Supplemental Material for

Comparative analysis of three pairs of surrogate redox partners for *in vitro* activity reconstitution of Class I cytochrome P450 enzymes

Xiaohui Liu[1†], Fengwei Li[1†], Tianjian Sun[1], Jiawei Guo[1], Xingwang Zhang[1,2], Xianliang Zheng[3], Lei Du[1], Wei Zhang[1], Li Ma[1*], Shengying Li [1,2]

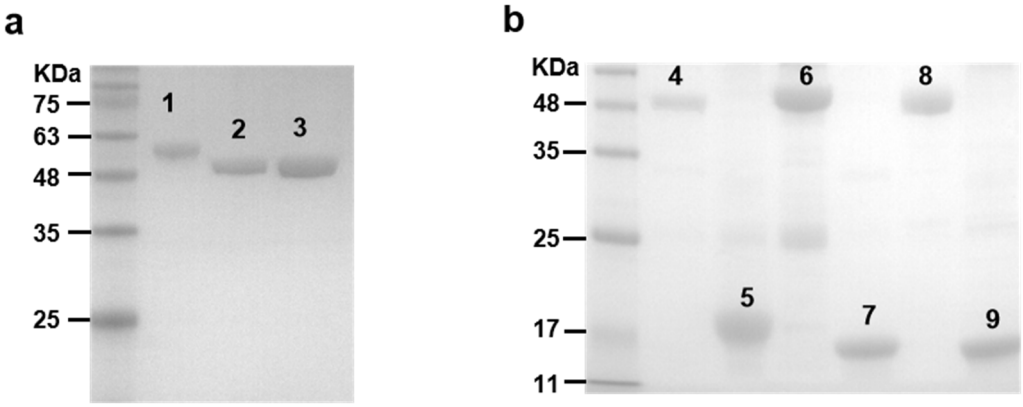
[1]State Key Laboratory of Microbial Technology, Shandong University, Qingdao, Shandong 266237, China.

[2]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, Shandong 266237, China.

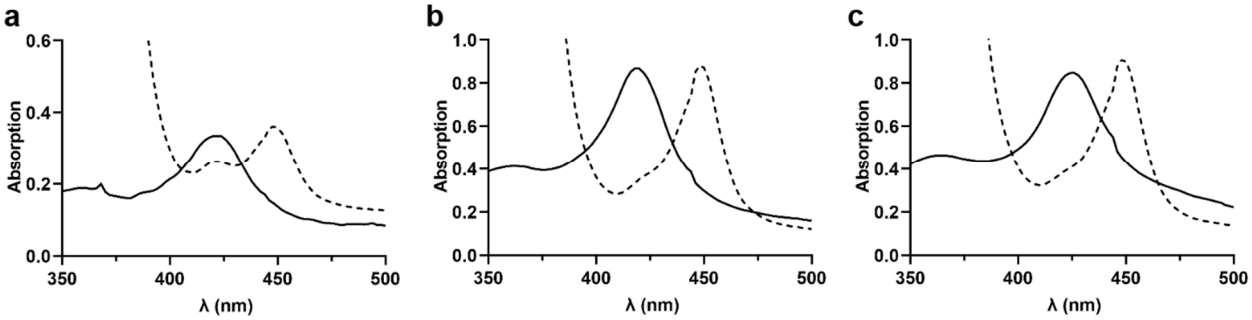
[3]Center For Biocatalysis and Enzyme Technology, Angel yeast co., LTD, Cheng Dong Avenue, Yichang, Hubei, 443003, China.

