Electronic Supplementary Material (ESI) for Polymer Chemistry.

Micelles from self-assembled Double-Hydrophilic PHEMA-Glycopolymer-Diblock Copolymers as multivalent Scaffolds for Lectin Binding

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Materials

All chemicals were purchased from Sigma Aldrich and used without further purifications unless stated otherwise.

Monomer Conversion

The conversion of HEMA to PHEMA was analysed by ^{1}H-NMR. We compared the integrals of the methacrylate protons δ=6.16 ppm to the protons of the ethylic residue δ=4.10 ppm. We calculated a monomer conversion of 90 %, which results in a theoretical DP of 45. For estimating the DP of the PGlcNAcEMA-block we analysed the mixture after polymerization and compared the integrals of the methacrylate protons at δ=6.00 ppm to the proton at the anomeric C-atom of GlcNAc (δ=4.40 ppm). For the PGlcNAcEMA-block a DP of approx. 10 is calculated. The respective ^{1}H-NMR spectra are shown in **Fig. S1** and **Fig. S2**.

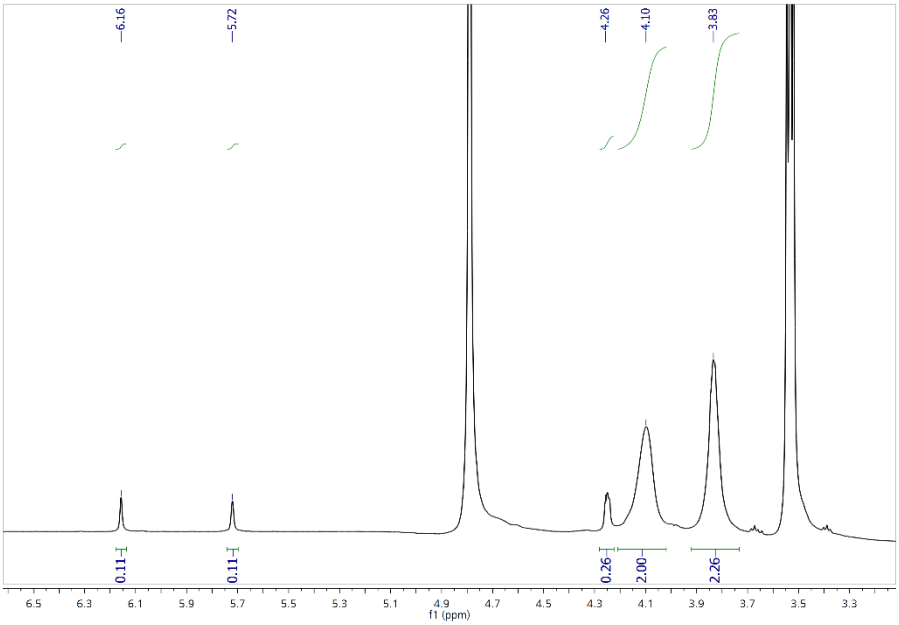

Figure S1: ^{1}H-NMR after polymerisation of PHEMA-block. ^{1}H-NMR (500 MHz, D_2O): δ= 6.16 (0.11 H, s, CH2=C- HEMA monomer), 5.72 (0.11 H, s, CH2=C- HEMA monomer), 4.26 (0.26H, t, CH2-CH2 HEMA monomer), 4.10 (2 H CH2-CH2 polymer), 3.83 (2H, t, CH2-CH2 polymer/monomer)

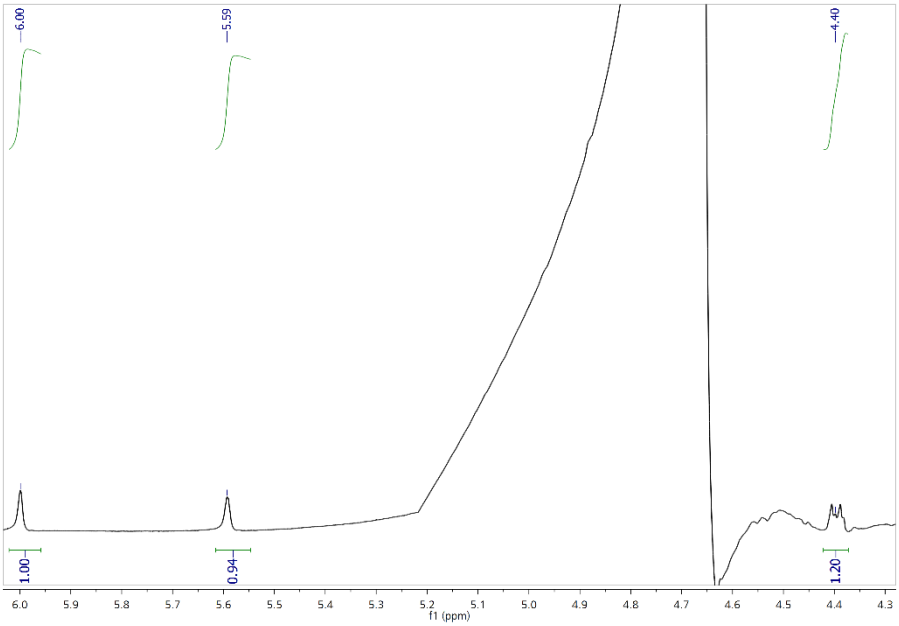

Figure S2: ^{1}H-NMR after polymerisation of PGEMA-block. ^{1}H-NMR (500 MHz, D_2O): δ= 6.00 (1 H, s, CH2=C- GlcNAcEMA monomer), 5.59 (1 H, s, CH2=C- GlcNAcEMA monomer), 4.4 (1.2 H, d, GlcNAc H-1 polymer/monomer)

Characterisation of PHEMA-b-PGlcNAcEMA diblock glycopolymer by GPC

Fig. S3 shows the RI traces for PHEMA and the diblock glycopolymer. After purification of PHEMA the Mn was determined by GPC giving an apparent molecular weight average of 6254 gmol^{-1} with polydispersity M_W/M_n of 1.7. The apparent molecular weight of the diblock glycopolymer was amounted to 7020 g mol^{-1} with polydispersity of 1.4. For GPC calibration dimethylformamide was used as solvent and polymethylmethacrylate as reference standard. Due to the poor comparability of polymethylmethacrylate to our polymers GPC can only be considered as relative method yielding only apparent molecular weights. Therefore we performed additional MALDI-TOF MS analysis.

