Solving the challenges in cancer research and protein mass spectrometry

Stephen Barnes, PhD

Department of Pharmacology & Toxicology

Seminar overview

- A perspective of mass spectrometry and science - basic mass spectrometry
- What regulates events in a cell, and in cancer?
- How can we detect events in cancer?
- Role and challenges of mass spectrometry

Over a 100 years of mass spectrometry

1886 Discovery of “canal rays” by Goldstein
1905 J.J. Thompson introduced the use of low pressure
1919 Francis Aston establishes isotopes of neon (20/22)
1931 Aston discovers U-235/U238 isotopes
1937 Aston notes the mass defect of elements up to fluorine - $e = mc^2$
1938 Hahn/Strassman observe uranium fission
1940 Nier begins isolation of U235 by mass spec
1943 Army takes over - Manhattan project (Lawrence)

Postwar - modern mass spectrometry begins
1952 First meeting of the ASMS

Biomedical Mass Spectrometry

Early work in mass spec concentrated on isotopes and isotope ratios ($^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{15}N/^{14}N$)

Rittenberg and Schoenheimer established many of the pathways of metabolism using these isotopes

The combination of gas chromatography and mass spectrometry was good for small molecules

BUT what about proteins, peptides and other heat labile molecules?

Evaporating peptides and macromolecules

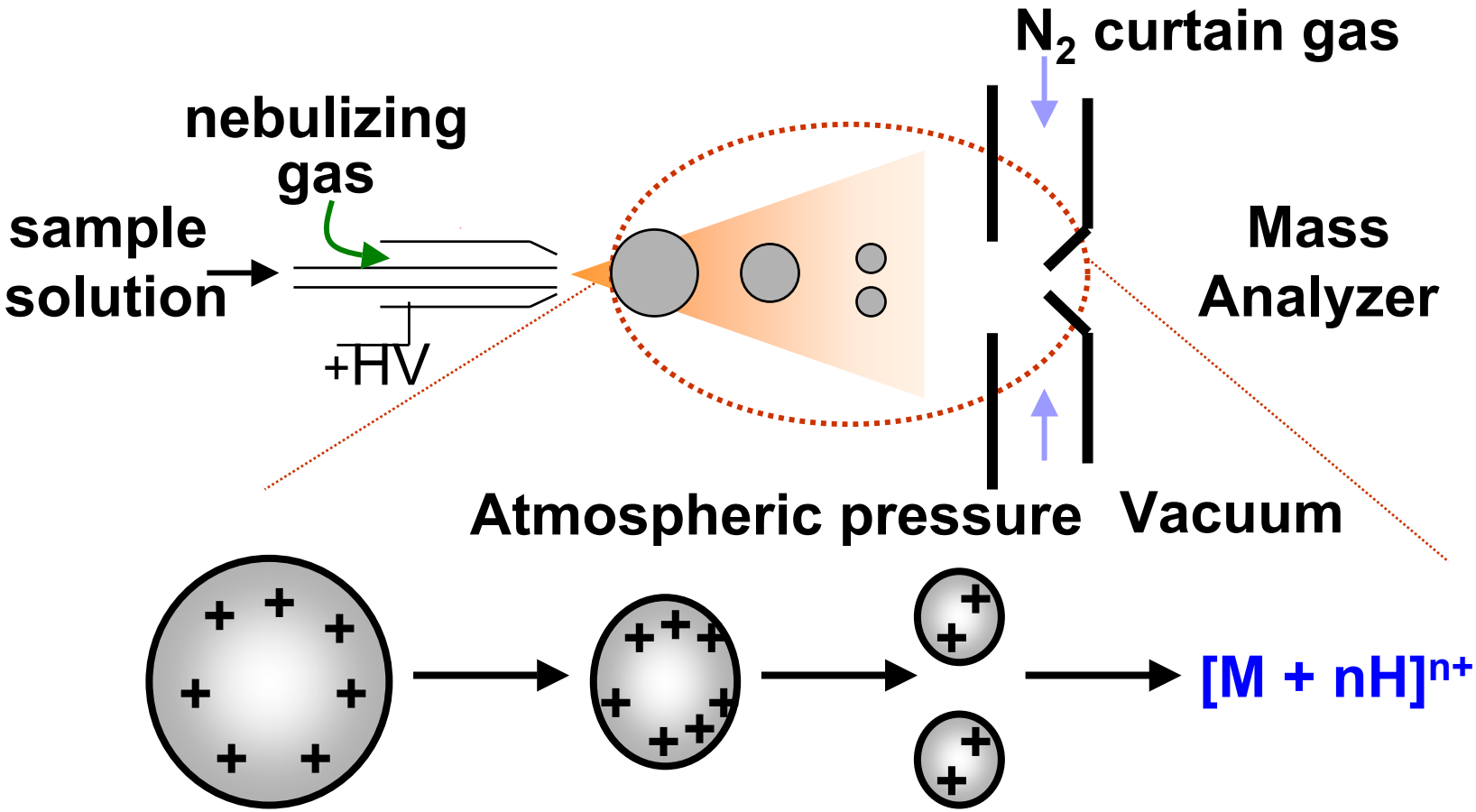
Fast atom bombardment **(FAB) - peptides**

Field desorption

Electrospray ionization **(ESI) - peptides, proteins, oligonucleotides, and small molecules**

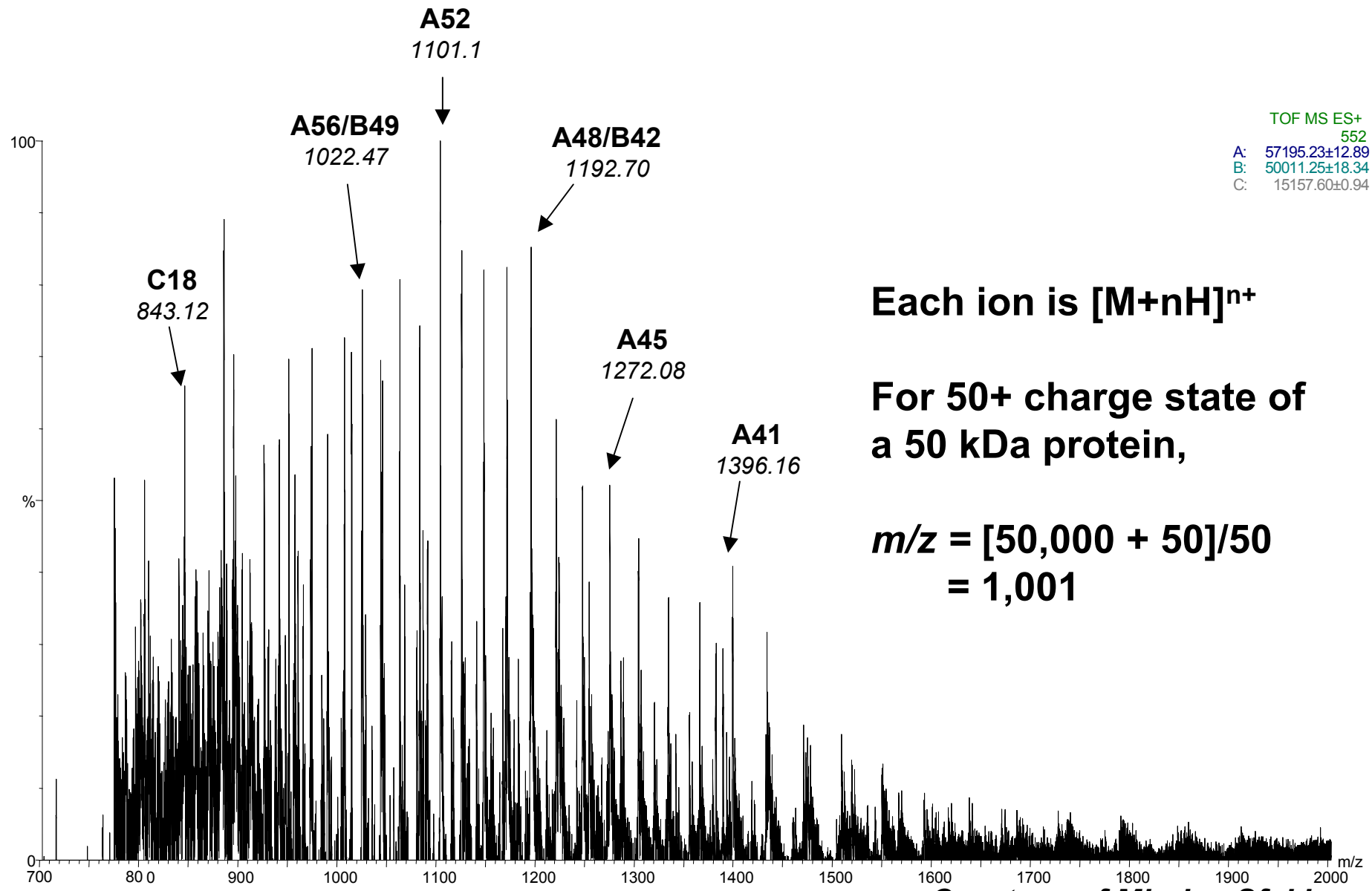
Laser desorption ionization **(LDI) - almost any molecule**

