Applications of mass spectrometry to cardiovascular research

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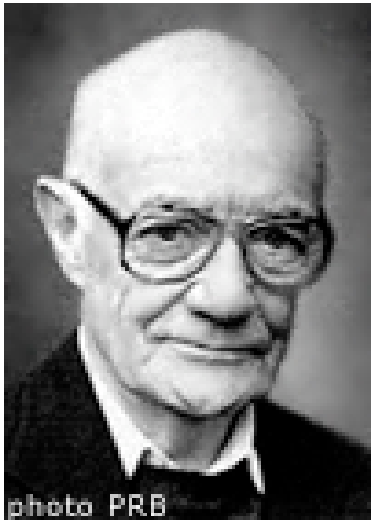
Overview of the talk

- **Vaporizing peptides, proteins and heat sensitive compounds**
- **Separating and identifying proteins**
- **Confirmation of protein identity and posttranslational modifications**

Congratulations to the Nobel Laureates - 2002



John Fenn

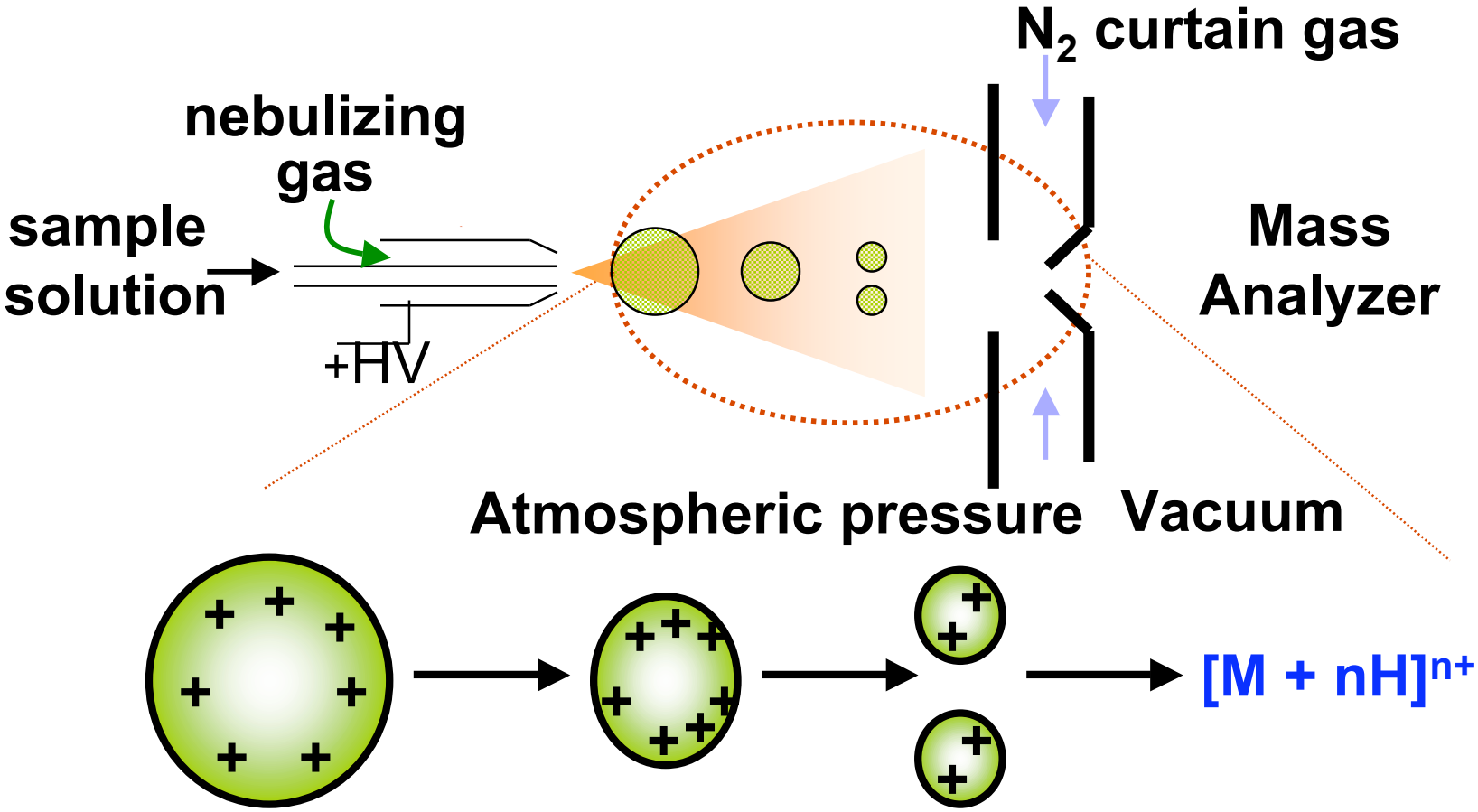
Koichi Tanaka

"*for the development of methods for identification and structure analyses of biological macromolecules*"

