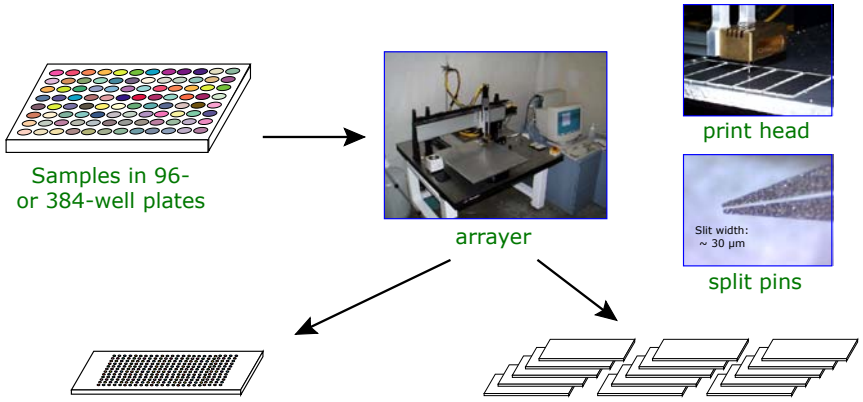
Extending Microarray Technology to Study Protein Function

Gavin MacBeath
Bauer Center for Genomics Research
Harvard University

MICROARRAYS: THE POWER OF MULTIPLEXING



simultaneous analysis

cDNA microarrays

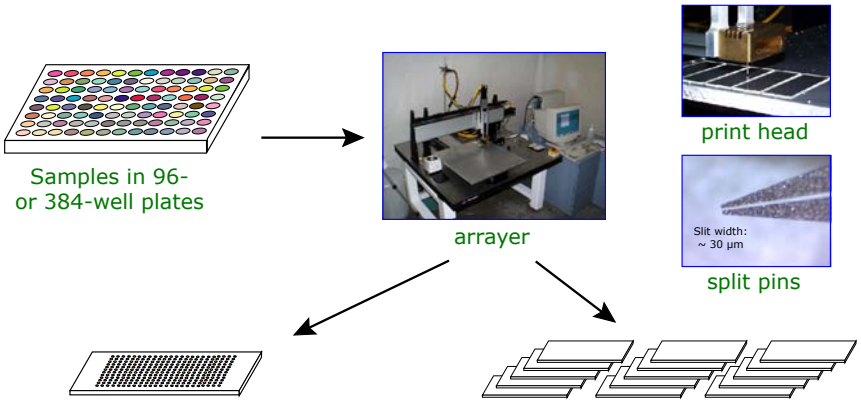
- quantitate relative abundance of >10,000 different mRNAs between two cell samples

repeated analysis

cDNA microarrays

- quantitate >10,000 mRNAs from cells under >100 different conditions
- cluster genes that are co-regulated

MICROARRAYS: THE POWER OF MULTIPLEXING



simultaneous analysis

protein microarrays

- screen >1000 proteins simultaneously

repeated analysis

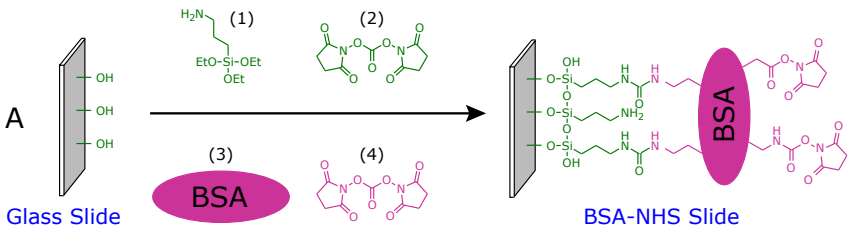
protein microarrays

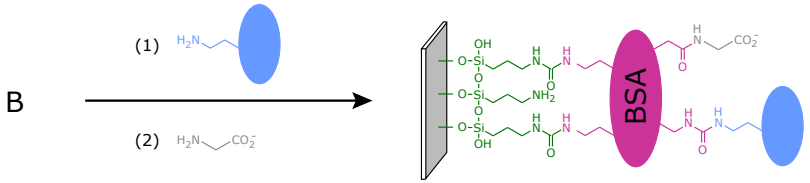
- screen >1000 proteins with >1000 proteins
- screen >100 protein-protein interactions with >10,000 small molecules (competition assay)

KEEP THE PROTEINS HAPPY

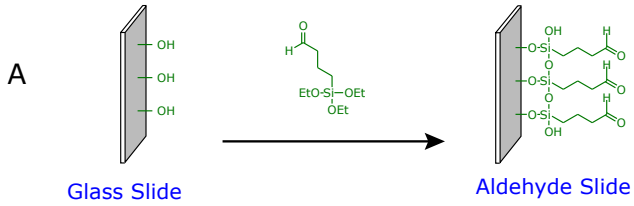
- hydrophilic surface
- amine-reactive chemistry
- constant hydration

