SUPPORTING INFORMATION for

Photoenzymatic epoxidation of styrenes

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Table of Contents

1. Chemicals

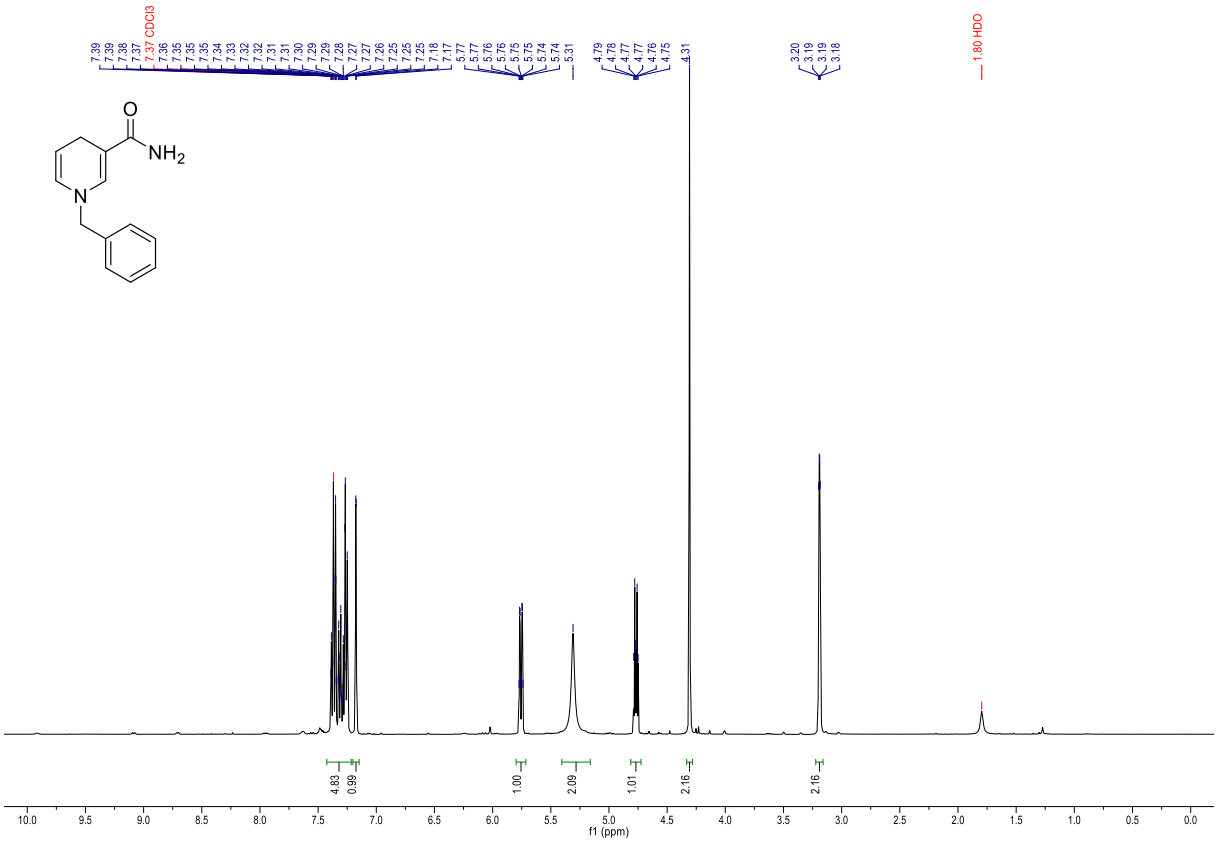
All chemicals were obtained from Sigma Aldrich in the highest purity available and used without further treatment. BNAH and the styrene oxides were synthesized following published procedures,[1,2] with the exception of styrene oxide, 4-fluorostyrene oxide, 4-chlorostyrene and 4-bromostyrene oxide, which were commercially available.

1.1 Synthesis of BNAH

1-Benzyl-1,4-dihydronicotinamide (BNAH) was synthesized exactly as previously described [1]. Briefly, a reaction mixture containing benzyl **bromide (1.1 equivalents**) and nicotinamide in acetonitrile (1 M) was refluxed overnight. After cooling, diethyl ether was added, the resulting white precipitate was filtered and further washed with diethyl ether, obtaining 1-benzyl-3-carbamoylpyridinium bromide. The reduction was carried out in distilled water with Na_2CO_3 (3 **equivalents**) and $Na_2S_2O_4$ (5 **equivalents**) at room temperature over 3 hours. The yellow precipitate formed was filtered, recrystallized in methanol-water, filtered and washed with cooled distilled water, and dried over P_2O_5 in a vacuum desiccator. The 1H NMR spectrum was recorded on a Bruker Avance III 400 spectrometer.

1-Benzyl-1,4-dihydronicotinamide BNAH

1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.22 (m, 5H), 7.17 (d, *J* = 1.7 Hz, 1H), 5.76 (ap dq, *J* = 8.1, 1.7 Hz, 1H), 5.31 (br s, 1H), 4.77 (dt, *J* = 8.0, 3.4 Hz, 1H), 4.31 (s, 2H), 3.19 (dd, *J* = 3.6, 1.7 Hz, 2H).



