Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting Information

A facile and fast method for the functionalization of polymersomes by photoinduced cycloaddition chemistry

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Materials and Methods

Horseradish peroxidase (type I) was from Sigma. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid ammonium Salt (ABTS), terephthalaldehydic acid, benzenesulfonyl hydrazide, aniline, *N*-hydroxysuccinimide, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl were from TCI. Methacrylate-terminated ABA block copolymer (MA-ABA) was bought from Polymer Source and used without further purificiation. The length of the blocks were determined (^{1}H-NMR) to be $A_{12}B_{80}A_{12}$. All other chemicals were purchased from Sigma.

Tetrazole **1** was prepared as detailed in literature.[1,2] This involved the formation of the diazoniumsalt of aniline by adding 2.0 ml of an aqueous $NaNO_2$ solution (2.5 M) slowly to a cooled mixture of 1.5 ml HCl and aniline (0.47 g, 5 mmole) in 8.0 ml of ethanol-water (1:1). Subsequently adding the yellow solution to 30 ml of the phenylsulfonylhydrazone in pyridine, precooled to -10°C in an ice-salt bath, gave a deep red solution. After addition the solution was extracted with 150 ml of EtOAc and 50 ml of 3M HCl was added to precipitate the product. The phenylsulfonylhydrazone was prepared by dissolving 0.75 g (5 mmole) of 4-formylbenzoic acid and benzenesulfonylhydrazide (0.90 g 26 mmole) in 50 ml EtOH, followed by the addition of water. The off-white precipitate that formed after ~ 2 minutes was filtered off, yielding the sulfonylhydrazone and was used directly.

The pinkish tetrazole was purified by recrystalization from hot ethanol. Yield: 0.72 g (55 %). ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 8.32 (d, 2H), 8.18 (m, 4H), 7.72 (m, 2H), 7.65 (m, 1H). ^{13}C-NMR (75 MHz, DMSO-d_6, δ): 166.6, 163.7, 136.1, 132.7, 130.4, 130.2, 130.1, 126.8, 120.0

UV-vis was performed on a Perkin-Elmer lambda 35 UV/Vis spectrometer. Fluorescence measurements were performed on a Fluorolog-3 spectrofluorometer from Horiba Jobin Yvon or on a Tecan infinite M200 pro plate reader using 96-well plates. Dynamic Light Scattering (DLS) was performed on a Malvern zetasizer. NMR-spectra were acquired at a 300 MHz Bruker Avance Ultrashield. Transmission Electron Microscope (TEM) was performed with a Philips CM300 FEGTEM.

General procedures

Polymersomes formation

Polymersomes were prepared by the film rehydration method and filtering the dispersion over 0.45 µm filters and then over 0.2 µm filters. Typically 5.0 mg of polymer was used for film formation. Attempts to functionalize polymersomes from pure methacrylate-terminated polymer did not yield stable polymersomes (i.e., they did not elute from the sepharose column). Mixing 5% of the polymer with hydroxy-terminated ABA of the same length did yield stable vesicles.

Enzyme modification

For enzyme modification 1.0 mg of EDC and 1.0 mg of sulfo-NHS were dissolved in 50 µl of DMSO and added to 0.33mg·ml^{-1} of tetrazole **1**. After half an hour, the solution was added to 1.0 ml of 5 mg·ml^{-1} horseradish peroxidase (0.11 mM) in PBS (50 mM pH = 7.4 or pH = 6.0) and incubated for 2 hours. The enzyme solution was purified over a PD-10 column, eluting with PBS, pH = 6.0, and centrifuged through a 100 kDa MWCO filter to remove higher-molecular weight aggregates and the

Activities were measured by the ABTS assay by taking 5 µl of the sample solution and addition of mixture of 150 µl of freshly prepared mixture of ABTS (5.0 mg·ml^{-1}, 9.1 mM in Milli-Q) and 5 µl H_2O_2 (0.3 % in Milli-Q). The absorbance was read at 470 nm and compared to the activity of a fresh solution of 1.0 mg·ml^{-1} enzyme. Concentrations were determined by the absorbance of the heme-group at 404 nm.

Light-induced conjugation procedures involving tetrazole 1

*Conjugation of tetrazole **1** to MA-ABA*

For UV, NMR and the intial fluorescence measurements conjugation of tetrazole **1** to MA-ABA, the polymer (10.0 mg, 0.17 µmole) was dissolved together with the appropriate equivalent of tetrazole **1** in 10.0 ml EtOH (0.90 mg for 1 equivalent). The solution was transferred to quartz cuvettes (10 mm pathlength) and, under stirring, irradidated at 254 nm with a handheld UV-lamp (UVP UVGL-58 6 watt / 0.12 Amps) at a distance of 10 cm. For measurement of the stoichiometry of the reaction the measurements an appropriate volume of stock solution of the tetrazole (0.8 mg · ml^{-1}, 3.0 mM) in EtOH was diluted to 0.20 ml and added to 2.50 ml of 0.125 mg · ml^{-1} (20.8 µM) MA-ABA. Samples were taken from the reaction mixtures (200 µl) and transferred to well plates and irradiated at 10 cm distance for the indicated times, with intermittent measuring.

