THE biodesign INSTITUTE
ARIZONA STATE UNIVERSITY

High Throughput Mass Spectrometric Immunoassays in Human Plasma Profiling and Targeted Protein Analysis

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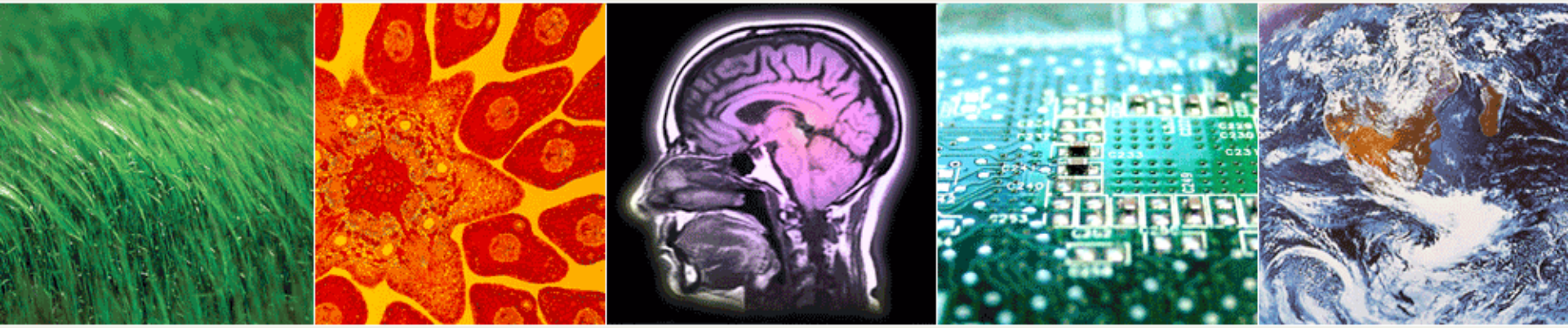
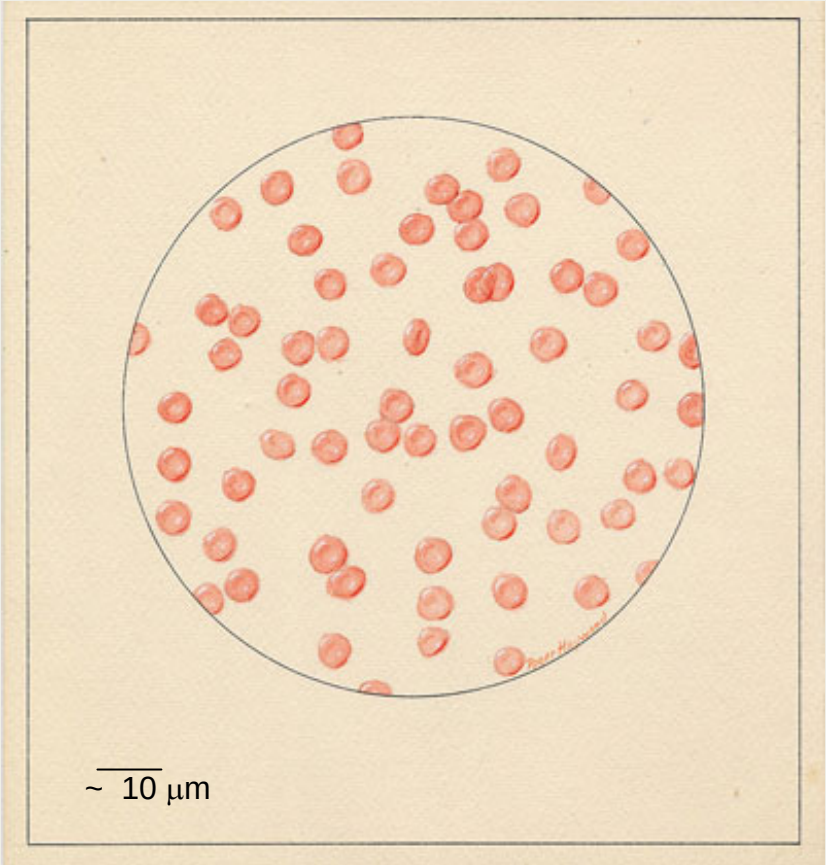
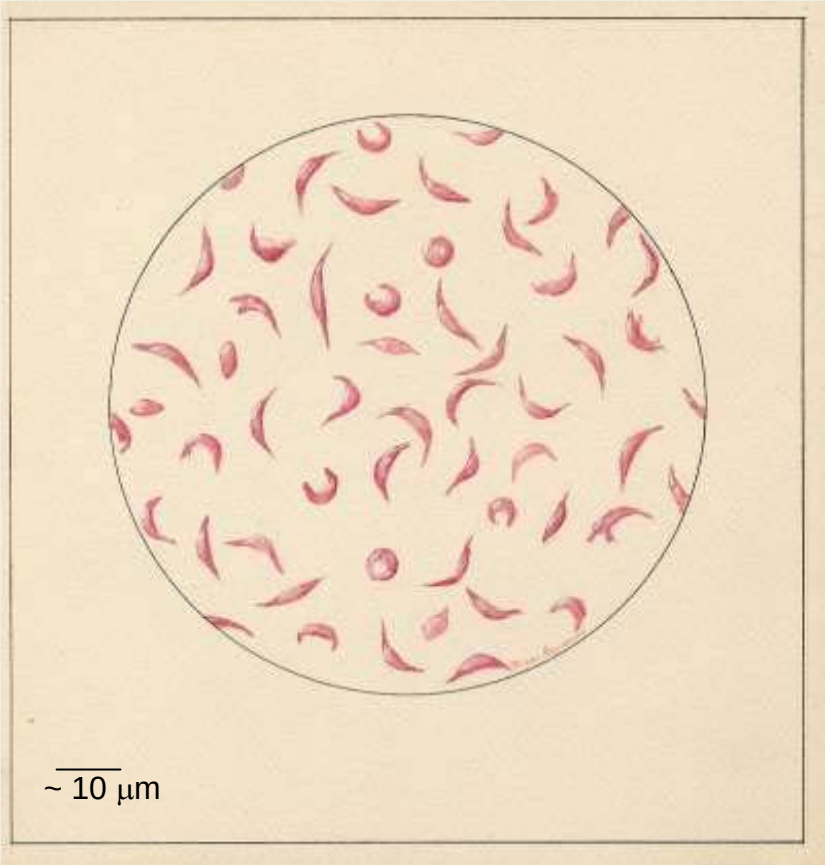
Center for Systems and Computational Biology

