Protein Microarrays

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NIH Proteomics Standards Meeting, January 3, 2005

What is a Protein Microarray?

Many Things are Called Protein Microarrays or Chips

- Affinity Resins for Fractionating Proteins (Ciphergen)
- Antibody Arrays
- Sets of Individual Proteins
- Protein Lysate Fractions
- Lysates from Tissues

What is a Protein Microarray?

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

Many Things are Called Protein Microarrays or Chips

- Affinity Resins for Fractionating Proteins (Ciphergen)
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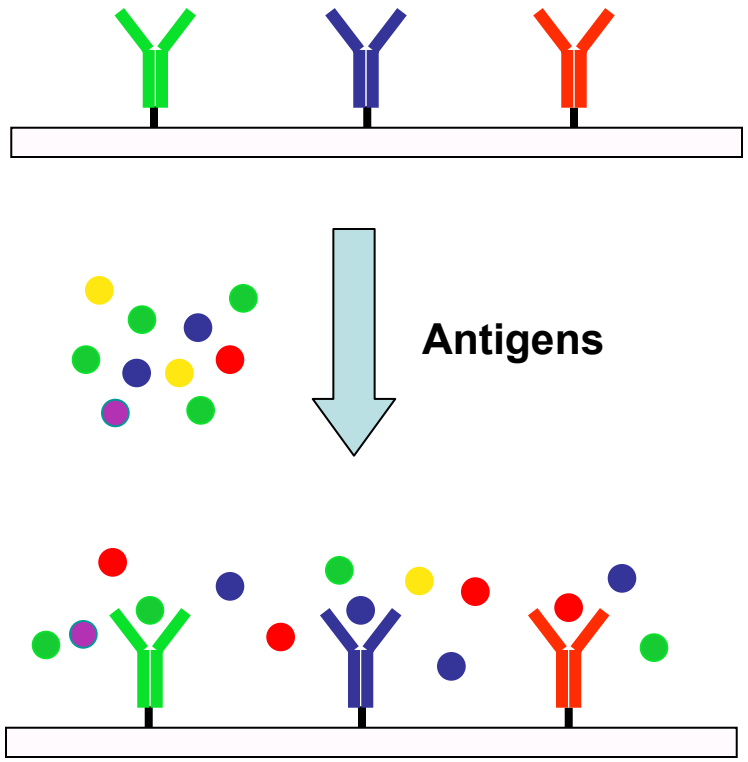
- Sets of Individual Proteins
- Protein Lysate Fractions
- Lysates from Tissues

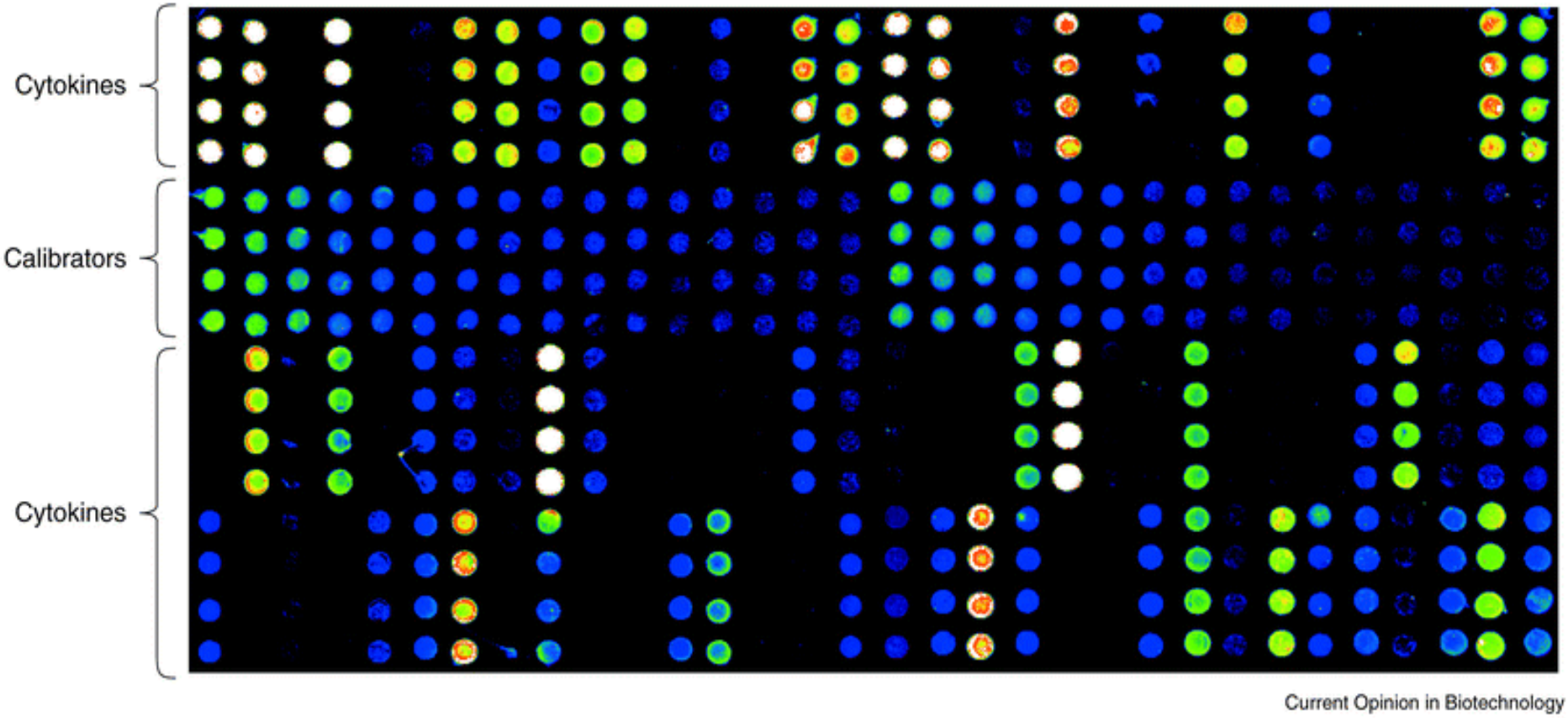
Two Major Types of Protein Microarrays

1) Antibody Microarrays

- Protein Profiling



Cytokine Detection withAntibody Microarray



Microarray assay of a human serum sample. A 15 μL sample of human serum was incubated for 30 min on a microarray with 75 different anticytokine antibodies printed in quadruplicate. Following washing and incubation with a mixture of secondary antibodies to each cytokine, detection was carried out using RCA. The fluorescent image was obtained using GenePix software on an Axon Microarray Scanner. The enlarged image shown represents one-eighth of the data acquired from a 1' × 3' microscope slide. Fluorescent intensities are represented in pseudocolor, with lowest intensities in blue and highest intensities in white.

Many Commercial Antibody Arrays Are Available

- **Arrays usually have 6-75 Antibodies**
- **Often Detect Cytokines**
- **Examples:**
  - BD Biosciences
  - Scheichler & Schuell
  - Zyomyx

