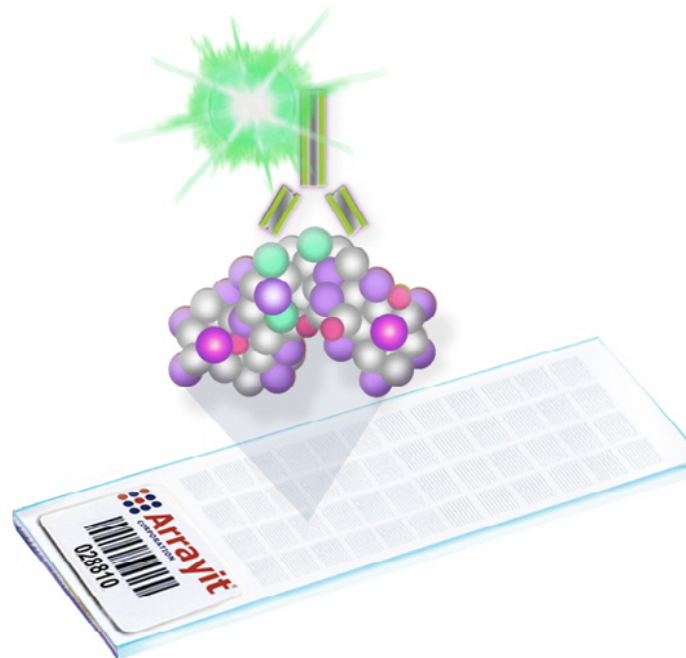


# Protein Microarrays

## Journal Club

Arnaud Monnard



August 26<sup>th</sup> 2014

Aguzzi lab

# Outline

## **Manufacturing of microarrays**

- **Contact Printing**
  - Pin Printing
  - Microstamping
  - Flow Printing
- **Lithography**
  - Photolithography
  - Electron Beam
  - Dip Pen Nanolithography
- **Non Contact Printing**
  - Thermal
  - Piezo
  - Valve
- **Cell Free**
  - PISA
  - NAPPA
  - In Situ Puromycin Capture

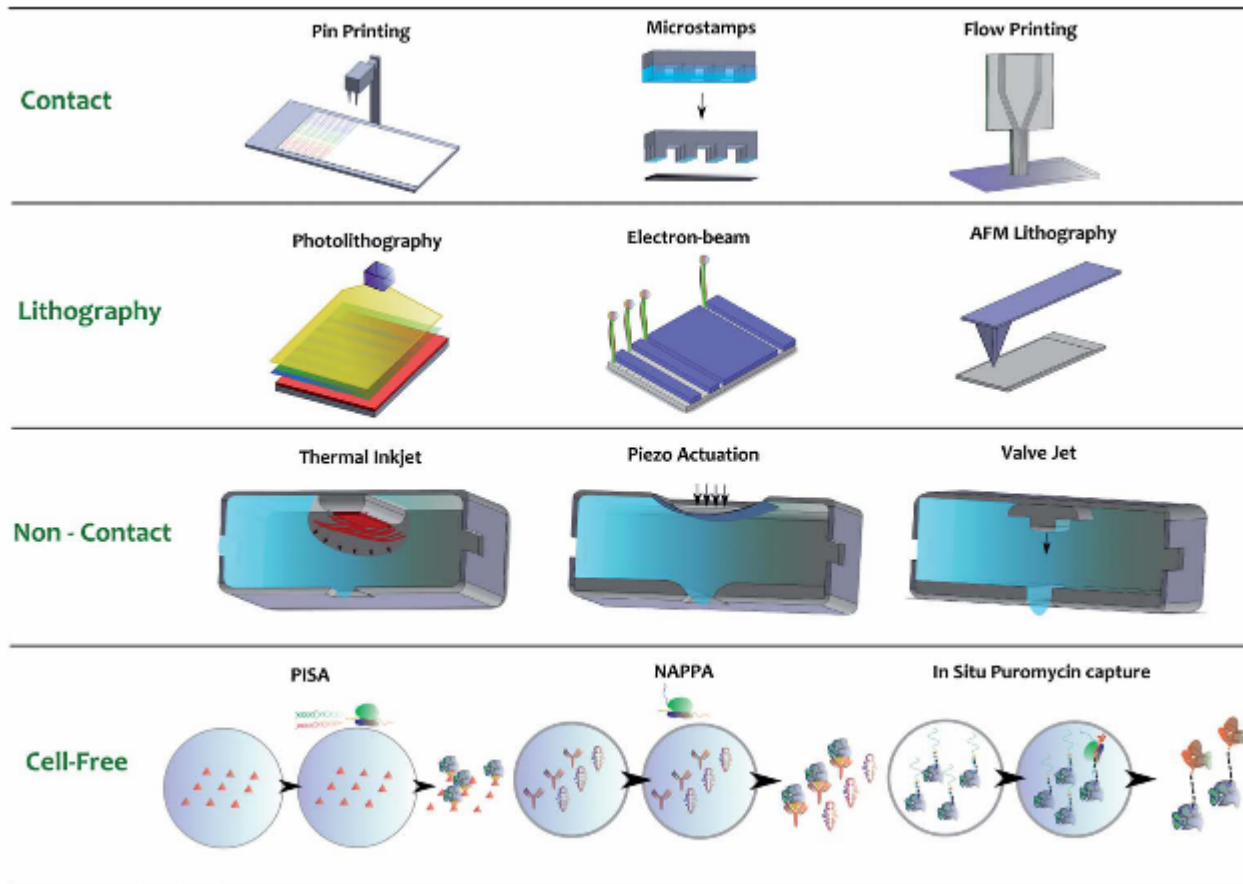
## **Publications**

- **Proof of Concepts**
  - MacBeath et al. Science 2000
  - Chen et al. BioTechniques 2006
- **Applications**
  - Jones et al. Nature 2006
  - Mok et al. Nature Protocols 2009
  - Gagni et al. Biosensors and Bioelectronics 2013

# Introduction

- First antibody array in 1983 followed by developement in the late 90's
- Use principles and technologies of DNA microarrays
  - Minimal sample consumption
  - High throughput
- Bypasses the limitation of DNA microarrays

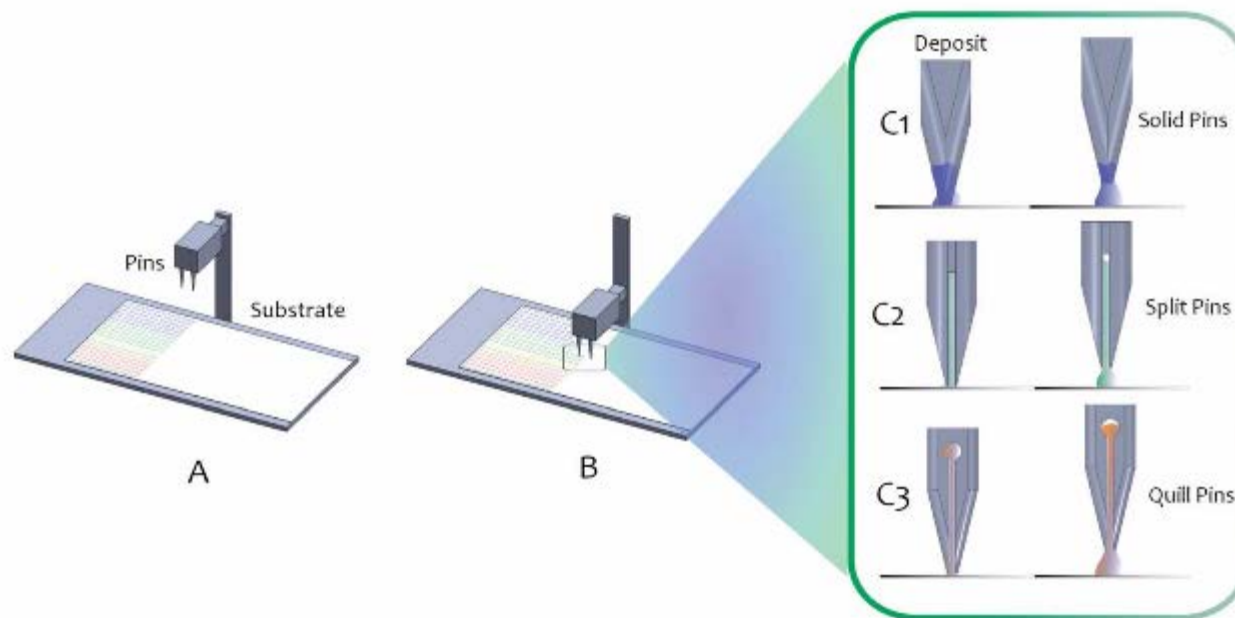
# Protein Microarray Manufacturing Technologies



