Supporting Information

Post-Translational Regulation of CYP450s Metabolism As Revealed by All-Atoms Simulations of the Aromatase Enzyme

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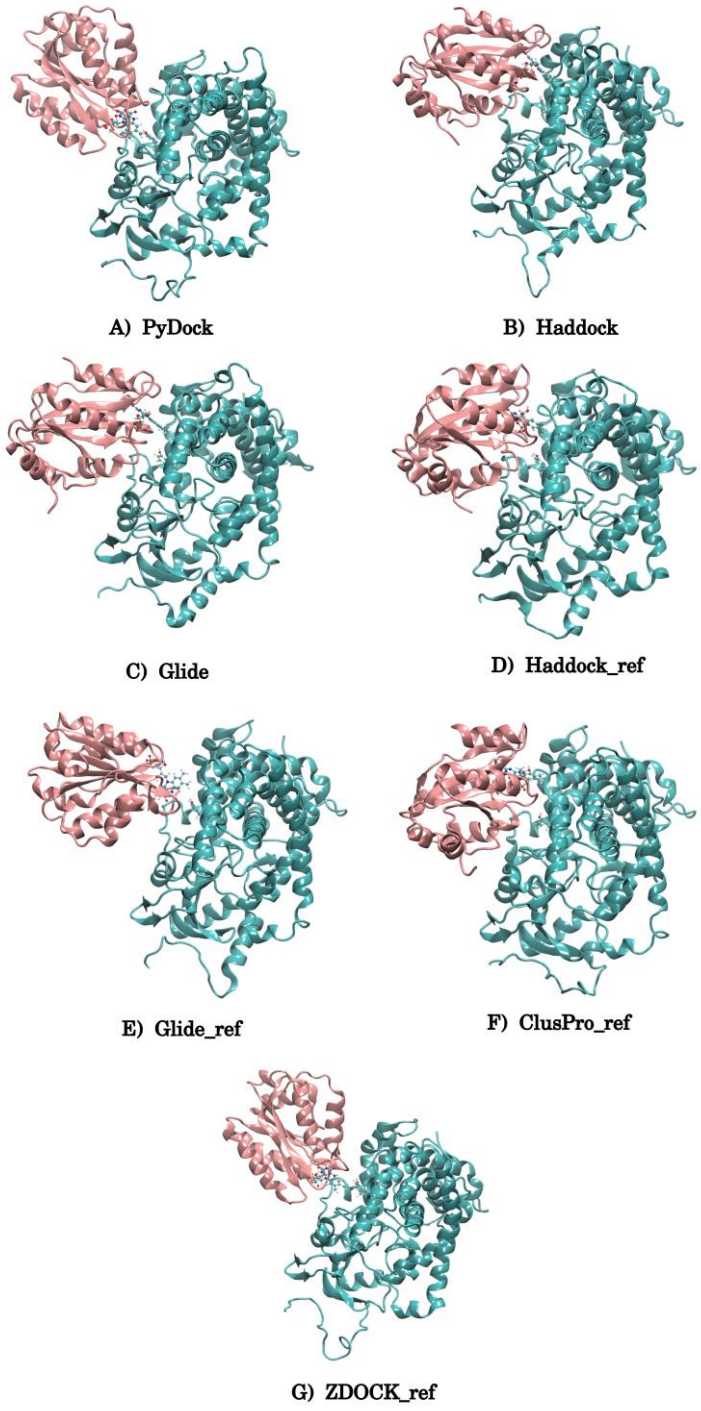


Figure S1. Equilibrated structures of FMN/HA obtained from ClusPro,[1] Glide,[2] Haddock,[3] PyDock[4] and ZDOCK[5] docking programs before and after the refinement by FiberDock server (indicated as _ref subscript).[6] HA is shown as a cyan ribbon, the FMN domain is represented as a pink ribbon, while FMN and Y361 cofactors are shown in ball and stick.

