BIOMARKERS ARC

Proteomics Approach to Identify Biomarkers of Metabolic Disease

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Boston University School of Medicine

Evans Center for Interdisciplinary Biomedical Research
Affinity Research Collaboratives (ARC) Celebratory Event
Tuesday, February 16, 2010

On the Road to Biomarker Discovery!

Mark E. McComb

Catherine E. Costello

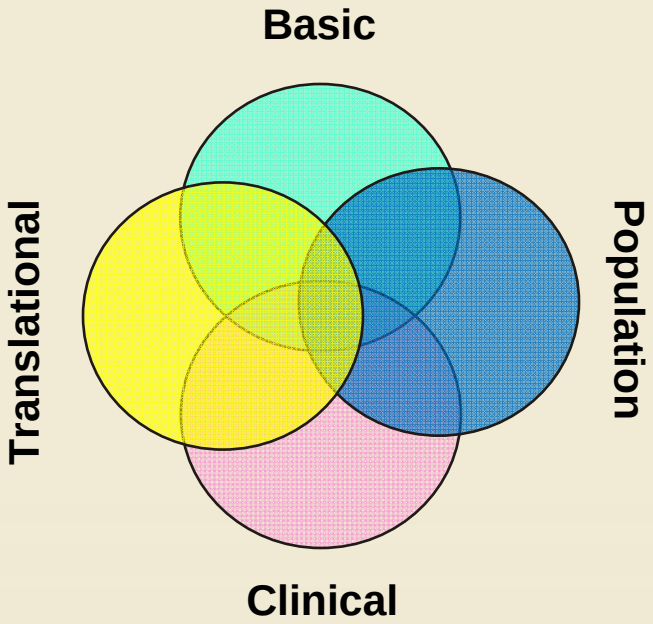
Richard A. Cohen

OPTMs and CVD
7+ years of successful collaboration!
Cardiovascular Proteomics Center

Biomarkers ARC: A Diverse Group of Investigators

ARC Member	Department
Mark E. McComb	Medicine
Catherine E. Costello	Biochemistry/Biophysics/ Chemistry
Richard A. Cohen	Medicine/Vascular Biology
Vasan Ramachandran	Medicine/Epidemiology
Wilson Colucci	Medicine/Cardiology
Robert Lafyatis	Medicine/Rheumatology
Joseph Vita	Medicine/Cardiology
Emelia Benjamin	Medicine/Cardiology
Daniel Levy	Medicine/Cardiology
Francis Farraye	Medicine
James Collins	Bioengineering
Susan Freed	Medicine/Diabetes-Metabolism
Jane Freedman	Medicine/Cardiology
Richard Myers	Neurology/Genome Science
M. Selim Unlu	Engineering/Nanoscience
Bennett Goldberg	Engineering/Nanoscience
Joseph Zaia	Biochemistry

Science in the Biomarkers ARC



We guarantee maximal debate on all topics of discussion!

Goals of the Biomarkers ARC

- **Foster discussion on Biomarker discovery**
  - **Definitions**
  - **Models**
  - **Approaches**

Questions > Answers

- **Obtain pilot project data**
  - **Answer questions on a project basis**
  - **Preliminary data for NIH funding**

Biomarker Models of CVD?

- **Correlate results between different models**
  - **Mouse to human**
  - **Genomics vs. proteomics**

Proteomics?

- **Cross model comparisons**
  - **Different groups at BUSM**
  - **Diabetes vs. PAH**

Meta-Analyses?

Elucidate and determine a panel of specific plasma markers for CVD

Biomarkers: Debatable Definitions

- Biomarkers constitute any multiplex of measurements that specifically detects normal or diseased physiology

- Biomarker come in 3 flavors:
  - early detection of disease
  - determine the effects of treatment
  - determine short and long term prognosis

Screening → Diagnosis → Prognosis

- Biomarkers can be defined through different means

Genomics — Proteomics
Metabolomics — PTM-omics

Biomarkers: A Process of Discovery

- **The road to biomarker discovery is well studied and typically involves 3 discrete steps: discovery, verification and validation.**

Discovery →	**Verification** **Qualification** →	**Validation**
Model →	**Cohort** →	**Population**
10s →	**100s** →	**1000s**
Many →	**Few** →	**Select**

During the discovery and validation processes incite must be gained into the understanding of the molecular mechanism of the disease

Metabolic Disease + Proteomics = Biomarker Discovery

- **Metabolic Disease**
  - **Diverse area of research at BUSM**
  - **Weak genomic component**
  - **Strong environmental link**
  - **Correlation with oxidative stress**

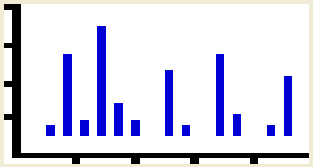
- **Proteomics within the Center for Biomedical Mass Spectrometry**
  - **Cardiovascular Proteomics Center**
  - **Mass Spectrometry Resource**

- **Hypothesis: metabolic changes in diseased tissue**
  - **may be detected by changes in plasma protein abundances**
  - **are betrayed by leakage of tissue-specific proteins**
  - **result in specific post-translational modifications**
  - **correlate with abnormal tissue metabolism**

Specific and systemic protein and PTM biomarkers exist simultaneously
MS + proteomics offers a powerful means to characterize these changes simultaneously

