AMGEN

Physical Models for Predicting Peptide Fragmentation Spectra

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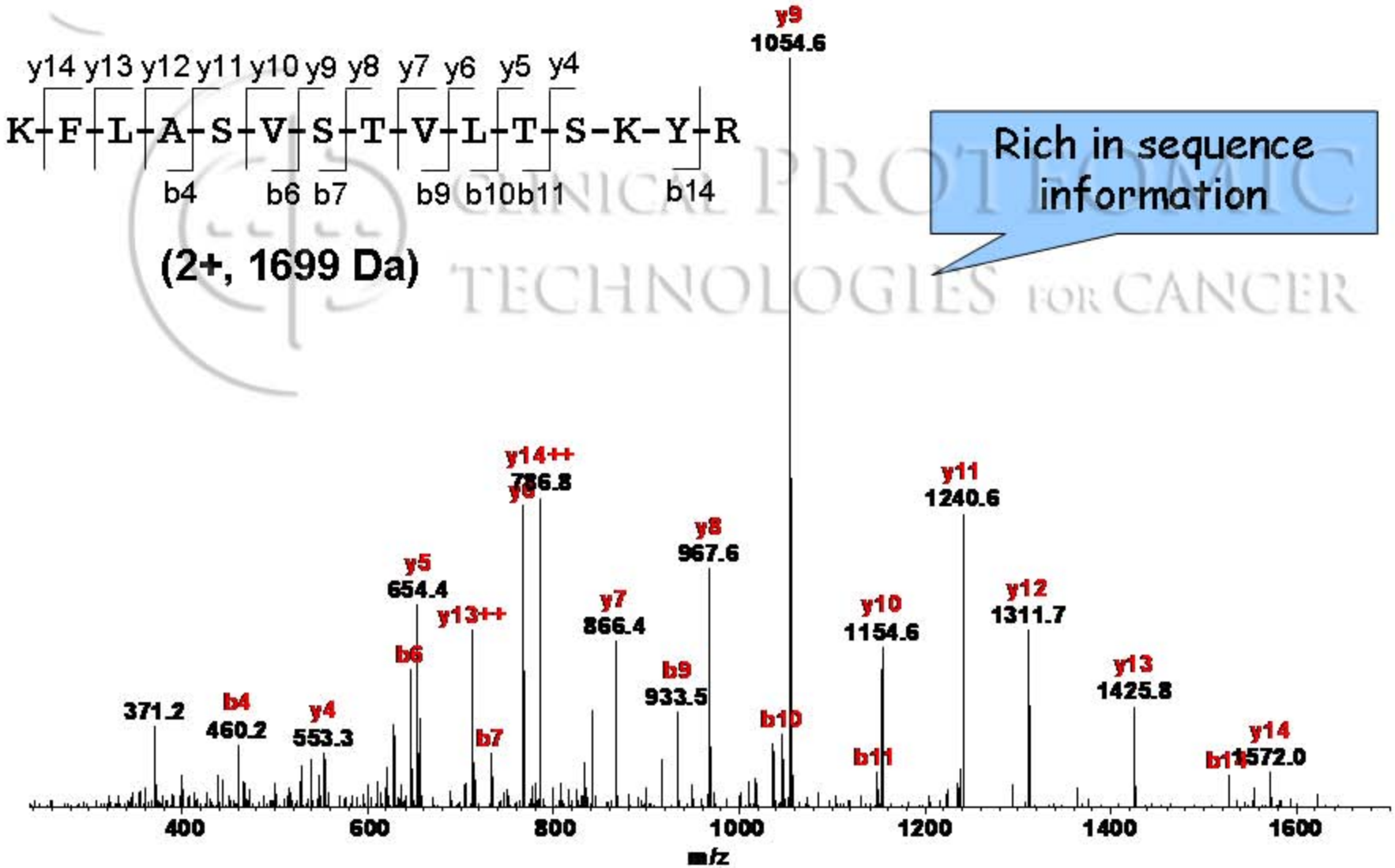
Amgen

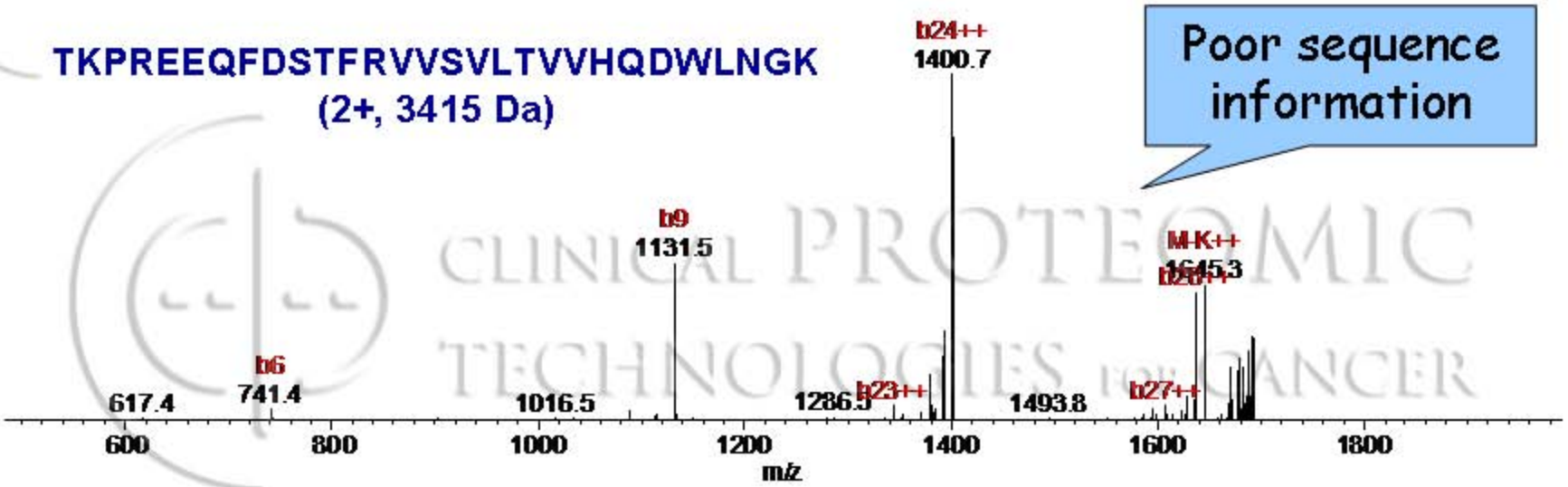
Strategies for Improving Reliability in Protein/Peptide Identification Workshop
NCI-NIST, November 15, 2007

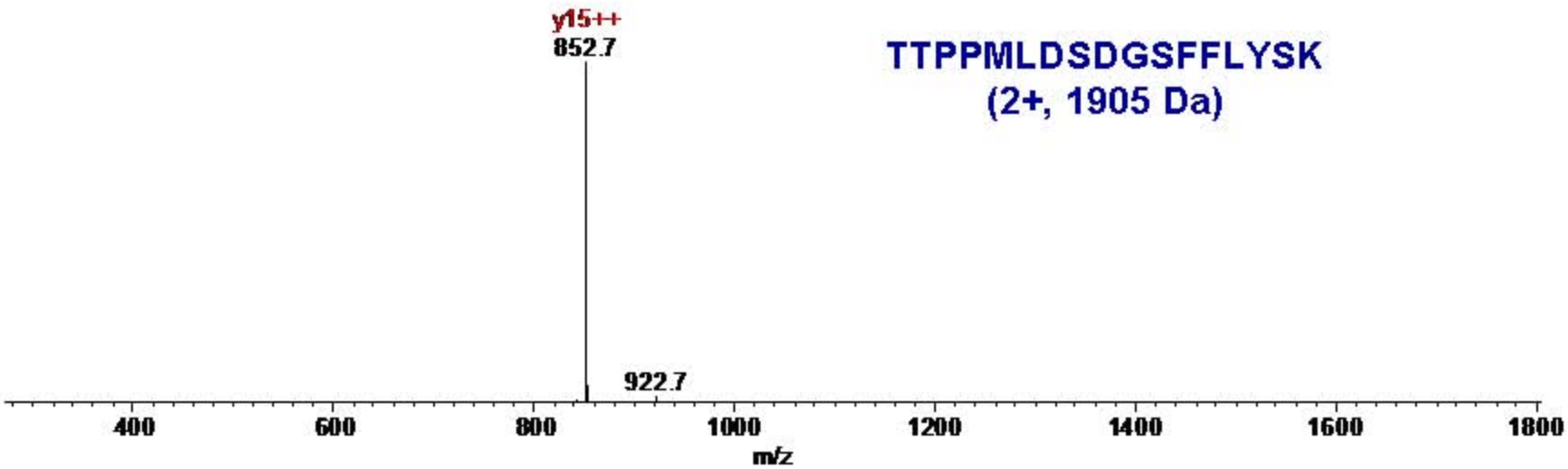
Peptide Identification by MS/MS



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

All b/y Fragments are not Created Equal!





CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

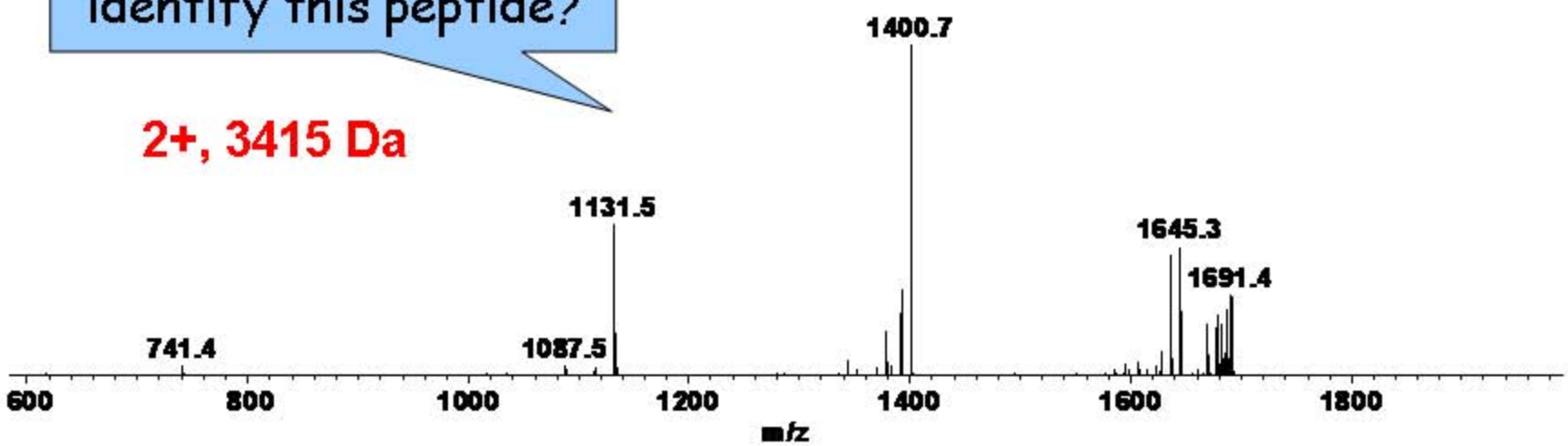
Protein Identification vs. Protein Characterization

- Protein Identification
  - Identify all proteins in a sample
- Protein Characterization
  - Identify all peptides in a sample

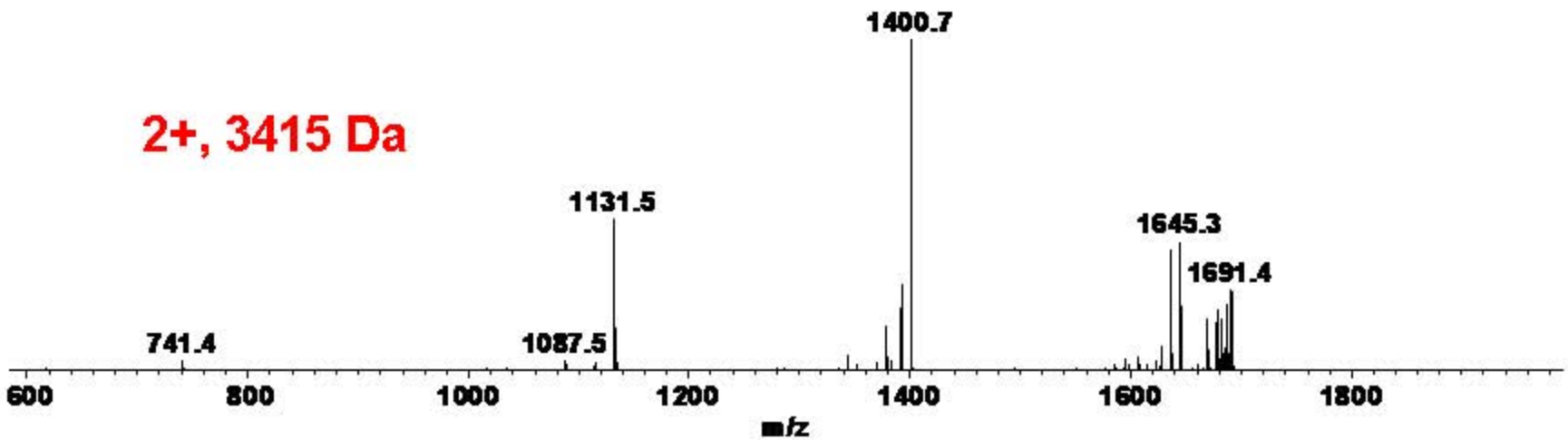
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Can we reliably identify this peptide?

