SUPPORTING INFORMATION:

Capillary HILIC-MS: a new tool for sensitive top-down proteomics

Andrea F.G. Gargano[a,b,c*], Liana S. Roca[a,c], Ryan T. Fellers [d], Max Bocxe [b], Elena Domínguez-Vega[b,e], Govert W. Somsen[a,b]

[a] Center for Analytical Science Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

[b] Vrije Universiteit Amsterdam, Amsterdam Institute for Molecules, Medicines and Systems, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

[c] Van 't Hoff Institute for Molecular Sciences, Science Park 904 1098 XH Amsterdam, Netherlands

[d] Departments of Chemistry, Molecular Biosciences and the Proteomics Center of Excellence, 2145 N. Sheridan Road, Evanston, Illinois 60208, United States

[e] Center for Proteomics and Metabolomics, Leiden University Medical Center, Postbus 9600, 2300 RC Leiden, The Netherlands

▪ TOC of the supporting information

Sections

*a.gargano@uva.nl

Dr. Andrea Gargano
Analytical Chemistry
Center for Analytical Science Amsterdam
Universiteit van Amsterdam

Tel: +31-20-525 7040
Science Park 904 |1098 XH Amsterdam
Building C2, room 248A

S.1 Retention in HILIC and RPLC vs Aliphatic Index

Here we report the linear regression between the % elution of solvent B of myoglobin, ubiquitin, cytochrome C, carbonic anhydrase, lysozyme, trypsinogen, RNAse A and their theoretical aliphatix index. Further details are available in Table 1 of the manuscript.

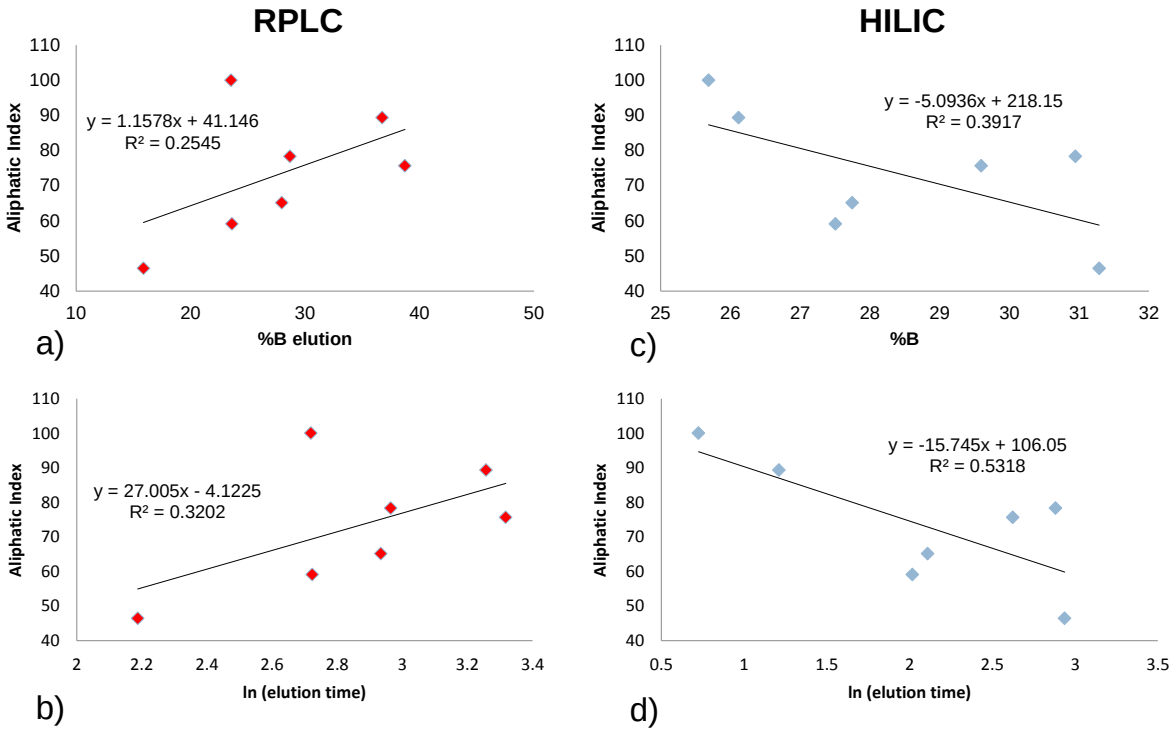


Figure S1: Scatter plots and linear regression of aliphatic index vs. percentage of elution solvent (a and c) and natural logarithm of the elution time (b,d) obtained during RPLC (a,b) and HILIC (C.d) for the proteins myoglobin, ubiquitin, cytochrome C, carbonic anhydrase, lysozyme, trypsinogen, RNAse A).

S.2 Breakthrough in HILIC

In Figure S2 we report the chromatograms used to calculate the protein area reported in Figure 2 of the manuscript.

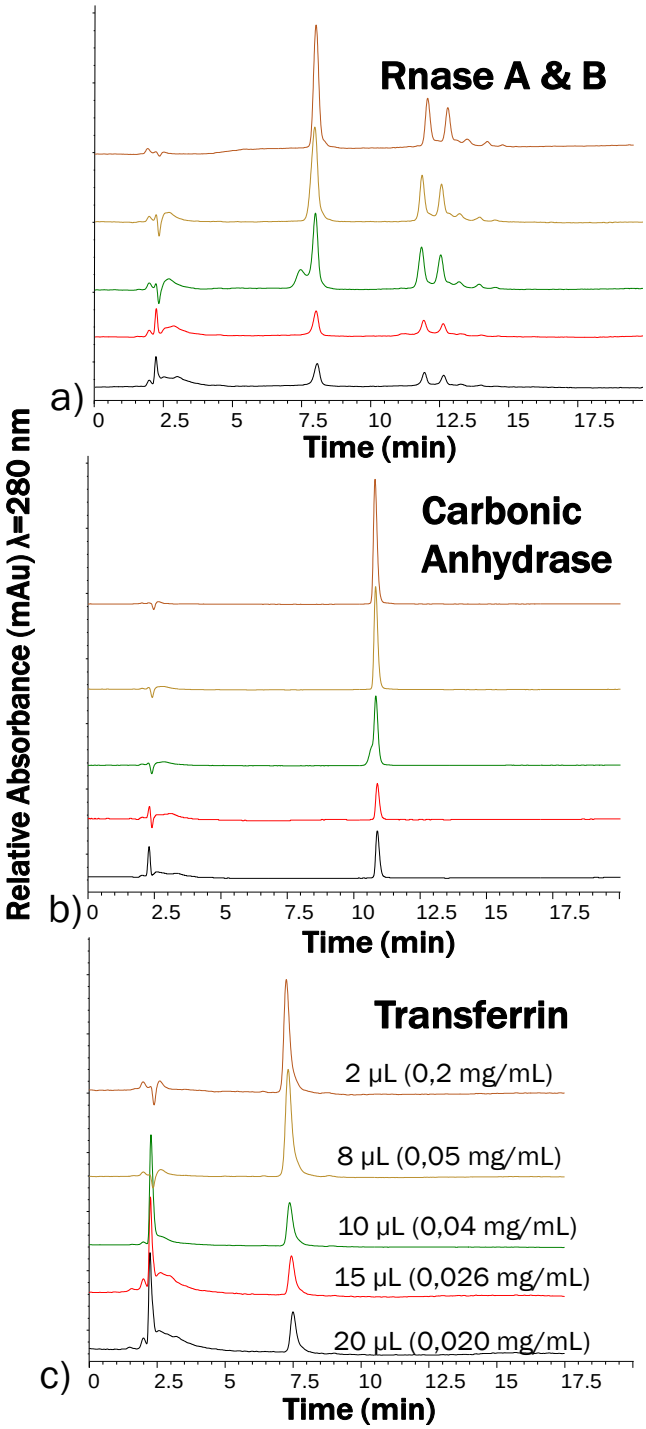


Figure S2: Effect of injection volume on HILIC of a) Ribonuclease A and B, b) Carbonic anhydrase, and c) Transferrin. Gradient solvents, see Figure 1. Linear gradient programming: a) and c) from 20% to 31% B in 1 min, to 45% B in 17 min, to 90% B in 1 min, 90% B for 20 min, and finally to 10% B in 1 min; b) 10%, to 20% B in 1 min, to 45% B in 17 min, to 90% B in 1 min, 90% B for 20 min, and finally to 10% B in 1 min. The Y axes of the different volume injection per protein are normalized to the response of the most intense peak at 2 µL.

