Supporting Information

Microdroplet Impact-Induced Spray Ionization Mass Spectrometry (MISI MS) for Online Reaction Monitoring and Bacteria Discrimination

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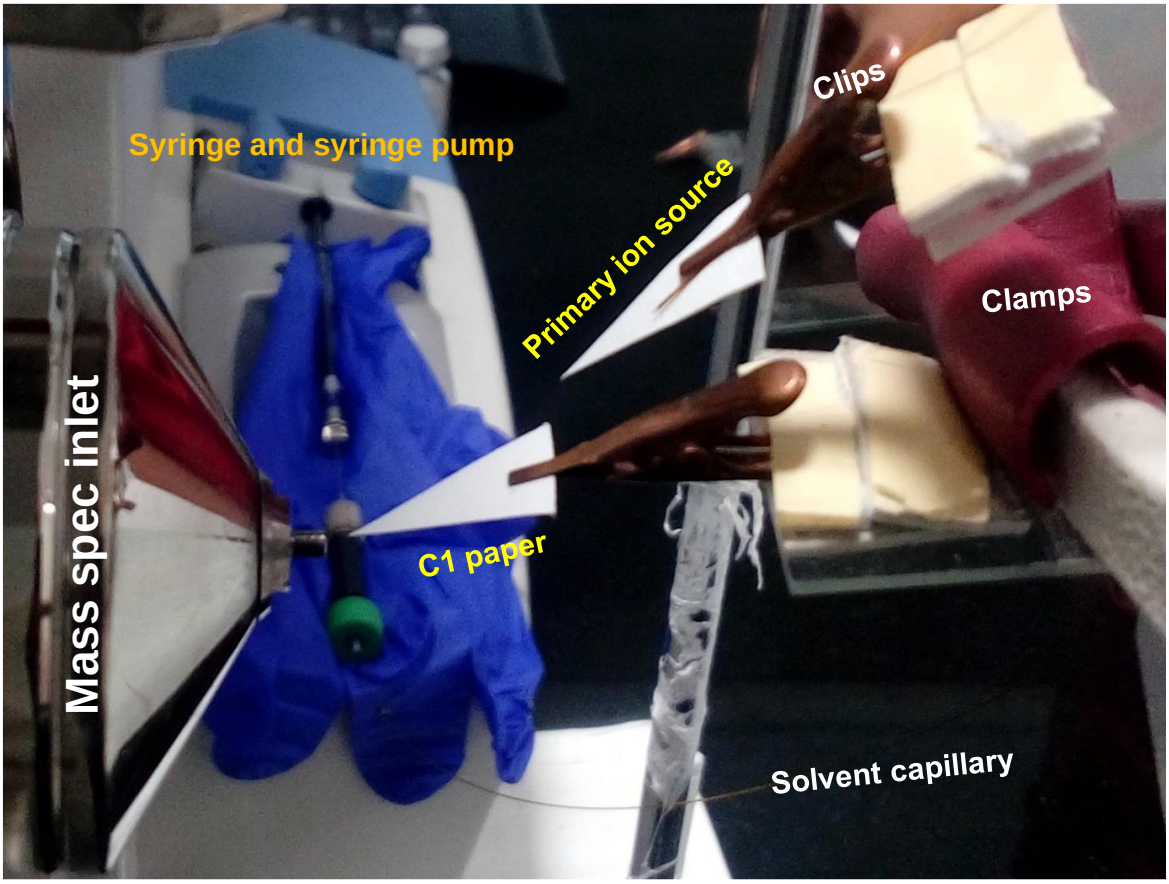

Figure S1. Optical image of the MISI MS in C1 mode. The primary ion source, paper in C1 mode, solvent capillary, mass spec inlet, clamp and clip are indicated in the picture.

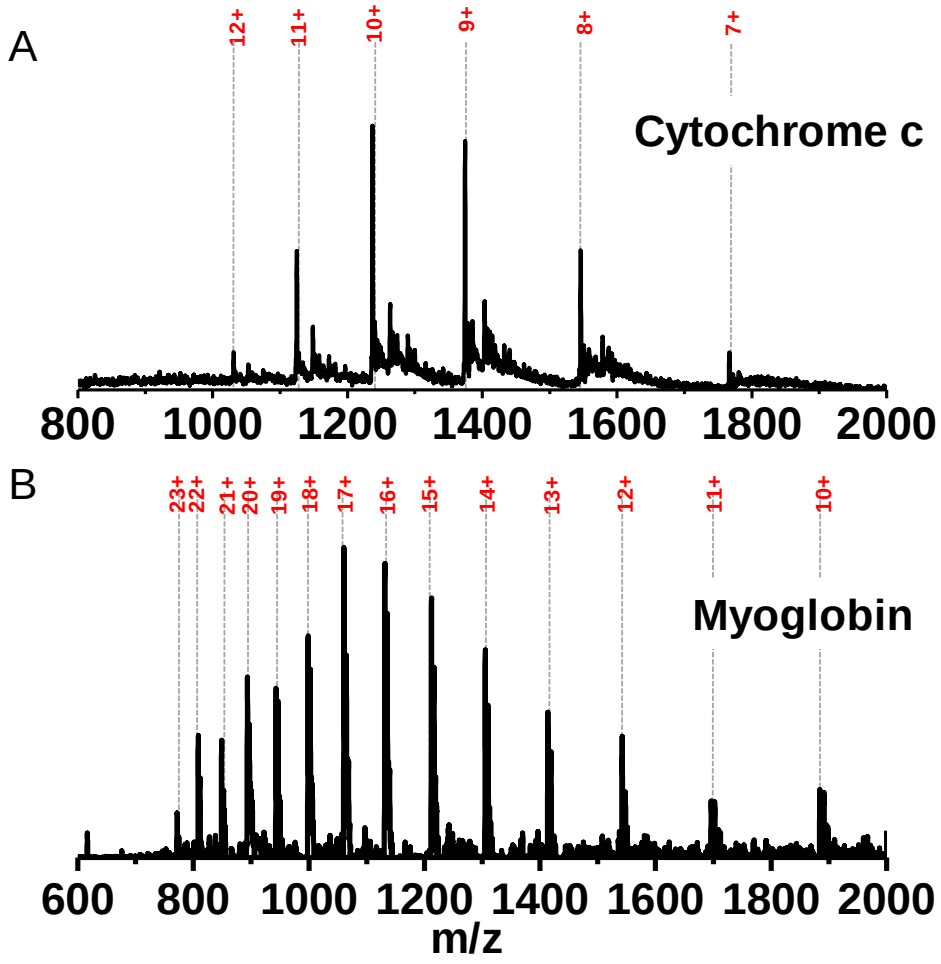

Figure S2. MISI mass spectra of A) cytochrome c, and B) myoglobin in C1 mode. The charge states of all the peaks for both the protein mass spectra are indicated in red. The solutions (0.1 mg/ml) were made in deionized water.

