An On-line Matrix Removal Platform for Coupling Gel-Based Separations to Whole Protein Electrospray Ionization Mass Spectrometry

*Ki Hun Kim, Philip D. Compton, John C. Tran and Neil L. Kelleher**

Departments of Chemistry, Molecular Biosciences and the Proteomics Center of Excellence, Northwestern University, 2145 N. Sheridan Road, Evanston, IL 60208, United States

Supporting Information

Supplementary Table 1 and 2

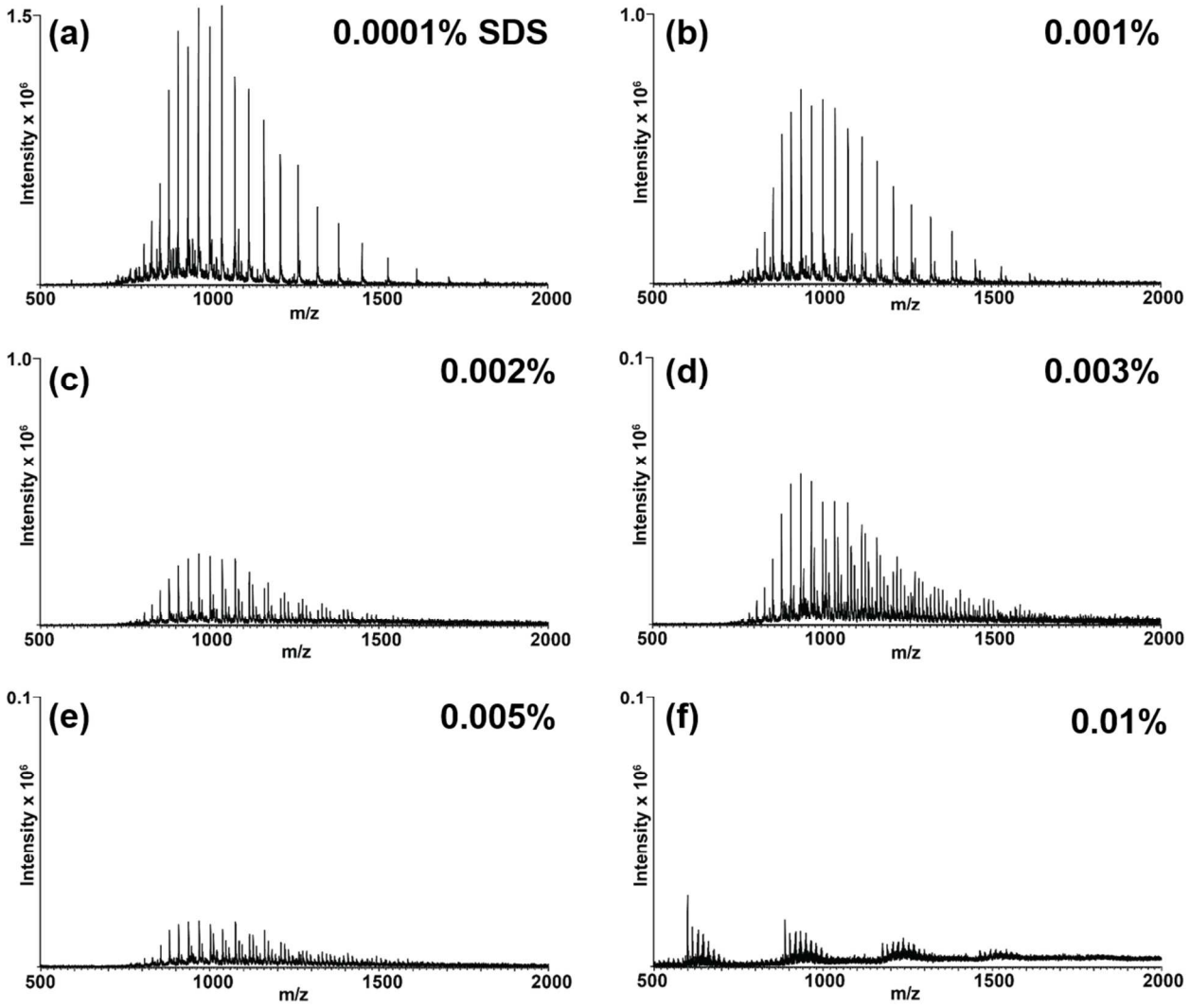
Supplementary Figure 1 and 2

Supplementary Table 1. The relative ratio of peak area for comparison between precipitation and the new matrix removal platform. The peak area from each protein standard obtained with the in-line device was set to 100%.

