Supporting Information II

Comparison of In-Solution, FASP, and S-Trap Based Digestion Methods for Bottom-Up Proteomic Studies

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Supplemental Figure 2: Boxplot displaying the GRAVY scores of proteins identified by each method

Supplemental Figure 3: Boxplot displaying the sequence coverage of proteins identified by each method

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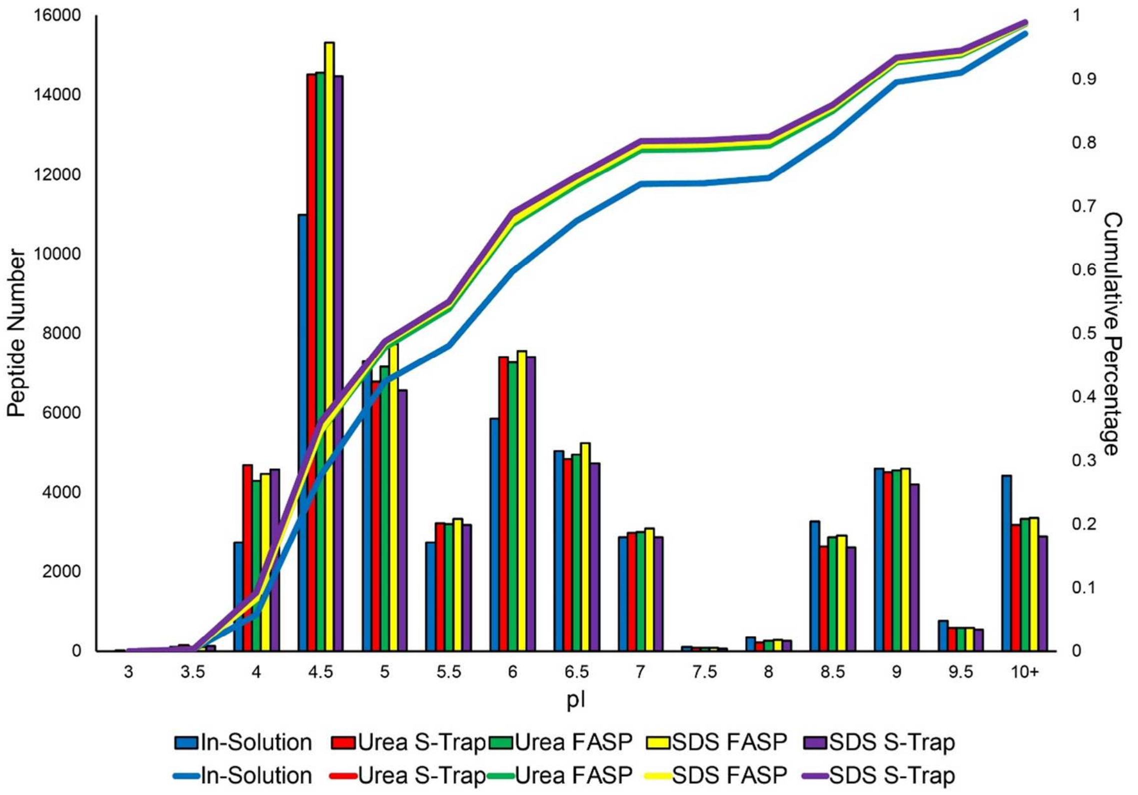
Supplemental Table 4: Cellular compartments that were overrepresented in each data set were analyzed. Unique cellular compartments are shown for each method.

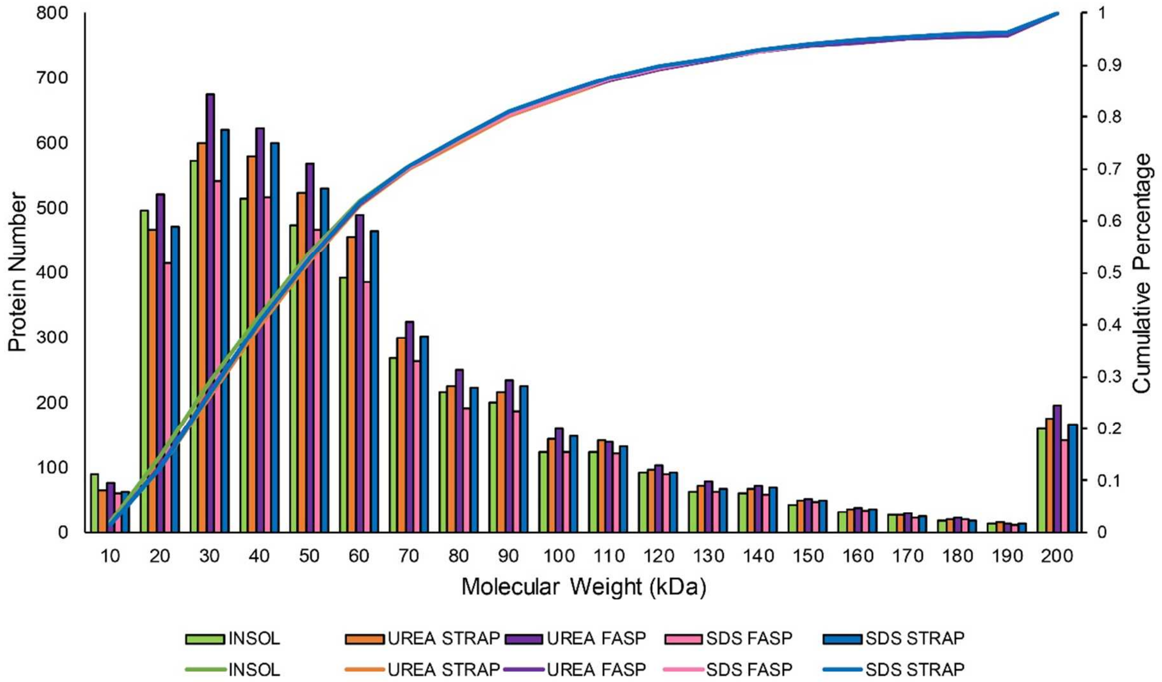
Supplemental Table 5: Biological processes that were overrepresented in each data set were analyzed. Unique biological processes are shown for each method.

Figure S1.
(A)



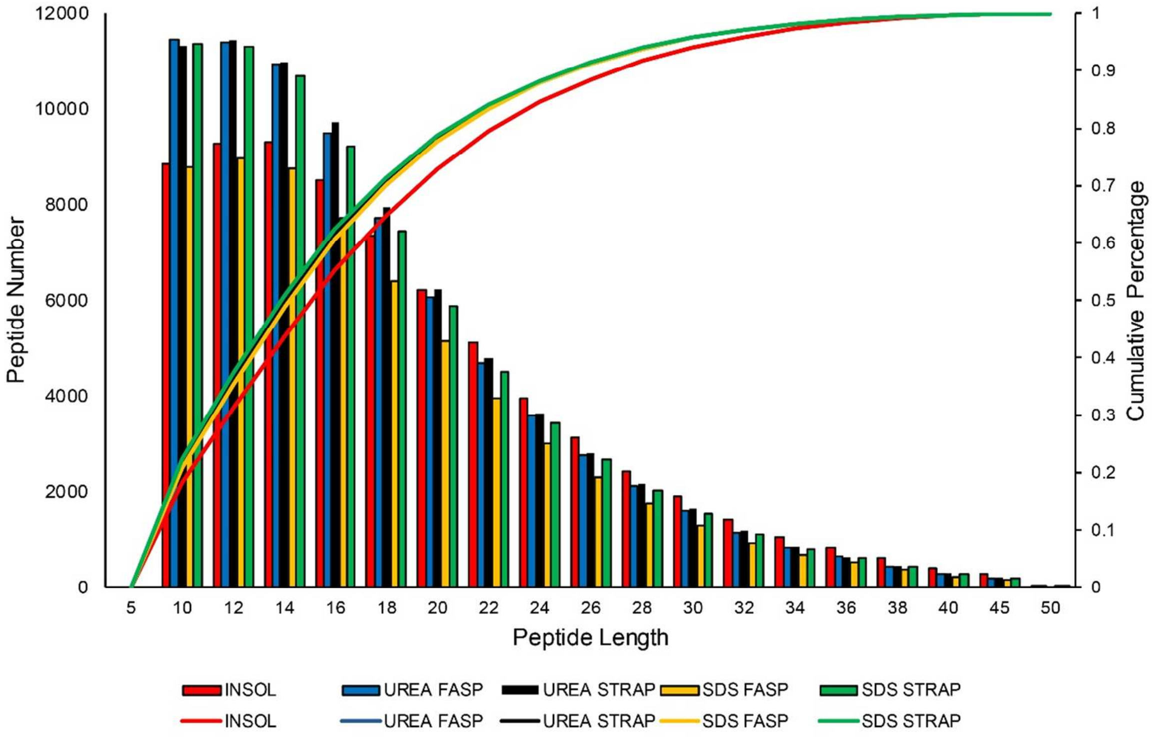
16000
14000
12000
10000
8000
6000
4000
2000
0
Peptide Number
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Cumulative Percentage
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10+
pI
In-Solution
Urea S-Trap
Urea FASP
SDS FASP
SDS S-Trap
In-Solution
Urea S-Trap
Urea FASP
SDS FASP
SDS S-Trap

(B)



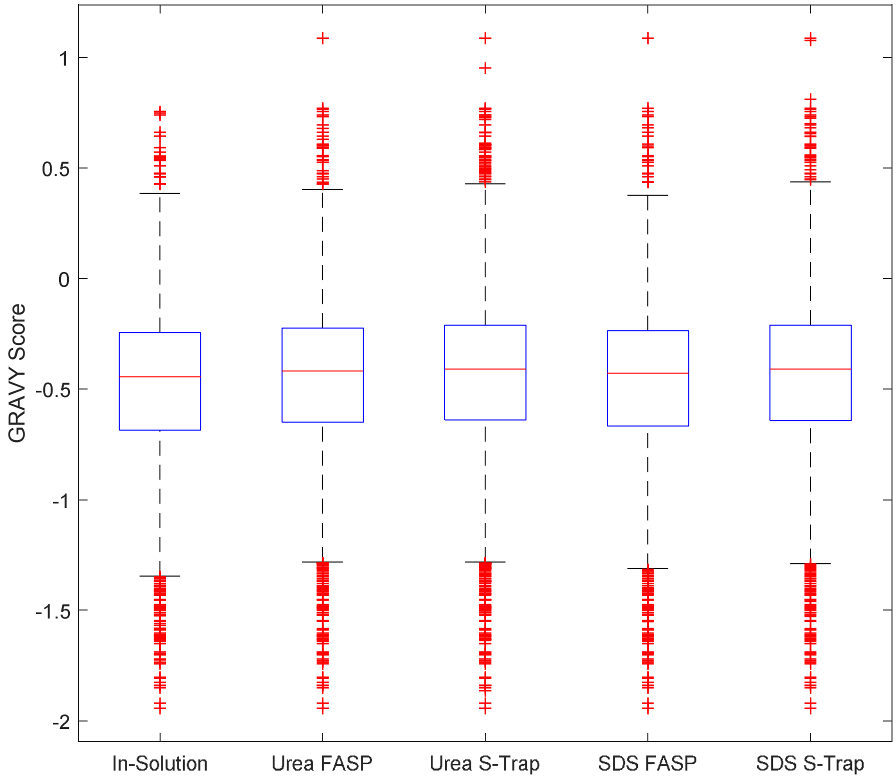
800
700
600
500
400
300
200
100
0
Protein Number
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Cumulative Percentage
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Molecular Weight (kDa)
INSOL
UREA STRAP
UREA FASP
SDS FASP
SDS STRAP
INSOL
UREA STRAP
UREA FASP
SDS FASP
SDS STRAP

(C)



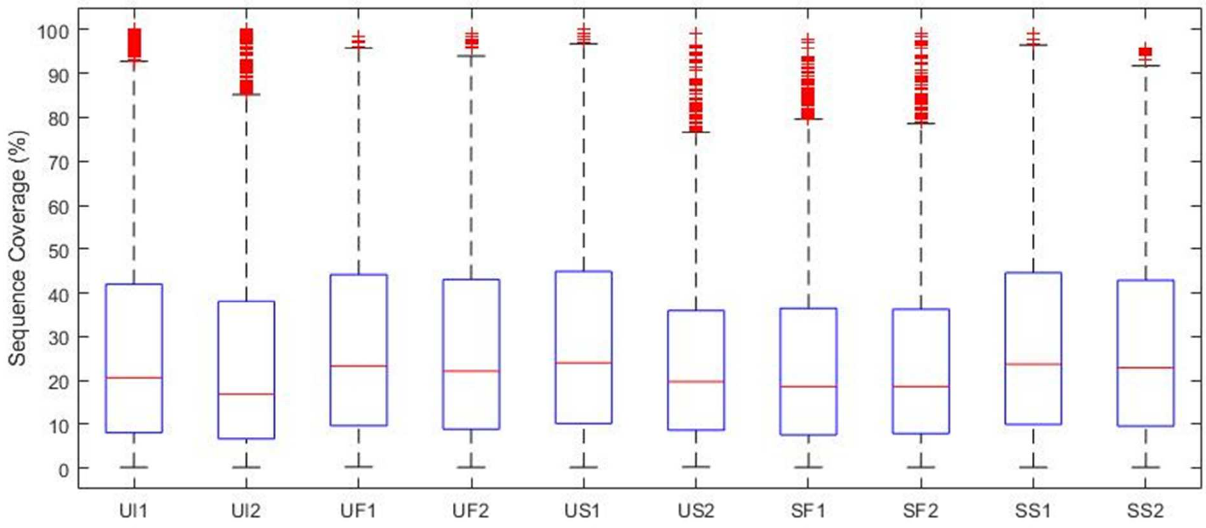
Supplemental Figure 1: Analysis of peptide trends. (A) Peptides were binned by isoelectric point and their cumulative distribution for each method was plotted. (B) Proteins identified by each method were binned by molecular weight and plotted against their cumulative distribution. (C) Peptides identified by each method were binned according to their length and plotted against their cumulative distribution.

Figure S2



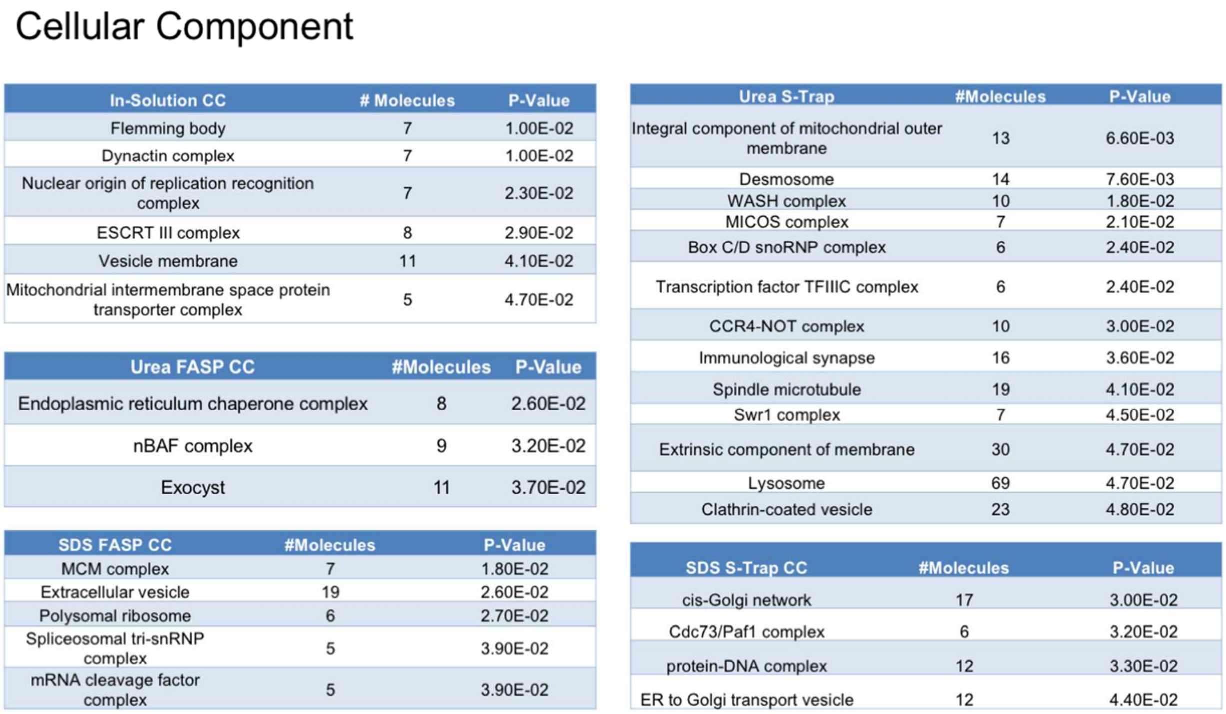
Supplemental Figure 2: Boxplot displaying the GRAVY scores of proteins identified by each method

Figure S3



Supplemental Figure 3: Boxplot displaying the sequence coverage of proteins identified by each method

Table S4



Cellular Component

In-Solution CC	# Molecules	P-Value
Flemming body	7	1.00E-02
Dynactin complex	7	1.00E-02
Nuclear origin of replication recognition complex	7	2.30E-02
ESCRT III complex	8	2.90E-02
Vesicle membrane	11	4.10E-02
Mitochondrial intermembrane space protein transporter complex	5	4.70E-02

Urea FASP CC	#Molecules	P-Value
Endoplasmic reticulum chaperone complex	8	2.60E-02
nBAF complex	9	3.20E-02
Exocyst	11	3.70E-02

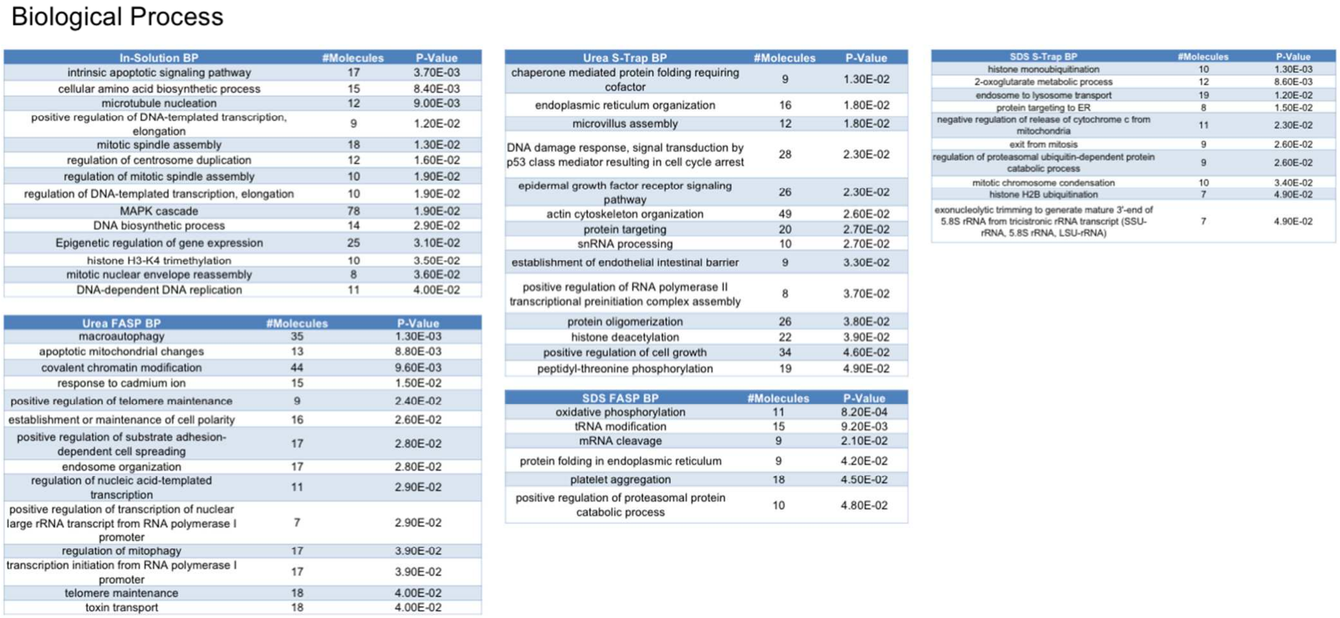
SDS FASP CC	#Molecules	P-Value
MCM complex	7	1.80E-02
Extracellular vesicle	19	2.60E-02
Polysomal ribosome	6	2.70E-02
Spliceosomal tri-snRNP complex	5	3.90E-02
mRNA cleavage factor complex	5	3.90E-02

Urea S-Trap	#Molecules	P-Value
Integral component of mitochondrial outer membrane	13	6.60E-03
Desmosome	14	7.60E-03
WASH complex	10	1.80E-02
MICOS complex	7	2.10E-02
Box C/D snoRNP complex	6	2.40E-02
Transcription factor TFIIIC complex	6	2.40E-02
CCR4-NOT complex	10	3.00E-02
Immunological synapse	16	3.60E-02
Spindle microtubule	19	4.10E-02
Swr1 complex	7	4.50E-02
Extrinsic component of membrane	30	4.70E-02
Lysosome	69	4.70E-02
Clathrin-coated vesicle	23	4.80E-02

SDS S-Trap CC	#Molecules	P-Value
cis-Golgi network	17	3.00E-02
Cdc73/Paf1 complex	6	3.20E-02
protein-DNA complex	12	3.30E-02
ER to Golgi transport vesicle	12	4.40E-02

Supplemental Table 4: Cellular compartments that were overrepresented in each data set were analyzed. Unique cellular compartments are shown for each method.

Table S5



Biological Process

In-Solution BP	#Molecules	P-Value
intrinsic apoptotic signaling pathway	17	3.70E-03
cellular amino acid biosynthetic process	15	8.40E-03
microtubule nucleation	12	9.00E-03
positive regulation of DNA-templated transcription, elongation	9	1.20E-02
mitotic spindle assembly	18	1.30E-02
regulation of centrosome duplication	12	1.60E-02
regulation of mitotic spindle assembly	10	1.90E-02
regulation of DNA-templated transcription, elongation	10	1.90E-02
MAPK cascade	78	1.90E-02
DNA biosynthetic process	14	2.90E-02
Epigenetic regulation of gene expression	25	3.10E-02
histone H3-K4 trimethylation	10	3.50E-02
mitotic nuclear envelope reassembly	8	3.60E-02
DNA-dependent DNA replication	11	4.00E-02

Urea FASP BP	#Molecules	P-Value
macroautophagy	35	1.30E-03
apoptotic mitochondrial changes	13	8.80E-03
covalent chromatin modification	44	9.60E-03
response to cadmium ion	15	1.50E-02
positive regulation of telomere maintenance	9	2.40E-02
establishment or maintenance of cell polarity	16	2.60E-02
positive regulation of substrate adhesion-dependent cell spreading	17	2.80E-02
endosome organization	17	2.80E-02
regulation of nucleic acid-templated transcription	11	2.90E-02
positive regulation of transcription of nuclear large rRNA transcript from RNA polymerase I promoter	7	2.90E-02
regulation of mitophagy	17	3.90E-02
transcription initiation from RNA polymerase I promoter	17	3.90E-02
telomere maintenance	18	4.00E-02
toxin transport	18	4.00E-02

Urea S-Trap BP	#Molecules	P-Value
chaperone mediated protein folding requiring cofactor	9	1.30E-02
endoplasmic reticulum organization	16	1.80E-02
microvillus assembly	12	1.80E-02
DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	28	2.30E-02
epidermal growth factor receptor signaling pathway	26	2.30E-02
actin cytoskeleton organization	49	2.60E-02
protein targeting	20	2.70E-02
snRNA processing	10	2.70E-02
establishment of endothelial intestinal barrier	9	3.30E-02
positive regulation of RNA polymerase II transcriptional preinitiation complex assembly	8	3.70E-02
protein oligomerization	26	3.80E-02
histone deacetylation	22	3.90E-02
positive regulation of cell growth	34	4.60E-02
peptidyl-threonine phosphorylation	19	4.90E-02

SDS FASP BP	#Molecules	P-Value
oxidative phosphorylation	11	8.20E-04
tRNA modification	15	9.20E-03
mRNA cleavage	9	2.10E-02
protein folding in endoplasmic reticulum	9	4.20E-02
platelet aggregation	18	4.50E-02
positive regulation of proteasomal protein catabolic process	10	4.80E-02

SDS S-Trap BP	#Molecules	P-Value
histone monoubiquitination	10	1.30E-03
2-oxoglutarate metabolic process	12	8.60E-03
endosome to lysosome transport	19	1.20E-02
protein targeting to ER	8	1.50E-02
negative regulation of release of cytochrome c from mitochondria	11	2.30E-02
exit from mitosis	9	2.60E-02
regulation of proteasomal ubiquitin-dependent protein catabolic process	9	2.60E-02
mitotic chromosome condensation	10	3.40E-02
histone H2B ubiquitination	7	4.90E-02
exonucleolytic trimming to generate mature 3'-end of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7	4.90E-02

Supplemental Table 5: Biological processes that were overrepresented in each data set were analyzed. Unique biological processes are shown for each method.