2+, 3415 Da

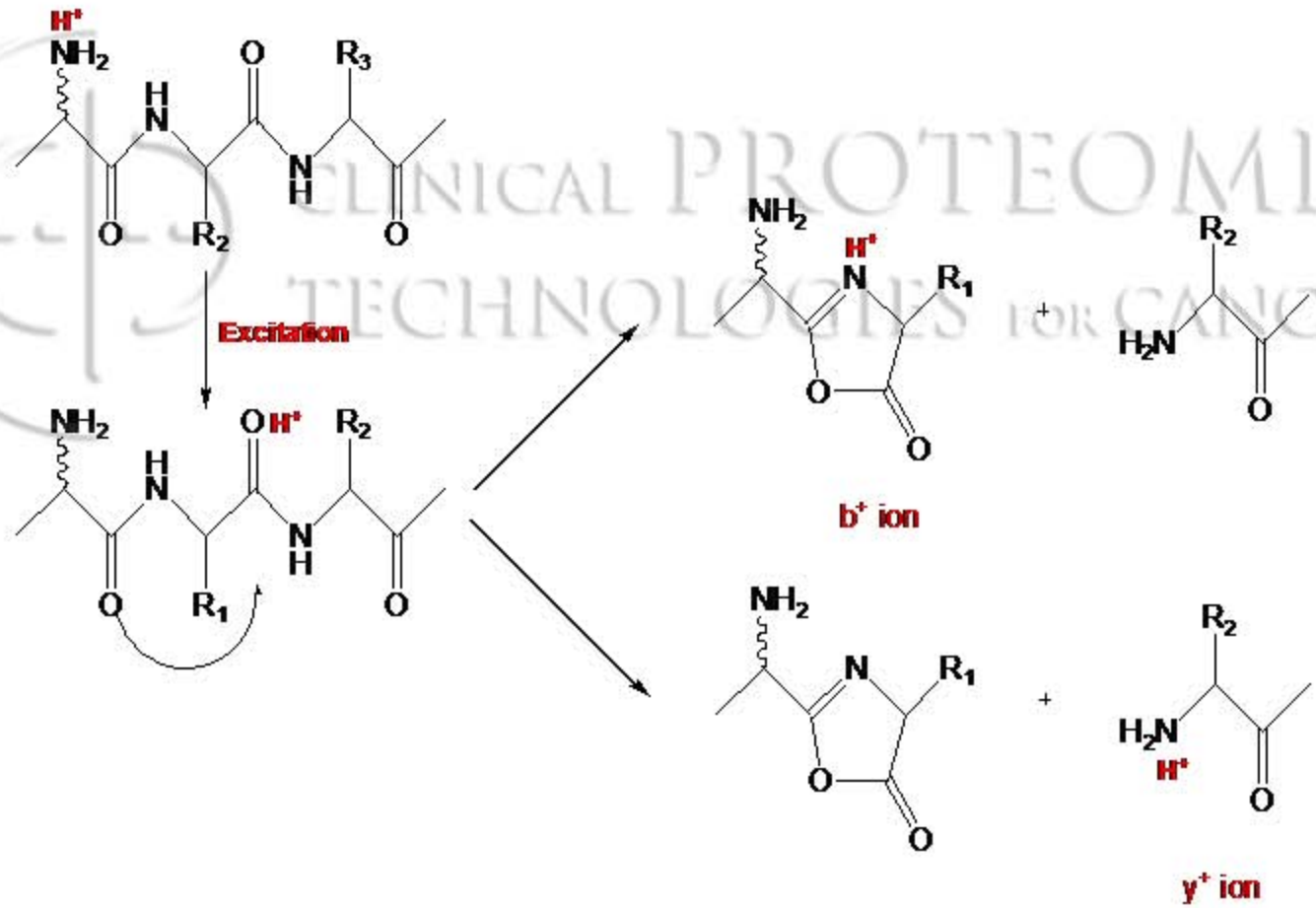


A Predictive Model for Peptide ID?



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

"Mobile Proton" Model
Charge-Directed Fragmentation



Wysocki et al., *J. Mass Spectrom.* (**2000**), 35, 1399-1406

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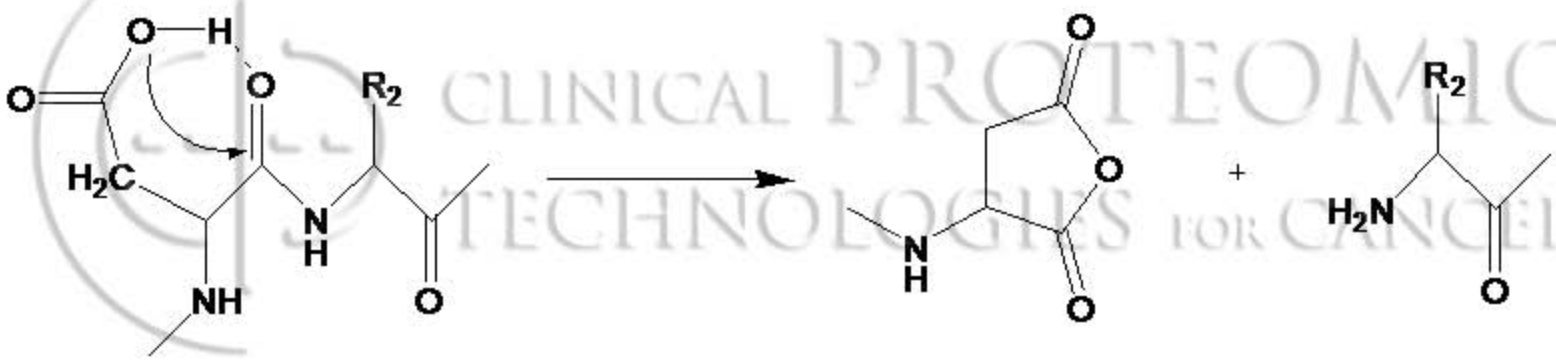
Charge-Remote Fragmentation



-D-X- b ion y ion

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Wysocki et al., *J. Mass Spectrom.* (**2000**), 35, 1399-1406

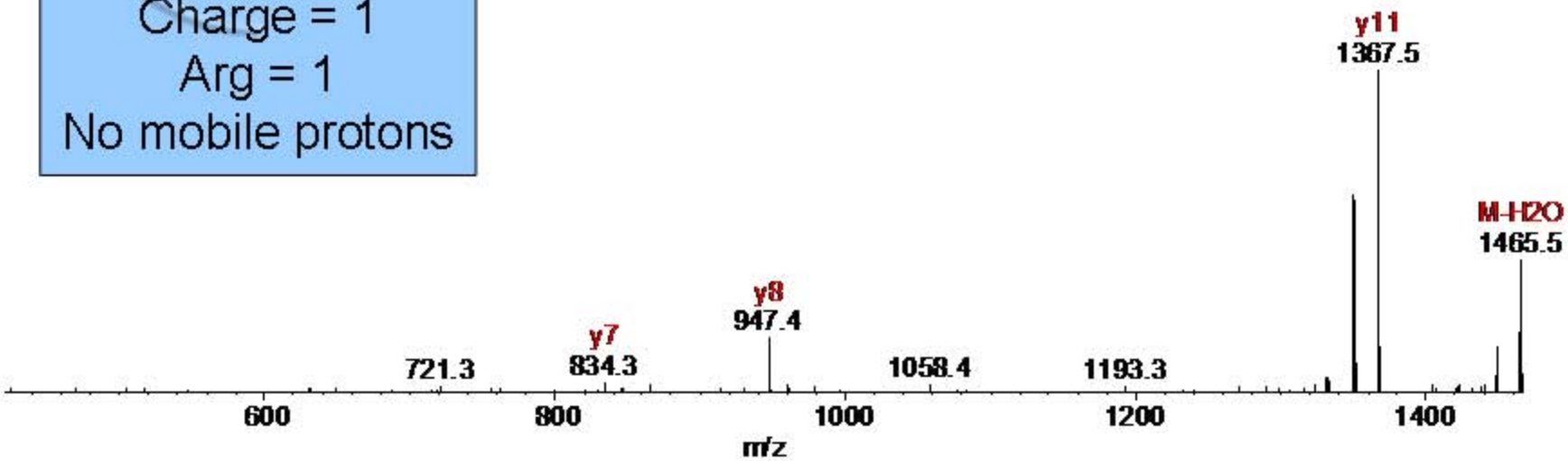
Charge-Remote Fragmentation

y11 y8

D-Q-Y-E-L-L-C-L-D-N-T-R

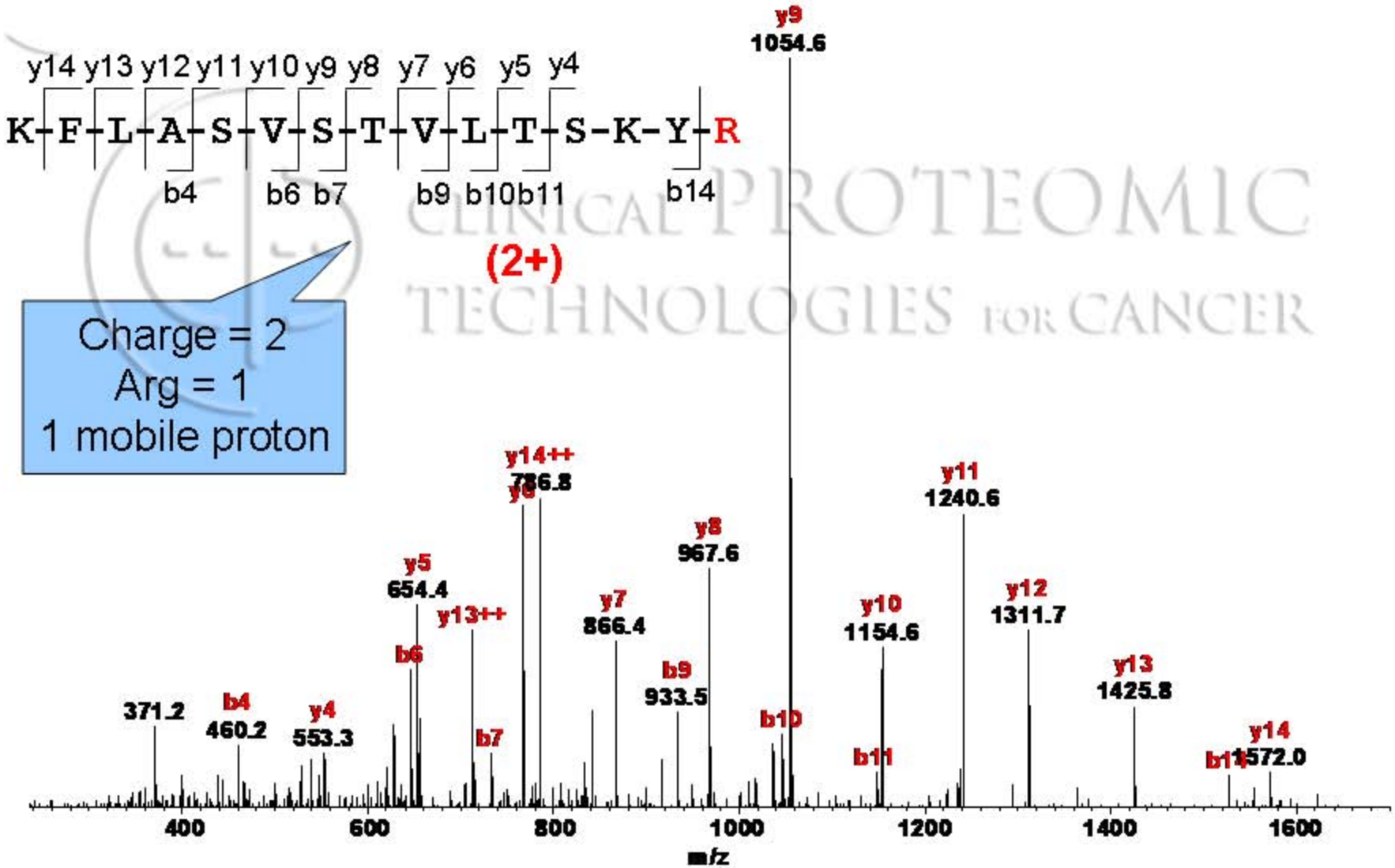
(H^+)

Charge = 1
Arg = 1
No mobile protons



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Charge-Directed Fragmentation



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Peptide Fragmentation

- Competition of many different pathways, including charge-directed and charge remote.
  - *Classical Chemical Kinetics*
- Knowledge of charge distribution is crucial in determining the peptide fragmentation behavior.
  - *Boltzmann Distribution*

