Direct detection of native proteins in biological matrices using extractive electrospray ionization mass spectrometry

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Supporting Information

Samples and chemical reagents for the experiments

Human urine, tear and saliva samples collected from volunteers were directly used without matrix clean up. The E. coli samples were cultured in a biological lab following the proper procedures (see the E. coli cultivation section below). Myoglobin (90% purity), Ribonuclease A (RNase A), α-chymotrypsin and human lysozyme (human milk extractions) were imported from Sigma-Aldrich (St. Louis, MO, US). L-glutathione and chicken egg lysozyme were purchased from Yuanju

Bio-Tech CO. Ltd. (Shanghai, P. R. China). Regular recombinant human insulin (medical grade) and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, P. R. China) with highest grade (HPLC grade or Analytical Reagent grade) available, and directly used without further treatment.

E. coli cultivation

All E. coli bacteria were provided by the Department of Biochemistry, East China Institute of Technology. Bacteria were grown in a 10 g/L nutrient broth at 37℃ for 12 hours. Medium culturing was terminated when the optical density (OD) value reached about 1.5 and then the yielded culture fluid was centrifuged at 4℃ with the rotational speed of 10000 rpm for 10 minutes to get rid of the medium. Isolated cells were flushed twice with water and then suspended in water, allowing an approximate concentration of $1x10^8$ cells/mL for further applications. The precipitation (1g) was first dissolved in a Tris HCL buffer solution (100 mL 10 mM, pH 8.0) and then subjected to ultrasonication. The ultrasonication power was 600W; the treatment time was 3 min. The slashed E. coli mixture was directly used without any further treatment.

Preparation of PBS buffer solution

Briefly, the buffer solution was prepared by mixing disodium hydrogen phosphate (0.2 mol/L) with citric acid (0.1mol/L) according to different proportion for different pH values. The strategy is shown in the Table S1.

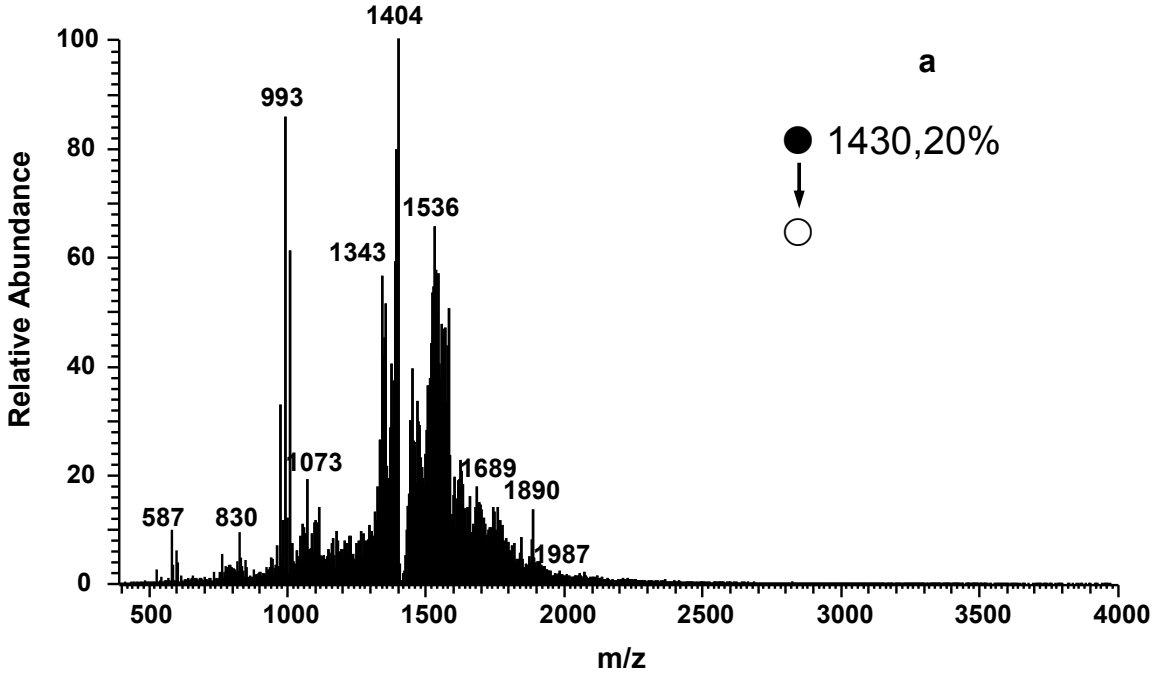
Table S1 Formula used to prepare the PBS buffer solutions for protein analysis.