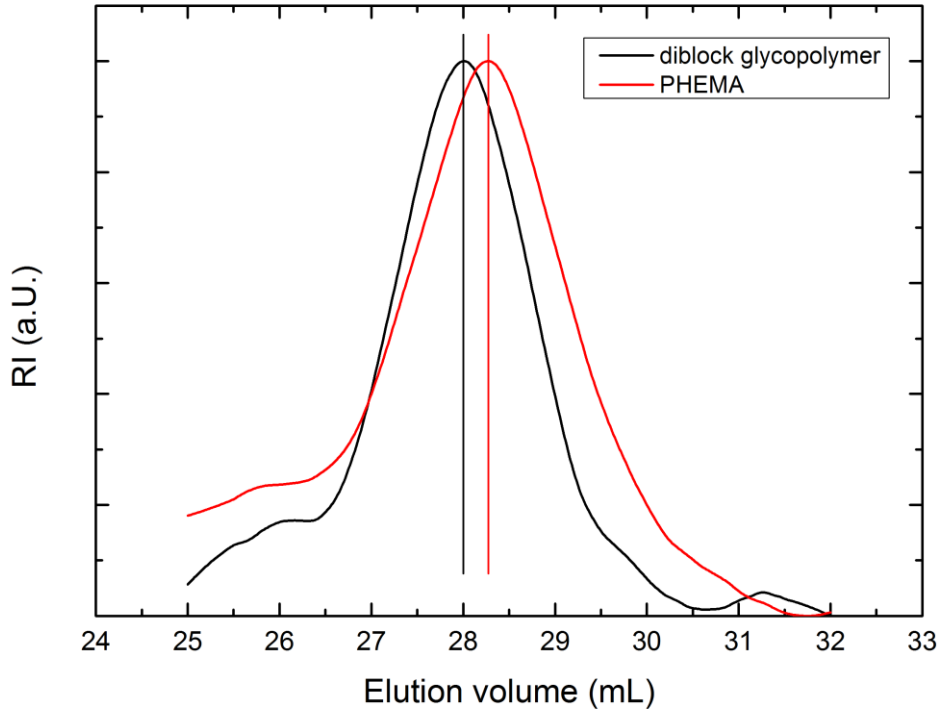

Figure S3: GPC analysis of PHEMA and PHEMA-b-PGlcNAcEMA glycopolymers.

Characterisation of PHEMA-b-PGlcNAcEMA diblock glycopolymer by MALDI-ToF-Analysis

Fig. S4 shows the mass spectrum of the PHEMA-b-PGlcNAcEMA diblock glycopolymer in the mass range of 3000-12000 Da. A total of seven molecular weight distribution series, each with a repeating unit of 130 Da corresponding to the HEMA block, were identified via Polytools™ and Polymerix ™. The criteria applied for the assignment of the oligomer masses to an appropriate copolymer composition are given in **Table S1**. The maximum and minimum values assumed for the HEMA and GlcNAcEMA portions were calculated on the basis of molecular weight average, polydispersity (PD) and degrees of polymerisation (DP) as obtained by GPC. The results for the molecular weight averages, Mn ((NiMi)/Ni) and Mw ((NiMi2)/NiMi Ni), polydispersity and mixture deconvolution as calculated with the Polymerix software tool (Sierra Analytics, Modesto, CA 95356 USA) are listed in **Table S2**. An average molecular weight Mn of 6545.561 Da and a low PD of 1.095, indicating a uniform size of the polymers, is determined. **Fig. S5** shows the assignment to the main series of the PHEMA-b-PGlcNAcEMA diblock glycopolymer, with S1 being the MNa^+, S5 the $MK^+$and S2 the MH^+ species. The bulk of the PHEMA-b-PGlcNAcEMA shows an average degree of polymerisation (DP) of approx. 39 for the HEMA unit and a DP of 4 for the GlcNAcEMA unit. PHEMA-b-PGlcNAcEMA series S1, S2, and S5 represent 36% of the spectrum while the PHEMA-b-PGlcNAcEMA series with a DP = 3 (S7) and a DP = 5 (S6) for GlcNAcEMA are of rather low intensity only; for DP values higher than 5 no reasonable results were obtained. The signals of the series S3 and S4 (both MNa^+ species) fit to

molecular weight distributions featuring the loss of an ethylenoxide (S3) and an acetylglucosamine unit (S4) respectively. The anticipated isotope pattern of a selected MNa^+-species PHEMA-b-PGlcNAcEMA oligomer, $C_6H_{11}O_2$ $[C_6H_{10}O_3]_{31}[C_{14}H_{23}NO_7]_4$ Br, is shown in **Fig. S6**. Both theoretical and determined m/z values as well as the resulting measurement error in mDa for this and two further exemplary oligomers, $C_6H_{11}O_2$ $[C_6H_{10}O_3]_{42}[C_{14}H_{23}NO_7]_4$ Br and $C_6H_{11}O_2$ $[C_6H_{10}O_3]_{52}[C_{14}H_{23}NO_7]_4$ Br, are shown in **Table S3**.