PROTEOMIC TECHNOLOGIES FOR CANCER

The Kinetic CID Model

Boltzmann Distribution

$$p_j = \frac{\exp(-E_j / RT_{eff})}{\sum_i \exp(-E_i / RT_{eff})}$$

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Arrhenius Equation

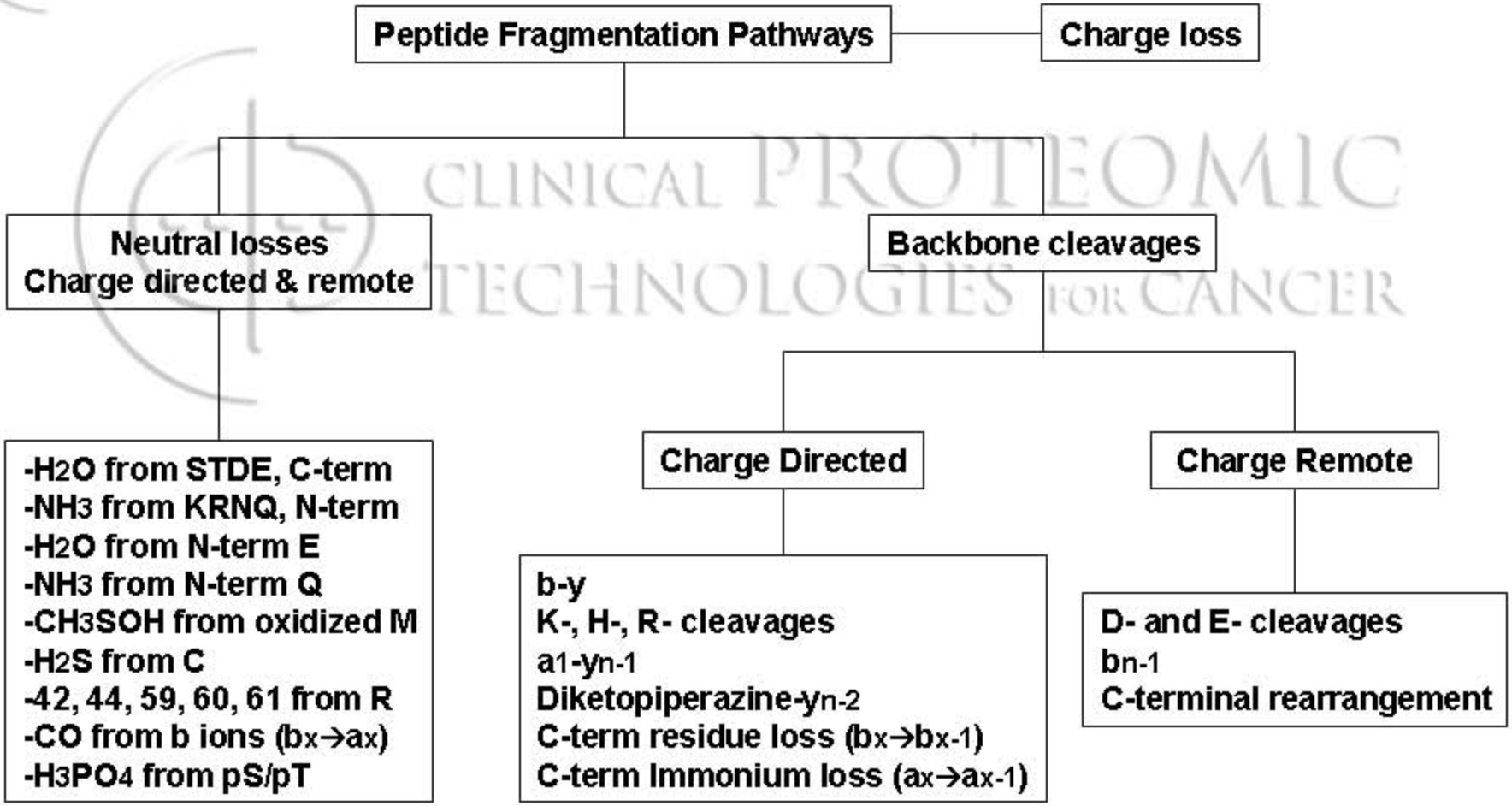
$$k = A \exp(-E_a / RT_{eff})$$

First-Order Kinetics

$$\frac{d[F_i]}{dt} = k_i [P]$$

Zhang, *Anal. Chem.* (**2004**), 76, 3908-3922
Zhang, *Anal. Chem.* (**2005**), 77, 6364-6373

Fragmentation Channels



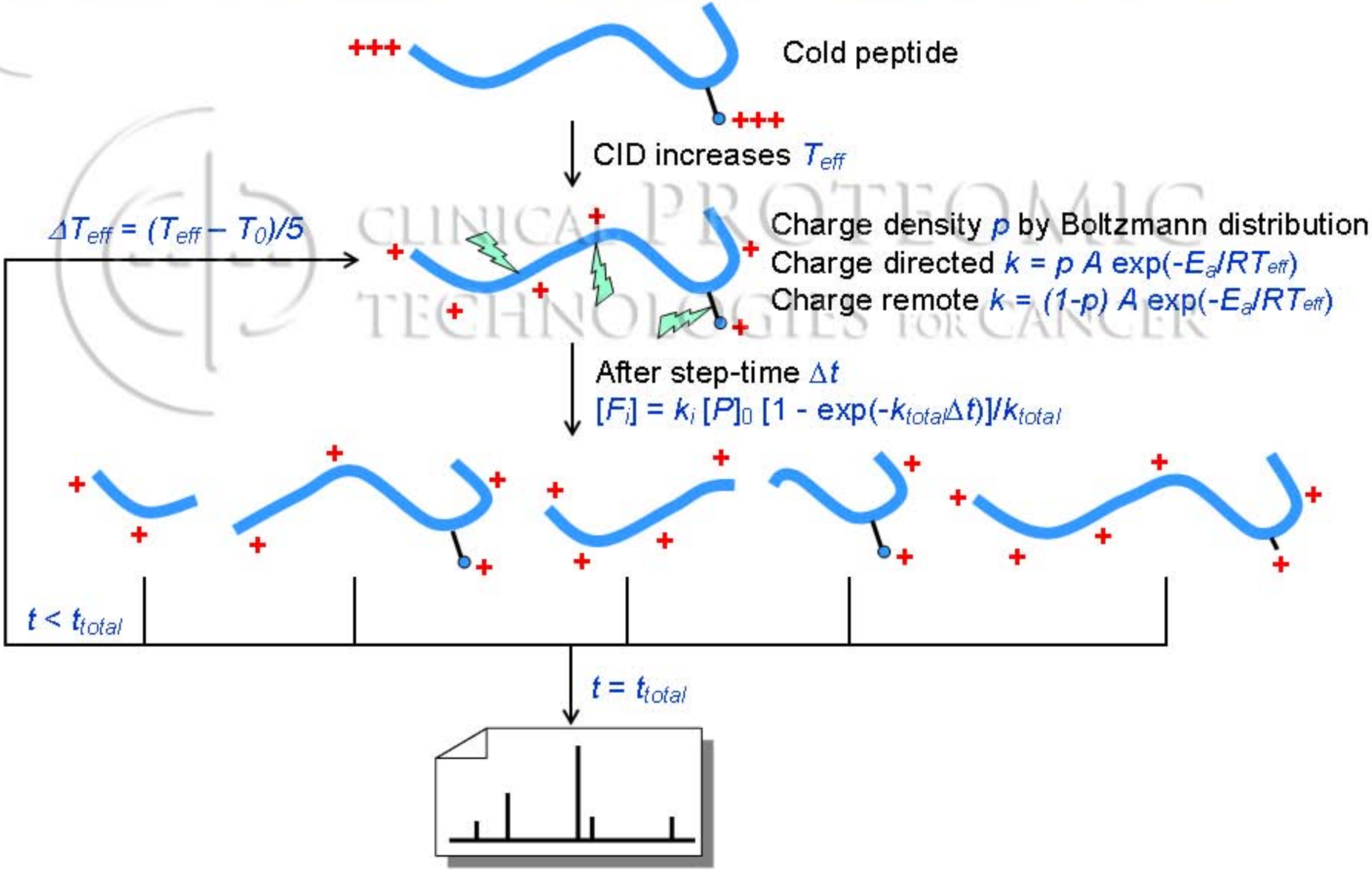
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Major Assumptions

- T_{eff} is related to the mass, charge and collision energy.
- Side chain of each residue has a constant gas-phase basicity (GB).
- GB of a backbone amide depends on the neighboring residues, and the effects are additive.
- Intra-molecular proton transfer is a fast process.
- E_a for each backbone cleavage depends on the neighboring residues, and the effects are additive.
- Same fragmentation mechanism has the same *A* factor.

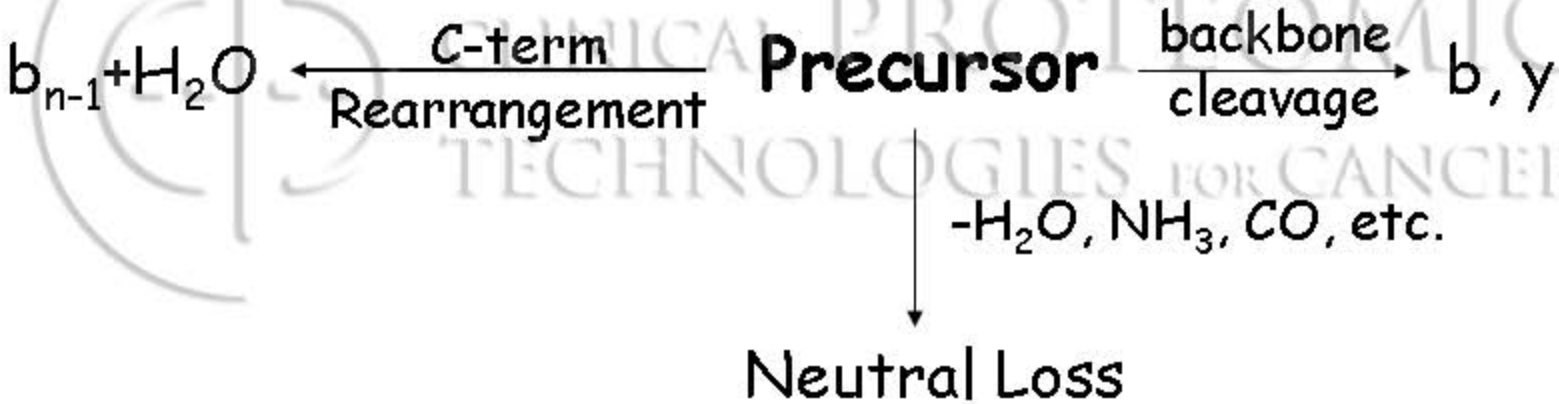
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

The Model



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

What Ions Are Predicted?



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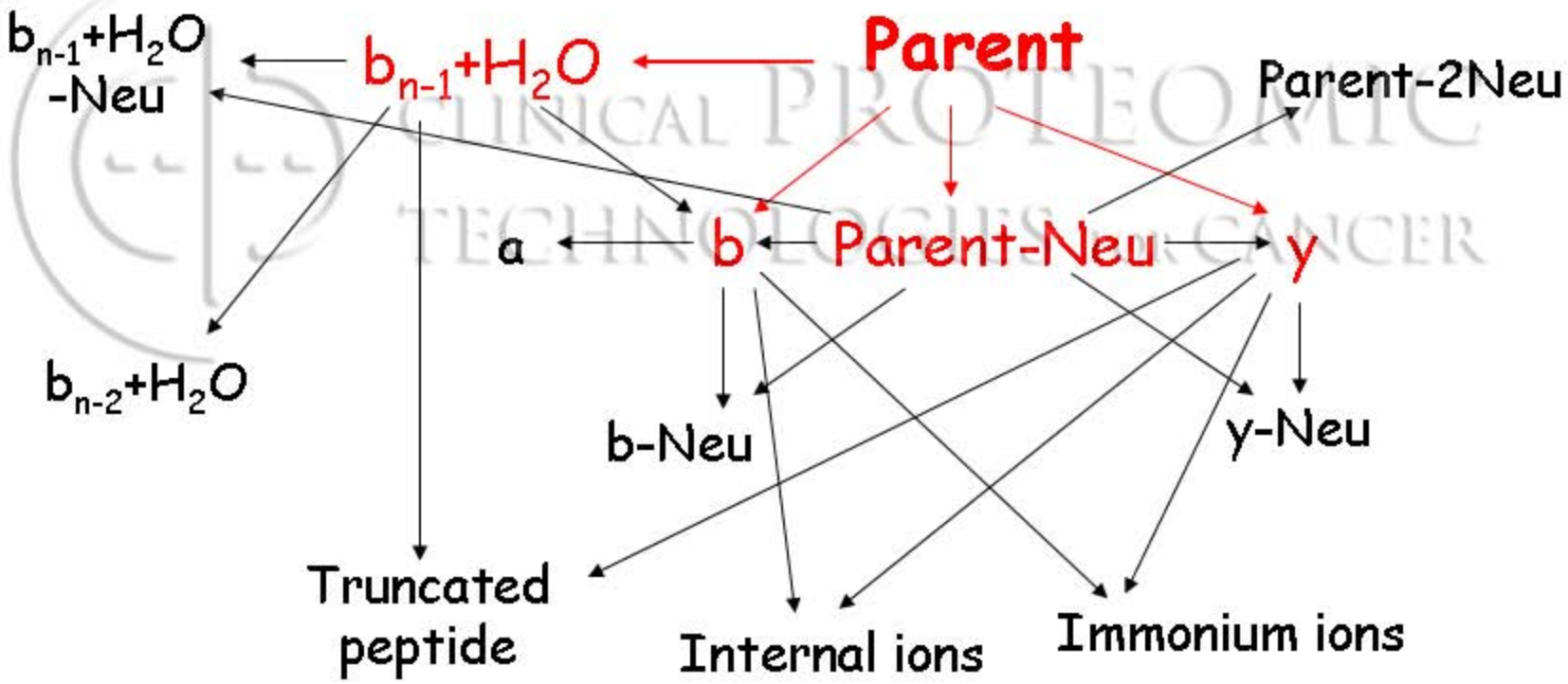
Ions Predicted within Two Generations



