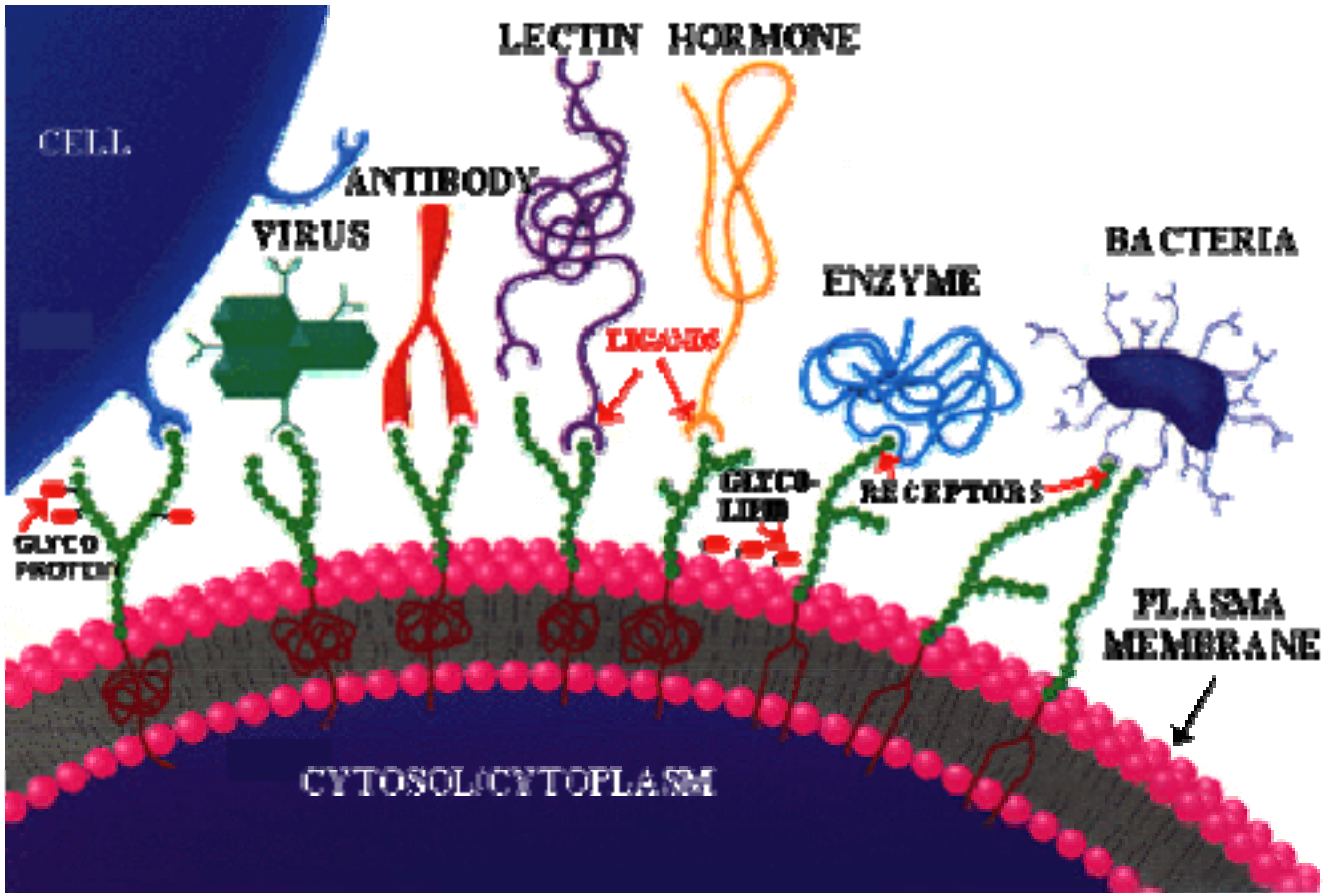
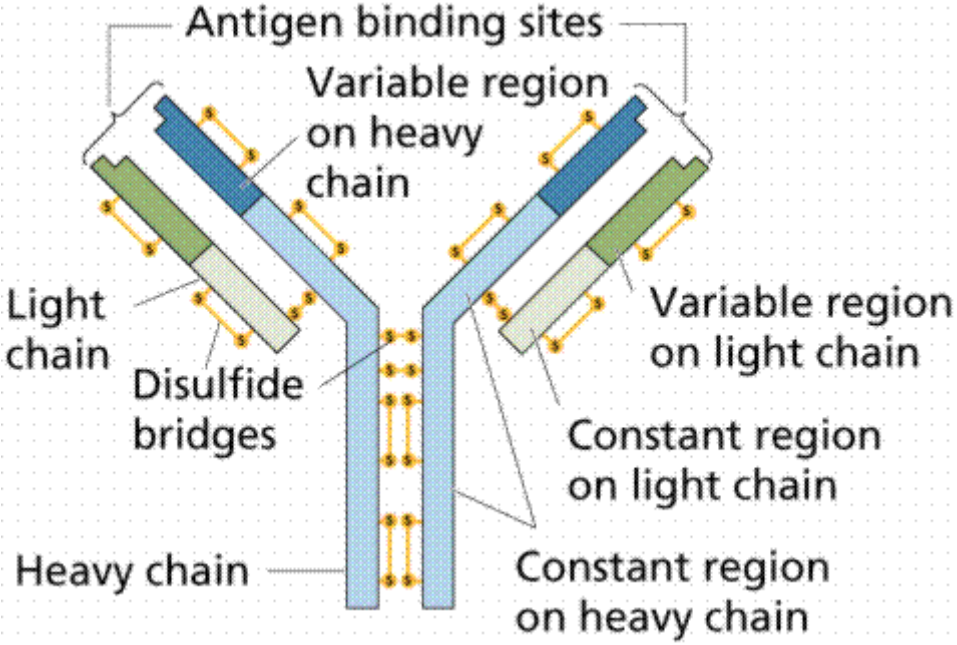
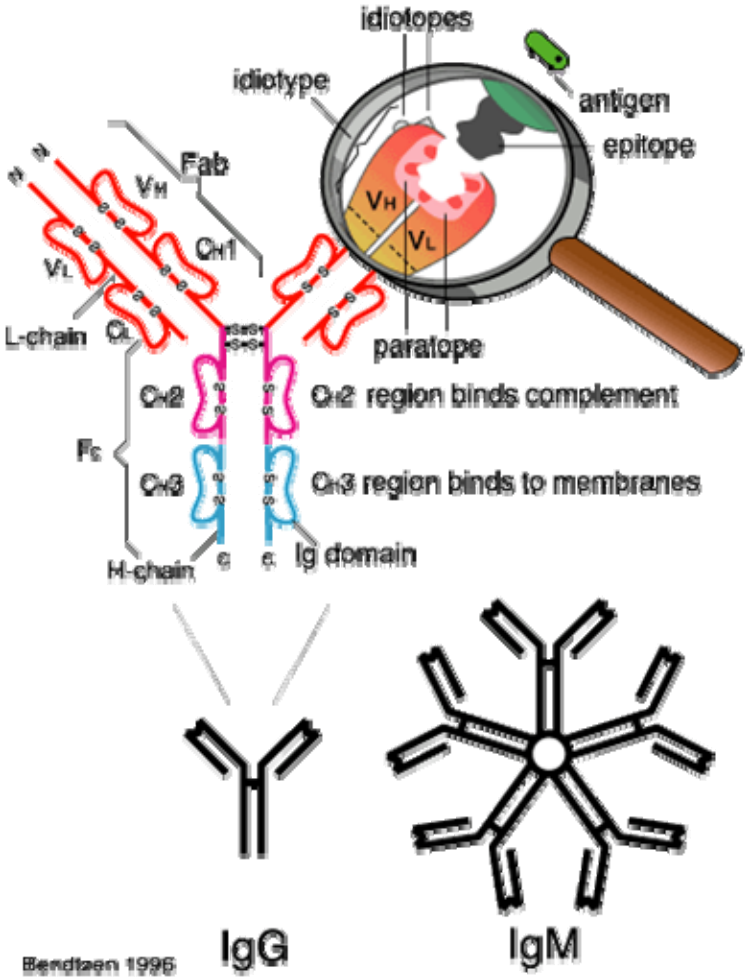
Molecular Recognition

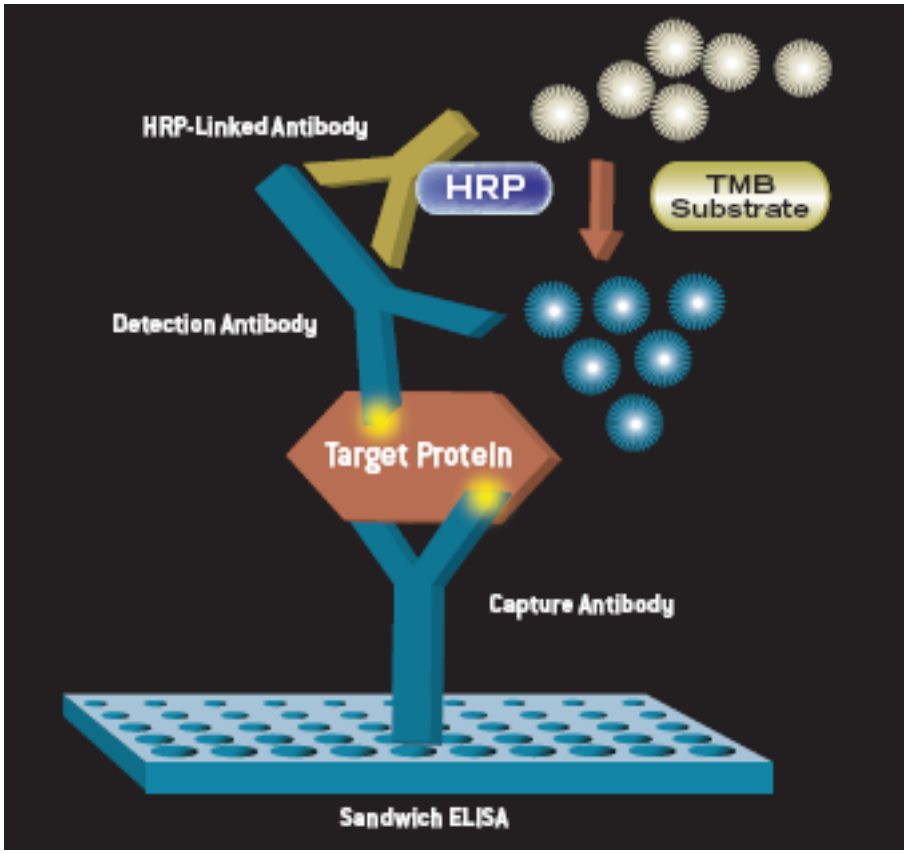


Antibody and Antigen



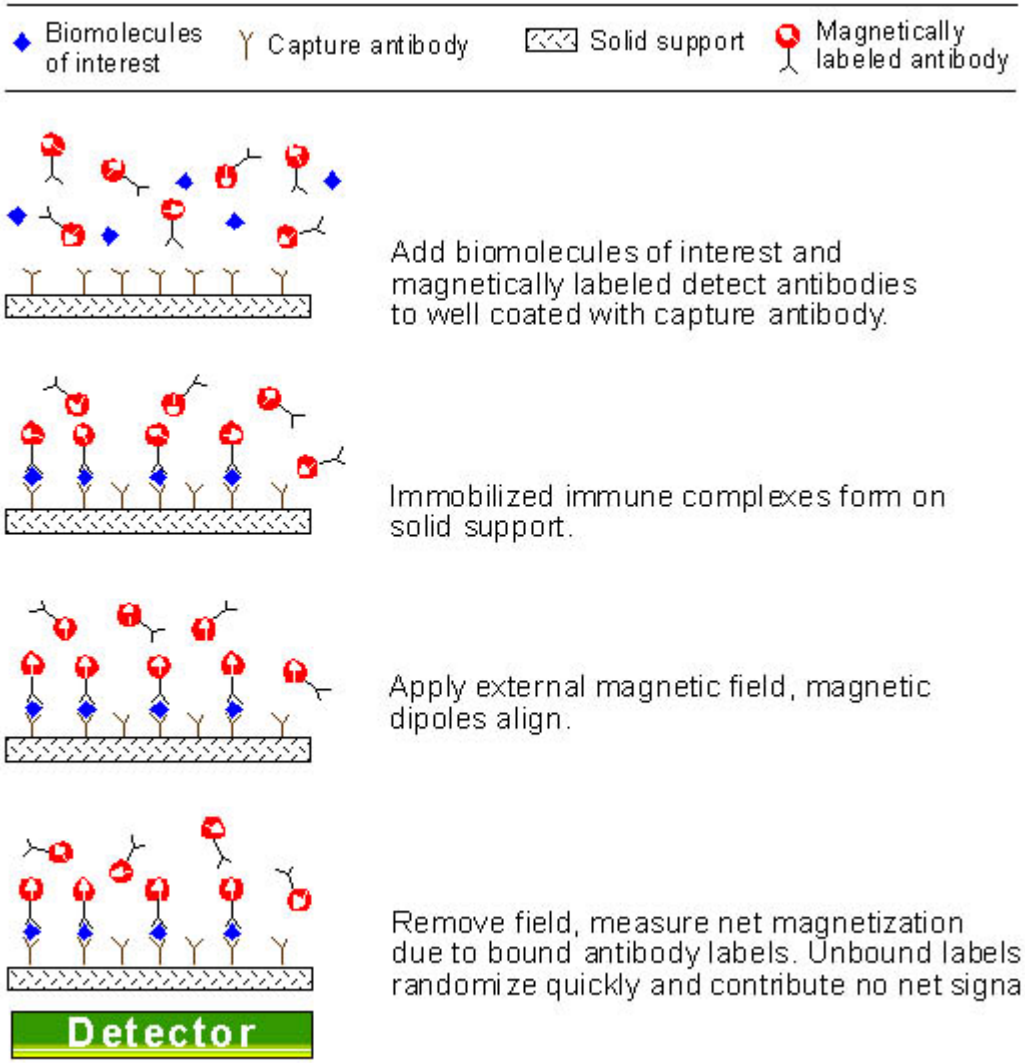


Enzyme-Linked ImmunoSorbent Assay (ELISA)

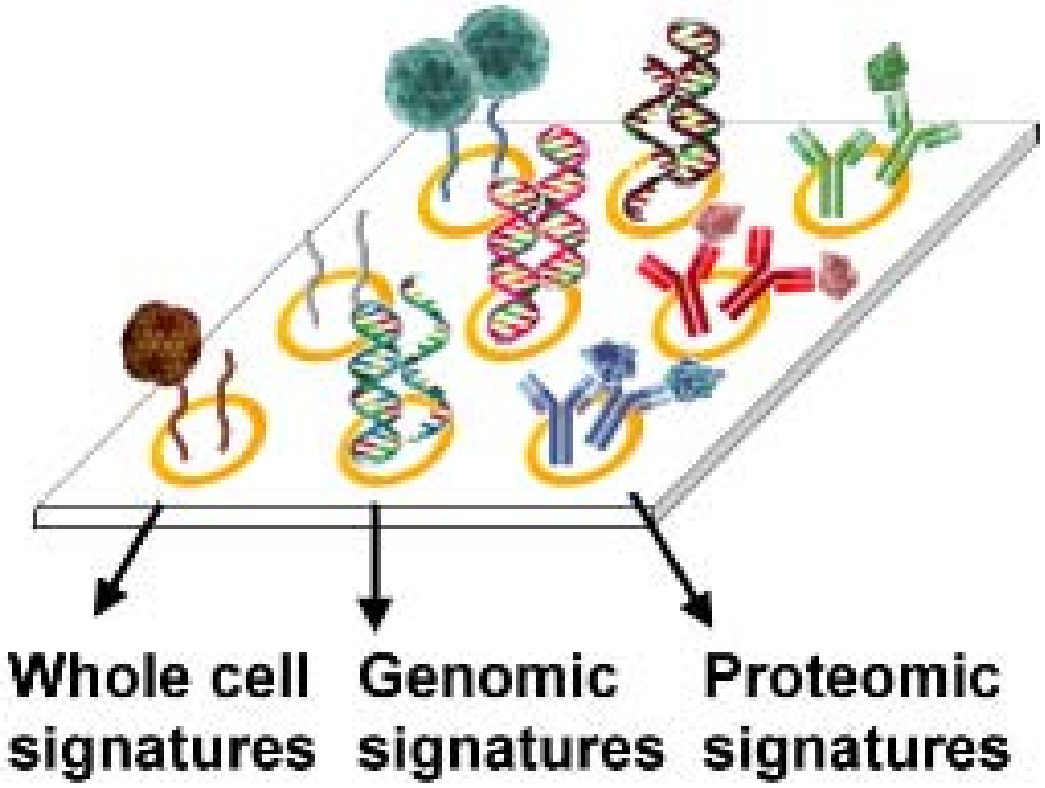


Labeling
BSA/PEG

Microarray


Biomolecules of interest
Capture antibody
Solid support
Magnetically labeled antibody
Add biomolecules of interest and magnetically labeled detect antibodies to well coated with capture antibody.
Immobilized immune complexes form on solid support.
Apply external magnetic field, magnetic dipoles align.
Remove field, measure net magnetization due to bound antibody labels. Unbound labels randomize quickly and contribute no net signal.
Detector

