Electronic Supplementary Material (ESI) for Analyst.

Effects of Charge States, Charge Sites and Side Chain Interactions on Conformational Preferences of a Series of Model Peptide Ions

Chunying Xiao, Lisa M. Pérez, and David H. Russell*

Department of Chemistry
Texas A&M University
College Station, Texas 77842
*russell@chem.tamu.edu

Supporting Information

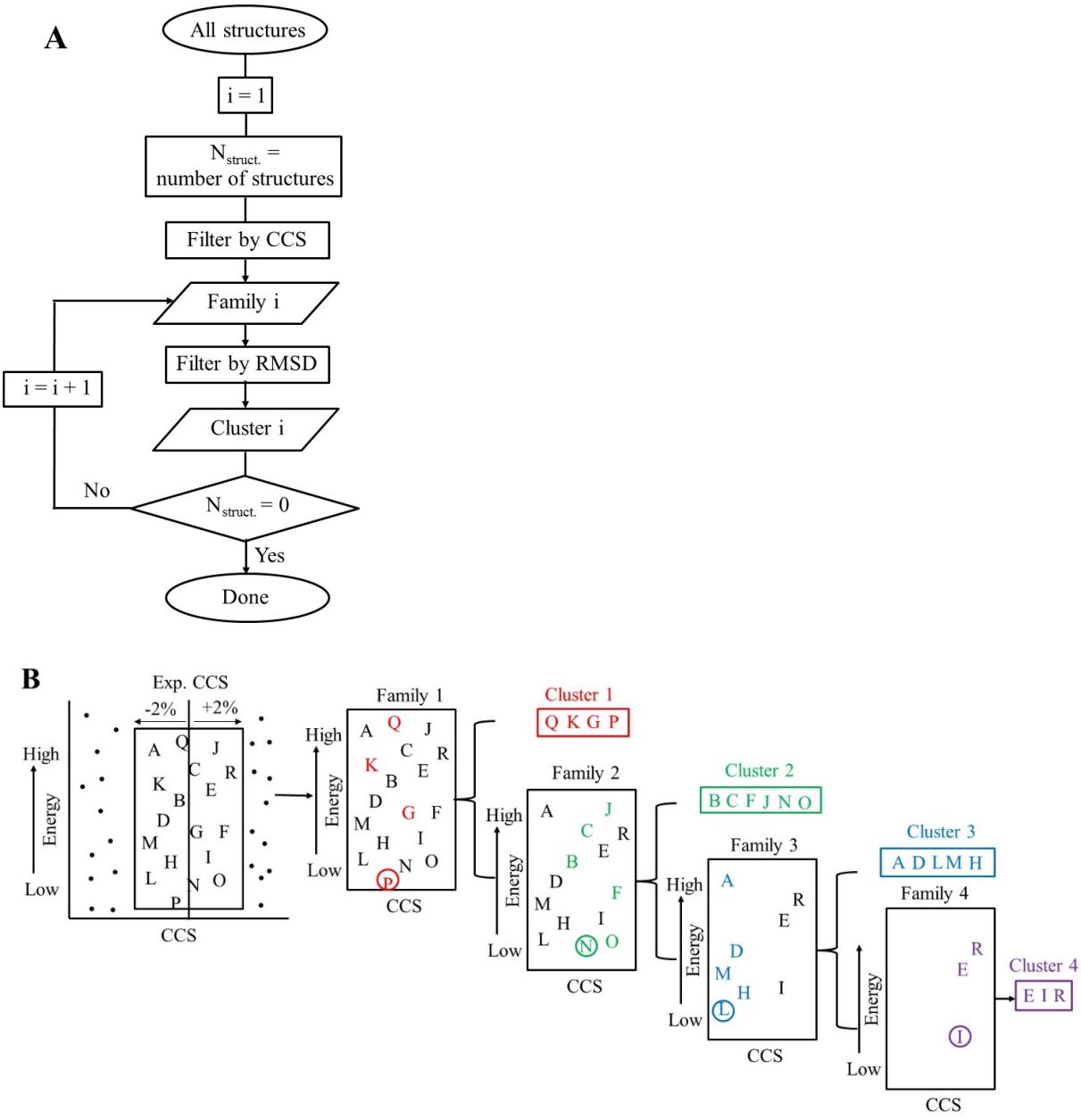
