Electronic Supplementary Material (ESI) for Faraday Discussions

Supplementary Information

Amino acids form prenucleation clusters: ESI-MS as a fast detection method in comparison to analytical ultracentrifugation

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Section S1 Supplementary Figures

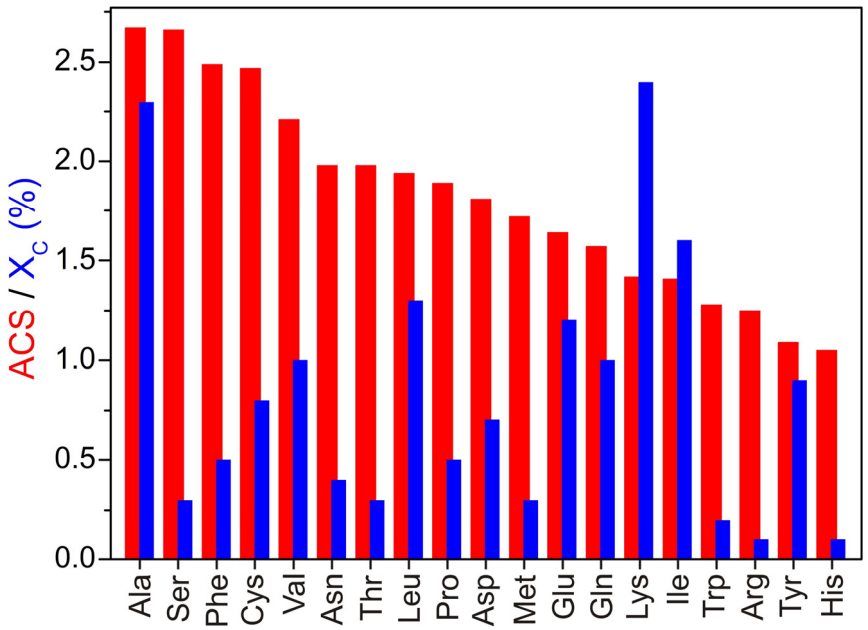


Figure S1 Average cluster sizes (ACS) calculated from ESI-MS spectra for 10^{-4} M solutions of different amino acids in their DL-form, as compared to apparent cluster percentages (X_C) derived from AUC measurements of 0.01 M solutions. Data are sorted with respect to the magnitude of the ACS value, in order to enable a direct comparison to the results of Nemes et al.[S1] Glycine is omitted due to the fact that clustering in the gas phase is markedly depressed in this case and no reliable mass spectra could hence be obtained. The pH of all samples was adjusted to 3.1 prior to measurement.

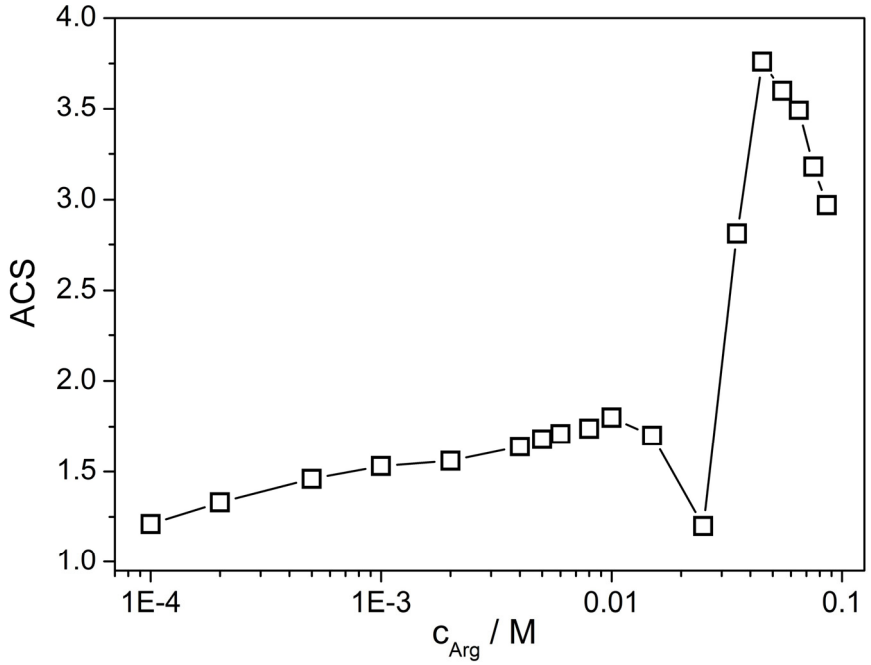


Figure S2 Average cluster size as a function of concentration for DL-Arg solutions at pH 3.1.

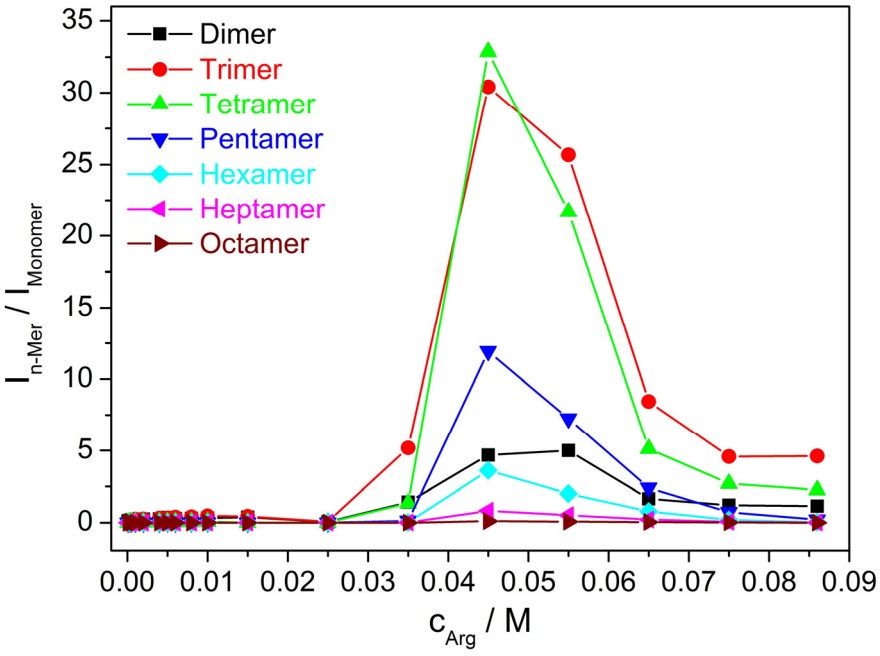


Figure S3 Peak intensities of the different arginine oligomers as depending on the solution concentration at pH 3.1, and normalised to the respective intensity of the monomer.

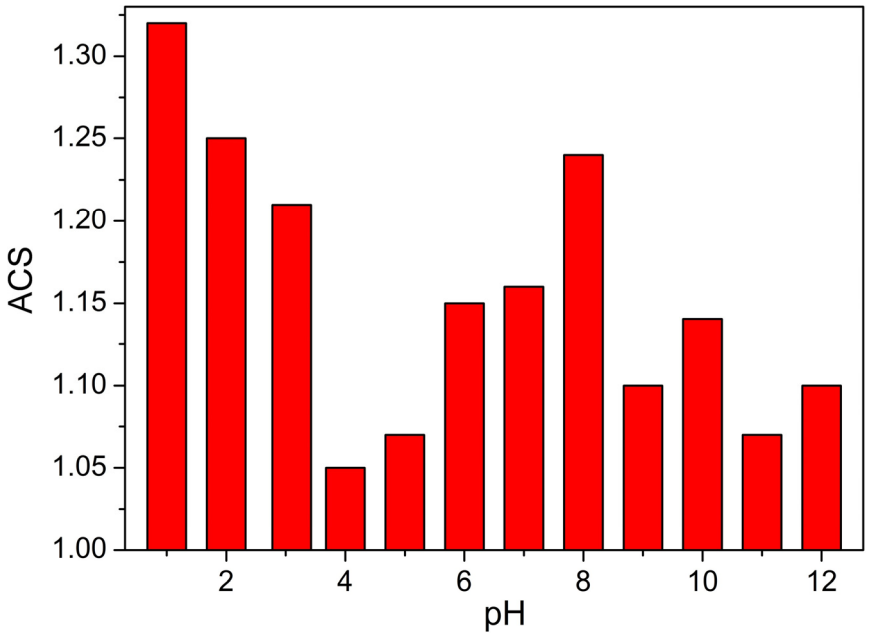


Figure S4 Average cluster size as a function of pH for 10^{-4} M solutions of DL-Arg.

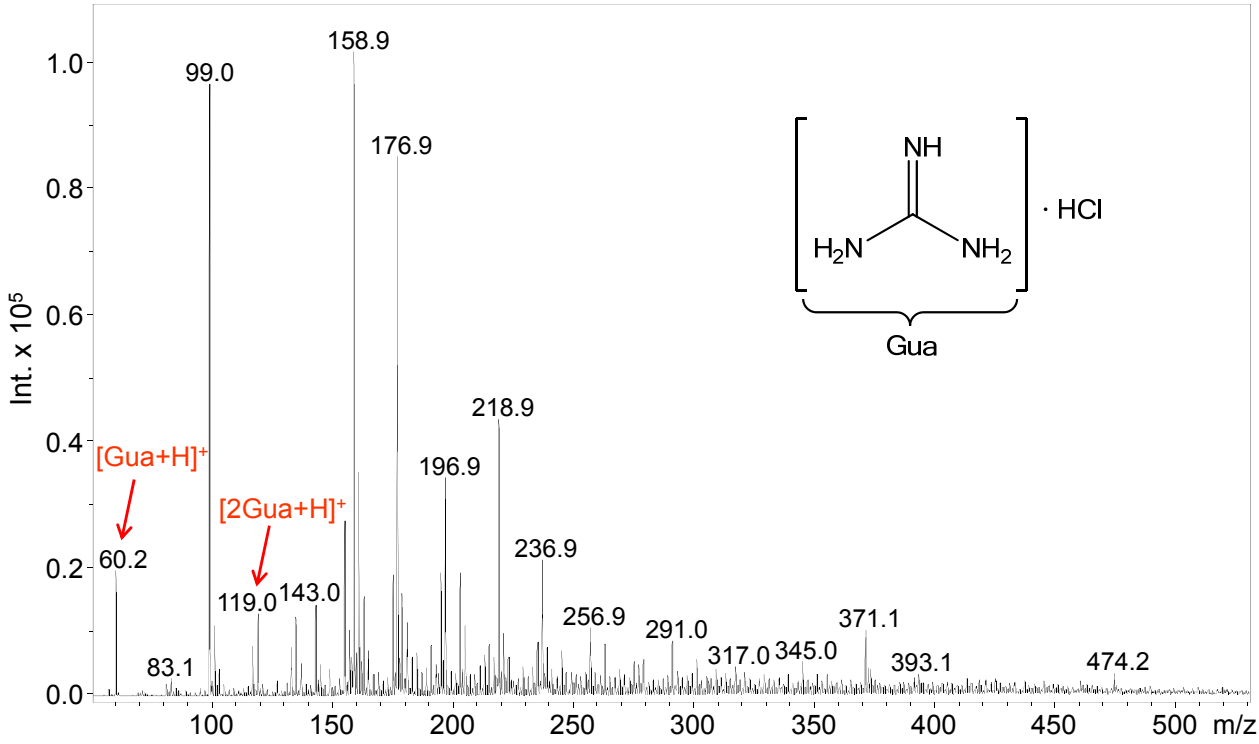


Figure S5 ESI-MS spectrum of a 10^{-4} M guanidinium (Gua) hydrochloride solution (adjusted to pH 3.1 by previous addition of concentrated acetic acid).

Electronic Supplementary Material (ESI) for Faraday Discussions

Section S2 Supplementary Tables

Table S1 ESI-MS results for DL-Arg solutions with different concentrations (all adjusted to pH 3.1).

c_{Arg} / M	Largest oligomer detected	Oligomer with highest abundance	ACS
$1 \cdot 10^{-4}$	Pentamer	Monomer	1.21
$2 \cdot 10^{-4}$	Pentamer	Monomer	1.33
$5 \cdot 10^{-4}$	Pentamer	Monomer	1.46
0.001	Pentamer	Monomer	1.53
0.002	Pentamer	Monomer	1.56
0.004	Pentamer	Monomer	1.64
0.005	Pentamer	Monomer	1.68
0.006	Pentamer	Monomer	1.71
0.008	Pentamer	Monomer	1.74
0.010	Pentamer	Monomer	1.80
0.015	Pentamer	Monomer	1.70
0.025	Pentamer	Monomer	1.20
0.035	Hexamer	Trimer	2.81
0.045	Octamer	Tetramer	3.76
0.055	Octamer	Trimer	3.60
0.065	Octamer	Trimer	3.49
0.075	Octamer	Trimer	3.18
0.086	Undecamer	Trimer	2.97

Table S2 ESI-MS results for 10^{-4} M solutions of DL-Arg at different pH values.

pH	Largest oligomer detected	Oligomer with highest abundance	ACS
1	Tetramer	Monomer	1.32
2	Pentamer	Monomer	1.25
3.1	Pentamer	Monomer	1.21
4	Trimer	Monomer	1.05
5	Trimer	Monomer	1.07
6	Tetramer	Monomer	1.15
7	Tetramer	Monomer	1.16
8	Tetramer	Monomer	1.24
9	Tetramer	Monomer	1.10
10	Trimer	Monomer	1.14
11	Trimer	Monomer	1.07
12	Trimer	Monomer	1.10

Table S3 ESI-MS results for different stereochemical configurations of arginine (10^{-4} M, pH 3.1).

Amino Acid	Largest oligomer detected	Oligomer with highest abundance	ACS
DL-Arg	Pentamer	Monomer	1.25
D-Arg	Hexamer	Monomer	1.25
L-Arg	Pentamer	Monomer	1.29

Table S4 Solubility of all 20 natural amino acids at their respective native pH and at pH 3.1 (both valid for the L-form and 25°C), and relative saturation levels of the solutions investigated by AUC (0.001 M for Tyr and 0.01 M in all other cases, pH 3.1). Values were calculated with the known dissociation constants of the amino acids and using neat solubility data reported by Amend and Helgeson.[S1]

Amino Acid	Solubility [M]		Saturation (c/c_{sol})
	native pH[S2]	*pH 3.1*	
DL-Ala	1.863	2.195	0.005
DL-Ile	0.242	0.282	0.036
DL-Leu	0.167	0.195	0.051
DL-Met	0.374	0.431	0.023
DL-Phe	0.170	0.179	0.056
DL-Pro	11.275	12.151	0.001
DL-Trp	0.065	0.077	0.129
DL-Val	0.500	0.578	0.017
DL-Asn	0.190	0.209	0.048
DL-Cys	0.211	0.224	0.045
DL-Gln	0.290	0.325	0.031
Gly	3.363	3.948	0.003
DL-Ser	3.473	3.920	0.003
DL-Thr	0.820	0.917	0.011
DL-Tyr	0.003	0.003	0.297
DL-Asp	0.038	0.280	0.036
DL-Glu	0.060	1.061	0.009
DL-Arg	1.123	soluble	---
DL-His	0.280	soluble	---
DL-Lys	1.684	soluble	---

Table S5 Hydrodynamic diameters calculated from the diffusion coefficient for amino acid clusters using the Stokes-Einstein equation.

Amino Acid	$d_{H,Cluster\ I}$ [nm]	$d_{H,Cluster\ II}$ [nm]
DL-Ala	4.6 ± 0.2	---
DL-Ile	6.6 ± 0.6	14 ± 1
DL-Leu	4 ± 4	11 ± 9
DL-Met	1.13 ± 0.08	2.5 ± 0.4
DL-Phe	4.4 ± 0.1	9 ± 1
DL-Pro	5 ± 2	10 ± 7
DL-Trp	1.6 ± 0.4	11 ± 14
DL-Val	1.5 ± 1.1	---
DL-Asn	4.3 ± 0.9	8.6 ± 0.2
DL-Cys	5.7 ± 0.7	8 ± 2
DL-Gln	4.3 ± 0.9	7 ± 1
Gly	1.3 ± 1	---
DL-Ser	1.08 ± 0.01	---
DL-Thr	1.51 ± 0.02	---
DL-Tyr	4.4 ± 0.2	---
DL-Asp	0.77 ± 0.08	---
DL-Glu	1.13 ± 0.08	2.5 ± 0.4
DL-Arg	0.86 ± 0.01	---
DL-His	9 ± 2	36 ± 10
DL-Lys	1.0 ± 0.7	---

References

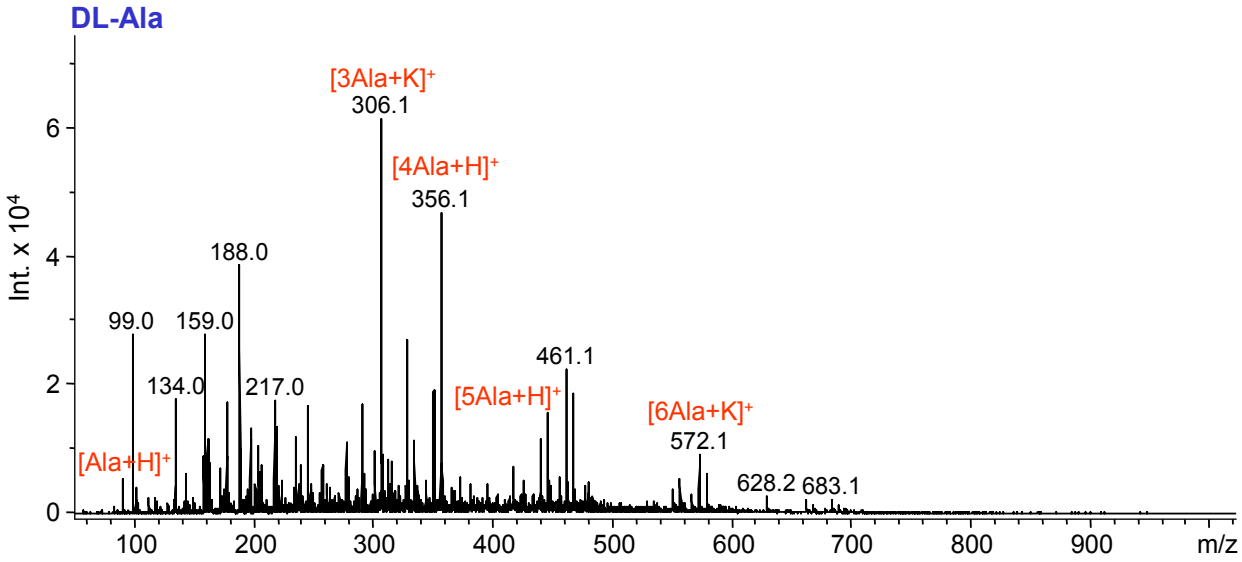
S1 P. Nemes, G. Schlosser and K. Vekey, *J. Mass Spectrom.*, 2005, **40**, 43.

