OMICS and Precision Medicine - Full Workflows for Lipidomics and Metabolic Profiling

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The Promise of Quantitative Systems Biology

• **Human Health**
  - Biomarkers will help monitor wellness, detect early disease, monitor disease progression, and stratify patients for more tailored care.

• **Agriculture and Food**
  - Bioengineering of key crops will enable the optimization of production, nutrition, taste, durability, and resistance to infectious agents.

• **Energy and Environment**
  - Bioengineering based on a systems level knowledge for the development of alternative bioenergy sources and improved carbon sequestration.
The Challenges in Systems Biology

Today's biomarker discovery  Tomorrow's personalized medicine

Oomics data are information rich and context dependent  NEED to compare complex biological results from diverse technologies

Vast amounts of omics data are generated by different labs  NEED to integrated environment to combine, compare, and share results

The Complexity of Systems Biology for Personalized | Precision Medicine

Leading to a systems-level understanding

Cardiovascular metabolism

Metabolomics hormones signaling

Proteomics proteins

Protein kinases Alzheimer’s disease

Transcriptomics RNAs

MicroRNA parasitology

Epigenetics epigenetics

DNA parasitology

Next-gen sequencing

Tuberculosis

OneOmics SWATH

Molecular biology

Molecular biology

Cancer

Transcriptomics

Inflammation

Molecular biology

DNA metastasis

Sciex

Illumina

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The OMICS Spectrum

Discovery

Targeted

Metabolomics

Proteomics

Genomics

Metabolites

Protein

DNA

Lipids, Sugars, Toxins

Wenk et al. Nature 2005
The OMICS Spectrum

Wenk et al. Nature 2005

OMICS Technologies

SCiEX Metabolomics Portfolio

Discovery

Global Untargeted Screening
- 1000's of features in 10's of samples
- SWATH® / IDA Acquisition
- Brings data analysis from weeks to days

Global Targeted Screening
- Profile over 500 known metabolites in 10's of samples
- SWATH® / IDA Acquisition
- SCiEX Accurate Mass Spectral Library: processing to visualization in minutes

Translation/Validation

Targeted Profiling
- 100s of putative biomarkers on 100-1000s of samples
- QTRAP® Systems: Industry standard robustness
- Kits / reagents / existing methods:
  - aTRAQ™ Reagent for AAA
  - AmpliFlex reagents for enhanced sensitivity
  - MRM assays for Vitamin D, Hormones/Steroids

Clinical Utilization

Validated Assays
- 10s of biomarkers and 100,000s of samples/yr.
- SCiEX Triple Quad™ 4500 MD
- MultiQuant™ Software MD
- Cliquid™ Software MD

# Putative biomarkers

# Samples
Applications
FluxOMICS and Pathways

Targeted Metabolomics - FluxOMICS

Quantitative and Qualitative Metabolomics for the Investigation of Intracellular Metabolism

Targeted Analysis on the QTRAP® 5500 System and Reverse-Phase Ion-Pairing Chromatography

Douglas McCloskey1 and Baljit K. Ubhi2
1Department of Bioengineering, University of California, San Diego, CA, USA, 2SCIEX, USA

Liquid chromatography mass spectrometry (LC-MS) provides a powerful analytical tool for understanding and monitoring intracellular metabolism by measuring the metabolome. The study of intracellular metabolism of model organisms, such as E. coli, is vital to further our biochemical knowledge, to develop new pharmaceuticals that target harmful pathogens, and to improve industrial applications that aim to metabolically engineer bacteria in order to produce commodity chemicals from renewable resources. Paramount to these endeavors is the ability to reliably and accurately measure the intracellular metabolome. For microorganisms, the compounds of most interest comprise intermediates of high flux pathways such as glycolysis, the pentose phosphate pathway, the citric acid cycle, amino acid metabolism, as well as energy and redox cofactors such as ATP and NADH (Figure 1). By measuring the absolute metabolite levels of such compounds, one is able to calculate reaction and pathway thermodynamics and infer in vivo enzyme kinetics. In addition, when microorganisms are grown on a specifically chosen labeled substrate (e.g., 13C-glucose) during a metabolic labeling experiment, the isotope enrichment distribution of intracellular compounds can be used to calculate the absolute flux through specific reactions of interest.

In this work, the QTRAP® 5500 system (a hybrid triple quadrupole linear ion trap mass spectrometer) was used to implement both quantitative and qualitative workflows aimed at measuring anionic and polar compounds of intracellular metabolism.

