Nanoparticle-Based Bio-Bar Codes for the Ultrasensitive Detection of Proteins

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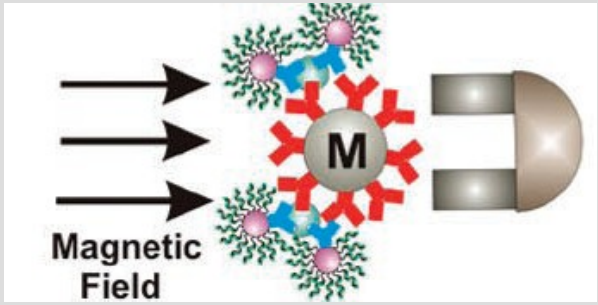


Overview

- Motivation/Introduction
- Experimental Design
- Results
- Conclusions
- Impact/Future work

Motivation

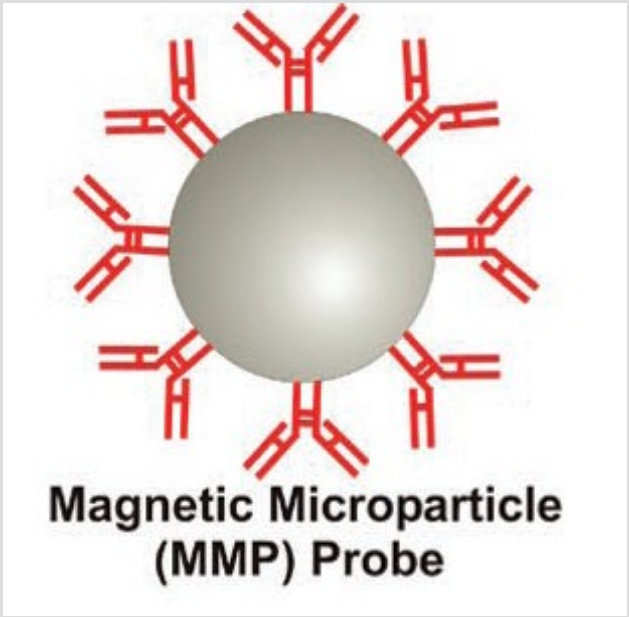
- Cancer screening and diagnostics – need for ultrasensitive protein detection
- PSA (Prostate Specific Antigen) is biomarker of breast and prostate cancer
- Ultrasensitive test could be used for screening and diagnosis
- Can amplify small amounts of DNA, but not protein

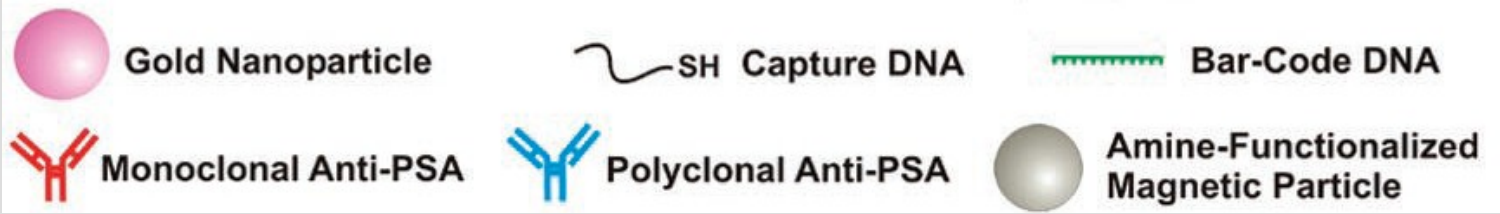
Introduction

- Basic idea similar to muno-PCR – use DNA as markers of protein, since we can amplify DNA to something measurable
- 1:1 DNA:Protein ratio

