ProteinChip® Technology: A new tool for proteome analysis and biomarker discovery

Kelly A. Conrads, Ph.D.

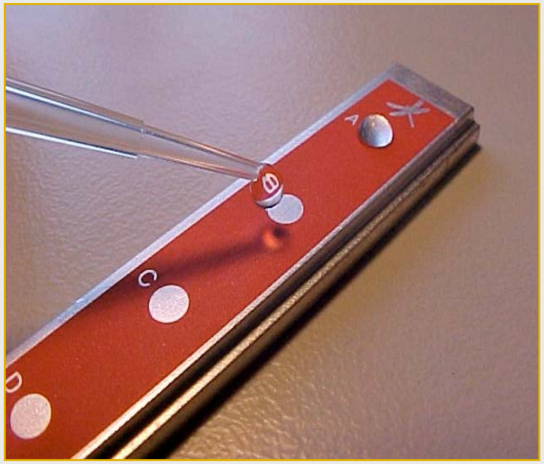
Ciphergen Biosystems, Inc.
kconrads@ciphergen.com

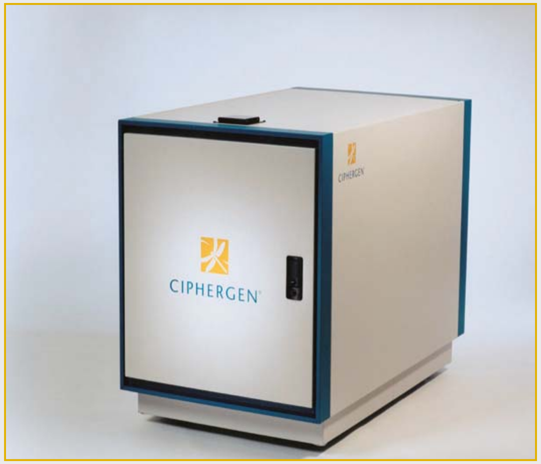
CIPHERGEN®

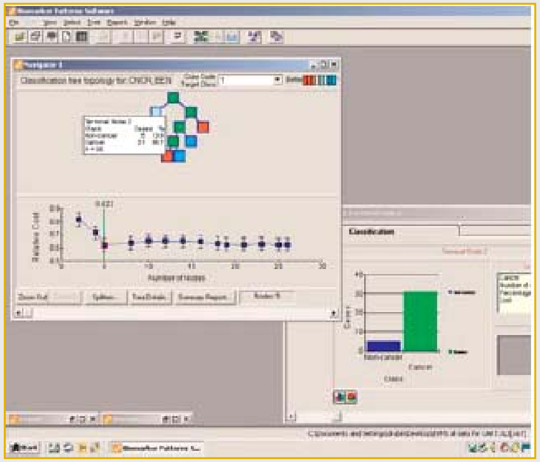
Outline

- Background on technology – What is SELDI?
- Applications
- Example biomarker discover study – Ovarian Cancer

ProteinChip® Platform: Components







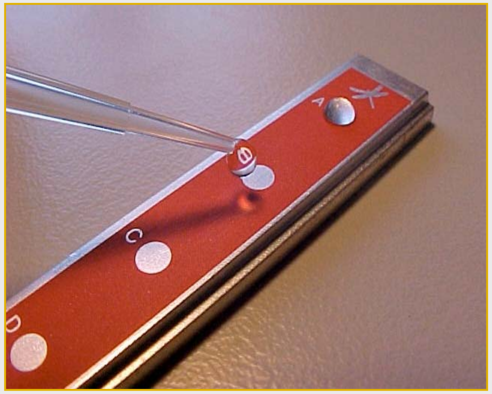
ProteinChip Arrays
» Separation

ProteinChip Reader
» Detection

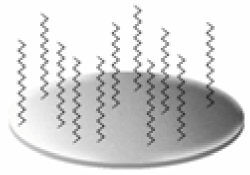
ProteinChip Software
» Analysis

CIPHERGEN®

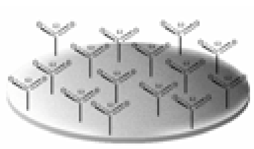
ProteinChip® Arrays: A Variety of Surfaces



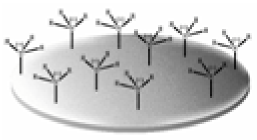
Chemical Surfaces – Protein Expression Profiling:



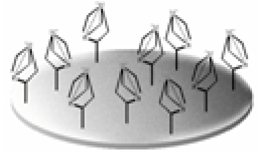
Hydrophobic



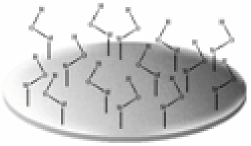
Anionic



Cationic

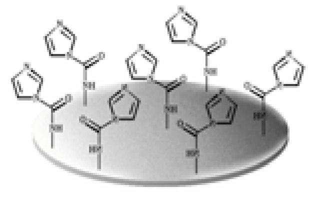


Metal Chelate

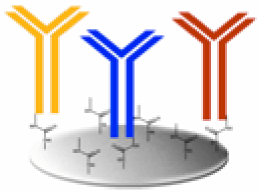


Normal Phase

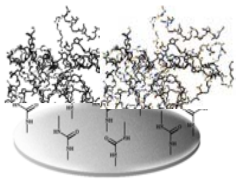
Biological Surfaces – Protein Interaction Assays:



PS-10 or PS-20



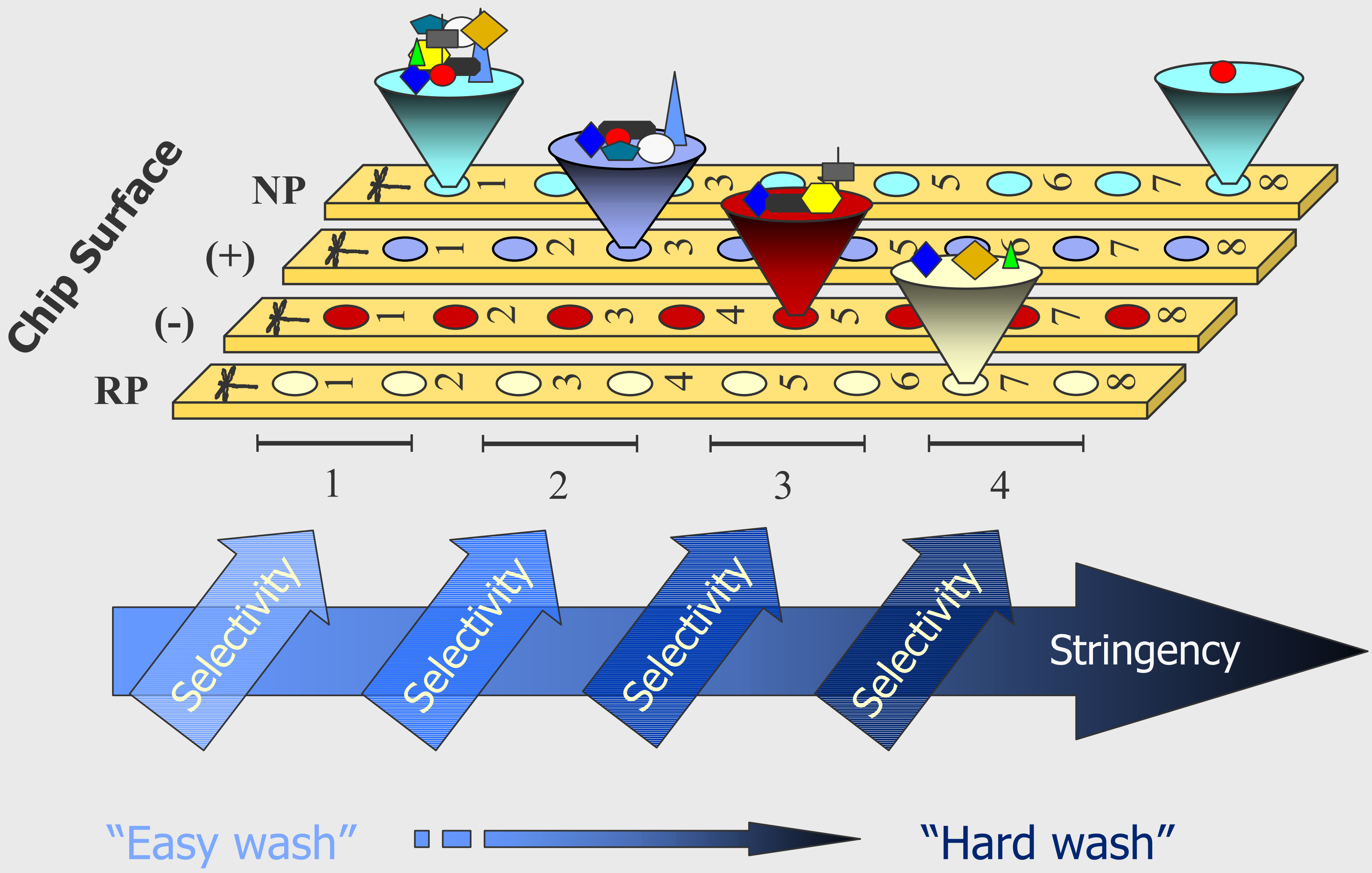
Antibody - Antigen



Receptor - Ligand

DNA - Protein

ProteinChip® Arrays: Retentate Chromatography



Selectivity

Selectivity

Selectivity

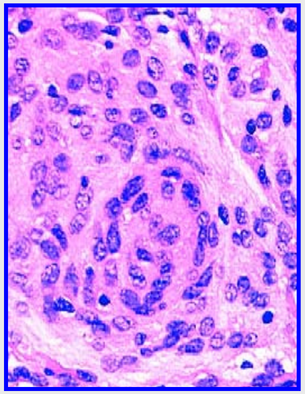
Selectivity

Stringency

"Easy wash"

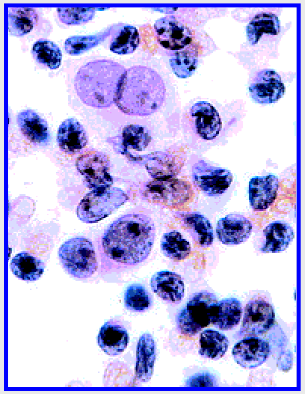
"Hard wash"

ProteinChip® Proteomics: Complexity of Samples



Tumor Tissue & Cultures

Biopsy
LCM
Nuclear Extracts
Cell lysates
Plant Extracts



Tumor Fluid

Seminal Plasma
Nipple Aspirates
Fine Needle
Aspirates

Fluids

Serum
Plasma
Urine
CSF
Blood eluates

ProteinChip® Array Preparation

Step 1: Complex protein sample is placed on a ProteinChip Array

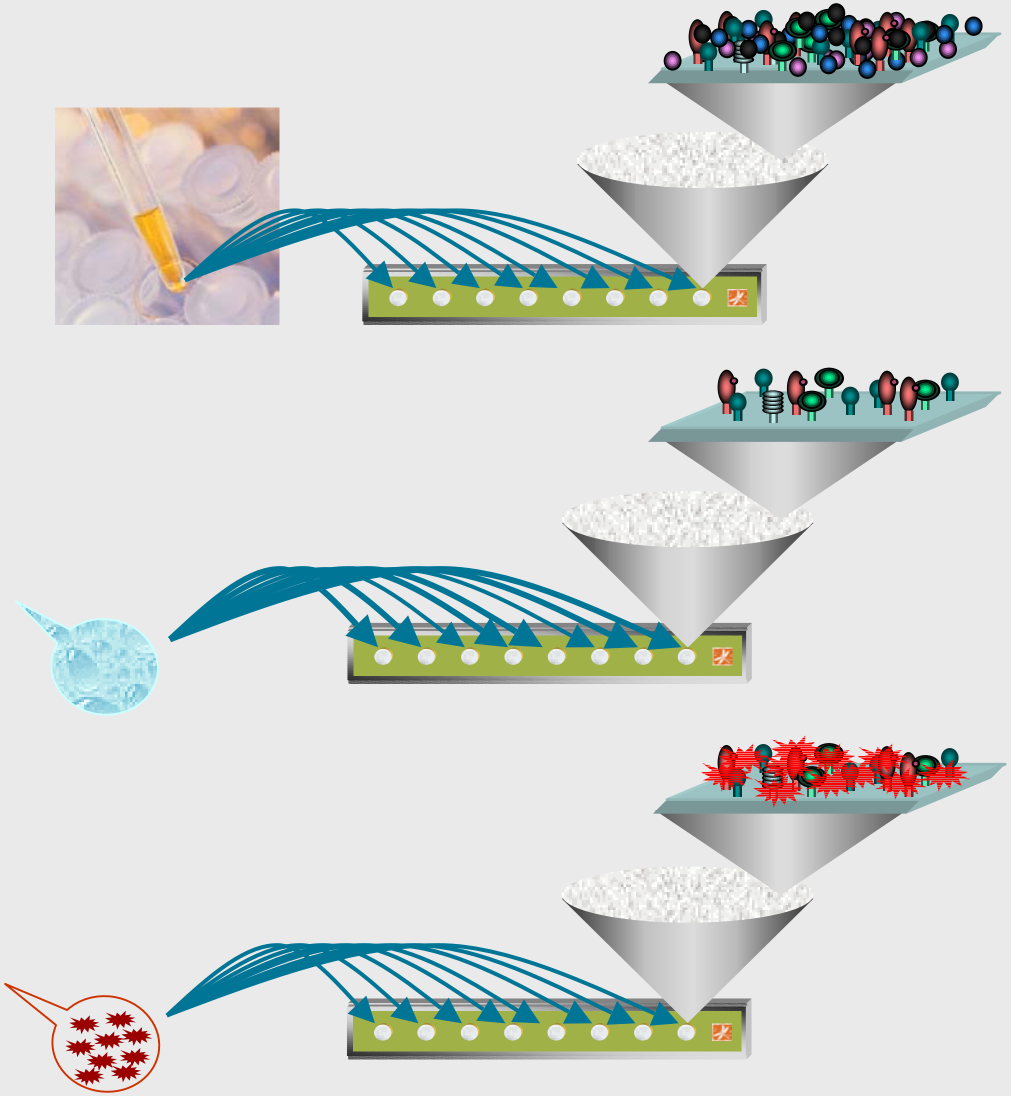
- Proteins bind to chemical or biological sites on the ProteinChip surface

Step 2: Remove unbound proteins

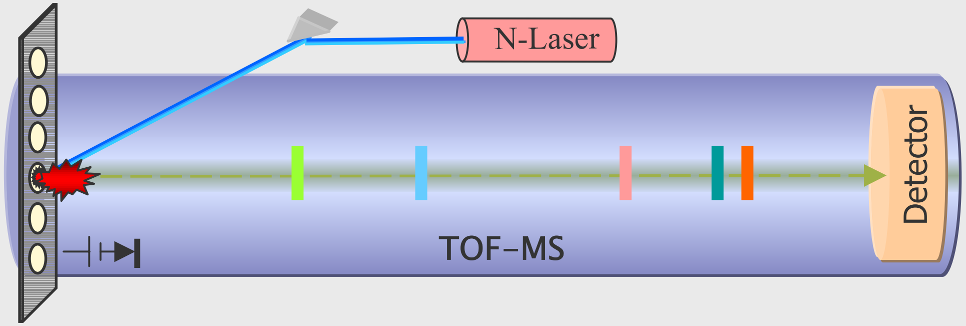
- Wash the ProteinChip with appropriate stringency buffer
- Bound proteins are retained

Step 3: Add Energy Absorbing Molecules or "Matrix"

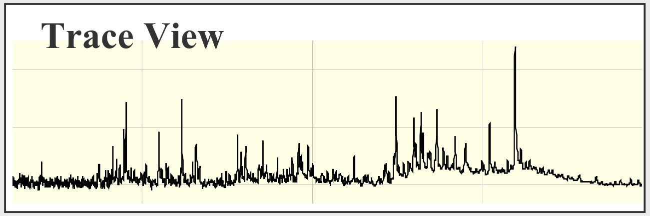
- EAM is applied to each spot to facilitate desorption and ionization in the TOF-MS Chip Reader

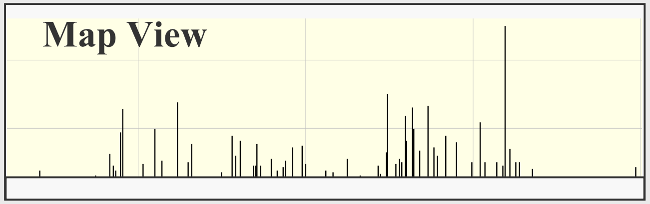


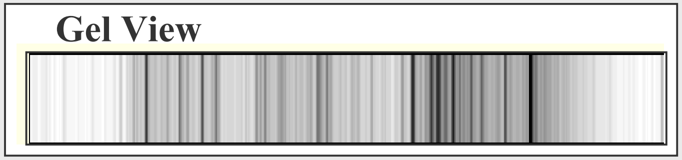
Detection Using SELDI



- Retained proteins are "eluted" from the ProteinChip Array by laser desorption/ionization
- Ionized proteins are detected and their mass accurately determined by Time-of-Flight Mass Spectrometry
- ProteinChip® software produces map of proteins showing their accurate mass and relative ion intensity







ProteinChip® Array Preparation: Sample Workflow

- **Sample deposit (2 min)**
- **Allow proteins to bind to surface (45-60 min)**
- **Wash (5-15 min)**
- **Matrix deposition (10 min)**
- **Chip introduction (1 min)**
- **Chip reading (15 min)**
- **Data analysis (30 min to …)**

Protein Discovery and Characterization
ProteinChip® Advantages

- **Capture, detect and analyze proteins directly from crude biological samples without tagging or labeling**
- **Wash step – analysis of protein in MS unfriendly mixtures**
- **Lower limit of detection: 1-50 fmole of protein**
- **Small sample size: 0.5 µl - 400 µl**
- **Medium to high throughput: 8, 24, 96, or 192 samples**
- **Rapid results: 10 minutes**
- **Significant result: Accurate molecular weight** *(500 Da- 300 kDa)*

ProteinChip® Applications

Research Proteomics
Basic Research

- Immunoassay development and screening
- Receptor-ligand assays
- Protein-protein interactions
- DNA-protein interactions

Process Proteomics
Protein Purificaiton

- Purification development and monitoring
- Peptide mapping and protein identification
- Analysis of post-translational modifications
- Epitope mapping

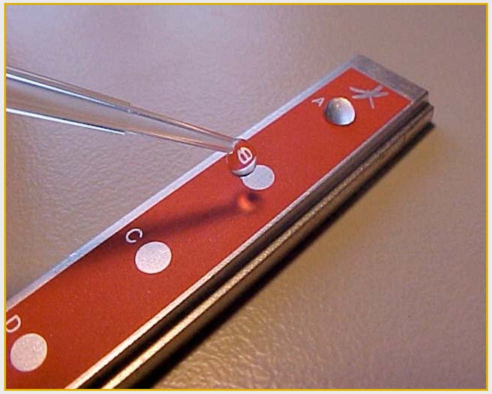
Clinical Proteomics
Profiling

- Target discovery and validation
- Disease monitoring (drug efficacy studies)
- Toxicology
- Diagnostics

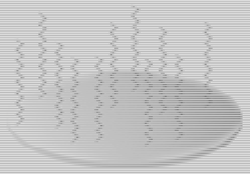
Interaction Discovery Mapping™

Protein-Protein Interactions

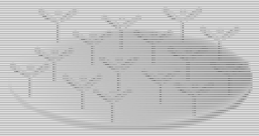
ProteinChip® Arrays: A Variety of Surfaces



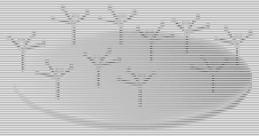
Chemical Surfaces – Protein Expression Profiling:



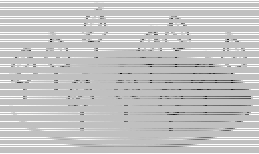
Hydrophobic



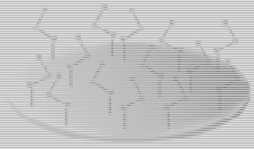
Anionic



Cationic

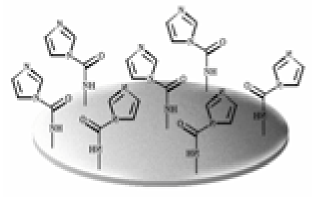


Metal Chelate

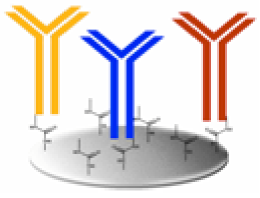


Normal Phase

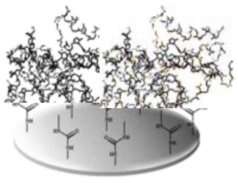
Biological Surfaces – Protein Interaction Assays:



