Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information for

Combination of the Lateral-Flow Immunoassay with Multicolor Gold Nanorod Etching for the Semi-Quantitative Detection of Digoxin

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Materials and Methods

Design of 3D Printed Tab, Tab Holder, and Positioner

The test line tab, tab holder, and positioner were designed to hold the cut-out LFA test line in place over a 96-well plate. All pieces were printed using an Ultimaker 3 FDM 3D printer (Ultimaker B.V., Geldermalsen, Netherlands) out of Ultimaker CPE filament (co-polyester).

Evaluation of CTAB Precipitation

A solution containing 0.25 M CTAB in Milli-Q water and a solution containing 0.25 M CTAB and 0.25 M Tween 20 in Milli-Q water were iced for 10 min and left overnight at room temperature. The solution containing only CTAB formed precipitate, and photos were taken using a Nikon D3400 DSLR camera. The GNR etching precursor suspensions were prepared as described in Section 2.2, except one suspension was prepared without any Tween 20. The etching reaction was performed as described in Section 2.2 using 0.5 mM H_2O_2. Photos and UV-Vis spectra were collected using the same protocol as described in Section 2.2.

Evaluation of Etching Components

The evaluation of each etching component was conducted by first preparing GNR etching suspensions following the same protocol as described in Section 2.2 with slight alterations to the GNR etching components. One etching suspension was prepared using all the same components. Another suspension was prepared without CTAB, while another suspension was prepared with 0.25 M CTAB instead of the standard 0.1 M CTAB. Additional experiments were conducted by removing NaBr or HRP. 40 µL of H_2O_2 in 3 mM NaOH was then added to each suspension to initiate the etching reaction. Finally, another experiment was prepared using all the same precursor components but run with no added H_2O_2. Photos and UV-Vis spectra were collected using the same protocol as described in Section 2.2.

RGB Analysis of GNR Suspensions

The Microsoft PowerPoint eyedropper tool was used to extract the RGB values for each GNR suspension in the photos taken as described in Section 2.7.

Results and Discussion

Design of 3D Printed Tab, Tab Holder, and Positioner

As depicted in Figure S1, the cut-out LFA test line is attached to the test line tab which is then inserted into the tab holder. The tab holder is then placed over a 96-well plate to submerge the test line during the H_2O_2 degradation step. The positioners are used to keep the tab holder steady while the whole assembly shakes on a well-plate shaker.

