Quantitative Targeted Proteomics of Mycobacterium Tuberculosis Disease Markers

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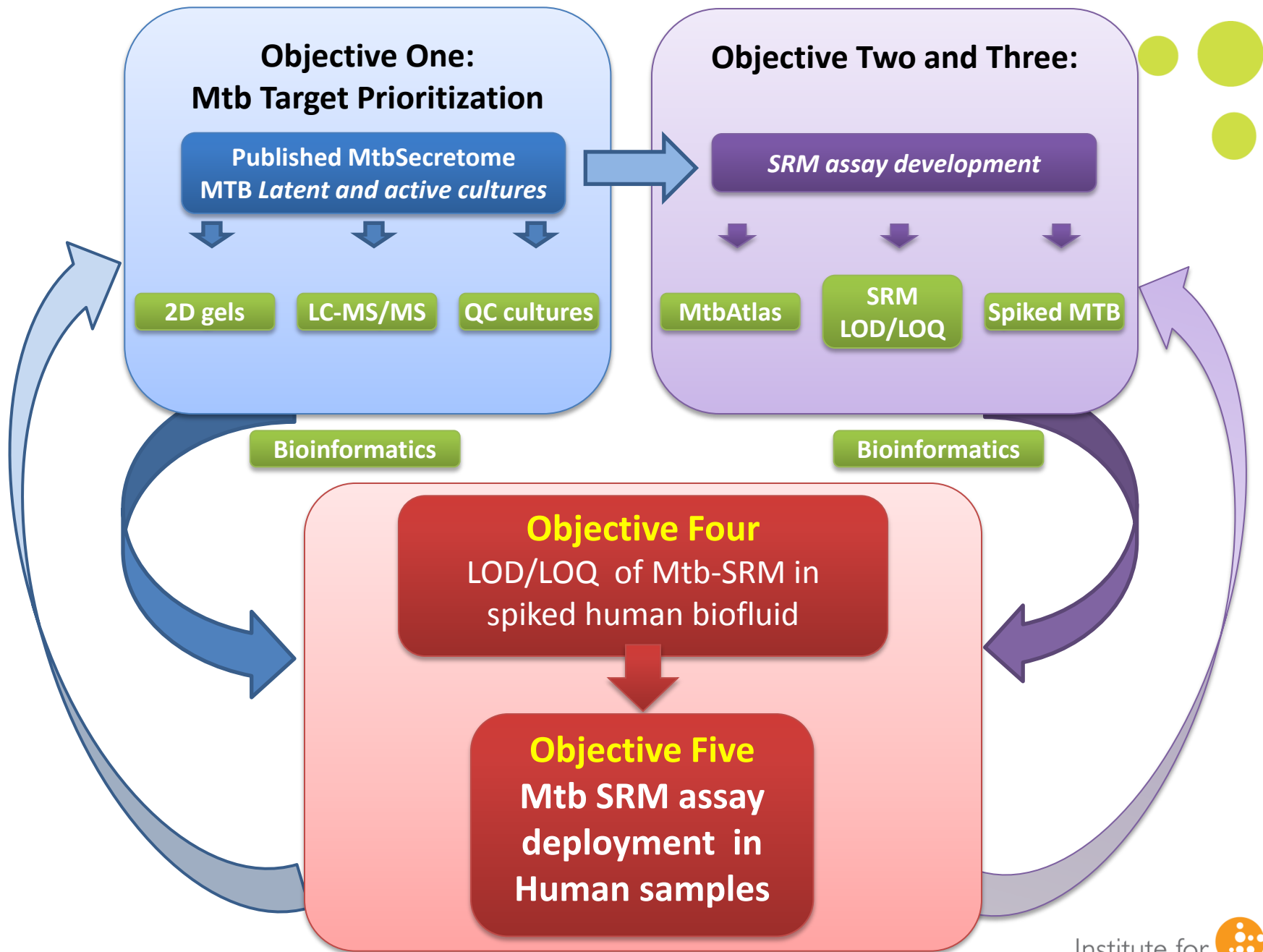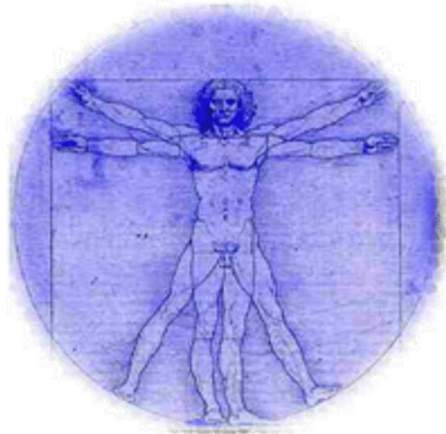
Human SRMAtlas

