Supplemental Materials

Cryo-EM reveals the architecture of the dimeric cytochrome P450 CYP102A1 enzyme and conformational changes required for redox partner recognition

Min Su[a,*], Sumita Chakraborty[b], Yoichi Osawa[b], and Haoming Zhang[b,*]

[a]Life Sciences Institute and [b]Department of Pharmacology, The University of Michigan, Ann Arbor, MI 48109

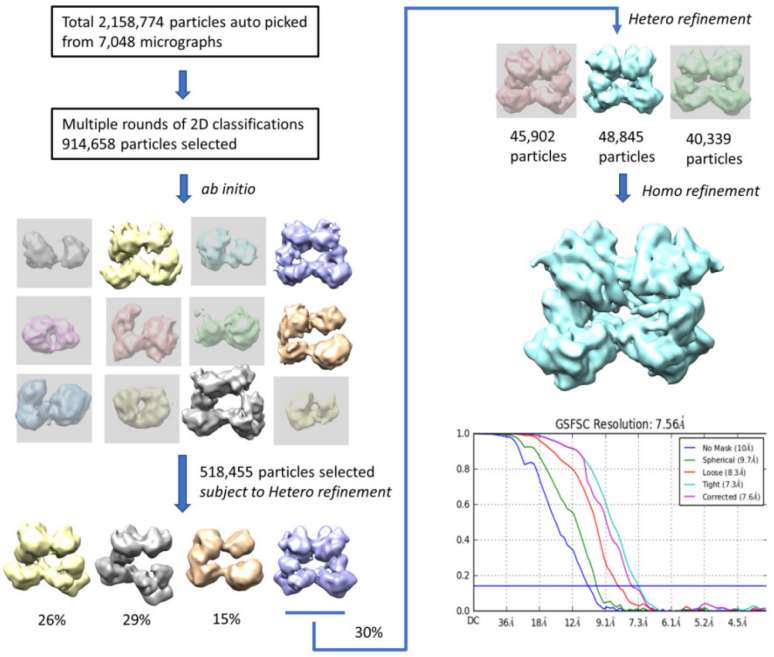
*Correspondence authors: Min Su, Life Sciences Institute, 210 Washtenaw Ave, Ann Arbor, MI 48109, Tel: 734-6478193, E-mail: minsu@umich.edu; Haoming Zhang, Department of Pharmacology, 1150 West Medical Center Drive, Ann Arbor, MI 48109, Tel: 734-764-6184, E-mail: haom@umich.edu

Running Title: complete architecture of full-length CYP102A1

This PDF file includes:

Figure S1. Cryo-EM workflow to obtain EM density maps of full-length CYP102A1 (A) and the Δ12 variant (B).