Microarray


Whole cell signatures
Genomic signatures
Proteomic signatures

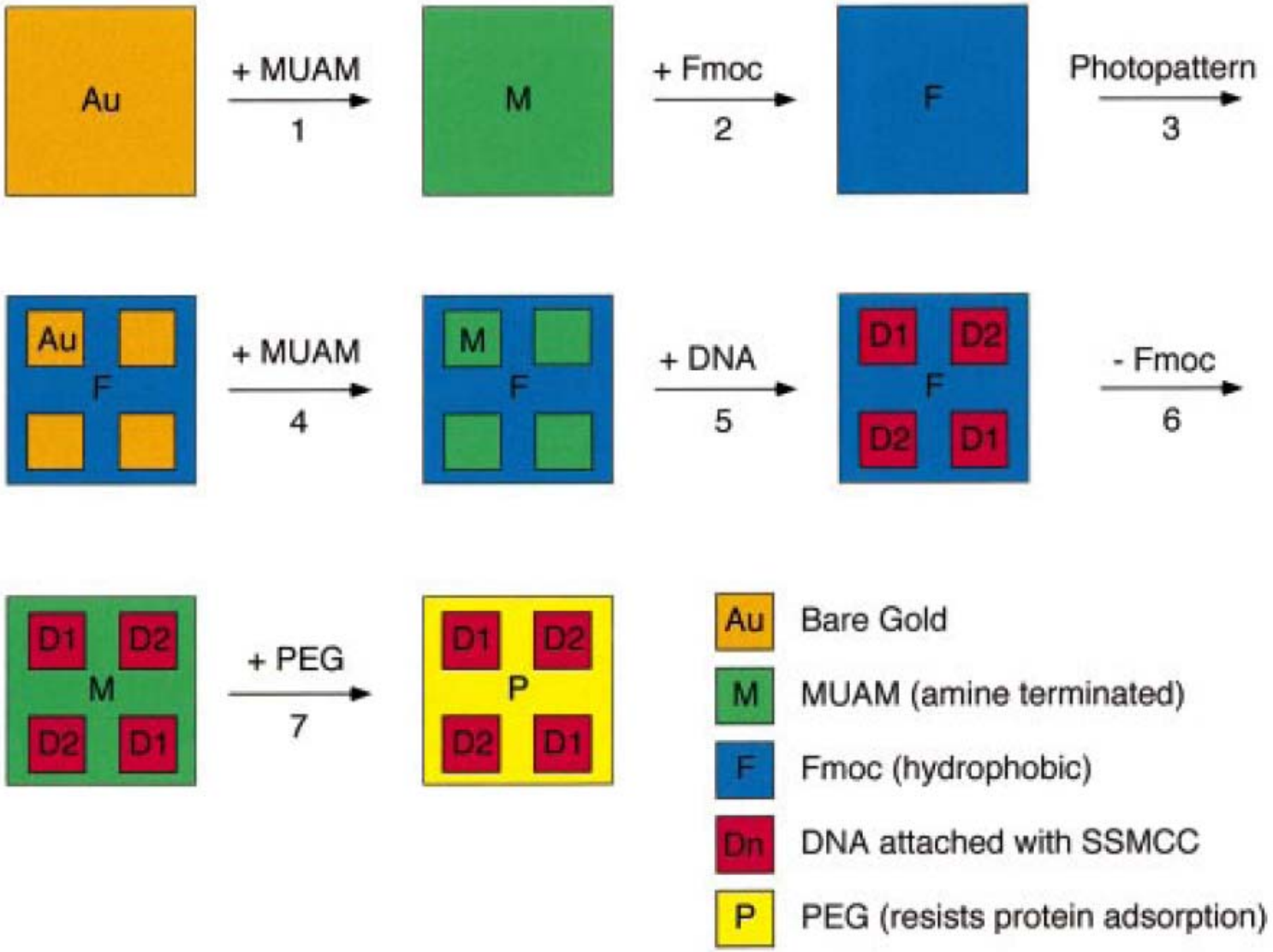


Figure 1. Fabrication scheme for the construction of multi-element DNA arrays. A clean gold surface is reacted with the amine-terminated alkanethiol MUAM, and subsequently reacted with Fmoc-NHS to create a hydrophobic surface. This surface is then exposed to UV radiation through a quartz mask and rinsed with solvent to remove the MUAM+Fmoc from specific areas of the surface, leaving bare gold pads. These bare gold areas on the sample surface are filled in with MUAM, resulting in an array of MUAM pads surrounded by a hydrophobic Fmoc background. Solutions of DNA are then delivered by pipet onto the specific array locations and are covalently bound to the surface via the bifunctional linker SSMCC. In the final two steps, the Fmoc-terminal groups on the array background are removed and replaced by PEG groups which prohibit the nonspecific binding of analyte proteins to the background.

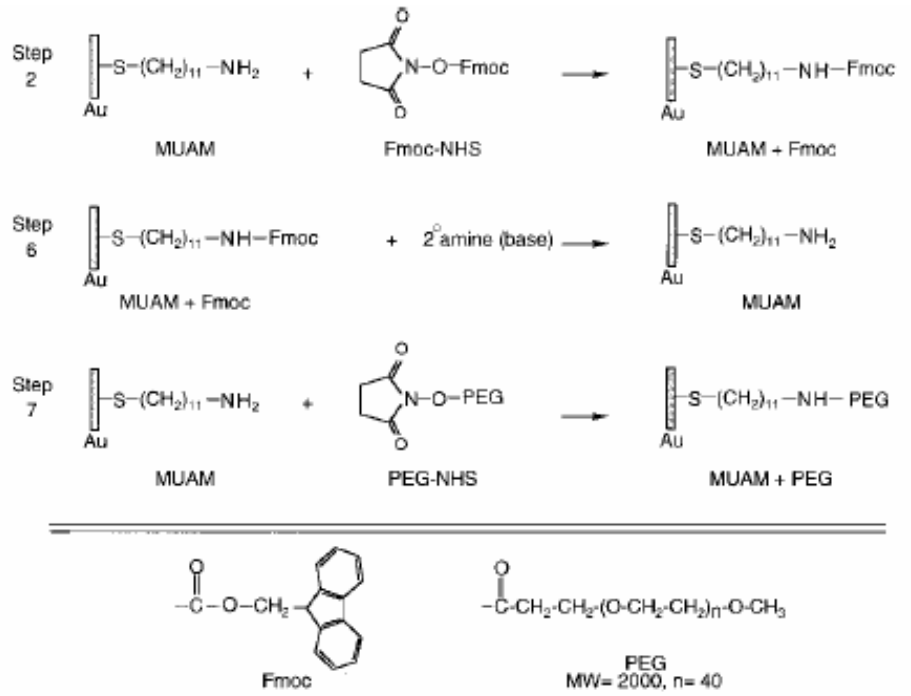


Figure 2. Surface reaction scheme showing the steps involved in the reversible modification of the array background. (Step 2) The starting amine-terminated alkanethiol surface (MUAM) is reacted with the Fmoc-NHS protecting group to form a carbamate linkage thus creating a hydrophobic Fmoc-terminated surface. (Step 6) After DNA immobilization (see Figure 3), the hydrophobic Fmoc group is removed from the surface with a basic secondary amine, resulting in the return of the original MUAM surface. (Step 7) In the final array fabrication step, the deprotected MUAM is reacted with PEG-NHS to form an amide bond that covalently attaches PEG to the array surface.

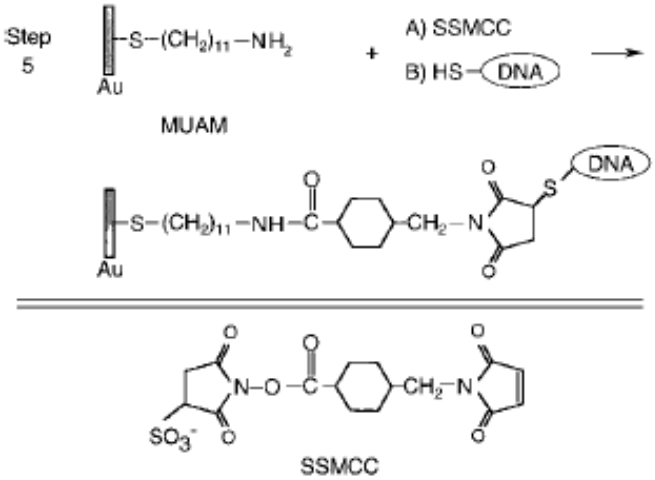
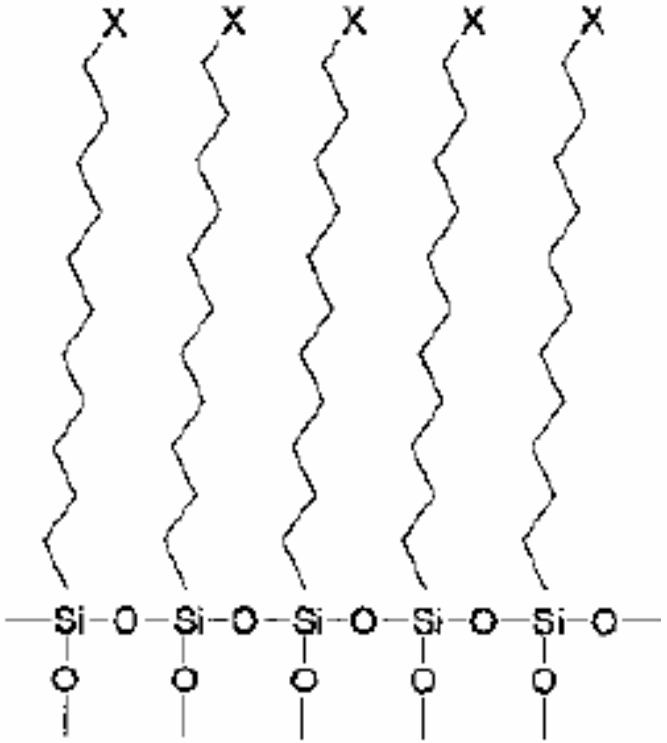
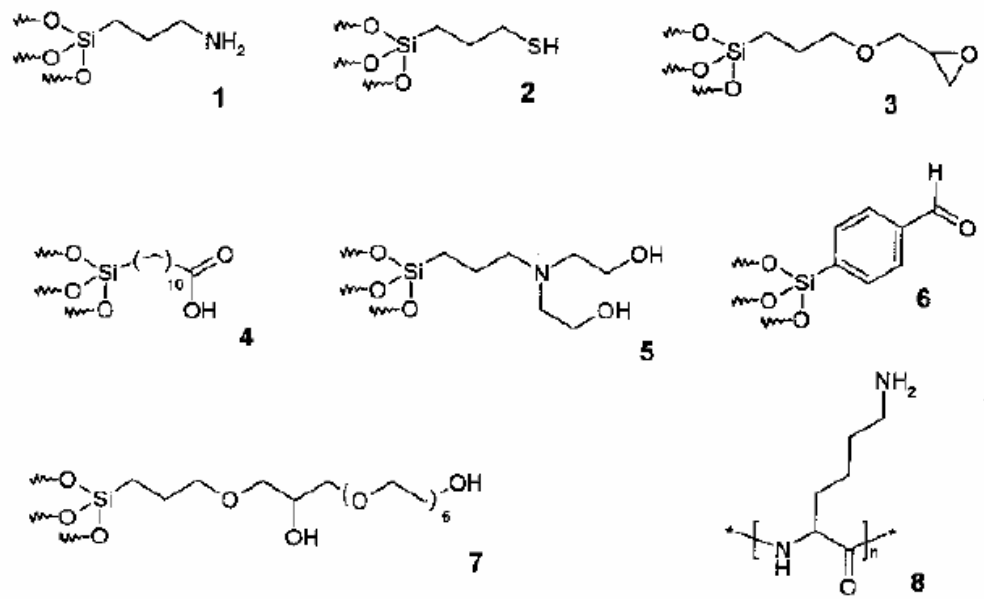


Figure 3. Surface reaction scheme showing the immobilization of thiol-terminated DNA to the array surface. In Step 5 of the DNA array fabrication, the heterobifunctional linker SSMCC is used to attach 5′-thiol modified oligonucleotide sequences to reactive pads of MUAM. This linker contains an NHSS ester functionality (reactive toward amines) and a maleimide functionality (reactive toward thiols). The surface is first exposed to a solution of the linker, whereby the NHSS ester end of the molecule reacts with the MUAM surface. Excess linker is rinsed away and the array surface is then spotted with 5′-thiol-modified DNA that reacts with the maleimide groups forming a covalent bond to the surface monolayer.

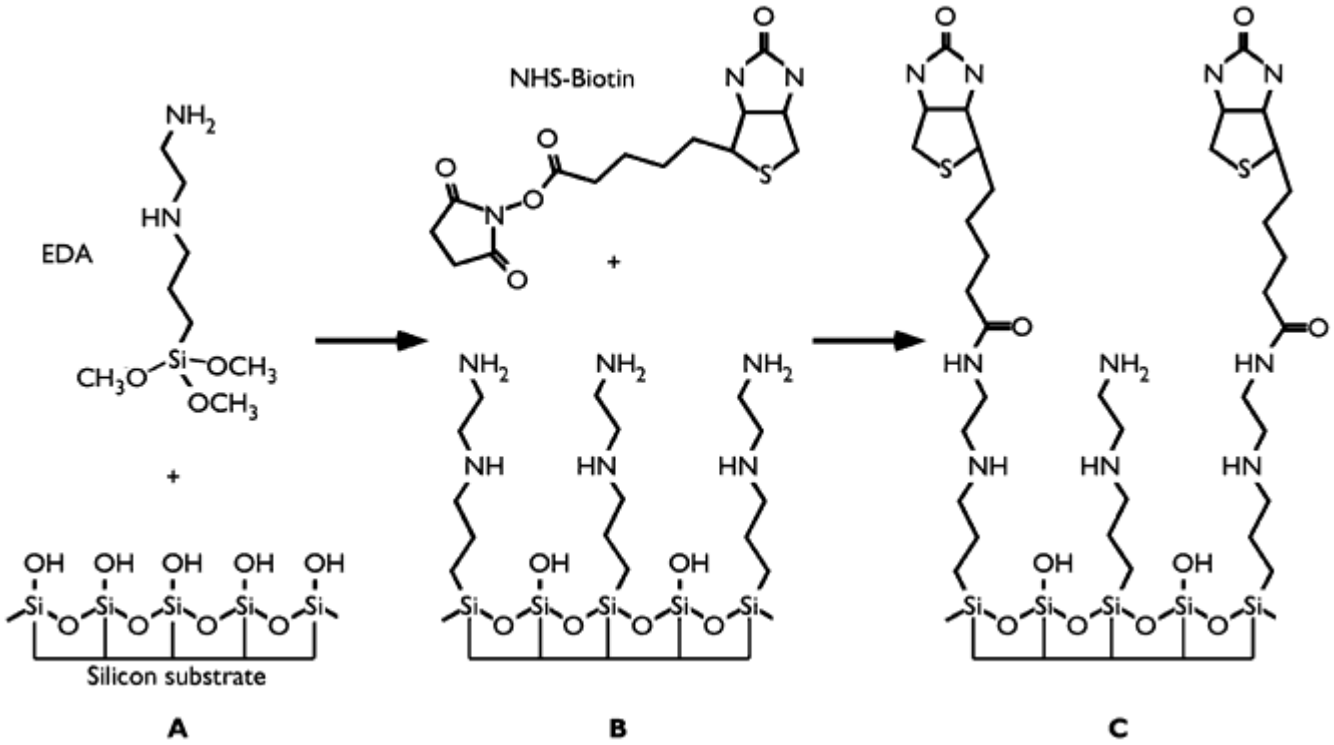
Glass Surface Modification





Scheme 2.2 Reagents for derivatization of glass surfaces. 1 APTES = aminopropyltriethoxysilane; **2** MPTS = 3-mercaptopropyltrimethoxysilane; **3** GPTS = glycidoxypropyltrimethoxysilane; **4** TETU = triethoxysilane undecanoic acid; **5** HE-APTS = bis(hydroxyethyl)aminopropyltriethoxysilane); **6** 4-trimethoxysilylbenzaldehyde; **7** GPTS/HEG = glycidoxypropyltrimethoxysilane-hexaethylene glycol; **8** poly(lysine).

