Supporting Information

Cellular interactions with photo cross-linked and pH sensitive polymersomes – biocompatibility and uptake studies

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Content of SI

1. Synthesis of the substances

Remark: This SI contains only the synthetic procedures. For characterisation data (including NMR), please see previously published results.[1]

Photo cross-linker:

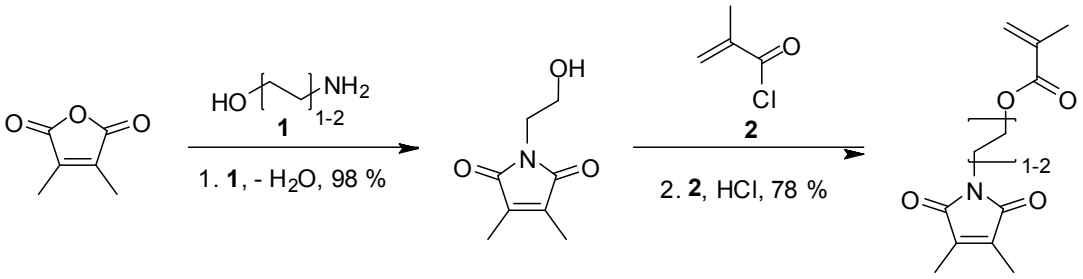


Figure 1-SI, Reaction scheme of the formation of the cross-linker.

Synthesis for step 1: We adopted a method by Kuckling et al.[2] Here, 5.00 g (39.7 mmol) anhydride are dissolved in 120 ml Toluene and 39.7 mol aminoethanol (C2 spacer) or aminobutanol (C4 spacer), respectively, 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.

For characterisation data (including NMR), see previously published results.[1]

Synthesis for step 2: We adopted a method by Armes et al.[3] Here, 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. For characterisation data (incl. NMR), see previously published results.[1]

PEG-Br macroinitiator

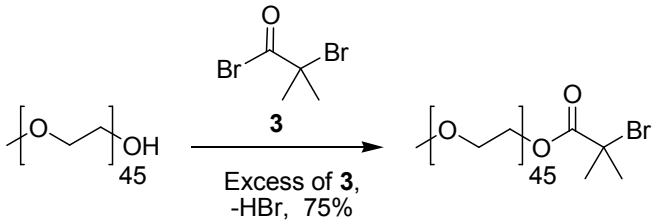


Figure 2-SI. Reaction scheme of the formation of PEG-Br macroinitiator.

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 %.

Poly(ethylene glycol)-*block*-poly(diethylaminoethylmetharcylate-*stat*-poly-3,4-dimethylmaleinimidoethylmetharcylate) (PEG-*b*-PDEAMA-*s*-PDMIEM)

and

Poly(ethylene glycol)-*block*-poly(diethylaminoethylmetharcylate-*stat*-poly-3,4-dimethylmaleinimidobutylmetharcylate) (PEG-*b*-PDEAMA-*s*-PDMIBM)

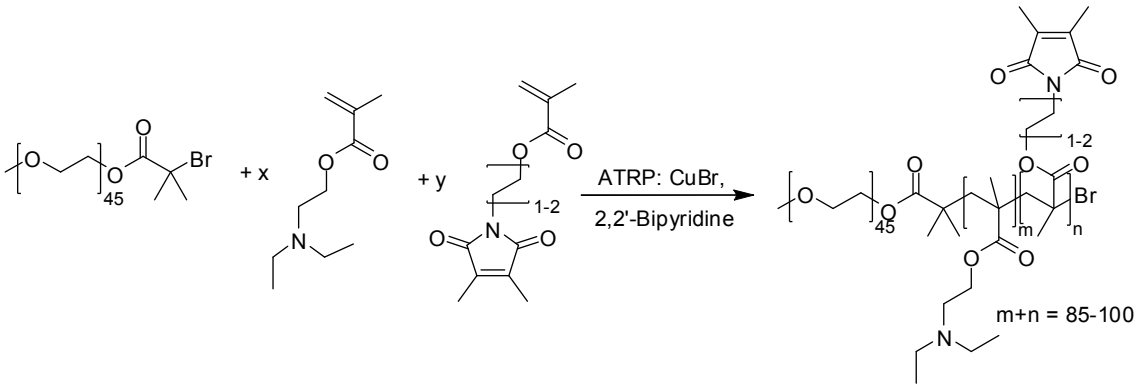


Figure 3-SI. Reaction scheme of the formation of poly(ethylene glycol)-*block*-poly(diethylaminoethylmetharcylate-*stat*-poly-3,4-dimethylmaleinimidoethylmetharcylate) (PEG-*b*-PDEAMA-*s*-PDMIEM).

