PROTEIN-PROTEIN INTERACTIONS

Guru Rao
BBMB
April 17, 2008

We will try to cover……

- What is
- Why pro
- Example
- Commo
- High thr
- 2D gels

nt

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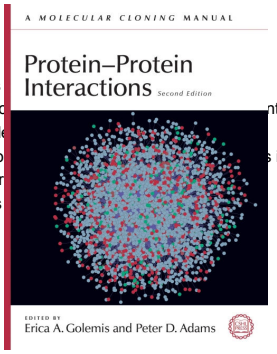

PROTEOME

A set of *PROT*eins encoded bya gen*OME* (Marc Wilkins and Keith Williams, Macquarie University, Sydney, Australia in 1995)

What is 'Proteomics'?

"Proteomics includes not only the identification and quantification of proteins, but also the determination of their localization, modifications, interactions, activities, and, ultimately, their function."

-Stan Fields in *Science*, 2001.

The cell can be thought of as a metropolitan city……

- Each person **(GENE)** contributes to making the city **(CELL)** thrive and function
- We then identify the names of all the people **(SEQUENCING)** and their occupation **(PROTEIN FUNCTION)**
- We then find out where they **work (CELLULAR LOCALIZATION)** and who they interact with in their workplace **(other proteins, nucleic acids, phospholipids, carbohydrates)**

Provart & McCourt, 2004

The genomes of many organisms have been sequenced…

Organism	Genome size (Mb)	(# of genes)
Escherichia coli	4.72	4,377
Saccharomyces cerevesiae	12.5	5,885
C. elegans	97.0	19.099
Arabidopsis thaliana	120	~20,000
Homo sapiens	~2900	~25,000?

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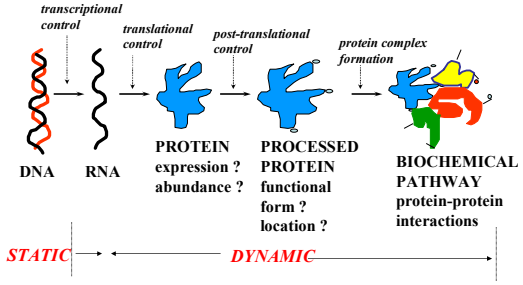
DNA makes RNA makes PROTEIN



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There is no 1:1 correspondence between number of genes and proteins

- The ~25,000 genes of the human genome are speculated to give rise to $1x10^6$ proteins through a series of post-translational modifications and gene splicing mechanisms.
- Although a population of these proteins can be expected to work in relative isolation, **the majority are expected to operate in concert with other proteins in complexes and networks** to orchestrate the myriad of processes that impact cellular structure and function.
- These processes include cell cycle control, differentiation, protein folding, signaling, transcription, translation, post-translational modification and transport.

POST-GENOMICS CHALLENGE

Understanding protein function

TYPES OF FUNCTION

PHENOTYPIC FUNCTION
- *physiology*
- *morphology*

⟷ *ENVIRONMENT*

CELLULAR FUNCTION
- *signal transduction*
- *metabolic pathway*

⟷ *CELLULAR LOCALIZATION*
EXPRESSION PATTERN

MOLECULAR FUNCTION
- *binding sites*
- *catalytic activity*
- *conformational changes*

⟷ *POST-TRANSLATIONAL MODIFICATIONS*
3D-STRUCTURE

THE INTERACTOME

Characterizing the interactions of proteins in a given cellular proteome, referred to as the **INTERACTOME**, is the next milestone in the path towards understanding the biochemistry of the cell

CONSEQUENCES OF PROTEIN-PROTEIN INTERACTIONS

Protein interactions can:

- Modulate the kinetic properties of enzymes through subtle changes in protein conformation.
- Create a new binding site, typically for small effector molecules.
- Inactivate or destroy a protein.
- Change the specificity of a protein for its substrate.
- Create a new function that is independent of the function of the component proteins
- Serve a regulatory role in either an upstream or a downstream action.

Protein interactions network is a critical link between genotype and phenotype……

GENOTYPE ⟷ **PHENOTYPE**
Your genes **What you look like**
Blueprint

↓

NETWORK INTERACTIONS

Perturbation of interactions can lead to diseased states

TYPES OF PROTEIN INTERACTIONS

Protein interactions fundamentally can be characterized as stable or transient and can be strong or weak.

STABLE INTERACTIONS

Stable interactions are those associated with proteins that are purified as multi-subunit complexes.

The subunits of the complex can be identical or different.

