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Supporting Information

A Chimeric Styrene Monooxygenase with Increased Efficiency in Asymmetric Biocatalytic Epoxidation

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1. Abbreviations

SMO	Styrene monooxygenase bi-enzymatic system (StyA and StyB) from *Pseudomonas sp.*
StyA	Epoxidase enzymatic unit of SMO
StyB	Reductase enzymatic unit of SMO
Cb-FDH	Formate dehydrogenase from *Candida boidinii*
KPi	Potassium phosphate buffer
NAD	Nicotinamide adenine dinucleotide
FAD	Flavin adenine dinucleotide
HCOONa	Sodium formate
MTBE	*tert*-butyl methyl ether
EtOAc	Ethyl acetate
Fus-SMO	N-His_6-StyA-linker-StyB
DCPK	Dicyclopropyl ketone

2. General information

Styrene (**1a**, 99.5%), (*S*)-styrene oxide ((*S*)-**2a,** >98%), racemic styrene oxide (*rac*-**2a**) and 4-methylstyrene (**1c,** >99%) were purchased from Fluka Chemicals. *trans*-β-Methyl styrene (**1b**, >97) was purchased from TCI Chemicals. (1*S*,2*S*)-1-phenylpropylene oxide (*1S,2S*-**2b**) and (1*R*,2*R*)-1-phenylpropylene oxide (*1R,2R*-**2b,** 97% purity 99% *ee*) were purchased from Sigma Aldrich. All chemicals and solvents were used without further purification.

Nicotinamide cofactor (NAD^+) was purchased from Melford Biolaboratories (Chelsworth, Ipswich, UK).

Flavin adenine dinucleotide (FAD) was purchased from TCI Chemicals.

Catalase from bovine liver was purchased by Sigma-Aldrich (lyophilized powder, >10000 U mg-1 of protein). Catalase was added to the reactions from a stock solution. The concentration of the catalase in the reaction mixture (ca. 2 µM) was calculated considering the MM of the monomer (60 kDa) since each monomer contains a catalytic iron site.

^1H NMR spectra were recorded on a Brucker (400 MHz) spectrometer in $CDCl_3$. All signals are expressed as ppm down field from tetramethylsilane.

Cb-FDH was expressed and purified as described previously.[1]

3. Preliminary study with StyA and StyB as single enzymes expressed in plasmid pSPZ10: expression, solubility and activity trials

3.1. *Expression of E. coli JM101 and E. coli Arctic express cells carrying pSPZ10-StyAStyB*

The pSPZ10 plasmid containing the styA and styB genes encoding for the SMO bi-enzymatic system of *Pseudomonas sp*. strain VLB120 was kindly donated by Prof. Sven Panke.[2] The expression of the protein was performed in two different *E. coli* host cells, as described below.

JM101: 800 mL of LB medium supplemented with kanamycin (50 µg mL^{-1}) were inoculated with 15 mL of an overnight culture and grown at 37 °C until an OD_{600} of 0.6-0.8 was reached. Enzyme expression was induced with DCPK (0.05% v v^{-1}), cells were grown for 16 h at 25 °C and then harvested by centrifugation.

Arctic express DE3: 800 mL of LB medium supplemented with kanamycin (50 µg mL^{-1}) and gentamycin (20 µg mL^{-1}), were inoculated with 15 mL of an overnight culture and grown at 37 °C until an OD_{600} of 0.6-0.8 was reached. Expression of the proteins was induced by the addition of DCPK (0.05% v v^{-1}). Cells were grown for 16 h at 15 °C and then harvested by centrifugation.

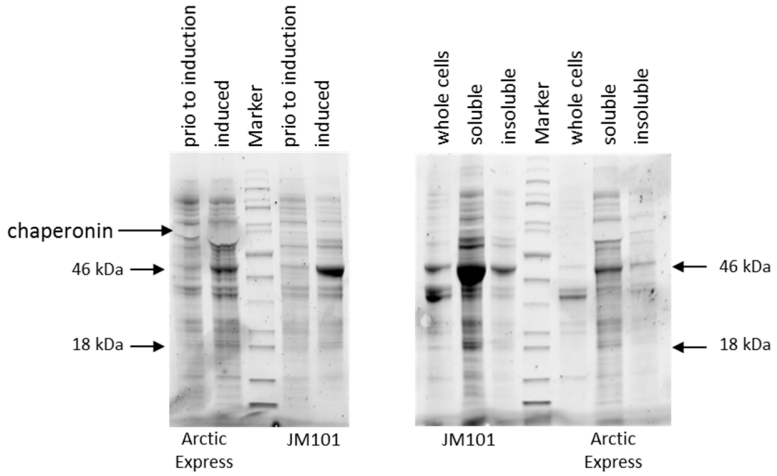


Figure S1. SDS-Page for the expression of pSPZ10-StyAStyB in *E. coli* JM101 and Arctic Express cells. The SDS-Page was visualized using a gel imaging system from Biorad which does not visualize proteins lacking of tryptophan residues (chaperonin from Arctic Express cells). Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific).

3.2. *Activity test: comparison between E. coli Arctic Express/pSPZ10-StyAStyB and E. coli JM101/pSPZ10-StyAStyB*

Reaction conditions: Fresh, frozen and lyophilized *E. coli* cells of JM101 and Arctic Express DE3 containing expressed StyA and StyB (25 °C, 16 h) were subjected to activity tests for the conversion of **1a** (10 mM) to **2a** as described below:

(a) JM101

(1) 3.2 g of wet fresh cells obtained from the expression trials (directly used for bio-catalytic reaction after harvesting of the *E. coli* culture) were resuspended in 18 mL of KPi buffer (50 mM, pH 7.0, final concentration of wet fresh cells ca. 180 mg mL^{-1}). An aliquot of 0.5 mL was used for each bio-catalytic reaction. (2) Frozen cell pellets were defrosted and the wet cells were resuspended in KPi buffer to obtain a final cell concentration of ca. 130 mg mL^{-1} (50 mM, pH 7.0). An aliquot of 0.5 mL was used for each biocatalytic reaction (3) Lyophilized cells (10 mg) were rehydrated in KPi buffer (0.5 mL, 50 mM, pH 7.0) and used for performing the activity check.

(b) Arctic Express DE3

(1) Fresh cells obtained from the expression test (2.3 g) were resuspended in KPi buffer (50 mM, pH 7; 18 mL, final concentration of wet fresh cells ca. 180 mg mL^{-1}). An aliquot of 0.5 mL was used for each test. (2) Frozen cells (2.24 g) were resuspended in KPi buffer (50 mM, pH 7; 18 mL, final concentration of wet cells ca. 130 mL^{-1}). An aliquot of 0.5 mL was used for each bio-catalytic reaction. (3) Lyophilized cells (10 mg) were rehydrated in 0.5 mL KPi buffer (50 mM, pH 7.0) and used for performing the activity check.

Bio-catalytic reactions were performed in a biphasic system (1:1, v v^{-1} buffer/decane) in 4 mL glass vials (final reaction volume 1 mL) in the presence of NAD^+ (1 mM, 0.1 eq.), HCOONa (100 mM, 10 eq.) and Cb-FDH (10 µM) for recycling of the NAD cofactor. The concentrations of coenzyme, co-substrate and recycling enzyme are calculated on the volume of the aqueous phase. The reactions were initiated by the addition of **1a** (10 mM, calculated on the volume of the organic phase) and incubated at 30 °C and 170 rpm on an orbital shaker for 16 h. The organic phase reaction mixture (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 500 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID as described in the chapter Analytical methods. The results are reported in Table S1.

Table S1. Results for the bio-catalytic conversion of **1a** (10 mM) to (*S*)-**2a** for the comparison of *E. coli* JM101 and Arctic Express cells as host organism for the expression of pSPZ10-StyAStyB.

entry	cells	JM101 **2a** [%][a)]	Arctic Express **2a** [%][a)]
1	wet fresh cells (180 mg mL^{-1})	37±1	72±13
2	frozen pellet (130 mg mL^{-1})	78±0.5	95±0
3	lyophilized whole cells (20 mg mL^{-1})	90±2	95±0.5

[a)] Reactions were performed in duplicates and the reported conversion is the average of two measurements.

4. Expression and activity test for the chimeric fused SMO (Fus-SMO) in *E. coli* BL21 DE3

4.1. *First expression trial of Fus-SMO*

The DNA sequence encoding for styA and styB (GenBank: AF031161.1) was optimized for the expression in *E. coli* and subcloned in pET28b between NdeI and XhoI to obtain the His_6-StyA-linker-StyB (MW = 69 kDa) construct (flexible linker AA sequence: ASGGGGSGGGGSGGGGSGGGGSGGGGSGAS.[3]

When transforming BL21 DE3 cells with the plasmid, colonies of various colors (pink/white/blue colors) grew and four of them were picked and subjected to expression and solubility trials. For each colony, 400 mL of LB medium were supplemented with Kanamycin (50 µg/mL) and inoculated with 8 mL of an overnight culture. The cells were grown at 37 °C until the cell density reached a value between 0.6 and 0.8 and split into 100 mL aliquots. To each aliquot IPTG was added (1 mM final concentration) and protein expression was carried out at 37 °C for 3h, 30 °C for 5 h or 25 °C overnight (o.n.). The cells were harvested by centrifugation, washed with KPi buffer (50 mM, pH 8) and lyophilized.

