Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information S1

Experimental Methods

Materials

All the water used in our experiments was from Milli-Q system (Millipore Inc., Milford, MA). Formic acid (FA) was purchased from Fluka (Buches, Germany) and acetonitrile (ACN, HPLC grade) was from Merck (Darmstadt, Germany). Acetic acid (AA), propionic acid (PA), triflouroacetic acid (TFA), Dithiothreitol (DTT), iodoacetamide (IAA), Triton X-100, protease cocktail, Myoglobin (horse heart), and lysozyme (chicken egg white) were obtained from Sigma (St. Louis, MO, USA). Cytochrome c (Cyt c) and RNase were obtained from Melonepharma (China). Fused silica capillaries with 75 μm i.d. were purchased from Polymicro Technologies.

LC-MS analysis

All of the experiments were performed by LTQ Orbitrap XL (Thermo, San Jose, CA) coupled with Accela 600 HPLC system (Thermo, San Jose, CA), and the enclosed ionization source is Coanda Effect Electrospray Ionization (CEESI, Haochuang Biotech, Hangzhou, China). As the inside of MS is high vacuum, the airflow from the acid solution can carry the gaseous acid into the enclosed ESI source (Figure S1).

Samples were automatically loaded onto the trap column (3 cm × 200 μm i.d.) packed with Jupiter C5 (5μm 300Å), followed with binary gradient separation by using the analytical column (12 cm × 75 μm i.d.) packed with Jupiter C5 (5μm 300Å). Mobile phases were 0.05% (v/v) TFA or 0.1% (v/v) FA in water (buffer A), and 0.05% (v/v) TFA or 0.1% (v/v) FA in ACN (buffer B). The flow rate was 300 nL/min for LC-MS analysis.

The MS parameters were set as: ion spray voltage, 1.9 kV; MS range, 500–2000 m/z; resolution, 60 000 at m/z 400; the 5 most intense precursors were selected for subsequent

fragmentation (CID) in a data-dependent acquisition (DDA) mode within the ion trap. The normalized collision energy for fragmentation was set as 35%. The dynamic exclusion settings: repeat count 1, repeat duration 120 s, and exclusion duration 240 s. The AGC targets were set as 100 000 and 5 000 for full MS scan (MS1) and tandem MS scan (MS2) separately, and the maximum injection time is 100 ms.

Standard intact protein sample analysis

The mixture of 0.6 μg standard proteins (RNase, Cyt c, lysozyme and myoglobin) was automatically loaded onto the trap column for LC-MS analysis. The binary separation gradient from 20% to 50% (v/v) buffer B was performed in 40 min.

Replicated analyses were performed three times at least for the following investigations, 0.1% FA and 0.05% TFA containing mobile phase without acid vapor assistance in electrospray, and 0.05% TFA containing mobile phase with FA, AA and PA vapor assistance in electrospray, respectively.

***E. coli* intact protein sample analysis**

Escherichia coli (*E. coli*, strain K12) was cultured in liquid Luria broth (LB), which containing 10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl (pH 7.4) for 6 h at 37℃. Then the mediums were centrifuged at 4000 × g for 6 min. The collected precipitation was washed three times by PBS to remove the residual medium. The *E. coli* cells were lysed in a buffer containing 50 mM Tris-HCl (pH 7.4), 8 M urea, 65 mM DTT, 1% protease cocktail (v/v), 1% Triton X-100 (v/v), 1 mM EDTA, 1 mM EDGA, then sonicated for 400 W × 120 s and centrifuged at 25 000 g for 30 min. The supernatant was precipitated with 5 volumes of ice-cold acetone/ethanol/acetic acid (v/v/v = 50/50/0.1) at −20 °C overnight. The protein precipitant was centrifuged at 15 000

g for 30 min, and the pellet was washed separately with acetone and 75% ethanol. After lyophilization, the proteins were re-dissolved into buffer containing 100 mM NH_4HCO_3 (pH = 8.2) and 8 M urea. The protein concentration was determined by BCA protein assay. The protein sample was reduced by 10 mM DTT at 60 ℃ for 1 h and then alkylated by 20 mM IAA in the dark at room temperature for 40 min.

The desalted samples of 0.7 μg *E. coli* proteins were analyzed at least three times at different investigating conditions as follow, 0.1% FA and 0.05% TFA containing mobile phase without acid vapor assistance in electrospray, and 0.1% FA and 0.05% TFA containing mobile phase with PA vapor assistance in electrospray, respectively. The separation gradient from 20% to 50% (v/v) ACN was performed in 90 min.

All the .raw data files were searched by ProSight PC 3.0 against an *E. coli* (strain K12) database from Uniprot. Iodoacetamide cysteine was set as a fixed modification. The mass tolerance for precursor search window was 1 kDa, and fragment tolerance was 20 ppm. Proteins with intact mass above 5 000 were chosen for the following analyses.

The consumption rates of the doping acids

We used electronic balance to calculate the weight reduction of liquid acid solution after doping acid vapor in 20 min. Then, the results were translated to volume/time through acid solution density.

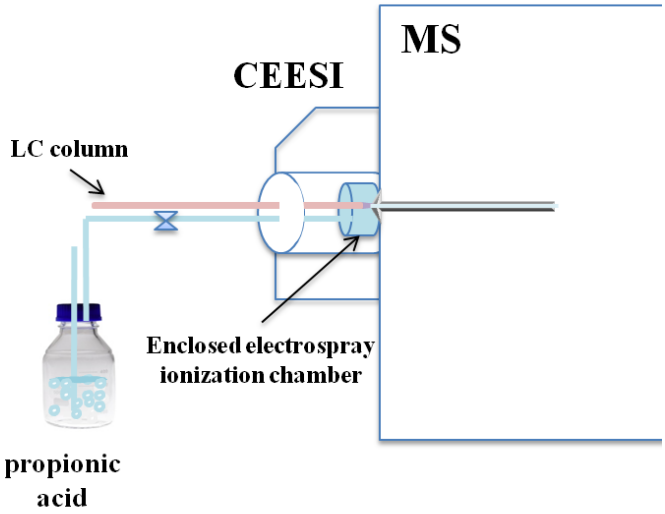


Figure S1. The schematic diagram of the acid vapor assisted electrospray within the enclosed ESI chamber of CEESI ion source (Haochuang Biotech., Hangzhou, China). The airflow from the acid solution carries the gaseous acid vapor into the enclosed ESI chamber during the electrospray process due to the MS is in high vacuum (the light blue zone), which means the electrospray ionization chamber is filled with high concentration of acid vapor. In our experiment, the rate of FA, AA and PA solvent gasifying and doping rates was 4.60 ± 0.06 (n=3) mL (liquid) /h, 2.64 ± 0.02 (n=3) mL (liquid) /h and 0.92 ± 0.01 (n=3) mL (liquid) /h, respectively, due to their different volatility.

