Supporting Information

Semi-rational directed evolution of a Deepsea-derived $P450_{S18}$ for Phenazines Construction

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Table of contents

1. Supplementary Figures

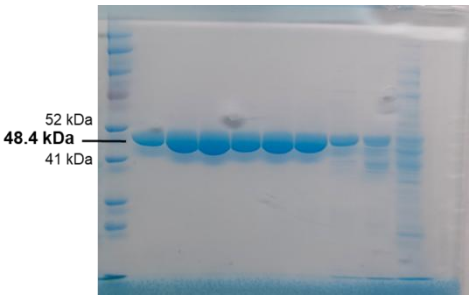

Figure S1. SDS-PAGE analysis of purified $P450_{S18}$ (48.4 kDa). Separation was performed using a 12% acrylamide gel and was stained with Coomassie Brilliant Blue.

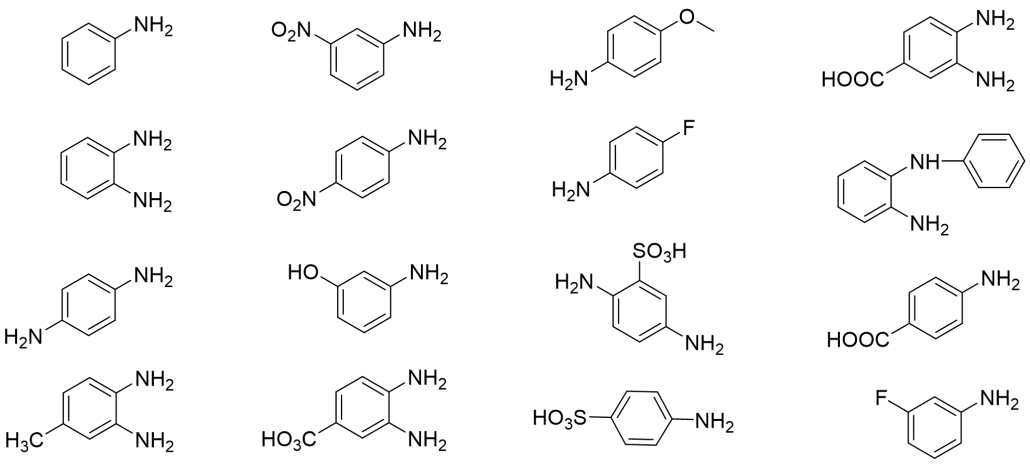

Figure S2. Substrates tested for $P450_{S18}$.

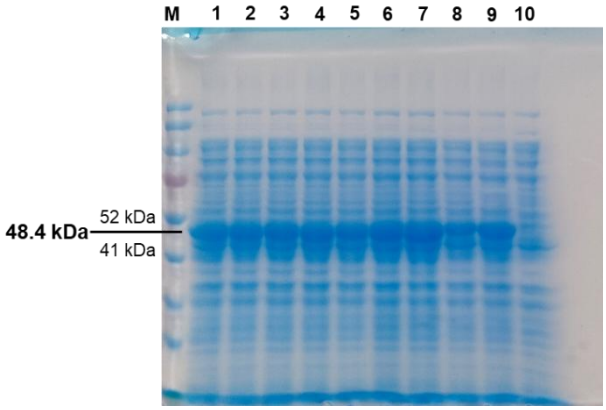

Figure S3. SDS-PAGE analysis of the crude enzyme of $P450_{S18}$ and its mutants. M: protein marker; lane 1-8: F82A, Q88A, F176A, P246A, R245A, F295A, T296A and F292A. lane 9: wild-type-$P450_{S18}$. lane 10: empty vector without the $P450_{S18}$ gene. Separation was performed using a 12% acrylamide gel and was stained with Coomassie Brilliant Blue

