Welcome

- Ask questions via the Q&A box
- Panelists may share info via the Chat Box
- Special thanks to the Scientific Committee:
  
  Dan Holmes, Cory Bystrom, Patrick Mathias, Shannon Haymond, Randall Julian, Peggi Angel, Livia Eberlin, Chris Anderton, Richard Drake, Kristine Glunde, Reid Groseclose, Jeff Spragins, Adam Rosebrock, Erin Baker, Chris Cox, Clifton Fagerquist, Rebecca Harris, Grace van der Gugten, Deborah French, Jacqueline Hubbard, Chris Shuford, Tim Collier, Mari DeMarco, Andy Hoofnagle, Jennifer Colby, Marilyn Huestis, Hema Ketha, Mark Marzinke, Richard van Breemen
Advances in Mass Spectrometry

John R. Yates
Departments of Molecular Medicine and Neurobiology
Scripps Research
Proteins are the workhorses of the cell
DNA -> mRNA -> Proteins -> Metabolites

• Heteropolymers of amino acids’
  a. “Linear” sequence of amino acids folds into a 3D structure
• Form the Structural and Functional elements of cells and tissues
• Enzymes – catalyze reactions, transmit signals, regulate processes
• Covalent modifications regulate activities and functions
• Protein sequences are derived from genes and genomes are sequenced

A key determinate to understanding protein function is discovering biological roles in a context.
“Shotgun Proteomics”

Protein Mixture

proteolysis

Peptide Mixture

Output Filtering and Re-Assembly

DTASelect

SEQUEST

Beowulf Compute Cluster
Cloud Computing

μLC

MS

MS/MS

Peptide Mixture

Output Filtering and Re-Assembly
High Resolution Separations
Integrated Multi-Dimensional Liquid Chromatography

50-100 micron FSC
5 μM
100-300 nL/min

Single Dimension UHPLC
50-100 micron ID
25-50 cm in Length
Peptide Fragmentation

C-terminal ions

N-terminal ions
(protonated mass 1410.6)

<table>
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<tr>
<th>mass^+</th>
<th>b-ions</th>
<th>y-ions</th>
<th>mass^+</th>
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<td>AFDSIMAETLK</td>
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<td>SPA</td>
<td>FDSIMAETLK</td>
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<td>403.5</td>
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<td>TLK</td>
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<td>1151.3</td>
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<td>LK</td>
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<td>1264.4</td>
<td>SPAFDSIMAETL</td>
<td>K</td>
<td>147.2</td>
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</table>
Genome Sequences Allow Fast and Accurate Lookup of Information

- Product/Price
- Inventory Adjustment
- Order more product

Amino Acid Sequence “Bar Code”

- Amino Acid Sequence
- Protein (Gene)
- Experimental Context
- Functional Context
- High Throughput
Top Down Proteomics Measures Intact Proteoforms

Discover, Characterize & Quantify Proteoforms

Courtesy of Neil Kelleher, NWU
Proteins Do Not Always Faithfully Recapitulate Gene Sequence

- Canonical sequence
- Post-translational modifications
- Endogenous proteolysis
- Alternative splicing/isoforms
- Natural variants, coding SNPs, and mutations

Courtesy of Neil Kelleher, NWU
Alternative splice forms yield different protein molecules and different functions.
What are Proteoforms?

Base Sequences

Site-Specific Features

- Coding SNP
- Glycosylation
- Phosphorylation

Proteoforms

Distinct protein forms arising from a single gene

Times Cited: 391
(from Web of Science Core Collection)

Courtesy of Neil Kelleher, NWU
Carbonic Anhydrase

Native Mode

Zinc 2+

Denaturing Mode

Courtesy of Neil Kelleher, NWU
Top Down by Tandem MS/MS

1. Detection of intact species
2. Isolation
   - m/z
3. Add energy to fragment the isolated species
   - collisions w/ gas
   - electron capture
   - photons (IR, UV)

Courtesy of Neil Kelleher, NWU
Outline

- Advances in Ionization methods
- Advances in Mass Analysis
- Advances in Applications
Advances in ionization
Direct Electrospray Ionization DESI

DESI Catalyzed Many Variations

a) PESI

b) Substrate Spray

c) Spray Desorption

d) DEMI

e) LMJ-SSP

f) nano-DESI

g) Plasma

h) Laser Ablation
Mass Spec Pen using Solvent Assisted Ionization


MALDESI- a combination of MALDI and ESI

“People Spray?”

