

Supplementary Information:

Unravelling the role of transient redox partner complexes in P450 electron transfer mechanics

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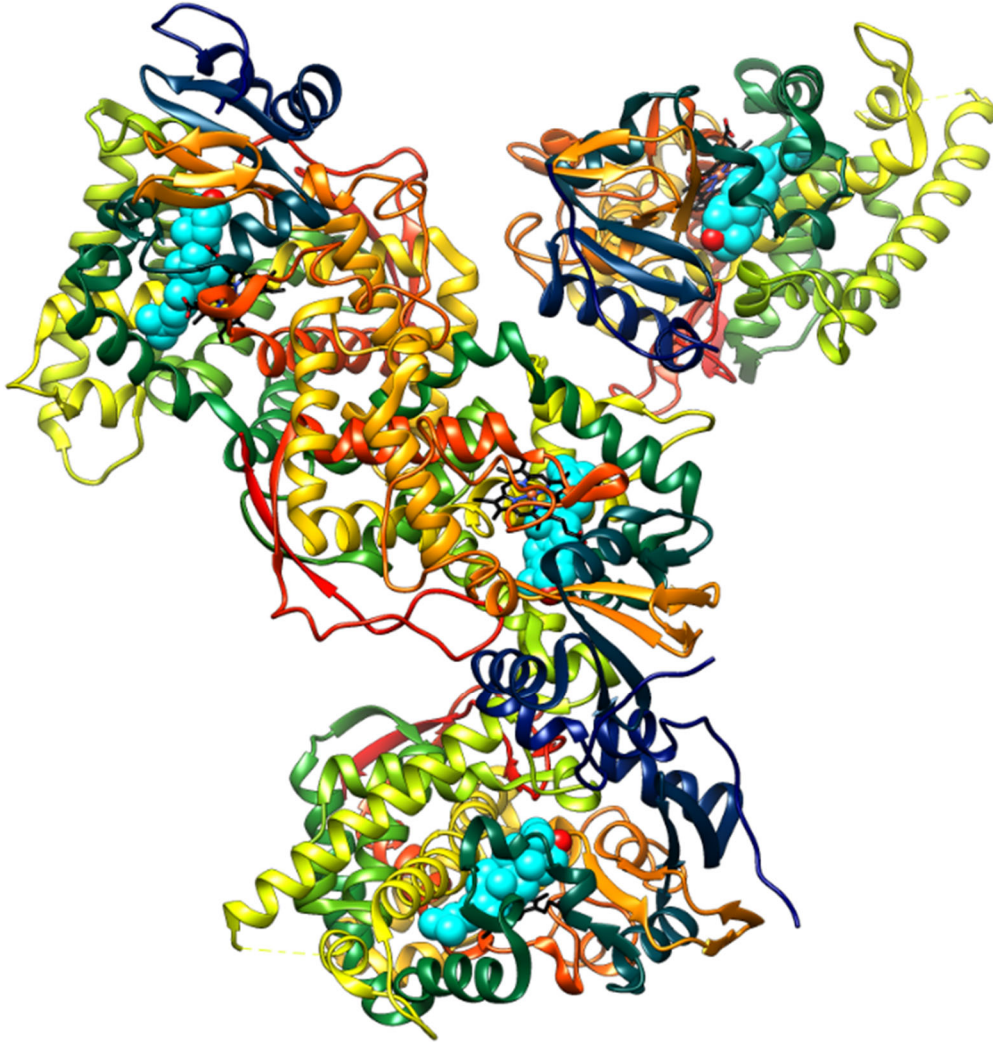
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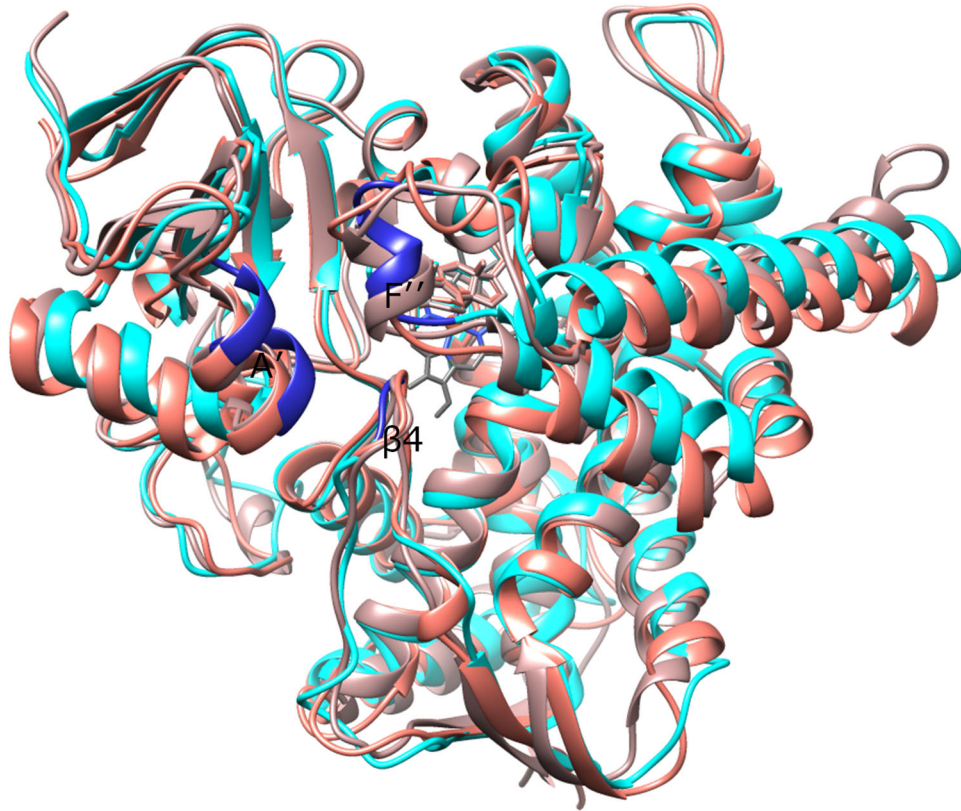
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Supplementary Table S1. Data collection and refinement statistics.