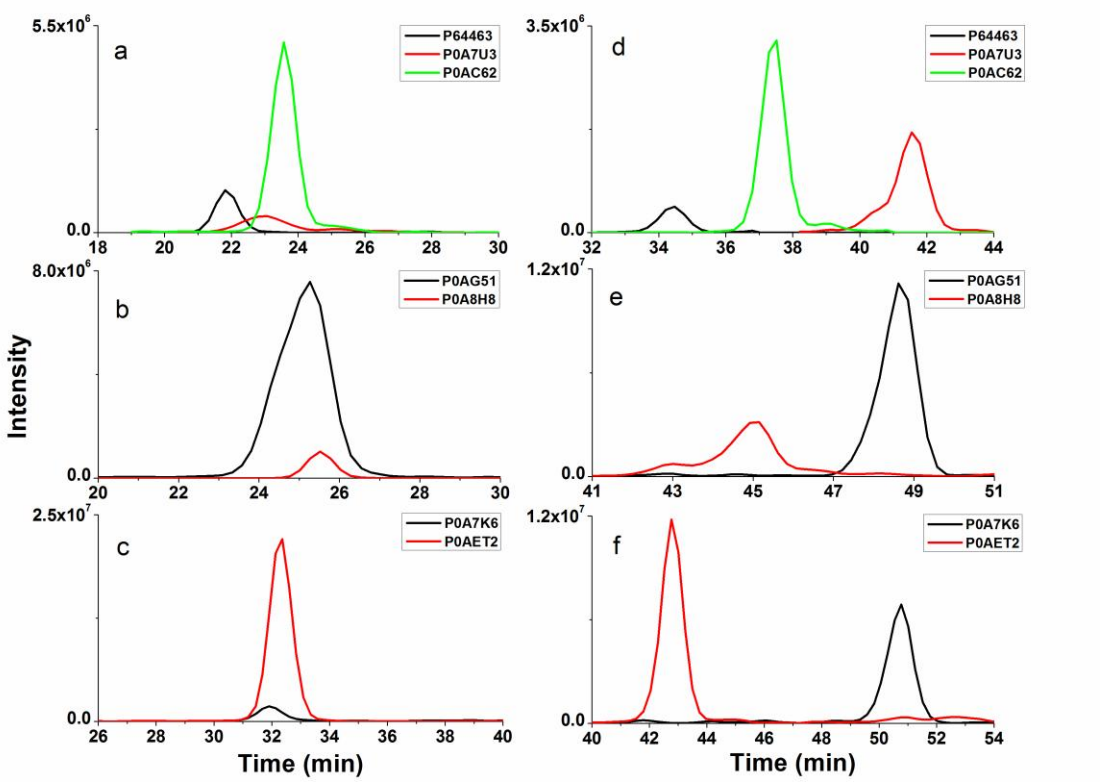


Figure S2. The extracted ion chromatograms (XICs) of three groups of adjacent intact proteins in LC-MS analyses with FA containing mobile phase (a, b, c) and TFA containing mobile phase with PA vapor assisted electrospray (d, e, f). Proteins were baseline separation by using TFA additive, in contrast to seriously co-eluting by using FA additive in the mobile phase.

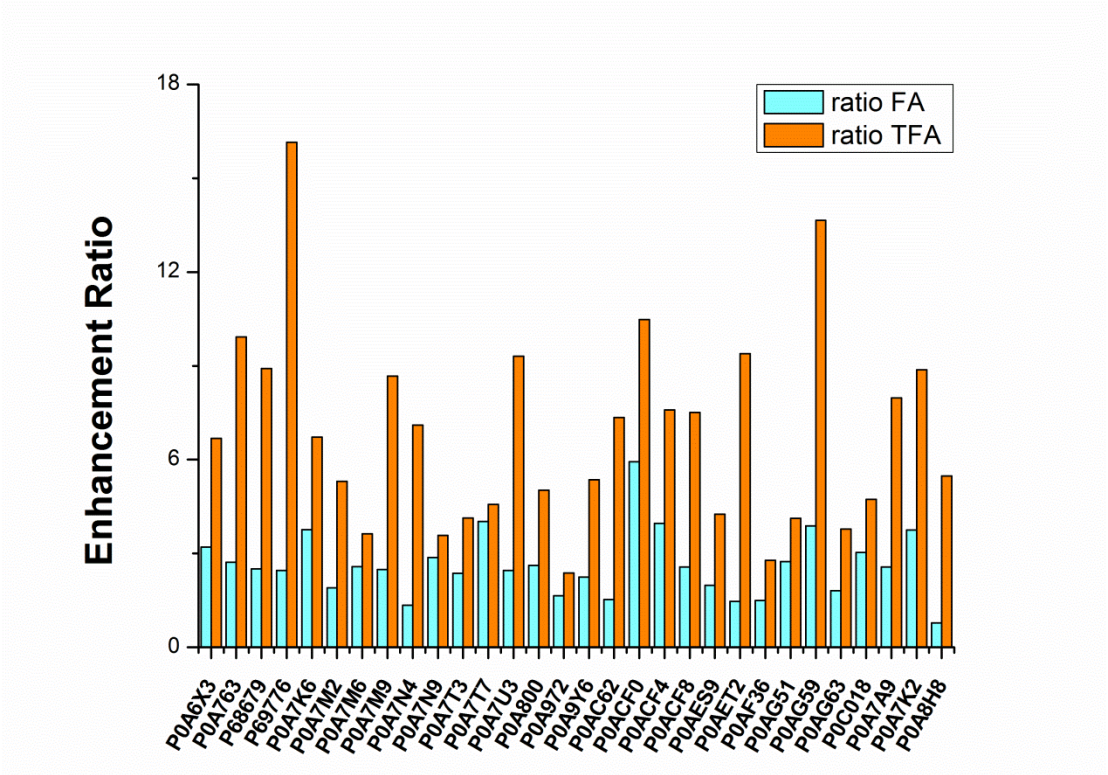


Figure S3. Intact proteins signal enhancement ratios by PA vapor doping in LC-MS analyses with FA and TFA containing mobile phases. “Ratio FA” and “Ratio TFA” means the signal intensity enhancement ratio by PA vapor doping in LC-MS analysis of intact proteins with FA and TFA containing mobile phase, respectively.

Table S1. The chromatographic resolution of four intact proteins at different condition (n=3) .

	RNase-Cyt C	Cyt c-Lysozyme	Lysozyme-Myoglobin
FA+air	1.43±0.16	0.26±0.09	2.27±0.09
TFA+air	2.83±0.06	1.16±0.05	3.22±0.05
TFA+FA	3.03±0.07	1.24±0.10	3.32±0.18
TFA+AA	3.09±0.14	1.32±0.03	3.38±0.07
TFA+PA	3.19±0.06	1.35±0.01	3.66±0.31

"FA+air" means FA additive in mobile phase without acidic vapor assisted electrospray. "TFA+air" "TFA+FA" "TFA+AA" and "TFA+PA" means TFA in mobile phase with air, FA, AA and PA vapors assisted electrospray, respectively.

Table S2. The average peak width at half peak height ($W_{0.5}$) of four standard proteins obtained at different conditions (n=3) .

	RNase	Cyt C	Lysozyme	Myoglobin
FA+air	1.07±0.11	1.23±0.21	2.90±0.07	1.64±0.24
TFA+air	1.06±0.09	0.93±0.06	1.01±0.06	0.99±0.04
TFA+FA	0.98±0.04	0.98±0.05	0.95±0.01	1.01±0.07
TFA+AA	0.95±0.01	0.99±0.05	0.96±0.04	1.04±0.05
TFA+PA	0.92±0.07	0.92±0.03	0.92±0.03	1.00±0.01

Table S3. The average peak intensity of four standard proteins obtained at different conditions (n=3) .

	RNase	Cyt C	Lysozyme	Myoglobin
FA+air	5.99E+07	8.37E+07	1.51E+08	4.06E+07
TFA+air	1.28E+07	4.34E+07	2.88E+07	2.49E+07
TFA+FA	6.49E+07	8.98E+07	1.21E+08	4.72E+07
TFA+AA	1.38E+08	2.33E+08	3.16E+08	1.50E+08
TFA+PA	1.06E+08	5.06E+08	2.77E+08	3.08E+08

Table S4. The average abundance weighted charge (q_{ave}) of four standard proteins obtained at different conditions (n=3) .

	RNase	Cyt c	Lysozyme	Myoglobin
FA+air	9.22	11.07	10.20	14.38
TFA+air	8.36	*	8.88	*
TFA+FA	8.16	12.15	8.85	14.67
TFA+AA	8.18	11.55	8.82	15.29
TFA+PA	8.10	9.57	8.55	12.66

q_{ave} was calculated via the following equation where *N* refers to the number of observed analyte charge states in a mass spectrum, q_i is the charge state of the *i*th state, and W_i is the intensity of the *i*th charge state[1].