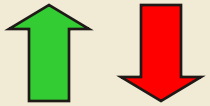
Cardiovascular Proteomics Center

Protein ID via database searching



Protein Quantitation
MS and other methods

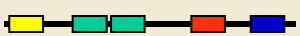
Measurement of differential expression



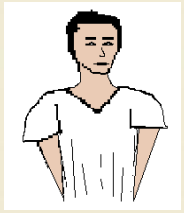
Proteomics

Characterization of PTMs

Correlation to genome

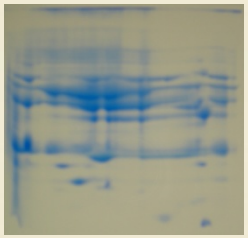


Correlation to phenotype



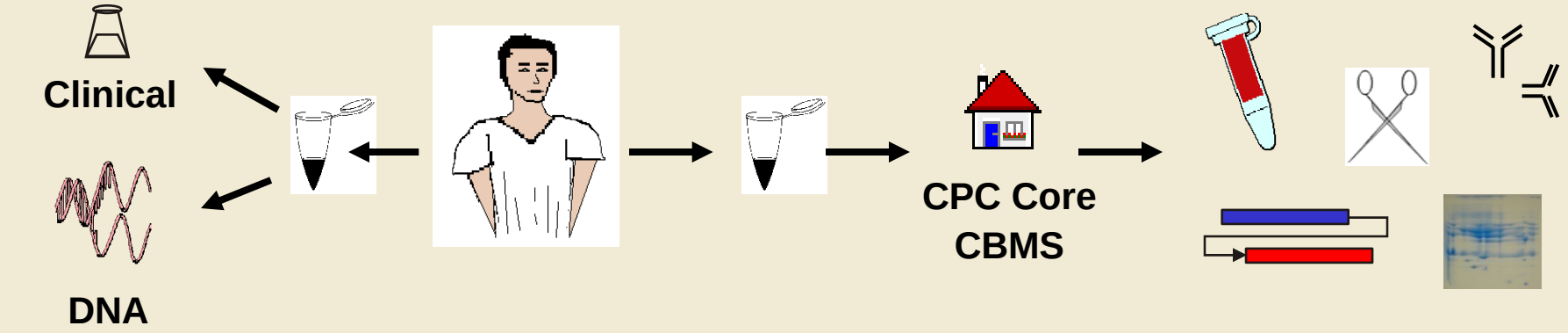
Advanced computational analysis

Multi-dimensional separation/ fractionation



Proteomics has evolved from protein identification to encompass a large number of fields of fundamental and applied sciences

MS Based Proteomics: A One Size Fits All Methodology



MS Results

ESI + MALDI MS	⟷	Mass of intact protein
ESI + MALDI MS	⟷	Peptide mass mapping
ESI + MALDI MS/MS	⟷	Sequence/ PTM id.
LC MS/MS	⟷	Automated sequencing/ id.
MRM	⟷	High throughput screening

