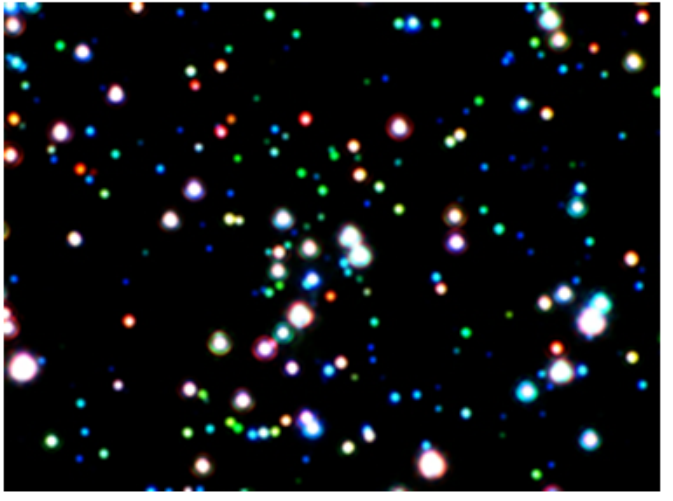
NANO means new structures and properties

- Optical, electrical, catalytic, and magnetic properties are size-dependent.



Dark-field image of Ag nanoparticles with various sizes and shapes.

DOI: 10.1002/anie.200904359

Nanotechnology

Gold Nanoparticles for Biology and Medicine

*David A. Giljohann, Dwight S. Seferos, Weston L. Daniel, Matthew D. Massich, Pinal C. Patel, and Chad A. Mirkin**

Keywords:
cytotoxicity · DNA · drug delivery · gold · nanoparticles

Biodetection:
Why is it important?

Genetic and Proteomic Analysis

Genomics and Proteomics

Forensics

Molecular Biology

Disease Identification

Protein Targets

Nucleic Acid Targets

Early Disease Diagnosis is the Best way to Cure or Prevent Serious Diseases

Pharmacogenomics

The study of how an individual's genetic inheritance affects the body's response to drugs.

Developing personalized drugs based on individual's genetic makeup

What are the conventional biodetection methods?

Protein Detection:

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Western Blot,
Protein chips…

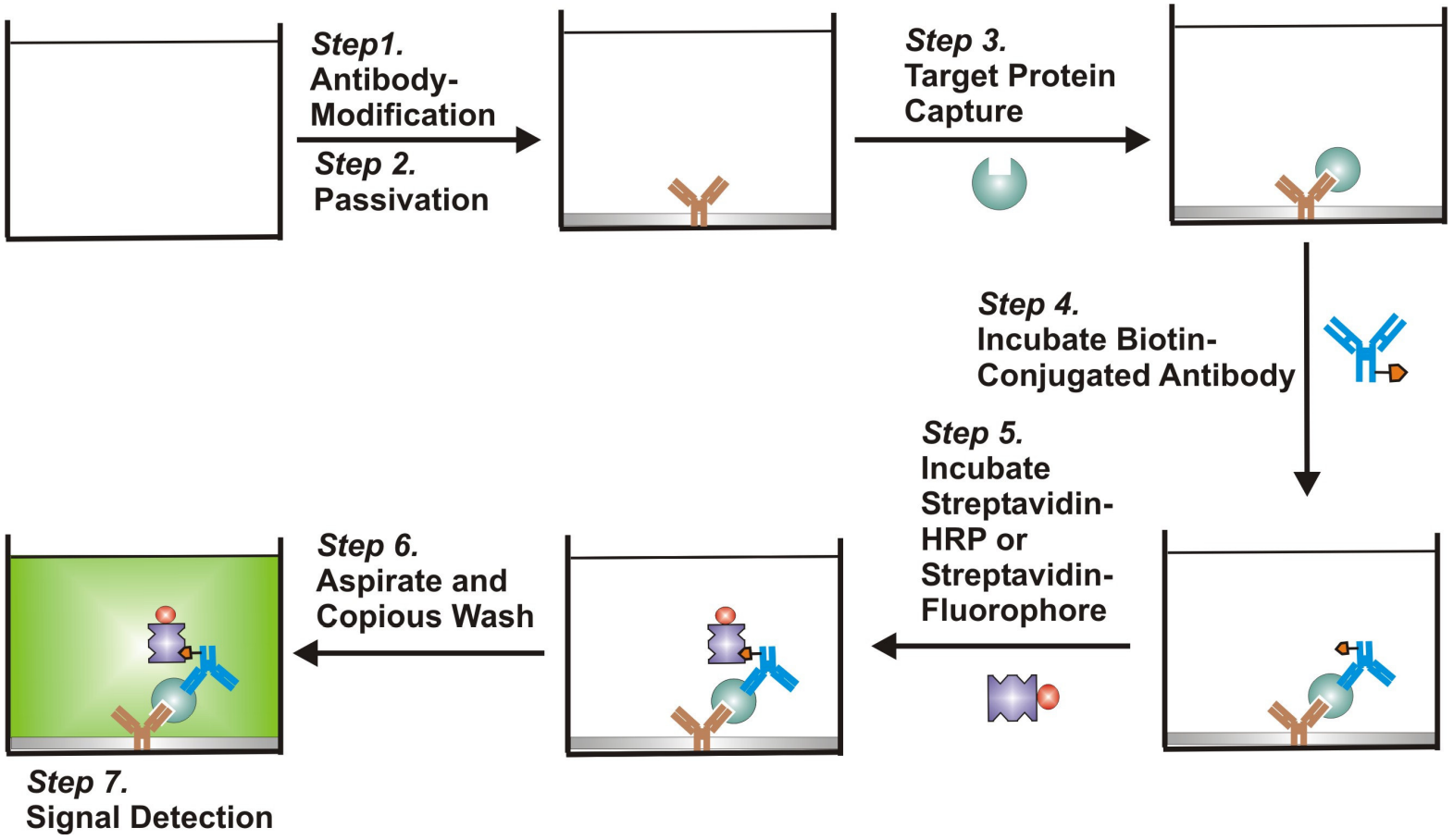
Nucleic Acid Detection:

Polymerase Chain Reaction (PCR) coupled with molecular fluorophore probes

DNA chips…

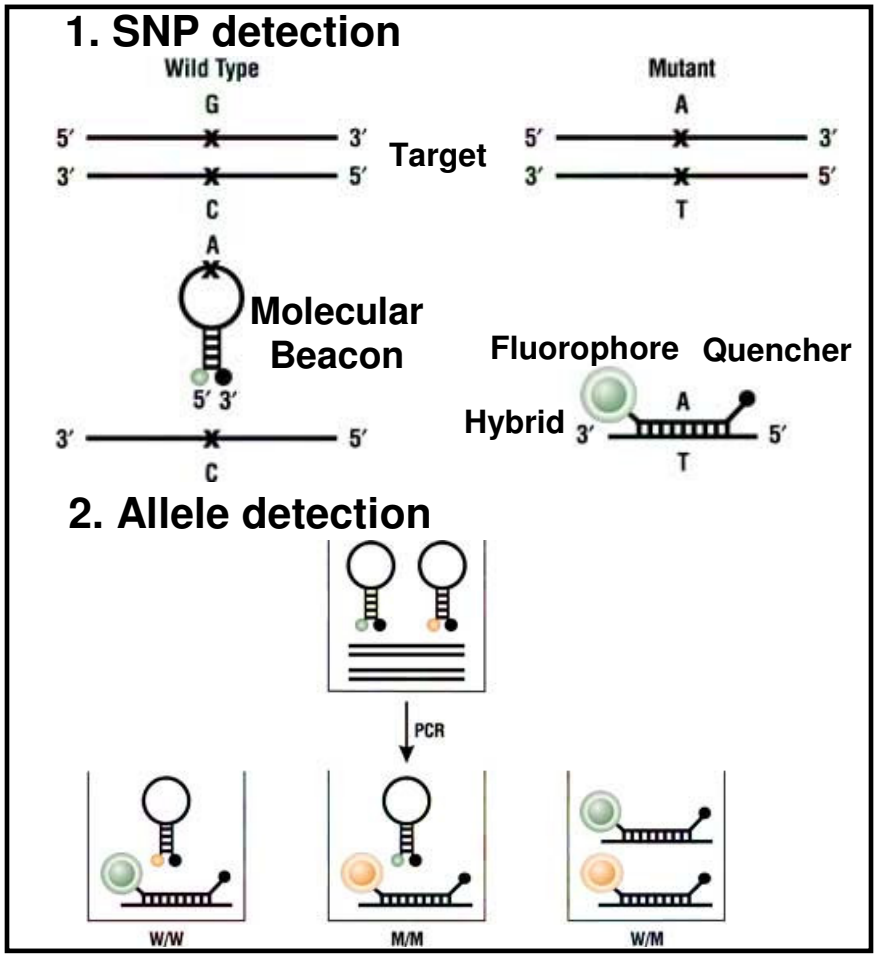
Enzyme **L**inked **I**mmuno**S**orbent **A**ssay **(ELISA)**

General detection Limit: ~ pM (~100 µL sample volume)
Limited multiplexing capability, low reproducibility, and ~3log quantification range

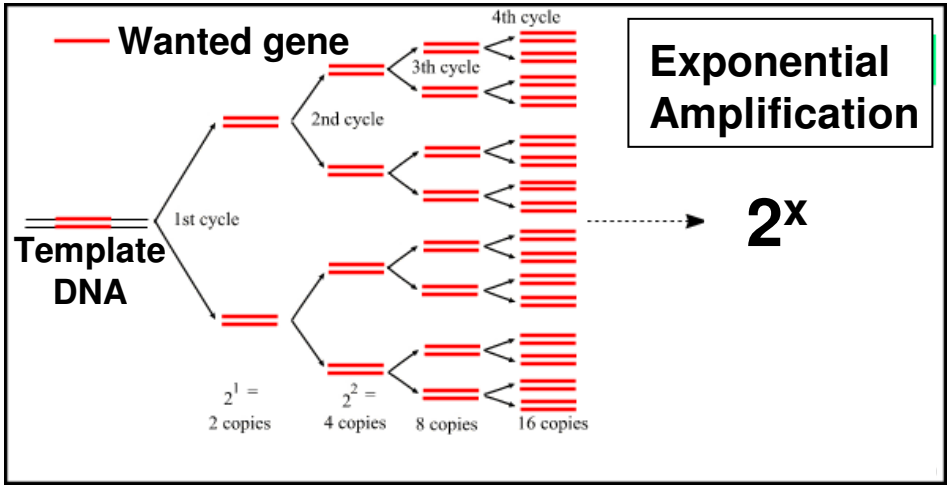


Molecular Fluorophore Method and Polymerase Chain Reaction (PCR)

Molecular Beacons



PCR



Detection limit: ~ 10 copies of DNA

Drawbacks

ELISA

Sensitivity (high fM ~ low pM)

