Electronic Supplementary Material (ESI) for Chemical Science.


# Supporting Information

## S1 Pipette fabrication and characterization

Quartz capillaries with 0.7-mm inner diameter (I.D.) and 1.0-mm outer diameter (O.D.) were used to fabricate ~150 nm and ~250 nm pipettes with a $CO_2$ laser-based pipette puller (P-2000, Sutter Instrument, Novato, CA). Pipettes with I.D. ~750 nm and ~950 nm were fabricated from quartz capillaries with 0.3-mm I.D. and 1.0-mm O.D. Prior to nanopipette fabrication, capillaries were treated with piranha solution ($H_2O_2$:$H_2SO_4$ = 1:3) to remove organic contaminants.

Nanopipette tip diameter and cone angle were characterized with scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) images obtained with a Quanta-FEG 600F scanning electron microscope (FEI, Hillsboro, OR). Image processing and analysis were performed with ImageJ (National Institutes of Health, Bethesda, MD).

## S2 Focused Ion Beam (FIB) milling of nanopipettes

A Zeiss Auriga® Modular Cross Beam work station (Oberkochen, Germany) was used to mill bare quartz nanopipettes to obtain long shank (taper) nanopipettes with a 250 nm I.D. The ‘Mill for depth’ function was selected in the SmartSEM® V05.05 XB operating software. A 5 μm depth and a 30 kV, 500 pA beam current was used.

**S3 Sample volume reproducibility**

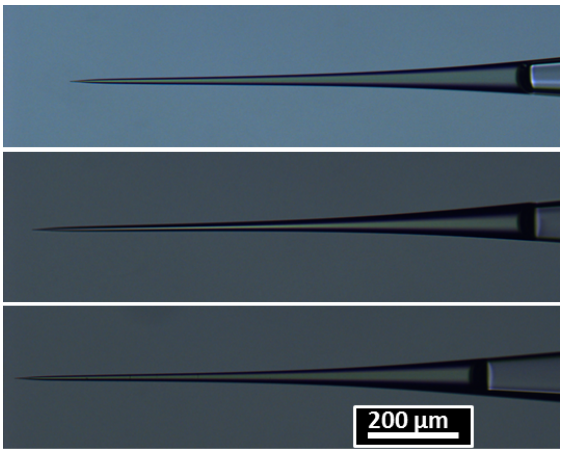


**Figure S1** Optical micrographs of three nanopipettes (same size and geometry) with purified water aspirated at a pressure difference of 44 kPa (aspiration time = 1 minute).

**Table S1** List of pipette parameters and volume of sample aspirated, of the pipettes shown in Figure S1, at identical conditions of pressure, time and solution viscosity.

| Pipette # | Radius (nm) | Height of ingress (µm) | Cone angle(°) | Volume (nL) | Average volume (nL) | Standard Deviation (nL) | RSD (%) |
|---|---|---|---|---|---|---|---|
| 1 | 72 | 1007 | 12 | 53 | 61 | 7 | 12 |
| 2 | 70 | 1041 | 14 | 66 | | | |
| 3 | 75 | 1014 | 13 | 63 | | | |

**S4 Behavior of fluids inside nanopipette shank and tip**

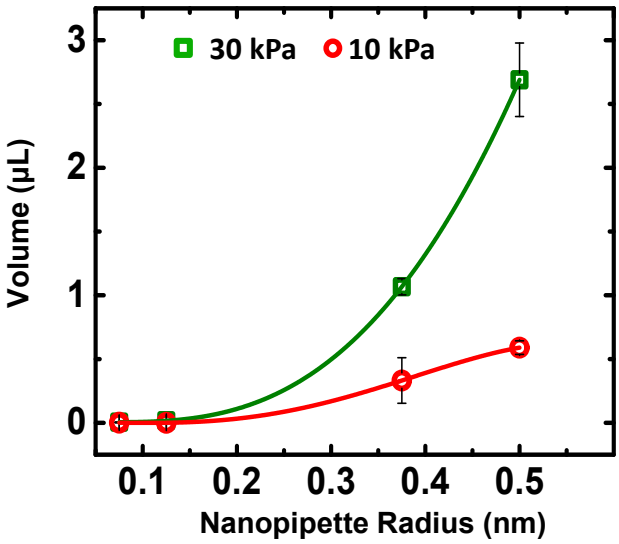


**Figure S2** Volume aspirated versus nanopipette radius at a constant pressure difference of 30 kPa ( ▫ ) and 10 kPa ( ○ ). Arbitrary lines were drawn to connect the data points.

