Supporting information

Effects of anions on the electrospray ionization of proteins in strong acids

Zhongbao Han[1], Huizi Zheng[1], Shuyao Wu[2], Liyan Liu[1]*, Lee Chuin Chen[3]*

[1]*College of Chemistry and Chemical Engineering, Shenyang Normal University, Shenyang 110034, China*

[2]*College of Chemistry, Liaoning University, Shenyang, 110036, China*

[3]*Faculty of Engineering, University of Yamanashi, 4-3-11, Takeda, Kofu, Yamanashi, 400-8511 Japan*

***To whom correspondence should be addressed:**

L. C. Chen, Email: leechuin@yamanashi.ac.jp, Phone: +81-55-220-8072

L. Liu, Email: liyanliu@synu.edu.cn, Phone: +86-138-8928-2570

Keywords: Protein, acid-induced protein compaction, molten globule state, high-pressure electrospray

Table of Contents

Figure	Description	Page
Figure S1	High-pressure ESI source	S-3
Figure S2	Electrical conductivities	S-4
Figure S3	Solution flow rates	S-5
Figure S4	MS of cytochrome *c* in HCl with incremental pH (pH 1-3)	S-6
Figure S5	MS of cytochrome *c* in TFA with incremental pH (pH 1-3)	S-7
Figure S6	MS of cytochrome *c* in HNO_3 with incremental pH (pH 1-3)	S-8
Figure S7	MS of cytochrome *c* in TCA with incremental pH (pH 1-3)	S-9
Figure S8	MS of cytochrome *c* in $HClO_4$ with incremental pH (pH 1-3)	S-10
Figure S9	Magnified MS for cytochrome *c* in $HClO_4$	S-11
Figure S10	MS of lysozyme in HCl with incremental pH (pH 1-3)	S-12
Figure S11	MS of lysozyme in TFA with incremental pH (pH 1-3)	S-13
Figure S12	MS of lysozyme in HNO_3 with incremental pH (pH 1-3)	S-14
Figure S13	MS of lysozyme in TCA with incremental pH (pH 1-3)	S-15
Figure S14	MS of lysozyme in $HClO_4$ with incremental pH (pH 1-3)	S-16
Figure S15	Figure S15: Magnified MS for lysozyme in $HClO_4$	S-17
Figure S16	MS of myoglobin in different acids with pH 2	S-18
Figure S17	MS of Ubiquitin in different acids with pH 2	S-19
Figure S18	Magnified MS for equine & bovine cytochrome *c*, lysozyme, myoglobin, ubiquitin	S-20
Figure S19	CD spectra for cytochrome *c* & myoglobin	S-21
Figure S20	Average charge states	S-22
Figure S21	Average charge for HCSD & LCSD at different pHs	S-23
Figure S22	Addition of NH_4OH (cytochrome *c*)	S-24
Figure S23	Addition of NH_4OH (myoglobin)	S-25
Figure S24	Addition of NH4OH (ubiquitin)	S-26
Figure S25	Addition of NH4OH (lysozyme)	S-27

Figure S1: High-pressure ESI source

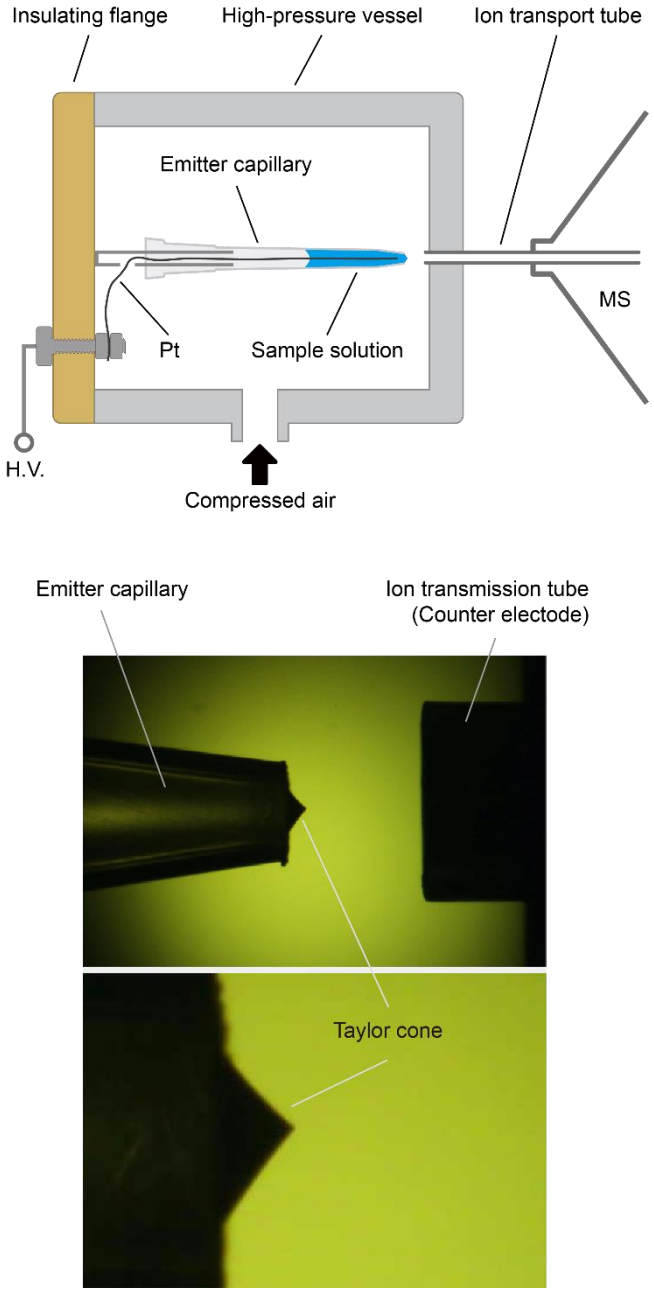

Figure S1. Schematic of the high-pressure nanoESI ion source. A micropipette tip (i.d. ~ 0.4 mm) is used as the emitter. A platinum wire inserted into the micropipette tip is used for liquid charging. The high-pressure ion source is connected directly to the mass spectrometer using an ion transmission tube with an inner diameter of 0.25mm. A stable Taylor cone can be formed for a highly conductive aqueous solution under super-atmospheric pressure (> 0.3 MPa gauge pressure). The schematic (upper figure) is adapted with permission from *Anal. Chem.*, 2023, 95, 14816–14821.

Figure S2: Electrical conductivities

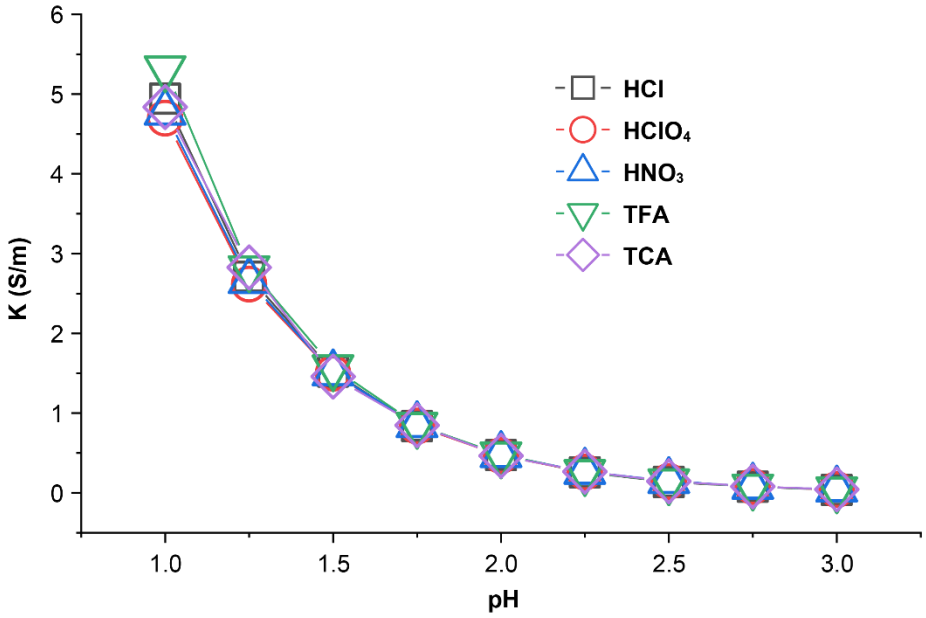

Figure S2. Measurement of electrical conductivities (K) of hydrochloric acids (HCl), trifluoroacetic acid (TFA), nitric acid (HNO_3), perchloric acid ($HClO_4$), and trichloroacetic acid (TCA) with different pHs.

Figure S3: Solution flow rates

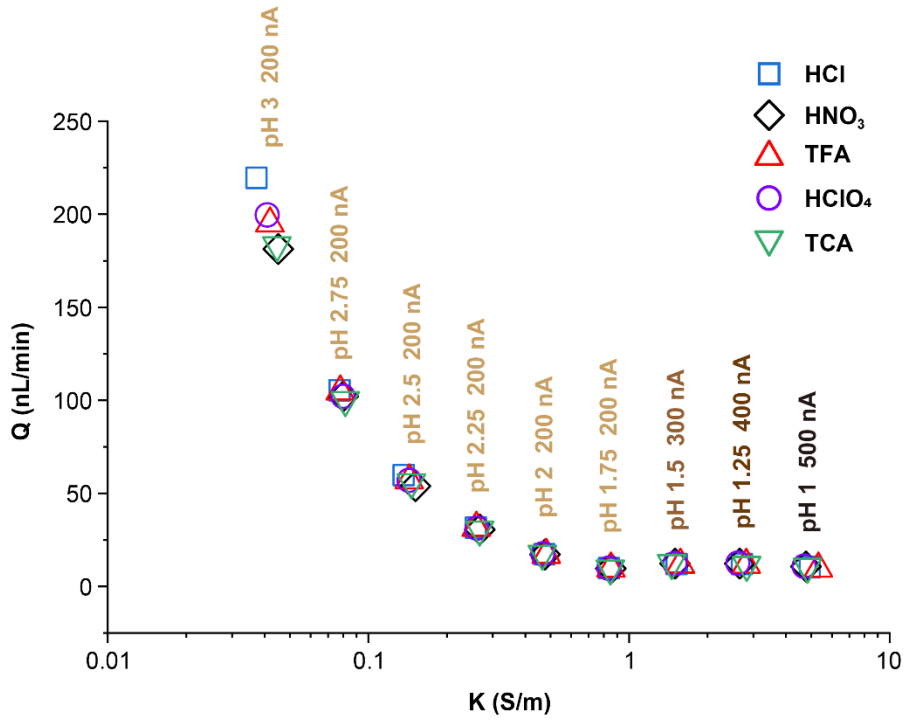

Figure S3. Calculated solution flow rate Q (nL/min) for solutions with different conductivity K (S/m) at different operating spray currents (200~500 nA).

