SUPPLEMENTARY INFORMATION

Designer Micelles Accelerate Flux Through Engineered Metabolism in *E. coli* and Support Biocompatible Chemistry

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Supplementary Materials

S1. General materials and methods

Optical densities of *E. coli* cultures were determined using a DU 730 Life Sciences UV/Vis spectrophotometer (Beckman Coulter) by measuring absorbance at 600 nm. *E. coli* were routinely cultured at 32 ˚C with shaking (220 rpm), unless stated otherwise.

Mass spectral data was acquired on an Advion Expression CMS mass spectrometer in electrospray ionization (ESI) mode. The capillary voltage was set to 3.5 kV, the drying gas temperature was set to 200 ˚C with a flow rate of 0.1 mL/min and a nebulizer pressure of 22 psi. Gas chromatography (GC) was carried out using an Agilent 7890A GC instrument equipped with an Agilent HP-MoleSeive column (30 m x 0.53 mm, 50 µm film). The GC inlet was maintained at 250 ˚C and the samples were injected in 10:1 split mode. The carrier gas was helium (1 mL/min). Samples were equilibrated for 90 s at 40 ˚C and then heated to 180 ˚C at a rate of 25 ˚C per minute before the oven temperature was held at 180 ˚C for a final 5 minutes. The total run time was 12.1 minutes. Analytes were detected using a flame ionization detector (FID) with a heater temperature of 300 ˚C.

All chemicals, solvents and iron catalysts were purchased from Sigma Aldrich. All water used experimentally was purified with a MilliQ (MQ) purification system. Luria-Bertani Lennox (LB) medium was prepared according to the following recipe: 10 g/L Bacto-tryptone, 5 g/L yeast extract and 10 g/L NaCl. LB was autoclaved at 121 ˚C for 20 min, cooled and stored at room temperature. For all quantitative measurements by GC, 1,3,5-trimethoxybenzene (TMB) was used as an internal standard. When quantifying metabolites by GC a standard curve was constructed over a range of analyte concentrations (10 µM-5.0 mM) providing linear relationships of $pA_{styrene}/pA_{TMB} = 1.167c_{styrene} + 0.01316$ (Figure S2) and $pA_4/pA_{TMB} = 1.401c_4 - 0.03179$ (Figure S3), (pA = peak area). Diastereomer ratios were calculated *via* integration of the corresponding product peaks by GC.

S2. Strains, media and culture conditions

E. coli NST74(K-12) [aroH367, tyrR366, tna-2, lacY5, aroF394(fbr), malT384, pheA101(fbr), pheO352, aroG397(fbr)] was purchased from the American Type Culture Collection (ATCC®, 31884™). Lyophilized cells were grown overnight in 5.0 mL of LB. The resulting saturated overnight culture (0.8 mL) was added to 0.8 mL of 1:1 v/v LB:glycerol solution. The resulting cell-stock was frozen in liquid nitrogen, stored at –80 ˚C and used as required. Plasmid pTrc99A_*PAL2-FDC1* was obtained from Professor David R. Nielsen (Arizona State University). Electrocompetent *E. coli* NST74(K-12) cells were prepared and transformed with

the modified *p*Trc99A plasmid harbouring the *PAL2* gene (*Arabidopsis thaliana*) inserted into the *NcoI* and *XBalI* restriction sites, and the *FDC1* gene (*Saccharomyces cerevisiae*) inserted into the *SalI* and *HindIII* restriction sites. Cells were recovered in 1 mL of LB for 1 h at 37 ˚C. Transformants were selected by plating on LB agar containing 100 mg/L ampicillin and incubating at 37 ˚C overnight. A single colony was picked and grown overnight in 5.0 mL of LB containing 100 mg/L ampicillin. The resulting saturated overnight culture (0.8 mL) was added to 0.8 mL of 1:1 v/v LB:glycerol solution. The resulting cell-stock was frozen in liquid nitrogen, stored at –80 ˚C and used as required.

MMI media was prepared according to the following procedure: Solid KH_2PO_4 (0.3 g) and K_2HPO_4 (1.0 g) were dissolved in 500 mL of MQ water and autoclaved at 121 ˚C for 20 min. Upon cooling to room temperature 100 mL of an autoclaved aqueous solution of $(NH_4)_2SO_4$ (40 g/L) and 100 mL of a filter-sterilized aqueous solution of MOPS (250 g/L) were added and the pH of the resulting solution was adjusted to 7.4 using NH_4OH (28% NH_3 in H_2O). To this mixture, 100 mL of an autoclaved solution of 20% w/v glucose solution, 10 mL of a filter-sterilized aqueous solution of $MgSO_4 \cdot 7H_2O$ (50 g/L) and 10 mL of ATCC Trace Mineral Supplement (Catalog No. MD-TMS) [consisting of EDTA (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (3.0 g/L), $MnSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (1.0 g/L), $FeSO_4 \cdot 7H_2O$ (0.1 g/L), $Co(NO_3)_2 \cdot 6H_2O$ (0.1 g/L), $AlK(SO_4)_2$ (0.01 g/L), H_3BO_3 (0.01 g/L), $Na_2MoO_4 \cdot 2H_2O$ (0.01 g/L), Na_2SeO_3 (0.001 g/L), $Na_2WO_4 \cdot 2H_2O$ (0.1 g/L) and $NiCl_2 \cdot 6H_2O$ (0.02 g/L)] were added. The total volume was adjusted to 1 L using autoclaved water. The media was then filter-sterilized and stored at room temperature.

LB Lennox media was prepared according to the following procedure: Bacto-tryptone (10 g), yeast extract (5 g) and NaCl (10 g) were dissolved in 1.0 L of MQ water, autoclaved at 121 ˚C for 20 min and stored at room temperature.

S3. Quantifying styrene production from engineered *E. coli* NST74 grown in the presence of surfactants and cyclodextrins

A –80 ˚C LB:glycerol stock of *E. coli* NST74 harbouring the *p*Trc99A-*PAL2*/*FDC1* plasmid was inoculated into 5.0 mL LB containing 100 mg/L ampicillin and incubated for 12-15 h (32 ˚C, 250 rpm). The saturated overnight culture was then diluted 1:50 into an appropriate volume of MM1 media containing 100 mg/L ampicillin. Day cultures were carried out in Erlenmeyer flasks sealed with glass stoppers in order to avoid the loss of styrene via evaporation (vapour density = 3.6 (vs air), vapour pressure = 4.3 mmHg at 37.7 ˚C). A culture:headspace volume ratio of 1:5 was used at all times to maintain an aerobic atmosphere.

Cultures were grown at 32 ˚C (220 rpm) to an OD_{600} =0.6–0.8, at which point protein expression was induced by the addition of IPTG to a final concentration of 0.2 mM. In addition, when appropriate, surfactant or cyclodextrin additives were also added at this point. The pH of the culture was monitored frequently (typically every 6–12 h), and readjusted to pH 7.4 if necessary using an autoclaved aqueous 6.0 M NaOH solution. Styrene production was monitored every 12 h over a period of 60 h. This was accomplished by first transferring 800 µL of culture to a 2.0 mL Eppendorf vial. To this sample, hexanes (720 µL) was added, followed by TMB (80 µL, 10 mM in hexanes). The biphasic mixture was then vortexed for 20 min and pelleted *via* centrifugation (10 min, 13000 rpm) to give a biphasic solution. The organic layer (1 µL) was analysed by GC. Styrene concentrations were calculated from the peak area of styrene detected at *ca.* 4.0 min using the equation $pA_{styrene}/pA_{TMB} = 1.167c_{styrene} + 0.01316$ (pA = peak area).

