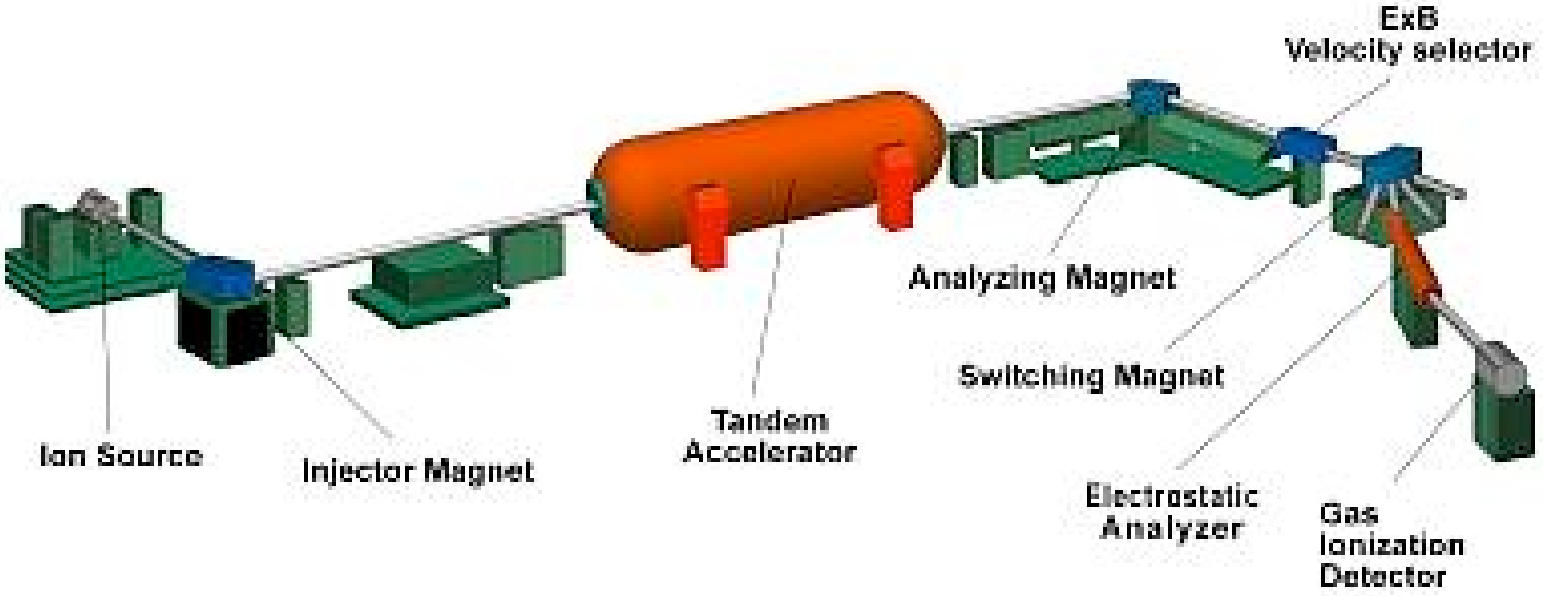
Really high sensitivity mass spectrometry

and

Discovery and analysis of protein complexes

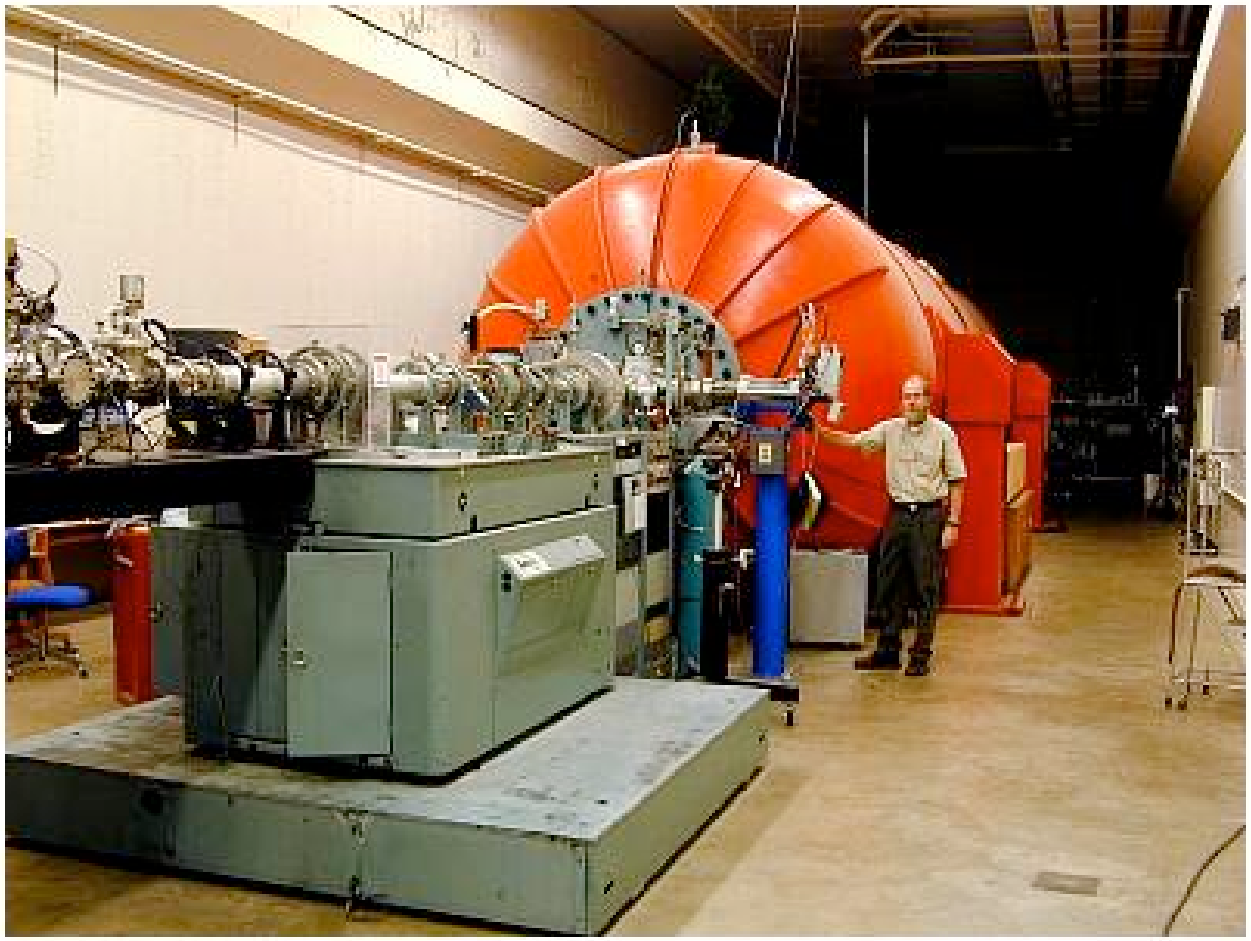
- The PRIME lab and AMS
- Importance of protein complexes in biology
- Methods for isolation of protein complexes
  - In solution
  - On a chip
  - In a gel (Paul Brookes)
- Analysis of protein complexes

