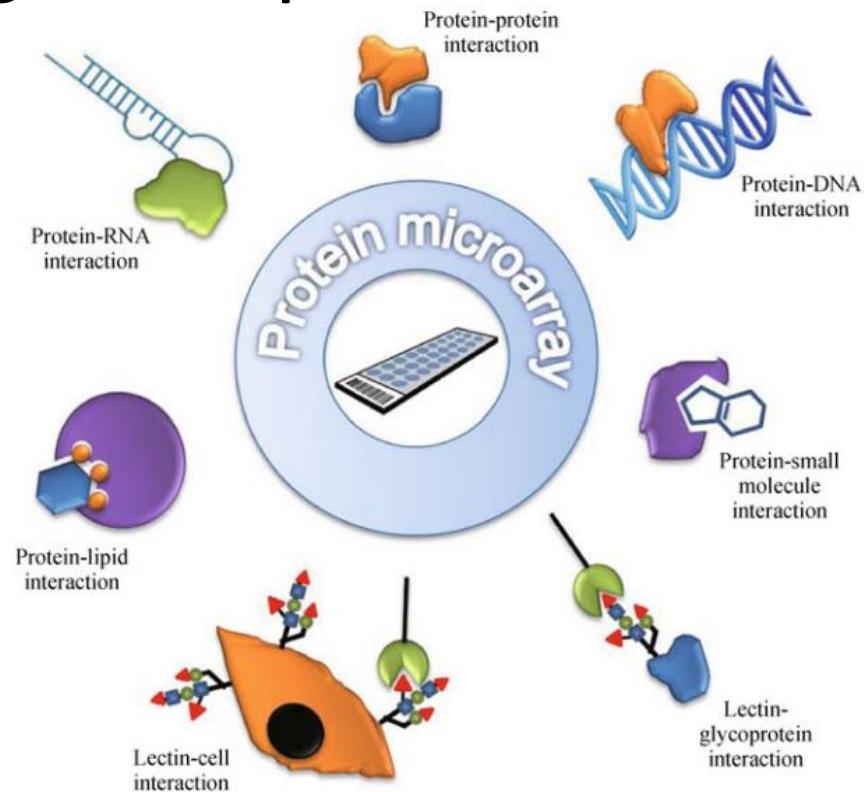
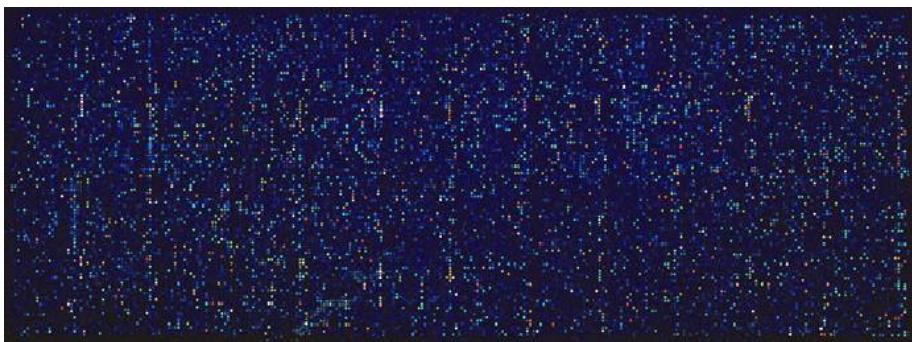


GENOMICA Y PROTEOMICA COMPUTACIONAL

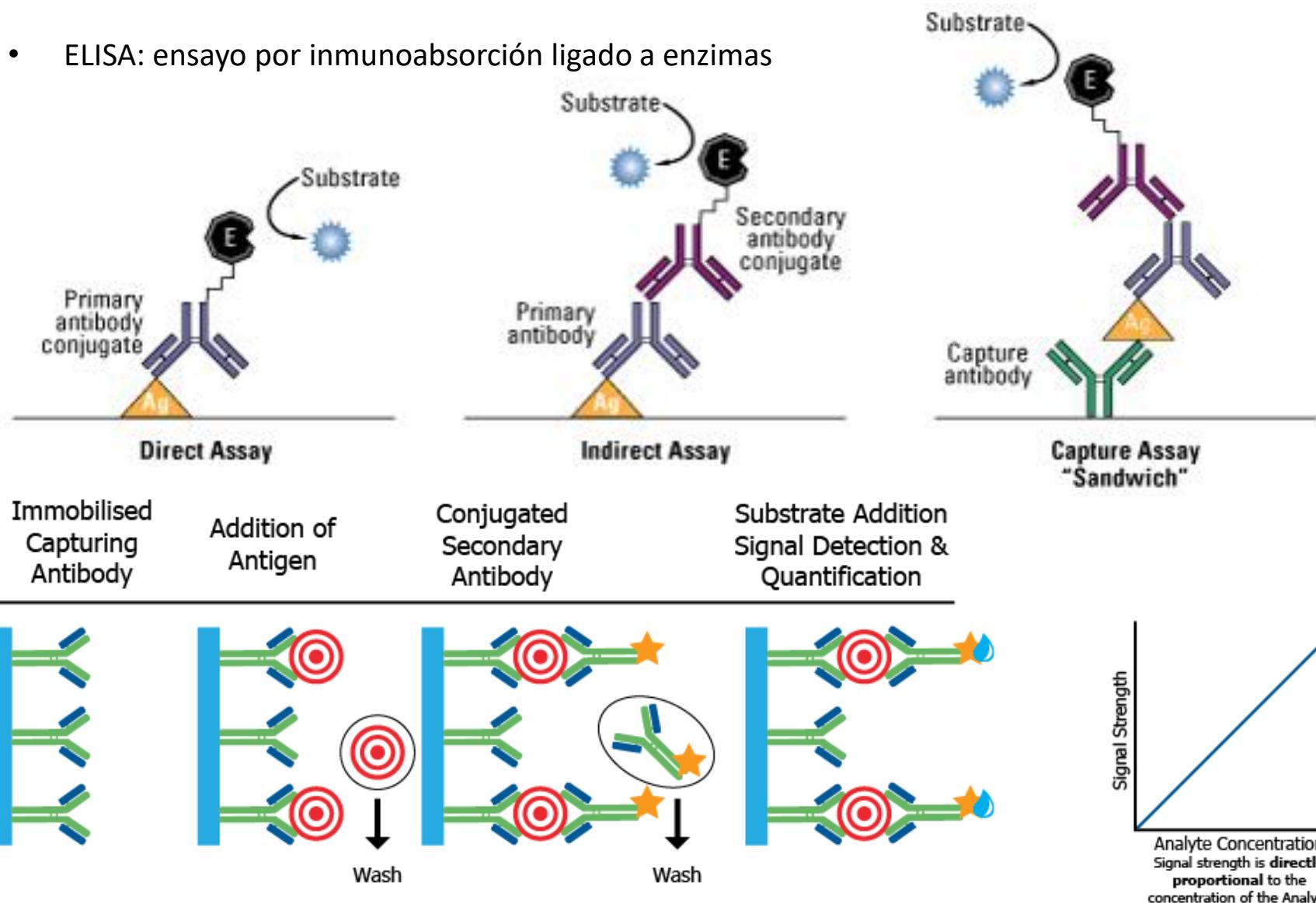
Clase 12: Microarreglos de proteínas

Dr. Frank Solano



ELISA

- ELISA: ensayo por inmunoabsorción ligado a enzimas

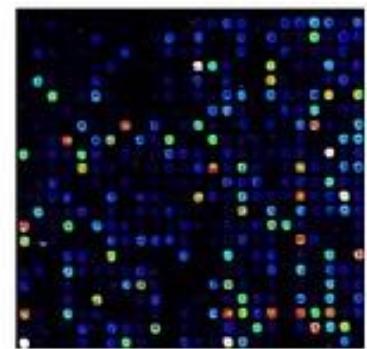


Microarreglos de proteínas vs ELISA

- Mas datos con menos muestra
 - Evalúa un gran número de interacciones con el mismo tamaño de muestra (multiplex), ELISA es monoplex, solo una por pozo
- Mayor sensibilidad
 - Detecta hasta 1 pg/ml mientras que un ELISA requiere 40 pg/ml
- Mayor rango de detección
 - Desde 25 a 250000 pg/ml, un aumento de 10000 veces, mientras en ELISA solo 100 o 1000 veces (diluciones)
- Precisión
 - Menores coeficientes de variación en la intensidad de la señal del spot entre arrays (menor a 5%), controles internos, dos replicados



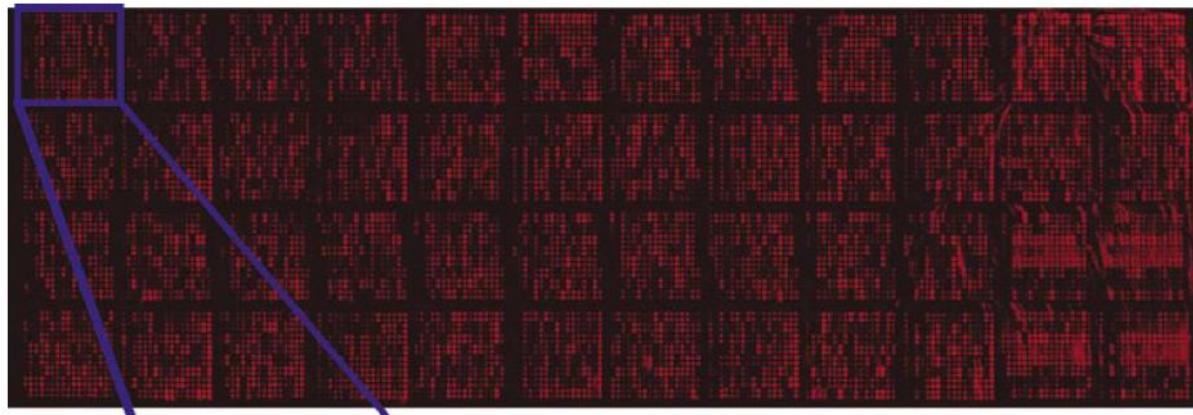
96 well plate 1 data point 7mm diameter



1 microarray "well" one to hundreds of 100 um size data points

Microarreglos de proteínas

- Herramientas para:
 - Evaluación de medicamentos, diagnóstico de enfermedades, mapeo de rutas metabólicas, interacciones proteína-proteína, desarrollo de vacunas, perfiles de enzimas-sustrato



Solo pueden detectar proteínas presentes en el chip (2-DE y MALDI-TOF)

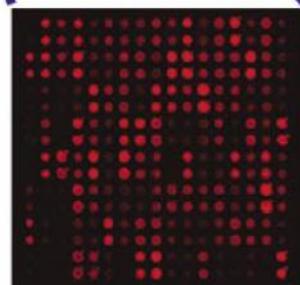


Figure 1. A typical protein microarray image. A yeast protein microarray is probed with anti-GST antibodies followed by detection with Cy5-conjugated secondary antibodies. An enlarged image of one of the 48 blocks is depicted below the protein chip.