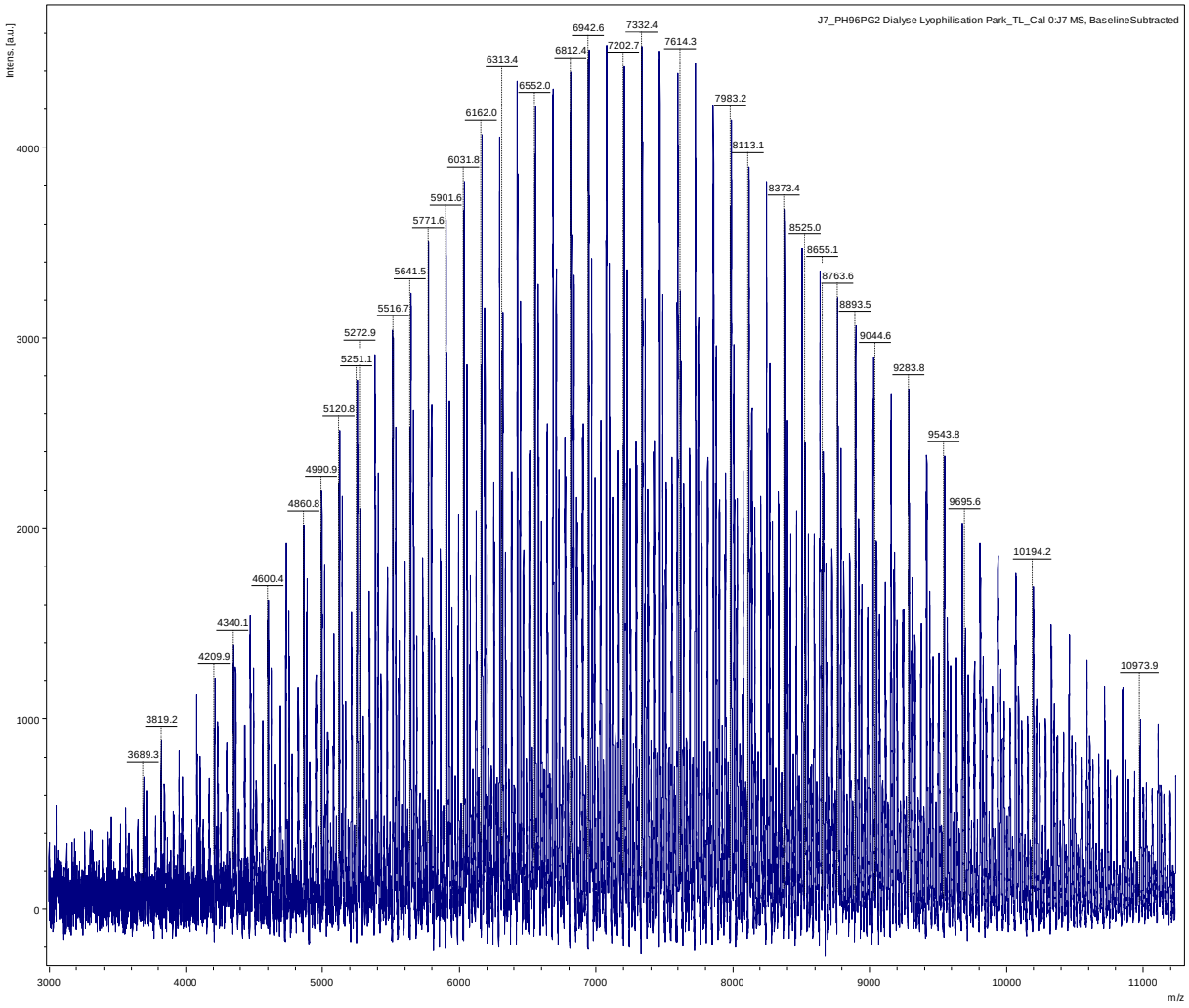

Figure S4: MALDI-ToF mass spectrum of PHEMA-b-PGlcNAcEMA diblock glycopolymer.

Table S1: Criteria applied to calculate the assignment of spectral features to individual series components

	Endgroup A	Repeat unit 1	Min/Max	Repeat unit 2	Min/Max	Endgroup B	Adduct	Charge	Loss of residues
S1	EMA	HEMA	8/75	GlcNAcEMA	4/4	Brom	Na	1	–
S2	EMA	HEMA	8/75	GlcNAcEMA	4/4	Brom	H	1	–
S3	EMA	HEMA	8/75	GlcNAcEMA	4/4	Brom	Na	1	Ethylenoxide
S4	EMA	HEMA	8/75	GlcNAcEMA	4/4	Brom	Na	1	Acetylglucosamine
S5	EMA	HEMA	8/75	GlcNAcEMA	4/4	Brom	K	1	–
S6	EMA	HEMA	8/75	GlcNAcEMA	5/5	Brom	Na	1	–
S7	EMA	HEMA	8/75	GlcNAcEMA	3/3	Brom	Na	1	–

Table S2: Copolymer Results Summary for PHEMA-b-PGlcNAcEMA diblock glycopolymer (Polymerix, Sierra Analytics)

