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Electronic Supplementary Information

Tunable nano-carriers from glycosaminoglycan block copolymers

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DOI: 10.1039/b000000x

S1. ^{1}H NMR Characterisation of the block copolymers

CS-*b*-PEG (300 MHz, D_2O, 293K) δ: 7.66 (d, J = 3.9 Hz, oxime N-C**H**), 6.96 (d, J = 5.3 Hz, oxime N-CH), 4.64-3.32 (m, H1-H6 protons of UA and GalNAc, CH_2 of PEG), 3.38 (s, CH_3O PEG). 2.07 (s, CH_3 of N-acetylglucosamine). N-CH protons are only visible in the ^{1}H NMR spectrum of the lowest molecular weight CS.

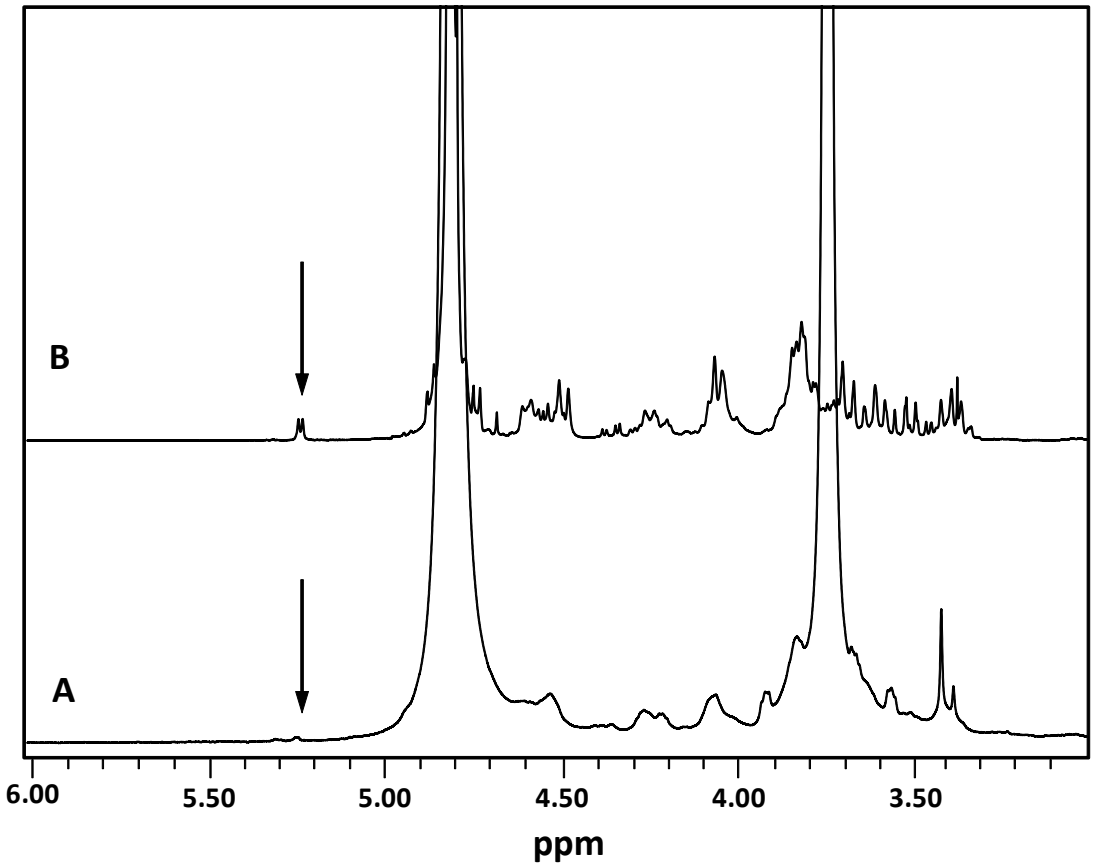

Figure S1. ^{1}H NMR (300 MHz, D_2O, 298K): (A) CS_{3k}-*b*-PEG and (B) precursor CS_{3k}.

