Supporting information

Structural dependence of the solution behavior of HPMA-based polymers

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Samples abbreviations

LH	linear homopolymers
LC	linear copolymer
LCd	linear copolymer with deprotected hydrazide groups
DH	linear two-arm homopolymer
DC	linear two-arm copolymer
SC-SA	star-like copolymer created from 18 kg·mol^{-1} arms
SC-LA	star-like copolymer created from 37 kg·mol^{-1} arms

Materials and Methods

Chemicals

Tert-butyl alcohol, dimethyl sulfoxide (DMSO), methanol, ethyl acetate, 2,4,6-trinitrobenzene-1-sulfonic acid (TNBSA), *N*, *N*-dimethylacetamide (DMAc), CuBr, 4-quinolinol, *N*, *N*-diisopropylethylamine (DIPEA), and azobisisobutyronitrile (AIBN) were purchased from Merck-Sigma Aldrich (Germany). Dendritic cores; PFD-G4-TMP-azide (48 end groups), PFD-G3TMP-azide (24 end groups), and PFD-G2-TMP-azide (12 end groups) dendrimers; PFD-G4acetylene-ammonium (16 end groups) and PFD-G3-acetylene-ammonium (8 end groups) dendrons were purchased from Polymer Factory (Sweden). PAMAM dendrimers were purchased from Dendritech (USA). Azo initiator V-70 was purchased from Wako Chemicals (Japan). All solvents were of practical grade or high-performance liquid chromatography (HPLC) grade.

Characterization

All samples were characterized using size exclusion chromatography at 25°C. The weight-average molecular weight M_w, number-average molecular weight M_n, dispersity $Đ$, intrinsic viscosity $[\eta]_w$, and hydrodynamic radius R_h of polymers were measured using SEC on an HPLC Shimadzu system equipped with an SPDM20A photodiode array detector (Shimadzu, Japan), differential refractometer (Optilab®rEX), viscometric detector (ViscoStar III), and multiangle light scattering (DAWN HELLEOS II) detectors (all from Wyatt Technology Co., USA). All samples were measured using column Superose 6 Increase (Cytiva) with a mobile phase of 0.2M phosphate buffer (pH 7.4) and the flow rate was 0.5 mL·min^{-1}. For the molar mass calculations, a value of dn/dc = 0.167 was used. Samples were prepared at a concentration of 3 mg/mL, and 100 µL of sample solution was injected. We state that for intrinsic viscosity measurements, this sample concentration is regarded as diluted.

The viscosity of the homopolymer solutions was determined using a rotational rheometer Physica MCR 501 (Anton Paar GmbH, Austria) with cone/plate geometry (diameter of 25 mm, cone angle of 2°). The shear rheological experiments were performed in the shear rate range of 1–100 s^{-1} at a room temperature of 25 °C. To minimize the evaporation effect during the measurement, the cone-plate geometry with each sample was covered by an aluminium lid.

Synthesis

Synthesis of monomers

N-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-(*tert*-butoxycarbonyl)-*N*'-(6-(methacryloylamino)hexanoyl)hydrazine (Ma-Acap-NHNH-Boc) were synthesized from methacryloyl chloride as described earlier. [1, 2]

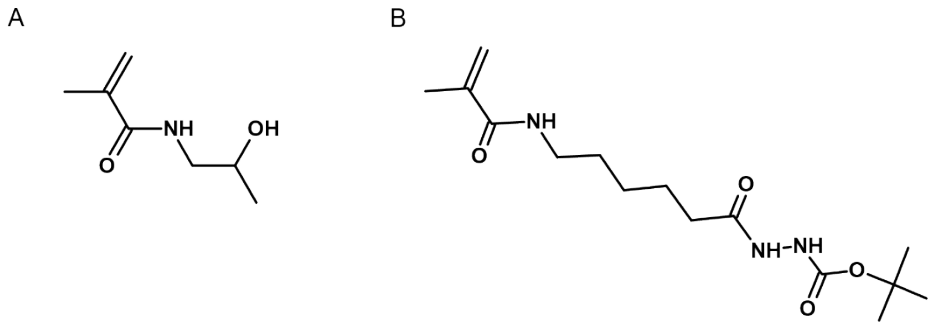

Figure S1: Monomer structures: HPMA (A), MaAcapNHNHBoc (B)

