Supporting Information

Imaging Single-Chain Nanoparticle Folding via High-Resolution Mass Spectrometry

Jan Steinkoenig,[a] Hannah Rothfuss,[a] Andrea Lauer,[a] Bryan T. Tuten,[a] Christopher Barner-Kowollik*[a,b]

[a] Preparative Macromolecular Chemistry, Institut für Technische Chemie und Polymerchemie, Karlsruhe Institute of Technology (KIT), Engesserstr. 18, 76128 Karlsruhe, Germany and Institut für Biologische Grenzflächen, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany. Email: christopher.barner-kowollik@kit.edu

[b] School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology (QUT), 2 George St, Brisbane, Australia. Email: christopher.barnerkowollik@qut.edu.au

Materials

All solvents for synthesis were obtained from Sigma-Aldrich, Acros Organics or Fischer and used without further purification. Absolute solvents were purchased from Acros Organics and stored under nitrogen and over molecular sieves. 2,2'-Azobis(2-methylpropionitril) (AIBN) was purchased from Acros (98%) and recrystallized twice from methanol. Methyl methacrylate (MMA, Sigma-Aldrich, 99%) and glycidyl methacrylate (GMA, Alfa Aesar, 97%) were deinhibited by passing through a column of basic aluminium oxide. Tris(pentafluorophenyl)borane ($B(C_6F_5)_3$) was purchased from TCI (97%) and stored in the glove box. THF (Scharlau, GPC grade) and MeOH (Roth, HPLC ultra gradient grade) for SEC-ESI MS analysis were used without further treatment. 2-cyano-2-propyl dodecyl trithiocarbonate (Sigma-Aldrich, 97%) and NaI (Sigma-Aldrich, 99%) were used as received.

Characterization Methods

Size exclusion chromatography-electrospray ionization mass spectrometry (SEC-ESI MS). Spectra were recorded on a LTQ Orbitrap XL Q Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an HESI II probe. The instrument was calibrated in the *m*/*z* range 74-1822 using premixed calibration solutions (Thermo Scientific). A constant spray voltage of 4.6 kV, a dimensionless sheath gas of 8, and a dimensionless auxiliary gas flow rate of 2 were applied. The capillary temperature and the S-lens RF level were set to 320 °C and 62.0, respectively. The Q Exactive was coupled to an UltiMate 3000 UHPLC System (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SD), autosampler (WPS 3000TSL), and a thermostated column department (TCC 3000SD). Separation was performed on two mixed bed size exclusion chromatography columns (Polymer Laboratories, Mesopore 250 × 4.6 mm, particle diameter 3 µm) with precolumn (Mesopore 50 × 4.6 mm) operating at 30 °C. THF at a flow rate of 0.30 $mL \cdot min^{-1}$ was used as eluent. The mass spectrometer was coupled to the column in parallel to (an UV-Detector (VWD 3400 RS), and) a RI-detector (RefractoMax520, ERC, Japan) in a setup described earlier.[1] 0.27 $mL \cdot min^{-1}$ of the eluent were directed through the RI-detector and 30 $\mu L \cdot min^{-1}$ infused into the electrospray source after postcolumn addition of a 100 µM solution of sodium iodide in methanol at 20 $\mu L \cdot min^{-1}$ by a micro-flow HPLC syringe pump (Teledyne ISCO, Model 100DM). A 50 µL aliquot of a polymer solution with a concentration of 2 $mg \cdot mL^{-1}$ was injected onto the HPLC system.

Size exclusion chromatography (SEC). SEC measurements were performed on a PL-SEC 50 Plus Integrated System, comprising an autosampler and a PLgel 5 µm bead-size guard column (50 × 7.5 mm) followed by a PLgel 5 µm Mixed E column (300 × 7.5 mm), three PLgel 5 µm Mixed C columns (300 × 7.5 mm), and a differential refractive index (RI) detector using THF as eluent at 35 °C

with a flow rate of 1 mL·min^{-1}. The SEC system is calibrated using linear poly(methyl methacrylate) standards ranging from 800 to 1.6×10^6 g·mol^{-1}. SEC calibration was carried out relative to poly(methyl methacrylate) calibrations (Mark Houwink parameters $K = 12.8 \cdot 10^{-5}$ dL·g^{-1}; $\alpha = 0.68$).

Nuclear magnetic resonance (NMR) spectroscopy. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM 400 (400 MHz) spectrometer. Chemical shifts are expressed in parts per million (ppm) and calibrated on characteristic solvent signals as internal standards.

Experimental Part

Poly(methyl methacrylate-stat-glycidyl methacrylate) (1). 3.53 mL methyl methacrylate (3.32 g, 33.2 mmol, 233 eq), 500 µL glycidyl methacrylate (0.53 g, 3.70 mmol, 26.0 eq), 2.80 mg AIBN (0.017 mmol, 0.12 eq) and 49.2 mg 2-cyano-2-propyl dodecyl trithiocarbonate (0.142 mmol, 1.00 eq) were placed in a dry glass vial with a septum cap, degassed by purging nitrogen through the reaction mixture for 30 min and then placed in an oil bath thermostated at 80 °C. After 60 min, the resulting p(MMA-stat-GMA) was recovered by precipitation in MeOH and dried under vacuum at 55 °C. The MMA:GMA=7:1 ratio was determined by NMR spectroscopy.

Single-chain folding of poly(methyl methacrylate-stat-glycidyl methacrylate) (1) to SCNP (2). A flame-dried Schlenk flask was charged with 40.0 mg poly(methyl methacrylate-stat-glycidyl methacrylate) (**1**) (0.053 mmol GMA, 1.00 eq) and dissolved in 200 mL dry dichloromethane. After 5 min of magnetic stirring, 13.3 mg $B(C_6F_5)_3$ (0.026 mmol, 0.49 eq) was added to the reaction mixture in one portion. The mixture was stirred for 72 h at ambient temperature. Subsequently, the mixture was washed with brine (50 mL), the phases were separated and concentrated under reduced pressure (avoiding evaporation to complete dryness). The polymer was recovered by precipitation in cold cyclohexane resulting in a white powder after filtration and drying under high vacuum.

NMR data

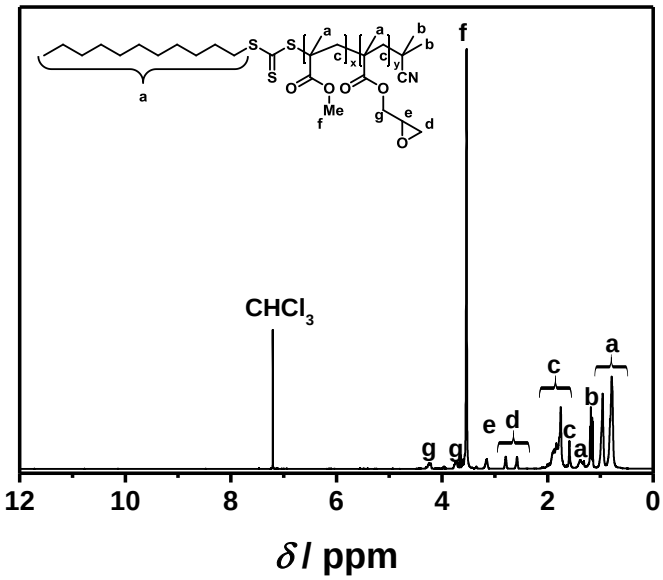


Figure S1 ^{1}H NMR (400 MHz, 298 K) of p(MMA-stat-GMA) (**1**) in $CDCl_3$. The peak assignment was in accordance to the literature.[1]

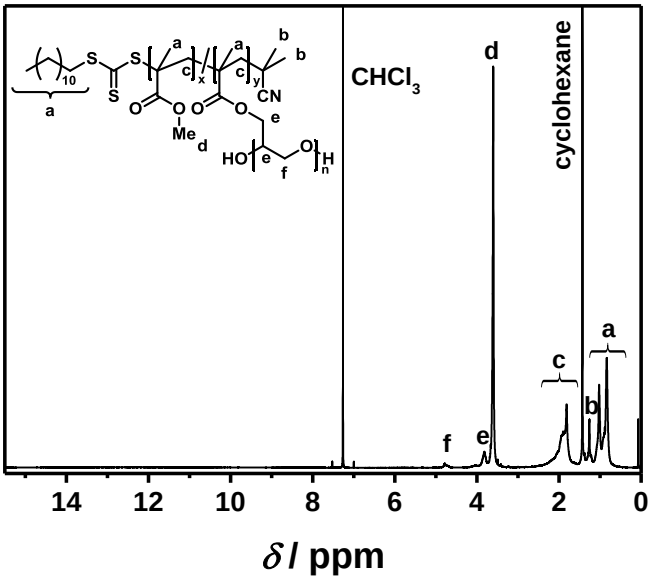


Figure S2 ^{1}H NMR (400 MHz, 298 K) of the SCNP (**2**) in $CDCl_3$.