Series / Label	**Mn**	**Mw**	**Mz**	**PD**	**% Series**	**% Spectrum**	
Total/Average	**6545,561**	**7164,613**	**7717,092**	**1,095**	**100**	**73,9**	
S1+ S3 + S4 + S6 + S7	**6567,225**	**7181,559**	**7730,428**	**1,094**	**70,16**	**51,85**	
S2	**6439,914**	**7101,378**	**7692,506**	**1,103**	**12,79**	**9,45**	
S5	**6535,647**	**7142,314**	**7680,666**	**1,093**	**17,06**	**12,61**	
S1+ S2+ S5	**6574,691**	**7188,653**	**7730,972**	**1,093**	**100**	**35,59**	
S3	**6619,896**	**7251,958**	**7808,854**	**1,095**	**100**	**11,92**	
S4	**6917,844**	**7508,295**	**8027,492**	**1,085**	**100**	**10,06**	
Series	**Alpha End Group**	**Repeat A**	**Repeat B**	**Omega End Group**	**Charge**	**Adduct**	
S1+ S3 + S4 + S6 + S7	**$C_6H_{11}O_2$**	**$C_6H_{10}O_3$**	**$C_{14}H_{23}NO_7$**	**Br**	**1**	**Na**	**$C_6H_{11}O_2$ [$C_6H_{10}O_3$]a[$C_{14}H_{23}NO_7$]b Br + Na $C_6H_{11}O_2$ [$C_6H_{10}O_3$]a[$C_{14}H_{23}NO_7$]b Br + Na, loss of EO $C_6H_{11}O_2$ [$C_6H_{10}O_3$]a[$C_{14}H_{23}NO_7$]b Br + Na, loss of N-Acetylglucosamin**
S2	**$C_6H_{11}O_2$**	**$C_6H_{10}O_3$**	**$C_{14}H_{23}NO_7$**	**Br**	**1**	**H**	**$C_6H_{11}O_2$ [$C_6H_{10}O_3$]a[$C_{14}H_{23}NO_7$]b Br + H**
S5	**$C_6H_{11}O_2$**	**$C_6H_{10}O_3$**	**$C_{14}H_{23}NO_7$**	**Br**	**1**	**K**	**$C_6H_{11}O_2$ [$C_6H_{10}O_3$]a[$C_{14}H_{23}NO_7$]b Br + K**

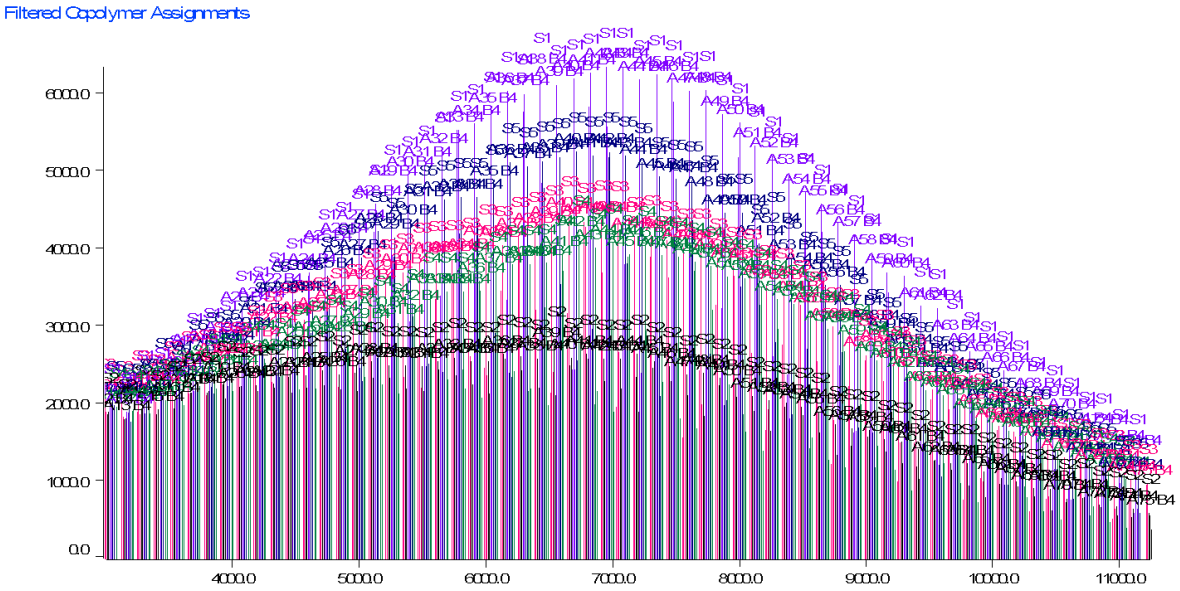

Figure S5: Assignment of PHEMA-b-PGlcNAcEMA diblock glycopolymer spectral features to the series S1-S6 specified in table S1 (Polymerix, Sierra Analytics)

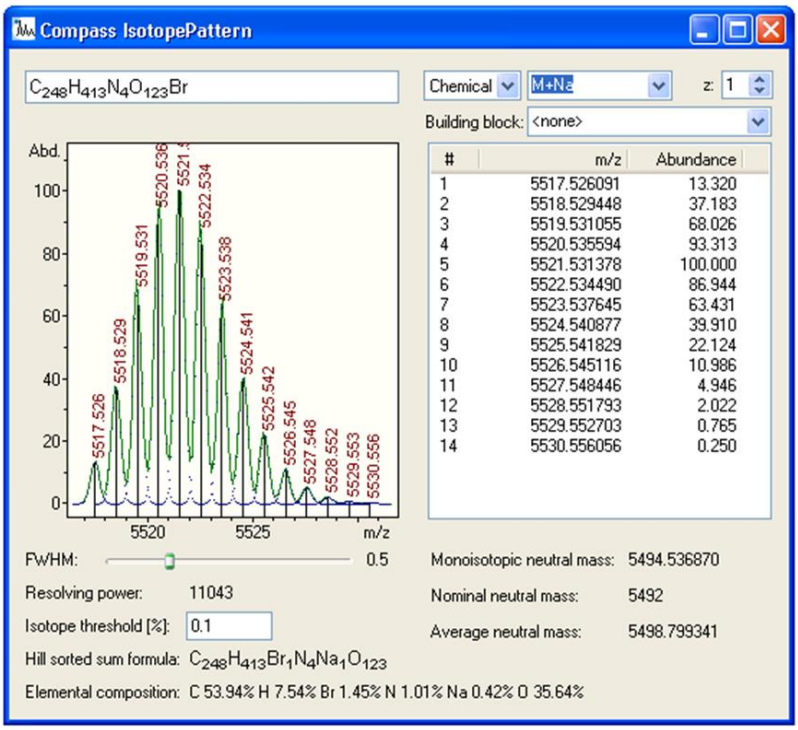

Figure S6: Isotope pattern of the PHEMA-b-PGlcNAcEMA oligomer $\mathbf{C_6H_{11}O_2\,[C_6H_{10}O_3]_{31}[C_{14}H_{23}NO_7]_4\,Br}$ (MNa^+ species)