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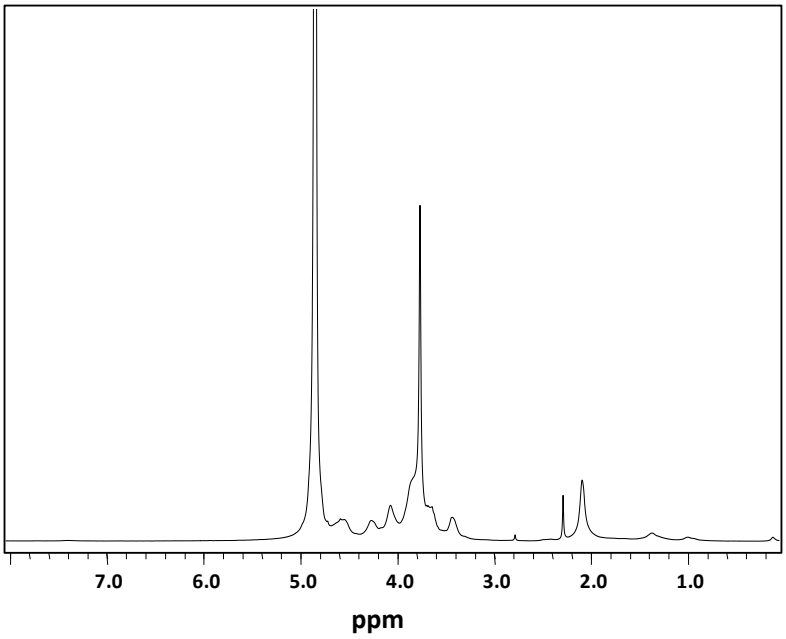

Figure S2. ^{1}H NMR (300 MHz, D_2O, 298K) of CS_{24k}-*b*-PEG.

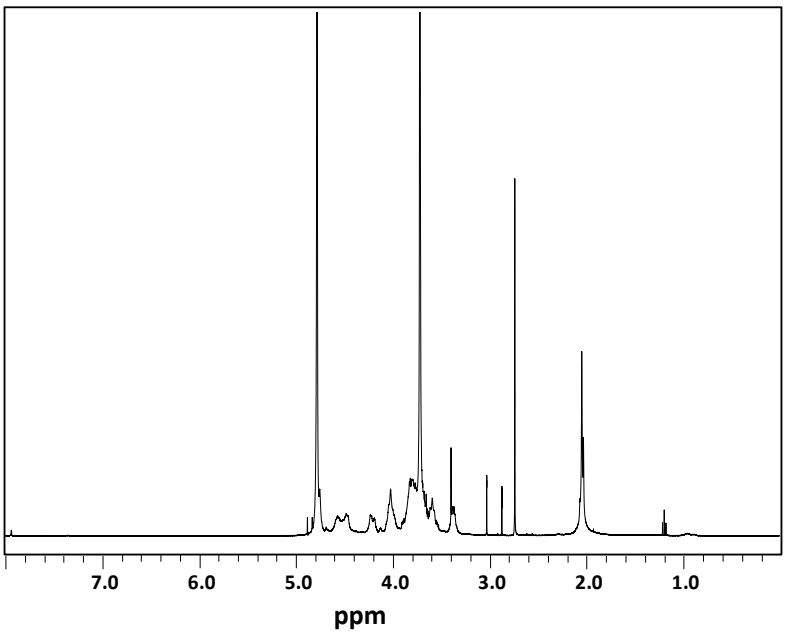

Figure S3. ^{1}H NMR (300 MHz, D_2O, 298K) of CS_{12k}-*b*-PEG

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HAS-*b*-PEG (300 MHz, D_2O, 293K) δ: 7 4.64-3.32 (m, H1-H6 protons of HAs, CH_2 of PEG), 3.38 (s, CH_3O PEG), 2.07 (s, CH_3 of N-acetylglucosamine).

