Supplemental Materials

Signal and Charge Enhancement for Protein Analysis by Liquid Chromatography-Mass Spectrometry with Desorption Electrospray Ionization

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Additional spectra and information are provided in this supplement.

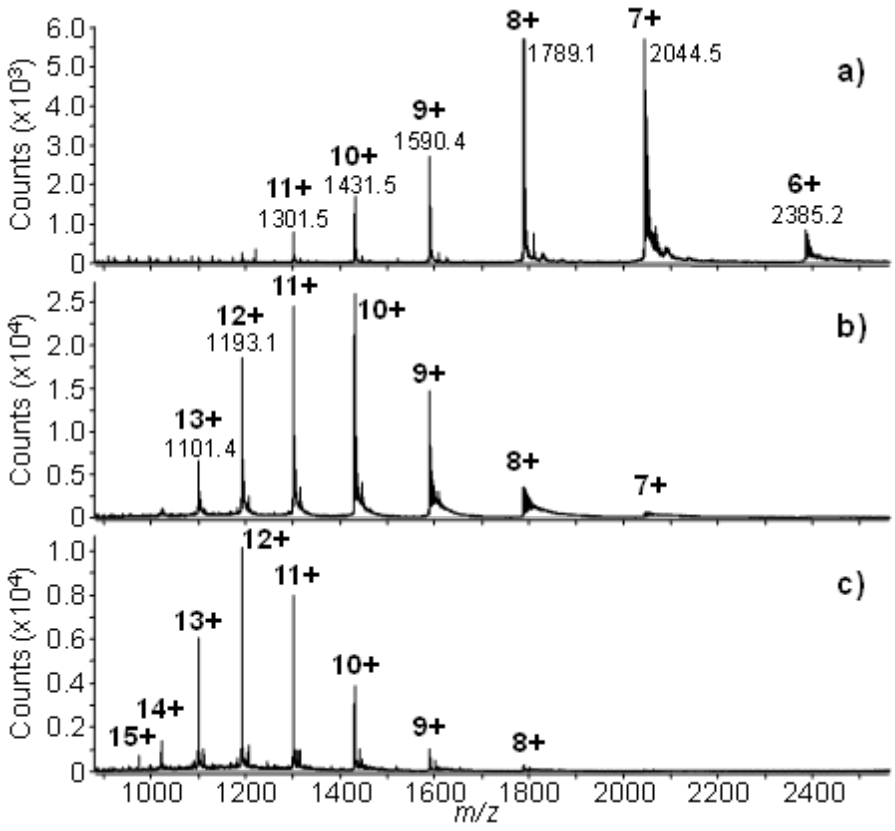

Figure S-1. a) ESI-MS spectrum of 10 μM lysozyme in 50/50/0.1 H_2O/ACN/TFA and the mass spectra in b) and c) were acquired by adding 0.5% *m*-NBA and 200 mM sulfolane, respectively. These ESI mass spectra were acquired with an Agilent 6220 LC-TOF instrument. The average charge shifted from +8 without any reagent to +10.7 with *m*-NBA and +11.7 with sulfolane. The signal intensity increased 4.3 fold with *m*-NBA and 1.6 fold with sulfolane when compared to the signal obtained without adding any reagent.

As shown in Figure S-2a, the direct infusion of insulin eluent by ESSI analysis, a variant form of ESI, after 100:1 splitting (10 μL/min) gave the mass spectrum with the normalized intensity of 1.54E5 for the most abundant peak at +4. When insulin eluent coming out of the LC system without splitting was sampled/ionized directly by a DESI spray probe consisting 50 mM *m*-NBA in CH_3OH/H_2O/HOAc (50:50:1 by volume), a mass spectrum with 2-fold higher intensity of protein peaks was observed along with the maximum charge state shifted from +4 to +5 (Figure S-2b). Similar phenomena were observed for ubiquitin (Figure S-3). In comparison to ESSI signal (Figure S-3a), the sensitivity enhancement using LC/DESI-MS by including *m*-NBA in the DESI spray solvent (Figure S-3b) is approximately 5-fold. In addition, the most abundant charge state shifted from +8 to +9 and the maximum charge state of +13 in Figure S-3b is more pronounced than that in Figure S-3a.