Figure S4: MS of cytochrome *c* in HCl with incremental pH (pH 1-3)

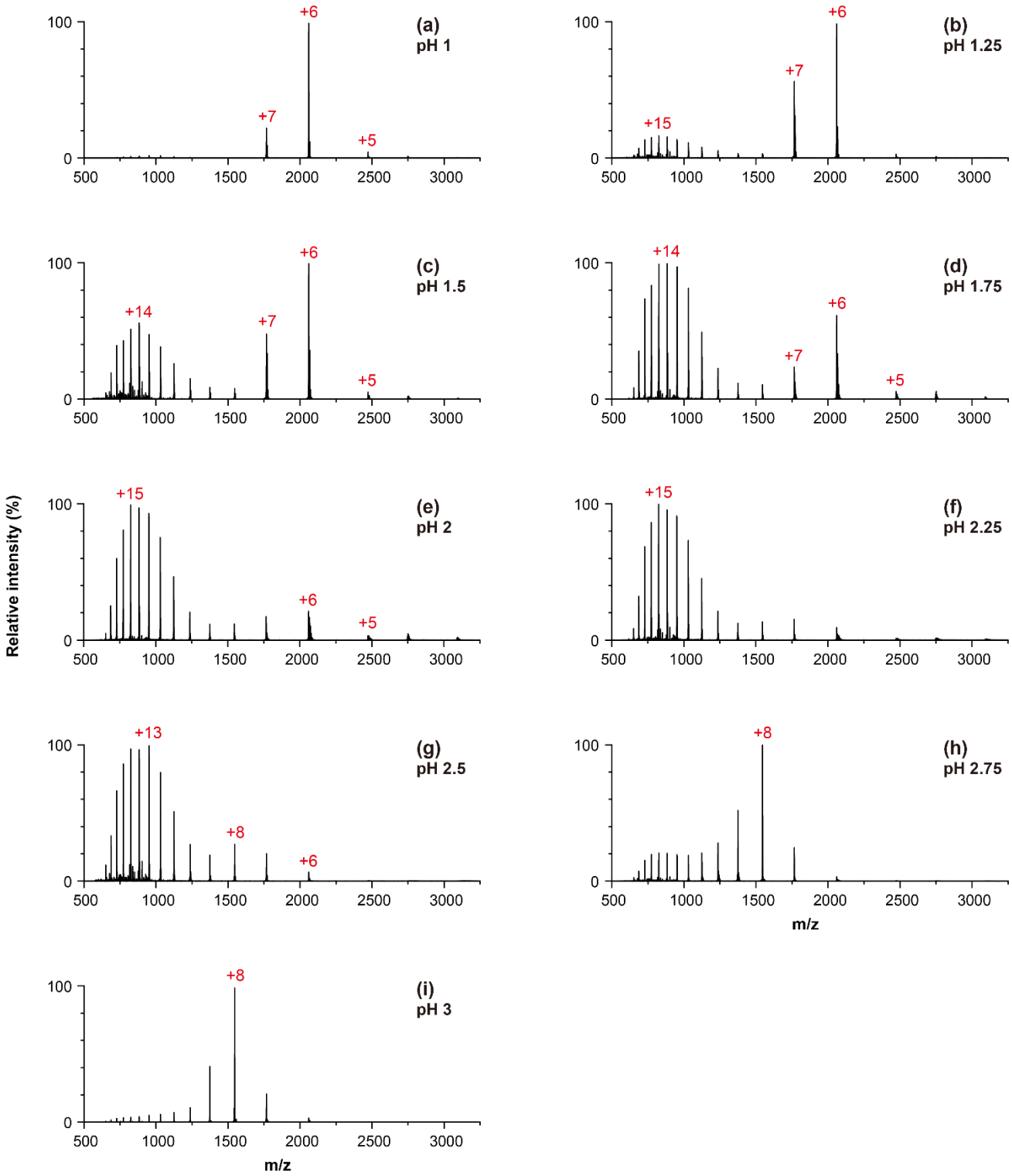

Figure S4. High-pressure ESI mass spectra of 10 μM equine cytochrome *c* in HCl aqueous solution with different pH values.

Figure S5: MS of cytochrome *c* in TFA with incremental pH (pH 1-3)

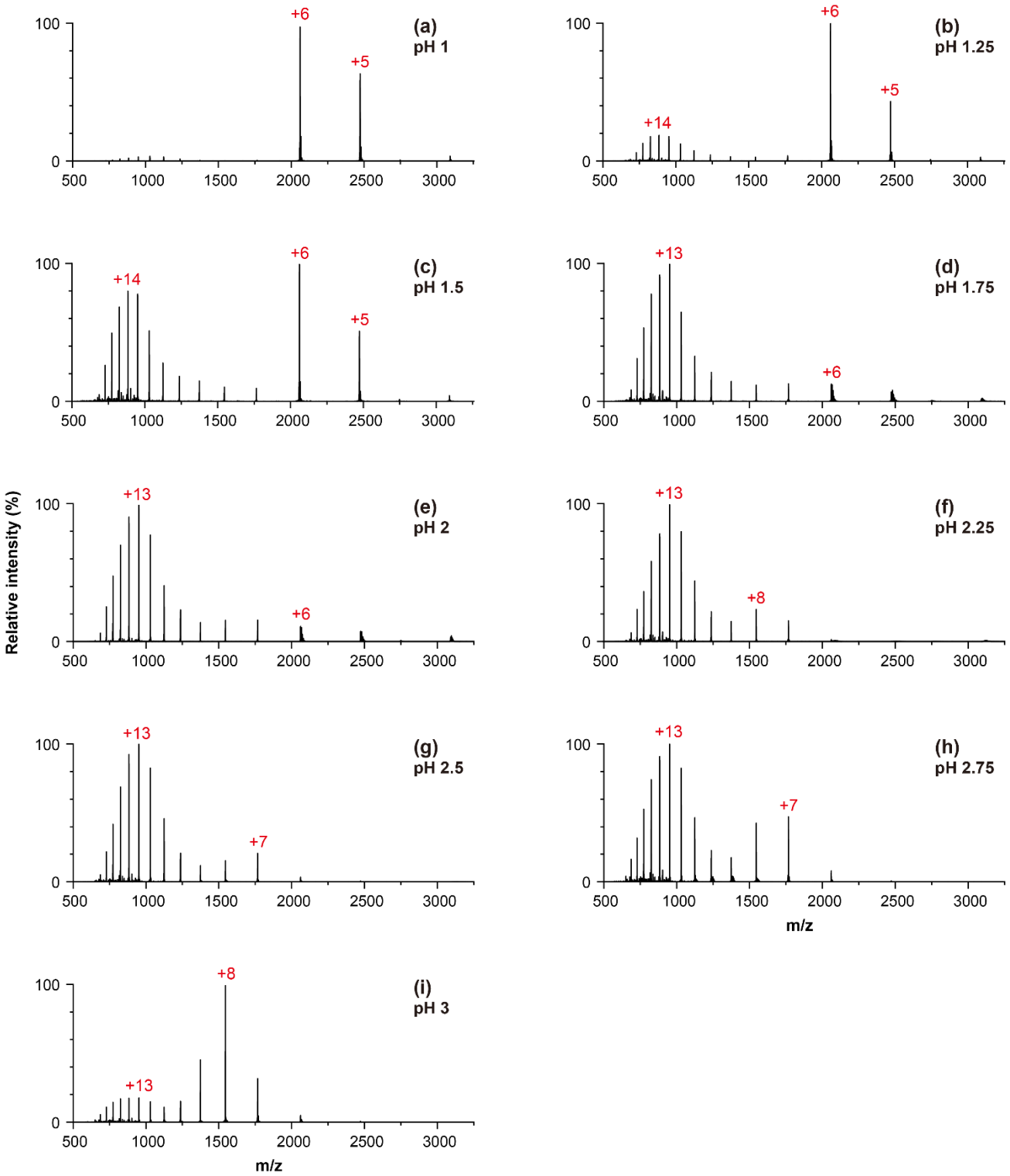

Figure S5. High-pressure ESI mass spectra of 10 μM equine cytochrome *c* in TFA aqueous solution with different pH values.

Figure S6: MS of cytochrome *c* in HNO_3 with incremental pH (pH 1-3)

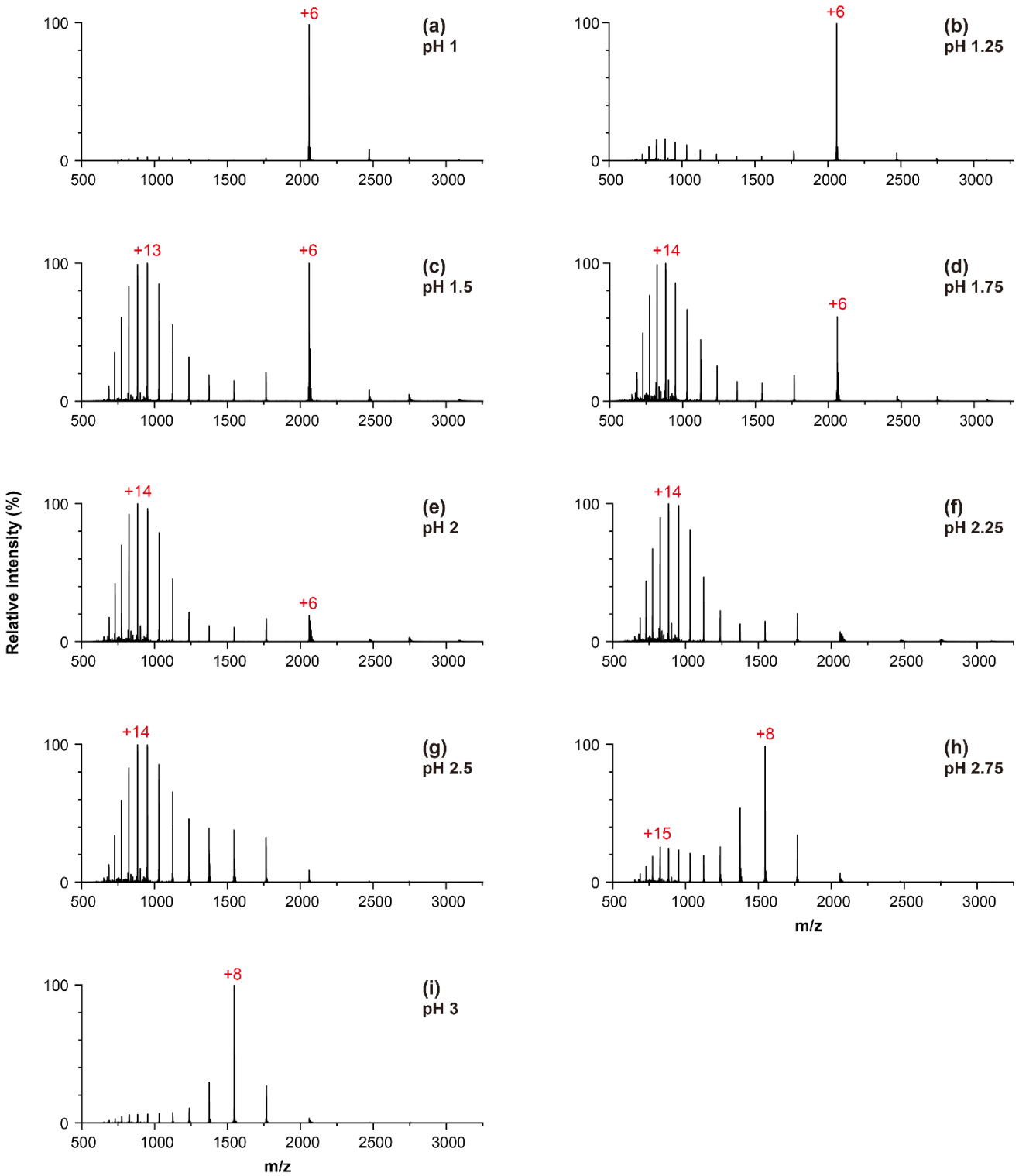

Figure S6. High-pressure ESI mass spectra of 10 μM equine cytochrome *c* in HNO_3 aqueous solution with different pH values.