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 $C\alpha$ between the four molecules is 0.35 Å.

a**b**

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Conservation:          4994 4   949994 99949 494          4 94 4 4 444 4 4 44 4 44 49 9   49 4 499499 9944444 44 494 49   9 44 4 4
6uez_chainA_p002      1  KGKLPPIYFSPPIPLGHAIAPGKSPIEFLFNAYEKY-GPVFSFTMVGKTFTYLLGSDAAALFNSKNDLNAEDVYSRLTFVFVGKGVAYDVPNPVFLFEQKMLKSLGLNIAHFKQHV 116
6fmo_chainA_p003      1  ---LPPVYPTVPFLGHIVQFGKNPLEFMQRCKRDLKSGVFTISIGQRTVIVGDPHEHSRRFFSPRNEILSPREVI-TFMTPVFGEGVAYAAPYPRMREQLNFLAEELTIAKFNQFV 113
7snmA.pdb_chainA_s001 1  -SNTPPPVKPGLLLLGHILEFGKNPHAFLMALRHEF-GDVAEFRMFHORMVLLTGSQASEAFYRAPDEVLDQGPAY-RIMTPIFGRGVVFDARIERKNQQLQMLPALRDKPMRTYS 114
Consensus_aa:
Consensus_ss:          hhh  hhhhhhhhhhhhh  eeeee  eeeee  hhhhhhhhh  hhh  hhhh  eee  hhhhhhhhhhhhh  hhhhhhhhh

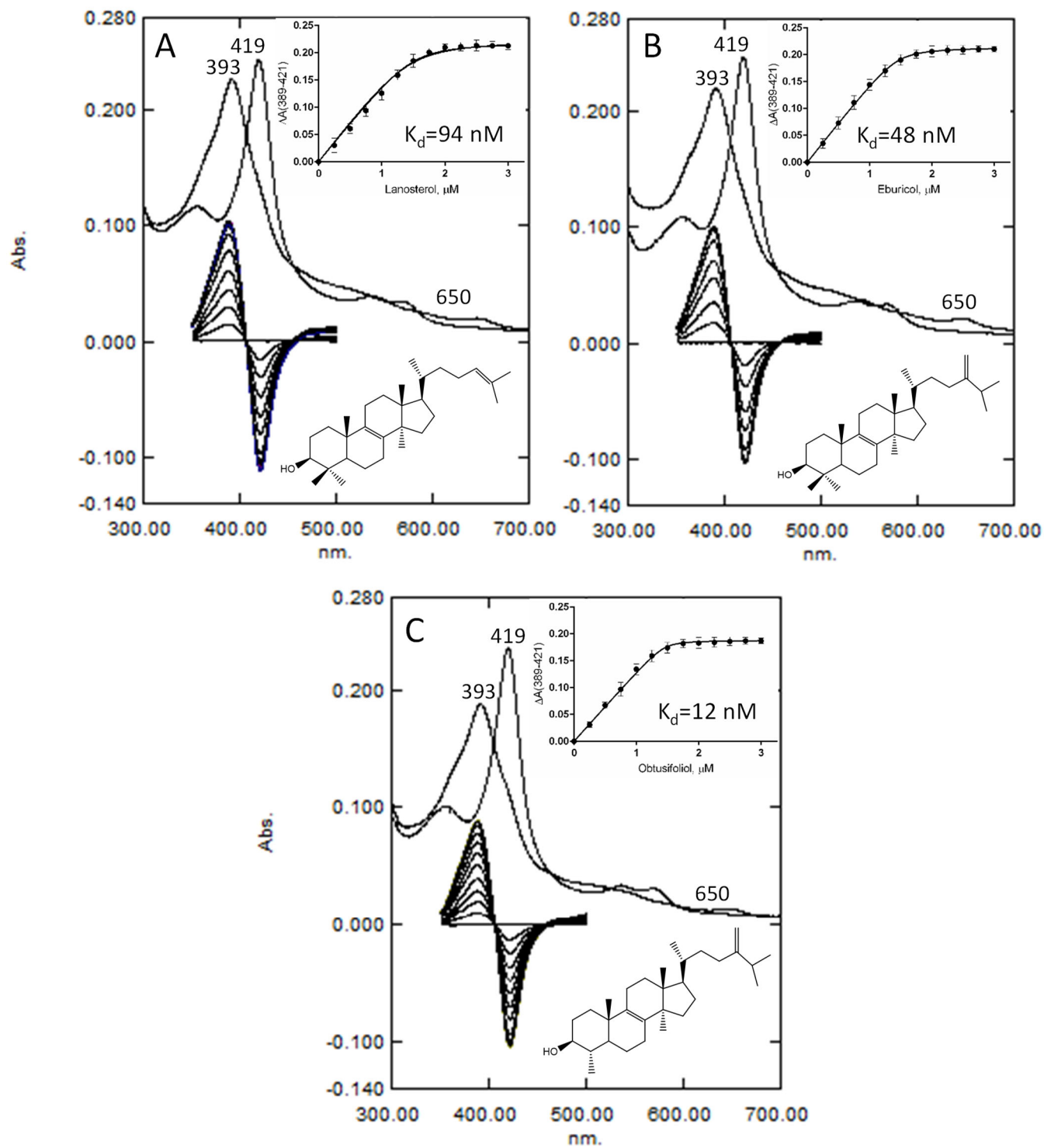
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6fmo_chainA_p003      114 PAIQHEVRKFMAENWKEDEGVINLLEDCGAMIINTACQCLFGEDLRKRLNARHFAQLLSKMES---SLIPAAVFMPWLLRLP--LPOSARCREARAELQKILGEIIVAREKEEASKD 225
7snmA.pdb_chainA_s001 115 EIIIAEVEAMLRD--WKDAGTIDLLELTKELTYTSSHCLLGAEFRHELN---TEFAGIYRDLEMGIOPIAYVFP--NLPLPVFKRRDQARVRLQELVTOIMERRARSQ--E--- 217
Consensus_aa:
Consensus_ss:          hhhhhhhhhhhhh  hh  hhhhhhhhhhhhhhhhh  h  hhhhhhhhhhhhhhhhhhhhhhh  hh  hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh

Conservation:          4 44 9449 9 99 44 494 99444 49944994 9449 9 4 4 4 4 944          4444 4 44 4444449 494 994 9944
6uez_chainA_p002      220 EKIDDILQTLLDATYKDGRPLTDDEVAGMLIGLLLAGQTSSTTSAWMGFLAR--DKTLQKKCYLEQKTVCGENLPPLTYDQLK--DLNLLDRCIKETLRLRPPIMIMMRMARTPQT 333
6fmo_chainA_p003      226 NNTSDLLGLLLKAVYRDGTRMSLHEVCGMIVAAMFAGQHTSTITTSWSMLHLMHPKNRKWLDRLKHEIDEF---PAQLNYDNVMDEMPFAERCVRESIRRDPLLLMVMRMVKAEVK 338
7snmA.pdb_chainA_s001 218 -RSTNVFQMLLDASYDDGSKLTPEIPCGMLIATIFAGHTSSGTTAWVLLELLR--REYLRRVRAEIDALFET-HGRVTFESLR--QMPOLENVIKEVLRLHPLLLLMRKVMKDFE 329
Consensus_aa:
Consensus_ss:          hhhhhhhhhhh  hhhhhhhhhhhhhhh  hhhhhhhhhhhhhhhhh  hhhhhhhhhhhhhhhhh  hhhh  hhhhhhhhhhhhhhh  hhhh  ee

Conservation:          9 44 44 9 44 49 4 44 4 4444 44449494          4 4 9949494949 99 94949 4 49 949 9          4 49 4944 9 4 4449 4
6uez_chainA_p002      334 VAGYTIPPGGHVCVSPTVNQRLKDSWVERLDFNPDRYLQDNPASGEKFAYVPFGAGRHRCIGENFAYVQIKTIWSTMLRLVFDLLIDGY-FPTVNYTMIHTPEN-P-VIRYKRRS- 446
6fmo_chainA_p003      339 VGSYVVPKGDIIIACSPLLSHHDEAFFPNRLNDPERD-----EKVDGAFIFGGAGVHKCIGQFALLQVKTILATAFREYDPQLLRDE-VPDPDXHTMVVGPTLNQCLVKYTRRKK 448
7snmA.pdb_chainA_s001 330 VQGMRIEAGKFVCAPSPVTHRIPELFPNELFDPDRYTPERAEDKLYGQAFGGRHKCSGNAFAMFQIKAIVCVLLRNVEFELAAAPESYRDDYRKMVVEPAS-PCLIRYR-RR- 443
Consensus_aa:
Consensus_ss:          e  eee  eeee  hhhh  hh  -c-c@A@ltFGAGRHKCIGSFAHIQIKTILthLRpYF-Lictb.hPcsDY+TMVVsPhs.P.LIRY+.R+.  eee  eeeee

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Supplementary Figure S2. a. Superimposition of the substrate-bound *M. capsulatus*, human, and *T. cruzi* CYP51s. The coloring is as in Fig. 3b. The secondary structural elements that form the substrate entry (helices A', F'', and the β 4 hairpin) are colored in blue on the *M. capsulatus* structure. **b. Structure-based sequence alignment (PROMALS3D).** Sequences are colored according to secondary structures (red: alpha-helix, blue: beta-strand). Conservation indices above 3 are shown.