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 and the corresponding amount of the cross-linker monomer (C2 or C4) 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 %

The polymers were characterized as described previously.[5]

Table 1-SI. Properties of the polymers forming the polymersomes

Name	M_n[a]	Block-length ratio[b]	Cross-Linker	
			Spacer[c]	mol-%[d]
C2-10	19.0	1:2.0	C2 (ethyl)	10
C2-20	21.5	1:2.2	C2 (ethyl)	20
C4-10	20.0	1:2.0	C4 (butyl)	10
C4-20	22.5	1:2.2	C4 (butyl)	20

[a] in kg/mol; M_n is determined from ^{1}H-NMR signal intensities, the PDI of the copolymers is 1.3 (1.4 for C4-10) [b] ratio hydrophilic / hydrophobic; determined via signal ratios in NMR spectra (described previously[1]) [c] referring to spacer in the crosslinking unit used [d] in mol-% in respect of the hydrophobic block length; determined via signal ratios in NMR spectra (described previously[1,5])

Crosslinking reaction of the vesicles

When irradiated, the polymersomes crosslinked, due to a dimerization of the crosslinking unit as shown in **Figure 4-SI**.

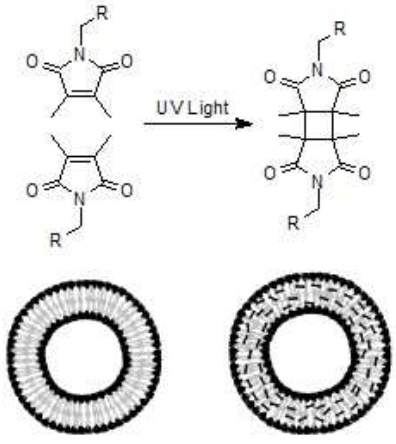


Figure 4-SI. Cyclobutane ring formation leading to polymersome crosslinking upon UV irradiation.

2. Preparation and Characterisation of Vesicles

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. Size dependency was achieved by an Auto-Titration using the apparatus mentioned.

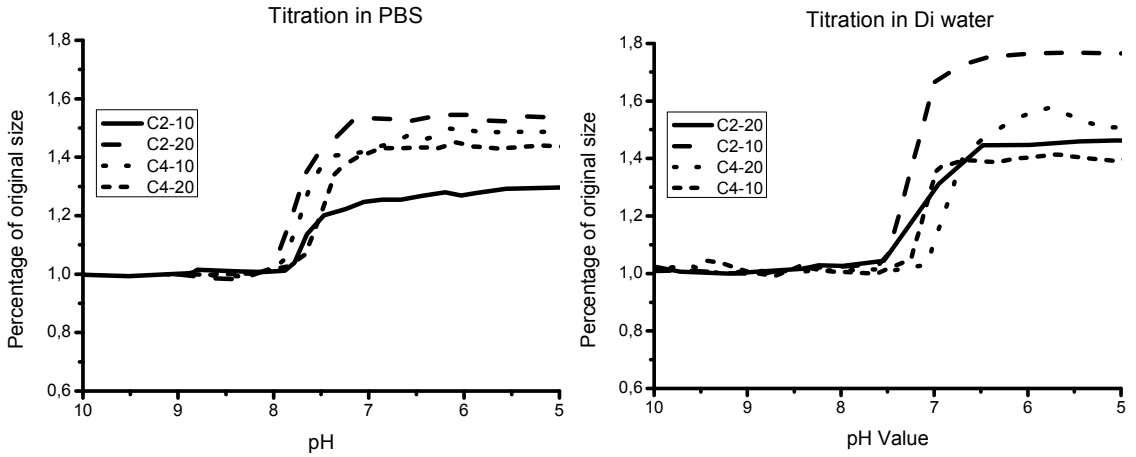


Figure 5-SI. pH titrations of our polymersomes to determine the pKa. The pKa was determined as the point, when a half-swollen state is reached.

Encapsulation of PEI-Mal into cross-linked vesicles

PEI-Mal (maltose- decorated hyperbranched poly(ethylene imine) molecules:We used a PEI core of 25 kDa and the final PEI possesses an open maltose shell which is finally described as structure B in reference 6 in SI. Our PEI-Mal was synthesized, characterized and dye-marked as previously described previously[6]. Briefly, 0.8 mg of dye were dissolved in 0.2 ml DMSO and 40 mg PEI-Mal dissolved in 1 ml deionized water. Both solutions were mixed and stirred overnight. Non-bound dye was removed using column chromatography Sephadex-25. Powders of the materials have been obtained by freeze drying process[6].

