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Cascade Reactions in Multicompartmentalized Polymersomes**

Ruud J. R. W. Peters, Maïté Marguet, Sébastien Marais, Marco W. Fraaije, Jan C. M. van Hest, and Sébastien Lecommandoux**

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Contents

1. Materials and Methods

Chemicals

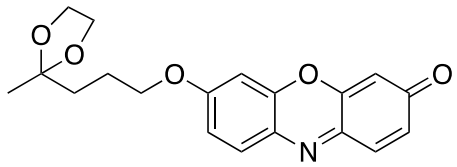
Recombinant histidine-tagged PAMO is a fusion protein of Phenylacetone monooxygenase and the NADPH-regenerating enzyme phosphite dehydrogenase (PTDH). The fusion enzyme is more solvent and temperature stable. The NADPH-cofactor regenerating function, however, is not used in this study. The protein was overexpressed as described elsewhere.[1] Alcohol dehydrogenase from *Saccharomyces* Cerivisae, Candida Antarctica Lipase B, Alcalase and N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide were obtained from Sigma-Aldrich. PS_{40}-*b*-$PIAT_{50}$ was purchased from Encapson B.V. Nijmegen. Poly(butadiene)$_{46}$-*b*-poly(ethylene oxide)$_{30}$ (PB_{46}-*b*-PEO_{30}) (P9095-BdEO, $M_{n\ PB}$=2,500 g/mol and $M_{n\ PEO}$=1,300 g/mol, PDI=1.04) was purchased from Polymer Source. Biotech cellulose ester dialysis tubing (1 MDa MWCO) was obtained from Spectrapor, Breda, The Netherlands. THF was distilled under Argon from sodium/benzophenone prior to use. “MilliQ” water was doubly deionized (18.2 MΩ). All other reagents and solvents were of the highest quality grade and purchased from commercial suppliers and used without further purification unless stated otherwise.

Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova 400 (400 MHz for ^{1}H) and on a Bruker DMC300 (75 MHz for ^{13}C). Inductively coupled plasma – mass spectrometry (ICP-MS) measurements were conducted on a Thermo Fisher Scientific Xseries I quadrupole machine using 5 mL sample solutions with a 0.49 mg/mL $InCl_3$ internal standard. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM 1010 microscope with an acceleration voltage of 60 kV. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano-Z (Malvern Instruments, Malvern, UK) and sizes obtained *via* the Cumulant algorithm (error indicated is standard deviation). Platereader data were obtained using 384-well flat black polystyrol plates (Greiner Bio-one) on a TECAN Infinite 200 PRO machine. High-resolution mass spectrometry (MS) measurements were performed with a JEOL AccuToF (electrospray ionization). Size-exclusion measurements were performed on an Amersham Ettan LC system (GE Healthcare, Diegem, Belgium) equipped with a fraction collector, using a self-packed Sephadex G-50 (GE Healthcare) column (50 mm length, 8 mm diameter) in 50 mM Tris/HCl buffer (pH 7.6) using a flow rate of 0.5 mL/min.

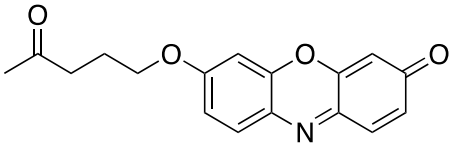
Synthesis

7-(3-(2-methyl-1,3-dioxolan-2-yl)propoxy)-3H-phenoxazin-3-one (I)



To a solution of 7-Hydroxy-3H-phenoxazin-3-one (100 mg, 0.47 mmol) in DMF (5 mL), was added 2-(3-chloropropyl)-2-methyl-1,3-dioxolane (176.4 µL, 1.173 mmol) and K_2CO_3 (162 mg, 1.173 mmol). The reaction mixture was stirred for 48 hours at 65 ºC. The mixture was diluted with ethyl acetate (30 mL) and extracted with 1 M NaOH (3 x 10 mL), after which the organic phase was dried over Na_2SO_4 and concentrated. Column chromatography (EtOAc : heptane, 3:1, R_f 0,48) yielded the product as an orange solid (64 mg, 40%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.68 (d, J = 8.9 Hz, 1H), 7.41 (d, J = 9.8 Hz, 1H), 6.92 (dt, J = 8.9, 2.4 Hz, 1H), 6.82 (dt, J = 3.9, 2.0 Hz, 1H), 6.79 (d, J = 2.6 Hz, 1H), 6.31 (t, J = 2.0 Hz, 1H), 4.09 (t, J = 6.4 Hz, 2H), 4.03 – 3.92 (m, 4H), 2.01 – 1.91 (m, 2H), 1.88 – 1.82 (m, 2H), 1.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.36, 163.27, 149.95, 145.78, 145.47, 134.77, 134.21, 131.64, 128.38, 114.18, 109.74, 106.76, 100.57, 69.13, 64.85, 35.45, 24.05, 23.73. HRMS (ESI) m/z calculated for $C_{19}H_{19}NO_5$: 342.13415 $[M+H]^+$, found: 342.13339

7-((4-oxopentyl)oxy)-3H-phenoxazin-3-one (1)



