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Supporting Information

Cofactor regeneration in polymersome nanoreactors: enzymatically catalysed Baeyer-Villiger reactions **

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I Materials and Methods

Materials

Recombinant histidine-tagged PAMO[1] and its self-sufficient analogue CRE2-PAMO[2-3] were over-expressed and purified as described previously. (1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate was purchased from SynAffix© and glucose-6-phosphate dehydrogenase from Sigma-Aldrich. PS_{40}-*b*-$PIAT_{50}$ and α-HOOC-PS_{52}-OH were kindly donated by H.-P. de Hoog and H.I.V. Amatdjais-Groenen, respectively.

THF was distilled under Ar from sodium/ benzophenone, CH_2Cl_2 and Et_3N were distilled under Ar from CaH_2. The water utilized in the self-assembly and dialysis of polymersomes and stomatocytes was double deionized with a *Labconco Water Pro PS* purification system (18.2 MΩ). All other reagents and solvents were of the highest quality grade available and acquired from commercial sources. Unless stated otherwise, chemicals were used without further purification.

Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on a *Bruker DMC300* (75 MHz for ^{13}C) and a *Varian Inova 400* (400 MHz for ^{1}H) spectrometer. ^{1}H NMR chemical shifts (δ) are reported in parts per million (ppm) relative to a residual proton peak of the solvent; δ = 7.26 for $CDCl_3$. Multiplicities are reported as s (singlet), d (doublet), t (triplet) and m (multiplet). Broad signals are indicated by the addition of br. Coupling constants are reported as *J* value in Hertz (Hz). ^{13}C NMR chemical shifts (δ) are reported in ppm, δ = 77.0 for $CDCl_3$.

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on an *ATI Matson Genesis Series FT-IR* spectrometer fitted with an ATR cell. The vibrations (ν) are given in cm^{-1}.

Molecular weights of the block copolymers were measured on a *Shimadzu size exclusion column* equipped with a guard column, a *Polymer Laboratories* gel 5 µm mixed D column and differential refractive index (RI) and UV (λ = 254 nm) detection. The system was eluted with tetrahydrofuran THF, analysis grade) using a flow rate of 1 mL/min at 35 °C. The calibration was performed with polystyrene standards ranging from 580 to 377,400 g mol^{-1}.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectra were recorded on a *Bruker Biflex III* spectrometer. Samples were prepared by adding together the solutions of the matrix trans-3-indoleacrylic acid (IAA, 20 mg/mL, 50 µL) or trans, trans-1,4-diphenyl-1,3-butadiene (DPB, 20 mg/mL, 50 µL), silver trifluoroacetate (Ag·TFA, 5 mg/mL, 50 µL) and polymer (2 mg/mL, 50

µL) in THF. Spots of 0.3, 0.6 and 0.9 µL were placed on the MALDI sample plate and air-dried before analysis.[4]

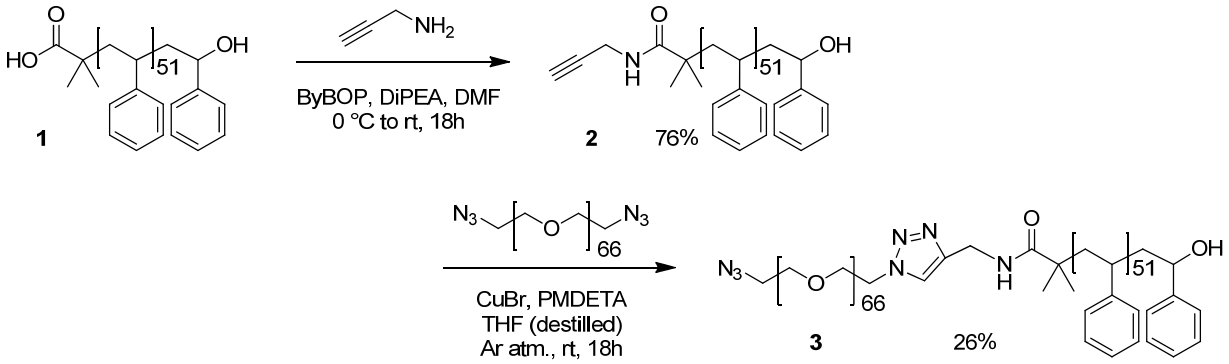
Transmission electron microscopy (TEM) was performed on a *JEOL JEM 1010* microscope with an acceleration voltage of 60 kV equipped with a charge-coupled device (CCD) camera. Sample specimens were prepared by placing a drop (10 µL) of a diluted aqueous vesicle solution on an *EM science* carbon-coated copper grid (200 mesh) for 15 min. The grid was purified from salts and other impurities by placing drop of MilliQ on it, which was immediately removed. The grid was finally air-dried for at least 3 hours and analysed without further treatment.