Electrospray Ionization (ESI)



1. Solvent evaporation
2. Coulombic repulsion

ESI spectrum of bacterially expressed protein



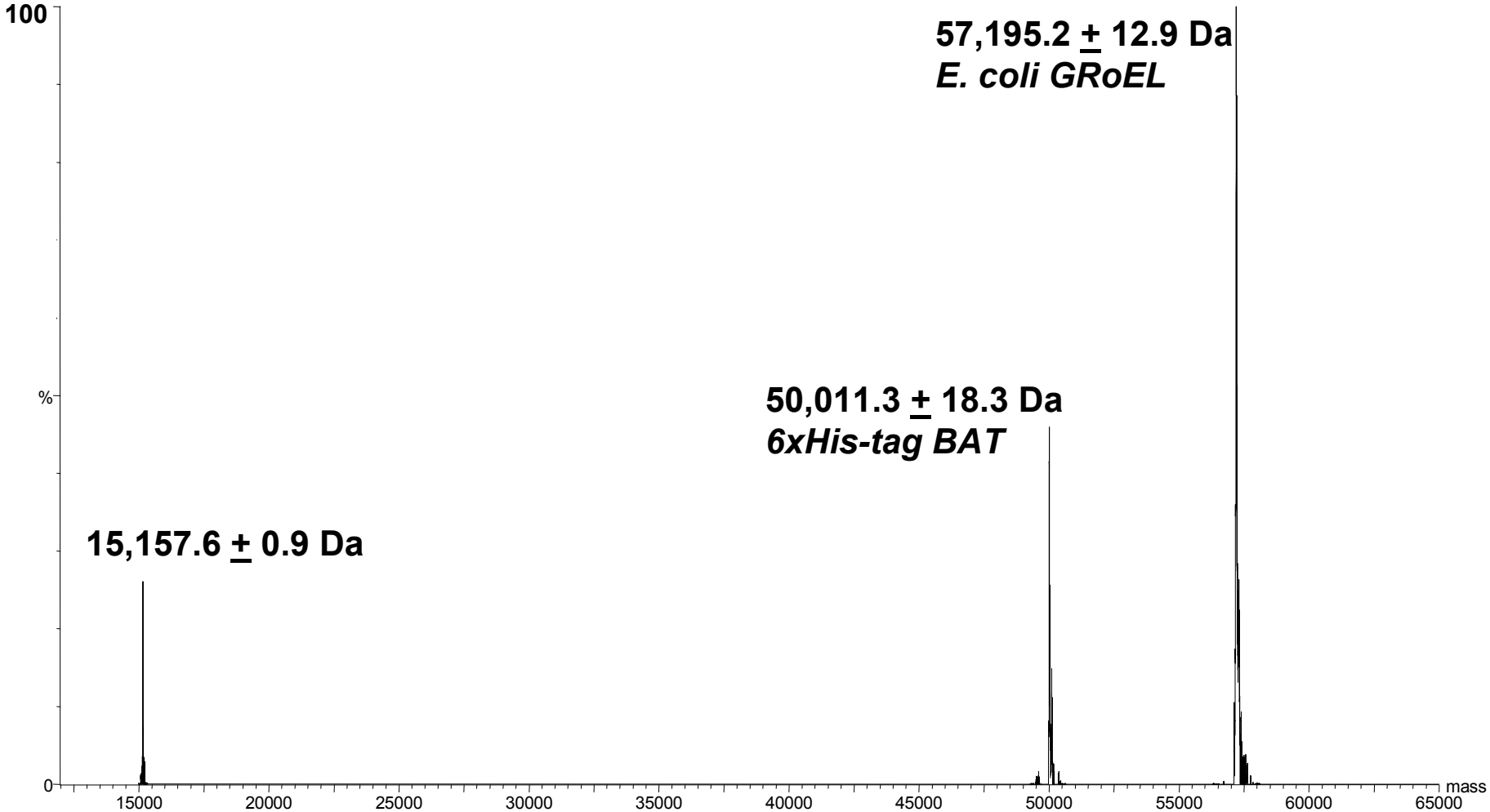
Each ion is $[M+nH]^{n+}$

For 50+ charge state of a 50 kDa protein,

***m/z* = [50,000 + 50]/50**
= 1,001

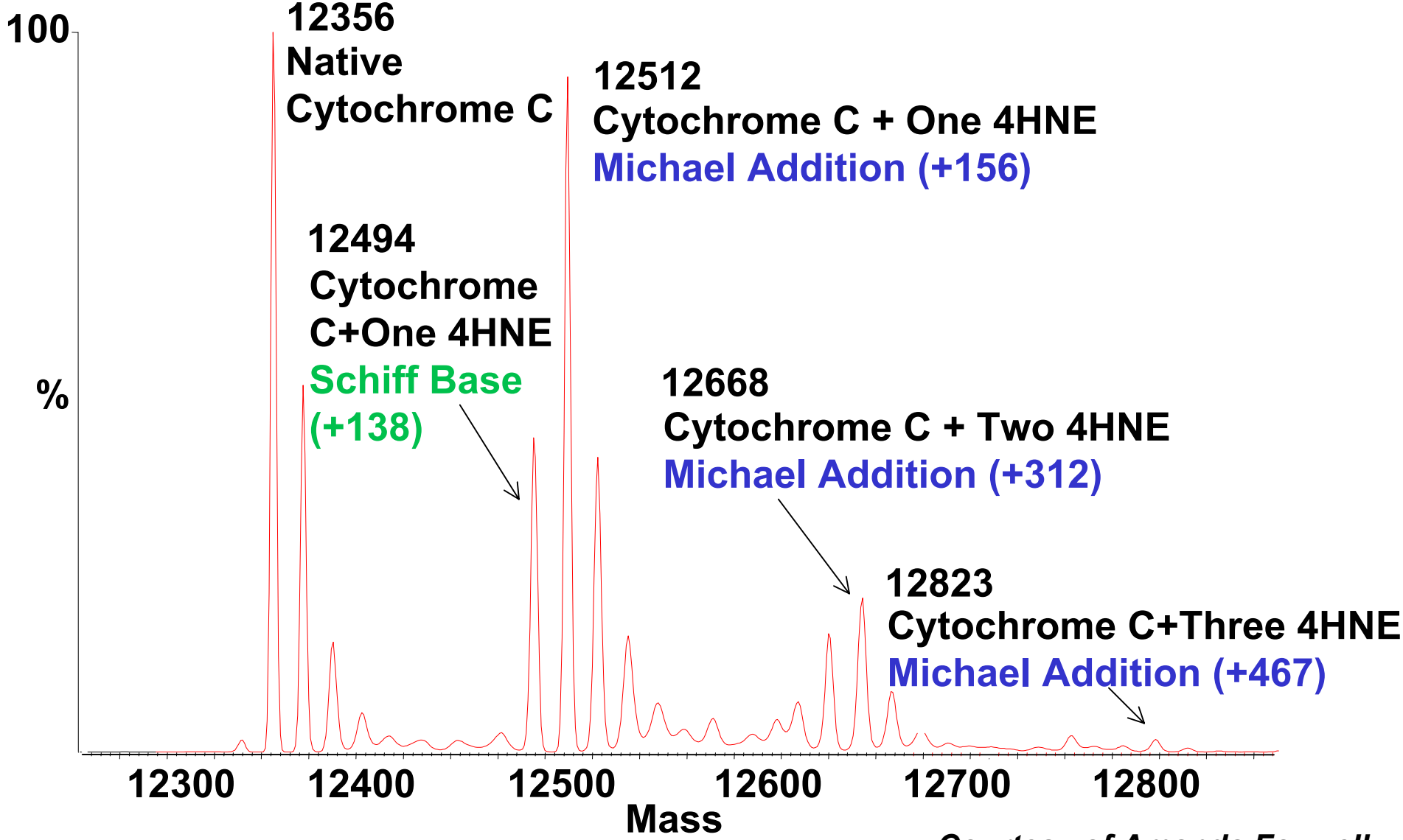