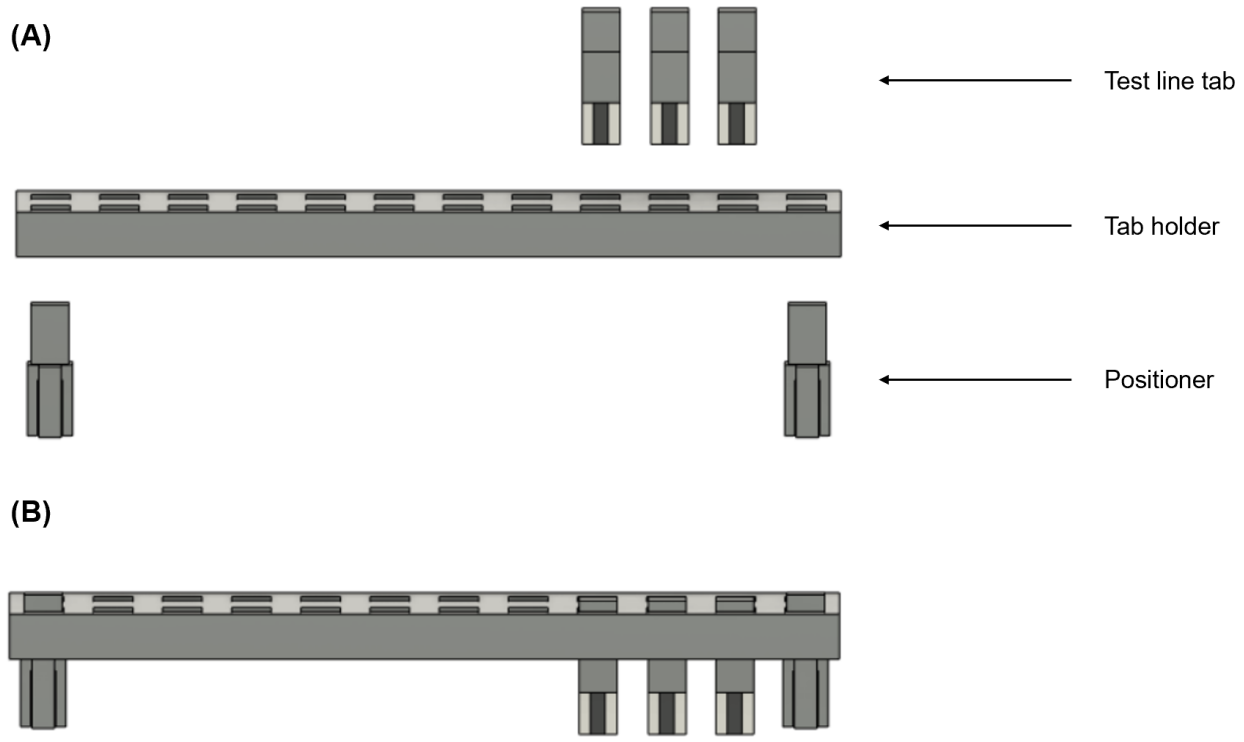

Figure S1. (A) CAD drawing depicting the test line tabs with cut-out LFA test lines attached, tab holder, and positioners used to hold the test lines in place during the H_2O_2 degradation step. **(B)** CAD drawing depicting the test line tabs, tab holder, and positioners assembled together.

Evaluation of CTAB Precipitation

Experiments conducted before the inclusion of Tween 20 were inconsistent due to the precipitation of CTAB. As shown in Figure S2, we found that the addition of Tween 20 significantly improved the solubility of CTAB, allowing the assay to be performed at room temperature without any heating steps. Furthermore, we confirmed that the inclusion of Tween 20 in the GNR etching suspension did not inhibit the etching reaction from occurring.

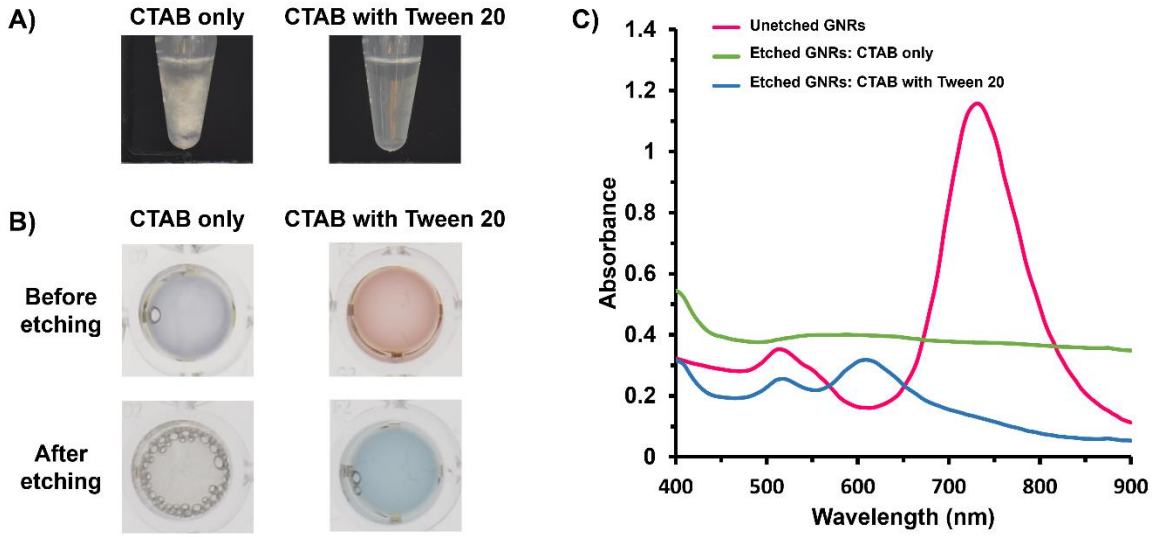

Figure S2. (A) Solutions containing 0.25 M CTAB. The solution without Tween 20 precipitated when iced, while the inclusion of Tween 20 prevented the formation of precipitate. **(B)** GNR etching suspensions with and without CTAB before and after the addition of H_2O_2. The suspension without CTAB formed precipitate, leading to the aggregation of the GNRs and the purple color before any H_2O_2 was added (labeled "Before etching"). The suspension containing Tween 20 did not form precipitate and was able to etch normally. **(C)** UV-Vis absorbance data of unetched GNRs and the GNR etching suspensions after the etching reaction was completed.

