

Supporting Information

Capillary Zone Electrophoresis–Electrospray Ionization-Tandem Mass Spectrometry for Top-Down characterization of *Mycobacterium marinum* Secretome

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Table S1 Measured conductivity of aqueous acetic and formic acid solutions

Formic Acid	
Concentration (V/V)	Conductivity (ms/cm)
0.1	0.96
1	4.01
5	7.38
10	9.39
20	12
30	11.9
50	9.17
70	5.6
100	0.51

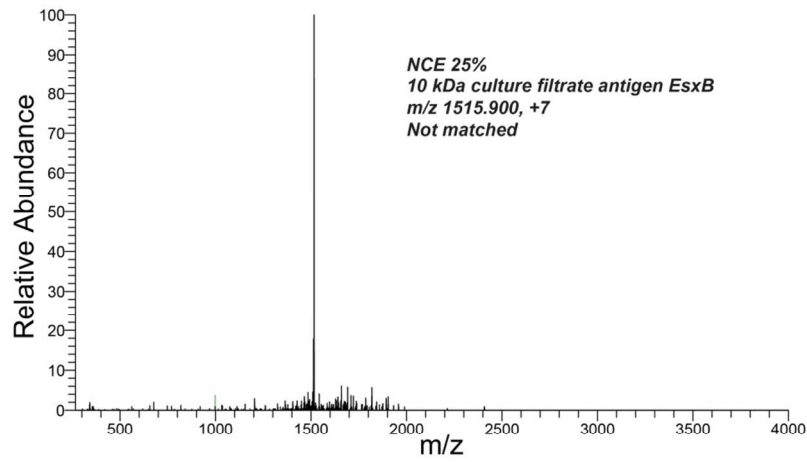
Acetic Acid	
Concentration (V/V)	Conductivity (ms/cm)
0.1	0.67
1	1.18
5	1.62
10	1.91
20	1.94
30	1.66
50	1.05
70	0.35
100	0.064

Table S2 Summary of CZE-ESI-MS cyt c analysis with different sampling buffers¹

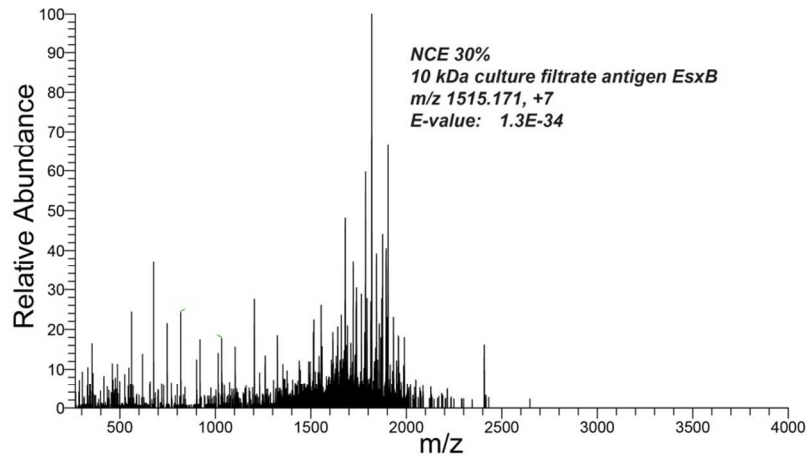
Sampling Buffer	Peak Width (s) (50% peak height)	Peak Intensity	Migration Time (min)
70% Acetic Acid	8.6 ± 2.5	(8.2 ± 5.3)E6	4.7±0.7
0.25% FA	7.4 ± 0.3	(5.0 ± 1.0)E6	3.8±0.1

¹CZE was coupled to LTQ XL mass spectrometer (Thermo Fisher Scientific) for cytochrome c characterization. A 40 cm LPA-coated capillary was used for separation. The voltage applied was 16 kV for CE separation and 1.5 kV for electrospray.