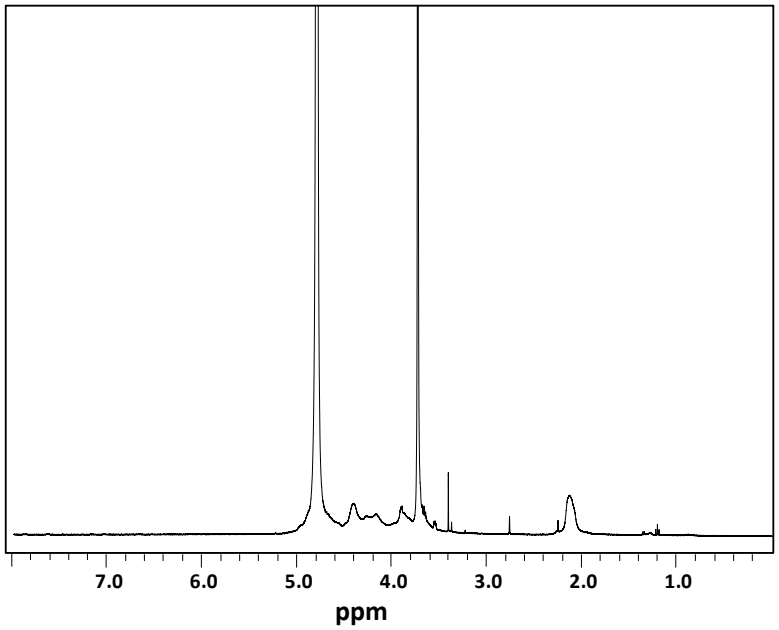

Figure S4. ^1H NMR (300 MHz, D_2O, 293K) of HAs_{36k}-*b*-PEG.

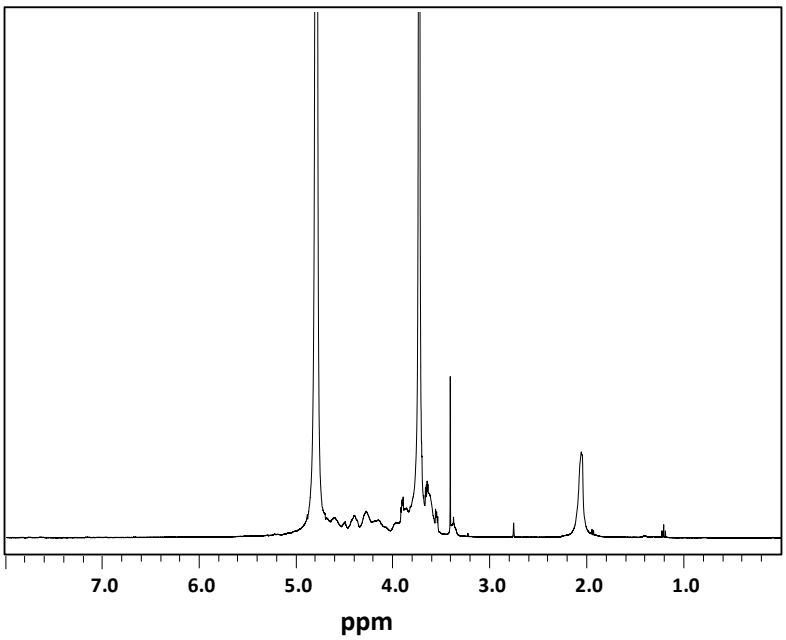

Figure S5. ^1H NMR (300 MHz, D_2O, 293K) of HAS_{16k}-*b*-PEG.

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S2. Molecular weight of the synthesised block copolymers

Briefly, we analysed each of the blocks (PEG and the respective GAG) before copolymerisation by ^{1}H NMR and GPC. The determined molecular weights were related to the ^{1}H NMR integrals. The number of the repeating units of the block copolymer was estimated from the ^{1}H NMR using the integral of the of the acetyl group (2.06 ppm) in GAG and compared it to the integral of the multiplet between 4.55 and 3.20 ppm that corresponds to the H1-H6 of the GAG and the CH2O and MeO groups of PEG.