Nonspecific Binding and Protein Denaturation on Surface

Reproducibility

Quantification

Limited Multiplexing

PCR

Complexity

Cost

Contamination

Limited Multiplexing

Selectivity

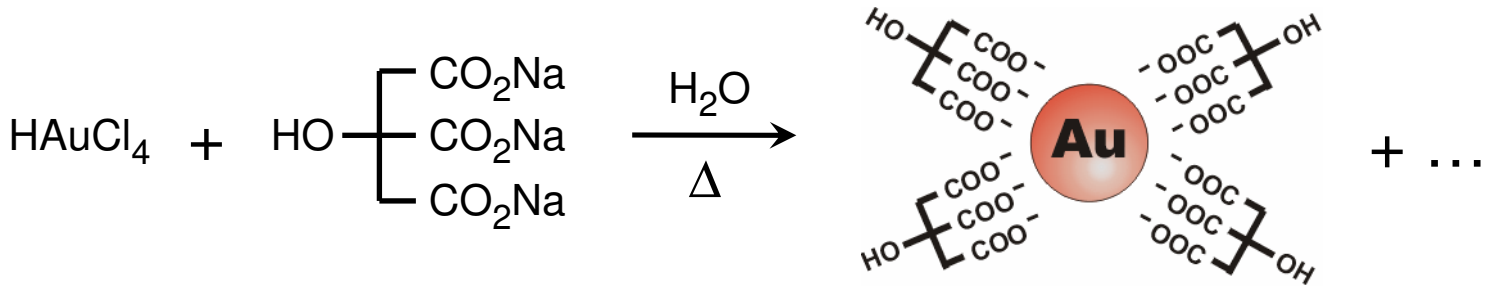
Portability

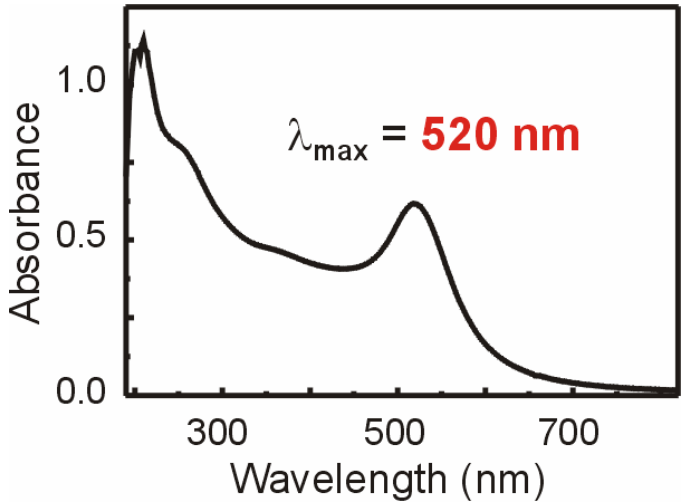
Optimization

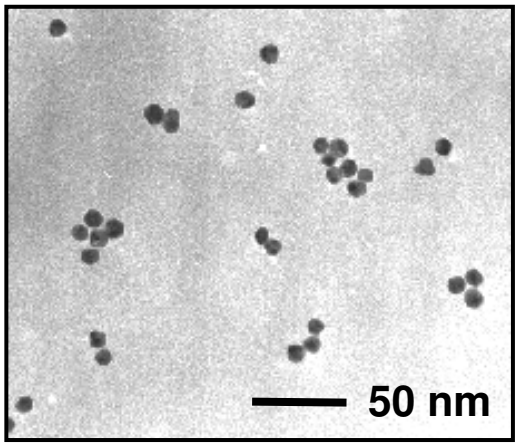
Challenges

Can we come up with a detection system that is good with respect to any bioanalytes including proteins, DNA, RNA, and small molecules and has sensitivity of PCR but does not have limitations of PCR and ELISA-based assays ?

Gold Nanoparticle Probes: Synthesis

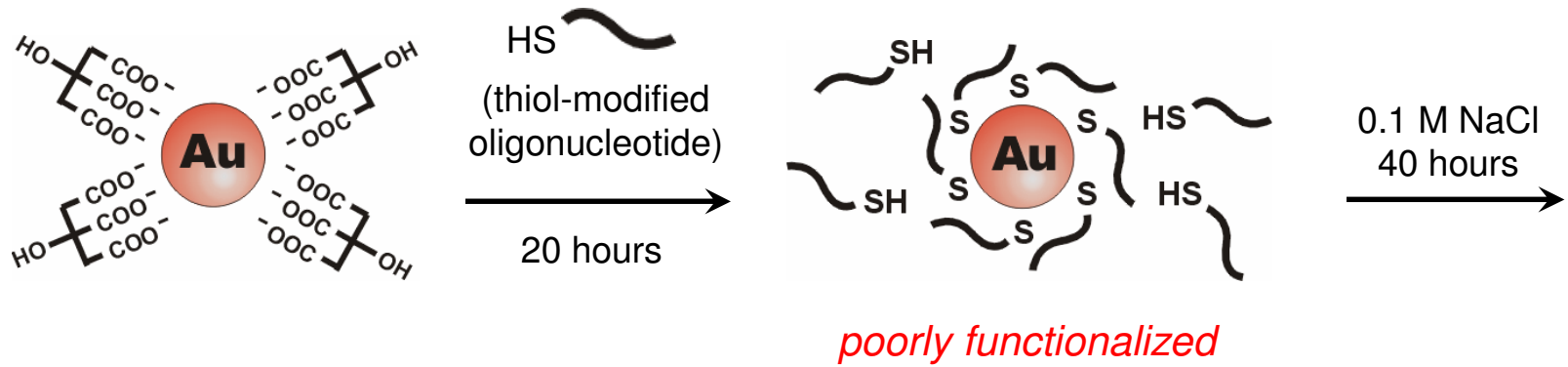




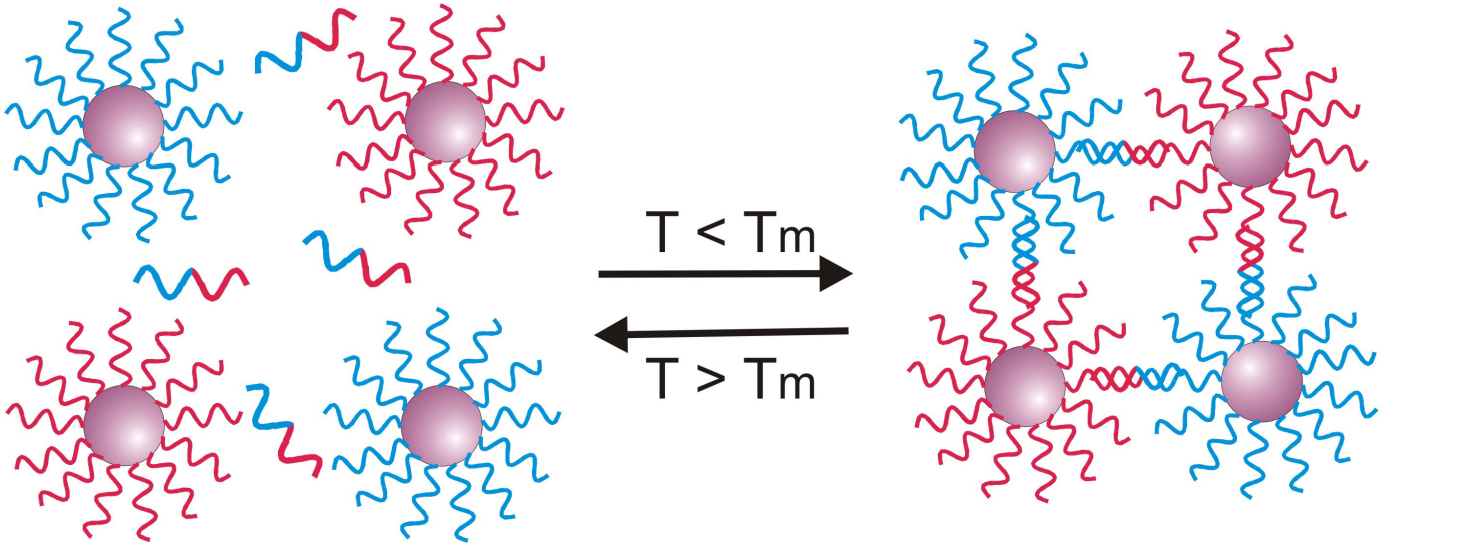


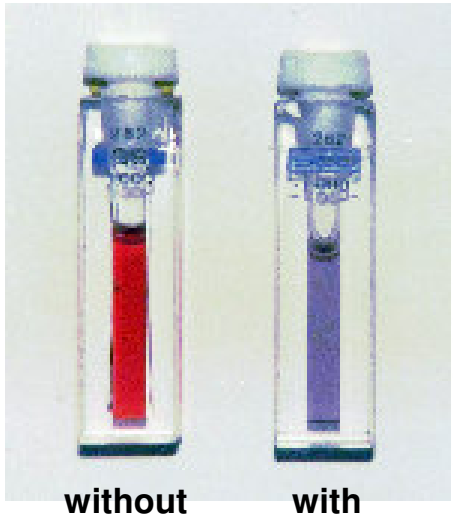
Frens, G. *Nature Phys. Sci.* **1973**, *241*, 20.