Outline

- Introduction: Goals of the program

- Development of SRM resource: Compendium of MS observable peptides and Mtb SRMAtlas: Unified transition resource

- Biomarker discovery
 – Identification
  – Validation

- Conclusions

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Objective One:
Mtb Target Prioritization

Published MtbSecretome
MTB *Latent and active cultures*

2D gels LC-MS/MS QC cultures

Bioinformatics

Objective Two and Three:

SRM assay development

MtbAtlas SRM LOD/LOQ Spiked MTB

Bioinformatics

Objective Four
LOD/LOQ of Mtb-SRM in spiked human biofluid

Objective Five
Mtb SRM assay deployment in Human samples

coPI-s David Sherman Seattle BioMed **, Ruedi Aebersold** ETH

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Human and MTB SRMAtlas

Human
20,333 proteins (20,277 2010 version)
32,562 proteins incl. isoforms
658,684 tryptic peptides (any length)
480,284 tryptic peptides (7-30aa)
439,213 proteotypic peptides (proteotypic)

MTB
4,012 proteins (Tuberculist v2.3)
3,972 distinct protein sequences
80,371 tryptic peptides (any length)
54,760 distinct peptides (7-30aa)
52,707 peptides (7-30aa, SSR 4-60)
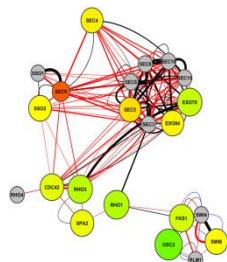52,273 peptides (proteotypic)

Q1	Q2	Q3	
Peptide Selection	Fragmentation	Fragment Selection	SRM Signal
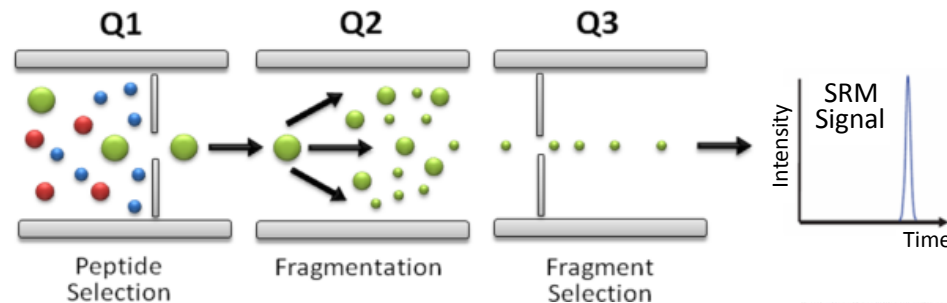
But where can we get this information?
Are all proteins available for analysis?

