Electronic Supplementary Information

Partial fusion of a cytochrome P450 system by carboxy-terminal attachment of putidaredoxin reductase to P450cam (CYP101A1)

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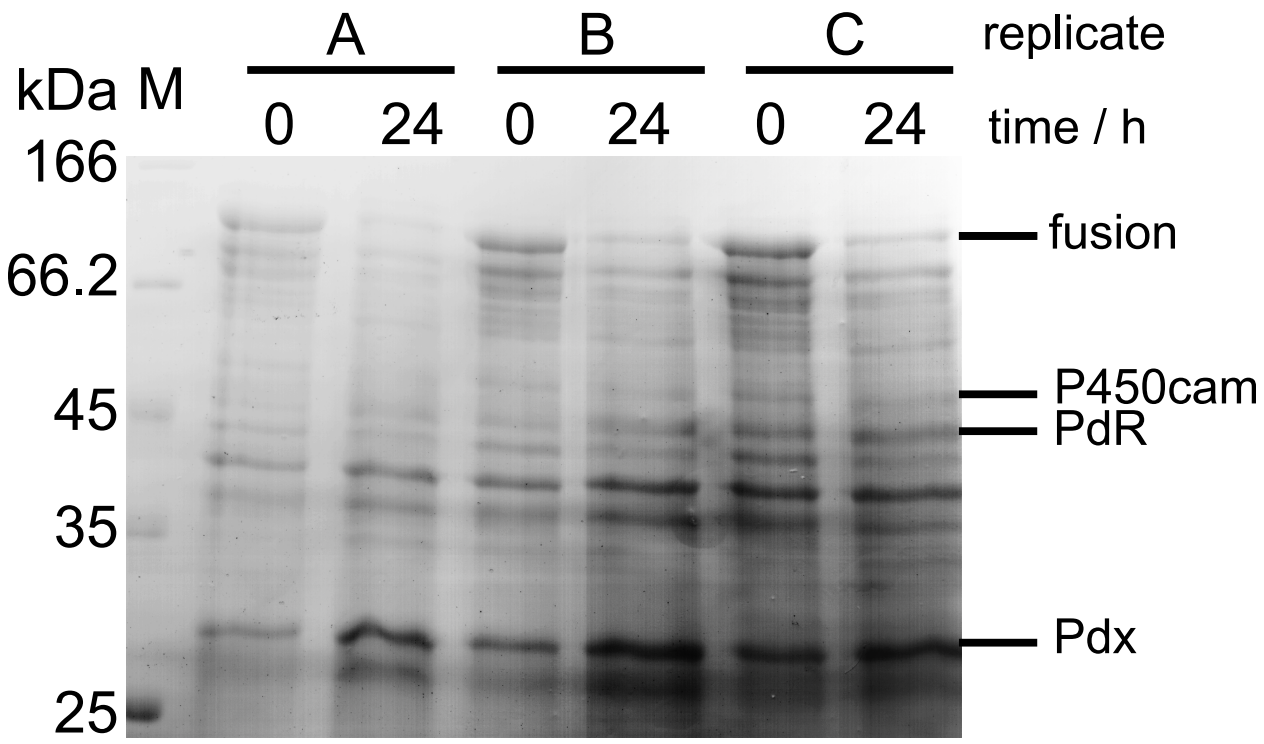

Fig. S1. Coomassie-stained SDS-PAGE analysis of fused P450cam-PdR expression during biotransformation of DL-camphor. This analysis indicated that all enzymes necessary to reconstitute P450cam monooxygenase activity were expressed, and that the fusion enzyme was present as an intact single polypeptide.

