Electronic Supplemental Information

Photo cross-linked and pH sensitive polymersomes for triggering the loading and release of cargo

Jens Gaitzsch,[a] Dietmar Appelhans,[a] David Gräfe,[a] Petra Schwille,[b] and Brigitte Voit[a,*]

[a]Leibniz Institute of Polymer Research Dresden, Hohe Straße 6, 01069 Dresden, Germany

[b]Technical University Dresden, Biotechnological Center, Tatzberg 47-49, 01307 Dresden, Germany

*corresponding author: voit@ipfdd.de

Content of ESI

Experimental Section

Materials. If not stated otherwise, all chemicals were used as received. All, anhydrous tetrehydrofurane (THF, Aldrich), anhydrous 2-butanone (Fluka) and triethylamine (Fluka) were stored over molecular sieve. Poly(ethylene glycol) methyl ether (MeO-PEG-OH; M_n ca. 2000; M_w/M_n = 1.05), DEAEM monomer, 2,2'-bipyridine, 2-bromoisobutyryl bromide, aminoethanol, methycryloylic chloride, copper-I-bromide, aluminium oxide (neutral, activated) and magnesium sulfate were purchased from Aldrich. 3,4-dimethylmaleic acid anhydrid, THF, toluene, chloroform and ethyl acetate were purchased from Acros. From merck, n-hexane was purchased. The dialysis membrane (MWCO 25 kDa) was purchased from Carl Roth (Germany).

The stock solutions of the dyes used were purchased from Avanti Polar Lipids (bodipy-chloresterol, 0.51 mM) and VWR (Cy-5 mono NHS ester, 1mg/ml).

Methods. The molecular weight distributions of the copolymers were assessed at 40 °C using a Polymer Laboratories PL-GPC50 Plus Integrated GPC system (Varian Inc., UK) equipped with a Polymer Laboratories pump, a PL ResiPore column (300 × 7.5 mm), a PL data stream refractive index detector and a PL-AS-RT Autosampler. The calibration was carried out using twelve polystyrene standards with M_n values ranging from 162 to 371,100 (Varian Inc., UK). The eluent was THF and the flow rate was 1.0 mL/min. The data were processed using Cirrus GPC offline GPC/SEC software (version 2.0).

^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance III 500 spectrometer operating at 500.13 MHz MHz (^{1}H) and 125.77 MHz (^{13}C), with $CDCl_3$ as solvent at room temperature. The copolymer compositions were determined from ^{1}H NMR spectra in dry $CDCl_3$, using the integrated signal assigned to the PEG block as an internal standard.

DLS studies of 2 g/L aqueous vesicle solutions were carried out over a range of pH at 25 °C using a ZETASIZER Nano series instrument (Malvern Instruments, UK) equipped with a multi-purpose autotitrator (MPT-2) and Dispersion Technology Software (version 4.00). The data was collected by the NIBS (non-invasive back-scatter) method using a Helium-Neon laser (4 mW, λ = 632,8 nm) and a fixed angle of 173°. More details about the evaluation of

the data in Figure 2a and Figure 2b. All data was obtained using Vol-% evaluation, assuming an RI of 1,5 for the polymer. The peak size given is the z-average within the measurements, except for radiation dependent measurements (Figure 2a), where the peak maximum was used.

The UV irradiation was carried out within a UVA Cube 100 (honle UV Technologies, Germany) equipped with a mercury lamp as UV source.

UV-Vis measurements were done at Cary 100 scan (Varian Inc., UK) and Lambda 800 (Perkin Elmer, USA). They were carried out in a range from 700 nm to 400 nm using 1 nm steps.

Vesicles from electroformation were imaged in a commercial ConfoCor2 system (Carl Zeiss, Germany) using a multi-track mode according to a protocol from Schwille et al.[1] Light from an Ar laser at 488 nm, and a He-Ne laser at 633 nm was reflected with a HFT UV/488/543/633 dichroic. A 40x numerical aperture 1.2 C-Apochromat water immersion objective was used, and the pinhole size was set to 90 µm in the green channel, although adjusted in the red channel for the same *z* thickness. Emitted fluorescence was separated with a secondary dichroic beam splitter 570 dichroic and passed through 505 nm or 650 nm long pass filters to be finally detected with a photomultiplier. Image processing and analysis was carried out with ImageJ and Zeiss LSM Image Browser.