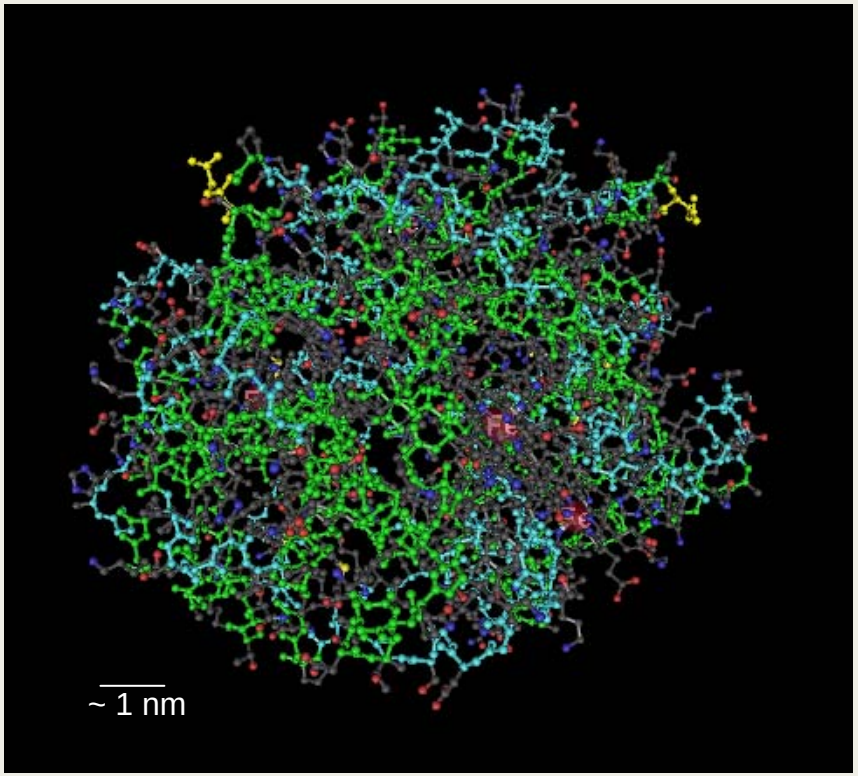


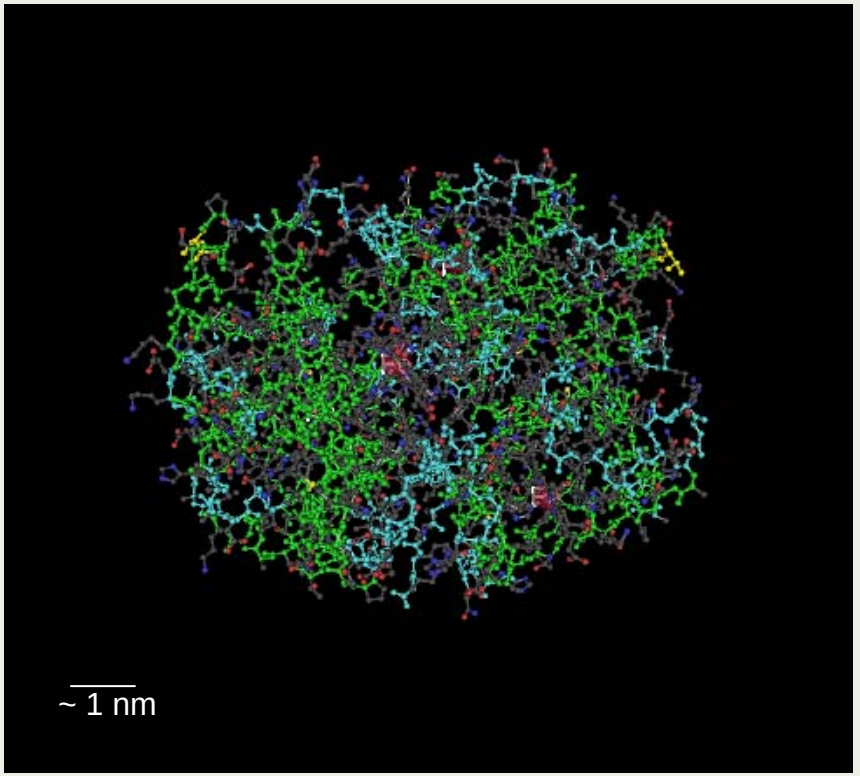




- Sickle Cell Anemia
  - ~ 2 million individuals world-wide
  - ~ 72,000 in US (~ 2 million carriers)
  - Mortality in neonates from opportunistic infection
    - Screening (in > 40 States) and prophylactic penicillin

Nano-Biotechnology (Genome-scale)





- Genetic Disorder
  - "Single Nucleotide Polymorphism" (SNP)
    - The change of one base in $3x10^9$
  - Results in "Point Mutation" (PM)
    - Glutamic acid – to - Valine

Subnano-Biotechnology (Atomic/Molecular-scale)

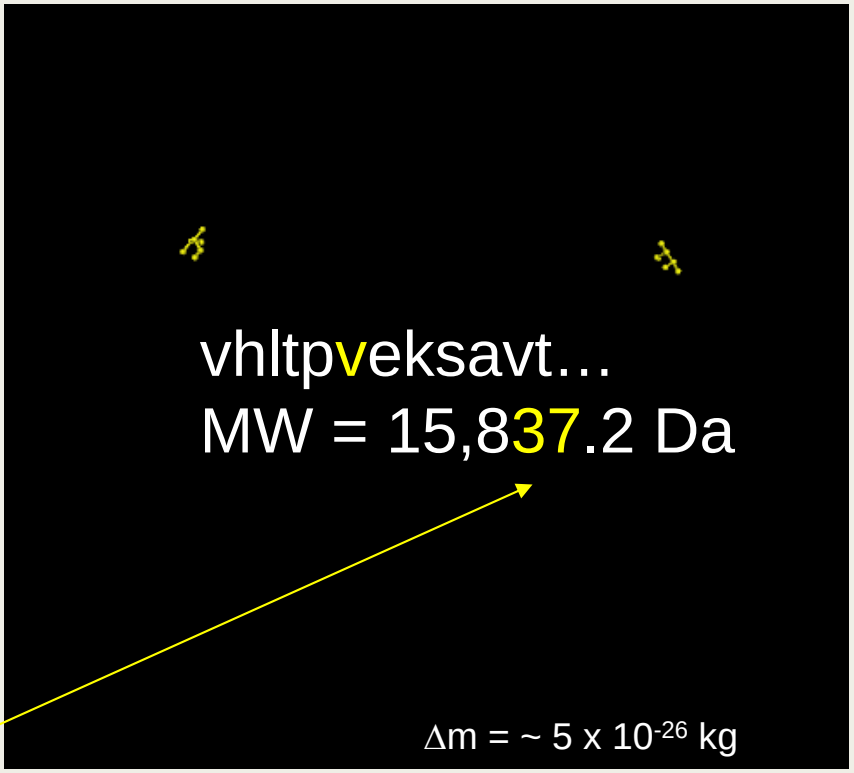
Hem A:
vlspadktnvkaawgkvgahageygaealermflsfpttktyfphfdlshgsaqvkghgkkvadaltnavahvddmpnalsalsdlhahklrvdpvnfkllshcllvtlaahlpaeftpavhasldkflasvstvltskyr

Hem B:
vhltpeeksavtalwgkvnvdevggealgrllvvypwtqrffesfgdlstpdavmgnpkvkahgkkvlgafsdglahldnlkgtfatlselhcdklhvdpenfrllgnvlvcvlahhfgkeftppvqaayqkvvagvanalahkyh

Hem A (Sickle Cell):
vlspadktnvkaawgkvgahageygaealermflsfpttktyfphfdlshgsaqvkghgkkvadaltnavahvddmpnalsalsdlhahklrvdpvnfkllshcllvtlaahlpaeftpavhasldkflasvstvltskyr

Hem B (Sickle Cell):
vhltpveksavtalwgkvnvdevggealgrllvvypwtqrffesfgdlstpdavmgnpkvkahgkkvlgafsdglahldnlkgtfatlselhcdklhvdpenfrllgnvlvcvlahhfgkeftppvqaayqkvvagvanalahkyh

Equate with an Intrinsic Property – Molecular Mass



Hem A:
vlspadktnvkaawgkvgahageygaealermflsfpttktyfphfdlshgsaqvkghgkkvadaltnavahvddmpnalsalsdlhahklrvdpvnfkllshcllvtlaahlpaeftpavhasldkflasvstvltskyr

Hem B:
vhltpeeksavtalwgkvnvdevggealgrllvvypwtqrffesfgdlstpdavmgnpkvkahgkkvlgafsdglahldnlkgtfatlselhcdklhvdpenfrllgnvlvcvlahhfgkeftppvqaayqkvvagvanalahkyh