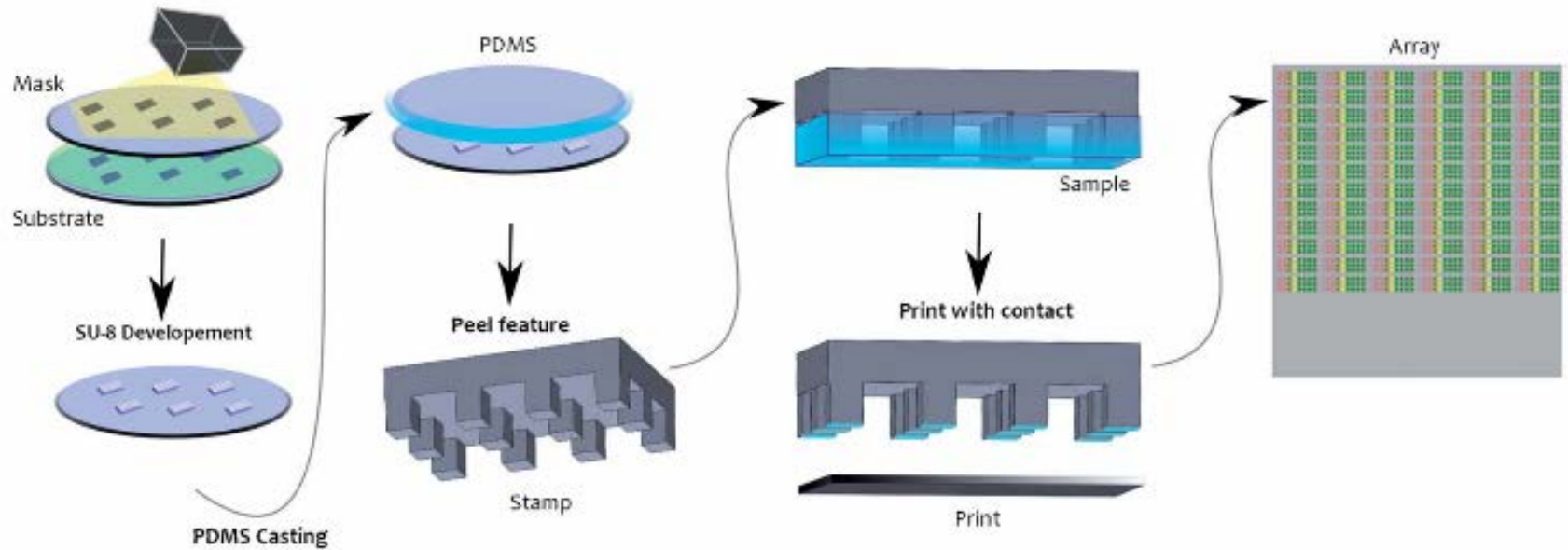
# Contact Printing



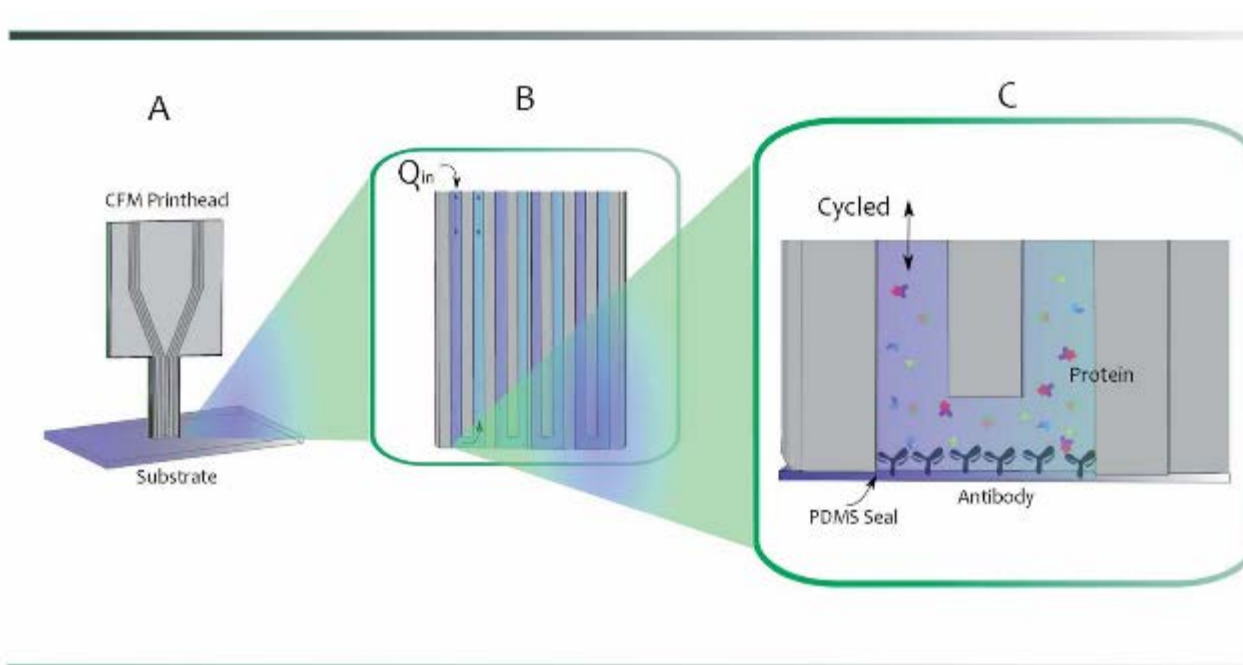
# Pin Printing



# Microstamping

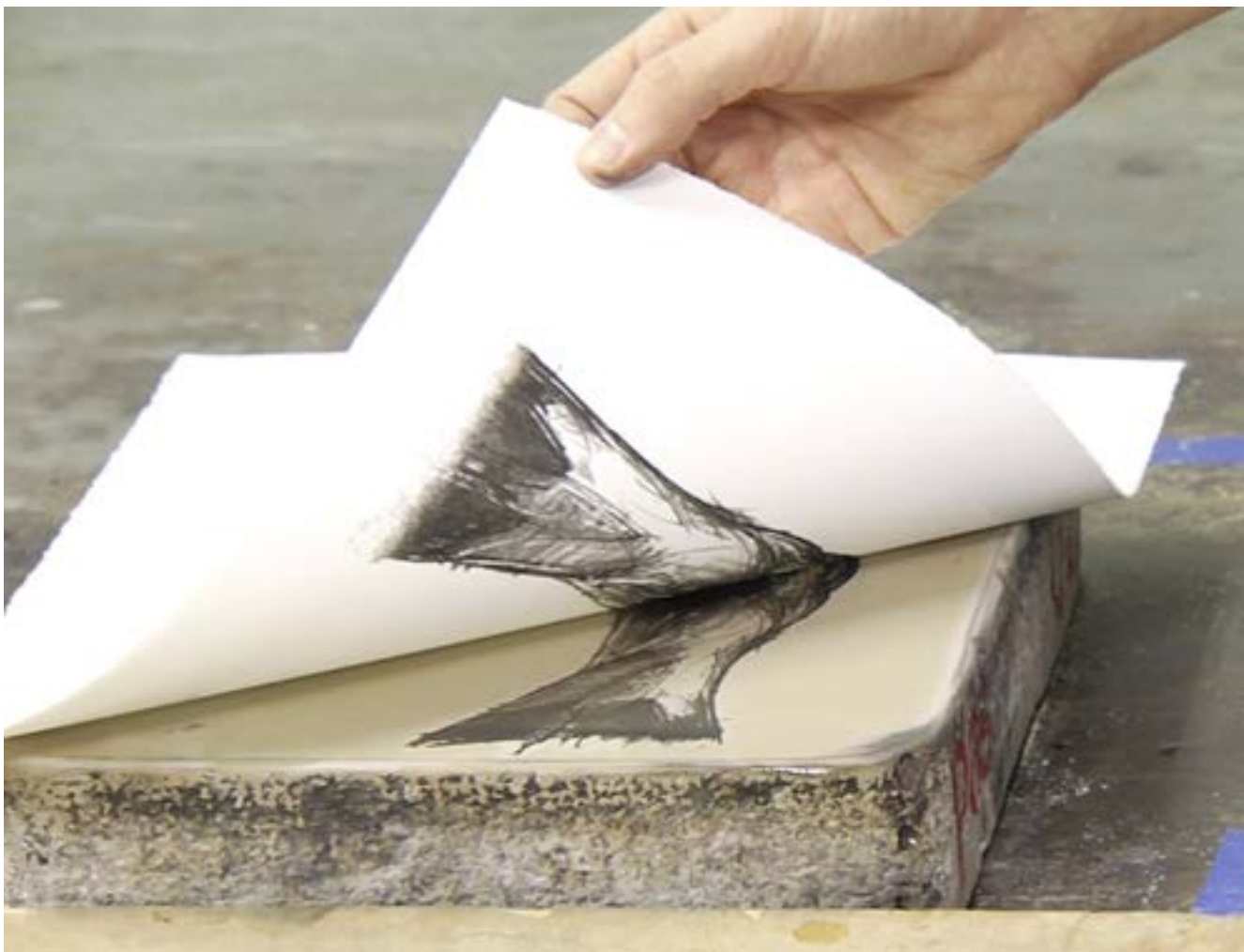


# Flow Printing

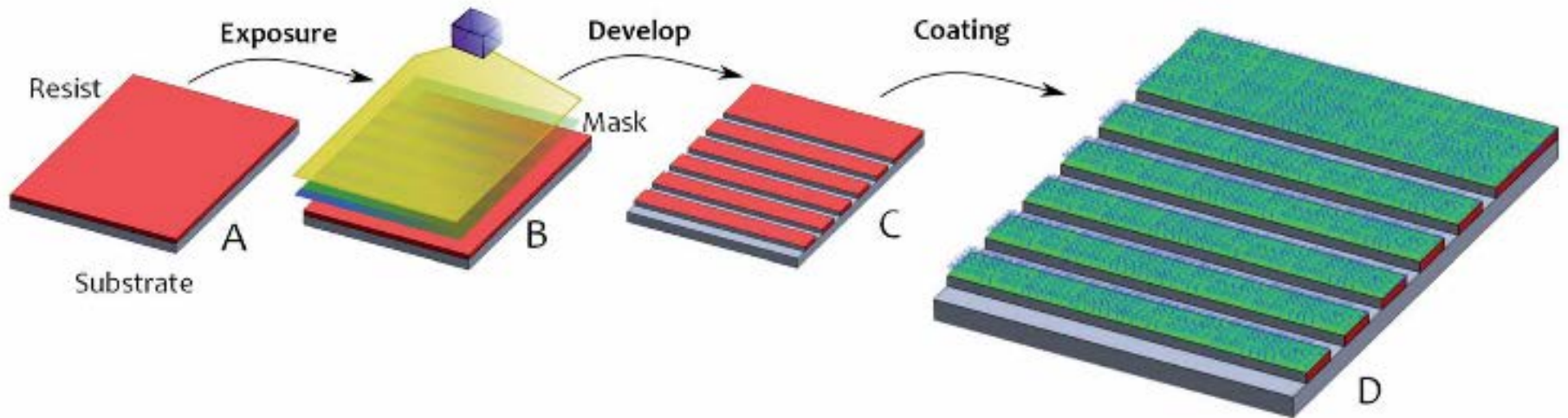




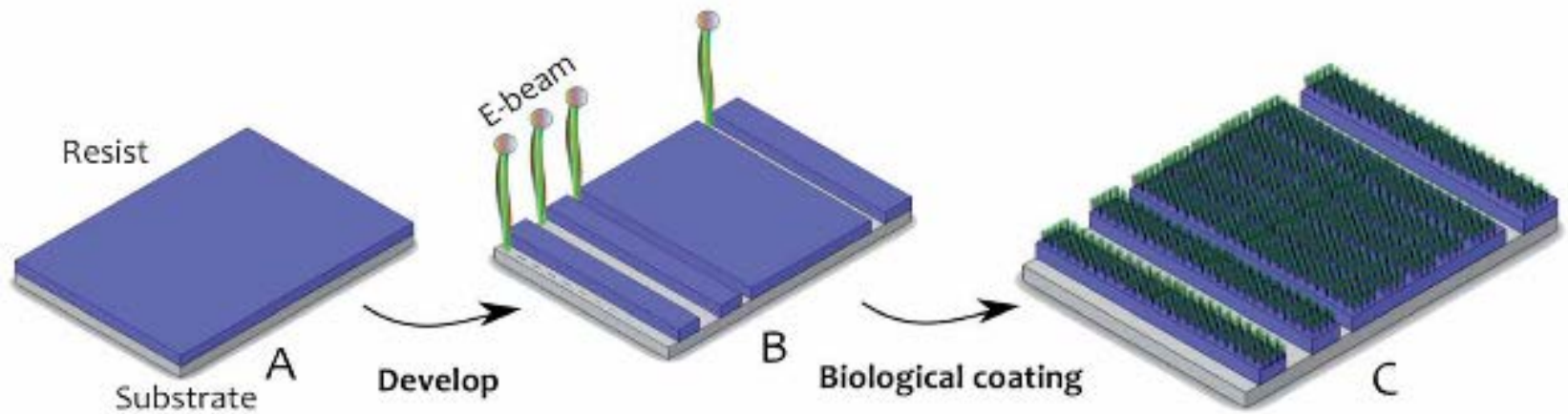
# Lithography



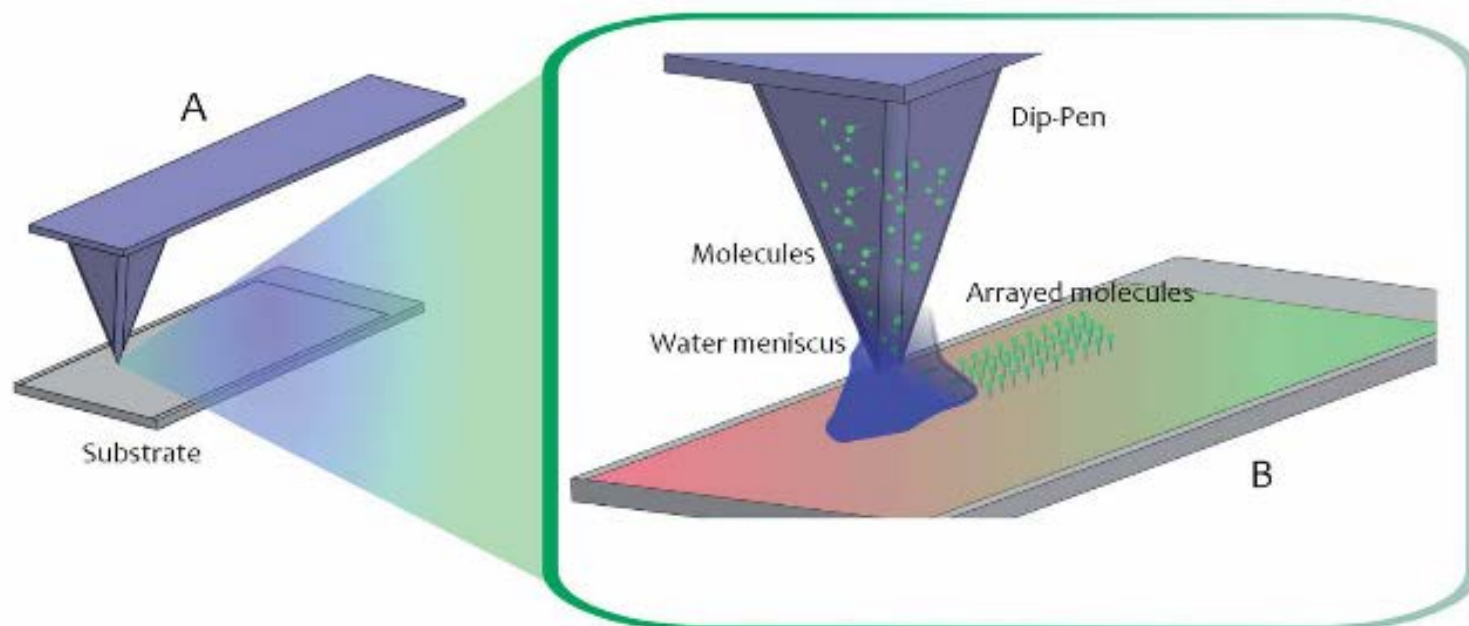