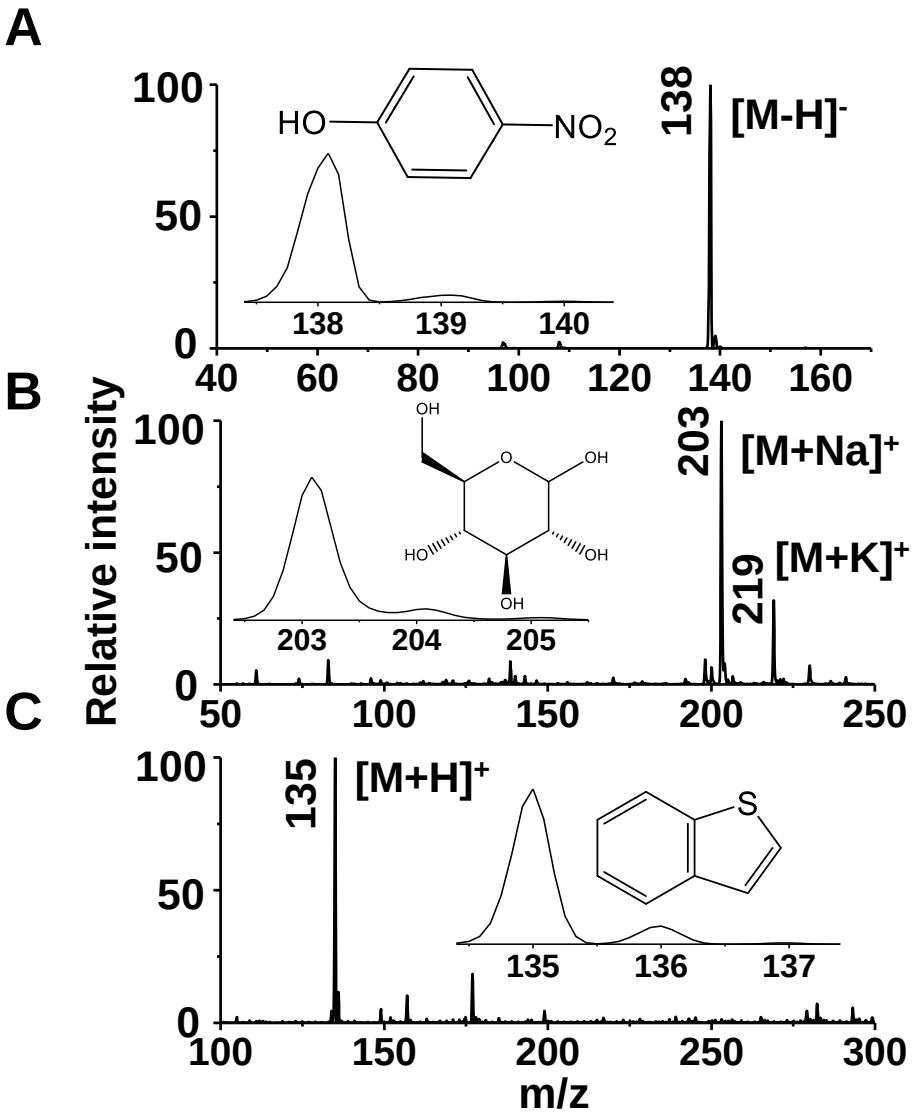

Figure S3. MISI mass spectra of A) nitrophenol, B) glucose and C) benzothiophene in C1 mode. Insets of each figure show the isotopic distribution of the corresponding peak as well as the molecular structure of the analyte used for the experiment.

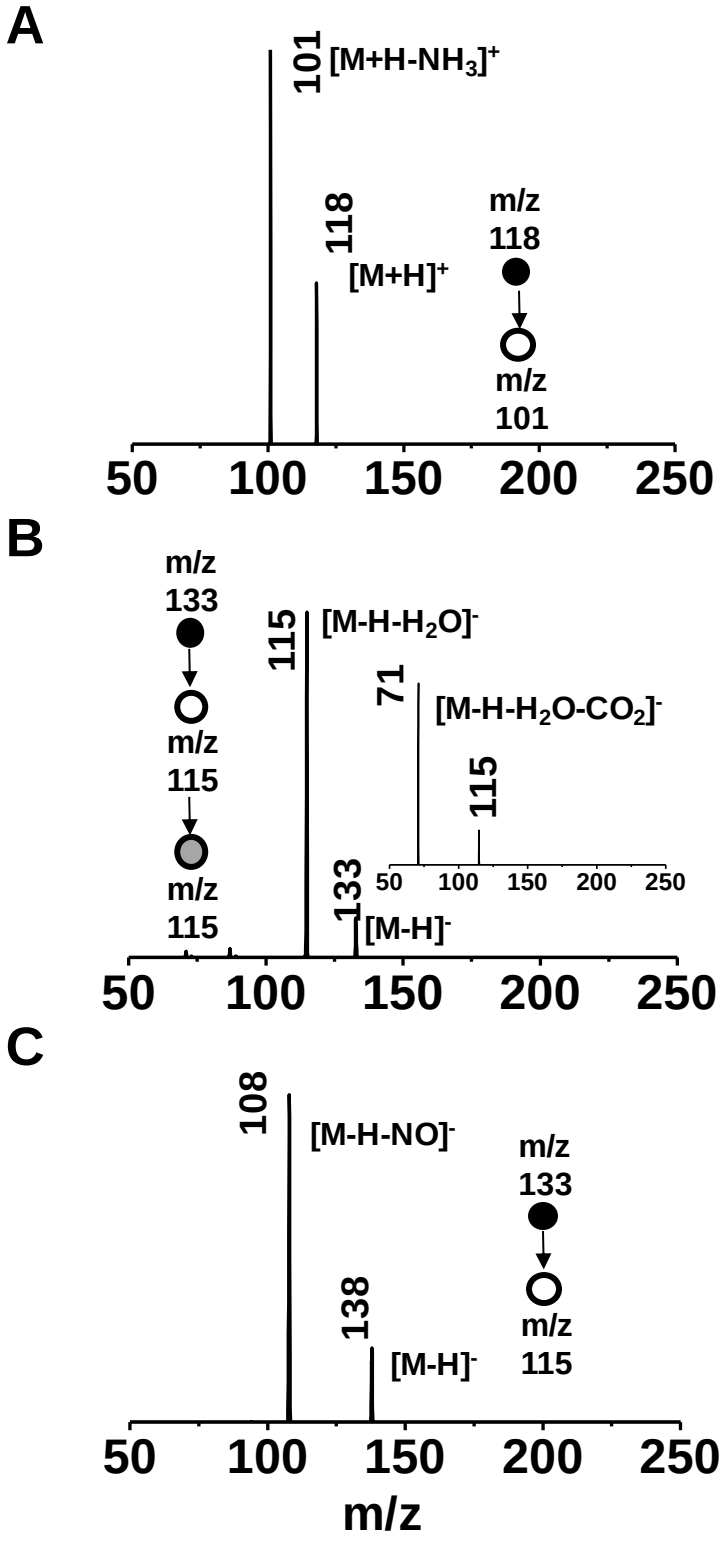

Figure S4. MS/MS spectrum of A) indole, B) malic acid, and C) p-nitrophenol in negative-ion mode. Inset of Figure B shows the MS^3 spectrum of the mass selected peak at *m/z* 115. The peak assignments are based on the neutral loss during the fragmentation of peaks.

Supporting information 1.

Cascaded mode of ionization-

To demonstrate the cascaded behavior, rhodamine 6G (20 µL of 10 µM) was chosen as an analyte. Figure S5A schematically represents the setup for cascade 1, 2 and 3 (C1, C2 and C3). The optical image of the setup is shown in Figure S6. The MISI mass spectrum of R6G in C3 mode is shown in Figure S5B. In it, we see a peak at *m/z* 443 which corresponds to the protonated ion. This was further confirmed by the MS^2 spectrum (Figure S7). Figure S8 presents the mass spectra of R6G for C1 to C3 modes where we see a gradual decrease in the absolute intensity. The absolute intensities of the selected peak at *m/z* 443 in C1-3 modes of ionization are presented in Figure S5D.