PS-10 or PS-20



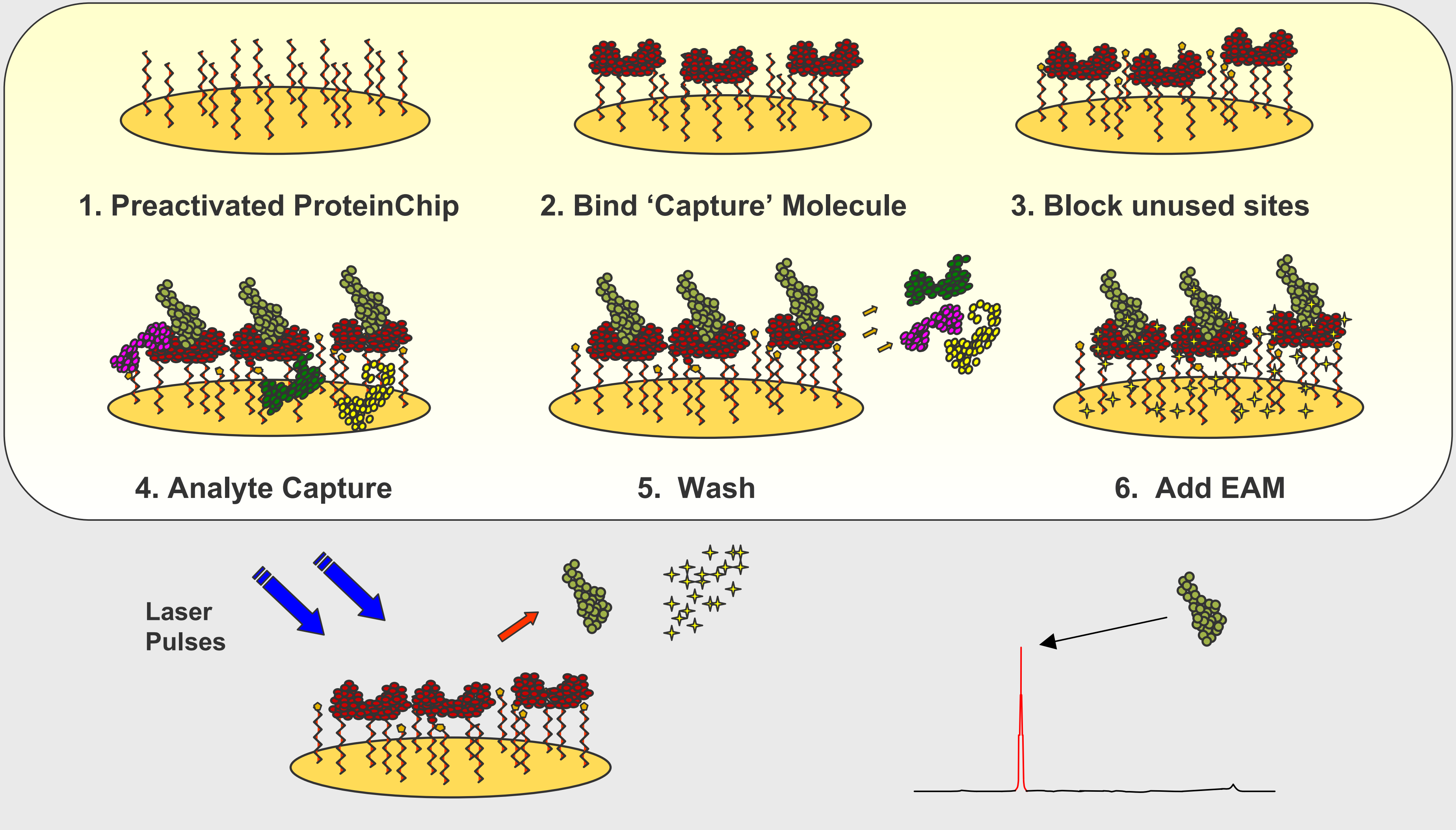
Antibody - Antigen



Receptor - Ligand

DNA - Protein

Protein-Protein Interaction Analysis



1. Preactivated ProteinChip

2. Bind ‘Capture’ Molecule

3. Block unused sites

4. Analyte Capture

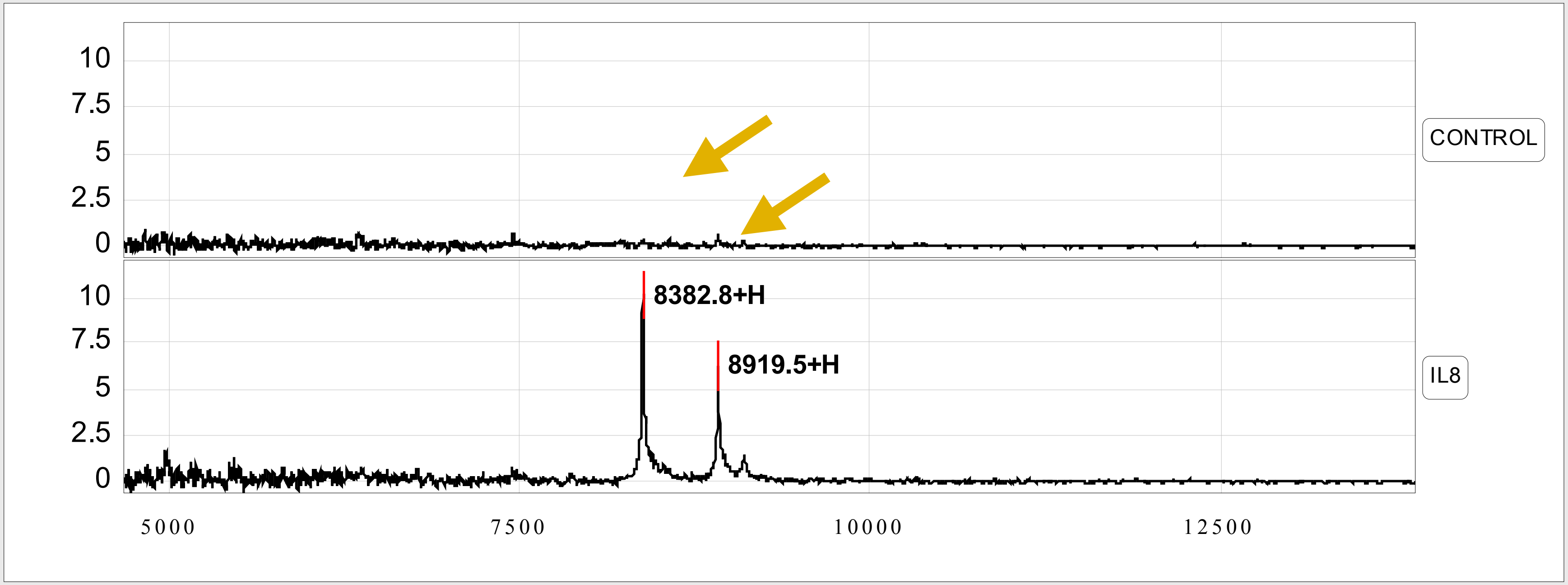
5. Wash

6. Add EAM

7. Laser Ionization / Desorption

8. Direct Mass Detection

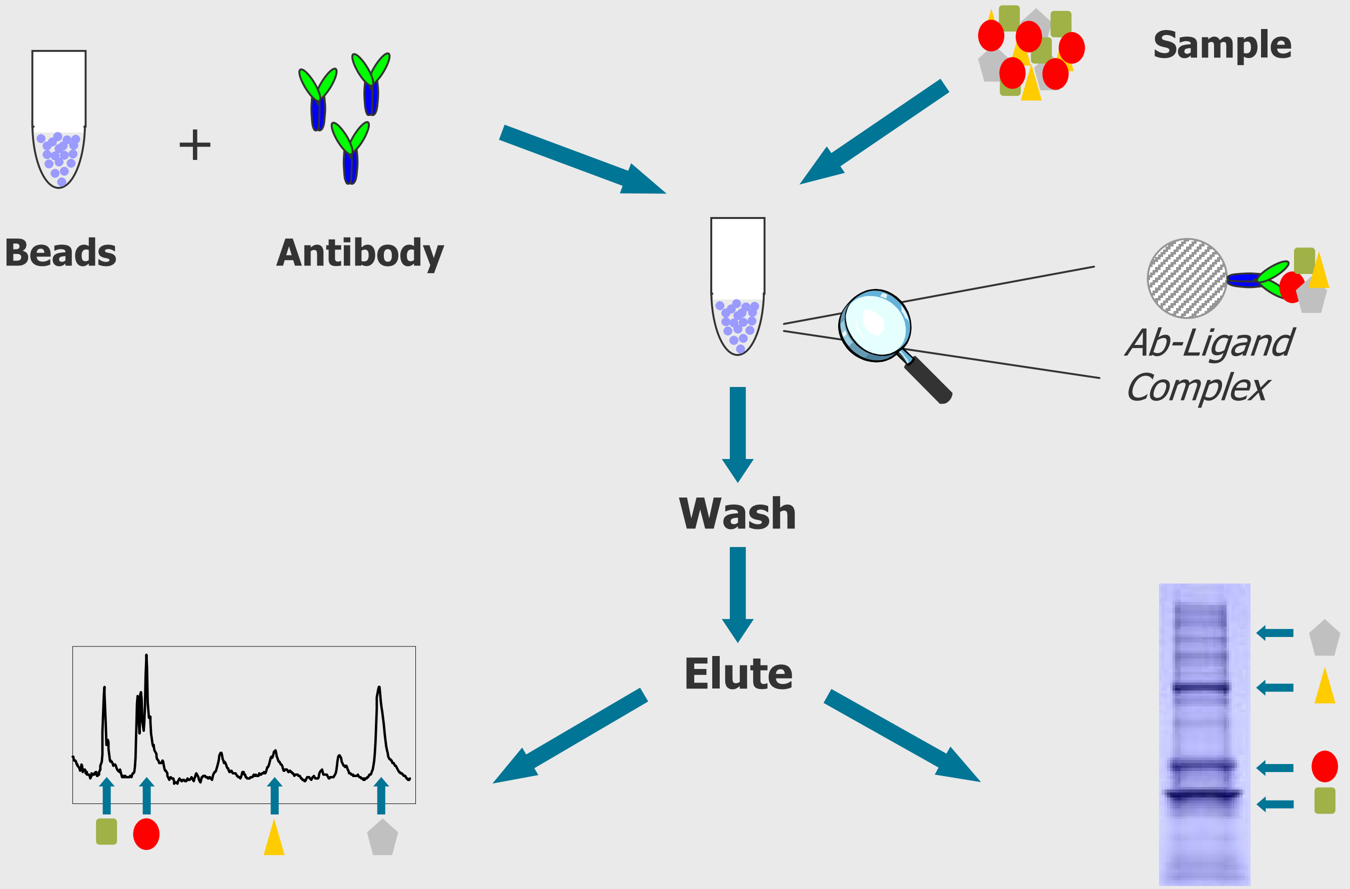
Antibody Capture of IL8



Two different isoforms detected using antibody capture

****Advantage over ELISA****

Protein-Protein Interaction Analysis



Beads + **Antibody**

Sample

Ab-Ligand Complex

Wash

Elute

Analyze captured proteins using ProteinChip® Array

ID captured proteins using SDS-PAGE & tryptic digest etc

Protein Interactions: Beads & Arrays

Arrays

- **Quick**
  - <4 hrs for typical capture experiment
- **High-throughput**
  - Up to 12 Arrays in parallel
- **Easily automated**
  - Simple to transfer protocols to robotics
- **Ability to work with small volumes**
  - 1-2 ul sample can be applied directly to the spot

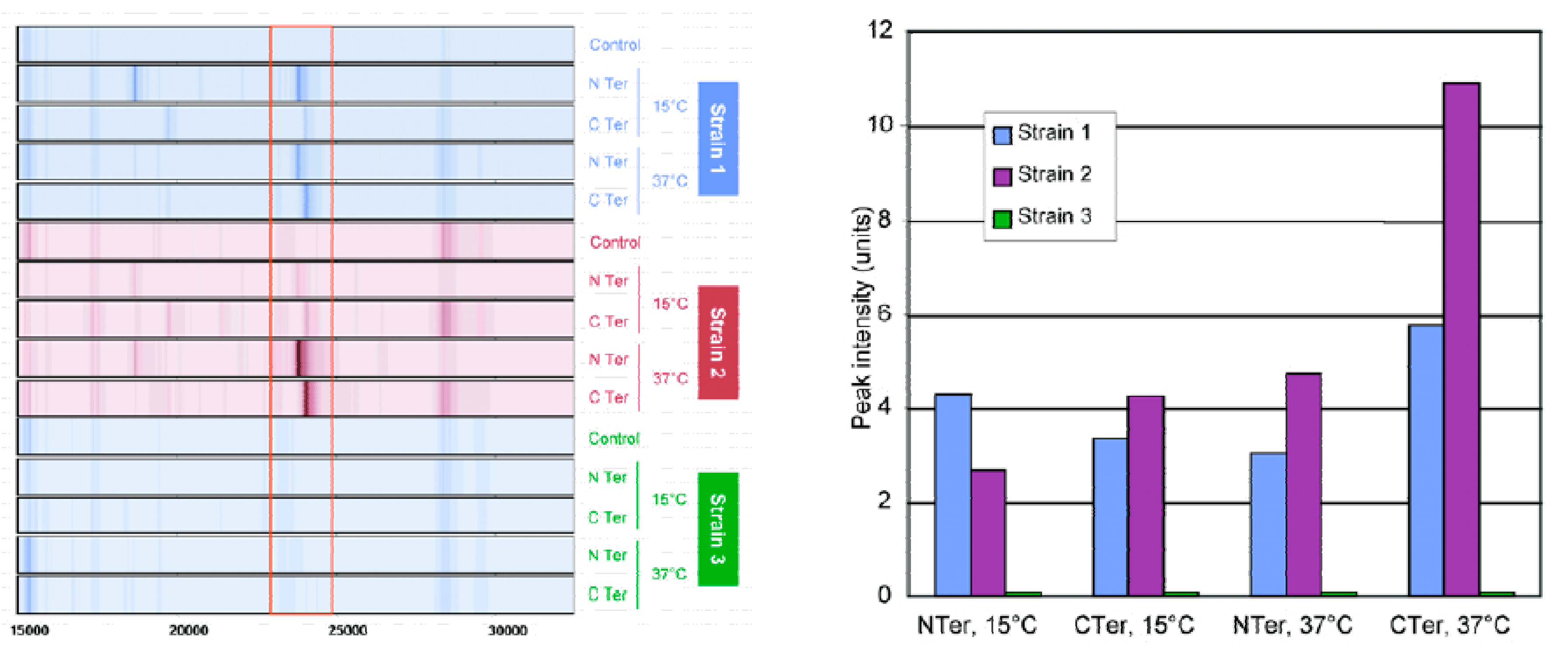
Beads

- **High capacity**
  - Large Dynamic Range
  - Ability to work with larger volumes
- **Ability to multiplex**
  - More than one antibody can be immobilized
- **Amenable to Orthagonal sample processing**
  - Coupling of affinity chromatography with subsequent purification steps
- **Semi-preparative Method**
  - Ability to ID captured proteins

Process Proteomics Applications

Protein Purification

Expression Optimization of a His-tagged Protein



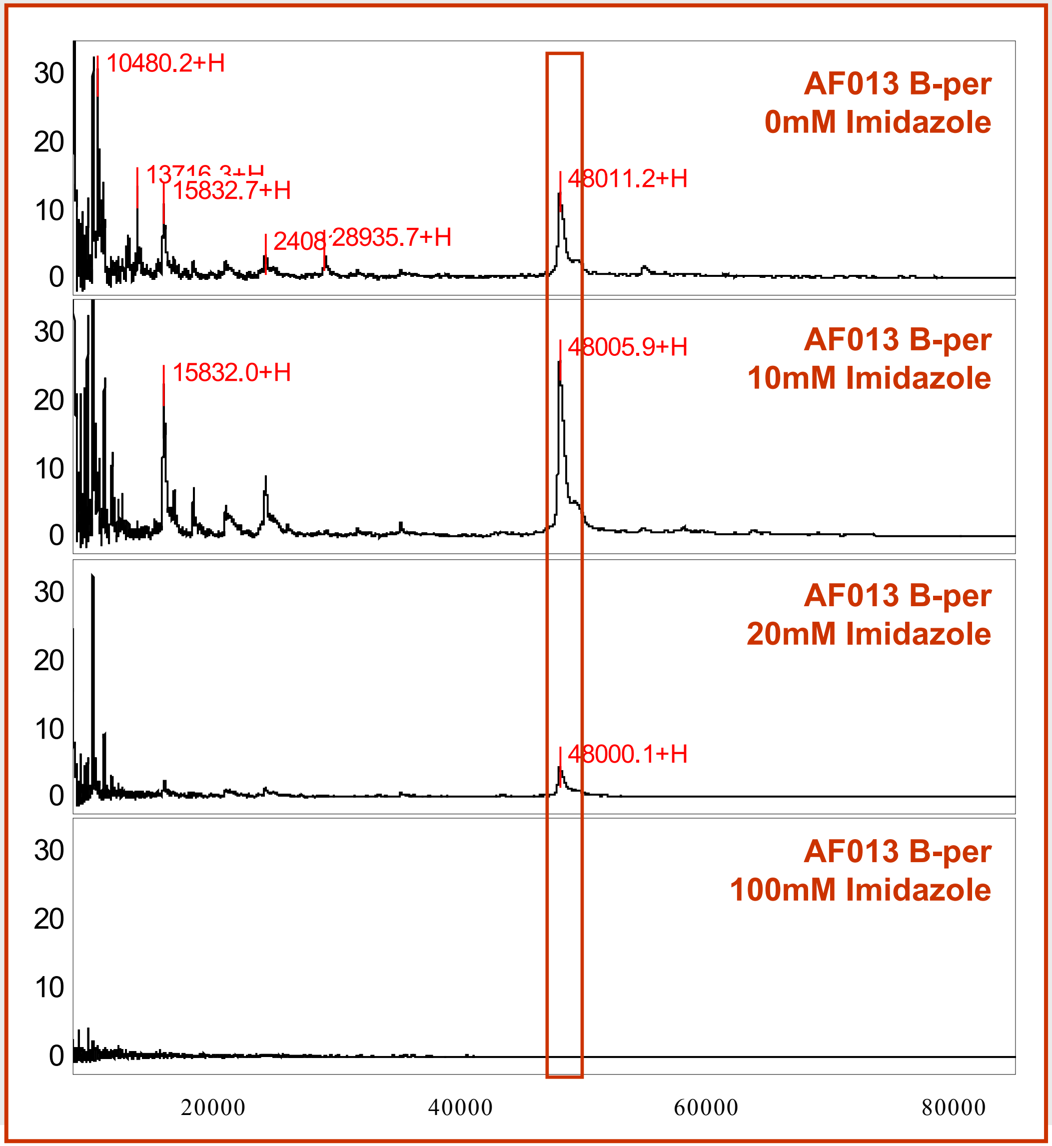
Objective: Determine the best conditions of expression of a recombinant 23.8 kDa His-tagged protein in a bacterial host strain

Conditions tested: 1. Strain, 2. Expression vector construct, 3. Temperature.

ProteinChip Array: Selective retention of the His-tagged target is found on IMAC-Cu+2

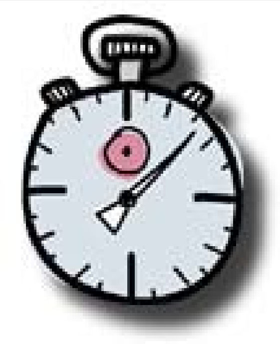
Results: C-Terminal vector construct in Strain 2 at 37°C attained the highest expression levels. Time spent for monitoring expression in 15 fermentation samples: Half a day.

