Revised; 20 June 2016

Chain-end and backbone analysis of poly(*N*-isopropylacrylamide)s using
sequential electron transfer dissociation and collisionally activated
dissociation

Selim Gerislioglu, Chrys Wesdemiotis*

Department of Chemistry, The University of Akron, Akron, OH 44325, USA

* Corresponding author. Tel.: +1 330 972 7699; fax: +1 330 972 6085. E-mail address:
wesdemiotis@uakron.edu (C. Wesdemiotis).

ABSTRACT

Electrospray ionization multistage mass spectrometry (ESI-MSⁿ) was employed for the structural characterization of poly(*N*-isopropylacrylamide) (pNIPAM) compounds, a widely used class of thermoresponsive materials. Experiments were performed on singly and doubly sodiated pNIPAMs terminated with thiopropionic acid or thiopropyl triethoxysilane substituents, using collisionally activated dissociation (CAD) and electron transfer dissociation (ETD) techniques. Single stages of CAD or ETD only caused small neutral losses diagnostic of the side chains and end groups. A more thorough structural characterization of the pNIPAMs was possible by applying a sequential stage of CAD on fragments generated by ETD, as no surviving charge-reduced precursor was present in the ETD spectra. The ETD fragments were selected such that they preserved differentiation of the two chain ends and contained features that led to random backbone cleavages. ETD fragments with an unpaired electron or with a particularly stable charge site fulfilled this requirement, giving rise to backbone fragments that made it possible to characterize the chain connectivity in addition to the end group and side chain structures. Whether a radical ion or an ion with a well stabilized charge site is used to obtain structurally diagnostic fragments by ETD-CAD depends on the end groups, the functional group being reduced in the ETD step and the structure of the ETD fragment chosen for consecutive CAD.

Keywords: polyacrylamides, pNIPAM, ETD, ETD coupled with CAD, end group analysis, polymer chain structure

1. Introduction

Tandem mass spectrometry (MS^2) is increasingly used to address analytical problems in polymer and materials science [1-3]. This technique provides a convenient and straightforward means to identify the end groups or central substituents of polymer chains [4-7], determine copolymer sequences [8-12] and distinguish (co)polymer architectures [13-16]. Although MS^2 experiments on synthetic macromolecular ions have mainly employed collisionally activated dissociation (CAD) [17], the closely related methods of electron capture dissociation (ECD) [18] and electron transfer dissociation (ETD) [19,20], which involve electron addition to multiply charged precursor ions formed by electrospray ionization (ESI), have emerged as promising alternatives to CAD, as they tend to break different bonds (compared to CAD), thus rendering complementary structure information for synthetic polymers [21-26].

ETD can be combined sequentially with CAD to improve the fragmentation extent and sequence coverage of the precursor ion [26-29]. In all cases reported thus far, the charge-reduced precursor ion resulting from the ETD step was activated further via CAD to increase the number of competitive and consecutive dissociation channels and gain more comprehensive structural information. Here, we introduce a variant of this strategy for precursor ions that result in ETD spectra where intact charge-reduced species do not survive or do not have the intensity necessary for further excitation. It involves CAD on high-mass ETD fragments formed by small neutral losses and having structural characteristics that promote further fragmentation, such as a new radical site or a different charge site as compared to the precursor ion; this will be illustrated for chain-end functionalized poly(*N*-isopropylacrylamide)s (pNIPAM)s.

pNIPAMs are widely used as smart materials because of their thermoresponsive properties [30]. Their lower critical solution temperature (LCST) is ideally suitable for the

manufacture of stimuli-responsive polymers for biomedical applications, like controlled drug delivery [31], bioseparations [32] and tissue engineering [32,33]. The LCST is tuned for the desired application by adjusting the hydrophilic or hydrophobic character of the polymer through introduction of the proper side chain [32] and end group [34] substituents, the choice of monomers in copolymeric materials [35] and the architecture of the final product [36]. Full structural characterization of these molecular properties is essential for creating materials with the physical and mechanical properties needed for the planned use.

MS² experiments can in principle provide the structural information sought [1-16]. As found in this study, however, single stages of CAD or ETD do not cause random fragmentation along the pNIPAM chain, which is needed to determine backbone connectivity and sequence. Both methods rather trigger losses of small molecules from the end groups and amide side chains, which suppress competitive dissociations in the backbone chain. Furthermore, ETD does not produce a surviving charge-reduced species to induce consecutive CAD [26-29]. Fortunately, the small neutral losses taking place upon ETD result in products containing an unpaired electron or a newly formed salt bridge which, as will be shown here, promote further fragmentation in a successive CAD stage, giving rise to fragmentation patterns that provide full backbone connectivity.