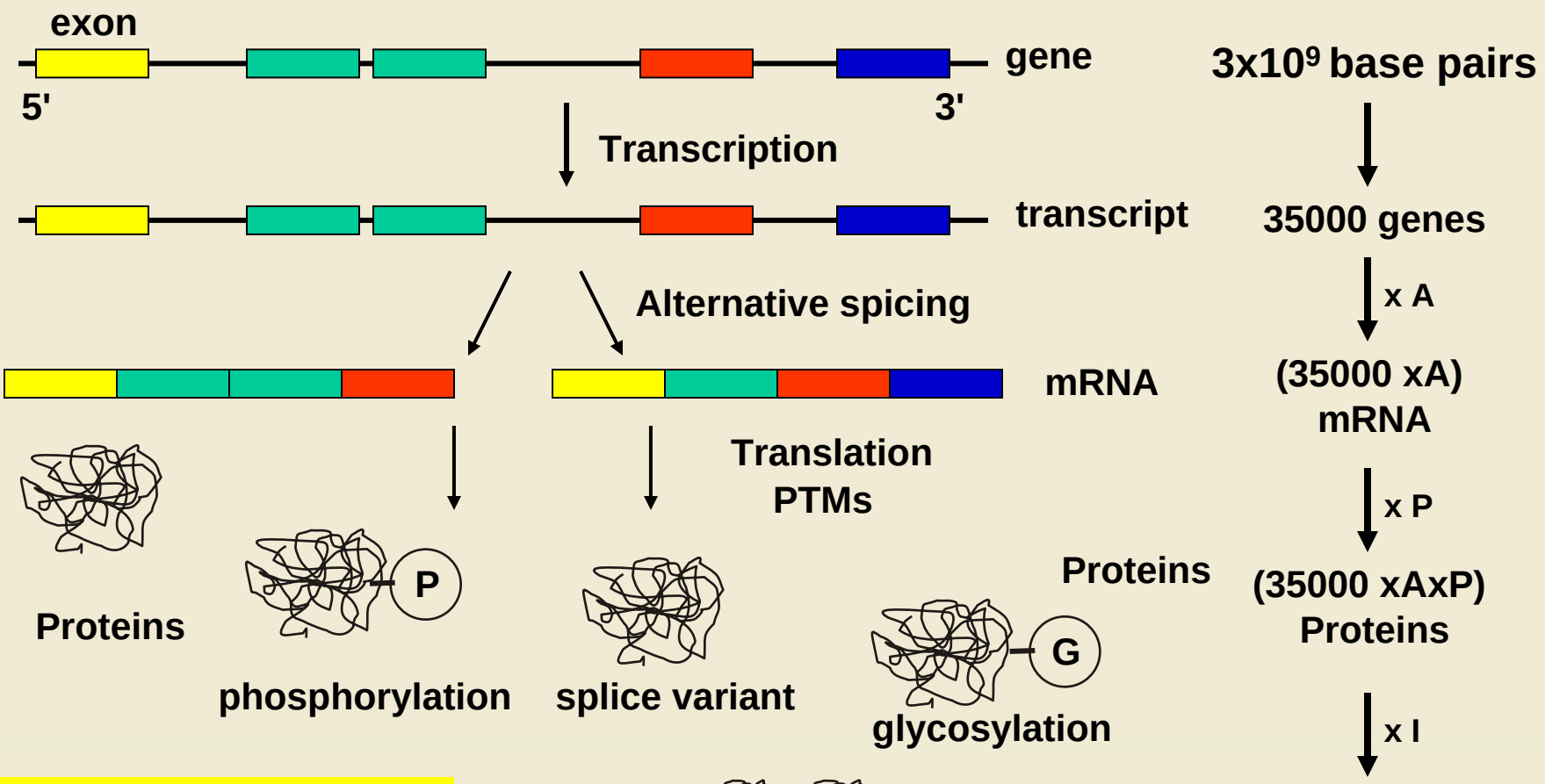
Protein Processing

↓

MS + MS/MS

Different approaches yield increasingly accurate results
Speed + Sensitivity, direct protein characterization
Post-translational modifications and unambiguous sequence determination
Correlate MS and MS/MS data with other analysis

From Genes to Proteins