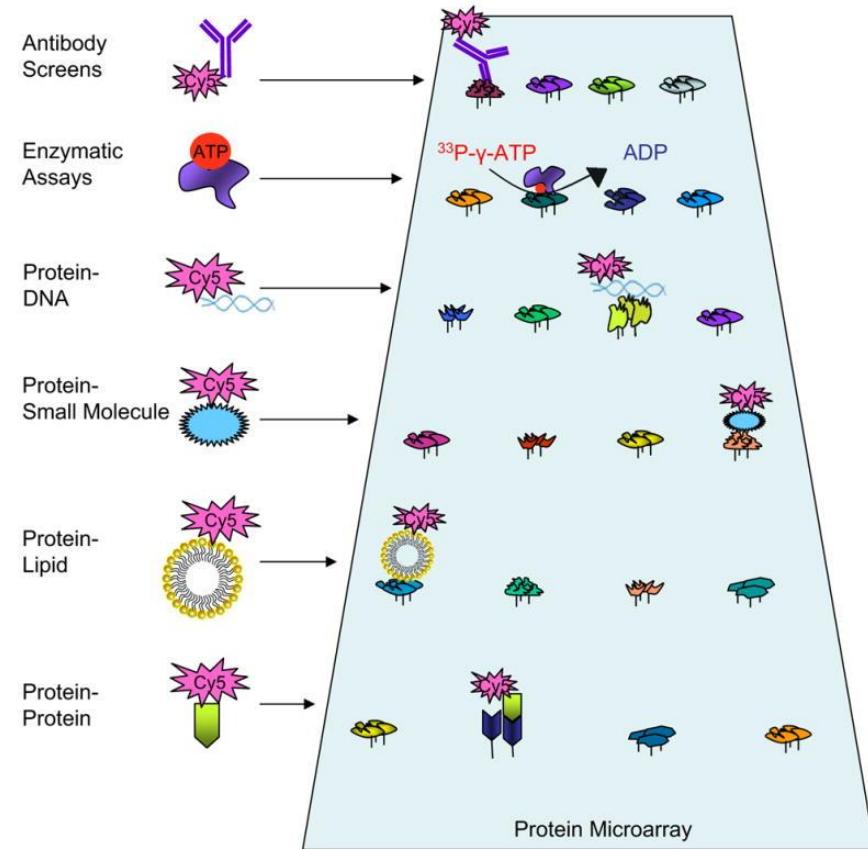
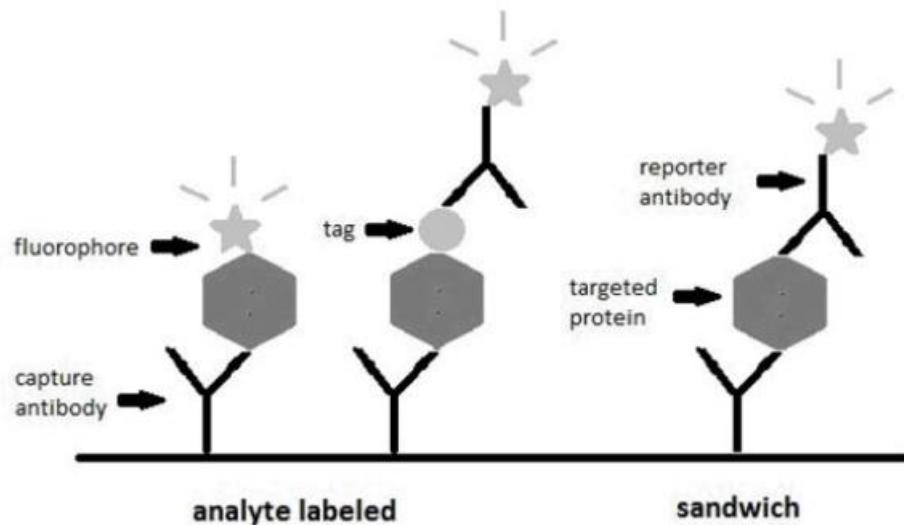
Tipos de microarreglos de proteínas

- Microarreglos analíticos

- Monitorear diferencias en perfiles de expresión del proteoma o diagnóstico clínico
- Antibodies o aptámeros en el chip, se agrega una mezcla líquida de proteínas
- Ej: medir anticuerpos, antígenos, citocinas en suero o saliva

Versiones miniatura y multiplex de un ELISA

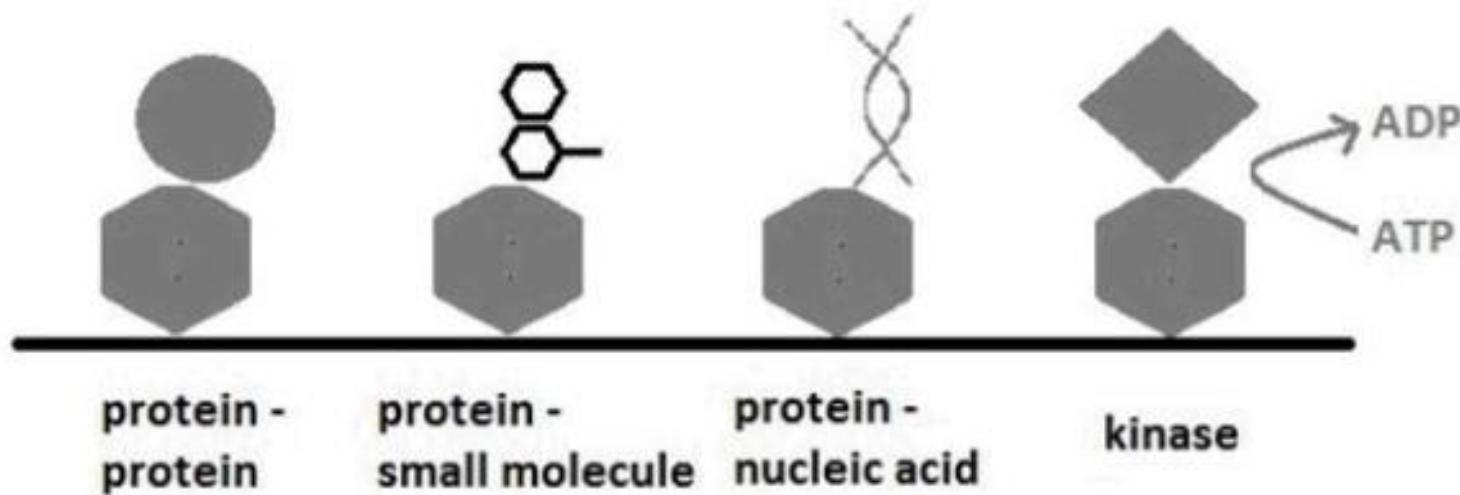
Reduce cantidad de muestra requerida,
antígeno, reactivos y tiempo



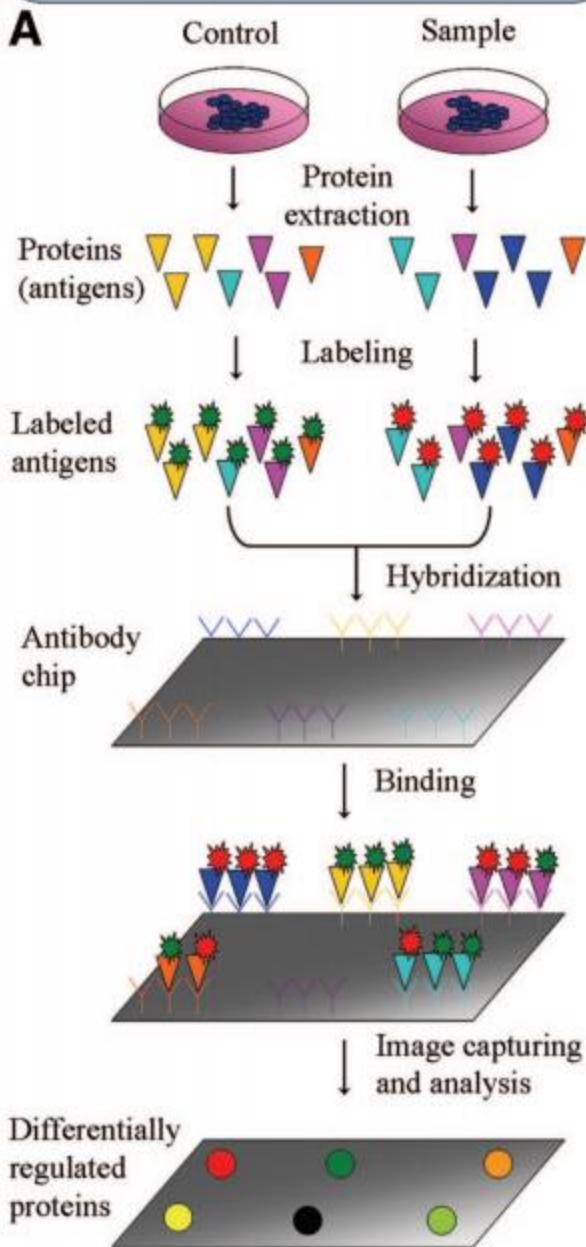
Tipos de microarreglos de proteínas

- Microarreglos funcionales

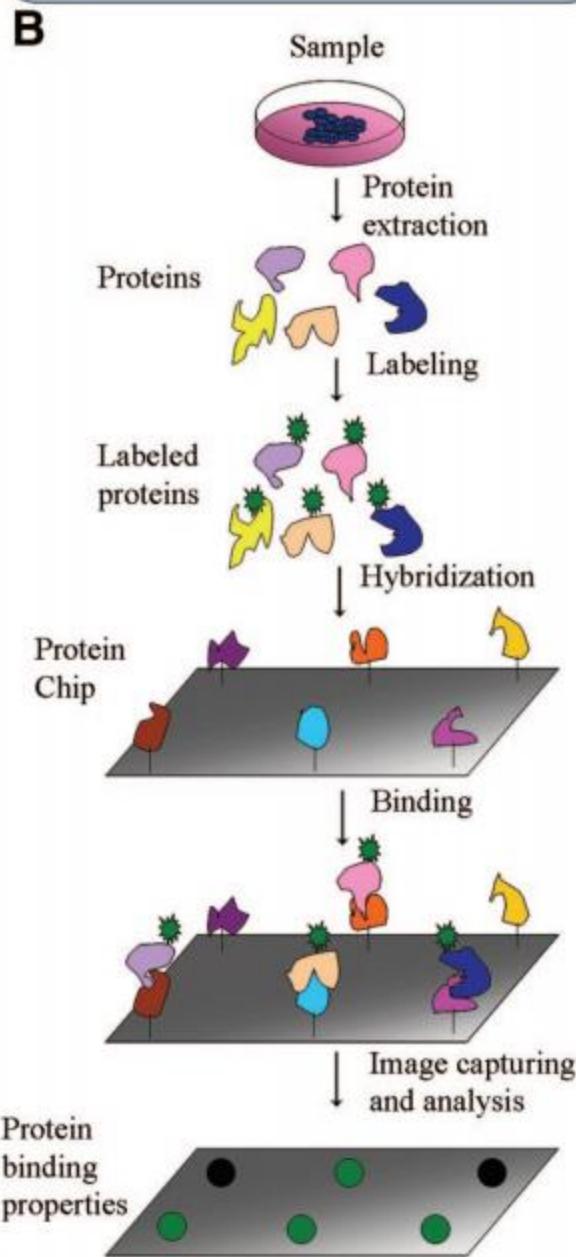
- Estudiar la actividad, las uniones y las modificaciones del proteoma en un solo experimento
- Interacciones proteína con proteína, con lípidos, con ADN o ARN
- Requiere proteínas en estado nativo