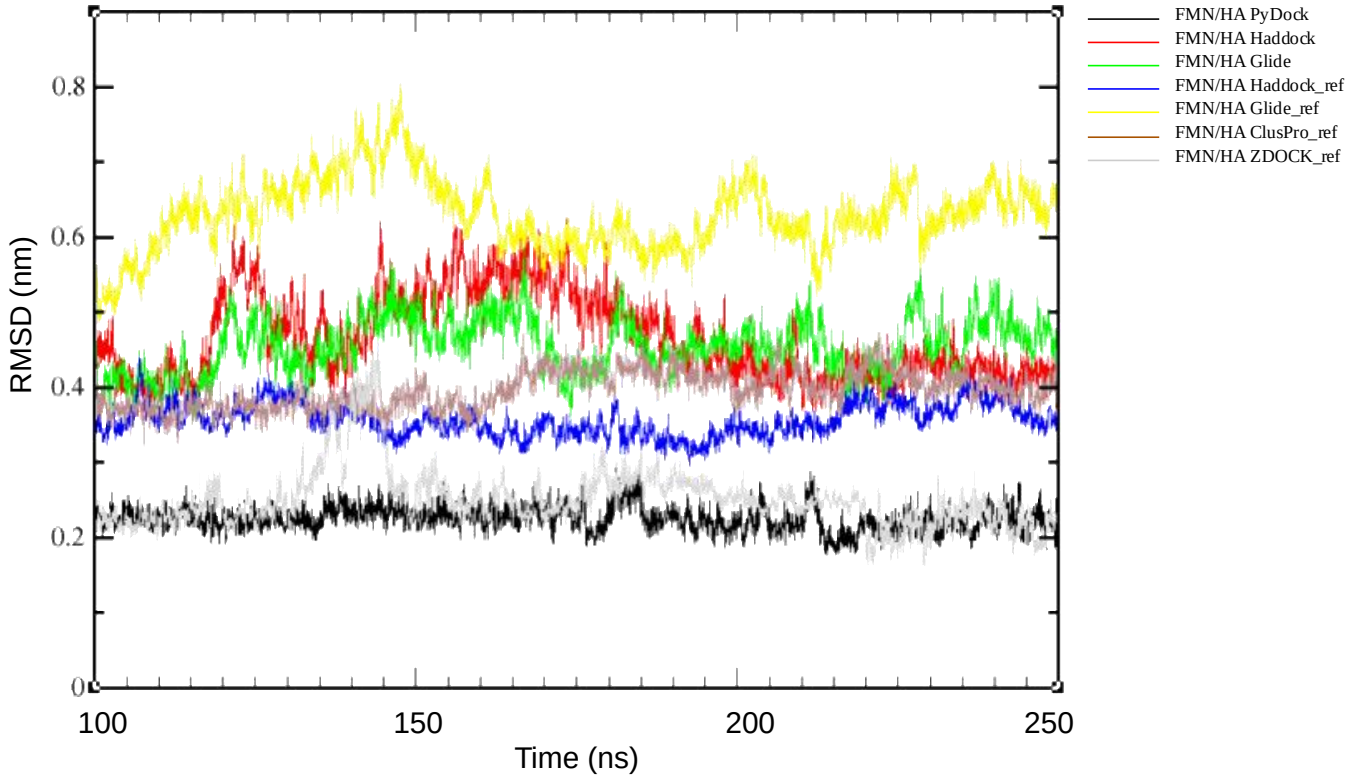


Figure S2. RMSD (nm) calculated on Cα atoms vs simulation time (ns) of FMN/HA models represented by black, red, green, blue, yellow, brown and gray lines, respectively. The reference structure for the RMSD calculation is the initial frame of the MD simulation.

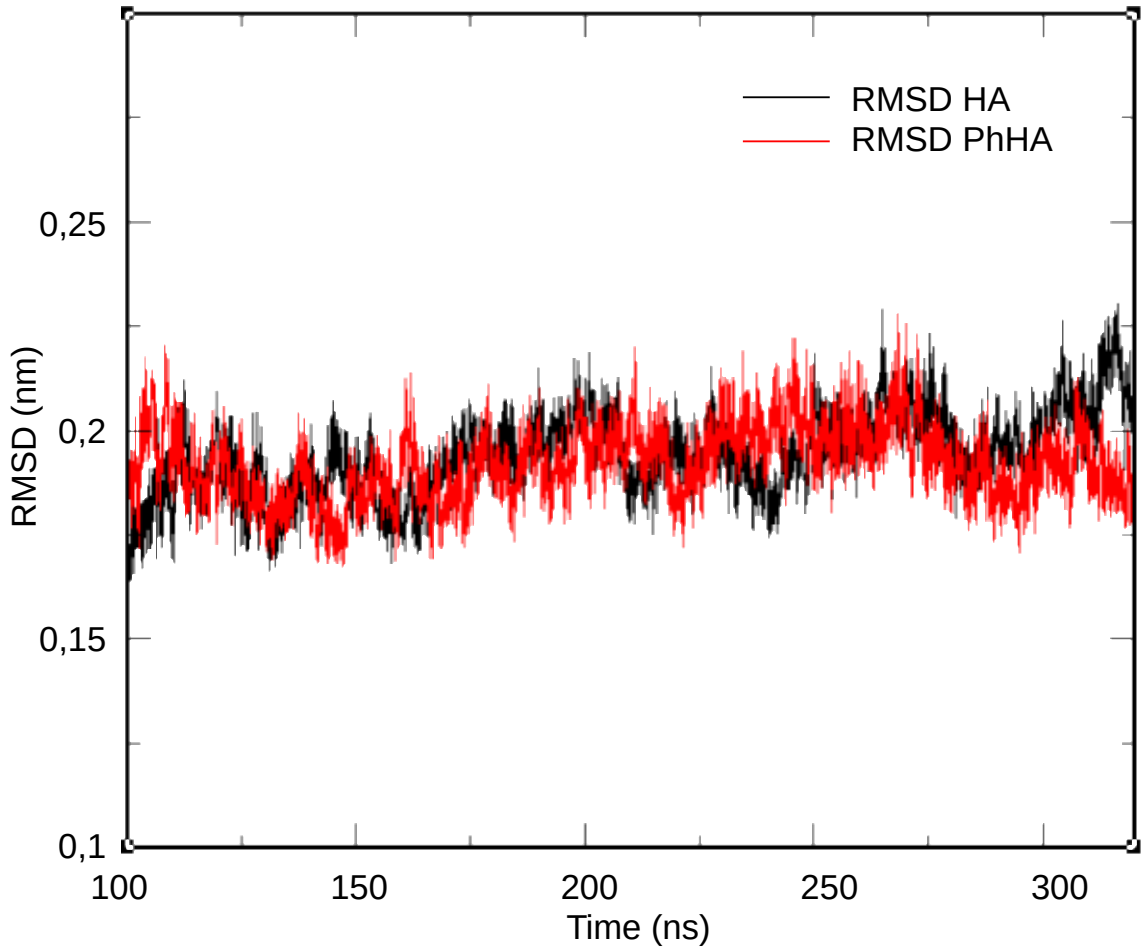


Figure S3. RMSD (nm) calculated on Cα atoms vs simulation time (ns) of HA and PhHA represented by black and red lines, respectively. The reference structure for the RMSD calculation is the initial frame of the MD simulation.