Experimental Design- Probe 1: MMPs

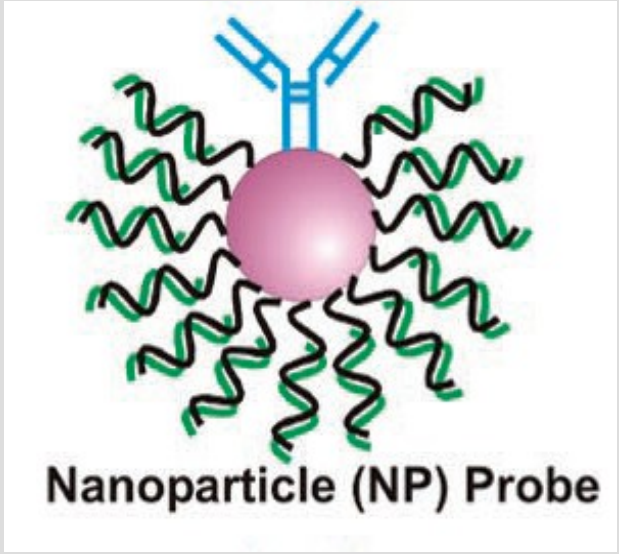
- 1um diameter
- Iron Oxide core, polyamine shell
- Functionalized with PSA antibodies

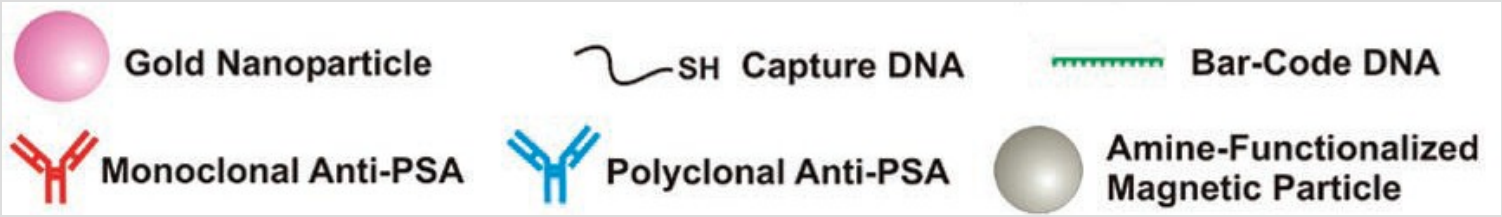




Experimental Design- Probe 2: NPs

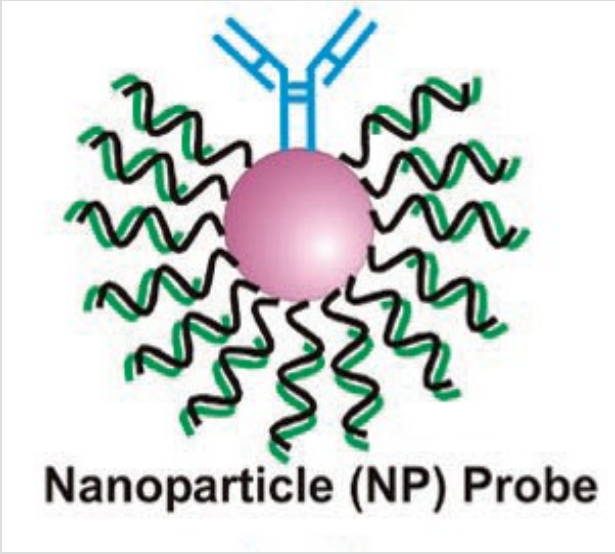
- 13nm diameter
- Made of Gold
- Functionalized with capture DNA and PSA antibodies

