Electronic Supplementary Information

Direct Sequencing Disulfide-linked Peptide with Electrospray Ionization Tandem Mass Spectrometry

*Gongyu Li, Jiying Pei, Yue Yin, and Guangming Huang**

Supplementary Experimental Settings.

Table S1. Electrolyte-enhanced corona discharge in ESI for increasing dissociation of disulfide in human insulin.

Scheme S1. Proposed cleavage pathways and corresponding identified MS/MS product ions derived from OCR-induced disulfide cleavage via electrolyte enhanced corona discharge during ESI. Except the conventional dissociation pathway (a), four additional CID types were observed as a result of disulfide cleavage (b, c, d and e). Then, the total cleavages were summarized in f), and 29 cleavages (12 cleavages beyond disulfide, 17 cleavages inside disulfide) out of total possible 49 cleavages were identified.

Fig. S1. Step voltage-ESL curves to study the effects of a) electrolyte, b) flow rate, c) distance of tip to counter electrode and d) tip size on ESL response. With regard to the electrolyte, water solution were added with various equal molar of 10 mM electrolyte (NH_4OAc, NaCl, $CaCl_2$, $FeCl_3$) respectively. In order to distinguish the effect of flow rate, water solution contained 50 mM NH_4OAc were used. In the compare of distance of tip to counter electrode and tip size, pure water added with 1% HOAc were used. The starting parameter for these experiments were set as follows, tip size 50 μm, flow rate 0.5 μL/min, distance of tip to counter electrode 5 mm. Note the regular variation of ESL signal when tip size was larger than 100 μm that was presumably due to the incompatibility of tip size with flow rate. PMT high voltage: -400 V.

Fig. S2. The dependence of disulfide cleavage in GSSG (8 μM) on the concentration of NH_4OAc. In order to clearly identify the *m/z* change with the increase of NH_4OAc in concentration, we only presented the highlighted *m/z* range (*m/z* 300 - 360) in main text.

Fig. S3. Dependence of GSH percentage on the concentration of NH_4OAc under different DC spray voltages by electrolyte-enhanced corona discharge in ESI.

Fig. S4. Dependence of GSH and GSO• percentage on the concentration of GSSG in the presence of 0.5 M NH_4OAc by electrolyte-enhanced corona discharge in ESI.

Fig. S5. ESI-CID-MS mass spectrum of radical additive insulin ion ($[M+ 5H+ OH]^{5•+}$, *m/z* 1166) with 100 mM NH_4OAc. Abundant sequence information could be obtained with the existence of concentrated NH_4OAc due to the selective OCR between disulfide and HO• generated from electrolyte-enhanced CD in ESI.

Fig. S6. The comparison of ESI-CID-MS mass spectrum of radical additive insulin ion ($[M+ 5H+ OH]^{5•+}$, *m/z* 1166) with (**Fig. 3c**) or without 100 mM NH_4OAc. Abundant sequence information could be obtained with the existence of concentrated NH_4OAc due to the selective OCR between disulfide and HO• generated from electrolyte-enhanced CD in ESI, while no sequence information was obtained without NH_4OAc.

Fig. S7. The comparison of ESI-CID-MS mass spectrum of native insulin ion ($[M+ 5H]^{5+}$, *m/z* 1162.5), radical additive insulin ion ($[M+ 5H+ OH]^{5•+}$, *m/z* 1166), and sodium adduct insulin ion ($[M+ 4H + Na]^{5+}$, *m/z* 1167). In order to trigger the OCR between disulfide and HO•, concentrated electrolyte of 100 mM NH_4OAc were added into the spray solution. Abundant sequence information could be obtained from the resulting spectrum of radical additive insulin ion (59% sequence coverage) but few information from other parent ions (26.5% and 14.3%).

Fig. S8. Reaction spectra derived from selectin binding peptide disulfide cleavage via dual channel ESI.

