

## Electronic Supporting Information

### A Multifunctional Microfluidic Droplet-Array Chip for Analysis by Electrospray Ionization Mass Spectrometer

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#### Chemicals and Materials.

Both methanol and glacial acetic acid (HPLC-grade) were obtained from Merck (Darmstadt, Germany). Deionized water was produced by a Millipore Ultrapure Water Purifier (Billerica, USA). All of other chemicals were of analytical grade and used as received without further purification. Reserpine, octadecyltrichlorosilane (ODS), tetradecane, myoglobin from equine skeletal muscle (Myo), cytochrome C from horse heart (Cyt-C), bovine serum albumin (BSA), dithiothreitol (DTT), iodoacetamide (IAA), and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, USA). Trypsin was obtained from Worthington Biochemical Co. (Lakewood, USA). Magnetic beads with C<sub>18</sub> coating were obtained from BaseLine Chromtech Research Centre (Tianjin, China). Magnetic iron was purchased from Junfeng Magnetic Materials Co. (Nd-Fe-B, Dongguan, China).

### **Test of Liquid Evaporation with Weight Loss Method**

A weight loss method was utilized to investigate the influence of tetradecane on the evaporation of liquid samples. The evaporation of tetradecane itself was first tested. 70  $\mu\text{L}$  of tetradecane was added into a tube and the tube was weighed ( $W_1$ ) with a precision balance. Before liquid adding, the weight of the empty tube ( $W_0$ ) was measured with the balance. Thus, the original weight of the added tetradecane can be calculated as ( $W_1 - W_0$ ). The weight of the tube ( $W_2$ ) was measured again after it was allowed to stand in the open air at 25  $^\circ\text{C}$  for 24 h. In repetitive experiments, the average weight loss of tetradecane was  $0.2 \pm 0.01$  mg ( $n=3$ ), corresponding to 0.4% of the total amount of tetradecane, which exhibits the non-volatility of tetradecane. We also tested the evaporation of 10  $\mu\text{L}$  of methanol water (methanol: water = 50 : 50, v : v) solution droplet under the protection of 50  $\mu\text{L}$  tetradecane with the same method. The average weight loss of methanol solution in 24 h was  $0.2 \pm 0.01$  mg ( $n=3$ ), which only occupies 2.3% of the weight of the whole droplet. This result indicates that tetradecane can effectively avoid droplet evaporation.

### **On-Chip Protein Digestion and Desalination.**

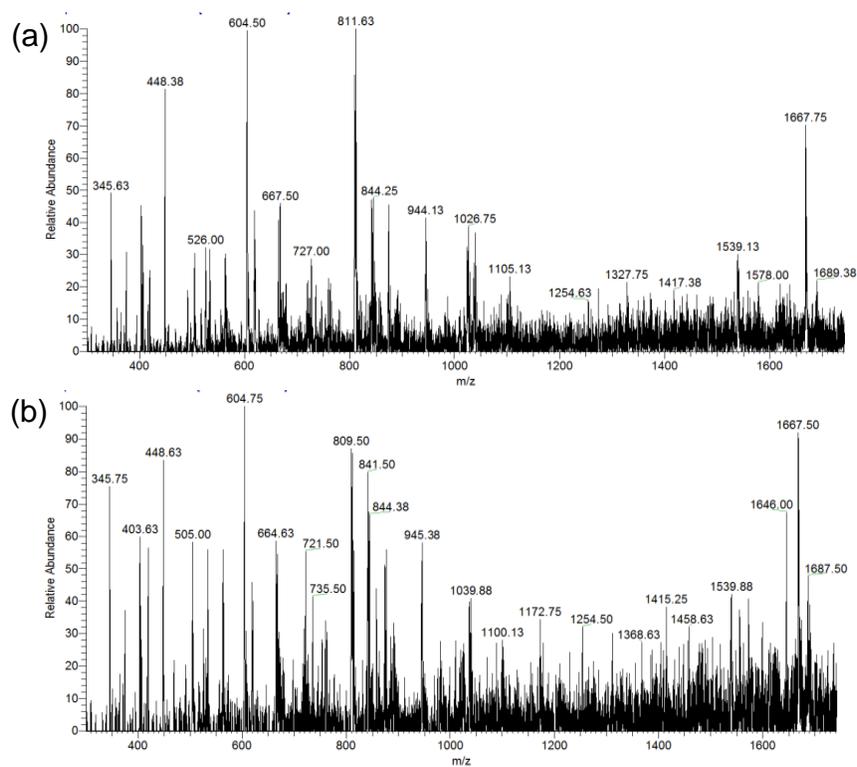
Figure 3 schematically showed the digestion and desalination workflows in myoglobin (Myo) and cytochrome C (Cyt-C) droplets. The digestion procedure included sample addition, reduction of disulfides, alkylation of free thiols, and proteolytic digestion. The liquid addition and removing operations for droplets were manually accomplished using pipettes. All of the samples mentioned were dissolved in 20 mM  $\text{NH}_4\text{HCO}_3$  aqueous solution. 2.0  $\mu\text{L}$  of  $10^{-4}$  mol/L myoglobin and cytochrome C solutions were deposited on two spots of the chip, respectively. Then, 2.0  $\mu\text{L}$  of  $4.5 \times 10^{-2}$  mol/L DTT solution was added to the myoglobin droplet, and the droplet was heated at 65  $^\circ\text{C}$  for 20 min. After the reduction reaction, 2.0  $\mu\text{L}$  of 0.1 mol/L IAA solutions was added to the myoglobin droplet, and the alkylation reaction of free thiols was performed in dark at 37  $^\circ\text{C}$  for 30 min. The digestion reaction was initiated by adding 0.5  $\mu\text{L}$  of 1 mg/mL trypsin solution to each protein droplet, and carried out at 45  $^\circ\text{C}$

