Supporting Information for

Spray-Capillary Based Capillary Electrophoresis Mass Spectrometry for Metabolite Analysis in Single Cells

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Abstract

Single-cell capillary electrophoresis mass spectrometry (CE-MS) is a promising platform to analyze cellular contents and probe cell heterogeneity. However, current single-cell CE-MS methods often rely on offline microsampling processes and may demonstrate low sampling precision and accuracy. We have recently developed an electrospray-assisted device, spray-capillary, for low-volume sample extraction. With the spray-capillary, low-volume samples (pL~nL) are drawn into the sampling end of the device, which can be used directly for CE separation and online MS detection. Here, we re-designed the spray-capillary by utilizing a capillary with a <15 μm tapered-tip so that it can be directly inserted into single cells for sample collection and on-capillary CE-MS analysis. We evaluated the performance of the modified spray-capillary by performing single-cell microsampling on single onion cells with varying sample injection time and direct MS analysis or online CE-MS analysis. We have demonstrated, for the first time, online sample collection and CE-MS for the analysis of single cells. This application of the modified spray-capillary device facilitates the characterization and relative quantification of hundreds of metabolites in single cells.

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Supplementary Figures:

Figure S1. Triplicate sample extraction from *A. cepa* single cells using the modified spray-capillary device. (A) Total ion chromatograms from three replicate single cell injections; (B) the summed spectrum of the eluted peak from each analysis. The spray-capillary used is a 360 μm O.D X50 μm I.D. long capillary (50 cm in length) with a tapered tip at the sample inlet end. For sample injection, the electrospray voltage was 3 kV, and the sample injection time was 5 s (NL: normalization level).

