

Supplementary Materials

Enantioselective Epoxidation by Flavoprotein Monooxygenases Supported by Organic Solvents

Enantioselective Epoxidation by Flavoprotein Monooxygenases Supported by Organic Solvents

Daniel Eggerichs ¹, Carolin Mügge ¹, Julia Mayweg ¹, Ulf-Peter Apfel ^{2,3} and Dirk Tischler ^{1,*}

¹ Microbial Biotechnology, Faculty of Biology and Biotechnology, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany; daniel.eggerichs@rub.de (D.E.); carolin.muegge@rub.de (C.M.); julia.mayweg@gmail.com (J.M.)

² Activation of Small Molecules, Faculty of Chemistry and Biochemistry, Ruhr-Universität Bochum, Universitätsstr 150, 44780 Bochum, Germany; ulf.apfel@rub.de

³ Fraunhofer UMSICHT, Division of Energy, Osterfelder Strasse 3, 46047 Oberhausen, Germany

* Correspondence: dirk.tischler@rub.de; Tel.: +49-234-32-22656

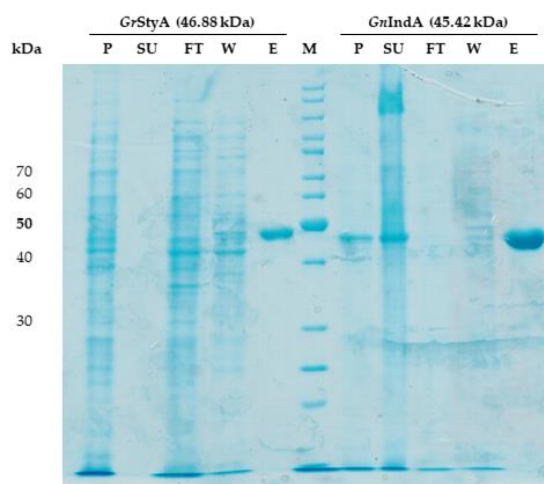


Figure S1. SDS gel of the protein production. Abbreviations: P: pellet, SU: supernatant, FT: flow through, W: wash, E: elution. Marker: Thermo Scientific unstained PageRuler (Lot #26614).

Table S1. Organic solvents used to optimize the enzymatic epoxidation of styrene. The solvents are ordered by their hydrophilicity in terms of their $\log P_{O/W}$ value.

Solvent (abbreviation)	$\log P_{O/W}$ ^a
Dimethyl sulfoxide (DMSO)	-1.35
Dimethyl formamide (DMF)	-1.01
Methanol (MeOH)	-0.77
Acetonitrile (AcN)	-0.34
Ethanol (EtOH)	-0.31
Acetone (-)	-0.24
2-Propanol (-)	+0.05
1-Propanol (-)	+0.25

Tetrahydrofuran (THF) +0.46

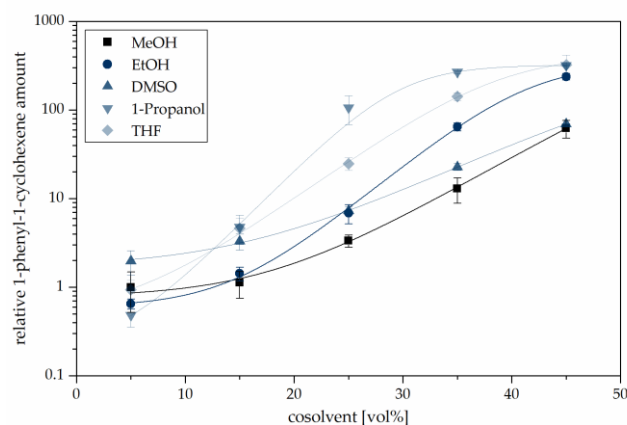
^a taken from Hansch *et al.* [28.]

Figure S2. Concentration of 1-phenyl-1-cyclohexane in aqueous solution in presence of different organic cosolvents normalized to the value for 5 vol% MeOH. Sigmoidal extrapolation of the data was performed using the following Boltzmann model: $y = A2 + (A1-A2)/(1 + \exp((x-x0)/dx))$. Variables: x: solvent concentration [vol%], y: relative amount of 1-phenyl-1-cyclohexane, A1, A2, x0, dx: constants.