Fig. S9. Dual channel electrolyte-enhanced corona discharge in ESI and tandem CID-MS experiments for GSSG sequence identification. First tip was loaded with disulfide peptide (for cleavage) and another tip was loaded with concentrated electrolyte (for HO• generation from corona discharge). When only high voltage for the first tip (single channel ESI) was turned on, disulfide cleavage was not evidenced (**a**). Then the high voltage for the next tip was turned on (dual channel ESI), GSSG cleavage was typically identified in (**b**).

Fig. S10. Single channel ESI and tandem CID-MS experiments for selectin binding peptide sequence identification. Both disulfide peptide and concentrated electrolyte (100 mM NH_4OAc) were loaded onto a single spray tip. When DC high voltage of 3 kV was turned on, disulfide cleavage was evidenced by the reaction spectra (**a**) and corresponding tandem MS spectrum was used to sequence the peptide (**b**). It should be noted that, single channel ESI protocol for disulfide cleavage is better than dual channel ESI in terms of reaction efficiency, which could be indicated by the ratio of $[M + 2H + OH]^{2•+}$ to $[M + 2H]^{2+}$. However, in terms of analyte signal intensity (dual channel, ~ 1.73E6 vs. single channel, ~ 6E3) and product purity (peaks existed in single channel ESI that could be denoted as impurities from concentrated electrolytes), dual channel ESI cleavage protocol is better than single channel ESI, which could also be verified by the signal to noise ratio of MS/MS spectrum. This could be explained by the charge competition from concentrated electrolyte in single channel ESI protocol, and this competition effect

could be effectively alleviated in dual channel ESI protocol which benefits from the separation of disulfide peptide and concentrated electrolyte into two individual spray emitters.

Supplementary Experimental Settings.

Chemicals. GSSG and selectin binding peptide were supplied by Sangon Biotech Co., Ltd (Shanghai, China). Other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). No further purifications were performed for all reagents. All solvents used in this study were of HPLC grade. Purified water (conductivity of 18.2 MΩ.cm) was obtained from Milli-Q® Reference System (Millipore Corp., USA).

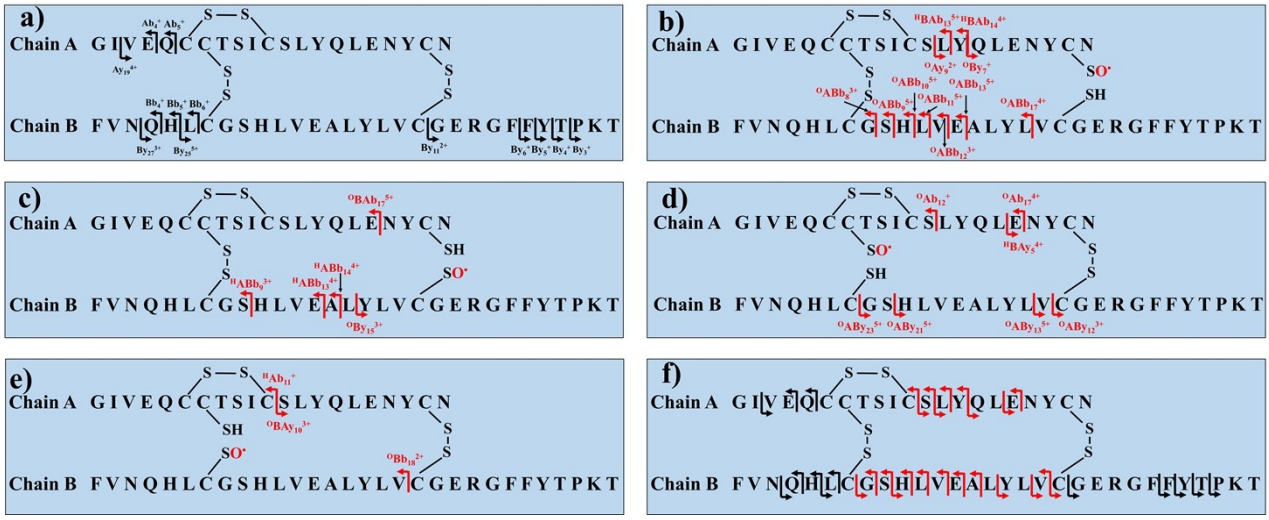
Experimental settings. All experiments were carried out with a LTQ-Velos Pro (Thermo Fisher Scientific, CA, USA) mass spectrometer. MS operation conditions are as follows: spray voltage 3 kV, CID energy 15%, max. ion injection time 100 milliseconds, MS inlet temperature of 275 °C (unless otherwise mentioned).