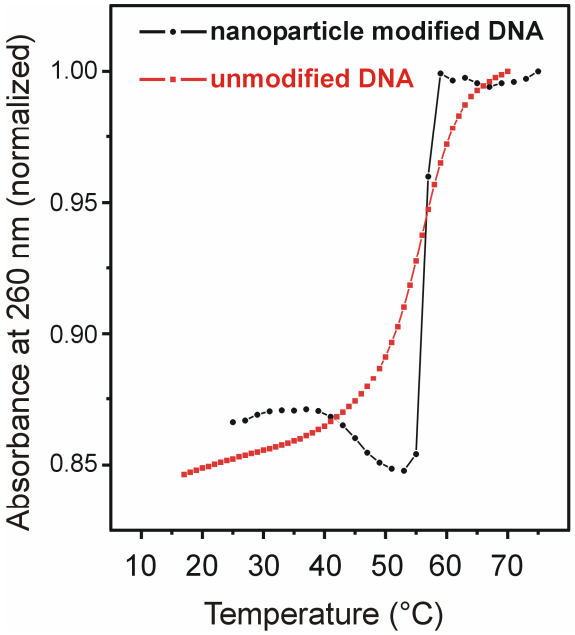
DNA-Nanoparticle Conjugates

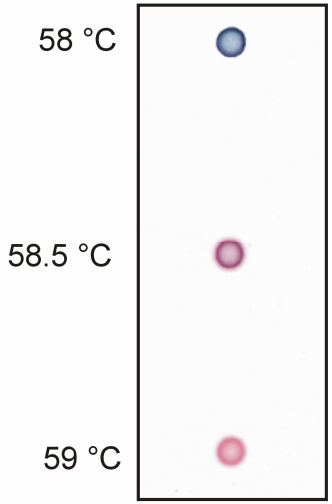


DNA-Directed Nanoparticle Assembly









C. A. Mirkin et al., *Nature* 1996

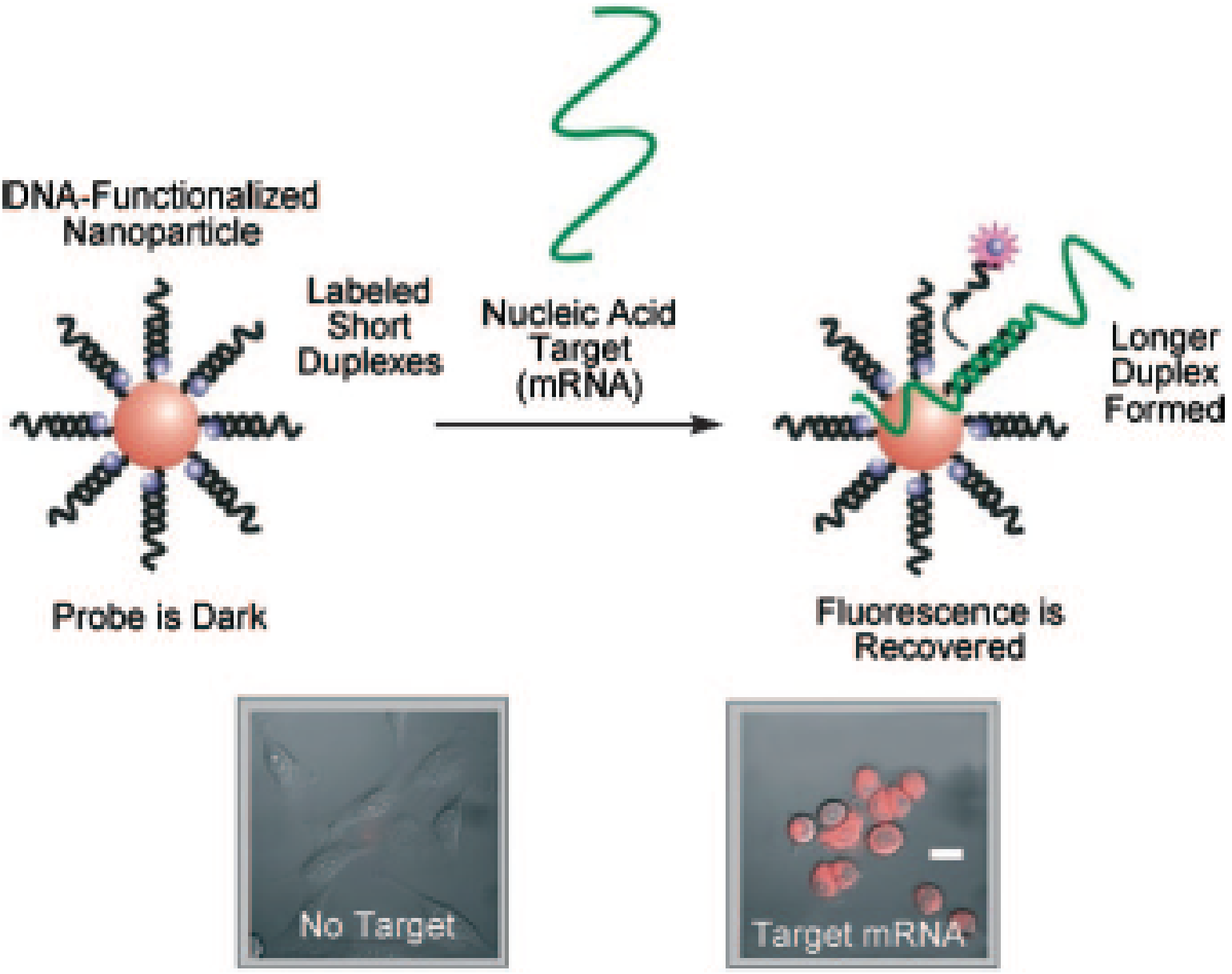
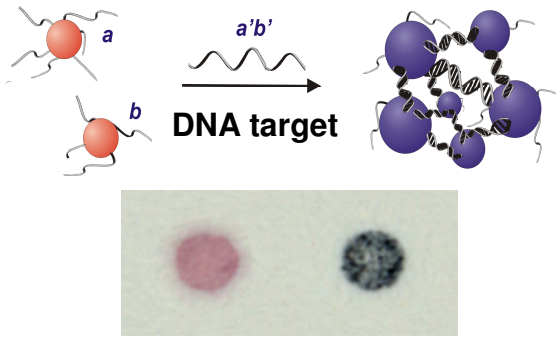


Figure 6. "Nanoflares" are gold nanoconjugates functionalized with oligonucleotide sequences complementary to a specific nucleic acid target (messenger RNA) hybridized to short fluorescent sequences. In the absence of a target the nanoflares are dark, because of quenching by the gold nanoparticle. In the presence of a target binding displaces the short flare through the formation of a longer (more energetically favorable) duplex. The result is a fluorescence signal inside the cell, which indicates the target has been detected. Scale bar: 20 μm. Adapted from Ref. [87], with permission from the American Chemical Society; Copyright 2007.

Nanoparticle-Based Biomolecule Detection Methods

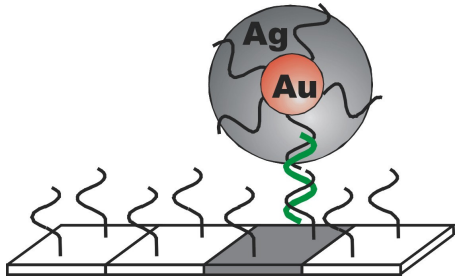


Simple & Easy

Spot test

Science, 1997

JACS, 1998

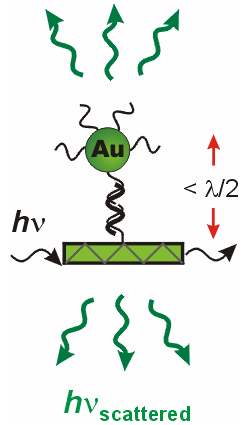


Signal Enhancement

Silver-Staining

Science, 2000: DNA

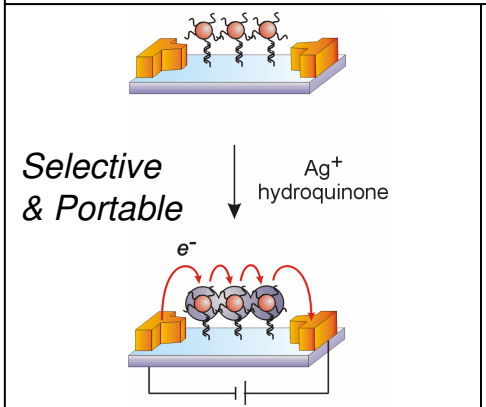
J. Poroski, **J.-M. Nam**, C. A. Mirkin, D. Appella, *Chem. Comm.* 16, 2101 (2005): PNA



