Electronic Supplementary Information for

Online Multi-Channel Microfluidic Chip-Mass Spectrometry and Its Application for Quantifying Noncovalent Protein-Protein Interactions

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1. Experimental details

Whatman silica gel-loaded paper (Grade SG81) was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK) and cut into a triangle shape, 5 mm long and 5.8 mm wide at the base. Phenylalanine (Phe) and citrulline (Cit) were obtained from Beijing Dingguo Changsheng Biotech. Co. Ltd. (Beijing, China) and ammonium acetate was obtained from Beijing Chemical Reagent Company (Beijing, China). Concanavalin A (Con A), Con A labelled with FITC (Con A-FITC), bovine serum albumin (BSA) and lysozyme were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isopropyl alcohol and water used in this work were of HPLC Grade (JT Baker, Phillipsburg, NJ, USA).

Fabrication of the hybrid capillary: the thinner silica capillary tube (5 cm × 20 μm i.d. × 90 μm o.d) was inserted in and fit together with the end of the thicker one (20 cm × 100 μm i.d. × 164 μm o.d.), followed by sealing the joints with room temperature vulcanizing-type silicone rubber (Wangda Adhesives, Tianchang, Anhui, China).

The microfluidic channels designed for generation of concentration gradients, with the dimensions of 0.8 mm width and 40 μm height, were fabricated from poly (dimethylsiloxane) (PDMS, Sylgard 184, Dow corning) by standard soft lithography techniques as reported.[1] Briefly, the layout of the device was fabricated by coating a negative photoresist SU-8 2050 (Microchem, Newton, MA, USA) on a silicon wafer. After photolithographic patterning of a photoresist coated silicon wafer with a commonly used design (Fig. S2),[2,3] a mold that carried a relief of the desired microstructure was generated. Premixed 10:1 ratio of PDMS prepolymer and curing agent was degassed in a vacuum chamber for 0.5 h and then poured onto the mold and cured in a 70 °C oven for 2 h. The inlets and "spy holes" of the channels were punched by a syringe needle with flat tip. Then the channels were treated with oxygen plasma (PDC-32G, Harrick Plasma, Ithaca, NY, USA) for 90 s and then sealed with a glass cover slip, through which an irreversible microstructure was formed.

When "clean-up" droplets were demanded, a hole was also punched between each two "spy holes" before sealing the channels with the glass. These holes were used to store eluting solution, from which the "clean-up" droplets were sampled.

In the Phe-Cit concentration gradient experiment, solutions of Cit and Phe at a concentration of 0.1 mM were infused respectively into the inlet #1 and #2 of the microfluidic concentration gradient generator, both at 5 μL/min. Isopropyl alcohol was employed as the spray solvent and supplied at the flow rate of 2.8 μL/min.

Before the generation of Con A concentration gradient, the microfluidic channels were blocked with 1% BSA for 1 h. When cascading two generators, the infuse rates were correspondingly increased to 15 μL/min, and other four outlets of the upstream generator were also connected to similar generator, to ensure that the flow rate in the downstream generator remained unchanged. Lysozyme at 2 μM concentration was chosen as the internal standard, since the *m*/*z* region for the MS spectra peak of lysozyme had no overlapping with that of Con A and the peak areas for lysozyme and ConA were comparable. A solution of Con A at 20 μM and lysozyme at 2 μM concentration in 100 mM ammonium acetate was infused into the inlet #2, while 100 mM ammonium acetate was infused into the inlet #1 of the upstream microfluidic concentration gradient generator, and the concentrations of Con A generated at the outlets were 0, 4, 8, 12, 16 and 20 μM. Thus, concentrations with 0.8 μM increments ranging from 0 to 20 μM could be generated at the outlets of downstream generator by switching the connection interface between two generators. For analysis of the proteins, 100 mM ammonium acetate in water was employed as the spray solvent.

Setup for the paper spray ionization of generated droplets was documented in our previous papers.[4,5] Herein, height difference between the channels and the capillary outlet was 100 mm; distance between the capillary outlet and the paper was 1.5 mm; horizontal distance between the capillary and the spraying tip of the paper substrate was 0.5 mm. The distance between the tip of the paper and the inlet of the mass spectrometer was set at 5 mm and the voltage applied to the paper was 4.5 kV.