Analytical protein microarray

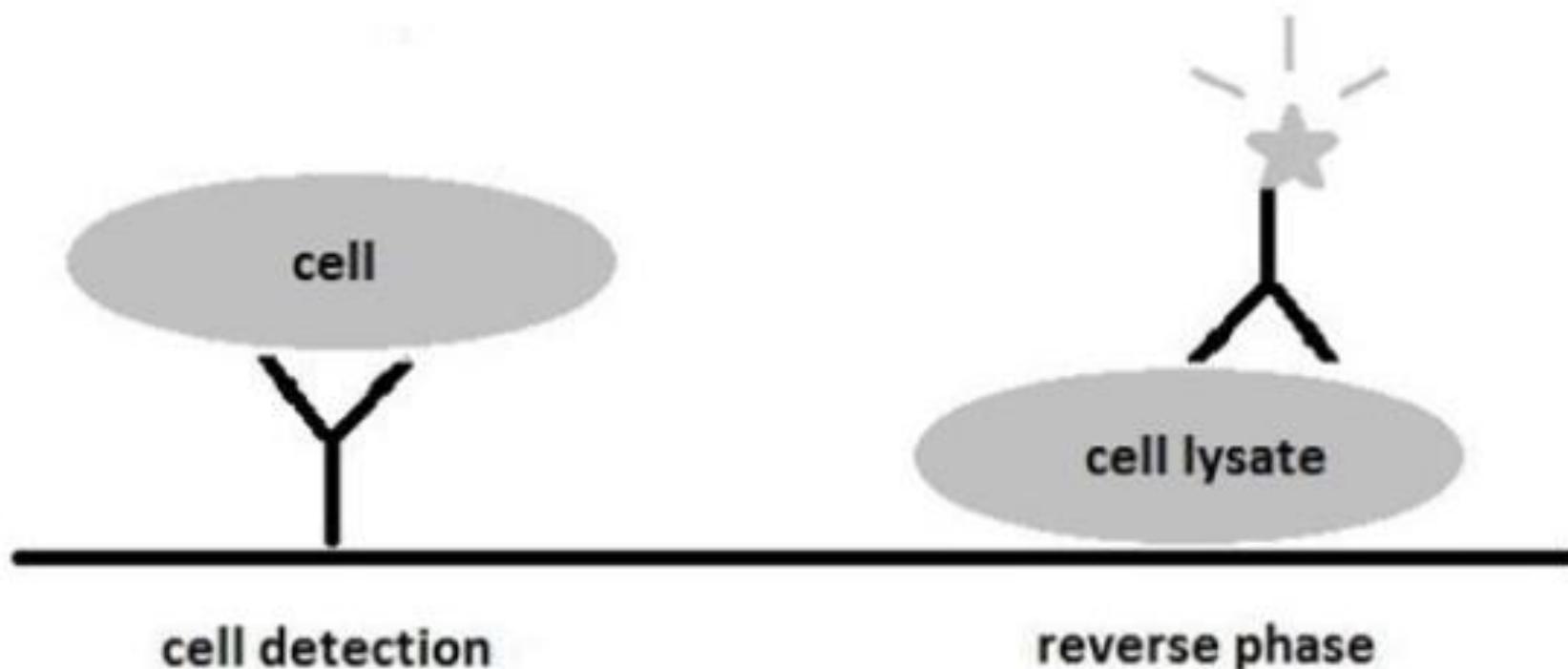


Functional protein microarray



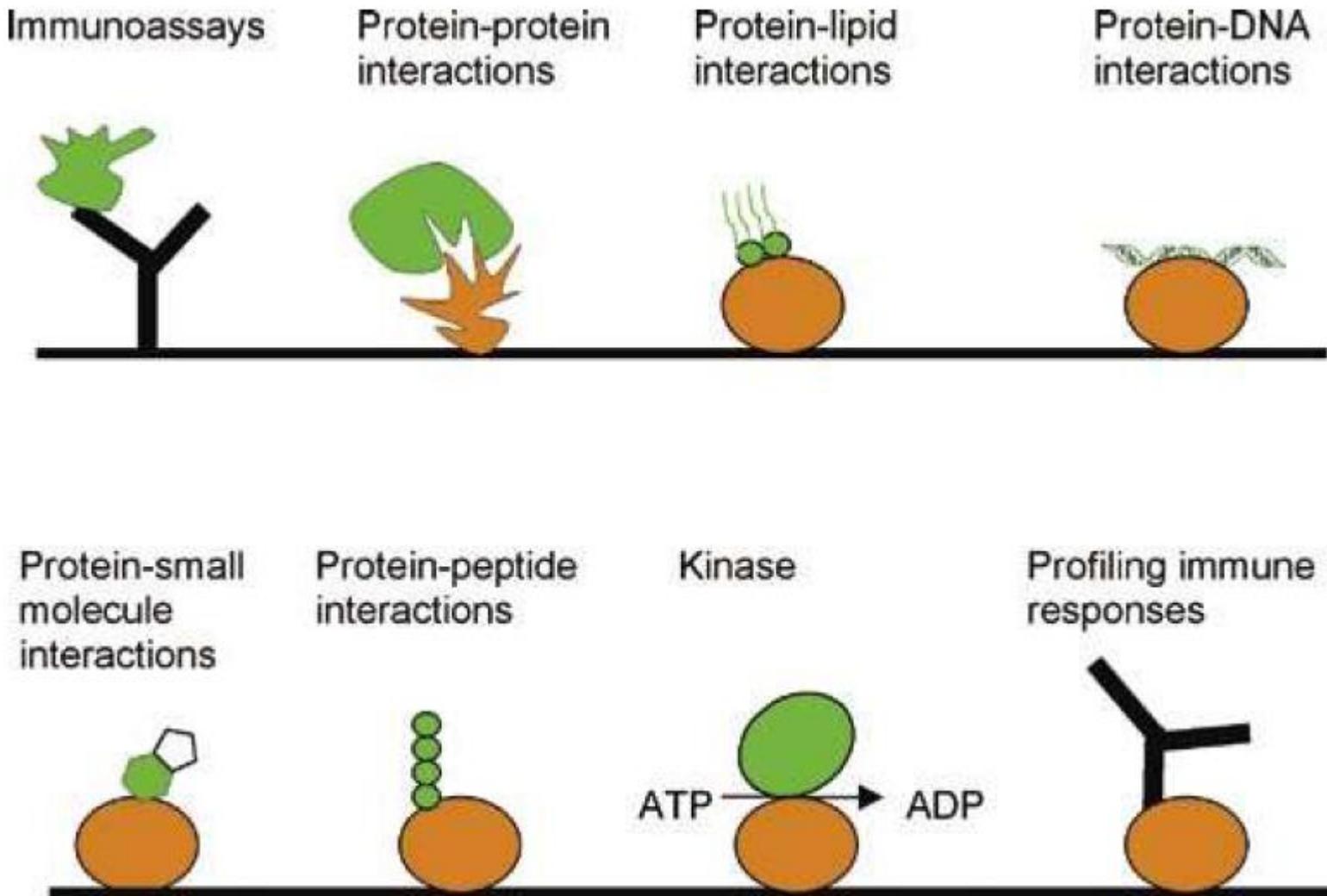
Tipos de microarreglos de proteínas

- Microarreglo en fase reversa



Microarray de proteínas

- Tipos de arrays



Microarray de proteínas



US006101946A

United States Patent [19]
Martinsky

[11] **Patent Number:** **6,101,946**
[45] **Date of Patent:** **Aug. 15, 2000**

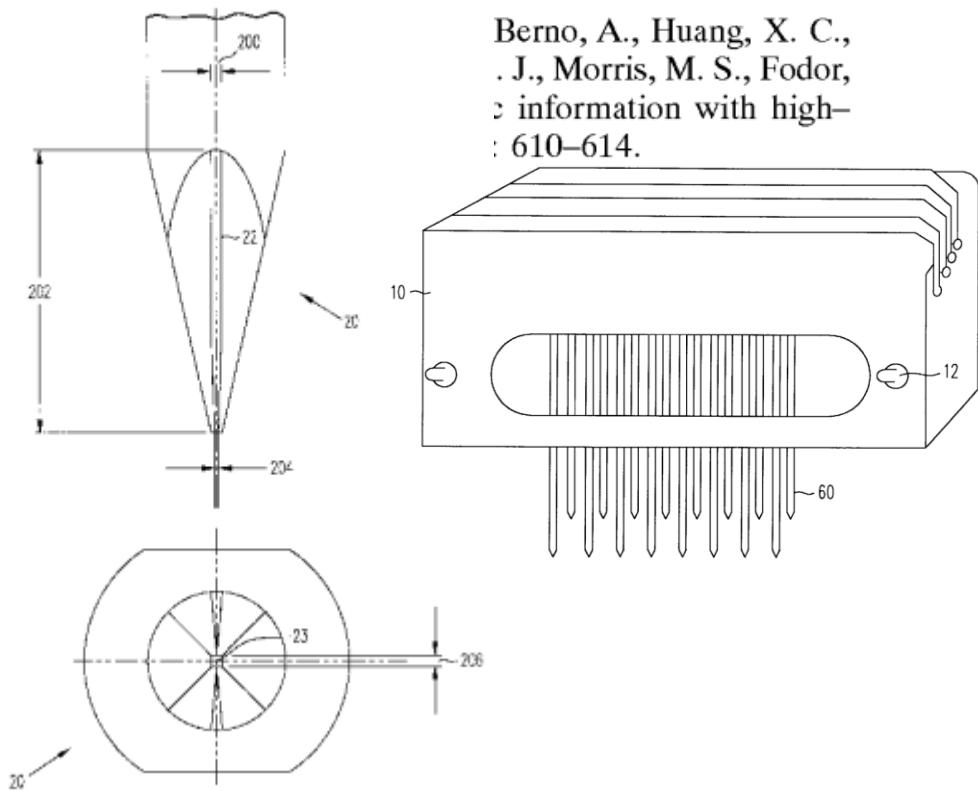
[54] **MICROARRAY PRINTING DEVICE
INCLUDING PRINTING PINS WITH FLAT
TIPS AND EXTERIOR CHANNEL AND
METHOD OF MANUFACTURE**

[75] Inventor: **Richard S Martinsky**, San Jose, Calif.

[73] Assignee: **TeleChem International Inc.**,
Sunnyvale, Calif.

[21] Appl. No.: **09/191,935**

[22] Filed: **Nov. 13, 1998**



Berno, A., Huang, X. C.,
. J., Morris, M. S., Fodor,
information with high-
: 610-614.