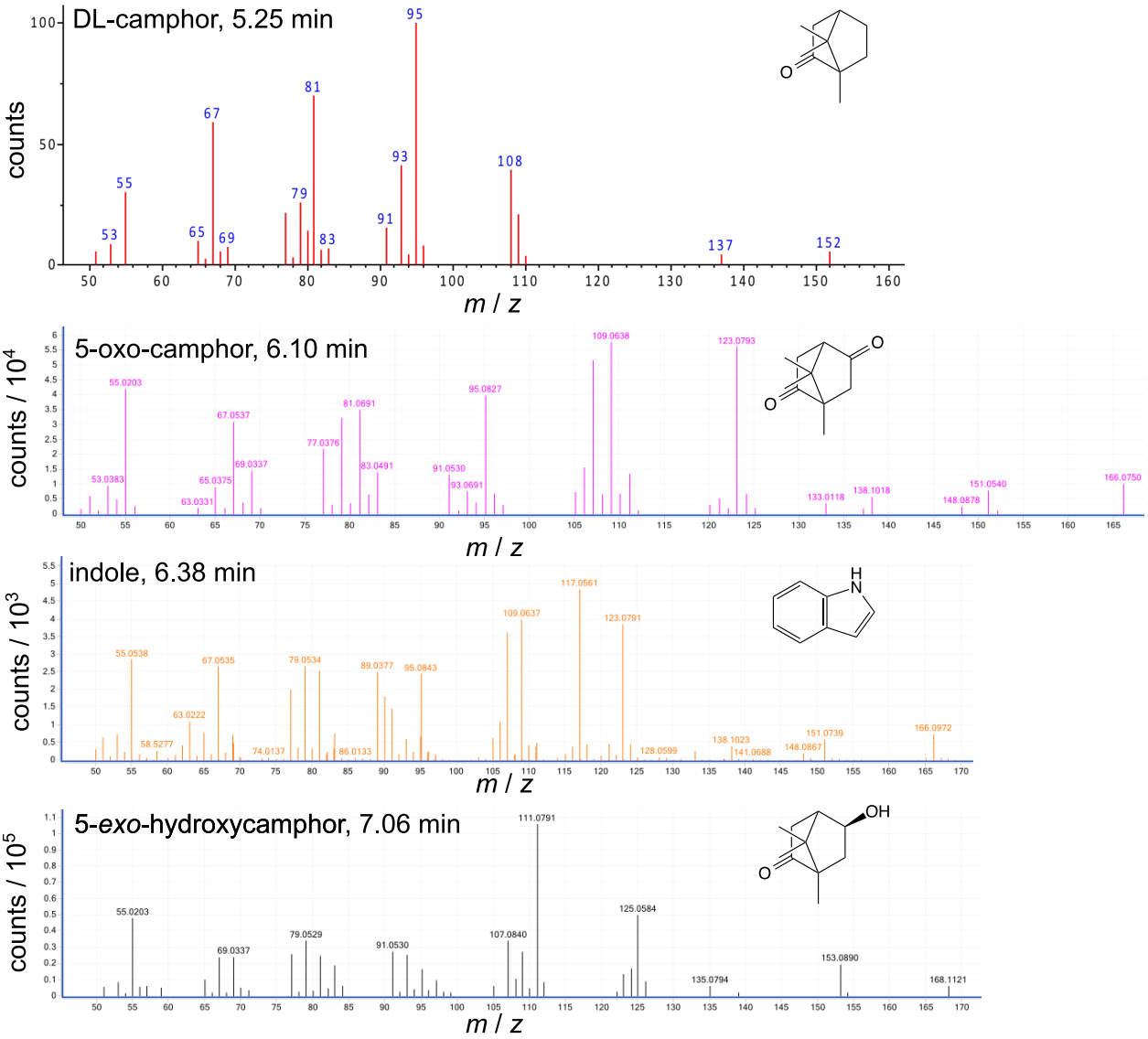

Fig. S2. Mass spectra of supernatant from whole cell biotransformation of DL-camphor, separated by gas chromatography. The identities of the DL-camphor substrate, the hydroxylation product (5-*exo*-hydroxycamphor), and further oxidation product (5-oxo-camphor**)** were confirmed by GC-MS. Another compound, unknown by GC analysis alone, was identified as indole by matching its mass spectrum with that in the NIST library. Spectra are labeled with compound name and chromatographic retention time on a 30 m DB-1 fused silica column.