Chain transfer agent synthesis

Chain transfer agent S-2-cyano-2-propyl-S'-ethyl Trithiocarbonate (TTc-A), and 2-cyano-5-oxo-5-(2-thioxo-1,3-thiazolidin-3-yl)pentan-2-yl ethyl carbontrithioate (TTc-TT) were synthesized according to the literature[3-5]. The synthesis of bifunctional CTAs was described by Hrochová et al. [6]

Linear polymer synthesis

Linear homopolymers LH1–LH9 were synthesized using the RAFT polymerization technique. Briefly, HPMA (300 mg, 2.1 mmol) was dissolved in 2.3 mL *t*-butanol, and azo initiator V-70 (6.46 mg, 2.10 µmol) and CTA TTc-A (8.61 mg, 41.90 µmol) were dissolved in 0.3 mL anhydrous DMAc, and the solutions were mixed in a polymerization ampoule. The reaction mixture was bubbled with argon for 10 minutes, and the reaction was carried out at 30°C for 72 h. The polymer product (LH1) was precipitated in an acetone/diethyl ethyl mixture and dried under a vacuum. Trithiocarbonate end groups were removed according to the literature. [7] LH2–LH9 were synthesized by the same procedures in a molar ratio of M:CTA:I 200–1200:2:1. Linear homopolymers LH10–LH15 were synthesized using the Cu-RDRP technique according to the literature. [8]

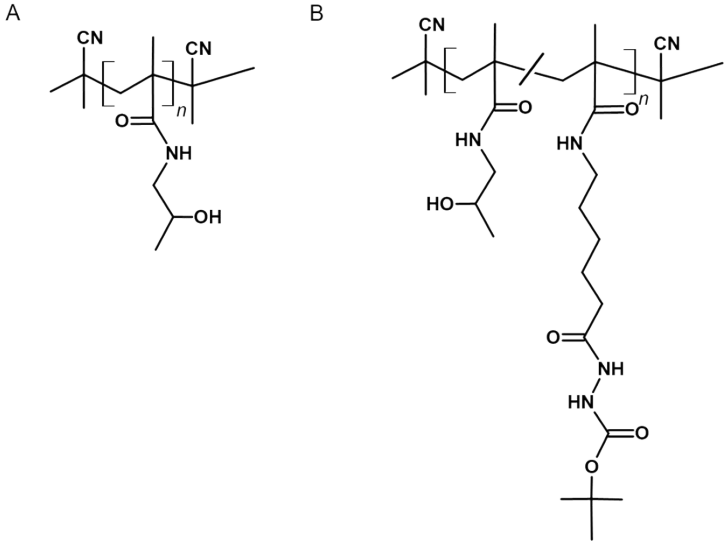

Figure S2: Scheme of homopolymers and copolymers based on HPMA

Linear and two-arm copolymers were synthesized as described earlier.[6] The molar ratio of comonomers HPMA: MaAcapNHNHBoc was 92:8 in all syntheses. Linear copolymers were synthesized in the range of molar ratio M:CTA:I 100–1200:2:1. Two-arm copolymers were synthesized in the range of molar ratio M:biCTA:I 200–1000:1:1. An example of synthesis is as follows (LC1): 300 mg HPMA (2.1 mmol) and 57 mg MaAcapNHNHBoc (0.18 mmol) was dissolved in 2.5 mL *t*-butanol, 9.35 mg CTA TTc-A (45.5 µmol) and 7.02 mg V-70 (22.7 µmol) was dissolved in 0.3 mL DMAc, both solutions were mixed in polymerization ampule, and bubbled by argon for 10 minutes to oxygen removing. The polymerization was carried out at 30°C for 72 h. The product was separated by precipitation into a mixture of diethyl ether/acetone and reprecipitation from methanol. The product was filtered and dried under a vacuum. Trithiocarbonate end groups were removed by reaction with azo initiator AIBN in DMAc, 80°C, 3h. [7]

Linear copolymers with free hydrazide groups (LCd) were prepared by deprotection reaction in Q-H_2O. 50 mg LC was dissolved in 2 mL Q-H_2O, and the solution was incubated in an oil bath at 100°C for 40 minutes. The unprotected copolymer was isolated by freeze-drying.