2. Experimental

2.1. Chemicals

Thiopropyl triethoxysilane terminated poly(*N*-isopropylacrylamide) (**1**) and thiopropionic acid terminated poly(*N*-isopropylacrylamide) (**2**) with average molecular weights (M_n) of 2750

and 2000 Da, respectively, as well as the solvents used for sample preparation (methanol, MeOH and tetrahydrofuran, THF) were purchased from Sigma-Aldrich, St. Louis, MO. All were used in the condition received without further purification.

2.2. Mass spectrometry experiments

All experiments were performed on a Bruker HCT Ultra II quadrupole ion trap mass spectrometer equipped with ESI (Bruker Daltonics, Billerica, MA). Sample solutions were prepared in MeOH/THF (1/1, v/v) at the concentration of 5 $\mu\text{g/mL}$ and injected into the ESI source using a syringe pump at a flow rate of 180 $\mu\text{L/h}$. MS^2 experiments via CAD or ETD were performed on singly, doubly or triply sodiated ions. MS^3 experiments via sequential ETD and CAD were carried out on doubly sodiated ions, which were sufficiently intense to permit two fragmentation stages. For CAD, the isolation width was kept at 1.0 and the amplitude of the excitation RF field between 0.23 and 0.30 (arbitrary units), depending on the precursor ion isolated. For ETD, fluoranthene radical anions (reagent ions) were produced in a negative chemical ionization (nCI) source filled with methane buffer gas (2.0-2.6 bar), which is located above the octapole lens that transfers ions from either the ESI or the nCI source to the ion trap. The reagent anion intensity was optimized at the following conditions: reagent ion ICC 100,000; ionization energy 70 eV; emission current 2.0 μA ; and reagent remove cutoff m/z 210. After accumulation of both types of species in the ion trap, the ion/ion reaction time and cutoff parameters were set within 50-170 ms and 150-170, respectively; these values were optimized during the MS^2 experiments to maximize the fragment ion abundances for the next fragmentation stage. MS and MS^n data were analyzed using Bruker's Compass DataAnalysis v.4.0 program.

3. Results and discussion

The polymers investigated were pNIPAM **1** and **2** (cf. Supplementary Material, Fig. S1), which carry a 3-mercaptopropyl triethoxysilane group at the α (initiating) chain end [37] and a 3-mercaptopropanoic acid group at the ω (terminating) chain end [38], respectively. The ESI mass spectra of both samples showed singly charged $[M + Na]^+$ and doubly charged $[M + 2Na]^{2+}$ oligomers (Fig. S1). Additionally, a few minor distributions were observed, arising from protonated $[M + H]^+$ and triply sodiated $[M + 3Na]^{3+}$ ions. MS² experiments were performed on singly, doubly and triply charged precursor ions. The CAD fragmentation patterns of all charge states and the ETD fragmentation patterns of the doubly and triply charged ions were, however, very similar (see, for example, Fig. 2 vs. S3 for CAD and Fig. 3a vs. S4 for ETD datasets). For brevity, only the data obtained from the doubly sodiated precursors will be discussed here.

3.1. MS² experiments via CAD

CAD of $[M + 2Na]^{2+}$ ions from triethoxysilane terminated pNIPAM (**1**) mainly leads to $[M + Na]^+$, as illustrated for the 10-mer in Fig. 1a. Fragments arising by elimination of the α end group (EG, C₉H₂₂O₃SSi, 238 Da) or of isopropyl amine (iPA) and propene (P) from the *N*-isopropylacrylamide pendants are also observed in trace amounts. Sequential CAD (MS³) on the major primary CAD product, viz. $[M + Na]^+$, increases substantially the dissociation extent via isopropyl amine, propene and α end group losses (Fig. 1b) and also causes some backbone fragmentation, leading to minor sodiated fragments with the connectivity CH₂=C(CONH-iC₃H₇)-[CH₂CH(CONH-iC₃H₇)]_{*n*-1}-H, which were labeled by y_n in Fig. 1b (vide infra for an explanation of the fragment nomenclature).

The small neutral losses dominating the CAD spectra of **1** are accounted for by charge-remote 1,5-H rearrangement and intramolecular nucleophilic displacement in the amide side chains, or by homolytic C-S bond cleavage at the α chain end, which result in the elimination of propene, isopropyl amine and the entire α end group, respectively, cf. Supplementary Material Scheme S1. On the other hand, previous studies have shown that backbone cleavages in linear polyacrylates proceed through homolytic C-C bond scissions along the chain, followed by typical radical-induced reactions, including backbiting, monomer evaporation (unzipping via consecutive monomer losses) and β scission of an H atom or the side chain [13].

In CAD, the internal energy gained by collisions is distributed over the entire molecule before fragmentation, thus promoting dissociations with low energy requirements [2,3]. The major dissociation pathways observed (Scheme S1) indeed involve energetically favorable 6-membered ring transition states or the cleavage of relatively weak C-S bonds, which require less energy than C-C bond cleavages in the polyacrylate chain [2,13], justifying their dominance upon CAD.

