Characterization of chemical noise in AP MALDI using LTQ/Velos and Orbitrap mass spectrometers

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Overview

MALDI-MS offers several advantages over ESI including a very high tolerance for contaminants and rapid off-line MS analysis of complex samples separated using multiplexed LC channels. The detection limit in MALDI-MS analysis of tryptic peptides is determined by the level of chemical noise, the latter being proportional to matrix concentration in a sample solution (Ref. 1). In MS instruments capable of counting single ions (e.g., quadrupole ion traps, TOF MS), chemical noise is typically presented by a 1-Da spaced "comb" of peaks with peak heights gradually diminishing with m/z.

Laser fluence in MALDI affects the intensity of both the noise and analyte peaks. Thus, it is important to select the laser fluence where the S/N ratio reaches its maximum, assuming the analytes signals from a spot under analysis are collected in a reasonable time (e.g., 1-10 sec). The limit of detection (LOD) for tryptic peptides in MALDI depends on matrix ionization efficiency, peptide hydrophobicity, analyte-to-matrix ratio, chemical purity of matrix and solvents, and method of sample preparation (e.g., methods yielding smaller crystals result in better sensitivity, sensitivity can be increased by using a post-spotting "cold-water" clean up, *etc*). In addition, S/N ratios attained in the MS scan across the dried-droplet sample spot are a function of mass spectrometer resolution. In the case of QIT instruments where mass resolution is proportional to the scan time, a selected target value for S/N determines the time required for the mass analysis.

Method

Mass spectra were obtained from samples containing matrix material (CHCA) and synthetic peptides using LTQ/Velos and LTQ-Orbitrap mass spectrometers. The 355-nm laser with 200-Hz repetition rate and MassTech's AP MALDI source were used in all MALDI experiments. The laser spot size on a dried-droplet sample was 300 micron. The 10 μL of acidified water-based solution containing synthetic peptides was added to 100 μL of matrix solution (acetonitrile/water 70%/30%, v/v). Aliquots containing 1-μL samples were placed onto a stainless-steel plate to form dried droplets. LTQ/Velos operated in either low-resolution (Normal Scan) or high-resolution (Zoom and Ultra-zoom Scan) modes.

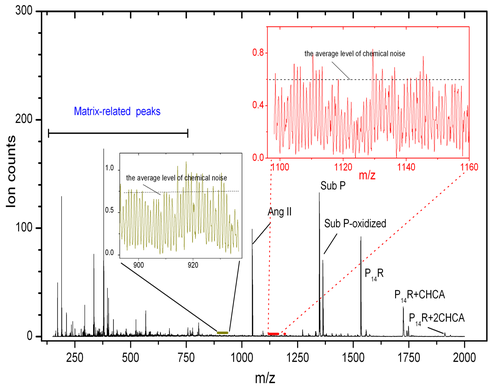
In ESI, a single peptide (bombesin) was dissolved in 50%/50% (v/v) acetonitrile/acidified water (1% acetic acid) in concentration of 10^{-7} M. The peptide solution was sprayed into the inlet of mass spectrometer through 10 micron fused silica ESI emitter. A syringe pump maintained 500 nL/min flow rate through the emitter. Voltage applied across the ESI tip was 2 kV, heated capillary inlet was kept at 240ºC.

The statistics of chemical noise in MALDI-MS was compared to that in ESI-MS analysis.

RESULTS

LTQ/Velos: Normal scan (low-res) mode

Peptides: 100 fmol of each added to 1 μL of CHCA (0.5 mg/mL) in 70%/30% MeCN/water. A few hundred of individual scans were summed up. One can see a regular "sinusoidal-like" pattern of chemical noise peaks



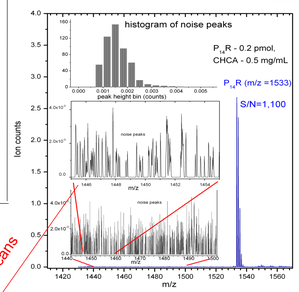
Normal Scan mode : ~140 ms/scan, mass resolution ~2,500.

LTQ/Velos: Zoom scan (high-res) mode

LTQ/Velos: Zoom scan mode, ~1600 ms/scan, mass resolution ~10,000.

Peptide: 200 fmol of $P_{14}R$ dissolved in 1 μL of solution (70%/30% MeCN/water) containing CHCA (0.5 mg/mL).

Over 100 individual scans were summed up.



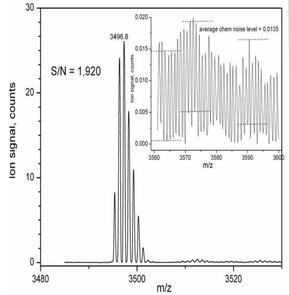
AP MALDI source

CHCA (0.5 mg/mL)

LTQ/Velos: Zoom scan mode (mass range extended mode), mass resolution ~10,000.

Peptide: 1 pmole of Ins chain B dissolved in 1 μL of solution (70%/30% MeCN/water) containing DHB (10 mg/mL).

Over 100 individual scans were summed up.