Compound **(I)** (77 mg, 0.225 mmol) was dissolved in acetone (8 ml), to which HCl (2 mL, 2 M) was added. The mixture was stirred overnight, after which the pH was adjusted to 8 via addition of a saturated $NaHCO_3$ solution. EtOAc (50 mL) was added to the mixture, after which the layers were separated. The organic phase was washed with 1 M NaOH (3 x 10 mL). The combined aqueous layers were washed with EtOAc (2 x 10 mL). After combining the organic phases, they were dried over Na_2SO_4 and evaporated. Column chromatography (DCM : MeOH, 99:1, R_f 0,52) yielded the product as an orange solid (62 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 8.9 Hz, 1H), 7.44 (d, J = 9.8 Hz, 1H), 6.94 (dd, J = 8.9, 2.6 Hz, 1H), 6.89 – 6.77 (m, 2H), 6.34 (d, J = 2.1 Hz, 1H), 4.11 (t, J = 6.2 Hz, 2H), 2.70 (t, J = 6.9 Hz, 2H), 2.22 (s, 3H), 2.14 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.77, 186.37, 162.97, 149.92, 145.75, 145.64, 134.79, 134.28, 131.69, 128.47, 113.99, 106.83, 100.69, 68.04, 39.64, 30.17, 23.08. HRMS (ESI) m/z calculated for $C_{17}H_{15}NO_4$: 298.10793 $[M+H]^+$, found: 298.10847

Formation of enzyme-loaded PS-*b*-PIAT nanoreactors

PS-*b*-PIAT nanoreactors were formed by dissolving 0.5 mg of the desired enzyme in 50 mM Tris/HCl buffer (2.5 mL, pH 8.0). This mixture was then gently agitated, while a solution of PS-*b*-PIAT (0.5 mL, 1 mg/mL) in THF for CalB and Alcalase, or dioxane for ADH, was added dropwise until an opaque suspension was obtained. The mixture was then left to self-assemble for 30 minutes before being transferred into a 1,000 kDa MWCO dialysis bag. The nanoreactors were dialysed for 48 hours against 50

mM Tris/HCl buffer (pH 8.0), with regular changes of the external dialysis buffer, to remove non-encapsulated enzymes from the solution.[2] After dialysis, volumes were corrected to 2 mL and the nanoreactor solutions were stored at 4 ºC and used within 3 days. Structural integrity was confirmed by TEM and DLS measurements.

Ruthenium-labeling of enzymes

To determine encapsulation efficiencies of ADH, CalB and Alcalase in PS-*b*-PIAT nanoreactors, 2 mg of protein was dissolved in 0.5 mL phosphate buffer (20 mM, pH 7.4). The proteins were first washed by centrifugation (3 x 5 min, 13000 rpm), using Amicon Ultra centrifugal filters with 10 kDa membranes. In between centrifugations, the supernatant was replenished with 400 µL phosphate buffer. After the final washing, the proteins were resuspended in 500 µL phosphate buffer, to which was added K_2CO_3 (60 µL, 1 mg/mL) and bis(2,2'-bipyridine)-(5-isothiocyanato-phenanthroline) ruthenium bis(hexafluorophosphate) (2.5 equivalents per protein, 1 mg/mL in phosphate buffer). The yellow/orange mixture was shaken overnight at 25 ºC. Labeled proteins were then purified by spin filtration using Amicon Ultra centrifugal filters 10 kDa membranes (5 x 5 min, 13000 rpm). In between washings, the supernatant was replenished with 400 µL of 50 mM Tris/HCl pH 8.0 buffer. After the last centrifugation step, the proteins were redissolved in a total volume of 500 µL and stored at 4 ºC.

Encapsulation efficiencies via ICP-MS analysis

Nanoreactors containing Ru-labeled enzymes were prepared as described above. After dialysis, the suspensions were lyophilized and then destructed in nitric acid (65%, 0.5 mL) for 3 hours at 80 ºC. After this, the samples were cooled down to room temperature and, after adding an internal standard of $InCl_3$ (1.5 mL, 0.49 mg/mL), the volume was adjusted to 5.0 mL with MilliQ. The Ru-counts of each sample were standardized using the internal standard counts and subsequently compared to samples with a known amount of Ru-labelled enzyme.

Enzymatic activity assays

For fluorimetry platereader experiments, experiments were prepared in triplicates of 60 µL and measured in 3 wells of a 384-wells plate. Reaction mixtures contained CalB nanoreactors (50 µL), NADPH (24 µL of 5 mM; 600 µM), NAD^+ (18 µL of 5 mM; 600 µM), substrate (**1**) (6 µL of 10 mM in DMSO; 300 µM), PAMO (40 µL of 100 µM; 20 µM) and either ADH enzyme (20 µL of 100 µM; 10 µM) or ADH nanoreactors (62 µL). The mixture was then completed with 50 mM Tris/HCl pH 8.0 buffer up to 200 µL total volume. Assays were performed at 25 ºC, while fluorescence emission intensity was measured at 590 nm, with an excitation wavelength of 561 nm.

For determination of relative reaction speeds between compartmentalized and non-compartmentalized cascade systems, the slopes of the reactions were compared between t=10200 sec and t=20100 sec and expressed as a percentage of the free enzyme cascade reaction. The conditions used were based upon nanoreactor

loading efficiencies: CalB 50 µL nanoreactors or 0.40 µM enzyme; ADH 62 µL nanoreactors or 0.26 µM enzyme; Alcalase 122.2 µL nanoreactors or 0.19 µM enzyme, here the same CalB concentrations were used for comparison.

Alcalase degradation assay

A solution of Alcalase (6.24 µL, 0.1 mg/mL, 0.19 µM final) was added to 113.76 µL of 50 mM Tris/HCl pH 8.0 buffer and shaken at 750 rpm at either 25 ºC or 37 ºC for 0, 2.5 or 8 hours. At the designated timepoints, 10 µL of this solution was mixed with 28 µL buffer and 2 µL N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (20 mM in DMSO; 1.25 mM final). The reaction was followed via the production of para-nitroaniline by a UV spectrophotometer measuring at 410 nm. The slope observed for the initial 30 seconds of the reaction was used to determine the residual enzymatic activity.