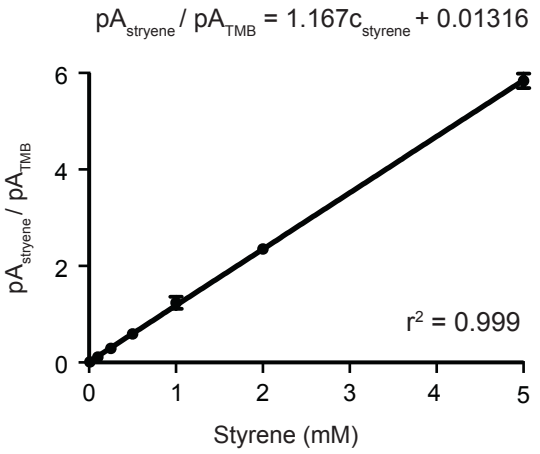

Figure S1: *Standard curve used to quantify styrene.*

S4. Calculating the relative rate-of-change of styrene production

Production time-course data was fit to the exponential equation $(\boldsymbol{I})$ from which values for y_0, φ and K could be extracted, affording a final equation where $y = c_{styrene}$ could be differentiated with respect to time ($\frac{dy}{dt}$). The resulting expression could be solved for t=12 to give a quantitative representation of the rate-of-change of styrene production at this time-point. For each surfactant, these values were then expressed relative to the rate-of-change of styrene production in the absence of surfactant ($\frac{f'(t=12)}{f'\text{control}(t=12)}$, Δdc/dt). An example of this calculation is shown below for 5% wt/v TPGS-750-M.

$$\boldsymbol{y = y_0 + (\varphi - y_0) * \left(1 - e^{-K*t}\right)} \qquad (\boldsymbol{I})$$

$$t = time, y = c_{styrene}, y_0 = c_{styrene}\ at\ 0\ h, \varphi = y\ at\ x = \infty, K = rate\ (mM^{-1}h^{-1})$$

$$5\%\ wt/v\ TPGS - 750 - M\text{:}\ \frac{dy}{dt} = 0.221511 * 0.96605^{x}\ ; x = 12, y = 0.1463$$

$$-ve\ control\text{:}\ \frac{dy}{dt} = 0.107569 * 0.929201^{x}\ ; x = 12, y = 0.0446$$

$$\frac{f'(t = 12)}{f'(t = 12)control} = \frac{0.1463}{0.0446} = \mathbf{3.28}$$

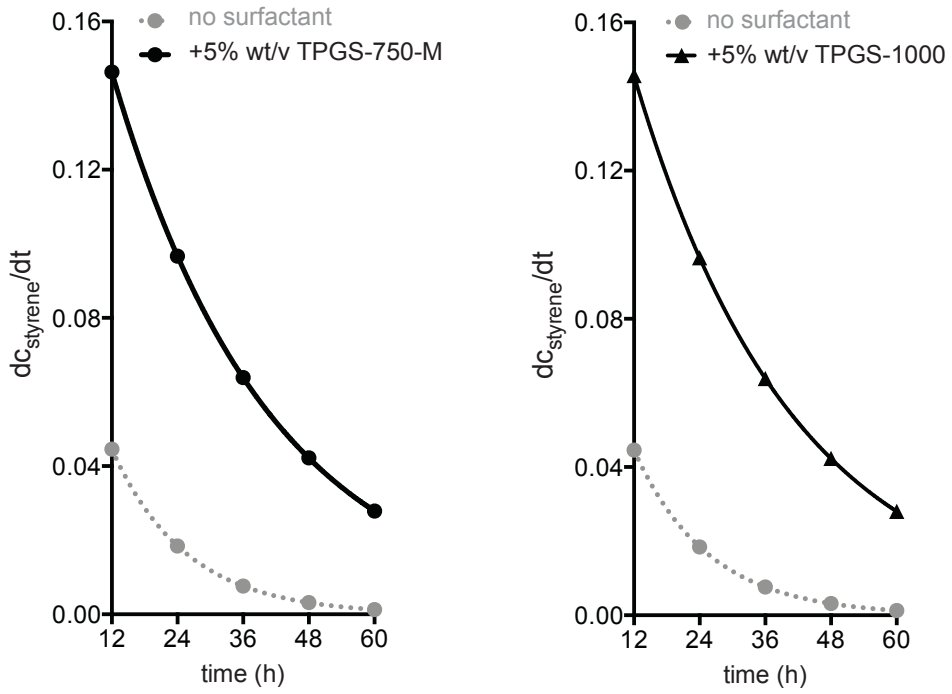

Figure S2: *Rate-of-change analysis.*

Table S1: *Surfactant and additive screen*

Entry	Surfactant Additive	Concentration (mM or %w/v)	Maximum styrene titre (mM ± S.D)	Δ*dc/dt* (t=12 h)
1	none	–	1.51 ± 0.02	1
2	α-cyclodextrin	3.2 mM	1.80 ± 0.07	1.05
3	ß-cyclodextrin	3.2 mM	1.80 ± 0.10	1.18
4	ß-OMe-cyclodextrin	3.2 mM	1.86 ± 0.16	1.24
5	calix[*8*]arene	3.2 mM	1.56 ± 0.09	1.04
6	cetrimonium bromide	3.2 mM	0.02 ± 0.004	–0.02
7	benzethonium chloride	3.2 mM	0.02 ± 0.004	–0.02
8	cetalkonium chloride	3.2 mM	0.04 ± 0.02	–0.02
9	cetylpyridinium chloride	3.2 mM	0.04 ± 0.02	–0.01
10	1-hexyl-3-methylimidazolium bromide	3.2 mM	0.10 ± 0.01	0.04
11	sodium dodecylbenzenesulfonate	3.2 mM	1.00 ± 0.08	0.0007
12	*poly*(ethylene glycol) 4-nonylphenyl 3-sulfopropyl ether potassium salt	3.2 mM	1.47 ± 0.11	0.05
13	sodium dodecylsulfonate	3.2 mM	1.29 ± 0.17	0.04
14	Tween-80	3.2 mM	1.97 ± 0.16	1.33
15	Triton X-100	3.2 mM	1.70 ± 0.44	1.10
16	Brij® 58	3.2 mM	1.96 ± 0.19	1.32
17	IGEPAL® CO-520	3.2 mM	2.06 ± 0.14	1.24
18	IGEPAL® CO-720	3.2 mM	1.73 ± 0.01	1.13
19	Pluronic® F-68	3.2 mM	2.42 ± 0.12	1.49
20	PTS	3.2 mM	2.70 ± 0.08	1.81
21	SPGS-550-M (nok)	3.2 mM	0.37 ± 0.07	–
22	TPGS-750-M	3.2 mM	2.51 ± 0.13	1.68
23	TPGS-1000	3.2 mM	2.64 ± 0.06	1.78
24	Vitamin E *O*-acetate	3.2 mM	1.38 ± 0.37	0.02
25	*n*-dodecane	3.2 mM	1.96 ± 0.06	1.13
26	PTS	2% wt/v	4.07 ± 0.18	2.95
27	TPGS-750-M	2% wt/v	4.62 ± 0.21	2.98
28	TPGS-1000	2% wt/v	4.36 ± 0.28	2.78
29	TPGS-750-M	5% wt/v	5.52 ± 0.35	3.28
30	TPGS-1000	5% wt/v	5.24 ± 0.23	3.27
31	TPGS-750-M	10% wt/v	3.95 ± 0.07	2.56
32	TPGS-1000	10% wt/v	4.54 ± 0.31	3.29
33	*n*-dodecane	20% wt/v	5.50	–

