Portable Reusable Accurate Diagnostics with nanoAntennas (PRADA) for Multiplexed Biomarker Screening

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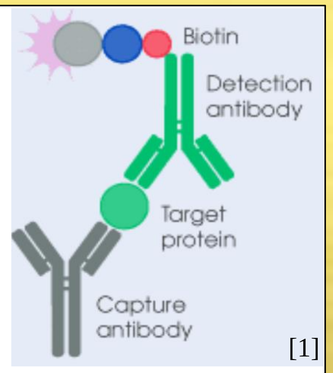
Introduction

- Precise detection of specific biomarkers in human fluids is compulsory for disease diagnosis, risk stratification, and treatment

Criteria for Biodiagnostic Devices

- **Multiplexed detection at low levels**
- **Easy sample prep and rapid read out**
- **Portable w/ low sample consumption**
- **Prolonged reagent shelf life**
- **Reusable**

- Currently, the most prevalent method is the enzyme-linked immunosorbent assay, or ELISA
- ELISA can only detect one biomarker at a time, requires large sample volumes and has high operational costs [1]



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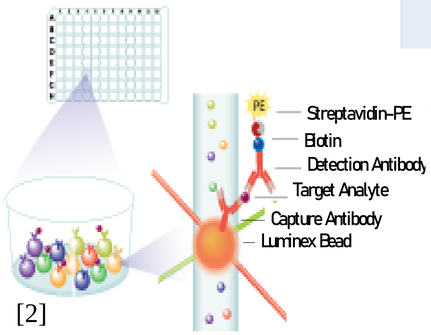
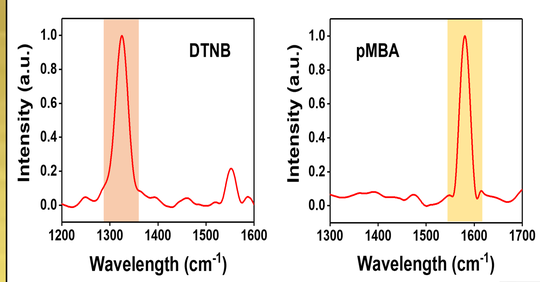


[2]

- The Luminex assay allows for multiplexing by using multicolored beads, but requires specialized equipment [2]
- PRADA eliminates disadvantages through the use of a sandwich assay with magnetic microbeads and gold nanostars, allowing multiplexed biomarker detection in resource limited settings
- Copper microbeads immobilized by a magnet for the duration of screening can be washed away afterward, allowing for reusability
- Biomarkers tested for here are cardiac troponin I (cTnI), a biomarker of myocardial infarction, and neuropeptide Y (NPY), a biomarker of anxiety and PTSD.

- Gold nanostars absorb near-infrared light in their centers and focusing the absorbed light at the tips of their branches
- This behavior leads to localized surface plasmon resonance, causing electromagnetic enhancement and increased sensitivity



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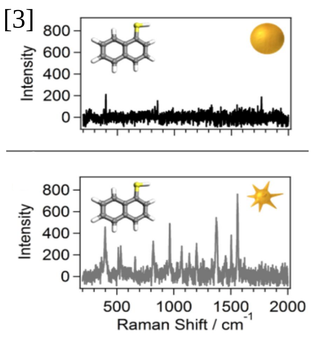


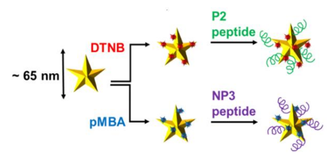
- The Raman tags used have characteristic peaks that do not overlap, supporting specific multiplexing

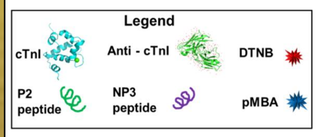
- Translation to a microfluidic enables multiplexed detection in small sample volumes
- The PDMS and glass microfluidic device can be reused over multiple cycles, reducing operational costs

Objective

- To verify PRADA as an inexpensive method of achieving quantitative, specific, and sensitive single- and multiplex biomarker detection

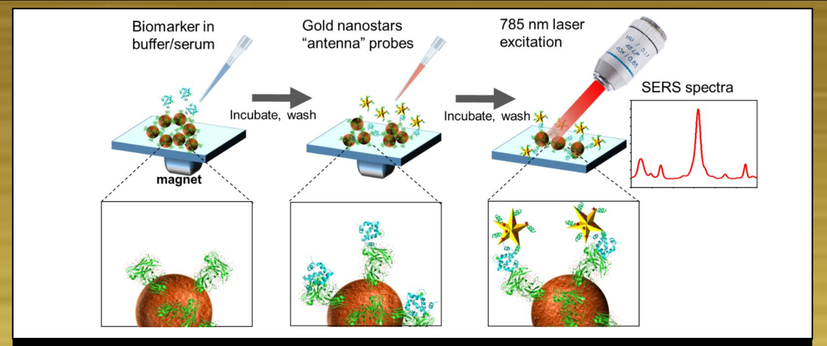
Methods



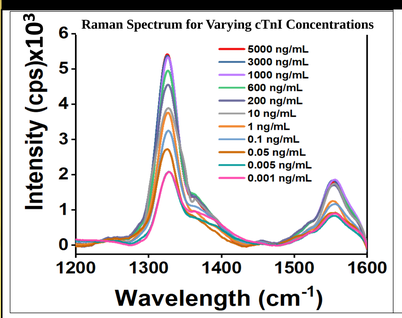


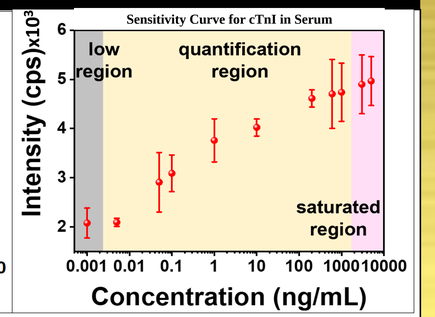
- Capture probes are formed by conjugating magnetic beads with biomarker specific antibodies

- Gold nanostars are conjugated with Raman tags and peptides to form detection probes
- Capture probes are held in place by a magnet and incubated with buffer or serum containing biomarkers of interest in different concentrations to prove sensitivity
- After removing the magnet, samples may be archived, and the microfluidic device is washed and used again to prove reusability

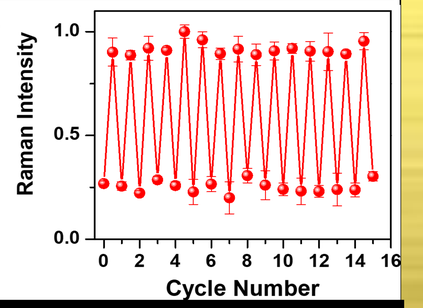


Results





- PRADA demonstrates a decrease in peak intensity corresponding to a decrease in concentration, with a limit of detection of 0.1 ng/mL
- After reuse of the same microfluidic device over 15 cycles, PRADA experienced less than 4% signal loss



Conclusions/Future Work

- PRADA is a sensitive and specific technique for quantitative biodiagnostics
- PRADA can be performed repeatedly on a microfluidic device with little effect on accuracy
- In the future, a sensitivity curve for NPY needs to be determined, screening for multiple biomarkers needs to be done, and a range of clinical patient samples need to be analyzed

References

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[3] Dorleta Jimenez de Aberasturi, Ana B. Serrano-Montes, Judith Langer, Malou Henriksen-Lacey, Wolfgang J. Parak, and Luis M. Liz-Marzán
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