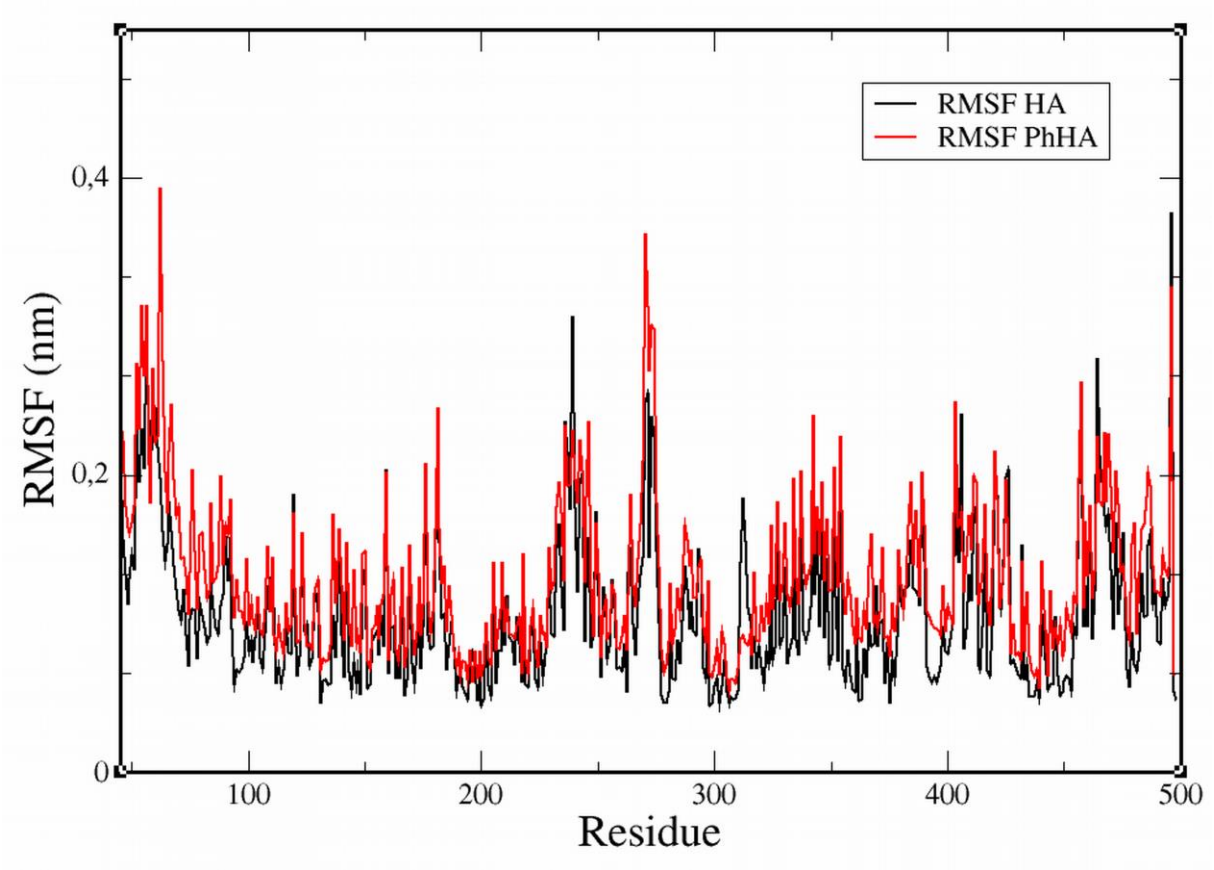


Figure S4. RMSF (nm) for HA and PhHA represented in black and red, respectively. Residues belonging to the transmembrane helix (1-44) are omitted for clarity.