Characterization of CD in ESI. The CD in ESI was characterized with a PMT supplied by Xianruimai Analytical Instruments Co., Ltd. (Changchun, China). Step voltage-ESL curves were used to study the effects of various influencing factors, including electrolyte, flow rate, distance of emitter to counter electrode and emitter size on ESL response. In order to distinguish the effect of flow rate, water solution contained 50 mM NH4OAc were used. In the compare of distance of emitter to counter electrode and emitter size, pure water added with 1% HOAc were used. The starting parameter for these experiments were set as follows, emitter size 50 μm, flow rate 0.5 μL/min, distance of emitter to counter electrode 5 mm. PMT high voltage: -400 V.

Dual Channel ESI experiment. Distance between two emitters (50 μm) is about 530 μm and the same spray DC voltage (5 kV) was applied on individual stainless steel connectors. The steel connectors are about 5 cm away from spray emitter. Individual flow rate, 0.5 μL/min.

Table S1. Electrolyte-enhanced corona discharge in ESI for increasing dissociation of disulfide in human insulin.

	$\mathbf{I}_{1162.5}$ ($[M + 5H]^{5+}$)	$\mathbf{I}_{1166}$ ($[M + 5H + OH]^{5\bullet+}$)	$\mathbf{I}_{1166}/\mathbf{I}_{1162.5}$
Insulin	7.92E+03	2.15E+02	2.71%
Insulin + 10 mM NH_4OAc	3.05E+03	2.70E+02	8.85%
Insulin + 100 mM NH_4OAc	2.45E+03	3.72E+02	15.18%
Insulin + 500 mM NH_4OAc	6.37E+02	2.10E+02	32.97%



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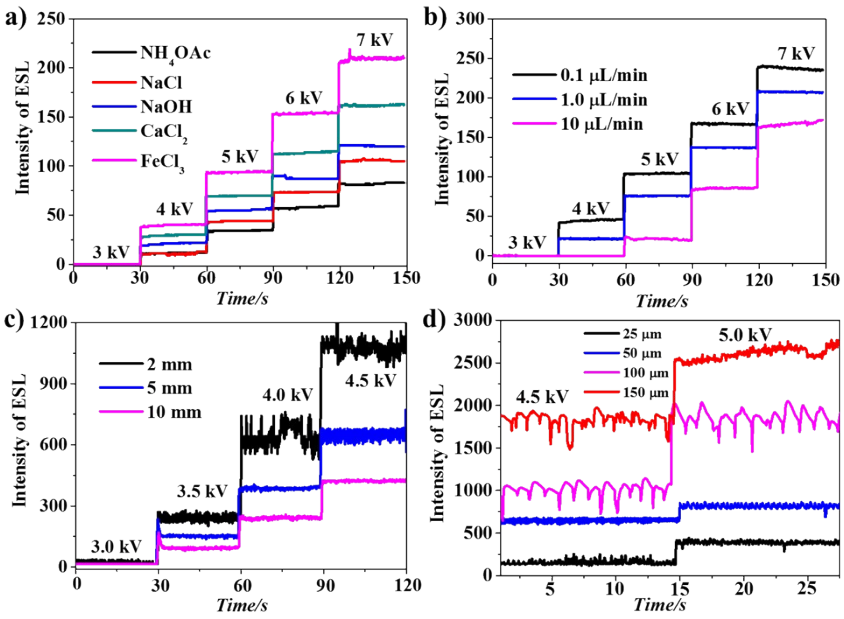