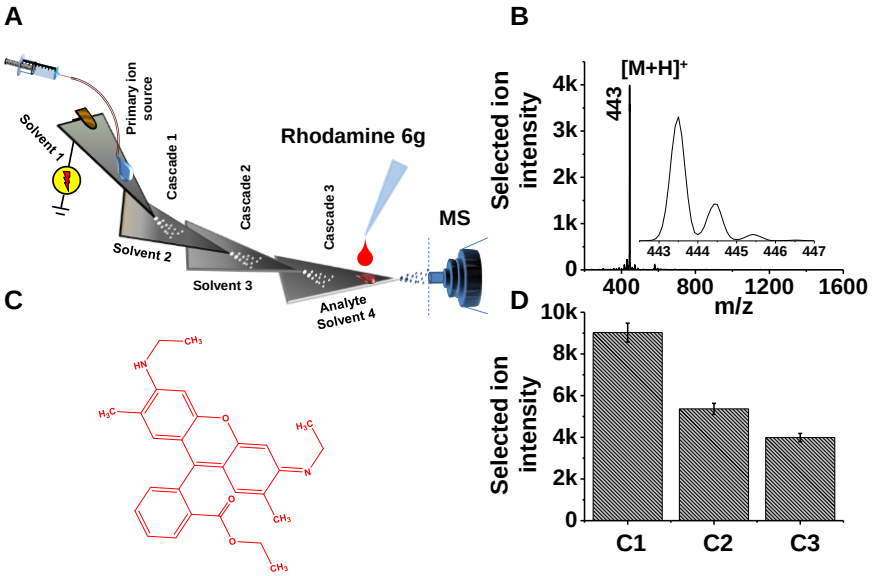

Figure S5. A) Schematic representation of cascade 1, 2 and 3. B) MISI mass spectrum of Rhodamine 6G in C3 mode. Inset represents the isotopic pattern of the peak at *m/z* 443 and the molecular structure of R6G. C) Molecular structure of rhodamine 6G. D) Ion intensity of the selected ion at *m/z* 443 for different cascade modes.

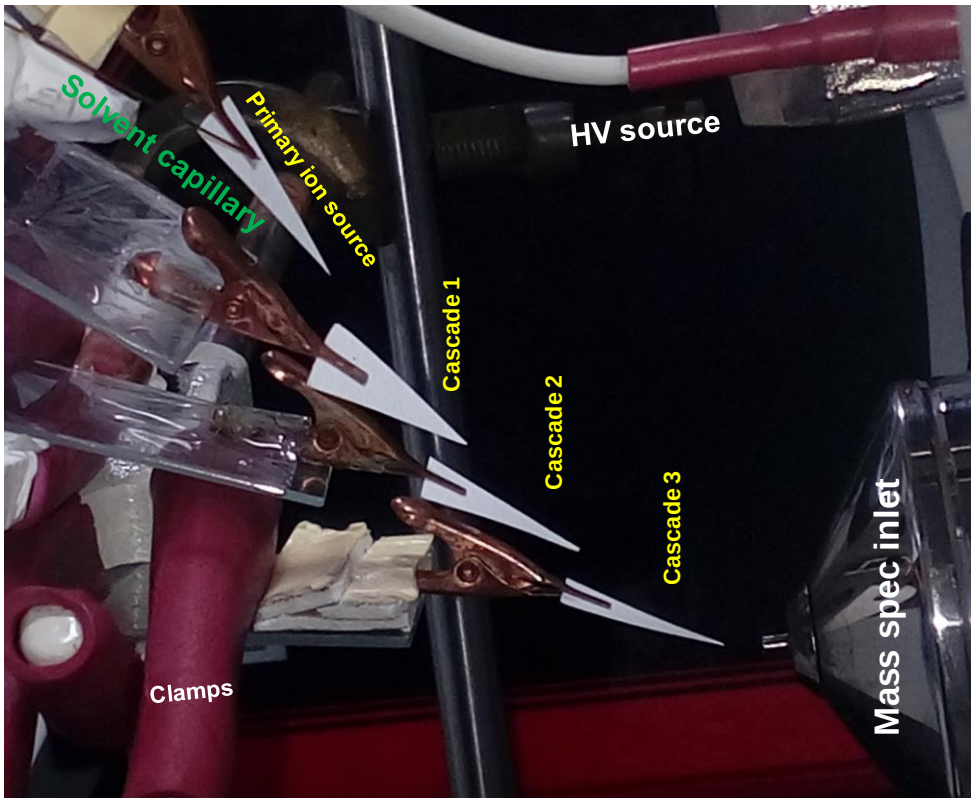

Figure S6. Optical image of MISI MS in C3 mode. the yellow traces indicate that the modes can be switched by removing or adding another paper triangle into the setup.

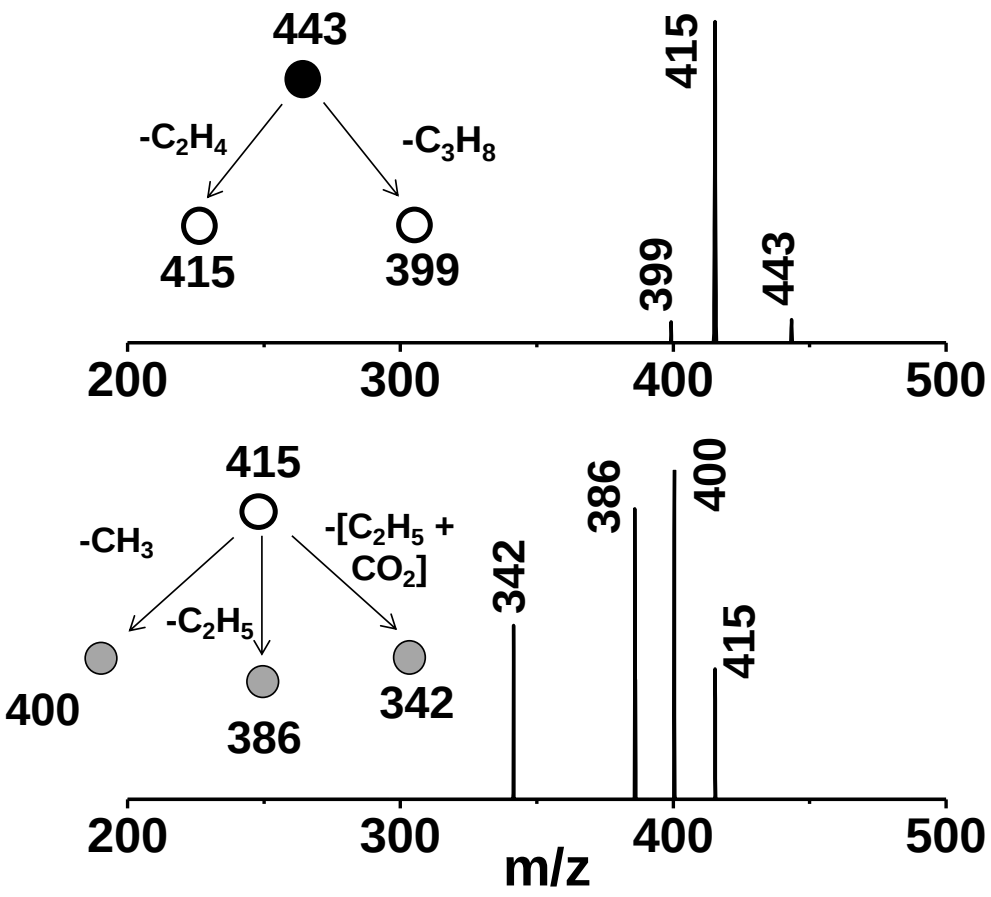

Figure S7. MS/MS spectra of the peak at *m/z* 443 corresponding to the protonated peak of R6G. The above spectrum presents the MS^2 fragmentation of the mass selected peak at *m/z* 443 whereas the bottom spectrum presents the MS^3 spectrum of the mass selected peak at *m/z* 415.