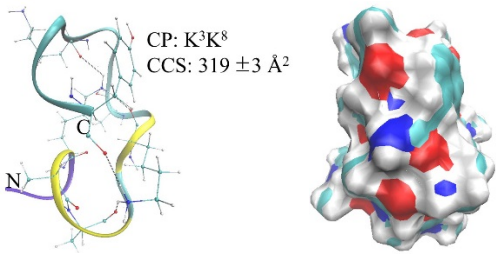
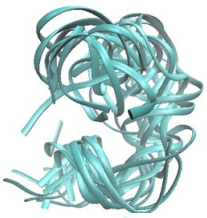
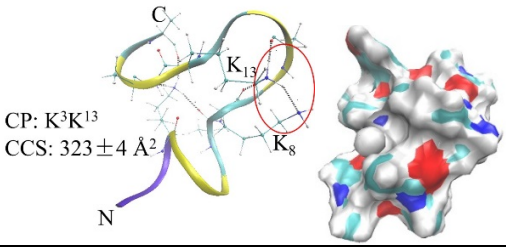
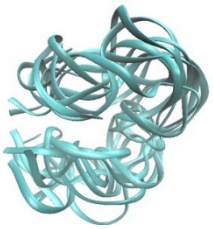
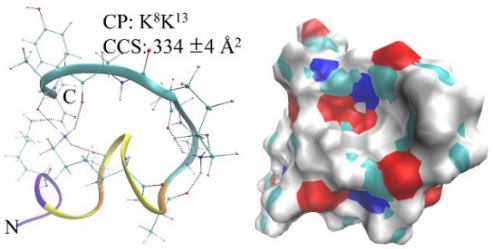
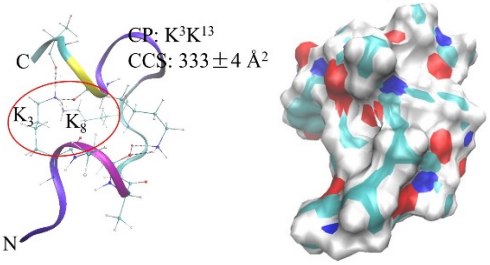
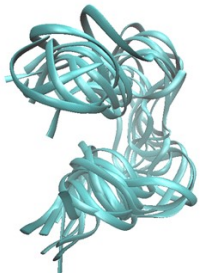
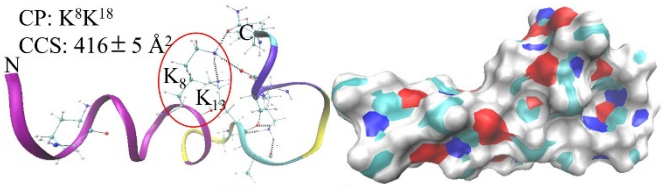
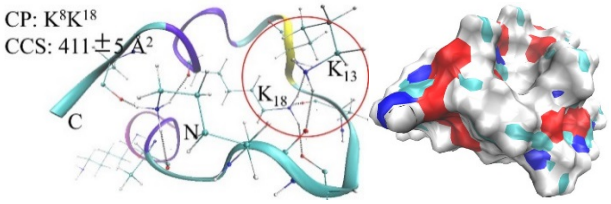
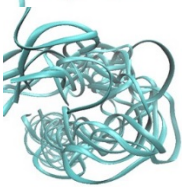