Inductively coupled plasma – mass spectrometry (ICP-MS) measurements were performed on a *Thermo Fisher Scientific Xseries I* quadrupole machine using 5.0 mL samples containing either 2.0 mg/L AgOAc or 0.49 mg/L $InCl_3$ solutions as internal standard.

Fluorescence measurements were performed on a *PerkinElmer LS 55* luminescence spectrometer using a quartz cuvette (1 cm path length). The excitation wavelength was set at 495 nm and the spectra were recorded using excitation and emission slits of 5 nm.

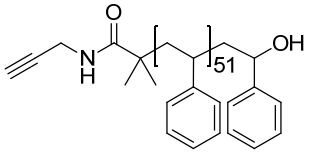
Enzymatic activities were analysed with gas chromatography (GC), performed on a *Shimadzu GC2010+* equipped with an *Altech EC-1* column (30 m, 0.32 mmID, 0.25 µm DF) or *HP-1* crosslinked methyl siloxane column (30 m, 0.32 mm, 0.25 µm FT) using FID detection.

II Synthetic procedures



Scheme 1. Synthesis of PS_{52}-*b*-PEG_{66}-N_3 (**3**) through coupling of propargylamine to α-carboxylic acid-polystyrene$_{52}$ **1**, followed by a Cu-catalysed 1,3-dipolar cycloaddition to N_3-PEG_{66}-N_3.

α-Propargylamide-polystyrene$_{52}$ (2)

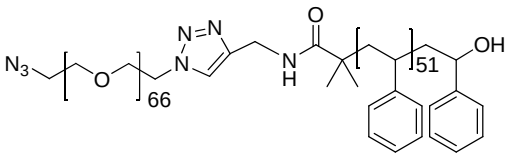


The polymer α-carboxylic acid-polystyrene$_{52}$ **1** (296 mg, 53.7 µmol) was dissolved together with propargylamine (10.1 µL, 157 µmol) and *N*,*N*-diisopropylethylamine (DiPEA, 43.9 µL, 257 µmol) in dimethylformamide (DMF, dried over molecular sieves, 7.5 mL). The solution was cooled to 0 °C and stirred for 10 min. Benzotriazole-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate (PyBOP, 92.0 µg, 1.77 µmol) was added and the mixture was stirred for 18 h, while slowly warming to room temperature.

The reaction mixture was diluted with CH_2Cl_2 (75 mL) and extracted with aqueous $NaHCO_3$ (5 wt%, 25 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4 and concentrated till dryness using the rotary evaporator. Finally, the product was precipitated from CH_2Cl_2 (5 mL) in MeOH (100 mL), filtrated and dried overnight in a vacuum exsiccator. The alkyne-functionalised polymer was obtained as a white solid (226 mg, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.22-6.30 (br s., arom H), 3.53 (m, HCCCH_2, 2H), 4.45 (m, $CH_2CH(C_6H_6)OH$, 1H), 2.30-1.15 (br s., PS backbone), 1.00-0.82 (br s., $C(O)C(CH_3)_2CH_2$), 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ : 144.9, 127.5, 125.2, 46.2-40.6, 39.9 ppm; FT-IR ν_{max} film: 3059, 3025, 2923, 2849, 1939, 1865, 1800, 1740, 1671 ($\nu_{C=O}$), 1601, 1492, 1452, 907, 758, 698 cm^{-1}; characterisation by GPC and MALDI-ToF is summarised in ***Table S1***.

