PROTEOMICS

Supporting Information for Proteomics

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Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells

Supplementary figures:

Figure 1. Overview of the experimental workflow. Same amount of starting material was applied to each approach in such a manner that each method was comparable. Details of the methods are described in the Materials and Methods part.

Figure 2. Physicochemical Properties of Identified Peptides.

Figure 3. Comparison of cellular component (A) and molecular function (B) of proteins identified using high pH RPLC, SCX LC and low pH RPLC fractionation methods.

Figure 4. LC profile of first dimension fractionation approaches (UV absorbance at 280nm).

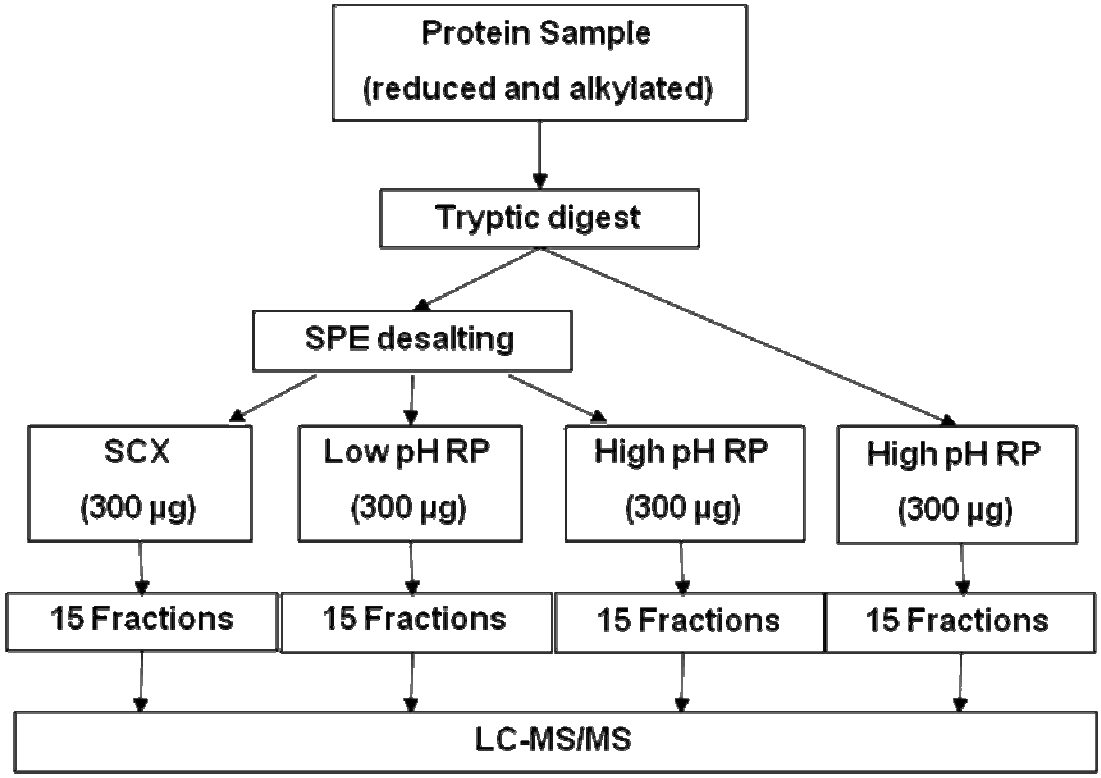
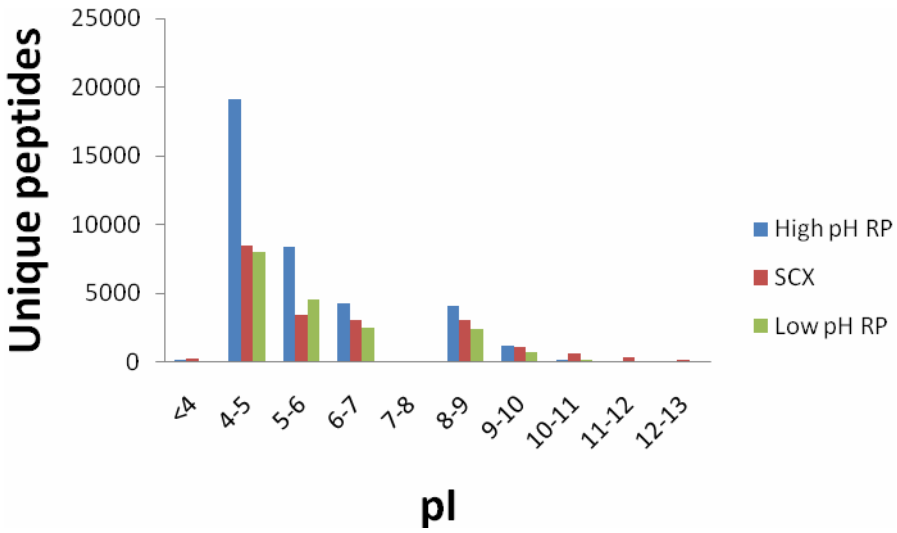

