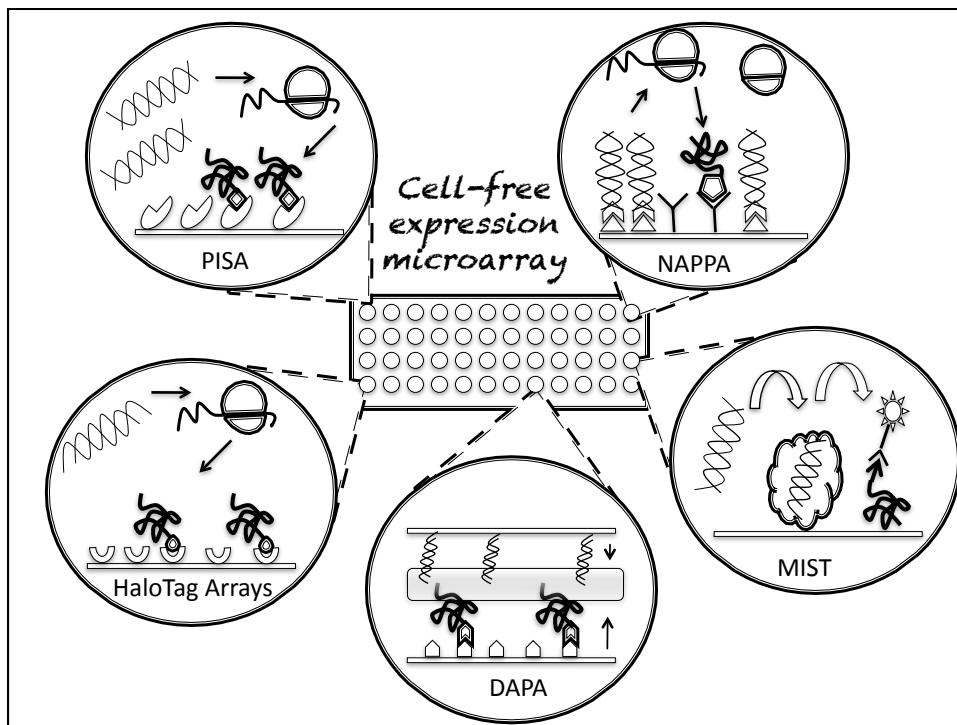


# Proteomics Course

## LECTURE-34 Applications of cell free protein microarrays



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IIT Bombay



## Lecture outline

- An overview of protein microarray experiment
- Applications
  - Biomarker screening
  - Immunological studies
  - Protein-protein interactions
- Challenges of data analysis: discussion

## An overview of protein microarray experiment

# **Applications**

## **I. Biomarker identification**

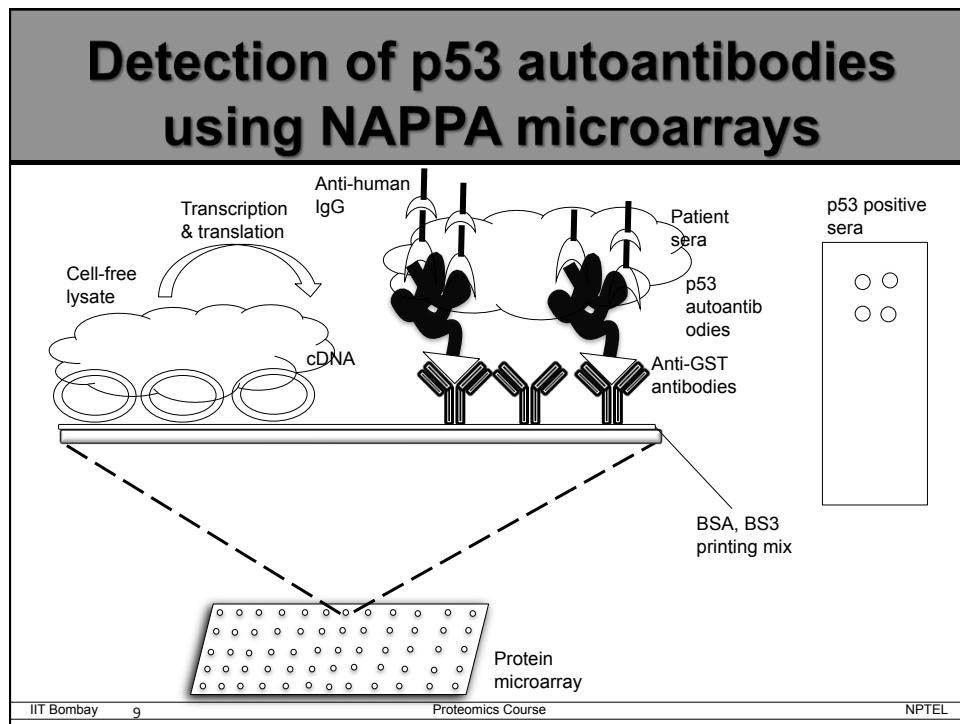
## Biomarker discovery using protein microarrays