Table S2. Enantiomeric excess of (S)-styrene oxide produced by *GnIndA* in presence of selected organic solvents.

Solvent	logP _{O/W} [28]	(S)-styrene oxide excess in presence of X vol% solvent		
		5	10	15
DMSO	-1.35	80.0 ± 2.1 %	-	81.1 ± 0.8 %
MeOH	-0.77	80.8 ± 1.5 %	-	80.1 ± 4.6 %
AcN	-0.34	-	80.3 ± 0.5 %	79.3 ± 1.4 %
EtOH	-0.31	82.8 ± 1.0 %	-	80.0 ± 2.1 %
Acetone	-0.24	-	80.4 ± 0.5 %	-

"-" = not measured.

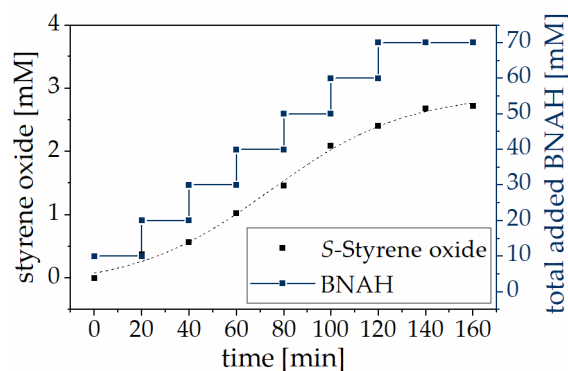


Figure S3. Continuously fed biotransformation of styrene to styrene oxide by *GrStyA*. The enzyme was supplied with 10 mM BNAH in solid form and 2 mM styrene every twenty minutes until minute 140. There is an increase in styrene oxide concentration until the BNAH supply was stopped indicating that the cosubstrate is limiting in the process. A maximal amount of 2.72 mM styrene oxide in solution (19.4% conversion) was reached. Sigmoidal extrapolation of the styrene oxide concentration was performed using the following Boltzmann model: $y = A2 + (A1-A2)/(1 + \exp((x-x0)/dx))$. Variables: x : solvent concentration [vol%], y : relative amount of 1-phenyl-1-cyclohexane, $A1$, $A2$, $x0$, dx : constants.

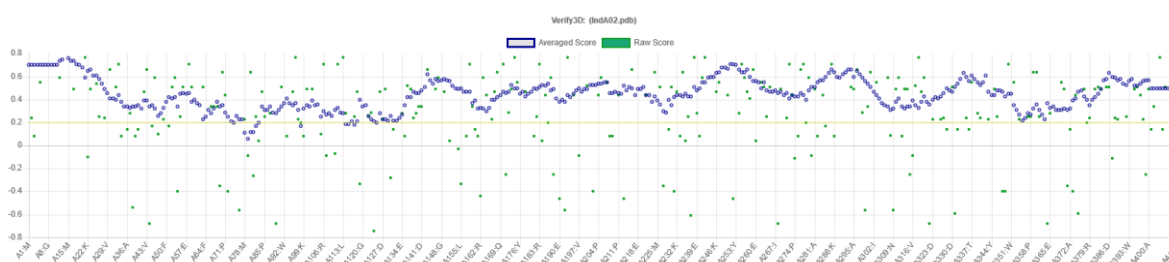


Figure S4. Results of the quality check of the structural *GrIndA* model by Verify 3D [36]. The structural model contains no amino acid clashes and sterical incorrect model secondary structure elements.