S3. GPC eluograms of the block copolymers

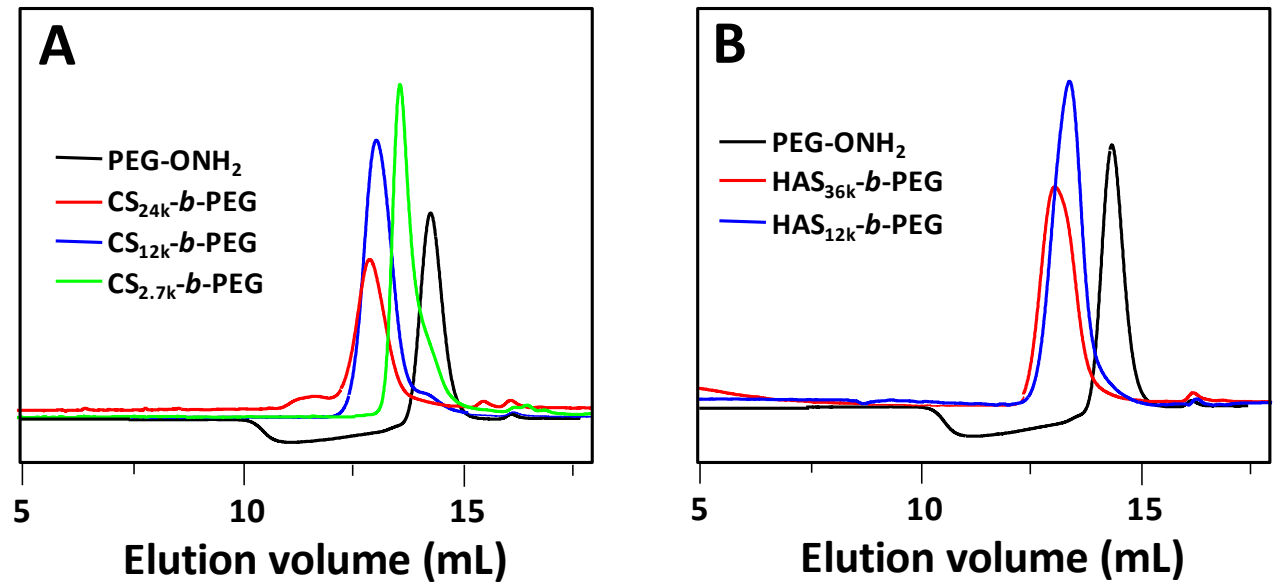

Figure S6. Comparative eluograms of the PEG-ONH$_2$ and CS-*b*-PEG (A) and HAS-*b*-PEG.

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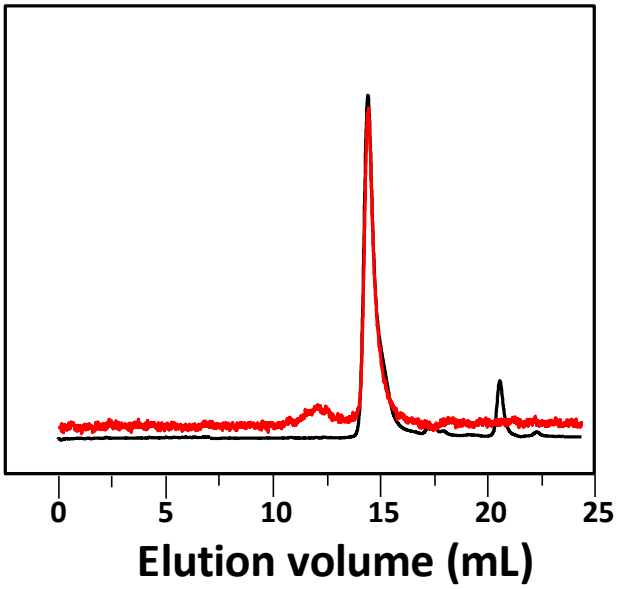

Figure S7. CS_{3k}-*b*-PEG eluogram (0.1 M NaN_3, 0.01 M NaH_2PO_4). Black line represents refractive index, and red line light scattering signals.

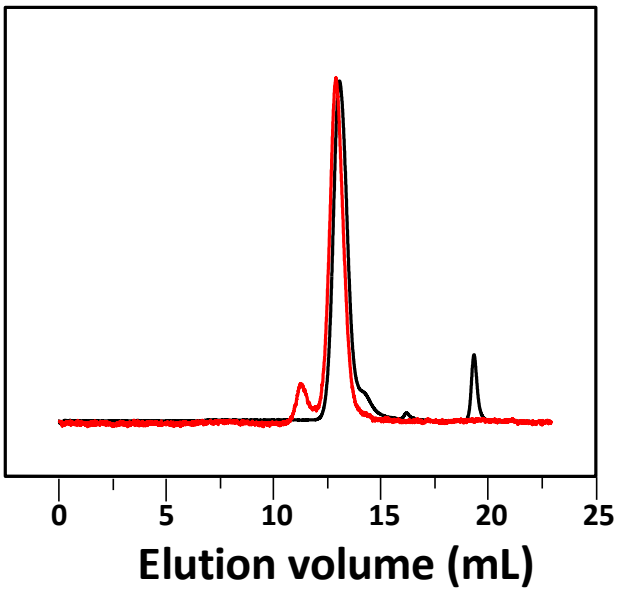

Figure S8. CS_{14k}-*b*-PEG eluogram (0.1 M NaN_3, 0.01 M NaH_2PO_4). Black line represents refractive index, and red line light scattering signals.

