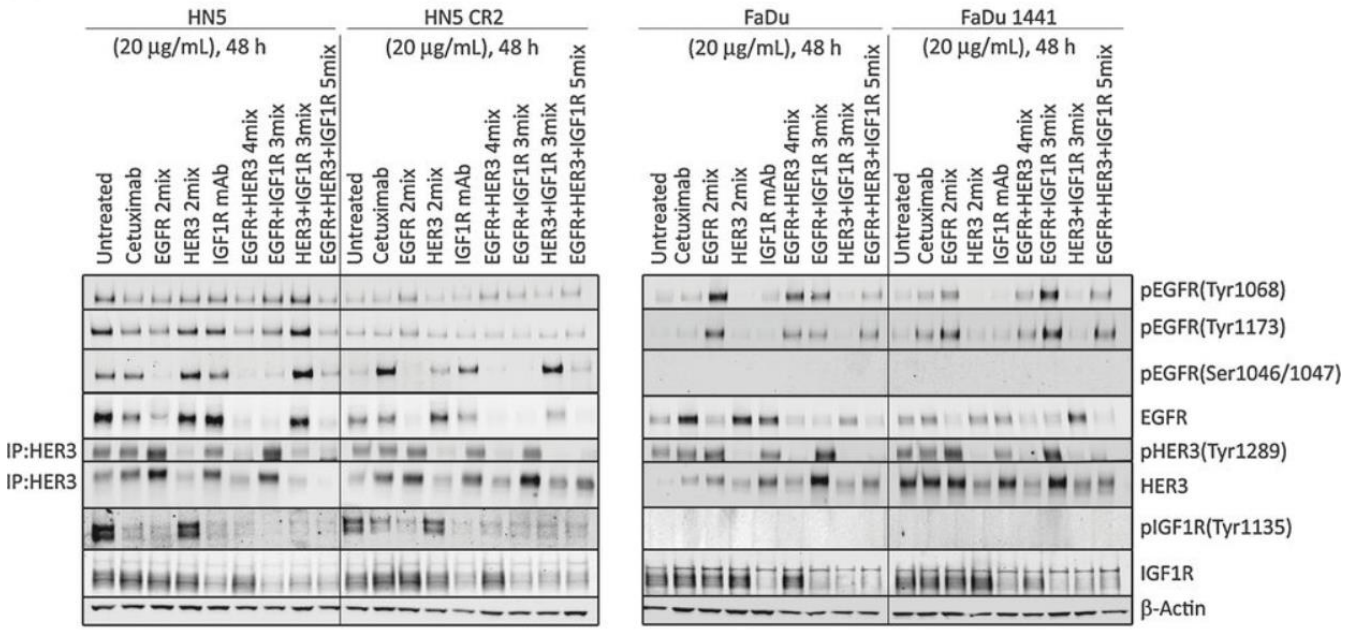
SureQuant: *Novel Turnkey Targeted Toolbox for Routine Quantitative Pathway Proteomics*

Aaron Gajadhar, PhD
Strategic Marketing Scientist – Quantitative Proteomics

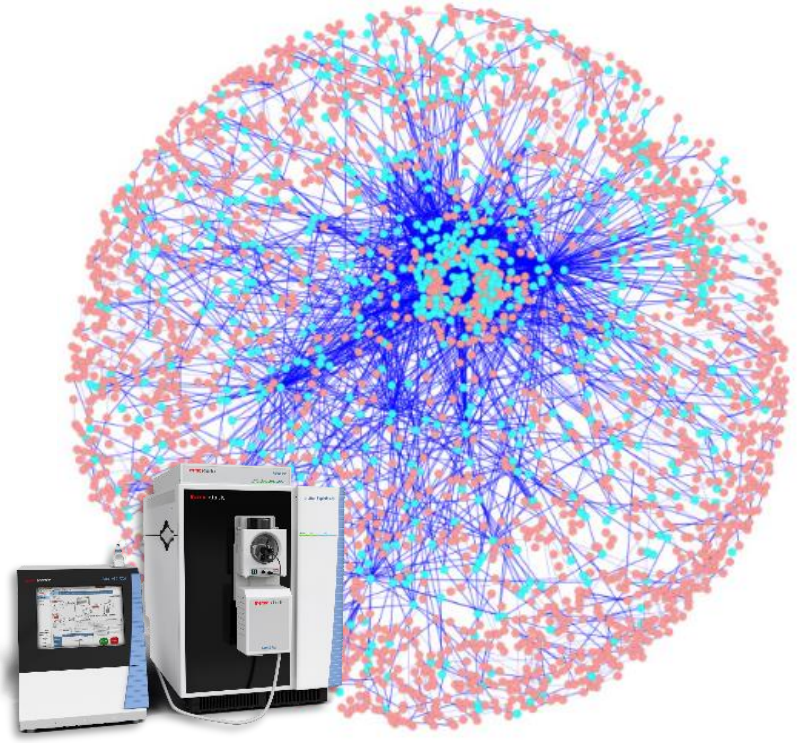
Fundamental Challenge of Biology is to Reliably Quantify Protein Components

Classical, mechanism-focused protein analysis



Mol Cancer Ther. 2016 Jul;15(7)

Systematic proteomic characterization



BMC Syst Biol. 2011 Aug 5;5:122

Need to know how the proteome profile is changing: Time, disease, stimulus, environment, …

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The Time *HAS* Come for Pathway-Driven Proteomics….

genomeweb

Tymora Developing Extracellular Vesicle-Based Phosphoproteomic Liquid Biopsies

- "…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."

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

- "….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."

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

- " 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."

Technology

Molecular Cell

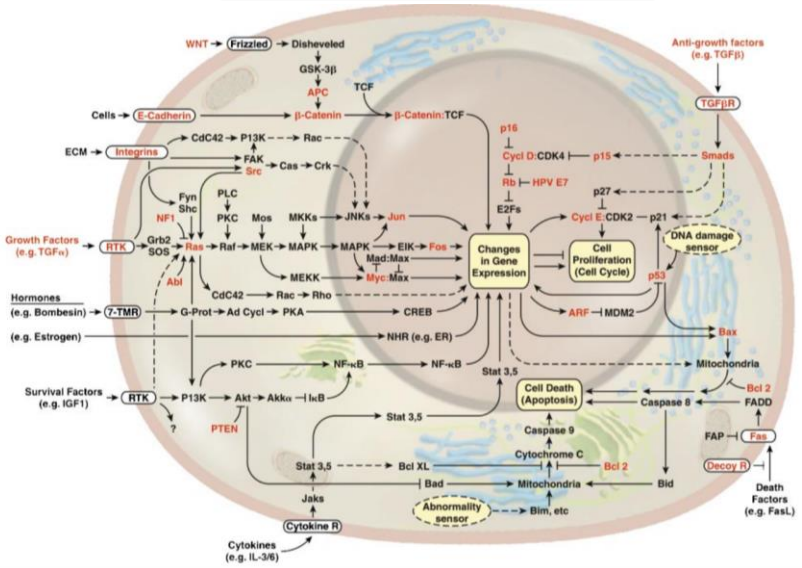
A Strategy to Combine Sample Multiplexing with Targeted Proteomics Assays for High-Throughput Protein Signature Characterization