Kwan-Ming Ng, JASMS, 2014, 25,1515–1520, Direct Analysis of Large Living Organism by Megavolt Electrostatic Ionization Mass Spectrometry
Advances in Mass Analysis

Advances have included:
Mass resolution and accuracy- low ppm is routine
Hybrids- combinations of mass analyzers
Orbitraps- Kingdon trap
Ion mobility mass spectrometry
WE ARE IN AN ERA OF ROUTINE
HIGH RESOLUTION AND MASS ACCURACY

1,000,000 resolution is achievable on new Orbitrap instruments.
Mass Cytometry

1. Cells are introduced
2. Cells are vaporized
3. Small ions filtered
4. Ions above 100 daltons proceed to Time of Flight
5. Ions are measured and counted
Mass Cytometry

Omatsky et al., JAAS 2008 (and others)
Bendall et al., 2011

DVS Sciences Inc.
Data-Independent Acquisition (DIA)

### Protein Identification Using Data Dependent or Independent MS/MS

#### Data-dependant acquisition

<table>
<thead>
<tr>
<th>Rep.</th>
<th># MS/MS spectra</th>
<th># peptide identifications</th>
<th># protein identifications (P2)</th>
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<td>86243</td>
<td>676</td>
<td>193</td>
</tr>
<tr>
<td>2</td>
<td>88619</td>
<td>745</td>
<td>201</td>
</tr>
<tr>
<td>3</td>
<td>86573</td>
<td>677</td>
<td>185</td>
</tr>
<tr>
<td>Avg</td>
<td>87,145</td>
<td>699</td>
<td>193</td>
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</table>

#### Data-independent acquisition

<table>
<thead>
<tr>
<th>Rep.</th>
<th># MS/MS spectra</th>
<th># peptide identifications</th>
<th># protein identifications (P2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112612</td>
<td>784</td>
<td>246</td>
</tr>
<tr>
<td>2</td>
<td>98264</td>
<td>739</td>
<td>213</td>
</tr>
<tr>
<td>3</td>
<td>108873</td>
<td>738</td>
<td>232</td>
</tr>
<tr>
<td>Avg</td>
<td>106,583</td>
<td>753</td>
<td>230 (16.1%)</td>
</tr>
</tbody>
</table>

Q1 Isolation Strategy

CONSIDERING QUANTITATION

Cycle time
= # steps X accum time

MS/MS Accumulation Time

Balance Specificity with Sensitivity

Total mass range = Q1 width x # steps
Sciex Triple TOF

(A) TurboV IS source

(B) Ion Optics

4 channel 40GHz TDC

15 kV Accelerator

Two-stage ion mirror

Produce More Ions

Focus More Ions

Detect More Ions

Resolve More Ions

Capture More Ions

Extend your TurboV™ Series with 1-2 Scans increase ion production by using enhanced gas flow dynamics and optimized heater configuration. Enhance resolution through improved ion focusing, along with efficient and rapid ion transmission to eliminate coronas, and high duty cycle for ion source sensitivity. Gain the benefits of lower intake needle with the dual channel TOF signal for refined TOF processing, while delivering lower bias voltage to improve robustness and lifetime. Qjet provides large capture holding for efficient transfer of ions from source to quadrupole configuration. Detailed real-time analysis uses a 2-stage range and 15 kV acceleration to improve resolution and sensitivity.