Profiling Types of Arrays

Antibodies - Rabbits, Mice

Phage Display-Present
Peptides on Bacteriophage

Nucleic Acid Aptamers -
In vitro Selection

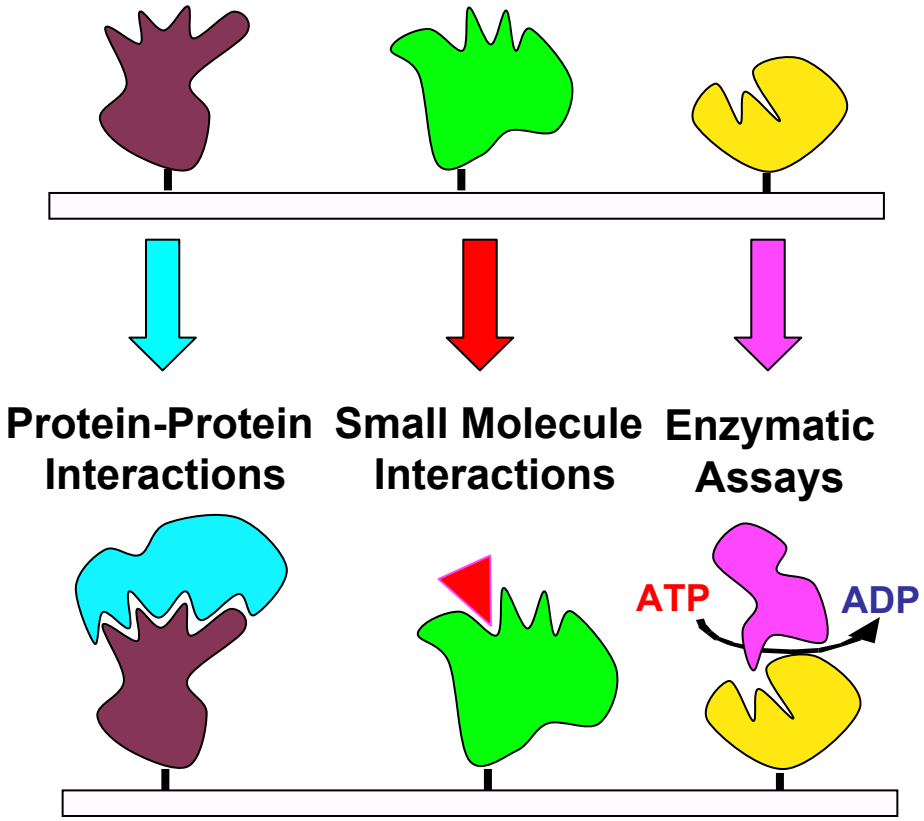
Major Challenge With Antibody Arrays

1) Antibody Specificity
   Haab et al. 20% of Antibodies Were Specific

2) Quantification

Two Major Types of Protein Microarrays

2. Functional Protein Microarrays

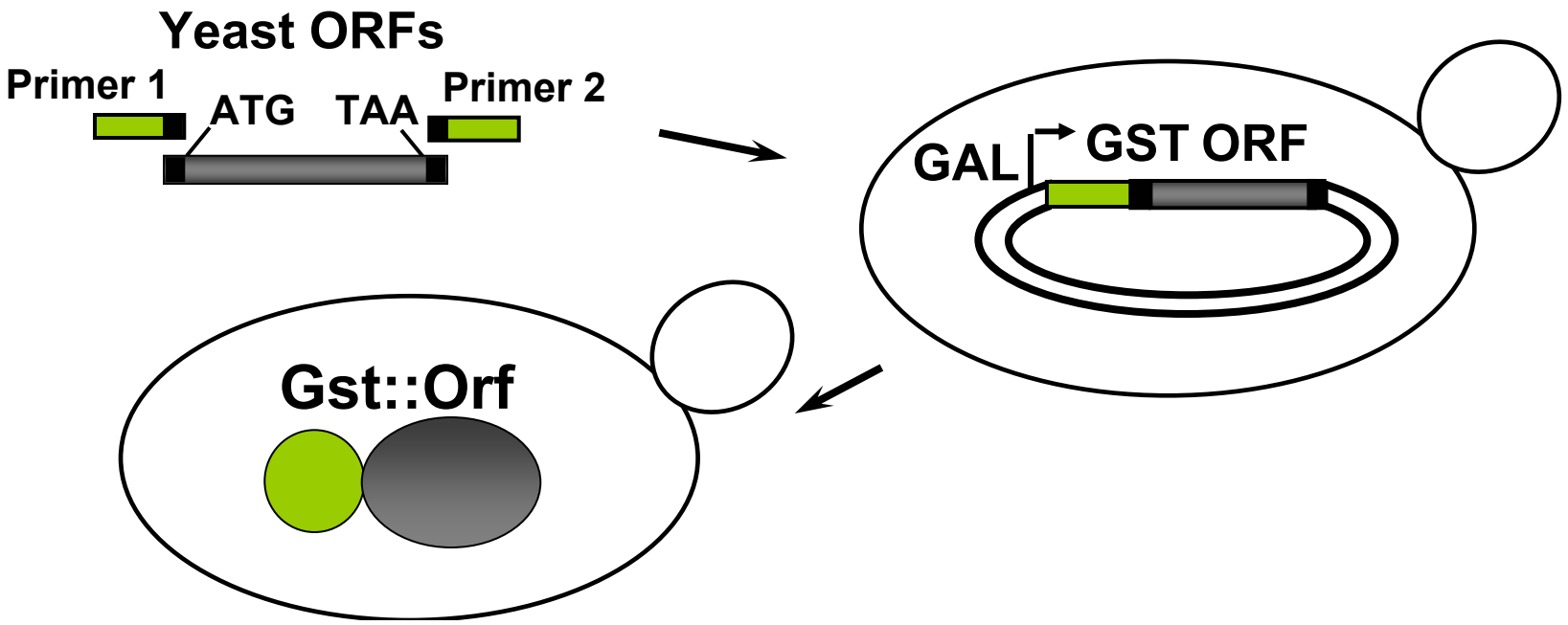


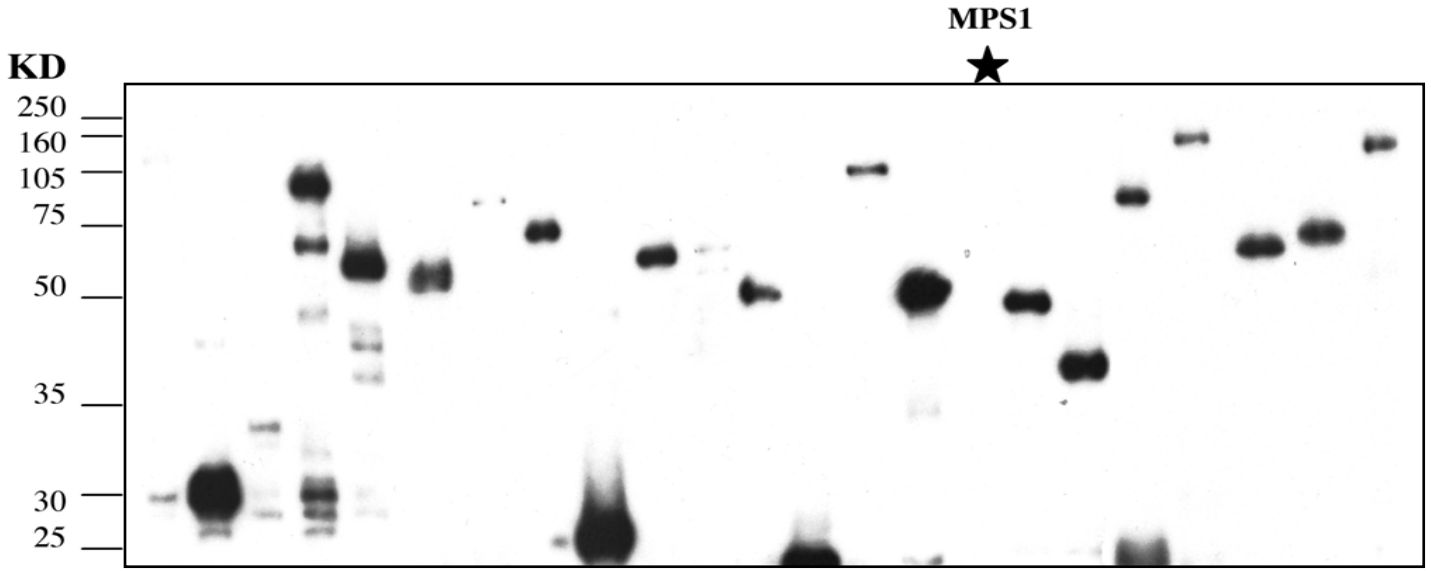
- Protein Biochemical Activities
- Protein Modification and Regulation
- Protein Pathways
- Drug Discovery and Development