Encapsulation of PS-*b*-PIAT nanoreactors in giant PB-*b*-PEO polymersomes

All the components for the nanoreactor cascade mixture were mixed together in similar ratios as described for the platereader experiments, yet to a final volume of 40 µL instead of 200 µL; CalB nanoreactors (10 µL), NADPH (4.8 µL of 5 mM; 600 µM), NAD+ (4.8 µL of 5 mM; 600 µM), substrate (**1**) (1.2 µL of 10 mM in DMSO; 300 µM), CRE3-PAMO (8 µL of 100 µM; 20 µM) and ADH nanoreactors (10 µL). As soon as the substrate, the final component added, was mixed in, the Eppendorf tube containing the mixture was placed in a Thermomixer® (Eppendorf) at 25°C and agitated with 800 rotations per minute (rpm). The nanoreactor cascade solution was submitted to 30 minutes of agitation to thoroughly mix all the components of the cascade together and dissolve the sucrose responsible for the 380 mOsm inside the final PB-*b*-PEO vesicles. Then, following a procedure published elsewhere,[3] 5 µL of the nanoreactor cascade solution was first poured in another Eppendorf tube containing 3 mg/mL PB-*b*-PEO in 500 µL toluene (step 1). The PB-*b*-PEO solution in toluene was previously stirred overnight to ensure a complete dissolution of the copolymer, as verified by DLS (no scattering intensity). In another tube (step 2), 30 µL of the same organic solution was poured over 30 µL of a 430 mOsm aqueous glucose solution and allowed to stabilize for 30 min. Finally, the first tube was emulsified with vigorous agitation by hand yielding inverted emulsion droplets. Then 50 µL of this emulsion was poured slowly over the interface tube. The sample was then immediately centrifuged at 25 °C at 500 g for 4 min and the aqueous polymersomes-in-polymersomes suspension was recovered in the lower phase. The samples were always kept in thermomixers at 25°C until the moment they were put in microscopy chambers and analyzed by spinning disk confocal microscopy.

Spinning disk confocal microscopy

Microscopy chambers were µSlides VI 0.4 (hydrophobic, uncoated, Ibidi, Germany). The spinning disk microscope was a Leica DMI6000 (Leica Microsystems, Wetzlar, Germany) equipped with a confocal Scanner Unit CSU-X1 (Yokogawa Electric Corporation, Tokyo, Japan) using for this experiment the objective HCX PL Apo 63X

oil NA 1.4 and an EMCCD camera Photometrics Quantem (Roper Scientific, Evry, France). Z-stack analysis was performed with a piezo (Physik Instrumente (PI), Karlsruhe, Germany). The diode laser used was a Cobolt Calypso of 100 mV (Cobolt AB, Solno, Stockholm) which emitted at 491 nm. A Semrock emission filter with a bandpass window in the red (**593 to 668 nm**) was used. Exposure time was set at 250 ms and the laser power was always set at 30% except when stated otherwise. The experiments were performed in the *Bordeaux Imaging Center of the University of Bordeaux Segalen*. (CNRS / *University of Bordeaux Segalen / Inserm)*.

Image processing and analysis for spinning disk confocal microscopy

Processing and analysis of spinning disk fluorescence confocal acquisitions were performed with ImageJ[4] software developed by the NIH and available as public domain software at http://rsbweb.nih.gov/ij/. It consisted in analysing the mean intensity of the complete 2D area of the multicompartmentalized structures. For Figure 4, a plot profile of the cross-section of the frames was also extracted, giving intensity with the distance on this cross-section. The analysed data was raw and unenhanced. Z-stacks were reconstructed to a three-dimensional structure with Imaris software (Bitplane, Zurick, Switzerland).

Colocalization studies via size-exclusion chromatography

For analysis 100 µL sample was applied onto the column with a flow rate of 0.5 mL/min at room temperature. After separation, fractions of 300 µL each were collected and analyzed via fluorescence measurements using a platereader (excitation 561 nm, emission 592 nm).