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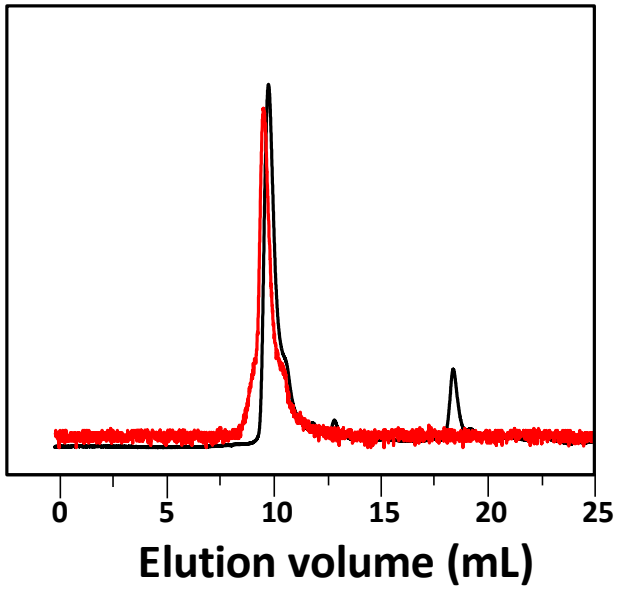

Figure S9. CS_{24k}-*b*-PEG eluogram (0.1 M NaN_3, 0.01 M NaH_2PO_4). Black line represents refractive index, and red line light scattering signal.

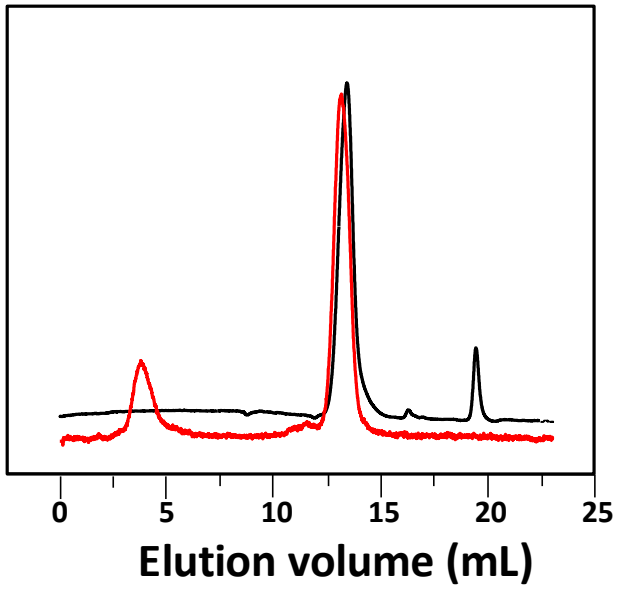

Figure S10. HAS_{12k}-*b*-PEG eluogram (0.1 M NaN_3, 0.01 M, NaH_2PO4). Black line represents refractive index, and red line light scattering signal.

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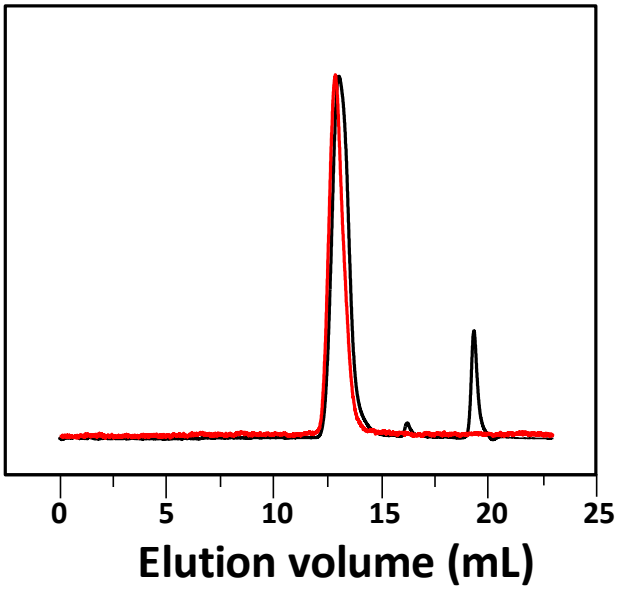

Figure S11. HAS_{36k}-*b*-PEG apparent molecular weight distribution (0.1 M NaN_3, 0.01 M, NaH_2PO4). Black line represents refractive index, and red line light scattering signal.