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Optimizing the Model

- 336 parameters (15 instrument dependent)
- Initial values from best estimates based on literature values
- >10,000 high-quality ion-trap spectra used in the training

$$Similarity = \frac{\sum \sqrt{I_m^1 I_m^2}}{\sqrt{(\sum I_m^1)(\sum I_m^2)}}$$

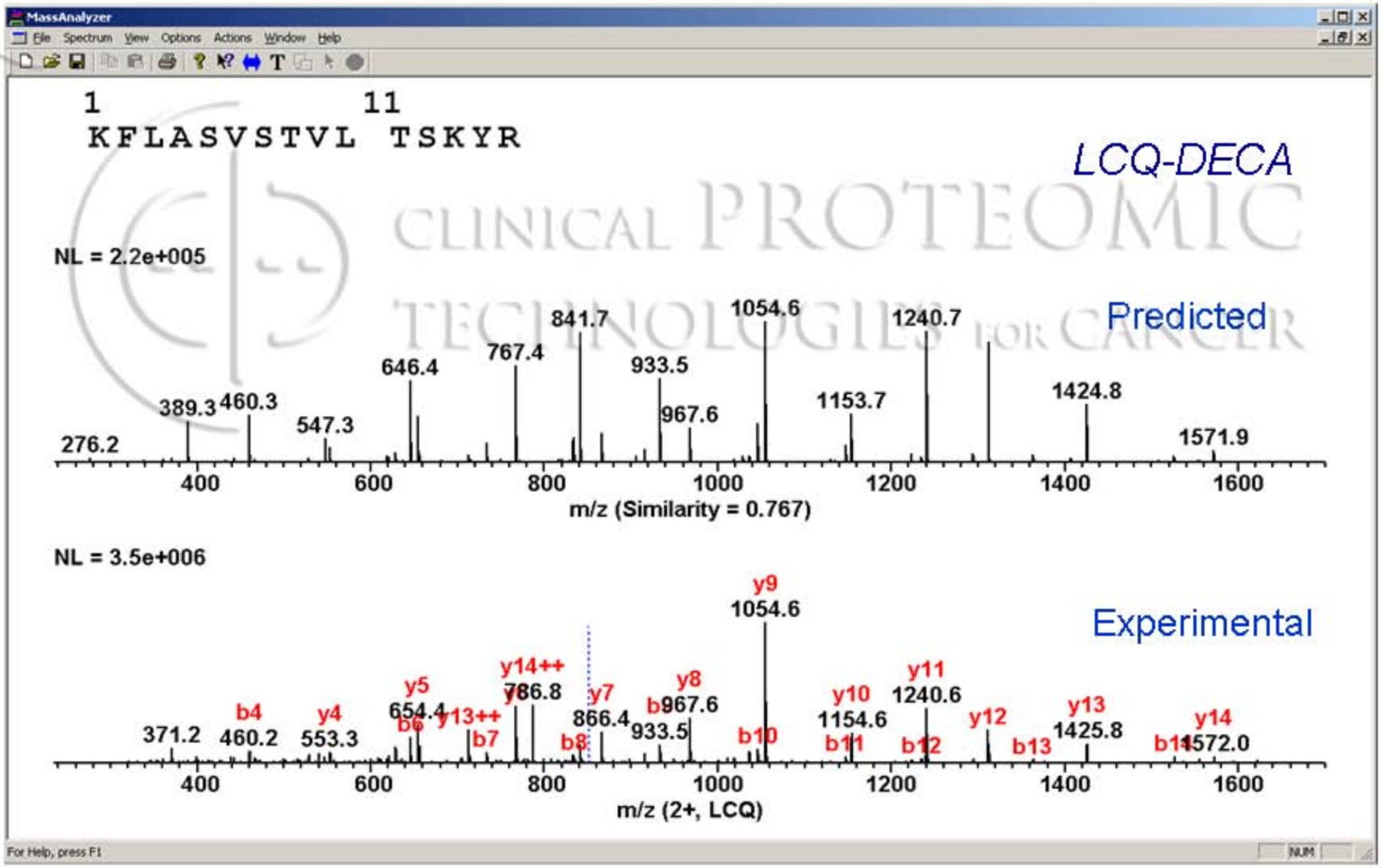
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Some Optimized Parameters

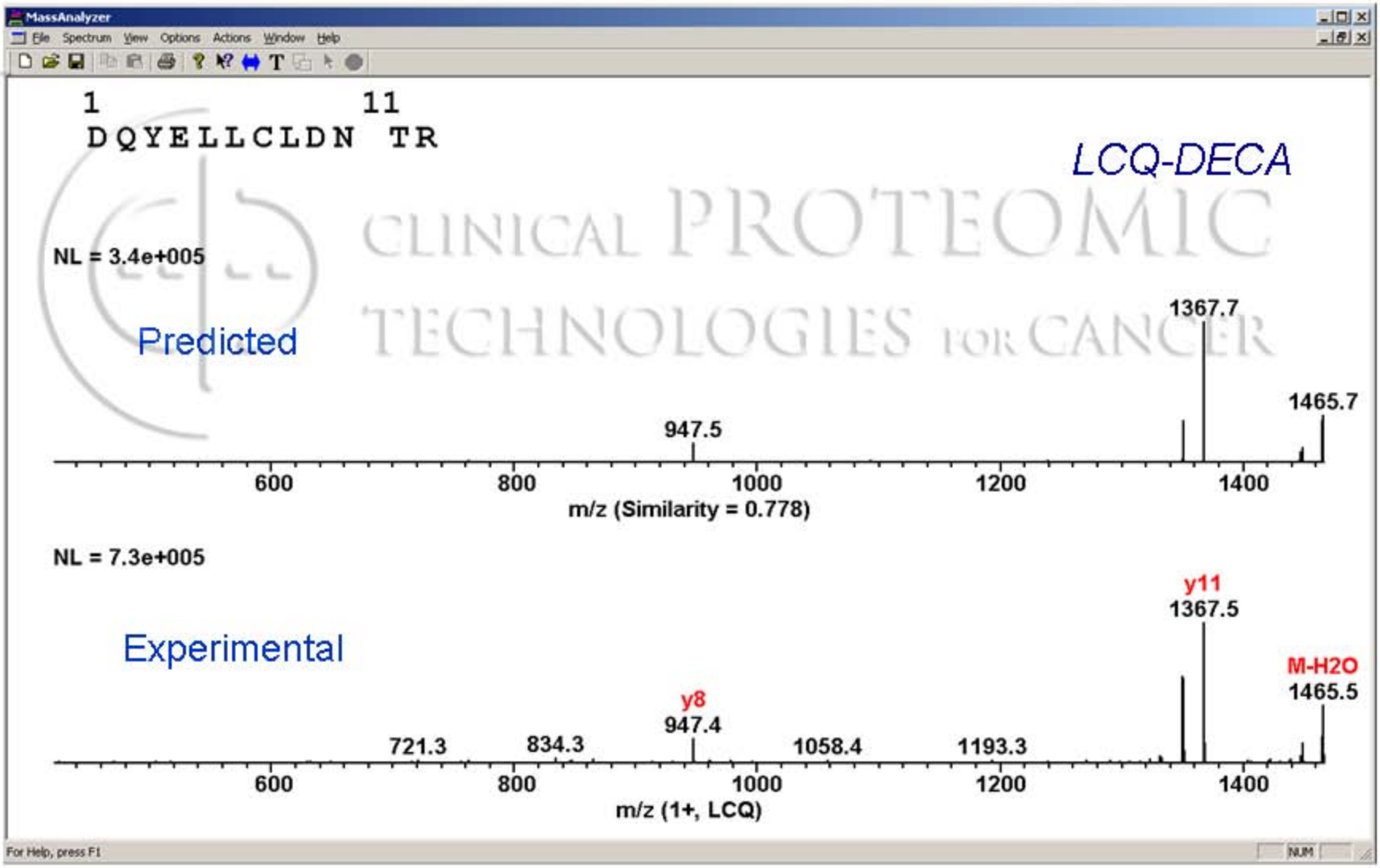
Residue	GB(SC)	GB(L)	GB(R)	EA(L-1)	EA(L)	EA(R)	EB(L)	EC(R)	EC(L)	Eb->a	ENL(d)	ENL(r)	Epyro(d)	Epyro(r)
A		883.4	0.0	0.0	168.5	0.0		185.0	0.0	130.2				
C	770.0	882.0	-1.2	4.9	171.7	-1.0		189.0	-3.2	135.6	152.5	121.6		
D	784.0	880.3	-1.0	4.4	170.4	0.3	152.9	175.6	1.1	148.3	77.4	118.9		
E	790.0	881.7	2.9	2.7	167.7	2.9	167.6	183.8	-1.3	132.2	86.5	144.4	36.7	184.8
F		881.8	0.7	0.3	165.2	-2.3		184.4	0.7	123.0				
G		883.2	1.9	2.4	181.2	0.0		206.8	-1.2	146.2				
H	929.7	883.6	-0.1	-3.1	166.4	-2.9	165.2	169.6	0.6	118.9				
I		881.1	-1.1	-1.6	159.4	-3.3		176.1	4.4	120.0				
J	830.0	880.6	-0.1	4.0	168.4	-0.8		189.7	-0.2	131.8	210.7	122.3		
K	927.6	880.4	-2.2	1.6	165.3	-4.7	161.1	169.5	2.0	187.0	206.6	221.9		
L		882.9	-0.2	-1.4	164.1	-1.3		180.4	0.9	123.4				
M	830.0	882.8	-0.5	0.4	166.8	-2.6		183.6	-3.5	125.2				
N	863.5	881.4	1.5	2.1	171.9	1.8		188.0	0.1	134.0	127.8	111.2		
O	854.0	882.8	0.4	6.2	173.9	3.8		187.0	1.0	137.4	205.4	91.2		
P		885.5	16.4	5.2	187.8	-0.4		174.3	2.1	126.7				
Q	864.6	882.9	6.2	2.9	168.4	7.1		189.1	-1.1	138.9	138.1	125.6	208.9	169.7
R	964.0	887.6	9.2	4.4	166.7	7.9	178.7	167.8	-6.9	133.9	197.4	111.2		
S	775.0	881.4	1.8	3.5	172.4	0.0		189.5	-1.2	138.7	37.8	124.0		
T	780.0	882.2	1.7	0.3	169.9	1.8		188.3	0.8	134.2	37.7	130.8		
U	860.0	882.4	0.9	5.0	171.4	1.4		188.3	-3.7	138.2	202.4	122.2		
V		882.9	-2.0	-0.3	161.9	-2.8		178.4	2.9	123.8				
W	897.6	883.1	3.0	-1.1	165.0	-2.0		184.0	2.5	123.3				
Y	790.0	882.3	0.0	0.3	166.5	-2.5		181.4	2.7	122.2				
N-term		911.9		30.4							297.5	127.7		
C-term			-97.4				169.6				51.5	125.5		
b ion			38.3			-13.6					177.7	101.6		
a ion			-200.0								246.5	81.9		
A(1/s)					6.4E+13		3.2E+10	1.6E+11	2.1e+09(directed)				1.4E+12	1.4E+12
									6.8e+08(remote)					

CENTRAL PROTEOMIC INSTITUTE FOR CANCER

A "Good" Spectrum

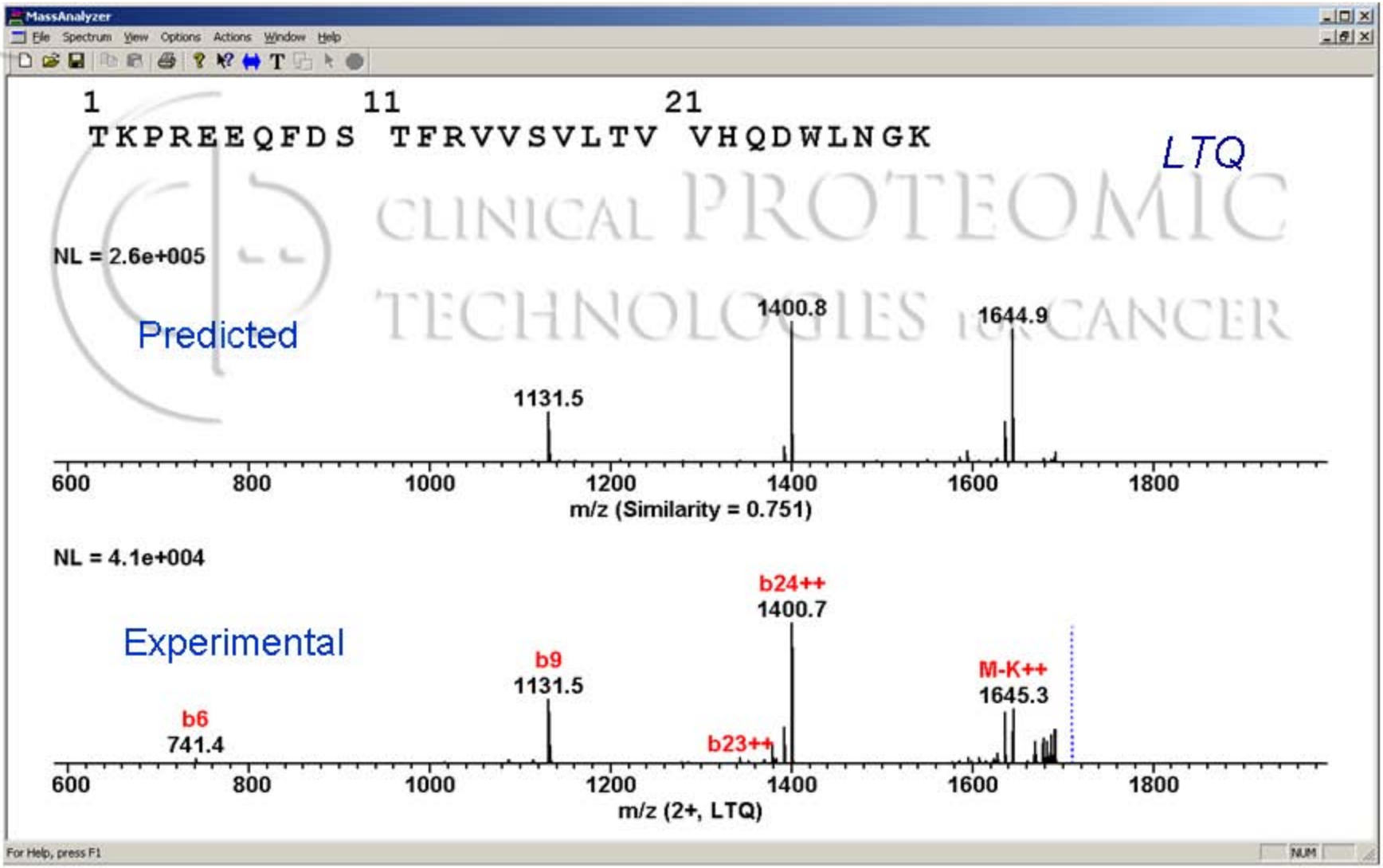


A "Bad" Spectrum?