pH	Volume of disodium hydrogen phosphate (0.2 mol/L) (mL)	Volume of Citric acid (0.1 mol/L) (mL)
4.4	8.82	11.18
4.6	9.35	10.65
4.8	9.86	10.14
5.0	10.30	9.70
5.2	10.72	9.28
6.4	13.85	6.15
6.6	14.55	5.45
6.8	15.45	4.55
7.0	16.47	3.53
7.2	17.39	2.61
7.4	18.17	1.83

EESI-MS for the direct detection of proteins in untreated biological matrix

The narrow CSD of proteins recorded in EESI-MS spectra features the sensitive detection of trace amounts of native proteins in raw biological samples, showing important applications in multiple disciplines such as proteomics and clinical

diagnosis. As a part of the innate immune system, lysozyme is abundant in a number of human secretions such as tears, saliva, and mucus. The EESI mass spectrum recorded from a fresh tear sample shows the narrow CSD of human lysozyme (Figure 2), which is comparable to that recorded using human lysozyme in an aqueous solution (pH 6.0) (Figure S7). The relative intensity of the peak at m/z 1835 in Figure 2 is even higher than that shown in Figure S7, probably because the lysozyme molecules maintained a more compact conformation in the tear sample (pH 7.4). Ions of high charge states such as +9 and +10 were abundantly detected from the human lysozyme samples, which is different from the CSD of chicken egg lysozyme. This indicates that the human lysozyme has 2 more basic groups than the chicken egg lysozyme. By using this method (inset of Figure 2), lysozyme contents in the tear samples collected from 3 healthy volunteers and 2 pink eye (pink eyes disease caused by the lack of lysozyme, for which the symptoms and signs are relatively non-specific[1]) patients were measured as 3~7 mg/mL and 50~220 μg/mL, respectively, showing the potential to diagnose conjunctivitis by quantitatively measuring the lysozyme levels in tears.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m/z
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1343
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1890
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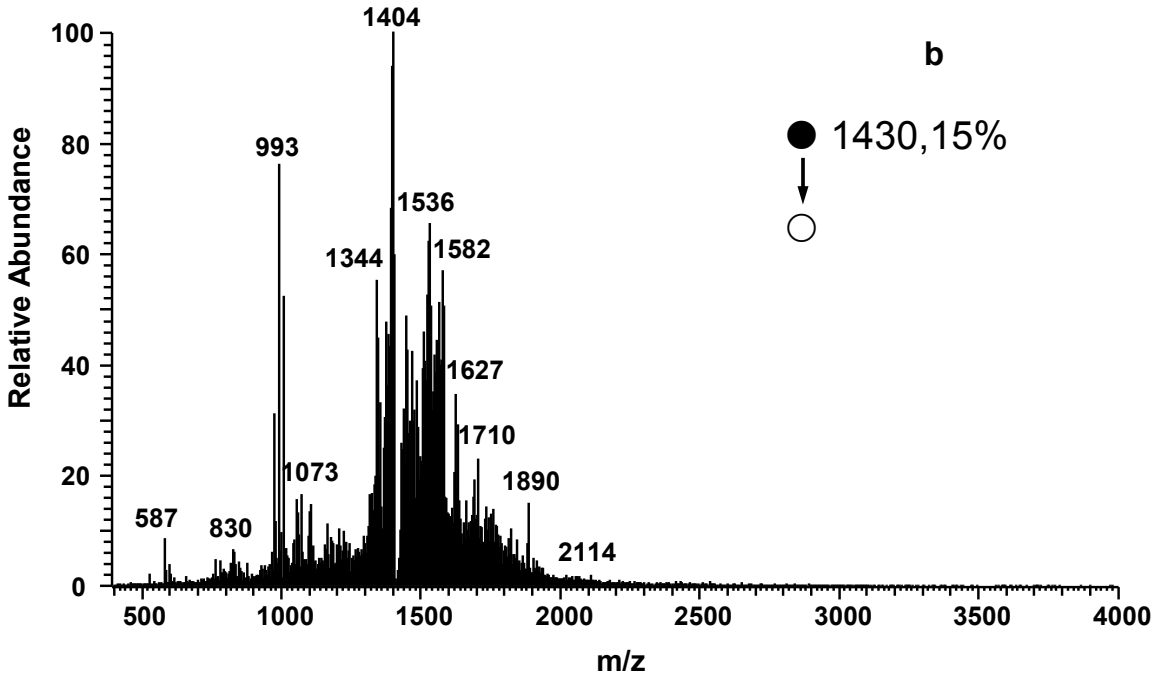
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1430,15%
Relative Abundance
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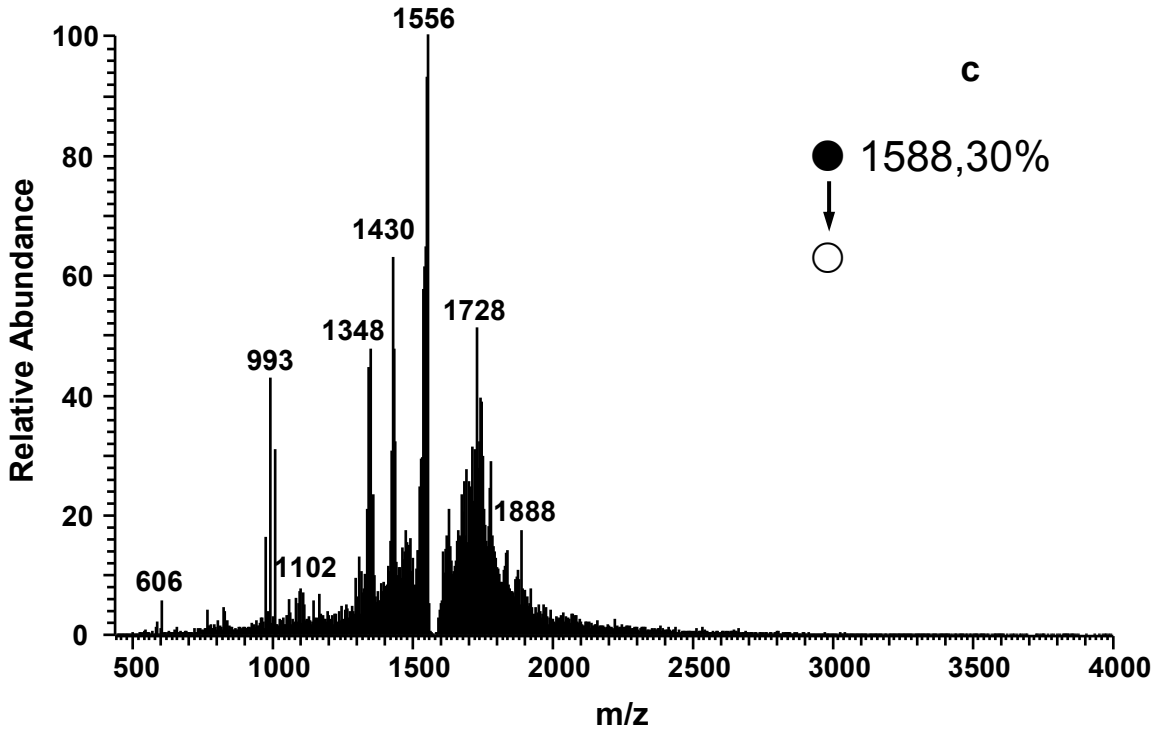