α-Azido-poly(ethylene glycol)$_{66}$-*b*-polystyrene$_{52}$ (3)



A flame-dried Schlenk-tube under nitrogen atmosphere was charged with polymer **2** (200 mg, 35.9 µmol) and diazido-poly(ethylene glycol)$_{66}$ (411 mg, 137 µmol)[5] in

distilled tetrahydrofuran (THF, 20 mL). The solution was degassed with N_2 for 15 min before copper bromide (CuBr, 13.3 mg, 92.7 µmol) and *N*,*N*,*N'*,*N"*,*N"'*-pentamethyl-diethylenetriamine (PMDETA, 19.4 µL, 93.0 µmol) in freshly distilled THF (1.0 mL) were added. The solution was flushed with N_2 for an additional 15 min, warmed to 40 °C and stirred for 48 h. The conversion was monitored using GPC analysis.

Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (20 mL) and extracted twice with aqueous ethylenediaminetetraacetic acid (EDTA, 65 mM, 20 mL). The water layers were washed once more with CH_2Cl_2 (20 mL), whereafter the combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*. The crude was purified by soxhlet extraction using MeOH (100 mL), followed by column chromatography over silica gel (CH_2Cl_2:MeOH, 10:0 to 9:1) to obtain a white solid (32 mg, 16%).

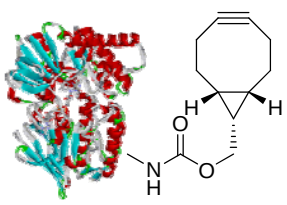
^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.22-6.30 (br s., arom H), 4.45 (m, $CH_2CH(C_6H_6)OH$, 1H), 3.72-3.60 (br s., PEG backbone), 2.30-1.15 (br s., PS backbone), 1.00-0.82 (br. s, $C(O)C(CH_3)_2CH_2$), 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ : 144.8, 127.5, 125.2, 70.1, 44.2-40.7, 39.9 ppm; FT-IR ν_{max} film: 3412, 2923, 2851, 2098, 1942, 1865, 1738, 1680 ($\nu_{C=O}$), 1601, 1492, 1453, 1107, 757, 698 cm^{-1}; characterisation by GPC and MALDI-ToF is summarised in ***Table S1***.

Table S1: Molecular characteristics of the polymers.

Entry	M_n (GPC)[a] (g mol^{-1})	PDI (GPC)[b] M_w/M_n	DP_n^{PS} (MALDI)[c]	M[d] calcd	m/z found	assigned
HO_2C-PS_{52}-OH	5350	1.14	52	5512	5535	$[M+Na]^+$
$HCCCH_2$-PS_{52}-OH	5560	1.12	52	5549	5597	$[M+K]^+$
N_3-PEG_{66}-*b*-PS_{52}-OH	6660[e]	1.06	52, 66 (PEG)	8821	7900	-

[a]Molecular weights measured by gel permeation chromatography (GPC). [b]The polydispersity index (PDI) was calculated by the quotient of M_w and M_n obtained by GPC measurements. [c]Calculated number average degree of PS polymerization from the molecular weight obtained by MALDI-ToF analysis. [d]The calculated molecular weight for the 100% isotope peak of M with the given DP_n. [e]GPC calibration is based on the hydrodynamic diameter of PS. PS containing block copolymers can give different M_n values than expected.

