Electronic Supplementary Material (ESI) for ChemComm.


# Bio-catalytic nanocompartments for *in situ* production of glucose-6-phosphate

***Supporting information***

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## Experimental details

### Chemicals

*Unless stated otherwise, all chemicals were purchased from Sigma Aldrich and used as received.*

### Preparation of phosphoglucomutase (PGM) CatCs and polymersomes/liposomes with 5(6)-Carboxyfluorescein (CF)

Polymersomes were prepared at room temperature from $PMOXA_6$-$PDMS_{34}$-$PMOXA_6$ triblock copolymer,[1] by film hydration method.[2] To produce a thin film, 5 mg of the block copolymer was dissolved in 1 mL of absolute ethanol and subsequently evaporated under reduced pressure (150 mbar, 40 $^0$C water bath, 40 rpm) to render a thin film.

To produce PGM containing CatCs, the polymer films were rehydrated with 1 mL PGM (55 µg/mL or 500 µg/mL), or 500 µg/mL labeled PGM (PGM-ATTO488) in **mixture solution A**: 33 mM Tris-HCl, 33 mM $KH_2PO_4$, and 13 mM $K_2HPO_4$ (pH 7.5).

To produce polymersomes with encapsulated CF, the films were rehydrated with 1 mL 50 mM 5(6)-Carboxyfluorescein (CF) in **buffer B** (150 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH=8.0). The polymer suspensions were stirred overnight at RT.

Meanwhile, liposomes were prepared according to a previously published procedure.[3] Shortly, 20 mg of L-α-phosphatidylcholine from soybean, type IV-S (Sigma) were solubilized in 1 mL $CHCl_3$ in a 2.5 mL green capped glass vial. The organic solvent was removed afterwards under a stream of Ar, followed by 3 hours drying under vacuum in a Varian 801 Thermocouple Vacuum Pressure/Harrick Plasma cleaner. The resulted lipid thin film was rehydrated with 300 µL 50 mM CF in buffer A, followed by gentle stirring overnight at RT. The following day, the lipid suspension was 5 times frozen/thawed using liquid $N_2$/37 $^0$C water bath.

The resulted suspensions were afterwards extruded through a 200 nm (CatCs/polymersomes) or 100 nm (liposomes) pore-sized polycarbonate (PC) membrane (Merck, Germany), using an Avanti mini-extruder (Avanti Polar Lipids, USA).

The non-encapsulated enzyme was removed by dialysis using 300 kDa cut-off dialyzing tubes (Spectrum Labs) placed in 1L beakers in dark containing **mixture solution A**. The dialysis solution was changed 3 times per day and the samples were dialyzed for two consecutive days.

Any free dye was separated from polymersomes or 1:10 diluted liposomes containing entrapped dye by eluting them twice in **buffer C** (300 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH=8.0) through HiTrap desalting columns (Sephadex G-25 Superfine, GE Healthcare, UK).

**Phosphoglucomutase fluorescent labeling**

Firstly, in four centrifugal filters (Vivaspin® 500, 10 KDa MWCO, Sartorius) 25 µL of the stock commercial PGM was added and complemented with 400 µL phosphate-buffered saline (PBS, pH 7.4), followed by their centrifugation at 13400 rpm for 15 min using an Eppendorf MiniSpin microcentrifuge. This process was repeated at least 5 times to ensure the complete removal of the ammonium salts. 100 µL ≈8 mg/mL PGM in PBS was mixed with 5 µL of 0.2 M sodium bicarbonate ($NaHCO_3$) solution (pH = 9.0), followed by 10 µL 10 mM ATTO488 NHS-ester (ATTO-TEC) in DMSO (1:8 molar excess of NHS-ester) and incubated overnight in dark under gentle mixing at room temperature. The next day, the reaction mixture was diluted with the **mixture solution A** up to a final volume of 1105 µL. Any free dye was separated from the conjugated enzyme using HiTrap desalting columns (Sephadex G-25 Superfine, GE Healthcare, UK) by eluting **mixture solution A**. A final concentration of 500 µg/mL labeled enzyme was determined (NanoDrop 2000c Spectrophotometer), which was used for the formation of CatCs.

**Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of PGM and PGM-ATTO488**

To prepare the loading samples, 3 µL of 500 µg/mL PGM or PGM-ATTO488 was mixed with 3 µL 4x Laemli Buffer (Bio-Rad) and 6 µL 33 mM Tris-HCl (125 µg/mL final concentration of enzyme and conjugated enzyme). 5 µL of BenchMark™ Protein Ladder (10-200 kDa, Invitrogen) or 10 µL of loading samples were loaded on commercially available SDS-polyacrylamide gels (4-20% Mini-PROTEAN® TGX™, BIO-RAD) and ran at 100 V in 1X SDS running buffer for approximately 1-2 hours. The gel was placed on a black UV tray and visualized using a Gel Doc™ EZ Imager (BIO-RAD). The gel was afterwards stained with InstantBlue™ Ultrafast protein stain (Sigma) for up to 1 hour, placed on a White tray and visualized again.

**Expression, purification and characterization of engineered α-HL**

The engineered α-hemolysin used herein has been expressed and purified via $Ni^{2+}$-NTA chromatography as previously described.[4] In order to avoid any interference from the engineered α-HL initial solubilization buffer during the concentration measurements, the protein samples were washed with PBS by passing them 5 times through centrifugal filters (Vivaspin® 500, 10 KDa MWCO, Sartorius) and the concentration of the engineered α-HL was determined using the BCA assay (Thermo Scientific), or directly via measuring the absorbance at 280 nm with a NanoDrop 2000c Spectrophotometer. To prepare the loading samples for the SDS-PAGE, 3 µL of stock engineered α-HL was mixed with 3 µL 4x Laemli Buffer (Bio-Rad) and 6 µL 33 mM Tris-HCl. 5 µL of BenchMark™ Protein Ladder (10-200 kDa, Invitrogen) or 10 µL of loading samples were loaded on commercially available SDS-polyacrylamide gels (4-20% Mini-PROTEAN® TGX™, BIO-RAD) and ran at 100 V in 1X SDS running buffer for approximately 1-2 hours and visualized.

