Electrostatic Spray Ionization Mass Spectrometry Imaging

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Figure SI-1. (a) ESTASI MS signal of 15 µM Ang I in 50% methanol, 49% water and 1% acetic acid obtained under a frequency of the square wave high voltage (0 to 9 kV) as 40; (b) ESTASI MS signal of 15 µM Ang I in 50% methanol, 49% water and 1% acetic acid obtained under a frequency of the square wave high voltage (0 to 9 kV) as 5. The analyte was delivered by a fused silica capillary under a flow rate of 60 µL/h. TIC – Total Ion Current chromatogram.
Figure SI-2. Schematic representation of the relative wetting capillary movements with respect to the moving substrate in ESTASI MSI for (a) x line scan, (b) y line scan and (c) 2D imaging.
Figure SI-3. (a) ESTASI MSI line scans over NO₂-Ang I spots (2 mm in diameter) with various sample amounts as indicated on the figures. (b) The averaged relative peak intensity of NO₂-Ang I within the 2 mm sample spots regions as a function of the amount of NO₂-Ang I in the sample spots. Error bar shows the standard deviation calculated from three experiments. The NO₂-Ang I spots were dried from droplets of NO₂-Ang I (1 µL) in methanol. The S/N ratio in the labelled region in Figure SI-3(a) is bigger than 3. \( I_{\text{NO2AngI}} \): integrated ion current from \( m/z \) 448.0 to \( m/z \) 449.0; \( I_{\text{AngI}} \): integrated ion current from \( m/z \) 433.0 to \( m/z \) 434.0. Solution in the wetting capillary 1: Ang I (15 µM) in 50% methanol, 49% water and 1% HAc. Experimental conditions: solution flow rate 60 µL/h, step size 50 µm, delay time 1s and translation rate 5 mm/s.
Figure SI-4. ESTASI MSI line scan over a NO₂-Ang I spot. The NO₂-Ang I spot was dried from a droplet of NO₂-Ang I (1 µL, 2 µM) in methanol. The S/N ratio in the labelled region is bigger than 3. \( I_{NO2AngI} \): integrated ion current from \( m/z \) 448.0 to \( m/z \) 449.0; \( I_{AngI} \): integrated ion current from \( m/z \) 433.0 to \( m/z \) 434.0. Solution in the wetting capillary 1: Ang I (15 µM) in 50% methanol, 49% water and 1% HAc. Experimental conditions: solution flow rate 60 µL/h, step size 50 µm, delay time 1s and translation rate 5 mm/s.
**Figure SI-5.** (a) ESTASI MSI line scan over a sample spot containing 25 pmole of NO$_2$-Ang I and 1 nmole of NaCl. (b) ESTASI MSI line scan over a sample spot containing 25 pmole of NO$_2$-Ang I and 10 nmole of NaCl. The labelled region in Figure SI-5(a) has S/N bigger than 3, while the region in Figure SI-5(b) is used for comparison. The NO$_2$-Ang I spots were dried from droplets (1 µL) of NO$_2$-Ang I and NaCl in methanol. $I_{NO2AngI}$: integrated ion current from $m/z$ 448.0 to $m/z$ 449.0; $I_{AngI}$: integrated ion current from $m/z$ 433.0 to $m/z$ 434.0. Solution in the wetting capillary 1: Ang I (15 µM) in 50% methanol, 49% water and 1% HAc. Experimental conditions: solution flow rate 60 µL/h, step size 50 µm, delay time 1s and translation rate 5 mm/s.