Scheme 2.1 2D schematic description of a polysiloxane monolayer on a glass surface (X = terminal functional


NHS-Biotin
EDA
Silicon substrate
A
B
C

Biotin-Streptavidin

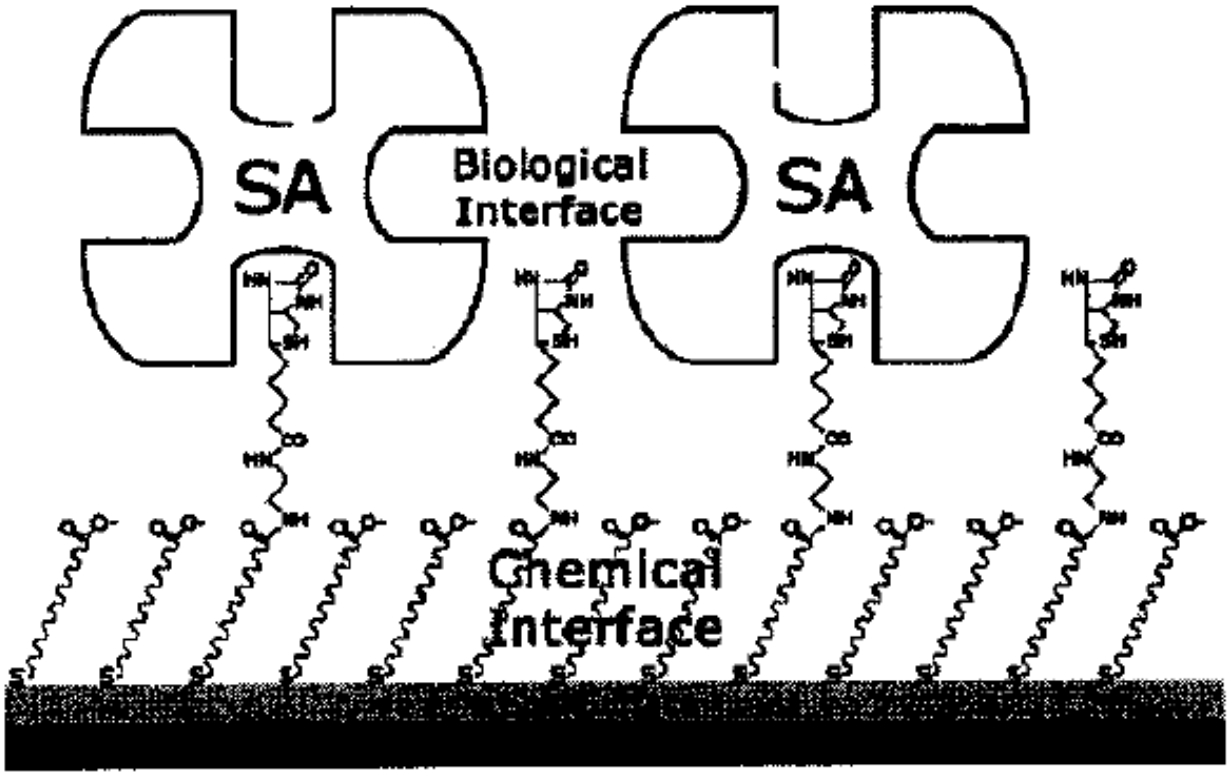
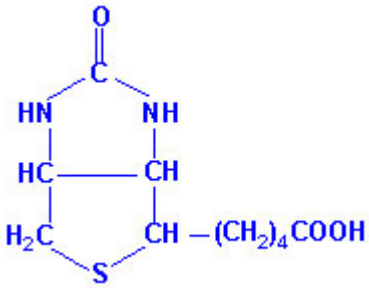
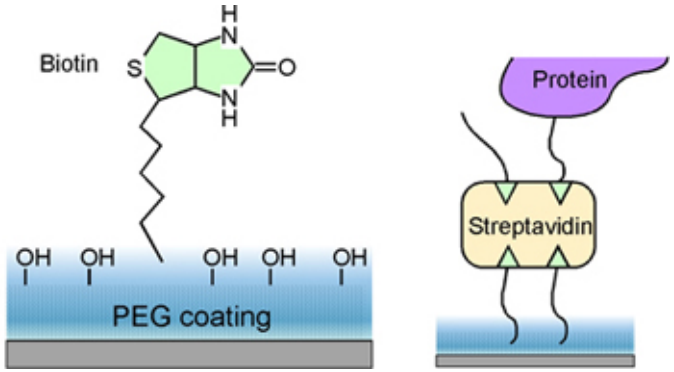


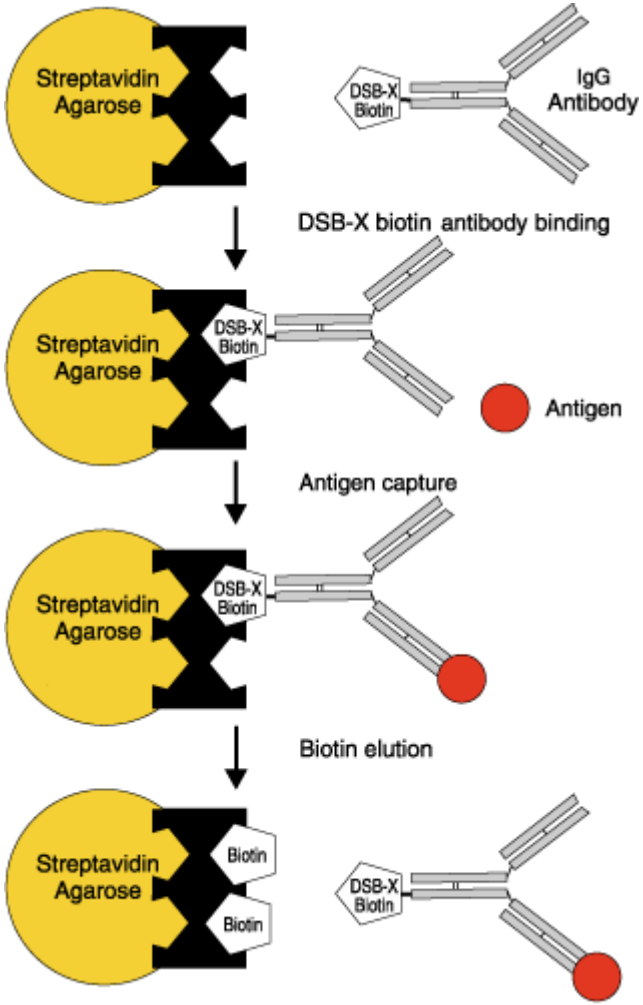
Figure 2.3 Schematic respresentation of a steptavidin sensor surface assembled on a reaction-controlled biotinylated SAM [28].

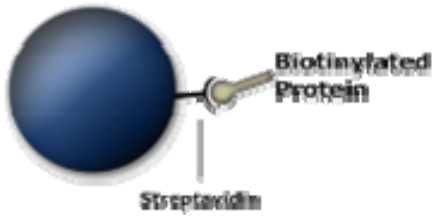
Biotin

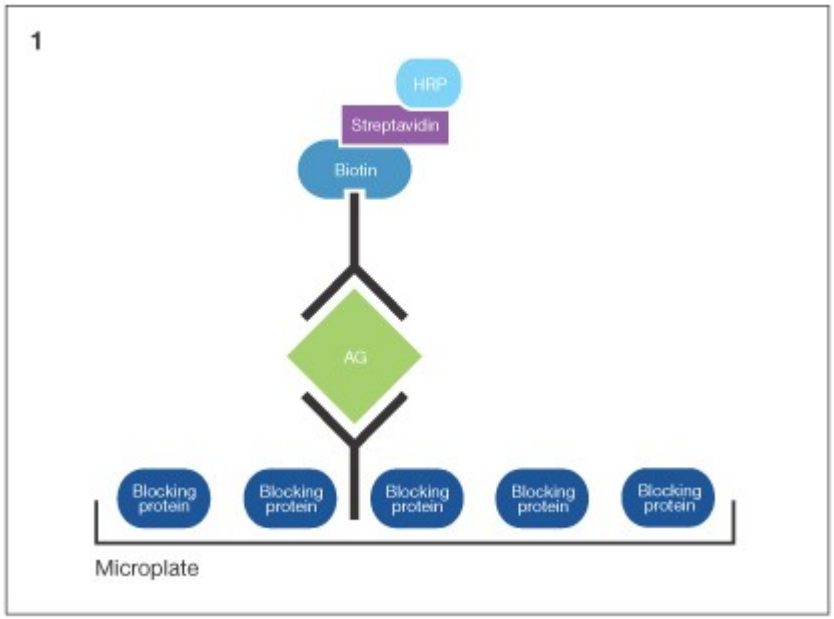


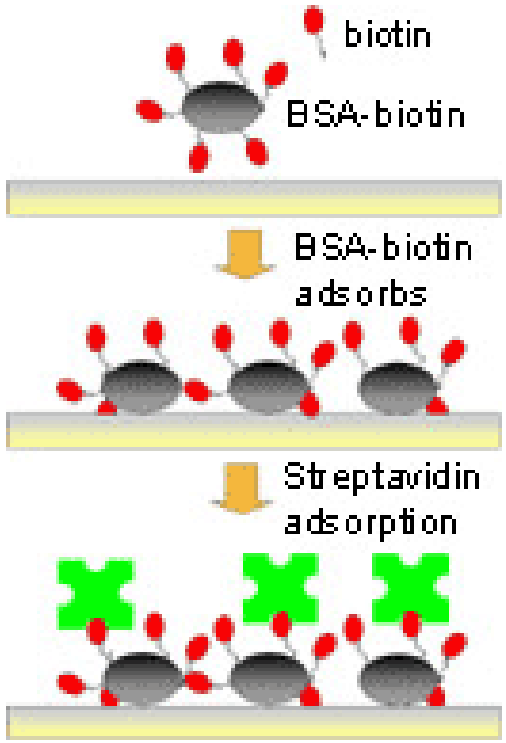
Avidin has a very strong affinity for biotin with a K_D (dissociation constant) of approximately 10^{-15} M^{-1}



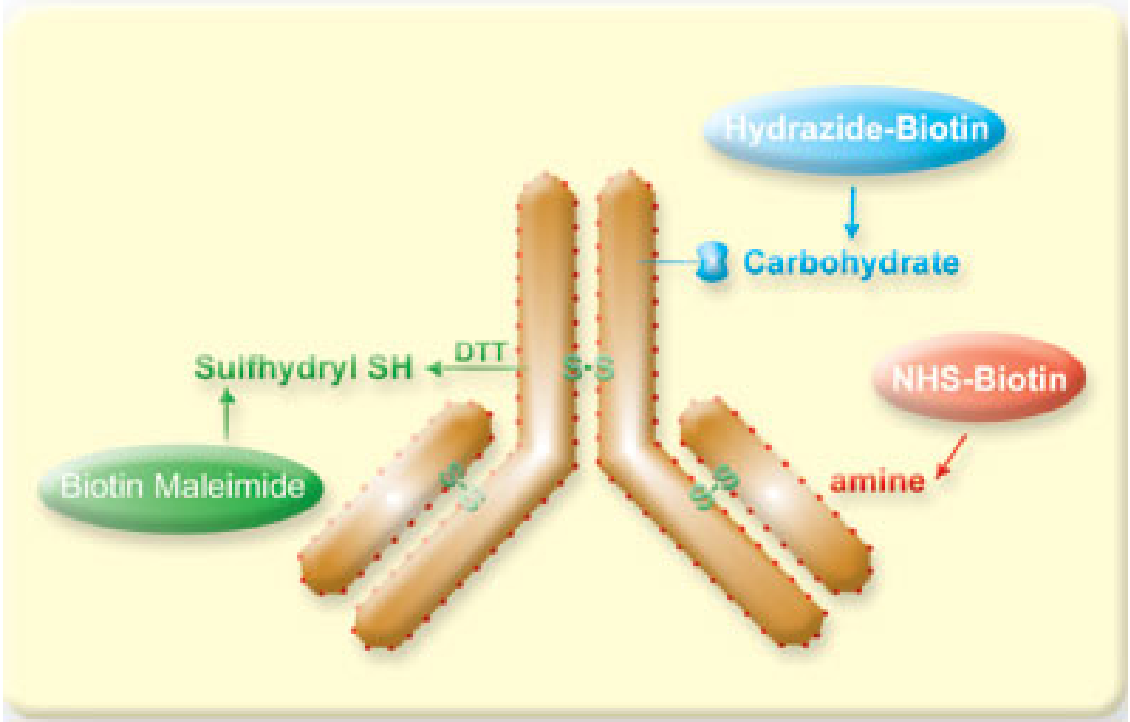

Streptavidin Agarose
DSB-X Biotin
IgG Antibody
DSB-X biotin antibody binding
Antigen
Antigen capture
Biotin elution
Biotin
Biotin


Biotinylated Protein
Streptavidin

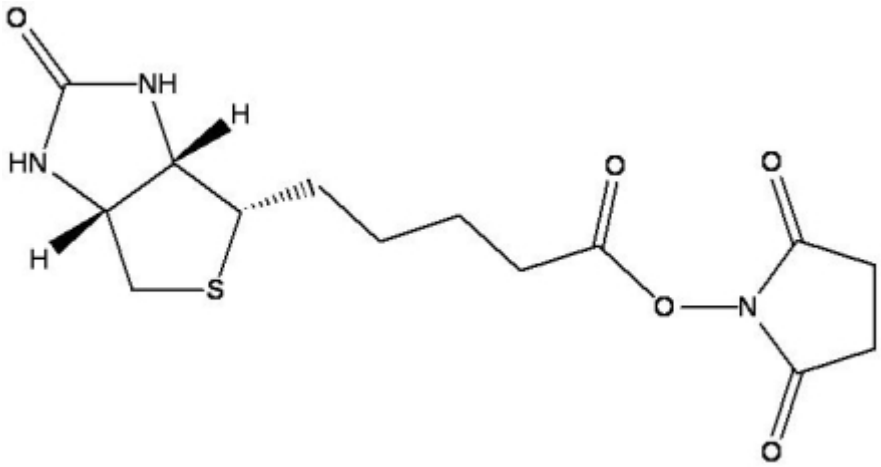

1
HRP
Streptavidin
Biotin
AG
Blocking protein
Blocking protein
Blocking protein
Blocking protein
Blocking protein
Microplate


biotin
BSA-biotin
BSA-biotin adsorbs
Streptavidin adsorption

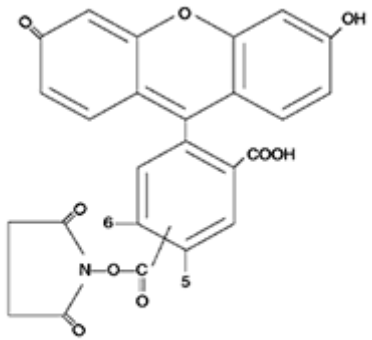
Protein Labeling



Amine Reactive Labeling

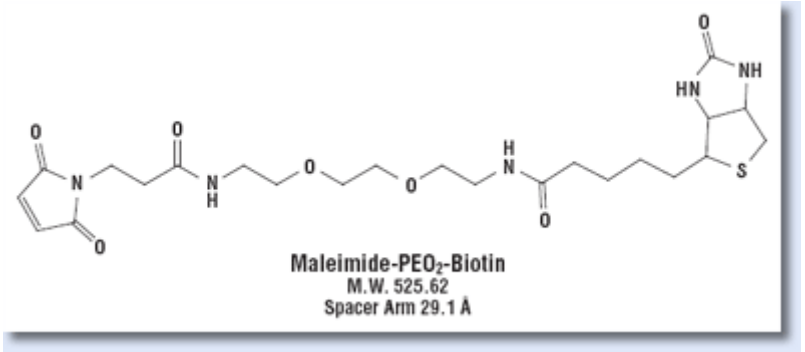


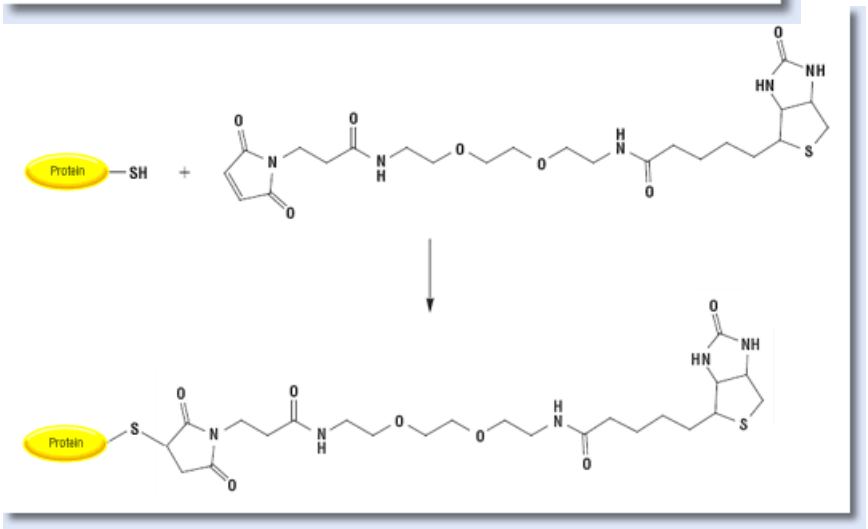
NHS ester

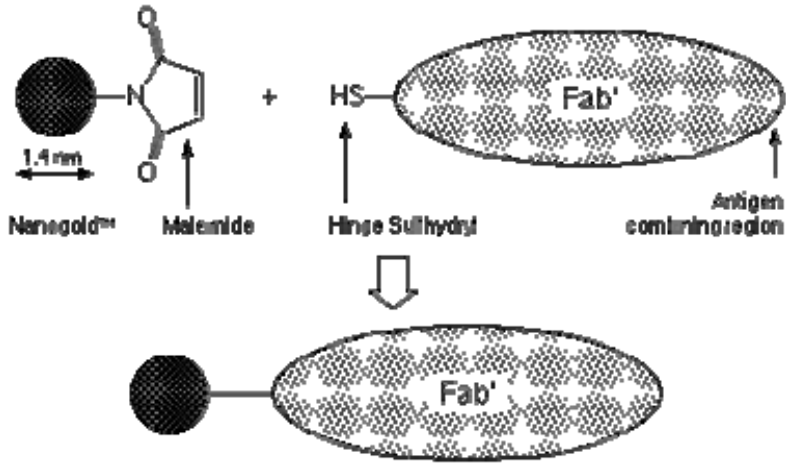


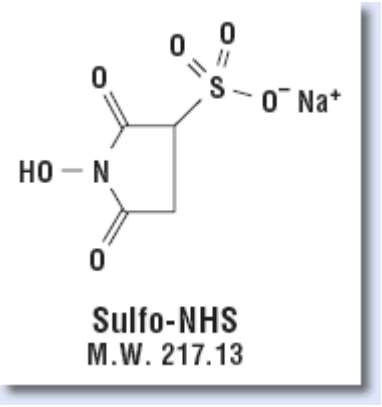
NHS-Fluorescein
MW 473.4

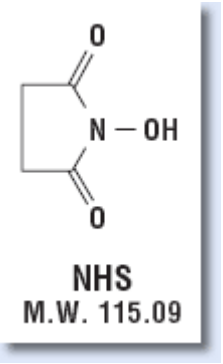
Sulfhydryl Labeling

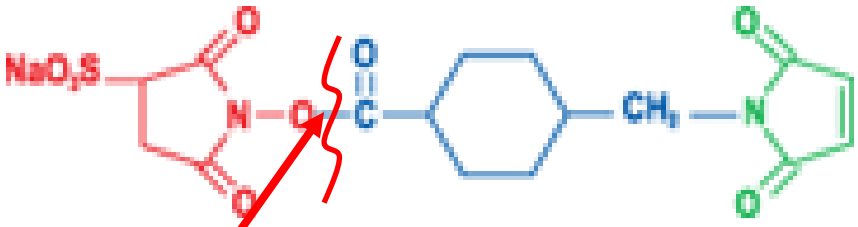












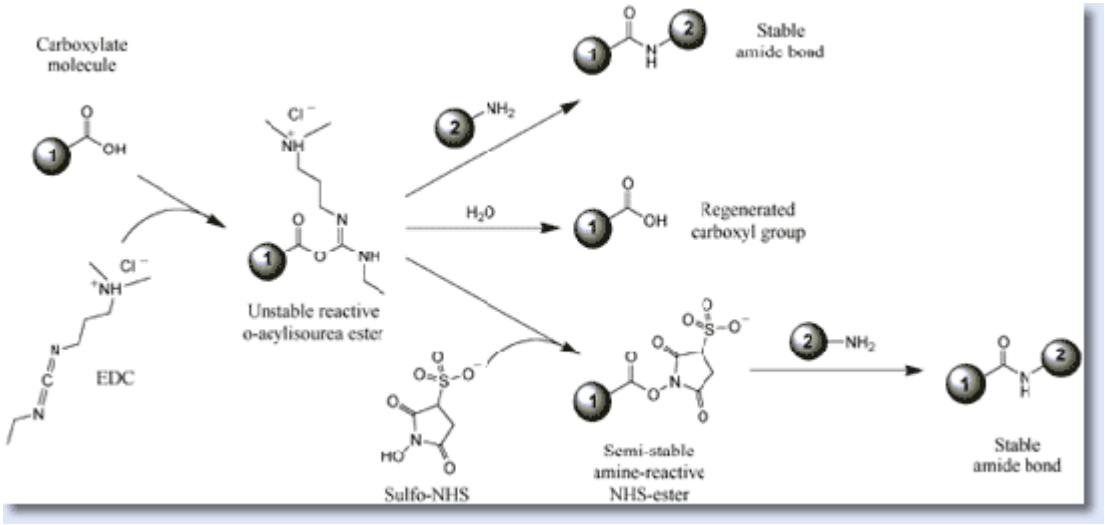
SulfoSuccinimidyl-4-(N-Maleimidomethyl)Cyclohexane-1-Carboxylate
SSMCC

