Electronic Supplementary Material (ESI) for Analyst.

Inline electrochemical reduction of NISTmAb for middle-up subunit liquid chromatography – mass spectrometry analysis

by

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Chromatography conditions and trap/release setup

Table S 1: Pump 1 liquid chromatography conditions

Pump 1:

Solvent A = 1% FA, 20% ACN - full reduction

Or

Solvent A = 1% FA – partial reduction

Time	Flow mL min^{-1}	%B
0	0.05	0
30	0.05	0

Table S 2: Pump 2 liquid chromatography conditions

Pump 2 – A – (100% H_2O, 0.1% FA)

Time	Flow	%B (100% ACN, 0.1% FA)
0	0.3	25.0
5	Switching valve setup from 1:6 to 1:2 position	
10	0.3	25.0
10.1	0.3	30.0
20	0.3	40.0
20.1	0.3	80.0
25.1	0.3	80.0
26.1	0.3	80.0
28.0	0.3	20.0
30	0.3	25.0

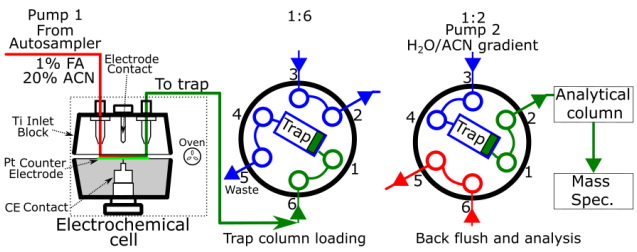

Figure S 1 Column trap/release setup for reference.

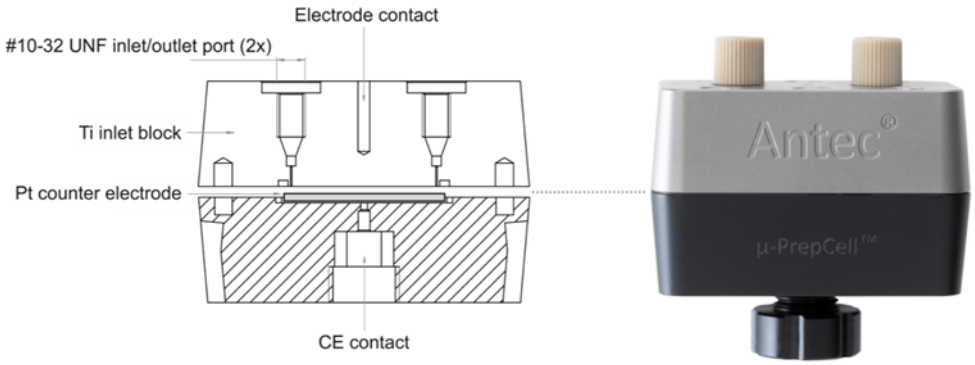

Figure S 2 EC µ-prep SS cell schematics and image

Table S 3: Mass Spectrometry conditions

Pump 2 – A – (100% H_2O, 0.1% FA)

Parameter	**Value**
Sheath gas flow	25
Aux gas flow	10
Sweep gas flow	0
Spray Voltage	3.8 kV
Capillary temperature	320
Aux temperature	150
S-Transfer lens	60
Mscans	5
Resolution setting	140,000 / 35,000
Scan range	*m/z* 600 – 5000
Maximum inject time	200
Intact protein mode	On

Table S 4: Theoretical mass comparisons

Figure	**Theoretical mass (monoisotopic mass)**	**Measured mass (mono)**	**Error (ppm)**
Figure 2E – singly reduced mass Lc	23109.273	23109.294	0.9
Figure 2F - triply reduced mass Lc	23111.289	23111.312	1
	Theoretical mass (Average)	**(average)**	**Mass Diff (Da)**
Figure 2J – G0F	50906.47	50900.32	6.15
Figure 2J – G1F	51068.61	51062.84	5.77
Figure 2J – G2F	51230.75	51226.62	4.13
			3 – unreduced
Figure 2K – G0F	50906.47	50904.19	2.28
Figure 2K – G1F	51068.61	51067.46	1.15
Figure 2K – G2F	51230.75	51229.34	1.41
			1 - unreduced
Figure 4D – G0F	50906.47	50906.59	-0.12
Figure 4D – G1F	51068.61	51068.68	-0.07
Figure 4D – G2F	51230.75	51230.43	0.32
			Fully reduced
	Theoretical mass (monoisotopic mass)	**(mono)**	**Error (ppm)**
Figure 5B – G0F	25220.464	25220.492	1.1
Figure 5B – G1F	25382.517	25382.553	1.4
Figure 5B – G2F	25544.565	25544.570	0.2

