1

2

Supporting Information for

3

Paper Spray Mass Spectrometry Based Method for Analysis of

4

Droplets in a Gravity-driven Microfluidic Chip

5

Yandong Zhang, Haifang Li, Yuan Ma, and Jin-Ming Lin[*]

6

Beijing Key Laboratory Microanalytical Methods and Instrumentation, Department of Chemistry,

7

Tsinghua University, Beijing 100084, P.R. China

8

9

1.   The Nozzle Structure for Droplet Generation

10

The nozzle structure for droplet generation was shown in **Figure S1**. An inlet hole of 640 μm

11

was drilled in the center of circular groove (2.0 mm in diameter, 100 μm wide) with drilling machine

12

and capillary tube with the same diameter (o.d.640 μm, i.d.340 μm) was connected to the inlet by

13

epoxy glue.

14

15
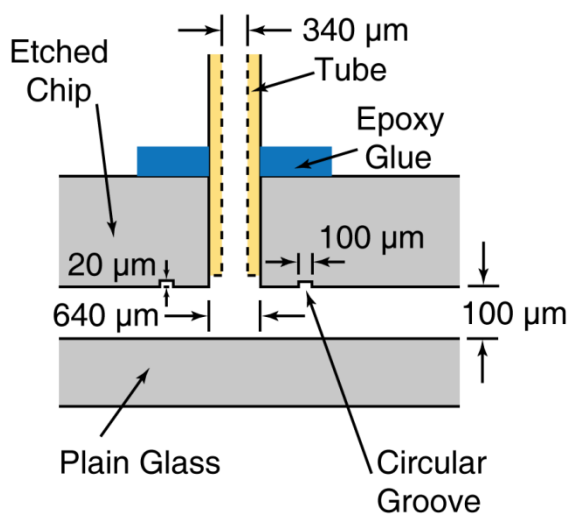
* To whom correspondence should be addressed. E-mail: jmlin@mail.tsinghua.edu.cn. Fax/Tel: +86 10 62792343

1 **Fig. S1** The schematic illustration of fabricated nozzle for droplet generation in our experiment. A

2 circular groove surrounded inlet was fabricated and capillary tube was connected to the chip for

3 solution infusion.

4

5 ## 2.   The Geometrical Structure of Fabricated Chips

6 Detailed configuration information for four chips fabricated in the experiment is shown in

7 **Figure S2**. The first chip contains one circular groove for droplet generation and two guiding lanes

8 for droplet motion control (**Figure S2a**). The second chip (**Figure S2b**) is designed to achieve rapid

9 mix of droplets on the chip. A narrow section (0.95 mm wide) in the mixing region (1.6 mm wide)

10 was fabricated to stop the motion of former droplet and thus made the two droplets contact in a

11 "head to tail" mode. Efficient mix by convection recirculation was achieved in coalesced droplet.

12 **Figure S2c** is the chip to measure the carry-over effect of our chip and paper-based droplet

13 collection. The chip 4 was fabricated to perform microreaction on chip. Functional parts including

14 reactant droplets generation, rapid mix, reaction region and quenching droplet generation were

