Purdue/UAB Botanicals Center for Age-related Diseases

Proteomics: its role in botanicals research

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Learning objectives

- What is proteomics?
- The rationale for proteomics-type approaches in botanicals research
- Qualitatively different proteomics methodologies:
  - What each is about
  - Which might be more useful for botanicals research
- 2-D electrophoresis proteomics:
  - What it involves: 2D separation, image analysis, MS
  - Some examples done here at UAB

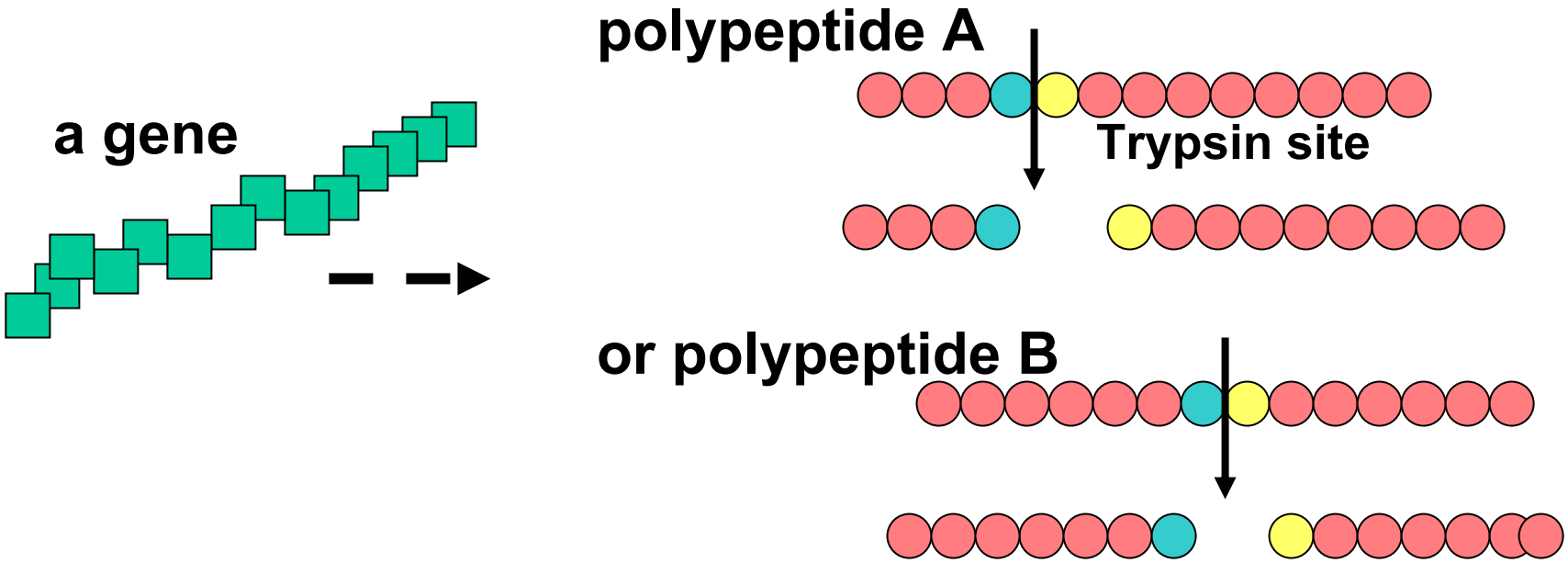
What is proteomics?

- **Genomics: study of genomes of a cell or organism**
- **Proteomics:**
  - **Original definition: study of the proteins encoded by the genome of a biological sample**
  - **Current definition: study of protein complement of a biological sample (cell, tissue, animal, biological fluid [urine, serum])**
  - **Usually involves high resolution of polypeptides at front-end, followed by mass spectrometry identification and analysis**

Multiple roles of proteomics technology in botanicals research

- Identify potential proteins involved in a botanical's effects in target tissues;
- Monitor the purification of a target protein;
- Characterize protein differences between disease and normal tissues--
  - does a botanical prevent these differences
- Assist in determining component of botanical that may have activity, by monitoring the proteins affected.

What are these tryptic mass "fingerprints," and why are they important?

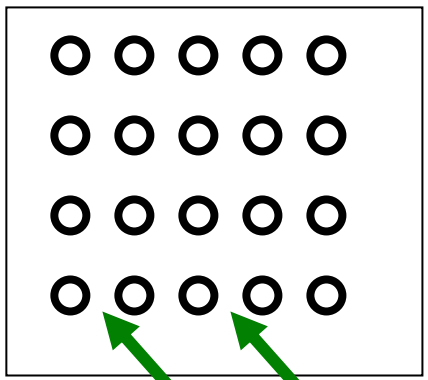


So, polypeptides of identical mass can have nonidentical tryptic "fingerprints."

If “regular” protein gel MS works great, why do we need “proteomics”?

- When you don’t have a clue; (see next page)
- When you have only a very small clue; (see next page)
- When you knock out a gene (and hence a protein) that you’re convinced is essential for life, and the animal pees as usual.