The synthesis of racemic epoxides was carried out as previously described [2]: the styrene derivative (2 mmol) was diluted in CH_2Cl_2 (10 mL) and mixed with distilled water (10 mL) containing $NaHCO_3$ (1 g); *m*-CPBA (2.2 mmol) was slowly added. The reaction mixture was stirred at room temperature for 3 h. The reaction was quenched with aqueous Na_2SO_3 (1.3 g in 10 mL) and left to stir for 20 min. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic phases were washed with $NaHCO_3$ (2 × 25 mL) and distilled water (25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to provide the product. The epoxide products were not further purified as NMR and GC/MS analyses were in agreement with literature[2]. Small peaks of impurities may appear on the GC chromatograms of the racemic epoxides.

2. Catalyst preparation

2.1. Cultivation of E. coli JM101 pSPZ10

Styrene monooxygenase A (StyA) from *Pseudomonas* sp. strain VLB120 production was based on a previous protocol.[3] 5 ml of autoclaved LB media containing 50 µg ml^{-1} kanamycin and 10 mg ml^{-1} glucose (both filter sterilized) were inoculated with E. coli JM101, with the pSPZ10 plasmid containing StyA genes, from a glycerol stock and incubated overnight. According to the original protocol, overexpression was performed in 2 l flasks with 0.5 l of autoclaved M9* medium. The medium was supplemented with 5 g l^{-1} glucose, 50 µg ml^{-1} kanamycin, 0.1 % (v/v) 1000x US* trace element solution, 1 mM $MgSO_4$ and 0.010 g l^{-1} thiamine HCl, which were all filter sterilized. The compositions of M9* medium and US* trace elements are shown below. The cells were grown at 30 °C while shaking at 250 rpm. StyA gene overexpression was induced by addition of 0.05% (v/v) dicyclopropyl ketone at an A_{450} of 0.4 AU. After 16 h of further incubation, the cells were harvested by centrifugation (15 min at 11 300 x g) and resuspended in 20 mM Tris buffer pH 7.5 containing 1 mM DTT, 1 mM $MgCl_2$ and 10 % (v/v) glycerol. The solutions were stored at -80 °C until further use.

M9* Minimal medium

18.0 g L^{-1} KH_2PO_4
9.0 g L^{-1} K_2HPO_4
0.5 g L^{-1} NaCl
1.0 g L^{-1} NH_4Cl

Adjust pH to 7.4

US* Trace elements

10 mM HCl
1.50 g L^{-1} MnCl
1.05 g L^{-1} $ZnSO_4$
0.30 g L^{-1} H_3BO_3
0.25 g L^{-1} $Na_2MoO_4 – 2\ H_2O$
0.15 g L^{-1} $CuCl – 2\ H_2O$
0.84 g L^{-1} $Na_2EDTA – 2\ H_2O$
4.87 g L^{-1} $FeSO_4 – 7\ H_2O$
4.12 g L^{-1} $CaCl_2 – 2\ H_2O$

2.2. Purification

0.1 mg ml^{-1} of DNase was added to the resuspended cell pellet. The solution was then passed twice through a cooled French press (1.9 bar, using a multi-shot Constant Cell Disruption Systems) and centrifuged (20 min at 11300 x g and 4 °C). The supernatant was collected and subsequently concentrated using Amicon Ultra centrifugal filter units (15 ml, 30 kDa MWCO) and was either frozen in liquid nitrogen in order to obtain the cell free extract sample, or further purified. A 5 ml HiTrap QFF column (GE Healthcare, UK) was used for the purification. StyA was eluted with a linear NaCl gradient (0 – 0.5 M elution at 0.36 M) using a 20 mM Tris buffer at pH 7.5. Samples containing StyA were pooled, desalted, concentrated and frozen in liquid nitrogen. The protein concentrations of both the cell free extract and the purified StyA were determined using a Bradford assay. The purity of the extracts was checked *via* a protein gel (SDS 12% PAGE)(Figure S1). 18% of the StyA activity remained. A purification table is shown in Table S1. Monooxygenase activity was confirmed (not quantified) *via* GC by the conversion of styrene to styrene oxide by the protein sample using the direct reduction of FAD by a NADH mimic (BNAH).[2] Reaction mixtures consisted of 1.7 mg mL^{-1} of the cell free extract, 5 mM styrene, 100 µM FAD, 600 U ml^{-1} of catalase and 10 mM BNAH (from a 1

M stock in DMSO) in a 100 mM KPi buffer at pH 7.0 at 30 °C. Frozen protein samples were stored at -80 °C.

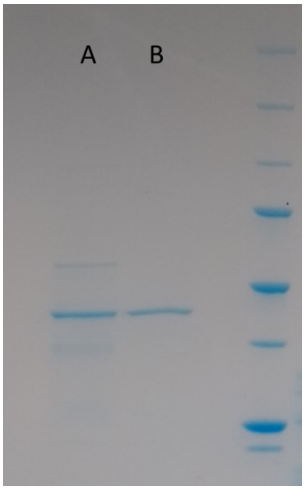


Figure S1: SDS PAGE of cell free extract containing StyA (lane A) and technically pure StyA (lane B).

Table S1: Purification table for the production of technically pure StyA.