S.3 Calibration curve for Cytochrome C and Ribonuclease on capillary HILIC

This subchapter present peak area data (Figure S3) and MS spectra (Figure S4) obtained from the injection of different mass quantity (from 5 to 400 ng using injection volumes varying between 1 and 20 µL) of a mixture of cytochrome C and ribonuclease A on a capillary HILIC method. The linear regression of mass quantity versus detected area demonstrates that this method can be used for quantitative purposes.

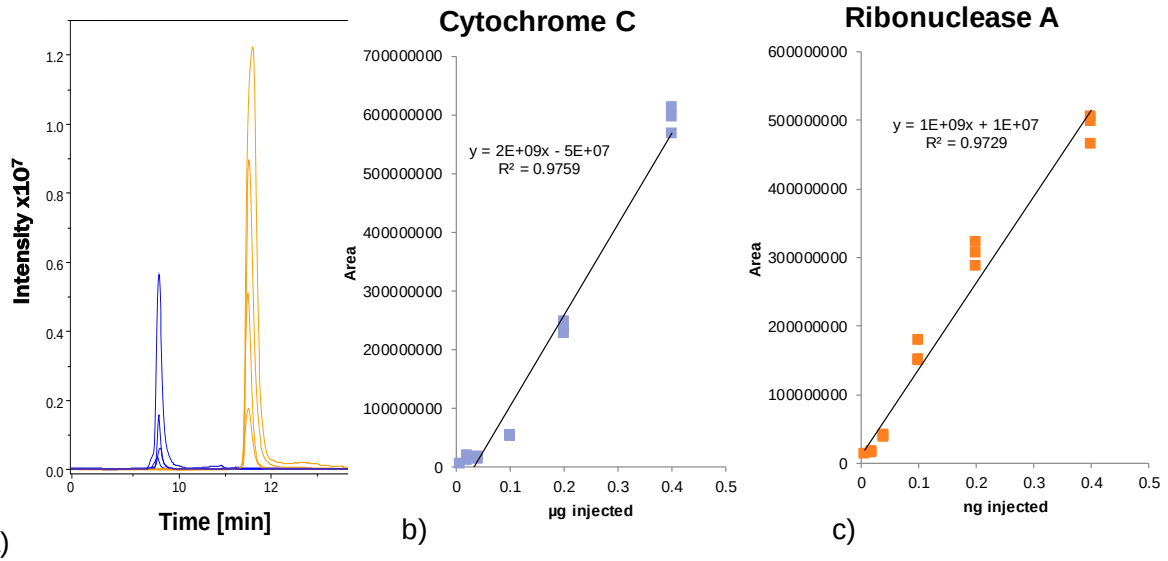


Figure S3: a) EICs obtained during capillary HILIC-MS of a mixture of cytochrome C (blue) and ribonuclease A (orange) after loading increasing protein mass (5-400 ng each) on the C4 trap column. b) and c) calibration curves obtained for both proteins by plotting peak area from TIC (triplicate injection) vs. loaded mass.