What's Needed to Make Functional Protein Arrays

- Expression Library
- Methods for Purifying Many Proteins
- Array Technology

Cloning & Expression Strategy

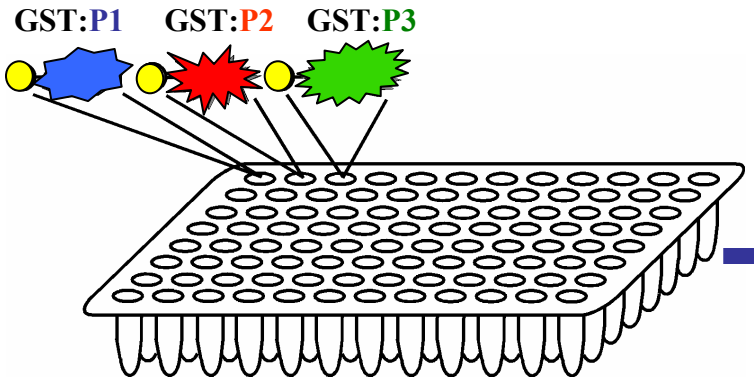




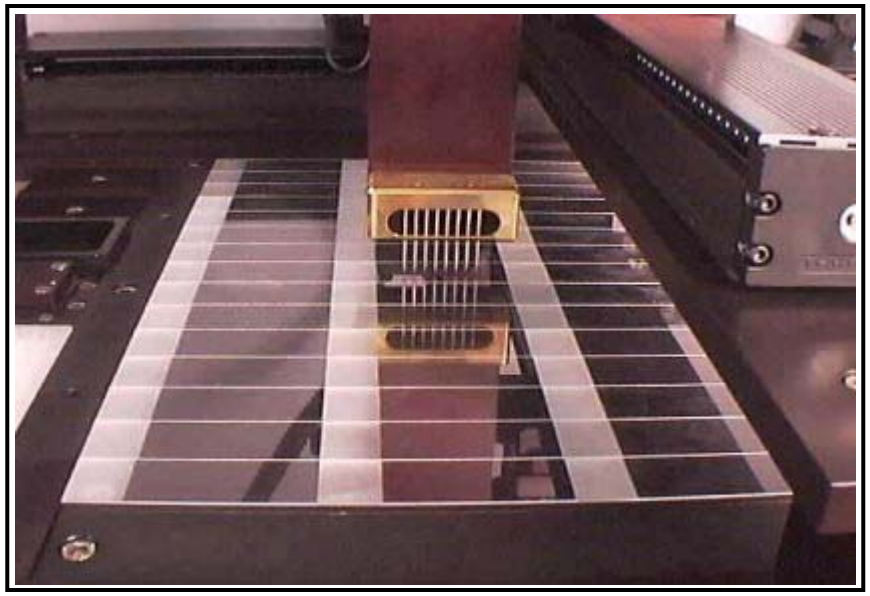
There are Many Expression Systems

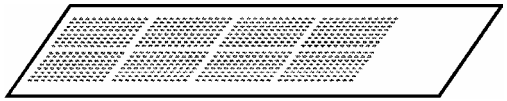
- E. coli
- Yeast
- Bacullovirus
- Plants
- Cell Free Systems (In vitro transcription/translation)