Figure S7: MS of cytochrome *c* in TCA with incremental pH (pH 1-3)

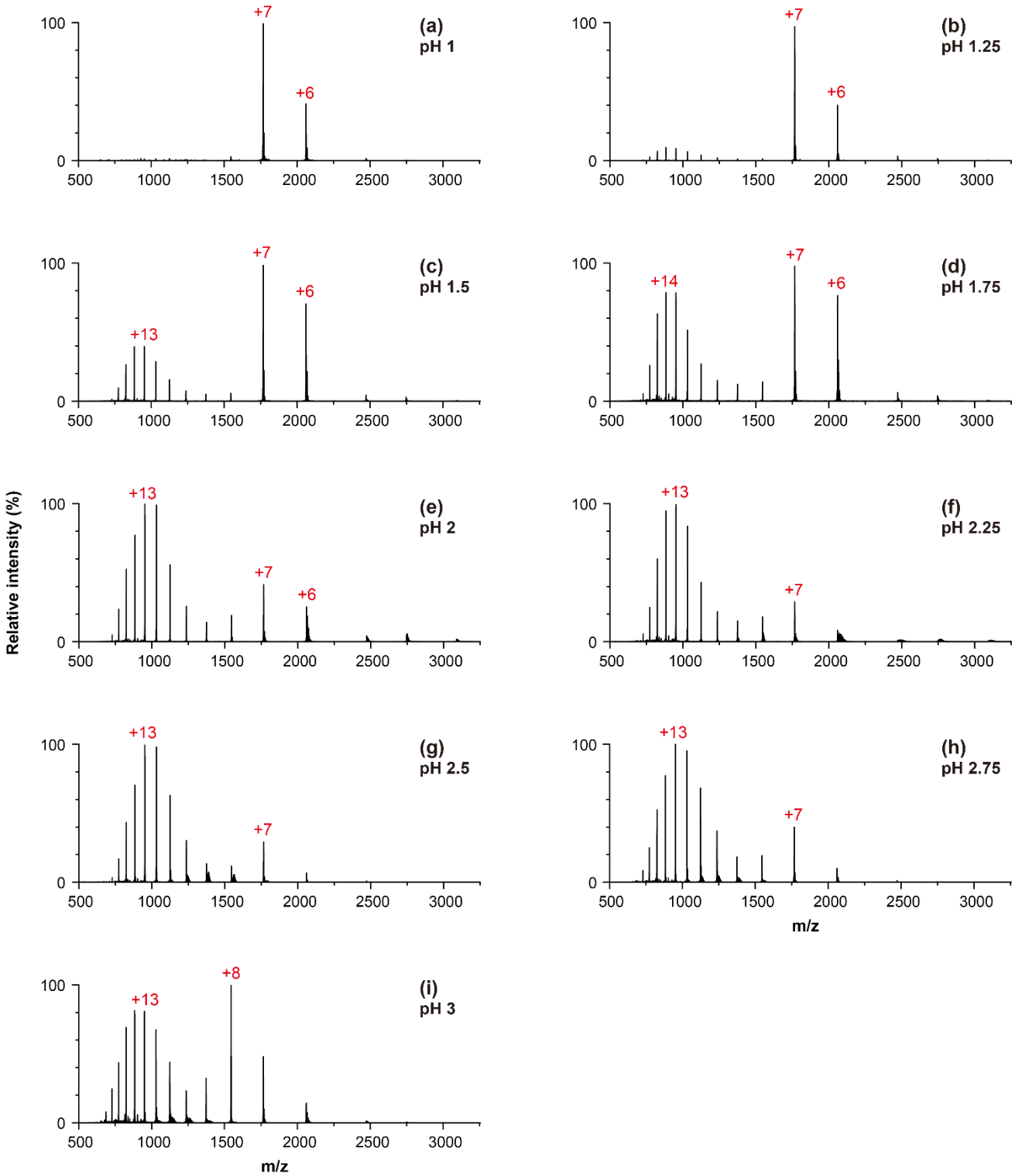

Figure S7. High-pressure ESI mass spectra of 10 μM equine cytochrome *c* in TCA aqueous solution with different pH values.

Figure S8: MS of cytochrome *c* in $HClO_4$ with incremental pH (pH 1-3)

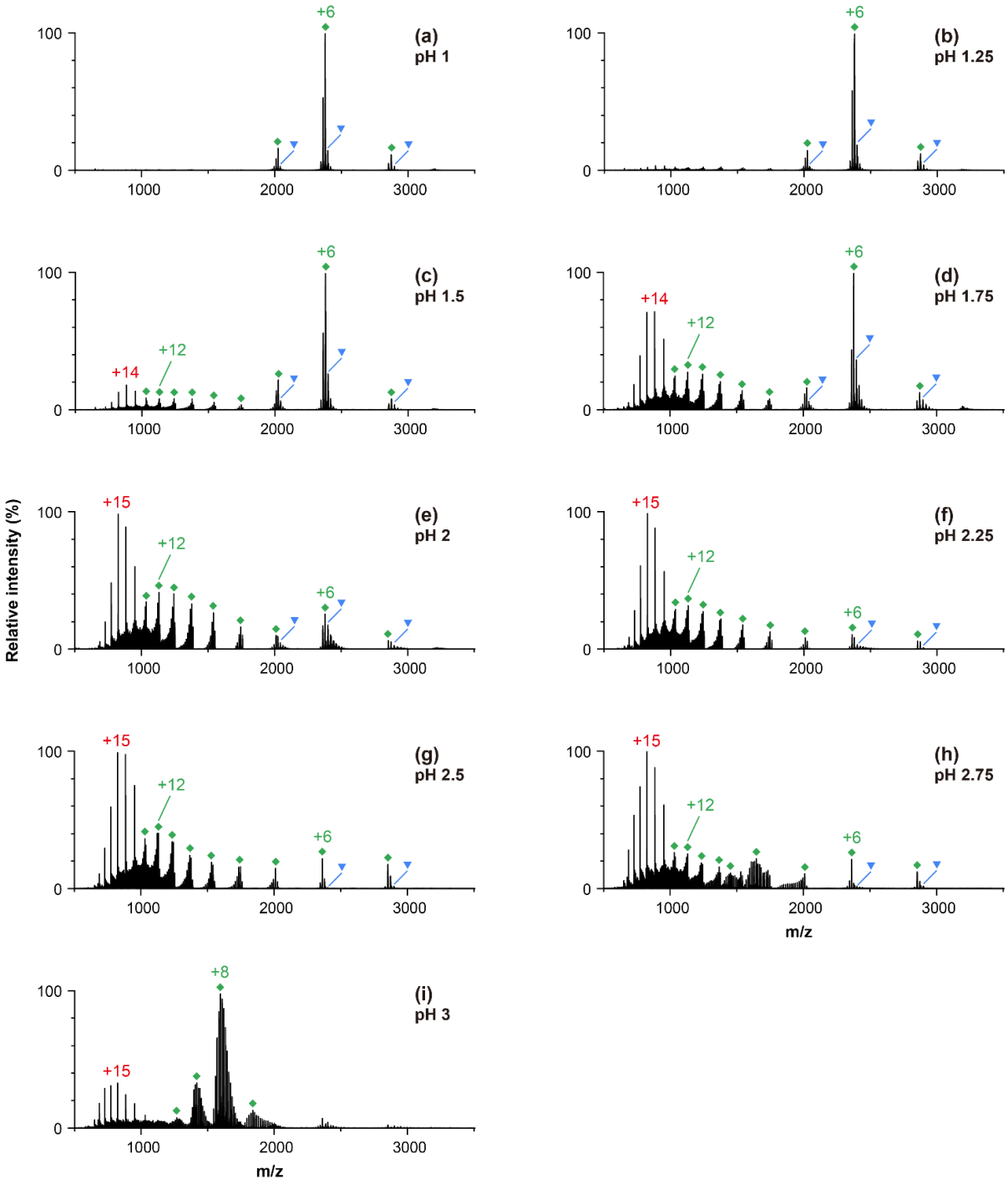

Figure S8. High-pressure ESI mass spectra of 10 μM equine cytochrome *c* in $HClO_4$ aqueous solution with different pH values. The green ◆ denotes protonated peaks with acid adducts, and the blue ▼ denotes peaks with adducts from acid, sodium, and sodium salt.

Figure S9: Magnified MS for cytochrome *c* in $HClO_4$

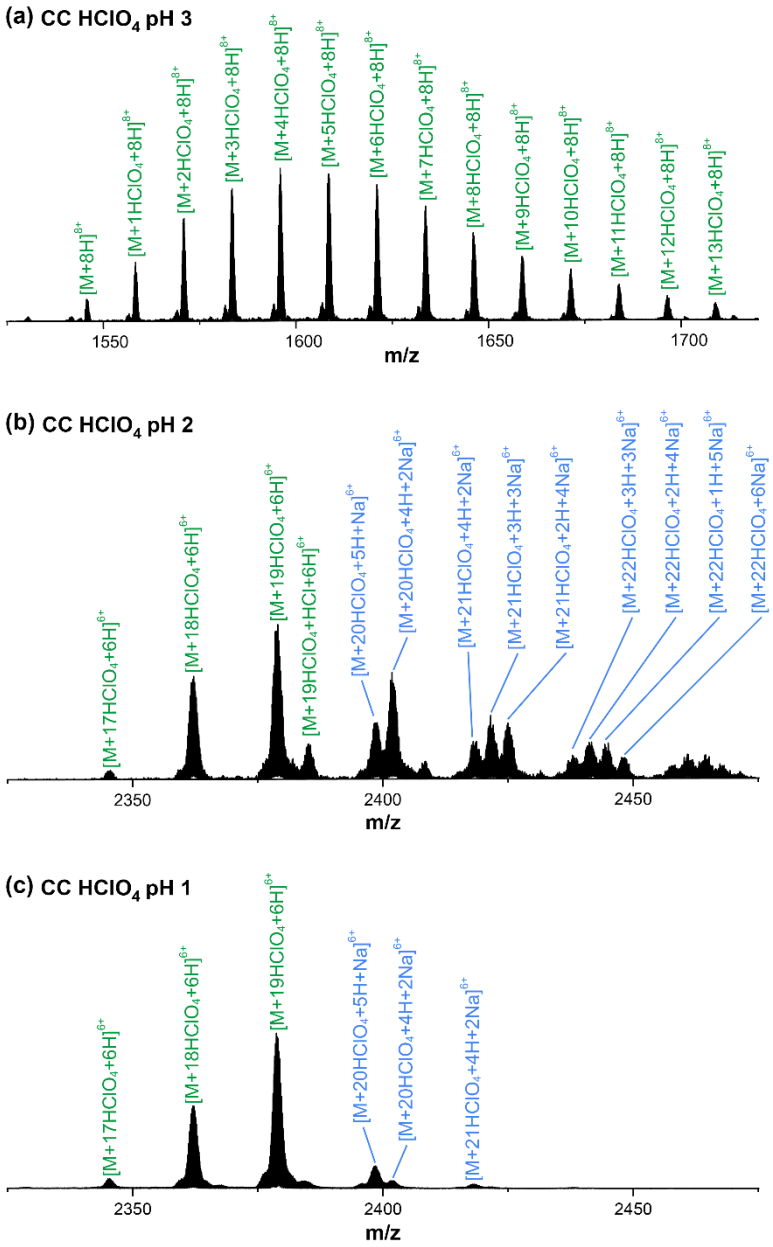

Figure S9. Magnified mass spectra for cytochrome *c* in perchloric acid (HClO4) with a) pH 3, b) pH 2, and c) pH 1.