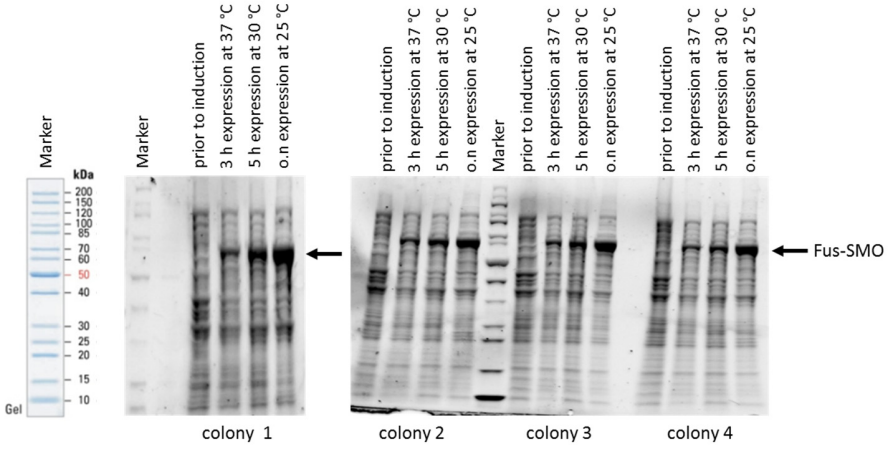


Figure S2. SDS-PAGE for the expression of Fus-SMO in *E. coli* BL21 DE3 as host. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific)

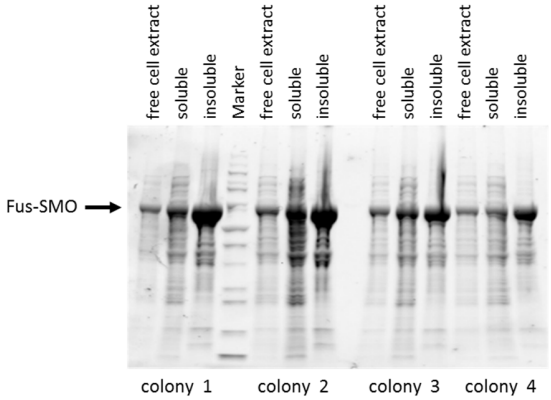


Figure S3. SDS-PAGE for verifying the soluble part of the expressed Fus-SMO when expression was performed at 25 °C overnight. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific)

4.2. *Preliminary tests with Fus-SMO as lyophilized whole cells for the conversion of **1a** to **2a***

Lyophilized cells (20 mg) obtained from the expression test were rehydrated in KPi buffer (50 mM, pH 7.5, 1 mL) in 20 mL glass vials. After that the cofactor NAD^+ (1 mM, 0.05 eq.) was added followed by addition of HCOONa (100 mM, 5 eq.) and purified *Cb*-FDH (10 µM). Furthermore, FAD (50 µM) was added to selected experiments. The concentrations of coenzymes, cosubstrate and recycling enzyme are calculated in relation to the volume of the aqueous phase. Decane (1 mL) was used as biphasic solvent and the biotransformations were initiated by the addition of **1a** (20 mM, calculated on the volume of the organic phase). The mixtures were shaken at 30 °C and 180 rpm on an orbital shaker for 17 h. The organic phase (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 500 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID and results are reported in Table S2.

Table S2. Conversions [%] of **1a** (20 mM) to (*S*)-**2a** for the activity test of lyophilized whole cells containing Fus-SMO with and without the addition of FAD (50 µM).

Entry	colony	conversion of **1a** after expression of Fus-SMO at 37 °C for 3 h [%]	
		- FAD	+ FAD
1	1	24	24
2	2	7	24
3	3	20	56
4	4	21	50
Entry	colony	conversion of **1a** after expression of Fus-SMO at 30 °C for 5 h [%]	
		- FAD	+ FAD
5	1	40	22
6	2	39	26
7	3	40	26
8	4	38	23
Entry	colony	conversion of **1a** after expression of Fus-SMO at 25 °C o.n. [%]	
		- FAD	+ FAD
9	1	55	52
10	2	74	99
11	3	53	52
12	4	52	48

4.3 Optimization of the expression using various concentrations of IPTG and testing the activity of Fus-SMO in BL21 DE3

800 mL of Luria-Bertani Broth were supplemented with kanamycin (50 µg mL^{-1}) and inoculated with 15 mL of an overnight culture of *E. coli*/pET28b-Fus-SMO (colony 1). The main culture was grown at 37 °C until the cell density reached an OD_{600} of 0.6-0.8. Then, the expression of the protein was induced by the addition of IPTG (0.1, 0.5 and 1 mM, respectively) and the cells grown for additional 16 h at 25 °C and harvested by centrifugation.

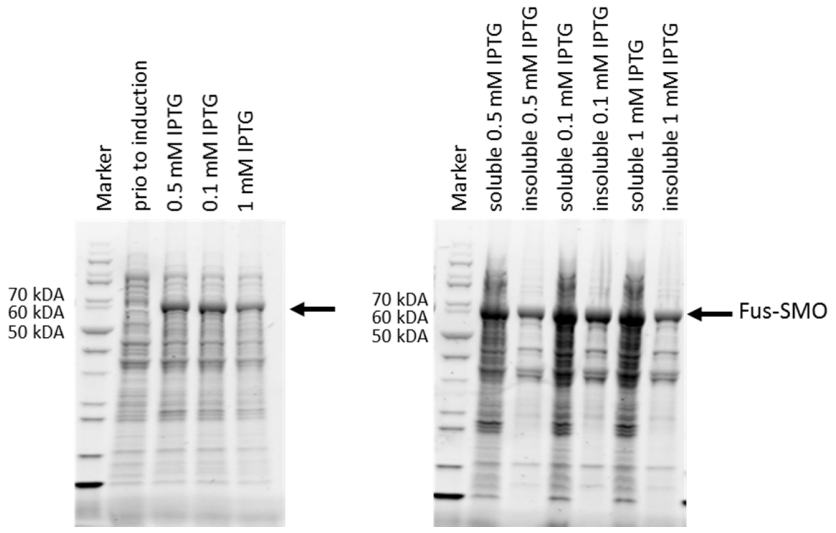


Figure S4. SDS-PAGE for the expression (left picture) of Fus-SMO using various concentrations of IPTG and solubility test (right picture). Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific).

Activity tests were first performed with the soluble fraction. The activities from all the expression conditions were assayed in order to understand the best condition for expression. Furthermore, the influence of different reaction vessels on the outcome of the biocatalytic reaction was also investigated.

*4.3.1 Reaction conditions for the conversion of **1a** to **2a** by the soluble part of expressed Fus-SMO:*

450-500 mg of cells (wet weight) obtained from the expression trials were resuspended in KPi buffer (pH 7.5, 50 mM, 3 mL) and lysed by sonication (5 sec. on, 10 sec. off, 5 min on time, 45 % amplitude). The cell debris was removed by centrifugation and only the supernatant was used for further activity tests as described below.

Note: the concentrations of coenzymes, cosubstrate and recycling enzyme are always calculated on the volume of the aqueous phase, whereas the concentration of the substrate is referred to the organic phase.

(A) Reactions performed in Eppendorf tubes (2 mL): To a 350 µL aliquot of the soluble fraction, NAD^+ (1 mM, 0.1 eq.), HCOONa (100 mM, 10 eq.), *Cb*-FDH (10 µM) and FAD (30 µM) were added (0.5 mL final volume of aqueous phase). Decane (0.5 mL) was used as biphasic solvent and the biotransformation was initiated by the addition of **1a** (10 mM). The reaction was shaken at 30 °C and 180 rpm on an orbital shaker for 20 h. The organic phase from the reaction mixture (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 250 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

(B) Reactions performed in 4 mL glass vials: To a 350 µL aliquot of the soluble fraction, NAD^+ (1 mM, 0.1 eq.), HCOONa (100 mM, 10 eq.), *Cb*-FDH (10 µM) and FAD (30 µM) were added (0.5 mL final volume of aqueous phase). Decane (0.5 mL) was used as biphasic solvent and the biotransformation was initiated by the addition of **1a** (10 mM). The mixture was shaken at 30 °C and 180 rpm on an orbital shaker for 20 h. The organic phase from the reaction mixture (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 250 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

(C) Reactions performed in 20 mL glass vials: To a 700 µL aliquot of the soluble fraction, NAD^+ (1 mM, 0.1 eq.), HCOONa (100 mM, 10 eq.), *Cb*-FDH (10 µM) and FAD (30 µM) were added (1 mL final volume of aqueous phase). Decane (1 mL) was used as biphasic solvent and the biotransformation was initiated by the addition of **1a** (10 mM). The mixture was shaken at 30 °C and 180 rpm on an orbital shaker for 20 h. The organic phase from the reaction mixture (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 500 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

Table S3. Bio-conversion [%] of **1a** (10 mM) to (*S*)-**2a** by the soluble fraction of Fus-SMO expressed with different IPTG concentrations using a variation of reaction vessels.

Entry	IPTG [mM]	2 mL eppendorf tubes **2a** [%]	4 mL glass vials **2a** [%]	20 mL glass vials **2a** [%]
1	0.1	40	>99.9	50
2	0.5	34	>99.9	55
3	1	41	>99.9	54

*4.3.2 Reaction conditions for the conversion of **1a** to (S)-**2a** by lyophilized whole cells containing Fus-SMO*

The reactions were performed in 4 mL glass vials, as this type of vessel showed highest conversion in the preliminary test (see above, Table S3). Lyophilized cells obtained from the expression trial using various concentration of IPTG for induction were tested for the activity towards the conversion of **1a**. Also the amount of lyophilized cells that is needed for the conversion of 20 mM substrate was investigated.

Lyophilized cells (2.5, 5 and 10 mg) were rehydrated in KPi buffer (0.5 mL, 50 mM, pH 7.5) in 4 mL glass vials. After that the cofactor NAD^+ (1 mM, 0.05 eq.) was added followed by addition of HCOONa (100mM, 5 eq.), *Cb*-FDH (10 µM) and FAD (50 µM). The concentrations of coenzymes, cosubstrate and recycling enzyme are calculated on the volume of the aqueous phase. Decane (0.5 mL) was used as biphasic solvent and the biotransformation was initiated by the addition of **1a** (20 mM, referred to the organic phase). The mixture was shaken at 30 °C and 180 rpm on an orbital shaker for 17 h. The organic phase from the reaction mixture (decane) was separated from the aqueous phase. The aqueous phase was further extracted with EtOAc (2x 250 µL). The combined organic phases (decane + EtOAc) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

Table S4. Bio-conversion [%] of **1a** (20 mM) to (*S*)-**2a** in 4 mL glass vials using lyophilized whole cells containing Fus-SMO expressed with different IPTG concentrations.

Entry	lyophilized cells [mg mL^{-1}]	0.1 mM IPTG **2a** [%][a)]	0.5 mM IPTG **2a** [%][a)]	1 mM IPTG **2a** [%][a)]
1	5	80.5±6.5	68.5±18.5	54.5±8.5
2	10	99.9±0.1	84.5±1.5	99.9±0.1
3	20	99.9±0.1	94.5±5.5	99.9±0.1

[a)]Reactions were performed in duplicates; the reported conversion is the average of two measurements

For all further experiments, the induction of Fus-SMO expression was done with 0.1 mM IPTG.

5. Solvent screening: bioconversion of 1a to (*S*)-2a by lyophilized cells containing Fus-SMO

For all experiments cells were used after expression with 0.1 mM IPTG at 25 °C for 16h.

Note: The concentrations of coenzymes, co-substrate and recycling enzyme are always calculated on the volume of the aqueous phase, the concentration of the substrate is referred to the organic phase.

(A) 1:1 ratio *n*-hexane or *n*-heptane/KPi buffer: Lyophilized whole cells (10 mg) were rehydrated in KPi buffer (0.5 mL, 50 mM, pH 8) in 4 mL glass vials. After that, the cofactor NAD^+ (1 mM, 0.05 eq.) HCOONa (100 mM, 5 eq.), *Cb*-FDH (10 µM) and FAD (50 µM) were added. Two organic solvents (*n*-hexane or *n*-heptane; 1:1, v v^{-1} with the buffer) were tested as biphasic solvents. Finally, the biotransformations were initiated by the addition of **1a** (20 mM). The reactions were shaken at 30 °C and 180 rpm on an orbital shaker for 24 h. The organic phase from the reaction mixture was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (2x 250 µL). The combined organic phases were dried with $MgSO_4$ and the conversions were measured by GC-FID.