2. TEM analysis

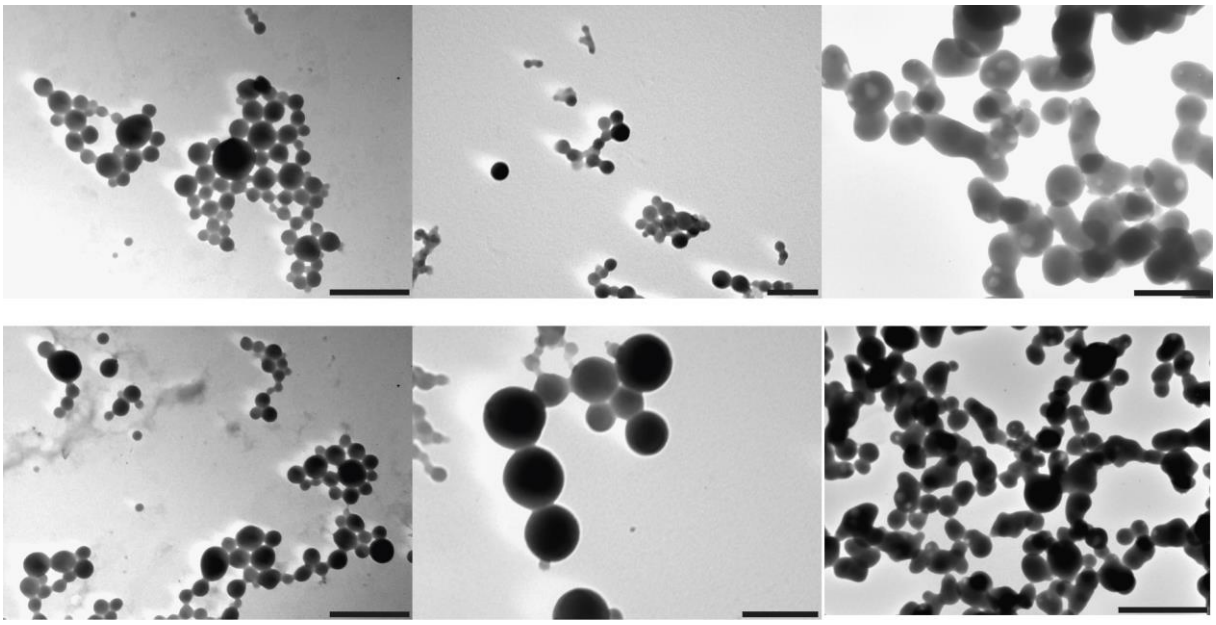


Figure S1. TEM micrographs of CalB PS-*b*-PIAT nanoreactors (left, scale bar 500 nm), ADH nanoreactors (middle, scale bar 200 nm) and Alcalase PS-*b*-PIAT nanoreactors (right scale bar 500 nm for top micrograph and 1000 nm for bottom micrograph).

3. Encapsulation efficiencies

Enzyme	Amount (mg)	Molecular Weight (kDa)	Encapsulated (nmol)	Concentration (µM)
CalB	0.5	33	3.24 (21.4 %)	1.62
ADH	0.5	150	1.71 (51.2 %)	0.85
Alcalase	0.5	27	0.62 (3.4%)	0.31

Table S1. Encapsulation efficiencies and incorporated quantities of enzymes in PS-*b*-PIAT nanoreactors determined via ICP-MS measurements and calculated final enzyme concentrations in PS-*b*-PIAT nanoreactor solutions. Measurements were performed in triplicate for ADH and Alcalase and in duplicate for CalB.

4. Enzymatic assays

Encapsulated enzymes	Enzymes in solution	Relative reaction rate (%)	Relative conversion (%)
-	PAMO, CalB, ADH	100	100
CalB	PAMO, ADH	83	81
CalB, ADH	PAMO	87	83

Table S2. Numerical data for Figure 2b, showing relative reaction rates and relative conversions for each of the displayed cascade reactions.

Encapsulated enzymes	Enzymes in solution	Relative reaction rate (%)	Relative conversion (%)
-	PAMO, CalB, ADH	100	100
-	PAMO, Alcalase, ADH	31	33
Alcalase	PAMO, ADH	71	71

Table S3. Numerical data for Figure 3a, showing relative reaction rates and relative conversions for each of the displayed cascade reactions.