# Photolithography



# Electron Beam



# Dip Pen Nanolithography

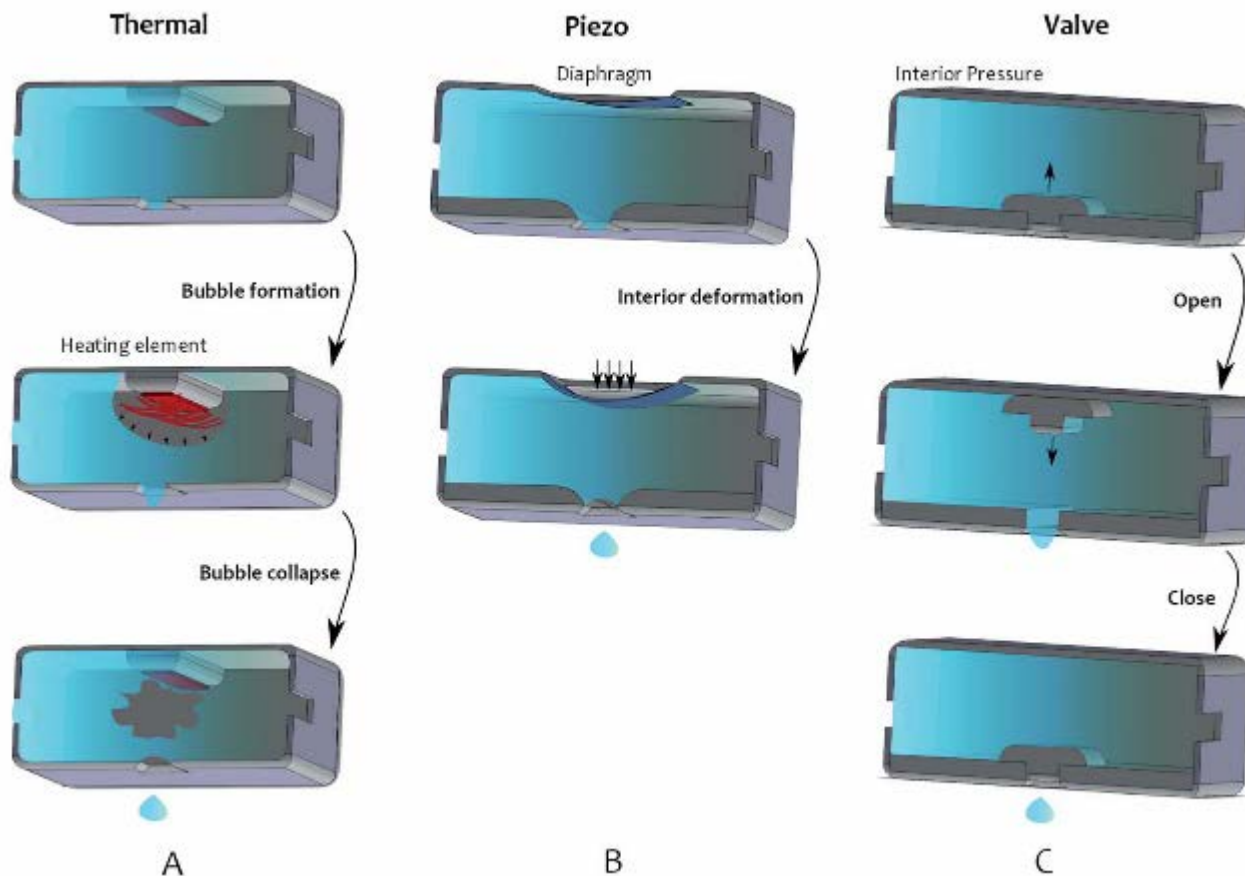


# Non-Contact Printing



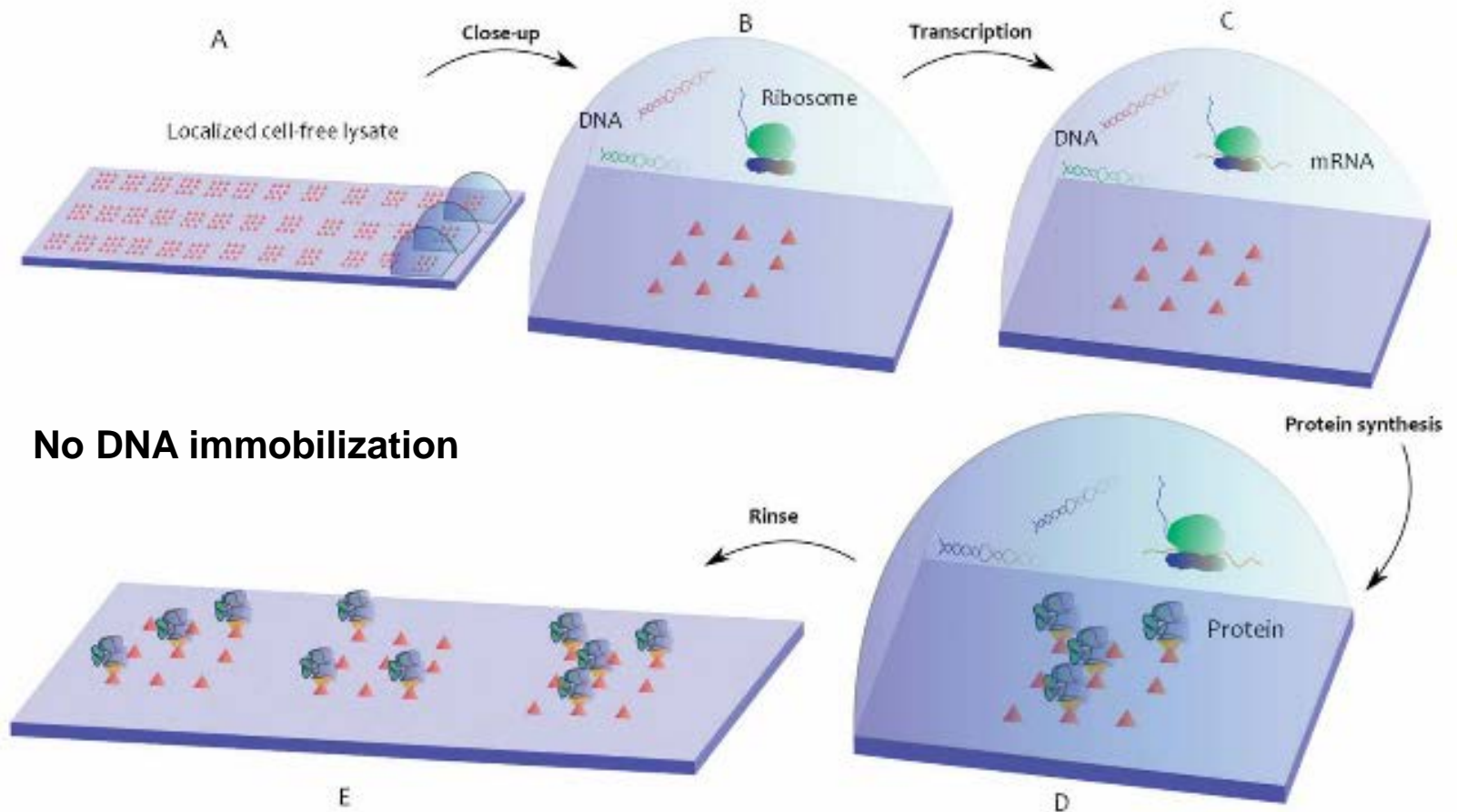


# Non contact Printing



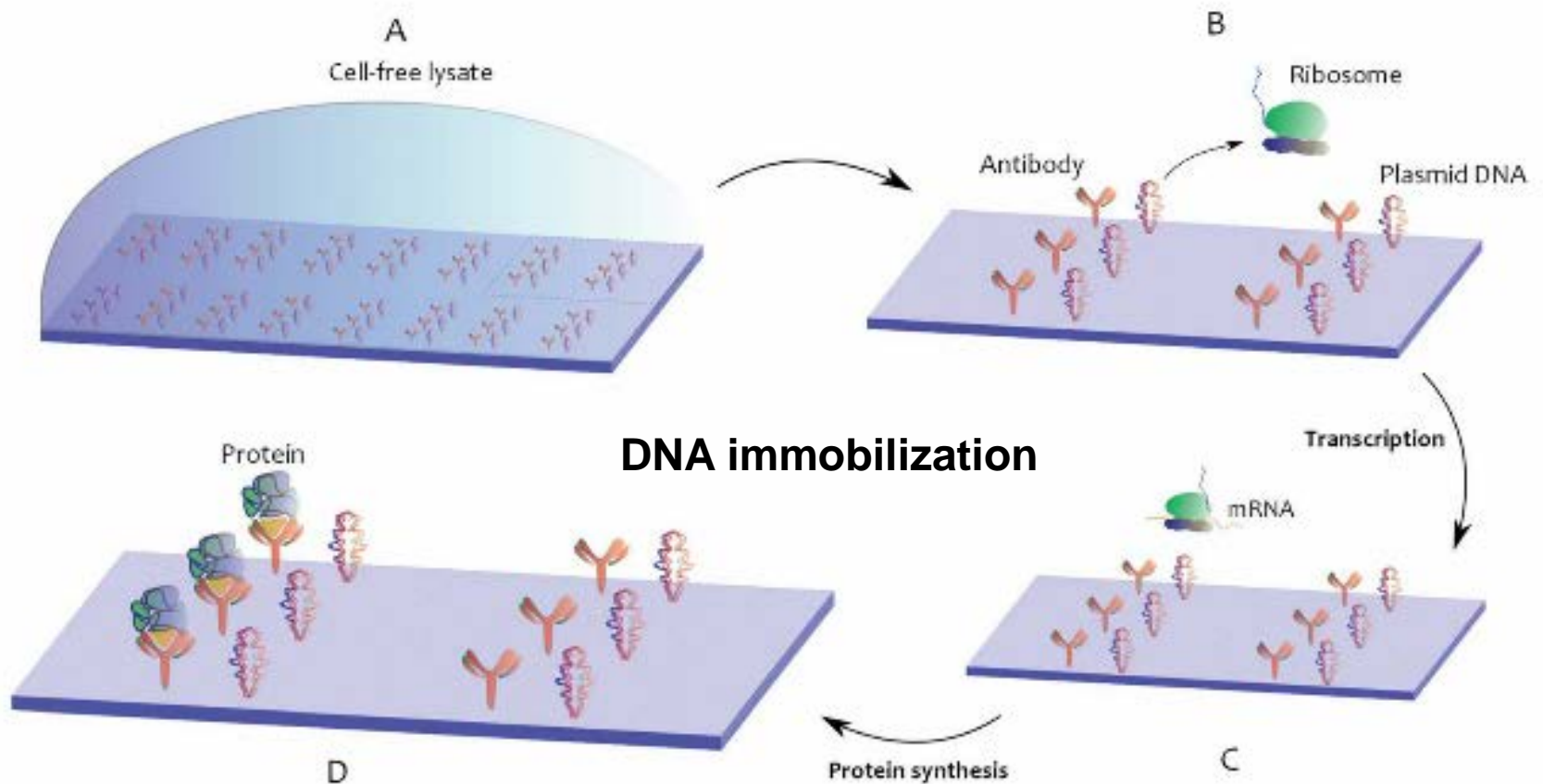
# Cell Free Systems

# Protein In Situ Array (PISA)

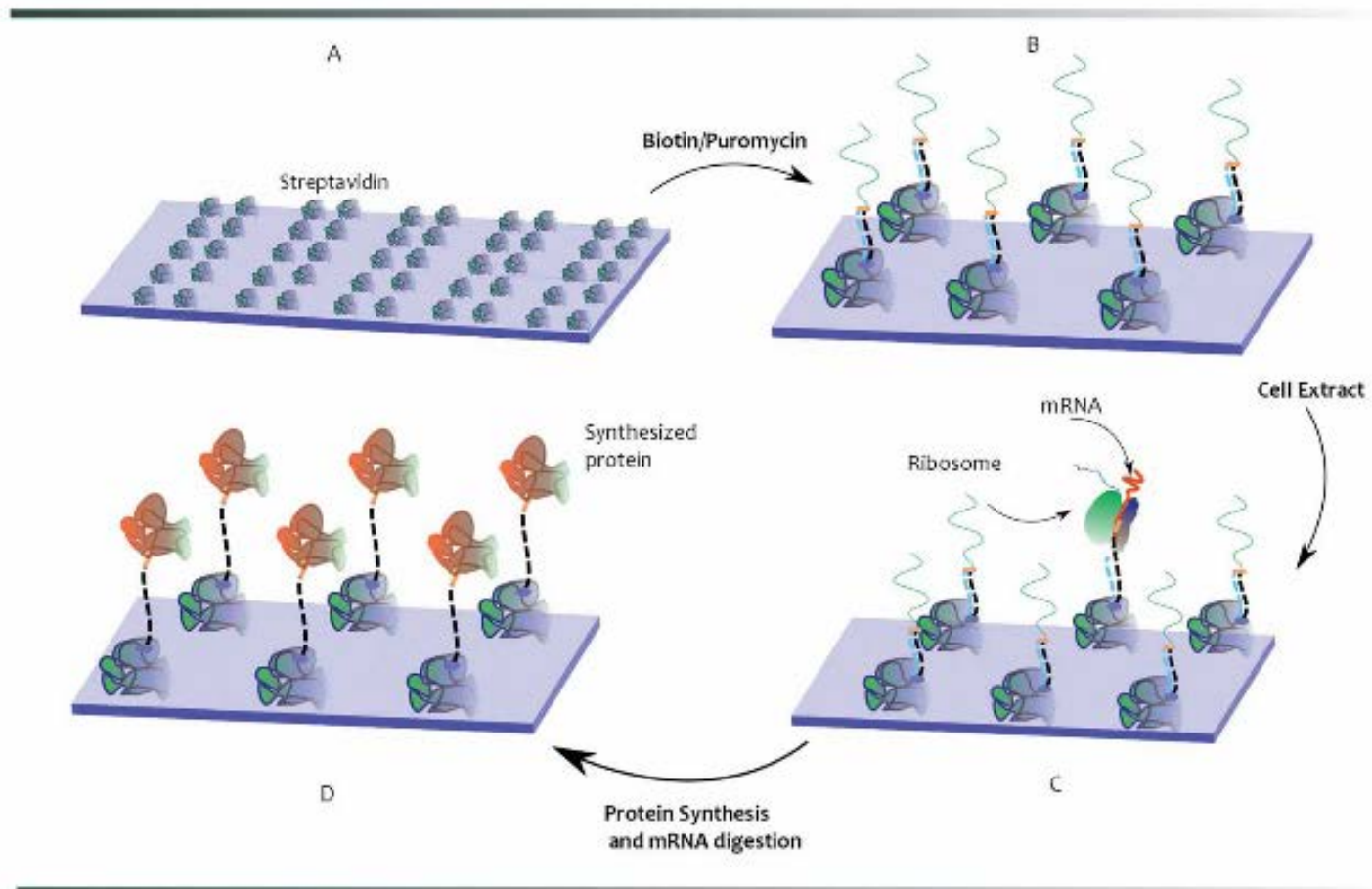