**PGM activity**

In a total volume of 200 µL, 1.3 µg/mL PGM (final concentration) was added over the enzyme cocktail mixture: 33 mM Tris-HCl (pH = 7.5), 33 mM $KH_2PO_4$, 13 mM $K_2HPO_4$, $9x10^{-2}$ mM EDTA, $3.6x10^{-4}$ mM G1,6-di-PO4, 0.36 mM $NADP^+$, 15 mM $MgCl_2$, 1 U G6PDH, without or with 0.4 mM G1P. Kinetic spectra were acquired by following the emission of NAPDH at 460 nm (excitation at 340 nm) on a SpectraMaxPlus 384 (Molecular Devices) using a 96-well black fluorescence microplate.

**Transmission electron microscopy (TEM)**

5 µL of sample solutions were adsorbed for 1 min on glow-discharged carbon-coated copper grids. The samples were blotted, washed and negatively stained with 2% uranyl acetate solution. Micrographs were obtained using a transmission electron microscope (Philips Morgagni 268D).

**Dynamic light scattering (DLS)**

The hydrodynamic $D_H$ of CatCs, polymersomes, and liposomes was determined using a Zetasizer Nano ZSP. Measurements were performed using diluted sample solutions using the same mixtures before and after fluorescence measurements, at 25 °C, 173° measurement angle. Data was analyzed by number distribution.

**Fluorescence correlation spectroscopy**

FCS measurements were performed at 20°C on a Zeiss LSM 510-META/Confcor2 laser-scanning microscope equipped with a 458 nm Argon/2 laser and a 40×water-immersion objective (Zeiss C/Apochromat 40X, NA 1.2). Excitation power was $P_L$ = 15 mW, and the excitation transmission was 10%. The diameter of the pinhole was set to 66 µm. For each measurement, 10 µL of sample solution, containing ATTO488, PGM-ATTO488, and CatCs containing PGM-ATTO488, were placed on glass slides. For FCS, spectra were recorded over 10 s, and each measurement was repeated 10 times.

**Fluorescence spectroscopy**

The fluorescence measurements were carried out on a LS 55 Fluorescence Spectrometer from Perkin Elmer controlled by FL Winlab Software. 1 cm path length quartz cuvette, placed in a thermostated stirred single cell holder. The reagents were continuously gentle mixed using a small stirrer. PGM loaded CatCs were excited at 340 nm monitoring their emission at 460 nm. Polymersomes/liposomes loaded with CF were excited at the wavelength of 495 nm and monitored their emission at 517 nm. Slits were set at 5.0 nm.

*Activity of PGM or PGM-ATTO488 loaded CatCs*: In a total volume of 3005 µL, 50 µL of PGM or PGM-ATTO488 loaded CatCs were added over a mixture containing: 33 mM Tris-HCl, 33 mM $KH_2PO_4$, 13 mM $K_2HPO_4$, $9x10^{-2}$ mM

EDTA, $3.6x10^{-4}$ mM G1,6-di-PO4, 0.36 mM $NADP^+$, 15 mM $MgCl_2$, 1 U G6PDH, 4 mM G1P (pH = 7.5), with or without 5 µL stock engineered α-HL.

*Insertion of engineered α-HL into the membrane of polymersomes/liposomes loaded with CF*: In a total volume of 3010 µL, 20 µL of CF loaded polymersomes or liposomes were added over 2980 µL **buffer C**, followed by the addition of 10 µL buffer C, or 0.1% (w/v) stock SDS, or stock engineered α-HL.

*Interaction of F-amphiphiles prior insertion of engineered α-HL into the membrane of polymersomes loaded with CF:* In a total volume of 3005 µL, 20 µL of CF loaded polymersomes were added over 2780 µL **buffer C**, followed by the addition of 200 µL 150 mM stock in double-distilled water solution of fluorinated FOS-choline (Anatrace), and 5 µL stock engineered α-HL.

*Interaction of F-amphiphiles post insertion of engineered α-HL into the membrane of polymersomes loaded with CF:* In a total volume of 3005 µL, 20 µL of CF loaded polymersomes were added over 2940 µL **buffer C**, followed by the addition of 5 µL stock engineered α-HL, and 40 µL 756 mM stock in double-distilled water solution of fluorinated FOS-choline.