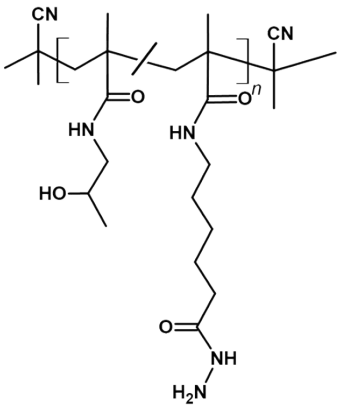

Figure S3: Deprotected linear copolymer (LCd) structure

Star-like polymer synthesis

Star-like polymers were synthesized by grafting to approach detailly described in Kostka *et al*. [5] Three different multifunctional dendritic cores were used for synthesis star-like polymers; PAMAM dendrimer generation 2, 3, and 4 with amino end groups, MPA dendrimer generation 2, 3, and 4 with azido end groups, and MPA dendron generation 3, and 4 with amino end groups. A star-like structure was synthesized by grafting linear copolymer arms with molecular weights of 18 kg·mol^{-1} (SC-SA) or 37 kg·mol^{-1} (SC-LA).

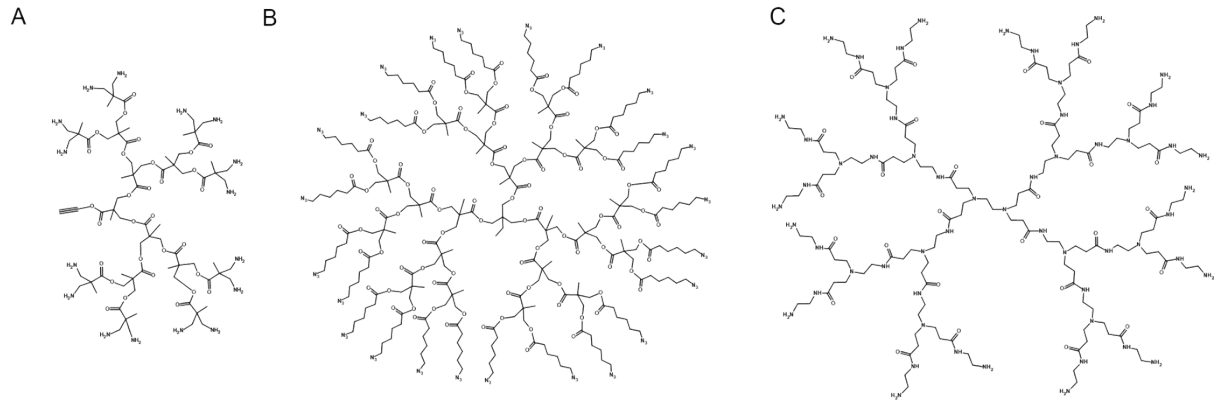

Figure S4: Scheme of dendritic cores. MPA dendron G4 (A), MPA dendrimer G3 (B), PAMAM dendrimer G3 (C)

Polymers characterization

Table S1: Characterization of linear homopolymers and copolymers

	M_w[g·mol^{-1}]	*Đ*	$[\eta]_w$ [mL·g^{-1}]	D_h [nm]
LH1	9 800	1.04	7.4	4.6
LH2	17 800	1.09	9.3	6.0
LH3	23 200	1.07	10.9	6.8
LH4	27 500	1.06	12.0	7.4
LH5	33 600	1.15	14.2	8.4
LH6	45 400	1.06	16.4	9.8
LH7	60 500	1.06	19.9	11.4
LH8	64 300	1.13	21.4	12.0
LH9	75 000	1.09	22.4	12.8
LH10	88 500	1.06	25.1	14.0
LH11	119 300	1.17	30.6	16.6
LH12	224 800	1.11	50.2	24.0
LH13	288 200	1.06	55.9	27.2
LH14	426 200	1.03	73.7	34.0
LH15	588000	1.31	92.2	39.8
LH16	1 050 000	1.72	144.7	55.0
LH17	1 358 200	2.06	168.8	62.0