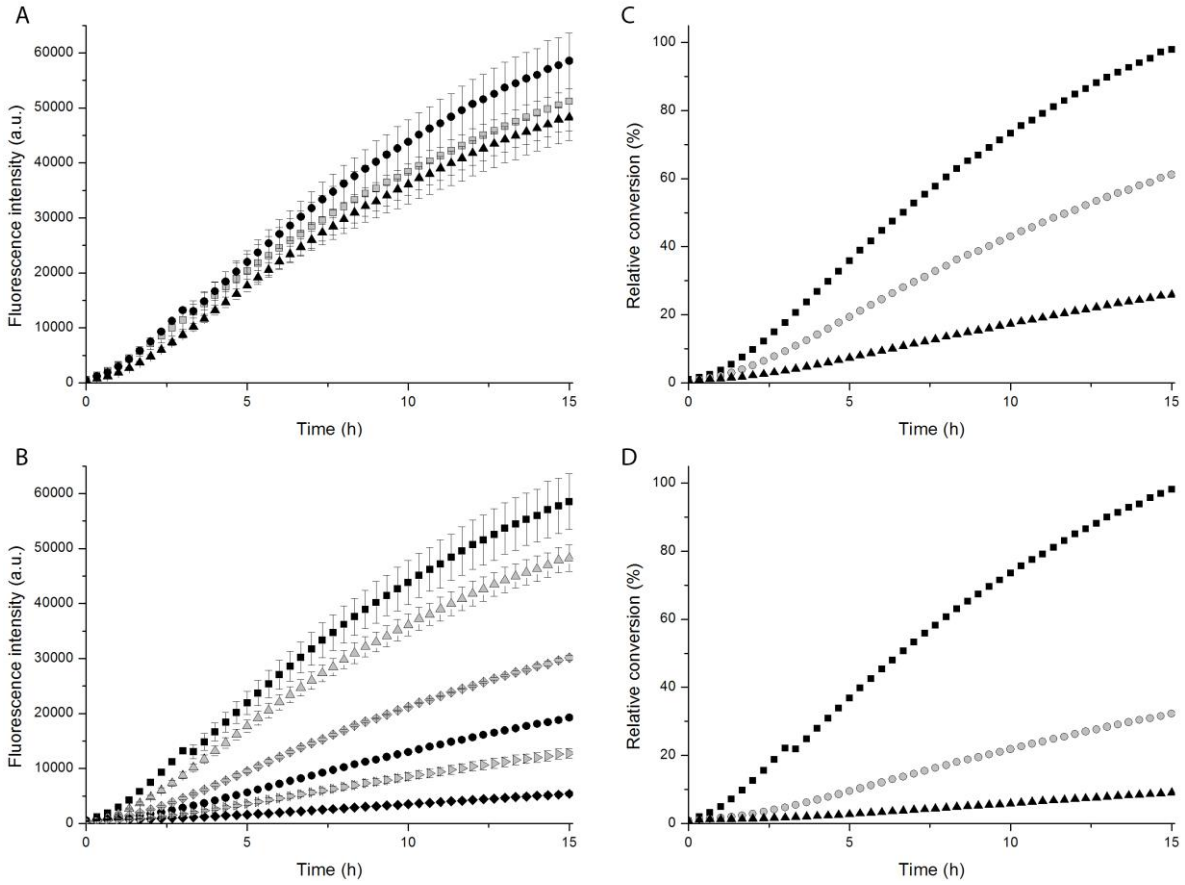


Figure S2. Ensemble fluorescence intensity measurements using Alcalase as a substitute for CalB in the cascade reaction. Reaction is performed at 25 ºC, where only esterase activity is observed for Alcalase. A) Absolute fluorescence intensity graphs for the comparison of cascade reactions performed with, from top to bottom, free CalB (squares), free Alcalase (spheres) and Alcalase nanoreactors (triangles) at equal enzyme concentrations. B-D) Assessment of proteolytic degradation in time by introducing a pre-incubation step at 37 ºC to allow degradation, before performing the Alcalase-containing cascade reactions at 25 ºC. B) Absolute fluorescence intensity graphs with different pre-incubation times, from top to bottom: 0 h (grey upward triangles, nanoreactors; black squares, free enzyme), 2.5 h (grey diamonds, nanoreactors; black spheres, free enzyme) and 8 h (grey right arrow, nanoreactors; black diamonds free enzyme). C) Normalized fluorescence intensity measurements for cascade reactions using Alcalase in PS-*b*-PIAT nanoreactors after, from top to bottom, 0 (squares), 2.5 (spheres) and 8 (triangles) hours pre-incubation at 37 ºC. D) Normalized fluorescence intensity measurements for cascade reactions using Alcalase in solution, from top to bottom, after 0 (squares), 2.5 (spheres) and 8 (triangles) hours pre-incubation at 37 ºC. Graphs were normalized based on the maximum conversion of the sample without pre-incubation.

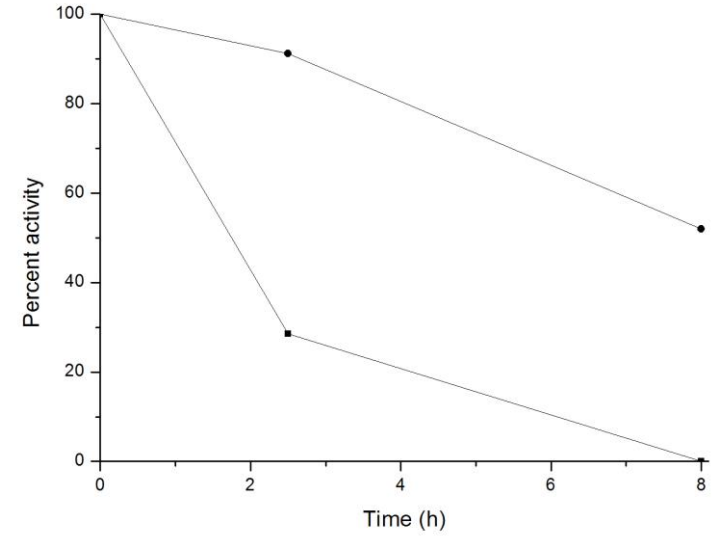


Figure S3. UV measurements of Alcalase proteolytic activity over time, using N-succinyl-AAPF-nitroanilide as substrate, after incubation for 0, 2.5, or 8 hours at 25 ºC (spheres), or 37 ºC (squares). Data were normalized based on the activity at t=0 hours.

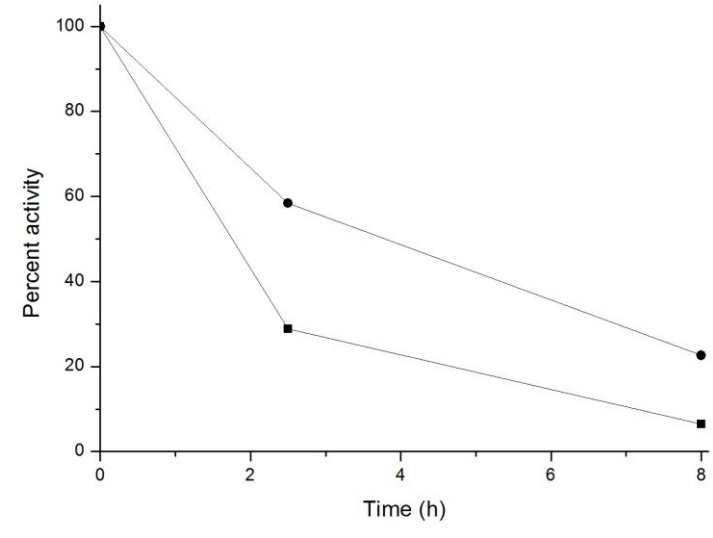


Figure S4. B) Time-dependent decrease in reaction rate of the cascade reaction after pre-incubation at 37 ºC for different periods of time, using either Alcalase nanoreactors (spheres) or Alcalase (squares) in the cascade.