Figure S1. **A**. General stategy to make clusters. **B**. An example of making clusters, showing details. The dots and letters indicate conformations generated by molecular dynamics simulations. The circles of different colors mark the lowest energy structures that were used as references when making clusters. Colored letters (red, green, blue and purple) in each family indicate the conformations having an RMSD of the backbone atoms less than a specified value when compared to the lowest energy conformation (circled).

Figure S2A

Peptides	Representative ribbons and surface structures	10 Representative ribbons
$[AK_3+2H]^{2+}$ Peak A Exp. CCS: 321 Å^2 Cluster 1 37%		
$[AK_3+2H]^{2+}$ Peak A Exp. CCS: 321 Å^2 Cluster 2 27%		
$[AK_3+2H]^{2+}$ Peak B Exp. CCS: 335 Å^2 Cluster 1 15%		
$[AK_3+2H]^{2+}$ Peak B Exp. CCS: 335 Å^2 Cluster 2 28%		
$[AK_4+2H]^{2+}$ Exp. CCS: 413 Å^2 Cluster 1 37%		
$[AK_4+2H]^{2+}$ Exp. CCS: 413 Å^2 Cluster 2 18%		

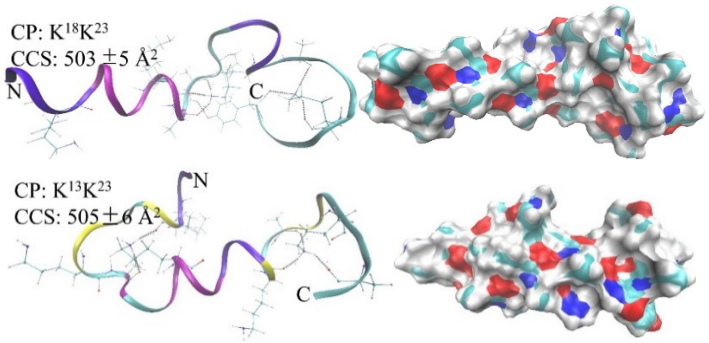
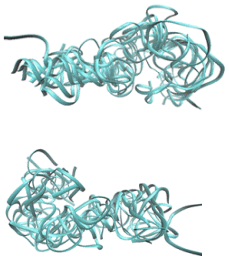
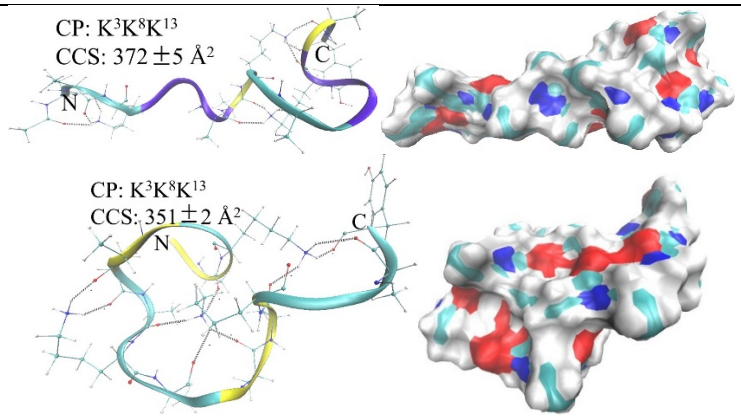
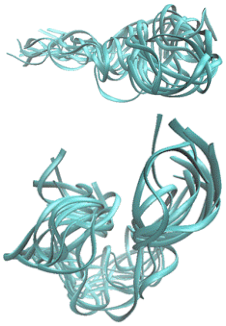
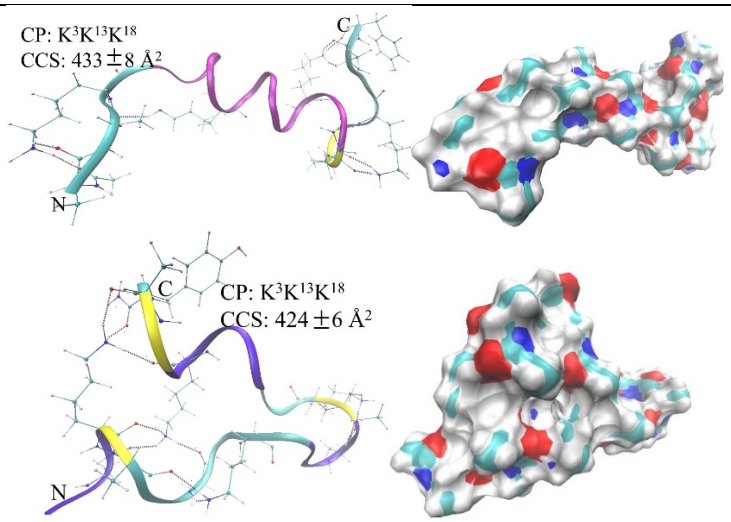
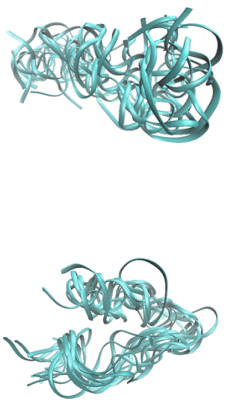
$[AK_5+2H]^{2+}$ Exp. CCS: 512 Å^2 Cluster 1 19% $[AK_5+2H]^{2+}$ Exp. CCS: 512 Å^2 Cluster 3 15%		