$$q_{ave} = \frac{\sum_{i}^{N} q_i W_i}{\sum_{i}^{N} W_i}$$

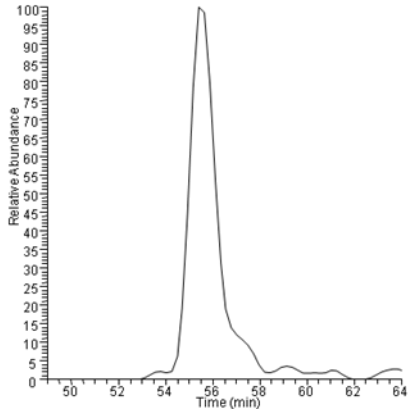
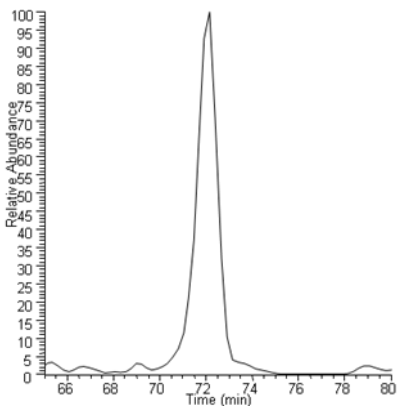
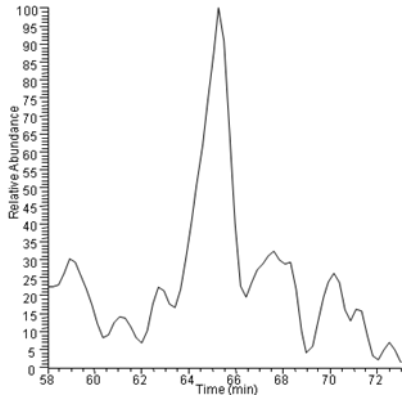
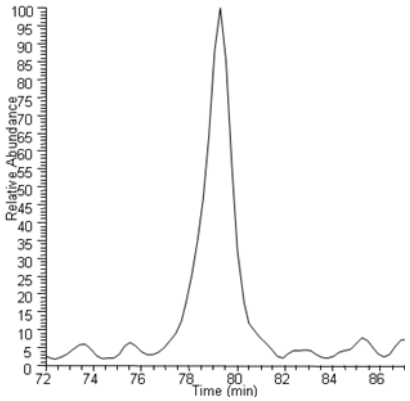
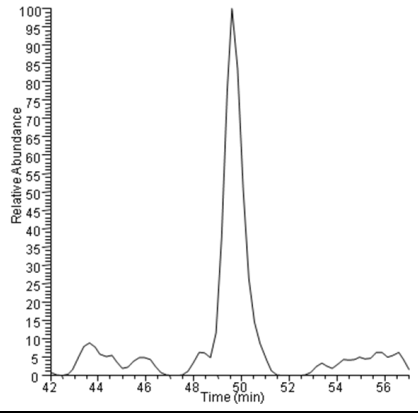
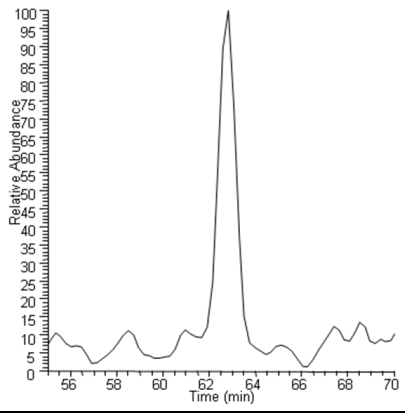
"*" refers to bimodal distribution in charge state distribution.

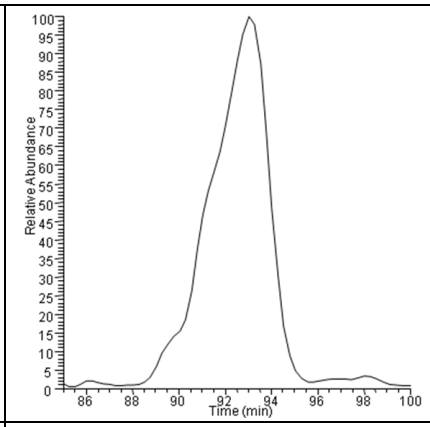
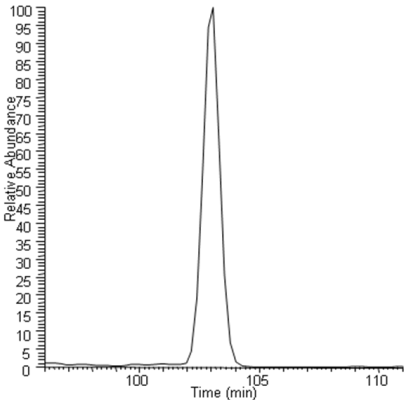
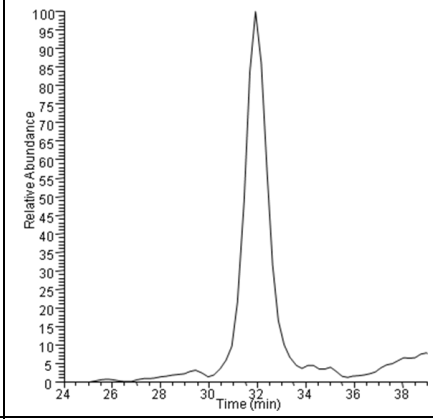
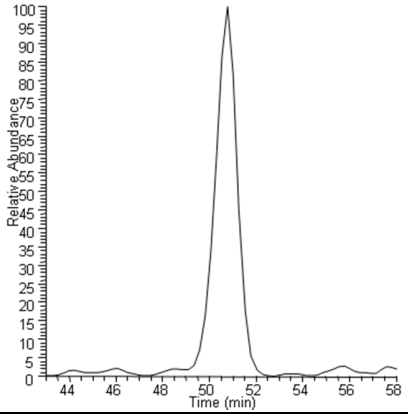
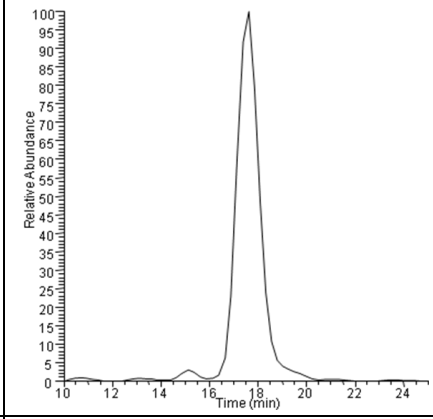
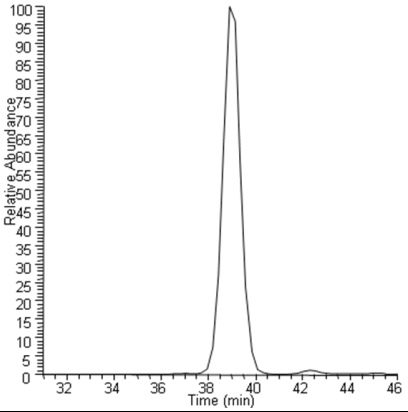
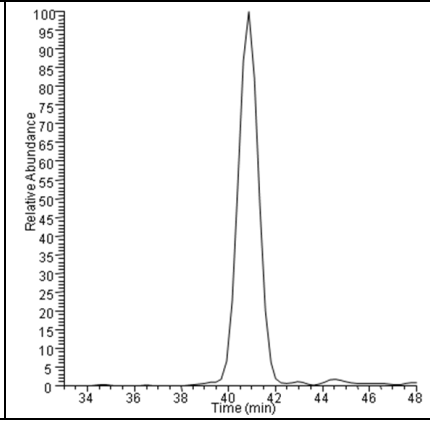
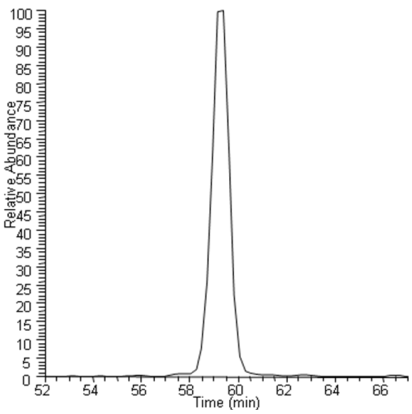
Table S5. The intensity and signal enhancement ratio of extracted ion chromatograms (XICs) of 44 randomly selected protein individuals (n=3).

