

DISCOVERY AND VALIDATION OF TARGETS AND BIOMARKERS BY MASS SPECTROMETRY-BASED PROTEOMICS

September, 2011

CAPRION PROTEOMICS



- **Leading proteomics-based service provider**
 - Biomarker and target discovery
 - Multiplexed MRM assays
 - Biomarker verification and validation
- **Located in:**
 - Montreal, Canada
 - Menlo Park, California
- **Founded in 2000**
- **49 employees (42 scientists)**
- **Extensive experience**
 - Over 30 industry and government partners
 - Over 70 large scale studies completed (clinical and pre-clinical)
 - All major therapeutic areas
 - Broad range of biological samples, including CSF, plasma, serum, urine, exudate, cells and tissues



CAPRION PRODUCTS, SERVICES AND INDUSTRY PARTNERS



Biomarker Services

- Biomarker discovery - CellCarta[®]
- Highly multiplexed assay development
- Validation assays

Drug Targets

- Oncology antibody therapy
- Metabolic disease secreted proteins
- Infectious disease targets

In-Vitro diagnostics

- Infectious disease
- Diabetes
- Oncology
- CNS



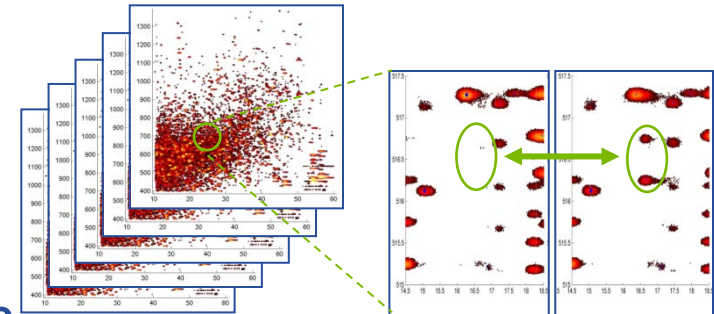
Québec Consortium for Drug Discovery



MS-BASED BIOMARKER DISCOVERY PROCESS

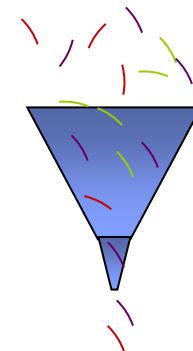
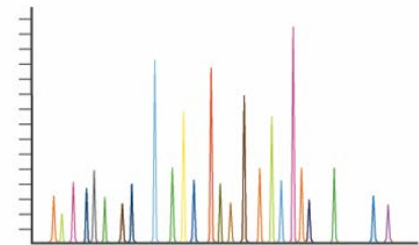
Biomarker Discovery

- Label-free, gel-free quantitative mass spectrometry
- Non-hypothesis based discovery approach
- Profile 1000's of proteins in 100's samples
- Identify differentially expressed proteins as candidate biomarkers