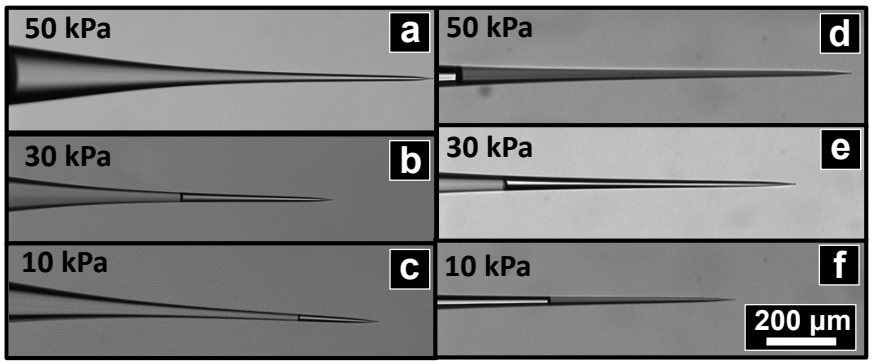


**Figure S3** Optical micrographs of ~150 nm (I.D.) short shank nanopipettes (a-c) and ~250 nm (I.D.) long shank nanopipettes (d-f) when subjected to pressure-assisted sampling of purified water at various pressures.

**Table S2** List of nanopipette tip diameter and water ingress (inside nanopipette) due to capillary action.

| Nanopipette tip diameter (nm) | Ingress due to capillary action (nL) |
|---|---|
| 150 | 0.0064 |
| 250 | 0.01 |
| 750 | 0.03 |
| 950 | 1.02 |

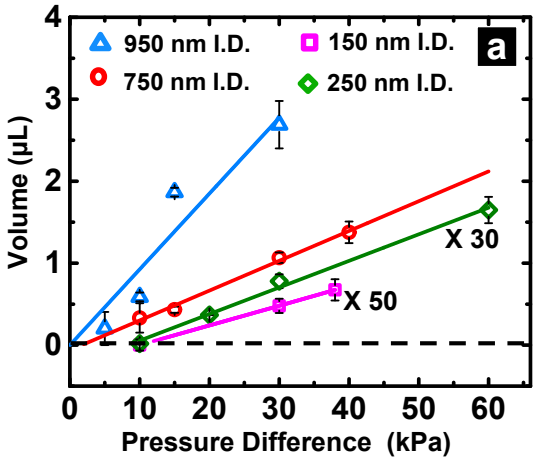


**Figure S4** Volume of sample aspirated as a function of pressure difference plot for tip diameter ~950 nm (I.D.) (△), ~750 nm (I.D.) (○), ~250 nm (I.D.) (◇), ~150 nm (I.D.) (□). Ingress of sample due to capillary action was subtracted from the total volume for this plot to compare ingress due to pressure driven aspiration only.

**S5 Mass spectrometric analysis of standard sample aspirated with nanopipettes**

Prior to application of the nanopipette sampling technique for mass spectrometric analysis of real biological sample such as *Allium cepa* and *Drosophila melanogaster*, standard mass spectrometry analyte solution was sampled and corresponding mass spectrum was acquired (**Figure S5**) to validate the pipette sampling method. For this analysis, sample deposition was performed by immersing the nanopipette in a drop of water, followed by application of a positive pressure. In this experiment, the sample delivery on to MALDI plate was performed by immersing the tip into a drop of water so as to maintain the integrity of the tip for subsequent STEM imaging. After sample deposition, STEM images of the tips were acquired and the integrity of the pipettes was verified. Determination of tip integrity is an important step to ensure the nanopipette tips do not break during sampling and the samples collected (and hence the mass spectrum) were obtained from a localized region of a sample.