*Corresponding Author E-mail: maliqd@sdu.edu.cn

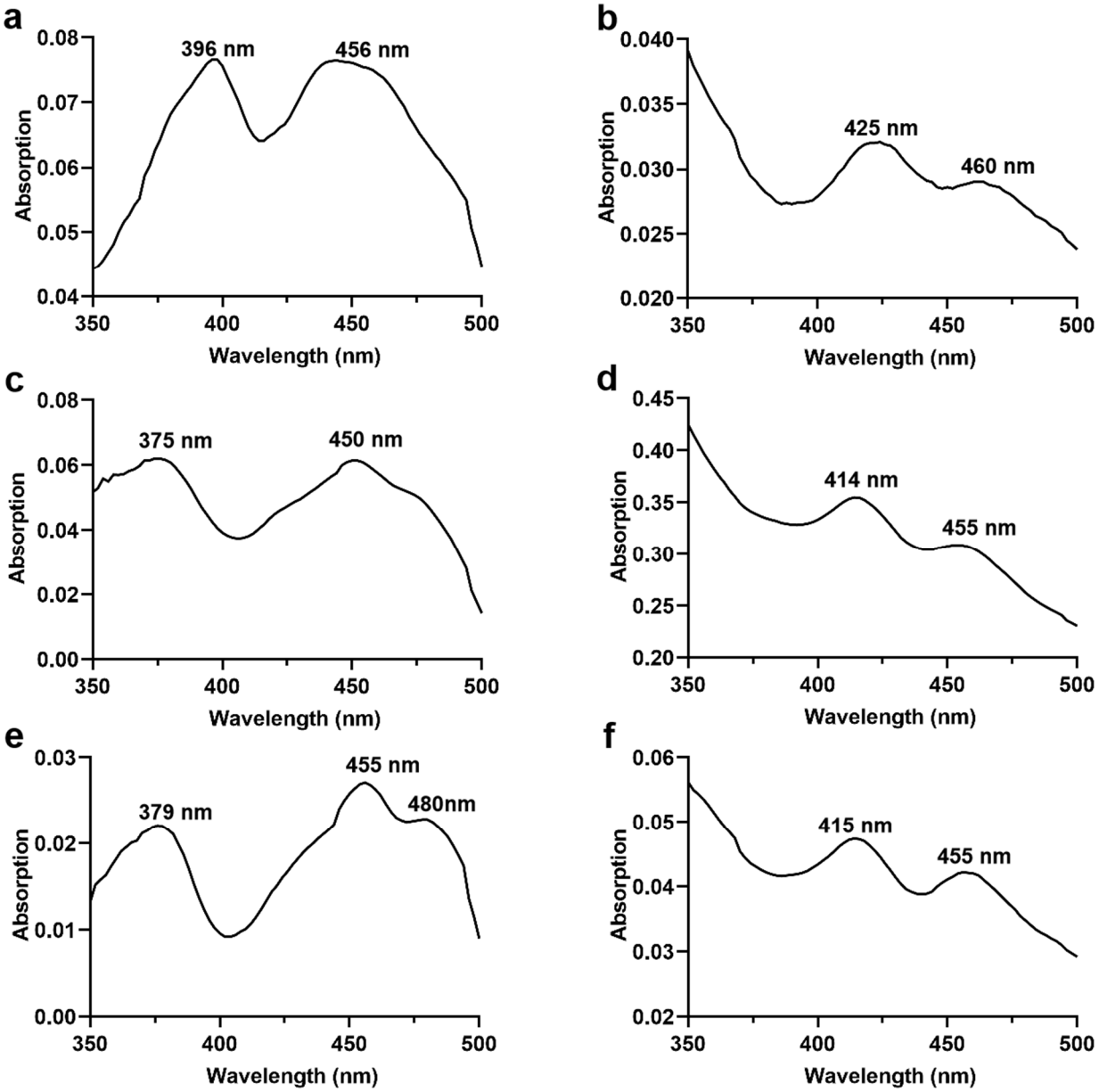
[†]These authors contributed equally.