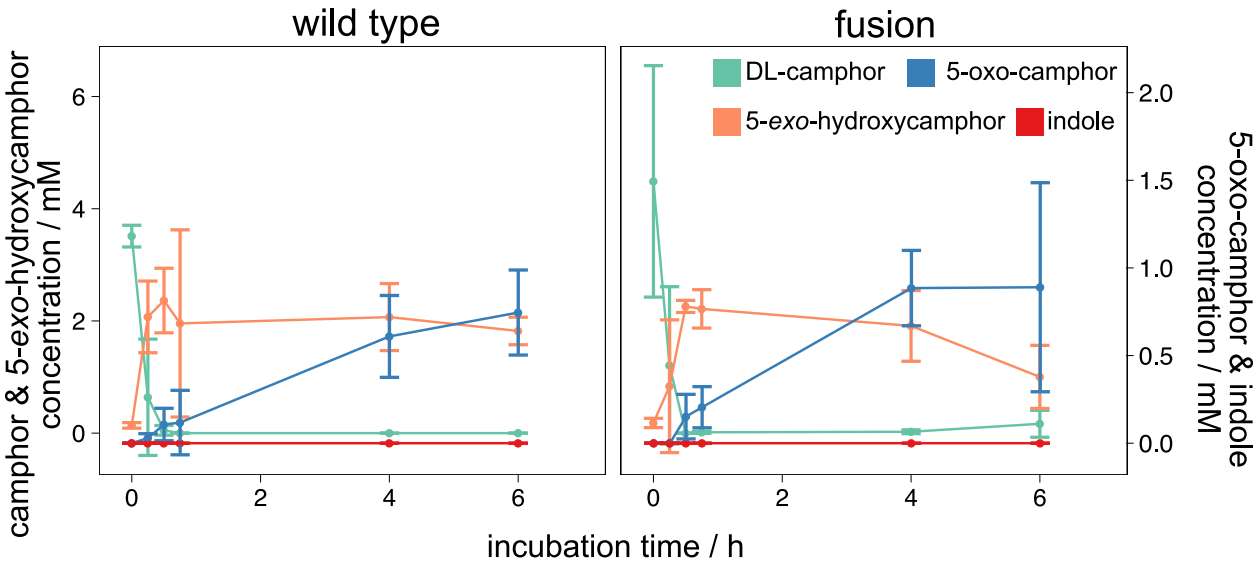

Fig. S3. *In vivo* activity of the fused P450cam-PdR system in $FB_{glycerol}$ medium. To test the camphor 5-hydroxylase activity of the fused P450cam-PdR system in the presence of Pdx, *E. coli* BL21 (DE3) were transformed with pFusionH2 or pRSFDuet::*camA*::*camC* and pUC18::*camB* and induced in $FB_{glycerol}$, a minimal medium, in the presence of 40 µM IPTG and 1 mM DL-camphor. GC analysis was performed on ethyl acetate extracts of culture supernatant (three biological replicates) at several time points until 24 h. The abundance of DL-camphor, 5-*exo*-hydroxycamphor, 5-oxo-camphor, and indole were followed over time. The results showed that, unlike in terrific broth (a rich medium) no build-up of indole occurred and, in sharp contrast, the fusion system showed a greater extent of further oxidation to form 5-oxo-camphor than the native system.