400 L/s

300 L/s

400 L/s
Variable Window SWATH® Acquisition

INCREASE SPECIFICITY – DECREASE NOISE

• Adjust Q1 selection window
  maintain roughly constant
  number of peptides to
  maintain specificity
  - Narrower window in m/z dense
    regions
  - Optimal cycle time maintained
    by adjusting accumulation time

• Reduce number of precursors
  in window for increased
  specificity of quantification

Optimized Q1
Windows

100 variable windows
Current optimal method
Available on SCIEX User Community
Industrialized Quantitative Proteomics

MICROFLOW SWATH® ACQUISITION

• Demonstrated throughput enabling ~150 proteomes per week
  − 1 hour total run time for up to 24 samples / day
  − Microflow LC provides higher throughput and robustness needs

• Quantified ~5000 proteins with CV <20%
  − SWATH® Acquisition provides robust high quality quantitation
  − 100 variable Q1 windows
  − High resolution MS/MS
  − Require loading ~4x more protein on column

Ion Mobility Mass Spectrometry

By Jeff Dahl - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=7025112
SLIM SUPER IM-MS

(A) Schematic diagram of the multipass SLIM SUPER IM-MS instrument used in this work; (B) photo of one of the two SLIM module surfaces; and (C) illustration of an ion switch (switch on, ion cycling; switch off, transmit ion to MS).

DOI: 10.1021/acs.analchem.7b00185
Cyclic T-Wave IMS – Q-TOF
timsTOF
trapped ion mobility separation – quadrupole isolation – time of flight mass spectrometer

Trapped Ion Mobility Separation
Fast switching quadrupole mass filter
UHR-TOF (R>50,000)

Courtesy of Bruker
Trapped Ion Mobility Mass Spectrometry

Courtesy of Bruker
Protein Identification on TIMSTOF

Hela standard, TimsTOF

Proteins

Peptides

PSM
Comparison of Proteomic Analyses

Ventral Rat Brain samples, alcohol addiction studies
TMT 6 plex experiment, 3 treated, 3 control
Proteins overlap shown,
8 fractions, 2hr gradients on TimsTOF, 4hr gradients on the Lumos
Half of the same material injected on the TIMSTOF

Lumos (8978 32 hrs)

TIMSTOF (8,974, 16 hrs)

Lumos with FAIMS (11,591 32 hrs)

Courtesy of Luis Navitidad, UTexas Austin
Informatics for Proteomics

• 20+ search programs published
• Several Commercial programs- SEQUEST, MASCOT, PEAKs, Byonic, “Skyline (DIA and Targeted)”
• Most have quantitation modules
• Emerging trends-
  • cloud computing
  • GPUs
ProLuCID GPU search engine

- 200ng HeLa sample
- 90min Gradient
- Total search time: 8 minutes
- Identified Proteins: 5,420
timsTOF Data Analysis Overview
Quantitation of Proteins
Quantitative Proteomics

Cell State 1

Arg/Lys-H

^{15}N

metabolic

label

Chemical tag-D

Proteolytic

Digestion

LC/LC-MS/MS

OR 1D/2D-GE

Cell Lysis

^{15}N/^{14}N combine

Tag combine

Proteolytic

Digestion

Chemical tag-H

Cell Lysis

^{14}N

unlabeled

Arg/Lys

Cell State 2

C. elegans
D. melanogaster
Rats
Mice
Mass spectrometry-based quantification methods

\[ \begin{array}{ccc}
15\text{N} & 18\text{O} & \text{TMT} \\
y & n/z
\end{array} \]
Mass spectrometry-based quantification methods

Methods Mol Biol. 2010;673:211-222
Isobaric Labeling for Quantitative, Multiplexed Proteomics

11-plex

12 x 3 hr = 36 hr
>8,000 proteins quantified across all 11 samples

MS1: Find

MS2: Identify

MS3: Quantify

12 x 3 hr = 36 hr
>8,000 proteins quantified across all 11 samples
3, 16-Plexes to Measure 8 Cell Lines, 2 Conditions, 3 Reps (48 samples)

**SmartTMT w/ Orbiter**

12 x 90 min runs

**16 proteomes in 18 hr = 1.1 hr/proteome**

8,800 proteins / 67 minutes = 131 proteins / min

2.2 proteins / second
Advances in Applications

• Single Cell proteomics

• Glycans

• Top down mass spectrometry

• Advanced OMICS (proteomics)
Single cell proteomics by mass-spec (SCoPE-MS)

