Supplemental Figures

Figure S1: Full wave length of APols in UV and visible

The measurement was blanked by water. The lower curve is from 1mg/mL APols, while the upper light blue curve is from 10mg/mL. Analysis was performed on Beckman spectrophotometer DU-640B. At 280nm, the absorbance is 0.024 for 1mg/mL APols and 0.25 for 10mg/mL.

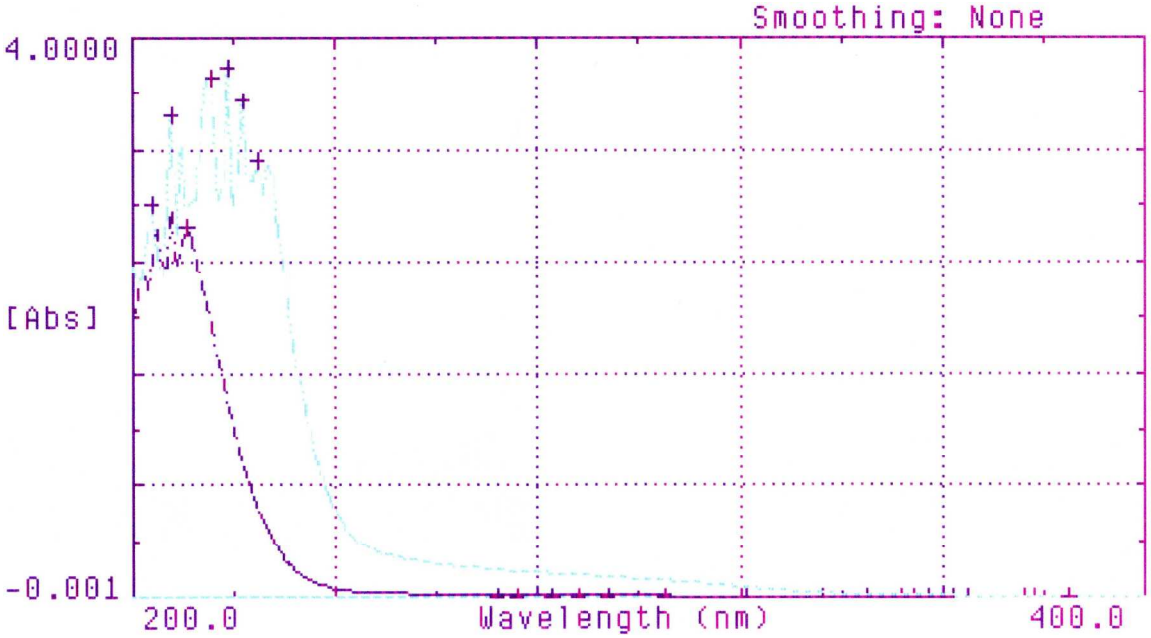
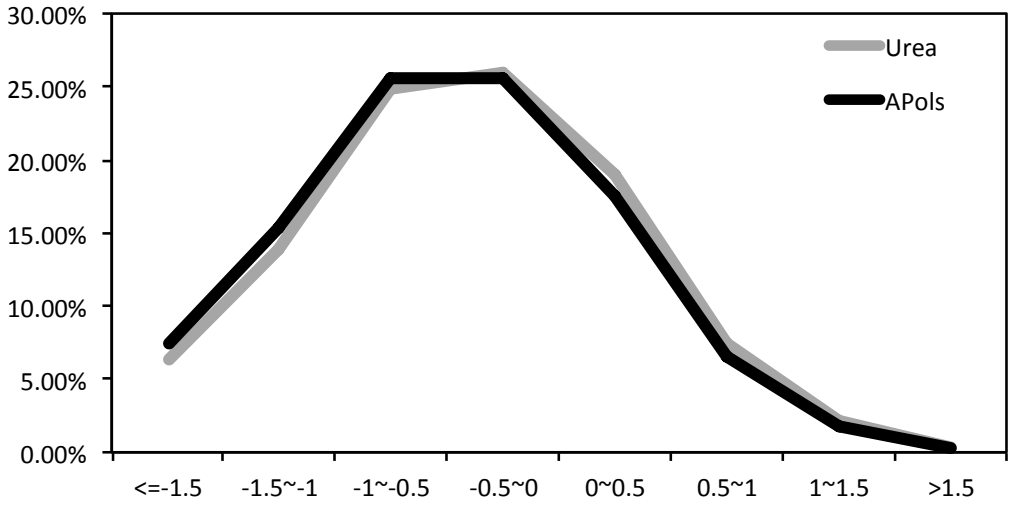