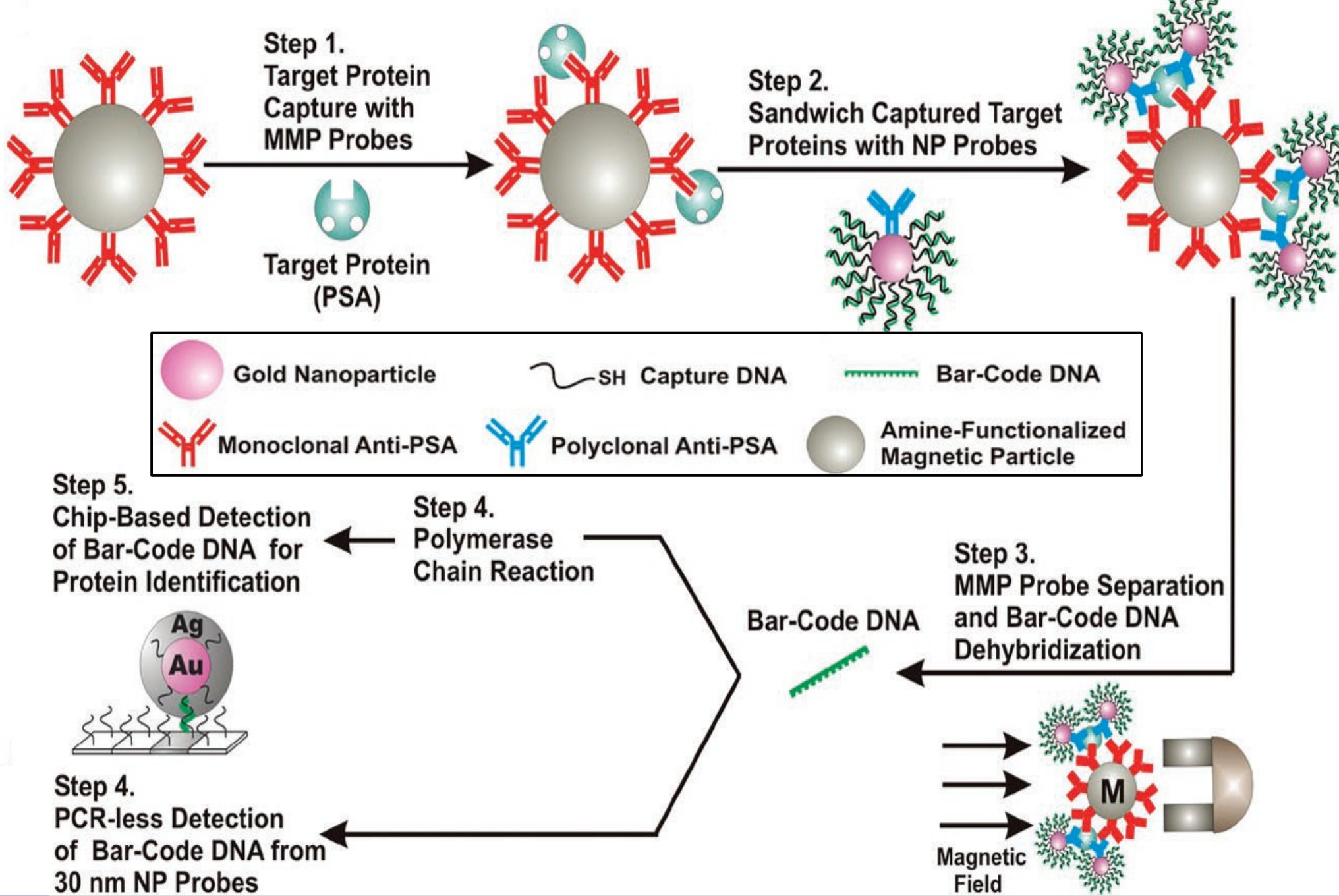


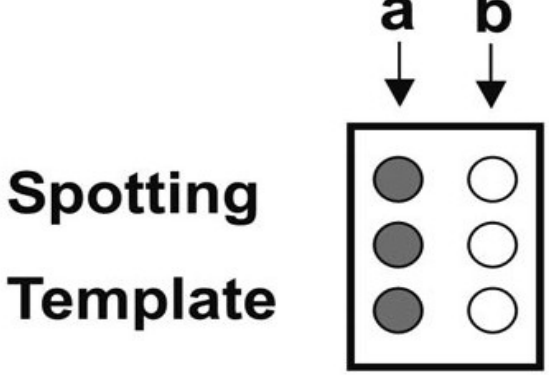


Expt'l Design – What is a bio-barcode?

