Supplemental materials

Improved Proteomic Analysis Pipeline for LC-ETD-MS/MS Using Charge Enhancing Methods

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Tabel S-1. Threshold for each algorithm when FDR was controlled at 5%.

FDR5%*	LCnoD[a]		TyNBA[b]		TynoD[c]	
	2+	3+	2+	3+	2+	3+
Mascot	-1.375	-0.575	-0.95	-0.2	-1.1	-0.25
Sequest	1.505	1.865	2.31	2.88	1.72	1.86
OMSSA	1.2	0.125	1.15	1.1	0.8	0.72
pFind	-1.915	-1.635	-1.5	-1.475	-1.82	-1.34
X!Tandem	-0.025	-0.145	0.04	-0.225	0.07	0.04

*Mascot Log10 (E-value), Sequest Xcorr, OMSSA Log10 (E-value), pFind Log10 (E-value) and

X!Tandem Log10 (E-value) were used to calculate FDR.

a.LCnoD stands for Lys-C digestion without further derivatization.

b.TyNBA stands for trypsin digestion and m-NBA derivatization.

c.TynoD strands for trypsin digestion without further derivatization.

Table S-2 Character annotation of AMJ2 data.

	TynoD	TyNBA	LCnoD
Average Charge	2.22	2.27	2.35
Peptide length[a]	13.10	13.51	13.60
Average GRAVY Score	-0.044	-0.069	-0.251
Average pI[b]	4.91	4.62	6.02
(+)%[c]	11.9	11	14.6
(-)%[d]	13.3	13.7	14.3

a) Number of amino acid residues (peptide length)
b) Average isoelectric point
c) The percentage of positively charged residues
d) The percentage of negatively charged residues

Table S-3 Charge distribution of precursor ions and the corresponding successful identification rate (pFind + X!Tandem) of Amj2 data.

|	2+	3+	4+	Overall
TynoD				
Spectra No.	13090	4258	502	17850
Spectra No.(FDR<5%)	7012	2002	109	9123
Successful Identification (%)	53.57	47.01	21.7	51.11
TyNBA				
Spectra No.	13581	5245	787	19506
Spectra No.(FDR<5%)	7118	2036	125	9279
Successful Identification (%)	52.41	38.81	15.88	47.57
LCnoD				
Spectra No.	8725	5304	1722	15751
Spectra No.(FDR<5%)	4271	2323	364	6958
Successful Identification (%)	48.95	43.80	21.14	44.17
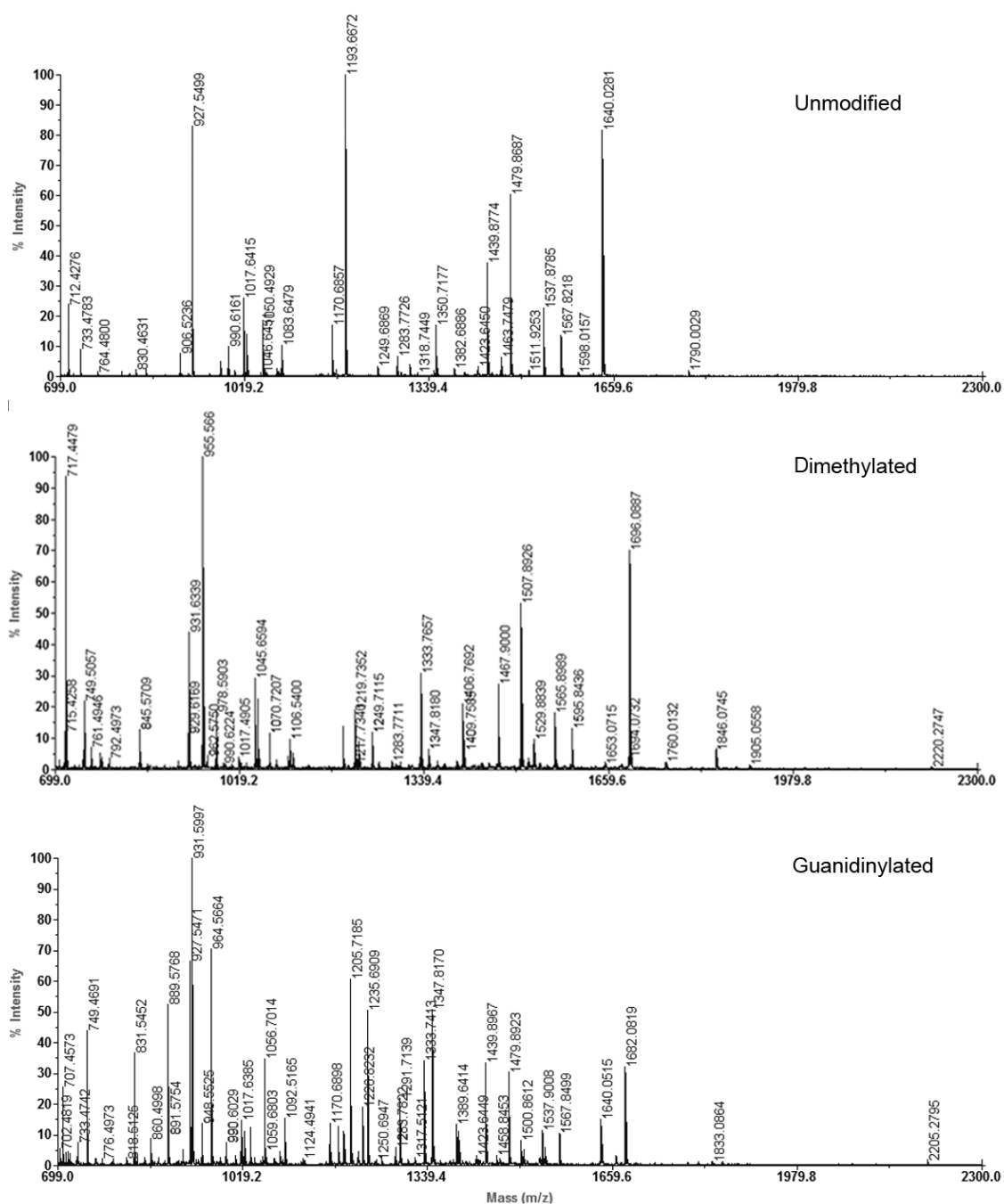
Figure S-1. MS spectra of natural, guandinlated and dimethylated tryptic BSA peptides.