## Detailed concept design of the CatC

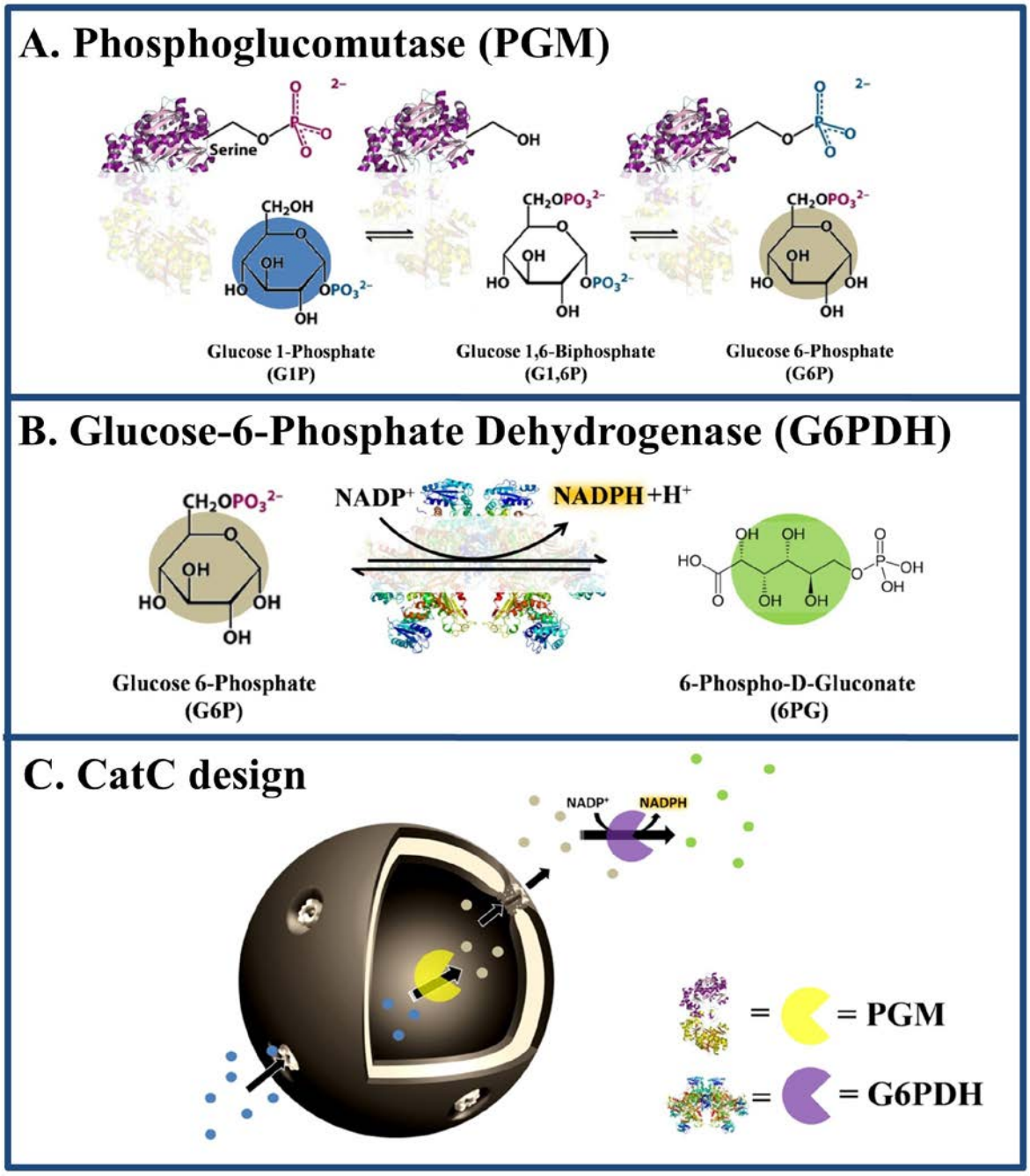


**Fig. S1 A.** The conversion of glucose 1-phosphate (G1P) to glucose 6-phosphate (G6P) is carried out by an important metabolic enzyme: phosphoglucomutase (PGM). A serine residue of PGM transfers its phosphoryl group to the C-6 hydroxyl group of glucose 1-phosphate. In this way, the glucose 1,6-biphosphate (G1,6-di-PO4) is formed as an intermediary product, with the role to shuttle its C-1 phosphoryl group to the same enzyme serine residue, resulting in the regeneration of PGM and formation of G6P as the final product. The presence of divalent cations

($Mg^{2+}$) activators has not been shown for simplicity reasons; **B.** The production of G6P, and thus the PGM enzyme activity, is indirectly monitored through the presence of glucose-6-phosphate dehydrogenase (G6PDH). G6PDH converts G6P to 6-glucose phosphogluconate (6-PG), while reducing nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH, the latter being fluorescent and easy to monitor spectrophotometrically. **C.** Concept design of a bio-catalytic nanocompartment (CatC), in which PGM (designated in yellow) is encapsulated and protected. The specific G1P substrate (blue solid circle) and products (G1,6-di-PO4 and G1P) of PGM can travel through the membrane of the nanoreactor due its permeabilization by an engineered α-hemolysin (engineered α-HL), which allows molecules up to 3-4 kDa to pass through, whilst keeping the PGM entrapped inside the inner cavity. G1,6-di-PO4 has not been drawn for simplicity reason and because it is an intermediary compound in the enzymatic reaction, whilst G1P is designated as a solid pale brown circle. Once out of the CatC through the engineered α-HL, G6P is converted to 6-PG (solid green circle) through the catalytic activity of G6PDH (purple), while reducing nicotinamide adenine dinucleotide phosphate ($NADP^+$) to fluorescently active NADPH.