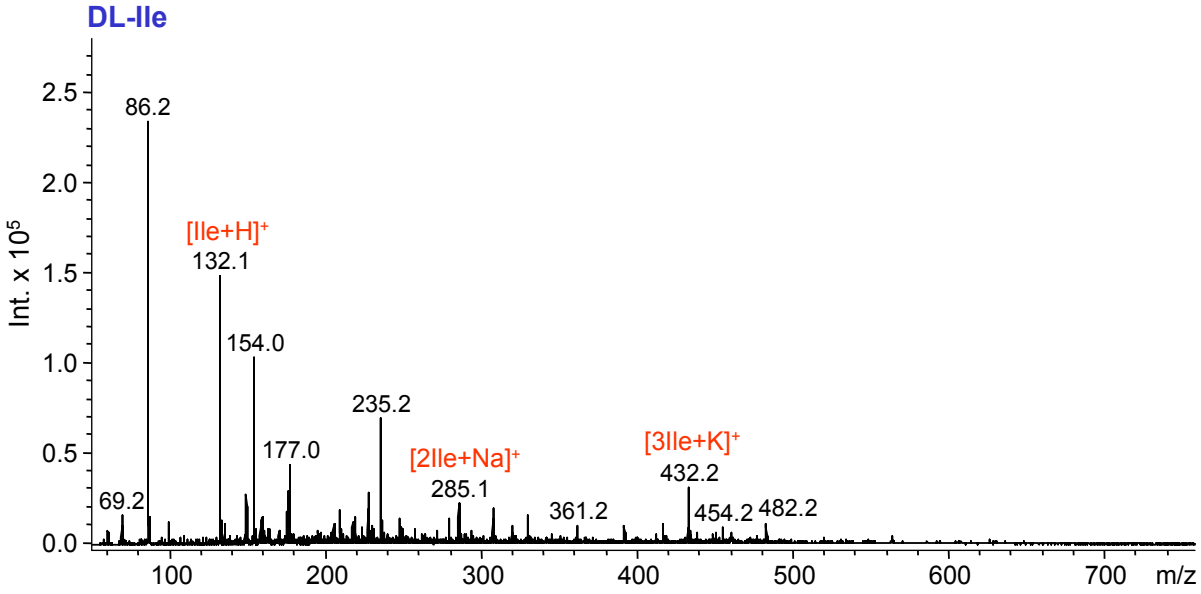
S2 J. P. Amend and H. C. Helgeson, *Pure Appl. Chem.*, 1997, **69**, 935.

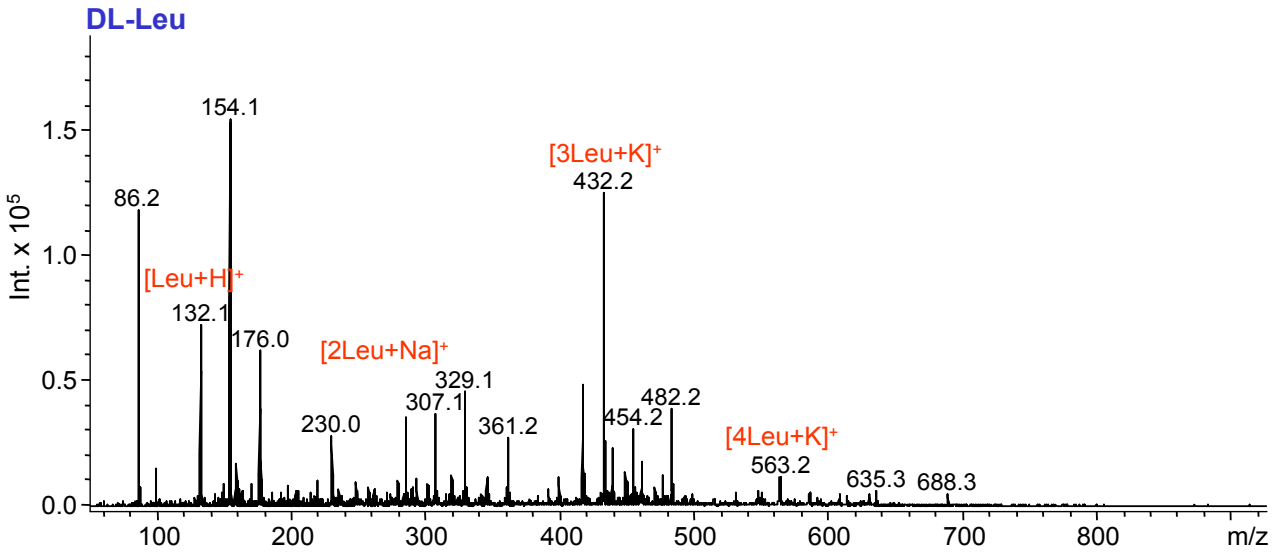
Section S3 ESI-MS Spectra of Amino Acid Solutions