Figure S10: MS of lysozyme in HCl with incremental pH (pH 1-3)

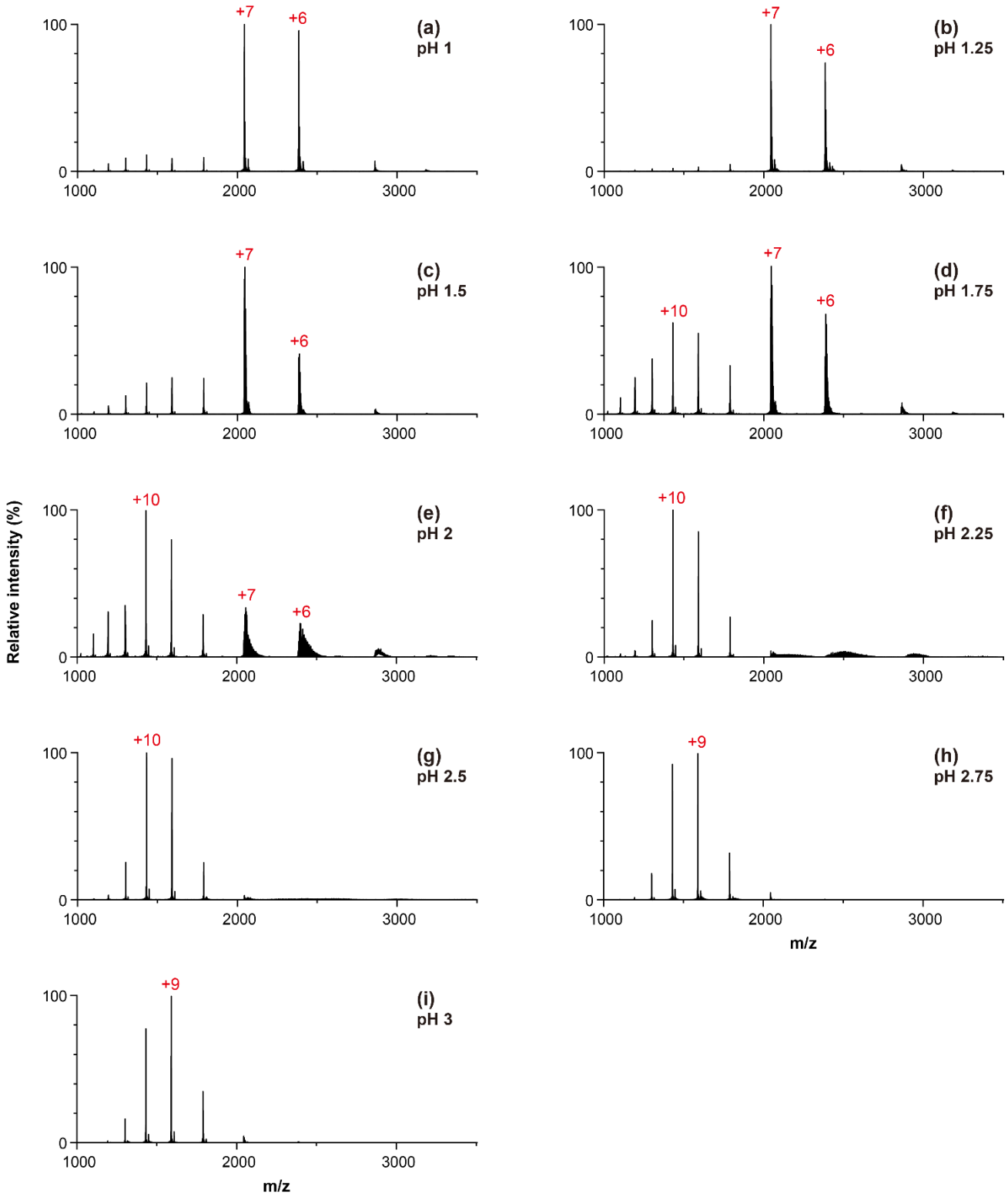

Figure S10. High-pressure ESI mass spectra of 10 μM lysozyme in HCl aqueous solution with different pH values.

Figure S11: MS of lysozyme in TFA with incremental pH (pH 1-3)

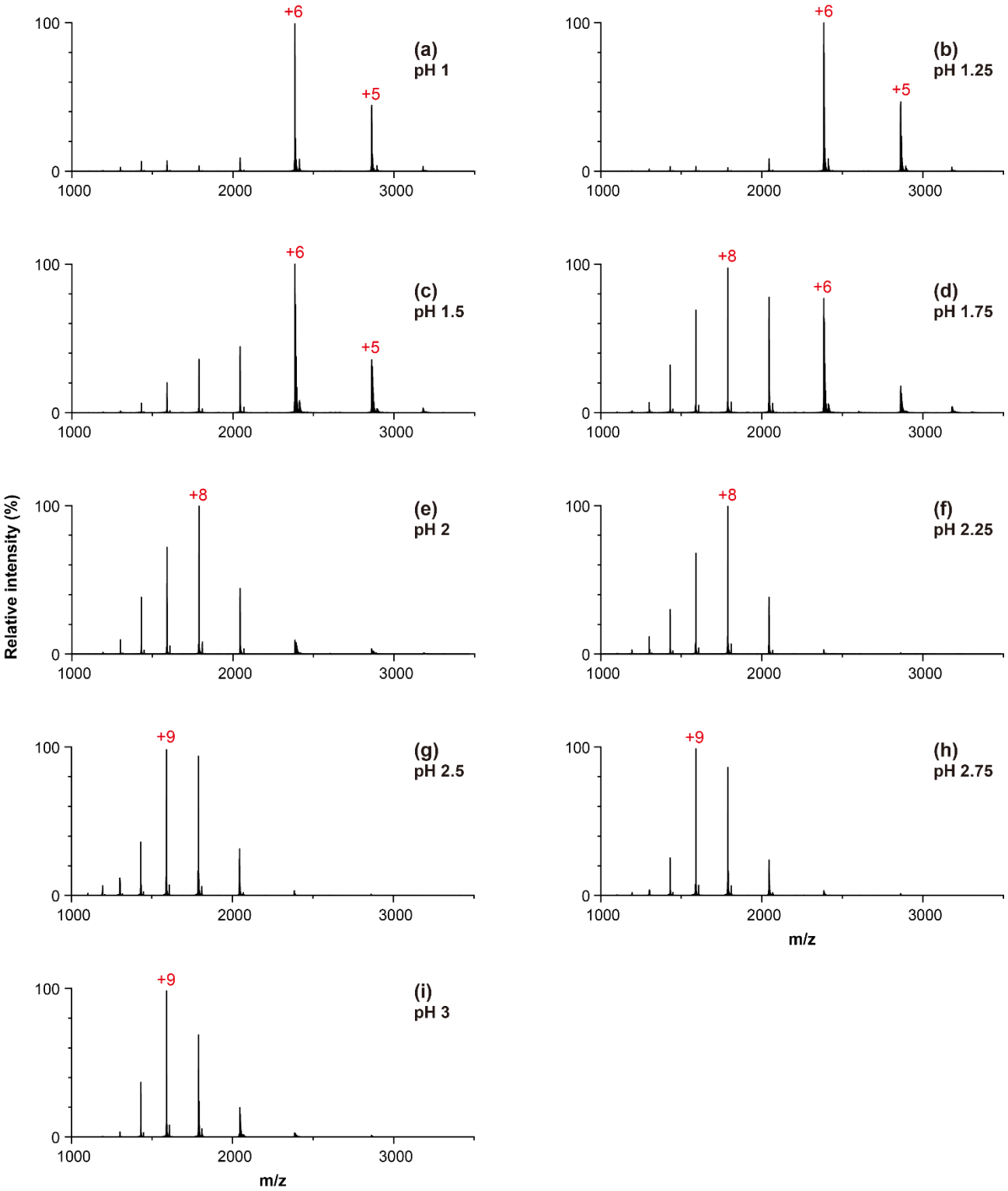

Figure S11. High-pressure ESI mass spectra of 10 μM lysozyme in TFA aqueous solution with different pH values.

Figure S12: MS of lysozyme in HNO_3 with incremental pH (pH 1-3)

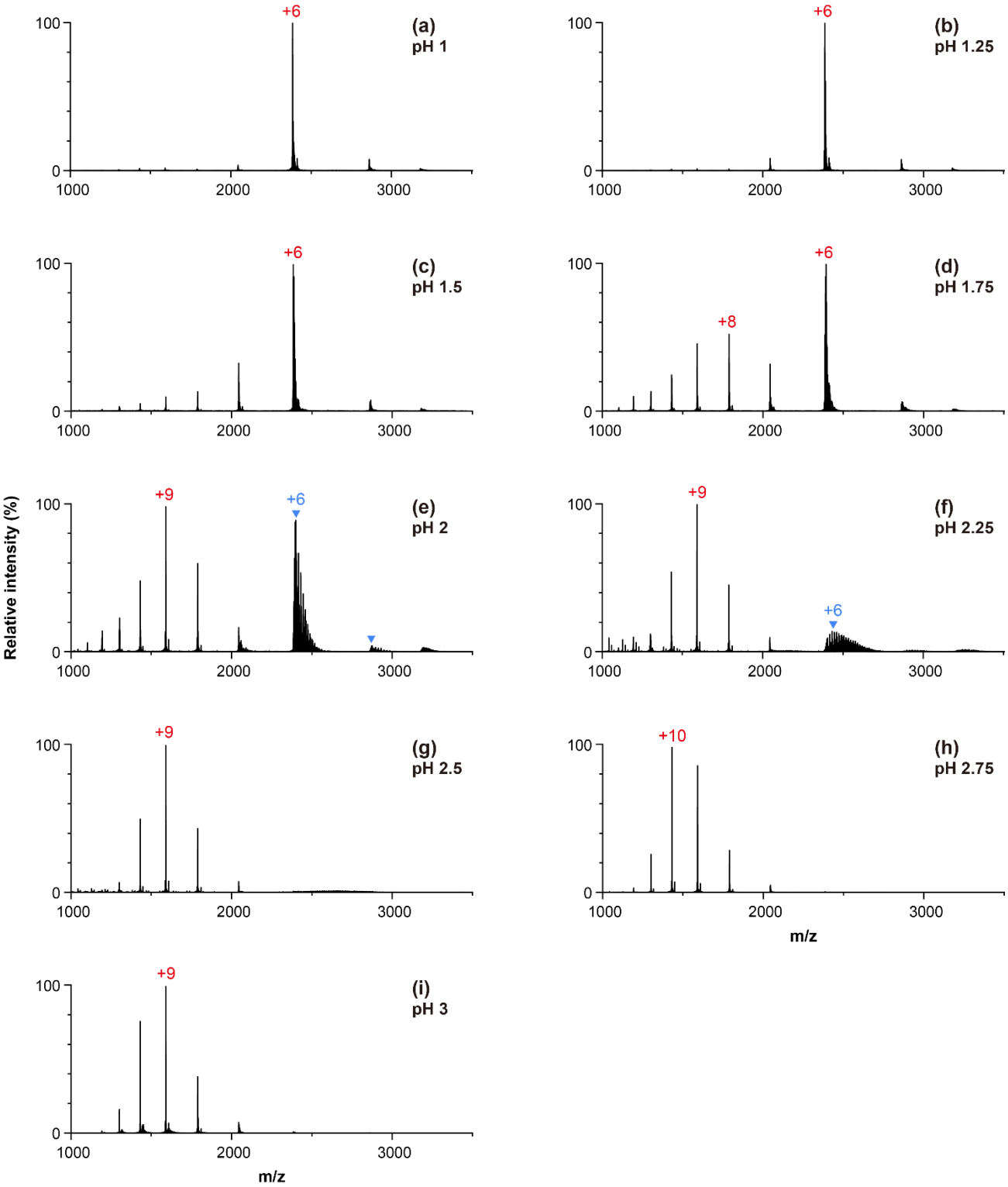

Figure S12. High-pressure ESI mass spectra of 10 μM lysozyme in HNO_3 aqueous solution with different pH values. The blue ▼ denotes peaks with adducts from acid, sodium, and sodium salt.