and

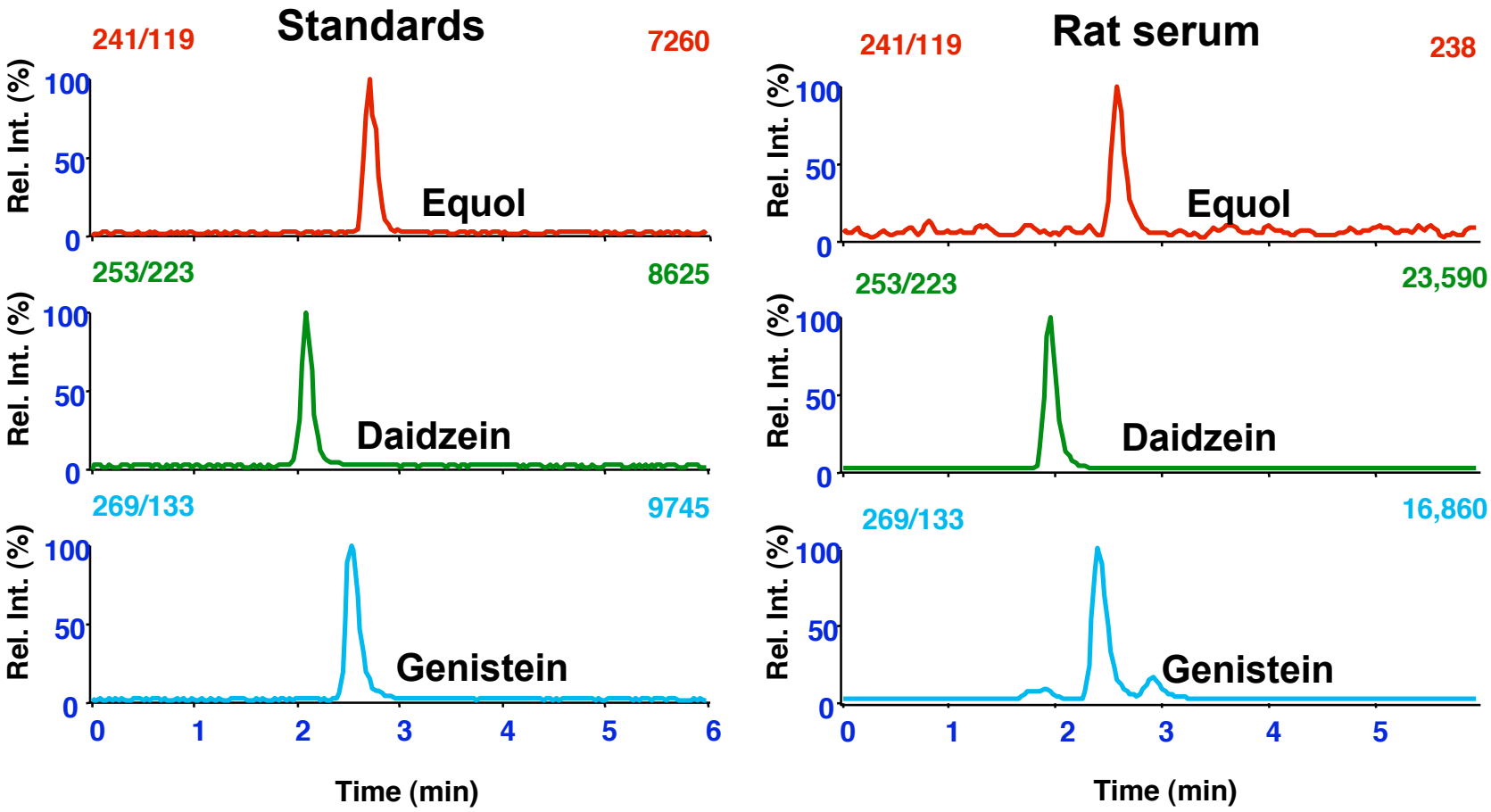
"*for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules*"

Electrospray Ionization (ESI)