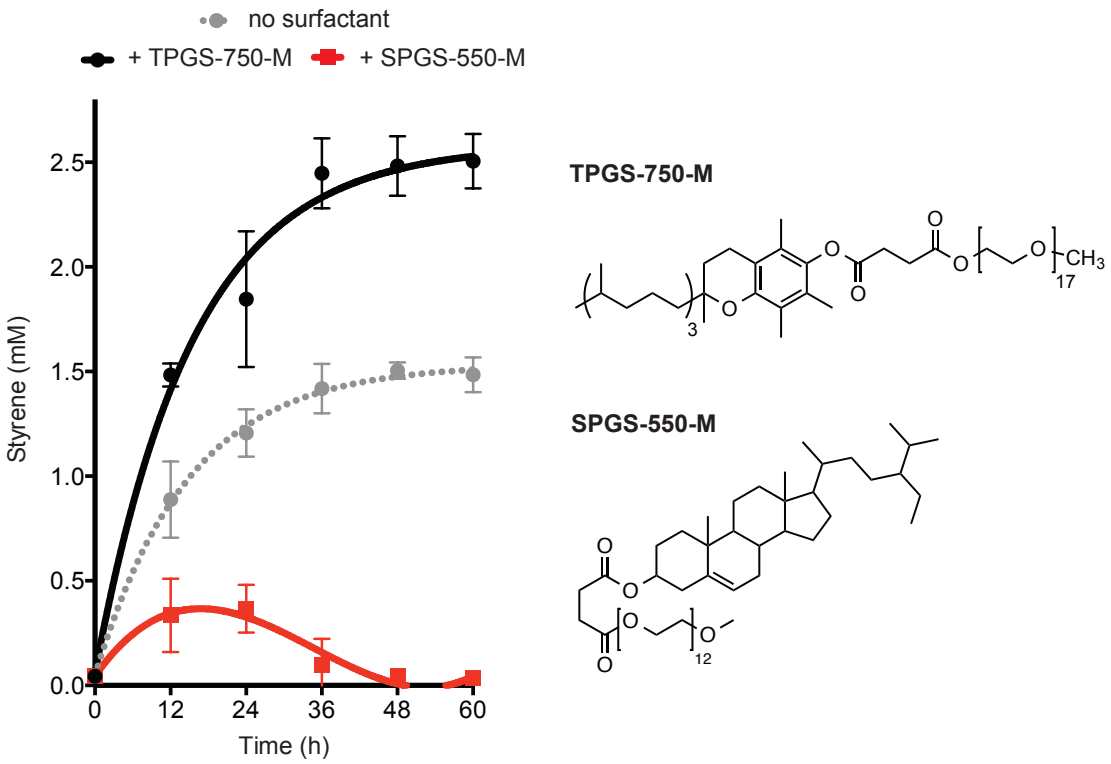

Figure S3: *Styrene production in the presence of TPGS-750-M and SPGS-550-M (nok).*

S5. Determining styrene toxicity to *E. coli* and growth-rescue experiments using TPGS-750-M and TPGS-1000

A –80 ˚C LB:glycerol stock of wild-type *E. coli* NST74 was inoculated into 5.0 mL LB and incubated for 12-15 h (32 ˚C, 250 rpm). The saturated overnight culture was then diluted 1:50 into 100 mL of MM1 media containing no antibiotics. Day cultures were carried out in Erlenmeyer flasks sealed with glass stoppers in order to avoid the loss of styrene via evaporation. Cultures were grown at 32 ˚C (220 rpm) to an OD_{600}=0.5–0.6 (typically 5-6 h after inoculation), at which point styrene was added at various concentrations (0 mM (0 µL), 1.0 mM (11.5 µL), 2.0 mM (23 µL), 3.0 mM (34.5 µL) or 4.0 mM (46.0 µL)) *via* microsyringe under aseptic conditions, as well as, where appropriate, either TPGS-750-M or TPGS-1000 (5.0 g, 5 % wt/v). Culture density (OD_{600}) was measured every hour for six hours. At OD_{600}<1.0, 1.0 mL aliquots were analysed directly, however at OD_{600}>1.0, 100 µL culture aliquots were diluted in 900 µL MQ water, analysed and the OD_{600} adjusted accordingly. Cells grown in the presence of 4.0 mM styrene and either TPGS-750-M or TPGS-1000 were then centrifuged (400 rpm, 15 min) and the cell pellet was washed with MQ water (3 x 100 mL). The cells were then suspended in a minimal volume of MQ water (*ca.* 1.0-5.0 mL) and then inoculated into MM1 media containing styrene (4 mM, 46.0 µL) and no surfactant, such

that the OD_{600} was 0.5-0.6. The cultures were then incubated at 32 ˚C for 6 h, and the OD_{600} was monitored every hour, as outlined above.

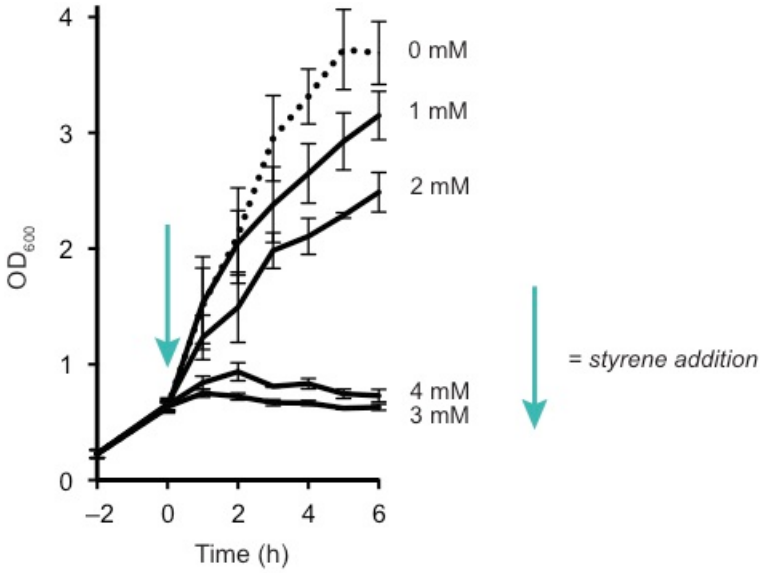

Figure S4: *Growth of E. coli NST74 in the presence of various concentrations of styrene.*

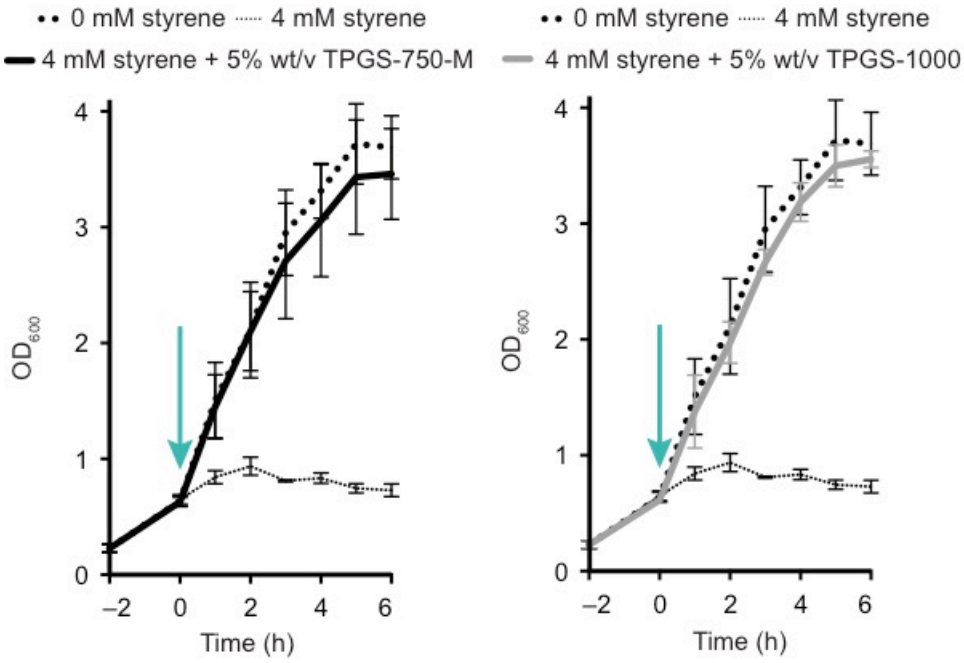

Figure S5: *Growth of E. coli NST74 in the presence of 4 mM styrene and either 5% wt/v TPGS-750-M or 5% wt/v TPGS-1000.*

