Dendritic Elastin-Like Peptides: The Effect of Branching on Thermoresponsiveness

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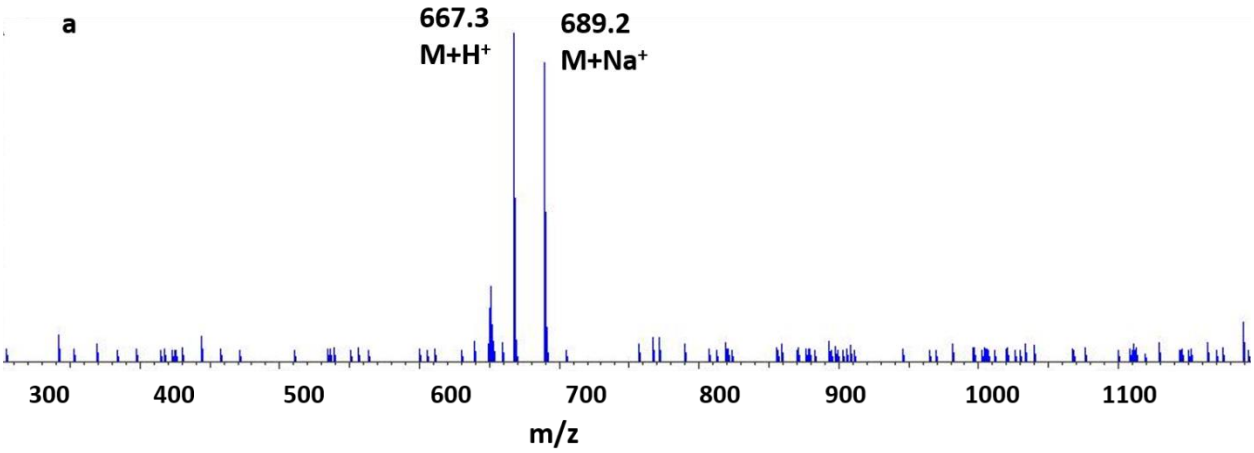
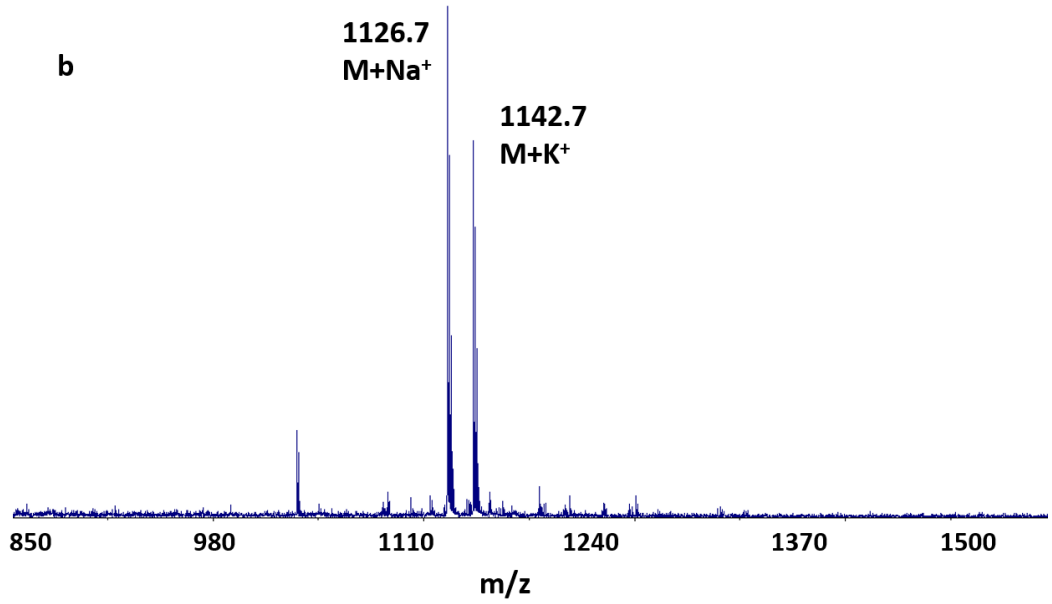
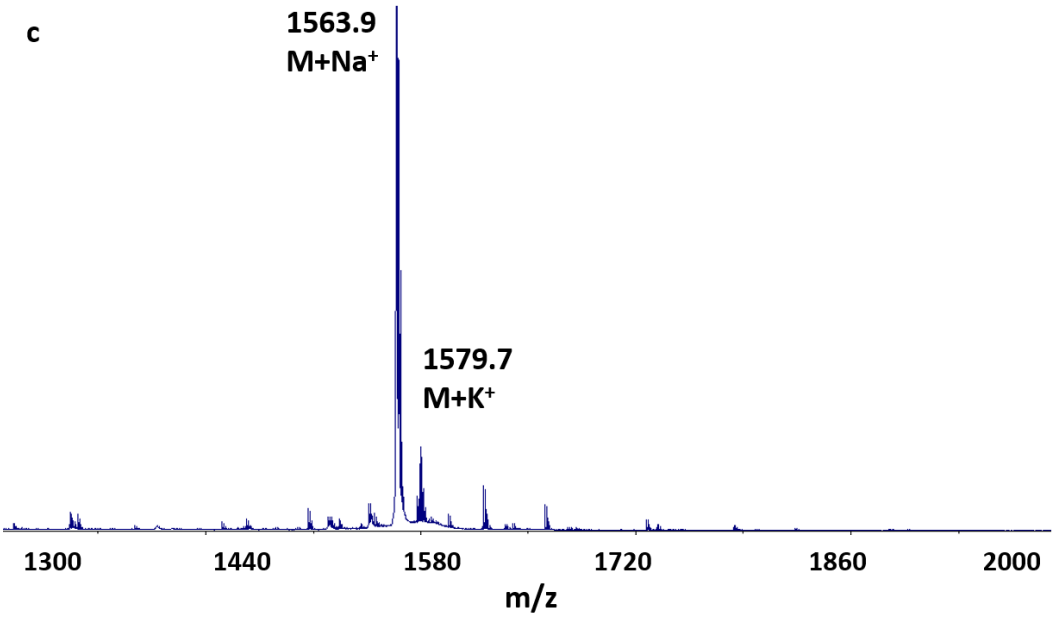
Supporting Information

Synthesis of Branched/Dendritic ELPs

MS spectra of capped (Figure S1) and uncapped (Figure S2) peptides.