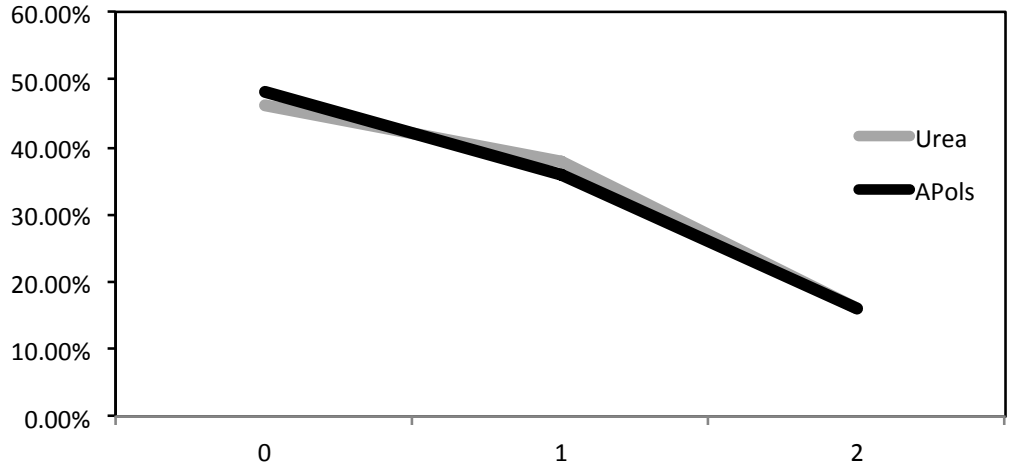
Figure S2: Comparison of identified membrane proteins from APols and Urea digestion methods

Comparison of some properies of the identified peptides from membrane protein preparations between the APols and Urea digestion. A: GRAVY; B: number of miss-cleavage site; C: Peptide length.

A:


30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Urea
APols
<=-1.5
-1.5~-1
-1~-0.5
-0.5~0
0~0.5
0.5~1
1~1.5
>1.5

B:


60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Urea
APols
0
1
2

C:

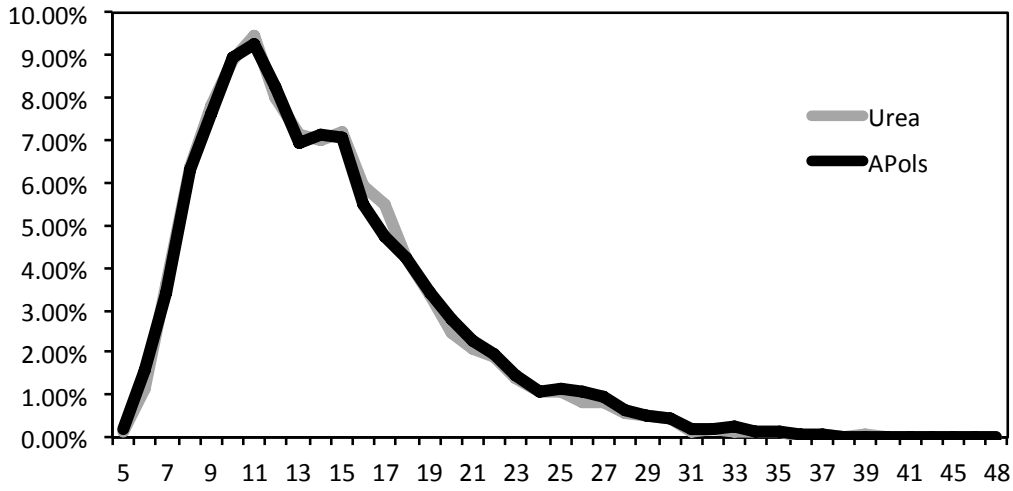


Figure S3: Transmembrane protein domain comparison between RIPA and APols.

Same amount of precipitated membrane protein pellet were analyzed Urea and APols workflow and identified by Maxquant. The first protein in each proteinGroup was subjected to transmembrane domain analysis by TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM/). The protein numbers with different number (y axis) of transmembrane domains (x axis) are compared.