Conjugation of HRP-tz to polymersomes

Polymersomes in PBS (100 µl, pH= 6.0) were added to 100 µl of the HRP-tetrazole **1** conjugate. The solution was transferred to a 96-well plate and irradiated at a distance of 10 cm. After reaction the solution was applied to a Sepharose-4B column and fractions were collected

in aliquots of 200 µl in a well plate for the acquisition of their emission spectra (λ_{ex} = 370 nm). Activity was measured by the ABTS assay as described above.

Conjugation of tertbutyl acrylate to HRPtz

For conjugation of tertbutyl acrylate, a stock solution of 1M in EtOH was made and 5 µl added to a once diluted sample of HRP-tz. The sample was irradiated and purified over a Sepharose-4B column.

Figure S1. For NMR the reaction of the tetrazole with the methacrylate functionalized ABA-block copolymer was carried out at a 1:2 ratio of tetrazole to ABA, because at higher ratios the dried material was insoluble in $CDCl_3$. The figure shows the ^{1}H-NMR spectrum of the polymer before conjugation (Figure S1A &B; upper spectra) and after conjugation (Figure S1A &B; lower spectra). The polymer before conjugation shows an intense peak a 1.95 ppm which corresponds to the methyl group on the methacrylate as well as the alkene protons at 6.15 ppm and 5.62 ppm (Figure S1B; magnified aromatic region). After 20 minutes reaction time these peaks have decreased in intensity (58 % for the methyl, 52 % for the double bonds) and a new peak has appeared at 1.26 ppm which is attributed to the same methyl-group but now attached to the tetrazoline ring (Figure S1A lower graph). The tetrazole peaks appear in the aromatic region from 7.0 – 8.3 ppm (Figure S1B lower graph). With respect to the starting tetrazole (inset) these appear at lower shifts, and appear to be broadened. ^{1}H-NMR (300 MHz, CDCl3) δ = 8.2 – 8.0 (br, pyrazoline COOH-Ph), 7.9 – 7.7 (br, pyrazoline COOH-Ph), 7.7 – 7.0 (br, pyrazoline N-Ph), 6.15 (br, CH3CCH_2), 5.62 (br, CH3CCH_2), 3.8 - 3.2 (br, NCH_2CH_2OH + NCH_2CH_2H + pyrazoline CCH2C), 2.4 – 2.0 (br, NCOCH_3), 1.95 (br, CH_3CCH_2), 1.7 -1.5 (br, $C_3H_6OC_2H_4$), 1.26 (br, pyrazoline CCH_3), 0.6 – 0.4 (br, $C_3H_6OC_2H_4$), 0.3 – -0.2 (br, $(CH_3)_2$SiO).

