Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary information

Whole-cell screening of oxidative enzymes using genetically-encoded sensors

Tsvetan Kardashliev[a], Alexandra Weingartner[b], Elvira Romero[c], Ulrich Schwaneberg[b], Marco W. Fraaije[c], Sven Panke[a] and Martin Held[a]*

[a]Department of Biosystems Science and Engineering, ETH Zurich, Mattenstrasse 26, 4058 Basel, Switzerland
[b]Institute of Biotechnology, RWTH Aachen University, Worringerweg 3, 52074 Aachen, Germany
[c]Faculty of Science and Engineering, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
** Corresponding author: Martin Held, e-mail: heldma@ethz.ch*

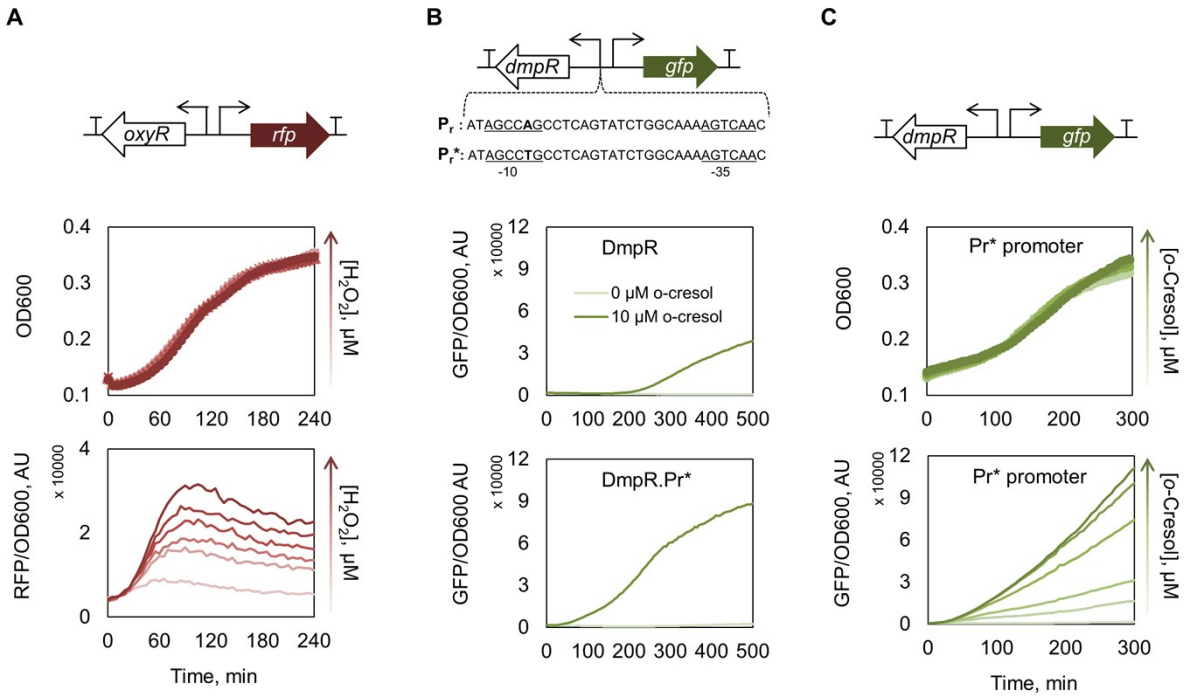

Figure S1 Basic characterization of genetically-encoded sensors for detection of hydrogen peroxide and phenolic compounds. A) Growth and normalized fluorescence curves for *E. coli* BW25113 [pOxyR_*rfp*] in the presence of between 0 and 100 μM exogenously added H_2O_2. B) Comparison of the fluorescence output of DmpR_*gfp* using either the native dmpR promoter P_r or the genetically engineered variant P_r* in the presence of 10 μM *o*-cresol. C) Growth and normalized fluorescence of *E. coli* BL21/pDmpR_*gfp* in the presence of 0 – 100 μM exogenously added *o*-cresol.