Adhesión de proteínas en el array

- Portaobjetos de vidrio recubierto de epoxisilano

Proteínas se unen por medio de los grupos amino, tiol e hidroxilo

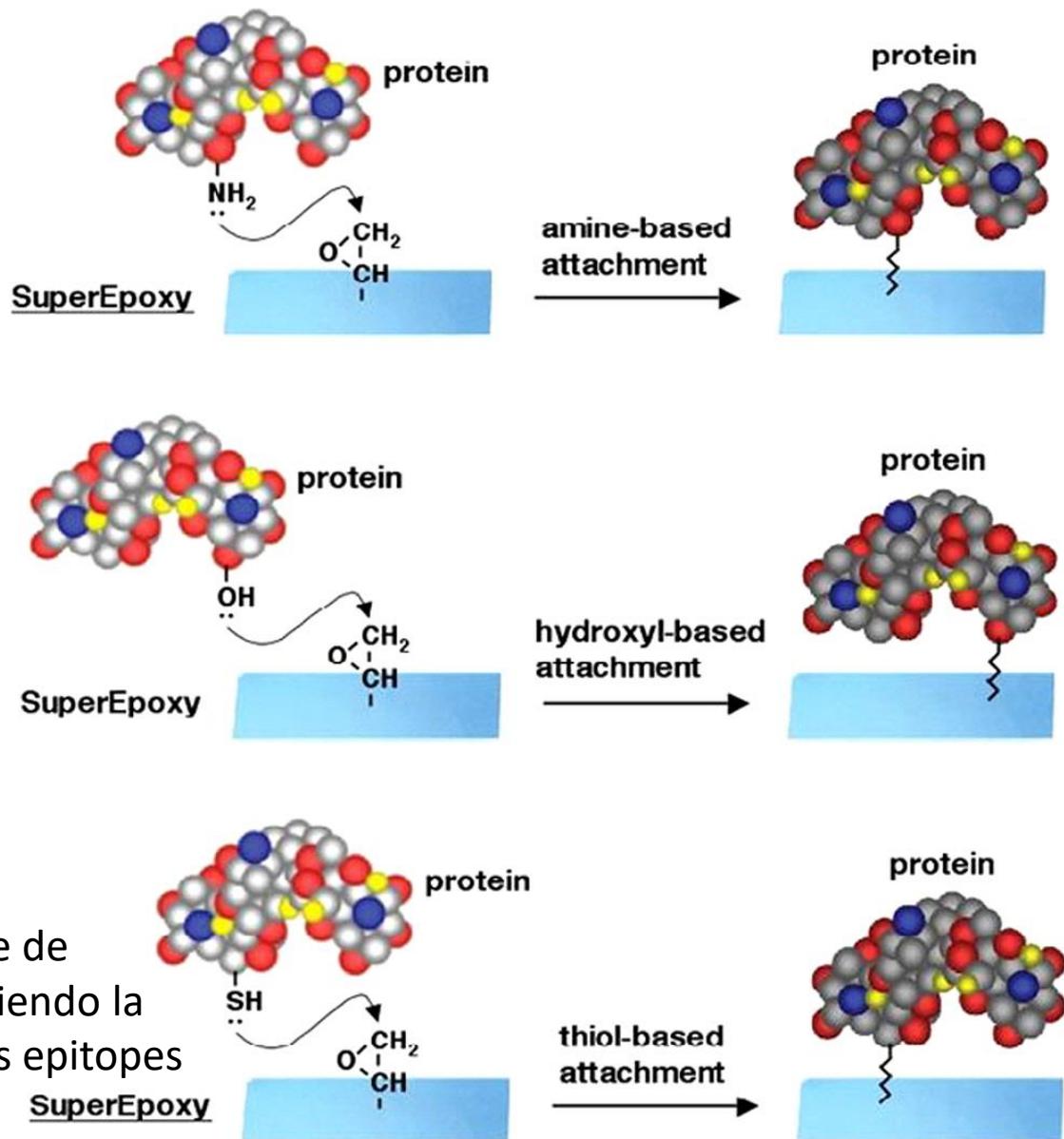
La reacción con el grupo epoxi es rápida, irreversible y no requiere calor o luz UV

Los grupos epoxi que no reaccionaron pueden ser desactivados después de la impresión de las proteínas para evitar la unión no específica

Une oligos, ARNm, PCR y BACs, así como proteínas

Adhesión de proteínas en el array

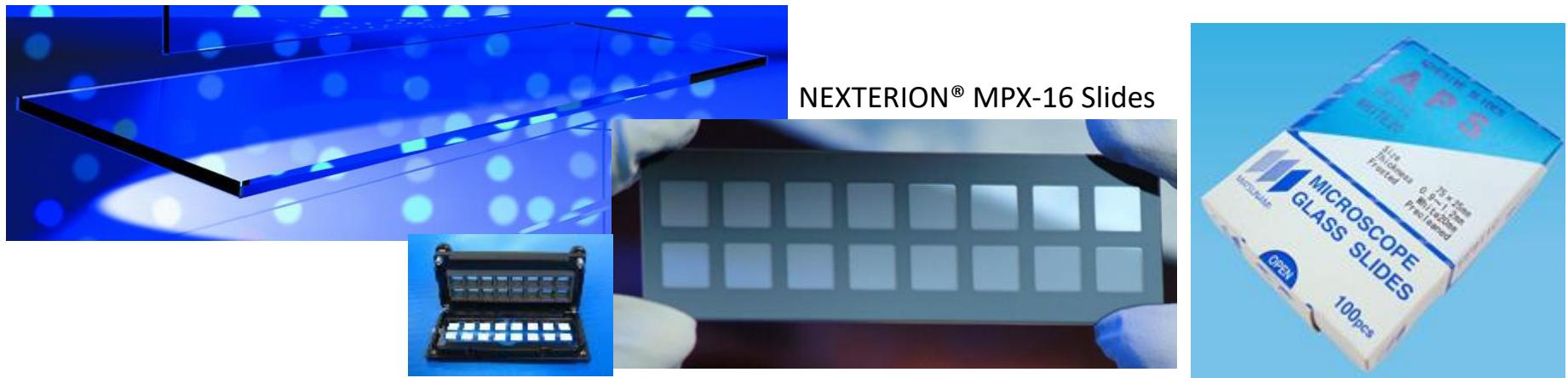
- Portaobjetos de vidrio recubierto de epoxisilano



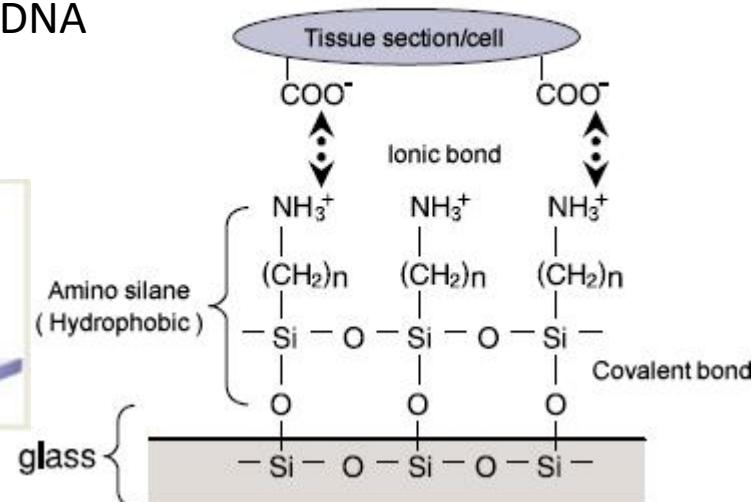
Las proteínas se unen a la superficie de manera espacial aleatoria, manteniendo la disponibilidad de todos los epitopos epitopes in each printed microarray spot

Adhesión de proteínas en el array

- Portaobjetos de vidrio recubierto de aminosilano



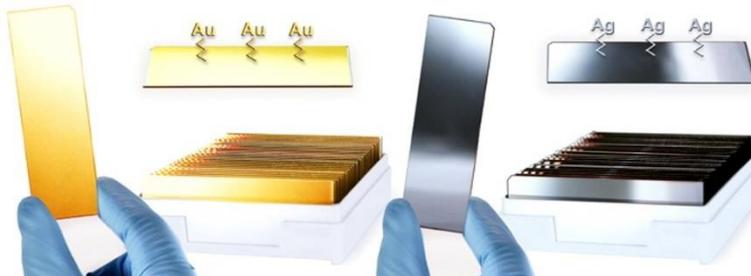
Para adherir BACS, oligonucleótidos \geq 40mers, pequeños fragmentos de proteínas (péptidos), y cDNA
Grupos fosfato negativos en el ADN



Adhesión de proteínas en el array



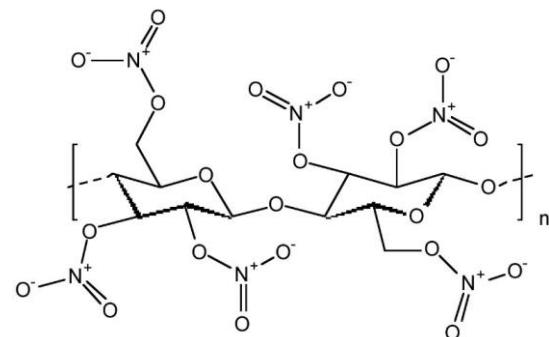
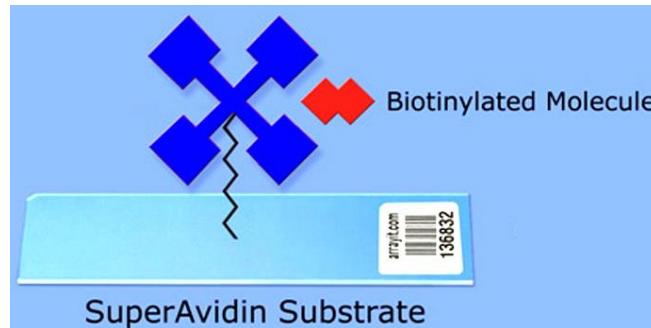
SuperClean 2 Microarray Substrate
Add their own chemical treatments and coatings



Protein A/G Microarray Substrates, bind human immunoglobulin proteins IgG1, IgG2, IgG3, IgG4 and all types of total IgG