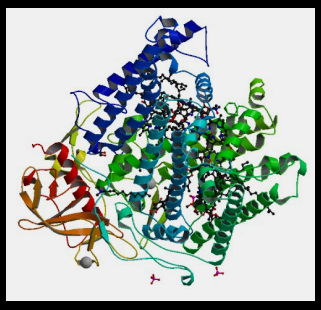
The photosynthetic reaction center is a stable multi-subunit complex.

The 26S proteasome is a large multi-subunit complex

TRANSIENT INTERACTIONS

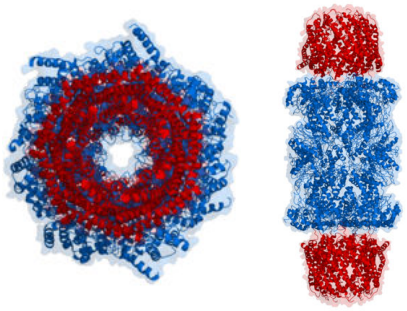
- Transient interactions are on/off or temporary in nature and typically require a set of conditions that promote the interaction.
- Transient interactions can be strong or weak, fast or slow.
- They affect cellular processes including protein modification, transport, folding, signaling, cell cycling, etc.
- Transient interactions can be captured by cross-linking or label transfer methods.

The photosynthetic reaction center of cyanobacteria



1982 Nobel prize to Robert Huber, Johann Deisenhofer & Hartmut Michel

An artist's model for the 26S proteasome, the protein degrading complex present in all eukaryotes and some bacteria



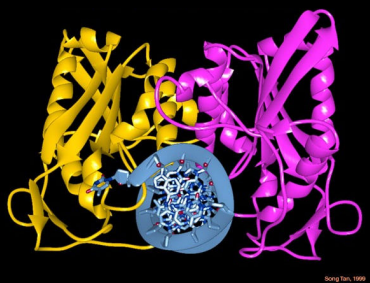
Protein:Nucleic Acid Interactions

Protein:nucleic acid interactions, (i.e., protein:RNA and protein:DNA interactions), are involved in several processes essential to normal cell function.

These processes include transcription, translation, regulation of gene expression, recognition, replication, recombination, repair, nucleic acid packaging and the formation of cellular machinery, such as ribosomes.

As with protein:protein interactions, disruption of protein:nucleic acid interactions leads to serious and often catastrophic consequences within the system.

BamH1 restriction endonuclease and DNA



IT'S ALL ABOUT COMMUNICATION !!!

Communication is important in life processes

Signs & symbols

Smoke signals

COMMUNICATION!!

Hand signals used by military

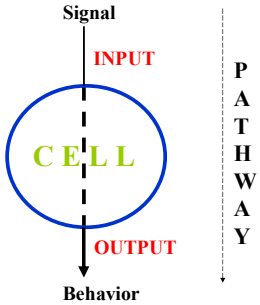
Whistles & horns

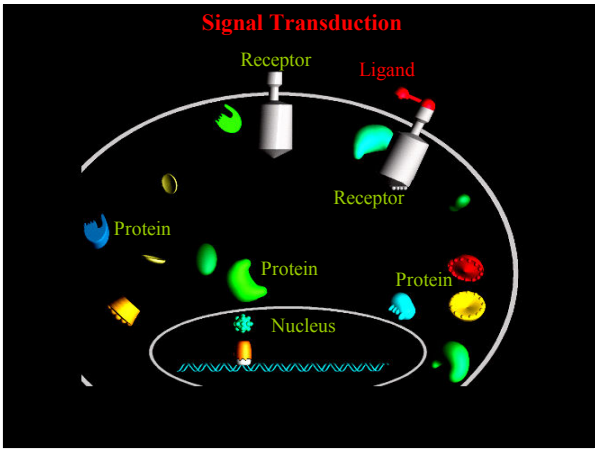
CELLULAR SIGNALING

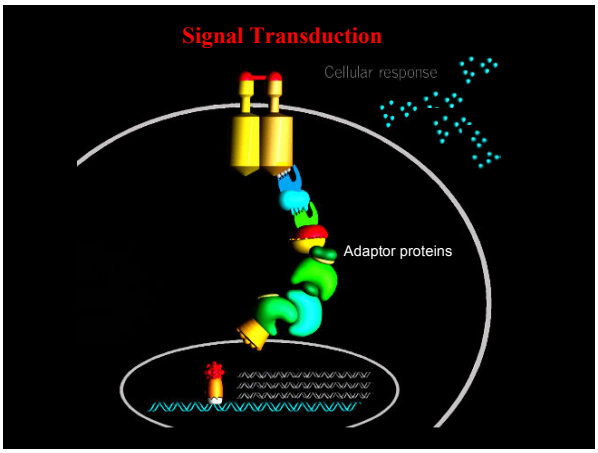
COMMUNICATION VIA PROTEIN-PROTEIN INTERACTIONS LEAD TO SPECIFIC CELLULAR RESPONSES