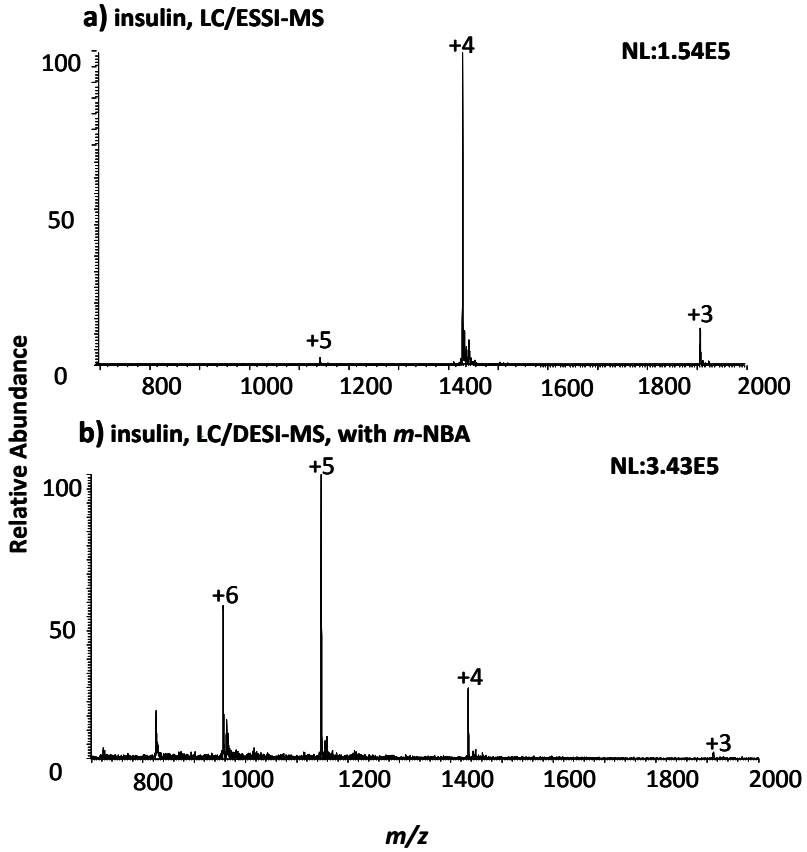

Figure S-2. Mass spectra of insulin following the HPLC separation obtained by (a) ESSI analysis after splitting the eluent (splitting ratio: 100:1), (b) DESI analysis of the jet eluent using the spray solvent of CH_3OH/H_2O/HOAc (50:50:1 by volume) containing 50 mM *m*-NBA.

