

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Offline Pentafluorophenyl (PFP)-RP prefractionation as an alternative to high-pH RP for comprehensive LC-MS/MS proteomics and phosphoproteomics

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Additional files available under “[Supplementary material](#)”.

ESM_1 Chromatographic Gradient Tables

Table S1a Hi-pH RP (pH8) TMT labeled peptide prefractionation

Time (min)	%B	Flow Rate ($\mu\text{L}/\text{min}$)
0	5	150
1.5	5	150
6	12	150
14	16.5	150
62	42	150
63	100	150
69	100	150
70	5	150
98	5	150

Table S1b PFP TMT labeled peptide prefractionation

Time (min)	%B	Flow Rate ($\mu\text{L}/\text{min}$)
0	5	135
1	5	135
1.5	16	135
61	55	135
62	100	135
68	100	135
69	5	135
97	5	135

Table S1c Hi-pH RP (pH8) phosphopeptide prefractionation

Time (min)	%B	Flow Rate ($\mu\text{L}/\text{min}$)
0	0	135
1.5	0	135
6	7	135
14	11	135
62	37	135
63	100	135
69	100	135
70	0	135
98	0	135

Table S1d PFP phosphopeptide prefractionation

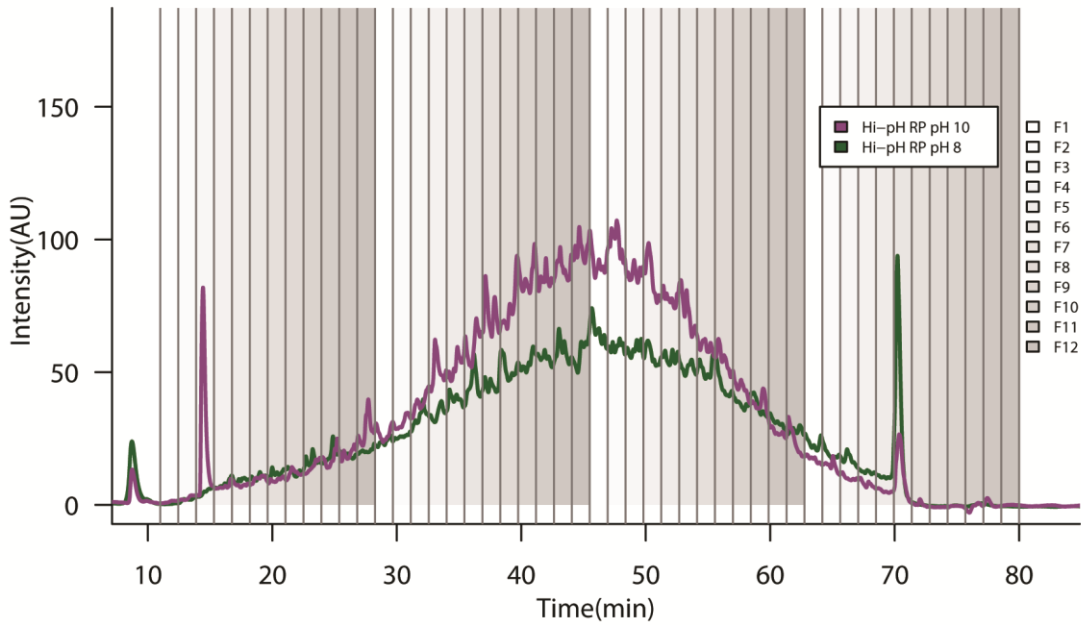
Time (min)	%B	Flow Rate ($\mu\text{L}/\text{min}$)
0	5	135
1	5	135
1.5	25	135
61.5	82	135
62	100	135
68	100	135
69	5	135
100	5	135

Table S1e Hi-pH RP (pH10) TMT labeled peptide prefractionation

Time (min)	%B	Flow Rate (μ L/min)
0	5	150
1.5	5	150
6	12	150
14	16.5	150
62	48	150
63	100	150
69	100	150
70	5	150
98	5	150

ESM_2

a



b

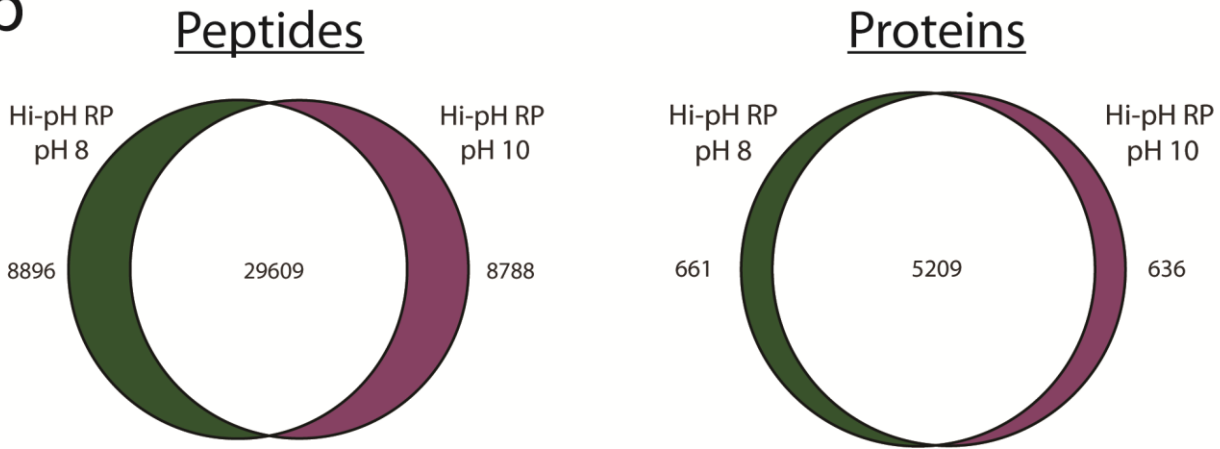


Fig. S1 pH 8 and pH 10 Hi-pH RP Peptide Identifications (a) Chromatograms are of 25 μ g TMT labeled peptides with fractional combinations overlaid; every 12th fraction was combined. (b) Proportional Venn diagrams illustrating the similarity of peptide and protein identifications from pH 8 and pH 10 Hi-pH RP