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Developing SRM Assays

Develop target lists

Highly curated "Gold Standard"

SRM transitions

APLAAGTWR
471.76, 774.43
471.76, 661.34

Query SRMAtlas for

6 peptides per protein

Synthesize peptides

Create fragmentation spectra
using quadrupole technology

Optimize fragmentation

Select new peptides
and repeat process

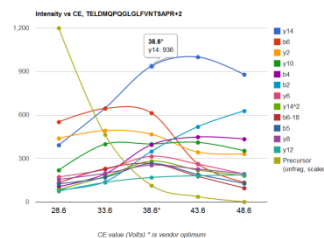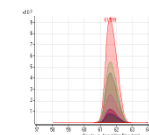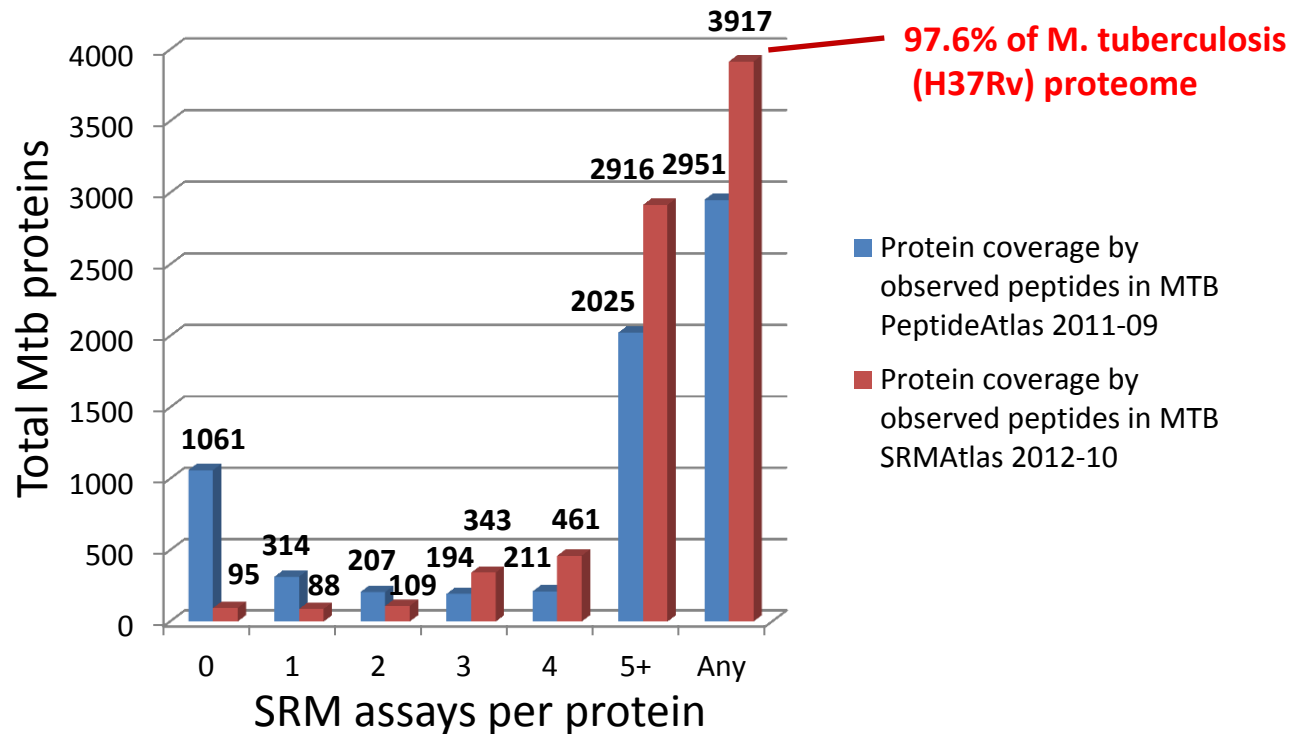Download data   program instrument