Purdue Rare Isotope Measurement Lab



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

Accelerator in PRIME Lab



Dr. David Elmore next to 10 MV accelerator

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

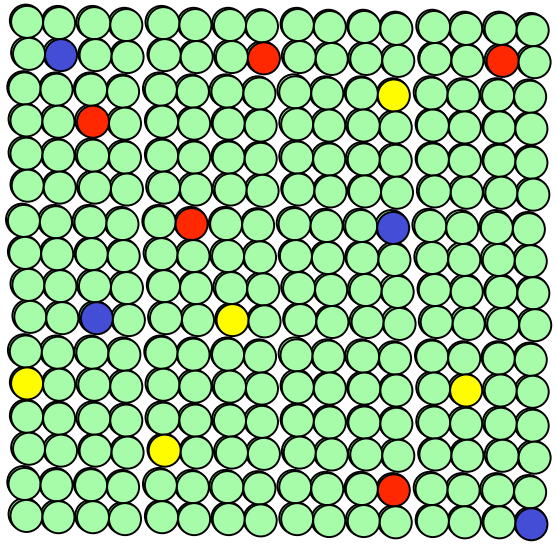
Diseases are due to single genes - by knocking out the gene, or designing specific inhibitors to its protein, disease can be cured

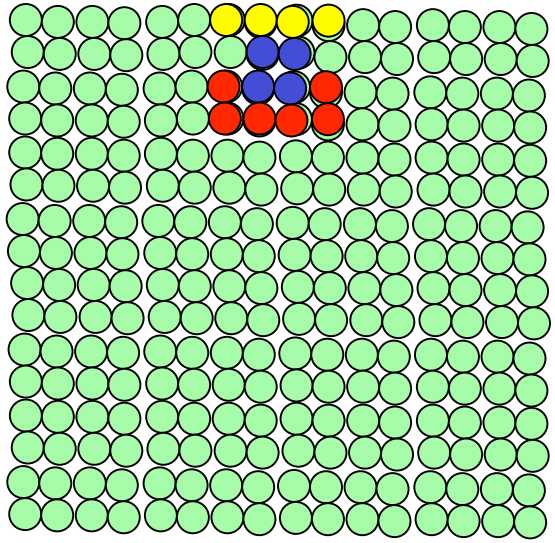
But the gene KO mouse didn't notice the loss of the gene

New paradigm

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

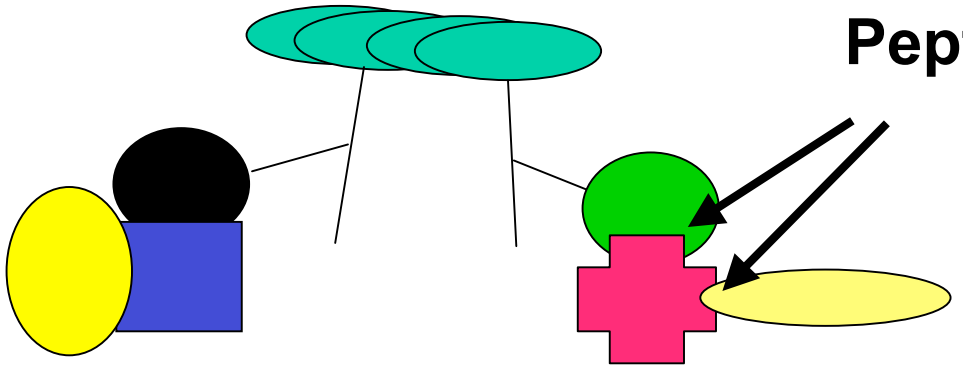
Proteins aren't random in cells





So, who's binding to whom?