Table S1 Partial DNA sequences of constructs pOxyR_*gfp*, pOxyR_*rfp* and pDmpR_*gfp* spanning genes encoding transcriptional regulators marked in red, promoter regions marked in grey, genes encoding reporter proteins marked in green and bidirectional terminator sequences in yellow.

	DNA Sequences
OxyR_*gfp*	ATTTAAATCGTAATTATTGGGGACCCCTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGT CATTACTGGATCTATCAACAGGAGTCCAAGACTAGTTTAAACCGCCTGTTTTAAAACTTTATCGAAATGGCCATCCATTCTTGCGCGGATGGCCTCTGCCA GCTGCTCATAGCGGCTGCGCAGCGGTGAGCCAGGACGATAAACCAGGCCAATAGTGCGGCGTGGTTCCGGCTTAATGCACGGCAGATAAACAACCCCA TCGCGTTTGCGCTCCGGCGGCACAGCCAGCGCTGGCAGTAAAGTGATCCCGCTACCTGCCGCCACCATGTTGCGCAGAGTTTCCAGGCTGGTCGCGCG GAAGTGTGTATCTTCATCCGCCCCGGCTTCAAAACAGAAACCCATTGCCTGATCGCGCAAACAGTGACCATCTTCCAGCATCAGCAGTTTTTCCCCTGCCA GATCGGCCATCGGTACGCATTCGCGGTTCGCCCACGGGTGATCTTCATAGATAGCCAGCAACATTGGCTCATCAAACAACGGCACTTCAATGAATGCTTC GCTCTCTTTCACCAGCGCGAGGATCACGCAATCGAGTTTGCCGCTGTCCAGTTGCGCCAGTAACTGGTGGGTCTGTGCTTCATGCAGATACATTTCCAGC TTTGGAAAGGTCTGGTGCAGCATAGGGATAATATGCGGTAGCAGGTACGGTCCAACTGTGGGAATCAAACCAATGTGCAGCGGTCCGGACATCGTCTC GCCCTGCTGGCTTGCCATCTCTTTAAGGACTTTCACCTCACGCAGCACGGTACGCGCCTGATCCACCAGCAGCATTCCCGCCTGGGTGAACAACACTTTA CGGCTGGTCCGCTCCAGCAACATCACGCCCAGCTCATCTTCCAGCTTACGAATTTGCCCGCTAAGCGTCGGCTGGCTAACGTGGCAGGAATCTGCCGCA CGCCGAAAATGGCGGTGTTCAGCCAATGCCACCAGGTACTCAAGATCACGAATATTCATTATCCATCCTCCATCGCCACGATAGTTCATGGCGATAGGTA GAATAGCAATGAACGATTATCCCTATCAAGCATTCTGACTGATAATTGCTCACAGCAGAATTCACACTTTACTTTTTAATTAGCAGGAGATTTAACATATG AGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGAGGAGAGGGTGAA GGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTATGGTGT TCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAA GATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGA AACATTCTCGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTC GCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCT GTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGAT GAGCTCTACAAAGGATCCTAATTAATTAAAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAA CGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACAGCTG

OxyR_*rfp*

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DmpR_*gfp*

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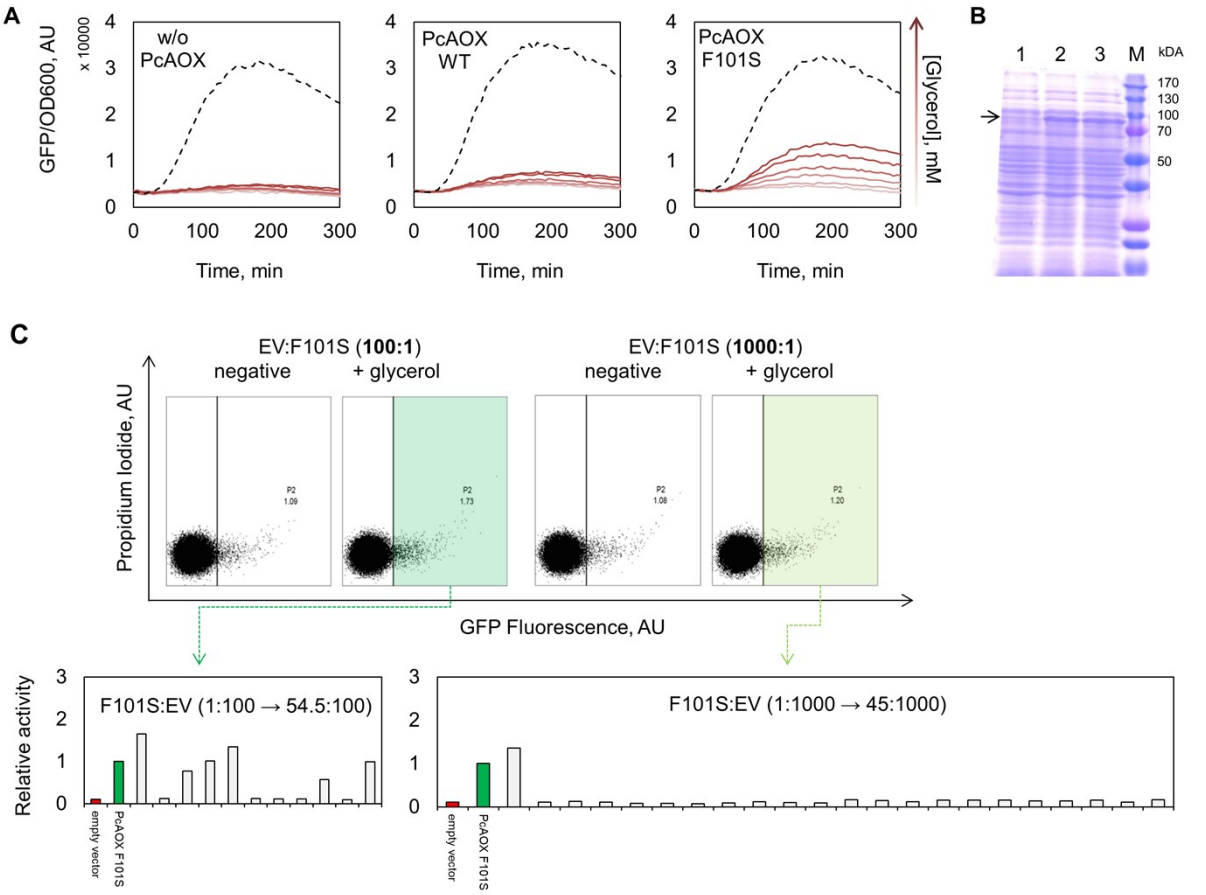

Figure S2. Whole-cell measurement of enzymatically produced hydrogen peroxide by alcohol oxidase, PcAOX. A) Specific fluorescence recorded over time for *E. coli* ΔglpK [pOxyR_gfp] expressing PcAOX variants in the presence of 0–500 mM glycerol. Dashed lines represent the fluorescent signal recorded for the same culture in the presence of 100 µM H_2O_2. B) SDS-PAGE of expression cultures (whole cell lysates) used for *in vivo* activity assays. Lane 1: *E. coli* ΔglpK [pOxyR_gfp, pBAD empty vector]. Lane 2: *E. coli* ΔglpK [pOxyR_gfp] + [pBAD_pcaox_WT]. Lane 3: *E. coli* ΔglpK [pOxyR_gfp, pBAD_pcaox_F101S], M – molecular size marker. Target region and band is marked with an arrow. C) Flow cytometric sorting of model libraries comprised of empty vector cells and cells synthesizing PcAOX F101S mixed at predefined ratios and re-screening of isolates from gate P2 in MTP.