(B) KPi buffer: Lyophilized whole cells (20 mg) were rehydrated in KPi buffer (1 mL, 50 mM, pH 8) in 4 mL glass vials. After that the cofactor NAD^+ (1 mM, 0.05 eq.), HCOONa (100 mM, 5 eq.), *Cb*-FDH (10 µM) and FAD (50 µM) were added. The biotransformations were initiated by the addition of **1a** (20 mM). The reactions were shaken at 30 °C and 180 rpm on an orbital shaker for 24 h. The organic compounds were extracted with MTBE (2 x 250 µL), the combined organic layers were dried over $MgSO_4$ and the conversions were measured by GC-FID.

(C) 5% styrene or 2-10% decane: Lyophilized whole cells (as reported in Table S5) were rehydrated in KPi buffer (50 mM, pH 8, for volumes see table) in 4 mL glass vials. Then, the cofactor NAD^+ (0.1 eq), HCOONa (5 eq.), *Cb*-FDH (10 µM) and FAD (50 µM) were added. In selected experiments, decane was used (2-10% v v^{-1}; 1 mL total reaction volume) as second phase or styrene (5% v v^{-1}; 1 mL total reaction volume) as neat substrate. The bio-transformations were initiated by the addition of **1a** (10 – 20 µmol, see Table S5) in the case of decane (2 to 10 % v v^{-1}) as organic solvent or **1a** (435 mM) in the case of styrene used as neat substrate. The reactions were shaken at 30 °C and 180 rpm on an orbital shaker for 24 h. The organic phase from the reaction mixture was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (2 x 400-500 µL). The combined organic layers were dried over $MgSO_4$ and the conversions were measured by GC-FID (Table S5).

Table S5. Bio-epoxidation of **1a** by Fus-SMO (20 mg mL^{-1}) in different solvent systems.

entry	buffer [µL]	cells [mg]	substrate [µmol]	organic solvent [µL]	organic solvent [% v/v]	conversion (*S*)-**2a** [%][a)]	(*S*)-**2a** formed [µmol]	productivity [µmol (*S*)-**2a**/mg cells]
1	500	10	10	500 (hexane)	50	>99±0.5	>9.9	>0.99
2	1000	20	20	-	-	67±5	13.3	>0.67
3	950	20	435	50 (styrene)	5	5±0	19.6	0.98
4	980	20	20	20 (decane)	2	68±2	13.6	0.68
5	960	20	20	40 (decane)	4	69±3	13.8	0.69
6	940	20	20	60 (decane)	6	69±0.5	13.6	0.68
7	920	20	20	80 (decane))	8	70±2	14.0	0.70
8	900	20	20	100 (decane)	10	72±3	14.4	0.72
9	500	10	10	500 (decane)	50	>99±0.5	>9.9	>0.99
10	500	10	10	500 (heptane)	50	>99±0.5	>9.9	> 0.99

[a)] Reactions were performed in duplicates and the reported conversion is the average of two measurements. The optical purity was measured for selected samples (one per each condition) by chiral HPLC. In all cases, the enantiomeric excess was >99% (*S)*.

6. Determination of the required reducing equivalents (i.e. equivalents of HCOONa) for the bio-conversion of 1a (20 mM) to (*S*)-2a using lyophilized whole cells containing Fus-SMO

Lyophilized whole cells of overexpressed Fus-SMO (5 mg, 10 mg mL^{-1}) were rehydrated in KPi buffer (0.5 mL, 50 mM, pH 8) in 4 mL glass vials. NAD^+ (1 mM, 0.05 eq.), HCOONa (0-100 mM, 0-5 eq.), *Cb*-FDH (10 µM), FAD (50 µM), *n*-heptane (0.5 mL) and **1a** (20 mM, referred to organic phase) were added and the reactions were incubated at 30 °C and 180 rpm on an orbital shaker for 24 h. The concentrations of coenzymes, cosubstrate and recycling enzyme are calculated on the volume of the aqueous phase. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (2x 250 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$ and the conversions were measured by GC-FID. The results for this experiment are summarized in Table S6.

Table S6. Conversions [%] of **1a** (20 mM) to (*S*)-**2a** by Fus-SMO (10 mg mL^{-1}) and various concentrations of hydride donor

entry	HCOONa [mM]	eq. HCOONa	*(S)*-**2a** [%][a]
1	0	0	21±8
2	2	0.1	22±3
3	4	0.2	19±1
4	8	0.4	28±1
5	12	0.6	23±0
6	16	0.8	34±1
7	20	1	37±6
8	40	2	63±1
9	60	3	93±0.5
10	80	4	99±1
11[b]	100	5	>99±0.1

[a] Reactions were performed in duplicates and the reported conversion is the average of two measurements. [b] The optical purity of the final product was measured by HPLC on a chiral column for sample entry 11. The enantiomeric excess was > 99% (*S*).

7. Influence of dioxygen in the headspace on the performance of Fus-SMO (expressed in *E. coli* BL21 DE3) and natural StyA-StyB (expressed in *E. coli* JM101, plasmid pSPZ10)

7.1. *Effect of oxygen pressure on the bio-catalytic conversion of **1a** (50 mM) in a pressurizable chamber*

Lyophilized *E.coli* cells containing Fus-SMO or natural StyA-StyB (pSPZ10 plasmid) (2.5 mg; 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 0.5 mL) in 20 mL glass vials. The buffer already contained the required amount of NAD^+ (1 mM, 0.02 eq.), HCOONa (250 mM, 5 eq.), FAD (50 µM) and *Cb*-FDH (10 µM). In certain experiments catalase (2 µM) was also added. The biotransformations were started by addition of *n*-heptane (0.5 mL) as the second solvent phase followed by the addition of **1a** (50 mM; calculated on the volume of the organic phase). The mixtures were shaken in a closed chamber (Fig. S5) on an orbital shaker (200 rpm) at 30 °C for 20 minutes. The reactions were quenched by freezing in liquid nitrogen. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous

phase. The aqueous phase was further extracted with MTBE (500 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

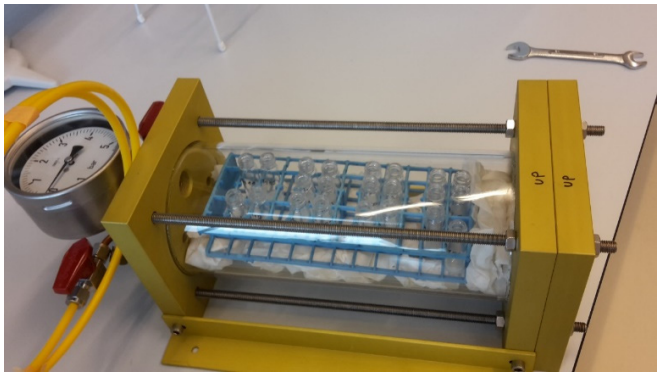


Figure S5. The oxygen chamber used in this study.

Table S7. Conversion [%] of **1a** (50 mM) to (*S*)-**2a** using lyophilized cells containing Fus-SMO (5 mg mL^{-1}) in a closed chamber. Reaction time: 20 minutes.

Entry	O_2 pressure [bar][a)]	*E. coli* BL21 DE3/Fus-SMO **2a** [%][c)]	*E. coli* BL21 DE3/Fus-SMO/catalase[b)] **2a** [%][c)]
1	Atmospheric pressure	16.3±0.6	21.2±2.9
2	Saturation with pure O_2	10.0±1.0	24.5±1.7
3	1	10.8±1.0	20.4±2.2
4	2	9.1±2.9	18.6±1.1
5	3	10.0±1.0	17.1±1.4
6	4	7.8±1.8	14.6±2.9

Table S8. Conversion [%] of **1a** (50 mM) to (*S*)-**2a** using lyophilized cells containing natural StyA and StyB (5 mg mL^{-1}) in a closed chamber. Reaction time: 20 minutes.

Entry	O_2 pressure [bar][a)]	*E. coli* JM101/pSPZ10-StyAStyB **2a** [%][c)]	*E. coli* JM101/pSPZ10-StyAStyB /catalase[b)] **2a** [%][c)]
1	Atmospheric pressure	11.8±2.3	14.0±2.6
2	Saturation with pure O_2	13.0±0.1	17.7±0.8
3	1	11.8±3.5	12.6±2.0
4	2	8.4±2.1	9.6±0.4
5	3	8.3±0.7	9.0±0.7
6	4	6.4±1.2	6.3±0.6

[a)]experiments were performed in a sealed pressurized chamber; for entry 2 the chamber was saturated with pure O_2 before reactions were started; [b)] final catalase concentration 2 µM; [c)]reactions were performed in duplicates and two independent experiments were performed; thus, the reported conversion is then the average of the 4 values. Errors are expressed as standard deviation.

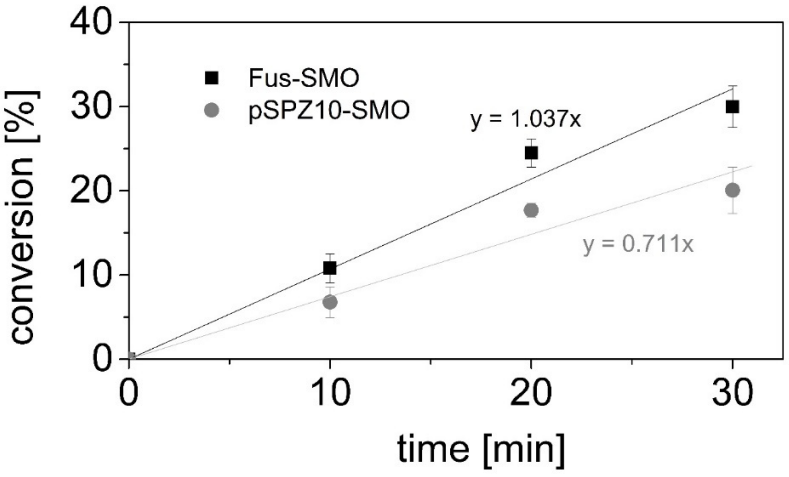
7.2. *Final comparison of the catalytic efficiency between the chimeric Fus-SMO and the natural StyA-StyB system (from plasmid pSPZ10)*

Lyophilized *E.coli* cells carrying Fus-SMO or natural StyA-StyB (plasmid pSPZ10) (2.5 mg; 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 0.5 mL) in 20 mL glass vials. The buffer already contained the required amount of NAD^+ (1mM, 0.02eq.), HCOONa (250 mM, 5 eq.), FAD (50 µM), *Cb*-FDH (10 µM) and catalase (2 µM). The biotransformations were started by the addition of *n*-heptane (0.5 mL) as the second solvent phase followed by the addition of **1a** (50 mM; calculated on the volume of the organic phase). The mixtures were shaken in a closed chamber on an orbital shaker (200 rpm) at 30 °C for 10, 20 and 30 minutes. The reactions were quenched by freezing in liquid nitrogen. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (500 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$ and the conversions were measured by GC-FID.