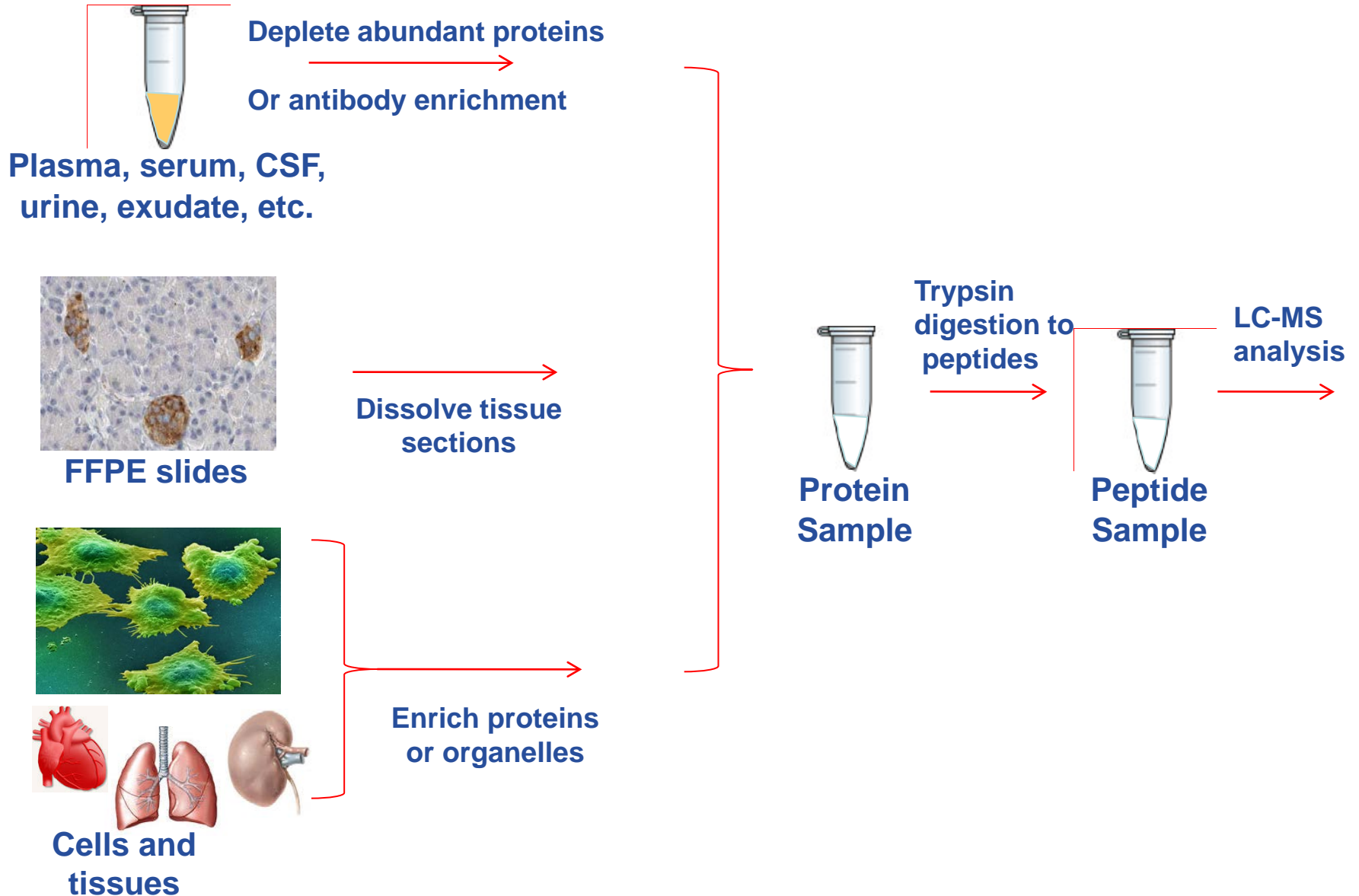


Multiplexed Assays

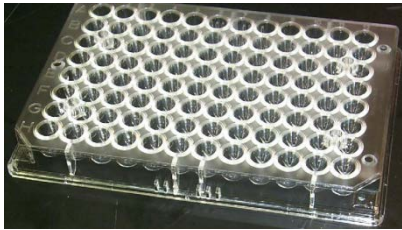
- Quantify 1 to 700 proteins in a single “MRM” assay
- Proteins from mass spec, literature, transcriptomics, etc.
- Rapid assay development
- Ab-free or ab enrichment
- Synthetic labeled standards for absolute quantification
- Profile candidate markers in 1,000's samples



