Electronic Supplementary Information

Detection of subtle extracellular glucose changes by artificial organelles in protocells

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1. Materials

O,O′-bis[*2*-(*N*-succinimidyl-succinylamino)ethyl] polyethylene glycol (NHS-PEG-NHS, Mn 2000, Sigma-Aldrich), fluorescein isothiocyanate-dextran (FITC-Dextran Mw 20 kDa, 40 kDa, 70 kDa, 150 kDa Sigma-Aldrich), alkaline phosphatase obtained from bovine intestinal mucosa (ALP, ≥ 10 DEA units/mg solid, Sigma-Aldrich), catalase obtained from bovine liver (CAT, 10000 - 40000 units/mg, aqueous suspension, Sigma-Aldrich), glucose oxidase obtained from aspergillus niger (GOx, 100000 - 250000 units/g solid, Sigma-Aldrich), bovine serum albumin (BSA, ≥ 98 %, Sigma-Aldrich), Cyanine 5 NHS-ester (Cy5, Lumiprobe), AF488 NHS-ester (AF488, Lumiprobe), Rhodamine B isothiocyanate (RhB, Sigma-Aldrich), 4 - 12 % NuPAGE Bis-Tris gel (Thermofisher), *2,3*-dimethylmaleic anhydride (98 %, Sigma-Aldrich), toluene (99.8 %, Sigma-Aldrich), *4*-amino-*1*-butanol (98 %, Sigma-Aldrich), *n*-hexane (Sigma-Aldrich), ethyl acetate (Sigma-Aldrich), tetrahydrofuran (THF, 98 %, Sigma-Aldrich), maleic imide (99 %, Sigma-Aldrich), methacryloyl chloride (97 %, Sigma-Aldrich), triethylamine (99.5 %, Sigma-Aldrich), magnesium sulfate (Sigma-Aldrich), MeO-PEG_{45}-OH (PEG_{45}, Mn 2000, Sigma-Aldrich), *α*-bromoisobutyryl bromide, (BIBB, 98 %, Sigma-Aldrich), *β*-(diethylamino)ethyl methacrylate (99 %, Sigma-Aldrich), copper(I) bromide (CuBr, 98 %, Sigma-Aldrich), *2,2′*-bipyridine (99.5 %, Sigma-Aldrich), *2*-butanone (99 %, Sigma-Aldrich), *4,4′*-azobis-(*4*-cyan-valeriansäure) (ACVA, 98 %, Sigma-Aldrich), *2*-mercaptothiazoline (98 %, Sigma-Aldrich), *1,4*-dioxane (99.8 %, Sigma-Aldrich), *N,N′*-dicyclohexylcarbodiimide (DCC (99 %, Sigma-Aldrich), *4*-(dimethylamino)pyridine (DMAP, 99 %, Sigma-Aldrich), sodium hydride (NaH, 60 % dispersion in mineral oil, Sigma-Aldrich), propanethiol (99 %, Sigma-Aldrich), carbon disulfide (CS_2, 99 %, Sigma-Aldrich), iodine (Sigma-Aldrich), sodium thiosulfate (Sigma-Aldrich), *N*-isopropylacrylamide (NIPAM, 97 %, Sigma-Aldrich), *2,2′*-azobis(*2*-methylpropionitrile) (AIBN, 98 %, Sigma-Aldrich), *1,6*-diaminohexane (Sigma-Aldrich), *3*-(*N*-morpholino)propane sulfonic acid (MOPS, 99.5 %, Sigma-Aldrich), *2*-(*N*-morpholino)ethanesulfonic acid (MES, 99.5 %, Sigma-Aldrich), *N*-ethyl-*N′*-(*3*-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 97 %, Sigma-Aldrich), $NaHCO_3$ (Sigma-Aldrich), dimethyl sulfoxide (DMSO, 99.5 %, Sigma-Aldrich), fluorescein diphosphate (FDP, 95 %, Thermofisher). Milli-Q water was employed to prepare all the solutions in this work.

2. Instrumentation

NMR Spectroscopy

^{1}H-NMR (500.13 MHz) spectra were recorded on Bruker Avance III 500 spectrometer (Bruker Biospin MRI GmbH, Ettlingen, Germany) at room temperature with $CDCl_3$ (δ = 7.26 ppm) as a solvent. The chemical shifts were referenced to the corresponding solvent signals and are expressed in ppm**.**

Gel Permeation Chromatography (GPC)

The number average molecular weight (M_n), weight average molecular weight (M_w), and molar mass distributions (Đ) of block copolymers were detected using a SEC equipped with a MALLS detector (MiniDAWN-LS detector, Wyatt Technology, USA) and a viscosity/refractive index (RI) detector (ETA-2020, WGE Dr. Bures, Germany). The column (PL MIXED-C with a pore size of 5 μm, 300 * 7.5 mm) and the pump (HPLC pump, Agilent 1200 series) were from Agilent Technologies (USA). THF, stabilized with 0.025 % BHT, was employed as an eluent, and the flow rate was set at 1 mL/min. The calibration was established based on polystyrene standards with Mn values ranging from 1300 to 377400 g/mol. The data were processed using Cirrus GPC offline GPC/SEC software (version 2.0).

Hollow Fiber Filtration (HFF)

Hollow fiber filtration (HFF) was carried out using KrosFlo Research Iii System (SpectrumLabs, USA), equipped with a polysulfone-based separation module (MWCO: 500 kDa, SpectrumLabs, USA). The transmembrane pressure was set at 130 mbar with a flow rate of 15 mL/min. All the samples were purified against 1 mM MOPS buffer at pH 8.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) measurements were carried out using Zetasizer Nano-series instrument (Malvern Instruments, UK). The measurements were performed at 25 ℃, and data were collected at the method of NIBS (non-invasive back-scatter), with a helium–neon laser (4 mW, l = 632.8 nm) at fixed scattering angle of 173 °. The data were analyzed using Malvern Software 7.11

UV lamp for Crosslinking of Psome

The UV irradiation of Psomes was carried out using EXFO Omnicure 1000 (Lumen Dynamics Group Inc., Mississauga, ON, Canada), equipped with a high-pressure mercury lamp as a UV source. The crosslinking process of Psomes samples was carried out at the volume of 2 mL for 180 second per time.

Fluorescence Spectroscopy

Fluorescence spectra were measured on a Fluorolog 3 (Horiba JobinYvon, USA) fluorescence spectrophotometer. Samples were analyzed at desired wavelength range in quartz cuvettes.

Transmission Electron Microscopy (TEM)

The TEM measurement was performed on the Libra 120 microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany), equipped with a charge coupled device (CCD) camera at an acceleration voltage of 120 kV. The samples were prepared by dropping 5 μL of polymersome-

in-proteinosome solution, prepared as described below, on a copper grid modified with holey carbon foil (Lacey type) for 5 min, and then the excess water was removed by a filter paper. Next, 2 µL of 2 % uranyl acetate solution was added to stain the sample for 2 min. After the removal of excess solution, grids were transferred into electron microscope. All images were acquired in bright field.

Cryogenic Transmission Electron Microscopy (cryo-TEM)

The cryo-TEM measurement was performed on the Libra 120 microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany), equipped with a charge coupled device (CCD) camera at an acceleration voltage of 120 kV. The samples were prepared by dropping 2 µL of Psomes solution, prepared as described below, on a copper grid modified with holey carbon foil (Lacey type). After 1 min sedimentation, the excess water was removed at the method of blotting with a filter paper, followed by plunging into liquid ethane (-178 ℃). Then, the frozen grids were transferred into electron microscope using a Gatan 626 cryo-holder. All images were recorded in bright field at -173 ℃. The average diameter and membrane thickness of Psomes were calculated by analyzing 150 - 200 particles from cryo-TEM images.

Confocal Laser Scanning Microscopy (CLSM)

CLSM investigations were performed on a Leica SP5 confocal microscope (Leica, Germany), equipped with an UV-Diode (405 nm), an Argon (458 nm, 476 nm, 488 nm, 496 nm, and 514 nm), a DPSS (561 nm), and a HeNe (633 nm), using a 63x oil immersion objective. Prepared samples, described below, were used by dropping 5 µL of fluorescently labelled solution onto glass slide, and the cover slide was required to seal the specimen.

Time of Flight Mass Spectrometry (MALDI-TOF MS)

Maldi-TOF measurement was conducted on a Bruker Autoflex Speed TOF/TOF in reflector or linear modes, respectively, and positive polarity by pulsed smart beam laser (modified Nd:YAG laser). The ion acceleration voltage was set to 20 k, and 2,5-dihydroxybenzoic acid as matrix substance was employed as matrix substance. Samples were prepared at the concentration of 1.0 mg/mL without salt.

Gel Electrophoresis

Samples (0.5 mg/mL) were prepared by dissolving protein in loading buffer (Laemmli sample buffer, 2x), followed by denaturing the protein at 85 °C for 5 min. After the addition of 10 ul of sample solution into separate channels of a 4 - 12 % NuPAGE Bis-Tris gel, gel electrophoresis was performed on a chamber system (XCell SureLock Mini-Cell Electrophoresis System, ThermoFisher Scienfitic) using a constant current and voltage (400 mA, 200 V, ca. 30 min). Next, the gel was stained by the staining solution (0.1 %, w/v, Coomassie R-250, in a mixture of H_2O/ethanol/acetic acid, 50:40:10, vol-%) for further visualization.

Microplate Reader

Fluorescence intensity was measured on a microplate reader (Infinite M Nano by Tecan, Switzerland), and samples were added on a 96-well microplate for the measurement at the desired wavelength range.

Asymmetrical Flow Field Flow Fractionation (AF4)

AF4 measurements were carried out on an Eclipse DUALTEC system (Wyatt Technology Europe, Germany) with a 0.001 M PBS buffer (at pH 8 and 6) as mobile phase and 0.02 % (w/v) NaN_3 to prevent the growth of algae and bacteria. The channel spacer, made of poly(tetrafluoroethylene), was 490 µm in thickness, and the channel dimensions were 26.5 cm in length and 0.6 - 2.1 cm in width. Regenerated cellulose membrane (MWCO: 10 kDa) was employed as accumulation wall (Superon GmbH, Germany). An Agilent Technologies 1260er series isocratic pump equipped with a vacuum degasser was used to control the flow rates. The detection system consisted of a MALS detector (DAWN HELEOS II, Wyatt Technology Europe, Germany) operating at a wavelength of 659 nm with online DLS detector (QELS module, Wyatt Technologies, USA) which is an add-on unit connected to the 99° angle of the MALS, a diode array detector SPD-M20A (Shimadzu Europe, λ = 280 nm) and a refractive index (RI) detector (Optilab T-rEX, Wyatt Technology Europe GmbH, Germany) operating at a wavelength of 658 nm. All injections were performed with an autosampler (1260 series, Agilent Technologies Deutschland GmbH). The data collection and calculation of molecular weights and radii were performed by Astra 7.3.219 software (Wyatt Technologies, USA). Cross flow rate (F_x) profile was optimized to achieve optimal fractionation of free ALP molecules from Psomes within the same elution. The following protocol was applied: detector flow was set to 0.5 mL/min, focusing was performed with focus flow (F_f) 2.5 mL/min for 4 min followed by an isocratic elution step with a F_x of 2 mL/min for 5 min followed by an exponential F_x gradient from 2 to 0 mL/min within 15 min. The last step proceeds without F_x (0 mL/min) for 15 min. Injections of two times 100 µL and two times 200 µL and one time 400 µL (for DLS) were performed for each sample. M_w and radius of gyration (R_g) of Psomes were calculated from the MALS data of detectors 6 to 17 applying a Berry fit.

Scaling parameter for AF4 data interpretation:

By plotting R_g vs M, ν can be determined by the slope of the plot, it gives information about the molecular shape in the used solvent

$R_g = K \cdot M^{\nu}$

ν = 0.33 (sphere)

ν = 0.5 - 0.6 (random coil macromolecule)

ν = 1 (rigid rod)

Apparent density for AF4 data interpretation:

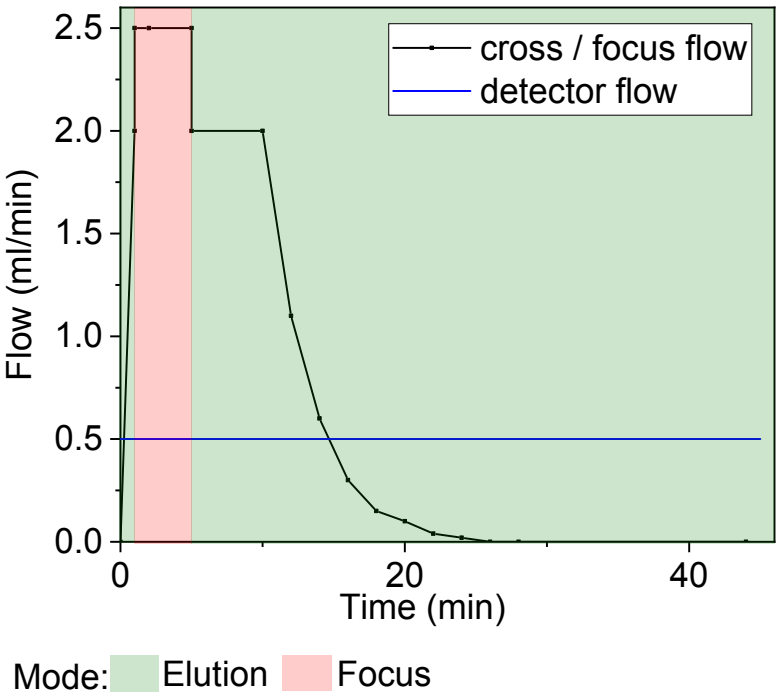
Give information about molecular density, is calculated by R_g and M_w (with V as volume fraction, α as geometrical correction, N_A as Avogadro's number):

$$d_{app,i} = \frac{M_i}{V\ (R_g)_i \cdot N_A} \cdot \alpha \qquad \text{with} \qquad \alpha = \frac{V_{sphere}(R_g)}{V_{sphere}(R)} = \frac{R_g{}^3}{R^3} = \frac{\left(\sqrt{\frac{3}{5}} \cdot R\right)^3}{R^3} = \left(\frac{3}{5}\right)^{\frac{3}{2}}$$

ρ Parameter for AF4 data interpretation:

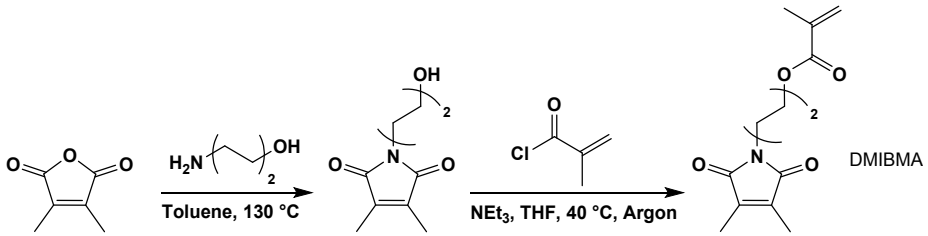
The ratio between R_g and R_h delivers valuable information about conformation and shape of molecules, some examples[1]:

Homogeneous sphere:	0.775
Random coil, linear chain (good solvent):	1.78
Hyperbranched polymer:	1.23
Rod (axial ratio = 2.5):	2.1



3. Experimental part

3.1 Synthesis of the cross-linker 4-(3,4-dimethylmaleic imido) butyl methacrylate (DMIBMA) via two-step process[2]



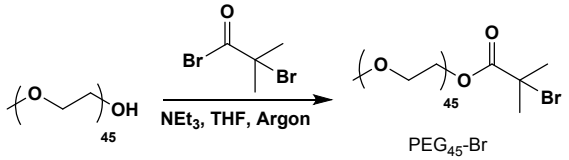
Step 1: *2,3*-dimethylmaleic anhydride (5.00 g, 39.70 mmol, Mw = 126 g/mol) was dissolved in toluene (120 mL) followed by the addition of *4*-amino-*1*-butanol (3.54 g, 39.70 mol, Mw = 89 g/mol). The reaction was performed by keeping the solution mixture at reflux for 3 h at a water trap. After the removal of solvent by rotary evaporation, the crude product was purified by using flash chromatography (*n*-hexane/ethyl acetate, 50:50 vol-%) to generate a white solid in 93 % yield.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 1.51 - 1.57 (m, 3 H), 1.66 (quin, 2 H), 1.95 (s, 6 H), 3.52 (t, 2 H), 3.65 (t, 2 H).

Step 2: *N*-(*4*-hydroxybutyl)-*2,3*-dimethyl maleic imide (4 g, 20.3 mmol, Mw = 197 g/mol) was dried in vacuum for 30 min and then set under an argon atmosphere. After the addition of anhydrous THF, the flask was cooled in an ice bath. Methacryloyl chloride (3.26 g, 31.2 mmol, Mw = 104.5 g/mol) dissolved in anhydrous THF (4 mL) was added dropwise to the mixture over 10 min. After triethylamine (3.7 g, 32.3 mmol, Mw = 101 g/mol) was added, the reaction was kept in oil bath at 40 °C for 4 h and quenched by pouring the reaction into water afterwards. The mixture was then extracted three times with ethyl acetate, and the extracts was combined and dried over anhydrous magnesium sulfate, followed by the removal of solvent by rotary evaporation. The raw product was purified by using flash chromatography (*n*-hexane/ethyl acetate, 67:33 vol-%), with yields of 69 %. Finally, a colorless liquid, the desired product *4*-(*3,4*-dimethylmaleic imido) butyl methacrylate (DMIBMA), can be obtained and kept in ethyl acetate in the dark at 4 °C for a long-term storage.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 1.67 - 1.69 (m, 4 H), 1.94 (s, 3 H), 3.53 (t, 2 H), 4.16 (t, 2 H), 5.55 (s, 1 H), 6.09 (s, 1 H).

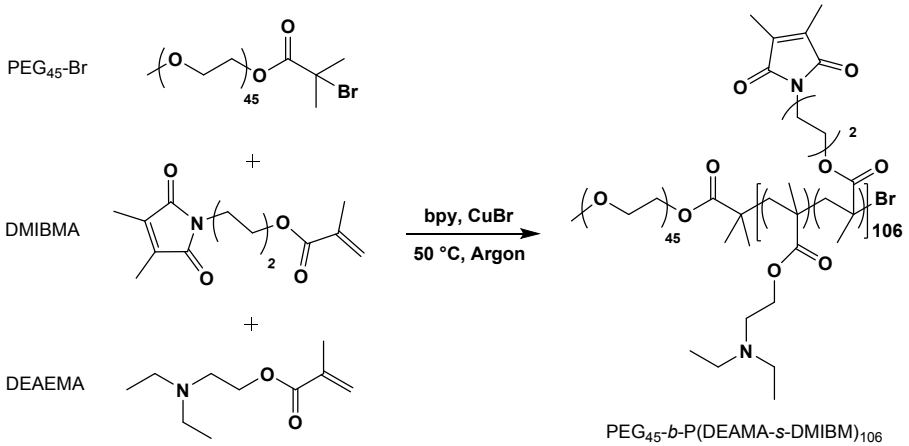
3.2 Synthesis of the PEG_{45}-Br macroinitiator[2]



MeO-PEG_{45}-OH (6 g, 3 mmol, Mw = 2000) was dried under vacuum at 60 °C for 30 min. After the addition of anhydrous THF (54 mL) under the argon atmosphere, the solution was cooled down to room temperature. Then, triethylamine (0.89 g, 4.8 mmol, Mw = 101 g/mol) was added, followed by adding *α*-bromoisobutyryl bromide (1.34 g, 5.8 mmol, Mw = 230 g/mol), dissolved in anhydrous THF (3.6 mL), dropwise to the reaction mixture over 10 min. The gloomy mixture was stirred at room temperature for 2 days under an argon atmosphere. Next, after the removal of salt by filter and solvent by rotary evaporation, the final product PEG_{45}-Br can be obtained by precipitating in *n*-hexane, generating a white solid in 80 % yield.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 1.93 (s, 6 H), 3.37 (s, 3 H), 3.63 (180 H).

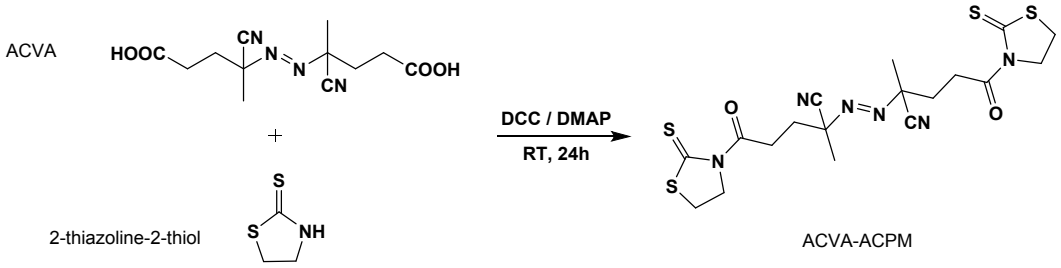
3.3 Synthesis of the PEG_{45}-*b*-P(DEAMA-*s*-DMIBM)$_{106}$[2]



The block copolymer (BCP) for Psomes formation was synthesized via ATRP polymerization. The above described macro-initiator $mPEG_{45}$-Br (220 mg, 0.1 mmol, Mw = 2150 g/mol), pH-responsive units *2*-(*N,N*-diethylaminoethyl) methacrylate (DEAEMA) (1.44 g, 7.77 mmol, Mw = 185 g/mol), photo cross-linking units *4*-(*3,4*-dimethylmaleic imido) butyl methacrylate (DMIBMA) (0.42 g, 1.58 mmol, Mw = 265 g/mol), CuBr (17 mg, 0.12 mmol, Mw = 143.5 g/mol), *2,2′*-bipyridine (32 mg, 0.2 mmol, Mw = 156 g/mol), and *2*-butanone (3 mL) were added to a Schlenk tube under an argon atmosphere, followed by degassing with four freeze-pump-thaw cycles. The reaction mixture was stirred at 50 °C for 17 h. To abort the reaction, the mixture was immersed into reaction flask cooled with a liquid nitrogen bath. After being diluted in THF (5 mL), the solution was filtered over a column of activated neutral aluminum oxide with THF as an eluent to remove any copper species. The raw product was concentrated by rotary evaporation and then washed by *n*-hexane for three times. Finally, a sticky polymer can be obtained after being dried in vacuum, with yields of 66 %.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 0.75 - 0.96 (m, 2 H), 0.98 - 1.11 (m, 3 H), 1.69 - 1.97 (m, 3 H), 1.97 - 2.07 (m, 3 H), 2.28 (s, 6 H), 2.51 - 2.64 (m, 4 H), 2.65 - 2.79 (m, 2 H), 3.39 (s, 3 H), 3.65 (s, 2 H), 3.70 - 3.84 (m, 2 H), 3.93 - 4.12 (m, 2 H).

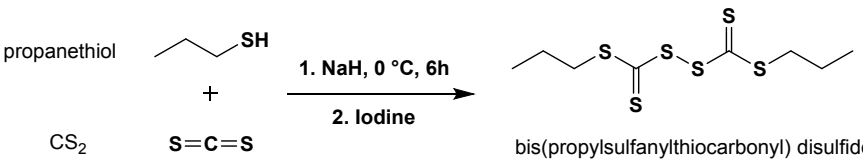
3.4 Synthesis of ACVA-ACPM[3]



4,4′-azobis (*4*-cyanovaleric acid) (ACVA) (4 g, 14.3 mmol, Mw= 280 g/mol) and *2*-thiazoline-*2*-thiol (4.5 g, 37.8 mmol, Mw= 119 g/mol) were dissolved in *1,4*-dioxane (150 mL), placed into 250 mL round-bottom flask, sealed and degassed under an argon atmosphere for 30 min. A solution of *N,N′*-dicyclohexylcarbodiimide (DCC) (6.8 g, 33.0 mmol, Mw = 206 g/mol) and *4*-(dimethylamino)pyridine (DMAP) (0.1 g, 0.82 mmol, Mw = 122 g/mol) in *1,4*-dioxane (75 mL) was added gradually. The reaction mixture was stirred vigorously at room temperature for 24 h. After being filtered and concentrated, the raw product was precipitated in cold diethyl ether, followed by drying under vacuum overnight. Finally, ACVA-ACPM was obtained as a yellow powder (5.3 g, 75 % yield).

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 4.60 (t, 2 H), 3.31 (t, 2 H), 3.12 - 3.66 (m, 2 H), 2.42 - 2.64 (m, 2 H), 1.76 (s, 3 H).

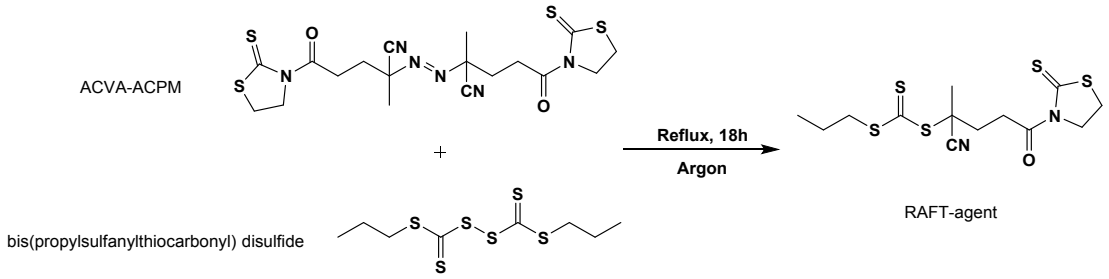
3.5 Synthesis of bis(propylsulfanylthiocarbonyl) disulfide[3]



A stirred suspension of sodium hydride (60 % dispersion in mineral oil) (4.7 g, 118 mmol, Mw = 24 g/mol) in diethyl ether (150 mL) was prepared, stirred, and cooled down in the ice bath, to which propanethiol (8.7 g, 114 mmol, Mw = 76 g/mol) was added gradually over 5 min. After the addition of carbon disulfide (CS_2) (9 g, 118 mmol, Mw = 76 g/mol), the reaction mixture was stirred for another 6 h. Then, the solution was filtrated and suspended in diethyl ether (75 mL). After the addition of iodine, the reaction mixture was stirred for another 1 h. Next, the solution was purified by filtration, then washed by sodium thiosulfate saturated solution, and dried over by sodium sulfate. Finally, an orange-colored liquid can be obtained after the removal of solvent by rotary evaporation, with yields of 78 %.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 3.30 (t, 4 H), 1.79 - 1.70 (m, 4 H), 1.02 (t, 6 H).

3.6 Synthesis of RAFT-agent[3]



Bis(propylsulfanylthiocarbonyl) disulfide (2 g, 6.8 mmol, Mw= 302 g/mol) and ACVA-ACPM (4.5 g, 9.34 mmol, Mw = 482 g/mol) were dissolved in ethyl acetate (75 mL) and then heated at reflux under an argon atmosphere for 18 h. After the removal of solvent, the final product can be obtained after the purification by flash chromatography (*n*-hexane/ethyl acetate, 50:50 vol-%), generating an orange/red oil in 75 % yield.

^{1}H-NMR (500.13 MHz, $CDCl_3$): δ_H (ppm) = 4.58 (t, 2 H), 3.66 - 3.49 (m, 2 H), 3.31 (td, 4 H), 2.66 - 2.43 (m, 2 H), 1.89 (s, 2 H), 1.78 - 1.69 (m, 2 H), 1.02 (t, 4 H).

3.7 Synthesis of PNIPAAm polymer via RAFT polymerization[3]

The described RAFT-agent (36.46 mg, 96.45 µmol, Mw = 378 g/mol), *N*-isopropylacrylamide (NIPAM) (0.905 g, 8.01 mmol, Mw = 113 g/mol), *2,2'*-Azobis(*2*-methylpropionitrile) (AIBN) (3.28 mg, 0.02 mmol, Mw = 164 g/mol), and *1,4*-dioxane (3 mL) were added to a Schlenk tube which was degassed with three freeze-pump-thaw cycles. Then the reaction mixture was heated at 70 °C for 8 h. Next, the solution was purified three times by precipitating in cold *n*-hexane/diethyl ether (33.5:66.5 vol-%). After drying under vacuum overnight, a light-yellow colored powder can be obtained in 90 % yield.

3.8 Cationization of BSA[3]

10 mL of *1,6*-diaminohexane (150 mg/mL) was prepared and adjusted to pH 6.5 by 10 M HCl, and combined to 100 mL of BSA aqueous solution (2 mg/mL BSA, in MES buffer pH 6.5, 10 mM). The cationization of BSA was initiated by the addition of *N*-ethyl-*N′*-(*3*-dimethylaminopropyl) carbodiimide hydrochloride (150 mg), and the solution was stirred for 12 h. Prior to the lyophilization, the solution was purified by dialysis (MWCO: 5000) against Milli-Q water at room temperature for 48 h. The final product can be obtained in white color in 95 % yield. Cationized BSA was characterized by MALDI-TOF MS.

3.9 Preparation of BSA-PNIPAAm bio-conjugates[3]

10 mL of mercaptothiazoline-functionalized PNIPAAm aqueous solution (2 mg/mL, Mn 7100 g/mol) was prepared and added dropwise to 10 mL of BSA-NH_2 (2 mg/mL, in 10 mM $NaHCO_3$ buffer, pH 8), with a polymer: protein molar ratio of around 10: 1. The solution was kept at room temperature and stirred for 12 h. To remove the salts and any unreacted PNIPAAm, the solution was purified by dialysis (MWCO: 50 kDa) against Milli-Q water at room temperature for 48 h, followed by lyophilization. Finally, bio-conjugates can be obtained in a white and fluffy state. Gel electrophoresis was used to compare the different states of modified BSA.

3.10 Protein labelling

The labelling of proteins was performed as follows: 10 mg of the proteins were dissolved in 5 mL of carbonate buffer (100 mM, pH 8.5), and 50 μL of fluorescent dye (Atto425-NHS, AF488-NHS, Cy5-NHS, and RhB isothiocyanate) solutions in DMSO (1 mg/mL) was added dropwise. Then, the mixed solution was stirred for 12 h at 4 ℃, followed by the dialysis (MWCO: 50 kDa) against Milli-Q water for 72 h. Any precipitates were removed by a filter (0.2 μm, Nylon filter) before the lyophilization.

3.11 Preparation of Psomes

10 mg of BCP was dissolved in HCl solution (pH 2), and any impurities were removed by a filter (0.2 μm, Nylon filter) after complete dissolution. The self-assembly process of BCP was triggered by the addition of 1M NaOH and pH was raised to pH 9. Then the mixed solution was stirred for three days in the dark, followed by filtering the as-formed Psome solution (0.8 μm, Nylon filter). To activate the photo-crosslinking between BCPs in the Psome membrane, the resulting Psomes solution was placed under UV irradiation for 180 s every 2 mL. The crosslinked Psomes solution was combined together for the subsequent purification process by HFF (MWCO: 500 kDa, 130 mbar).

The Psomes comprising ALP were prepared following the above procedure except that 1 mL of ALP solution (2 mg/mL) was added to the dissolved BCP solution before raising the pH to trigger the self-assembly of BCP.

3.12 Preparation of empty proteinosomes[3]

Proteinosomes were fabricated as previously reported. Firstly, 45 μL of BSA-PNIPAAm bio-conjugate solution (5 mg/mL) was prepared by dissolving the building blocks in aqueous carbonate buffer (pH 8.5, 10 mM). After the addition of PEG-bis(*N*-succinimidyl succinate) (0.5 mg, Mw = 2000 Da) into BSA-PNIPAAm solution, the mixture was combined with *2*-

ethyl-*1*-hexanol (683 μL) at a constant aqueous/oil volume fraction of 0.066, followed by shaking the mixture vigorously by hand for 30 s. The as-formed emulsion was kept in the dark for 12 h for equilibrium. Next, a phase transfer procedure was followed to remove the oil phase, starting with discarding the clear upper phase, extracting existing oil phase from the dispersion by 1 mL of 75 % ethanol, and then concentrating the dispersion by centrifugation (3420 g, 3 min). Then, the same extracting process was repeated again but extracted the dispersion with 50 % ethanol, 25 % ethanol, and Milli-Q water successively. Finally, aqueous dispersions of the proteinosomes were obtained and kept at 4 °C for subsequent experiments.

3.13 Preparation of polymersome-in-proteinosomes system (PsomesP)[3]

PsomesP bearing GOx, CAT, and Psomes-ALP were prepared following the same protocol as empty proteinsomes, with a subtle adjustment in the concentration of BSA-PNIPAAm. Briefly, 10 μL of BSA-PNIPAAm bio-conjugates (15 mg/mL, in 20 mM sodium carbonate buffer, pH 8.5), 7 μL of CAT (0.1 mg/mL, in 20 mM sodium carbonate buffer, pH 8.5), 8 μL of GOx (6.6 mg/mL, in 20 mM sodium carbonate buffer, pH 8.5), 20 μL of purified Psomes-ALP (2 mg/mL), and 0.5 mg of PEG-bis(*N*-succinimidyl succinate) were mixed together. After the addition of 683 μL of *2*-ethyl-*1*-hexanol, which was, then, kept at 4 °C for subsequent experiments.

3.14 Reversible swelling-shrinking behavior of Psomes[4]

To check the swelling-shrinking behavior of crosslinked Psomes, the pH of Psomes solution was regulated by 1 M HCl or 1 M NaOH between pH 6 and pH 8 repeatedly. At each pH value, the size of Psomes were measured by DLS. This process was repeated for 5 cycles.

3.15 Purification of Psomes-ALP by the hollow fibre filtration[4]

Hollow fiber filtration system (HFF), equipped with a polyethersulfone-based separation module (MWCO: 500 kDa, SpectrumLabs, USA), was conducted to remove non-encapsulated enzymes. To validate this purification process, enzyme was labelled with AF488. After the self-assembly and photo-crosslinking process of Psomes-ALP-AF488, 10 mL of unpurified Psomes-ALP-AF488 was combined with 10 mL of MOPS buffer (1 mM, pH 8), and transferred into a cone tube, which was connected to the circulating system of the HFF system. The sample was constantly refilled with fresh MOPS buffer until no fluorescence can be detected in the collected liquid waste. The transmembrane pressure was kept at 130 mbar throughout the whole purification process. All the non-encapsulated enzymes can be removed when the extraction volume reached 200 mL (**Figure S18**).

3.16 Encapsulation efficiency of ALP

To calculate the encapsulation efficiency of ALP in Psomes, the enzyme was labelled with AF488. The calibration curve was plotted by determination of the change in fluorescence (excitation (ex)/emission (em)): 475 nm/520 nm) of different mass concentration of ALP-AF488 (mass concentrations of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 mg/mL). The corresponding equation can be simulated and is given in **Figure S19**. The encapsulation efficiency of ALP-AF488 by Psomes can be calculated by measuring the fluorescence intensity of purified Psomes-ALP-AF488 and then comparing the result with calibration curve. The measurement was performed three times and the statistical average is presented in **Table S3.**

3.17 Enzyme assay of Psomes-ALP

The activity of Psomes-ALP was studied by the ALP assay using nonfluorescent FDP as a substrate. For the evaluation of pH effect on ALP activity, 10 µL of purified Psomes-ALP solution (1mg/mL, BCP) or native ALP (40 µg/mL) was mixed with 100 µL of MOPS buffer (5mM), and the pH was adjusted to selected points (pH 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0). After the addition of 60 µL of FDP (10 µM), the changes in fluorescence intensity was monitored by microplate reader (ex/em: 480 nm/515 nm) over time. The relative activity of ALP was compared by different slopes of the fluorescence increase in the original linear phase. For normalization of ALP activity, the highest value was fixed as 100 % to normalize the other determined enzyme activities within one experiment series.

3.18 Release behavior of ALP from Psomes-ALP

To check if Psomes can be a good carrier to retain the loaded enzymes, the loading behavior of ALP in Psomes was studied by the method of dialysis. ALP labelled with AF488 was encapsulated into Psomes via *in-situ* loading process and purified by HFF to remove non-encapsulated enzyme. 7 mL of Psomes-ALP solution (1 mg/mL of BCP for Psomes formation) was transferred into dialysis bag (MWCO: 1000 kDa) and dialyzed against MOPS buffer (1 mM, pH 6 and 8) over time. The fluorescence intensity of the samples taken from the dialysis bag at selected time points (0, 8, 16, and 24 h) was evaluated by fluorescence spectrometer. Native ALP labelled with AF488 was set as a reference in dialysis bag.

3.19 Enzymatic cascade in PsomesP

Measurement after the oxidation of glucose

125 µL of PsomesP solution (at the concentration of PsomesP prepared following the step **3.13**) was diluted to 750 µL by MOPS buffer (1mM), and adjusted to pH 7.4. After the addition of selected volumes (0, 0.24, 0.47, 0.93, 1.5, 1.875, and 3.75 µL) of glucose (100 mg/mL), the pH value was determined by pH meter and recorded manually every 10 min. The result of GOx-mediated pH drop is presented in **Fig. 4b**.

After 5 h mixing the glucose, as mentioned above, the acidized solution was transferred into a quartz cuvette (chamber volumes 1.4 mL). After the addition of 75 µL of FDP (10 µM), the changes in fluorescence intensity were monitored by fluorescence spectrophotometer (ex/em: 480 nm/515 nm) every 60 s. The result of Psomes-ALP activity in PsomesP is presented in **Fig. 4c**.

Measurement in the course of the oxidation of glucose – Consideration of dynamic intracellular processes for oxidation of glucose and enzymatic activity of Psomes-ALP

125 µL of PsomesP solution, as mentioned above, was diluted to 750 µL by MOPS buffer (1mM), and adjusted to pH 7.4. After the addition of 1.875 µL of glucose at selected time points (0, 40, 80, and 120 min), followed by adding 75 µL of FDP (10 µM), the changes in fluorescence intensity were monitored by fluorescence spectrophotometer (ex/em: 480 nm/515 nm) every 60 s. The result of Psomes-ALP activity in PsomesP is presented in **Fig. S20.**

Detection limit of BEC

125 µL of PsomesP solution (at the concentration of PsomesP prepared following the step **3.13**) was diluted to 750 µL by MOPS buffer (1mM), and adjusted to pH 7.4. 5h after the addition of selected volumes (0, 0.24, 0.47, 0.93, 1.5, 1.875µL) of glucose (100 mg/mL), 75 µL of FDP (10 µM) was added, and the changes in fluorescence intensity were monitored by fluorescence spectrophotometer (ex/em: 480 nm/515 nm) every 60 s. The results of Psomes-ALP activity in PsomesP are presented in **Fig. S23.**

3.20 Influence of loaded ALP concentration on Psomes-ALP activity in presence of defined concentration of GOx, Cat and glucose

To achieve this goal Psomes-ALP were fabricated by 1 eq (2 mg/mL), 0.5 eq (1 mg/mL), 0.25 eq (0.5 mg/mL), and 0 eq ALP through in-encapsulation approach with HFF purification step. 0.0625 mg/mL as glucose concentration was used in each experiment.

7 µL of CAT (0.1 mg/mL), 8 µL of GOx (6.6 mg/mL), and selected volumes of purified Psomes-ALP (20 µL, 1 eq, 2mg/mL) were mixed together. The volume was diluted to 750 µL by MOPS buffer (1mM), and then pH was adjusted to pH 7.4. 5h after the addition of 0.47 µL (of glucose (100 mg/mL), 75 µL of FDP (10 µM) was added, and the changes in fluorescence intensity were monitored by fluorescence spectrophotometer (ex/em: 480 nm/515 nm) every 60 s. The effect of ALP concentration on the activity was studied following the above procedure using 0, 0.25, and 0.5 eq of Psomes-ALP (20 µl) was added before the enzymatic assay. The results of Psomes-ALP activity are presented in **Fig. S22**

4. Supplementary Figures and Tables

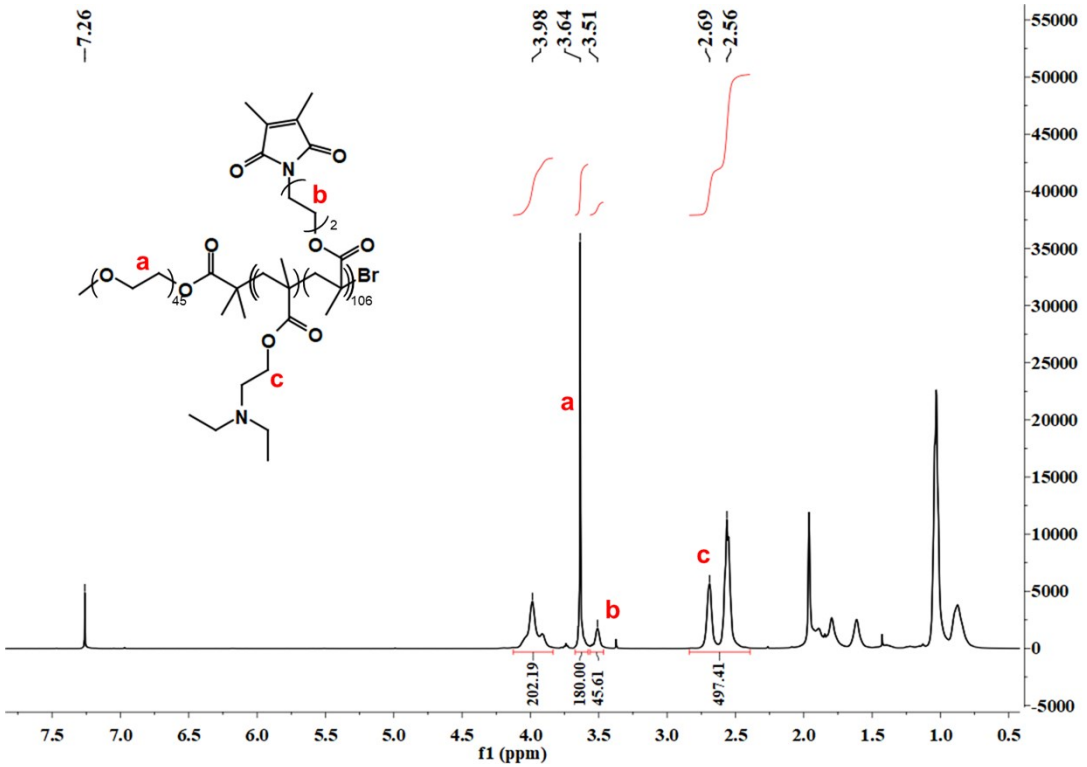


Figure S1. ^{1}H-NMR spectrum of PEG_{45}-*b*-P(DEAMA-*s*-DMIBM)$_{106}$ in $CDCl_3$.

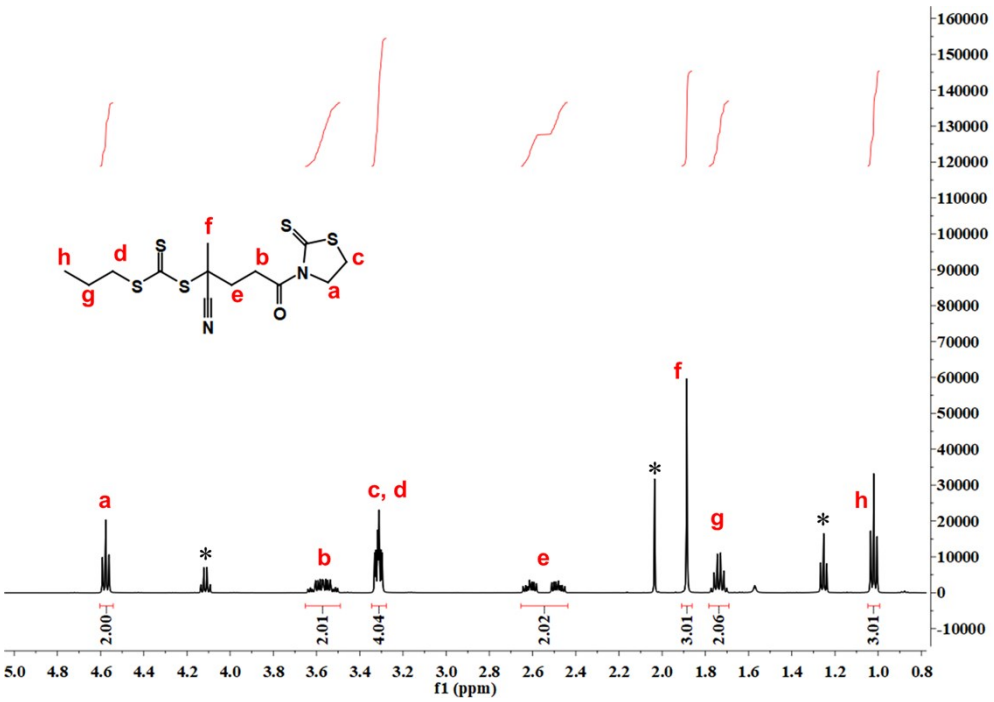


Figure S2. ^{1}H-NMR spectrum of mercaptothiazoline-activated trithiol-RAFT agent *4*-cyano-*4*-(propylsulfanylthiocarbonyl) sulfanylpentanoic acid mercaptothiazoline amide in $CDCl_3$. The residual ^{1}H-NMR signals (*) belong to the solvent ethyl acetate.

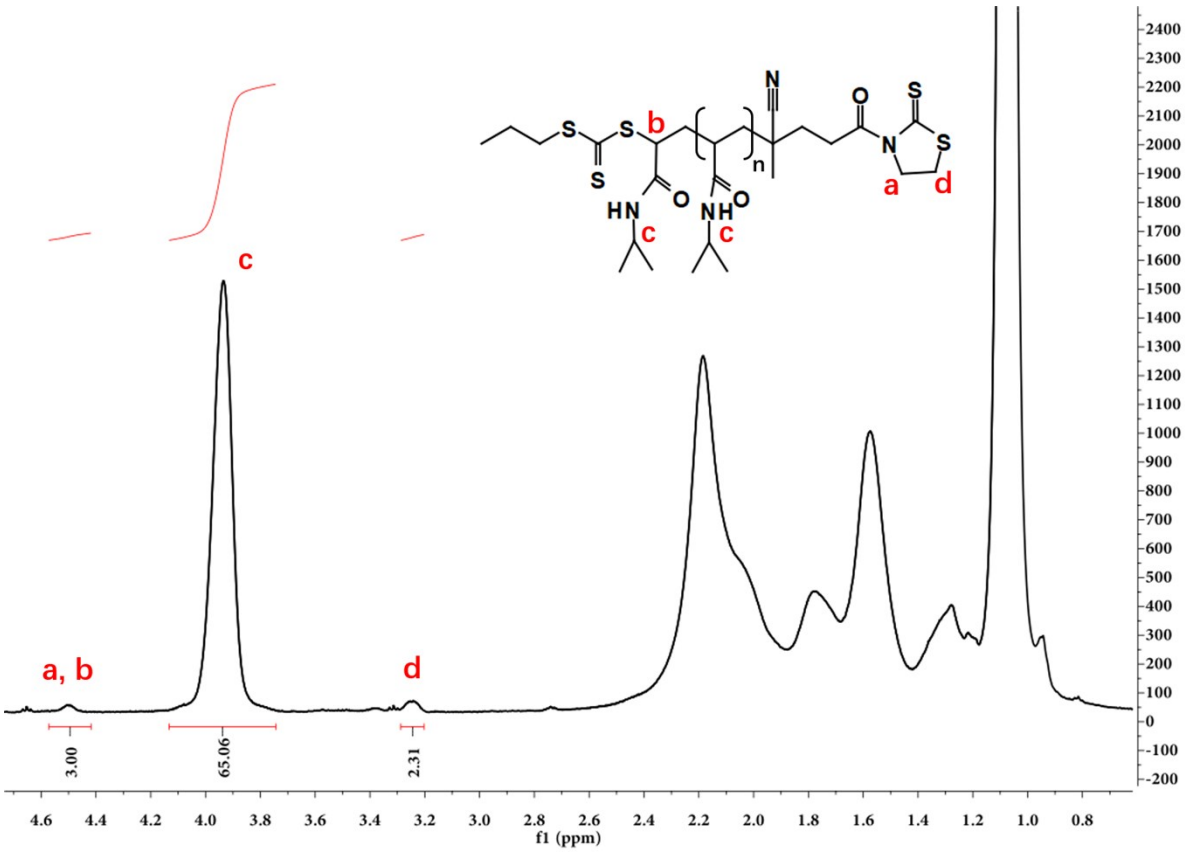


Figure S3. ^{1}H-NMR spectrum of mercaptothiazoline-activated PNIPAAm in $CDCl_3$.

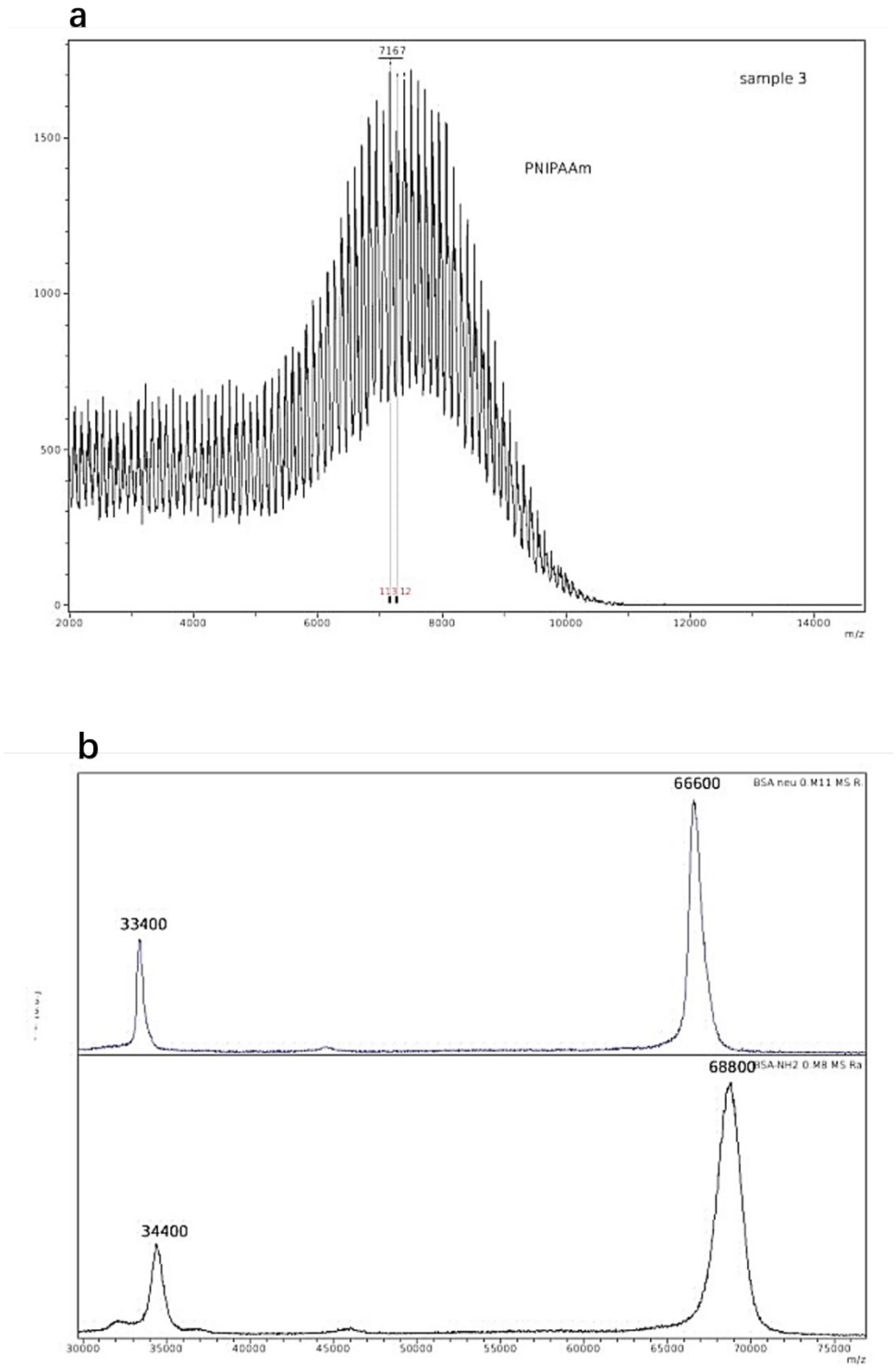


Figure S4. MALDI-TOF MS spectra of PNIPAAm **(a)**, and BSA **(b)** before (upper) and after (down) cationization. The detected molecular weight of BSA and BSA-NH_2 were 66600 Da and 68800 Da, and the number of NH_2 can be calculated to be ca.19.

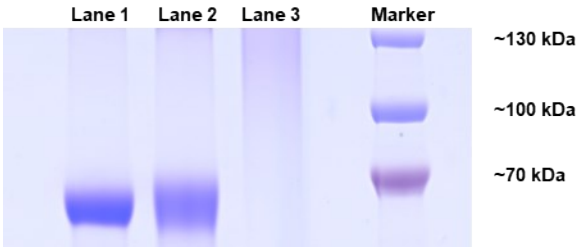


Figure S5. Gel electrophoresis study for BSA-PNIPAAm bio-conjugates. Lane 1, native BSA; Lane 2, BSA-NH_2; Lane 3, BSA-PNIPAAm bio-conjugates.

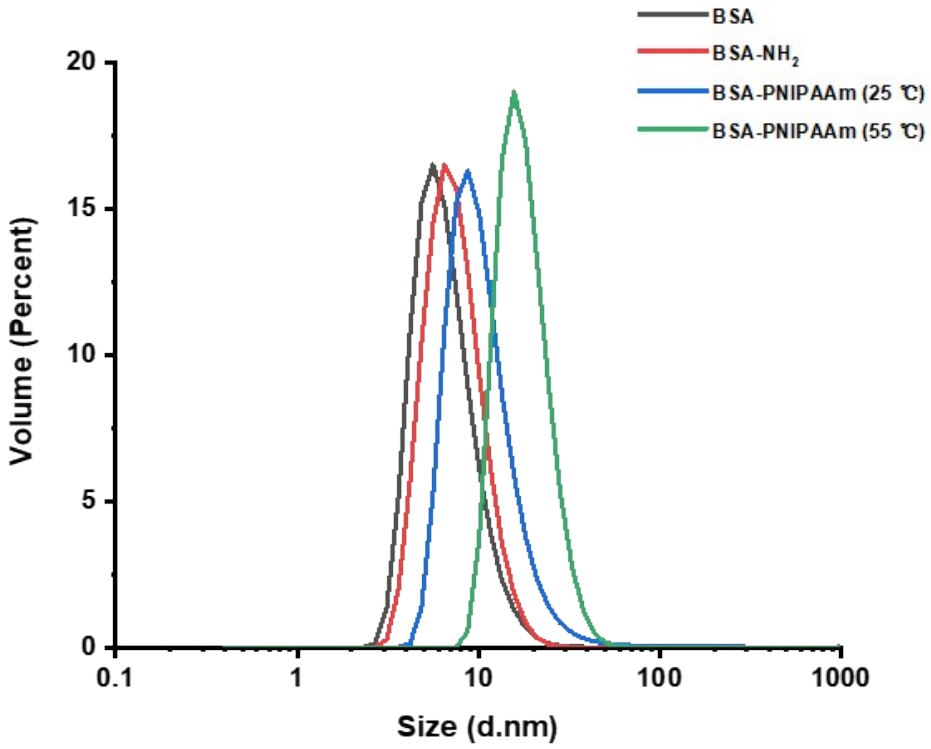


Figure S6. DLS profiles for aqueous BSA, BSA-NH_2 and BSA-NH_2/PNIPAAm bio-conjugates recorded at 25 ℃. Profile for aqueous BSA-NH_2/PNIPAAm at 55 ℃ is also shown. Samples were measured at the concentration of 1 mg/mL.

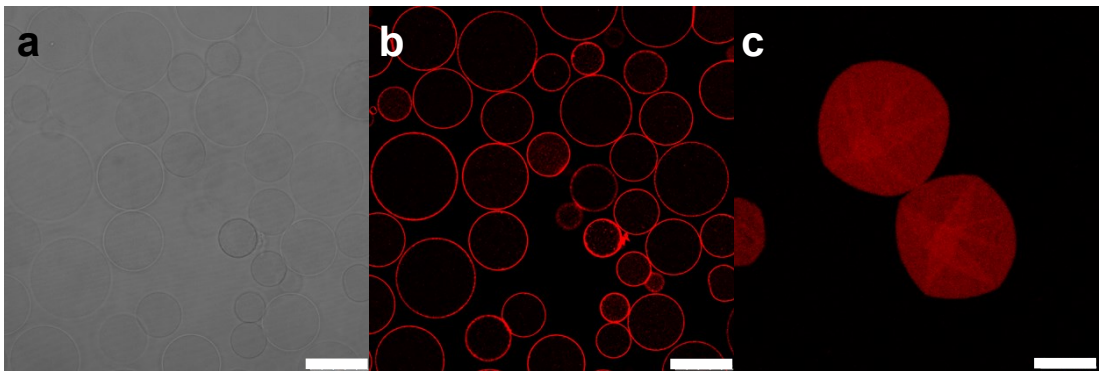


Figure S7. Optical microscopy image (a) and corresponding confocal laser scanning microscopy image of BSA-NH_2-RhB/PNIPAAm-based proteinosomes in aqueous solution **(b)**, and after drying in the air on a glass slide overnight **(c)**. Scale bars in a - c are 25, 25 and 10 µm, respectively.

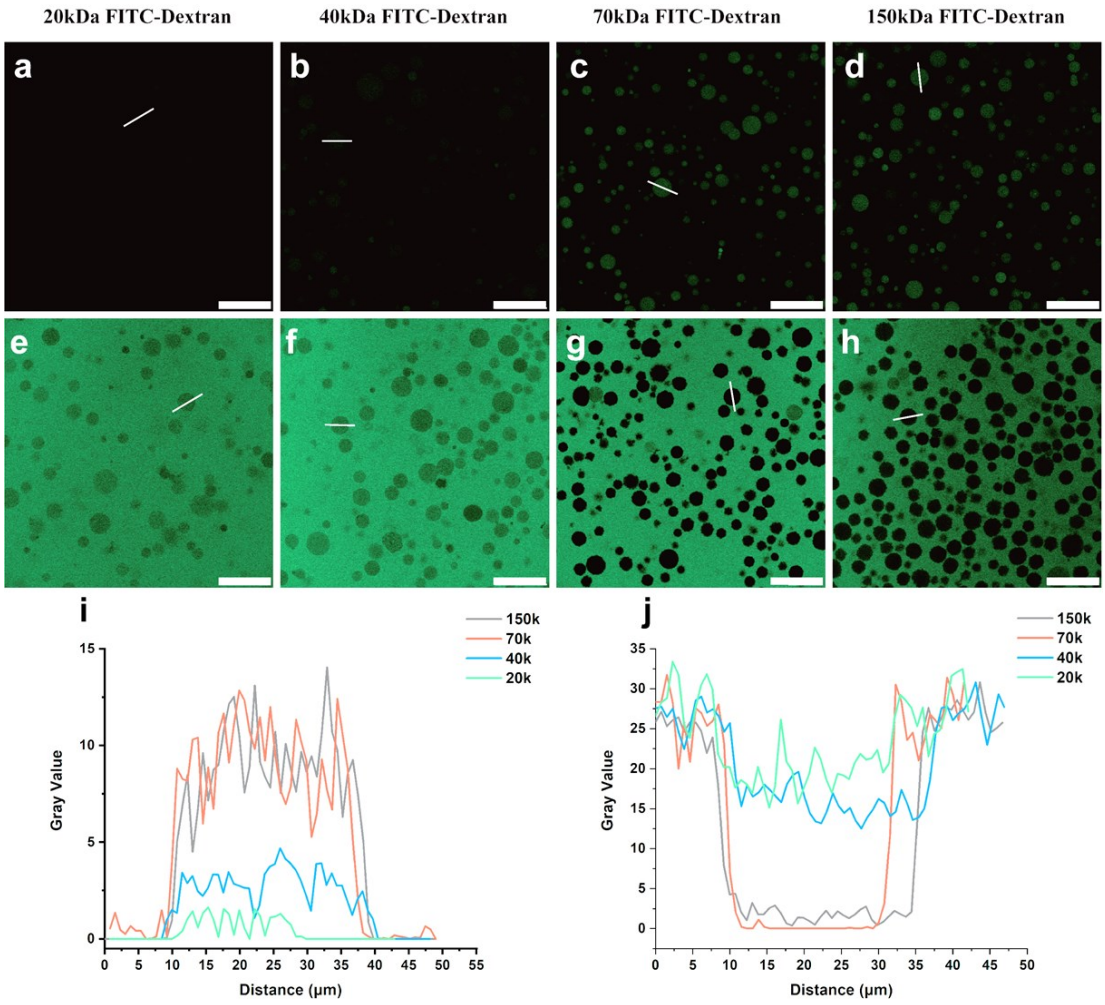


Figure S8. Permeability study of proteinosomes. Confocal laser scanning microscopy images and fluorescence intensity line profiles **(i-j)** recorded from proteinosomes with *in-situ* loaded **(a-d)** and *post* loaded **(e-h)** FITC-Dextran (molecular weight: 20 kDa, 40 kDa, 70 kDa, and 150 kDa). Corresponding fluorescence intensity profiles (**i-j**) across a single proteinosome (dotted lines in **a-d**, and **f-i** respectively). Scale bar, 75 µm.

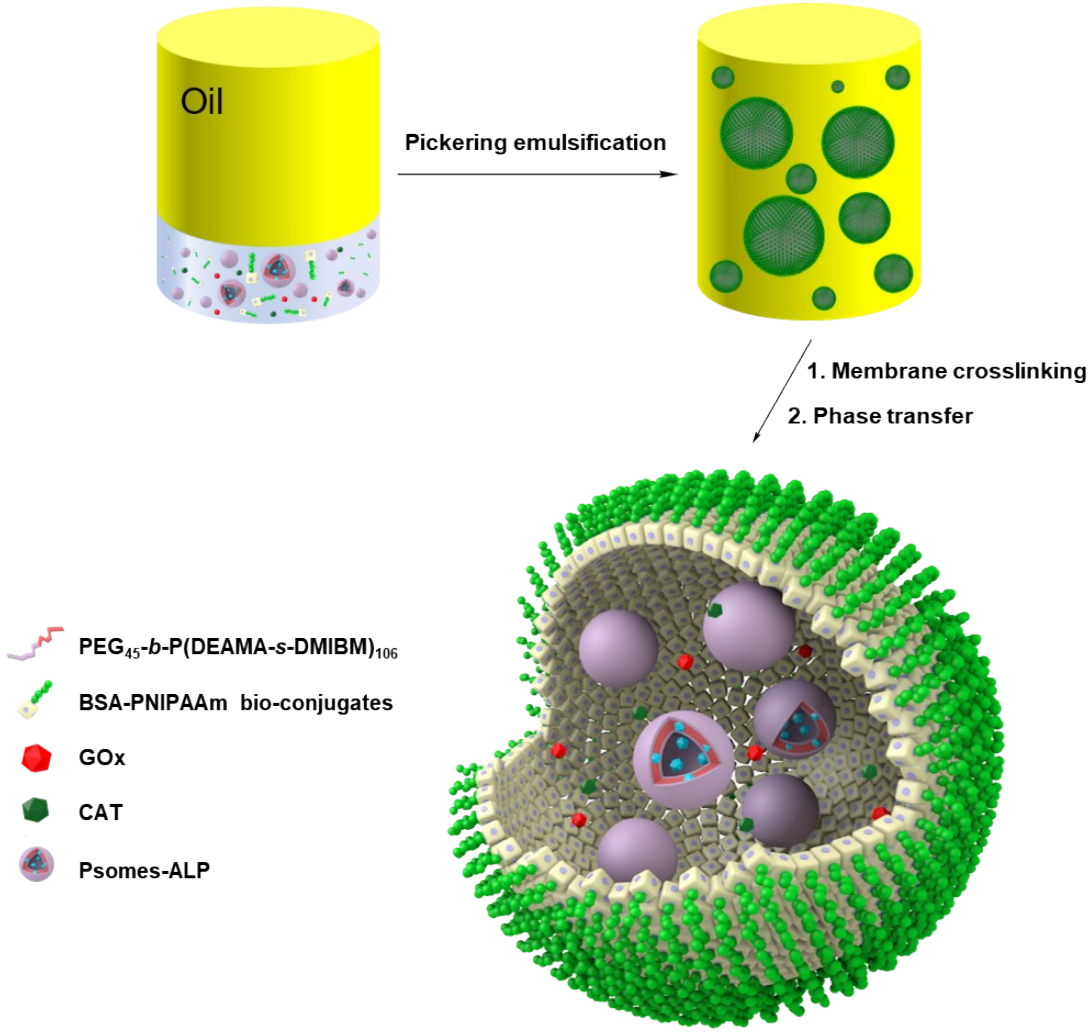


Figure S9. General procedure for the construction of a polymersome-in-proteinosome protocell. Prepared by water droplet/oil interfacial assembly, membrane crosslinking, and phase transfer, this hierarchical structure was designed to mimic the advanced architecture of a biomimetic eukaryotic cell.

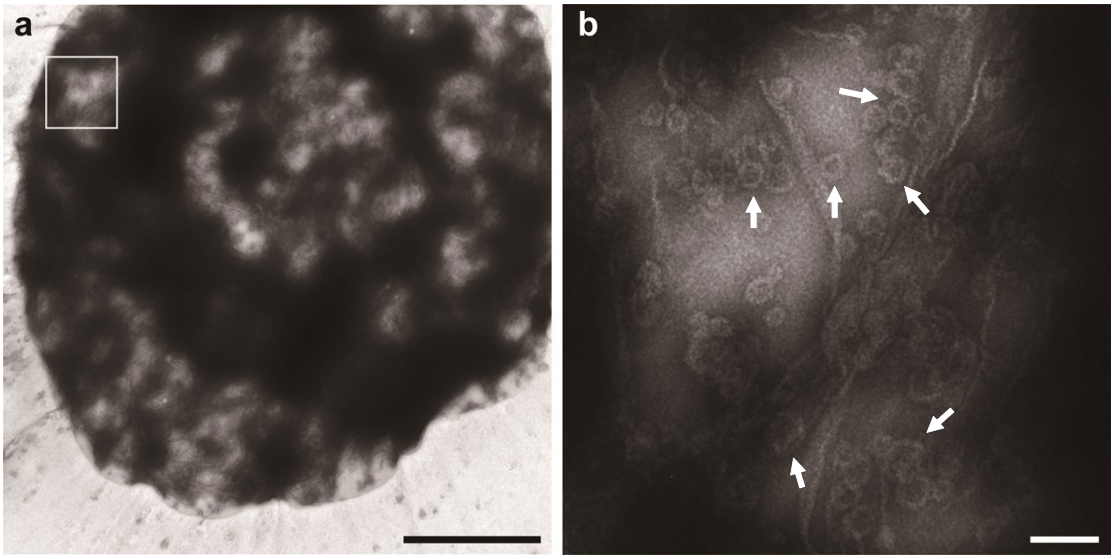


Figure S10. TEM images of a polymersome-in-proteinosome multicompartment structure **(a)** and its magnification image presenting the intact structure of Psomes (white arrows point to the resided Psomes) **(b)**. Both of two samples were stained with 2 % uranyl acetate. Scale bars in **a** and **b** are 2 μm and 250 nm, respectively.

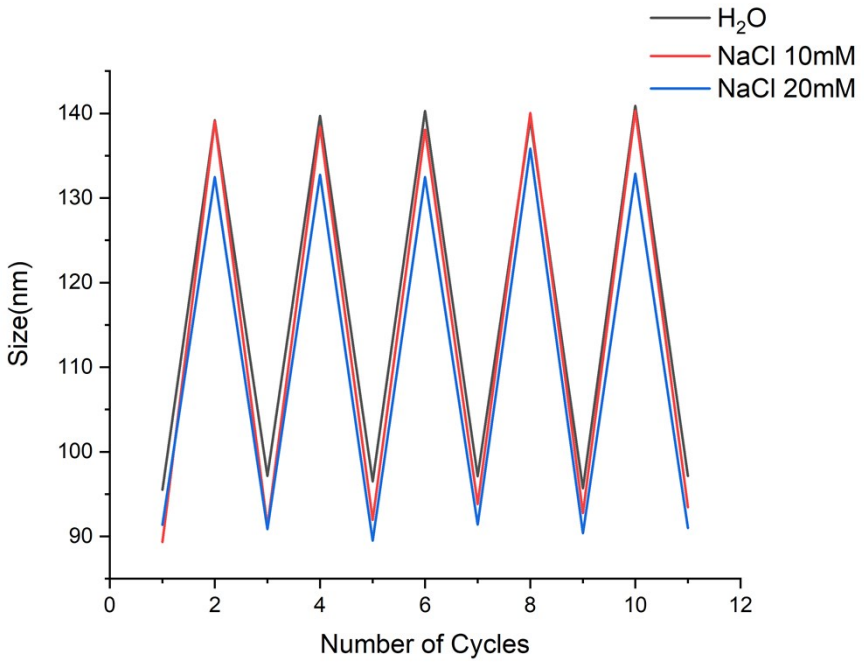


Figure S11. Swelling-shrinking cycles of Psomes between pH 6 and 8 studied in H_2O and NaCl solution monitored by DLS. Samples were measured at the concentration of 1 mg/mL.

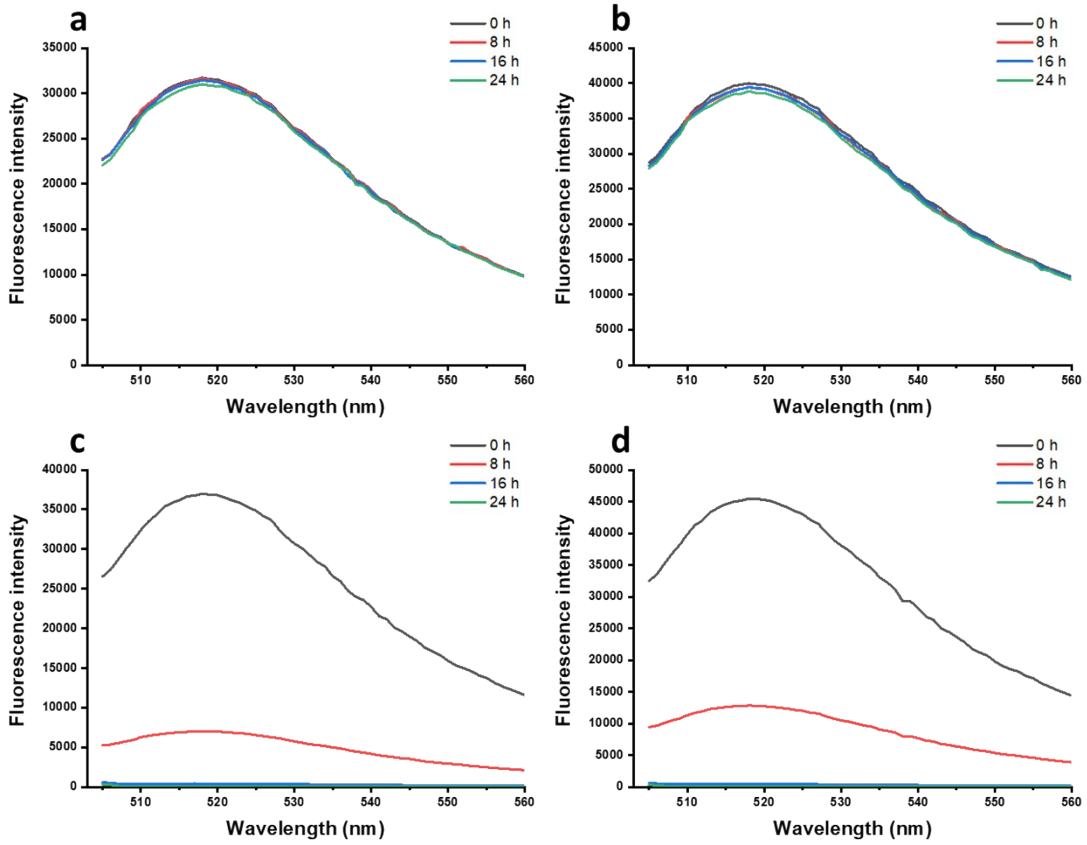


Figure S12. The release behaviors of AF488-labelled ALP encapsulated in Psomes, dialyzed (MWCO: 1000 kDa) against MOPS buffer (1 mM) at pH 6 **(a)** and pH 8 **(b)**. The fluorescence intensity of the samples taken from the dialysis bag was monitored at selected time points (0, 8, 16, and 24 h) (ex: 475 nm, emission range: from 505nm to 560 nm). Native ALP labelled with AF488 was set as a reference, and release behaviors were evaluated by dialyzing against MOPS buffer at pH 6 **(c)** and pH 8 **(d)**. Each experiment was carried out for 3 times at least.

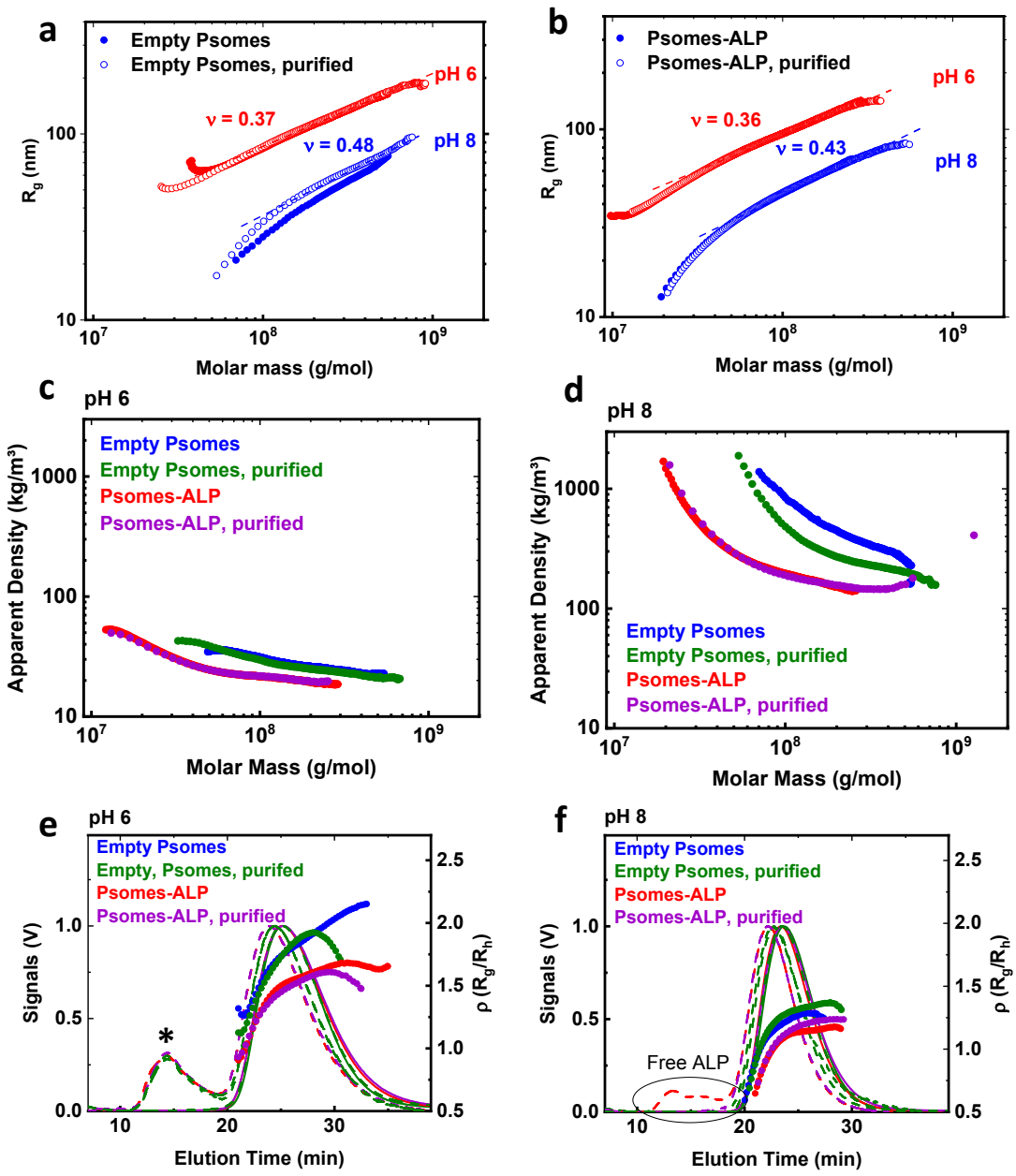


Figure S13. Conformation studies of Psomes-ALP by AF4-LS, compared with empty Psomes. Conformation plots (molar masses vs. Rg) at pH 6 (red) and pH 8 (blue) of **(a)** empty Psomes (filled circles) and purified, empty Psomes (empty circles) and **(b)** Psomes-ALP (filled circles) and purified Psomes-ALP (empty circles). Apparent densities as a function of molar mass of empty Psomes and Psomes-ALP at **(c)** pH 6 and **(d)** pH 8. AF4 fractograms (normalised detector signals; LS - solid line and RI - dashed line) and ρ parameters (Rg/Rh), of empty Psomes and Psomes-ALP at **(e)** pH 6 and **(f)** pH 8. Samples were measured at concentration of 1 mg/mL in PBS buffer (1mM, pH 6 and 8). * unexpected assembled few BCP at pH 6.

Figure S13f – Only in the case of non-purified sample "Psomes-ALP" one can recognize a second peak from isolated, non-encapsulated ALP in comparison to monomodal sample "Psomes-ALP, purified", as expected.

Figure S13e – Uniform RI signals (*) between 10 and 20 min elution time are visible as a second peak. Obviously, it implies the presence of assembled BCP in all samples at pH 6 after (non-)HFF purification and fabrication of all

samples at pH 8 first. This existence is unusual and exclude the release of ALP at pH 6 as discussed in the main text. We could determine molecular weight (approx. 10^6 g/mol, approx. 30 BCP) for assembled BCP, but no R_g and R_h values for assembled BCP online. We could not observe such behavior in a previous study at acidic pH (e.g. Gumz et al., *Adv. Sci.*, 2019, **6**, 1801299). Assembled BCP are not visible at pH 8 (**Figure S13f**). Cryo-TEM study of non-purified Empty Psomes sometimes reveals the presence of smaller micelles for non-purified samples. This is one possible explanation for us.

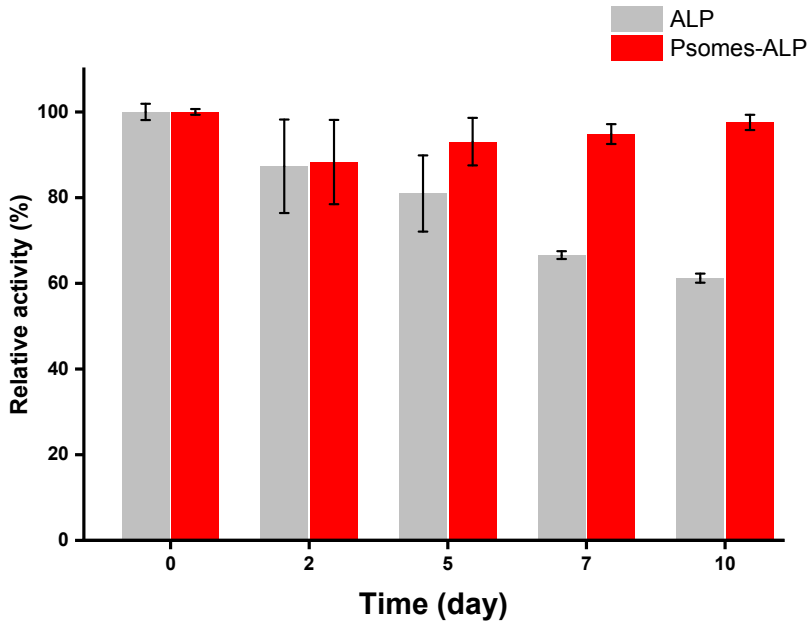


Figure S14. Stability study of encapsulated ALP, compared with native ALP, as a function of time. The stability of ALP at different conditions were compared by their enzymatic activity via the established ALP assay using non-fluorescent FDP as a substrate. The fluorescence intensity of F (product of hydrolyzed FDP) was determined by microplate reader (ex/em: 480 nm/515 nm). Each experiment was carried out for 3 times at least. Data are presented as mean values ± SD, error bars indicate standard deviations (n = 3).

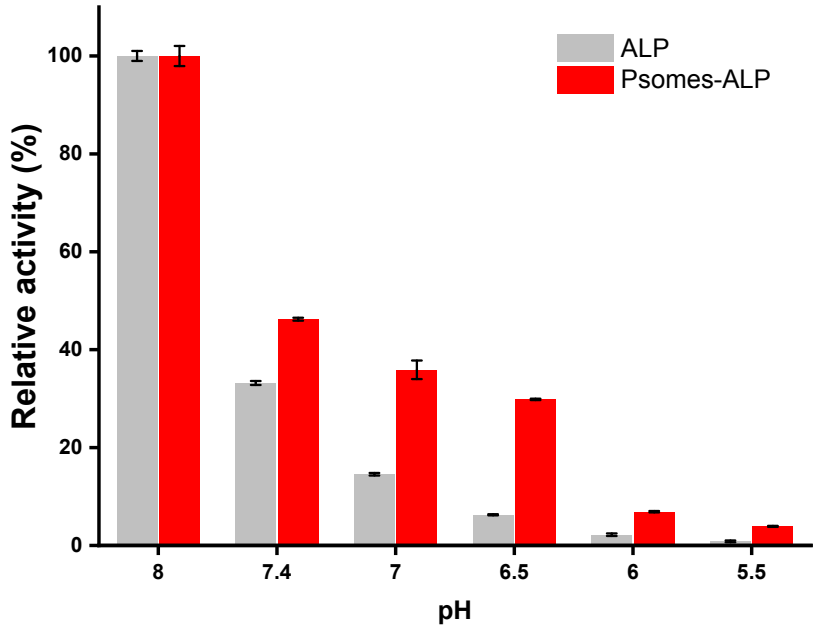


Figure S15. Relative enzymatic activity as a function of pH for ALP and ALP encapsulated in Psomes as artificial organelles at room temperature evaluated by ALP assay using FDP as a substrate. The fluorescence intensity of F (product of hydrolyzed FDP) was determined by microplate reader (ex/em: 480 nm/515 nm). Each experiment was carried out for 3 times at least. Data are presented as mean values ± SD, error bars indicate standard deviations (n = 3).

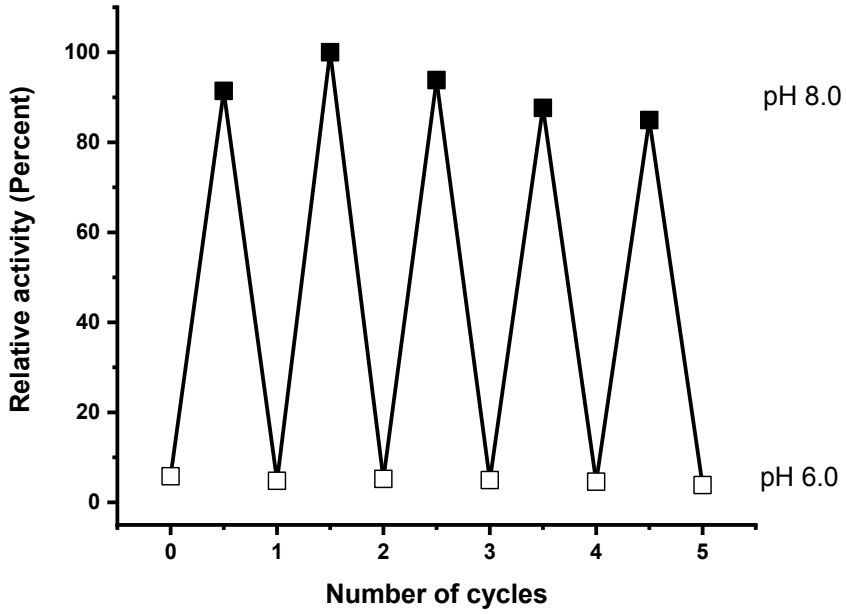


Figure S16. Reversible regulation towards the activity of Psomes-ALP at pH 6 and pH 8.

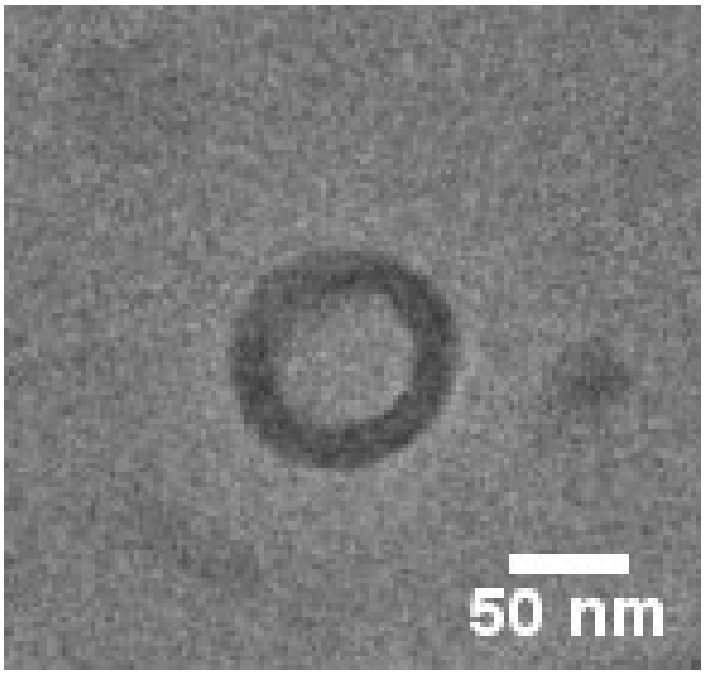


Figure S17. Cryo-TEM image of empty Psomes. Scale bar 50 nm.

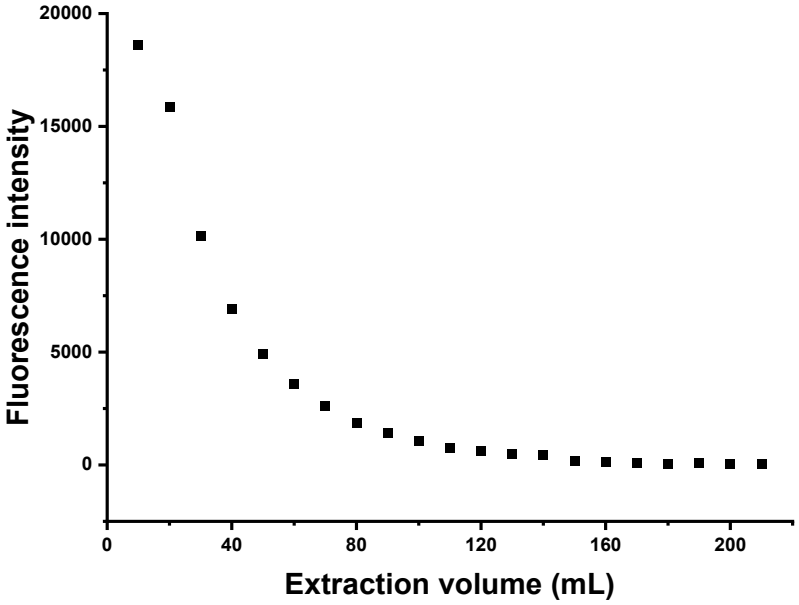


Figure S18. Purification process of Psomes-ALP-AF488 by HFF. The fluorescence intensities of liquid waste were detected by microplate reader (ex/em: 475 nm/520 nm) every 10 mL.

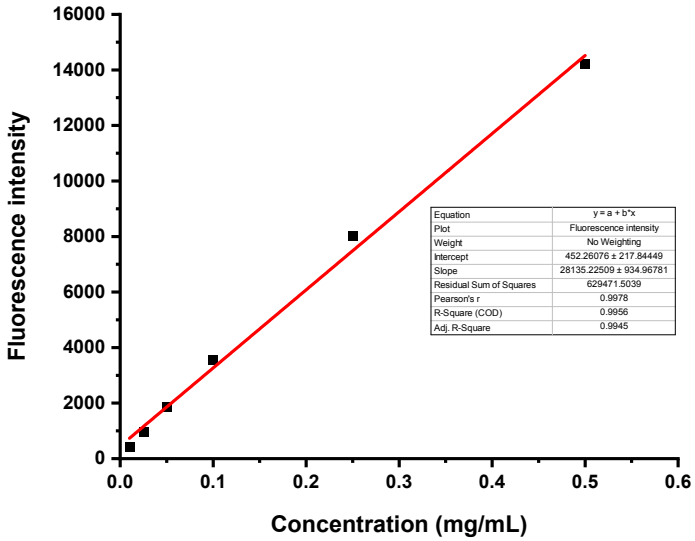


Figure S19. Change in fluorescence intensity as a function of mass concentration of ALP-AF488. The fluorescence intensity of encapsulated ALP-AF488 was measured by microplate reader (ex/em: 475 nm/520 nm).

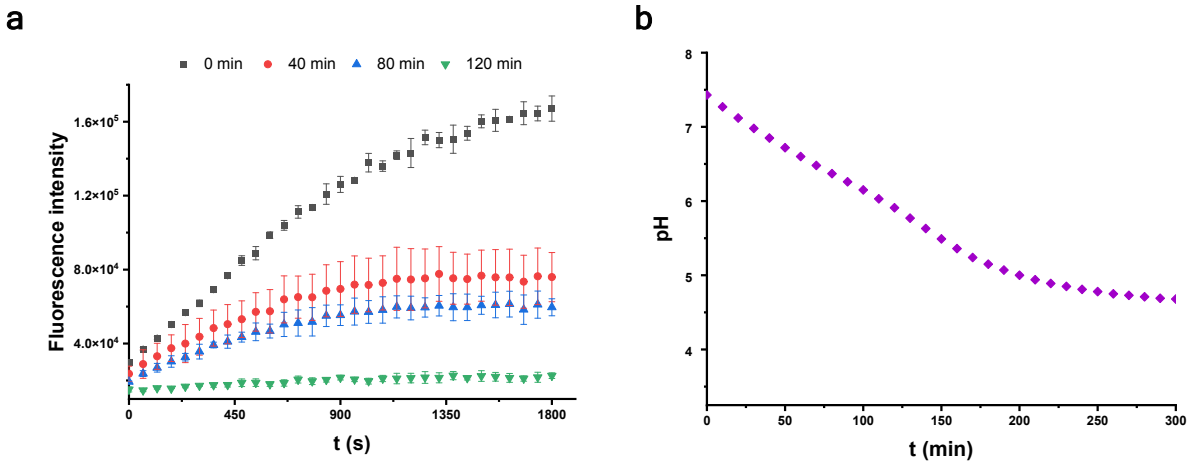


Figure S20. (a) Changes in fluorescence intensity of F (product of hydrolysed FDP by Psomes-ALP) were detected after incubation for different reaction times (0, 40, 80, and 120 min) following the addition of glucose (0.25 mg/mL in Fig. 4c). Each experiment was carried out for 3 times at least. Data are presented as mean values ± SD, error bars indicate standard deviations (n = 3). (b) The pH changes was detected after the addition of glucose (0.25 mg/mL). Psomes-ALP were prepared by 2 mg/mL ALP.

One can smoothly recognize that there is a decreasing enzymatic activity between 0 min and 120 min. After 120 min Psomes-ALP do not work below pH 6, while gradual differentiation can be done between 40 and 80 min, when reaching pH values between 6.8 for 40 min and 6.2 for 80 min.

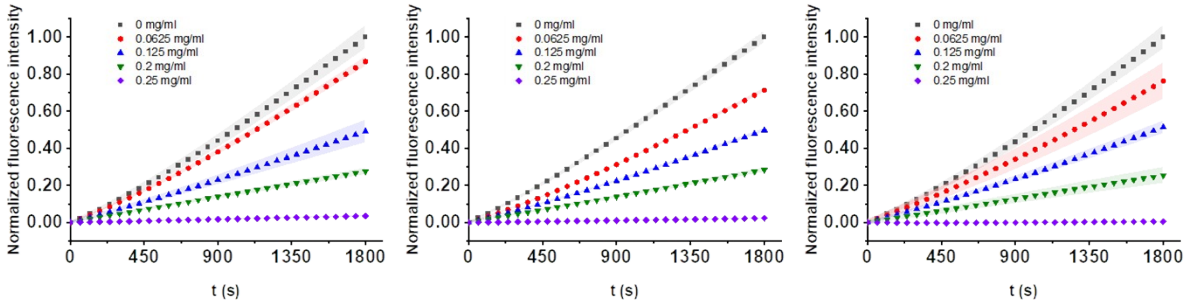


Figure S21. Time profile for BEC with AO "Psomes-ALP" showing changes in in fluorescence intensity of hydrolysed FDP in the presence of different glucose after 30 min for three independent experiments. Each experiment for selected glucose concentration was carried out for 3 times at least. Data are presented as mean values ± SD, error bars indicate standard deviations (n = 3). Psomes-ALP was prepared with 2 mg/mL of ALP.

With the established protocol for BEC with AO "Psomes-ALP" reproducible results can be obtained to differ subtle glucose concentration from 0.0625 to 0.25 mg/mL. This indicates that the highest concentration on glucose (0.25 mg/mL) thoroughly results in the deactivation of AO and follow the functional principle of fluctuation-detecting artificial organelles. The use of lower concentrated ALP in Psomes-ALP does not work smoothly due to low concentration of membrane-integrated ALP in Psomes-ALP (**Figure S22**). Those Psomes-ALP do not work in BEC as well.

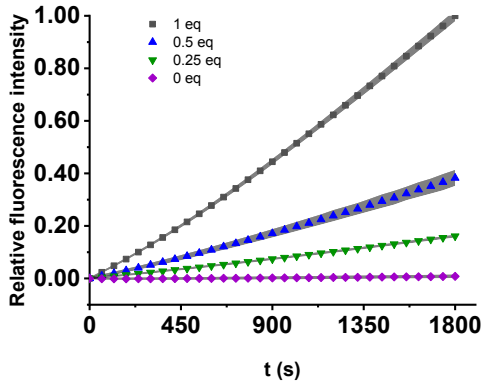


Figure S22. Function of AO "Psomes-ALP" in BEC works only when enough ALP is integrated in the membrane of Psomes-ALP. 1 eq (2 mg/mL) ALP was finally selected for the fabrication of AO "Psomes-ALP" in BEC. 0.5 eq (1 mg/mL), 0.25 eq (0.5 mg/mL) and 0 eq of ALP for preparing Psomes-ALP are not suited for the final application in BEC. Glucose concentration for the experiment series is 0.0625 mg/mL.

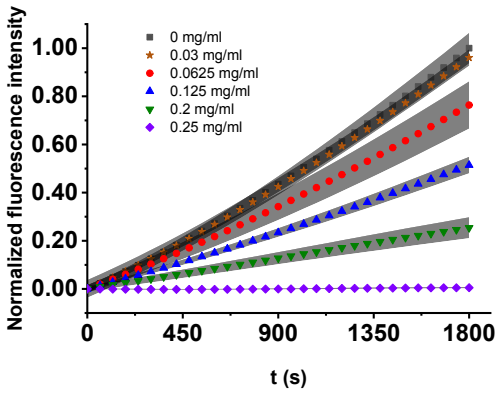


Figure S23. Determination of "Lower Limit of Detection" of BEC. Concentration of 0.03 mg/mL glucose cannot be differed from 0.0 mg/mL glucose. Data are presented as mean values ± SD, error bars indicate standard deviations (n = 3). Psomes-ALP were prepared with 2 mg/mL of ALP, finally, for integrating Psomes-ALP in BEC.

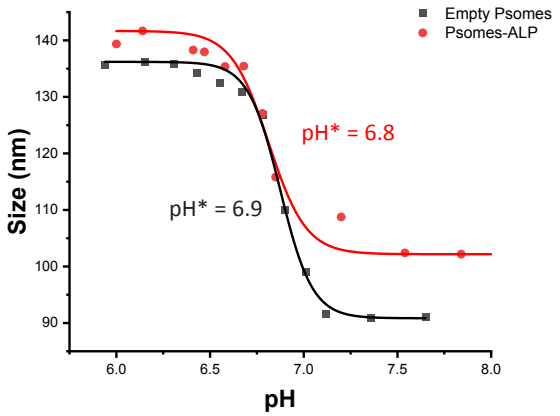


Figure S24. pH-dependent DLS titration curves of empty Psomes and Psomes-ALP for determining the critical pH value (pH*), measured in 20 mM NaCl.

Polymer	DEAMA units[a]	DMIBM units[a]	PEG units	BLR[c]	M_w (kg/mol)[b]	M_n (kg/mol)[b]	M_w (kg/mol)[a]	Đ[b]
BCP A	82.9	22.8	45	2.36	37.4	30.3	23.6	1.23

Table S1. Composition, block ratio, molecular weight, dispersity of block copolymers

The composition of BCP. [a] Determined from signal intensities in the ^{1}H-NMR spectra. [b] Studied by GPC. [c] BLR = block length ratio between hydrophobic and hydrophilic parts.

Table S2. Diameter and membrane thickness of empty Psomes and Psomes-ALP

Sample	DLS		Cryo-TEM	
	Diameter (nm)		Diameter (nm)	Membrane thickness (nm)
Empty Psomes	91.0 ± 0.1		71.3 ± 18.7	13.9 ± 2.5
Psomes-ALP	93.1 ± 0.4 [1]	102.2 ± 1.8 [2]	71.5 ± 19.3	16.6 ± 2.1

Diameter measured by DLS, before [1] and after [2] the purification of HFF; For cryo-TEM study, both diameter and membrane thickness were calculated by analyzing 150 - 200 particles.

Table S3. Determination of encapsulation efficiency for ALP in Psomes after HFF purification

Sample	Calculated concentration before HFF purification (mg/mL)	Calculated concentration after HFF purification (mg/mL)	Encapsulation efficiency (%)
Psomes-ALP-AF488	0.189 ± 0.006	0.029 ± 0.005	18.16

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[1] W. Burchard, in Branched polymers II, Springer, 1999, pp. 113-194.

[2] J. Gaitzsch, D. Appelhans, L. G. Wang, G. Battaglia, B. Voit, *Angew. Chem. Int. Ed.* 2012, **51**, 4448.

[3] X. Huang, M. Li, D. C. Green, D. S. Williams, A. J. Patil and S. Mann, Nat. Commun., 2013, **4**, 1-9.

[4] S. Moreno, P. Sharan, J. Engelke, H. Gumz, U. Oertel, B. Voit, S. Banerjee, R. Klajn, P.

Wang, A. Lederer, D. Appelhans, *Small.* 2020, **16**, 2002135.