

Comparison of bottom-up proteomic approaches for LC-MS analysis of complex proteomes

Analytical Methods

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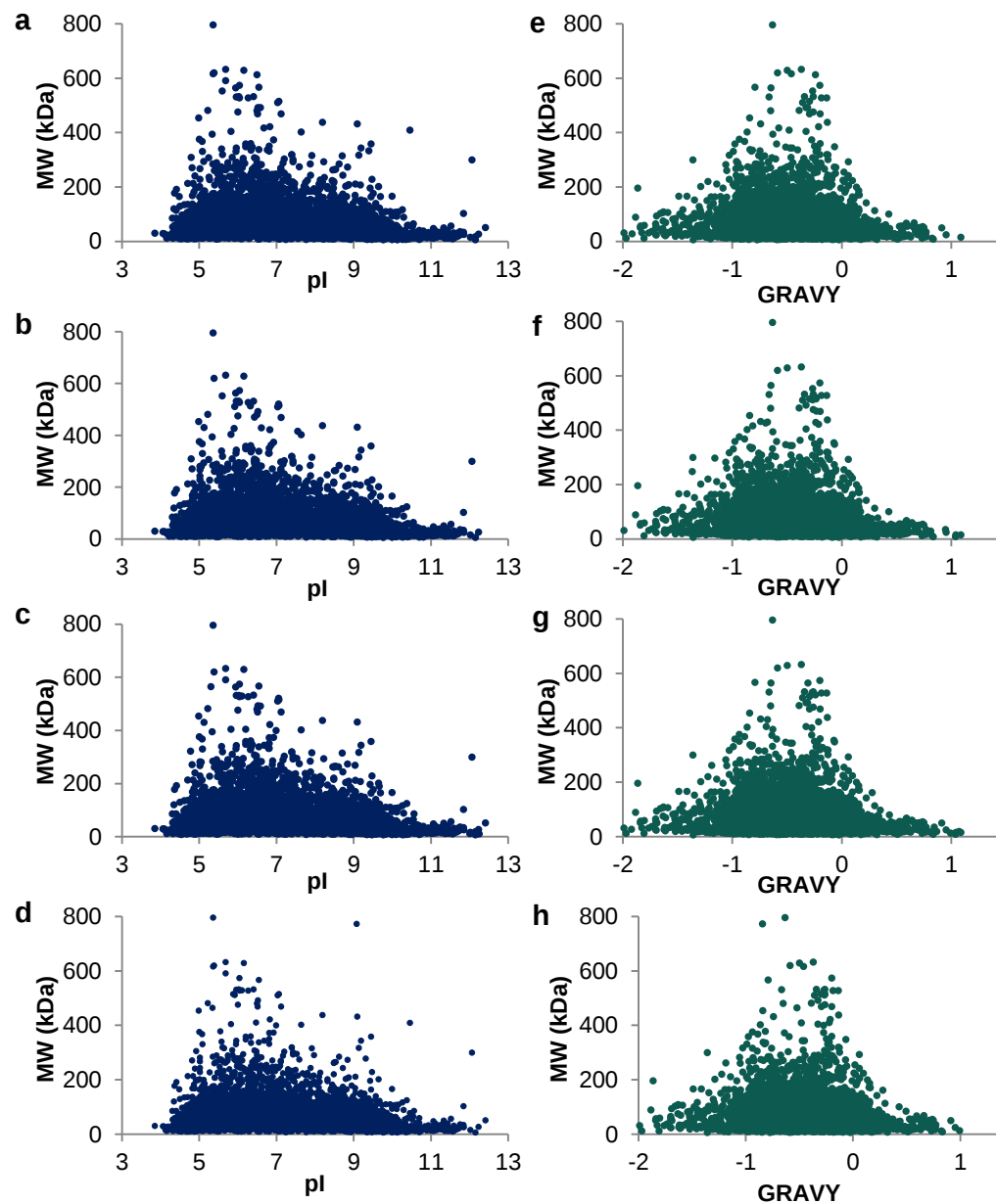
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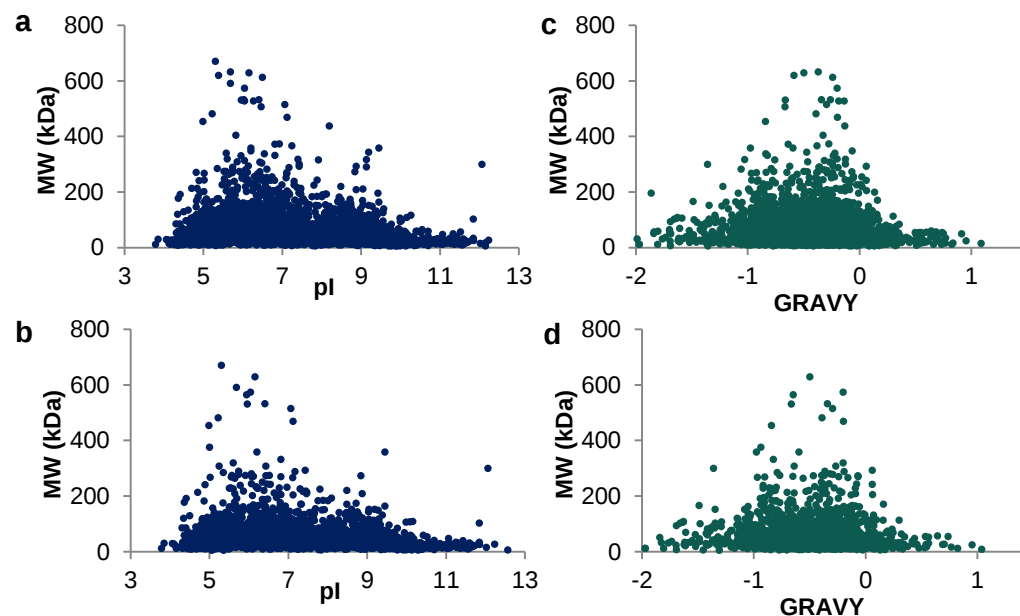
	Lysis Buffer	Digestion	Fractionation
Protein Extraction Samples			
Label Compatible	Label Compatible	In-gel	SDS-PAGE
Lysis-M	Lysis-M	In-gel	SDS-PAGE
Modified RIPA	Modified RIPA	In-gel	SDS-PAGE
SDT	SDT	In-gel	SDS-PAGE
Protein Digestion Samples			
In-solution	Label Compatible	In-solution	*N/A
Filter-aided	Label Compatible	Filter-aided	*N/A
Fractionation Samples			
SCX Spin Tip	Label Compatible	In-solution	SCX Spin Tip
C18 SepPak	Label Compatible	In-solution	C18 SepPak
SDS-PAGE	Label Compatible	In-gel	SDS-PAGE