ANALYSIS OF ANY BIOLOGICAL SAMPLE IS POSSIBLE



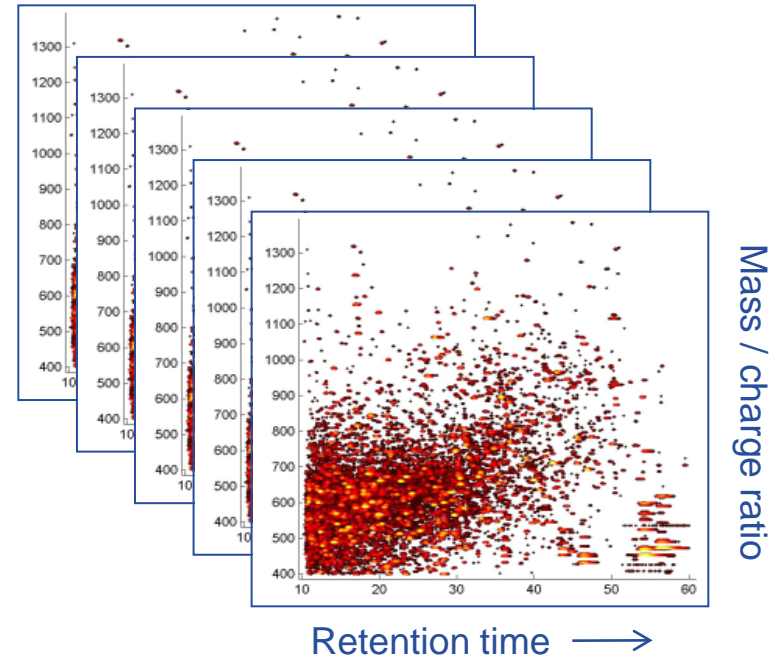
LC-MS ANALYSIS AND MATCHING PEPTIDES ACROSS ALL SAMPLES



Peptide
samples

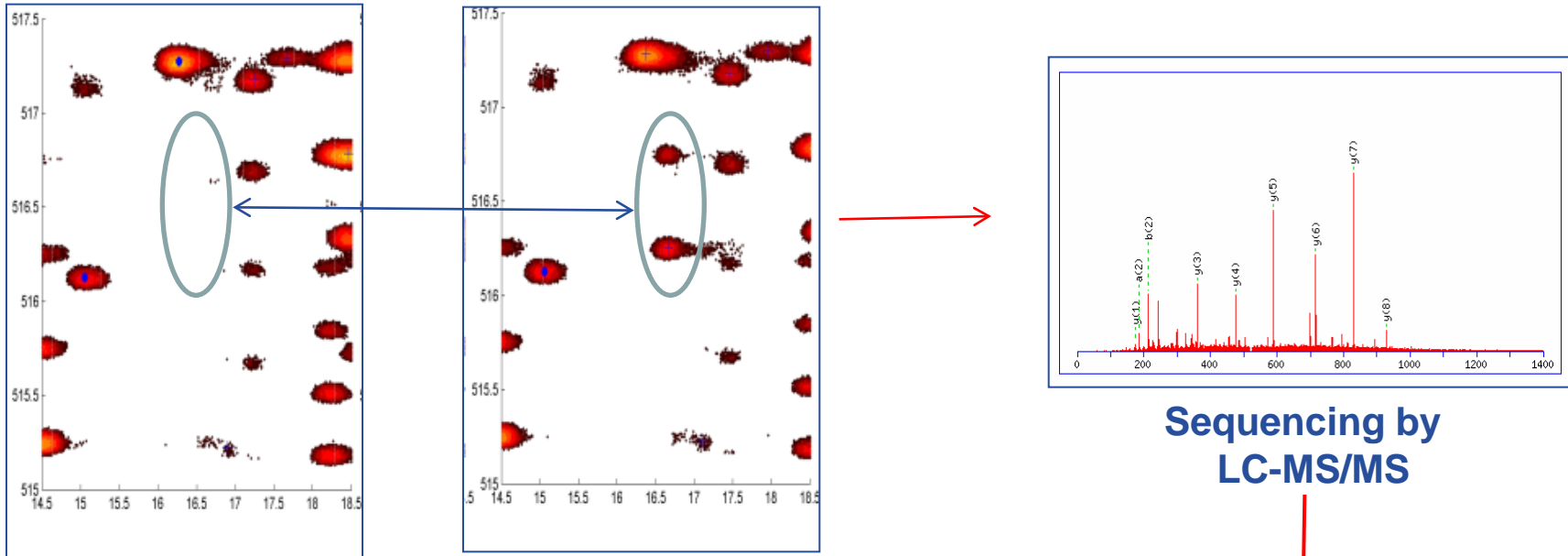


RP-HPLC and
QTOF or Orbitrap Mass
Spectrometry



10,000-20,000 peptides
observed, matched
and quantified

EXPRESSION PROFILING AND PROTEIN ID



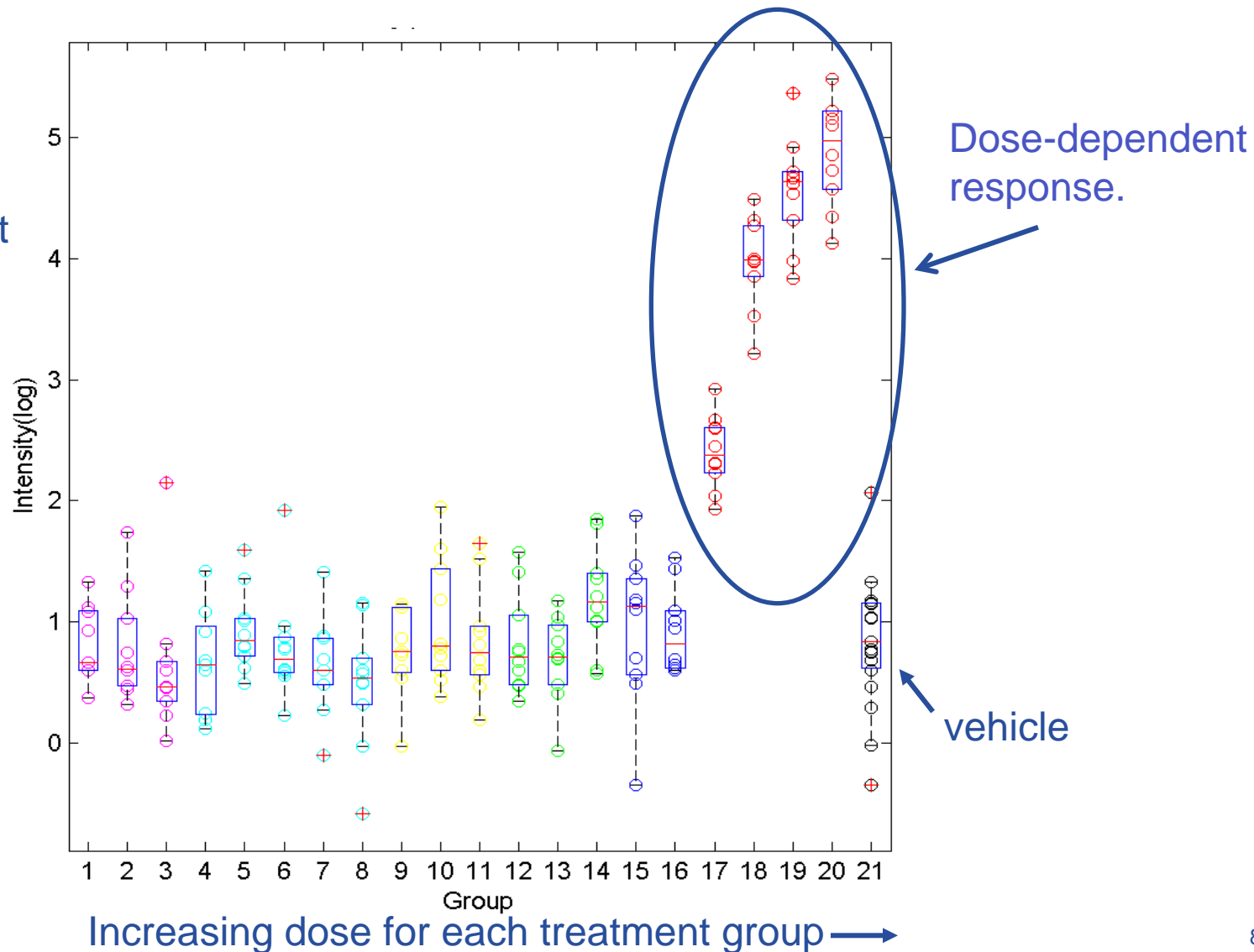
Statistical analysis to identify differentially expressed peptides

```
>gi|41327732|ref|NP_958439.1|  
epidermal growth factor  
receptor isoform b [Homo  
sapiens]  
MRPSGTAGAALLALLAALCPASRALEEKVCQGTSNKL  
TQLGTFEDHFLSLQRMFNCEVVLGNLEITYVQRNYDL  
SFLKTIQEVAGYVLIALNTVERIPLLENLQIIRGNMYYE  
NSYALAVLSNYDANKTGLKELPMRNLQEIILHGAVRFSN  
NPALCNVESIQWRDIVSSDFLSNMSMDFQNHLGSCQKC  
DPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSP  
SDCCHNQCAAGCTGPRESDCLVCRKFRDEAT..... . .
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CASE 1: DOSE RESPONSE STUDY

SINGLE PEPTIDE RESULT ACROSS 220 SAMPLES