- Protein microarrays have greatly accelerated biomarker discovery by simultaneous and rapid investigation of thousands of proteins
- Biomarkers have potential for –
  - early identification of disease state
  - monitoring treatment
  - following disease prognosis

## Case study-1

### Detection of p53 autoantibodies using NAPPA microarrays

Anderson, K. A., Ramachandran, N., Wong, J., Raphael, J. V. et al., Application of protein microarrays for multiplexed detection of antibodies to tumor antigens in breast cancer. *J. Proteome Res.* 2008, 7, 1490–1499

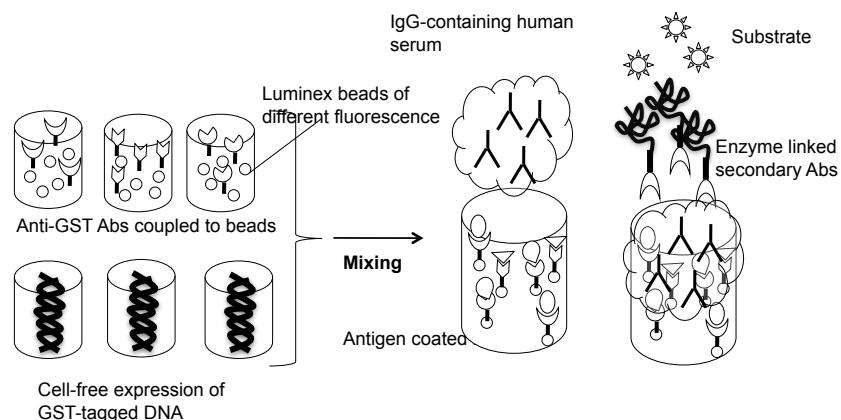


## Case study-2

### Bead-based assay for multiplexed detection of antibodies to EBNA-1 and p53

*Wong, J., Sibani, S., Lokko, N. N., LaBaer, J., Anderson, K. S., Rapid detection of antibodies in sera using multiplexed self-assembling bead arrays. J. Immunol. Methods 2009, 350, 171–182.*

## multiplexed detection of antibodies to EBNA-1 and p53



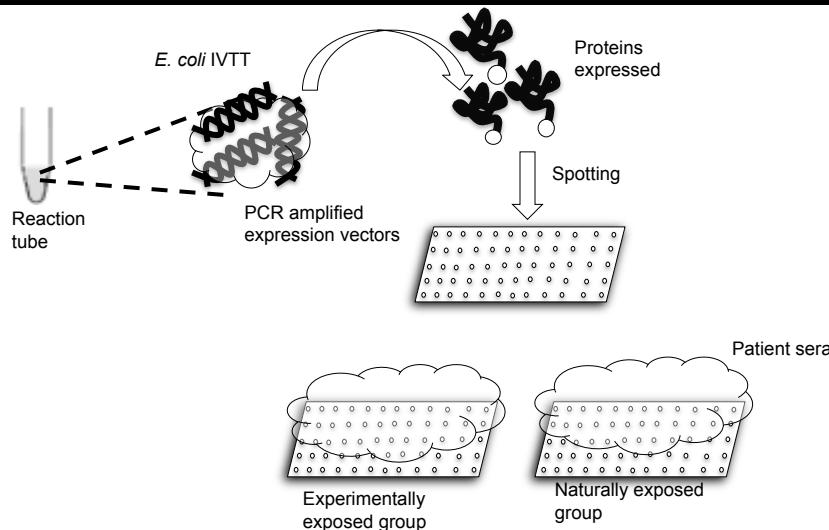
## II. Immunological studies

## Case study-3

### Detection of potential immunogenic proteins of *Plasmodium falciparum*

Doolan, D. L., Mu, Y., Unal, B., Sundaresh, S. et al., Profiling humoral immune responses to *P. falciparum* infection with protein microarrays. *Proteomics* 2008, 8, 4680–4694.

