

Design, Development, and Method Evaluation of Aptamer-Based Aflatoxin Detection
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Aflatoxins-silent, deadly toxins produced by fungi like *Aspergillus flavus* and *A. parasiticus*-pose a serious global threat to food safety. These carcinogenic compounds infiltrate food and feed commodities, endangering both human and animal health by causing liver cancer, immunosuppression, and developmental disorders. Amidst this crisis, my PhD research set out to address a critical need: the development of a rapid, sensitive, and cost-effective method for detecting Aflatoxin B₁ (AFB₁), the most potent of the aflatoxins.

The journey began with the innovative use of DNA aptamers-short, single-stranded oligonucleotides that fold into precise 3D structures to bind specific targets with high affinity and specificity, similar to antibodies. Unlike antibodies, however, aptamers are thermally stable, inexpensive to synthesize, and do not require cold-chain storage-making them ideal candidates for field-based diagnostics.

In the first phase of the study, I employed a gold nanoparticle (AuNP)-based SELEX platform for the selection of AFB₁-specific aptamers. This SELEX method allowed real-time monitoring of aptamer enrichment cycles through visible color changes, greatly enhancing the speed and precision of aptamer screening. Next-generation sequencing (NGS), using both MiSeq and NovaSeq platforms, was applied to analyze the evolving aptamer pools. With the help of advanced bioinformatics tools like MEME, MAST, and AptaSUIITE, I identified highly enriched aptamer motifs from a large sequence pool.

17 aptamers were characterized using AuNPs-based colorimetric assays, revealing strong binding affinities in the nanomolar range, and fluorescence techniques confirmed specific interactions with AFB₁. Circular Dichroism (CD) Spectropolarimetry showed aptamer structures underwent stable conformational changes upon target binding. Cross-reactivity assays demonstrated high specificity of the top aptamers-CPMA1, L1, L2, and L3-towards AFB₁, with minimal interference from other mycotoxins. Computational modelling and molecular dynamics simulations revealed stable aptamer-AFB₁ complexes and detailed the molecular interactions that governed their specificity. Aptamer L2 exhibited the strongest binding affinity, making it a promising candidate for assay development.

In the final phase, I developed an aptamer-based LFA using L2 aptamer and thiolated AuNPs. A carefully designed cDNA probe enabled specific AFB₁ detection without binding interference. Optimization steps achieved a detection limit of 0.648 μ M, with clear dose-dependent response, high specificity, and minimal cross-reactivity.

In conclusion, my thesis presents a complete, multi-disciplinary approach-from aptamer discovery to real-world application-laying the foundation for rapid, sensitive, and affordable aflatoxin detection tools. The work not only advances the field of aptamer-based biosensors but also contributes meaningfully to global food safety efforts.