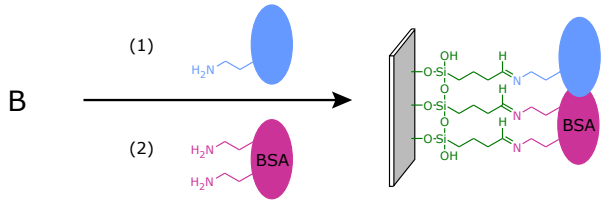
BSA-NHS SLIDES



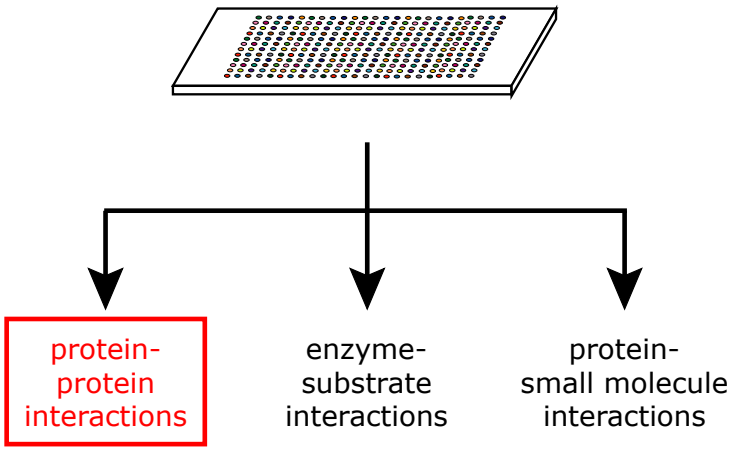


ALDEHYDE SLIDES

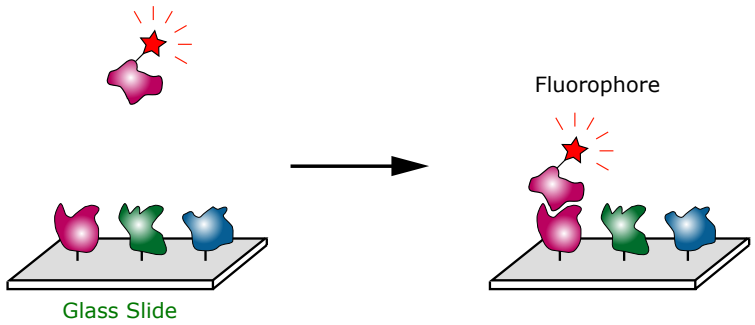




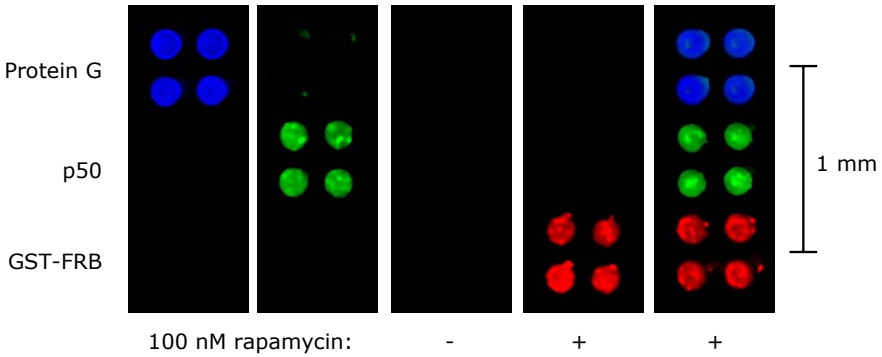
PROTEIN MICROARRAYS



PROTEIN-PROTEIN INTERACTIONS

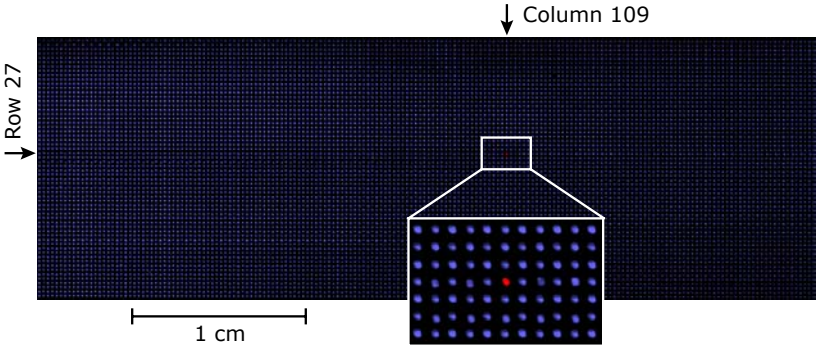


PROTEIN-PROTEIN INTERACTIONS



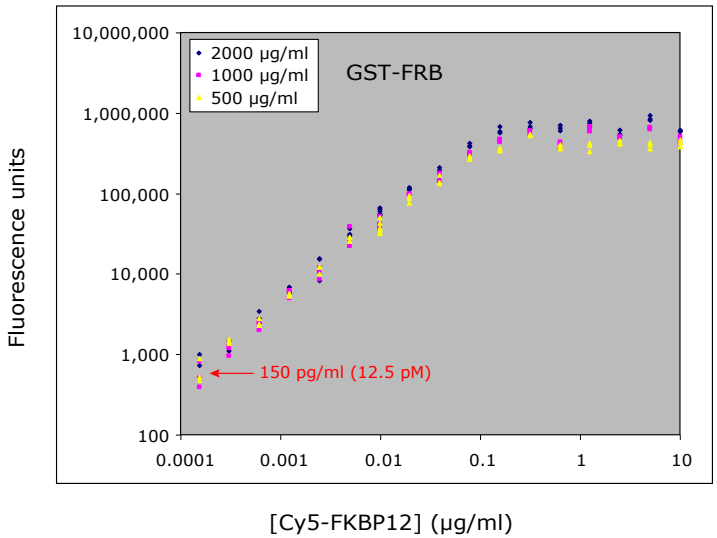
0.50 µg/ml BODIPY-FL-IgG
0.05 µg/ml Cy3-IκBα
0.50 µg/ml Cy5-FKBP12

