

Aptamer-Mediated Colorimetric Sensing of Caffeine via Gold Nanoparticles- A Prototype for Rapid Detection of Opioid-like Small Molecules

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Purpose: Drug overdoses have become a public health crisis in the United States, with approximately 68% involving opioids, particularly synthetic opioids such as fentanyl. Because overdose confirmation requires hospital-based testing, there is a critical need for low-cost, rapid, and sensitive biosensors that could be integrated into portable devices. This study tested whether mixing caffeine with caffeine-aptamer-functionalized gold nanoparticles (AuNPs) would produce a rapid, measurable color change. **Procedure:** We investigated whether 20 nm AuNPs coated with caffeine aptamers could selectively bind caffeine. Aptamers were stabilized on the AuNPs using a binding buffer. Two caffeine concentrations were tested with the AuNP-caffeine aptamer conjugate. After incubation, a salt challenge was performed, and the resulting color changes were measured using a spectrophotometer. Two similar Theobromine concentrations were tested as a potential interferent. **Results:** The AuNP-caffeine aptamer conjugate was initially red and remained red after caffeine addition. Following a salt challenge, samples containing caffeine exhibited nanoparticle aggregation and a visible color change from red to blue, quantified by increased absorbance at 650 nm and decreased absorbance at 520 nm. Identical salt challenge of the Theobromine samples produced no color change, demonstrating high selectivity. **Conclusion:** These results indicate that aptamer-based small-molecule recognition can trigger AuNP aggregation and a visible color change. This principle could be adapted for the development of portable opioid overdose detection devices for use outside hospital settings, allowing emergency responders to rapidly identify overdoses and initiate life-saving treatment.

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