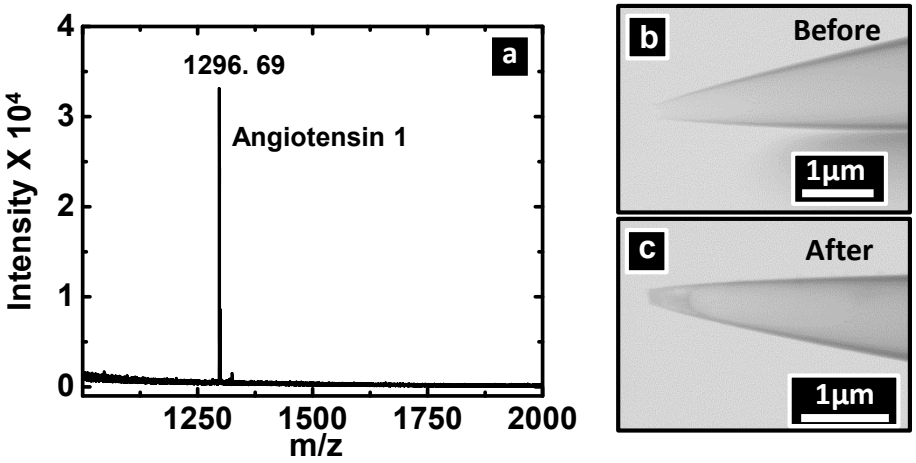


**Figure S5** MALDI-MS spectra of 20 µM solution of angiotensin I aspirated into a 112 nm I.D. nanopipette. Volume of analyte aspirated was 32 nL, or 644 fmol, of angiotensin I. (Matrix: α-cyano-4-hydroxycinnamic acid). Scanning transmission electron micrograph

of the nanopipette before sampling (b) and after sampling (c). The I.D. before and after sampling was ~112 nm.

**Table S3** Tentative Peak assignments in mass spectrum of a single *Allium cepa* epidermal cell.

| Assignment | Exact mass | Observed mass | Δppm |
|---|---|---|---|
| Glutamine+Sucrose+K | 527.1490 | 527.1424 | 12.5 |
| Cyanidin malonyl glucoside | 535.1087 | 535.1300 | 39.87 |
| Trisaccharide+K | 543.1328 | 543.1193 | 24.8 |
| Tetrasaccharide+K | 705.1875 | 705.1815 | 2.6 |
| Pentasaccharide+K | 867.2384 | 867.2274 | 12.6 |
| Hexasaccharide+K | 1029.2913 | 1029.3200 | 27.8 |
| Heptasaccharide+K | 1191.3440 | 1191.3266 | 14.6 |

**S6 Spiking study to further support the identity of oligosaccharides**

For the spiking studies, a standard solution of dextran was prepared in water and was analyzed by MALDI-MS. Another spot of *Allium cepa* cytoplasm was prepared and analyzed for oligosaccharides independently by MALDI-MS after application of DHB matrix. Then the spot was spiked with above mentioned dextran stock by application of a microliter of the standard onto the dried cytoplasm/matrix spot. The spot was again analyzed for oligosaccharides under identical conditions as the previous spot. Shown below are some of the representative mass spectra of a sample before (in green) and after (in black) spiking with dextran standard. The spectra were zoomed-in to confirm the presence of only one peak at m/z corresponding to the oligosaccharides. Ofnote, for the black trace, internal calibration was not performed. The green trace was internally calibrated as per the procedure detailed in the paper.

