Electronic Supplementary Material (ESI) for Analyst.


Supporting Information for

# Molecular Profiling of Single Axon and Dendrite in Living Neurons using Electrosyringe-assisted Electrospray Mass Spectrometry

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Figure S1. The fluorescent image of nano-capillary immersed in 100 mM $NH_4HCO_3$ solution with fluorescein for 60 s without the application of negative voltage. The contrast was adjusted for better visualization of weak fluorescence at capillary wall.

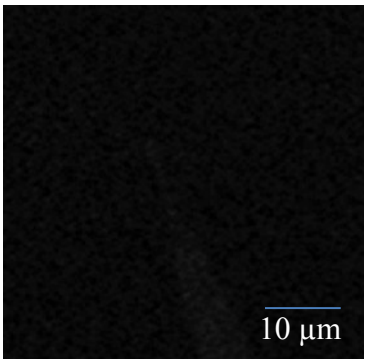


Figure 2. The simulation of the fluorescein distribution in nano-capillary during the electrochemical loading for 60 s using the diffusion module in Comsol software . (A) the simulation model: the diffusion coefficient of fluorescein is set as $10^{-6}$ $cm^2/s$; the influx of fluorescein at the orifice of capillary is set as $10^{-19}$ mole (100 µM, 1 fL); the boundary condition of all the other interfaces is set as the insulation; (B) the simulated concentration of fluorescein in nano-capillary; a small region with high concentration is observed at the tip associated with a diluted region across the capillary.

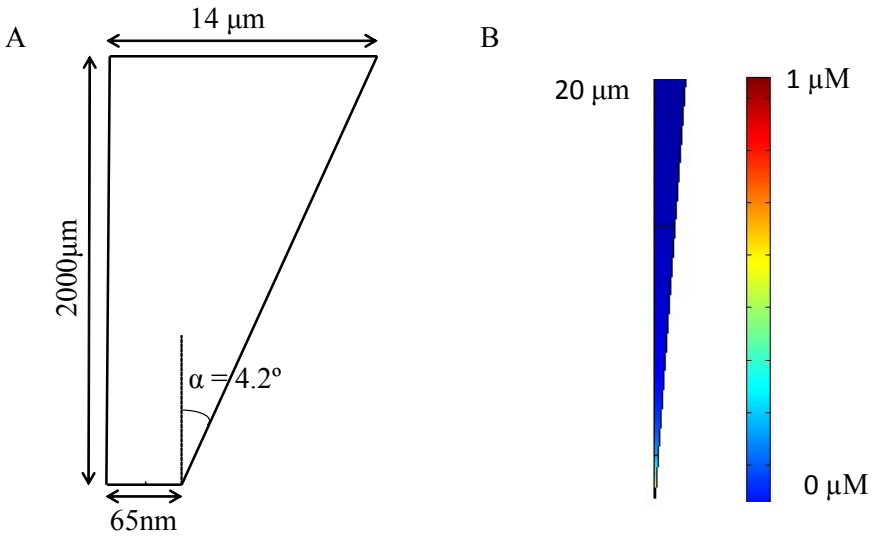

Figure S3. The integrated peak intensity of sucrose with (A) the application voltage for 60 s; (B) application time with the voltage of -3 V. The capillary used here had the tip opening of ~ 130 nm. The error bar presented the standard deviation from three independent measurements.

