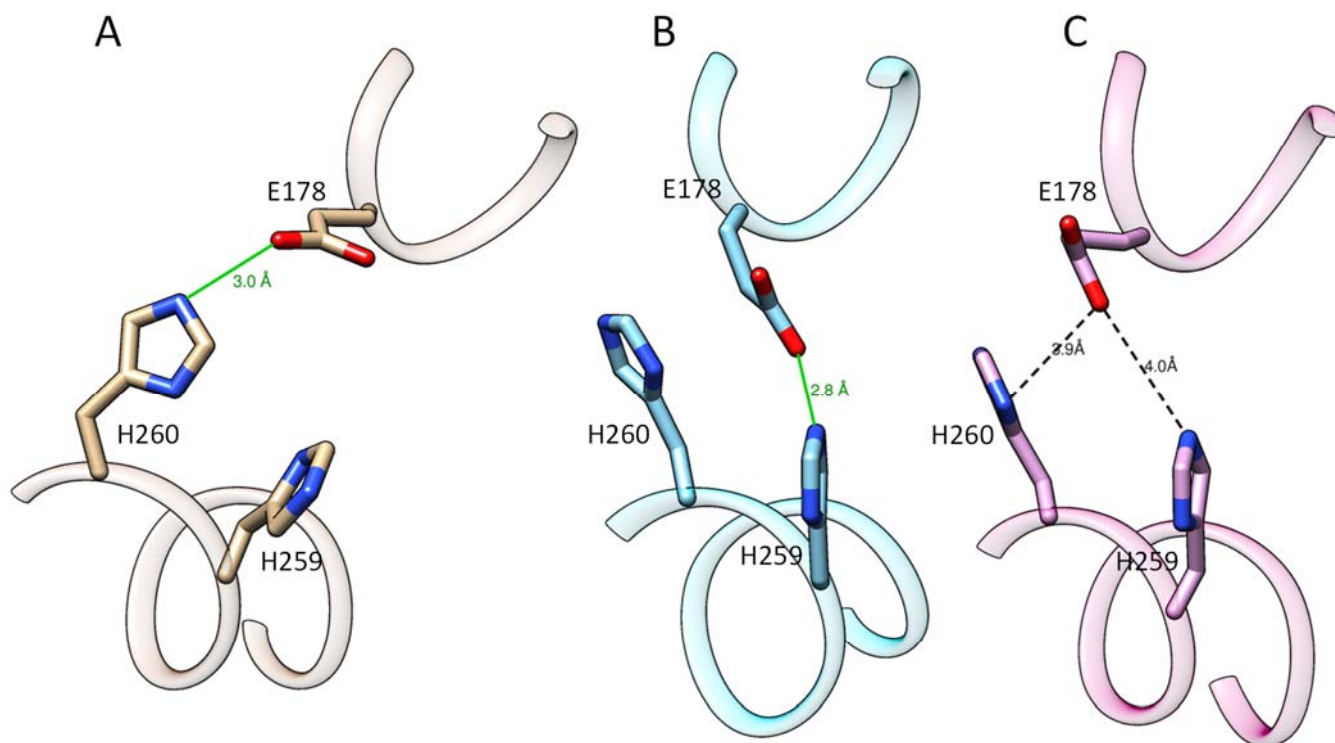
PDB ID	7SNM
<i>Data collection</i>	
Wavelength, Å	1.000
Space group	P ₁ 2 ₁ 1
Cell dimensions	
a, b, c, Å	73.2 206.20 81.4
α, β, γ, °	90.0 114.1 90.0
Molecules per asymmetric unit	4
Resolution (upper shell), Å	74.45-2.55 (2.62-2.55)
Solvent content, %	47.7
R _{merge} (upper shell)	0.065 (0.637)
CC (1/2) (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)
<i>Refinement</i>	
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
<i>Model</i>	
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 C α 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 μ M. Inset: The titration curves fitted to the quadratic (Morrison) equation.