Printing the Yeast Proteome

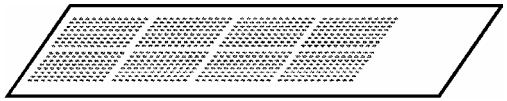


Source Plate

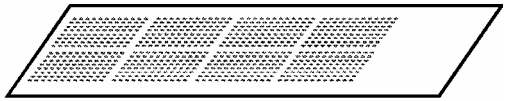




Protein-Protein



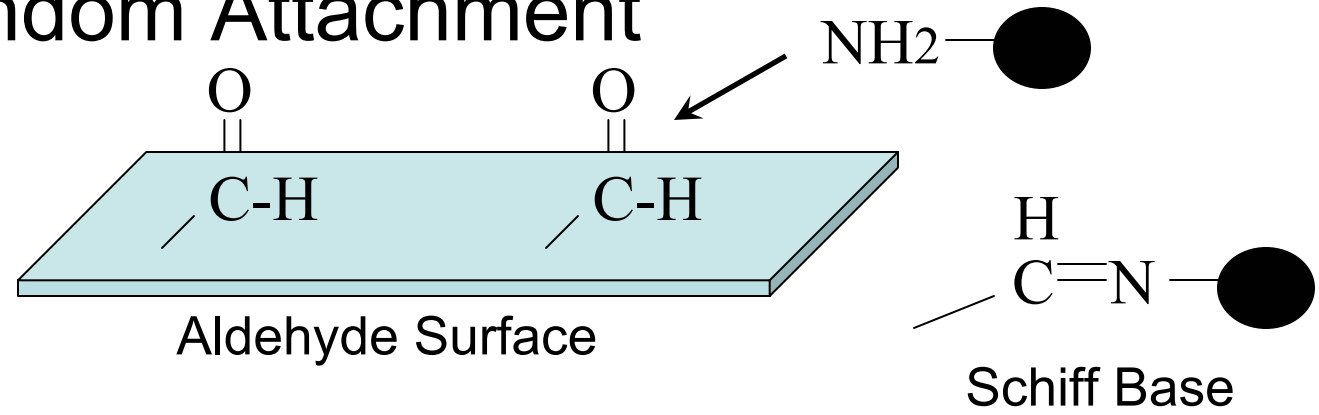
Protein-Lipid



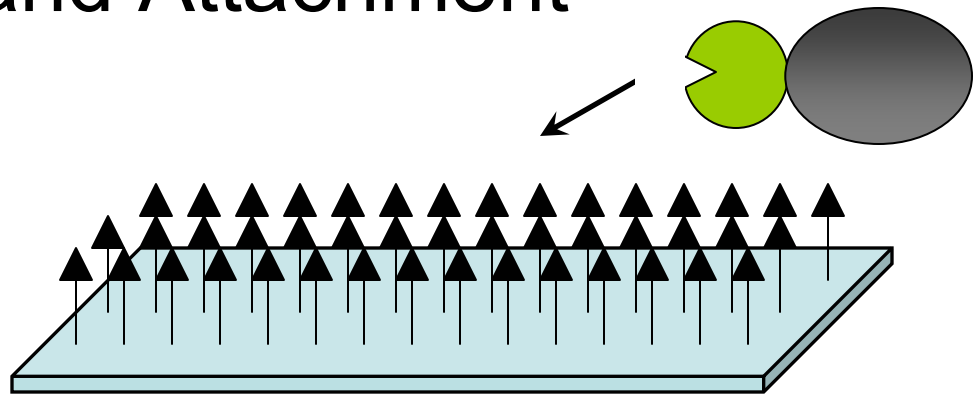
Protein-DNA

Glass Slides

1) Random Attachment



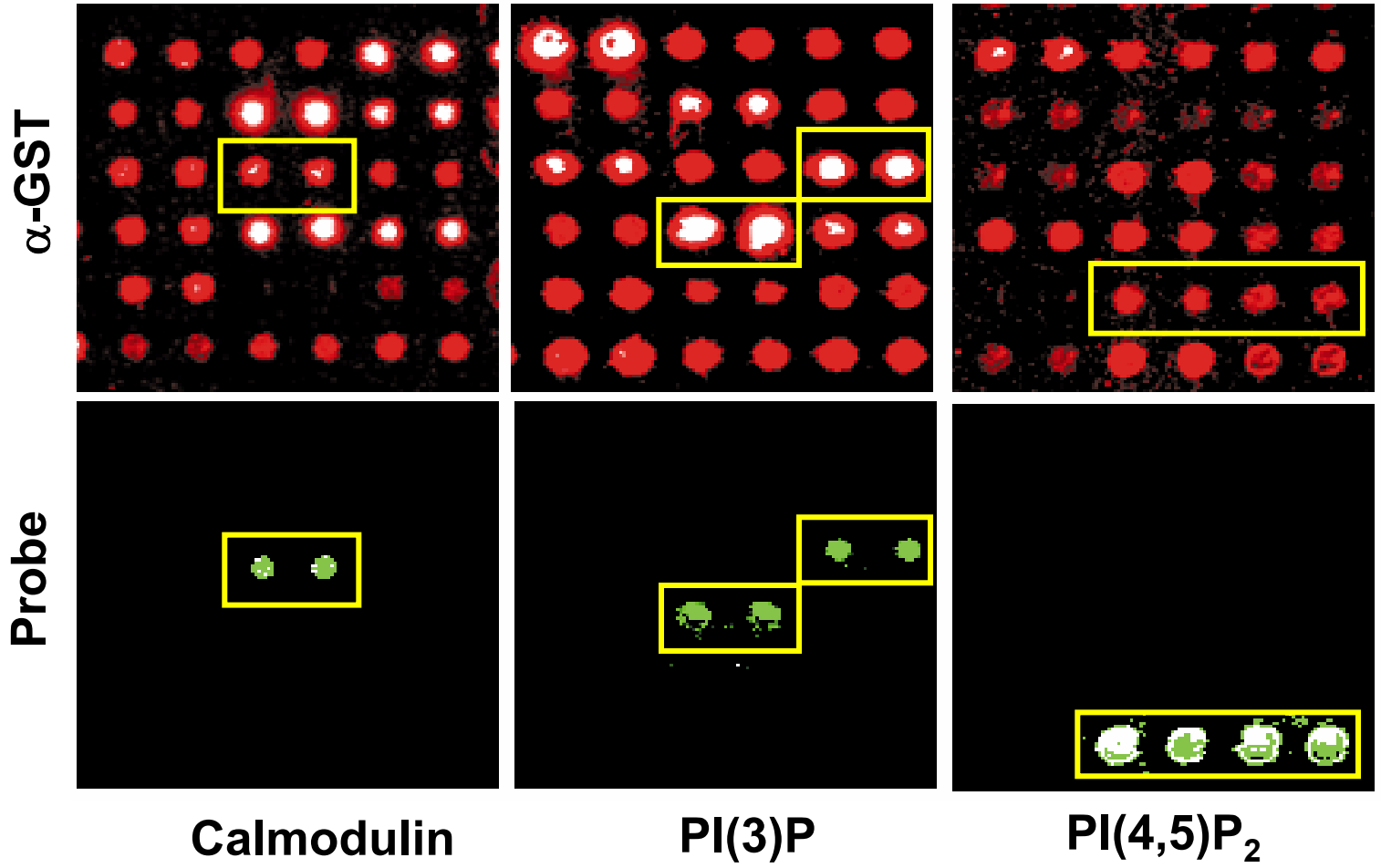
2) Ligand Attachment



Screens Thus Far

- 20 Protein-Protein Interactions
- 8 Protein-Lipid Interactions
- 3 Nucleic Acids (dsDNA, ssDNA, polyA-mRNA)
- 4 Small Molecule Screens
- 3 Posttranslational Modifications
- 14 Antibodies
- 89 Kinase Probings

Biochemical Assays on Proteome Chips



Functional Protein Arrays Commercially Available

1) Yeast proteome

2) Human 2K array

General Issues for Standardization

1) Protein content & array platforms vary widely (Expression systems; slides, etc)

2) Protein quality may vary from prep to prep.

3) Negative results harder to interpret than DNA arrays

General Issues for Standardization (cont.)

4) Ideally, measurements should be quantitative

Ab arrays - Each Ab must be standardized

Func. Protein Array - like to get affinity constants

5) Field is still maturing

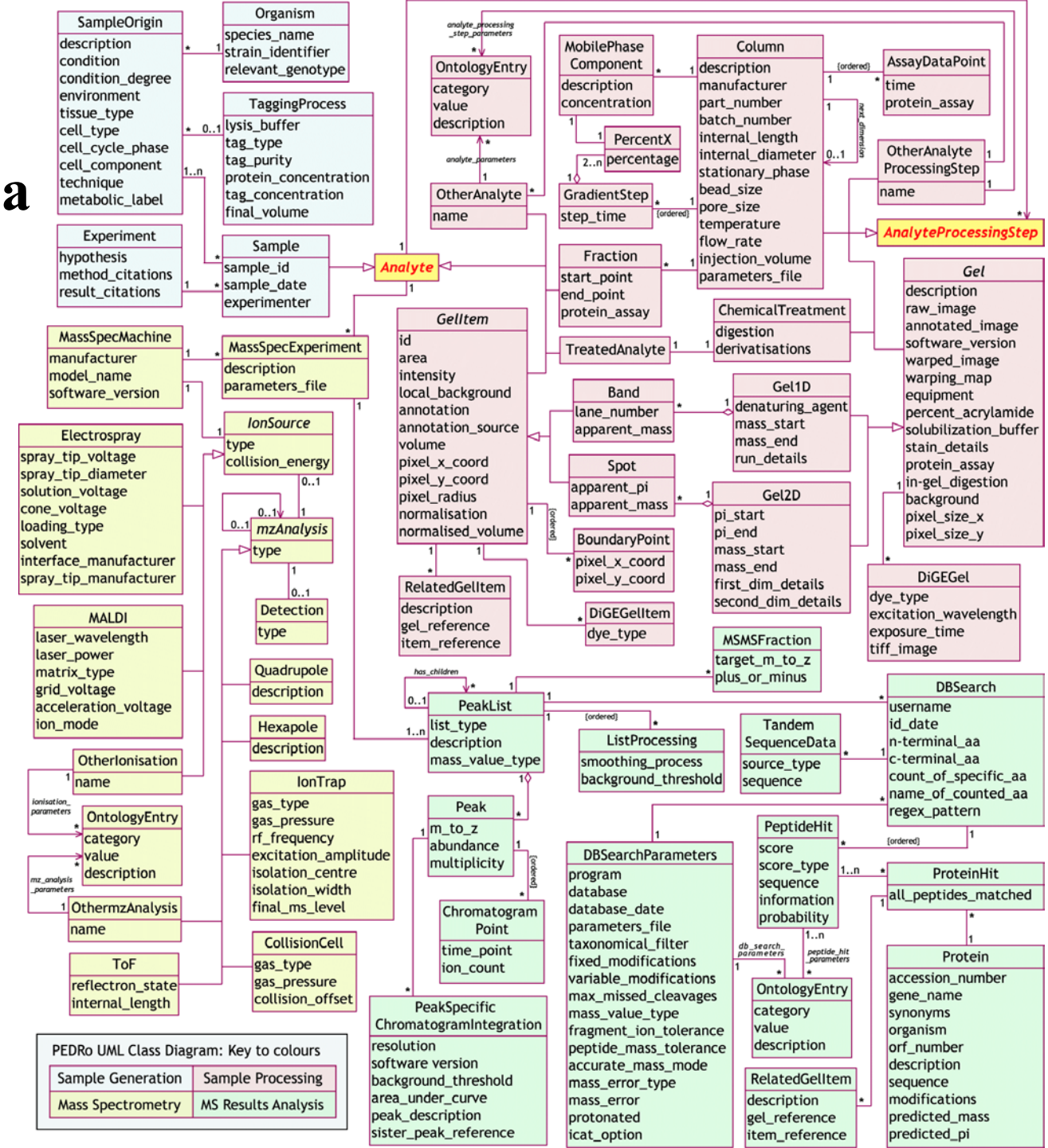
Informal QC Standards That Currently Exist:

- Antibody Arrays:
  Immunoblot Analysis
- Functional Protein Arrays:
  Assess Protein Levels and Purity
- Validation
- Provide Hit List

Formal Community Standards That Currently Exist:

0

PEDRo Schema



Formal Community Standards That Should Exist:

- People should have access to all primary and minimally processed data.
- Source, quality and amount of the proteins (e.g. Antibodies) should be documented.
- Results need to be validated statistically or by other means.