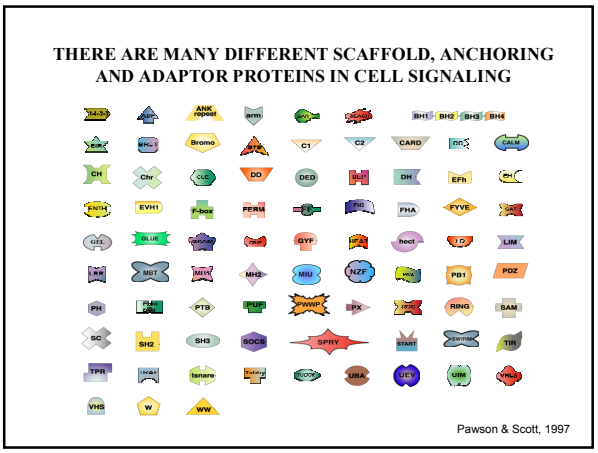
WHAT IS SIGNAL TRANSDUCTION ?

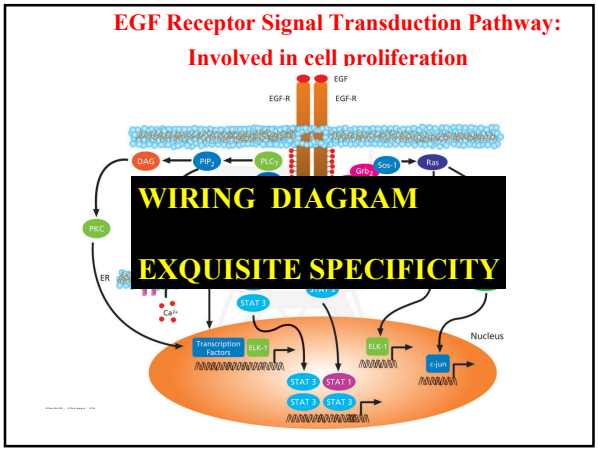
The process of converting an external signal or stimulus to a biological or cellular response