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S4. Optimisation of the complexation with PLL

Table S1. Characteristics of the GAG-*b*-PEG complexes with PLL. The best results (in bold) are discussed in the manuscript.

GAG	10^3Mn, g/mol (M_w/M_n)	Concentration GAG-*b*-PEG/PLL [g/L]	Used volumes GAG *b*-PEG /PLL [µL]	Degree of sulfation (Elemental analysis)	R_h (nm)	PDI	Zeta (mV)
Chondroitin sulfate (CS)	24 (1.33)	0.7/1.2	**250/750**	0.9	**94±8**	**0.08±0.02**	**0.3**
			300/600		79	0.08	-
			600/600		46	0.09	-
			900/600		68	0.09	-5.0
			1000/750		80	0.048	-2.6
	14 (1.39)	1.2/0.7	250/750	0.9	41	0.08	-
			300/600		**40±1**	**0.05±0.04**	**-1.2**
			600/600		38	0.17	
			900/600		33	0.20	-3.9
			600/300		42	0.17	-12
	2.7 (1.20)	0.8/1.2	**250/750**	0.7	129	0.56	-
			300/600		**208**	**0.49**	**0.4**
			600/600		252	0.53	-
Hyaluronan sulfate (HAS)	36 (1.50)	0.7/1.2	250/750	3	50	0.24	
			300/600		32	0.22	
			600/600		**25±1**	**0.16±0.05**	**0.4**
			900/600		20	0.19	-12.7
			600/300		23	0.36	-14.4
	12 (1.8)	0.72/1.2	250/750	1.7	28	0.16	-
			300/600		26	0.21	-
			600/600		**22±1**	**0.09±0.02**	**-0.11**
			900/600		20	0.16	-2.0
			600/300		22	0.20	-2.0
	19 (1.45)	0.8/1.5	250/750	3	40	0.31	-
			300/600		26	0.23	-
			600/600		**36±3**	**0.16±0.00**	**0.6**
	13 (2.6)	1.0/1.0	**250/750**	1	35±4	0.10±0.08	-
			300/600		53	0.14	-
			600/600		31	0.12	-0.5

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S5. Additional SEM, TEM and STEM images

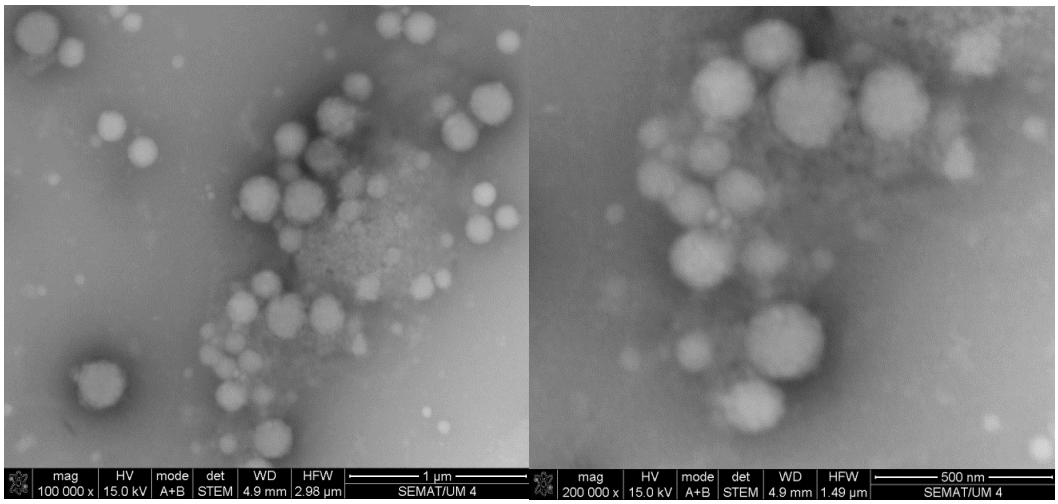

Figure S12. STEM images of IPECs from CS_{24k}-*b*-PEG and PLL.