IMAC-Ni ProteinChip® Array



- Various amounts of imidazole were added to the binding step to determine what amount would improve the specific capture
- Protein binds to the IMAC-Ni surface, seen at 48 kDa
- The presence of low amounts of imidazole (10 mM) doesn’t interfere with binding
- There are a significant number of endogenous impurity proteins binding to the IMAC surface
- The number of impurity proteins decreases as the amount of imidazole increases
- 8.5-85 kDa MW region is shown

Process Proteomics: Key Benefits

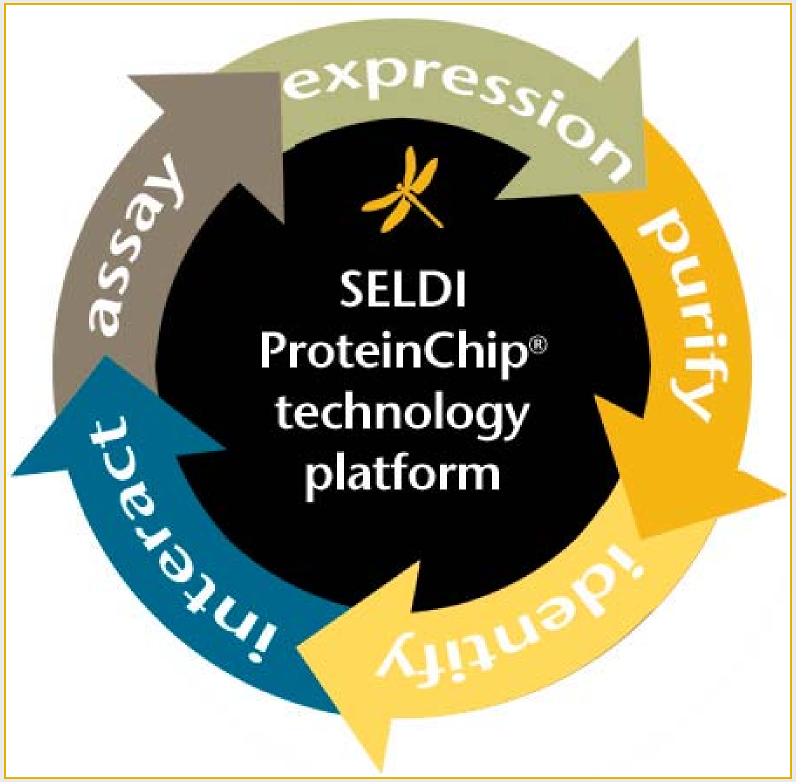


- **A versatile technology**
  - Analytical capabilities for crude fermentation/CC samples through purification and final formulation
- **Significantly reduce analysis/purification time**
  - Rapid, ‘on-chip’ analytical and purification method development
  - Small sample requirement allows development to begin earlier in the process
  - Reduce number of SDS-PAGE, Westerns, ELISAs, HPLC runs
- **Get more information from the sample**
  - Accurate MW tracking of target and impurities

Expression Difference Mapping™ Applications

Biomarker Discovery

Pattern Track™ Process



Pattern Track™ Process

Discovery

Discovery Pilot Study: biomarker scouting study to find biomarker candidates

Multiple Biomarker Candidates

□◆◆●○●○□ □◆
●○◆●○◆●○◆

- Maximize number of conditions to discover biomarkers
- Univariate analysis to identify biomarker candidates

Validation Study: biomarker study using optimal conditions from Pilot Study to validate biomarker candidates

Validated Biomarkers

□◆●○◆

- Minimize number of conditions to validate biomarkers
- Multivariate analysis to classify patient groups based on multiple biomarker patterns
- Biomarker Patterns™ Software selects the best biomarker candidates

Purification

Purified Biomarkers

□◆●○◆

- SELDI Assisted Purification

Identification

Identified Biomarkers

■◆●●◆

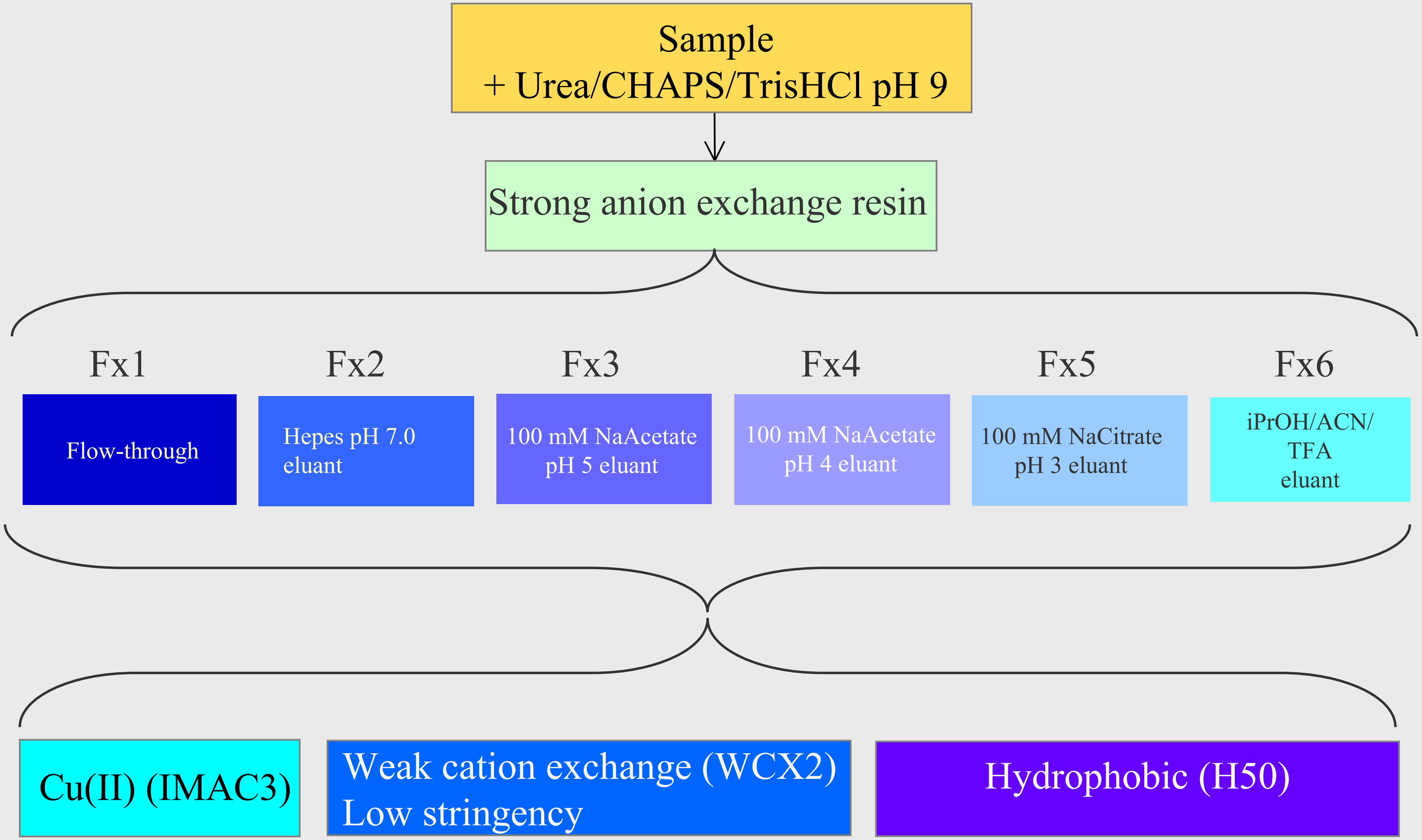
- SEND ID on-chip peptide mapping
- SELDI Tandem Mass Spectrometry

Assay

Quantitative Predictive Multi Biomarker Pattern Assays

- ProteinChip System, Series 4000

Fractionation



Sample
+ Urea/CHAPS/TrisHCl pH 9

Strong anion exchange resin

Fx1	Fx2	Fx3	Fx4	Fx5	Fx6
Flow-through	Hepes pH 7.0 eluant	100 mM NaAcetate pH 5 eluant	100 mM NaAcetate pH 4 eluant	100 mM NaCitrate pH 3 eluant	iPrOH/ACN/ TFA eluant

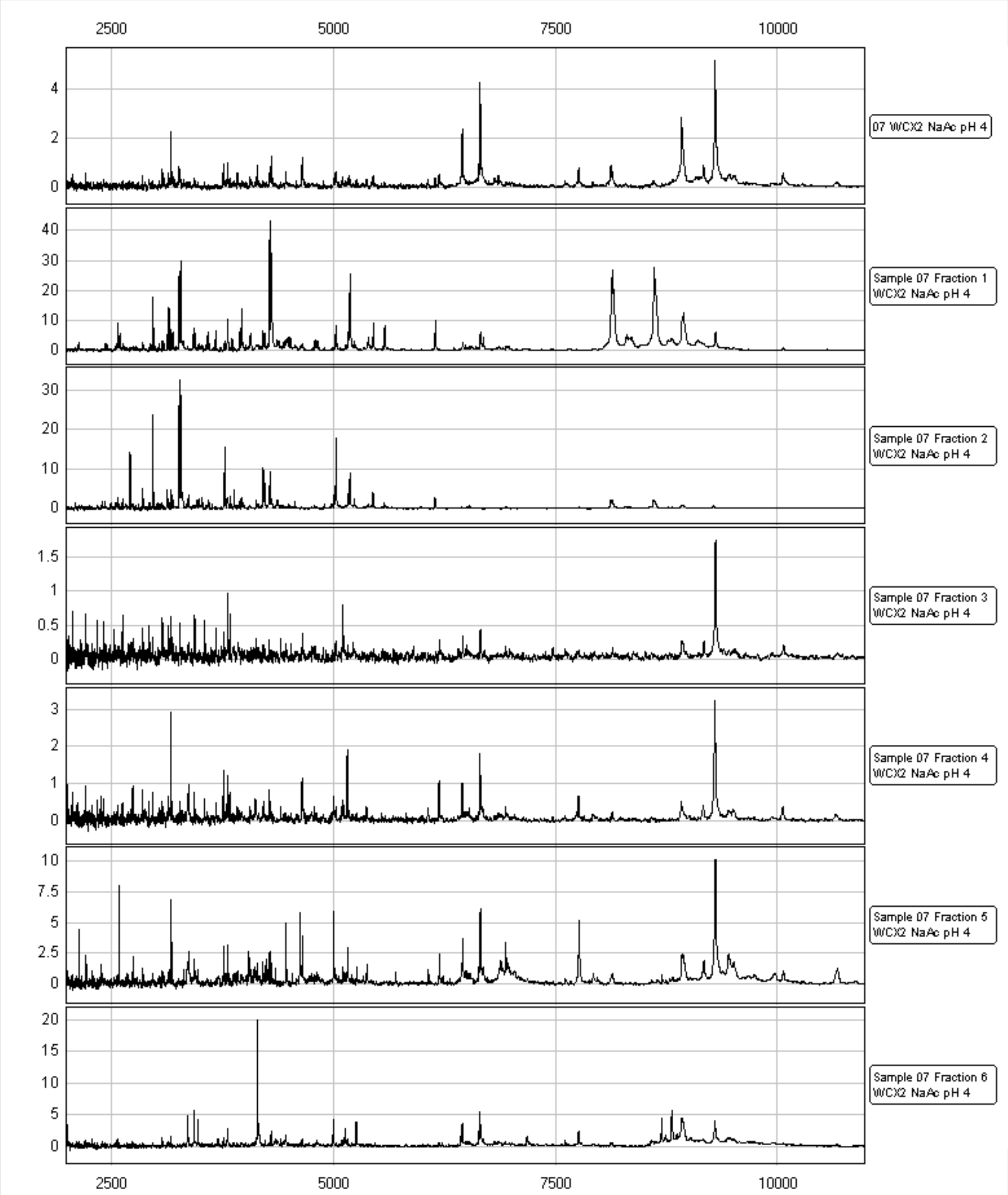
Cu(II) (IMAC3)

Weak cation exchange (WCX2)
Low stringency

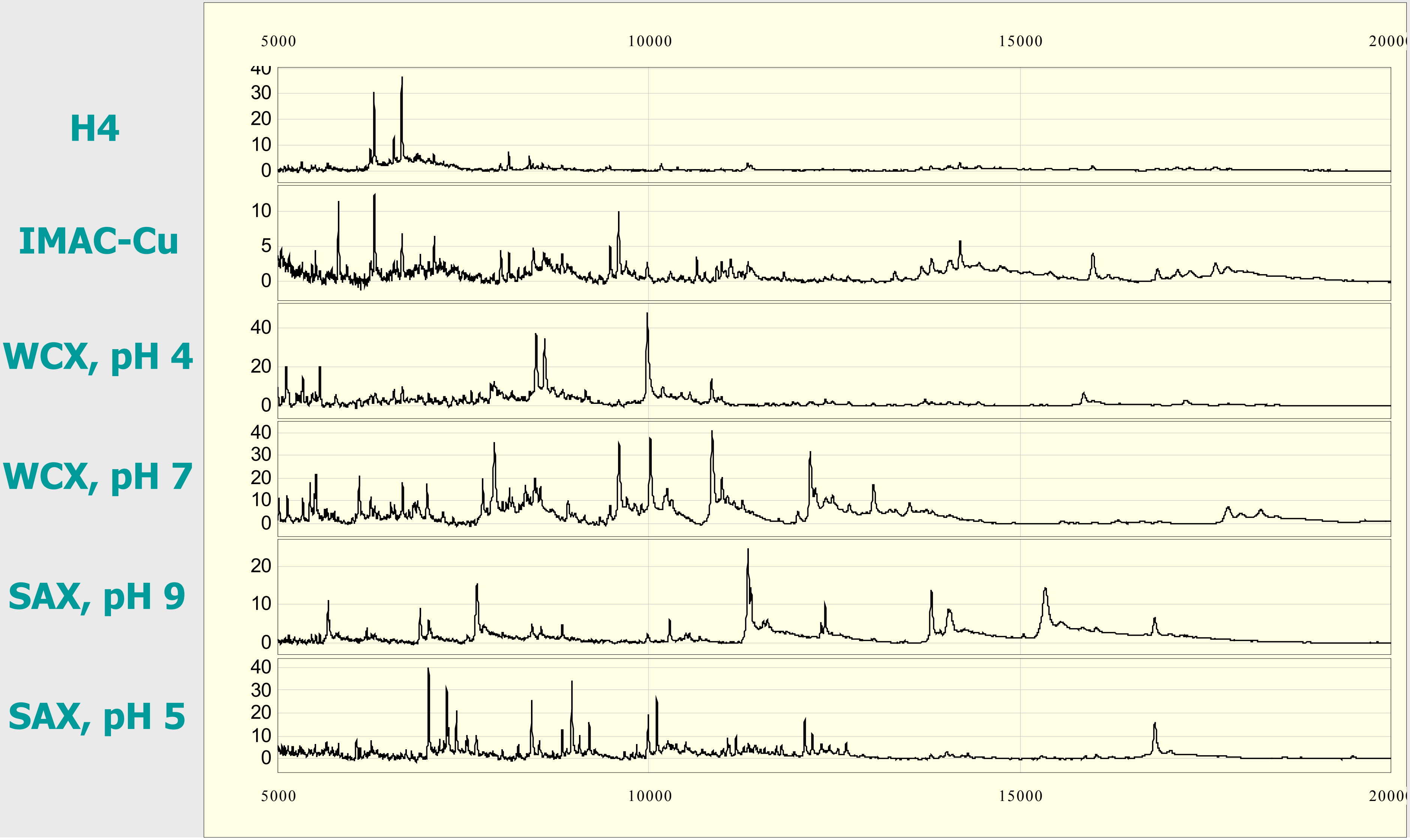
Hydrophobic (H50)

Benefits of Fractionation

- **Fractionation expands the investigated part of the proteome:**
- **Neat = 48 peaks**
- **6 Fractions = 180 unique peaks, or 132 new peaks.**