Figure S1 EESI-MS/MS spectra of signals detected from either urine samples spiked with lysozyme or lysozyme solutions. a) CID data of ions of m/z 1430 detected from the urine sample; b) CID data of ions of m/z 1430 detected from the standard lysozyme solution; c) CID data of ions of m/z 1588 detected from the urine sample; d) CID data of ions of m/z 1588 detected from the standard lysozyme solution;

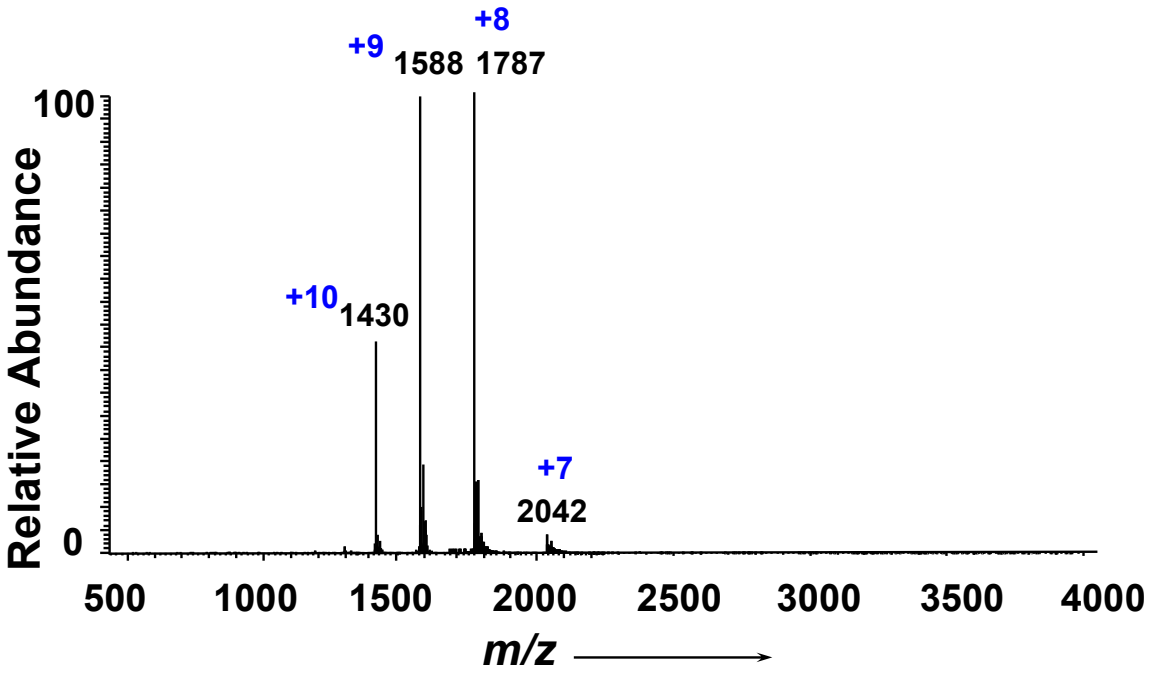

Figure S2 . EESI-MS spectrum recorded using a lysozyme solution (pH 5.5), providing signals corresponding to 7 ~ 10 charges, respectively.