Proteins don’t act alone

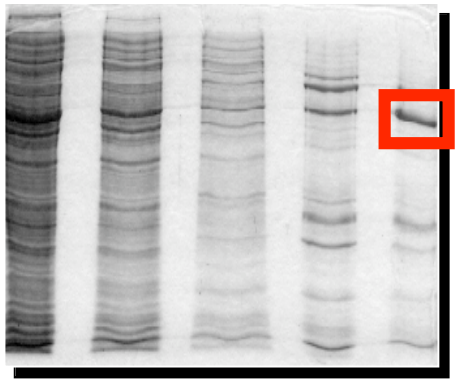


Signal transduction complex lying in anticipation

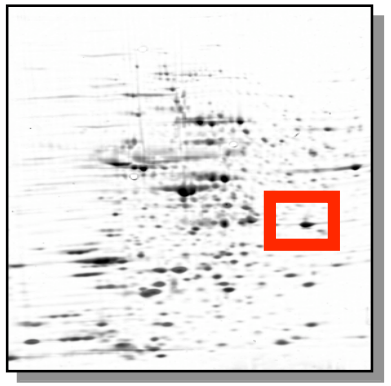
How to discover protein brotherhoods

Old method:
Yeast 2-hybrid screen

New method:
Recover protein complexes



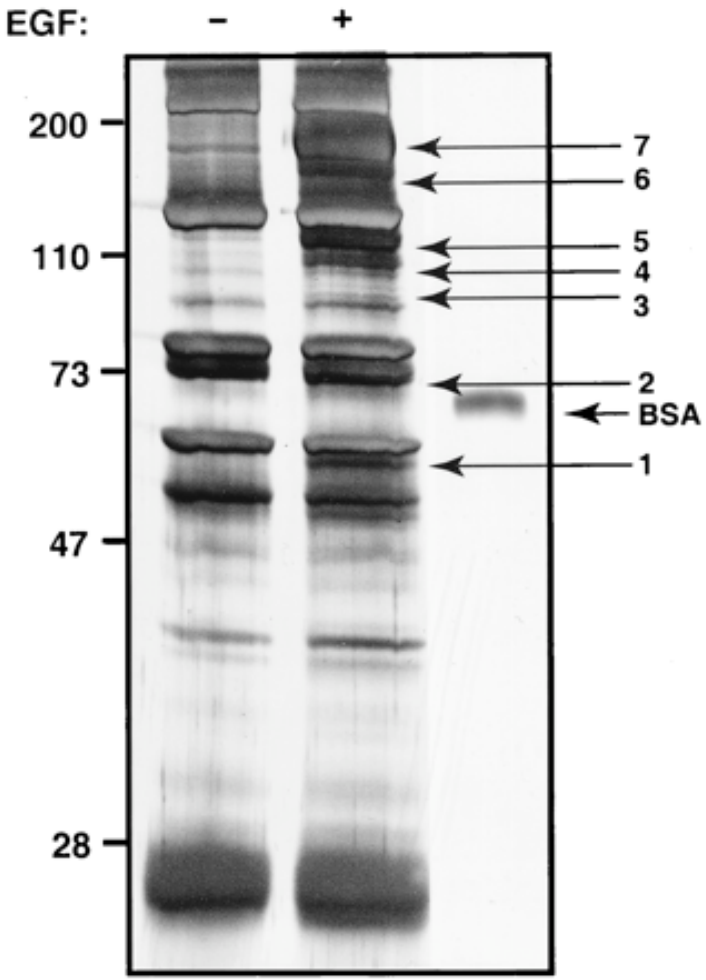
SDS-PAGE



IEF/SDS-PAGE

Affinity isolation of EGF-responsive proteins

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

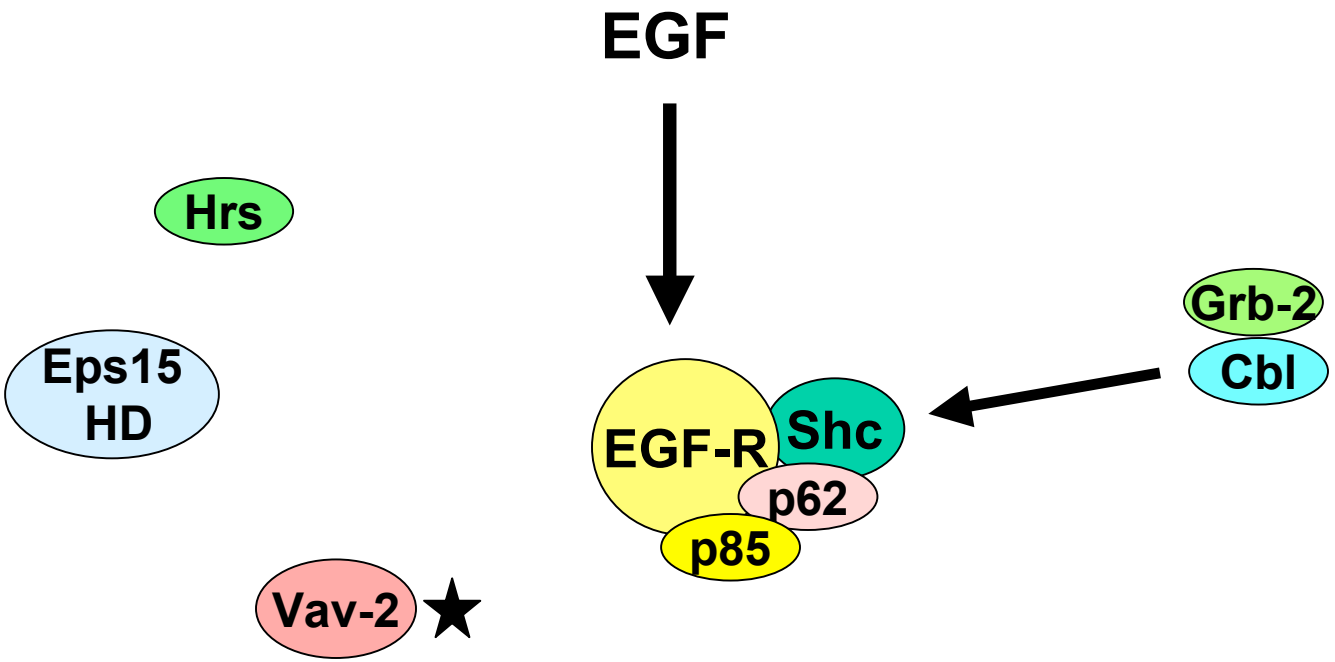


EGF-induced tyrosine phosphorylation in HeLa cells. ***Serum-deprived HeLa S3 cells (5 x 10^9) were either left untreated or treated with 1 □g/ml EGF for 5 min.***

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.

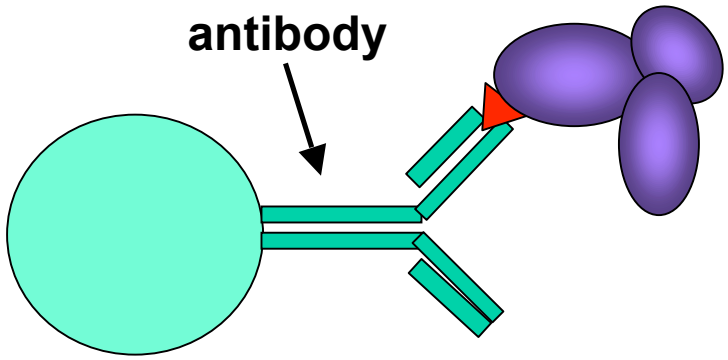
Numbers indicate the positions of the bands that were excised for enzymatic digestion by trypsin and subsequent mass spectrometric analysis.

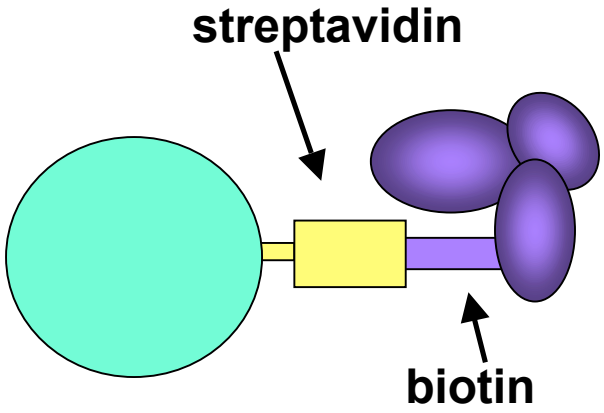
EGF-stimulated, tyrosine-phosphorylated proteins identified by mass spec