(A) WT25_50ul_50cm #888 RT: 17.47 AV: 1 NL: 2.12E5
T: FTMS + p NSI d Full ms2 1515.90@hcd25.00 [266.00-4000.00]



(B) WT30_50ul_50cm #895 RT: 17.73 AV: 1 NL: 1.29E5
T: FTMS + p NSI d Full ms2 1515.89@hcd30.00 [266.00-4000.00]



(C) WT35_50ul_50cm #1018 RT: 19.92 AV: 1 NL: 7.36E3
T: FTMS + p NSI d Full ms2 1516.18@hcd35.00 [266.00-4000.00]

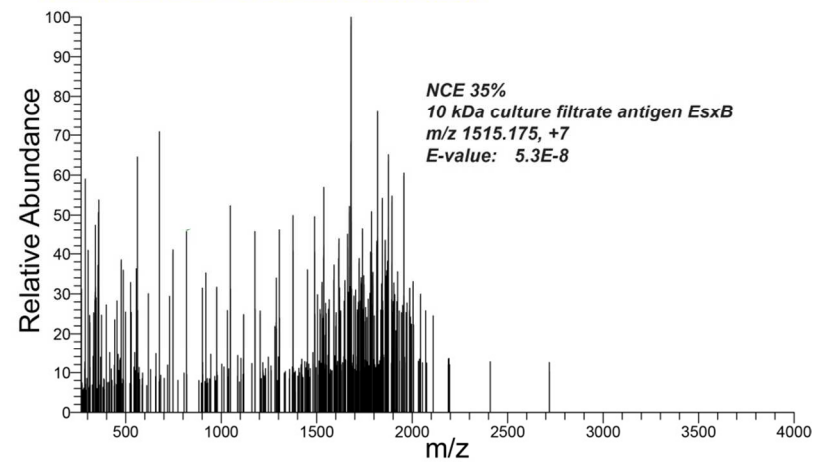


Fig. S1 HCD fragmentation of the 10-kDa culture filtrate antigen EsxB with NCE of (A) 25%. (B) 30%. (C) 35%.

WT28_50ul_50cm #1388 RT: 30.22 AV: 1 NL: 8.34E4
T: FTMS + p NSI Full ms [600.00-2000.00]

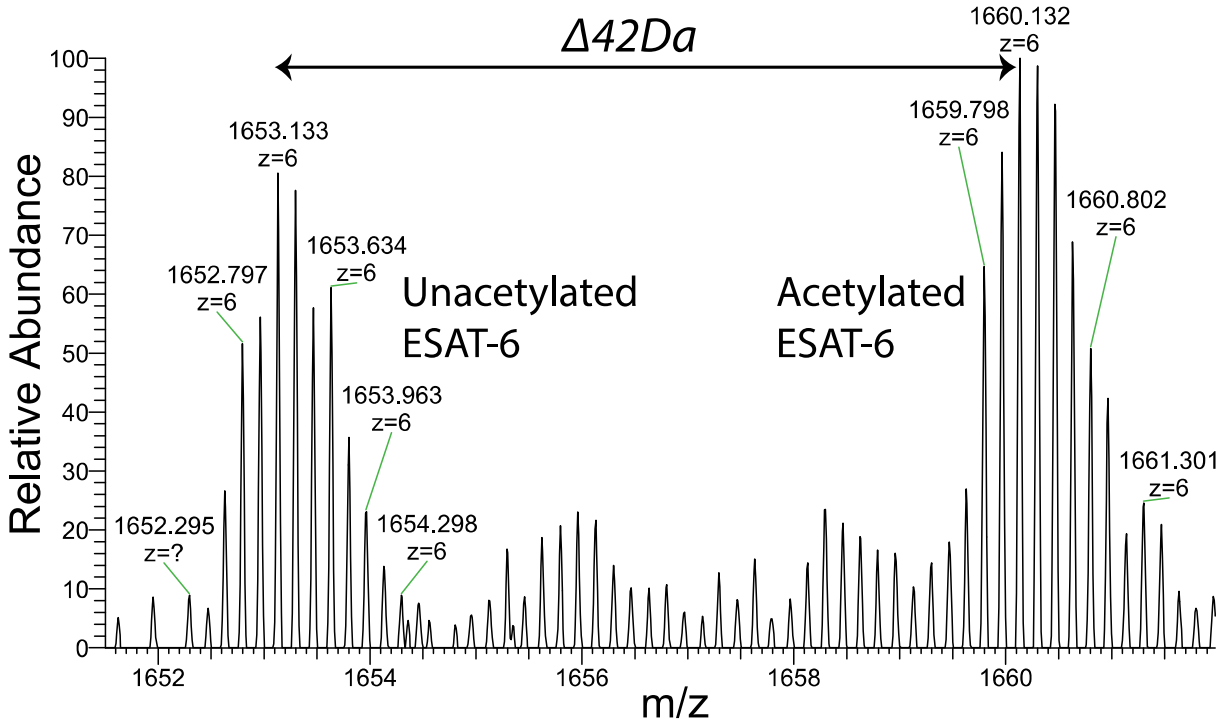


Fig. S2 Spectrum of both unacetylated ESAT-6 and acetylated ESAT-6.