$[M + 2Na]^{2+}$ ions from the acid terminated pNIPAM (**2**) dissociate much more efficiently upon CAD than their congeners from **1**. Already a single CAD stage (MS^2) generates abundant singly and doubly charged fragments via competitive and consecutive losses of isopropyl amine, propene or the entire ω end group ($C_3H_6O_2S$, 106 Da), as attested by the spectrum of the 10-mer in Fig. 2. The fragmentation pattern is very similar with that of **1** (i.e., both undergo very similar neutral losses). The much higher fragment ion yield from **2** vis à vis **1** is attributed to the different end groups. The acid functionality at the chain end of **2** can bind a Na^+ ion much more strongly than the ether groups at the chain end of **1**. This reduces the tendency of $[2 + 2Na]^{2+}$ to lose Na^+ , thus facilitating alternative dissociation channels, such as the small neutral losses in

Scheme S1. Additionally, the proton of the acid functionality may become mobile upon CAD, opening a low-energy, charge-induced fragmentation channel for side chain losses that is unavailable to $[1 + 2\text{Na}]^{2+}$, cf. Scheme S2. The favorable energetics of all these small neutral losses [2,13] suppress backbone cleavages in $[2 + 2\text{Na}]^{2+}$.

The CAD characteristics of sodiated **1** and **2** clearly indicate that facile rearrangement fragmentations at the pNIPAM side chains hinder backbone cleavages, especially when the polymer chains also carry labile C-S bonds at their termini. Using multiple CAD stages does not change significantly the preference for dissociations at the side chains or end group (cf. Figs 1a and 1b). Based on our findings, CAD of pNIPAMs unveils useful information about the side chain and end group functionalities, but the fragmentations occurring at the latter sites suppress the random backbone cleavages needed to characterize the chain connectivity (architecture) of such polymers or to determine the sequence of pNIPAM copolymers. ETD was therefore examined for its suitability to deduce the latter structural features.

3.2. MS^2 experiments via ETD

ETD experiments on $[M + 2\text{Na}]^{2+}$ ions from **1** and **2** did not yield the desired backbone cleavages, cf. Fig. 3. The major fragments observed in the ETD spectra are formed by elimination of small neutrals, as was the case in CAD. In contrast to CAD, however, there are substantial differences in the ETD pathways of doubly sodiated **1** and **2**. For example, $\text{Na}^\bullet$ loss to form $[M + \text{Na}]^+$ proceeds much more efficiently for **1** than for **2** (Fig. 3), whereas the opposite is true for $\text{H}^\bullet$ loss to form $[M + 2\text{Na} - \text{H}]^+$. Moreover, certain fragments are only observed from one of the pNIPAMs, including $[M + 2\text{Na} - \text{EG} - \text{H}]^+$ (only from **1**) and $[M + 2\text{Na} - \text{iPA} -$

CO]⁺• (only from **2**). These differences are accounted for if the electron is transferred to distinct sites of pNIPAM **1** and **2**. The most likely electron attachment site on the polymer chain of [**1** + 2Na]²⁺ is the carbonyl group of a side chain (Scheme 1a). The resulting charge-reduced species [**1** + 2Na]⁺• can lose a C₃H₇• radical and the α end group (+ H•) through the radical- and charge-catalyzed dissociations rationalized in Scheme 1a. Conversely, the absence of end group loss from [**2** + 2Na]²⁺ provides evidence that electron attachment occurs primarily at the carbonyl group of the acid chain end, possibly because it is more readily accessible and to a lesser extent hydrogen-bonded than the amide groups. This is corroborated by the high relative intensity of the fragment formed by H• loss which can readily proceed from the acid-reduced species, cf. Scheme 1b (left). Further, the O[•] site created in [**2** + 2Na]⁺• after electron addition to the acid chain end can facilitate intramolecular H⁺/Na⁺ migration as well as nucleophilic displacement at the adjacent amide through a favorable six-membered ring transition state (Scheme 1b, right), resulting in the elimination of carbon monoxide plus isopropyl amine (combined mass of 87 Da) to form [M + 2Na – iPA – CO]⁺•, the main ETD product of acid terminated **2**.

In addition to the small neutral losses discussed, the ETD spectrum of triethoxysilane terminated [**1** + 2Na]²⁺ shows two singly charged product ions with higher mass than the precursor ion. Based on their *m/z* values, these ions correspond to the association product of the precursor ion and the fluoranthene reagent anion, viz. [M + 2Na + C₁₆H₁₀]⁺• (*m/z* 1617.0), and the methanol adduct of the reduced precursor ion, viz. [M + 2Na + CH₃OH]⁺• (*m/z* 1446.9). A methanol adduct is also observed for the main ETD product of **2**, viz. [M + 2Na – iPA – CO]⁺• (*m/z* 1227.7). The substantial differences between the CAD and ETD spectra of the same ion (Fig. 1a vs. 3a and Fig. 2 vs. 3b) further indicate that ETD is not accompanied by CAD under the experimental conditions used.