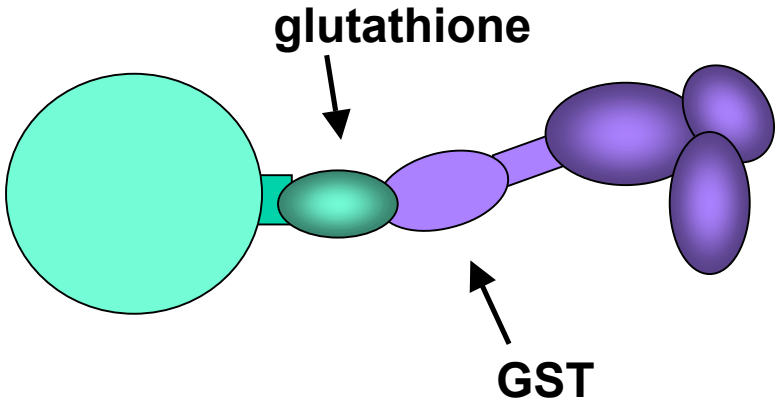


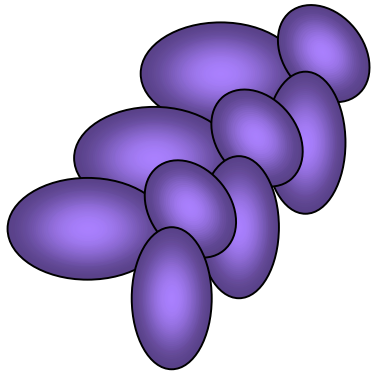
See protein interactions at www.bind.ca

Affinity methods for recovering complexes



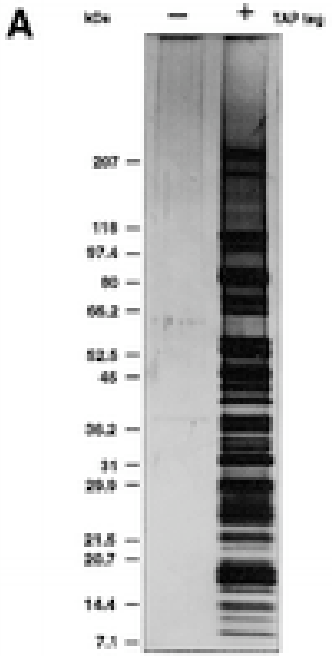






Multiprotein complex

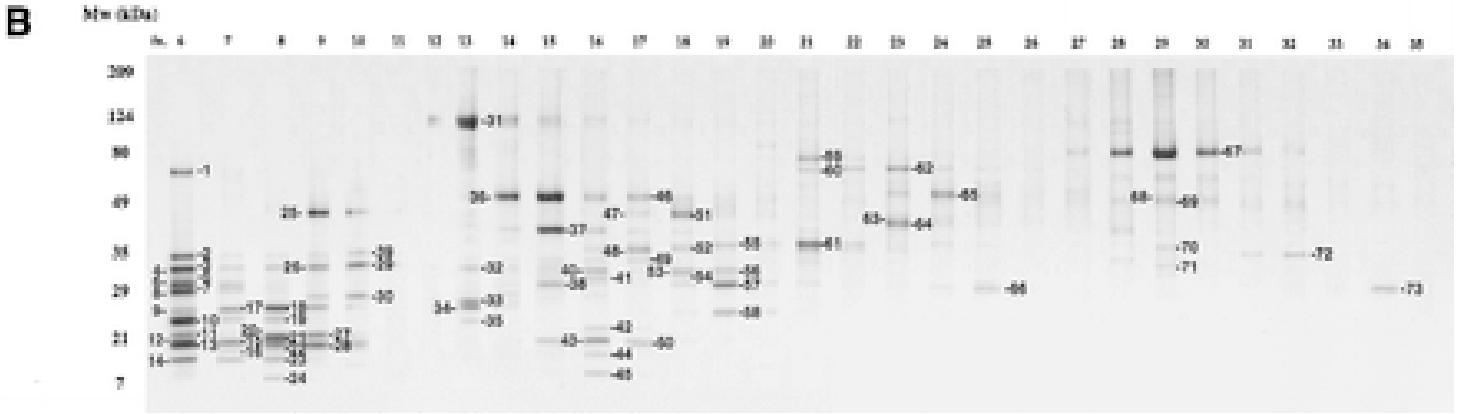
Recovering a ribosomal protein complex



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

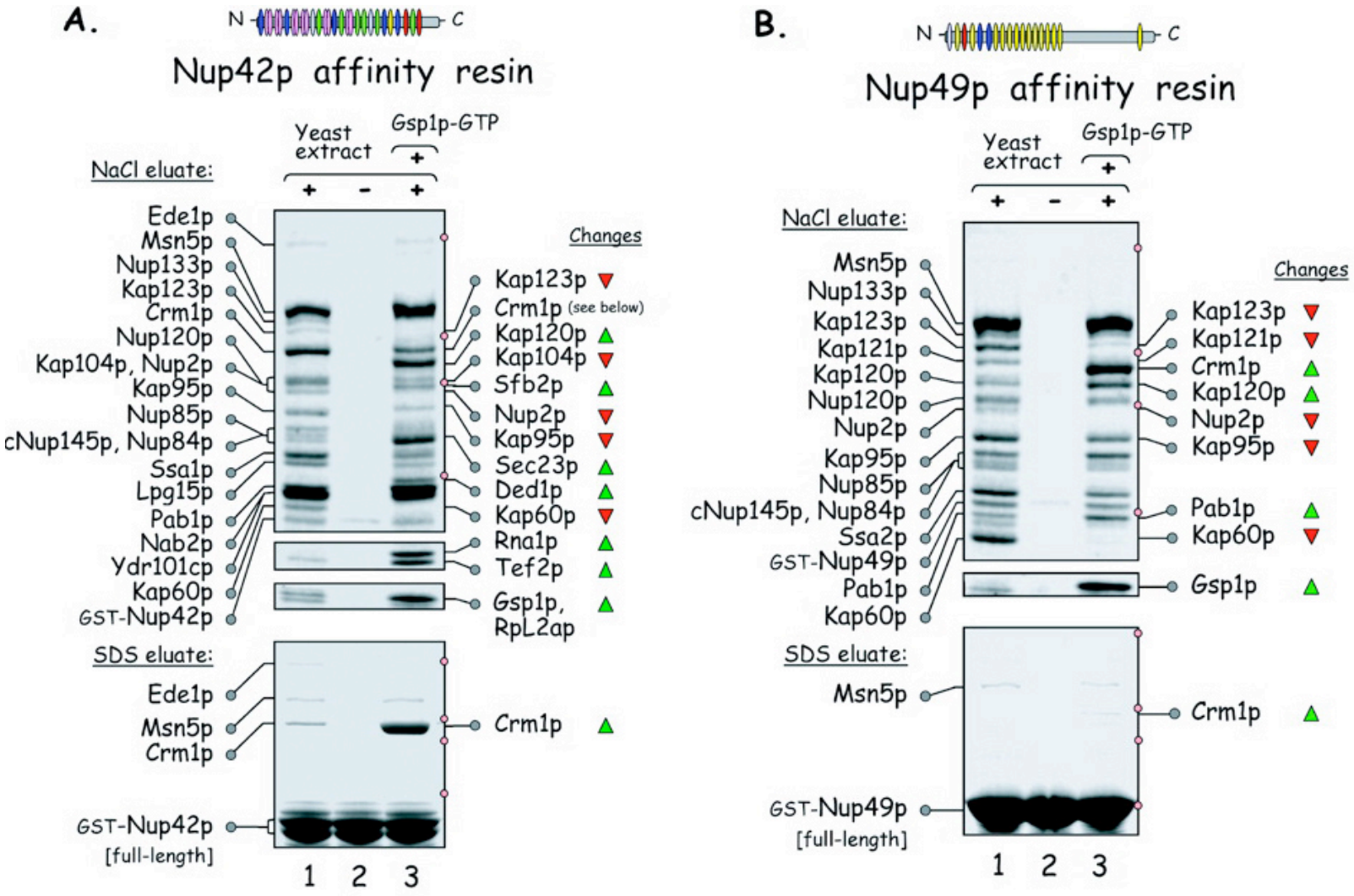
In B, these proteins were separated by reverse-phase HPLC and were subjected to trypsin fingerprint analysis by MALDI-TOF