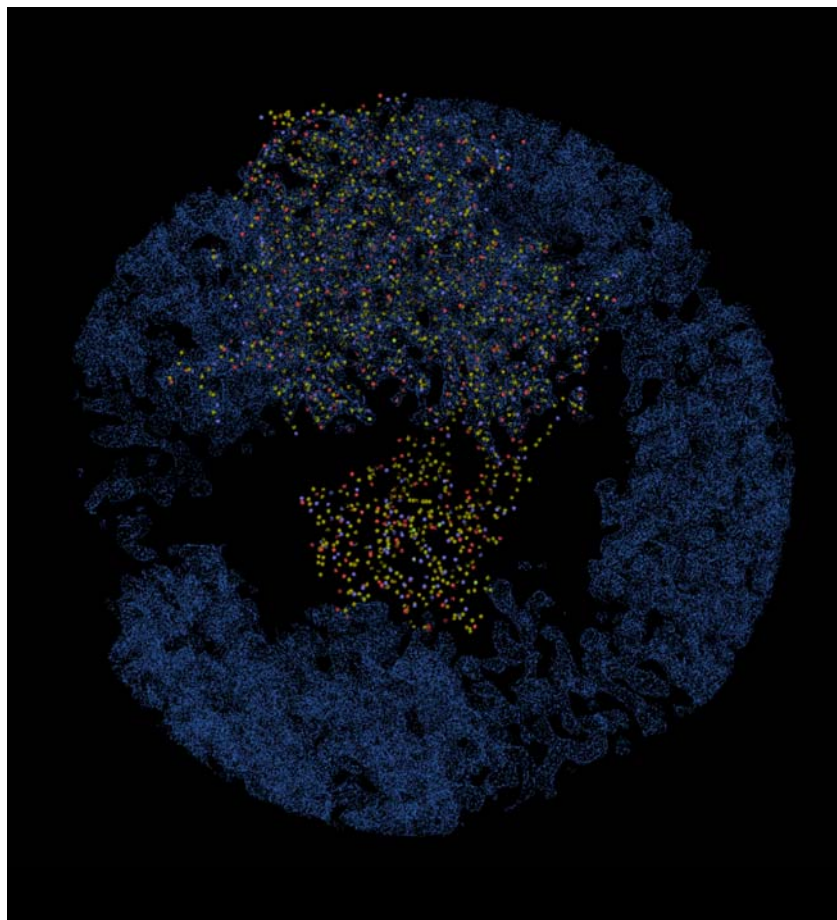


Supplementary Figure S3. Rearrangements in the proton relay network in *M. capsulatus* CYP51. Ligand-free (salt bridge E178-H260, 3.0 Å) (A), lanosterol-bound (salt bridge E178-H259, 2.84 Å) (B) structures of *M. capsulatus* CYP51, and a snapshot from MD simulations of the CYP51-ferredoxin complex, $|\text{Fe-Fe1}|=16.6$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

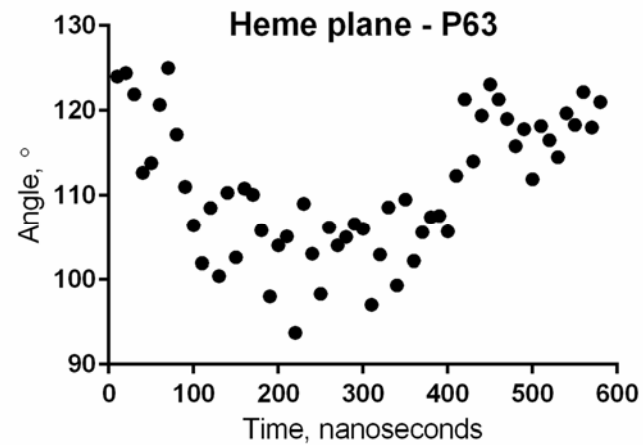
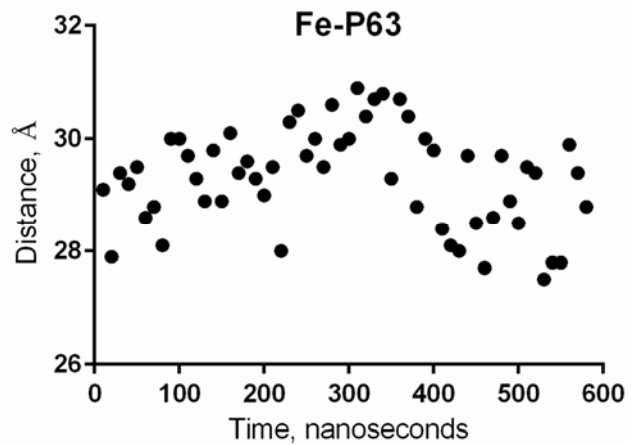
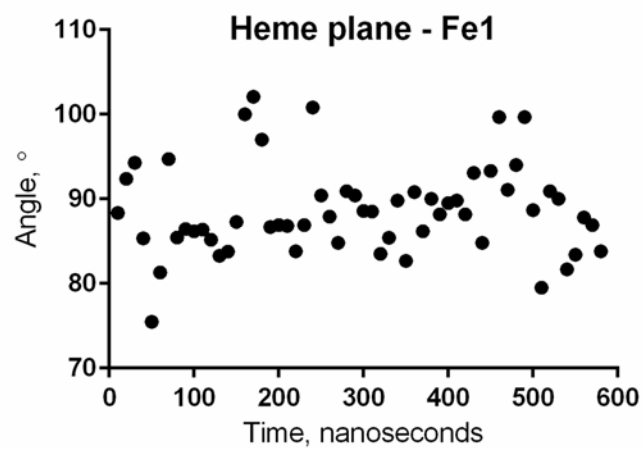
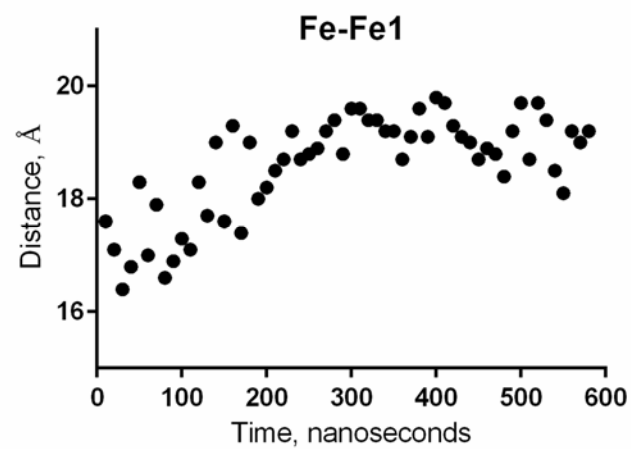