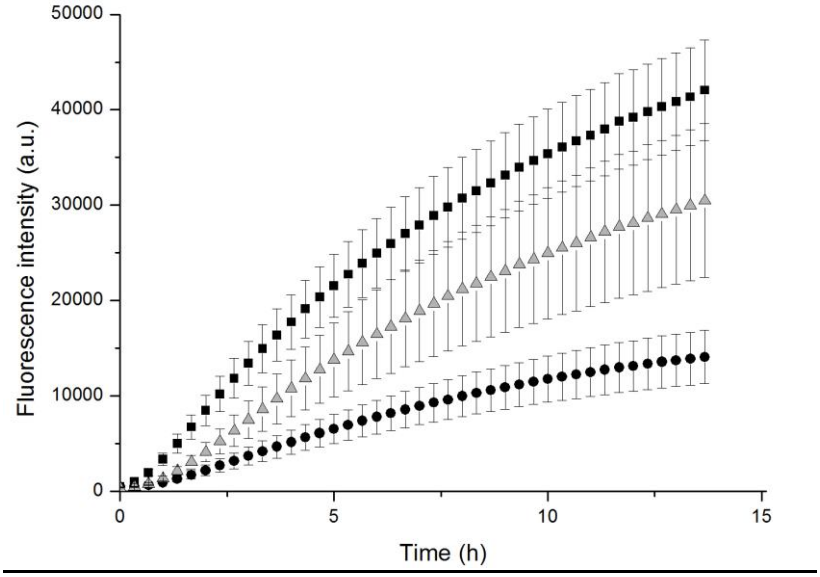


Figure S5. Ensemble fluorescence intensity measurements at 37 ºC showing absolute fluorescence intensity graphs for Figure 3a, displaying the comparison of cascade reactions with free CalB (black squares), Alcalase nanoreactors (grey triangles) and free Alcalase (black spheres) at equal enzyme concentrations.

5. Spinning disk confocal microscopy data

Time	Mean intensity[a]	Standard error[b]
Initial (t = 0h)	1109.8	0.90 (n = 5)
Intermediate (t = 7h55m)	1161.8	2.54 (n = 10)
Full conversion (t∞)	1483.4	46.2 (n = 5)

Table S4. Numerical data for Figure 4, showing mean intensities, standard error, and number of vesicles used to determine these averages.
[a] Mean fluorescence intensity displayed in arbitrary units. [b] Standard error is standard deviation divided by the square root of the number of samples (n).

t	Mean Intensity	Area (pixels)	Minimal Intensity	Maximal Intensity
t_0	**1110.0**	4838	977	1449
	1110.6	2044	986	1372
	1106.5	7482	957	1366
	1111.9	13994	967	1456
	1109.8	3968	976	1366
Time point	**Average Mean Intensity**	**Standard deviation**	**Standard error**	**Number of vesicles**
Negative control, at 33h07 as "t_0"	**1109.8**	**2.02**	**0.90**	**5 vesicles**

Table S5. Representative raw data for the negative control experiment, without PAMO enzyme. As no increase in fluorescence is observed even after 33 h of measurement time, this fluorescence level is also used as "t_0".

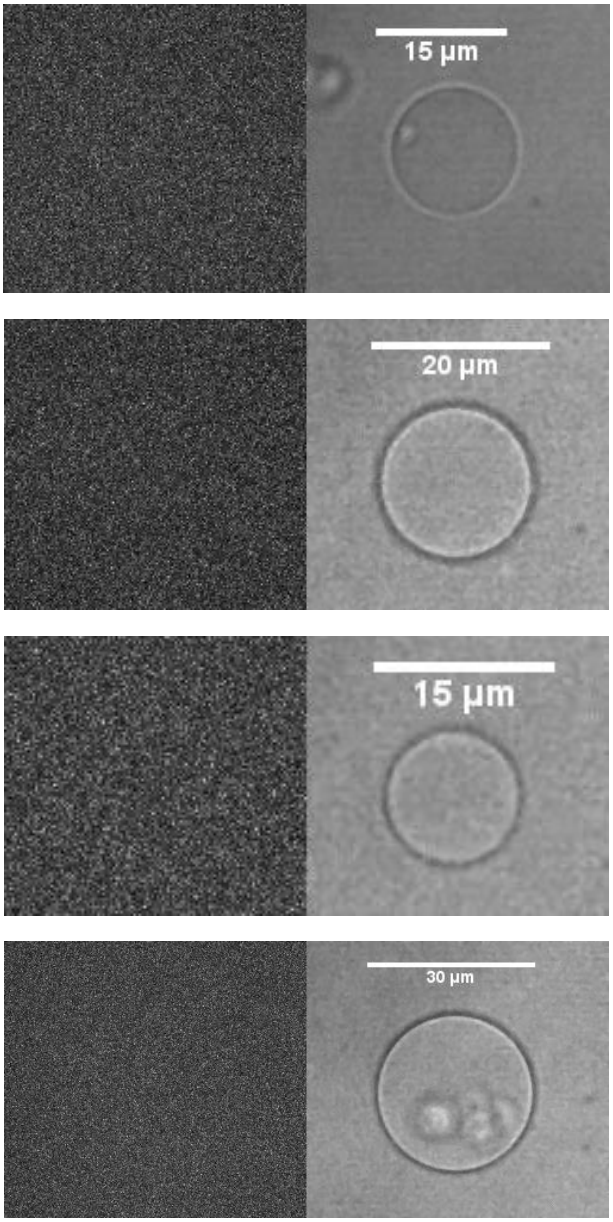


Figure S6. Additional raw images for "t_0" (negative control, without PAMO enzyme) which are relative to the data presented in Table S2.