Gene microarray analysis: the good, the bad, and the usually ugly

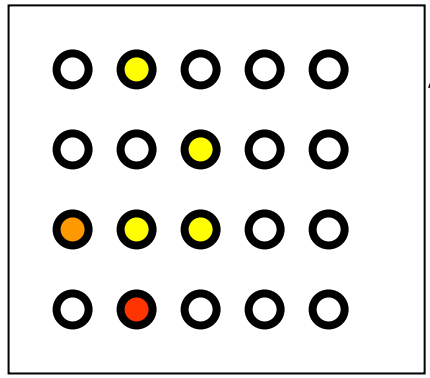


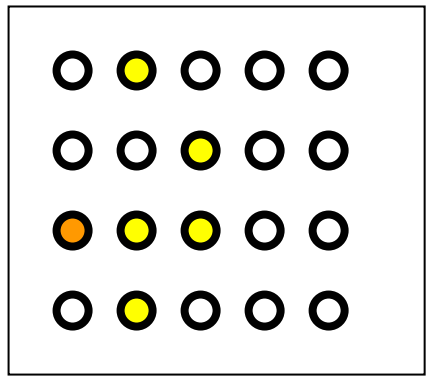
Dots of cDNA's

A gene "chip" or microarray

mRNA's, then
fluorescent probe for RNA's

the good: high-throughput





Analysis

The bad: list of LOTS of
gene accession numbers

The UGLY: still don't know for sure
what proteins are involved, or what's going on

"Potential" proteins
Involved in the disease

Introduction to high resolution protein separation in proteomics

- Types of high-resolution protein separations
- Advantages and limitations of the types
- How combinations of other protein separations or enrichments can enhance proteomics methods

Types of Proteomics Technologies

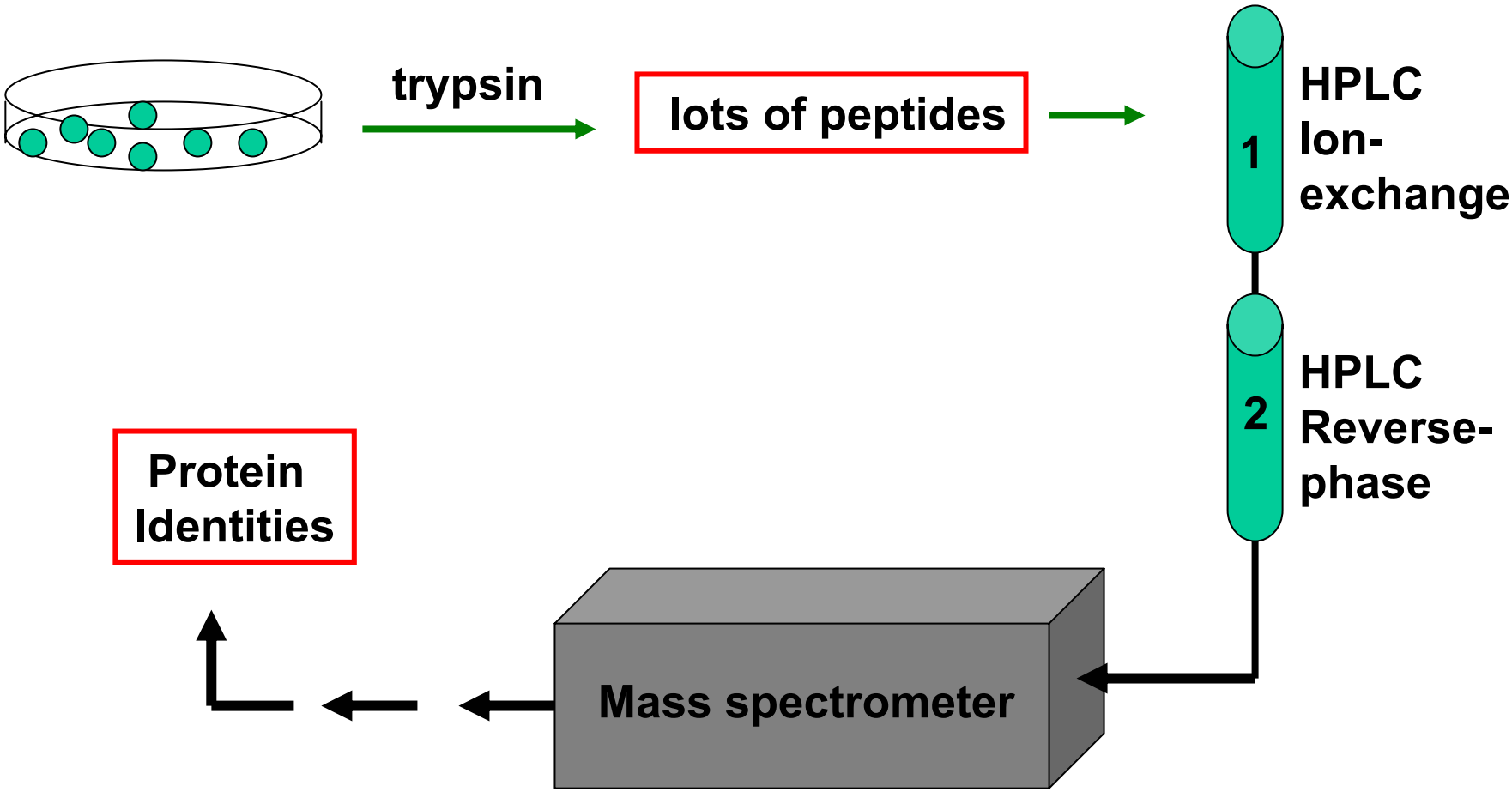
I. 2-dimensional electrophoresis (2-DE) & mass spectrometry