Structural element	<i>M. capsulatus</i>	Human D231A/H314A	<i>T. cruzi</i> I105F
	Substrate (Lanosterol)	Substrate (Lanosterol)	Substrate (Obtusifolol)
B' helix	Y79 I81 M82 F86	Y131 L134 T135 F139	Y103* F105 M106 F110
B'' η-turn	V90 V91 F92	V143 A144 Y145	V114 A115 Y116
C helix	K99 Q102 L103 M105 L106 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 <i>L324***</i>	P376 I377 M378 <i>L379***</i>	P355 L356 L357 <i>M358***</i>
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*)

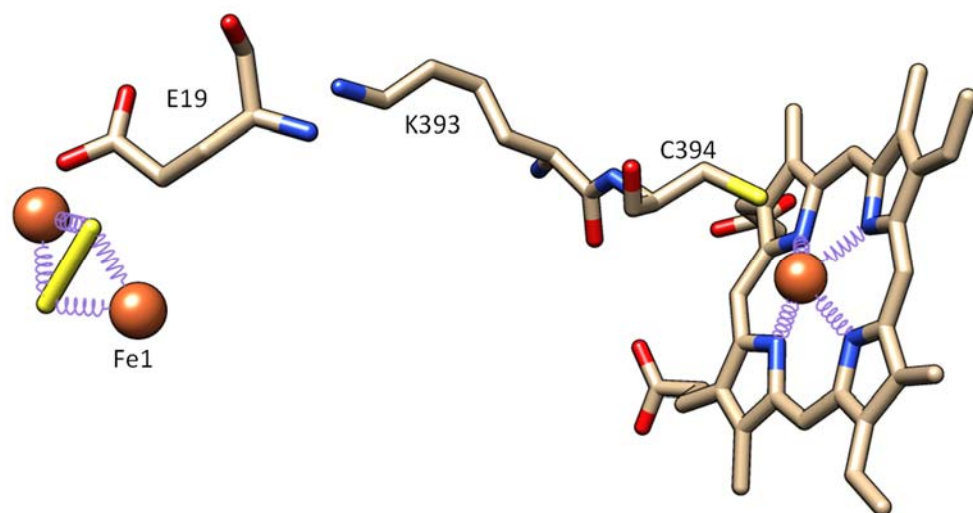


Supplementary Figure S4. A Coot snapshot of the 2F₀-F_c density map contoured at 1.5 σ , map radius 50 Å.