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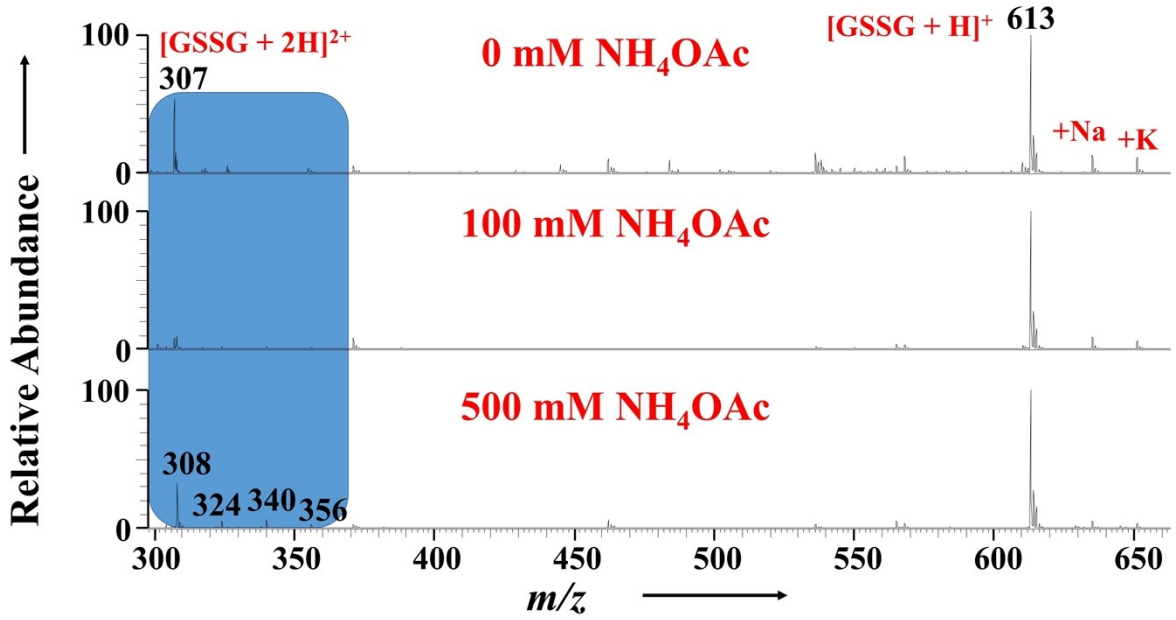

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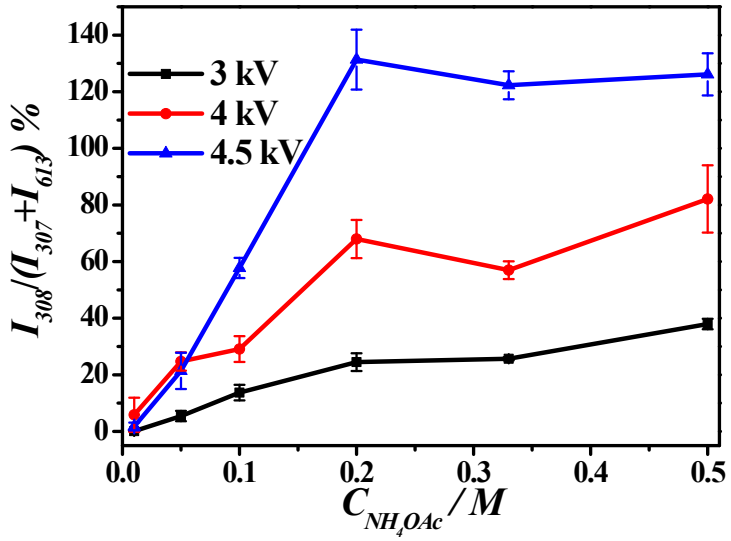