<i>G. nectariphilus</i> IndA	--MRKFTIVGGGQSGMLV <u>A</u> I <u>G</u> LLKA <u>G</u> HQVRVVQNR <u>T</u> GAEITA <u>C</u> KVLSSQCM <u>S</u> NSVQNE <u>S</u>	58
<i>G. rubripertincta</i> StyA	-MSKSIAIVGAGTAGLHLGLYLQQH <u>V</u> ESTIFSDKRPEEYRDVRLNNTVAHHAVTVVEREN	59
<i>P. putida</i> S12 StyA	-MKKRIGIVGAGTAGLHLGLFLRQH <u>D</u> VVTVVYTRDKPDEYSGLRLLNNTVAHNAVTVQREV	59
	: : * * * * : : * * : : : : * * : : : *	
FireProt	PpStyA -MKKRIGIVGAGTAGLHL <u>A</u> LFLRQH <u>D</u> VVTVVYTRDKPDEY <u>S</u> GLRLLNNTVAH <u>N</u> AVTVQREV <u>S</u>	59
HotSpot Wizard	PpStyA -MKKRIGIVGAGTAGLHL <u>G</u> LFLRQH <u>D</u> VVTVVYTRDKPDEY <u>S</u> GLRLLNNTVAH <u>N</u> AVTVQREV <u>V</u>	59
<i>G. nectariphilus</i> IndA	DL <u>G</u> IDFWSDSCPPVEGINFMVNPKEKPEG ----KLIDWTGKLD-HKAYAVDQVRVKMPRWL	113
<i>G. rubripertincta</i> StyA	AL <u>G</u> VNHWQDA--GYFGHYYYIGVPG -----MPIEFYGDLAGSPRAVDYRIYLP TLM	109
<i>P. putida</i> S12 StyA	ALDVNEWPSEEFYFGHYYYV -GGP-----QPMRFYGD LK-APSRAYDYRLYQ PMLM	109
	: : : * : : : : * * : : * * : : * * : :	
FireProt	PpStyA AL <u>G</u> VNEWPSEEFYFGHYYYV -GGP-----QPMRFYGD LK-APSRAYDYRLY <u>F</u> PMLM	109
HotSpot Wizard	PpStyA AL <u>D</u> VNEWPSEEFYFGHYYYV -GGP-----QPMRFYGD LK-APSRAYDYRLYQ PMLM	109
<i>G. nectariphilus</i> IndA	<u>E</u> EFQKLGGO-LVIKDAGIAELETYAREDDLIV <u>V</u> SGK <u>G</u> EIGO <u>F</u> TR <u>D</u> AT <u>R</u> SPYDKPQRAL	172
<i>G. rubripertincta</i> StyA	ND <u>V</u> IDRGGK-IEYRNIGLEDLDELSAAYDLVVIGTGK <u>G</u> GLGT <u>F</u> AR <u>D</u> ED <u>S</u> SPYSE PQRHL	168
<i>P. putida</i> S12 StyA	RALEARGGK-FCYDAVSAEDLEGLSEQYDLLVCTGKYALGKVF EKQSEN S PF EKPQRAL	168
	* : : : * * : : * * : : * * : : * * : : *	
FireProt	PpStyA <u>E</u> ALEARGGK-FCYDAV <u>S</u> EDLEGLSEQYDLLV <u>V</u> CTGKYAL <u>G</u> KV <u>F</u> E <u>K</u> QSEN S PF EKPQRAL	168
HotSpot Wizard	PpStyA <u>R</u> ALEARGGK-FCYDAVSAEDLEGLSEQYDL <u>L</u> VVCTGKYALGK <u>V</u> F <u>E</u> KQSEN S PF EKPQRAL	168
<i>G. nectariphilus</i> IndA	ALTYVKGMTPREP <u>H</u> SAVEFNLI PGVGEY <u>F</u> VFPSL <u>T</u> TGPCEIMVLEGI <u>G</u> GPMDCWADV K	232
<i>G. rubripertincta</i> StyA	CVGLFKGIAPQE -TRAVTFCIAPNGEMIEIPTVSNFGDATA LVLENHFG <u>G</u> DLEVLAKTR	227
<i>P. putida</i> S12 StyA	CVGLFKGIKEAP-IRAVTMSFSPGHGELIEIPTLSFNGMSTALVLENHIGSDLEVAHTK	227
	: : * * : : * * : : * * : : * * : : * * : : *	
FireProt	PpStyA CVGLFKGIKEAP-IRAVTMSFSPGHGELIEIPTL <u>S</u> FNGMS <u>A</u> LVLENH <u>G</u> SDLEVLAH <u>K</u>	227
