

Supplemental Materials

Enhancing Sensitivity of Liquid Chromatography/Mass Spectrometry of Peptides and Proteins Using Supercharging Agents

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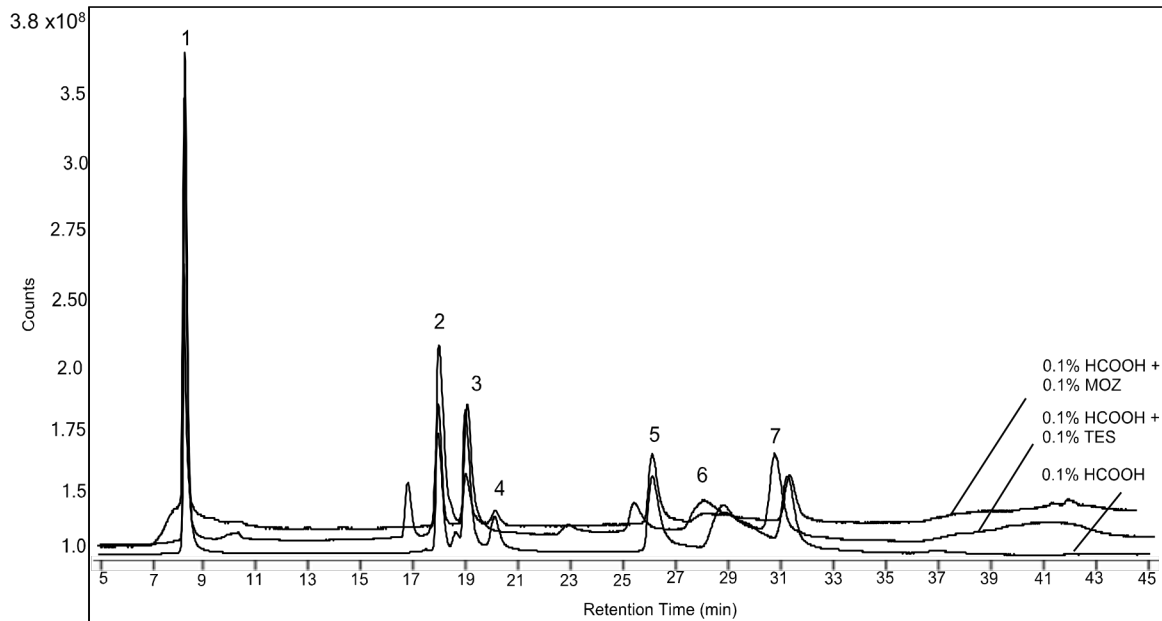
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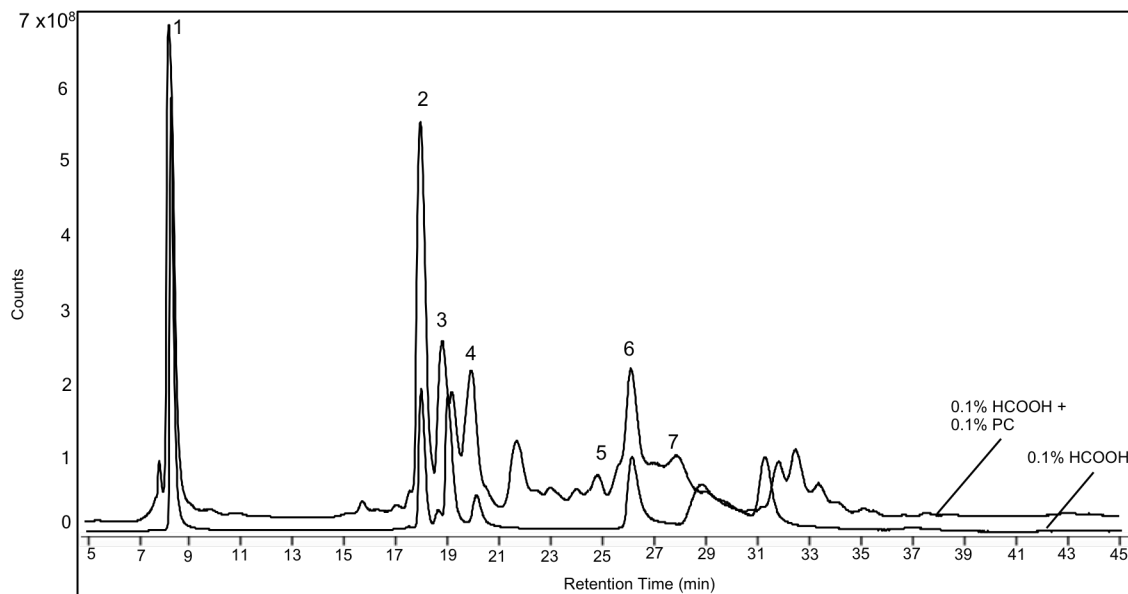
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Submitted to:
International Journal of Mass Spectrometry
November 2017