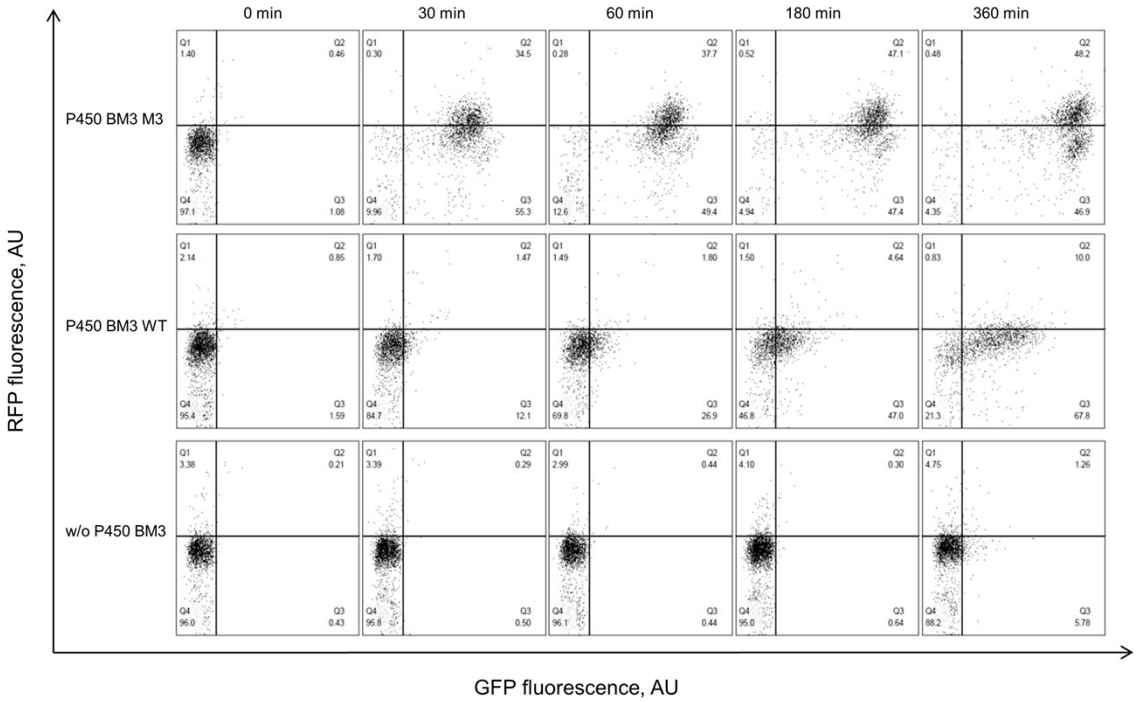

Figure S3 Flow cytometry detection of cytochrome P450 activity. The fluorescence intensity of RFP and GFP in whole-cell biocatalysts expressing P450 BM3 monooxygenases (P450 BM3 WT and M3) is triggered by *o*-cresol and H_2O_2. Fluorescence signals were recorded over 360 min by flow cytometry and the data (10'000 events) are presented in dot plots split in 4 quadrants. The fraction (in %) of the total recorded events that fall in each quadrant is indicated in the figure.

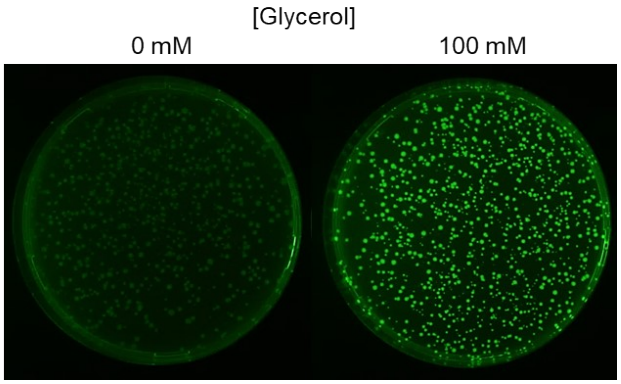

Figure S4 Survival of recombinant *E. coli* assayed on solid support*. E. coli* Δ*glpK* [pOxyR_*gfp*, pBAD_*pcaox*_F101S] incubated on agar plates in the absence of glycerol (left) and with 100 mM glycerol (right). The agar plates were seeded with roughly 10^3 CFUs and no reduction in the number of colony forming units was observed the presence of the glycerol acting as a substrate of PcAOX.

