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
Experimental Procedure

Step 1. Target Protein Capture with MMP Probes

Step 2. Sandwich Captured Target Proteins with NP Probes

Step 3. MMP Probe Separation and Bar-Code DNA Dehybridization

Step 4. Polymerase Chain Reaction

Step 5. Chip-Based Detection of Bar-Code DNA for Protein Identification

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- Gold Nanoparticle
- SH Capture DNA
- Bar-Code DNA
- Monoclonal Anti-PSA
- Polyclonal Anti-PSA
- Amine-Functionalized Magnetic Particle

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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.

PCR-less Detection

300 fM 30 fM 3 fM

30 aM control

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′ CAACTTCATCCACGTTCAGCTAGTGAACACAAGTTTG-A10-(CH2)3-SH 3′

- **Bio-Barcode strand is:**
  5′ ACACAACTGTTACTAGCGTGGATGAAGTTG 3′

![Diagram of the binding process between a gold nanoparticle and a bar-code DNA](image)
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