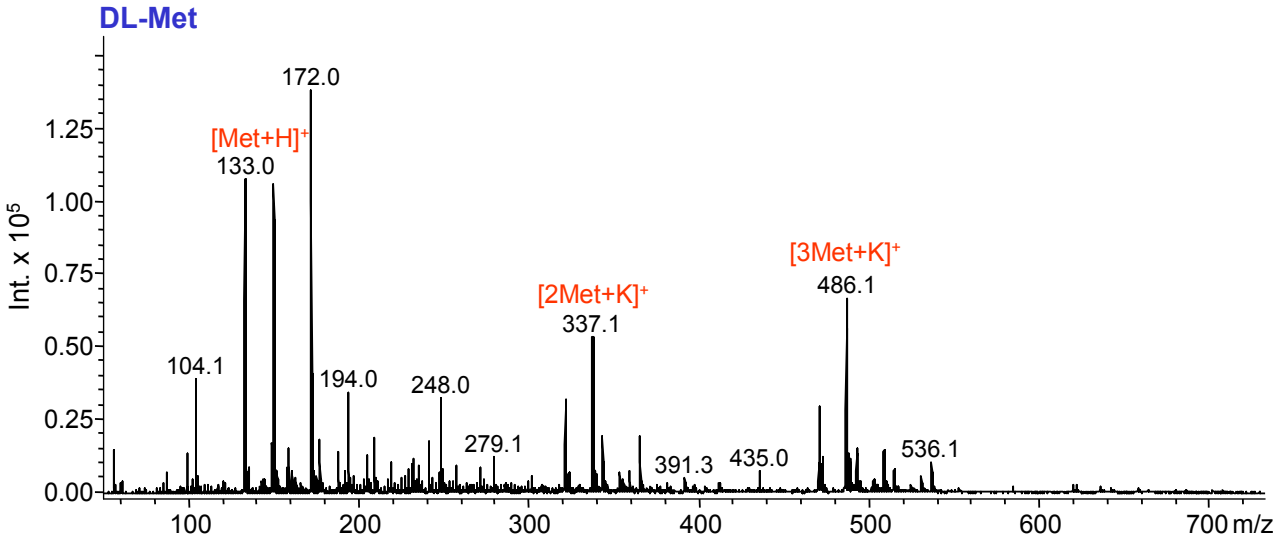
S3.1 Screening at pH 3.1 and 10^{-4} M

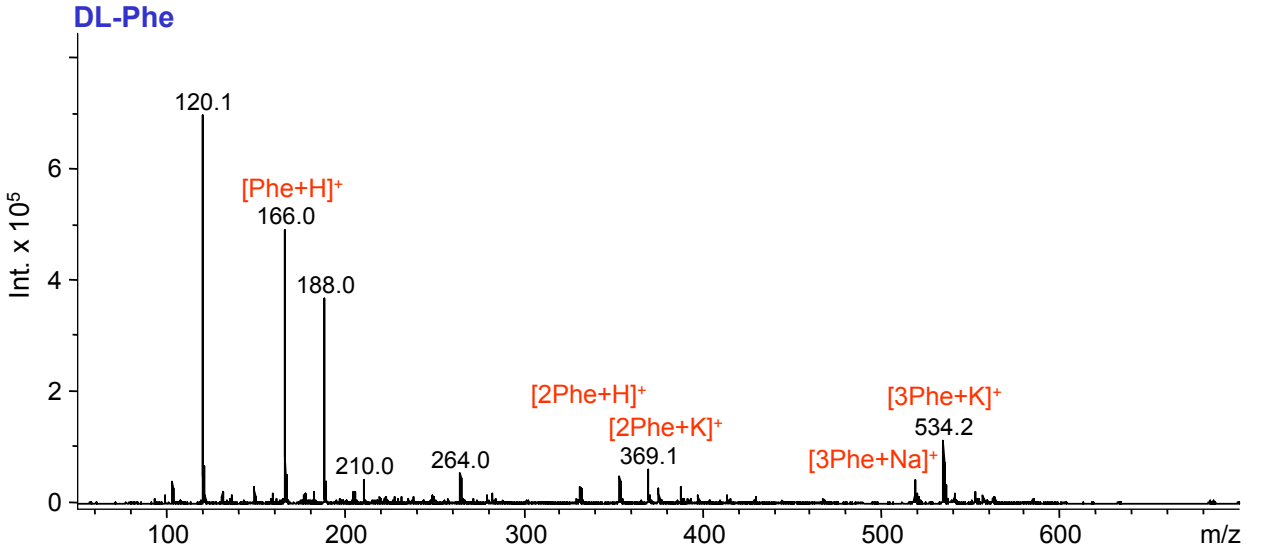
Non-polar / Hydrophobic Amino Acids

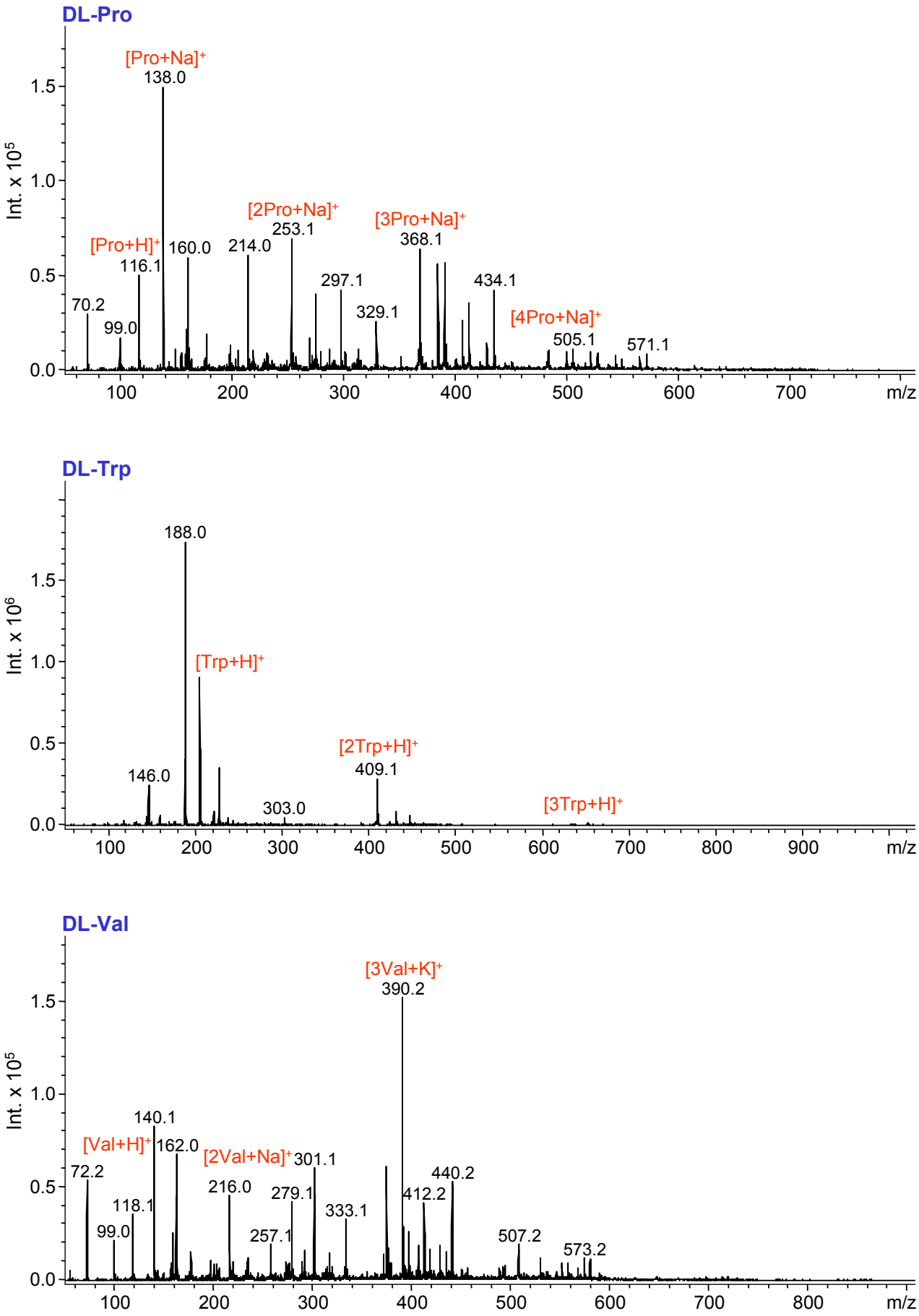




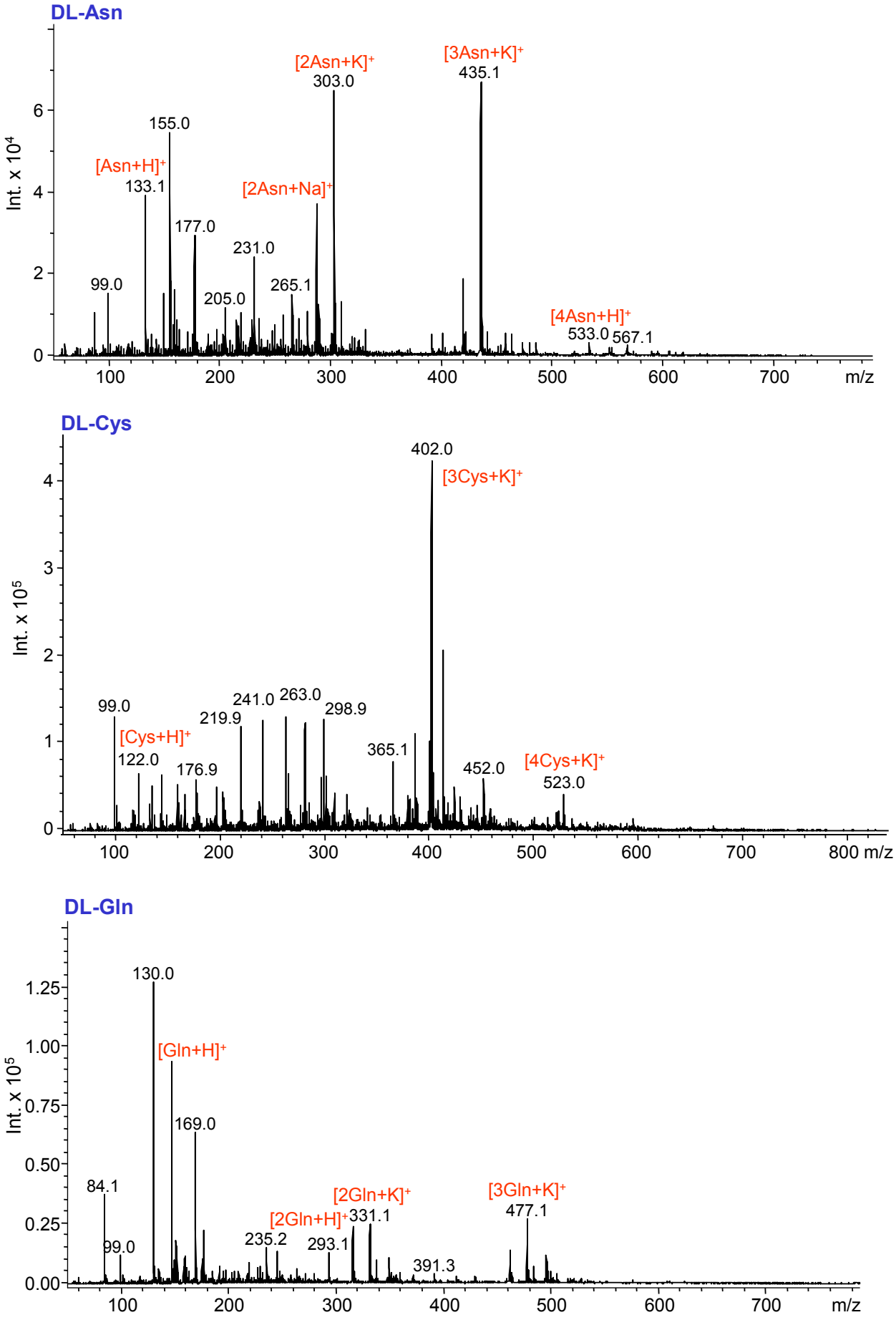


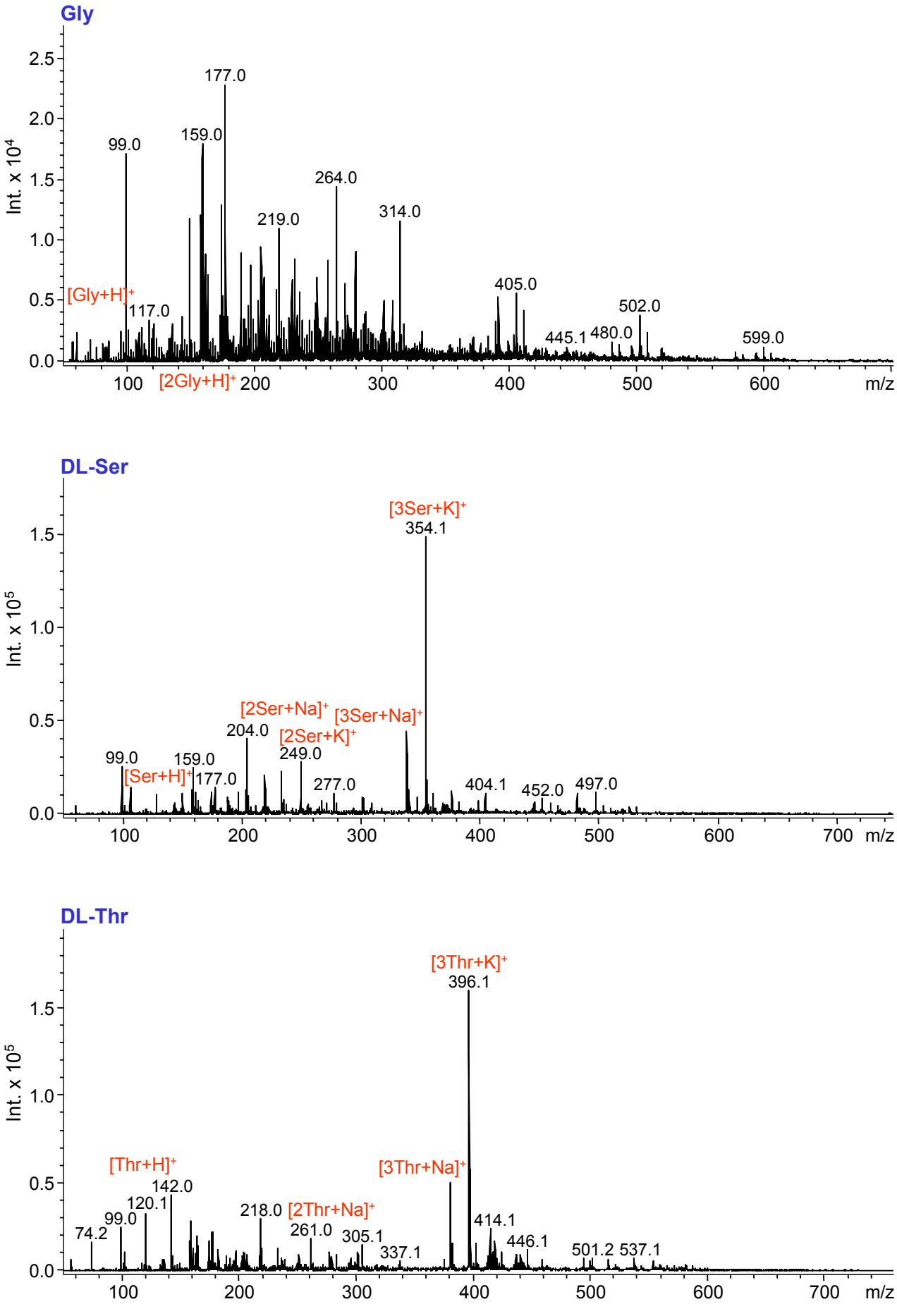


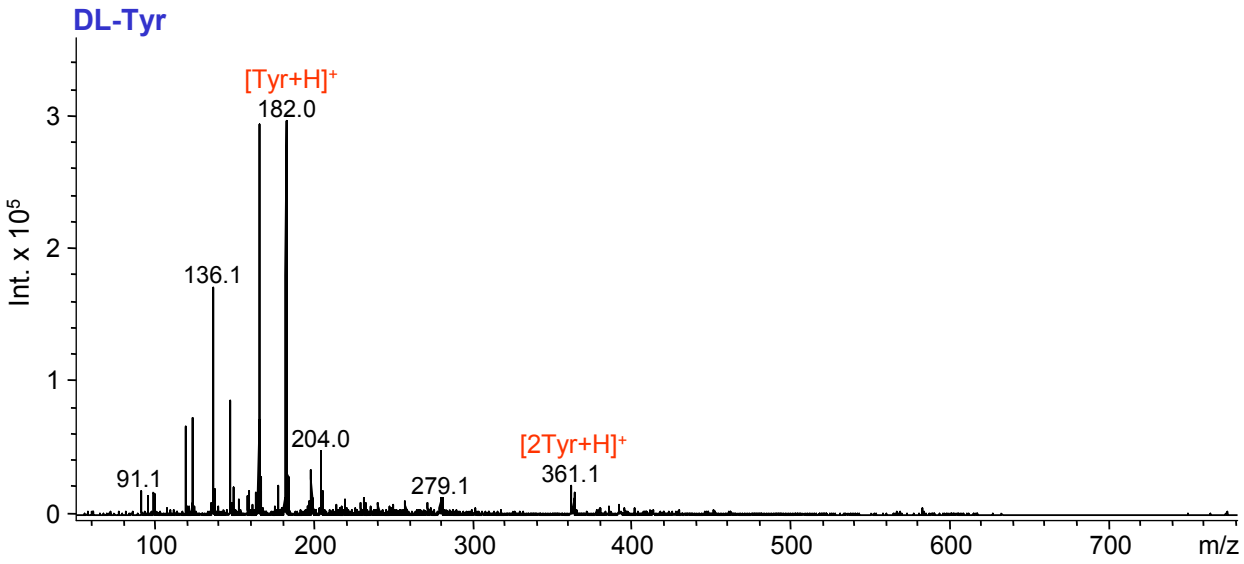




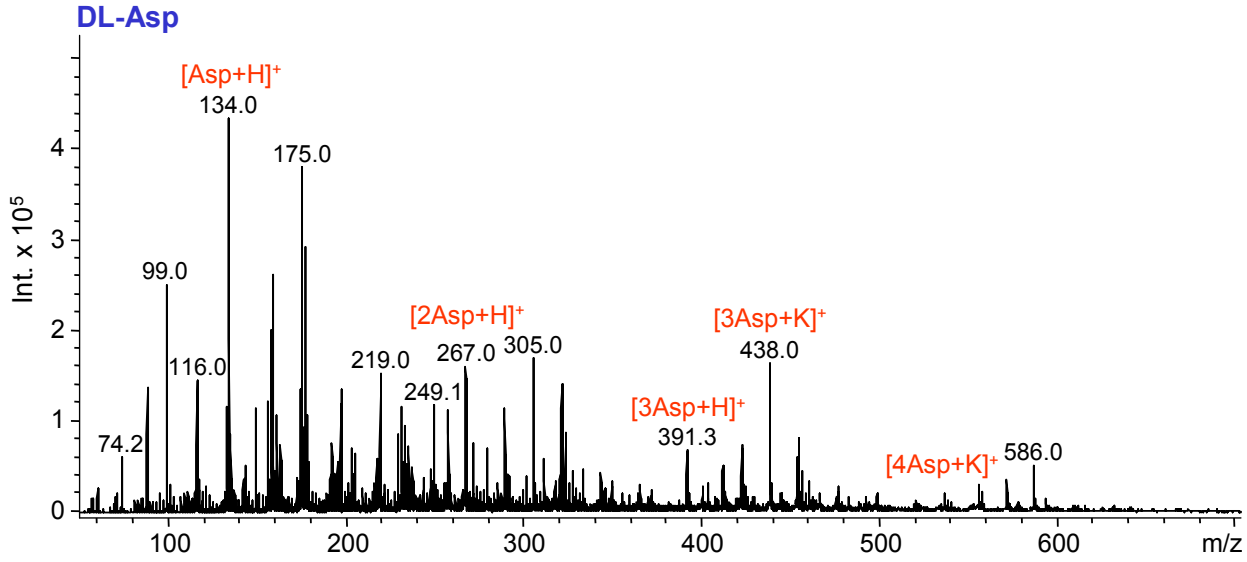
Polar / Neutral Amino Acids