Figure S13: MS of lysozyme in TCA with incremental pH (pH 1-3)

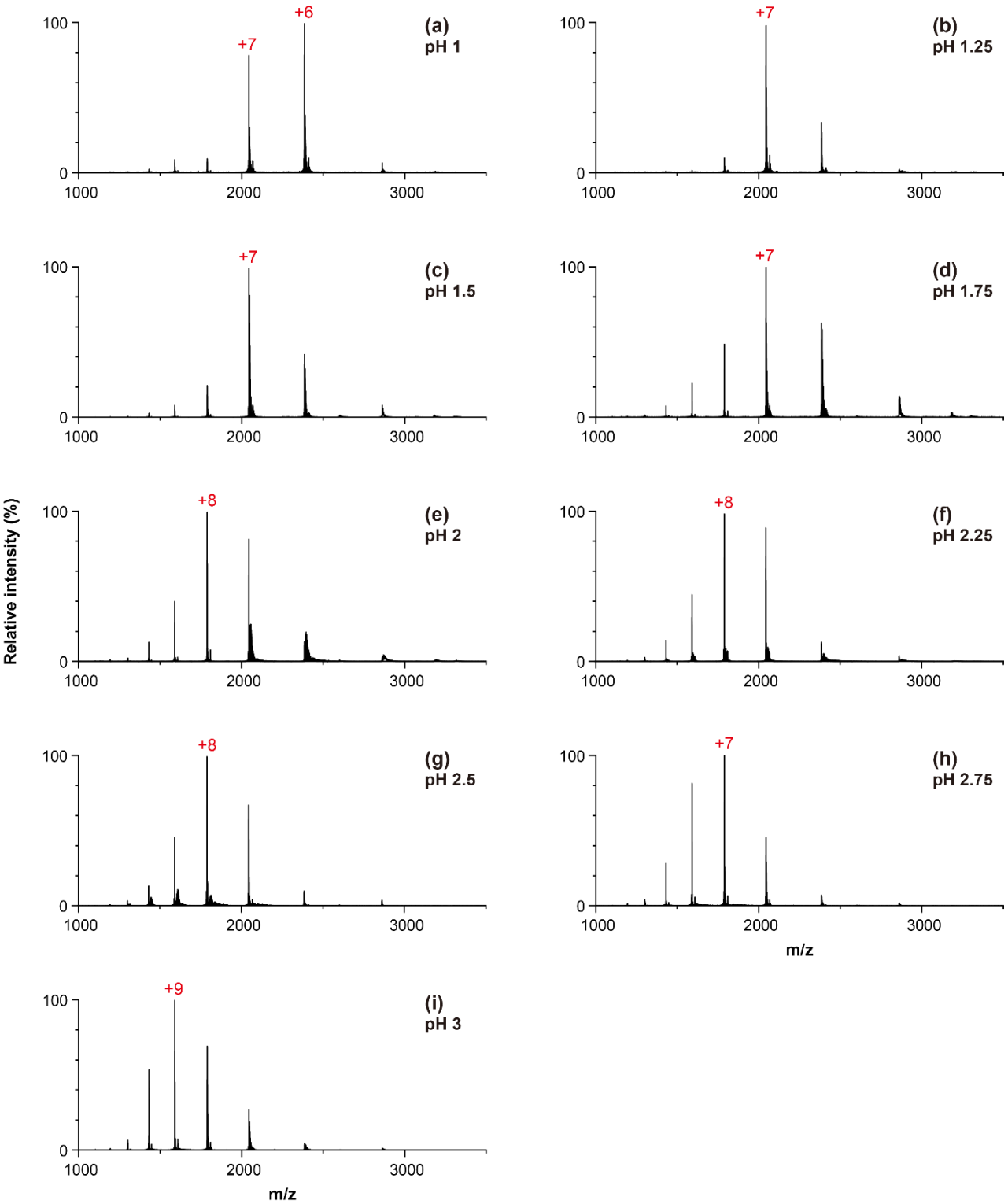

Figure S13. High-pressure ESI mass spectra of 10 μM lysozyme in TCA aqueous solution with different pH values.

Figure S14: MS of lysozyme in $HClO_4$ with incremental pH (pH 1-3)

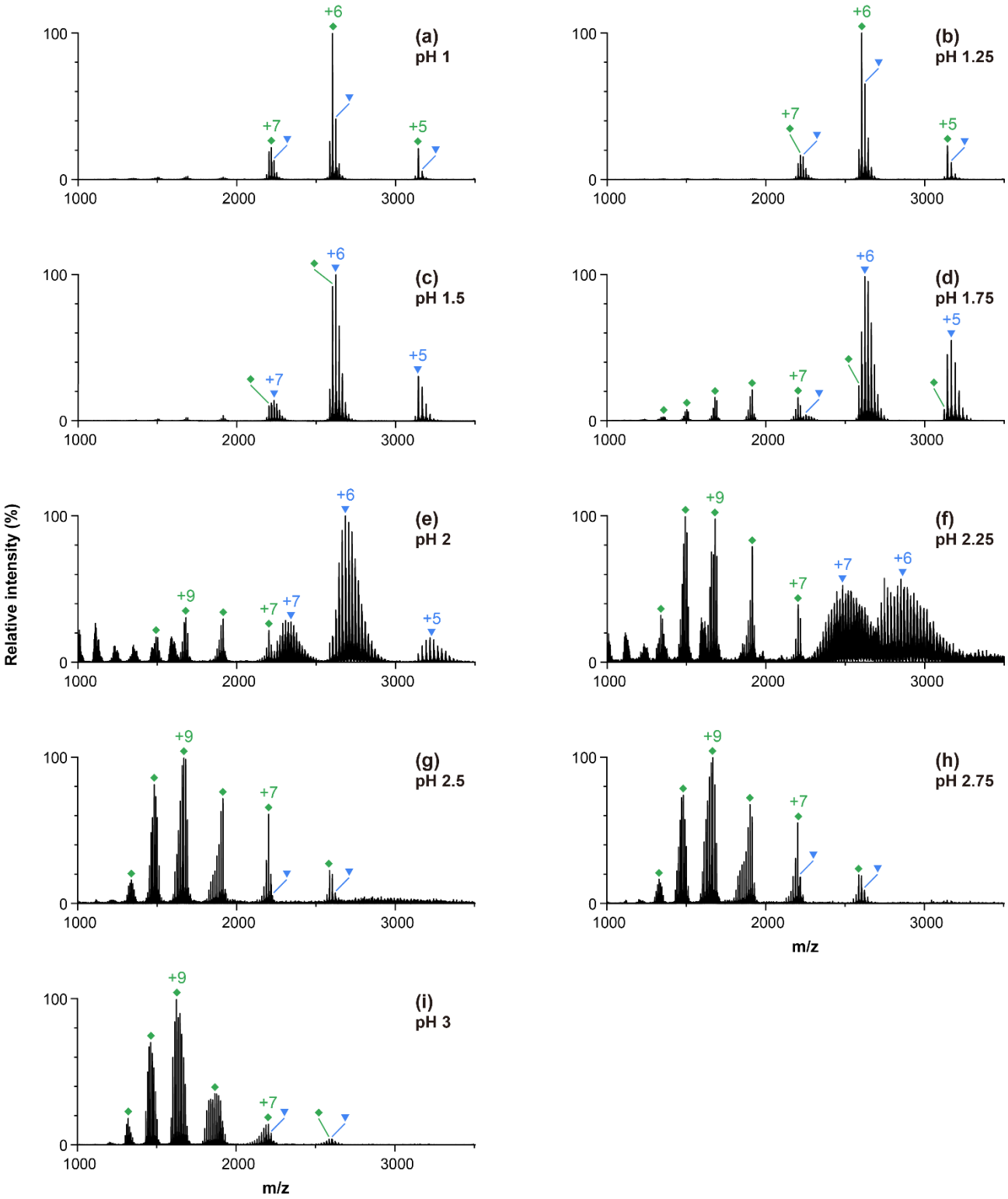

Figure S14. High-pressure ESI mass spectra of 10 μM lysozyme in $HClO_4$ aqueous solution with different pH values. The green ◆ denotes protonated peaks with acid adducts, and the blue ▼ denotes peaks with adducts from acid, sodium, and sodium salt.