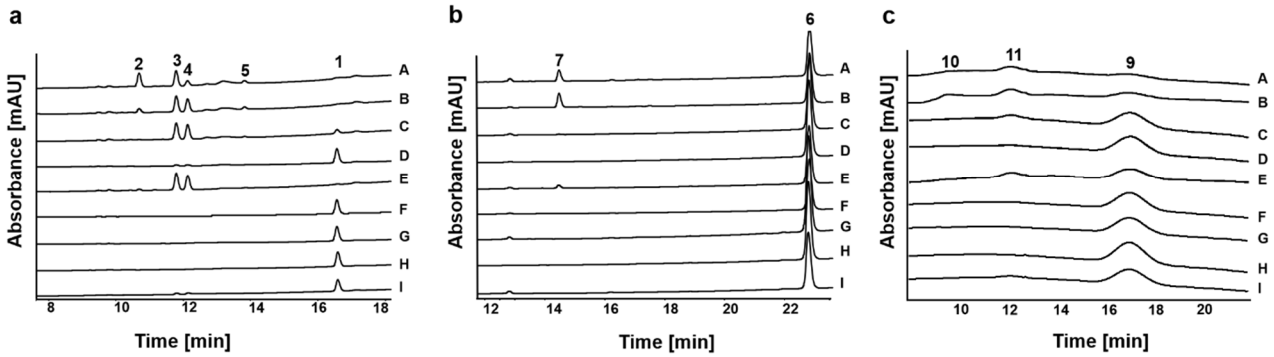
Supplementary Fig 1. SDS-PAGE analysis of purified cytochrome P450s (a) and redox partners (b). Lane 1, CYP-sb21; 2, P450sca-2; 3, PikC; 4, *Sel*Fdx1499; 5, *Sel*FdR0978; 6, AdR; 7, Adx; 8, PdR; 9, Pdx. Proteins were stained with Coomassie blue in 12.5% gel.



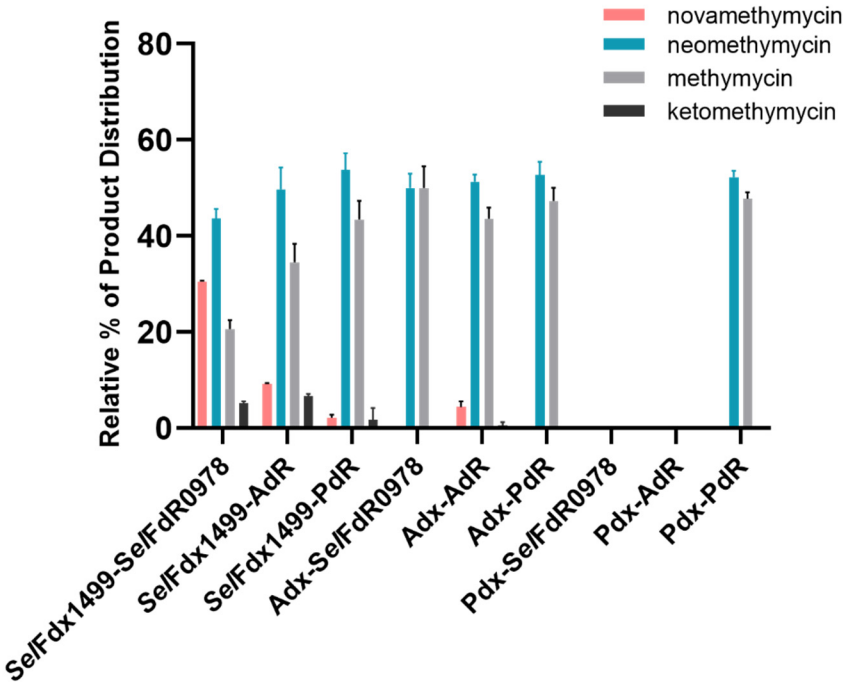
Supplementary Fig 2. CO-bound reduced difference spectra of purified P450s. (a) PikC, (b) P450sca-2, (c) CYP-sb21. *Dotted line*, P450 absorbance spectra in ferrous CO-complexed state; *Solid line*, ferric state. This assay was also employed to determine the concentration of functional cytochrome P450 enzyme using the extinction of 91,000 $M^{-1}cm^{-1}$ [1,2].



