Development of a Botulism Neurotoxin Sensitive Lateral Flow Assay Biosensor for Clinical Applications and Medical Settings

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Abstract

One of the many great challenges that medical diagnostics face is the need for sensitive, reliable, and rapid detection of molecules in very complex solutions such as blood or urine. DNA-based biosensors have shown great promise in terms of sensitivity and reliability for target detection, but the need for rapid testing has considerably slowed their use in practical applications within the medical world. In the research to be conducted, we explore the incorporation of DNA-based biosensors into a lateral flow assay format (similar to the common at-home pregnancy test for human chorionic gonadotropin in urine). To facilitate this, we are developing a gold nanoparticle decorated with a functional DNA probe that recognizes and binds to botulism neurotoxin variant A (BoNTA). This conjugate then wicks across a nitrocellulose membrane to specific capture points, allowing rapid visual assessment of the BoNTA contamination of a sample. In the future, we aim to demonstrate that this represents a generic platform for detection that could be used with any existing DNA aptamer-based biosensing technique and can be applied to many medical settings, including small clinics, without the need for technicians to operate the biosensor.

Background

Lateral flow assays

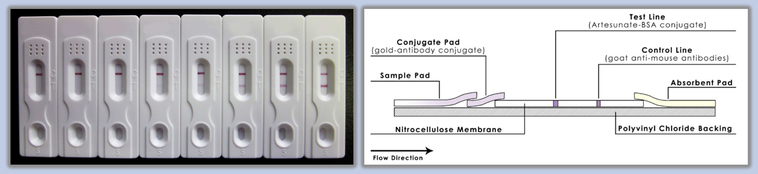


Figure 1. (left) Dipsticks showing color changes corresponding to concentrations of artemisinin in distilled water. (Right) Assembly of colloidal gold-based dipstick[1]

Lateral Flow Assays (LFAs) are rapid assay methods that allow for visual conformation of a desired target. LFAs are also known as immunochromatography assays, aptamer chromatography assays, dipsticks, and strip tests. LFAs allow for a fast preliminary diagnosis of a wide variety of analyte.[2] One of the most commonly used LFAs are household pregnancy tests. These assays specifically test for the presence of human chorionic gonadotropin in a potential mother's urine. Aside from being used in household products, LFAs are also be used in clinical, veterinary, agricultural, food industry, biodefence, and environmental applications. The range of different molecules that a LFA can detect within these applications is quite extensive. A few examples of these molecules include…

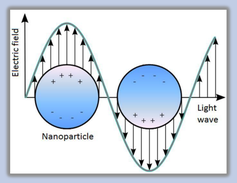
- Illegal drugs (cocaine, heroin, opioids) which can be applied in the crime and justice setting
- Banned athletic substances (growth factors, anabolic substances, peptide hormones) in sports competitions
- Bacterial and/or viral antigens (LPS, peptidoglycan, foreign nucleic acid) in diagnostics and food/drink industry
- Heavy metals (cadmium, arsenic, lead, uranium) in water and soil treatments
- Early and late stage cancer molecules which are vastly important to cancer treatment options

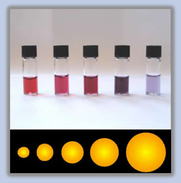
Not only are these assays fast and diverse, but they are also low in cost.[3] By using sophisticated yet easily constructible technology, these simple assays can be created so that almost anyone can successfully utilize them. In a potential medical setting, this can eliminate the training required to become a specialized technician for the operation, application, and interpretation of the results for these tests. With the simplicity, rapidness, diversity, and reliability of these assays, increased use of LFA could greatly aid in many of the previously mentioned applications. However, LFA development hinges on creating a molecular system with high target specificity and clear optical readout. Here, we investigated the use of gold nanoparticles and DNA aptamers to solve those challenges.

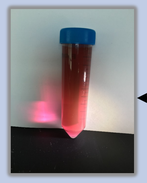
Gold Nanoparticle (AuNP) Properties

A main component of a lateral flow assay is gold nanoparticles (AuNPs). These particles have been extensively used in biological and technological applications because of their unique optical properties. Because these gold particles are smaller than visible light, this gives them a red or purple color rather than gold. In our lateral flow assays, this allows for the visualization of the test and control line in the form of a red/purple line depending on the molecules present in the analyte.[4]

Gold Colloid Plasmon Resonance







Colloid Size	Colloid Conc.
Approx. 50nm	$4.3x10^{-12}$ M

Figure 2. (Left) Schematic of localized surface plasmon resonance (LSPR) where the free conduction electrons in the metal nanoparticle are driven into oscillation due to strong coupling with incident light.[5] (Middle) Solutions of gold nanoparticles of various sizes. The size difference causes the difference in colors.[6] (Right) Bonham lab produced AuNPs