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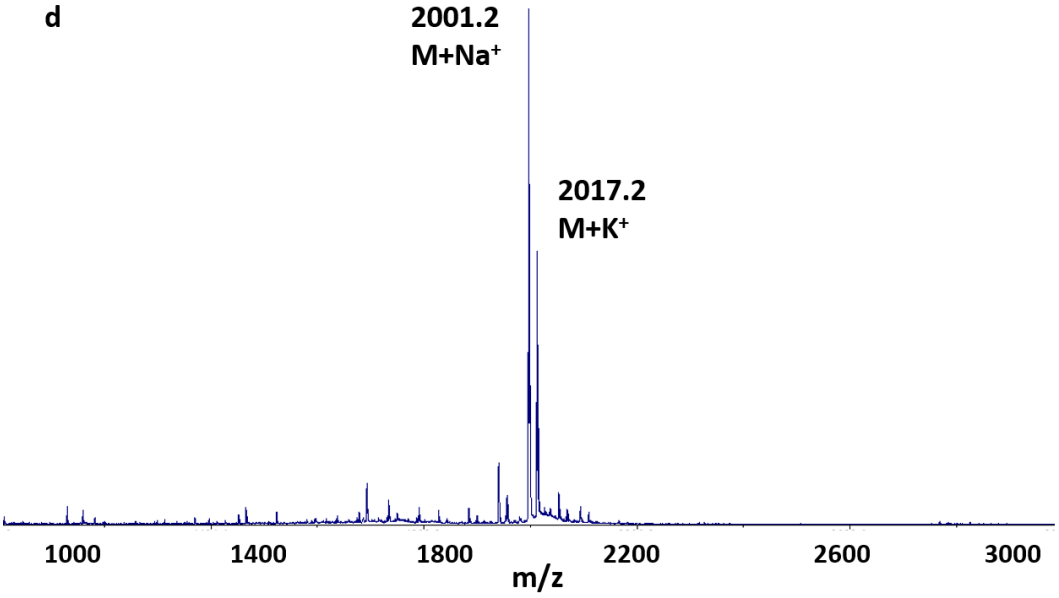
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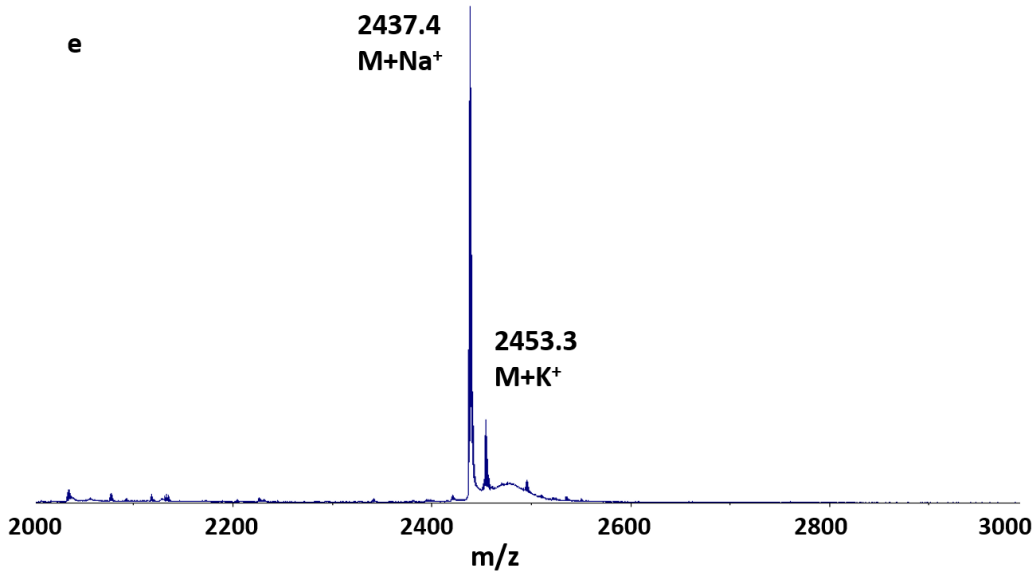
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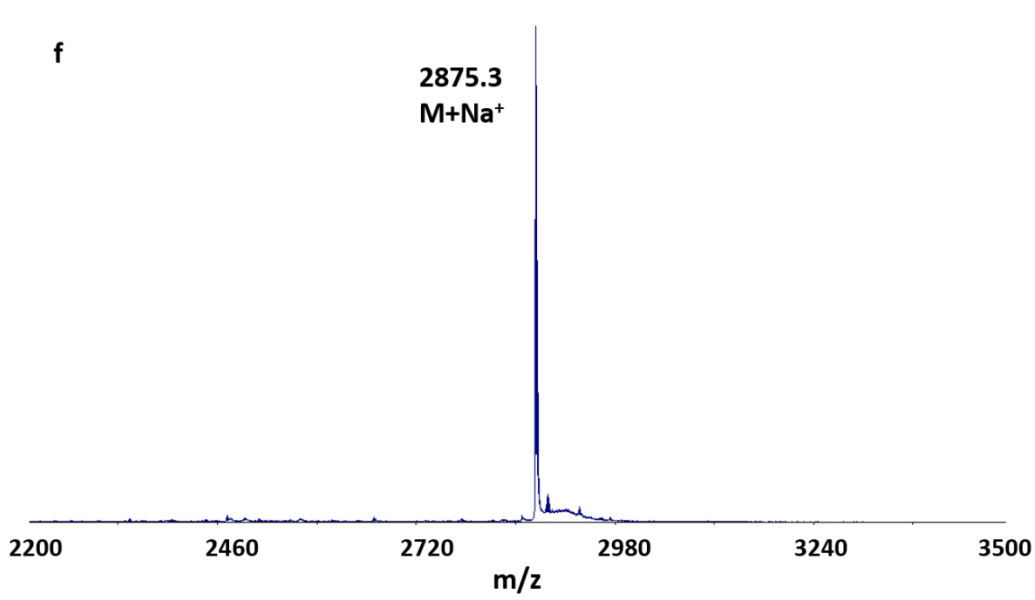
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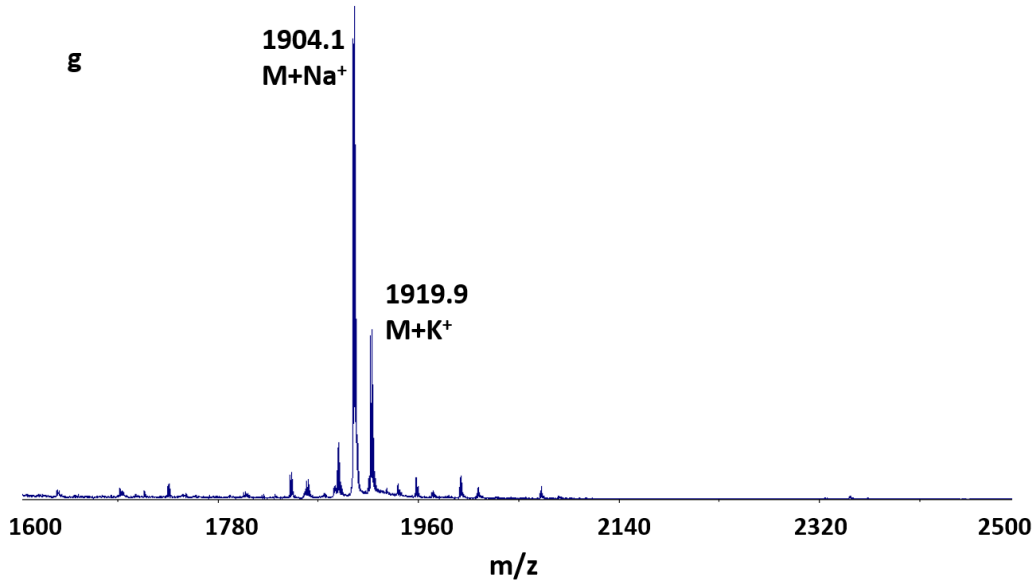
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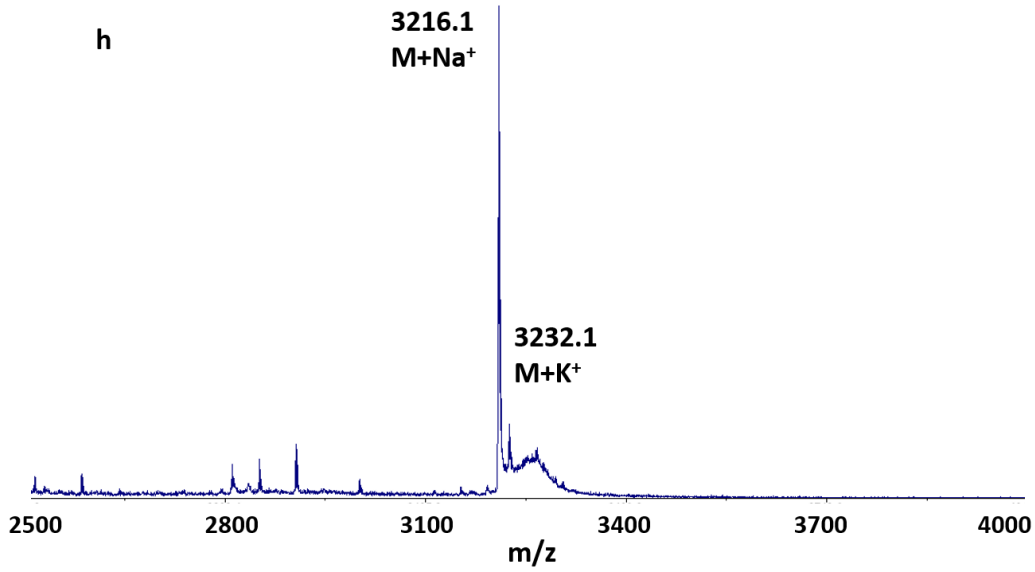