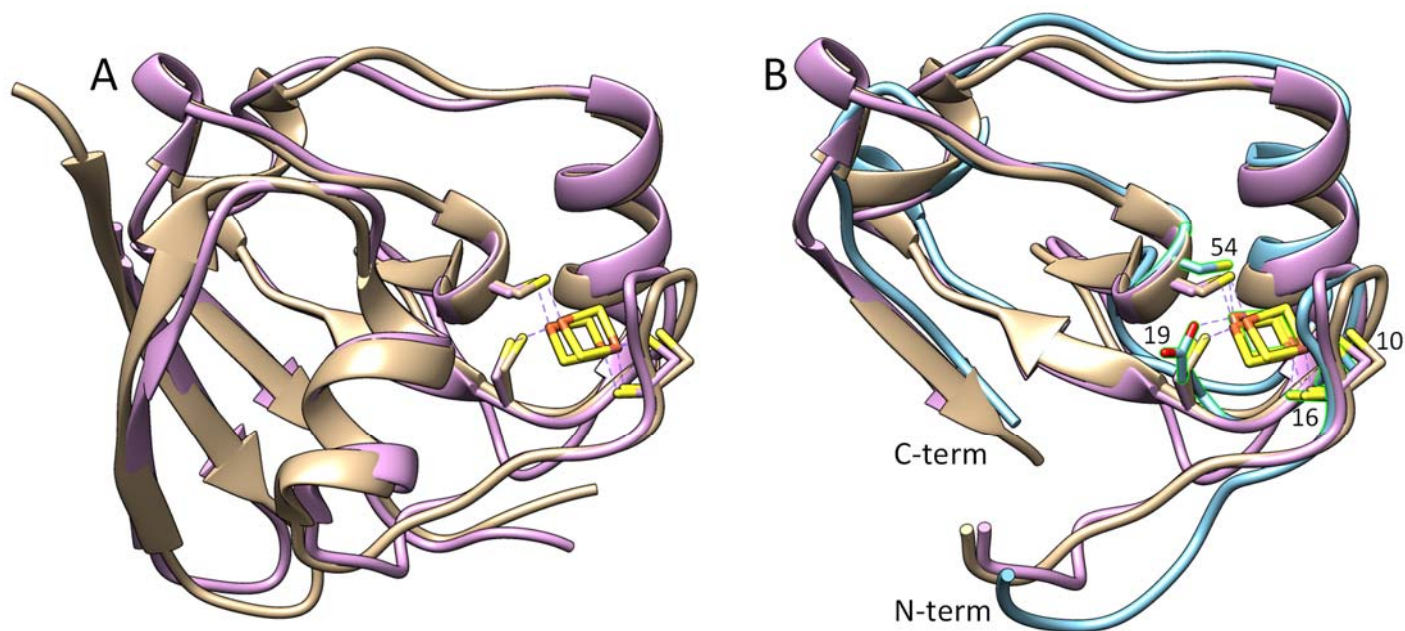
The dots correspond to one molecule of P450 (top, proximal face down) and one molecule of ferredoxin (bottom, the docked pose, selected for MD simulations). The electron density is clearly seen for the P450 domain only, while any density close to the proximal P450 surface (where the ferredoxin domain 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.