II. Liquid chromatography & mass spectrometry (LC-MS or LC/LC MS/MS)

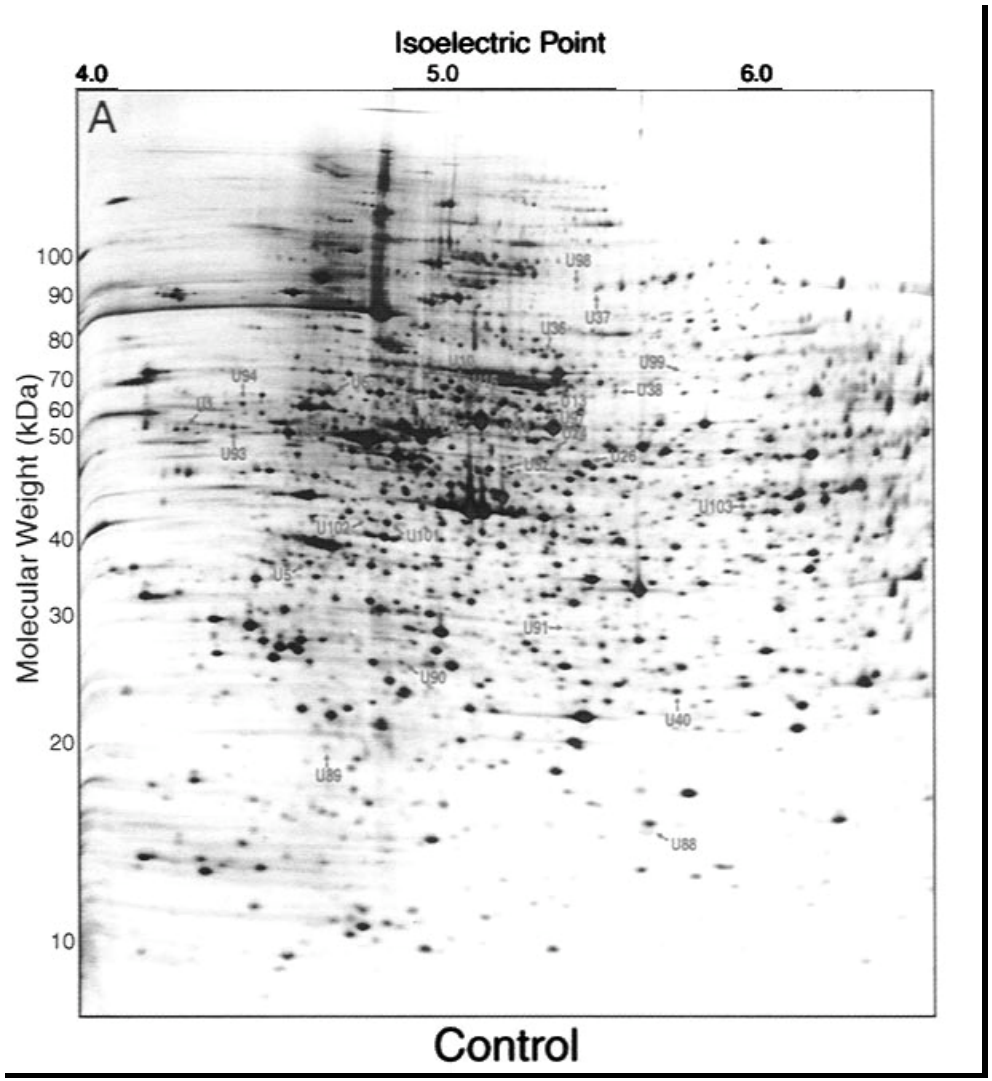
III. "Chip" technology:

2-D array of recombinant polypeptides or antibodies on a single microscope slide; the entire chip is probed with a labelled "ligand" (protein, lipid, drug)

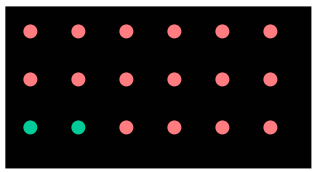
MultiDimensional Protein Identification Technology



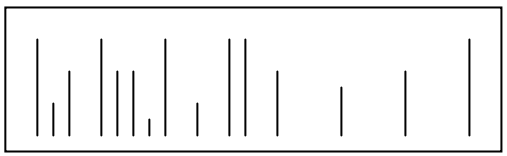
2-dimensional electrophoresis: separation of polypeptides according to two different parameters



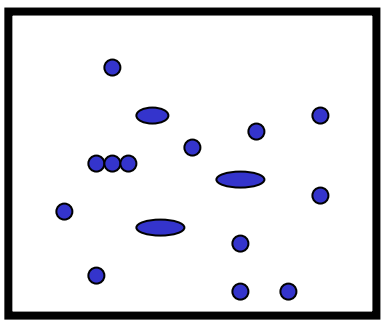
When to use which technology?