15 fabricated (**Figure S2d**).
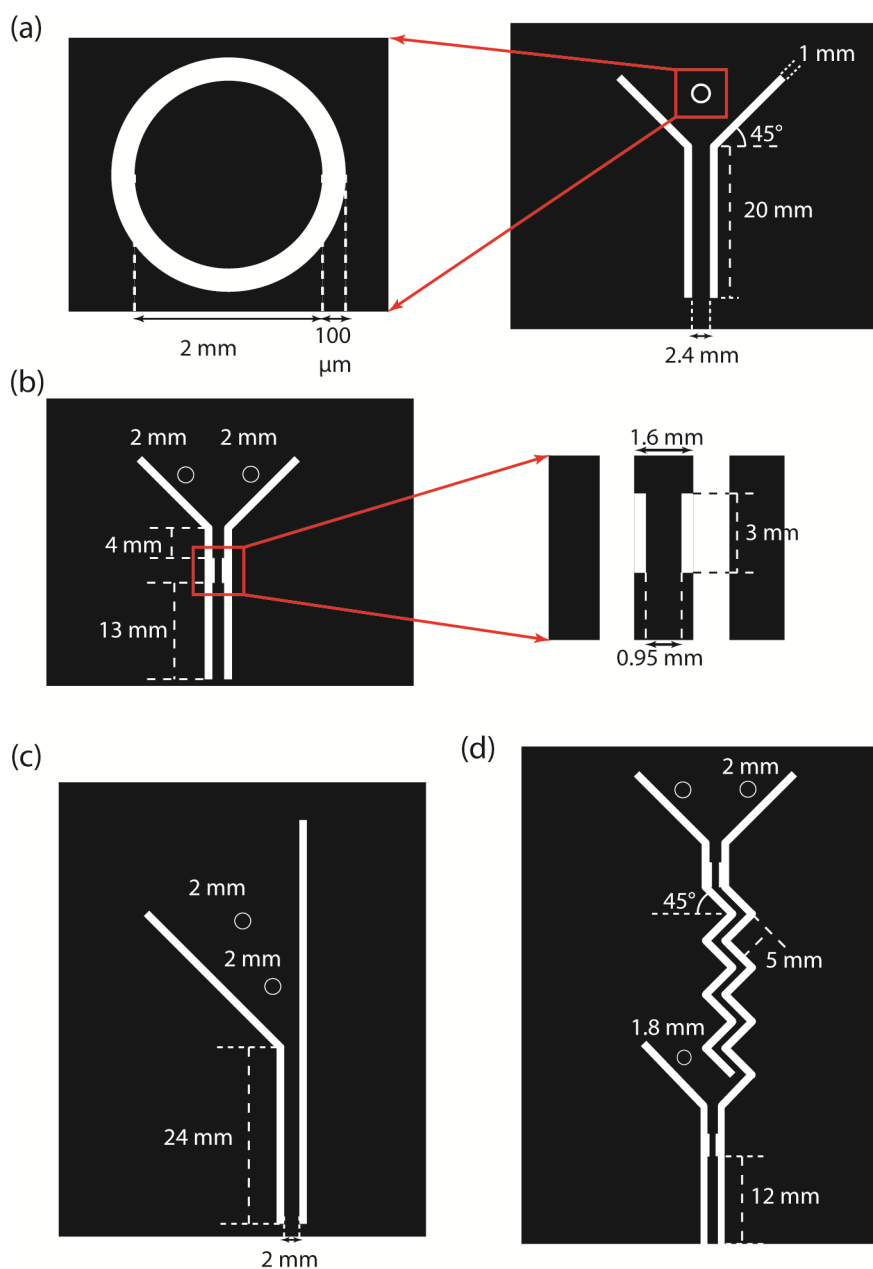
1
2 **Figure S2** The geometrical structure of four chips used in the experiment. (a) The chip for single
3 droplet experiment. A detailed structure of the nozzle for droplet generation is shown in the inset
4 (not to scales). (b) The chip for droplet mix experiment. The inset shows the narrow section which
5 makes the two droplets contact in head-to-tail mode thus rapid mix can occur by convection effect in
6 the mixing region. (not to scales) (c) The chip fabricated for carry-over measurement. (d) The chip
7 fabricated to perform microreaction on chip. Functional parts including reactant droplets generation,
8 rapid mix, reaction region and quenching droplet generation were fabricated.
9

1 ## 3. Carry-over Measurement

2   A microfluidic chip containing two droplet nozzles (**Figure S2c**) were used: one nozzle was for

3 Rhodamine B/methanol solution, the other is for blank methanol solution. These two nozzles were

4 adjusted to generate the droplet alternatively by turning on the pump in sequence. Thus, after the

5 Rhodamine B droplet flows through the chip, the blank methanol washes the chip. These two

6 droplets were analyzed by paper spray ionization and the target signal (Rhodamine B *m/z* 444) is

7 used to measure the carry over effect. Less than 5% carry-over occurred for different concentration

8 of Rhodamine B solution in this process. (**Figure S3**). It should be noted that since for each droplet a

9 new paper substrate is used, this measurement is only for the microfluidic chip itself. It should be

10 also noted that since on quantification method is used, this measurement only gives out the

11 approximate carryover between each droplet.