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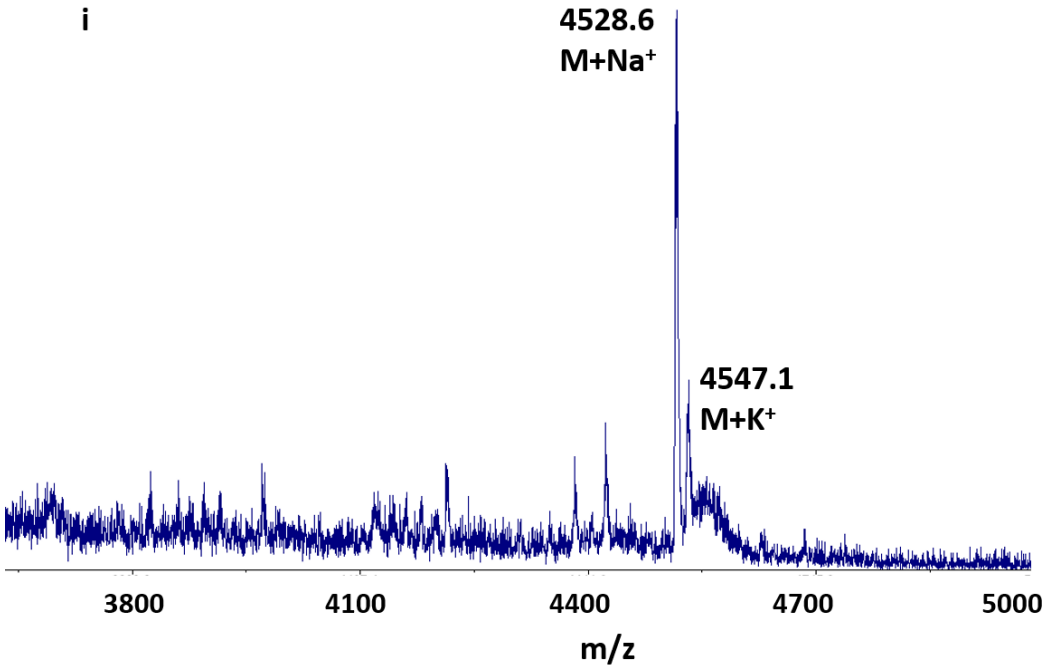
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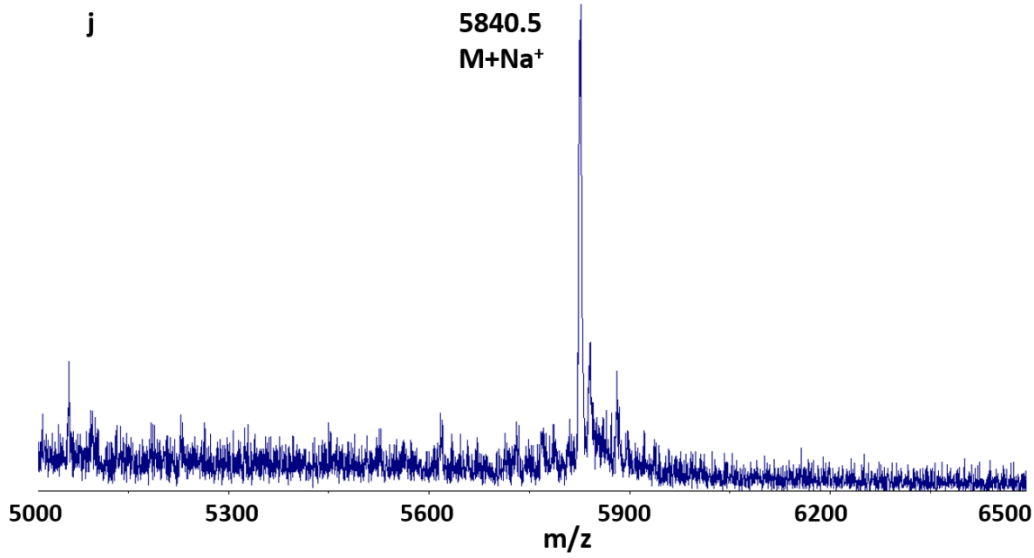
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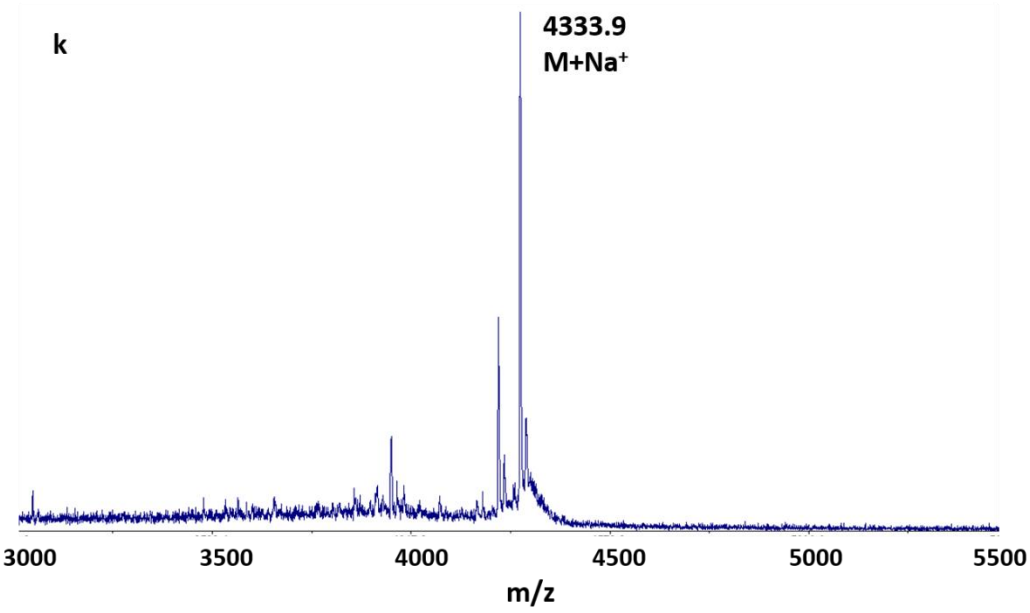
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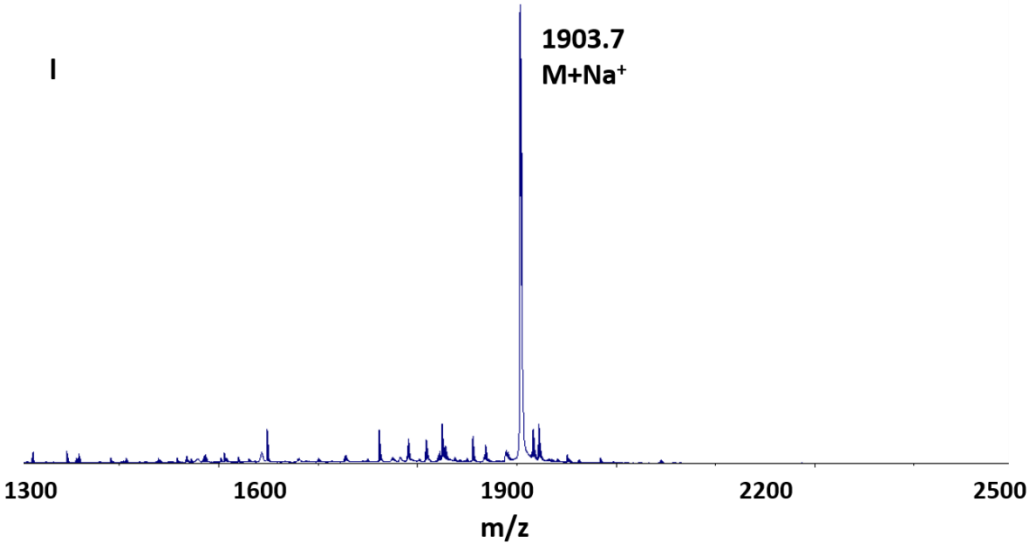
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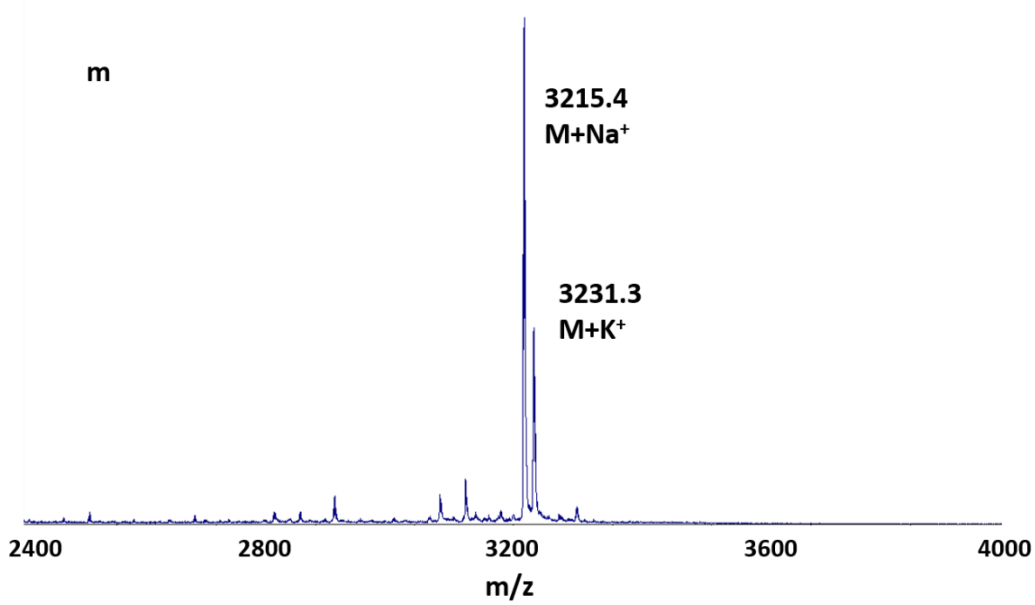
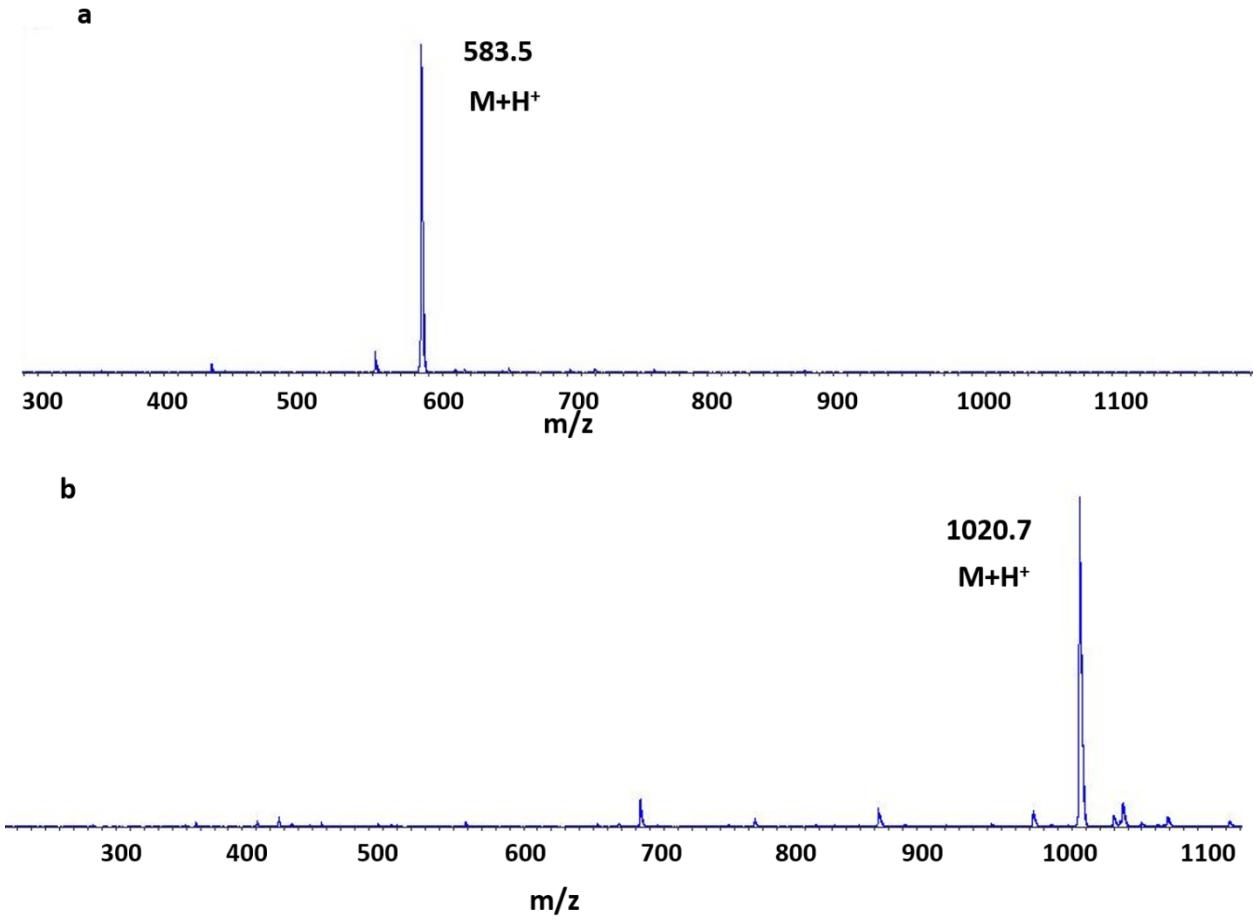
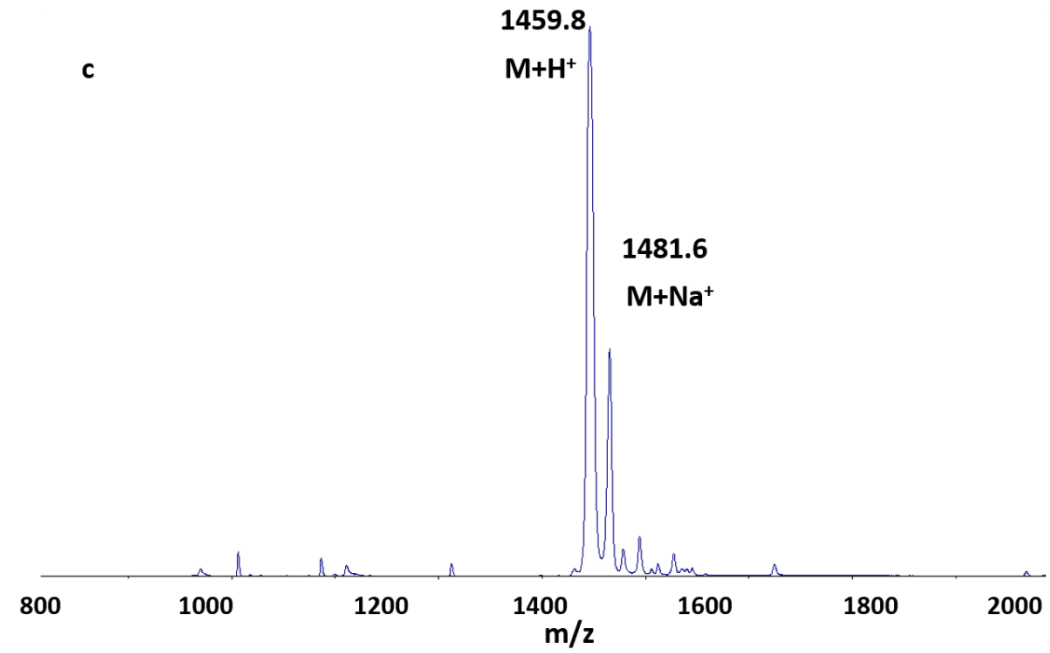