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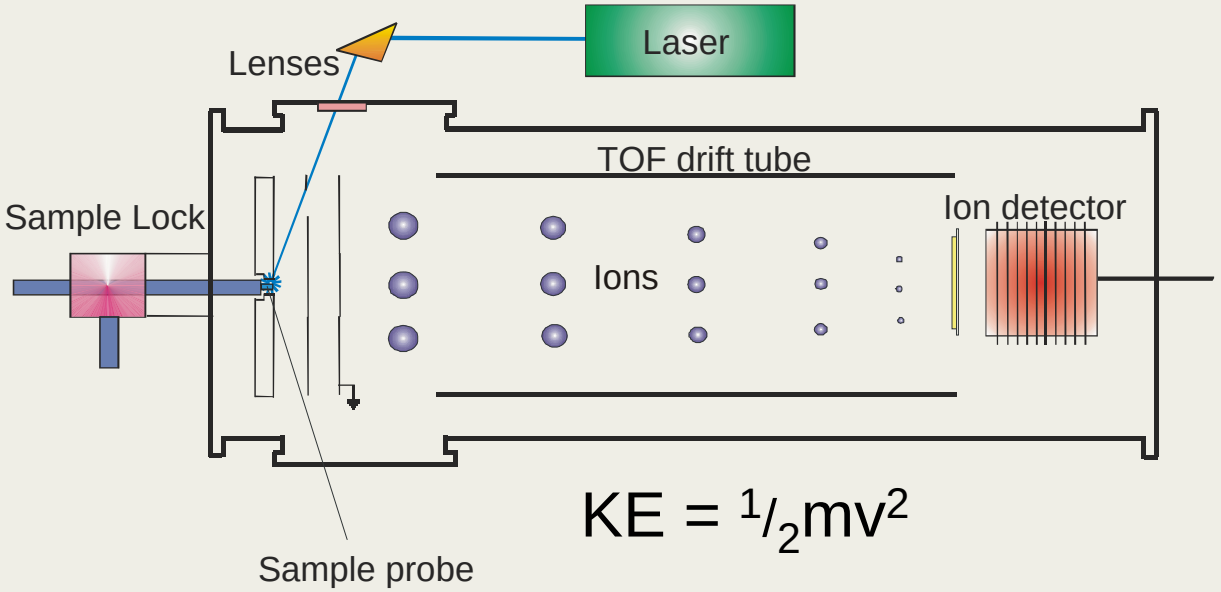
vlspadktnvkaawgkvgahageygaealermflsfpttktyfphfdlshgsaqvkghgkkvadaltnavahvddmpnalsalsdlhahklrvdpvnfkllshcllvtlaahlpaeftpavhasldkflasvstvltskyr

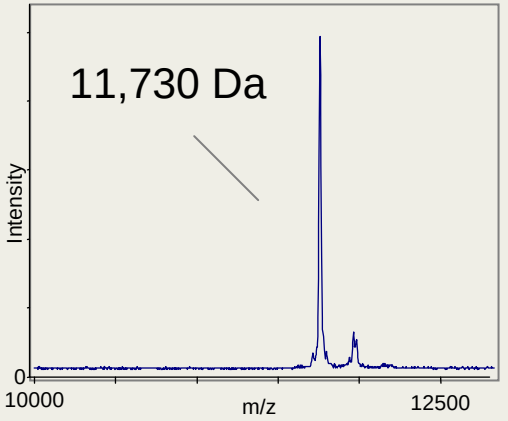
Hem B (Sickle Cell):
vhltpveksavtalwgkvnvdevggealgrllvvypwtqrffesfgdlstpdavmgnpkvkahgkkvlgafsdglahldnlkgtfatlselhcdklhvdpenfrllgnvlvcvlahhfgkeftppvqaayqkvvagvanalahkyh

An Enabling Technology (ca. 1990)

- Matrix-Assisted Laser Desorption/Ionization (MALDI)
  - Delayed Extraction-Time of Flight Mass Spectrometry (DE-TOFMS)
  - Delayed Extraction-Reflectron Time of Flight Mass Spectrometry (DE-reTOFMS)
  - Delayed Extraction-Time of Flight Mass Spectrometry^2 (DE-TOF/TOFMS)



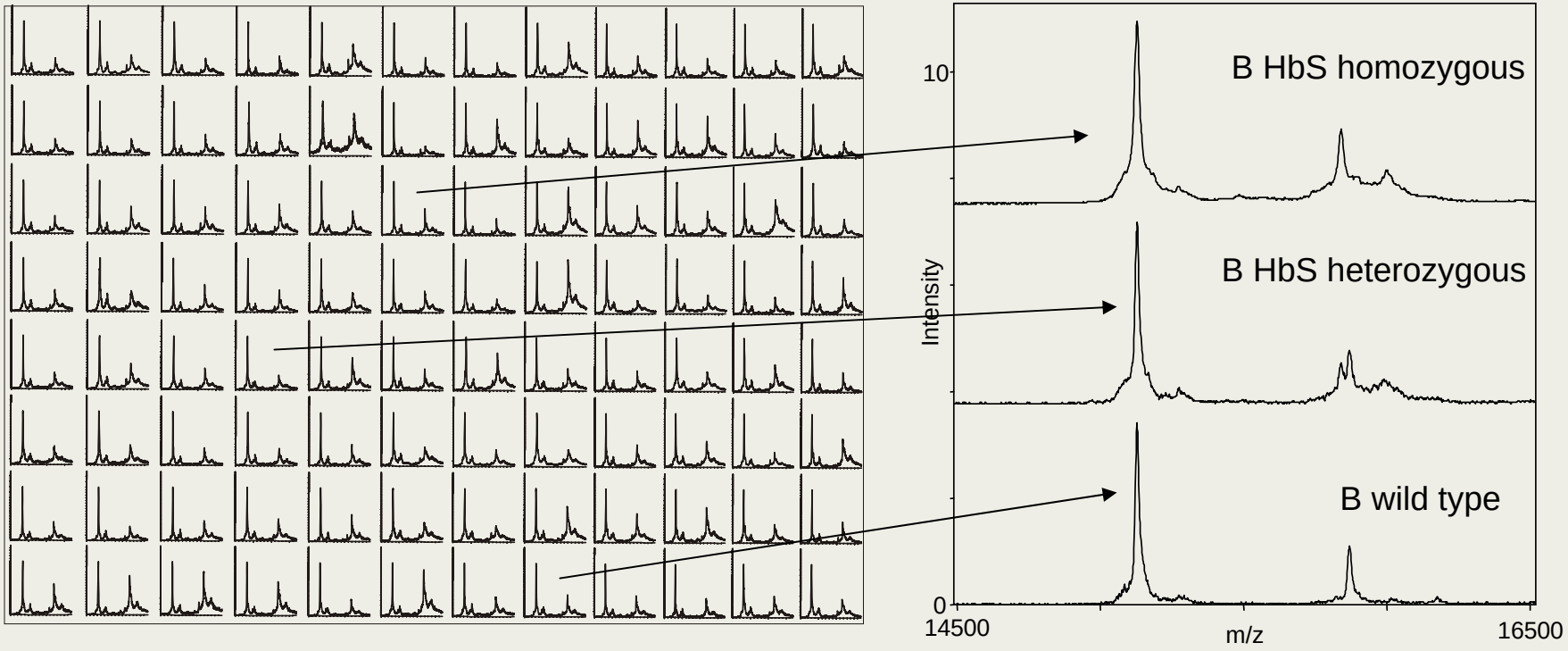
$$KE = {}^{1}/_{2}mv^{2}$$



- Fast: ~ 5-minute from sample-to-data
- Sensitive: 10^8 – 10^{10} molecules into instrument
- Multiple proteins/peptides in a single spectrum
- Accurate: Direct measure of protein @ exact MW