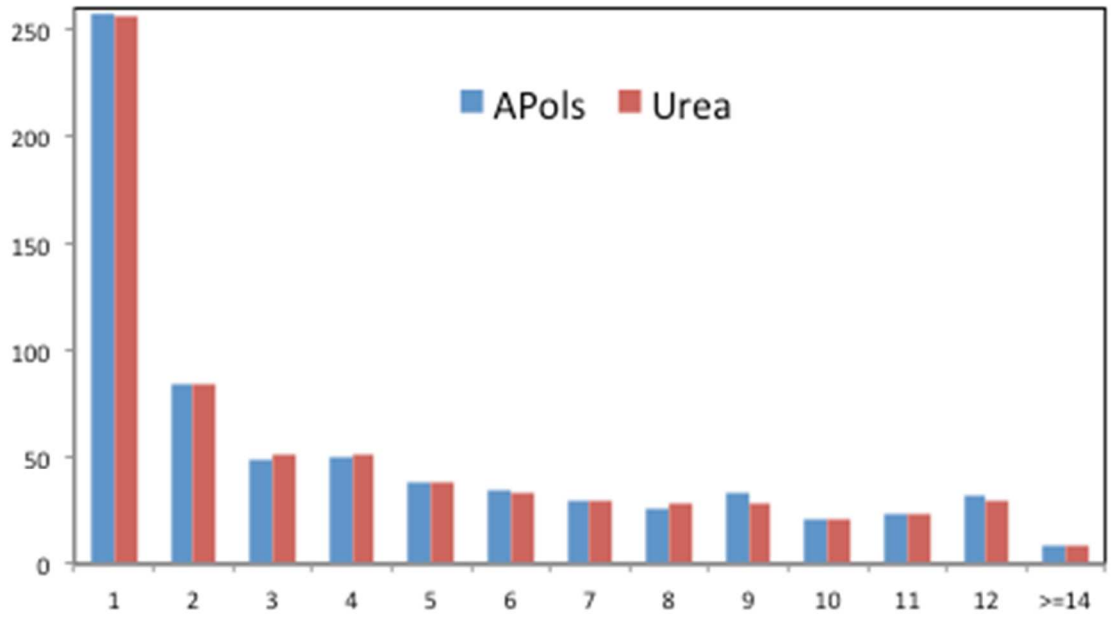


Figure S4: Transmembrane protein domain comparison between three FASP, ALS and APols.

Same amount of precipitated membrane protein pellet were analyzed by three workflows (2% SDS followed by FASP, ALS and APols digestion) and identified by Maxquant. Transmembrane protein domain analysis was the same as Figure S8.

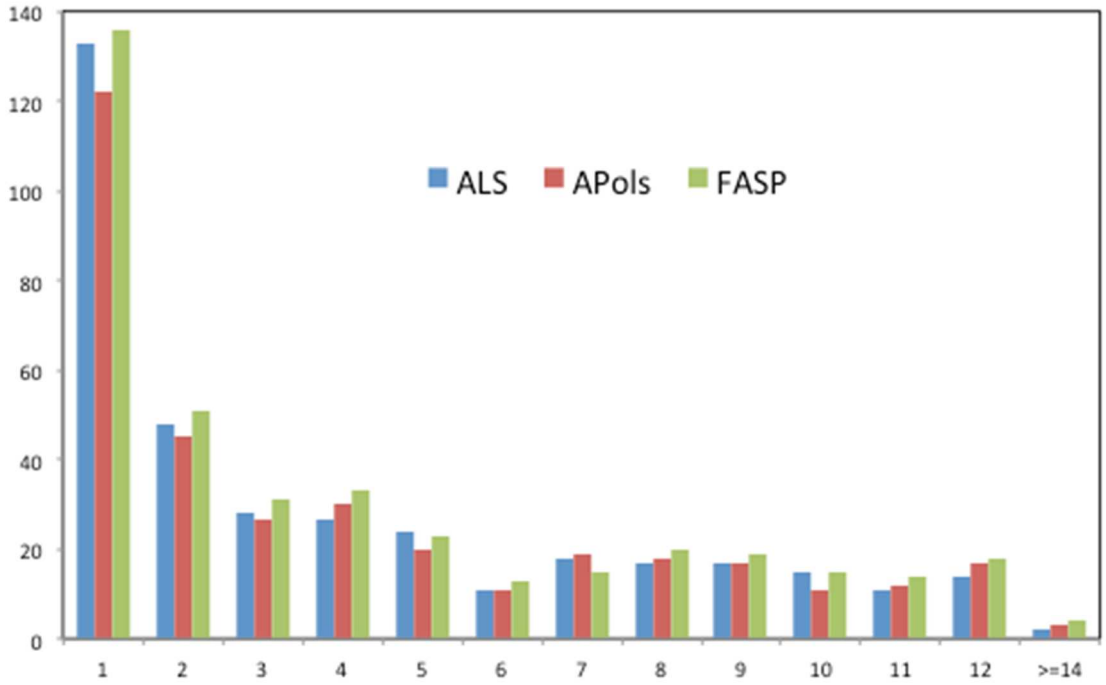


Figure S5: Full scan of APols

Full MS spectrum of APols ionization. All polymer ions are singly charged. The m/z interval between main high abundance ions is 68, composed of two secondary mass differences of 34.