Figure S2B

Peptides	Representative ribbons and surface structures	10 Representative ribbons
$[AK_3+3H]^{3+}$ Exp. CCS: 363 Å^2 Cluster 1 20% $[AK_3+3H]^{3+}$ Exp. CCS: 363 Å^2 Cluster 2 26%		
$[AK_4+3H]^{3+}$ Exp. CCS: 424 Å^2 Cluster 1 14% $[AK_4+3H]^{3+}$ Exp. CCS: 424 Å^2 Cluster 2 23%		

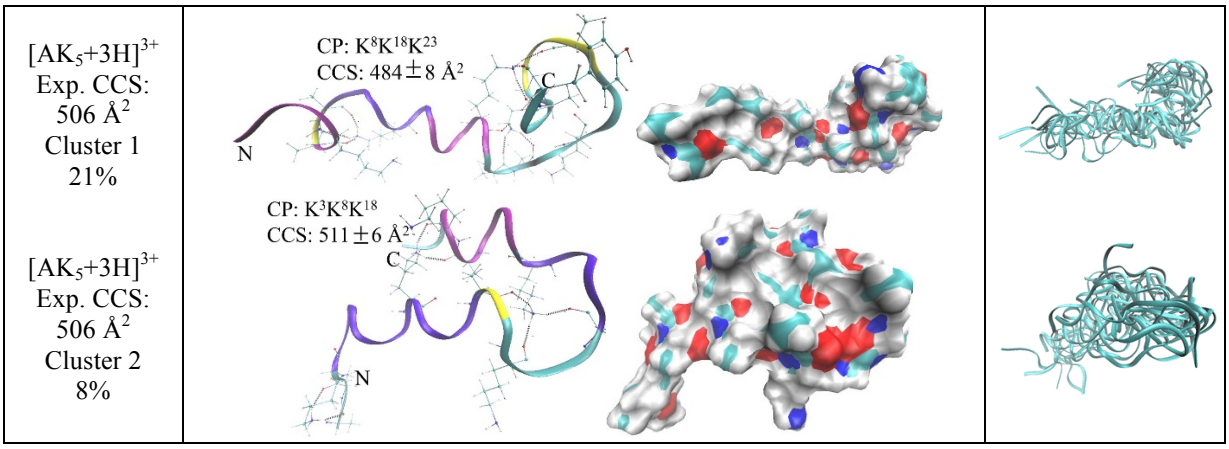
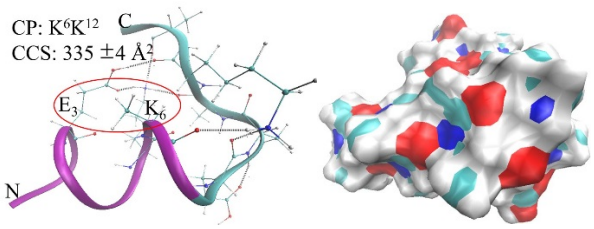
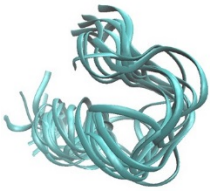
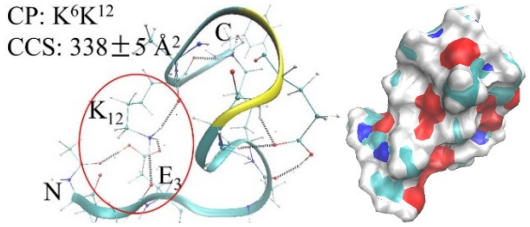
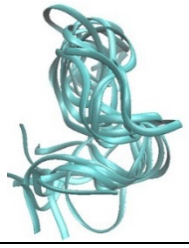
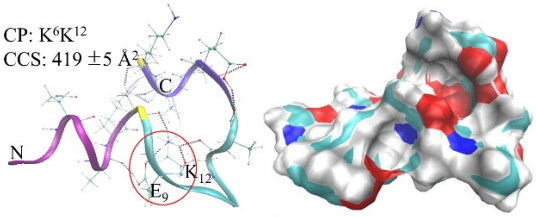
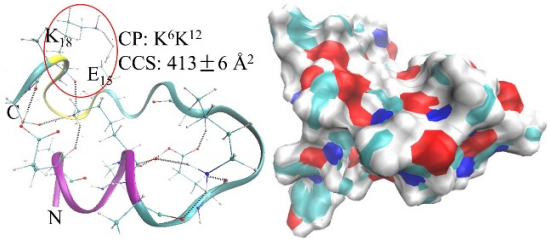
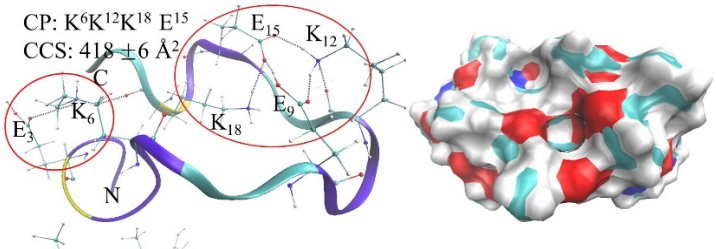
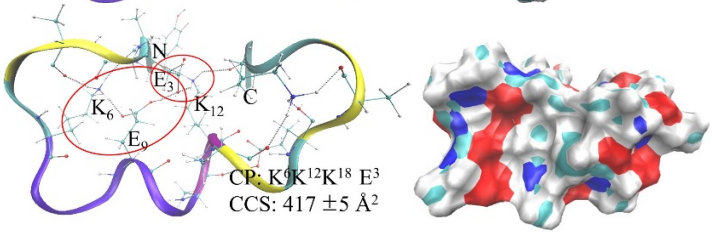
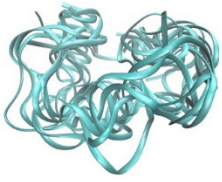


