Supplementary Information

A stationary phase with a positively charged surface allows for minimizing formic acid concentration in the mobile phase, enhancing electrospray ionization in LC-MS proteomic experiments

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Table S1. MS1 and DDA settings for all experiments.

Analysis of iRT and Alberta peptides		
MS1 settings	Resolution at 200 m/z	60,000
	AGC target	1×10^6
	Maximum injection time	60 ms
	Scan range	300 to 1500 m/z
Analysis of trastuzumab digest with a reduced formic acid concentration in the mobile phase		
MS1 settings	Resolution at 200 m/z	60,000
	AGC target	1×10^6
	Maximum injection time	60 ms
	Scan range	300 to 1500 m/z
DDA and MS2 settings	Charge states of precursors	≥2 and ≤5
	Isolation window	2.5 m/z
	Normalized collision energy for HCD	27
	Resolution at 200 m/z	15 000
	AGC target	2×10^5
	Maximum injection time	100 ms
	Exclusion time	3 s
	Max. number of precursors	2
Effect of formic acid concentration in analysis of digest of bacterium & in analysis of a complex eukaryotic cell		
MS1 settings	Resolution at 200 m/z	60,000
	AGC target	3×10^6
	Maximum ion time	110 ms
	Scan range	350 to 1500 m/z
DDA and MS2 settings	Charge states of precursors	≥2 and ≤5
	Isolation window	1.8 m/z
	Normalized collision energy for HCD	27
	Resolution at 200 m/z	15 000
	AGC target	2×10^5
	Maximum injection time	50 ms
	Exclusion time	20 s
	Max. number of precursors	10
Effect of formic acid concentration in analysis of a complex HeLa cells lysate digest usig nano-flow LC-MS		
MS1 settings	Resolution at 200 m/z	70,000
	AGC target	3×10^6
	Maximum ion time	100 ms
	Scan range	350 to 1600 m/z
DDA and MS2 settings	Charge states of precursors	≥2 and ≤7
	Isolation window	2.0 m/z
	Normalized collision energy for HCD	28
	Resolution at 200 m/z	17,500
	AGC target	1.0×10^5
	Maximum ion time	60 ms
	Exclusion time	20 s
	Max. number of precursors	10

Table S2. pH measured at different concentrations of formic acid.

Concentration of formic acid in the mobile phase (%)	Measured pH of solution
0.10	2.63
0.08	2.70
0.06	2.74
0.04	2.84
0.02	3.00
0.01	3.20

Table S3. Retention time of iRT and Alberta peptides when separated using mobile phase acidified with varied concentrations of formic acid on BEH and CSH columns.

Retention time (min) of iRT peptides when separated on CSH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	4.96	4.97	4.97	5.71	5.71	5.71	6.16	6.16	6.17	7.06	7.05	7.07	8.69	8.69	8.69
0.08	4.98	4.97	4.97	5.73	5.72	5.72	6.16	6.16	6.16	7.08	7.08	7.08	8.68	8.66	8.69
0.06	4.97	4.93	4.97	5.72	5.7	5.72	6.15	6.12	6.15	7.11	7.08	7.10	8.67	8.65	8.67
0.04	4.97	4.96	4.97	5.74	5.72	5.73	6.14	6.15	6.14	7.16	7.16	7.16	8.66	8.66	8.66
0.02	4.99	5.00	5.00	5.77	5.78	5.78	6.15	6.16	6.16	7.31	7.30	7.31	8.68	8.68	8.69
0.01	5.06	5.06	5.06	5.85	5.85	5.85	6.20	6.20	6.20	7.49	7.47	7.48	8.76	8.76	8.76

Retention time (min) of iRT peptides when separated on BEH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	5.71	5.71	5.72	6.74	6.74	6.73	6.99	6.99	6.99	7.81	7.81	7.8	9.78	9.78	9.78
0.08	5.69	5.70	5.69	6.71	6.70	6.70	6.96	6.95	6.95	7.80	7.78	7.78	9.75	9.75	9.75
0.06	5.66	5.67	5.66	6.68	6.67	6.67	6.93	6.91	6.92	7.76	7.75	7.76	9.67	9.68	9.68
0.04	5.61	5.63	5.62	6.63	6.63	6.63	6.86	6.86	6.87	7.73	7.73	7.73	9.61	9.6	9.61
0.02	5.56	5.56	5.57	6.59	6.57	6.58	6.80	6.79	6.79	7.73	7.72	7.73	9.51	9.51	9.51
0.01	5.52	5.50	5.51	6.53	6.53	6.53	6.72	6.72	6.72	7.72	7.72	7.71	9.41	9.41	9.41