A



B

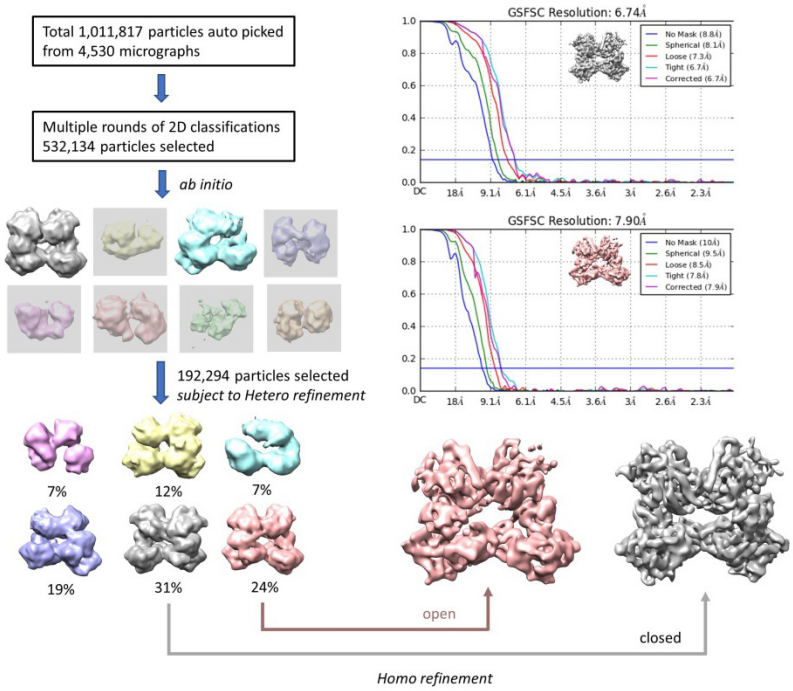


Table S1. Cryo-EM data collection and image processing

Data collection and processing	**FL (closed)**	**FL (open I)**	**FL (open II)**	**Δ12 (closed)**	**Δ12 (open)**
Microscope	Titan Krios				
Camera	K2 Summit direct electron detector				
Magnification (nominal)	29,000× (50,000×)				
Voltage (kV)	300				
Electron exposure ($e^-/Å^2$)	6				
Exposure per frame (sec)	0.2				
Number of frames collected	40				
Defocus range (μm)	-1 to -3				
Pixel size (Å)	1.01				
Micrographs (no.)	7048			4530	
3D processing package	cryoSPARC				
Symmetry imposed	C1				
Initial particle images (no.)	2,158,744			1,011,817	
Final particle images (no.)	155,536	134,798	150,351	59,611	46,150
Map resolution corrected (FSC=0.143)	7.6	8.3	8.5	6.7	7.9
Modeling					
Modeling package	Chimera (fitmap; rigid body fitting)				
Initial model used	4KEW,1BVY,4DQK				
Correlation coefficient	0.64	0.53	0.52	0.73	0.65

Figure S2. Structural alignment of the BMP dimer model of full-length CYP102A1 in closed state (Fig. 2B) with the crystal structure of BMP dimer (PDB ID: 1BVY) determined by Dr. Poulos and co-workers (1). The RMSD value for the alignment is 0.755 Å. The BMP dimer model is shown in blue ribbon, whereas the crystal structure of the BMP dimer is shown in green ribbon**.** It is of note that the BMP dimer model was built with the crystal structure of the heme domain of the A82F variant (PDB:4KEW), whereas the crystal structure of the BMP dimer was determined with a truncated form of wile type CYP102A1 BMP/FMN construct.

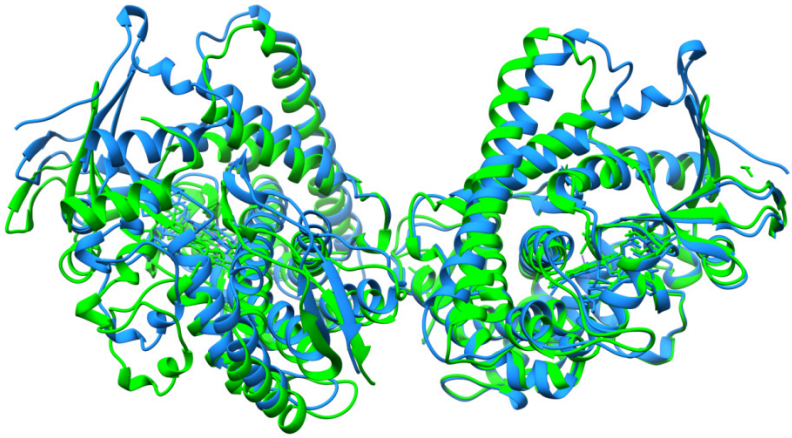


Figure S3. Comparison of the ET complex models in Open State I (A) and Open State II (B) with that determined by X-ray crystallography for a truncated form of wild type CYP102A1BMP/FMN construct (PDB:1BVY) (1). The structures were aligned with Chimera. The model complex is colored in orange, whereas the crystal structure is colored in green. The heme in the crystal structure is displayed as red stick. Key structural elements are labeled as: Helix C, G, H, and I; HBL, heme-binding loop; PG, peptide Pro383-Gln387.