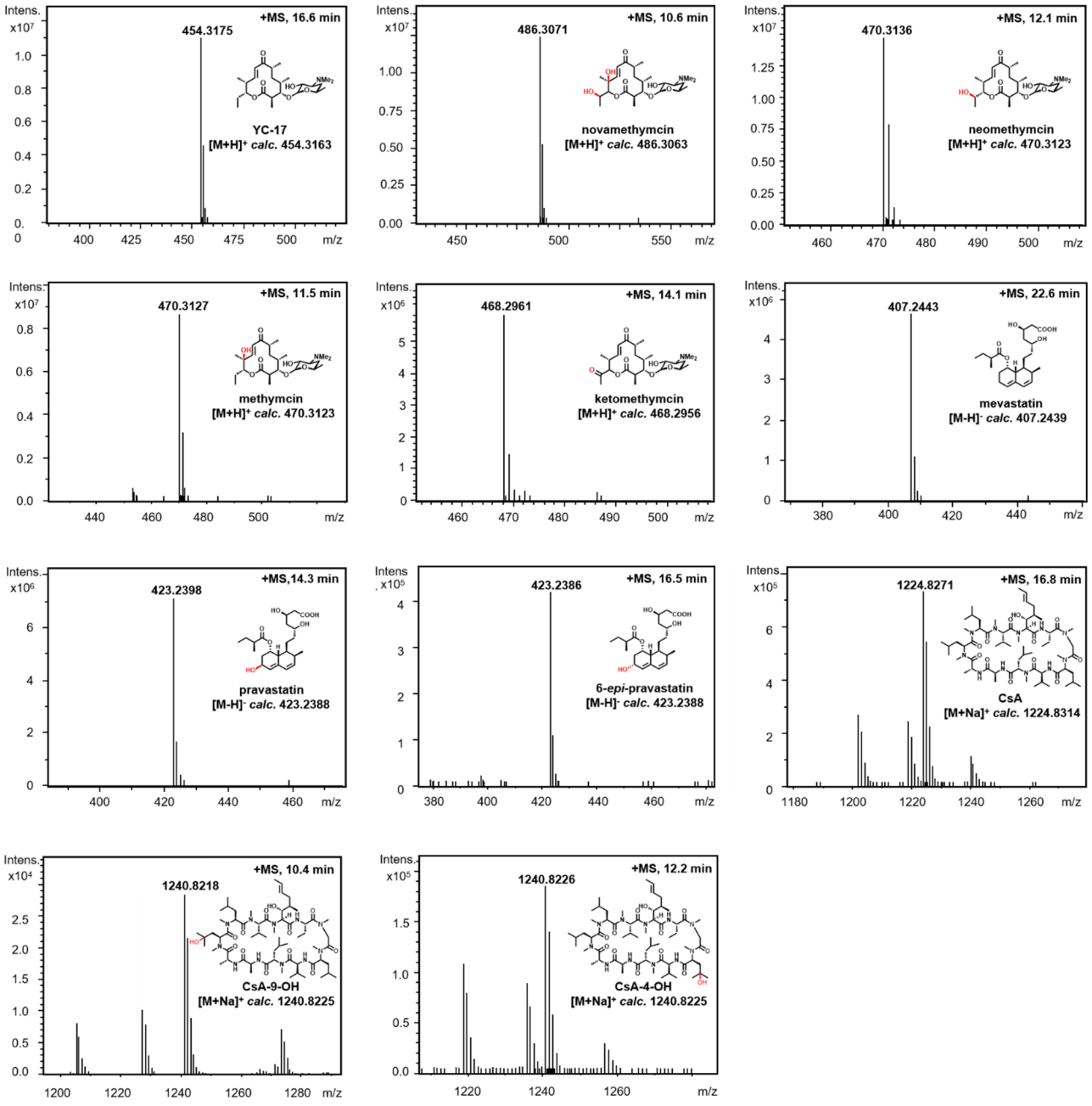
Supplementary Fig 3. UV/Vis absorption spectra of the selected redox partner proteins. (a) *Sel*FdR0978, (b) *Sel*Fdx1499, (c) AdR, (d) Adx, (e) PdR, and (f) Pdx.