Protein Sample
(reduced and alkylated)
Tryptic digest
SPE desalting
SCX
(300 µg)
Low pH RP
(300 µg)
High pH RP
(300 µg)
High pH RP
(300 µg)
15 Fractions
15 Fractions
15 Fractions
15 Fractions
LC-MS/MS

Figure 1.

(A)


Unique peptides
25000
20000
15000
10000
5000
0
<4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
12-13
pI
High pH RP
SCX
Low pH RP

(B)

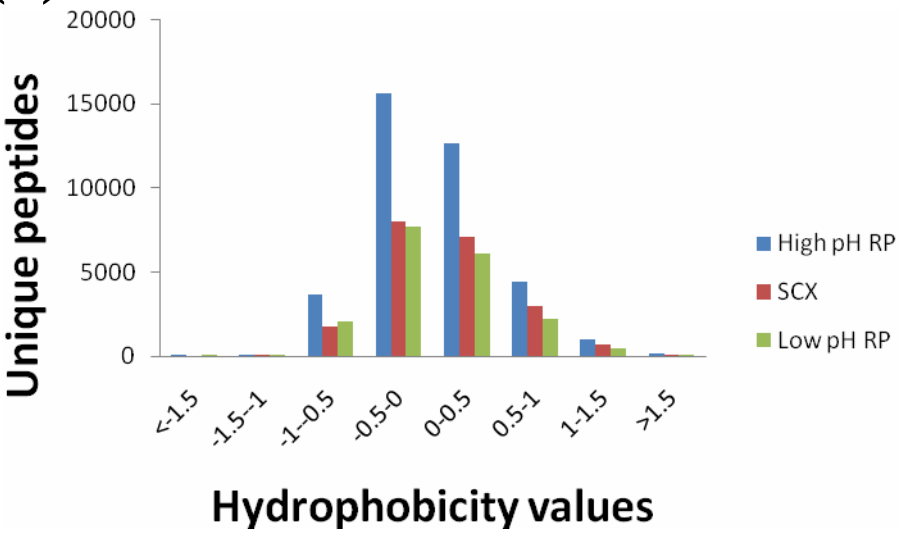

Unique peptides
20000
15000
10000
5000
0
<-1.5
-1.5--1
-1--0.5
-0.5-0
0-0.5
0.5-1
1-1.5
>1.5
Hydrophobicity values
High pH RP
SCX
Low pH RP

Figure 2.