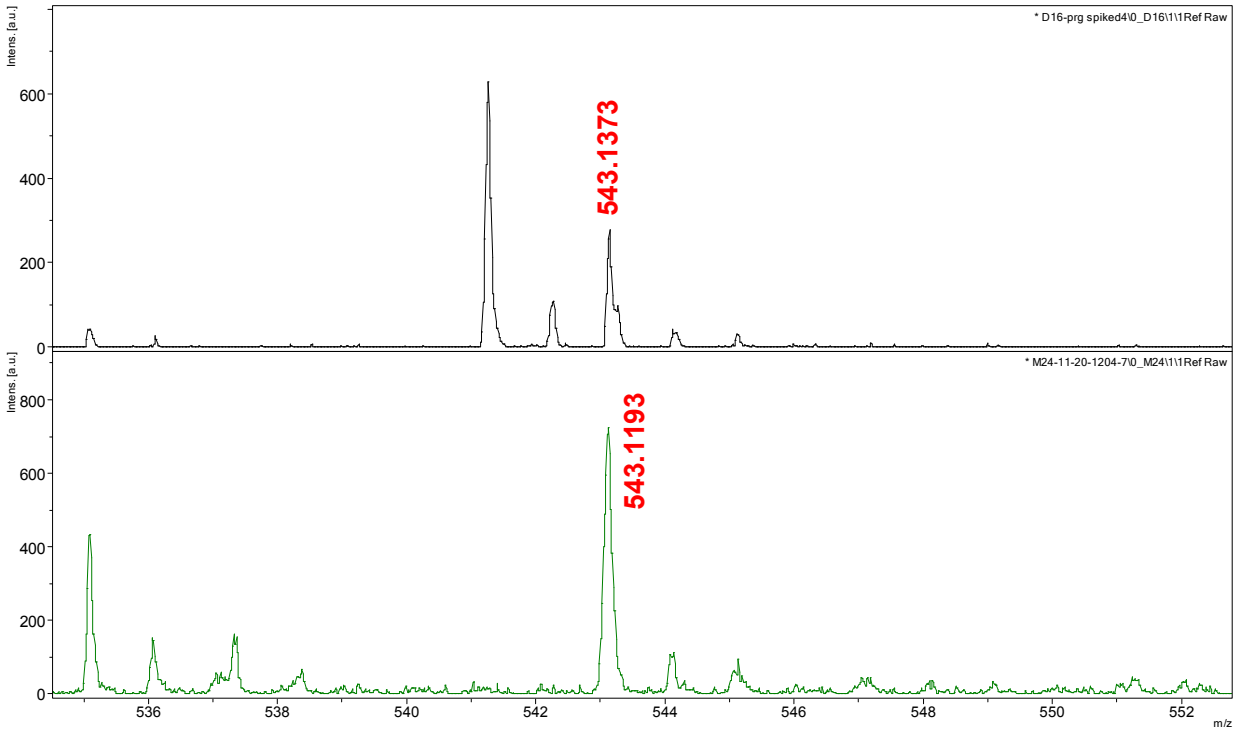

**Figure S6** MALDI-MS spectra of trisaccharide+K in *Allium cepa* cytoplasm before (green) and after (black) spiking with dextran standard.

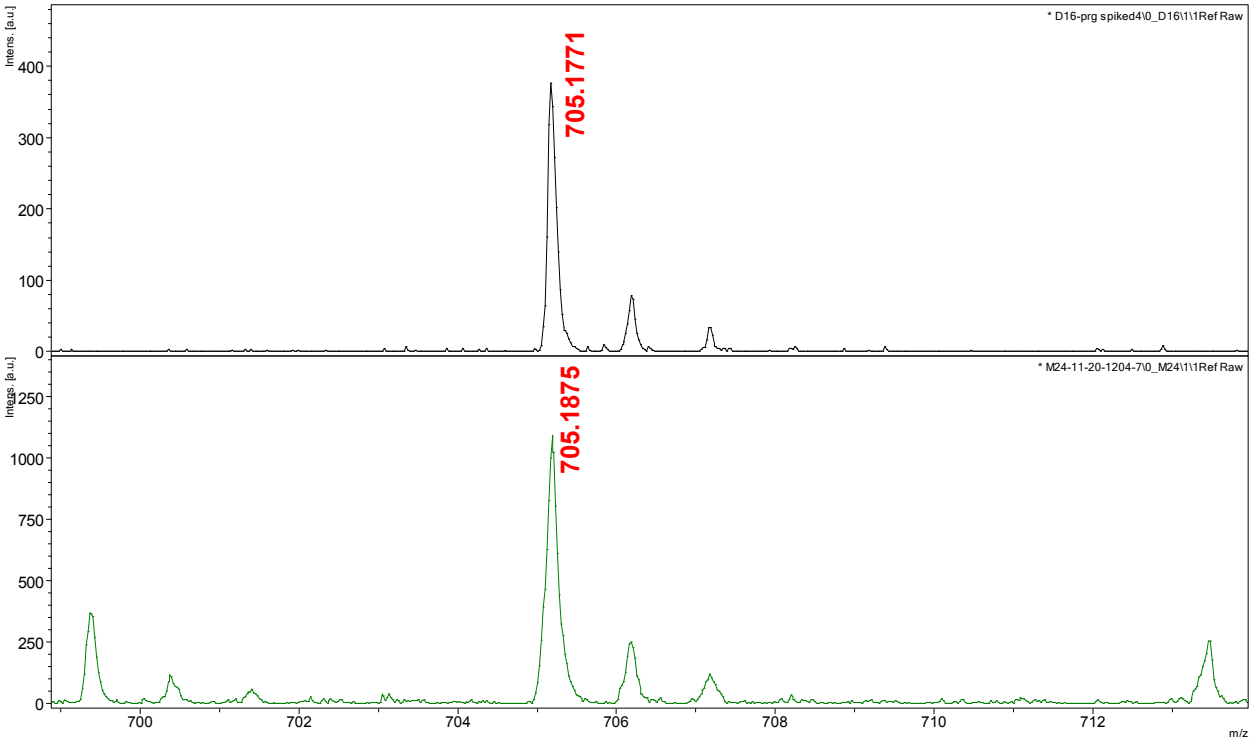


**Figure S7** MALDI-MS spectra of tetrasaccharide+K in *Allium cepa* cytoplasm before (green) and after (black) spiking with dextran standard.