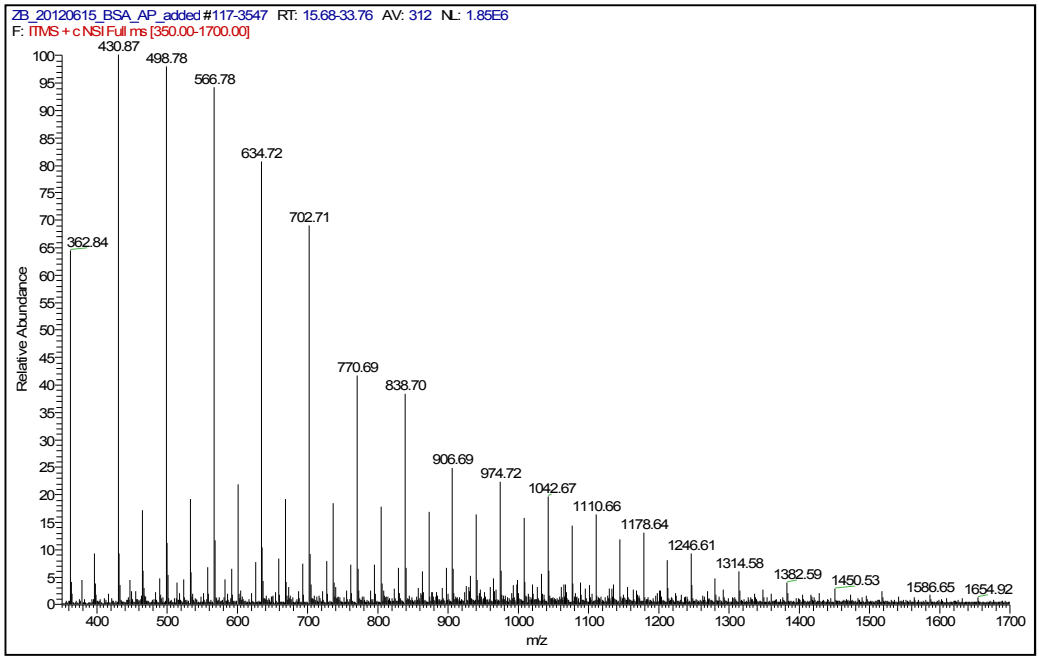
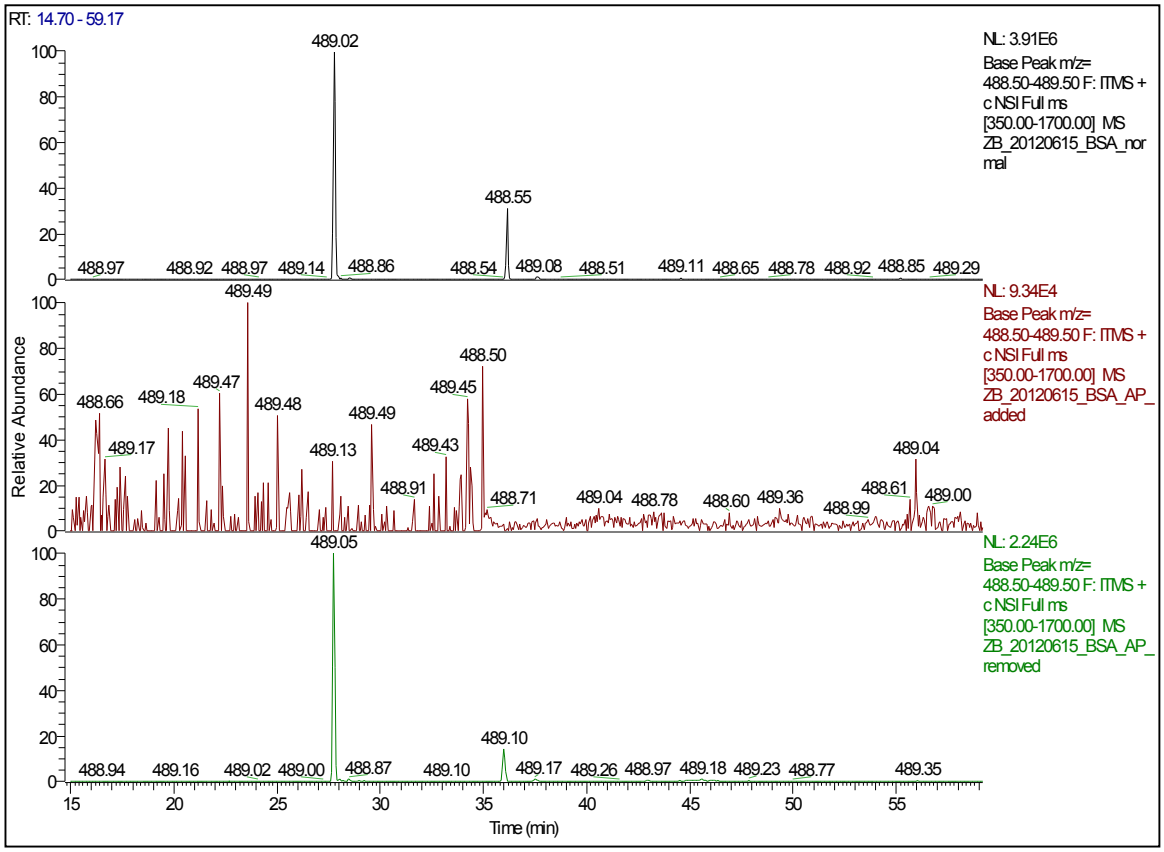
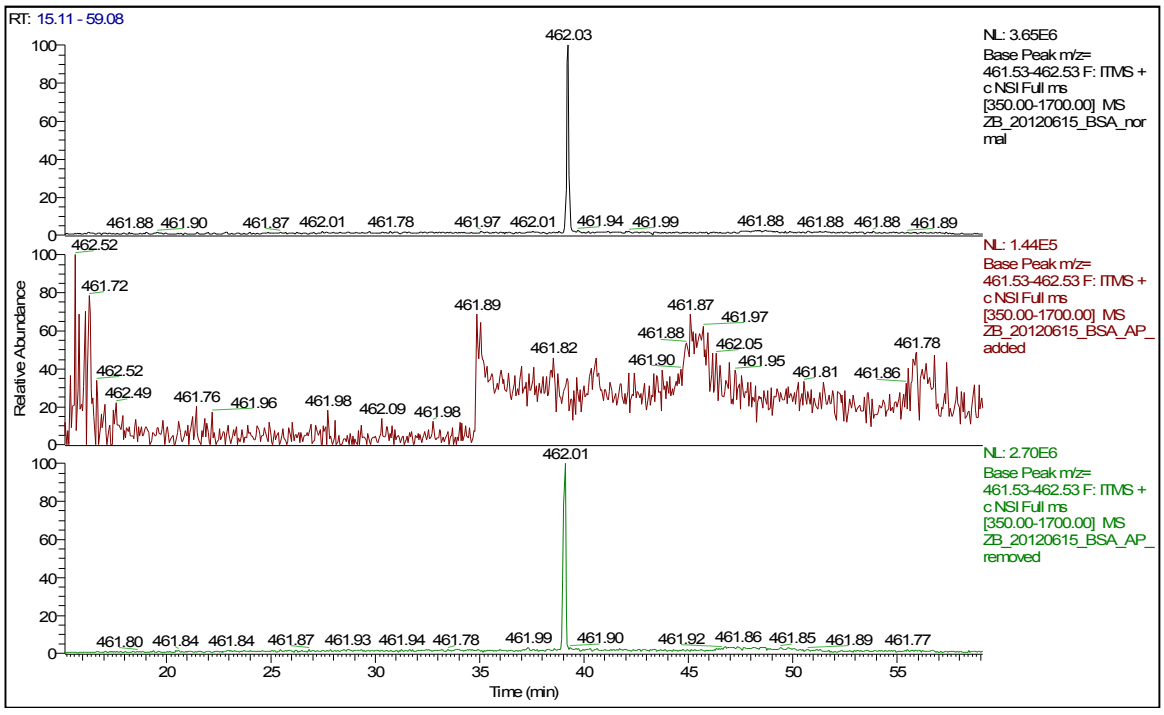


Figure S6: Ion suppression of some peaks

Comparison of some peptide peaks between the three samples shows that APols produces limited time-window ion suppression on MS, and it can be removed efficiently by acidification and centrifugation.


RT: 14.70 - 59.17
NL: 3.91E6
Base Peak m/z= 488.50-489.50 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_normal
NL: 9.34E4
Base Peak m/z= 488.50-489.50 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_AP_added
NL: 2.24E6
Base Peak m/z= 488.50-489.50 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_AP_removed
Relative Abundance
Time (min)

B:


RT: 15.11 - 59.08
NL: 3.65E6
Base Peak m/z= 461.53-462.53 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_normal
NL: 1.44E5
Base Peak m/z= 461.53-462.53 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_AP_added
NL: 2.70E6
Base Peak m/z= 461.53-462.53 F: ITMS + c NSI Full ms [350.00-1700.00] MS ZB_20120615_BSA_AP_removed
Relative Abundance
Time (min)

C:

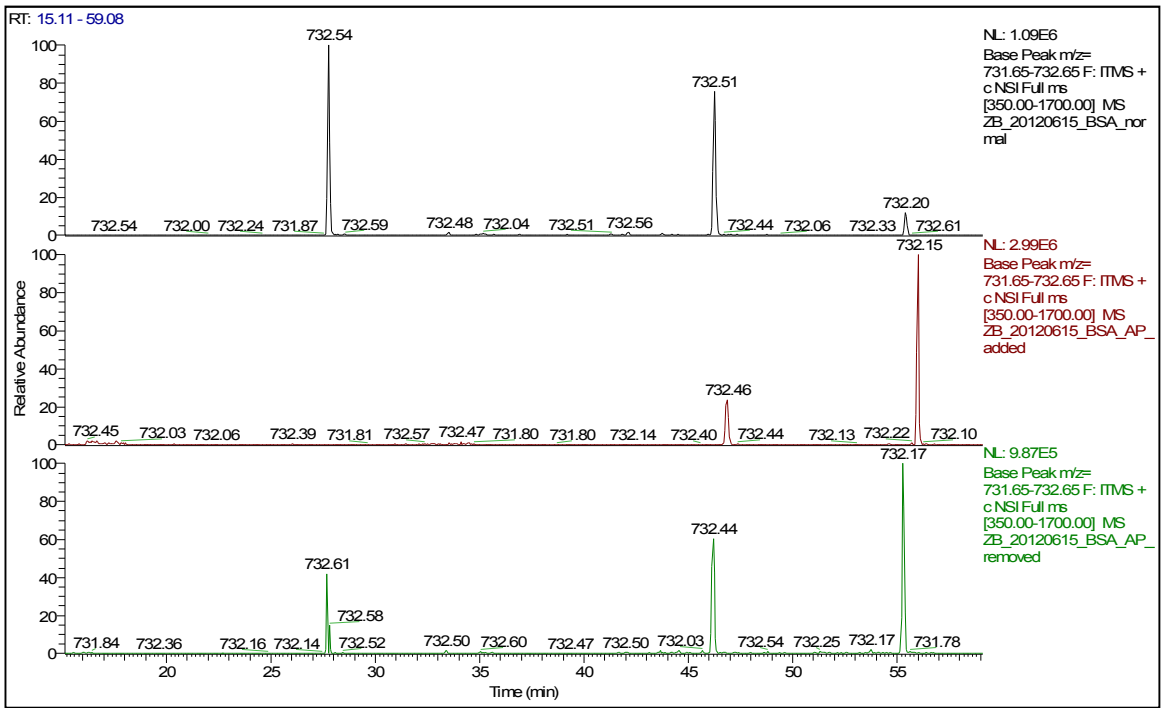


Figure S7: Chromatography of Depletion test from precipitated APols

800μg MP was digested in the presence of 10mg/mL APols. After APols was precipitated and washed twice, then eluted with 80% ACN and 100% isopropanol. The eluted were dried down and reconstituted in 50μL 0.5% FA, while the supernatant were diluted into 500μL with 0.5% FA. The supernatant were analyzed by 5~35% gradient of ACN (A). The eluent was analyzed by 5~35%(B) and 20~50% gradient (C).

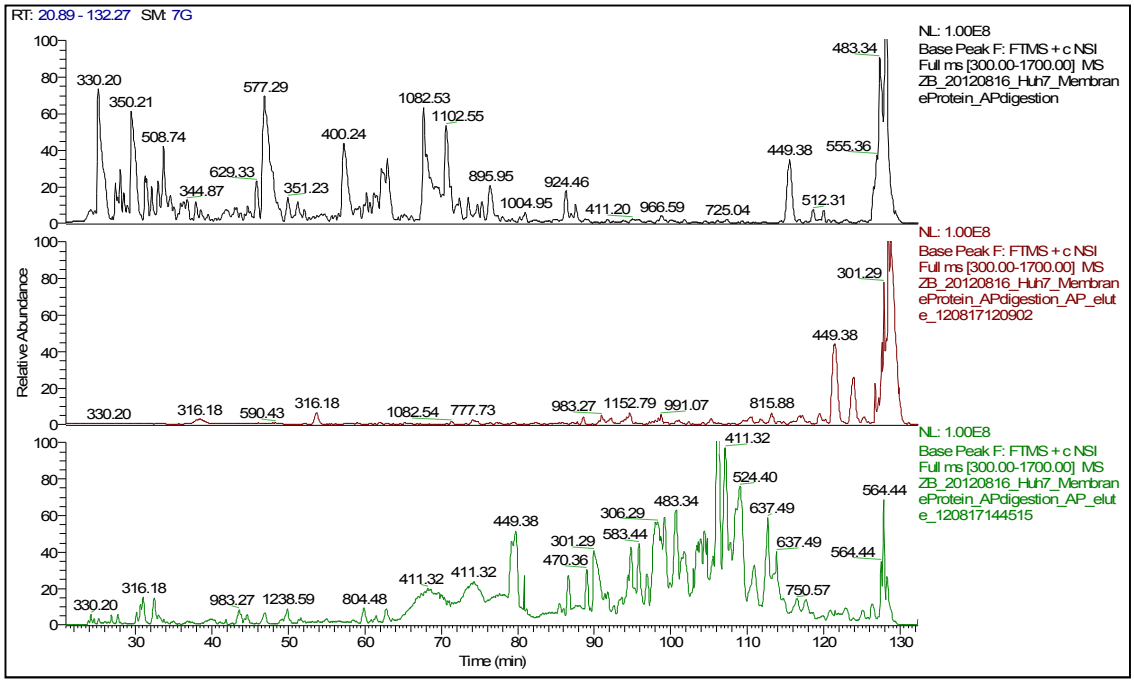
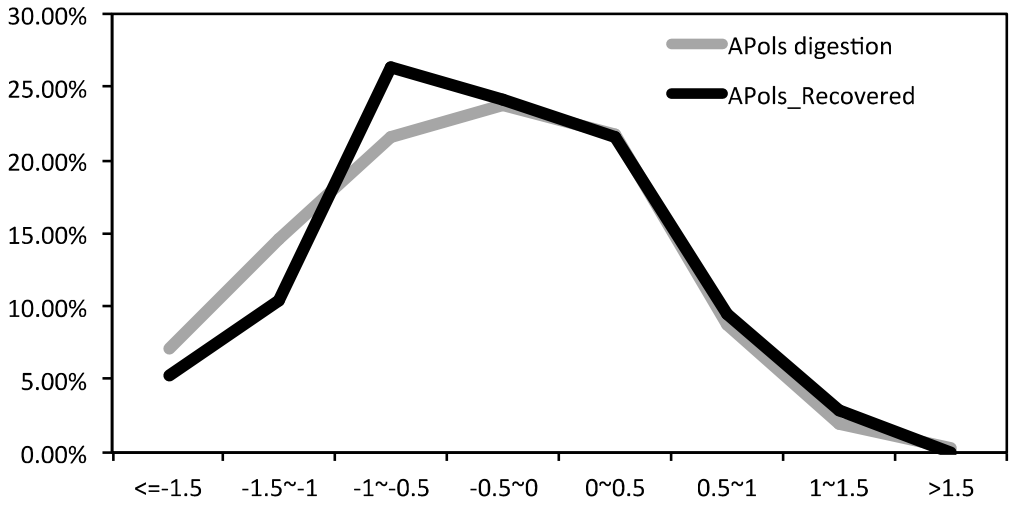


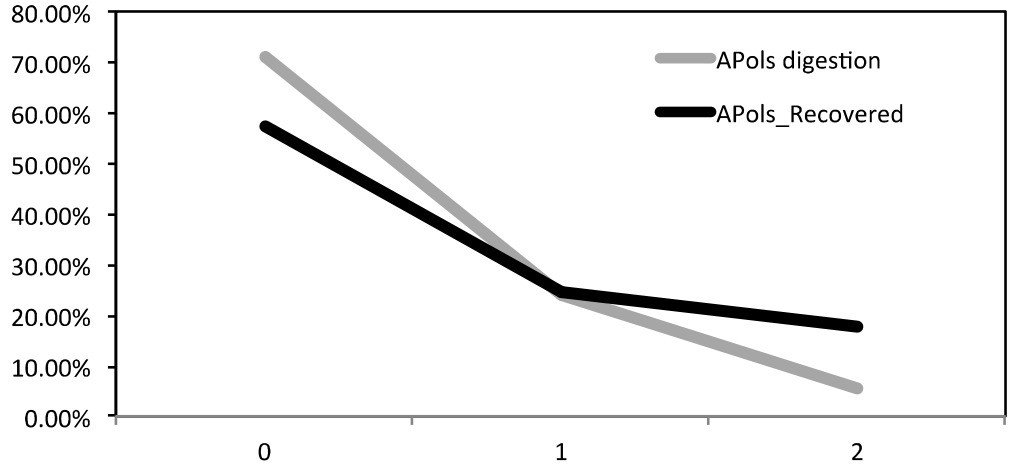
Figure S8: Comparison between peptides between normal digestion and that recovered from precipitated APols.

Comparison of some properties of the identified peptides digested in APols from membrane protein preparation between the normal elution and the peptides recovered from hash wash of APols precipitation (80% ACN and 100% isopropanol). A: GRAVY; B: number of miss-cleavage site; C: Peptide length.

A:


30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
APols digestion
APols_Recovered
<=-1.5
-1.5~-1
-1~-0.5
-0.5~0
0~0.5
0.5~1
1~1.5
>1.5

B:


80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
APols digestion
APols_Recovered
0
1
2

C:

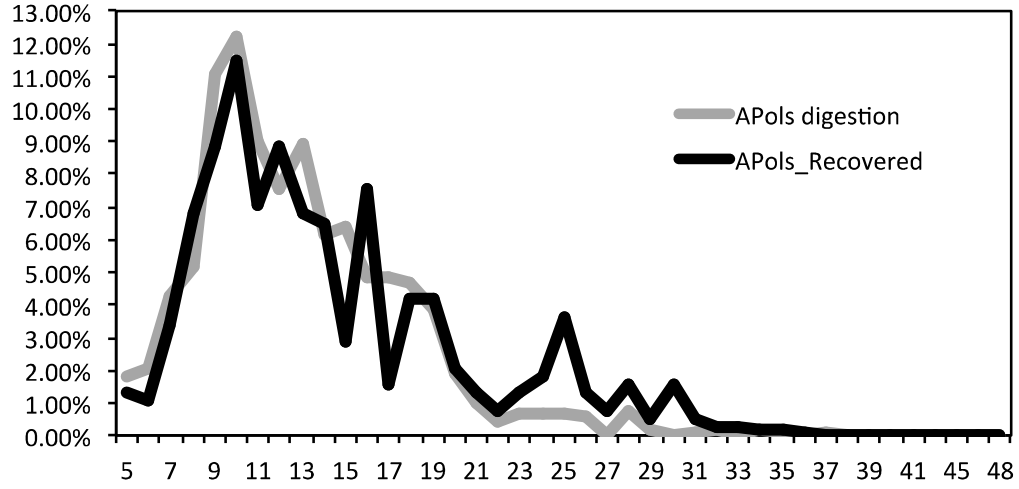
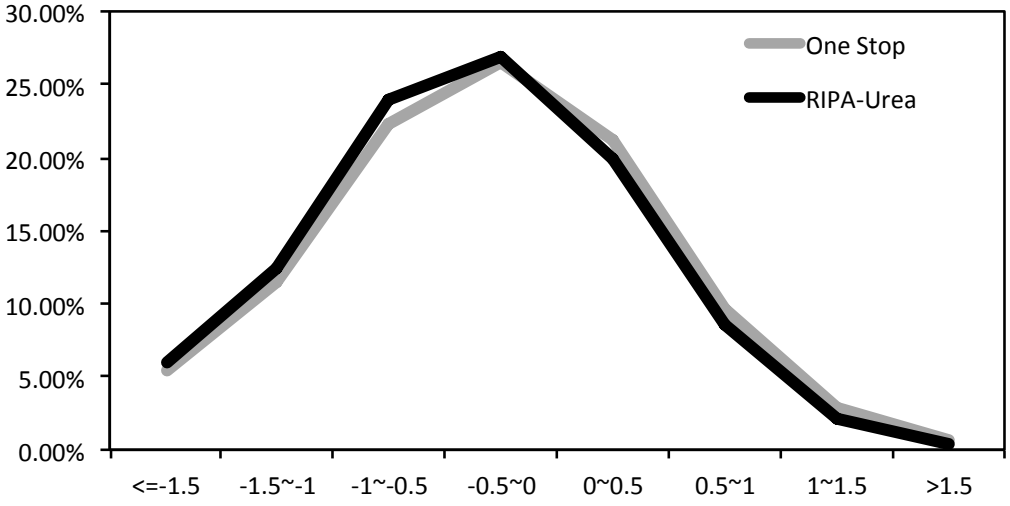