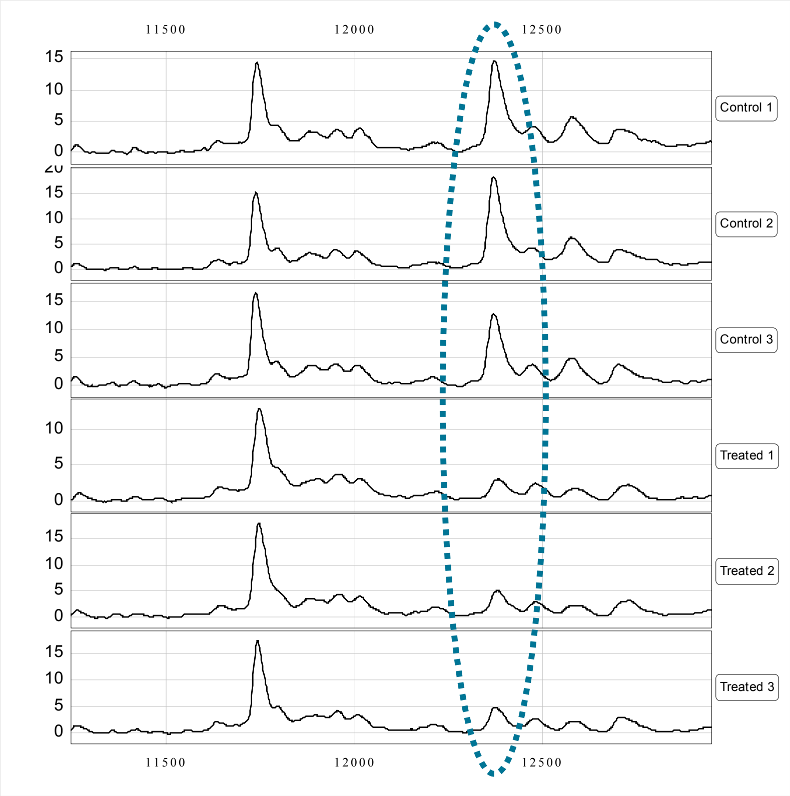
Discovery: Surface Specificity



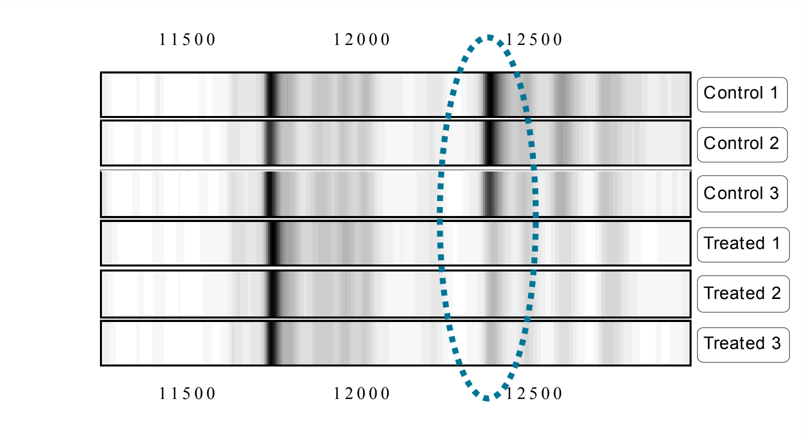
CIPHERGEN®

Discovery: Visualizing Biomarkers

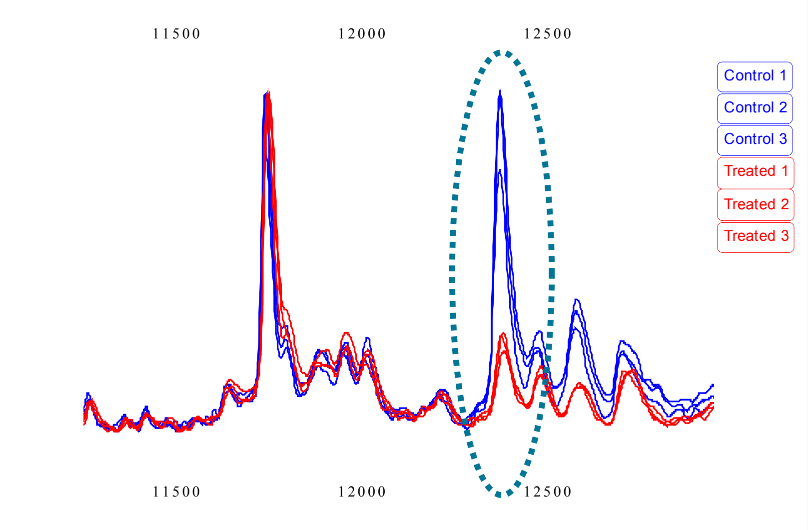
Spectral View



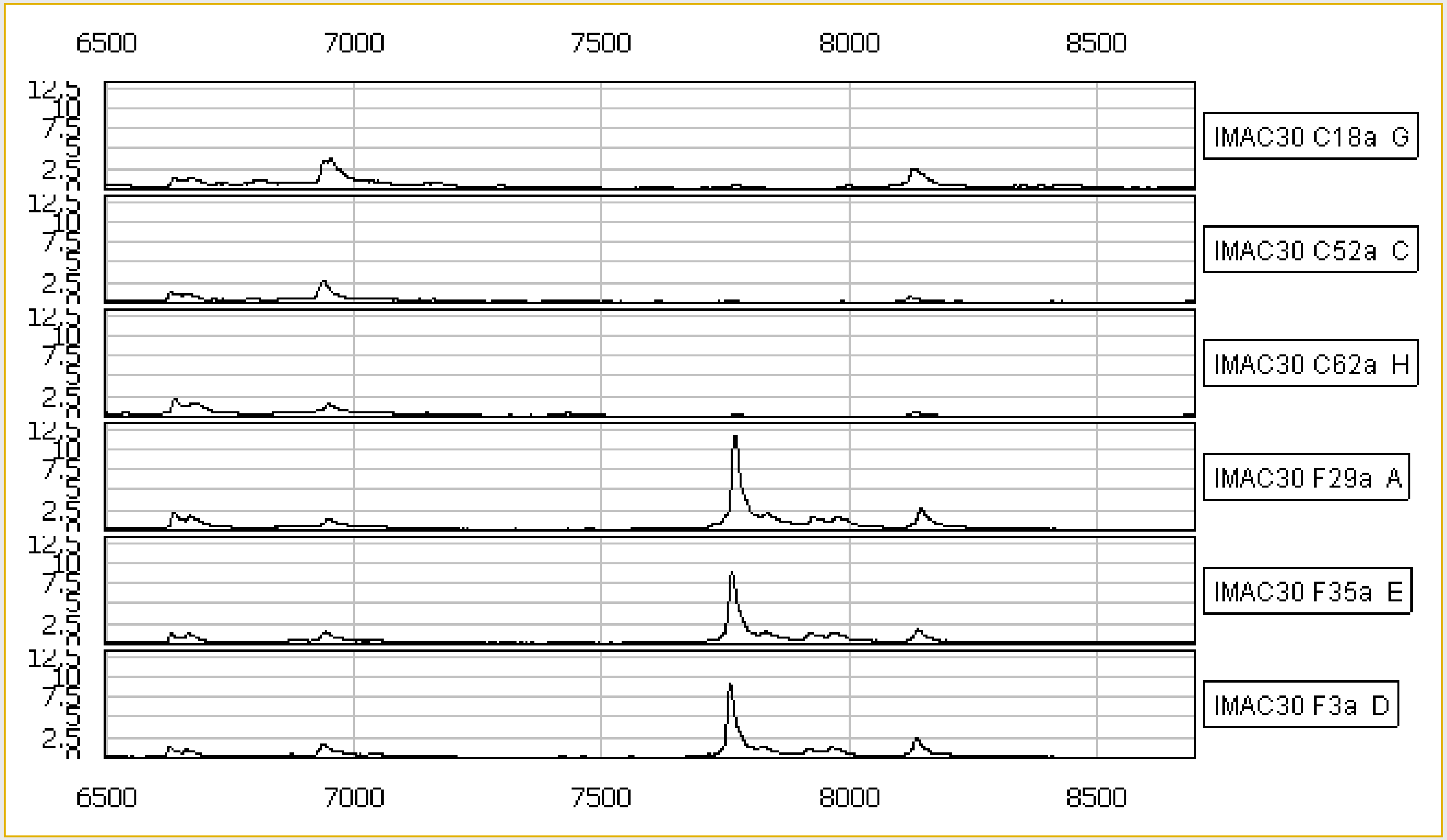
Gel View



Overlay View



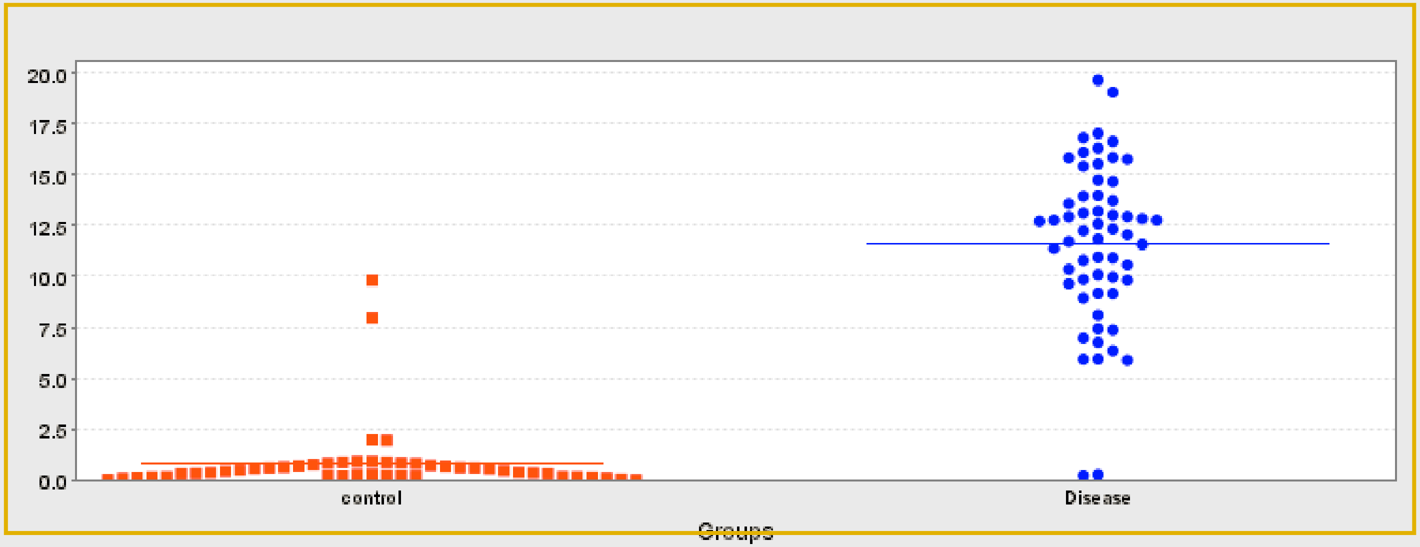
7.8 kDa Peak is Differentially expressed

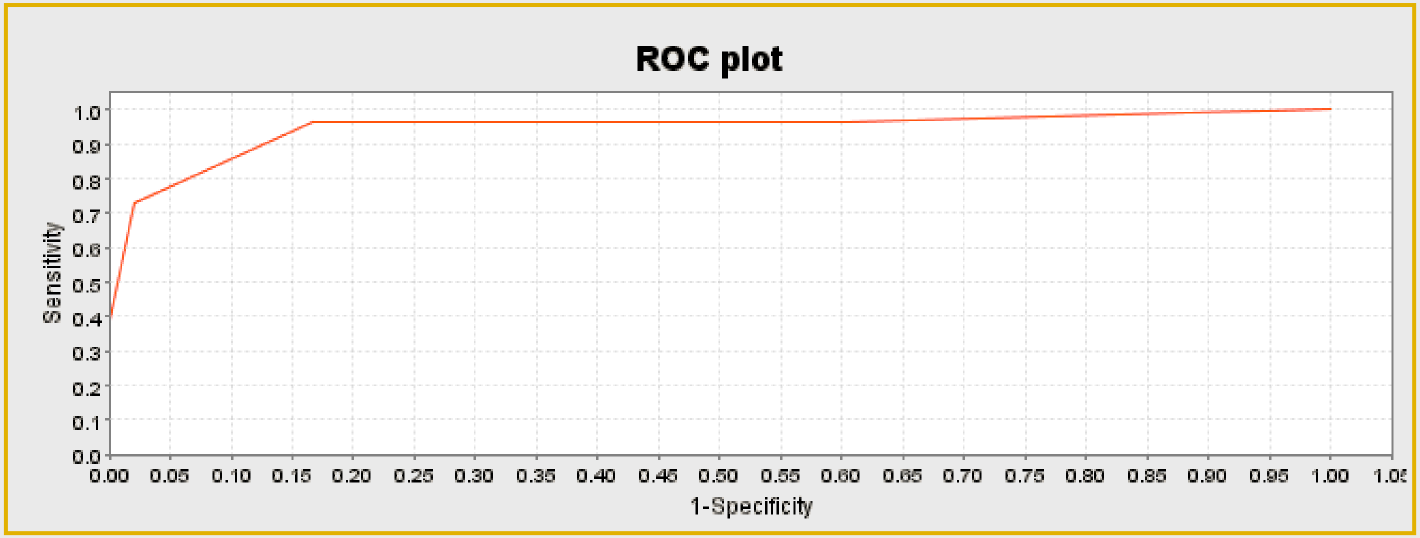


7.8 kDa Peak is Differentially expressed

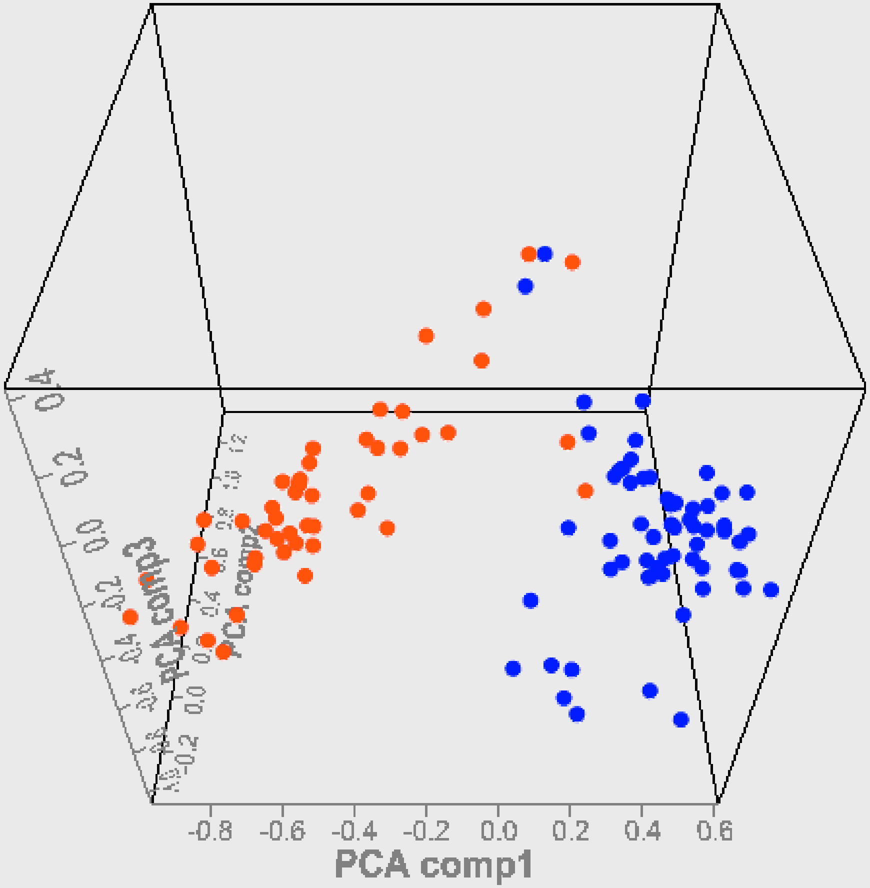
7.8 kDa peak discovered on IMAC30-Cu^{2+} array, Low Mass

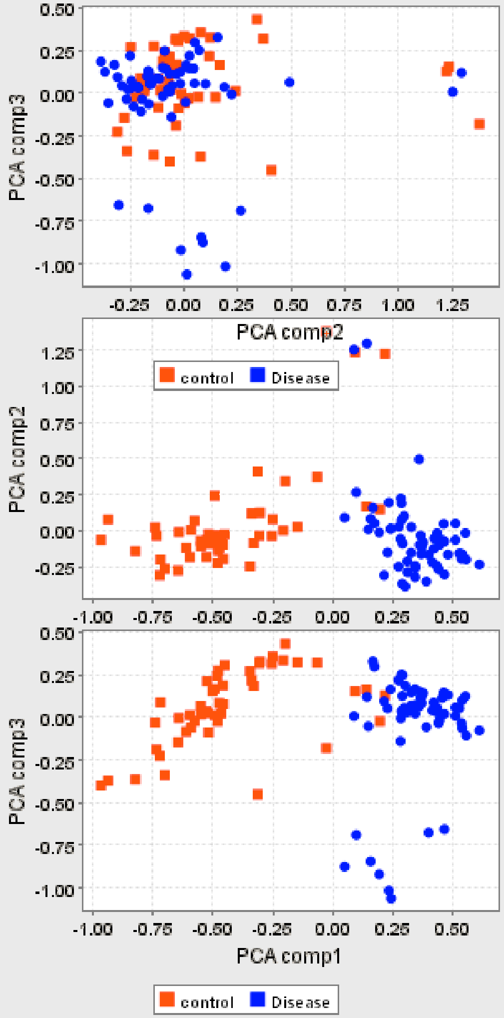
p-value < 0.001
AUROC = 0.947





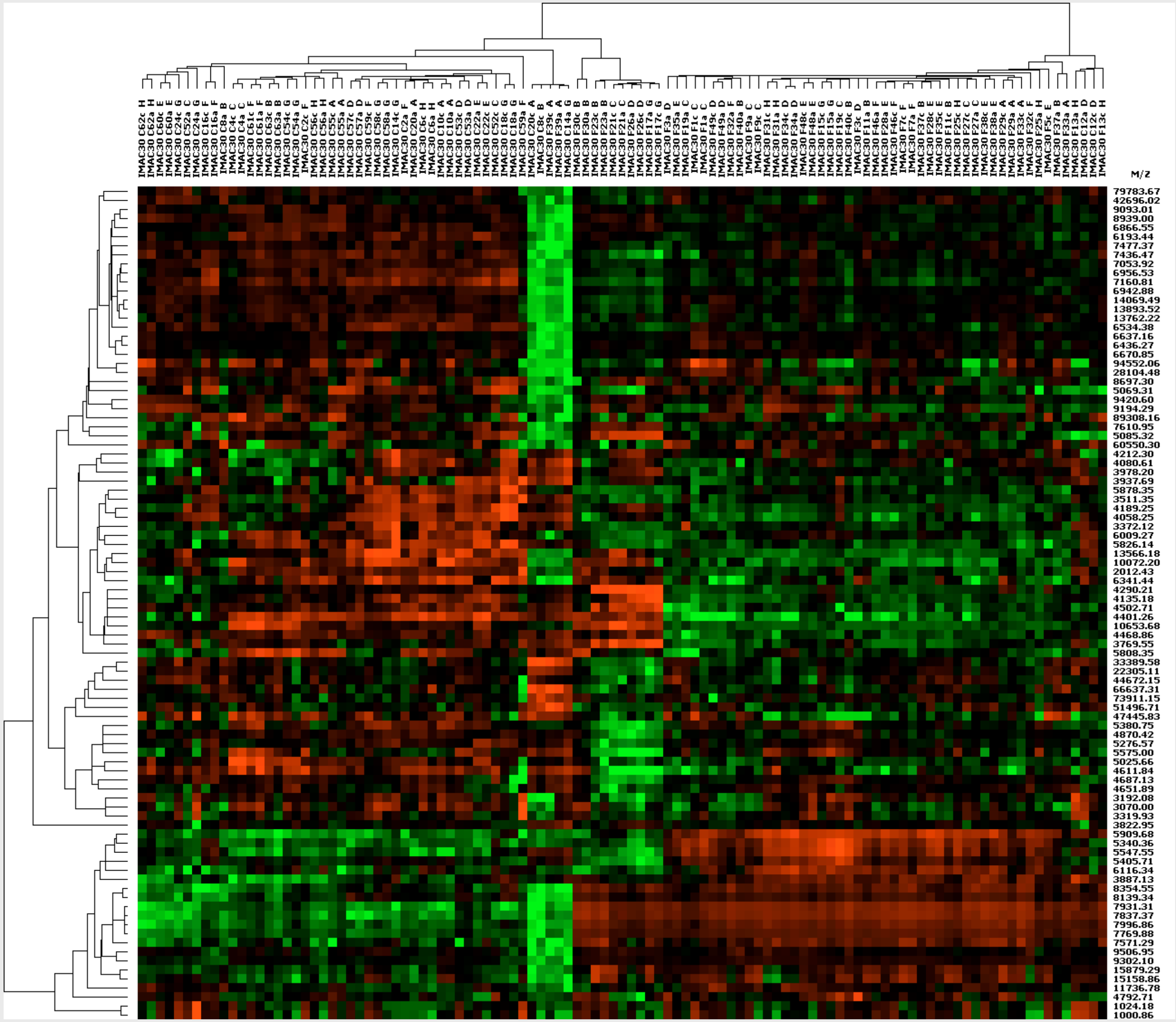
Principle Component Analysis





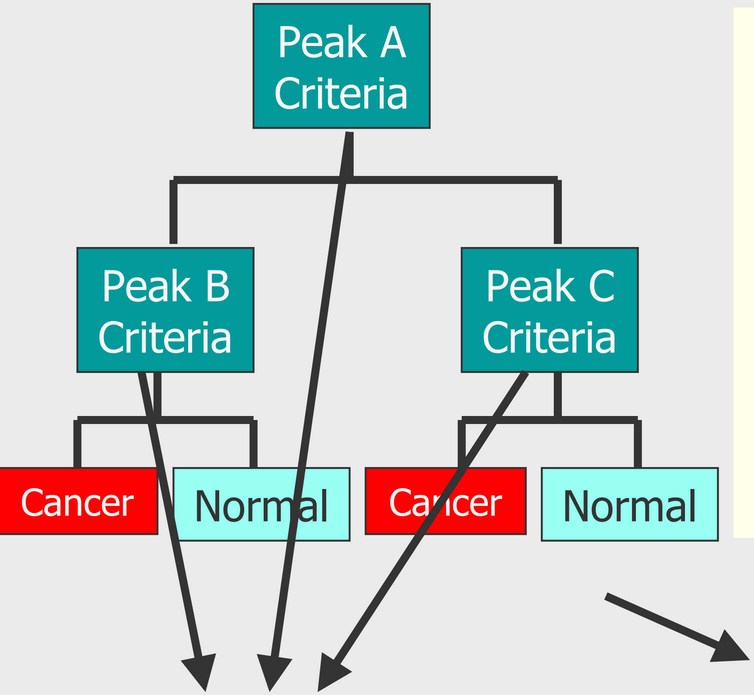
Heat Map Hierarchical Clustering

Hierarchical clustering and heat map creation can be performed using the CE Data Analysis Module.



CIPHERGEN®

Multiple Biomarker Diagnostic Assays


Peak A Criteria

Peak B Criteria

Peak C Criteria

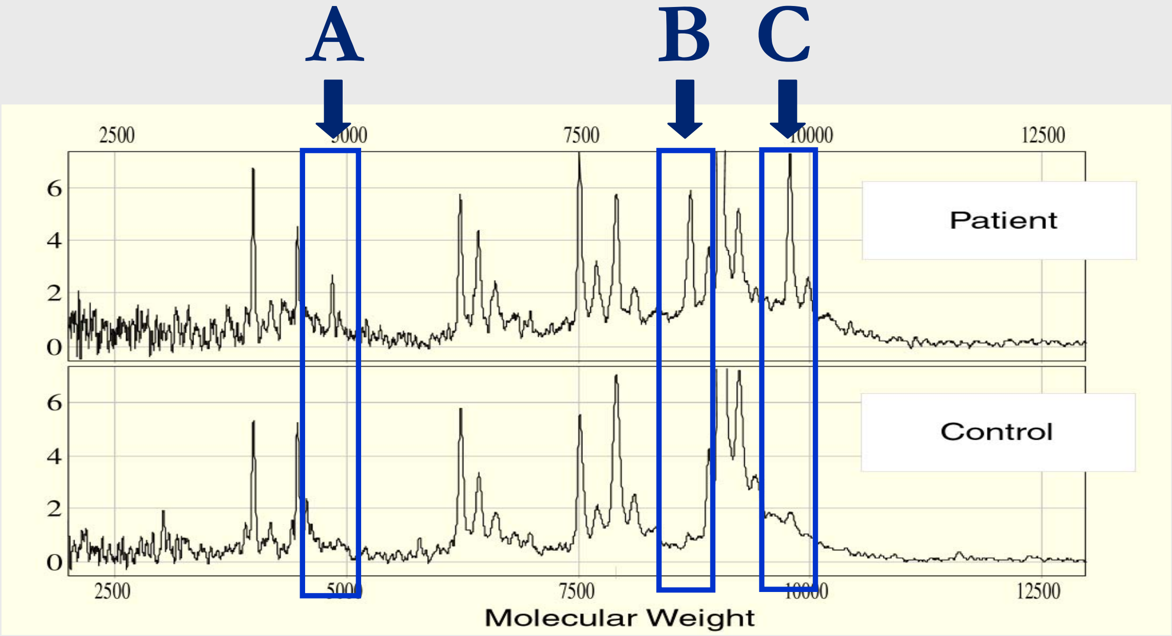
Cancer

Normal

Cancer

Normal

ID the biomarkers, Link to biology of disease

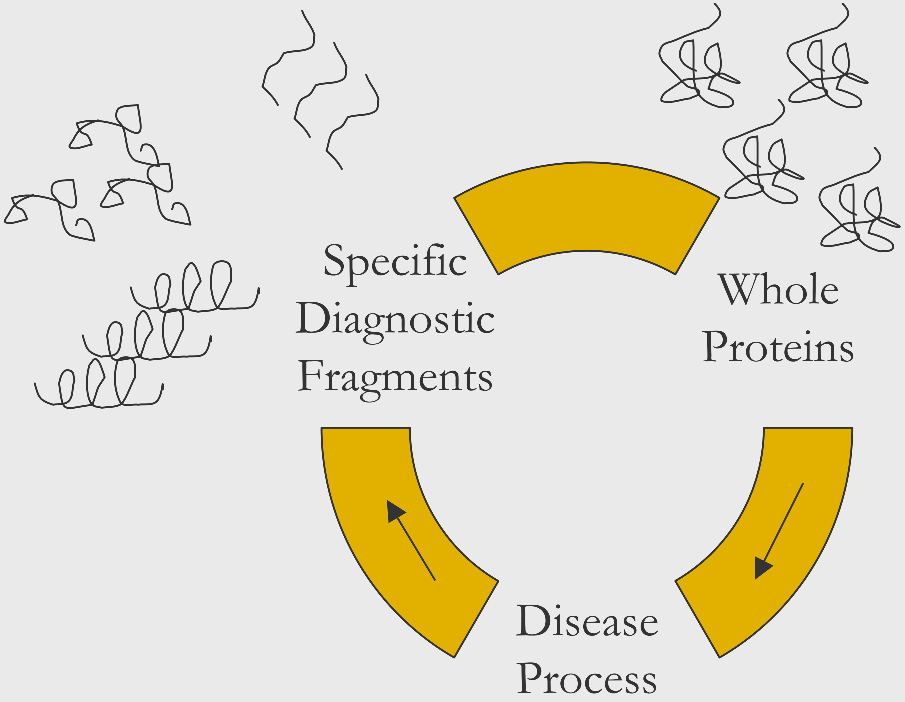


	Clinical Dx Sensitivity "True Positives"	Clinical Dx Specificity "True Negatives"
Single Biomarker	**65%**	**35%**
Multi-Biomarker Pattern	**>90%**	**>90%**

Improve Predictive Accuracy and Diagnostic Value

Host Response Protein Amplification Cascade: Common Proteins Yield Uncommon Fragments

- **Apolipoprotein A1**
- **Transthyretin**
- **Inter alpha-trypsin inhibitor 4**
- **Haptoglobin a**
- **Serum amyloid A**
- **Vitamin D Binding Protein**
- **C3 anaphylotoxin**



Specific Diagnostic Fragments

Whole Proteins

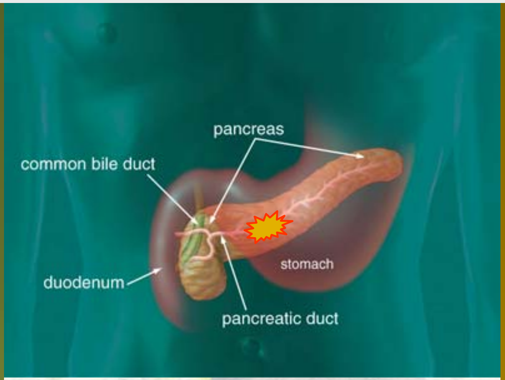
Disease Process

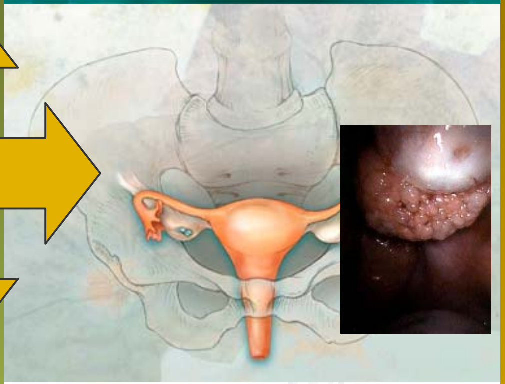
Specific Disease Processes Cleave and Modify Common Proteins into Uncommon Fragments with Diagnostic Utility

Common Proteins → **Disease Processes** → **Specific Diagnostic Fragments**

Inter Alpha Trypsin Inhibitor 4

(ITIH4)





Pancreatic Cancer

nvhsgstffkyylqgakipkpeasfspr

Ovarian Cancer

mnfrpgvlssrqlglpgppdvpdhaayhpfr

Diabetes

srqlglgppdvpdhaayhpfr

Biomarkers found using SELDI technology

- Cancer
  - Breast
  - Prostate
  - Bladder
  - Leukemia
  - Lung
  - Brain
- Pharmaceutical testing
  - Toxicity markers
  - Non-responders
- Other
  - Acute renal failure
  - Acute heart failure
  - Exposure to airborne toxins
- Neuropsychiatric
  - Depression
  - Schizophrenia
  - Alzheimer’s disease
  - Parkinson’s
  - Huntington’s
- Infectious diseases
  - Yersinia pestis
  - Mycobacterium
  - Caulobacter
  - Streptococcus
  - Botulism
  - Prions

A Novel Multi-Marker Assay for Ovarian Cancer

Zhang, Z. *et al*. (2004) *Cancer Research* **64**, 5882-5890.