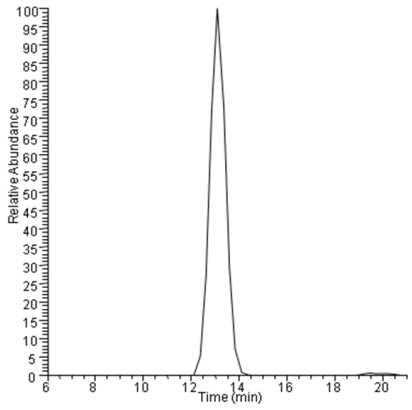
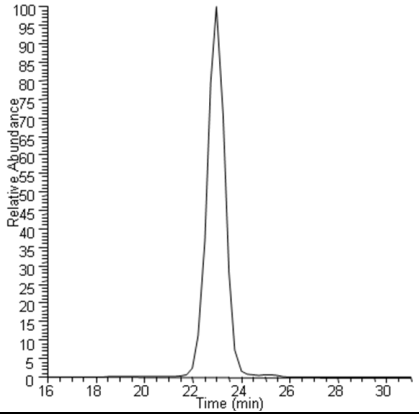
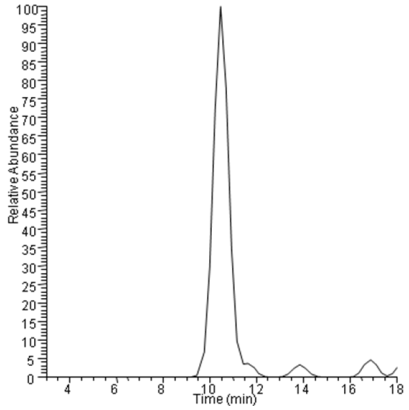
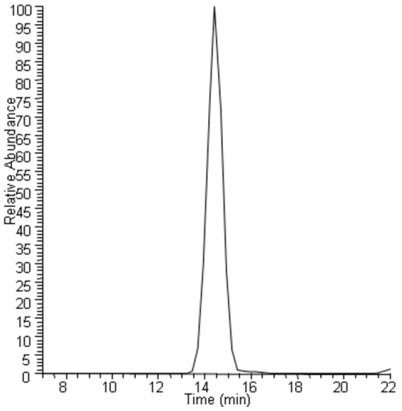
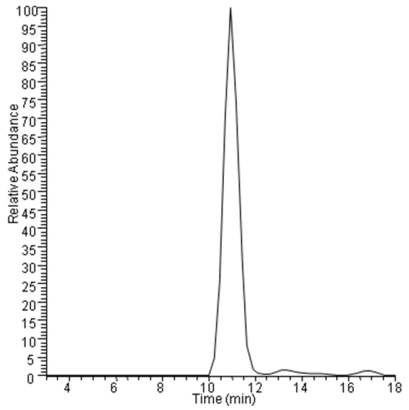
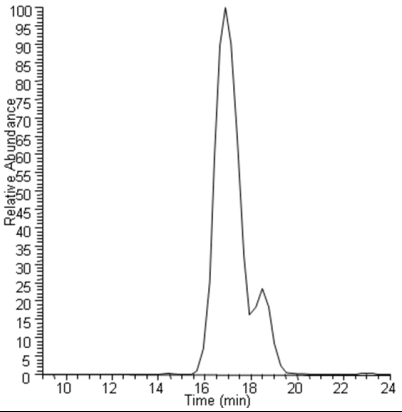
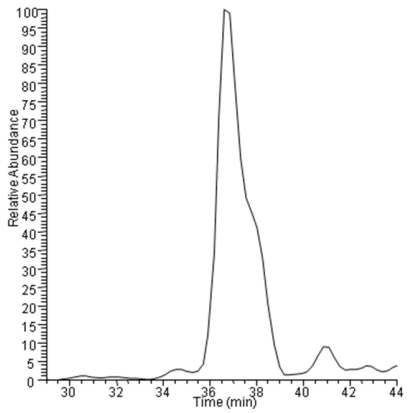
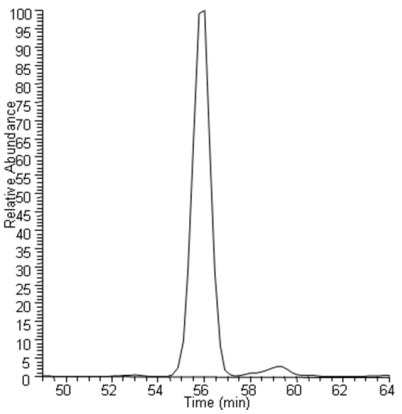
Accession Number	intensity TFA+air	intensity TFA+PA	ratio
P0A6X3	1.71E+06	1.14E+07	7
P0A763	6.06E+05	6.01E+06	10
P0A7A9	5.25E+05	4.19E+06	8
P0A7K2	3.84E+06	3.40E+07	9
P0A7K6	1.72E+06	1.16E+07	7
P0A7M2	4.43E+06	2.35E+07	5
P0A7M6	8.95E+06	3.25E+07	4
P0A7M9	2.18E+06	1.89E+07	9
P0A7N4	3.17E+06	2.26E+07	7
P0A7N9	2.35E+06	8.42E+06	4
P0A7T3	5.03E+06	2.08E+07	4
P0A7T7	3.32E+06	1.52E+07	5
P0A7U3	4.33E+05	4.03E+06	9
P0A800	2.40E+06	1.20E+07	5
P0A972	4.03E+06	9.60E+06	2
P0A9Y6	2.36E+06	1.27E+07	5
P0AC62	1.25E+06	9.19E+06	7
P0ACF0	4.34E+06	4.55E+07	10
P0ACF4	2.33E+06	1.77E+07	8
P0ACF8	1.25E+06	9.41E+06	8
P0AES9	2.50E+07	1.06E+08	4
P0AET2	2.66E+06	2.49E+07	9
P0AF36	4.26E+06	1.18E+07	3
P0AG51	4.61E+06	1.90E+07	4
P0AG59	1.33E+06	1.82E+07	14
P0AG63	3.29E+06	1.24E+07	4
P0C018	2.70E+06	1.28E+07	5
P68679	1.06E+06	9.44E+06	9
P69776	1.33E+06	2.15E+07	16
P69428	2.94E+05	2.68E+06	9
P61175	7.96E+05	6.31E+06	8
P60624	2.96E+05	4.24E+06	14
P0AG44	1.85E+06	8.03E+06	4
P0AFX0	8.71E+05	4.09E+06	5
P0ADZ4	1.81E+06	8.51E+06	5
P0ADZ0	6.85E+05	5.44E+06	8
P0AB61	6.01E+05	3.09E+06	5
P0AA04	5.99E+05	4.37E+06	7
P0AB55	9.18E+05	4.45E+06	5