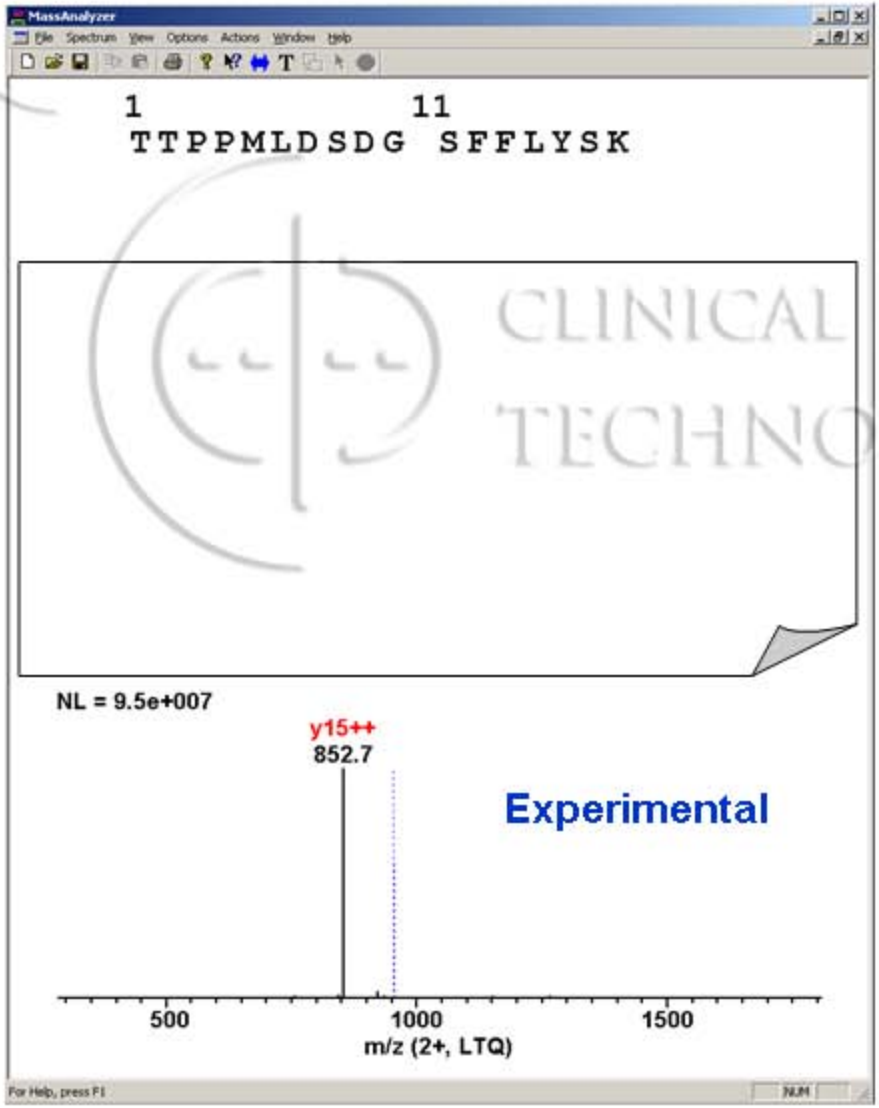


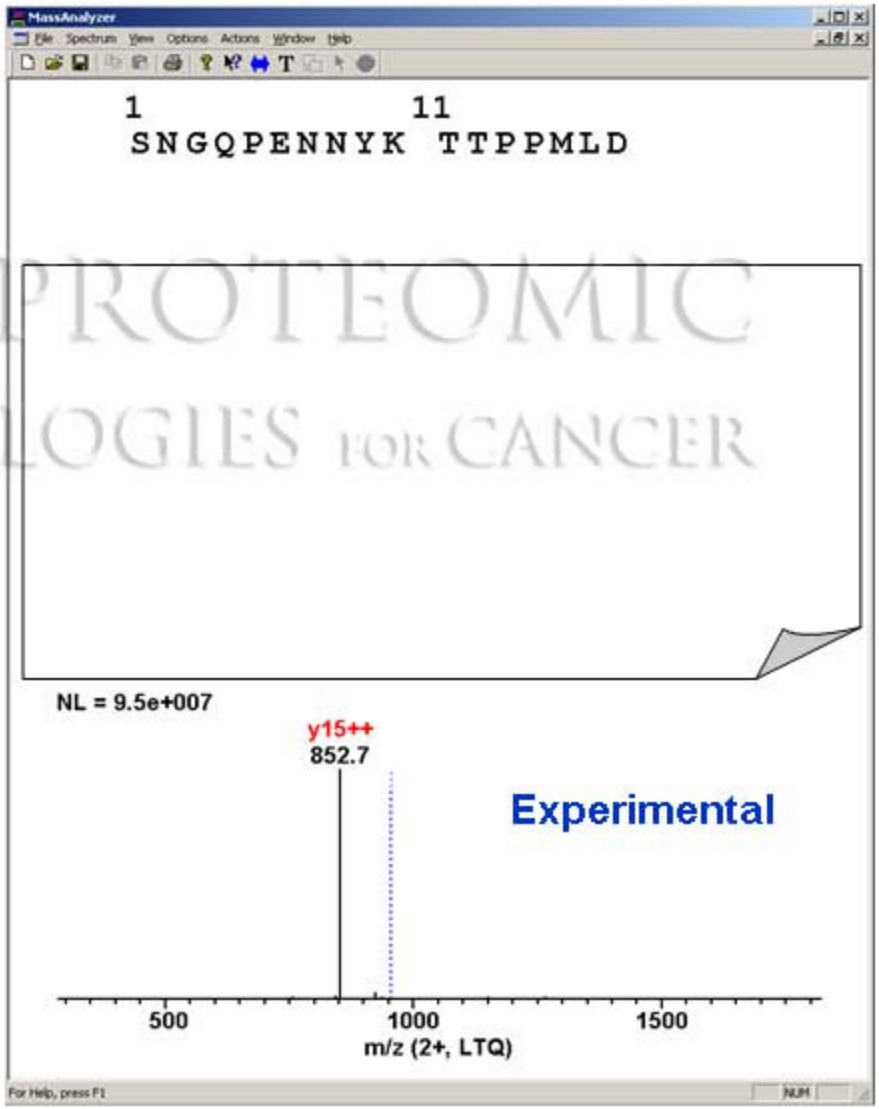
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

“Lack of information” is also information !



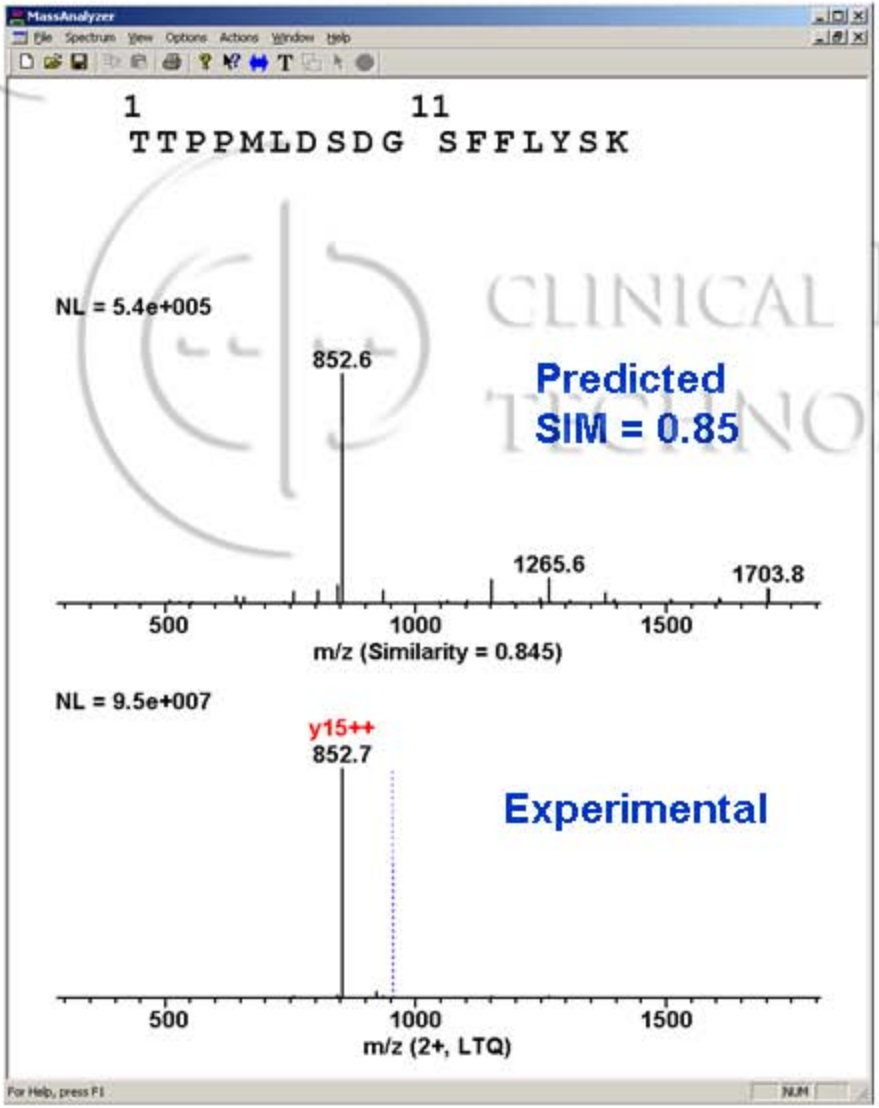
Can you distinguish these two peptides?

