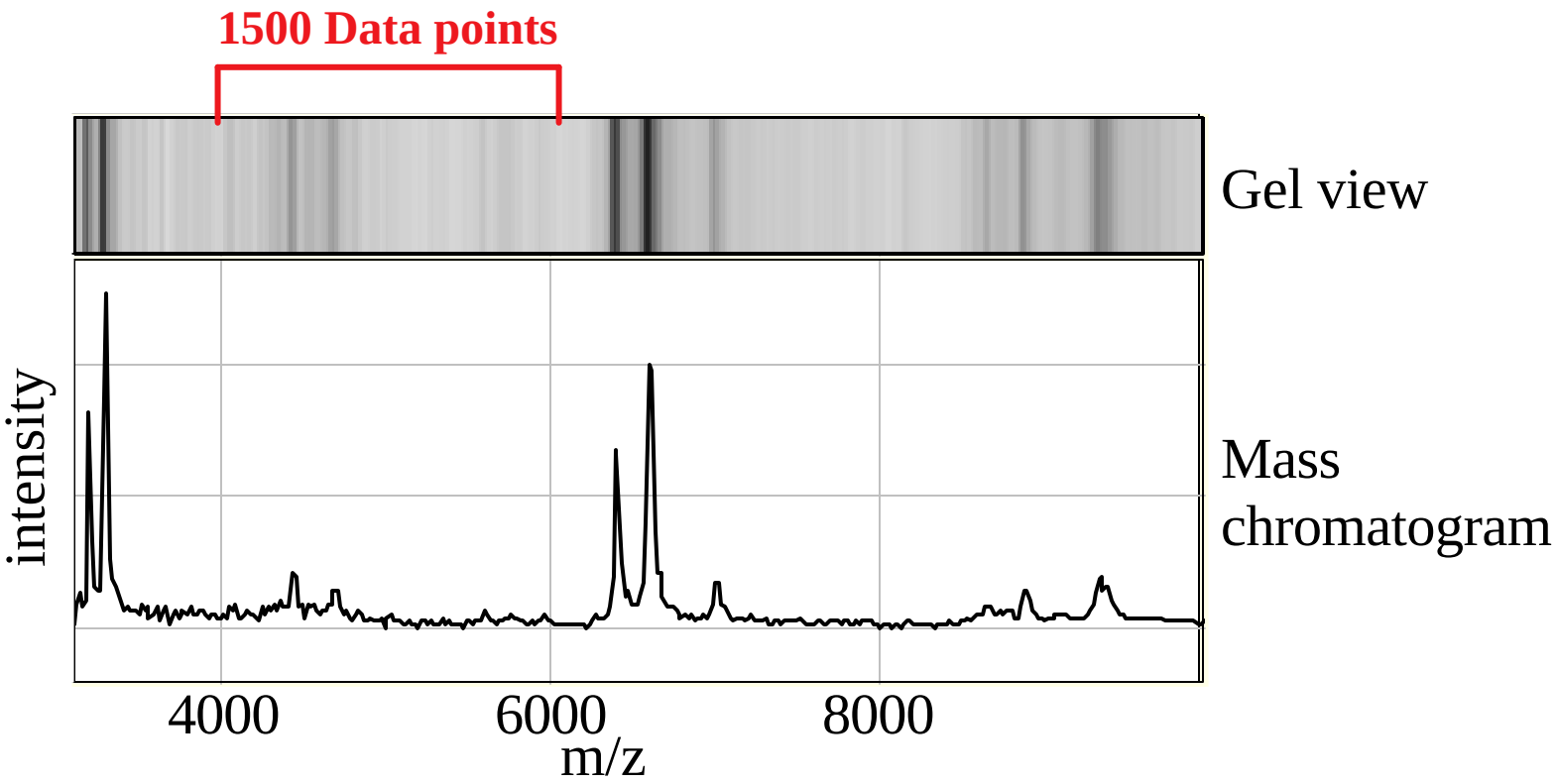
Typical serum profile from SELDI analysis