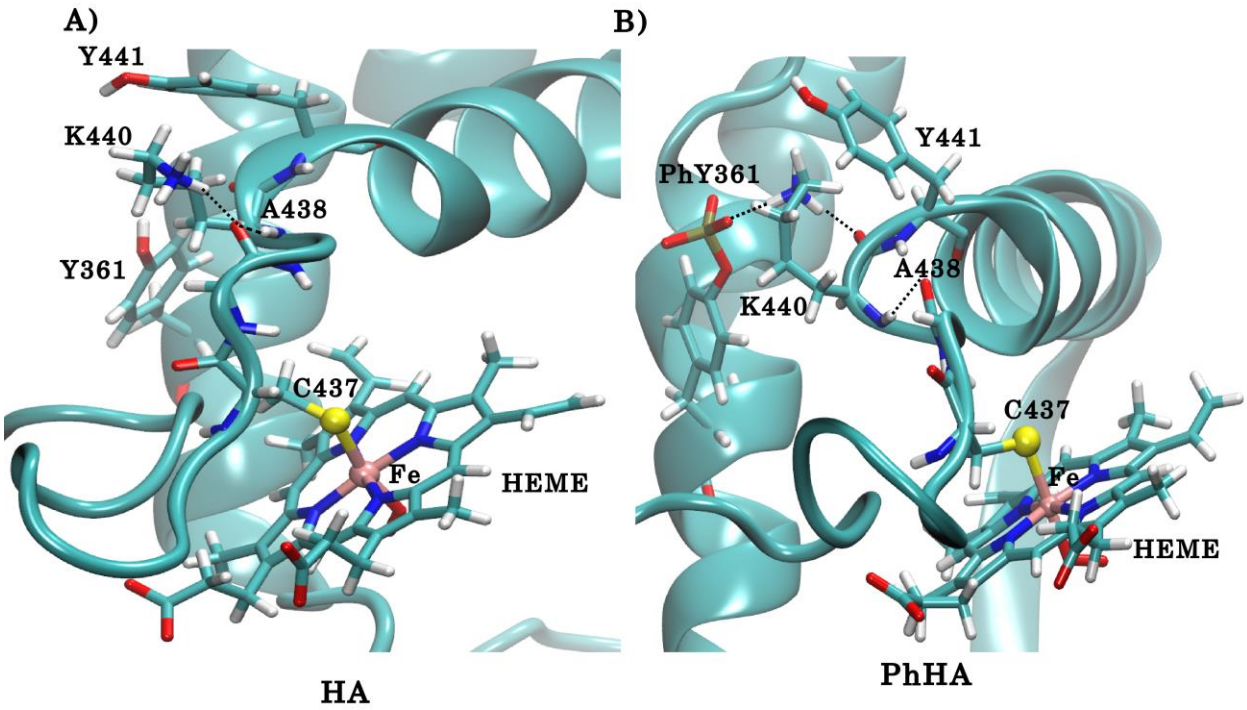


Figure S5. (A) Hydrogen (H)-bond network lining the heme proximal cavity in HA. H-bonds K440@Nz..OC@A438=1.8 ± 0.2 Å (persistency 83%) and K440@Nz..OP@PhY361=1.8 ± 0.3 Å (persistency 88%) in HA (A) and PhHA (B), respectively.

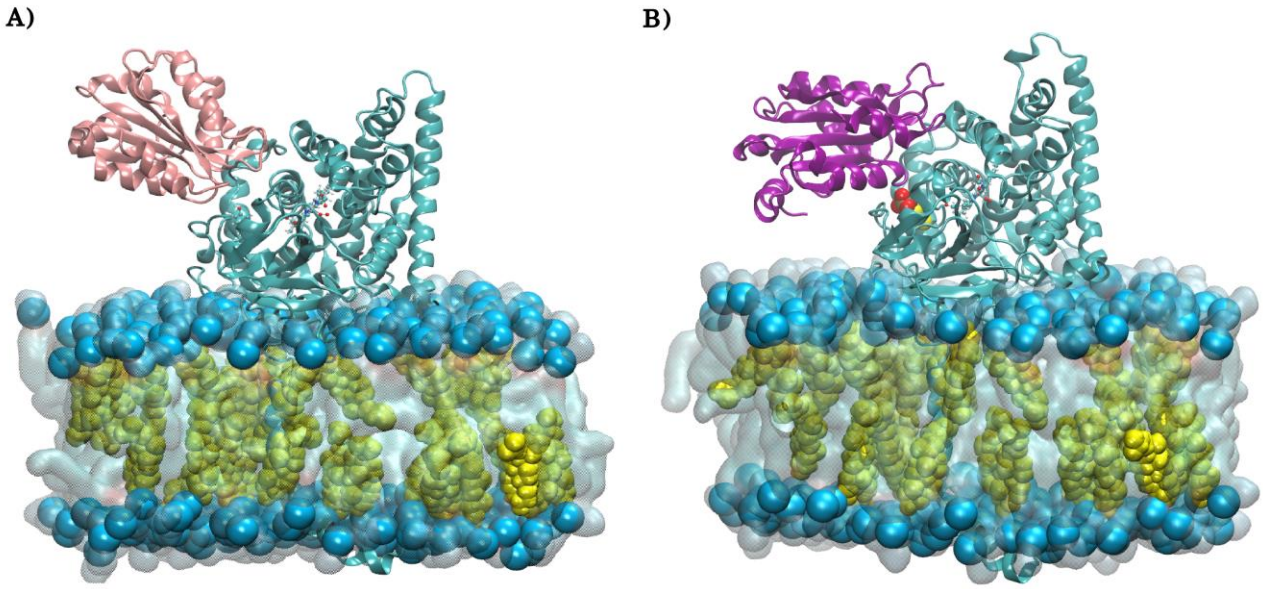


Figure S6. Equilibrated structure of (A) FMN/HA and (B) FMN/PhHA adducts as extracted from the cluster analysis of classical MD trajectory embedded in a mixed 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/cholesterol (CHL) membrane. Phosphorylated Y361 is shown as yellow and red van der Waals (vdw) spheres, HA is shown as a cyan ribbon, POPC molecules are shown as transparent surface with the phosphorus atoms highlighted as light blue vdw spheres, CHL molecules are shown as yellow vdw spheres. A different orientation of the FMN domain represented in pink in FMN/HA and purple in FMN/PhHA, respectively.