Purification step	Total protein content (mg)
Cell free extract	115
Cell free extract > 30 kDa	81.1
Technically pure StyA	12.7

2.3. Reaction procedure

In general, reaction mixtures contained 5 mM substrate, 6.25% (v/v) DMSO, 20 µM StyA, 200 µM FAD, 20 mM EDTA and 600 U ml^{-1} catalase in a 100 mM KPi buffer at pH 7.0. 500 µl reactions solutions were incubated in 1.5 ml glass screw cap vails held upside down in a temperature controlled water bath. The mixture was stirred by a 6 mm Teflon magnetic bar at 300 rpm. The vials were illuminated through the bottom of the glass vials using a lightningcure^{tm} LC8 - L9588 spot light source (Hamamatsu, Japan). In general, the intensity of the lamp was set to 20%. A schematic representation of the setup is shown in Figure S2. Samples were extracted by injecting 250 µl of ethyl acetate, containing 5 mM of dodecane, directly in the vial using a syringe. This method minimized the evaporation of the volatile styrenes. The organic phase was dried over $MgSO_4$, mixed for several seconds, centrifuged and subsequently analysed by gas chromatography.

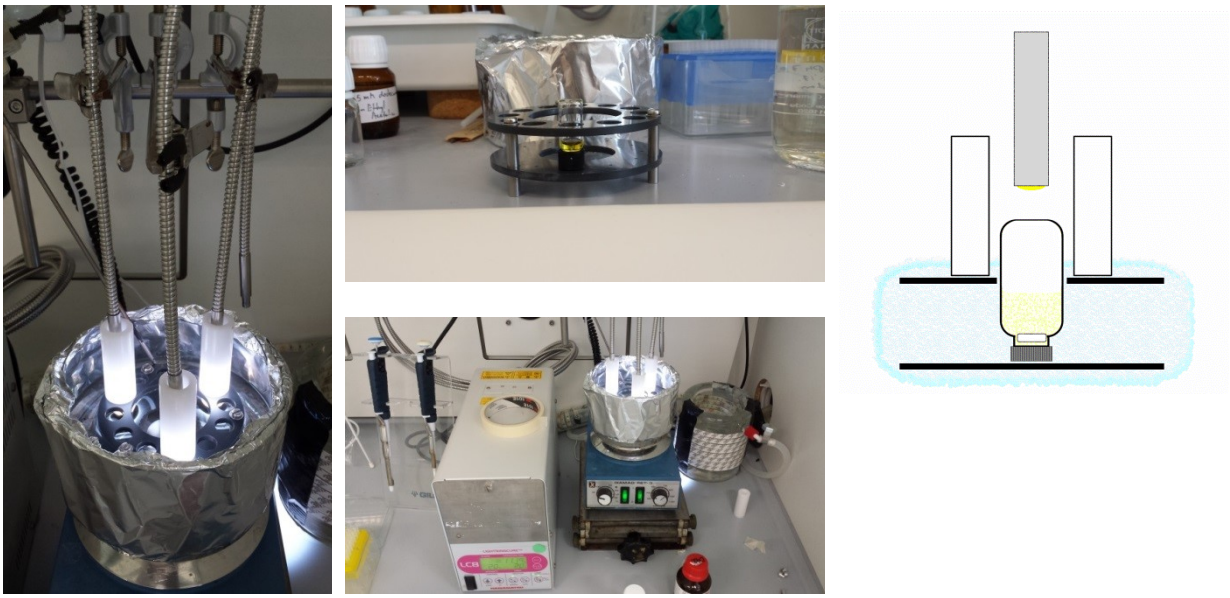


Figure S2: Representation of the photoreactor setup.

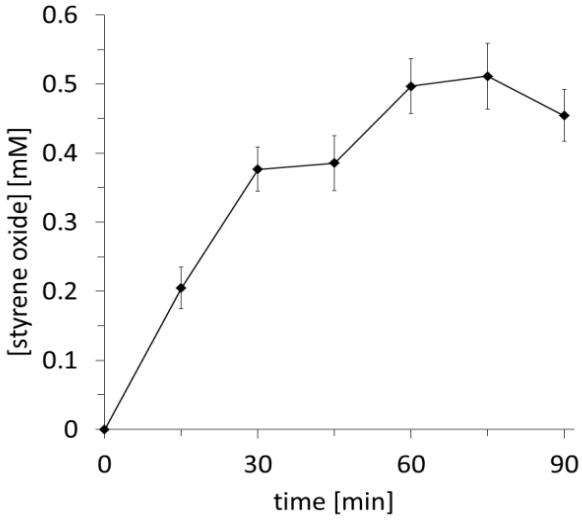


Figure S3: Time-course of the first photobiocatalytic epoxidation of styrene to (S)-styrene oxide. General conditions: [styrene]$_0$ = 5 mM, [cell free extract] = 1.7 g l^{-1} containing 4 µM StyA, [FAD] = 200 µM, [EDTA] = 20 mM, catalase = 600 U ml^{-1}, 100 mM KPi buffer pH 7.0, 30 °C, stirring at 300 rpm, light intensity of 20%. Error bars show the standard deviation for three independent experiments.

3. Gas chromatography

Measurements were performed on Shimadzu GC-2010 Plus gas chromatographs with an AOC-20i Auto injector with FID (Shimadzu, Japan), using hydrogen as the carrier gas. The following columns were used:

Column A: Chirasil Dex CB: length: 25 m, inner diameter: 0.32 mm, film thickness: 0.25 µm

Column B: Hydrodex ß-6TBDM, length: 50 m, inner diameter: 0.25 mm, film thickness: 0.15 µm

Column C: Lipodex E, length: 50 m, inner diameter: 0.25 mm, film thickness: 0.25 µm

The calibration curve of 4-chlorostyrene was used for the quantification of 3-chlorostyrene. The calibration curve of *trans*-ß-methylstyrene was used for the quantification of 3-methylstyrene and α-methylstyrene.