Table S2 Amino acid sequence analysis of PcAOX muteins identified in this study, based on DNA sequence analysis of corresponding genes. Silent mutations are marked with an asterisk (*).

PcAOX	Amino acid substitutions
A9	F101S H632Q
B9	F101S A41V R321H
C8	F101S A18A* L132L* G255R T316A K569R
D11	F101S T69T* L93M
E1	F101S H170N R224S H637H*
E8	F101S G14S A151S T252I L343L* K390R P570A L601M E622D
G12	F101S G119G* L281M Q294H I434T I620I*

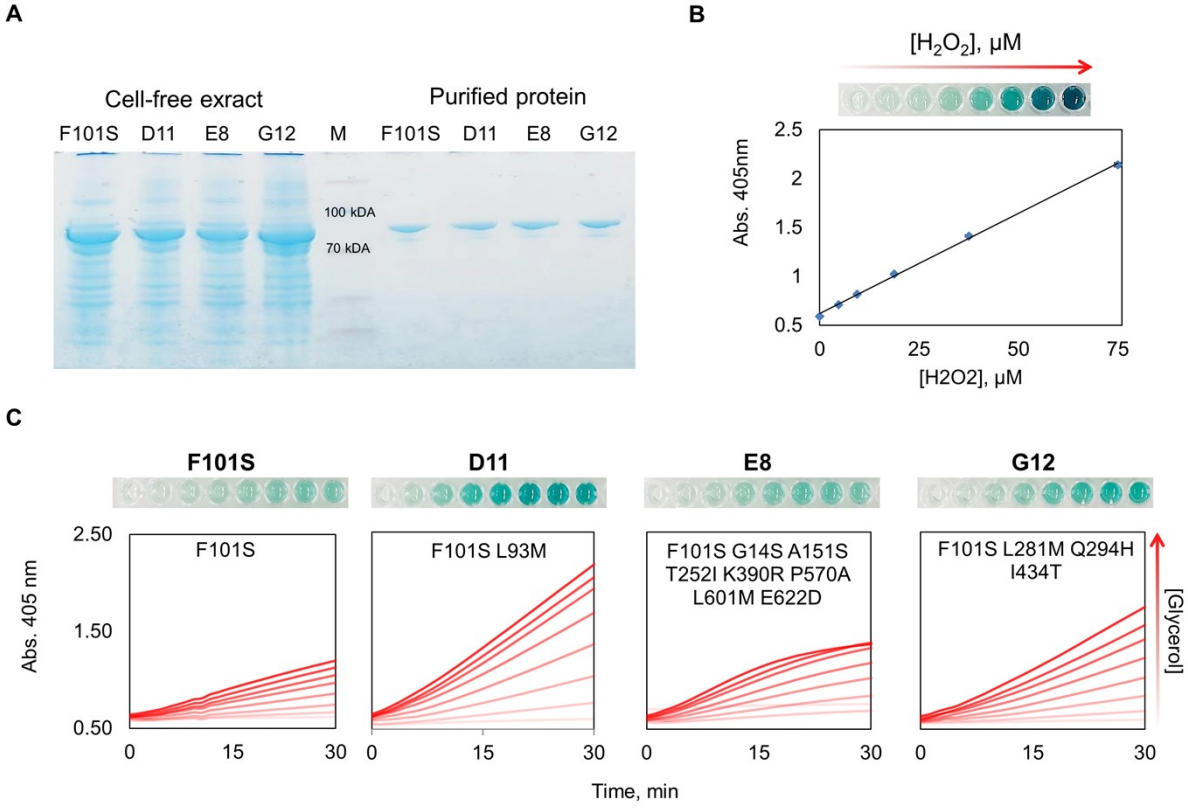

Figure S5. *In vitro* characterization of PcAOX variants. A) Purification of PcAOX variants. B) ABTS/HRP-assay signal in response to various concentrations of H_2O_2. C) Progress curves of ABTS/HRP-coupled oxidase assays carried out with equimolar concentrations of purified PcAOX variants and glycerol added at concentration ranging between 0 and 1.5 M.