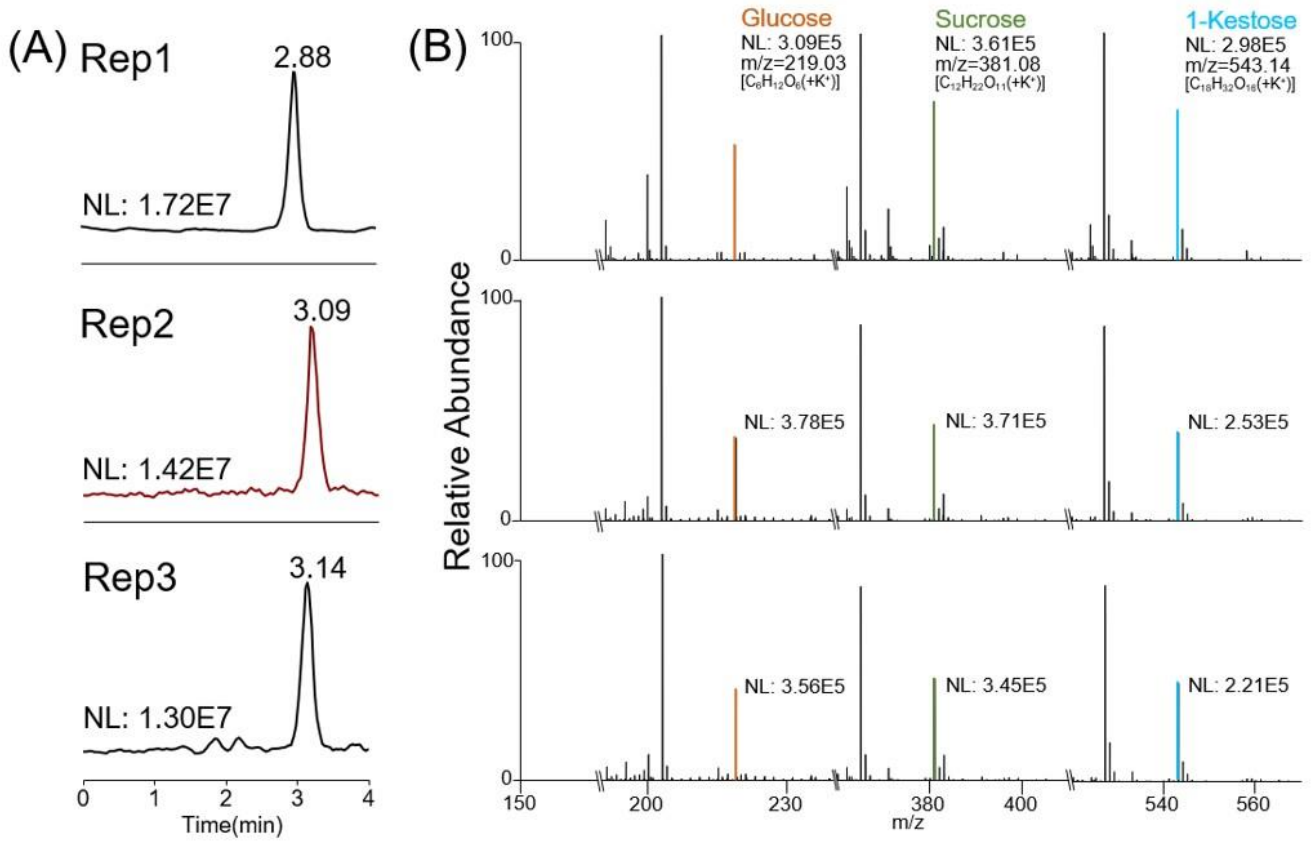

Figure S2. Comparison between control (buffer) and normal (onion) trial. (A) Normalized base peak chromatogram of control and onion trial. (B) Normalized averaged spectrum of control trial and onion trial. NL: normalization level.

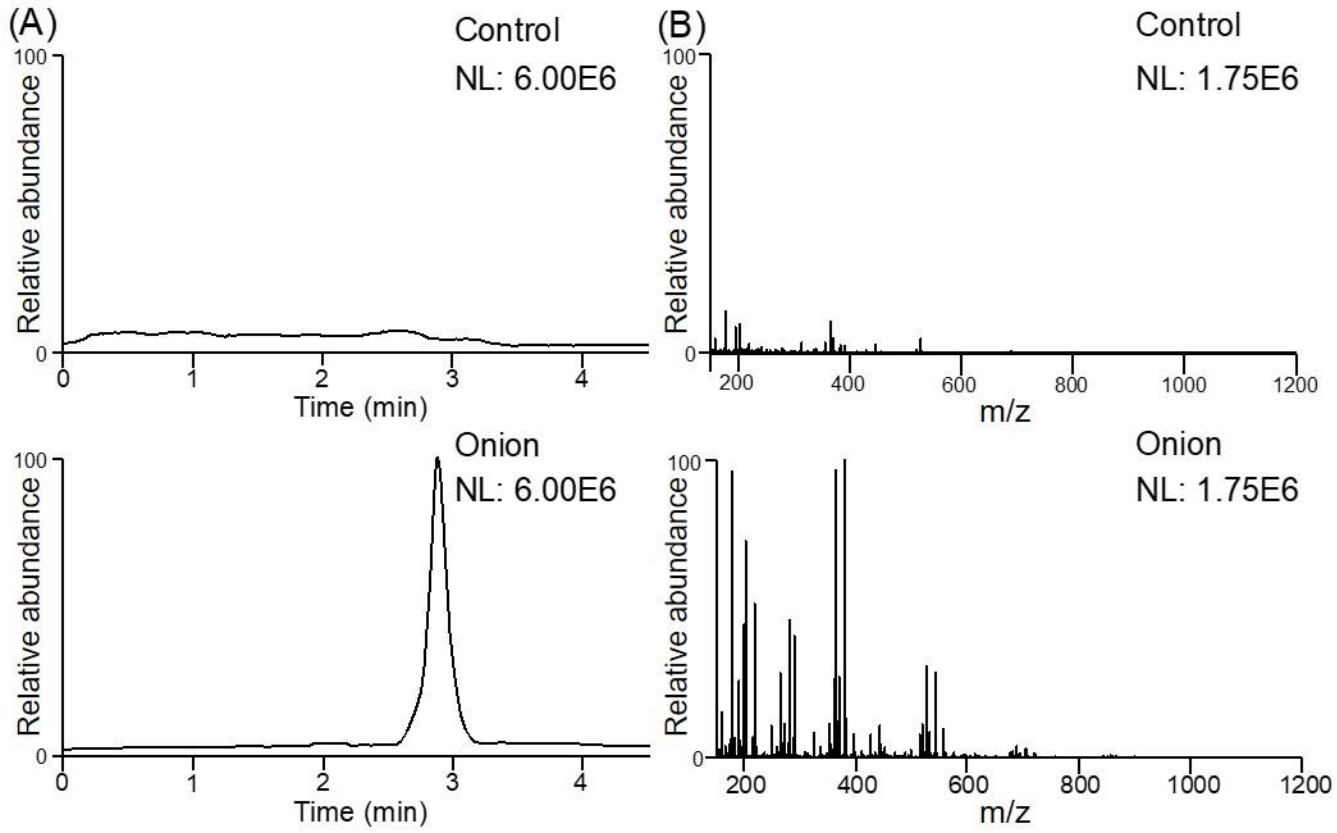

Figure S3. MS/MS identification of (A) phenylalanine, (B) glucose with sodium adduct, and (C) malic acid with sodium adduct.