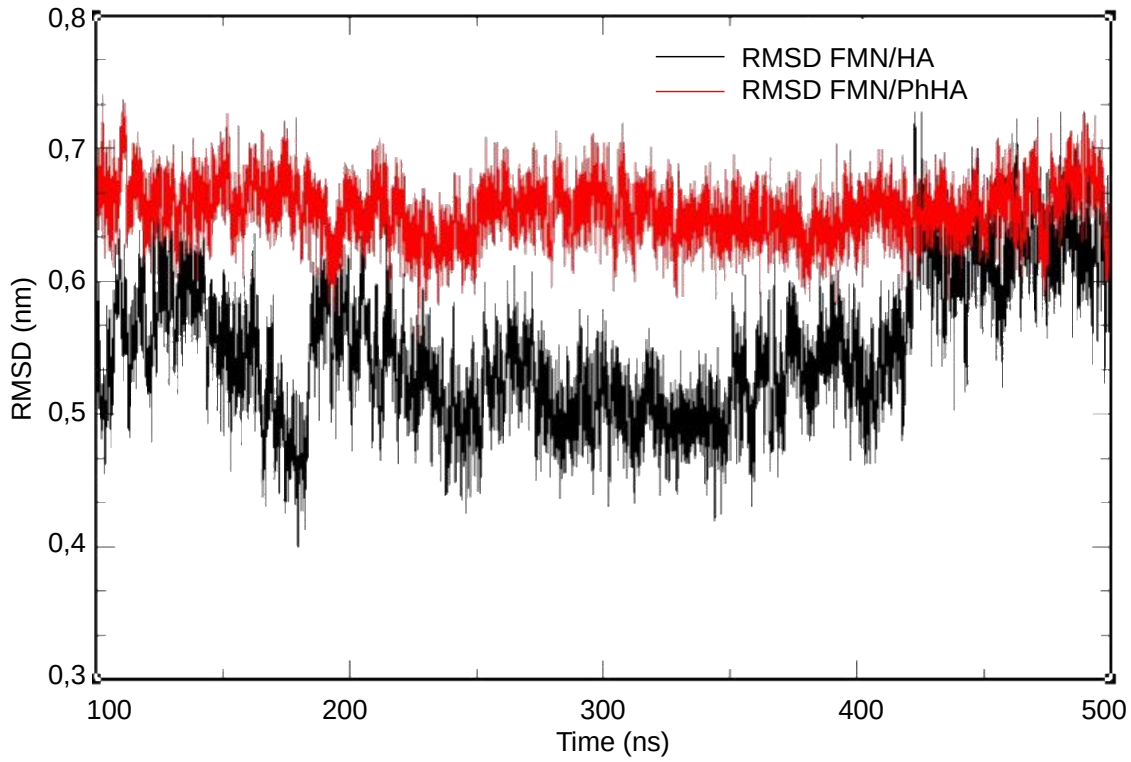


Figure S7. RMSD (nm) calculated on Cα atoms vs simulation time (ns) of FMN/HA and FMN/PhHA represented by black and red lines, respectively. The reference structure for the RMSD calculation is the initial frame of the MD simulation.