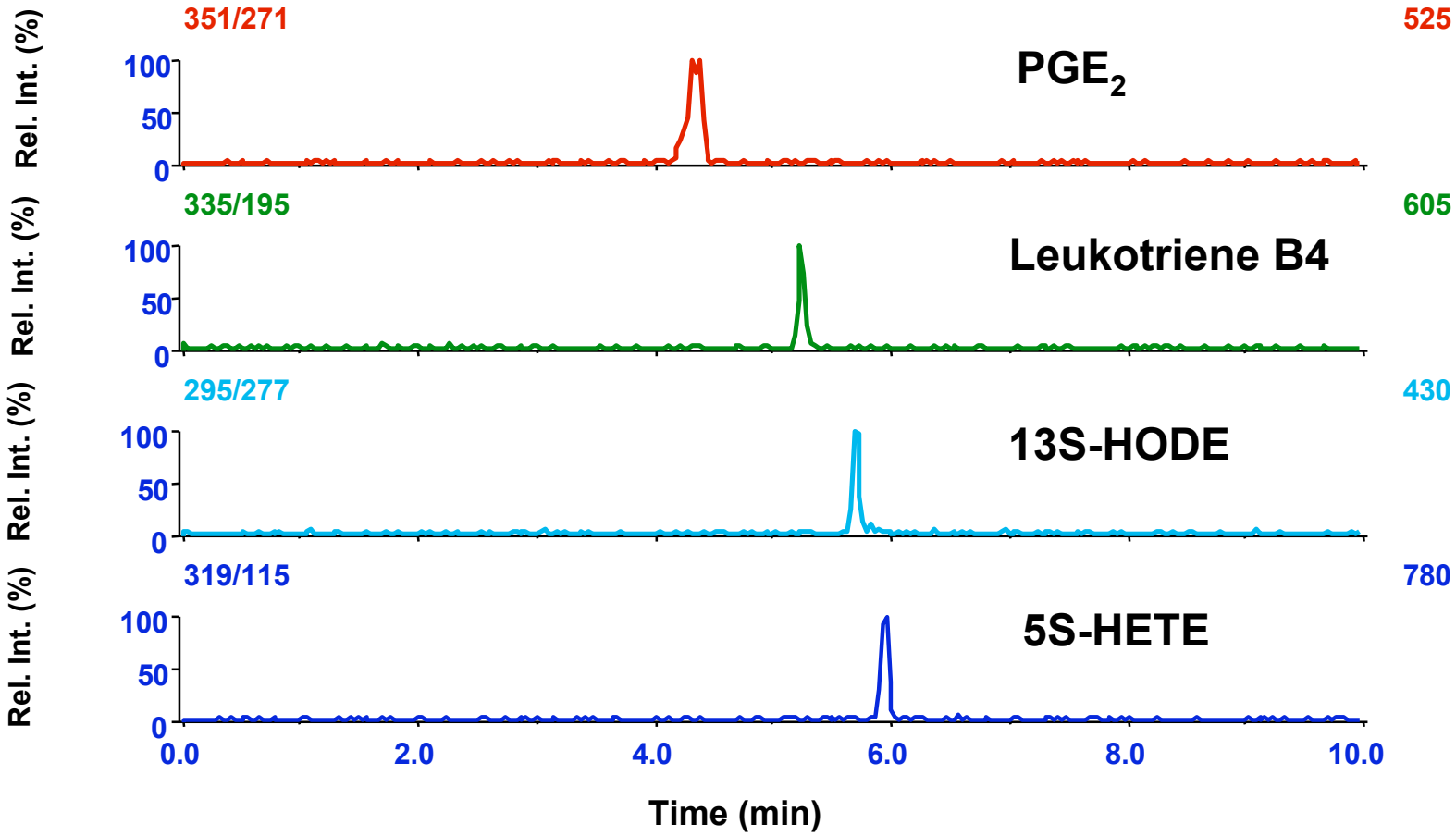


1. Solvent evaporation
2. Coulombic repulsion

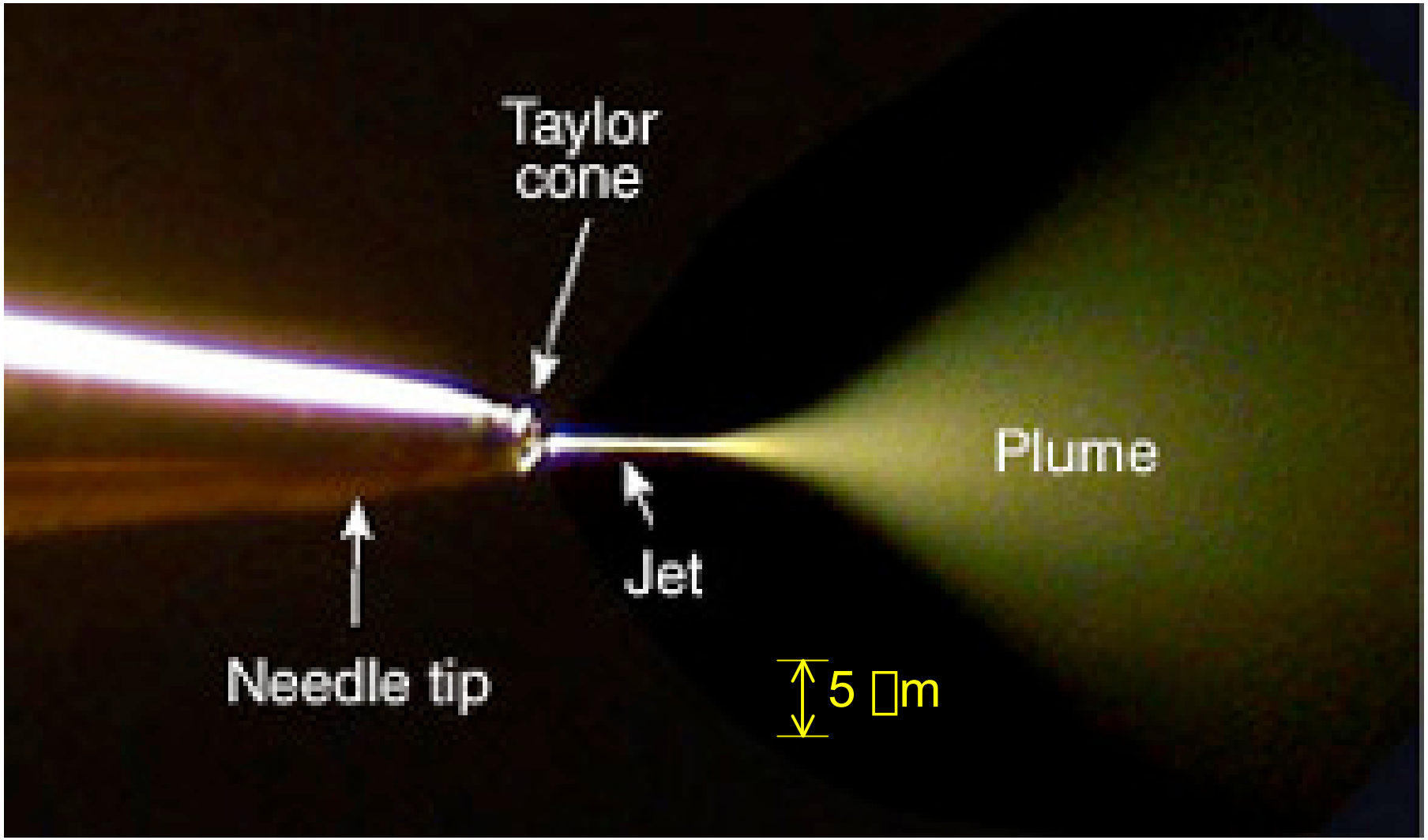