[1] B. Liu, Y.-Y. Zhang, X.-H. Zhang, B.-Y. Du, Z.-Q. Fan, *Polym. Chem.* **2016**, *7,* 3731-3739.

SEC Data

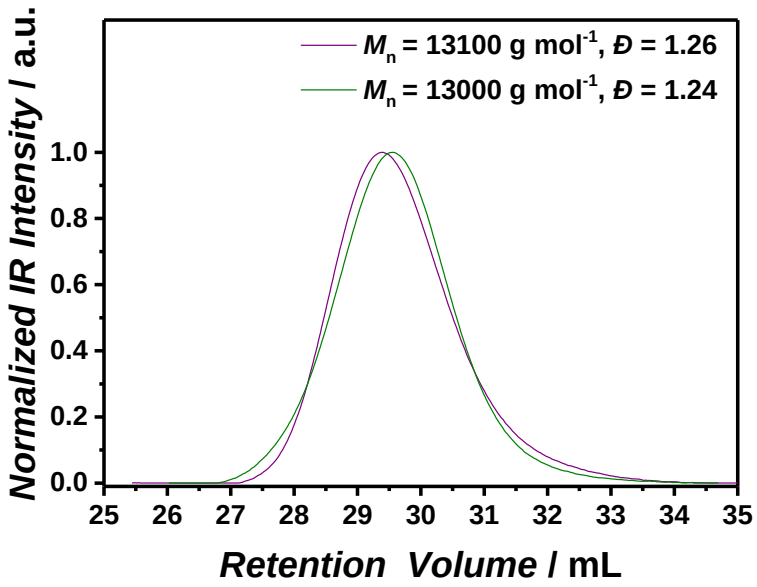


Figure S3 SEC traces for p(MMA-stat-GMA) (**1**) (purple line) and SCNP (**2**) obtained by using a syringe pump to inject $B(C_5F_6)_3$ with a flow rate of 1 mL·h^{-1} into a solution containing p(MMA-stat-GMA) (**1**) in dry 100 mL dichloromethane.

MS Data

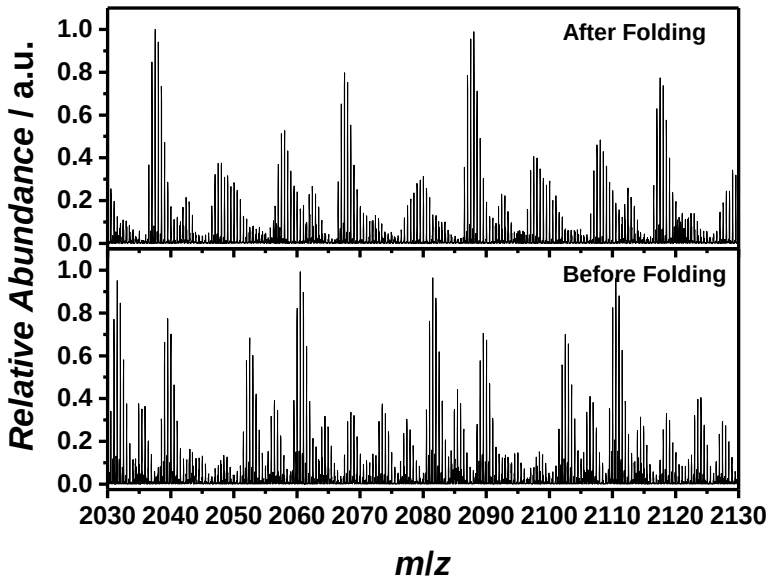


Figure S4 (Top) SEC-ESI Orbitrap mass spectrum after successful folding resulting in SCNP (**2**) formation; (bottom) SEC-ESI Orbitrap mass spectrum prior to the folding of p(MMA-stat-GMA) (**1**). The spectrum ranges from *m*/*z* 2030 to *m*/*z* 2130 illustrating double charged species of the precursor polymer and the SCNP.

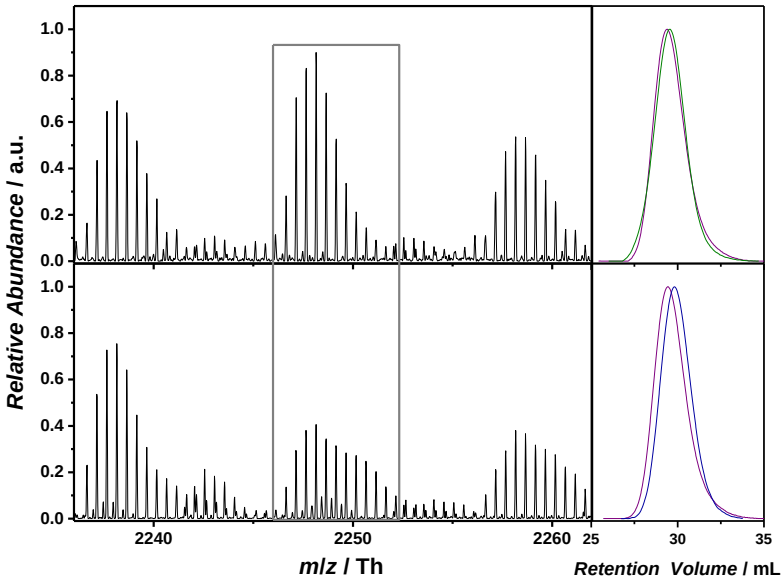


Figure S5 (Top) Syringe pump addition of catalyst: SEC-ESI Obitrap mass spectrum obtained of a SCNP (**2**, green line) sample evidenced by a slight shift in the SEC elugramm; (bottom) direct addition of catalyst: SEC-ESI Orbitrap mass spectrum with a strong shift in the SEC elugramm. The obtained ESI mass spectra are identical except of a broadening of the isotopic pattern in specific mass areas.

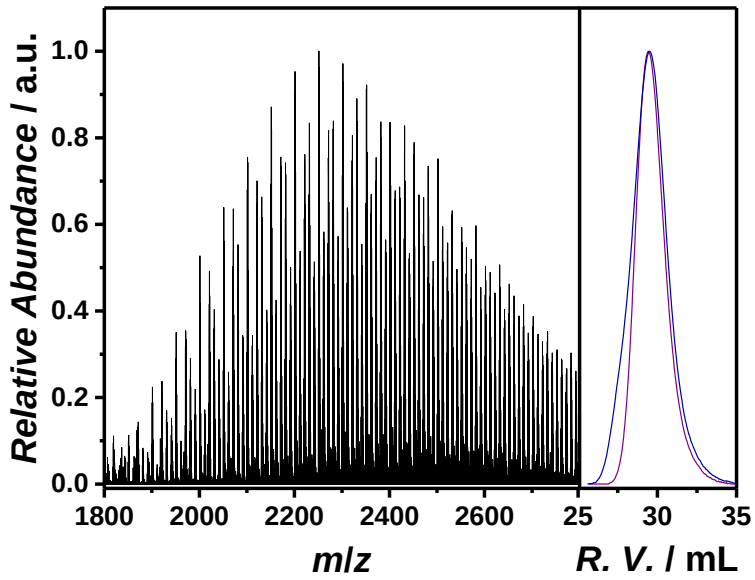


Figure S6 SEC-ESI Orbitrap mass spectrum (negative mode) obtained of p(MMA-stat-GMA) (**1**), in which all glycidyl moieties were ring-opened by water. The SEC trace depicts no shift from the parent polymer (purple trace) to the ring-opened polymer (blue trace). The ionization is by virtue of complexation to iodide.

SEC-ESI MS data of p(MMA-stat-GMA) (1)

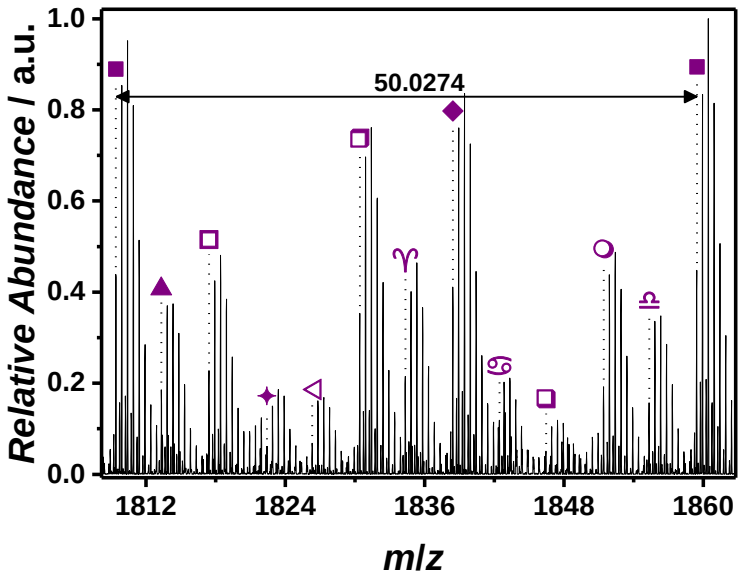


Figure S7 Zoomed SEC-ESI Orbitrap mass spectrum (positive mode) of p(MMA-stat-GMA) (**1**) between *m*/*z* 1808 and *m*/*z* 1862 obtained by summing all species between 14.71 mL and 15.13 mL retention volume. Labeled are the most abundant species and the repeating unit of pMMA (*m*/*z*(exp) 50.0274; *m*/*z*(theo) 50.0257). The minor distributions (slightly shifted isotopic pattern) stems from complexation by virtue of a second NaI and was not labeled in order to keep a good readability of the spectrum.