Courtesy of Mindan Sfakianos

MaxEnt deconvolution of MWs



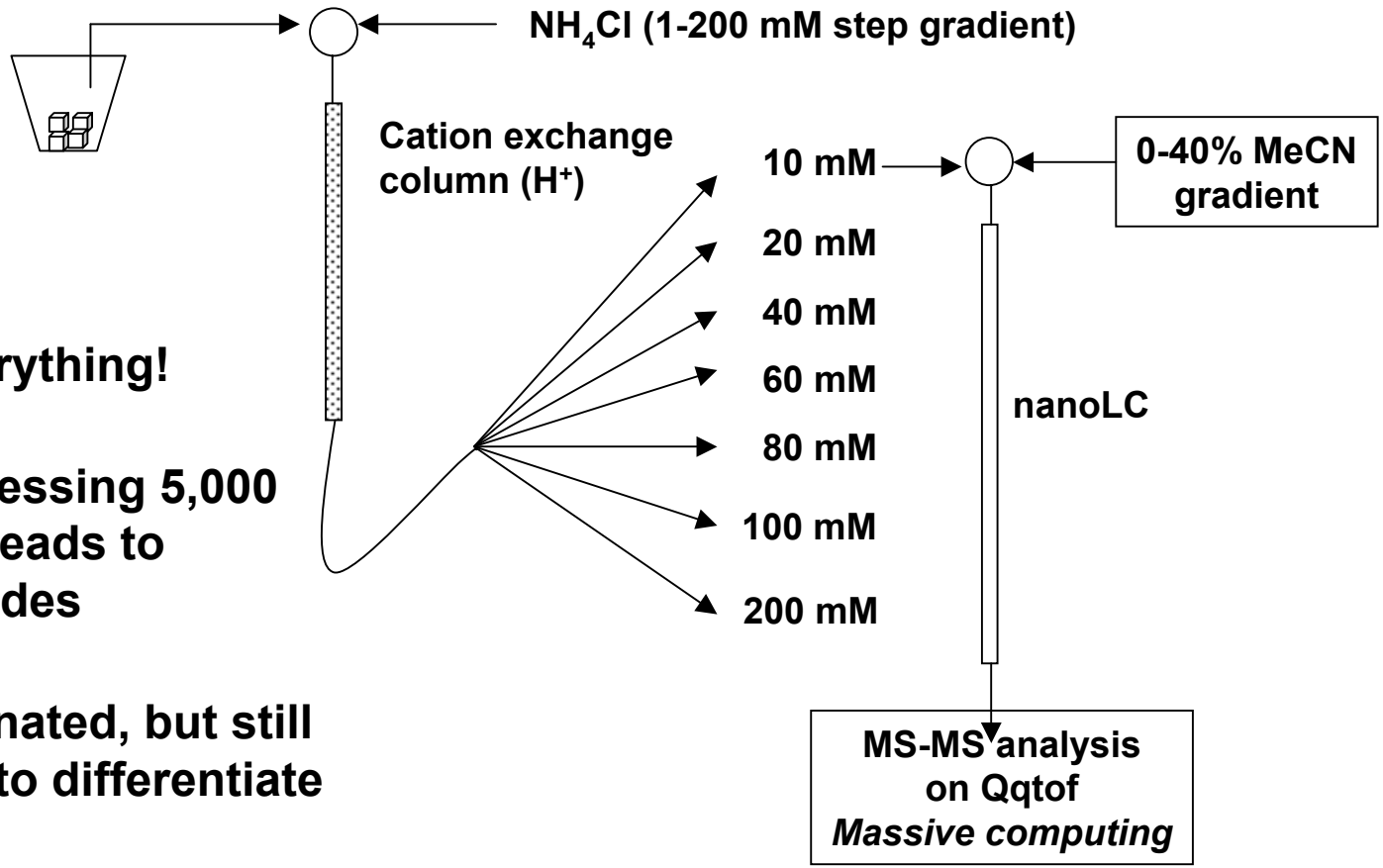
Courtesy of Mindan Sfakianos

LC/MS of 4HNE-Modified Cytochrome C



Courtesy of Amanda Foxwell

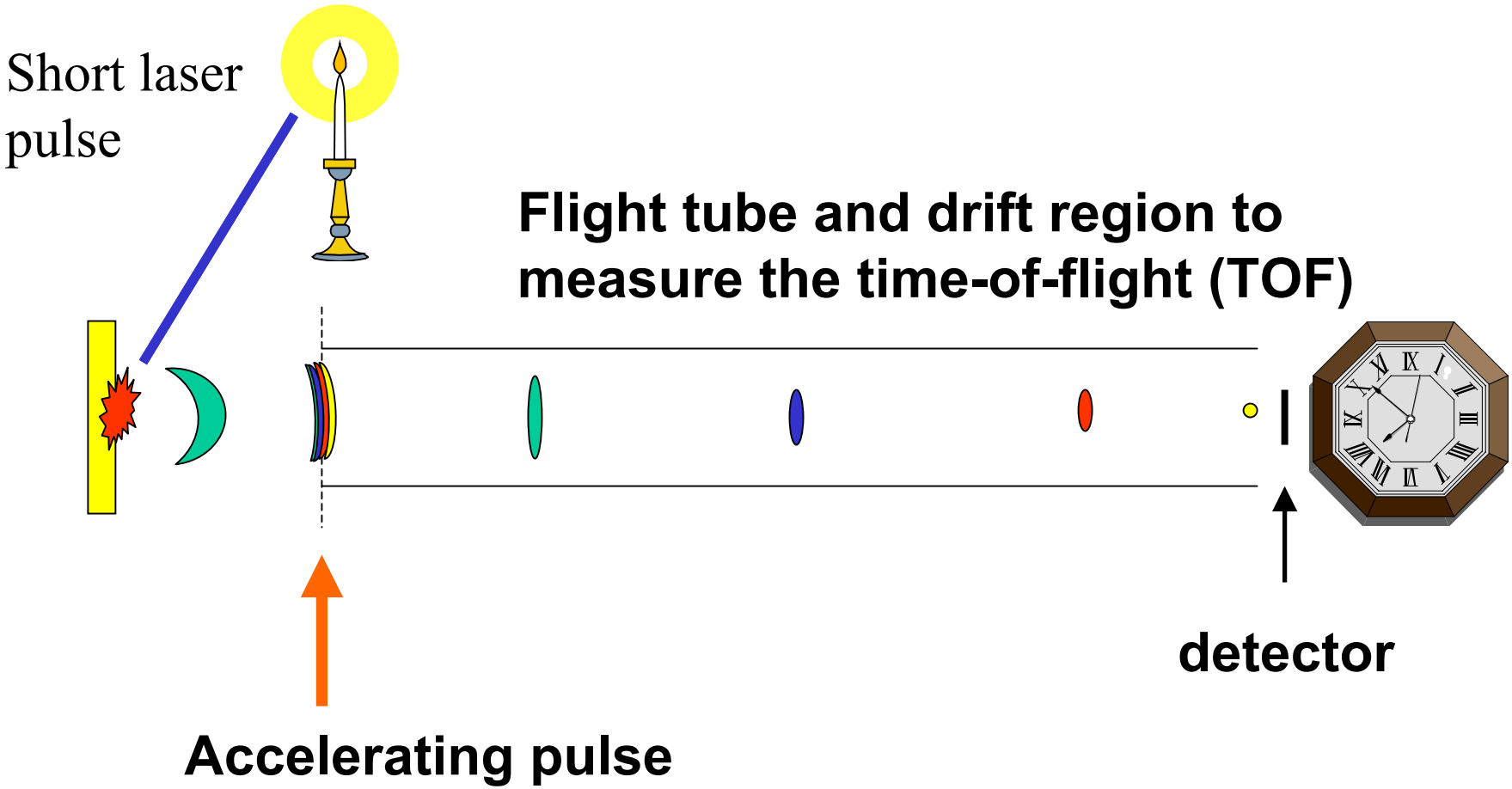
MUDPIT - MUlti-Dimensional Protein Identification Technology