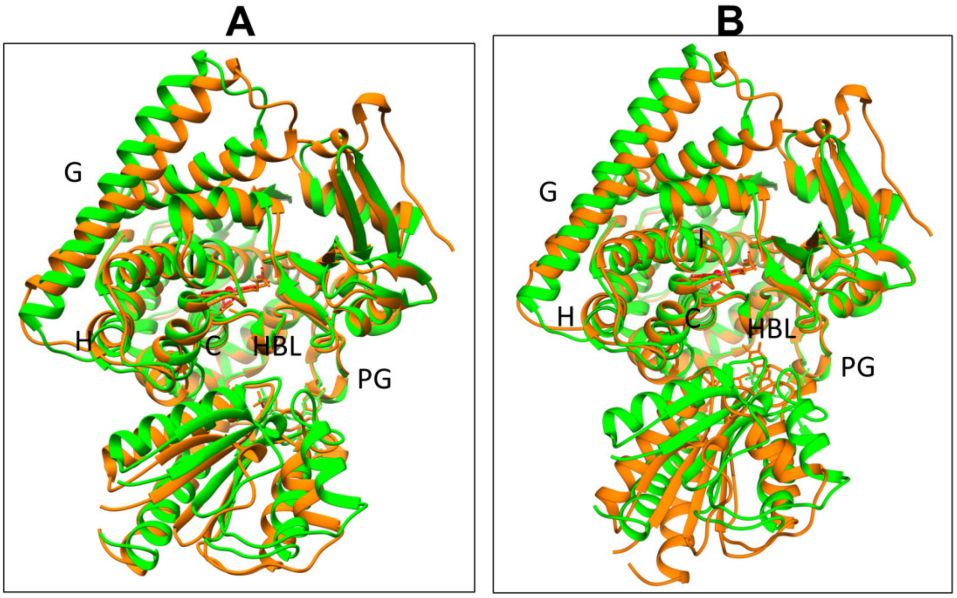


Figure S4. Conformational changes in the FMN domain from closed-to-open conformational transition. The structure of full-length CYP102A1 in closed state (Fig. 2B) is aligned at the heme domain with the structure of full-length CYP102A1 in Open State I (Fig. 3C). The backbones of the FMN domains are depicted in orange (closed state) and blue (Open State I) ribbons. Cofactors FMN are depicted as orange and blue sticks respectively. The green, red and black arrows indicate the direction in which the linker, hinge and FMN cofactor move from closed to open state**.**