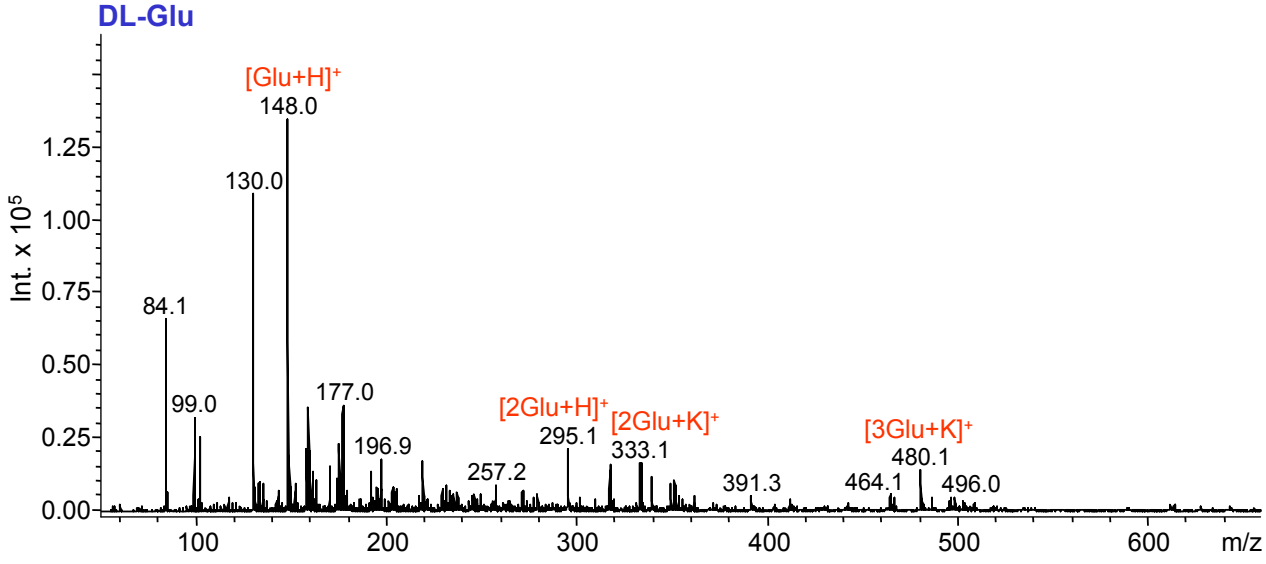




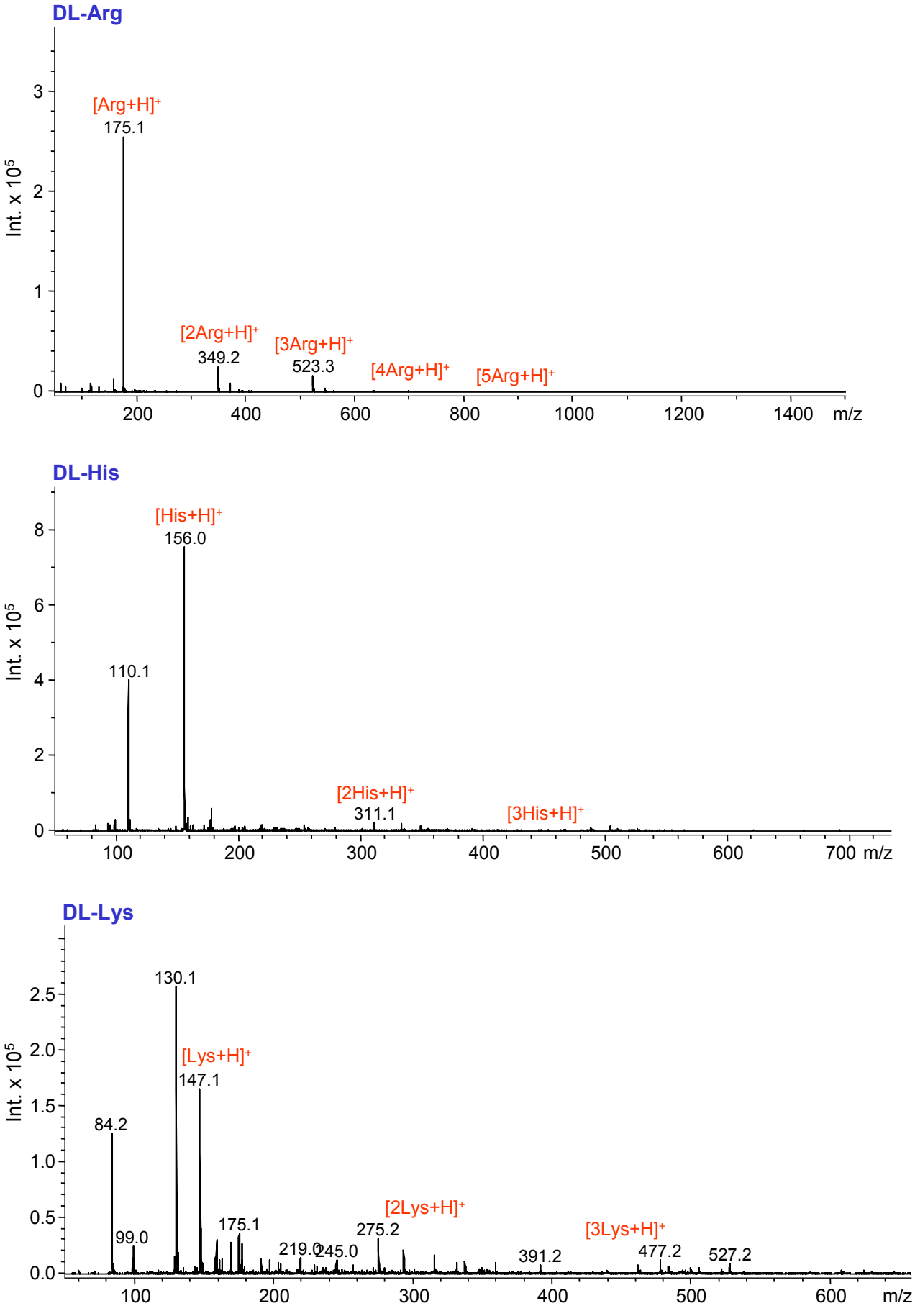


Acidic Amino Acids

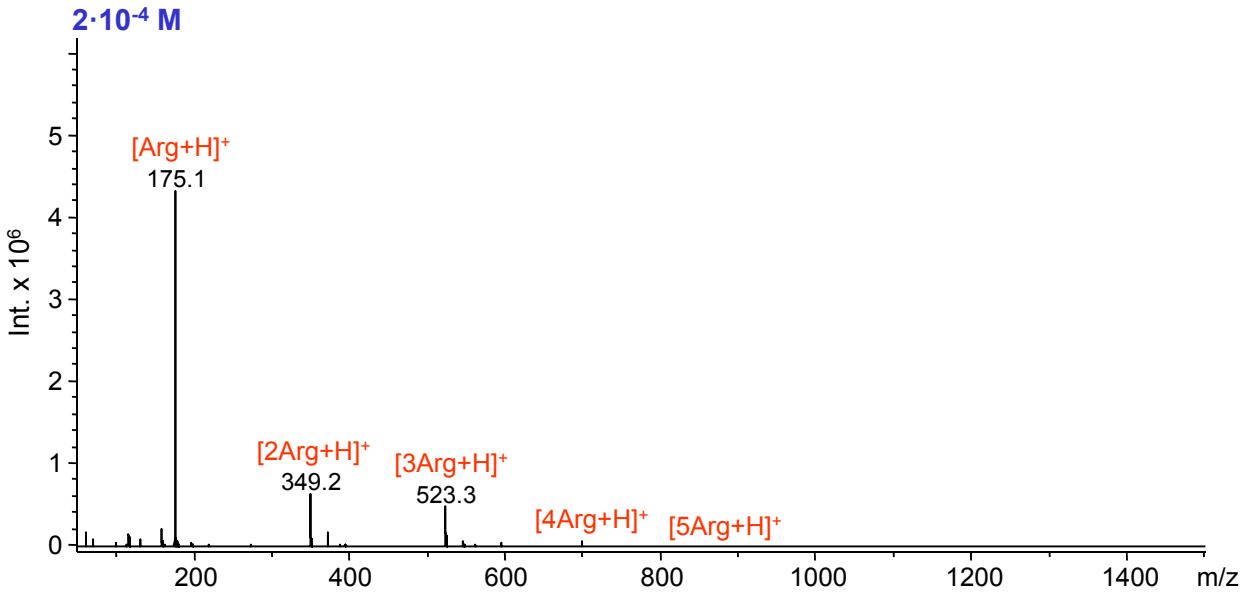


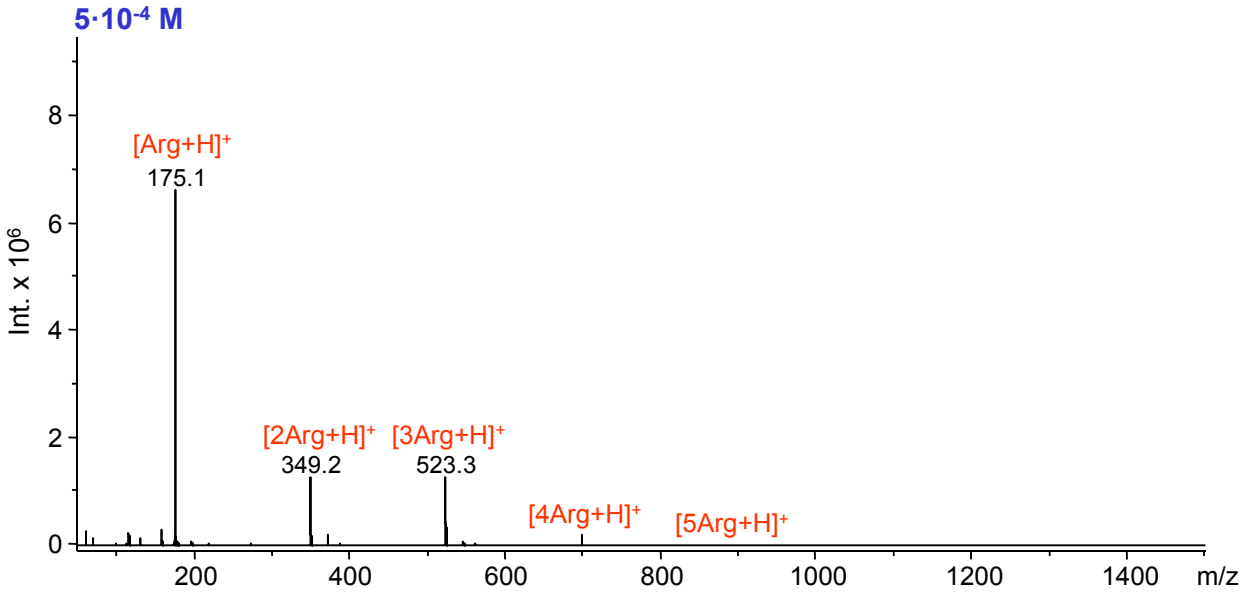


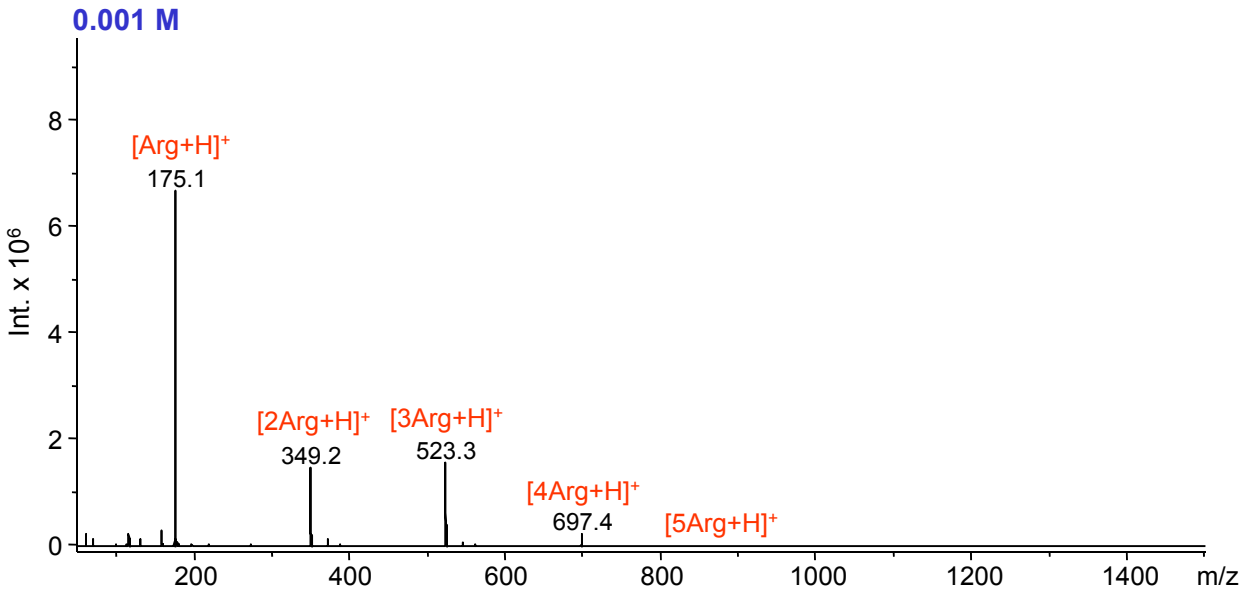
Basic Amino Acids

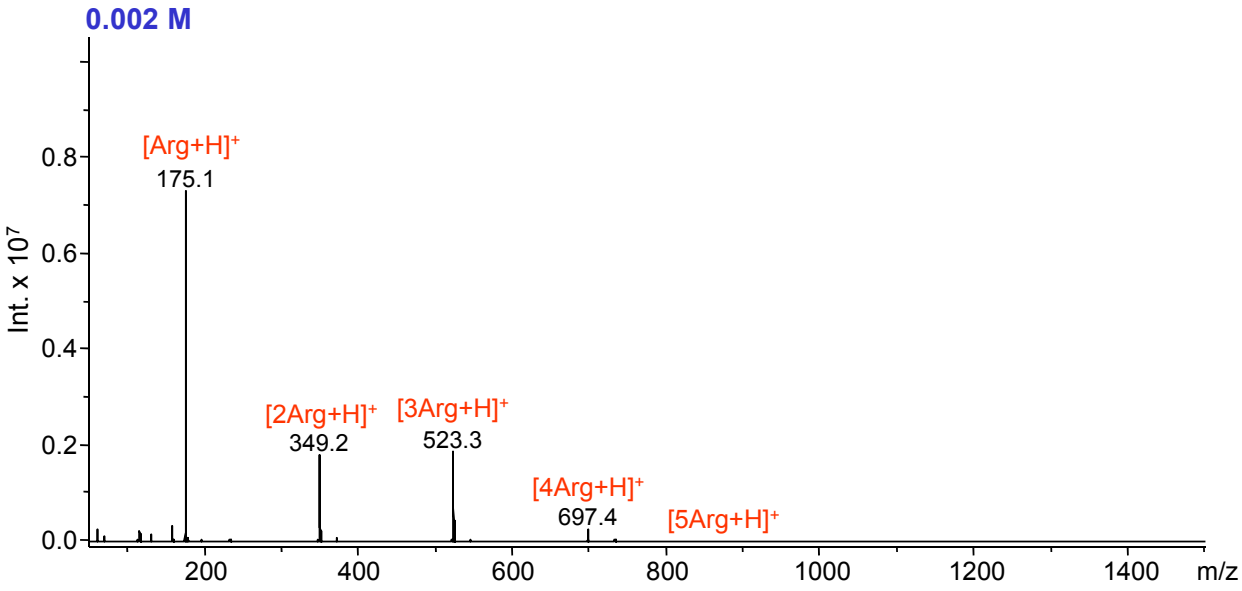


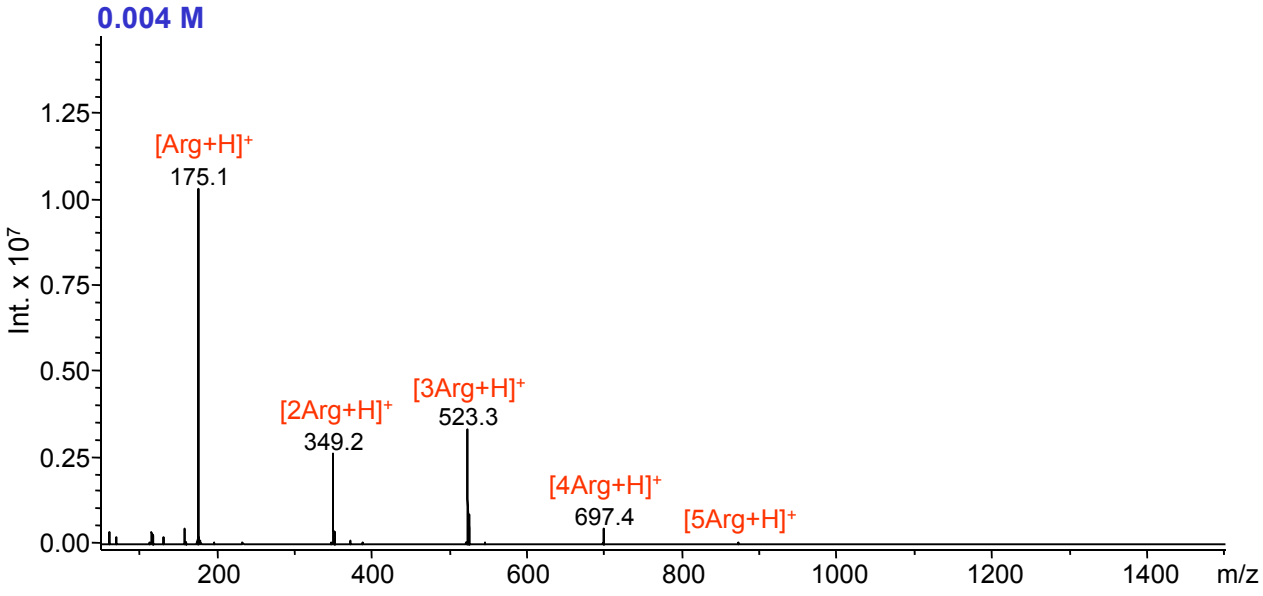
S3.2 Concentration Series of DL-Arg at pH 3.1