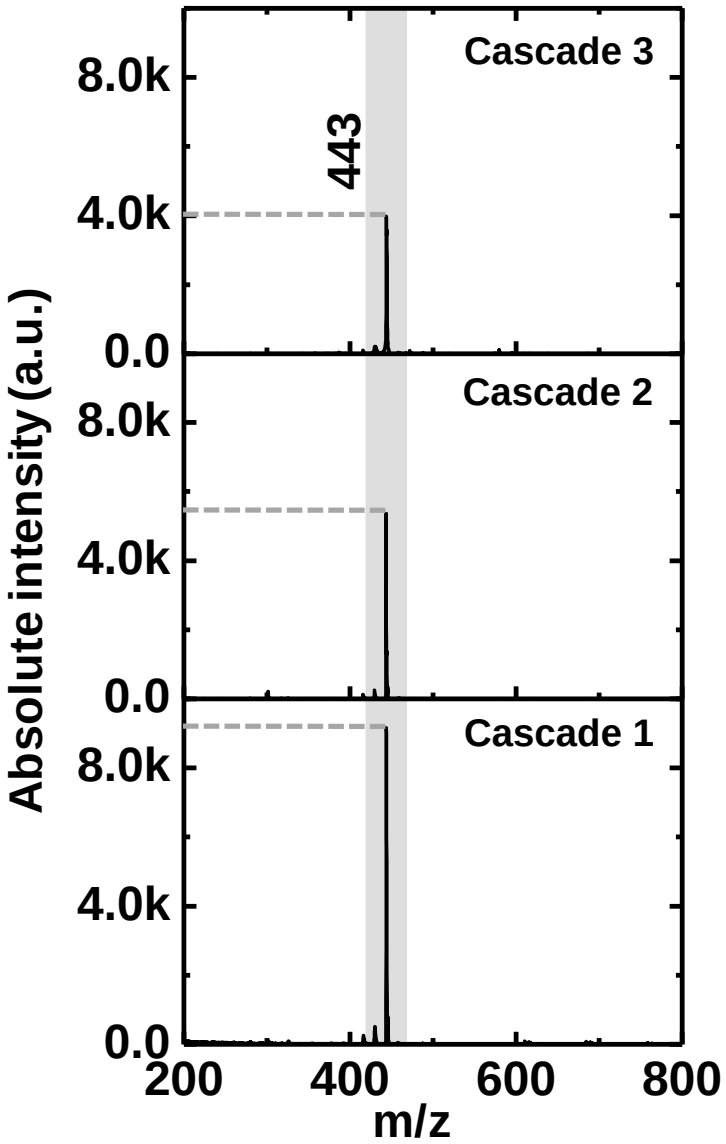

Figure S8. MISI mass spectrum of R6G in C1-3 mode. The intensity variation of the shaded peak at *m/z* 443 is shown. The intensity loss during the cascaded process may due to the charge loss.

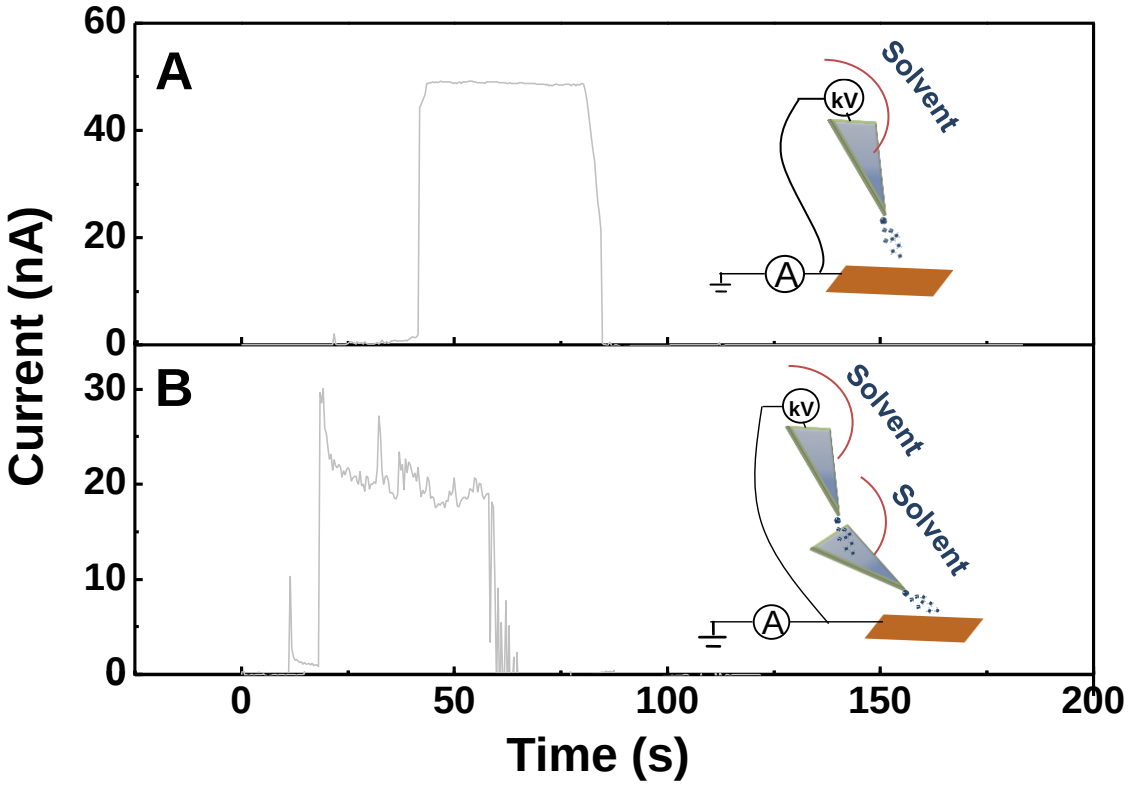

Figure S9. Spray current measurement in A) kVPSI, B) MISI. Inset of each trace presents the arrangements used for the measurement. The intensity fluctuations in the latter two spectra are probably due to the cascaded process in which the charges are also lost during the charge transfer processes over the surface.

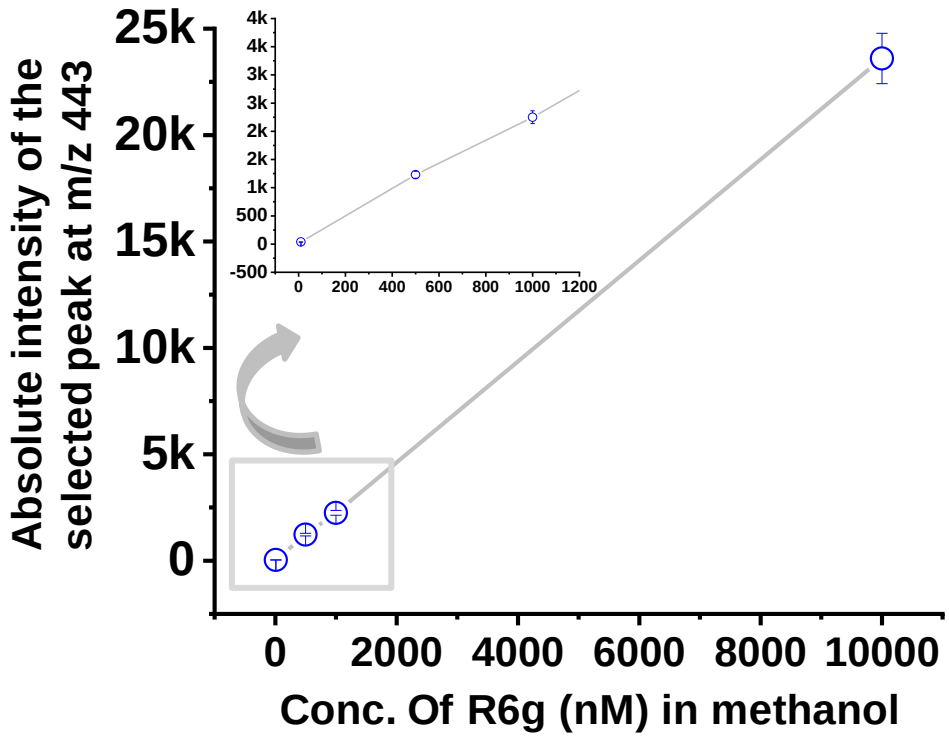

Figure S10. Intensity vs concentration plot using R6G as the analyte. The inset shows the zoomed-in view of the low concentration regime.