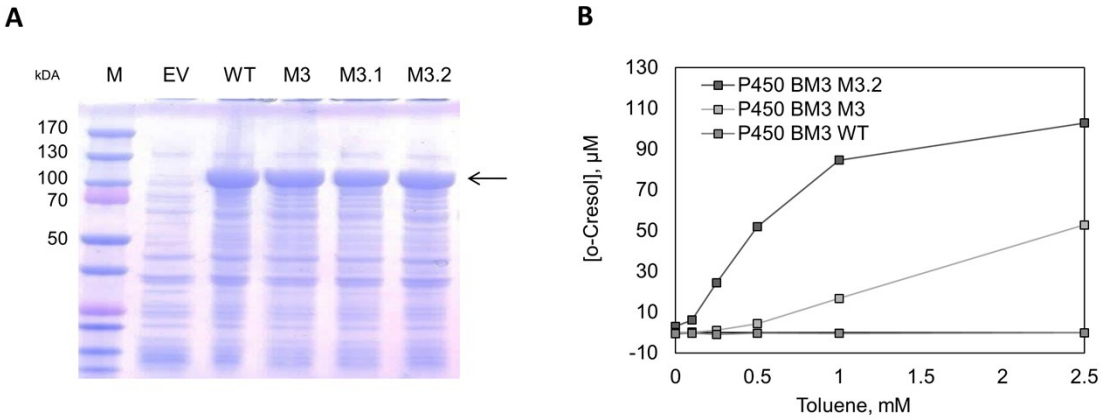

Figure S6 Characterization of P450 BM3 variants. A) SDS-PAGE analysis of *E. coli* BL21 Gold (DE3) lacI^{q1} equipped with [pOxyR_*rfp*] and [pDmpR_*gfp*] expressing either wildtype P450 BM3 (WT) or variants thereof (M3, M3.1 and M3.2). EV: Empty vector control. B) Measurement of *o*-cresol production by *E. coli* BL21 Gold (DE3) lacI^{q1} equipped with pOxyR and pDmpR and expressing P450 BM3 variants. Product quantification in the medium after 3 h reaction in MTP was achieved using the 4-AAP colorimetric assay following published protocols.[1,2]