### Detection of potential immunogenic proteins of *Plasmodium falciparum*



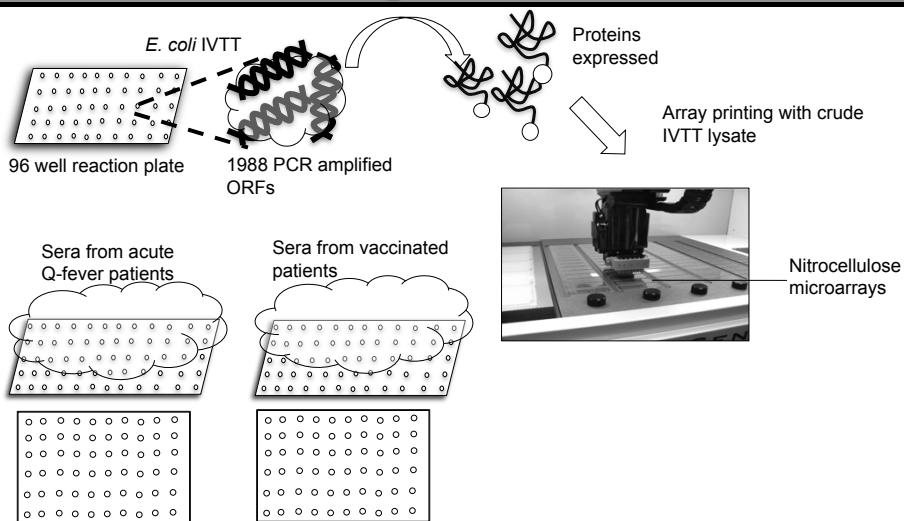
## Case study-4

### Identification of immunogens of Q-fever-causing *Coxiella burnetti*

Beare, P. A., Chen, C., Bouman, T., Pablo, J. et al., Candidate antigens for Q fever serodiagnosis revealed by immunoscreening of a *Coxiella burnetti* protein microarray. *Clin. Vaccine Immunol.* 2008, 15, 1771–1779.

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### Identification of immunogens of Q-fever-causing *Coxiella burnetti*



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### III. Protein Interaction

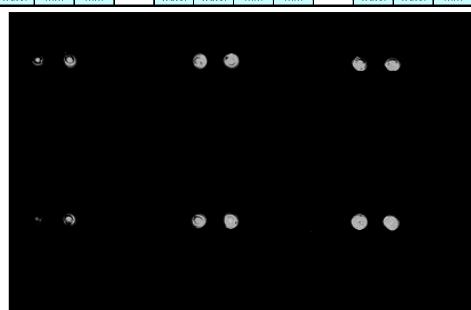
### Protein-protein interactions (Jun-Fos)

CDK2	CDK2	CDK4	CDK4
JUN	JUN	p53	p53
p21	p21	pCITE	pCITE
water	water	MM	MM

CDK2	CDK2	CDK4	CDK4
JUN	JUN	p53	p53
p21	p21	pCITE	pCITE
water	water	MM	MM

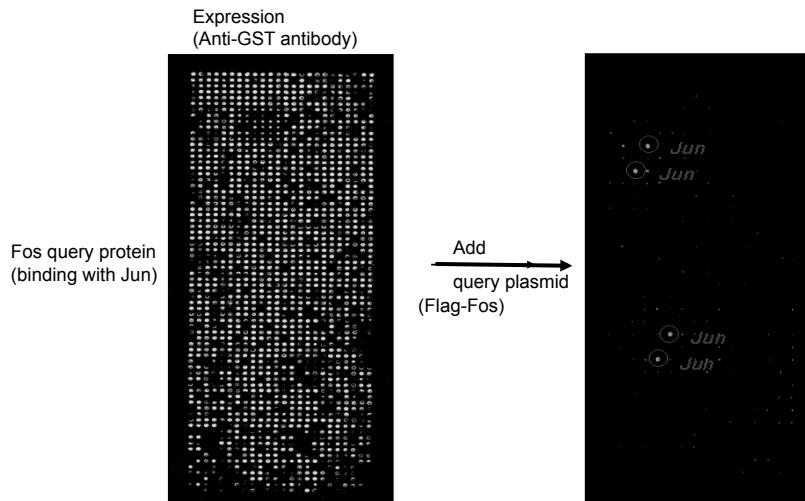
CDK2	CDK2	CDK4	CDK4
JUN	JUN	p53	p53
p21	p21	pCITE	pCITE
water	water	MM	MM