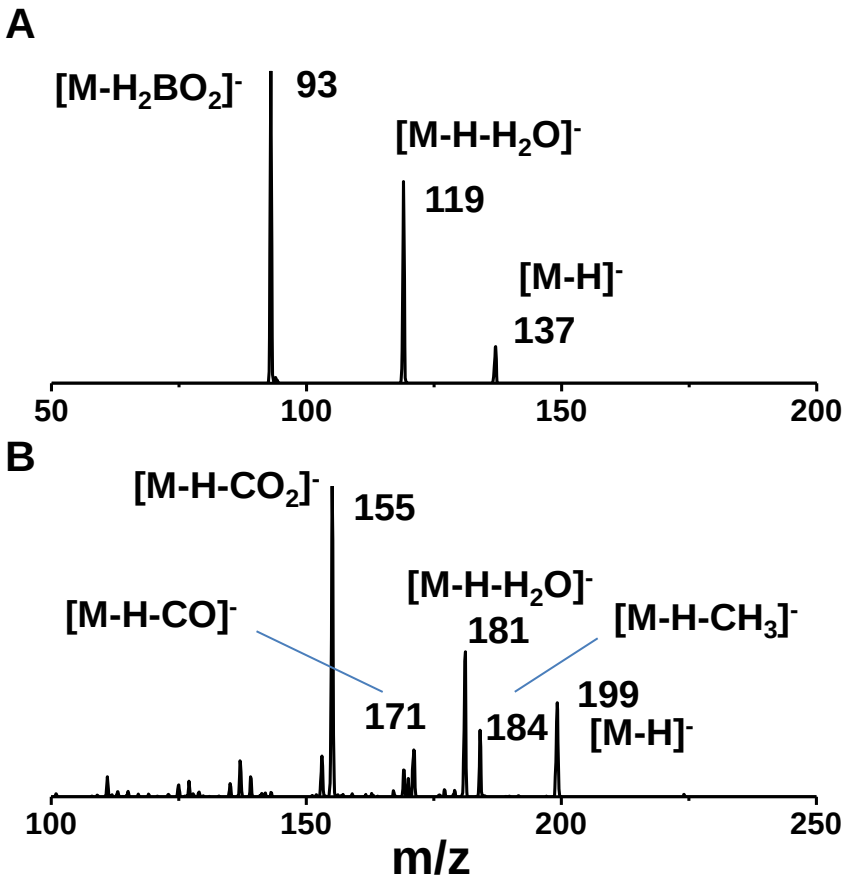

Figure S11. MSMS spectra of deprotonated 4-hydroxyboronic acid and product peak at *m/z* A) 137 and B) 119 in the negative-ion mode.

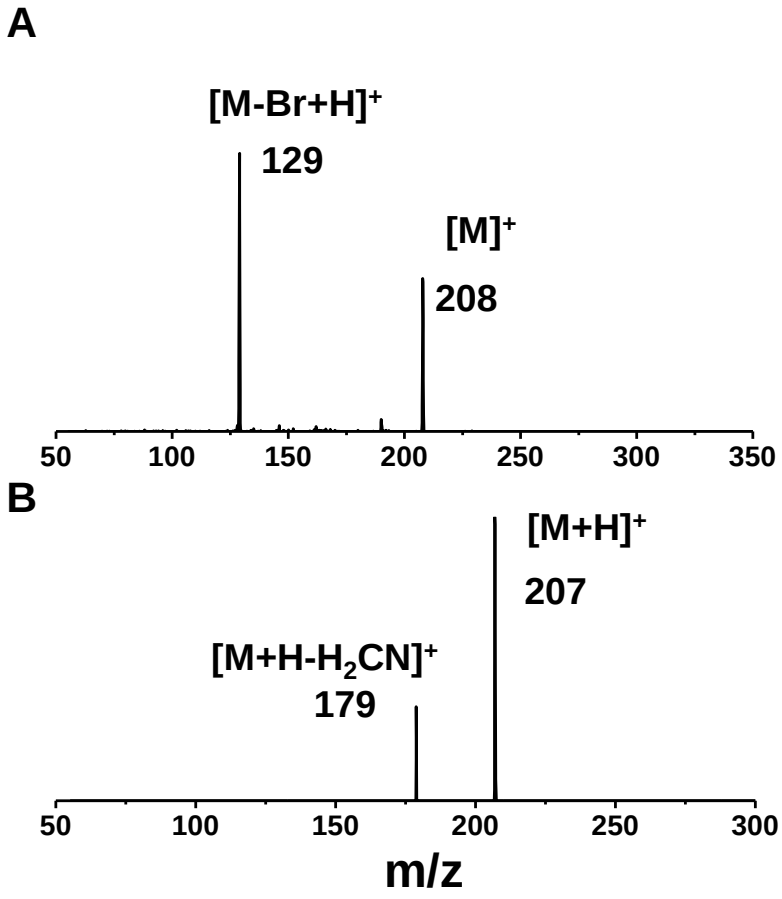

Figure S12. MSMS spectra of 6-bromoquinoline and product peak at *m/z* A) 208 and B) 207 in the positive-ion mode.

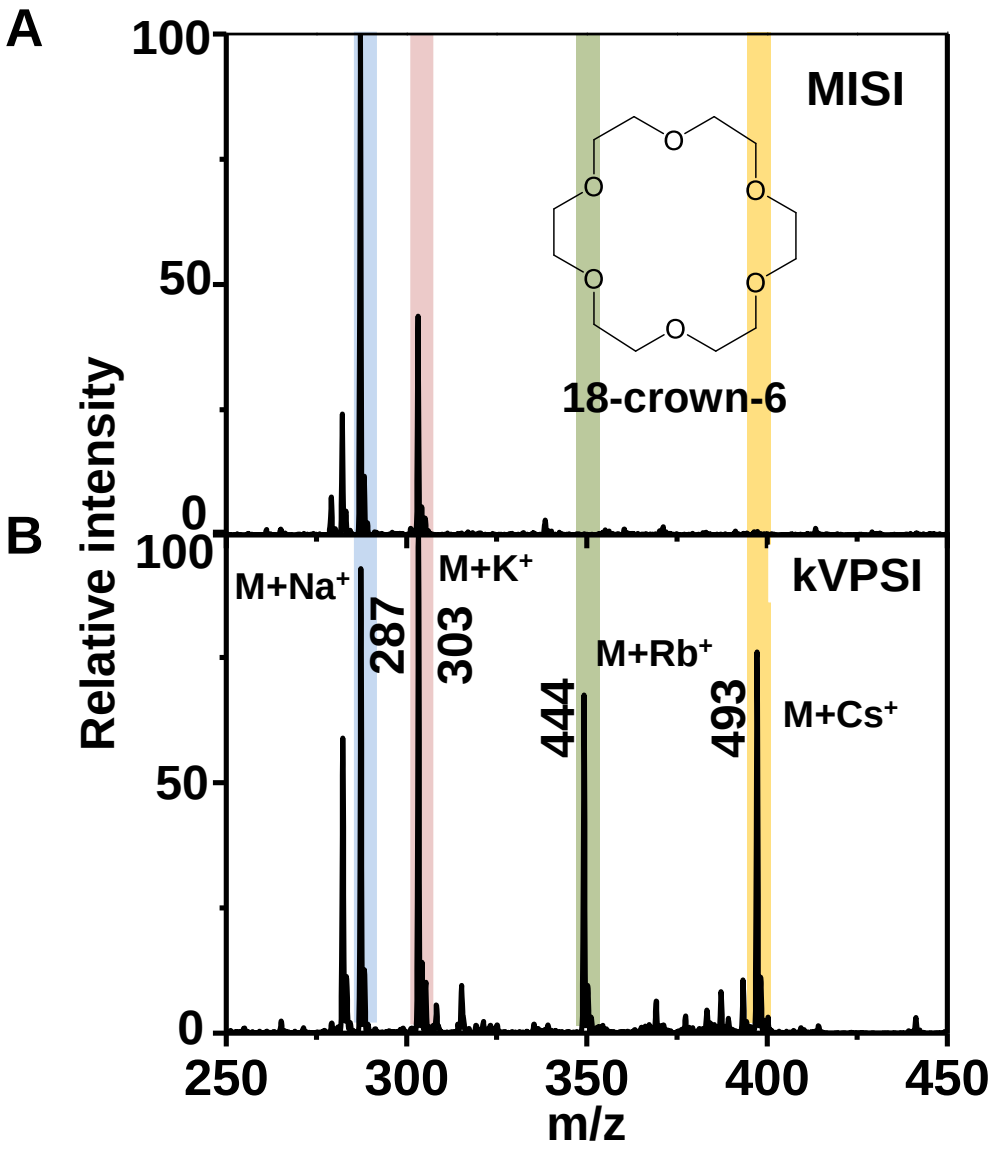

Figure S13. Comparative mass spectrum using A) MISI and B) kVPSI of ion encapsulation reaction between alkali metal ions with 18-crown-6. The kVPSI mass spectrum is recorded for the bulk reaction. Inset of Figure A shows the molecular structure of crown ether.