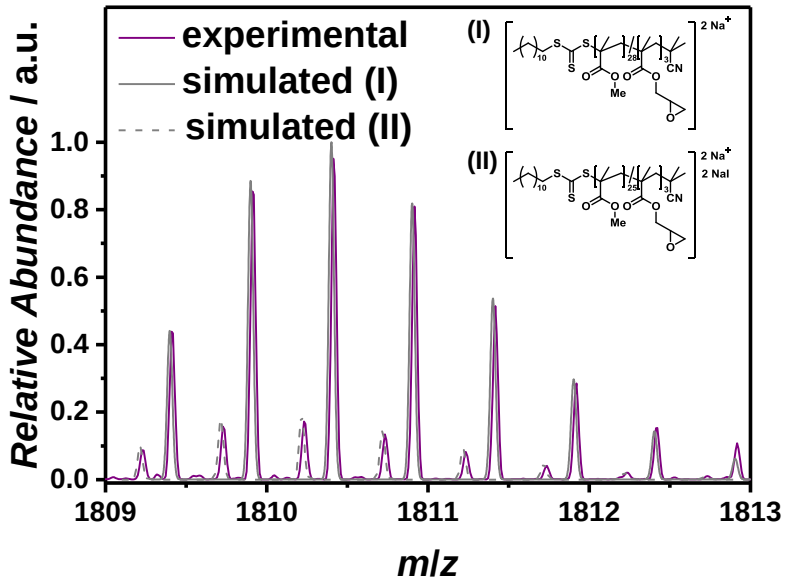
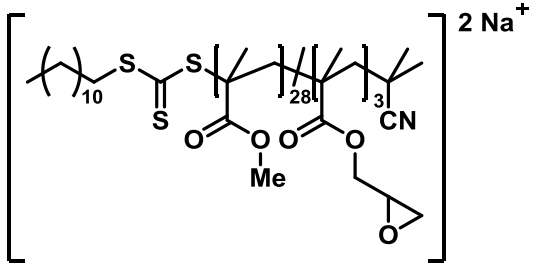
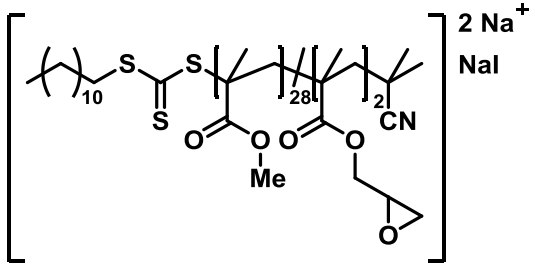
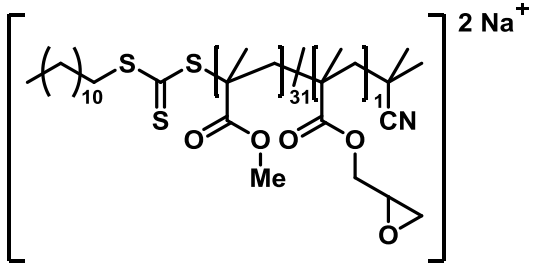
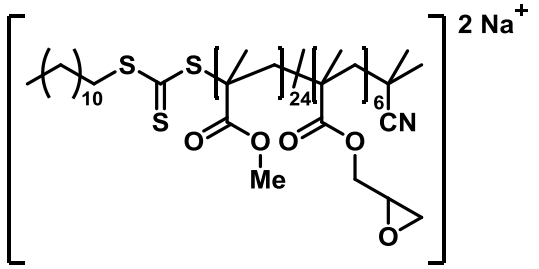
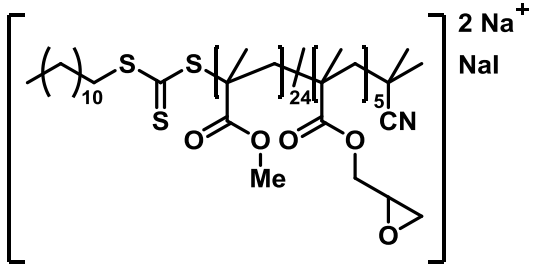
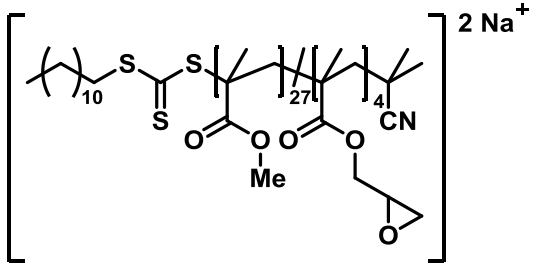
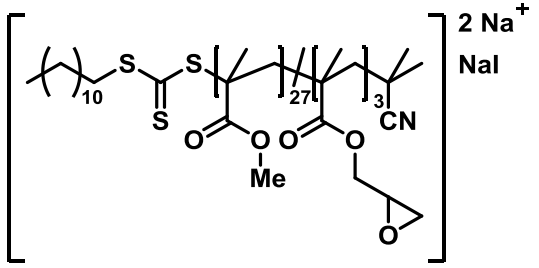
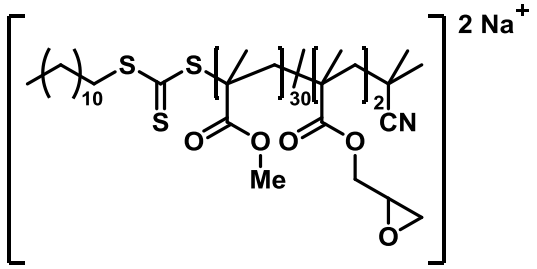
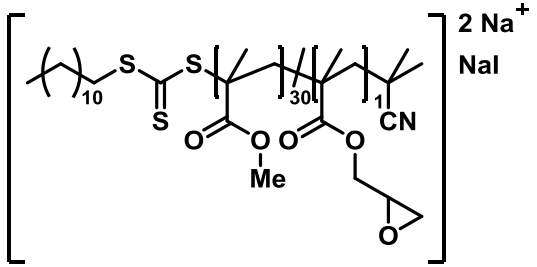
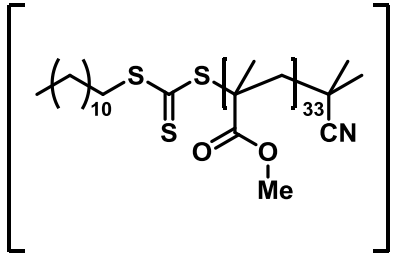
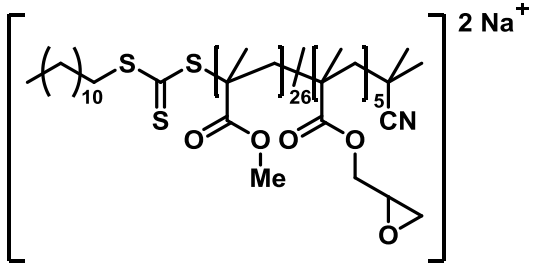


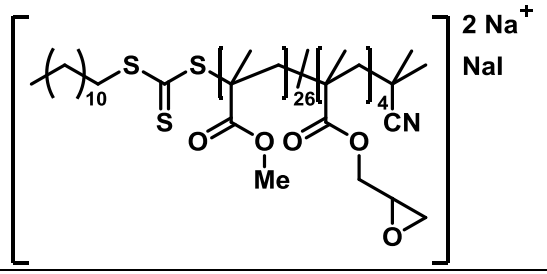
Figure S8 Isotopic simulation of a selected peak at *m*/*z* 1813 comparing the experiment (purple line) and the simulation (grey line) of (**I**) p(MMA_{28}-stat-GMA_3) ionized by virtue of complexation to sodium and (**II**) p(MMA_{26}-stat-GMA_3) ionized by virtue of complexation to sodium and formation of adducts to sodium iodide with a resolution of 48000.

Table S1 Peak assignment of the SEC-ESI Orbitrap mass spectrum of p(MMA-stat-GMA) (**1**) showing the labels (corresponding to the species in ***Figure S7***), the resolution (obtained by the Xcalibur software), the experimental *m*/*z* and theoretical *m*/*z* values, Δ*m*/*z* and the proposed chemical structures. The SEC-ESI Orbitrap mass spectrum was integrated from 14.71 mL to 15.13 mL to obtain a sufficient signal to noise ratio. The minor distributions (slightly shifted isotopic pattern) stems from a second complexation by virtue of NaI and was not labeled in order to keep a good readability of the spectrum.