CIPHERGEN®

Study participants

Johns Hopkins University: Zhen Zhang, Ph.D., Jinong Li, Ph.D., Lori J. Sokoll, Ph.D., Alex J. Rai, Ph.D., Jason M. Rosenzweig, Bonnie Cameron, Daniel W. Chan, Ph.D.

MD Anderson: Robert C. Bast Jr., M.D., Yinhua Yu, M.D.

Duke: Andrew Berchuck, M.D.

Royal Hospital for Women (Sydney): Carolien van Haaften-Day, Ph.D., Neville F. Hacker, M.D.

Groningen University Hospital: Henk W. A. de Bruijn, Ph.D., Ate G. J. van der Zee, M.D.

Bart's and The London, Queen Mary School of Medicine, London University: Ian J. Jacobs, M.D.

Ciphergen: Xiao-Ying Meng, M.Sc., Eric T. Fung, M.D., Ph.D.

CIPHERGEN®

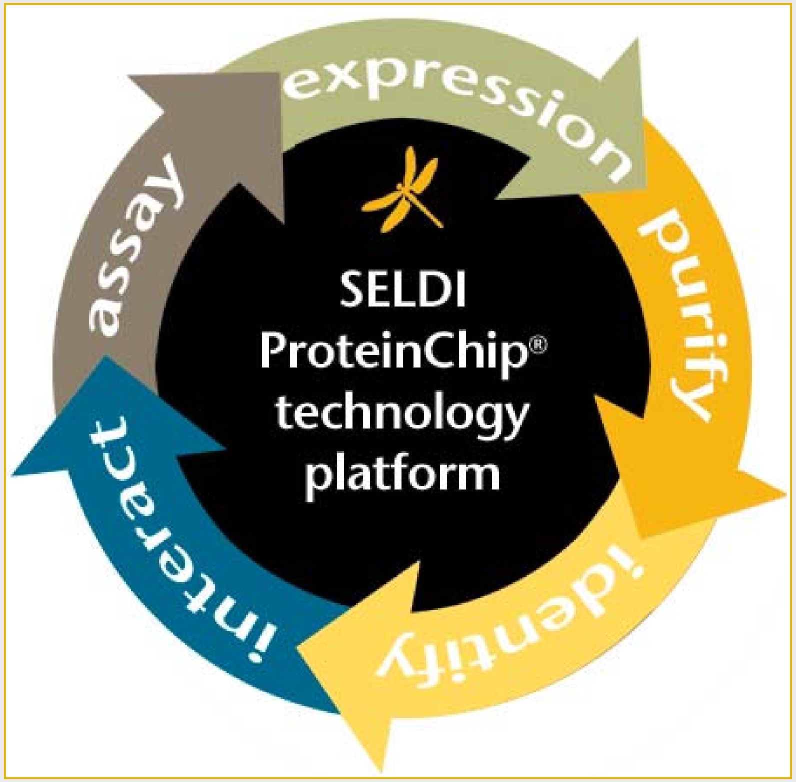
Basic statistics of ovarian cancer

- **Prevalence 40/100,000 (1 in 2500)**
- **23,000 new cases diagnosed annually**
- **14,000 deaths annually**
- **Overall 5 year survival 20-30%**
- **75% of cases are diagnosed in late stage (stage III/IV)**
- **90% cure rate in stage I/IIa**
- **Therefore, detection in earlier stages critical in improving overall survival**

Diagnosing early stage ovarian cancer

- **Low prevalence of disease places extremely high requirements on screening test**
- **CA-125 is the only generally accepted tumor marker for ovarian cancer**
  - Elevated in ~80% of all patients with ovarian cancer
  - Elevated in <50% of patients with early stage ovarian cancer
  - Not useful for screening
  - Used typically for:
    - *Initial diagnosis in symptomatic women in conjunction with other tests (e.g., transvaginal ultrasound, computed tomography)*
    - *Monitoring of response to therapy*

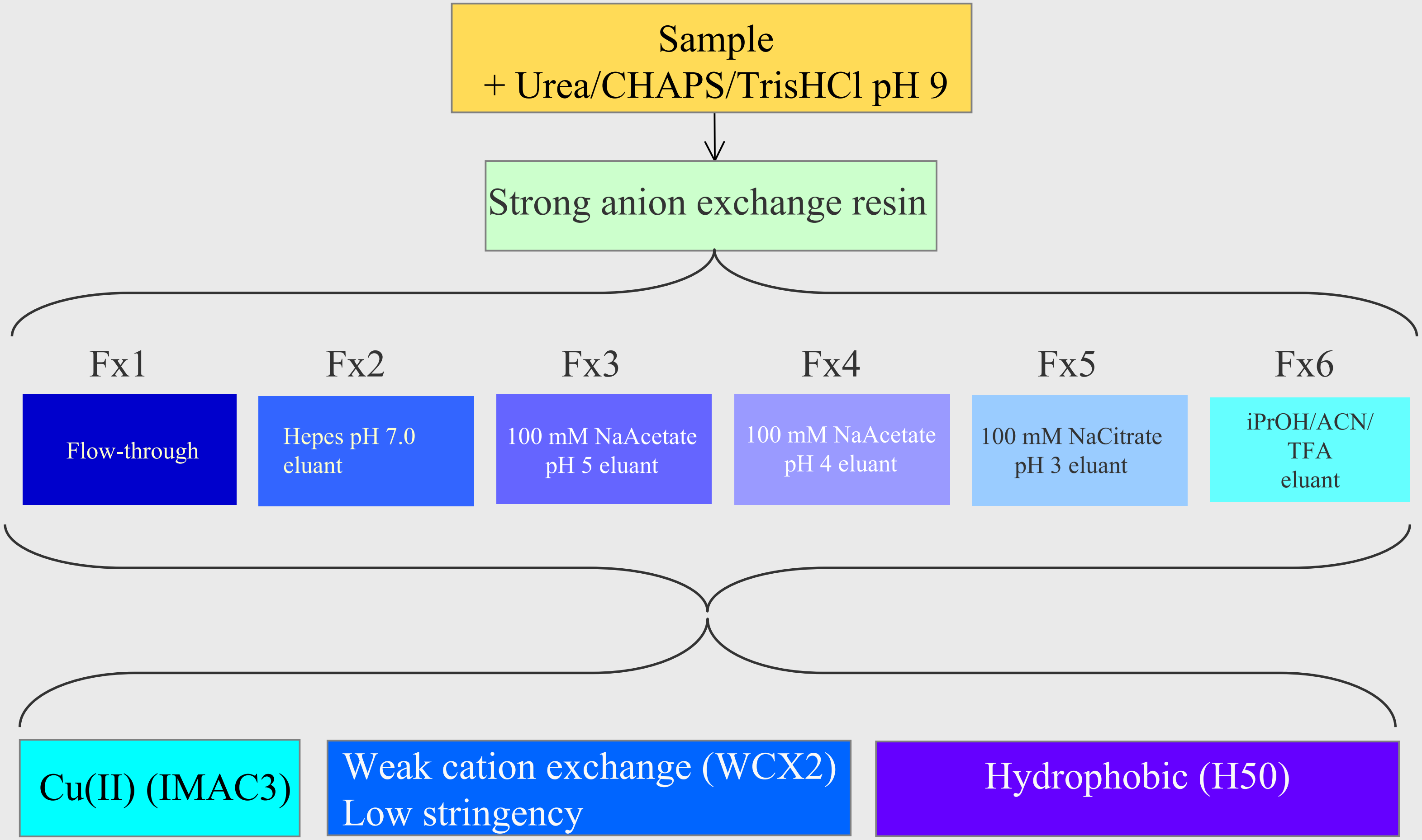
Discovery: Expression Differences



Methodology Outline

- **Protein expression profiling for all samples was performed simultaneously**
- **Protocol: anion exchange fractionation of serum followed by binding of fractions to ProteinChip arrays**
- **Each sample was bound to each array in triplicate**
- **Instrument performance was monitored using**
  - Insulin (test for instrument resolution)
  - Immunoglobulin (test for instrument sensitivity)
- **Assay performance was monitored by processing a standard serum sample**

Fractionation



Sample
+ Urea/CHAPS/TrisHCl pH 9

Strong anion exchange resin

Fx1	Fx2	Fx3	Fx4	Fx5	Fx6
Flow-through	Hepes pH 7.0 eluant	100 mM NaAcetate pH 5 eluant	100 mM NaAcetate pH 4 eluant	100 mM NaCitrate pH 3 eluant	iPrOH/ACN/ TFA eluant

Cu(II) (IMAC3)

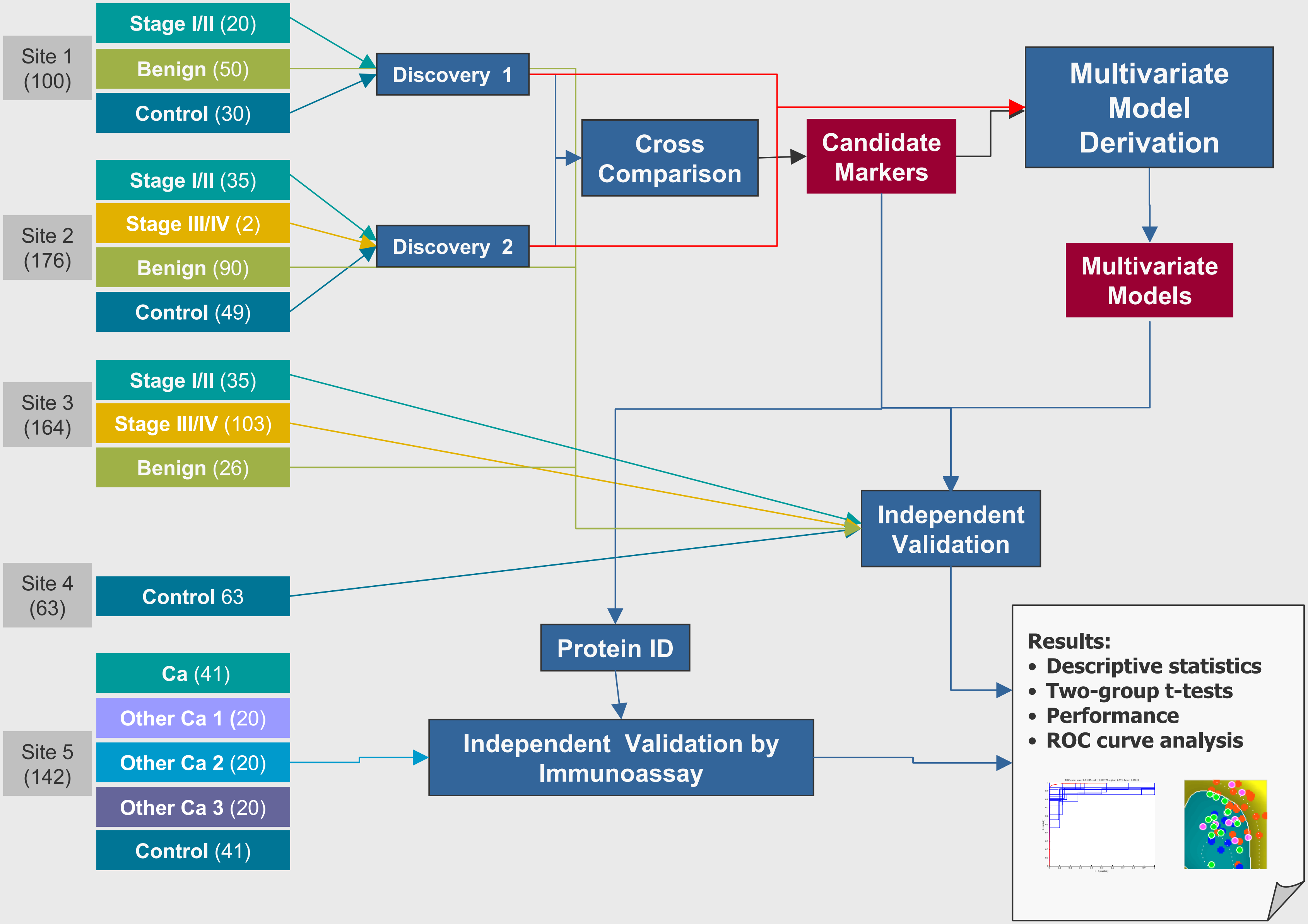
Weak cation exchange (WCX2)
Low stringency

Hydrophobic (H50)

Data mining

- **Data pre-processing**
  - Mass calibration, baseline subtraction, total ion current normalization
  - Peak selection – automated followed by manual confirmation
- **Data analysis**
  - Unified Maximum Separability Analysis (UMSA): Modified support vector machine algorithm
  - Bootstrap re-sampling technique on site A and site B data separately to determine best set of peaks from independent analysis
  - Biomarkers for inclusion are those that
    - *Are deemed significant repeatedly during the bootstrap process*
    - *Change in the same direction between both sites*
  - Validate biomarkers on independent data set

Study design for biomarker discovery



Site 1 (100)
- **Stage I/II** (20)
- **Benign** (50)
- **Control** (30)

Site 2 (176)
- **Stage I/II** (35)
- **Stage III/IV** (2)
- **Benign** (90)
- **Control** (49)

Site 3 (164)
- **Stage I/II** (35)
- **Stage III/IV** (103)
- **Benign** (26)

Site 4 (63)
- **Control** 63

Site 5 (142)
- **Ca** (41)
- **Other Ca 1** (20)
- **Other Ca 2** (20)
- **Other Ca 3** (20)
- **Control** (41)

Discovery 1

Discovery 2

Cross Comparison

Candidate Markers

Multivariate Model Derivation

Multivariate Models

Independent Validation

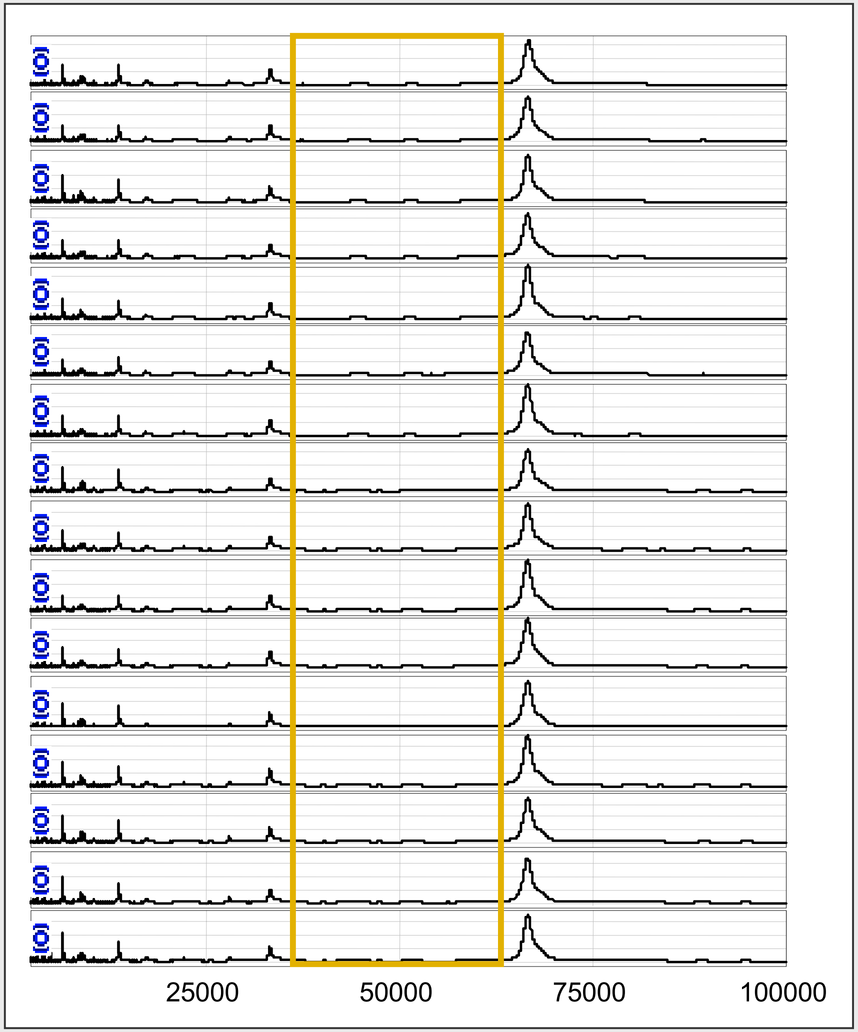
Protein ID

Independent Validation by Immunoassay

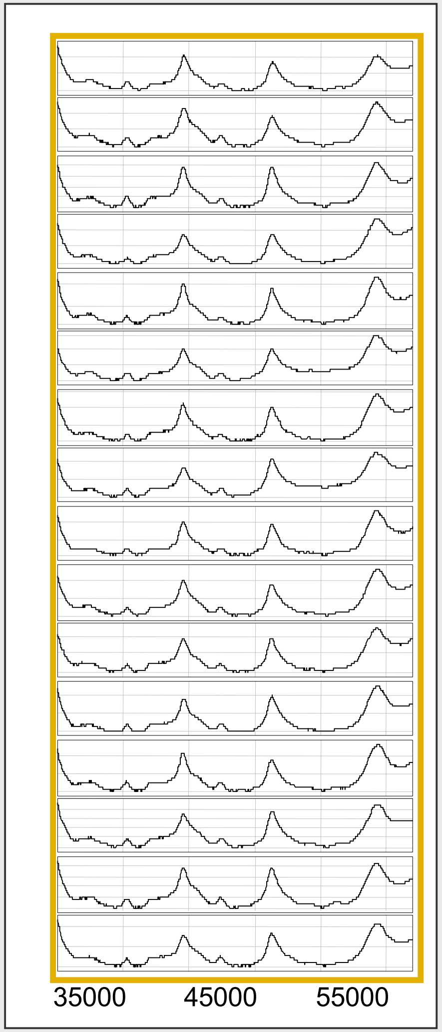
Results:
- **Descriptive statistics**
- **Two-group t-tests**
- **Performance**
- **ROC curve analysis**