Deploy, verify and validate SRM assays

Mtb Proteome SRM Assay Availability

unmapped proteins:
> Rv3367 GTDGNPG
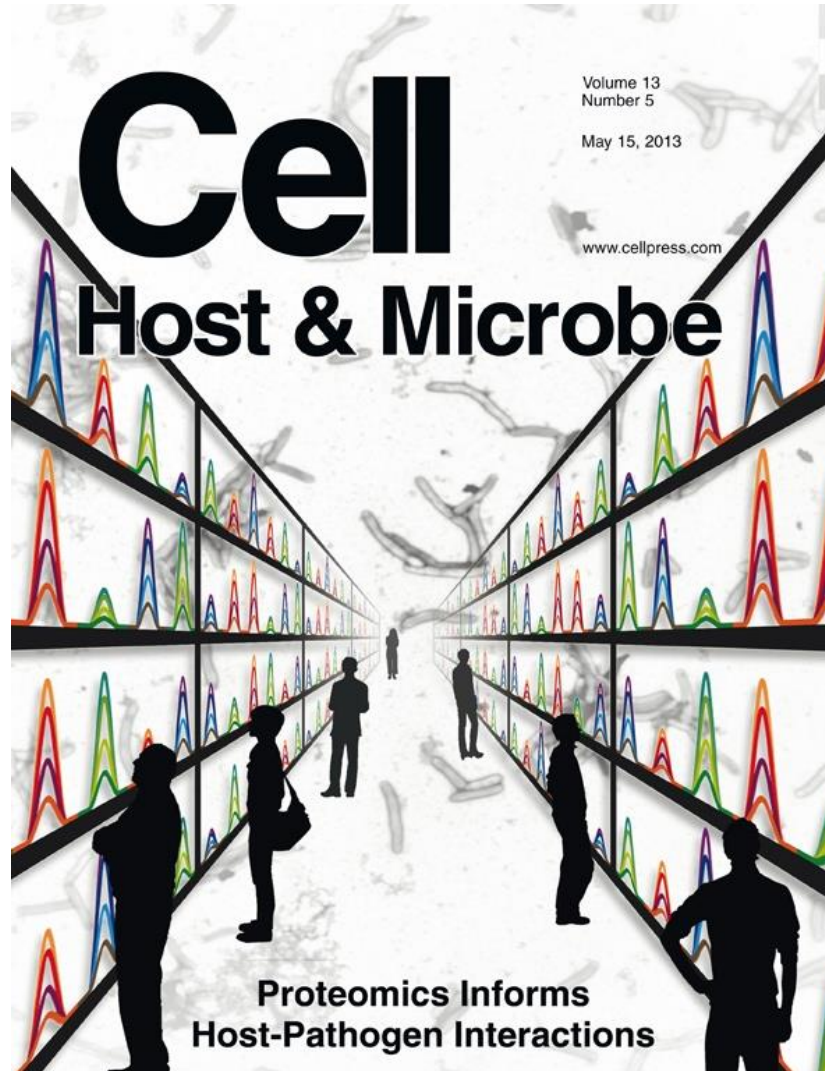
The Mtb Proteome Library: A Resource of Assays to Quantify the Complete Proteome of Mycobacterium tuberculosis
Schubert et al..
Cell Host & Microbe, Volume 13, Issue 5, 602-612, 15 May 2013

SRMAtlas: transition and spectra visualization

www.srmatlas.org

Biomarker Discovery Stages

Stage I Qualitative profiling

- Proteotypicity/selectivity
- Observability
- Inter-peptide signal interferences