A)

LCnoD

B)

TyNBA

C)

TynoD
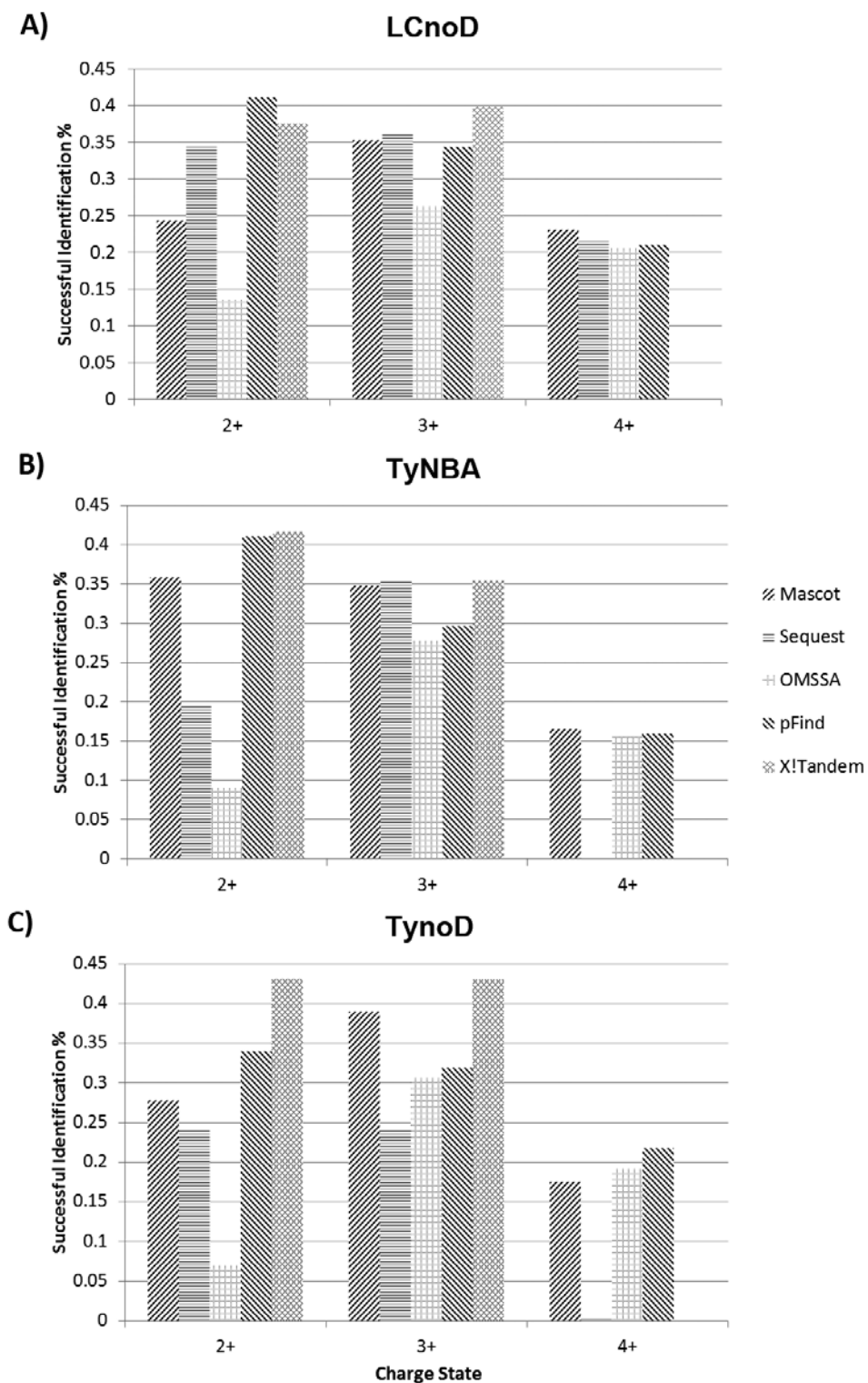
Figure S-2. Percentage of successful identification of each charge state for Lys-C (A), *m*-NBA (B)