LC1	7 800	1.04	6.0	3.8
LC2	16 000	1.04	8.4	5.6
LC3	23 900	1.04	10.2	6.8
LC4	30 000	1.05	11.7	7.6
LC5	41 100	1.07	13.6	8.8
LC6	50 100	1.05	14.9	9.8
LC7	65 800	1.07	18.3	11.4
LC8	83 300	1.09	21.5	13.0
LC9	86 800	1.13	21.8	13.2
LC1d	7 900	1.04	5.7	3.8
LC2d	16 100	1.04	8.5	5.6
LC3d	24 400	1.04	10.7	6.8
LC4d	31 000	1.06	11.9	7.8
LC5d	41 700	1.06	14.5	9.2
LC6d	49 900	1.07	16.3	10.0
LC7d	68 400	1.07	19.7	11.8
LC8d	85 600	1.09	21.9	13.2
LC9d	99 400	1.10	23.4	14.2

Table S2: Characterization of linear two-arm homopolymers and copolymers

	M_w[g·mol^{-1}]	*Đ*	$[\eta]_w$ [mL·g^{-1}]	D_h [nm]	
DH1	27 600	1.12	12.8	7.6	**CTA1**
DH2	56 900	1.13	20.8	11.4	**CTA1**
DH3	69 500	1.19	22.3	12.4	**CTA1**
DH4	72 800	1.16	24.3	13.0	**CTA1**
DH5	104 100	1.18	30.4	15.6	**CTA1**
DH6	29 300	1.16	12.7	7.6	**CTA2**
DH7	50 700	1.20	19.4	10.6	**CTA2**
DH8	84 100	1.20	23.1	12.8	**CTA2**
DH9	96 800	1.14	27.1	14.8	**CTA2**
DH10	113 400	1.21	31.9	16.4	**CTA2**
DH11	29 300	1.18	13.4	7.8	**CTA3**
DH12	55 300	1.13	20.6	11.2	**CTA3**
DH13	68 300	1.18	23.3	12.4	**CTA3**
DH14	80 500	1.16	26.2	13.8	**CTA3**
DH15	130 900	1.14	35.0	17.8	**CTA3**
DH16	34 000	1.27	14.4	8.4	**CTA4**
DH17	51 800	1.19	20.0	10.8	**CTA4**
DH18	72 900	1.21	24.2	12.8	**CTA4**
DH19	97 900	1.12	29.6	15.2	**CTA4**
DH20	125 100	1.22	34.8	17.4	**CTA4**
DC1	13 050	1.10	7.9	5.0	**CTA3**
DC2	32 500	1.12	12.4	8.0	**CTA3**
DC3	36 500	1.08	13.7	8.6	**CTA3**
DC4	89 500	1.18	24.0	13.8	**CTA3**

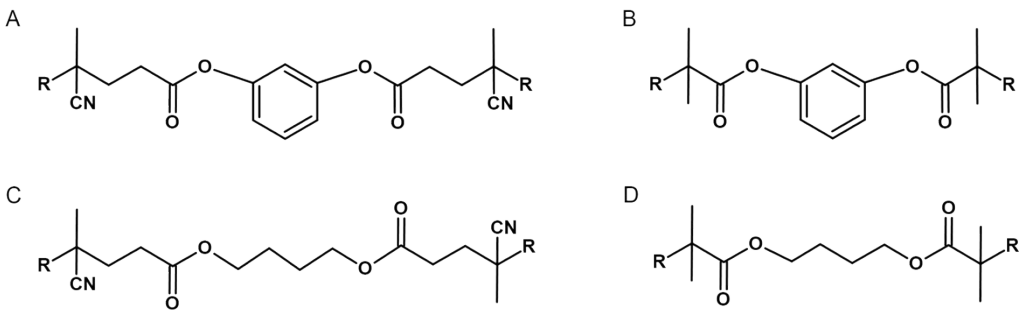

Figure S5: Scheme of degradable linker in the middle of linear two-arm homopolymers and copolymers. Structure incorporated from CTA1 (A), CTA2(B), CTA3 (C), and CTA4 (D).