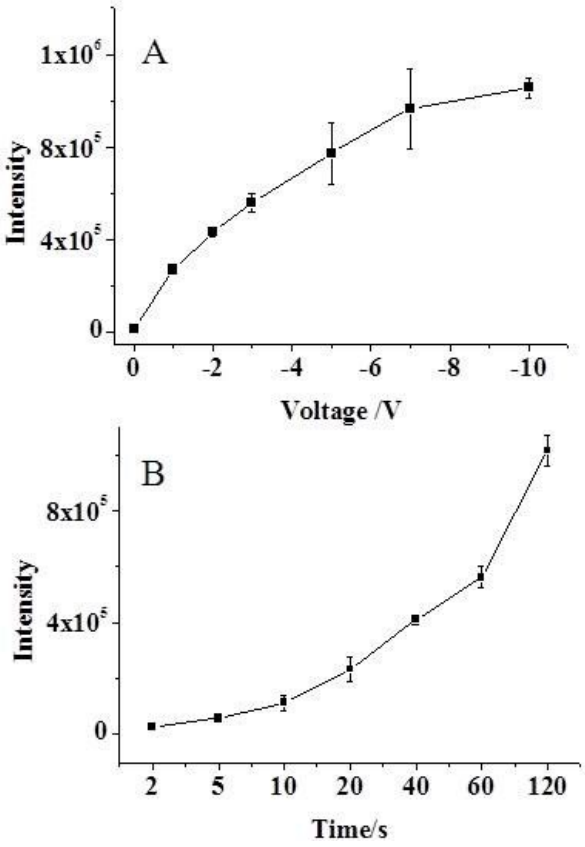

Figure S4. The mass spectrum of the electrosprayed solution from the capillary without the application of negative voltage at the metal wire for the electrochemical ingression. The capillary used here had the tip opening of ~ 130 nm. The solution was 100 mM $NH_4HCO_3$ with 1 mM $Et_4NHSO_4$, $(CH_2)_6N_4$, glucose, sucrose and ascorbic acid.

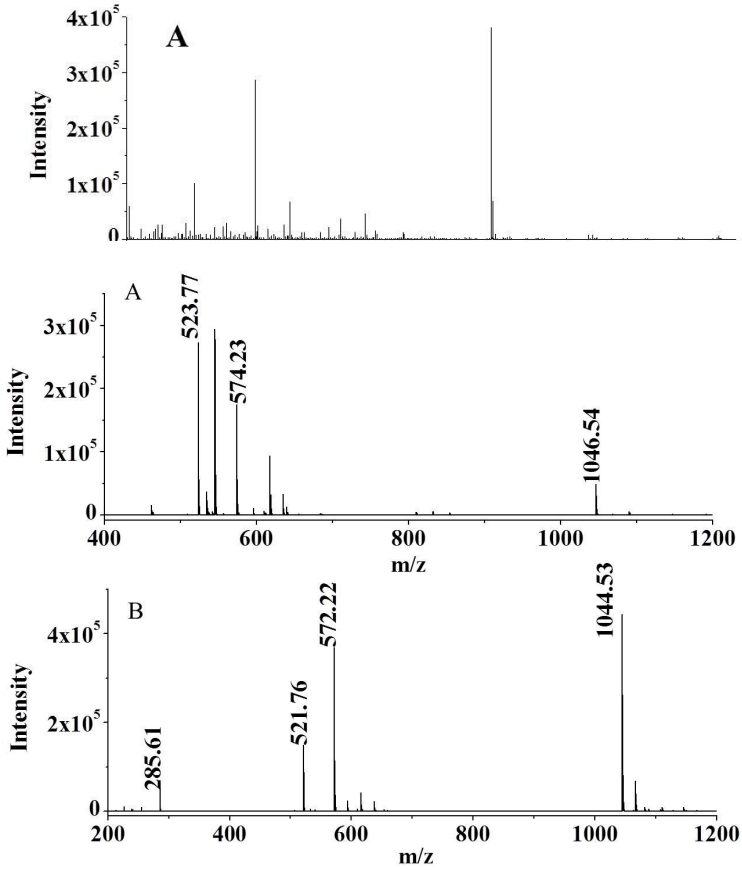


Figure S5. The mass spectrum of 100 mM $NH_4HCO_3$ with (A, B) 1 mM met-enkephalin and Angiotensin II loaded into the nano-capillary assisted by electrosyringe. (A) positive mode; (B) negative mode. The substance identification was shown in Table S1 of the Supporting Information.

Figure S6. (A) The intensity of glucose in mass spectrums using nano-capillary (black) and micro-capillary (red). The solutions with different concentration of glucose were manually filled into the capillary and electrosprayed. (B) the intensity of glucose in mass spectrum using nano-capillary. The solution with 1 mM glucose was electrochemically loaded into the nano-capillary by the application of -3 V for different time. The intensity labelled with light magenta was collected from the solution with 1 mM glucose directly filled in the nano-capillary. The error bar presented the standard

deviation from three independent measurements.

