Ultrasensitive protein detection using proximity initiated nucleic acid target amplification with digital biosensing

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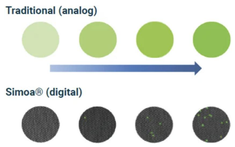
INTRODUCTION

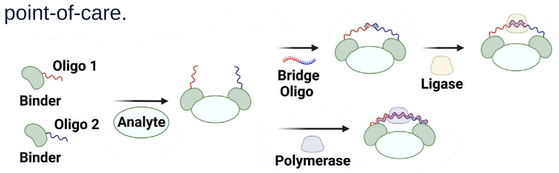
Proteins can provide a **real-time dynamic window into cancer states**. Longitudinal monitoring of diseases for diagnostic and prognostic decisions, such as liquid biopsies, requires rapid, cost-effective and ultrasensitive protein detection methods that can be applied at the point-of-care.

Digital detection: Approaches that allow detection of single-molecule targets increase the potential sensitivity and allow absolute quantification for disease detection. Digital assays like single-molecule arrays (SiMoAs) increase sensitivity 1000x over other methods.[1]



Analog vs. digital sensing.[1]

Proximity assays: These assays rely on antibody pairs that responds to the presence of a protein when the antibodies are brought into near proximity by a target. They are powerful tools for ultrasensitive protein detection, but current proximity assays require enzymatic amplification and are not suitable for point-of-care.



Proximity assay principles: antibody-oligonucleotide conjugates bind to the analyte protein, which triggers signal amplification such as an amplicon for PCR or rolling circle amplification for bulk fluorescence detection.[2]

Amplify-then-digitalize approach: Through the combination of non-enzymatic amplification methods with digital detection using nanoparticles, we can achieve rapid and point-of-care detection without sacrificing sensitivity.

This toehold-mediated approach allows linear amplification with digital detection of IL6 using the proximity-initiated nucleic acid target amplification (PINATA) assay.

AIMS

Objective: To develop a rapid and affordable method for ultrasensitive protein detection that could be used for point-of-care applications.

Point-of-care assay development:

- Room temperature, rapid, and ultrasensitive detection
- Portable, cost-effective detection instrument
- One-pot, single-step reaction
- <2 hour testing of low sample volumes

Novel assay design for ultrasensitive protein detection: Protein signal is transduced into nucleic acid reporter, amplified using non-enzymatic DNA circuits, then digitally detected using photonic resonator absorption microscopy.

METHODS

Photonic Resonator Absorption Microscopy (PRAM)

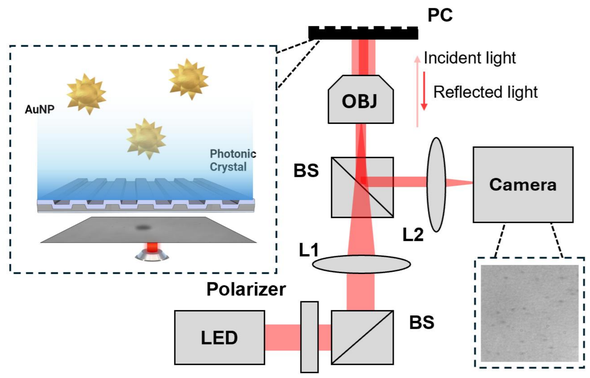


Figure 1: Photonic resonator absorption microscope light path. LED reflects off red PC unless quenched by AuNP. Gold nanoparticles bound to PC surface in evanescent field can be imaged and digitally counted.

Proximity Initiated Nucleic Acid Target Amplification (PINATA)

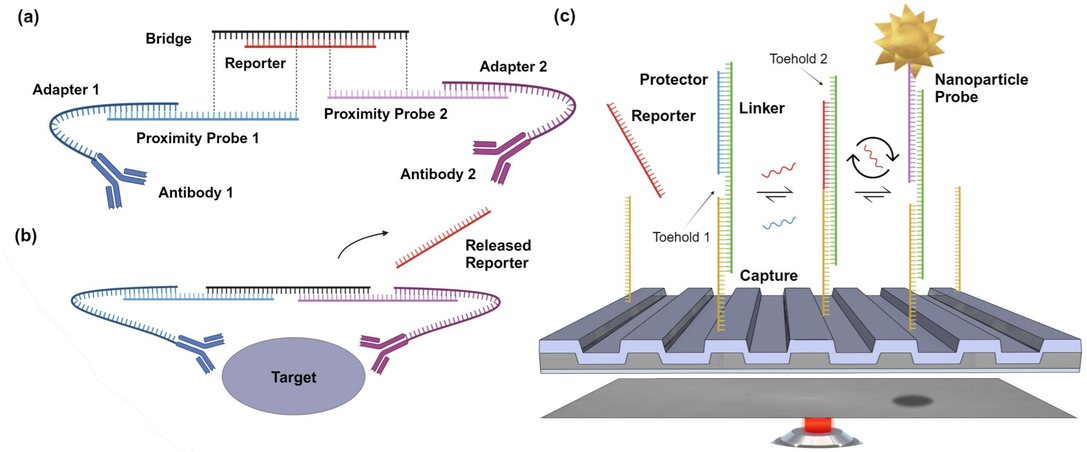


Figure 2: PINATA schematic. **(a)** Pre-annealed and purified BR duplex has two toehold regions that can react with proximity probes to displace reporter when **(b)** target protein is present to be **(c)** amplified and digitally counted.