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

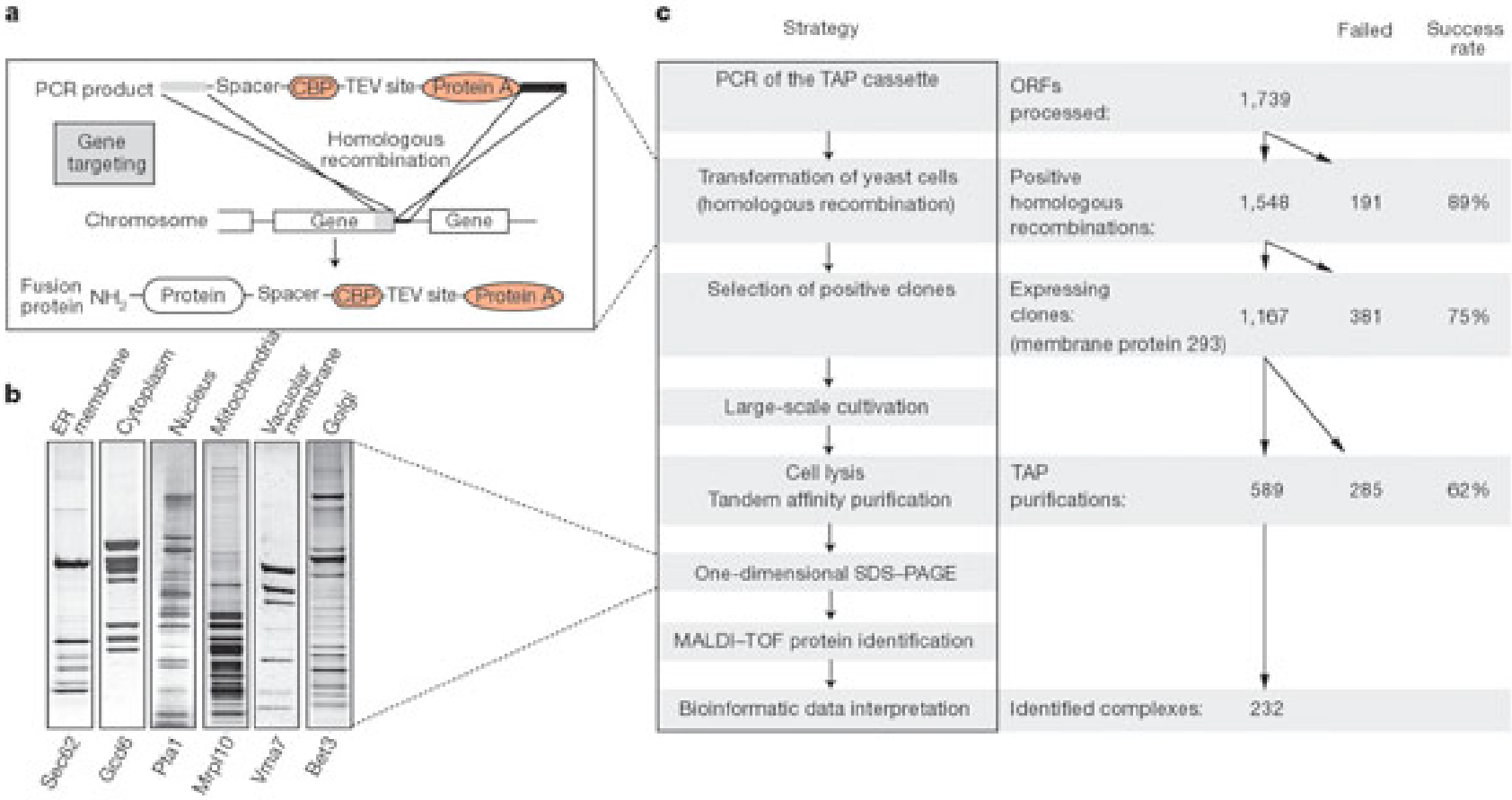


Affinity purification of nucleoporin interaction proteins

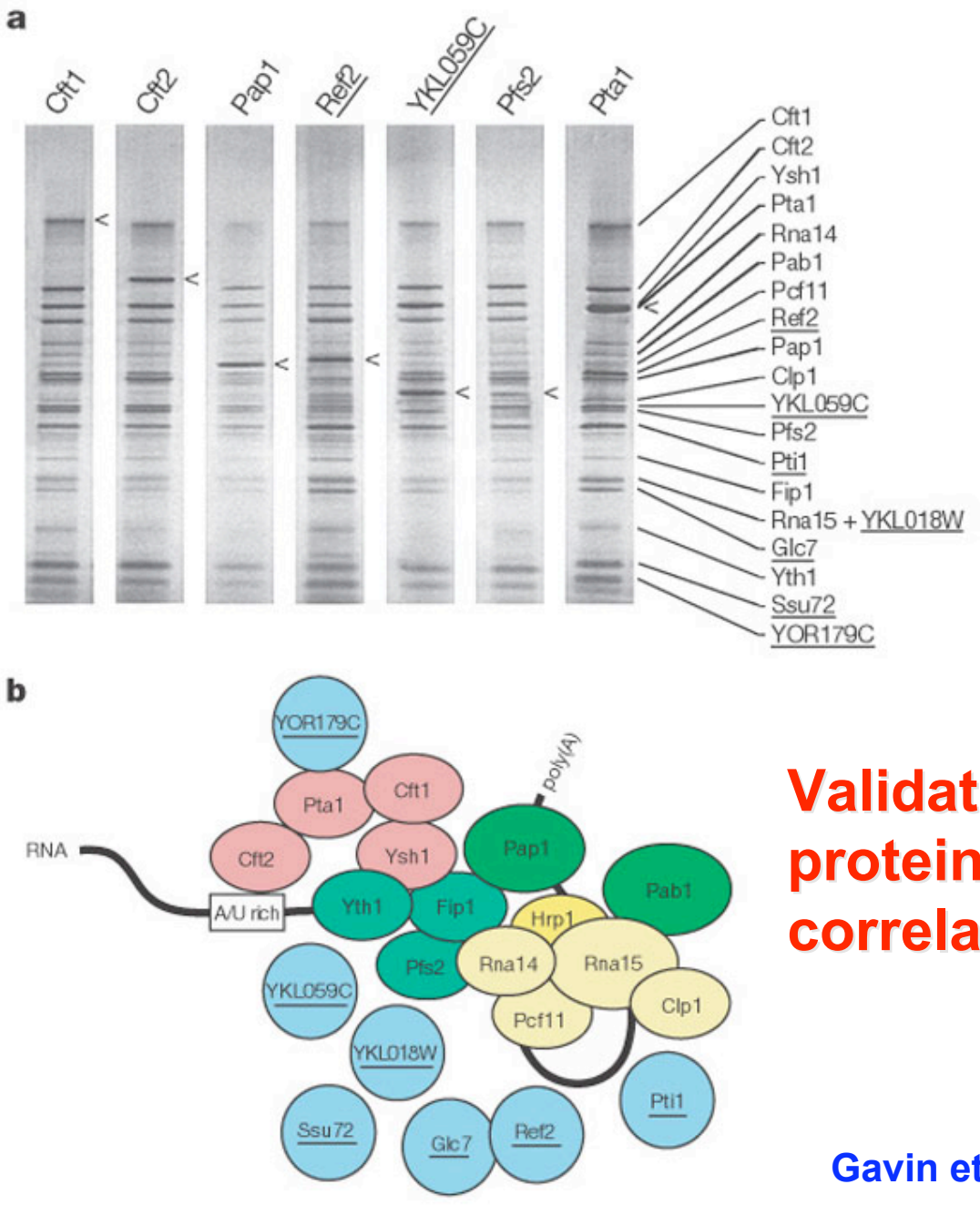
Allen et al.,J Biol Chem 276: 29268 (2001)



Tap-Tag isolation of protein complexes

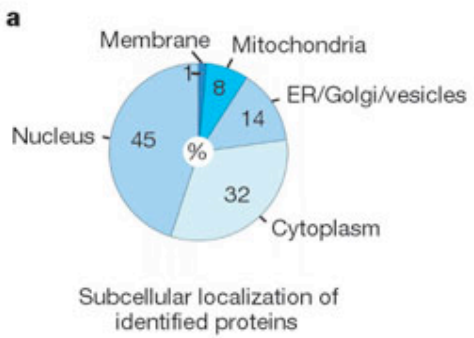


Gavin et al.,Nature 415: 141 (2002)

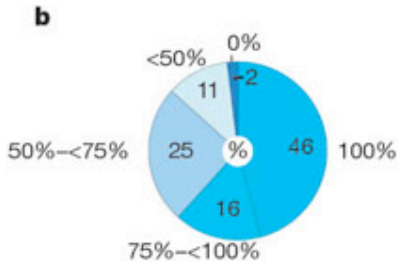


Validation of partners in protein complexes - cross correlation analysis

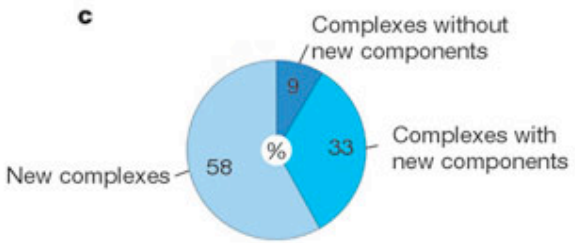
Gavin et al.,Nature 415, 141 (2002)



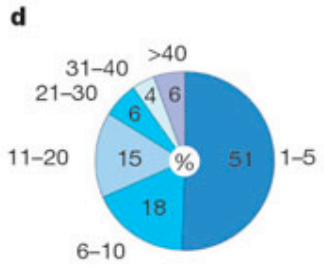
Subcellular localization of identified proteins



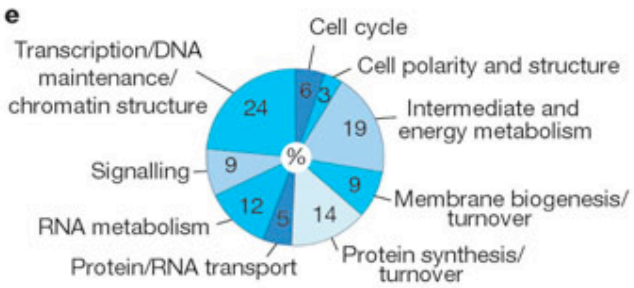
Coverage of described complexes



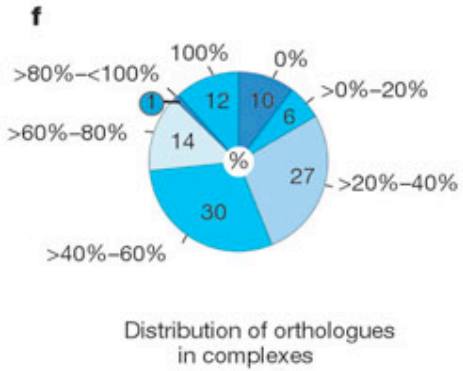
Novelties in complexes



Number of proteins per complex



Distribution of complexes according to function



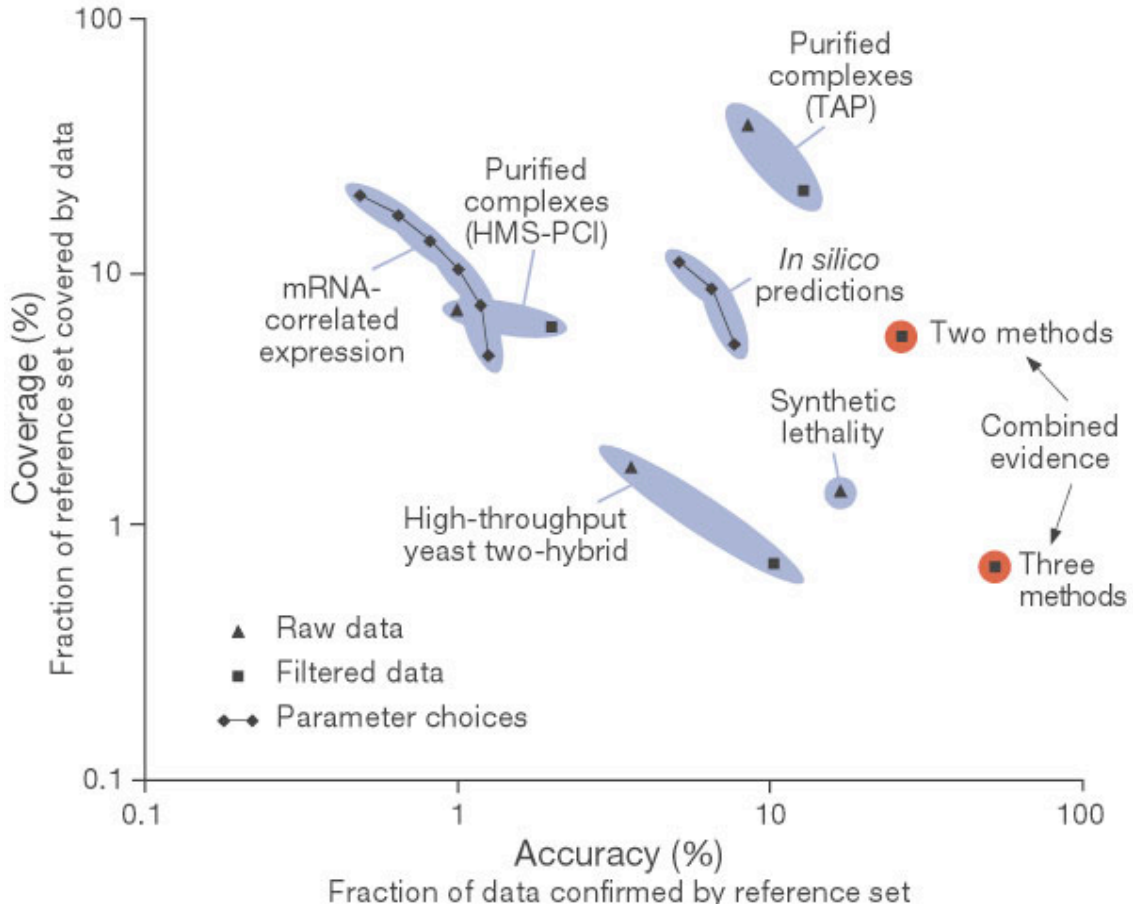
Distribution of orthologues in complexes

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

Gavin et al., Nature 415, 141 (2002)

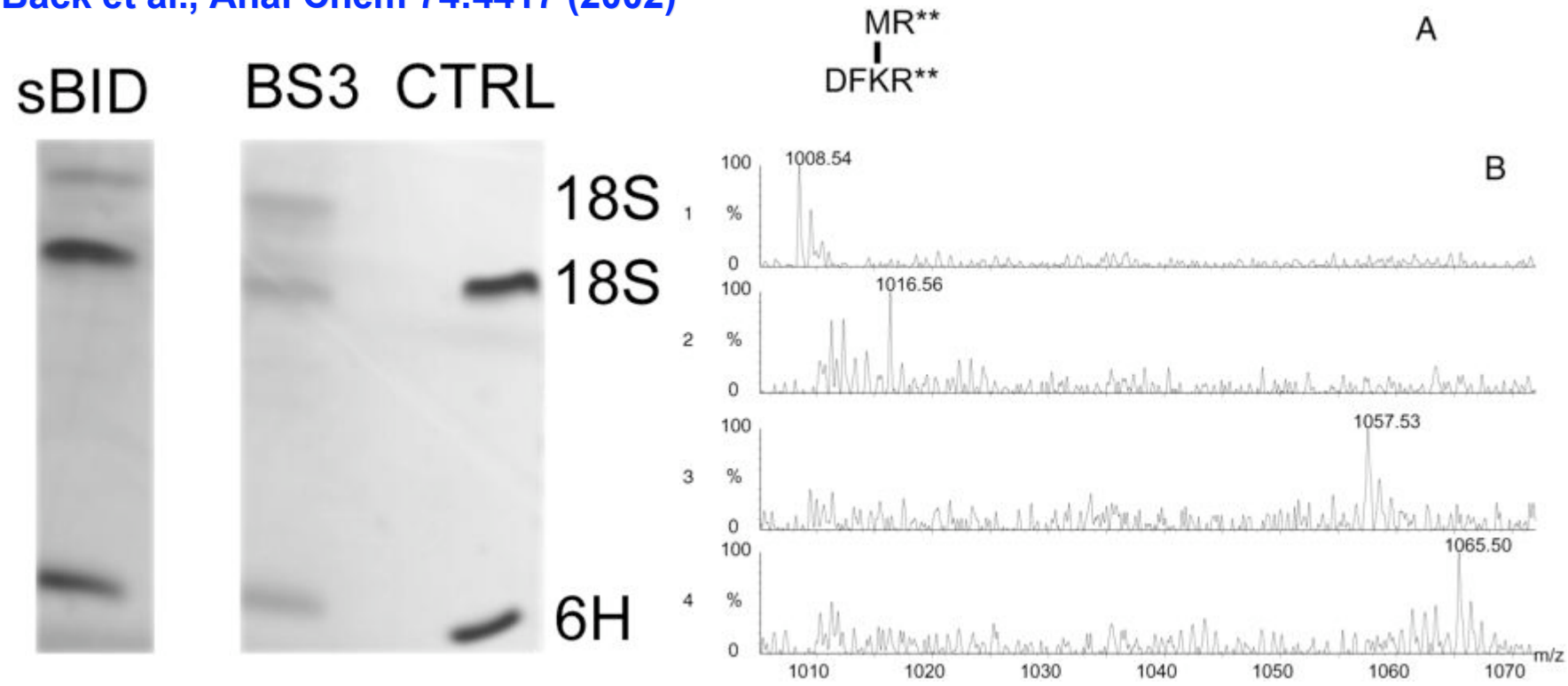
Comparison of the effectiveness of protein-protein interaction methods