- 40bp DNA sequence
- Binds to capture sequence on NP
- Can easily be separated from the capture sequence and be PCR amplified if necessary




Step 1.
Target Protein
Capture with
MMP Probes
Target Protein
(PSA)
Step 2.
Sandwich Captured Target
Proteins with NP Probes
Gold Nanoparticle
SH Capture DNA
Bar-Code DNA
Monoclonal Anti-PSA
Polyclonal Anti-PSA
Amine-Functionalized
Magnetic Particle
Step 5.
Chip-Based Detection
of Bar-Code DNA for
Protein Identification
Step 4.
Polymerase
Chain Reaction
Step 3.
MMP Probe Separation
and Bar-Code DNA
Dehybridization
Bar-Code DNA
Ag
Au
M
Magnetic
Field
Step 4.
PCR-less Detection
of Bar-Code DNA from
30 nm NP Probes

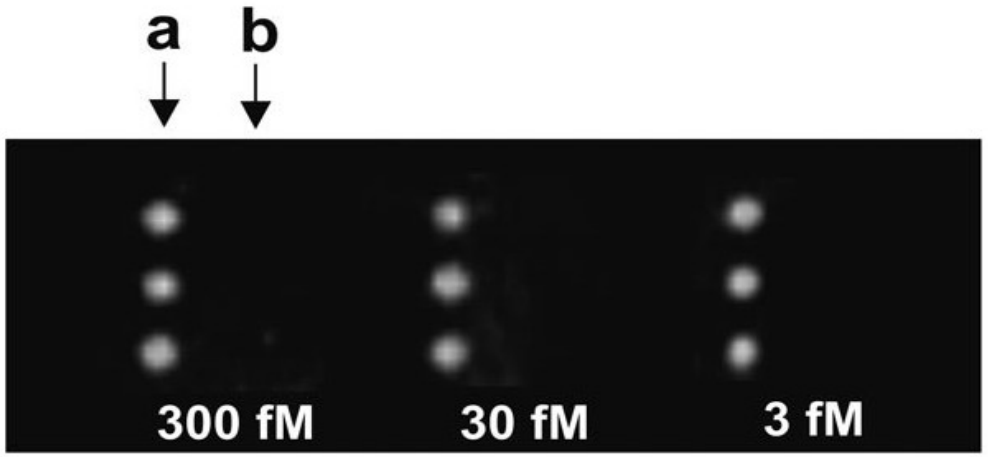


Spotting

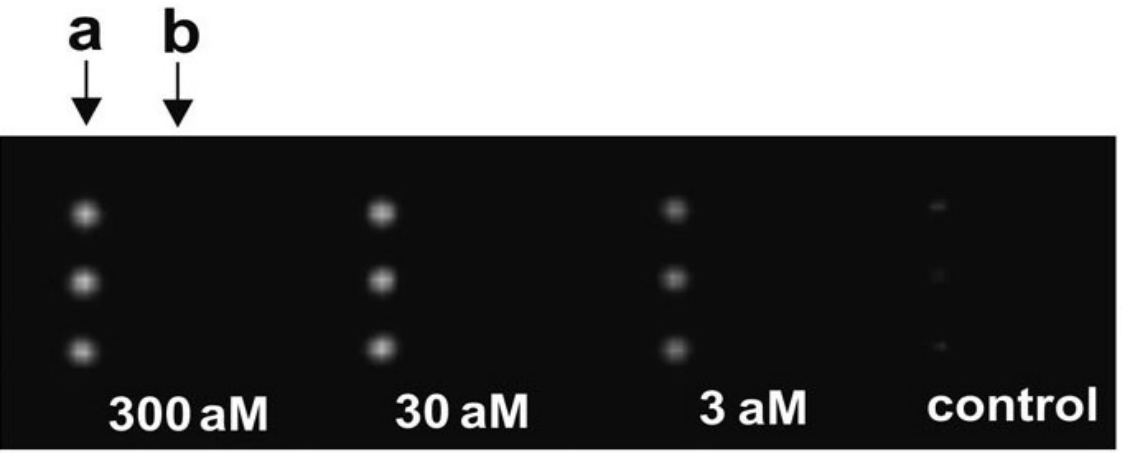
Template

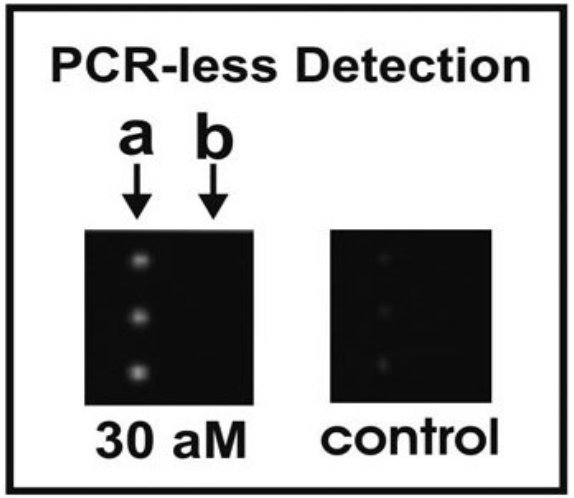
a: Complementary Capture DNA

b: Noncomplementary Capture DNA



Background proteins B-galactosidase and antidinetrophenyl were added to all 7 samples