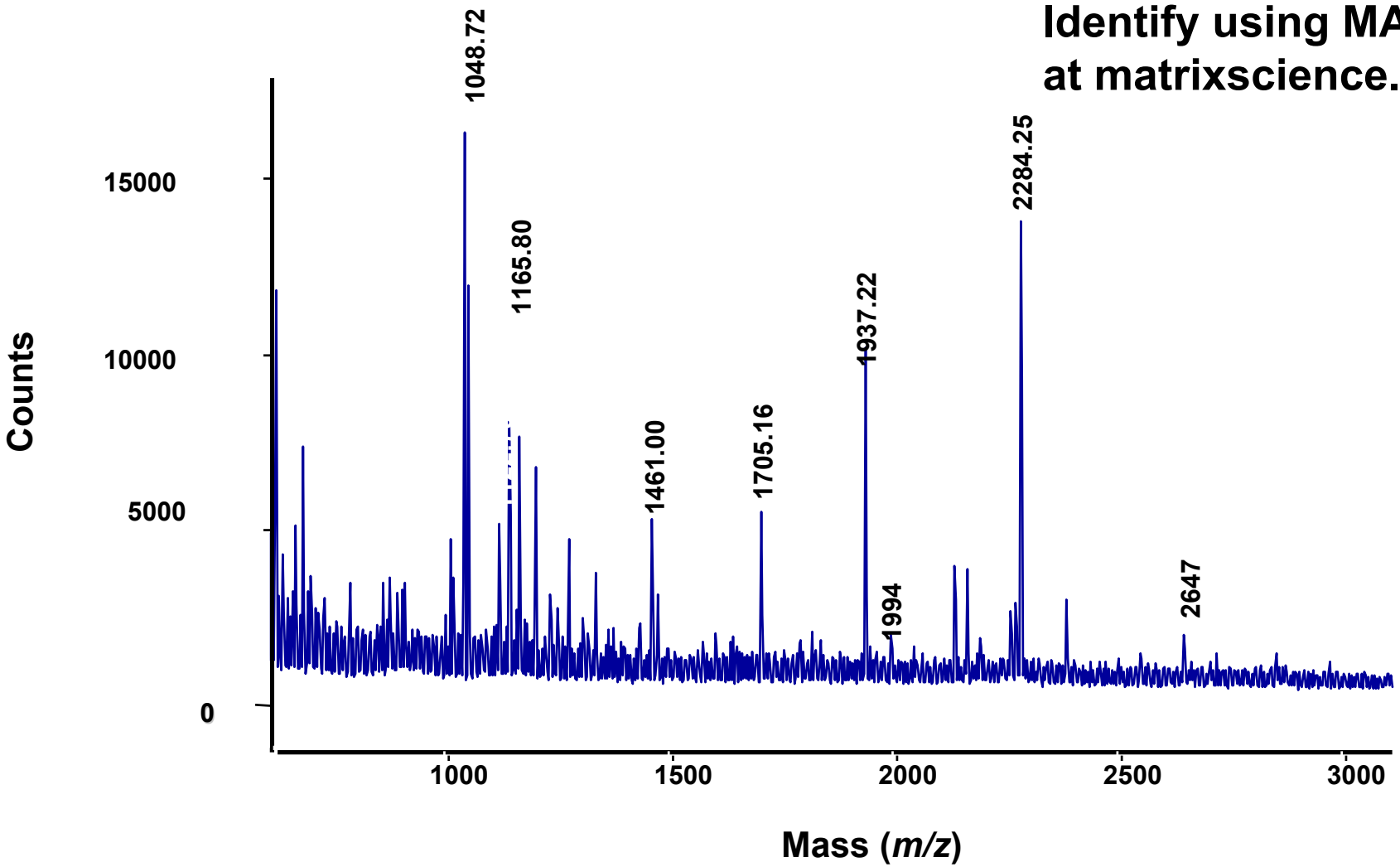
- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

John Yates

Matrix-Assisted Laser Desorption Ionization (MALDI)



MALDI-TOF spectrum of a trypsinized protein

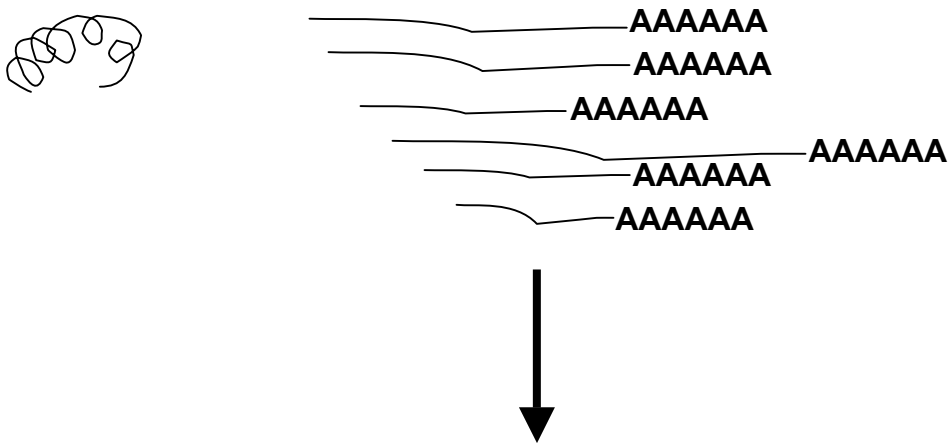


Courtesy of Mindan Sfakianos

What does the genome provide?