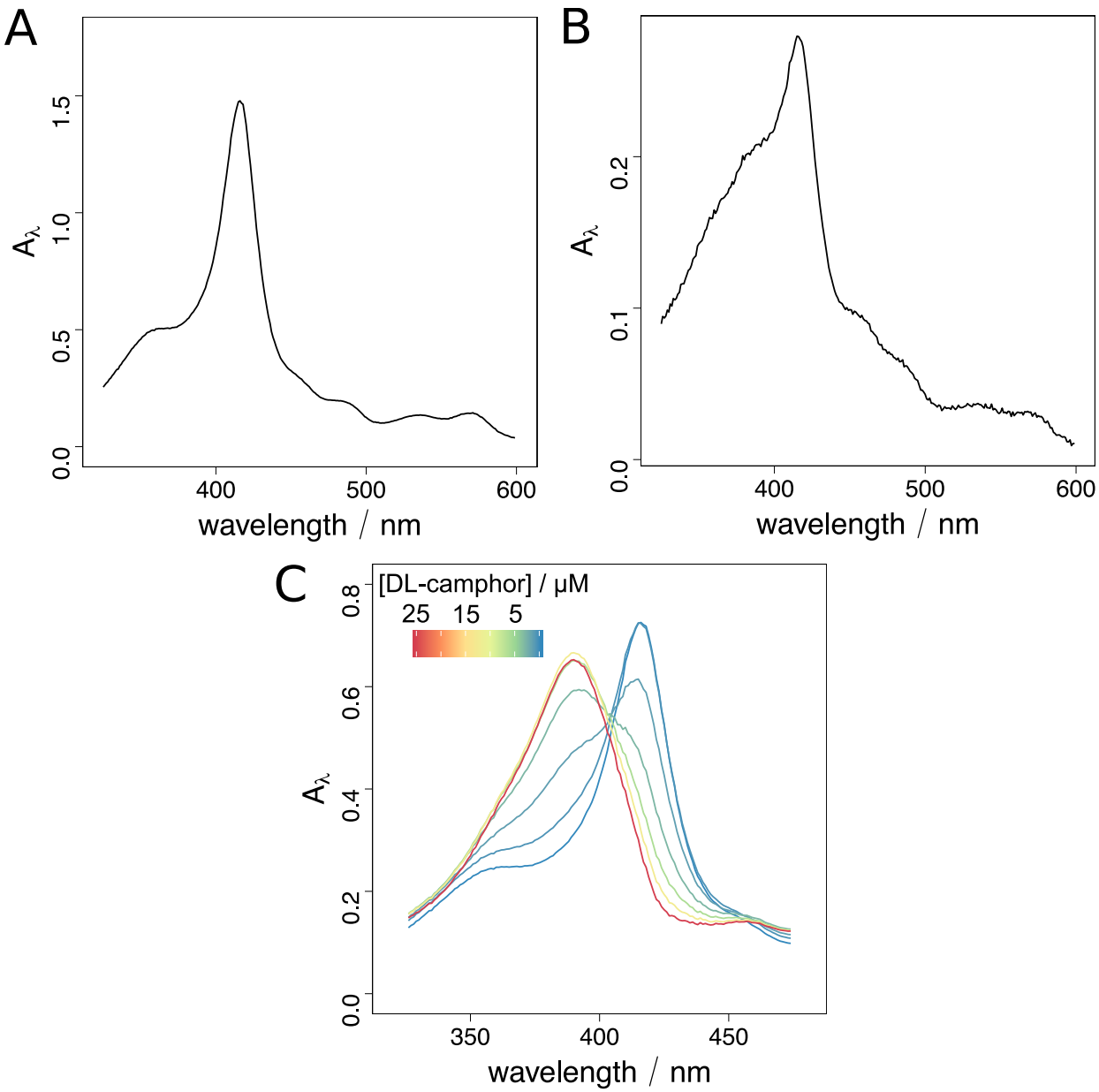

Fig. S4. UV-visible spectra of the fused P450cam-PdR enzyme in the presence and absence of camphor. In the absence of camphor, the enzyme's heme iron is in its low-spin state and presents an electronic spectrum (A) with a characteristic major band at 419 nm. When even trace camphor is present in solution, such as immediately after FPLC purification (B), a band at 390 nm is observed, indicating the displacement of the heme axis water ligand by the substrate and a shift to the high-spin state. Titration of camphor (C) results in a dose-dependent increase in the intensity of the 390 nm band.

