SureQuant: Novel Turnkey Targeted Toolbox for Routine Quantitative Pathway Proteomics

Aaron Gajadhar, PhD
Strategic Marketing Scientist – Quantitative Proteomics
Fundamental Challenge of Biology is to Reliably Quantify Protein Components

Classical, mechanism-focused protein analysis

Systematic proteomic characterization

Need to know how the proteome profile is changing: Time, disease, stimulus, environment, …
The Time HAS Come for Pathway-Driven Proteomics….

Tymora Developing Extracellular Vesicle-Based Phosphoproteomic Liquid Biopsies

Early Results From Second Side-Out Study Indicate Benefits of Molecularly Guided Cancer Treatment

MSK Researchers Develop High-Sensitivity Mass Spec Assay for Clinical Cancer Work

Molecular Cell

A Strategy to Combine Sample Multiplexing with Targeted Proteomics Assays for High-Throughput Protein Signature Characterization

...profile tumor signaling pathways via blood or urine samples, similar to how liquid biopsies make use of ctDNA analysis...

...number of research groups are exploring the use of phosphoproteomics analysis to aid selection of patient treatments and track their response to therapy.

...using molecular data to guide patient treatment extends progression-free survival in metastatic breast cancer patients.

...bolster the case for phosphoproteomic data specifically, as this was the primary type of data used for guiding therapy.

... We are working on clinical tests to measure signaling in cancer specimens as part of diagnostic clinical tests....

...more quantitative measurements than antibody assays and the ability to profile pathways more broadly, covering molecules for which there are not good antibodies.

... We targeted 131 peptides corresponding to 69 cancer-related proteins

... We utilized TOMAHAQ to assay expression of 69 protein targets across the entire NCI-60

... BAZ1B, also a tyrosine-kinase….expression was shown to correlate with drug response sensitivity....
Quantitative LC-MS for Pathway Proteomics - Promises & Pitfalls

- **Extreme sensitivity and inherent specificity**
- **Quantitate 100s - 1000s of proteins**
- **Identify PTMs and protein interactions**
- **Compatible with enrichment and isolation methods**
- **Detect protein isoforms and modifications**

- **Dynamic range of target abundance**
- **Quantitative reproducibility 'Missing values’**
- **Tradeoffs for discovery vs. targeted workflows**
- **Tedious, time-consuming assay development**
- **Lack of truly integrated and standardized workflows**
SureQuant Targeted Mass Spec Assay Kits

Validated, modular reagents for multiplexed target protein quantitation

Thermo Scientific™ SureQuant™ Mass Spec Assay Kits

**Highlights**

- **Complete**—includes all reagents for successful monitoring of MS system performance, sample preparation, and absolute quantitation of target proteins/peptides
- **Verified**—antibodies, peptides, and control lysate are rigorously tested for specificity and successful quantitation of each target peptide
- **Multiplex**—able to quantitate different targets (including isoforms and phosphorylated states) from the AKT/mTOR pathway
- **Flexible**—modular format allows for immune-enrichment only, or in combination with relative or absolute quantitation

ThermoFisher

Prepare Cell or Tissue Lysate

Multiplex IP AKT Total or Phospho Pathway Proteins

Add Internal Standards (30 heavy peptides)

MS Sample Prep

Directed Discovery MS Analysis (DDA)

System Suitability Standard (7 x 5 Mix)

Run Calibration Curve (PRM)

Run Unknown Samples by targeted PRM MS Analysis

Skyline Data Analysis
The Next Leap Towards Turnkey Pathway Quantitation

Two new ‘data aware’ intelligence-driven systems to meet the evolving needs of the protein mass spectrometry community

Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer

Robust workhorse for proteome analysis and quantitation

Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ Mass Spectrometer

Highest performance, flexibility, usability for protein structure and quantitation studies
Thermo Scientific™ SureQuant™ IS Targeted Protein Quantitation Solution

Fills the gap for large-scale target profiling with superior quantitative performance

Discovery

Data Dependent Acquisition (DDA)

High Resolution DIA (HR-DIA)

TMT Quantitation

Identification

Quantitative performance (accuracy, precision, dynamic range, reproducibility)

Targeted

For Scale

SRM/PRM

For Quality

SRM/PRM

# of targets

1000s

10

500

100

7

10

ThermoFisher

SCIENTIFIC
Introducing **SureQuant** Targeted Protein Quantitation

Intelligent Data Acquisition Strategy for Sensitive, Precise and Reproducible Quantitation