Use of LC-MS to measure isoflavones



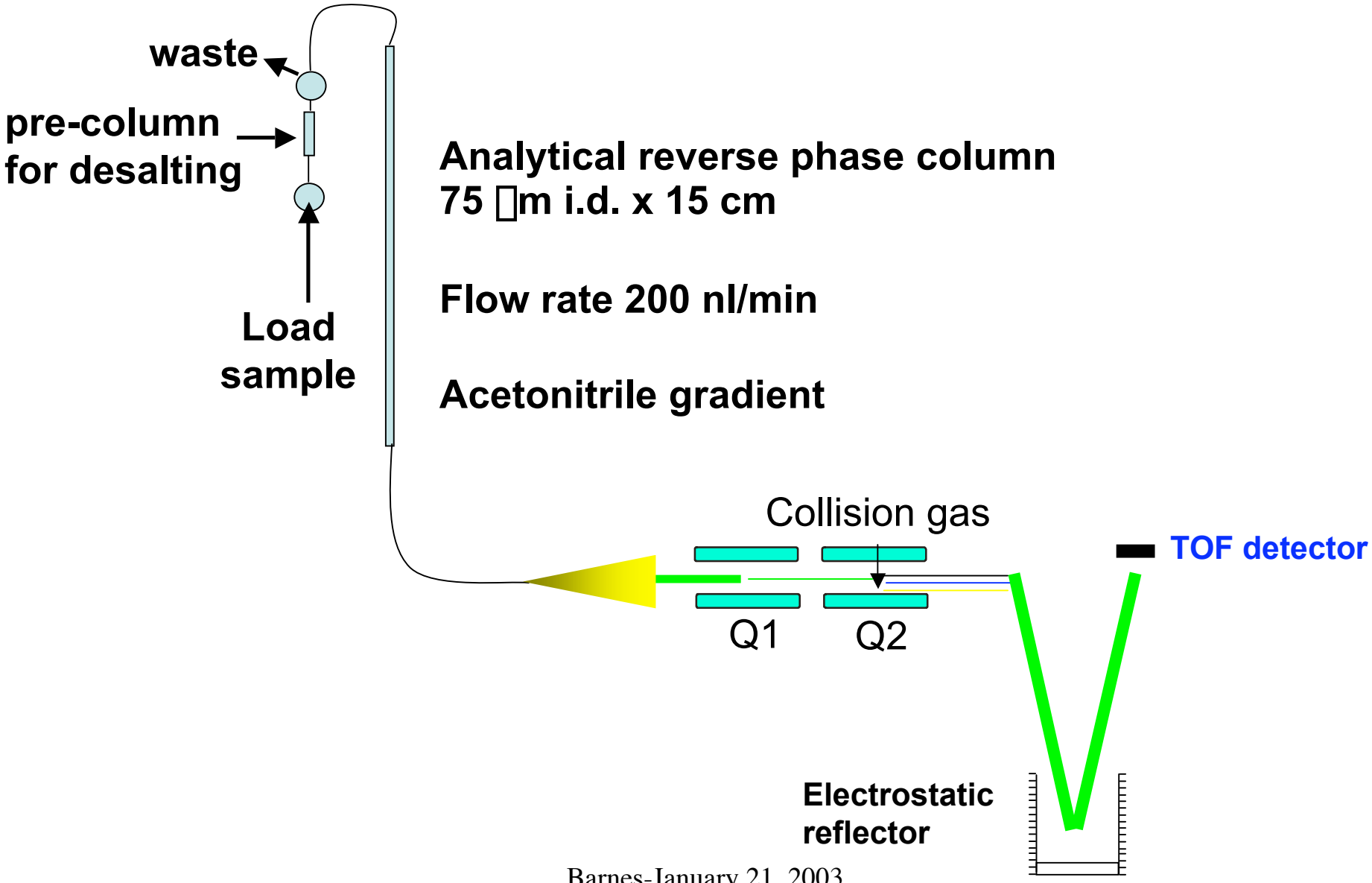
LC-MS analysis of prostanoids



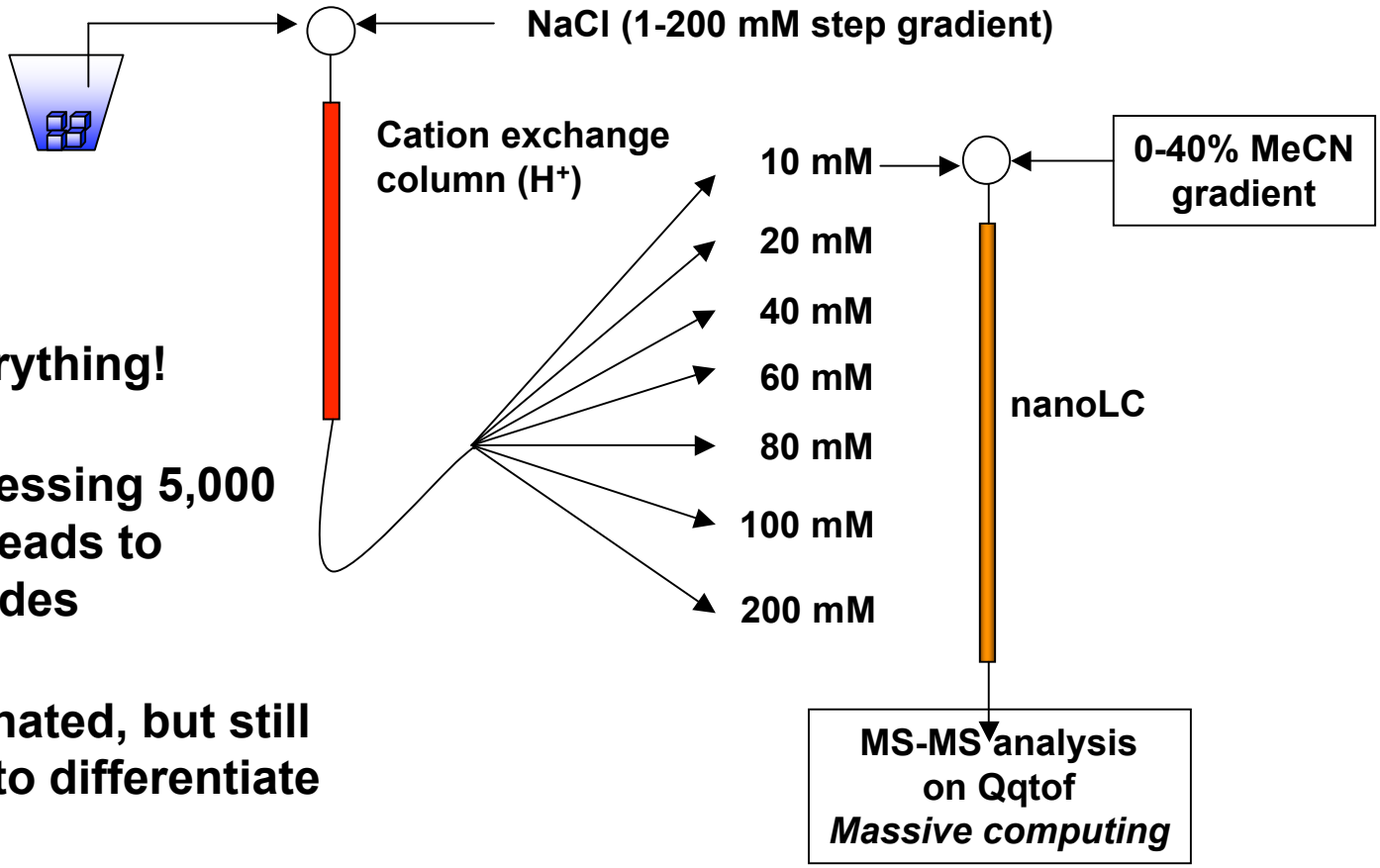