Stage II Quantitative profiling

- Detection limit
- Linearity of response
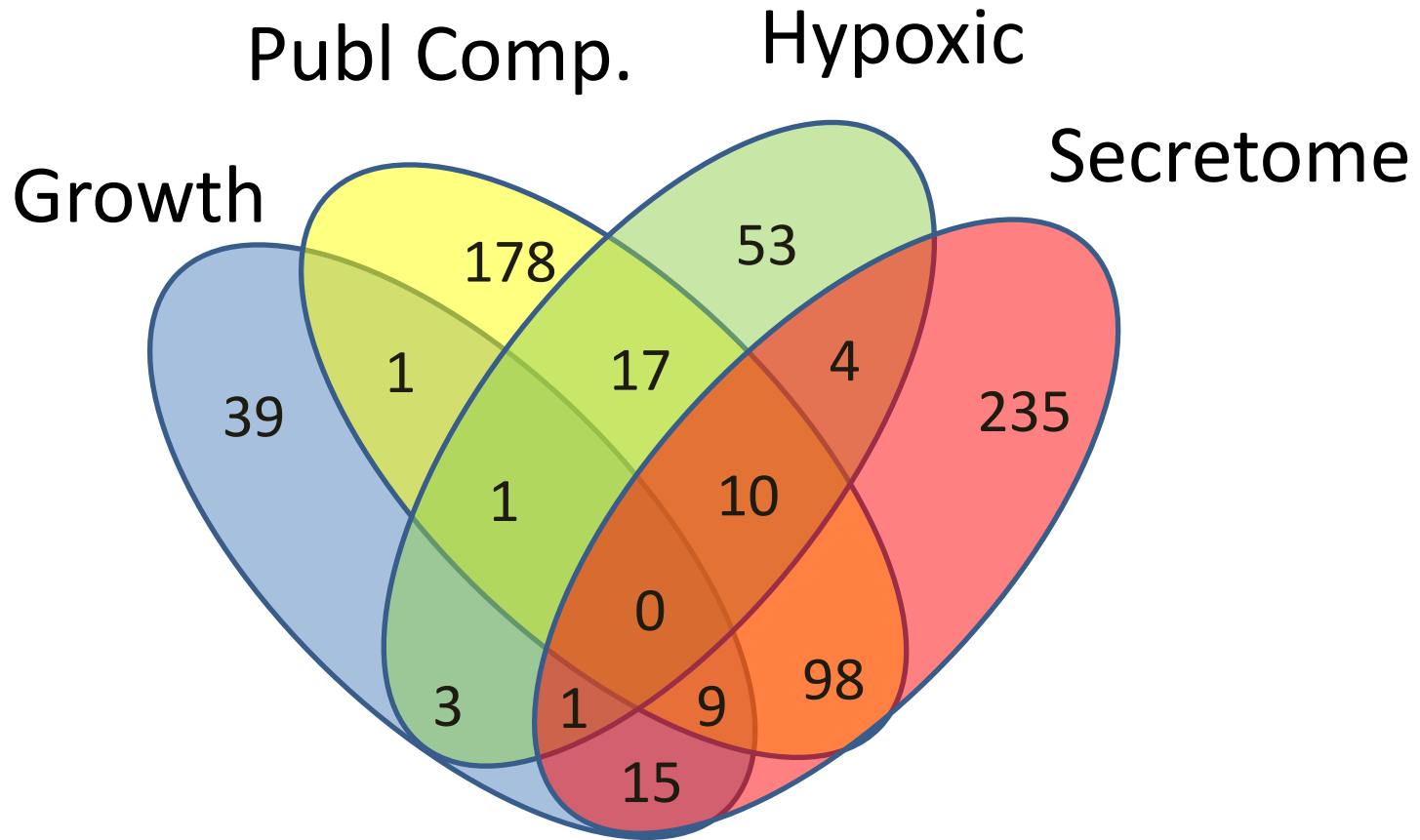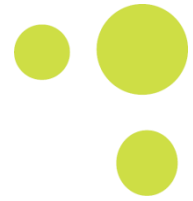- Technical reproducibility

Stage III Biomarker discovery and validation

- Detection in sample matrix
- Peptide interferences
- Dynamic range
- Chemical stability