Bicyclononyne (BCN)-functionalised phenylacetone monooxygenase (PAMO)



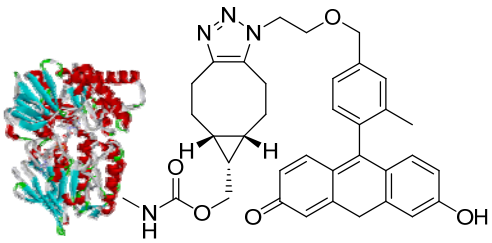
Prior to the enzyme functionalization, the Tris/HCl buffer (50 mM, pH 7.5) for storage of phenylacetone monooxygenase (PAMO) was replaced by phosphate buffer (20 mM, pH 7.4) using a Amicon Ultra centrifugal filter with 10 kDa cut-off membranes. A solution of PAMO (100 µL of 100 µM) was concentrated by centrifugation (5 min, 10.000 rpm, 4 °C) and the supernatant was redissolved in

phosphate buffer (300 µL). This procedure was repeated for a total of six washings and ended by redissolving PAMO till a concentration of 100 or 300 µM for respectively method a and b.

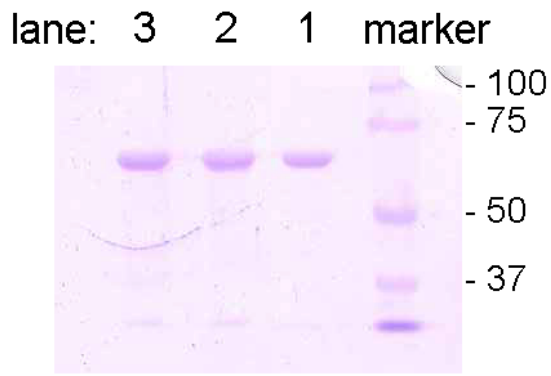
Method a: (1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (2.0 µg, 7 nmol) was dissolved in dimethylsulfoxide (DMSO, 1.0 µL) and added to a solution of PAMO (70 µL of 100 µM) in phosphate buffer (20 mM, pH 7.4) basified with Na_2CO_3 (70 µg). The reaction mixture stood overnight at 4 °C, whereafter it was further diluted with phosphate buffer (200 µL), transferred to a 10 kDa membrane Amicon Ultra centrifugal filter and concentrated by centrifugation (5 min, 10.000 rpm, 4 °C). The supernatant was redissolved in phosphate buffer (200 µL) and centrifuged again. This step was repeated five times. Finally, the bicyclononyne-functionalised PAMO was dissolved in phosphate buffer (20 mM, pH 7.4) to yield a 200 µM enzyme concentration.

Method b: (1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (2.9 µg, 10 nmol) was dissolved in dimethylsulfoxide (DMSO, 1.0 µL) and added to a solution of PAMO (33 µL of 300 µM) in phosphate buffer (20 mM, pH 7.4). The reaction mixture was shaken at room temperature for 2.5 h, whereafter it purified as described above.

Conjugation of ClickGreen-N_3 to BCN-functionalised PAMO



BCN-functionalised PAMO (0.78 µL of 200 µM) in phosphate buffer (20 mM, pH 7.4) synthesized by either method a or method b[6] was further diluted with the same buffer (1.12 µL) and subsequently with charged with ClickGreen-N_3 (0.30 µg of 1 mg/ mL in DMSO, 0.78 nmol).[7] The mixture was reacted for 3 h at room temperature. The unbound ClickGreen-N_3 was removed by extensive washing *via* centrifugation using a 10 kDa membrane Amicon Ultra centrifugal filter. The integrity of the enzymes was examined with 10% (w/v) polyacrylamide gel followed by Coomassie staining (Figure S1). The degree of ClickGreen-N_3 conjugation was determined *via* a calibration line of the compound using fluorescence spectroscopy.



Lane 1: unmodified PAMO

Lane 2: BCN-PAMO (method a) reacted with ClickGreen-N_3

Lane 3: BCN-PAMO (method b) reacted with ClickGreen-N_3

Figure S1. The conjugation of ClickGreen-N_3 to PAMO functionalised with BCN using two methods.

The maximum emissions of the fluorescence spectra of different concentrations of ClickGreen-N_3 (λ_{ex} = 495 nm) at λ_{em} = 506.5 nm were combined into a calibration curve, shown in Figure S2a. The fluorescence traces of the two experiments where ClickGreen-N_3 was coupled to the BCN-functionalised PAMO (two different methods) are depicted in Figure S2b.