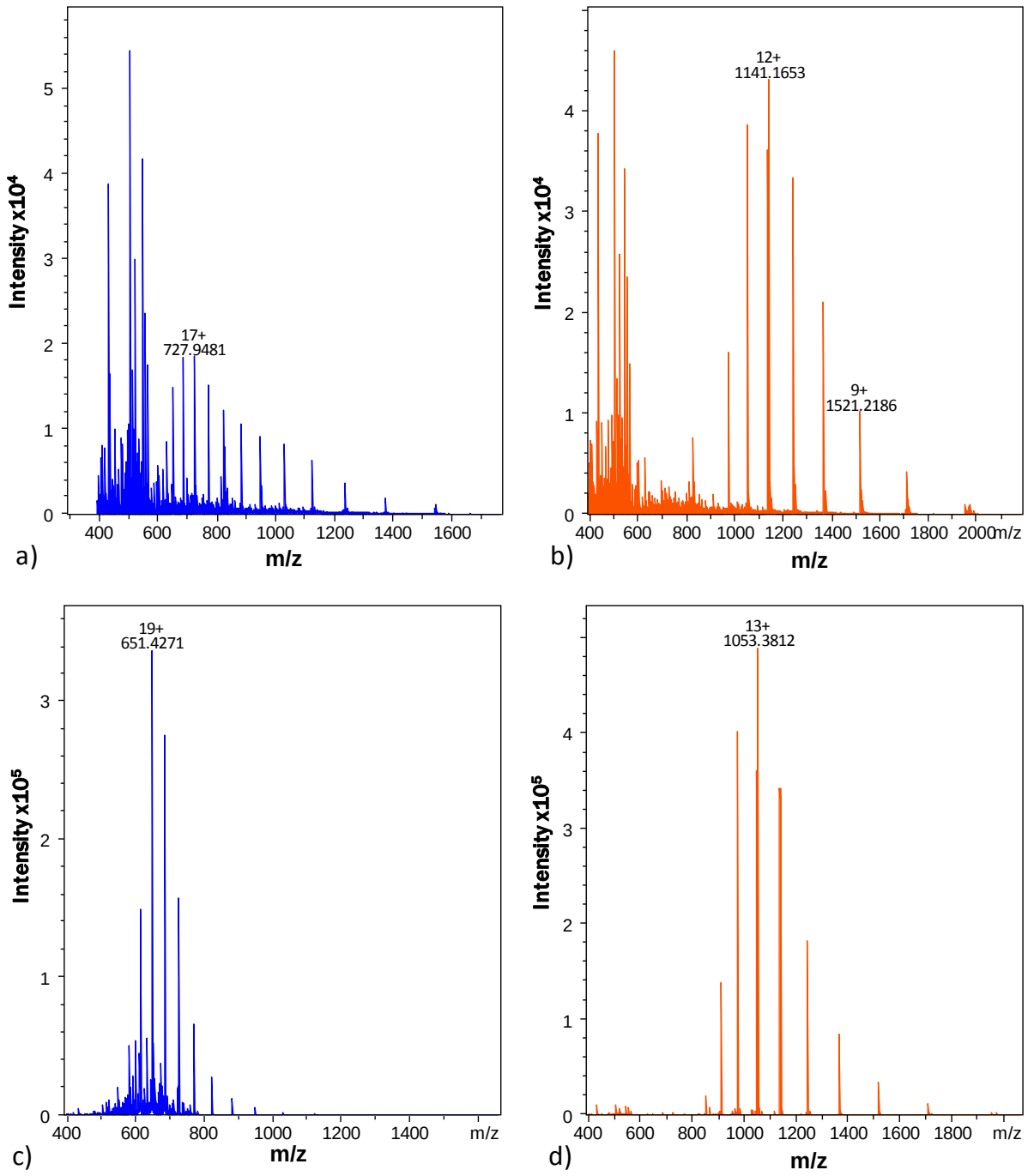
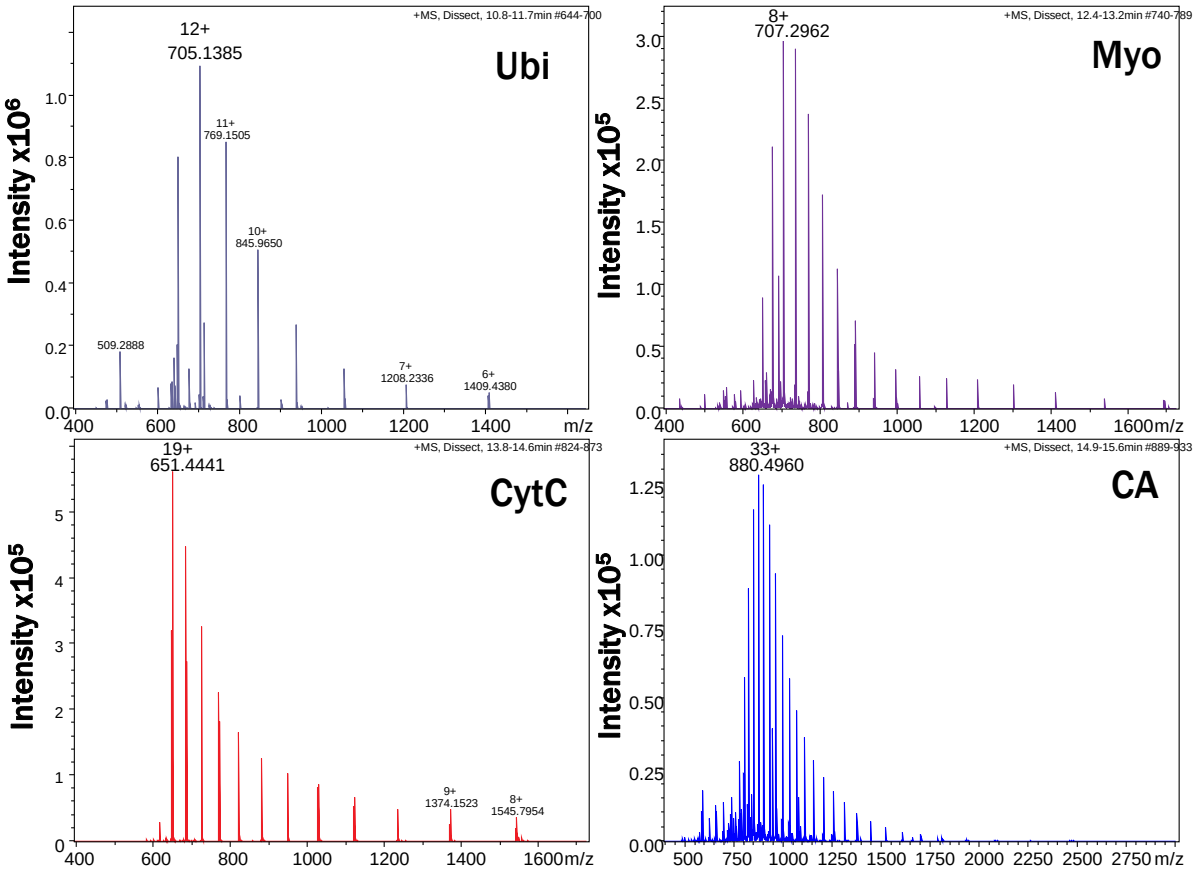
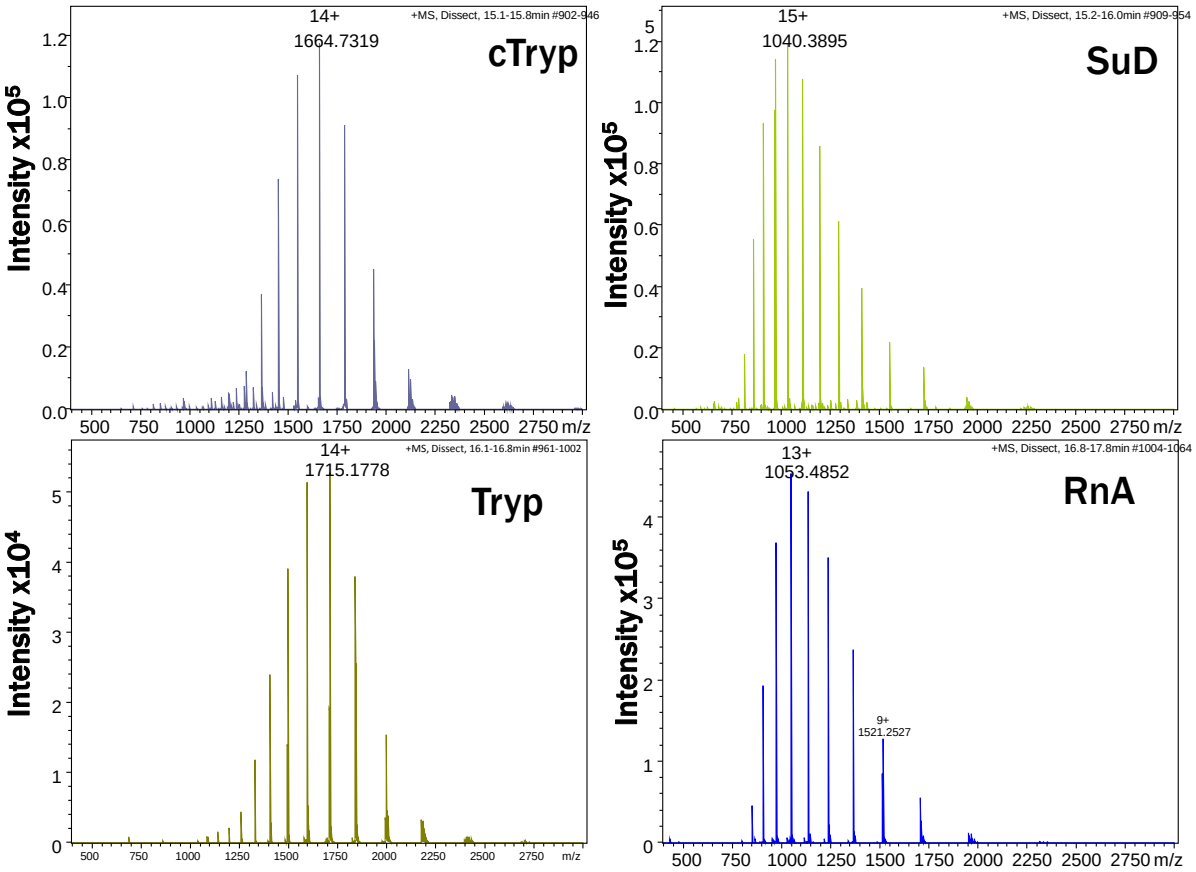


Figure S4: Mass spectra obtained during capillary HILIC-MS loading a mixture of cytochrome C (blue) and ribonuclease A (orange). a) and b) report the spectra at 5 ng loaded on the C4 trap column and c) and d) for 400 ng.