The most popular NH_2- and SH- crosslinker

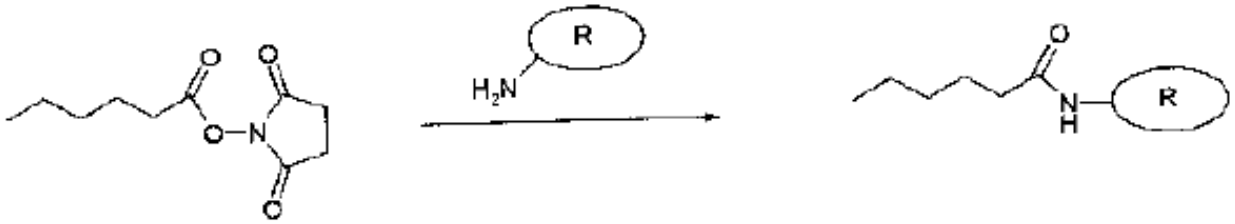
N-hydroxysuccinimide

NH_2 => amide

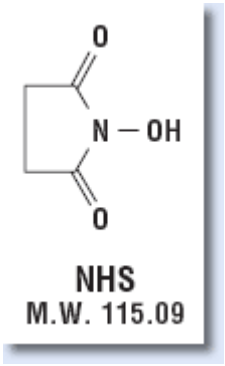
$R_1NH_2 + NHS\text{-}R_2 = >R_1NHC=OR_2$



N-hydroxysuccinimide (NHS)

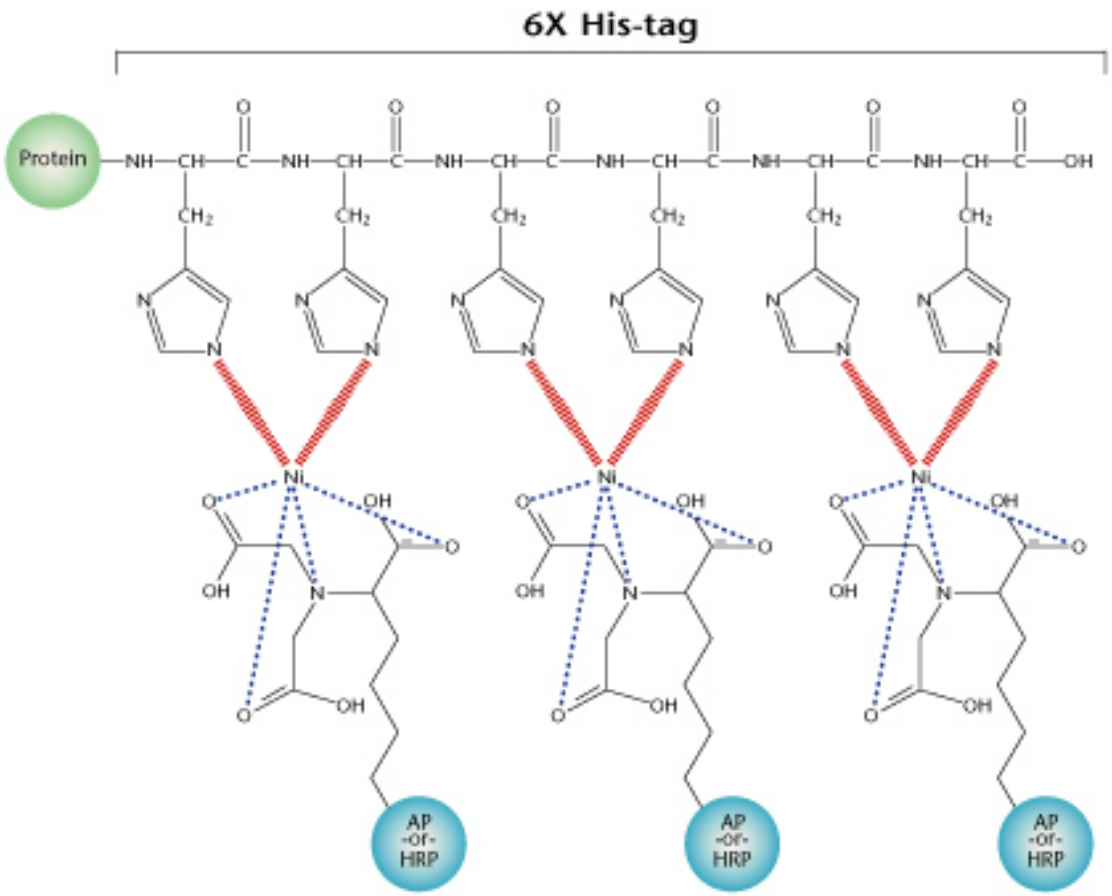


Scheme 2.6 Surface coupling reaction of NHS-esters with the amino residues of the side-chains of polypeptides (lysine units). R, protein.

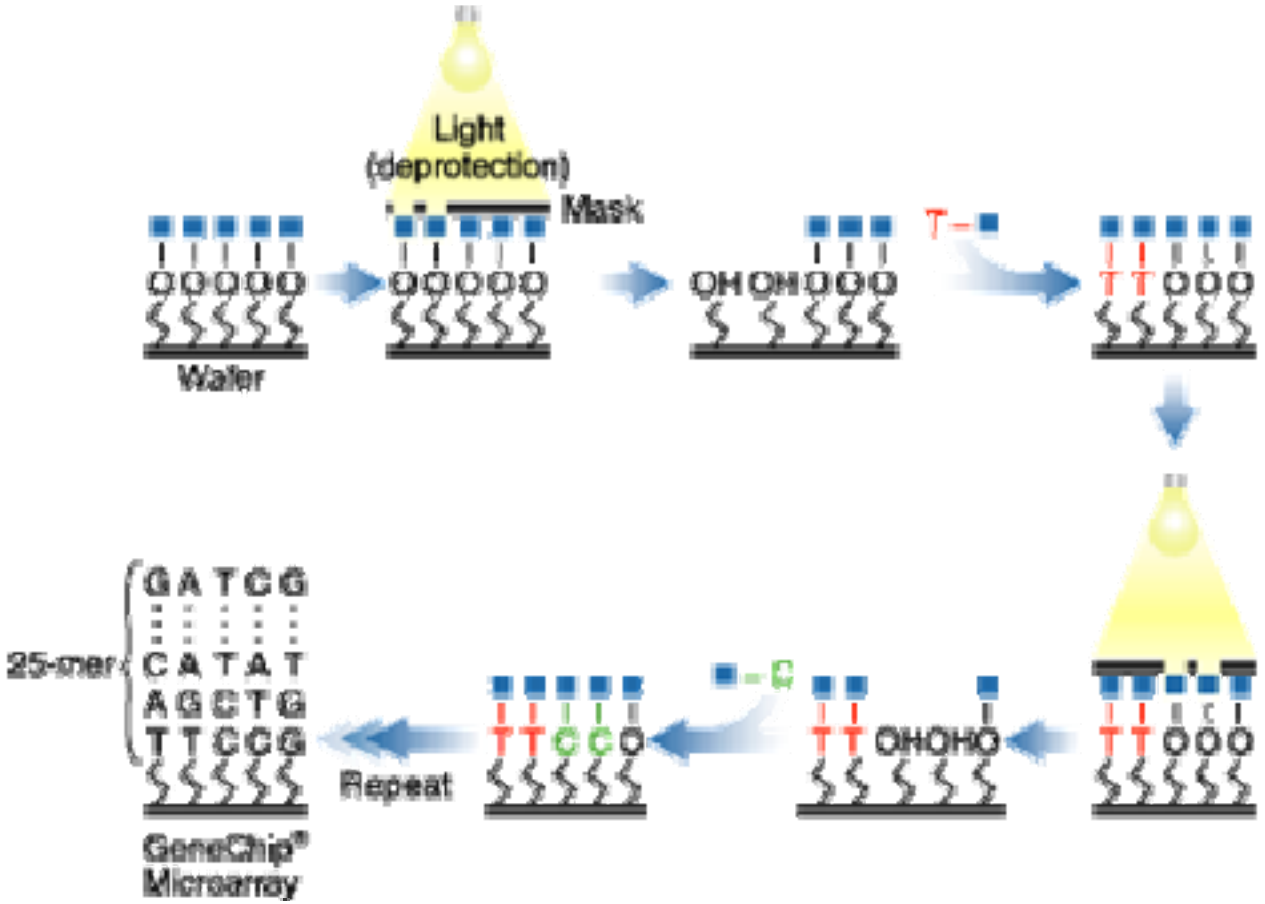


N-hydroxysuccinimide

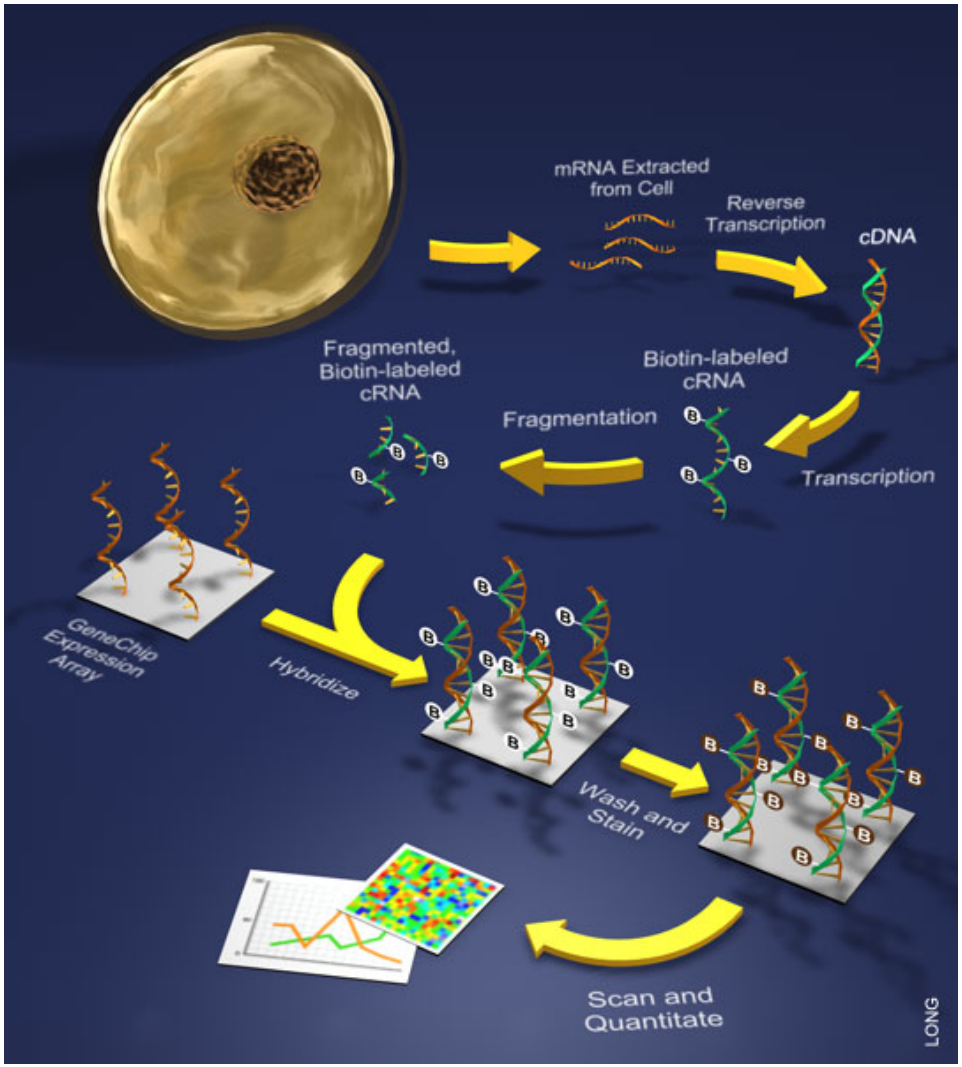
His Tag



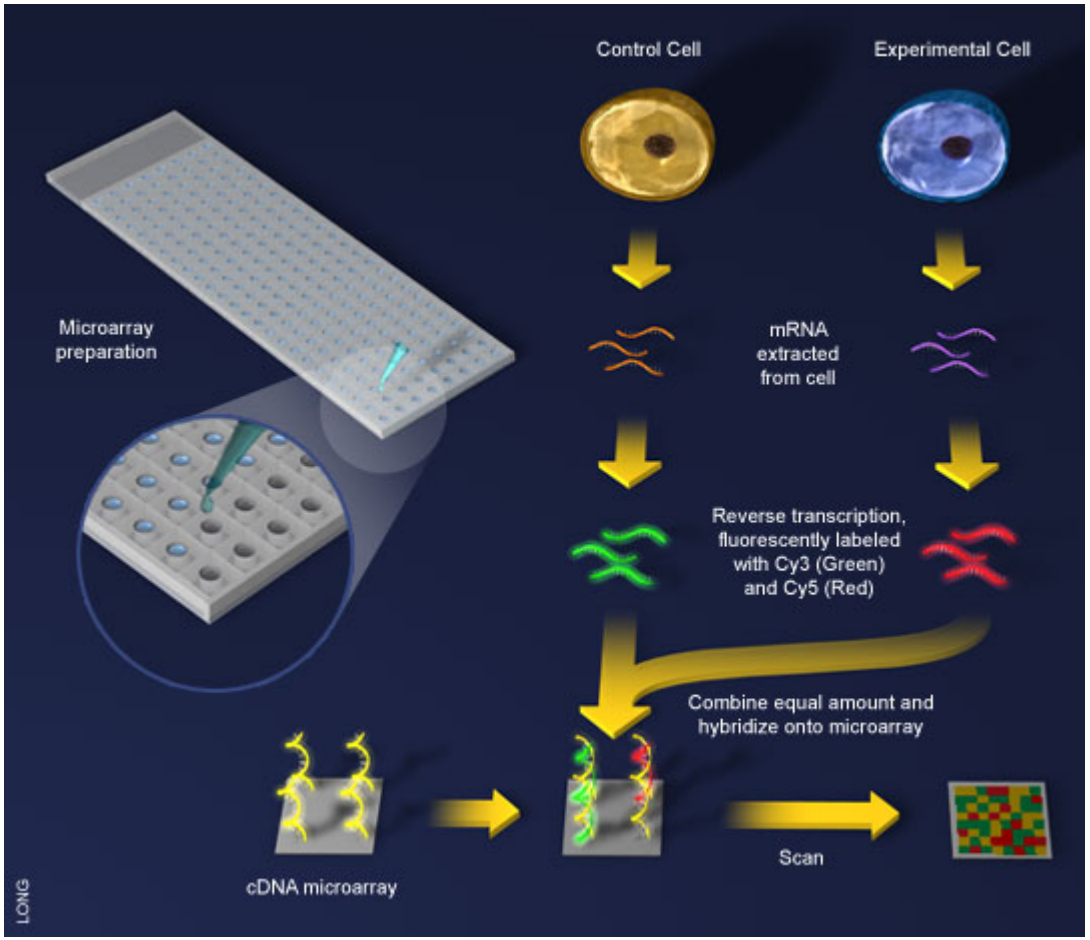
GeneChip


Light
(deprotection)
Mask
Wafer
T –
– C
Repeat
25-mer
GATCG
CATAT
AGCTG
TTCCG
GeneChip®
Microarray