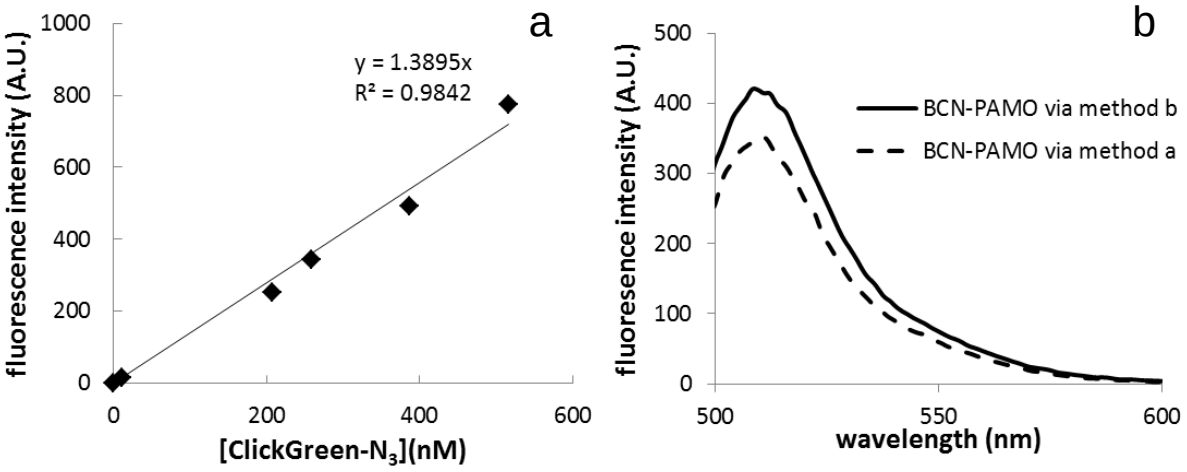


Figure S2. a) Calibration curve of ClickGreen-N_3 at λ_{ex} = 495 nm in which the intensity at λ_{em} = 506.5 nm is plotted against the concentration; b) emission spectra of ClickGreen-N_3 conjugated to BCN-functionalised PAMO.

For recording the emission spectra in Figure S2b, samples with known concentrations of PAMO were utilised. Combination of these data with the calibration curve indicated that 81% of PAMO was functionalised with BCN using method a against 97% applying method b (Table S2), when assuming that only one amine per enzyme was available for reaction.

Table S2. Conjugation of ClickGreen-N_3 *via* the SPAAC reaction to determine the degree of BCN-functionalisation of PAMO.

Entry	[enzyme] (nM)	intensity at 506.5 nm (A.U.)	Calculated [ClickGreen-N_3] (nm)	Ratio [enzyme]/[ClickGreen-N_3]
BCN-PAMO via method a	311	351	253	0.81
BCN-PAMO via method b	311	421	303	0.97

III Polymersome preparation procedures

Preparation of coenzyme regenerator enzyme-phenylacetone monooxygenase (CRE2-PAMO) loaded polymersomes

For vesicles containing CRE2-PAMO in their lumen, CRE2-PAMO (39 µL of 100 µM) was mixed with Tris/HCl buffer (2.46 mL, 50 mM, pH 9.0). To this solution, PS_{40}-*b*-$PIAT_{50}$ (0.50 mg) dissolved in distilled dioxane (500 µL) was gently added in a drop wise fashion. The vesicles were allowed to self-assemble for 30 min. Dioxane and non-encapsulated CRE2-PAMO were removed by size exclusion chromatography over Sephadex G200 in Tris/HCl (50 mM, pH 9.0) buffer. The polymersome containing fractions were combined and concentrated till 100 µL using an Amicon UltraFree-MC centrifugation (3000 rpm, 4 °C) filter with a cut-off of 200 nm. The polymersomes were stored at 4 °C before use.