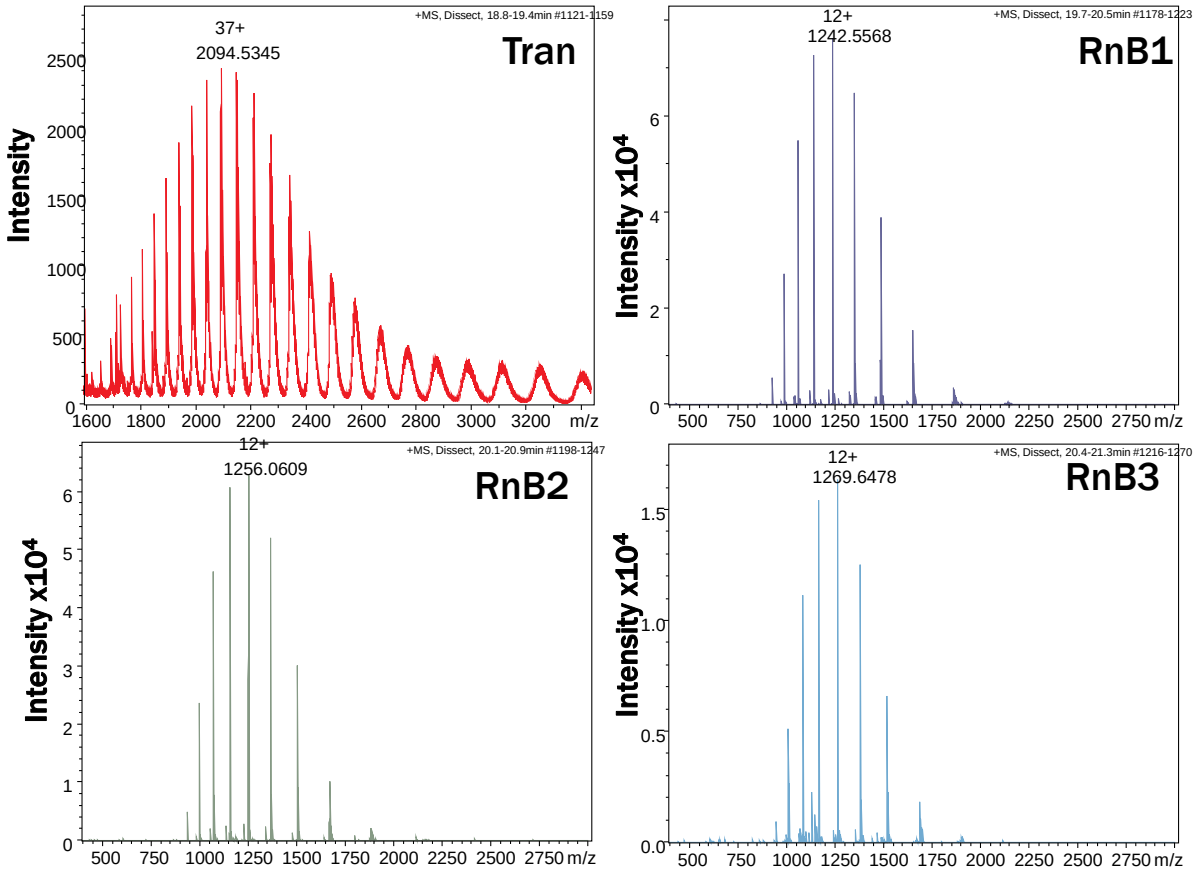
S.4 MS spectra of Figure 5

Here we reported the MS spectra (Figure S5), extracted ion current chromatogram (Figure S6) as well as peak capacity calculation (Table S1) of the analysis of a mixture of protein standards using an HILIC separation method.




cTryp
14+
1664.7319
+MS, Dissect, 15.1-15.8min #902-946
SuD
15+
1040.3895
+MS, Dissect, 15.2-16.0min #909-954
Tryp
14+
1715.1778
+MS, Dissect, 16.1-16.8min #961-1002
RnA
13+
1053.4852
9+
1521.2527
+MS, Dissect, 16.8-17.8min #1004-1064
Intensity x10⁵
Intensity x10⁴
m/z

85


Tran
37+
2094.5345
+MS, Dissect, 18.8-19.4min #1121-1159
RnB1
12+
1242.5568
+MS, Dissect, 19.7-20.5min #1178-1223
RnB2
12+
1256.0609
+MS, Dissect, 20.1-20.9min #1198-1247
RnB3
12+
1269.6478
+MS, Dissect, 20.4-21.3min #1216-1270
Intensity
Intensity x10⁴
m/z

86

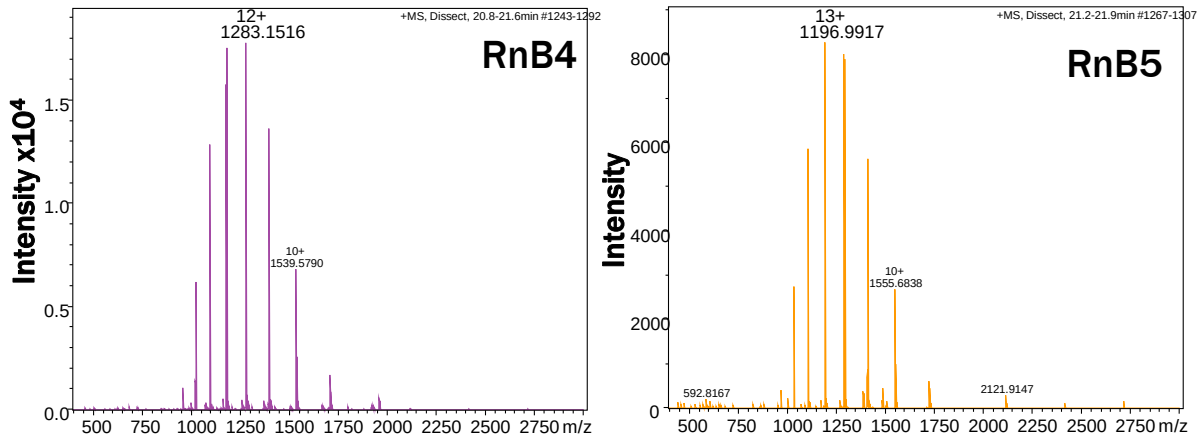


Figure S5: Mass spectra obtained during capillary HILIC-MS of a mixture of proteins. The TIC of the separation is reported in Figure 5 of the manuscript. The experimental conditions are reported in the experimental section of the manuscript and in Figure 5.

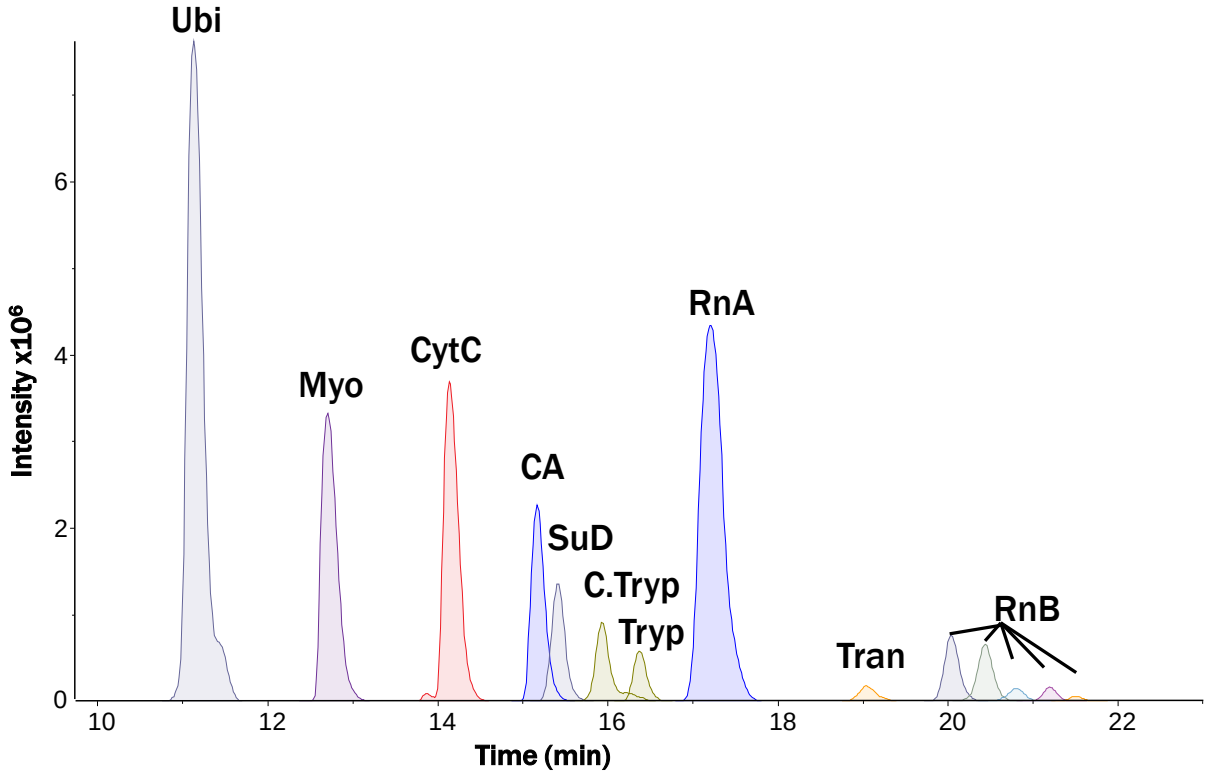


Figure S6: Extracted-ion chromatograms obtained during capillary HILIC-MS of a mixture of proteins. The experimental conditions are reported in the experimental section of the manuscript and in Figure 5.