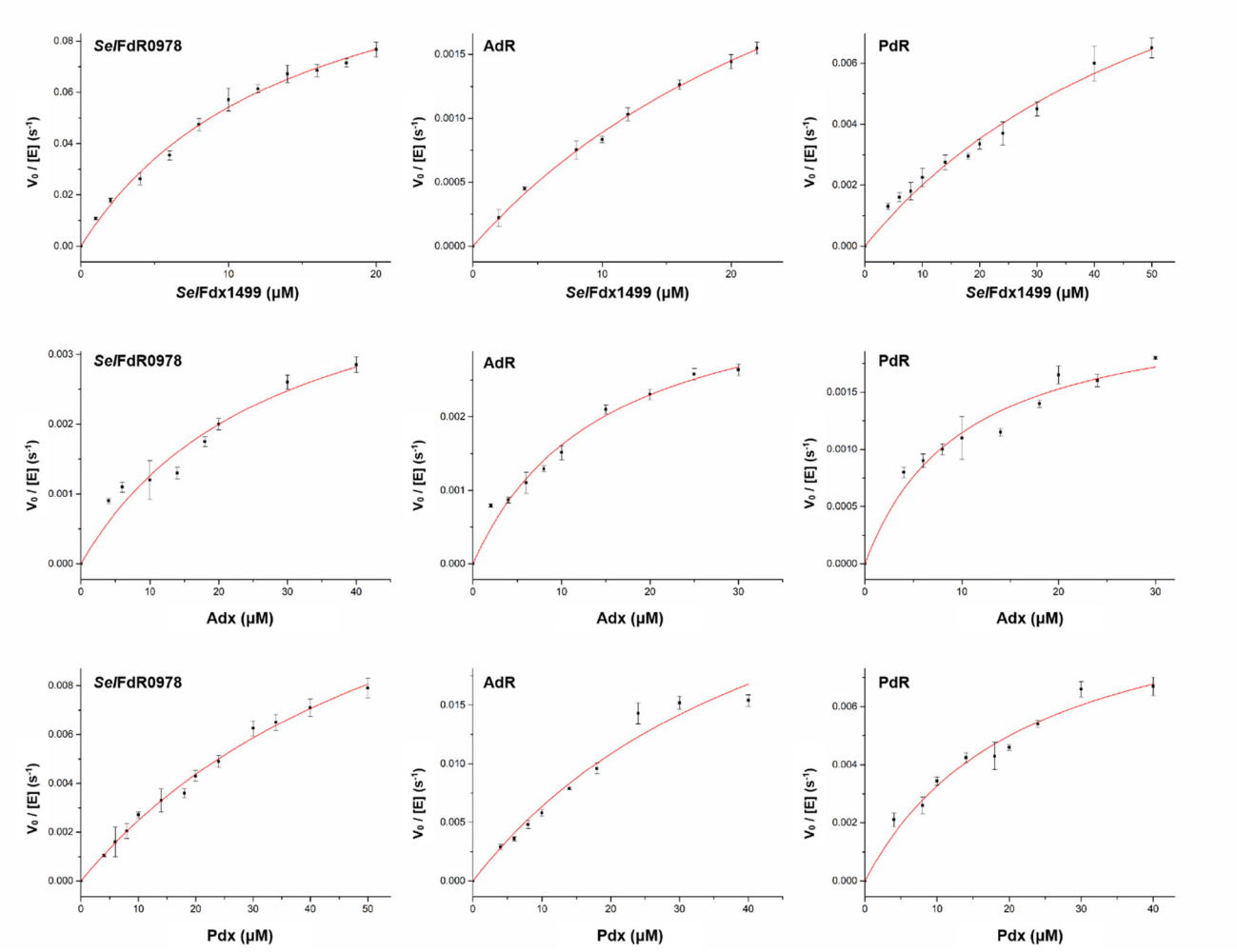
Supplementary Fig 4. HPLC analysis of three P450 enzymatic reactions. (a) PikC hydroxylates YC-17 (**1**) at either C10 or C12 position, giving rise to methymycin (**3**), neomethymycin (**4**), novamethymycin (**2**), and ketomethymycin (**5**). (b) P450sca-2 converts mevastatin (**6**) into pravastatin (**7**). (c) CYP-sb21 hydroxylates CsA (**9**) into CsA-9-OH (**10**) and/or CsA-4-OH (**11**) to different extents. A, *Sel*Fdx1499/*Sel*FdR0978; B, *Sel*Fdx1499/AdR; C, *Sel*Fdx1499/PdR; D, Adx/*Sel*FdR0978; E, Adx/AdR; F, Adx/PdR; G, Pdx/*Sel*FdR0978; H, Pdx/AdR; I, Pdx/PdR.