Multiplexing

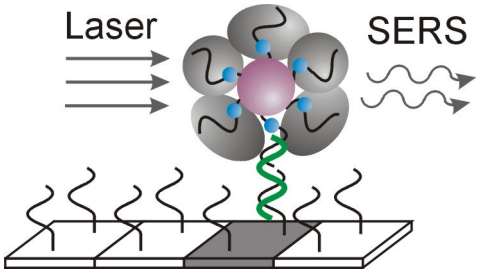
White light

JACS, 2000



Electrical

Science, 2002

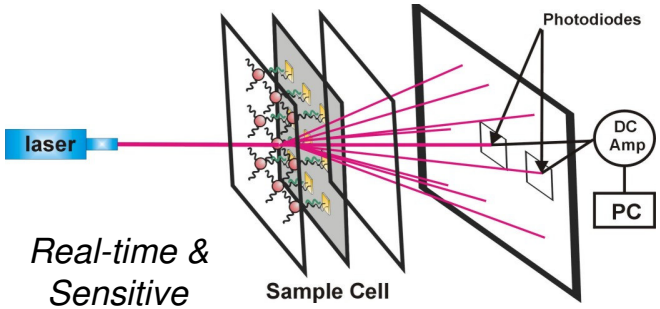


Multiplexing and Sensitivity

Raman

Science, 2002

Y. W. Cao, R. Jin, **J.-M. Nam**, C. S. Thaxton, C. A. Mirkin, *J. Am. Chem. Soc.* **125**, 14676 (2003).



Real-time & Sensitive

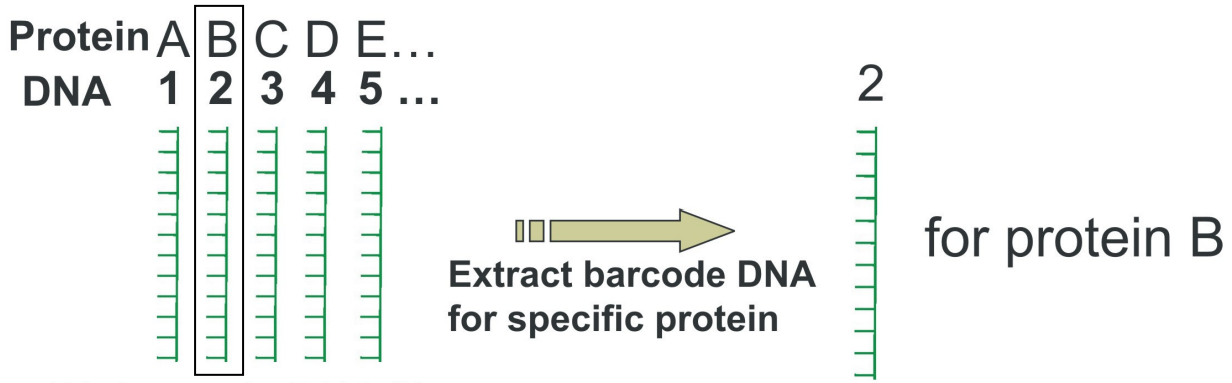
Laser & Diffraction Grating

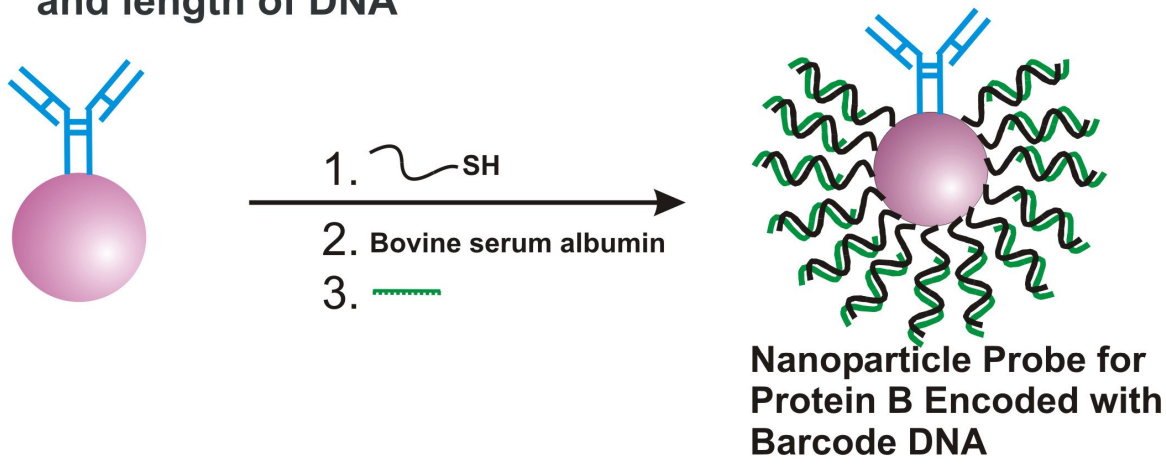
JACS, 2003

R. C. Bailey, **J.-M. Nam**, C. A. Mirkin, J. T. Hupp, *J. Am. Chem. Soc.* **125**, 13541 (2003).

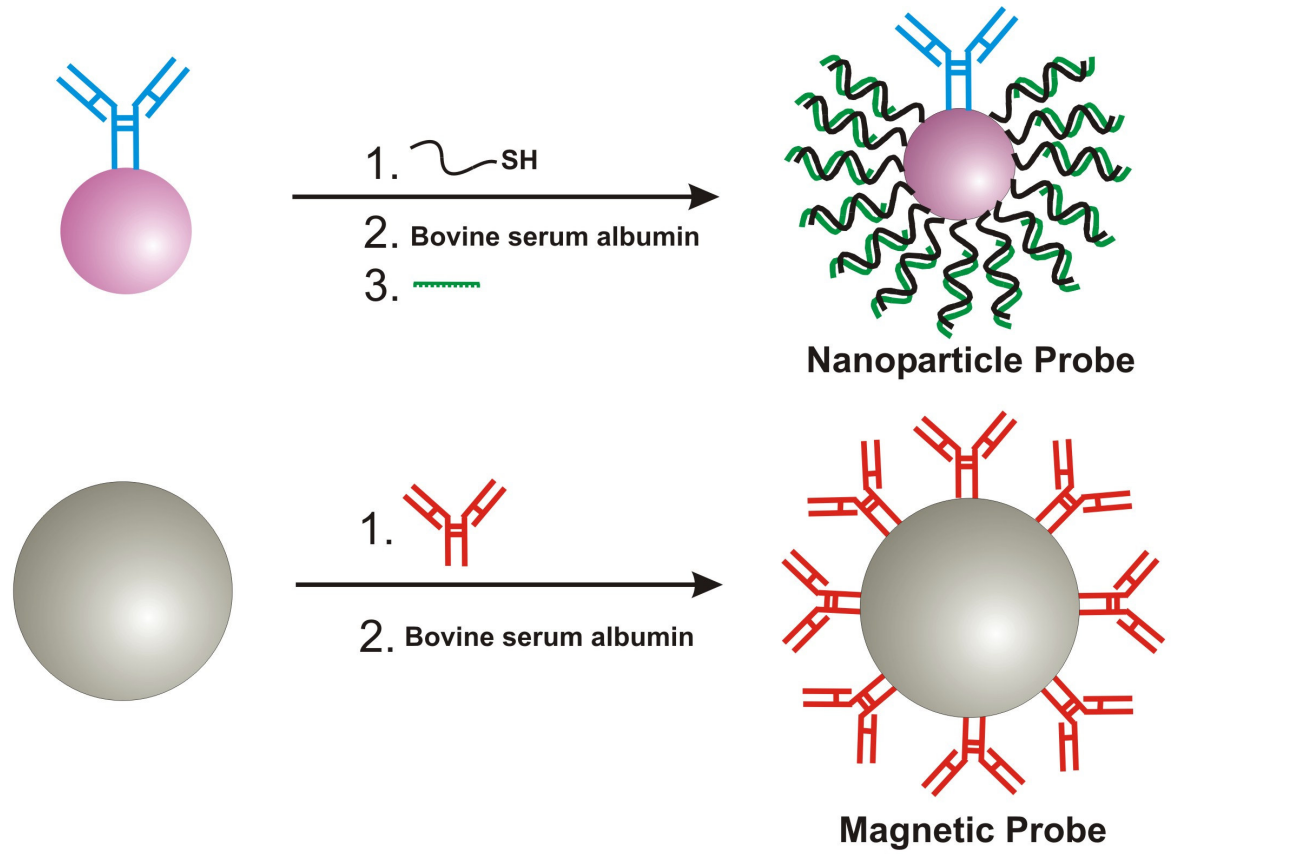
None thus far reached PCR-like sensitivity (~ 10 copies in entire sample)

Bio-Bar-Code Amplification Assay

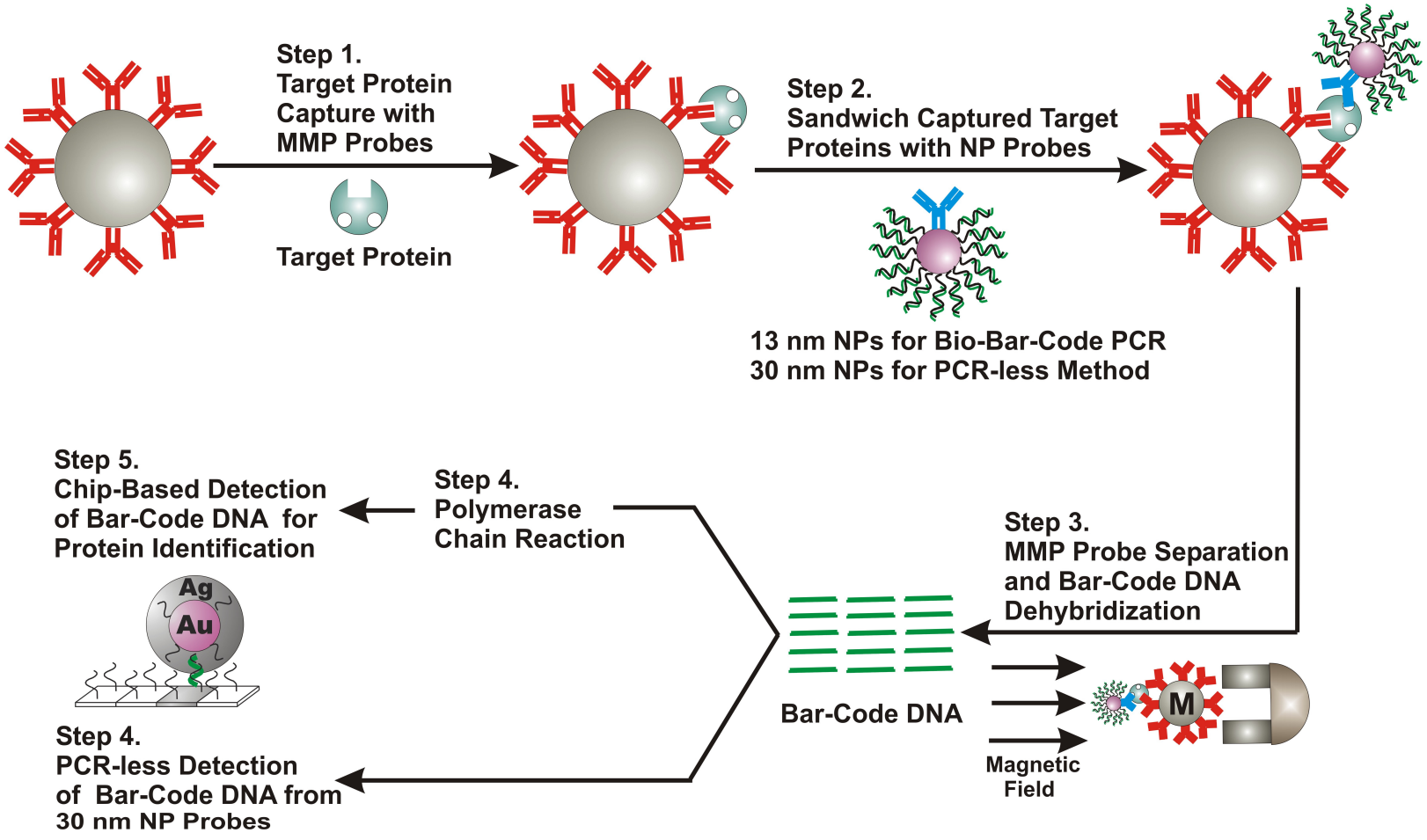




Probe Design and Preparation

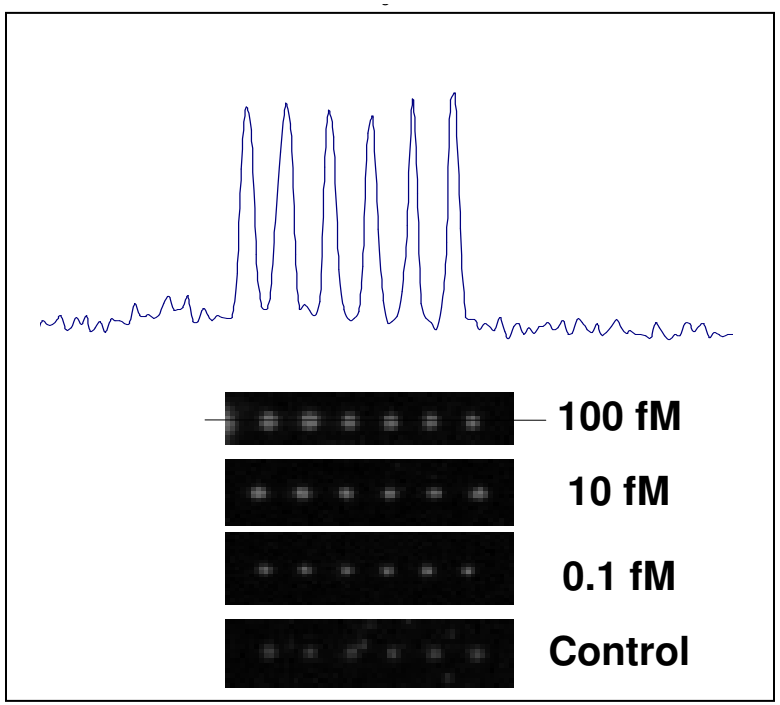


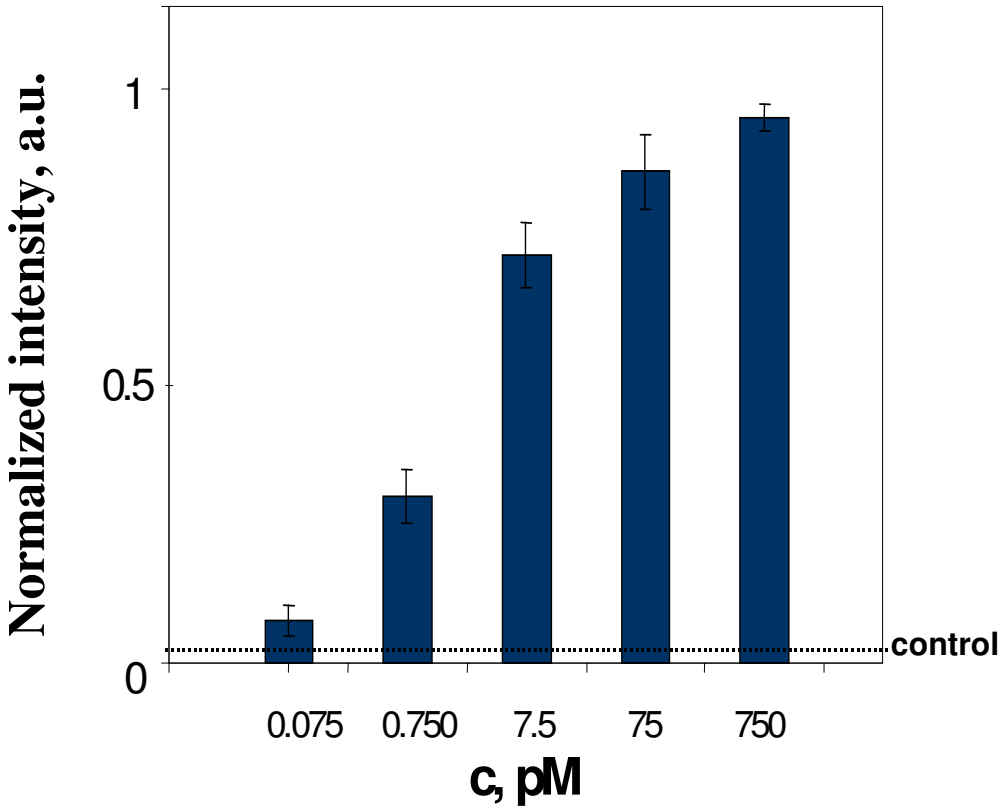
Protein Detection and Bar-Code DNA Amplification/Identification



Jwa-Min Nam, C. Shad Thaxton, Chad. A. Mirkin, ***Science*** 301, 1884 (2003).