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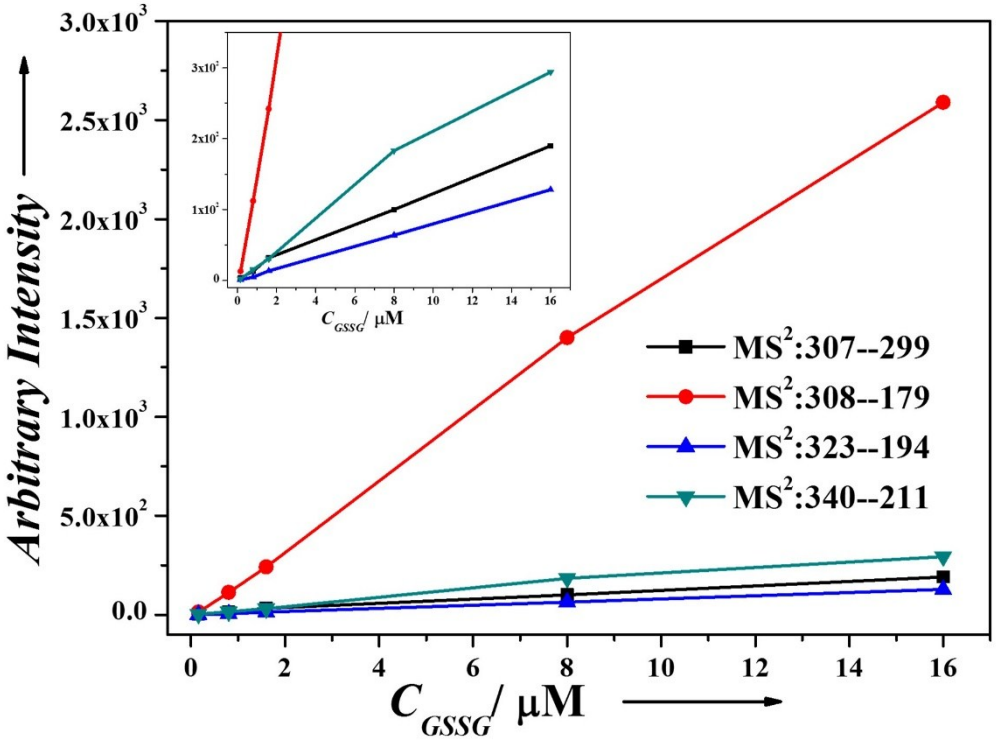