Fos query protein  
(binding with Jun)



Fos query

## Protein-protein interactions (Jun-Fos)



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## Case study-5

### Identification of novel protein-protein interactions using NAPPA microarray

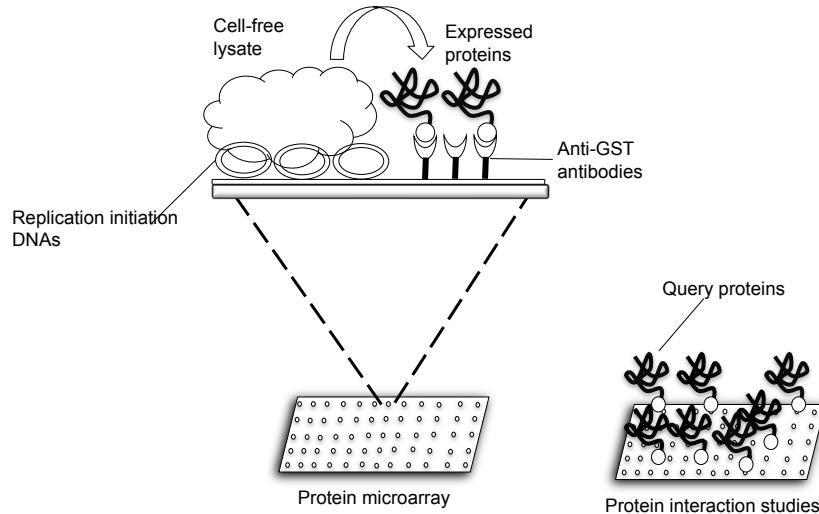
Ramachandran, N., Hainsworth, E., Bhullar, B., Eisenstein, S. et al., Self-assembling protein microarrays. *Science* 2004, 305, 86–90.