NanoElectrospray



LC-MS of peptide mixtures



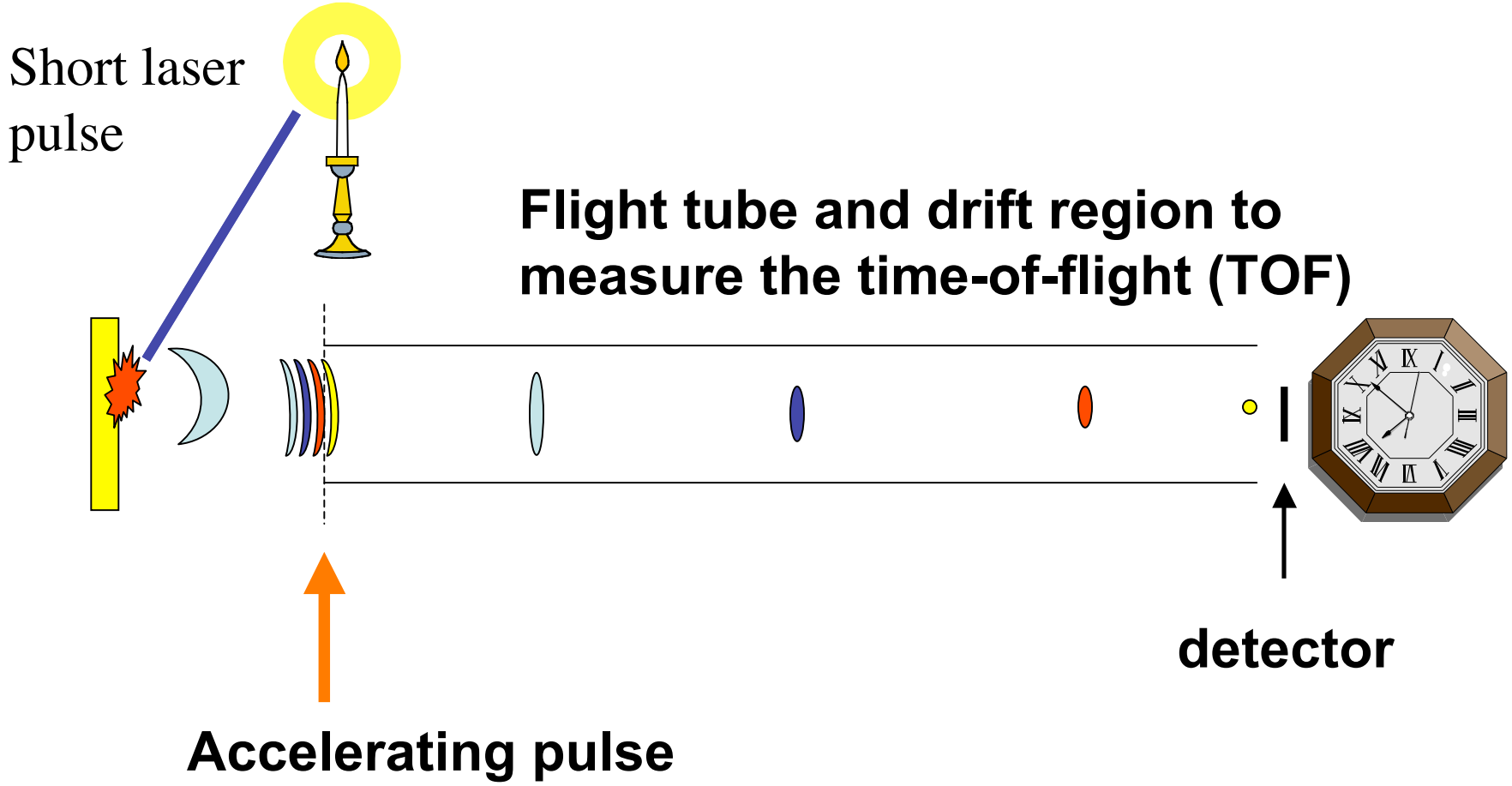
MUDPIT - MUlti-Dimensional Protein Identification Technology



- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

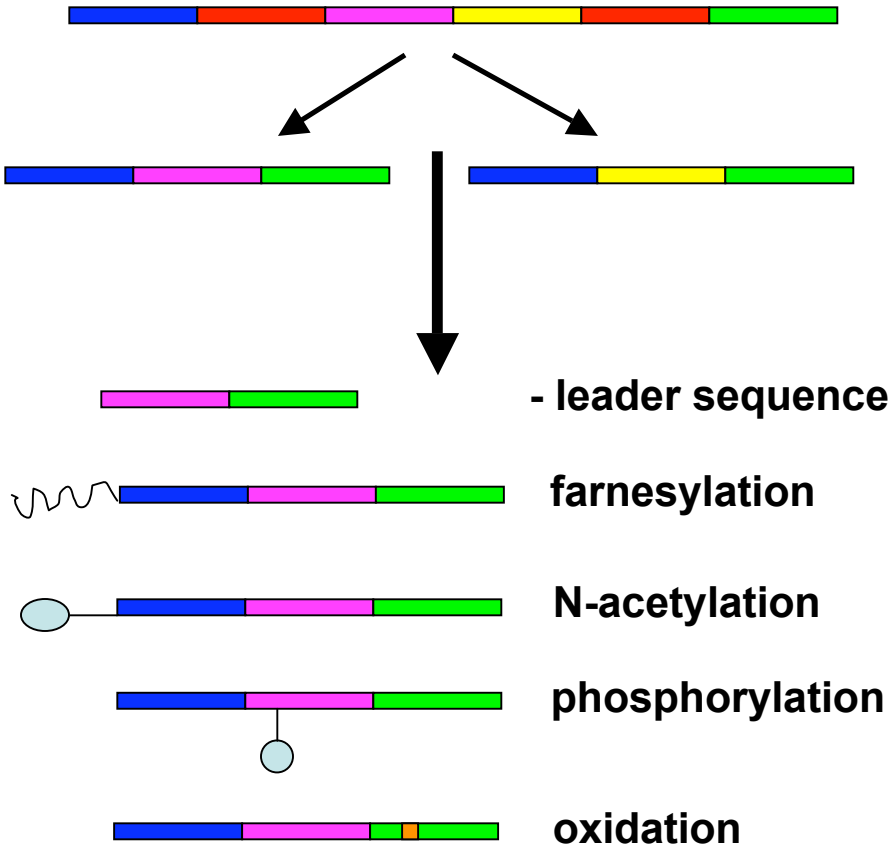
John Yates

Matrix-Assisted Laser Desorption Ionization (MALDI)



Posttranslational modifications

Differential mRNA splicing accounts for a small part of the protein forms arising from a gene - the remainder are due to chemical alterations of the protein AFTER it's been translated



Posttranslational modifications

- some are permanent (removal of N-terminal Met, N-glycosylation at asparagine, farnesylation on cysteines)
- some are transient (phosphorylation, O-glycosylation on Ser, Thr and Tyr)
- others are unintended (nitration, oxidation)

How can we detect PTMs?

Combination of:

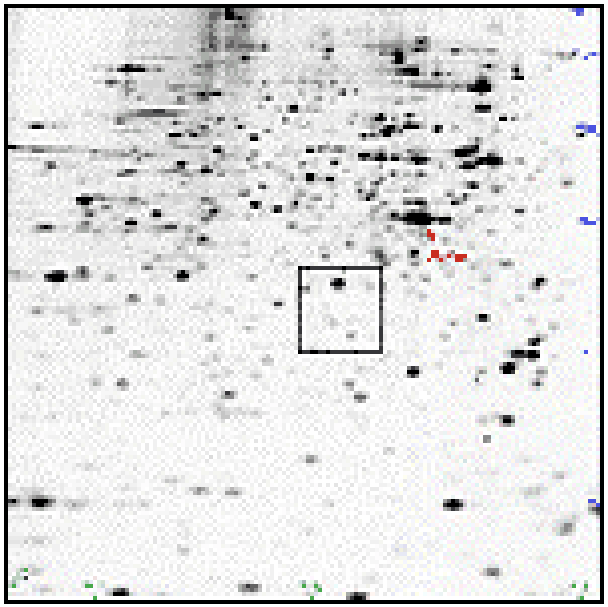
- **isoelectric focusing (pI changes of single proteins) and electrophoresis**
- **affinity isolation (to detect all proteins with a specific chemical grouping)**
- **mass spectrometry (molecular weight changes)**