Figure S1. The MS spectra of capped peptides: (a) G1-5 obtained by ESI-MS, and (b) G1-10, (c) G1-15, (d)G1-20, (e)G1-25, (f) G1-30, (g) G2-5, (h) G2-10, (i) G2-15, (j) G2-20, (k) G3-5, (l) Ac-$[K(Ac)GLPGL]_3$-NH_2, (m) Ac-$[K(Ac)GLPGLGLPGL]_3$-NH_2 obtained by MALDI-TOF MS.

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1000
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1400
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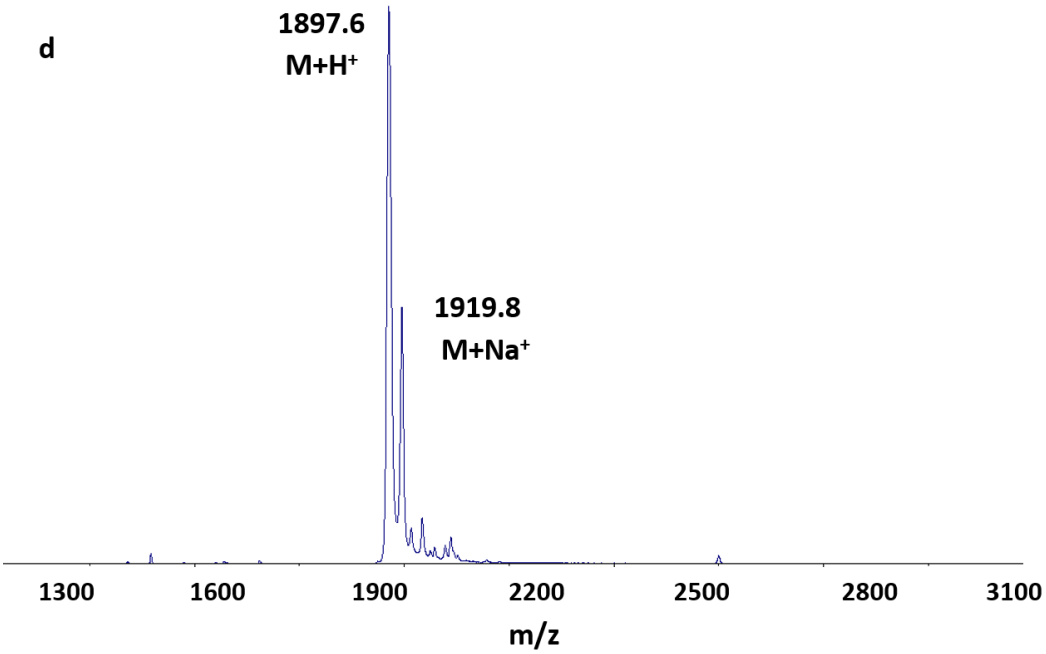
1800
2000