MS³ experiments via ETD-CAD

The fragmentations induced by CAD or ETD of pNIPAMs were diagnostic for the structures of their side chain and end group functionalities, but less informative about the corresponding backbone structures. The sequential application of ETD and CAD was therefore tested as a possible solution to this problem.

The two major ETD products from the triethoxysilane terminated $[\mathbf{1} + 2\text{Na}]^{2+}$ ion are two closed-shell fragments with no unpaired electrons, viz. $[\mathbf{1} + \text{Na}]^+$ and $[\mathbf{1} + 2\text{Na} - \text{EG} - \text{H}]^+$ (Fig. 3b). Consecutive CAD of $[\mathbf{1} + \text{Na}]^+$ mainly causes bond cleavages at the end group and side chains (cf. Fig. 1b); on the other hand, MS³ (ETD-CAD) on $[\mathbf{1} + 2\text{Na} - \text{EG} - \text{H}]^+$ produces the desired backbone fragments, as attested for the 10-mer in Fig. 4.

Fragment $[\mathbf{1} + 2\text{Na} - \text{EG} - \text{H}]^+$ is a doubly sodiated singly charged species that contains a resonance-stabilized amide enolate salt bridge, cf. Scheme 1a. It has been well documented that such highly stabilized charge sites promote charge-remote fragmentations [39]. All product ions in the MS³ spectrum of $[\mathbf{1} + 2\text{Na} - \text{EG} - \text{H}]^+$ (Fig. 4) are reconciled by the pathways summarized in Scheme 2, which involve charge-remote homolytic bond cleavages in the pNIPAM chain followed by H[•] transfer between the incipient fragments [2]. The availability of amide protons and the strong binding interactions between Na⁺ ions and amide carbonyl groups [40,41] enables the formation of transient Na⁺-bound and/or hydrogen-bonded complexes between the dissociating radicals, in which interfragment H[•] transfer can occur before complete dissociation. This mechanism results in the formation of unsaturated and saturated end groups (Scheme 2). Only the transfer of tertiary H atoms to the secondary radicals is observed in these reactions due to more competitive energetics compared to the alternative transfer of secondary H atoms to the primary radicals [2]. Further, since the O⁻Na⁺ salt bridge can move along the chain via proton

shifts from the amide side chains, most fragments are observed both singly sodiated (no salt bridge), as well as doubly sodiated (with the salt bridge), cf. Fig. 4 and Scheme 2.

The pNIPAM chain includes two types of bonds, viz. CH₂-CH and CH-CH₂. Cleavages at the CH₂-CH bonds give rise to a-type fragments containing the α chain (a_n^{*} and a_n) end and z-type fragments containing the ω chain end (z_n^{**}), cf. Scheme 2 [2]. The contiguous a_n^{*}, a_n and z_n^{**} series (n = 3-8) produced from [1 + 2Na – EG – H]⁺ confirm the position of the respective end groups (Fig. 4) and the linear chain connectivity of **1**. Conversely, cleavages at the CH-CH₂ bonds give rise to b-type fragments with the α chain (b_n^{**} and b_n) end and y-type fragments with the ω chain end (y_n^{*} and y_n), cf. Scheme 2 [2]. Series b_n and y_n (as well as b_n^{**} and y_n^{*}) are isomeric and, thus, cannot be used to elucidate the chain ends; they still substantiate the linear chain connectivity of **1**. It is noteworthy that the number and intensity of fragments with 9 repeat units from the 10-mer precursor are significantly lower than those of the smaller fragments, consistent with the presence of a six-membered ring with two repeat units at the α chain end, in which the described decompositions cannot take place.

The same multistage fragmentation method was applied to two ETD products of the acid terminated pNIPAM **2**, viz. closed-shell fragment [2 + 2Na – H]⁺ and radical ion [2 + 2Na – iPA – CO]^{•+} (cf. Fig. 3b). The former ion contains a carboxylate salt bridge (Scheme 1b, left) which in analogy to the behavior of [1 + 2Na – EG – H]⁺ should promote charge-remote fragmentations in the pNIPAM chain; however, the MS³ (ETD-CAD) spectrum of [2 + 2Na – H]⁺ (Fig. S2) does not support this expectation. Instead, this ion dissociates almost exclusively by expulsion of small molecules, with loss of the end group being the major fragment. This result is attributed to the facile homolytic cleavage of the CH(A)-S bond (A = amide side chain) at the ω chain end, whose low bond dissociation energy [42] must suppress C-C bond cleavages in the polymer

backbone. The end group lost from $[2 + 2\text{Na} - \text{H}]^+$ includes the Na^+ ion (128 Da), providing evidence that at least one sodium ion is localized on the thiopropionic acid end (see structure in Fig. S2).