Table S 5: Partially reduced Lc fragment assignments (Figure 6A)

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution	Adjusted Theo mass (-2Da)	ppm
b_{117}	12542.201	5.09E+04	12544.201		4	9 - 12	12542.187	1.1
Precursor	23111.289	4.81E+04			1	23 - 23		
y_{96}	10569.106	4.32E+04	10569.103	0.32	5	10 - 14		
b_{116}	12395.132	4.26E+04	12397.133		4	8 - 11	12395.118	1.1
	23093.326	3.81E+04			1	23 - 23		
b_{115}	12282.050	2.90E+04	12284.049		4	8 - 11	12282.034	1.2
y_{53}	5840.808	2.06E+04	5840.779	5.09	3	6 - 8		
	10551.090	1.62E+04			3	11 - 13		
y_{54}	5969.823	1.52E+04	5969.821	0.26	2	7 - 8		
y_{51}	5654.679	1.38E+04	5654.678	0.21	3	6 - 8		
	23075.304	1.38E+04			1	23 - 23		
y_{97}	10716.174	1.37E+04	10716.171	0.25	3	11 - 13		
b_{114}	12134.979	1.36E+04	12136.981		3	8 - 10	12134.970	1.1
y_{81}	9073.352	1.14E+04	9073.351	0.13	2	10 - 11		
b_5	588.258	7.26E+03	588.258	-0.08	1	1 - 1		
	1070.907	6.98E+03			1	1 - 1		
y_{39}	4300.088	6.61E+03	4300.089	-0.20	2	5 - 6		
y_{82}	9172.419	6.18E+03	9172.419	-0.05	3	10 - 12		
	12524.188	5.57E+03			3	10 - 12		
y_{95}	10472.053	5.20E+03	10472.050	0.30	2	11 - 12		
	10698.157	5.03E+03			2	12 - 13		
y_{41}	4500.202	4.77E+03	4500.205	-0.81	2	5 - 6		
	23128.256	4.61E+03			1	23 - 23		
	12116.970	4.19E+03			2	9 - 10		
y_{56}	6184.911	4.15E+03	6184.912	-0.09	2	7 - 8		
b_{160}	17270.530	4.09E+03	17272.525		2	15 - 16	17270.511	1.1

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution	Adjusted Theo mass (-2Da)	ppm
	23057.303	4.00E+03			1	23 - 23		
	9055.337	3.84E+03			3	10 - 12		
	12377.120	3.68E+03			2	10 - 11		
	5951.811	3.68E+03			1	7 - 7		
b_{132}	14037.953	3.42E+03	14039.953		2	12 - 13	14037.939	0.96
	1103.549	3.25E+03			1	1 - 1		
	1135.031	3.25E+03			1	1 - 1		
y_{52}	5753.741	3.10E+03	5753.747	-0.96	1	7 - 7		
	22882.224	3.05E+03			2	22 - 23		
y_{60}	6571.070	3.05E+03	6571.067	0.43	2	7 - 8		
	10533.076	3.04E+03			2	12 - 13		
	1495.752	2.94E+03			1	2 - 2		
	4727.325	2.89E+03			2	5 - 6		
	23138.299	2.69E+03			1	23 - 23		
	22865.182	2.49E+03			2	22 - 23		
	12313.087	2.48E+03			2	10 - 11		
	764.727	2.30E+03			1	1 - 1		
y_{55}	6097.882	2.26E+03	6097.880	0.41	2	7 - 8		
	12054.957	2.22E+03			2	9 - 10		
b_{113}	12037.946	2.08E+03	12037.912	2.82	2	9 - 10		
	23037.292	2.02E+03			1	23 - 23		
	764.869	2.01E+03			1	1 - 1		
	13919.874	1.86E+03			2	11 - 12		
	12167.023	1.85E+03			1	10 - 10		
	1396.683	1.68E+03			1	2 - 2		
y_{99}	10976.326	1.65E+03	10976.324	0.19	2	12 - 13		
	570.247	1.61E+03			1	1 - 1		