m/z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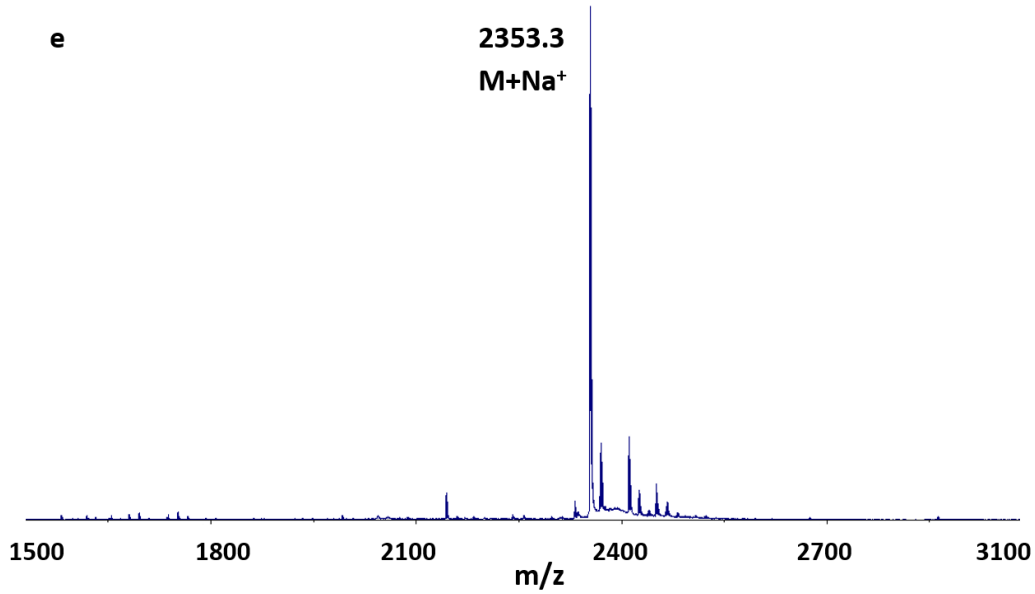
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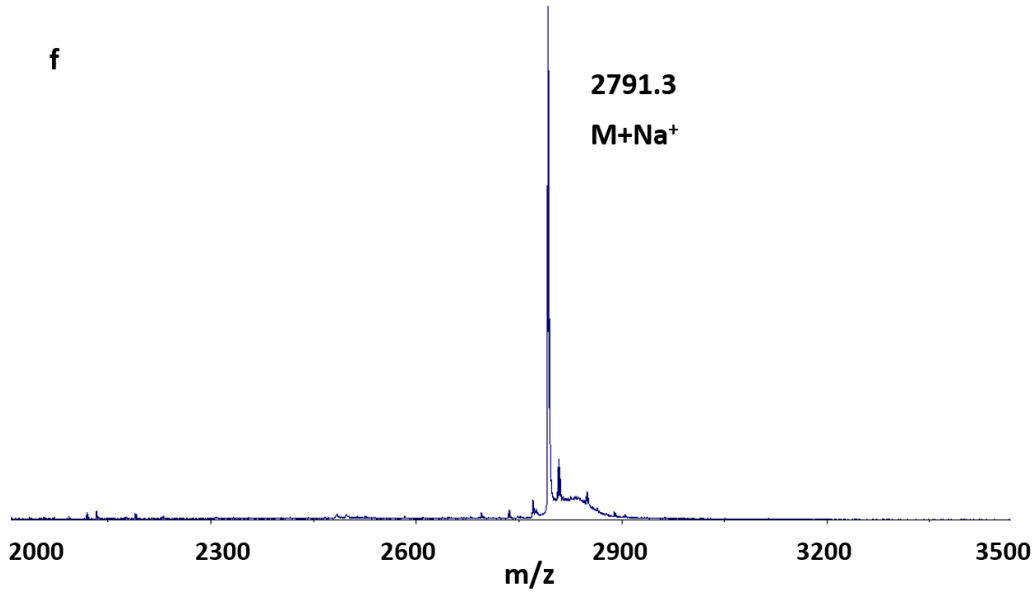
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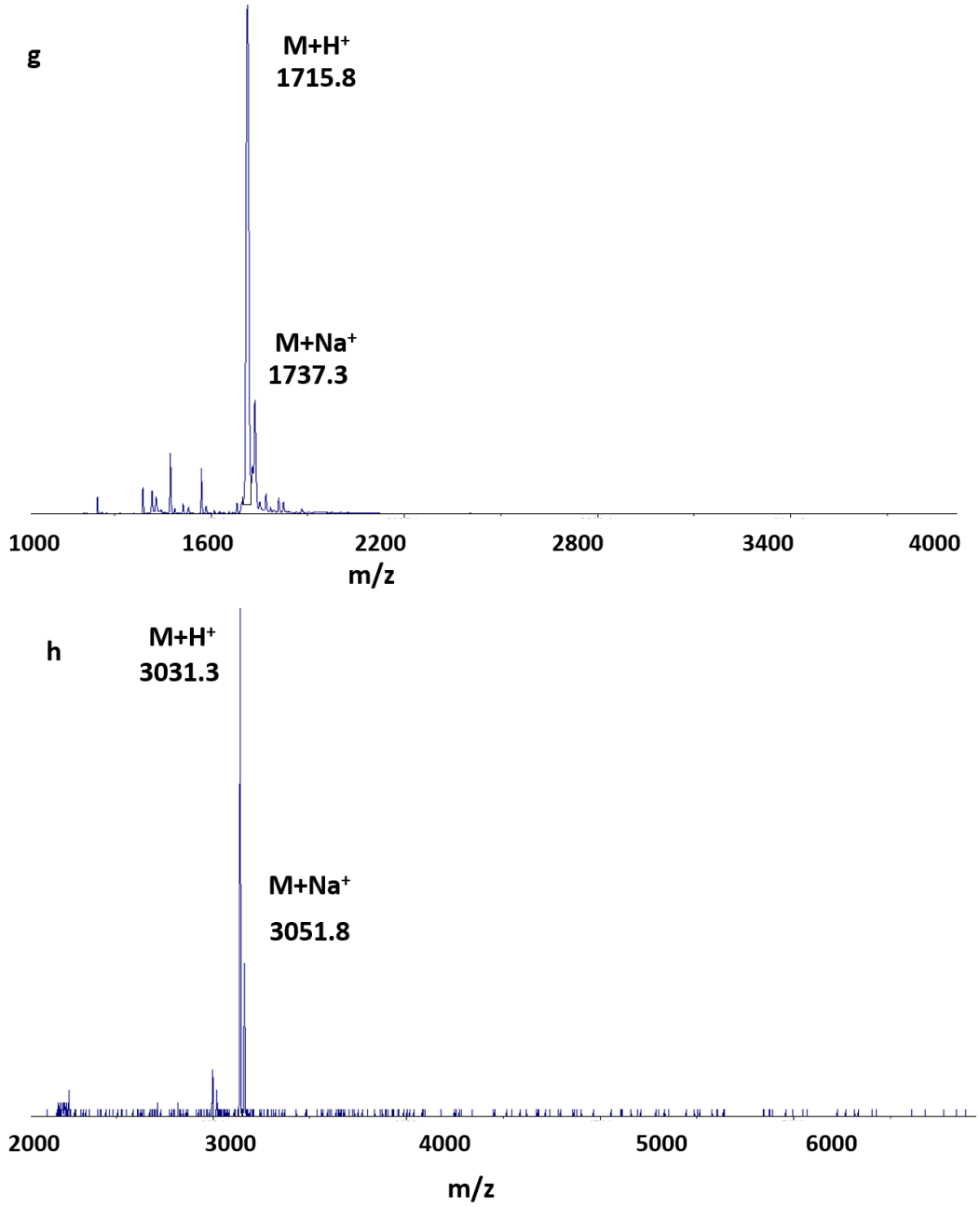
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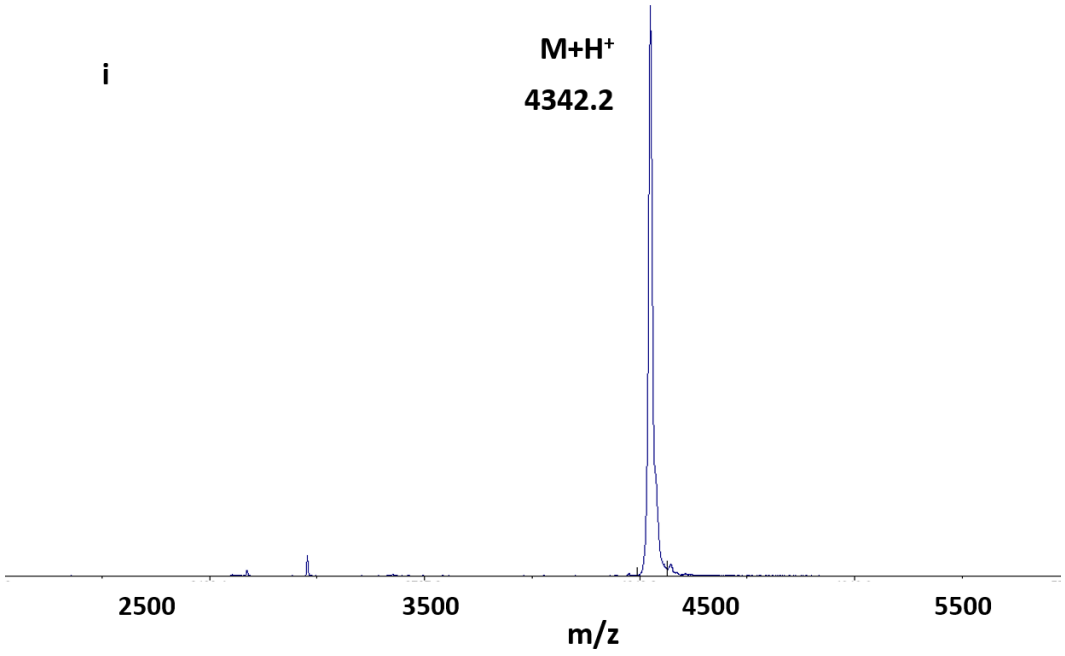
5000
6000
m/z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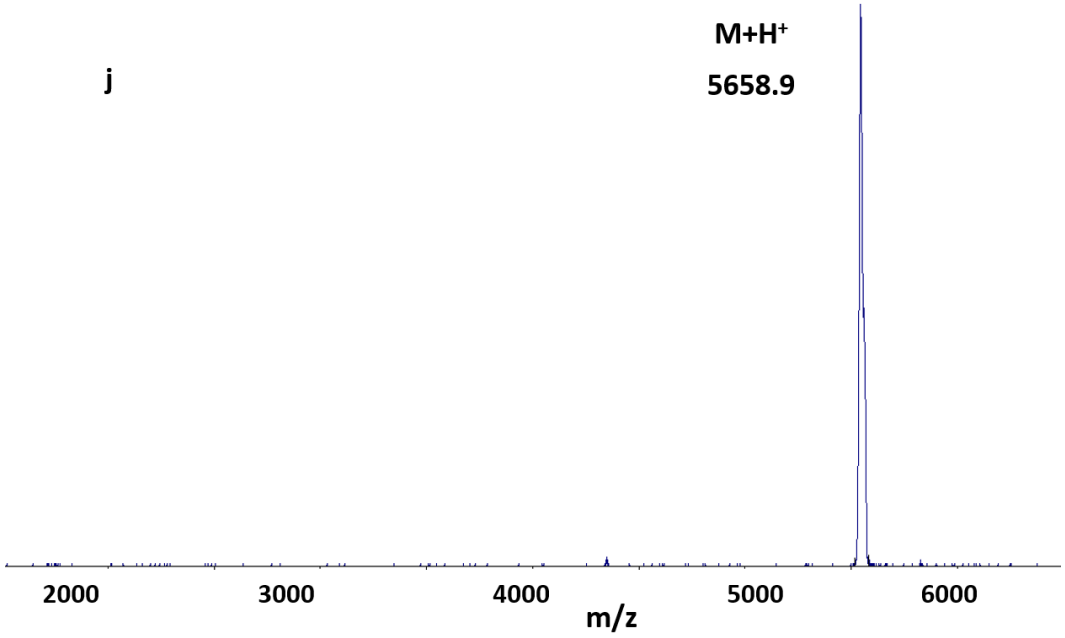
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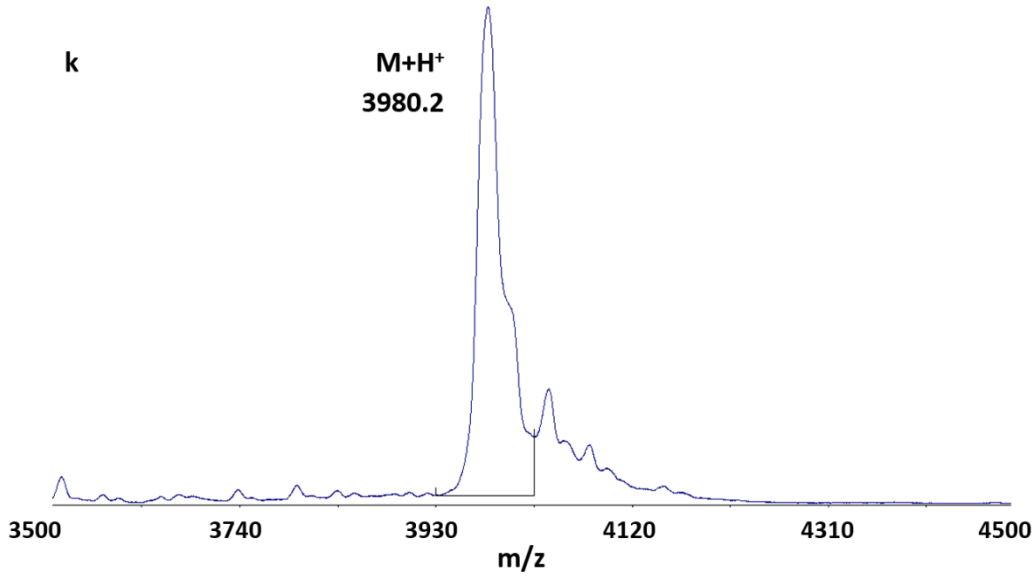