PROTEIN-PROTEIN INTERACTIONS

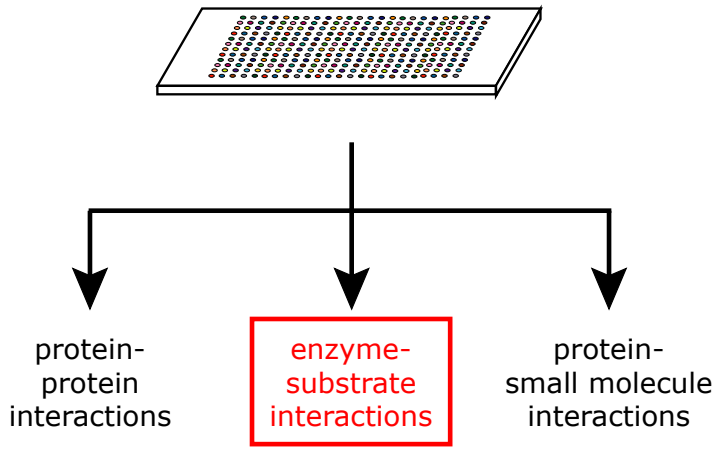


0.50 µg/ml BODIPY-FL-IgG
0.50 µg/ml Cy5-FKBP12 + 100 nM rapamycin

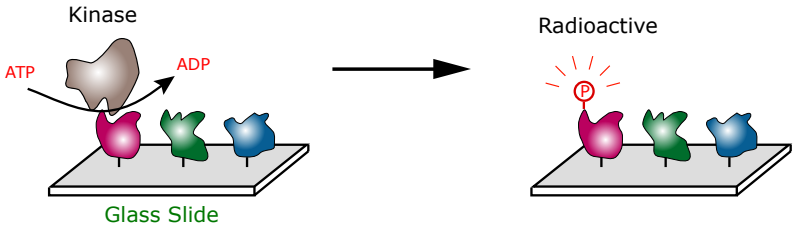
TITRATION ON GLASS SLIDE



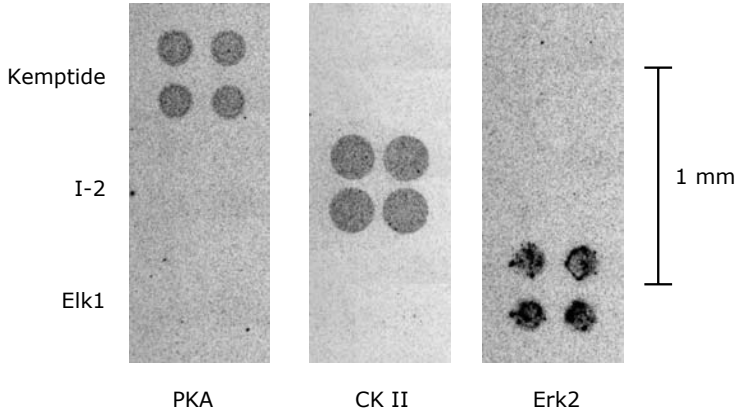
PROTEIN MICROARRAYS



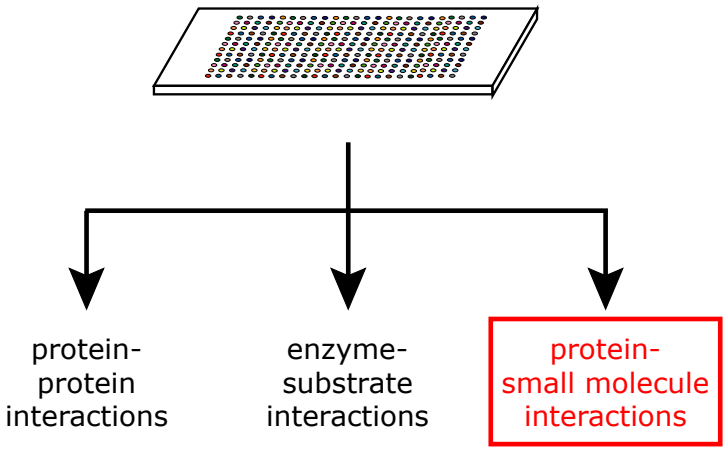
ENZYME-SUBSTRATE INTERACTIONS



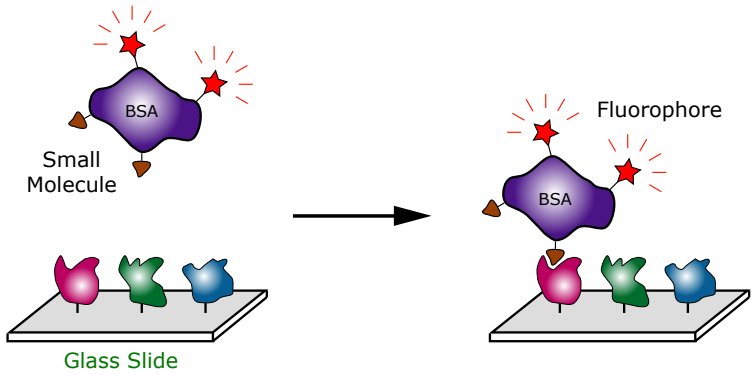
IDENTIFYING SUBSTRATES OF PROTEIN KINASES



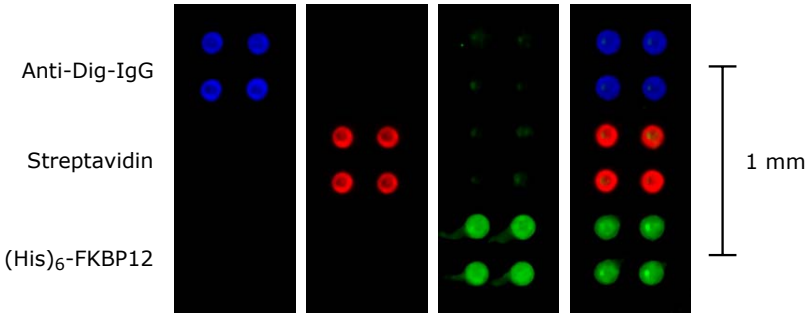
PROTEIN MICROARRAYS



PROTEIN-SMALL MOLECULE INTERACTIONS

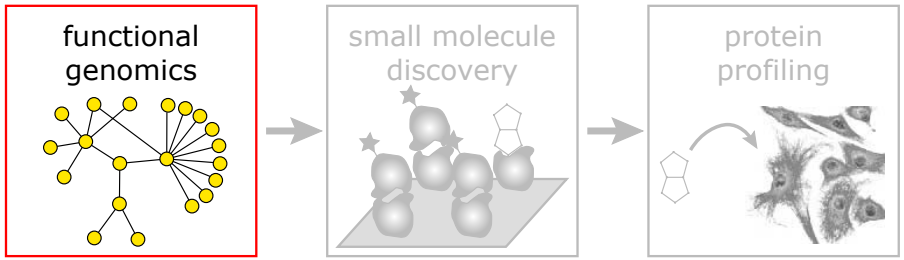


IDENTIFYING THE TARGETS OF SMALL MOLECULES



10 µg/ml A_{488}-BSA-digoxigenin
5 µg/ml Cy3-BSA-AP1497
5 µg/ml Cy5-BSA-biotin

PROTEIN MICROARRAYS



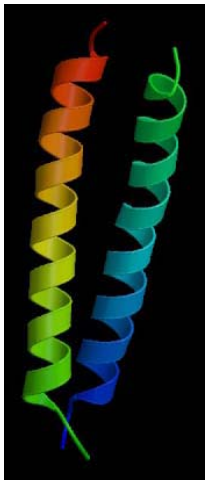
FUNCTIONAL GENOMICS

subsets of related proteins

- informatics (sequence motifs)
- literature searches

protein domains	full-length proteins
• coiled coils John Newman	• cancer proteins Joshua LaBaer, Pascal Braun
• protocadherins Viara Grantcharova	• c. *elegans* development Viara Grantcharova