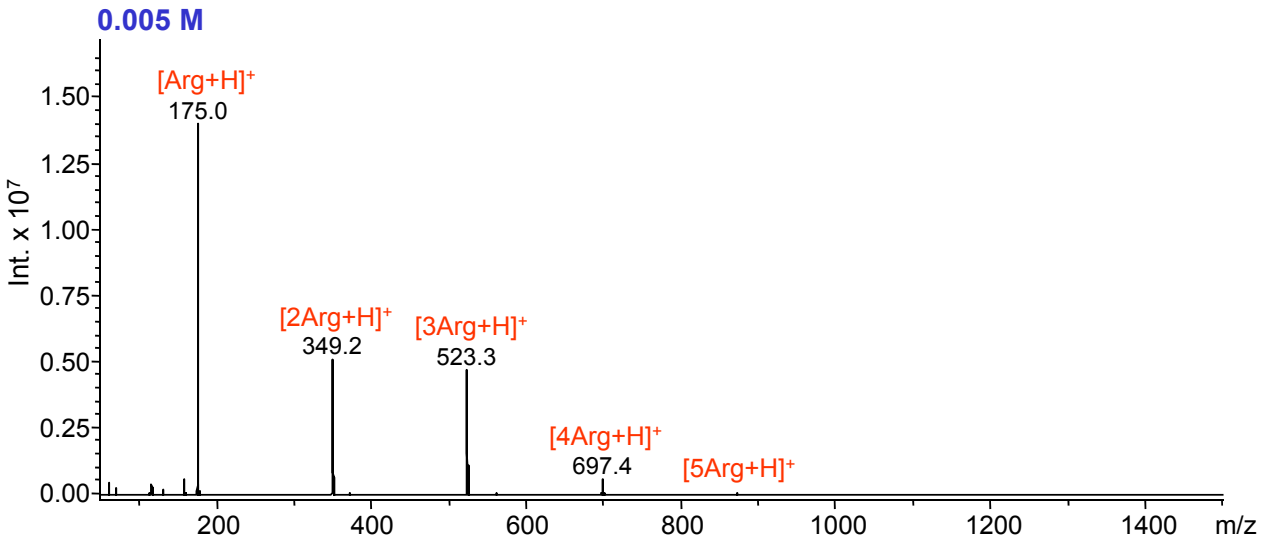


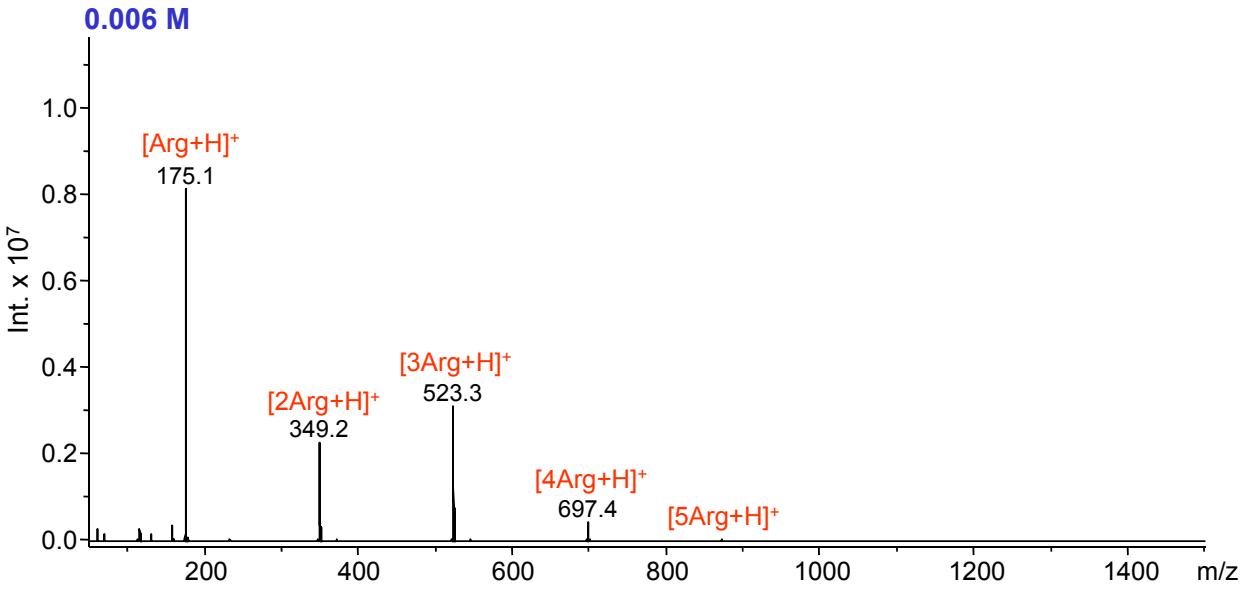


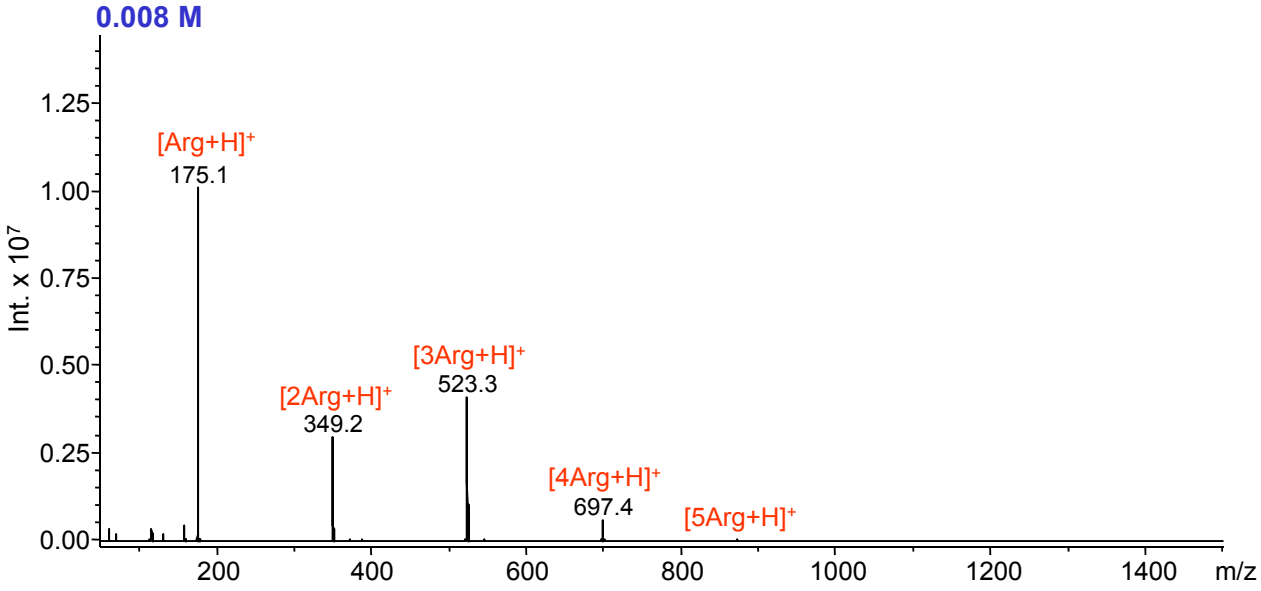


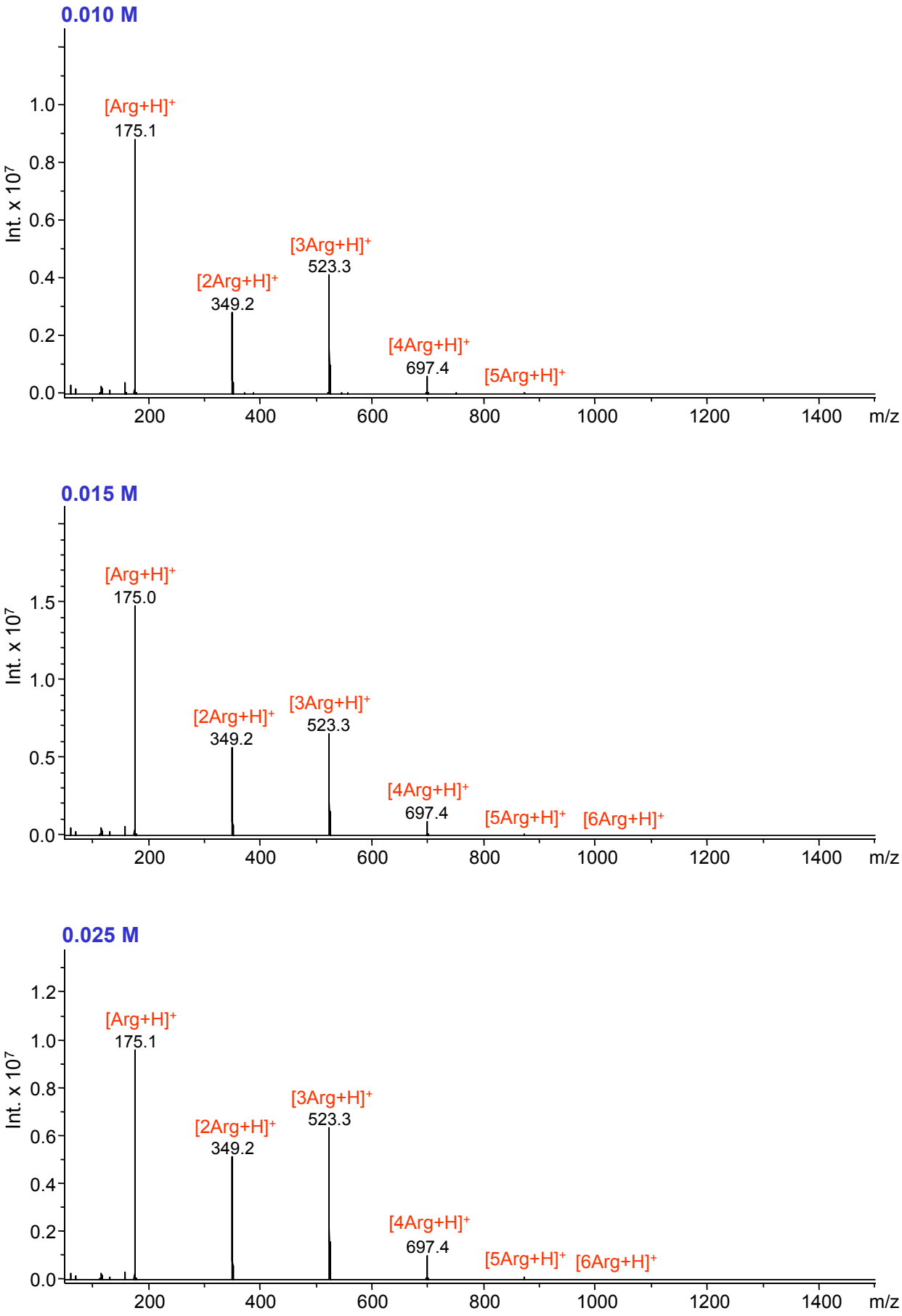


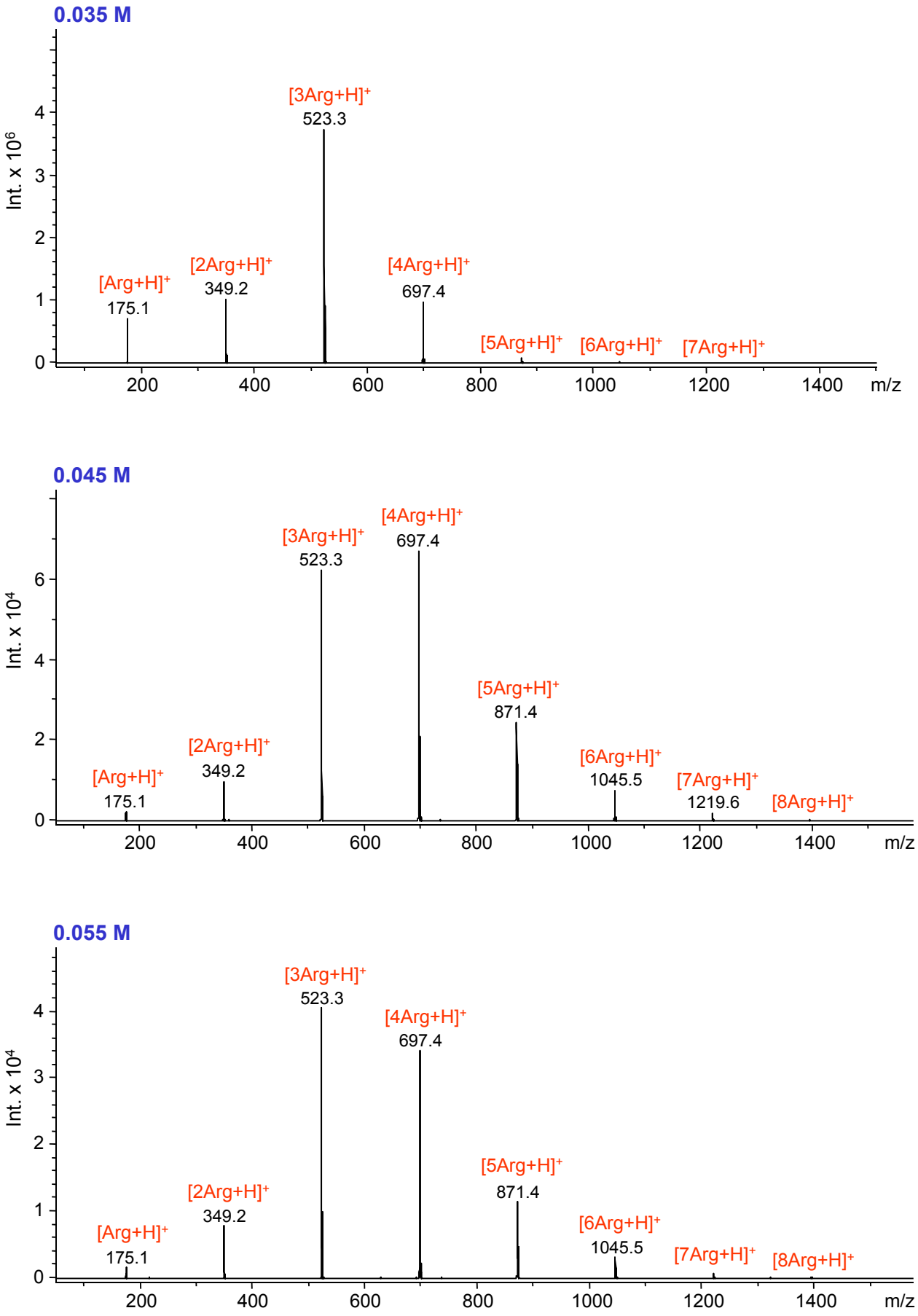


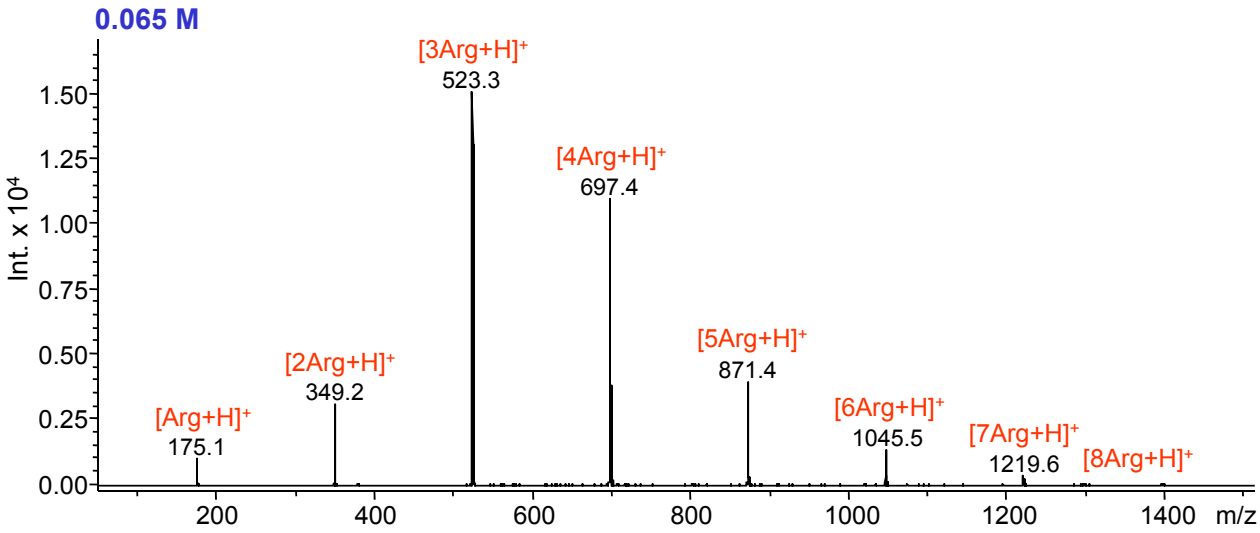


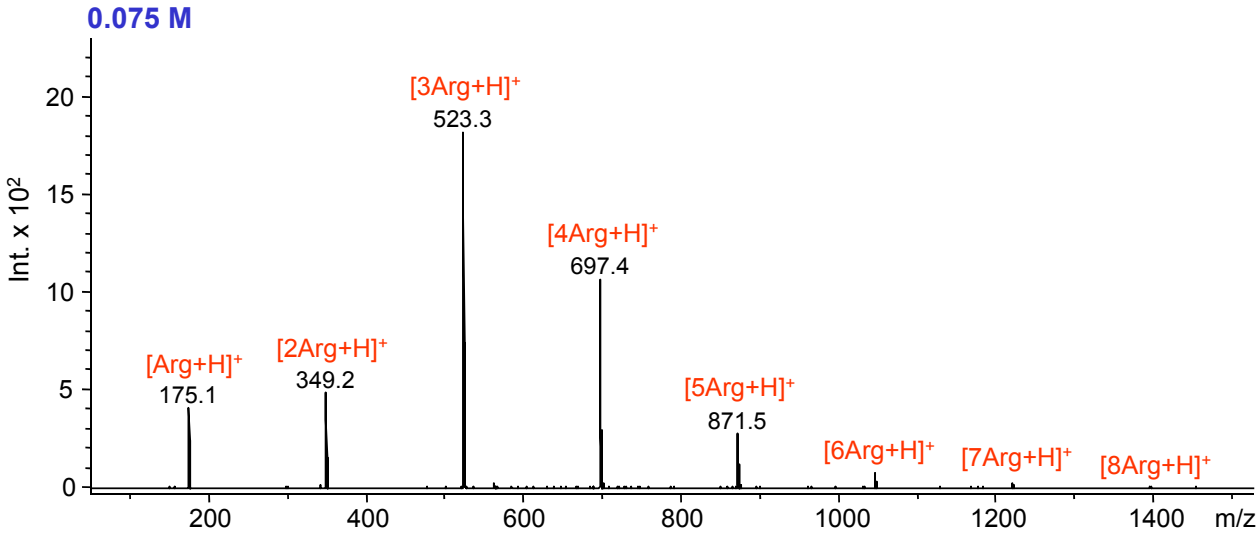


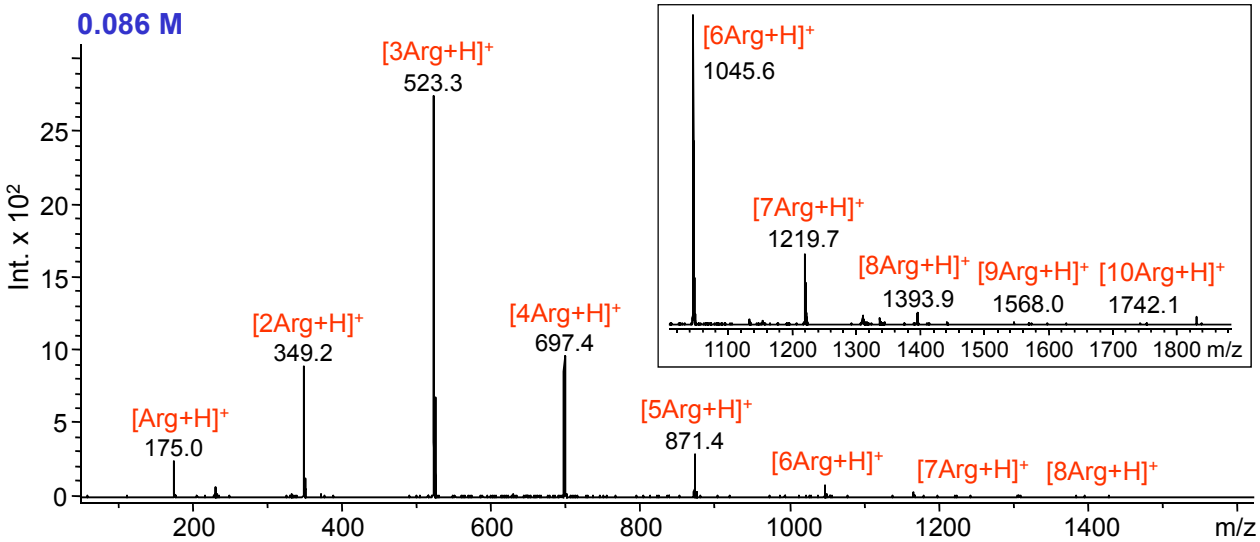




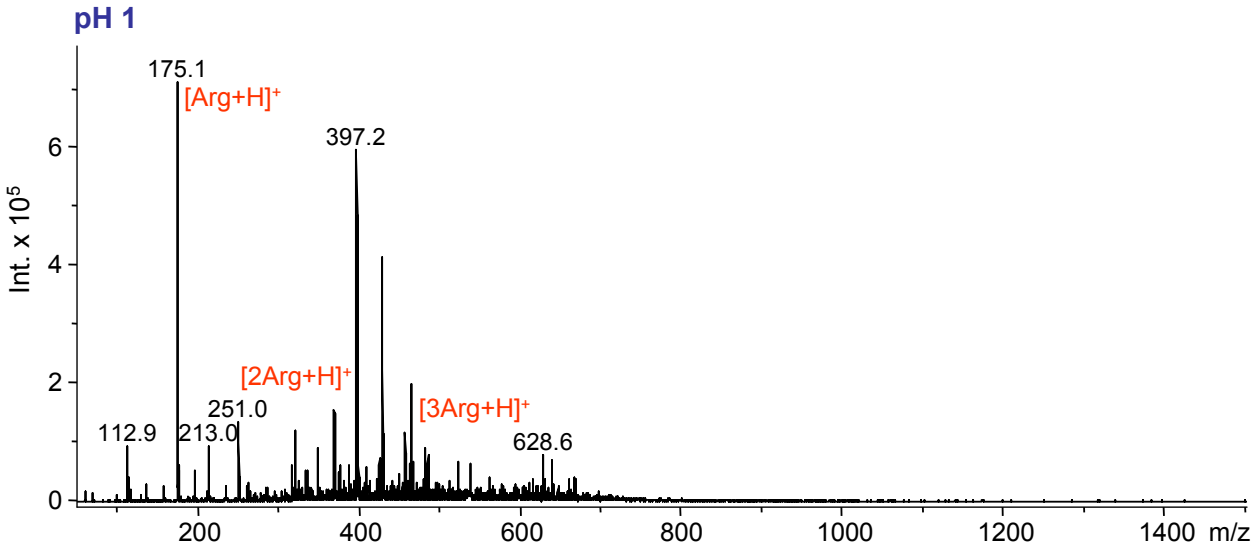


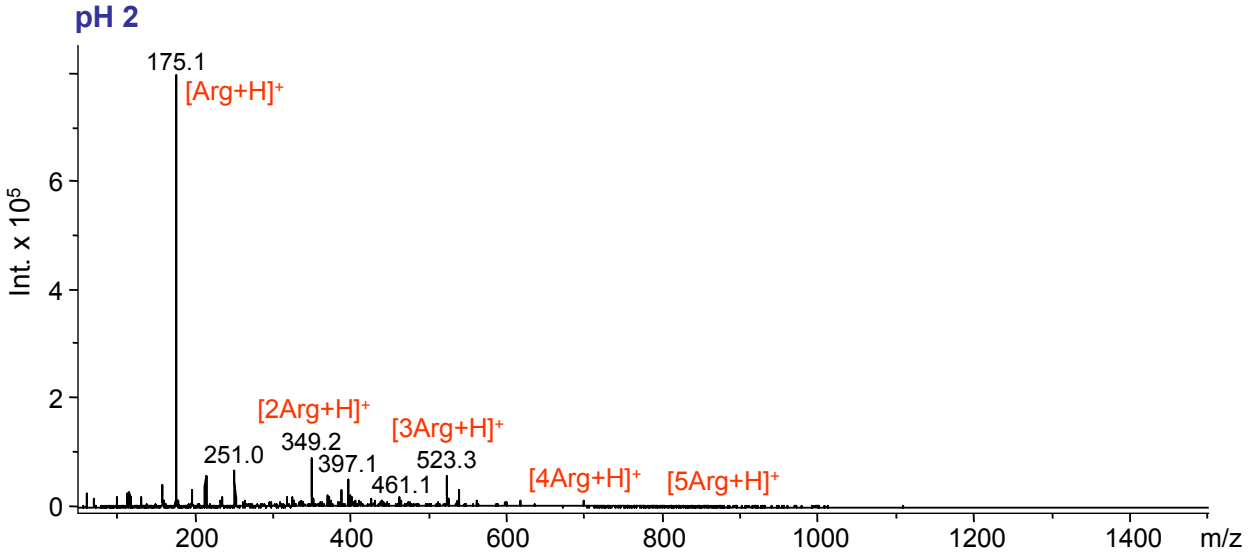


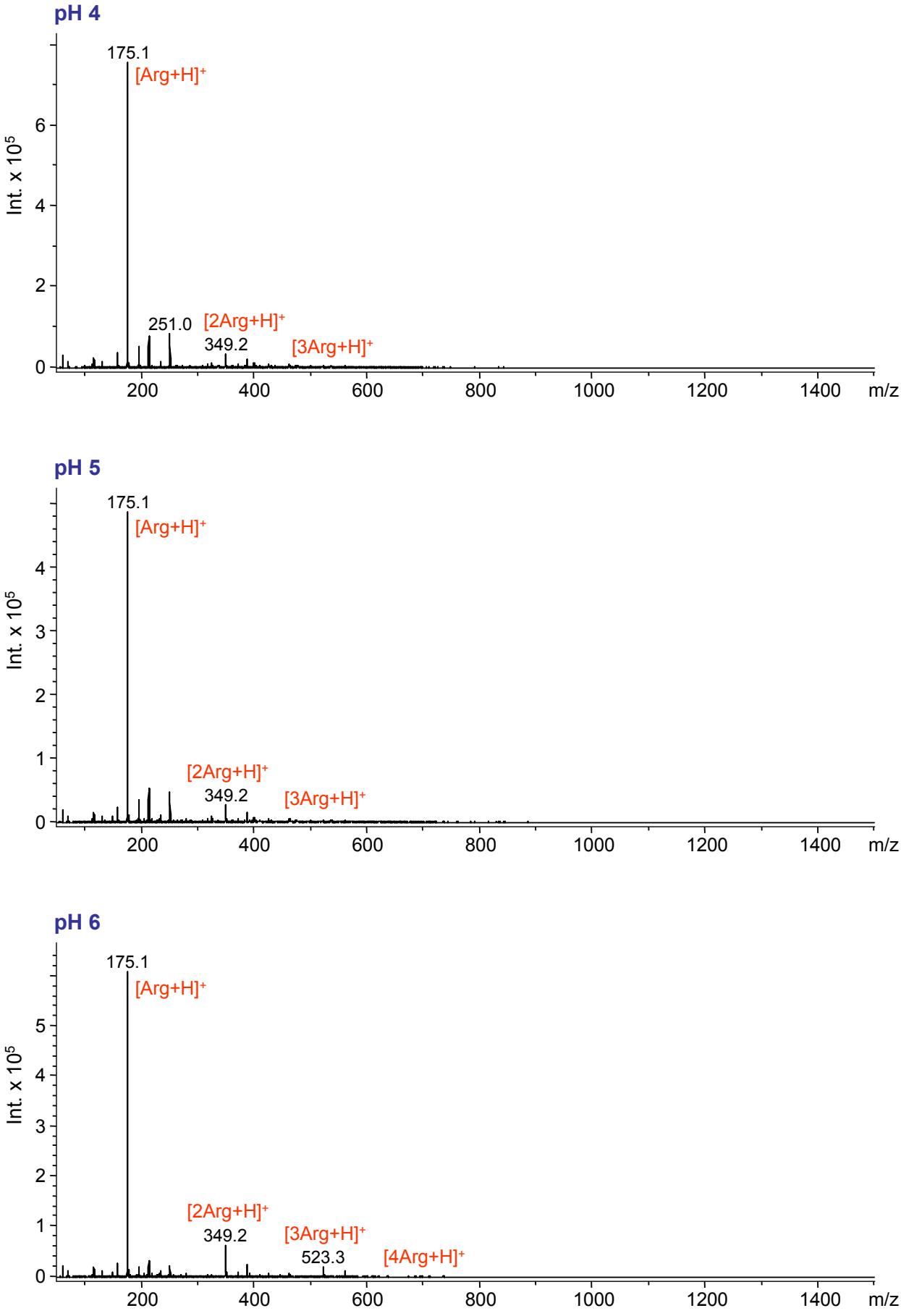


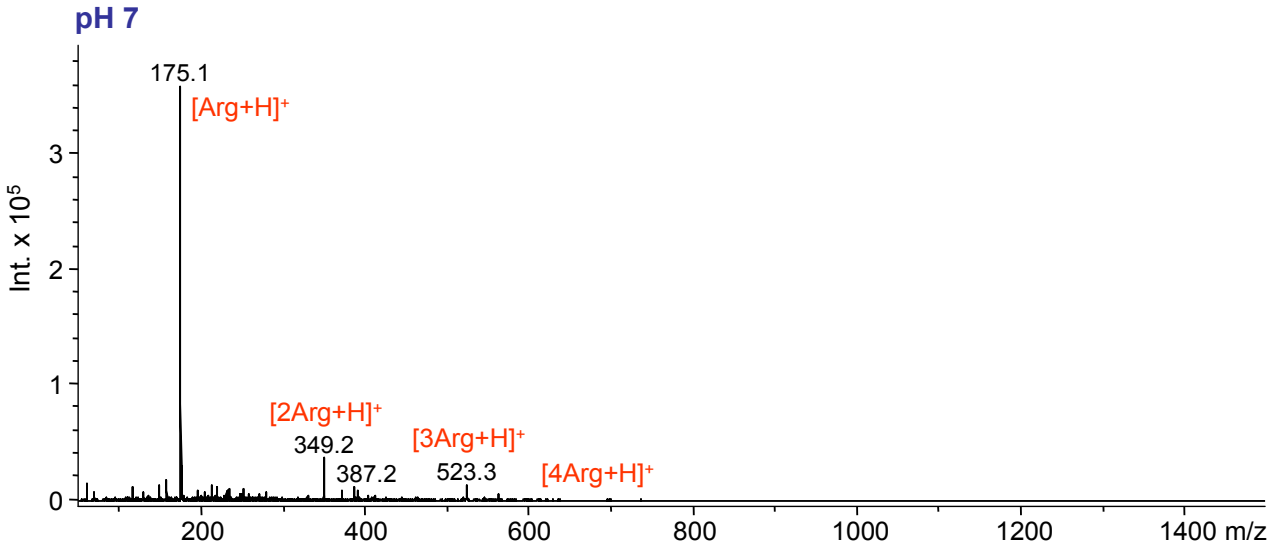


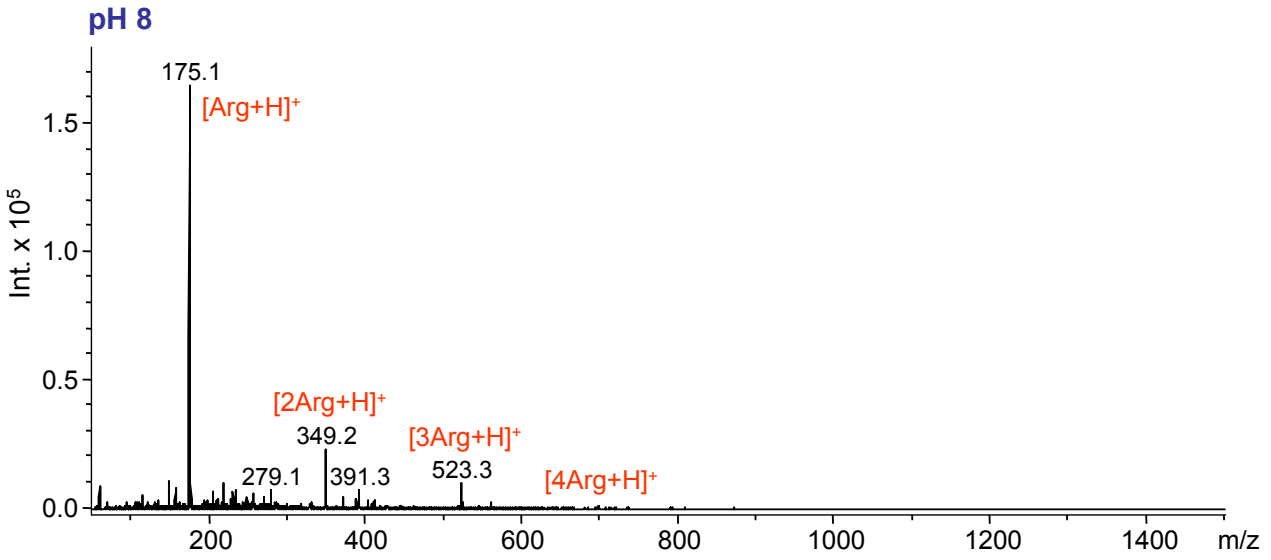
S3.3 Variation of pH for DL-Arg Solutions at 10^{-4} M

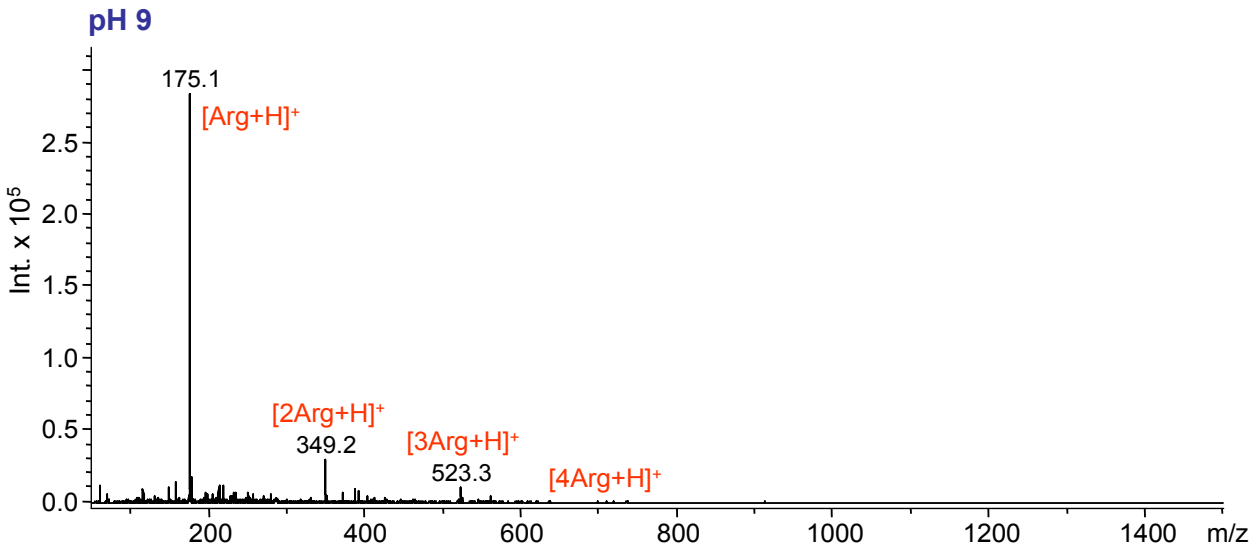


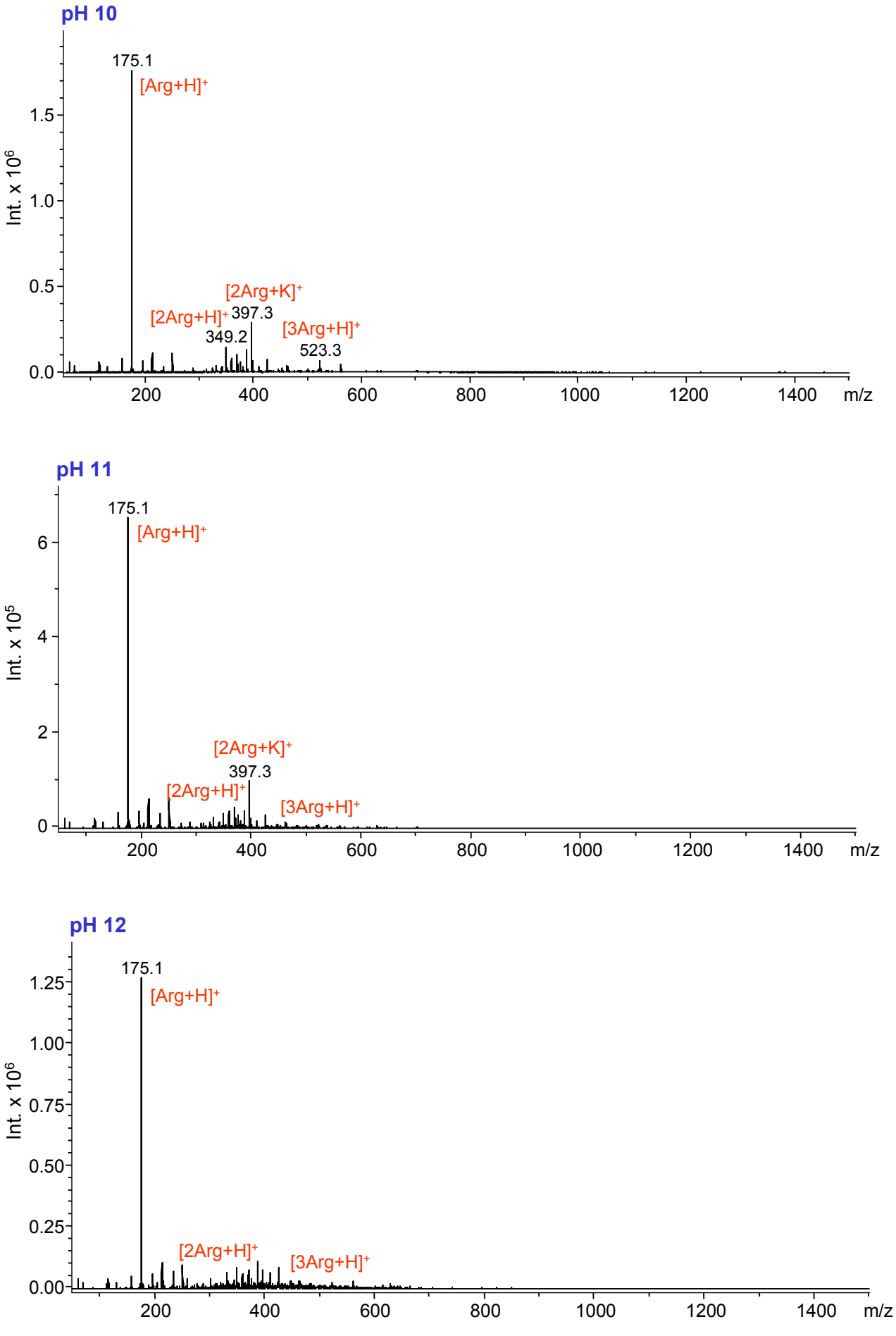




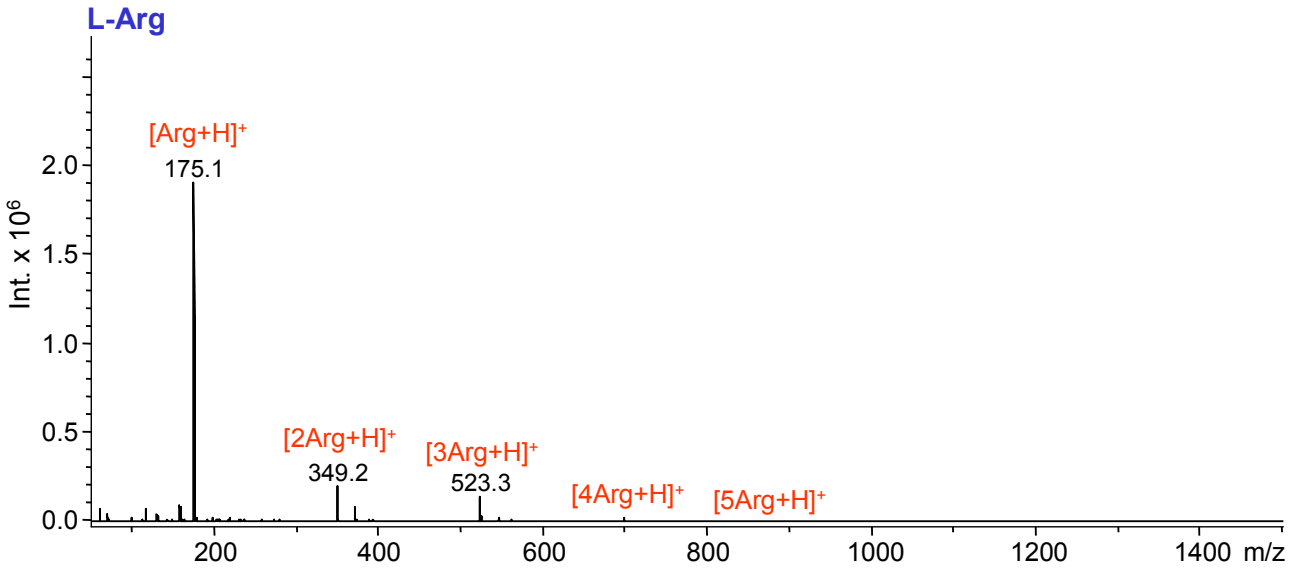


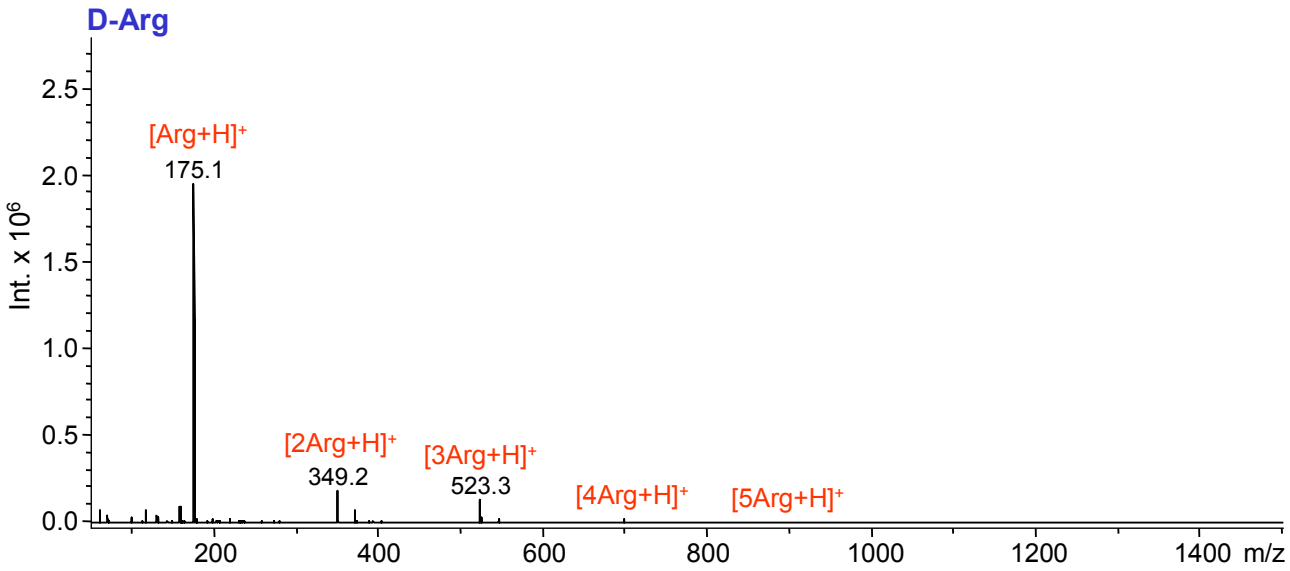






S3.4 Spectra of Optically Pure Arg Solutions at pH 3.1 and 10^{-4} M



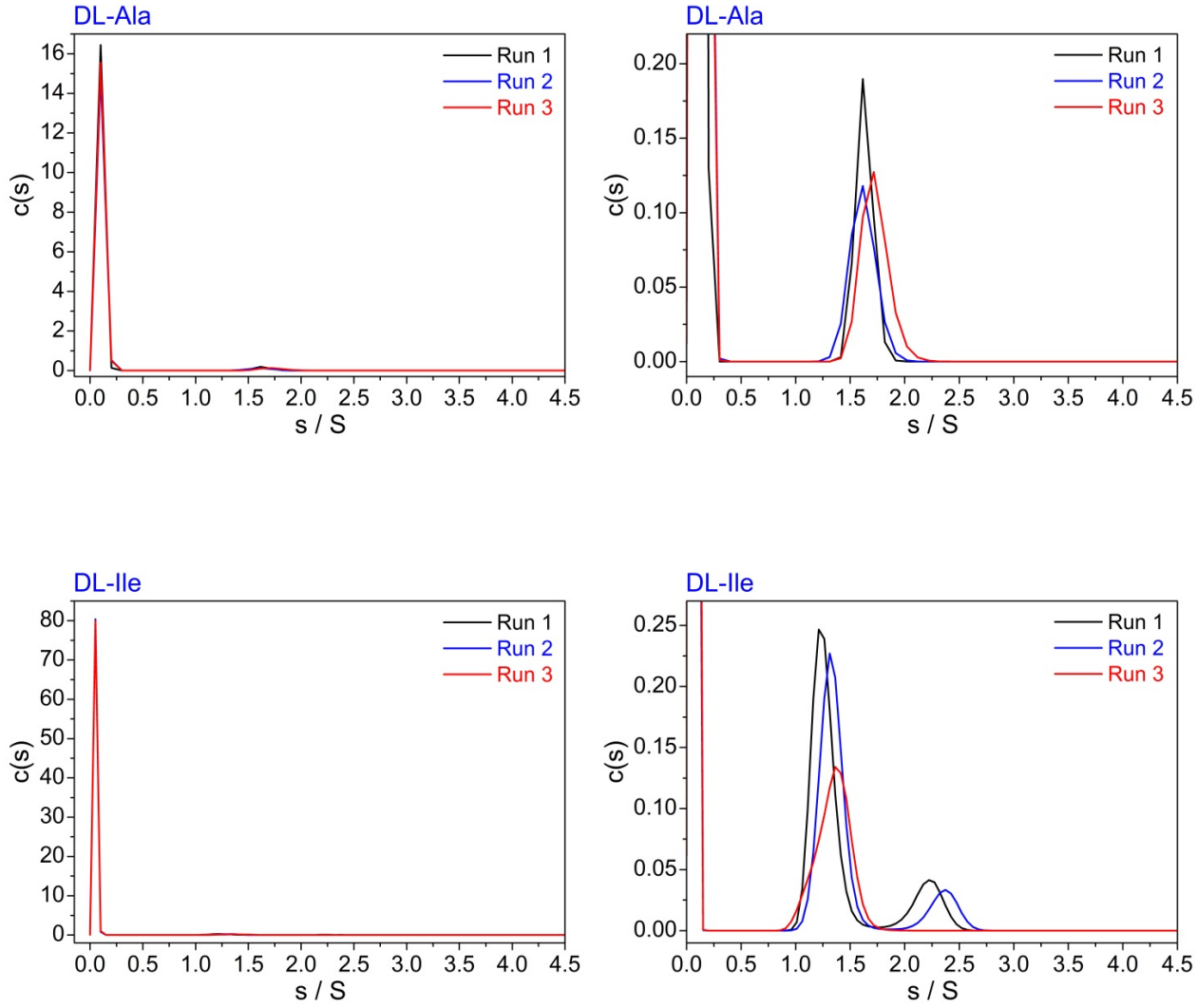


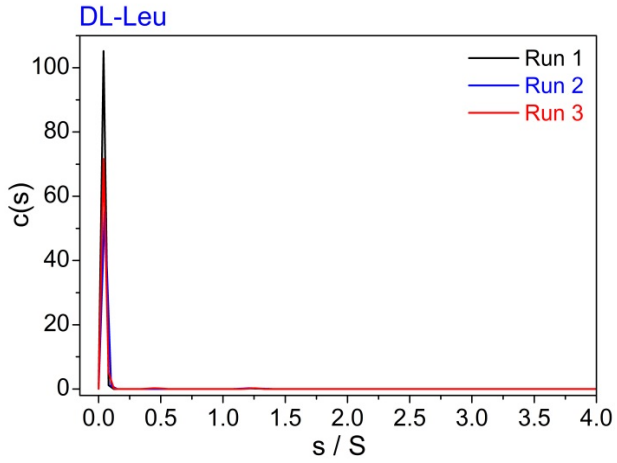
Section S4 Sedimentation Coefficient Distributions

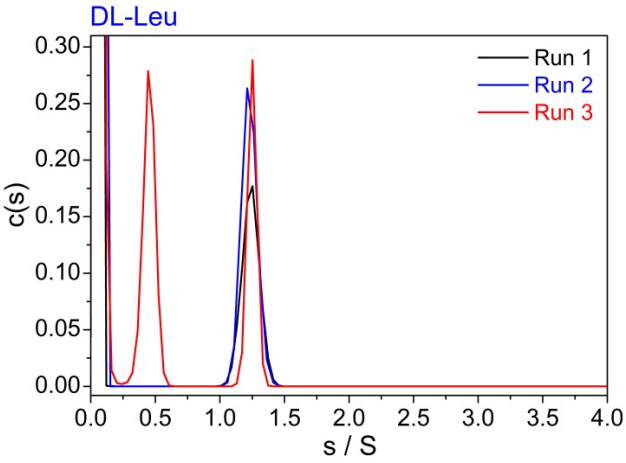
S4.1 Non-Polar/Hydrophobic Amino Acids

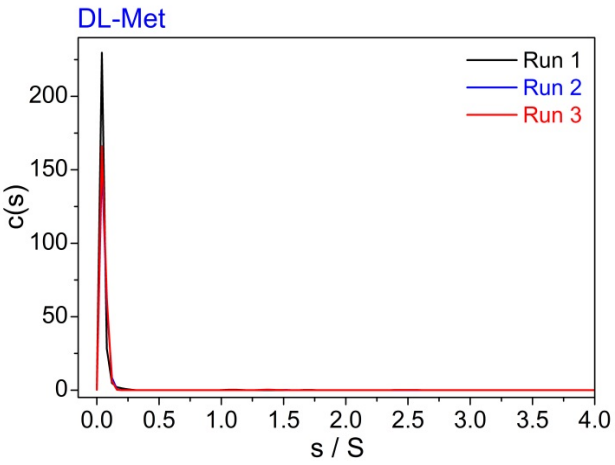
Left: Overview of the distribution, showing an intense monomer signal in all cases.

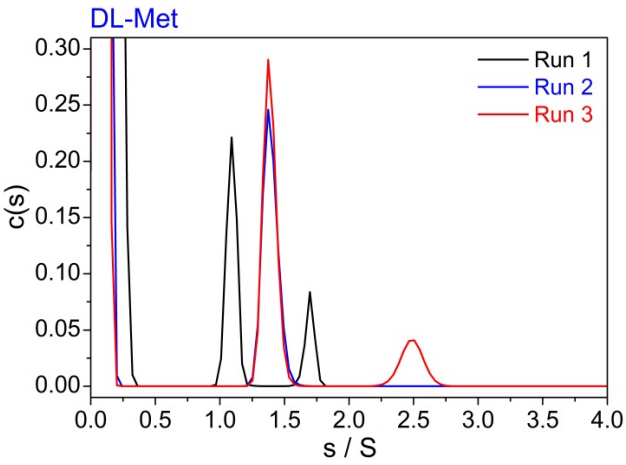
Right: Zoom into the s-region typical for amino acid clusters.

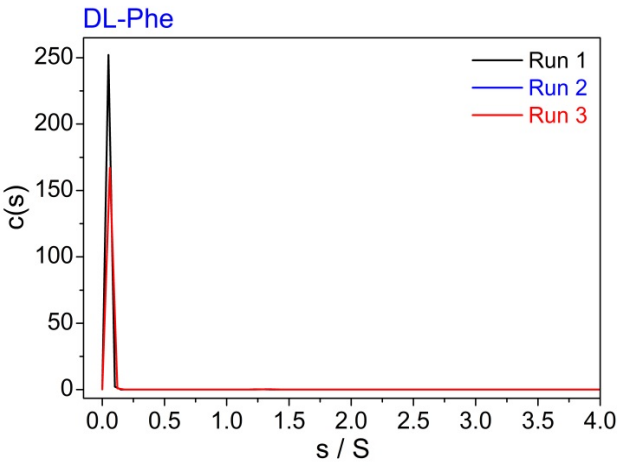


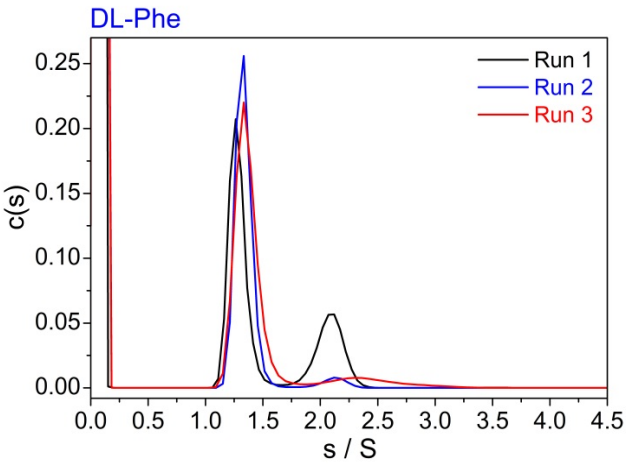


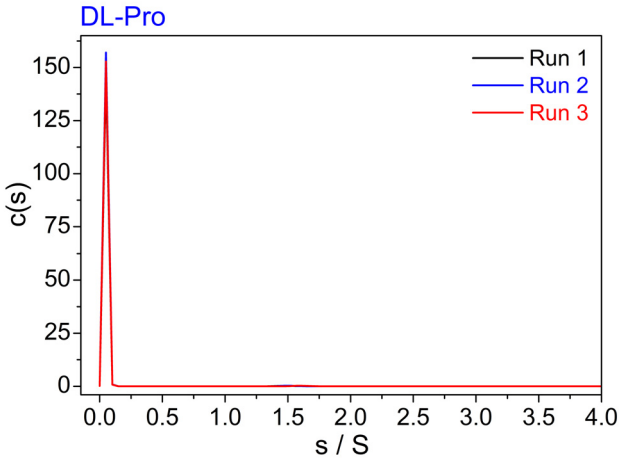


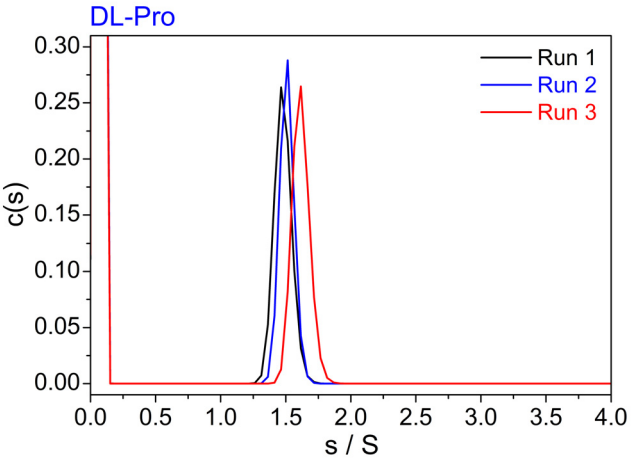


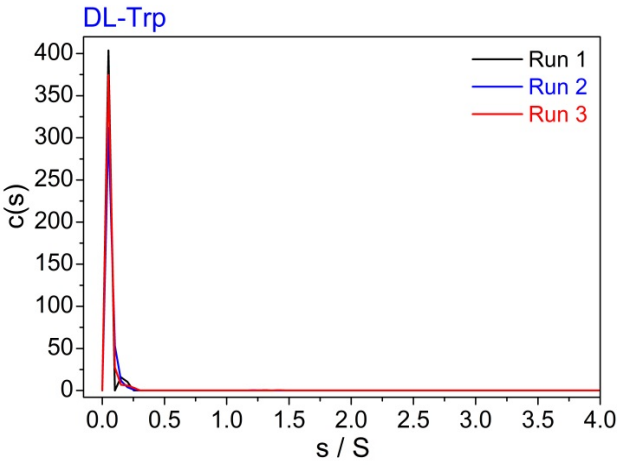


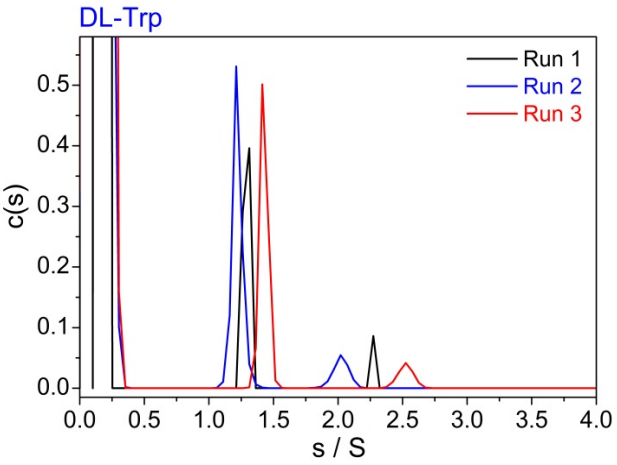


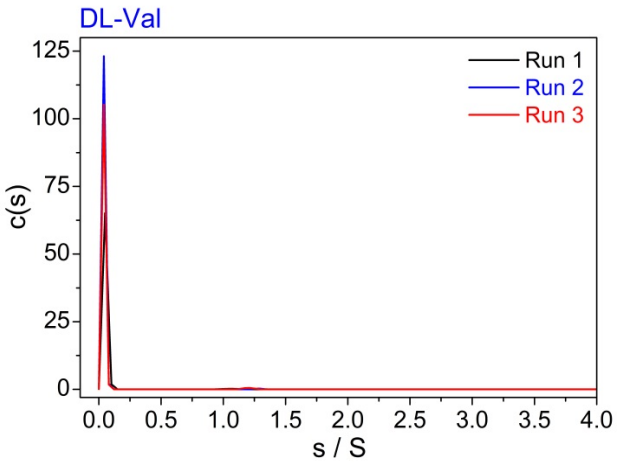


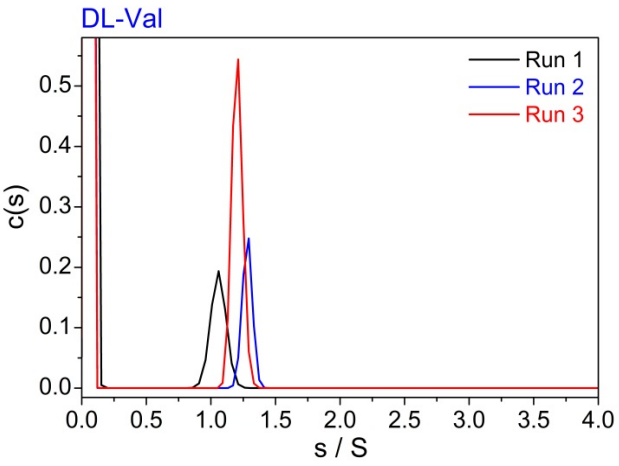




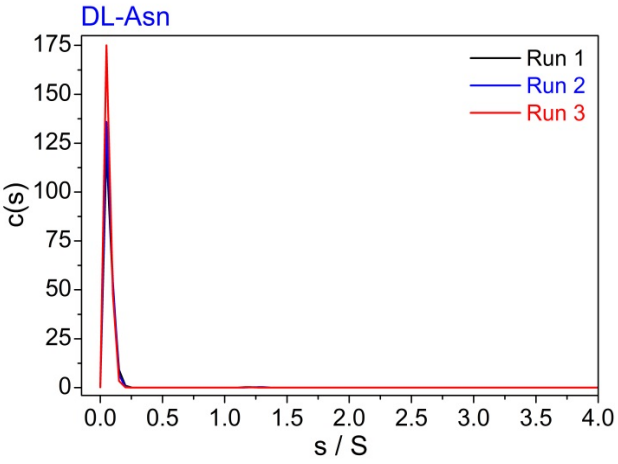


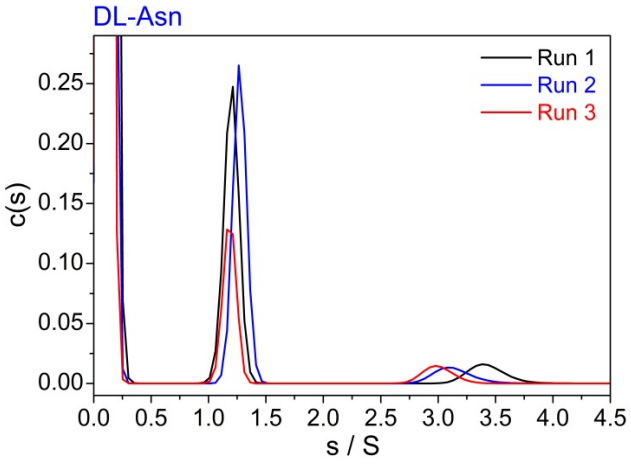


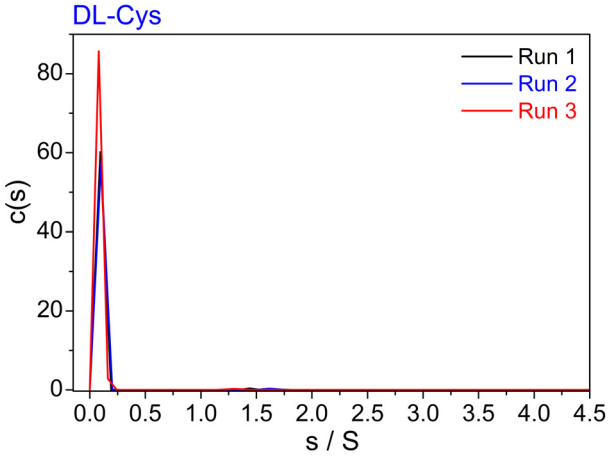


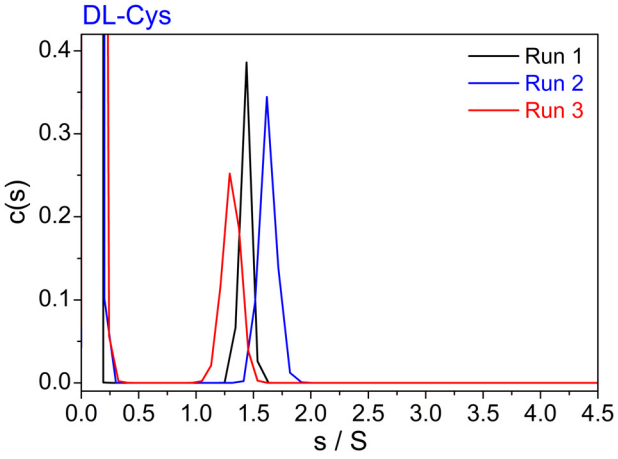


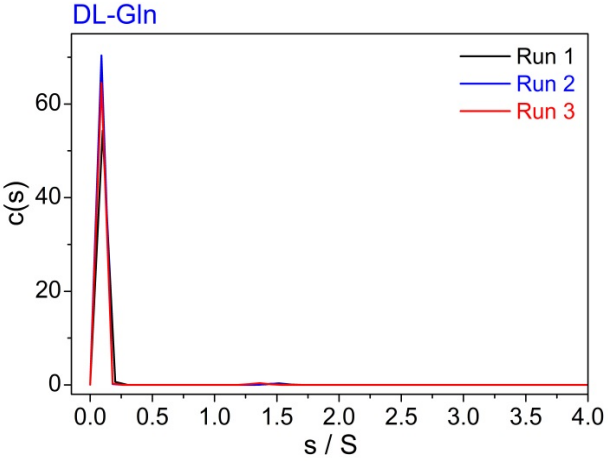
S4.2 Polar/Neutral Amino Acids

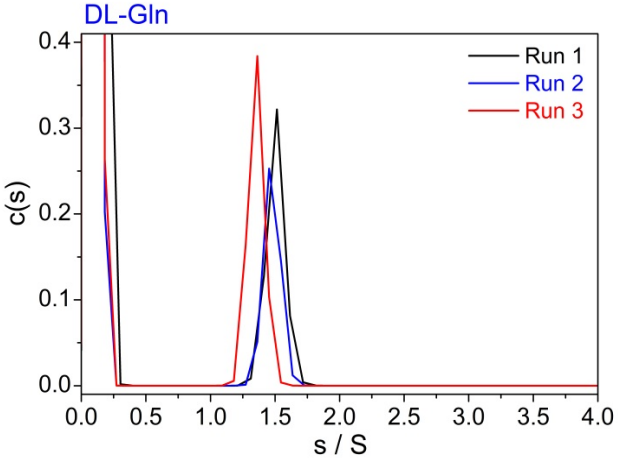


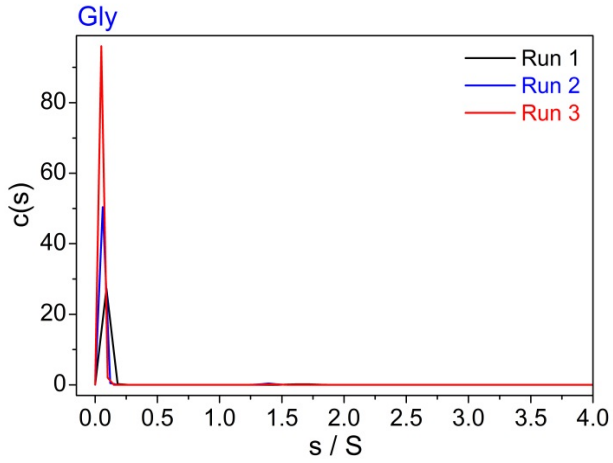


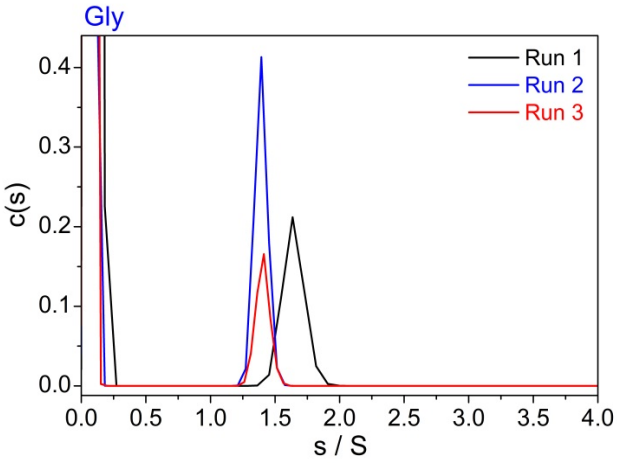


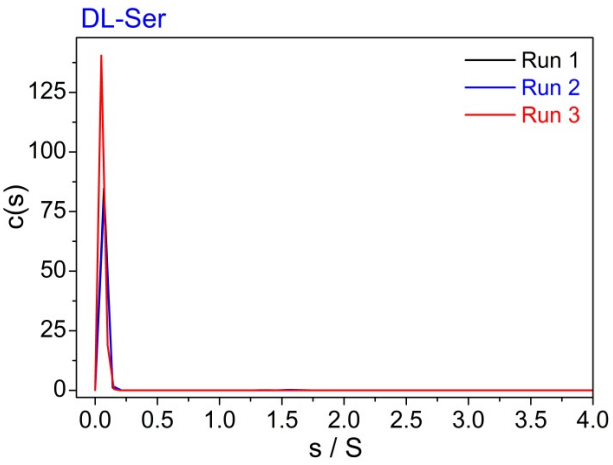


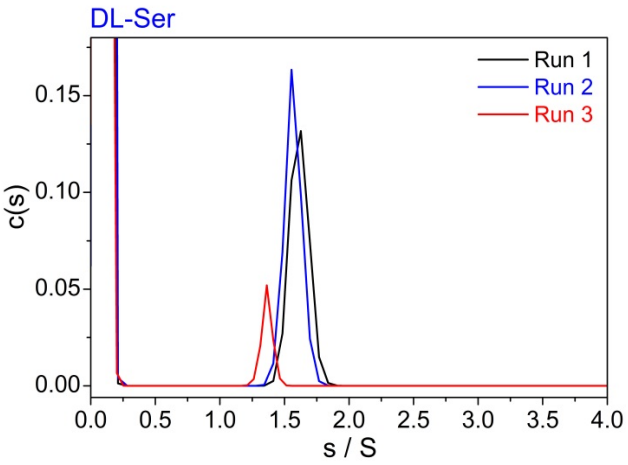


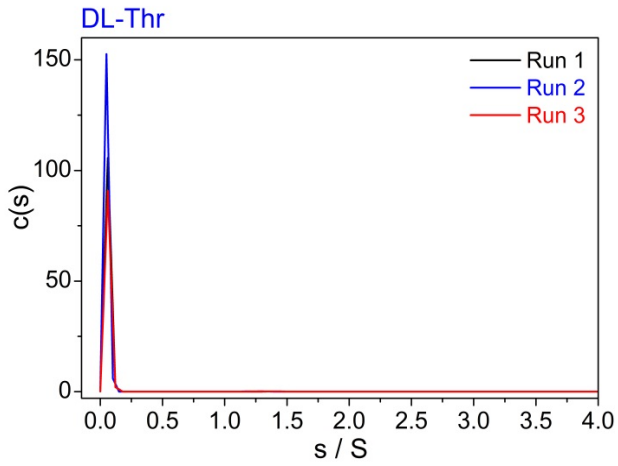


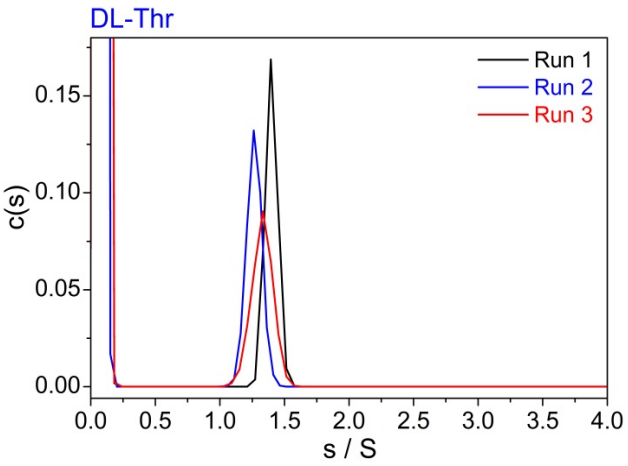


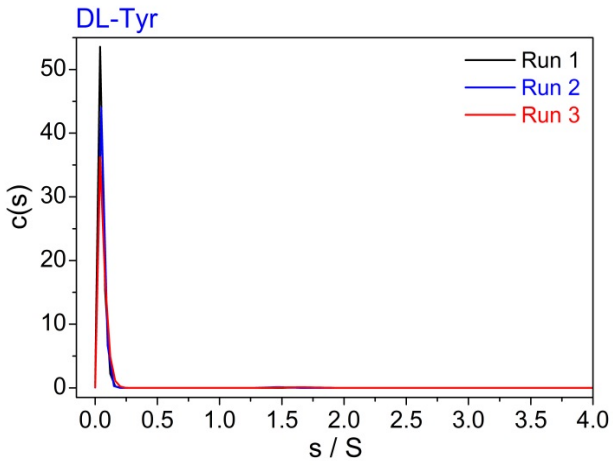


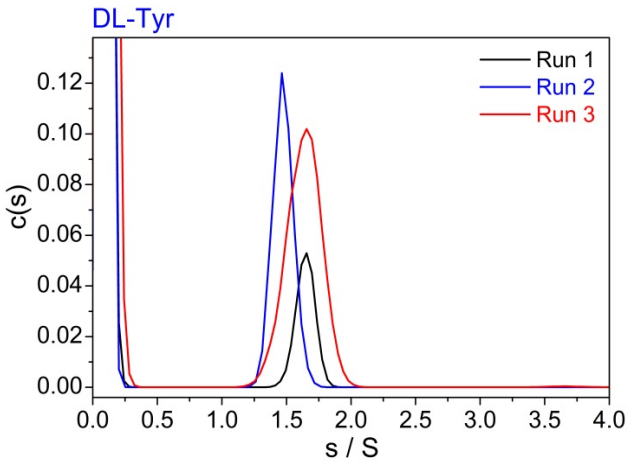




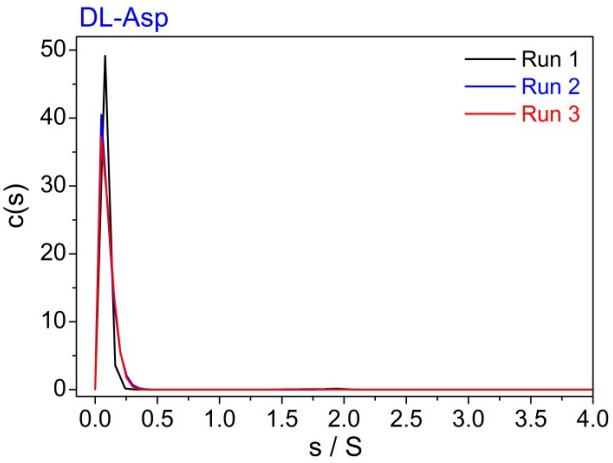


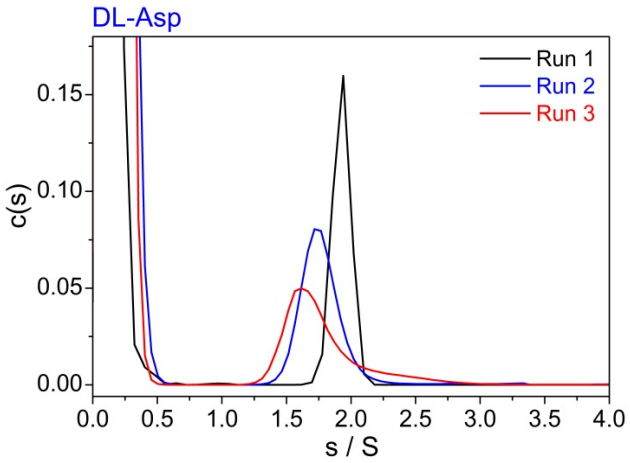


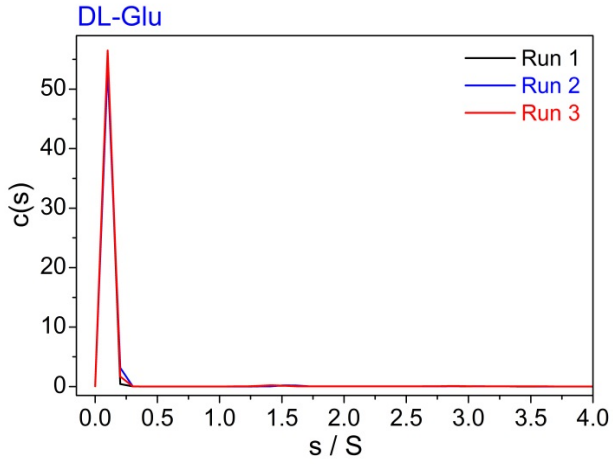


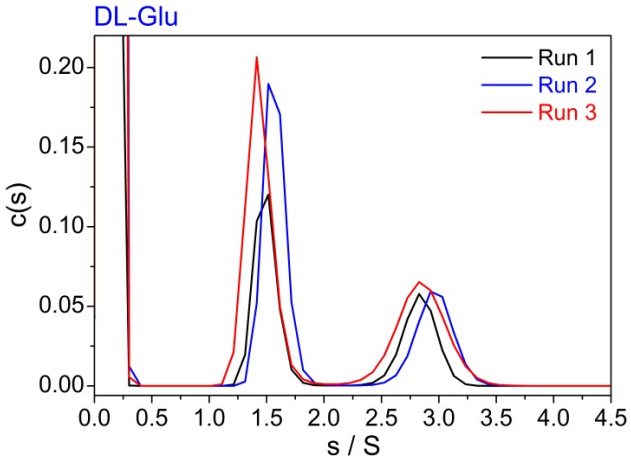


S4.3 Acidic Amino Acids









S4.4 Basic Amino Acids