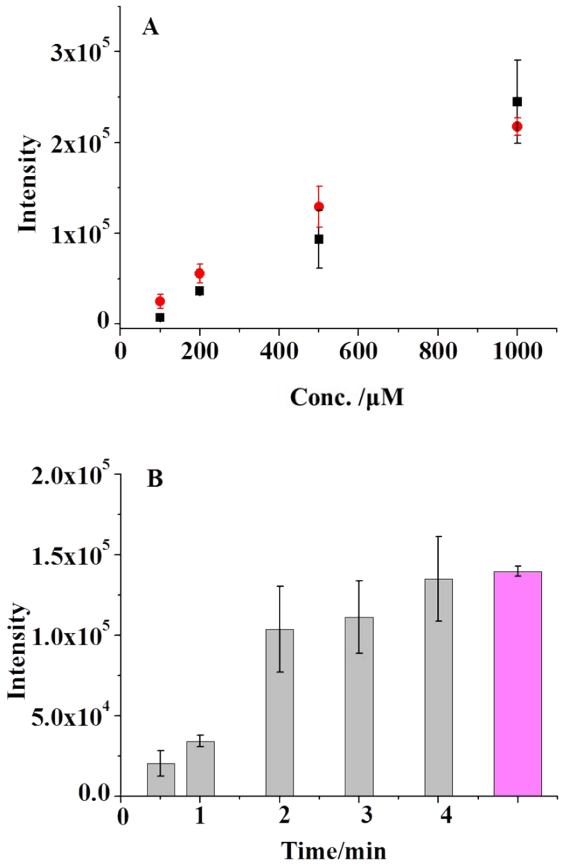


Figure S7. The negative-mode mass spectrum from the nano-capillary inserted into single cell without the application of negative voltage.

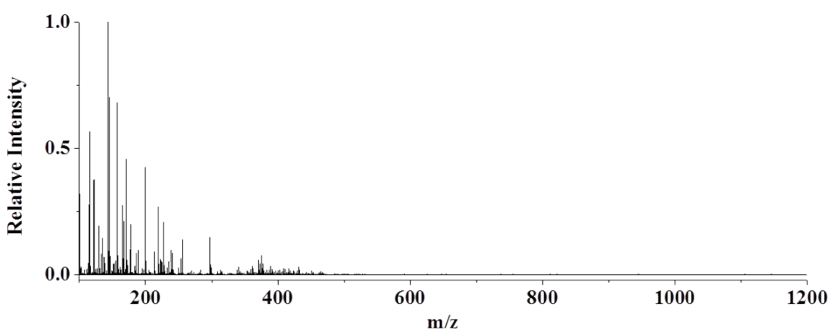

Figure S8. The negative-mode mass spectrum from the nano-capillary that was positioned near the neuron to sample extra-capillary buffer.

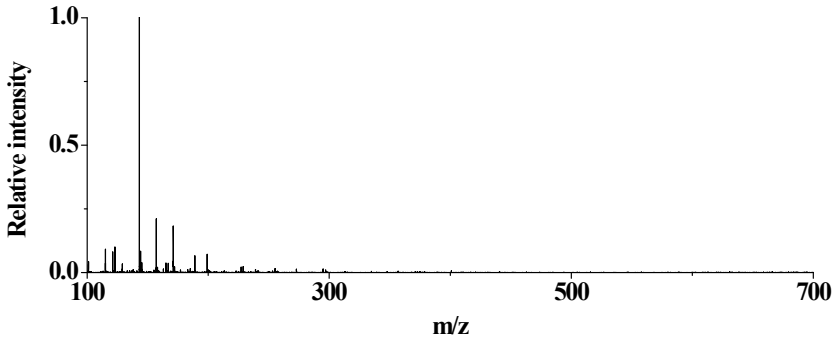


Table S1: The substance tentative assignment in the mass spectrums for the analysis of $Et_4NHSO_4$, $(CH_2)_6N_4$, glucose, sucrose, ascorbic acid, met-enkephalin and Angiotensin II in 100 mM $NH_4HCO_3$ that were electrochemically loaded into the nano-capillary.

| Species | Exact Mass | MS mode | Representative m/z peaks in mass spectra |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Ascorbic Acid | 176.03 | - | 175.02 (M-H) |
| $(CH_2)_6N_4$ | 140.11 | + | 141.11 (M+H) |
| $Et_4NHSO_4$ | 227.12 | + | 130.16 ($C_8H_{20}N^+$) |
| Glucose | 180.06 | + | 198.10 ($M+NH_4$) |
| | | - | 179.06 (M-H) |
| Sucrose | 342.12 | + | 360.15 ($M+NH_4$) |
| | | - | 341.11 (M-H) |
| Met-enkephalin | 573.23 | + | 574.23 (M+H) |
| | | - | 572.22 (M-H), 285.61 (M-2H) |
| Angiotensin II | 1045.53 | + | 1046.54 (M+H), 523.77 (M+2H) |
| | | - | 1044.53 (M-H), 521.76 (M-2H) |