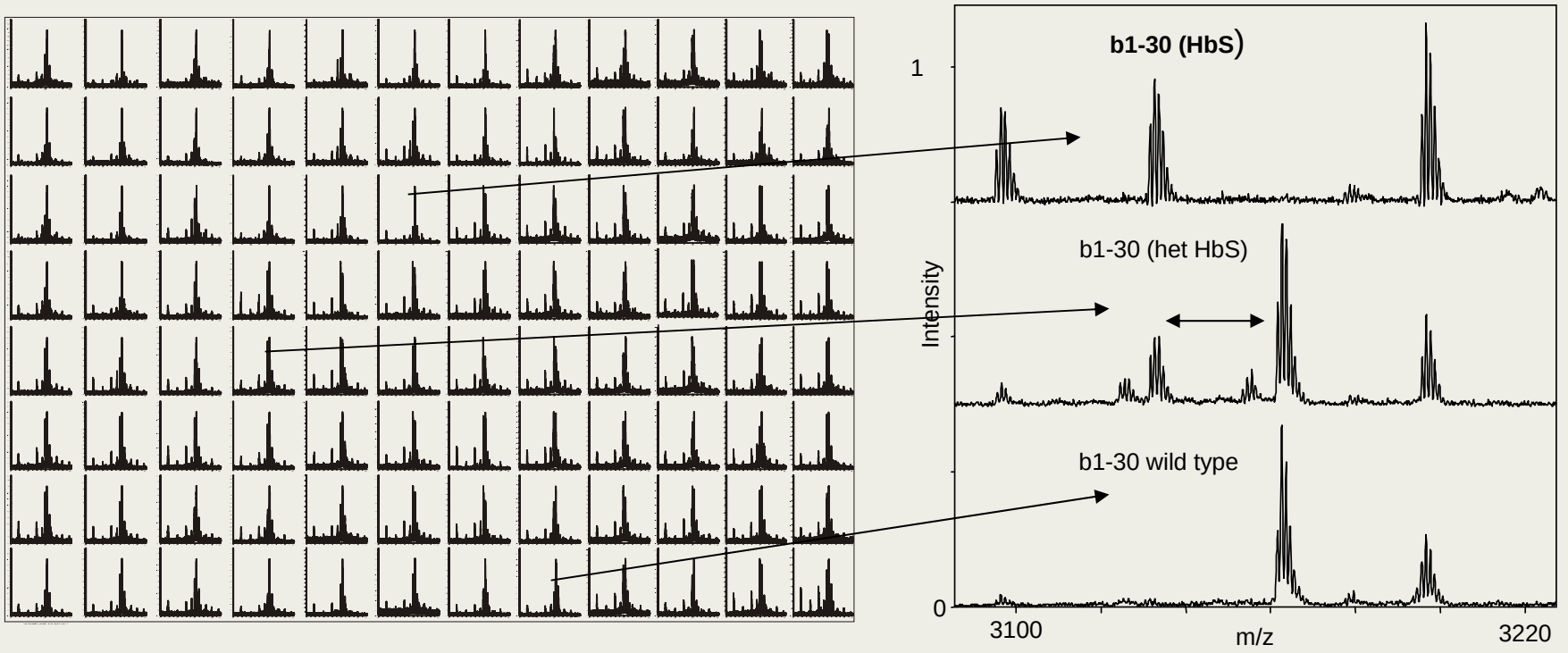
Neonate Screening – MALDI-TOFMS for Intact MW

- Neonatal Screening – predominantly HbF (a-, b- and g chains)
  - HbS, HbC, HbE (~ 700 known mutations)
  - Blotter spots (Equivalent of ~ 0.1 uL WB)
  - Qualitative Screen – MALDI-TOFMS



Neonate Screening – Mass Map for Location of PM

- Neonatal Mass Mapping
  - Map with trypsin-active targets (~15 minute digest)
  - View b-chain 1-30 signal
  - 3/95 HbS heterozygous: 1/95 HbS homozygous



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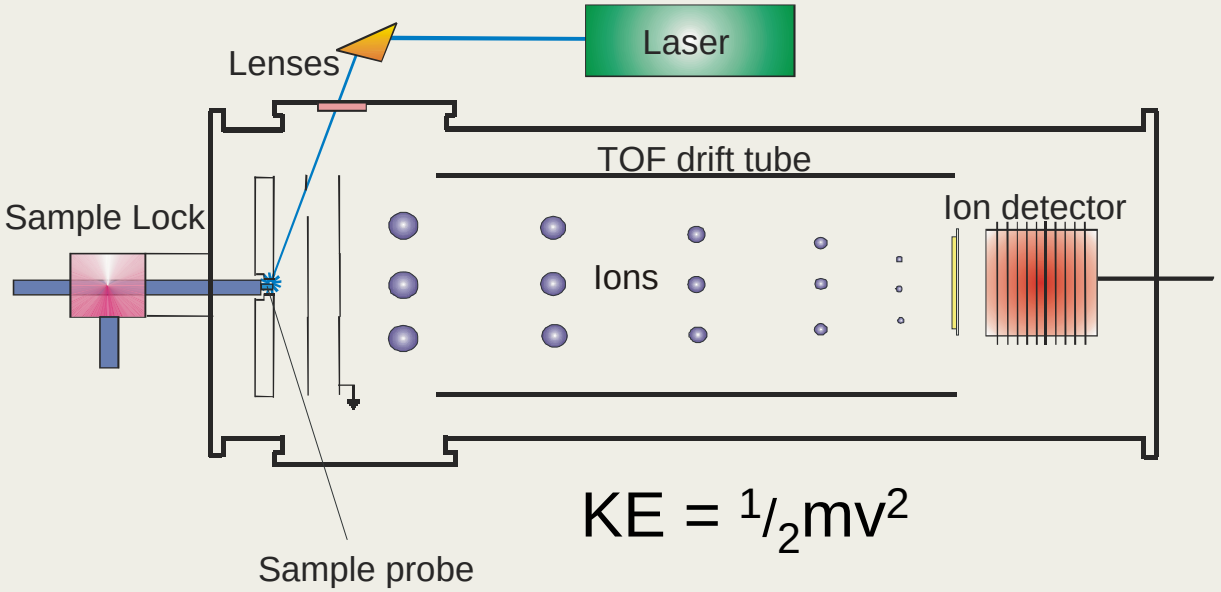
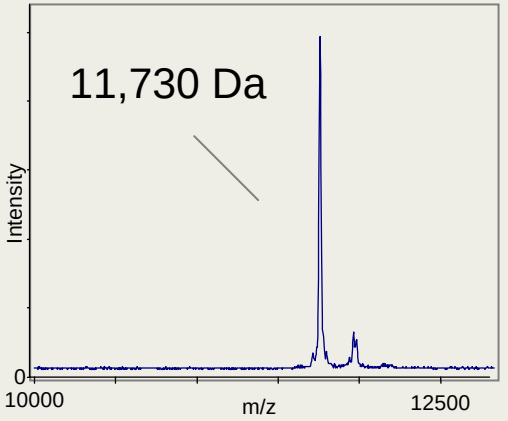




$$KE = {}^{1}/_{2}mv^{2}$$

The use of mass spectrometry to analyze human proteins is not the issue.
Making the technical interface with thousands of Individuals.
Formation of Intrinsic Bioprobes, Inc.

Intrinsic Bioprobes, Inc. (IBI)

- Based in Tempe, AZ (Arizona C Corporation)
- Founded 1996
- Spin-out of Arizona State University
- Privately Held
- Five-years Profitability
- Support from NIH/NSF (> 20 Grants & Contracts)
- > 20 Issued Patents; > 25 Pending
- ~ 50 Peer-reviewed Manuscripts
- 2005 Small Company Innovator of the Year: Arizona Governor's Celebration of Innovation