Key Features of the QTRAP® 5500 System for Qualitative and Quantitative Metabolomics
Targeted Metabolomics - FluxOMICS

Figure 1. Metabolomics of Intracellular Pathways for Investigations into the Biochemistry of Microorganisms. High flux pathways such as those shown are key to generating a biological picture and targeted metabolomics strategies provide a robust quantitative strategy for monitoring changes.

Targeted Metabolomics - FluxOMICS
Figure 3. Simultaneous Quantitative Analysis with Qualitative Confirmation of L-Glutamate using the QTRAP® 5500 System. A) The primary and secondary transitions (blue and green) for glutamate are monitored, along with the uniformly heavy carbon labeled analog (red). A full scan MS/MS spectrum (EPI scan) is triggered when the primary and secondary transitions reach a predefined threshold. B) To confirm the identity of the MRM signal, the MS/MS was matched with a greater than 95% match to the reference spectrum (taken for pure standards for glutamate).

Figure 4. Qualitative Method for Characterizing the Isotopomers. Enabled by the QTRAP 6500® System. The advantage of the qualitative method is highlighted in this example measuring unlabeled and fully labeled maleic acid (maleyl L) in E. coli. Several transitions corresponding to the isotopomer distribution are monitored per compound. An Enhanced Resolution (ER) scan is triggered when one of the isotopomer transitions reaches a predefined threshold and provides MS and isotope ratio information. An EPI scan is also triggered and provides information regarding the location of the heavy label (if present).
The Lipidizer™ Platform: A Revolutionary Tool for Understanding the Role of Lipids in Disease

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Industrializing Lipidomics
Lipidyzer™ Platform

- Robust MS System
- Class separation using DMS
- Unique internal standard strategy with validated kits
- Data Visualization including heat maps, box & whisker plots, etc

Demonstrating the Power of Lipidyzer™ Platform
Benefits

- Specificity – Differential Mobility Spectrometry (DMS)
- Eliminating Quantitative Bias – Novel Internal Standards
- Coverage – Complex Lipid Metabolism
- Sensitivity and Precision - Assay
- Robustness – Assay and Platform
- Ease of Use - Platform

- Samples Sets used for Biological Validation
Complex Lipids are like a Matrix

• Lipid are present in classes that have concentrations and compositions (important for level of metabolism)
  - Concentration = sum of the FAs for any given class (column)
  - Composition = relative abundances of each FA (or species) across many classes (rows)

• When FA metabolism is altered there is the ability to change FA composition of all classes
Lipid are present in classes that have concentrations and compositions (important for level of metabolism)
- Concentration = sum of the FAs for any given class (column)
- Composition = relative abundances of each FA (or species) across many classes (rows)

When FA metabolism is altered there is the ability to change FA composition of all classes
When lipid class metabolism is altered there is the ability to change all members of the class

What is needed from a Lipid Platform

1) Specificity
- A non-specific method (e.g. PC 36:2) does not allow mapping to the elements of the matrix

2) Quantitation
- A non-quantitative approach does not allow accurate summing of the rows and columns

3) Comprehensive Coverage
- A partially complete matrix is difficult to interpret
**Broad Range of IS to Normalize Quantitative Data**

- Diversity of fatty acid chain lengths and degrees of unsaturation result in differential fragmentation efficiency which impacts quantitation
- Multiple IS that reflect the diversity of lipid molecular species

| PHOSPHATIDYLCHOLINE (PC) INTERNAL STANDARD MIX |
|-----------------|----------------|---|
| STRUCTURE       | FATTY ACID     | POS % |
| 16:0 sn-1        | Palmitoleic acid | 5 |
| 18:1 sn-2        | Oleic acid     | 20 |
| 18:2 sn-2        | Linoleic acid  | 20 |
| 18:3 sn-2        | α-Linoleic acid| 5  |
| 20:3 sn-2        | Dihomo-γ-linoleic acid | 5 |
| 20:4 sn-2        | Arachidonic acid| 20 |
| 20:5 sn-2        | Eicosapentaenoic acid | 5 |
| 22:4 sn-2        | Eicosatetraenoic acid | 5 |
| 22:5 sn-2        | Docosapentaenoic acid | 5 |
| 22:6 sn-2        | Docosahexaenoic acid | 10 |
| d8:16:0 sn-1     | Labeled palmitic acid | 100 |

- Each lipid class has multiple internal standards at concentrations that reflect those found in biology