Figure S15: Magnified MS for lysozyme in $HClO_4$

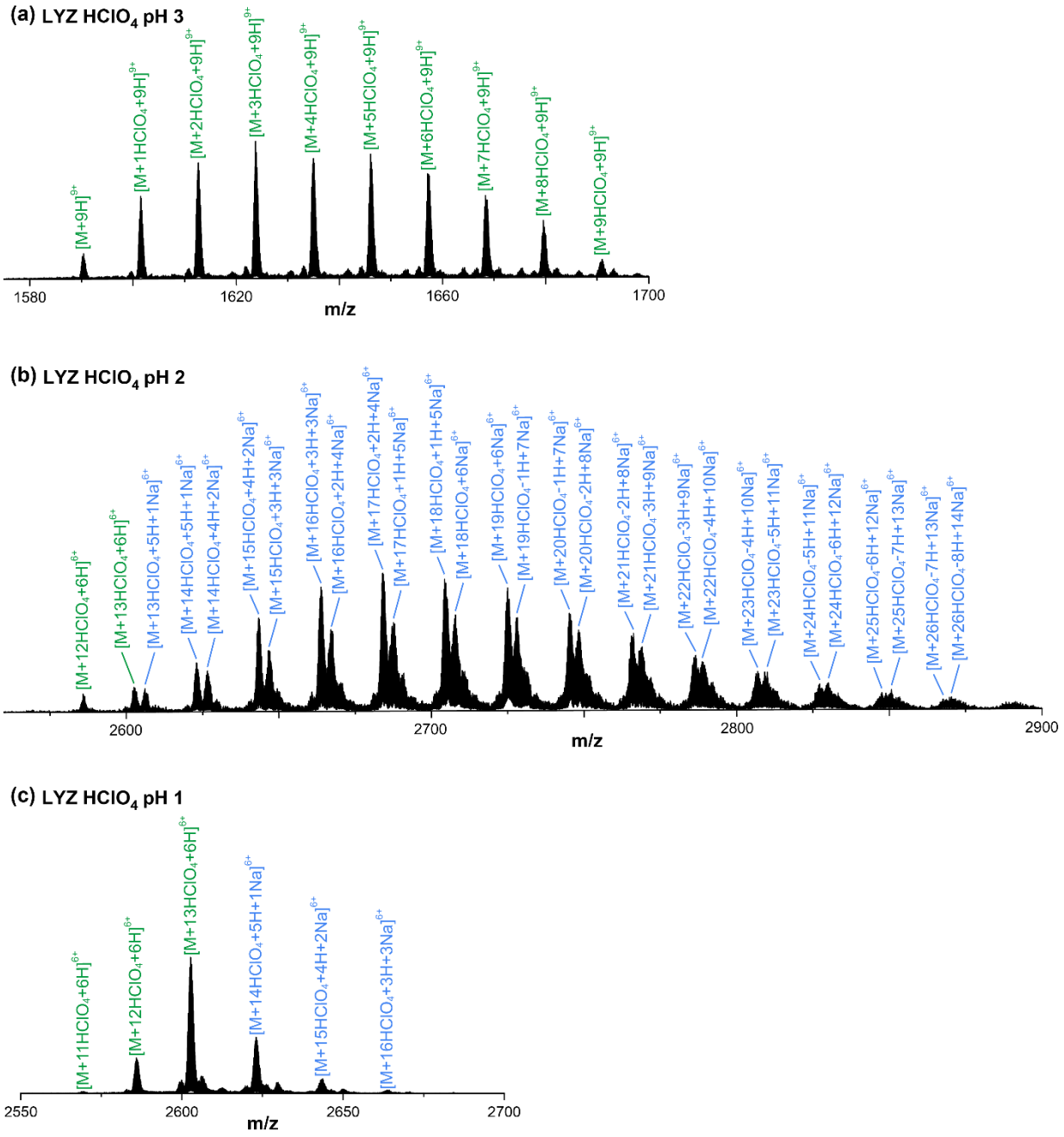

Figure S15. Magnified mass spectra for lysozyme in perchloric acid (HClO4) with a) pH 3, b) pH 2, and c) pH 1.

Figure S16: MS of myoglobin in different acids with pH 2

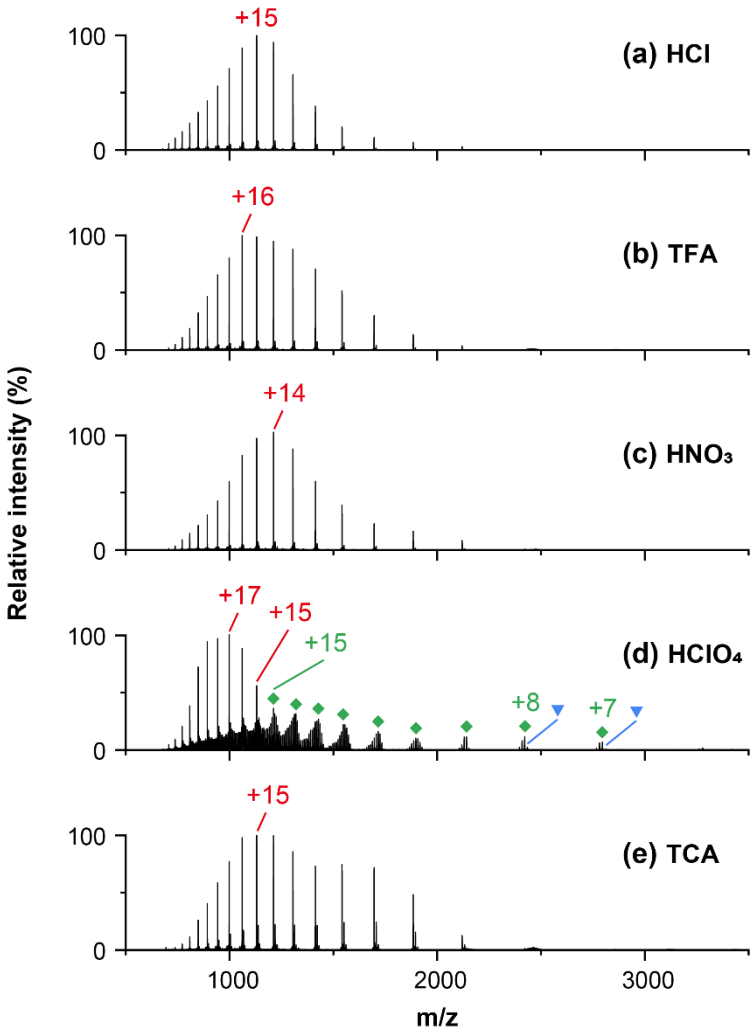

Figure S16. High-pressure ESI mass spectra of 10 μM myoglobin in different acidic solutions with pH 2. The green ◆ denotes protonated peaks with acid adducts, and the blue ▼ denotes peaks with adducts from acid, sodium, and sodium salt.

Figure S17: MS of Ubiquitin in different acids with pH 2

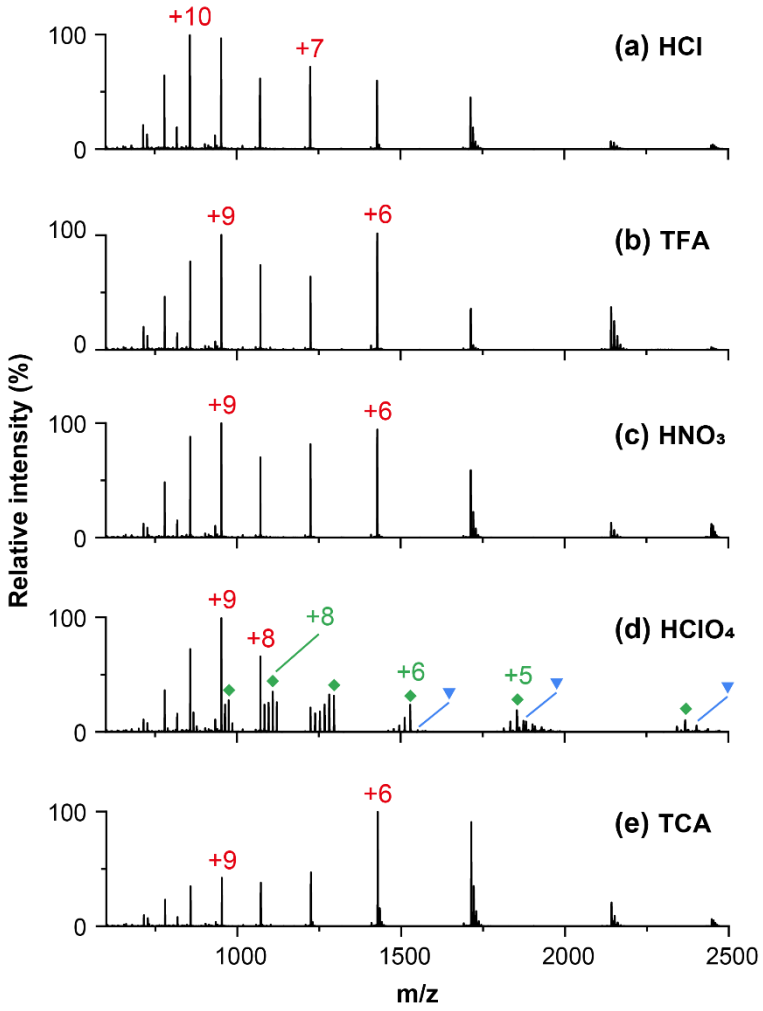

Figure S17. High-pressure ESI mass spectra of 10 μM ubiquitin in different acidic solutions with pH 2. The green ◆ denotes protonated peaks with acid adducts, and the blue ▼ denotes peaks with adducts from acid, sodium, and sodium salt.

Figure S18: Magnified MS for equine & bovine cytochrome c, lysozyme, myoglobin, ubiquitin

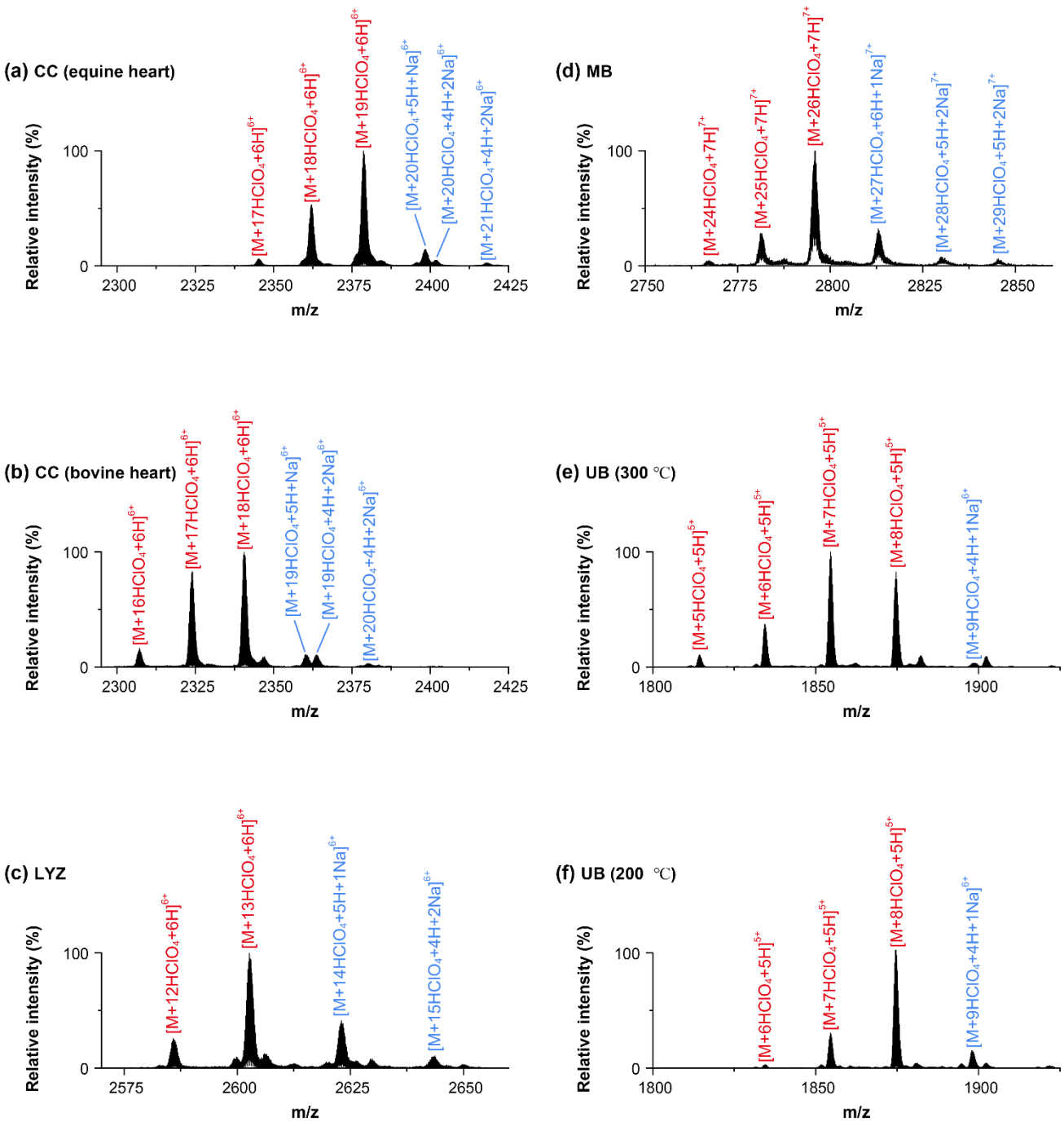

Figure S18. Magnified mass spectra for a) equine cytochrome *c*, b) bovine cytochrome *c*, c) lysozyme, d) myoglobin, and e) ubiquitin in perchloric acid ($HClO_4$) with pH 1.