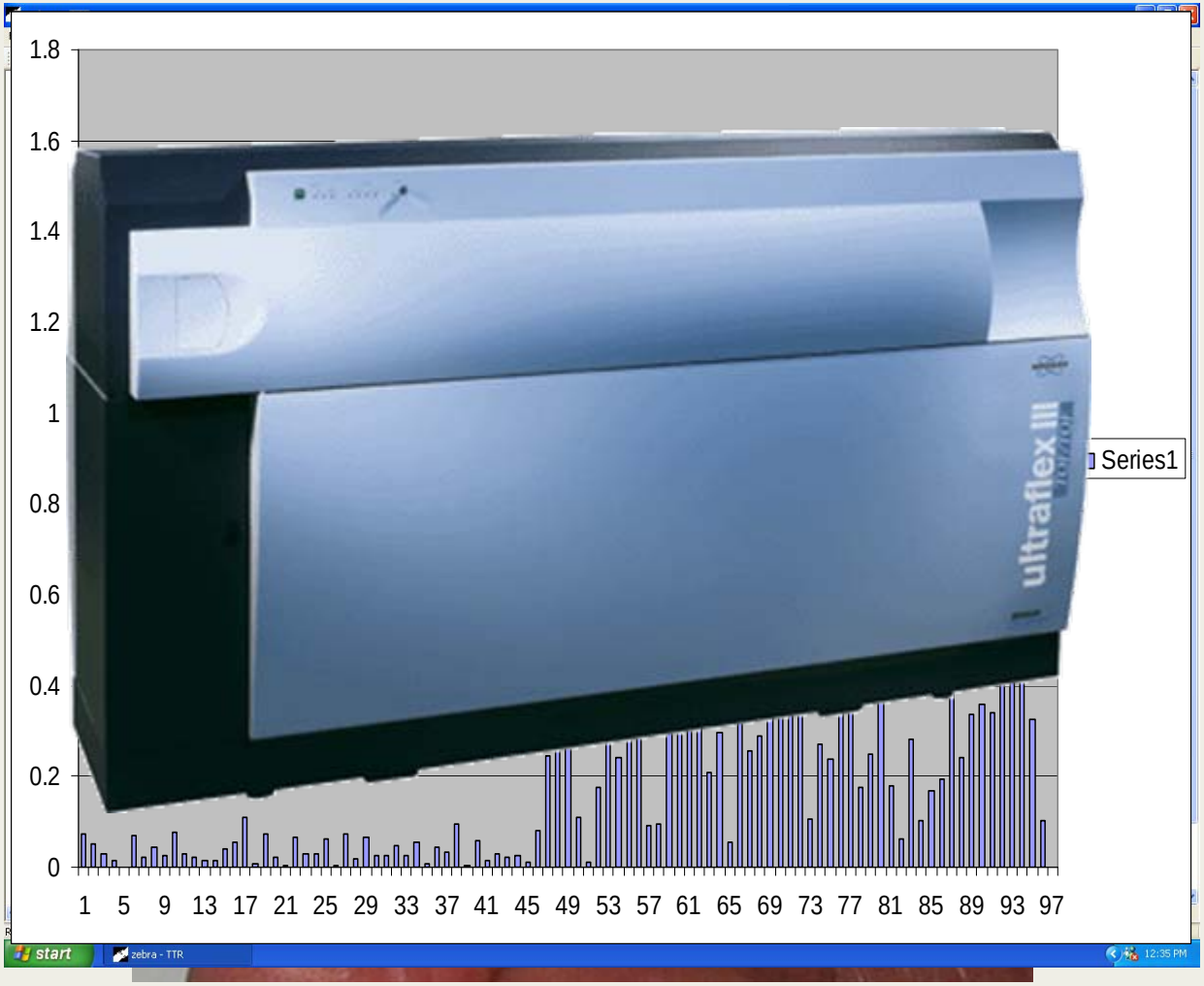
IBI's Enabling Technologies (ca. 2007)

MSIA™ + BRP™ + Robotics/MS

MASSAY® Integrated System

- Nelson, R. W., *et al*, Mass Spectrometric Immunoassay, Anal. Chem., 67, 1153 (1995).
- Dogruel, D., *et al*, Rapid tryptic mapping using enzymatically active mass spectrometer probe tips, Anal. Chem., 67, 4343 (1995).
- Niederkofler, E. E., *et al*, Determination of β-2-m levels in plasma using a high-throughput mass spectrometric immunoassay system, Anal. Chem., 73, 3294 (2001).
- Kiernan, U. A., *et al*., High-throughput protein characterization using mass spectrometric immunoassay, Anal. Biochem., 301, 49 (2002).

MASSAY® Workflow



Data Acquisition
Elute - Stamp
Prep for Elute
~ 15 minutes
~ 30 minutes

Technology Development – to – Application

- Technologies Originally Developed for Clinical/Diagnostic Application
  - Rigorous qualitative characterization between individuals
  - Quantification (variant-specific)
  - Multiplexed (multi-analyte) high information content analyses
  - Scaled and economical application
- Fills a "Technical Void" in Biomarker Discovery/Validation
  - Unique Outcome
  - Blind Spot between Proteomics (Peptides) and Diagnostics (ELISA)
- Conceptual and Technical basis for Population Proteomics

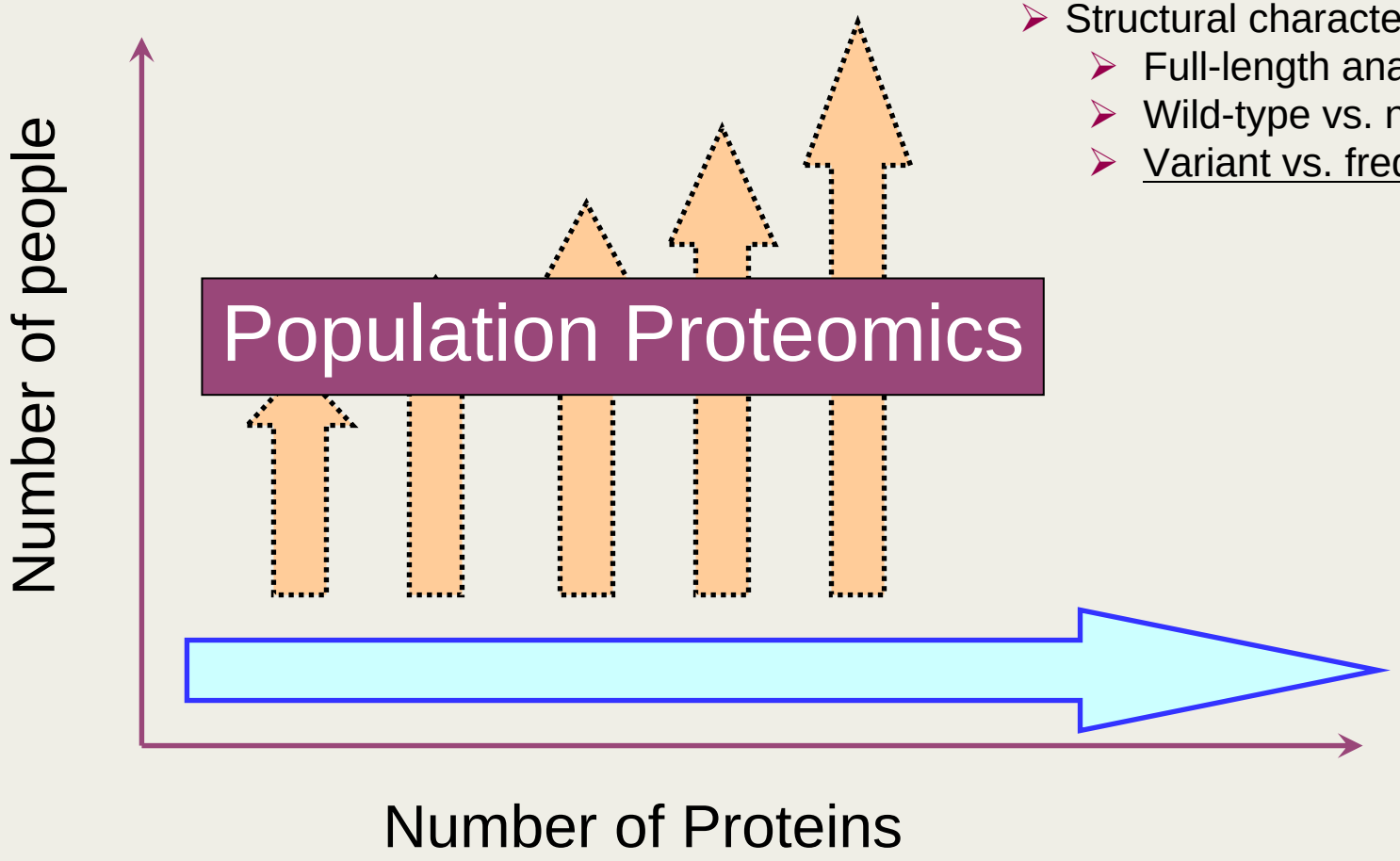
- **Nedelkov, D., Kiernan, U. A., Niederkofler, E. E., Tubbs, K. A., and Nelson, R. W., Investigating diversity in human plasma proteins, *PNAS* 102, 10852-857 (2005).**
- **Nedelkov, D., Kiernan, U. A., Niederkofler, E. E., Tubbs, K. A., and Nelson, R. W., Population proteomics: The concept, attributes, and potential for cancer biomarkers research. *Mol Cell Proteomics.* 5, 1811-1818 (2006).**