*Whole proteome digestion samples were analyzed with a 3 hour LC run time instead of one of the other evaluated fractionation methods.

Electronic Supplementary Information 1 Sample preparations for evaluated extraction, digestion, and fractionation methods



Electronic Supplementary Information 2 Distribution of MW, pI range and hydrophobicity for proteins identified with each lysis buffer (a, e) Label Compatible, (b, f) SDT, (c, g) Lysis M, (d, h) Modified RIPA. Positive Gravy scores denote hydrophobic character whereas negative Gravy score denote hydrophilic character.



Electronic Supplementary Information 3 Distribution of MW, pI range and hydrophobicity for proteins identified with each digestion method (a, c) Filter aided, (b, d) In solution. Positive Gravy scores denote hydrophobic character whereas negative Gravy score denote hydrophilic character.

a SDS-PAGE		Fraction							
Fraction		1	2	3	4	5	6	7	8
	1								
	2	121							
	3	65	192						
	4	48	95	156					
	5	46	62	74	109				
	6	40	73	90	94	110			
	7	39	57	77	90	87	182		
	8	34	48	58	57	69	93	157	

b SCX Spin Tips		Fraction							
Fraction		1	2	3	4	5	6	7	8
	1								
	2	741							
	3	796	697						
	4	605	570	733					
	5	748	513	642	550				
	6	858	574	674	549	778			
	7	558	446	535	452	583	579		
	8	751	540	657	532	732	945	579	

c C18 SepPak		Fraction							
Fraction		1	2	3	4	5	6	7	8
	1								
	2	73							
	3	51	632						
	4	53	701	453					
	5	52	642	407	1230				
	6	49	639	407	1098	1399			
	7	48	514	360	817	996	1136		
	8	28	185	155	206	213	199	190	

Electronic Supplementary Information 4 Protein identification overlap between fractions for (a) SDS-PAGE (b) SCX Spin Tips (c) C18 SepPak. Fractions number is indicated on top and left.