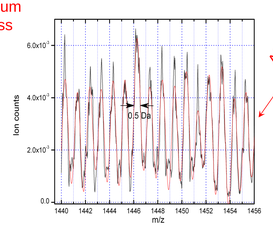


SubAP MALDI source

DHB (10 mg/mL)

High-res Zoom scan in LTQ/Velos. After averaging of a few thousand LTQ scans, the noise "carpet" of narrow (~0.07-0.1 Da wide) individual peaks of modestly varying heights transforms into a familiar sinusoidal pattern featuring ~0.5-Da wide peaks.

Red trace – the noise spectrum after low-pass FFT filtering



After collecting 3,000 Zoom scans

Zoom scan (high-res) : under conditions of high mass resolution, individual noise peaks do not generally start to overlap when one sums up ~100 MS scans taken at moderate laser fluences (20% above threshold) , but rather spread (yet not evenly) over the m/z scale centering around some "nominal" mass intervals separated by 1 Da.

In the figure above (a sample with low concentration of CHCA) , one can see a "carpet" comprising individual noise peaks of not very different heights.

Peptide/DHB samples (DHB in high concentration, 1-Torr MALDI source) at high laser fluence (100% above threshold) shows a "hump" noise pattern after averaging of 100 MALDI MS spectra.

At high matrix concentration or after extensive averaging chemical noise patterns have the same appearance in both **AP** and **Sub AP** (low-pressure) MALDI MS sources.

LTQ/Velos: Ultra-zoom scan.

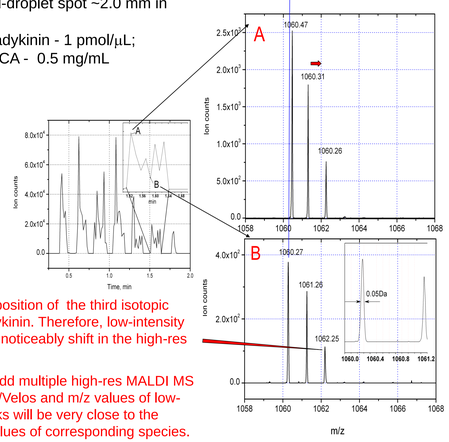
Selected m/z interval: target m/z +/-8 m/z. High intensity peaks are shifted to larger m/z values compared to corresponding theoretical values, whereas peaks with very low intensity (<500 ion counts) remain close to corresponding theoretical values.

When one gradually sums up noise peaks spread over the 16-Da wide mass interval (note that noise comprises low-intensity peaks), the "noise pattern" evolves as follows: individual noise peaks - noise "carpet" - "sinusoidal" or "hump-like" noise pattern.

A red arrow indicates a shift in peak positions (compared to theoretical ones) for high-intensity peaks (ultra-zoom mode)

The UV laser beam (0.3 mm) scans across dried-droplet spot ~2.0 mm in diameter.
Sample: Bradykinin - 1 pmol/μL;
CHCA - 0.5 mg/mL

Total ion current

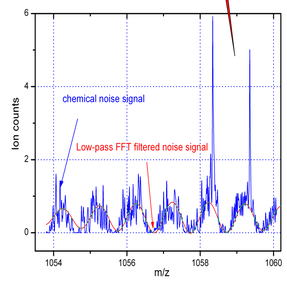


No shift in a position of the third isotopic peak of bradykinin. Therefore, low-intensity peaks do not noticeably shift in the high-res LTQ mode.

So one can add multiple high-res MALDI MS scans in LTQ/Velos and m/z values of low-intensity peaks will be very close to the theoretical values of corresponding species.

Chemical noise pattern in the averaged spectrum. A thousand of ultra zoom scans were summed up.

Two narrow peaks originate from the MALDI-related ISD process, particularly H2 abstraction from the first and second isotopes of Bradykinin



Location of analyte peaks on a slope of the noise "hump" shifts the position of the peak maximum. That is why denoising procedure increases not only S/N but also improves mass accuracy (see Refs. 2, 3).

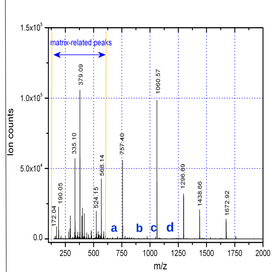
The ion signals corresponding to chemical noise were recorded at three different settings of the LTQ/Velos mass spectrometer: normal scan (resolution R ~2,500); zoom scan (R~ 10,000), and ultra-zoom scan (R~20,000) modes.

The results showed that looking with a "magnifying glass" or under "zoom-in" conditions at the chemical noise pattern, one reveals that the latter is built up from scores of individual peaks. At first glance the noise peaks "pop up" rather randomly on the m/z scale, yet the statistics shows that the peaks prefer certain "allowed zones" separated by 1-Da intervals. When the limited number of MALDI MS spectra is averaged, the noise peaks in zoom and ultra-zoom modes formed a "grass carpet" comprising single-ion peaks. The peak height of the noise peaks (many of the peaks originate from single ions) theoretically supposed to be about the same because their height is determined by a single-ion response of the LTQ ion detector consisting of a conversion dynode and Secondary Electron Multiplier (SEM).