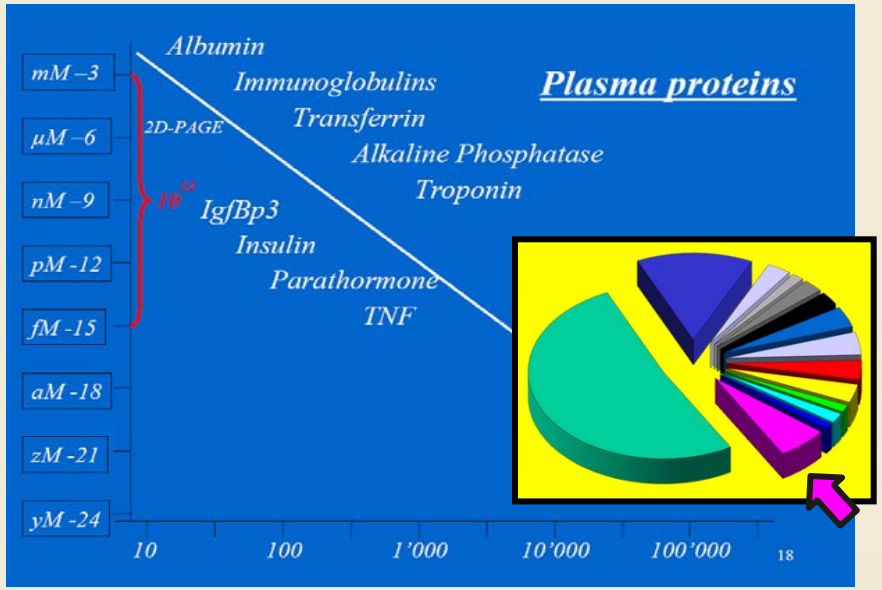
Proteins, protein interactions and post-translational modifications increase the complexity of the proteome.

J. Mass Spectrom. 36, 1083-1091, 2001
Mol. Cell Prot. 3, 311-326 (2004)

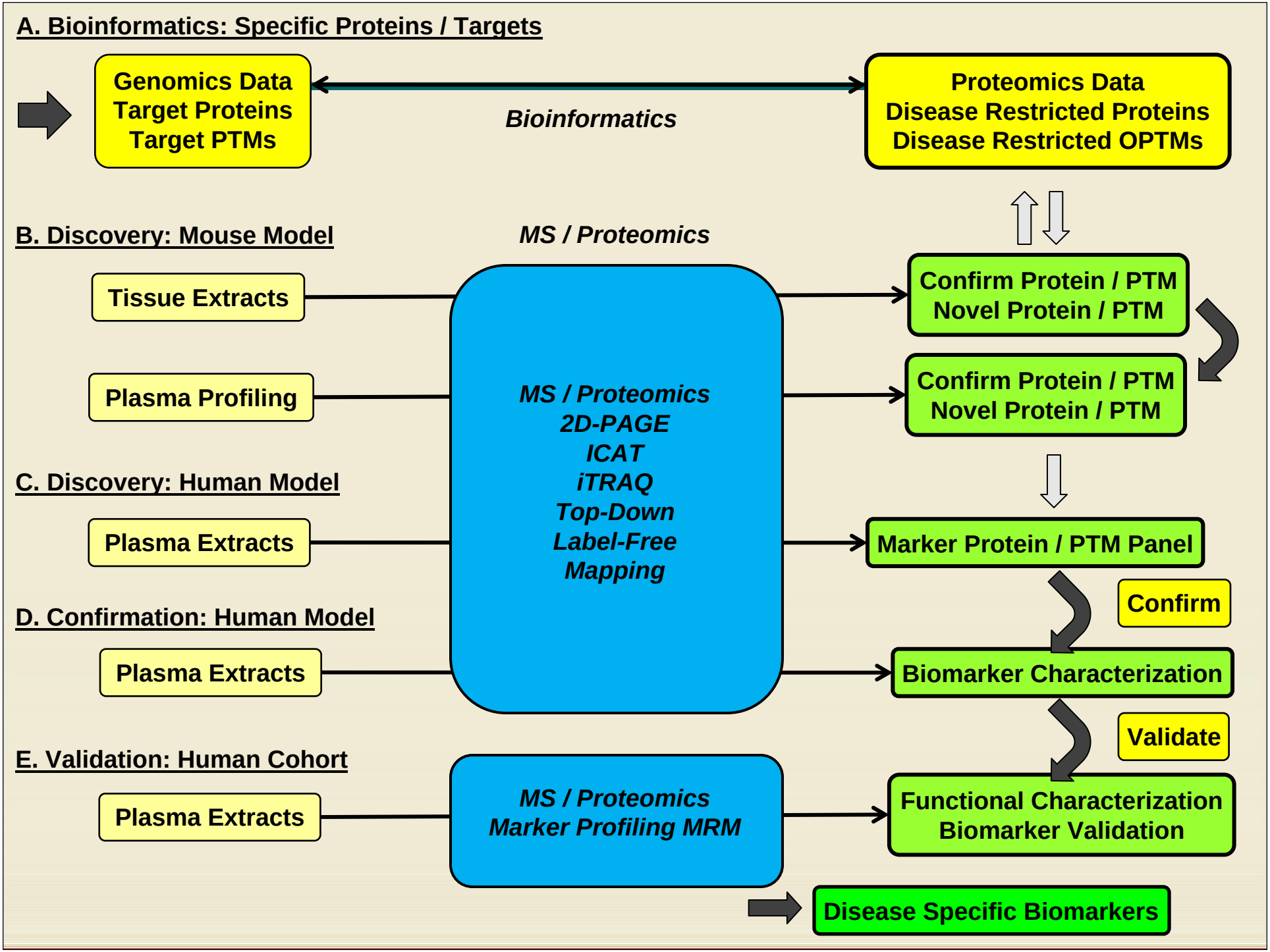
Discovery Based Plasma Proteomics: Tread Carefully