**Specificity Offered by SelexION® Technology**

**Removal of Isobaric Interferences**

- Problem: The Q1 isolation window during MS/MS is ~1.2 Da, which increases number of potential isobars

**Ionogram**: Separation by Lipid Head Group in the Gas Phase

Differential Mobility Spectrometry-Driven Shotgun Lipidomics Anal. Chem. 2014. 86. 9662-9669 10.1021/ac5021744
**How Does SelexION™ Technology Separate Ions?**

Differential Mobility Spectrometry (DMS) separates molecules using planar geometry

Separation waveform (SV): radially displaces ions towards one or the other electrode, depending upon high and low mobility characteristics

Compensation voltage (COV): restores the trajectory for a given ion or range of ions to allow them to transmit through the DMS device and enter the mass spectrometer

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**Separation of Lipid Classes Using SelexION™ Technology**

**Effects of Modifier on COV Values**

- **a.** NO MODIFIER
- **b.** 3-PRPANOL
- **c.** 2-PRPANOL
- **d.** 1-PRPANOL
- **e.** CHLOROFORM
- **f.** HEXANE

Differential Mobility Spectrometry-Derived Microarray Lipidomics

The Lipidyzer Eliminates Quantitative Bias
Multiple internal standards per class provide accurate quantitation

Full Coverage of Complex Lipid Metabolism
Coverage and Depth

• Over 1100 molecular species across 13 lipid classes
• Lipidyzer™ Platform provides 6 measurements:
  1. Lipid Class Concentration
  2. Lipid Species Concentration
  3. Fatty Acid Concentration
  4. Lipid Class Composition
  5. Lipid Species Composition
  6. Fatty Acid Composition

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Lipid Classes</th>
<th>Number of Species*</th>
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<tbody>
<tr>
<td>Neutral Lipids</td>
<td>Triacylglycerols (TAG)</td>
<td>502</td>
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<tr>
<td></td>
<td>Diacylglycerols (DAG)</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Free Fatty Acids (FFA)</td>
<td>28</td>
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<tr>
<td></td>
<td>Cholesterol Esters (CE)</td>
<td>34</td>
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<tr>
<td>Polar Lipids</td>
<td>Phosphatidylcholines (PC)</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylethanolamines (PE)</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td>Lyso phosphatidylcholines (LPC)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Lyso phosphatidylethanolamines (LPE)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Sphingomyelins (SM)</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Ceramides (CER)</td>
<td>56</td>
</tr>
</tbody>
</table>

*The Ceramides listed above includes the further three classes, DCER, HCER and LCER.
Lipidyzer™ Platform Sensitivity and Precision

5 Day Study

• Validated across 4 instruments and 3 labs the instruments detected similar numbers of lipid species and with similar precision
• >675 lipid species with RSD <20% in this control sample

Lipidyzer™ Platform Robustness

Precision: 6 Day Study in Plasma (Total Class Concentration)

• 11/13 Classes <10% RSD over 6 days

*Note: DCERs are present at exceedingly low levels in plasma
Ease of Use

Lipidomics Workflow Manager Software

• Automates the entire workflow
  ‒ Kit registration (concentration info)
  ‒ System tuning and testing
  ‒ Experimental design, data collection and processing
• Data Visualization including heat maps, QC charts and quantitative data tables

Biological Validation of Lipidyzer™ Platform

Sample Set 1

• Preeclampsia - a leading cause of maternal perinatal morbidity and mortality
  ‒ Shallow invasion of cytotrophoblast (CTB) cells into mother’s uterus
  ‒ Failed vascular transformation of the spiral arteries is the hallmark of the placental defects in preeclampsia
  ‒ Response: high blood pressure & proteinuria
• Understand normal CTB development and how this goes awry in preeclampsia
• Pilot study of 12 preterm labor (PTL) vs 12 severe preeclampsia women (SPE).
  ‒ Gestational age-matched plasma samples, 25-37 wks
• Validated TAGs and DAGs upregulation in SPE

Professor Katherine Williams & Susan Fisher, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco (UCSF).
Biological Validation of Lipidyzer™ Platform

Sample Set 2

• Patients with hypertriglyceridemia (high triacylglycerols, TAGs x14), hypercholesterolemics (high cholesteryl esters, CEs x14) and controls (x12) – 40 samples total.

