Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information –

Cross-linked and pH Sensitive Supported Polymer Bilayers from Polymersomes – Studies Concerning Thickness, Rigidity and Fluidity

Jens Gaitzsch,[a,b,c] Dietmar Appelhans,[a] Andreas Janke,[a] Maria Strempel,[d] Petra Schwille[d,e] and Brigitte Voit*[a,b]

[a] Leibniz-Institut für Polymerforschung Dresden e.V., Hohe Straße 6, 01067 Dresden, Germany.

[b] Organic Chemistry of Polymers, Technische Universität Dresden, 01062 Dresden, Germany.

[c] University College London, Department of Chemistry, 20 Gordon Street, London WC1H 0AJ

[d]Technische Universität Dresden, Biotechnological Center, Tatzberg 47-49, 01307 Dresden, Germany

[e]MPI of Biochemistry, Am Klopferspitz 18, 82152 Martinsried

Email: voit@ipfdd.de

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1 Experimental Section

1.1 Materials and Instruments

Materials. If not stated otherwise, all chemicals were used as received. All, anhydrous tetrahydrofurane (THF, Aldrich), anhydrous 2-butanone (Fluka) and triethylamine (Fluka) were stored over molecular sieve. Poly(ethylene glycol) methyl ether (MeO-PEG-OH; Mn ca. 2000; Mw/Mn = 1.05), diethylaminoethylmethacrylate (DEAEM) monomer, 2,2'-bipyridine, 2-bromoisobutyryl bromide, 2-aminoethanol, 4-aminobutanol, methacryloylic chloride, copper-I-bromide, aluminium oxide (neutral, activated) and magnesium sulphate were purchased from Aldrich. 3,4-dimethylmaleic acid anhydride, THF, toluene, chloroform and ethyl acetate were purchased from Acros. From Merck (Germany), n-hexane was purchased. Atto 647N attached to DOPE lipid was purchased from ATTO-TEC (Germany)

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

The UV irradiation was carried out within an EXFO Omnicure 1000 spot curing system (Lumen Dynamics Group Inc., Canada) equipped with a high pressure mercury lamp (High Pressure 100 Watt Mercury Vapor Short Arc and a 320-500 nm filter) as UV source

Fluorescence-labelled films 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 20x numerical aperture 1.2 C-Apochromat water immersion objective was used, and the pinhole size was set to 90 µm in the red channel. Emitted fluorescence was separated with a secondary dichroic beam splitter 570 dichroic and passed through a 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.

Atomic force microscopy (AFM): Images were taken using a DIMENSION ICON from Bruker-Nano (USA).

Plasma cleaning: The wafers (Si) and glass slides were plasma cleaned with a PDC002 (Harric Plasma, USA) at the „high" setting (740 V, 40 mA, 29.6 W applied to the RF coil) for 120 seconds for each sample.

1.2 Synthesis of the compounds

1.2.1 Synthesis of the photo cross-linker

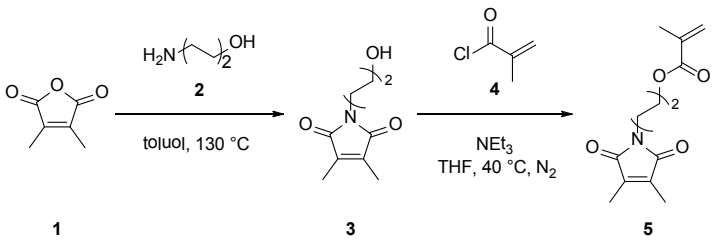

Figure 1-SI: Reaction scheme for the preparation of the cross-linker 3,4-dimethyl maleic imidobutyl methacrylate (**5**) in a two-step sequence.

Synthesis for step 1: We adopted a method by Kuckling et al.[2] Here, 1.71 g (14 mmol) aminobutanol (**2**) are dissolved in 100 ml Toluene and 1.2 g maleic acid anhydride (**1**) 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 *n*-hexane / ethyl acetate (50:50 vol-%) mixture and gives a colorless oil in 98 % yield.

^{1}H - NMR: 1.51 – 1.57 (m, 3H); 1.66 (quin, J_{HH} = 7.3 Hz, 2H); 1.95 (s, 6H); 3.52 (t, J_{HH} = 7.1 Hz, 2H); 3.65 (t, J_{HH} = 6.5 Hz, 2H).

^{13}C - NMR: 8.45 (2 CH_3); 24.99 (CH_2); 29.55 (CH_2); 37.44 (CH_2); 61.90 (CH_2); 136.93 (2 C); 172.19 (2 C).