Data analysis window: 0-20,000 Daltons = 15,500 data points

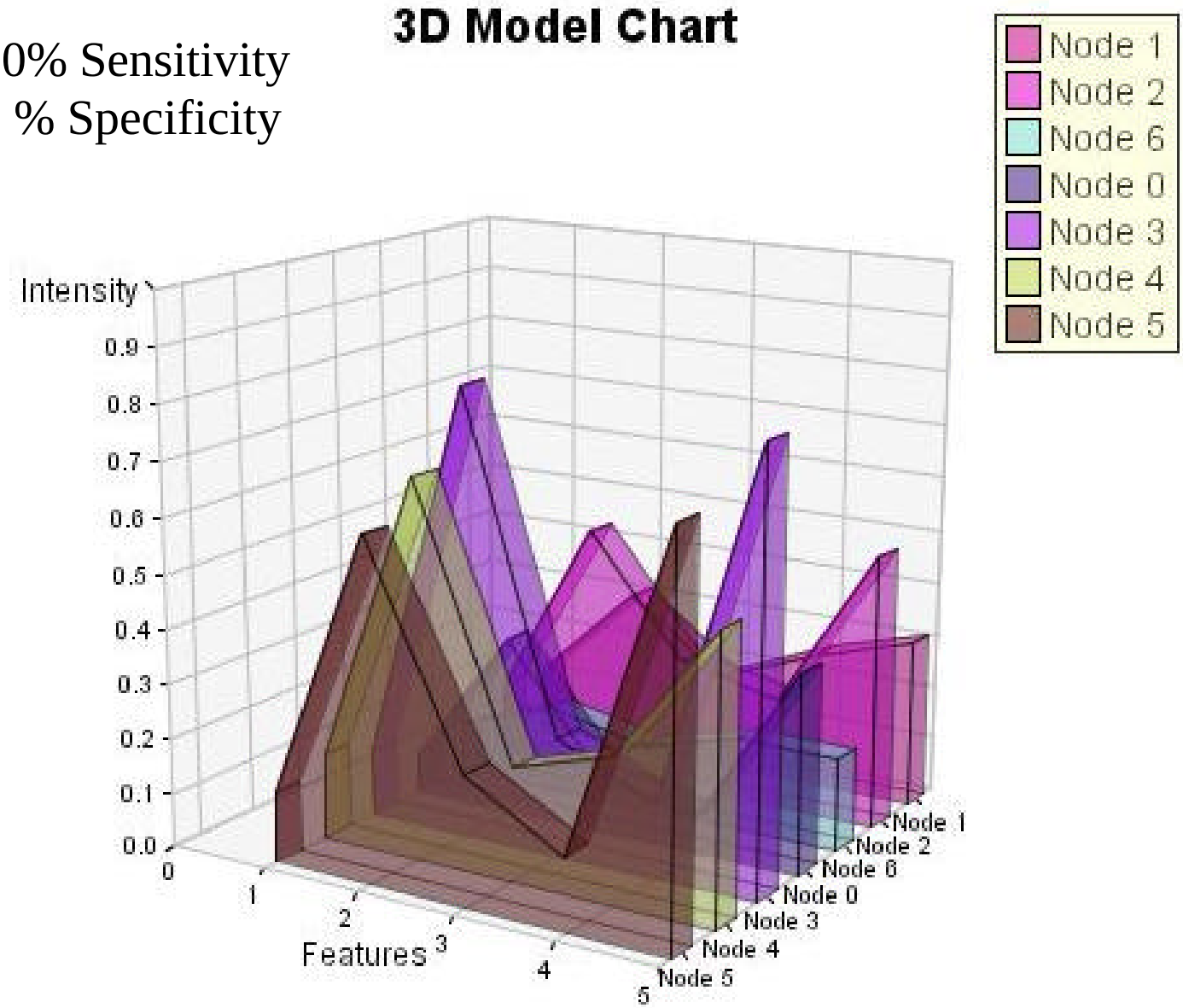
OVARY UNAFFECTED TRAINING SET [a]	
DISEASE STATUS	**N**
No Evidence of Ovarian Cysts	37
Benign Ovarian Cysts < 2.5 cm	11
Benign Ovarian Cysts > 2.5 cm	2
TOTAL	**50**