Table S2. The substance tentative assignment in the mass spectrums for the analysis of $Et_4NHSO_4$, $(CH_2)_6N_4$, glucose, sucrose and ascorbic acid in 10 mM PBS that were electrochemically loaded into the nano-capillary.

| Species | Exact Mass | MS mode | Representative m/z peaks in mass spectra |
|---|---|---|---|
| Ascorbic Acid | 176.03 | - | 175.02 (M-H) |
| $(CH_2)_6N_4$ | 140.11 | + | 141.11 (M+H) |

| | | | |
|---|---|---|---|
| $Et_4NHSO_4$ | 227.12 | + | 130.16 ($C_8H_{20}N^+$) |
| Glucose | 180.06 | + | 203.05 (M+Na) |
| | | - | 179.06 (M-H) |
| Sucrose | 342.12 | + | 365.11 (M+Na) |
| | | - | 341.11 (M-H) |

Table S3. The substance tentative assignment from the negative-mode mass spectrum for the analysis of single *Allium cepa* cell assisted by electrosyringe.

| m/z (measured) | m/z (calculated) | Delta m/z (mDa) | Possible Substance | Formula |
|---|---|---|---|---|
| 179.0560 | 179.0561 | -0.1 | Glucose/Fructose | $C_6H_{12}O_6(-H^+)$ |
| 341.1090 | 341.1089 | 0.1 | Sucrose | $C_{12}H_{22}O_{11}(-H^+)$ |
| 463.0881 | 463.0882 | -0.1 | Quercetin glucoside | $C_{21}H_{20}O_{12}(-H^+)$ |
| 503.1617 | 503.1618 | -0.1 | Fructan(DP3) | $C_{18}H_{32}O_{16}(-H^+)$ |
| 625.1427 | 625.1410 | 1.7 | Quercetin diglucoside | $C_{27}H_{30}O_{17}(-H^+)$ |
| 665.2159 | 665.2146 | 1.3 | Fructan(DP4) | $C_{24}H_{42}O_{21}(-H^+)$ |
| 827.2689 | 827.2674 | 1.5 | Fructan(DP5) | $C_{30}H_{52}O_{26}(-H^+)$ |
| 989.3208 | 989.3202 | 0.6 | Fructan(DP6) | $C_{36}H_{62}O_{31}(-H^+)$ |
| 1151.3808 | 1151.3731 | 7.7 | Fructan(DP7) | $C_{42}H_{72}O_{36}(-H^+)$ |

Table S4. The substance tentative assignment from the negative-mode mass spectrum for the analysis of single HeLa cell.

| m/z (measured) | m/z (calculated) | Delta m/z (mDa) | Possible Substance | Formula |
|---|---|---|---|---|
| 114.0560 | 114.0561 | -0.1 | Proline | $C_5H_9NO_2(-H^+)$ |
| 116.0715 | 116.0717 | -0.2 | Valine | $C_5H_{11}NO_2(-H^+)$ |
| 130.0871 | 130.0874 | -0.3 | Leucine/Isoleucine | $C_6H_{13}NO_2(-H^+)$ |