Protein	Precipitation/Removal device (Std. Dev.)
Ubiquitin	29% (8.4%)
Myoglobin	55% (1.4%)
Carbonic Anhydrase	10% (24.4%)
Transferrin	17% (49.5%)

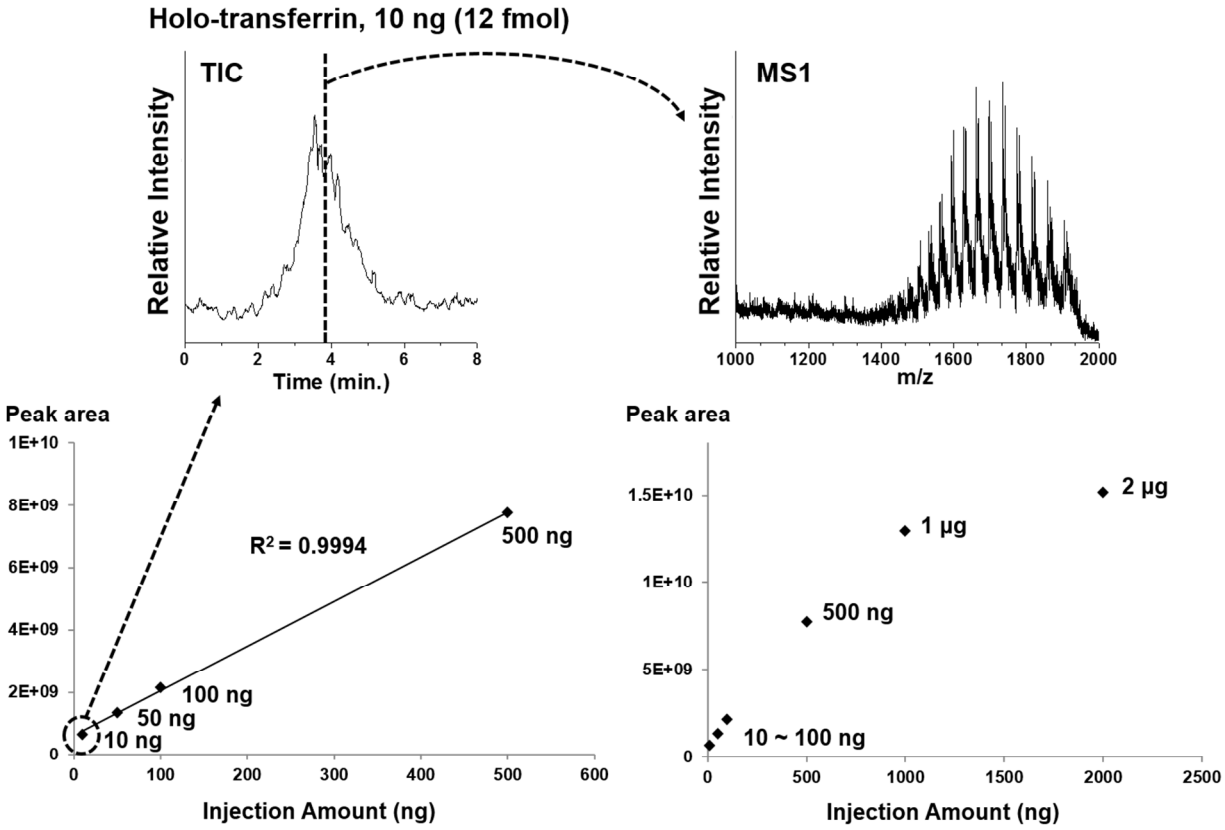
Supplementary Table 2. The mass accuracy of each histone subunit identified from high-resolution tandem mass spectrometry. All four subunits were observed within a 10 ppm mass tolerance. Molecular weight values are reported for the neutral, most abundant isotopic peak.

Histone subunit	Theoretical MW (Da)	Observed MW (Da)	Mass error (Da)	ppm	Representative E-value
H2A	14004.30	14004.38	-0.08	-5.7	4×10^{-11}
H2B	13774.95	13774.97	-0.02	-1.5	3×10^{-18}
H3	15272.89	15272.92	-0.03	-2.0	9×10^{-14}
H4	11236.15	11236.22	-0.07	-6.2	6×10^{-27}



Supplementary Figure 1. The mass spectra of carbonic anhydrase in solutions containing various concentrations of SDS. Carbonic anhydrase was diluted to 0.1 mg/mL (3.4 pmol/μL) in 0.1% formic acid and SDS aqueous solution then introduced directly without a removal device. The mass spectrum from 0.0001% (3.47 x 10^{-6} M) of SDS showed no significant adduct peaks or suppression of electrospray ionization in spectrum (a). Adduct peaks by bound SDS and decrease of signal was observed from 0.001% (3.47 x 10^{-5} M) of SDS and its effect was increased up to

0.005% in spectra (b)-(e). Above 0.01% (3.47 x 10^{-4} M) of SDS concentration, no proteins or SDS adduct peaks were observed and all peaks originated from SDS and sodiated cluster ions, $[(SDS)_n + Na]^+$ in spectrum (f).



Supplementary Figure 2. The calibration curve of transferrin standard obtained from the matrix removal device and a total ion chromatogram and selected mass spectrum at the center of peak. Good linearity was observed from 10 ng to 500 ng of material injected and the device was able to produce molecular weight information even at the 10 ng level of injection. All of experimental conditions were equal to the experimental conditions as described in the main text.