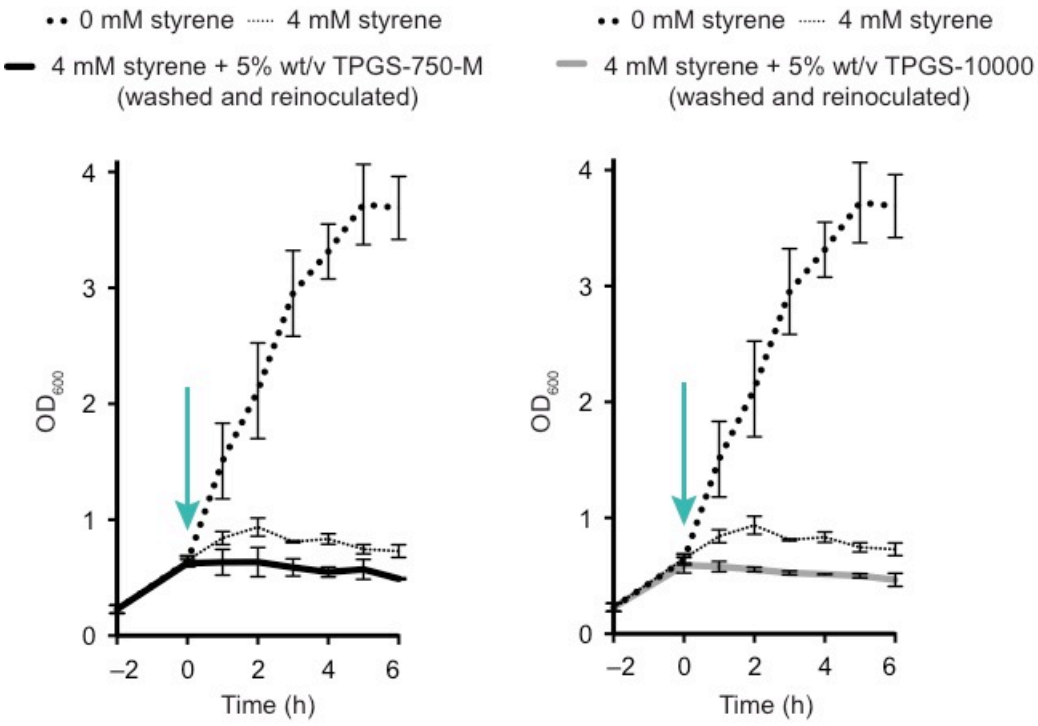

Figure S6: *Growth of E. coli NST74 from cultures containing either 5% wt/v TPGS-750-M or 5% wt/v TPGS-1000 inoculated into media containing 4 mM styrene and no surfactant.*

S6. Growth and cell-count assays of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1*

Initial culturing of this strain was carried out as outlined in Section S3. The culture density (OD_{600}) was analysed over 60 h in 12 h time-points. After 60 h, 100 µL of each culture (containing 2% or 5% wt/v of either TPGS-750-M or TPGS-1000, and control cultures containing no surfactant) were added to 900 µL of MM1 media. These aliquots were subjected to serial 10-fold dilutions (10^2–10^8). Aliquots (100 µL) of six different dilutions (10^2, 10^3, 10^4, 10^5, 10^6, 10^7) were plated onto individual LB agar plates containing ampicillin (100 mg/L). The plates were incubated at 37 ˚C overnight. These plates were used to calculate the number of colony-forming units (CFU's) in each original reaction mixture. These experiments were carried out in triplicate from separate overnight cultures to give an estimation of standard error.

Table S2: *E. coli NST74_pTrc99A-PAL2/FDC1 viable cell calculations after 60 h*

Entry	Additive (wt/v)	cfu/mL (x10^7, ± S.D)	log_{10}(cfu/mL x10^3, ± S.D)
1	none	0.02 ± 0.02	1.57 ± 0.08
2	TPGS-750-M (2%)	0.79 ± 0.38	3.80 ± 0.25
3	TPGS-750-M (5%)	3.27 ± 0.45	4.46 ± 0.06
4	TPGS-1000 (2%)	1.30 ± 0.36	3.76 ± 0.25
5	TPGS-1000 (5%)	0.17 ± 0.12	3.16 ± 0.28

S7. Slime isolation and BHI-sucrose-Congo Red exo-polysaccharide assay

Cultures of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* (100 mL) grown in the presence of 5% wt/v TPGS-750-M were transferred into two 50 mL Falcon tubes and centrifuged (4000 rpm, 15 min). The cell-containing supernatant was decanted to yield *ca.* 12 g of a gel-like pellet. An aliquot of the slime pellet was transferred to a 1 mL Eppendorf tube and 70% $EtOH_{(aq)}$ was added drop-wise. The slime was examined for precipitation of protein/DNA by eye. To confirm whether *E. coli* NST74 was capable of secreting exo-polysaccharide slime we used the BHI-sucrose-Congo Red assay as described by Freeman et al. [1]. To do this, after 60 h, 100 µL of cultures of *E. coli* NST74 was removed and added to 900 µL of MM1 media. These aliquots were subjected to serial 10-fold dilutions (10^2–10^8). Aliquots (100 µL) of three different dilutions (10^5, 10^6, 10^7) were plated onto individual sterile plates consisting of brain heart infusion broth (BHI, 37 g/L), agar (10 g/L), sucrose (50 g/L) and Congo Red (0.8 g/L). Congo red was prepared as a 8 g/L stock solution in MQ water, autoclaved at 121 ˚C for 20 min and added to the final solution under aseptic conditions during cooling. The plates were incubated at 37˚C overnight, and then at room temperature for 24 h before examining for black colony formation.

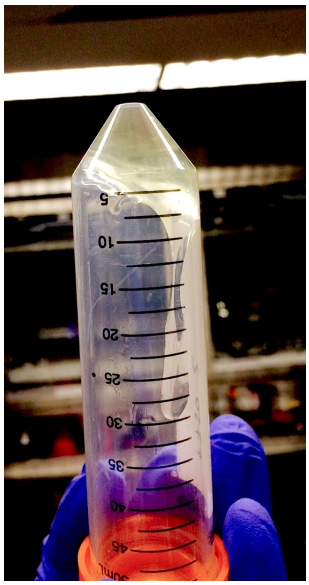

Figure S7: Slime produced by *E. coli* in response to 5% wt/v TPGS-750-M.

Figure S8: *Growth of E. coli* NST74 on BHI-sucrose-Congo Red agar plates.

S8. Whole-cell negative staining procedures for transmission electron microscopy

Culture aliquots (1 mL) were centrifuged (12000 rpm, 10 min), the supernatant was discarded and the cell pellet was re-suspended in 1 mM of phosphate-buffered saline (PBS) solution. The sample (5µl) was then adsorbed for one minute to a carbon-coated grid that had been made hydrophilic by a 30 second exposure to a glow discharge. Excess liquid was removed with a filter paper (Whatman #1) and the samples were stained with 0.75% uranyl formate for 30 seconds. After removing the excess uranyl formate with a filter paper the grids were examined in a JEOL 1200EX Transmission electron microscope or a TecnaiG² Spirit BioTWIN and images were recorded with an AMT 2k CCD camera.

S9. Plastic-mounted cell slice image procedure for transmission electron microscopy

Initial culturing of this strain was carried out as outlined in Section S3. A routine fixative of 2.5% glutaraldehyde, 1.25% paraformaldehyde and 0.03% picric acid in 0.1 M sodium cacodylate buffer (pH 7.4) was used. Culture aliquots (1 mL) were centrifuged (12000 rpm, 10 min) and the supernatant discarded. The cell pellet was fixed for at least 2 hours at RT in the above fixative, washed in 0.1M cacodylate buffer and post-fixed with 1% osmiumtetroxide (OsO_4) and 1.5% potassiumferrocyanide ($KFe(CN)_6$) for one hour, washed in water (x3) and incubated in 1% aqueous uranyl acetate for one hour. This was followed by two washes in water and subsequent dehydration in grades of alcohol (10 min each; 50%, 70%, 90%, 100% (2x10 min)). The samples were then put in propyleneoxide for one hour and infiltrated in a 1:1 mixture of propyleneoxide and TAAB Epon (Marivac Canada Inc., St. Laurent, Canada). The following day the samples were embedded in TAAB Epon and polymerized at 60 ˚C for 48 h. Ultrathin sections (*ca.* 60 nm) were cut on a Reichert Ultracut-S microtome, picked up onto copper grids stained with lead citrate, examined in a JEOL 1200EX Transmission electron microscope or a TecnaiG² Spirit BioTWIN and images were recorded with an AMT 2k CCD camera.