Experiments regarding the stability of the system and the Phe-Cit concentration gradient experiment were performed with a Shimadzu LCMS-2010A mass spectrometer (Shimadzu, Kyoto, Japan), while mass spectra of the proteins were recorded by a Bruker microQ-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany).

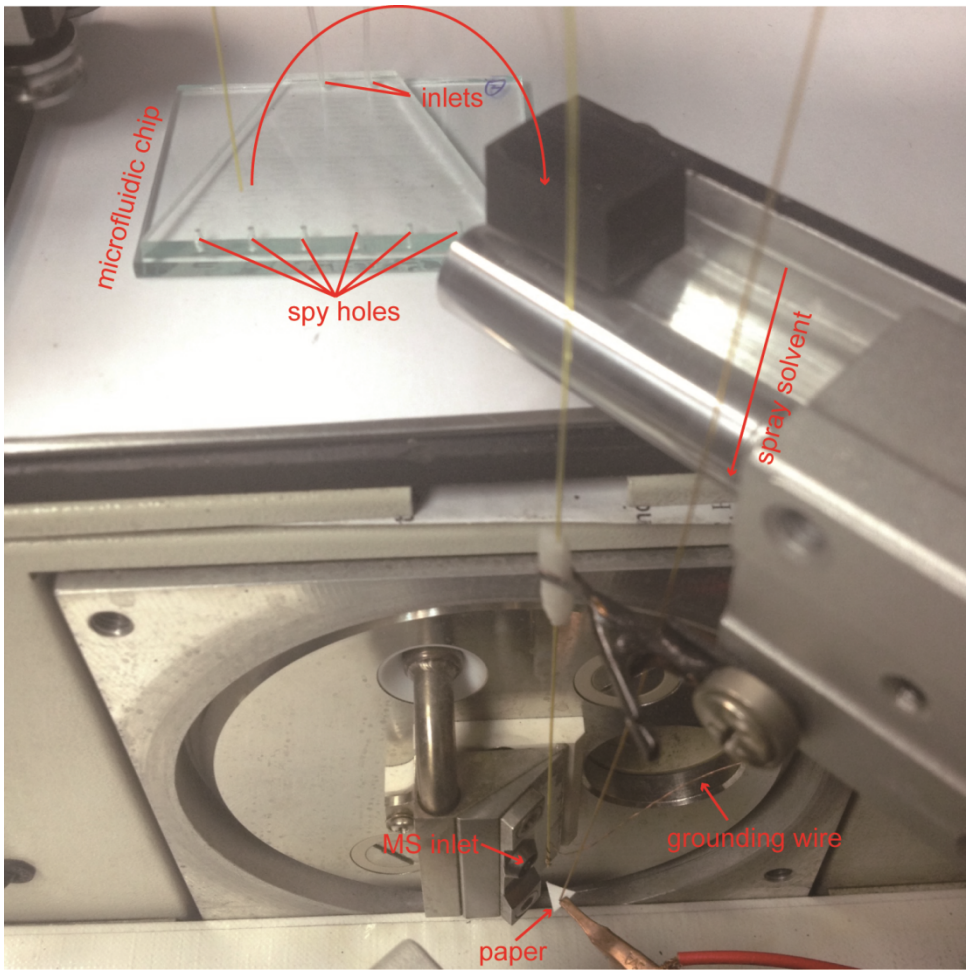

Fig. S1 Image of the "Chip-MS" platform interfaced by paper spray ionization.

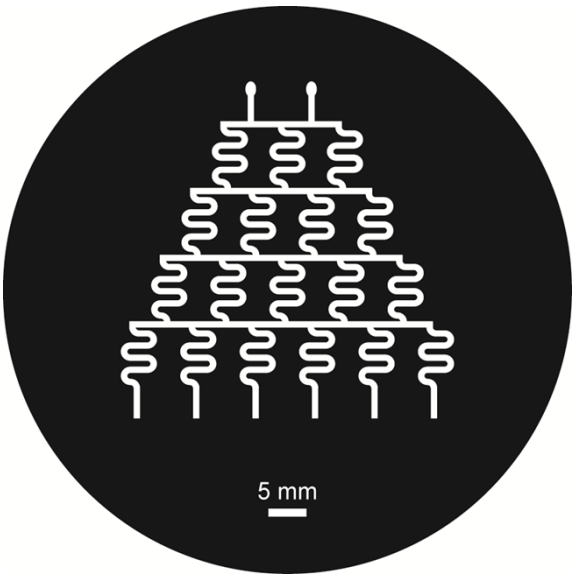

Fig. S2 Mask design for the photolithographic patterning of the mold that carried a relief of the microfluidic concentration gradient generator.