Evaluation of Etching Components

As shown in Figure S3, we evaluated the importance of each etching component by performing experiments in the absence of different components described in Section 2.2 and by varying the concentration of CTAB. We found that without any CTAB added to stabilize the GNRs, the GNRs would aggregate in the presence of NaBr and citrate buffer salt. However, we found that too high of a concentration of CTAB would limit etching, likely because the positively-charged CTA surfactant would associate strongly with tribromide and prevent it from etching the GNRs. We also showed that limited etching could occur without additional NaBr added, as tribromide could be formed from the bromide ions from the CTAB. However, for more substantial etching, additional bromide ions needed to be provided. Finally, we found that without HRP and H_2O_2, no etching was observed, likely because no tribromide was formed.

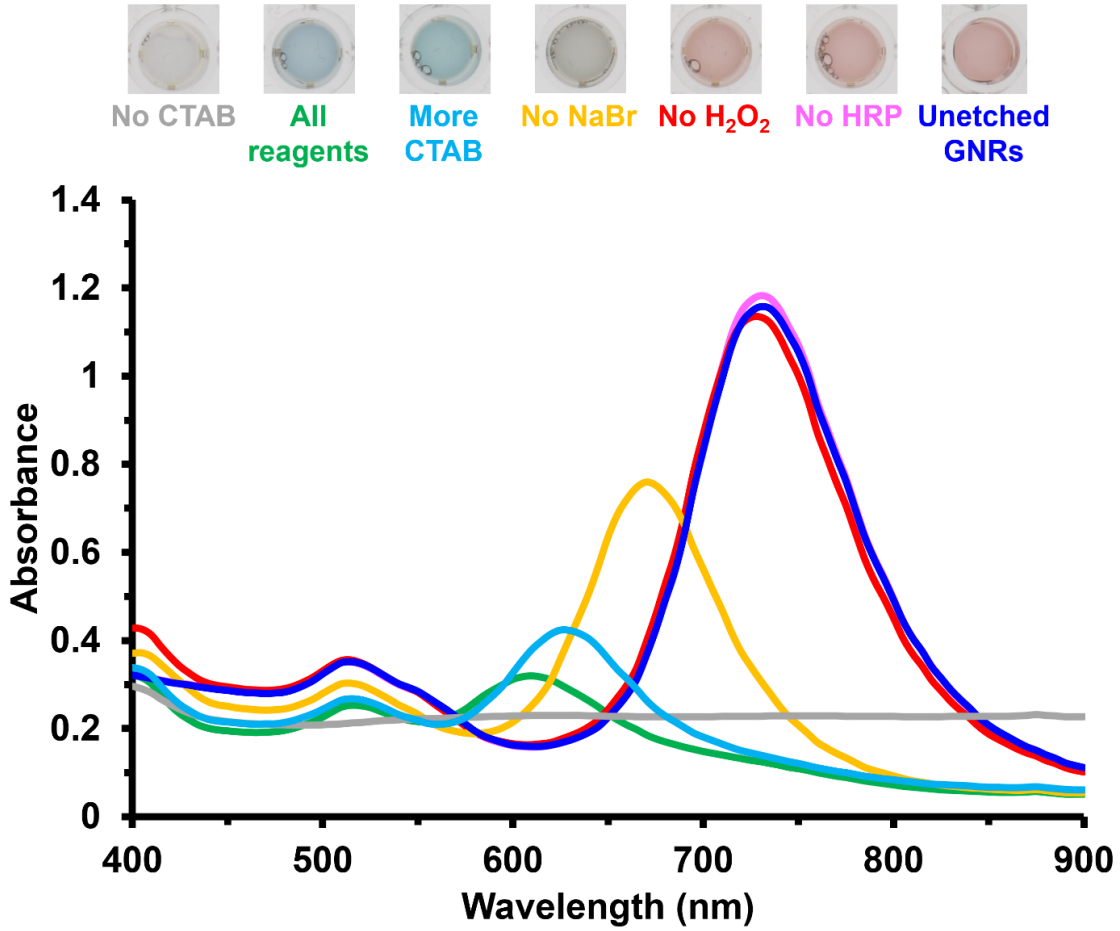

Figure S3. Evaluation of etching components. Photos displaying the final colors and UV-Vis absorbance data are shown. The suspension labeled "All reagents" contained the final optimized GNR etching suspension. The suspension labeled "No CTAB" contained no CTAB to stabilize the GNRs, leading to their aggregation and the light gray-purple color. The suspension labeled "more CTAB" contained 0.25 M CTAB rather than 0.1 M CTAB, leading to limited etching. The suspension containing no NaBr led to little etching, while the suspensions containing no HRP and no H_2O_2 led to no etching.