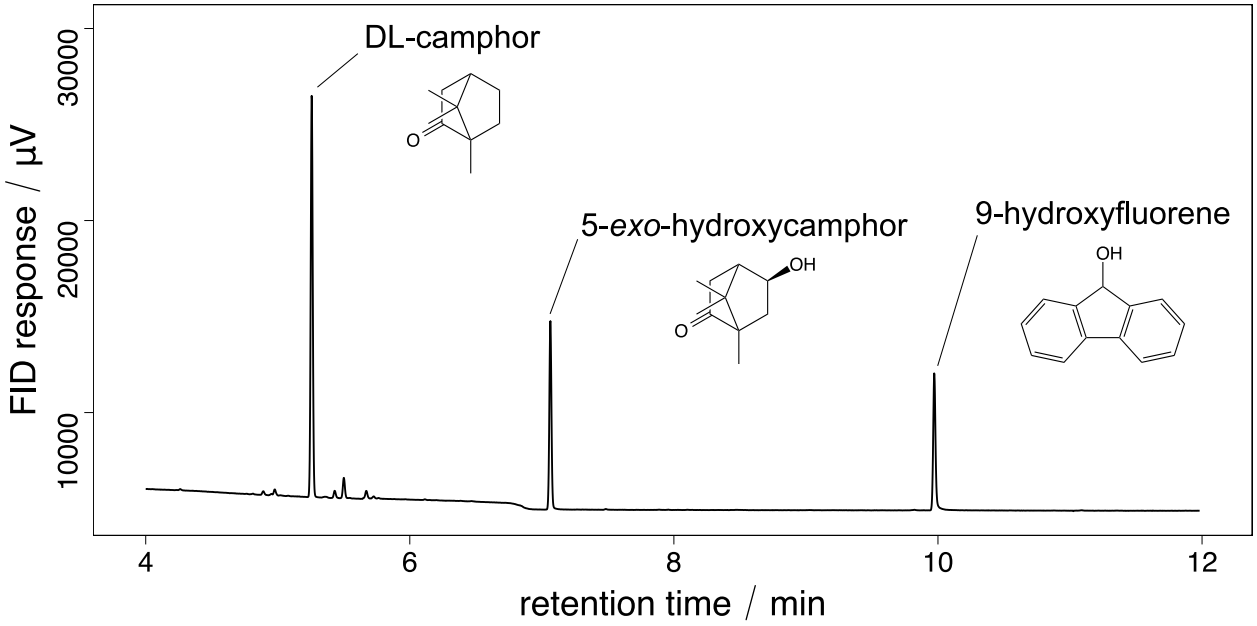

Fig. S5. Representative GC analysis of products of camphor oxidation catalyzed by the fused P450cam-PdR system *in vitro*. The peak integral of the 5-*exo*-hydroxycamphor product was compared to that of the 9-hydroxyfluorene internal standard to obtain an estimate of coupling of NADH consumption to product formation as mediated by the fused P450cam-PdR system. Analysis was carried out in triplicate. FID = Flame ionization detector.

Table S1. Primers and oligonucleotide sequences used in this work. Restriction sites are underlined; linker regions are italicized.

Name	Sequence
camA_F	taa att gag ctc aac gca aac gac aac gtg gtc atc gtg
camC_R	tta att gag ctc *ggt ttt ttt cgc cgc* tac cgc ttt ggt ag
hemH_F	tta ata cat atg atg cgt cag act aaa acc ggt atc ctg
hemH_R	aat aat ctc gag tta gcg ata cgc ggc aac aag att agc
pFusion_F	*ggc gcc ttc cgg* gag ctc atg aac gca aac gac aac gtg gtc atc
pFusion_R	*c gga ctg ctc ggt gga* gac gtc tac cgc ttt ggt agt cgc cgg
L2_F	gac gtc *tcc acc gag cag tcc gcc aag gag gcc ccc gcc gag acc ctg ggc gcc ttc cgg* gag ctc
L2_R	gag ctc *ccg gaa ggc gcc cag ggt ctc ggc ggg ggc ctc ctt ggc gga ctg ctc ggt gga* gac gtc