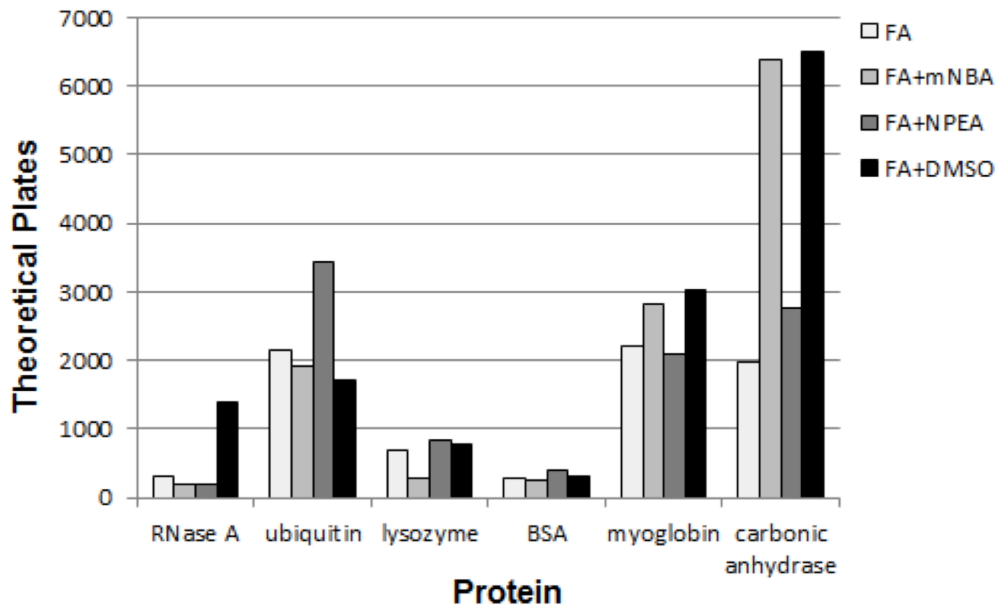
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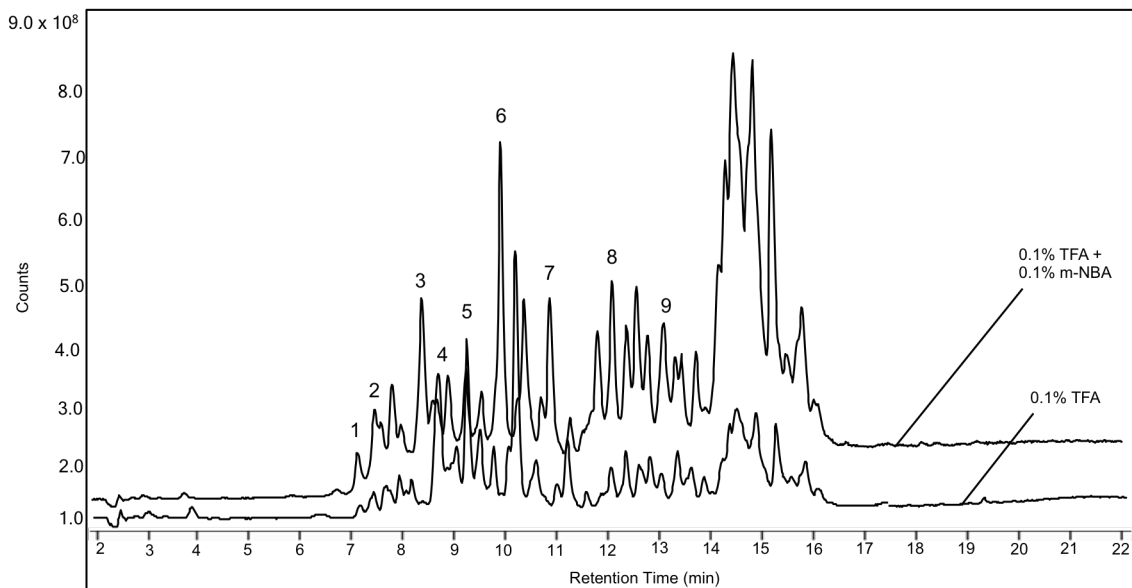
Supplemental Figure S1. LC/MS of protein mixture containing (1) 17 μM RNase A, (2) 13 μM ubiquitin, (3) 7.7 μM lysozyme, (4) 30 μM insulin, (5) 6.5 μM myoglobin, (6) 15 μM BSA, and (7) 7.6 μM carbonic anhydrase with 0.1% formic acid and 0.1% TES and 0.1% MOZ.