Recommendations

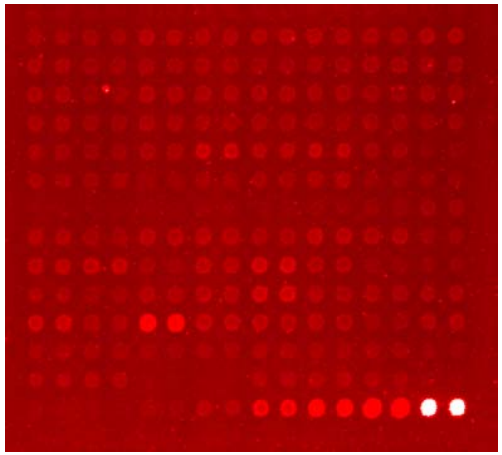
1) Need to Establish a Data Repository (analogous to GEO)
2) Need to Establish Mimimum Reporting Standards (MIAME)
3) Interactions should be deposited in a public database (e.g. BIND)

Comparison Between Protein and DNA Arrays

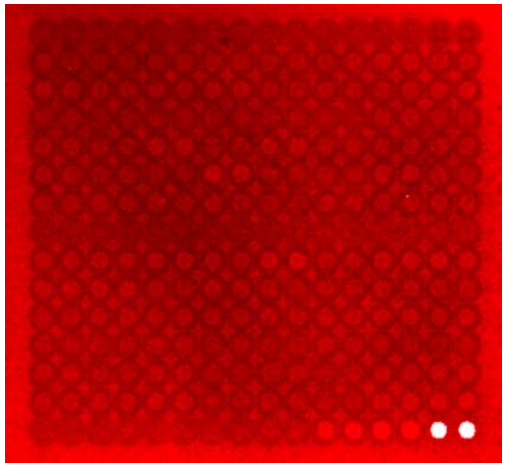
	Funct. Protein array	**DNA array**
Common problems	Smudges, uneven probing (position artifacts)	
Aim	Varied; e.g.Binding assay	Specific probe amount
Design	Diverse formats: Surfaces; prot. source	Few standard formats
Probes	Pure	Mixture
Array features	Uneven amount	Even amount
Color	Mostly one channel	One or two channels
Factors for the intensities	1. Binding affinity 2. Feature amount on the array	Amount of specific probes
Non-specific binding	-Sticky tags -Anti-spots	Trivial

Non-specific binding

All spots react



Anti-spots



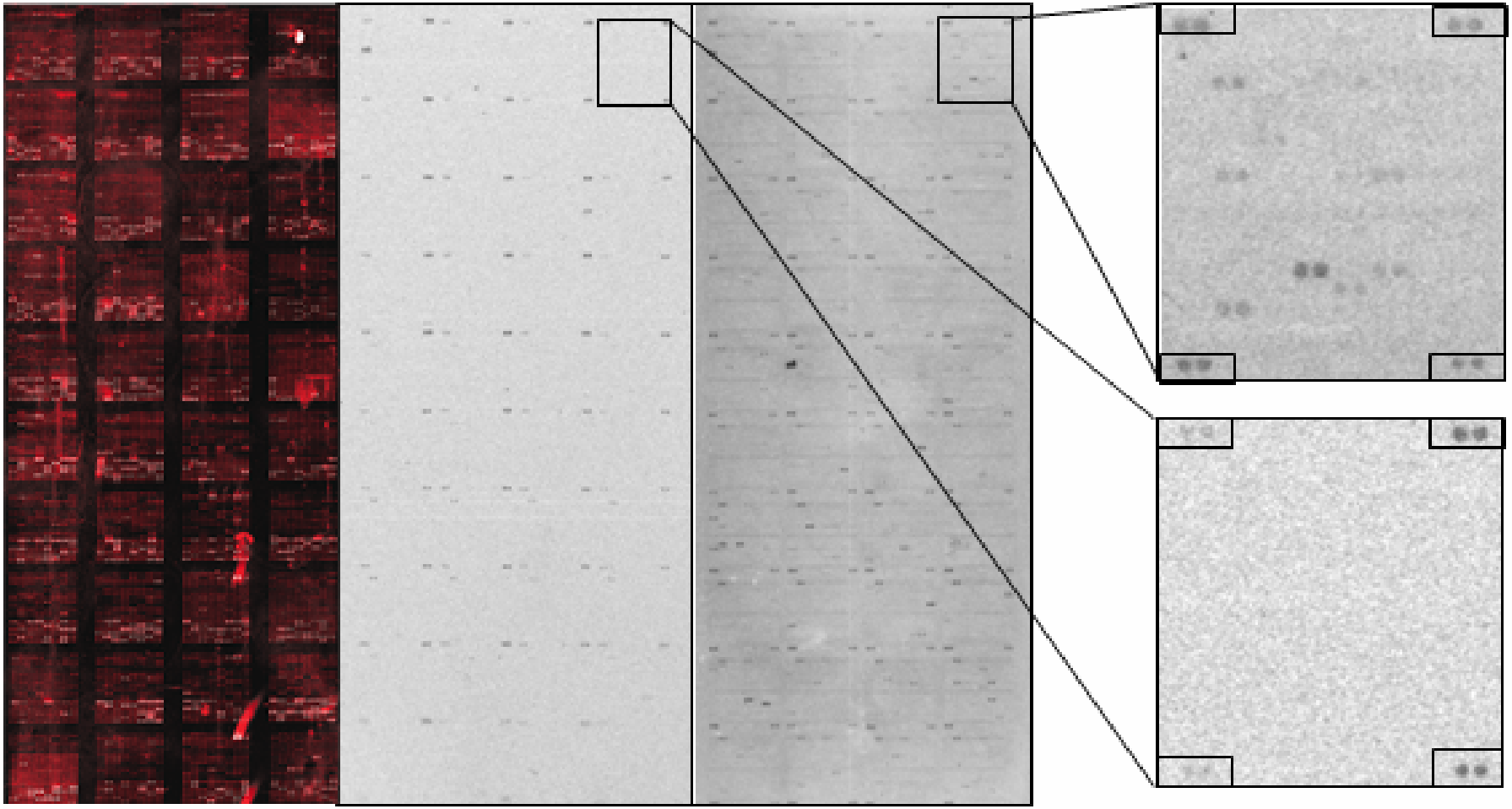
- Raw intensities can be misleading
- Negative control is necessary
- Subtract signals from neighboring spots; normalize to negative control probings

- “Negative” signals
- Blocking problems
- Reprobing

Next Steps

- Have protein microarray experts establish standards in conjunction with the microarray community
- Deposit interactions in database
- Start discussions now

Kinase Assays on Protein Chips



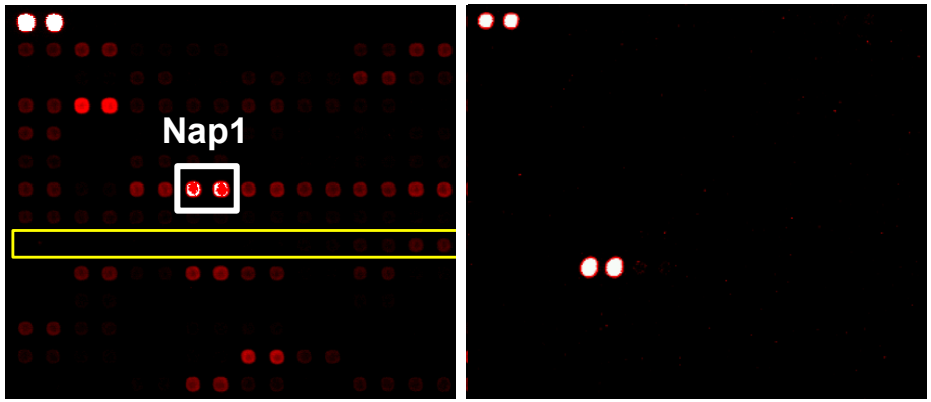
Anti-GST

Auto-Phosphorylation

Tpk1

Antibody Probing of the Yeast Proteome Microarray

	Antibody	# of +s
Monoclonal (3 Yeast + 3 Control)	α-Sed3, α-Cox4	1
	α-Pep12	4
Anti-Peptide Polyclonal (6)	α-Hda1	8
	α-Mad2	1
Anti-FL Protein Polyclonal (2)	α-Nap1	1770
	α-Cdc11	7