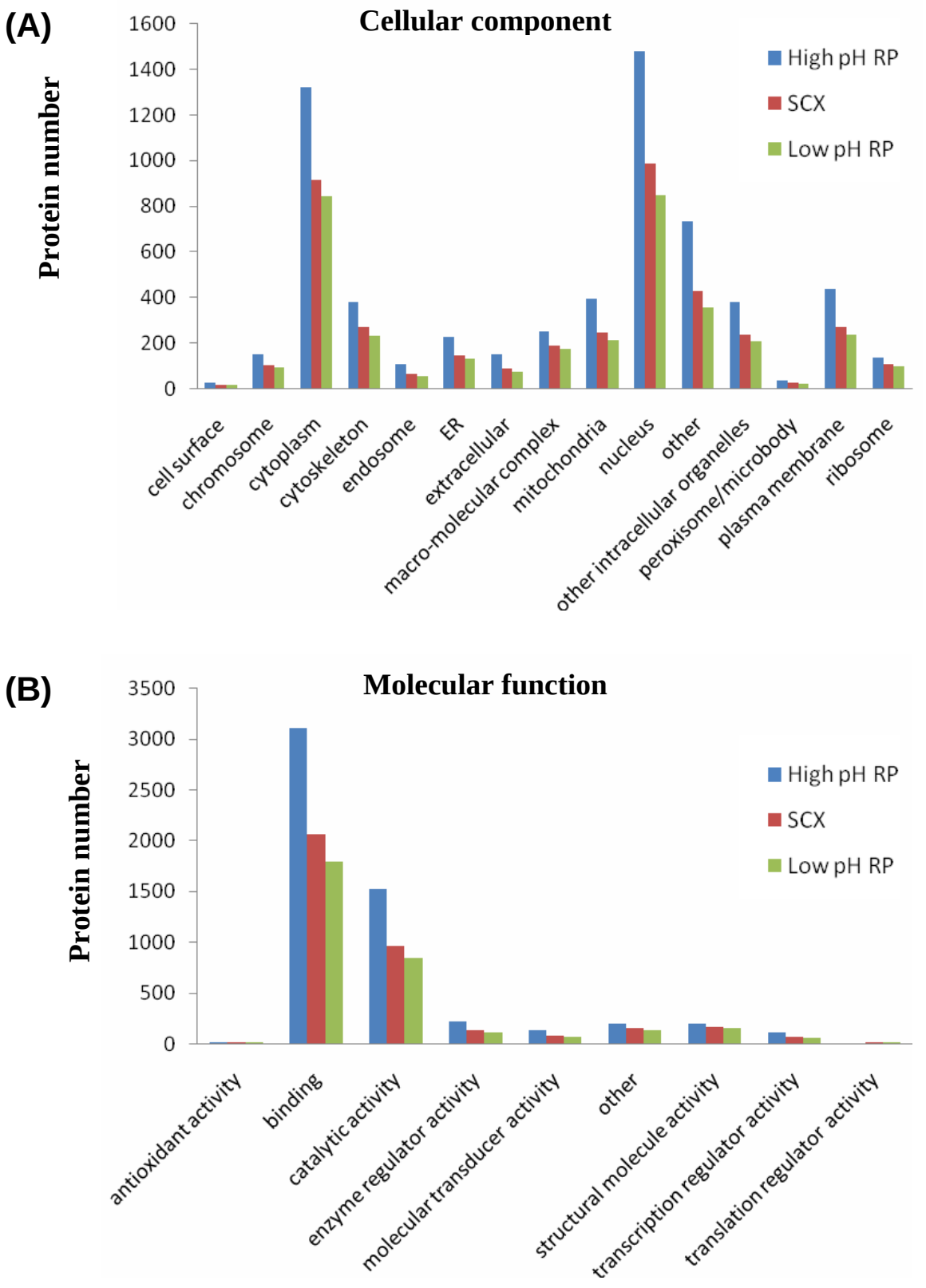

(A)
Cellular component
Protein number
1600
1400
1200
1000
800
600
400
200
0
High pH RP
SCX
Low pH RP
cell surface
chromosome
cytoplasm
cytoskeleton
endosome
ER
extracellular
macro-molecular complex
mitochondria
nucleus
other
other intracellular organelles
peroxisome/microbody
plasma membrane
ribosome
(B)
Molecular function
Protein number
3500
3000
2500
2000
1500
1000
500
0
High pH RP
SCX
Low pH RP
antioxidant activity
binding
catalytic activity
enzyme regulator activity
molecular transducer activity
other
structural molecule activity
transcription regulator activity
translation regulator activity

Figure 3.