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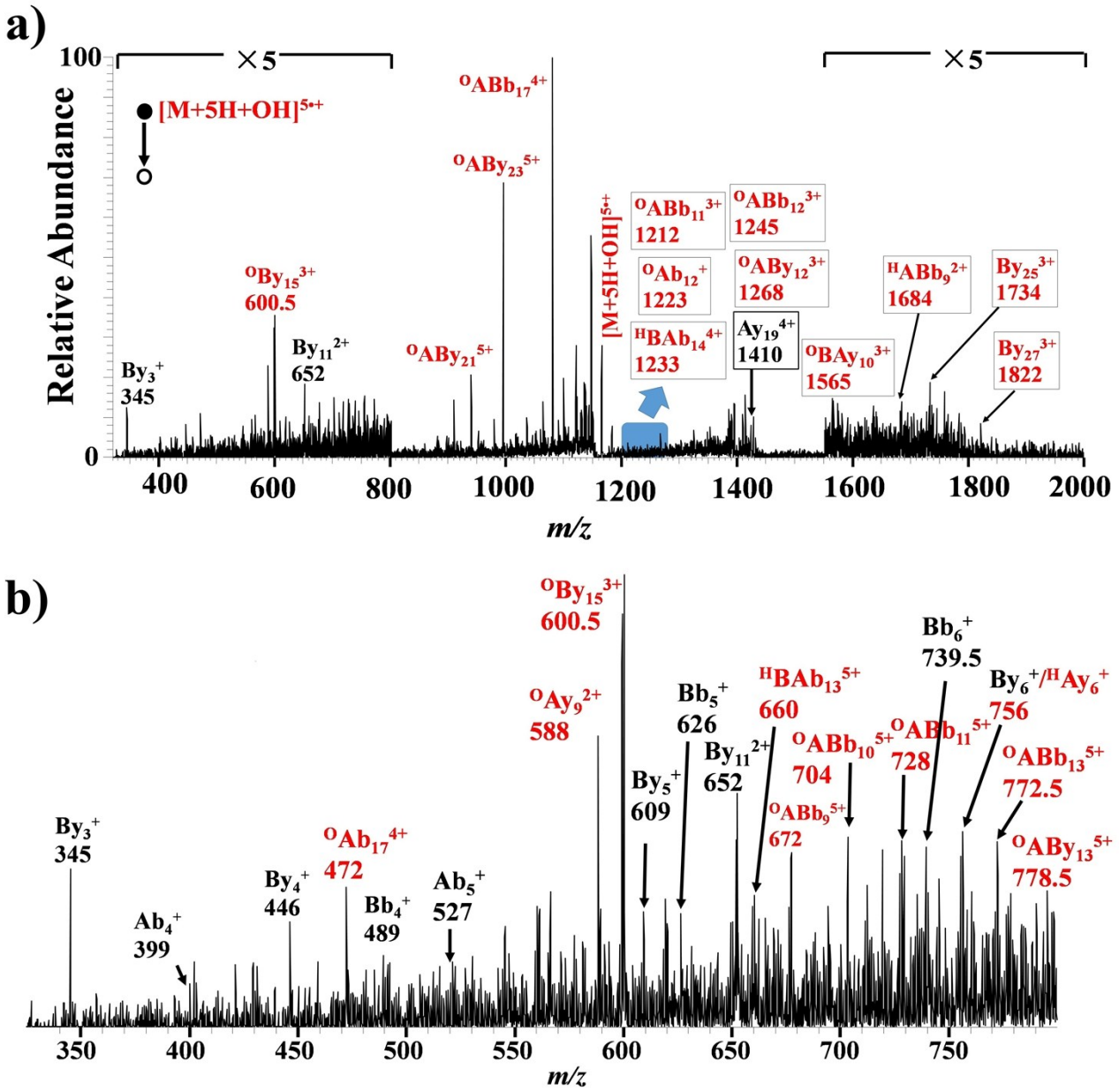

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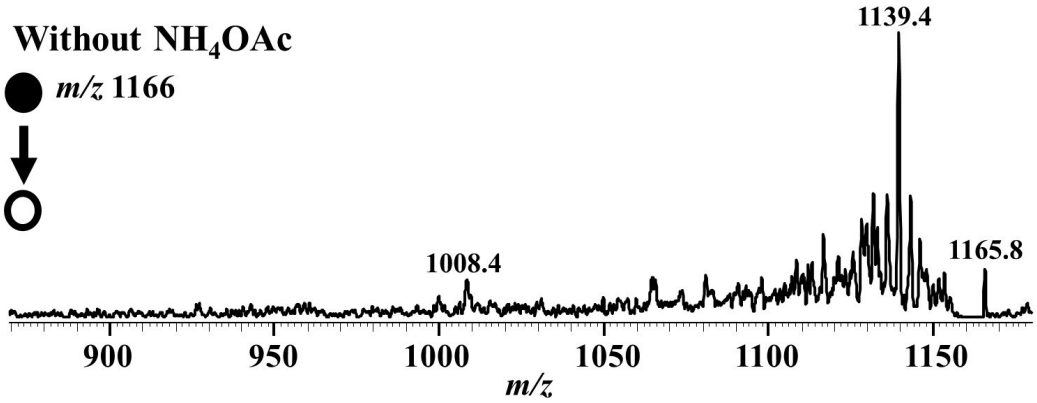