Table S3: Copolymer Result Details for three exemplary PHEMA-b-PGlcNAcEMA oligomers (Polymerix, Sierra Analytics)

	Repeat A Count	Repeat B Count	Computed mono m/z	Found mono m/z	Error in mDa	Computed Cluster m/z	Found Cluster m/z	Error in mDa
Series Label S1	31	4	5517,526	5517,966	439,9	5521,77	5521,816	46,4
	42	4	6948,219	6948,681	462,4	6953,319	6952,918	-401,6
	52	4	8248,849	8249,256	407,4	8254,729	8253,846	-882,6
Series Label S2	31	4	5495,544	5495,735	190,6	5499,788	5500,847	1058,5
	42	4	6926,237	6926,7	462,9	6931,338	6931,899	561,4
	52	4	8226,867	8227,269	402,2	8232,747	8232,889	142,2

Characterisation of PHEMA-b-PGlcNAcEMA diblock glycopolymer by FESEM and AFM

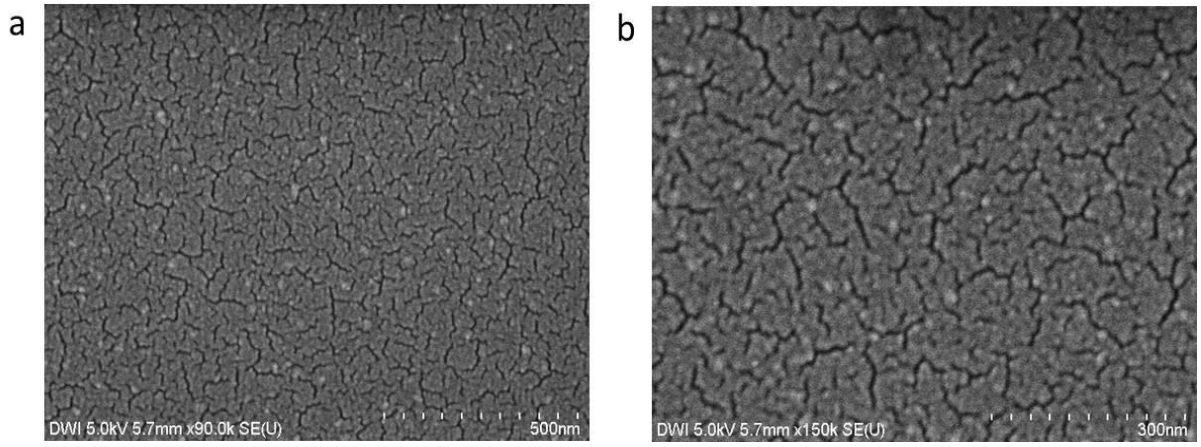

Figure S7: FESEM micrographs of PHEMA-b-PGlcNAcEMA glycopolymers.

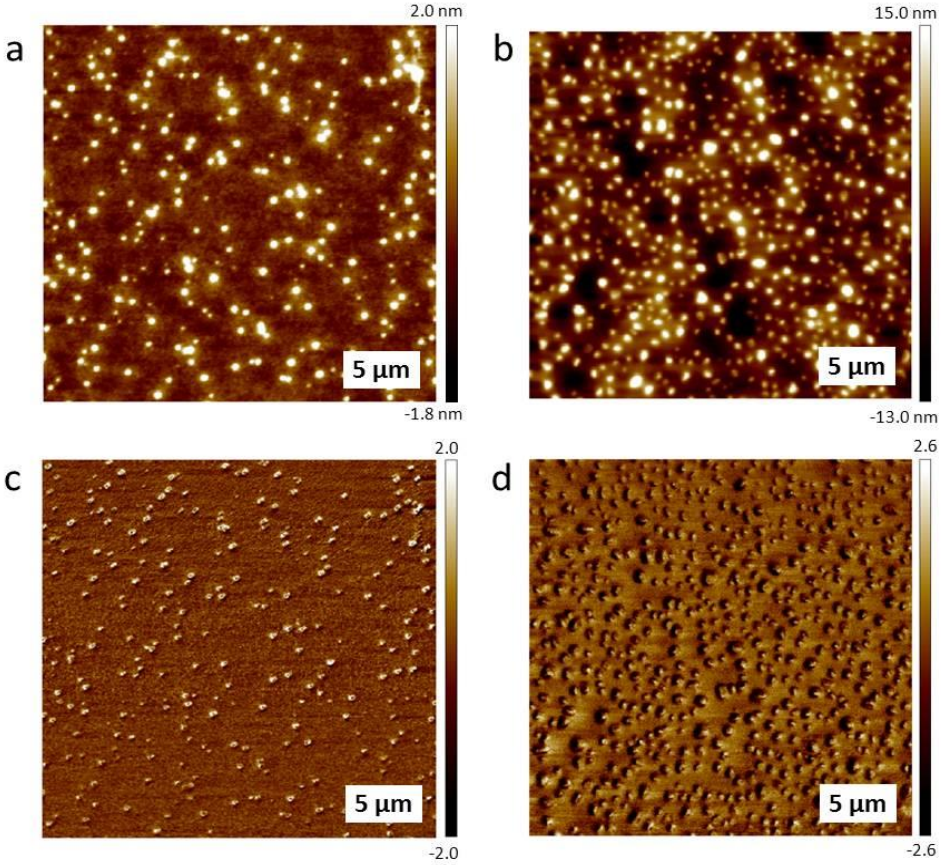

Figure S8: AFM height (top) and phase (bottom) images of PHEMA-b-PGlcNAcEMA glycopolymers before (a,c) and after (b,d) GS-II addition.