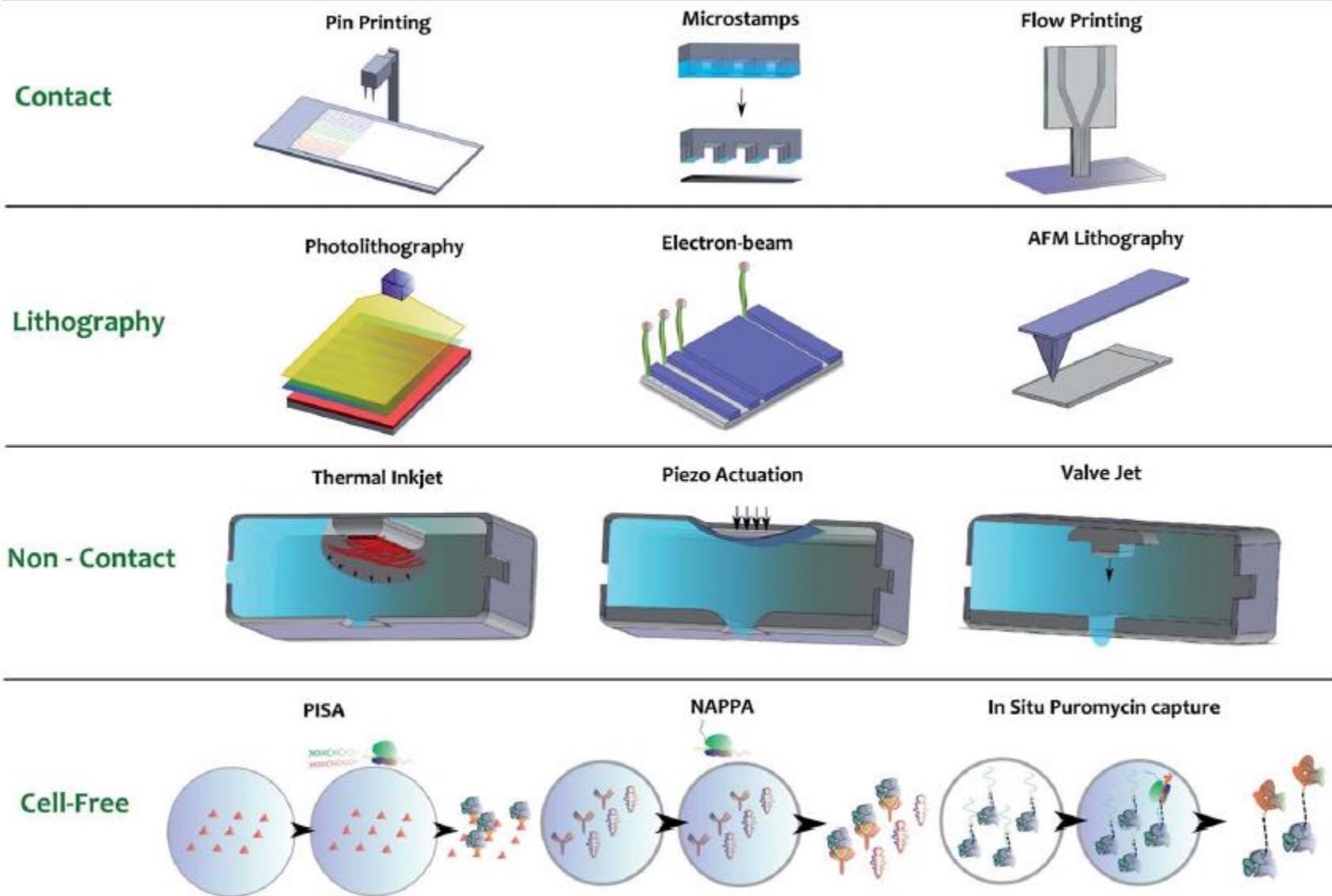


Binds proteins and other biomolecules through an efficient hydrophobic interaction



SuperNitro Microarray Substrates attach DNA, protein, carbohydrate and any other molecule that has been shown to bind nitrocellulose in traditional filter assays

Fabricación del microarreglo de proteínas



Microarray de proteínas



- Oferta de productos



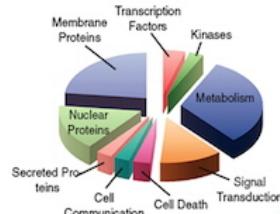
Arrayit Corporation (ARYC) leads and empowers the genetic, research, pharmaceutical, and diagnostic communities through the discovery, development and manufacture of proprietary life science technologies and consumables for disease prevention, treatment and cure. - Powerful Science for Life™



Products - Protein Microarrays

Arrayit sets the standard in the microarray industry with ArrayIt® Brand Protein Microarrays. Please review our product list below, and click on a product for protocols and pricing. Click here for [scientific publications](#) using Arrayit protein microarrays.

[HuProt™ v2.0 19K Human Proteome Microarrays](#)



Arrayit HuProt™ v2.0 19K Human Proteome Microarrays contain 19,275 human proteins representing most of the human proteome and 119 mouse proteins for total 19,394 proteins. All major protein functional families are represented for protein-protein, small molecule-protein, antibody binding specificity, immune profiling, DNA and RNA binding studies on a proteomic scale, advanced contact printing technology and proprietary surface chemistry, cold room manufacturing and linear drive robotics, universal 25 x 76 mm glass substrate slide format, 1 HuProt™ v2.0 19K microarray per kit.

[Whole Human Blood Collection Cards](#)



Arrayit whole human blood collection cards facilitate the collection, sampling and storage of whole blood and serum components for protein microarrays, immunoassays, allergy testing and other downstream applications that require high quality whole blood samples. Cards contain 4 x 12.5 mm circles on a proprietary collection matrix to enable the collection of 4 x 2-3 drops (50 µl) of whole blood in each of the 4 collection regions. Buffers, columns, lancets, and collection kits simplify the rapid and affordable deployment of Arrayit whole blood cards for a wide range of life sciences and research applications.

[AllerSpot™ Companion Animal Testing Service](#)



Arrayit AllerSpot™ Companion Animal Testing Service using a 62-allergen microarray based on rigorous scientific studies and environmental research on various factors affecting the prevalence rates of allergies. Based on this information, we have identified a test for the most common allergens inhaled and ingested by our companion animal pets including dogs and cats. Ask your veterinarian about AllerSpot™.

[Pathogen Antigen Microarrays](#)

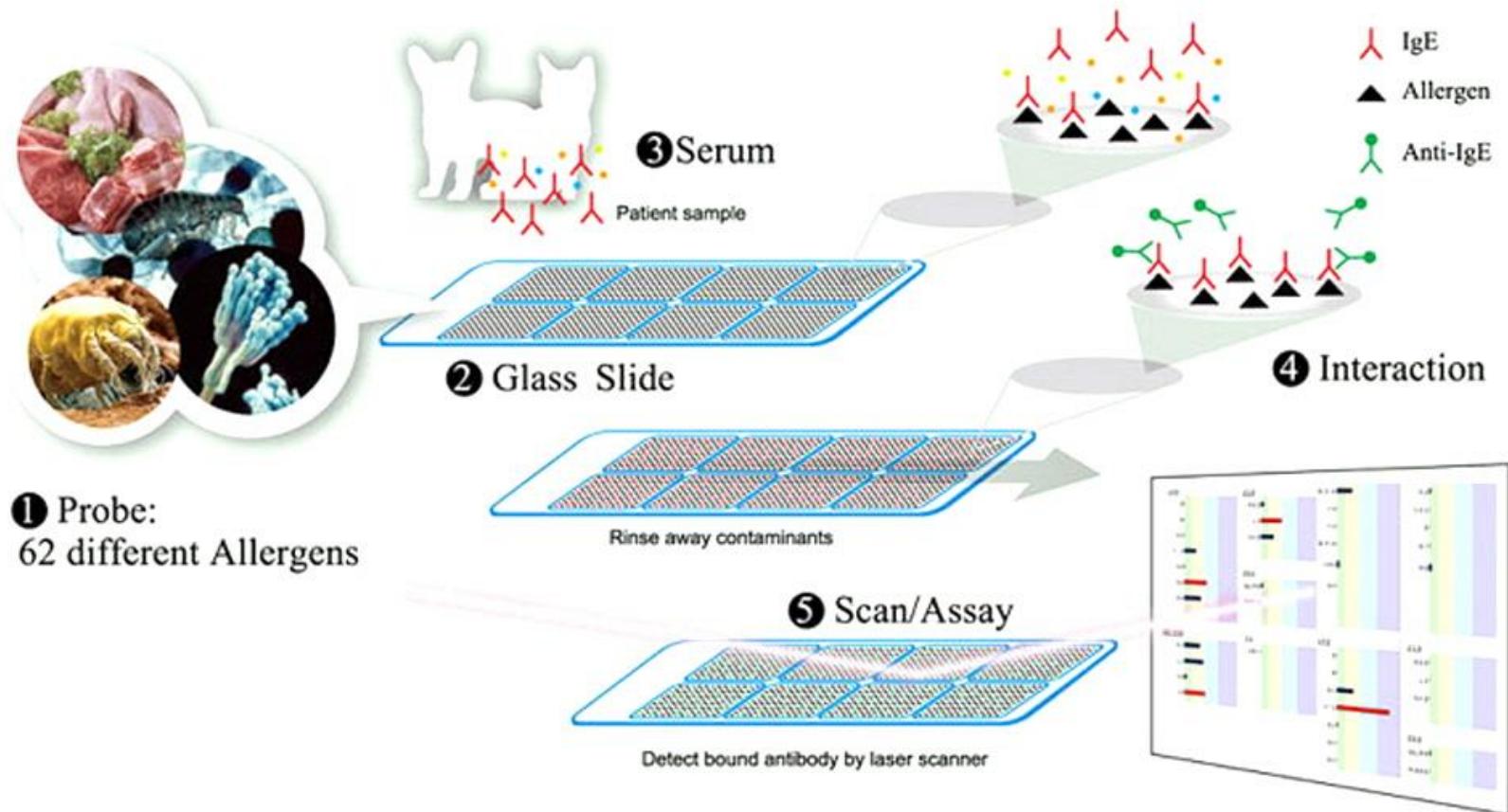


Arrayit Antigen Microarrays, manufactured with Arrayit's patented microarray manufacturing technology 6,101,946, are designed for reactive antibody screening, immune response profiling and biomarker research. Each microarray contains antigens and extensive controls for reactions with 1 µl of human serum or plasma immunology, infectious disease, microbiology, and cell biology. For research use only.

Microarray de proteínas



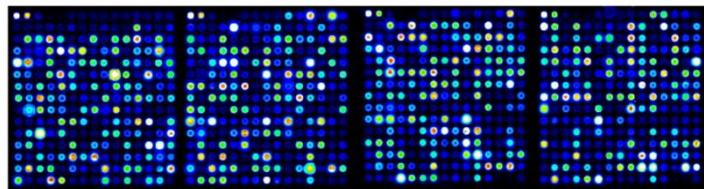
- Microarray de alérgenos en mascotas
 - Detección de 62 anticuerpos IgE (respuesta hipersensitiva) en la sangre de mascotas, los alérgenos más comunes que son inhalados o ingeridos por mascotas



Microarray de proteínas

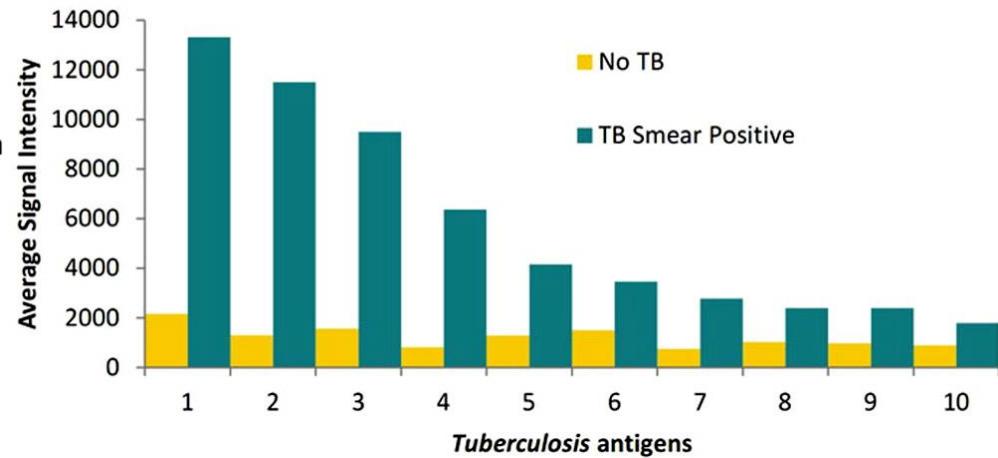


- Microarray de antígenos de patógenos humanos
 - Detección de anticuerpos en el suero contra microorganismos patógenos
 - *Brucella melitensis, Chlamydia muridarum, Francisella tularensis, Mycobacterium tuberculosis, Plasmodium falciparum, Salmonella enterica non-typhoidal, Salmonella enterica typhi, Staphylococcus aureus, y Toxoplasma gondii, etc....*