- Experimental Design
- Sample Collection
- Storage
- Abundant Protein Removal
- Pre-fractionation
- Secondary fractionation
- 2D separation
- Quantitation via Abs/Fl/Ab
- ID via MS

>10,000 proteins over 10^{-3}M to 10^{-12}M expression



Plasma: an exceptional problem due to the dynamic range/diversity of proteins
Strict attention to detail must be made in all aspects of experimental design
Post discovery, other methods of confirmation and validation are mandatory


A. Bioinformatics: Specific Proteins / Targets
Genomics Data
Target Proteins
Target PTMs
Bioinformatics
Proteomics Data
Disease Restricted Proteins
Disease Restricted OPTMs
B. Discovery: Mouse Model
MS / Proteomics
Tissue Extracts
Plasma Profiling
C. Discovery: Human Model
Plasma Extracts
D. Confirmation: Human Model
Plasma Extracts
MS / Proteomics
2D-PAGE
ICAT
iTRAQ
Top-Down
Label-Free
Mapping
Confirm Protein / PTM
Novel Protein / PTM
Confirm Protein / PTM
Novel Protein / PTM
Marker Protein / PTM Panel
Confirm
Biomarker Characterization
Validate
Functional Characterization
Biomarker Validation
E. Validation: Human Cohort
Plasma Extracts
MS / Proteomics
Marker Profiling MRM
Disease Specific Biomarkers

Pilot Projects: Human Models of Metabolic Disease

- **Bed rest and diet induced insulin resistance (Vita/Cohen):**
  - **40 subjects: 20 control, 20 treated with anti-inflammatory**
  - **+/- salsalate: inhibitor for NFkB and insulin resistance**
  - **Samples before and after 5 days of bed rest**

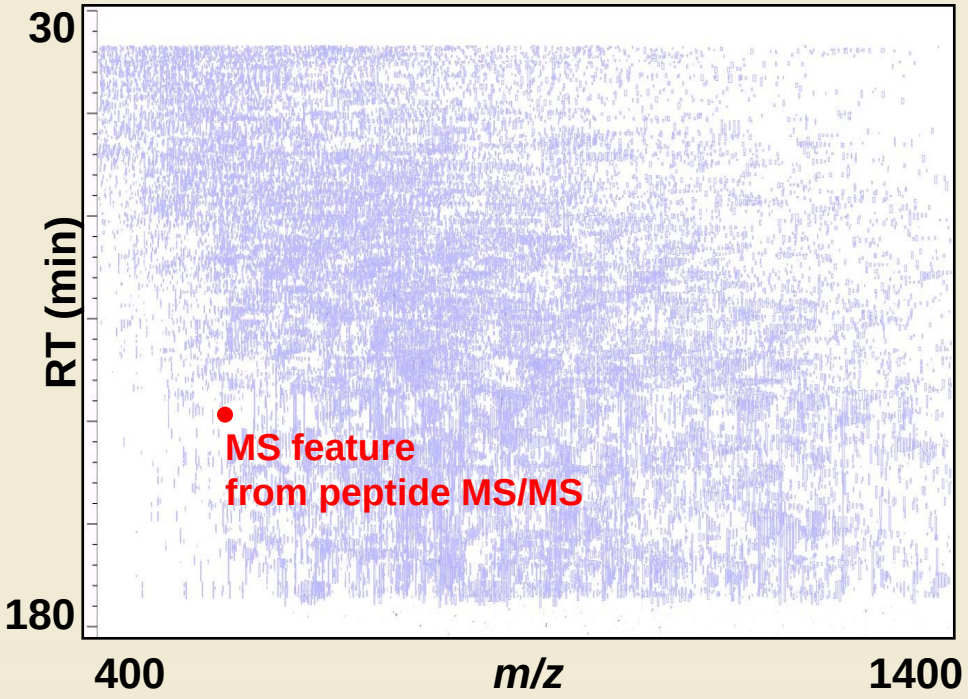
- **Heart failure in mice to men (Colucci):**
  - **80+ subjects: 40 control, 40 with heart failure**
  - **Paired samples: de-compensation, re-compensation**
  - **Transgenic mouse: overexpression of growth factor driven G-protein (Gq-mouse) shows significant changes in plasma peptides**