Protein treatment

Fig. S9 shows a chromatogram of the purification. The absorption at 280 nm as well as the conductivity is recorded.

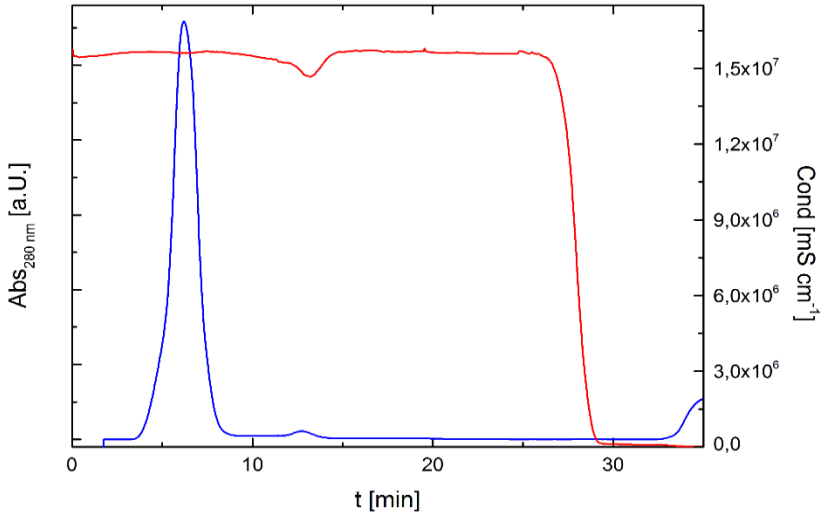

Figure S9: Chromatogram of SEC for purification of FITC-GS-II. The blue curve shows the absorption at 280 nm, the red curve monitors the conductivity. The large peak represents the FITC-GS-II conjugate followed by a small peak and a drop of conductivity caused by the free dye.

CMC determination

Fig. S10 depicts the results for some measured concentrations of block copolymer.

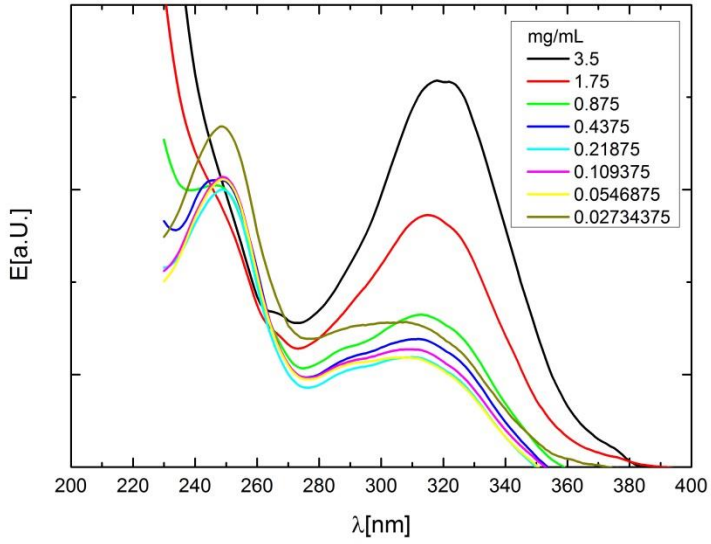

Figure S10: Absorption spectra of various diblock glycopolymer in a solution containing 5 mg mL^{-1} BZA. Note the decrease of the absorption band at 315 nm with decreasing concentrations of polymer. This indicates a hydrophobic surrounding at higher polymer concentrations due to formation of micelles. At lower concentration of polymer micelles are not formed therefore the ketonic form with its absorption band at 250 nm is more pronounced. Below concentrations of 0.4 mg mL^{-1} of polymer there are now more spectral changes observed, resulting in a CMC in this range.

Two-focus fluorescence correlation spectroscopy (2fFCS)

Fig. S11 presents the unnormalised ACFs and CCFs plotted against the lag time for GS-II in aqueous buffer solution (LB). Because a one-component model did not fit the data sufficiently well, residual FITC (not chemically attached to GS-II) was considered as second component for fitting. For better accuracy a diffusion coefficient of $D = 4.26 \cdot 10^{-6}\ \mathrm{cm^2/s}$ (measured for fluorescein in LB) was used as fixed bound. The fraction of free dye was reduced from more than 50 percent to approx. 30 percent via SEC. The diffusion coefficient of GS-II was determined to $D = (0.349 \pm 0.004) \cdot 10^{-6}\ \mathrm{cm^2/s}$, corresponding to a hydrodynamic radius of $R_h = 6.8 \pm 0.1$ nm. **Fig. S12** shows the unnormalised ACFs and CCFs plotted against the lag time for GS-II after addition of PHEMA-b-PGlcNAcEMA. The diffusion coefficient of GS-II decreased to $D = (0.154 \pm 0.002) \cdot 10^{-6}\ \mathrm{cm^2/s}$, corresponding to a hydrodynamic radius of $R_h = 15.4 \pm 0.2$ nm. An additional (significant) fraction of unbound GS-II was considered by performing a two-component fit with unbound GS-II as second component and a three-component fit with unbound GS-II and residual FITC as further components, respectively. But it was not possible to apply these fits to the data successfully. When the two-component model was used, the fraction of free dye does not change, so that it can be assumed that little or no unbound GS-II remained.
Fig. S13 presents the unnormalised ACFs and CCFs plotted against the lag time for BSA in aqueous buffer solution (LB). The data were fitted with a one-component model including triplet blinking. A corresponding triplet relaxation time of 8 µs was obtained. Furthermore the fits result in a diffusion coefficient of $D = (0.601 \pm 0.010) \cdot 10^{-6}\ \mathrm{cm^2/s}$. **Fig. S14** shows the unnormalised ACFs and CCFs plotted against the lag time for BSA after addition of PHEMA-b-PGlcNAcEMA. Again the data were fitted with a one-component model including triplet blinking. The triplet relaxation time did not change and the diffusion coefficient only displayed an insignificant decrease to $D = (0.588 \pm 0.007) \cdot 10^{-6}\ \mathrm{cm^2/s}$.