Figure S2. MS spectra of uncapped peptides: (a) G1-5U, (b) G1-10U obtained with ESI-MS, and (c) G1-15U, (d)G1-20U, (e)G1-25U, (f) G1-30U, (g) G2-5U, (h) G2-10U, (i) G2-15U, (j) G2-20U, (k) G3-5U obtained with MALDI-TOF MS.

LogD calculations

Because of their high molecular weights, typical software packages were not capable of calculating logP or logD values for several peptides. Thus, a traditional residue addition model was used for most residues, and a fragment addition model was incorporated to calculate contributions from branching lysine residues.[1] Each lysine has a reported logD contribution of -2.27. Notably, this value refers to an internal lysine residue with a free ε-amine.

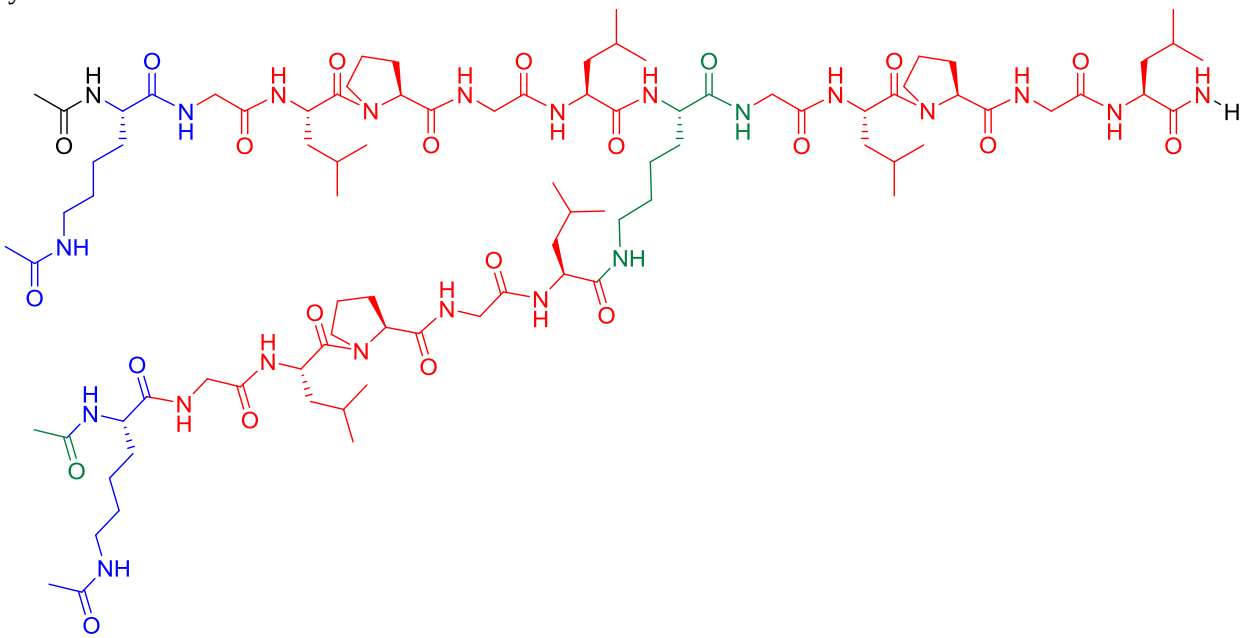


Figure S3. G2-5 is used as example to clarify the logD calculation. Different components are shown in different colors.

Capped G2-5 is used here as an example (Figure S3). The residues in red are three (GLPGL) repeating units, each of which has a logD contribution of (0.80(2) – 0.22(2) + 0.15 = 1.31). The C- and N-termini in black contribute –1.18 to the logD value as has been reported. Each of the lysine residues in blue has capped ε amines. The logD contribution for each of these residues was calculated as follows from the sum of logD contributions for a regular (uncapped) amine plus the logD contribution from uncapped amines to capped amines, which is also the difference between the blocked and unblocked peptides.

– 2.27 (general lysine residue)
+ 2.07 (comes from –1.18 + 3.25, which is conversion from uncapped to capped amine)
= –0.20

The remaining fragments (in green), combine to make another capped lysine with the logD contribution of (–0.20). The logD of uncapped peptides was analyzed and calculated in a similar way. The logD of peptides was calculated according to:

logD(capped) = 1.31(# of GLPGL) – 1.18 (blocked peptide) – 0.20*(# of capped lysine residues)
logD(uncapped)= 1.31(# of GLPGL) – 3.25 (unblocked peptide) – 2.27*(# of free lysine residues)

ChemSketch was also used to predict logP values for some capped peptides, but G2-15, G2-20, and G3-5 were above the 255-atom limit in this software. Table S1 shows a comparison between the logD calculated from the equation above and the logP obtained from ChemSketch. Considering all of the amines were capped, and all C-termini were uncharged amides, logD and logP of the capped peptides are assumed to be very close. Therefore, we believe that logP predictions in Chemsketch should be similar to our logD predictions. As listed in Table S1, the logD/P values obtained from two different methods are reasonably close.

Table S1. Summary of the calculated logD and and logP obtained with ChemSketch

	G1-5	**G1-10**	**G1-15**	**G1-20**	**G1-25**	**G1-30**	**G2-5**	**G2-10**
logD (this paper)	-0.1±0.4	1.2±0.4	2.6±0.4	3.9±0.5	5.2±0.5	6.5±0.5	2.2±0.6	6.1±0.7
logP (Chemsketch)	-0.9±0.9	0.7±1.0	2.2±1.0	3.8±1.1	5.4±1.1	7.0±1.2	0.3±1.1	5.1±1.2

Circular Dichroism measurements
The following figures show circular dichroism data of linear (Figure S4) and branched (Figure S5) capped peptides and of linear and branched uncapped peptides (Figure S6). Measurements were carried out in the same conditions as described in the main text.

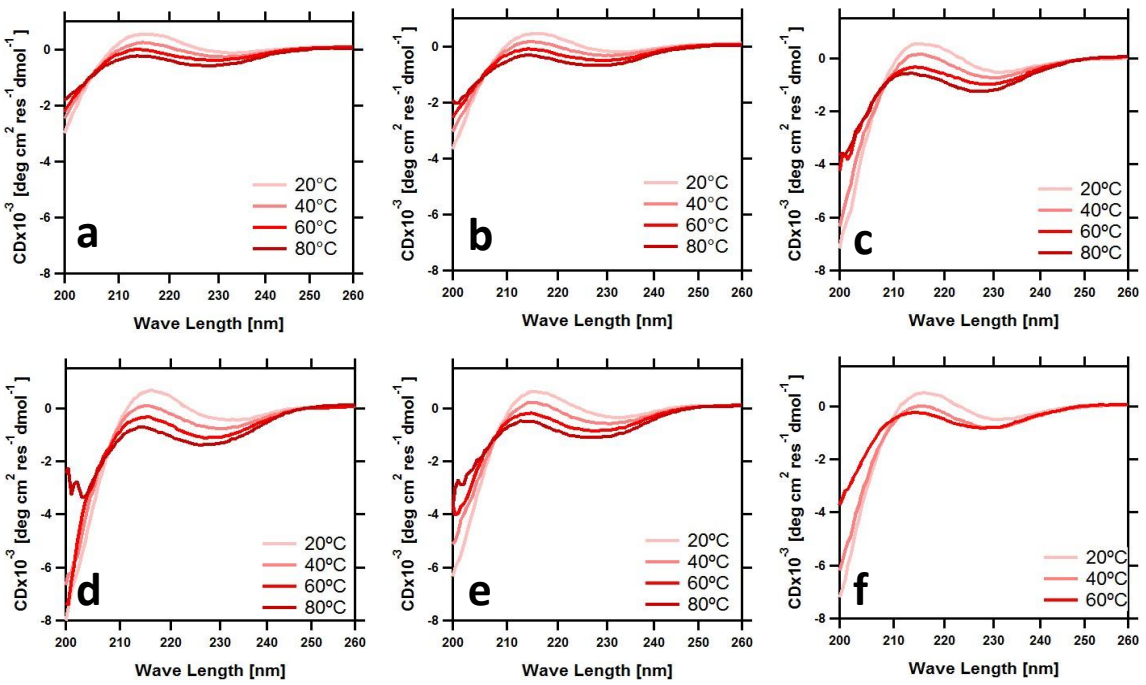


Figure S4. Temperature/wave length scan of linear capped peptides. G1-5 (a), G1-10 (b), G1-15 (c),G1-20 (d), G1-25(e), G1-30(f). Measurements were taken at 0.5-1 mg/mL in PBS (2.5 mM).

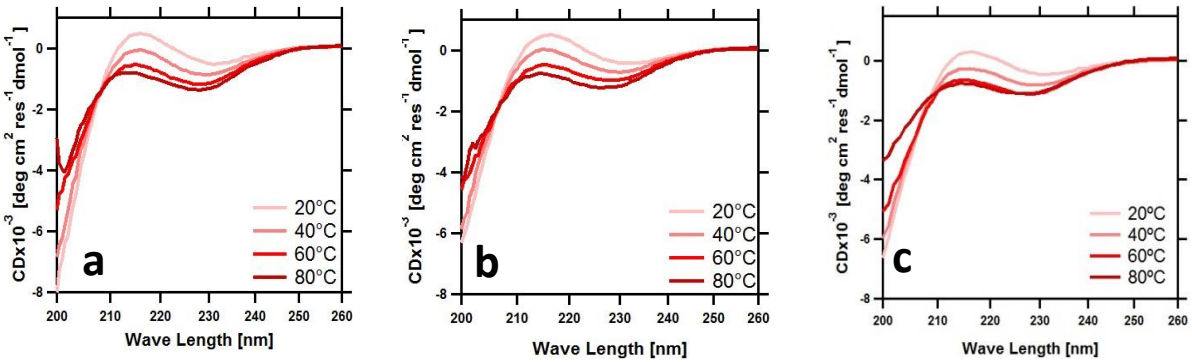


Figure S5. Temperature/wavelength scan of branched capped peptides. G2-5 (a), G2-15 (b), G3-5 (c). Measurements were taken at 0.5-1 mg/mL in PBS (2.5 mM).

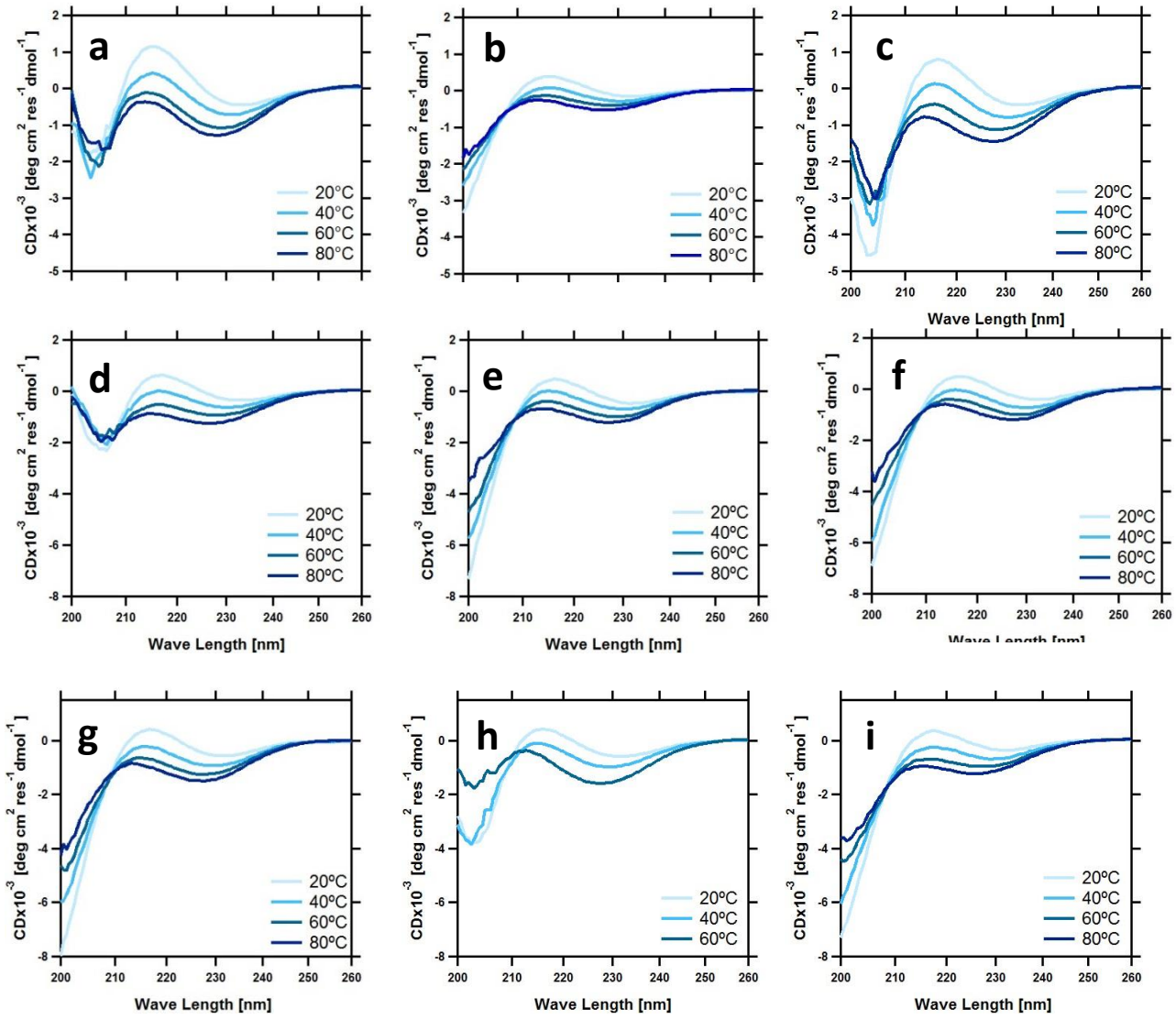


Figure S6. CD spectra of uncapped peptides at different temperatures. G1-5U (a), G1-10U(b), G1-15U(c), G1-20U(d), G1-25U(e), G1-30U(f) G2-15U (g), G2-20U (h), G3-5U (i). Measurements were taken at 0.5-1 mg/mL in PBS (2.5 mM).

Van't Hoff endpoint determination

The endpoints of the transition, $[\theta]^F$ and $[\theta]^U$, represent the values for the fitted minimum and maximum points of the transition (high-temperature folded form, $[\theta]^F$, and low-temperature unfolded form, $[\theta]^U$). They were chosen as the initial and the final points of the transition in the inspected temperature range where $[\theta]^F$ and $[\theta]^U$ were the values of the mean molar residue ellipticity at 80 °C and 20 °C, respectively.

DLS measurements

DLS measurements were performed using a Zetasizer Nano-ZS (Malvern, UK). Samples were dissolved in PBS (pH 7.4) to a final concentration of 5 mg/mL. Samples were equilibrated for 5 min at the measuring temperature prior to data collection. Correlograms were collected at 173° for at least 10 runs of 10 s. The recorded correlograms were analyzed with the CONTIN procedure using the software provided with the instrument.