COILED COILS



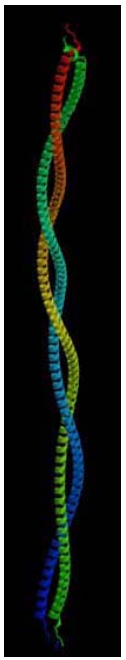
myc/max heterodimer

J Mol Biol (1998) **281**, 165.

Mediate homo- and hetero-dimerization

Found in:
- structural proteins
- motor proteins
- transcription factors
- membrane fusion proteins

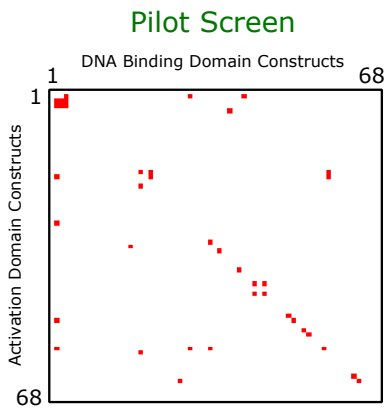
In yeast, MULTICOIL predicts:
- ~300 proteins with 2-stranded coiled-coils
- ~250 proteins with 3-stranded coiled coils



tropomyosin homodimer

J Mol Biol (1986) **192**, 111.

COILED-COIL SCREEN: YEAST TWO-HYBRID



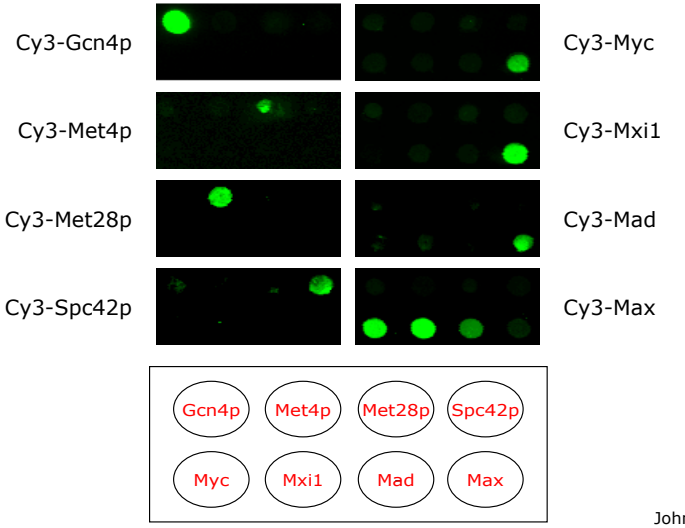
20 homotypic

19 heterotypic

3 intra-protein

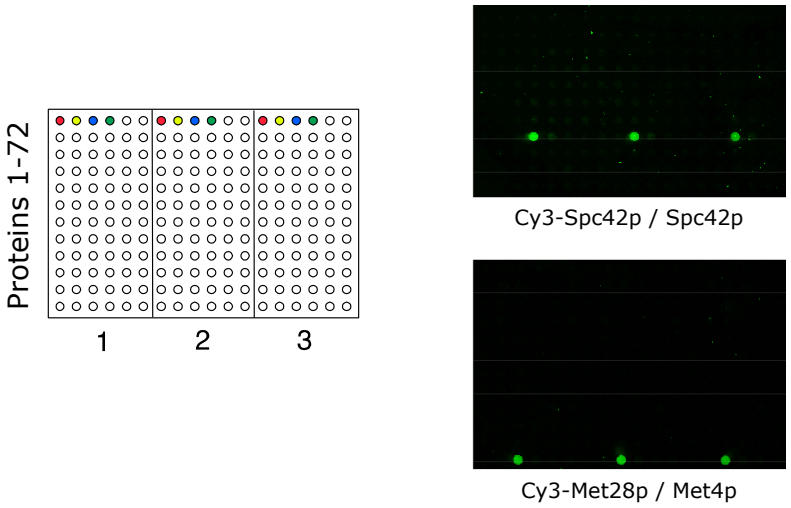
Newman, Wolf & Kim (2000) *PNAS* **97**, 13203

