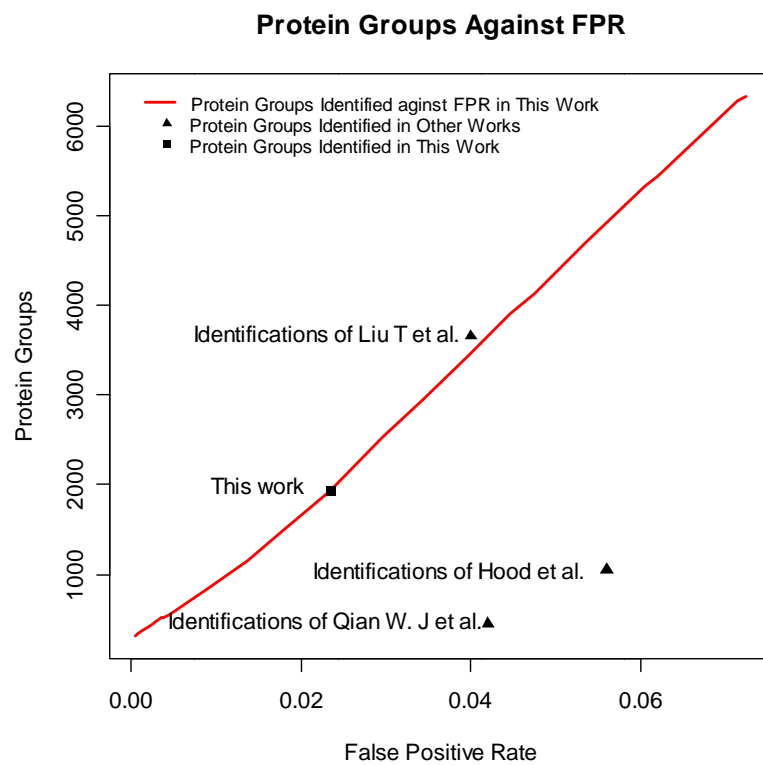**Figure S1:** Identification results against FPR. FPR(%) was calculated as described in Table S2. Protein identifications of different works are plotted in the figure.

Figure S2. Comparison with HPPP data. The proteins from two databases (IPI.HUMAN.3.07 of this work and IPI.HUMAN.2.21 of HPPP datasets) with more than 95% similarity are considered to be the same one. This work's result has an overlap of 1201 with HPPP all 9504 identifications, 732 with multi-peptide 3020 identifications (Omenn, G. S,et al.,2005，ref 51), and 364 with 889 identifications (States, D. J et al., 2006, ref 52).
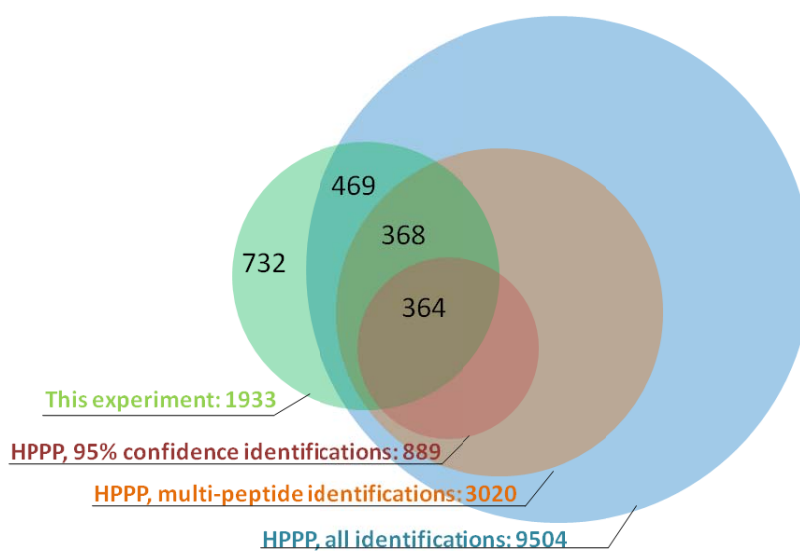
Figure S3. Comparison with earlier published data of our lab (ref 35). **A**: Protein groups identified in two datasets; **B**: Protein groups increase in different p*I* ranges than the dataset before of our lab. The increase is calculated as percentage.

Figure S4. Protein group numbers with more than one unique peptide identified in each fraction. Each rectangle comprises three fractions eluted from one RP cycle. Stars mark RP cycles with increasing trends.
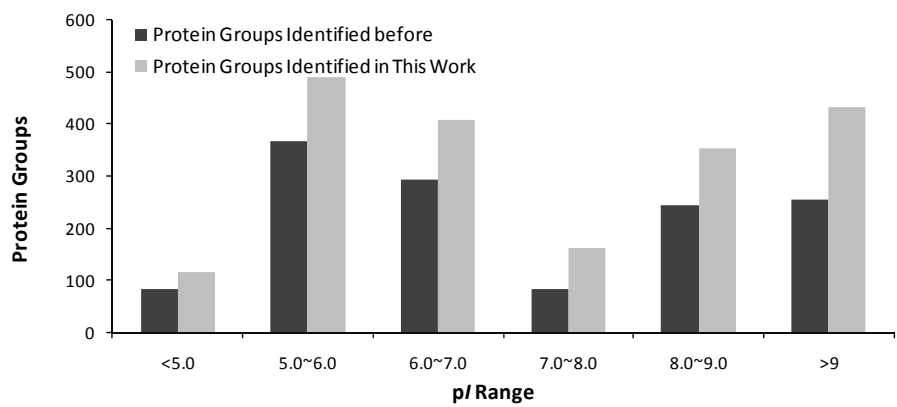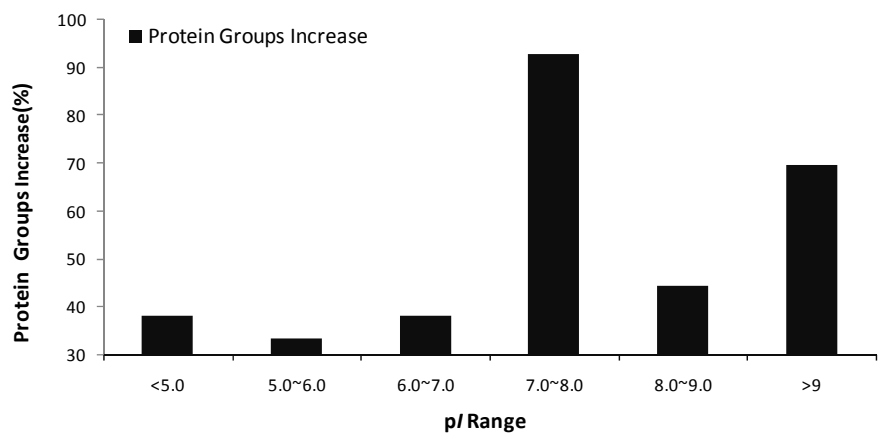
Figure S1.

Protein Groups Against FPR

Legend:
- Protein Groups Identified aginst FPR in This Work
- ▲ Protein Groups Identified in Other Works
- ■ Protein Groups Identified in This Work

Identifications of Liu T et al. ▲

This work ■

Identifications of Hood et al. ▲

Identifications of Qian W. J et al. ▲

Y-axis: Protein Groups
X-axis: False Positive Rate

Figure S2.

469
368
732
364

This experiment: 1933
HPPP, 95% confidence identifications: 889
HPPP, multi-peptide identifications: 3020
HPPP, all identifications: 9504

Figure S3.

A.

B.

Figure S4.