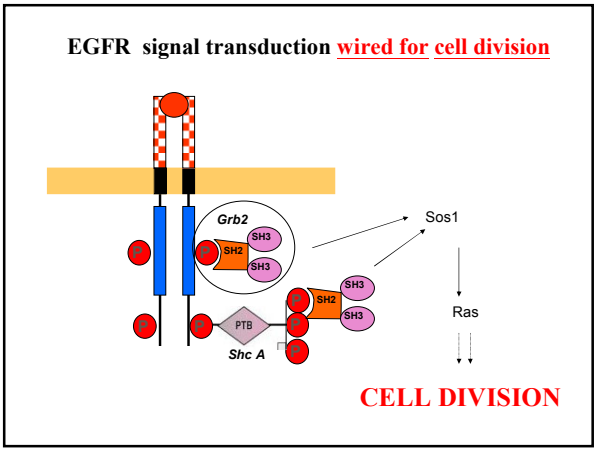


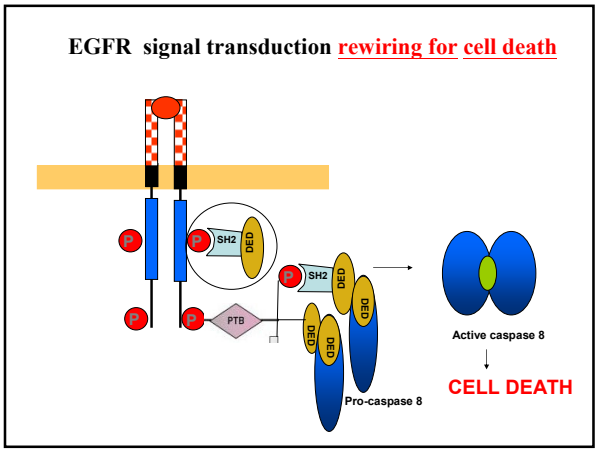


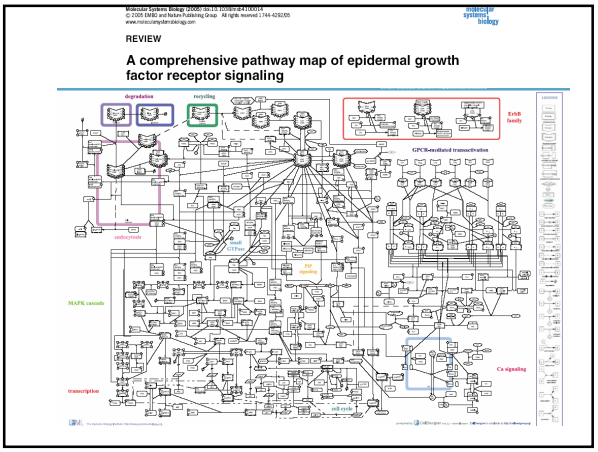

Signal Transduction
Cellular response
Adaptor proteins


THERE ARE MANY DIFFERENT SCAFFOLD, ANCHORING AND ADAPTOR PROTEINS IN CELL SIGNALING
Pawson & Scott, 1997


EGF Receptor Signal Transduction Pathway:
Involved in cell proliferation
WIRING DIAGRAM
EXQUISITE SPECIFICITY
Nucleus


EGFR signal transduction wired for cell division
Grb2
Sos1
Ras
Shc A
CELL DIVISION

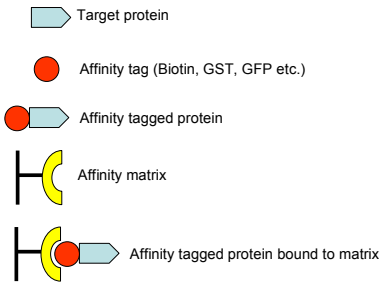

EGFR signal transduction rewiring for cell death
Active caspase 8
Pro-caspase 8
CELL DEATH


REVIEW
A comprehensive pathway map of epidermal growth factor receptor signaling

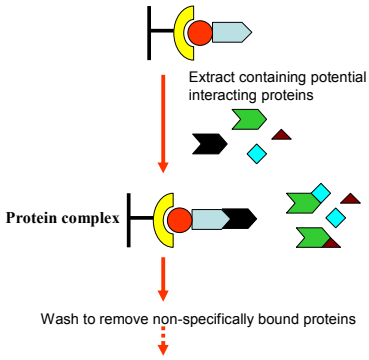
METHODS AND TECHNOLOGIES FOR DETECTING PROTEIN-PROTEIN INTERACTIONS

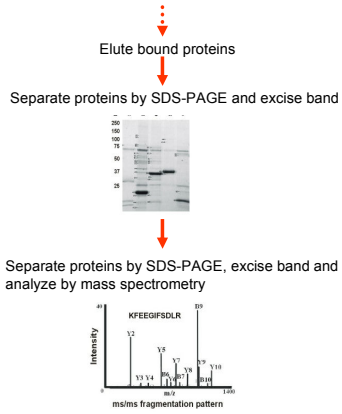
- AFFINITY TAGGING OF PROTEINS
- CO-IMMUNOPRECIPITATION
- PROTEIN CROSS-LINKING
- PROTEIN ARRAYS
- YEAST 2-HYBRID
- MASS SPECTROMETRY

"Pulling down" interacting proteins with affinity tags

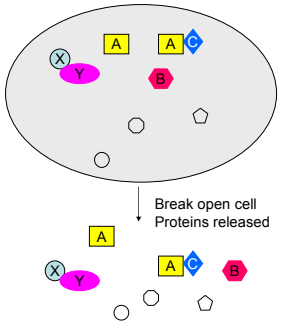


"Pulling down" interacting proteins with affinity tags





Co-immunoprecipitation

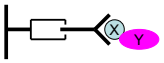


Antibodies available for Protein X and Protein C

Co-immunoprecipitation

Addition of antibody (Ab) to Protein X will pull down the XY pair and addition of antibody to Protein C will pull down the AC pair

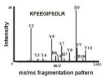
Apply to Protein G affinity column that specifically recognizes the antibody



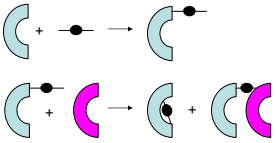
The disadvantage with this method is that artifactual interactions may be introduced upon cell lysis

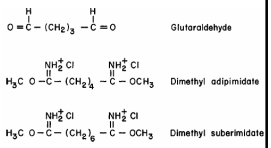
Elute bound protein

Identify components by SDS-PAGE



Protein Crosslinking



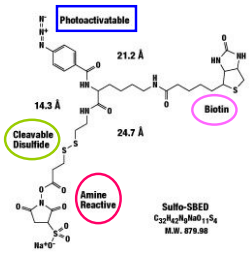


Chemical crosslinking offers a direct method of identifying both transient and stable interactions.