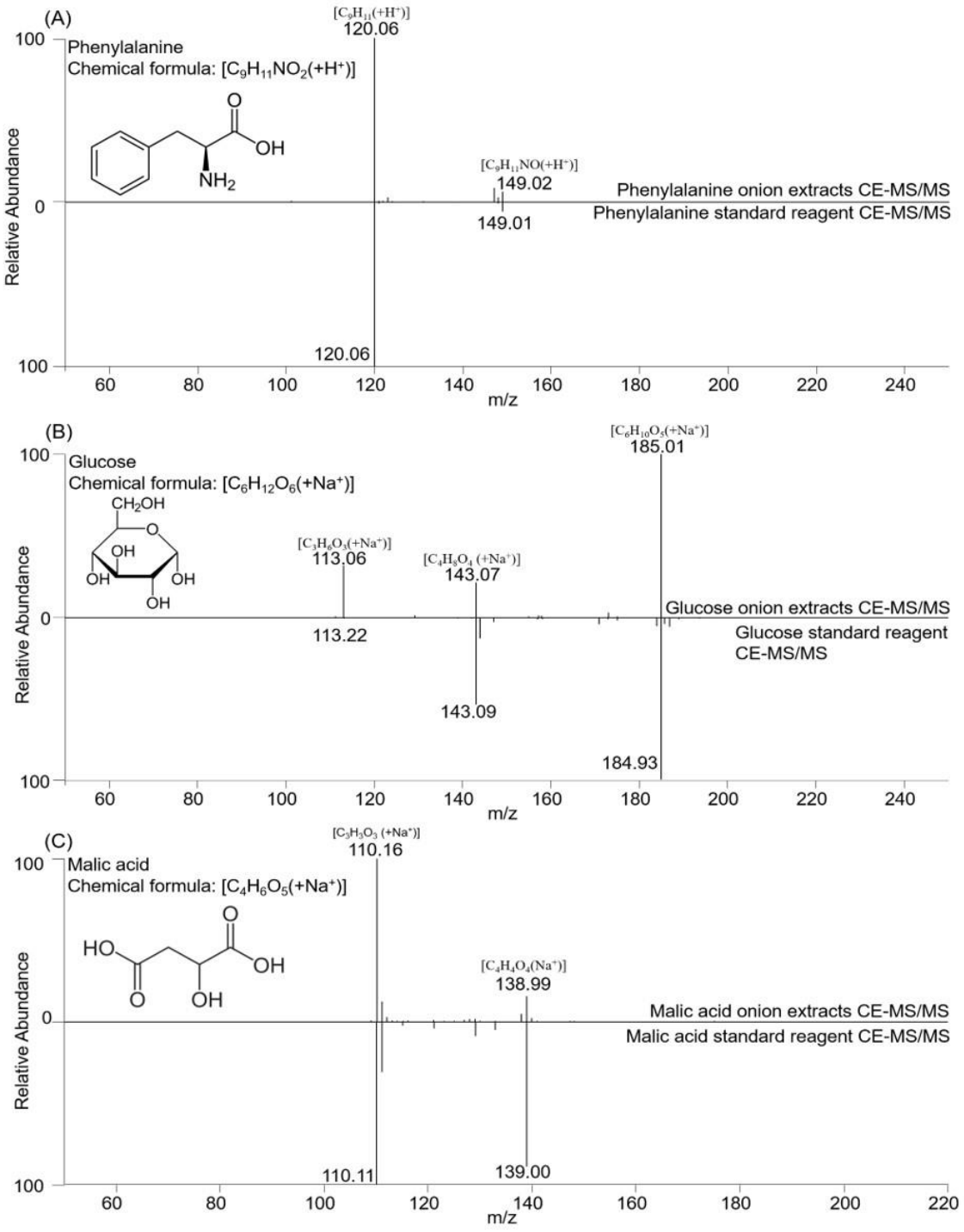

Figure S4. MS/MS spectra of (A) nystose (DP4) with sodium adduct, (B) sucrose with potassium adduct, (C) arginine, and (D) tyrosine.