- **Scleroderma induced pulmonary hypertension (PAH) (Lafyatis):**
  - **Patients with scleroderma +/- PAH and controls**
  - **Microarray data mRNA on blood monocytes shows upregulation of serum biomarkers including inflamatory cytokines (IL-6, TNF-apha)**

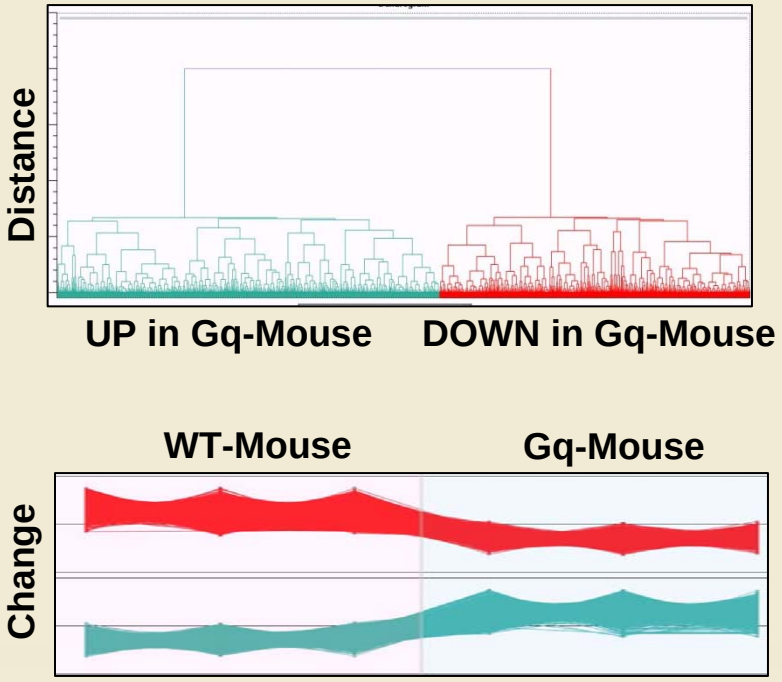
Can we correlate proteomics with phenotype, genomics and other markers?
Underlying theme of metabolic disease: can we see a correlation between models?

Pilot Project: Gq-Mouse Model of Heart Failure: n=3 across 2 groups

Label-Free LC-MS/MS Differential Analysis



Clustering and Change in Features



1º data analysis
30,292 Features: 2,584 Anova <0.05

10,120 MS/MS: 9,521 peptides, 1,363 proteins

➡

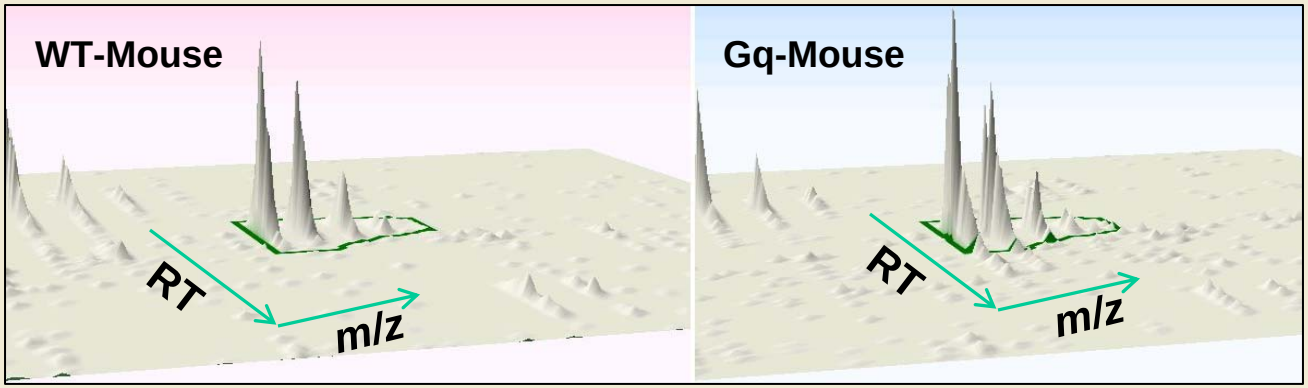
Peptide expression
364 (232) UP, 525 (289) DOWN

Protein expression
95 (97) UP, 119 (34) DOWN

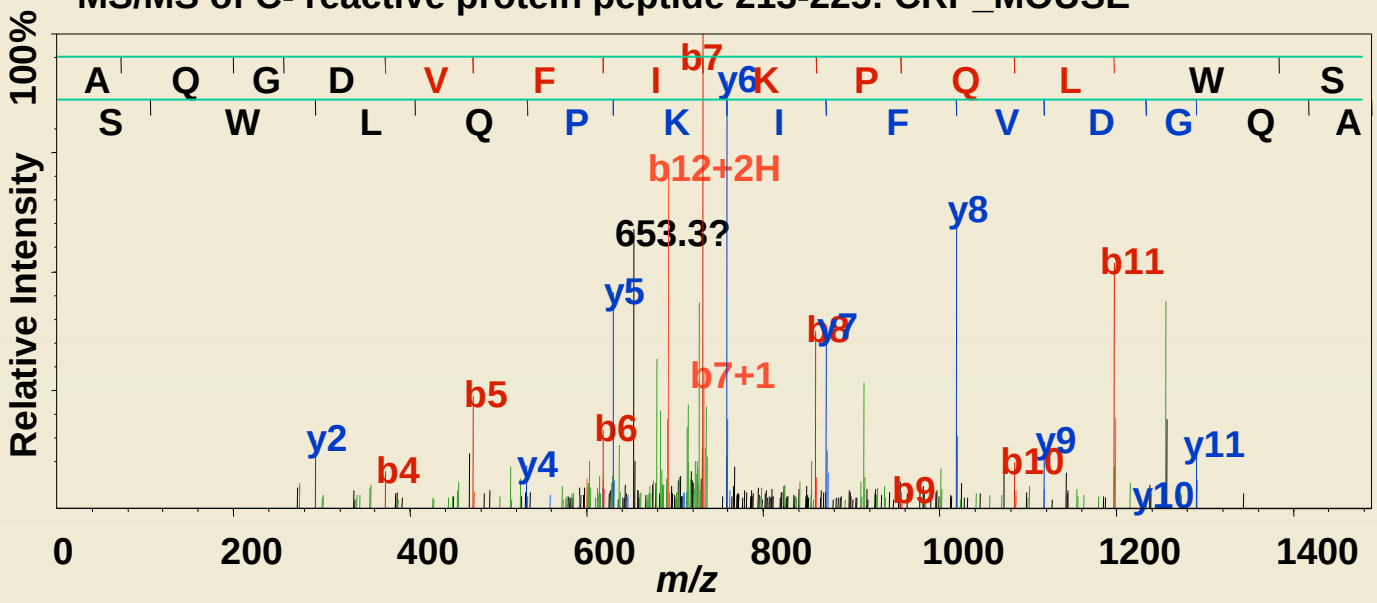
Differential peptide/protein/PTM expression all point towards potential markers