Chip: To identify all possible targets of a drug, or ligand, or protein; HIGH THROUGHPUT



LC/LC MS/MS: To catalogue a new Proteome; HIGH THROUGHPUT



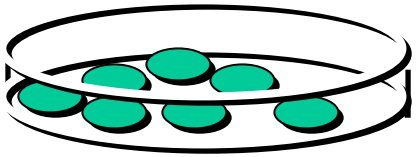
2-D gels (& MS):

- **The only method that separates intact proteins;**
- **The only method that shows the relative levels of expression of proteins;**
- **The only method that readily indicates posttranslational modifications;**
- ***Least high throughput, but most informative?***

The Elements of 2-DE Proteomics

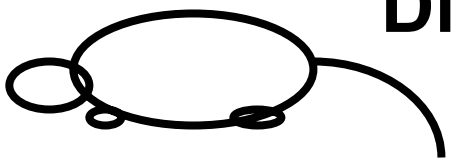
- Sample preparation
- Fractionation to enrich for suspected proteins?
- 2-DE
- Image analysis to identify gel “spot” differences between untreated & treated
- MALDI-TOF MS of trypsin-digest of spots of interest, to identify and characterize the protein

Biological sample preparation:



Harvest, rinse, and pellet the cells;

or



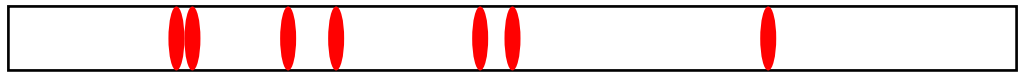
Dissect out tissue, organ, or fluids;

- Homogenize/lyse in buffer that unfolds the proteins w/o adding or disturbing the charges:
  - High urea usually 5-8 M
  - Sometimes 2 M thiourea
  - 1-4% CHAPS or other detergent
  - Beta-mercaptoethanol or other reductant, such as TBP
  - Inhibitors: of proteases, kinases, & phosphatases
- Clarify by centrifugation to get rid of crud or gorp;
- Protein assay;

What 2-D electrophoresis involves:

- 1st dimension: **Isoelectric focussing**

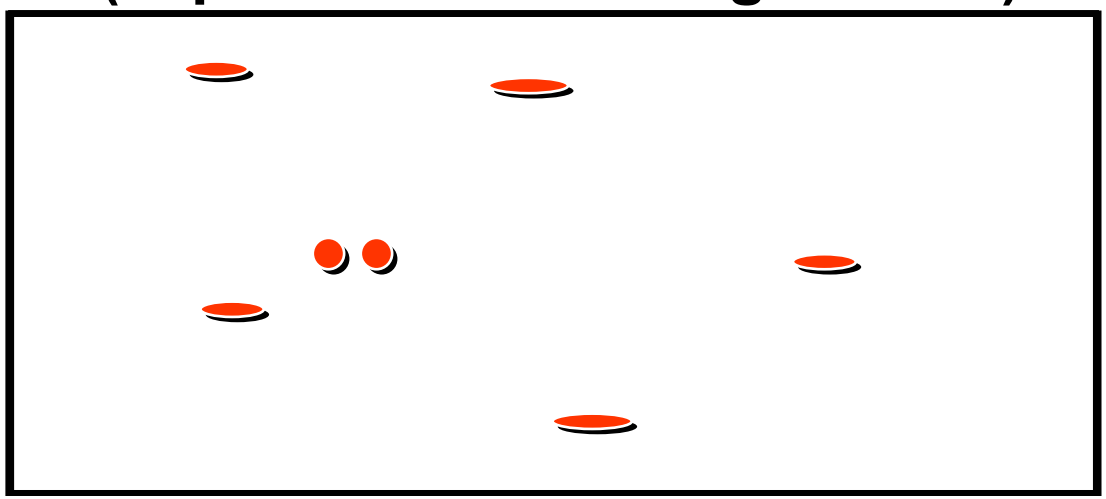
(separation according to charge)



pH 3 **pH 10**

- 2nd dimension: **(SDS)-PAGE**

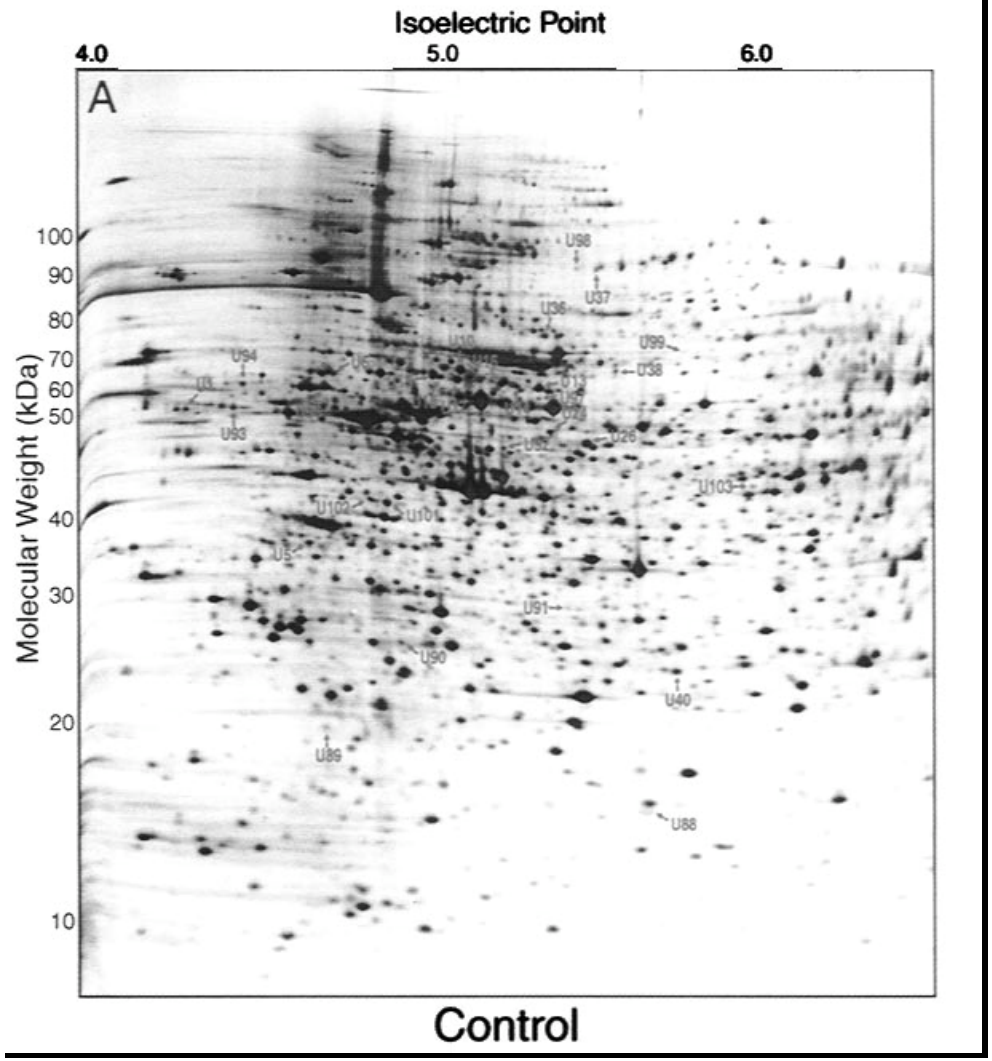
(separation according to size)