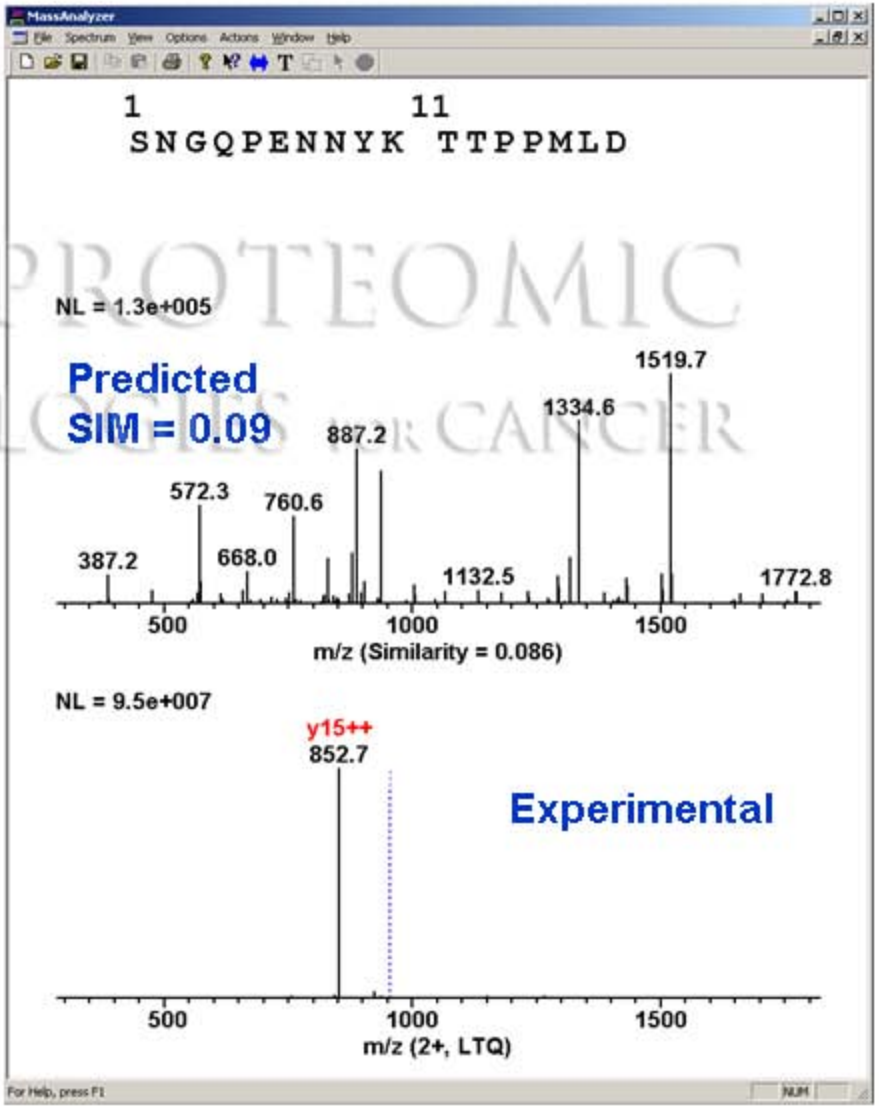




CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

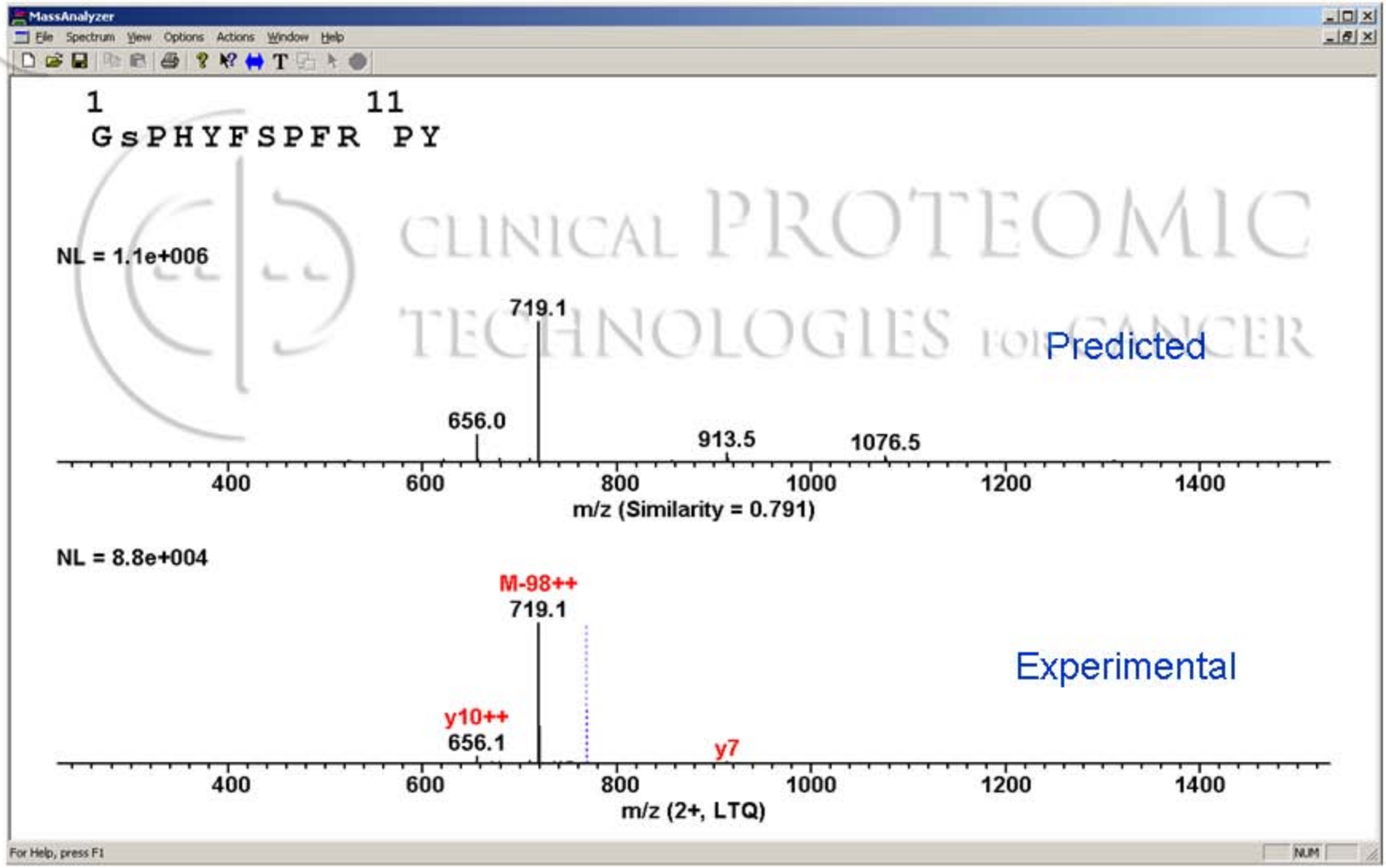
Peptide Distinguished





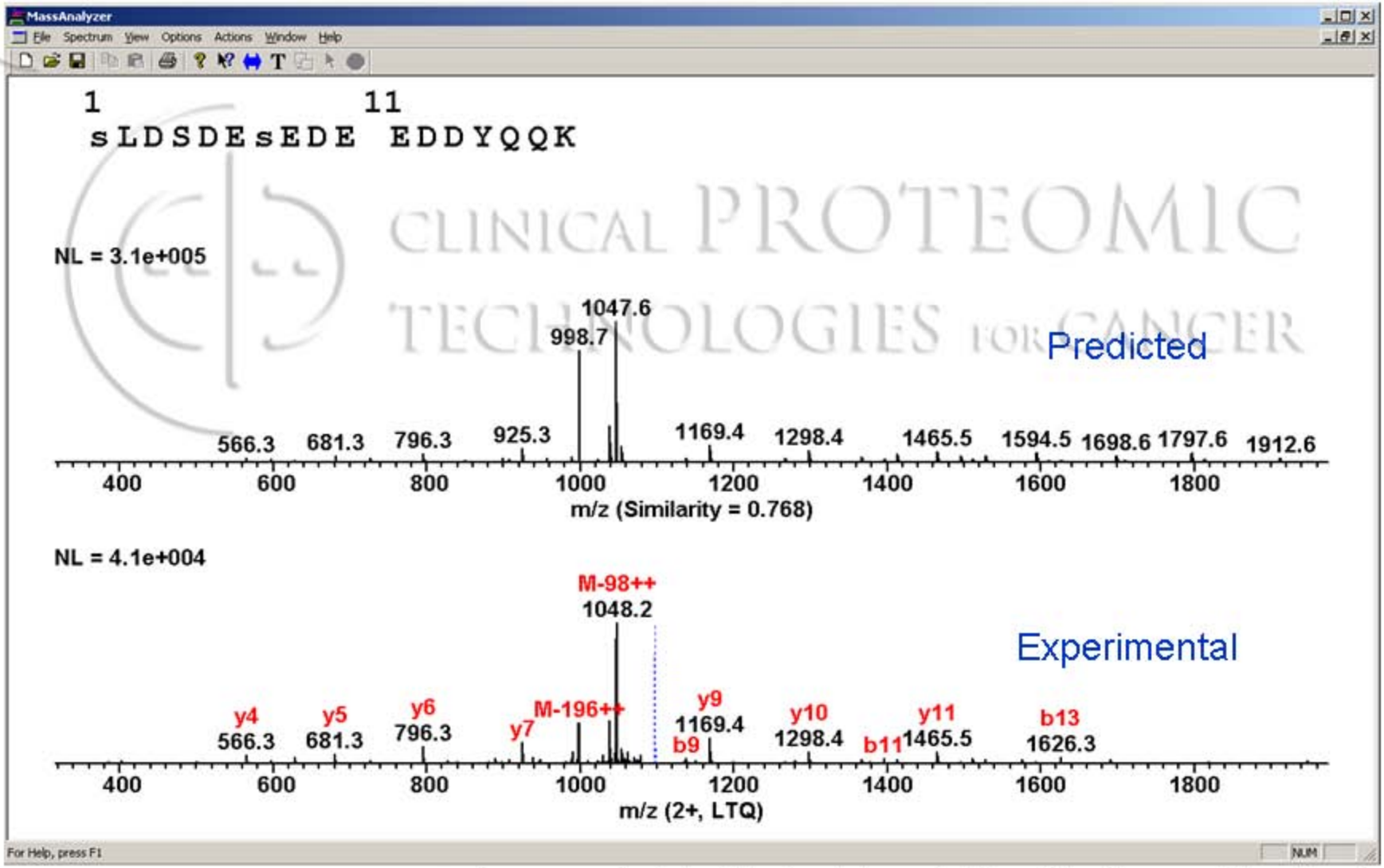
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Phosphopeptide



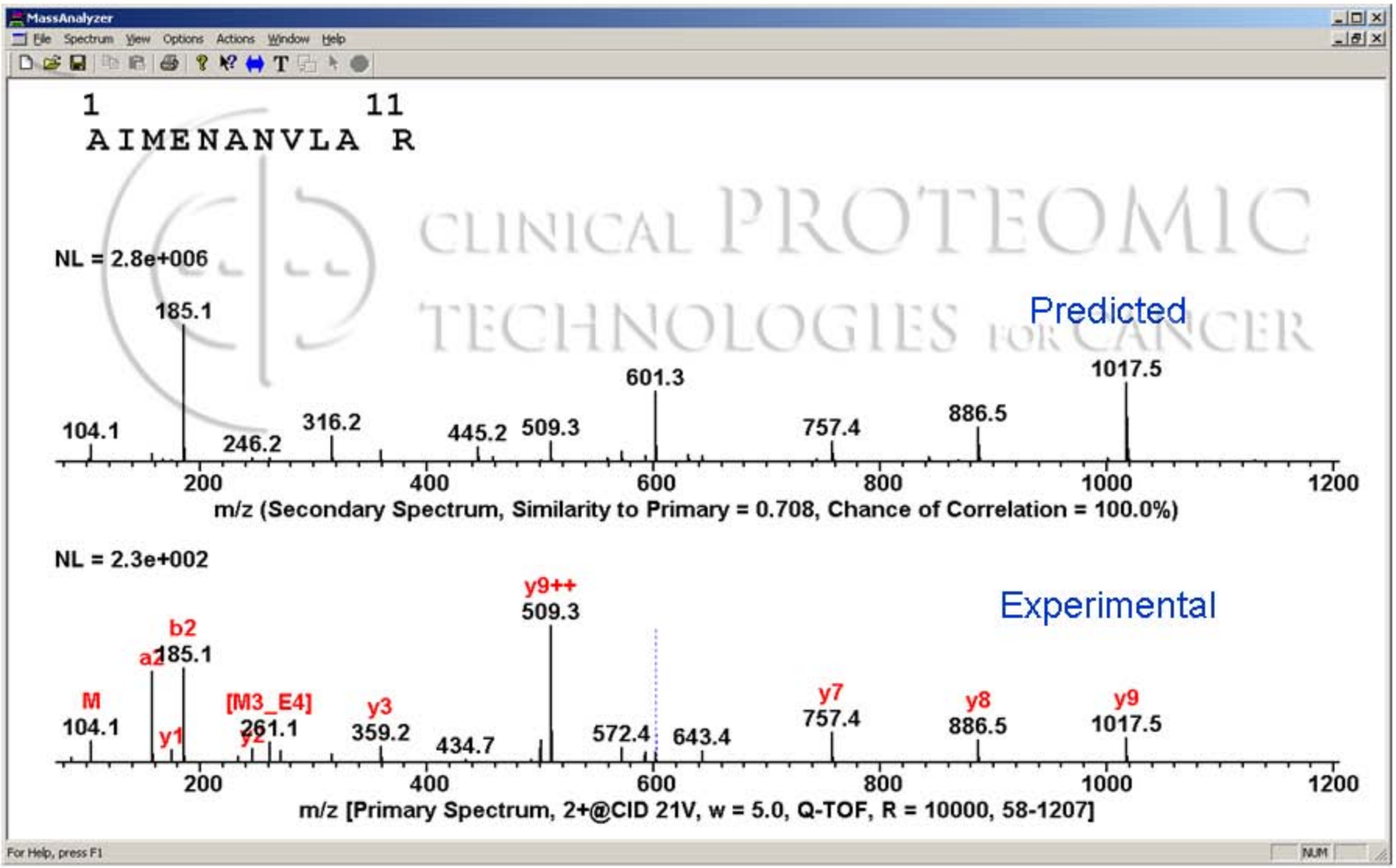
Data generated by Kimberly Lee & Wen Yu (Amgen Washington)

Phosphopeptide



Data generated by Kimberly Lee & Wen Yu (Amgen Washington)

Q-TOF



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Issues in the Model

- Computation speed
  - A long step time has to be used for computation speed, with sacrifice of accuracy

$$\frac{d[F_i]}{dt} = k_i[P]$$

$$[F_i]_{\Delta t} = \frac{k_i[P]_0}{k_{total}}[1 - \exp(-k_{total}\Delta t)]$$

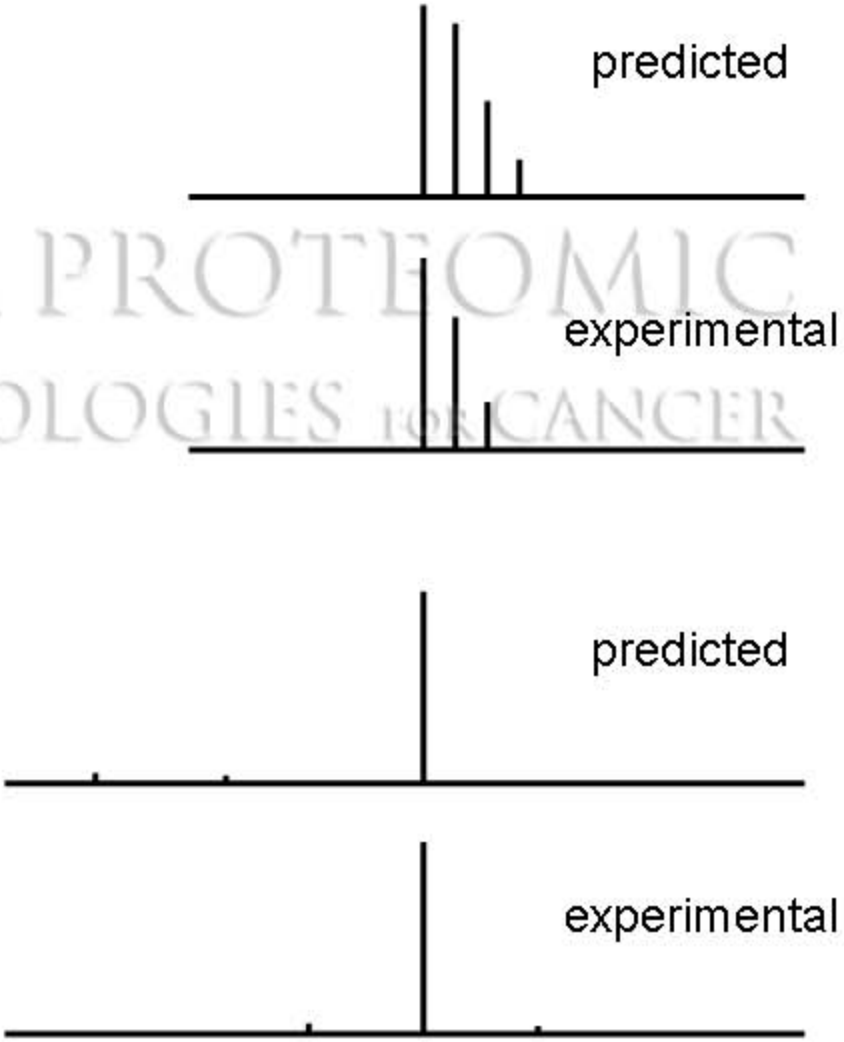
Step time Δt -

- Theoretically infinitely small
- Practically set as time required for temperature to decrease ~120K