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Bull      :SSSEDKITVHFINRDGETLTGKIGDSSLDDVYNNLDIDGFGAEETLACSTGH-LIEQHIFEK EATDEENDMLDLAYGLTDRSRLGCQICLTKNONMTVRVPDAVSARESIDMGMSKIE---: 128
Pig       :SSSEDKITVHFINRDGKLTGKIGDSSLDDVYNNLDIDGFGAEETLACSTGH-LIEQHIFEK EATDEENDMLDLAYGLTDRSRLGCQICLTKNONMTVRVPEAVADARESIDLGNSSKLE---: 128
Cat       :SSSEDKITVHFVNRDGETLTAKGKVGDSLLDVYNNLDIDGFGAEETLACSTGH-LIEQHIFEK DAEDEENDMLDLAYGLTDRSRLGCQICLTKSNONMTVRVPDVADARQSIDVGKNS-----: 124
Human     :SSSEDKITVHFINRDGETLTGKIGDSSLDDVYNNLDIDGFGAEETLACSTGH-LIEQHIYEK DAEDEENDMLDLAYGLTDRSRLGCQICLTKSNONMTVRVPETVADARQSIDVGKTSANLYFQ: 131
Marmota   :SSSEDKITVHFINNDGVTLTGKIGDSSLDDVYNNLDIDGFGAEETLACSTGH-LIEQHIYEK EATDEENDMLDLAYGLTDRSRLGCQICLTKSNONMTVRVPEAVADARQSIDVGKNS-----: 124
Ketobacter :-----YRTHVD-----RQIGAMCMGEAEIDFQDDHNQH PNDAPT-----IASLEQANR AKHC NOIRAVEQQ-----: 66
Pseudomonadales :-----FRTHVD-----TQIGAMCMGEAEHIFQDANNEQ DSTPL-----MONLEQAQQ VRNC NOIR ELNHHEGTPS-----: 71
Alcanivoracaceae :-----FRTHVD-----TQIGAMCMGEAEHIFQDANNEQ DSTPL-----MONLEQAQQ VRNC NOIR ELNHHEGTPS-----: 71
Spirochaeta :-----FRTHVD-----EDIGAMCMGEAEIA GEDEKAE EESPR-----KDLRKKAEER VRYC NRVR EEG-----: 64
Candidatus Microthrix :-----WRTHVD-----RDIGAAQGEAEIQE SKRGE T DERPP-----DEARAAADA VKYC THS HEDPTPEDPN - PEDPQ-----: 76
Gammaproteobacterium :-----FLTHVD-----MDIGATQGEAEIDFH DESGKT KAIVS-----EGEVEKARL AKYC TOIK VKRGSV-----: 67
Methylococcus Powlak :-----FLTHVD-----RDIGATQGEAEIDFH DDKGT T KAIVS-----EGEVEKARL EKYC TKIK VKRVTL-----: 67
Methylospirillum :-----FRTHVD-----MDIGGNCMAEAEIH DDSGKT QTHCE-----LELLEKARA AKYC TOIK ALDDEVP-----: 68
Methylococcaceae FWC3 :-----FRTHVD-----MDIGGNCMAEAEIH DDSGKT QTHCE-----LELLEKARA AKYC TOIK ALDDEAS-----: 68
Methyloprfundus :-----FKTHVD-----MDIGGNCMAEAEIQ DDKGK S QDTPN-----NTLLKKAQA AEYC TSIK IQD-----: 64
Methylomonas lenta :-----FTTHVD-----KEIGATQGEAEIEH DDAGN T QEQPQ-----LDLLFKAQQ EKYC TKIK KLN-----: 64
Methylobacterium wino :-----FRTHVD-----REIGATQGEAEIEH DDAGN T QENPA-----LDLLNKAQA EKYC TOIK KLK-----: 64
Methylovulvum :-----FOITHVD-----RAIGASCTGEAEIEH DDAGN T QESPP-----LDLLAKARQ EKYC AKIK KST-----: 64
Methylobacter marinus :-----FOITHVD-----REIGATQGEAEIEH DDAGN S QENPP-----LDLLSKARQ EKYC TKIK ELK-----: 64