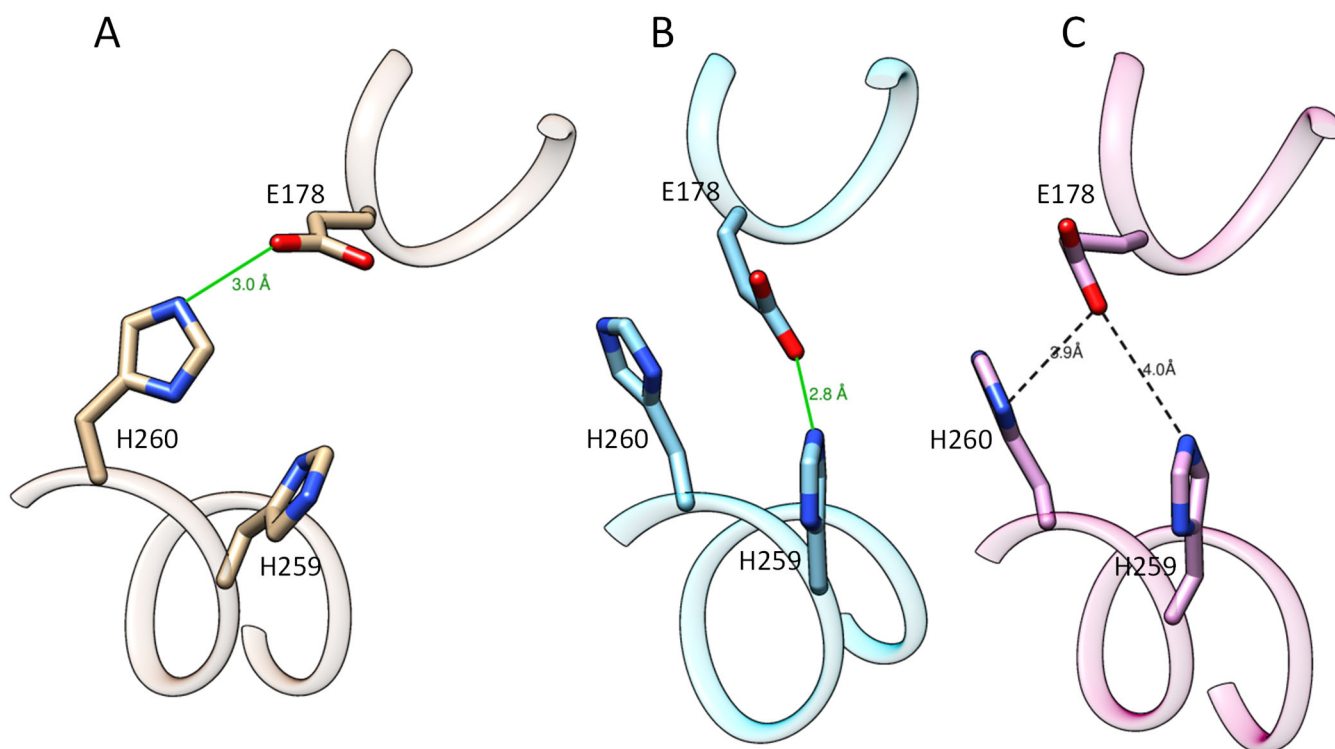


Supplementary Figure S3. Spectral changes observed during titration of *M. capsulatus* CYP51-fx with (A) lanosterol (B) eburicol, and (C) obtusifoliol. Absolute (top) and difference (bottom) spectra. The P450 concentration was ~ 2 μM . Inset: The titration curves fitted to the quadratic (Morrison) equation.