Table S3: Characterization of star-like copolymers with 18 kg·mol^{-1} polymer arms

	M_w[g·mol^{-1}]	*Đ*	$[\eta]_w$ [mL·g^{-1}]	D_h [nm]	*No. of arms on the core*	*Core / M_n (arm)*
SC-SA1	66 100	1.12	17.6	11.2	3.3	MPA-NH_2, 18kg·mol^{-1}
SC-SA2	81 300	1.08	18.3	12.2	4.2	MPA-NH_2, 18kg·mol^{-1}
SC-SA3	87 200	1.04	18.9	12.8	4.7	MPA-NH_2, 18kg·mol^{-1}
SC-SA4	63 500	1.10	17.6	11.2	3.2	MPA-NH_2, 18kg·mol^{-1}
SC-SA5	110 800	1.02	19.5	14.0	6.0	MPA-NH_2, 18kg·mol^{-1}
SC-SA6	126 300	1.02	19.6	14.6	6.9	MPA-NH_2, 18kg·mol^{-1}
SC-SA7	121 000	1.01	19.1	14.2	6.7	MPA-NH_2, 18kg·mol^{-1}
SC-SA8	102 700	1.12	19.2	13.4	5.1	MPA-N_3, 18kg·mol^{-1}
SC-SA9	103 300	1.15	19.1	13.4	5.0	MPA-N_3, 18kg·mol^{-1}
SC-SA10	127 300	1.09	19.9	14.6	6.5	MPA-N_3, 18kg·mol^{-1}
SC-SA11	159 100	1.08	20.0	15.8	8.2	MPA-N_3, 18kg·mol^{-1}
SC-SA12	103 600	1.15	19.0	13.4	5.0	MPA-N_3, 18kg·mol^{-1}
SC-SA13	138 200	1.12	19.8	15.0	6.9	MPA-N_3, 18kg·mol^{-1}
SC-SA14	169 500	1.07	19.9	16.2	8.8	MPA-N_3, 18kg·mol^{-1}
SC-SA15	217 900	1.13	20.8	17.8	10.7	MPA-N_3, 18kg·mol^{-1}
SC-SA16	329 500	1.12	20.2	20.2	16.3	MPA-N_3, 18kg·mol^{-1}
SC-SA17	137 200	1.12	18.9	14.8	6.8	MPA-N_3, 18kg·mol^{-1}
SC-SA18	170 700	1.15	19.8	16.0	8.2	MPA-N_3, 18kg·mol^{-1}
SC-SA19	187 300	1.10	19.7	16.6	9.5	MPA-N_3, 18kg·mol^{-1}
SC-SA20	249 500	1.07	19.6	18.2	13.0	MPA-N_3, 18kg·mol^{-1}
SC-SA21	403 100	1.11	20.0	21.4	20.4	MPA-N_3, 18kg·mol^{-1}
SC-SA22	405 300	1.04	18.8	21.2	21.7	MPA-N_3, 18kg·mol^{-1}
SC-SA23	77 610	1.15	19.6	12.2	3.7	PAMAM, 18kg·mol^{-1}
SC-SA24	98 350	1.11	19.9	13.4	4.9	PAMAM, 18kg·mol^{-1}
SC-SA25	164 300	1.10	20.2	16.0	8.3	PAMAM, 18kg·mol^{-1}
SC-SA26	194 500	1.11	20.7	17.0	9.7	PAMAM, 18kg·mol^{-1}
SC-SA27	210 400	1.09	20.5	17.4	10.7	PAMAM, 18kg·mol^{-1}
SC-SA28	408 700	1.11	19.4	21.4	20.4	PAMAM, 18kg·mol^{-1}
SC-SA29	165 900	1.09	21.5	16.4	8.5	PAMAM, 18kg·mol^{-1}
SC-SA30	155 500	1.07	20.8	15.4	8.1	PAMAM, 18kg·mol^{-1}
SC-SA31	160 300	1.05	19.8	15.8	8.5	PAMAM, 18kg·mol^{-1}
SC-SA32	230 800	1.10	20.1	17.8	11.7	PAMAM, 18kg·mol^{-1}
SC-SA33	281 000	1.07	19.6	19.0	14.6	PAMAM, 18kg·mol^{-1}
SC-SA34	530 600	1.14	18.7	23.0	25.9	PAMAM, 18kg·mol^{-1}

Table S4: Characterization of star-like copolymers with 37 kg·mol^{-1} polymer arms