• Validated TAG changes with clinical findings

• Validated CE changes with clinical findings

<table>
<thead>
<tr>
<th>CHEMICAL_NAME</th>
<th>HMDB</th>
<th>KEGG</th>
<th>LIPID_MAPS</th>
<th>HIGH_CE_NORMAL(FOLD)</th>
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<tbody>
<tr>
<td>CE(18:4)</td>
<td>-</td>
<td></td>
<td></td>
<td>5.4032</td>
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<tr>
<td>CE(14:1)</td>
<td>HMD810367</td>
<td>LMST01020021</td>
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<td>4.8496</td>
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<tr>
<td>CE(16:1)</td>
<td>HMD800658</td>
<td>LMST01020006</td>
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<td>CE(14:0)</td>
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<td>LMST01020004</td>
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<td>LMST01020013</td>
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<td>2.2789</td>
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</table>

Biological Validation of Lipidyzer™ Platform

Sample Set 2 Continued

• Novel findings in down regulated HCER & LCER highlighting altered glycosphingolipid metabolism
Biological Validation of Lipidyzer

Sample Set 3

- Weight Loss Study
  - Clinical manifestation of inflamed adipose tissue
  - Insulin resistance → leading to metabolic syndrome
  - Calorie-restricted diet over 8 wks (900 kCals per day)
  - Serum taken before and after weight loss
- Decreased lipogenesis (including decreased TAGs)
- Increased FFAs

Quickest Route to Success

Comparison to a Traditional Discovery Platform

- Lipid changes with age that are prevented by dietary restriction may be responsible for age-related neuronal damages, decreased cognition functions and increased neurological disorders
- Aging Study - Young (10), old (10) and old dietary-restricted (8) mice
- Discovery data already collected on a QExactive compared to Lipidyzer™ Platform
- QE data collection & processing and 1-2 weeks
- Lipidyzer™ Platform data collection & processing less than 1 day

*non-validated matrix

Identified lipids with non-zero values in more than 50% of the samples

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>LC-MS</th>
<th>Lipidyzer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>96</td>
<td>226</td>
</tr>
<tr>
<td>SM</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>SE</td>
<td>1</td>
<td>0</td>
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<tr>
<td>PS</td>
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<td>PI</td>
<td>15</td>
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<tr>
<td>PE</td>
<td>32</td>
<td>30</td>
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<tr>
<td>PC</td>
<td>101</td>
<td>41</td>
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<tr>
<td>LPE</td>
<td>7</td>
<td>5</td>
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<td>LPC</td>
<td>34</td>
<td>17</td>
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<tr>
<td>LdMePE</td>
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<tr>
<td>dMePE</td>
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<td>0</td>
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<tr>
<td>DG</td>
<td>6</td>
<td>12</td>
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<tr>
<td>ChE</td>
<td>9</td>
<td>24</td>
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<tr>
<td>CerG2GNAc1</td>
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<td>0</td>
</tr>
<tr>
<td>CerG1</td>
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<td>0</td>
</tr>
<tr>
<td>Cer</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>FFA</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>TOTAL</td>
<td>351</td>
<td>395</td>
</tr>
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</table>

Professor Mike Synder & Kevin Contrepois, Department of Genetics, Stanford
**Quickest Route to Success**

Significant changes between Young, Old and Calorie Restricted

- Univariate statistical analysis
  - Non-parametric Wilcoxon t test – FDR corrected q value < 0.05
  - Fold change > 1.5

**Lipidyzer™ demonstrated:**

- Fastest route to successful data ready for interpretation
- Larger number of detected species
- Quantitative data on all species detected
- Allows mapping data to biochemical pathways

<table>
<thead>
<tr>
<th>Young</th>
<th>Old</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS (128 lipids)</td>
<td>Lipidyzer (179 lipids)</td>
<td></td>
</tr>
<tr>
<td>LPC(20:4)</td>
<td>PC(18:1/20:4)</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

**Demonstrating the Power of Lipidyzer™ Platform**

- Specificity – Differential Mobility Spectrometry (DMS)
- Eliminating Quantitative Bias – Novel Internal Standards
- Coverage – Over 1100 Molecular Species across 13 lipid classes
- Sensitivity and Precision – Quantitate ~700 species <20% CVs
- Robustness – <10% CVs over 6 days
- Ease of Use – Lipidomics Workflow Manager

- Biological Validation of the Lipidyzer Platform
  - Preeclampsia Pilot Study
  - Hypertriglyceridemia Study
  - Weight Loss Study
  - Aging Pilot Study
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