Figure S19: CD spectra for cytochrome *c* & myoglobin

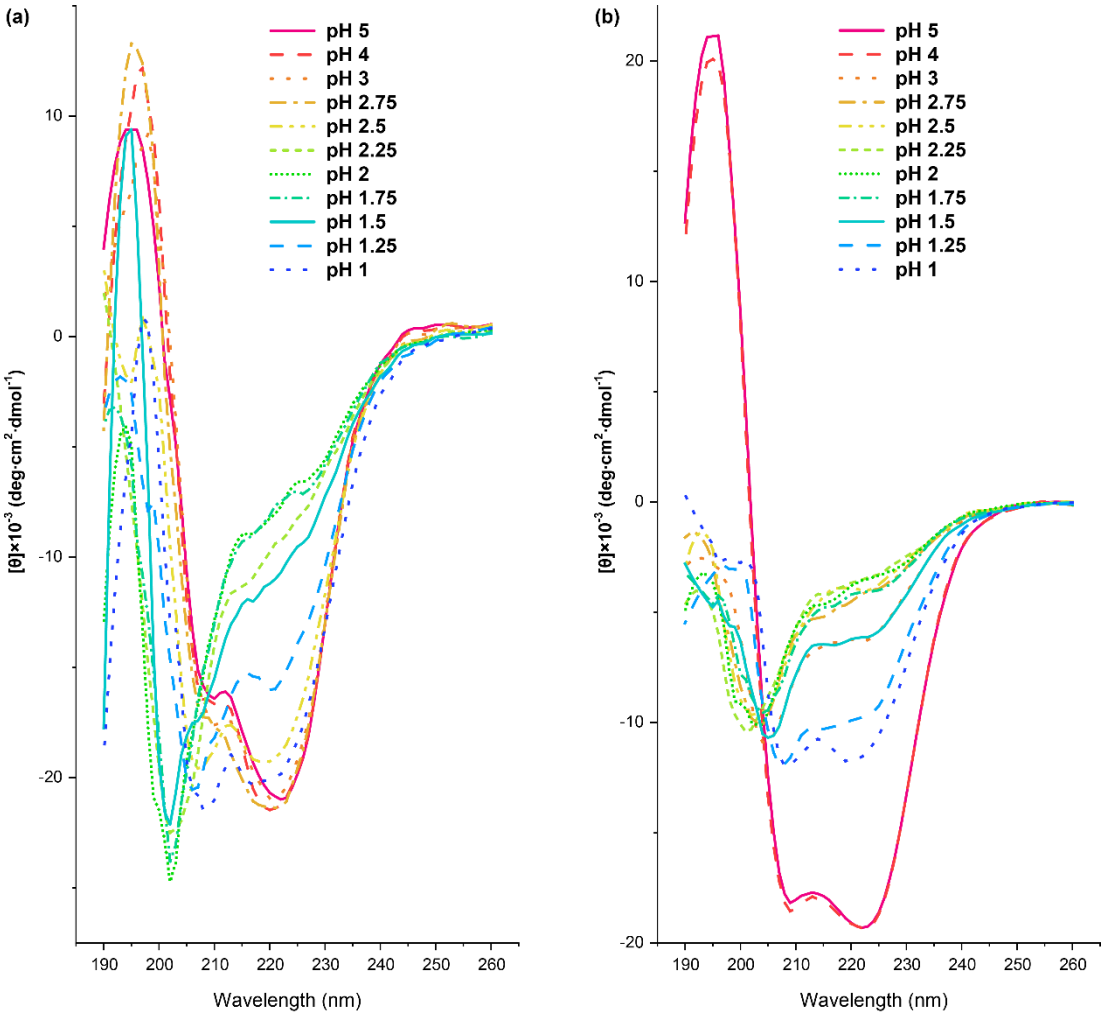

Figure S19. CD spectrum of cytochrome *c* (a) and myoglobin (b) in trifluoroacetic acid solution with different pHs. The vertical axis is the mean residue ellipticity.

Figure S20: Average charge states

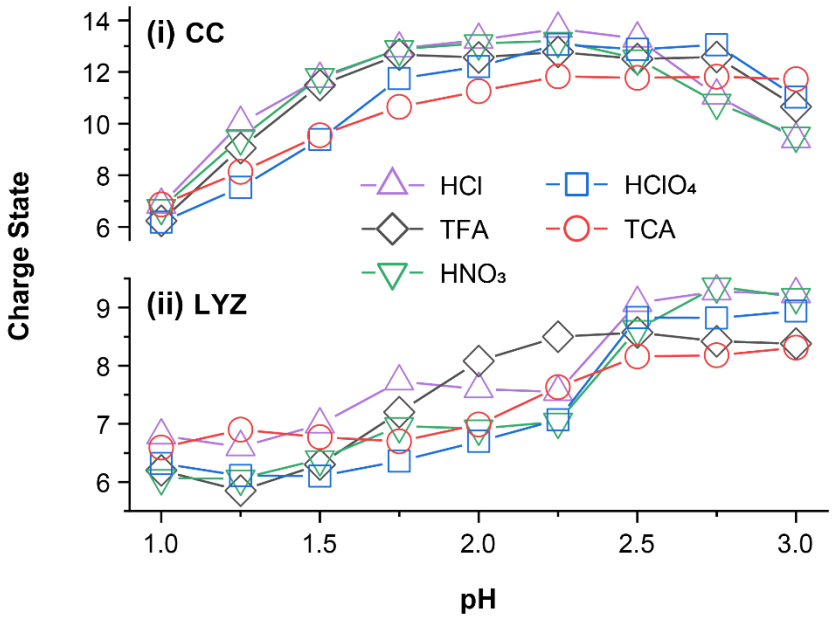

Figure S20. a) Average charge state of cytochrome *c* (i) and lysozyme (ii) versus pH for different acids (HCl: purple △, TFA: gray ◇, HNO_3: green ▽, $HClO_4$: blue □, TCA: red ○).

Figure S21: Average charge for HCSD & LCSD at different pHs

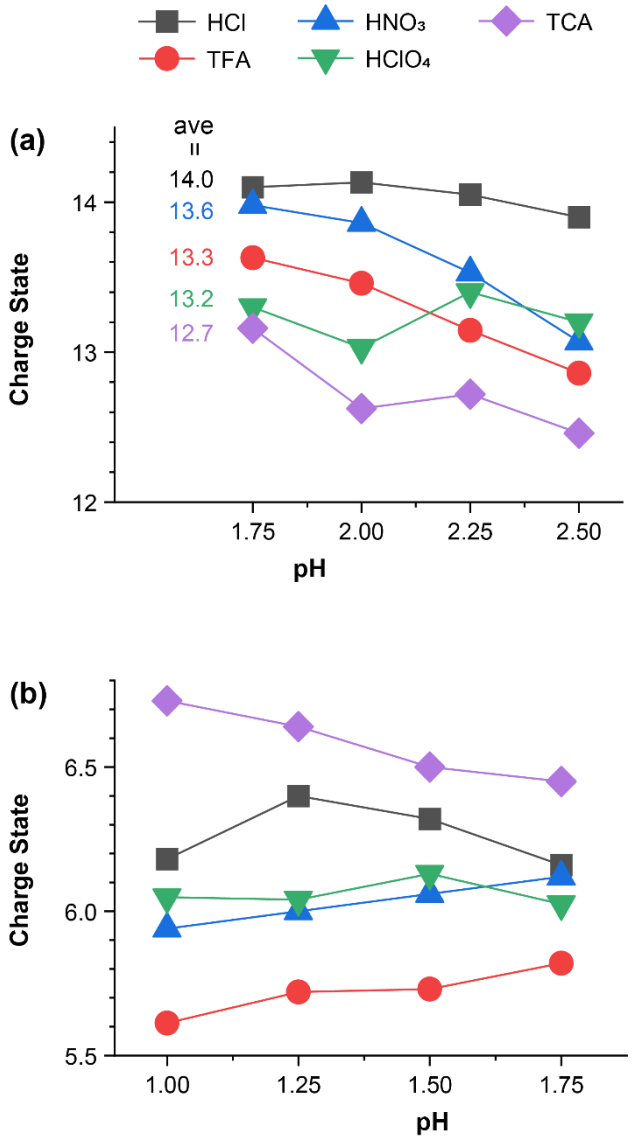

Figure S21. a) Average charge state for the high-charge-state distribution (HCSD) of equine cytochrome *c* for pH from 1.75 to 2.5. b) Average charge state for the low-charge-state distribution (LCSD) for pH from 1 to 1.75.

Figure S22: Addition of NH_4OH (cytochrome *c*)

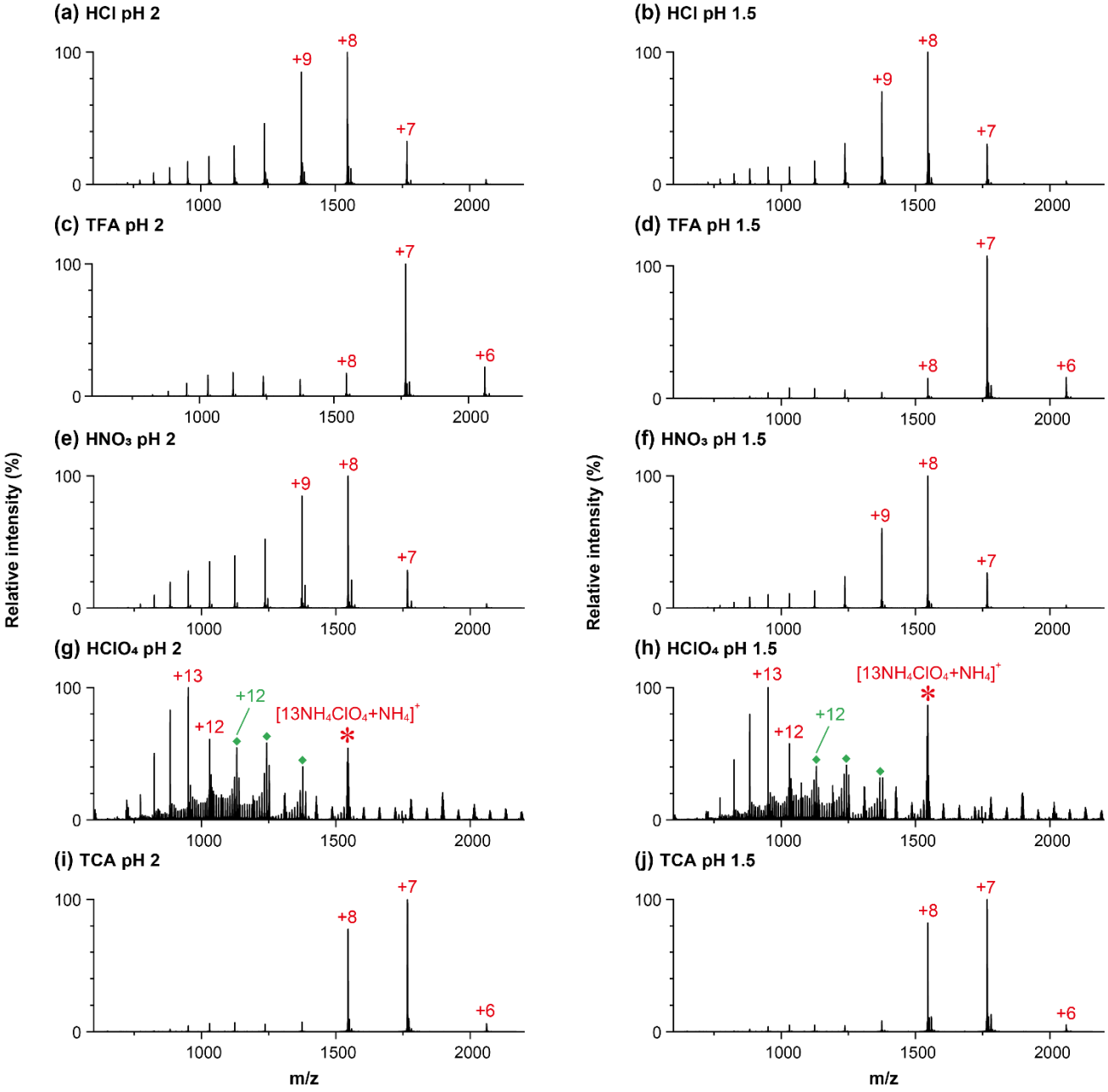

Figure S22. Mass spectra of equine cytochrome *c* prepared by titrating the pH 1 acid solutions with NH_4OH to pH 2 (left) and pH 1.5 (right).

Figure S23: Addition of NH_4OH (myoglobin)

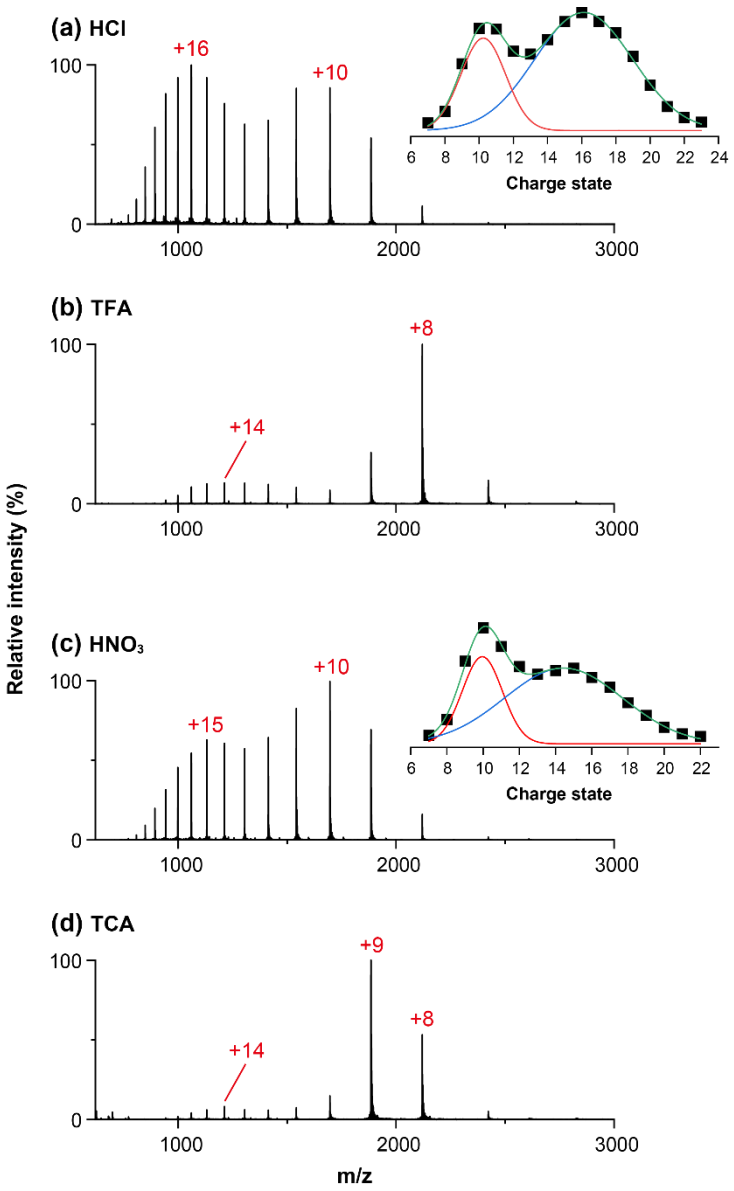

Figure S23. Mass spectra of myoglobin in HCl, TFA, HNO_3, and TCA with the presence of ammonium salt. pH = 1.5. The solution is prepared by titrating the pH 1 acid solution to 1.5 using NH_4OH. For (a) HCl and (c) HNO_3, the fitting method is used to estimate the average value for high charge and low charge state distribution (insets). Assume Gaussian distribution.

Figure S24: Addition of NH_4OH (ubiquitin)

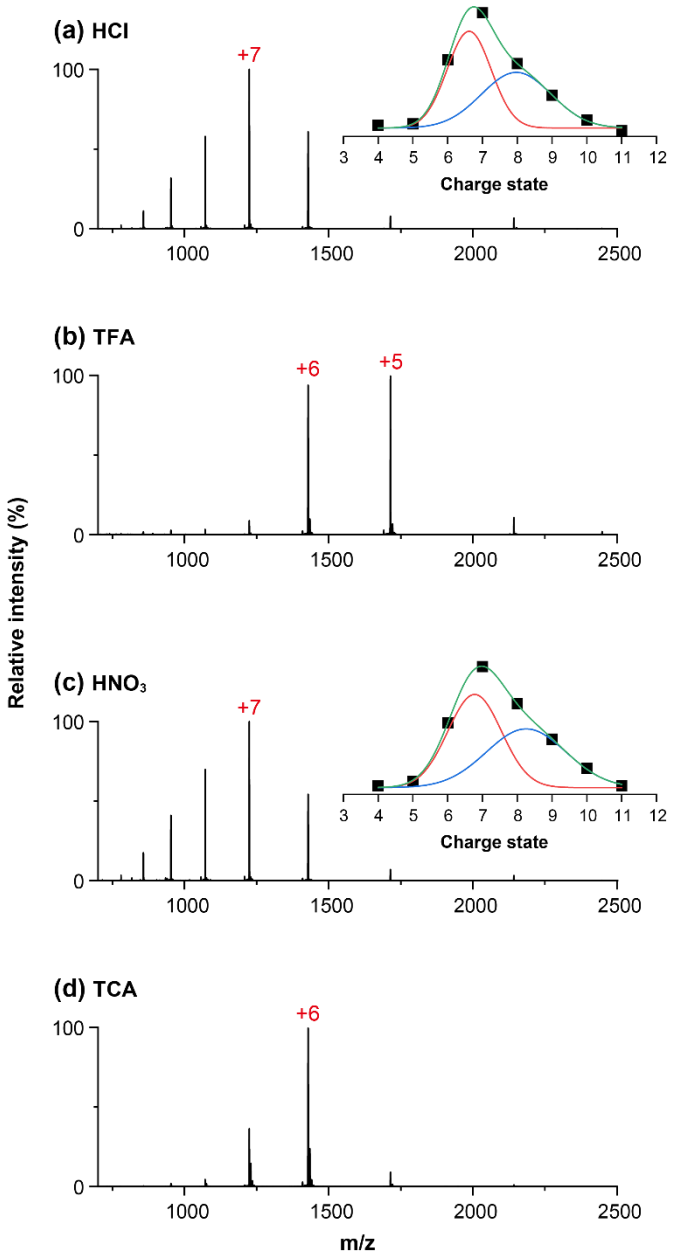

Figure S24. Mass spectra of ubiquitin in HCl, TFA, HNO_3, and TCA with the presence of ammonium salt. pH = 1.5. The solution is prepared by titrating the pH 1 acid solution to 1.5 using NH_4OH. For (a) HCl and (c) HNO_3, the fitting method is used to estimate the average value for high charge and low charge state distribution (insets). Assume Gaussian distribution.

Figure S25: Addition of NH_4OH (lysozyme)

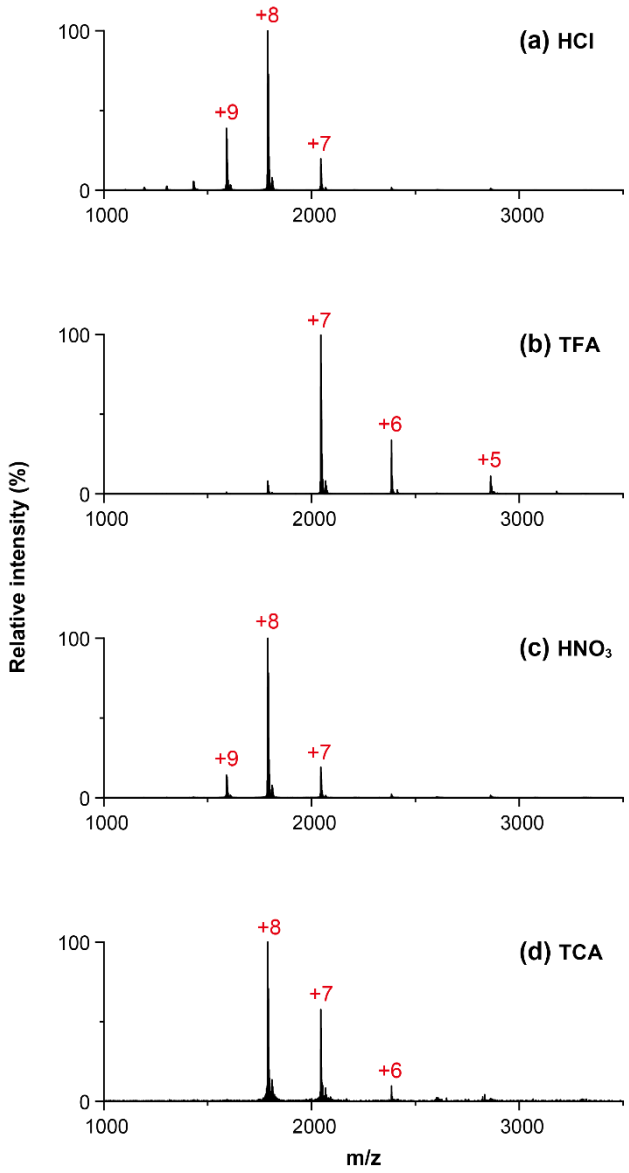

Figure S25. Mass spectra of lysozyme in HCl, TFA, HNO_3, and TCA with the presence of ammonium salt. pH = 1.5. The solution is prepared by titrating the pH 1 acid solution to 1.5 using NH_4OH.