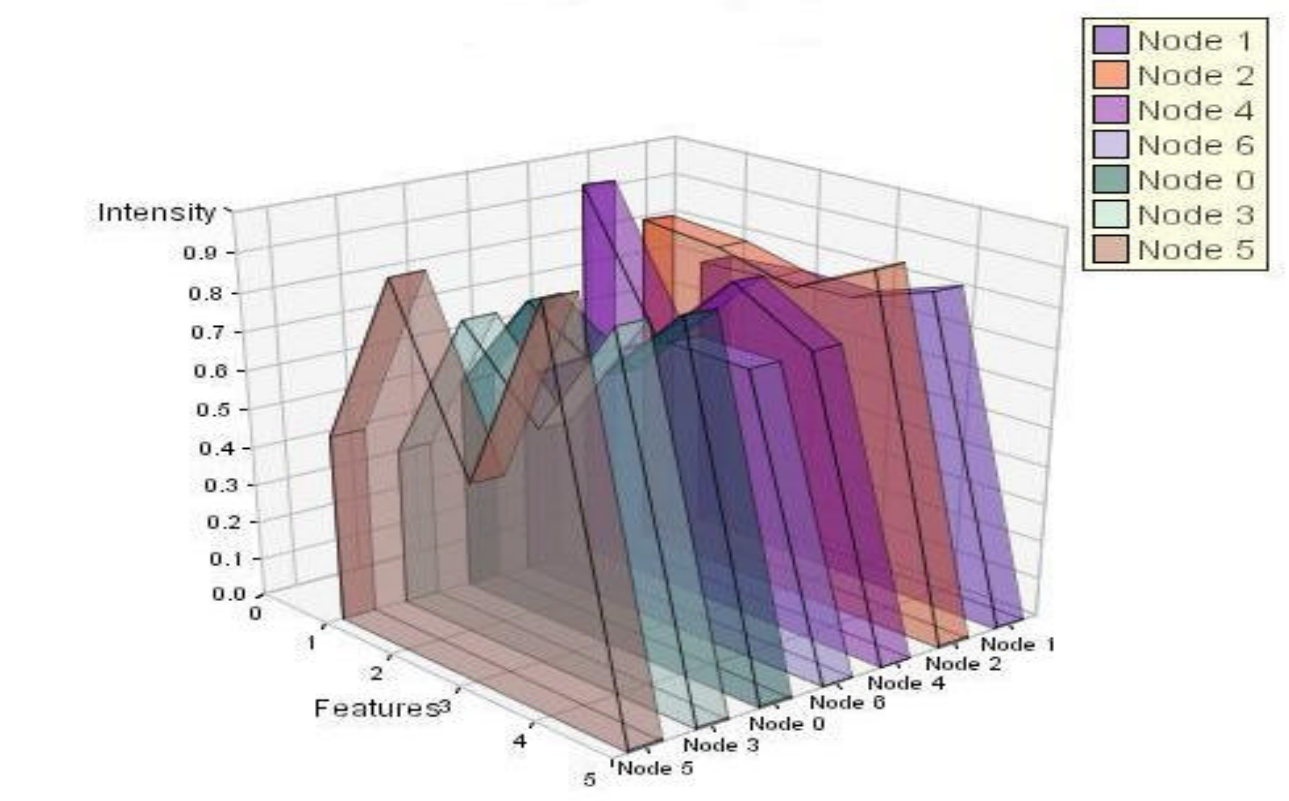
OVARY CANCER TRAINING SET [b]	
DISEASE STATUS	**N**
Surgically Staged Ovarian Cancer **Stage II, III, IV**	44
Surgically Staged Ovarian Cancer **Stage I**	6
TOTAL	**50**

a = Sera donated before 3-dimensional color Doppler flow ultrasound confirmation of benign cysts. All patients followed 5 years after sera collection.

b = Sera donated before surgical staging and diagnosis.