3.1. Styrene – styrene oxide

Method: Column A

Ramp [°C min^{-1}]	Temperature [C]	Hold time [min]
-	100	12.5
20	225	1

Retention times

Compound	Retention time (min)
Styrene	3.4
DMSO	3.6
S-Styrene oxide	8.6
R-Styrene oxide	9.1
Phenylacetaldehyde	9.7
Dodecane	11.0

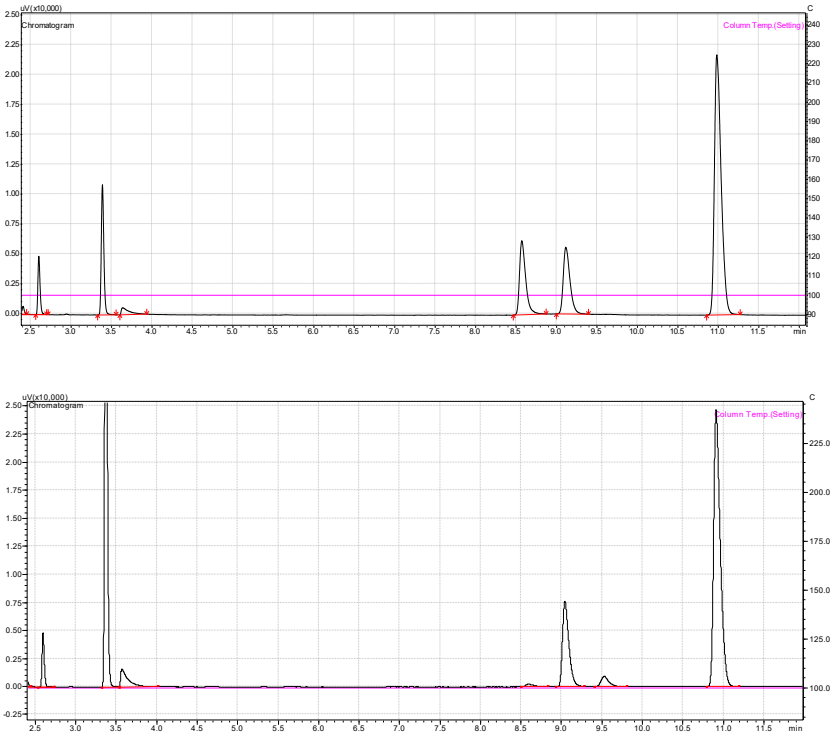


Figure S4: Gas chromatograms of styrene and racemic styrene oxide (top) and of a reaction sample (bottom).

3.2. 3-Methylstyrene – 3-methylstyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	100	24
20	220	1

Retention times

Compound	Retention time (min)
3 methyl Styrene	6.1
Dodecane	8.7
Synthesis by-product	16.0
S-3 methyl Styrene oxide	20.3
DMSO	21.6
R-3 methyl Styrene oxide	22.6

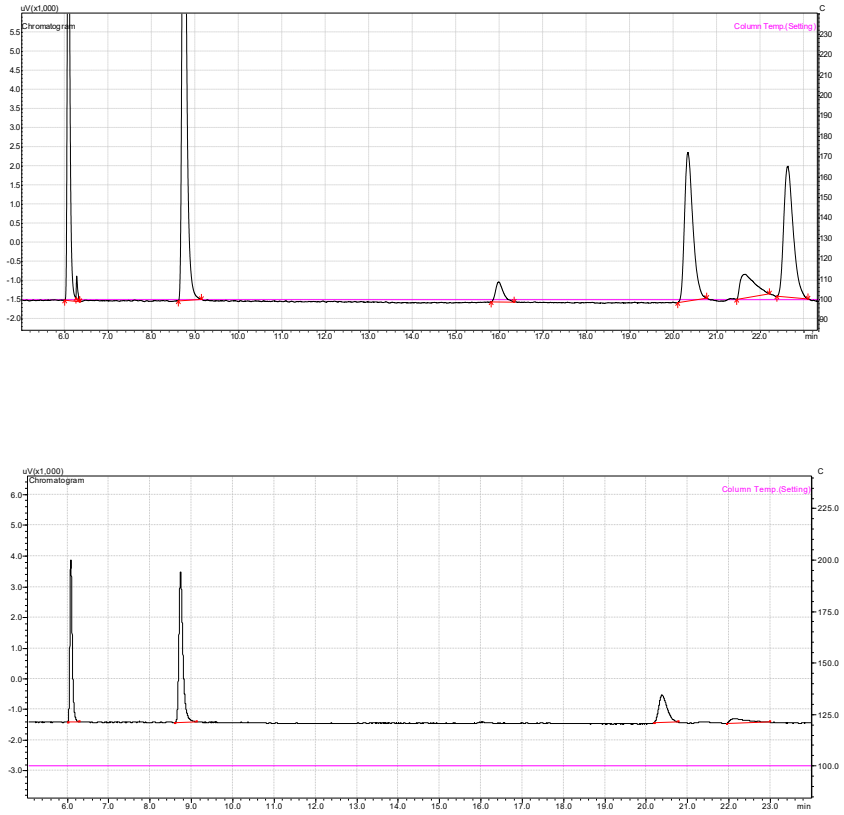


Figure S5: Gas chromatograms of 3-methylstyrene and racemic 3-methylstyrene oxide (top) and of a reaction sample (bottom).