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution	Adjusted Theo mass (-2Da)	ppm
	6442.008	1.51E+03			1	7 - 7		
	1279.604	1.39E+03			1	2 - 2		
	23149.230	1.37E+03			1	23 - 23		
	12264.039	1.37E+03			1	10 - 10		
	17252.512	1.27E+03			1	16 - 16		
	22756.132	1.26E+03			1	22 - 22		
	1543.755	1.25E+03			1	2 - 2		
	22737.122	1.09E+03			1	22 - 22		
	1378.672	1.09E+03			1	2 - 2		
	10455.046	1.06E+03			1	12 - 12		
y_{11}	1236.592	1.03E+03	1236.592	-0.09	1	2 - 2		
	22522.013	1.03E+03			1	22 - 22		
y_{79}	8857.259	9.82E+02	8857.258	0.18	1	10 - 10		
	9154.397	9.74E+02			1	11 - 11		
	12638.251	9.71E+02			1	11 - 11		
y_{118}	12875.383	9.13E+02	12875.381	0.10	1	14 - 14		
	22624.097	9.10E+02			1	22 - 22		
	17140.486	8.95E+02			1	16 - 16		
	23046.300	8.61E+02			1	23 - 23		
	17121.475	8.31E+02			1	16 - 16		
	16927.398	8.03E+02			1	15 - 15		
y_{40}	4413.173	7.72E+02	4413.173	-0.05	1	6 - 6		
y_{98}	10829.257	7.17E+02	10829.255	0.19	1	12 - 12		
y_{78}	8744.175	6.94E+02	8744.174	0.11	1	10 - 10		
	23021.248	6.83E+02			1	23 - 23		
	17437.610	6.55E+02			1	16 - 16		
	4471.224	6.12E+02			1	6 - 6		

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution	Adjusted Theo mass (-2Da)	ppm
	10958.317	5.95E+02			1	13 - 13		
	6554.053	5.83E+02			1	8 - 8		

Table S 6: Fully reduced Lc fragment assignments (Figure 6C)

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution
y_{96}	10569.122	1.19E+05	10569.103	1.85	4	9 - 12
	7845.925	8.75E+04			3	7 - 9
y_{135}	14834.174	8.26E+04	14834.164	0.72	4	13 - 16
	8133.074	7.59E+04			3	7 - 9
y_{97}	10716.177	5.34E+04	10716.171	0.57	4	9 - 12
y_{124}	13483.650	4.96E+04	13483.641	0.64	3	12 - 14
Precursor	23113.343	4.29E+04			1	23 - 23
	23095.331	3.82E+04			1	23 - 23
b_{78}	8279.148	2.60E+04	8279.141	0.83	3	7 - 9
y_{128}	14024.851	2.46E+04	14024.840	0.72	4	12 - 15
b_{75}	7950.973	2.43E+04	7950.966	0.84	3	7 - 9
y_{136}	14962.234	2.00E+04	14962.222	0.78	2	14 - 15
	7631.797	1.91E+04			3	7 - 9
b_{73}	7750.853	1.84E+04	7750.850	0.45	3	7 - 9
	8019.992	1.81E+04			3	7 - 9
y_{139}	15249.382	1.77E+04	15249.370	0.79	2	14 - 15
	23076.316	1.76E+04			1	23 - 23
b_5	588.258	1.75E+04	588.258	-0.08	1	1 - 1
	14816.154	1.60E+04			3	13 - 15