100% Sensitivity
95 % Specificity





<u>NEW MODEL</u>:

BLINDED TEST RESULTS:

100% Specificity and Sensitivity

50/50 Cancers, 63/63 Unaffected or Benign

Artificial Intelligence-Based Proteomic Pattern Diagnostics
Ovarian Cancer Results

BLINDED TEST DATA (N= 116 PATIENTS)

NED (5 Year follow up)	**22/24 (92%)**
Benign gynecologic and non-gyn inflammatory (cysts, fibroids) (RA, colitis, sinusitis)	**41/42 (98%)**

TOTAL SPECIFICITY: 63/66 = 95%

Ovarian cancer Stage II, III, IV	**32/32 (100%)**
Ovarian cancer Stage I	**18/18 (100%)**

TOTAL SENSITIVITY: 50/50 = 100%

POSITIVE PREDICTIVE VALUE: 95% VS. 20% FOR CA125

SERUM PROTEOMIC PATTERN DIAGNOSTICS

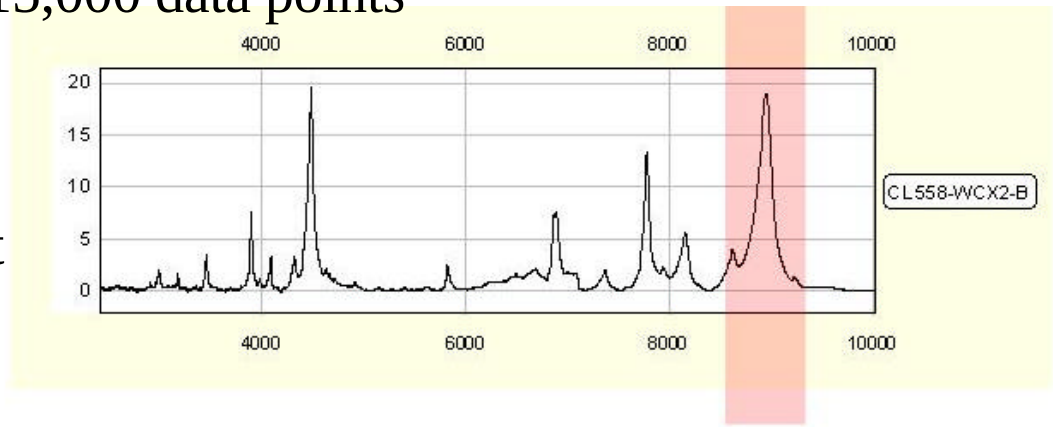
BLINDED TEST RESULTS: PROSTATE CANCER

N= 266

		Predicted Diagnosis by Proteomic Pattern Analysis	
Actual Diagnosis (serum PSA ng/ml)	**N**	**CANCER (% total)**	**BENIGN (% total)**
Prostate Cancer (> 4.0)	**38**	**36 (95%)**	**2 (5%)**
Benign (< 4.0)	**75**	**5 (7%)**	**70 (93%)**
Benign (>4.0)	**153**	**46 (30%)**	**117 (70%)**

Ciphergen

15,000 data points

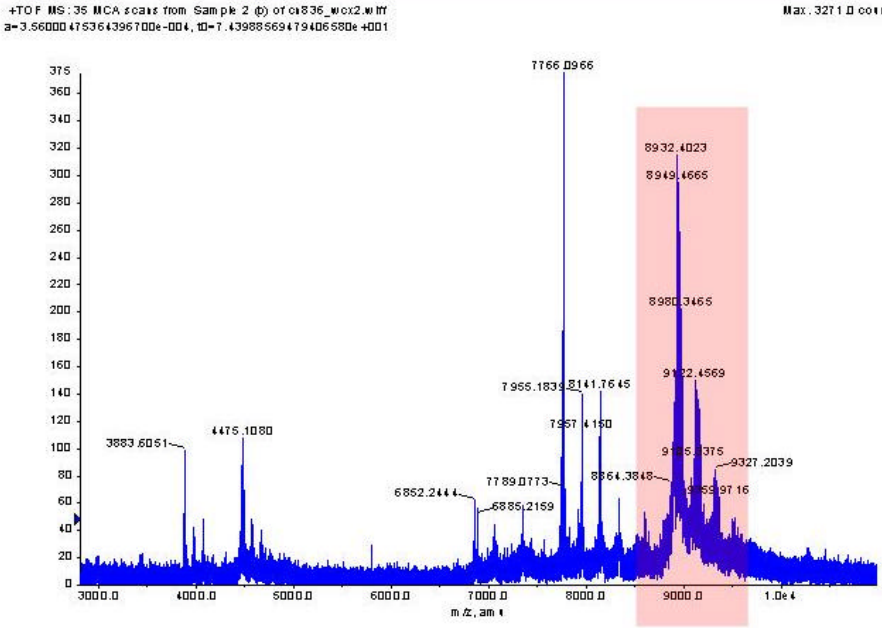


- Low Resolution
- No protein peak ID
- MALDI-TOF ions do not necessarily reflect relative abundance in sera