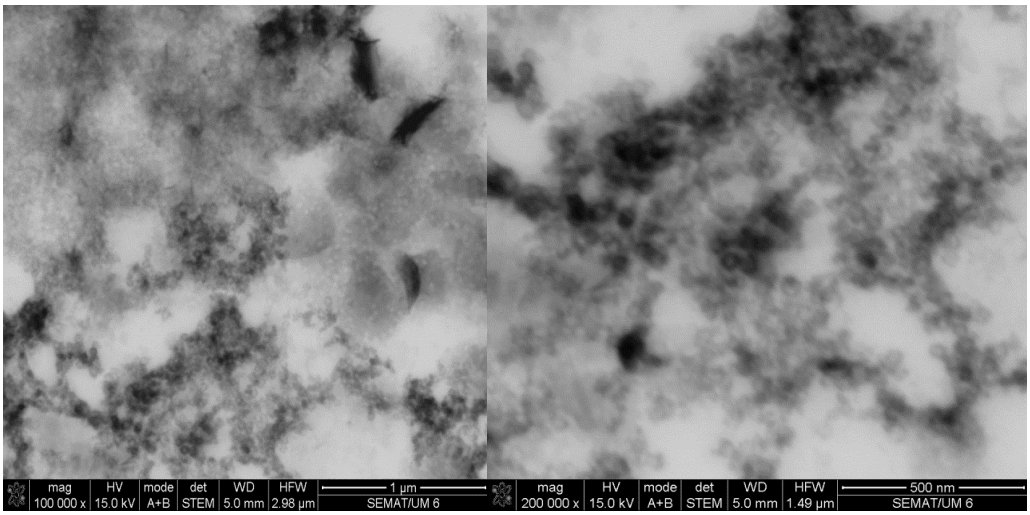

Figure S13. STEM and SEM images of IPECs from HAS_{12k}-*b*-PEG and PLL.

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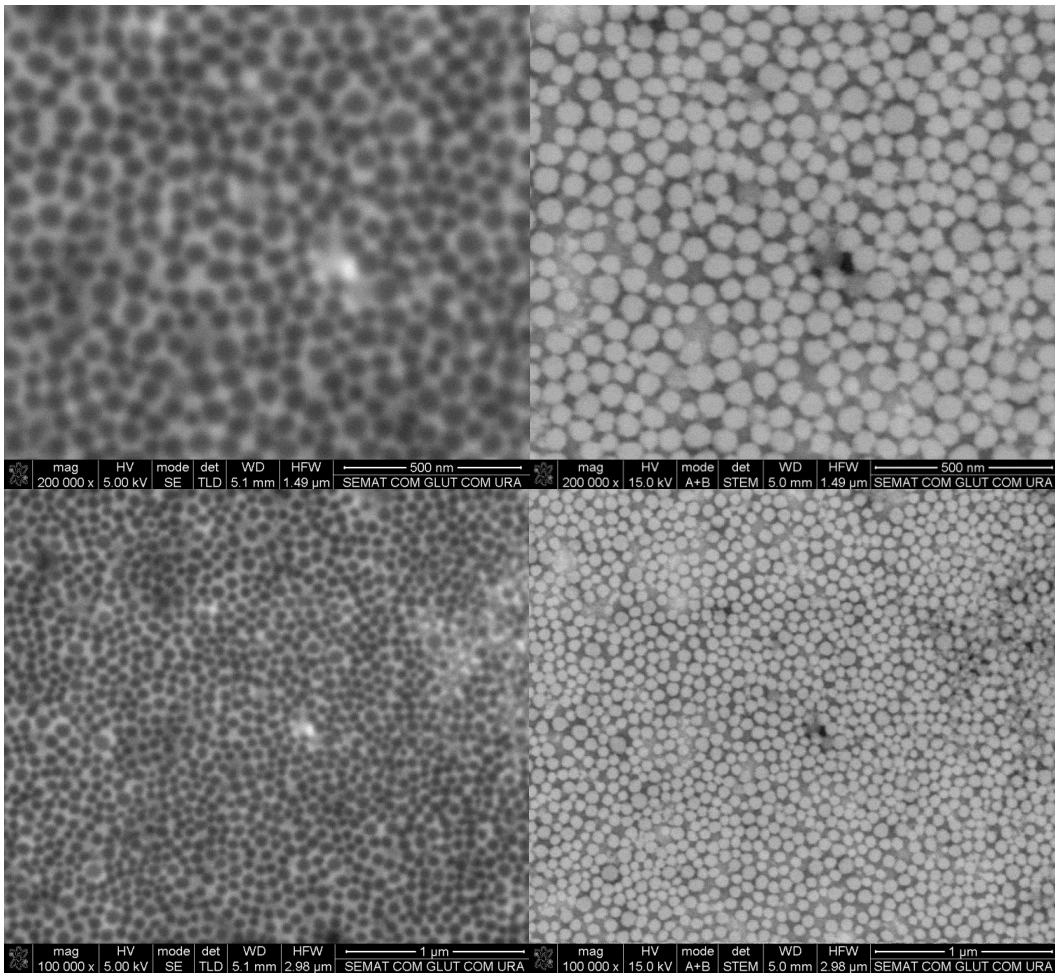