High m.w.

Low m.w.

A real 2-D gel



Find this and other 2-D gels at http:www.expasy.org

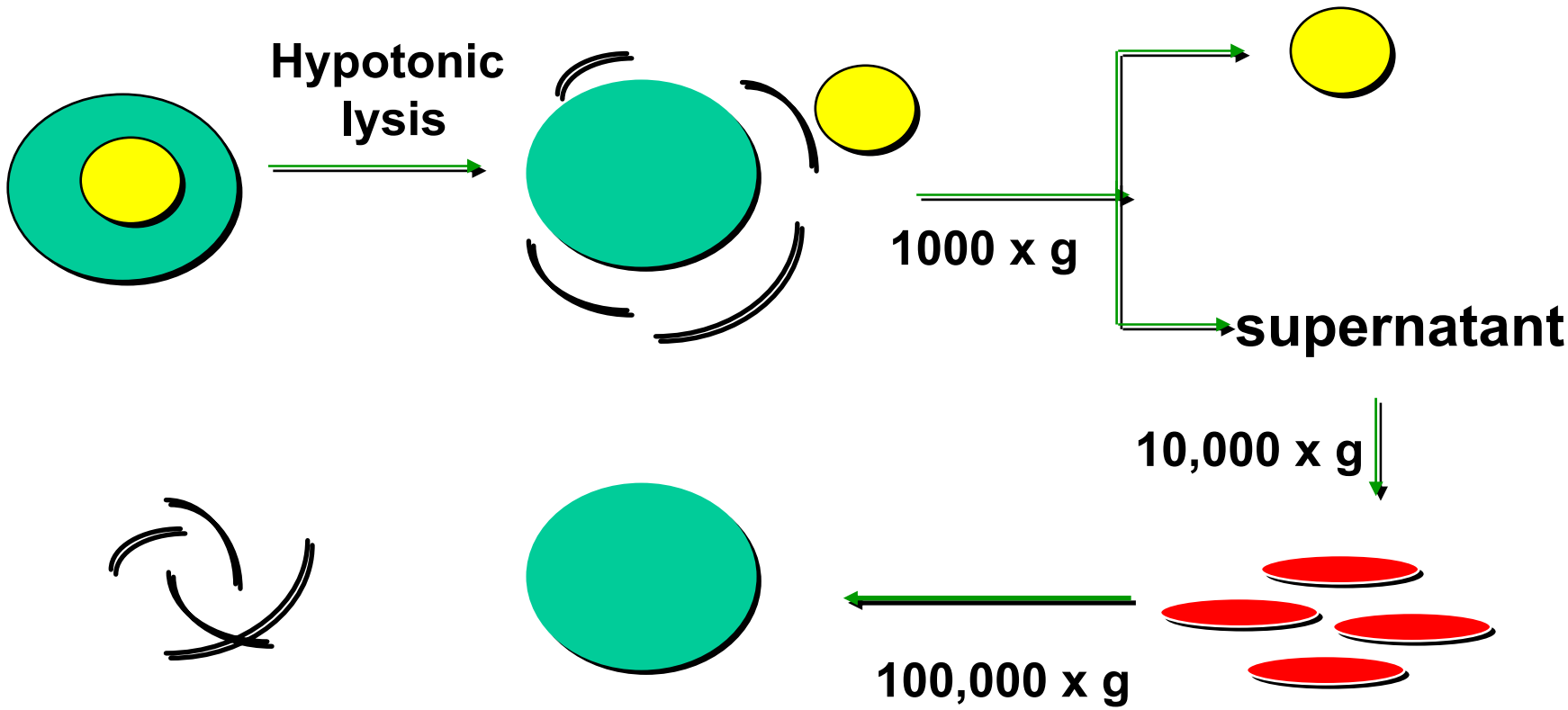
Lewis et al., [2000] Molec. Cell, 6)

(from Natalie Ahn's lab)