	M_w[g·mol^{-1}]	*Đ*	$[\eta]_w$ [mL·g^{-1}]	D_h [nm]	*No. of arms on the core*	*Core / M_n (arm)*
SC-LA1	118 100	1.17	26.7	15.6	2.7	MPA-NH_2, 37 kg·mol^{-1}
SC-LA2	144 500	1.14	27.8	17.0	3.4	MPA-NH_2, 37 kg·mol^{-1}
SC-LA3	141 200	1.13	28.4	17.0	3.4	MPA-NH_2, 37 kg·mol^{-1}
SC-LA4	185 800	1.13	29.4	18.8	4.4	MPA-NH_2, 37 kg·mol^{-1}
SC-LA5	191 000	1.13	29.2	19.0	4.6	MPA-NH_2, 37 kg·mol^{-1}
SC-LA6	194 900	1.10	29.3	19.2	4.8	MPA-NH_2, 37 kg·mol^{-1}
SC-LA7	152 200	1.29	28.5	17.4	3.2	MPA-N_3, 37 kg·mol^{-1}
SC-LA8	150 300	1.11	28.0	17.4	3.7	MPA-N_3, 37 kg·mol^{-1}
SC-LA9	212 800	1.17	30.2	20.0	4.9	MPA-N_3, 37 kg·mol^{-1}
SC-LA10	247 500	1.11	30.9	21.2	6.0	MPA-N_3, 37 kg·mol^{-1}
SC-LA11	158 800	1.19	28.3	17.6	3.6	MPA-N_3, 37 kg·mol^{-1}
SC-LA12	193 900	1.20	29.2	19.0	4.4	MPA-N_3, 37 kg·mol^{-1}
SC-LA13	231 600	1.15	30.8	20.6	5.4	MPA-N_3, 37 kg·mol^{-1}
SC-LA14	343 200	1.17	33.1	24.0	7.9	MPA-N_3, 37 kg·mol^{-1}
SC-LA15	369 400	1.08	32.0	24.6	9.2	MPA-N_3, 37 kg·mol^{-1}
SC-LA16	191 700	1.21	28.8	18.8	4.3	MPA-N_3, 37 kg·mol^{-1}

SC-LA18	377 000	1.27	32.6	24.6	8.0	MPA-N_3, 37 kg·mol^{-1}
SC-LA19	457 200	1.13	31.7	26.2	10.9	MPA-N_3, 37 kg·mol^{-1}
SC-LA20	440 000	1.13	30.8	25.6	10.5	MPA-N_3, 37 kg·mol^{-1}
SC-LA21	635 400	1.12	30.8	29.0	15.3	MPA-N_3, 37 kg·mol^{-1}
SC-LA22	119 100	1.07	28.0	16.0	3.0	PAMAM, 37 kg·mol^{-1}
SC-LA23	173 600	1.12	30.8	18.8	4.2	PAMAM, 37 kg·mol^{-1}
SC-LA24	252 000	1.18	30.3	21.0	5.8	PAMAM, 37 kg·mol^{-1}
SC-LA25	271 500	1.11	31.8	22.0	6.6	PAMAM, 37 kg·mol^{-1}
SC-LA26	394 000	1.11	31.6	24.8	9.6	PAMAM, 37 kg·mol^{-1}
SC-LA27	464 400	1.08	31.9	26.4	11.6	PAMAM, 37 kg·mol^{-1}
SC-LA28	422 700	1.09	30.2	25.2	10.5	PAMAM, 37 kg·mol^{-1}
SC-LA29	238 000	1.05	32.1	21.2	6.1	PAMAM, 37 kg·mol^{-1}
SC-LA30	264 900	1.07	31.4	21.8	6.7	PAMAM, 37 kg·mol^{-1}
SC-LA31	359 000	1.09	31.9	24.2	8.9	PAMAM, 37 kg·mol^{-1}
SC-LA32	502 200	1.08	32.3	27.2	12.6	PAMAM, 37 kg·mol^{-1}
SC-LA33	604 900	1.09	31.4	28.6	15.0	PAMAM, 37 kg·mol^{-1}
SC-LA34	620 900	1.07	32.1	29.2	15.7	PAMAM, 37 kg·mol^{-1}
SC-LA35	913 200	1.10	32.3	33.2	22.4	PAMAM, 37 kg·mol^{-1}
SC-LA36	592 500	1.19	30.7	28.0	13.5	PAMAM, 37 kg·mol^{-1}