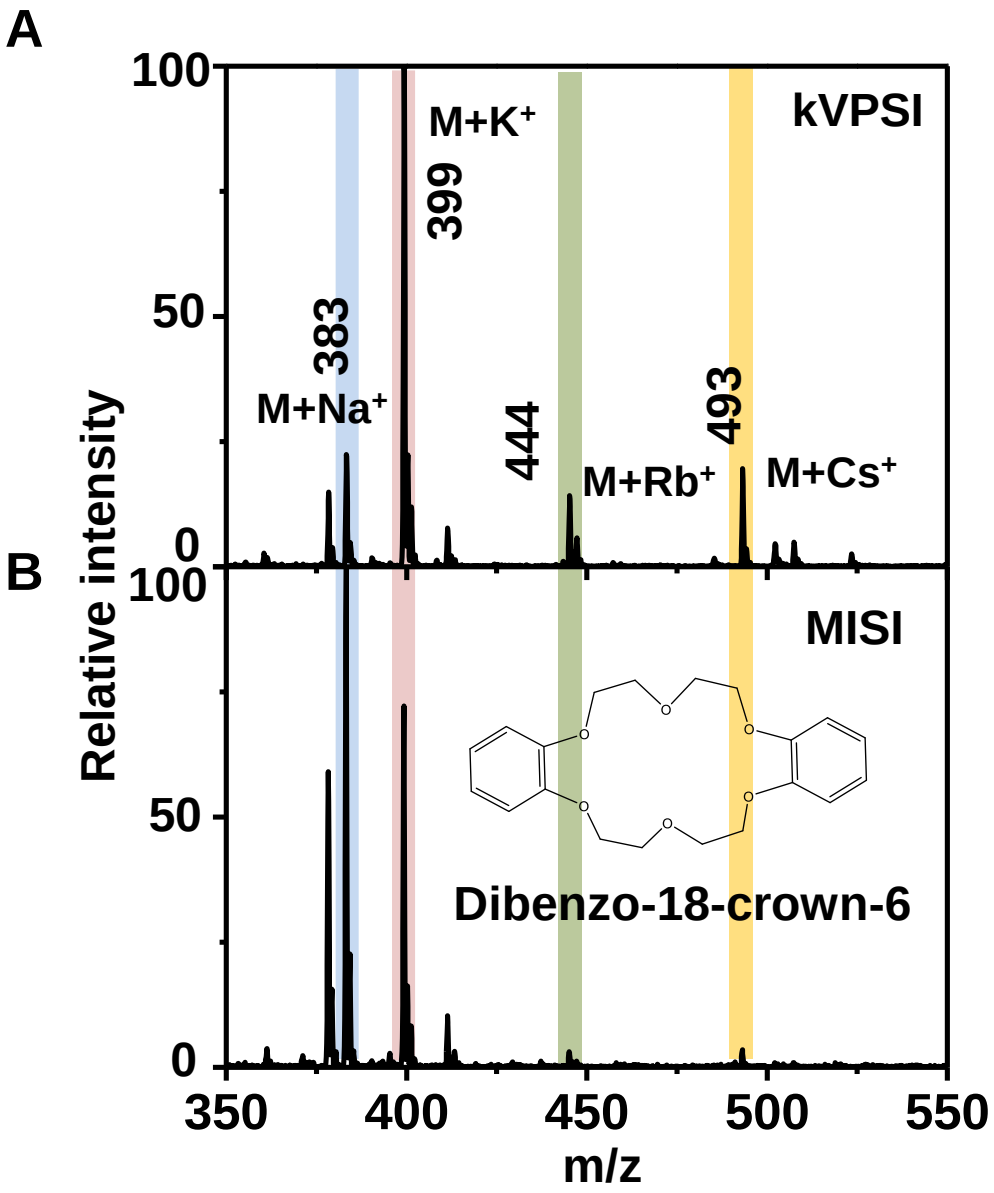

Figure S14. Comparative mass spectrum using A) kVPSI and B) MISI of ion encapsulation reaction between alkali metal ions with dibenzo-18-crown-6. The kVPSI mass spectrum is recorded for the bulk reaction. Inset of Figure B shows the molecular structure of the crown ether.

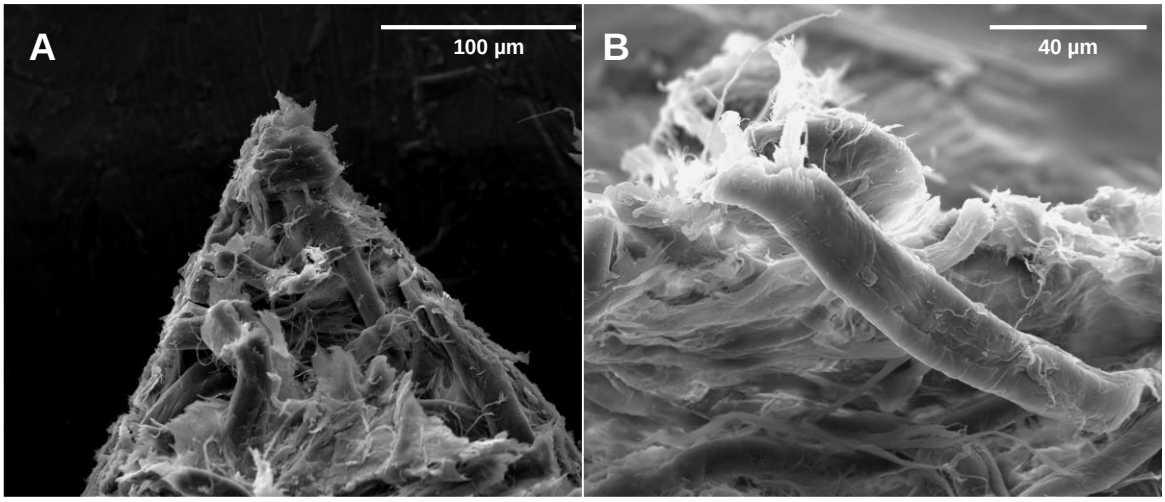

Figure S15. SEM images of paper surface. The scale bar is shown at the top of the images.

