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Ultra-sensitive detection method with droplet based microfluidic device coupled to MALDI-TOF

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We present an automated microfluidic droplet generator/depositor for MALDI-TOF analysis allowing attomol detection of peptides from sub-nanomolar solutions: a sensitivity which is a paramount importance for proteomics and diagnosis field.

Droplet based microfluidic devices offer many advantages in integration of multiple biological or chemical processes, essential tools for biomarker/biological fluids analysis [1]. However the detection mainly relies on optical methods such as fluorescence which implies a labelling of target molecules but also suffers from a lack of sensitivity. At the opposite mass spectrometry is a highly sensitive technic based on a label free detection of analytes but interfacing droplet-based microfluidic and mass spectrometry is challenging and up to now there are few studies reported [2-3].

We focus on the integration of a droplet based microfluidic system with a MALDI-TOF. We develop methods which prevent the use of surfactants: a crippling factor since surfactants led to high background noise, and then are not compatible with high sensitivity detection method. To circumvent this effect, we designed a chip which prevents droplets coalescence by spacing each droplet with a controlled volume of oil (Figure 1). This simple geometry allows to finely tune the amount of spotted droplets and controlled the coalescence. The microfluidic chip outlet is then connected to a capillary, and the droplets are transferred to a MALDI plate mounted on a motorized xy-stage. To estimate the potential of the platform, we developed a full optimized method (sample composition, matrix composition, droplet generation, deposition and analysis) of relevant proteomics biomarker: a native peptide Angiotensin II involved in several diseases [4-5]. First, we compared our system with a standard MALDI deposition procedure *i.e.* manual pipetting. The spectra of Figure 2 show 500nl of a solution of 10fmol/ μ l Angiotensin II *-left* pipette deposited and *-right* platform deposited. In both cases, the 1046.2 m/z peak of Angiotensin II is visible but in the former case the intensity is increased by one order of magnitude. A closer look to the deposits shows a concentration effect with the platform deposition (see insets figure 2).

In a second step, we made a sensitivity test by depositing different concentrations of analytes. The Figure 3 shows the average peak intensity as a function of n the quantity deposited in femtomole. We record good intensity signal down to 100 attomoles (see inset figure 3). Below this threshold the spectra are very noisy (Figure 4-*left*).

In order to increase the sensitivity of the method, we developed a multi-spotting deposit. By repeated deposition of droplets, we increase drastically the amount of peptides on the spot, and can then reach very low level of detection. As a proof the Figure 4 shows the spectra of sub-nM solution before (*left*) and after the multideposit (*right*).

We developed a method which allows to generate single droplet in a microchip coupled with a MALDI-TOF analysis. We control the volume of sample deposit on each spot, and by multi-spotting we reached a very high sensitivity compatible with physiological concentration of proteomic biomarkers.

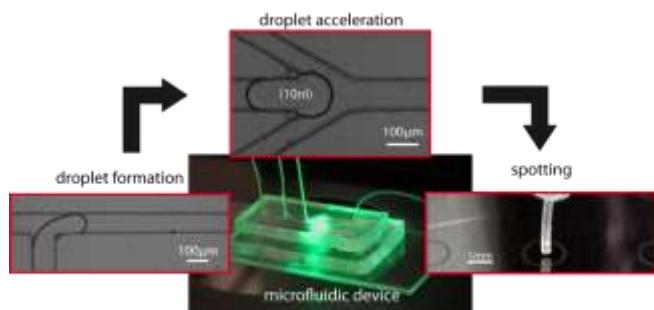


Figure 1. Principle of the droplet formation and MALDI deposition

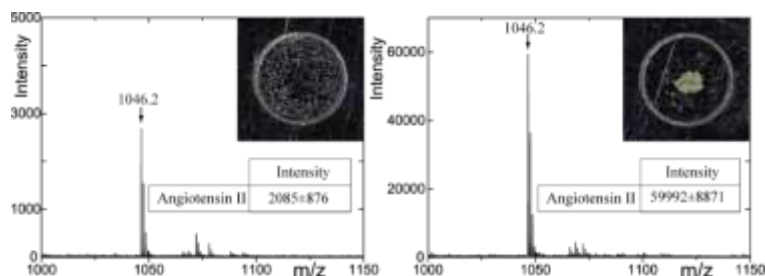


Figure 2. MALDI spectra. *Left.* Standard pipette deposit. *Right.* Microfluidic platform deposit. Inserts: Dried mixture of peptide/matrix before laser desorption.

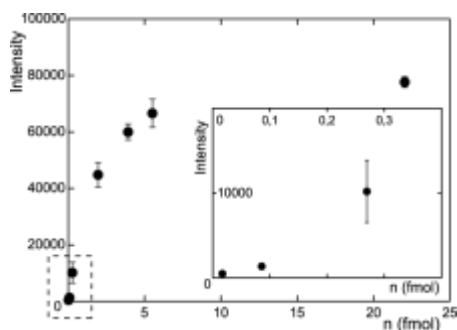


Figure 3. Average MS signal intensity as a function of the amount n (n from 22 femtomoles to 17 attomoles) of Angiotensin II deposited on the MALDI plate by the microfluidic platform deposit.

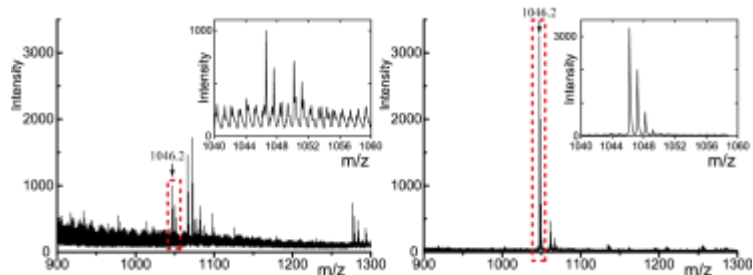


Figure 4. MALDI spectra of a 100 attomoles solution of Angiotensin II. *Left.* Microfluidic platform deposit. *Right.* Microfluidic platform multi-spotting deposit. Inserts: zoom on Angiotensin II peaks.

REFERENCES:

1. "Digital microfluidics." Choi, Kihwan, Alphonsus HC Ng, Ryan Fobel, and Aaron R. Wheeler., *Annual review of analytical chemistry*, 5: 413-440 (2012).
2. "Recent advances in microfluidics combined with mass spectrometry: technologies and applications." Gao, D., Liu, H., Jiang, Y., & Lin J. M., *Lab on a Chip*, 13(17), 3309-3322 (2013).
3. "Interfacing droplet microfluidics with matrix-assisted laser desorption/ionization mass spectrometry: label-free content analysis of single droplets." Küster, S. K., Fagerer, S. R., Verboket, P. E., Eyer, K., Jefimovs, K., Zenobi, R., & Dittrich, P. S., *Analytical chemistry*, 85(3), 1285-1289 (2013).
4. "Identification of neutrophil-derived proteases and angiotensin II as biomarkers of cancer cachexia." Penafuerte, C. A., Gagnon, B., Sirois, J., Murphy, J., MacDonald, N., & Tremblay, M. L. *British journal of cancer*, 114, 680-687 (2016).
5. "Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients." Huang F, Guo J, Zou Z, Liu J, Cao B, Zhang S, Li H, Wang W, Sheng M, Liu S, Pan J., *Nature communications*, 5:3595 (2014).