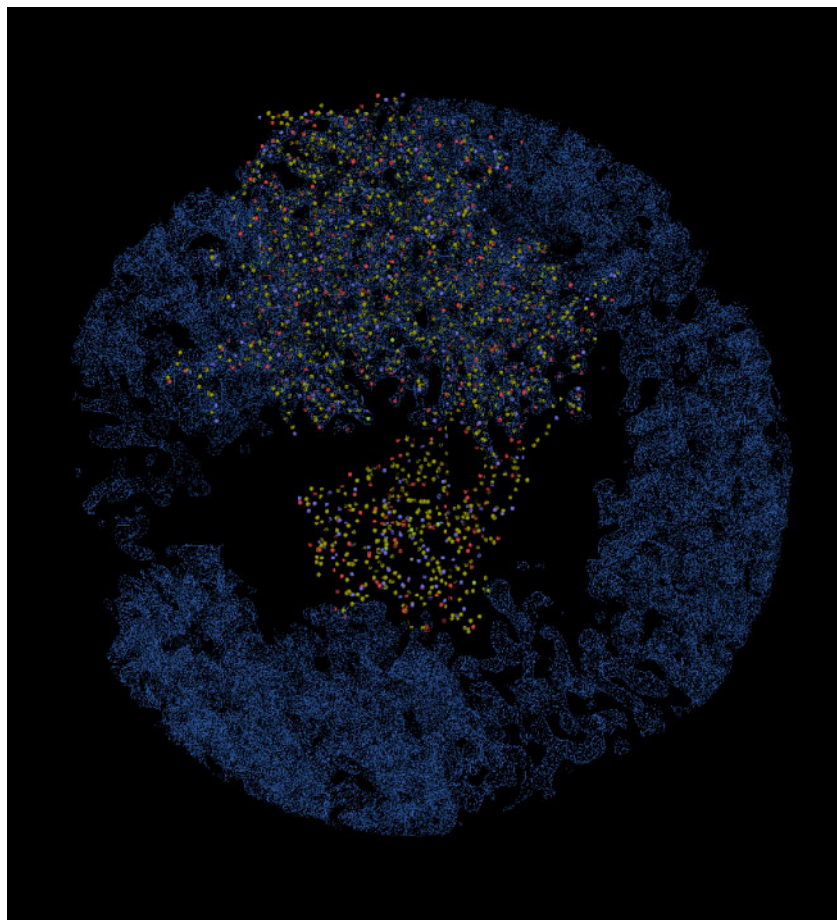
Supplementary Table S2. Active site cavity lining residues in the substrate-bound bacterial (*M. capsulatus*, 7SNM), protozoan (*T. cruzi*, 6FM0), and mammalian (human, 6UEZ) CYP51 structures

Structural element	<i>M. capsulatus</i>	Human D231A/H314A	<i>T. cruzi</i> I105F
	Substrate (Lanosterol)	Substrate (Lanosterol)	Substrate (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 <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*)

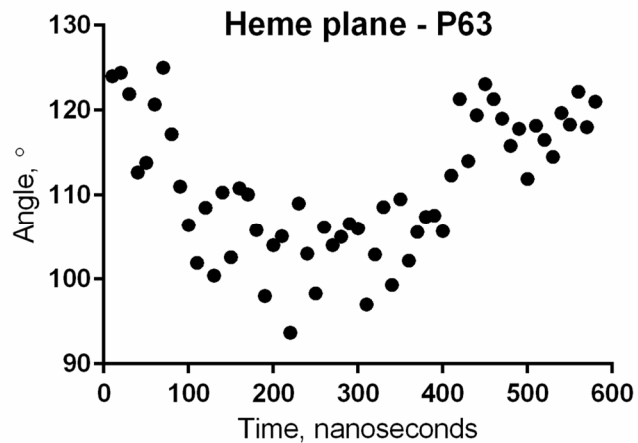
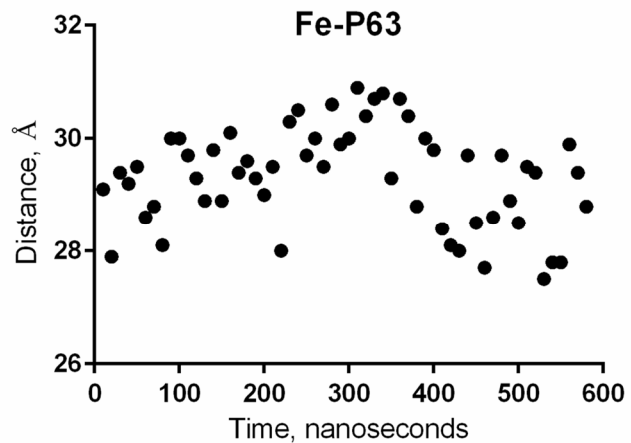
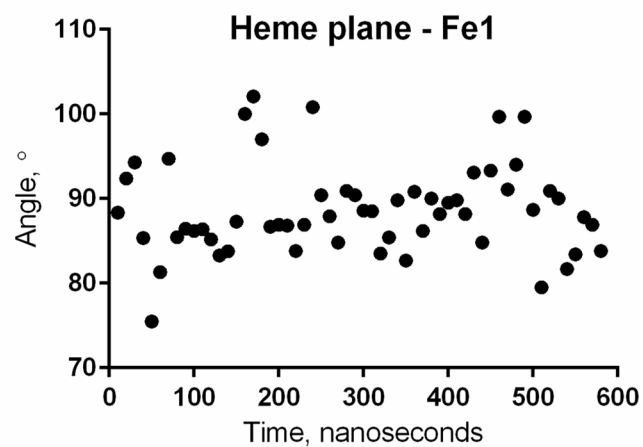
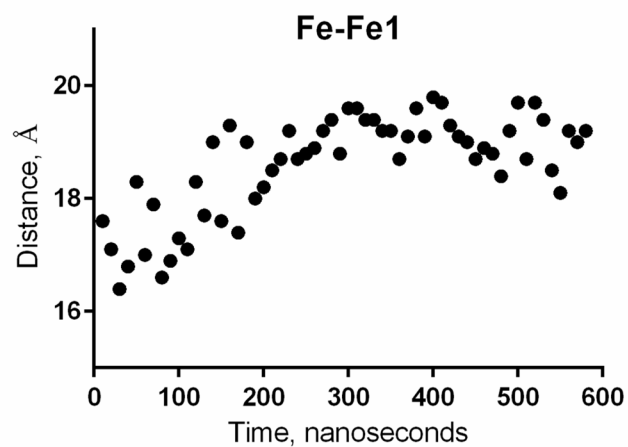