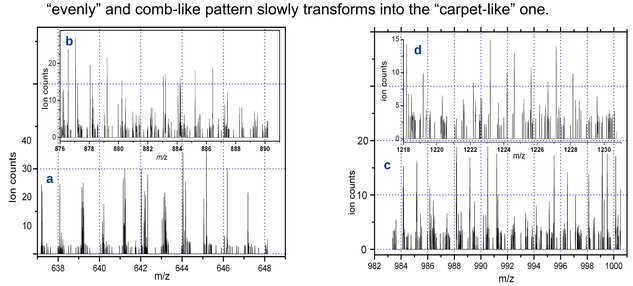
Summing up 1000s of high-resolution MALDI spectra eventually brings the overlaps in the individual noise peaks. The "carpet" grows into a series of "humps" resulting in a formation of a sinusoidal pattern similar to the one observed in low-resolution mass spectra.

Orbitrap (R~ 80,000 at m/z =1,000)

Peptides: 100 fmole each,
CHCA - 0.5 mg/mL.
Averaging: ~500 AP MALDI MS scans.



A comb-like noise pattern in the averaged MALDI MS spectrum is visibly observed till m/z~1,200. The averaged noise peak height diminishes with m/z. At m/z >1,200, noise peaks (not shown here) start to spread over mass intervals more "evenly" and comb-like pattern slowly transforms into the "carpet-like" one.

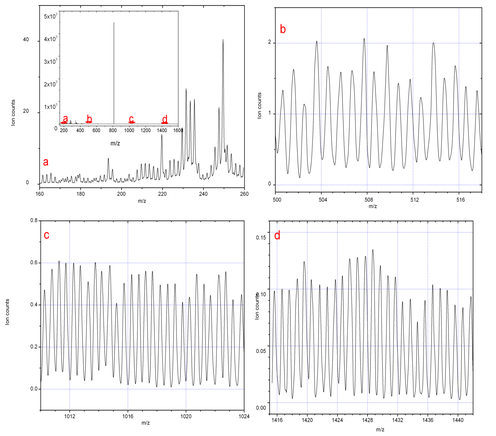


Chemical noise in ESI-MS

LTQ/Velos, Normal Scan mode. Nano-ESI source.

Chemical noise pattern after summing up ~1,000 MS scans.

Peptide: Bombesin, 10^{-7} M in 50%/50% MeCN/water + 1% acetic acid.



A low-resolution ESI-MS spectrum shows a sinusoidal pattern of noise peaks.

Conclusion

The nature of 1-Da spaced chemical noise in MALDI-MS and ESI-MS seems to be the same. High-resolution scans shows that the 0.5-Da wide evenly spaced (1-Da peak-to-peak distance) noise "humps" observed in low-resolution MS scans, are built up, in fact, from scores of individual "noise" peaks.

A striking similarity between the ESI and MALDI noise patterns casts a doubt that the chemical noise in MALDI-MS is related to matrix clusters/adducts. The same can be said about the cluster-related noise origin in ESI-MS. It is hard to accept the existence of multitude of protonated/sodiated single-charged clusters built of water or other solution molecules capable to yield hundreds distinct species which masses densely populate a narrow (~0.4-0.6 Da) mass interval at each "nominal" mass. Most likely, the background noise originates from ambient contamination with bio-organic species (poor proton acceptors) that can be found in solvents or MALDI matrices. Ion mobility (IM) studies typically demonstrate that the IM-m/z curve for single-charged peptides overlaps with IM-m/z curve for the chemical noise, which can be interpreted as chemical similarity between the peptides and species that yield the noise background.

A necessary condition for the observation of analyte ions in Orbitrap or FTICR-MS is a "bunching" of ions with the same m/z. The bunching necessitates a presence of at least ~50 single-charged ions with the same m/z in the ICR or Orbitrap cells and impose the "instrumental" cut off for low-abundance and poorly ionizable species. The bunching limitation most likely prevents a formation of typical comb-like pattern at higher m/z (m/z>1,200) despite summing up 1000s Orbitrap spectra.

Chemical noise peaks randomly (yet not evenly) appear on the m/z scale, preferring to cram around 1-Da spaced "nominal" masses. The averaging of 100s spectra recorded in high-res MS instruments counting single ions (e.g., LIT with ultra zoom scan or high-res qTOF) leads to a formation of a noise "carpet". After averaging of over 1000 individual MS spectra, the peaks start to overlap leading to a formation of 1-Da separated "humps". This highlights the notion that higher mass resolution brings higher S/N ratios (the noise level is counted against chemical background) due to the fact that working with higher resolution delays the moment when noise peaks scattered over 0.5-Da intervals start to overlap with each other.

References

1. Hioki Y *et al* "An improved sample preparation method for the sensitive detection of peptides by MALDI-MS", *J Mass Spectrom* **48**(11):1217 (2013)
2. V. Andreev *et al*, "Universal Denoising and Peak Picking Algorithm", *Anal Chem* **75**, 6314 (2003)
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