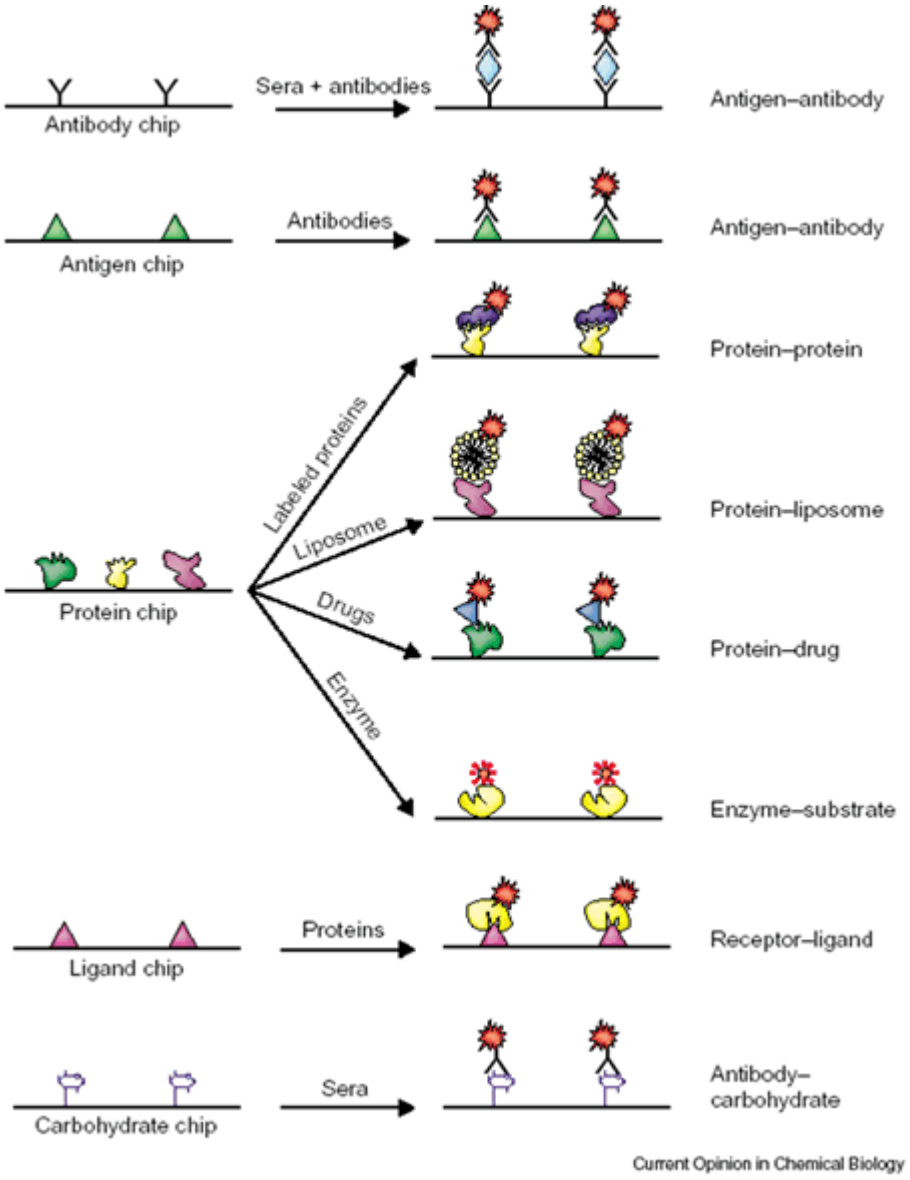
Scheme


mRNA Extracted from Cell
Reverse Transcription
cDNA
Biotin-labeled cRNA
Transcription
Fragmentation
Fragmented, Biotin-labeled cRNA
GeneChip Expression Array
Hybridize
Wash and Stain
Scan and Quantitate
LONG

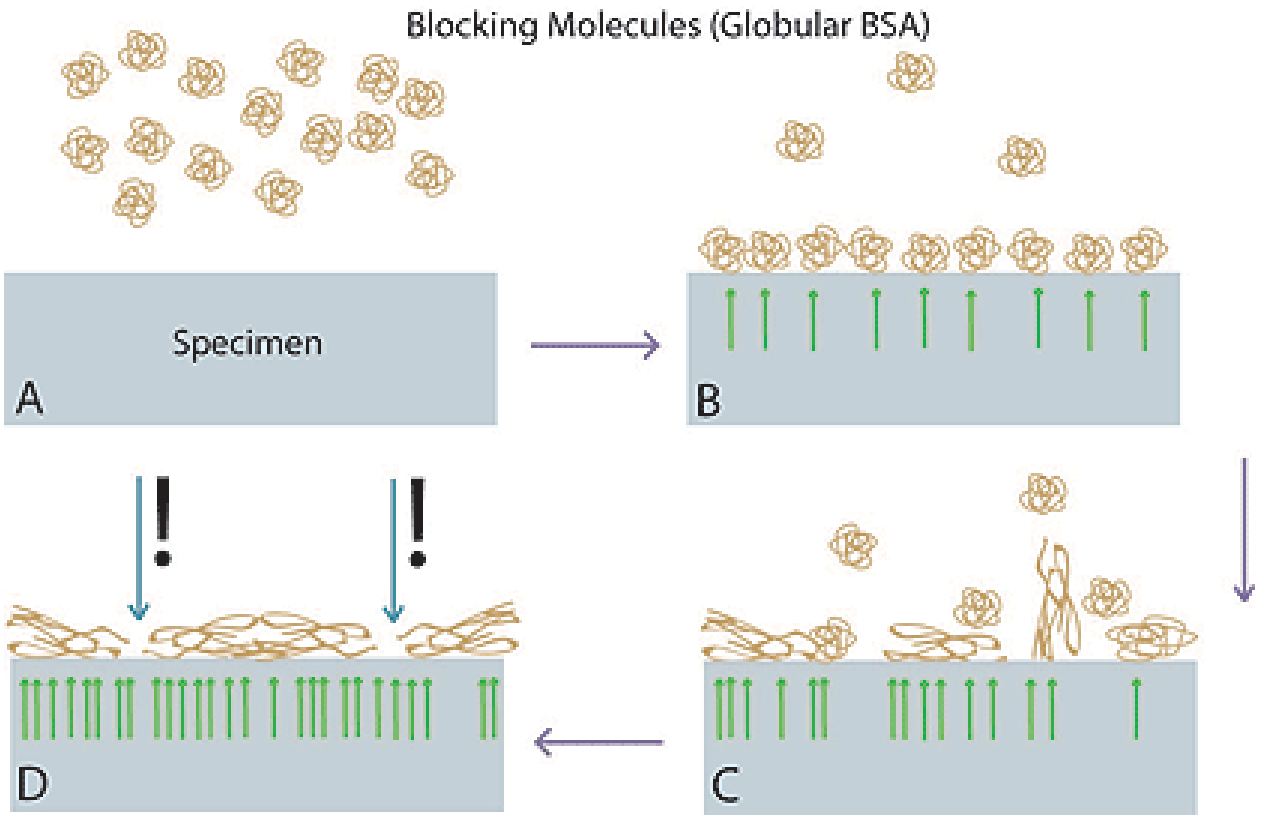
cDNA Microarray



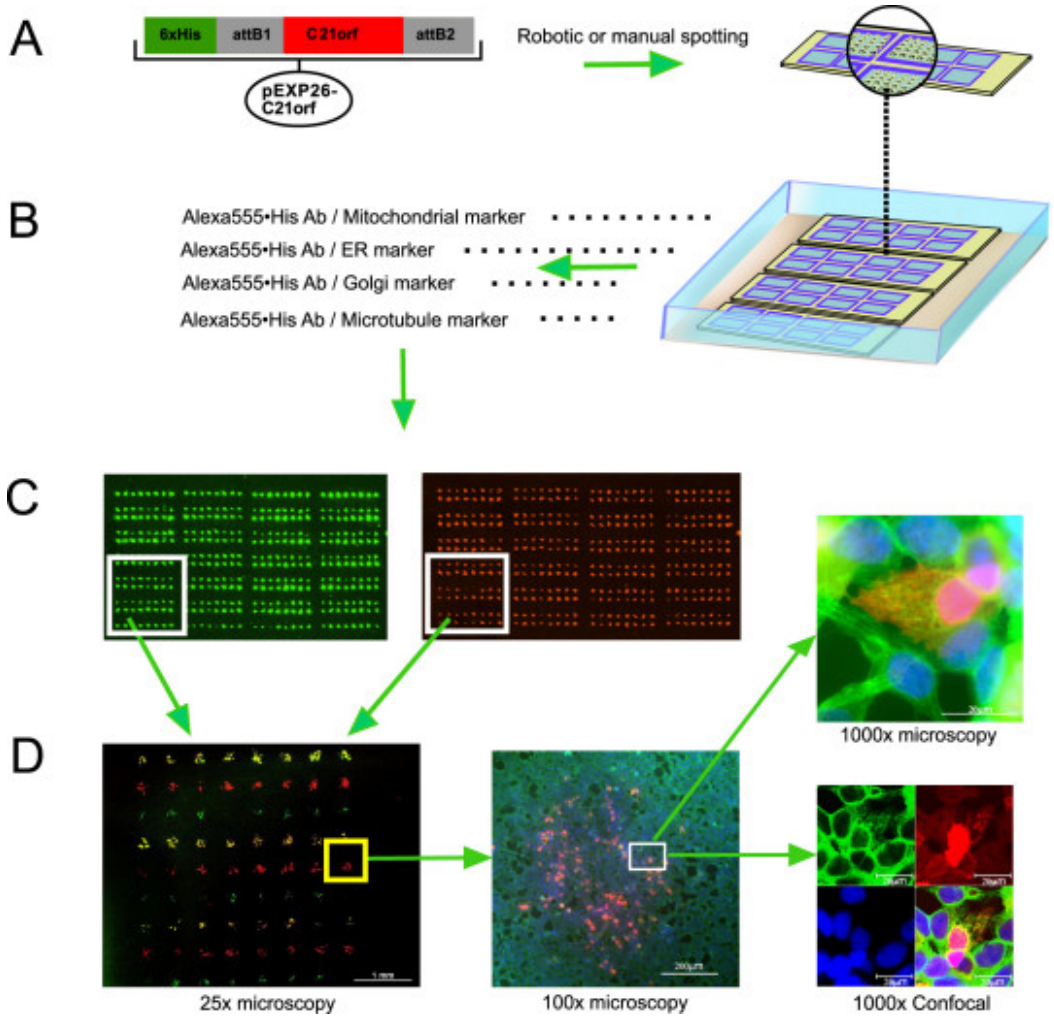
Protein Array



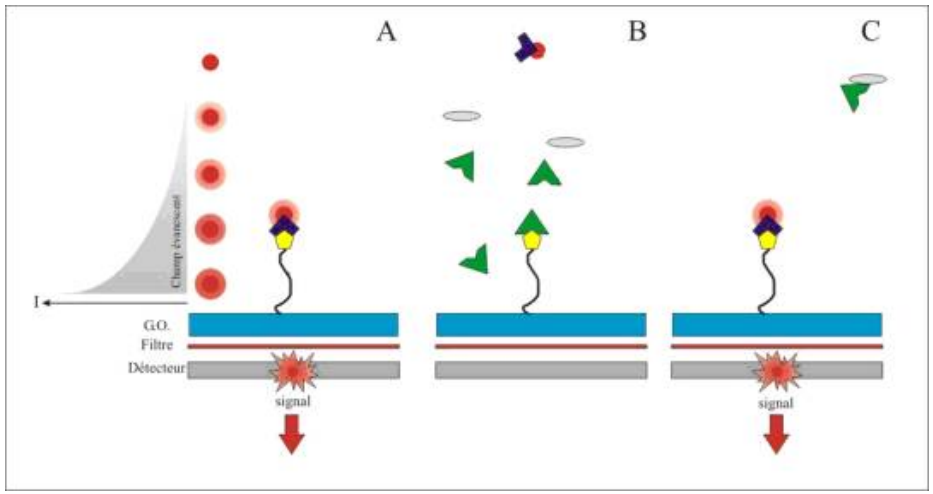
BSA Blocking

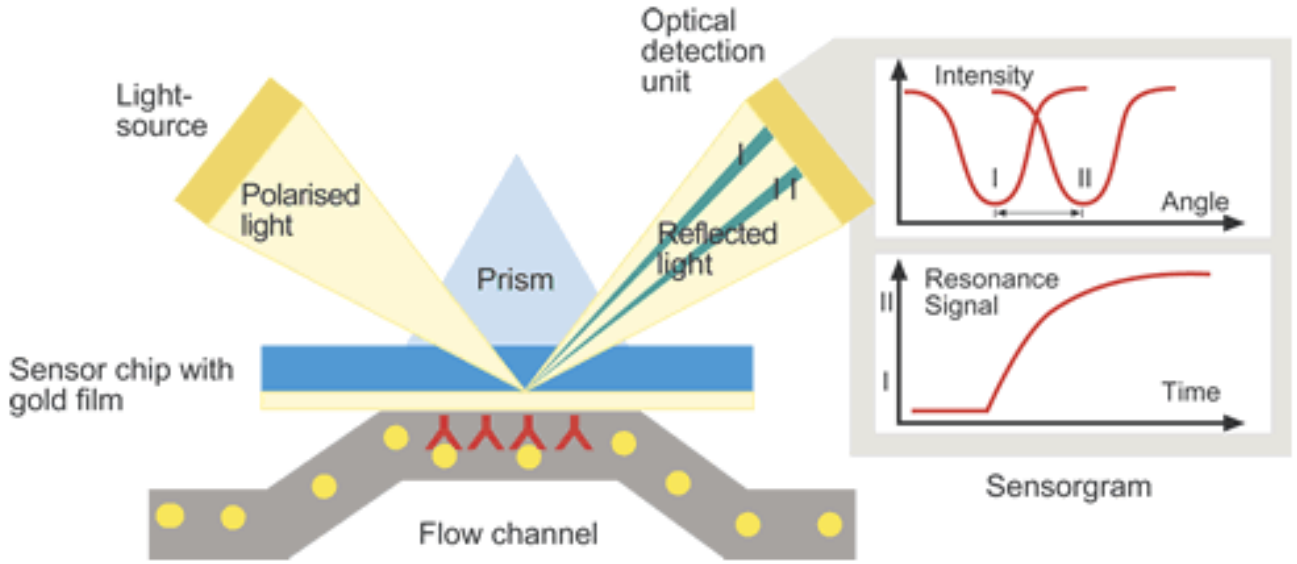


Cell Array

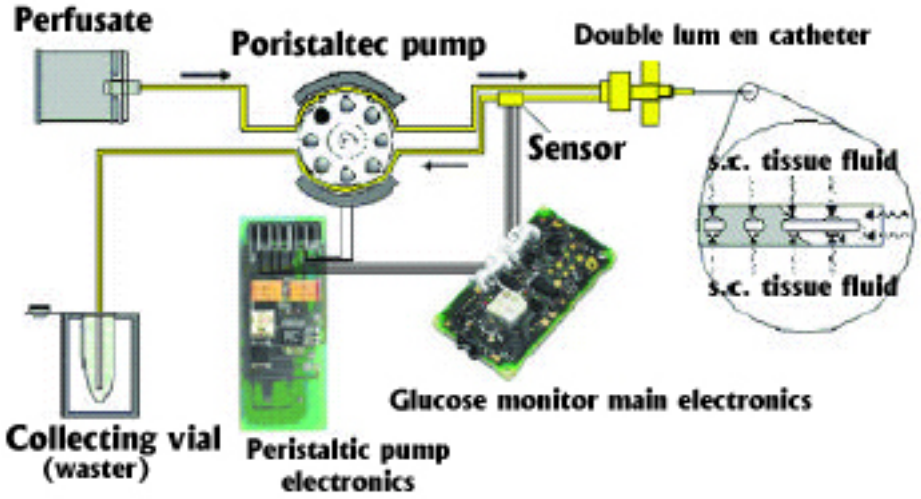

A
6xHis
attB1
C21orf
attB2
pEXP26-C21orf
Robotic or manual spotting
B
Alexa555•His Ab / Mitochondrial marker
Alexa555•His Ab / ER marker
Alexa555•His Ab / Golgi marker
Alexa555•His Ab / Microtubule marker
C
D
1000x microscopy
25x microscopy
100x microscopy
1000x Confocal

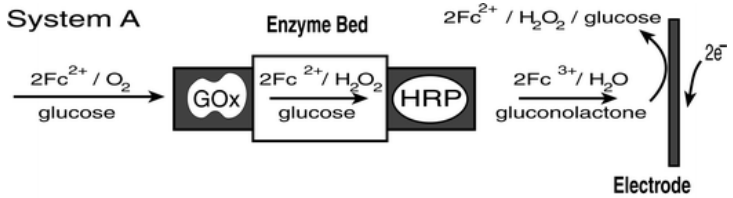
Surface Plasmon Resonance (SPR)