Supplementary Figure S4. Rearrangements in the proton relay network in *M. capsulatus* CYP51. Ligand-free (salt bridge E178-H260, 3.0 Å) (A), lanosterol-bound (salt bridge E178-H259, 2.84 Å) (B) structures of *M. capsulatus* CYP51, and a snapshot from MD simulations of the CYP51-ferredoxin complex, $|\text{Fe-Fe1}|=16.6$ Å. (E178 is freed, proton delivery route activated) (C).

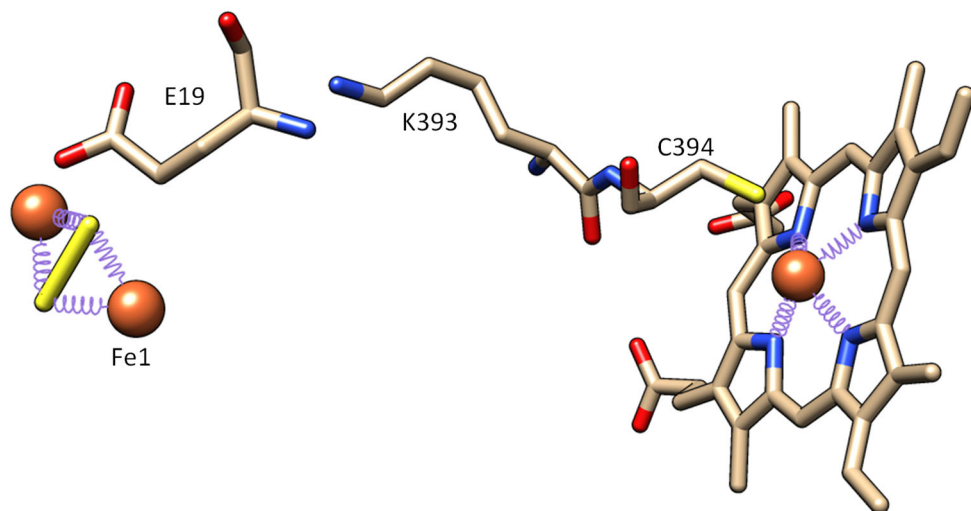


Supplementary Figure S5. A Coot snapshot of the 2F₀-F_c density map contoured at 1.5 σ , map radius 50 Å.

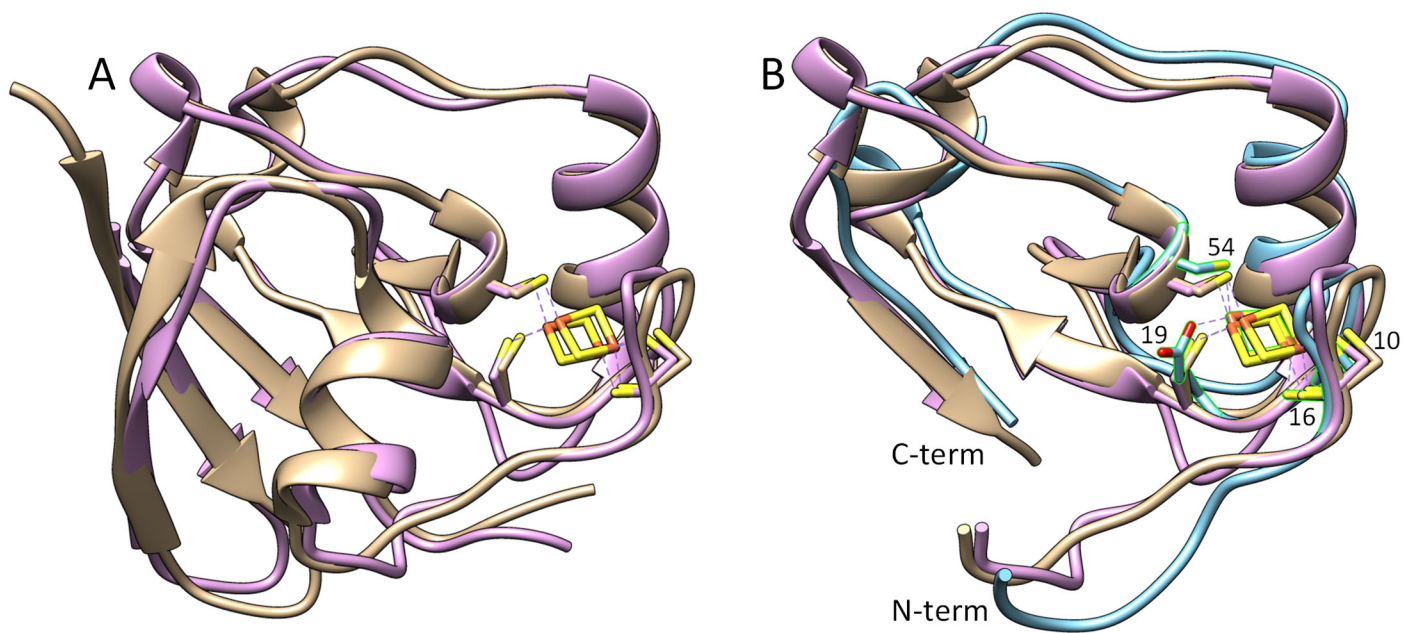
The dots correspond to one molecule of P450 (top, proximal face down) and one molecule of ferredoxin (bottom, the docked pose, selected for MD simulations). The electron density is clearly seen for the P450 domain only, while any density close to the proximal P450 surface (where the ferredoxin domain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