Figure S2. Backbone and surface structures of the most populated clusters for $[AK_n+2H]^{2+}$and $[AK_n+3H]^{3+}$ generated by molecular dynamics simulations. All the conformers shown were generated by VMD. The second column contains the lowest energy structures in clusters and the third column contains 10 lowest energy ribbon structures in each cluster. "N" and "C" indicate the N- and C-terminus respectively. "CP" in the second column indicates charge sites. The superscript on K in the second column indicates the residue that is protonated. For example, K^3K^8 indicates the third and eighth residues, lysines, are protonated. The second column contains the backbone and surface representative structure. On the backbone structures, all the polar side chains and the residues that are involved in the connection with side chains are shown. Side chain interactions are marked with red circles. The dashed black lines (---) represent H-bonds. Colors in the backbones indicate different secondary structures: ■ (Purple): α-helix, ■ (violet): ^{3}10-helix, ■ (yellow): turn, ■ (orange): β-sheet, ■ (cyan): random coil; different color on peptide surface indicates different atoms: ■ (cyan): C; ■ (red): O; ■ (blue): N; ■ (grey): H. Charge sites, experimental CCSs and calculated CCSs are also listed. The third column lists ten representative backbone structures in each cluster. Only the clusters having abundances exceeding 12% (percentage cutoff) are presented. If only one cluster has a population higher than 12%, the second cluster is also presented. In the event that no clusters have a percentage higher than 12%, the two most populated clusters are presented. The second column contains representative ribbon (backbone) structures that illustrate how polar side chains affect the conformation of peptide ions, and how side chain interactions affect the 3-D shape of the molecule. The third column reveals the similarities in backbone conformations (10 lowest energy structures)

Figure S3A

Peptides	Representative ribbons and surface structures	10 Representative ribbons
$[AEK_2^{EN}+2H]^{2+}$ Exp. CCS: 333 Å^2 Cluster 1 55%		
$[AEK_2^{EN}+2H]^{2+}$ Exp. CCS: 333 Å^2 Cluster 2 16%		
$[AEK_3^{EN}+2H]^{2+}$ Exp. CCS: 418 Å^2 Cluster 1 31%		
$[AEK_3^{EN}+2H]^{2+}$ Exp. CCS: 418 Å^2 Cluster 2 20%		
$[AEK_3^{EC}+2H]^{2+}$ Exp. CCS: 418 Å^2 Cluster 1 28%		
$[AEK_3^{EC}+2H]^{2+}$ Exp. CCS: 418 Å^2 Cluster 2 13%		