CANCER PROTEOMIC TECHNOLOGIES FOR CANCER

Issues in the Model

- Computation speed
  - A long step time has to be used for computation speed, with sacrifice of accuracy
- Similarity score
  - Isotope peaks weigh the same as any other peaks
  - High intensity peaks weight too much



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Issues in the Model

- Computation speed
  - A long step time has to be used for computation speed, with sacrifice of accuracy
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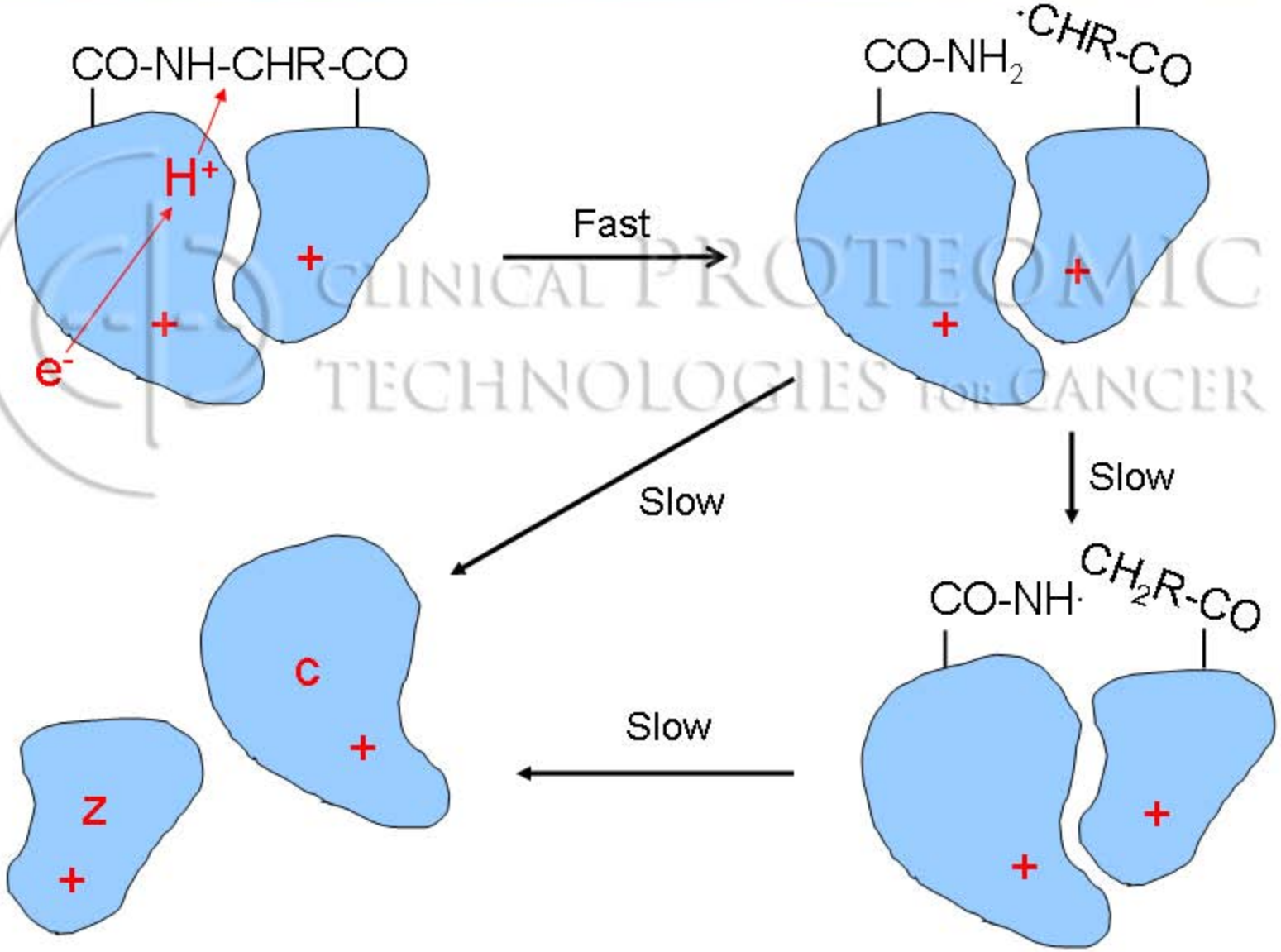
$$\frac{\sum \sqrt{I_m^1 I_m^2}}{\sqrt{(\sum I_m^1)(\sum I_m^2)}}$$

Need a better score function

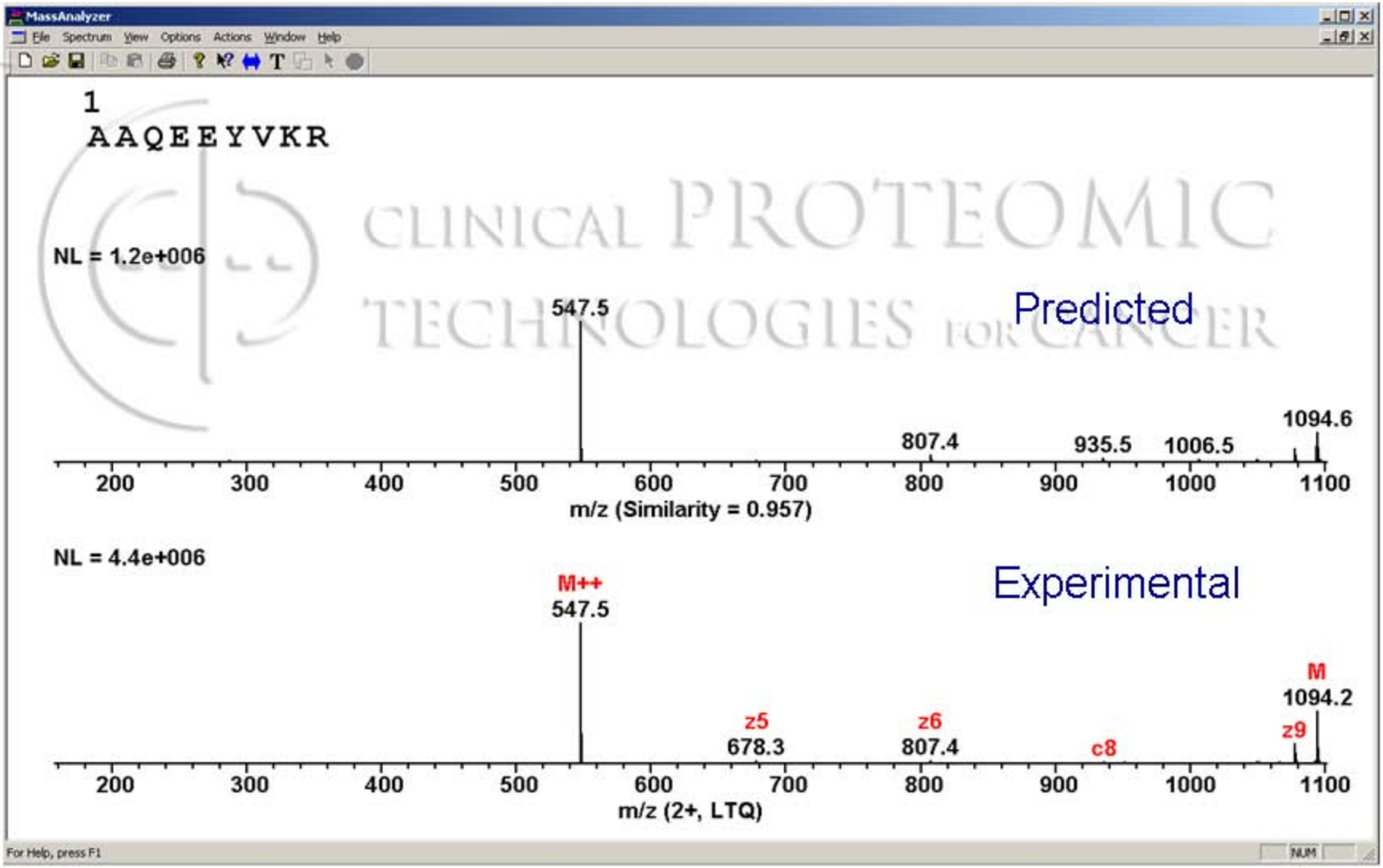
CLINICAL PROTEOMIC TECHNOLOGIES for CANCER

Electron-Transfer Dissociation



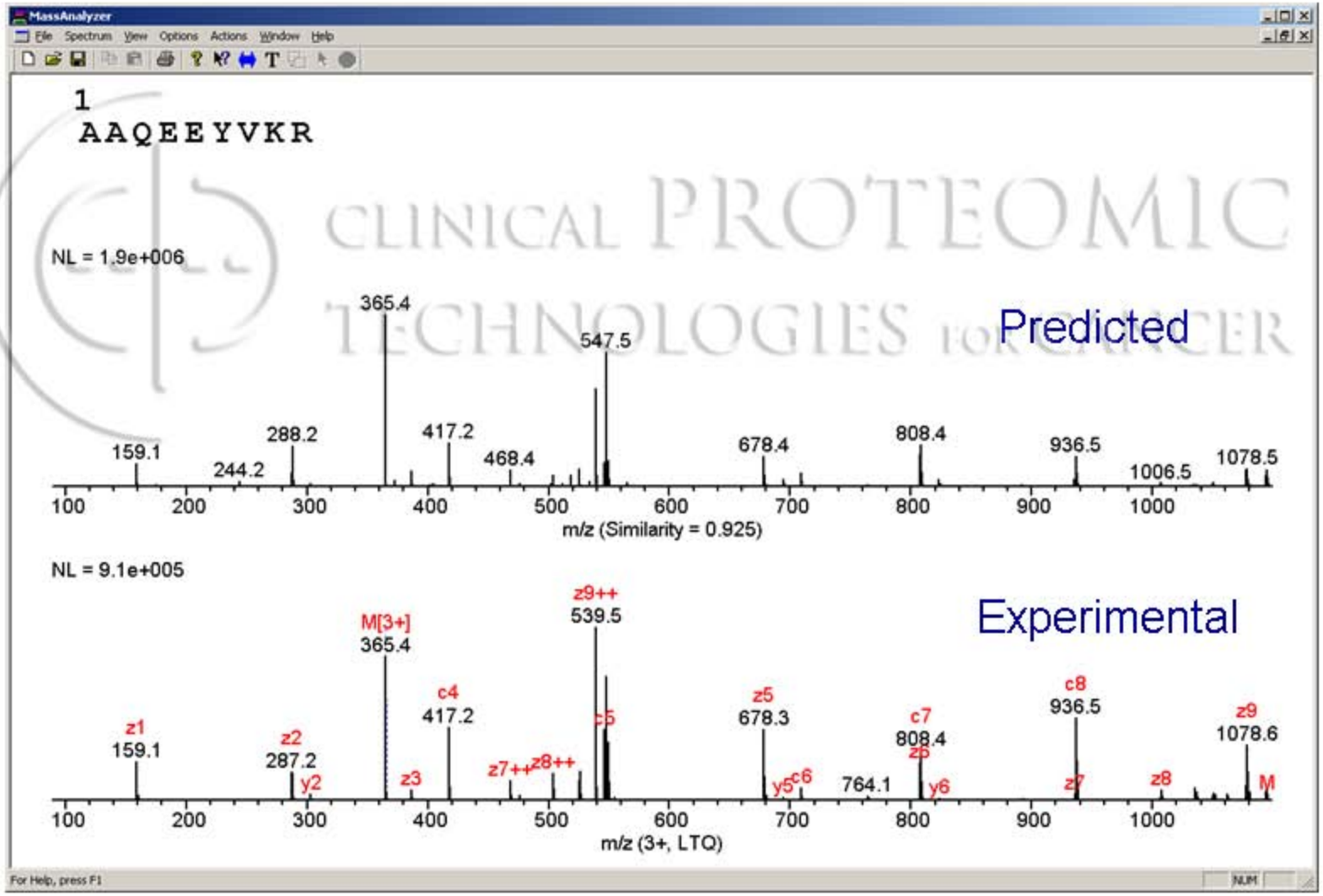
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