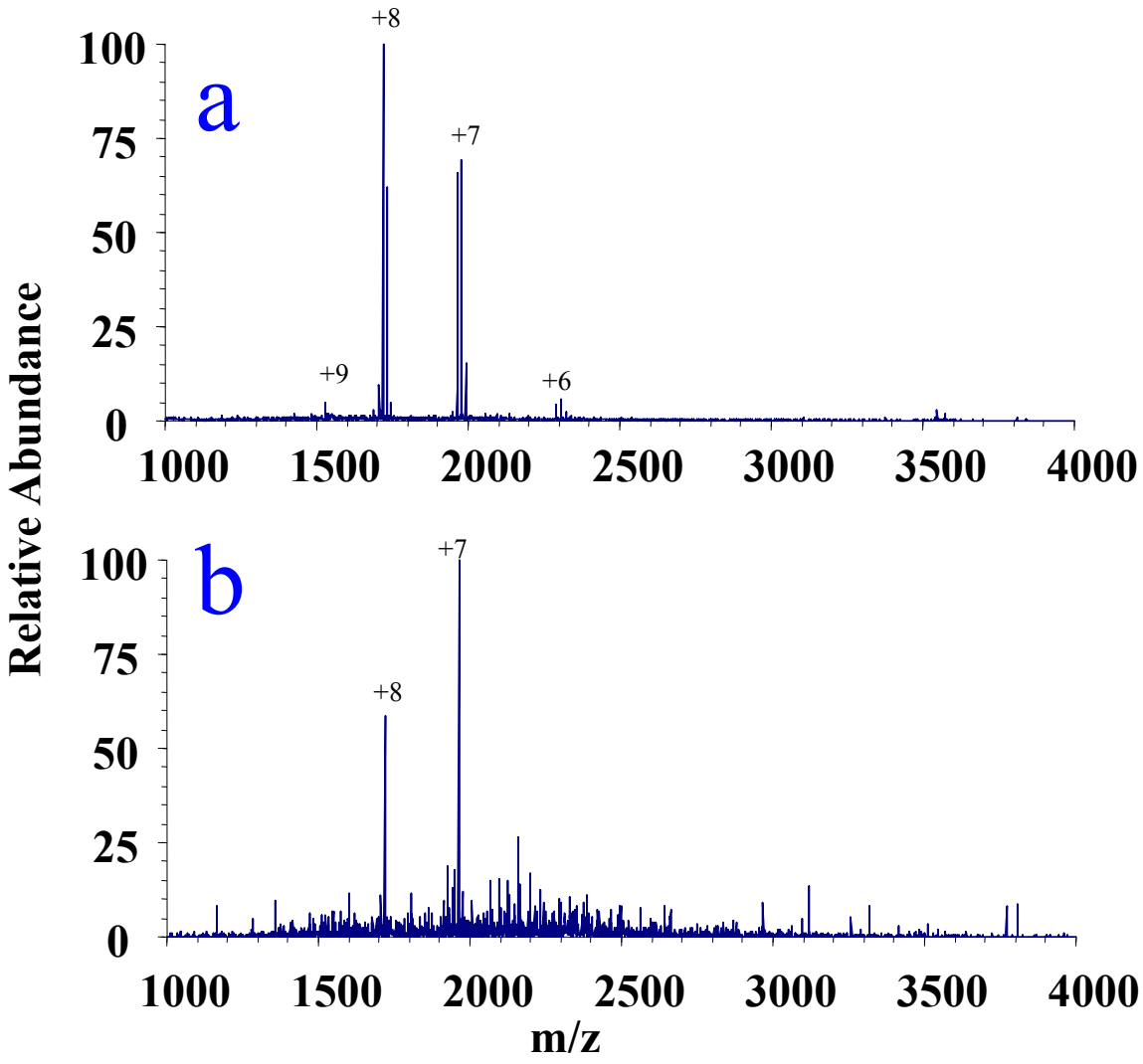

Figure S3 EESI-MS spectrum recorded using Rnase A protein. a) dilute NH_4Ac aqueous solution (1 mol/L); b) undiluted PBS buffer solution. The comparable major peaks were detected without sensitivity loss.