Separating proteins in two dimensions

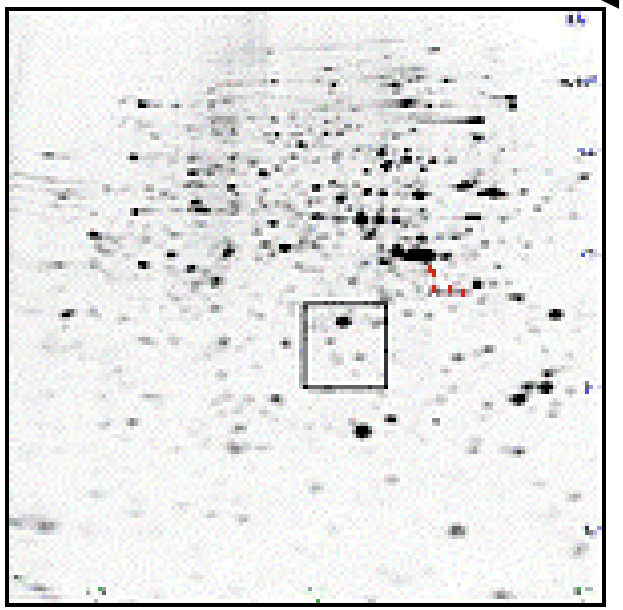
SDS-PAGE

IEF

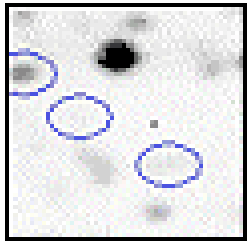
Experimental Control:
Full Image



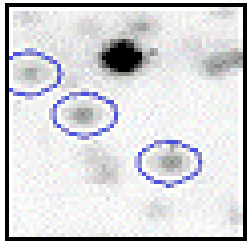
Experimental Result:
Full Image



Experimental
Control:
Region Outlined



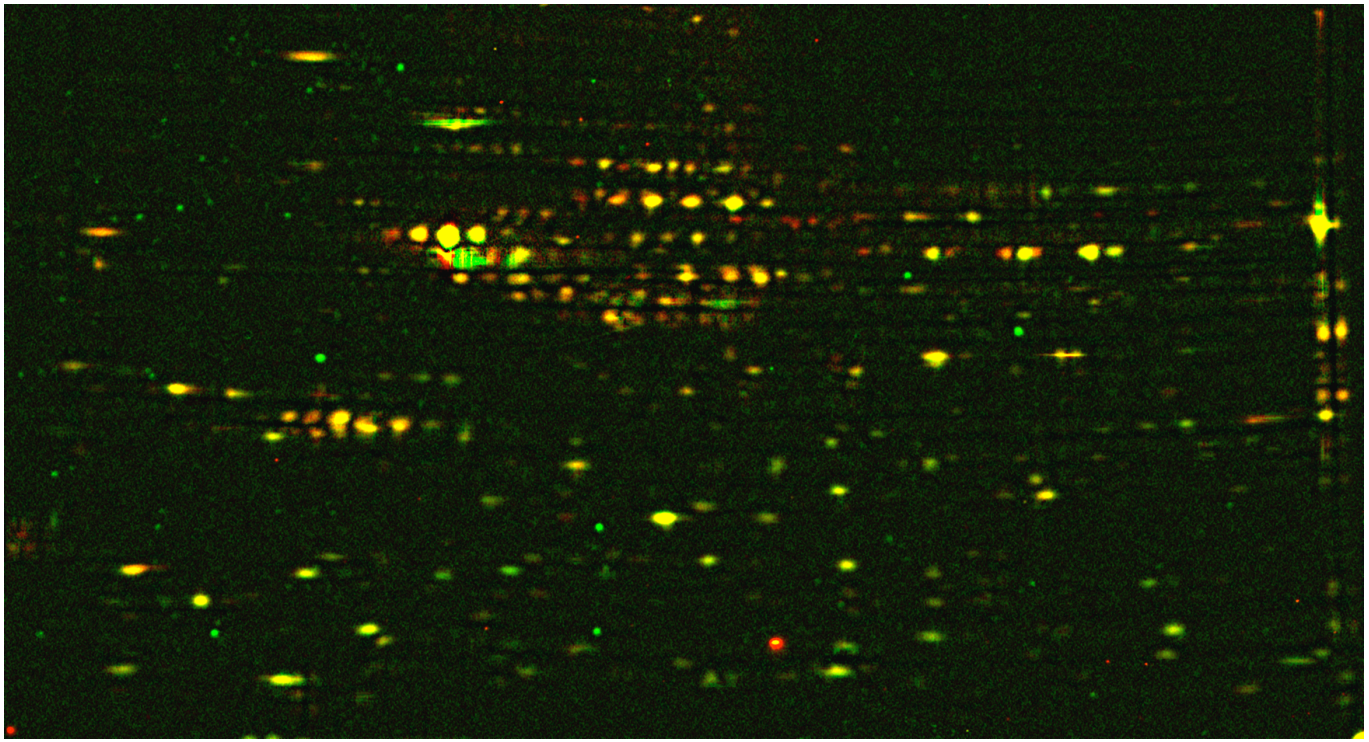
Experimental
Result:
Region Outlined



Detecting a modification using 2DE

- **Adding a phosphate residue to a serine, threonine or tyrosine residue increases the acidity of the protein, i.e., its isoelectric point (pI) decreases with only a small change in MW**
  - **This causes the protein to shift horizontally in 2DE**
- **Adding a ubiquitin residue to a specific lysine residue adds over 8 kDa to the MW**
  - **This causes the protein to be shifted upward (higher MW)**

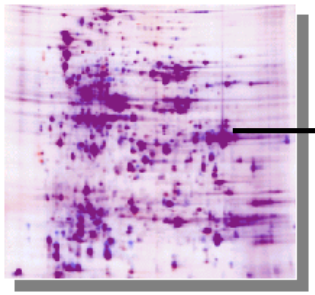
Differential protein labeling with Cy3 and Cy5 Superimposed images from the same gel of normal and cancer cell lines from the breast



Courtesy of Helen Kim and Jessy Deshaine

We've identified a spot - what next?

Peptide finger print mapping



Eppendorf tube

destain

Speed-Vac

trypsin

1:20

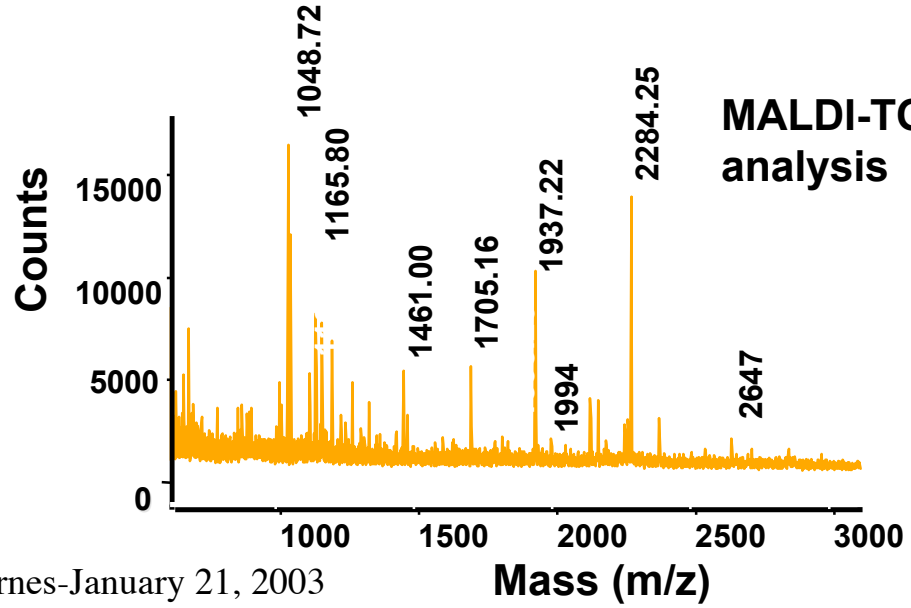
Incubate

overnight

Water Bath 37°C

MALDI plate

Desalting Ziptip



Peptide fingerprint mapping

- **Assuming a 1 Da mass measurement accuracy, "theoretically" it takes on average 2.1-2.3 peptide masses to uniquely identify a protein from a database of all possible peptides (generated *in silico*) resulting from action of a specific peptidase**
  - **In practice, 4-6 peptides are generally needed**

- **Analysis of a 2DE protein spot requires 100 fmol (5 ng of a 50 kDa protein)**
  - **Smaller amounts can be detected, but coverage is low**

