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# **Electronic Supplementary Information**

# Fine-tuning the pH response of polymersomes for mimicking and controlling cell membrane functionality

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## **Content of ESI**

		Page
Experimental		3
Materials and metho	ds	3
Polymer synthesis		3
Polymersome forma	tion	4
Additional figures a	and tables	4
Tab. ESI-1	Composition, block ratio, molecular weight, dispersity and entirely hydrophobic fraction <i>ehf</i> of the used block copolymers as determined by <sup>1</sup> H-NMR and GPC.	4
Fig. ESI- 1	DLS titration data of polymersomes assembled from different block copolymers. For the sake of easier comparison the diameters at basic conditions are normalised to 100%. The lines represent mathematical fit functions as described in the main text in Scheme 1.	5
Fig. ESI- 2	Relative diameters of polymersomes assembled from different block copolymers for cyclic switching between pH 8 and 5.	5
Fig. ESI- 3:	$pH^*$ values of polymersomes assembled from single block copolymers (dark blue) and joint assemblies of mixtures of two single polymers (light blue). For mixed polymersomes the individual components are mixed in 1 to 1 ratio prior to the self-assembly process. Error bars refer to the error of the fit function used (see main text, Scheme 1).	6
Fig. ESI- 4	CryoTEM images of polymersomes assembled from different block copolymers. Comparison of diameters from DLS and CryoTEM measurements of polymersomes assembled from polymers of different composition. Error bars refer to standard deviation of the determined size distributions.	6
Fig. ESI- 5	Zeta-Potential curves at different concentrations of sodium chloride (1 and 10 mM) for polymersomes assembled from BCP-32 and BCP-50, respectively.	7
Fig. ESI- 6	Typical GPC traces of selected block copolymers.	7
References		7

#### **Experimental**

**Materials and methods.** Crosslinking experiments were carried out in a custom made UV chamber connected to an OmniCure S2000 (Excelitas Technologies) device equipped with a high pressure mercury lamp at an irradiation level of 40 W/cm<sup>2</sup>. <sup>1</sup>H-NMR spectra were recorded in CDCl<sub>3</sub> on a Bruker Avance III 500 spectrometer using the solvent residual peak as reference. Combined measurements of size and zeta-potential were recorded on Zetasizer nano-Series (Malvern Instruments) equipped with an automatic titrator using 0.1 M hydrochloric acid. Additional DLS measurements were carried out using a DynaPro Nanostar (Wyatt) equipped with a He-Ne-laser (120 mW,  $\lambda$  = 658nm) at a fixed angle of 90°. CryoTEM images were recorded on a Libra 120 (Carl Zeiss). Samples were absorbed on Lacey-type gold grids, frozen in liquid ethane using a grid plunger (Leica Microsystems) and transferred into the microscope using a Gatan 626 cryo holder. GPC measurements were conducted in THF (flow rate 1 ml/min) using Series 1200 pump (Agilent) connected to a MiniDAWN light scattering detector (Wyatt) on a Mixed-C column (Polymer Laboratories).

Polymer synthesis. 2.2'-Bipyridine (SigmaAldrich), 2-butanone (Merck), CuBr (Aldrich), diethylaminoethyl methacrylate (DEAEMA) (Aldrich), dimethylaminoethyl methacrylate (DMAEMA) (Aldrich), n-butyl methacrylate (nBMA) (Aldrich) and ZelluTrans dialysis membranes (Roth) were used as received. mPEG-Br macroinitiator and dimethylmaleic imidobutyl methacrylate (DMIBMA) were synthesized as reported in the literature.<sup>[1,2]</sup> The polymerisation of the »standard« block copolymer BCP-20 was carried out as described by Gaitzsch with slight modifications.<sup>[1]</sup> In short mPEG-Br macroinitiator (107 mg, 0.05mmol, 1.07 eq ), 2,2'-bipyridine (14.5 mg, 0.093mmol, 2 eq ), DEAEMA (604 mg, 3.257mmol, 70 eq.) and DMIBMA (247 mg, 0.931mmol, 20 eq.) are added to a Schlenk tube equipped with a stirring bar. The compounds are dissolved in 1.5 ml of 2-butanone and completely frozen in liquid nitrogen. Now CuBr (6.7 mg, 0.047mmol, 1 eq.) is added, the mixture is degassed using four freeze-pump-thaw-cycles, backfilled with argon and stirred over night at 50°C. To end the polymerization the tube is opened, the reaction mixture is diluted with THF and filtrated over aluminium oxide to remove all copper species. The mixture is transferred to a dialysis membrane (regenerated cellulose, MWCO=2 kDa) und dialysed against acetone (technical grade) for three days exchanging the solvent twice a day. Afterwards the solvent is removed under reduced pressure and the final product is dried in vacuo. For the synthesis of more hydrophobic block copolymers (BCP-32 to -57) the amount of DEAEMA is reduced (70x eq.) and x eq. of *n*BMA are added. For BCPs containing DMAEMA the same principle is applied (BCP-M11 to -M36).