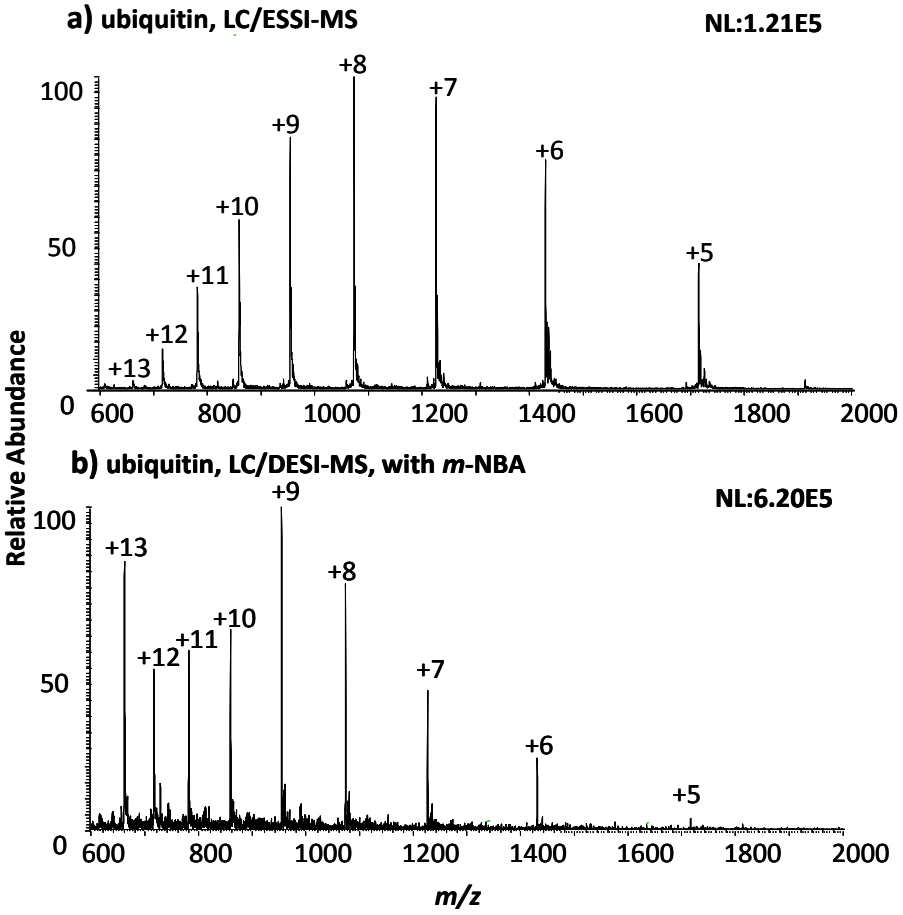

Figure S-3. Mass spectra of ubiquitin following the HPLC separation obtained by (a) ESSI analysis after splitting the eluent (splitting ratio: 100:1), (b) DESI analysis of the jet eluent using the spray solvent of CH_3OH/H_2O/HOAc (50:50:1 by volume) containing 50 mM *m*-NBA.

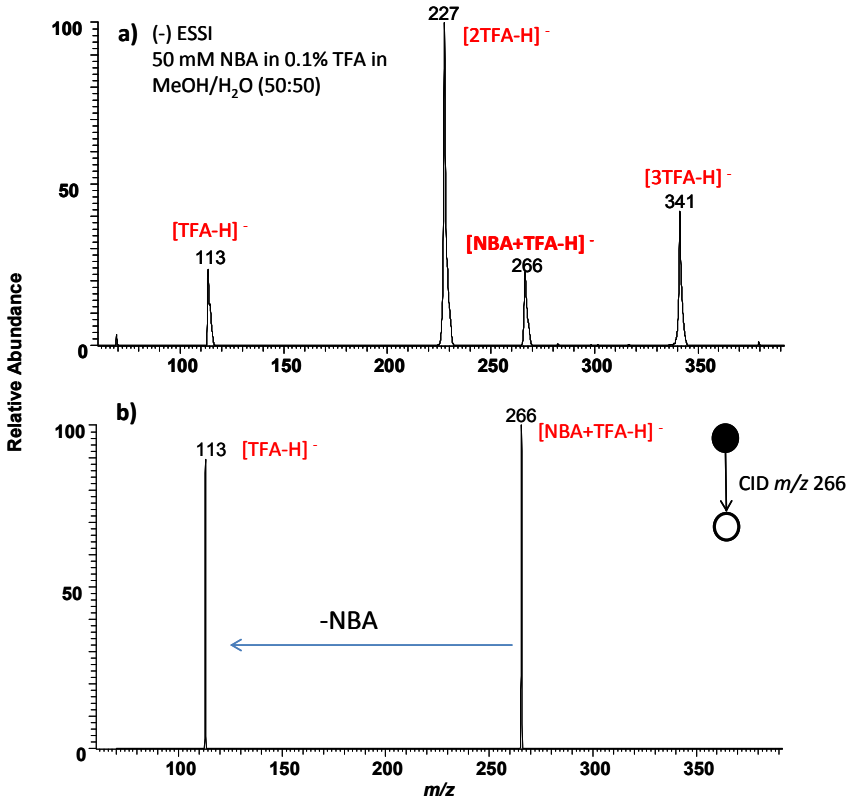

Figure S-4. (a) Negative ion ESSI-MS spectrum showing the formation of adduct of TFA anion with NBA (*m/z* 266); (b) CID of *m/z* 266.