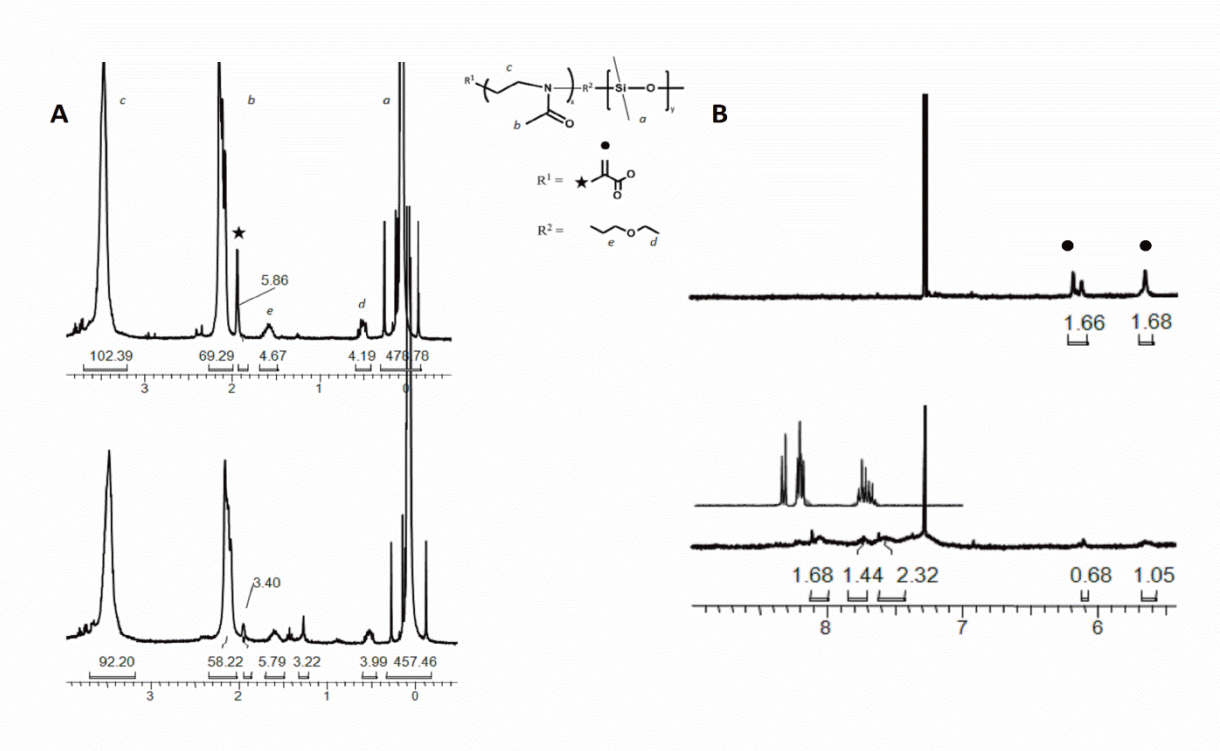


Figure S2. A convincing way to show the complete consumption of tetrazole by equimolar amounts of methacrylate-conjugated ABA was TLC (Figure S2; 25 % ethyl acetate / 75 % hexane). Figure S2A shows three spots (at 254 nm) of which the lane *x* shows the reaction mixture before reaction. A faint band at R_f = 0.4 is visible corresponding to the tetrazole (the polymer is not visible because of its low extinction coefficient). Both spot *y* and *z* show the reaction mixture after the reaction, showing intense fluorescence from the tetrazole-ABA conjugate at the base when irradiated at 365 nm (Figure S2B). Cutting up the plates and immersing them in 1M tert-butyl acrylate dissolved in EtOH, followed by irradiation at 254 nm for 5s, revealed the tetrazole, which is depicted in Figure S2C (lane *x*). As is clear from figure S2D no remaining tetrazole was observed (lane *z*).

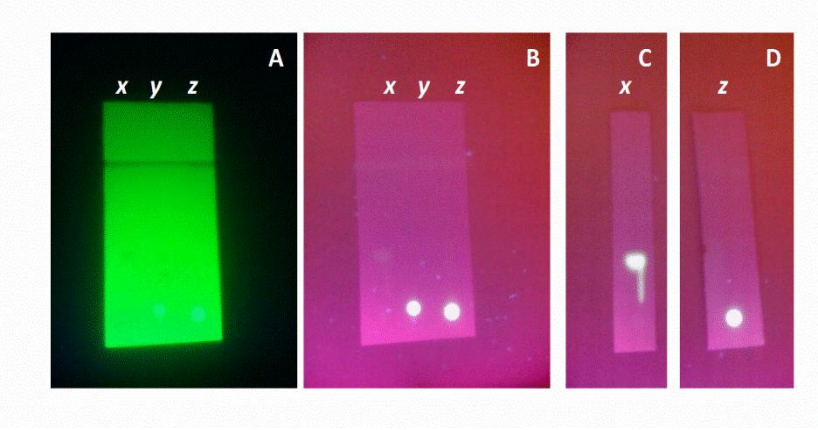


Figure S3. Formation of product from tetrazole **1** and methacrylate-terminated ABA polymer by irradiating a solution in ethanol at 254 nm. A: Product formation by measuring the increase in UV-absorbance at λ = 370 nm. The increase was measured at different ratios of double bond to tetrazole (indicated in the figure), showing that the rate is independent of the concentration of the tetrazole. B: product formation measured by the fluorescence of the pyrazoline moiety at λ_{em} = 370 nm and λ_{em} = 482 nm, measured in an excess of double-bond (squares) or excess tetrazole (circles). The dashed line is the average of the UV traces, indicating that the fluorescence increase occurs simultaneously.

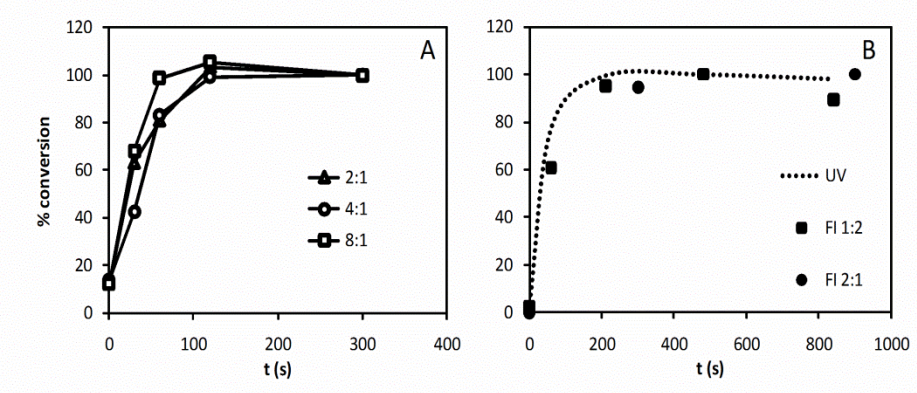


Figure S4. A. Size distribution of ABA polymersomes containing 5 % of methacrylate terminated ABA; B & C. Representative TEM-images of the same polymersomes, showing the membrane-delineated compartments. The samples were stained with 1 % phosphotungstic acid.