Anti-Nap1

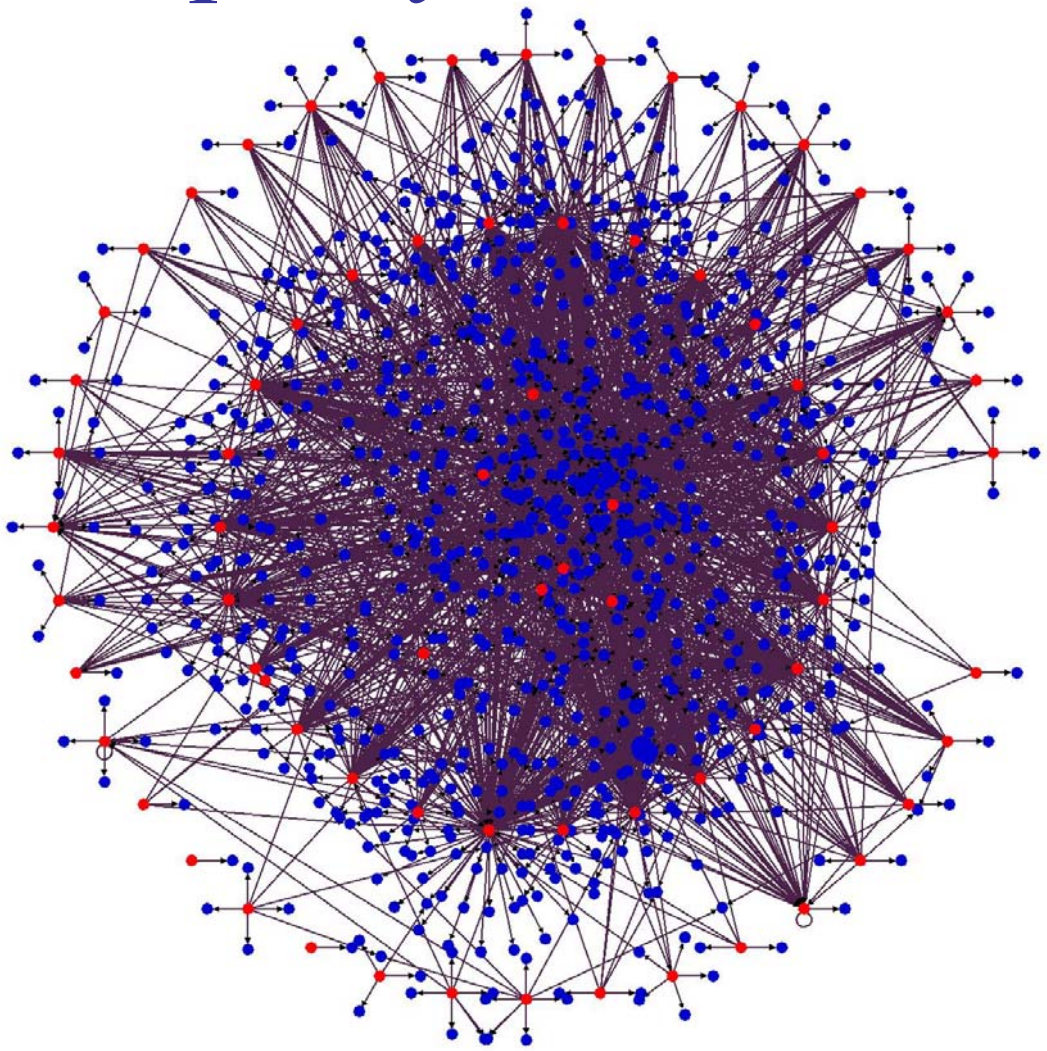
α-Sed3p

Protometrix

Software Issues

- DNA array software can be used but the protein chips often have unique features not present in DNA arrays

Phosphorylome Network



Yeast Phosphorylome Map

122 Protein Kinase Homologs

- 14 Uncharacterized
- 50% Have no known *in vivo* substrates
- <160 Known kinase-substra
  phosphorylations

In Vitro Phosphorylome Summary

ARK1 (8) CKA1(26) CBK1 (1) ELM1 (5) ERK2 (43)
GIN4 (5) HRR25 (13) KIN1 (3) KIN2 (28) KNS1 (8)
MEK1 (33) PBS2 (6) PKK2 (5) PHO85-ALONE (6)
PHO85-PCL1 (4) PHO85-PCL2 (9) PHO85-PCL9 (11)
RIM11 (19) SLT2 (8) STE11 (2) STE20 (100) SWE1 (7)
TPK1 (130) TPK2 (30) TPK3 (82) VHS1 (16) YGL059C
(101) YMR291W (1) YOL128C (9) FUS3 (8) PTK2 (202) IPL1
(2) YCK3 (1) YAK1 (4) PRR1 (7) PKA (41) PRR2 (24)
SKM1 (26) SKS1 (27) CDC5 (21) CLA4 (30) CTK1 (10)
MKK1 (12) CDC15 (18) CDC28-Clb5 (56) DBF2 (85) IME2 (74)
PAK1 (17) RAD53 (32) YGR052W (10) KSP1 (190) YCK1 (85)
IRE1 (88) HAL5 (35) SAT4 (23) SSK22 (25) MCK110
(112) DUN1 (31) YKL171W (53) IKS1 (19) FUN31 (27)
KIN28 (14) PRK1 (61) RCK2 (46) CMK2 (14) KCC4 (10)
KIN4 (30) YOR267C (20) BCK1 (85) SRB10 (15) HSL1
(59) YPL141C (66) YDR466W(11) RIM15 (26) RIM15dead
DBF2dead HSL1dead RAD53dead

Conclusions

1) Construct protein microarray containing nearly an entire proteome
2) Screen for diverse activities: interactions with proteins, DNA, small molecule; antibody specificity; kinase substrates
3) Unbiased screens yield unexpected results. Examples:
   - Arg5,6
   - Many novel substrates of kinases
4) Construct an in vitro phosphorylome map

Advantages of Protein Chips

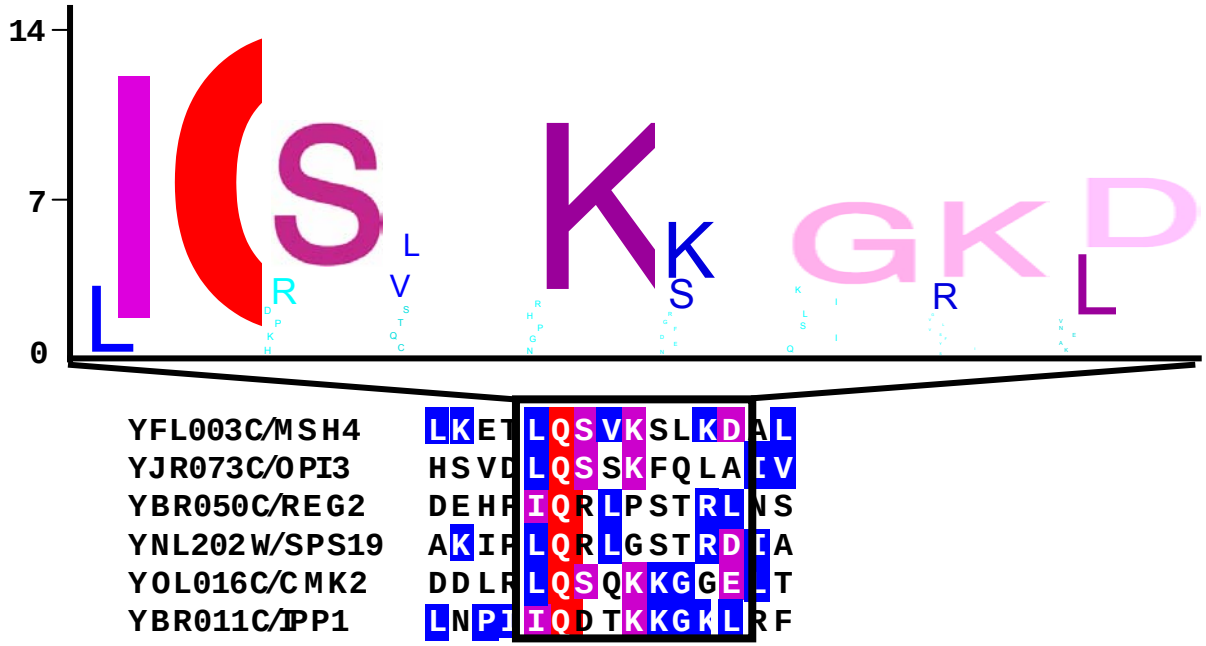
- Can screen many proteins simultaneously
- Small amounts of proteins and reagents
- High throughput
- Diverse applications-biochemical assays, posttranslational modifications, small molecule screening