HotSpot Wizard	PpStyA CVGLFKGIKEAP- <u>I</u> RAVTMSFSPGHGEL <u>I</u> EIPTL <u>S</u> FNGMSTALVLENH <u>I</u> GS DLEVL AHTK	227
<i>G. nectariphilus</i> IndA	---TPEQHLEKSLGILKTFLPWE <u>V</u> ERSKNVELT ---D <u>P</u> NGILAGRFP <u>P</u> VRHPVATL <u>S</u> SG	286
<i>G. rubripertincta</i> StyA	YDDDPRAFLDLLDKLRTYYPITADRINEDEFDLANSSLDLQGA VTPG <u>V</u> RHGHVKLDNG	287
<i>P. putida</i> S12 StyA	YDDDPRAFLDLMLEKLGKHHPSVAERIDPAEFDLANSSLDILQGGVVP <u>A</u> FRDGHATL <u>N</u> NG	287
	* . : : : . * . : : * * . * * * * * * *	
FireProt	PpStyA YDDDPRAFLDLMLEK <u>L</u> KHH <u>P</u> SV <u>E</u> RIDPAEFDLANSSLDILQGGVVP <u>F</u> RDGHATL <u>N</u> NG	287
HotSpot Wizard	PpStyA YDDDPRAFLDLMLEKLGKHHPSVAERIDPAEFDLANS <u>S</u> LDILQGGVVP <u>A</u> FRDGHATL <u>N</u> NG	287
<i>G. nectariphilus</i> IndA	RK <u>V</u> LGLGDA <u>V</u> CLN <u>D</u> PI <u>T</u> GQGSNNASKAAAVYLKSILDHGD -----RAYD <u>A</u> FMRATF	338
<i>G. rubripertincta</i> StyA	KI <u>A</u> ALLGDA <u>H</u> AHVDPVVGQGNMASYAAHVLGEEIVGNN -----V <u>D</u> E <u>H</u> FFEVVN	337
<i>P. putida</i> S12 StyA	KTIIGLDIQATVDPVLGQGANMASYA AWILGEEILAH S -----VYDLRFSEHLE	337
	: : * * * * : : * * : : * * : : * * : : *	
FireProt	PpStyA K <u>T</u> IIGLDI <u>A</u> TVDPVLGQGANMASYA AWILGEEILAH S -----V <u>D</u> E <u>R</u> FSEHLE	337
HotSpot Wizard	PpStyA K <u>T</u> IIGLDI <u>I</u> QATV <u>D</u> VPVLGQGANMASYA AWILGEEILAH S -----VYD <u>L</u> R FSEHLE	337
<i>G. nectariphilus</i> IndA	ERFW- <u>D</u> YAQVVRWTN <u>M</u> L---Q <u>P</u> PP <u>F</u> ILE <u>I</u> MGTACAVPELAHRMANAFDDPRDFPFWF	394
<i>G. rubripertincta</i> StyA	AR- <u>R</u> AVRVLGATRW <u>T</u> NY <u>M</u> LKNL <u>R</u> E <u>L</u> PN <u>S</u> LVEFL <u>G</u> A <u>V</u> SLDRGLADKFTTFNFYPERQWDIF	396
<i>P. putida</i> S12 StyA	RR-RQDRVLCATRWTFN <u>T</u> LSALSALPPEFLAFLQILS QSREMADEFTDNFNYP ERQWDRF	396
	. . . * * * : : : : * * : : * * : : *	
FireProt	PpStyA RR-RQDRVLCATRWTFN <u>L</u> SALSALPPEFL <u>F</u> LQILS QSREMADEFT <u>S</u> YFNYP ERQWDRF	396
HotSpot Wizard	PpStyA RR-RQDRVLCATRWTFN <u>T</u> LSALSALPPEFLAFLQ <u>L</u> ILS QSREMADEFTDNFNYP ERQWDRF	396
<i>G. nectariphilus</i> IndA	ADPDAAASYLADLRKAA ----- 411	
<i>G. rubripertincta</i> StyA	SSPESMSSWVESNAGSQEDDDVRFAGAAV -- 427	
<i>P. putida</i> S12 StyA	SSPERFGWCNQYAPTIAA ----- 415	
FireProt	PpStyA SSPERF <u>Q</u> WCNQYAPTIAA ----- 415	
HotSpot Wizard	PpStyA SSPERFGWCNQYAPTIAA ----- 415	