COILED-COIL INTERACTIONS: PROTEIN MICROARRAYS



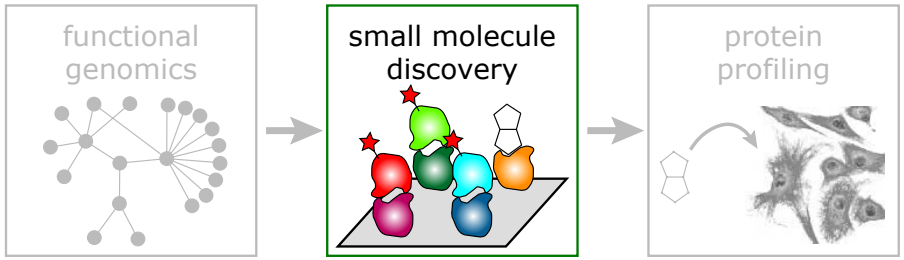
John Newman

PILOT COILED-COIL SCREEN: PROTEIN MICROARRAYS

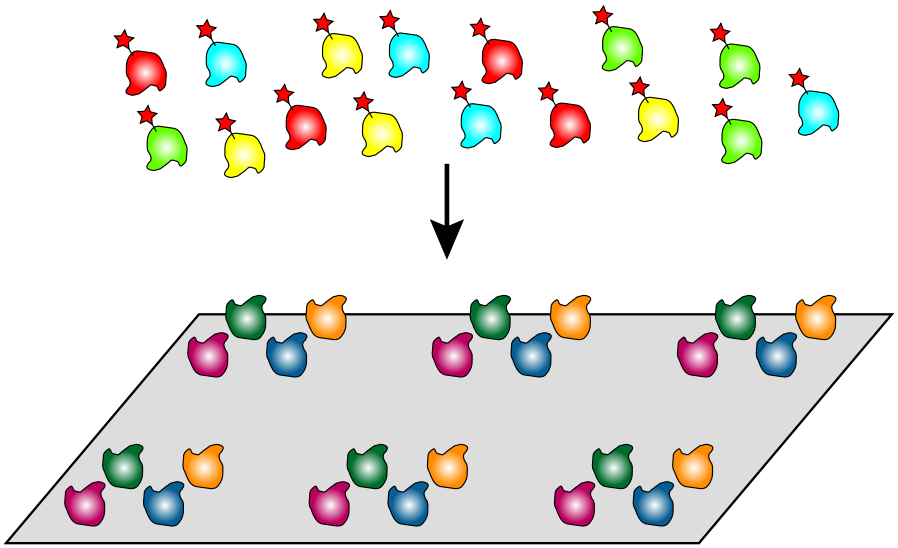


John Newman

PROTEIN MICROARRAYS

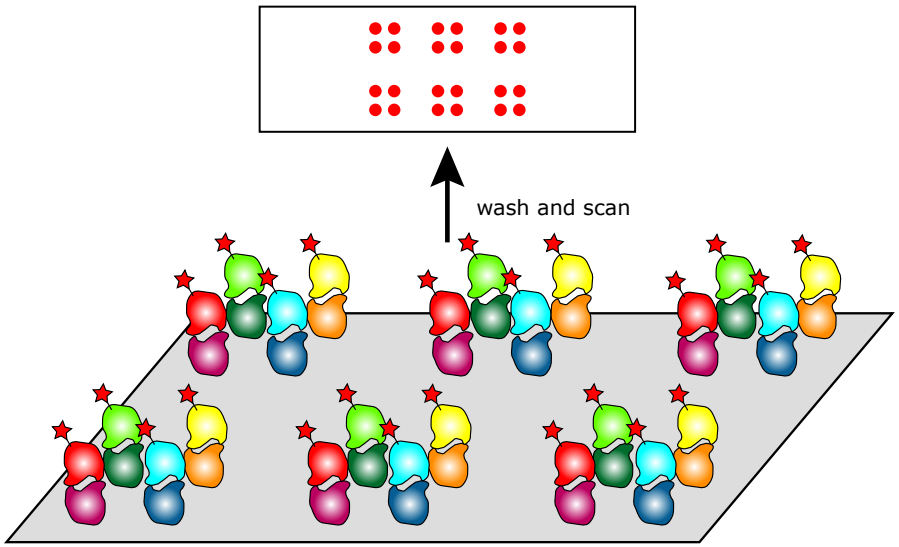


SCREENING METHOD 2



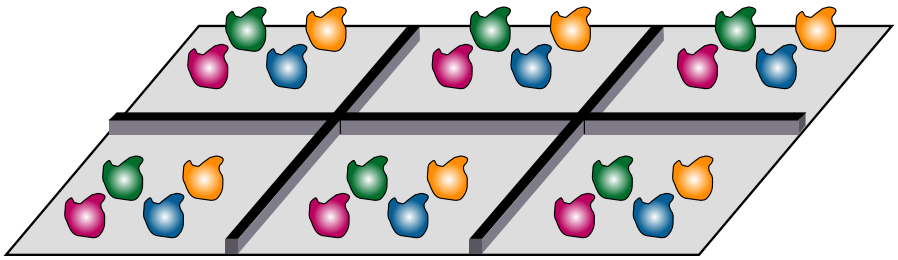
protein microarrays

SCREENING METHOD 2



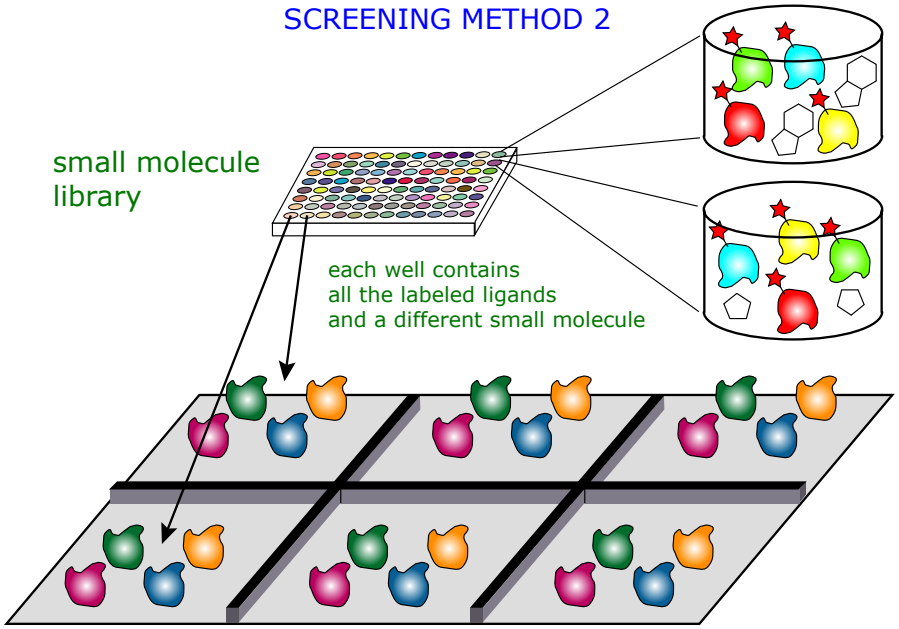
protein microarrays

SCREENING METHOD 2



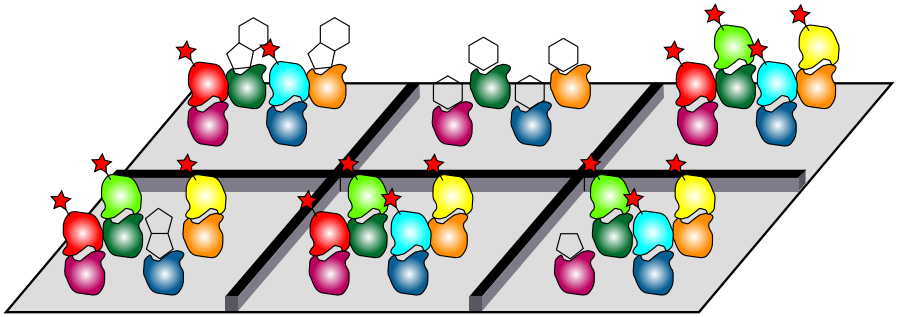
protein microarrays