In sharp contrast to $[2 + 2\text{Na} - \text{H}]^+$, the radical ion $[2 + 2\text{Na} - \text{iPA} - \text{CO}]^{+\bullet}$ dissociates extensively via backbone cleavages (Fig. 5). Here, all MS^3 (ETD-CAD) fragments can be accounted for by random backbiting events that transfer the radical site created initially at the ω end group to the tertiary (amide-substituted) C-atoms of the pNIPAM backbone, from where the unpaired electron can induce homolytic β C-C bond scissions (Schemes 3 and S3). Cleavage of the C-C bond proximal to the α chain end (Scheme 3) would generate $b_n^\bullet$ radicals and $y_n^{*\bullet}$ molecules; only b_n and $y_n^{*\bullet}$ fragments are observed, however, strongly suggesting that dissociation proceeds through Na^+ - and/or hydrogen-bonded complexes in which interfragment $\text{H}^\bullet$ transfer takes place. On the other hand, cleavage of the C-C bond proximal to the ω chain end (Scheme S3) would generate a_n molecules and $z_n^{*\bullet}$ radicals and, after interfragment $\text{H}^\bullet$ transfer, $a_n^\bullet$ and $z_n^{*\bullet}$; in this case, all four types are formed.

In addition to the fragments mentioned, closed-shell z-type ions are detected that are missing the amide side chain (marked by $z_n^* - \text{A}$ in Fig. 5). These most likely originate by consecutive decomposition of the $z_n^{*\bullet}$ fragments through a favorable six-membered ring transition state, cf. Scheme S4. A notable difference between the MS^3 characteristics of **1** and **2** is that the salt bridge of $[2 + 2\text{Na} - \text{iPA} - \text{CO}]^{+\bullet}$ does not move along the chain but remains at the ω chain end; as a consequence all fragments carrying the α chain end (a_n , $a_n^\bullet$ and b_n) are only observed in singly sodiated form, while all fragments carrying the ω chain end ($y_n^{*\bullet}$, z_n^* , $z_n^{*\bullet}$ and $z_n^* - \text{A}$) are consistently doubly sodiated. The COO^-Na^+ salt bridge does not tautomerize to

an amide enolate salt bridge because of its higher thermodynamic stability, endowed by the higher gas-phase acidity of carboxylic acids vs. amides [43].

4. Conclusions

Single- and double-stage CAD on pNIPAM ions with thioethers groups produce only or predominantly fragments characteristic of the side chain and end group substituents. MS² experiments utilizing ETD result in less sequential decompositions, but again cause mainly small neutral losses from the side chains or end groups. Unfortunately, these activation modes do not render sufficient structural information on the backbone. Consecutive fragmentation via CAD of select ETD products does, however, cause extensive fragmentation in the polyacrylamide backbone, thus allowing for full structural characterization. The increased fragmentation efficiency resulting by MS³ experiments utilizing sequential ETD and CAD steps is partly the result of the higher internal energy deposited in the combined ETD-CAD venue than in a single stage of ETD or CAD. The main reason is a new structural feature in the initial ETD fragment, which opens new dissociation pathways unavailable to the original precursor ion. This new structural feature can be a mobile radical site that can migrate to the polymer backbone (through H rearrangements) to induce β C-C bond scissions, or a particularly stable, salt-bridge containing charge site that promotes random charge-remote fragmentations in the polymer backbone. Which feature is instilled upon ETD and the ensuing fragmentation pathways enabled in this process depend on the end groups.

Acknowledgements

This project was supported by the National Science Foundation (grant CHE-1308307).

References

1. A.C. Crecelius, A. Baumgaertel, U.S. Schubert, Tandem mass spectrometry of synthetic polymers, *J. Mass Spectrom.* 44 (2009) 1277-1286.
2. C. Wesdemiotis, N. Solak, M.J. Polce, D.E. Dabney, K. Chaicharoen, B.C. Katzenmeyer, Fragmentation pathways of polymer ions, *Mass Spectrom. Rev.* 30 (2011) 523-539.
3. V. Scionti, C. Wesdemiotis, Tandem mass spectrometry analysis of polymer structures and architectures, in: *Mass Spectrometry in Polymer Chemistry*, C. Barner-Kowollik, T. Gruending, J. Falkenhagen, S. Weidner (Eds.), Wiley-VCH, Weinheim, 2012, pp. 57-84.
4. T. Yalcin, W. Gabryelski, L. Li, Structural analysis of polymer end groups by electrospray ionization high-energy collision-induced dissociation tandem mass spectrometry, *Anal. Chem.* 72 (2000) 3847-3852.
5. K.M. Wollyung, C. Wesdemiotis, A. Nagy, J.P. Kennedy, Synthesis and mass spectrometry characterization of centrally and terminally amine-functionalized polyisobutylenes, *J. Polym. Sci. Part A: Polym. Chem.* 43 (2005) 946-958.
6. S.M. Weidner, J. Falkenhagen, K. Knop, A. Thünemann, Structure and end-group analysis of complex hexanediol-neopentylglycol-adipic acid copolyesters by matrix-assisted laser desorption/ionization collision-induced dissociation tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 23 (2009) 2768-2774.
7. C. Barrere, C. Chendo, T.N.T. Phan, V. Monnier, T. Trimaille, S. Humbel, S. Viel, D. Gigmes, L. Charles, Successful MALDI-MS analysis of synthetic polymers with labile end-groups: The case of nitroxide-mediated polymerization using the MAMA-SG1 alkoxyamine, *Chem. Eur. J.* 18 (2012) 7916-7924.