Known “Biomarker” CRP Peptide/ Protein Expression Changes

LCMS Feature: Precursor peptide ion for CRP_MOUSE



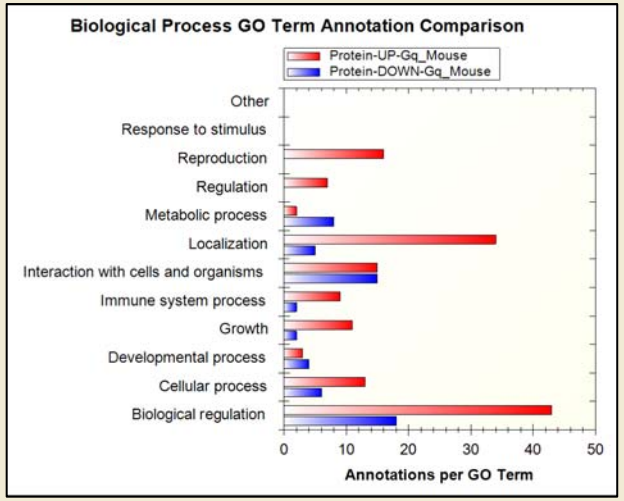
MS/MS of C- reactive protein peptide 213-225: CRP_MOUSE

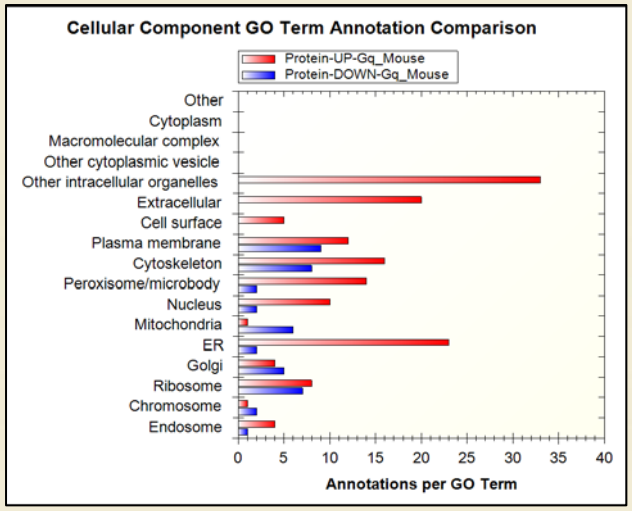


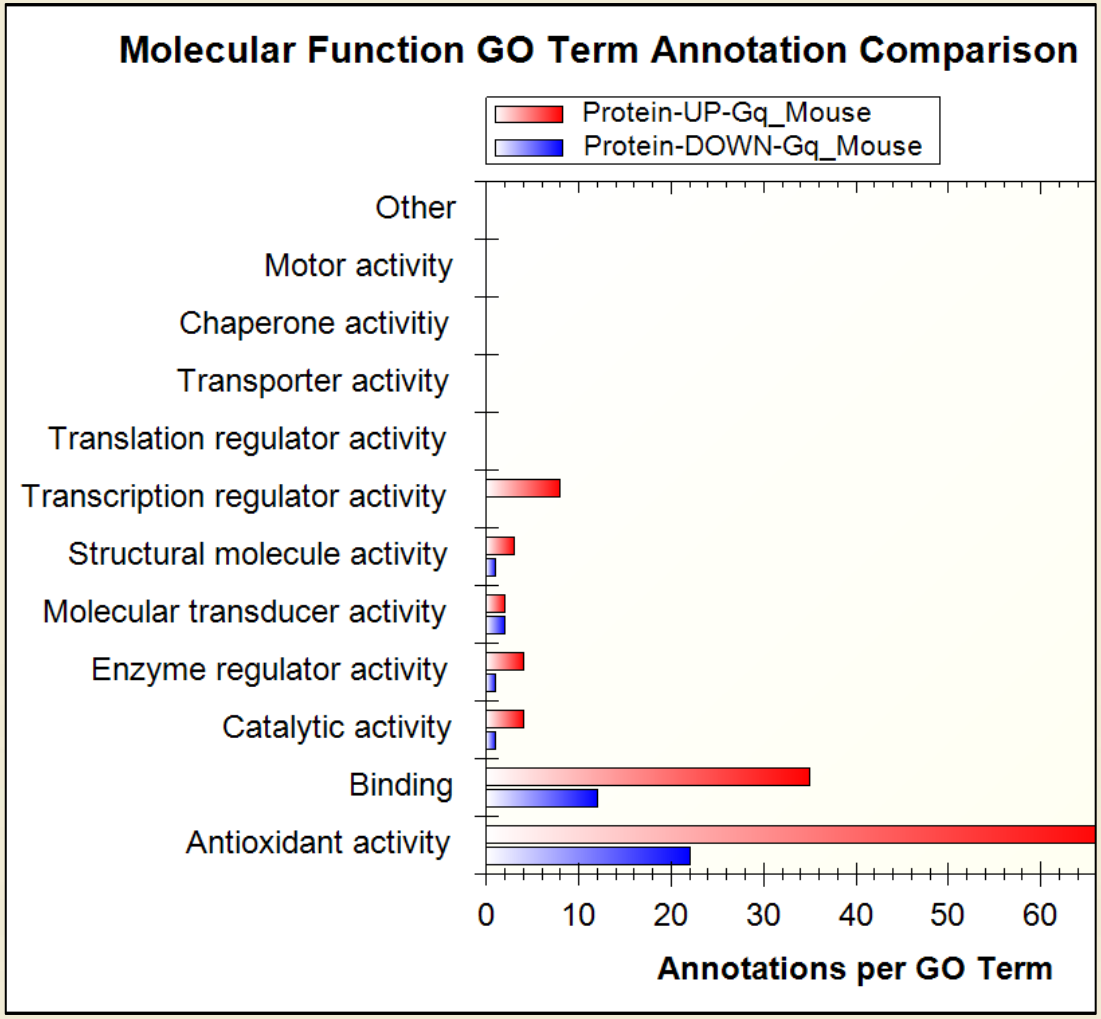
CRP: associate with increased risk of CVD, diagnostic/prognostic values limited
Known markers validate approach, multiple peptides observed, including PTMs

Global Changes Observed in Gq-Mouse Model

GO Term Enrichment: Biological Processes, Cellular Component and Molecular Function



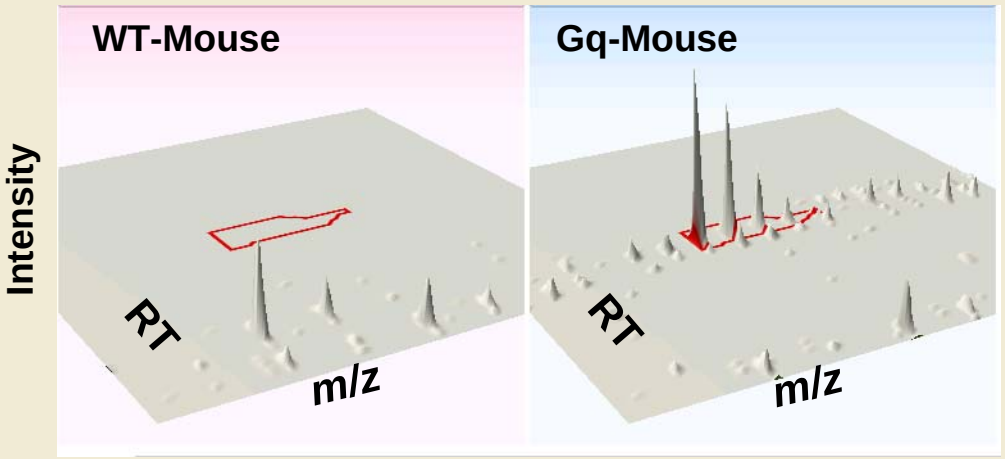




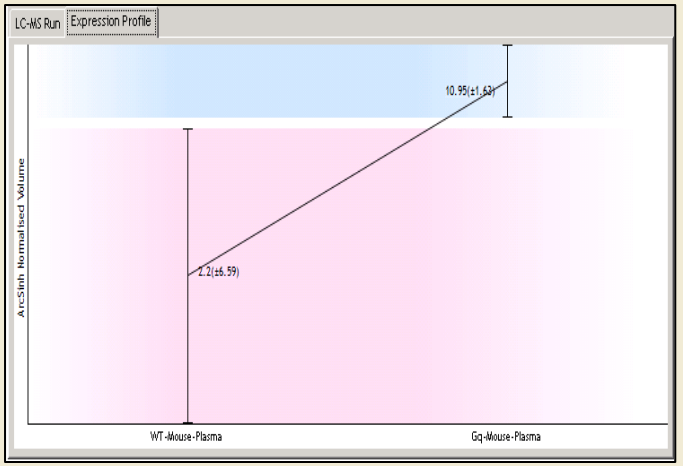
Rather than limit ourselves to one marker, we may gain incite into global changes