Malaria Reactive Antigen Microarray probed with infected serum

TB Smear Positive vs No TB



Microarray de proteínas



- Microarray de anticuerpos contra proteínas del suero humano
 - 380 anticuerpos monoclonales contra proteínas nativas del plasma



\$982

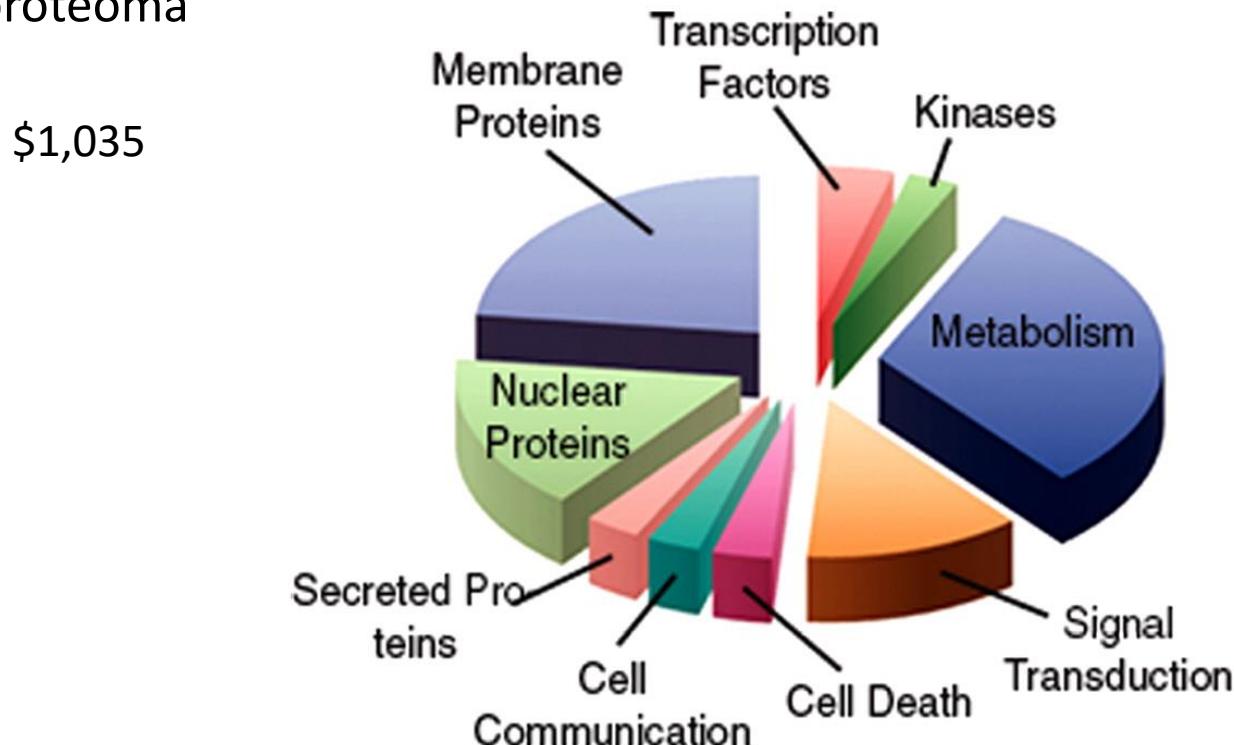
Enfermedades, toxicidad
y respuesta a drogas



Microarray de proteínas



- Microarray del proteoma humano
 - HuProt v2.0 19K: más de 19 mil proteínas humanas, por duplicado
 - Análisis global de la respuesta inmune, interacción de proteínas con moléculas, especificidad de anticuerpos, unión de ADN y ARN al proteoma



Microarray de proteínas

- Microarray del proteoma humano

DISCOVERY SERVICES ▾

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SUP



NEXTGEN PROTEOMICS

HuProt™

Human Proteome Microarray v3.1

Overview

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[Applications](#)

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The HuProt™ Human Proteome Microarray contains the world's largest number of unique, individually-purified human proteins. That's **thousands** more than the not-so-close second.

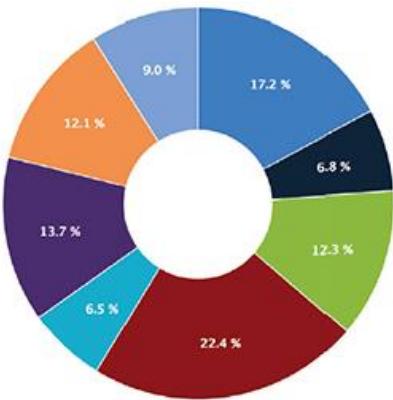
The comprehensive content of the HuProt™ microarray allows thousands of interactions to be profiled in high-throughput.

Explore:

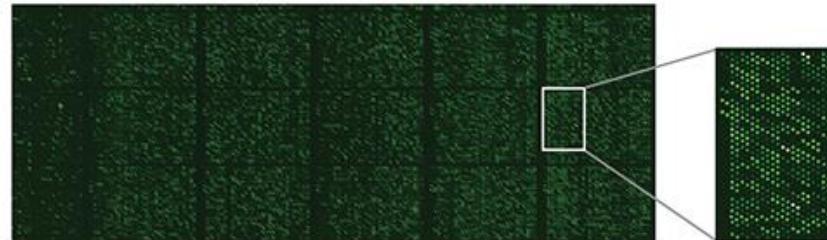
- Antibody Specificity
- Serum/Biomarker Profiling
- Small Molecule Profiling
- Protein-Protein Interaction
- DNA/RNA Binding
- Enzyme Substrate ID

in 16,152 genes, covering ~81% of the proteome.

Below: CDI HuProt™ microarray comprising GST fusion proteins probed with anti-GST antibody.



Categories
Kinases
Cell Death
Membrane Proteins
Nuclear Proteins
Metabolism
Signal Transduction
Secreted Proteins
Transcription Factors



Microarray de proteínas

- Microarray para tuberculosis



MTB Array



NEXTGEN PROTEOMICS

Mycobacterium Tuberculosis Proteome Microarray

One-third of the world population is thought to have been infected with *M. tuberculosis*, with the disease claiming upwards of 1.5 million lives annually (WHO) – and the increase in multi-drug resistant strains of TB (MDRTB) is increasing mortality rates. **The need for new, potent, robust drug intervention is urgent.**

CDI Laboratories offers the **Mycobacterium Tuberculosis Proteome Microarray** featuring 4,262 full-length recombinant proteins encompassing >95% of the MTB proteome – a powerful, high-throughput analytical tool.

Explore

- protein-protein interactions
- small molecule-protein binding
- serum profiling (patient/population)

Research Applications

- Protein - protein interactions: infection, immunity and resistance mechanisms, construction of signaling pathways
- Protein - nucleic acid interactions: gene regulation / protein function
- Protein - small molecule interactions: signal regulation; drug target screening
- Protein - lipid interactions: infection, drug resistance and other mechanisms, regulation of lipid metabolism
- Protein microarray-based enzymology research: protein function, signal regulation
- Antibody evaluation: antigen identification, specificity validation, application optimization

Microarray de proteínas

- Microarray del proteoma humano

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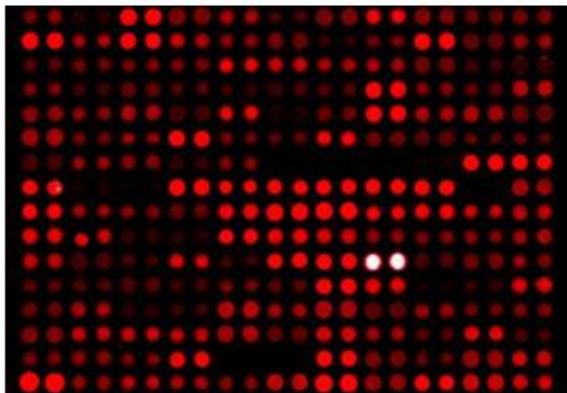
[Home](#) > [Life Sciences](#) > [Protein Biology](#) > [Protein Assays and Analysis](#) > [Protein Microarrays](#)

Protein Microarrays

[Protein Assays and Analysis](#)

Protein Microarrays

- Human Protein Microarrays Overview
- Immune Response Biomarker Profiling (IRBP)
- Small Molecule Profiling
- Enzyme Substrate Profiling
- Antibody Specificity Profiling



Purified and arrayed under native conditions

The ProtoArray Human Protein Microarray is a high-density microarray that contains thousands of unique, full-length human proteins, including kinases, phosphatases, GPCRs, nuclear receptors, and proteases, spotted in duplicate on a 1 inch x 3 inch thin nitrocellulose coated glass slide. ProtoArray human protein microarray contains over 9,000 unique human proteins individually purified and arrayed under native conditions to maximize functionality.

Microarray de proteínas

- Microarrays



1-888-494-8555

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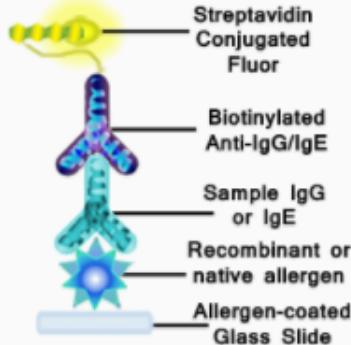


Antibody
Array
Overview



Protein Arrays

RayBio® Protein Arrays are a versatile and innovative series of products featuring immobilized proteins spotted onto a glass slide support. Protein arrays allow a much broader view of protein activity than a traditional ELISA. RayBio arrays come in smaller, research specific panels as well as broad screening panels.