Scanometric Response of Bar-Code DNA

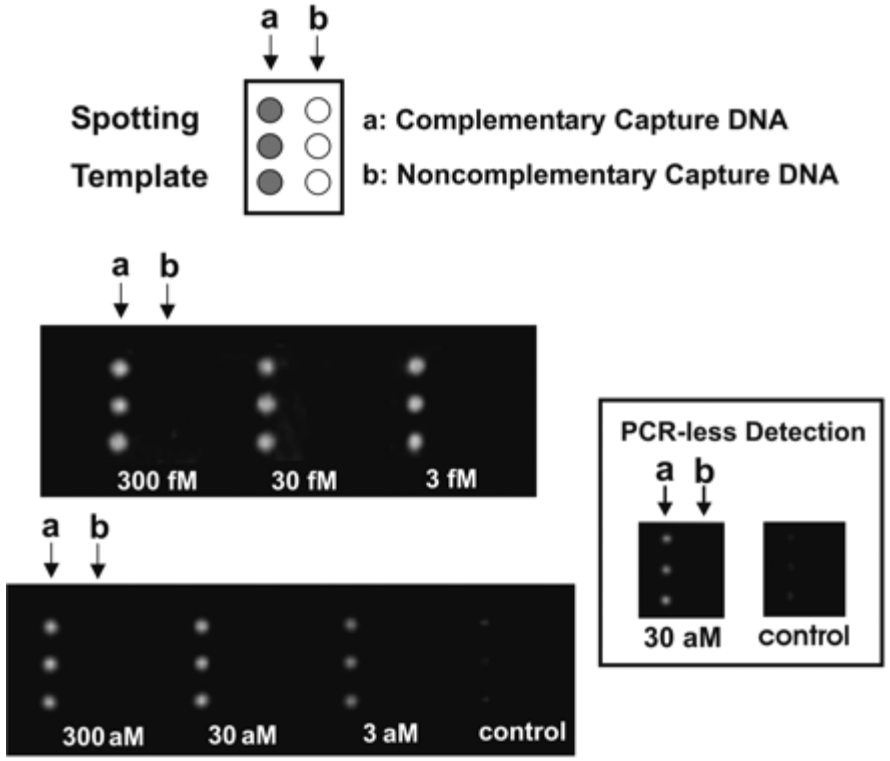


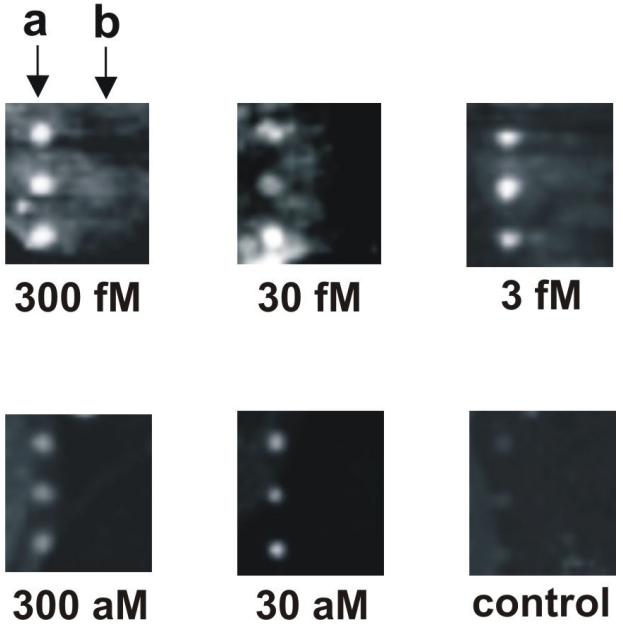


Detection limit: ~ 400 probes

Storhoff, J. J. et al., *Biosensors and Bioelectronics* **2004**, *19*, 875.

PSA in 0.1 M PBS solution with background proteins after PCR (total volume: 10 μl)





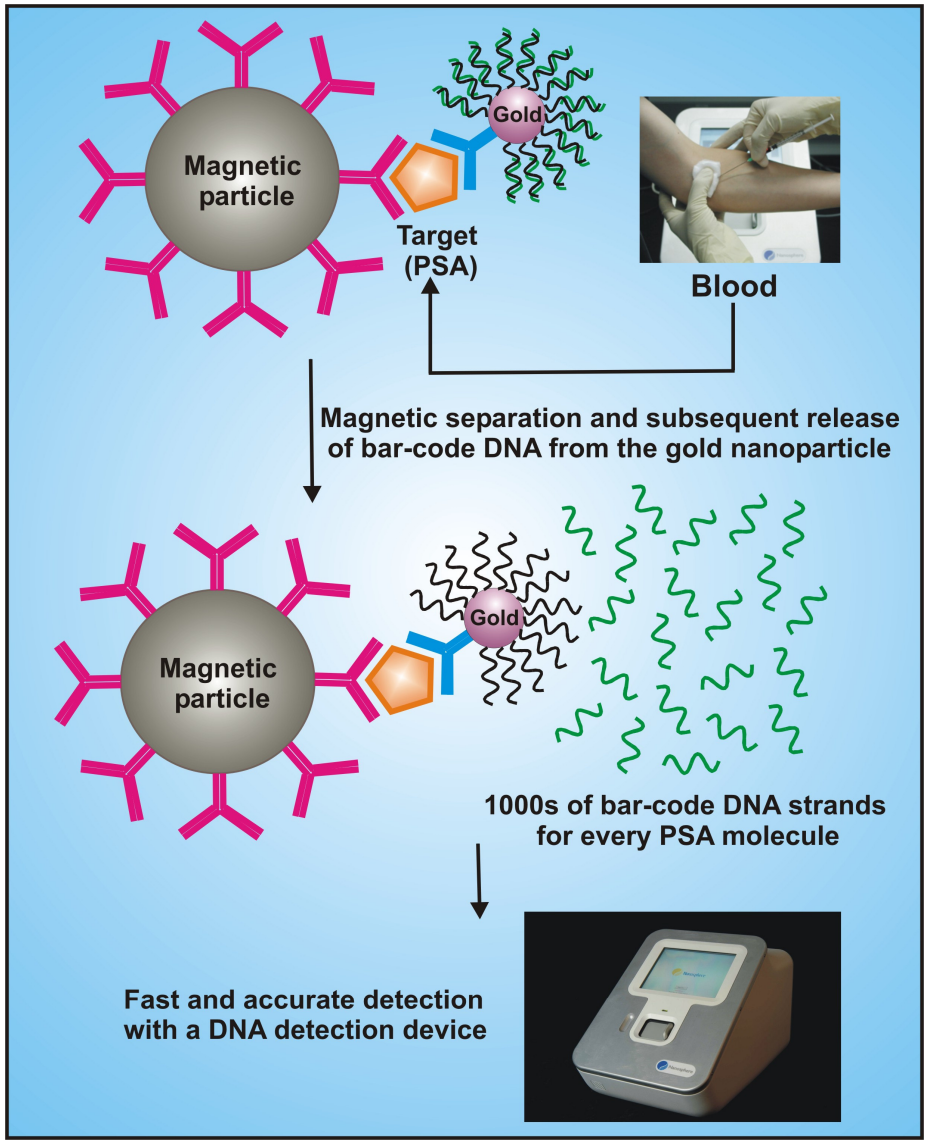
PSA in goat serum (total volume: 10 μl)

In buffer solution

10^6 more sensitive than conventional PSA assays
(~ 20 copies of proteins in entire sample)

***still need PCR and signal saturated from 3 fM**

Nam, J.-M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**



The bio-bar-code assay works because…

- The target binding portion of the assay is homogeneous.

- The use of a large number of bio-bar-code-loaded NP probe provides a high ratio of bar-code DNA to labeling antibody that greatly increases assay sensitivity.

-Both gold nanoparticle probes and magnetic particles are dynamically interacting

- Synthetic DNA is easier to detect on a chip than proteins.

- Large concentrations of the probes can be used to efficiently bind protein targets and to reduce the time required for high sensitivity detection experiments (large number of probes and large number of antibodies per probe).

- Silver enhancement on immobilized gold nanoparticles.

- Accurate and sensitive chip reader that measures scattered light from developed silver spots.

- Magnetic separation is quick and effective.

Detection Limits of Protein Assays (Chemical Review 2005)

	assay	target	protein in saline	protein in serum
nanostructure-based methods	optical[72] (Au nanoshells)	rabbit IgG	0.88 ng/mL (~4.4 pM)[a]	0.88 ng/mL (~4.4 pM)[a]
	optical[74] (Au nanoparticles)	IgE and IgG1	~20 nM	
	magnetic relaxation[98] (iron oxide nanoparticles)	adenovirus (ADV) and herpes simplex virus (HSV)	100 ADV/ 100 uL	50 HSV/ 100 uL
	scanometric[79] (Au nanoparticles with Ag amplification)	mouse IgG	200 pM	
	Raman[82] (Au nanoparticles with Raman labels)	prostate-specific antigen		30 fM
	surface plasmon resonance[83,84] (triangular Ag particles on surfaces)	streptavidin(S A) and anti-biotin (AB)	~1 pM SA and ~700 pM AB	
	electrical[110] (single-walled carbon nanotubes)	10E3 antibody to U1A RNA splicing factor	~1 nM	
	electrical[20] (Si nanowires)	streptavidin	10 pM	
	bio-bar-code amplification[75] (Au nanoparticles with Ag amplification)	prostate-specific antigen	30 aM (3 aM)[b]	(30 aM)[b]
molecular fluorophore methods	enzyme-linked immunosorbent assay	various	pM range	pM range
electrochemical methods	electrochemical amplification[137] (oligonucleotide reporter molecules)	IgG	13 fM	
enzyme-based amplification methods	immuno-PCR[76]	bovine serum albumin	2 fM	
	rolling circle amplification[77]	prostate-specific antigen	3 fM	

The Bio-Bar-Code Assays for Protein Targets

PSA (Prostate & Breast Cancer Marker)

Our sensitivity: 30 aM

Benchmark Sensitivity: ~ 3 pM

Other Sensitivity: ~ fM (Immuno-PCR)

*** Tested in serum samples**

***Science* 2003**

ADDL c-Tau (Alzheimer's Disease Markers)

Our sensitivity: 8 aM (ADDL) and 80 aM (c-Tau)

Benchmark Sensitivity: 100 pM (ADDL) & 0.2 pM (total Tau)

***Never been detected in human CSF samples before**

***PNAS* 2005:**

2nd Most Downloaded PNAS Paper in Feb 2005

p24 (HIV Marker)

Our sensitivity: 6.3 aM

Benchmark Sensitivity: ~ 420 fM

Other Sensitivity (PCR-Based RNA Test): ~ 50 copies/ml

***Tested in blood samples (sensitivity: ~ 22 RNA copies/ml)**

Nature Biotechnol.