3.3. 4-Fluorostyrene – 4-fluorostyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	100	15
20	220	1

Retention times

Compound	Retention time (min)
4 Fluoro styrene	4.5
Isomerisation by-product	8.0
Dodecane	8.7
S-4 Fluoro styrene oxide	11.9
Product impurity	12.8
R-4 Fluoro styrene oxide	13.7

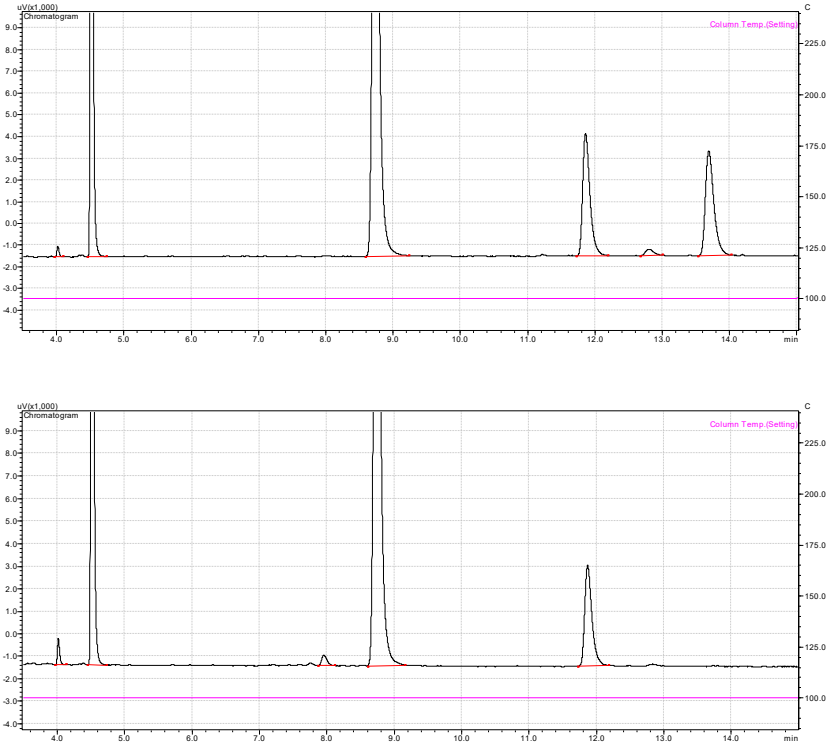


Figure S6: Gas chromatograms of 4-fluorostyrene and racemic 4-fluorostyrene oxide (top) and of a reaction sample (bottom).

3.4. 3-Chlorostyrene – 3-chlorostyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	120	23
20	220	1

Retention times

Compound	Retention time (min)
Dodecane	5.5
3 Chloro styrene	6.0
DMSO	9.0
Synthesis by-product	11.2
S-3 Chloro styrene oxide	16.7
R-3 Chloro styrene oxide	21.9

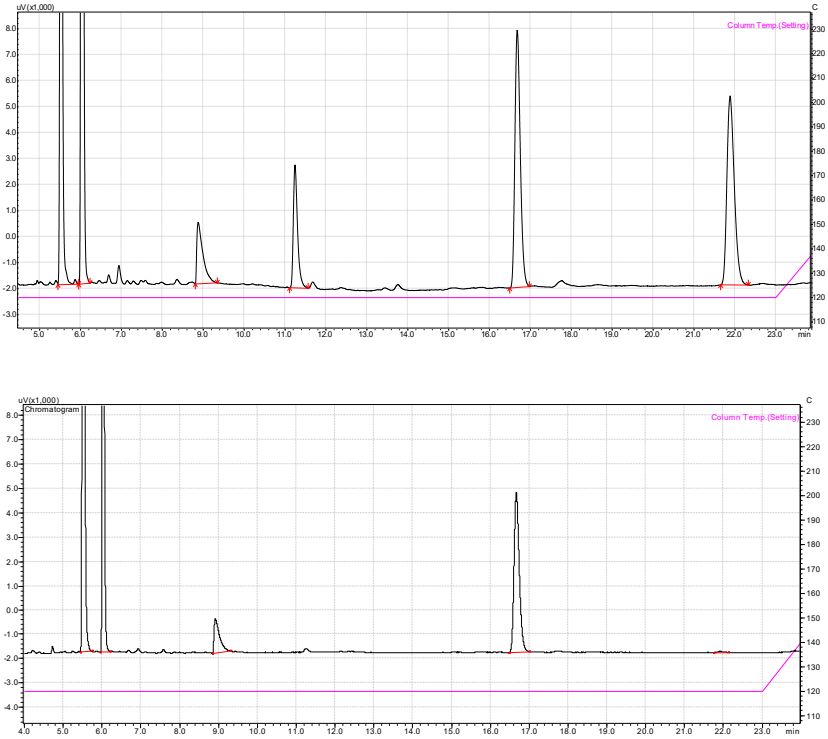


Figure S7: Gas chromatograms of 3-chlorostyrene and racemic 3-chlorostyrene oxide (top) and of a reaction sample (bottom).