## Supporting results

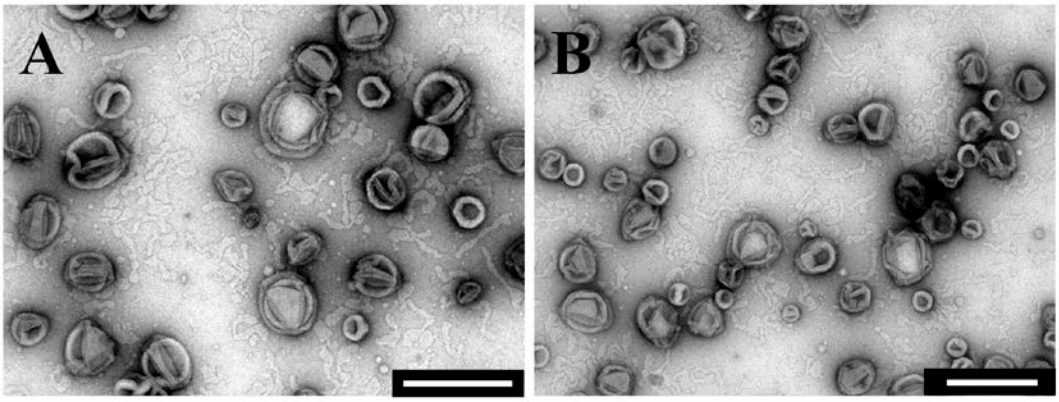


**Fig. S2** Representative TEM micrographs depicting $A_6B_{34}A_6$ CatCs formed by the polymer film rehydration with 55 µg/mL PGM and addition of $6.12x10^{-4}$% SDS (A), or in the presence of the 0.70 µg/mL engineered α-HL (B). Scale bar: 200 nm.

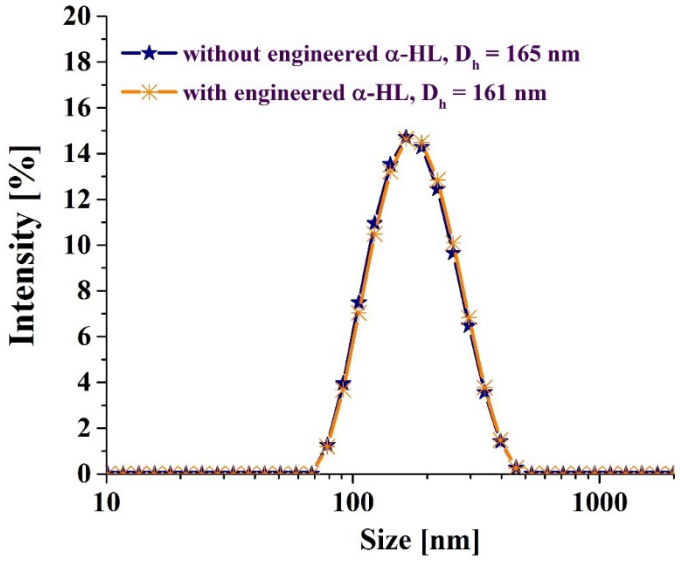


**Fig. S3** Dynamic light scattering (DLS) intensity distribution of $A_6B_{34}A_6$ CatCs resulted by the polymer film rehydration with 55 µg/mL PGM and addition of $6.12x10^{-4}$% SDS (blue curve), or in the presence of the 0.7 µg/mL engineered α-HL (orange curve)

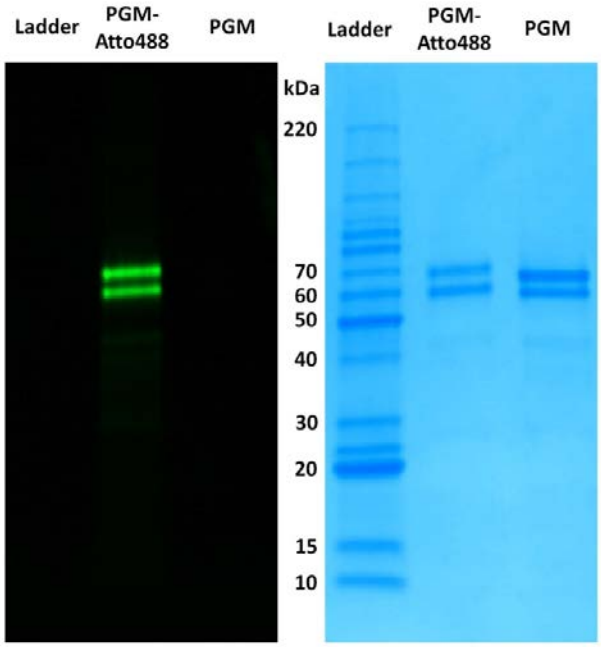


**Fig. S4** SDS PAGE of the fluorescently labeled phosphoglucomutase (PGM-ATTO488, left), followed by the InstantBlue™ staining of the same SDS PAGE to compare the ≈125 µg/mL PGM-ATTO488 to the non-labeled ≈125 µg/mL phosphoglucomutase.