Retention time (min) of Alberta peptides when separated on CSH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.10	5.92	5.92	5.91	5.53	5.53	5.53	5.45	5.44	5.43	5.68	5.67	5.67
0.08	5.90	5.91	5.91	5.5	5.51	5.50	5.42	5.42	5.42	5.64	5.65	5.65
0.06	5.91	5.90	5.90	5.49	5.5	5.49	5.39	5.39	5.38	5.6	5.61	5.6
0.04	5.90	5.90	5.90	5.47	5.48	5.47	5.36	5.36	5.36	5.56	5.57	5.57
0.02	5.92	5.92	5.93	5.46	5.46	5.46	5.32	5.32	5.34	5.51	5.51	5.52
0.01	5.98	5.97	5.97	5.46	5.47	5.47	5.30	5.30	5.30	5.48	5.48	5.48

Retention time (min) of Alberta peptides when separated on BEH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.10	6.54	6.54	6.54	6.46	6.47	6.46	6.53	6.54	6.54	6.87	6.88	6.87
0.08	6.52	6.52	6.52	6.43	6.43	6.43	6.49	6.49	6.49	6.82	6.81	6.82
0.06	6.49	6.48	6.49	6.39	6.38	6.38	6.42	6.43	6.42	6.74	6.74	6.73
0.04	6.44	6.44	6.44	6.31	6.32	6.31	6.35	6.35	6.35	6.65	6.64	6.65
0.02	6.37	6.36	6.37	6.21	6.21	6.21	6.2	6.2	6.21	6.47	6.47	6.49
0.01	6.30	6.30	6.30	6.11	6.11	6.11	6.07	6.07	6.08	6.31	6.32	6.32

Table S4. USP symmetry factor of iRT and Alberta peptide peaks when separated using mobile phase acidified with varied concentrations of formic acid on BEH and CSH columns.

USP symmetry factor of iRT peptides when separated on the CSH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	1.200	1.180	1.190	1.200	1.215	1.218	1.189	1.208	1.186	1.170	1.182	1.170	1.056	1.049	1.060
0.08	1.230	1.094	1.136	1.190	1.173	1.191	1.168	1.184	1.184	1.148	1.168	1.168	1.034	1.034	1.046
0.06	1.055	1.054	1.067	1.157	1.186	1.190	1.150	1.150	1.151	1.150	1.149	1.169	1.019	1.028	1.033
0.04	1.031	1.035	1.041	1.173	1.141	1.137	1.155	1.147	1.162	1.144	1.117	1.125	1.023	1.033	1.004
0.02	0.993	0.986	0.993	1.103	1.073	1.093	1.096	1.116	1.113	1.125	1.117	1.098	1.000	1.023	1.004
0.01	0.990	1.000	0.980	1.045	1.030	1.040	1.058	1.055	1.053	1.166	1.149	1.149	0.993	0.995	0.981

USP symmetry factor of iRT peptides when separated on the BEH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	1.320	1.330	1.290	1.509	1.472	1.509	1.415	1.420	1.420	1.280	1.310	1.298	1.302	1.246	1.280
0.08	1.338	1.309	1.301	1.472	1.472	1.518	1.398	1.381	1.379	1.312	1.312	1.298	1.290	1.285	1.290
0.06	1.300	1.322	1.307	1.470	1.490	1.482	1.410	1.415	1.433	1.357	1.348	1.342	1.220	1.242	1.269
0.04	1.320	1.310	1.310	1.482	1.490	1.473	1.416	1.398	1.398	1.401	1.410	1.403	1.335	1.320	1.310
0.02	1.388	1.365	1.359	1.509	1.518	1.473	1.390	1.416	1.372	2.534	2.389	2.412	1.230	1.257	1.250
0.01	1.320	1.360	1.320	1.280	1.310	1.280	1.350	1.390	1.380	2.290	2.320	2.330	1.180	1.170	1.200

USP symmetry factor of Alberta peptides when separated on the CSH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.10	1.230	1.250	1.240	1.280	1.260	1.280	1.190	1.230	1.250	1.230	1.220	1.220
0.08	1.257	1.253	1.253	1.260	1.250	1.268	1.188	1.250	1.200	1.230	1.240	1.260
0.06	1.231	1.220	1.220	1.260	1.239	1.232	1.238	1.227	1.227	1.210	1.220	1.240
0.04	1.220	1.240	1.240	1.219	1.256	1.253	1.230	1.200	1.191	1.250	1.220	1.220
0.02	1.148	1.197	1.190	1.189	1.208	1.240	1.160	1.160	1.140	1.197	1.230	1.210
0.01	1.150	1.130	1.160	1.180	1.150	1.190	1.080	1.100	1.080	1.200	1.200	1.200