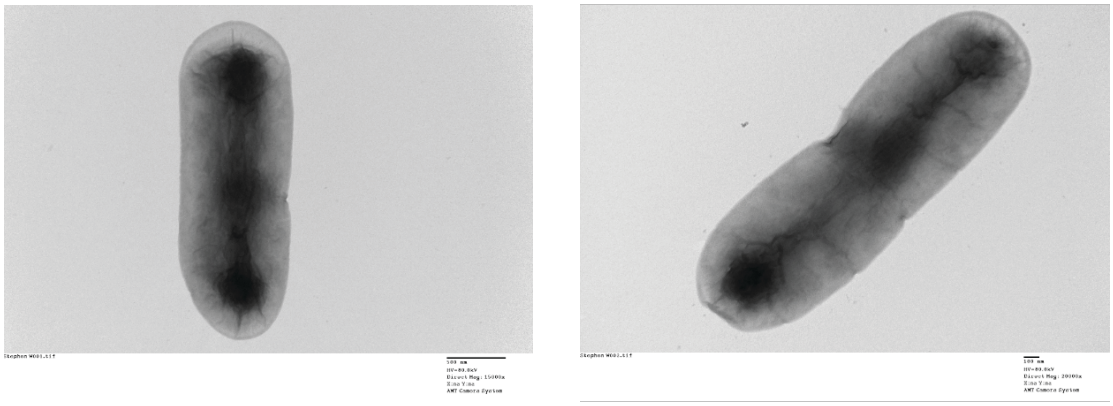

Figure S9: Negative stained cells of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing no surfactant.

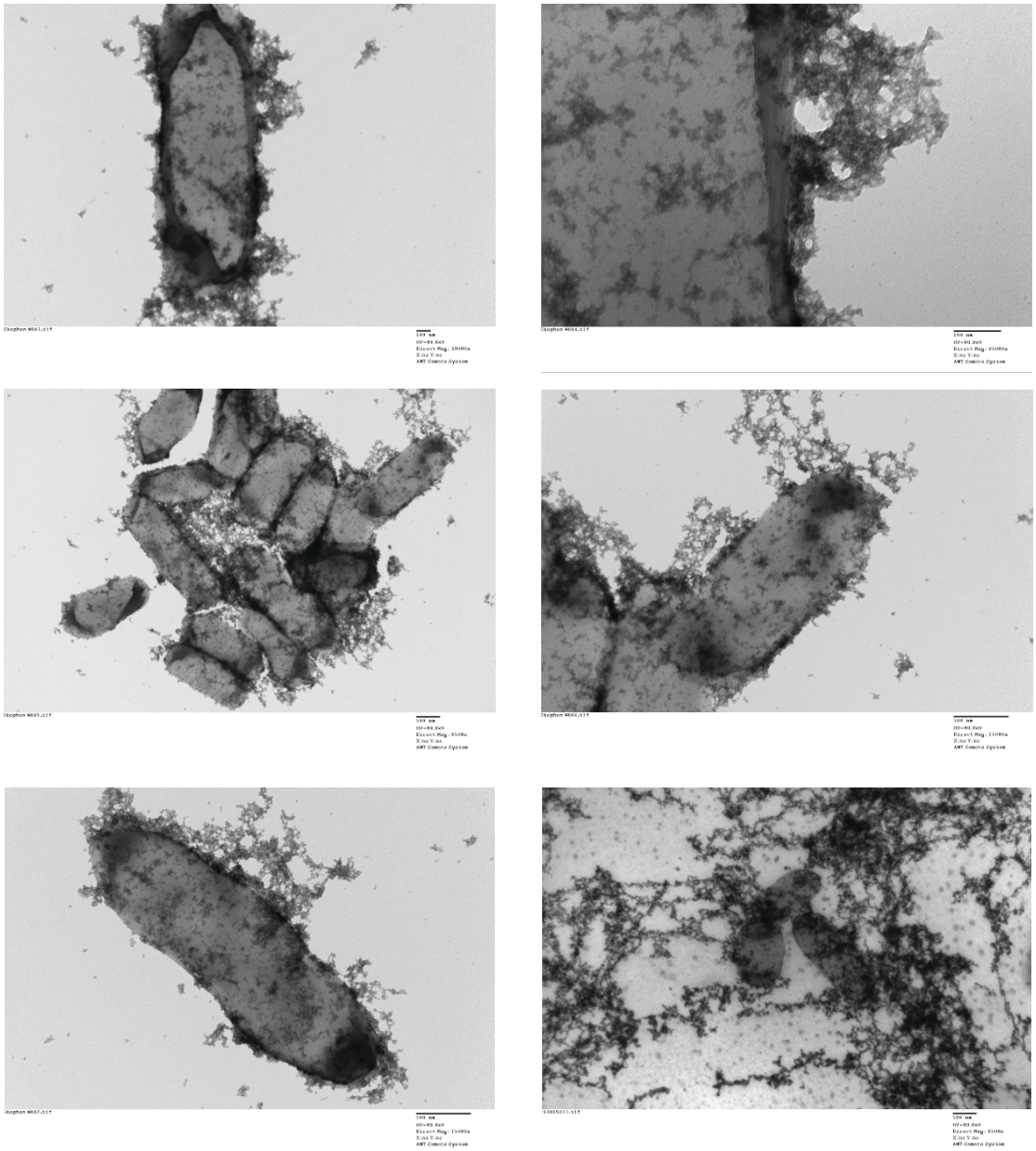

Figure S10: Negative stained cells of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing 5% wt/v TPGS-750-M.