2. Volume and the time interval of sampled microdroplets

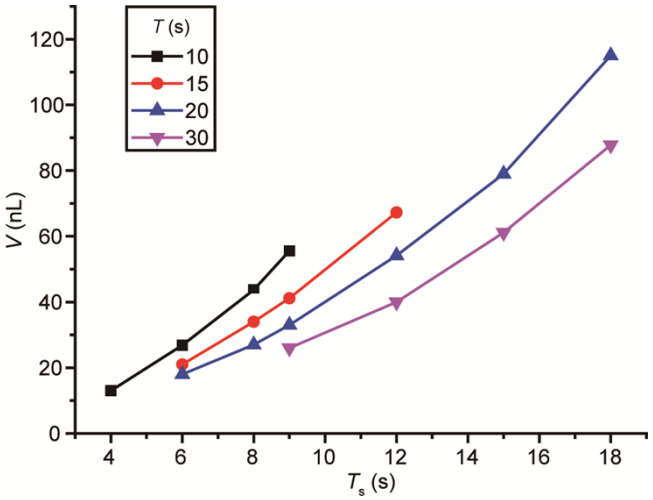

Fig. S3 Droplet volume (V) versus sample aspiration time (T_s) with different time interval (T). The time interval between generated microdroplets (T) was namely the sum of sample aspiration time (T_s) time span between samplings of the capillary from microchannels (T_i). The values of T_s and T_i could both be set and adjusted by the computer program.

The droplet volume generated at the outlet of the capillary was controllable by altering the electric field and setup of the system, as mentioned in our earlier articles.[2,3] Under the condition introduced in the "Experiments Details" section, the droplet volume for solutions in water generated at the outlet of the capillary was about 50 nL. Therefore, to make the sample volume match this value, we set T_s and T_i at 11.6 s and 8.4 s respectively (T = 20 s).

The generated droplets were measured with an average volume at 53.5 nL, thus the flow rate in the hybrid capillary was 277 nL/min.