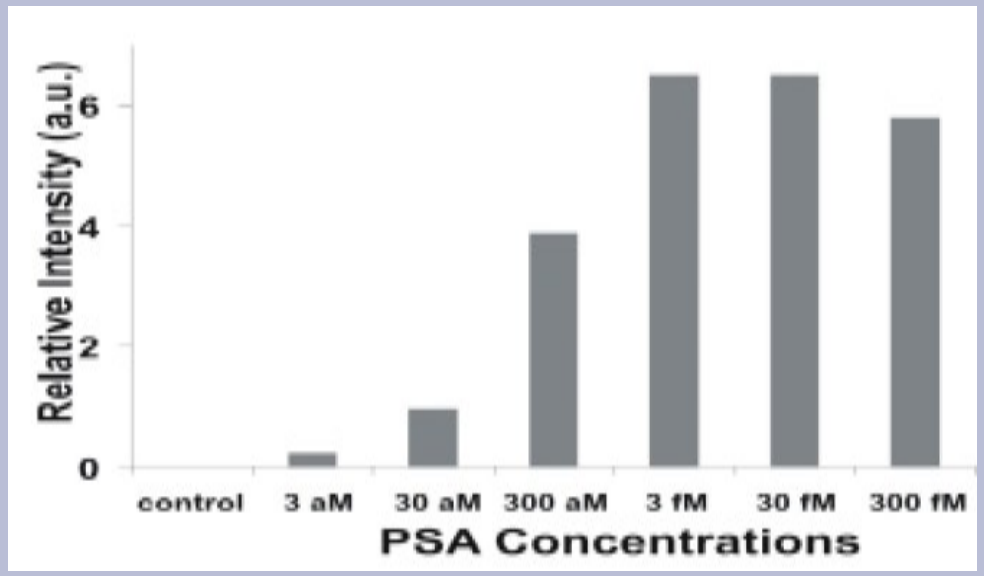




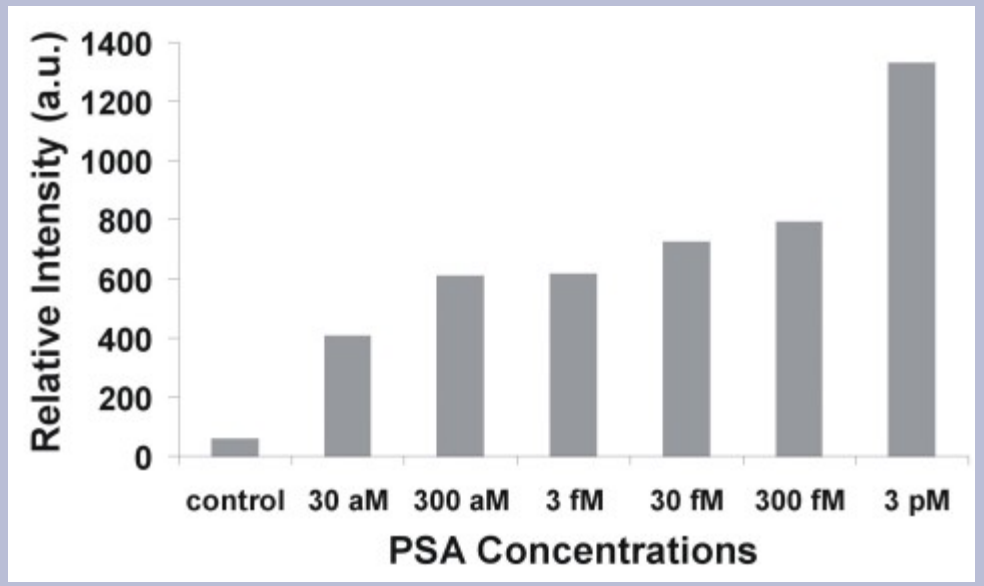
Control = no PSA added

Is PCR necessary?