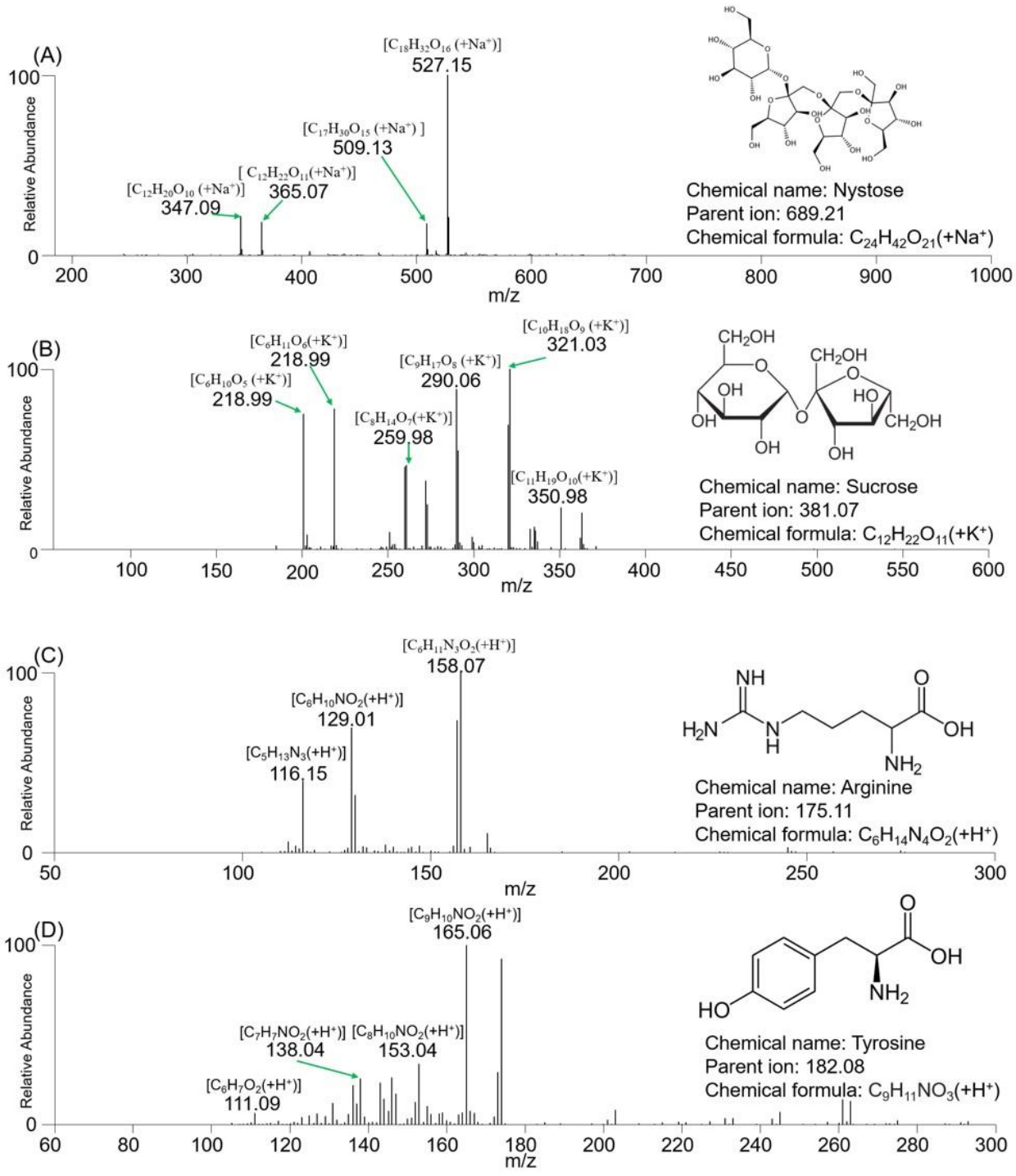

Figure S5. CE separation of standard metabolites and comparison with same metabolites from onion extracts.