Each data point represents one plasma sample



CASE 2: VERTEX PRESS RELEASE: PREDICTIVE BIOMARKERS OF RESPONSE



- **Goal: Predict response to drug before treatment with Telaprevir (personalized medicine)**
- **Proteomics analysis of 240 plasma samples collected from HCV patients**
 - Pre-dose samples from phase 3 clinical trials
 - Clinical outcome provided
- **Candidate biomarkers identified**
 - 34 proteins distinguish responders and non-responders to SOC + Telaprevir
 - 10 proteins distinguish responders and non-responders to SOC alone
 - Significant number of proteins correlated to the underlying severity of liver damage
- **Follow-on study in progress**
 - Multiplexed MRM assay developed
 - Verify and validate biomarker candidates in larger population



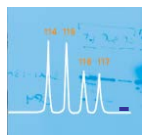
MRM-MS Assays

Multiplexed Assays for Biomarker Verification and Validation

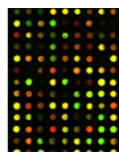
MULTIPLEXED MRM ASSAYS: RAPID VALIDATION OF CANDIDATE BIOMARKERS



Literature

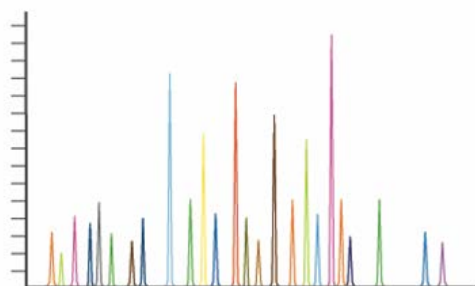