SCREENING METHOD 2



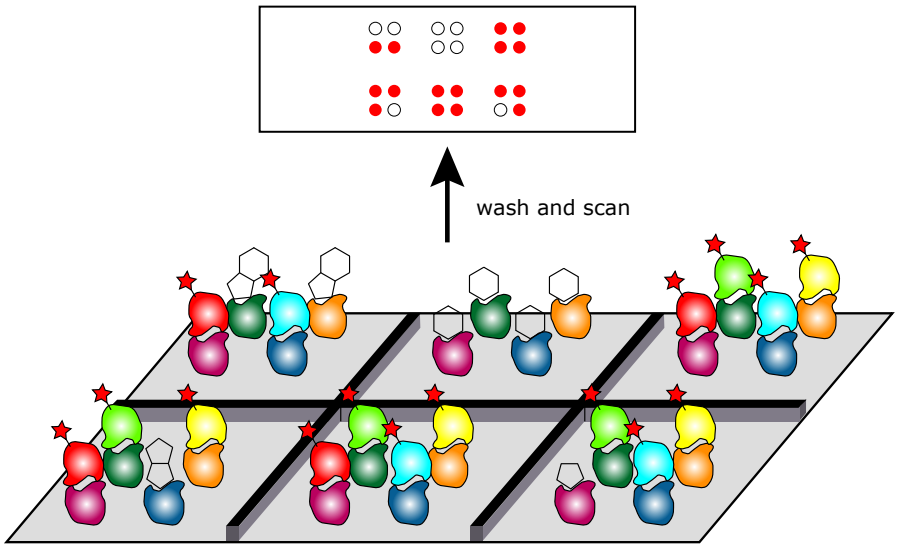
protein microarrays

SCREENING METHOD 2



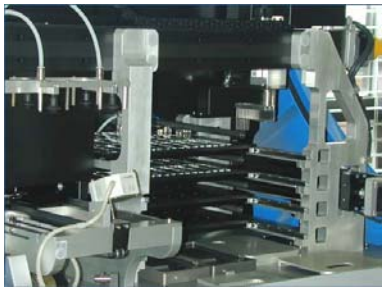
protein microarrays

SCREENING METHOD 2



protein microarrays

THE MICROARRAY WORLD



Glass slides

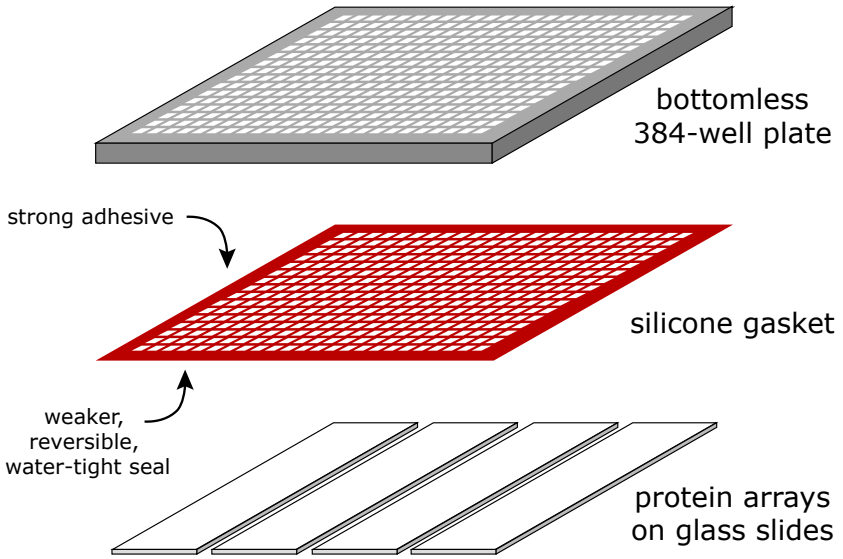
- 2.5 cm x 7.5 cm
- >10,000 spots per plate
- spacing varies

THE HTS WORLD

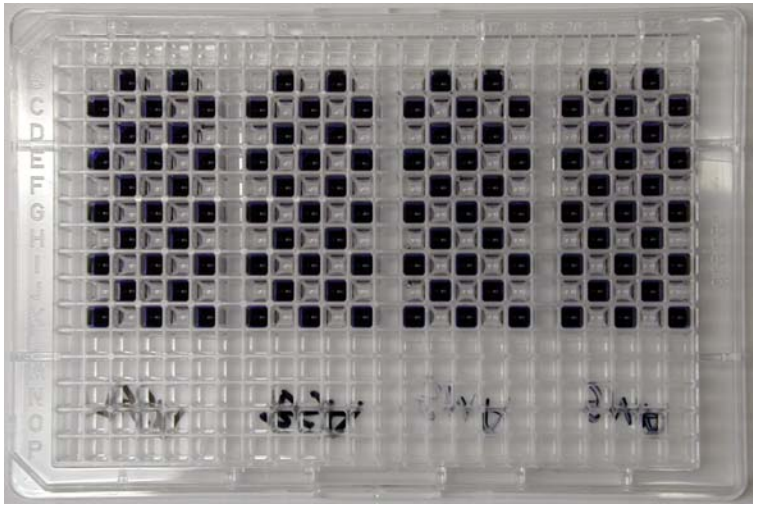
Microtiter plates

- 8.5 cm x 12.5 cm
- 96, 384, or 1536 wells per plate
- 9 mm, 4.5 mm, or 2.25 mm spacing