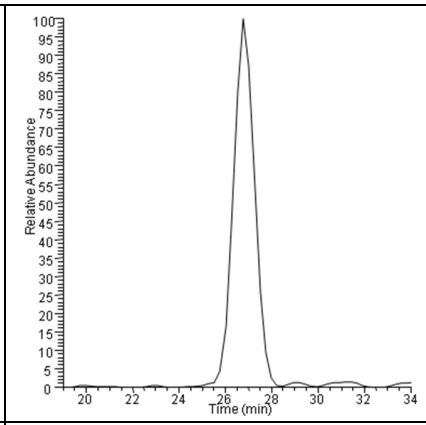
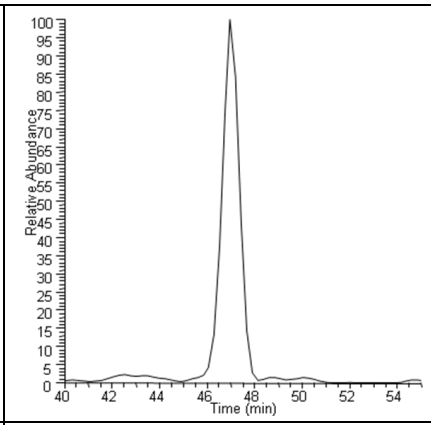
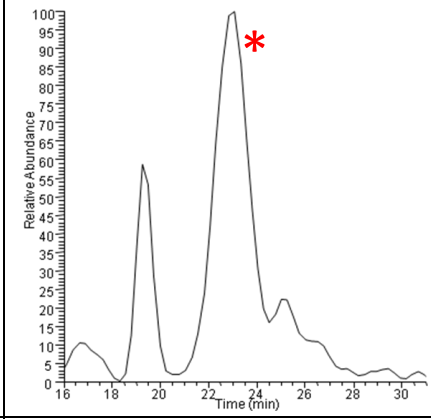
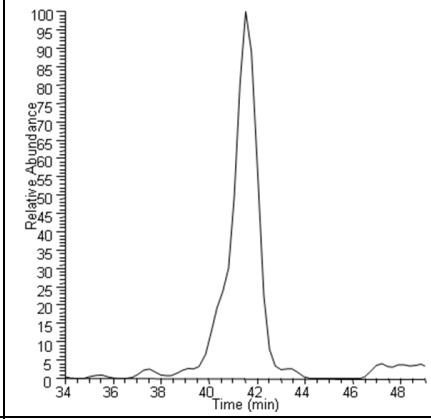
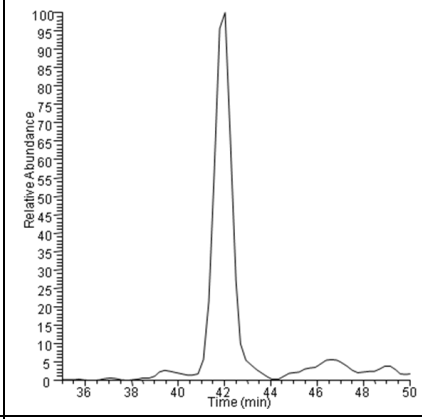
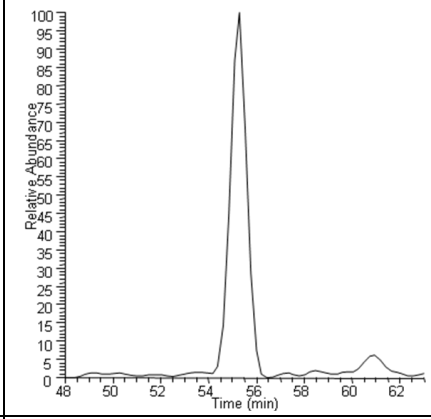
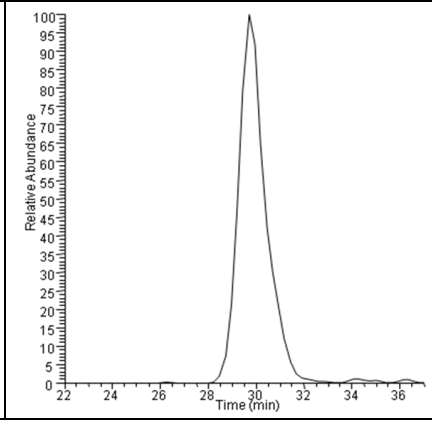
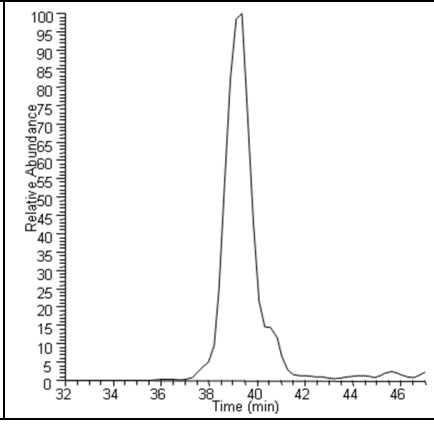
P0A8P3	3.42E+05	3.25E+06	10
P0A7X3	6.43E+05	8.44E+06	13
P0A7R9	7.04E+05	1.15E+07	16
P0A7J3	5.82E+05	4.38E+06	8
P0A8H8	2.30E+05	1.26E+06	5

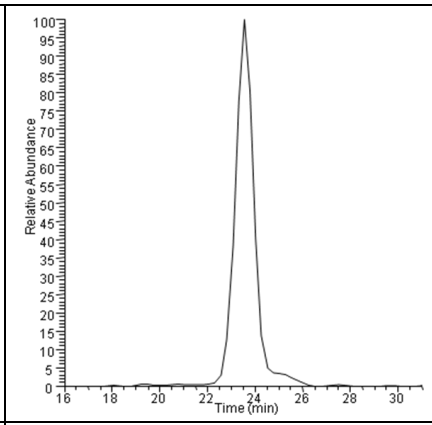
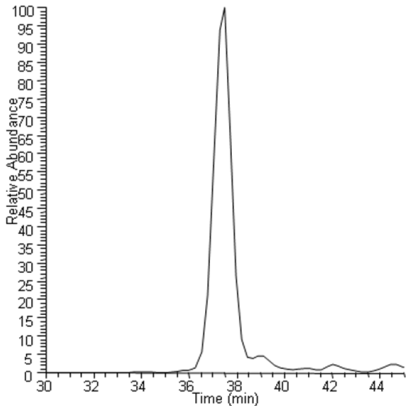
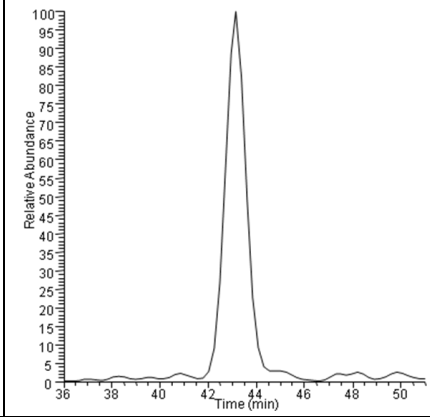
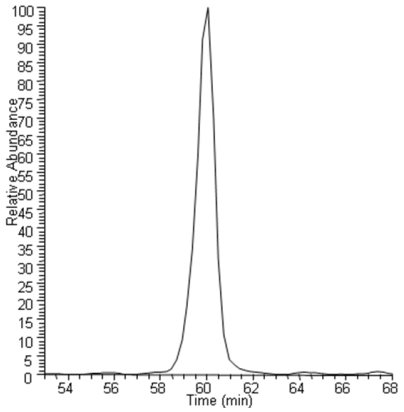
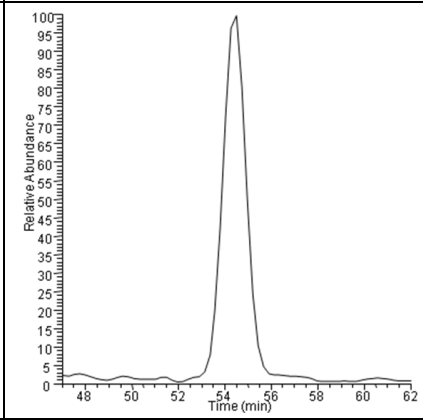
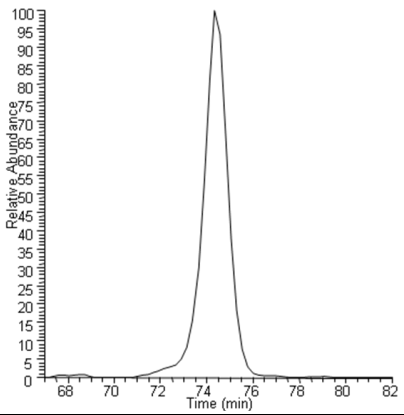
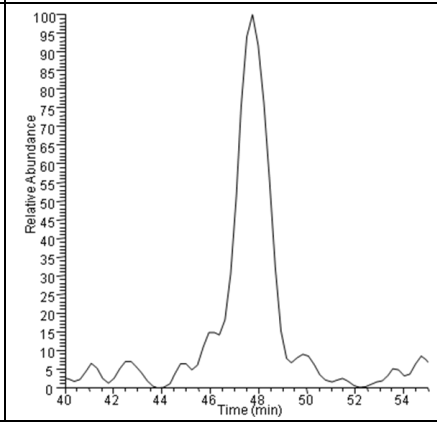
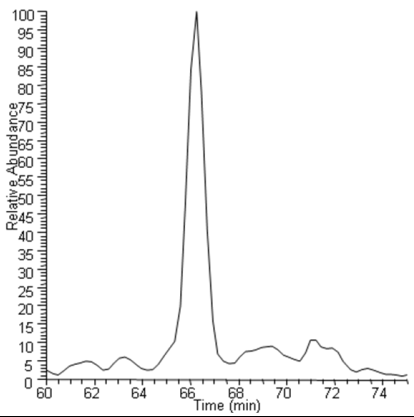
Table S6. The average half-peak width ($W_{0.5}$) and peak shape of 36 intact proteins extracted from LC-MS analyses of *E. coli* protein sample by using FA containing mobile phase and TFA containing mobile phase with PA vapor assisted electrospray (n=3).