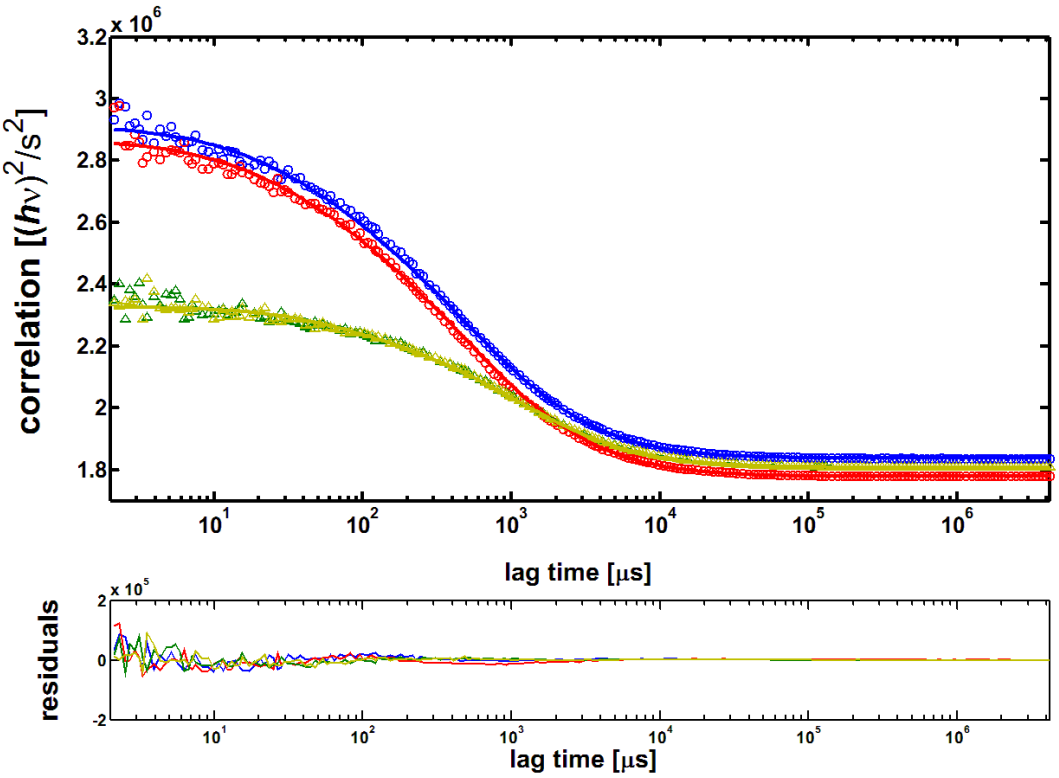

Figure S11: Autocorrelation (blue, red) and cross correlation curves (green, yellow) for GS-II diffusion in aqueous buffer solution. Solid lines are fits to the data (circles, triangles).

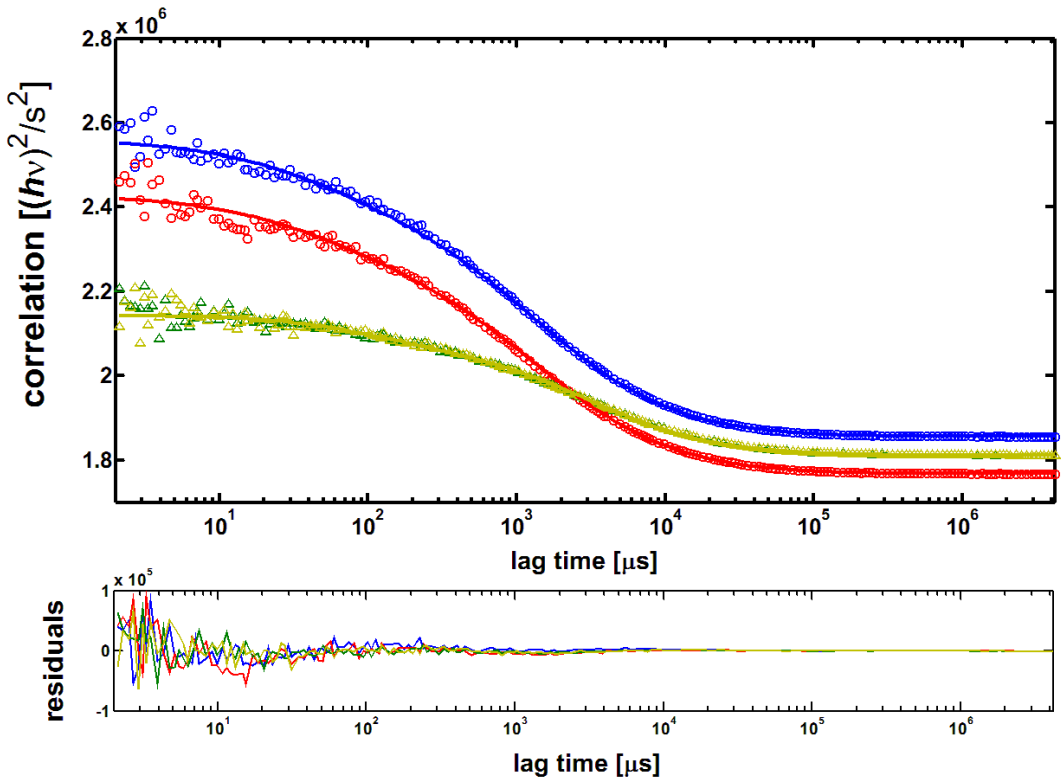

Figure S12: Autocorrelation (blue, red) and cross correlation curves (green, yellow) for GS-II diffusion after addition PHEMA-b-PGlcNAcEMA in aqueous buffer solution. Solid lines are fits to the data (circles, triangles).