The ABI QSTAR® Pulsar Hybrid LC/MS/MS System is a high performance hybrid quadrupole time-of-flight mass spectrometer

ABI QSTAR

900,000 data points



ABI QqTof Qstar Ovarian Results:

100% Sensitive and Specific

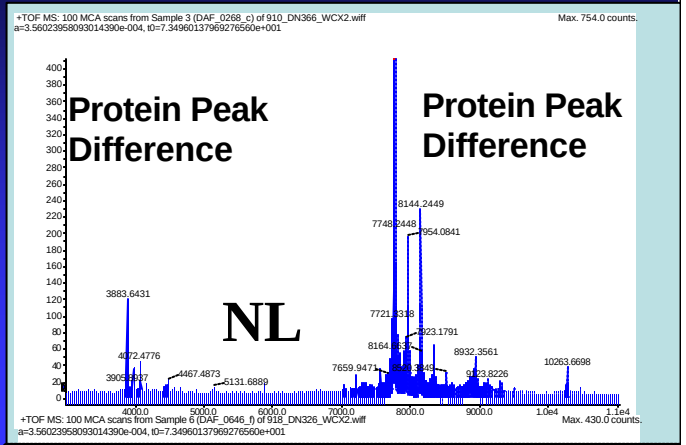
- Q-STAR VALIDATION : Direct comparison to Cipheregen PBS II using the same WCX chips

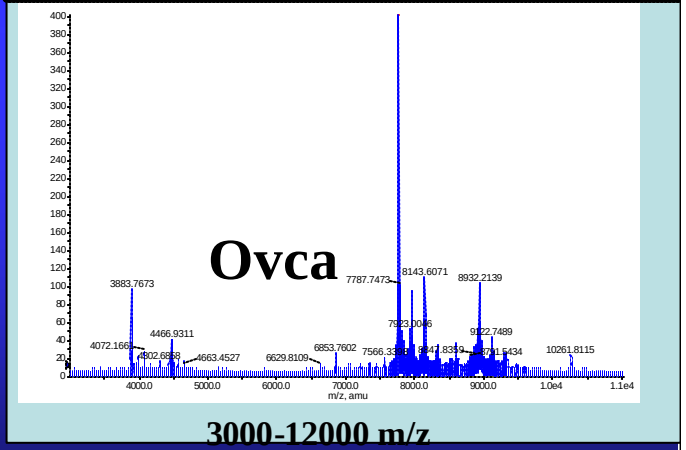
- Independent analysis by Dr. Tim Veenstra and Dr. Thomas Conrads (Director, NCI Biomedical Proteomics Program)

- Serum sample: National Ovarian Cancer Early Detection Program (NOCEDP), Northwestern University; Director: Dr. David Fishman

- Total Number of samples:

Unaffected = 95, Ovarian Cancer = 153





3000-12000 m/z

Samples were divided into 3 groups:

A. 84 training samples (28 Unaffected and 56 Ovarian Cancer)

B. 87 testing samples (30 Unaffected and 57 Cancer)

C. 87 blind validation samples (37 Unaffected and 40 Cancer)

Results:

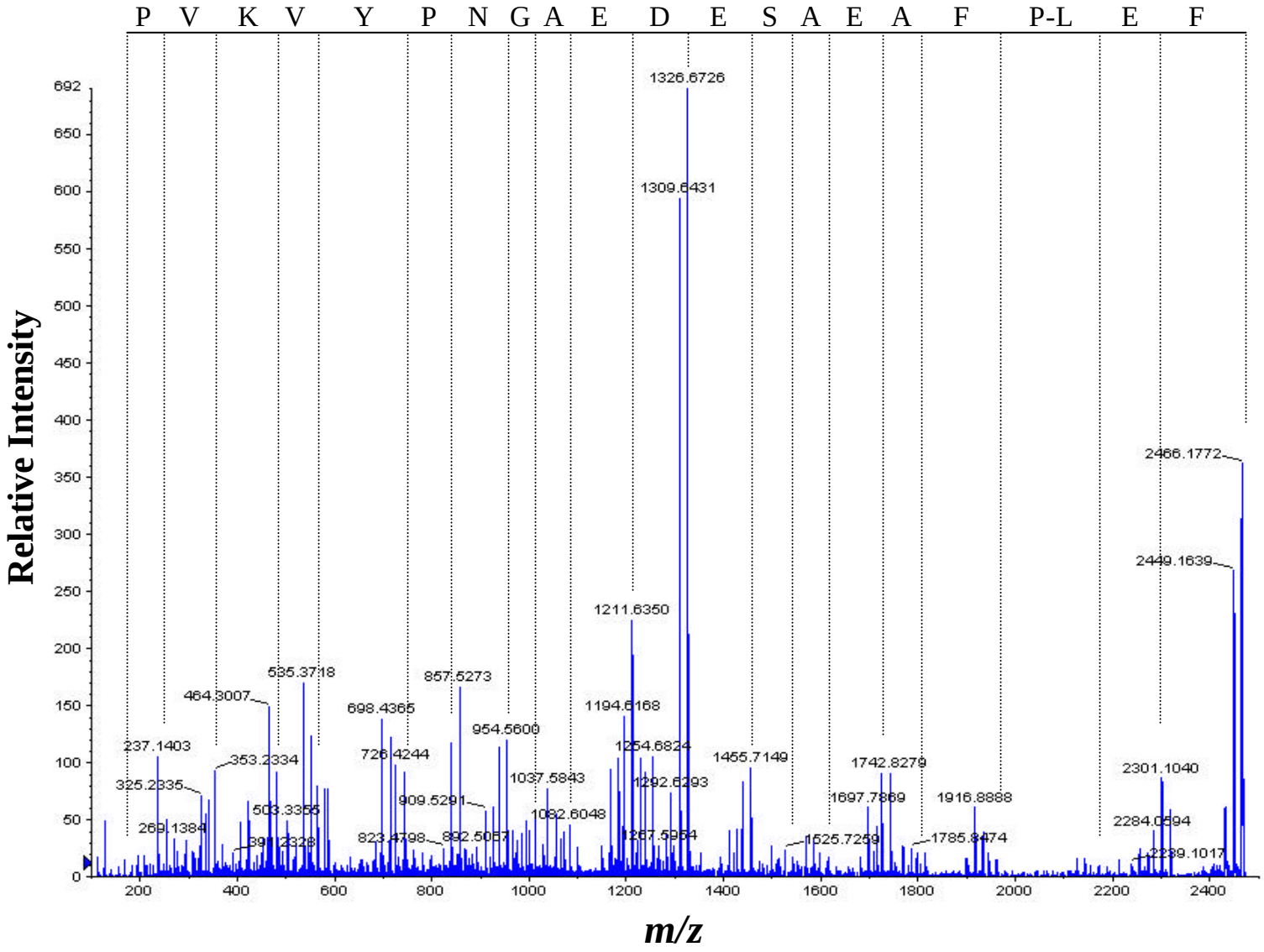
- Two different models generated 100% sensitivity and specificity.
- These same model correctly classified 100% of the blinded samples.

Key Ions (m/z) values from one model:

1276.8612	2374.2444	4292.900	7060.1210	8605.678	8706.065	9870.9375

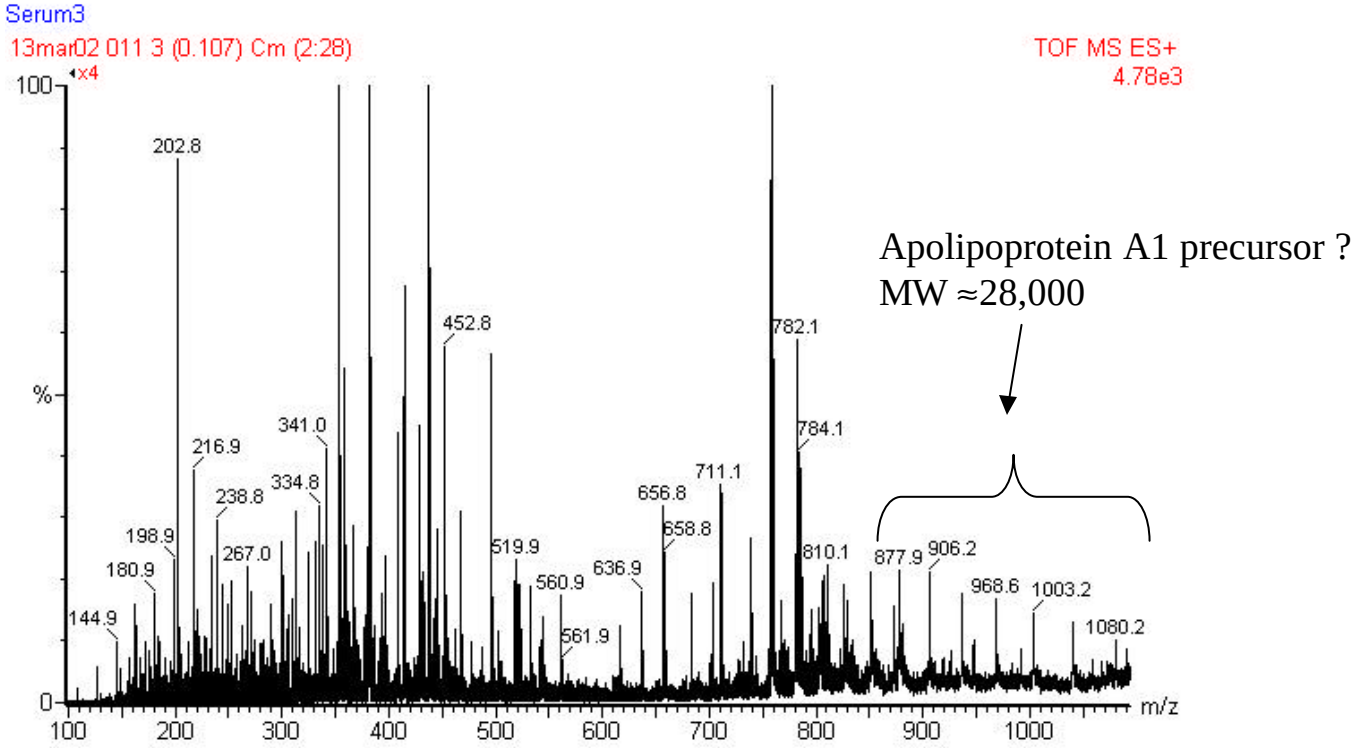
Protein Peptide ID directly from complex Q-Star Spectra