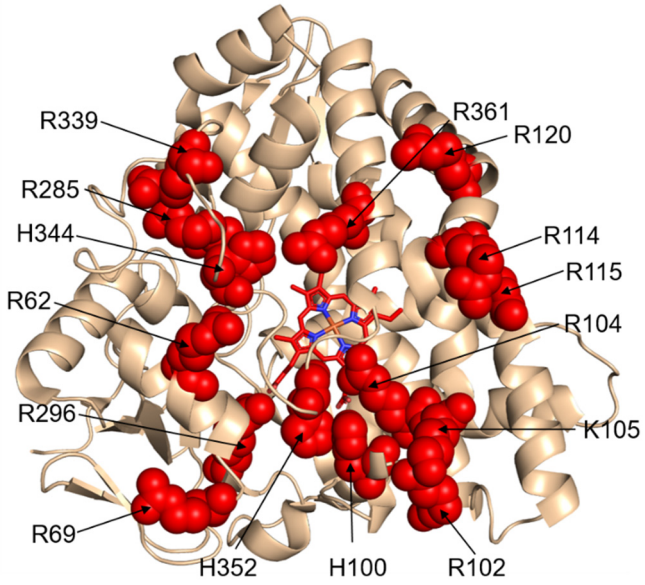
Supplementary Fig 5. Products distribution of PikC when coupled with 9 different combinations of RPs. The diagram shows the relative percentage of products distribution (%) obtained from the *in vitro* conversions of YC-17 by PikC in the presence of NAD(P)H recycling system.



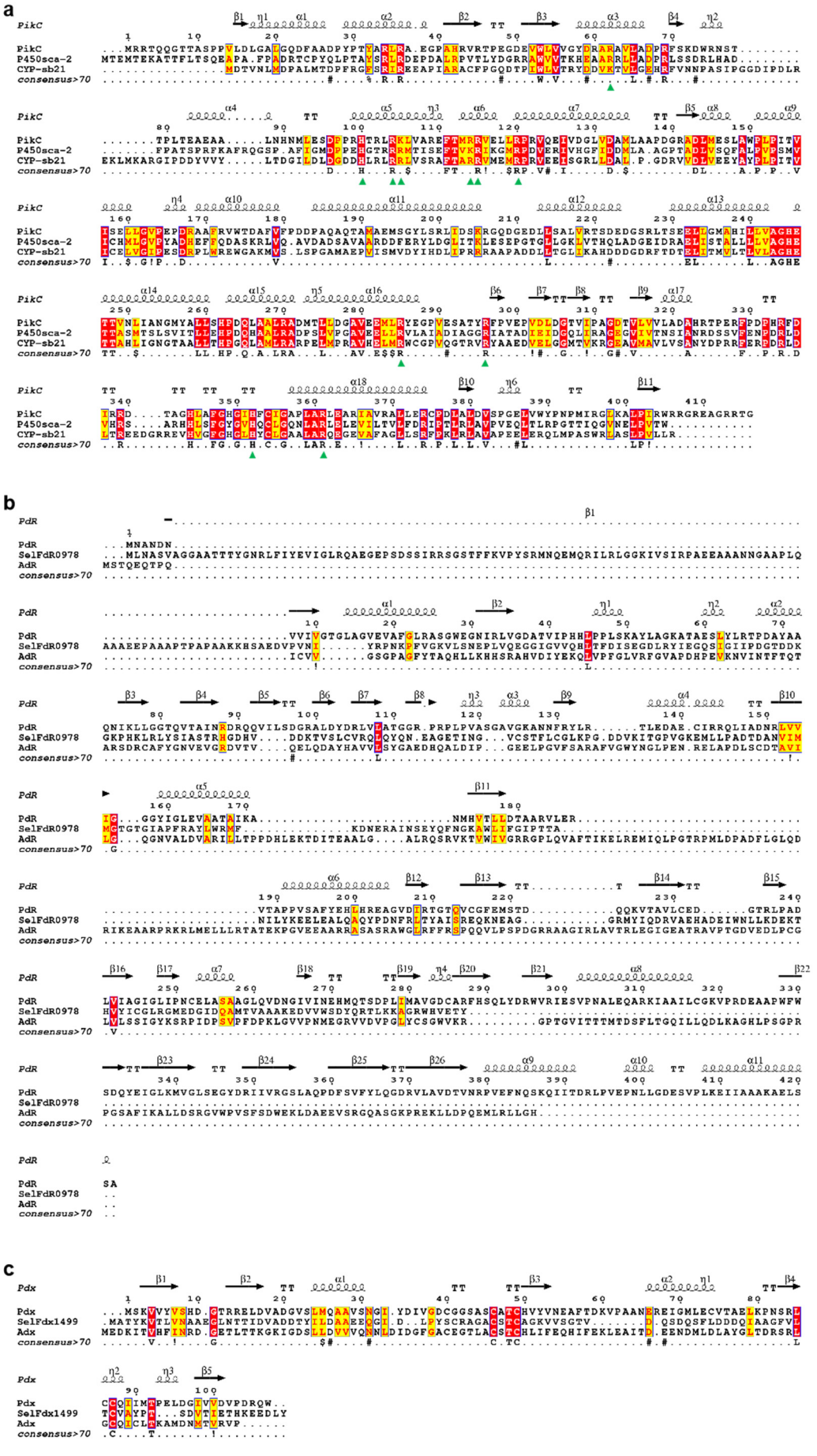
Supplementary Fig 6. High resolution mass spectra of the substrates and products associated with the three P450-mediated reactions.



