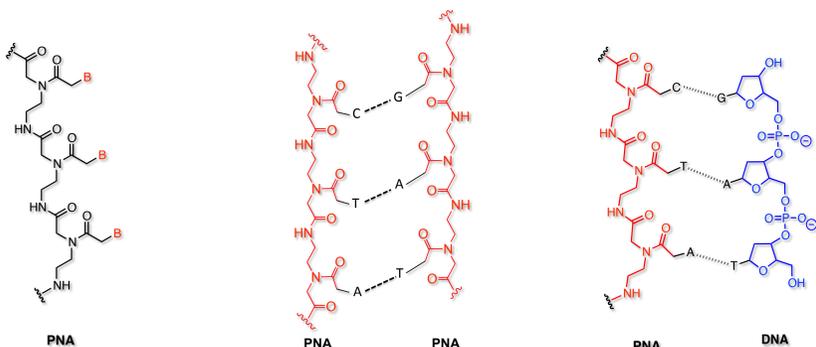


Abstract

Current HIV detection methods have many drawbacks in clinical and point-of-care (POC) settings. The gold standard assay to detect HIV in a blood sample is RT PCR. While highly accurate in detection and quantification of HIV, RT PCR is slow, requires expensive equipment, and requires highly trained personnel, all hindrances in clinical or POC settings. The objective of this project is to develop a cost-effective, convenient PNA-based microfluidic assay to detect and quantify HIV RNA. This ELISA-like sandwich assay is based upon complementary binding between PNA and nucleic acids. The goals for this summer were optimizing the conjugation of the surface probe PNA to Bovine Serum Albumen (BSA), and fluorescence experiments to identify nonspecific interactions between the various reagents in the microfluidic channels.

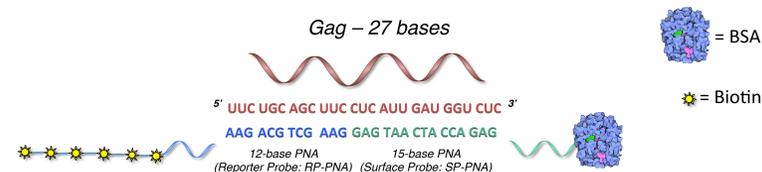
What are PNAs?

- Peptide Nucleic Acids (PNAs) are polymers consisting of nucleic acid bases on a pseudo-peptide backbone that replaces the traditional phosphodiester backbone of nucleic acids.
- PNAs interact with PNA, DNA and RNA by Watson-Crick complementary base pairing



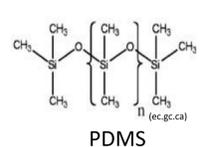
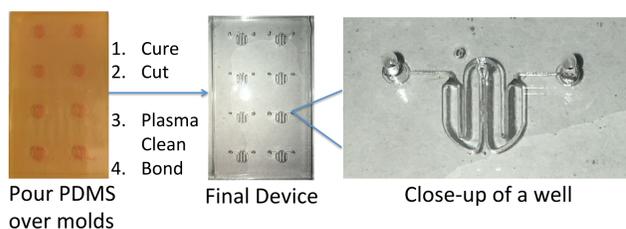
Target HIV RNA Sequence and Probe Design:

- A PNA surface probe and a PNA reporter probe were designed to target portions of Gag, an HIV RNA sequence
- PNAs synthesized via solid phase peptide synthesis



Microfluidic Device Fabrication

- Devices fabricated out of polydimethyl siloxane (PDMS)
- An inlet and an outlet port in each well allow for loading of samples
- The volume of each well is ~18 μ l

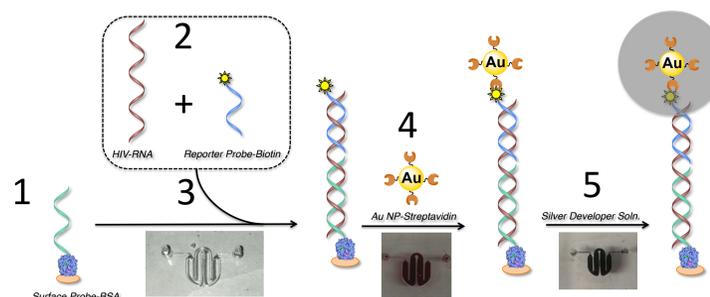


PNA-Based HIV Detection Assay

Assay Protocol:

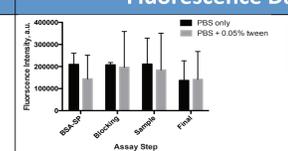
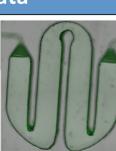
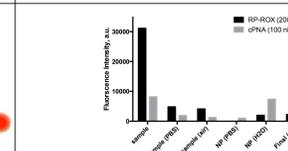
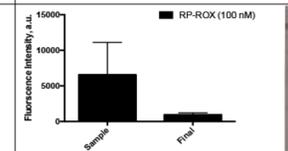
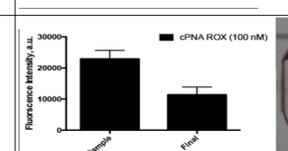
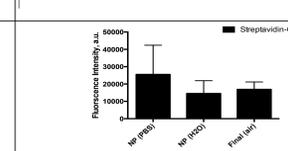
- Surface probe PNA immobilized to surface of microfluidic channels
- Reporter probe PNA mixed with target nucleic acid sequence and annealed
- Reporter probe/nucleic acid complex binds to surface probe on the channel surface
- Streptavidin conjugated Au nanoparticles bind to biotin on reporter probe
- Silver developer solution amplifies the signal of the Au nanoparticles and gives a qualitative readout