SR Atlas

Autocalib

SRMQuant Atlas

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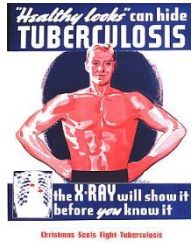Revolutionizing Science. Enhancing Life.
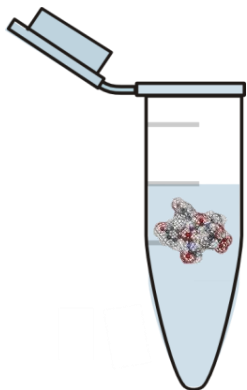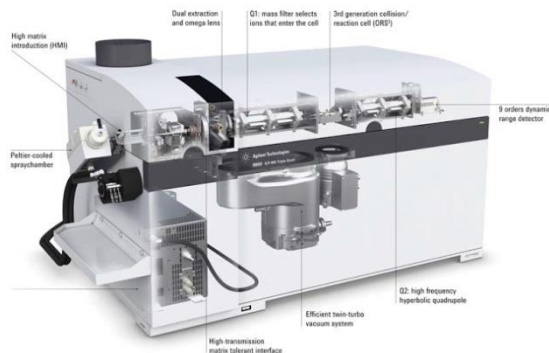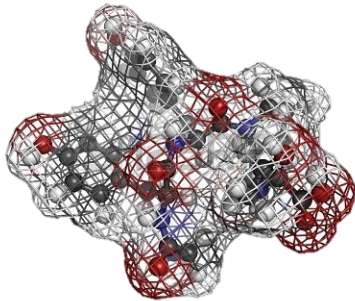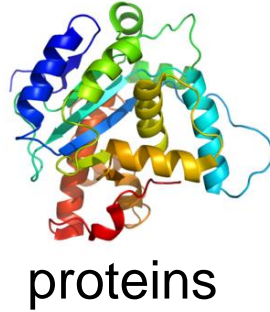
Overall Mtb Target List Overlap

Total of 505 targets selected

Proteomic profiling of TB patient samples

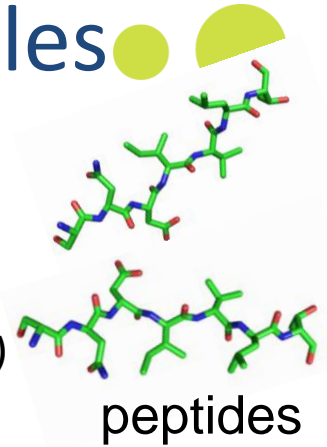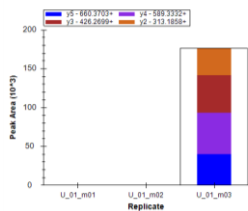Patient samples

sputum
urine
plasma

tryptic digestion

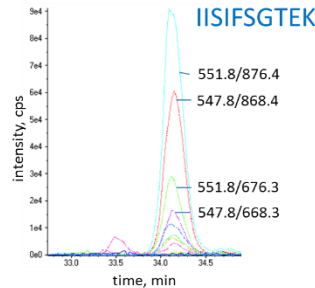*clean-up:*
solid phase extraction (SPE)
Sep-Pak tC18 column

proteins

peptides

OGE

clean-up: SPE
tC18 elution plate

data analysis

IISIFSGTEK

551.8/876.4
547.8/868.4

551.8/676.3
547.8/668.3

SRM MS analysis
of 24 fractions

fractionation

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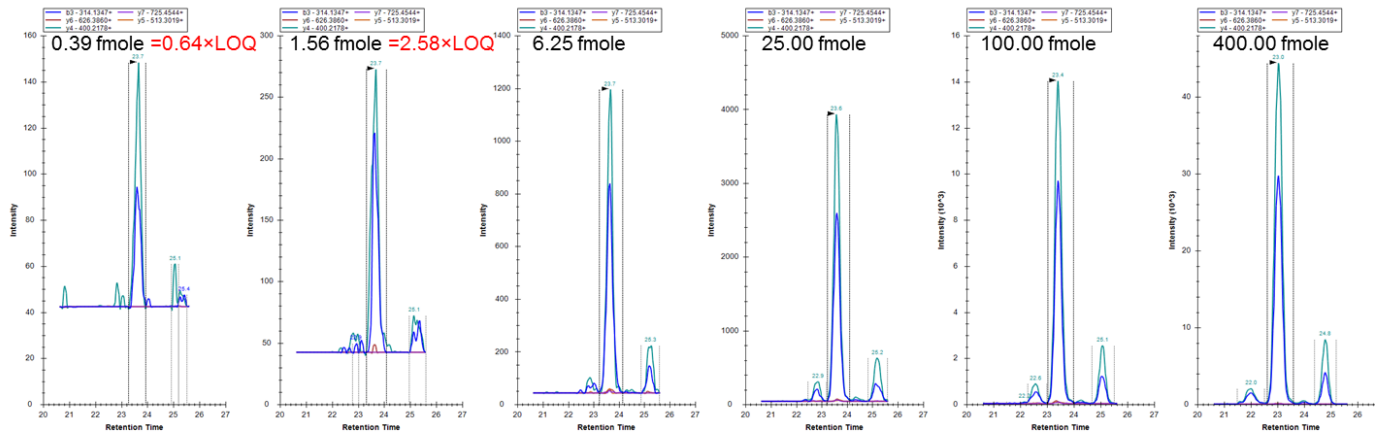Revolutionizing Science. Enhancing Life.