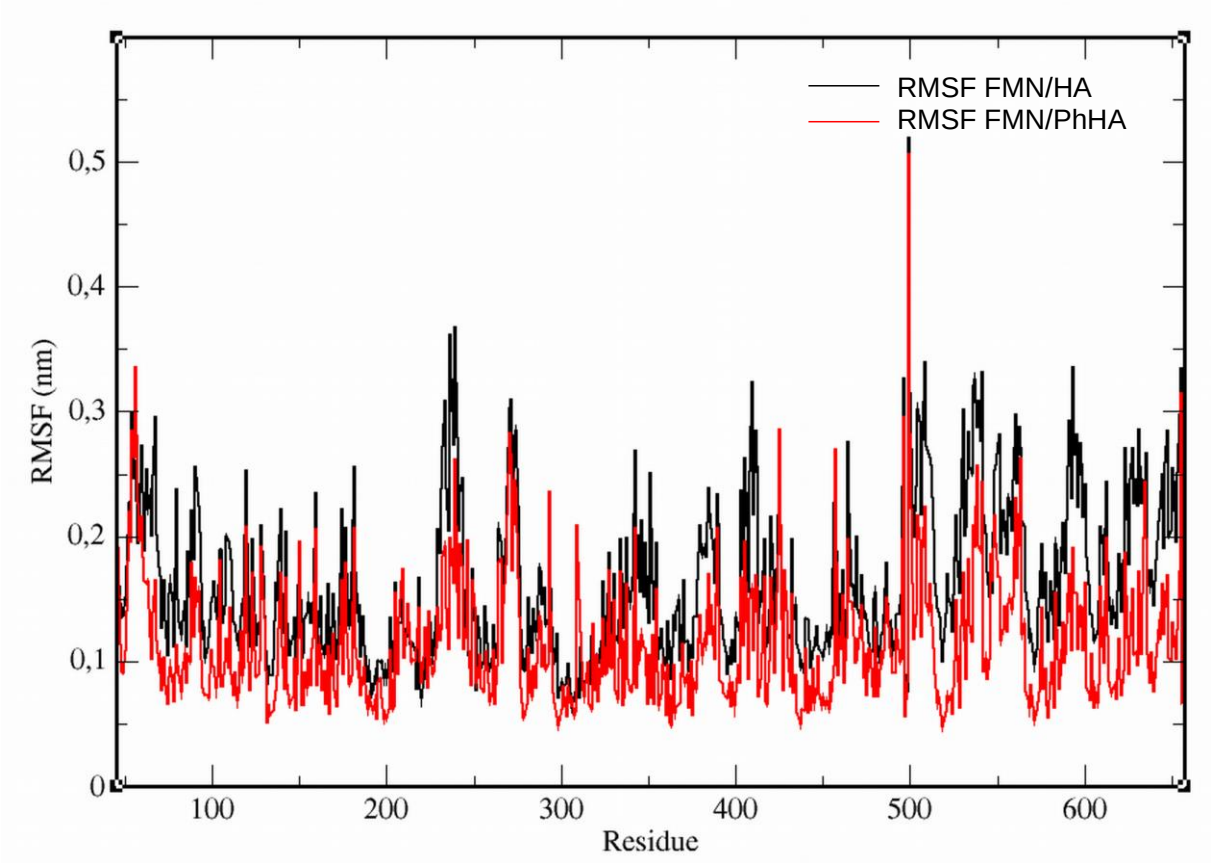


Figure S8. RMSF (nm) of FMN/HA and FMN/PhHA adducts represented in black and red, respectively.

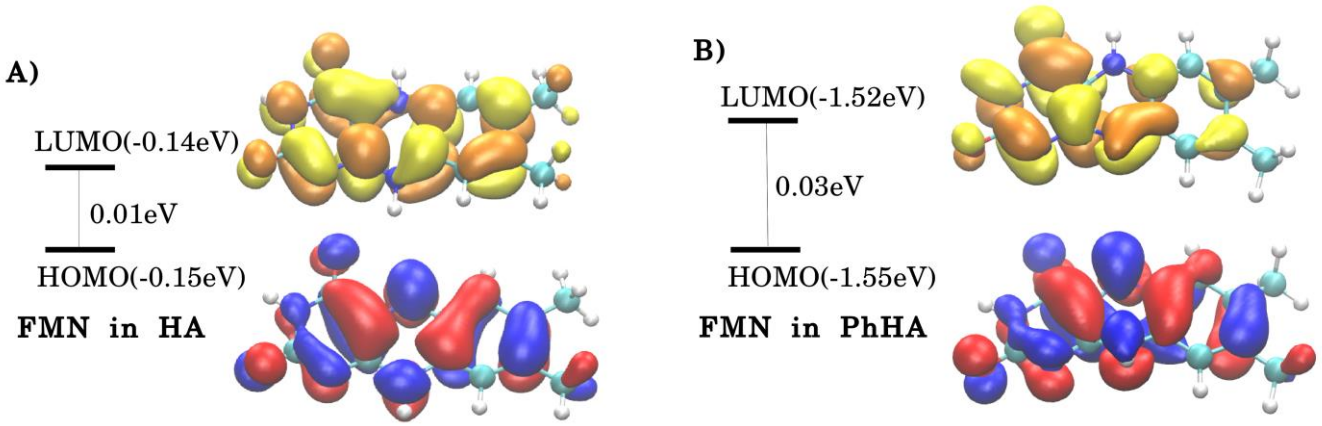


Figure S9. Graphical and energetic representation of the HOMO and LUMO orbitals of FMN in FMN/HA (A) and FMN in FMN/PhHA (B), along with their relative energies (eV).