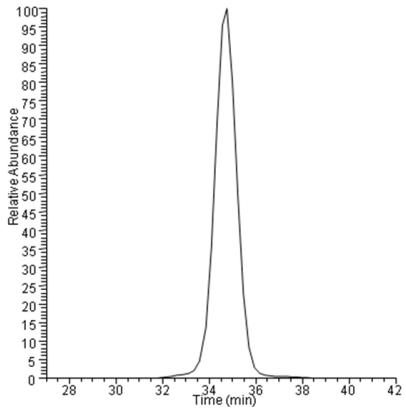
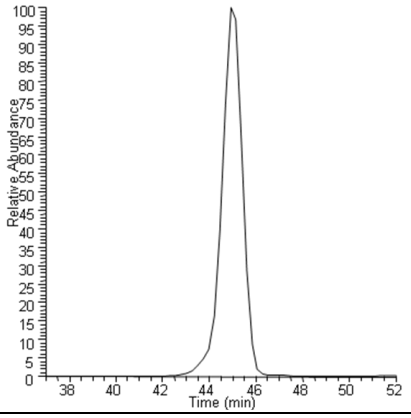
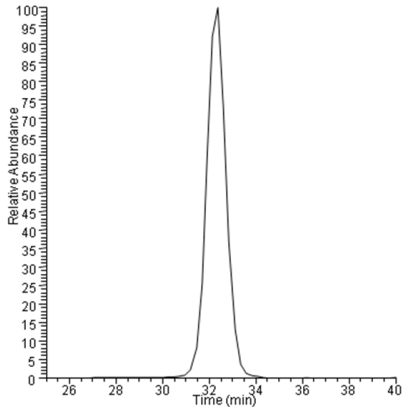
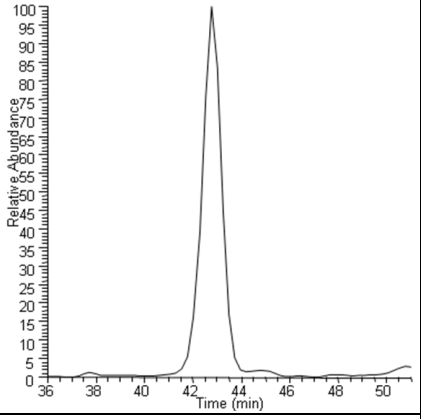
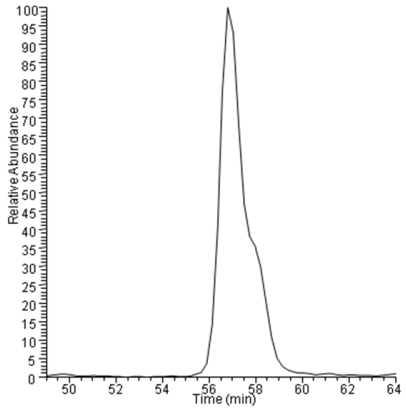
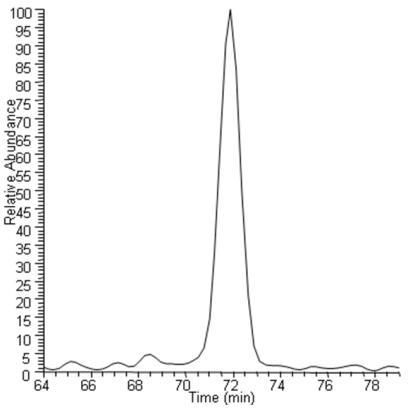
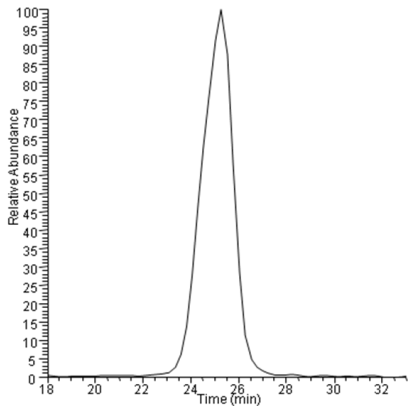
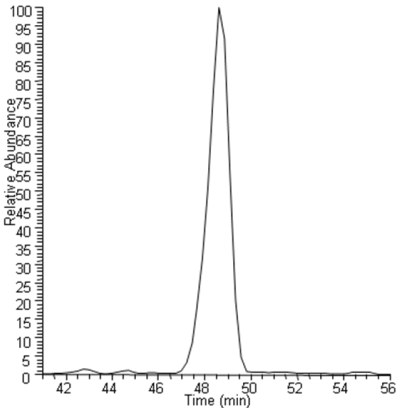
Protein	FA+air		TFA+PA		Mark
	Peak Shape	$W_{0.5}$	Peak Shape	$W_{0.5}$	
P0A6X3		1.27 ± 0.09		0.99 ± 0.04	○
P0A763		1.55 ± 0.08		1.16 ± 0.23	○
P0A7A9		0.94 ± 0.07		0.87 ± 0.04	○

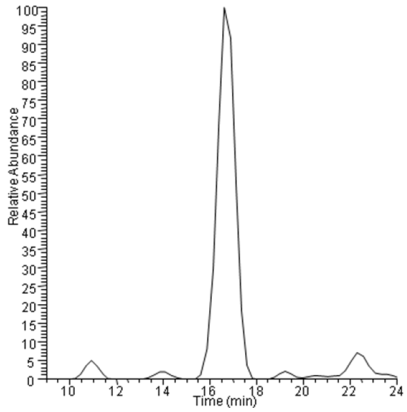
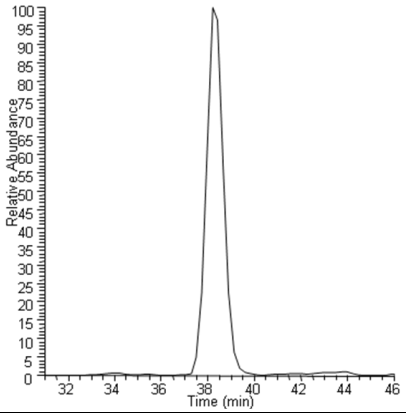
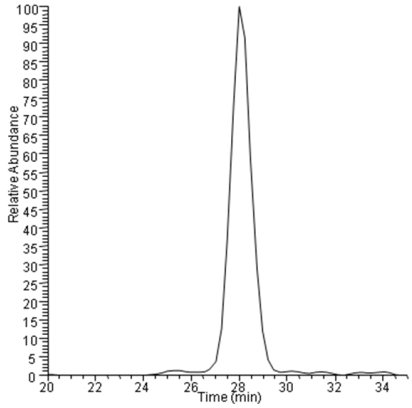
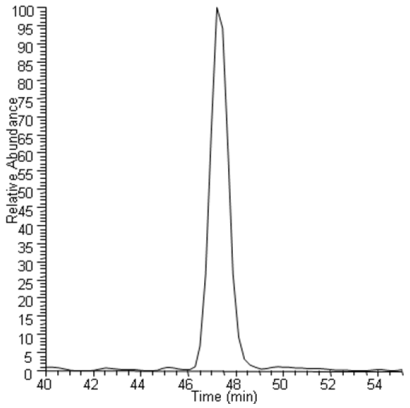
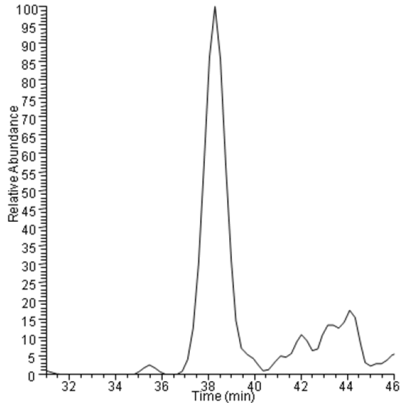
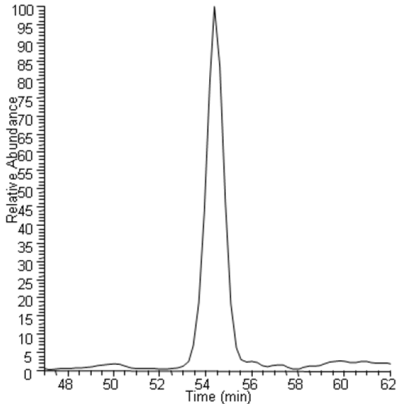
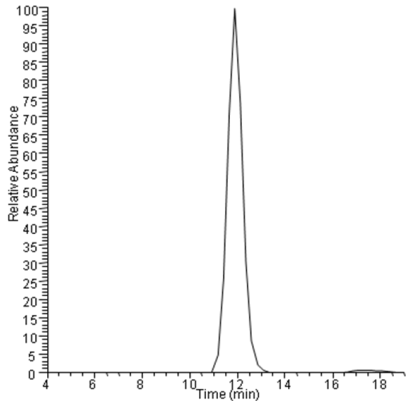
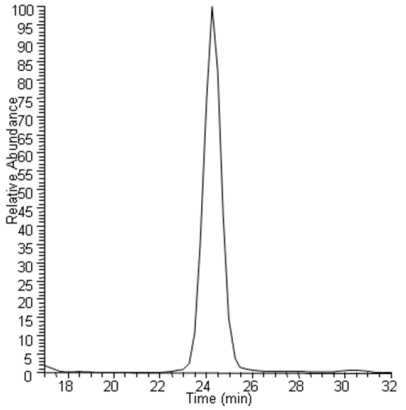
P0A7K2		2.50 ± 0.52		0.72 ± 0.04	○
P0A7K6		1.11 ± 0.13		1.01 ± 0.00	○
P0A7M2		0.97 ± 0.04		0.85 ± 0.01	○
P0A7M6		0.98 ± 0.02		0.83 ± 0.04	○