Label	Resolution	*m*/*z*(exp)	*m*/*z*(theo)	Δ*m*/*z*	Structure
■	48000	1809.4139	1809.3987	0.0152	
▲	48000	1813.3304	1813.3143	0.0162	
□	40000	1817.4307	1817.4143	0.0164	

✦	46000	1822.4063	1822.3883	0.0180	
◁	43000	1826.3174	1826.3039	0.0135	
❒	46000	1830.4180	1830.4039	0.0141	
♈	49000	1834.3356	1834.3196	0.0160	

◆	49000	1838.4356	1838.4196	0.0160	
♋	49000	1842.3539	1842.3352	0.0187	
▼	45000	1846.4538	1846.4352	0.0185	
❍	44000	1851.4204	1851.4092	0.0112	

♎	47000	1855.3399	1855.3248	0.0150	

SEC-ESI MS data of SCNP (2) obtained by direct addition of $B(C_6F_5)_3$

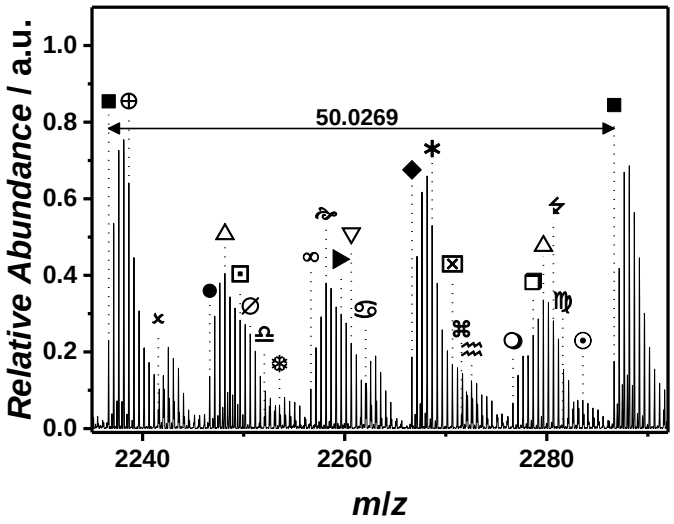


Figure S9 Zoomed SEC-ESI Orbitrap mass spectrum (positive mode) of SCNPs (**2**) between *m*/*z* 2235 and *m*/*z* 2292 obtained by summing all species between 14.42 mL and 15.92 mL retention volume. Labeled are the most abundant species and the repeating unit of pMMA (*m*/*z*(exp) 50.0269; *m*/*z*(theo) 50.0257). The minor distributions (slightly shifted isotopic pattern) stems from complexation by virtue of a second NaI and was not labeled in order to keep a good readability of the spectrum. The spectrum is identical with Figure 2a.

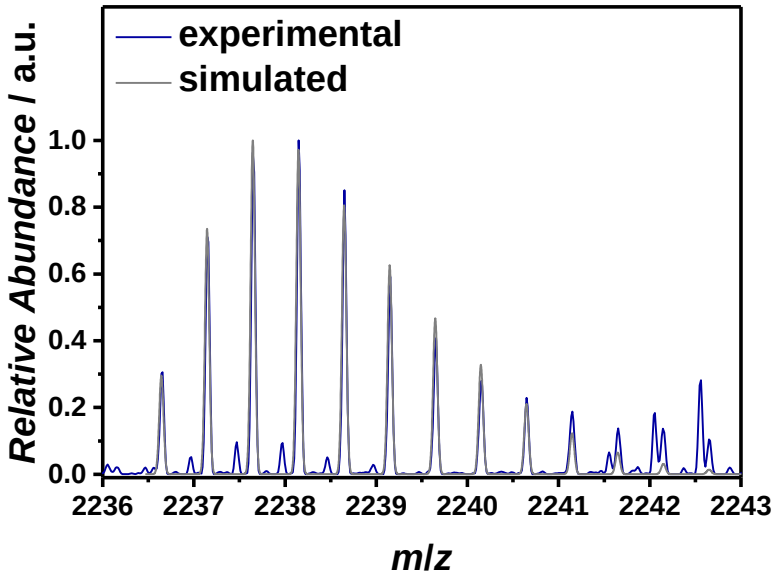


Figure S10 Isotopic simulation of a selected peak at *m*/*z* 2238 comparing the experiment (blue line) and the simulation (grey line) of SCNPs (**2**) with a resolution of 40000. Species ■ and species ⊕ have an approximated ratio of ■:⊕ = 5:1.

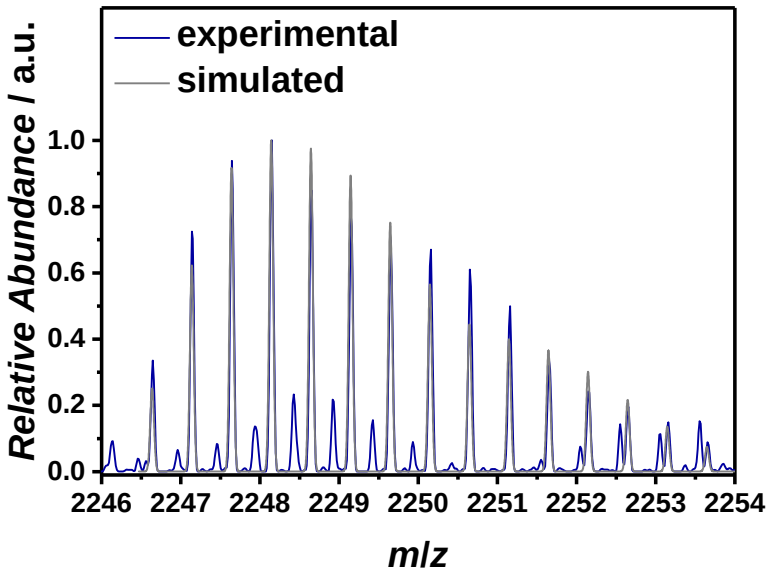


Figure S11 Isotopic simulation of a selected peak at *m*/*z* 2248 comparing the experiment (blue line) and the simulation (grey line) of SCNP (**2**) with a resolution of 40000. Species ●, △, ⊡ and species ∅ have an approximated ratio of ●:△:⊡:∅ = 10:3:4:3.

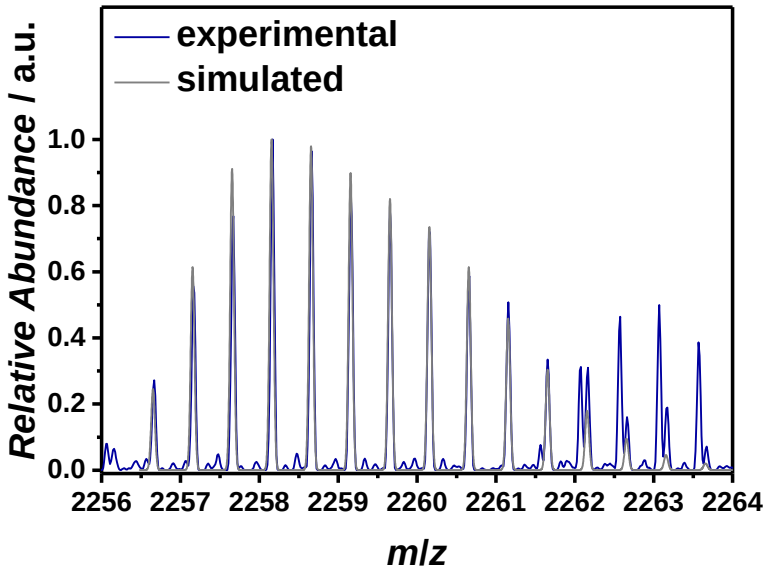


Figure S12 Isotopic simulation of a selected peak at *m*/*z* 2258 comparing the experiment (blue line) and the simulation (grey line) of SCNP (**2**) with a resolution of 40000. Species ∞, ≈, ▶ and species ▽ have an approximated ratio of ∞:≈:▶:▽ = 10:3:4:3.