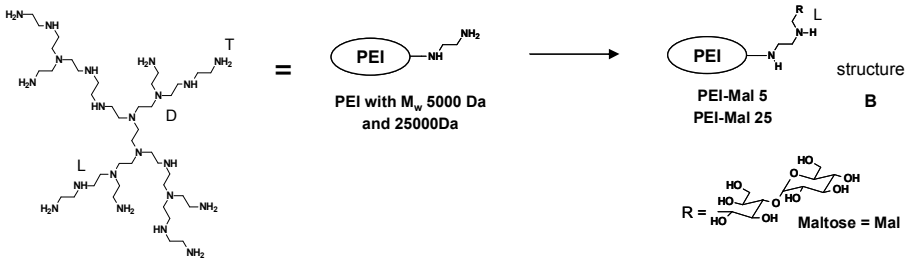


Figure 6-SI. Structure of PEI-Mal (structure B)

Encapsulation process:

The polymer is dissolved as described above and then 2 mg of PEI-Mal (molecular weight of the PEI core: 25000 kDa) are added to the solution. Subsequently, the vesicles were created as described above. Now the vesicles are eventually cross-linked, as described above. The solution is now to be cleaned from any non-enclosed PEI-Mal and from PEI-Mal which leaves the vesicles upon the cleaning process using a hollow fibre filtration system.[1]

Hollow fibre filtration as cleaning process for the Polymersome / PEI-Mal mixture

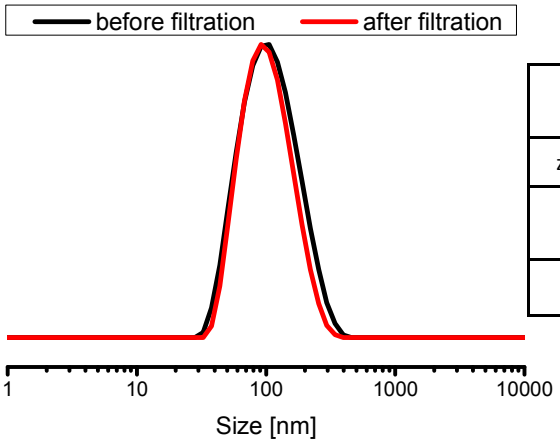
2.5 ml of the polymersome solution are diluted to 5.0 ml with pH = 9 water to measure the initial UV spectrum. Afterwards, the solution is transferred into a 30 ml cone tube, which is attached to the hollow fibre filtration system. The tube is filled with pH = 9 water afterwards. During the extraction process, the cone tube is constantly refilled with pH = 9 water, since extracted “waste” constantly leaves the system. To take samples, the fluid level is extraction is continued until 5.0 ml remain in the cone tube.

To proof, that vesicles are not destroyed, we performed DLS studies after the hollow fibre filtration. (Table 2-SI)

Table 2-SI. Characterisation of vesicles after hollow fibre filtration. The starting data was z-Average of 148 nm and a PDI of 0.17 for the vesicles

TMP /mbar	Cross-linked?	z-Average	PDI
250	No	149 nm	0.16
250	Yes	154 nm	0.19
750	No	151 nm	0.18
750	Yes	149 nm	0.15

Polymersome filtration to reach sterilization



Property	Before filtration	After filtration
z-Average	94 nm	93 nm
Peak diameter	117 nm	109 nm
PDI	0.18	0.16

Figure 7-SI. Influence of filtrating the polymersomes through a 200 nm filter on their size and distribution. The DLS curve on the left and table with results on the right show that no influence of filtration can be observed.

Sterilisation through a 200 nm filter was used to sterilize polymersomes. The polymersomes used are on average roughly 100 nm in size. To verify, that no material was lost, the polymersome solutions were studied with DLS before and after the filtration to see, whether a significant change can be observed (**Figure 6-SI**). As it could be expected, the DLS trace after filtration is narrowed down slightly from larger sized structures. This means, a small amount of larger polymersomes left the solution upon filtration. As a result, the PDI of the solution shrinks from 0.18 to 0.16, proving a more narrow distribution than before. Additional key data from DLS also undermine the conclusion from the bare DLS trace. Both, z-average and Peak diameter decrease slightly upon filtration, but only the peak diameter somewhat significantly. Although all changes are determinable, they are of a minor nature only. Hence, filtration through a 200 nm filter is a reasonable sterilisation method for the crosslinked polymersomes studied and was used in all further experiments. In addition to DLS study, visual inspection showed that the solution remained turbid.

References:

(1) Gaitzsch, J.; Appelhans, D.; Wang, L. G.; Battaglia, G.; Voit, B. *Angew. Chem. Int. Ed.* **2012**, *51*, 4448.

(2) Vo, C. D.; Kuckling, D.; Adler, H. J. P.; Schonhoff, M. *Colloid Polym. Sci.* **2002**, *280*, 400.

(3) Save, M.; Weaver, J. V. M.; Armes, S. P.; McKenna, P. *Macromolecules* **2002**, *35*, 1152.

(4) Adams, D. J.; Butler, M. F.; Weaver, A. C. *Langmuir* **2006**, *22*, 4534.

(5) Gaitzsch, J.; Appelhans, D.; Grafe, D.; Schwille, P.; Voit, B. *Chem. Commun.* **2011**, *47*, 3466.

(6) Appelhans, D.; Komber, H.; Quadir, M. A.; Richter, S.; Schwarz, S.; van der Vlist, J.; Aigner, A.; Muller, M.; Loos, K.; Seidel, J.; Arndt, K. F.; Haag, R.; Voit, B. *Biomacromolecules* **2009**, *10*, 1114.