Table S9. Time study for the conversion of **1a** (50 mM) using lyophilized cells containing Fus-SMO or separated StyA and StyB.

Entry	Time [min]	*E. coli* BL21 DE3/Fus-SMO [a)]	*E. coli* JM101/pSPZ10-StyAStyB [a)]
1	10	10.8±1.7	6.8±1.8
2	20	24.5±1.7	17.7±0.8
3	30	30.0±2.5	20.1±2.8

Experiments were performed in a sealed chamber flushed with oxygen; [a)] reactions were performed in duplicates and two independent experiments were performed; thus, the reported conversion is then the average of the 4 values. Errors are expressed as standard deviation.



$$\frac{initial\ activity\ Fus_{SMO} - initial\ activity\ StyAStyB}{initial\ activity\ StyAStyB} \times 100 = 46\%$$

Figure S6. Time study for the conversion of **1a** (50 mM) by *E. coli* BL21 DE3/Fus-SMO (5 mg mL^{-1}) and *E. coli* JM101/pSPZ10-StyA-StyB (5 mg mL^{-1}). Time range was 0, 10, 20 and 30 minutes. Reactions were carried out in 40 mL glass vials (in a sealed chamber flashed with pure molecular oxygen). The bio-transformations were performed in a biphasic system KPi (pH 8, 50 mM)/*n*-heptane (1:1, v v^{-1}, 1 mL total reaction volume) containing NAD^+ (1mM), FAD (50 µM), HCOONa (250 mM, 5 eq.) and Cb-FDH (10 µM). The mixture were incubated at 30 °C, 200 rpm up to 30 min. Two independent experiments were carried out and both as duplicate; hence, the reported conversions are the average of 4 measurements. Error bars represent the standard deviations.

7.3. *Purification of His_6-Fus-SMO, determination of the activity of purified His_6-Fus-SMO and comparison with literature data of bi-enzymatic StyA-StyB*

7.3.1 Purification His_6 Fus-SMO

Expression was conducted as previously described in section 4.3 (0.1 mM IPTG, 25 °C overnight).

Wet cells containing Fus-SMO (ca. 12 g) were resuspended in lysis buffer (ca. 65 mL; 50 mM KH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8) and disrupted by sonication (10 min, amplitude 45%, pulse on 10 s, pulse off 10 s). The suspension was centrifuged (18000 rpm, 1 h, 4 °C). The supernatant was filtered through a 0.45 µm filter and loaded onto a Ni^{2+} column (5 mL, GE Healthcare) that was previously conditioned with lysis buffer. The column was washed with washing buffer (50 mM KH_2PO_4, 300 mM NaCl, 25 mM imidazole, pH 8) and the protein eluted with elution buffer (50 mM KH_2PO_4, 300 mM NaCl, 300 mM imidazole, pH 8). Purity was analyzed by SDS-Page and fractions showing >95% purity were combined and dialyzed overnight against potassium phosphate buffer (6 L, pH 8, 20 mM). The enzyme solution was concentrated and the concentration was measured spectrophotometrically by the method of Bradford (Bovine Serum Albumine standard). 128 mg of protein were obtained from ca. 12 g of wet cells, equal to 3.2 L of culture, without further optimization.

The purified protein shows a yellow color which corresponds to a natural loading of FAD of ca. 25%. The loading of FAD was analyzed by UV-Vis-spectroscopy at 450 nm by using the characteristic extinction coefficient of free FAD (ε=11300 M^{-1} cm^{-1}).

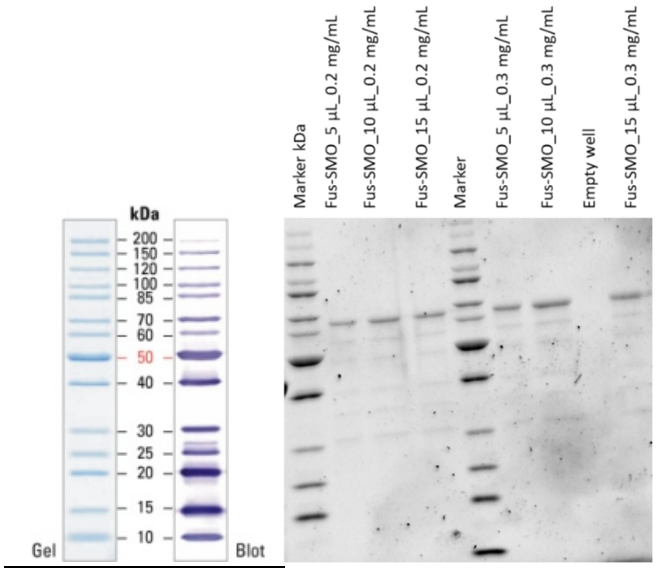


Figure S7. SDS-PAGE for the purification of Fus-SMO in *E.coli* BL21 DE3 as host. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific)

7.3.2 Typical assay for the determination of the initial activity of purified His_6 Fus-SMO

The epoxidation activity of Fus-SMO with **1a** and **1c** was measured according to the procedure reported by Otto *et al*. [4] as well as Tischler *et al*. [5] The quantification of the product formation *vs* time was performed by quenching samples at different time points and determining the conversion by GC-FID. A typical sample assay contained (2 mL Eppendorf tube): Fus-SMO (3 µM), FAD (15 µM), HCOONa (150 mM), catalase (650 U), *Cb*-FDH (20 µM), styrene (**1a**) or 4-methylstyrene (**3a**) (2 mM, from a 50 mM stock solution in heptane) in a 1 mL total volume of Tris-HCl buffer (pH 8.5, 50 mM). The mixture was incubated at 37 °C for 1 min and the reaction was started by the addition of NADH (50 mM). The samples were shaken at 37 °C, 1400 rpm for 1, 2, 3, 4 and 5 min, respectively. Reactions were stopped by freezing in liquid nitrogen followed by extraction with MTBE (2 x 500 µL). The organic phase was separated from the aqueous layer, dried over $MgSO_4$ and injected into GC-FID. Results are reported in **Figure S8.**

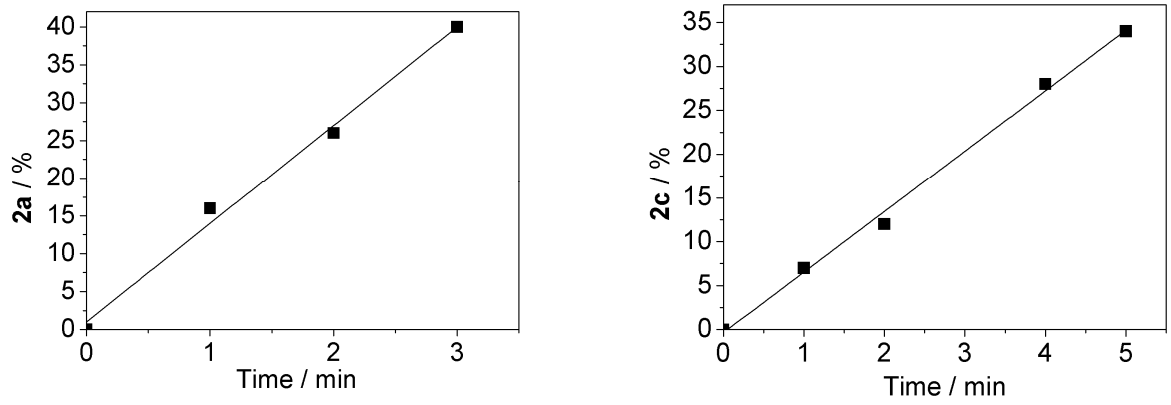


Figure S8. Initial activity purified Fus-SMO for the quantitative formation of enantiopure **2a** and **2c.** Slope conversion **2a**: 14.2±0.8; Slope conversion **2c**: 6.6±0.2

- Calculation of the activity of Fus-SMO:

$$Activity\ FusSMO = \left[\frac{U}{\mu mol\ FusSMO}\right] = \frac{\frac{Slope}{100} \times 2\left[\frac{mmol\ product\ formed}{min \times l}\right] \times 10^3 \left[\frac{\mu mol}{mmol}\right]}{3\left[\frac{\mu mol\ FusSMO}{l}\right]}$$

- Activity of Fus-SMO for epoxidation of **1a**

$$Activity = \left[\frac{U}{\mu mol\ FusSMO}\right] = \frac{\frac{14.2}{100} \times 2 \times 10^3}{3} = 95\ (std\ \pm 5)$$

- Activity of Fus-SMO for epoxidation of **1c**

$$Activity = \left[\frac{U}{\mu mol\ FusSMO}\right] = \frac{\frac{6.6}{100} \times 2 \times 10^3}{3} = 44\ (std\ \pm 2)$$

8. Co-expression of Fus-SMO and FDH in *E. coli* BL21 DE3

LB medium (800 mL), supplemented with kanamycin (50 µg mL^{-1}) and ampicillin (100 µg mL^{-1}), was inoculated with 15 mL of a pre-culture and grown at 37 °C until the cell density reached an OD_{600} value of 0.6-0.8. The expression was induced by the addition of IPTG (0.1 mM final concentration) and the cells were grown for further 16 h at 25 °C prior to harvesting by centrifugation at 4500 rpm. The whole cells were then washed with KPi buffer (pH 8, 50 mM), lyophilized (ca. 2g dry weight) and stored at -20 °C.

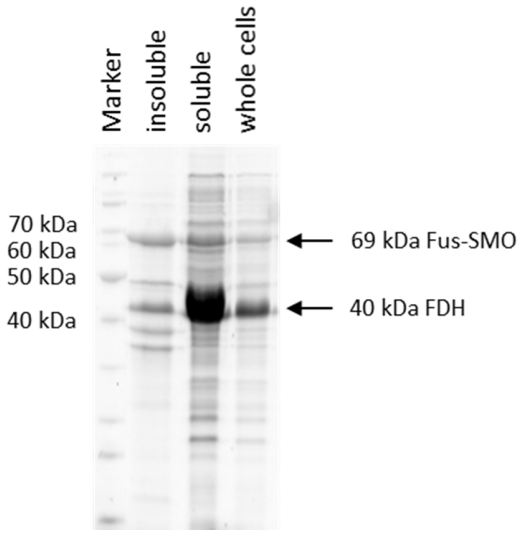


Figure S9. SDS-Page for the co-expression of Fus-SMO and FDH. Marker: PageRuler™ Unstained Protein Ladder (ThermoFisher Scientific).

8.1. *Activity test for the co-expressed cells*

Lyophilized whole cells containing Fus-SMO and *Cb*-FDH (5 mg) were rehydrated in KPi buffer (pH 8, 50 mM, 0.5 mL) in 4 mL glass vials. NAD^+ (1 mM, 0.05 eq.), FAD (50 µM), HCOONa (100 mM, 5 eq.) and heptane (0.5 mL) were added. The biotransformations were started by the addition of substrate (**1a** or **1b,** 20 mM) and shaken at 30 °C, 180 rpm on an orbital shaker for 24 h. The concentrations of coenzymes, co-substrate and recycling enzyme are always calculated on the volume of the aqueous phase, the concentration of the substrate is referred to the organic phase. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (2 x 250 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$ and analyzed by GC-FID and chiral HPLC.