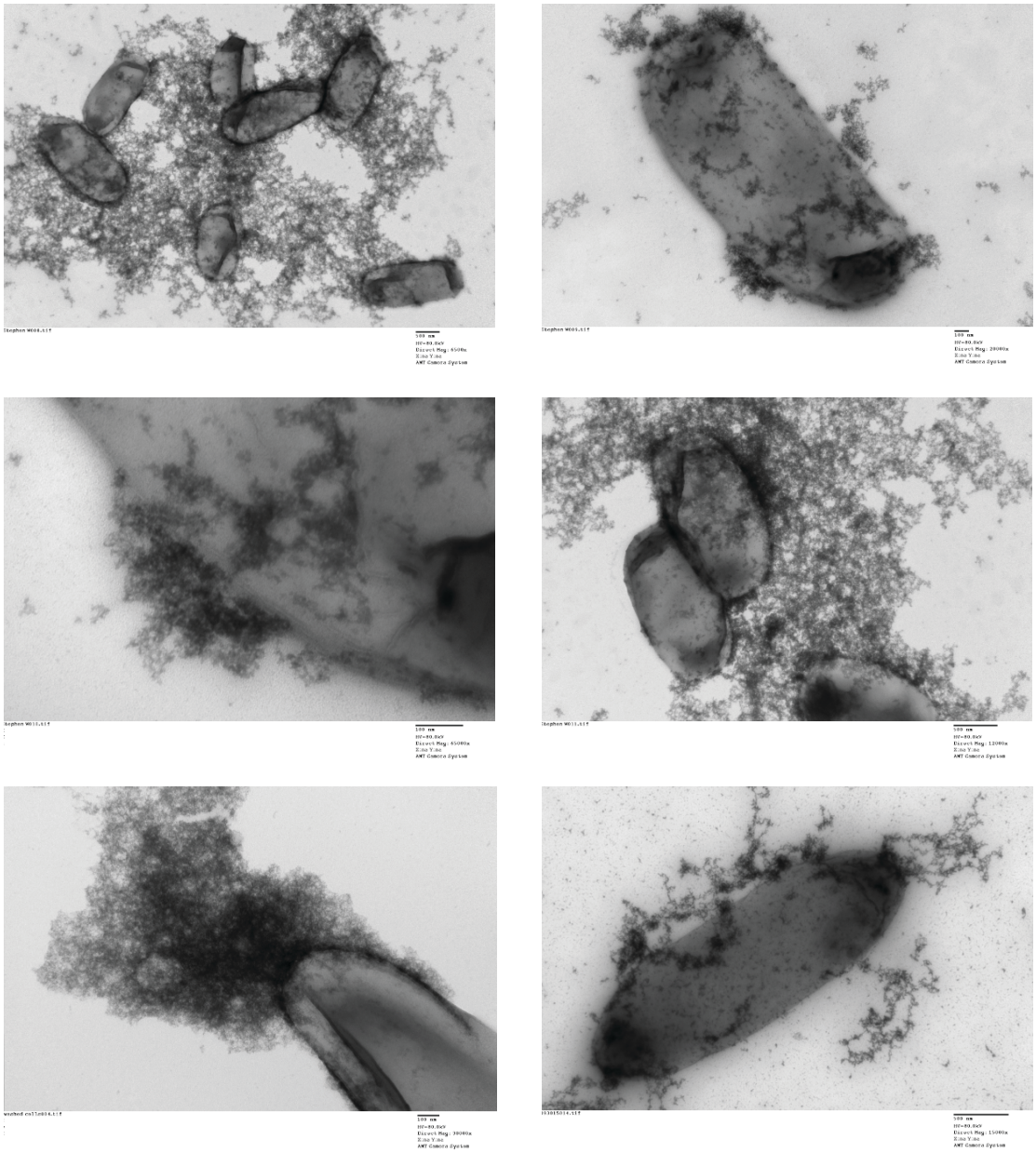

Figure S11: Negative stained cells of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing 5% wt/v TPGS-1000.

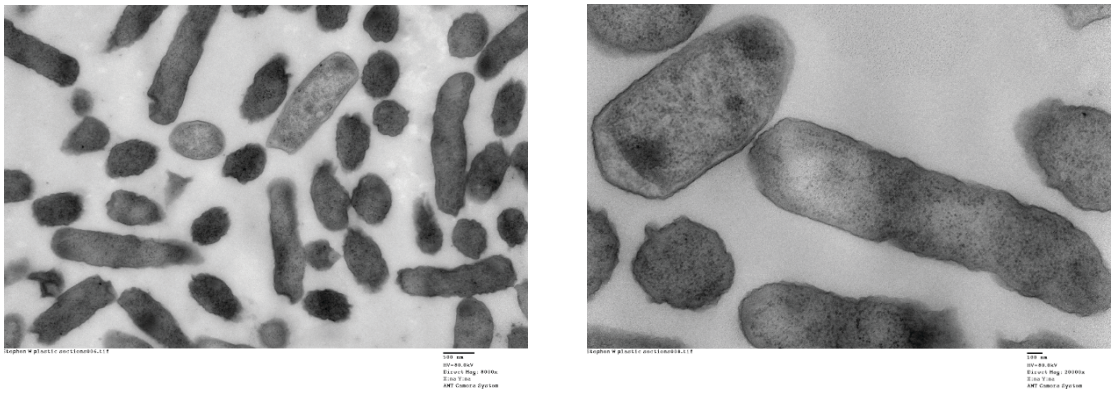

Figure S12: Plastic-mounted cell slice images of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing no surfactant.

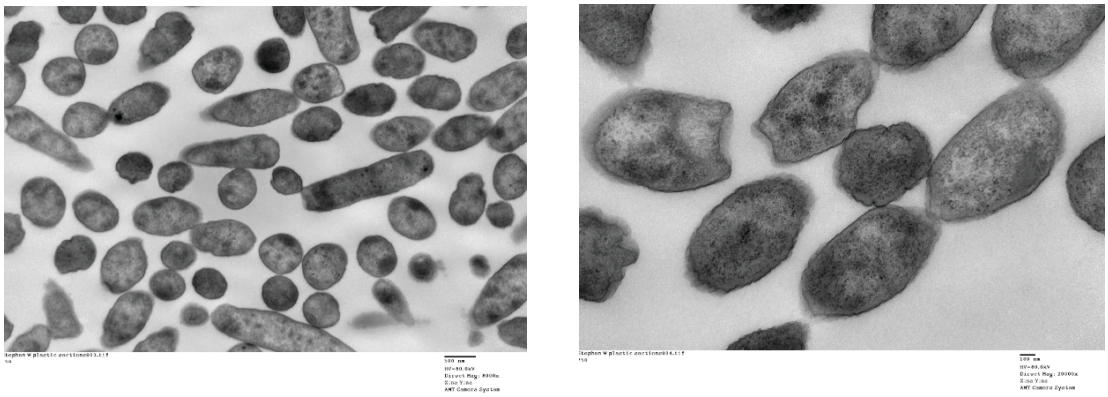

Figure S13: Plastic-mounted cell slice images of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing 5% wt/v TPGS-750-M.

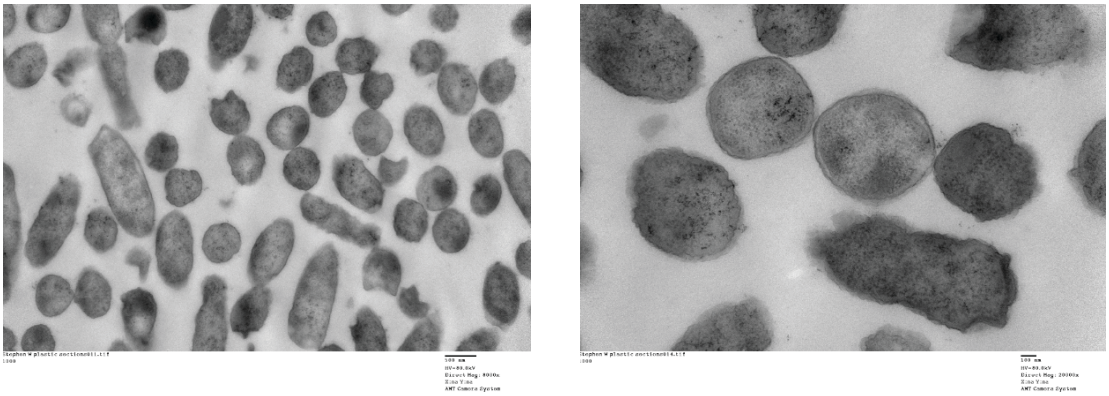

Figure S14: Plastic-mounted cell slice images of *E. coli* NST74_pTrc99A-*PAL2*/*FDC1* from cultures containing 5% wt/v TPGS-1000.

S10. Determining Critical Micelle Concentration (CMC)

This assay was adapted from that described by Tahirat *et al.* [2]. An appropriate volume of a stock solution of TPGS-750-M (2% wt/v) or TPGS-1000 (2% wt/v) in MM1 media was added to a 12.5 mL scintillation vial containing an appropriate volume of MM1 media to give surfactant concentrations ranging from 0–0.01% wt/v and a final volume of 2 mL. To this solution, five drops of a 1.6 x 10^{-3} M stock solution of 1-(2-pyridylazo)-2-naphthol (PAN) in *n*-hexanes was added. The mixture was then gently mixed and the hexanes were allowed to evaporate at room temperature over a period of ten minutes. A 1 mL volume of each solution was transferred to a 1 mL cuvette and the absorbance of the solution at 470 nm was measured using a DU 730 Life Sciences UV/Vis spectrophotometer (Beckman Coulter). The CMC of each surfactant was determined as the point at which the otherwise water-insoluble dye PAN dissolved, causing a sharp increase in the absorbance of the solution at 470 nm.