Disadvantage

- In Vitro Assay

Calmodulin-Binding Proteins

- 12 Known or Suspected Targets
- 33 New Binding Proteins
- Derived New Consensus Binding Site

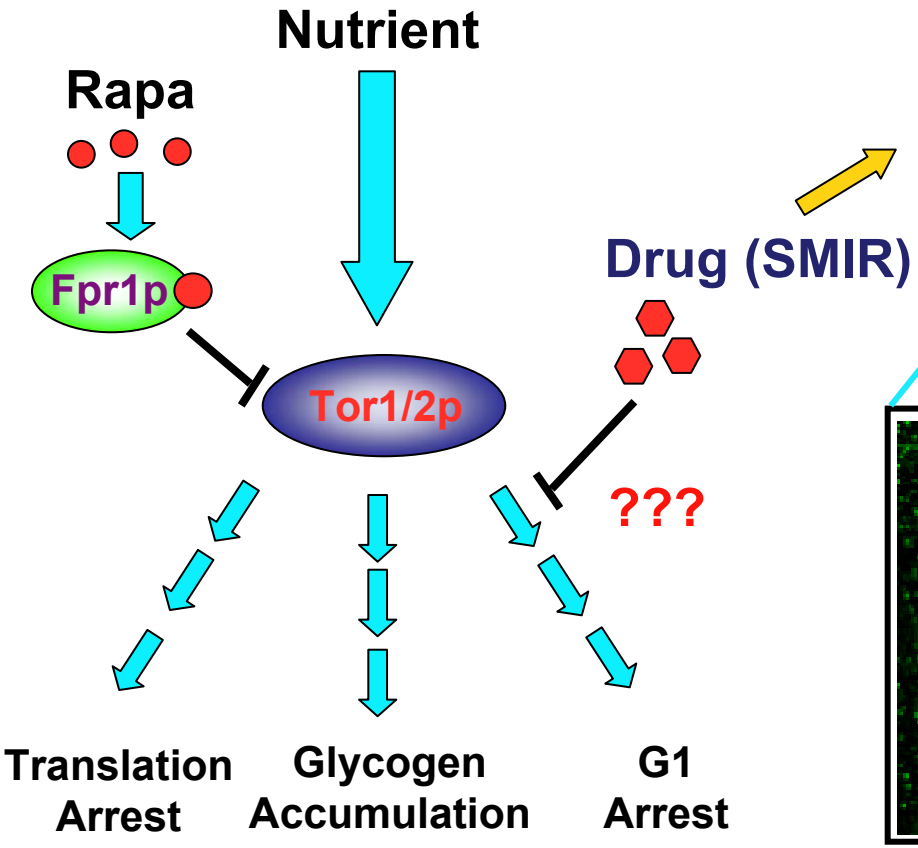


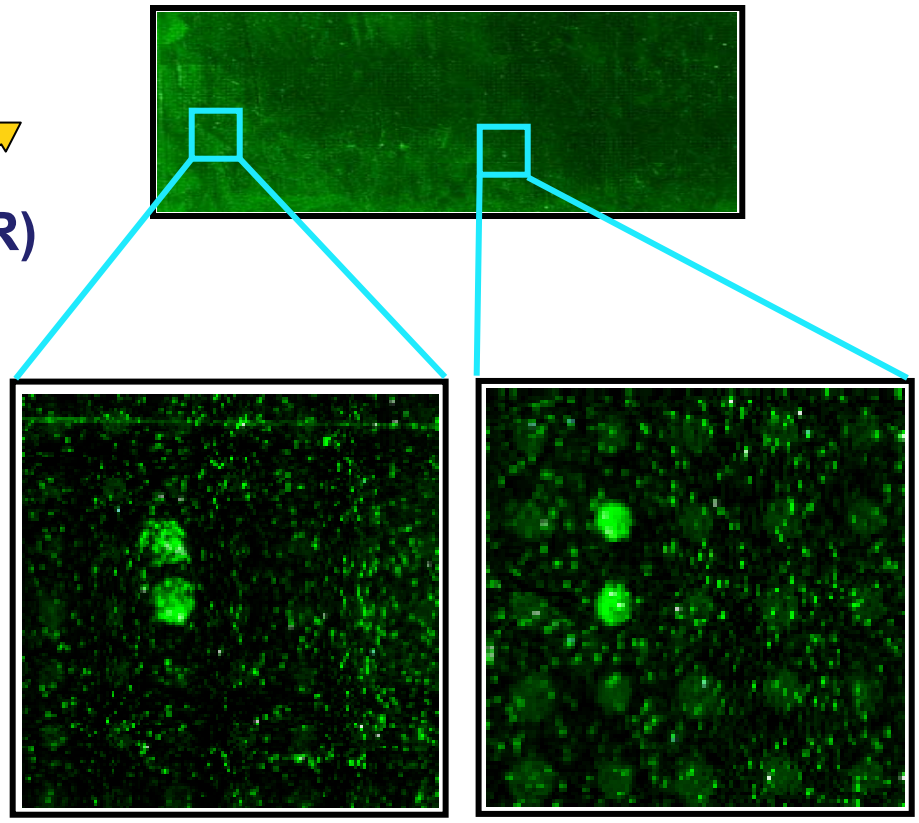
Summary of Genomic DNA Screen

- ~200 Proteins bound DNA probe
- 8 Novel ChIP chiped
  - 5 No loci enriched
  - 3 Showed enrichment:

    Mtw1, Dig2, Arg5,6

Identification of Drug Targets

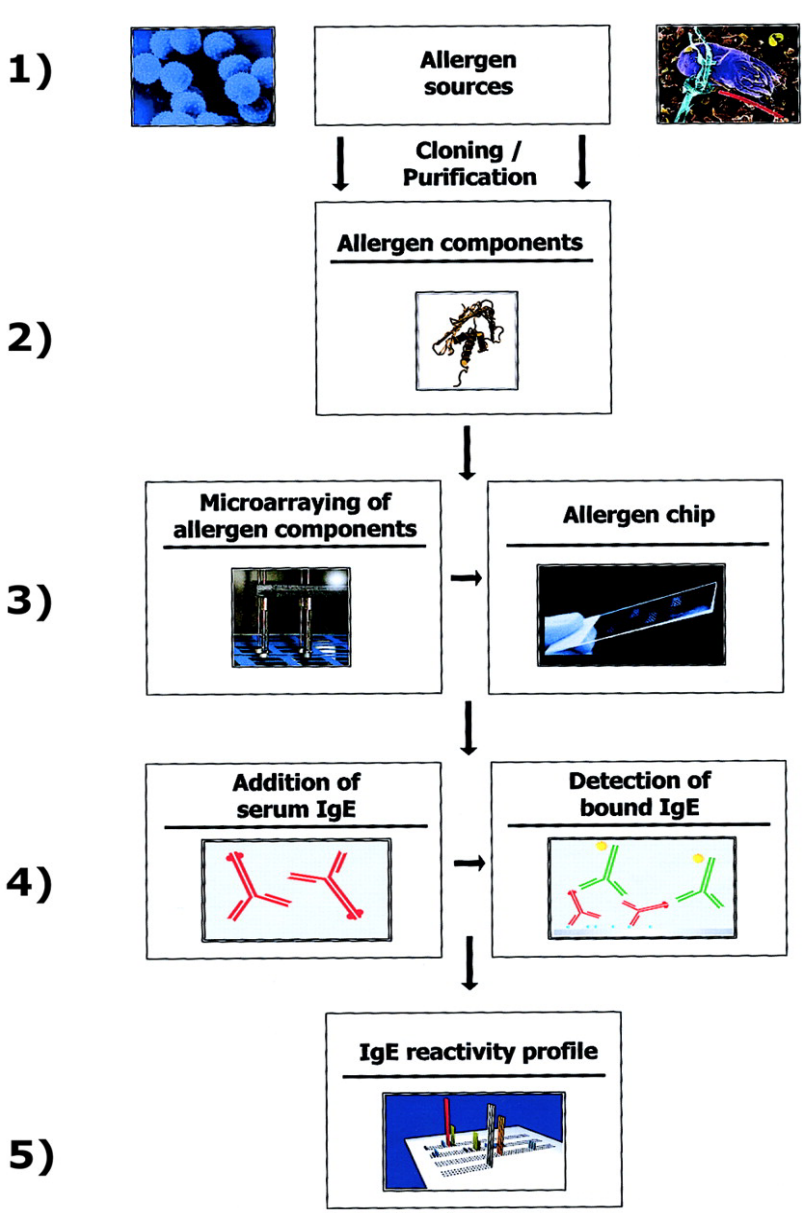


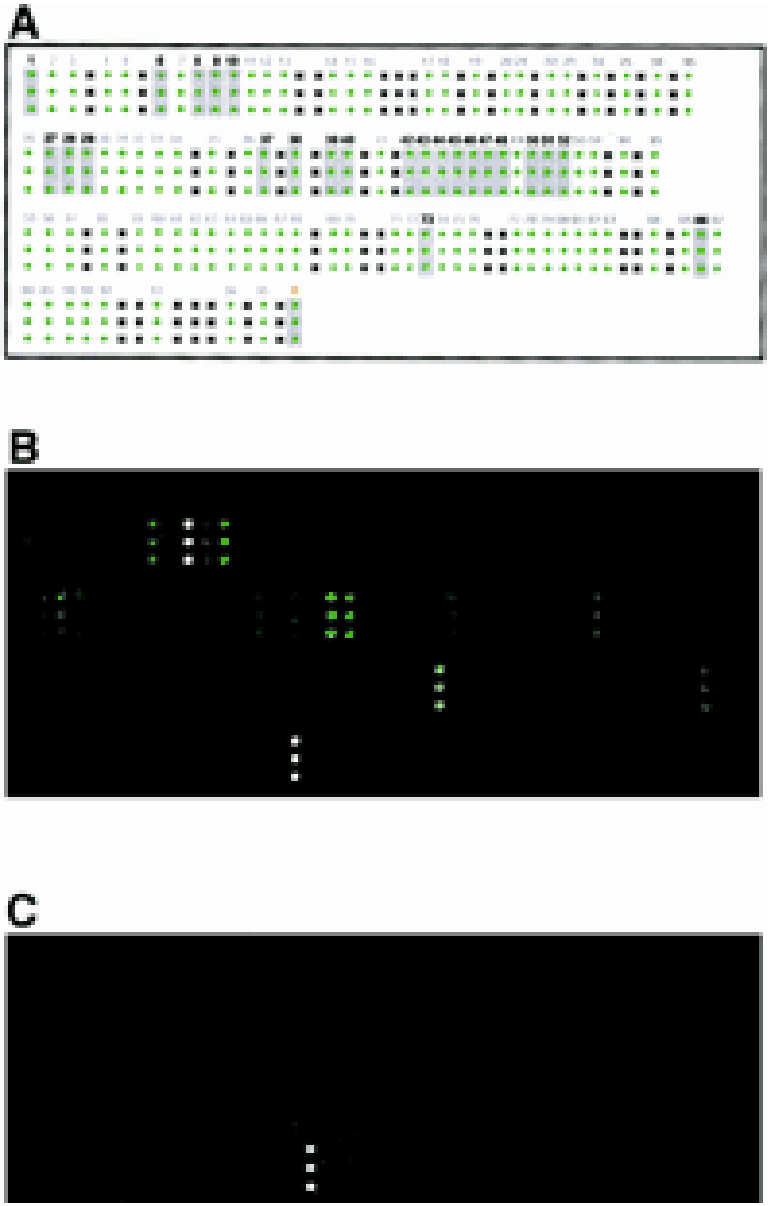


J. Huang, H. Zhu, S. Schreiber, M. Snyder

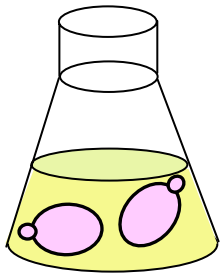
SMIR3 8 Targets
SMIR4 30 Targets

Human Allergen Microarray

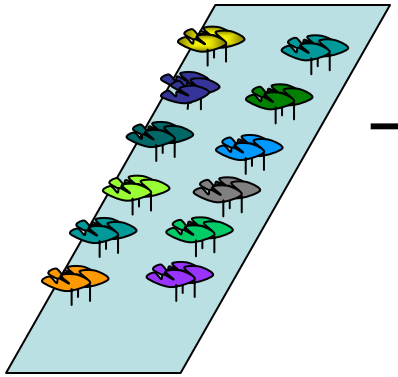




Global Analysis of Kinase Substrates



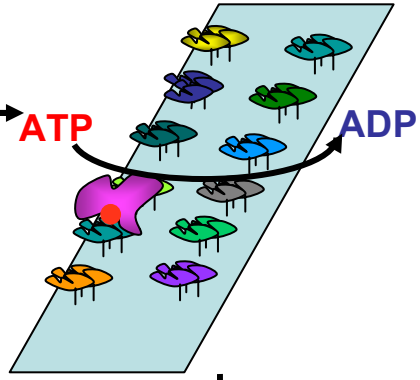
Overexpress and Purify Kinase



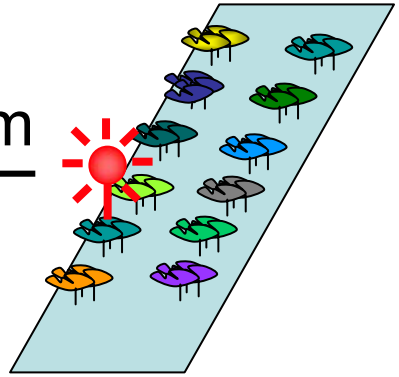
Proteome Array

Incubate with Kinase and $^{33}P\gamma$-ATP

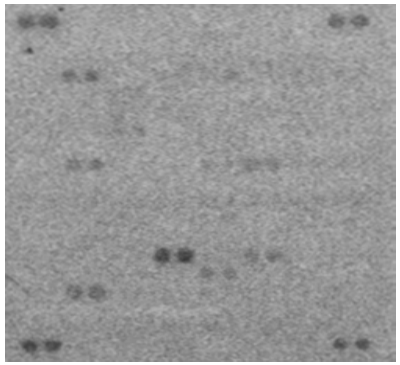
30°C



Wash



Expose to Film



In Vitro Phosphorylome Summary

- 84 unique kinases and several isoforms with different cyclins for 89 specific hit lists

- 3291 total phosphorylation events on 1238 individual targets

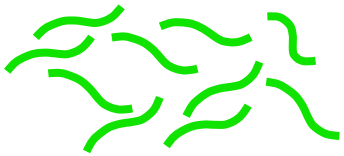
- On average kinase phosphorylated 48 proteins on chip (Range 1- 202)

- Most substrates were phosphorylated by only one kinase

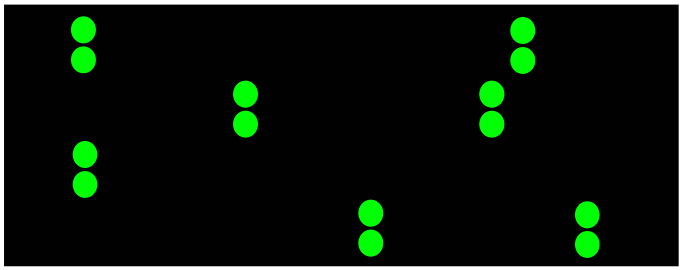
- Identified at least 13 known kinase-substrate phosphorylations

Identification of New DNA Binding Activities

Cy3 labeled genomic DNA



Probe proteome chip



~200 Positives