3. Mass Spectra obtained from the phenylalanine-citrulline concentration gradient experiment

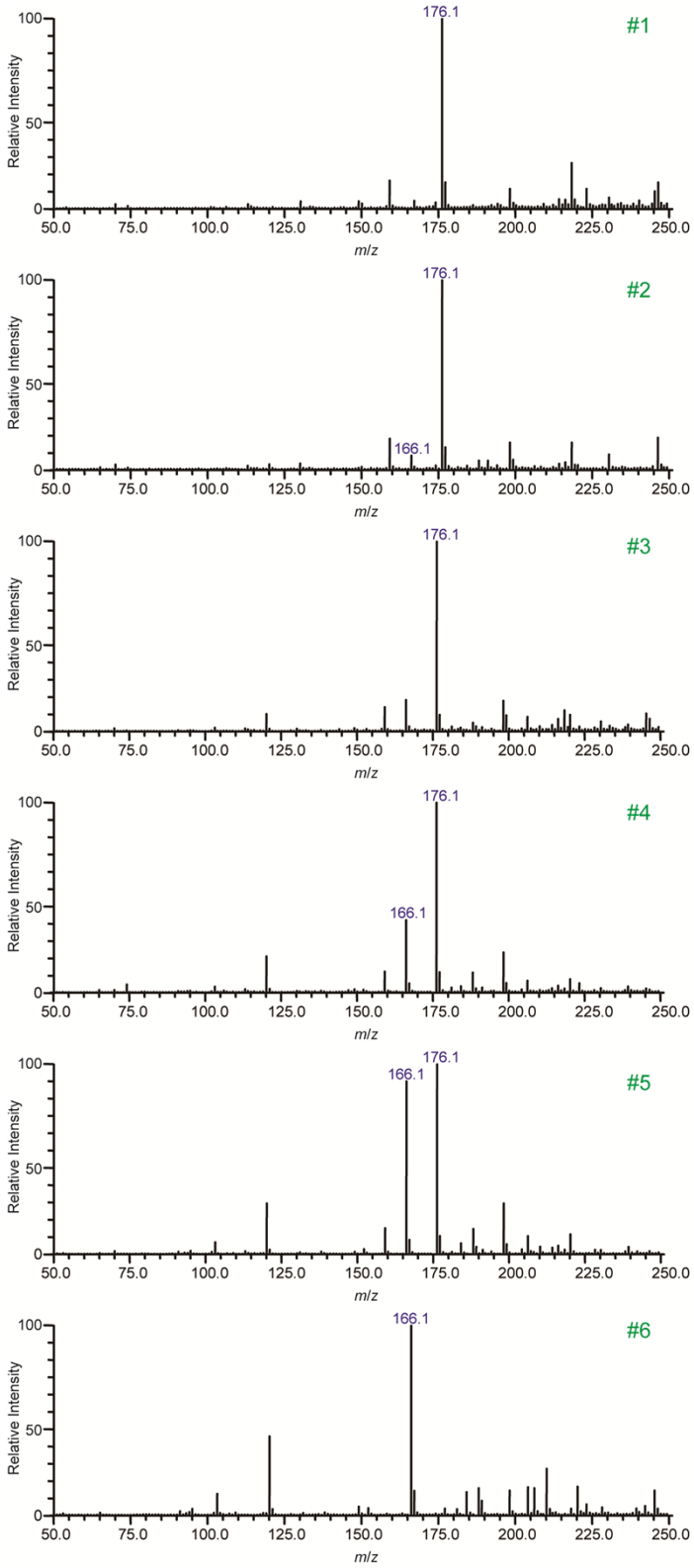

Fig. S4 Mass spectra for the droplets generated from microfluidic channel #1~6 in the Phe-Cit concentration gradient experiment.

4. Derivation of Eq. (2):

$$R = \frac{R_T}{R_D}$$

The analytical concentration of dimer ($[D_0]$) is equal to the concentration of the dimer ($[D]$) added to twice the concentration of the tetramer ($[T]$):

$$[D_0] = [D] + 2[T]$$

When an internal standard was spiked into the sample:

$$[D] = \frac{I_D R_{Std}}{I_s \ R_D}[Std]$$

$$[T] = \frac{I_T R_{Std}}{I_s \ R_T}[Std]$$

$$\frac{[D_0]}{[Std]} = \frac{[D] + 2[T]}{[Std]} = \frac{I_D \ R_{Std}}{I_{Std} \ R_D} + 2\frac{I_T \ R_{Std}}{I_{Std} \ R_T}$$

$$\frac{I_T}{I_{Std}} = \frac{R_T \ [D_0]}{R_{Std}[Std]} - \frac{R \ I_D}{2I_{Std}}$$

Derivation of Eq. (3):

$$\frac{[D]}{[T]} = \frac{I_D}{I_T}R$$

$$[D] = \frac{\frac{I_D}{I_T}R[D_0]}{\frac{I_D}{I_T}R + 2}, [T] = \frac{[D_0]}{\frac{I_D}{I_T}R + 2}$$

$$K_a = \frac{[T]}{[D]^2} = \frac{\frac{I_D}{I_T}R + 2}{\left(\frac{I_D}{I_T}R\right)^2[D_0]}$$

This above equation can be solved for the variable (I_D/I_T):