USP symmetry factor of Alberta peptides when separated on the BEH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.1	1.381	1.406	1.372	1.528	1.446	1.490	1.650	1.550	1.630	1.651	1.620	1.640
0.08	1.388	1.432	1.433	1.518	1.518	1.509	1.629	1.639	1.622	1.568	1.605	1.633
0.06	1.407	1.416	1.415	1.500	1.509	1.490	1.672	1.634	1.680	1.631	1.680	1.675
0.04	1.410	1.460	1.450	1.563	1.500	1.570	1.693	1.666	1.704	1.680	1.670	1.660
0.02	1.280	1.294	1.280	1.508	1.525	1.491	1.805	1.828	1.833	1.758	1.738	1.741
0.01	1.280	1.290	1.280	1.500	1.520	1.490	1.800	1.820	1.830	1.750	1.730	1.740

Table S5. Peak width ($w_{50\%}$) of iRT and Alberta peptide peaks when separated using mobile phase acidified with varied concentrations of formic acid on BEH and CSH columns.

Peak width ($w_{50\%}$, s) of iRT peptides when separated on CSH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	1.56	1.56	1.56	1.50	1.50	1.50	1.50	1.44	1.50	1.62	1.62	1.56	1.56	1.56	1.56
0.08	1.62	1.56	1.56	1.50	1.50	1.50	1.50	1.44	1.50	1.56	1.62	1.62	1.62	1.56	1.56
0.06	1.56	1.56	1.56	1.50	1.50	1.50	1.50	1.50	1.50	1.62	1.62	1.62	1.56	1.56	1.56
0.04	1.56	1.62	1.62	1.50	1.50	1.50	1.50	1.50	1.50	1.62	1.62	1.62	1.56	1.62	1.62
0.02	1.68	1.68	1.68	1.56	1.56	1.62	1.56	1.56	1.56	1.74	1.74	1.68	1.68	1.68	1.68
0.01	1.74	1.74	1.74	1.74	1.68	1.68	1.62	1.62	1.62	1.80	1.80	1.80	1.74	1.74	1.74

Peak width ($w_{50\%}$, s) of iRT peptides when separated on BEH column

$C_{(formic\ ac.)}$ %	GAG			YIL			TPV			ADV			GTF		
0.10	2.22	2.22	2.22	2.22	2.22	2.16	2.04	2.04	2.04	1.92	1.92	1.92	2.22	2.22	2.22
0.08	2.22	2.22	2.27	2.22	2.22	2.22	2.10	2.10	2.10	1.92	1.92	1.98	2.22	2.22	2.27
0.06	2.28	2.22	2.28	2.22	2.22	2.22	2.04	2.10	2.10	1.92	1.92	1.98	2.28	2.22	2.28
0.04	2.28	2.28	2.28	2.22	2.22	2.22	2.10	2.10	2.10	1.98	1.92	1.98	2.28	2.28	2.28
0.02	2.34	2.34	2.34	2.28	2.28	2.22	2.10	2.10	2.10	2.40	2.40	2.34	2.34	2.34	2.34
0.01	2.52	2.46	2.52	2.22	2.22	2.22	2.16	2.16	2.16	2.28	2.28	2.28	2.52	2.46	2.52

Peak width ($w_{50\%}$, s) of Alberta peptides when separated on CSH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.10	1.50	1.50	1.50	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
0.08	1.50	1.38	1.50	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
0.06	1.50	1.50	1.50	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
0.04	1.50	1.50	1.50	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
0.02	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.44	1.44	1.44	1.44
0.01	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.50	1.50	1.50

Peak width ($w_{50\%}$, s) of Alberta peptides when separated on BEH column

$C_{(formic\ ac.)}$ %	ac-GGG $^{1+}$			ac-KYG $^{2+}$			ac-GGA $^{3+}$			ac-KYA $^{4+}$		
0.1	2.04	2.04	2.10	2.28	2.34	2.34	2.82	2.82	2.76	2.58	2.58	2.52
0.08	2.10	2.10	2.10	2.34	2.34	2.34	2.94	2.88	2.88	2.58	2.58	2.58
0.06	2.10	1.50	2.10	2.40	2.40	2.40	3.00	3.00	2.94	2.64	2.70	2.70
0.04	2.10	2.16	2.16	2.46	2.47	2.46	3.00	3.00	3.00	2.76	2.70	2.76
0.02	2.04	1.98	2.04	2.88	2.82	2.82	3.72	3.84	3.72	3.24	3.30	3.24
0.01	2.04	1.98	2.04	2.88	2.82	2.82	3.72	3.84	3.72	3.24	3.30	3.24