**Synthetic Heavy Peptides**

**Sample**

<table>
<thead>
<tr>
<th>#1</th>
<th>#2</th>
<th>#n</th>
<th>Mix</th>
<th><strong>LC-MS Analysis</strong></th>
</tr>
</thead>
</table>

**SureQuant method**

1. **MS1 Survey Scan**
   - (Precursor m/z, intensity threshold)

2. **ENDO Peptides**

3. **IS peptides (trigger)**

4. **Pseudo Spectral Matching**
   - Fast, Low Res MS2 of Trigger
   - MS/MS IS
   - (≥ Min. number fragment m/z)

5. **‘QUANT MODE’**
   - High Quality MS2 of Target

---

ThermoFisher SCIENTIFIC
Overall SureQuant Acquisition Workflow – **Embedded Application Specific Kits**

1. **Survey Run**
   - Ready-to-use template
   - Internal standards in matrix
   - LC-MS Analysis
     - Directed DDA
     - *Inclusion list optimal IS precursor ions*
   - Survey Run Analysis
     - Kit specific Skyline analysis template
     - Export reports
     - *Intensity response of precursor ion*
     - ‘Optimal’ fragments / selected peptide precursor

2. **SureQuant**
   - Ready-to-use template
   - Internal standards
   - Sample
   - Import parameters
   - LC-MS Analysis
     - SureQuant
   - Target Quantification
     - Kit specific Skyline analysis template
SureQuant Workflow – Validated Method Templates

Optimized Methods Enable Focus on Science not Set-Up

- Ready-to-use templates validated for application specific SureQuant peptide kits
- SureQuant available on all TNG Orbitrap instruments with Tune v3.3 or higher
SureQuant Acquisition Drives Enhanced Productivity by Intelligent Detection of Targets

**Parameters / conditions**

- **PRM**
  - 15k / 20 ms
  - 2.5-min monitoring window

- **SureQuant**
  - Watch mode: 7.5k / 10 ms
  - Quant mode: 60k / 116 ms
  - 10-ppm tolerance MS1 (w/ lock mass)

- **Sample**
  - 250 ng cell line digest
  - 50 fmol internal standards

**Proportion MS2 productive scans (% MS2 acquisition time)**

- **PRM** = 10-15 %
- **SureQuant** ≈ 80-90 %
SureQuant’s Intelligent Acquisition Robustness Overcomes Analytical Fluctuations

- SureQuant target detection occurs independent of RT scheduling or poor chromatography conditions allowing reliable and robust target quantification.

Original elution profile

5 min delayed elution profile

Expected elution window

Delayed Elution

Heavy Peptide Detection

PRM

SureQuant
Application Example #1 - AKT/mTOR pathway analysis

- Many genetic alterations in cancer cells modify the protein expression from AKT, RAS and TP53 pathways.
- Quantitative measurement of alterations in the expression of pathway proteins and post-translational modifications (PTM) is necessary for classifying disease states, monitoring cancer progression and determining treatment response.
- SureQuant analysis of the AKT/mTOR pathway (12 protein/30 peptide targets)
- Capillary flow LC-MS setup (1.2 uL/min)
- Detection of targets without IP enrichment?

AKT/mTOR protein pathway

Targeted MS Assay
SureQuant Allows Sensitive Detection of AKT/mTOR Proteins Without IP Enrichment