This technique involves the formation of covalent bonds between two proteins by using bifunctional reagents containing reactive end groups that react with functional groups—such as primary amines and sulfhydryls—of amino acid residues.

If two proteins physically interact with each other, they can be covalently cross-linked. The formation of crosslinks between two distinct proteins is a direct and convincing evidence of their close proximity

Photoaffinity Crosslinking



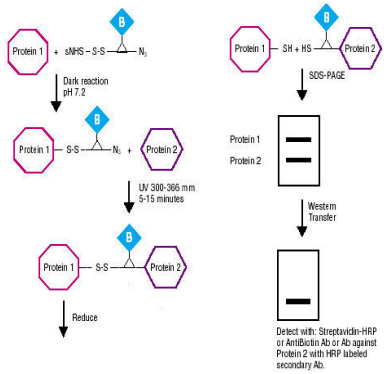
Amine-reactive NHS-ester group – for labeling a purified "bait" protein at the N-terminus and side chain of lysine residues

UV light-activatable aryl azide group – for crosslinking nonspecifically to the protein side chains and backbone of the interacting protein after allowing protein binding to occur

Cleavable disulfide bond (S-S) – can be reduced to release the crosslinker from the original "bait" protein

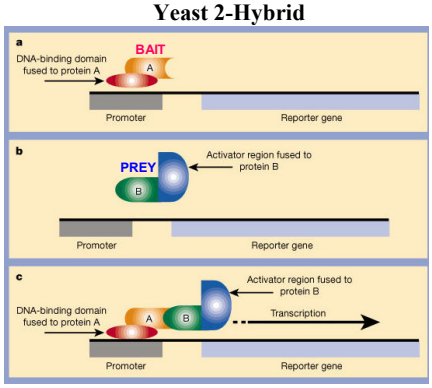
Biotin group – remains attached to target interacting protein after cleaving the disulfide bond, thereby tagging the previously unknown interacting protein(s) for affinity purification and detection.

Pierce catalog

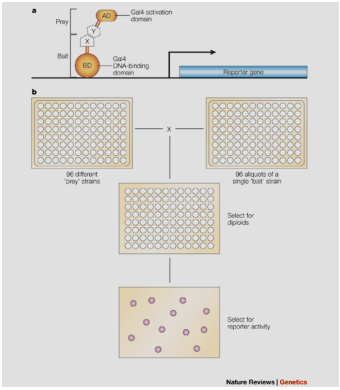


Pierce catalog

Yeast 2-Hybrid



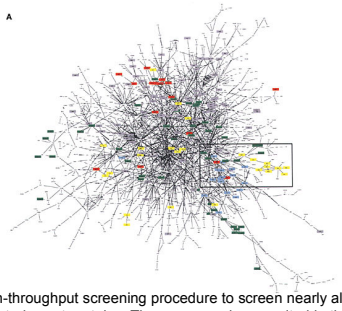
High Throughput 2-hybrid



High-throughput experiments have resolved genome scale networks of protein-protein interactions (PPIs; interactomes) in……

- **Yeast (Saccharomyces cerevisiae, Uetz et al., 2000),**
- **Fruitfly (Drosophila melanogaster, Giot et al., 2003)**
- **Nematode worm (Caenorhabditis elegans, Li et al.,2004)**
- **and Human (Homo sapiens; Miller et al.,2005; Rual et al., 2005; Gandhi et al., 2006).**

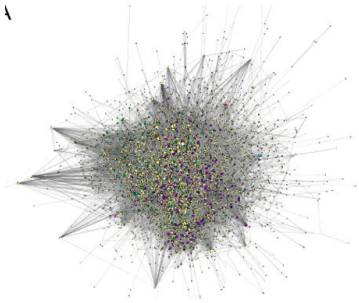
A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*
Nature **403**, 623-627 (10 February 2000)



Used a high-throughput screening procedure to screen nearly all of the 6,000 predicted yeast proteins. These approaches resulted in the detection of 957 putative interactions involving 1,004 *S. cerevisiae* proteins.

Plant Physiology, October 2007, Vol. 145, pp. 317–329, www.plantphysiol.org

A Predicted Interactome for Arabidopsis

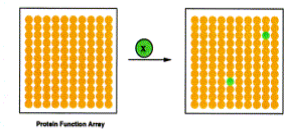


A giant hairy ball of 19,979 interactions

What is a Protein Microarray?