Preparation of glucose-6-phosphate dehydrogenase (G6PDH) loaded polymersomes

A solution of PS_{40}-*b*-$PIAT_{50}$ (0.50 mg) in distilled THF (500 µL) was slowly dripped into phosphate buffer (2.47 mL, 20 mM, pH 7.4) containing glucose-6-phosphate dehydrogenase (G6PDH, 31 µL of 1000 U/mL). The turbid solution stood without perturbation for 30 min before purification *via* size exclusion chromatography over Sephadex G200 in phosphate buffer (20 mM, pH 7.4). The polymersome containing solution was concentrated till 100 µL with a 200 nm cut-off Amicon UltraFree-MC centrifugation (3000 rpm, 4 °C) filter. The dispersion was stored at 4 °C before use..

Preparation of azido-functionalised G6PDH loaded polymersomes

PS_{40}-*b*-$PIAT_{50}$ (0.45 mg) was dissolved together with PS_{52}-*b*-PEG_{66}-N_3 (10 wt%, 50 µg) in distilled THF (500 µL) and subsequently gently dripped into a solution of G6PDH (31 µL of 1000 U/mL) in phosphate buffer (2.47 mL, 20 mM, pH 7.4). The same purification procedure as for (G6PDH)-loaded polymersomes was followed.

Immobilisation of BCN-PAMO on azido-functionalised G6PDH loaded polymersomes

A suspension of N_3-functionalised polymersomes containing G6PDH in their lumen (100 µL of 5.0 mg/mL polymer, of which 0.5 mg/mL N_3-groups) was charged with BCN-functionalised PAMO (23.2 µL of 200 µM). The solution was shortly vortexed and then placed at 4 °C for 16 h.

The reaction mixture was transferred to an Amicon UltraFree-MC eppendorf filter with a cut-off of 100 nm. The polymersomes were centrifuged to dryness (5 min, 3000 rpm, 4 °C) and the supernatant was redispersed in 500 µL phosphate buffer (20 mM, pH 7.4). This procedure was repeated for a total

of five washings. Finally, the product was redissolved in the same phosphate buffer (100 μL) and stored at 4 °C until further use.

IV Enzymatic activity studies

Ru-labelling of enzymes

The Tris/HCl buffer (20 mM, pH 7.5), in which the enzymes were stored, was replaced by phosphate buffer (20 mM, pH 7.4) using a 10 kDa membrane Amicon Ultra centrifugal filter. The enzymatic solution (100 µL of 100 µM for CRE2-PAMO and PAMO; 93 U of 1000U/mL for G6PDH) was concentrated by centrifugation (5 min, 10.000 rpm) and the supernatant was replenished with MilliQ (300 µL). This procedure was repeated for a total of six washings. Finally, the enzyme in question was redissolved in the same phosphate buffer to obtain a concentration of 100 µM.

To the enzyme solution was added respectively Na_2CO_3 (6.0 µL, 10 mg/mL MilliQ) and bis(2,2'-bipyridine)-(5-isothiocyanato-phenanthroline) ruthenium bis(hexafluorophosphate) (0.1 eq with respect to the total amount of primary amines of the N-terminus and all present lysines for CRE2-PAMO and PAMO, 0.05 eq with respect to the total amount of primary amines of the N-terminus and all present lysines for G6PDH; 1.00 mg/mL MilliQ; 20 µL for CRE2-PAMO (21 NH_2), 16 µL for PAMO (17 NH_2), 3.3 µL for G6PDH (70 NH_2)). The bright yellow solution was shaken at room temperature for 2.5 h.

The unbound ruthenium complex was removed by dialysis against phosphate buffer (20 mM, pH 7.4) using an Amicon Ultra centrifugal filter with 10 kDa cut-off membranes. The enzymatic solution was concentrated by centrifugation (5 min, 10.000 rpm) and replenished with phosphate buffer. After performing another five washing steps, the Ru-labelled enzyme was redissolved in phosphate buffer. BCN-PAMO was reacted with the Ru-complex prior to the BCN-functionalisation, as described above.