# NAPPA



# In Situ Puromycin Capture





**Printing Proteins as Microarrays for High-Throughput Function Determination**

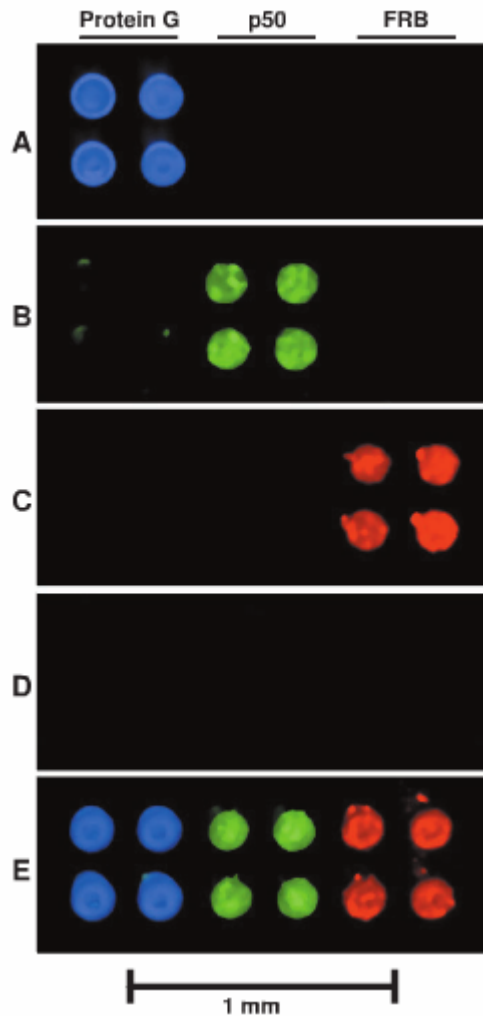
Gavin MacBeath and Stuart L. Schreiber

*Science* **289**, 1760 (2000);

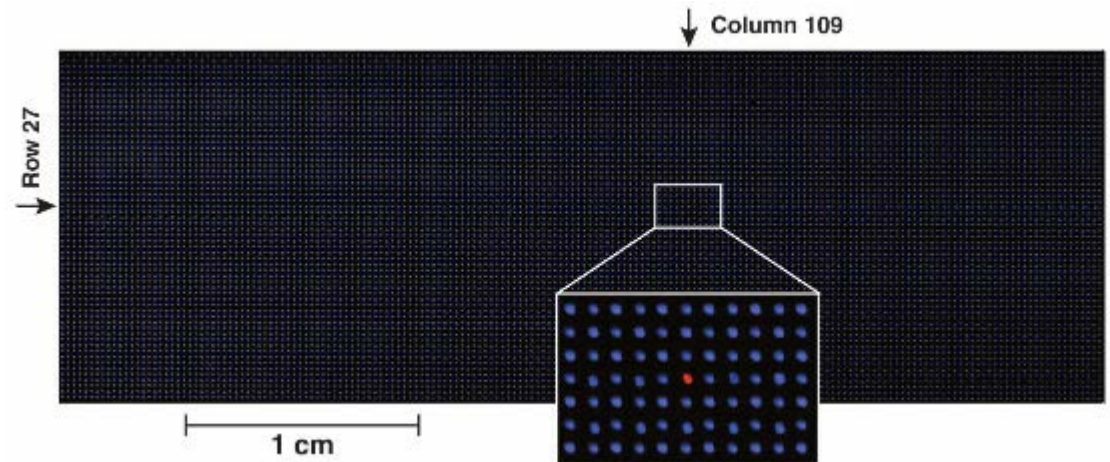
DOI: 10.1126/science.289.5485.1760

# Proof of Concept

## Protein-Protein interaction



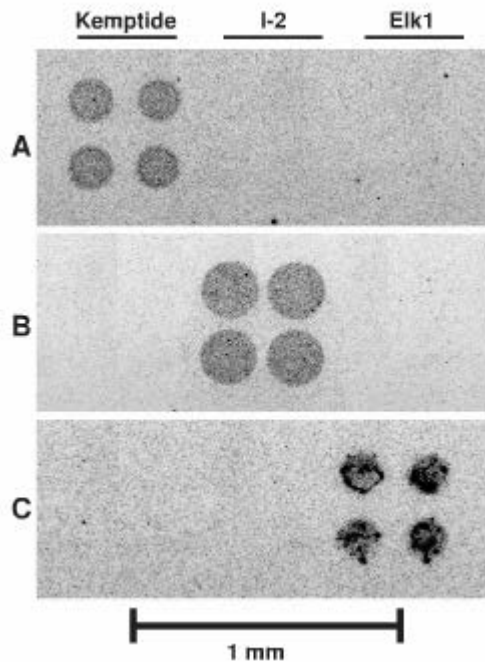
