Electronic Supplementary Material (ESI) for Analyst.

Charge site assignment in native proteins by ultraviolet photodissociation (UVPD) mass spectrometry

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Supporting Information: Supplemental figures show the distribution of a and x ions upon UVPD of melittin (3+); ESI mass spectrum of acetylated melittin; collisional cross sections and relative energies (kcal/mol) of the twenty lowest energy conformations of 3+ melittin for each of the three different charging schemes studied by simulated annealing; modelled conformations of melittin (3+) for different charge site permutations; map of potential ion pairs of ubiquitin.

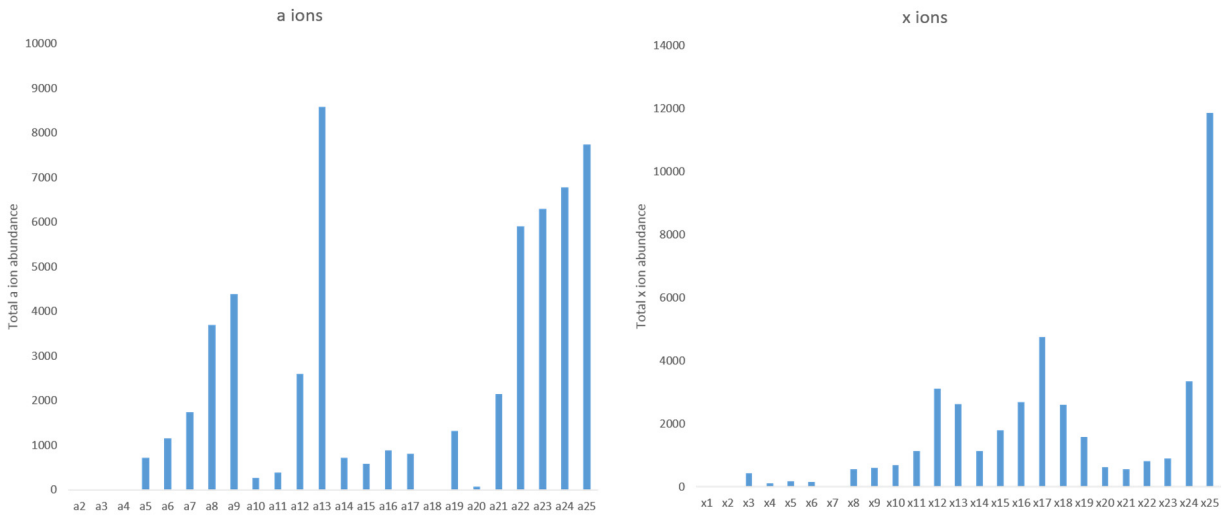


Figure S1: Relative abundance of total a_n and x_n ions from triply charged melittin