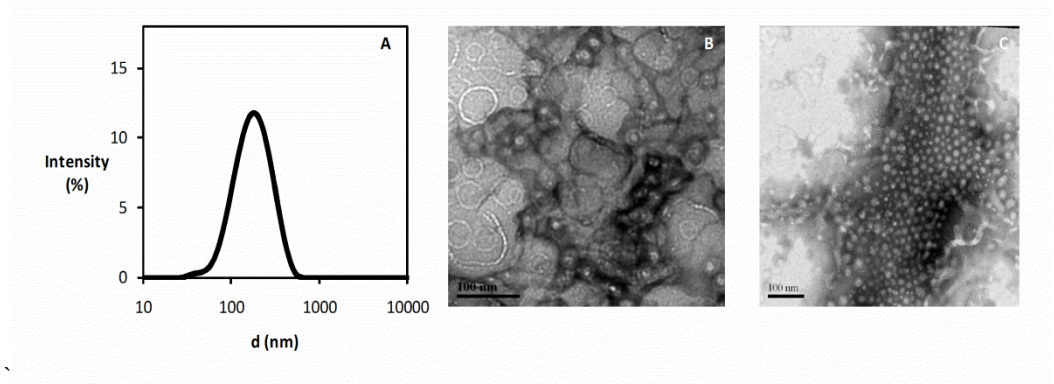


Figure S5. A. Size distribution of 5% MA-ABA polymersomes before (dots) and after (black line) reaction with HRP-tz and filtration over sepharose 4B. B. TEM image of HRP-functionalized polymersomes after purification.

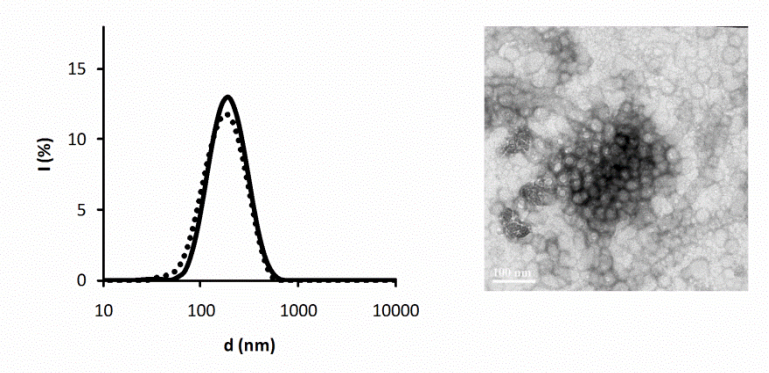
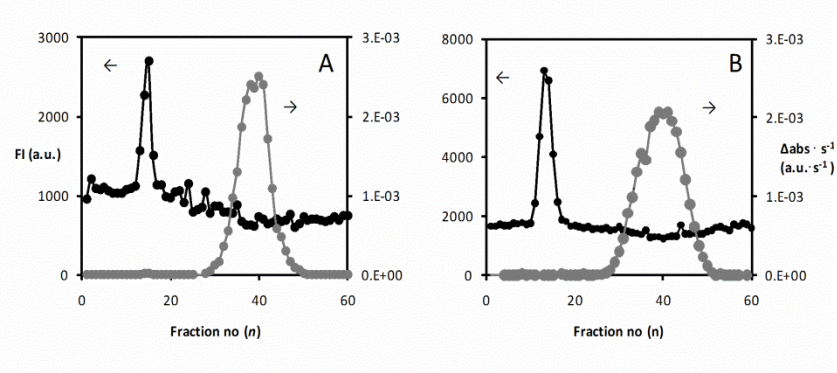


Figure S6. Full elution profile (fractions 1 – 60) of 5% ABA MA-polymersomes incubated with HRP-tz. The black circles indicate the fluorescence intensity of the fractions and the grey circles indicate the HRP activity. A. Elution profile of tetrazole-HRP where tetrazole was coupled at pH = 7.4 to HRP. Apart from a small peak of activity coinciding with the fluorescent fractions a large fraction of unreacted HRP elutes between fractions 30 and 50. B: Elution profile of tetrazole HRP where tetrazole was coupled at pH = 6.0.



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