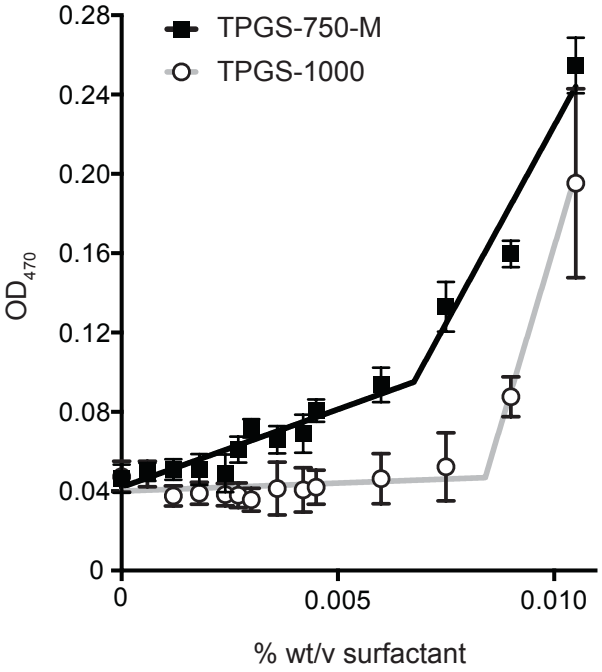

Figure S15: *Determining the critical micelle concentration (CMC) for TPGS-750-M and TPGS-1000 in MM1 media using the PAN absorbance assay.*

S10. Plasmid Stability Test

This assay was performed as described by Luo et al. [3]. Initial culturing of this strain was carried out as outlined in Section S3. After 60 h, 100 µL of each culture containing 2% or 5% wt/v of either TPGS-750-M or TPGS-1000, and control cultures containing no surfactant were removed and added to 900 µL of MM1 media. These aliquots were subjected to serial 10-fold dilutions (10^2–10^8). Aliquots (100 µL) of six different dilutions (10^2, 10^3, 10^4, 10^5, 10^6, 10^7) were plated onto individual LB agar plates containing no antibiotic. The plates were then incubated at 37 ˚C overnight. After this time, 100 colonies were picked from these plates using a sterile toothpick and stabbed into a LB-agar plate containing ampicillin (100 mg/L) forming grids (as shown in Figure S16). These plates were then incubated at 37 ˚C for 24 h. Regrowth of the cells on these LB+Amp plates indicated retention of the pTrc99A plasmid, which was used as a proxy for membrane integrity. Control experiments were carried out using wild-type *E. coli* NST74, and *E. coli*_pTrc99A-*PAL2*/*FDC1* heated to 95 ˚C for 15 min and then plated directly onto LB+Amp plates. All experiments were carried out in triplicate from separate overnight cultures to give an estimation of standard error.

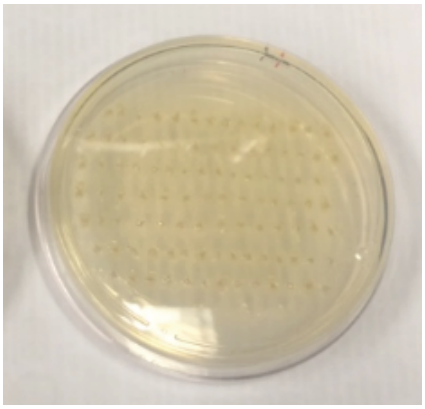

Figure S16: A grid of colonies on LB+Amp agar plates.

Table S3: *Plasmid stability test*

Entry	Surfactant Additive	% Survival
1	none (–plasmid)	0
2	none (heat lysis)	0
3	none	100
4	+2% wt/v TPGS-750-M	52
5	+2% wt/v TPGS-1000	44
6	+5% wt/v TPGS-750-M	96
7	+5% wt/v TPGS-1000	9

S11. Biocompatible Cyclopropanation in *E. coli*-associated Micelles

Day cultures of *E. coli* NST74_*p*Trc99A-*PAL2*/*FDC1* were grown as described in Section S3. Day cultures were grown aerobically at 32 ˚C (220 rpm) to an OD_{600} =0.6–0.8. Protein expression was induced by the addition of IPTG to a final concentration of 0.2 mM. At this point TPGS-750-M (2% wt/v), *meso*-tetraphenylporphyrin iron(III) chloride (FeTPPCl) or iron(III) phthalocyanine chloride (FePcCl) (63 μM, 2.5 mol%) and EDA (1.5 mM, 0.6 equiv.) were added to the cultures and the Erlenmeyer flasks were sealed using glass stoppers. Reactions were incubated at 32 ˚C (220 rpm) for 60 h. The pH of the culture was monitored frequently (typically every 6–12 h) and readjusted to pH 7.4 if necessary using autoclaved aqueous 6.0 M NaOH. Further portions of EDA (1.5 mM, 0.6 equiv.) were added 12, 24, 36 and 48 h post-induction. After 60 h, an aliquot (0.8 mL) of the culture was transferred to a 2.0 mL Eppendorf vial. To this sample, hexanes (720 μL) was added, followed by TMB (80 μL,

10 mM in hexanes). The biphasic mixture was then vortexed for 20 min and then pelleted *via* centrifugation (10 min, 13000 rpm) to give a biphasic solution. The organic layer (10 μL) was analysed by GC. Styrene concentrations were calculated from the peak area of styrene detected at *ca.* 4.0 min using the equation $pA_{styrene}/pA_{TMB} = 1.167c_{styrene} + 0.01316$ (pA = peak area). Cyclopropane **4** concentrations were calculated from the peak area of *cis*-/*trans*-**4** detected at *ca.* 7.3 and 7.6 min using the equation $pA_{4}/pA_{TMB} = 1.401c_{4} - 0.03179$ (pA = peak area). Diastereomeric ratios were calculated *via* integration of the relative peaks of *cis*-/*trans*-4 in the GC trace.

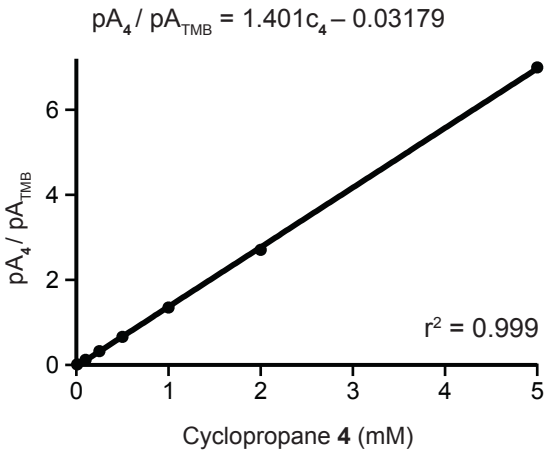

Figure S17: *Standard curve used to quantify cyclopropane **4**.*

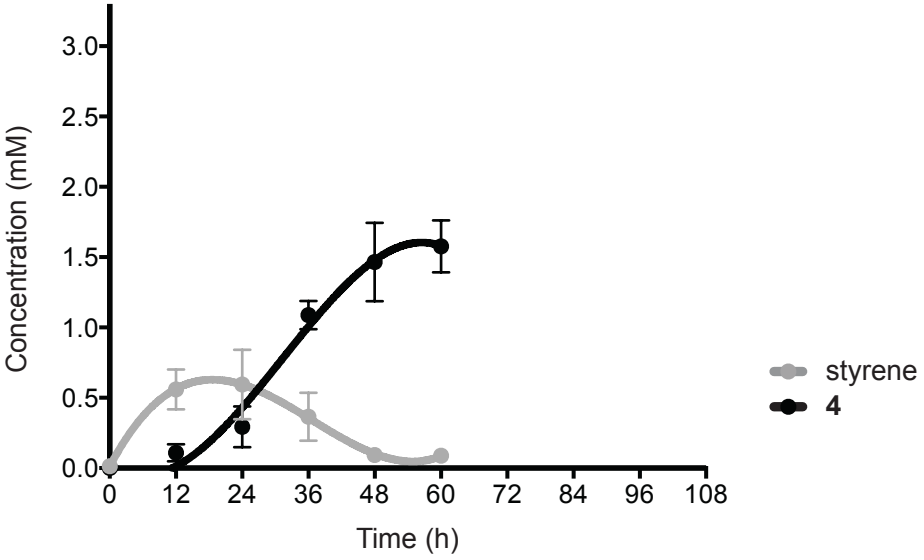

Figure S18: *Metabolite production time-course during fermentations using 2.5 mol% FePcCl and no surfactant.*