t	Mean Intensity	Area (pixels)	Minimal Intensity	Maximal Intensity
Intermediate timepoint ≈7h55	**1145.4**	2468	996	1424
	1163.6	28514	989	2231
	1168.8	28514	982	1735
	1167.6	21000	975	5158
	1166.4	21000	987	1607
	1153.8	7156	983	1603
	1160.4	28960	980	1959
	1173.1	28960	991	2040

	1159.6	14626	981	1682
	1159.6	8248	992	2223
Time point	**Average Mean Intensity**	**Standard deviation**	**Standard error**	**Number of vesicles**
Intermediate timepoint ≈7h55	**1161.8**	**8.03**	**2.54**	**10**

Table S6. Representative raw data for the full multicompartmentalized cascade reaction at intermediate timepoint t=7h55.

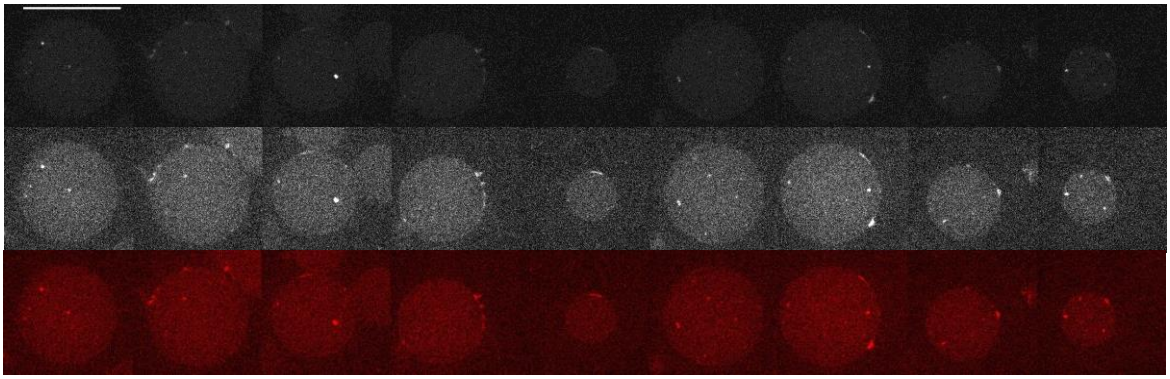


Figure S7. Spinning disk confocal fluorescence acquisitions of multiple multicompartmentalized polymersomes at intermediate time point (7h55). Scale bar is 40µm. Images in the top row correspond to the raw data acquisitions. Image in the middle row are enhanced and images at the bottom are enhanced and coloured acquisitions (red) for the reader's better visualization.

t	**Mean Intensity**	**Area (pixels)**	**Minimal Intensity**	**Maximal Intensity**
t_∞, full conversion, ≈32h35	1427.8	4964	1109	1930
	1646.1	64028	1100	5419
	1525.8	24347	1057	2194
	1402.2	5736	1060	2012
	1415.2	5555	1089	2192
Time point	**Average Mean Intensity**	**Standard deviation**	**Standard error**	**Number of vesicles**
t_∞, full conversion, ≈32h35	**1483.4**	**103.2**	**46.2**	**5 vesicles**

Table S7. Representative raw data for the full multicompartmentalized cascade reaction at "t∞, full conversion"

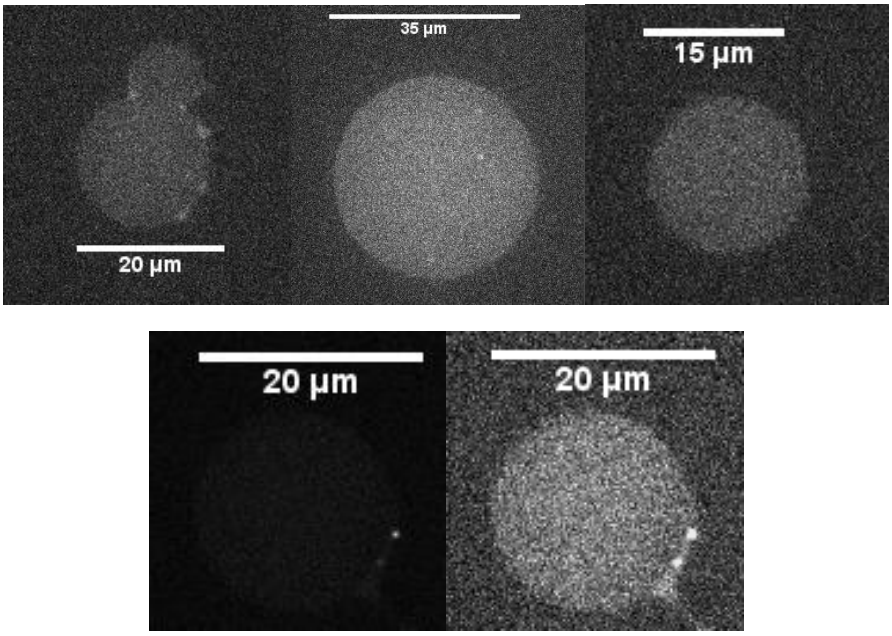


Figure S8. Additional pictures for "t_∞, full conversion" as numerically described in Table S4. All pictures are raw acquisitions, except for the bottom right, which is presented as a raw (left) and enhanced image (right).