MHC7 Differential Expression in Gq-Mouse Model

LCMS Feature: Precursor peptide for MYH7_MOUSE



Expression Change: ANOVA <0.05

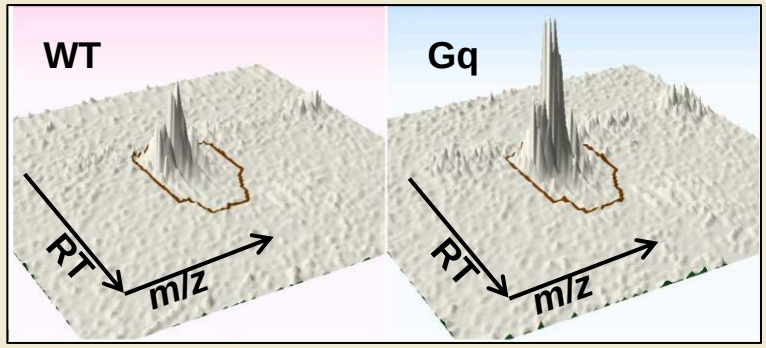


#	Max fold	Anova	Score	Protein	Sequence	PTM	UP	DN	F-2	F-10	F-Inf	Description
15256	2.09	0.028	11.81	MYH1_MOUSE	HDCDLLREQYEEEQEAKAELQR	X	x		x			Myosin-1, Mus musculus (Mouse)
21441	2.61	0.003	5.6	MYH14_MOUSE	TPNVGGPGGPQVEWTAR			x	x			Myosin-14 - Mus musculus (Mouse)
13779	5.09	0.001	10.44	MYH3_MOUSE	SEFKLEIDDLSSSVESVSK			x	x			Myosin-3 - Mus musculus (Mouse)
14416	323.01	0.018	4.19	MYH7_MOUSE	MCRTLEDQMNEHR	X	x			x		Myosin-7 (Myosin heavy chain 7) (Myosin heavy chain, cardiac muscle beta isoform) (MyHC-beta) (Myosin heavy chain slow isoform) (MyHC-slow) - Mus musculus (Mouse)
54895	11.31	0.016	12.5	MYO7A_MOUSE	HEPINHSDMVDK		x			x		Myosin-VIIa - Mus musculus (Mouse)

Specific protein isoforms relative to theoretical targets are observed to change
PTMs associated with oxidative stress also show significant changes in abundance

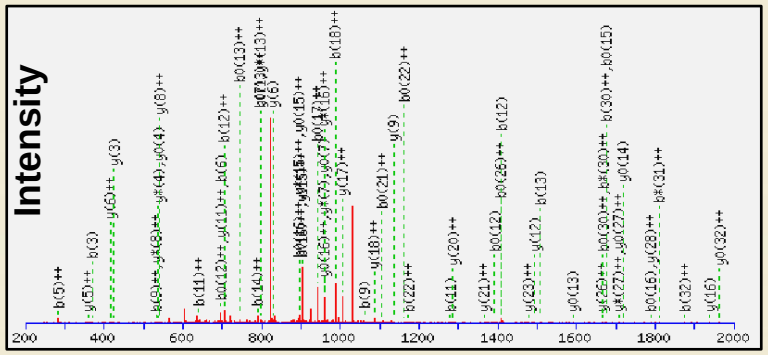
Specific PTMs Associated with Oxidative Stress

Increase in HNE Modified Albumin Peptide



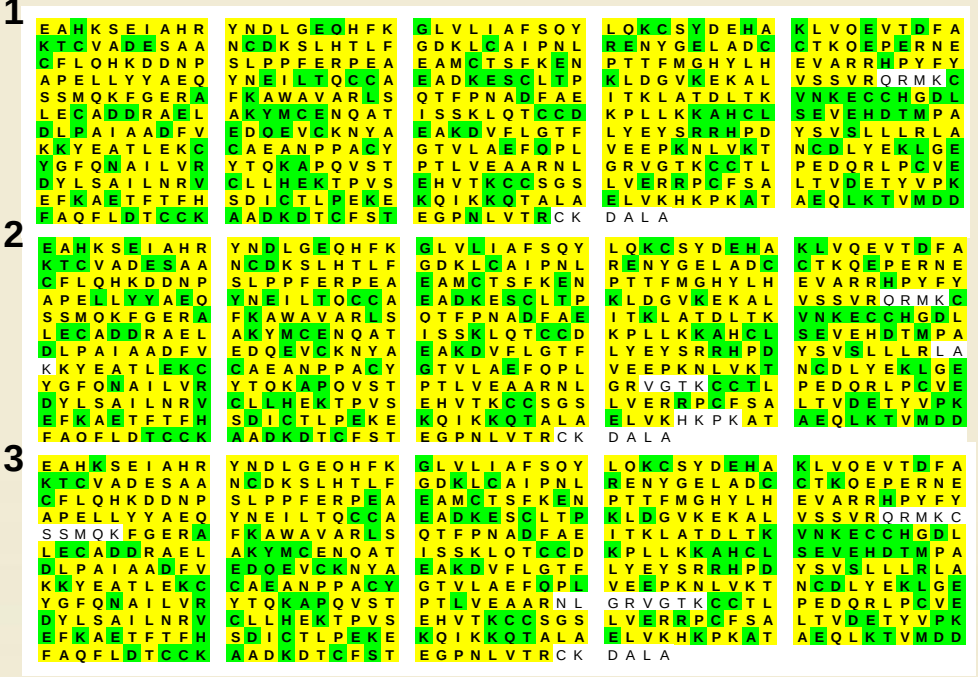
MS/MS of HNE Modified Peptide

AHcLSEVEHDTMPADLPAIAADFVEDQEVckNYAEAKDVFLGTFLYEYSR



m/z

OPTM Mapping: Gq-Mouse ALBU_MOUSE Sequence



Global changes in PTMs map to specific locations on protein sequences
Select PTMs correlate well with other models of oxidative stress: GenMod SC+PAH

Going Forward

- Continue to foster discussion in the realm of biomarkers
  - Rapidly changing field with endless potential for discovery
  - Investigate alternative approaches, methodology, models
- Near term evaluation of pilot projects
  - Identify both protein and PTM changes associated with each model
  - Correlate proteomics data with ancillary data
  - Perform cross project comparison
  - Meta-analysis comparison with different models of CVD
- Build new collaborations
  - All are welcome

Develop a CVD specific protein and PTM panel of putative markers

Acknowledgments

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- Robin MacDonald
- Katya Ravid
- All the enthusiastic members of the Biomarkers ARC