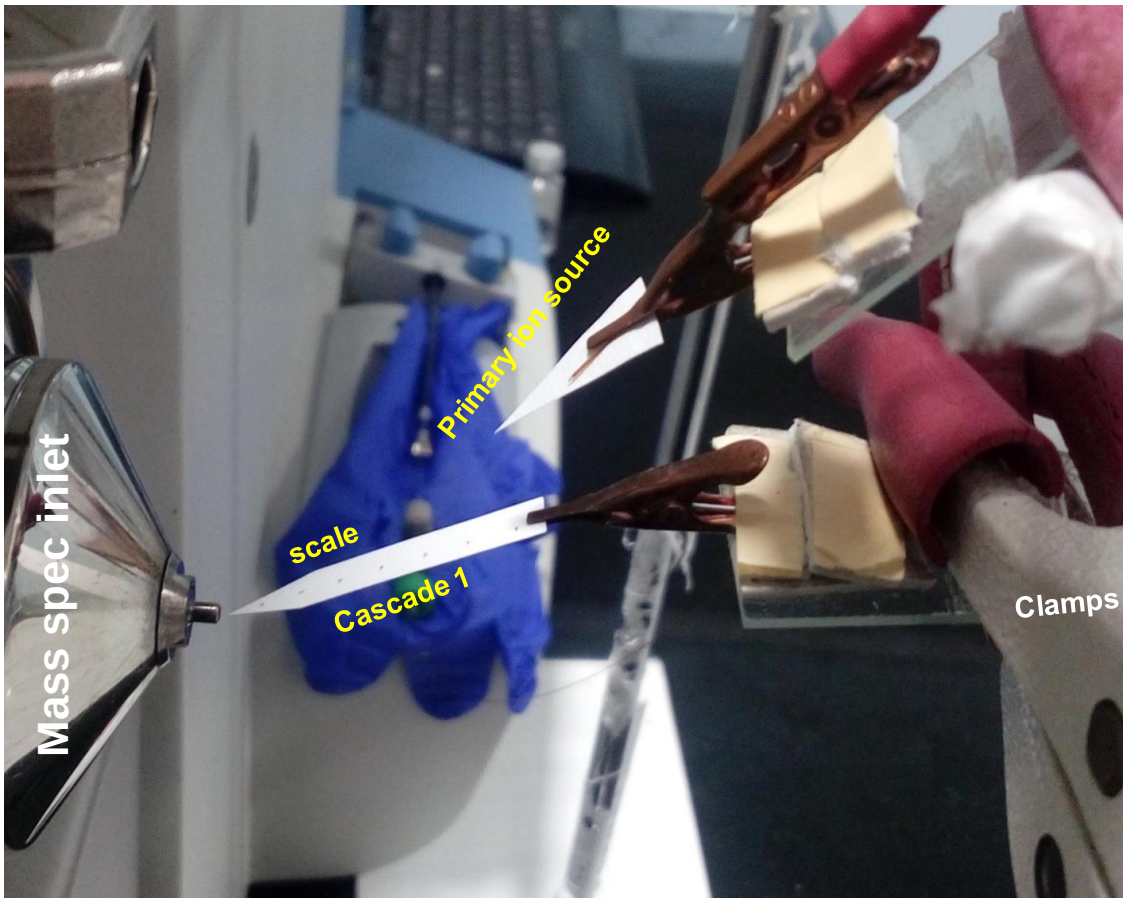

Figure S16. Photograph of the setup for the measurement of encapsulation kinetics. Marks over the second paper are to provide a measure of scale.

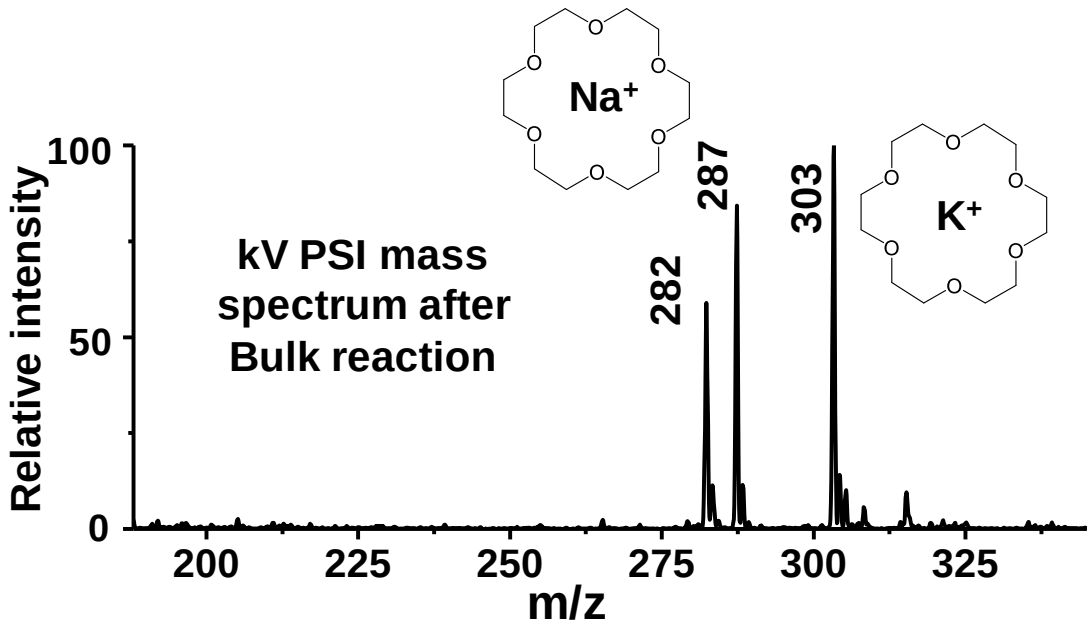

Figure S17. kVPSI mass spectrum of the bulk reaction. Intensity ratio of peak at *m/z* 303 and 287 is 119 %. The final concentration of each reagent was 10 μM in water. The reaction mixture was kept at room temperature for 15 min before the mass spectrometric measurement.

Supporting information 2.

Fluid terminal velocity-

Experimental details- The fluid terminal velocity over the paper surface was measured by measuring the time to flow a liquid due to the capillary action of the paper. The experiment was conducted on a labelled paper, as shown in Figure S18. The pencil marks do not interfere with the fluid flow. The flow was initiated using a fused silica capillary by flowing methanol containing some R6G in it. R6G was added to visualise the system. The flow rate was set to 100 µL/min using a syringe and a pump. Three independent experiments gave time as 2.54, 2.60 and 3.20 s to travel a distance of 1 cm. The average time taken was 2.78 s. The velocity was found to be 0.36 cm/s.

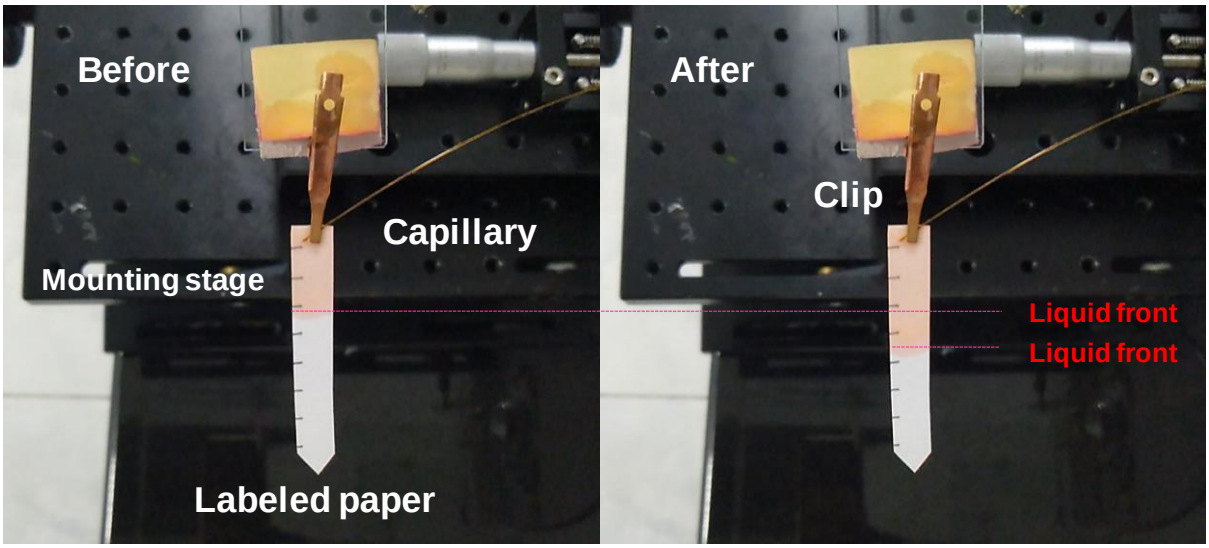

Figure S18. Optical images of the fluid terminal velocity experiment. The change in the liquid front is clearly visible and indicated in red trace. The paper has marks to indicate to scale. each gap in the paper corresponds to 1 cm. The flow rate is set to 100 µL/min. The average terminal velocity of three measurements was 0.36 cm/s.