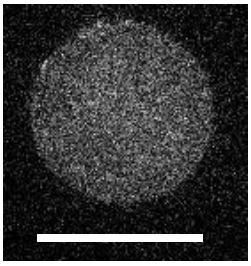


Figure S9. Spinning disk confocal fluorescence micrograph of a multicompartmentalized polymersome containing only CalB nanoreactors and free ADH enzyme in the lumen of the large compartment (scale bar 20 µm).

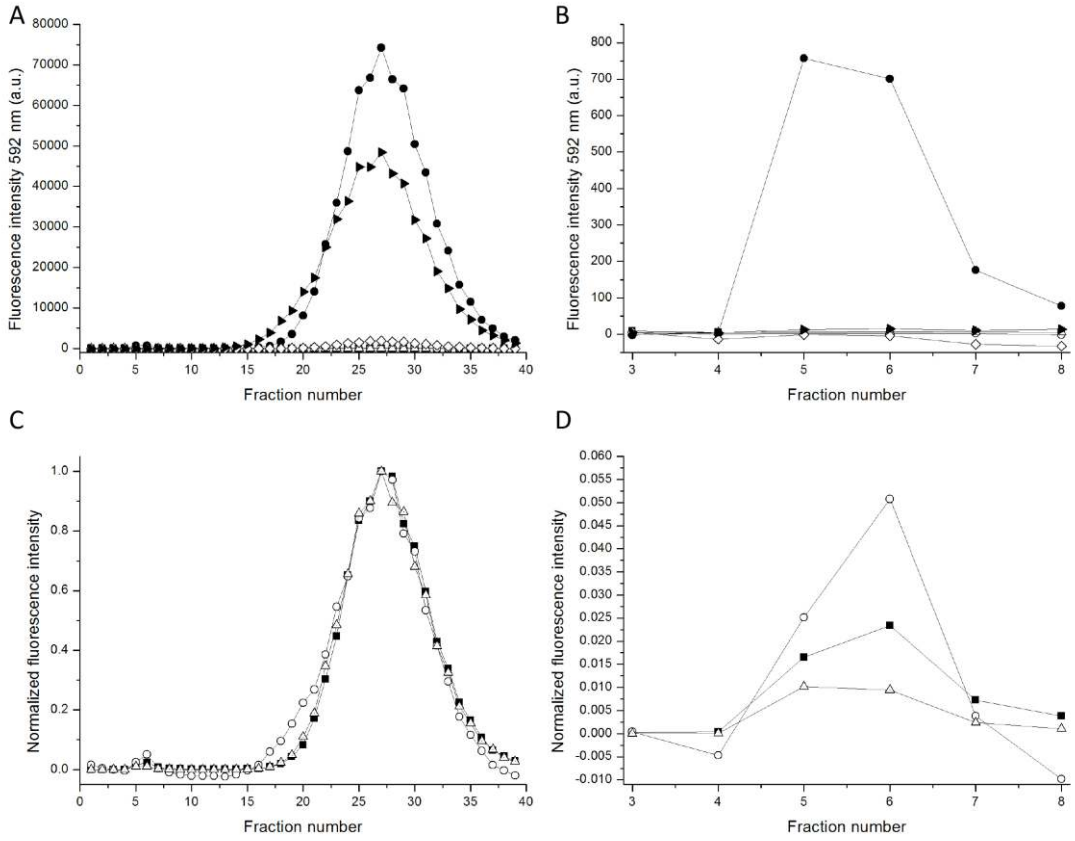


Figure S10. Fluorescence spectroscopy measurements of size-exclusion chromatography fractions of cascade reaction mixtures, showing colocalization of fluorescent resorufin with ADH enzyme or ADH nanoreactors. The enzymes and nanoreactors elute simultaneously (fractions 4-7) as both have higher molecular weights than the column exclusion limit (~30 kDa), resorufin elutes in fractions 20-35. A) Control samples of just ADH in solution (diamonds), ADH nanoreactors (open spheres), CalB in solution (black arrowheads), CalB nanoreactors (open triangles), all showing no resorufin colocalization. Also displayed are reaction mixtures containing both ADH and CalB in solution with substrate (black spheres), or without substrate (black squares), in which only the substrate-containing sample shows strong emission at 592 nm. B) Zoom of same graph, showing fractions 3-8 in which protein and nanoreactors elute, showing resorufin colocalization with ADH. C) Normalized graphs for reaction mixtures containing both ADH and CalB nanoreactors (spheres), ADH in solution with CalB nanoreactors (black squares), and both ADH and CalB in solution (triangles), all showing product conversion and interaction of fluorescent product with ADH nanoreactors or ADH in solution (panel D for zoom).

Movie S1.

Movie S1 caption: Movie of the 3D representation of structure at full conversion, vesicle at t_∞ shown in Figure 3.

References

1. Torres Pazmiño, D. E. *et al.* Efficient biooxidations catalyzed by a new generation of self-sufficient Baeyer-Villiger monooxygenases. *ChemBioChem* **10**, 2595–8 (2009).
2. Van Dongen, S. F. M. *et al.* Cellular integration of an enzyme-loaded polymersome nanoreactor. *Angewandte Chemie International Edition* **49**, 7213-7216 (2010).
3. Marguet, M., Edembe, L. & Lecommandoux, S. Polymersomes in polymersomes: multiple loading and permeability control. *Angewandte Chemie International Edition* **51**, 1173–1176 (2012).
4. Image j (http://rsb.info.nih.gov/ij/) (2008). URL http://rsb.info.nih.gov/ij/.