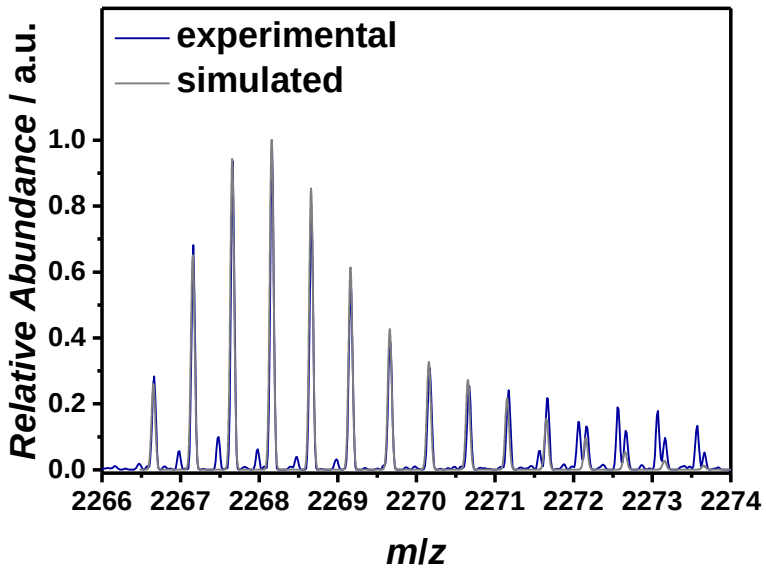


Figure S13 Isotopic simulation of a selected peak at *m*/*z* 2268 comparing the experiment (blue line) and the simulation (grey line) of SCNP (**2**) with a resolution of 40000. Species ◆, ∗ and species ☒ have an approximated ratio of ◆:∗:☒ = 5:1:1.

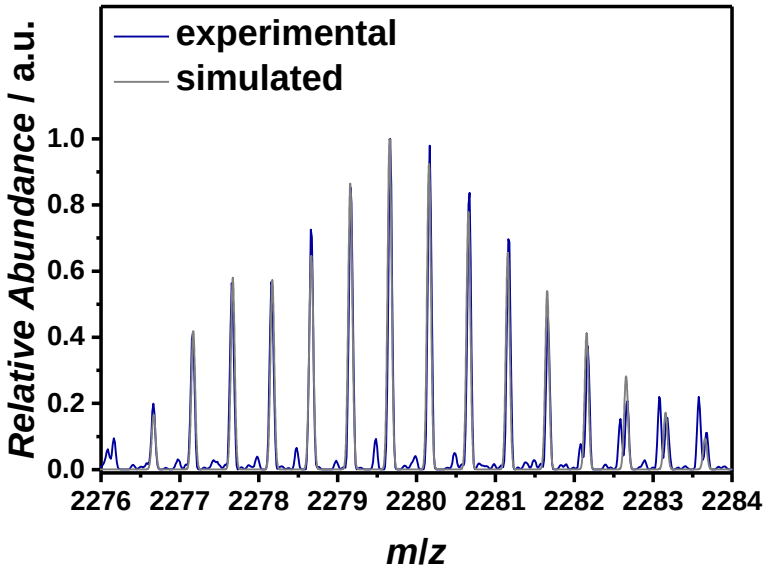
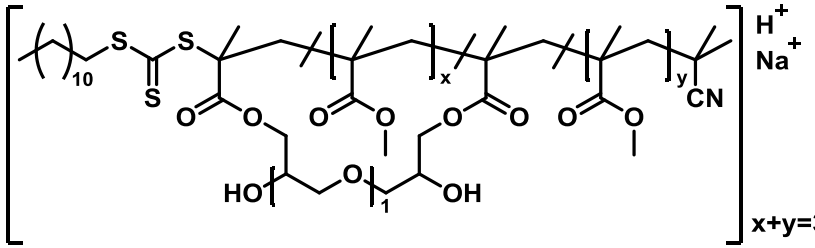
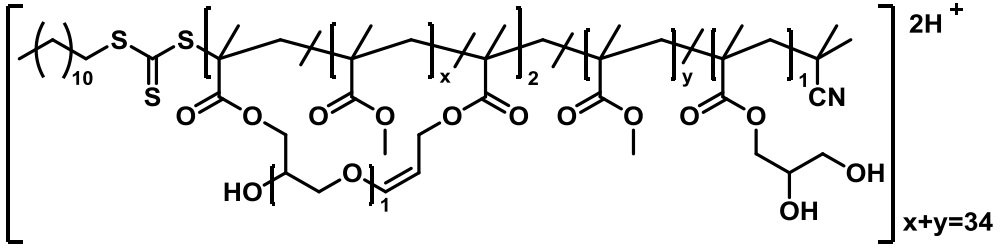
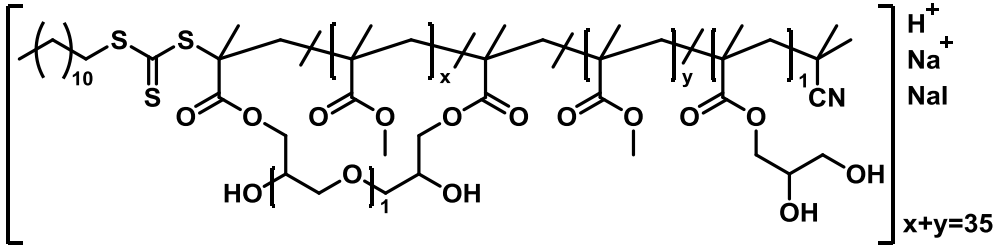
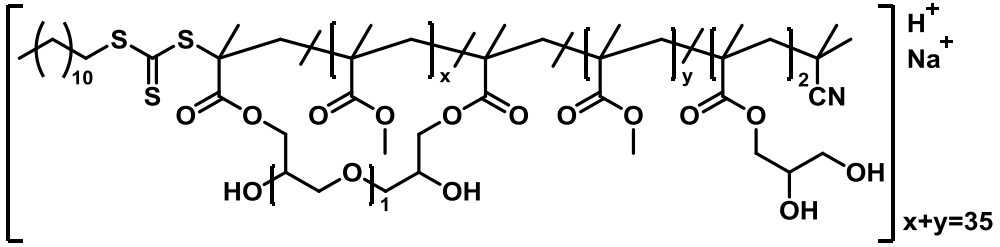
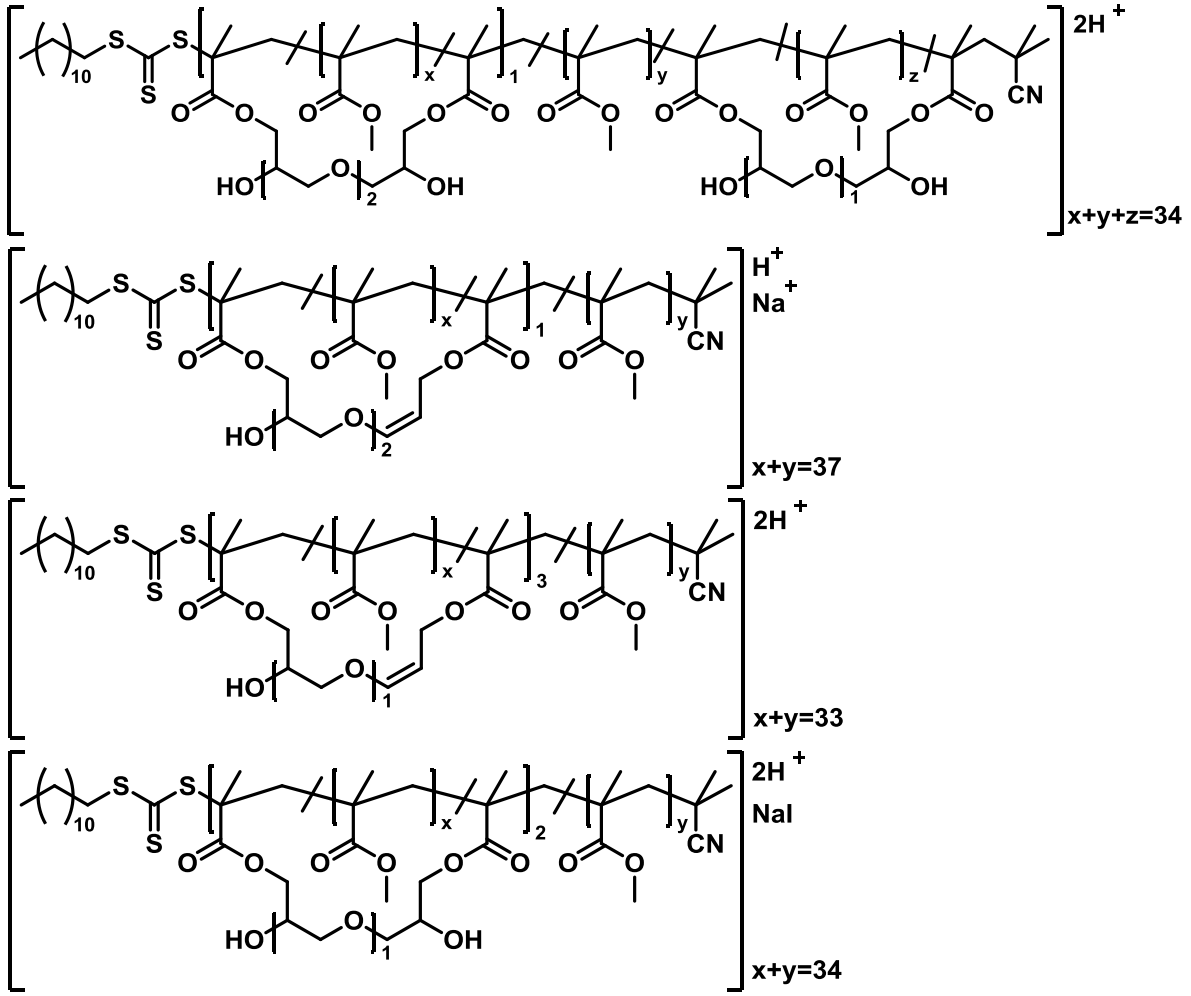