Figure S5. Multiple sequence alignment of *Gn*IndA, *Gr*StyA and *Pp*StyA with 22 related SMOs and IMO (see also [11], data not shown here) in comparison with the results from the FireProt and HotSpot Wizard webserver. The conservation of amino acids among those 25 enzymes is indicated in the fourth row. For the FireProt results the sequence of *Pp*StyA is displayed including the suggested mutations highlighted in green. If present in one of the sequences above, the respective amino acid is also highlighted in green there. The crucial amino acids assigned by the HotSpot Wizard are highlighted in yellow in the sequence of *Pp*StyA. Amino acids exchanges which were classified as “neutral or beneficial” are highlighted in yellow in the respective sequences except the amino acid matches a suggested mutation by the FireProt server. Amino acids conserved in the IMO sequences are underlined. Enzyme sequences used for alignment: SMOs: *R. opacus* 1CP (ANS32444), *Rhodococcus* sp. ST-5 (BAL04132), *Rhodococcus* sp. ST-10 (BAL04129), *N. ramosa* DSM11499 (WP_022977994), *S.*

fribergensis Kp5.2 (WP_039579272), *P. agarilytica* NO2 (GAC06215), *M. litorale* DSM 23545 (WP_027855270), *Pseudomonas* sp. LQ26 (ADE62390), *Pseudomonas* sp. Y2 (CAA04000), *P. fluorescens* ST (CAB06823), *P. taiwanensis* VLB120 (AAC23718), *P. putida* SN1 (ABB03727), *P. putida* CA-3 (ABX24519); IMOs: *Burkholderia* sp. IDO3 (APT36898), *R. opacus* 1CP (ACR43974), *N. farcinica* IFM 10152 (BAD56093), *P. aurescens* TC1 (ABM07034), *S. auratus* AGR0001 (EJJ03821), *V. paradoxus* EPS (ADU39062), *V. paradoxus* EPS (ADU39063), *D. acidovorans* SPH-1 (ABX34433).

Table S3. FireProt mutations in comparison with their abundance among IMOs and SMOs and corresponding amino acids in the sequence of *GrStyA* and *GnIndA*.

FireProt mutation in <i>P. putida</i> S12	$\Delta\Delta G$ [kJ mol ⁻¹]	Corresponding residue in		Abundance of residue in related enzymes	
		<i>G. rubripertincta</i> CWB2	<i>G. nectarophilus</i> DSM15620	SMOs (15 enzymes.)	IMOs (9 enzymes)
Evolutionary mutations					
G18A	-3.20	A18	G17	1	5
D25G	1.93	G25	G24	6	8
S40R	-4.66	R40	S39	2	3
N51F	-12.88	H51	F50	0	9
V59R	-0.91	N59	R58	0	9
D62G	-6.29	G62	G61	7	9
R110E	1.87	N110	E114	1	3
C142A	1.09	G142	A146	0	7
V151L	-2.12	L151	L155	5	9
K154R	-1.21	R154	R158	4	8
Q155D	0.42	D155	D159	4	8
S202T	0.30	S202	T208	3	8
I216P	-5.14	F216	P221	2	9
A276T	-3.33	G276	T275	4	9
N285P	-4.66	D285	P284	1	6
Q297V	2.90	H297	V296	0	9
Y328F	2.12	F328	Y329	5	6
L330E	0.06	E330	E331	6	1
T354M	-19.35	M354	M355	7	2
Energy mutations					
S40P	-7.07	R40	T39	0	0
A49F	-14.27	A49	C48	0	1
N64F	-12.76	N64	D63	0	0
Q105F	-9.79	L105	M109	0	2
A126P	-11.85	L126	I130	0	1
G149M	-8.59	G149	G153	0	0
T208F	-24.67	T208	E213	0	0
T226F	-6.11	T226	V231	0	0
G244W	-8.10	R244	K246	0	0
S249F	-9.49	I249	W251	0	0
A251Y	-6.65	A251	Y253	2	2
T289V	-10.52	I289	K288	0	1
T354W	-17.05	M354	M355	0	0
A367F	-7.62	E367	E365	0	0
D384E	-9.61	T384	N382	1	0
N385W	-11.73	N385	A383	0	0
G403W	-9.79	S403	A401	0	0