## Single spot detection



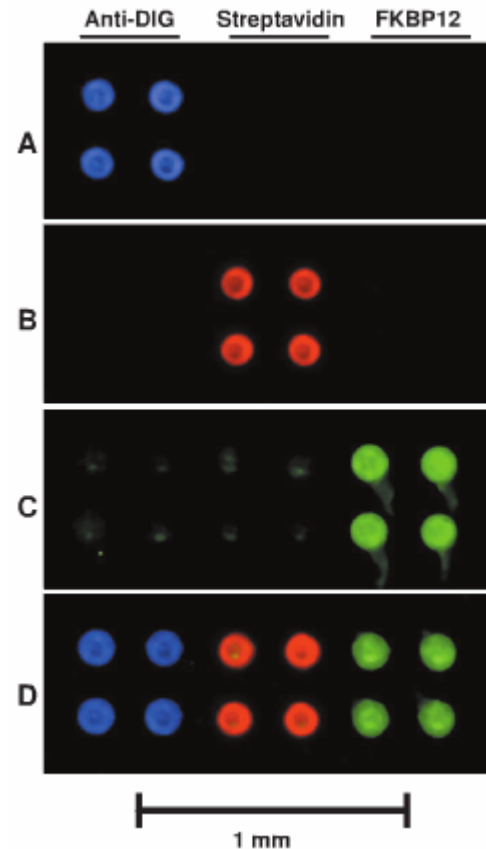
← No rapamycin

# Proof of Concept

Detection of kinase substrates



Detection of small molecules targets



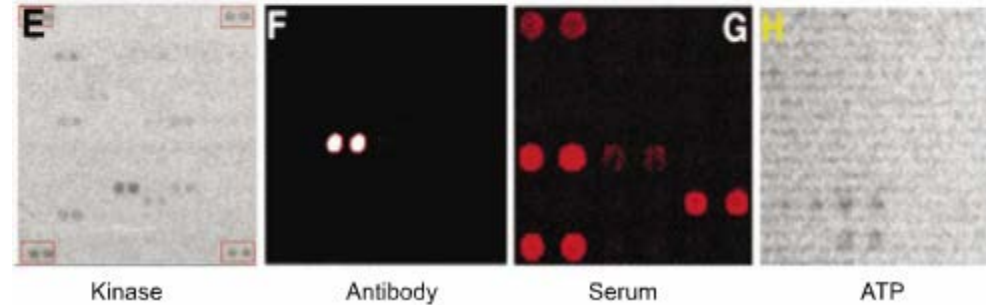
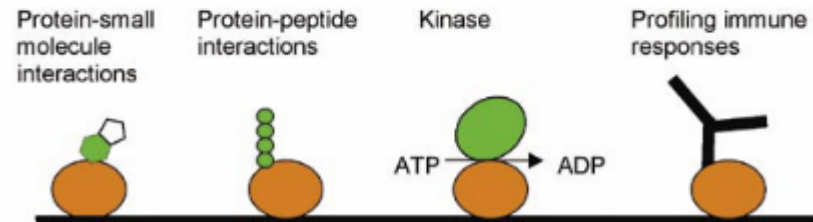
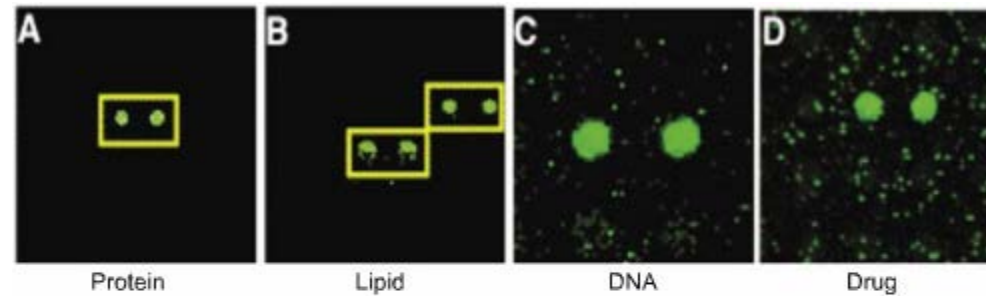
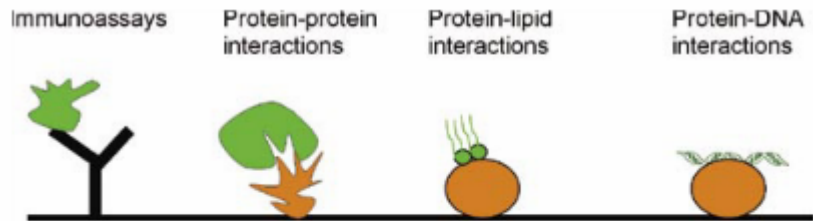
# Techniques Essay

## Protein Microarrays

**Chien-Sheng Chen and Heng Zhu**

Department of Pharmacology and Molecular Sciences/High-Throughput Biology Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA

# Applications



## ARTICLES

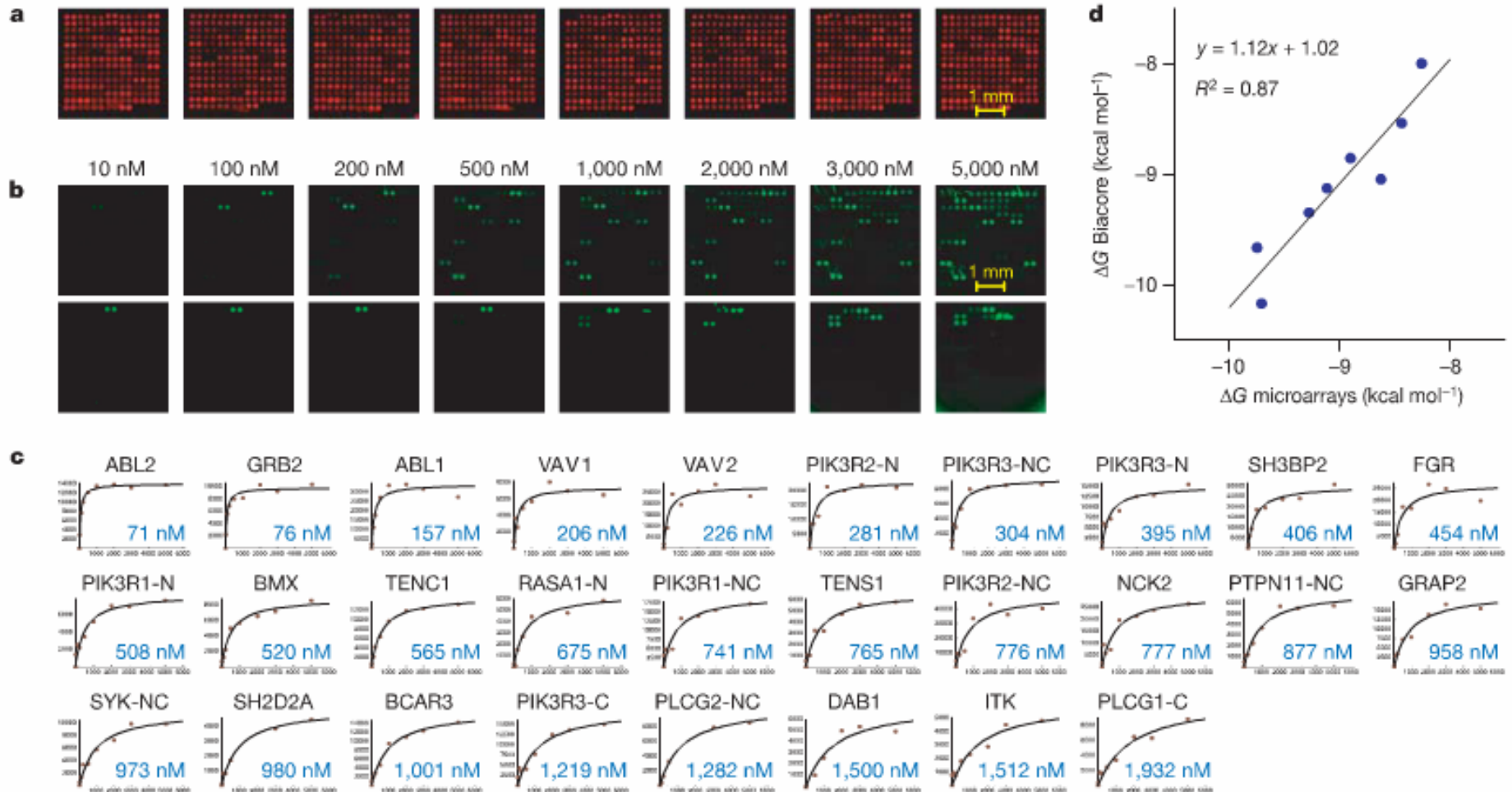
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# **A quantitative protein interaction network for the ErbB receptors using protein microarrays**

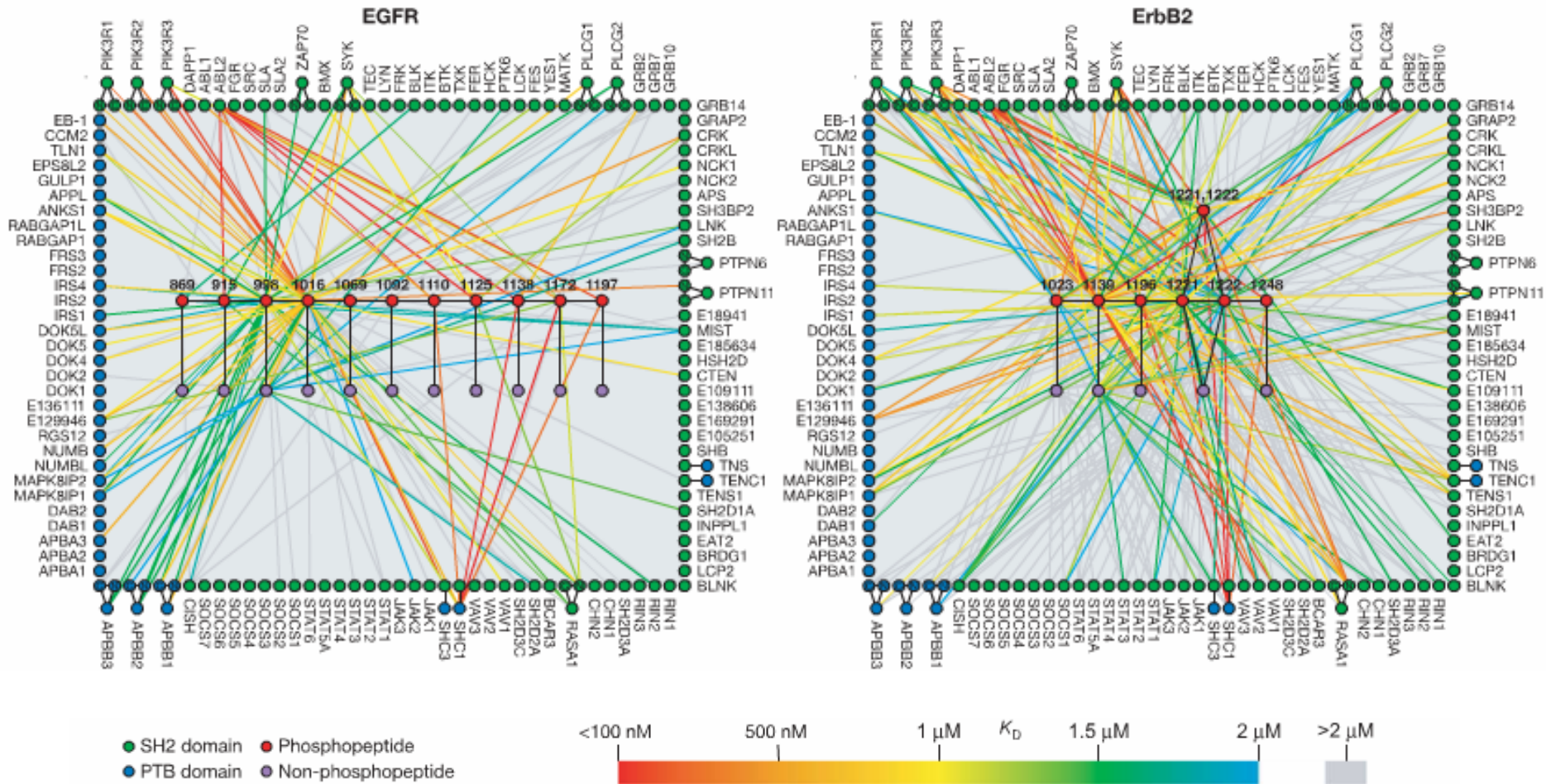
Richard B. Jones<sup>1\*</sup>, Andrew Gordus<sup>1,2\*</sup>, Jordan A. Krall<sup>1</sup> & Gavin MacBeath<sup>1</sup>



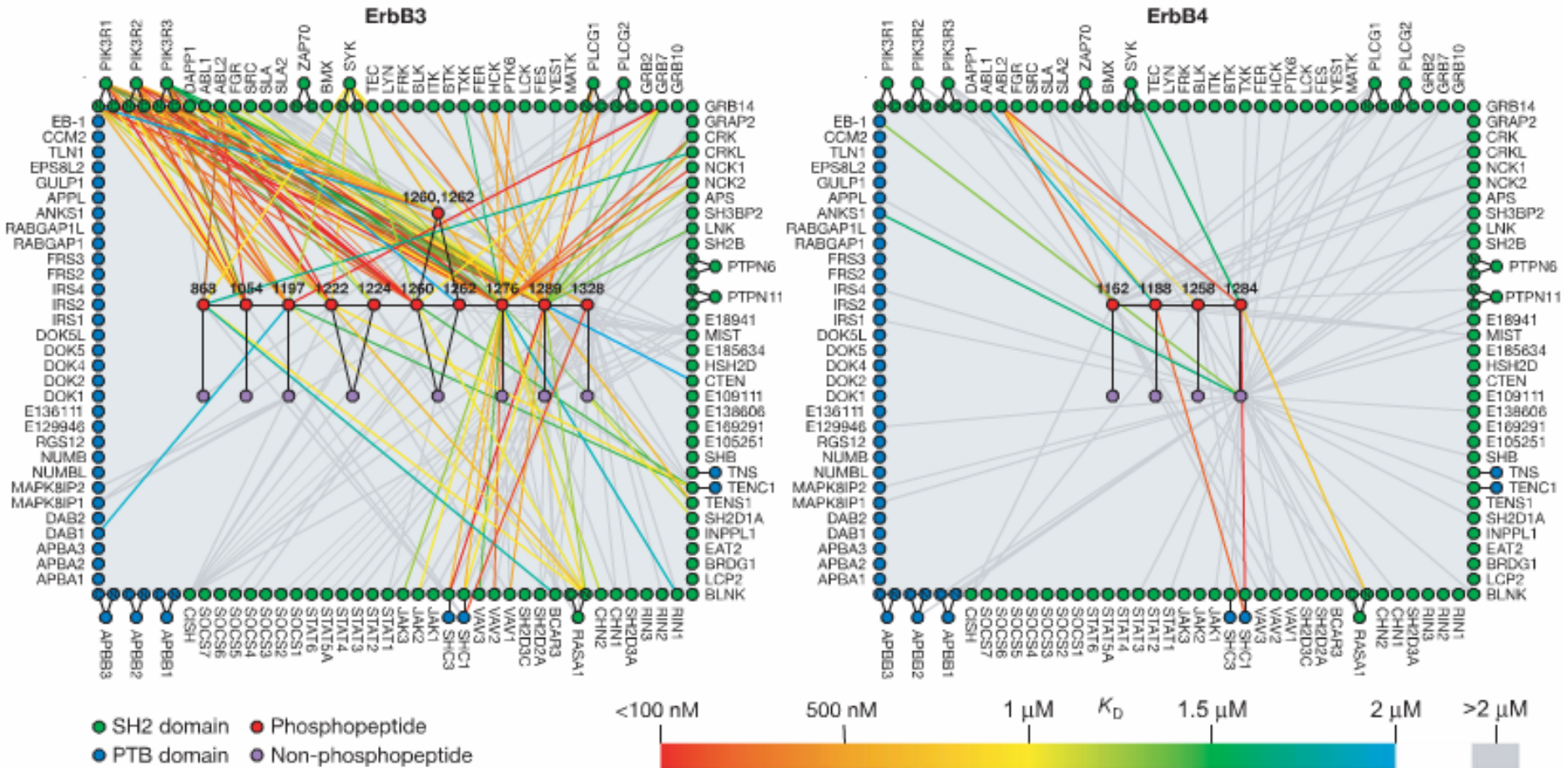
# Binding Affinity



# Quantitative Interaction

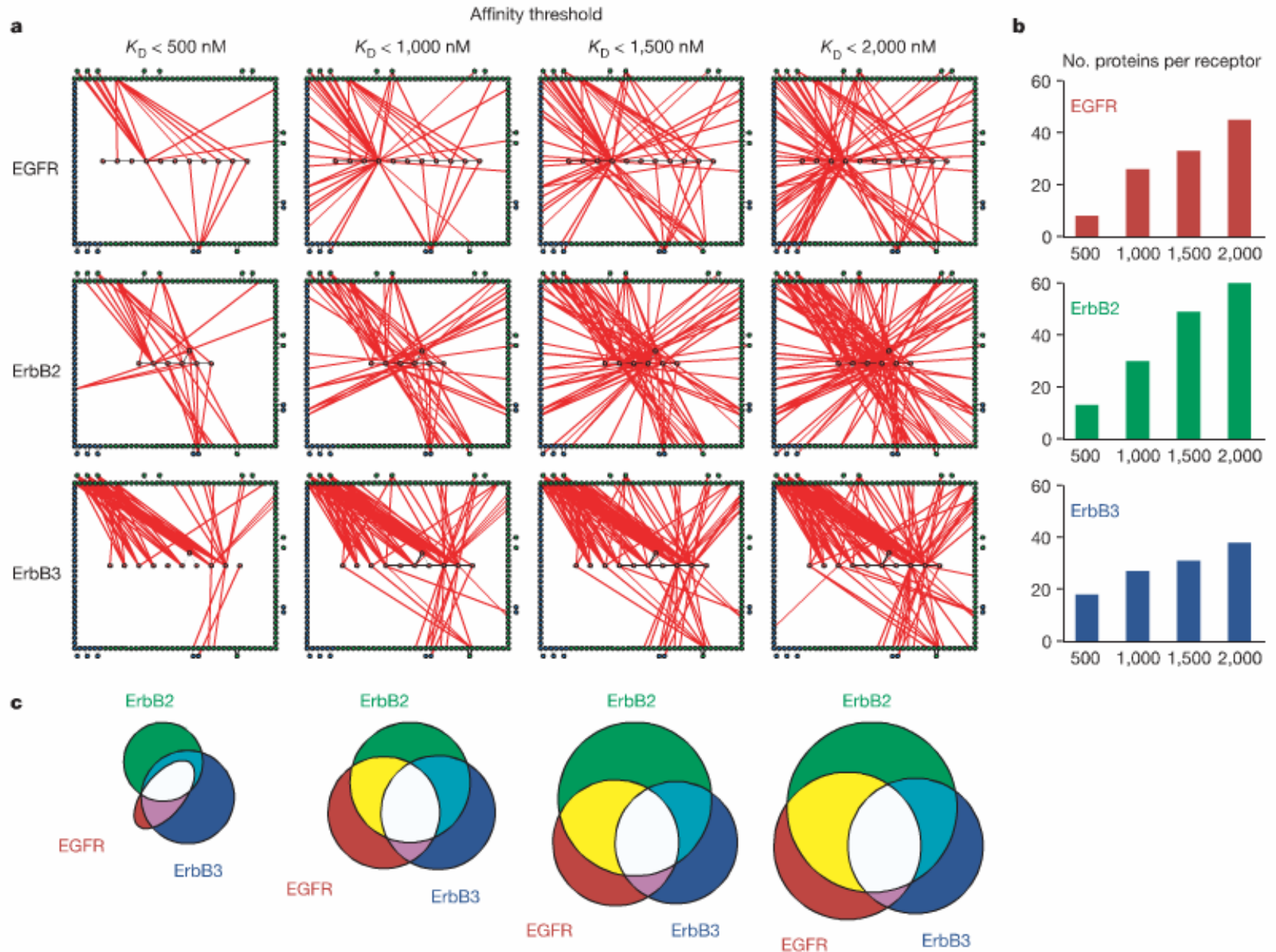


# Quantitative Interaction

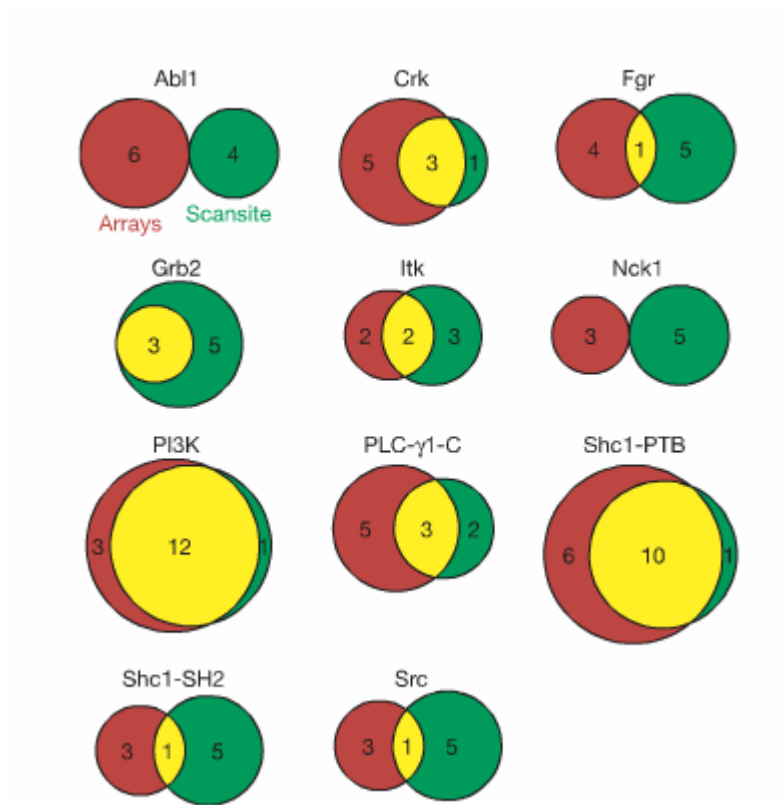




# System-level View



# Available Domains



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**PROTOCOL**

# Global identification of protein kinase substrates by protein microarray analysis

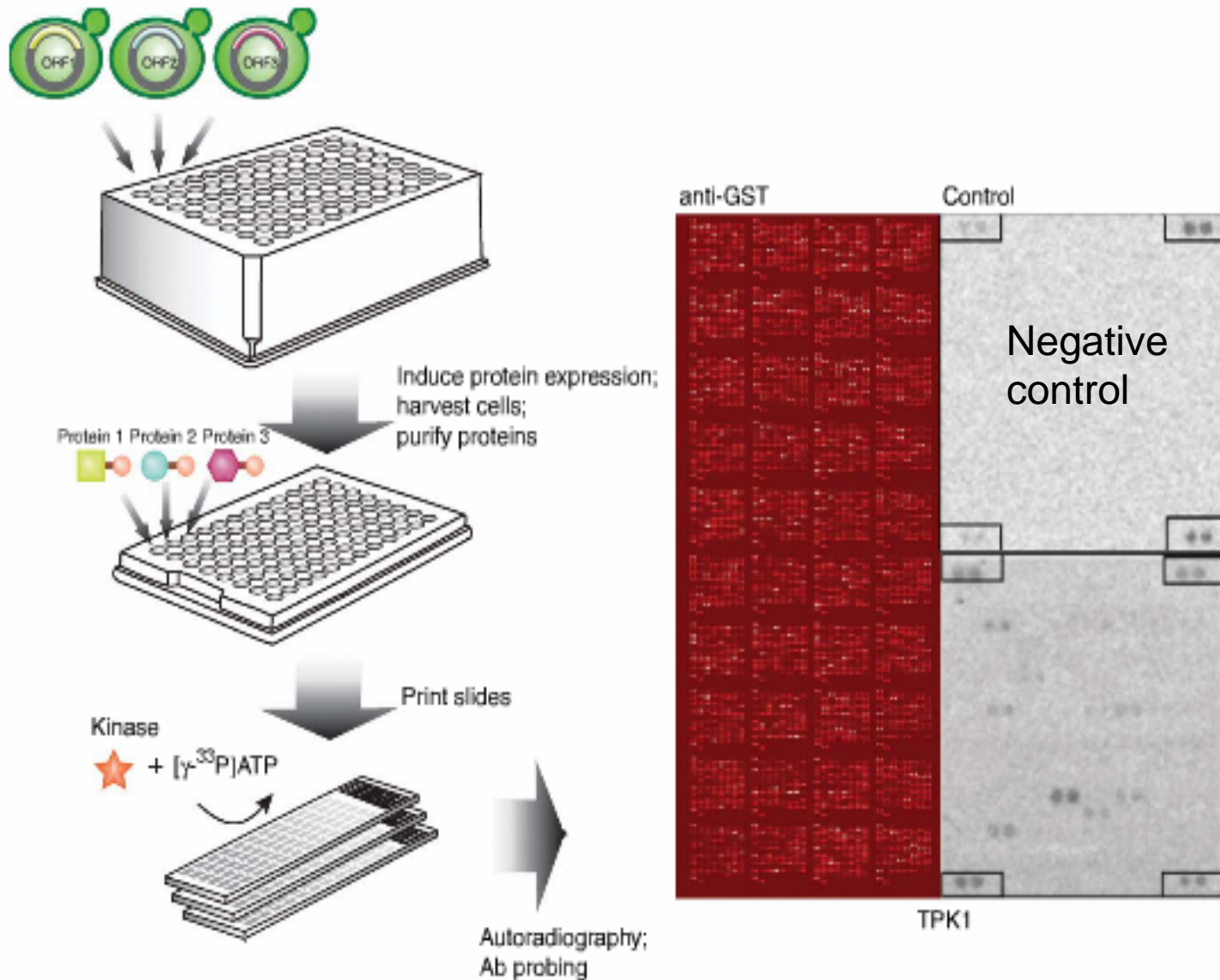
Janine Mok<sup>1-3</sup>, Hogune Im<sup>1-3</sup> & Michael Snyder<sup>1, 2</sup>

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<sup>1</sup>Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA. <sup>2</sup>Present addresses: Stanford Genome Technology Center, Stanford University, Palo Alto, California, USA (J.M.); Department of Genetics, Stanford University, Palo Alto, California, USA (H.I. and M.S.). <sup>3</sup>These authors contributed equally to this work. Correspondence should be addressed to M.S. (MPSnyder@stanford.edu).

Published online 19 November 2009; doi:10.1038/nprot.2009.194

# Yeast Kinase Identification





Contents lists available at [SciVerse ScienceDirect](#)

## Biosensors and Bioelectronics

journal homepage: [www.elsevier.com/locate/bios](http://www.elsevier.com/locate/bios)