Methylopusculum :-----FOITHVD-----REIGATQGEAEIEH DDAGN T QENPM-----LDLLVKARQ EKYC TKIK SVSTELNHYEE - KE-----: 73
Methylosarcinia lacus :-----FOITHVD-----REIGATQGEAEIEH DDAGN T QDNPP-----LDLLTKARQ EKYC SKIK ELNQNP - KES - KNGLIS-----: 76
Methylococcaceae NSM :-----FOITHVD-----REIGATQGEAEIEH DDAGN T QENPP-----LDLLAKARQ EKYC TKIK ESQ-----: 64
Methylospirillum :-----IKTHVD-----MDIGGNCMAEAEIH DDKGR T DENPS-----PEWVEKLRA EKYC TRIK EDGETEGGRC - PFH-----: 74
Methylospirillum mobilis :-----FOITHVD-----MDIGGNCMAEAEIH DEEGR T DAQAD-----HALLEKARQ EKYC TOIK TQTG-----: 65
M. capsulatus :-----FRTHVD-----RDIGGNCMAEAEIH DEDGR T SETPD-----PVLVGAALA ERFK ARIK LPQRDPATRD - RSLSPSGED-----: 80
Methylospirillum ozyz :-----FRTHVD-----FDIGGNCMAEAEIEH DDKGH T QETPD-----AVLLDKARE EKHC ARIR VEEGS-----: 66
Methylospirillum :-----FRTHVD-----FDIGGNCMAEAEIH DEKGR T DGNPN-----VMLLKARS EKHC AGIR VIDG-----: 65
Methylospirillum :-----FRTHVD-----REIGAHNGEAEIEH DEKGR E DERPA-----ATLIEKARA EKYC NRIS VNESKP-----: 67
Cellvibrionales :-----TLTHVD-----AQIGAMCMGEAEIDF DAASV H QNEVQ-----AGDEEKVHR VHYC NGIR EAVQESAGL - A-----: 72
Spongibacter tropic :-----LQTHVD-----TVIGAMCMGEAEIK DEDGI Q KPQFD-----AGQREAVR VKYC NOIR VHE-----: 64
Stenotrophobium :-----FRTHVD-----RGIGAMCMGEAEIEH DSNNN E RKPEIK-----AEEMEKVEQ VKFC NSIK VEE-----: 64
Sinimaribacterium :-----FRTHVD-----RDIGGNCMAEAEIH DANGQ H KPELT-----EQAREQVTR VRYC NSIK VEGPTP-----: 67
Alphaproteobacterium :-----LRTHVD-----RQIGAVNGEAEIEH GADGV H KEAVD-----PGDKAKVDQ VELC TOIK VEE-----: 64
Oleomonas :-----ITTHVD-----RQIGAVNGEAEIEH GDDGI K KEEVS-----EAELEKVQL ALHC NAIR VRHGP-----: 66
Zavarzinia comparanso :-----ITTHVD-----RGIGAMCMGEAEIDFH GEDGV R KEAVT-----EAEMAASQS INYC NAIR ERR-----: 64
Sandaracinus NAT131 :-----VRTHVD-----RQIGAVNGEAEIEH AESGEAE EAHPP-----AEQYALLAR AEHC NOIR EQGDERCPF - - H-----: 71
Myxococcales bacteriu :-----LNTHVD-----LDIGGAVNGEAEIEH DDETGVH HTDRP-----DPKHNDVRA ADYC QRTIQ EEEALPLDD - - ASLPSGCPVAH-----: 82
Minicystis rosea :-----YRTHVD-----LDIGGAAQGEAEIEH DGLGK - R IDTTP-----DASLSAKVEK AQFC TKTIK EAI-----: 64

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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 [Fe₂-S₂] cluster are marked with red stars.