and trypsin (C) strategy. Successful identification ratio was calculated by number of successful

identifications dividing by all PSMs.
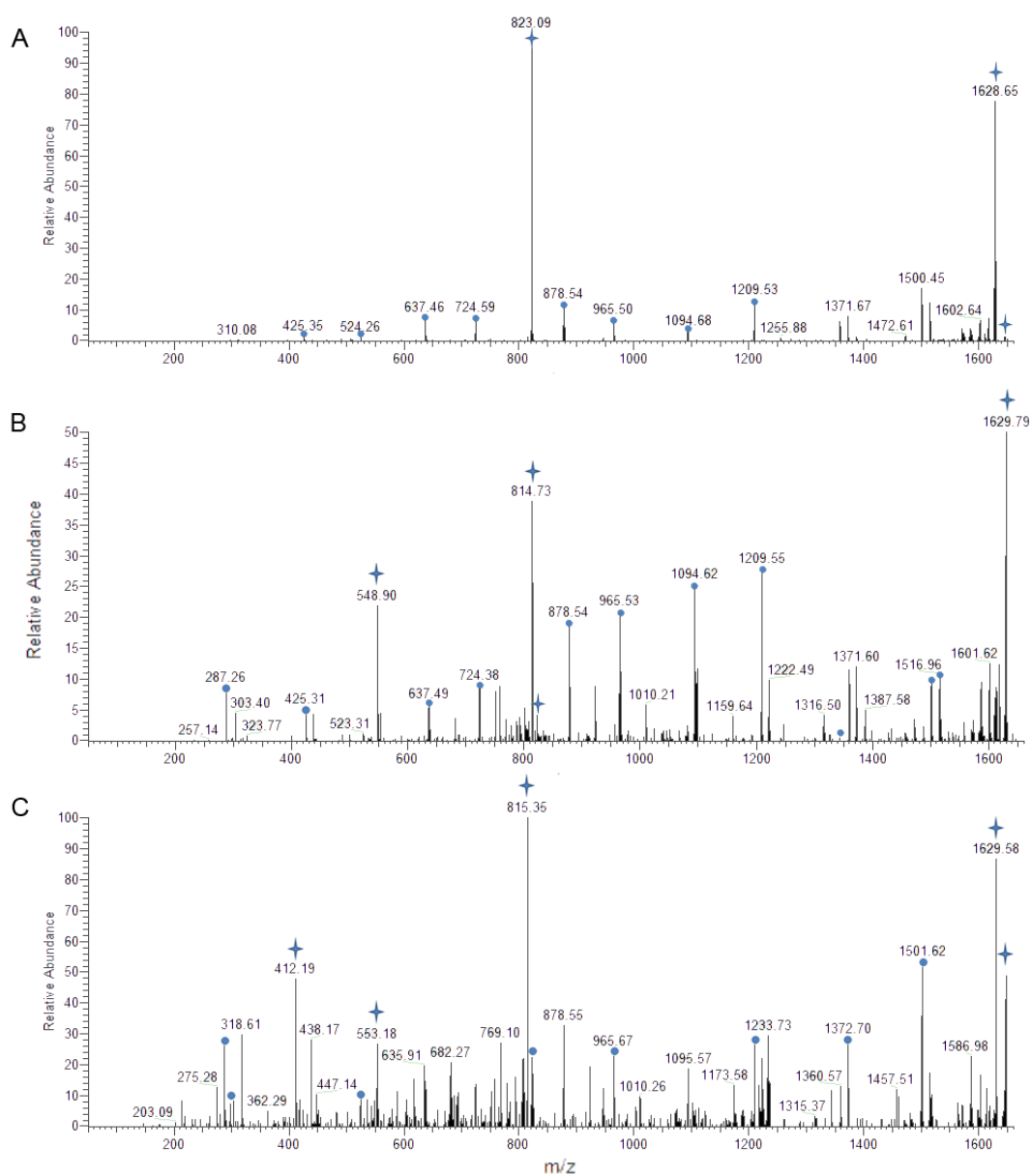
Figure S-3. ETD spectra of doubly (A), triply (B) and quadruply (C) charged "K.QEYDESGPSIVHRK.C".

All three spectra with a peptide-prophet score of 1 indicate good data consistency. Four-pointed star

indicates peaks belonging to precursor, charge reduced products and neutral loss species are

included. Rotundity indicates hydrogen-rearranged fragment ions.