Allergen Protein Arrays

Immobilized Proteins	Recombinant or extracted native allergen proteins
Species Detected	Human
Spot Replicates	Triplicate
Applications	Screening specific allergens based on IgG and/or IgE levels present in serum
Solid Support	Glass Slide

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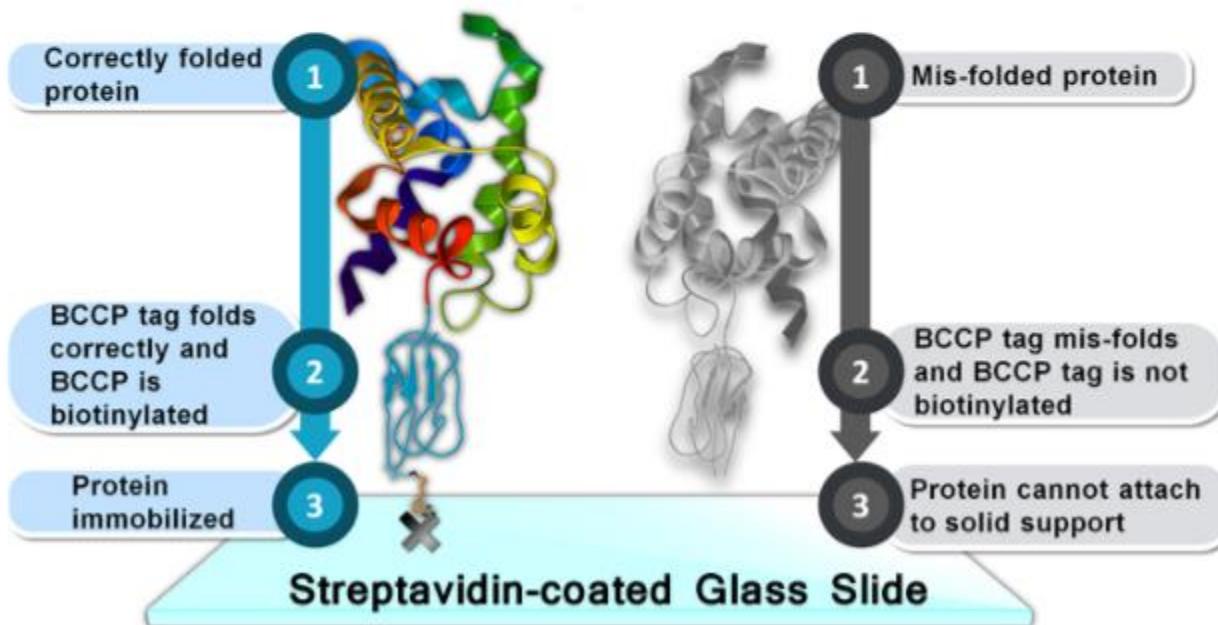
Microarray de proteínas

- Microarray del inmunoma humano

Immune Protein Array Features

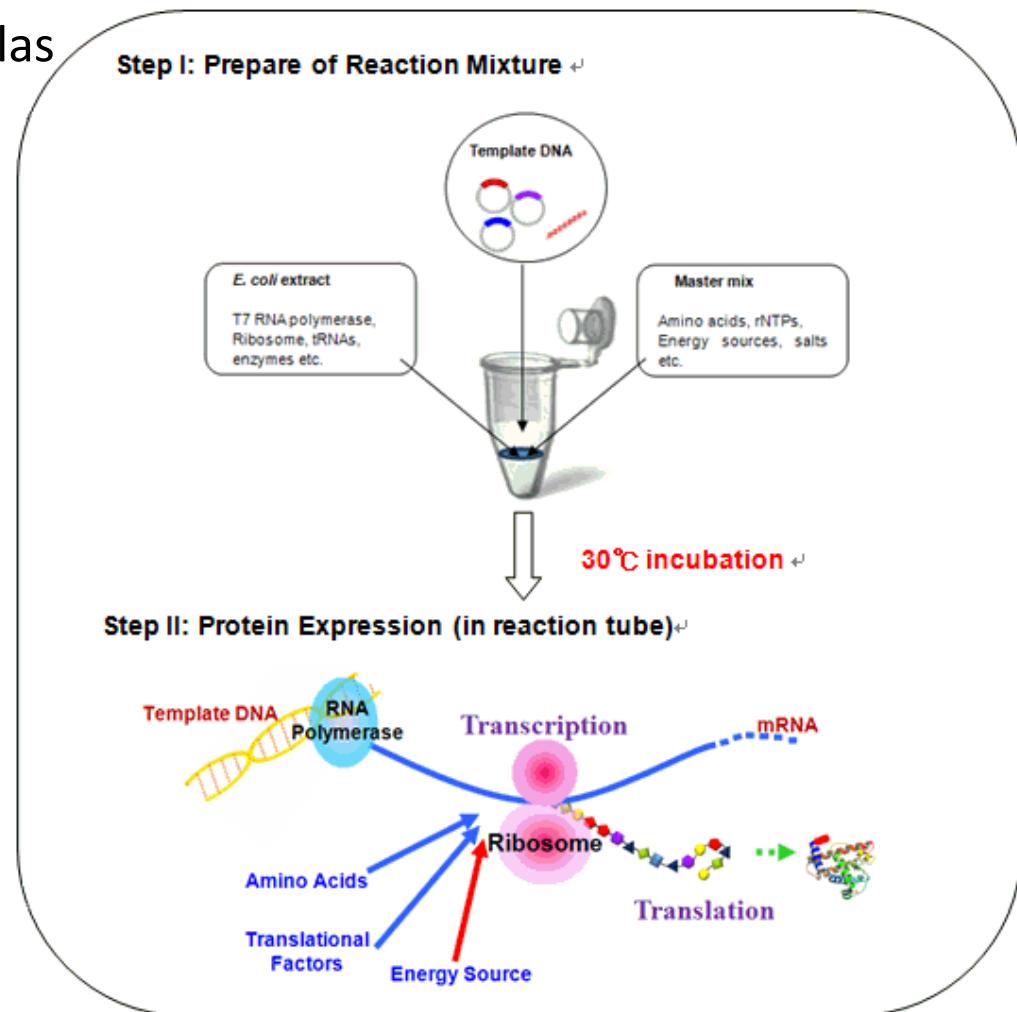
Correctly Folded, Functional Proteins

Each 75mm x 25mm glass slide is spotted with 4 identical protein arrays (also called "subarrays"), each with **1631 correctly folded and functional spotted proteins!** Each protein is attached to the streptavidin-coated slide exclusively via a biotinylated affinity tag, specifically the biotin carboxyl carrier protein (BCCP) domain of the *E. coli* acetyl CoA carboxylase. This affinity tag was chosen because the BCCP domain must be folded into its native 3-dimensional structure in order to become biotinylated. If the expressed protein mis-folds the BCCP tag will also mis-fold, preventing biotinylation. If the protein folds correctly then folding of the BCCP tag should be unhindered and biotinylation will result. This property allows only correctly folded proteins to be bound to the slide.



Microarray de proteínas

- Producción de proteínas para el microarray
 - Transcripción-traducción in vitro
 - Sistema *E. coli* libre de células
 - Proteínas humanas se expresan en levadura



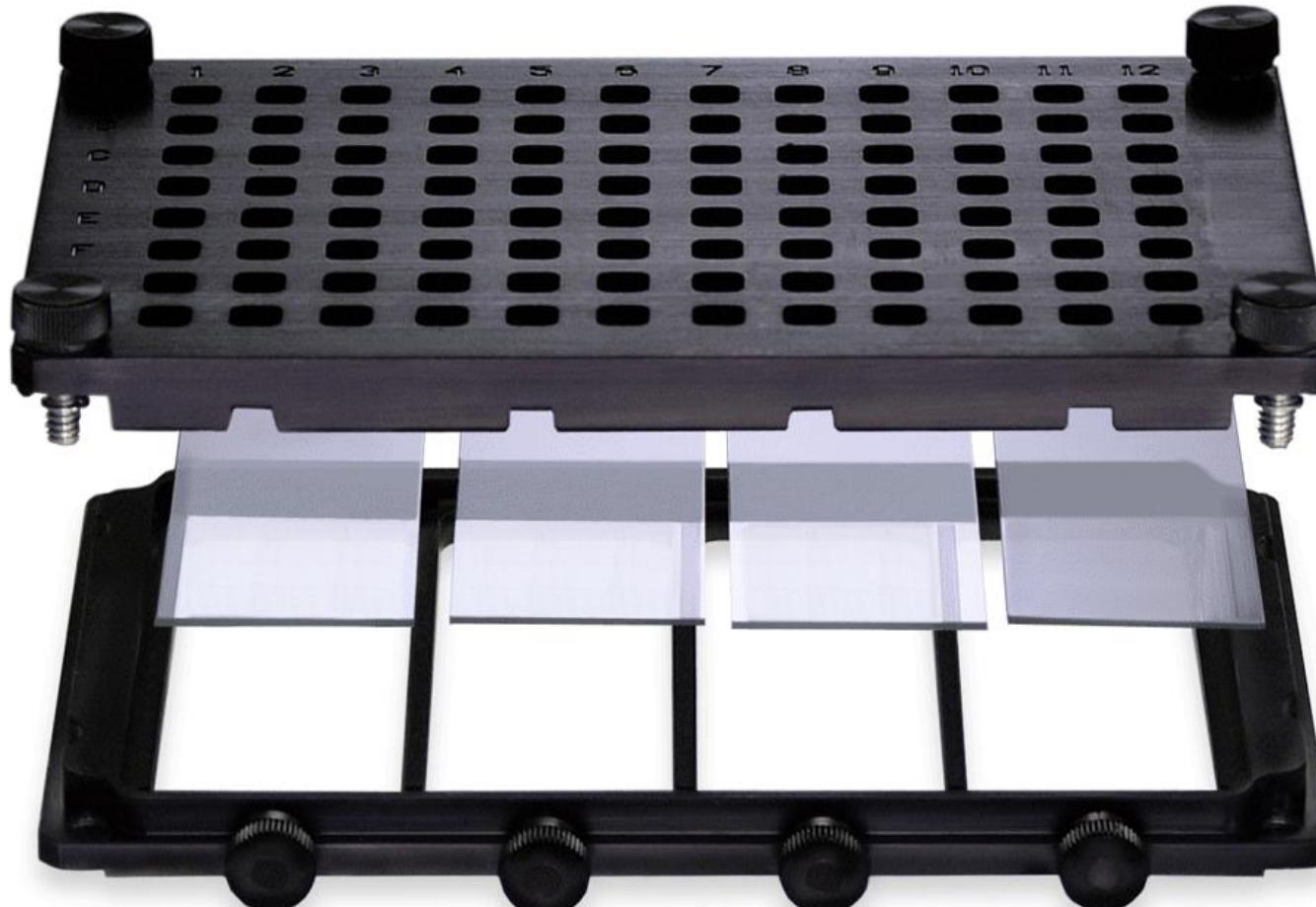


Microarray de proteínas

[http://www.arrayit.com/Products/Protein
Microarrays/protein_microarrays.html](http://www.arrayit.com/Products/Protein_Microarrays/protein_microarrays.html)