RGB Analysis of GNR Suspensions

To further support the claim that there are distinguishable visual differences between the GNR suspensions at different digoxin concentrations, the RGB values of each GNR suspension were extracted. As seen in Figure S4, there are clear differences in the R, G, and B values across different digoxin concentrations.

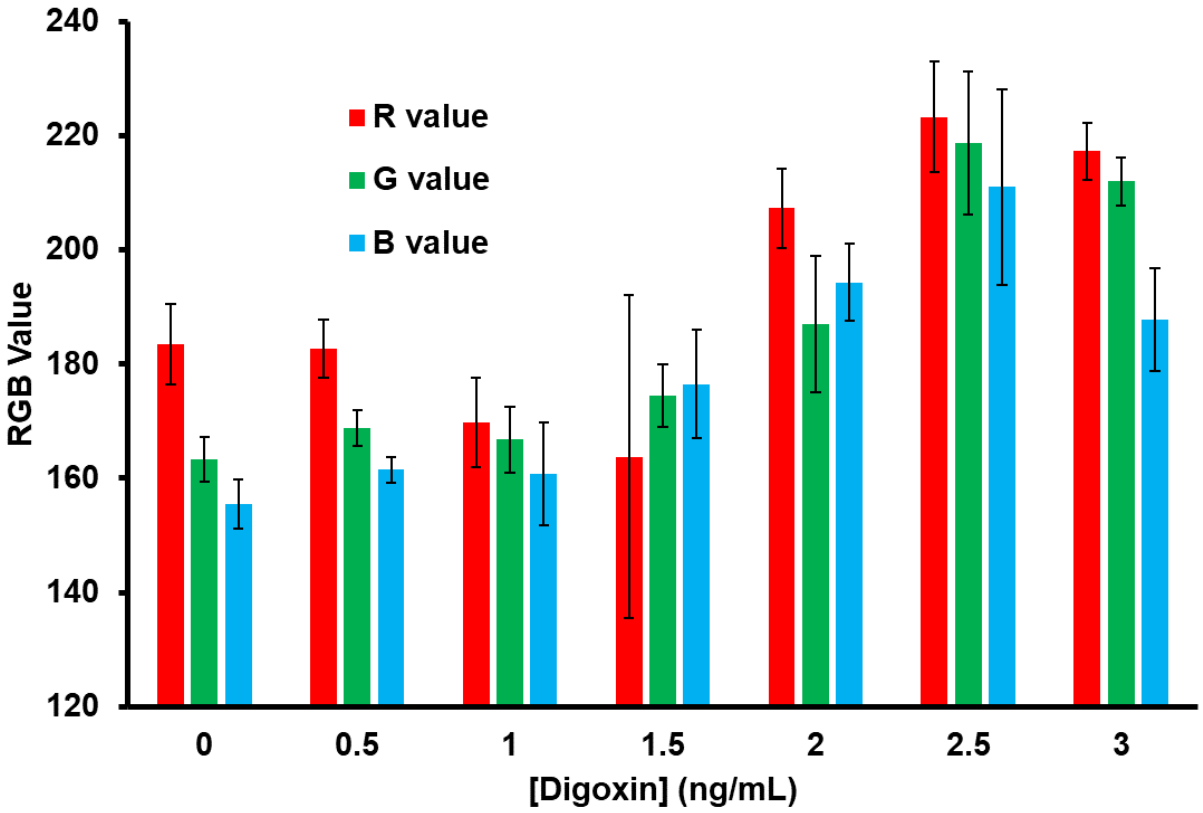

Figure S4. RGB analysis of GNR suspensions resulting from different digoxin concentrations. The RGB values for each GNR suspension were extracted using the Microsoft PowerPoint eyedropper tool. Data is presented as mean ± SD (n = 4).