8. P. Rizzarelli, C. Puglisi, G. Montaudo, Sequence determination in aliphatic poly(ester amide)s by matrix-assisted laser desorption/ionization time-of-flight and time-of-flight/time-of-flight tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 19 (2005) 2407-2418.
9. C. Wesdemiotis, F. Pingitore, M.J. Polce, V.M. Russel, Y. Kim, C.M. Kausch, T.H. Connors, R.E. Medsker, R.R. Thomas, Characterization of a poly(fluorooxetane) and poly(fluorooxetane-co-THF) by MALDI mass spectrometry, size exclusion chromatography, and NMR spectroscopy, *Macromolecules* 39 (2006) 8369-8378.
10. P. Terrier, W. Buchmann, B. Desmazieres, J. Tortajada, Block lengths and block sequence of linear triblock and glycerol derivative diblock copolyethers by electrospray ionization - collision-induced dissociation mass spectrometry, *Anal. Chem.* 78 (2006) 1801-1806.
11. A.M. Yol, J. Janoski, R.P. Quirk, C. Wesdemiotis, Sequence analysis of styrenic copolymers by tandem mass spectrometry, *Anal. Chem.* 86 (2014) 9576-9582.
12. L. Charles, C. Laure, J.F. Lutz, R.K. Roy, MS/MS sequencing of digitally encoded poly(alkoxyamine amide)s, *Macromolecules* 48 (2015) 4319-4328.
13. K. Chaicharoen, M.J. Polce, A. Singh, C. Pugh, Characterization of linear and branched polyacrylates by tandem mass spectrometry, *Anal. Bioanal. Chem.* 392 (2008) 595-607.
14. A.M. Yol, D.E. Dabney, S.-F. Wang, B.A. Laurent, M.D. Foster, R.P. Quirk, S.M. Grayson, C. Wesdemiotis, Differentiation of linear and cyclic polymer architectures by MALDI tandem mass spectrometry (MALDI-MS²), *J. Am. Soc. Mass Spectrom.* 24 (2013) 74-82.
15. A.M. Yol, C. Wesdemiotis, Multidimensional mass spectrometry methods for the structural characterization of cyclic polymers, *React. Funct. Polym.* 80 (2014) 95-108.

16. X. Liu, K. Lin, A.M. Kasko, C. Wesdemiotis, Tandem mass spectrometry and ion mobility mass spectrometry for the analysis of molecular sequence and architecture of hyperbranched glycopolymers, *Analyst* 140 (2015) 1182-1191.
17. L. Sleno, D.A. Volmer, Ion activation methods in tandem mass spectrometry, *J. Mass Spectrom.* 39 (2004) 1091-1112.
18. R.A. Zubarev, N.L. Kelleher, F.W. McLafferty, Electron capture dissociation of multiply charged protein cations. A nonergodic process, *J. Am. Chem. Soc.* 120 (1998) 3265-3266.
19. J.E.P. Syka, J.J. Coon, M.J. Schroeder, J. Shabanowitz, D.F. Hunt, Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry, *Proc. Nat. Acad. Sci.* 101 (2004) 9528-9533.
20. S.J. Pitteri, S.A. McLuckey, Recent developments in the ion/ion chemistry of high-mass multiply charged ions, *Mass Spectrom. Rev.* 24 (2005) 931-958.
21. B.A. Cerda, D.M. Horn, K. Breuker, F.W. McLafferty, Sequencing of specific copolymer oligomers by electron-capture-dissociation mass spectrometry, *J. Am. Chem. Soc.* 124 (2002) 9287-9291.
22. S. Koster, M.C. Duursma, J.J. Boon, R.M.A. Heeren, S. Ingemann, R.A.T.M. van Benthem, C.G. de Koster, Electron capture and collisionally activated dissociation mass spectrometry of doubly charged hyperbranched polyesteramides, *J. Am. Soc. Mass Spectrom.* 14 (2003) 332-341.
23. B. Bogdanov, X.N. Zhao, D.B. Robinson, J.H. Ren, Electron capture dissociation studies of the fragmentation patterns of doubly protonated and mixed protonated-sodiated peptoids, *J. Am. Soc. Mass Spectrom.* 25 (2014) 1202-1216.