- Contains all the genes (and hence the proteins) that are needed throughout life
- However, only a select group of genes are expressed at any one moment
- Individual cells have an even more restricted set of expressed genes

The biochemistry of the cell



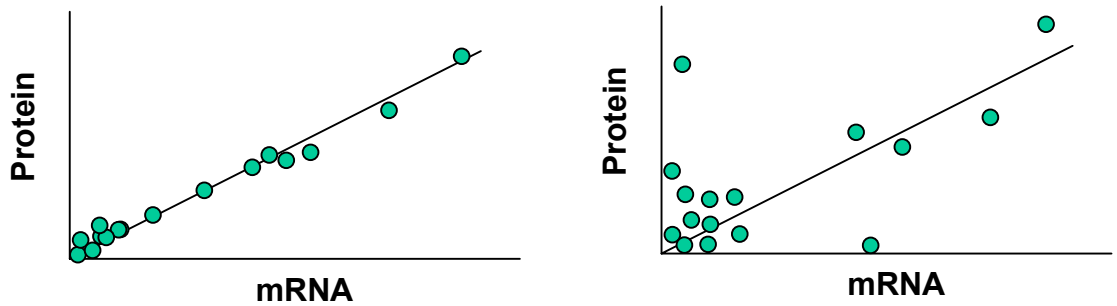
Proteins:

enzymes
structural proteins
transporters
channels
transcription factors

These are what the genes make - failure in their properties is what causes disease

The proteome versus the genome

- **They are not correlated except for high abundance proteins**



Why? The genes are used to make the proteins - they represent the dy/dt of the amount of proteins, not the y (the amount)

The cell tries to maintain a given set of protein concentrations - controlled but changing *homeostasis* or *program*

The proteome in the cancer cell

- **Inappropriately expressed genes (and hence proteins) for a cell at that stage of development**
- **Because of chromosome instability, novel mRNAs (and hence proteins)**
- **Altered posttranslational modifications (enzymatically and chemically-driven)**
- **Altered antigen processing - novel peptides**

Changes in the cancer cell proteome

- If a gene is mutated, then a protein at any level of expression is important in the context of cancer

- A cancer-causing event could be the over- or undermodification of a critical protein target

  – Enzyme catalyzed - amplified signal
  – Chemically modified - targets are the most abundant proteins

Importance of a protein modification

I = [concentration] x [modifiability]
x [susceptibility] x [biochemical impact]
x [biological impact]

The first three terms relate to how much of the protein is present, the number of modifiable groups, and the affinity for reaction with the modifying agent

The *biochemical impact* has two terms - the change in activity caused by the modification, and protein-protein interactions (complexes)

The *biological impact* depends on the importance of a modified activity or complex formation on function

How do we assess the cancer proteome?

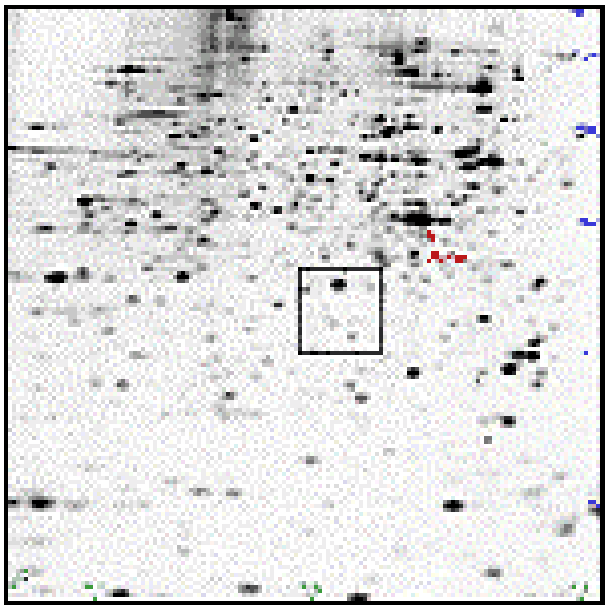
- **2D-isoelectric focusing-SDS-PAGE (with differential display - 2D-DIGE)**
- **Laser desorption ionization (LDI)-mass spectrometry**
  - ***Surface enhanced LDI (SELDI)***
  - ***Matrix assisted LDI (MALDI)***
  - ***Protein array MALDI***
- **HPLC-electrospray MS-MS (MUD-PIT)**