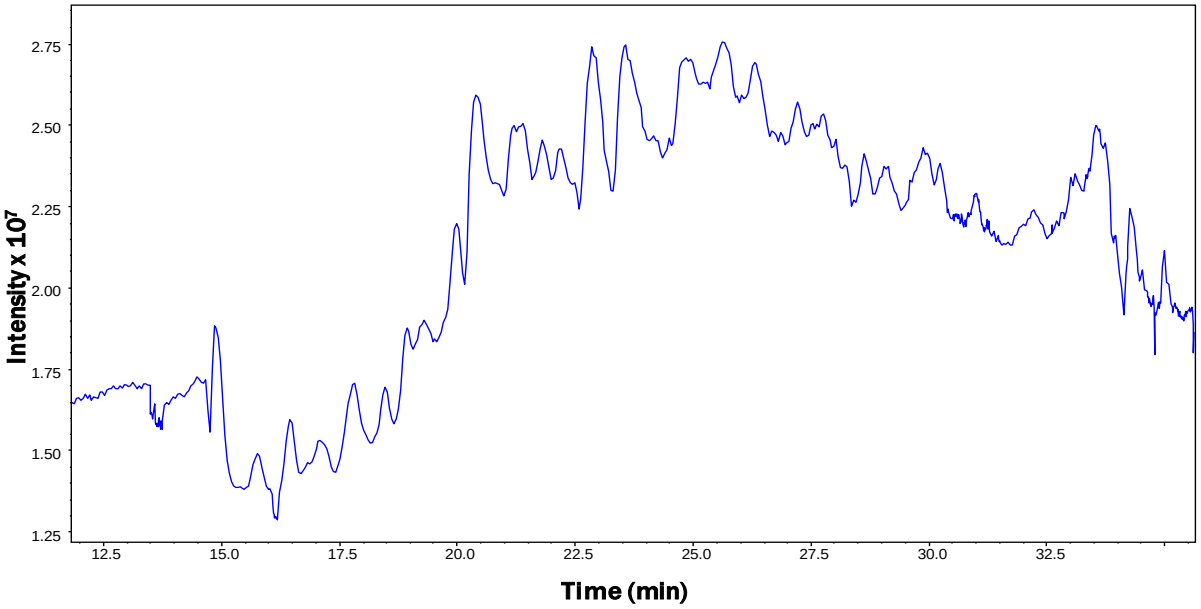
Table S1: Retention time, peak width at half height and peak capacity for the separation of the protein mixture reported in Figure 5 of manuscript and Figure S6.

Protein	RT [min]	Area	I	S/N	Max. m/z	FWHM [min]
Ubi	11.2	1.1E+08	7.57E+06	23697	705.1385	0.2
Myo	12.7	4.4E+07	3.28E+06	6207	707.2962	0.2
CytC	14.2	4.7E+07	3.63E+06	12029	651.4441	0.2
CA	15.2	2.6E+07	2.25E+06	2583	880.4960	0.2
SuD	15.4	1.6E+07	1.36E+06	2375	1664.7319	0.2
C.Tryp	16	1.2E+07	9.08E+05	1034	1714.0359	0.2
Tryp	16.4	6.7E+06	5.87E+05	1143	1715.1778	0.2
RnA	17.2	8.5E+07	4.34E+06	9120	1053.4852	0.3
Tran	19.1	2.8E+06	1.82E+05	53	2094.5345	0.2
RnB1	20.1	9.7E+06	7.57E+05	1526	1242.5568	0.2
RnB2	20.4	8.8E+06	6.64E+05	1294	1256.0609	0.2
RnB3	20.8	2.3E+06	1.55E+05	326	1269.6478	0.2
RnB4	21.2	2.1E+06	1.69E+05	361	1283.1516	0.2
RnB5	21.5	7.9E+05	6.50E+04	172	1196.9917	0.2
					Av. FWHM	0.21
					nc*	43.6

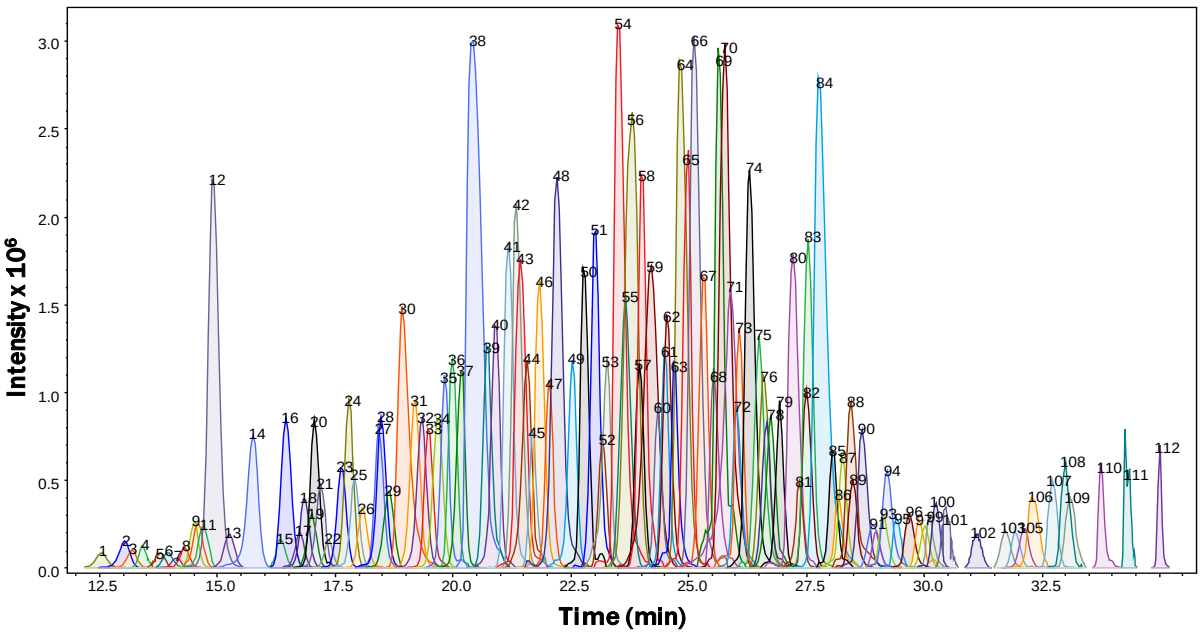
* Peak Capacity (n_c) was calculated according to $n_c = \frac{t_G}{1.7 \cdot (\bar{w}_{1/2h})} + 1$ using 15 min as t_G (based on the effective elution window).