DNA Aptamer based versus Antibody Based LFAs

Various techniques exist that offer high rates of detection, including PCR, cytometry, or cell enrichment. However, these are time-consuming, expensive, and may require target enrichment or production of other biomolecules (i.e. antibodies). LFAs serve as a highly sensitive, rapid, and inexpensive tool which can help provide a solution to these problems. However, many LFAs so far have used antibodies in their construction and have a relatively long assay time or multiple washing and separation steps. DNA-aptamer based LFAs have high specificity, low molecular weight, easy and reproducible production, versatility in application, easy discovery and manipulation, and eliminate the need for washing and/or waiting for an extended amount of time.[3]

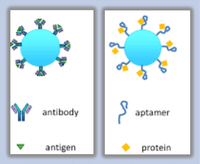


Figure 3. Comparison of aptamer and antibody AuNPs[7]

DNA Aptamer Based Lateral Flow Assay

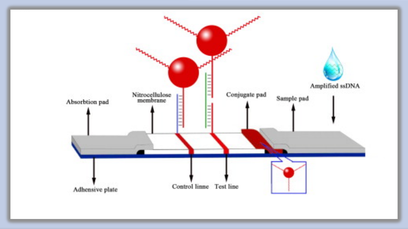
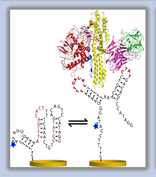


Figure 4. Schematic of our LFA. AuNPs can be seen with DNA-aptamer probes attached. These AuNP-conjugate probes will then interact with the target DNA in the analyte and then bind to the test line. Any AuNPs that do not bind with target DNA will interact with the control line further down the assay.[8]

Aptamer Discovery for Novel Detection



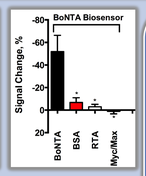
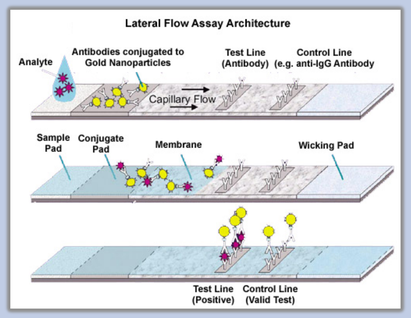


Figure 5. (Left) Botulism Aptamer Biosensor design. (Right) Signal strength and specificity of botulism biosensor challenged with off-target substances.[9]

Research in the Bonham lab has revolved around aptamer biosensors which can sense a variety of targets. Targets that have a biosensor already created are…

- a breast cancer biomarker c-Myc
- the inflammation marker NFκB
- Cytokine IP-10 associated with tissue rejection
- Botulism and Ricin
- Tobramycin, an antibiotic
- Uranium
- Mycoplasma pneumoniae

Action of a Lateral Flow Assay



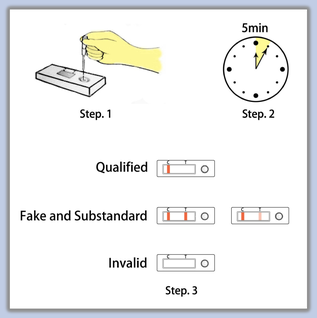


Figure 6. (Left) Schematic demonstrating the method of a antibody based LFA.[2] (Right) A schematic of the assay steps and results.[1]

Assuming the presence of the target DNA in your analyte, the LFA works in just a few steps:

1. Desired analyte added to the sample pad
2. Analyte travels across conjugate pad and target DNA will interact with the AuNP-DNA probe conjugate
3. Target DNA bound AuNP-DNA conjugate travels across the nitrocellulose membrane and interacts with the DNA probe test line. The AuNP conjugate will stick and a red line will be visible
4. Any unbound AuNP-DNA probe conjugates will flow past the test line and interact with the control line to give second visible red line

Bonham Lab Constructed Lateral Flow Assays

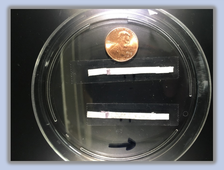


Figure 7 (Left) Two lab constructed LFAs with penny to convey dimensions. (Right) Lab constructed LFA with AuNPs flowing across nitrocellulose to control line.

Future Directions

Currently we have been constructing LFAs that are sensitive to DNA from an E. Coli strain. Optimization of the test and control line visibility/definition as well as the optimum buffer amount are being tested. In time, we hope to incorporate Botulism toxin into one of our LFA designs. To further tests the limits of our assays, we intend to conduct tests with pure Botulism toxin and contaminated cows blood.

References

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Acknowledgements

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