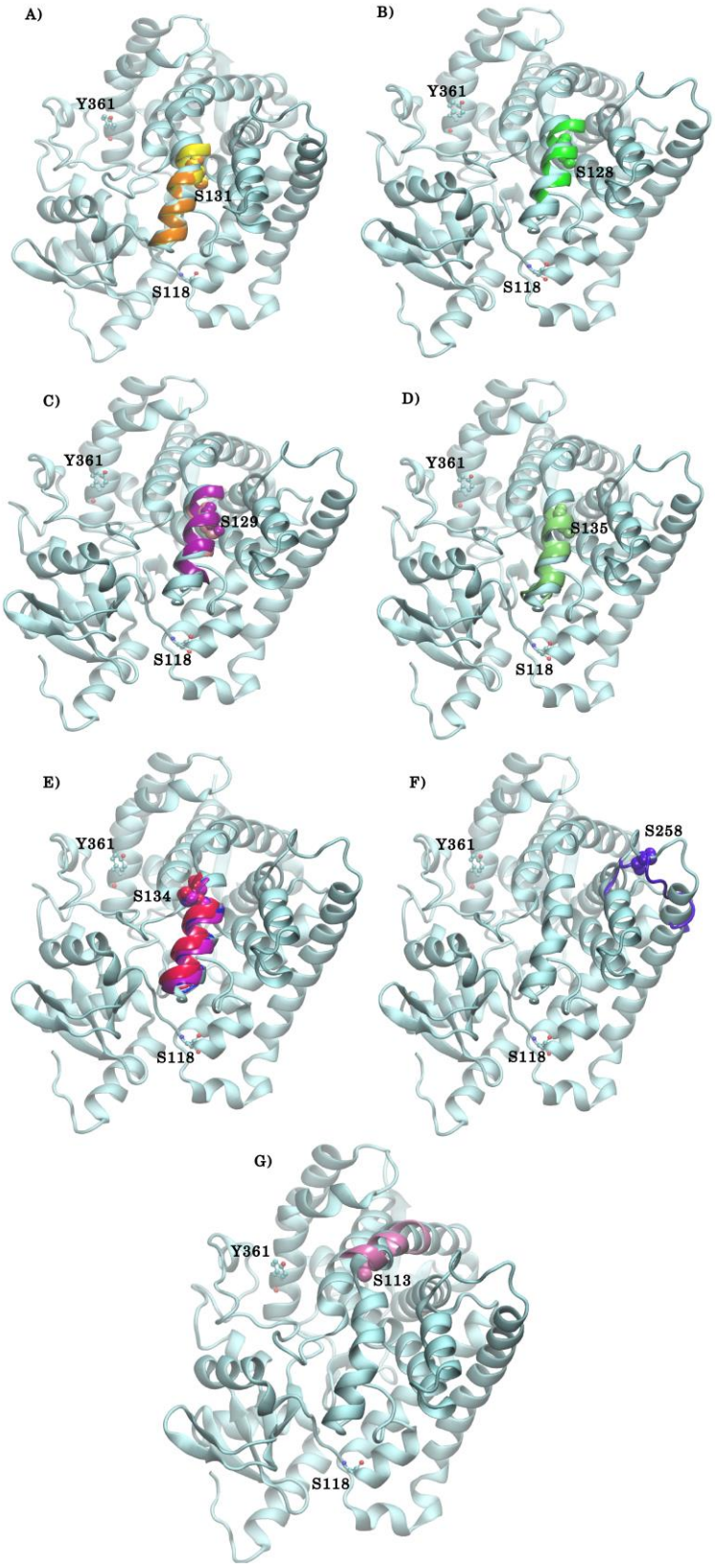
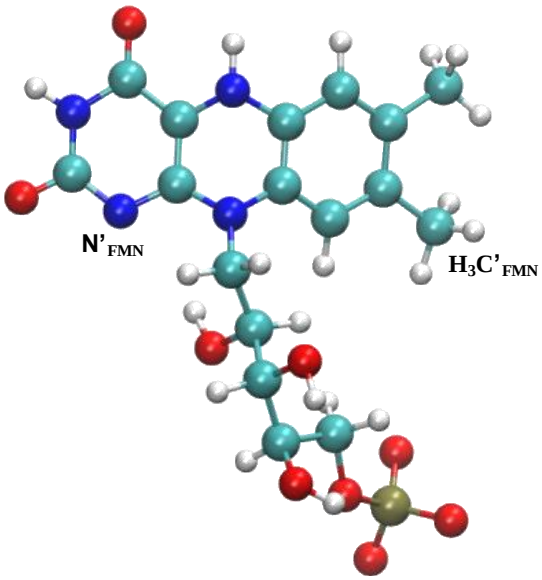


Figure S10. Phosphorylation sites describing the positions reported in Table 2 of the main text determining an enhanced catalysis of CYP450s. Orange, yellow, green, pink, purple, lime, dark blue, magenta, red, violet, and light pink show an alignment of the helix phosphorylated in A) CYP2A6, CYP2A13, B) CYP2B6, C) CYP2C8, CYP2E1, D) CYP2D6, E) CYP3A4, CYP3A5, CYP3A7, F) CYP17A1, G) CYP11A1, respectively, and on the corresponding helix of HA.

Table S1. Van der Waals and electrostatic non-bonded interaction energies (kcal/mol) of FMN/HA after the MD simulations of the structures obtained by the different docking programs listed. Standard deviations are reported.

Energy of FMN/HA	Pydock	Haddock	Glide	Haddock_ref	Glide_ref	ClusPro_ref	ZDOCK_ref
Van der Waals	-115.2±27.5	-109.9±23.4	-86.22±15.6	-110.2±23.3	-59.6±13.6	-110.6±27.8	-103.9±21.1
Electrostatic	-41.6±4.1	-51.5±6.4	-30.1±4.4	-51.4±6.2	-21.8±3.8	-50.7±6.2	-49.7±5.8

Table S2. Bond distances between FMN cofactor and the iron of the heme moiety (*Å*) in FMN/HA as measured along the MD simulations of the models obtained by the different docking programs listed.



FMN COFACTOR

Distance (d)	Pydock	Haddock	Glide	Haddock_ref	Glide_ref	ClusPro_ref	ZDOCK_ref
d(N'$_{FMN}$-Fe$_{HEM}$)[a]	18.1±0.2	21.1±0.7	20.3±0.6	21.3±0.6	20.4±0.6	22.7±0.4	19.8±0.6
d(H$_3$C'$_{FMN}$-Fe$_{HEM}$)[a]	12.4±0.4	16.4±1.3	15.2±0.8	15.8±1.0	20.3±1.2	17.8±0.9	14.6±0.7

[a]We considered either distances between the N'$_{FMN}$ and Fe and that between H$_3$C'$_{FMN}$ and Fe of the heme since the FMN cofactor is aromatic and as a result the electron, which has to be donated to the heme, is delocalized.[7]

Table S3. Residues involved into persistent H-bonds observed in FMN/HA after the MD simulations of the structures obtained by the different docking programs listed.

