Electronic Supplementary Information

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

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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/cm². 1H-NMR spectra were recorded in CDCl₃ 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, λ = 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. The polymerisation of the »standard« block copolymer BCP-20 was carried out as described by Gaitzsch with slight modifications. 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 (70-x eq.) and x eq. of nBMA are added. For BCPs containing DMAEMA the same principle is applied (BCP-M11 to -M36).
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 μm) 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 μm) 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 ¹H-NMR and GPC (please see Fig- ESI-6 for GPC traces).

| Polymer  | *n*<sub>DEAEMA</sub><sup>1</sup> | *n*<sub>DMIBMA</sub><sup>1</sup> | *n*<sub>BMA</sub><sup>1</sup> | block ratio<sup>1</sup> | *ehf*<sup>1</sup> (%) | *M<sub>n</sub><sup>1</sup> (g/mol)<sup>1</sup> | *M<sub>n</sub><sup>2</sup> (g/mol)<sup>2</sup> | *M<sub>w</sub>/M<sub>n</sub>  

| 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  
| 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 ¹H-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).

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

References