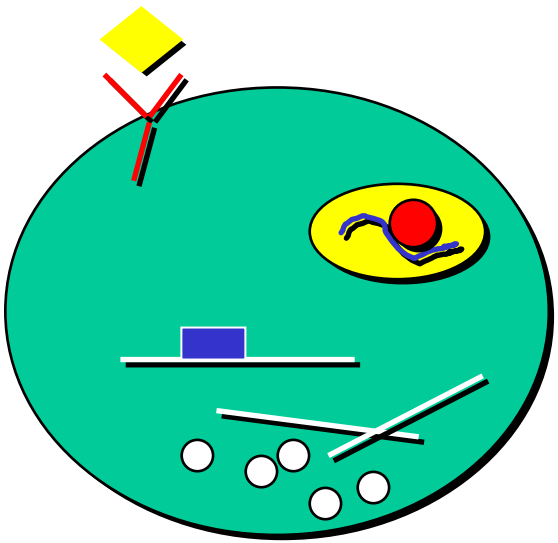
From this set of gels, 25 novel phosphorylations were identified as part of the MAP-kinase signalling pathway.

SMART proteomics, part I: Reduce the protein complexity of biological samples.

Fractionate, fractionate, fractionate

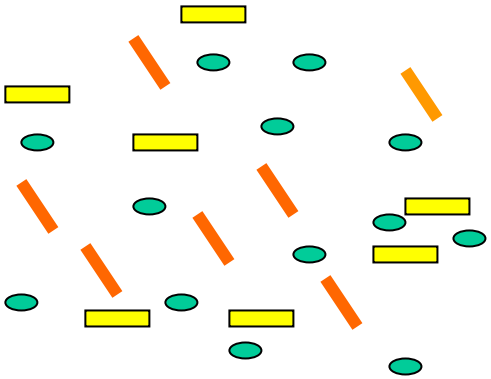


SMART proteomics, part II:
"know" your system.

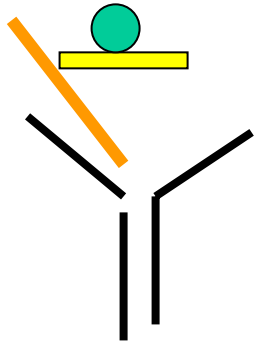


- Interactions?
- Protein location?
- Different assembly states?
- Nature of modifications?
- How are others studying the system?
  - how are your competitors studying the system??

Incorporate nearest neighbor analysis:



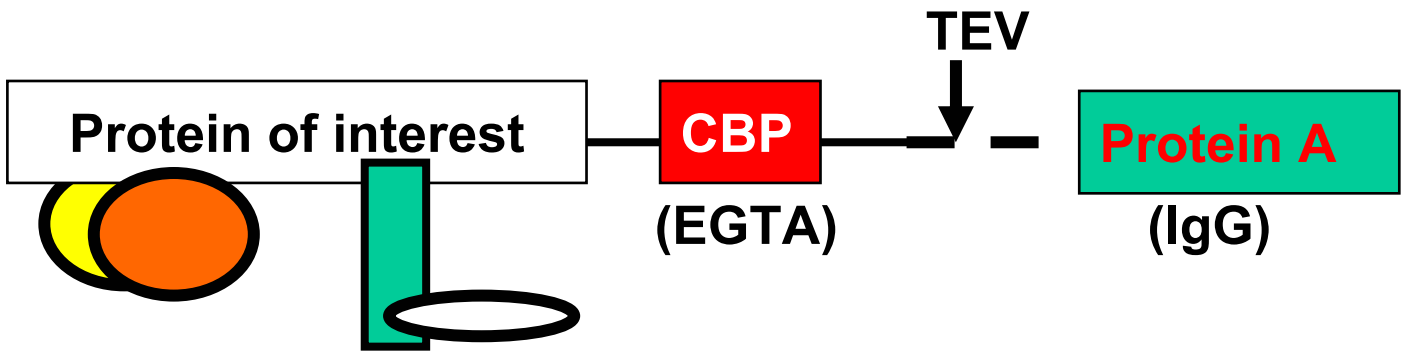
A whole cell lysate: 6,589 polypeptides floating around



An immune complex of 3 proteins:

Which would you rather run on a 2-D gel

Tandem-affinity purification: the newest neighbor analysis:



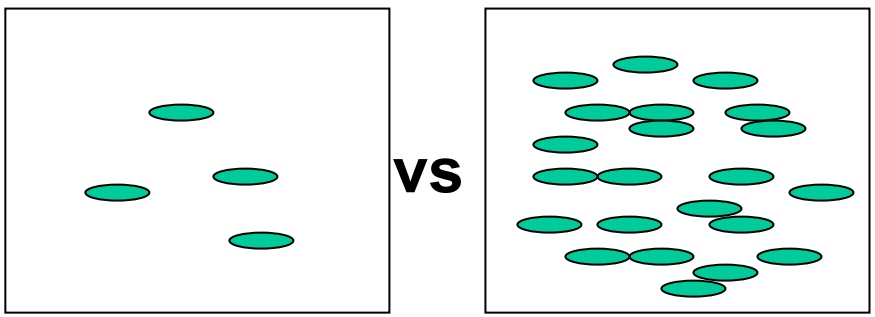
Use molecular biology to enhance proteomics analysis

Use one of the following:

- **GST-fusion protein**
- **HA-tagged protein**
- **FLAG-tagged protein**
- **Histidine-tagged protein**