Proteomics

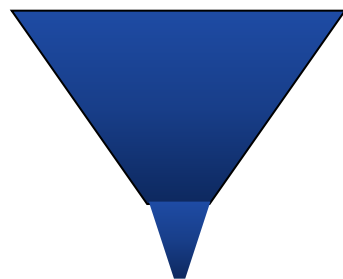


Transcriptomics

**Candidate
Biomarker
list**



**Multiplexed
MRM assay
(up to 700
proteins)**



**Biomarker
verification
funnel**

**Develop ELISA or MRM
assay for large scale
biomarker validation**

**Cost and time effective
alternative to antibody-based
approaches**

- Highly multiplexed
- Rapid assay development
- Competitive pricing
- Quantitative measurements
- Amenable to GLP analysis

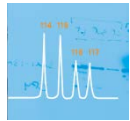
**Custom assays:
Up to 700 proteins per assay**

**Off-the-shelf products in development:
Preclinical (rat) Tox Panel
PCSK9 assay (active/inactive)**

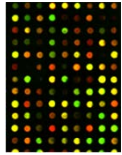
MRM ASSAY DEVELOPMENT STRATEGY



1. Literature



2. Proteomics

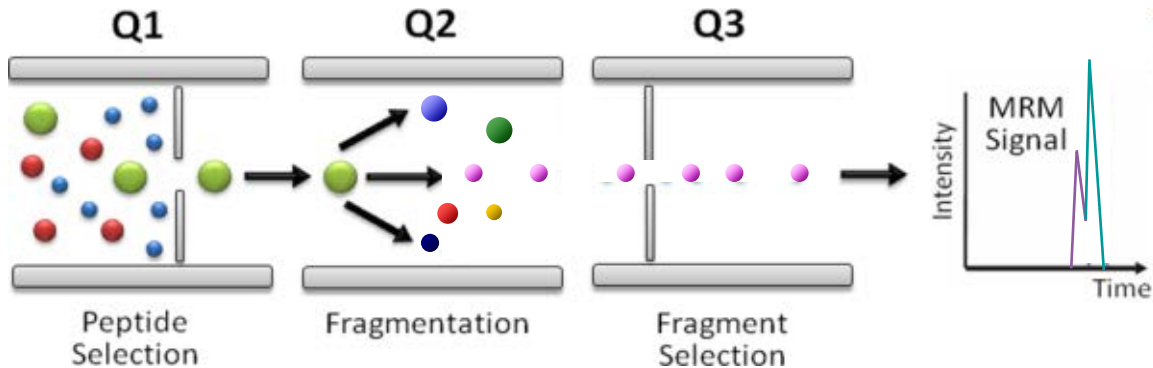


3. Transcript Profiling

Protein Sequence

MSAIQAAWPSGTECIAKYNFHGTAEQD
 LPFCK**GDVLTIVAVTK**DPNWKAKNKV
 GREGIIPANYVQKREGVKAGTKLSLMP
 WFHGKITREQAER**LLYPPETGLFLVRE**
 STNYPGDYTLCVSCDGKVEHYRIMYHA