CIPHERGEN®

Reproducibility



Mass Range = 2-100 kDa



Mass Range = 35-62 kDa

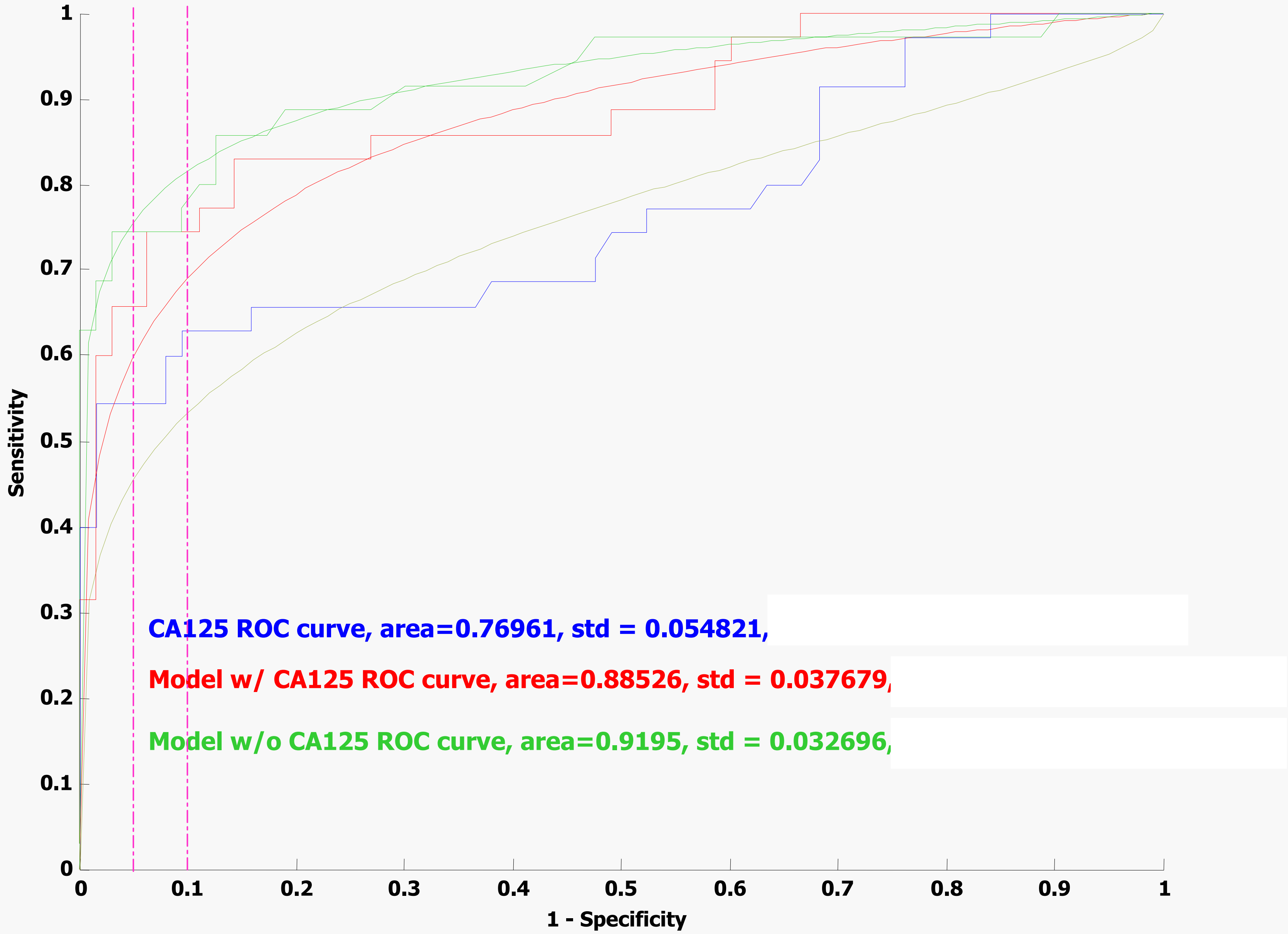
Average CV's range from 10-25%

CIPHERGEN®

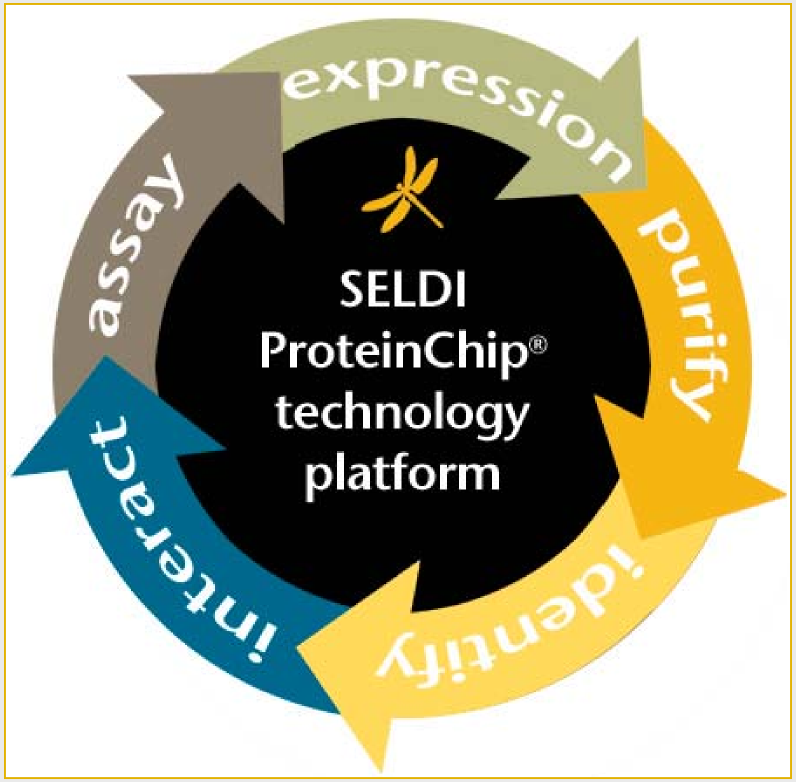
Performance of individual biomarkers in discovery set & validation set

Comparison	All epithelial ovarian cancer	Stage I/II invasive ovarian cancer
CA125, Discovery set	<.00001	<.00001
CA125, Validation set	.00001	<.00001
Marker 1, Discovery set	<.00001	<.00001
Marker 1, Validation set	<.00001	<.00001
Marker 2, Discovery set	.00002	.00004
Marker 2, Validation set	<.00001	<.00001
Marker 3, Discovery set	<.00001	.059178
Marker 3, Validation set	<.00001	.079999

P value estimated for two-group T test between healthy controls and ovarian cancer patient population

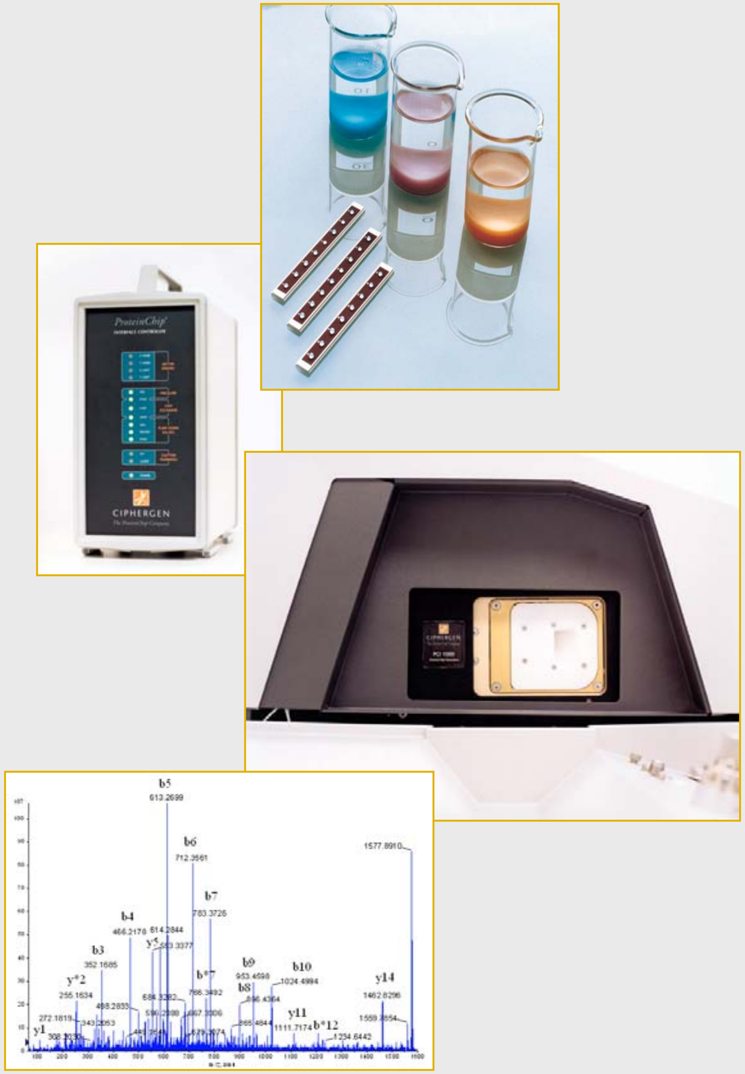

Sensitivity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1 - Specificity
CA125 ROC curve, area=0.76961, std = 0.054821,
Model w/ CA125 ROC curve, area=0.88526, std = 0.037679,
Model w/o CA125 ROC curve, area=0.9195, std = 0.032696,

Purification and identification



ProteinChip® Assisted purification and ID

- Spin column chromatography, with monitoring of fractions using the ProteinChip® Biomarker System
- Protease digestion
- Matching of peptide fingerprint with database
- Confirmation using the Ciphergen ProteinChip Interface installed on the ABI/Sciex QSTAR™ tandem MS



Apolipoprotein A1

- Major lipoprotein in HDL’s
- Negative acute phase reactant protein
- Total apolipoprotein A has previously been reported to be decreased in patients with ovarian cancer (Kuesel et al, 1992)

Transthyretin

- **Marker identified by profiling is an N-terminal truncation of transthyretin (pre-albumin)**
- **Transthyretin has been reported to be decreased in ovarian cancer patients previously (Mahlck et al, 1994)**
- **Biological function**
  - Homotetramer
  - Transports thyroid hormone, retinol binding protein (RBP)
  - Decreases in pre-albumin lead to decreases in RBP
  - Decreased levels of RBP leads to malignant transformation of ovarian epithelial cells (in culture)
- **No reports of truncated forms**

Peptide fragment of inter alpha trypsin inhibitor IV (ITIH4)

- **ITIH4 Known to be cleaved by Kallikrein enzymes**
- **Kallikrein enzyme levels known to change in Ovarian Cancer**
- **Hypothesis: kallikreins, or other cancer-related proteases, may be responsible for generating fragments found in this study**
- **Test hypothesis using IDM platform**

Specificity of marker panel

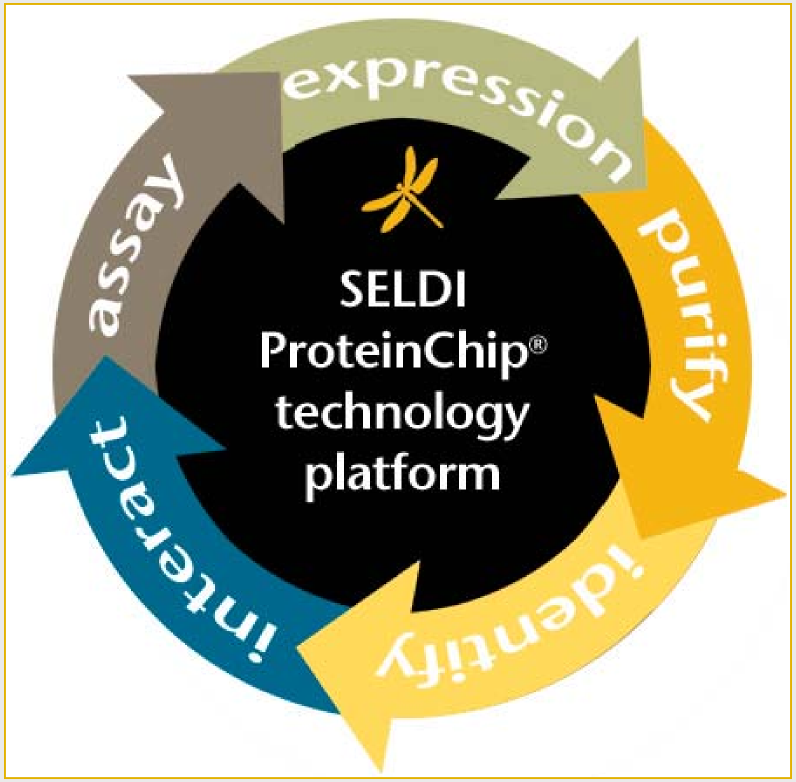
	Ovarian cancer	Breast cancer	Prostate cancer	Colon cancer
CA125	**0.000000**	**0.39**	**0.07**	**0.86**
Apolipoprotein A1 (intact)	**0.0004**	**0.77**	**0.02**	**0.69**
Transthyretin (intact)	**0.00005**	**0.51**	**0.22**	**0.01**

Additional specificity conferred by:

Use of combination of markers

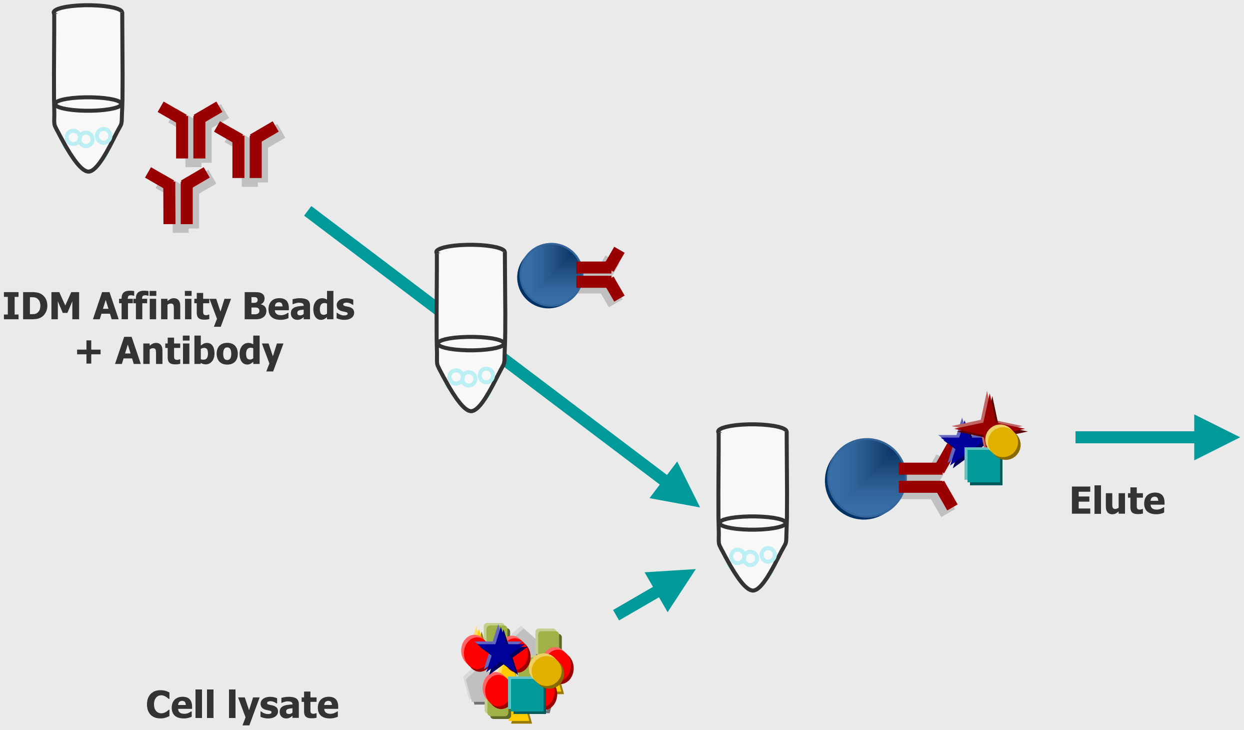
Nature of cleavage products

Interacting Proteins



Secondary interaction discovery

Confirm existing interactors, discover new ones

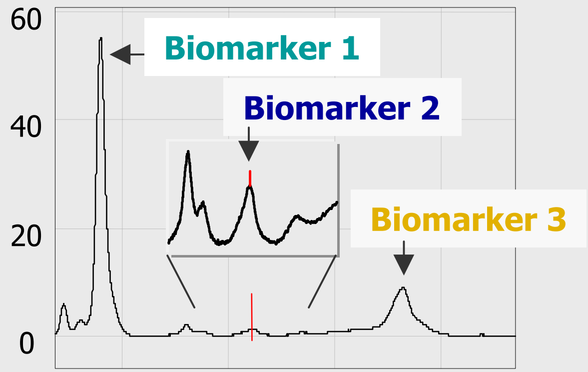


IDM Affinity Beads + Antibody

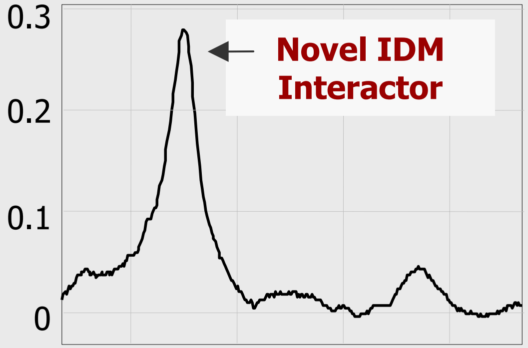
Cell lysate

Elute

Analysis on ProteinChip® Array



Low mass range

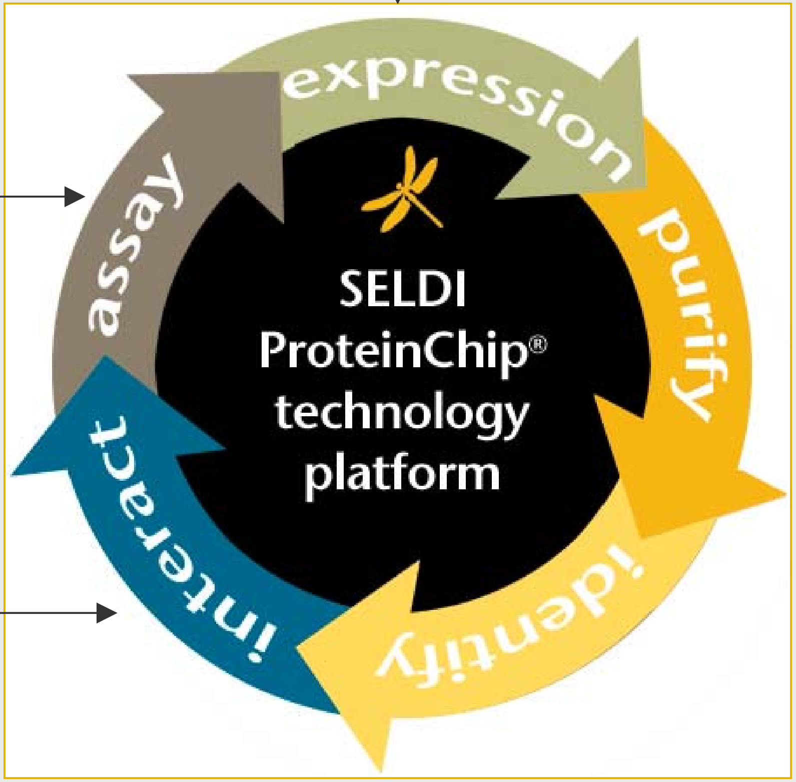


High mass range

Ongoing research

- **Expansion of sample sets (>1500 total)**
  - University of Leuven (prognosis and treatment response)
  - Groningen (prognosis and treatment response)
  - Bart's College (screening study)
  - Mayo Clinic/National Cancer Institute (initial diagnosis)
- **Validation of existing marker set**
- **Combination of markers, novel and previously characterized**
- **Discovery of additional markers by**
  - New generation protein expression profiling protocol
  - Interaction mapping
  - Kallikrein substrate experiments
- **Development of multiplexed ProteinChip assays for markers**

Pattern Track™ Process



Discovery assay

Diagnostic assay

Interaction assay

ProteinChip® System, Series 4000

Personal edition

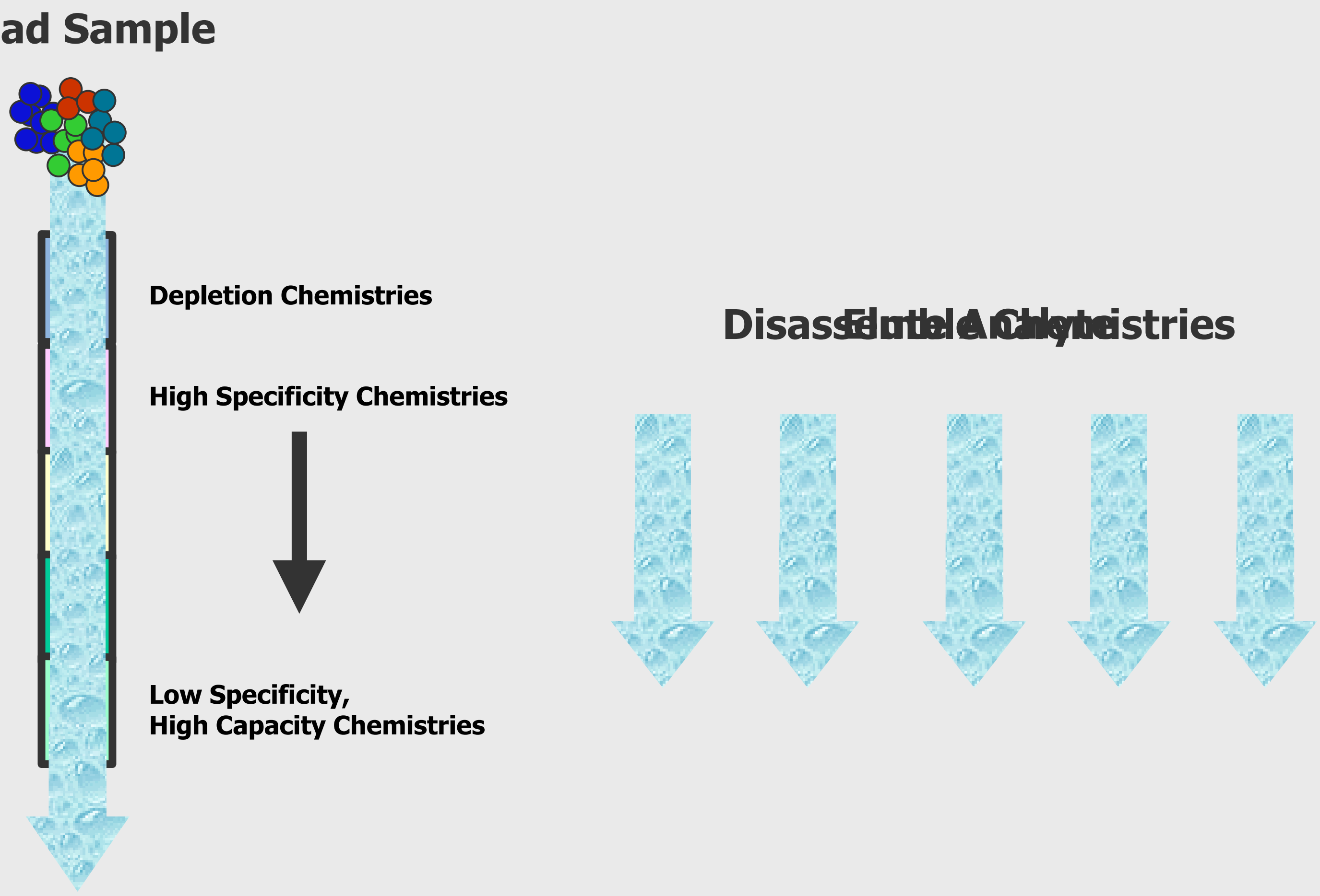
Enterprise edition

ProteinChip System, Series 4000

Superior Quantitation	**■Raster laser design for maximum spot coverage** **■Auto laser energy setting** **■Extended Linear Dynamic Range** **■Improved ProteinChip Arrays and protocols**
Highest Sensitivity	**■New detector** **■New Ion Source increases ion efficiency** **■Detector blanking reduces noise** **■Innovative flight tube design**
Highest Resolution/Resolving power	**■Improved fractionation tools increase resolution up to 3000 proteins**
Highest Throughput	**■Unattended runs of up to 168 ProteinChip Arrays**
Improved *low abundance* biomarker discovery	**■New Deep Proteome™ Tools to explore the last 1% of the serum proteome**