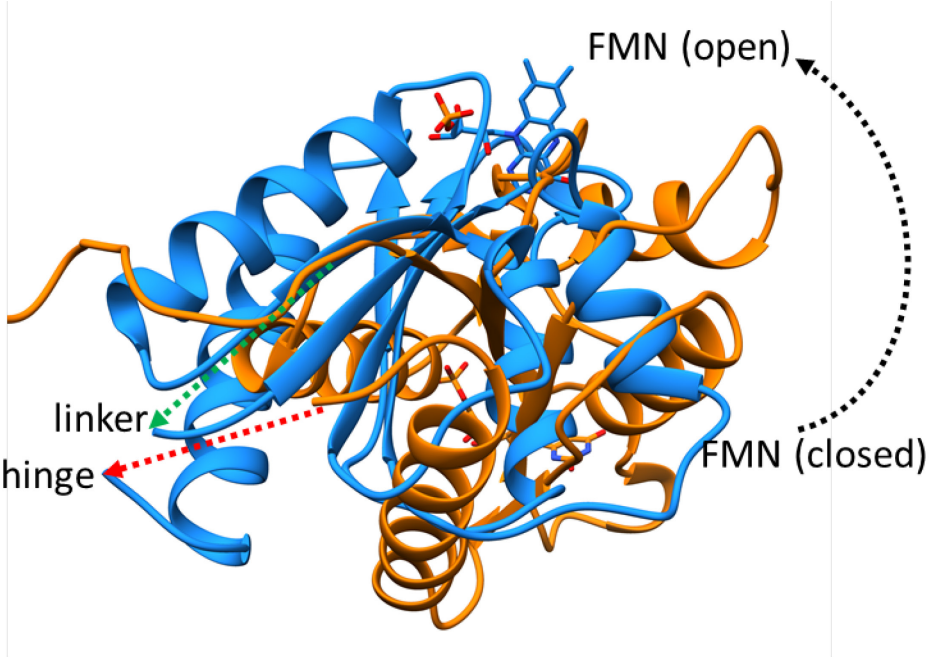


Figure S5. Comparison of the 3D model of the full-length enzyme with that of the Δ12 variant in closed state. The 3D model of the full-length enzyme (Figure 2B) was aligned with that of the Δ12 variant (Figure 4B) using Chimera. The RMSD value for the alignment is 1.168 Å. The 3D model of the full-length enzyme is depicted as green ribbons, whereas the 3D model of the Δ12 variant is in blue ribbons. Cofactor heme, FMN, and FAD are displayed as red, orange, and yellow sticks.

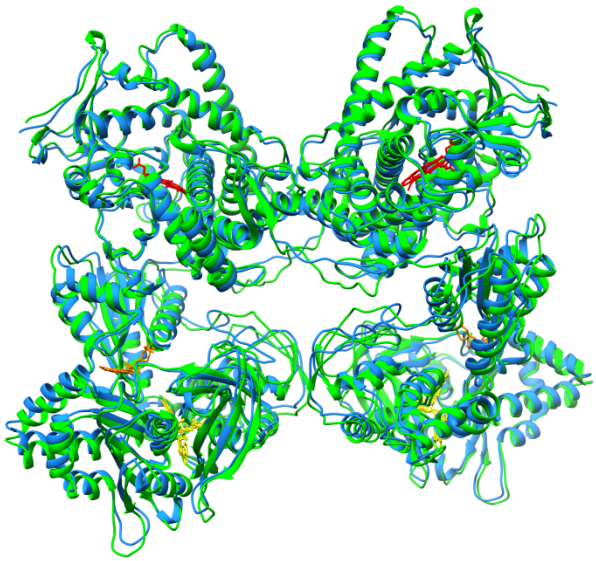


Figure S6. Comparison of the ET complex of the Δ12 variant with those of the crystal structure (PDB ID: 1BVY) (A) and the 3D model of full-length enzyme (open state I) (B). The 3D model of the Δ12 variant is displayed as colored ribbons with the BMP in orange and FMN in blue, whereas the crystal structure or the 3D model of full-length is shown as grey ribbons. Cofactor heme and FMN of the variant are depicted as red and pink sticks respectively.