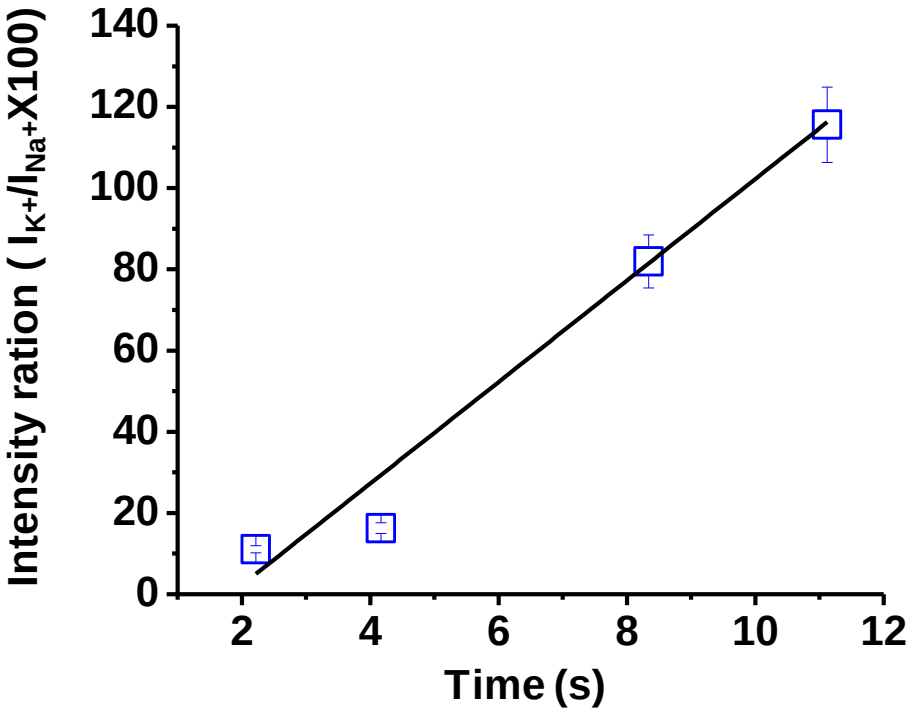

Figure S19. Mass spectral intensity ratio of K^+ to Na^+ added peaks of 18-crown-6 vs time.

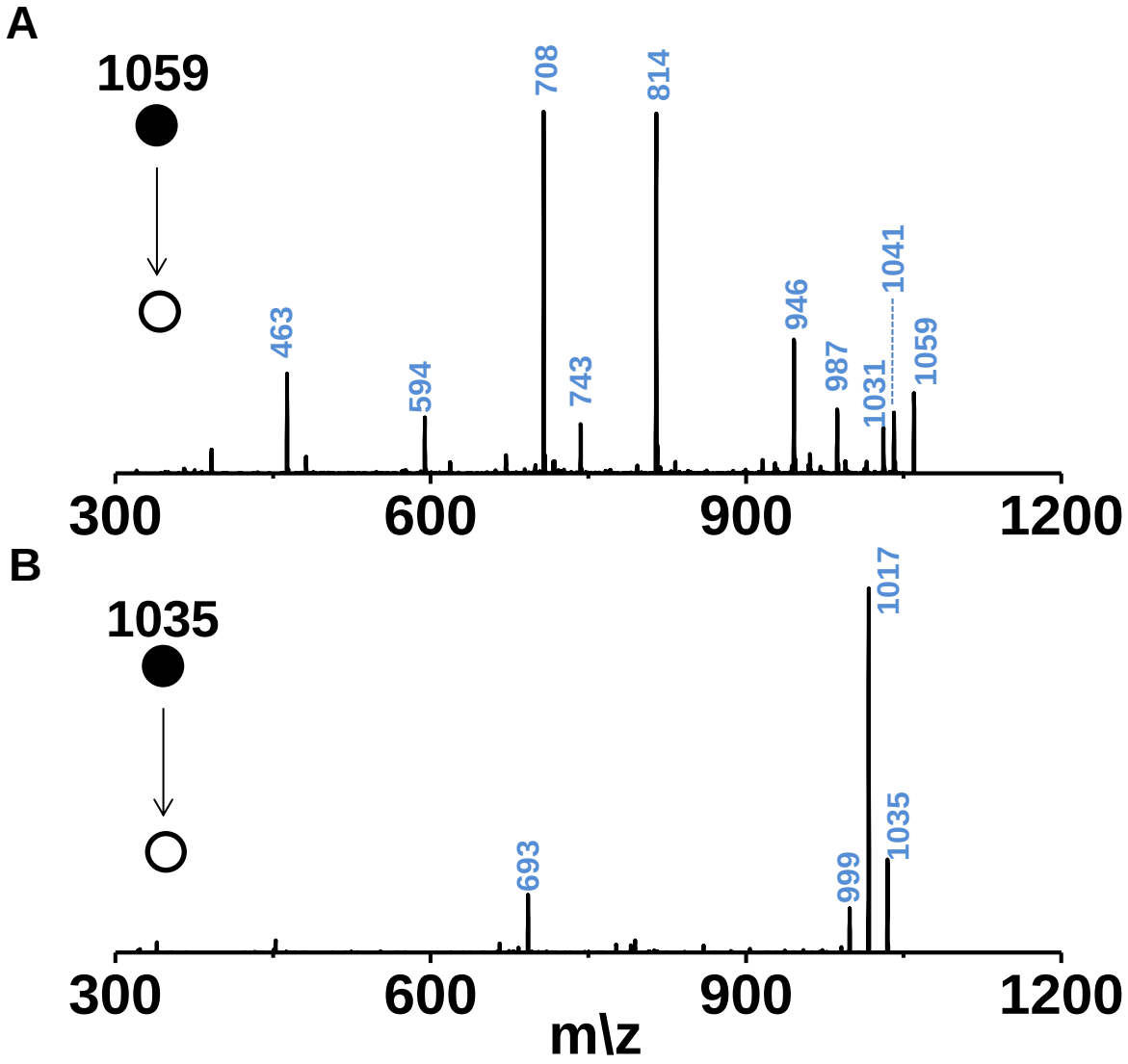

Figure S20. MS/MS spectrum of the peaks: A) *m/z* 1059 in positive-ion mode and B) *m/z* 1035 in negative-ion mode. The peaks are labeled in blue.

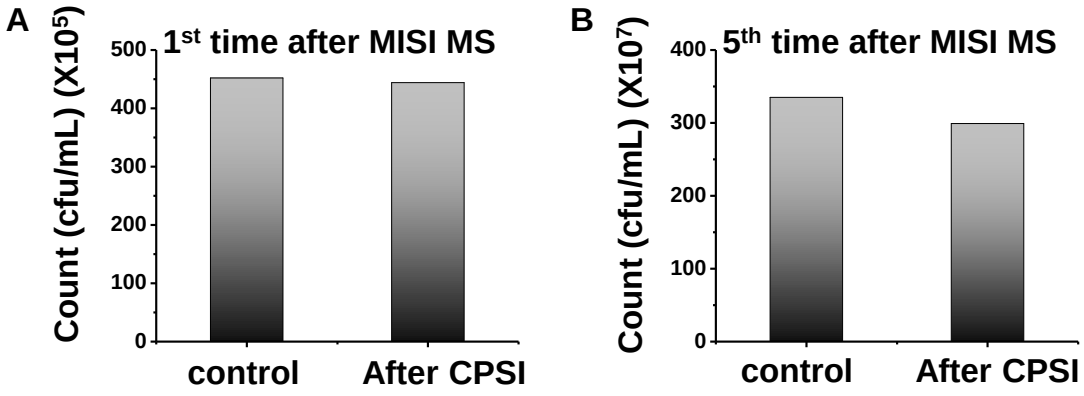

Figure S21. Effect of MISI on bacterial viability after A) 1st time and B) 5th time measurements. Bars represents the colony-forming units of the bacteria over the paper surface in control vs MISI-measured sample. Each measurement was conducted for 1-2 min and the paper was held for 10 min at rest in between the mass spec measurements.

Figure S22. Optical image of the petri dish containing three triangularly cut filter papers after 5-time measurements of bacterial lipids using MISI MS. Samples were incubated for 24 hrs after MISI measurements. Bacteria used for this image was *B. subtilis*.