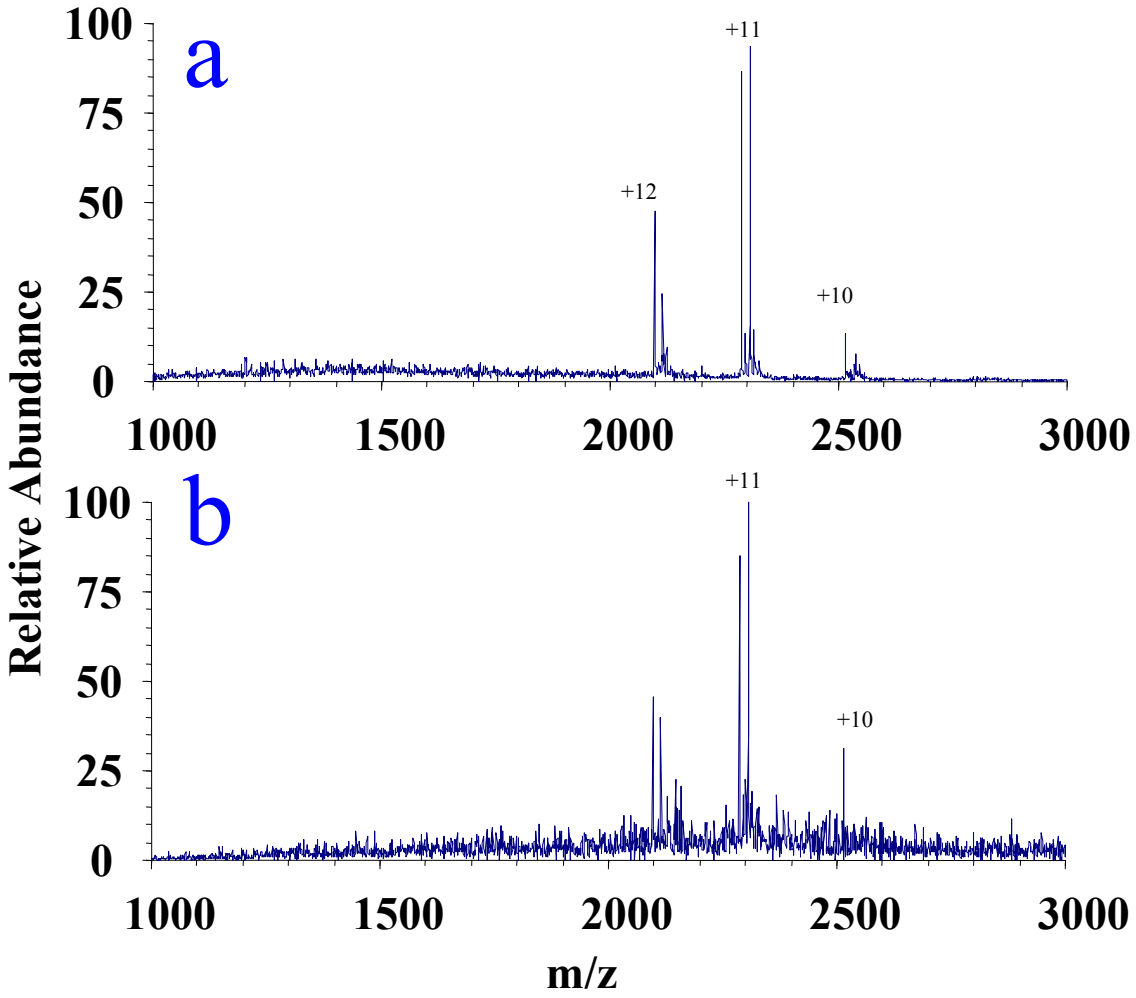

Figure S4 EESI-MS spectrum recorded using α-Chymotrypsin protein. a) dilute NH_4Ac aqueous solution (1 mol/L); b) undiluted PBS buffer solution. The comparable major peaks were detected without sensitivity loss.