Synthesis for step 2: We adopted a method by Abd-El-Aziz et al.[3] Here, 2 g (10.1 mmol) maleic imide **3** are dried in vacuum and then set under a nitrogen atmosphere. 117 ml dry THF are added and the flask is cooled with ice. 1.63 g (15.6 mmol) methacryloyl chloride (**4**) are dissolved in 3 ml dry THF before they are added to the mixture. After 1.6 g (15.8 mmol) dry triethylamine are added, the mixture becomes gloomy and the ice is removed. The reaction is carried out for 2.5 h at 40 °C and aborted by pouring the reaction into water. The water is extracted three times with diethyl ether. 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 *n*-hexane / ethyl acetate / triethylamine (74.9:24.9:0.2 Vol-%) mixture to give a colorless oil in 78 % yield.

^{1}H - NMR: 1.67 – 1.69 (m, 4H); 1.94 (s, 3H); 3.53 (t, J_{HH} = 6.6 Hz, 2H); 4.16 (t, J_{HH} = 6.0 Hz, 2H); 5.55 (s, 1H); 6.09 (s, 1H).

^{13}C - NMR: 8.51 (2 CH_3); 18.15 (CH_3); 25.24 (CH_2); 25.90 (CH_2); 37.35 (CH_2); 63.92 (CH_2); 125.25 (CH_2); 136.24 (C); 137.01 (2 C); 167.26 (C); 172.10 (2 C).

1.2.2 Synthesis of the PEG-Br macroinitiator

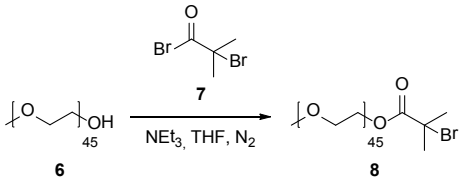

Figure 2-SI: Reaction scheme for the preparation of the PEG_{45}-Br macroinitiator (**8**).

We adopted a method by Armes et al.[4] Here, 5.00 g (2.5 mmol) MeO-PEG_{45}-OH (**6**) are dried in a flask at vacuum and 60 °C for 1 h. The flask is flushed with nitrogen before 45 ml dry THF are added. 1.12 g (4.9 mmol) 2-bromoisobutyric acid bromide (**7**) is dissolved in 3 ml dry THF before added to the solution. The flask is now cooled with ice and 0.74 g (4 mmol) dry triethylamine are added. The gloomy mixture is stirred for 4 d at room temperature. The final

macro initiator is precipitated in CO_2-cooled ether and three times recrystallized 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).

1.2.3 Synthesis of the polyethyleneglycol$_{45}$-*block*-poly(diethylaminoethylmethacrylate-*stat*-3,4-dimethylmaleinimidobutylmethacrylate)$_{100}$ (PEG$_{45}$-*b*-P(DEAMA-*s*-DMIBM)$_{100}$)

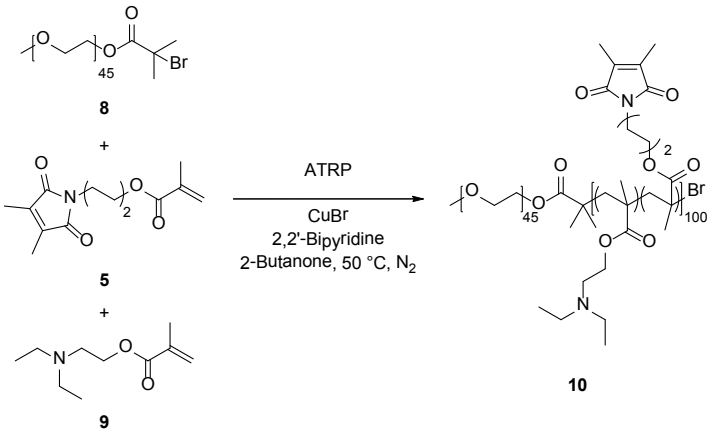

Figure 3-SI: Reaction scheme for the preparation of the final block copolymers PEG$_{45}$-*b*-P(DEAMA-*s*-DMIBM)$_{100}$ **10**.