FMN/HA	FMN domain	HA
PYDOCK	Asp211	Lys108
	Glu145	Tyr441
	FMN226	Tyr441
	Asp150	Lys354
	Gln153	Lys354
	Glu95	Lys420
HADDOCK	Asp154	Lys108
	Asp150	Tyr441
	FMN226	Gln351
GLIDE	Asp157	Lys108
	Asp157	Arg425
	Asp147	Tyr424
HADDOCK_ref	Asp157	Lys108
	Glu118	Asn110
	Asp150	Arg145
	Glu182	Lys354
GLIDE_ref	Glu182	Tyr441
CLUSPRO_ref	Asp157	Lys108
	Glu145	Gln351
	Glu95	Lys150
ZDOCK_ref	Asp211	Lys108
	Gln90	Tyr424
	Glu145	Tyr441
	Asp150	Asn421
	Gln90	Asn421

Table S4. Residues involved into persistent hydrogen bonds observed during MD simulations of the FMN/HA and FMN/PhHA adducts. The interactions established between PhHA and FMN domain, following after phosphate addiction on the equilibrated HA/CPR model and FMN repositioning during the MD simulations have been highlighted in sky blue. Atom labels have been assigned according to the Amber force field labelling scheme.

FMN domain	**HA**	**Persistence (%)**	**Average distance (*Å*)**
Asp150@OD1	Lys354@HZ2	98.2	2.7
Asp157@OD2	Lys352@HZ1	83.6	2.8
Glu95@OE1	Lys420@HZ1	77.8	2.7
Glu96@OE2	Lys420@HZ2	73.1	2.7
Asp211@O	**Lys108@HZ1**	65.1	2.8
FMN226@H1	**Tyr441@OH**	59.6	2.8
Glu45@OE1	Tyr441@HH	54.8	2.7
Gln153@OE1	Lys354@HZ1	53.9	2.8
Asn151@HD21	Asn421@OD1	45.5	2.8
Gln153@OE1	Lys354@HZ2	32.3	2.8
Asp150@OD2	Lys354@HZ1	31.7	2.8
FMN domain	**PhHA**	**Persistence (%)**	**Average distance (*Å*)**
Asp150@OD2	Lys354@HZ2	100	2.8
Asp116@OD1	Lys420@HZ1	99.2	2.8
Glu96@OE1	**Lys108@HZ2**	98.0	2.8
Glu118@OE1	Lys420@HZ2	97.3	2.8
Asn151@OD1	Lys354@HZ3	96.0	2.8
Tyr143@HH	Ile347@O	95.9	2.7
Glu95@OE2	**Arg425@HH22**	94.4	2.8
Tyr87@HH	Asn421@OD1	88.3	2.8
Glu95@OE1	**Arg425@HH12**	85.3	2.8
Glu119@OE1	Lys420@HZ3	75.3	2.8
Glu95@OE2	**Arg425@HH12**	44.9	2.9
Thr180@OG1	Lys150@HZ2	44.6	2.9
Asn178@HD21	Ser153@OG	24.0	2.9
Thr180@OG1	Lys150@HZ1	21.2	2.9
Asn151@HD21	Asn421@OD1	18.3	2.9

Table S5. Residues involved into persistent hydrogen bonds observed during MD simulations between membrane components and HA/PhHA systems.

Membrane lipid	**HA**	**Persistence (%)**	**Average distance (*Å*)**
PC17@O34	Ser46@HG	100.0	2.6
PC23@O34	Ser61@HG	99.5	2.6
PC26@O22	Tyr41@HH	98.7	2.7
PC44@O33	Arg79@HE	95.7	2.7
CHL10@O1	Tyr76@HH	95.2	2.8
PC11@O34	Trp67@HE1	90.7	2.8
PC143@O33	Lys243@HZ3	89.6	2.8
CHL1@O1	Asn40@HD22	86.7	2.9
PC2@O22	His62@H	84.3	2.8
PC23@O12	Gly57@H	82.6	2.8
PC95@O33	His62@H	75.2	2.8
PC17@O34	Gly49@H	44.5	2.9
PC23@O12	Ile56@H	38.6	2.9
Membrane lipid	**PhHA**	**Persistence (%)**	**Average distance (*Å*)**
PC23@O34	Ser61@HG	100.0	2.6
PC26@O34	Ser46@HG	99.8	2.5
PC11@O34	Arg64@HH12	99.3	2.7
PC2@O22	His62@H	91.7	2.8
PC20@O34	Asn40@HD22	91.7	2.8
CHL10@O1	Tyr76@HH	90.2	2.8
PC95@O33	His62@HE2	86.2	2.8
PC44@O33	Arg79@HH21	86.1	2.7
PC23@O12	Gly57@H	82.3	2.8
PC134@O34	Lys243@HZ3	80.8	2.8
PC11@O34	Trp67@HE1	80.1	2.8
PC143@O33	Lys243@HZ1	80.0	2.8
PC11@O34	Arg64@HH22	59.5	2.8
PC44@O33	Arg79@HE	58.2	2.8
CHL14@O1	Arg64@HH11	35.9	2.9
PC143@O34	Lys243@HZ1	35.1	2.8
PC23@O12	Ile56@H	29.1	2.9
PC134@O33	Lys243HZ3	26.9	2.8
PC44@O34	Arg79@HH21	23.8	2.8

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