To be submitted

Prion (Mad Cow Disease Marker)

Our sensitivity: 100 aM (fluorophore-based method)

Benchmark Sensitivity: ~ pM

***Never been detected in real Mad Cow samples**

Unpublished result

Cardiac Troponin I (Heart Disease Marker)

Our sensitivity: 50 aM

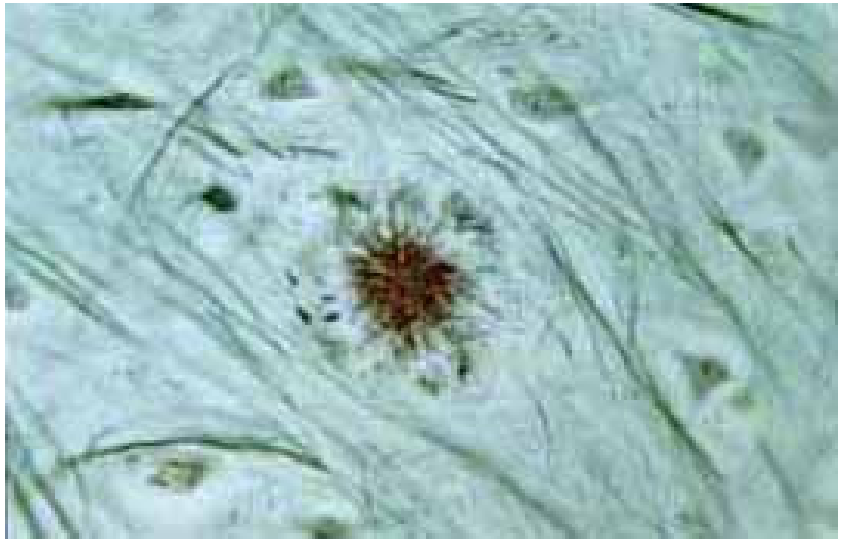
Benchmark Sensitivity: ~ 42 pM

Other Sensitivity: 3.35 pM

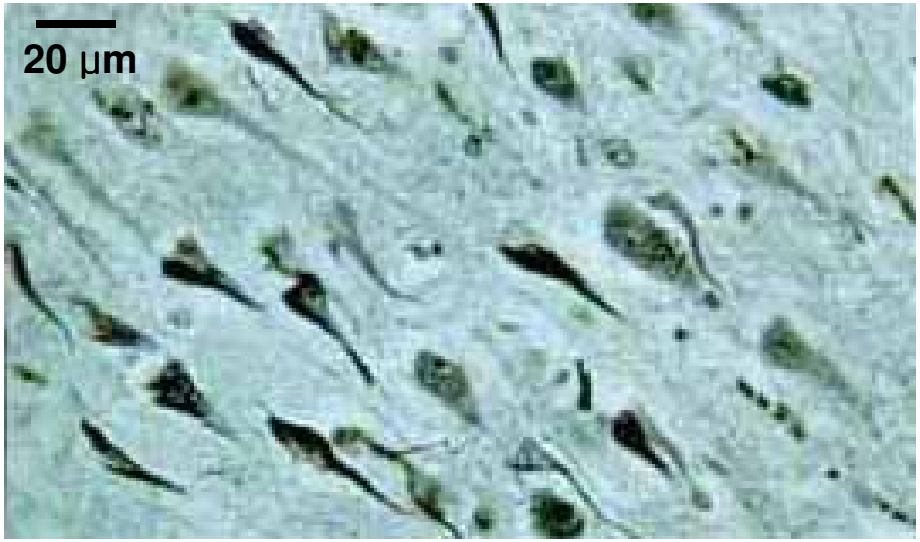
***Routinely used in Emergency Room (~ 1 billion dollar market)**

Unpublished result

Hallmarks of Alzheimer's Disease (from brain slices)

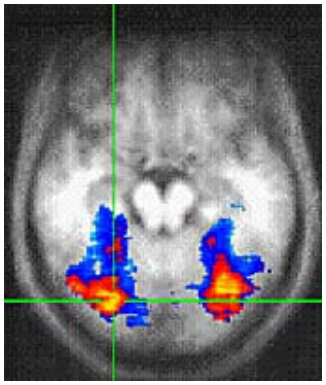


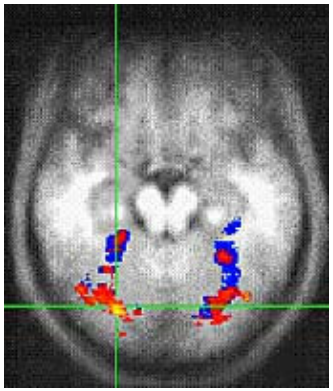
Plaques (CERAD)
The brain of the Alzheimer's patient will show accumulations of a normal body-protein - beta-amyloid - which has been transformed into a form which is toxic to the brain. This transformed beta-amyloid is found between the nerve-cells of the brain and becomes surrounded by the remnants of the cells which it has killed forming so-called plaques.



Tangles (Braak & Braak)
The other marker of Alzheimer's Disease is known as a Neurofibrillary Tangle. This tangle is caused by the build-up of a protein called Tau inside the nerve-cells of the brain.

Current Diagnostic Method: Visual Encoding





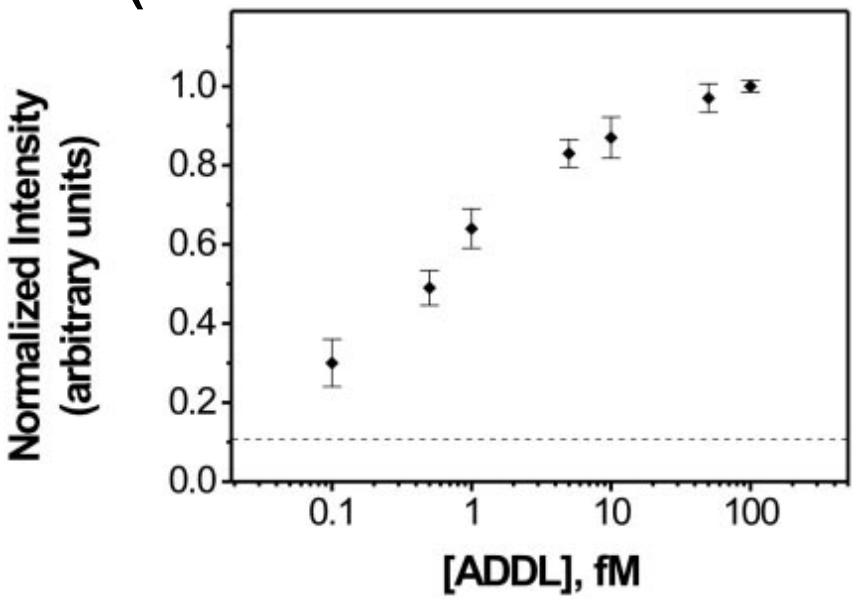
In this process the patient first lies down on a platform with an electrical device around his or her head and then is gently pushed inside the MRI machine. The patient can then look at a screen on which various pictures are flashed up – in this case, a series of repeated images of landscapes and street-scenes, interspersed every now and then with completely new and unfamiliar images. Meanwhile the MRI machine can take cross-sectional photographs – "slices" – of the person's brain whilst they are carrying out these tasks - a process known as *Visual Encoding*.

In Alzheimer's patients, there appears to be much less activity in this area (see figure below) than in healthy people (see figure above)

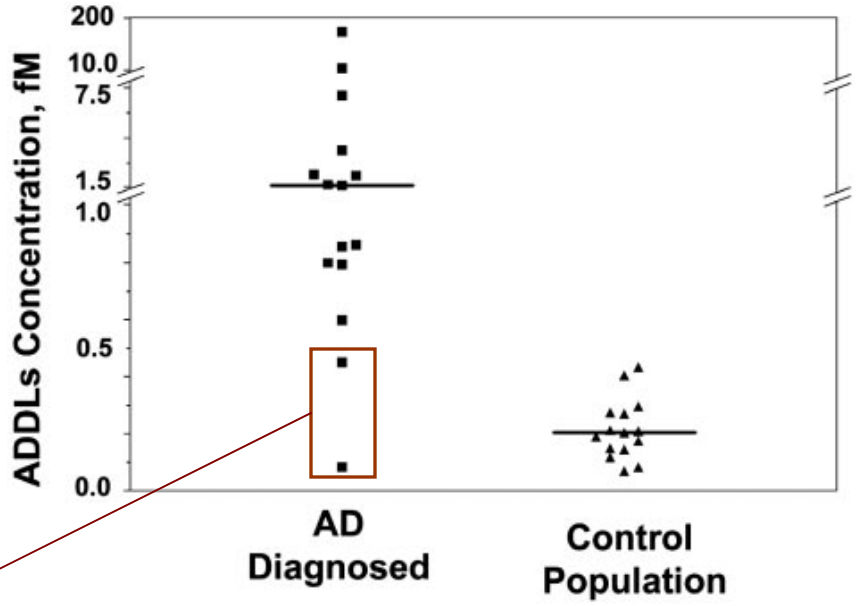
And "the localized surface plasmon resonance method for ADDL detection" by Van Duyne Group (Nano. Lett. 2004, ASAP Article)

ADDL & AD

ADDLs in buffer
(Detection Limit: ~500 ADDLs)



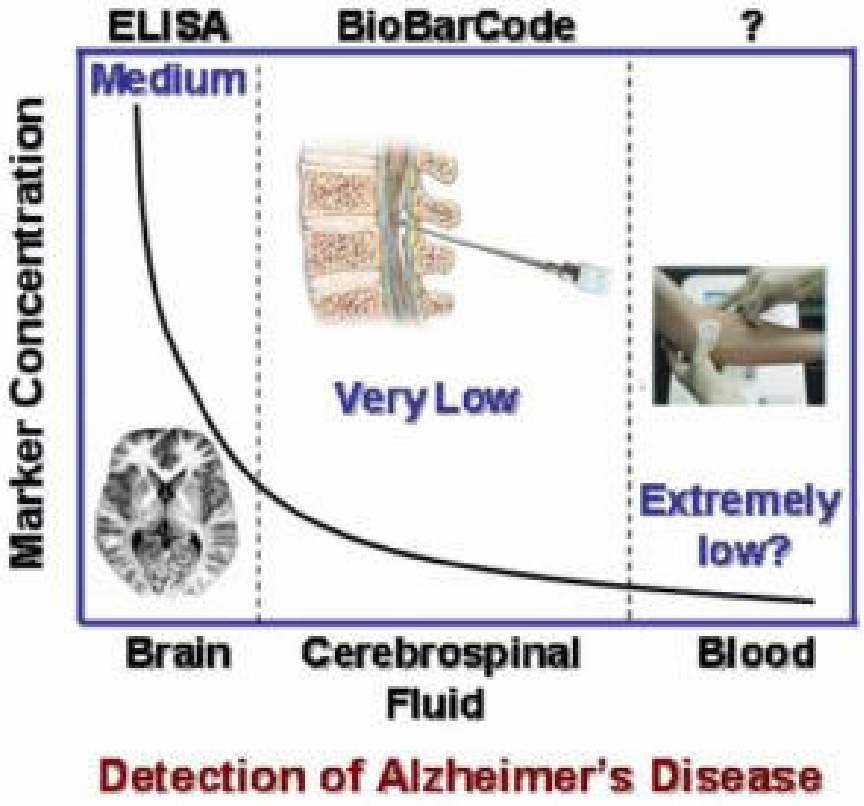
ADDLs in Clinical Subjects



One subject was an aged male that scored very high on the Mini Mental State Examination (MMSE), which is inconsistent with the later pathological stages of the disease, whereas the other subject had pathological signs of infarctions in addition to AD. These two AD subjects that are outliers in our assay could be false positives of current diagnostic methods that consider both clinical and pathological data.

The assays for control subjects showed a consistently lower level of ADDLs than the assays for subjects diagnosed with the disease.

Bio-Barcode-Based Alzheimer's Disease (AD) Diagnosis



"The first example of human fluid-based AD diagnosis"

The association of Alzheimer's Disease with a protein in the brain called amyloid-beta-derived diffusible ligand, or ADDL.