| | | | | |
|---|---|---|---|---|
| 132.0312 | 132.0302 | 1.0 | Aspartic acid | $C_4H_7NO_4$ ($-H^+$) |
| 145.0615 | 145.0619 | -0.4 | Glutamine | $C_5H_{10}N_2O_3$ ($-H^+$) |
| 146.0483 | 146.0459 | 2.4 | Glutamic acid | $C_5H_9NO_4$ ($-H^+$) |
| 179.0549 | 179.0561 | -1.2 | Glucose | $C_6H_{12}O_6$ ($-H^+$) |
| 180.0661 | 180.0666 | -0.5 | Tyrosine | $C_9H_{11}NO_3$ ($-H^+$) |
| 306.0781 | 306.0765 | 1.6 | Reduced glutathione | $C_{10}H_{17}N_3O_6S$ ($-H^+$) |
| 341.1100 | 341.1089 | 1.1 | Lactose | $C_{12}H_{22}O_{11}$ ($-H^+$) |
| 403.0018 | 402.9949 | 6.9 | UDP | $C_9H_{14}N_2O_{12}P_2$ ($-H^+$) |
| 426.0253 | 426.0221 | 3.2 | ADP | $C_{10}H_{15}N_5O_{10}P_2$ ($-H^+$) |
| 442.0176 | 442.0171 | 0.5 | GDP | $C_{10}H_{15}N_5O_{11}P_2$ ($-H^+$) |
| 482.9646 | 482.9613 | 3.3 | UTP | $C_9H_{15}N_2O_{15}P_3$ ($-H^+$) |
| 505.9905 | 505.9885 | 2.0 | ATP | $C_{10}H_{16}N_5O_{13}P_3$ ($-H^+$) |
| 521.9802 | 521.9834 | -3.2 | GTP | $C_{10}H_{16}N_5O_{14}P_3$ ($-H^+$) |
| 540.0583 | 540.0533 | 5.0 | Cyclic ADP-ribose | $C_{15}H_{21}N_5O_{13}P_2$ ($-H^+$) |
| 606.0728 | 606.0743 | -1.5 | UDP-Glc-NAc | $C_{17}H_{27}N_3O_{17}P_2$ ($-H^+$) |
| 662.1021 | 662.1018 | 0.3 | NAD | $C_{21}H_{27}N_7O_{14}P_2$ ($-H^+$) |

Table S5. The substance tentative assignment from the negative-mode mass spectrum for the analysis of single axon, dendrite and body in neuron cell.

| m/z (measured) | m/z (calculated) | Delta m/z (mDa) | Possible Substance | Formula | Location |
|---|---|---|---|---|---|
| 118.0504 | 118.0510 | -0.6 | Threonine | $C_4H_9NO_3$ ($-H^+$) | Body/axon/ dendrite |
| 124.0092 | 124.0074 | 1.8 | Taurine | $C_2H_7NO_3S$ ($-H^+$) | Body/axon/ dendrite |
| 128.0356 | 128.0353 | 0.3 | Pyroglutamic acid | $C_5H_7NO_3$ ($-H^+$) | Body/axon/ dendrite |

| | | | | | |
|---|---|---|---|---|---|
| 132.0299 | 132.0302 | -0.3 | Aspartic acid | $C_4H_7NO_4$ ($-H^+$) | Body/axon/ dendrite |
| 140.0118 | 140.0118 | 0.0 | Phosphoethanolamine | $C_2H_8NO_4P$ ($-H^+$) | Body/axon/ dendrite |
| 146.0464 | 146.0459 | 0.5 | Glutamic acid | $C_5H_9NO_4$ ($-H^+$) | Body/axon/ dendrite |
| 191.0212 | 191.0197 | 1.5 | Citric acid/Isocitric acid | $C_6H_8O_7$ ($-H^+$) | Body/axon/ dendrite |
| 235.0757 | 235.0758 | -0.1 | Met-Ser/Ser-Met | $C_8H_{16}N_2O_4S$ ($-H^+$) | Body/axon/ dendrite |
| 306.0764 | 306.0765 | -0.1 | Glutathione | $C_{10}H_{17}N_3O_6S$ ($-H^+$) | Body/axon/ dendrite |
| 346.0565 | 346.0558 | 0.7 | AMP | $C_{10}H_{15}N_5O_7P$ ($-H^+$) | Body/axon/ dendrite |
| 445.0586 | 445.0531 | 5.5 | CDP-Ethanolamine | $C_{11}H_{20}N_4O_{11}P_2$ ($-H^+$) | Body |
| 487.0989 | 487.1001 | -1.2 | Citicoline | $C_{14}H_{26}N_4O_{11}P_2$ ($-H^+$) | Body |
| 540.0473 | 540.0538 | -6.5 | Cyclic ADP-ribose | $C_{15}H_{21}N_5O_{13}P_2$ ($-H^+$) | Body /dendrite |
| 662.1060 | 662.1018 | 4.2 | NAD | $C_{21}H_{27}N_7O_{14}P_2$ ($-H^+$) | Body/axon/ dendrite |