+ Cell/tissue homogenate

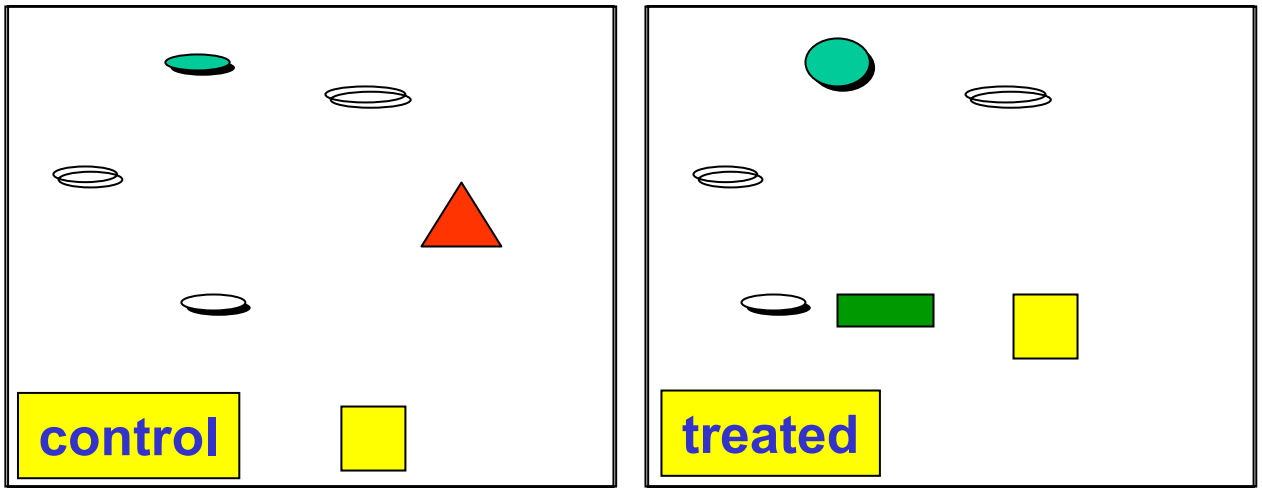
+ Antibody to recombinant protein



vs

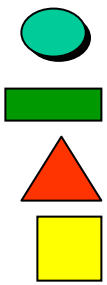
“Immune complex,” enriched for your protein 10,000 -fold

Critical part of 2-D gel proteomics: Image analysis



Either manually or with software: "compare" the the images.

Types of information:



------ Upregulation of gene

------ Posttranslational modificatiion

------ Downregulation of gene

------ Aberrant processing

Image analysis: part II

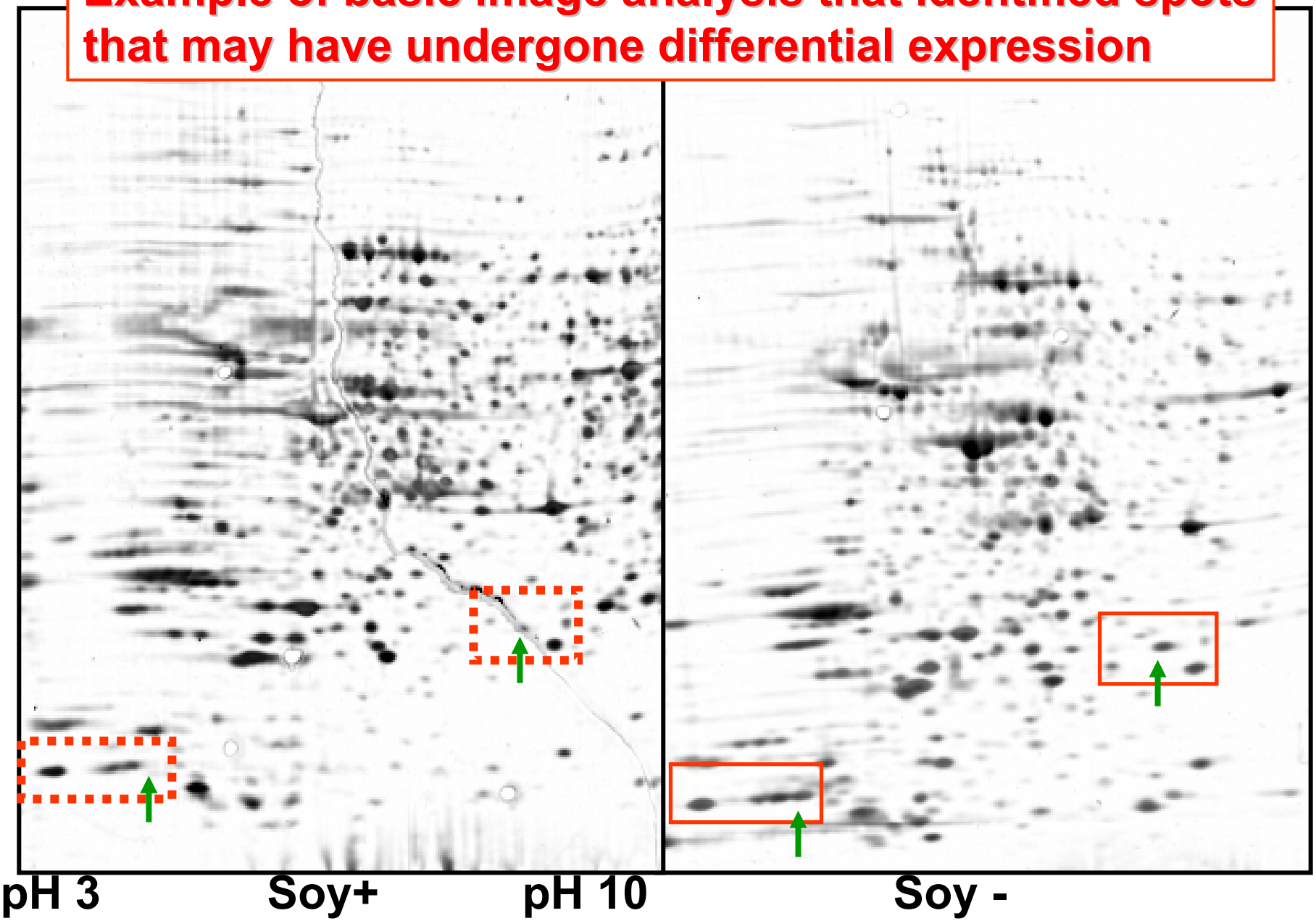
several software packages available;
none perfect;
we use PDQuest (BioRad) (thus far)