- " 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**

LC-MS Pathway Proteomics



Cell. 2000 Jan 7;100

- **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**

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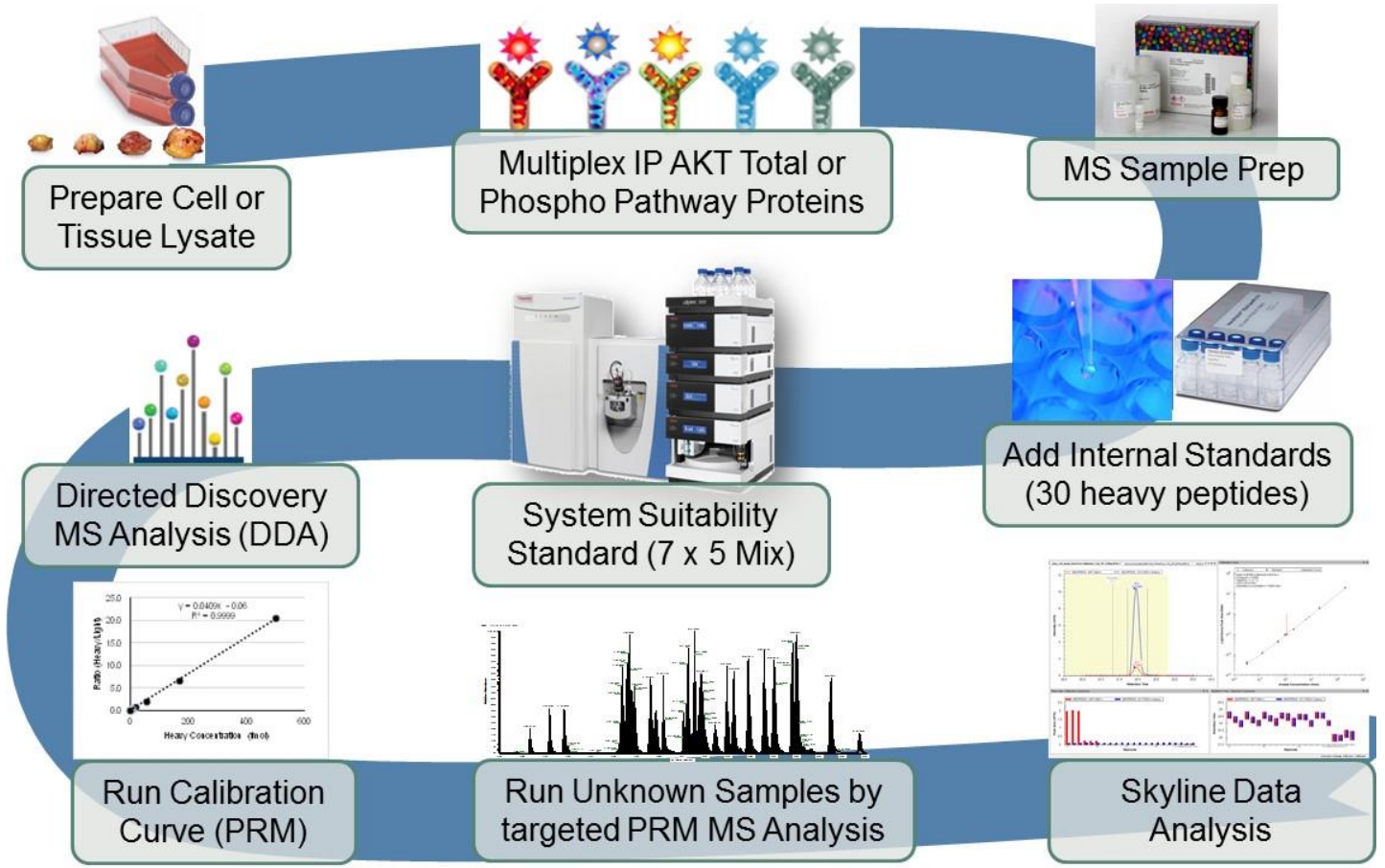
SureQuant Targeted Mass Spec Assay Kits

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



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

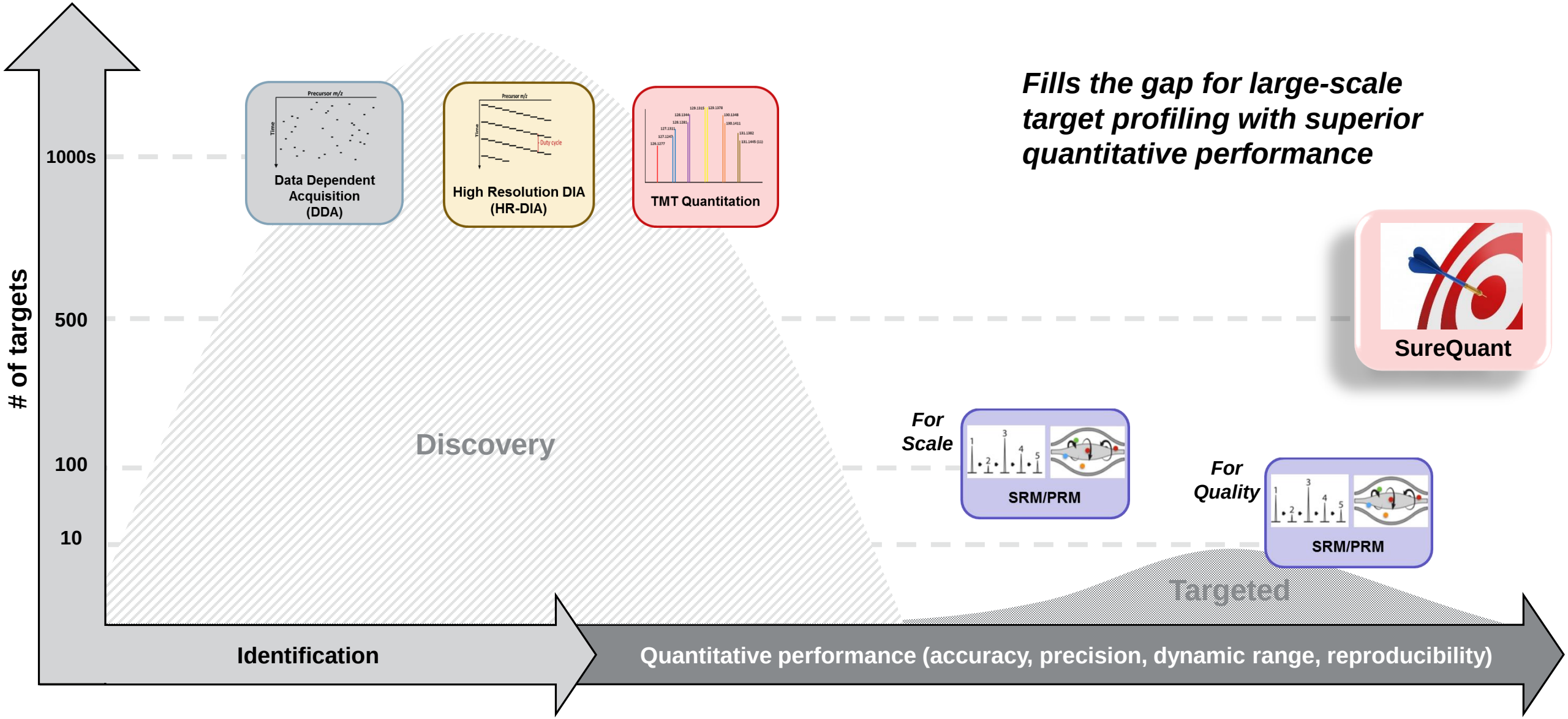
Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer

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



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Introducing **SureQuant** Targeted Protein Quantitation

Intelligent Data Acquisition Strategy for Sensitive, Precise and Reproducible Quantitation

Synthetic Heavy Peptides

#1
#2
....
#n

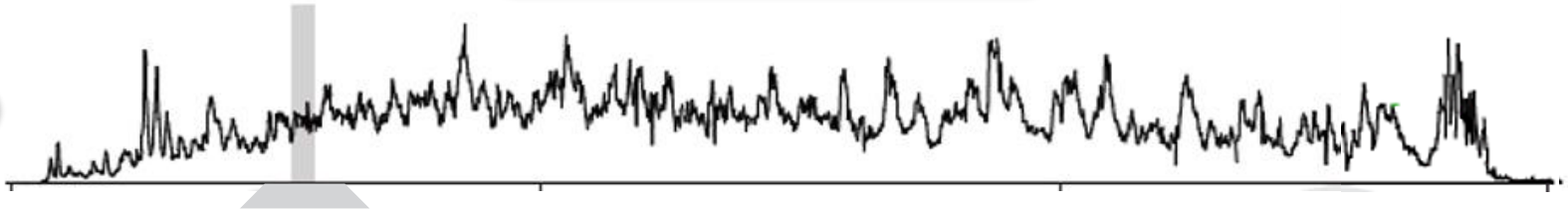
Mix

Sample

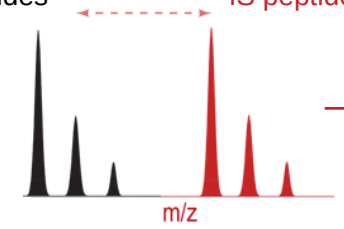
LC-MS Analysis

SureQuant method

1



2 ENDO Peptides — IS peptides (trigger)



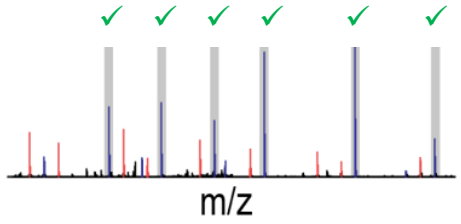
MS1 Survey Scan
(Precursor m/z, intensity threshold)

3

'WATCH MODE'
Fast, Low Res MS2
of Trigger

Pseudo Spectral Matching

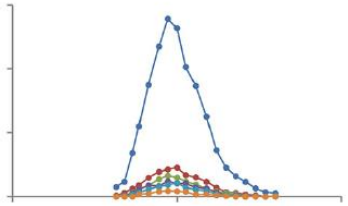
4



MS/MS IS
(≥ Min. number fragment m/z)

5

'QUANT MODE'
High Quality MS2
of Target



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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

Skyline

Kit specific Skyline analysis template

Export reports

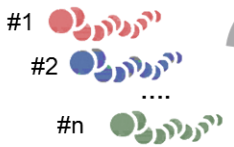
Targeted Mass — *Intensity response of precursor ion*

Targeted Mass Trigger — *'Optimal' fragments / selected peptide precursor*

2 SureQuant

Ready-to-use template

Internal standards



Sample

Import parameters

LC-MS Analysis

SureQuant

Target Quantification

Skyline

Kit specific Skyline analysis template

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SureQuant Workflow – Validated Method Templates

Optimized Methods Enable Focus on Science not Set-Up

System Template

SureQuant

Kit Specific method

SureQuant AKT/mTOR

SureQuant MultiPathway Phosphorylation

SureQuant PQ500 (Biognosys)

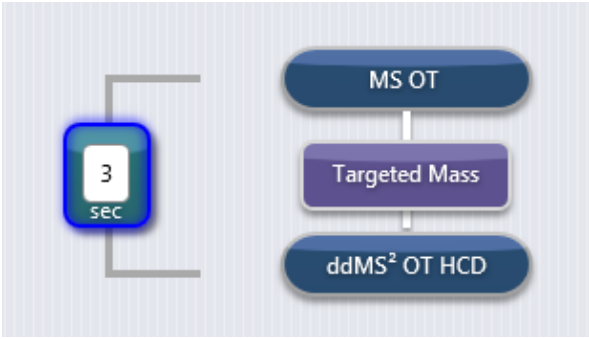
Custom panel

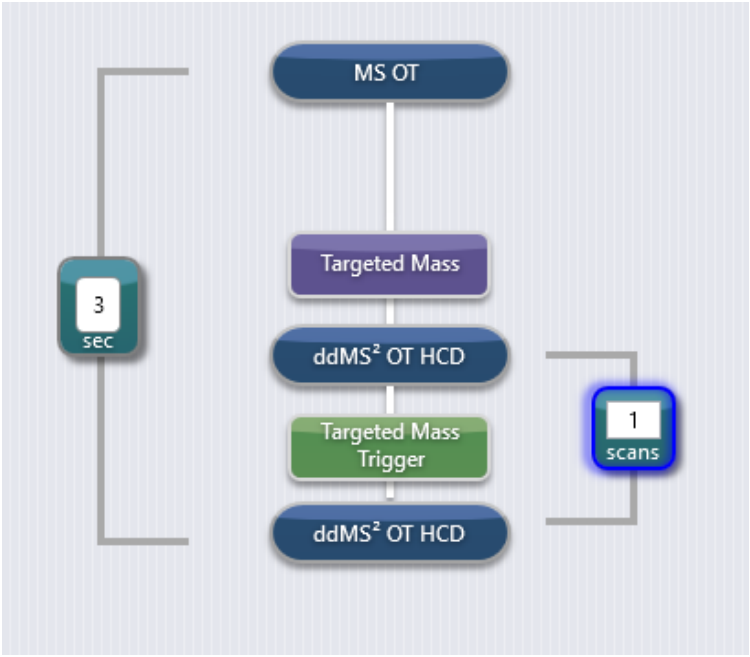
Generic template

SureQuant Custom Panel

Survey Run

SureQuant





- Ready-to-use templates validated for application specific SureQuant peptide kits
- SureQuant available on all TNG Orbitrap instruments with Tune v3.3 or higher

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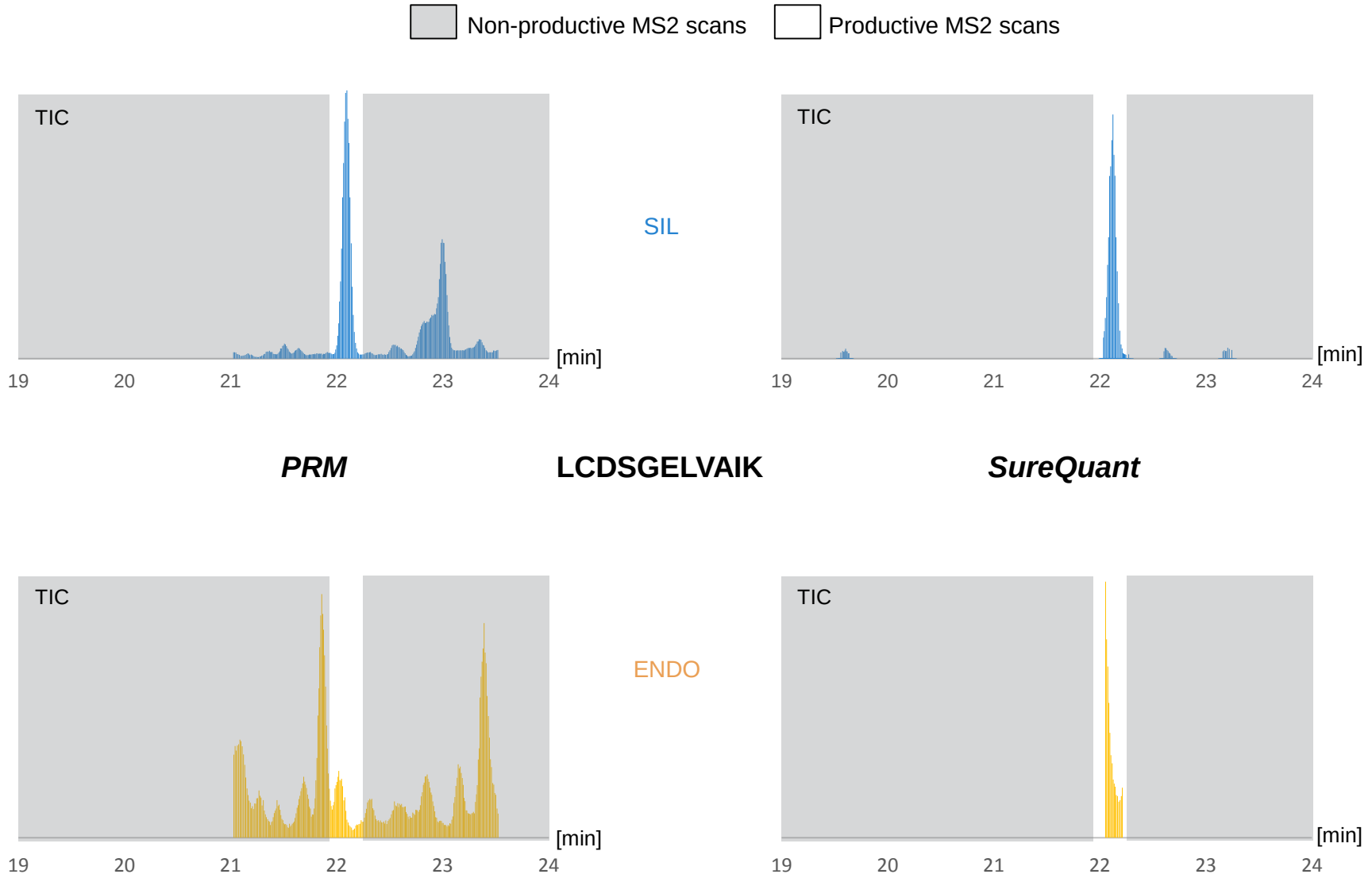
SureQuant Acquisition Drives Enhanced Productivity by Intelligent Detection of Targets



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 %

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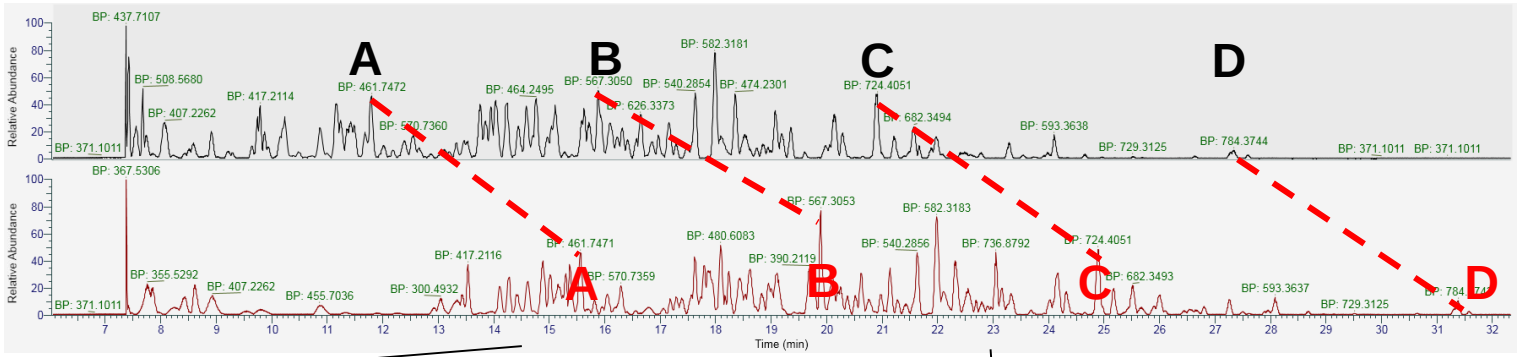
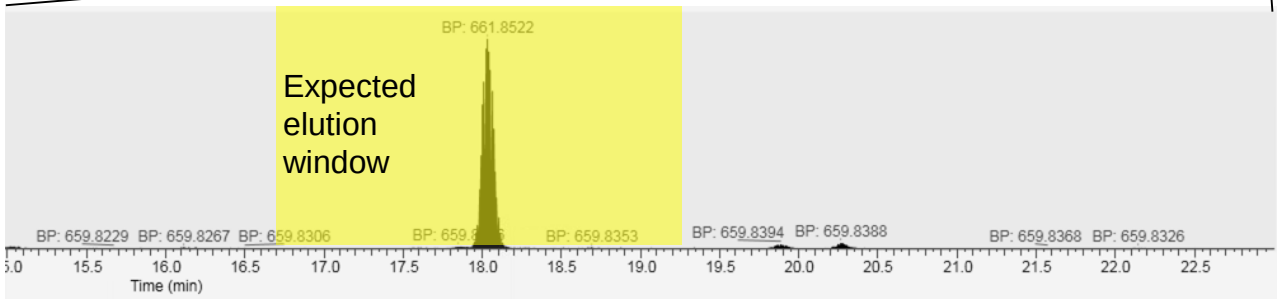
SureQuant's Intelligent Acquisition Robustness Overcomes Analytical Fluctuations

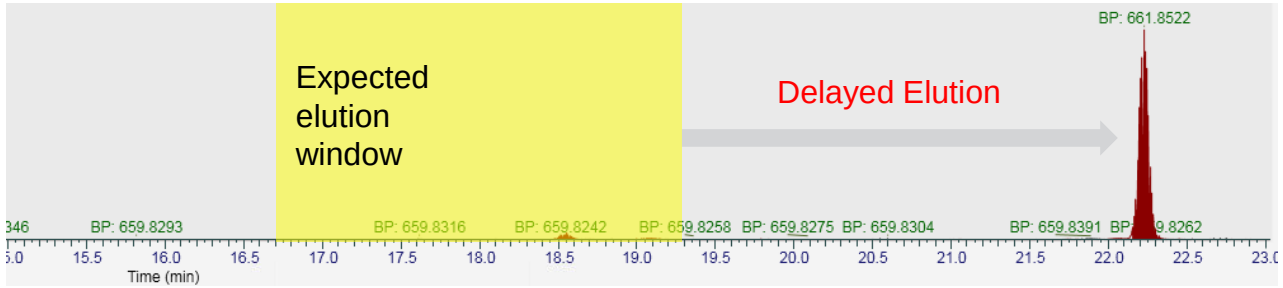
- SureQuant target detection occurs independent of RT scheduling or poor chromatography conditions allowing reliable and robust target quantification

Original elution profile

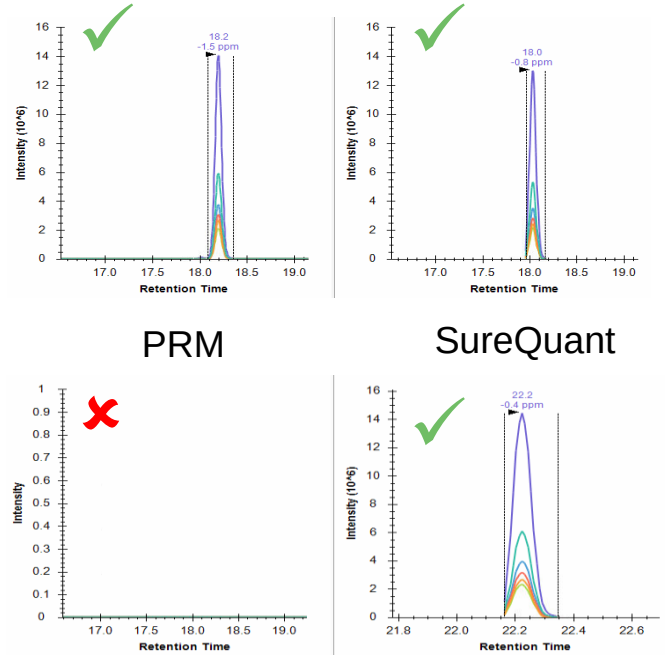
5 min delayed elution profile







Heavy Peptide Detection



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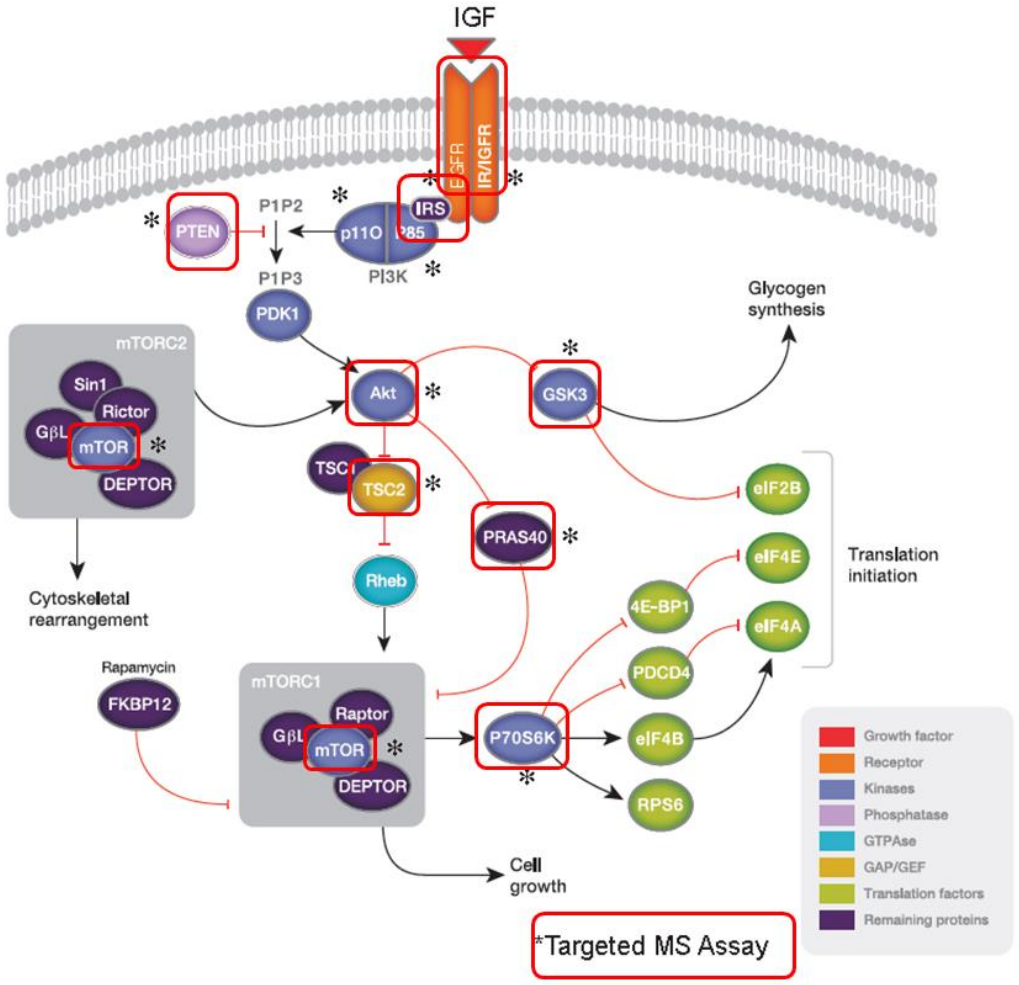
Application Example #1 - AKT/mTOR pathway analysis

- 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



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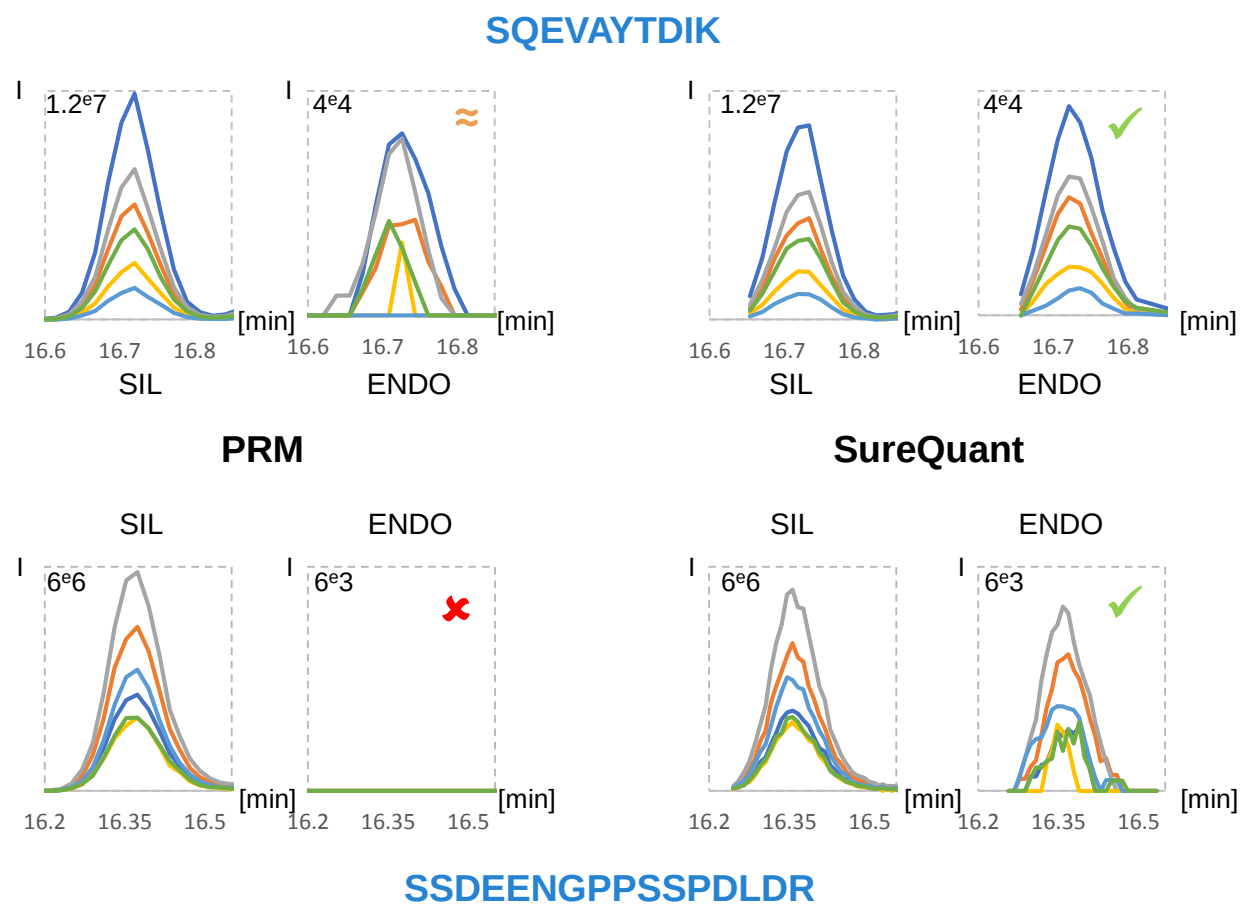
SureQuant Allows Sensitive Detection of AKT/mTOR Proteins Without IP Enrichment

Protein	Peptide	PRM 15K-20ms	SureQuant 60K-116ms
GSK3β	TPPEAIALC[Carbamidomethyl]SR	1	20
AKT2	LPFYNQDHER	0	17
IGF1R	AENGPGPGVLVLR	1	15
PTEN	IYNLC[Carbamidomethyl]AER	0	15
GSK3β	LC[Carbamidomethyl]DSGELVAIK	5	15
GSK3β	LLEYTPTAR	0	15
GSK3α	VTTVVATLGQGPER	6	15
AKTS1	GALAEAAR	5	13
AKT1	NDGTFIGYK	0	13
AKT2	SDGSFIGYK	0	13
IGF1R	IDIHSC[Carbamidomethyl]NHEAEK	1	12
PTEN	AQEALDFYGEVR	10	11
MTOR	LFDAPEAPLPSR	11	11
MTOR	TLDQSPELR	2	10
AKT1	YSFQTHDR	0	10
KS6B1	FEISETSVNR	0	9
TSC2	SNPTDIYPSK	0	9
GSK3α	SQEVAYTDIK	1	9
TSC2	GQPEGPLPSSSPR	0	8
AKTS1	SSDEENGPPSSPDLDR	0	8
IGF1R	TTINNEYNYR	0	7
MTOR	ETSFNQAYGR	2	6
MTOR	GNNLQDTLR	0	6
TSC2	GYTISDSAPSR	0	5
PTEN	NNIDDVVR	0	4
KS6B1	DGFYPAPDFR	0	1
AKT3	LVPPFKPQVTSETDTR	0	0
IRS1	SVSAPQQIINPIR	0	0
AKT3	TFHVDTPEER	0	0
IRS1	TGIAAEEVSLPR	0	0
Number of peptides detected:		11	26