The Human Dilemma

- A protein is not necessarily the same species in or between individuals
  - Gene- and transcriptional-level modifications
  - Posttranslational modifications
  - Possibility of multiple, closely-related species (Microheterogeneity)

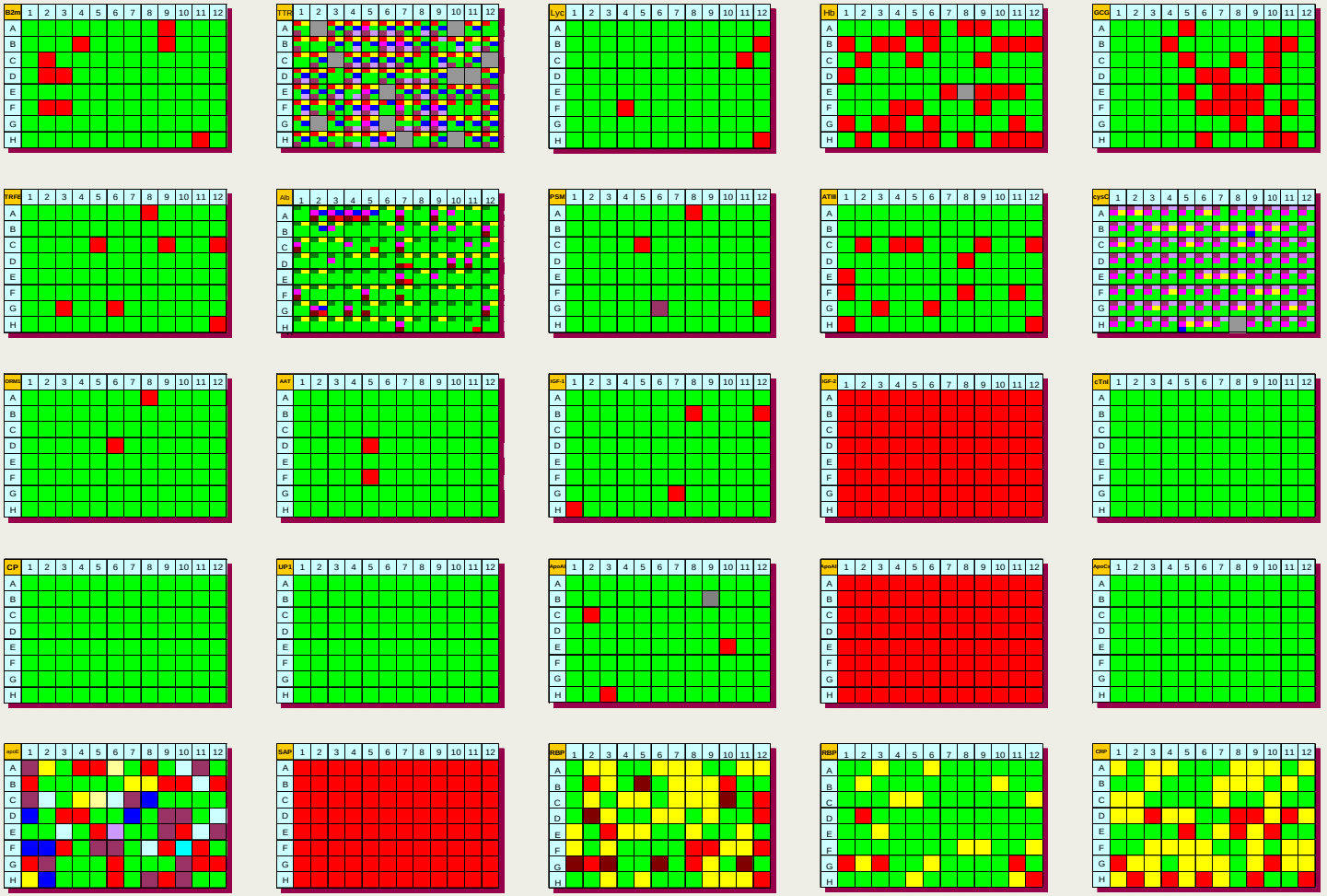
Population Proteomics: Goals

- Population Proteomics
  - Large # samples
  - Small - Large # proteins
- Structural characterization
  - Full-length analysis
  - Wild-type vs. non-wt
  - Variant vs. frequency



Overview of Results [PNAS 2005]

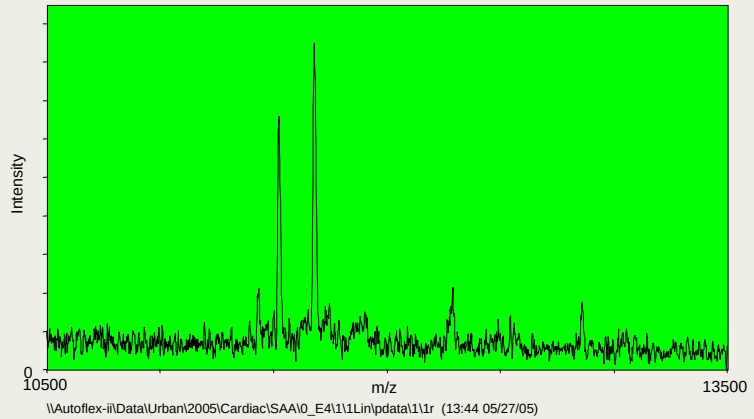
- 96 Healthy Individuals – 25 Plasma proteins – wt. / variant subsets
- > 80 variants observed in Population: 1- 100% frequency

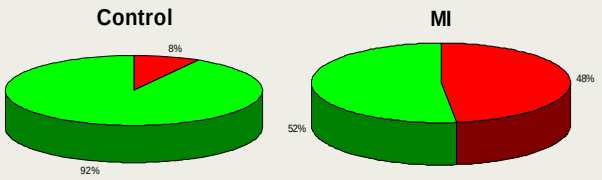


- Population Studies Lay the Foundation for Disease-State Comparison
  - Cancer
  - Metabolic
  - Alcohol/Drug Abuse
  - Cardiovascular
    - Myocardial Infarction
    - "Targeted Biosignature"
      - Screening relative to foundational data
      - Biomarker discovery/verification – unique protein variants
      - Knowledge assembly – design of multiplexed MSIA
      - Challenge
        - Translation to Clinic

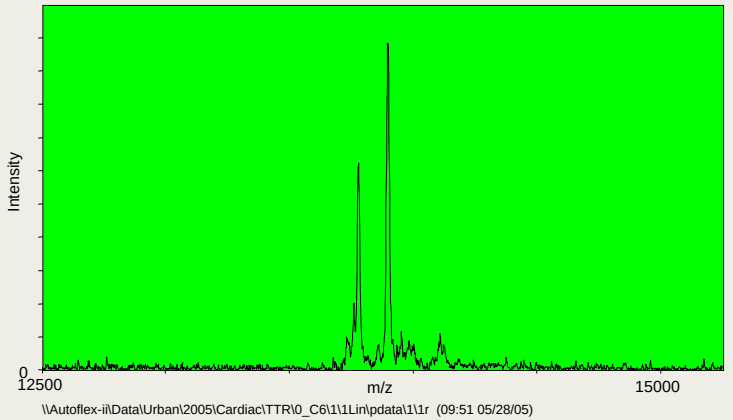
Putative Myocardial Infarction Markers

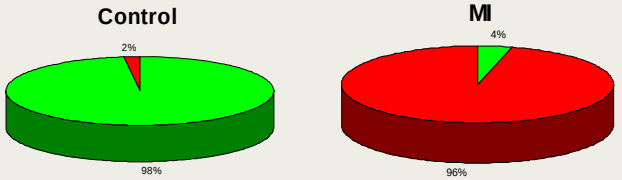
- Ratio of SAA1α to SAA1α(v)