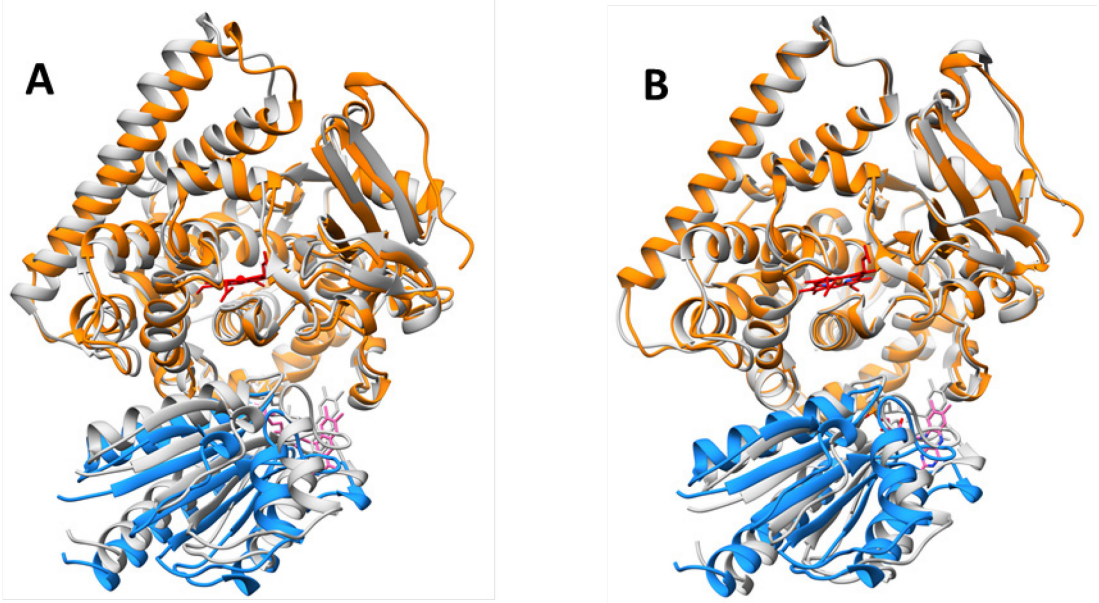


Figure S7. SEC-MALS data showing deletion variants elute as homogenous homodimer. The horizontal bars on the right Y-axis indicate molecular weight (M_w) in kDa. The three deletion variants eluted as homogenous peaks at ~11.2 ml with M_w of 237±6.3, 235±3.6 and 225±11 kDa for Δ4, Δ6, and Δ12 variants respectively. These M_w values are in agreement with the M_w values expected for respective homodimer at 239, 239 and 237 kDa, suggesting that these variants remain as homodimers in solution.

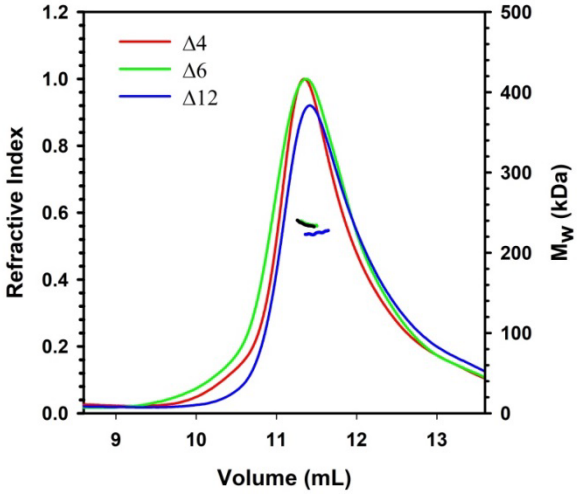


Figure S8. Comparison of the BMR domain model of full-length CYP102A1 in closed state (Fig. 2B) with the crystal structure of rat POR (PDB ID:1JA0) (2). The structures were aligned with Chimera. The RMSD value of the alignment is 1.001 Å. The BMR domain model is shown in blue ribbon, whereas the crystal structure of rat POR is shown in green ribbon.