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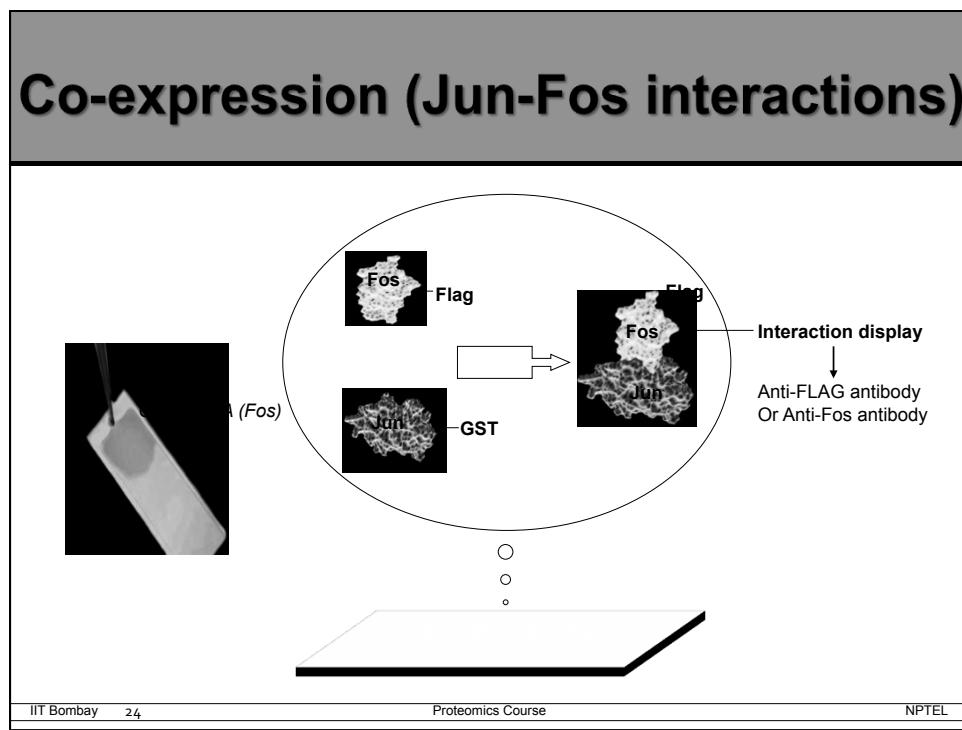
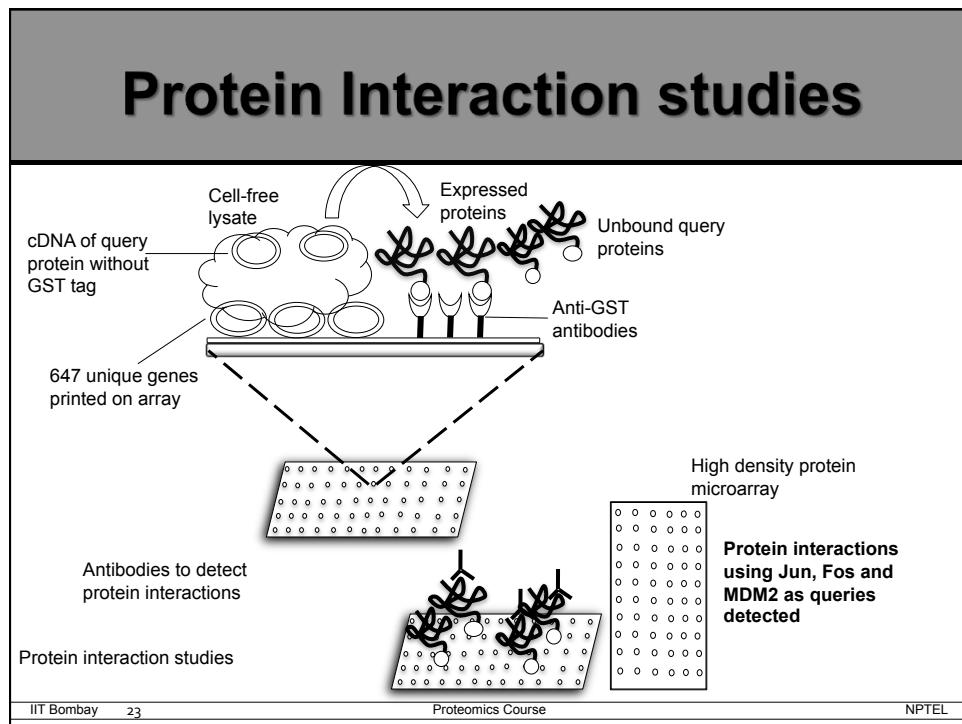
## Protein interaction studies



## Case study-6

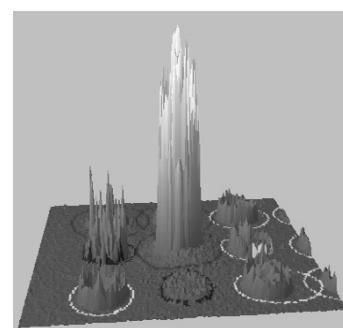
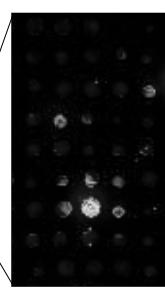
### High density NAPPA array approach for studying well characterized gene pairs

Ramachandran, N., Raphael, J. V., Hainsworth, E., Demirkiran, G. et al., Next-generation high-density self-assembling functional protein arrays. *Nat. Methods* 2008, 5, 535–538.



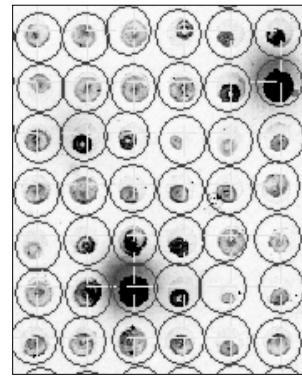
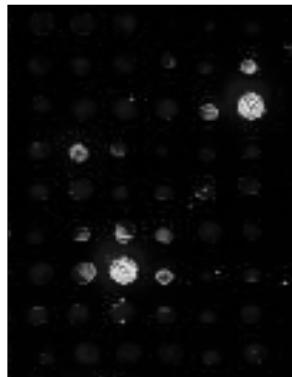
# Microarray data analysis challenges