Bright-field microscopy was carried out at a Zeiss Axiovert 200 (Carl Zeiss, Germany) microscope, equipped with an Zeiss C-Apochromat LD 40x objective (Carl Zeiss, Germany). Imaging was carried out using a Andor iXon EMCCD camera (Andor Technology, USA).

Synthesis of the substances

Photo cross-linker:

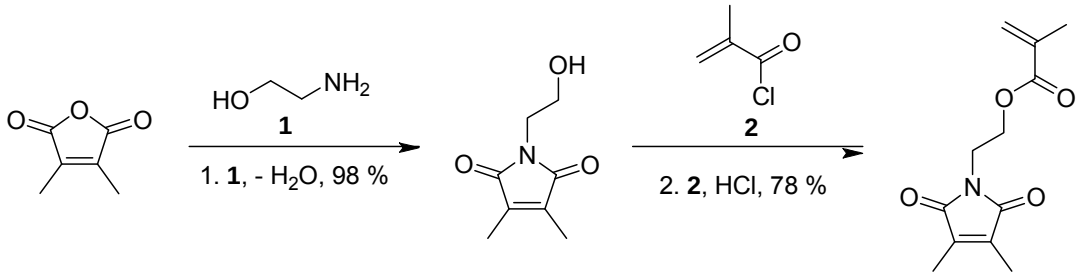

Figure 1-ESI: Reaction scheme of the formation of the cross-linker.

Synthesis for step 1: We adopted a method by Kuckling et al.[2] Here, 5,00 g (39,7 mmol) anhydrid are dissolved in 120 ml Toluene and 2,42 g (39,7 mol) aminoethanol are added. The mixture is kept at reflux for 2 h at a water trap and the solvent is removed afterwards at reduced pressure. The crude product is purified using flash chromatography with a hexane / ethyl acetate (50:50 Vol-%) mixture and gives a white solid in 98 % yield.

^{1}H - NMR: 1.98 (s, 6 H), 2.32 (b, 1H), 3.70 (t, J = 5.2 Hz, 2 H), 3.77 (t, J = 4.9 Hz, 2 H)

^{13}C - NMR: 8.66 (2 CH_3), 40.80 (CH_2), 61.10 (CH_2), 137.27 (2 C), 172.59 (2 C)

Synthesis for step 2: We adopted a method by Armes et al.[3] Here, 2,00 g (11,8 mmol) maleic imide are dried in vacuum and then set under a nitrogen atmosphere. 100 ml dry THF are added and the flask is cooled with ice. 1,85 g (17,7 mmol) methacryloyl chloride are dissolved in 3 ml dry THF before they are added to the mixture. After 2,10 g (18,3 mmol) dry triethylamine are added, the mixture becomes gloomy and the ice is removed. The reaction is carried out for 2 h at 40 °C and aborted by pouring the reaction into water. The water is extracted three times with ethyl acetate. All organic phases are dried over magnesium sulfate, the solids are removed and the solvent is removed at reduced pressure. The crude product is purified using flash chromatography with a hexane / ethyl acetate (67:33 Vol-%) mixture to give a colorless oil in 78 % yield.

^{1}H - NMR: 1.85 (s, 3 H), 1.93 (s, 6 H), 3.77 (t, J = 5.2 Hz, 2 H), 4.22 (t, J = 5.36 Hz, 2 H), 5.49 – 5.52 (m, 1 H), 6.00 – 6.03 (m, 1 H),

^{13}C - NMR: 8.67 (2 CH_3), 18.19 (CH_3), 36.84 (CH_2), 61.99 (CH_2), 126.03 (2 C), 135.85 (C), 137.35 (CH_2), 167.05 (C), 171.78 (2 C)

PEG-Br macroinitiator

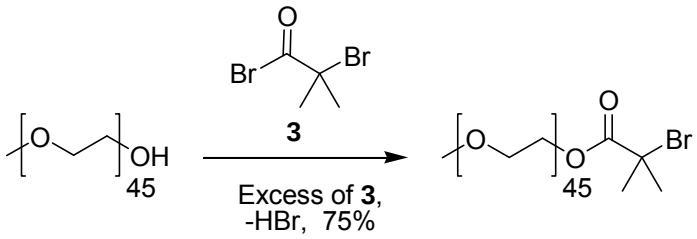

Figure 2-ESI: Reaction scheme of the formation of PEG-Br macroinitiator.