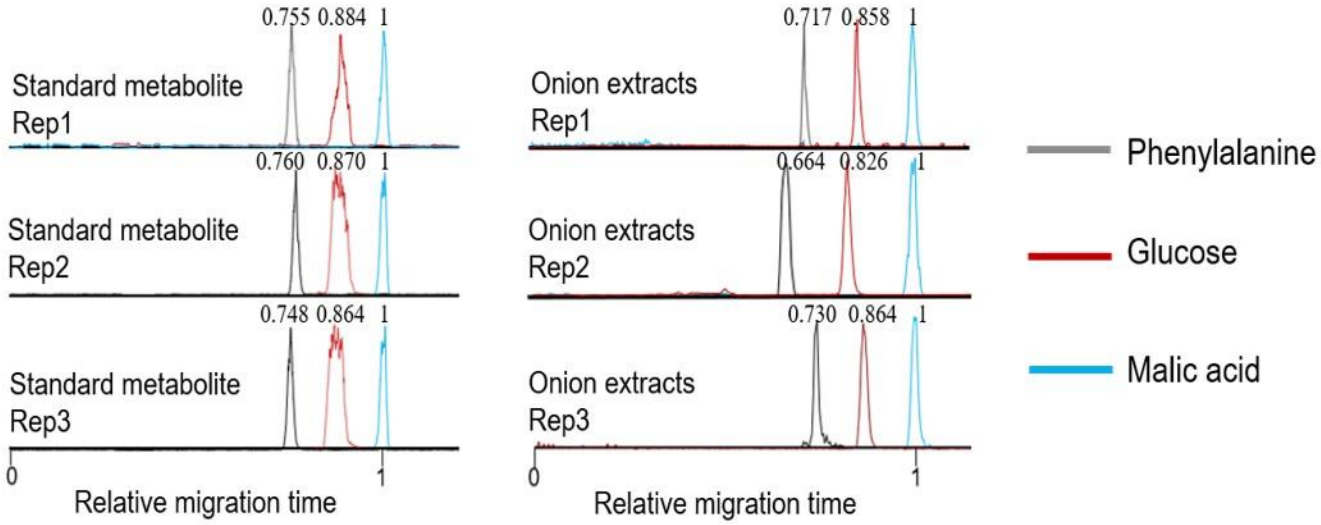

Table S1. Characterization of metabolites using spray-capillary CE-MS system.

Metabolite	Replicate #	Peak area	RSD (Peak area)	Relative migration time	RSD
Malic acid $[C_4H_6O_5(+Na^+)]$	R1	2.98E5	17.0%	1	N/A
	R2	2.62E5		1	
	R3	3.66E5		1	
Phenylalanine $[C_9H_{11}NO_2(+H^+)]$	R1	2.62E5	35.3%	0.664	4.89%
	R2	5.20E5		0.728	
	R3	5.49E5		0.717	
Cycloalliin $[C_6H_{11}NO_3S(+H^+)]$	R1	1.08E7	16.3%	0.752	3.85%
	R2	9.72E6		0.806	
	R3	1.33E7		0.802	
Glucose $[C_6H_{12}O_6(Na^+)]$	R1	1.64E7	16.8%	0.824	2.63%
	R2	1.40E7		0.867	
	R3	1.96E7		0.858	
Methyl cysteine sulfoxide $[C_4H_9NO_3S\ (+H^+)]$	R1	1.58E5	3.78%	0.748	3.79%
	R2	1.49E5		0.801	
	R3	1.48E5		0.798	
Adenosine $[C_{10}H_{13}N_5O_4(+H^+)]$	R1	7.67E4	10.7%	0.459	8.61%
	R2	7.82E4		0.540	
	R3	6.39E4		0.529	

Table S2. Relative migration time comparison between standard metabolites and corresponding onion metabolites.

Trials	Replicate #	Relative migration time	
		Phenylalanine	Glucose
Standard metabolites mixture	Rep1	0.755	0.884
	Rep2	0.760	0.870
	Rep3	0.748	0.864
Average (Standards)	N/A	0.754	0.872
RSD (Standards)	N/A	0.80%	1.18%
Onion extracts	Rep1	0.717	0.858
	Rep2	0.664	0.826
	Rep3	0.730	0.864
Average (Onion extracts)	N/A	0.703	0.849
RSD (Onion extracts)	N/A	4.97%	2.41%