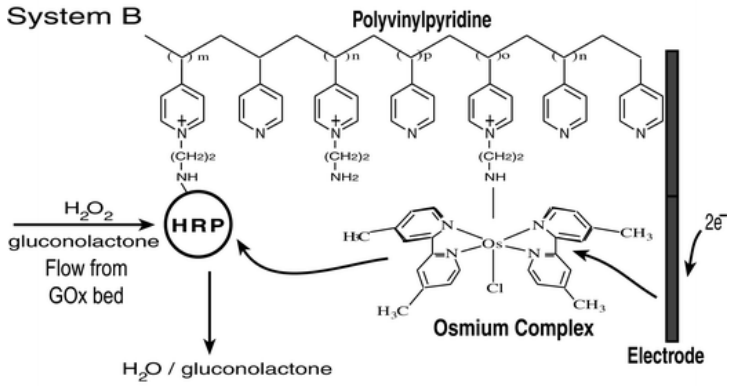


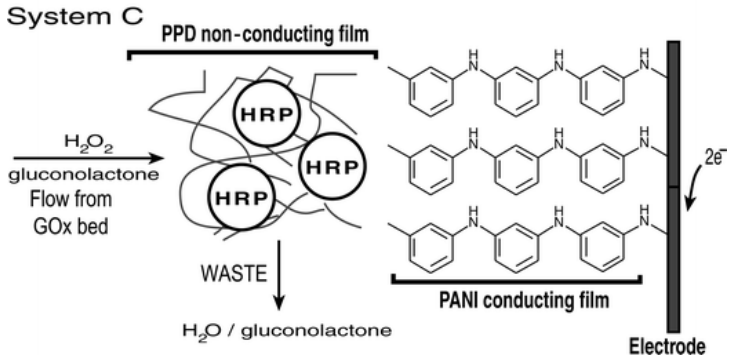


Glucose Sensor

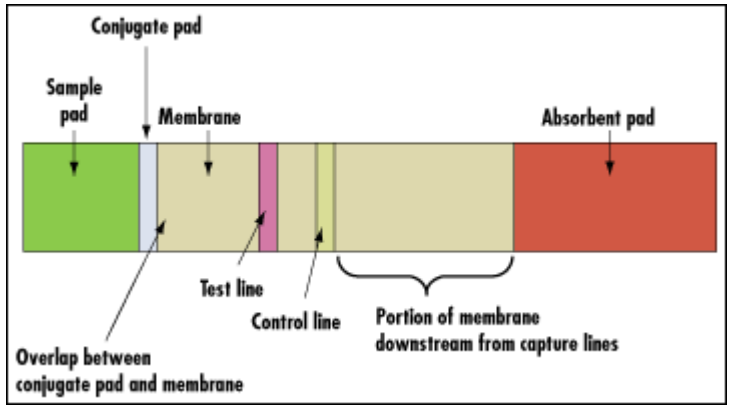






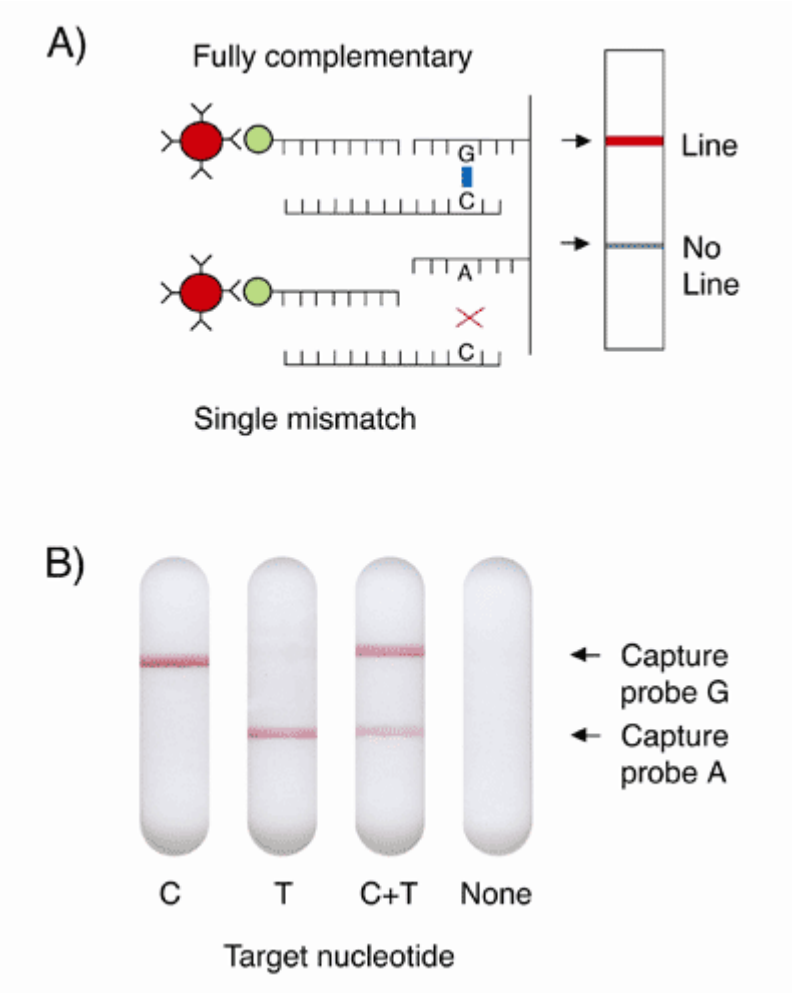


hCG immunoassay

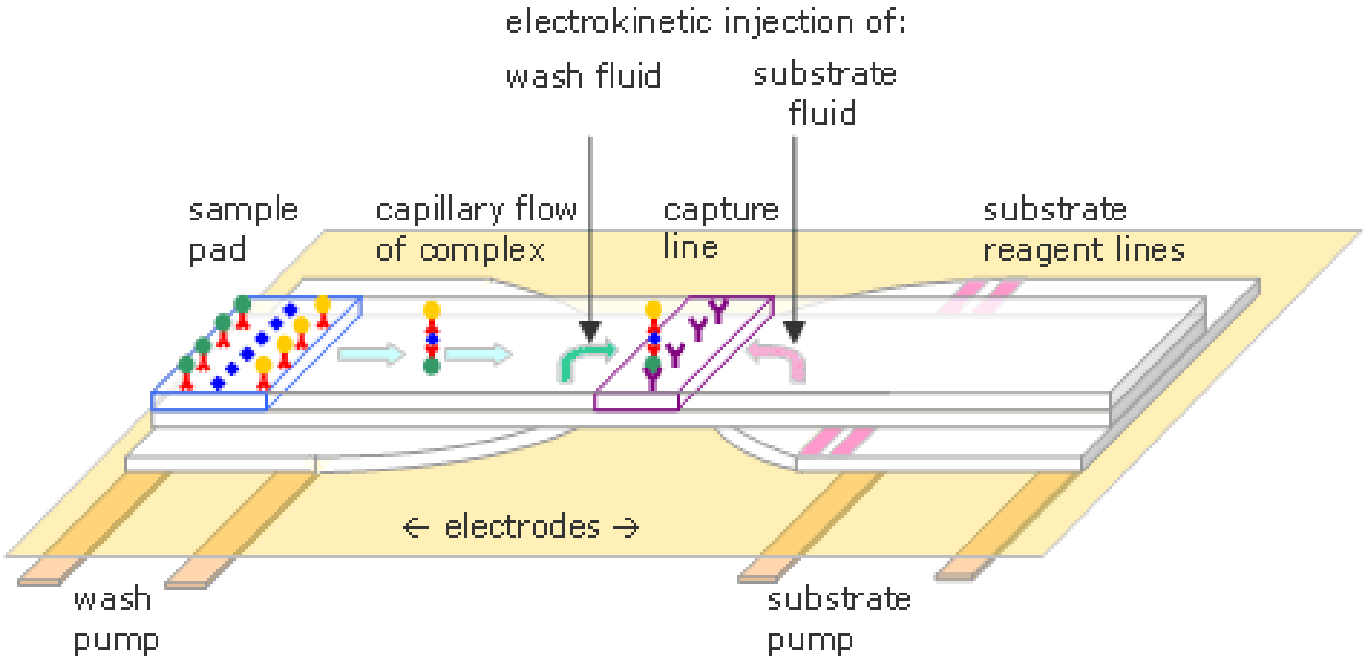


human chorionic gonadotropin (hCG)

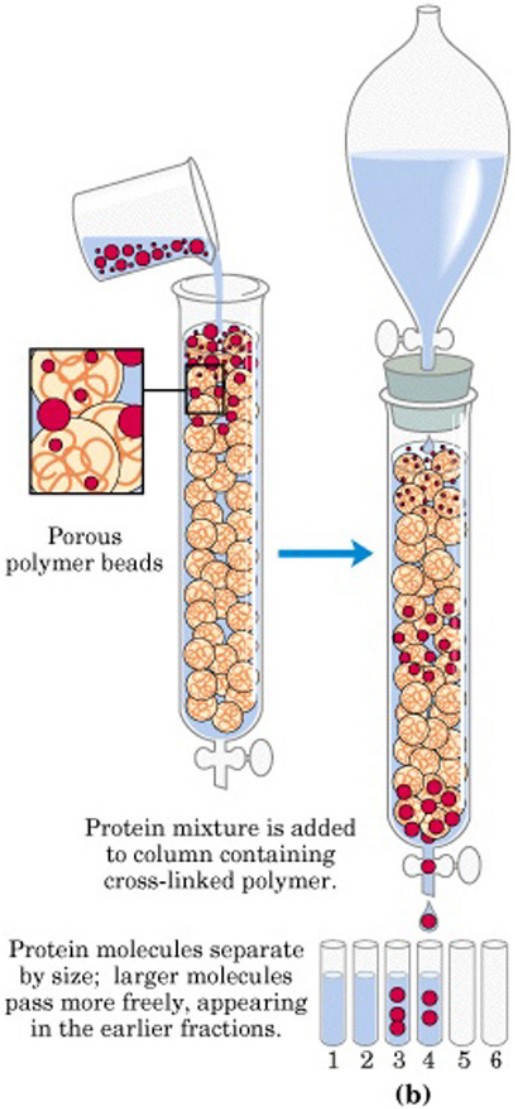
Nucleotide Sensor

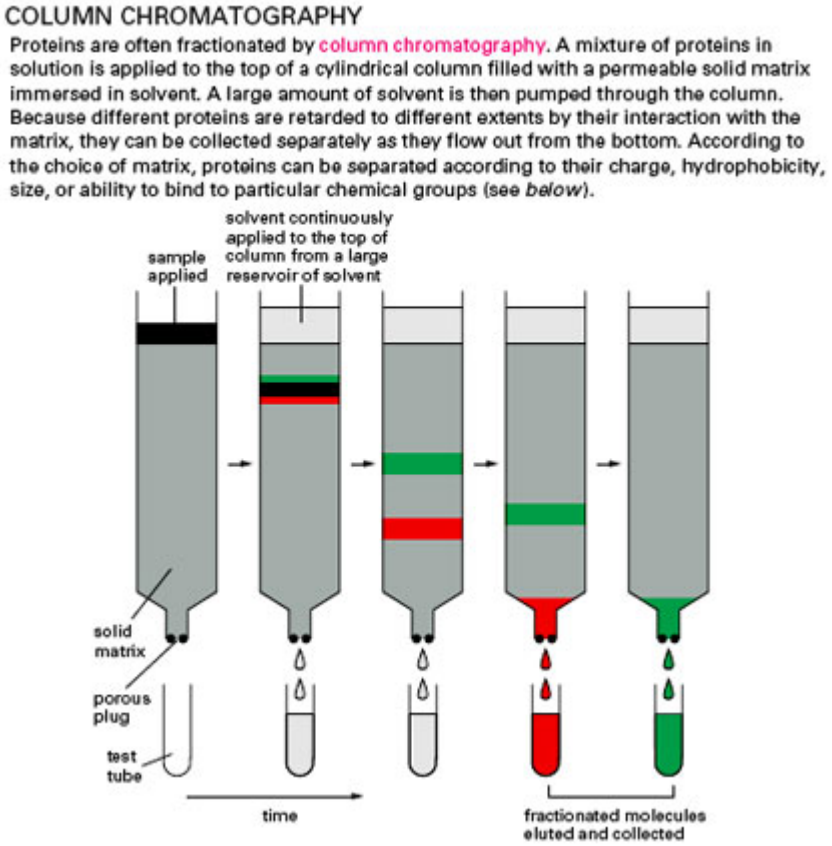


Microfluidic Immunoassay

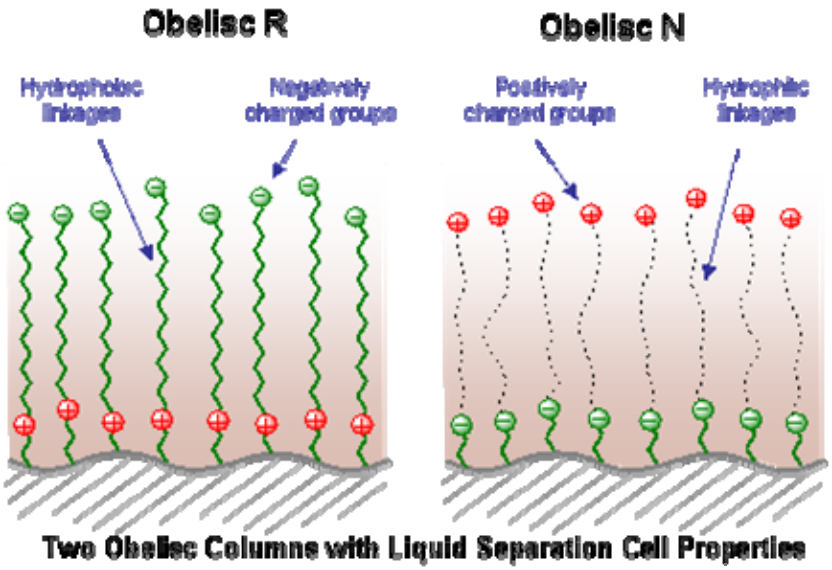


Chromatography

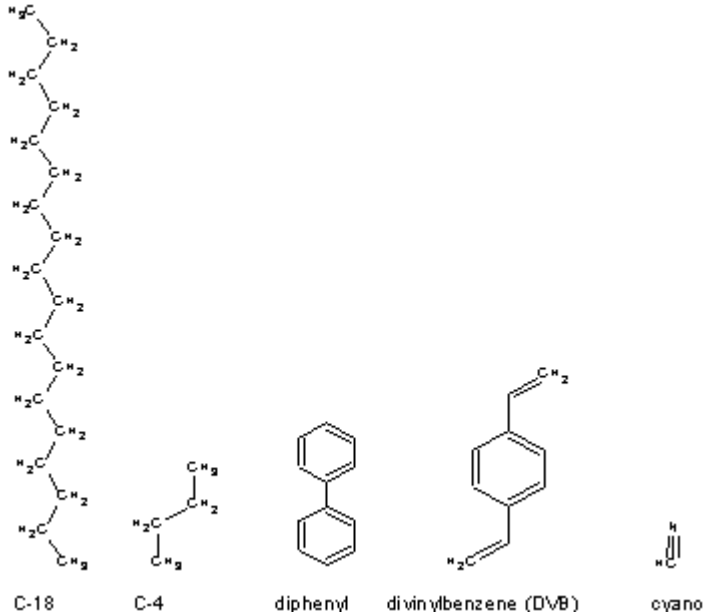




Reverse Phase



Two Obelisc Columns with Liquid Separation Cell Properties



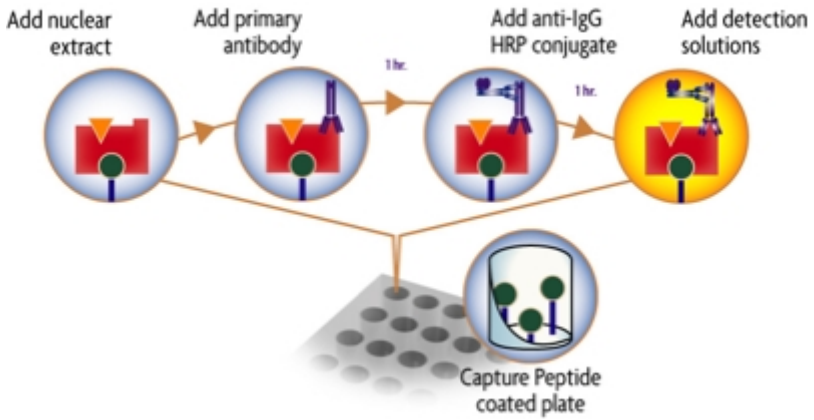
C-18 C-4 diphenyl divinylbenzene (DVB) cyano

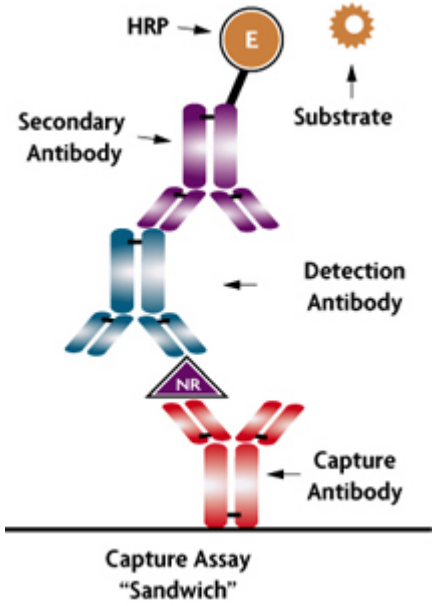
Nanomaterials for Biodiagnostic

- Nucleic Acid
  - Genetic information for identification
  - Diseases, bacterium, virus, pathogen
  - PCR with molecular fluorophore, State of the Art
  - Expansive, Non-portable, Non-multiplexing
- Proteins
  - Cancers and diseases, unusual high concentration of marker
  - ELISA (~pM) with molecular fluorophore
  - No PCR version

ELISA (Enzyme-Linked Immunosorbent Assay)

is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. It utilizes two antibodies, one of which is specific to the antigen and the other of which is coupled to an enzyme. This second antibody gives the assay its "enzyme-linked" name, and will cause a chromogenic or fluorogenic substrate to produce a signal.





Why Nanomaterials?