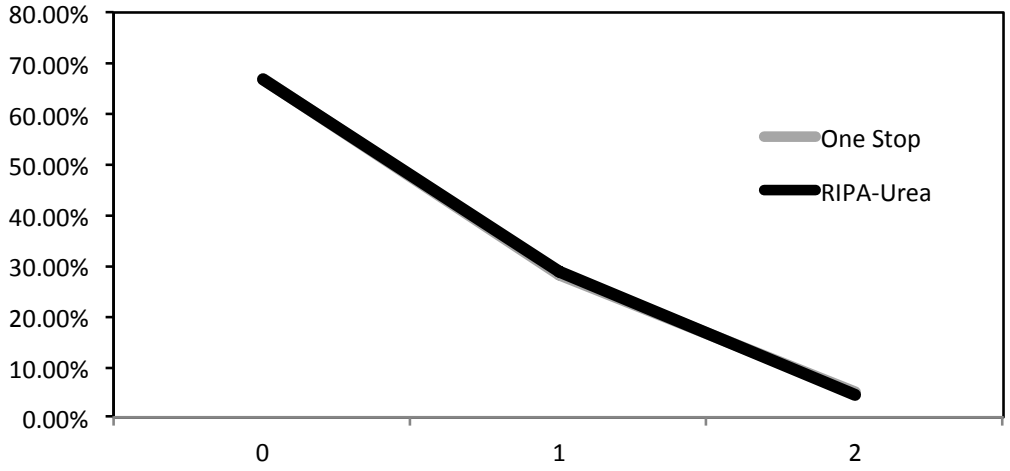
Figure S9: Comparison between One-Stop and conventional in-solution digestion

Comparison of some characters of the identified peptides between the one-stop strategy and classical RIPA-Urea one. A: GRAVY; B: number of miss-cleavage site; C: Peptide length.

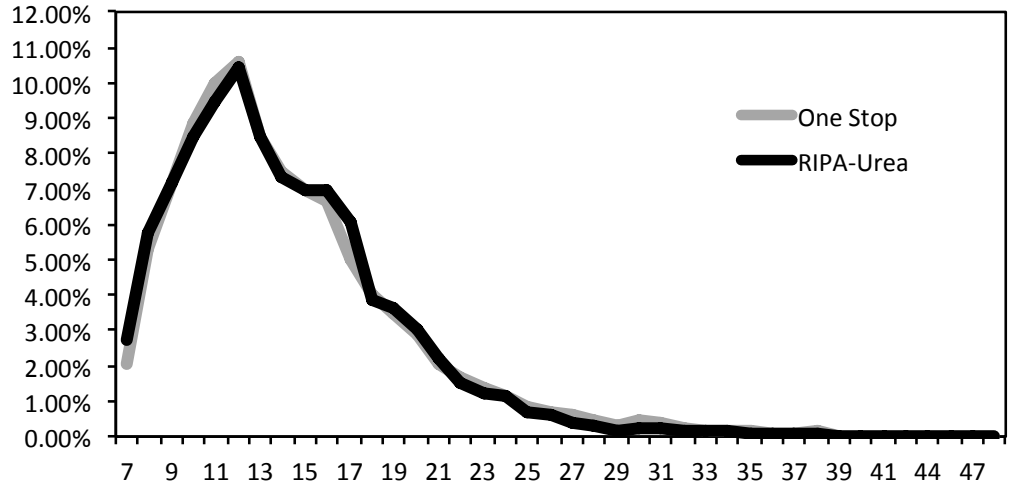
A:



B:


80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
0
1
2
One Stop
RIPA-Urea

C:


12.00%
11.00%
10.00%
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4.00%
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2.00%
1.00%
0.00%
7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 44 47
One Stop
RIPA-Urea