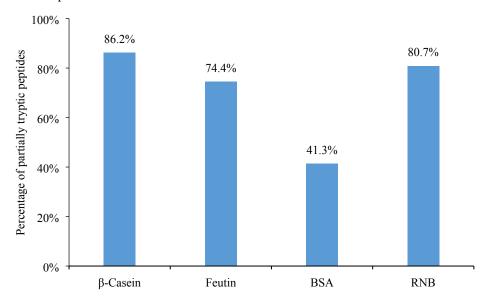
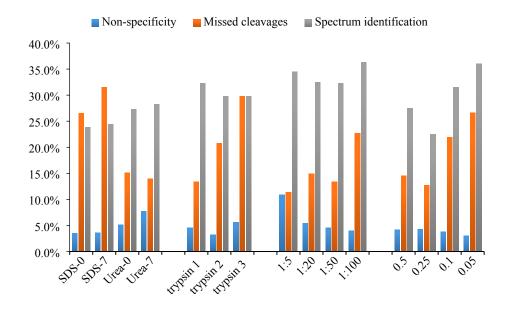
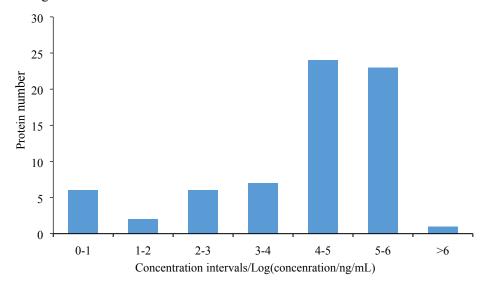
Supplementary figure 1. The percentage of partially tryptic peptides varies greatly in different standard proteins.



Supplementary figure 2. The partially tryptic cleavage rate, missed cleavages rate and spectrum identification rate under various experimental conditions.



Supplementary figure 3. The concentration distribution of proteins occurring nonspecific trypsin cleavage.



Supplementary table 3. Overview of the Three Commercial Trypsins Compared in This Study

no.	name	manufacturer	side activity	origin	modification	part no.	price (Y)	ratios
1	Sequencing Grade Modified Trypsin	Promega	none	porcine	TPCK, <sup>a</sup> Dimethylated	V5113	344/100µg	1:20 to 1:100
2	Trypsin Gold, Mass spectrometry Grade	Promega	none	porcine	TPCK, <sup>a</sup> Dimethylated	V5280	614/100μg	1:20 to 1:100
3	Trypsin	Sigma	chymotrypsin ≤0.1 units/mg protein	bovine	TPCK <sup>a</sup>	T1426	$0.96/100 \mu g$	1:20 to 1:100

 $<sup>^{</sup>a}$ L-1-tosylamido-2-phenylethyl chloromethyl ketone.  $^{b}$ 20 mM Ca $^{2+}$  will inhibit auto-digestion.