3.5. 4-Chlorostyrene – 4-chlorostyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	110	13
5	120	7
20	220	1

Retention times

Compound	Retention time (min)
Dodecane	6.8
4- Chloro styrene	7.5
DMSO	13.4
Isomerisation by-product	13.8
S 4-Chloro styrene oxide	20.2
R 4- Chloro styrene oxide	20.7

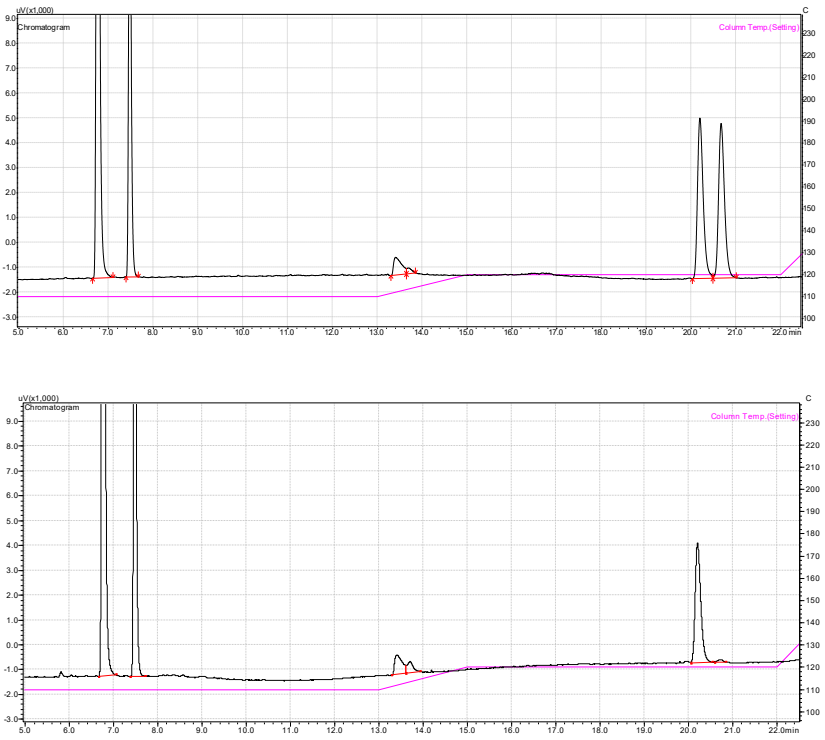


Figure S8: Gas chromatograms of 4-chlorostyrene and racemic 4-chlorostyrene oxide (top) and of a reaction sample (bottom).

3.6. 4-Bromostyrene – 4-bromostyrene oxide

Method: Column B

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	100	4
15	175	2.2
10	205	2
25	250	2

Retention times

Compound	Retention time (min)
Dodecane	9.9
4-Bromo styrene	11.1
R 4-Bromo styrene oxide	14.2
S 4-Bromo styrene oxide	14.9

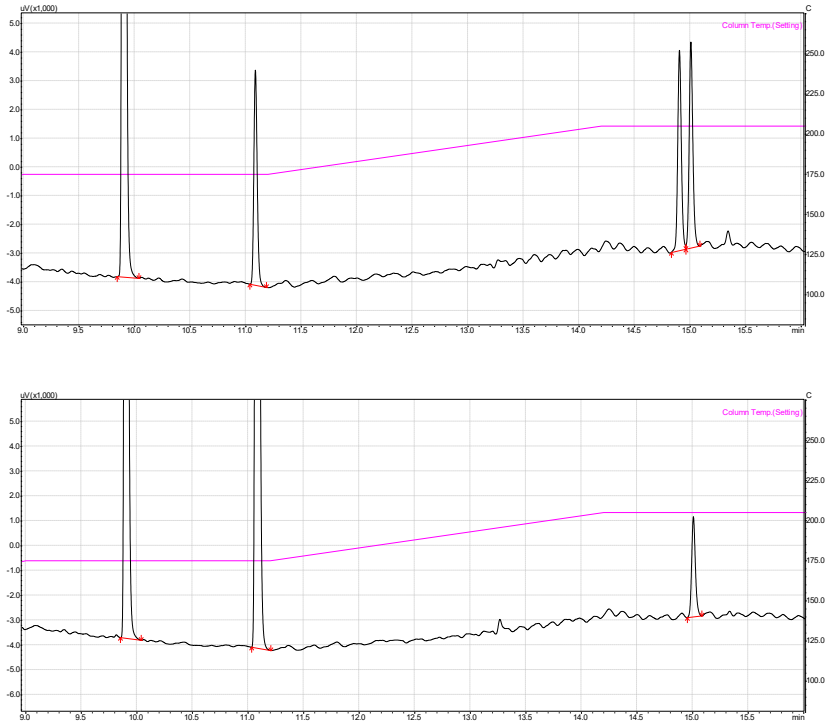


Figure S9: Gas chromatograms of 4-bromostyrene and racemic 4-bromostyrene oxide (top) and of a reaction sample (bottom).