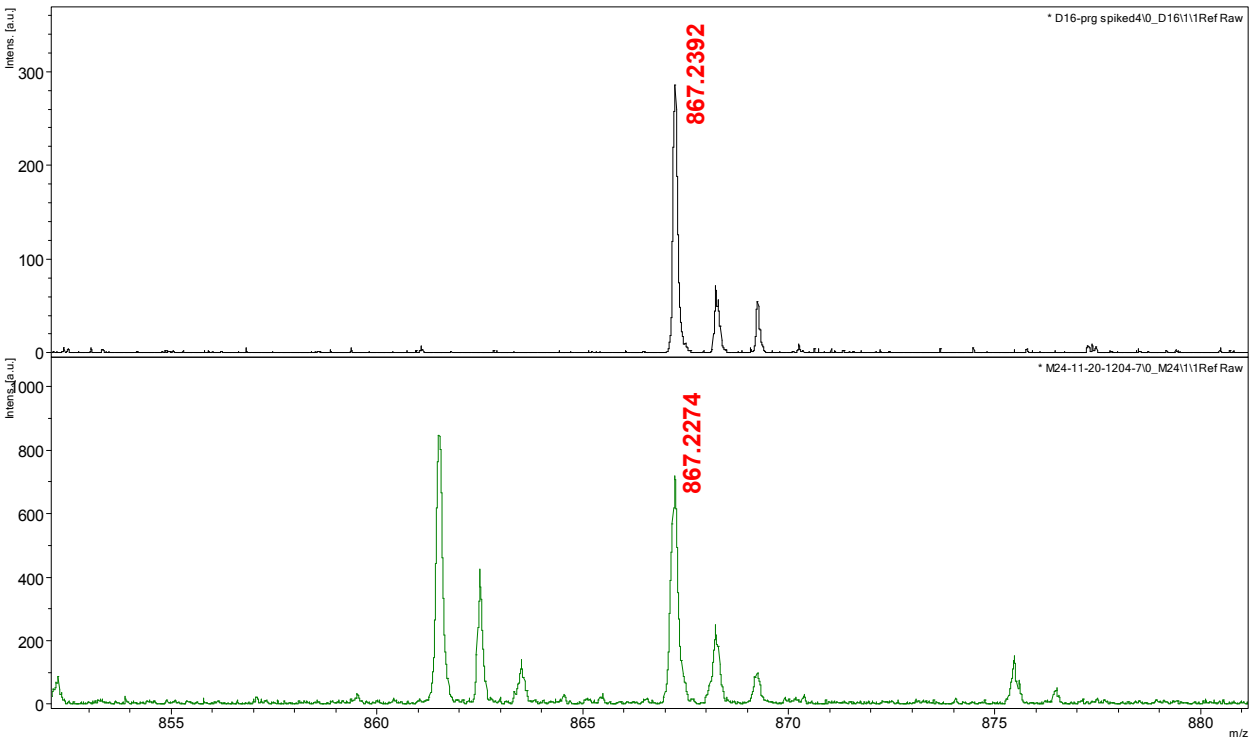


**Figure S8** MALDI-MS spectra of pentasaccharide+K in *Allium cepa* cytoplasm before (green) and after (black) spiking with dextran standard.

**Table S4** Tentative Peak assignments for lipids from *D. melanogaster* hemolymph

| Assignment | Exact mass | Observed mass | Δppm |
|---|---|---|---|
| PS 32:4 [M+Na] | 750.4317 | 750.4174 | 19.0 |
| PS 32:3 [M+Na] | 752.4473 | 752.4616 | 19.0 |
| PS 32:2 [M+Na] | 754.4630 | 754.4815 | 24.5 |
| PC 34:3 [M+H] | 756.5540 | 756.5410 | 17.2 |
| PC 34:2 [M+H] | 758.5694 | 758.5789 | 12.5 |
| PC 34:1 [M+H] | 760.6010 | 760.5923 | 11.4 |
| PS 34:3 [M+Na] | 780.4786 | 780.4902 | 14.9 |

| | | | |
|---|---|---|---|
| PS 34:2 [M+Na] | 782.4943 | 782.5068 | 16.0 |
| PS 34:5 [M+K] | 792.4212 | 792.4290 | 9.8 |
| PS 34:4 [M+K] | 794.4369 | 794.4491 | 15.3 |

**Table S5** Tentative Peak assignments for lipids from Rat brain tissue section

| **Assignment** | **Exact mass** | **Observed mass** | **Δppm** |
|---|---|---|---|
| PC 32:0 [M+H] | 734.5694 | 734.5625 | 9.3 |
| PC 34:1 [M+H] | 760.5851 | 760.5852 | 0.1 |
| PC 36:4 [M+H] | 782.5694 | 782.5857 | 20.8 |
| PC 36:1 [M+H] | 788.6164 | 788.6247 | 10.5 |