 SKL**SIDEEVYFENL**KMQLVEHYTSDAD
 GLCTRLIKPKVMEGTVAQAQDEFYRSGW
 ALNMKELKLLQTIGK**GFPGDVMLGDYR**
 GNKVAVKCIKNDATA...

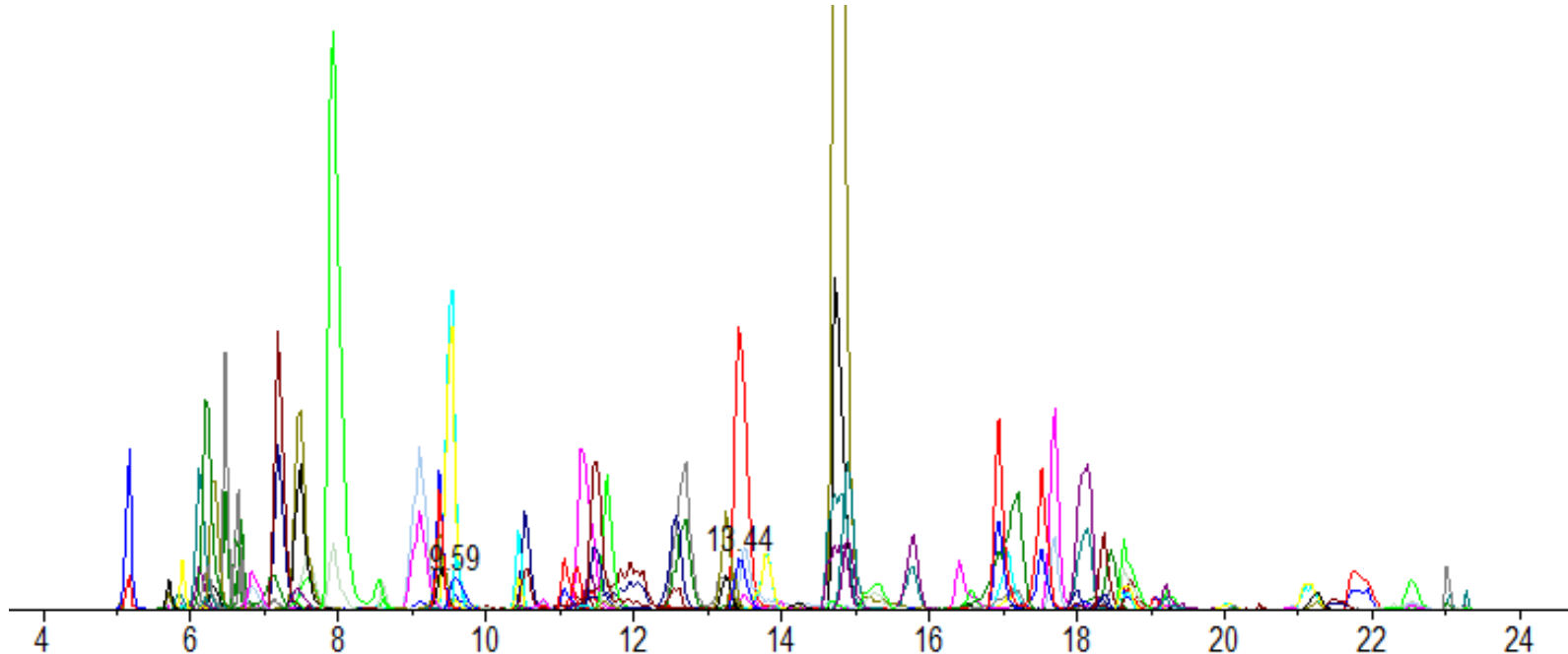
Candidate markers for MRM assay development can come from proteomics or other sources, including the literature



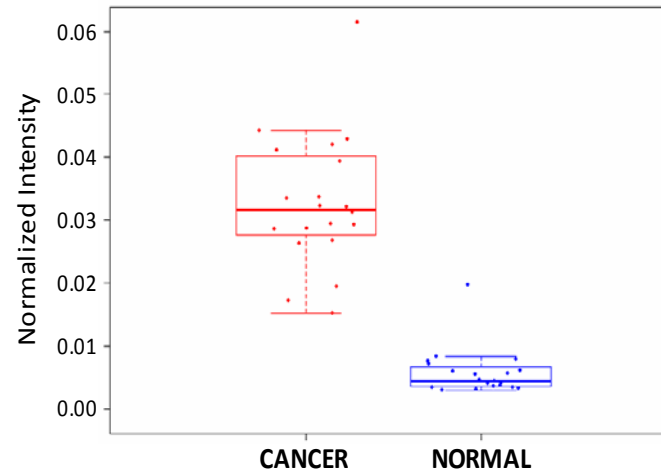
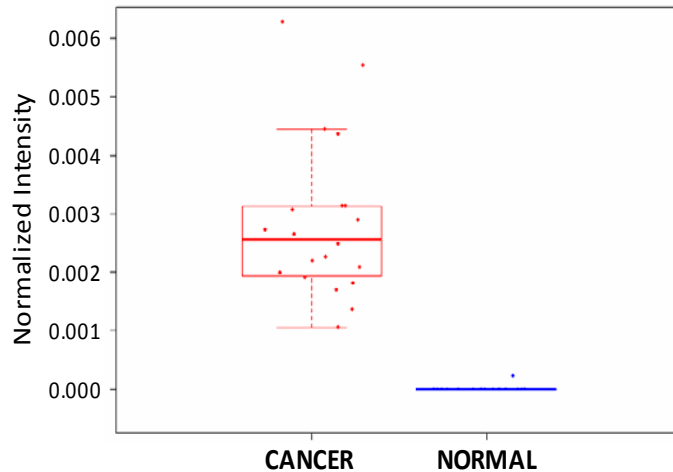
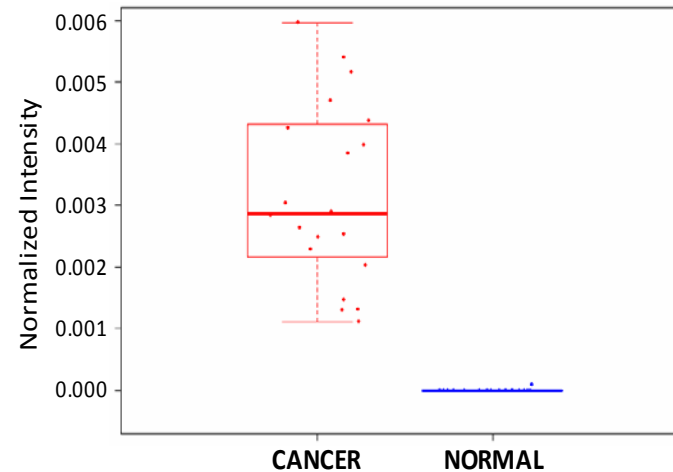
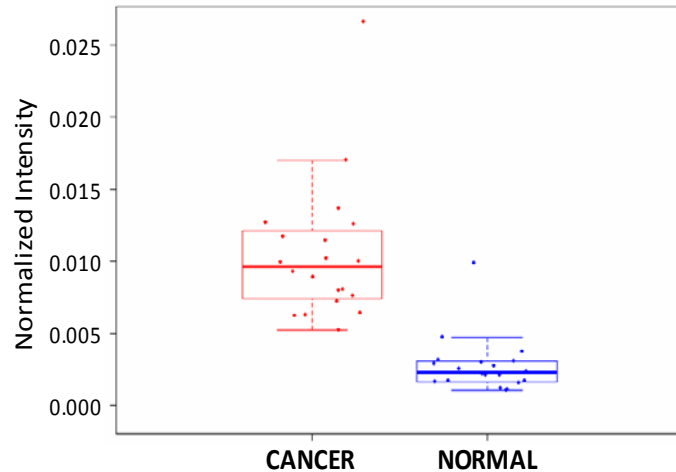
Double selection improves signal/noise and reduces interference

EXAMPLE OF MRM ASSAY DEVELOPED FROM LIST OF 90 LUNG CANCER Dx CANDIDATES

- No antibody enrichment
- Plasma depleted of high abundance proteins
- 64 of the targeted proteins (71%) successfully detected in un-spiked plasma samples (cancer and controls)

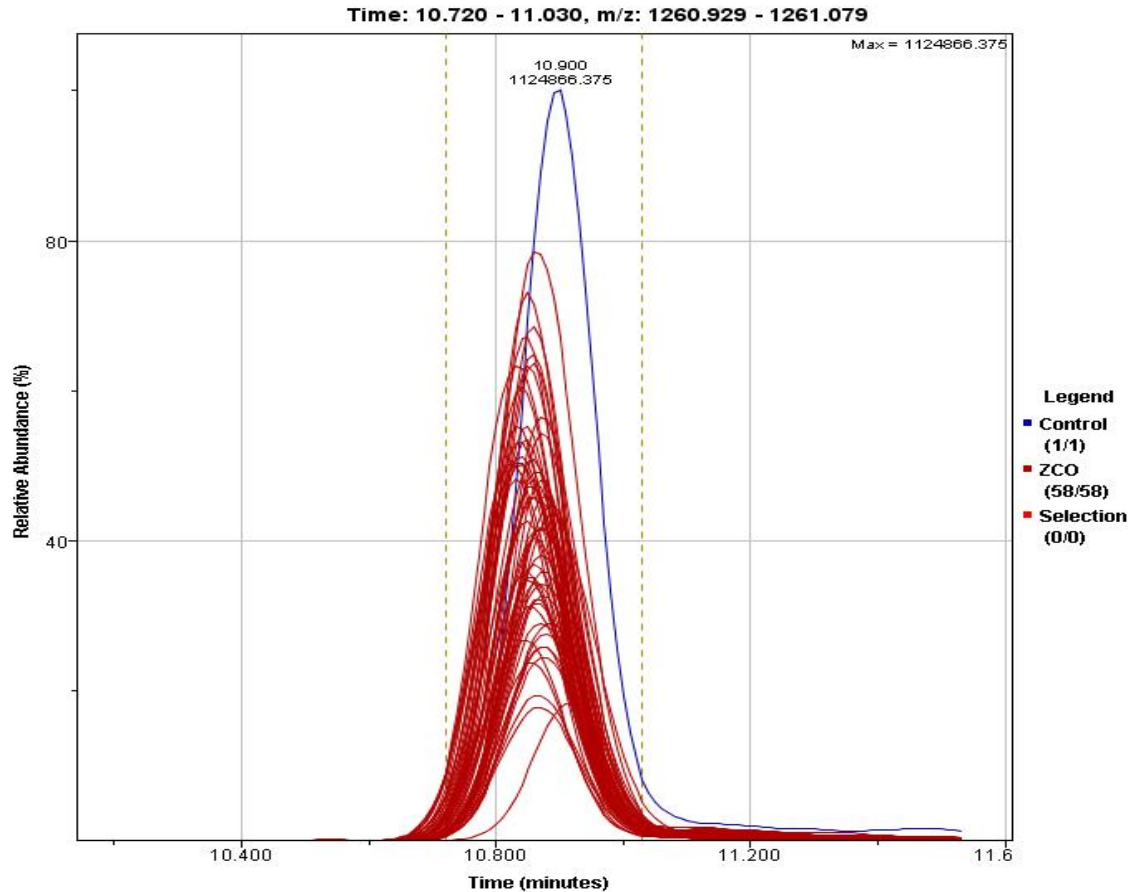


CANDIDATE CLASSIFIERS IDENTIFIED



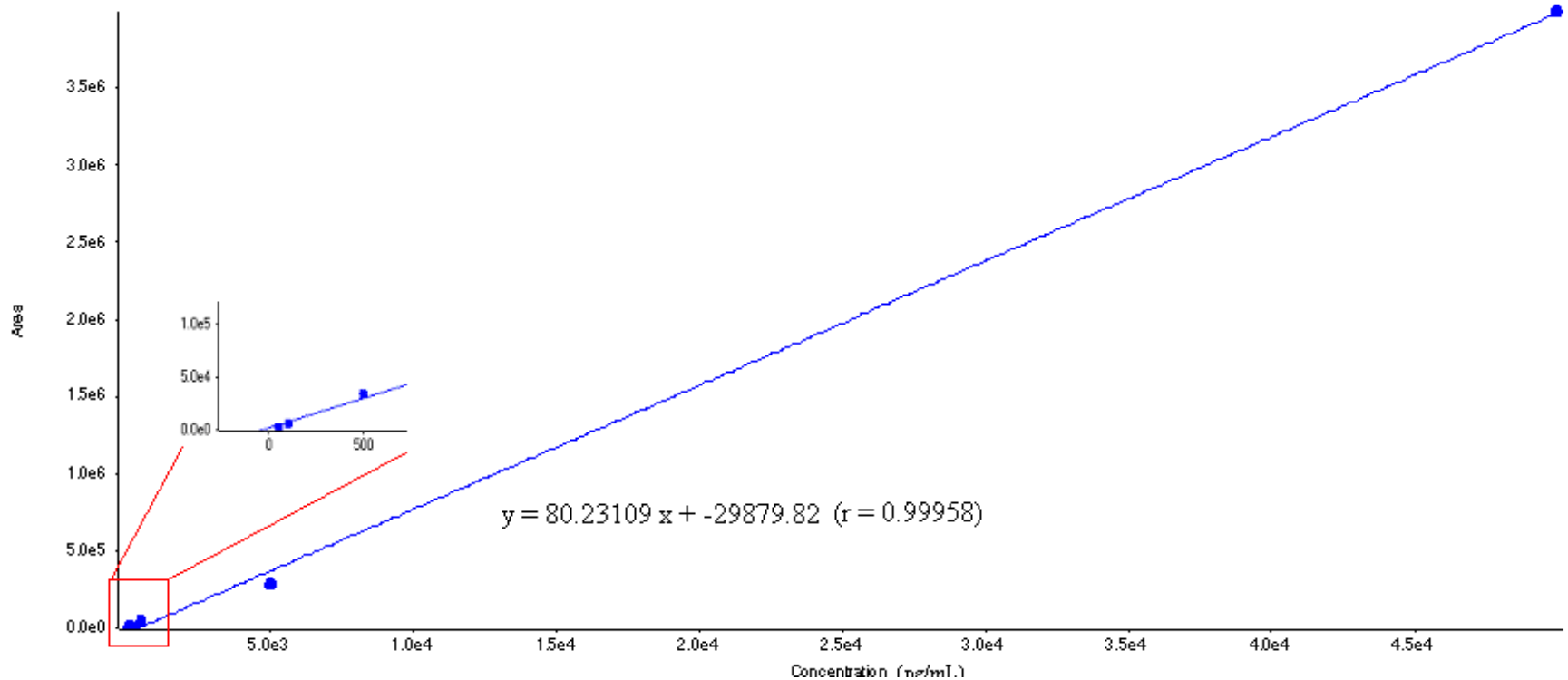
Current study: Larger set of proteins (350 candidate markers) and more samples (~400) from multiple sources

CUSTOM SOFTWARE FOR PEAK DETECTION AND QUANTIFICATION