ETD of a 2+ Peptide



CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

ETD of a 3+ Peptide



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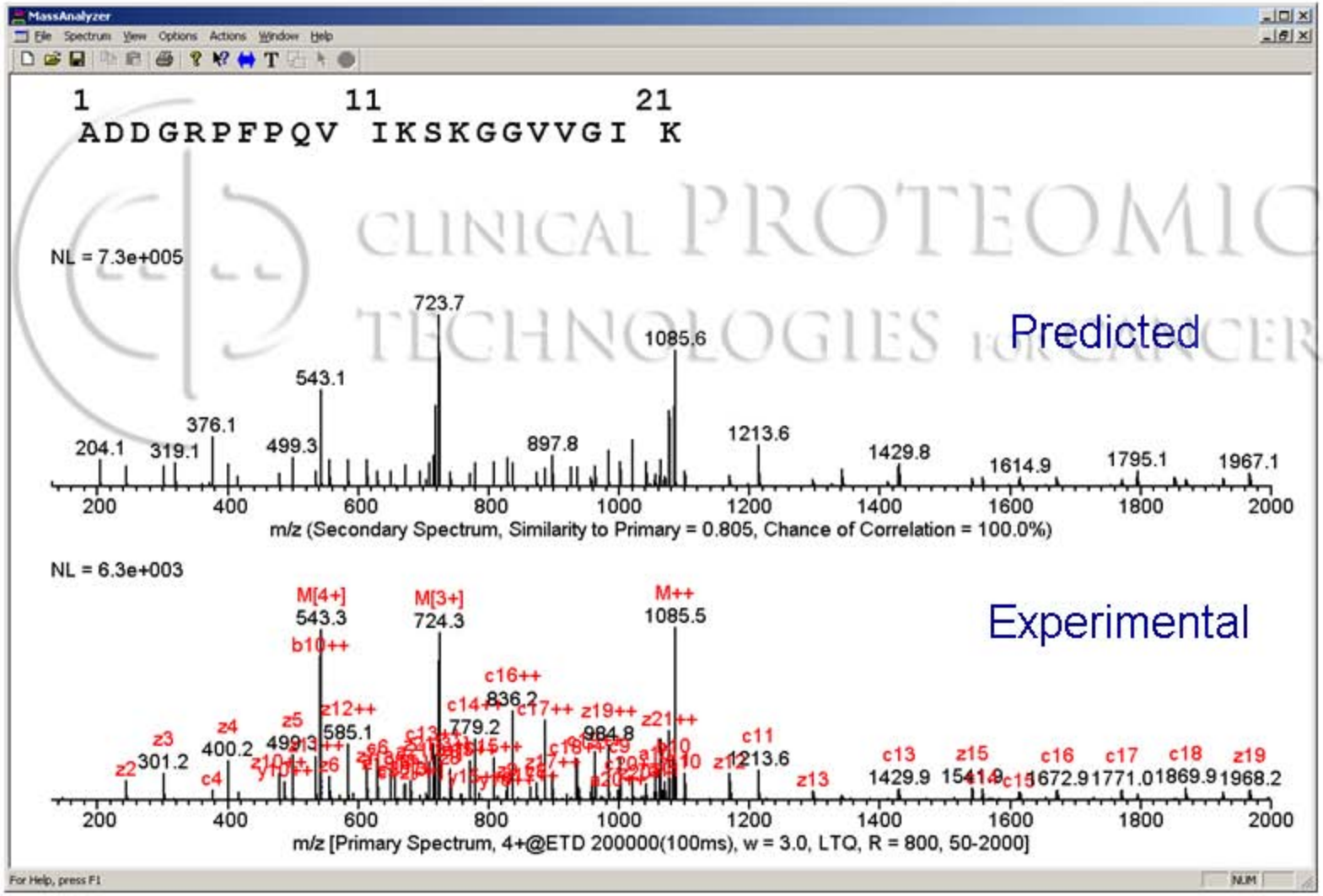
ETD of a 4+ Peptide



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Potential Applications in Proteomics for Peptide Identification?

CENTER FOR PROTEOMIC TECHNOLOGIES FOR CANCER

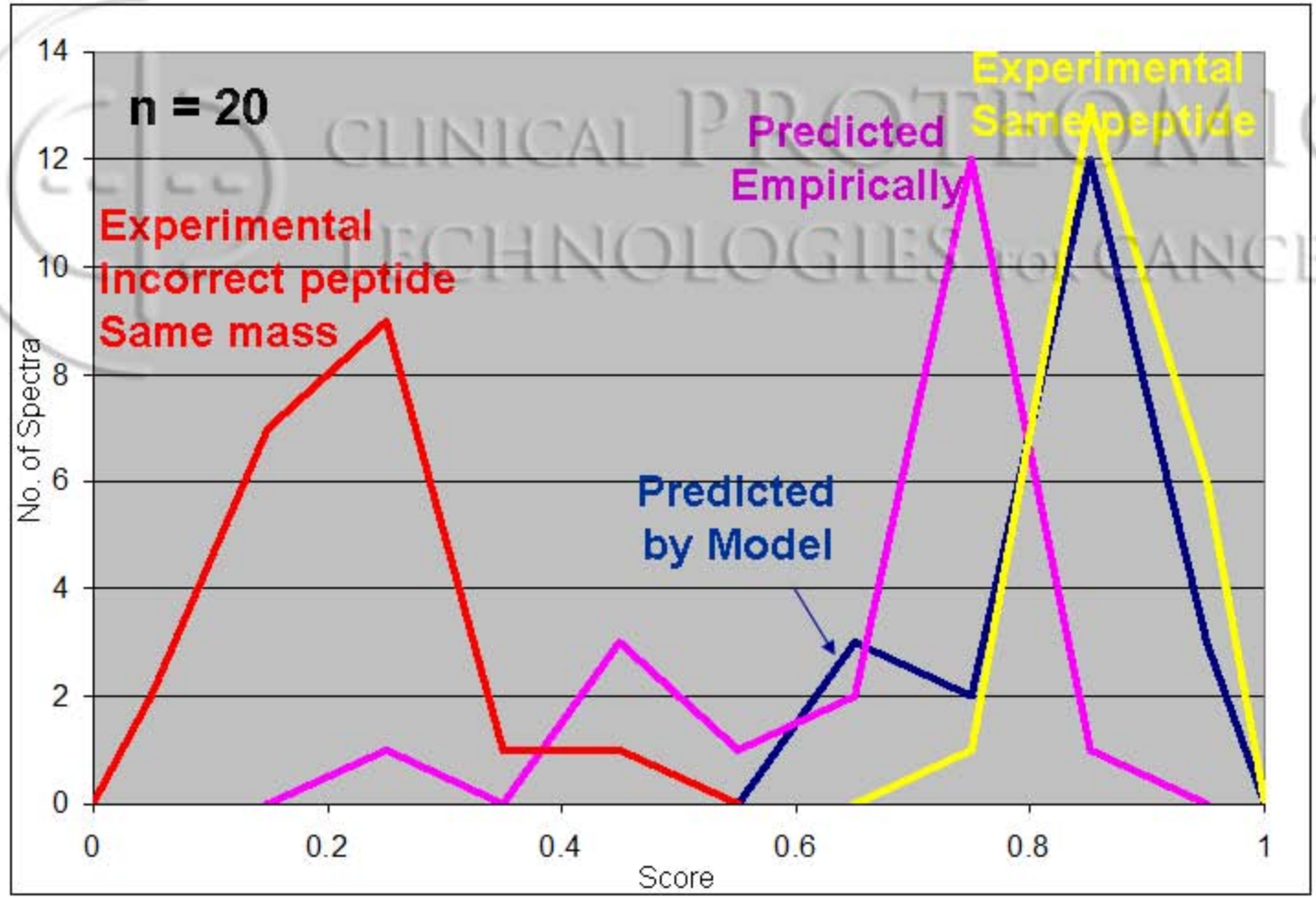
Applications in Proteomics

- Challenge
  - Too slow for database search with on-the-fly predictions (~100 spectra/second)
- Potential solutions
  - Used for validating results after a loose-criteria database search
  - Pre-calculate theoretical mass spectral library

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

What as Reference Spectra in a Spectral Library?

Distribution of similarity scores for different reference spectra



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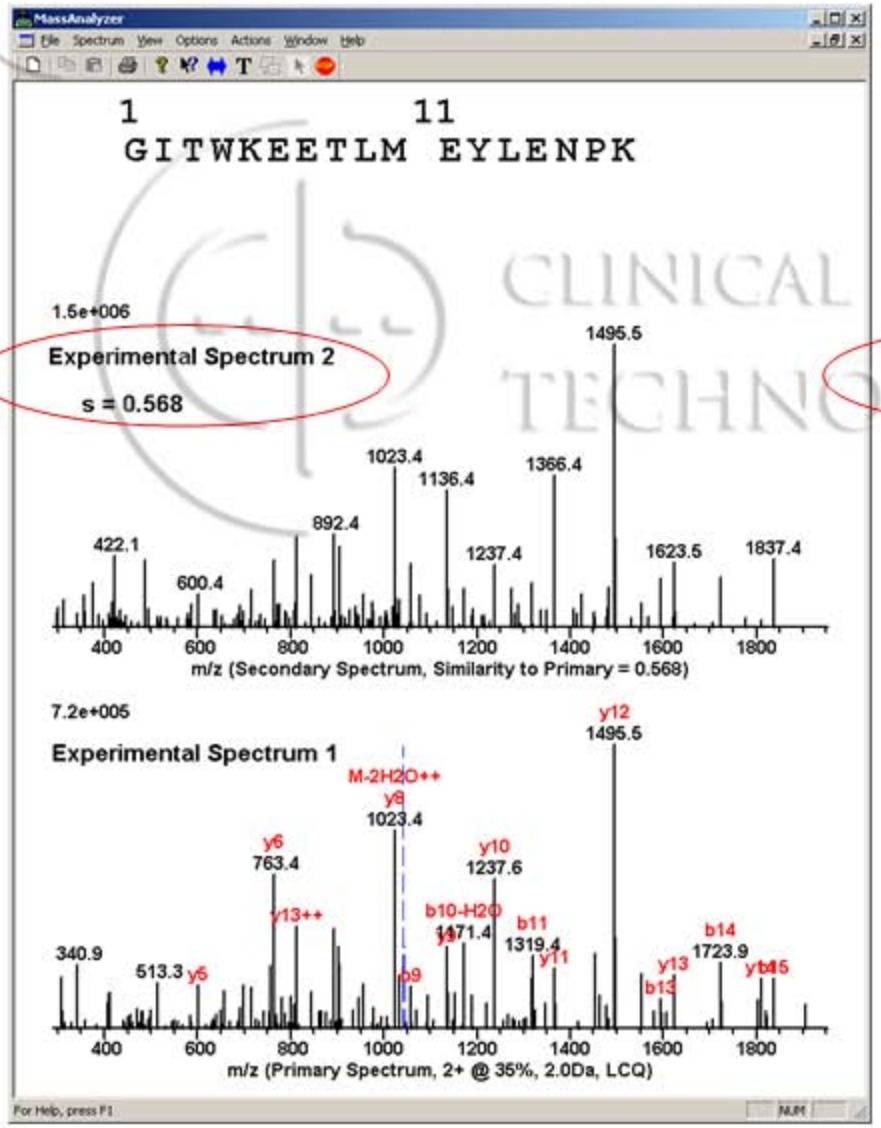
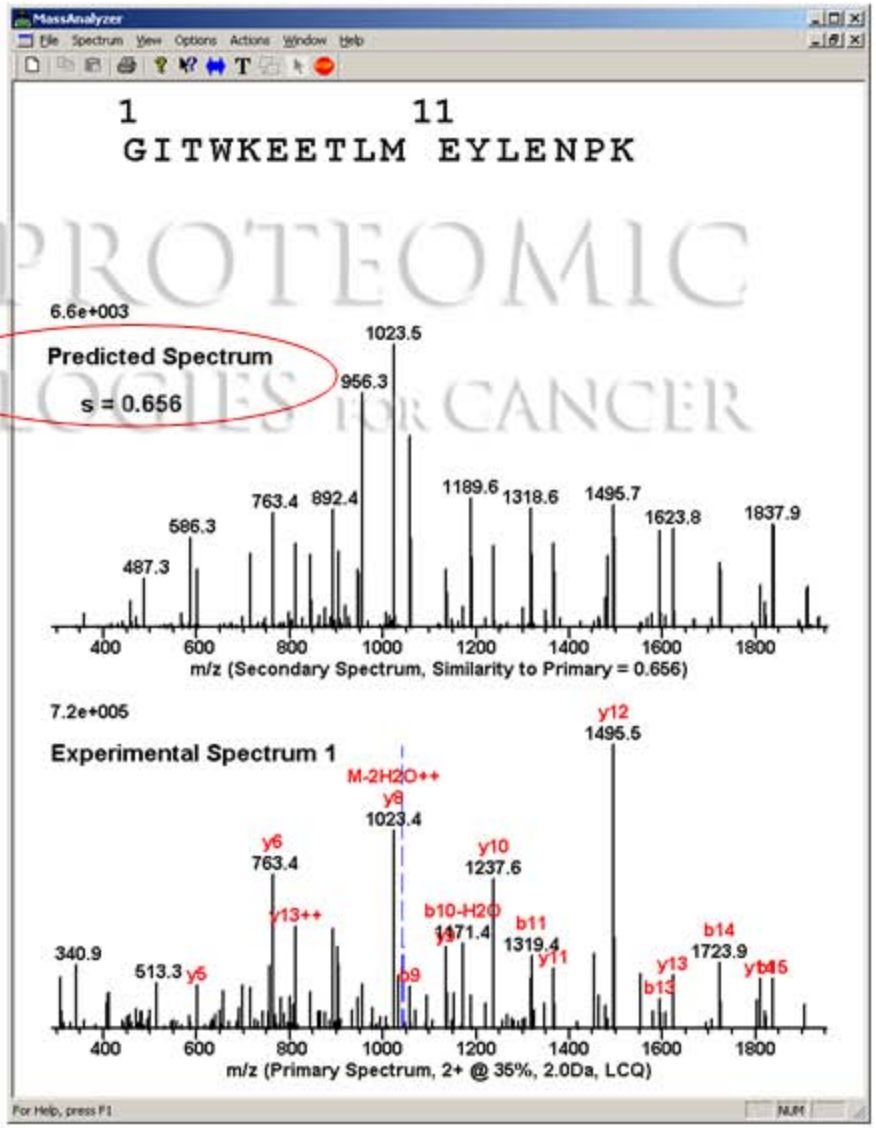
Theoretical or Experimental Spectra?





CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Theoretical Spectra as Reference Spectra?

- Not perfect but
  - Noise free!
  - No incorrect peptide assignment.
  - Fast way to get large amount of reference spectra.
  - Include spectra with few sequence ions.
  - May be better than low-quality experimental spectra such as those from shotgun experiments.
  - Definitely better than no experimental spectra at all.

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Conclusions

- Physical modeling is a practical method to predict peptide fragmentation spectra, including CID and ETD.
- The CID model is successfully used for reliable peptide identification, especially for spectra with limited sequence information.
- The model can be used for more reliable peptide identification in proteomics experiments.

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER