Discovery and analysis of protein complexes

- **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**

Collapse of the single target paradigm

Old paradigm

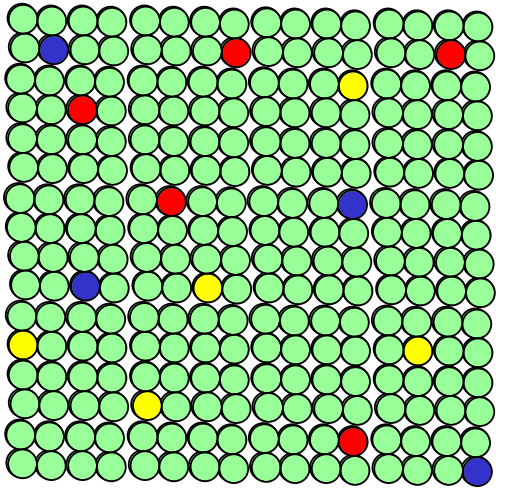
Diseases are due to single genes - by knocking out the gene, or designing specific inhibitors to its protein, disease can be cured

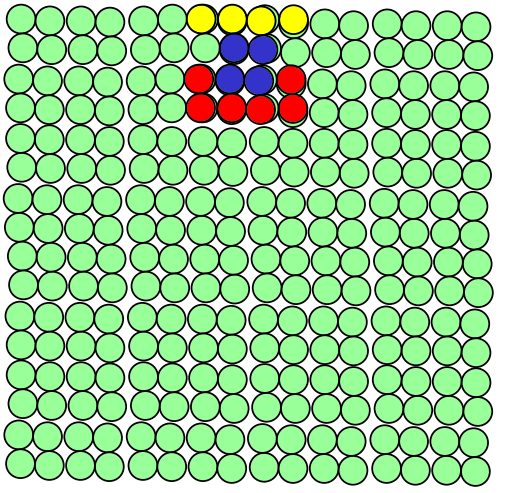
But the gene KO mouse didn't notice the loss of the gene

New paradigm

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

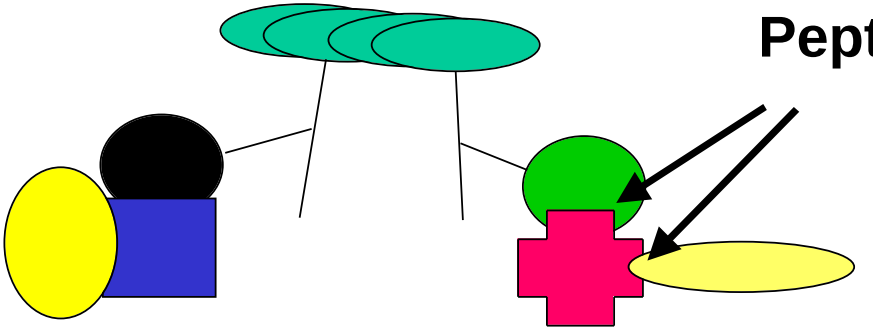
Proteins aren’t random in cells





So, who’s binding to whom?

Proteins (and spies) don’t act alone



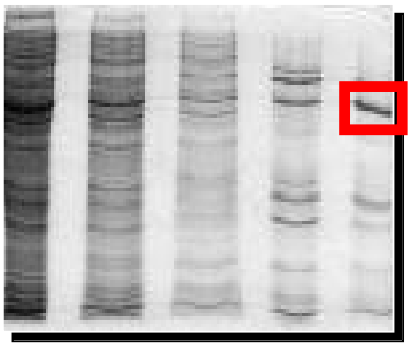
Peptidomimetic targets

Signal transduction complex lying in anticipation

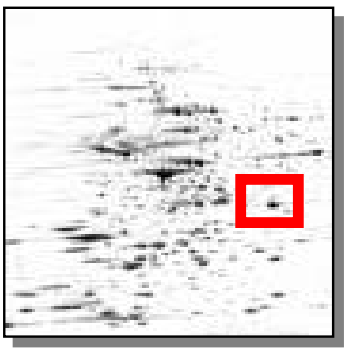
How to discover protein brotherhoods

Old method:
Yeast 2-hybrid screen

New method:
Recover protein complexes



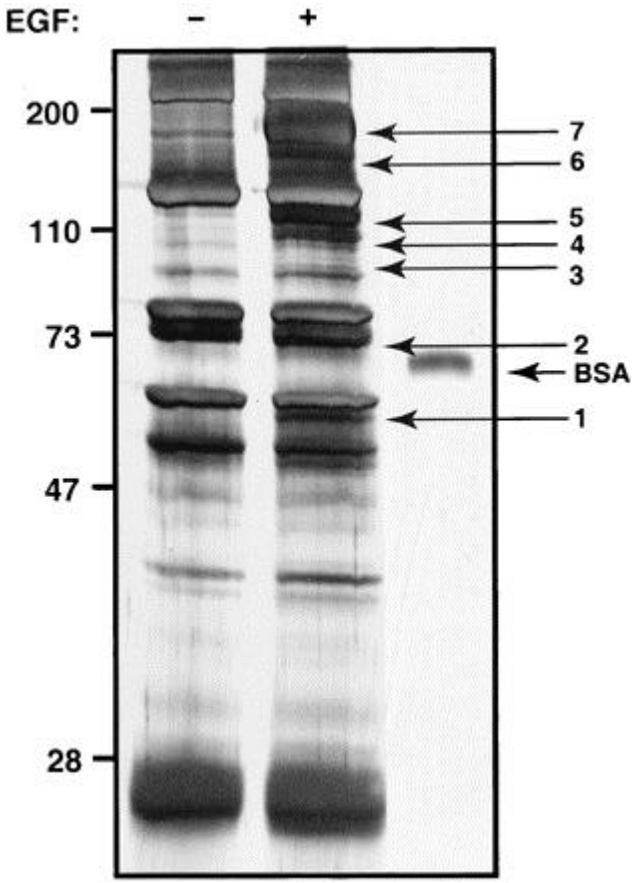
SDS-PAGE



IEF/SDS-PAGE

Affinity isolation of EGF-responsive proteins

Pandey et al., PNAS 97: 179-184 (2000)



EGF-induced tyrosine phosphorylation in HeLa cells. ***Serum-deprived HeLa S3 cells (5 x 10^9) 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.

Peptide masses of 110 kD band

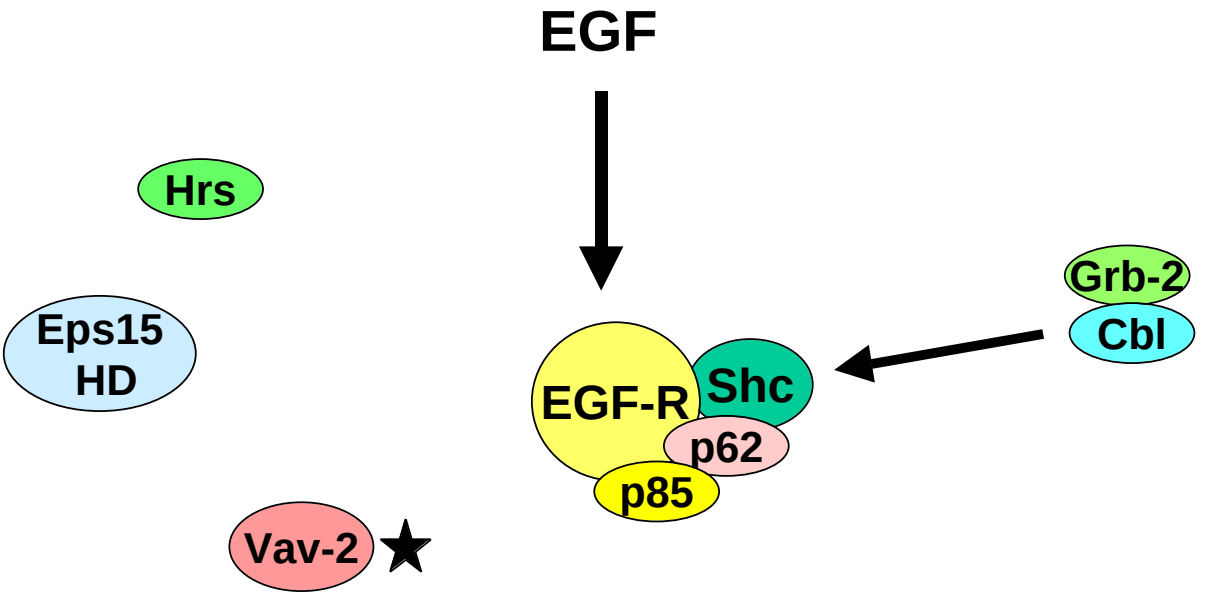
- 926.36
- 1046.51
- 1065.43
- 1226.62
- 1315.57
- 1713.81
- 1770.99

MALDI analysis of tryptic peptides

Protein is Vav-2, a human oncogene

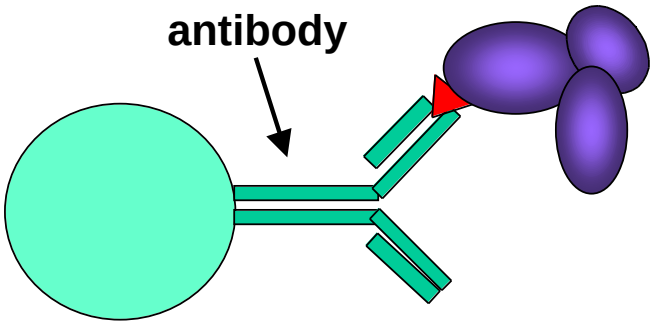
- has many conserved domains(e.g., SH2 and SH3) typical of signal transduction complexes

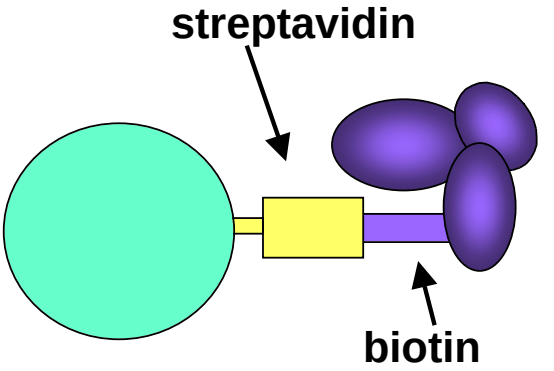
EGF-stimulated, tyrosine-phosphorylated proteins identified by mass spec