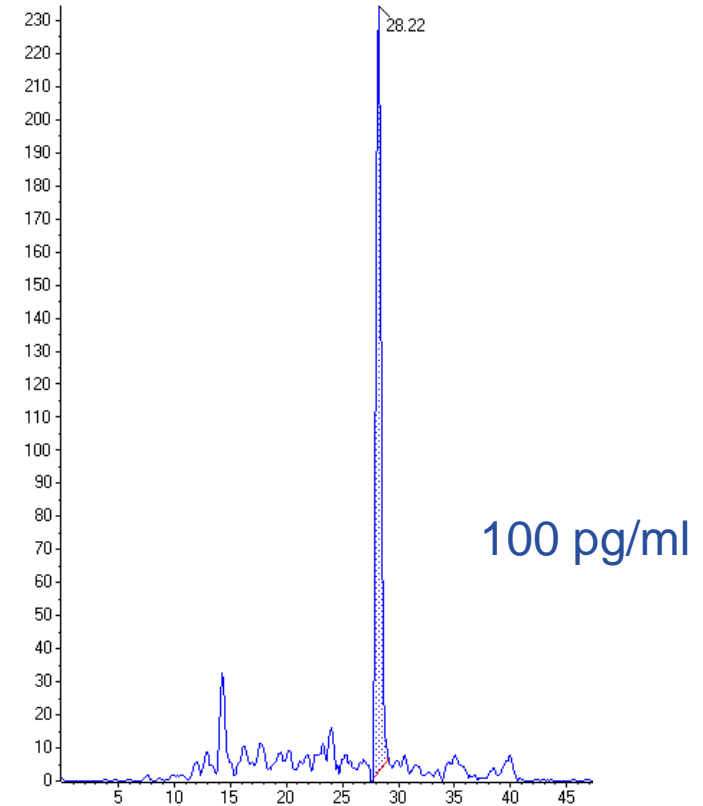
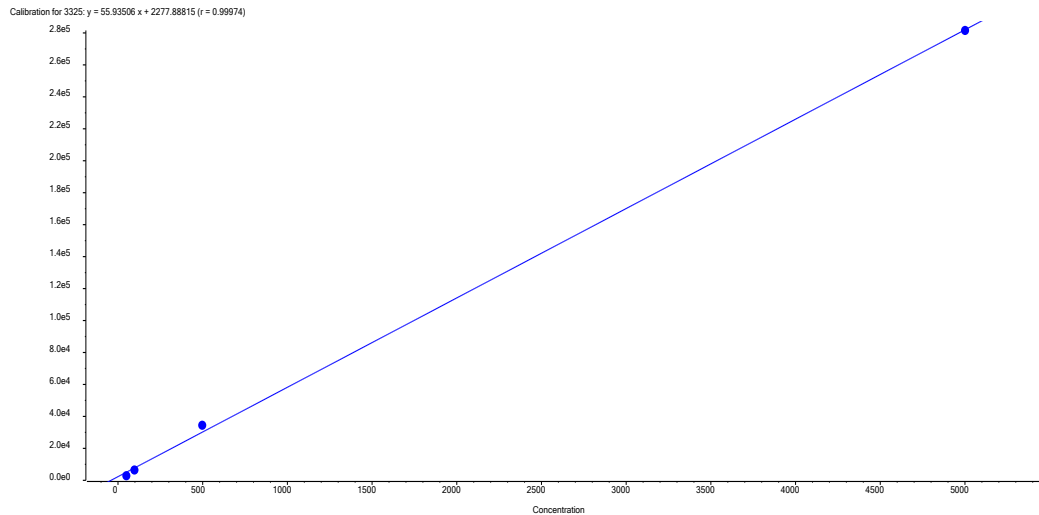
AUTOMATION OF PROCESS INCREASES QUALITY AND CONFIDENCE IN DATA

¹³C, ¹⁵N-LABELED STANDARDS CAN BE USED



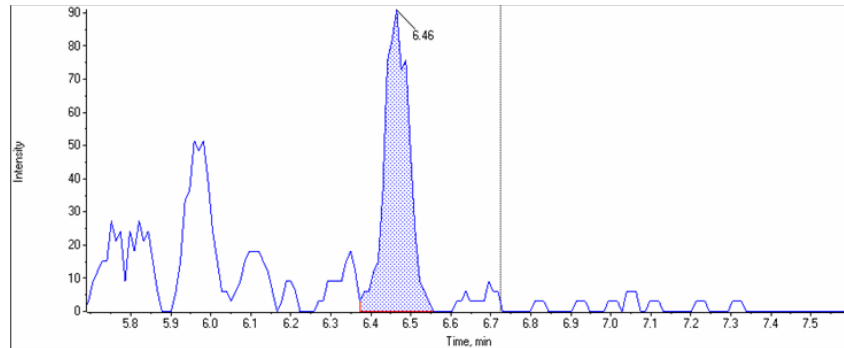
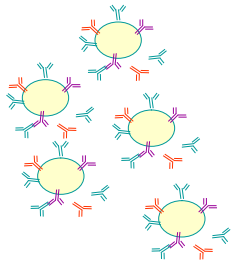
Synthetic labeled standards can be used to quantify biomarkers over 4 orders of magnitude

QUANTIFICATION OF ENDOGENOUS PEPTIDES IN PLASMA

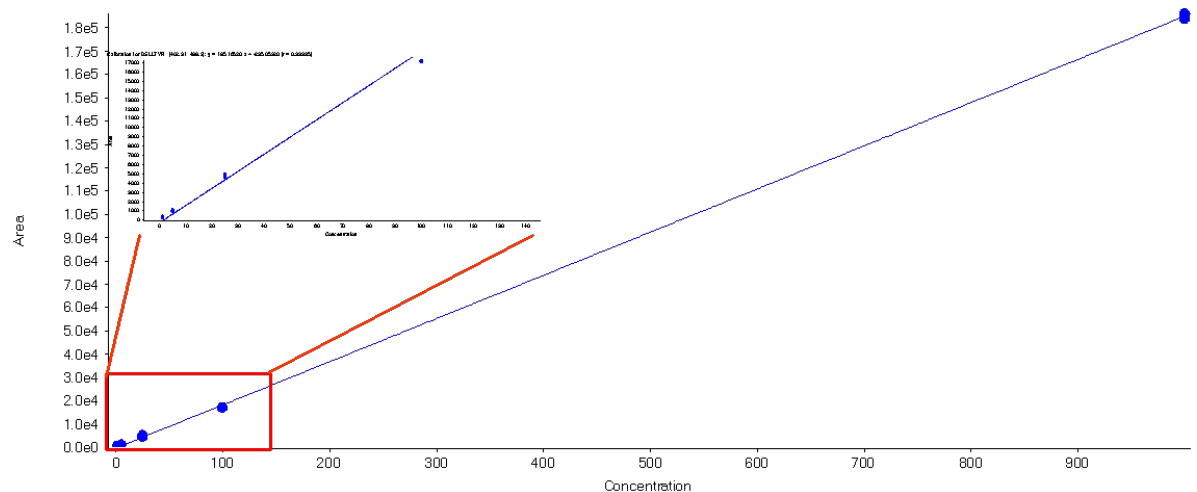


- Protein precipitation followed by SPE cleanup of endogenous peptides
- 500 ul rat plasma
- LOQ 1-10 pg/ml (CV ~15%)

ANTIBODIES CAN BE USED TO ENRICH SPECIFIC PROTEINS WHEN NECESSARY

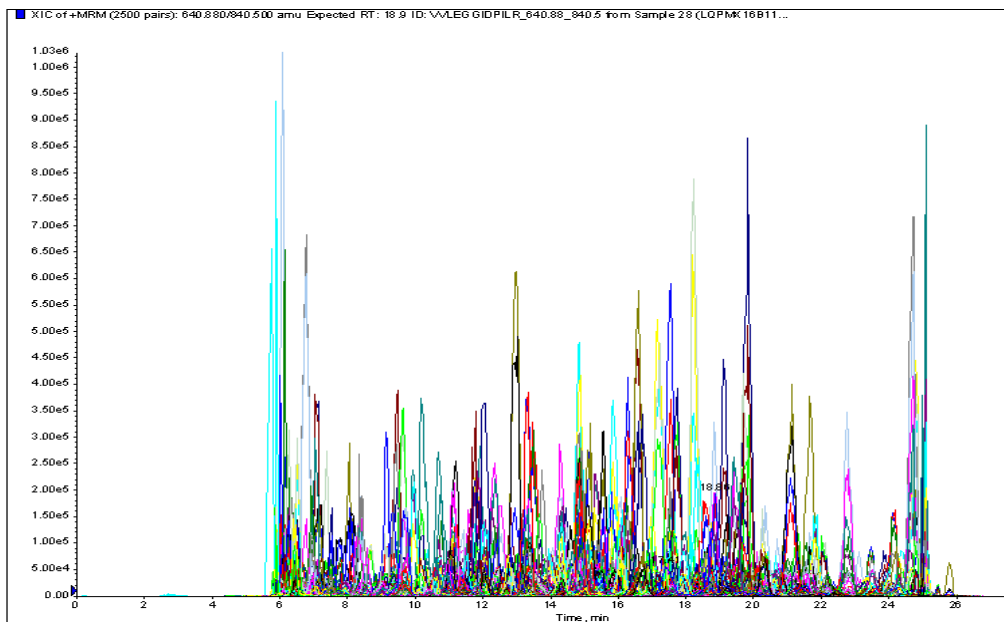


- Ab's conjugated to magnetic beads
- Immuno-enrichment followed by trypsin digestion
- 250 ul plasma
- LLOQ 100 pg/ml (CV ~15%)



VERY HIGHLY MULTIPLEXED ASSAYS ARE POSSIBLE BY MRM

- 1498 peptides in one assay
- 700 proteins



Highly reproducible results

# of transitions measured	Median Int CV of detected transitions
250	2.4
500	3.1
1000	4
1500	5.2
2000	6.4
2500	7.6

CAPRION MRM ADVANTAGES



Over 60 industry and government sponsored studies performed

- Majority of projects involved multiplexed analysis of over 50 analytes

Comprehensive sample preparation capabilities and experience

- Reproducible high-abundance protein depletion and cell fractionation capabilities
- Plasma, serum, CSF, urine, exudate, cells and tissues

Optimized process for minimal variability

- Low process variability, including GLP study option
- Optimized instrumentation (Waters nano-Acquity LC/ ABSciex QTRAP 5500)

Proprietary software suite for enhanced quality and throughput

- Automated peptide selection tool for rapid assay development
- In-house database of experimentally detected peptides
- Assay development throughput >9000 peptides per week, including collision energy optimization.
- Signal interference detection and assay quality assessment software
- Automated data processing through Elucidator
- Automated bio-statistical analysis