Separating proteins in two dimensions

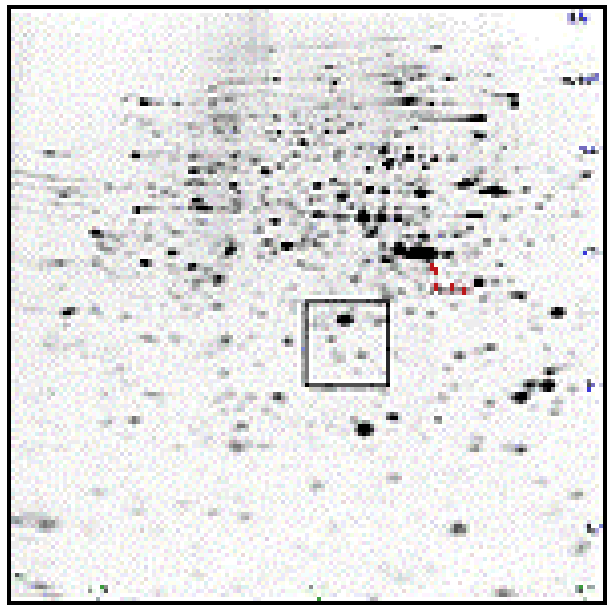
SDS-PAGE

IEF

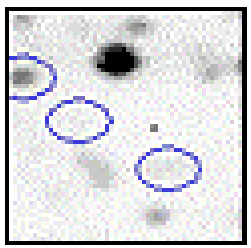
Experimental Control:
Full Image



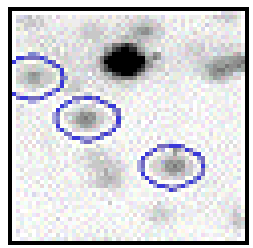
Experimental Result:
Full Image



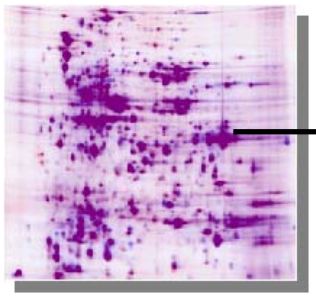
Experimental
Control:
Region Outlined



Experimental
Result:
Region Outlined



Protein analysis 2002



Eppendorf tube

destain

Speed-Vac

trypsin

1:20

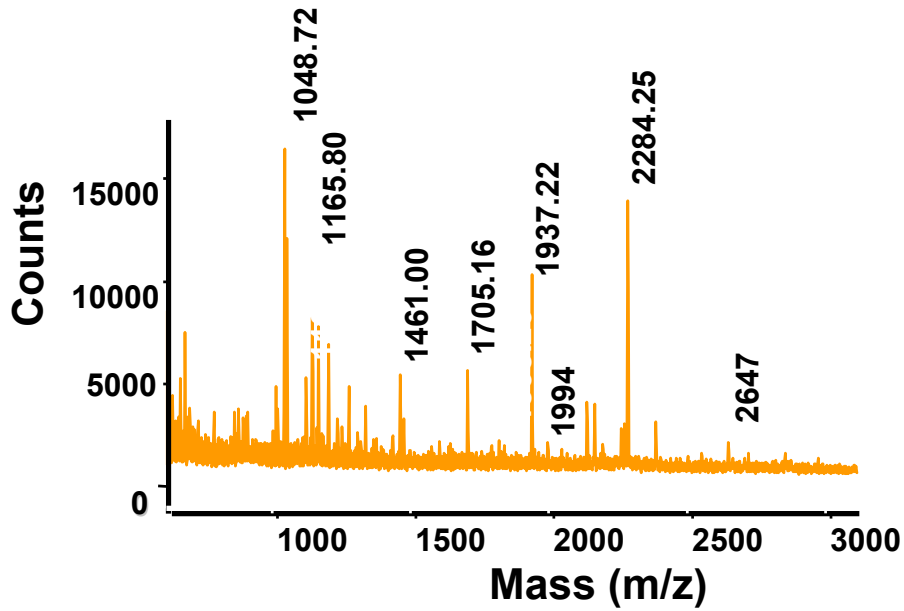
Incubate

overnight

Water Bath 37°C

MALDI plate

Desalting Ziptip



How can mass spec meet the challenges in cancer?

- Identifying the mutations in well known proteins associated with cancer, e.g., p53
- Investigating novel proteins and peptides produced by tumors
- Identifying and quantitating the posttranslation modifications of proteins
- Protein-protein complexes

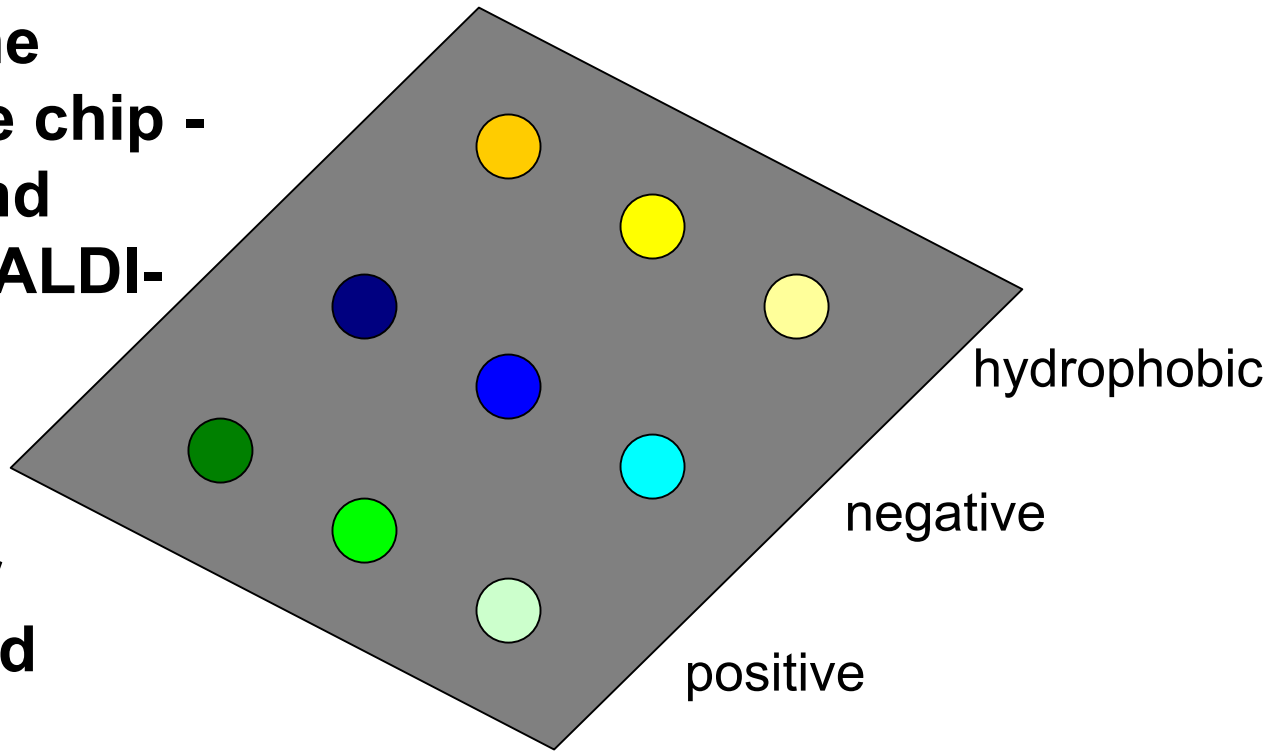
Breast cancer and mass spectrometry at ASMS 2002

- **Laser capture and MALDI reveals 100 proteins that differ between normal and tumor tissue**
- **Ductal carcinoma *in situ* - 250 proteins differentially expressed relative to normal (used Q-tof) - most independent of differences detected by DNA microarrays**
- **HER2 +ve/-ve cell lines differ with 7.4% of proteins underexpressed and 10.8% overexpressed (2D-DIGE)**

Surface enhanced laser desorption ionization (SELDI)

Selective binding of proteins to the surface of the chip - add matrix and analyze by MALDI-TOF-MS

***Future*: Ab or protein coated onto chip**

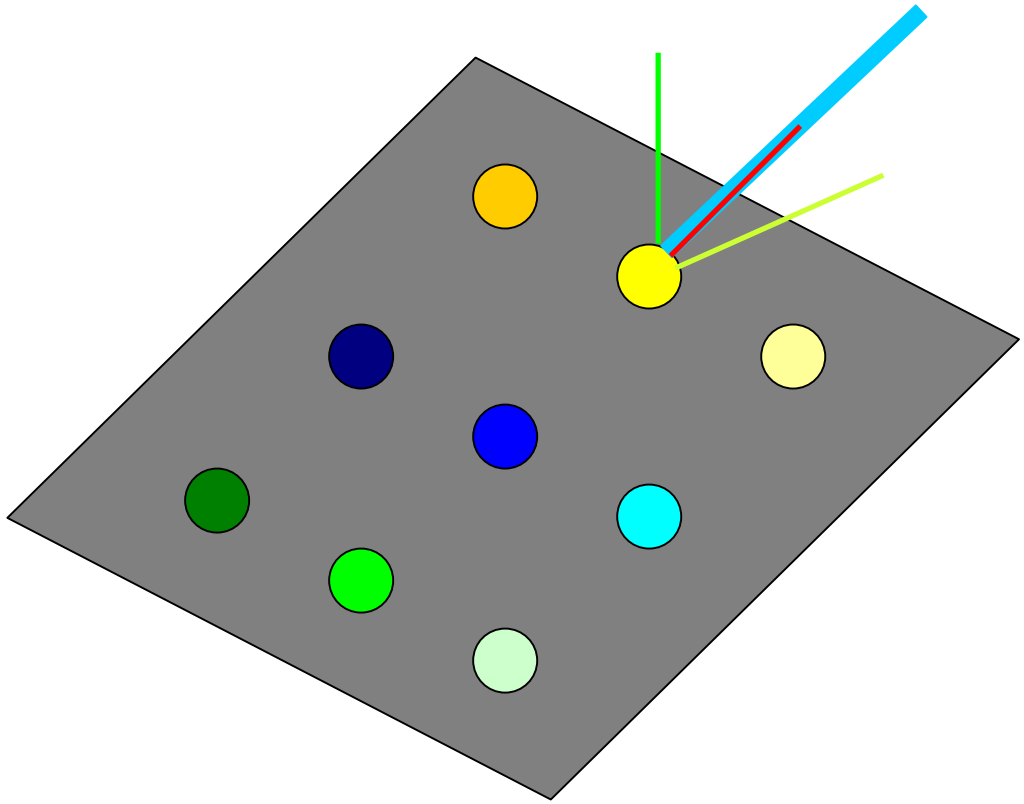


Identifying posttranslational modifications

- **Better to pull them out of the haystack**
  - ***Antiphosphotyrosine antibody affinity***
  - ***IMAC procedure (cf, recovery with Ni-column)***
  - ***β-elimination of phosphoserine and phosphothreonine with ethanedithiol - biotin***
  - ***the glycome***