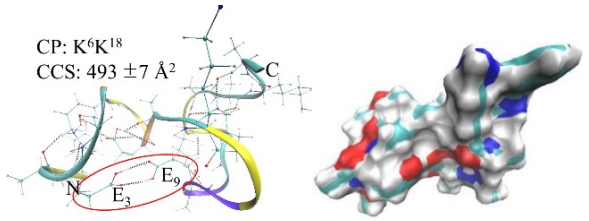
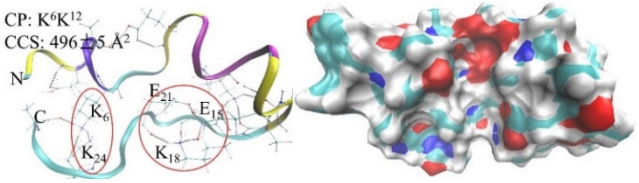
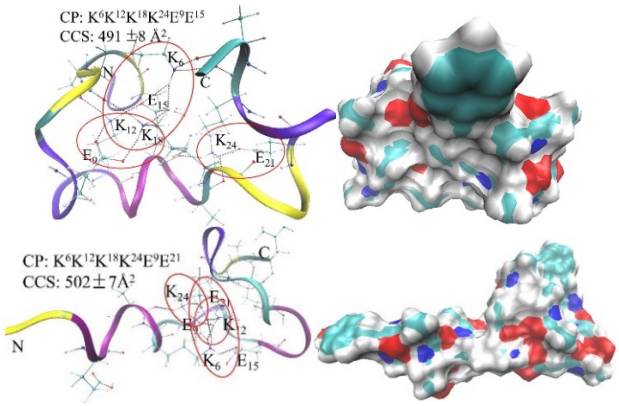
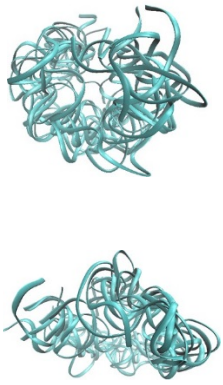
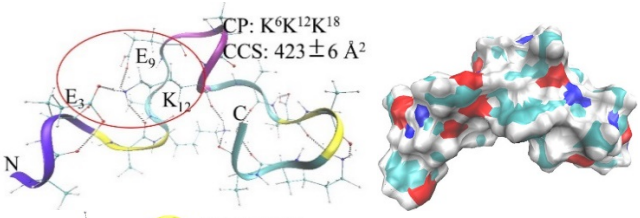
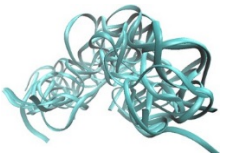
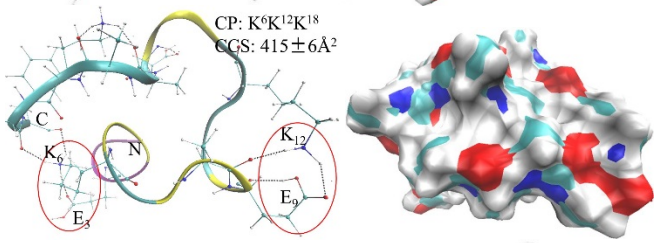
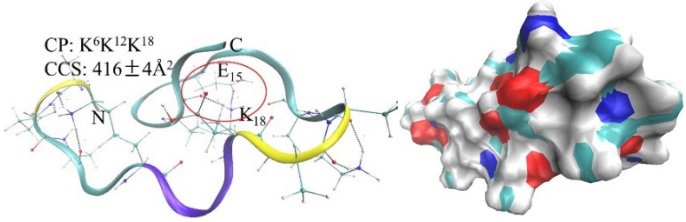
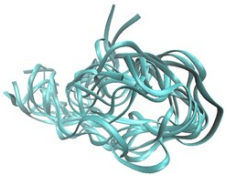
$[AEK_4{}^{EN}+2H]^{2+}$ Exp. CCS: 501 Å^2 Cluster 1 12%		
$[AEK_4{}^{EN}+2H]^{2+}$ Exp. CCS: 501 Å^2 Cluster 6 11%		
$[AEK_4{}^{EC}+2H]^{2+}$ Exp. CCS: 501 Å^2 Cluster 1 37% $[AEK_4{}^{EC}+2H]^{2+}$ Exp. CCS: 501 Å^2 Cluster 3 22%		