Number of spectra including ≥ 5 ref. fragments

0 ≤LOD | 1-2 | ≥ 3 Detection >>> Quantification

30 min Gradient Method



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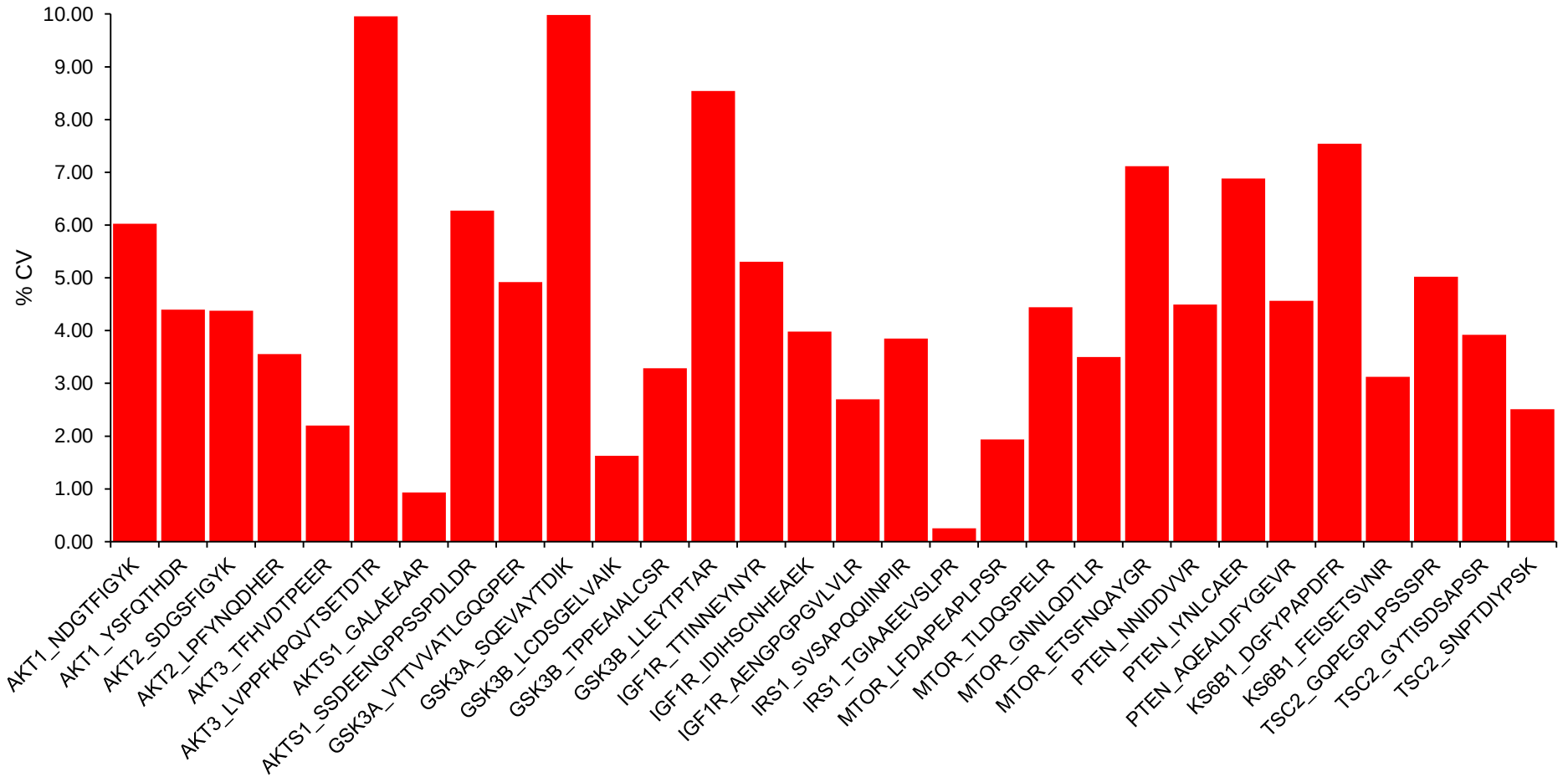
Rapid Pathway Analysis – Sufficient Peak Sampling Ensures Precise Quantitation

- The entire pathway (12 proteins/30 peptides) was quantified with a ***10 min gradient*** (n=3 replicates)



100%

Peptides quantified <10% CV

67%

Peptides quantified <5% CV

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Customer Application Example - Global pTyr Profiling In Human Tumor Specimens

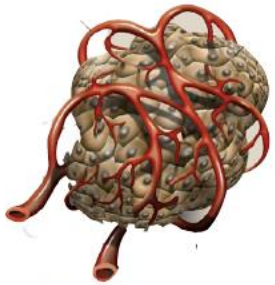
Collaboration with Forest White Lab, MIT

Tyrosine phosphorylation analysis of tumors uncovers druggable targets

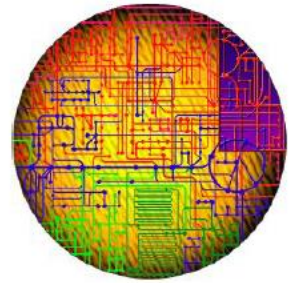
Tyrosine kinases

0.3% of genome

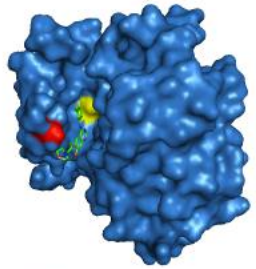
30% of oncoproteins



Human tumor



Signaling targets



Therapeutics

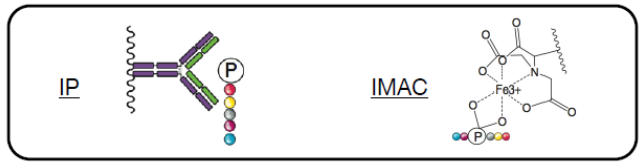
SureQuant triggered global pTyr profiling in colon tumor specimens

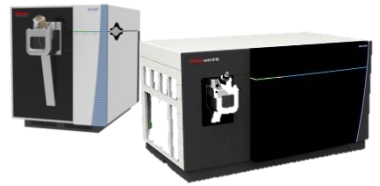
385 pTyr heavy peptide standards

Mix

Tumor specimen

pY peptide enrichment





SureQuant Method

For research use only

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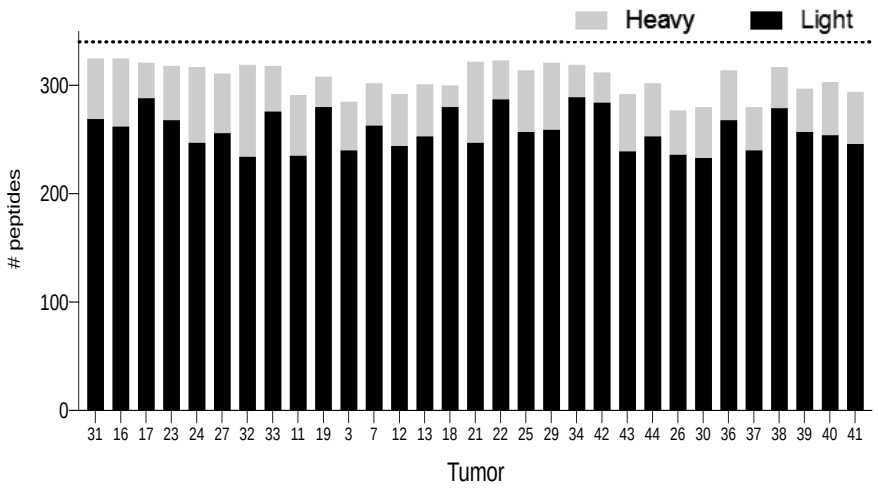
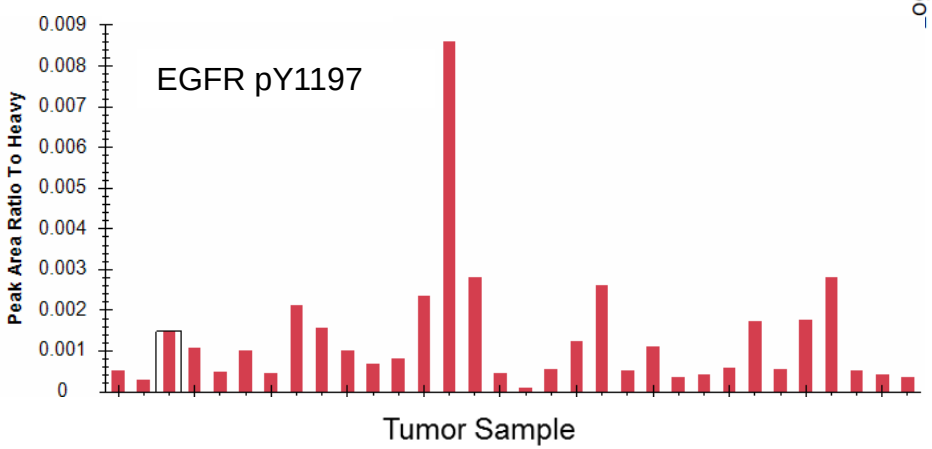
SureQuant pTyr Signaling Analysis Offers Reproducible and Complementary Insights