Table S10. Results for the biotransformation of **1a** (20 mM) and **1b** (20 mM) using lyophilized whole cells containing co-expressed Fus-SMO and *Cb*-FDH.

Entry	product	conversion [%]	*ee* [%]	*de* [%]
1	(*S*)-**2a**	>99	>99	-
2	(1*S*,2*S*)-**2b**	75	>99	>98

8.2. *Time study for the bio-catalytic epoxidation of **1a** and **1b** (50 mM) to enantiopure (S)-**2a** and (1S,2S)-**2b** using lyophilized whole cells of co-expressed Fus-SMO and Cb-FDH.*

Lyophilized whole cells containing co-expressed Fus-SMO and *Cb*-FDH (2.5 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 0.5 mL) in a 20 mL glass vial. NAD^+ (1 mM), FAD (50 µM), HCOONa (5 eq.), catalase (2 µM), *n*-heptane (0.5 mL) and the substrates **1a** (50 mM) or **1b** (50 mM) were added. The concentration of cells, coenzyme and recycling enzyme are calculated on the volume of the aqueous phase, while the concentration of the substrate on the organic phase. The reactions were incubated at 30 °C and 200 rpm on an orbital shaker and monitored over time (0.5, 1, 2, 3, 4, 5, 6 and 24 h). The reactions were quenched by freezing in liquid nitrogen**.** The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (500 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$. The conversions were analysed by GC-FID.

Table S11. Time study for the conversion of **1a** and **1b** (50 mM) by lyophilized cells of co-expressed Fus-SMO and *Cb*-FDH

Entry	Time [h]	*S*-**2a** [%]	*1S,2S*-**2b** [%]
1	0.5	27.1±1.2	17.6±0.5
2	1	51.4±5.3	35.8±1.6
3	2	70.9±3.4	57.4±0.5
4	3	83.4±4.3	68.6±1.8
5	4	86.6±3.4	75.4±3.0
6	5	91.8±2.5	81.1±2.2
7	6	93.8±1.3	82.5±1.6
8	24	98.8±1.2	90.4±1.4

Reactions were performed as duplicates in two independent experiments; hence, the reported conversion is the average of 4 values. Error is expressed as the standard deviation.

8.3. *Experiments with increased substrate concentration for the bio-epoxidation of **1a** and **1b** (50 mM - 1 M) using lyophilised E. coli cells co-expressing Fus-SMO/Cb-FDH*

General procedure. Lyophilised whole cells containing co-expressed Fus-SMO and *Cb*-FDH (2.5 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 0.5 mL) in a 20 mL glass vial. NAD^+ (1 mM), FAD (50 µM), HCOONa (5 eq.), catalase (2 µM), *n*-heptane (0.5 mL) and the substrates **1a** (from 50 to 1 M) or **1b** (from 50 mM to 1 M) were added. The concentration of cells, coenzyme and recycling enzyme are calculated on the volume of the aqueous phase, while the concentration of the substrate is calculated on the volume of on the organic phase. The reactions were incubated at 30 °C and 200 rpm on an orbital shaker for 24 h. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (500 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$. The conversions were analysed by GC-FID.

Table S12. Conversion [%] of **1a** and **1b** to enantiopure **2a** and **2b** by lyophilized cells of co-expressed Fus-SMO and *Cb*-FDH in 20 mL glass vials

Entry	Substrate [mM]	**2a** [%]	**2a [mM]**	**2b** [%]	**2b [mM]**
1	50	97.7±1.3	48.9±0.7	96.1±2.8	48.1±1.4
2	75	74.0±2.2	55.5±1.7	74.8±1.2	56.1±0.9
3	100	58.7±0.9	58.7±0.9	64.4±0.2	64.4±0.2
4	150	43.2±1.0	64.8±1.5	52.3±1.6	78.5±2.4
5	170	33.9±1.9	57.6±3.2	51.1±0.3	86.9±0.5
6	200	29.1±0.3	58.2±0.6	46.7±1.5	93.4±3.0
7	250	23.7±0.1	59.3±0.3	39.3±0.5	98.3±1.3
8	500	10.7±0.4	53.5±2.0	13.0±0.6	65.0±3.0
9	1000	4.8±0.1	48.0±1.0	5.0±0.3	50.0±3.0

Reactions were performed as duplicates; hence, the reported conversion is the average of 2 values. Error is expressed as the absolute difference between the two measurements.

General procedure. Lyophilised whole cells containing co-expressed Fus-SMO and *Cb*-FDH (5 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 1 mL) in a 100 mL Erlenmeyer flask. NAD^+ (1 mM), FAD (50 µM), HCOONa (5 eq.), catalase (2 µM), *n*-heptane (1 mL) and the substrates **1a** (from 50 mM to 1 M) or **1b** (50 mM to 1 M) were added. The concentration of cells, coenzyme and recycling enzyme are calculated on the volume of the aqueous phase, while the concentration of the substrate is calculated on the volume of the organic phase. The reactions were incubated at 30 °C and 200 rpm on an orbital shaker for 24 h. The organic phase from the reaction mixture (*n*-heptane) was separated from the aqueous phase. The aqueous phase was further extracted with MTBE (2 x 500 µL). The combined organic phases (*n*-heptane + MTBE) were dried with $MgSO_4$. The conversions were analysed by GC-FID.

Table S13. Conversion [%] of **1a** and **1b** to enantiopure **2a** and **2b** by lyophilized cells of co-expressed Fus-SMO and *Cb*-FDH in 100 mL Erlenmeyer flask

Entry	Substrate [mM]	**2a** [%]	**2a [mM]**	**2b** [%]	**2b [mM]**
1	50	96.4±2.6	48.2±1.3	68.0±4.0	34.0±2.0
2	75	52.4±0.2	39.3±0.2	53.8±7.9	40.4±5.9
3	100	40.9±1.3	40.9±1.3	53.6±7.4	53.6±7.4
4	150	31.2±0.2	46.8±0.3	40.3±0.8	60.5±1.2
5	170	29.4±1.4	50.0±2.4	39.0±0.3	66.3±0.5
6	200	24.3±0.4	48.6±0.8	32.4±1.5	64.8±3.0
7	250	20.1±1.1	50.3±2.8	21.1±0.2	52.8±0.5
8	500	11.6±0.9	58.0±4.5	13.4±2.2	67.0±11.0
9	1000	4.7±0.2	47.0±2.0	6.6±0.2	66.0±2.0

Reactions were performed as duplicates; hence, the reported conversion is the average of 2 values. Error is expressed as the absolute difference between the two measurements.

8.4. *Study on the influence of the mode of dioxygen transfer in the biphasic biocatalytic epoxidation: sealed system with air headspace versus system with continuous flow (i.e. bubbling) of dioxygen*

General procedure for the epoxidation applying a sealed system with air headspace: Lyophilised whole cells containing co-expressed Fus-SMO and *Cb*-FDH (125 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 25 mL) in a 500 mL three necks round-bottom flask. NAD^+ (1 mM), FAD (50 µM), HCOONa (5 eq.), catalase (2 µM), *n*-heptane (25 mL) and the substrate **1a** (50 mM) were added. The concentration of cells, coenzyme and recycling enzyme are calculated on the volume of the aqueous phase, while the concentration of the substrate is calculated on the volume of the organic phase. A set of reactions was conducted at room temperature under magnetic agitation for 30 min, 45 min, 60 min and 24 h. A vigorous agitation (ca. 700-800 rpm) was set during the reaction in order to obtain an emulsion without a visible separation between the aqueous phase and the organic phase. In this way, we can minimize the mass transfer resistance of styrene from the organic phase to the aqueous phase as well as of styrene oxide in the contrary direction. Thus, any possible kinetic limitation in the mass transfer of dioxygen from the gas phase to the liquid phases can be studied under these conditions.

The rate of the epoxidation reaction expressed as mass of styrene oxide produced per minute is reported in Figure S10 (black line).

General procedure for the epoxidation applying continuous flow of dioxygen (bubbling): Lyophilised whole cells containing co-expressed Fus-SMO and *Cb*-FDH (125 mg, 5 mg mL^{-1}) were rehydrated in KPi buffer (pH 8, 50 mM, 25 mL) in a 100 mL three necks round-bottom flask. NAD^+ (1 mM), FAD (50 µM), HCOONa (5 eq.), catalase (2 µM), *n*-heptane (25 mL) and the substrate **1a** (50 mM) were added. The concentration of cells, coenzyme and recycling enzyme are calculated on the volume of the aqueous phase, while the concentration of the substrate is calculated on the volume of the organic phase. The flow of molecular oxygen was supplied to the reaction *via* a needle placed through a septum in one neck of the round-bottom flask. That permitted the bubbling of pure dioxygen (ca. 1 mL min^{-1}) directly into the reaction mixture. Another small needle was placed through another septum in the flask in order to prevent any overpressure. A set of reactions was conducted at room temperature under magnetic agitation for 30 min, 45 min, 60 min and 24 h. A vigorous agitation (ca. 700-800 rpm) was set during the reaction in order to obtain an emulsion without a visible separation between the aqueous phase and the organic phase. In this way, we can minimize the mass transfer resistance of styrene from the organic phase to the aqueous phase as well as of styrene oxide in the contrary direction. Thus, any possible kinetic limitation in the mass transfer of dioxygen from the gas phase to the liquid phases can be studied under these conditions.

The rate of the epoxidation reaction expressed as mass of styrene oxide produced per minute is reported in Figure S10 (grey line).