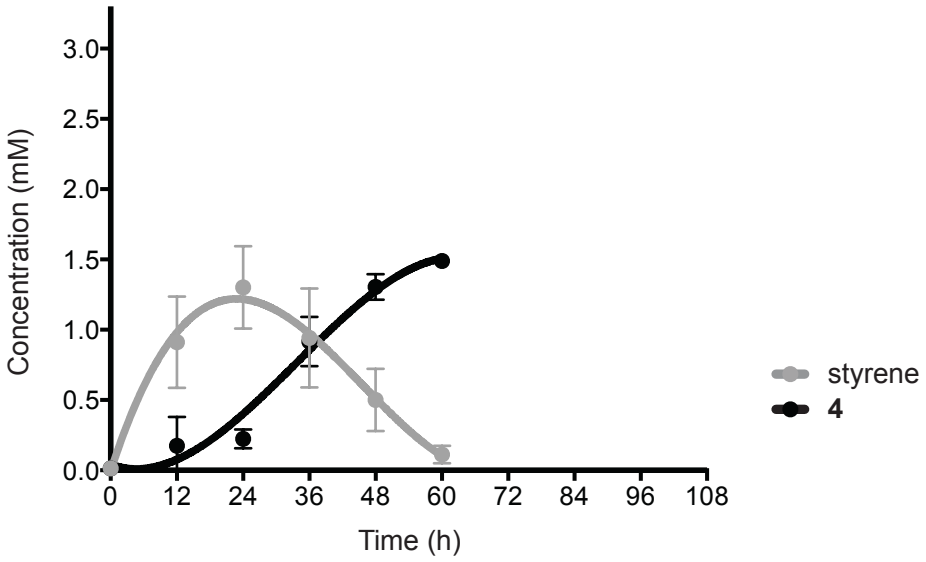

Figure S19: *Metabolite production time-course during fermentations using 2.5 mol% FeTPPCl and no surfactant.*

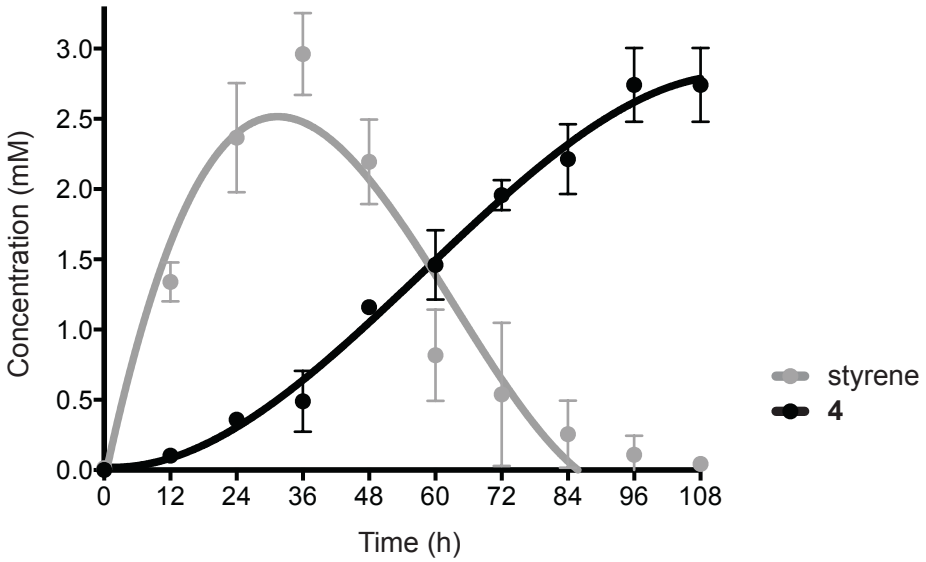

Figure S20: *Metabolite production time-course during fermentations using 2.5 mol% FePcCl and 2% wt/v TPGS-750-M.*

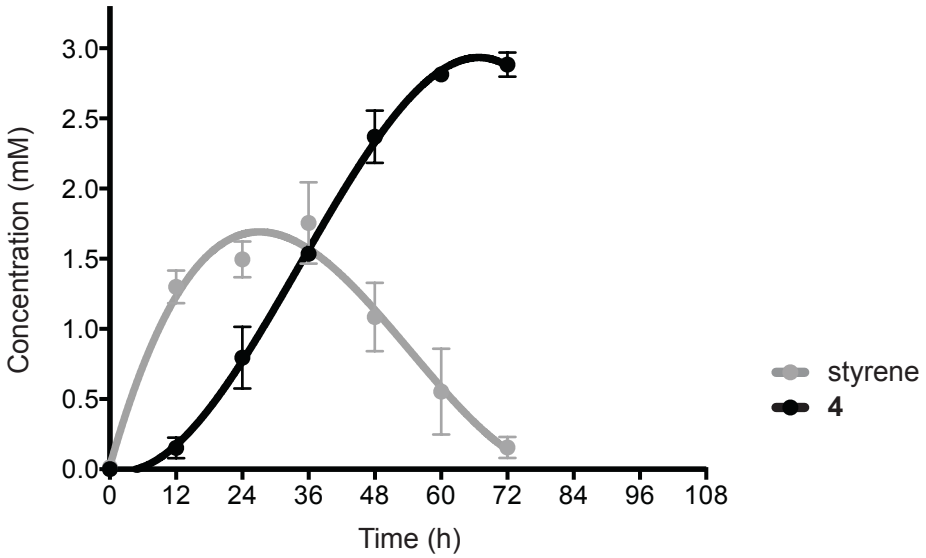

Figure S21: *Metabolite production time-course during fermentations using 2.5 mol% FeTPPCl and 2% wt/v TPGS-750-M.*

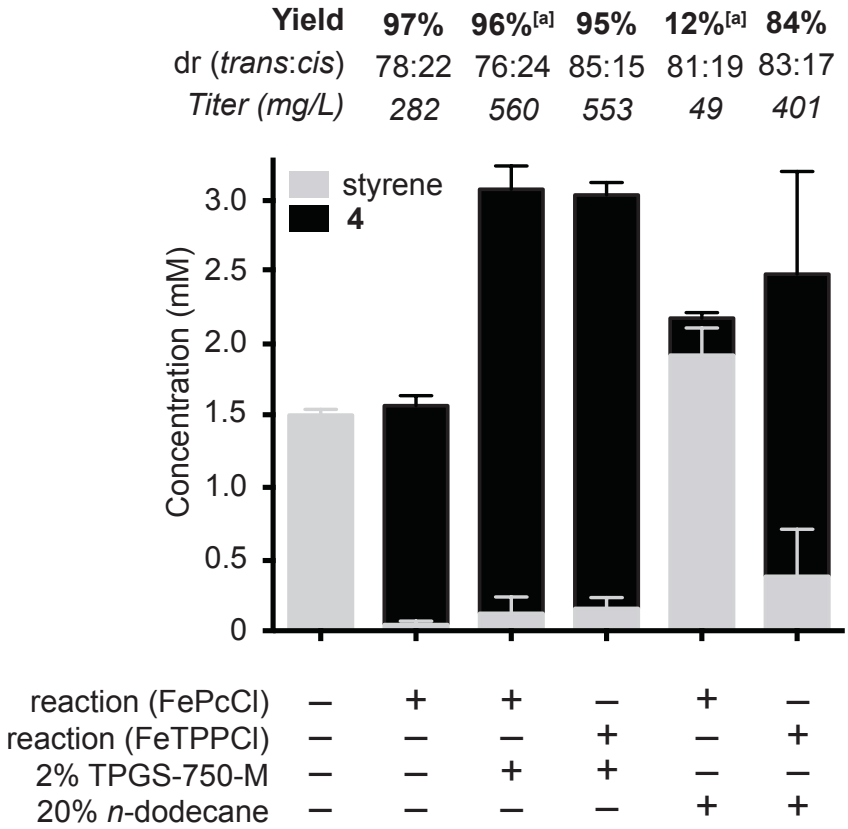

Figure S22: *End-point production levels of styrene and cyclopropane **4** in the presence and absence of TPGS-750-M or n-dodecane. [a] 96 h.*

S13. References

[*1*] D. J. Freeman, F. R. Falkiner, C. T. Keane, *J. Clin. Pathol.* **1989**, *42*, 872–874.

[*2*] N. Tahirat, A. Luis, L. Mindy, *J. High School Res.* **2011**, *2*:1.

[*3*] W. Jin, J. Huang, X. Zhu, L. Huang, J. Cai, Z. Xu, *Food Sci. Biotechnol.* **2013**, *22*, 207–214.