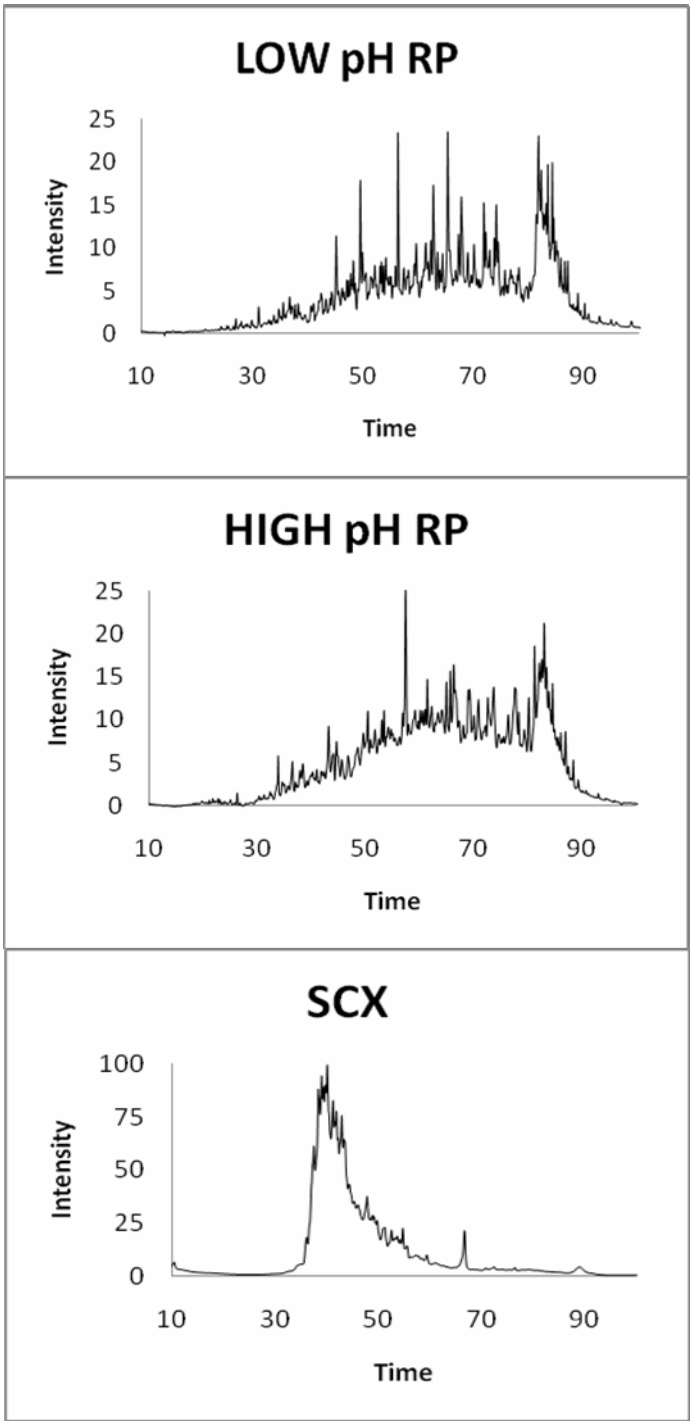

LOW pH RP
Intensity
25
20
15
10
5
0
10
30
50
70
90
Time
HIGH pH RP
Intensity
25
20
15
10
5
0
10
30
50
70
90
Time
SCX
Intensity
100
75
50
25
0
10
30
50
70
90
Time

Figure 4.