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution
	7732.837	1.39E+04			3	7 - 9
	13338.601	1.17E+04			3	12 - 14
y_{53}	5840.805	1.12E+04	5840.779	4.44	1	7 - 7
y_{54}	5969.822	1.05E+04	5969.821	0.13	1	7 - 7
	7862.930	1.05E+04			2	8 - 9
y_{120}	13119.514	1.04E+04	13119.503	0.86	2	12 - 13
y_{130}	14288.960	1.02E+04	14288.951	0.63	2	13 - 14
	15231.362	9.78E+03			2	14 - 15
	629.338	9.70E+03			1	1 - 1
y_{99}	10976.331	9.68E+03	10976.324	0.68	3	10 - 12
y_{127}	13861.792	9.42E+03	13861.777	1.10	2	13 - 14
y_{51}	5654.680	9.34E+03	5654.678	0.25	2	6 - 7
	9069.456	8.83E+03			3	8 - 10
y_{125}	13611.711	8.77E+03	13611.699	0.81	2	12 - 13
b_{77}	8151.082	8.56E+03	8151.082	0.03	2	8 - 9
	23133.278	8.37E+03			1	23 - 23
	1350.524	8.19E+03			1	1 - 1
	1086.526	8.15E+03			1	1 - 1
	14944.223	7.89E+03			2	14 - 15
	8115.062	7.84E+03			2	8 - 9
	1024.504	6.99E+03			1	1 - 1
b_7	803.348	6.74E+03	803.348	-0.19	1	1 - 1
y_{123}	13426.632	6.20E+03	13426.619	0.94	2	13 - 14
	13757.780	6.07E+03			2	13 - 14
	1033.279	5.95E+03			1	1 - 1
	8000.979	5.74E+03			2	8 - 9
	14186.907	5.32E+03			2	13 - 14

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution
y_{138}	15162.341	5.30E+03	15162.338	0.20	2	14 - 15
y_{137}	15075.334	4.92E+03	15075.306	1.83	1	14 - 14
y_{98}	10829.264	4.91E+03	10829.255	0.83	2	10 - 11
b_{117}	12544.208	4.62E+03	12544.201	0.52	2	12 - 13
y_{95}	10472.050	4.35E+03	10472.050	0.02	2	10 - 11
	10551.097	4.30E+03			2	11 - 12
	7827.914	4.26E+03			2	8 - 9
b_{69}	7288.606	3.98E+03	7288.602	0.60	2	7 - 8
	23151.324	3.93E+03			1	23 - 23
	2857.195	3.80E+03			1	3 - 3
	7732.254	3.79E+03			1	7 - 7
	7932.962	3.73E+03			2	8 - 9
	23059.287	3.63E+03			1	23 - 23
y_{39}	4300.091	3.63E+03	4300.089	0.43	1	5 – 5
	13464.630	3.47E+03			2	13 - 14
	2767.470	3.30E+03			1	3 - 3
	2914.538	3.30E+03			1	3 - 3
	8261.132	2.73E+03			1	9 - 9
	7518.712	2.71E+03			1	8 - 8
b_{71}	7536.718	2.63E+03	7536.718	-0.08	1	8 - 8
	23141.279	2.58E+03			1	23 - 23
y_{141}	15463.515	2.17E+03	15463.502	0.82	1	15 - 15
b_{86}	9251.536	2.15E+03	9251.527	1.00	1	9 - 9
	12398.141	2.12E+03			1	12 - 12
y_{52}	5753.746	2.09E+03	5753.747	-0.11	1	7 - 7
	12528.202	2.01E+03			1	12 - 12
y_{56}	6184.911	1.99E+03	6184.912	-0.06	1	8 - 8

Ion Name	Monoisotopic Mass	Sum Intensity	Theoretical Mass	Corrected Delta Mass (ppm)	Number of Charge States	Charge State Distribution
	5950.813	1.93E+03			1	7 - 7
b_{72}	7649.805	1.91E+03	7649.802	0.42	1	8 - 8
	13283.586	1.88E+03			1	13 - 13
	13612.704	1.78E+03			1	14 - 14
	15445.509	1.75E+03			1	15 - 15
	3455.737	1.73E+03			1	4 - 4
	15444.475	1.67E+03			1	14 - 14
	12525.187	1.34E+03			1	13 - 13
	22886.248	1.17E+03			1	23 - 23
	7617.809	1.11E+03			1	9 - 9
b_{83}	8824.353	1.06E+03	8824.353	0.00	1	10 - 10