## Comparison of AP-MALDI/MS with SubAP-MALDI (1 Torr) Mass Spectrometry

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1. Experimental setups:



The atmospheric pressure (AP) MALDI data (using an ion funnel, or i-Funnel, interface to increase ion collection efficiency, at the left) were compared to the data from SubAP-MALDI (operated near 1 Torr pressure) interfaced to the same i-Funnel (at the right). Thermo LTQ mass spectrometer (MS) was used for data acquisition in both setups.

In both setups the experiments have been conducted using the same samples, MALDI laser spot size, sample scanning speed and MS operational parameters. The laser energy and the extraction voltage on the sample plate were adjusted in both setups to get the best spectral data quality.

## 2. Major Results:

The mixture of Insulin (1 pmol/ $\mu$ l) and four peptides (100 fmol/ $\mu$ l each) with DHB matrix (7 mg/ml). LTQ operated in Normal Scan mode (5 scans/sec)



The mass spectra of Insulin (1 pmol/  $\mu l$ ) with DHB matrix (7 mg/ml) in high resolution (Zoom - 1 scan/sec) mode:



The mass spectra of four peptides (100 fmol/ $\mu$ l each) with CHCA matrix (0.5 mg/ml):

20000

10000

Total ion current (TIC), counts



## AP-MALDI

SubAP-MALDI

S/N = 2,480

1534.7

- (1) One double-charged peptide peak is observed: m/z 767.4 ( $P_{14}R$ ) (1) Do
- (2) CHCA matrix adducts for some peptides are observed: m/z 1722\* and m/z 1911\*\*

Notes:

(3) Very intense matrix peaks compared to the peptide peaks



(674.6) (767.2

(Substance P) and m/z 767.2 ( $P_{14}R$ )

chemical noise le

- (2) There are no CHCA matrix adducts (m/z 1722 and m/z 1911 peaks are not observed)
- (3) Matrix peaks are much less intense compared to peptide peaks (in contrast to the AP-MALDI case where matrix peaks are much more intense)

## 3. <u>Conclusions</u>:

- With similar samples and operational conditions, a signal-to-noise (S/N) ratios are about 20-fold higher in SubAP-MALDI (1 Torr) experiments compared to AP-MALDI ones. This SubAP-MALDI advantage over AP-MALDI becomes even more substantial when compared to regular AP-MALDI ( i.e., one without i-Funnel) as the ion funnel technology results in about an order of the magnitude increase of the AP-MALDI ion signal.
- The difference in S/N ratio between SubAP-MALDI compared to AP-MALDI has a tendency to increase with increase of mass of peptides/proteins.
- No matrix adducts are observed in SubAP-MALDI experiments while small intensity CHCA matrix adduct ions (M+H +190 and M+H+380 Da) are observed in the AP-MALDI spectra.
- The optimal laser energy/fluence is 2 times lower for SubAP-MALDI compared to AP-MALDI, which may reflect the difference in processes of ion formation for SubAP-MALDI and AP-MALDI.