We adopted a method by Weaver et al.[5] Here, PEG$_{45}$-Br (220 mg; 0.1 mmol) **8** and 2,2'-bipyridine (32 mg; 0.2 mmol) are mixed in a flask and dried for 5 min in vacuum and flushed with nitrogen. Then CuBr (17 mg; 0.1 mmol) are added and another 30 min dried in vacuum and again flushed with nitrogen. In an additional flask diethylaminoethyl methacrylate (1.44 g; 7.77 mmol) (**9**) and the cross-linker monomer (0.42 g; 1.58 mmol) **5** are dried 30 min in vacuum and also flushed with nitrogen afterwards. These monomers are then solved in 3 ml 2-butanone, the resulting solution degassed and added to the solids afterwards. The mixture is stirred for 17 h at 50 °C. To abort the reaction, the mixture 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 %

1.3 Vesicle Formation

Vesicle formation. At first, the polymer was dissolved (1 mg/ml) in previously deionized acidified water (pH 2) and the solution was filtrated through a 0,2 µm Nylon filter (Carl Roth, Germany). Vesicle formation was induced by adding a solution of sodium hydroxide (pH 13) until pH 9 was reached and the solution was stirred for three days.

1.4 Fluorescence labelling of the films

In order to find the best incubation time of the polymersome solution with the plasma-cleaned glass slide, various times were tested (15 s, 30 s, 45 s, 60 s, 90 s). After each test, the resulting films were incubated with Atto 647-N DOPE solution for staining reasons (see Materials and Methods section of the main paper).

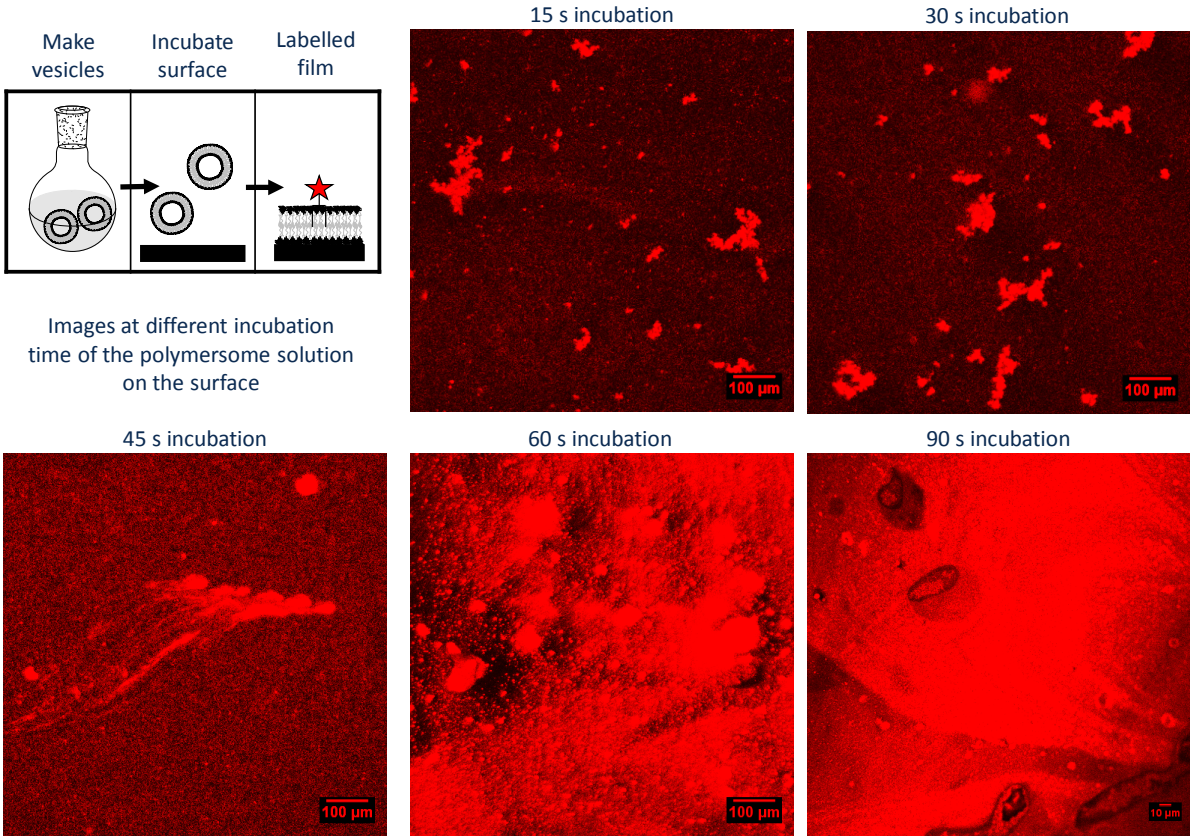

Figure 4-SI: Growth of the polymer bilyer on a plasma-cleaned glass surface after different incubation times with the polymersome solution. Since the films were continuous over a large range, an incubation time of 90 s was chosen.

2 References

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