MICROARRAYS IN WELLS OF PLATES

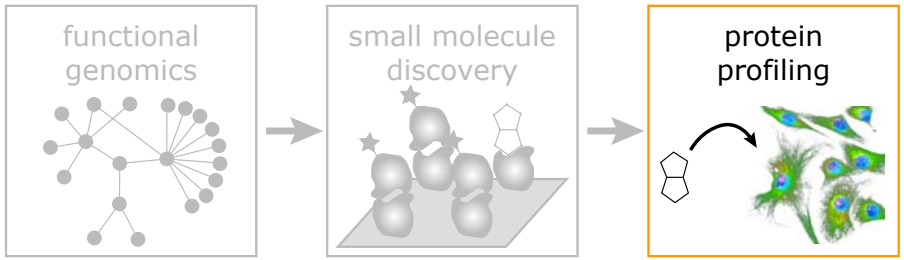


MULTIPLEXED SCREENING

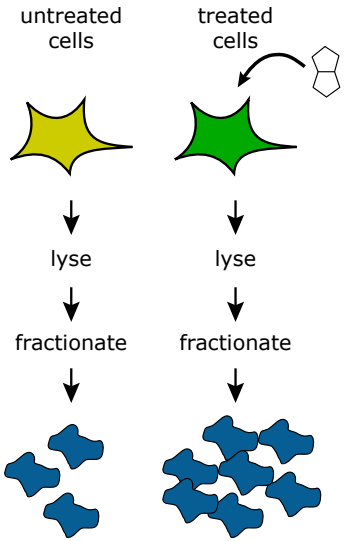


- bottomless 384-well plate
- 64 wells per slide x 4 slides per plate = 256 wells per plate
- 256 wells per plate x 100 proteins per well = 25,600 assays per plate

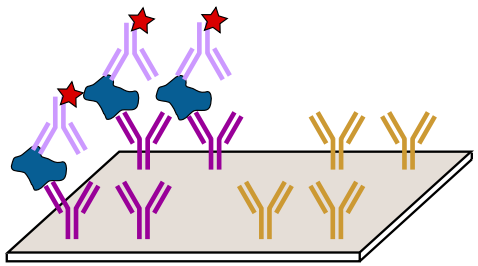
PROTEIN MICROARRAYS



ANTIBODY ARRAYS: SANDWICH ELISA

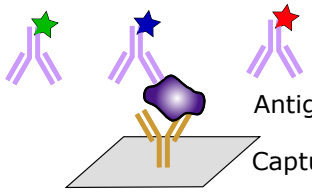


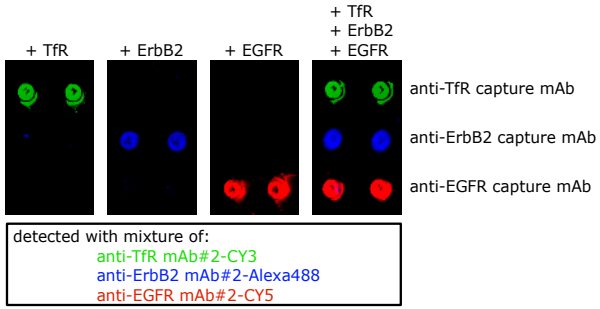
1. apply unlabeled protein
2. detect with labeled secondary antibody



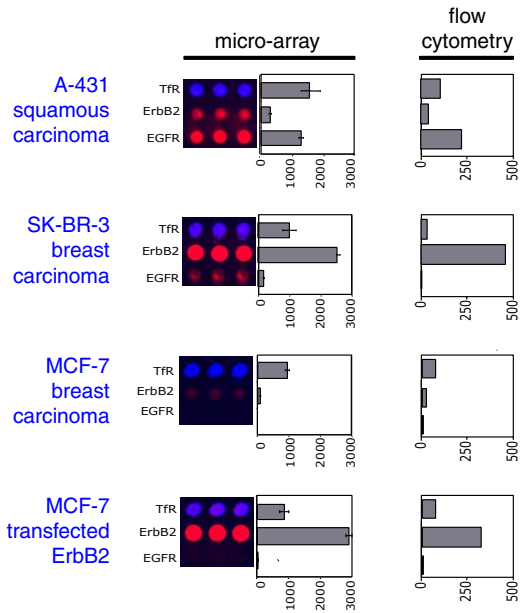
ANTIBODY ARRAYS: MICRO-SANDWICH ASSAY

Flourescent 2' Antibodies



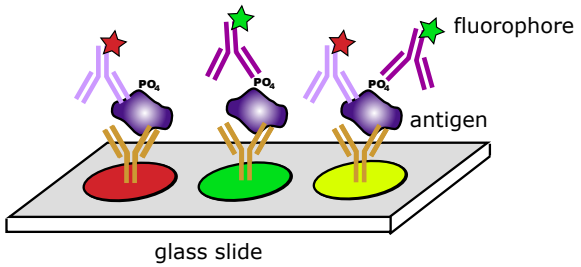


MICRO-SANDWICH DETECTION TUMOR CELL PROFILING

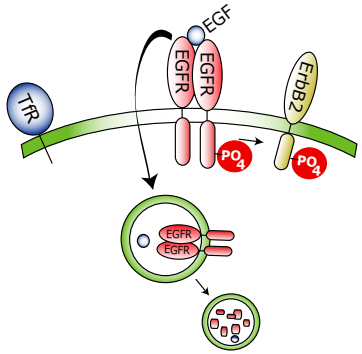


ANTIBODY ARRAYS: MICRO-SANDWICH ASSAY

Ratiometric analyses of protein modification

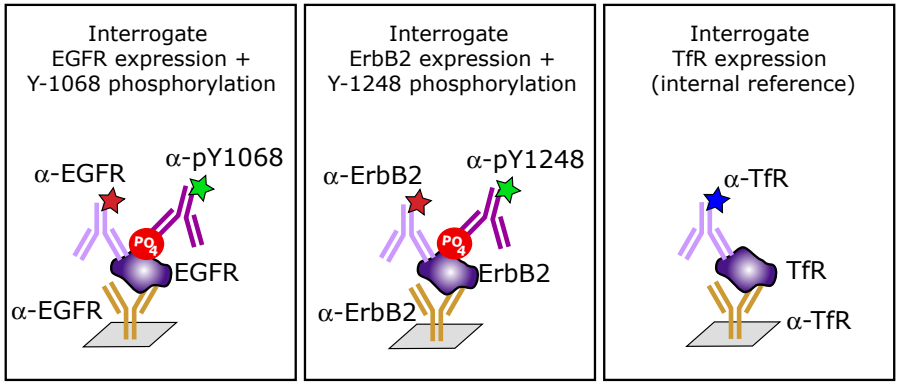


PHOSPHORYLATION AND REGULATION OF GROWTH FACTOR RECEPTORS



- EGFR is phosphorylated in response to EGF
- ErbB2 is phosphorylated by EGFR
- EGFR is down-regulated
- TfR remains unchanged