Von Mering et al., Nature 417: 399 (2002)



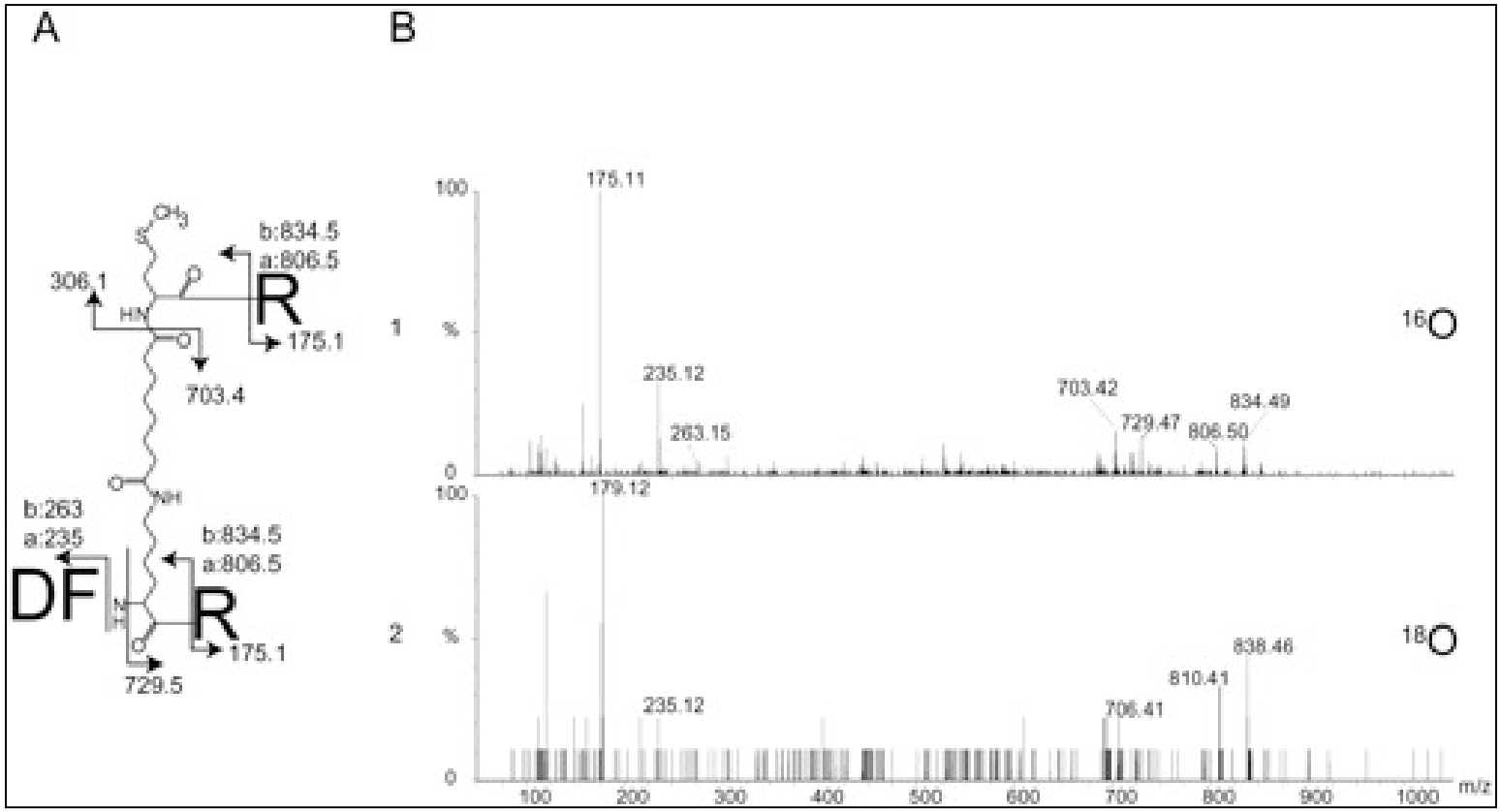
Analysis of bridged protein complexes

Back et al., Anal Chem 74:4417 (2002)



Digestion of chemically linked proteins results in a bridged peptide. If the hydrolysis is carried out in H_2O^{18}, then there will be four O^{18} and hence the MW will increase by 8

Tandem MS of bridged peptide



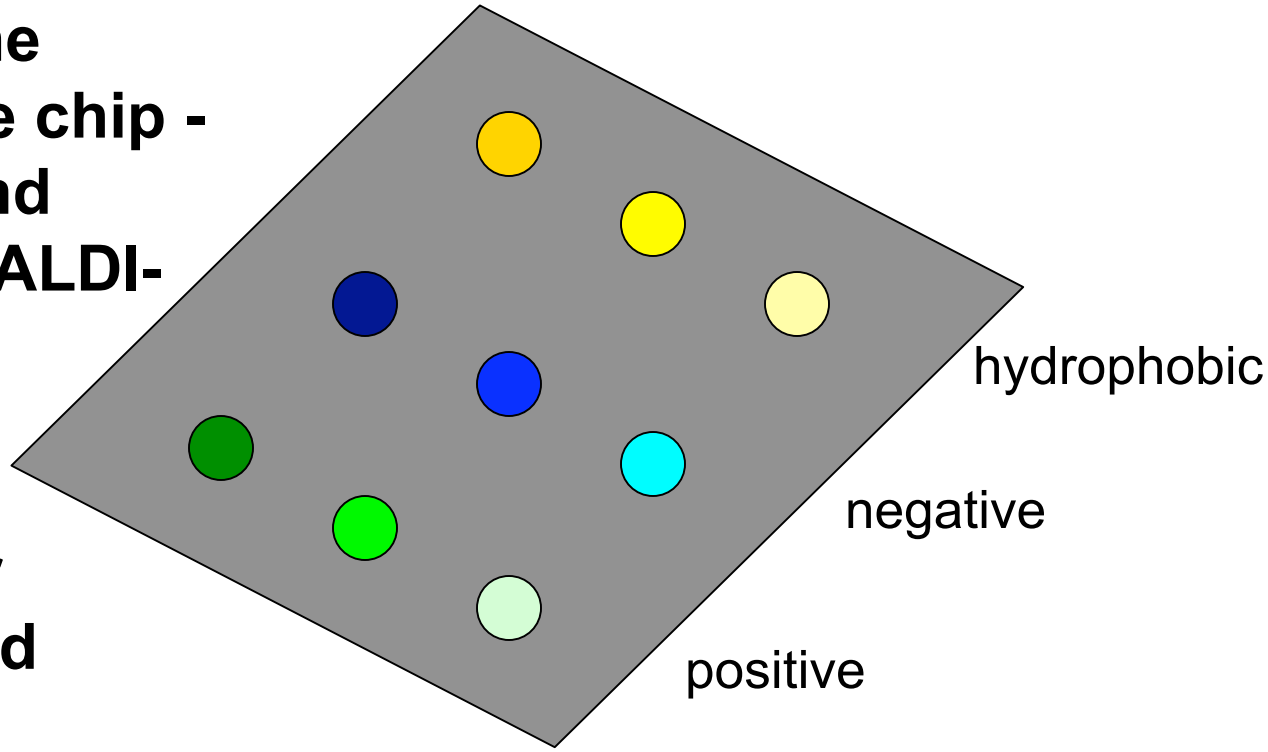
Note the increase in the Arg fragment (*m/z* 175) to *m/z* 179