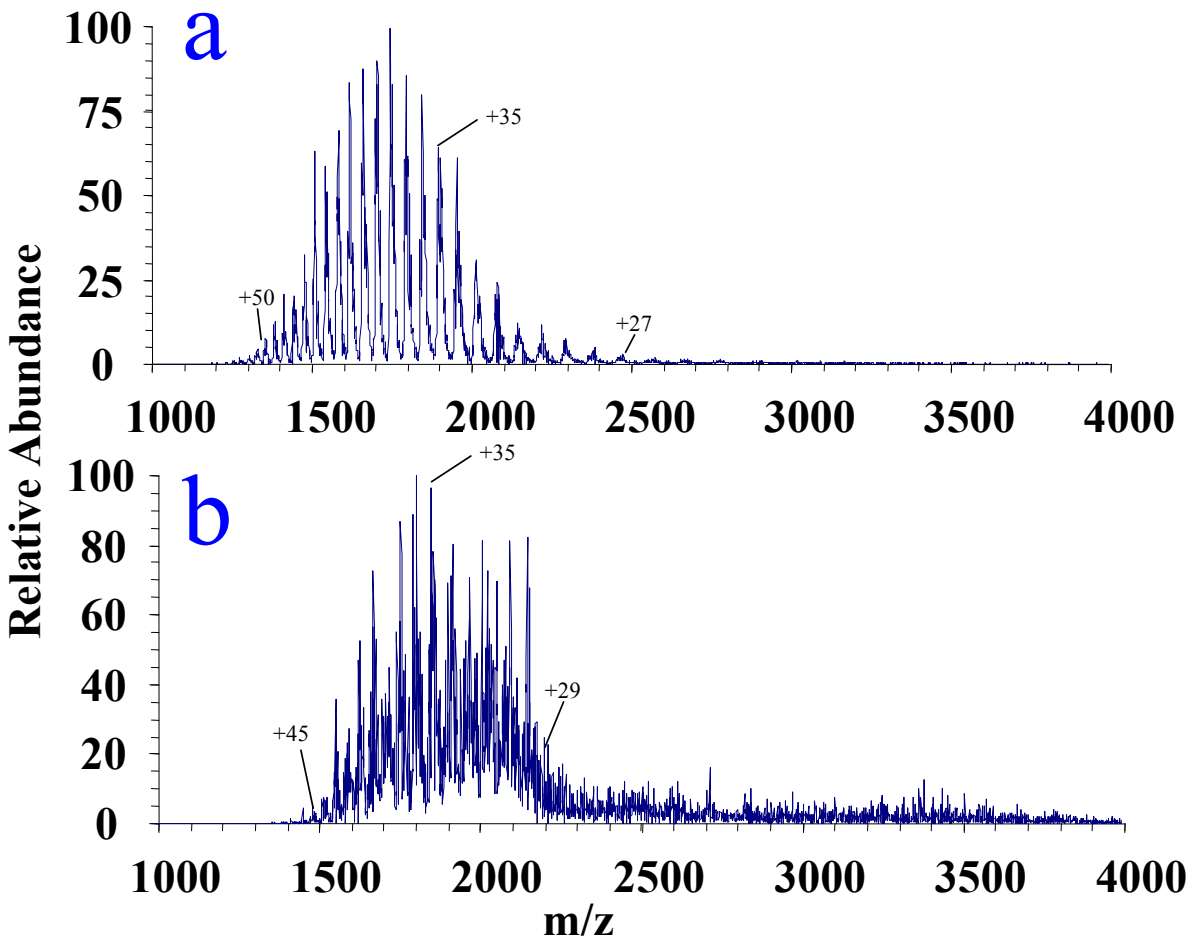

Figure S5 EESI-MS spectrum recorded using human serum albumin protein. a) dilute NH_4Ac aqueous solution (1 mol/L); b) undiluted PBS buffer solution. The comparable major peaks were detected, but showing slight sensitivity loss.