- Molecular fluorophores
  - Limited spectral response
  - photostability
- Nanomaterials
  - Small size (1-100 nm)
  - Chemically tailorable physical properties
  - Unusual target binding properties
  - Structure robustness

Tailorable Physical Properties

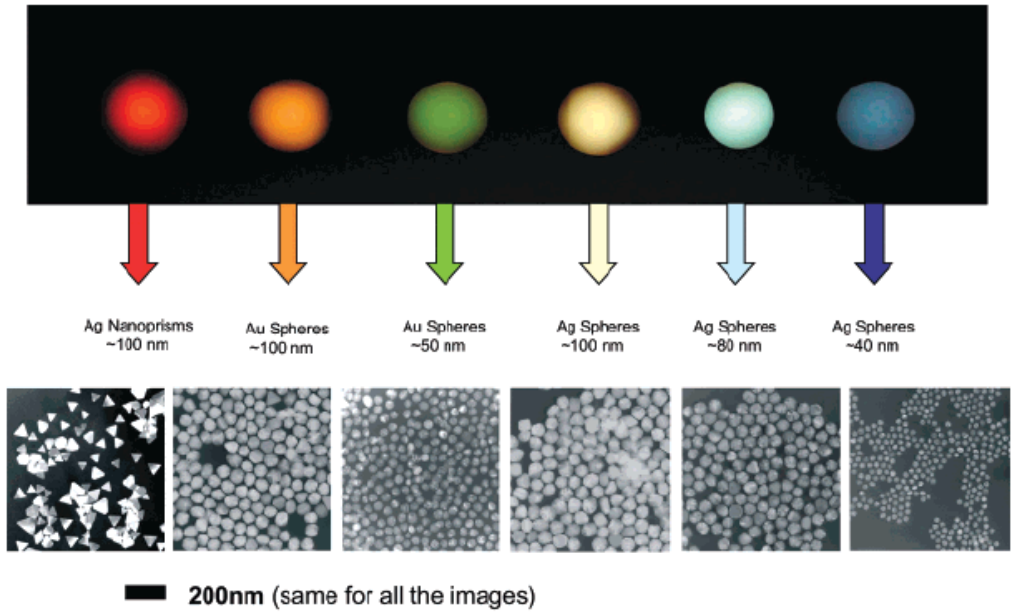


Figure 1. Sizes, shapes, and compositions of metal nanoparticles can be systematically varied to produce materials with distinct light-scattering properties.

Nanomaterial Detection

- Optical
- Electrical and electrochemical
- Magnetic
- Nanowire and Nanotubes
- Nanofabrication

Colorimetric Detection of DNA

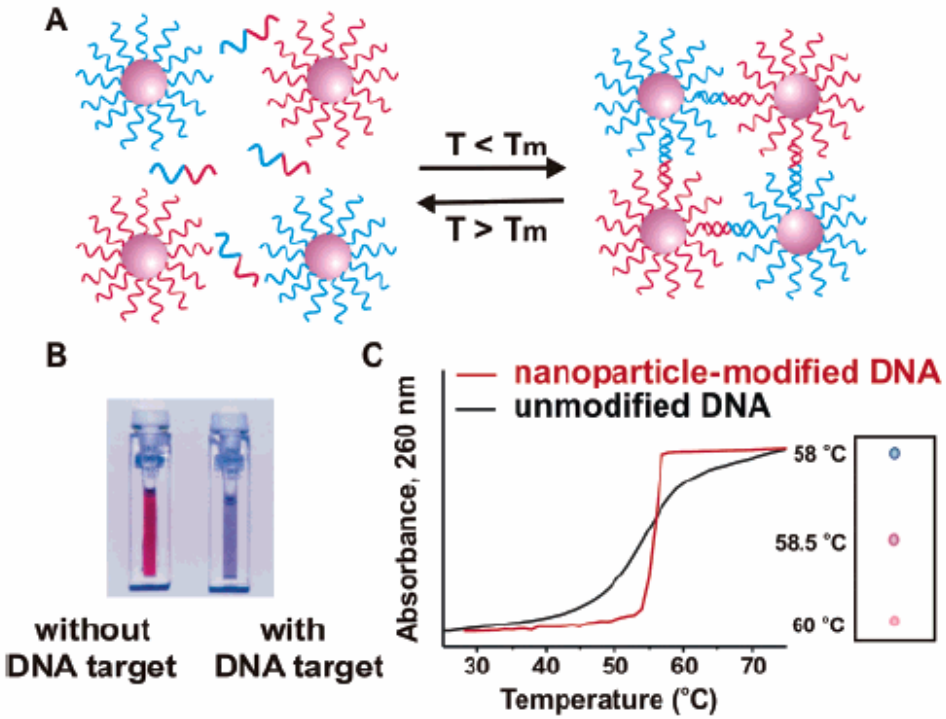


Figure 2. In the presence of complementary target DNA, oligonucleotide-functionalized gold nanoparticles will aggregate (A), resulting in a change of solution color from red to blue (B). The aggregation process can be monitored using UV−vis spectroscopy or simply by spotting the solution on a silica support (C). (Reprinted with permission from *Science* (http://www.aaas.org), ref 29. Copyright 1997 American Association for the Advancement of Science.)

A DNA-based method for rationally assembling nanoparticles into macroscopic materials

Chad A. Mirkin, Robert L. Letsinger, Robert C. Mucic & James J. Storhoff

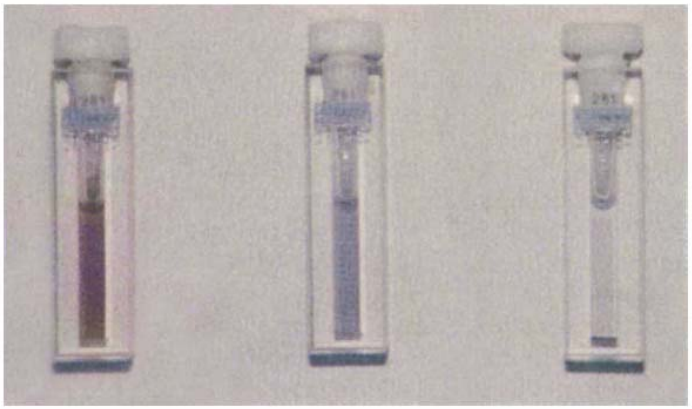
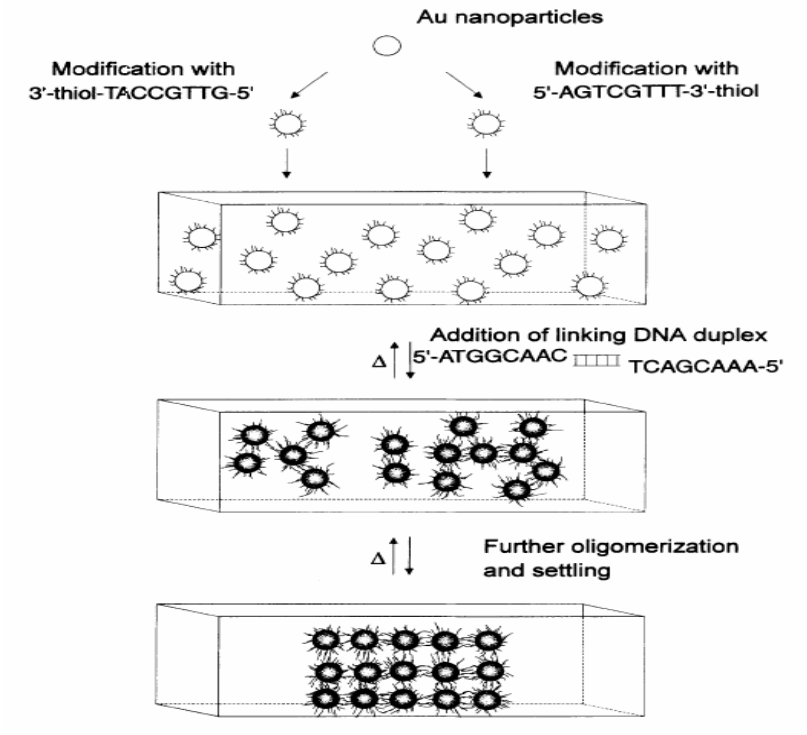
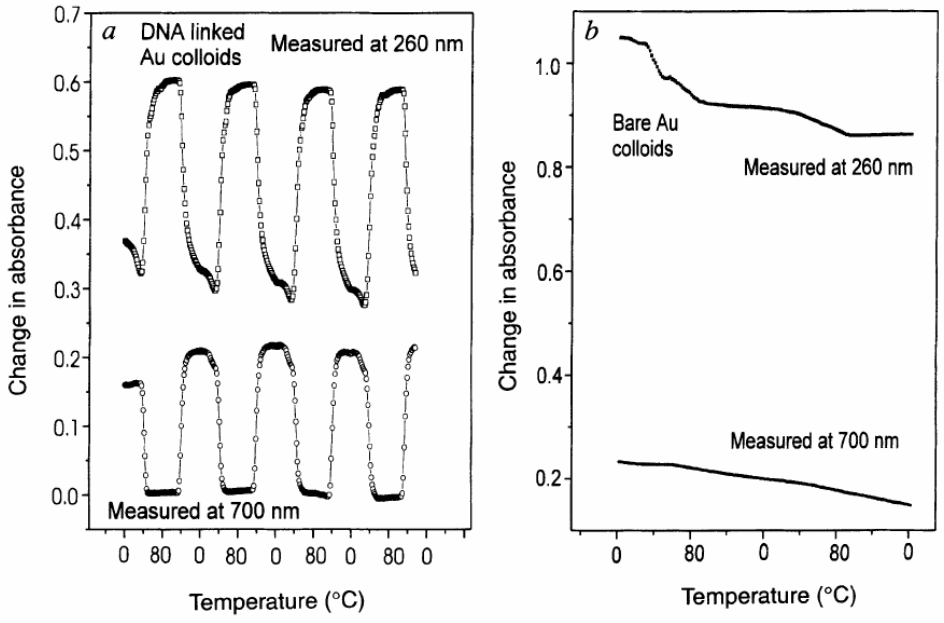


FIG. 2 Cuvettes with the Au colloids and the four DNA strands responsible for the assembly process. Left cuvette, at 80 °C with DNA-modified colloids in the unhybridized state; centre, after cooling to room temperature but before the precipitate settles; and right, after the polymeric precipitate settles to the bottom of the cuvette. Heating either of these cool solutions results in the reformation of the DNA-modified colloids in the unhybridized state (shown in the left cuvette).





Nature, 1996, 382, 607

Selective Colorimetric Detection of Polynucleotides Based on the Distance-Dependent Optical Properties of Gold Nanoparticles

Robert Elghanian, James J. Storhoff, Robert C. Mucic,
Robert L. Letsinger,* Chad A. Mirkin*

SCIENCE • VOL. 277 • 22 AUGUST 1997

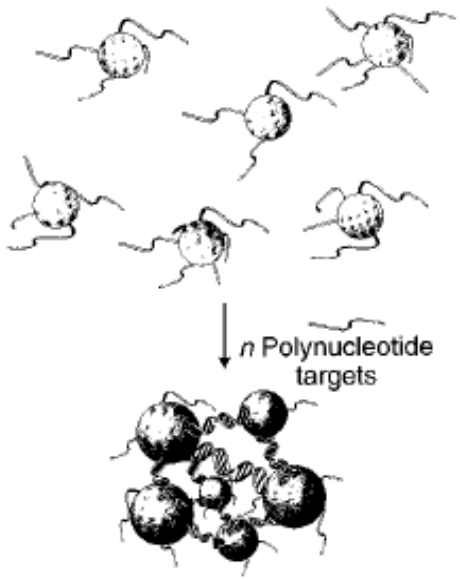


Fig. 1. Schematic representation of the concept for generating aggregates signaling hybridization of nanoparticle-oligonucleotide conjugates with oligonucleotide target molecules. The nanoparticles and the oligonucleotide interconnects are not drawn to scale, and the number of oligomers per particle is believed to be much larger than depicted.

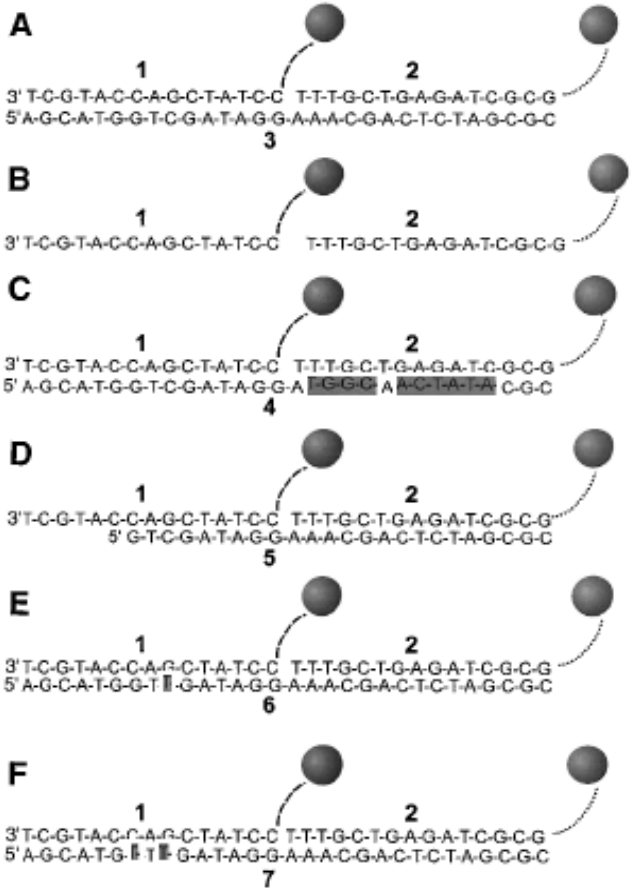


Fig. 2. Mercaptoalkyloligonucleotide-modified 13-nm Au particles and polynucleotide targets used for examining the selectivity of the nanoparticle-based colorimetric polynucleotide detection system. (**A**) Complementary target; (**B**) probes without the target; (**C**) a half-complementary target; (**D**) a 6-bp deletion; (**E**) a 1-bp mismatch; and (**F**) a 2-bp mismatch. For the sake of clarity, only two particles are shown; in reality a polymeric aggregate with many particles is formed. Dashed lines represent flexible spacer portions of the mercaptoalkyloligonucleotide strands bound to the nanoparticles; note that these spacers, because of their noncomplementary nature, do not participate in hybridization. The full sequences for the two probes, **1** and **2**, which bind to targets **3** through **7**, are

1-5′SH-$(CH_2)_6$- [CTA-ATC-CGC-ACA-G]
spacer

[CC-TAT-CGA-CCA-TGC-T]
probe

2-5′SH-$(CH_2)_6$-[ATG-GCA-ACT-ATA-C]
spacer

[GC-GCT-AGA-GTC-GTT-T]
probe

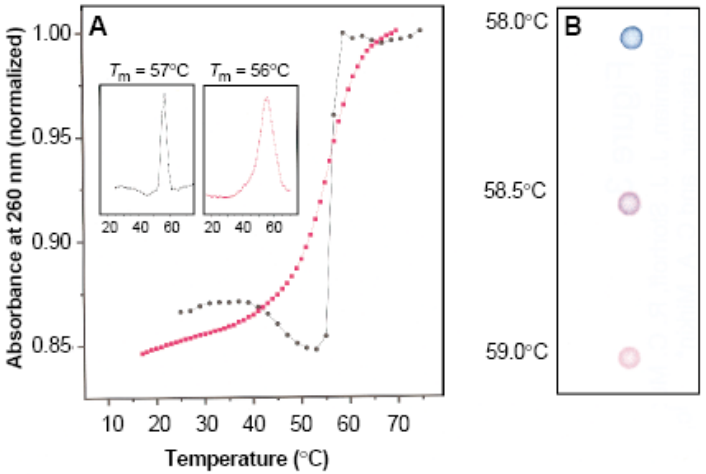
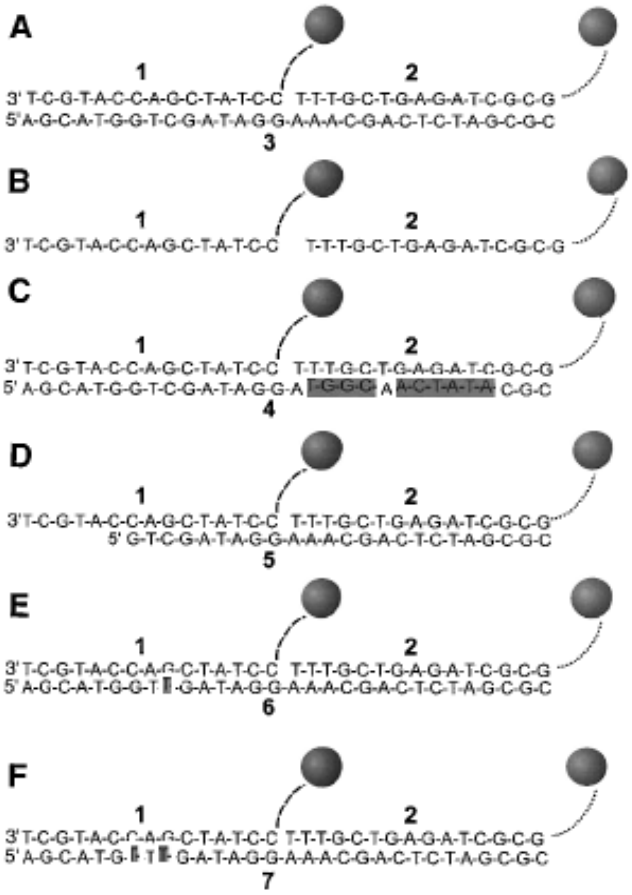


Fig. 3. (**A**) Comparison of the thermal dissociation curves for complexes of mercaptoalkyloligonucleotide-modified Au nanoparticles (black circles) and mercaptoalkyloligonucleotides without Au nanoparticles (red squares) with the complementary target, **3**, in hybridization buffer (0.1 M NaCl, 10 mM phosphate buffer, pH 7.0). For the first set (black circles), a mixture of 150 μl of each colloid conjugate and 3 μl of the target oligonucleotide in hybridization buffer (0.1 M NaCl, 10 mM phosphate, pH 7.0) was frozen at the temperature of dry ice, kept for 5 min, thawed over a period of 15 min, and diluted to 1.0 ml with buffer (final target concentration, 0.02 μM). The absorbance was measured at 1-min intervals with a temperature increase of 1°C per minute. The increase in absorption at 260 nm (A_{260}) was ~0.3 absorption units (AU). In the absence of the oligonucleotide targets, the absorbance of the nanoparticles did not increase with increasing temperature. For the second set, the mercaptoalkyloligonucleotides and complementary target (each 0.33 μM) were equilibrated at room temperature in 1 ml of buffer, and the changes in absorbance with temperature were monitored as before. The increase in A_{260} was 0.08 AU. (**Insets**) Derivative curves for each set (*15*). (**B**) Spot test showing T_c (thermal transition associated with the color change) for the Au nanoparticle probes hybridized with complementary target. A solution prepared from 150 μl of each probe and 3 μl of the target (0.06 μM final target concentration) was frozen for 5 min, allowed to thaw for 10 min, transferred to a 1-ml cuvette, and warmed at 58°C for 5 min in the thermally regulated cuvette chamber of the spectrophotometer. Samples (3 μl) were transferred to a C_{18} reverse phase plate with an Eppendorf pipette as the temperature of the solution was increased incrementally 0.5°C at 5-min intervals.