Improved Resolution/Resolving power

MultiSelect™ Fractionation



Load Sample

Depletion Chemistries

High Specificity Chemistries

Low Specificity,
High Capacity Chemistries

Disassemble Chemistries

Elute Analyte

Improved sensitivity with multiple chemistry approach

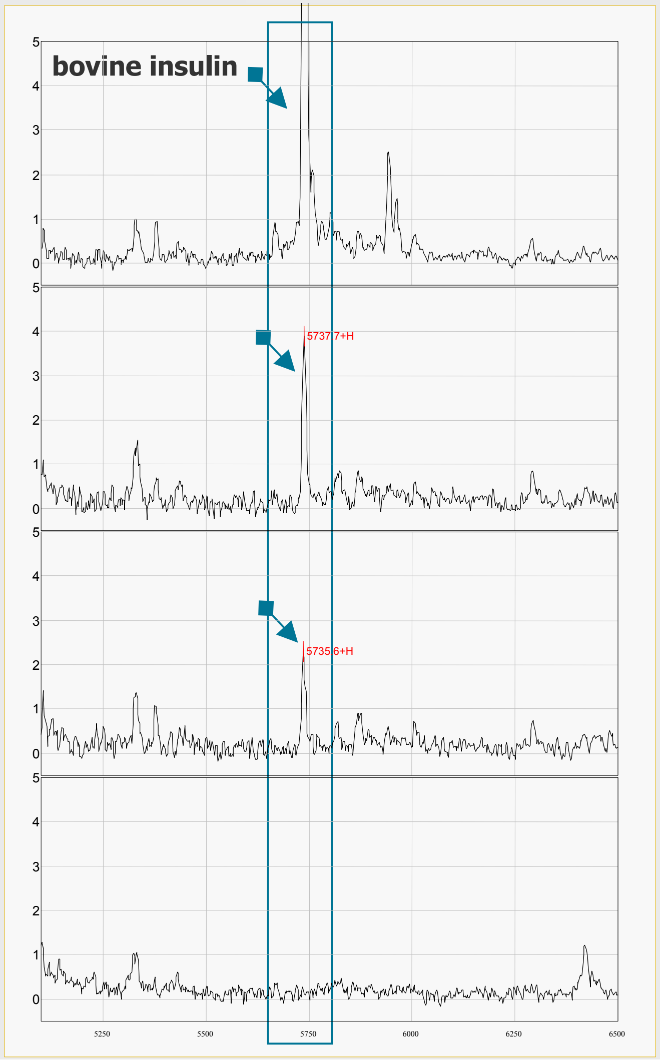
100 fmol/ul

10 fmol/ul

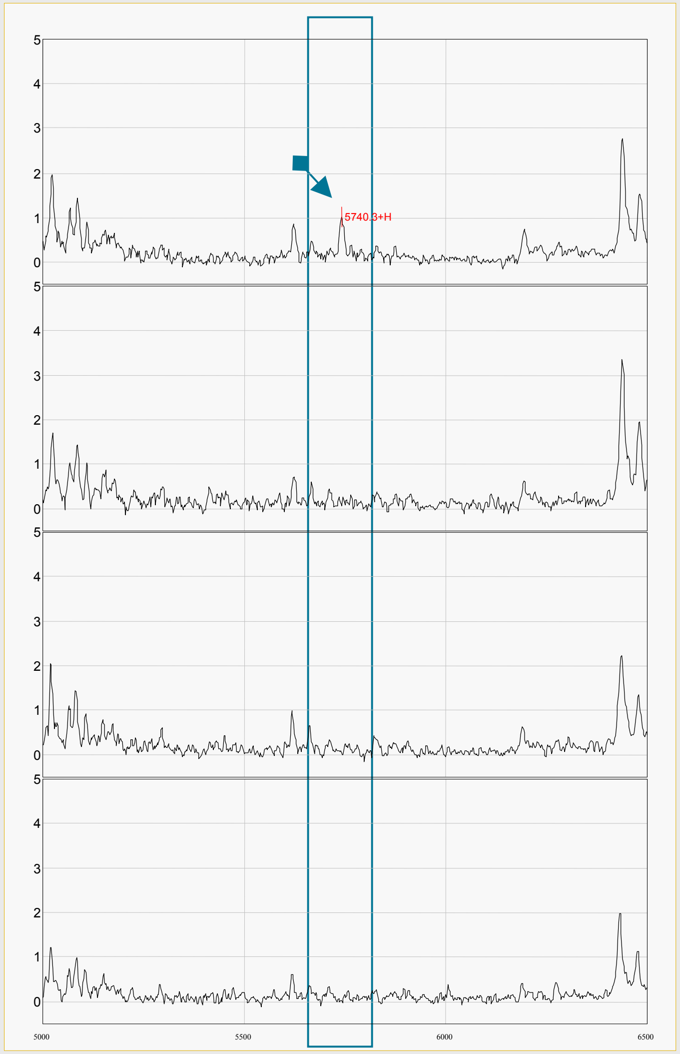
1 fmol/ul

Control

Multiple Chemistry Pre-fractionation



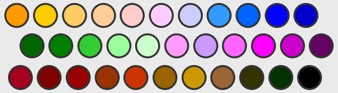
Single Chemistry Pre-fractionation

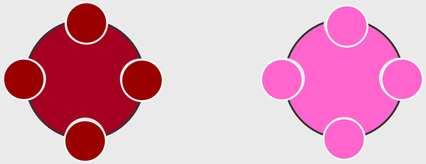


Protein Equalizer™ Beads

Affinity Bioseparation of Proteins via Combinatorial Ligands

Define Equalizer Bead Library





Bind

Wash away Excess

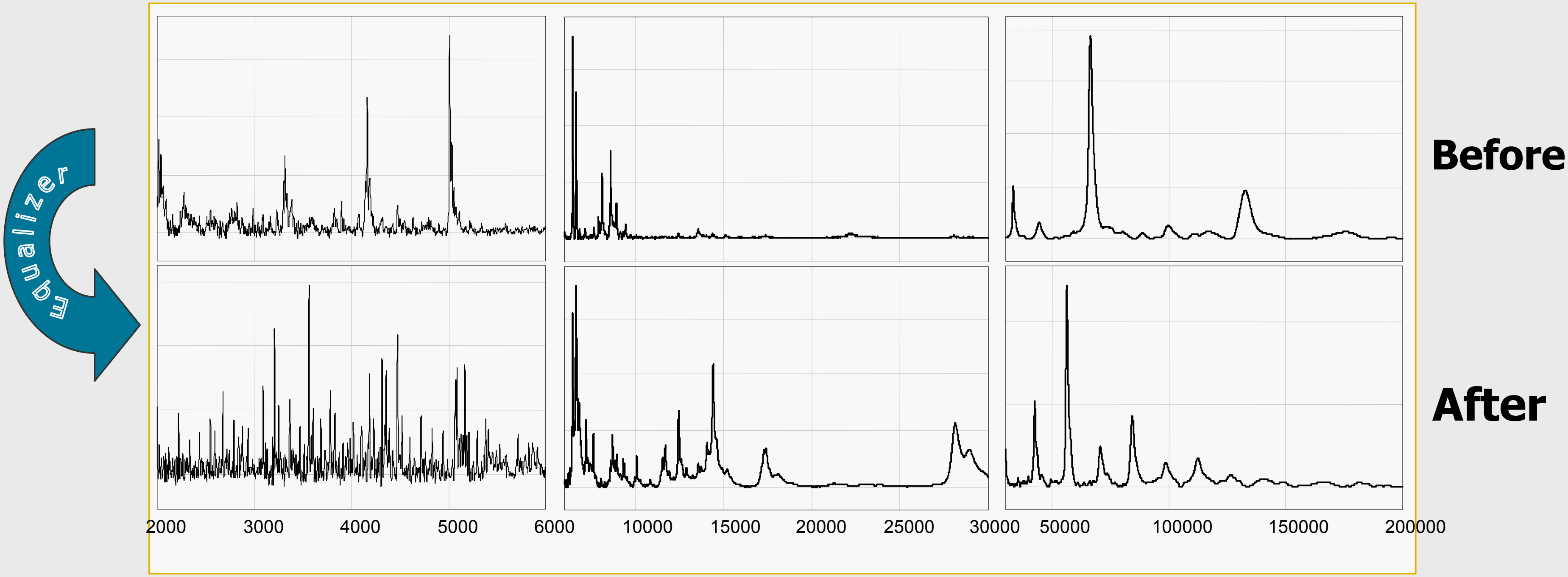
Elute Equalized Sample

- Each bead binds a unique protein
- Capacity of every bead is the same

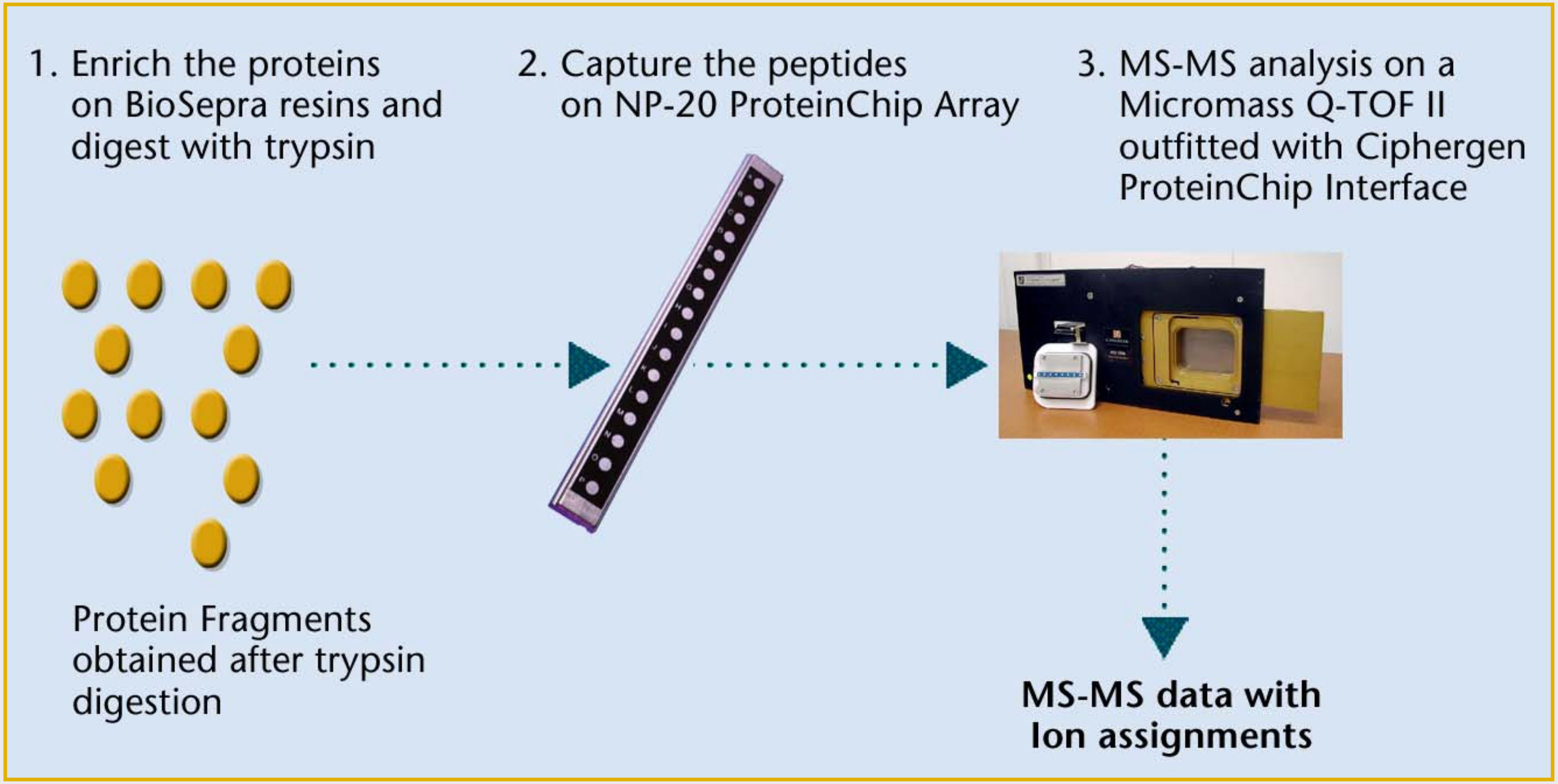
4 Yellow
4 Blue
3 Green
1 Red
1 Pink

Effect of Protein Equalizer™ Beads on serum proteins

Unfractionated Serum profiled Before and After processing on Protein Equalizer Beads



SELDI-MS Platform Protein Identification Using ProteinChip Interface

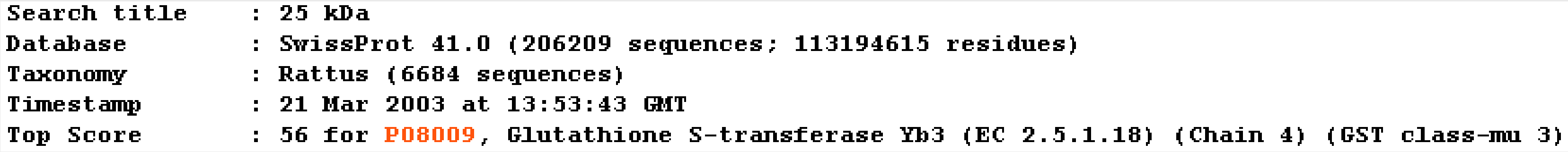


1. Enrich the proteins on BioSepra resins and digest with trypsin
2. Capture the peptides on NP-20 ProteinChip Array
3. MS-MS analysis on a Micromass Q-TOF II outfitted with Ciphergen ProteinChip Interface

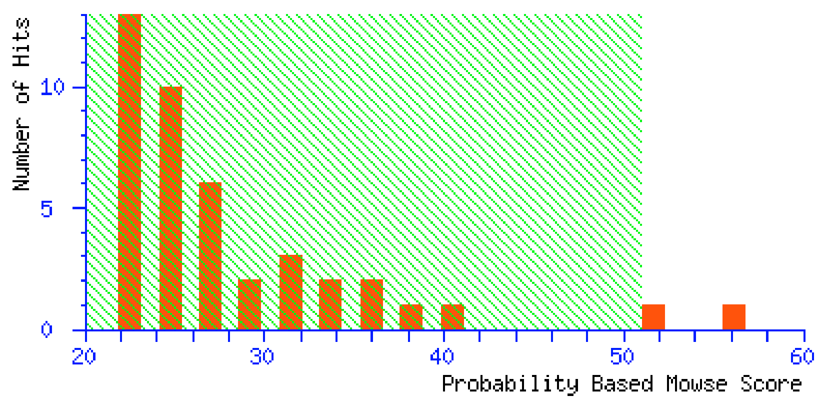
Protein Fragments obtained after trypsin digestion

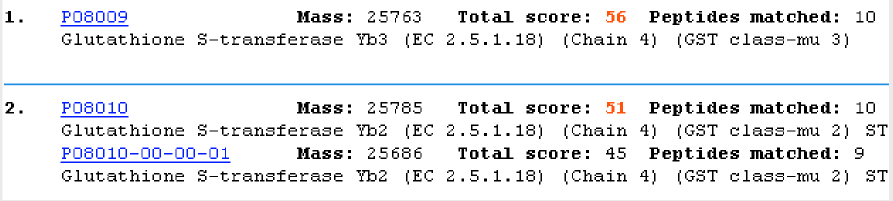
MS-MS data with Ion assignments

Protein Identification (Mascot Search)

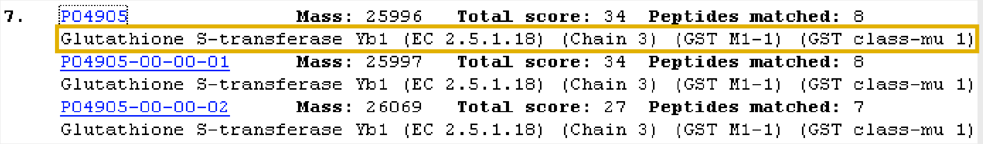


```
Search title     : 25 kDa
Database         : SwissProt 41.0 (206209 sequences; 113194615 residues)
Taxonomy         : Rattus (6684 sequences)
Timestamp        : 21 Mar 2003 at 13:53:43 GMT
Top Score        : 56 for P08009, Glutathione S-transferase Yb3 (EC 2.5.1.18) (Chain 4) (GST class-mu 3)
```

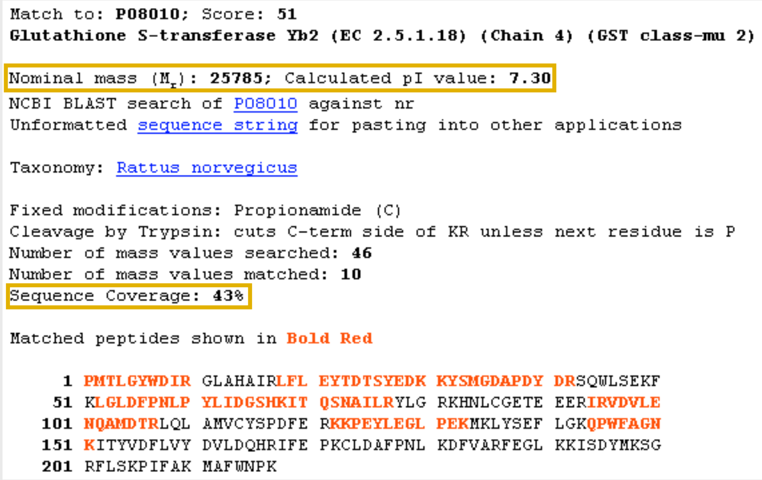




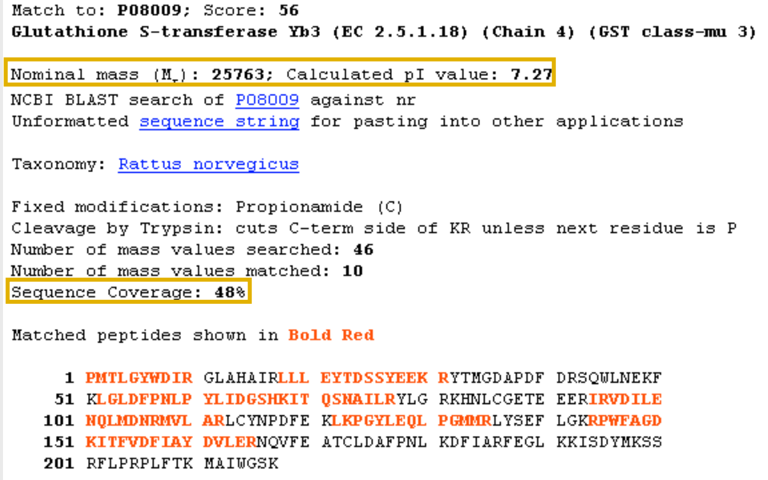
```
1.   P08009               Mass: 25763   Total score: 56   Peptides matched: 10
     Glutathione S-transferase Yb3 (EC 2.5.1.18) (Chain 4) (GST class-mu 3)

2.   P08010               Mass: 25785   Total score: 51   Peptides matched: 10
     Glutathione S-transferase Yb2 (EC 2.5.1.18) (Chain 4) (GST class-mu 2) ST
     P08010-00-00-01      Mass: 25686   Total score: 45   Peptides matched: 9
     Glutathione S-transferase Yb2 (EC 2.5.1.18) (Chain 4) (GST class-mu 2) ST
```



```
7.   P04905               Mass: 25996   Total score: 34   Peptides matched: 8
     Glutathione S-transferase Yb1 (EC 2.5.1.18) (Chain 3) (GST M1-1) (GST class-mu 1)
     P04905-00-00-01      Mass: 25997   Total score: 34   Peptides matched: 8
     Glutathione S-transferase Yb1 (EC 2.5.1.18) (Chain 3) (GST M1-1) (GST class-mu 1)
     P04905-00-00-02      Mass: 26069   Total score: 27   Peptides matched: 7
     Glutathione S-transferase Yb1 (EC 2.5.1.18) (Chain 3) (GST M1-1) (GST class-mu 1)
```



```
Match to: P08010; Score: 51
Glutathione S-transferase Yb2 (EC 2.5.1.18) (Chain 4) (GST class-mu 2)

Nominal mass (Mr): 25785; Calculated pI value: 7.30
NCBI BLAST search of P08010 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Rattus norvegicus

Fixed modifications: Propionamide (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 46
Number of mass values matched: 10
Sequence Coverage: 43%

Matched peptides shown in Bold Red

    1 PMTLGYWDIR GLAHAIRLFL EYTDTSYEDK KYSMGDAPDY DRSQWLSEKF
   51 KLGLDFPNLP YLIDGSHKIT QSNAILRYLG RKHNLCGETE EERIRVDVLE
  101 NQAMDTRLQL AMVCYSPDFE RKKPEYLEGL PEKMKLYSEF LGKQPWFAGN
  151 KITYVDFLVY DVLDQHRIFE PKCLDAFPNL KDFVARFEGL KKISDYMKSG
  201 RFLSKPIFAK MAFWNPK
```