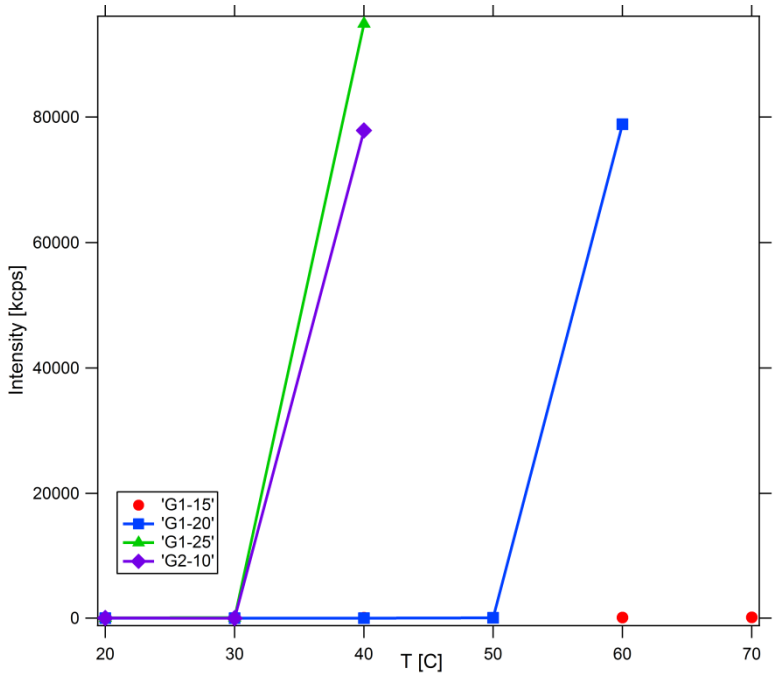
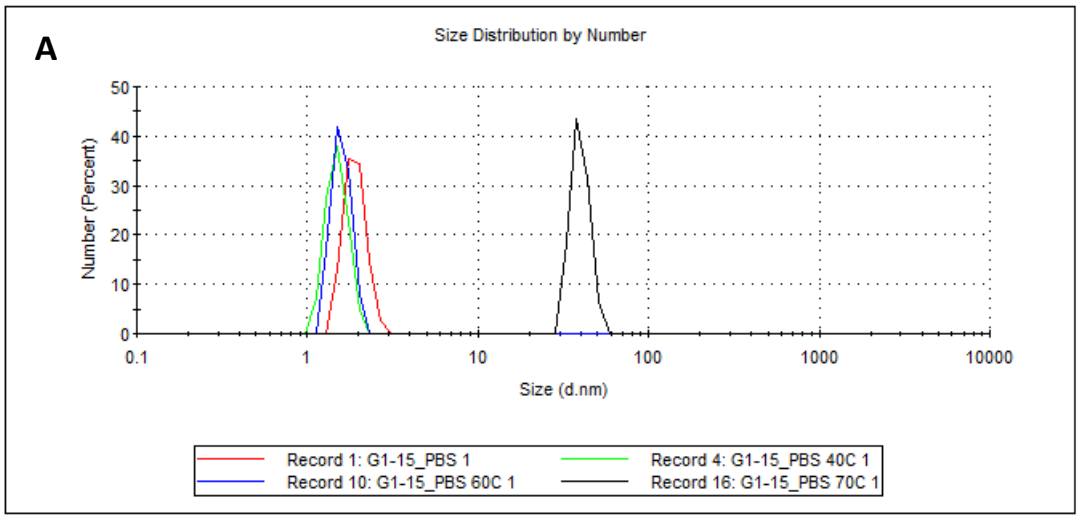


Figure S7: Temperature dependence of the average scattered intensity. Solid lines are drawn as guides to the eye to emphasize the sharp increase in intensity (attributed to coacervate formation) above the peptide's T_t.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Size Distribution by Number
Number (Percent)
Size (d.nm)
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1
10
100
1000
10000
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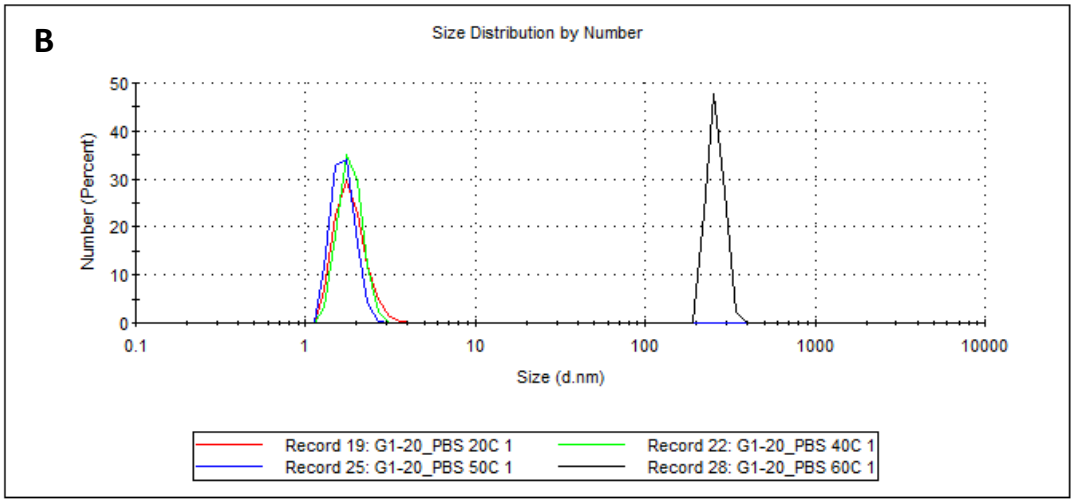
Record 4: G1-15_PBS 40C 1
Record 10: G1-15_PBS 60C 1
Record 16: G1-15_PBS 70C 1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Size Distribution by Number
Number (Percent)
Size (d.nm)
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1
10
100
1000
10000
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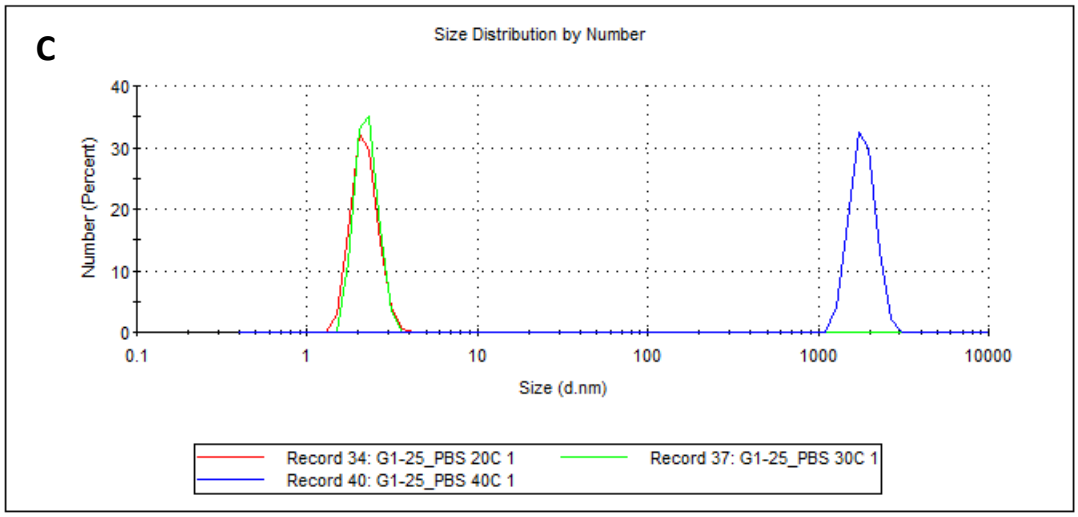
Record 22: G1-20_PBS 40C 1
Record 25: G1-20_PBS 50C 1
Record 28: G1-20_PBS 60C 1


C
Size Distribution by Number
Number (Percent)
Size (d.nm)
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100
1000
10000
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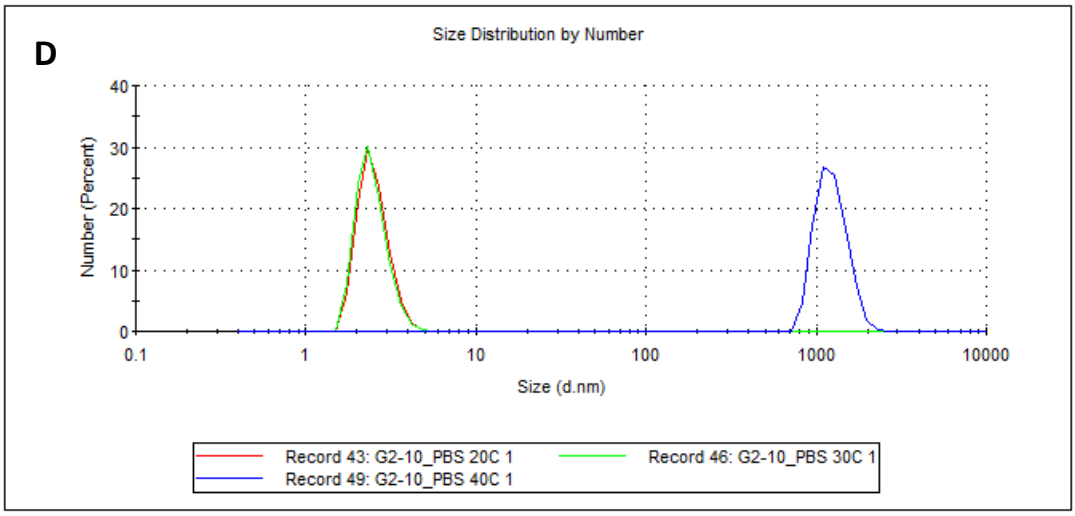

Figure S8: Size distribution of G1-15 (A), G1-20 (B), G1-25 (C) and G2-10 (D) in PBS measurements. The temperatures above which large aggregates are detected correspond to the T_t^0 determined by UV-Vis and turbidity measurements.

References

(1) Tao, P.; Wang, R.; Lai, L. *J. Mol. Model.* **1999**, *5*, 189-195.