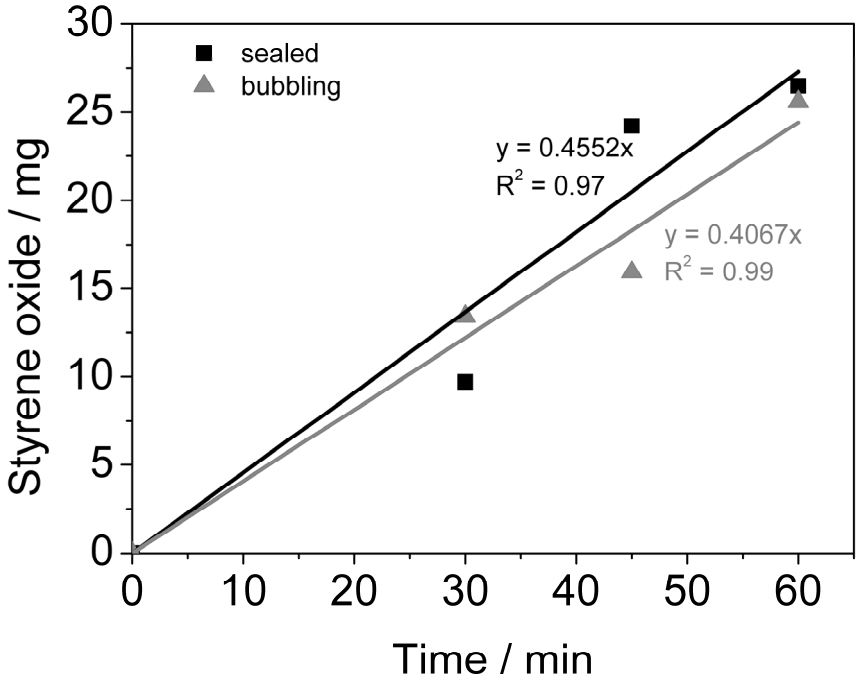


Figure S10. Comparing different modes of dioxygen supply: seal system with air headspace (black line) and continuous flow (grey line)

$$Relative\ difference\ in\ styrene\ oxide\ productivity = \frac{0.4552 - 0.4067}{0.4552} \times 100 = 10\%$$

9. Analytical methods

9.1. *Method for the determination of the conversion by GC-FID*

Column: Agilent J&W DB1701 (30 m, 250 µm, 0.25 µm). Carrier gas: H_2

Parameter: T injector 250 °C; constant pressure 14.50 psi; temperature program: 80 °C, hold 6.5 min; gradient 10 °C min^{-1} up to 160 °C, hold 5 min; gradient 20 °C min^{-1} up to 200 °C, hold 2 min; gradient 20 °C min^{-1} up to 280 °C, hold 1 min.

9.2. *Method for the determination of the enantiomeric excess (ee) and diastereomeric excess (de) by chiral HPLC*

Column: Daicel Chiralcel OD (0.46 cm x 25 cm)

HPLC program: constant oven temperature 25 °C; eluent composition: isocratic Hexane/Isopropanol 99:1; flow rate: 0.5 mL min^{-1}.

The absolute configuration of the products (*S*)-**2a** and (1*S*,2*S*)-**2b** were identified by comparison with authentic optically active reference compounds.

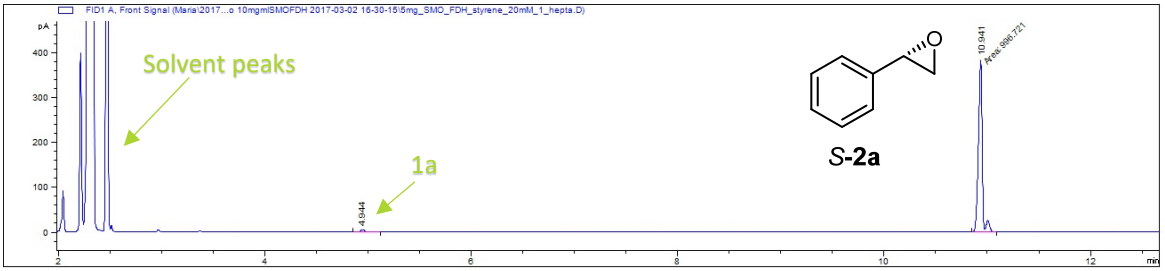
10. GC and HPLC chromatograms and NMR spectra

10.1. *Characterization of (S)-**2a** in analytical and preparative scale*

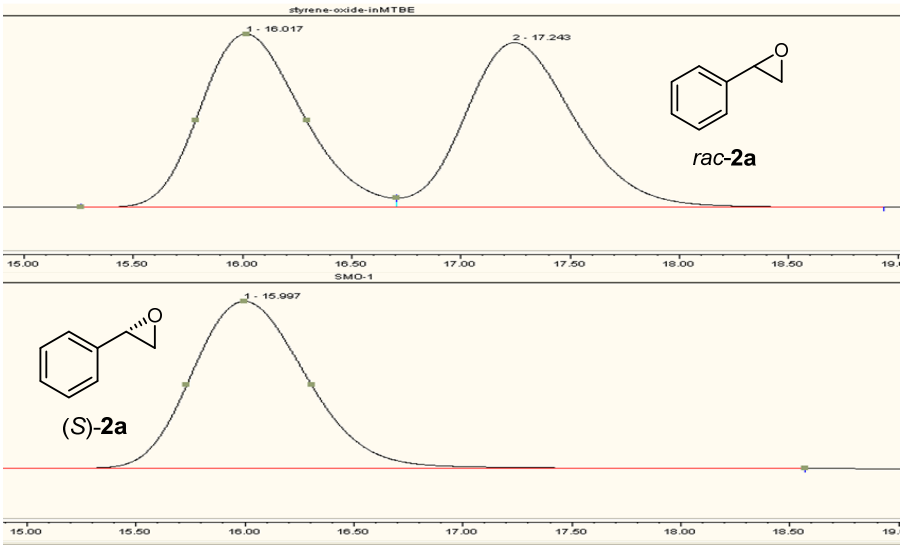
Analytical scale: conversion of **1a** (20 mM) to (*S*)-**2a** by lyophilized whole *E.coli* BL21 (DE3) overexpressing Fus-SMO

Note: product (*S*)-**2a** seems to be split in two peaks. By GC-MS and NMR, we have determined that the second apparent peak is still the same product (*S*)-**2a**. The same applies to all the chromatograms reported below.

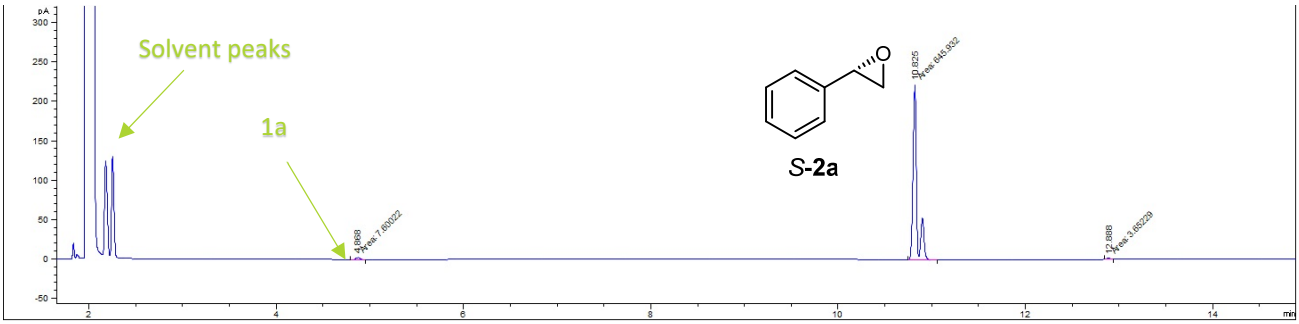
Analytical scale: conversion of **1a** (20 mM) to (*S*)-**2a** by lyophilized whole *E.coli* BL21 (DE3) co-expressing Fus-SMO and Cb-FDH



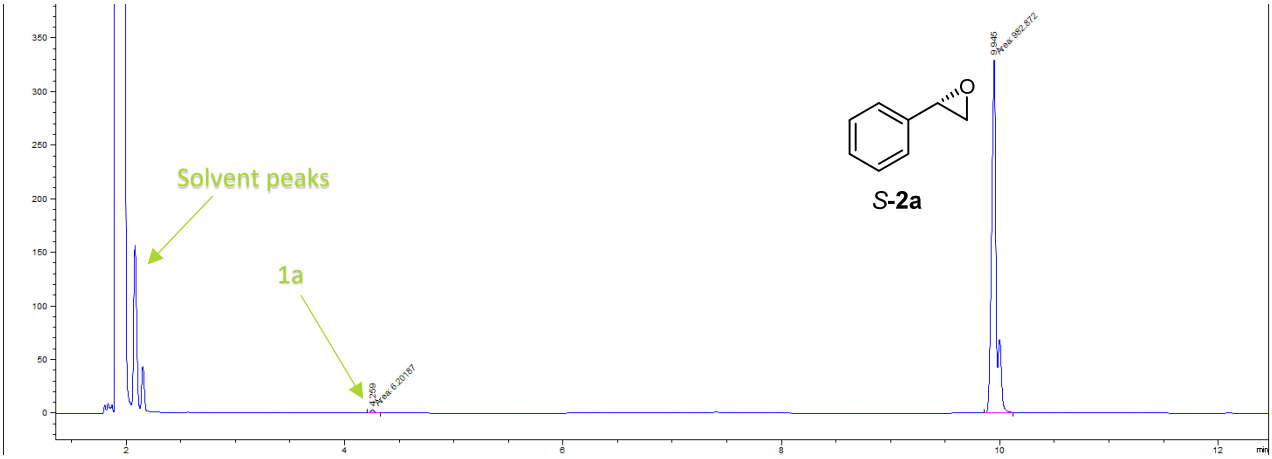
Determination of *ee* for (*S*)-**2a** by chiral HPLC: conversion in analytical scale of **1a** (20 mM) to (*S*)-**2a**



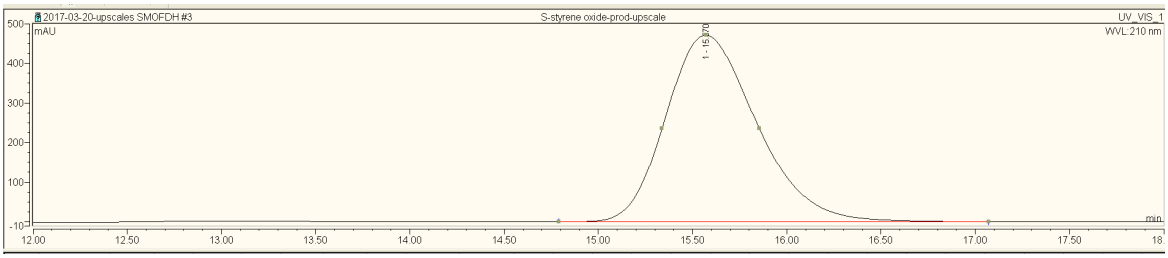
First preparative scale experiment: conversion of **1a** (20 mM) to (*S*)-**2a** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH.