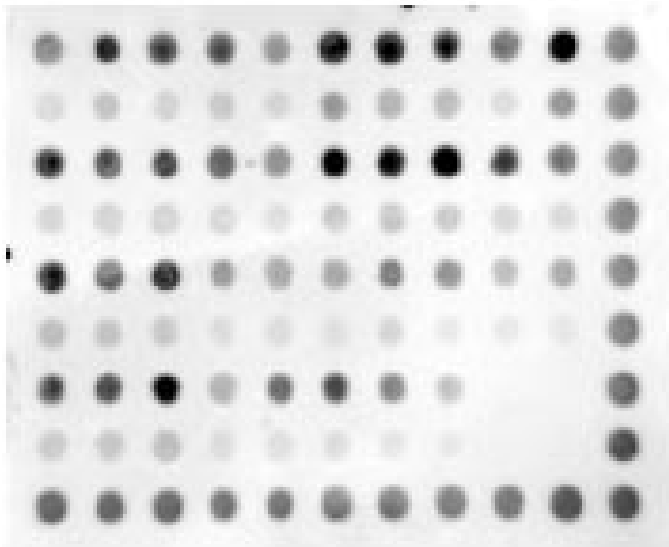
Chip affinity MALDI-MS for the glycome

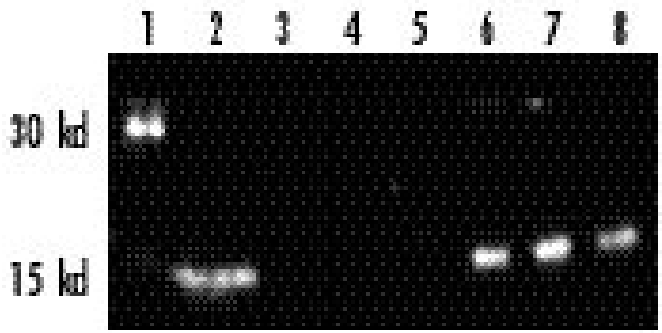
Selective binding of lipid-glycosyl groups to the surface of the MALDI plate - apply the test protein or mixture of proteins. Wash off unbound proteins and then carry out on plate trypsinolysis to identify the protein



Protein-protein binding

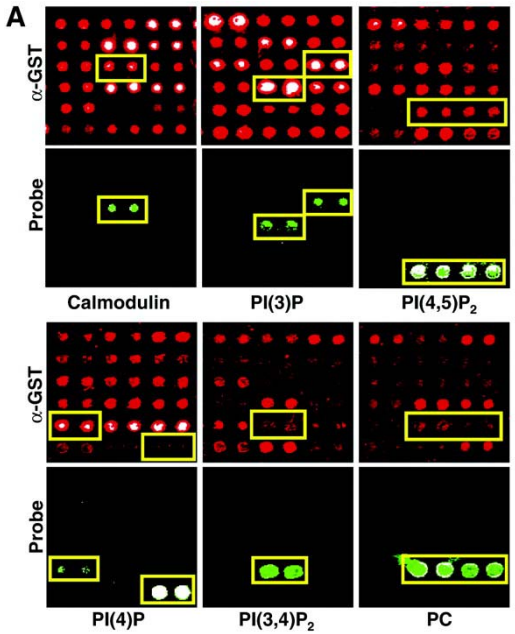
http://www.panomics.com/products/transignal_SH3/index.html



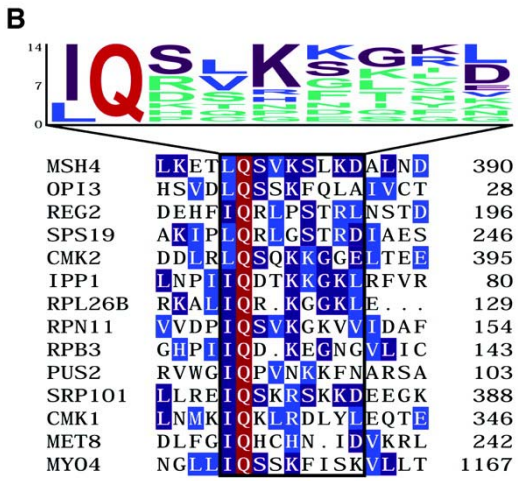


Spotted array of 39 different SH3 domains

Application of protein chip to calmodulin binding and lipid binding proteins



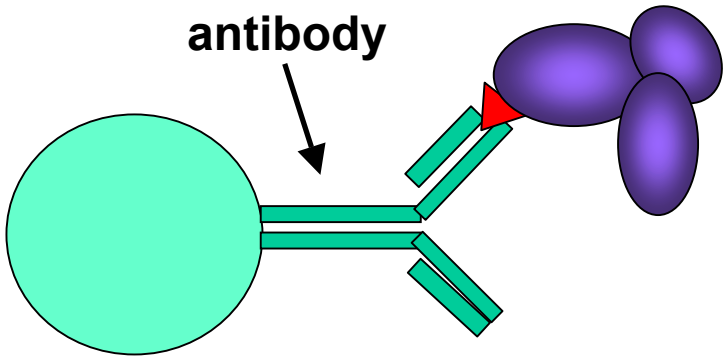
A. Positive signals in duplicate (green) are in the bottom row of each panel; the top row shows the amounts of the yeast protein preparations probed with anti-GST (red).

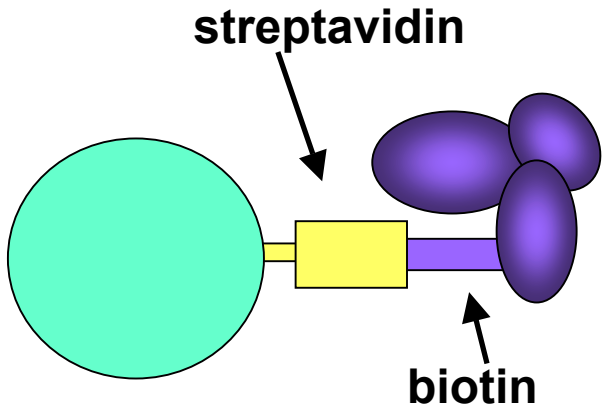


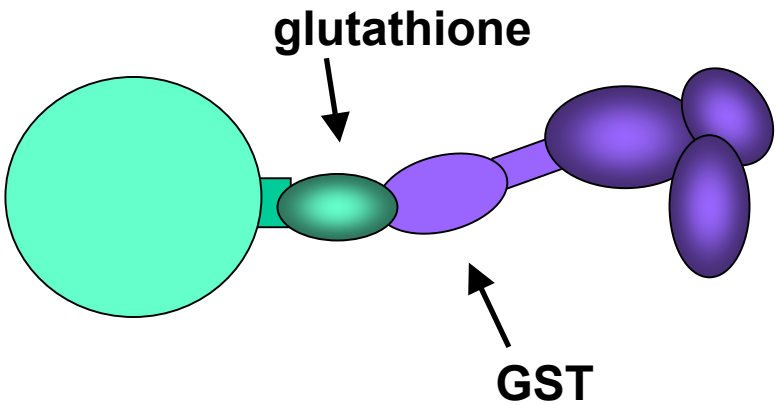
B. A putative calmodulin-binding motif. Fourteen of 39 positive proteins share a motif whose consensus is (I/L)QXK(K/X)GB, where X is any residue and B is a basic residue. *The size of the letter indicates the relative frequency of the amino acid indicated*