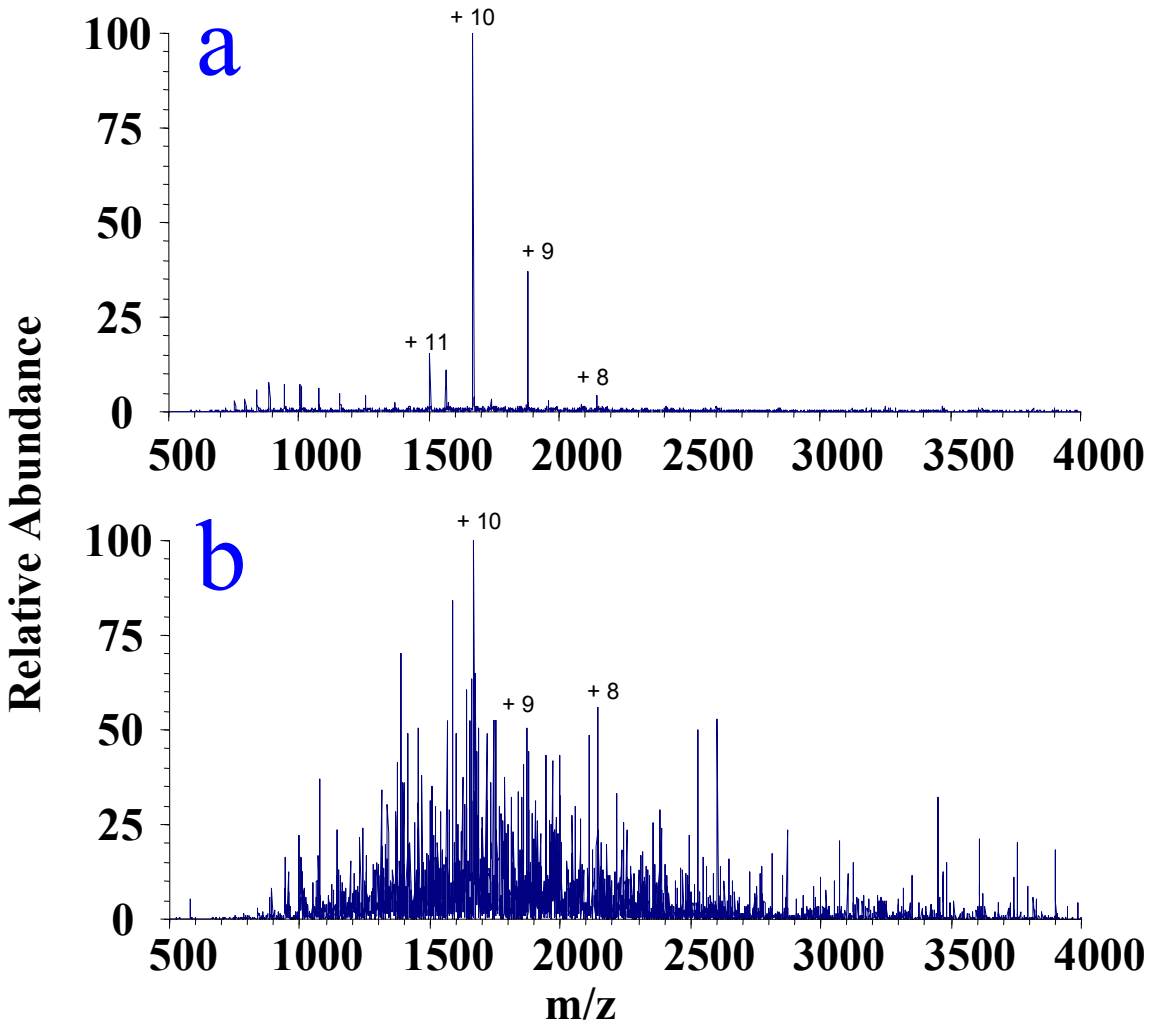

Figure S6 EESI-MS spectrum recorded using hemoglobin (bovine) protein. a) dilute NH_4Ac aqueous solution (1 mol/L); b) undiluted PBS buffer solution. The EESI-MS spectrum shows that the protein forms strong salt associated complexes which were dominant in the spectrum, although the major signals (e.g., +10, +9) were still detected. These data, however, proved the "soft" nature of the EESI process since the fragile non-covalently bound complexes were detected with high abundances. Note that no tetramer ions were detected, probably because that the noncovelently bound tetramers were dissociated into dimers prior to the EESI analysis or the tetramer ions were beyond the mass range of the LTQ instrument in our experiments.

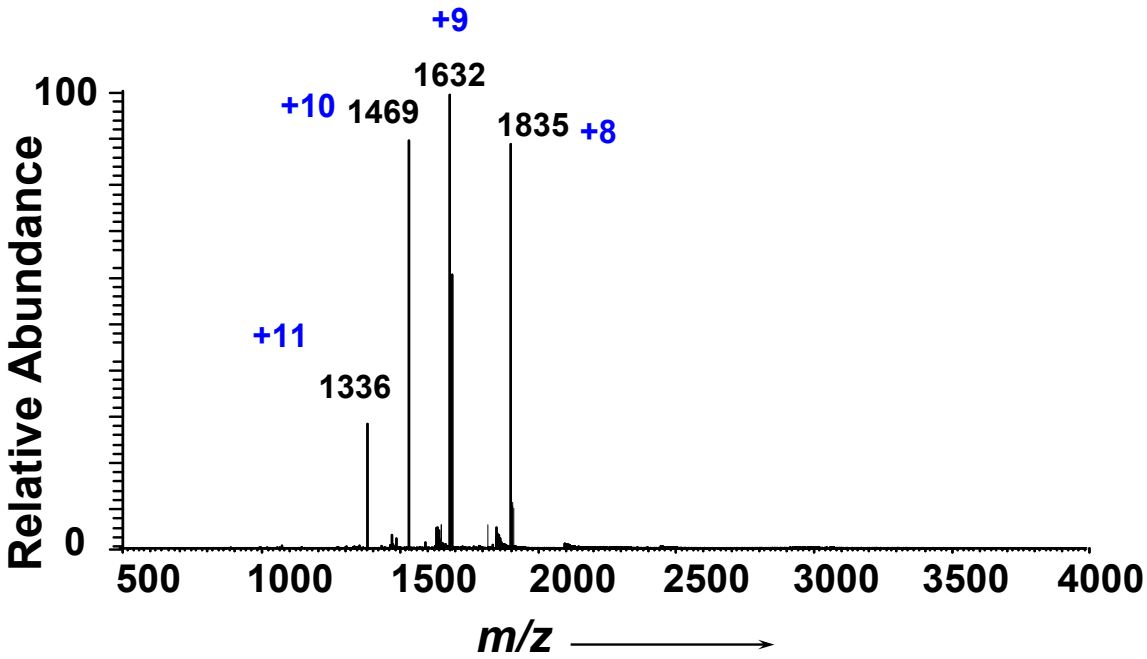

Figure S7 EESI-mass spectrum recorded using human lysozyme water solution (pH 6.0), showing a narrow CSD.

Table S2 RSD values for 6 measurements of lysozyme by EESI-MS

CnM / Measurements	10	50	100	250	500	2500	5000	10000	25000	50000
1	43	121	135	178	218	343	432	878	2120	3990
2	42	118	130	187	207	349	455	837	2210	4430
3	41	120	136	180	206	331	439	869	1900	4370
4	42	122	132	186	199	335	445	887	2010	4200
5	45	123	137	192	207	332	427	905	1930	3870
6	45	116	139	182	211	339	436	918	1980	3910
Mean value	43	120	134.8	184.1	208	338.1	439	878	2120	3990
Standard Deviation	1.67	2.60	3.31	5.15	6.26	6.9	9.9	28.5	118.4	240.0
RSD (%)	3.89	2.17	2.46	2.80	3.01	2.05	2.26	3.2	5.9	5.8

References:

1. Bielory, L. Differential diagnoses of conjunctivitis for clinical allergist-immunologists. *Annals of Allergy Asthma & Immunology* **98**, 105-115 (2007).