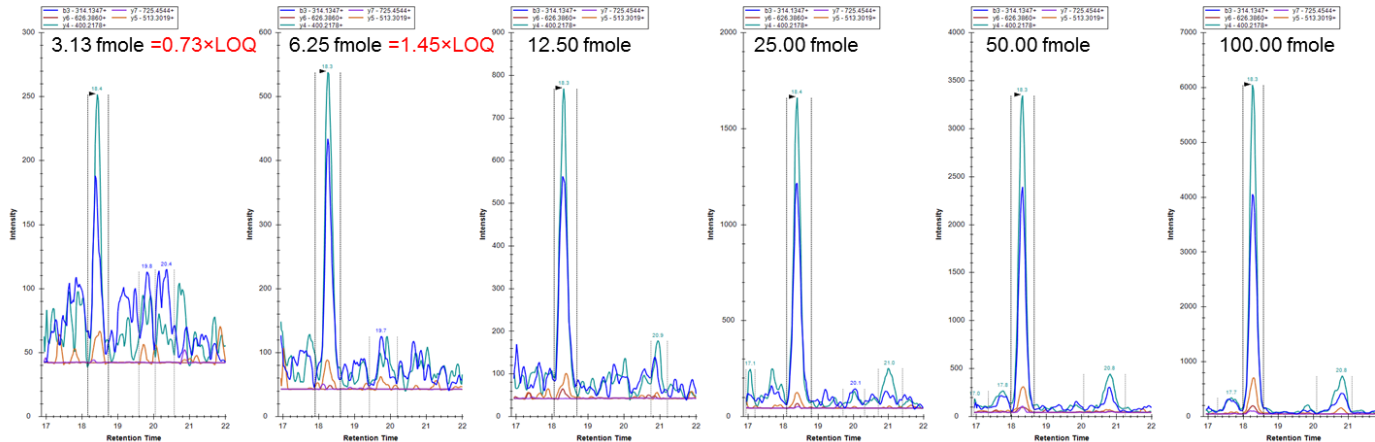# ANALYTICAL CHALLENGES

Sample matrix signal response

a) Neat solution titration series

79.2% peptides demonstrated quantitative in neat solution remains quantitative in human sample matrix

b) Matrix-matched titration series (SPEVLLGSAR)