### Development of a high-sensitivity immunoassay for amyloid-beta 1–42 using a silicon microarray platform

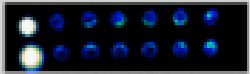








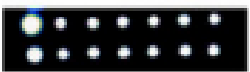


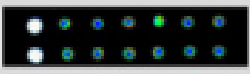
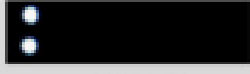

Paola Gagni, Laura Sola, Marina Cretich\*, Marcella Chiari

*Consiglio Nazionale delle Ricerche, Istituto di Chimica del Riconoscimento Molecolare (ICRM), Via Mario Bianco, 9 20131 Milano, Italy*

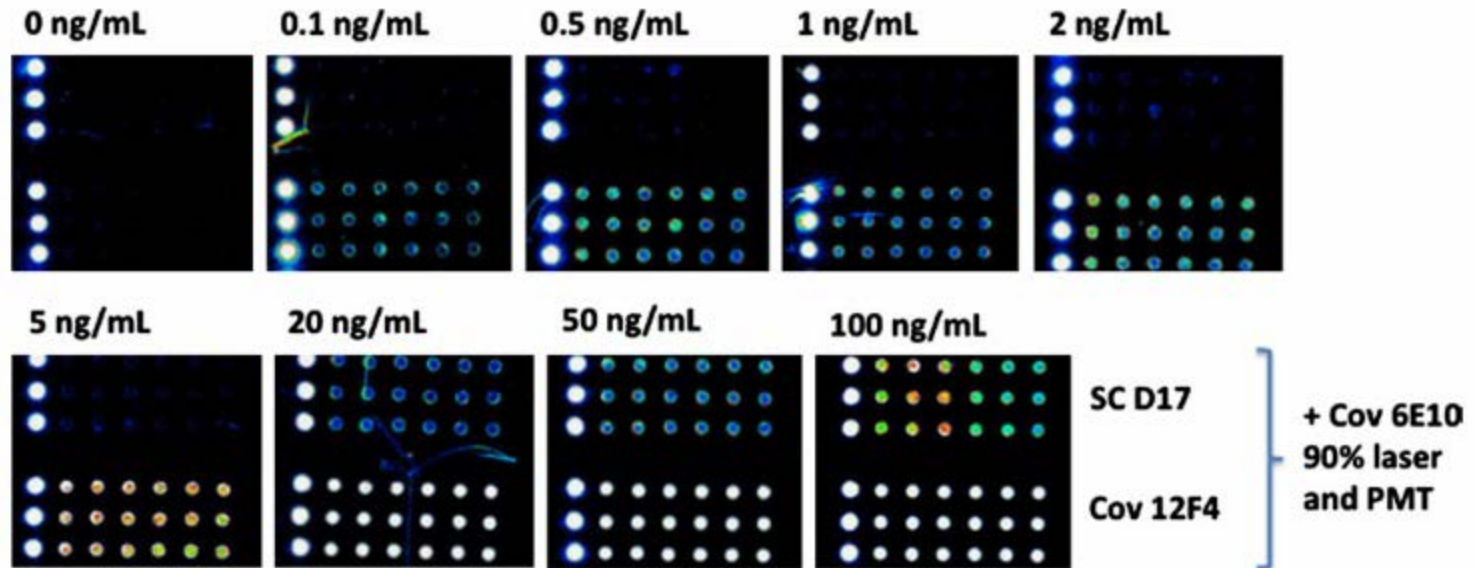




# Capture Ab selection

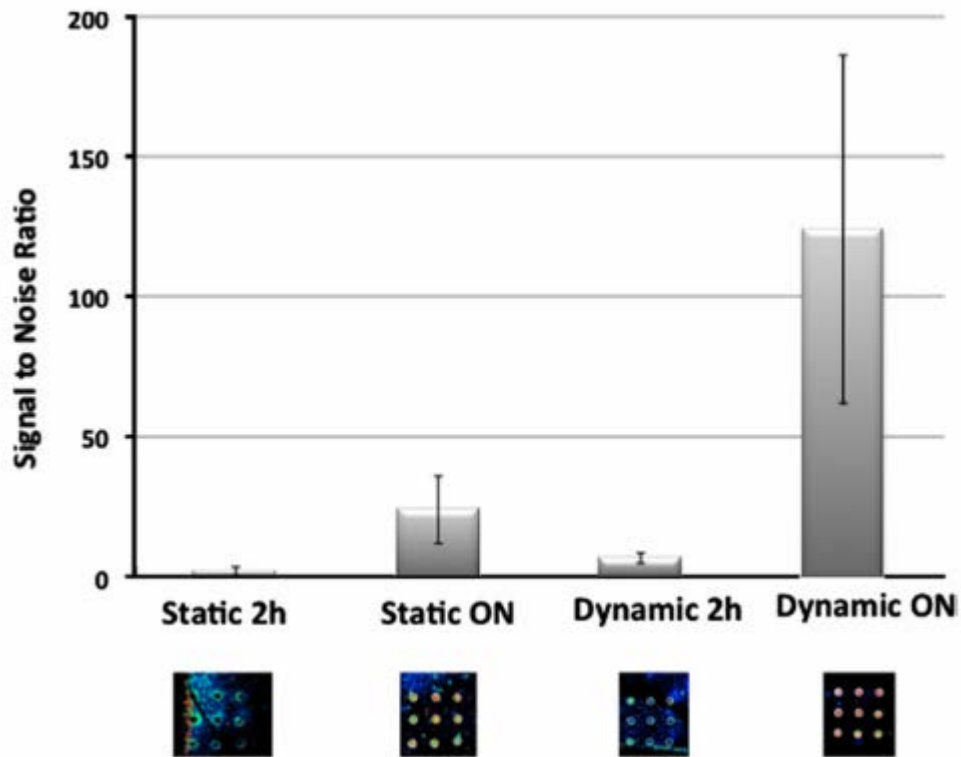
Incubation 1 hour/Cov-6E10 (80% laser and PMT)			
	A-Beta 1-42 100 ng/mL	A-Beta 1-39 100 ng/mL	Blank
SC-D17	 5540±1274	 42±26	 13±11
NT-11H3	 2290±412	 51±37	 23±10
NT-8G7	 506±210	 47±8	 47±13
Cov-4G8	 63219±751	 13818±260	 1208±117
Cov-12F4	 21010±1698	 260±65	 275±166

# AB 42 Detection



Artificial CSF

# Signal to Noise ratio



**Human CSF**

# Conclusions

- 15 years after the first landmark papers and proof of concepts the technique is limited to a few applications
- Use of arrays is discouraged by
  - The laborious preparation processes
  - Cost and equipment
  - Complexity of data generated
- Protein folding and missfolding remain a challenge and an obstacle for protein chip
- Protein chips are already available commercially, but major publications validating their use are still missing

# Questions?