3

**Polymersome formation.** The block copolymer is solved in diluted hydrochloric acid at pH 2 at a concentration of 1 mg/ml. The solution is passed through a syringe filter (Nylon, 0.2  $\mu$ m) and titrated with sodium hydroxide solution until pH 8-9 is reached. The mixture is stirred in the dark for three to four days, passed through a syringe filter (cellulose ester, 0.8  $\mu$ m) and crosslinked in small aliquots for 90 s each.

### Additional figures and tables

**Tab. ESI- 1:** Composition, block ratio, molecular weight, dispersity and entirely hydrophobic fraction *ehf* of the used block copolymers as determined by <sup>1</sup>H-NMR and GPC (please see Fig- ESI-6 for GPC traces).

Polymer	<b>n</b> <sub>DEAEMA</sub> 1	<b>n</b> <sub>DMIBMA</sub> 1	nnBMA <sup>1</sup>	block	ehf	M <sub>n</sub>	Mn	$M_w/M_n^2$
				ratio <sup>1</sup>	(%) <sup>1</sup>	(g/mol) <sup>1</sup>	(g/mol)²	
BCP-0	102	0	0	1 : 2.3	0	21 000	-	-
BCP-20	83	21	0	1 : 2.3	20	23 100	28 750	1.19
BCP-22	86	24	0	1 : 2.4	22	24 400	-	-
BCP-24	78	24	0	1 : 2.2	24	23 000	37 900	1.10
BCP-32	70	20	13	1 : 2.3	32	22 300	20 500	1.23
BCP-44	54	22	20	1 : 2.1	44	20 800	26 900	1.18
BCP-50	48	20	28	1:2.1	50	20 300	29 100	1.11
BCP-55	41	20	30	1 : 2.0	55	19 300	-	
BCP-57	46	23	38	1 : 2.3	57	22 200	27 300	1.12
	n <sub>DEAEMA</sub>	<b>n<sub>DMIBMA</sub></b>	<b>n</b> <sub>DMAEMA</sub>					
BCP-M11	67	22	11	1 : 2.2	-	22 100	32 200	1.06
BCP-M22	55	24	22	1 : 2.2	-	22 200	35 500	1.07
BCP-M27	49	24	27	1:2.2	-	21 800	-	-
BCP-M36	42	25	36	1 : 2.2	-	22 200	31 100	1.14

<sup>1</sup> as determined by <sup>1</sup>H-NMR-Spectroscopy

<sup>2</sup> as determined by GPC



Fig. ESI- 1: DLS titration data of polymersomes assembled from different block copolymers. For the sake of easier comparison the diameters at basic conditions are normalised to 100%. The lines represent mathematical fit functions as described in the main text in Scheme 1.



**Fig. ESI- 2:** Relative diameters of polymersomes assembled from different block copolymers for cyclic switching between pH 8 and 5.



**Fig. ESI- 3:** *pH*\* values of polymersomes assembled from single block copolymers (dark blue) and joint assemblies of mixtures of two single polymers (light blue). For mixed polymersomes the individual components are mixed in 1 to 1 ratio prior to the self-assembly process. Error bars refer to the error of the fit function used (see main text, Scheme 1).



Fig. ESI- 4: CryoTEM images of polymersomes assembled from different block copolymers. Comparison of diameters from DLS and CryoTEM measurements of polymersomes assembled from polymers of different composition. Error bars refer to standard deviation of the determined size distributions.



**Fig. ESI- 5:** Zeta-Potential curves at different concentrations of sodium chloride (1 and 10 mM) for polymersomes assembled from BCP-32 and BCP-50, respectively.



Fig. ESI-6: Typical GPC traces of selected block copolymers.

#### References

- [1] J. Gaitzsch, D. Appelhans, D. Gräfe, P. Schwille, B. Voit, *Chemical Communications* 2011, **47**, 3466-3468.
- [2] J. Du, S. P. Armes, *The Journal of the American Chemical Society* 2005, **127**, 12800–12801.