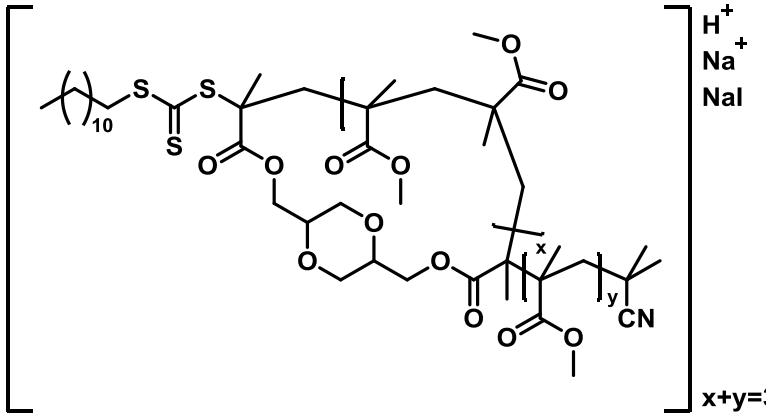
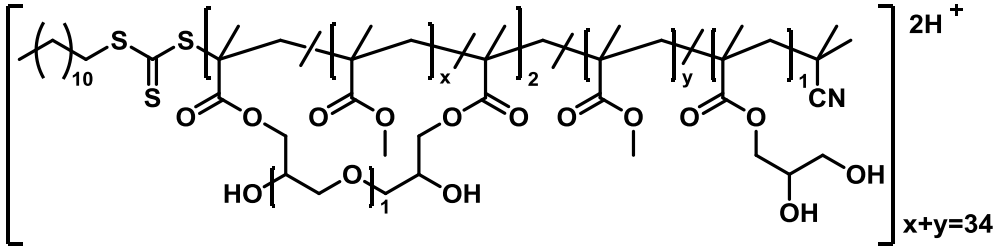
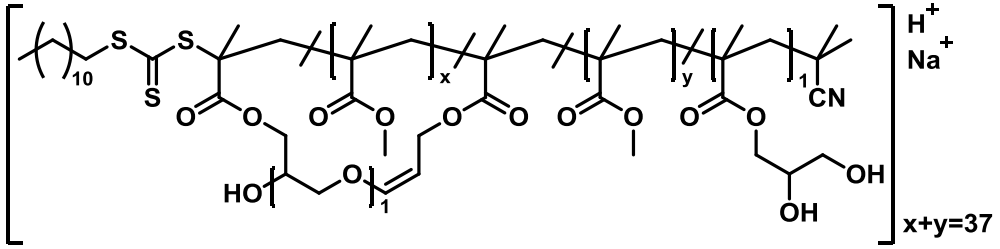
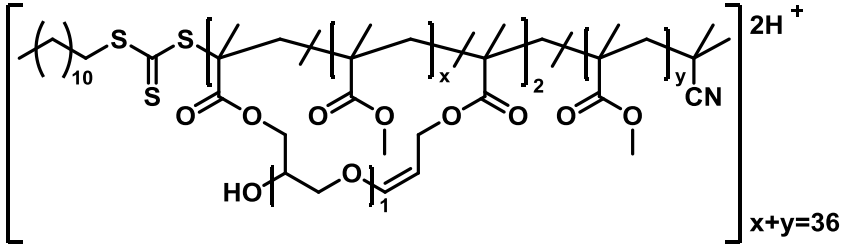
Figure S14 Isotopic simulation of a selected peak at *m*/*z* 2258 comparing the experiment (blue line) and the simulation (grey line) of SCNP (**2**) with a resolution of 40000. Species ❍, ❒, △ and species ↯ have an approximated ratio of ❍:❒:△:↯ = 2:2:3:1.

Table S2 Peak assignment of the SEC-ESI Orbitrap mass spectrum of SCNP (**2**) showing the label (corresponding to the species in ***Figure S9***), the resolution (obtained by the Xcalibur software), the experimental *m*/*z* and theoretical *m*/*z* values, Δ*m*/*z* and the proposed structures. SEC-ESI Orbitrap mass spectrum was integrated from 14.42 mL to 14.92 mL to obtain sufficient signal to noise ratio.

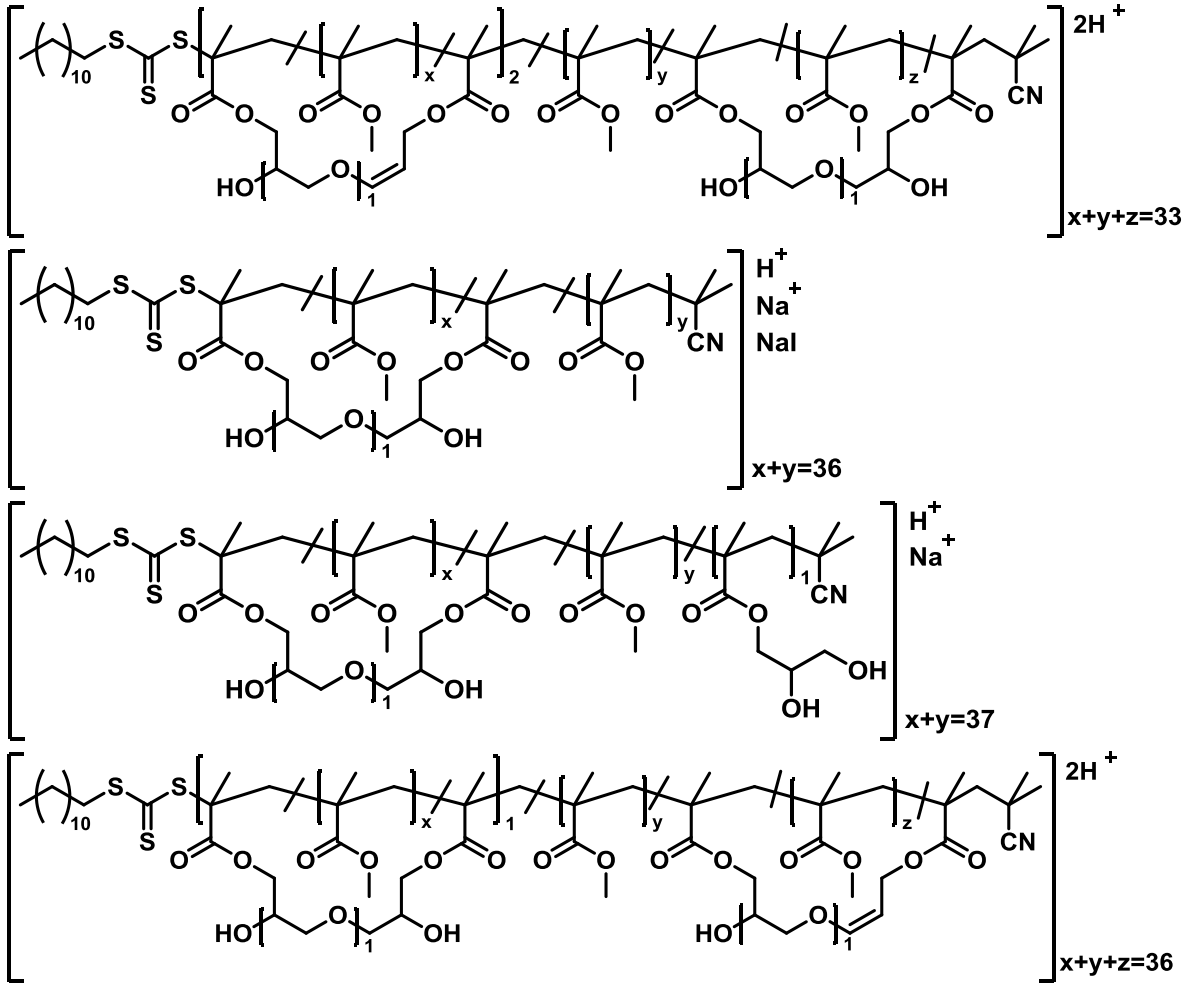
Label	Resolution	*m*/*z*(exp)	*m*/*z*(theo)	Δ*m*/*z*	Structure
■	40000	2236.6506	2236.6436	0.0070	
⊕	44000	2239.6546	2239.6454	0.0093	
×	44000	2241.5566	2241.5489	0.0077	
●	42000	2246.6452	2246.6385	0.0067	