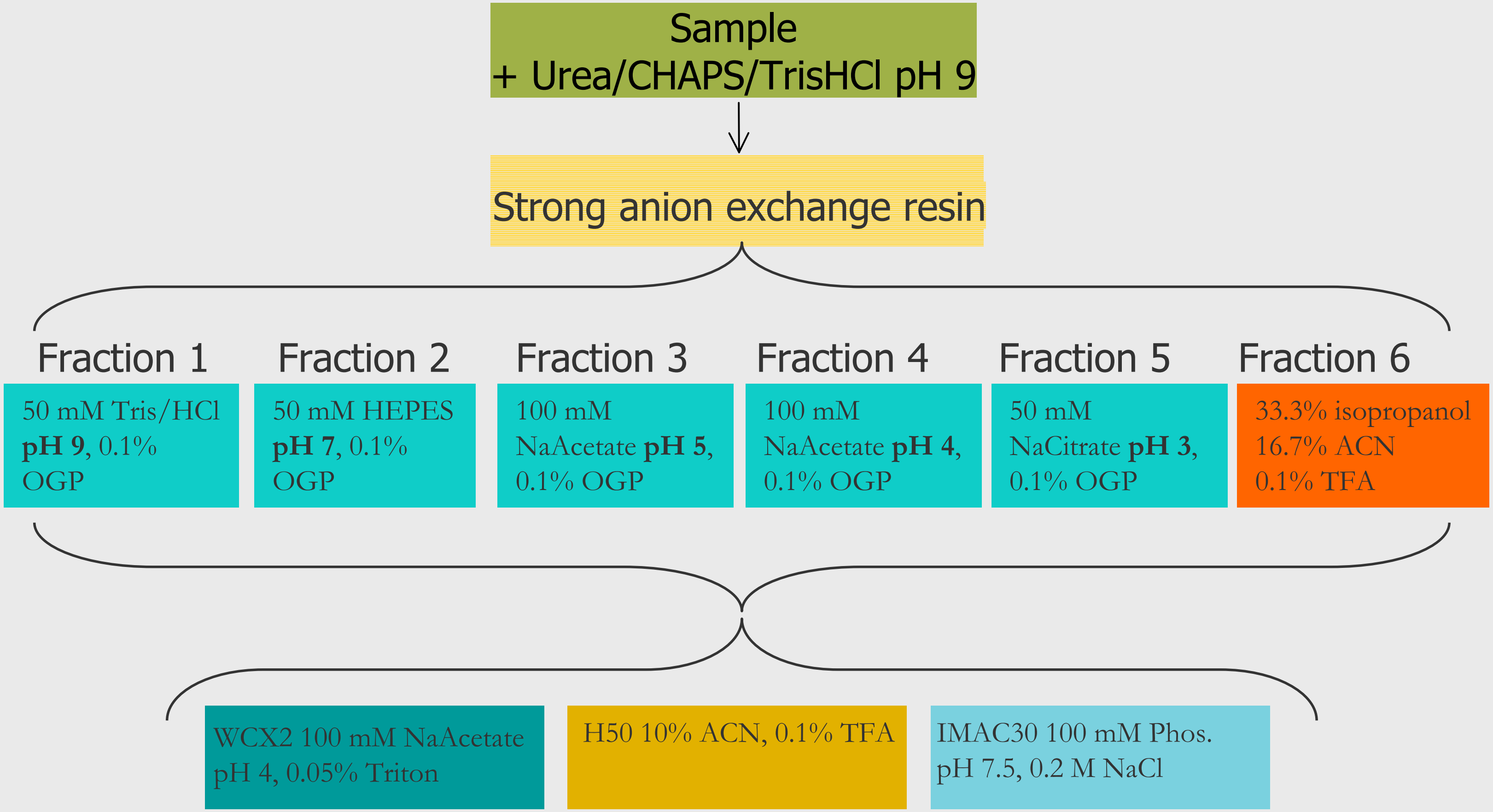


```
Match to: P08009; Score: 56
Glutathione S-transferase Yb3 (EC 2.5.1.18) (Chain 4) (GST class-mu 3)

Nominal mass (Mr): 25763; Calculated pI value: 7.27
NCBI BLAST search of P08009 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Rattus norvegicus

Fixed modifications: Propionamide (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 46
Number of mass values matched: 10
Sequence Coverage: 48%

Matched peptides shown in Bold Red

    1 PMTLGYWDIR GLAHAIRLLL EYTDSSYEEK RYTMGDAPDF DRSQWLNEKF
   51 KLGLDFPNLP YLIDGSHKIT QSNAILRYLG RKHNLCGETE EERIRVDILE
  101 NQLMDNRMVL ARLCYNPDFE KLKPGYLEQL PGMMRLYSEF LGKRPWFAGD
  151 KITFVDFIAY DVLERNQVFE ATCLDAFPNL KDFIARFEGL KKISDYMKSS
  201 RFLPRPLFTK MAIWGSK
```

CIPHERGEN®

Fractionation - scheme

- **Employed fractionation scheme:**



Sample
+ Urea/CHAPS/TrisHCl pH 9

Strong anion exchange resin

Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
50 mM Tris/HCl **pH 9**, 0.1% OGP	50 mM HEPES **pH 7**, 0.1% OGP	100 mM NaAcetate **pH 5**, 0.1% OGP	100 mM NaAcetate **pH 4**, 0.1% OGP	50 mM NaCitrate **pH 3**, 0.1% OGP	33.3% isopropanol 16.7% ACN 0.1% TFA

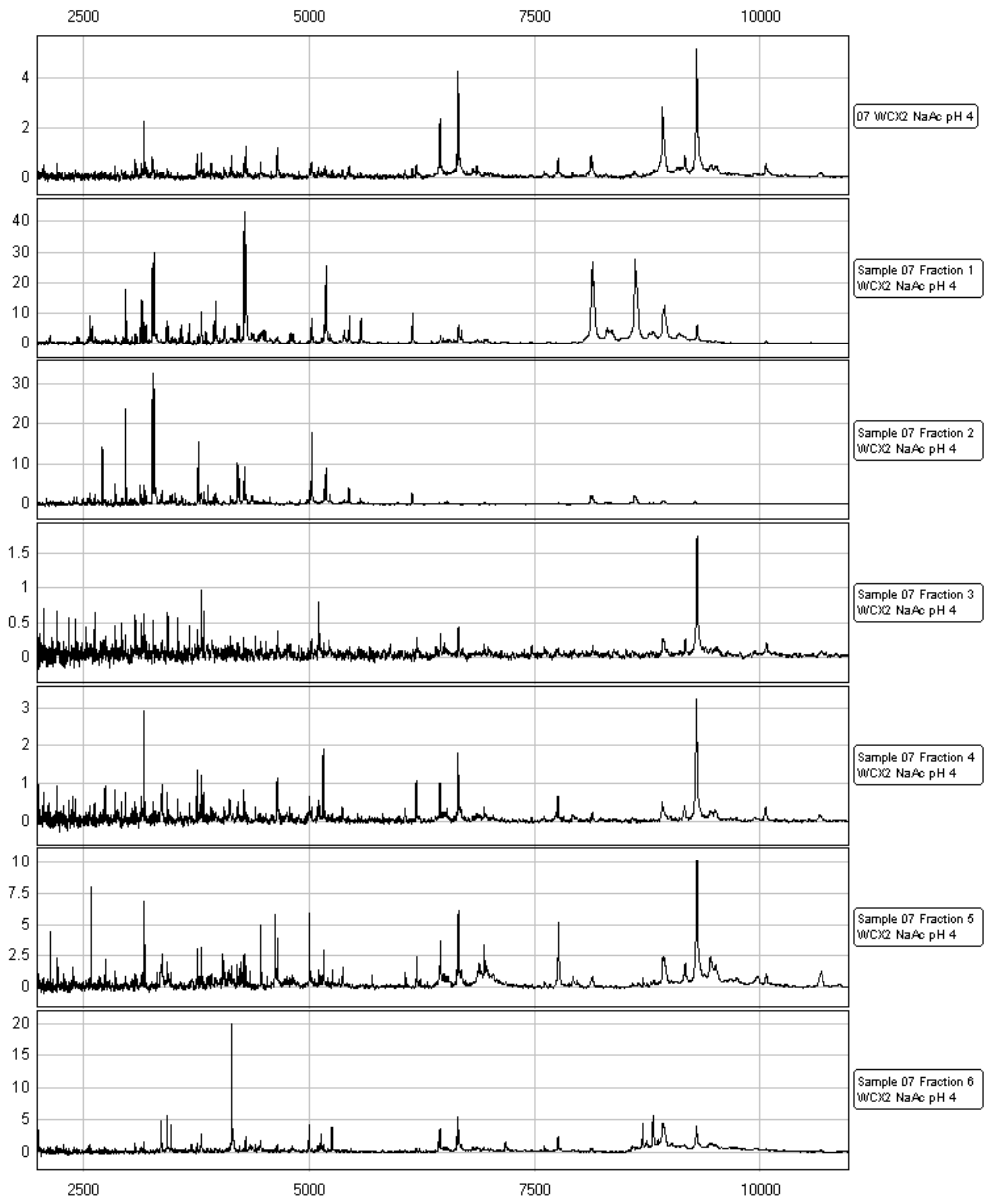
WCX2 100 mM NaAcetate pH 4, 0.05% Triton

H50 10% ACN, 0.1% TFA

IMAC30 100 mM Phos. pH 7.5, 0.2 M NaCl

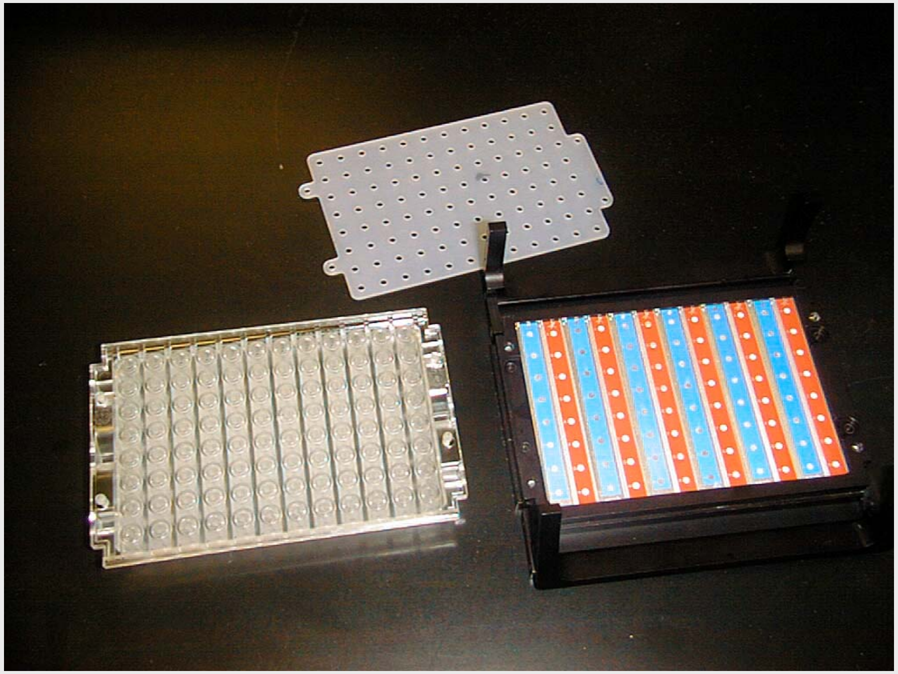
Results – Fractions

- **Fractionation expands the investigated part of the proteome:**
- **Neat = 48 peaks**
- **6 Fractions = 180 unique peaks, or 132 new peaks.**



CIPHERGEN®

The ProteinChip® Bioprocessor



SELDI Citations

Over 200 SELDI Papers:
Nature, Science, The Lancet…..

http://www.ciphergen.com/pub/searchPub.asp

Publications: *Search database*

◄Search Again

190 total documents returned: ProteinChip Citations (✓ denotes recommended readings)

ProteinChip Citations

view	190. **A data-analytic strategy for protein biomarker discovery: profiling of high-dimensional proteomic data for cancer detection**	Yasui, Y., Pepe, M., Thompson, M.L., Adam, B.-L., Wright, G.L., Qu, Y., Potter, J.D., Winget, M., Thornquist, M., Feng, Z.	2003
view	189. **Haptoglobin-alpha Subunit As Potential Serum Biomarker in Ovarian Cancer: Identification and Characterization Using Proteomic Profiling and Mass Spectrometry**	Ye, B., Cramer, D.W., Skates, S.J., Gygi, S.P., Pratomo, V., Fu, L., Horick, N.K., Licklider, L.J., Schorge, J.O., Berkowitz, R.S., Mok, S.C.	2003
view	188. **A panel of cerebrospinal fluid potential biomarkers for the diagnosis of Alzheimer's disease**	Carrette, O., Demalte, I., Scherl, A., Yalkinoglu, O., Corthals, G., Burkhard, P., Hochstrasser, D.F., Sanchez, J.-C.	2003
view	187. **Stereoselective Inactivation of Torpedo californica Acetylcholinesterase by Isomalathion: Inhibitory Reactions with (1R)- and (1S)-Isomers Proceed by Different Mechanisms**	Doorn, J.A., Thompson, C.M., Christner, R.B., Richardson, R.J.	2003
view	186. **Estradiol Control of Expression and Levels of Estradiol-Binding Proteins in the Medial Preoptic Area, Medial Hypothalamus and Pituitary**	Gao, G., Herbert, Z., Kong, J., Gabrielson, N., Mautz, A., Wu, D., Jirikowski, G.F., Caldwell, J.D.	2003
view	185. **Modern Tumor Marker Discovery in Urology: Surface Enhanced Laser Desorption and Ionization (SELDI)**	Gretzer, M.B., Partin, A.W., Chan, D.W., Veltri, R.W.	2003
view	184. **Putative protein markers in the sera of men with prostatic neoplasms**	Lehrer, S., Roboz, J., Ding, H., Zhao, S., Diamond, E.J., Holland, J.F., Stone, N.N., Droller, M.J., Stock, R.G.	2003
view	183. **Extraction of oxytocin and arginine-vasopressin from serum and plasma for radioimmunoassay and surface-enhanced laser desorption-ionization time-of-flight mass spectrometry**	Cool, D.R., DeBrosse, D.	2003
view	182. **Presenilin-1, Nicastrin, Amyloid Precursor Protein, and gamma-Secretase Activity Are Co-localized in the Lysosomal Membrane**	Pasternak, S.H., Bagshaw, R.D., Guiral, M., Zhang, S., Ackerley, C.A., Pak, B.J., Callahan, J.W., Mahuran, D.J.	2003
view	181. **beta-Secretase Cleavage at Amino Acid Residue 34 in the Amyloid beta Peptide Is Dependent upon gamma-Secretase Activity**	Shi, X.-P., Tugusheva, K., Bruce, J.E., Lucka, A., Wu, G.-X., Chen-Dodson, E., Price, E., Li, Y., Xu, M., Huang, Q., Sardana, M.K., Hazuda, D.J.	2003
view	180. **Use of ProteinChip™ Array Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS) to Identify Thymosin Beta-4, A Differentially Secreted Protein from Lymphoblastoid Cell Lines**	Diamond, D.L., Zhang, Y., Gaiger, A., Smithgall, M., Vedvick, T.S., Carter, D.	2003
view	179. **Analysis of complex autoantibody repertoires by surface-enhanced laser desorption/ionization-time of flight mass spectrometry**	Grus, F.H., Joachim, S.C., Pfeiffer, N.	2003
view	178. **Applications of high-throughput methods to cancer metastases**	Keller, E.T., Yao, Z.	2002
view	177. **Macrophage proteomic fingerprinting predicts HIV-1-associated cognitive impairment**	Luo, X., Carlson, K.A., Wojna, V., Mayo, R., Biskup, T.M., Stoner, J., Anderson, J., Gendelman, H.E., Meléndez, L.M.	2003

Over 51 papers featuring SELDI at the 2004 AACR meeting (NCI, JHU, MD Anderson, Stanford etc. covering biomarker applications for all majors cancers

20 Million Possible Diagnostic Patterns from 12 Common Proteins

- **Apoliprotein A1**
- **Transthyretin**
- **Inter alpha-trypsin inhibitor 4**
- **Haptoglobin**
- **Serum amyloid A**
- **Vitamin D Binding Protein**
- **C3 anaphylotoxin**
- **Apolipoprotein A4**
- **C4 Anaphylatoxin Des-Arg**
- **Alpha-1 antichymotrypsin**
- **Apolipprotein C1**
- **Human Serum Albumin**

12 Most Common Proteins

X 5 Fragments (estimate)

- Fragments !

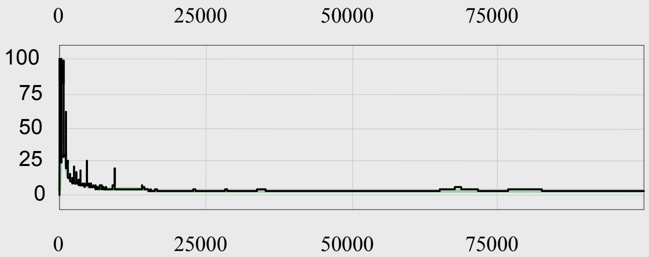
60x59x58x57…

20 Million Possible Diagnostic Patterns

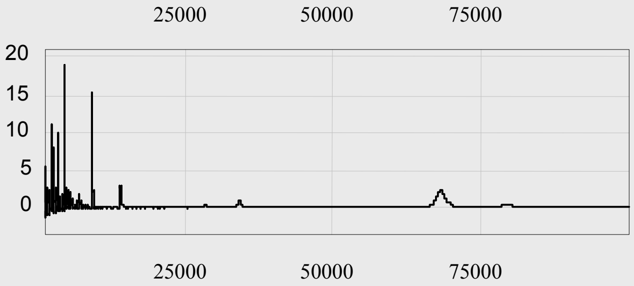
Individual markers have low correlation to each other

	CA125	Marker 1	Marker 2
CA125	1		
Marker 1	-0.26	1	
Marker 2	-.015	.32	1
Marker 3	.05	-0.42	-.07

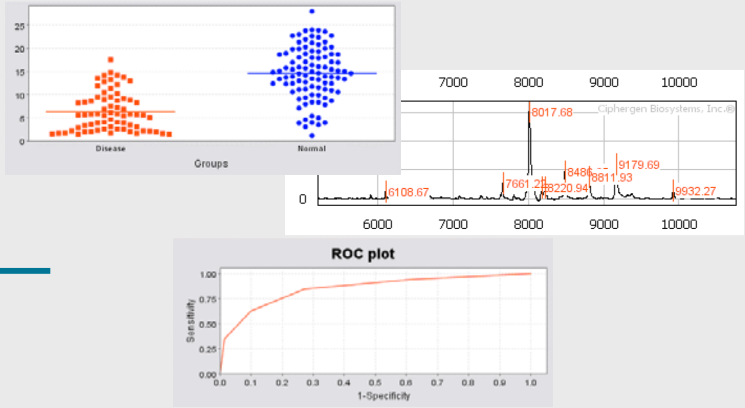
Flowchart of data analysis



Preprocess
Baseline subtract
Normalize
Calibrate



Univariate analysis
Biomarker Wizard or
CiphergenExpress™
Cluster Analysis,
Detect peaks,
Calculate P-values,
ROC/AUC



Multivariate analysis
CiphergenExpress
Heirarchical
Clustering, PCA;
Biomarker Patterns
Software