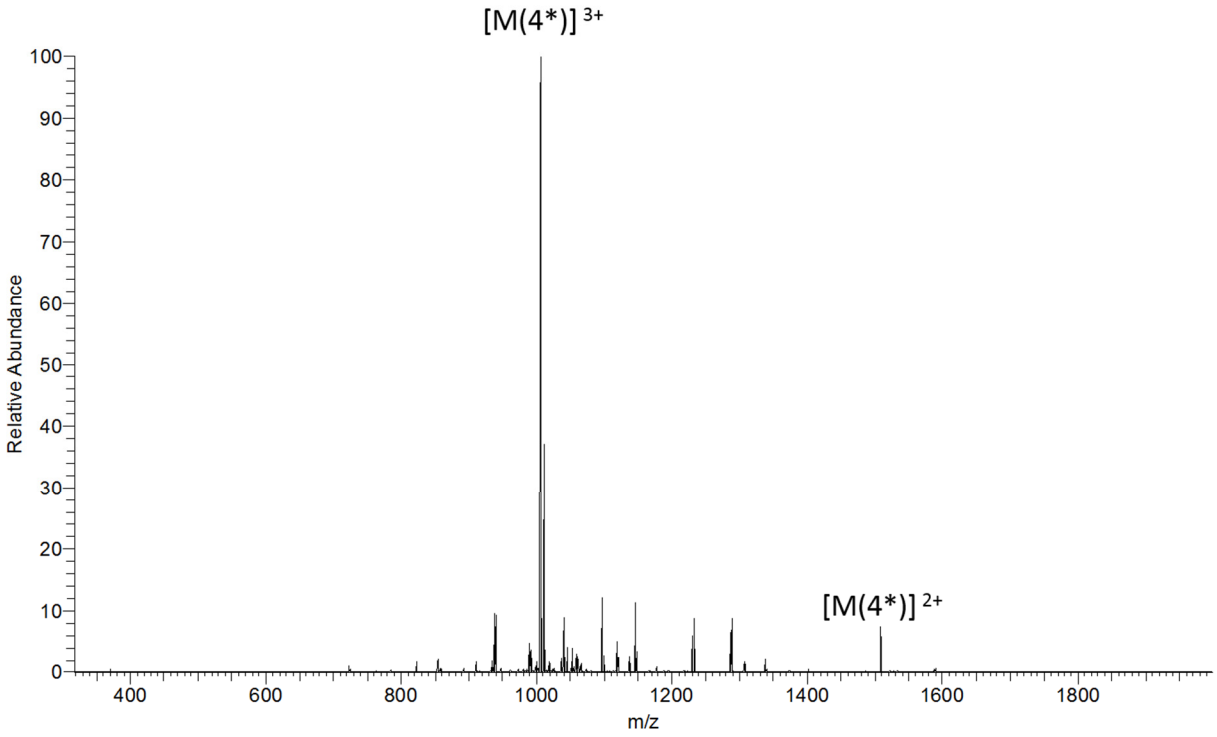


Figure S2: Mass spectrum of acetylated melittin. The 3+ is the dominant charge state of the quadruply acetylated peptide. The * symbol denotes a single acetyl group.

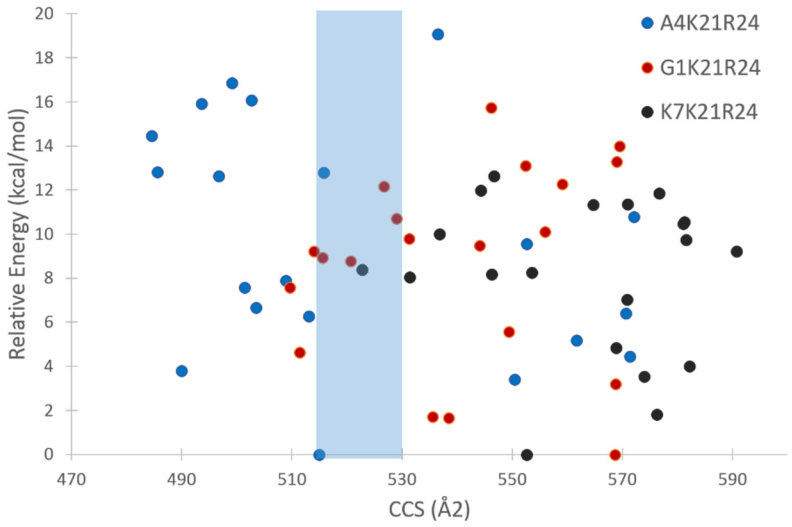


Figure S3**:** Collisional cross sections and relative energies (kcal/mol) of the twenty lowest energy conformations of 3+ melittin for each of the three different charging schemes studied by simulated annealing. The reported CCS is the average CCS from five different frames in all cases. Note that comparison of relative energy between charge site isomers is not appropriate, and the relative energy of each the conformations associated with the different charging schemes are normalized to the lowest energy conformation for that charge site isomer. The CCS space ± 1.5% of the experimental CCS is highlighted in blue.

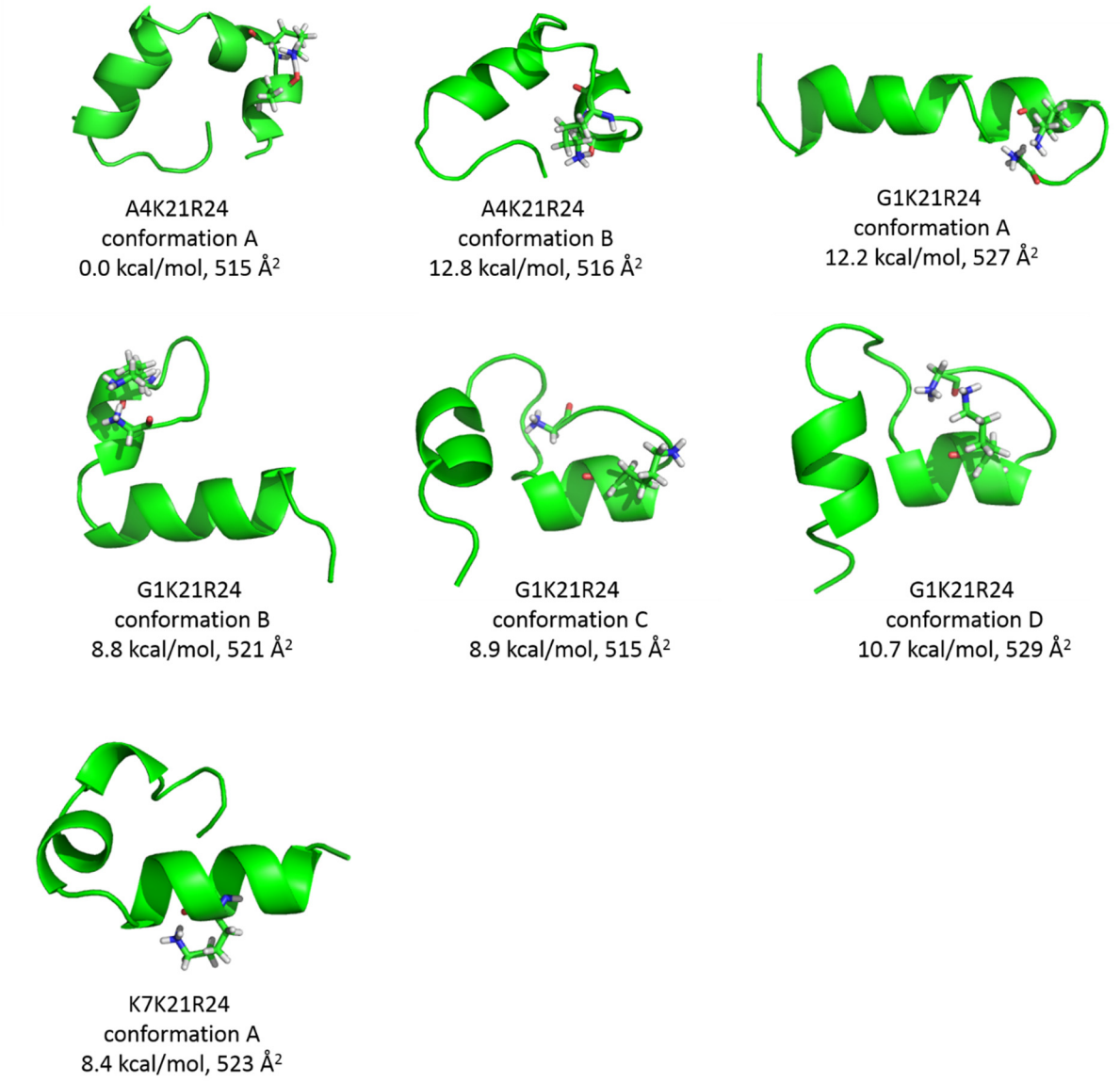
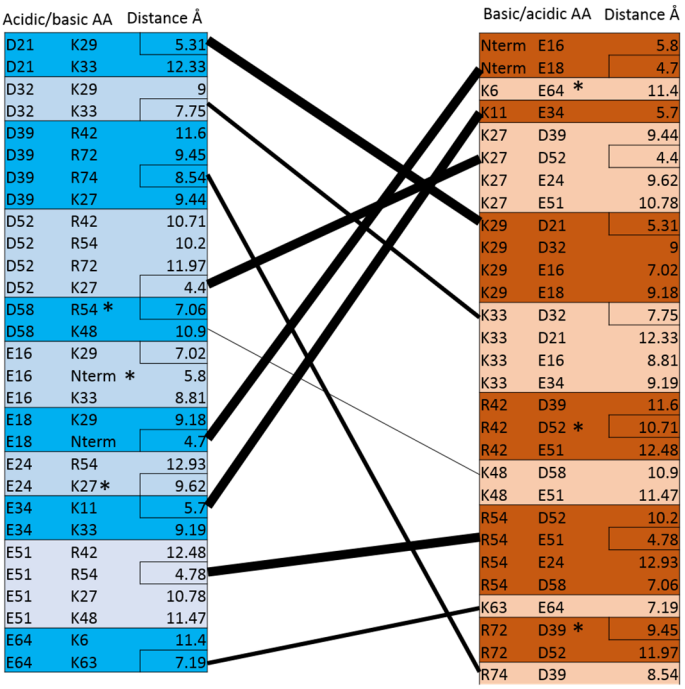


Figure S4: Conformations of A4K21R24 and G1K21R24 that have collisional cross sections between 515 and 530 Å2, in accordance with the measured CCS of 3+ melittin when sprayed from a 50:50 water/methanol mixture.



Acidic/basic AA		Distance Å
D21	K29	5.31
D21	K33	12.33
D32	K29	9
D32	K33	7.75
D39	R42	11.6
D39	R72	9.45
D39	R74	8.54
D39	K27	9.44
D52	R42	10.71
D52	R54	10.2
D52	R72	11.97
D52	K27	4.4
D58	R54 *	7.06
D58	K48	10.9
E16	K29	7.02
E16	Nterm *	5.8
E16	K33	8.81
E18	K29	9.18
E18	Nterm	4.7
E24	R54	12.93
E24	K27 *	9.62
E34	K11	5.7
E34	K33	9.19
E51	R42	12.48
E51	R54	4.78
E51	K27	10.78
E51	K48	11.47
E64	K6	11.4
E64	K63	7.19

Basic/acidic AA		Distance Å
Nterm	E16	5.8
Nterm	E18	4.7
K6	E64 *	11.4
K11	E34	5.7
K27	D39	9.44
K27	D52	4.4
K27	E24	9.62
K27	E51	10.78
K29	D21	5.31
K29	D32	9
K29	E16	7.02
K29	E18	9.18
K33	D32	7.75
K33	D21	12.33
K33	E16	8.81
K33	E34	9.19
R42	D39	11.6
R42	D52 *	10.71
R42	E51	12.48
K48	D58	10.9
K48	E51	11.47
R54	D52	10.2
R54	E51	4.78
R54	E24	12.93
R54	D58	7.06
K63	E64	7.19
R72	D39 *	9.45
R72	D52	11.97
R74	D39	8.54

Figure S5: Acidic and basic residues of ubiquitin and the distance separating potential ion pairs. The * symbol denotes residues whose nearest ion pairing neighbor is engaged in a closer bond. Ion pair distances were calculated from the 1D3Z structure using the VMD software. The nearest ion pairs are denoted by connecting lines, the heaviness of which symbolized the relative closeness of the pair.