Figure S14. SEM (left) and S-TEM (right) images of IPECs from CS_{14k}-*b*-PEG and PLL.

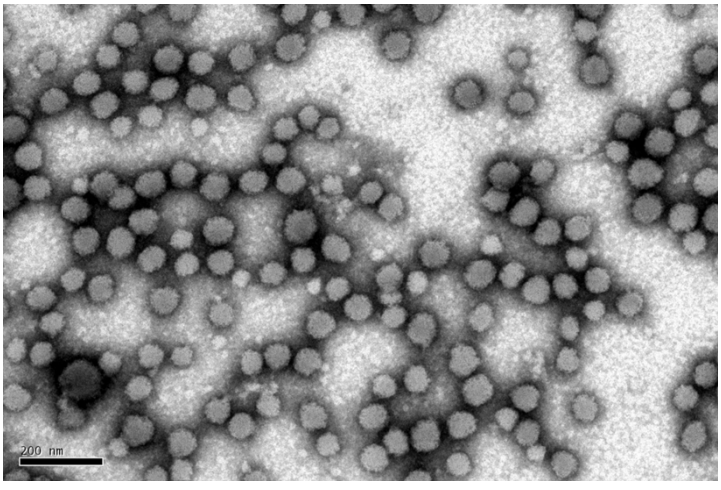

Figure S15. TEM images of IPECs from CS_{14k}-*b*-PEG and PLL.

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S6. Optimisation of the FGF-2 complexation

Table S2. Characteristics of the CS_{34k}-*b*-PEG complexes with FGF-2. Equal volumes of the polymer solutions were mixed.

FGF concentration [g/L]	CS_{34k}-*b*-PEG concentration [g/L]	Mass ratio CS_{34k}-*b*-PEG/FGF	R_h (nm)	PDI
0.075	0.160	2.10	75	0.27
0.160	0.170	1.00	51	0.28
0.500	**0.150**	**0.30**	**98**	**0.22**
0.300	0.075	0.25	136	0.35
0.500	0.075	0.15	151	0.29

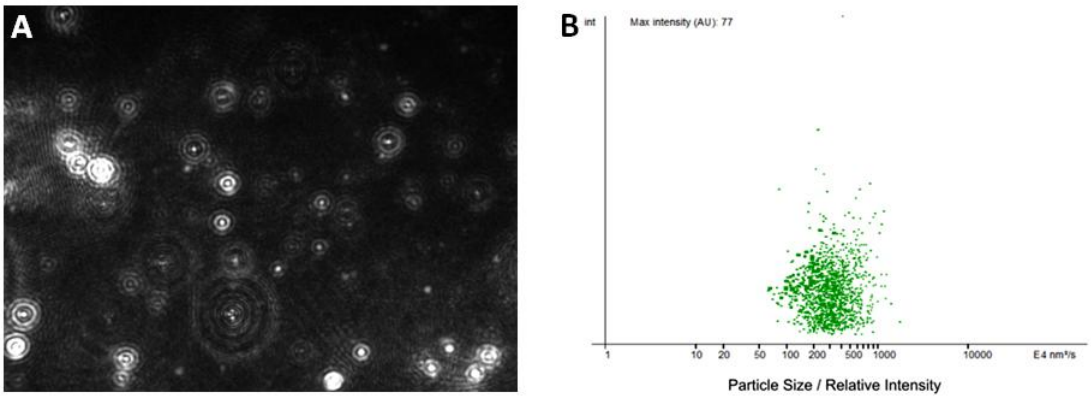

Figure S16. NTA analysis of IPECs from CS_{24k}-*b*-PEG and FGF-2 at ratio = 0.3: A) 35 s video frame; B) Particle size versus relative intensity.