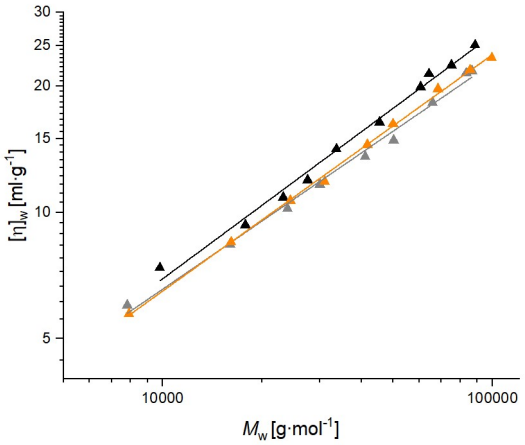

Figure S6: Dependence of intrinsic viscosity of linear HPMA-based homopolymers and copolymers on molar mass. Black - homopolymers, gray – copolymers with protected hydrazides, orange – copolymers with deprotected hydrazides. Values of [η] are from SEC from viscometric detector. Molar mass was determined using SEC with MALS and dRI detector. (linear fit with R^2>0.99 for all three curves)

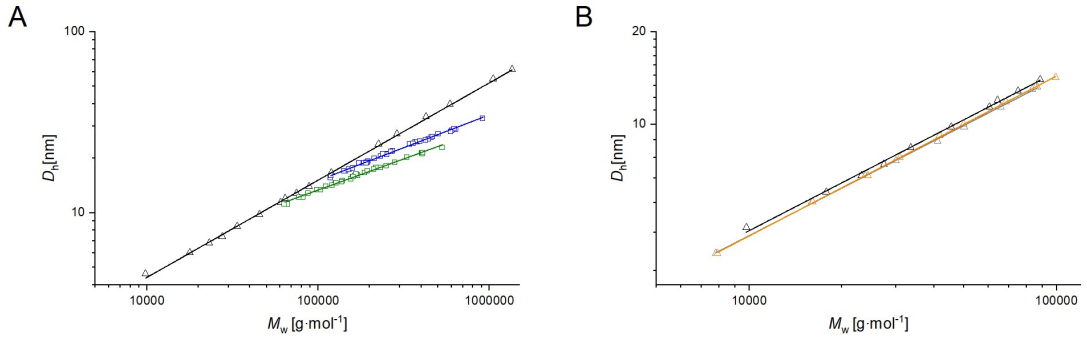

Figure S7: Hydrodynamic radius as a function of Molar mass: A) Linear homopolymer (black), SC-SA polymers (green), and SC-LA polymers (blue). B) *HPMA-based linear homopolymers and copolymers. Black -homopolymers, gray – copolymers with protected hydrazides, orange – copolymers with deprotected hydrazides.* D_h *values are determined by SEC from a viscometric detector. (linear fit was with* R^2*>0.99 for all plots)*

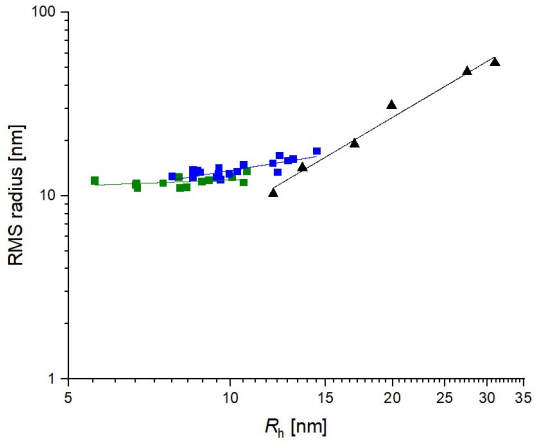

Figure S8: Dependence of RMS radius on hydrodynamic size of polymers. Black: linear homopolymers, blue: star-like copolymers with MPA dendritic core and arms with M_n*=37 000 g·mol*$^{-1}$ (SC-LA1 – SC-LA21)*, green: star-like copolymers with MPA dendritic core and arms with* M_n*=18 000 g·mol*$^{-1}$ (SC-SA1 to SC-SA22)*.*

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