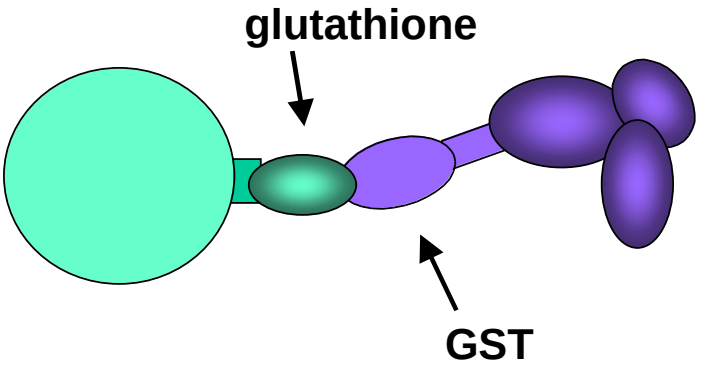


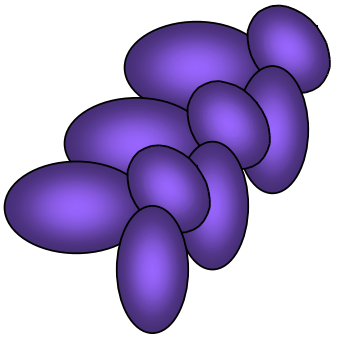
See protein interactions at www.bind.ca

Affinity methods for recovering complexes



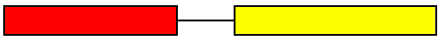






Multiprotein complex

Preparation of protein array

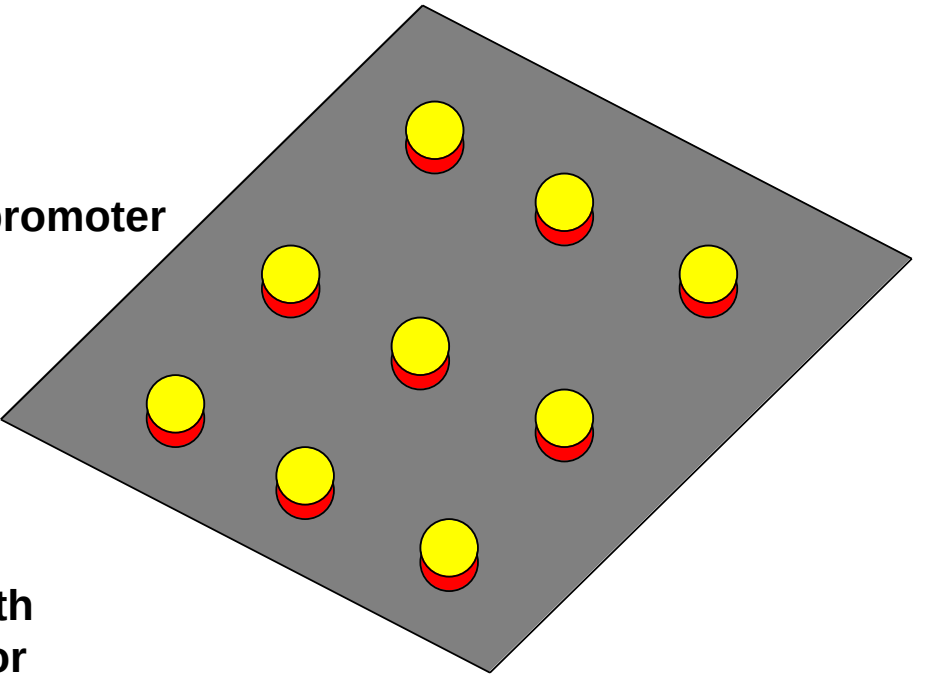


Protein fused to GST-6xHis

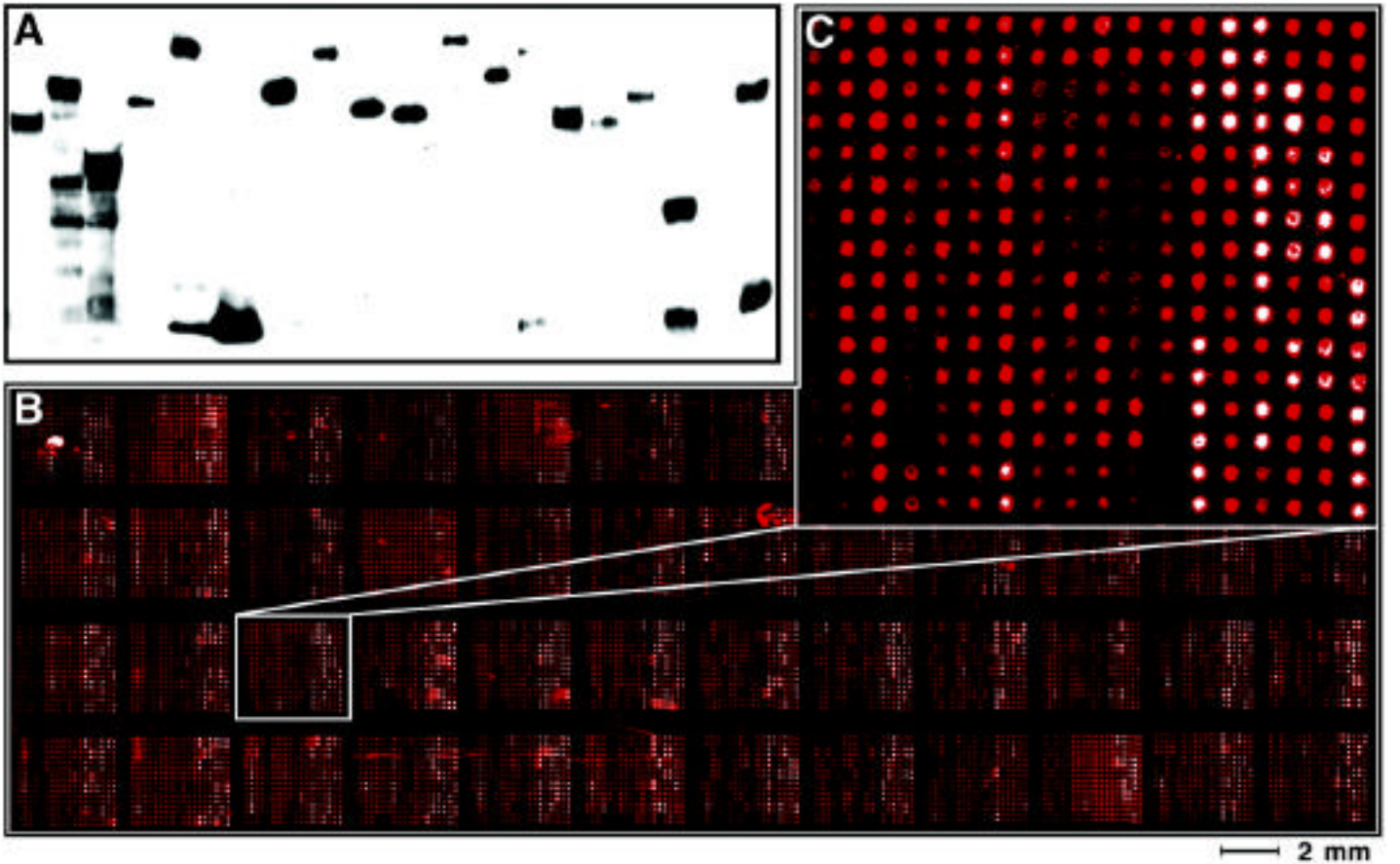
Expressed in yeast with Gal1 promoter

Extracted and absorbed on glutathione-agarose beads