3.7. trans-ß-Methylstyrene - trans-ß-methylstyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	100	15
20	220	1

Retention times

Compound	Retention time (min)
Trans ß methyl styrene	6.8
Dodecane	8.5
Synthesis by-product	9.5
S S Trans ß methyl styrene oxide	11.9
R R Trans ß methyl styrene oxide	13.1

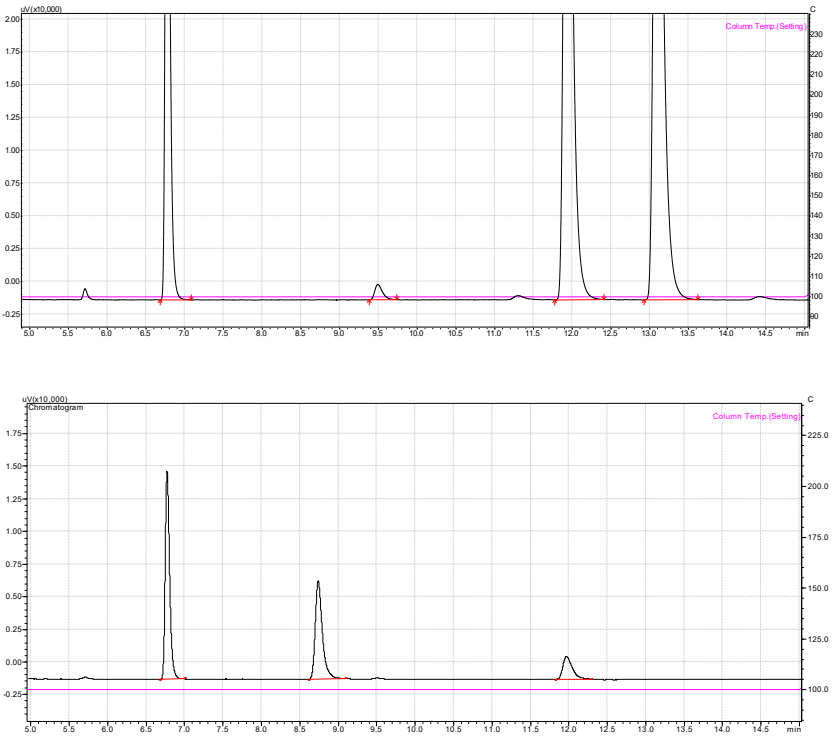


Figure S10: Gas chromatograms of trans-ß-methylstyrene and racemic trans-ß-methylstyrene oxide (top) and of a reaction sample (bottom).

3.8. α-Methylstyrene - α-methylstyrene oxide

Method: Column C

Ramp [°C min^{-1}]	Temperature [°C]	Hold time [min]
-	75	42
20	220	1

Retention times

Compound	Retention time (min)
α methyl styrene	11.1
Dodecane	22.8
S α methyl styrene oxide	38.4
R α methyl styrene oxide	39.9

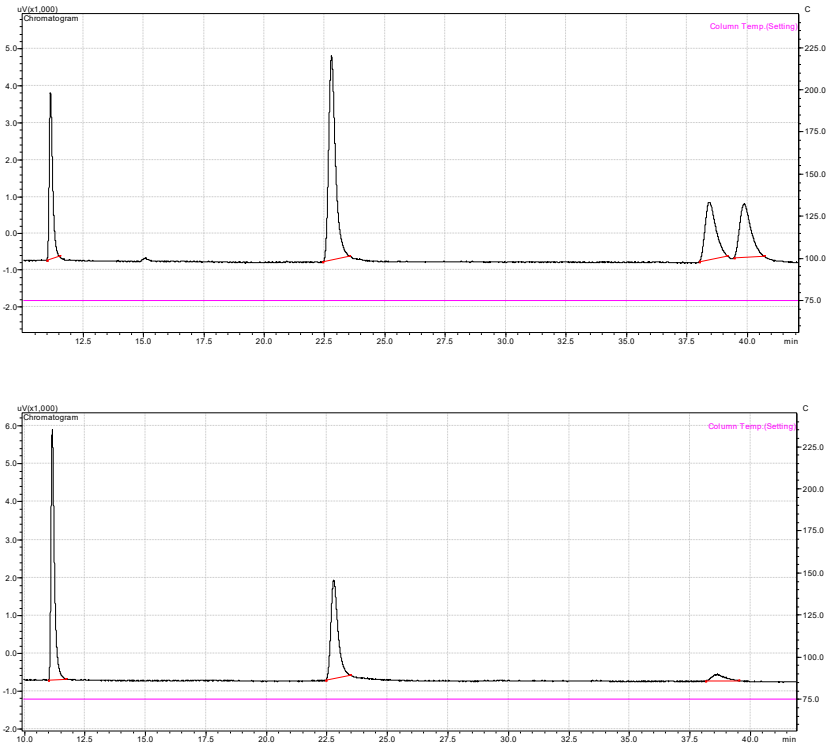


Figure S11: Gas chromatograms of α-methylstyrene and racemic α-methylstyrene oxide (top) and of a reaction sample (bottom).