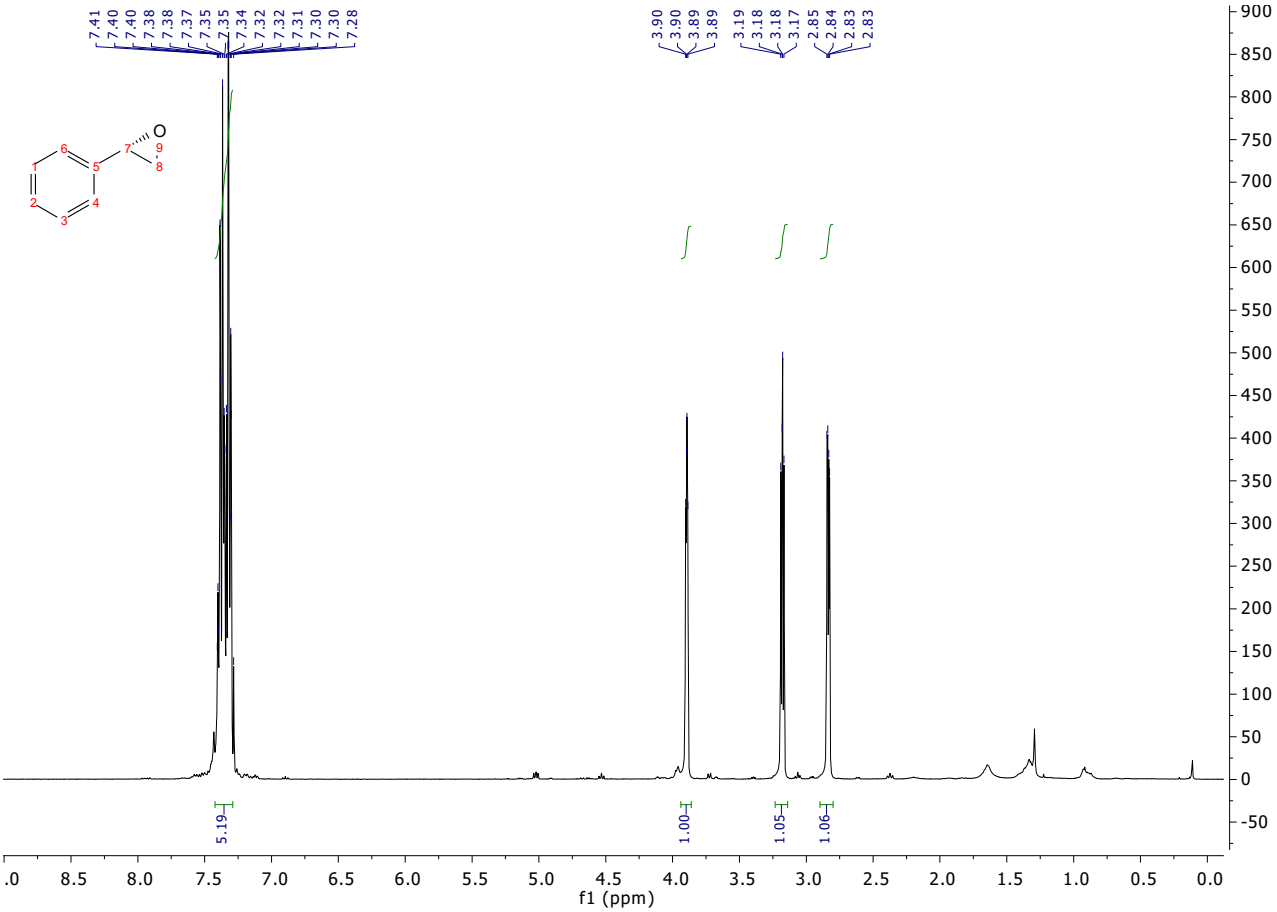
Second preparative scale experiment: conversion of **1a** (50 mM) to (*S*)-**2a** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH.



Determination of *ee* for (*S*)-**2a** by chiral HPLC: conversion in preparative scale of **1a** to (*S*)-**2a** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH.

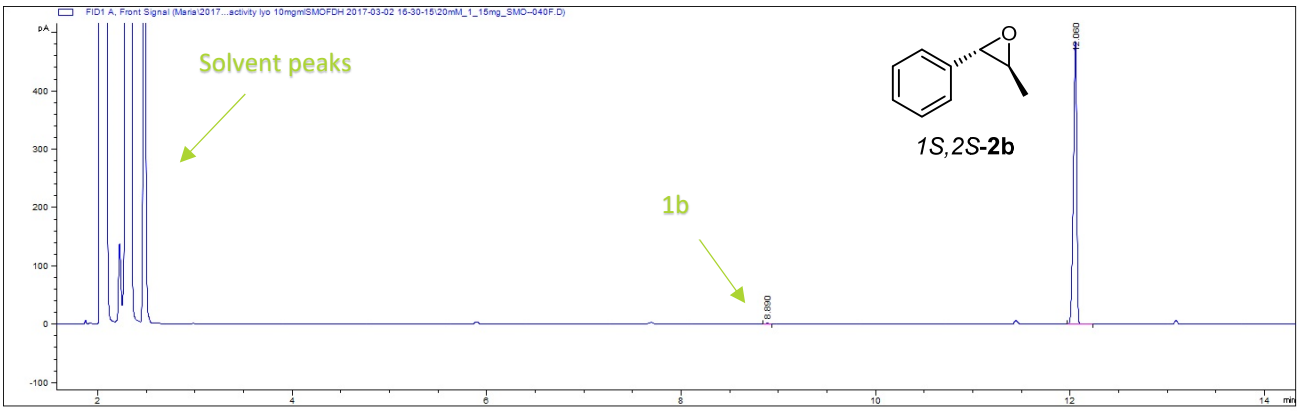


^{1}H-NMR: preparative scale bio-conversion of **1a** to (*S*)-**2a** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH.

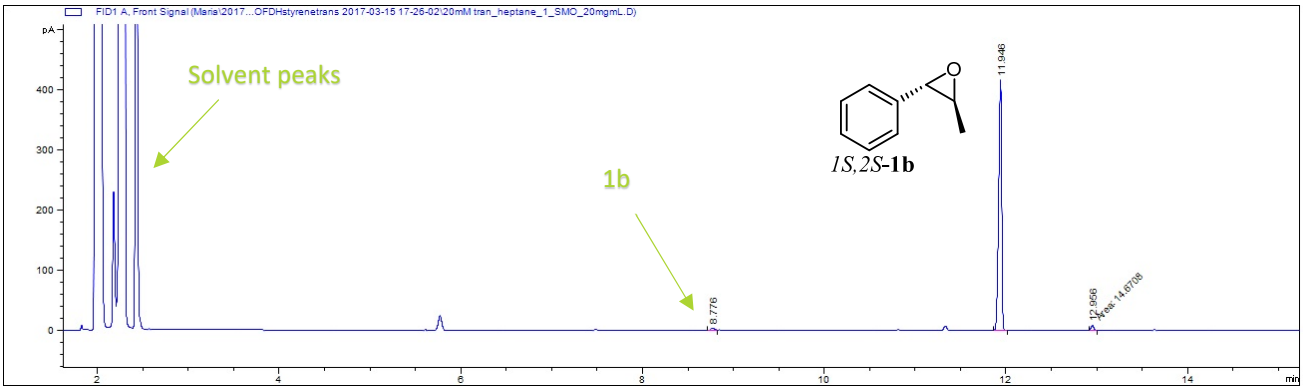


10.2. *Characterization of* (*1S,2S*)-***2b*** *in analytical and preparative scale*

Analytical scale: conversion of **1b** (20 mM) to **2b** by lyophilized *E.coli* BL21 DE3 overexpressing Fus-SMO

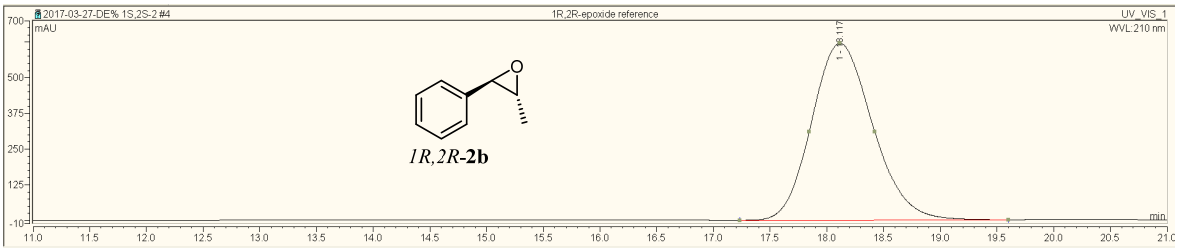


Analytical scale: conversion of **1b** (20 mM) to **2b** by lyophilized *E.coli* BL21 (DE3) co-expressing Fus-SMO and *Cb*-FDH

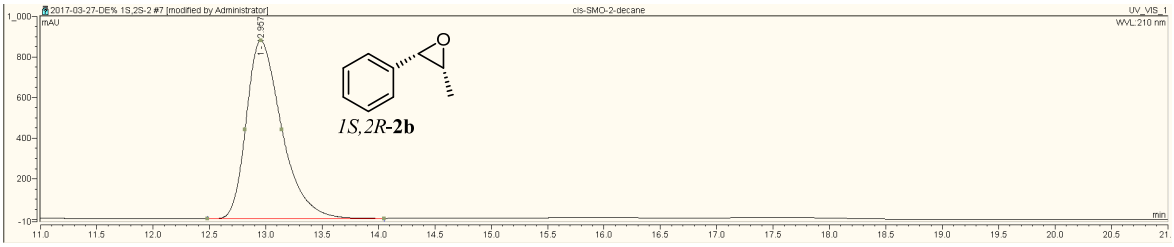


Determination of *de* and *ee* for (1*S*,2*S*)-**2b** by chiral HPLC for the conversion of **2b** (20 mM) in analytical scale: a) (*1R,2R*)-**2b** reference purchased from Sigma Aldrich; b) (*1S,2R*)-**2b** obtained from the enzymatic epoxidation of *cis*-β-methyl styrene (**1c**); c) (*1S,2S*)-**2b** reference purchased from Sigma Aldrich; d) (*1S,2S*)-**2b** obtained from the enzymatic epoxidation of **1b**.