Proteins eluted and reacted with aldehyde-treated glass slides or nickel-coated (for the 6xHis tag)

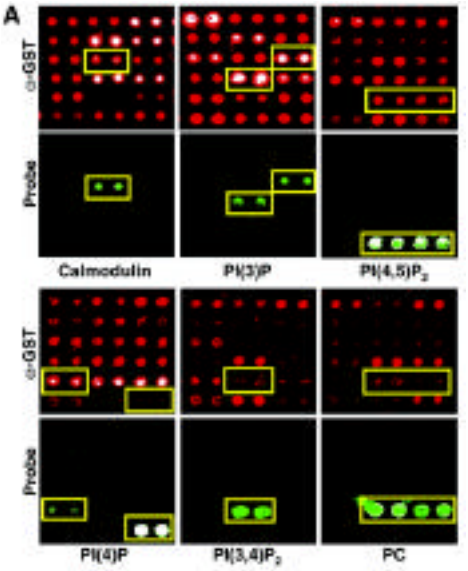


Spotted array of 80% of the yeast proteome

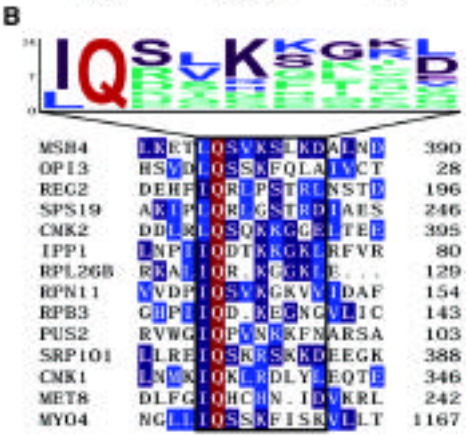


6566 protein samples representing 5800 unique proteins were spotted in duplicate on a single nickel-coated microscope slide. The slide was probed with anti-GST. [Zhu & Snyder, *Science* 293, 2101 (2001)]

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).