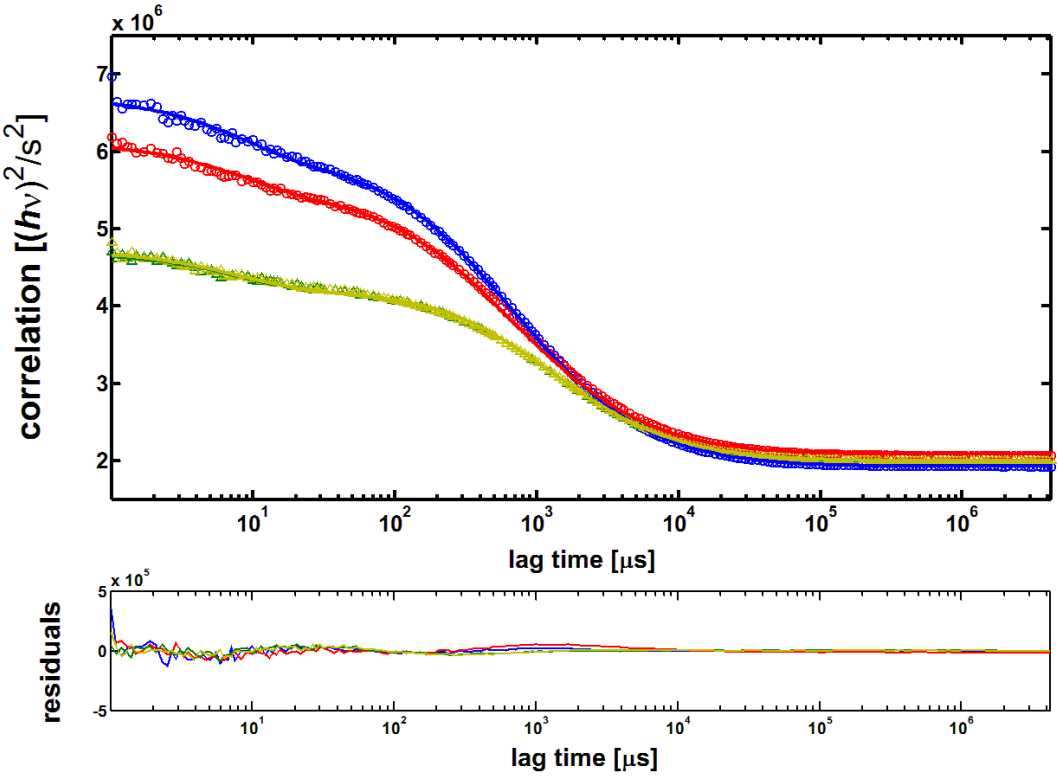

Figure S13: Autocorrelation (blue, red) and cross correlation curves (green, yellow) for BSA diffusion in aqueous buffer solution. Solid lines are fits to the data (circles, triangles).

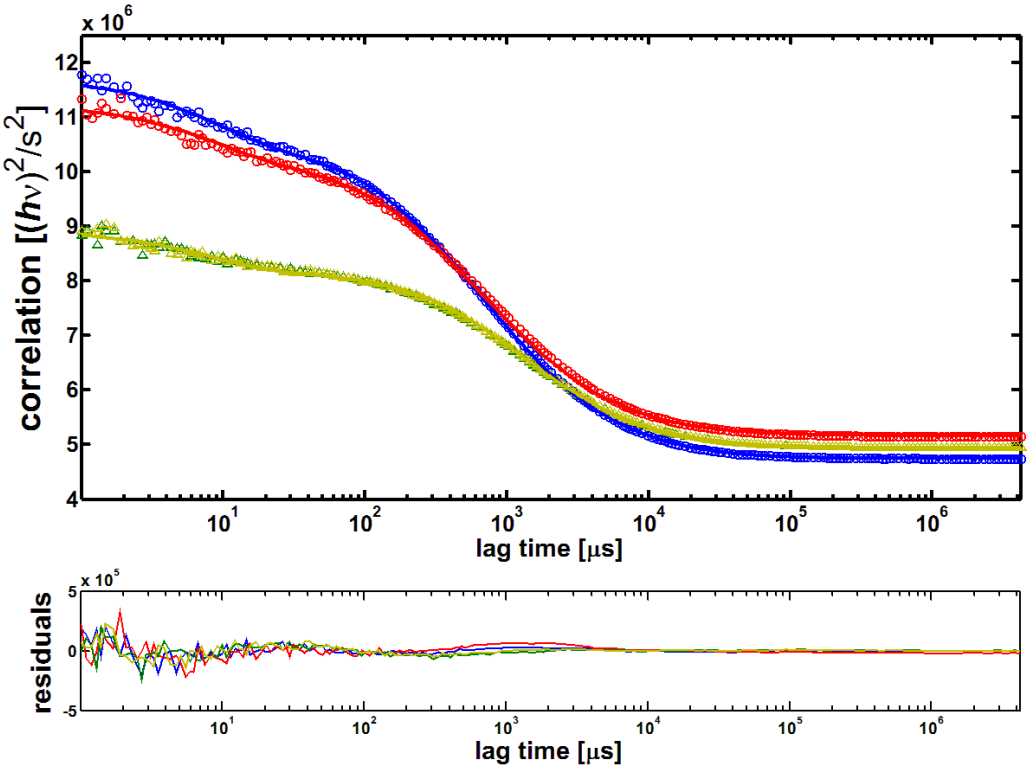

Figure S14: Autocorrelation (blue, red) and cross correlation curves (green, yellow) for BSA diffusion after addition PHEMA-b-PGlcNAcEMA in aqueous buffer solution. Solid lines are fits to the data (circles, triangles).