A high density array containing 100s to many thousands of proteins positioned in an addressable format

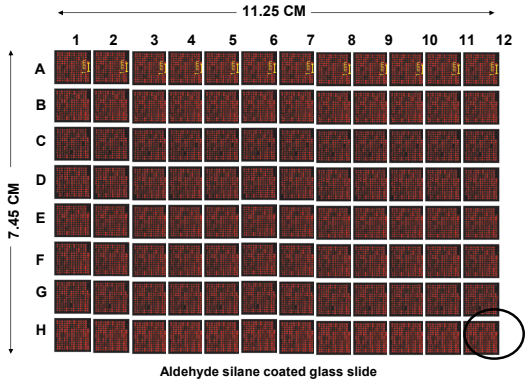
PROTEIN ARRAYS



Facilitate the high throughput analysis of protein function

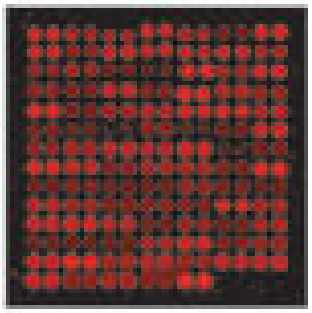
- **protein-protein interactions**
- **protein-small molecule interactions**
- **enzyme-substrate reactions.**

A quantitative protein interaction network for the ErbB receptors using protein microarrays (Jones et al, Nature 2006)