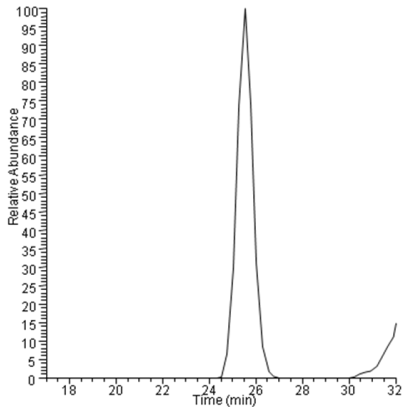
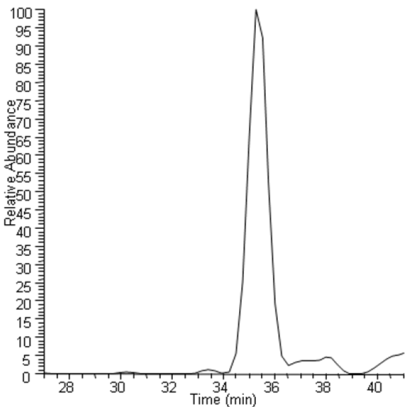
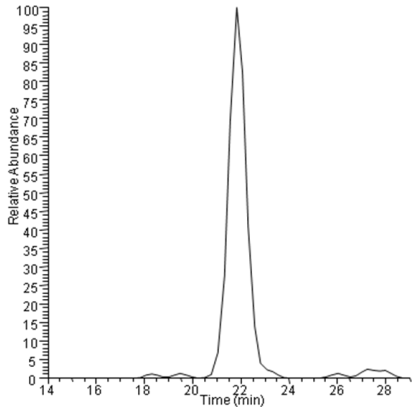
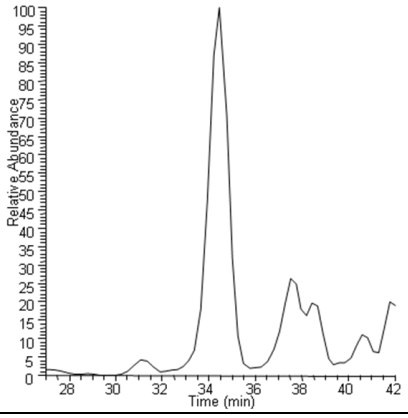
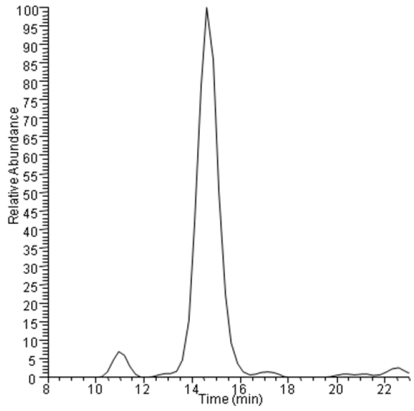
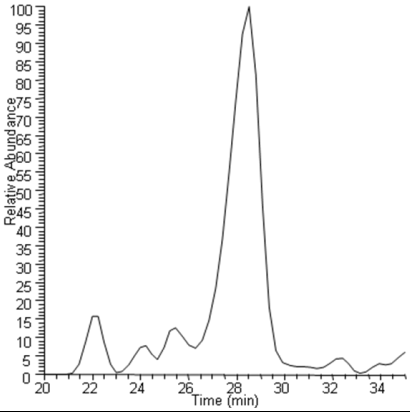
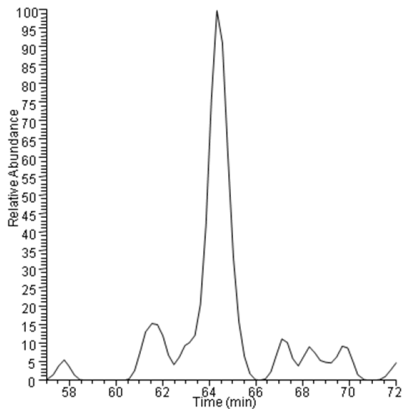
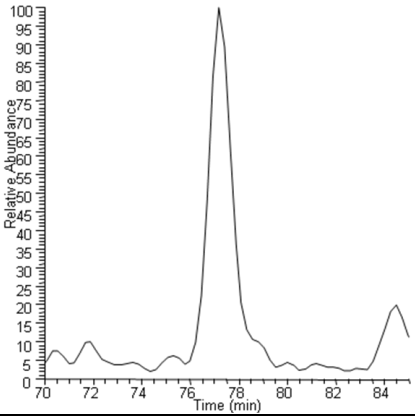
P0A7M9		0.74 ± 0.02		0.81 ± 0.02	
P0A7N4		0.74 ± 0.02		0.73 ± 0.01	○
P0A7N9		0.74 ± 0.01		1.22 ± 0.09	
P0A7T3		1.22 ± 0.09		0.86 ± 0.06	○