for 16 h. In the digestion of cytochrome C, no DTT or IAA solutions were required to be added to the droplet since no disulfide bond exists in cytochrome C molecule.

The desalination of peptides using C<sub>18</sub> immobilized magnetic beads was accomplished according to a SPE procedure modified from the manufacturer's instructions of ZipTipC<sub>18</sub><sup>32</sup>. In order to enhance the peptide extraction efficiency, the beads were previously activated with 100% acetonitrile solution for 1 h, and were equilibrated with an aqueous solution containing 0.1% TFA and 50% acetonitrile for 5 min. Then, the beads were solved in 0.1% acetic acid aqueous solution. The digested peptide droplet was treated with adding 0.1 μL of formic acid to adjust the pH to 4.0, then followed with 2.0 μL of 5 μg/μL C<sub>18</sub> immobilized magnetic bead solution. After incubation for 30 min at room temperature, the suspension was removed from the droplet by a pipette while a magnetic iron was placed under the chip substrate to immobilize the magnetic beads on the chip surface to facilitate the phase separation. The remained beads were washed by adding 8.0 μL of 0.1% acetic acid aqueous solution and remove the suspension after 5 min. Then the bound peptides were eluted from the magnetic beads with 5.0 μL of an eluent containing 0.1 % acetic acid, 50% acetonitrile, 30% methanol and 20% water for 30 min. Finally, 2.5 μL of the suspension was transferred to another droplet array chip for MS detection with spot diameter of 2.0 mm using a pipette. Additional 2.5 μL of 0.5% formic acid aqueous solution was added to each droplet on the chip to improve the electrospray ionization efficiency.

