Using dynamic ejection of high abundance ions to enhance the dynamic range of LTQ-FT proteomics measurements

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Finite trap capacity limits dynamic range

Concentration range exceeds dynamic range of trap

Increased ion fill time doesn’t result in more ions in the trap
Detecting more of the less abundant ions

Dynamic ejection of a highly abundant species

Increased trapping time results in more low abundant ions
Ejection of multiple high abundant ions further extends dynamic range
Dynamic range enhancement applied to mass spectrometry (DREAMS)

• Implement DREAMS with a hybrid ion trap/FT-ICR mass spectrometer
  – Automated gain control (AGC) regulates ion injection time to improve gains from DREAMS
  – High resolution and mass measurement accuracy with enhanced dynamic range
• More identified low abundant peptides

Ejection of highly abundant ions with dipolar excitation

Dipolar excitation frequency for ion ejection is proportional to $1 / m/z$

$$\Omega_0 = \frac{q_u \omega_0}{\sqrt{8}}$$

LTQ ion optics

Lowest pressure region prior to ion trap
Highest DREAMS resolution

Thermo Fisher Scientific, Finnigan LTQ Hardware Manual, Revision B, p. 2-14
Hyperbolic DREAMS quadrupole replaces octapole ion guide

- Stable DREAMS ejection calibration
- Increased DREAMS resolution with increased RF frequency

External RF power supply

2.5 kV at 2.0 MHz RF

Q00 Ion Guide

Q0 Ion Guide

Ion Detection
Ion funnel increases ion current to the ion trap, reducing ion injection times.
Ion funnel efficiently focuses ions through conductance limit

Skimmer samples small region of the inlet ion plume

Scan events for implementing DREAMS

Scan 1
IT pre-scan

Scan 2
• Identify most abundant peaks
• Calculate ejection waveform
• Apply DREAMS waveform

Scan 3
IT DREAMS scan

Scan 4
FT DREAMS scan

Scan 5
IT zoom scan during FT scan
Turn off DREAMS waveform
DREAMS control reacts to software events from LTQ COM

Determine timing relationship between hardware triggers and software events

DREAMS waveform to hyperbolic quadrupole
LTQ-FT DREAMS timing chart

- FT DREAMS scan
- Zoom Scan
- Pre-scan
- IT DREAMS scan
- AGC pre-scan
- Scan 4 (FT)
- Scan 5
- Scan 1
- Scan 2
- Scan 3
- DREAMS ON
- DREAMS OFF
- DREAMS waveform, time

DREAMS ON

DREAMS OFF

DREAMS ON
Selecting more peaks for ejection increases ion accumulation time.
Increased S/N for non-ejected ions

Test peptide mixture

Number of peaks selected for ejection

S/N

0 5 10 15 20 25 30

Increased S/N for non-ejected ions

Top 3 ejected

Top 5 ejected

No DREAMS ejection

$m/z$: ~1722, 1022, 524

$m/z$:

400 800 1200 1600 2000

Targeted for ejection in subsequent scans

$m/z$: ~1722, 1022, 524

$m/z$:
LC-MS proteomic samples characterization of DREAMS

- “Depleted” human plasma
  - Depletion of Top 12 abundant proteins
  - Alkylation and tryptic digestion
- Mouse plasma tryptic digest
- Gradient of 100 min
  - Solvent A: 0.2% acetic acid, 0.05% TFA
  - Solvent B: 90% acetonitrile, 0.1% TFA
  - Balance water
Accurate mass and time (AMT) Tag based approach to assign peptides to observed LC-MS features

- Decon2LS for deisotoping high resolution LC-MS data
- VIPER for grouping and comparing deisotoped data to AMT tag DBs

Increased Number of Identified Unique Peptide Mass Tags using DREAMS

Top 7 peaks ejected
Depleted human plasma

FDR based on 11 Da shift
decoy database

- With DREAMS, average 1817
- Standard, average 1600

FDR Standard ~1.5%
FDR DREAMS ~1.1%
MASS ACCURACY AND RETENTION TIME HISTOGRAMS

100 min LC-LTQ FT experiments with depleted human plasma

Identical mass measurement accuracy with and without DREAMS

Increased LC-MS features with DREAMS
Example mass tags identified only with DREAMS LC-MS

Depleted human plasma

Top 7 peaks ejected

MFTTAPDQVDKEDEDQFQSNK, MTID: 6702475

TGIVSGFGR, MTID: 21053333
More Low Abundance Peptides Identified with DREAMS

Top 7 peaks ejected
Depleted human plasma

Using DREAMS doubled the identified low abundant peptides

Common in 5 DREAMS LCMS runs and 5 standard LCMS runs

Peptides identified only in DREAMS LCMS runs
Increased number of identified peptides and proteins with DREAMS

Percentage of identified peptides and proteins relative to control LC-MS runs without DREAMS

<table>
<thead>
<tr>
<th></th>
<th>% Peptides</th>
<th>% Proteins</th>
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</thead>
<tbody>
<tr>
<td>Human plasma</td>
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<tr>
<td>DREAMS Top 7</td>
<td>111%</td>
<td>108%</td>
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<tr>
<td>Mouse plasma</td>
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<td>DREAMS Top 20</td>
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<tr>
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<tr>
<td>DREAMS Top 30</td>
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<td>146%</td>
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Proteins considered identified with 2 or more peptides
Future Directions

• Automate determination of peptide abundances in DREAMS scans
• Active control of LTQ-FT DREAMS scans
• Incorporate parent ion isolation with DREAMS quadrupole during MS/MS events (replacing zoom scan)
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