Supplementary Information

The role of active site Phe87 in modulating the organic co-solvent tolerance of cytochrome P450 BM3 monooxygenase

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Table S1: Total RMSD (Å) and backbone RMSD (numbers in brackets; Å) over active site residues (P25, V26, R47, Y51, S72, A74, L75, V78, F81, A82, F/A87, T88, L188, T260, I263, A264, T268, A328 and M354) between different crystallographic structures of cytochrome P450 BM3 monooxygenase. For pair-wise comparison between WT (F87) and its F87A counterpart, phenyl ring at Cβ was removed. Comparison between two monomers of the same crystallographic structure was shaded light green.

		1BU7		**2J1M**		**2J4S**		**2X80**		**2X7Y**	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
1BU7	**A**	–									
	B	1.28 (0.77)	–								
2J1M	**A**	1.00 (0.80)	1.40 (0.93)	–							
	B	1.20 (0.54)	1.67 (0.87)	0.89 (0.40)	–						
2J4S	**A**	0.96 (0.49)	1.60 (0.54)	1.20 (0.97)	1.34 (0.79)	–					
	B	0.58 (0.50)	1.00 (0.42)	1.19 (0.90)	1.50 (0.75)	1.07 (0.20)	–				
2X80	**A**	0.93 (0.62)	1.25 (0.34)	1.03 (0.77)	1.27 (0.73)	0.95 (0.45)	0.93 (0.37)	–			
	B	0.99 (0.80)	1.05 (0.26)	1.23 (0.98)	1.65 (0.91)	1.16 (0.53)	0.71 (0.45)	0.96 (0.33)	–		
2X7Y	**A**	0.79 (0.53)	1.66 (1.06)	1.09 (0.80)	1.42 (0.57)	1.26 (0.83)	1.07 (0.84)	1.26 (0.94)	1.17 (1.08)	–	
	B	0.65 (0.46)	1.56 (0.93)	1.01 (0.67)	1.23 (0.47)	1.17 (0.75)	1.01 (0.74)	1.07 (0.79)	1.22 (0.96)	0.66 (0.25)	–

Figure S1: DMSO tolerance profile of cytochrome P450 BM3 WT (F87; closed circle) and its F87A variant (opened circle) (Wong *et al.*, 2004). The vertical dashed lines represent 14% (v/v) DMSO (left) and 28% (v/v) DMSO (right) concentrations.

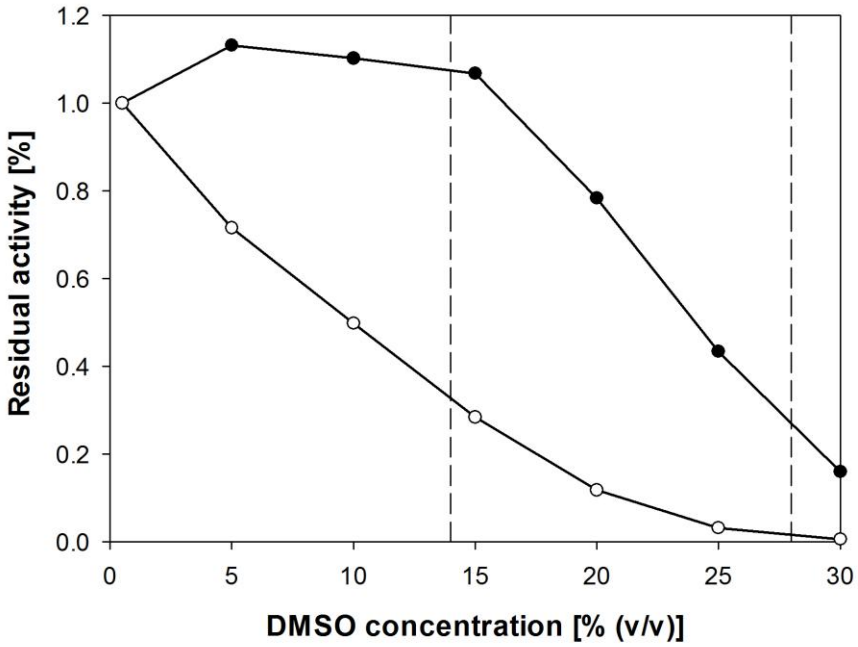


Figure S2: UV-vis difference spectra of P450 BM3 F87A in phosphate buffer (blue), in 14% (v/v) DMSO (red) and in 30% (v/v) DMSO (black). The vertical dashed line represents wavelength of 418 nm.