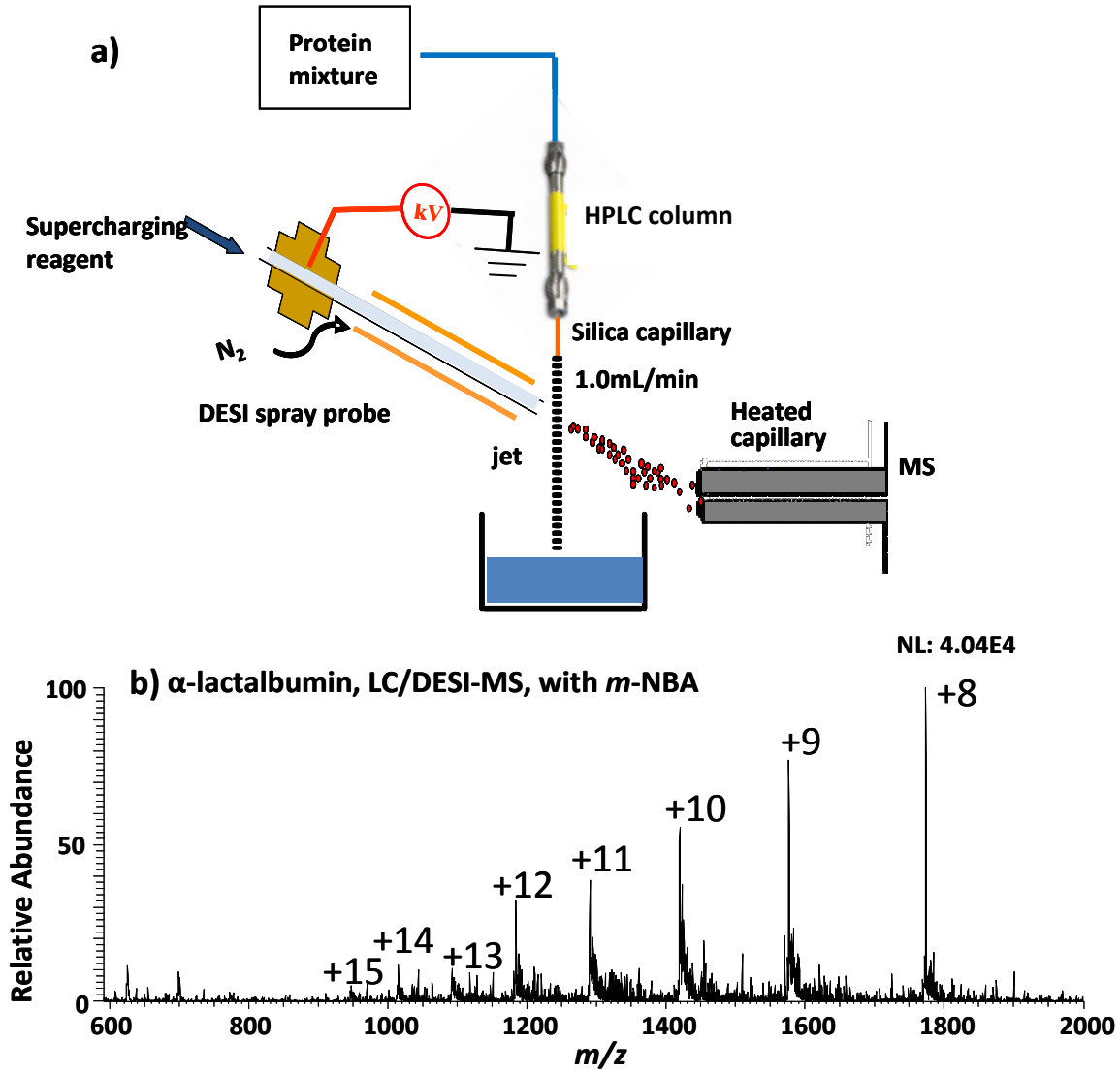

Figure S-5. (a) Scheme showing a variant apparatus configuration of LC/DESI-MS apparatus in which a DESI spray probe was used to ionize analytes of interests in the eluent directly from LC column. The outlet of the column was connected with a short piece of fused silica capillary (4.2 cm) to facilitate the generation of a free jet eluent; (b) mass spectrum of α-lactalbumin following the HPLC separation obtained by DESI analysis of the jet eluent using the spray solvent of CH_3OH/H_2O/HOAc (50:50:1 by volume) containing 50 mM *m*-NBA.