12
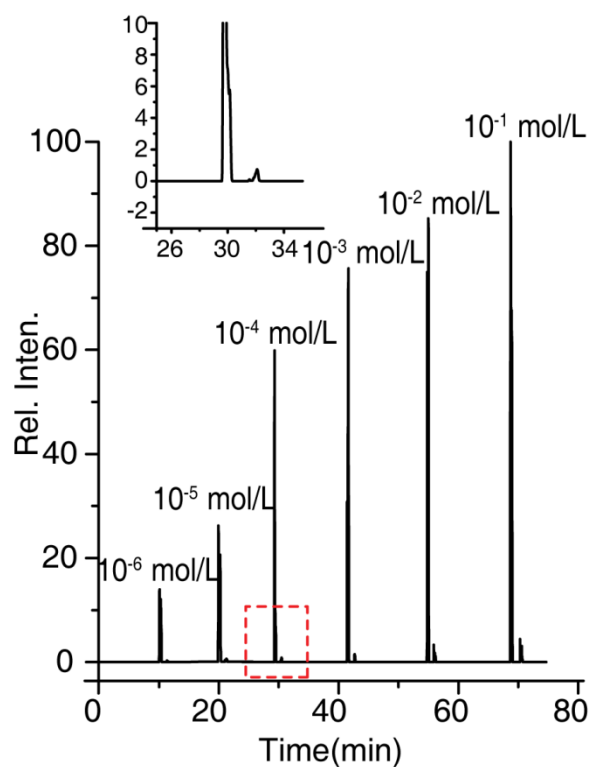
13

14

15 **Figure S3** The carry-over measurement of our chip and paper-based absorption. For each

1   concentration, less than 5% leftover was observed. The inset is the TIC for droplet of 10^{-4} M

2   rhodamine/methanol and the blank droplet generated consecutively.

3

1   **4. The Matrix Effect of MS Analysis of Acetylcholine (ACH) Hydrolysis**

2   **Reaction Solution.**

3   The final droplet of acetylcholine hydrolysis in our experiment contains very high concentration,

4   which will interfere with conventional electrospray process because of ion signal suppress. We

5   examined the mass spectrum of reaction solution obtained by standard capillary emitter and

6   compared with the mass spectrum from paper spray ionization. The hydrolysis reaction was

7   performed in a tube in an offline way (ACh/methanol 2 mM, KOH/methanol 5 mM, HCl/methanol

8   0.1 M, reaction time 20 s). As shown in **Figure S4**, in paper spray ionization, target ions can be

9   easily identified in a clean background. In contrast, the mass spectrum from standard ESI emitter has

10 strong noise signals. And if the solution is diluted to 100-fold, the noise signals become stronger and

11 target peaks are hard to be identified which is a typical phenomenon for matrix effect. In conclusion,

12 paper spray has apparently desalt the sample thus are suitable for direct analysis of real

13 microreaction sample for which conventional ESI is not suitable.