Preparation of Ru-labelled enzyme loaded polymersomes

PS_{40}-*b*-$PIAT_{50}$ (0.50 mg) or a combination of PS_{40}-*b*-$PIAT_{50}$ (0.45 mg) and PS_{52}-*b*-PEG_{66}-N_3 (10 wt%, 50 µg) was dissolved in distilled THF (500 µL). The polymer solution was gently added to Ru-labelled enzyme (39 µL of 100 µM for CRE2-PAMO; 31 µL of 1000 U/mL for G6PDH) in phosphate buffer (2.47 mL, 20 mM, pH 7.4). The vesicles were allowed to self-assemble for 30 min. Further treatment was equivalent to the procedure described for the encapsulation of non-functionalised enzymes.

ICP-MS analysis of bio-hybrid polymersomes

The polymersome suspensions, containing Ru-labelled enzymes, were lyophilized and subsequently destructed for 3 h in nitric acid (65%, 0.50 mL) at 80 °C. After cooling the samples to room temperature, an internal standard of AgOAc (1.50 mL of 2.00 mg/L) or $InCl_3$ (1.50 mL of 0.49 mg/mL) was added. The total volume of each sample was adjusted to 5.00 mL with MilliQ. The measured

values of Ru in ppb were expressed as molarities by standardising Ru-counts on Ag- or In-counts and by comparing the results to samples containing a known amount of Ru-labelled enzyme.

Activity assay for CRE2-PAMO

The enzyme CRE2-PAMO (39 µL of 100 µM in Tris/HCl pH 7.5; 1.3 µM) was added to a mixture of phenylacetone (7.5 µL of 1.00 M in DMSO; 2.5 mM), β-nicotinamide adenine dinucleotide phosphate (NADPH, 30 µL of 10 mM in Tris/HCl pH7.5; 0.10 mM), sodium phosphite (30 µL of 500 mM in MilliQ; 5.0 mM) and 1,4-dioxane (dioxane, 0.50 mL) in Tris/HCl buffer (2.40 mL, 50 mM, pH 9.0). The mixture was shaken at 30 °C for 5 h.

Once finished, the crude reaction was extracted twice with ethyl acetate (EtOAc, 500 µL). The organic layers were separated by centrifugation (5 min, 12.000 rpm), combined and dried over Na_2SO_4. The organic phase was directly analysed by GC, which indicated 100% substrate conversion.

Activity assay for CRE2-PAMO loaded polymersomes

The polymersomes with CRE2-PAMO in their lumen (95 µL, 5.0 mg polymer/mL in Tris/HCl) were suspended in Tris/HCl buffer (98 µL, 50 mM, pH 9.0) and were added to a mixture of phenylacetone (1.77 µL of 1.00 M in DMSO; 8.40 mM), NADPH (9.08 µL of 10 mM in Tris/HCl pH 7.5; 0.43 mM) and sodium phosphite (7.09 µL of 500 mM in MilliQ; 16.8 mM). The mixture was shaken at 30 °C until GC analysis indicated completion of the reaction.

The progress of the reaction was monitored with reaction mixture aliquots of 50 µL. These aliquots were extracted twice with EtOAC (60 µL and 100 µL), using centrifugation (5 min, 12.000 rpm) to separate the organic layer. The combined organic phases were dried over Na_2SO_4 and the substrate conversion was analysed directly by GC.

Activity assay for PAMO

To Tris/HCl buffer (50 mM, pH 9.0) was added phenylacetone (5 µL of 1000 mM in DMSO, 10 mM), (NADPH, 10 µL of 10 mM in Tris/HCl pH7.5; 0.20 mM) glucose-6-phosphate (20 µL of 500 mM in MilliQ; 20 mM). This mixture was charged with the enzymes G6PDH (5 µL of 1000 U/mL in phosphate buffer pH 7.4) and PAMO (20 µL of 100 µM in Tris/HCl pH 7.5, 4.0 µM) and subsequently shaken at 30 °C for 30 min.