△	42000	2248.1500	2248.1492	0.0008
⊡	40000	2249.6551	2249.6467	0.0084
∅	40000	2250.6579	2250.6423	0.0152
⏚	40000	2251.5541	2251.5632	0.0090

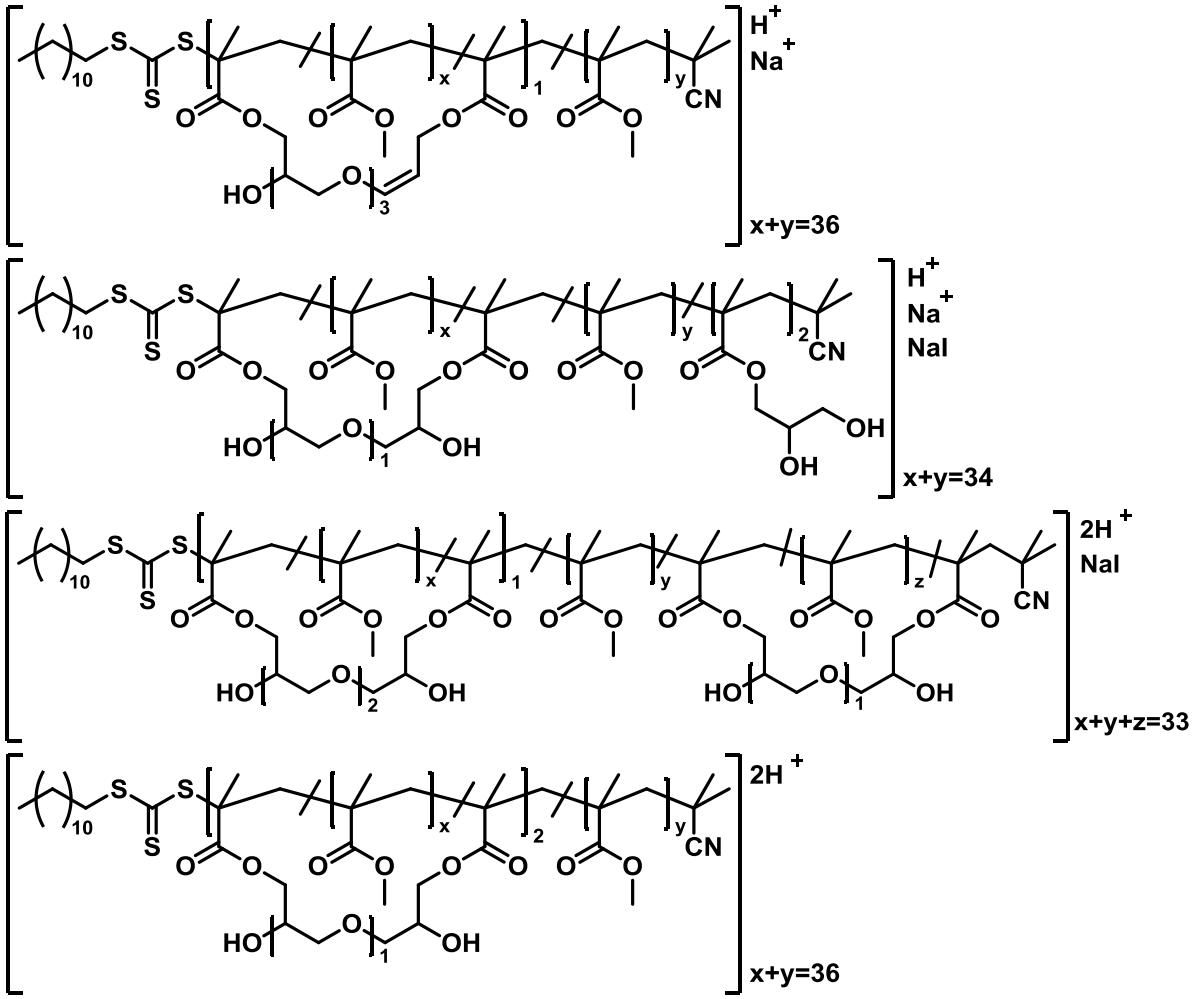


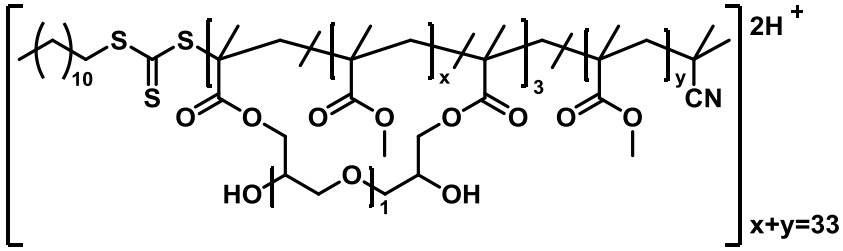
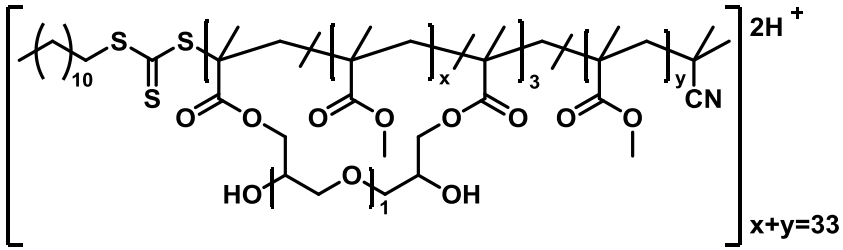
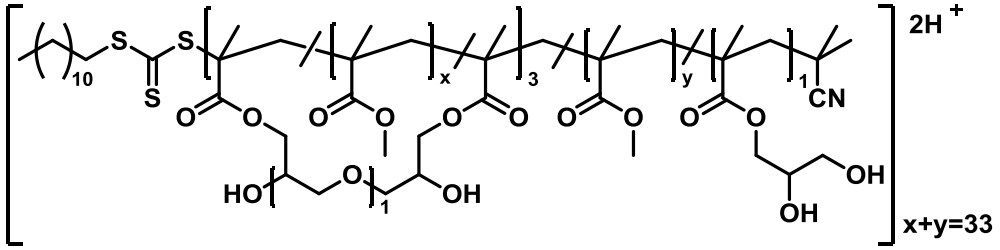
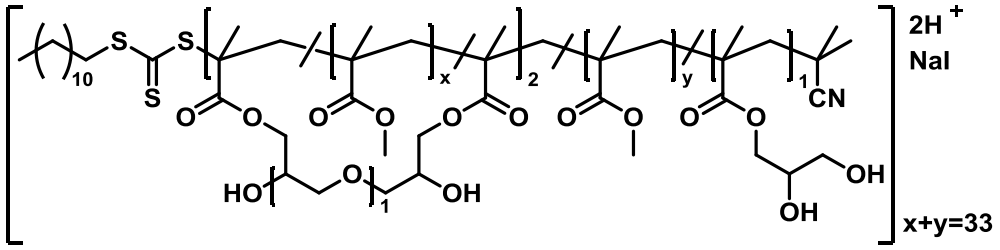
❄	42000	2252.5548	2252.5592	0.0045	
∞	40000	2256.6660	2256.6528	0.0131	
≈	43000	2258.6646	2258.6520	0.0126	
▶	41000	2259.6600	226604	0.0004	

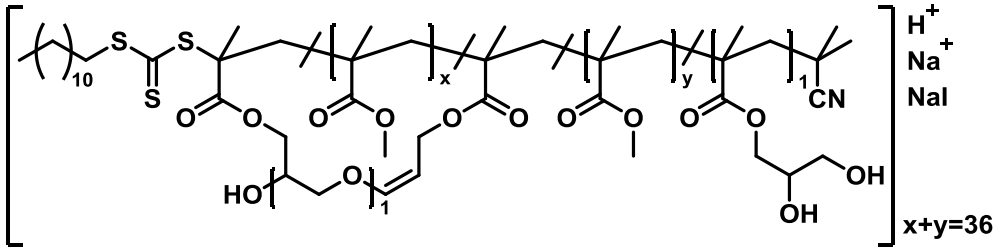
▽	40000	2260.6594	2260.6506	0.0088
♋	48000	2261.5678	2261.5678	0.0032
◆	42000	2266.6604	2266.6542	0.0062
∗	44000	2268.6648	2268.6663	0.0015



☒	40000	2270.6725	2270.6520	0.0205
⌘	39000	2271.5674	2271.5594	0.0080
≈	44000	2272.5667	2272.5685	0.0018
❍	40000	2276.6685	2276.6611	0.0074



❒	40000	2278.6612	2278.6667	0.0055	
△	40000	2279.6684	2279.6573	0.0111	
ϟ	42000	2281.6689	2281.6559	0.0130	
♍	46000	2281.5771	2281.5737	0.0034	

⊙	43000	2283.5826	2283.5729	0.0097	

SEC-ESI MS data of SCNP (2) obtained by slow addition of $B(C_6F_5)_3$ (syringe pump)