Synthesis: We adopted a method by Armes et al.[3] Here, 5,00 g (2,5 mmol) PEG_{45}-OH are dried in a flask at vacuum and 60 °C for 30 min. The flask is flushed with nitrogen before 50 ml dry THF are added. 1,43 g (6,25 mmol) 2-bromoisobutyric acid bromide is dissolved in 3 ml dry THF before added to the solution. The flask is now cooled with ice and 0,38 g (3,75 mmol) dry triethylamine are added. The gloomy mixture is stirred for 40 h at room temperature. The final macro initiator is precipitated in CO_2-cooled ether and three times recrystallised in ethanol until a white solid is obtained. Yield: 74 %.

^{1}H - NMR: 1.93 (s, 6 H), 3.37 (s, 3 H), 3.63 (180 H)

^{13}C - NMR: 30.73 (2 CH_3), 58.96 (C), 65.08 (CH_3), 70.53 (CH_2), 171.54 (C)

Polyethylenglycol-*block*-Polydiethylaminoethylmethacrylat (PEG-*b*-PDEAMA)

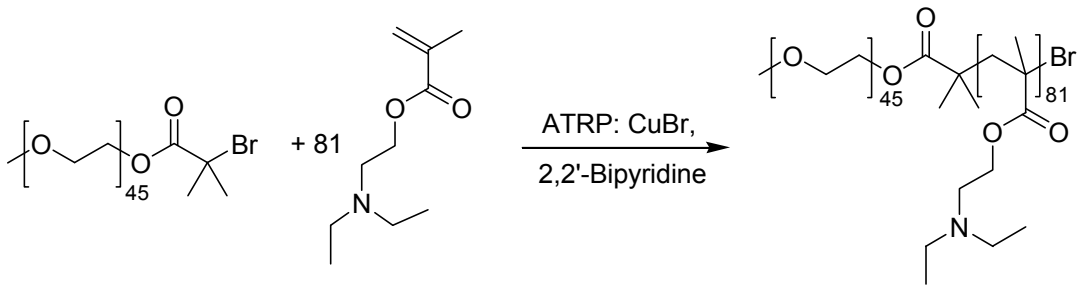

Figure 3-ESI: Reaction scheme of the formation of polyethylenglycol-*block*-polydiethylaminoethylmethacrylat (PEG-*b*-PDEAMA).

Synthesis: We adopted a method by Weaver et al.[4] Here, 220 mg (0,1 mmol) PEG_{45}-Br and 32 mg 2,2'-bipyridine (0,2 mmol) are mixed in a flask and dried for 5 min in vacuum and flushed with nitrogen. Then 15 mg (0,1 mmol) CuBr are added and another 30 min dried in vacuum and again flushed with nitrogen. In an additional flask 1,52 g (9,22 mmol) diethylaminoethylmethacrylate are dried 30 min in vacuum and also flushed with nitrogen afterwards. This monomer is then solved in 3 ml 2-butanone and the solution degassed and added to the solids afterwards. The mixture is stirred for 17 h at 50 °C. To abort the reaction, it is diluted in 3 ml THF and with additional THF filtrated over activated neutral aluminium oxide to remove any copper species. From the resulting gloomy solution the solvent is removed at reduced pressure. The crude product is washed with n-hexane and water before it is dried in vacuum to give a sticky polymer. Yield: 63 %

^{1}H - NMR: 0.85 - 0.98 (m, 2 H), 0.99 - 1.11 (m, 3 H), 1.55 – 2.08 (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), 3.93 – 4.12 (m, 2 H)

^{13}C - NMR: 12.19 (2 CH_3), 25.60 (CH_2), 30.32 (CH_3), 44.72 (C), 45.14 (CH_3), 47.67 (CH_2), 50.53 (CH_2), 63.26 (CH_2), 67.49 (CH_3), 70.58 (CH_2), 177.31 (C=O)