14
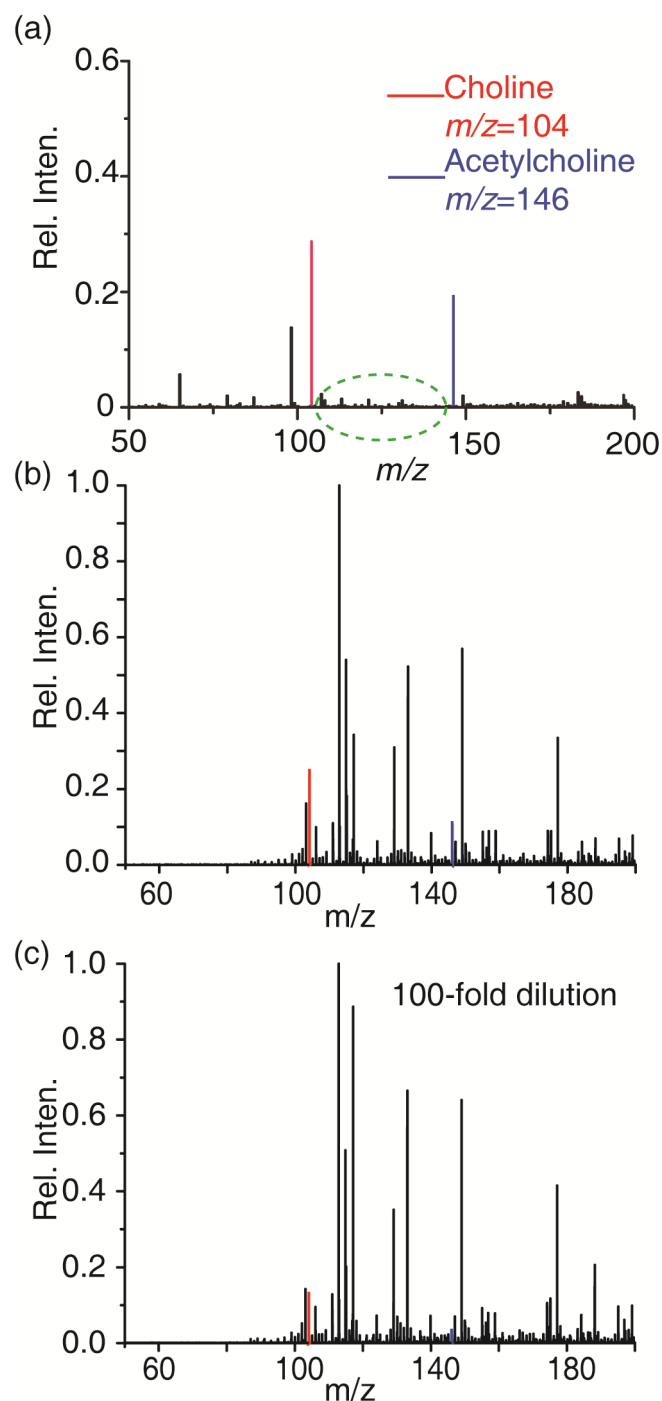
1

2 **Figure S4.** The matrix effect of MS analysis of acetylcholine hydrolysis reaction solution. (a) The

3 mass spectrum obtained from paper spray ionization. Target singal (m/z 146, 104) can be clearly

4 identified. (b) The mass spectrum of this reaction from standard ESI emitter. Strong noise signals are

5 observed. (c) The mass spectrum obatained by 100-fold dilution of reaction solution. At this

6 condition, target peaks are hard to identify because of stronger noise signals.

7

8

9

10

11

5. The Calibration of Concentration Gradient in Droplet Sequence

In the experiment, we used relative time-based method, a widely used method in flow injection analysis, to calibrate the concentration gradient. Basically, a calibration curve between the concentrations of sample solution (Rhodamine B, 0.1 mM-10 mM) and their signal intensity was obtained. Then a sample (Rhodamine B/methanol solution, 50 mM) was injected through the flow injection system under the same condition and the flow-out curve of signal intensity was plotted versus flow-out time to obtain the concentration gradient profile. The concentration could then be calculated with the calibration curve. In our experiment, we used two methods the get the concentration gradient profile. The fluorescent intensity of Rhodamine B was used to calibrate the concentration gradient at the detection window the flow tube in FIA-chip system. Besides, the relative intensity of a certain area in the droplet was also recorded to get the concentration gradient in droplet sequence. For the on-chip acetylcholine hydrolysis, since the KOH sample zone was injected under the same conditions as Rhodamine B sample, the gradient profile curve for Rhodamine B is used to calibrate the concentration gradient of KOH.

1   ## 6.   The Reaction Kinetics of Alkaline Hydrolysis of Acetylcholine

2   The alkaline hydrolysis of acetylcholine is commonly considered a secondary reaction,

3   according to which, the second-order rate constant k can be determined from the following equation.

4   (**Scheme S1(1), (2)**) where $[OH^-]_0$ and $[ACh]_0$ represents the initial concentration of OH^- and

5   acetylcholine, $[ACh]$ and $[OH^-]$ represent the respective concentration at reaction time t. The

6   concentration of acetylcholine at reaction time t (20 s), $[ACh]$, can be calculated from $[ACh]/[Ch]$,

7   which can be obtained from the area ratio of acetylcholine to choline (I_{ACh}/I_{Ch}) in each mass

8   spectrum (**Figure 5c**).

9   It should be noted that $[ACh]/[Ch]$ and I_{ACh}/I_{Ch} are not necessarily the same, taking into account

10   the differences in ionization efficiency, instrumental transmission and detection of these two

11   analytes. These differences can be included in the relative response factor R, which can be

12   determined by MS titration method.[1] For simplification reason, the factor R is simply assumed to be

13   1 in our experiment, which is reasonable because of the high structure similarity of acetylcholine and

14   choline.

$$r = -\frac{d[ACh]}{dt} = k[ACh][OH^-] \tag{1}$$

$$\ln I = k[-([OH^-]_0 - [ACh]_0)t] \tag{2}$$

$$I = \frac{[ACh][OH^-]_0}{([OH^-]_0 - [ACh]_0 + [ACh])[ACh]_0} \tag{3}$$

$$\frac{[ACh]}{[Ch]} = \frac{[ACh]}{[ACh]_0 - [ACh]} \tag{4}$$

$$[ACh] = \frac{\frac{[ACh]}{[Ch]}[ACh]_0}{1 + \frac{[ACh]}{[Ch]}} \tag{5}$$

$$\frac{I_{ACh}}{I_{Ch}} = R\frac{[ACh]}{[Ch]} = \frac{[ACh]}{[Ch]} \tag{6}$$

15

16   **Scheme S1** kinetic deduction of alkaline hydrolysis of acetylcholine

1

Reference:

3

4   (1) Boeri Erba, E.;Barylyuk, K. V.;Yang, Y.;Zenobi, R. *Anal. Chem.* **2011**, 83, 9251-9259.

5

6