Coupling Microdroplet Microreactors with Mass Spectrometry: 

Reading the Contents of Single Droplets On-line

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S1. a) Scheme of microfluidic device for MS analysis of microdroplets. Poly(dimethyl siloxane) channels are moulded and sealed using soft lithography. Electrodes were fabricated from solder (Indalloy 51962, Indium Corporation of America) using microsolidic techniques. Extra channels for the electrodes were included in the mold used to fabricate the fluidic channels. After assembly, devices were placed on a hot plate at 150°C. When device temperature equilibrated, solder was introduced in previously punched holes, filling the cavity completely aided by capillary forces. Before removing the device from the hotplate, while the solder was still liquid, tin-coated copper (Rowan Cable Products Ltd.) was inserted in the holes to serve as electrical contact. Glass syringes (Hamilton gastight) were connected to PEEK unions (1/16" OD, 0.75 mm bore) using a PEEK tubing sleeve (1/16" OD, 0.75 mm ID). Teflon tubing (1/16" OD, 0.75 mm ID) was used to connect these unions to PEEK reducing unions (1/16" to 1/32" OD, 0.25 mm bore). The reducing unions were connected to PEEK tubing (1/32" OD, 0.38 mm ID) that was glued onto the device. PEEK tubing, teflon tubing and PEEK unions were obtained from Valco Instruments Company Inc. Device surface was conditioned prior to gluing the tubing using an air plasma for 20 min (Emitech K–1050X, 100 W). Optical glue (NOA 81, Norland Products Inc.) was applied around inserted tubes and exposed to UV radiation for at least 5 min (BLAK-RAY NON-UV semiconductor inspection lamp, model B100AP). Devices were kept at high temperature (above 100°C) prior to use. To serve as emitter, fused silica (Composite Metal Services, 0.375 mm OD, 0.075 mm ID) was connected to the device through a PEEK union (1/32" OD, 0.25 mm bore) using a PEEK tubing sleeve (1/32" OD, 0.38 mm ID). The length of the fused silica emitter was 10 cm approximately, with small variations for the different experiments. Liquid injection was controlled by syringe pumps (Harvard apparatus PhD 2000). b) Micrograph of microfluidic device in operation. Aqueous droplets are formed in fluorous oil (30% wt. 1,1,2,2-perfluorooctanol in FC-77, 3M) using flow focusing. In the absence of an electric field, they flow past a lateral aqueous stream. When an electric field is applied they coalesce with the lateral stream and their contents are analyzed by MS. Oil flow rate 700 µl/h, aqueous flow rate 60 µl/h, lateral water flow rate 250 µl/h. Transmission images are obtained using a CCD camera (Phantom v7.2, Phantom Cameras, USA).
S2.  

a) MS analysis of bradykinin encapsulated in microdroplets and extracted in a continuous fashion. The spectrum shows a clear signal assigned to doubly charged bradykinin ([M+2H]^{2+} = 530.79 m/z, monoisotopic) after tandem MS. Positive mode electrospray MS data was recorded using a Synapt HDMS instrument (Waters, Milford MA, USA). 

b) Ion current for the mass range associated with bradykinin showing peaks corresponding to the extraction of single droplets. We used electric pulses to extract and analyze individual droplets while monitoring the process using high-speed video (recorded at 250 fps, played back at 25 fps). [Bradykinin] = 500 µM, bradykinin flow rate 60 µl/h, oil flow rate 700 µl/h, lateral water flow rate 250 µl/h, droplet frequency ~10 Hz, droplet volume ~2 nl. In a) droplets were extracted continuously using a square wave voltage (2 kV, 3.3 kHz). Pulse details in b): voltage 1.85 kV, width 40 ms, frequency 0.3 Hz. MS scan rate 3 Hz.
Figure 3. a-c) MS identification of single droplets. (top) Micrographs showing droplets of angiotensin (a), bradykinin (b) and both (c) before extraction (video recorded at 100 fps, played back at 15 fps). Scale bars are 200 µm. (lower panel) MS data corresponding to each extraction event showing the signals associated with angiotensin (a, [M+2H]+ = 648.354 m/z), bradykinin (b, [M+2H]+ = 530.79 m/z) and both peptides (c). d) Ion currents for the signals corresponding to doubly charged angiotensin (upper) and bradykinin (lower). We used electric pulses to extract periodically individual droplets of either compound. We observe peaks corresponding to individual droplets allowing us to identify droplets based on their composition. We also observe concurrent peaks for both compounds due to the simultaneous extraction of two droplets. [Bradykinin] = 500 µM, [Angiotensin] = 500 µM, peptide flow rate 20 µl/h each, oil flow rate 200 µl/h each, lateral water flow rate 800 µl/h, droplet frequency ~5 Hz, droplet volume ~2 nl. Extra oil is injected between droplets after droplet formation to improve separation (800 µl/h). Pulse details: voltage 3 kV, width 30 ms, frequency 0.1 Hz. MS scan rate 3 Hz.
Figure 4. Integration of fluorescence screening and MS analysis of microdroplets. 

a) Schematic diagram of the experimental setup for fluorescence intensity measurement. Light for fluorescence measurements is carried to the chip using an optical fiber. The excitation light is coupled into the fiber using a dichroic mirror. The fluorescence signal gathered by the optical fiber is measured by a photomultiplier tube. This signal is used as a trigger for the pulse generator that controls the extraction. High-speed imaging is used to determine the extraction parameters and monitor the process. A 20mW, 488nm DPSS laser (Picarro Cyan) was coupled to the optical fibre via a long-pass dichroic mirror (FF500-Di01, Semrock, USA) which reflects the laser light into a fibre-coupling lens of focal length 11 mm (Thorlabs Inc, UK). Light is carried to and from the device using a 50 micron core step-index multimode optical fibre (AFS50/125 Y, Throlabs Inc, UK). Fluorescent light returning from the device is collimated by the coupling lens and passes through the dichroic mirror to the PMT. A 536nm bandpass filter (FF01-536/40m Semrock, USA) is placed in front of the PMT to block non-fluorescein emission light. The output of the PMT is fed into the pulse generator (TGP110, Thurbly Instruments), the generated pulses are amplified through a high-voltage amplifier (609E, Trek Inc) connected to the device.

b) Micrograph of the microfluidic device in operation showing the fluorescence emission of fluorescein within a droplet as it passes the optical fiber carrying the laser light. The path of the laser light leaving the fiber can clearly be seen as a green fluorescent emission across the droplet. [Fluorescein] = 12mM.

c) Ion currents for the signals corresponding to angiotensin (top) and fluorescein-labelled angiotensin (bottom). When electric pulses were generated periodically (left) we observe peaks for both compounds. When the electric pulses were triggered by the fluorescence signal (right) we observe no peaks for angiotensin and regularly spaced peaks for its fluorescent analogue. We used high-speed imaging to confirm droplet selection. [Angiotensin] = 500 µM, [Angiotensin-FAM] = 500 µM, peptide flow rate 50 µl/h each, oil flow rate 2000 µl/h.
each, lateral water flow rate 2000 µl/h, droplet frequency ~8 Hz, droplet volume ~3 nl. Pulse details: voltage 0.8 kV, width 30 ms, delay 100 ms, frequency 0.1 Hz. MS scan rate 3 Hz.