*Steps 1-4 followed by a phosphate buffered saline (PBS) wash



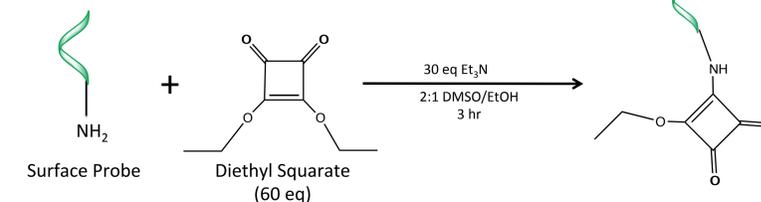
Fluorescence Experiments

- A series of experiments with fluorescent molecules was designed to determine why signal was observed in the negative control wells
- Each well subjected to the same wash conditions as a normal assay
- Fluorescence readings taken with a plate reader, and fluorescence images taken by confocal microscopy

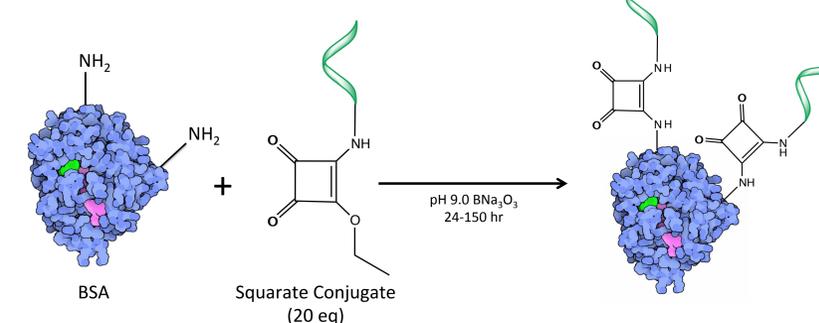
Question	Steps Examined	Fluorescence Data
Is BSA getting washed off the channel surface?	1. 2. N/A 3. N/A 4. N/A 5. N/A	 
Does the reporter probe or target sequence stick to a BSA-blocked surface?	1. or 2. N/A 3. N/A 4. N/A 5. N/A	 <p>No observable fluorescence with confocal microscopy</p>
Does the reporter probe interact with the surface probe?	1. 2. N/A 3. 4. N/A 5. N/A	 
Does a complementary sequence bind the surface probe?	1. 2. N/A 3. 4. N/A 5. N/A	 
Is streptavidin adsorbing to a BSA-blocked surface?	1. 2. N/A 3. N/A 4. N/A 5. N/A	 <p>No observable fluorescence with confocal microscopy</p>

BSA-Surface Probe Conjugation

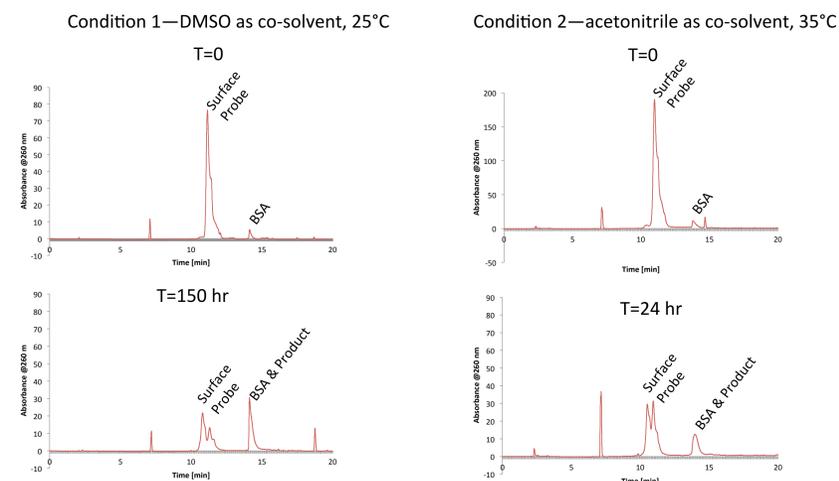
Step 1: Coupling of Squarate to PNA



Step 2: SP-Squarate Coupling to BSA



HPLC Monitoring of Step 2:



A 10-90% gradient of 9:1 MeCN/H₂O over 15 minutes was employed in separation

Future Work

- Develop an imaging protocol for quantitative detection of viral RNA
- Detect HIV RNA in patient samples
- Improve conjugation of BSA to surface probe
- Characterize streptavidin-coated Au nanoparticle

Acknowledgements and References

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