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

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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 soltutions: 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/µl 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.

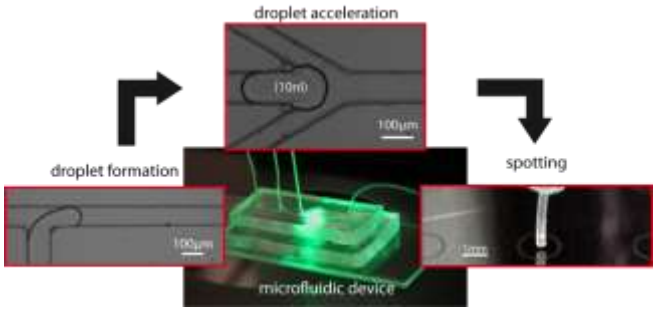

Figure 1. Principle of the droplet formation and MALDI deposition

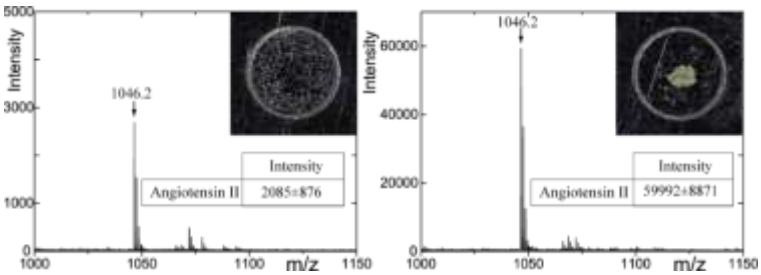

Figure 2. MALDI spectra. *Left*. Standard pipette deposit. *Right*. Microfluidic platform deposit. Inserts: Dried mixture of peptide/matrix before laser desorption.

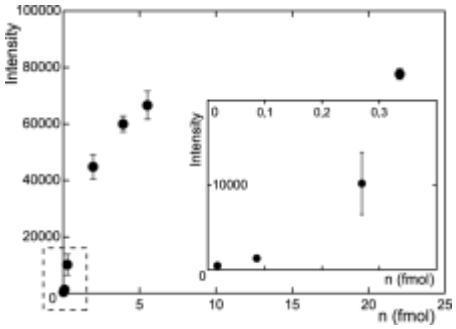

Figure 3. Average MS signal intensity as a function of the amount n (n from 22 femtomoles to 17 attomoles) of Angiotensin II deposited on the MALDI plate by the microfluidic platform deposit.

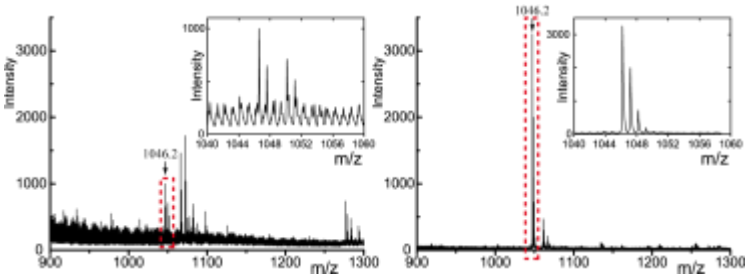

Figure 4.
MALDI spectra of a 100 attomoles solution of Angiotensin II. *Left*. Microfluidic platform deposit. *Right*. Microfluidic platform multi-spotting deposit. Inserts: zoom on Angiotensin II peaks.

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