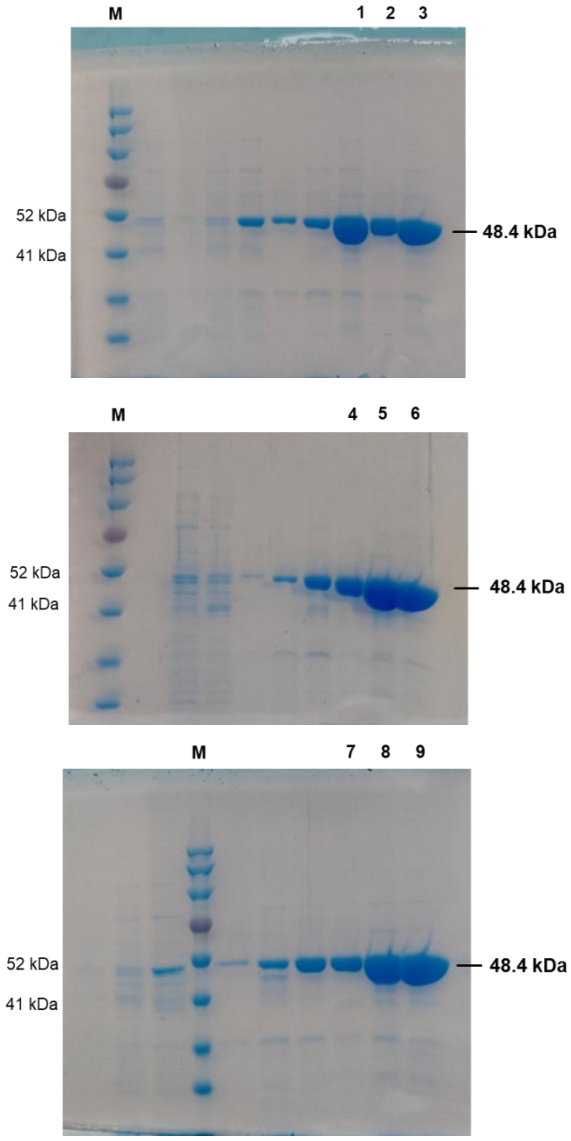

Figure S4. SDS-PAGE analysis of $P450_{S18}$ and its mutants. M: protein marker; lane 1-8: F82A, Q88A, F176A, P246A, R245A, F295A, T296A and F292A. lane 9: wild-type-$P450_{S18}$. Separation was performed using a 12% acrylamide gel and was stained with Coomassie Brilliant Blue.

2. Supplementary Tables

Table S1. Bacteria and plasmids used in this study.

Strains or plasmids	Description	Reference or source
E. coli		
E. coli DH5*α*	*dam*$^+$, *dcm*$^+$, F$^-$ Φ80/*lacZ*ΔM15 Δ(*lacZYA-argF*) U169, *recA1*, *endA1*, *hsdR17* (r_K^-, m_K^+), *phoA*, *supE44* λ– *thi-1 gyrA96*, *relA1*, for general cloning and preparing methylated DNA	Stratagene
E. coli BL21 (DE3)	F-, *omp*T, *hsdSB*(r_{B-},m_{B-}), *gal*, *dcm*(DE3)	Novagen
Plasmids		
pET28a	Kan^R, expression vector	Novagen
pET28a::P450$_{S18}$	pET28a harboring P450$_{S18}$	This study
pET28a::P450$_{S18}$ F176A	pET28a harboring P450$_{S18}$ F176A	This study
pET28a::P450$_{S18}$ P246A	pET28a harboring P450$_{S18}$ P246A	This study
pET28a::P450$_{S18}$ R245A	pET28a harboring P450$_{S18}$ R245A	This study
pET28a::P450$_{S18}$ F295A	pET28a harboring P450$_{S18}$ F295A	This study
pET28a::P450$_{S18}$ T296A	pET28a harboring P450$_{S18}$ T296A	This study
pET28a::P450$_{S18}$ F292A	pET28a harboring P450$_{S18}$ F292A	This study
pET28a::P450$_{S18}$ Q88A	pET28a harboring P450$_{S18}$ Q88A	This study
pET28a::P450$_{S18}$ F82A	pET28a harboring P450$_{S18}$ F82A	This study

Table S2. The primer pairs used in this study.

		Primer pairs used for inactivation (5'-3') [a]	Restriction sites	Size (bp)
Protein expression in	$P450_{S18}$-FP	GGAATTCCATATGaattcaggtaagcaaatac	Nde I	1281
E. coli BL21 (DE3)	$P450_{S18}$-RP	CCGCTCGAGttacttaacctttatattc	Xho I	
Site-directed	F176A-FP	gacatgattgatgcaGCAggcgcaacaggcccac		
mutagenesis	F176A-RP	gtgggcctgttgcgccTGCtgcatcaatcatgtc		
	P246A-FP	ctgaacctttacggGCAattgtggcaattgcc		
	P246A-RP	ggcaattgccacaatTGCccgtaaaaggttcag		
	R245A-FP	attctgaacctttaGCAccgattgtggcaattg		
	R245A-RP	caattgccacaatcggTGCtaaaaggttcagaat		
	F295A-FP	ctatccgtttgcacccGCAacaggggcgttaacag		
	F295A-RP	ctgttaacgcccctgtTGCgggtgcaaacggatag		
	T296A-FP	ccgtttgcacccttGCAggggcgttaacagcg		
	T296A-RP	cgctgttaacgccccTGCaaagggtgcaaacgg		
	F292A-FP	cgtaggtactatccgGCAgcacccttacaggg		
	F292A-RP	ccctgtaaagggtgcTGCcggatagtacctacg		
	Q88A-FP	ggtgtaggtggtgtgGCAgggatggatggggaag		
	Q88A-RP	cttccccatccatcccTGCcacaccacctacacc		
	F82A-FP	gaataaaagaaagcttgGCAggtgtaggtggtgtgcaag		
	F82A-RP	cttgcacaccacctacaccTGCcaagctttcttttattc		

[a]Underlined letters represent restriction site