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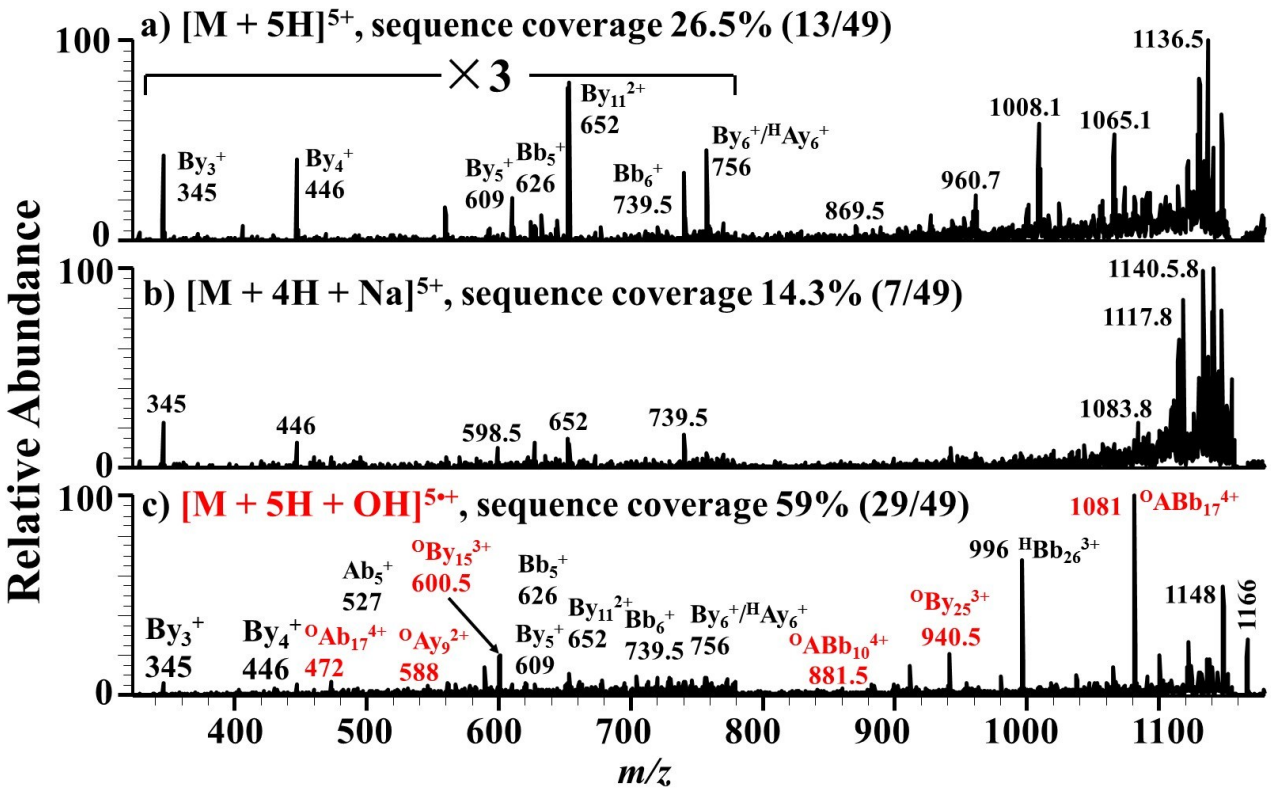