#### **MS analysis of Cyt-C enzymatic hydrolyzate with methanol and acetonitrile buffers.**

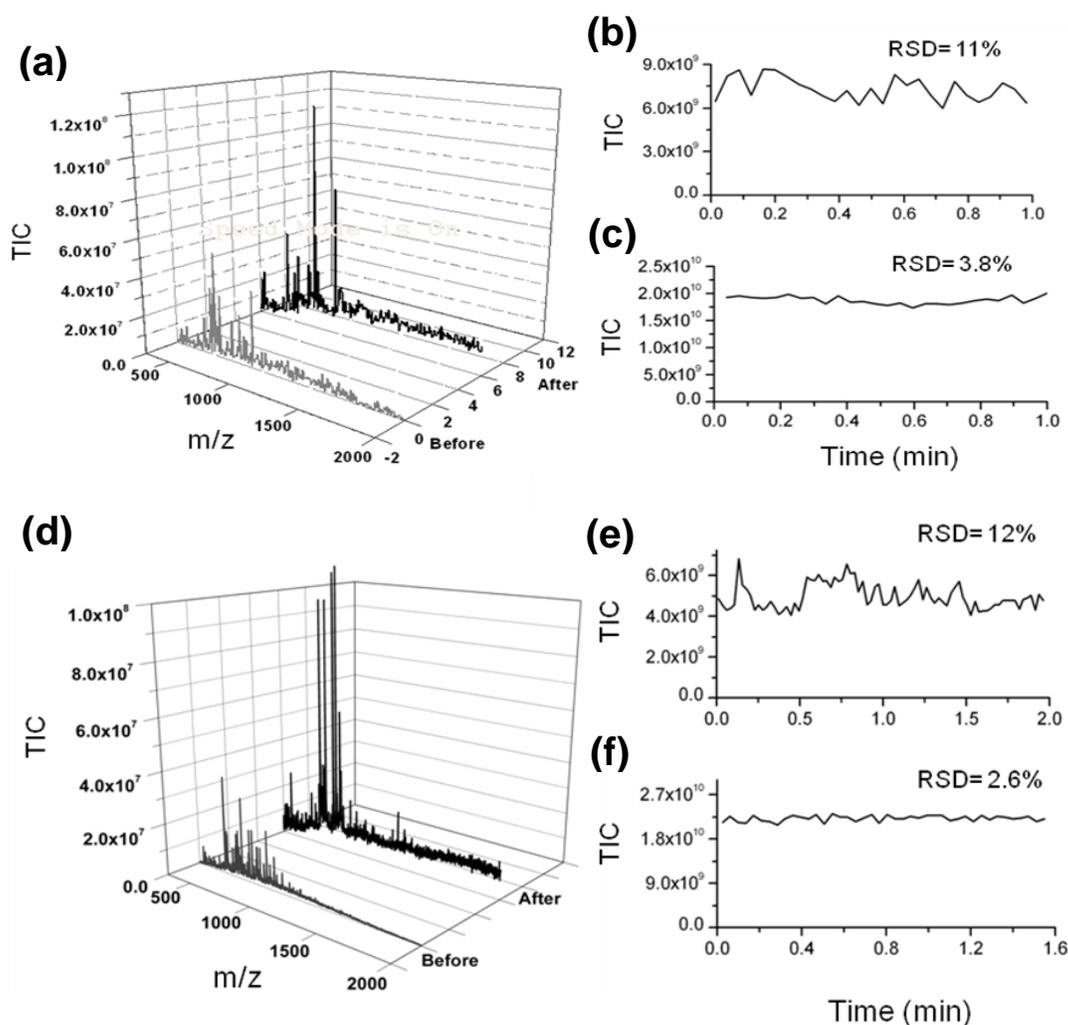
Typical MS analysis results of 10<sup>-6</sup> M Cyt-C enzymatic hydrolyzate (Cp) with the two buffers of methanol and acetonitrile are as shown in Fig. S1. More signal peaks can be detected when the methanol buffer was used. In addition, signal intensities of the highest abundant peak (m/z 604) of Cp in methanol and acetonitrile buffers were 6E5 and 1E6, respectively. This result indicates that methanol buffer is more suitable for electrospray in the present system.



**Fig. S1** Mass spectra of  $10^{-6}$  M Cyt-C enzymatic hydrolyzate with buffer solution (a) 50% methanol-50% water solution and (b) 50% acetonitrile-50% water solution, respectively.

## MS analysis of Cyt-C and Myo enzymatic hydrolyzates with and without desalination.

The comparative MS analysis results of  $10^{-6}$  M Cyt-C enzymatic hydrolyzate (Cp) and Myo enzymatic hydrolyzate (Mp) before and after the desalination are shown in Fig. S2. The total ion current intensities of the desalted peptides of Myo and Cyt-C increased 3 times and 1.5 times than those without the desalination, respectively. The RSD of the signal intensities of the Myo and Cyt-C peptides were improved from 12% and 11% to 2.6% and 3.8%, respectively. This result indicates that the desalination is effective to improve the electrospray by using the present semi-closed 2D droplet array system.



**Fig. S2** (a) Mass spectra of  $10^{-6}$  M Cyt-C enzymatic hydrolyzate before (front) and after (back) the desalination. Total ion current of  $10^{-6}$  M Cyt-C enzymatic hydrolyzate before (b) and after (c) the desalination, respectively. (d) Mass spectra of  $10^{-6}$  M Myo enzymatic hydrolyzate before (front) and after (back) the desalination. Total ion current of  $10^{-6}$  M Myo enzymatic hydrolyzate before (e) and after (f) the desalination, respectively.

**Table S1** Detailed information on the tryptic digestion products of Cyt-C and Myo. (Mass spectra searching are based on Swiss-Prot database)

Cyt-C		Myo	
10-24	IFVQKCAQCHTVEKG	12-18	LNVWGKV
29-39	TGPNLHGLFGR	17-33	KVEADIAGHGQEV LIRL
40-54	KTGQAPGFTYTDANK	32-44	RLFTGHPETLEKF
56-62	KGITWKE	70-79	LTALGGILKK
62-73	EETLMEYLENPK	104-114	YLEFISDAIHH
74-81	KYIPGTKM	134-141	KALELFRN
81-88	MIFAGIKK	140-147	RNDIAAKY