Detection of ADDL in spinal fluid.

Samples of the spinal fluid of 30 people, 15 who had Alzheimer's disease and 15 who did not.

Assay results show that at least some ADDL in all the patients, which is an indication that everyone may have a baseline level of the protein.

The concentration of ADDL increases as the disease gets worse, so the progression of the illness could be followed.

Proc. Natl. Acad. Sci. USA **102**, 2273, (2005): **From the Cover**

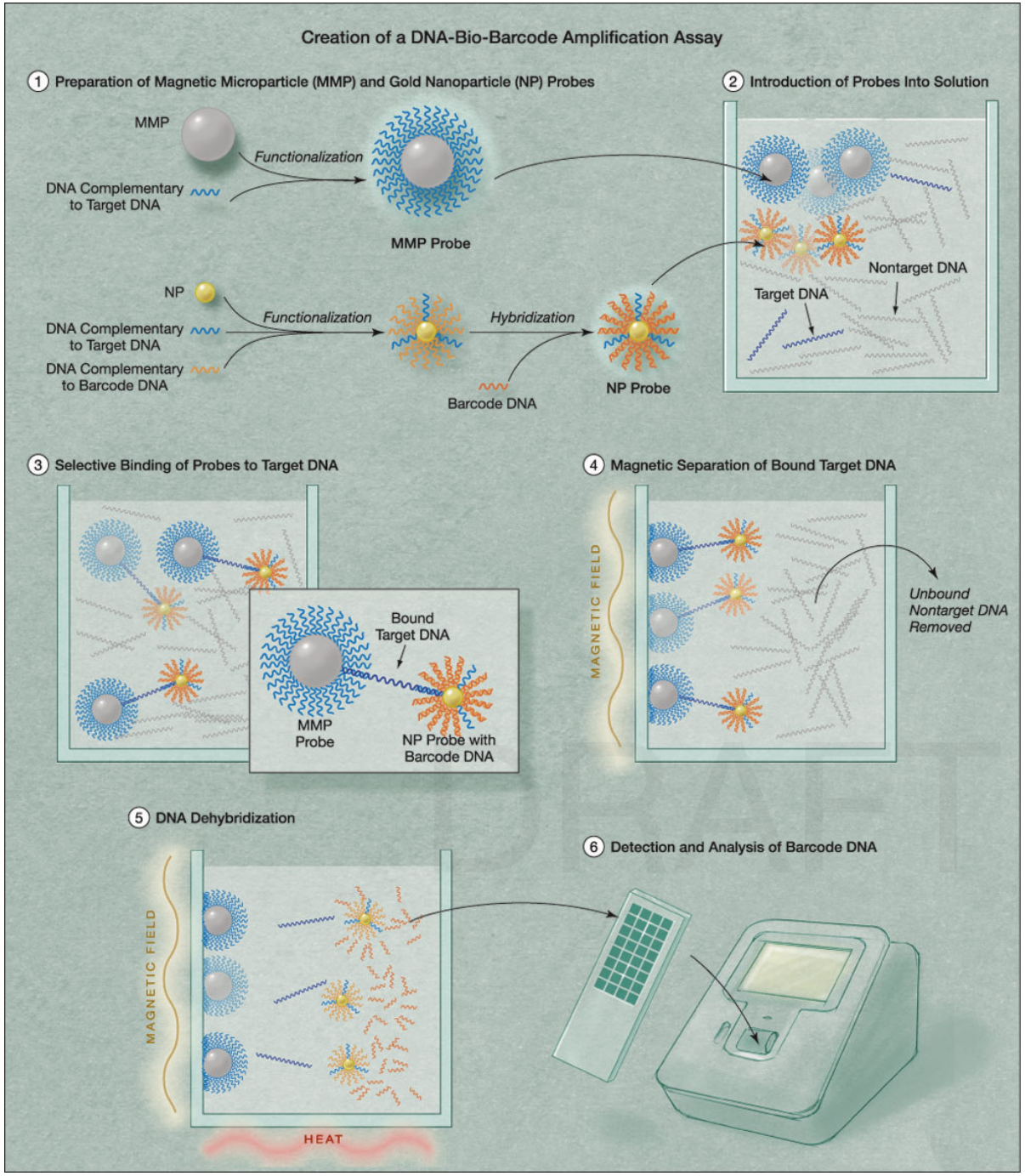
Cover Story, Chemical & Engineering News (2005)

Associated Press (AP) Feb 22, 2005 (CNN, ABC, NBC, CBS, Fox, USA Today, Forbes, NY Times, LA Times, Washington Post, BBC, Business Week, and 2005 NSF Congress Hearing)

Bio-Bar-Code Amplification Assay for DNA

Objectives

- PCR-like sensitivity with bio-bar-code method for DNA detection without the need for enzymes (simple but sensitive).
- Quantitative assay with wide detecting range
- High Selectivity (Differentiate Single Base Mismatch)
- With less effort, cost, and time



Journal of American Chemical Society, 2004, *126*, 5932.

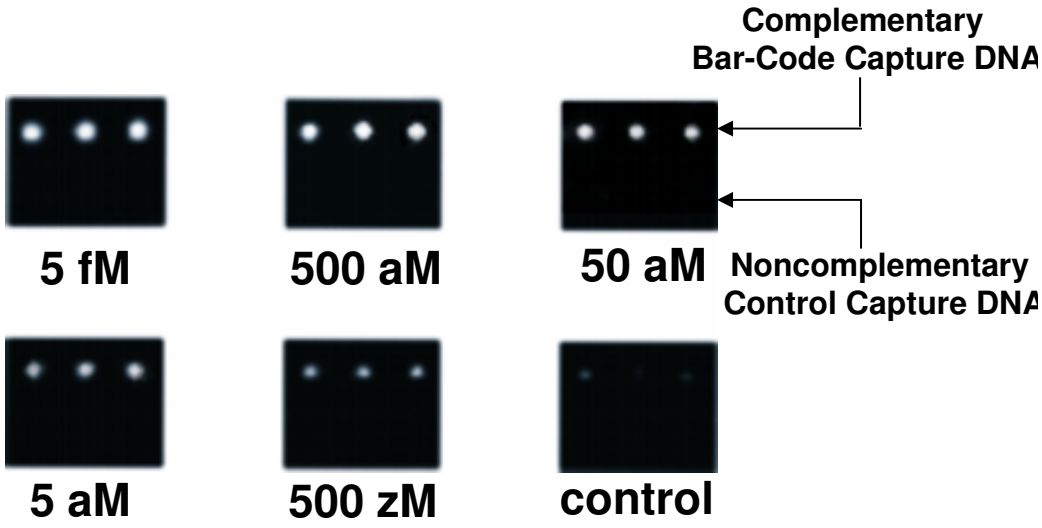
Journal of American Medical Association, 2004, *292*, 1291.

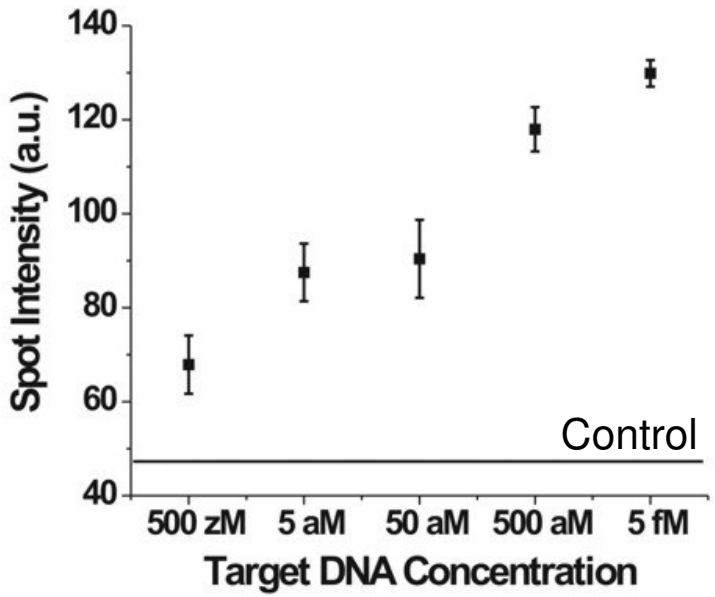
Journal of Chemical Education, 2004, *81*, 1386.

Chicago Tribune, Bar-Code Technology Tapped for DNA Tests (April 28, 2004).

The New York Times, Tiny is Beautiful: Translating 'Nano' into Practical (Feb 22, 2005).

Bio-Bar-Code-Based DNA Detection with 30 nm NP Probes





ME-Fluoroscence Measurement: actual ratio is 70(Bar):1(Tar)

~360 DNA strands on each 30 nm NP

Overall, stronger signals and higher sensitivity than 20 nm probes.

Detection limit is 500 zM (~10 target DNA strands)

*** The detection limit of scanometric method: ~ 50 fM**

*** The detection limit of fluorescence microscope (Tan et al; JACS 2003): ~ 800 aM**

Single base mismatch (SNP)

At 500 aM target DNA concentration
(after incubating at 45 °C for 3 min)

G Mismatch **Perfect Match**

G Mismatch: 5´GGATGATTGTTAAATGATAAGGAT 3´

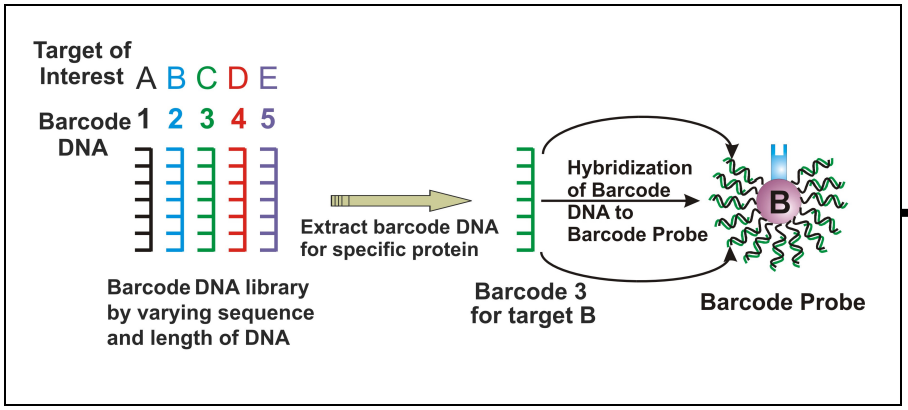
Perfect Match: 5´GGATTATTGTTAAATGATAAGGAT 3´