$$\frac{I_D}{I_T} = \frac{1 + \sqrt{1 + 8K_a[D_0]}}{2K_a[D_0]R}$$

5. Validation of the concentration gradient

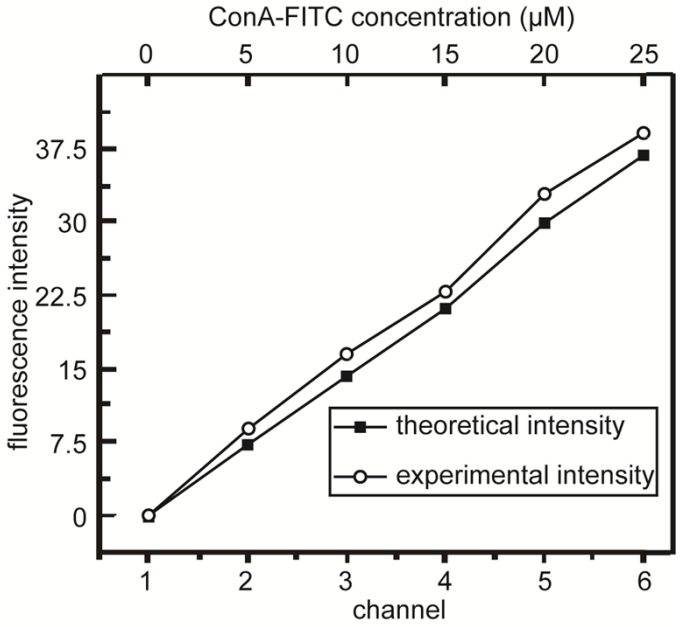

Fig. S5 The fluorescent intensities generated from the concentration gradient of Con A-FITC were quantified and compared with the theoretical data. The background fluorescence of the medium and the device had been subtracted. A solution of 25 μM Con A-FITC in 100 mM ammonium acetate was infused into the inlet #2, while 100 mM ammonium acetate was infused into the inlet #1.

6. Comparison between paper spray ionization and conventional ESI for MS analysis of Con A

As shown in Fig. S6, besides the peaks representing the dimer and the tetramer, peaks representing the monomer of Con A could also be found in the mass spectra generated by conventional ESI-MS, while the peaks representing the monomer were negligible with paper spray ionization (Fig. 3). This is an evidence for the softness of paper spray ionization, which thus should be more suitable for the determination of the association constant.

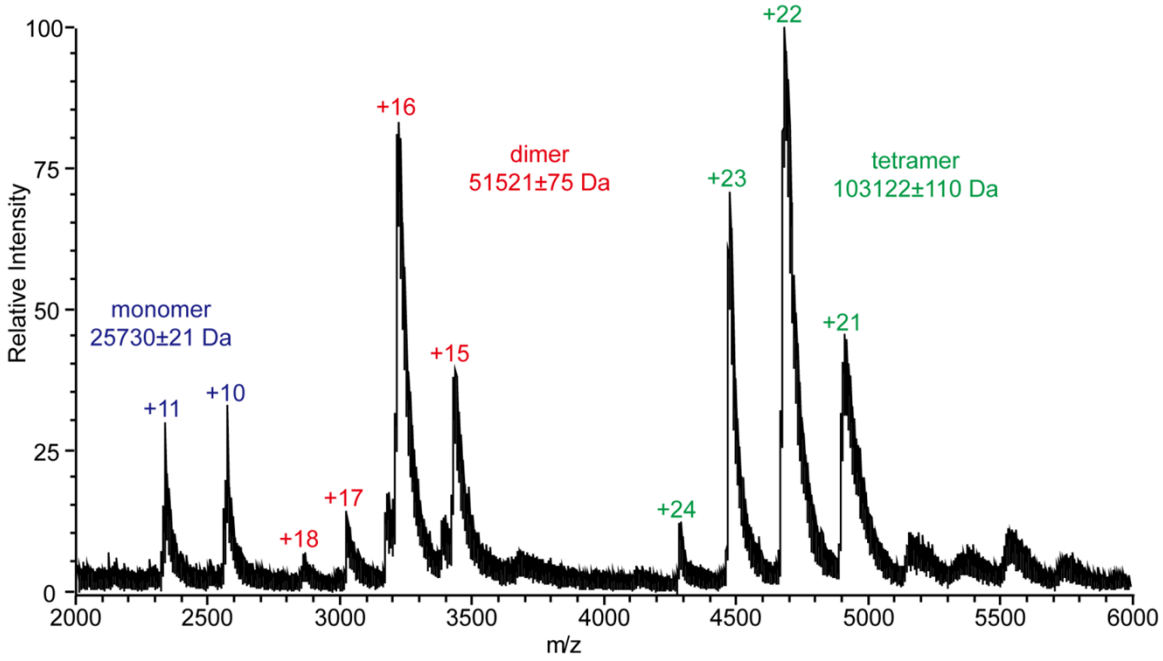

Fig. S6 Mass spectra of 10 μM Con A in 100 mM ammonium acetate generated with conventional ESI-MS.

References

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