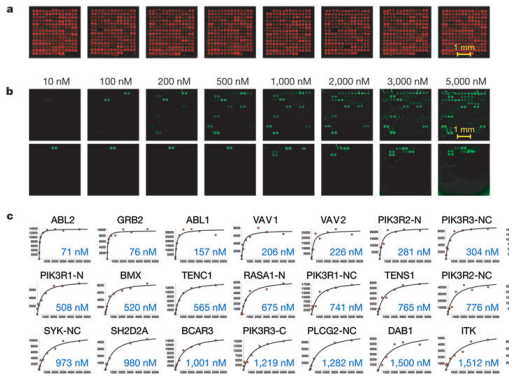


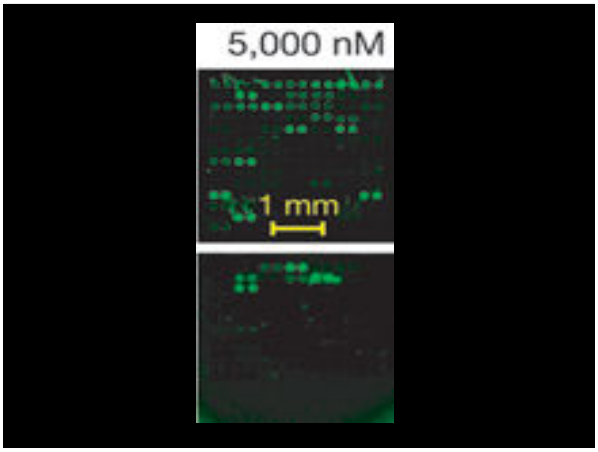
Aldehyde silane coated glass slide

MICROARRAY



96 wells, PROTEINS SPOTTED AT A CONCENTRATION OF 40 uM USING A PIEZOELECTRIC NONCONTACT MICROARRAYER





Functional Protein Arrays Commercially Available

1) Yeast proteome

2) Human 2K array

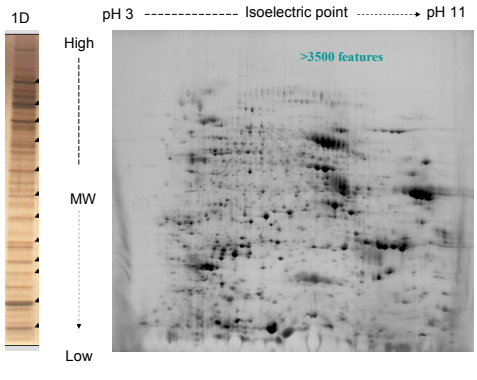
inVtirogen Corporation

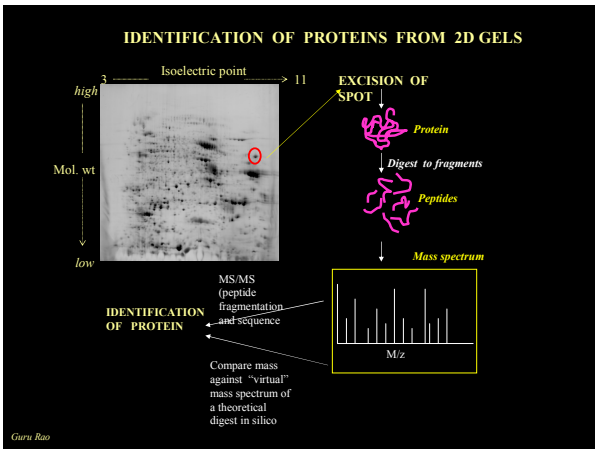
Links to Protein Interaction Databases

http://proteome.wayne.edu/PIDBL.html

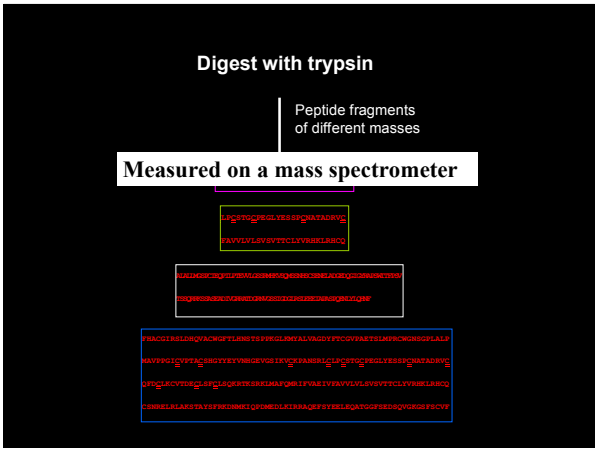
Finley Lab Interactions Databases:
- ***Drosophila Interactions Database (DroID)***
- ***Campylobaster jejuni Interactions Databases***

Gene or Protein Interactions Databases in the reseach community:
- BioGRID- A Database of Genetic and Physical Interactions
- DIP - Database of Interacting Proteins
- MINT - A Molecular Interactions Database
- IntAct - EMBL-EBI Protein Interaction
- MIPS - Comprehensive Yeast Protein-Protein interactions
- Yeast Protein Interactions - Yeast two-hybrid results from Fields' group
- PathCalling- A yeast protein interaction database by Curagen
- SPiD - *Bacillus subtilis* Protein Interaction Database
- AllFuse - Functional Associations of Proteins in Complete Genomes
- BRITE - Biomolecular Relations in Information Transmission and Expression
- ProMesh - A Protein-Protein Interaction Database
- The PIM Database - by Hybrigenics
- Mouse Protein-Protein interactions
- Human herpesvirus 1 Protein-Protein interactions
- Human Protein Reference Database
- BOND - The Biomolecular Object Network Databank. Former **BIND**
- MDSP - Systematic identification of protein complexes in *S. cerevisiae* by mass spectrometry
- Protcom - Database of protein-protein complexes enriched with the domain-domain structures
- Proteins that interact with GroEL and factors that affect their release
- DPIDB - DNA-Protein Interaction Database
- YPD™ - Yeast Proteome Database by Incyte





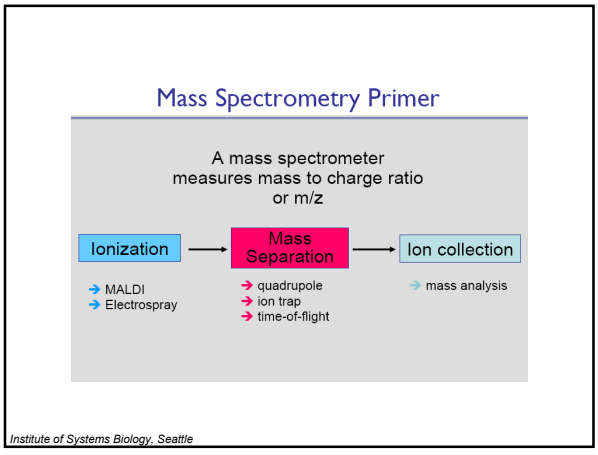
Protein "cut" with trypsin



What is a mass spectrometer?

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted to ions i.e. molecules that have been electrically charged

A machine used to weigh molecules



Sample Ionization

Ionisation usually occurs by the addition or abstraction of a proton, an electron or another ion.

- ***Protonation***: Proton is added to a molecule producing a net positive charge of 1+ for every proton added (to basic residues)

$$M + H^+ \longrightarrow MH^+$$

- ***Deprotonation***: Net negative charge of 1- is achieved through removal of a proton from a molecule.

$$M - H^+ \longrightarrow [M-H]^-$$

- ***Cationization***: Similar to protonation. A charged complex is produced by non-covalently adding a positively charged ion to a neutral molecule.