- Arrayit

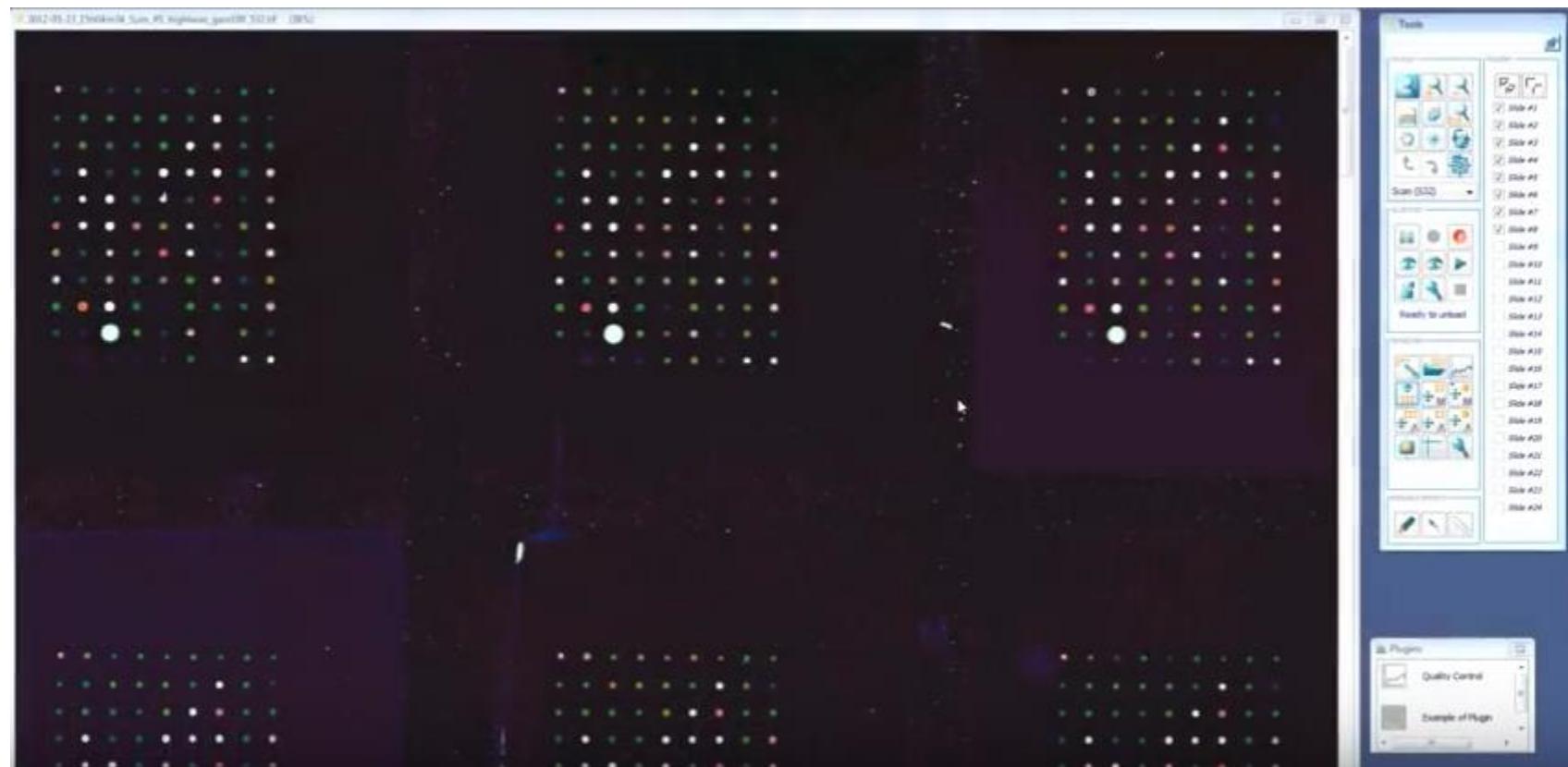
Formato de 96 microarrays: 24 microarrays por chip x 4 chips por bloque



Microarray de proteínas



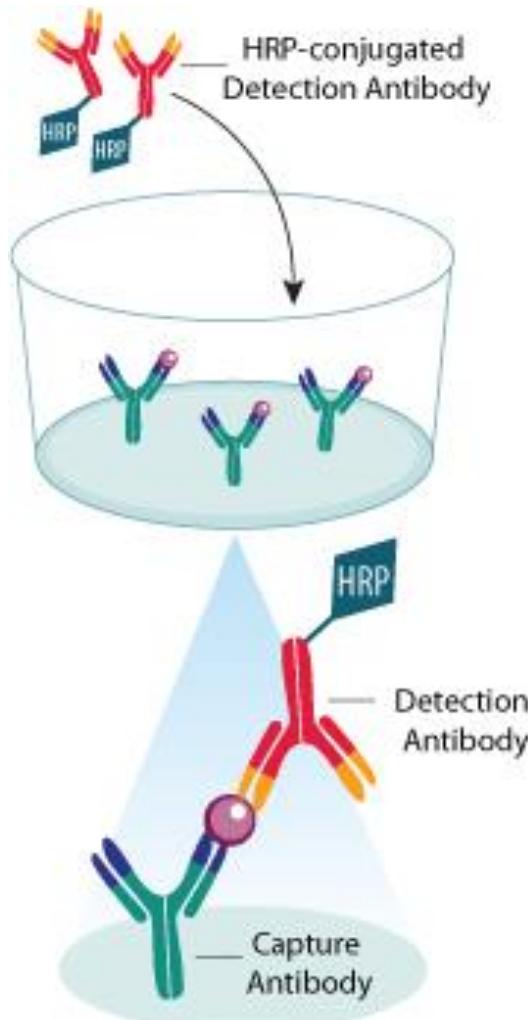
- Arrayit: 24 microarrays por chip x 4 chips por bloque



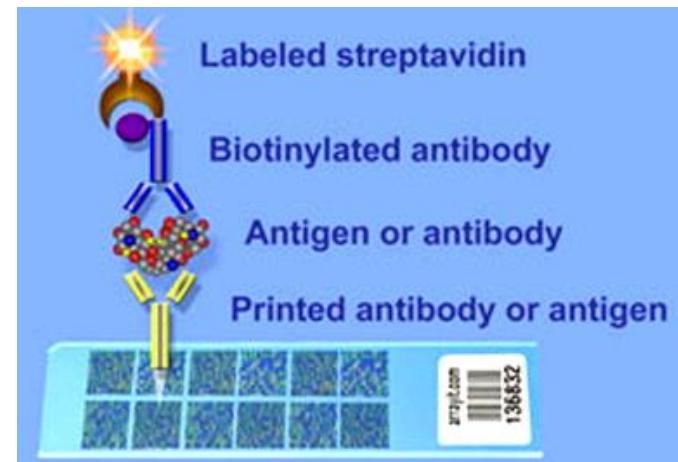
24

Microarray de proteínas

- Arrait: detección



Biotina-
estreptavidina

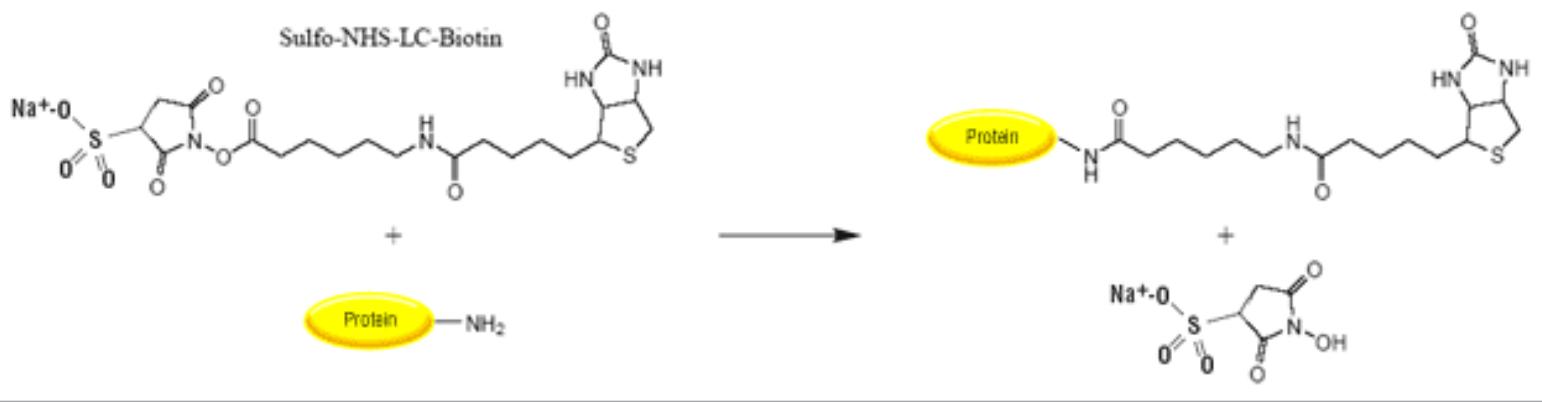
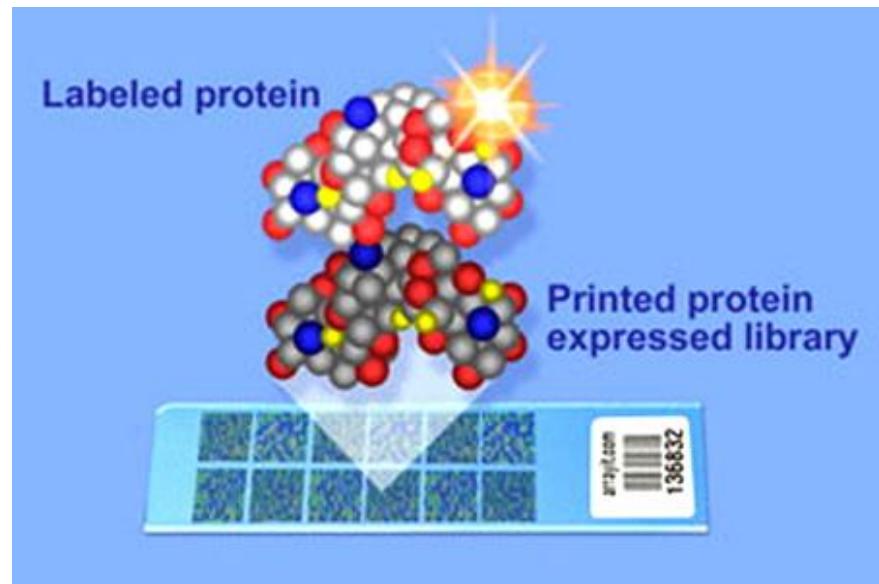


Parejas de
anticuerpos

Microarray de proteínas

- Arrait: detección

Thermo EZ-Link Sulfo-NHS-LC-Biotin:
Reactivio para marcar proteínas con biotina
(lisinas y N-terminal de las proteínas)



Microarray de proteínas



- Arrait: costos

M. tuberculosis reactive antigen microarray, 32 microarray kit	15MA010K	\$2,900
M. tuberculosis reactive antigen microarray, 80 microarrays	15MA0002	\$4,000
M. tuberculosis reactive antigen microarray, 80 microarray kit	15MA002K	\$4,900
M. tuberculosis reactive antigen microarray, 160 microarrays	15MA0003	\$6,400
M. tuberculosis reactive antigen microarray, 160 microarray kit	15MA003K	\$7,300
M. tuberculosis reactive antigen microarray, 320 microarrays	15MA0004	\$11,200
M. tuberculosis reactive antigen microarray, 320 microarray kit	15MA004K	\$12,400
M. tuberculosis reactive antigen microarray, 800 microarrays	15MA0005	\$22,400
M. tuberculosis reactive antigen microarray, 800 microarray kit	15MA005K	\$24,200



\$179,430

Microarray de proteínas



- Arrait: costos

\$71,605