Back et al., Anal Chem 74:4417 (2002)

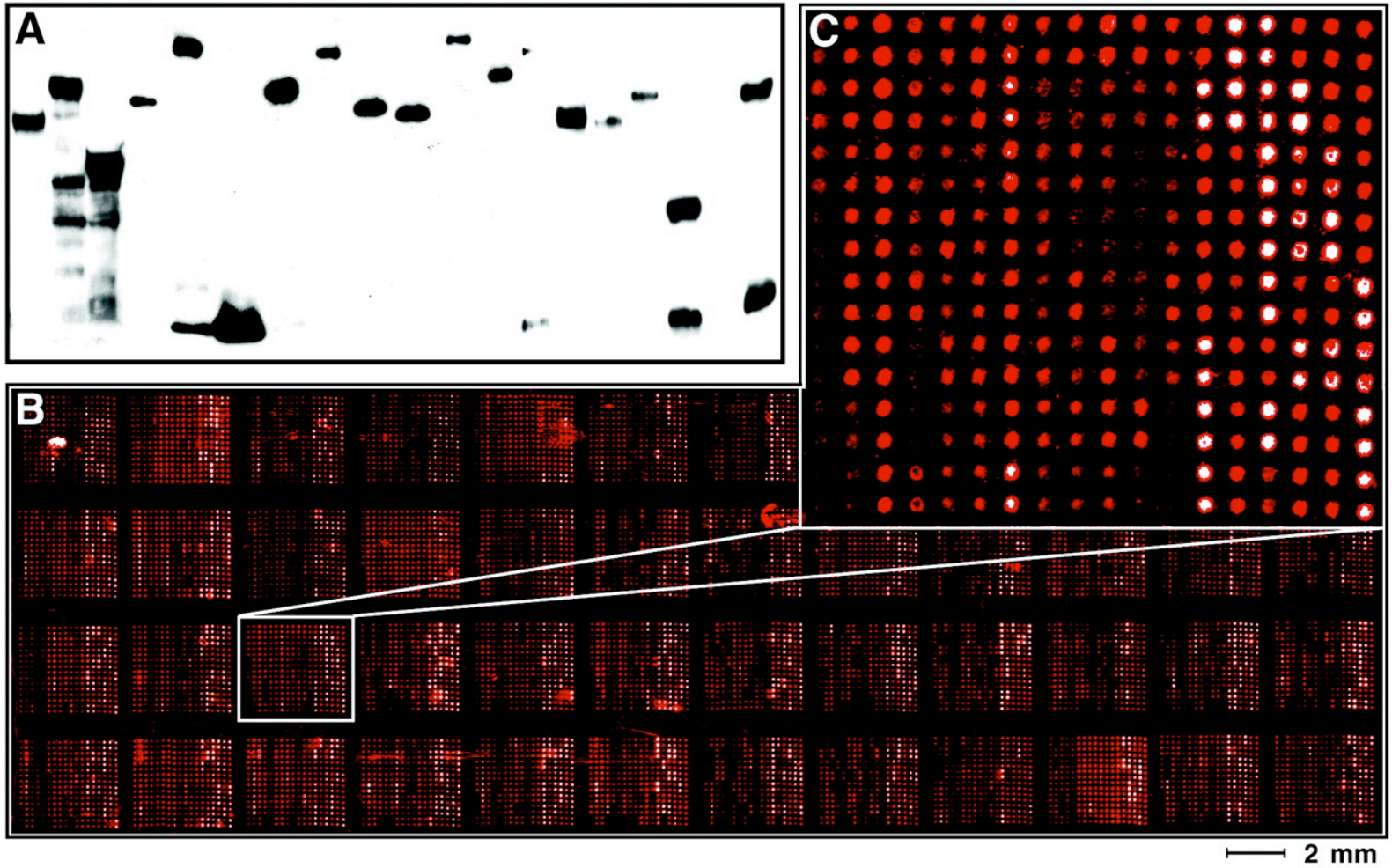
Surface enhanced laser desorption ionization (SELDI)

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**

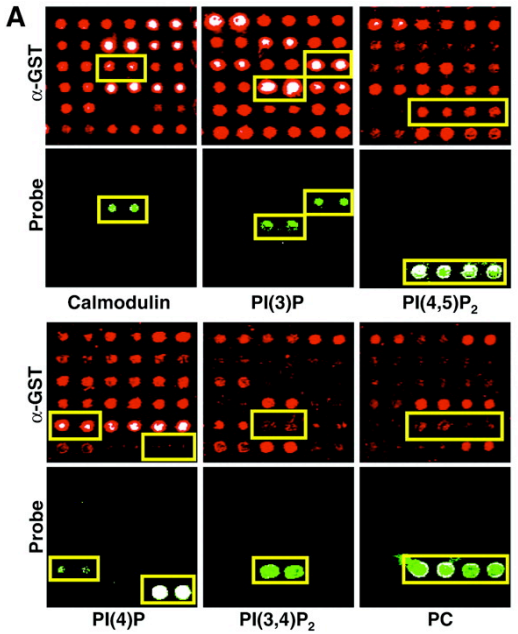


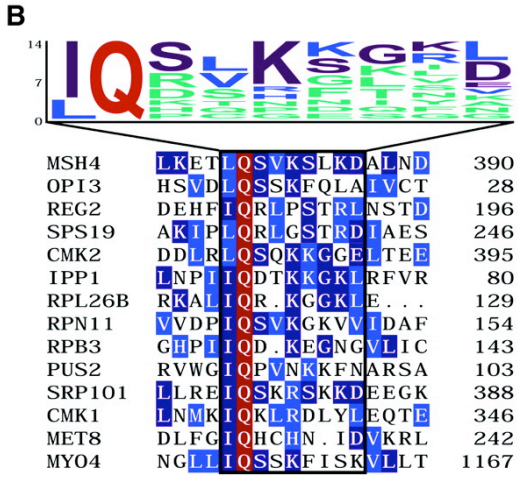
Spotted array of 80% of the yeast proteome



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)

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).

Zhu & Snyder, *Science* 293, 2101 (2001)

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*