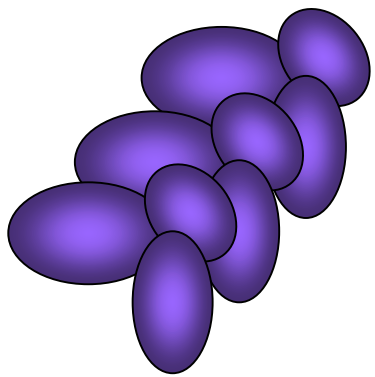
From Snyder & Zhu

Affinity methods for recovering complexes









Multiprotein complex

Importance of the microenvironment near a cancer cell and mass spec

95% of breast tumors are ringed by inflammatory cells

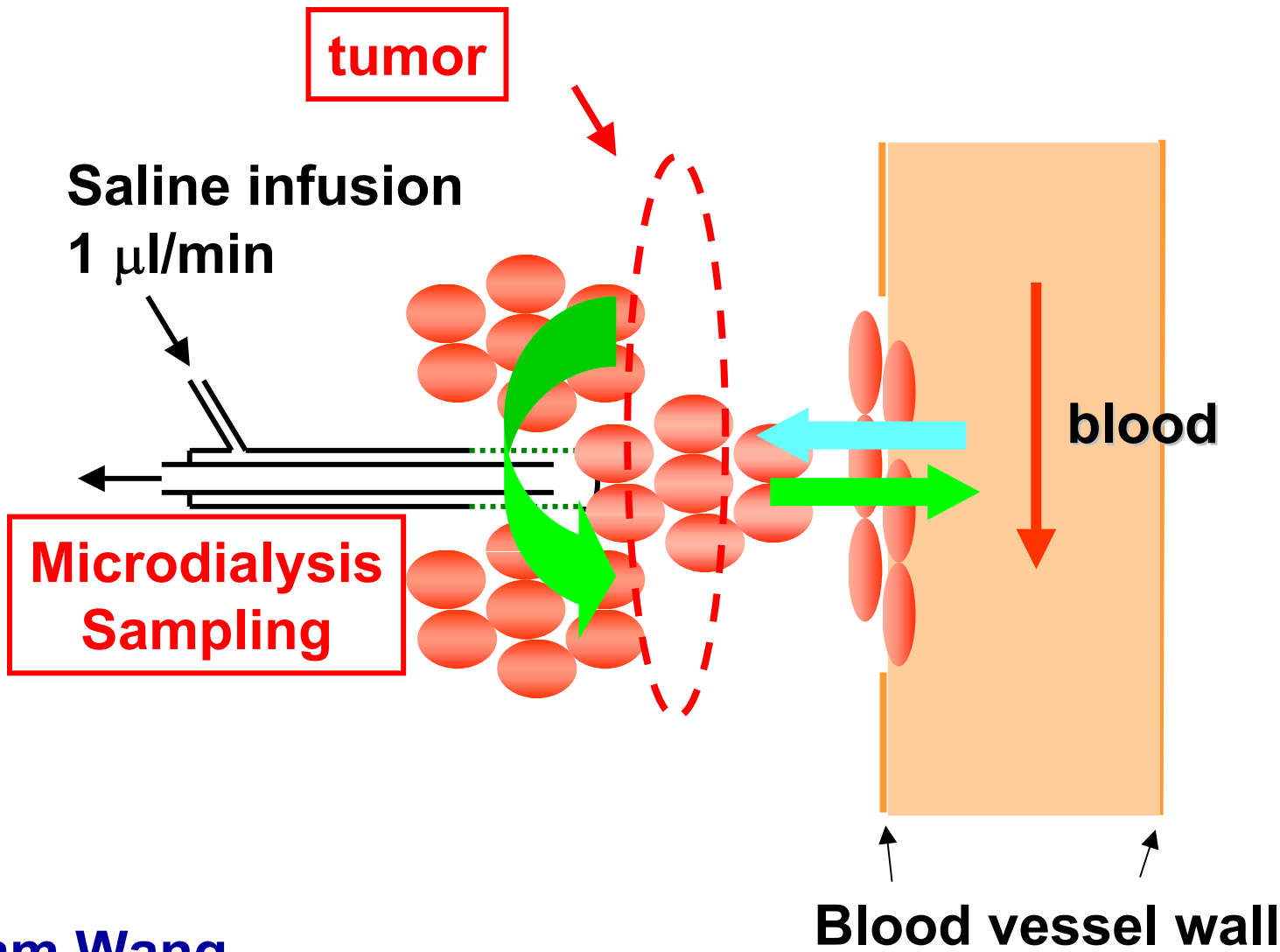
- contribution of inflammatory cytokines and ROS and RNS

An interstitial fluid (rather than blood) surrounds the tumor cells

- Invasiveness is a function of interstitial metalloproteinase activity

- what are the peptides that are found in this space? Do they have biological activity?

Interstitial fluid sampling from tumors

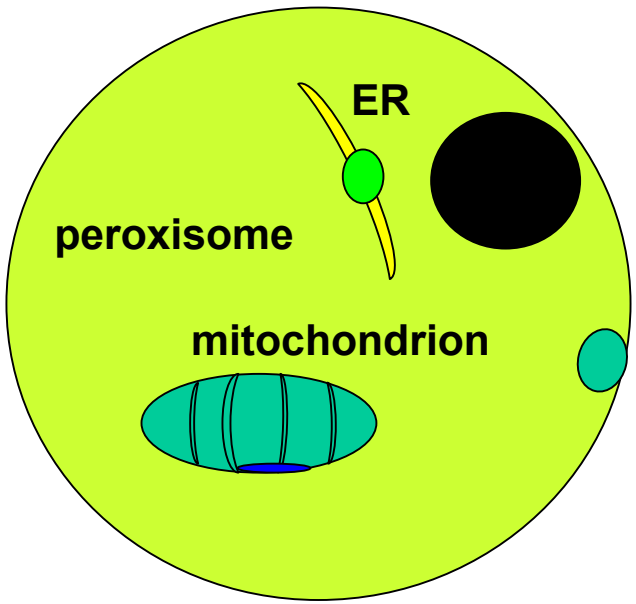


Sam Wang

Proteins are different from mRNAs

It's all about location, location

For mRNA it doesn't matter.
But where a protein is is everything



Summary of talk

- **Proteomics has become a mature and robust science**
- **Studying a single gene or protein isn't enough these days - you need to know its place in the cell network**
- **The technology to study proteins and protein complexes in cancer is ready for exploitation**

New areas in mass spectrometry

- **High speed analysis with TOF-TOF instruments - thousands of MS and MS-MS (i.e., sequencing) experiments per hour *(planned experiments with Tim Townes to identify transcription factor complexes)***

- **Ultimate resolution with FT-ICR-MS *(concept of top-down sequencing of whole proteins)***

- **Cell-by-cell identification of protein distribution *(currently at 1 μm resolution)***

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