Figure S3B

Peptides	Representative ribbons and surface structures	10 Representative ribbons
$[AEK_3{}^{EN}+3H]^{3+}$ Exp. CCS: 428 Å^2 Cluster 1 33%		
$[AEK_3{}^{EN}+3H]^{3+}$ Exp. CCS: 428 Å^2 Cluster 2 12%		
$[AEK_3{}^{EN}+3H]^{3+}$ Exp. CCS: 428 Å^2 Cluster 8 12%		

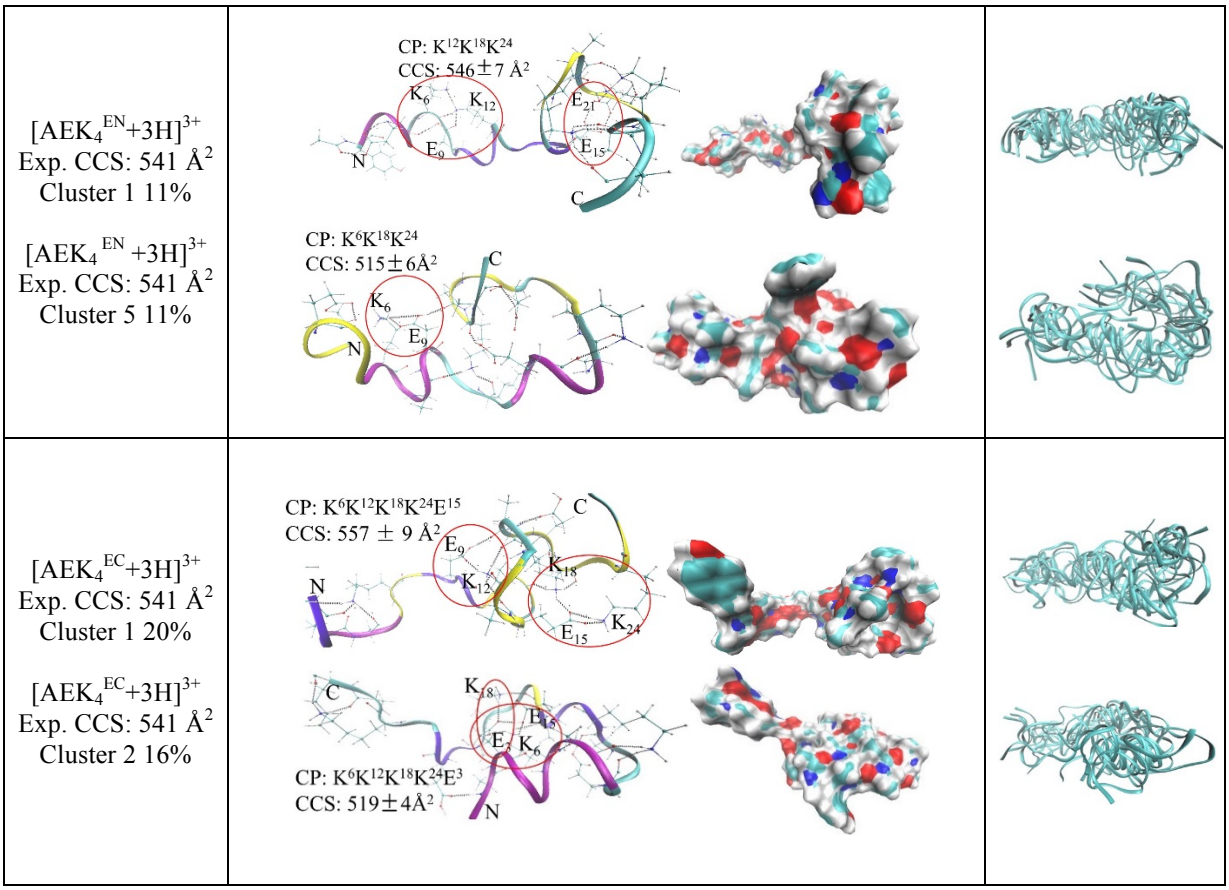


Figure S3. Structures of the most populated clusters for $[AEK_n+2H]^{2+}$ and $[AEK_n+3H]^{3+}$ generated by molecular dynamics simulations. In column 1, "AEK_n^{EN}" indicates that only lysines are protonated and all the glutamic acids are neutral (EN); "AEK_n^{EC}" indicates that all lysines are protonated and some of glutamic acids ((n-2) for doubly-charged ions and (n-3) for triply-charged ions) are negatively charged (EC). CP indicates charge site. The superscript