S.5 HILIC- MS/MS analysis of E.coli Lysate

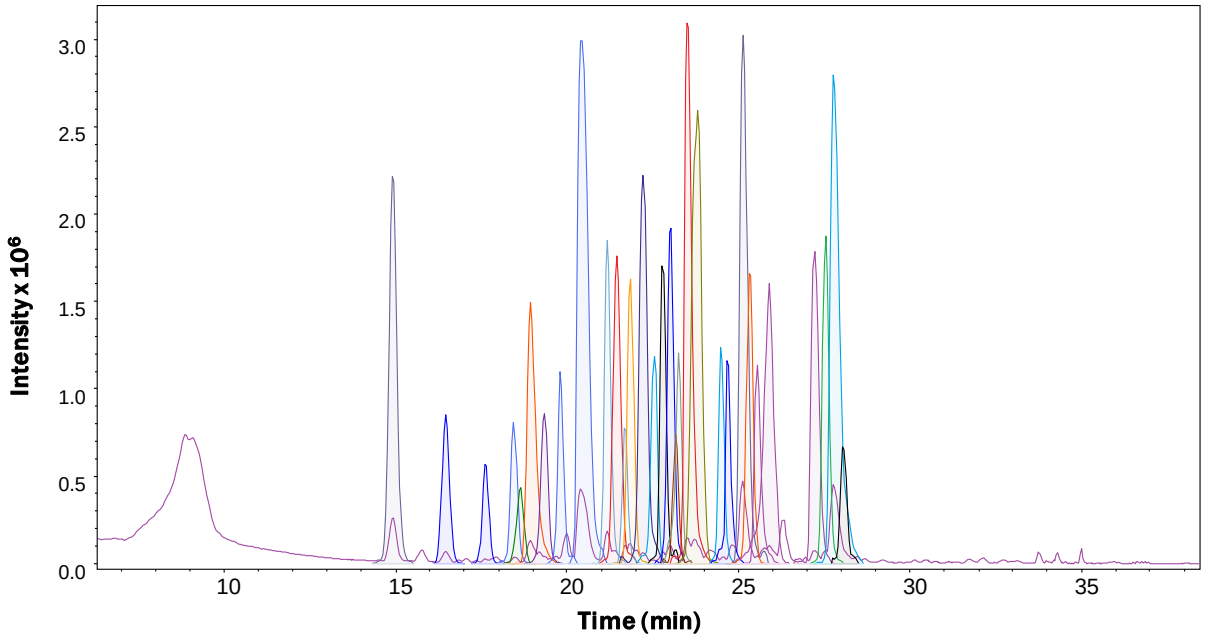
This subchapter gathers results from the HILIC-MS analysis of an E.coli Lysate (10 ug on column). Figure S7 reports TIC and dissected chromatograms, Figure S8 representative MS spectra at different time point of the LC-MS separation and table S2 the calculation used to determine the peak capacity of the separation. Figure S9 reports the protein identified performing MS/MS online (CID) and analyzing the data using TDportal



a)



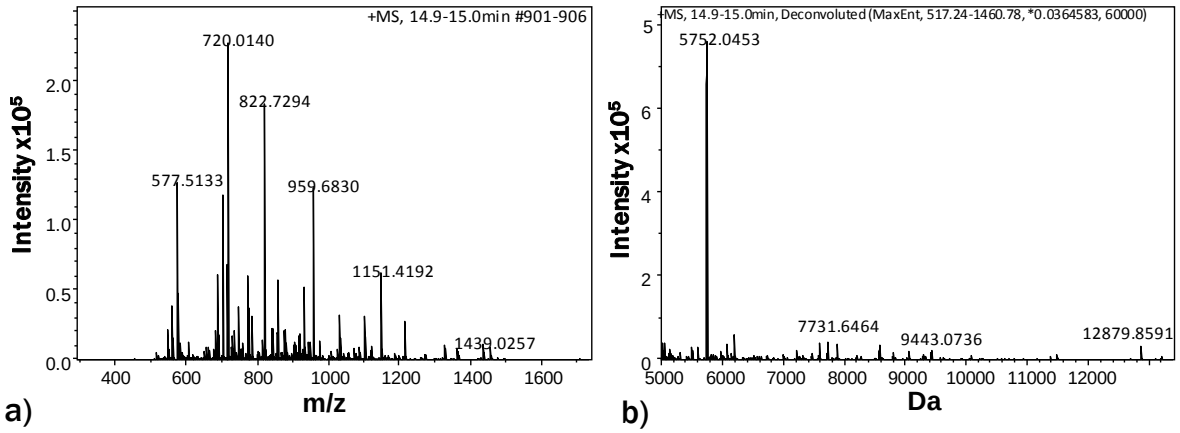
b)



c)

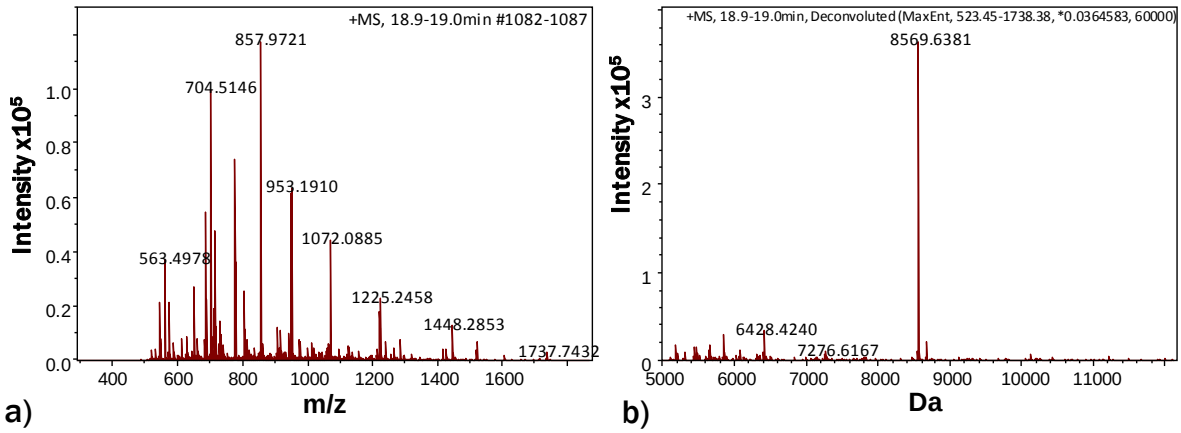
Figure S7: a) Total-ion and b) Extracted-ion chromatograms of the 112 (protein)-mass features (S/N 10) obtained using the dissect mass list algorithm (Bruker Data Analysis software) and c) overlay of base peak chromatogram and extracted ion chromatogram of the 28 identified proteoforms using CID and TD portal for analysis of capillary HILIC-MS/MS of an E.coli lysate. For experimental conditions see experimental section of the manuscript and Figure 6.