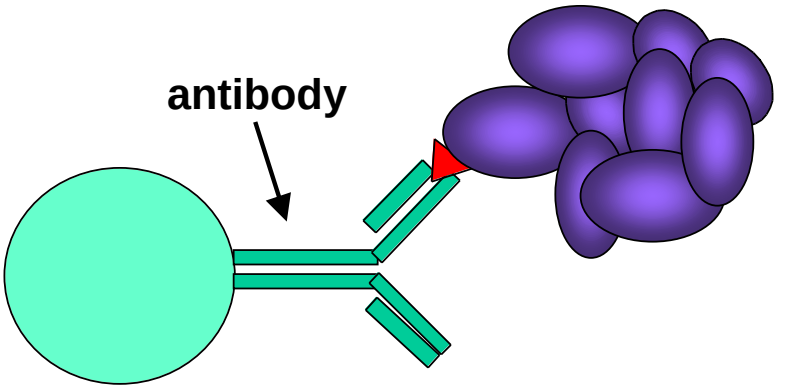
B. A putative calmodulin-binding motif. Fourteen of 39 positive proteins share a motif whose consensus is (I/L)QXK(K/X)GB, where X is any residue and B is a basic residue. *The size of the letter indicates the relative frequency of the amino acid indicated*

Analyzing bound proteins

- ***2D-electrophoresis of proteins***
- **Reverse phase nanoLC-MSMS of peptides**
- **Ion exchange/reverse phase LC-LC-ESI-MSMS**
- **Isotope-coded affinity tagging LC-ESI-MSMS**
- **CE- or reverse phase nanoLC/MALDI-TOF-MS**
- ***MALDI and SELDI***

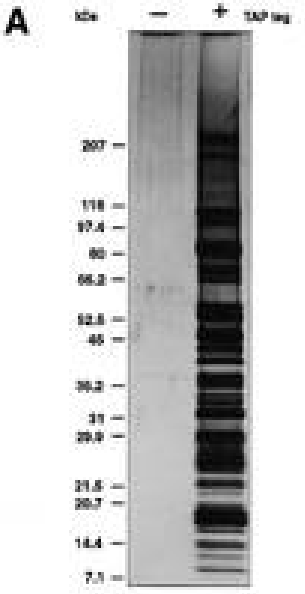
Identification of ribosomal proteins

Nucleolar protein Nop7p expressed in yeast with (+) and without (-) affinity tag



Harnpicharnchai et al., Mol. Cell 8: 505 (2001)

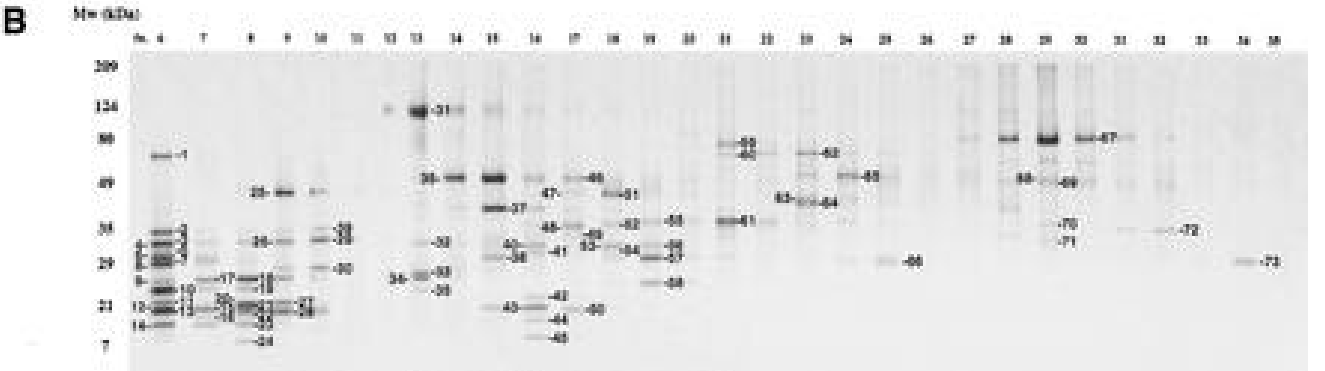
Recovering a ribosomal protein complex



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

In B, these proteins were separated by formic acid HPLC and were subjected to trypsin fingerprint analysis by MALDI-TOF

Harnpicharnchai et al., Mol. Cell 8: 505 (2001)



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**