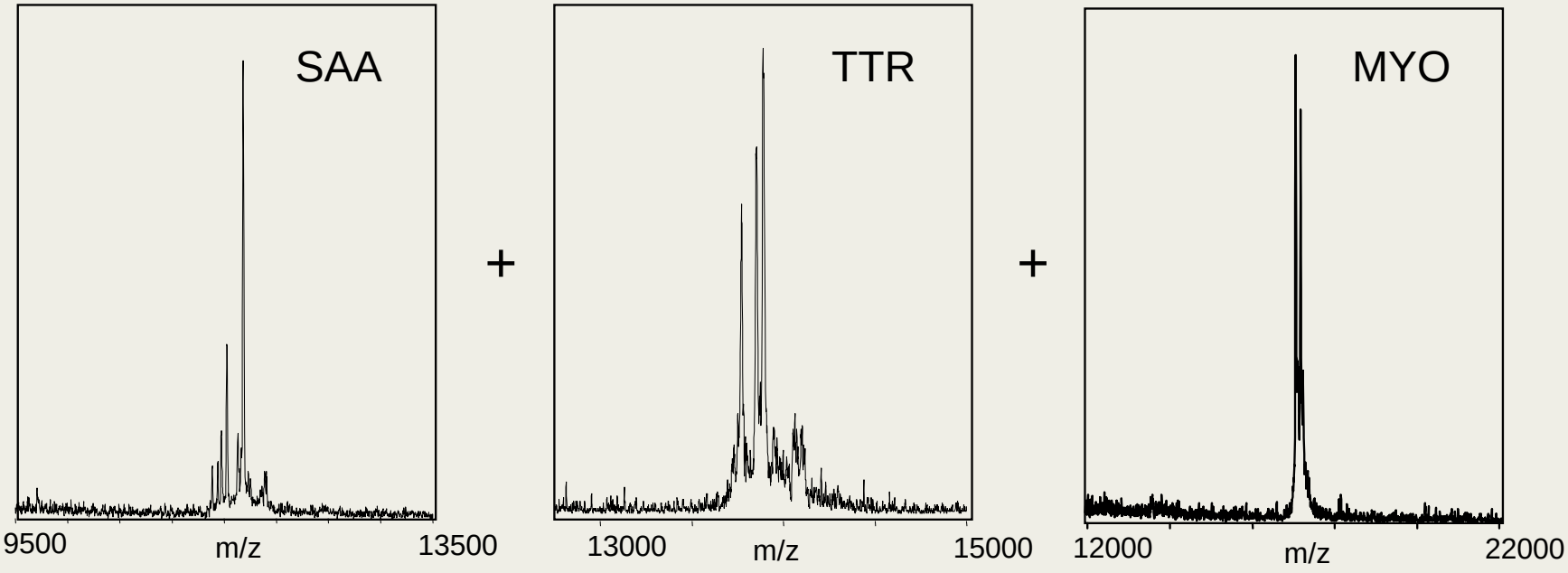
- Sulfonation of TTR



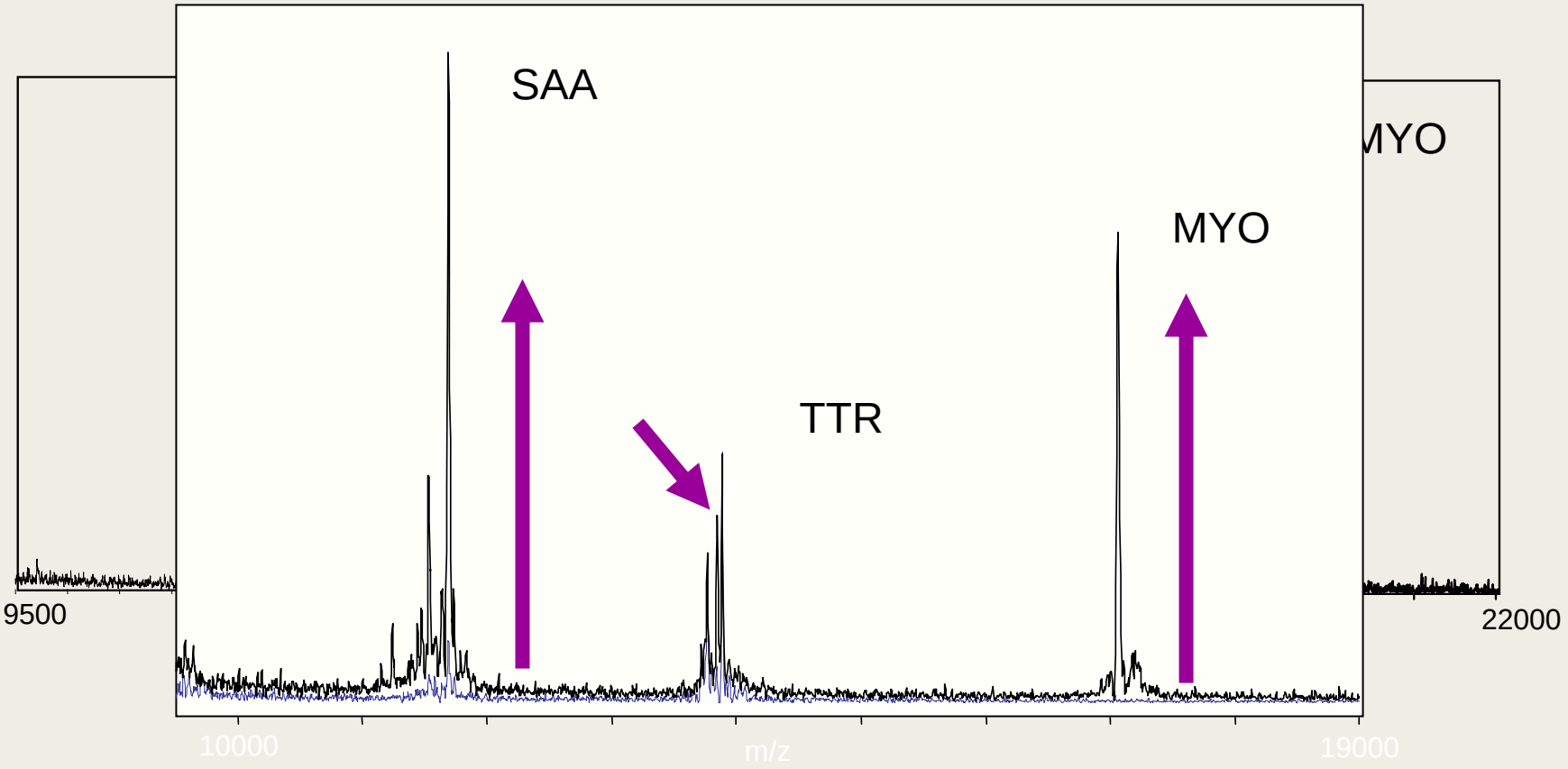


Multiplexed MI MSIA

Two Putative Biomarkers + Known MI Biomarker



Targeted Biosignature - MI Healthy

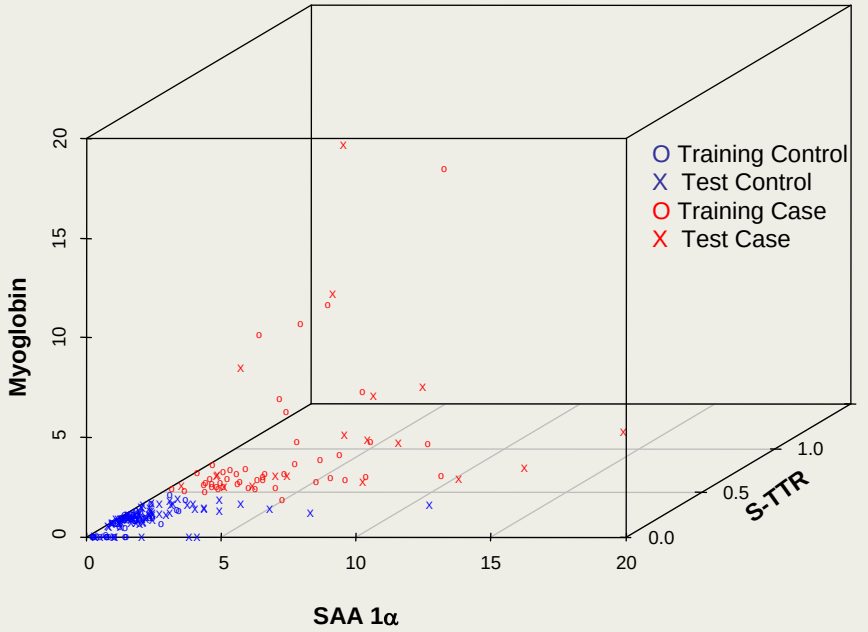


Single MSIA-Tip; Three Antibodies (SAA, TTR, MYO)