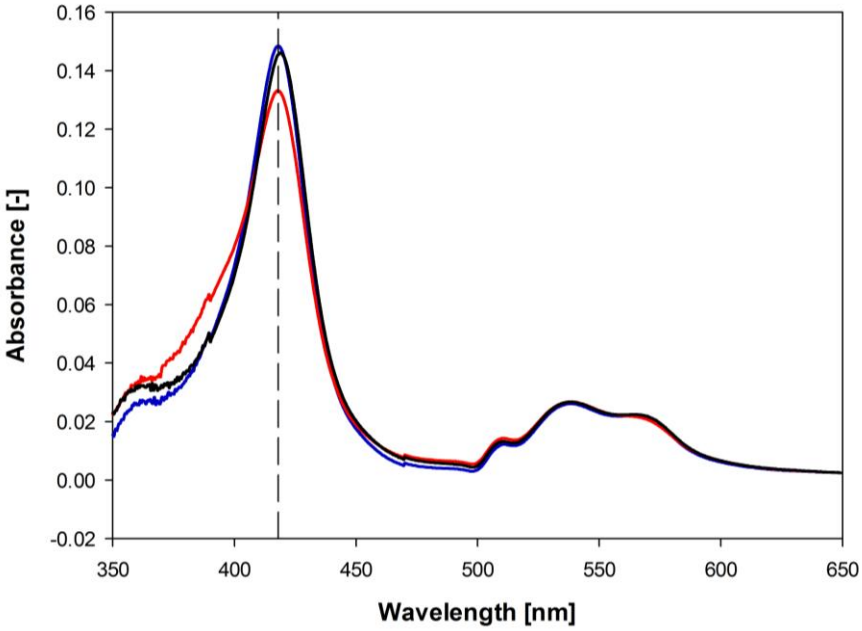
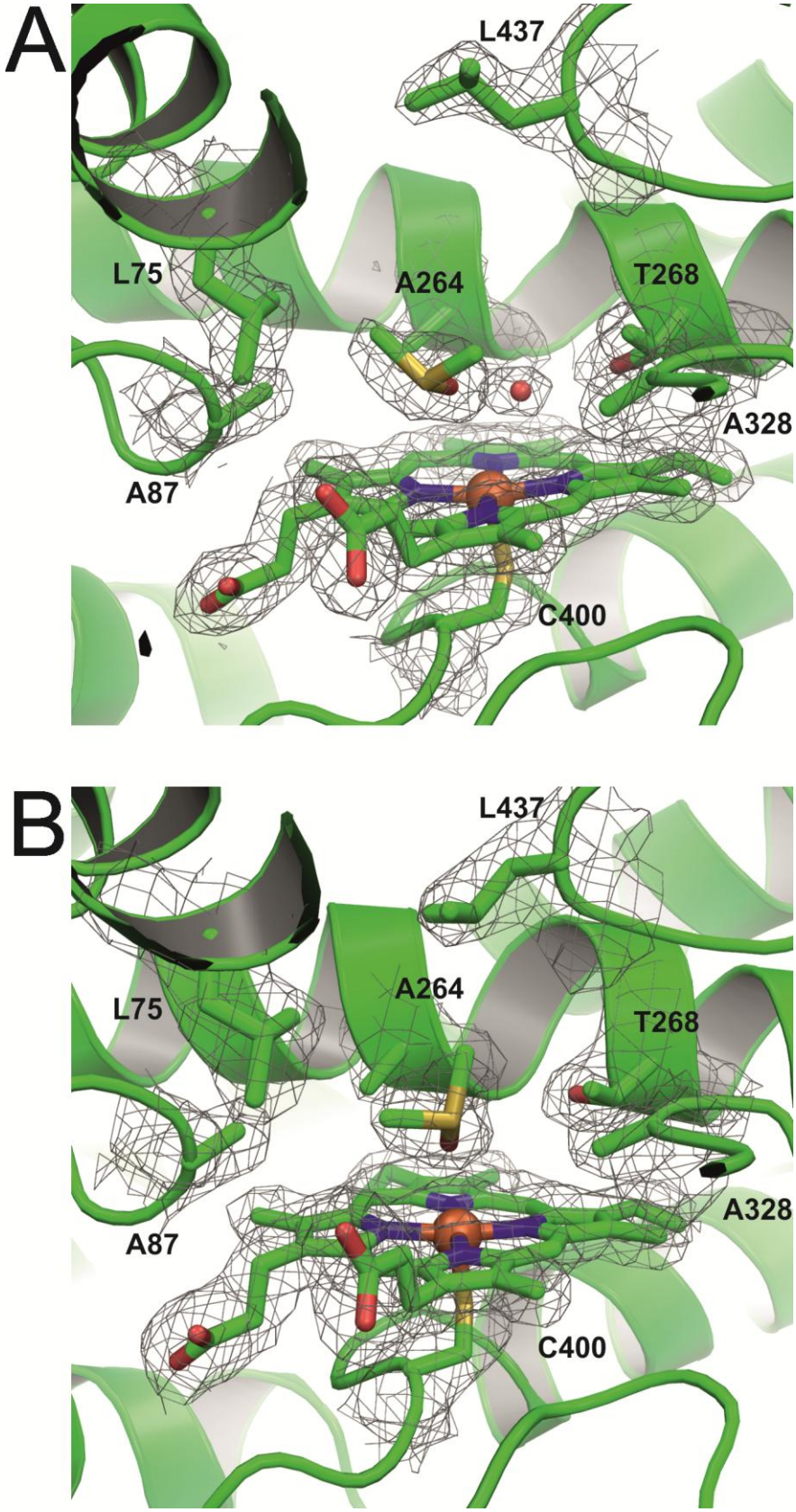


Figure S3: Active sites of F87A variant in the presence of 14% (v/v) DMSO (**A**) and 28% (v/v) DMSO (**B**)



Reference

Wong, T. S., Arnold, F. H. & Schwaneberg, U. (2004). *Biotechnol Bioeng* **85**, 351-358.