- Multiplexed LC-MS/MS for single cells
- Quantify > 1000 proteins / cell
- Automated analysis of > 1000 cells / week per instrument

Opportunities to grow by leaps and bounds

Specht & Slavov, J. of Proteome Research (2018)
Budnik et al Genome Biology (in press)
Strategy for assessing site occupancy and proportion of high mannose and complex type glycosylation

Liwei Cao et al., *Nat. Commun.*, 2017
Top-Down Proteomics of KRAS4b from DLD-1 Colorectal Cancer Cell Lines

Doubling time:
- DLD-1 PAR KRAS (WT/G13D): ~24 hours
- DLD-1 MUT KRAS (-/G13D): ~24 hours
- DLD-1 WT KRAS (WT/-): ~48 hours (2x slower)


Courtesy of Neil Kelleher, NWU
Can cTnI Phosphorylation Be Related to Heart Diseases?

Proteoforms in Heart Disease

G1 Normal Heart (NOR)
G2 Mild hypertrophy (HYP)
G3 Severe hypertrophy/dilation (SHD)
G4 Congestive heart failure (CHF)

Courtesy of Ying Ge, UWisc
Dynamic System-Wide Molecular Response to Acute Exercise in Insulin Sensitive and Resistant Individuals

Kévin Contrapois1,#, Kegan J Moneghetti2,3,4,5,#, Si Wu1,#, Daniel Hornburg1,#, Sara Anadi1,#, Ming-Shian Tsai1,#, Eric Wei1, Brittany Lee-McMullen1, Jennifer Quintela1, Songic Chen1, Jeffrey W. Christie2,3,6, Brunilda Balliu6, Shalina Taylor7, Matthew Durant1, David A Knowles1,8, Melanie Ashland1, Amir Bahmani1, Brooke Enslen1, Myriam Amsallem2,3, Yukari Kobayashi2,3, Monika Avina1, Dalia Perelman1, Sophia Miriam Schüessler-Florenza Rose1,9,10, Wenyu Zhou1, Euan A Ashley1,3,11, Stephen B Montgomery1,6, Hassan Chaib1, Francois Haddad2,3,12* and Michael P. Snyder1,12*

#These authors contributed equally, co-first authors.
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10Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA
11Center for Inherited Disease, Stanford University, Stanford, CA, USA
ABSTRACT

Exercise testing is routinely used in clinical practice to assess fitness - a strong predictor of survival - as well as causes of exercise limitations. While these studies often focus on cardiopulmonary response and selected molecular pathways, the dynamic system-wide molecular response to exercise has not been fully characterized. An integrative analysis revealed an orchestrated choreography of biological processes across key tissues. Most of these processes were dampened in insulin resistant participants. We discovered biological pathways involved in exercise capacity and developed prediction models revealing potential resting blood-based biomarkers of fitness. Finally, we identified individuals with outlier molecular profiles and show the potential use of this approach to detect subclinical conditions.
Summary

• It’s possible to do quantitative comparisons between states.

• Multiplexing improves throughput making possible larger studies in blood or urine that require more up-front fractionation.

• Mass spectrometers are evolving faster than we can raise money to acquire.
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• Rohan Thakur Bruker- TIMSTOF
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• Neil Kelleher Northwestern U- Top down
• Ying Ge - University of Wisconsin
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THE PARTNERSHIP FOR CLEAN COMPETITION (PCC) FUNDS GRANTS, MICRO-GRANTS, AND FELLOWSHIPS.

WORLDWIDE FUNDING

DECISIONS IN 4-5 MONTHS
*Micro-grants in days

AWARDS FROM $10K - $1M (USD)

PRE-APPLICATION DEADLINES:
MARCH 1
JULY 1
NOVEMBER 1

FULL APPLICATIONS DUE THE FOLLOWING MONTH:
APRIL 1, AUGUST 1, OR DECEMBER 1

/PCCANTIDOPING
CLEANCOMPETITION.ORG
Both Phos and Protein Measurements!

Courtesy of Steve Gygi HMS