4. Catalase activity assay

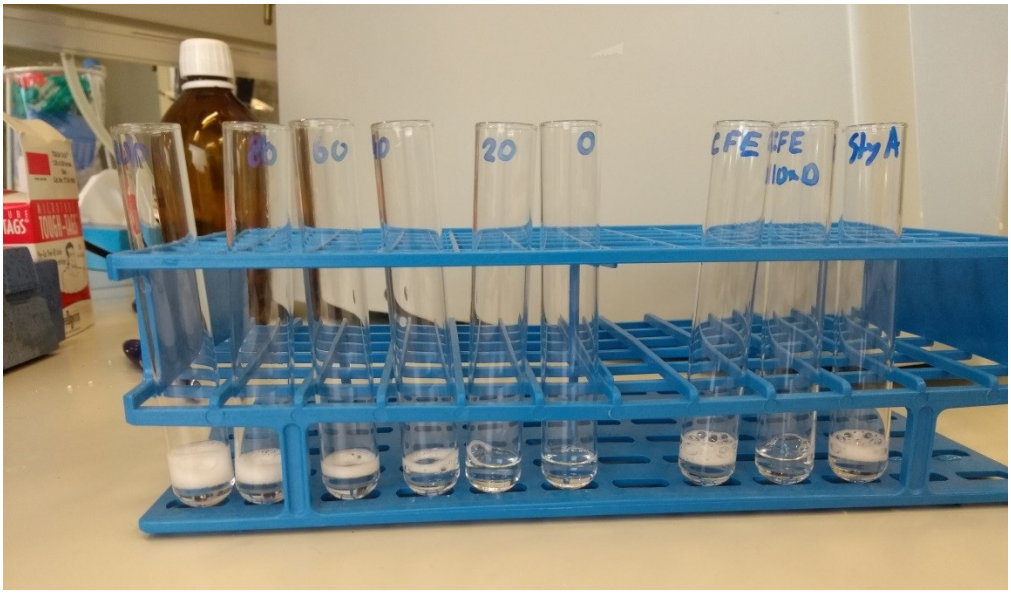


Figure S12: Catalase activity assay, performed as reported by Iwase *et al* [4]. Reaction mixtures all contained 10% H_2O_2 and 0.33 % Triton X-100 in 600 µl water. To those test tubes we further added 300 µL of 1000 to 0 U mL^{-1} catalase (first six test tubes), cell free extract (1.1 mg mL^{-1} and 0.11 mg mL^{-1}) or technically pure StyA (55 µM). Catalase would dismutate the hydrogen peroxide forming oxygen causing foam with the Triton X-100. These experiments show the presence of catalase already in the cell free extract and the technically pure StyA.

5. Photostability of the catalysts

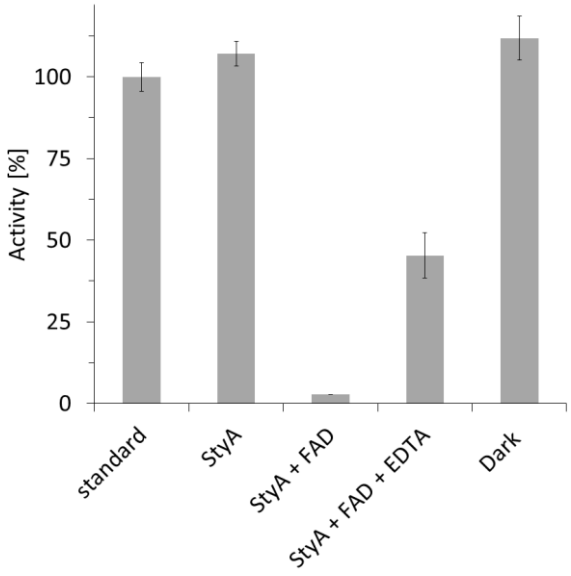


Figure S13. Control reactions to determine the robustness of the system. 1: Standard reaction. 2: StyA illuminated for 30 min before starting the reaction. 3: StyA and FAD illuminated for 30 min before starting the reaction. 4: StyA, FAD and EDTA illuminated for 30 min before starting the reaction. 5: Total reaction mixture incubated in the dark for 30 min before starting the reaction. General conditions: $[styrene]_0$ = 5 mM, [StyA] = 20 µM, [FAD] = 200 µM, [EDTA] = 20 mM, catalase = 600 U ml^{-1}, 100 mM KPi buffer (pH 7), 30 °C, stirring at 300 rpm, light intensity of 20% for 1 h. 100% corresponds to a product formation rate of 0.47 mM h^{-1}. Error bars show the standard deviation for three independent experiments.

6. Spectra

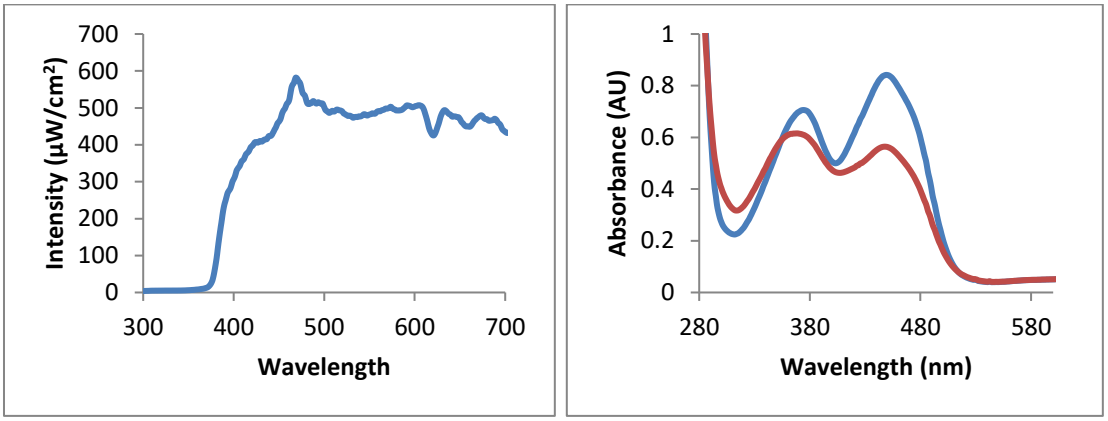


Figure S14: Left: Emission spectrum of the Hamamatsu light setup at 10 % intensity. Right: absorption spectra of the FAD before (blue) and after (red) illumination under the reaction conditions. The spectra correspond to the degradation of the FAD as reported before [5].

6. References

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