RESULTS

Feasibility: Biotin-streptavidin antibody-antigen mimic

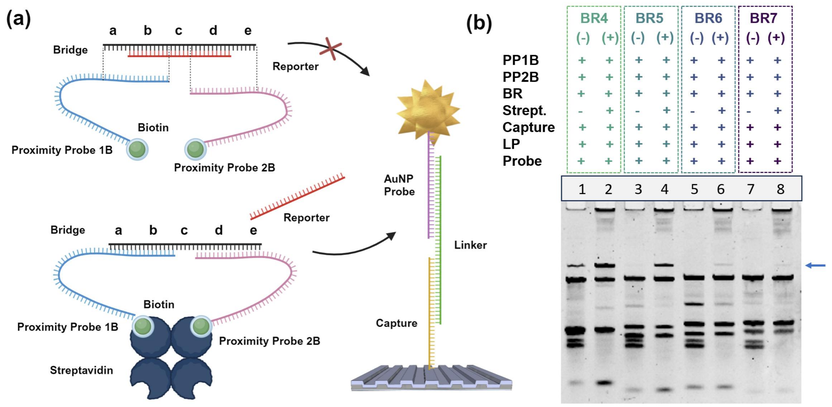


Figure 3: PINATA optimization. **(a)** Region "c" was tuned for unpaired nucleotides to control reporter displacement and **(b)** tested with amplifying sequences in Native PAGE.

IL-6 testing in Native PAGE

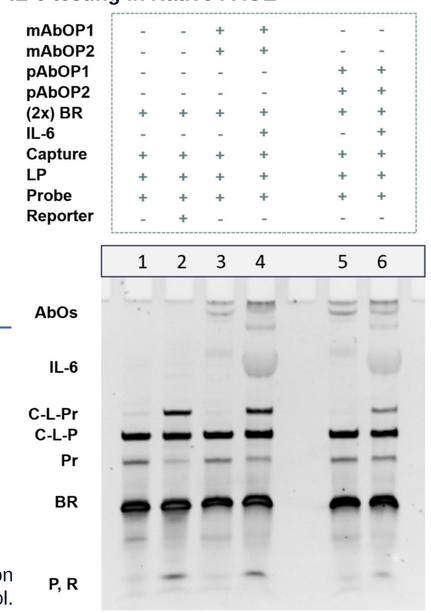


Figure 4: Comparison of monoclonal and polyclonal AbOs for PINATA assays in PAGE.

IL-6 testing on PRAM using PINATA

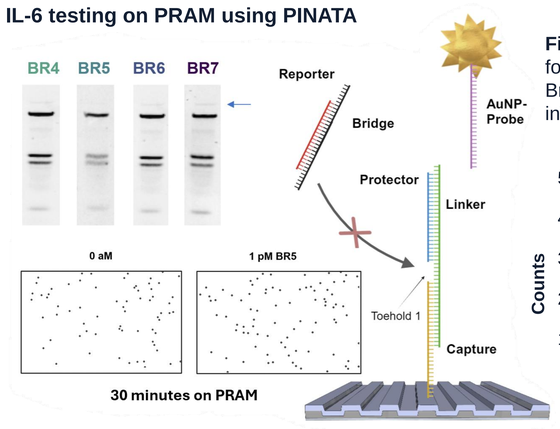
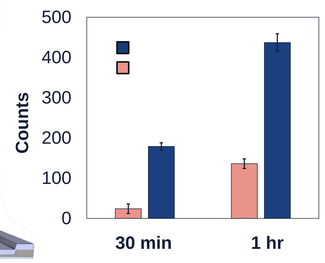


Figure 5: Testing of amplification for the background BR control. Bridge-reporter alone does not initiate amplification



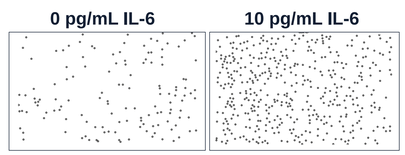


Figure 6: Testing of human IL-6 using PINATA on PRAM. 10 pg/mL concentration can be detected in just a thirty-minute reaction time. Counted images from PRAM shown.

CONCLUSIONS

- Protein presence is converted to nucleic acid signal and tested with amplify-then digitalize approach
- Enzyme-free amplification does not require expensive thermocycling or hands-on steps.
- Flexible approach will allow antibodies to be easily exchanged for various protein biomarkers of interest
- Demonstrates a limit of detection of less than 10 pg/mL for human protein marker interleukin 6 (IL-6)
- Antibodies demonstrate high selectivity in ELISAs against other cytokines (IL2, IL10, etc)

ACKNOWLEDGEMENTS

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Tumor Engineering and Phenotyping Shared Resource (TEP)

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