24. V. Scionti, C. Wesdemiotis, Electron transfer dissociation versus collisionally activated dissociation of cationized biodegradable polyesters, *J. Mass Spectrom.* 47 (2012) 1442-1449.
25. G. Hart-Smith, A review of electron-capture and electron-transfer dissociation tandem mass spectrometry in polymer chemistry, *Anal. Chim. Acta* 808 (2014) 44-55.
26. B.C. Katzenmeyer, L.R. Cool, J.P. Williams, K. Craven, J.M. Brown, C. Wesdemiotis, Electron transfer dissociation of sodium cationized polyesters: Reaction time effects and combination with collisional activation and ion mobility separation, *Int. J. Mass Spectrom.* 378 (2015) 303-311.
27. D.L. Swaney, G.C. McAlister, M. Wirtala, J.C. Schwartz, J.E.P. Syka, J.J. Coon, Supplemental activation method for high-efficiency electron-transfer dissociation of doubly protonated peptide precursors, *Anal. Chem.* 79 (2007) 477-485.
28. Y. Gao, S.A. McLuckey, Electron transfer followed by collision-induced dissociation (NET-CID) for generating sequence information from backbone modified oligonucleotide anions, *Rapid Commun. Mass Spectrom.* 27 (2013) 249-257.
29. Z. Zhang, S.J. Browne, R.W. Vachet, Exploring salt bridge structures of gas-phase protein ions using multiple stage of ETD and CID, *J. Am. Soc. Mass Spectrom.* 25 (2014) 604-613.
30. D. Roy, W.L.A. Brooks, B.S. Sumerlin, New directions in thermoresponsive polymers, *Chem. Soc. Rev.* 42 (2013) 7214-7243.
31. A.K. Bajpai, S.K. Shukla, S. Bhanu, S. Kankane, Responsive polymers in controlled drug delivery, *Prog. Polym. Sci.* 33 (2008) 1088-1118.

32. Y. Akiyama, Y. Shinohara, Y. Hasegawa, A. Kikuchi, T. Okano, Preparation of novel acrylamide-based thermoresponsive polymer analogues and their application as thermoresponsive chromatographic matrices, *J. Polym. Sci. Part A: Polym. Chem.* 46 (2008) 5471-5482.
33. H. Hatakeyama, A. Kikuchi, M. Yamato, T. Okano, Patterned biofunctional designs of thermoresponsive surfaces for spatiotemporally controlled cell adhesion, growth, and thermally induced detachment, *Biomaterials* 28 (2007) 3632-3643.
34. Q. Duan, Y. Miura, A. Narumi, X. Shen, S.-I. Sato, T. Satoh, T. Kakuchi, Synthesis and thermoresponsive property of end-functionalized poly(*N*-isopropylacrylamide) with pyrenyl group, *J. Polym. Sci. Part A: Polym. Chem.* 44 (2006) 1117-1124.
35. J. Buller, A. Laschewsky, J.-F. Lutz, E. Wischerhoff, Tuning the lower critical solution temperature of thermoresponsive polymers by biospecific recognition, *Polym. Chem.* 2 (2011) 1486-1489.
36. A.P. Vogt, B.S. Sumerlin, Tuning the temperature response of branched poly(*N*-isopropylacrylamide) prepared by RAFT polymerization, *Macromolecules* 41 (2008) 7368-7373.
37. O. Moriya, S. Yamamoto, T. Masuda, M. Kashio, T. Sugizaki, Preparation of thermoresponsive grafted polysilsesquioxane from polyacrylamides having methoxysilyl end group, *Polym. J.* 40 (2008) 1042-1048.
38. J. Cao, S. Liu, Y. Chen, L. Shi, Z. Zhang, Synthesis of end-functionalized boronic acid containing copolymers and their bioconjugates with rod-like viruses for multiple responsive hydrogels, *Polym. Chem.* 5 (2014) 5029-5036.

39. C. Cheng, M.L. Gross, Applications and mechanisms of charge-remote fragmentation, *Mass Spectrom. Rev.* 19 (2000) 398-420.
40. A.L. Heaton, R.M. Moision, P.B. Armentrout, Experimental and theoretical studies of sodium cation interactions with the acidic amino acids and their amide derivatives, *J. Phys. Chem. A* 112 (2008) 3319-3327.
41. P. Wang, G. Ohanessian, C. Wesdemiotis, The sodium ion affinities of asparagine, glutamine, histidine and arginine, *Int. J. Mass Spectrom.* 269 (2008) 34-45.
42. R.T. Sanderson, *Chemical bonds and bond energy*, Academic Press, New York, 1971.
43. C.E. Plummer, M.L. Stover, S.S. Bokatzian, J.T.M. Davis, D.A. Dixon, C.J. Cassady, An experimental and computational study of the gas-phase acidities of the common amino acid amides, *J. Phys. Chem. B* 119 (2015) 9661-9669.