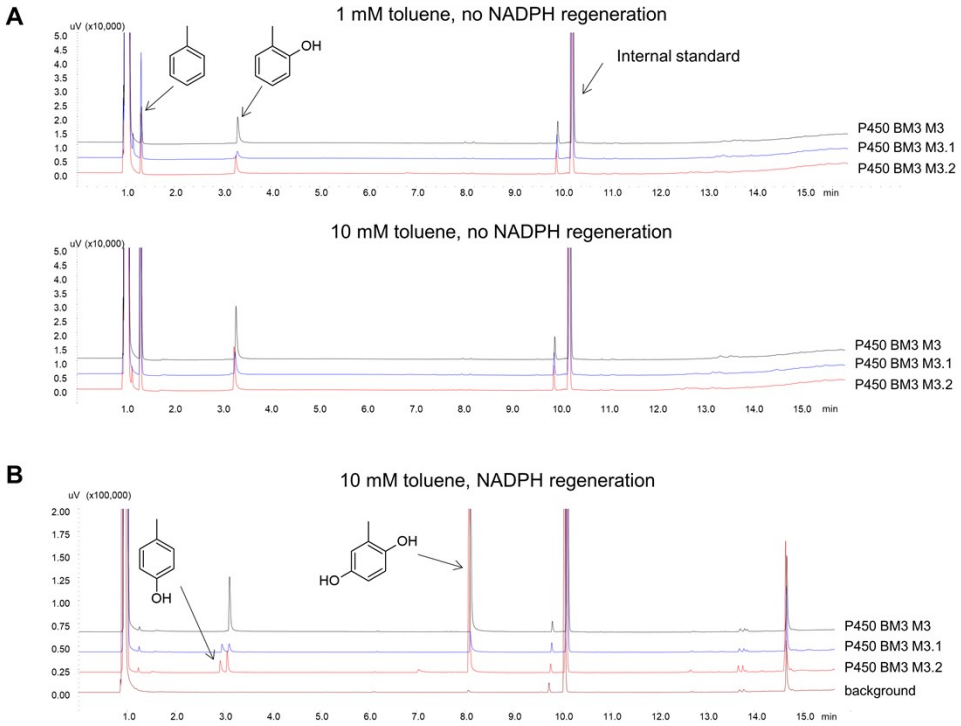

Figure S7 Gas chromatogram traces of toluene hydroxylation products formed by P450 BM3 muteins *in vitro*. The biotransformations were carried at out at initial toluene concentrations of 1 and 10 mM without (A) and with cofactor regeneration (B). The reaction products were analyzed either immediately after depletion of the NADPH cofactor (A) or after 24 h (B). Similarly to its parent P450 BM3 M3, mutant M3.2 produced 1,4-hydroqionone as an overoxidation product. Unlike M3, mutant M3.2 also produced a second monohydroxylated product (likely, *p*-cresol) as a side product.

Table S3 List of Chemicals

Chemical name	CAS Number	Supplier
2-Methylphenol (*o*-cresol)	95-48-7	Sigma-Aldrich
Agar	9002-18-0	Applichem
Agarose	9012-36-6	VWR
Aminolevulinic acid hydrochloride	5451-09-2	Sigma Aldrich
Ammonium Chloride (NH_4Cl)	12125-02-9	Roth
Ampicillin sodium salt	69-52-3	Sigma-Aldrich
Calcium chloride dihydrate ($CaCl_2*H_2O$)	10035-04-8	Merck
Casamino acids	n/a	BD BactoTM
D(+)-galactose	59-23-4	Sigma-Aldrich
D(+)-glucose	50-99-7	Sigma-Aldrich
D(+)-maltose	6363-53-7	Sigma-Aldrich
Dipotassium hydrogen phosphate (K_2HPO_4)	7758-11-4	Roth
Disodium hydrogen phosphate (Na_2HPO_4)	7558-79-4	Roth
Dimethyl sulfoxide (DMSO)	67-68-5	Sigma-Aldrich
Ethanol	64-17-5	Sigma-Aldrich
Ethylenediaminetetraacetic acid disodium salt (EDTA)	6381-92-6	Chemie Brunschwig AG
Glycerol	56-81-5	Applichem
Hydrogen peroxide solution (H_2O_2)	7722-84-1	Sigma-Aldrich
Isopropyl-D-thiogalactopyranoside (IPTG)	367-93-1	Sigma-Aldrich
Kanamycin sulfate	25389-94-0	Sigma-Aldrich
L(+)-arabinose	9328-37-0	Sigma-Aldrich
L-aspartic acid	56-84-8	Sigma-Aldrich
L-glutamine	56-85-9	Sigma-Aldrich
L-methionine	63-68-3	Sigma-Aldrich
L-serine	56-45-1	Sigma-Aldrich
L-tyrosine	60-18-4	Sigma-Aldrich
Magnesium sulfate heptahydrate ($MgSO_4*7H_2O$)	10034-99-8	Roth
Potassium dihydrogen phosphate (KH_2PO_4)	7778-77-0	Roth
Sodium chloride (NaCl)	7647-14-5	Applichem
Sodium L-lactate	867-56-1	Fluka
Streptomycin sulfate	3810-74-0	HUBERLAB.AG
Thiamine hydrochloride	67-03-8	Sigma-Aldrich
Toluene	108-88-3	Fluka
Tryptone	n/a	Becton Dickinson