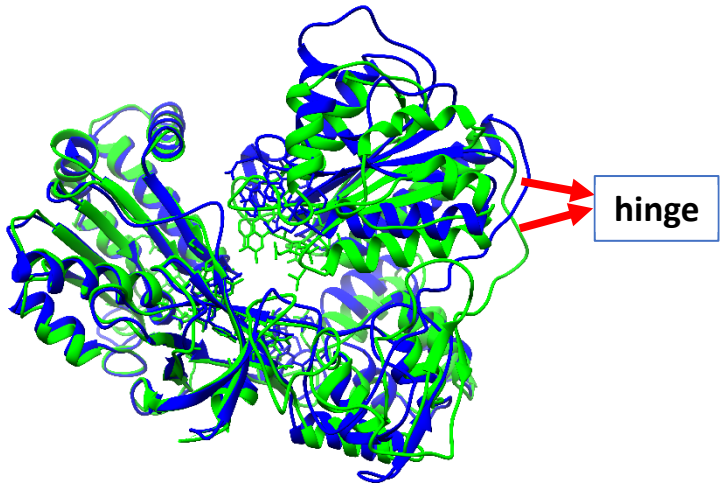


Figure S9. An alternative ET pathway from FMN to the heme as revealed in Open State II. The ET complex model in Open State II (Fig. 3F) is displayed as colored ribbons with the heme domain in orange and the FMN domain in blue. The red and green arrows point to the linker and hinge respectively. The expanded view illustrates an alternative ET pathway from FMN to the heme via the heme-binding loop.

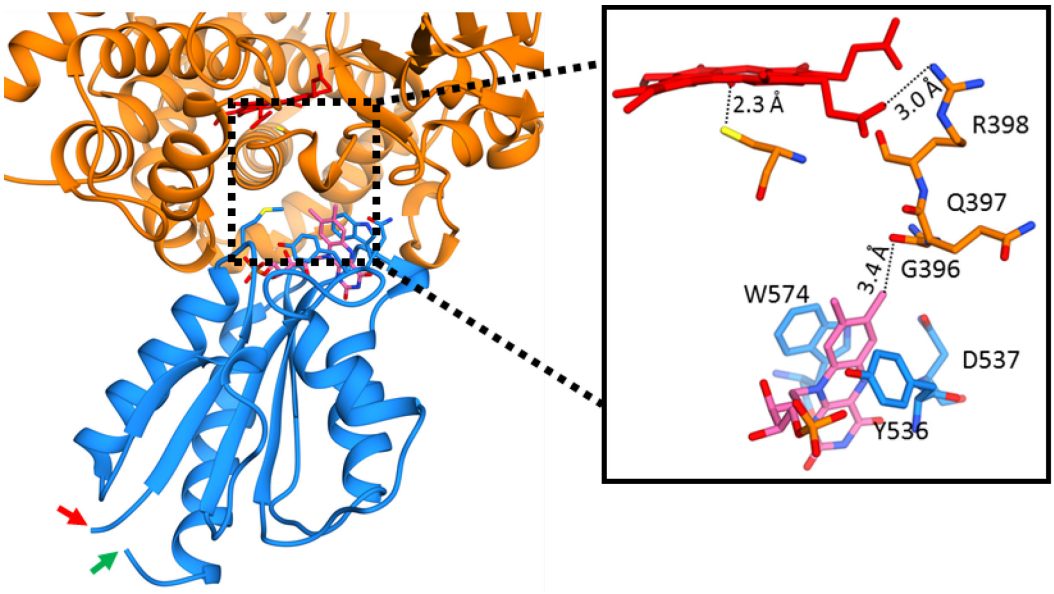


Table S2. PCR primers for construction of deletion mutants in the linker region of CYP102A1

Mutant	Deleted AA	5'-Forward Primer	5'-Reverse Primer
Δ4	ΔTEQS	GCGAAAAAAGTTCGGAAAAAGGC	GGATGGGCTCGGAATGCC
Δ6	ΔSTEQSA	AAAAAAGTTCGGAAAAAGGCGGAAAATG	TGGGCTCGGAATGCCACC
Δ12	ΔGGIPSPSTEQSA	AAAAAAGTTCGGAAAAAGGC	CAGCGGAATCTTTTTGCTTTTC

References

1. Sevrioukova IF, Li H, Zhang H, Peterson JA, & Poulos TL (1999) Structure of a cytochrome P450-redox partner electron-transfer complex. *Proc Natl Acad Sci U S A* 96(5):1863-1868.
2. Hubbard PA, Shen AL, Paschke R, Kasper CB, & Kim JJ (2001) NADPH-cytochrome P450 oxidoreductase. Structural basis for hydride and electron transfer. *J Biol Chem* 276(31):29163-29170.