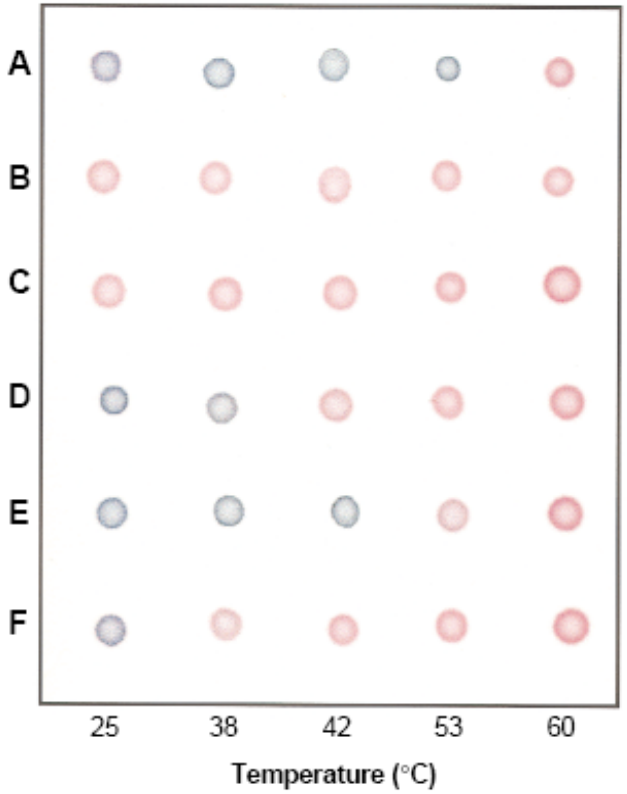


Fig. 4. Selective polynucleotide detection for the target probes shown in Fig. 2: (**A**) complementary target; (**B**) no target; (**C**) complementary to one probe; (**D**) a 6-bp deletion; (**E**) a 1-bp mismatch; and (**F**) a 2-bp mismatch. Nanoparticle aggregates were prepared in a 600-μl thin-walled Eppendorf tube by addition of 1 μl of a 6.6 μM oligonucleotide target to a mixture containing 50 μl of each probe (0.06 μM final target concentration). The mixture was frozen (5 min) in a bath of dry ice and isopropyl alcohol and allowed to warm to room temperature. Samples were then transferred to a temperature-controlled water bath, and 3-μl aliquots were removed at the indicated temperatures and spotted on a C_{18} reverse phase plate.

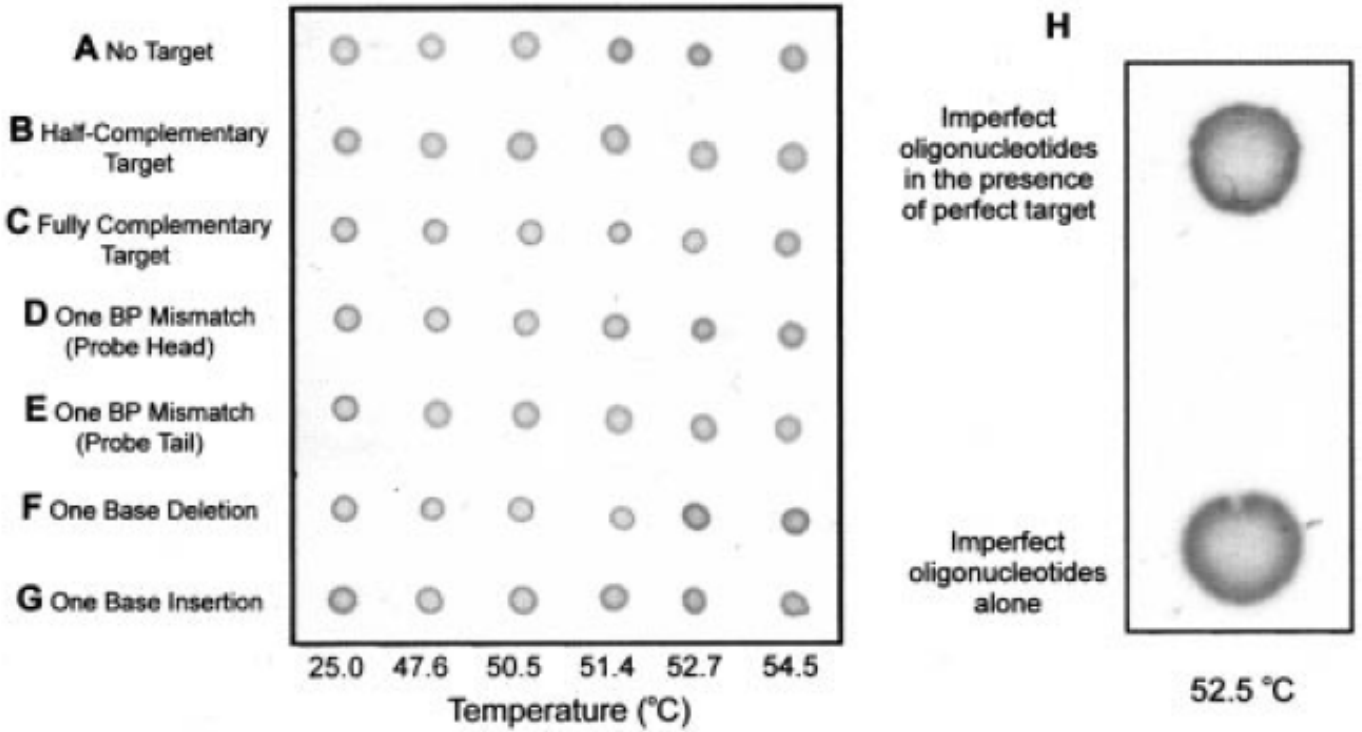


Figure 5. (A−G) The spot method for polynucleotide detection which demonstrates the selectivity of the Au nanoparticle based detection system toward single base imperfections. The probes and corresponding polynucleotide targets are listed in Figure 2. (H) Spot test demonstrating the detection and differentiation by color of a polynucleotide target in the presence of polynucleotides with single base imperfections.

1nM => 50pM

Rapid Aggregation of Gold Nanoparticles Induced by Non-Cross-Linking DNA Hybridization

Kae Sato, Kazuo Hosokawa, and Mizuo Maeda*

8102 ■ J. AM. CHEM. SOC. 2003, *125*, 8102−8103

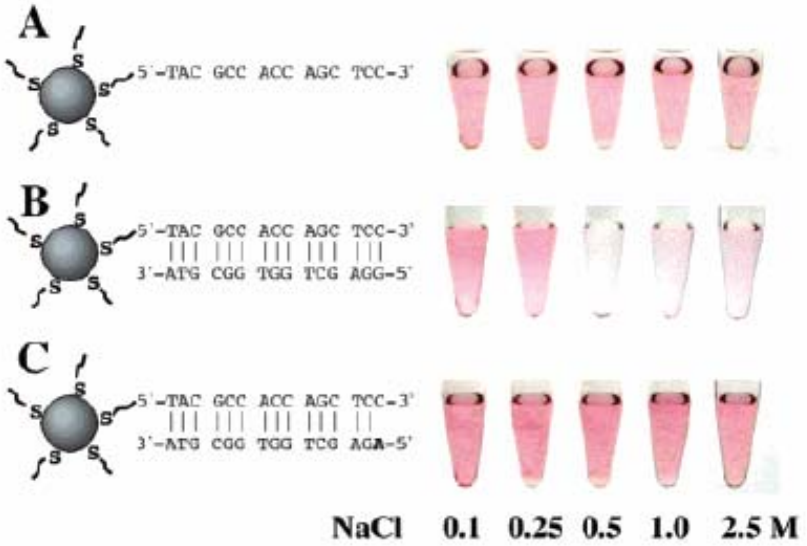


Figure 1. Aggregation behaviors of the DNA-gold nanoparticles at various NaCl concentrations at room temperature: (A) without a target DNA, (B) with the complementary target, and (C) with a target containing a single-base mismatch at its 5′ terminus. The final concentrations of the particle, the probe DNA, and the targets were 2.3, 500, and 500 nM, respectively.

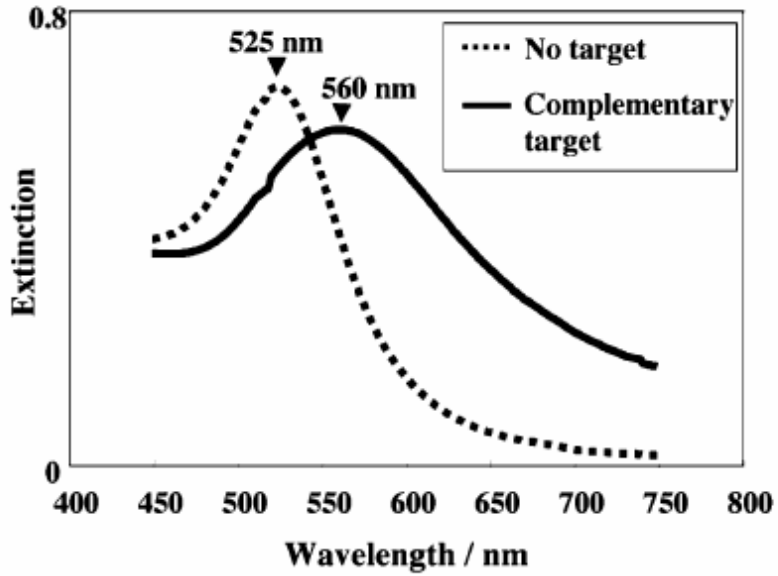


Figure 2. Visible spectra corresponding to Figure 1A (dotted line, no target) and 1B (solid line, complementary target) at 0.5 M NaCl.

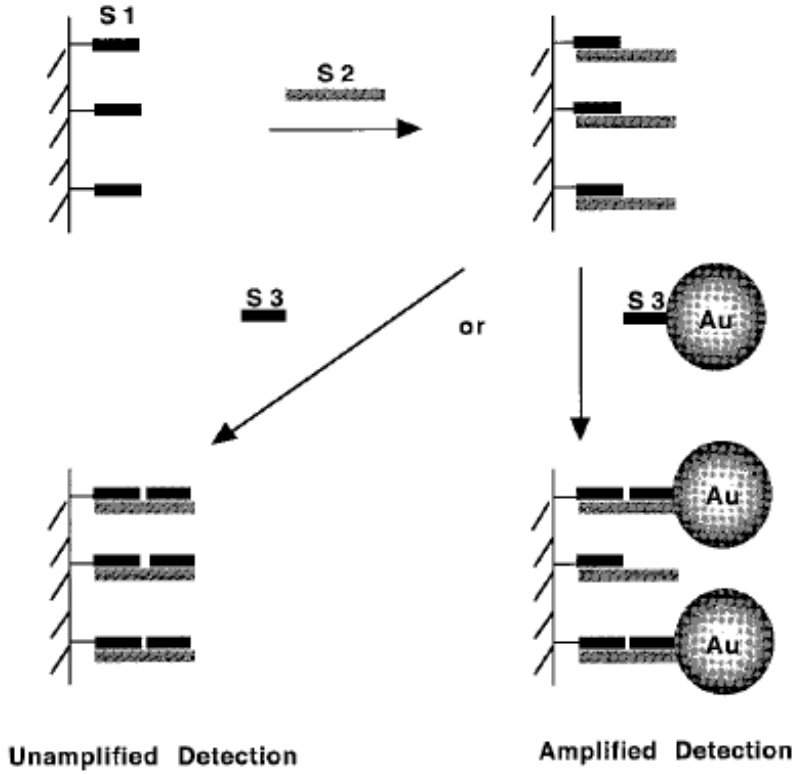
60-500 nM

J. Am. Chem. Soc. **2000,** *122,* 9071−9077

Colloidal Au-Enhanced Surface Plasmon Resonance for Ultrasensitive Detection of DNA Hybridization

Lin He, Michael D. Musick, Sheila R. Nicewarner, Frank G. Salinas, Stephen J. Benkovic, Michael J. Natan, and Christine D. Keating*

Scheme 1. SPR Surface Assembly



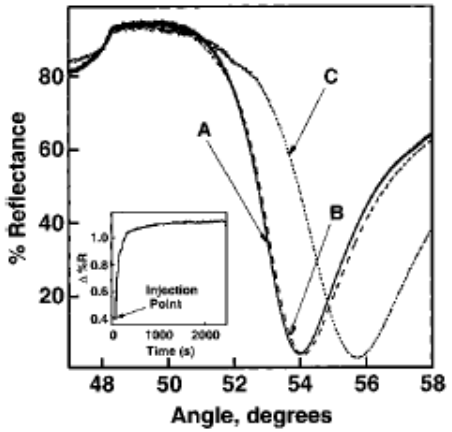
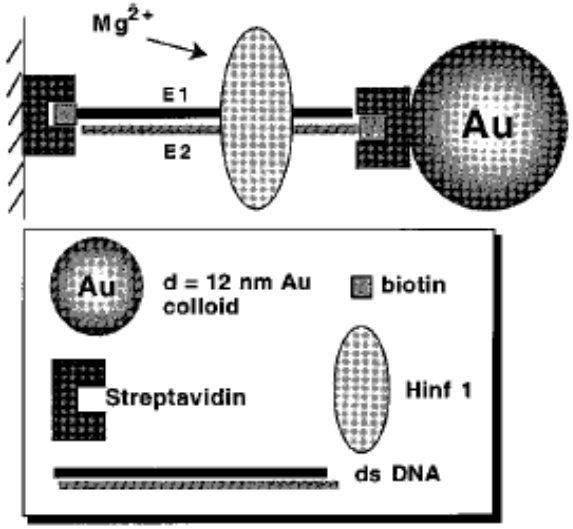


Figure 1. SPR curves of surfaces prepared in sequential steps as illustrated in Scheme 1: a MHA-coated Au film modified with a 12-mer oligonucleotide S1(A), after hybridization with its complementary 24-mer target S2 (B), and followed by introduction of S3:Au conjugate (C) to the surface. Inset: surface plasmon reflectance changes at 53.2° for the oligonucleotide-coated Au film measured during a 60-min exposure to S3:Au conjugates.

Scheme 2. SPR Surface Assembly in the Digestion Experiment



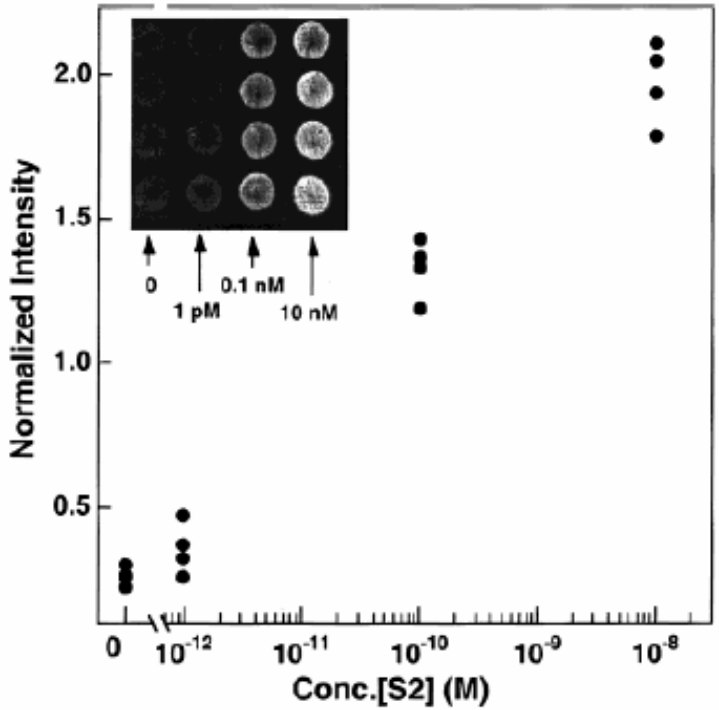


Figure 5. Plot of normalized intensity of SPR reflectance as a function of logarithmic concentration of the analyte 24-mer oligo (S2). Each spot represents one data point at the corresponding concentration. CCD parameters: exposure time = 0.3 s, 16 bit resolution, spot size = 4.5 mm in diameter. Inset: a 2-D SPR image of a Au surface derivatized with 20 μL of buffer blank, 1 pM, 0.1 nM, and 10 nM S2 oligos (from left to right, respectively).