With PCR



Without PCR



Conclusions

- Two-probe barcode system is sensitive down to 3 attomolar (6 orders of magnitude more than other current assays)
- Excellent selectivity – little signal when PSA is absent, no detectable signal from noncomplimentary DNA
- PCR step is unnecessary for >=30 attomol concentration

Conclusions - advantages

- Nothing is immobilized – faster binding kinetics
- High ratio of bar-code DNA to protein yields high assay sensitivity
- Simple to attach and release bar code from NP – simple wash step
- No need for secondary antibodies
- Use of MMPs reduces background signal

Future Directions

- Potential for detecting many antigens in one solution with high sensitivity

Summary

- Magnetic Microparticle probes use antibodies to bind PSA
- Nanoparticle probes are encoded with Bio-bar code DNA and sandwich PSA
- Magnetic separation isolates bound nanoparticle probes
- Bar Code DNA is isolated easily
- Sensitive to 3 attomolar

Questions?

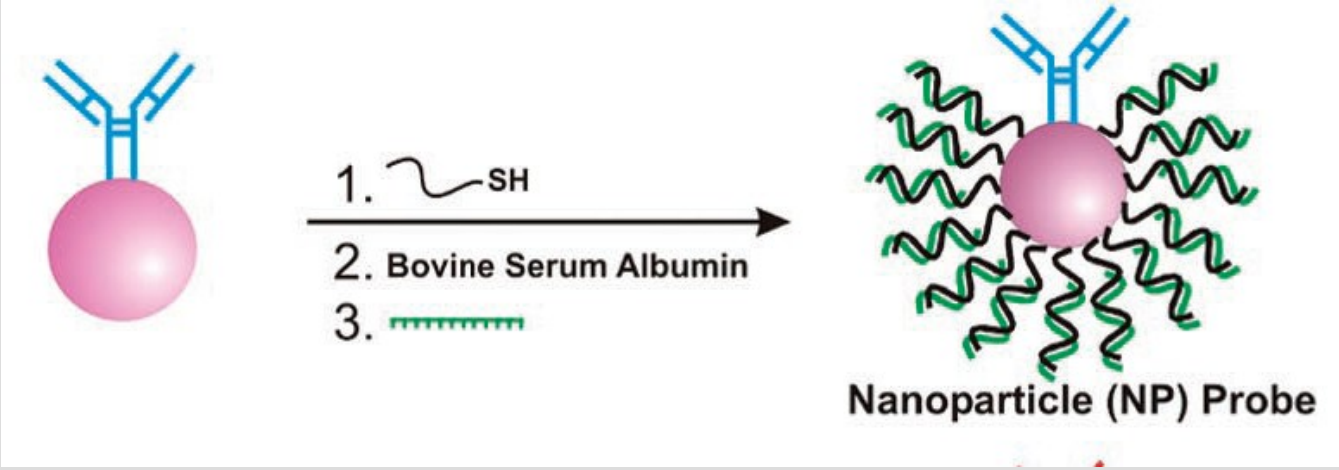
More Bio-Barcode

- Capture strand is:

5′ CAACTTCATCCACGTTCAACGCTAGTGAACACAGTTGTGT-A10-(CH2)3-SH 3′

- Bio-Barcode strand is:

5' ACACAACTGTGTTCACTAGCGTTGAACGTGGATGAAGTTG 3″



CHIP detection

- 5’ alkylthiol-capped DNA capture strand (20bp) attached to glass microscope slide
- Gold NPs were functionalized with 3’ alkylthiol-capped oligonucleotides (20bp)
  - Both are complementary to half of the target bio-bar-code DNA sequence
- bar-code DNA amplicons are added to NP probes
- Thermal cycled to hybridize
- Added to chip w/ immobilized capture strand, hybridized again
- Imaged with silver enhancement solution

No PCR

- Number of DNA strands on NP can be increased by increasing NP size
- For PCR, 30nm gold particle is used in place of 13nm.
- If ~100 DNA strands fit on a 13nm, 532 could fit on a 30nm