Supplementary Figure S6. Molecular-dynamics trajectories. The pbd files were written each 10 ns. The maximal Fe-Fe1 distance of 19.8 Å (at 400 ns of the 600 ns run) is perfectly compatible with electron transfer, the calculated K_{ET} value being 1.51/sec (or 90.8 electron transfer events per minute).



Supplementary Figure S7. Possible ET path in *M. capsulatus* CYP51-fx.



Supplementary Figure S8. 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      :SSSEDKITVHFVNRDGETLTTGKIGDSDLVYVQNNLDIDGFGA*E*TLACSTH-L*E*EQHIFEK*EA*TDDEENDMLDLAYGLTDRSRLGCQ*LT*KNMTMTVVRVPEAVDARES*IDMGMNSSKIE*~::~128
Pig       :SSSEDKITVHFVNRDGETLTTGKIGDSDLVYVQNNLDIDGFGA*E*TLACSTH-L*E*EDHIFEK*EA*TDDEENDMLDLAYGLTDRSRLGCQ*LT*KNMTMTVVRVPEAVADARES*IDLGNSSKLE*~::~128
Cat       :SSSEDKITVHFVNRDGETLTTGKIGDSDLVYVQNNLDIDGFGA*E*TLACSTH-L*E*EDHIFEK*DA*TDDEENDMLDLAYGLTDRSRLGCQ*LT*KNMTMTVVRVPEAVADARQSI*DVGKNS*~::~124
Human     :SSSEDKITVHFVNRDGETLTTGKIGDSDLVYVQNNLDIDGFGA*E*TLACSTH-L*E*EDHIFEK*DA*TDDEENDMLDLAYGLTDRSRLGCQ*LT*KNMTMTVVRVPEAVADARQSI*DVGKNSAENLYFQ*::131
Marmota   :SSSEDKITVHFVNRDGETLTTGKIGDSDLVYVQNNLDIDGFGA*E*TLACSTH-L*E*EDHIFEK*EA*TDDEENDMLDLAYGLTDRSRLGCQ*LT*KNMTMTVVRVPEAVADARQSI*DVGKNS*~::~124
Ketobacter :-----YR*H-----RQ*G*AMCMGE*H*E*Q*DDHNO*H*PNDAPT*-----IASIEQANR*AKH*NO*G*AVEGQ*-----: 66
Pseudomonadales :-----FR*H-----TQ*G*AMCMGE*H*E*Q*DANNE*Q*DS*PL*-----MQNLEQAQQ*VRN*NO*G*ELNHHEGTPS*-----: 71
Alcanivoracaceae :-----FR*H-----TQ*G*AMCMGE*H*E*Q*DANNE*Q*DS*PL*-----MQNLEQAQQ*VRN*NO*G*ELNHHEGTPS*-----: 71
Spirochaeta :-----FR*Y-----ED*G*AVCMGE*E*E*E*GEDEK*A*EESPR*-----KDLRKAER*VRV*NRV*E*EEG*-----: 64
Candidatus Microthrix :-----WR*Y-----RD*G*AAOCGE*Q*E*E*SKRGE*T*DERPP*-----DEARAAADA*VKY*TH*S*HEDPTPEDPN*--PEDPQ*-----: 76
Gammaproteobacterium :-----FL*E-----MD*G*ATOCGE*SD*E*H*DESGK*T*KAIVS*-----EGEVEKARL*AKY*TQ*K*VKRGSV*-----: 67
Methylococcus PowLak :-----FL*E-----RD*G*ATOCGE*AD*E*H*DDKGT*T*KAIVS*-----EGEVEKARL*EKY*TK*H*VKRVTL*-----: 67
Methyloctetradococcus :-----FR*A-----MD*G*GNQNAE*E*E*H*DDSGK*T*QTHCE*-----LELLEKARA*AKY*TQ*H*ALDDEVP*-----: 68
Methylococcaceae FW3 :-----FR*A-----MD*G*GNQNAE*E*E*H*DDSGK*T*QAHCE*-----LELLEKARA*AKY*TQ*H*ALDDEAS*-----: 68
Methyloprfundus :-----FR*D-----MD*G*ANQNAE*E*E*Q*DDKGG*S*QDTPN*-----NTLLKKAQA*AEY*TS*K*IQD*-----: 64
Methylomonas lenta :-----FL*Q-----KE*G*ATQTE*E*E*Q*DDAGN*T*QE*Q*P*-----LDLLKFAQQ*EKY*TK*K*KLN*-----: 64
Methylomicrobium wino :-----FR*Y-----RE*G*ATQTE*E*E*Q*DDAGN*T*QENPA*-----LDLLNKARQ*EKY*TQ*K*KLK*-----: 64