## Image analysis



Evaluate spot morphology

## Issues in microarray data analysis



## Microarray data analysis challenges: discussion

## Summary

- An overview of protein microarray experiment
- Applications
  - Biomarker screening
  - Immunological studies
  - Protein-protein interactions
- Challenges of data analysis: discussion

## References

- He M, Wang MW. Arraying proteins by cell-free synthesis. *Biomol Eng.* 2007 Oct;24(4):375-80.
- Dobaño C, Widera G, Rabussay D, Doolan DL. 2007. Enhancement of antibody and cellular immune responses to malaria DNA vaccines by in vivo electroporation. *Vaccine.* 2007 Sep 4;25(36):6635-45.
- Davies DH, Liang X, Hernandez JE, Randall A, Hirst S, Mu Y, Romero KM, Nguyen TT, Kalantari-Dehaghi M, Crotty S, Baldi P, Villarreal LP, Felgner PL. Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. *Proc Natl Acad Sci U S A.* 2005 Jan 18;102(3):547-52.
- Doolan DL, Mu Y, Unal B, Sundaresh S, Hirst S, Valdez C, Randall A, Molina D, Liang X, Freilich DA, Oloo JA, Blair PL, Aguiar JC, Baldi P, Davies DH, Felgner PL. Profiling humoral immune responses to *P. falciparum* infection with protein microarrays. *Proteomics.* 2008 Nov;8(22):4680-94. doi: 10.1002/pmic.200800194.
- Wong J, Sibani S, Lokko NN, LaBaer J, Anderson KS. Rapid detection of antibodies in sera using multiplexed self-assembling bead arrays. *J Immunol Methods.* 2009 Oct 31;350(1-2):171-82. doi: 10.1016/j.jim.2009.08.013.

## References

- Harini Chandra, Sanjeeva Srivastava. 2010. Cell-free synthesis-based protein microarrays and their applications. PROTEOMICS. Volume 10, Issue 4, pages 717–730, No. 4 February 2010
- Harini Chandra, Panga Jaipal Reddy and Sanjeeva Srivastava. 2011. Expert Review of Proteomics. Protein microarrays and novel detection platforms. February 2011, Vol. 8, No. 1, Pages 61-79 10.1586/epr.10.99
- Mingyue Hea, Ming-Wei Wangb. 2007. Arraying proteins by cell-free synthesis. Biomolecular Engineering. Volume 24, Issue 4, October 2007, Pages 375–380.
- Alison M. Jackson, Joe Boutell, Neil Cooley, Mingyue He. 2004. Cell-free protein synthesis for proteomics. *Briefings in Functional Genomics and Proteomics* (2004) 2 (4): 308-319.
- Deb K. Chatterjee, Kalavathy Sitaraman, Cassio Baptista, James Hartley, Thomas M. Hill, David J. Munroe. 2008 Protein Microarray On-Demand: A Novel Protein Microarray System. Volume 3 | Issue 9. PLoS one.
- Joshua LaBaer , Niroshan Ramachandran. 2005. Protein microarrays as tools for functional proteomics. Current Opinion in Chemical Biology. Volume 9, Issue 1, February 2005, Pages 14–19.

## References

- Niroshan Ramachandran, Dale N. Larson, Peter R. H. Stark, Eugenie Hainsworth, Joshua LaBaer. 2005. Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS Journal. Volume 272, Issue 21, pages 5412–5425, November 2005.
- Farid Khan , Elizabeth Palmer , Mingyue He , Michael J . Taussig , and Mingwei Wang. 2007. Functional Protein Microarrays in Drug Discovery. Chapter 8. Protein In Situ. Arrays through Cell-Free Protein Synthesis Pages 133–143. ISBN: 978-0-8493-9809-4.
- Niroshan Ramachandran, Dale N. Larson, Peter R. H. Stark, Eugenie Hainsworth, Joshua LaBaer. 2005. Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS Journal. Volume 272, Issue 21, pages 5412–5425, November 2005.
- Luis Berrade, Angie E. Garcia, Julio A. Camarero. 2011. Protein Microarrays: Novel Developments and Applications. Pharmaceutical Research. July 2011, Volume 28, Issue 7, pp 1480-1499.
- Oda Stoevesandt, Michael J Taussig and Mingyue He. 2009. Protein microarrays: high-throughput tools for proteomics. Expert Review of Proteomics. April 2009, Vol. 6, No. 2, Pages 145-157.

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