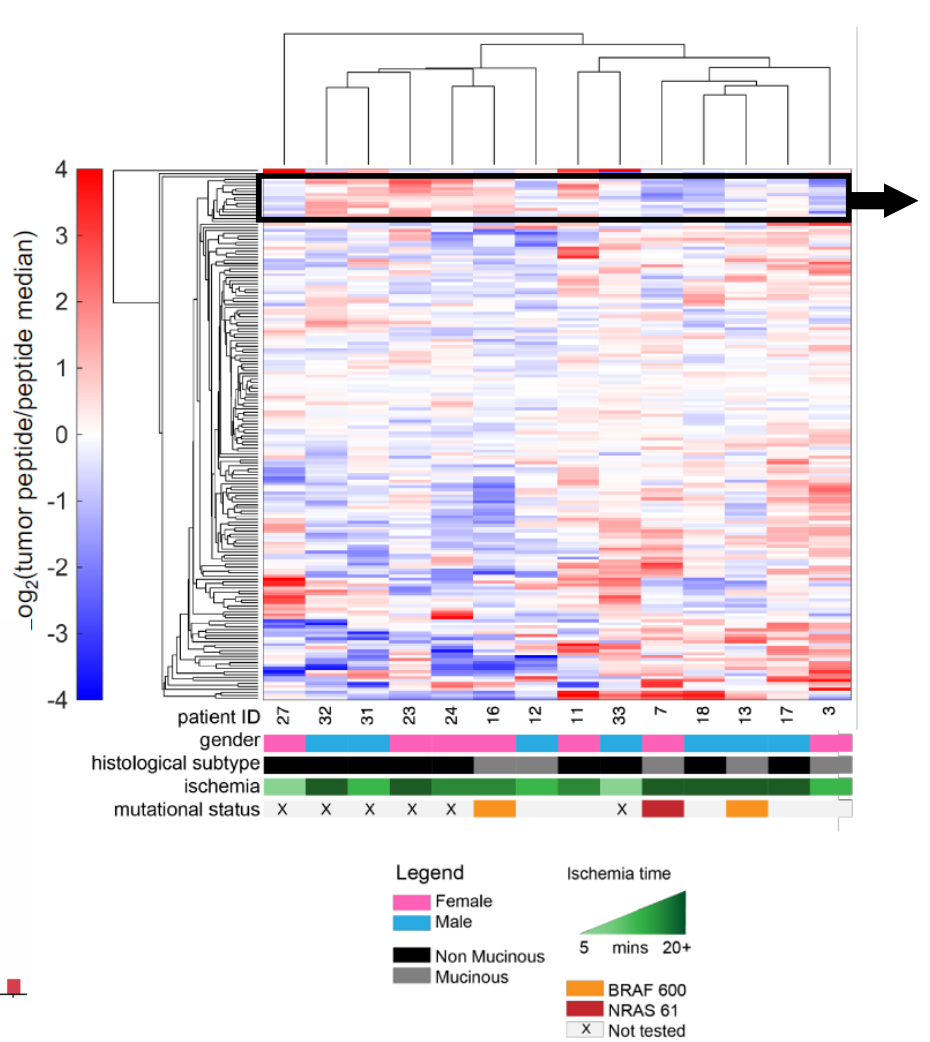
Reproducible target quantitation

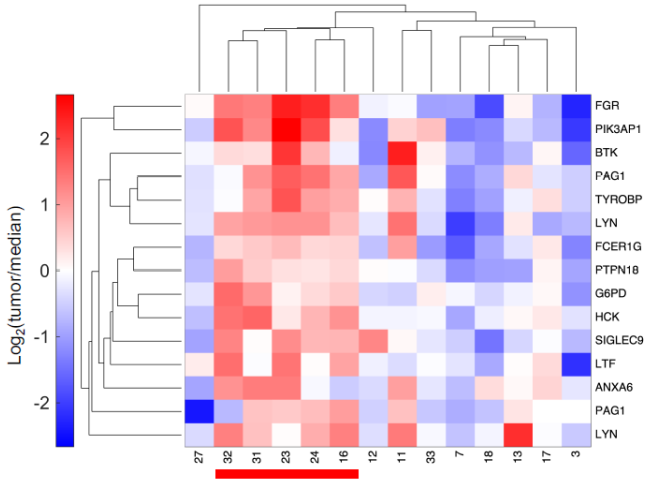


Activation of actionable targets can be monitored across colon tumors

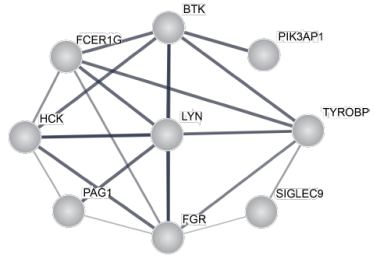


Signaling heterogeneity uncovers biological insight not available from genetic subtyping

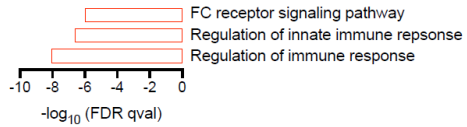




Network of immune response related peptides



GO Biological Process Analysis



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SureQuant Large-Scale Target Profiling with Superior Quantitative Performance

	SureQuant	PRM/SRM	PRM/SRM
	up to 500 pairs of H/L targets/30 min		
Value Prop	*Highest targeting efficiency *Complete quan profile every analysis *Maintain sensitivity *AND* target scale	*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)
Quantification Performance (Precision/Accuracy)	★★★★	★★★★	★★☆☆
Sensitivity (LLOQ)	★★★★	★★★★	★☆☆☆
Scale (# Targets)	★★★★	★☆☆☆	★★★★
Efficiency (Productive Scans)	★★★☆	★☆☆☆	★☆☆☆
Load-and-play (Minimal adjustment)	★★★☆	★☆☆☆	★☆☆☆

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Acknowledgements

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supporting the global business of science

Applications Team

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