Supplemental Figure S2. LC/MS of protein mixture containing (1) 17 μM RNase A, (2) 13 μM ubiquitin, (3) 7.7 μM lysozyme, (4) 30 μM insulin, (5) 6.5 μM myoglobin, (6) 15 μM BSA, and (7) 7.6 μM carbonic anhydrase with 0.1% formic acid and with and without 0.1% PC.



Supplemental Figure S3. Number of theoretical plates for LC/MS of proteins with 0.1% formic acid and supercharging agents.



Supplemental Figure S4. LC/MS of BSA tryptic digest in 0.1% TFA with and without 0.1% *m*-NBA. Tryptic peptides: (1) ATEEQLK, (2) YICDNQDTISSK, (3) VPQVSTPTLVEVSR, (4) LFTFHADICTLPDTEK, (5) HLVDEPQNLIK, (6) ECCDKPLLEK, (7) YNGVFQECCQAEDK, (8) TVMENFVAFVDK, (9) QTALVELLK

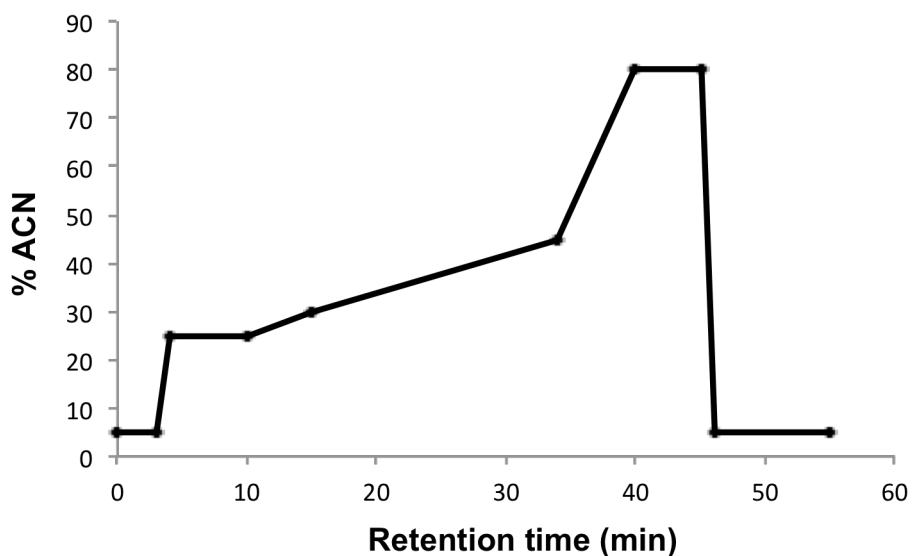
Retention time (min)

protein	TFA	+ <i>m</i> -NBA	+ 3-NPEA	+ TES	+ MOZ	+ PC
RNase A	18.20	18.53	18.32	17.06	18.20	16.33
Ubiquitin	24.29	24.82	24.48	23.47	24.42	22.37
Lysozyme	28.18	28.92	28.41	27.59	28.27	N/D
BSA	33.56	34.43	33.80	33.00	33.68	31.05
Myoglobin	37.36	37.84	37.66	36.83	37.41	35.24
Carbonic Anhydrase	38.45	38.63	38.55	38.34	38.46	39.18

Retention time (min)

Protein	HCOOH	+ <i>m</i> -NBA	+ 3-NPEA	+ TES	+ MOZ	+ PC
RNase A	7.95	7.85	7.68	7.84	7.89	7.84
Ubiquitin	17.99	18.01	17.62	16.74	18.03	17.81
Lysozyme	20.10	20.26	19.80	19.11	20.30	19.81
BSA	28.12	28.47	28.12	25.76	26.59	26.09
Myoglobin	26.65	26.93	26.54	28.61	28.78	27.89
Carbonic Anhydrase	32.26	32.75	32.03	31.33	32.11	32.62

Supplemental Figure 5. Protein retention times with TFA (top) or formic acid (bottom) as the mobile phase additive.



Supplemental Figure 6. LC gradient showing % ACN change as a function of time.

Protein	+ mNBA	+ NPEA	+ TES	+ MOZ	+ PC
RNase A	2.5	7	1.3	no change	1.2
Ubiquitin	3	6	1.25	no change	2.5
Lysozyme	5	8	1.7	no change	1.3
BSA	no change	no change	no change	no change	1.8
Myoglobin	no change	no change	no change	no change	1.4
Carbonic Anhydrase	no change	3	no change	no change	no change

Supplemental Figure 7. Signal enhancement (fold increase) with addition of supercharging agents to 0.1% FA ion pairing agent.