ESM_3 Spreadsheet of Peptides Identified in pH 8 and pH 10 Hi-pH RP Analyses (see separate files)

ESM_4 Spreadsheet of Proteins Identified in pH 8 and pH 10 Hi-pH RP Analyses (see separate files)

ESM_5

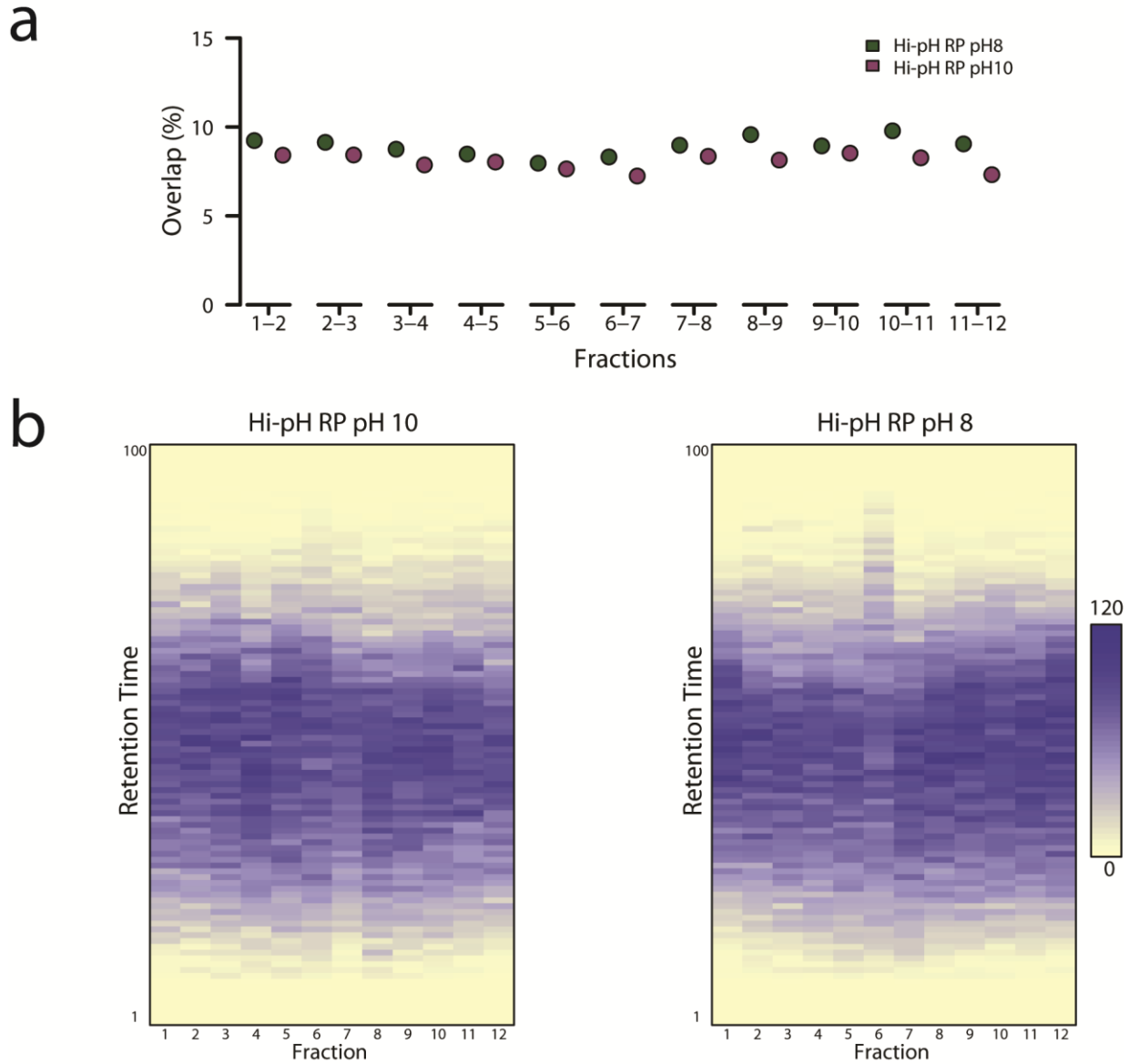


Fig. S2 pH 8 and pH 10 Hi-pH RP Fractional Overlap and Orthogonality (a) Overlap between each adjacent fraction is expressed as a percentage of the total peptides identified between the pair of fractions. (b) Heat maps of unique peptide retention time distributions plotted for each combined fraction in pH 8 and pH 10 Hi-pH RP analyses. Each peptide was allocated to 1 of 100 bins based on its average retention time between 1 and 138 minutes. The white-blue scale represents 0-120 peptides in each bin.

ESM_6 Spreadsheet of Peptides Identified in PFP RP Analyses (see separate files)

ESM_7 Spreadsheet of Proteins Identified in PFP RP Analyses (see separate files)

ESM_8 Spreadsheet of Peptides Identified in Hi-pH RP Analyses (see separate files)

ESM_9 Spreadsheet of Proteins Identified in Hi-pH RP Analyses (see separate files)

ESM_10 Spreadsheet of Phosphorylated Peptides Identified in PFP and Hi-pH RP Analyses (see separate files)