Fig. 1. (a) MS² (CAD) spectrum of the $[M + 2Na]^{2+}$ ion from the 10-mer of triethoxysilane terminated pNIPAM **1** (m/z 707.3) and (b) MS³ (CAD) spectrum of the $[M + Na]^+$ ion (m/z 1391.9) formed by CAD of $[M + 2Na]^{2+}$; iPA = isopropyl amine (59 Da); P = propene (42 Da); EG = end group (3-thiopropyl triethoxysilane, C₉H₂₂O₃SSi, 238 Da).

Fig. 2. MS² (CAD) spectrum of the $[M + 2Na]^{2+}$ ion from the 10-mer of acid terminated pNIPAM **2**; iPA = isopropyl amine (59 Da); P = propene (42 Da); EG = end group (3-thiopropionic acid, C₃H₆O₂S, 106 Da).

Fig. 3. MS² (ETD) spectra of the [M + 2Na]²⁺ ion from the 10-mer of (a) triethoxysilane terminated pNIPAM **1** (m/z 707.3) and (b) acid terminated pNIPAM **2** (m/z 641.3); iPA = isopropyl amine (59 Da); EG = end group of **1** (3-thiopropyl triethoxysilane, C₉H₂₂O₃SSi, 238 Da); C₁₆H₁₀ = fluoranthene, 202 Da). The products marked by & are methanol adducts of (a) [M + 2Na]⁺⁺ and (b) [M + 2Na - iPA - CO]⁺⁺.

Fig. 4. MS³ (ETD-CAD) spectrum of the $[M + 2Na - EG - H]^+$ ion from the 10-mer of triethoxysilane terminated pNIPAM **1** (m/z 1175.8). The $[M + 2Na - EG - H]^+$ ion was generated by ETD of $[M + 2Na]^{2+}$ (m/z 707.3) and induced to fragment via CAD. “A” abbreviates the amide side chain CONH- iC_3H_7 . The fragment nomenclature is explained in the structure at the top of the spectrum [2]; a_n/b_n designate fragments containing the α chain end and y_n/z_n fragments containing the ω chain end. A double prime indicates fragments with one saturated chain end (cf. Scheme 2) and an asterisk fragments containing both Na ions (i.e., a $O^- Na^+$ salt bridge plus a Na^+ charge).

Fig. 5. MS³ (ETD-CAD) spectrum of the $[M + 2Na - iPA - CO]^{+*}$ ion from the 10-mer of acid terminated pNIPAM **2** (m/z 1195.7). The $[M + 2Na - iPA - CO]^{+}$ ion was generated by ETD of $[M + 2Na]^{2+}$ (m/z 641.3) and induced to fragment via CAD. “A” abbreviates the amide side chain $CONH-iC_3H_7$. The fragment nomenclature is explained in the structure at the top of the spectrum [2]. A superscripted $*$ denotes a radical ion and an asterisk fragments containing both Na ions (i.e., a O^-Na^+ salt bridge and a Na^+ charge).

Scheme 1. (a) Dissociations in the triethoxysilane terminated radical ion $[1 + 2Na]^{++}$ formed by electron addition to an amide side chain and leading to the elimination of an $iso-C_3H_7^\bullet$ radical (left) or the α end group plus $H^\bullet$ (right). $R = (CH_2)_3Si(OC_2H_5)_3$; $iso-C_3H_7^\bullet$ can be cleaved after electron addition to any of the side chains, whereas the charge-induced elimination of the α end group shown can only take place in the $[1 + 2Na]^{++}$ population that accepted the electron near the α chain end. (b) Dissociations in the acid terminated radical ion $[2 + 2Na]^{++}$ formed by electron addition to the carboxyl end group and leading to the elimination of a $H^\bullet$ radical (left) or isopropyl amine plus CO (right). The notation rH^+/Na^+ designates intramolecular proton and sodium ion migration. All species contain a second Na^+ charge which has been omitted to avoid congestion.

Scheme 2. Dissociation of pNIPAM ion $[1 + 2\text{Na} - \text{EG} - \text{H}]^+$ via Na^+ -bound radicals.

Additional stabilization via interfragment hydrogen bonds is possible. "A" abbreviates the amide side chain $\text{CONH-iC}_3\text{H}_7$. See Fig. 4 for an explanation of the fragment nomenclature.

Scheme 3. Dissociation of pNIPAM ion $[2 + 2\text{Na} - \text{iPA} - \text{CO}]^{+\bullet}$ to b_n and $y_n^{*\bullet}$ fragments via backbiting, β C-C bond scission and interfragment $\text{H}^\bullet$ transfer through noncovalent (Na^+ - and/or hydrogen-bonded) complexes. “A” abbreviates the amide side chain $\text{CONH-iC}_3\text{H}_7$. See Fig. 5 for an explanation of the fragment nomenclature and Scheme S3 for the pathway to $a_n/a_n^\bullet$ and $z_n^{*\bullet}/z_n^\bullet$ fragments through isomeric noncovalent complexes.

Chain-end and backbone analysis of poly(*N*-isopropylacrylamide)s using sequential electron transfer dissociation and collisionally activated dissociation

Graphical abstract