Methylolulvum :-----FO*G*-----RA*G*ASQTE*E*E*Q*DDAGN*T*QESPP*-----LDLLAKARQ*EKY*AK*I*KST*-----: 64
Methylobacter marinus :-----FO*G*-----RE*G*ATQNAE*D*E*H*DDAGN*S*QENPP*-----LDLLSKARQ*EKY*TK*S*ELK*-----: 64
Methyloricopusculum :-----FO*G*-----RE*G*ATQTE*E*E*H*DDAGT*T*QENPM*-----LDLLVKARQ*EKY*TK*S*VSTELNHYEE*--KE*-----: 73
Methylosarcinia lacus :-----FO*G*-----RE*G*ATQTE*E*E*H*DDAGN*T*QDNPP*-----LDLLTKARQ*EKY*SK*K*ELNQNP*--RES*--KNGLIS*~::~: 76
Methylococcaceae NSM :-----FO*G*-----RE*G*ATQTE*E*E*H*DDAGN*T*QENPP*-----LDLEKARQ*EKY*TK*K*ESQ*-----: 64
Methylomagnum ishizav :-----IK*A-----WD*G*ANQTE*E*E*Q*DDKGR*T*DENPS*-----PEWVEKRA*EKY*TR*K*EDGE*TEGGR*--PFH*~::~: 74
Methylospira mobilis :-----FO*E-----MD*G*GAC*GE*E*E*H*DEEGR*T*DAQAD*-----HALLEKARQ*EKY*TQ*R*PQTG*-----: 65
M. capsulatus :-----FR*Y-----RD*G*GNQNAE*E*E*H*DEEGR*T*SETPD*-----PVLVGAALA*ERF*AR*K*LPQRDPATRD*--RSLSPSGED*~::~: 80
Methyloterricola ozyz :-----LR*E-----FD*G*SNQTE*E*E*Q*DDKGH*T*QETPD*-----AVLLDKARE*EKH*AR*R*VEEGS*-----: 66
Methylomudiphilus :-----FO*E-----FD*G*ANQNAE*E*E*H*DEKGR*T*DGMPN*-----VMLLQKARS*EKY*AG*S*VIDG*-----: 65
Methylocaldum szegedi :-----FR*L-----FR*G*AHQGE*E*E*H*DEKGR*T*DERPA*-----ATLEKARA*EKY*NR*S*VNESK*-----: 67
Cellulibrionales :-----TL*H-----AQ*G*AMOCGE*DY*E*F*DAASV*H*QNEVQ*-----AGDEEKVHR*VHY*NG*S*BAVQAESAGL*--A*~::~: 72
Spongibacter tropic :-----LO*E-----TV*G*AMOCGE*E*E*H*DEEGL*Q*KP*FD*-----AGQREAVR*VKY*NQ*R*VER*-----: 64
Stenotrophium :-----FR*Y-----RG*G*AMOCGE*E*E*H*DSNNN*E*RKPEIK*-----AEEMEKEVQ*VKI*NS*K*VHE*-----: 64
Sinimarimbacterium :-----FR*Y-----RD*G*ANQNAE*E*E*H*DANGQ*H*KPELT*-----PGAREQVTR*VRV*NS*K*VEGPTP*-----: 67
Aliphilus aromaticiv :-----LR*Y-----RQ*G*AVCMGE*S*AH*E*Q*GADGV*H*KEAVD*-----PGDKARVDQ*VEI*TS*K*VEE*-----: 64
Oleomonas :-----IT*H-----RQ*G*AVCMGE*S*AH*E*Q*GDDGI*K*KEEVS*-----EAELKQVL*ALH*NA*R*VRHG*-----: 66
Zavarzinia comparanso :-----IT*A-----RG*G*AVCMGE*DE*H*GEDGV*LR*KEAVT*-----EAEMAARQS*INV*NA*R*ERR*-----: 64
Saxdarcinus NAT131 :-----VR*R-----RQ*G*AVCMGE*S*E*H*AESGEAE*EAHPP*-----AEQYALLAR*AEH*NO*V*EQGERCP*--H*~::~: 71
Myxococcales bacteriu :-----LN*A-----LD*G*AVCMGE*E*E*Y*DEETKVA*H*DRP*-----DPKHNDVRA*ADY*QRT*Q*EBEALPLD*--ASLPSGCFVAH*~::~: 82
Minicystis rosea :-----YR*S-----LD*G*AA*V*G*E*E*E*RD*GLGR*--R*IDTTP*-----DASLSA*V*EK*AO*E*TKT*Q*EAI*-----: 64

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Supplementary Figure S9. 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.