For workup, the crude reaction mixture was extracted twice with EtOAc (500 µL). The organic layers were separated by centrifugation (5 min, 12.000 rpm), combined and dried over Na_2SO_4. The mixture was directly analysed by GC to observe a substrate conversion of 100%.

Activity assay for G6PDH loaded polymersomes; ratio G6PDH:PAMO is 1:30

A suspension of polymersomes, containing G6PDH in their lumen (95 µL, 5.0 mg polymer/mL), in phosphate buffer (20 mM, pH 7.4) was further diluted in Tris/HCl buffer (440 µL, 50 mM, pH 9.0). This dispersion was subsequently added to a mixture of phenylacetone (5.07 µL of 1.00 M in DMSO; 8.40 mM), NADPH (10.1 µL of 10 mM in Tris/HCl pH 7.5; 0.17 mM) and glucose-6-phosphate (20.3 µL of 500 mM in MilliQ; 16.8 mM). PAMO (20.3 µL of 100 µM in Tris/HCl pH 7.5; 3.4 µM) was finally added and the reaction mixture was shaken at 30 °C.

The reaction progress was analysed in a similar fashion to the procedure described for the CRE2-PAMO loaded vesicles.

Activity assay for G6PDH loaded polymersomes; ratio G6PDH:PAMO is 1:15

A dispersion of polymersomes in phosphate buffer (20 mM, pH 7.4), containing G6PDH in their lumen (95 µL, 5.0 mg polymer/mL), was further diluted in Tris/HCl buffer (176 µL, 50 mM, pH 9.0). This suspension was subsequently added to a mixture of phenylacetone (2.50 µL of 1.00 M in DMSO; 8.40 mM), NADPH (5.0 µL of 10 mM in Tris/HCl pH 7.5; 0.17 mM) and glucose-6-phosphate (10 µL of 500 mM in MilliQ; 16.8 mM). PAMO (10 µL of 100 µM in Tris/HCl pH 7.5; 3.4 µM) was finally added and the reaction mixture was shaken at 30 °C.

The reaction progress was analysed in a similar fashion to the procedure described for the CRE2-PAMO loaded vesicles.

The activity assay for G6PDH filled polymersomes containing 10 wt% of N_3PEG-*b*-PS anchor was performed analogously to the described evaluation of G6PDH loaded vesicles.

Activity assay for BCN-functionalised PAMO

BCN-functionalised PAMO (22 µL of 45.6 µM; 2.0 µM) was diluted with Tris/HCl buffer (448 µL, 50 mM, pH 9.0) and subsequently added to a mixture of phenylacetone (5.0 µL of 500 mM in DMSO; 5.0 mM), NADPH (10 µL of 10 mM in Tris/HCl pH 7.5; 0.20 mM), glucose-6-phosphate (10 µL of 500 mM in MilliQ; 10 mM) and G6PDH (5.0 µL of 1000 U/mL in Tris.HCl pH 7.5, 5U). The mixture was shaken at 30 °C.

After 5h of reaction time, the crude was extracted twice with EtOAc (500 µL). The organic layers were separated by centrifugation (5 min, 12.000 rpm), combined and dried over Na_2SO_4. The enzymatic activity was then analysed by GC, which showed quantitative substrate conversion.

Activity assay for PAMO-functionalised polymersomes with G6PDH-loaded compartment

Vesicles with G6PDH in their lumen and PAMO immobilised onto their surface (95 µL, 5.0 mg polymer/mL) in phosphate buffer (20 mM, pH 7.4) were suspended in Tris/HCl (176 µL, 50 mM, pH

9.0). This mixture was added to a solution of phenylacetone (2.5 µL of 1.00 M in DMSO; 8.40 mM), NADPH (5.0 µL of 10 mM in Tris/HCl pH 7.5; 0.17 mM) and glucose-6-phosphate (10 µL of 500 mM in MilliQ; 16.8 mM). The suspension was shaken at 30 °C, while the reaction was monitored using GC analysis.

The GC samples were prepared similar to the above described procedure for G6PDH-loaded nanoreactors.

V References

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