14.9-15.0 min



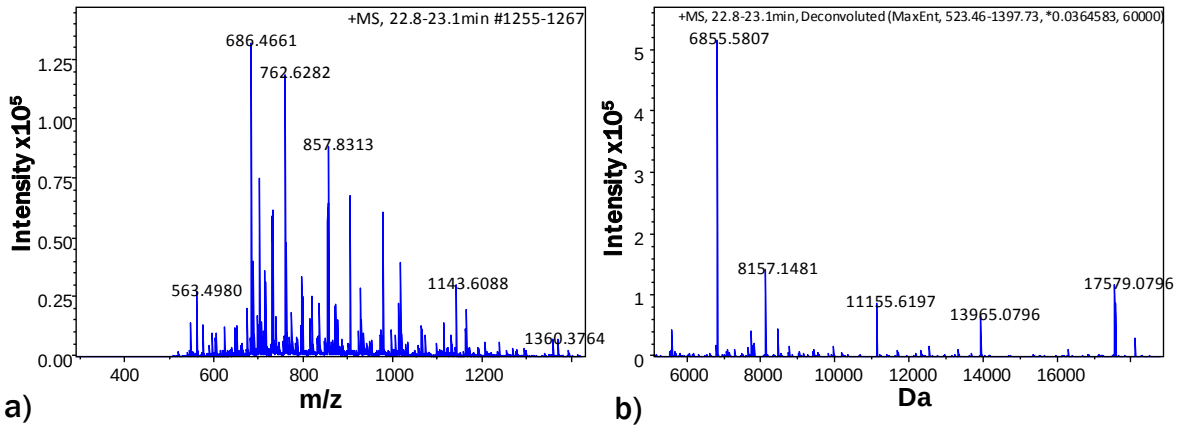
127

18.9-19.0 min



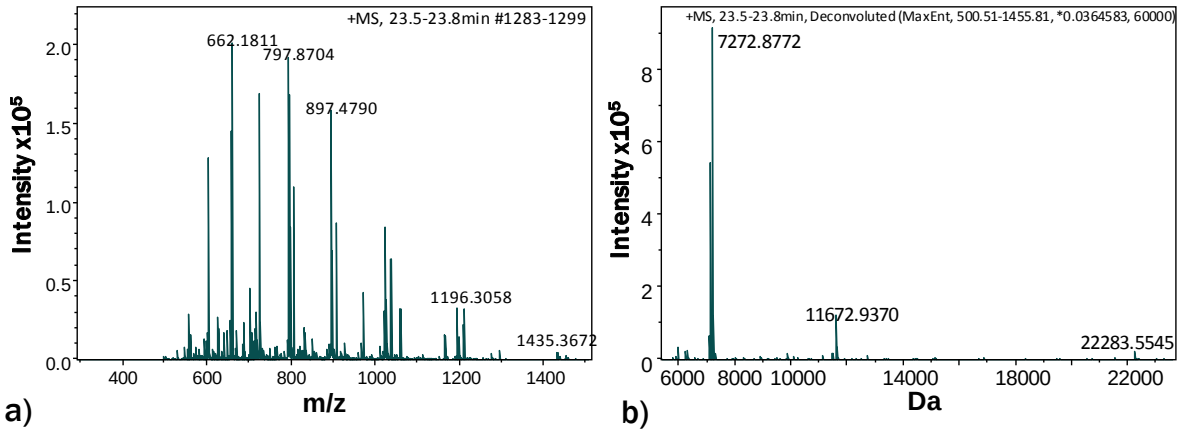
128

22.8-23.1 min



129

23.5-23.8 min



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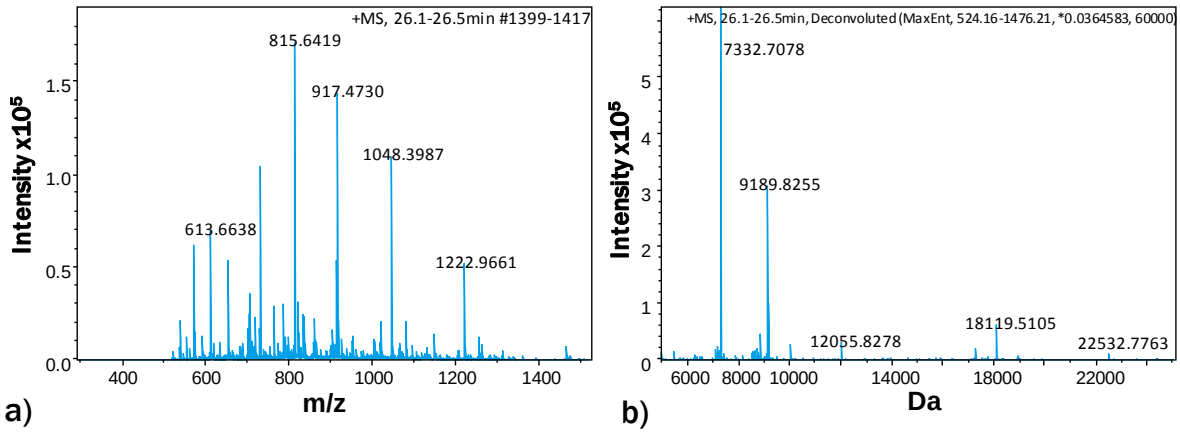
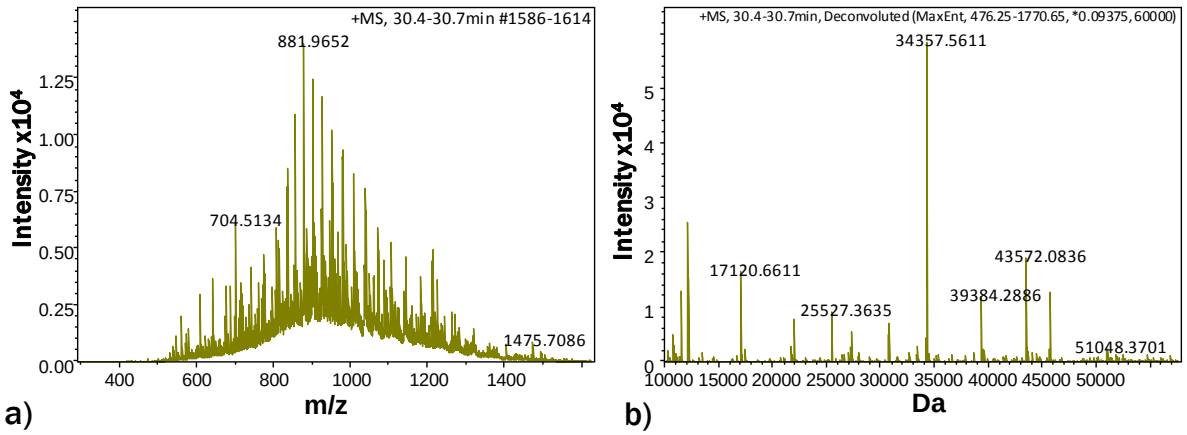
26.1-26.5 min



131

30.4-30.7 min



132

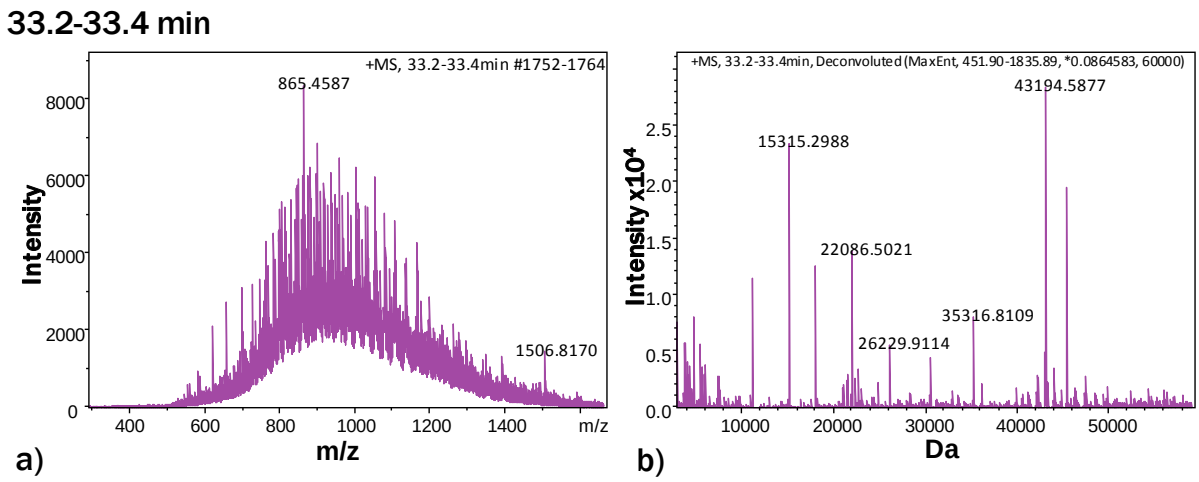


Figure S8: a) Mass spectra recorded in the indicated time intervals during capillary HILIC-MS of an E.coli lysate (see Figures 6 and S7). b) Deconvoluted mass spectra corresponding to mass spectra depicted in a).

Table S2: Retention time, peak area and width at half height and peak capacity for the separation of the EColi reported in Figures 6 and S7, analyzed using the dissect algorithm (Bruker DataAnalysis) using a S/N threshold of 10. Results from Promex analysis are reported in a separated excel file.