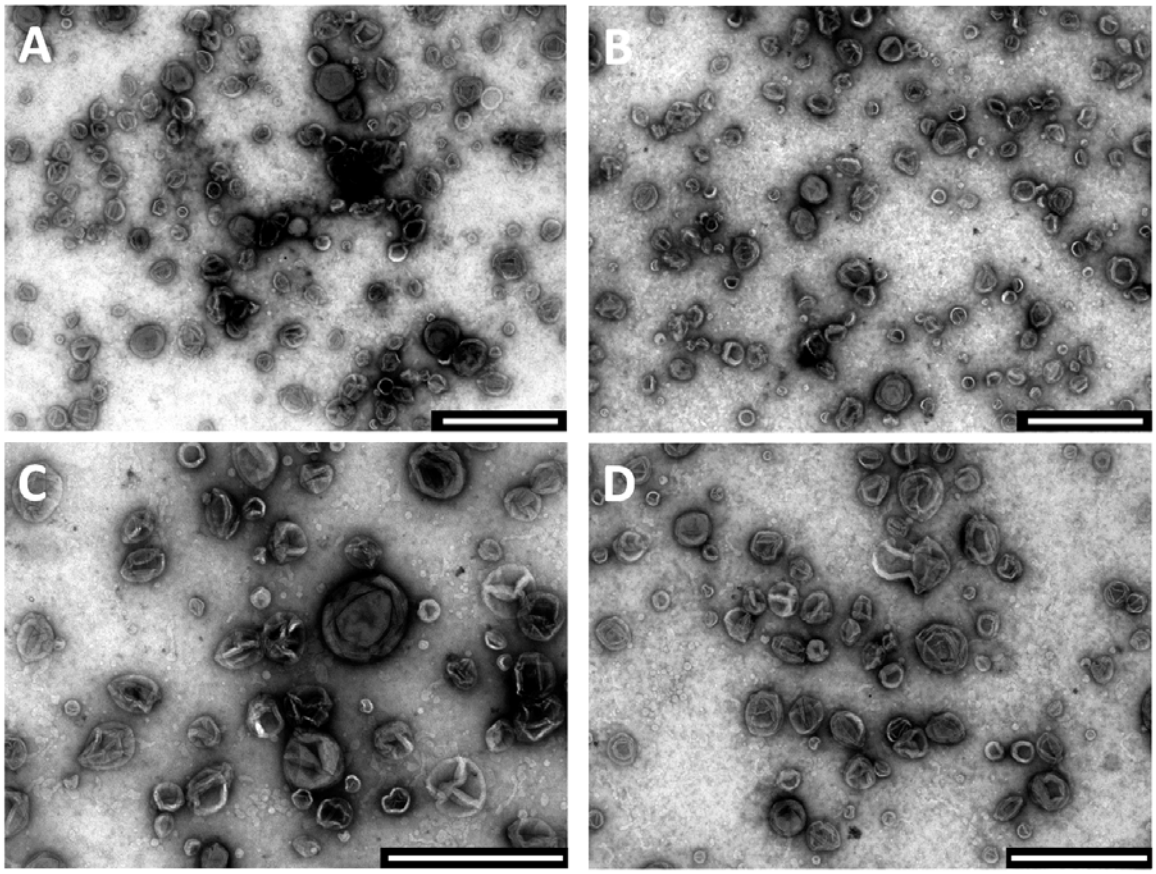


**Fig. S5** TEM micrographs of nanoreactors (CatCs) resulted by the polymer film rehydration with 500 μg/mL PGM (upper side) and with 500 μg/mL PGM-ATTO488 (lower side) in the in the absence (A, C) or presence of engineered α-HL (B, D). Scale bar: 500 nm.