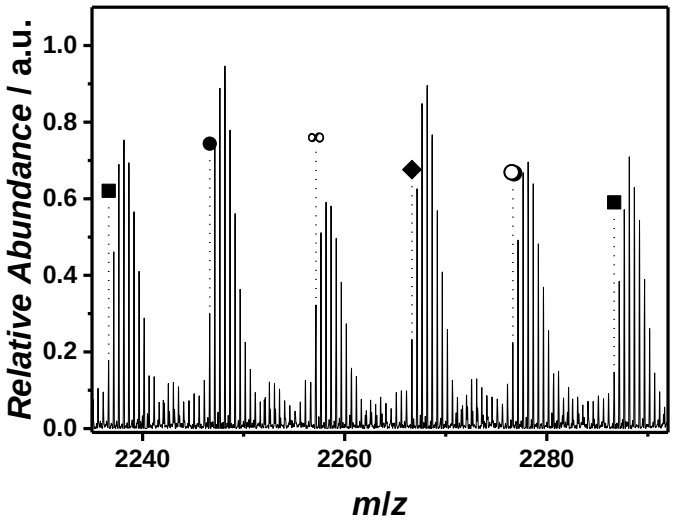


Figure S15 Zoomed SEC-ESI Orbitrap mass spectrum (positive mode) of SCNPs (**2**) between *m*/*z* 2235 and *m*/*z* 2292 obtained by summing all species between 14.32 mL and 15.18 mL retention volume. The labelling is identical to Fig. S9.

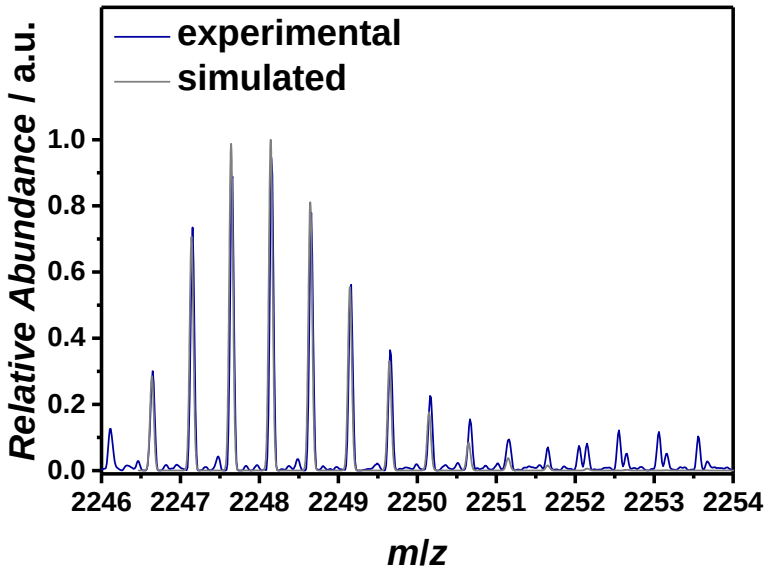


Figure S16 Isotopic simulation of a selected peak at *m*/*z* 2248 comparing the experiment (blue line) and the simulation (grey line) of SCNP (**2**) with a resolution of 40000. Species ● and species △ have an approximated ratio of ●:△ = 10:1.

SEC-ESI MS/MS data of SCNP (2)

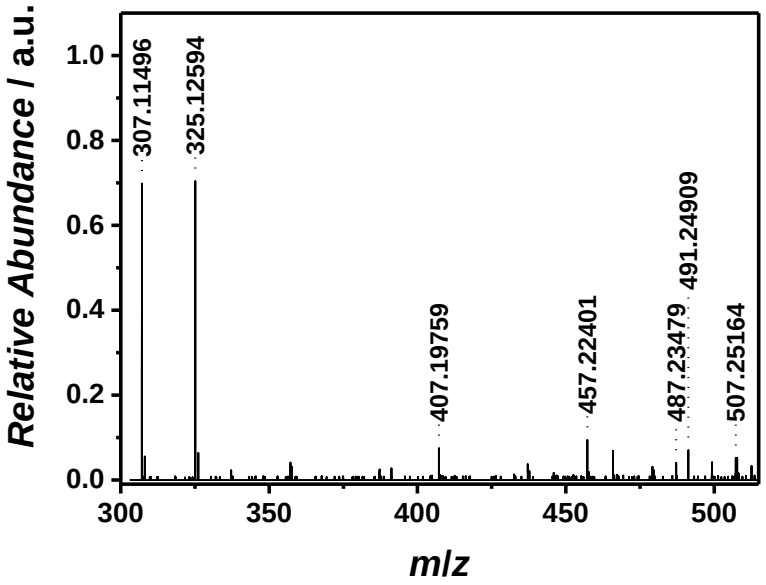
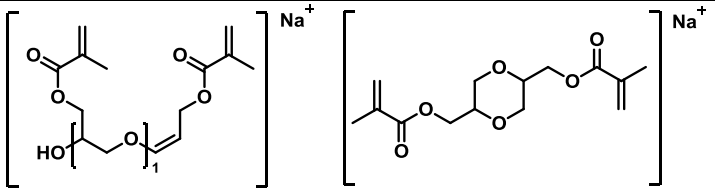
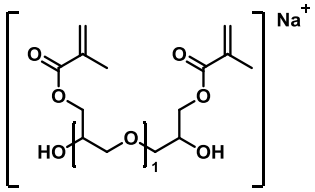
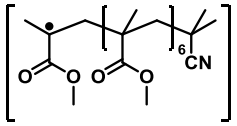
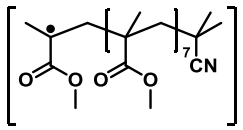
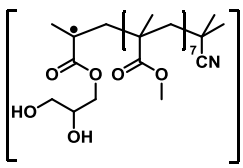
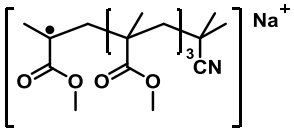
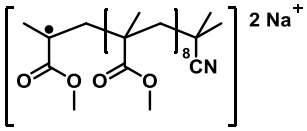


Figure S17 SEC-ESI MS/MS (tandem MS in positive mode) of a double charged species at 2238 *m*/*z* with a higher-energy collision dissociation (HCD) of 25 eV in the relevant range from 300 *m*/*z* and 515 *m*/*z*.

Table S3 Peak assignment of SEC-ESI MS/MS experiment of SCNP (**2**) at 2238 m/z with a higher-energy collision dissociation (HCD) of 25 eV showing the experimental *m*/*z*, the theoretical *m*/*z* values, and Δ*m*/*z* and the proposed structure.

***m*/*z*(exp)**	***m*/*z*(theo)**	**Δ*m*/*z***	**Structure**
307.11496	307.11495	0.00001	
325.12594	325.12591	0.00003	
407.19759	407.19731	0.00028	
457.22401	457.22395	0.00006	
487.23479	487.23452	0.00026	

491.24909	491.24896	0.00013	
507.25164	507.25017	0.00147	

SEC-ESI MS data of SCNP (2) with 10% GMA in comparison with 15% GMA

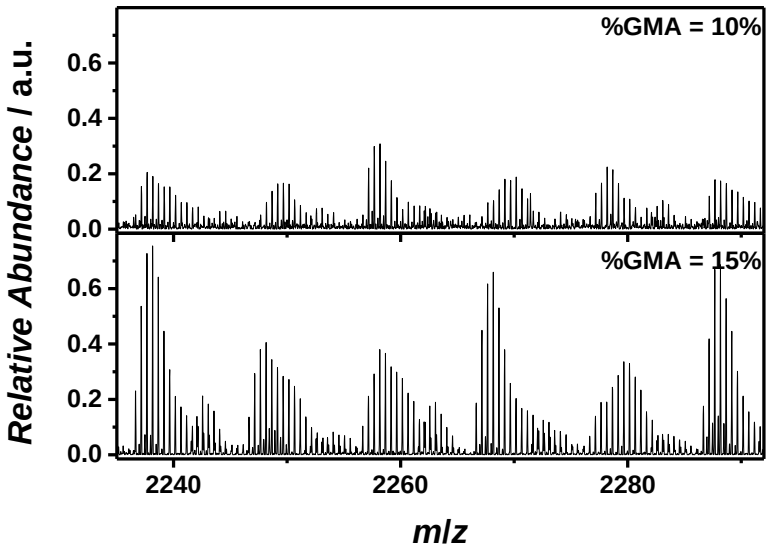


Figure S18 (Top) SEC-ESI Orbitrap mass spectrum of SCNP (**2**) with 10% GMA; (bottom) SEC-ESI spectrum of SCNP (**2**) with 15% GMA. The spectrum ranges from *m*/*z* 2235 to *m*/*z* 2292 illustrating double charged species.

References

(1) Gruendling, T.; Guilhaus, M.; Barner-Kowollik, C. *Anal. Chem.* **2008**, *80*, 6915–6927.