Elements of basic image analysis (will be demonstrated)

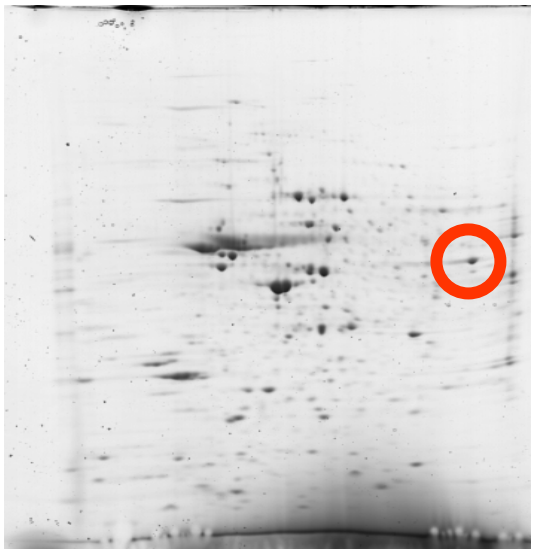
1. Superimpose the 2D displays of spots
2. Determine total spot number for each display
3. Quantify spot intensities
4. Identify spots that may have " moved" horizontally; these

are candidates for those may have alterations in charge.

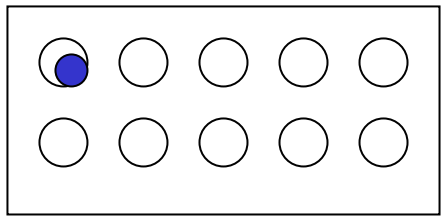
Example of basic image analysis that identified spots that may have undergone differential expression



Protein identification by MALDI-TOF mass spectrometry:



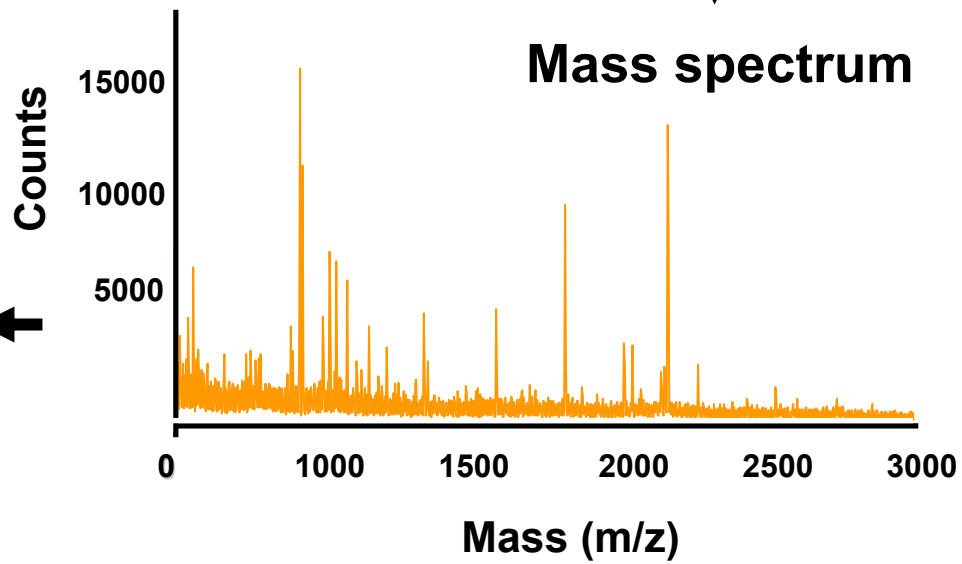
In-gel digestion with trypsin



96-well plate

MALDI-TOF mass spectrometry

Mass spectrum



Www.matrixscience.com

How to predict or interpret 2-D gel data:

I. Existing databases and web-links:

www.expasy.org

more 2D gels than you thought possible

helpful links:

proteomics tools

II. Keep up with the literature:

Electrophoresis

Proteomics

Molecular & Cellular Proteomics

III. Use genomics information:

The gene accession # can predict electrophoretic paramters-- m.w. & pI; can help in setting up 2D gel conditions

Take home messages

- **Proteomics technologies can play major role in analysis of targets of botanicals;**
- **2D electrophoresis can indicate both differential expression or posttranslations effected by botanicals;**
- **Enrichment for a suspected protein enhances proteomics analysis by reducing the complexity of the polypeptide pattern;**
- **Molecular biology techniques can enhance proteomics analysis by increasing the specificity of the polypeptide mixture**