	RT [min]	Area	I	S/N	Max. m/z	FWHM [min]
1	12.6	1.60E+06	8.78E+04	17	630.85	0.3
2	13.1	2.47E+06	1.50E+05	40	577.5137	0.2
3	13.2	1.56E+06	1.02E+05	25	874.3068	0.2
4	13.5	1.65E+06	1.23E+05	13	966.1186	0.2
5	13.8	1.27E+06	7.36E+04	12	991.2663	0.3
6	14	1.49E+06	8.96E+04	15	1136.9735	0.3
7	14.2	9.02E+05	6.04E+04	16	844.1617	0.2
8	14.3	1.74E+06	1.20E+05	16	881.1343	0.2
9	14.5	3.72E+06	2.60E+05	28	577.5133	0.2
10	14.6	4.10E+06	2.49E+05	11	577.5133	0.2
11	14.7	3.72E+06	2.36E+05	16	577.5133	0.2
12	15	3.70E+07	2.20E+06	563	577.5132	0.3
13	15.3	3.08E+06	1.94E+05	16	704.5152	0.3
14	15.8	1.34E+07	7.55E+05	234	894.042	0.3
15	16.4	2.34E+06	1.58E+05	34	722.3809	0.2
16	16.5	1.32E+07	8.45E+05	141	563.4981	0.2
17	16.8	3.05E+06	2.03E+05	14	704.5154	0.2
18	16.9	5.85E+06	3.89E+05	46	805.8578	0.2
19	17	4.32E+06	3.02E+05	24	825.0143	0.2
20	17.1	1.13E+07	8.24E+05	44	563.4982	0.2
21	17.2	6.86E+06	4.71E+05	41	563.4982	0.2
22	17.4	2.17E+06	1.61E+05	25	1239.9847	0.2
23	17.7	8.10E+06	5.69E+05	58	577.5134	0.2
24	17.8	1.33E+07	9.44E+05	47	577.5134	0.2
25	18	7.46E+06	5.18E+05	50	929.1927	0.2
26	18.1	4.77E+06	3.29E+05	25	577.5134	0.2
27	18.5	1.15E+07	7.86E+05	52	857.8172	0.2
28	18.5	1.31E+07	8.52E+05	97	688.4818	0.2
29	18.7	6.93E+06	4.31E+05	47	706.754	0.2
30	19	2.76E+07	1.46E+06	281	857.9721	0.3
31	19.2	1.48E+07	9.43E+05	191	866.1252	0.2
32	19.4	1.34E+07	8.46E+05	181	889.9748	0.2
33	19.5	1.09E+07	7.83E+05	90	744.1219	0.2

34	19.7	1.27E+07	8.43E+05	141	563.4977	0.2
35	19.8	1.44E+07	1.07E+06	92	828.4368	0.2
36	20	1.63E+07	1.17E+06	185	940.9608	0.2
37	20.2	1.52E+07	1.11E+06	54	704.5143	0.2
38	20.5	6.74E+07	2.99E+06	504	939.8827	0.4
39	20.8	1.80E+07	1.24E+06	28	672.2585	0.2
40	20.9	1.93E+07	1.37E+06	35	672.2585	0.2
41	21.2	2.63E+07	1.82E+06	311	820.6326	0.2
42	21.4	3.19E+07	2.05E+06	92	820.6327	0.2
43	21.5	2.91E+07	1.75E+06	177	655.6571	0.3
44	21.6	1.81E+07	1.18E+06	53	578.5168	0.2
45	21.7	9.39E+06	7.64E+05	128	1010.722	0.2
46	21.9	2.32E+07	1.62E+06	224	1133.6168	0.2
47	22.1	1.33E+07	1.04E+06	23	1133.6168	0.2
48	22.2	3.78E+07	2.22E+06	337	844.7874	0.3
49	22.6	1.64E+07	1.18E+06	101	656.3504	0.2
50	22.8	2.24E+07	1.67E+06	81	857.8322	0.2
51	23	2.80E+07	1.91E+06	126	857.8322	0.2
52	23.2	9.16E+06	7.22E+05	84	991.8394	0.2
53	23.3	1.67E+07	1.17E+06	26	991.8394	0.2
54	23.5	5.17E+07	3.08E+06	400	809.1109	0.2
55	23.7	2.51E+07	1.53E+06	124	1047.8528	0.3
56	23.8	5.22E+07	2.54E+06	150	1047.8528	0.3
57	24	1.53E+07	1.15E+06	117	921.8032	0.2
58	24	3.09E+07	2.22E+06	26	563.4987	0.2
59	24.2	3.97E+07	1.70E+06	210	852.9732	0.3
60	24.4	1.07E+07	9.04E+05	50	1041.4763	0.2
61	24.5	1.50E+07	1.22E+06	32	839.4573	0.2
62	24.6	1.88E+07	1.42E+06	49	1228.597	0.2
63	24.7	1.46E+07	1.14E+06	99	918.5813	0.2
64	24.9	4.96E+07	2.85E+06	400	904.022	0.3
65	25	2.98E+07	2.31E+06	143	904.022	0.2
66	25.2	5.00E+07	2.99E+06	547	912.8745	0.3
67	25.3	2.43E+07	1.65E+06	117	857.6933	0.2
68	25.6	1.56E+07	1.08E+06	76	887.403	0.2
69	25.7	4.31E+07	2.88E+06	38	784.5971	0.2
70	25.8	5.24E+07	2.95E+06	107	936.2339	0.3
71	25.9	3.63E+07	1.59E+06	117	887.4035	0.3

72	26.1	1.21E+07	9.06E+05	50	784.5971	0.2
73	26.1	1.80E+07	1.35E+06	112	802.4307	0.2
74	26.3	3.51E+07	2.26E+06	361	815.6419	0.2
75	26.5	2.04E+07	1.31E+06	37	766.8293	0.2
76	26.6	1.71E+07	1.08E+06	40	577.5139	0.2
77	26.7	1.20E+07	8.23E+05	20	577.5139	0.2
78	26.8	1.32E+07	8.61E+05	33	923.8084	0.2
79	27	1.12E+07	9.39E+05	41	783.2922	0.2
80	27.2	2.94E+07	1.75E+06	184	636.6124	0.3
81	27.4	6.20E+06	4.83E+05	22	954.5173	0.2
82	27.5	1.37E+07	9.90E+05	41	923.8079	0.2
83	27.6	2.75E+07	1.87E+06	92	843.7888	0.2
84	27.8	5.48E+07	2.75E+06	510	909.8408	0.3
85	28.1	9.27E+06	6.60E+05	23	824.9659	0.2
86	28.2	5.61E+06	4.08E+05	18	798.7695	0.2
87	28.3	8.42E+06	6.19E+05	39	1061.5579	0.2
88	28.5	1.58E+07	9.35E+05	50	980.8937	0.2
89	28.5	6.71E+06	4.97E+05	24	871.2022	0.2
90	28.7	1.24E+07	7.86E+05	58	851.95	0.2
91	28.9	4.12E+06	2.47E+05	22	983.9128	0.3
92	29	2.66E+06	2.11E+05	14	608.5286	0.2
93	29.2	4.38E+06	2.97E+05	18	765.7498	0.2
94	29.2	8.89E+06	5.46E+05	44	653.0486	0.3
95	29.5	3.50E+06	2.72E+05	17	885.0077	0.2
96	29.7	4.57E+06	3.11E+05	26	564.502	0.2
97	29.9	3.89E+06	2.65E+05	19	577.5137	0.2
98	30	3.43E+06	2.36E+05	19	549.4832	0.2
99	30.1	3.38E+06	2.81E+05	29	902.1686	0.2
100	30.3	4.55E+06	3.71E+05	24	751.215	0.2
101	30.5	4.29E+06	2.67E+05	32	881.9657	0.1
102	31.1	2.75E+06	1.94E+05	23	859.4501	0.2
103	31.7	3.45E+06	2.20E+05	50	780.5398	0.2
104	31.9	2.74E+06	1.99E+05	12	1303.0196	0.2
105	32.1	3.46E+06	2.22E+05	41	1007.9323	0.2
106	32.3	6.92E+06	3.94E+05	14	549.4842	0.3
107	32.7	6.93E+06	4.88E+05	44	898.9514	0.2
108	33	7.99E+06	5.94E+05	17	844.9189	0.2
109	33.1	6.36E+06	3.91E+05	24	865.4195	0.3

110	33.8	5.37E+06	5.61E+05	82	974.7796	0.1
111	34.3	6.21E+06	5.22E+05	27	1073.4674	0.2
112	35	4.25E+06	6.76E+05	106	1053.4034	0.1
113	35.9	6.00E+05	5.98E+04	18	650.2339	0.2
					Av. FWHM	0.219
					nc*	195.43

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148 * Peak Capacity (n_c) was calculated according to $n_c = \frac{t_G}{1.7 \cdot (\bar{w}_{1/2h})} + 1$ using 25 min as t_G (based on the effective

149 elution window).