Polyethylenglycol-*block*-polydiethylaminoethylmethacrylat-*stat*-poly-3,4-dimethylmaleinimidoethylmethacrylat (PEG-*b*-PDEAMA-*s*-PDMIEM)

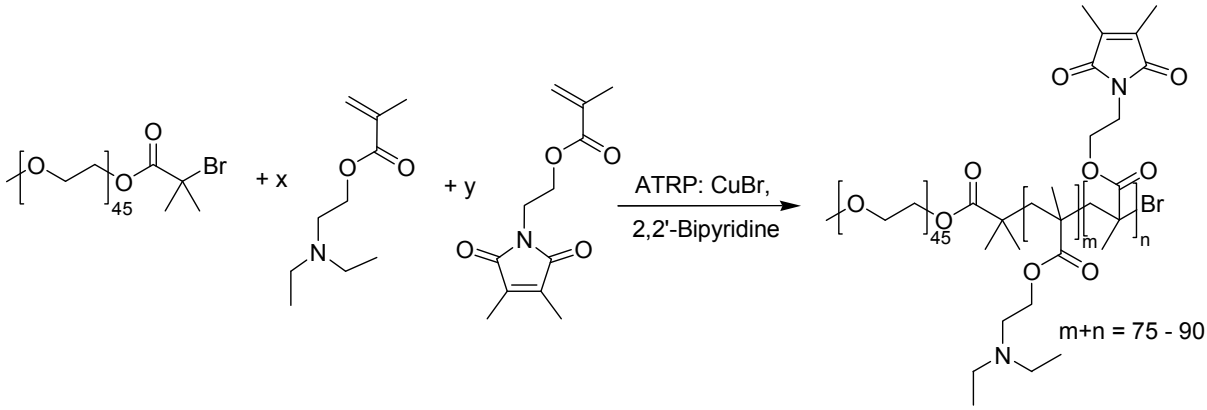

Figure 4-ESI: Reaction scheme of the formation of Polyethylenglycol-*block*-polydiethylaminoethylmethacrylat-*stat*-poly-3,4-dimethylmaleinimidoethylmethacrylat (PEG-*b*-PDEAMA-*s*-PDMIEM).

Synthesis: Same approach as for PEG-b-PDEAMA, but DMIEM is added to DEAEM accordingly.

^{1}H - NMR: 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)

^{13}C - NMR und DEPT: 8.73 (CH_3), 12.19 (2 CH_3), 25.60 (CH_2), 30.32 (CH_3), 44.72 (C), 45.14 (CH_3), 47.67 (CH_2), 50.53 (CH_2), 50.58 (CH_2), 63.26 (CH_2), 67.49 (CH_3), 70.58 (CH_2), 137.42 (C), 177.31 (C=O)

The polymers could be obtained in a clean manner, as indicated by GPC measurements:

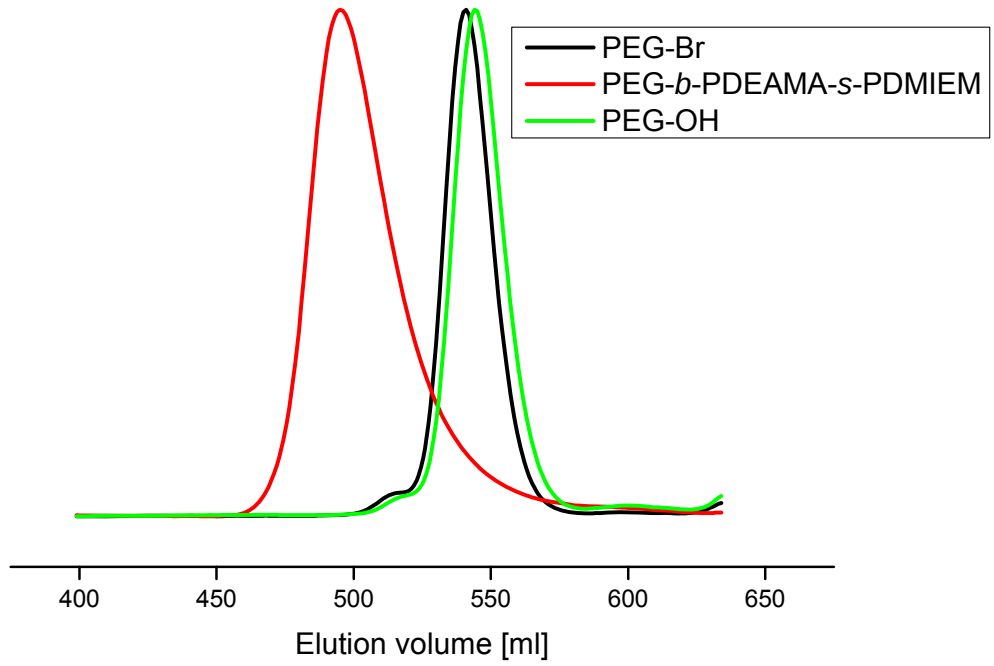

Figure 5-ESI: GPC traces of pure PEG-OH, the PEG-Br macroinitiator as well as the final PEG-*b*-PDEAMA-*s*-PDMIEM block-copolymer.

Evaluation of NMR spectra to determine polymer composition

The ratio of all blocks was calculated using integrals of specific ^{1}H NMR signals.

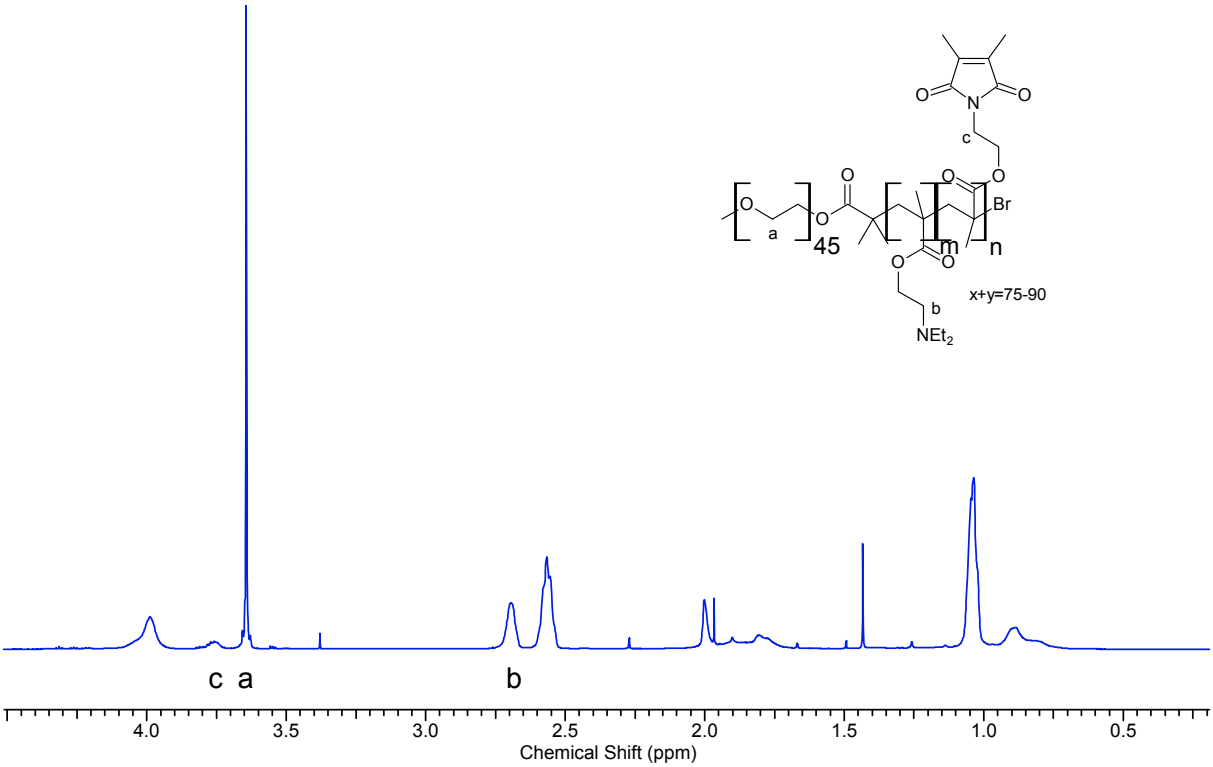

Figure 6-ESI: NMR Spectrum of PEG-b-PDEAEM-s-PDMIEM with labelled ^{1}H NMR signals used to determine the final structure. The corresponding atoms are labelled in the polymer.