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide</th>
<th>PRM 15K-20ms</th>
<th>SureQuant 60K-116ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSKβ</td>
<td>TPEIAIA[L]C(Carbamidomethyl)SR</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>AKT2</td>
<td>LPFYQNDHER</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>IGF1R</td>
<td>AENGPGP[V]LVR</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>PTEN</td>
<td>YNL<a href="Carbamidomethyl">C</a>AER</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>GSKβ</td>
<td>L<a href="Carbamidomethyl">C</a>DSGE[ALVA]K</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>GSKβ</td>
<td>LLEYTP[AR]</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>GSK3α</td>
<td>VTTYVA[T]LQGQPER</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>AKTS1</td>
<td>GALAAAR</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>AKT1</td>
<td>NDGTFIGYK</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>AKT2</td>
<td>SGDSFIGYK</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>IGF1R</td>
<td>IDIHSC(Carbamidomethyl)[N]HEAEK</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>PTEN</td>
<td>AQEALDFYG[ER]</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>MTOR</td>
<td>LF[AD]APEALPSR</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>MTOR</td>
<td>TLQ[DS]PELR</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>AKT1</td>
<td>YSFQTHDR</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>K56B1</td>
<td>FEIES[SV]NR</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>TSC2</td>
<td>SNPTDIYPSK</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>GSK3α</td>
<td>SQE[AYTDIK</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>TSC2</td>
<td>GQ[EGPLP]SSS(PR)</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>AKTS1</td>
<td>SDE[ENGPP]SSPDLDR</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>IGF1R</td>
<td>T[IN]E[N]YR</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>MTOR</td>
<td>ETSF[NGAYGR]</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>MTOR</td>
<td>GNNLQ[DT]LR</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>TSC2</td>
<td>GY[IT]DSAPSR</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>PTEN</td>
<td>NNIDI[DV]R</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>K56B1</td>
<td>DG[YP]APD[FR]</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>AKT3</td>
<td>LVPPF[K]QP[VT]SET[D]TR</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IRS1</td>
<td>SV[SAPQ][QINI[PR]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AKT3</td>
<td>TF[HV][D]P[EE]R</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IRS1</td>
<td>TGI[AA][E][V][SL]PR</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of peptides detected: 11 26

Number of spectra including ≥ 5 ref. fragments

- 0
- 1-2
- ≥ 3

≤LOD  Detection >>> Quantification

30 min Gradient Method

SQE[AYTDIK]

SSDE[ENGPP]SSPDLDR

Number of spectra including ≥ 5 ref. fragments

- 0
- 1-2
- ≥ 3

≤LOD  Detection >>> Quantification
• The entire pathway (12 proteins/30 peptides) was quantified with a **10 min gradient** (n=3 replicates)
Customer Application Example - Global pTyr Profiling In Human Tumor Specimens

**Tyrosine phosphorylation analysis of tumors uncovers druggable targets**

**SureQuant triggered global pTyr profiling in colon tumor specimens**

**Tyrosine kinases**
- 0.3% of genome
- 30% of oncoproteins

For research use only
SureQuant pTyr Signaling Analysis Offers Reproducible and Complementary Insights

**Reproducible target quantitation**

![Graph showing reproducible target quantitation](image)

**Signaling heterogeneity uncovers biological insight not available from genetic subtyping**

![Heatmap showing signaling heterogeneity](image)

**Activation of actionable targets can be monitored across colon tumors**

![Graph showing EGFR pY1197](image)
SureQuant Large-Scale Target Profiling with Superior Quantitative Performance

**SureQuant**
- up to 500 pairs of H/L targets/30 min
- *Highest targeting efficiency*
- *Complete quant profile every analysis*
- *Maintain sensitivity AND target scale*

**Value Prop**
- *Favoring sensitivity*
  - up to 70-80 pairs of H/L targets in 30 min (60k – 116ms)
- *Favoring target number*
  - up to 400-450 pairs of H/L targets in 30 min (7.5k – 10ms)

<table>
<thead>
<tr>
<th>Quantification Performance (Precision/Accuracy)</th>
<th>SureQuant</th>
<th>PRM/SRM</th>
<th>PRM/SRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (LLOQ)</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
</tr>
<tr>
<td>Scale (# Targets)</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
</tr>
<tr>
<td>Efficiency (Productive Scans)</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
</tr>
<tr>
<td>Load-and-play (Minimal adjustment)</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
<td>★★★★☆☆☆☆</td>
</tr>
</tbody>
</table>
Acknowledgements

70,000 Thermo Fisher employees in 50+ countries supporting the global business of science

Applications Team
- Sebastien Gallien
- Bhavin Patel
- Khatereh Motamed
- Daniel Lopez-Ferrer
- Andreas Huhmer

Product Management Team
- Tabiwang Arrey
- Christian Thoeing
- Kerstin Strupat
- Markus Kellmann
- Alexander Harder
- Derek Bailey
- Graeme McAlister
- Romain Huguet
- Shannon Eliuk
- Vlad Zabrouskov

Marketing Team
- Julian Saba
- Sally Webb
- Maciej Bromirski
- Scott Peterman
- Lisa Thomas
- Ken Miller