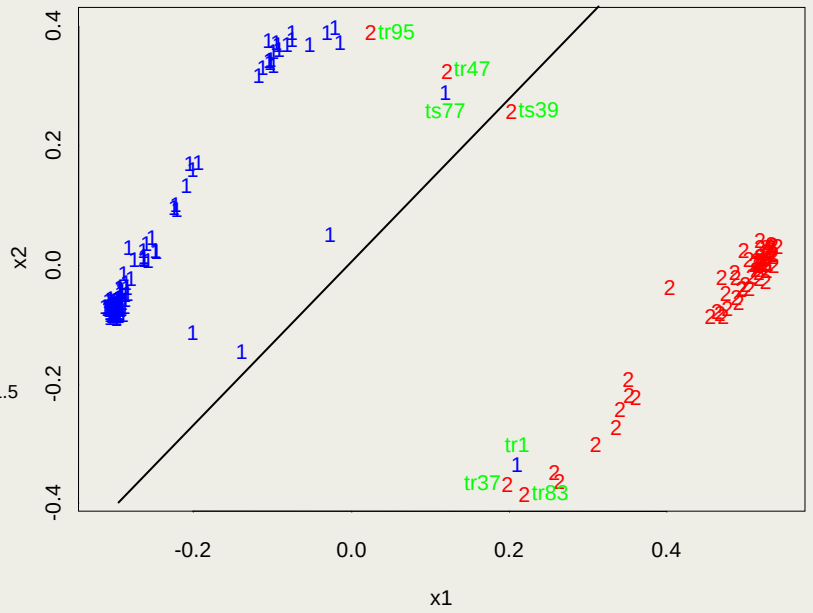
Multiplexed Multivariant Analysis

- **Results of Tri-marker Assay using Multivariate Analysis**
- **Random Forest: 48/48 Training Set; 19/77 Challenge Set**
- **Combines Multiplexed Targeted Analysis with Machine Learning Approaches**

Visualization of Intensities at Markers



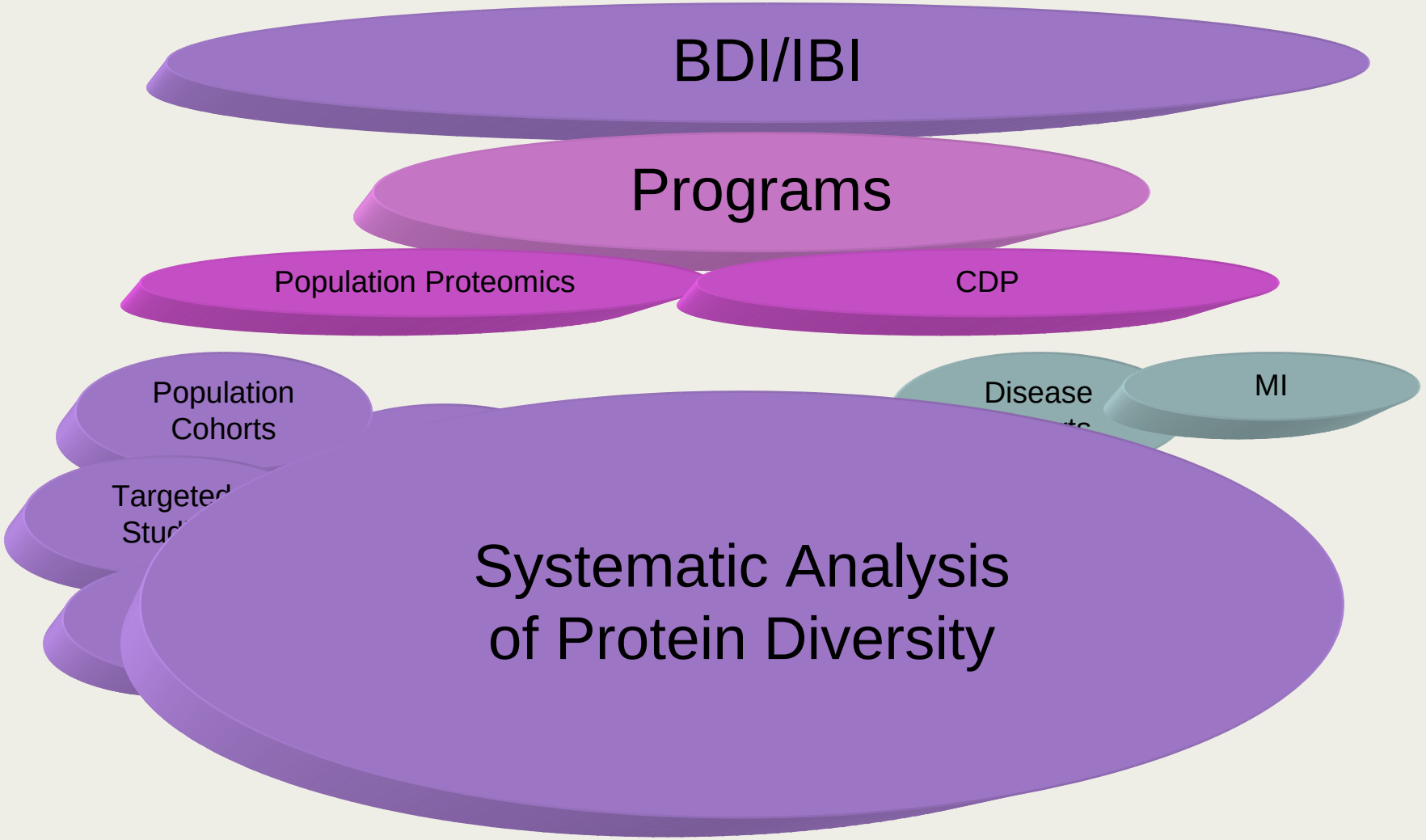
Proximity of samples



Requirement	Sens	Spec	PVP	PVN	Predicted Error (48/48)	False Readings # Case = 19 # Control = 77
3- Marker MV	100	98.7	95.0	100	1.8%	FP = 1, FN = 0

- Kiernan, U. A., Nedelkov, D., Nelson, R. W., "Multiplexed Mass Spectrometric Immunoassay: A Novel Approach to the Determination of Myocardial Infarct", *J. Proteome Res.* 5, 2928 – 2934 (2006).

Production of a Human Protein Knowledge Base



Closing Thoughts

- We are all Different
  - Linking protein ID from common database is inherently incorrect
  - Even operating on DNA-level, MS analysis needed for PTM
  - Microheterogeneity escapes immunometric detection
- Presumptuous to Overlook Subtle Changes in Protein Structure
  - Cause/contribute/indicative/preventative of a number of diseases
    - Sickle-cell anemia
    - Cancer (e.g., p53)
    - Cystic fibrosis
    - Diabetes
    - Cardiovascular disease (Apo-AI Milano)
- Determining/Indexing Diversity
  - 1000s' of individuals
  - More proteins – and include known biomarkers
    - Establish basal "Healthy" range
    - Challenge with disease cohorts
- Targeted Proteomics Approaches Applied in Systematic Studies
  - Fill a "Technical Void" in Biomarker Discovery/Validation
  - Long-Term Clinical and Diagnostic Applications

Collaborations

INTRINSIC BIOPROBES, INC.

Technologies and Applications

Institute for Population Proteomics

Institute for Population Proteomics

... understanding protein diversity

Tempe, Arizona

THE biodesign INSTITUTE
ARIZONA STATE UNIVERSITY

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- Dr. Allan L. Bieber
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