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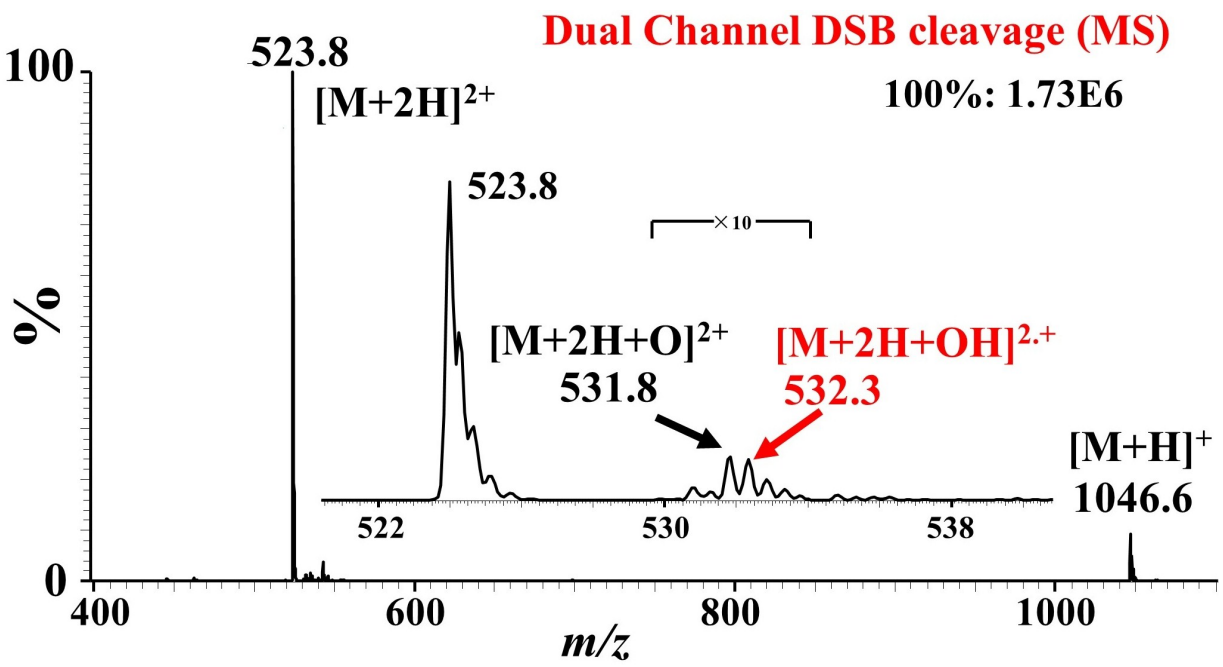

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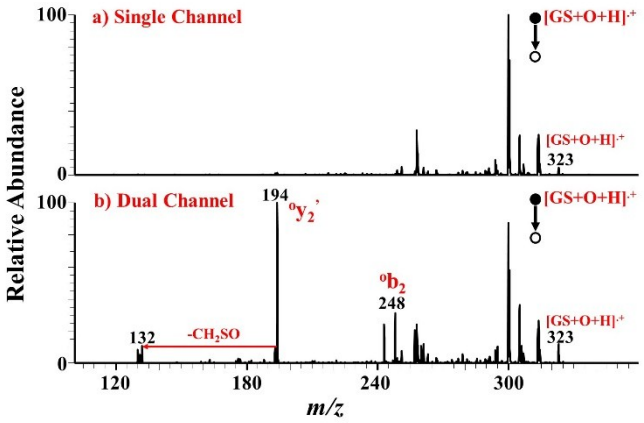

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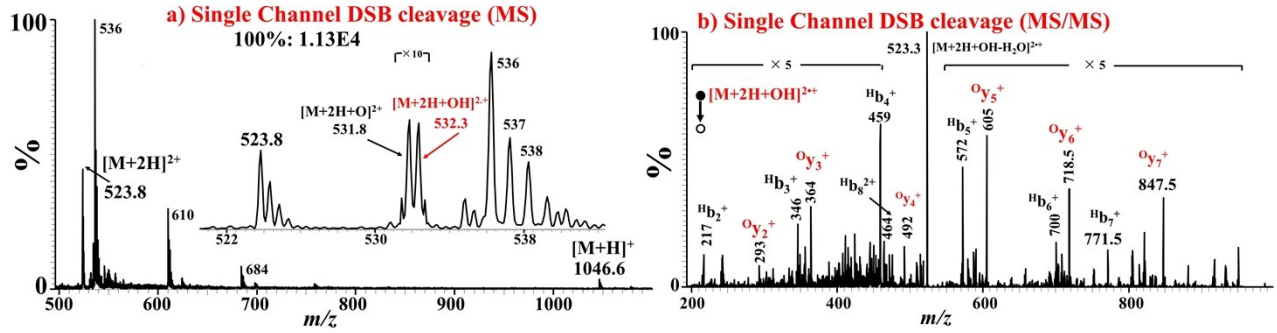

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