RATIOMETRIC ANALYSES OF EXPRESSION AND PHOSPHORYLATION

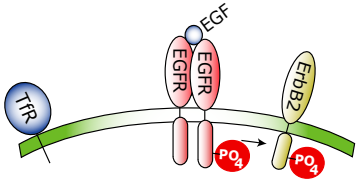


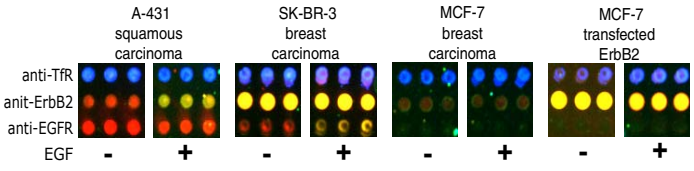
Capture and detection antibodies against different epitopes

Blue - Alexa488; Green - Cy3; Red - Cy5

RATIOMETRIC PROFILING IN TUMOR CELL LINES

EFFECT OF EGF

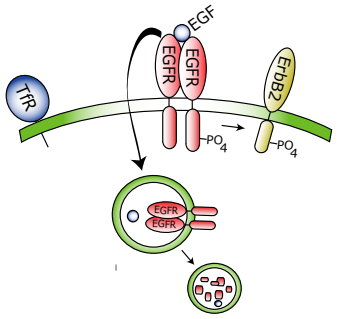


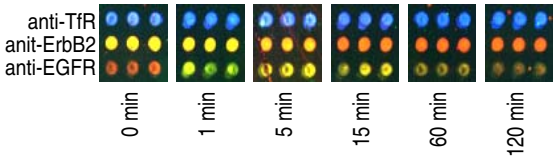


- RED = growth factor receptor expression
- GREEN = growth factor receptor phosphorylation
- BLUE = transferrin receptor expression
- YELLOW = ratio expression/phosphorylation

RATIOMETRIC ANALYSES OF EXPRESSION AND PHOSPHORYLATION

EGF time course on SK-BR-3 cells

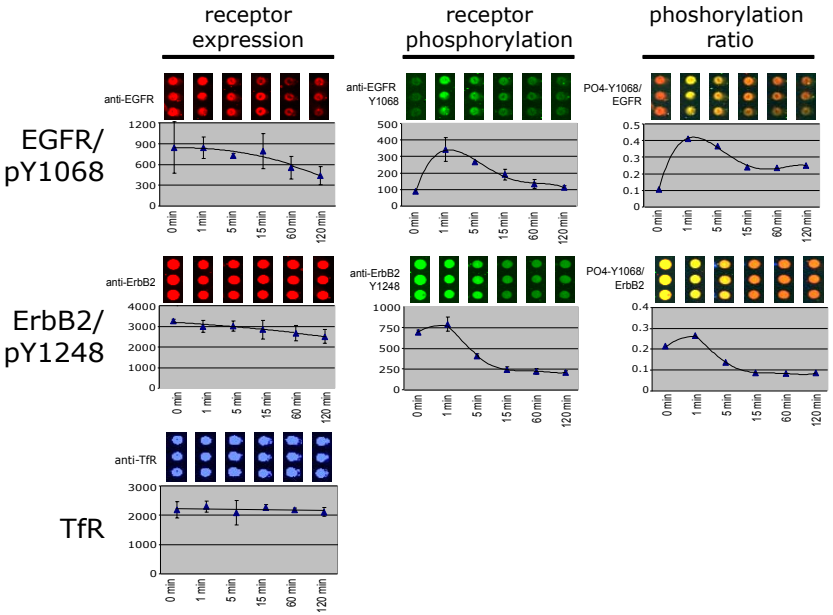




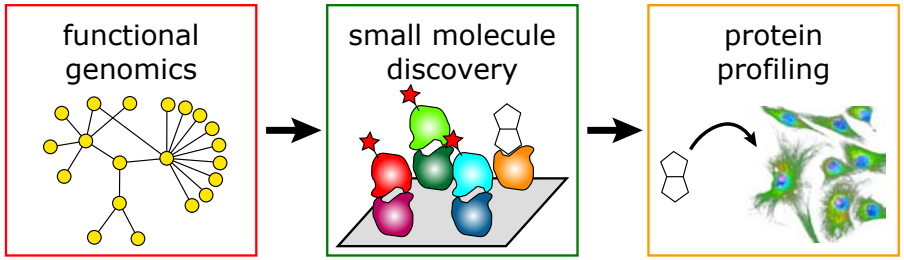
time of EGF treatment

RATIOMETRIC ANALYSES OF EXPRESSION AND PHOSPHORYLATION

EGF time course on SK-BR-3 cells



PROTEIN MICROARRAYS



SUMMARY

- rapid discovery of protein interactions
- screening of small molecules *in solution*
- screening for *specificity*
- system-wide analysis at the *protein level*

ACKNOWLEDGEMENTS

Protein Microarrays

Prof. Peter Kim (Whitehead Institute)
Dr. John Newman (Whitehead Institute)

Dr. Viara Grantcharova
Lioudmila Zaslavskaia

Antibody Microarrays

Prof. Peter Sorger (MIT)
Dr. Ulrik Nielsen (MIT)
Dr. Michael Cardone (MIT)

Dr. Raghida Bu-Khalid

Harvard Center for Genomics Research
DARPA