Table S4 List of strains

Strain	Application	Source/ Reference
E. coli DH5α	Cloning strain	Thermo Fischer Scientific
E. coli CloneCatcher™ Gold DH5G	Cloning strain	Genlantis
E. coli Top10	Expression of PcAOX	Thermo Fischer Scientific
E. coli BL21 (DE3) $lacI^{q1}$	Expression for P450 BM3 variants and whole cell activity assay	Blanusa et al.[3]
E. coli B3926 (Δ*glpK*)	KEIO strain; whole cell activity assay strain for PcAOX	Baba et al.[4]

Table S5 List of plasmids

Plasmid name	Ori	Resistance	Insert	Reference
pALXtreme-1a	pBR322	Kanamycin	T7 promoter; multiple cloning site	Blanusa et al.[3]
pALXtreme-1a-*p450_bm3*_WT	pBR322	Kanamycin	T7 promoter; P450 BM3 wildtype	Blanusa et al.[3]
pALXtreme-1a- *p450_bm3*_M3	pBR322	Kanamycin	T7 promoter; P450 BM3 M3	Dennig et al.[5]
pALXtreme-1a-*p450_bm3*_M3.1	pBR322	Kanamycin	T7 promoter; P450 BM3 M3.1	This study
pALXtreme-1a-*p450_bm3*_M3.2	pBR322	Kanamycin	T7 promoter; P450 BM3 M3.2	This study
pBAD	ColE1	Ampicillin	araBAD promoter; multiple cloning site	Invitrogen
pBAD-*pcAOX*_WT	ColE1	Ampicillin	araBAD; His-SUMO-PcAOX wildtype	Nguyen et. al.[6]
pBAD-*pcAOX*_F101S	ColE1	Ampicillin	araBAD; His-SUMO-PcAOX F101S	Nguyen et. al.[6]
pBAD-*pcAOX*_D11	ColE1	Ampicillin	araBAD; His-SUMO-PcAOX variant D11	This study
pBAD-*pcAOX*_E8	ColE1	Ampicillin	araBAD; His-SUMO-PcAOX variant E8	This study
pBAD-*pcAOX*_G12	ColE1	Ampicillin	araBAD; His-SUMO-PcAOX variant G12	This study
pDmpR-*sfgfp*	p15A	Ampicillin	Pr promoter; *dmpR;* Po promoter ; *sfgfp*	This study
pDmpR*-*sfgfp*	p15A	Ampicillin	Pr* promoter; *dmpR;* Po promoter ; *sfgfp*	This study
pOxyR-*sfgfp*	pSC101	Streptomycin	P_{oxyR} promoter; *oxyR;* P_{oxyS} promoter ; *sfgfp*	This study
pOxyR-*rfp*	pSC101	Streptomycin	P_{oxyR} promoter; *oxyR;* P_{oxyS} promoter ; *mcherry*	This study

Supplementary References

1 N. Lülsdorf, L. Vojcic, H. Hellmuth, T. T. Weber, N. Mußmann, R. Martinez and U. Schwaneberg, *Appl. Microbiol. Biotechnol.*, 2015, **99**, 5237–5246.

2 T. S. Wong, N. Wu, D. Roccatano, M. Zacharias and U. Schwaneberg, *J. Biomol. Screen.*, 2005, **10**, 246–252.

3 M. Blanusa, A. Schenk, H. Sadeghi, J. Marienhagen and U. Schwaneberg, *Anal. Biochem.*, 2010, **406**, 141–146.

4 T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner and H. Mori, *Mol. Syst. Biol.*, 2006, **2**, 2006.0008.

5 A. Dennig, N. Lülsdorf, H. Liu and U. Schwaneberg, *Angew. Chem. Int. Ed.*, 2013, **52**, 8459–8462.

6 Q.-T. Nguyen, E. Romero, W. P. Dijkman, S. P. de Vasconcellos, C. Binda, A. Mattevi and M. W. Fraaije, *Biochemistry*, 2018, **57**, 6209–6218.