of K in the second column indicates the residue that is protonated and the superscript of E indicates the residue that is deprotonated. The polar side chains and the residues that are involved in the side chain interactions are also represented. Red circles indicate the side chain interactions. The color coding and cluster selection criteria is the same as in **Figure S2.**

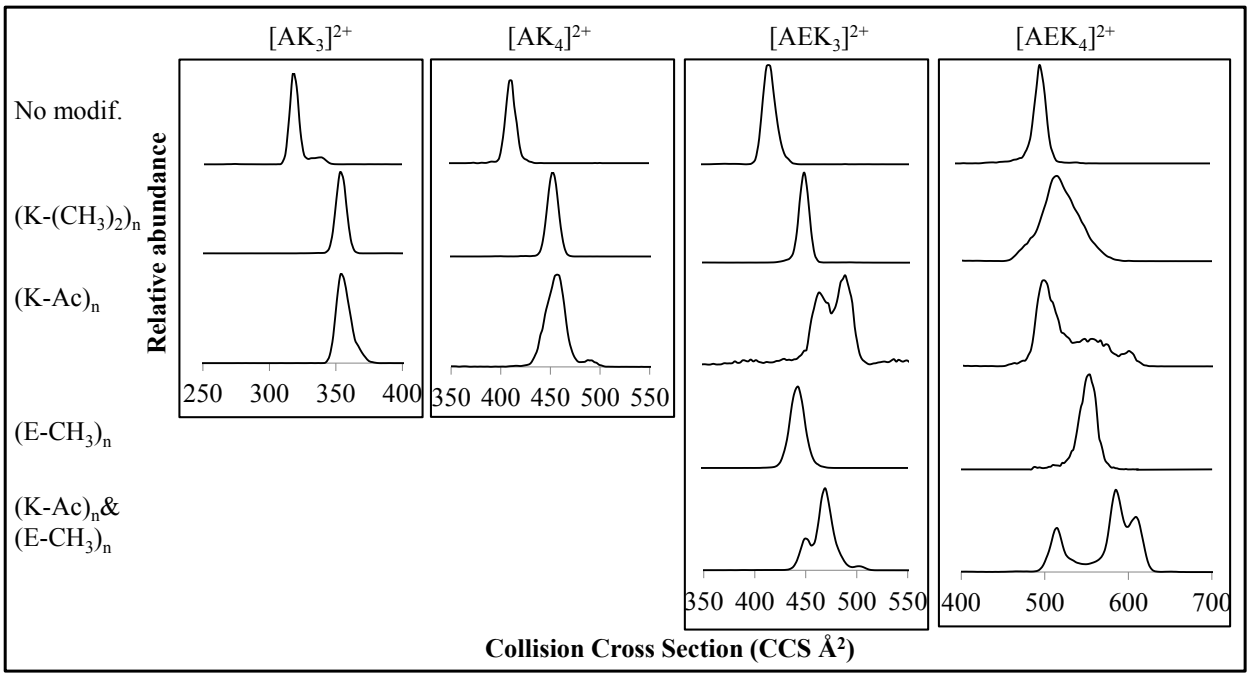


Figure S4. Collision cross section profiles of $[M + 2H]^{2+}$ ions for chemically modified AK_n and AEK_n ions. K-$(CH_3)_2$ indicates the methylation of lysine side chains; K-Ac indicates the acetylation of lysine side chains; E-CH_3 indicates the methylation of glutamic acid side chain. For each peptide, all the functional groups are methylated and/or acetylated.