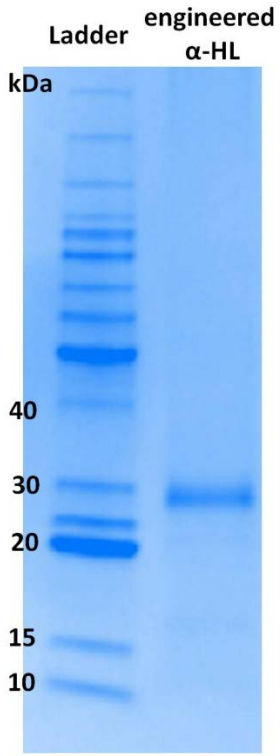


**Fig. S6** SDS Page of the engineered α-HL, which migrated as a majoritary band around 29 kDa.

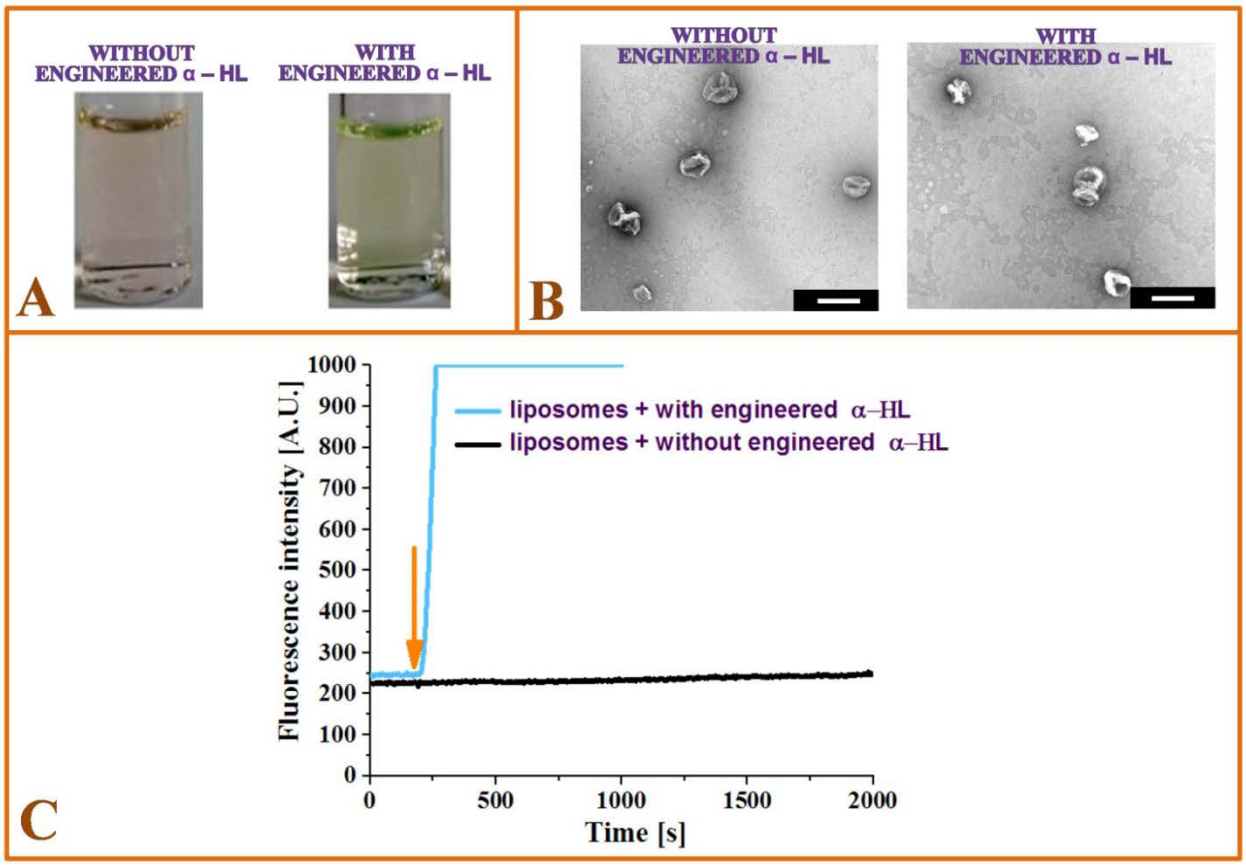


**Fig. S7 A.** Visible color change in the liposome suspension due to the addition of the engineered α-hemolysin; **B**. Morphology and stability of liposomes with and without engineered α-HL (scale bar: 100 nm); **C**. After the addition of 1.3 µg/mL of engineered α-HL (after around 200 s, as indicated by the orange arrow) dye leakage and increase in fluorescence upon dilution from the liposomes was immediatedly observed (light blue curve). No dye leakage upon addition of $6.12 \times 10^{-4}$% (w/v) SDS only (without engineered α-hemolysin) occurred during 2000 s (black curve). As a result, we managed to confirm that the engineered α-HL is able to successfully insert in the membrane of liposomes and allow the dye to be released and increase its fluorescence upon dilution.**

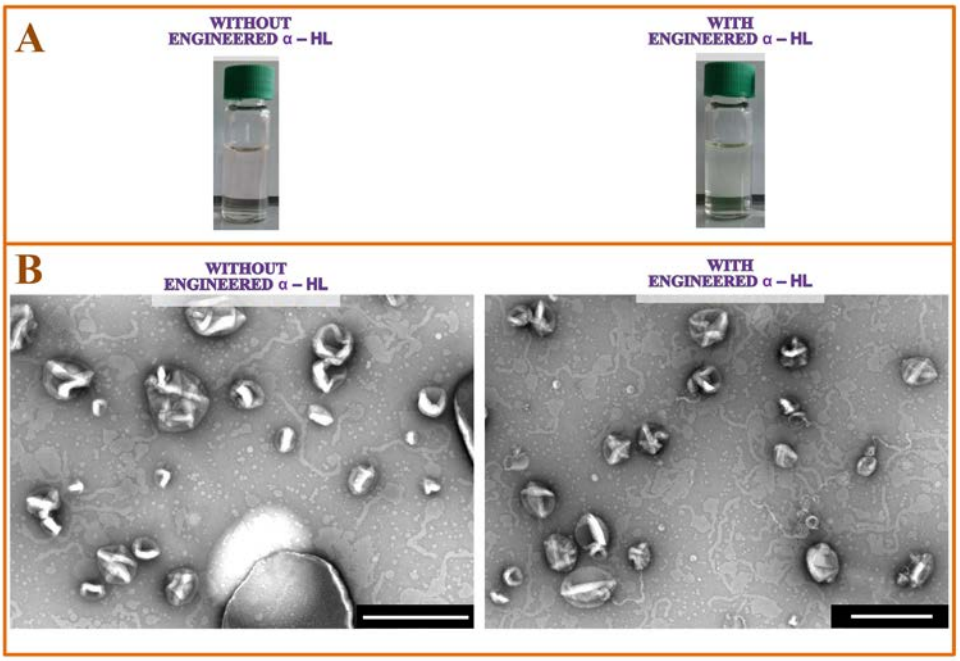


**Fig. S8 A.** Visible color change in the polymersome suspension due to the addition of the engineered α-hemolysin; **B**. Morphology and stability of polymersomes with and without engineered α-HL (scale bar: 500 nm).**

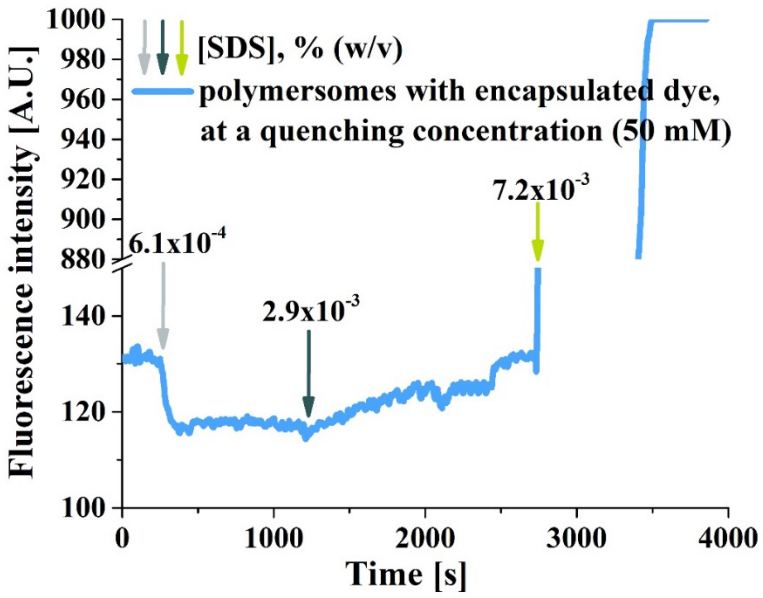


**Fig. S9** Time-driven fluorescence of a $A_6B_{34}A_6$ polymersome sample with encapsulated carboxyfluorescein (CF) to which SDS was added from a 0.1% (w/v) SDS and the integrity of the membrane was monitored via the release of the dye, correlated with the increase in fluorescence intensity. The addition of SDS is indicated by each arrow with the final calculated SDS concentrations. This experiment was done as a quick run on a single polymersome sample with encapsulated CF to which SDS was added at the indicated final concentrations and the change in fluorescence intensity was monitored in time. To confirm this result, fluorescence spectra was acquired for three different $A_6B_{34}A_6$ polymersome samples with encapsulated CF, which were mixed with SDS at the same concentrations and presented in **Fig. S10** **