Figure S1. Base peak (BP) chromatogram of five iRT peptides separated on CSH column using mobile phase acidified with 0.10% (A) and 0.01% (B) of formic acid. Base peak chromatogram of four Alberta peptides separated on CSH column using mobile phase acidified with 0.10% (C) and 0.01% (D) of formic acid.

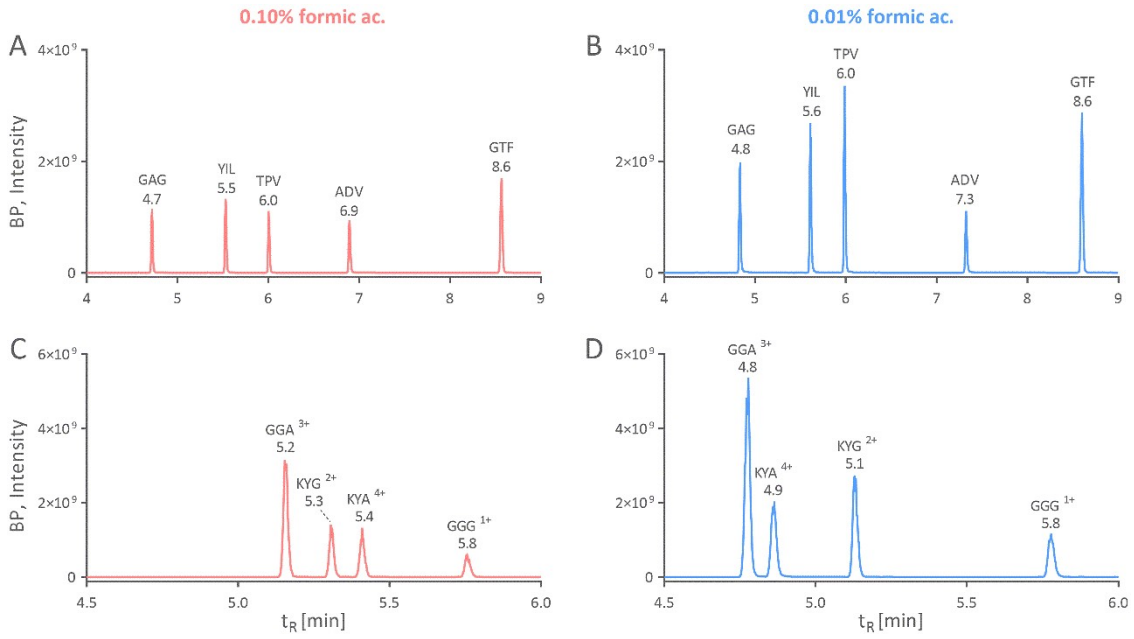

Figure S2. Intensity distribution for peptides identified from the tryptic digest of Jurkat cells exclusively in low formic acid concentration versus those identified in both conditions.

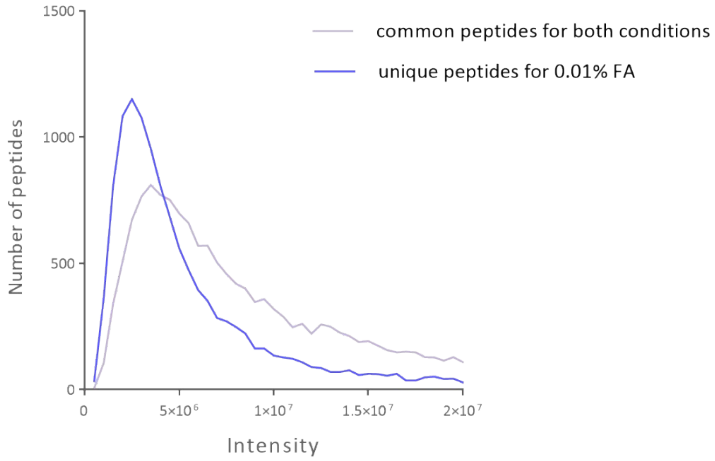

Figure S3. Percentage of peptides with artificial modifications identified against total identified peptides containing that particular amino acid when using 0.10% and 0.01% of formic acid as mobile phase acidifiers.