Supplementary Fig 7. The steady-state kinetic curves of cytochrome *c* (cyt *c*) toward 9 pairs of RPs. Identification of electron transfer using cyt *c* as the final electron acceptor. The assays were carried out on a SpectraMax M2 plate reader (USA) at 30℃ by varying the concentrations of Fdxs in 50 mM sodium phosphate buffer (pH 7.4).



Supplementary Fig 8. The conservative basic amino acids on the proximal surface of the three P450 enzymes. Basic amino acids R, H or K in different P450s were mapped onto the proximal surface of PikC, which form a conservative positively charged surface.


a
PikC
PikC
P450sca-2
CYP-sb21
consensus>70
b
PdR
PdR
SelFdR0978
AdR
consensus>70
c
Pdx
Pdx
SelFdx1499
Adx
consensus>70

Supplementary Fig 9. Protein sequence alignment of the studied P450s (a), Fdxs (b) and FdRs (c). The secondary structure alignments are based on the models of PikC (PDB ID: 2BVJ), PdR (PDB ID: 1Q1R), and Pdx (PDB ID: 1VJI). The conservative basic amino acids R, H and K on the proximal surface of P450s are stamped by green triangles. Sequence alignment based on their tertiary structures was prepared using T-COFFEE online service [3,4] and the figure was output by ESPript 3.0 [5].

Supplementary Table 1. Strains and plasmids used in this study

***E. coli* strain or plasmid**	**Relevant characteristics**	**Reference/Source**
Strains		
DH5α	Cloning host	Life Technologies
BL21(DE3)	Expression host	Life Technologies
Plasmids		
pET28b-*pikC*	Plasmid pET28b harboring *pikC* gene	Wei Zhang et al. Z
pET28b-*P450sca-2*	Plasmid pET28b harboring *P450sca-2* gene	Wei Zhang et al. [6]
pET28b-*cyp-sb21*	Plasmid pET28b harboring *cyp-sb21* gene	Li Ma et al. [7]
pET28b-*selfdR*0978	Plasmid pET28b harboring *selfdR*0978 gene	Li Ma et al. [7]
pET28b-*selfdx*1499	Plasmid pET28b harboring *selfdx*1499 gene	Li Ma et al. [7]
pCwori^{+}-*adR*	Plasmid pCwori^{+} harboring *adR* (44-108) gene, added His-tag	This work
pET30a-*adx*	Plasmid pET30a harboring *adx* gene, added His-tag	This work
pET28b-*pdx*	Plasmid pET28b harboring *pdx* gene	Wei Zhang et al. [6]
pET19b-*pdR*	Plasmid pET19b harboring *pdR* gene	Wei Zhang et al.[6]

Supplementary Table 2. Primers used in this study

Oligonucleotide	Primer sequence (5'-3')	Function of underlined bases
Adx-F	CGGGGTACCATGGAAGATAAAATAACAGTCC	*Kpn* I
Adx-R	CCCTCGAGTTAAGGTACTCGAACAGTC	*Xho* I
AdR-F	GGAATTCCATATGAGCACTCAAGAACAAACTC	*Nde* I
AdR-R	CCCTCGAGGTGCCCCAGCAGCCGCAGCA	*Xho* I

References

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