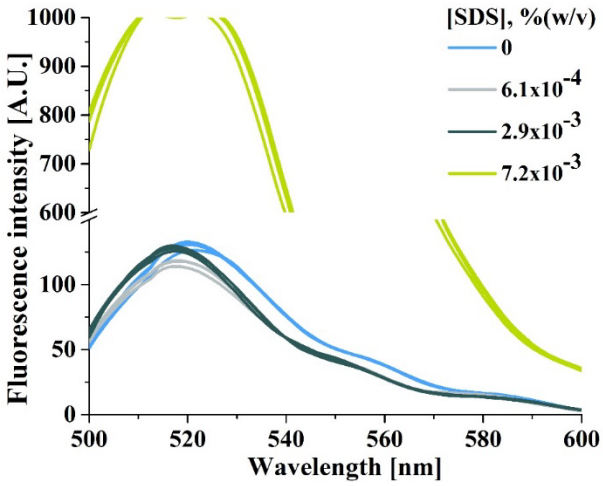


**Fig. S10** Fluorescence spectra of the three independent preparations of $A_6B_{34}A_6$ polymersome samples with encapsulated CF over which SDS was added from a 0.1% (w/v) SDS and the integrity of the membrane was monitored via the release of the dye, correlated with the increase in fluorescence intensity: intrinsic fluorescence of $A_6B_{34}A_6$ polymersome samples with encapsulated CF (blue spectra), upon the presence of $6.1x10^{-4}$% (w/v) SDS, upon the presence of $2.9x10^{-3}$% (w/v) SDS (green spectra), and upon $7.2x10^{-3}$% (w/v) SDS (light green spectra). **

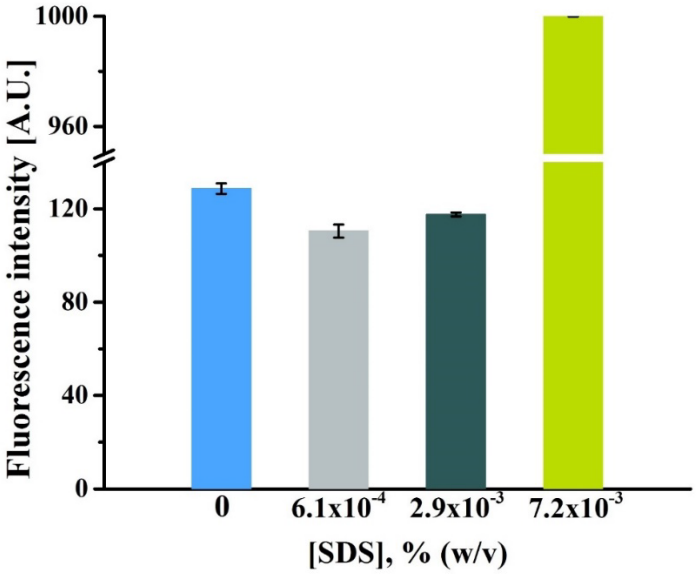


**Fig. S11** Fluorescence emission maxima of the three independent preparations of $A_6B_{34}A_6$ polymersome samples with encapsulated carboxyfluorescein (CF) over which SDS was added from a 0.1% (w/v) SDS and the integrity of the membrane was monitored via the release of the dye, correlated with the increase in fluorescence intensity, respresented as bars with errors. A slight decrease in fluorescence intensity is observed in the presence of $6.1x10^{-4}$% (w/v) SDS (grey bar) due to a slight dilution of the $A_6B_{34}A_6$ polymersome samples with encapsulated CF (blue

bar). The presence of 2.9x10$^{-3}$% (w/v) SDS induces the first signs of dye leakage through the membrane (dark green bar), while the presence of 7.2x10$^{-3}$% (w/v) SDS induces an even higher leakage of the dye (light green bar).**