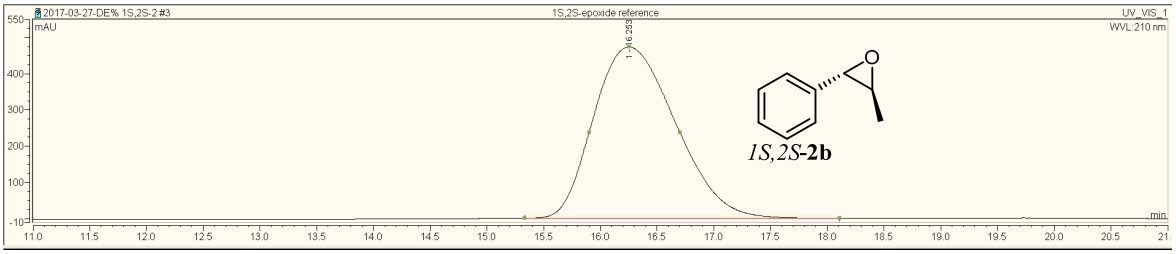
a) (*1R,2R*)-**2b** reference



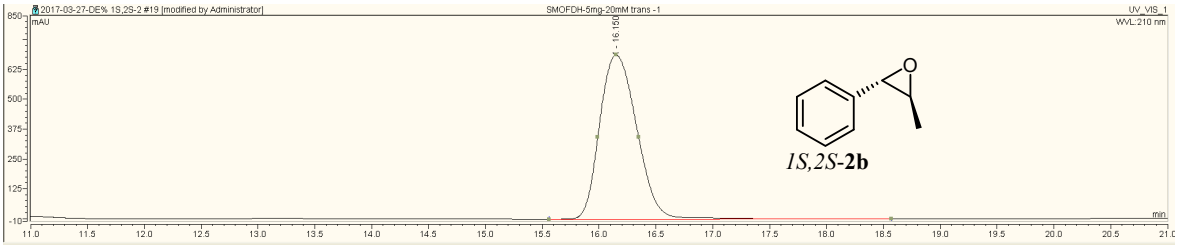
b) (*1S,2R*)-**2b** reference



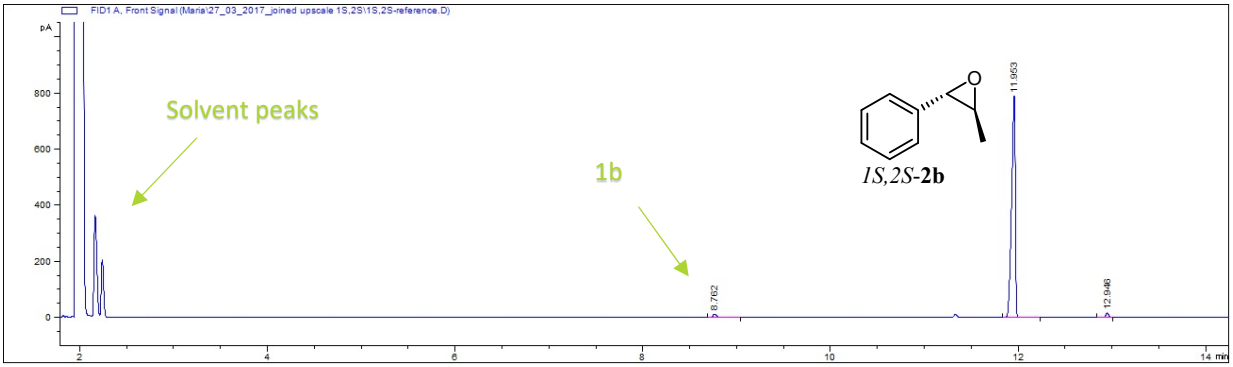
c) (*1S,2S*)-**2b** reference



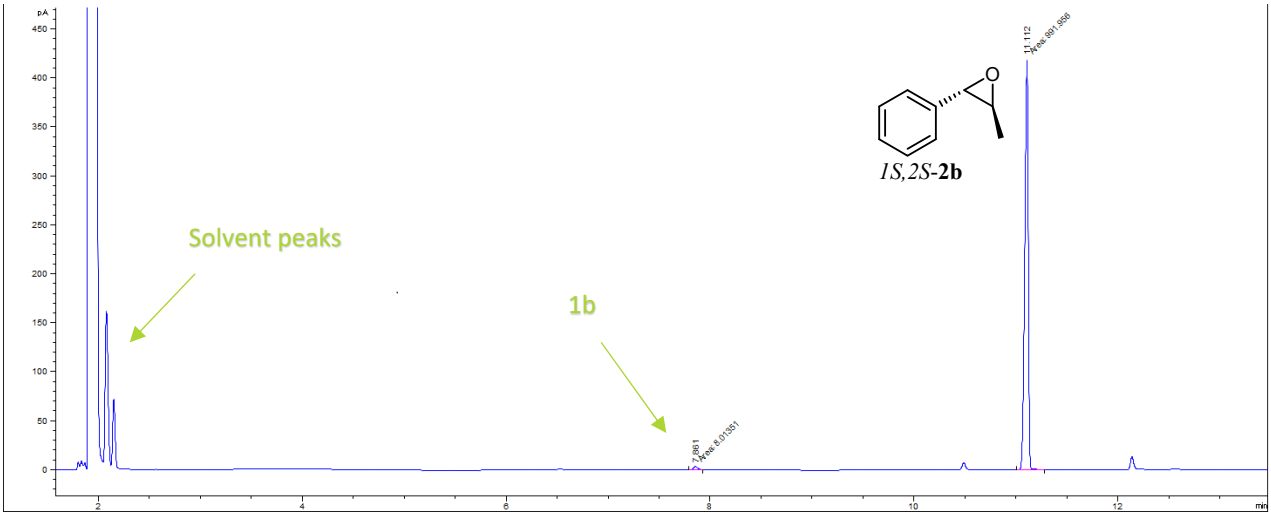
d) (*1S,2S*)-**2b** analytical scale



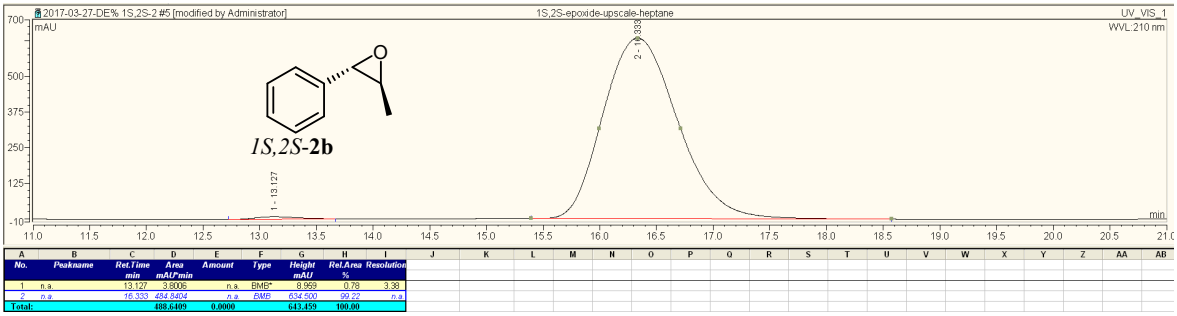
First preparative scale experiment: conversion of **1b** (20 mM) to (*1S,2S*)-**2b** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH



Second preparative scale experiment: conversion of **1b** (50 mM) to (*1S,2S*)-**2b** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH

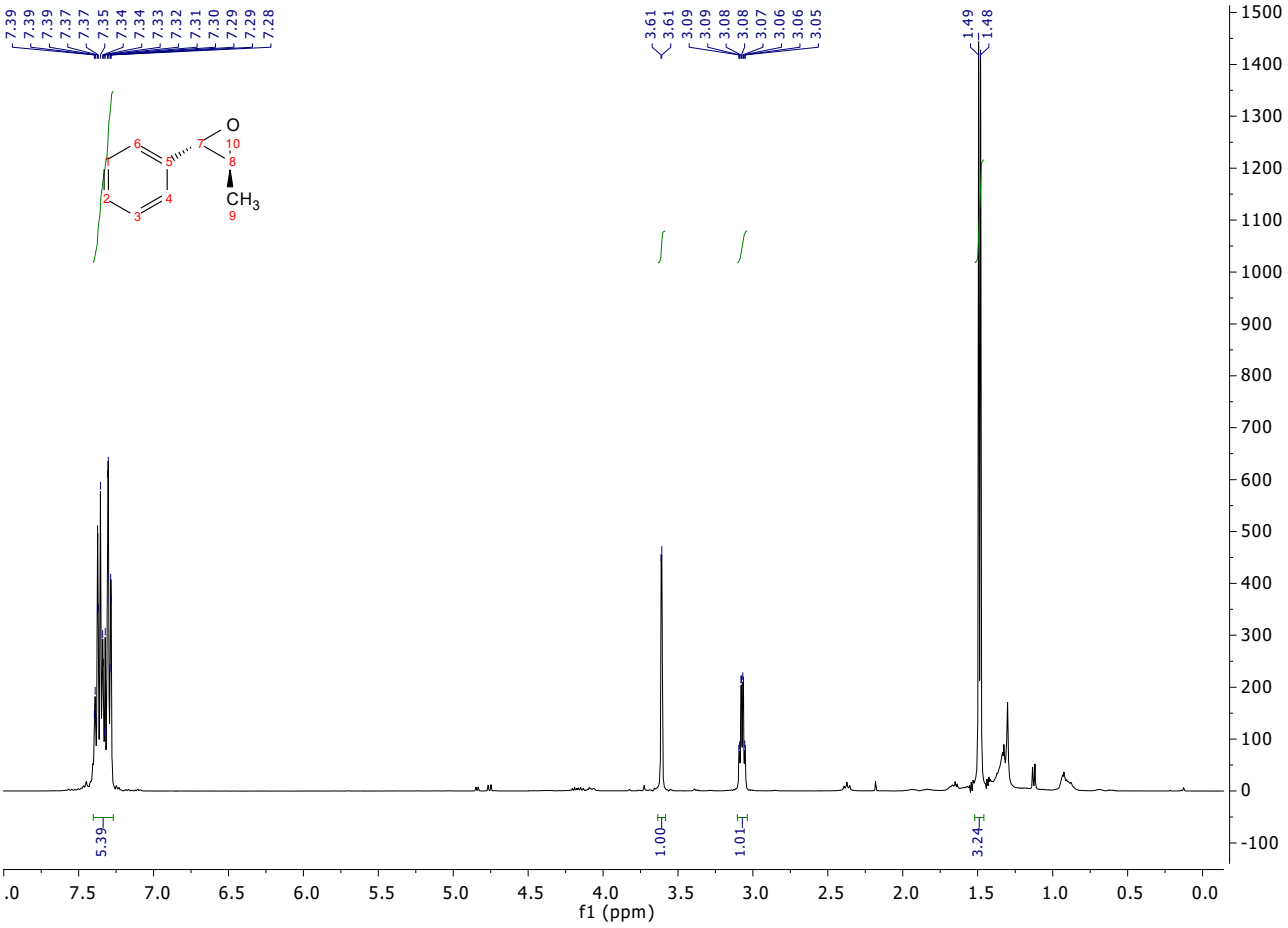


Determination of *de* and *ee* for (1*S*,2*S*)-**2b** by chiral HPLC for the conversion of **1b** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH (preparative scale)



No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	13.127	3.8006	n.a.	BMB*	8.959	0.78	3.38
2	n.a.	16.333	484.8404	n.a.	BMB	634.500	99.22	n.a.
Total:			488.6409	0.0000		643.459	100.00	

^{1}H-NMR preparative scale: bio-conversion of **1b** to (1*S*,2*S*)-**2b** by lyophilized *E.coli* BL21 DE3 co-expressing Fus-SMO and *Cb*-FDH.



11. References

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[2] a) S. Panke, B. Witholt, A. Schmid, M. G. Wubbolts, *Appl. Environ. Microbiol.* **1998**, *64*, 2032-2043; b) S. Panke, V. de Lorenzo, A. Kaiser, B. Witholt, M. G. Wubbolts, *Appl. Environ. Microbiol.* **1999**, *65*, 5619-5623; c) S. Panke, M. G. Wubbolts, A. Schmid, B. Witholt, *Biotechnol. Bioeng.* **2000**, *69*, 91-100.

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