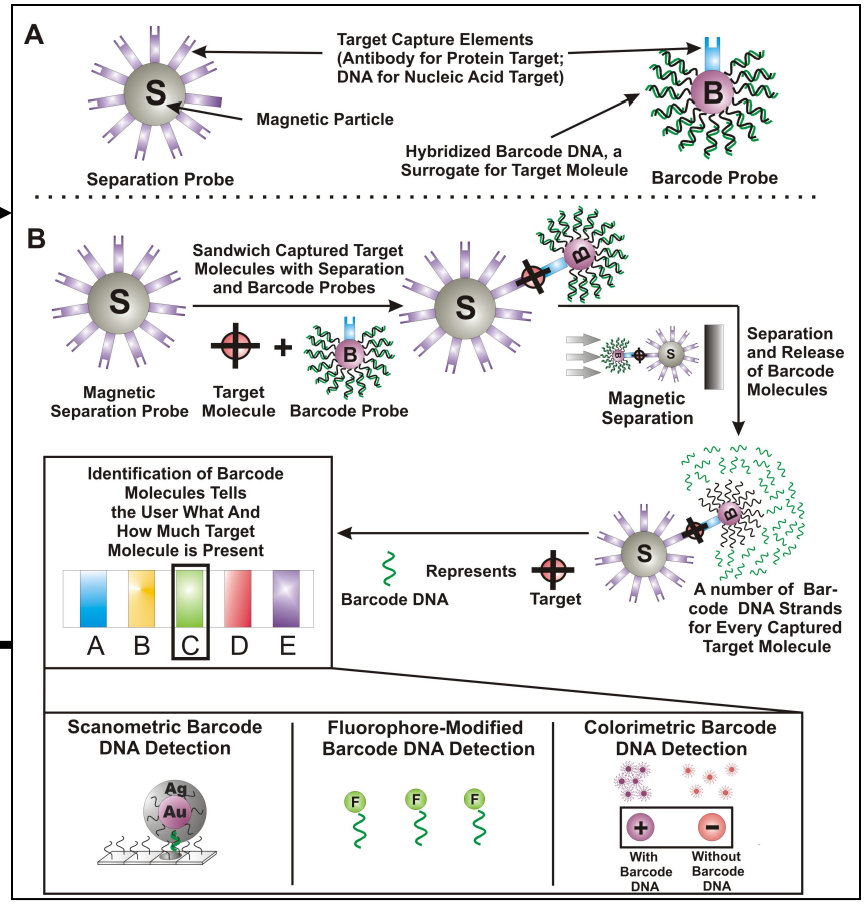
Detection Limits of Nucleic Acid Assays (Chemical Review 2005)

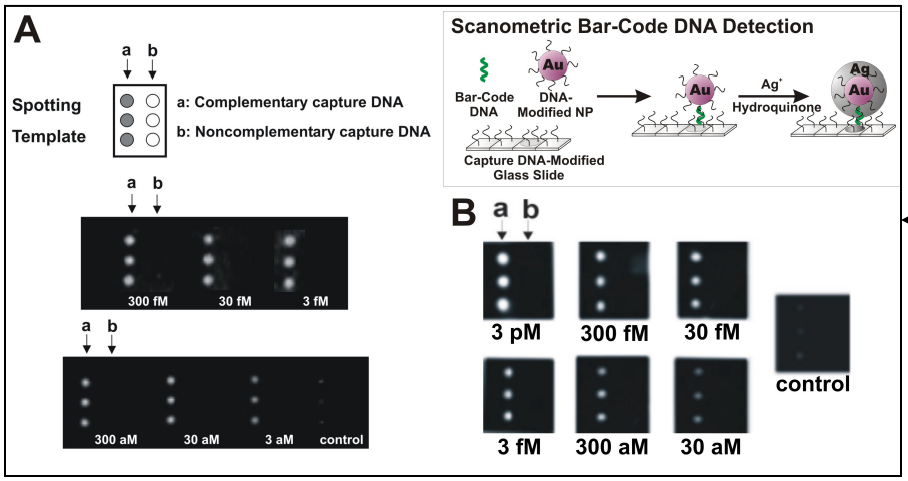
	assay	ss DNA	PCR products	genomic DNA
nanostructure-based methods	colorimetric[29] (cross-linked Au nanoparticles)	~10 nM		
	colorimetric[36] (non-cross-linked Au nanoparticles)	60 nM		
	magnetic relaxation[97] (iron oxide nanoparticles)	20 pM		
	electrochemical[96] (nanoparticles)	270 pM		
	scanometric[35,66,67] (Au nanoparticles with Ag amplification)	50 fM	100 aM[b]	200 fM
	Raman spectroscopy[68] (Au nanoparticles with Ag amplification)	~1 fM		
	electrical[93] (Au nanoparticles with Ag amplification)	500 fM		
	electrical[99] (Si nanowire)	10 fM		
	electrical[103] (carbon nanotube)	54 aM		
	resonant light-scattering[61-66] (metal nanoparticles)	170 fM[b]		33 fM
	fluorescence[56] (ZnS and CdSe quantum dots)	2 nM		
	surface plasmon resonance[41] (Au nanoparticles)	10 pM		
	quartz crystal microbalance[94] (Au nanoparticles)	~1 fM		
	laser diffraction[42] (Au nanoparticles)	~50 fM		
	fluorescence[45] (fluorescent nanoparticles)	~1 fM		
	bio-bar-code amplification[71] (Au nanoparticles with Ag amplification)	500 zM		
other non-enzymatic based methods	fluorescence[35] (molecular fluorophores)		~600 fM[b]	
	fluorescence (dendrimer amplification)[134]		2.5 ug	
	electrochemical amplification[136] (electroactive reporter molecules)	100 aM		

Bio-Barcode Amplification Assay

***Target molecules can be proteins, DNA, RNA, metal ions, etc.**







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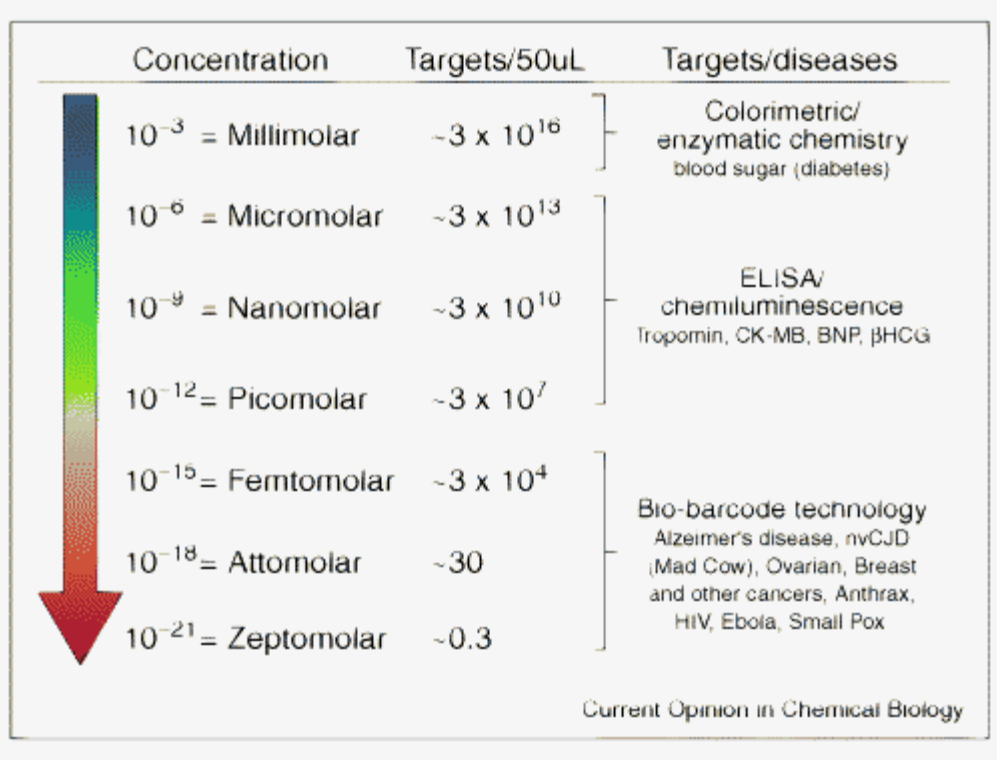
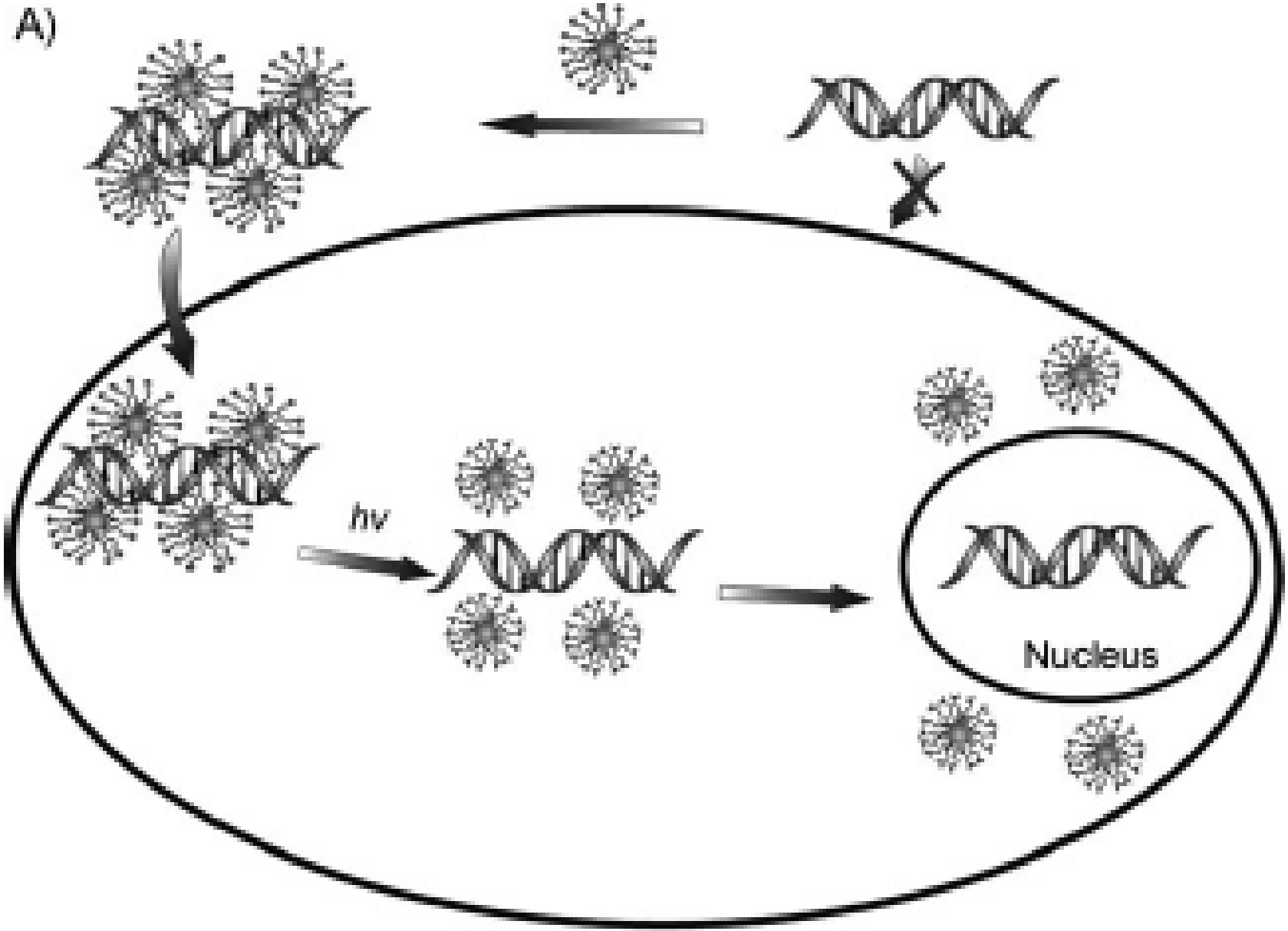
Biomolecule detection technology



The bio-barcode assay provides access to a target concentration range well below that of conventional ELISAs. This ultra-sensitivity provides the ability to utilize new markers for disease screening in biodiagnostics.

Current Opinion in Chemical Biology, **10**, 11-19 (2006)


A)
hv
Nucleus
Positively charged function
Negatively charged function
Photocleavable linker