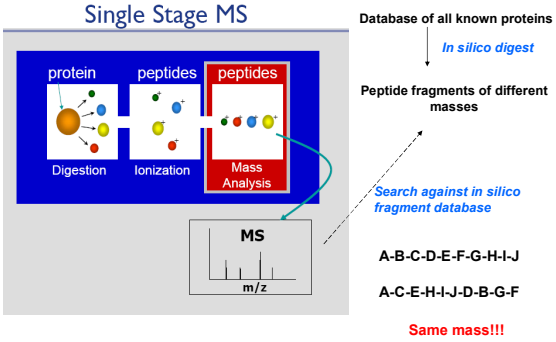
Mass to Charge ratio
m/z

Mass spectrometers measure *m/z* values of molecular ions

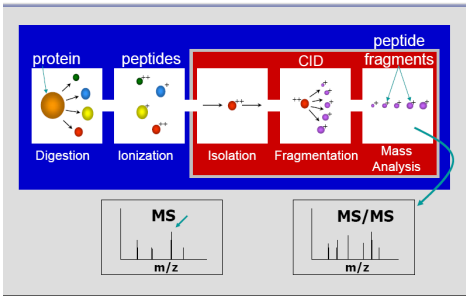
Typically one proton is added in MALDI and multiple protons in the case of ESI

Peptide of molecular mass 2000
One proton added, $[M + H]^{1+}$, m/z = 2001
Two protons added, $[M + 2H]^{2+}$, m/z = 1001

Single Stage MS

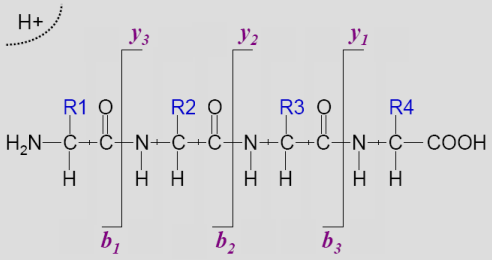


Tandem MS



Institute of Systems Biology, Seattle

Fragment Ions

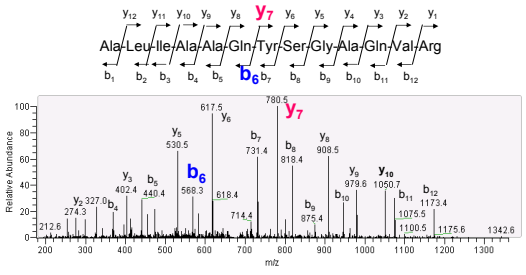


Fragmenting a peptide

A-L-I-A-A-Q-Y-S-G-A-Q-V-R

	b-ions	y-ions
B12	A----------L-I-A-A-Q-Y-S-G-A-Q-V-R	Y1
B11	A-L----------I-A-A-Q-Y-S-G-A-Q-V-R	Y2
B10	A-L-I----------A-A-Q-Y-S-G-A-Q-V-R	Y3
B9	A-L-I-A----------A-Q-Y-S-G-A-Q-V-R	Y4
B8	A-L-I-A-A----------Q-Y-S-G-A-Q-V-R	Y5
B7	A-L-I-A-A-Q----------Y-S-G-A-Q-V-R	Y6
B6	A-L-I-A-A-Q-Y----------S-G-A-Q-V-R	Y7
B5	A-L-I-A-A-Q-Y-S----------G-A-Q-V-R	Y8
B4	A-L-I-A-A-Q-Y-S-G----------A-Q-V-R	Y9
B3	A-L-I-A-A-Q-Y-S-G-A----------Q-V-R	Y10
B2	A-L-I-A-A-Q-Y-S-G-A-Q----------V-R	Y11
B1	A-L-I-A-A-Q-Y-S-G-A-Q-V----------R	Y12

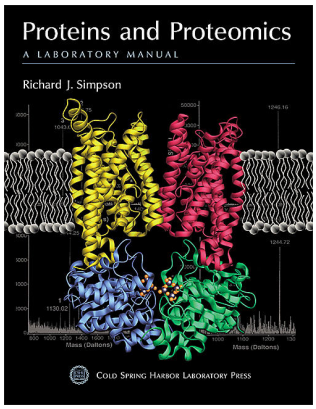
Collision-Induced Dissociation of a Peptide to Produce a Product Ion Spectrum



Fragmenting a Peptide

A-P-N-D-F-N-L-K
(MH+ 918.5)

B-ions		Y-ions
72.0	A ——— P-N-D-F-N-L-K	847.4
169.1	A-P ——— N-D-F-N-L-K	750.4
283.1	A-P-N ——— D-F-N-L-K	636.3
398.2	A-P-N-D ——— F-N-L-K	521.3
545.2	A-P-N-D-F ——— N-L-K	374.2
659.3	A-P-N-D-F-N ——— L-K	260.2
772.4	A-P-N-D-F-N-L ——— K	147.1



THANK YOU!!!