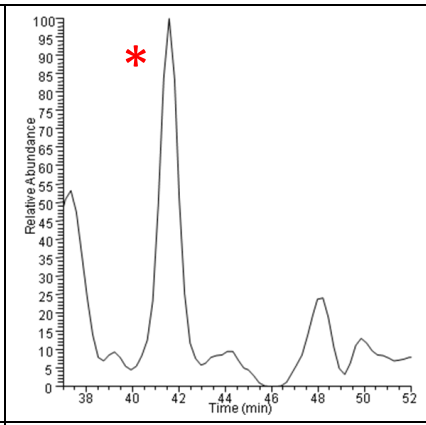
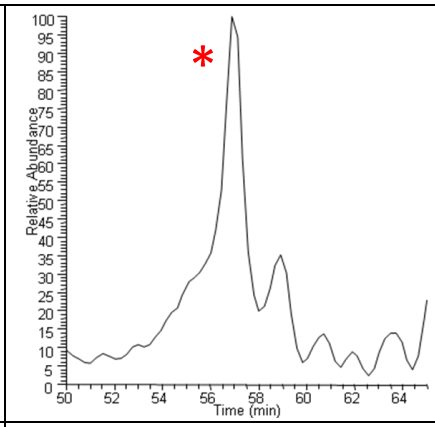
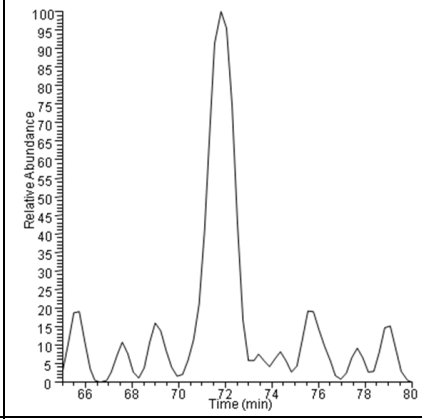
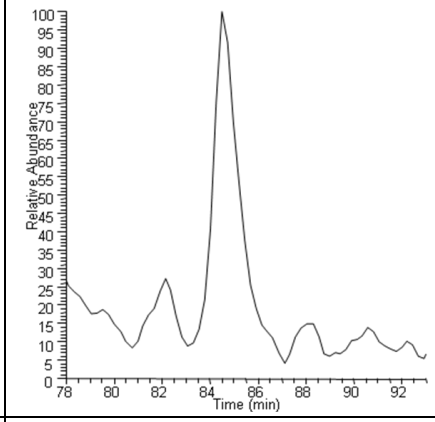
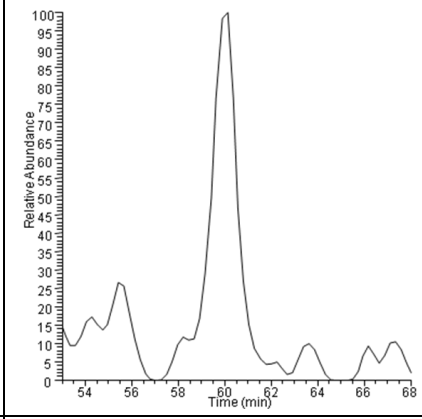
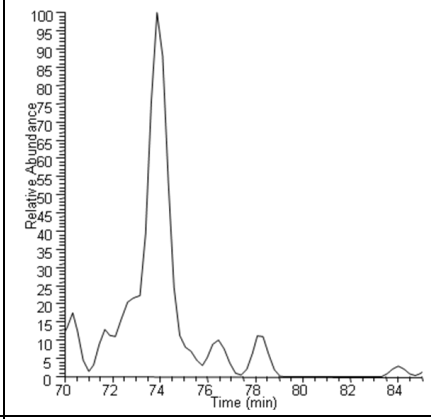
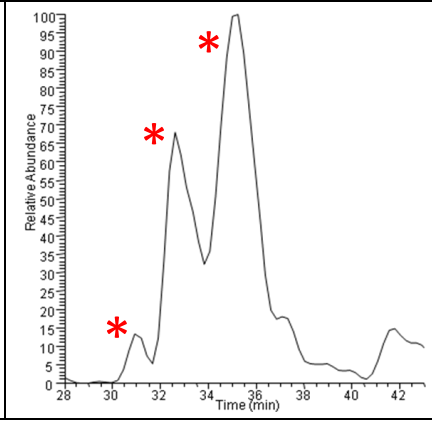
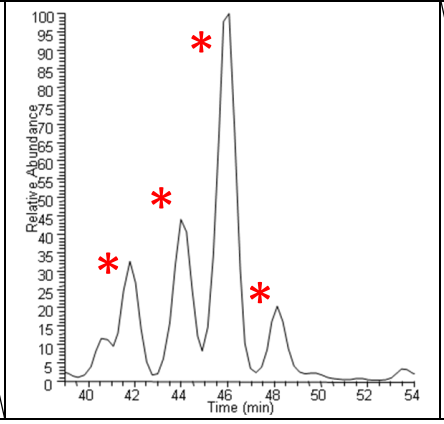
P0A7T7		1.06 ± 0.08		0.82 ± 0.02	○
P0A7U3		1.43 ± 0.17		0.99 ± 0.03	○
P0A800		0.83 ± 0.01		0.80 ± 0.01	○
P0A9Y6		1.11 ± 0.03		1.17 ± 0.07	

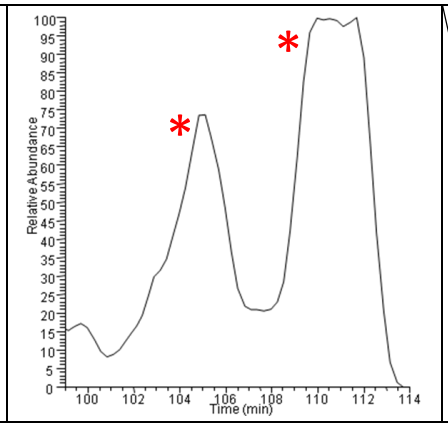
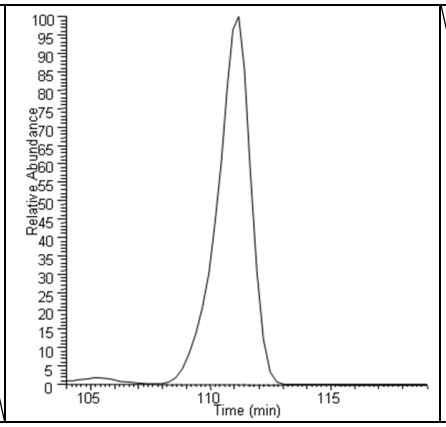
P0AC62		0.88 ± 0.08		0.80 ± 0.04	○
P0ACF0		0.94 ± 0.04		0.93 ± 0.07	○
P0ACF4		1.11 ± 0.05		1.20 ± 0.10	
P0ACF8		1.27 ± 0.23		0.83 ± 0.04	○

P0AES9		1.08 ± 0.06		0.86 ± 0.08	○
P0AET2		0.93 ± 0.04		0.89 ± 0.03	○
P0AF36		1.18 ± 0.09		1.01 ± 0.10	○
P0AG51		1.64 ± 0.37		1.07 ± 0.12	○

P0AG59		0.90 ± 0.03		0.78 ± 0.04	○
P0AG63		1.00 ± 0.05		0.88 ± 0.04	○
P0C018		0.99 ± 0.07		0.86 ± 0.02	○
P68679		0.77 ± 0.08		0.85 ± 0.02	

P0A8H8		0.83 ± 0.04		1.03 ± 0.2	
P64463		0.86 ± 0.03		0.90 ± 0.03	
P0AFW2		0.94 ± 0.09		1.61 ± 0.41	
P0AF93		1.02 ± 0.06		1.00 ± 0.05	○

P0AEU7		0.92 ± 0.02		0.90 ± 0.13	○
P0AED0		1.15 ± 0.12		1.30 ± 0.14	
P0A8J4		1.00 ± 0.14		0.90 ± 0.07	○
P0A972					

P69776				

Mark means that it marks "○" if $W_{0.5}$ in "TFA+PA" is narrower than "FA+air".

"*" refers to the relevant peak of the extracted protein when multiple peaks come up.

Table S7. The properties of 36 intact proteins extracted from LC-MS analyses of *E. coli* protein sample by using FA containing mobile phase and TFA containing mobile phase with PA vapor assisted electrospray (n=3)*.

	Molecular Weigh	Hydropathy**	pI	Mark***
P0A6X3	11159.58	-0.51	6.97	○
P0A763	15453.79	-0.08	5.54	○
P0A7A9	19691.03	-0.27	5.03	○
P0A7K2	12287.51	0.30	4.60	○
P0A7K6	13125.19	-0.53	10.62	○
P0A7M2	9000.87	-0.65	11.42	○
P0A7M6	7268.98	-0.66	9.98	○
P0A7M9	7865.92	-0.65	9.46	
P0A7N4	6442.43	-0.97	11.03	○
P0A7N9	6367.62	-0.80	10.25	
P0A7T3	9184.95	-0.33	10.54	○
P0A7T7	8980.85	-0.78	10.60	○
P0A7U3	10423.71	-0.62	10.52	○
P0A800	10230.39	-0.52	4.87	○
P0A9Y6	7397.76	-0.19	6.54	
P0AC62	9131.63	-0.31	6.71	○
P0ACF0	9529.19	-0.23	9.57	○
P0ACF4	9219.99	-0.04	9.69	
P0ACF8	15530.03	-0.75	5.44	○
P0AES9	11850.15	-0.21	5.06	○
P0AET2	12035.00	-0.24	5.73	○
P0AF36	9628.81	-0.98	4.69	○
P0AG51	6537.65	-0.14	10.96	○
P0AG59	11573.26	-0.80	11.16	○
P0AG63	9698.28	-0.29	9.64	○
P0C018	12761.93	-0.40	10.42	○
P68679	8494.73	-1.09	11.15	
P0A8H8	7301.42	-0.62	4.49	
P64463	7271.76	-0.28	7.94	
P0AFW2	6503.26	-1.14	10.86	
P0AF93	13603.14	0.09	5.36	○
P0AEU7	17677.28	-0.47	9.69	○
P0AED0	16056.15	-0.06	5.08	
P0A8J4	9821.12	-0.17	5.50	○
P0A972	7458.81	-0.21	8.09	
P69776	8318.30	-0.31	9.30	

*The properties of proteins are required from the website http://www.expasy.org/.

**The lower the value is, the more hydrophilic protein is.

***It marks "○" if $W_{0.5}$ in "TFA+PA" is narrower than "FA+air", according to Table S6.

Reference

[1] A. T. Iavarone, J. C. Jurchen, E. R. Williams, *Anal Chem* **2001**, *73*, 1455-1460.