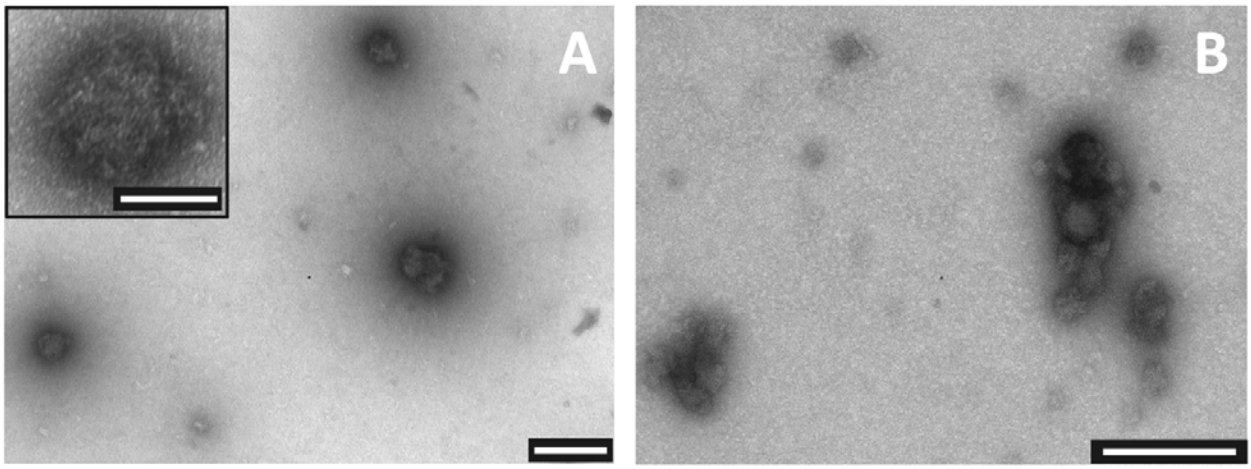


**Fig. S12** Transmission electron microscopy (TEM) of 10 mM F-amphiphile aggregates in the absence (A) or presence of engineered α-HL (B). Scale bar: 200 nm. The inset represents a zoom of a 10 mM F-amphiphiles aggregates (scale bar: 100 nm).

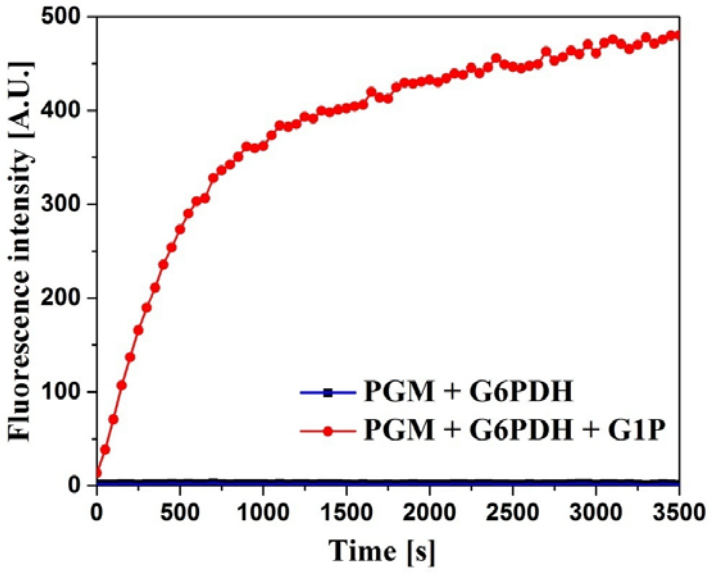


**Fig. S13** Activity of bulk 1.3 µg/mL PGM without G1P substrate (dark blue curve), or with 0.45 mM G1P substrate (red curve).*

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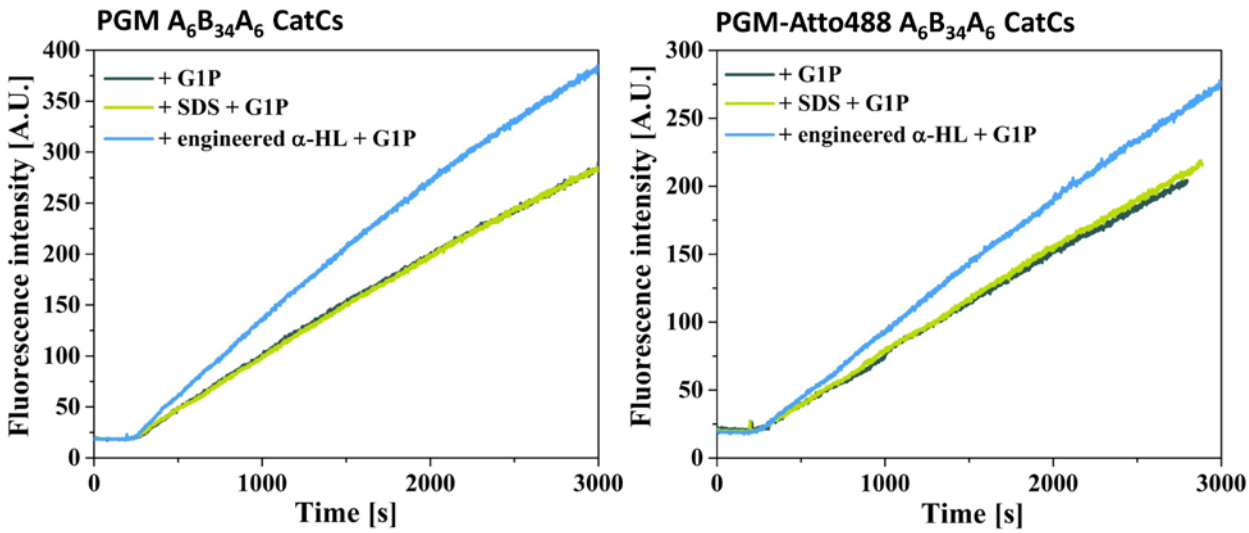


**Fig. S14** Activity of the nanoreactors (CatCs) resulted by the polymer film rehydration with 500 µg/mL PGM (left) and with 500 µg/mL PGM-ATTO488 (right). Fluorescence intensity increase upon addition of 4 mM G1P (final concentration) over the CatCs (dark green curve), CatCs with SDS (light green curve), and CatCs with engineered α-HL (blue curve). The activity of the CatCs without having a permeabilized membrane, is due to PGM non-specifically attached on the membrane of the CatCs accessed by the G1P substrate.*

* *Experiments based on enzymatic reactions were carried out at pH = 7.5, RT.*

** *Experiments based on dye release were carried out at pH = 8.0, RT.*

**References**

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