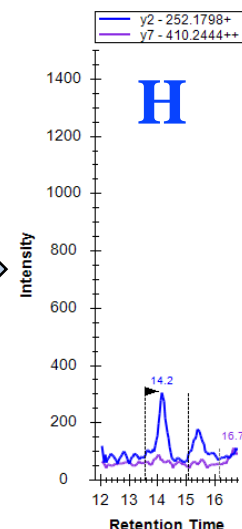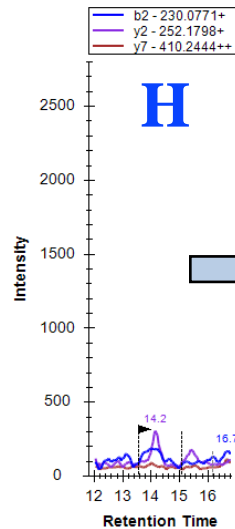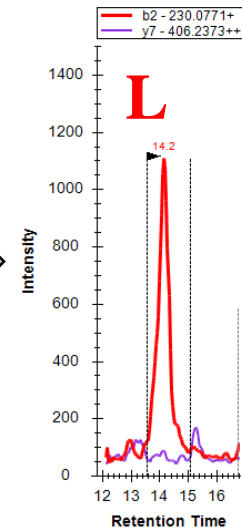| (n=62)	Calibration Curve (Neat Solution)	Calibration Curve (Urine Matrix)	MMu/NS ratio
LOD$_{median}$	0.776	2.012	2.59

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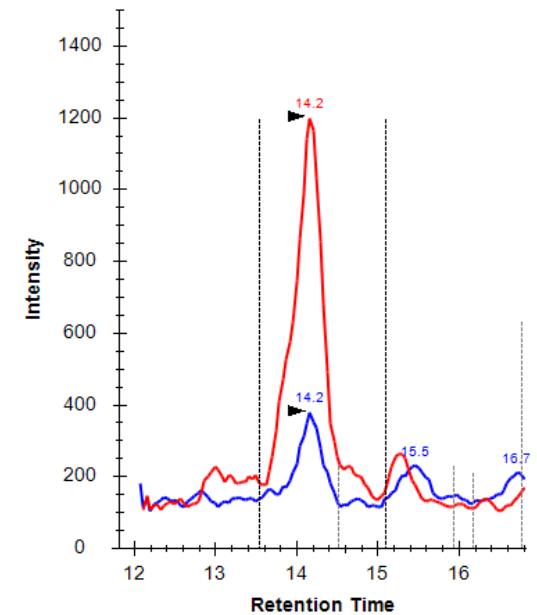Revolutionizing Science. Enhancing Life.

Interference removal of Mtb target protein SRM

Body Fluid Matrix (urine)
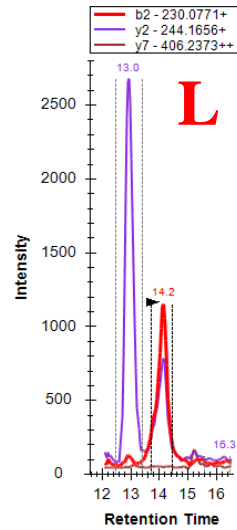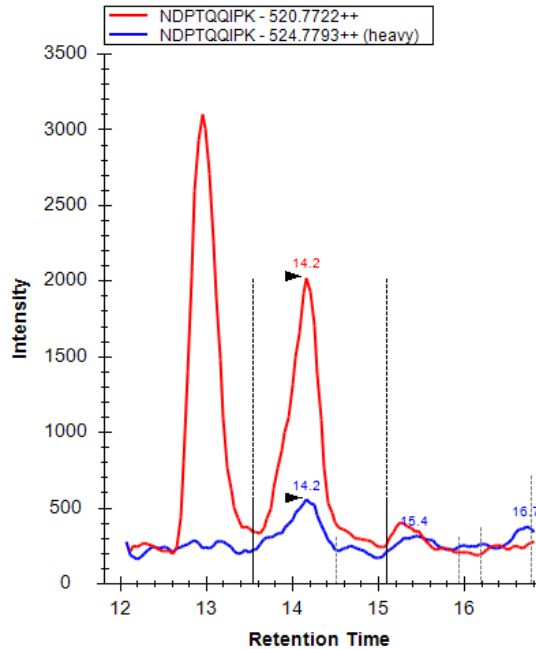
Conclusions

- **Quantitative profiling** applied to biomarker discovery

- Developed sample processing for **sputum, plasma and urine**

- Analysed sputum samples after OGE fractionation, plasma and urine with minimal fractionation

- SRM analysis and biomarker verification in progress...

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Acknowledgements

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