Adrenocorticotropic hormone 2 clip 18-39 tandem MS spectrum

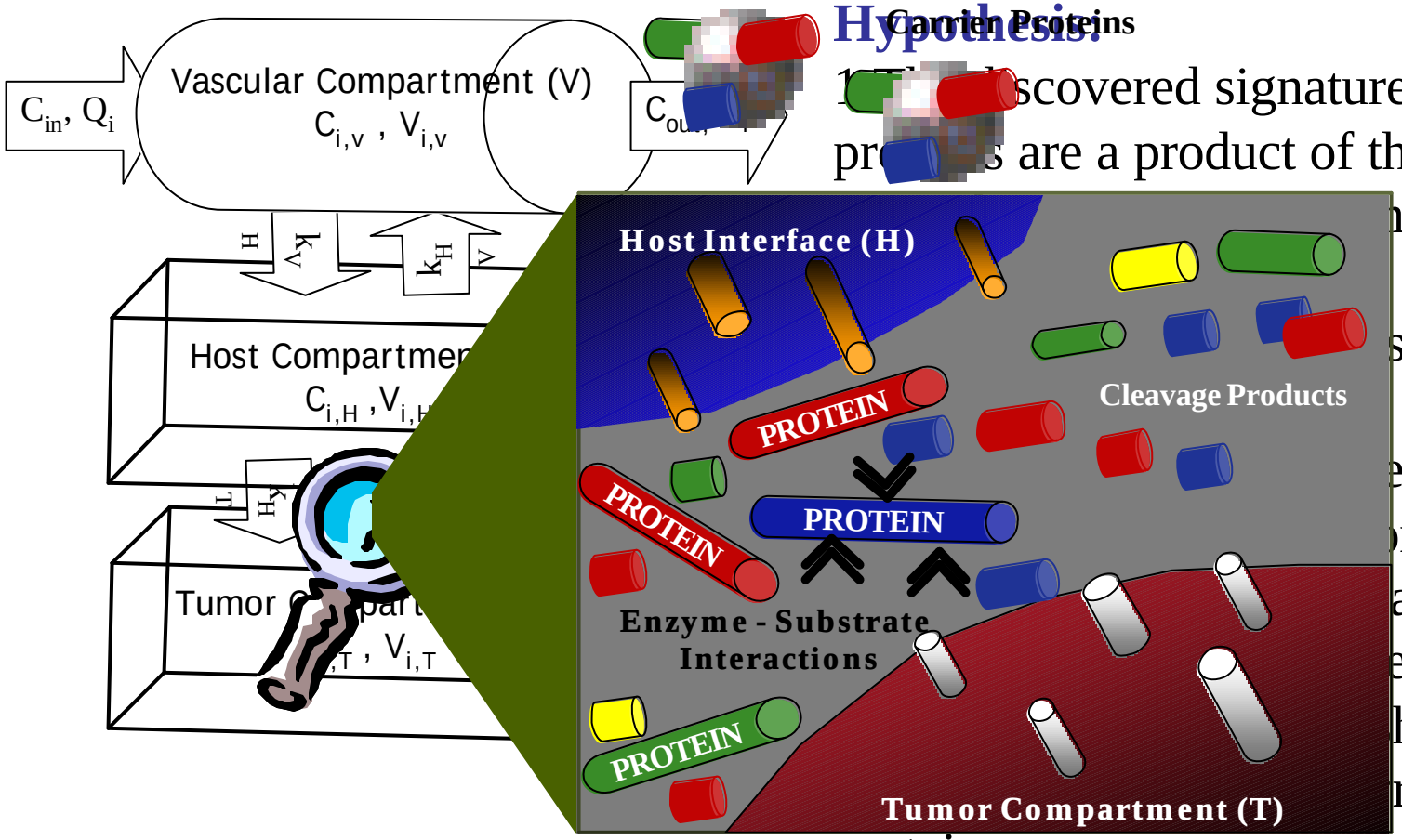


Human serum - Micromass LCT and ESI-Chip

500 x diluted in 1:1 ACN:H20, 0.2% formic acid



Source of Ions Comprising Diagnostic Signature Subset



Hypothesis:

1. The discovered signature proteins are a product of the tumor environment,

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Nth Dimensional Proteomic Pattern Analysis:

A New Paradigm for Diagnostics

- **Requires less than 1 microliter of raw unfractionated serum**
- **MALDI-TOF profiles obtained in less than 5 minutes**
- **High throughput: Capacity to analyze more than 400 samples/day**
- **Nth-Dimensional analysis virtually instantaneous once training sets are defined**
- **Validated sera added into an ever-expanding training population: models get better and better as more data is analyzed**
- **Analysis compatible with web-centric platforms**

Sistare Vasculitis Results 8-5-02

Vasculitis Samples
Negative = 21 samples
Positive = 16 samples

37 Total samples
19 Testing set (10 Negative and 9 Positive)
18 Training set (11 Negative and 7 Positive)
100% specificity and sensitivity

Validation with Renal Positive and Negative
Classified 15/15 (100%) Renal Negative as negative
Classified 8/11 (72.7%) Renal Positive as negative

Model:

Node	Count	State	StateSum	Error	475.495	875.625	1067.490	980.226	9551.137	2527.856	11683.457	6266.976	17988.113	4037.875
0	11	0	2	2	0.997	0.806	0.756	0.843	0.024	0.241	0.016	0.102	0.000	0.156
1	6	1	5	1	1.000	0.719	0.656	0.710	0.018	0.192	0.013	0.083	0.000	0.117
2	1	0	0	0	0.840	0.652	0.675	1.000	0.017	0.191	0.012	0.072	0.000	0.119