Really high sensitivity mass spectrometry and Discovery and analysis of protein complexes

• The PRIME lab and AMS
• Importance of protein complexes in biology
• Methods for isolation of protein complexes
  – In solution
  – On a chip
  – In a gel (Paul Brookes)
• Analysis of protein complexes
Purdue Rare Isotope Measurement Lab

Accelerator mass spectrometry for rare isotopes, $^{10}$Be, $^{14}$C, $^{26}$Al, $^{36}$Cl, $^{41}$Ca, $^{129}$I

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Accelerator in PRIME Lab

Dr. David Elmore next to 10 MV accelerator

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Inside Accelerator in PRIME Lab

If an animal is given 50 nCi of a $^{14}$C-labeled compound and 0.01% is absorbed and reaches the brain, then 20 mg of tissue is sufficient to provide enough signal to give a 1000:1 signal-to-noise ratio.
Collapse of the single target paradigm

Old paradigm

*Diseases are due to single genes - by knocking out the gene, or designing specific inhibitors to its protein, disease can be cured*

But the gene KO mouse didn’t notice the loss of the gene

New paradigm

*We have to understand gene and protein networks - proteins don’t act alone - effective systems have built in redundancy*

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Proteins aren’t random in cells

So, who’s binding to whom?
Proteins don’t act alone

Signal transduction complex lying in anticipation

Peptidomimetic targets
How to discover protein brotherhoods

Old method: Yeast 2-hybrid screen

New method: Recover protein complexes

SDS-PAGE

IEF/SDS-PAGE

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EGF-induced tyrosine phosphorylation in HeLa cells. Serum-deprived HeLa S3 cells (5 x 10⁹) were either left untreated or treated with 1 µg/ml EGF for 5 min.

Cleared cell lysates were immunoprecipitated with a mixture of monoclonal anti-phosphotyrosine antibodies, washed, and resolved by SDS/PAGE. The gel was then silver-stained.

Numbers indicate the positions of the bands that were excised for enzymatic digestion by trypsin and subsequent mass spectrometric analysis.
EGF-stimulated, tyrosine-phosphorylated proteins identified by mass spec

See protein interactions at www.bind.ca

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Affinity methods for recovering complexes

Antibody

Streptavidin

Glutathione

GST

Multiprotein complex
Recovering a ribosomal protein complex

In A, the proteins pulled down by untagged (-) and tagged (+) Nop7p were analyzed by SDS-PAGE.

In B, these proteins were separated by reverse-phase HPLC and were subjected to trypsin fingerprint analysis by MALDI-TOF.

Affinity purification of nucleoporin interaction proteins


A. Nup42p affinity resin

B. Nup49p affinity resin

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Tap-Tag isolation of protein complexes


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Validation of partners in protein complexes - cross correlation analysis

Summary of protein complexes discovered in yeast by the Tap-Tag method

Comparison of the effectiveness of protein-protein interaction methods

Analysis of bridged protein complexes

Digestion of chemically linked proteins results in a bridged peptide. If the hydrolysis is carried out in H$_2$O$^{18}$, then there will be four O$^{18}$ and hence the MW will increase by 8.


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Tandem MS of bridged peptide

Note the increase in the Arg fragment ($m/z$ 175) to $m/z$ 179


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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
Spotted array of 80% of the yeast proteome

6566 protein samples representing 5800 unique proteins were spotted in duplicate on a single nickel-coated microscope slide. The slide was probed with anti-GST. Zhu & Snyder, *Science* 293, 2101 (2001)

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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.

Zhu & Snyder, Science 293, 2101 (2001)

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