Δ masses from modifications

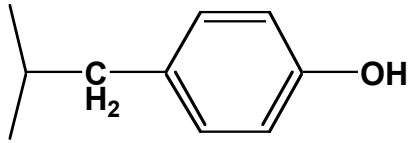
Change (Da)	Chemical type
-79	5' dephospho
-58	Desmosine (from Lysine)
-48	decomposed carboxymethylated Methionine
-44	decarboxylation of gamma carboxy Glutamate
-43	gamma-glutamyl semialdehyde (from arginine)
-42	Ornithine (from Arginine)
-34	Lysinoalanine (from Cysteine)
-34	Lanthionine (from Cysteine)
-34	Dehydroalanine (from Cysteine)
-30	Homoserine formed from Met by CNBr treatment

□ masses from modifications

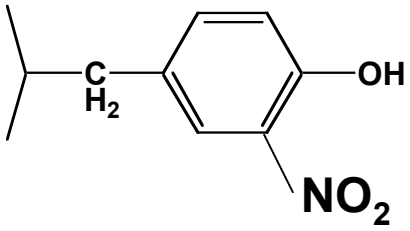
□ Da	Chemical change
78	3-Bromination (of Tyrosine with ^{79}Br)
78	L-O-bromination of Phe with ^{79}Br
80	L-O-bromination of Phe with ^{81}Br
80	Sulphonation (SO_3H) (of PMC group)
80	Sulphation (of O of Tyrosine)
80	Phosphorylation (O of Serine, Threonine, Tyrosine and Aspartate, N-epsilon of Lysine)
80	3-Bromination (of Tyrosine with ^{81}Br)

For all the others, http://www.abrf.org/index.cfm/dm.home

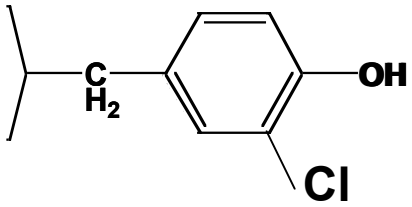
Tyrosine modifications



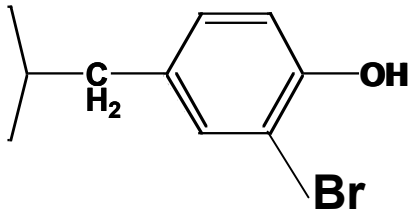
tyrosine



3’-nitrotyrosine

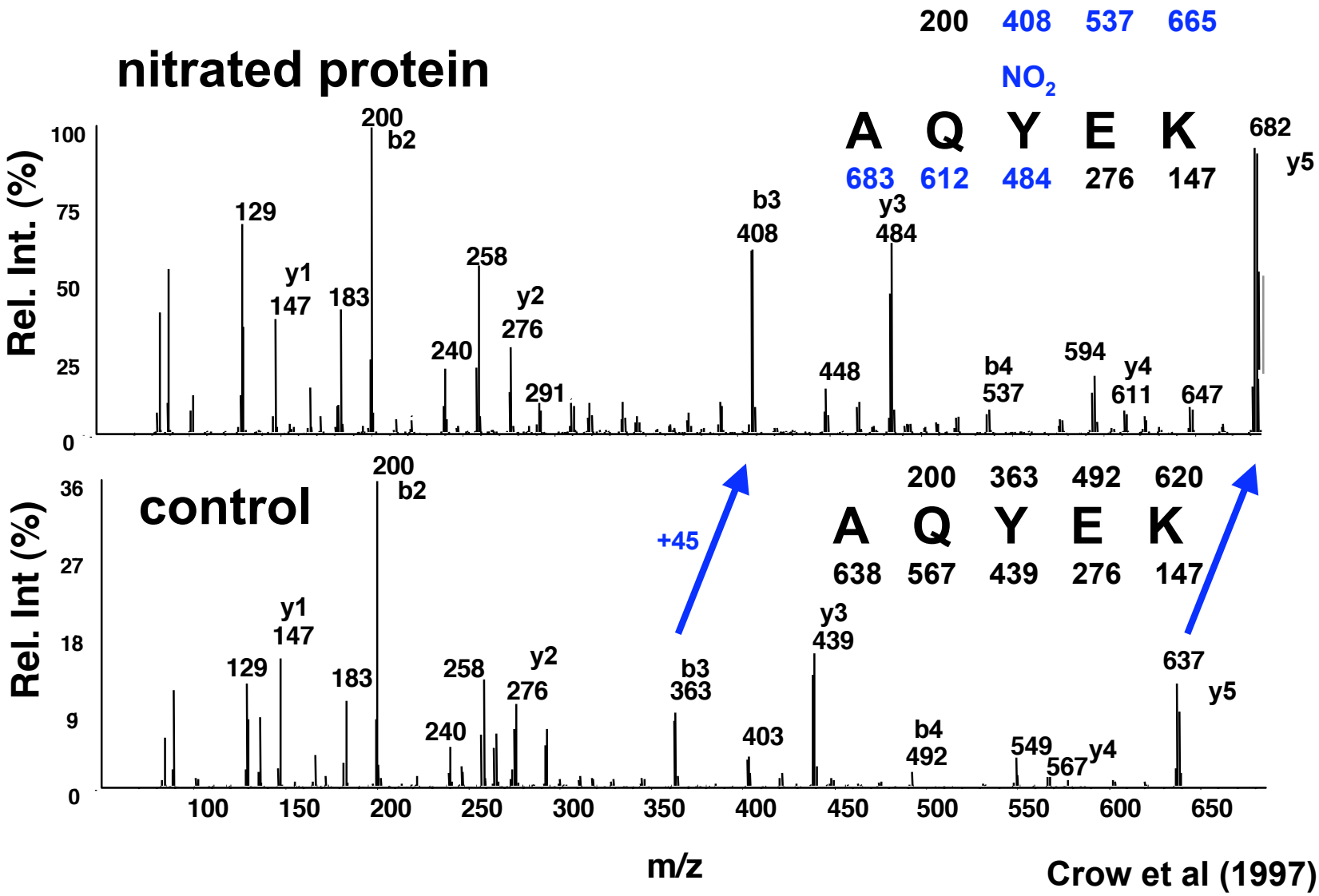


3’-chlorotyrosine



3’-bromotyrosine

Site-specific nitration of a tyrosine-containing peptide using CID MS-MS spectra



Acknowledgments

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