Table 1-ESI: Calculations concerning the block-length ratio (hydrophilic (a) / hydrophobic (b and c)) (blr) and the amount of cross-linker present in the polymer.

No.	Integral "a"	Integral "b"	Integral "c"	blr[a]	Cross-linker[b]
1	1	1,01	0	1:2,0	0 mol-%
2	1	1,01	0,02	1:2,1	2 mol-%
3	1	0,86	0,04	1:1,8	5 mol-%
4	1	0,84	0,09	1:1,9	10 mol-%
5	1	0,79	0,14	1:1,9	15 mol-%

[a] blr = block length ratio; calculated via: blr = a : (2xb + 2xc); during the course of polymerization one have used 2 equivalents of DEAEM and DMIEM in total against the repeating unit in the PEG-macro initiator. [b] relative amount of cross-linker polymerised within the hydrophobic block (c/(b+c).

The intensity of signal “a” from the PEG part is taken as a reference, since the total amount of ethylene glycol units is known (45 units). Since PEG has a symmetric structure, the integral of peak “a” represents 4 H atoms per monomer repeating unit. In contrast, the integrals of the signals “b” and “c”, referring to PDMIEM and PDEAEM, respectively, only represent 2 H atoms each. Due to this, an integral ratio from “a” to “b+c” from 1:1 means a block-length ratio of 1:2, concerning the repeating units. The share of integral “c” within the hydrophobic block, meaning integrals “b+c”, is the amount of cross-linker polymerised into the substance.

Preparation and Characterisation of Vesicles

Preparation of vesicles in water:

A solution of 0,2 weight-% polymer in acid (pH = 2) water is prepared and stirred until the whole polymer is dissolved. The final solution is passed through a 0,2 µm nylon filter to remove any remaining particles, including dust. Now, 1 M NaOH is added through a 0,2 µm nylon filter until pH 10 is reached. If vesicles are formed, the solution becomes gloomy. The vesicles were characterized using DLS.

Preparation of vesicles with electroformation:

To a solution of 0,2 weight-% Polymer in 2 ml chloroform 1 µl of bodipy-chol (green dye) is added. 10 µl of this solution are slowly spread upon an indium-tin oxide (ITO) coated coverslide, resulting in a thin polymer film. This crystal is covered with another ITO coated slide and the resulting chamber is filled with Millipore® water. Both crystals are equipped with copper sheets, which are connected to a power supply. The vesicles are grown at 3V. The frequency of the alternating current is kept at 10 Hz for 30 minutes, at 5 Hz for the following 30 minutes, and during the following hour kept at 0,6 Hz.

Parallel, a solution 20 ml Millipore® water with 1 µl Cy-5 mono NHS ester (red dye) is prepared. After imaged at a LSCM, the chamber is poured with the solution of the red dye and the vesicles are again imaged at LSCM, as described previously.

Cross-Linking of vesicles:

A solution of vesicles prepared in water is placed in the UV chamber and irradiated for the minutes mentioned in the main text (Figure 2a). This procedure results in stock solutions of cross-linked polymersomes used for further acidification in different experiments (Figure 2a and 2b) finally investigated by (in-situ) DLS.

pH-sensitivity of cross-linked vesicles:

A stock-solution of cross-linked polymersomes was titrated automatically connected to a DLS measurement cell taken after the pH value remained constant.

Reversible swelling upon repeated changes in pH:

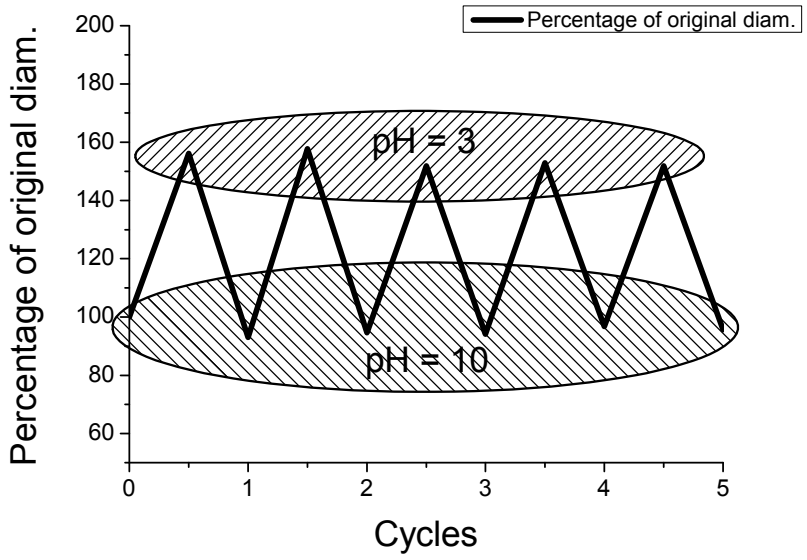

Figure 7-ESI: Development of vesicle-diameters (z-average) from DLS measurements upon repeated changes of the pH value.

To a polymer with 15 mol% cross-linker, little amounts of 1M HCl or 1M NaOH were added to reach pH 3 or 10, respectively. This cycle was repeated 5 times.

Encapsulation of dye into cross-linked vesicles:

To a stock solution containing cross-linked polymersomes with 10 or 15 mol-% cross-linker an equal volume of rhodamin B-solution (c = 4 mg/l) is added. This mixture is titrated with 1 M HCl to obtain pH 4 and stirred for 5 h. The non-encapsulated rhodamin B is removed by dialysis against a membrane with MWCO = 25000 for 3 days.

The same procedure was carried with pure water instead of the polymer solution (presented in Figure 3a in the main paper).

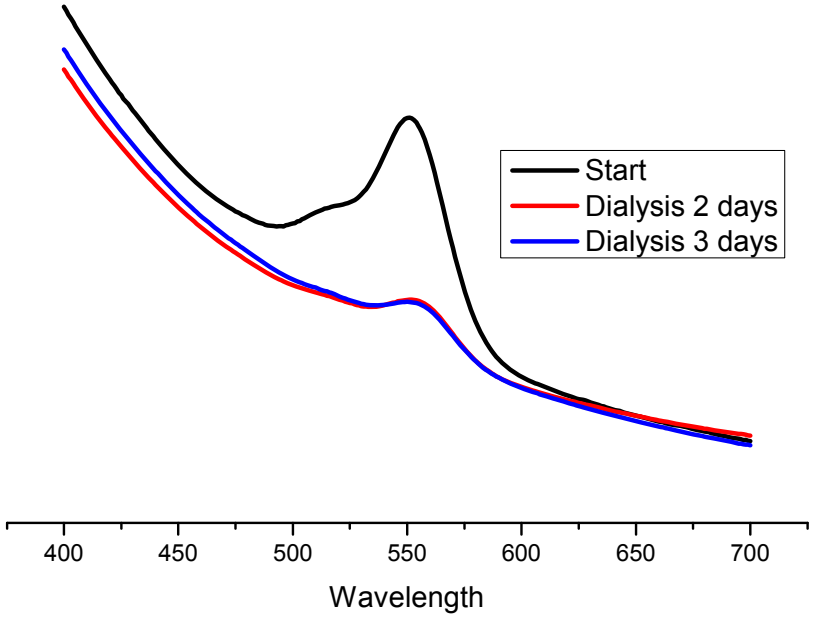

Figure 8-ESI: UV-Vis monitored dialysis of solution containing rhodamin B / cross-linked polymersome with 15 mol-% cross-linker solution after the entrapment of the dye at > pH 7. Finally, no further escape of the dye from polymersome is given after two days. Result of vesicle with 10 mol-% cross-linker is shown in Figure 3b in the main paper.

References:

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3. M. Save, J. V. M. Weaver, S. P. Armes and P. McKenna, *Macromolecules*, 2002, **35**, 1152-1159.
4. D. J. Adams, M. F. Butler and A. C. Weaver, *Langmuir*, 2006, **22**, 4534-4540.