Supplementary Information (SI) for Analytical Methods.
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Supporting Information

A label-free colorimetric aptasensor for multiple rapid detection of leuco-malachite green and leuco-crystal violet

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Conflict of Interest: The authors declare no competing financial interest.

1. Experimental sections

1.1 Preparation of AuNPs

AuNPs (particle size: 18 nm) were synthesized by citrate reduction method. In a 500 mL flask, 1 mL of 1% (m/V) $HAuCl_4$ was mixed with 99 mL of deionized water. After thorough mixing, the mixture was boiled. Then 2.2 mL of 1% (m/V) sodium citrate was added to the boiling $HAuCl_4$ solution. When the solution turned red and the color no longer changed, heating was stopped and the solution was gradually cooled to room temperature. Continuous stirring was maintained throughout the experiment. The obtained solution was stored at 4 ℃ for future use.

1.2 Optimization of detection system

PDDA concentration. Various PDDA concentrations (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 nM) were individually incubated with 1.62 nM AuNPs for 15 minutes. Subsequently, the absorption spectrum ranging from 400 to 800 nm was scanned, and the absorbance ratio (A_{670}/A_{520}) was calculated. The PDDA concentration corresponding to the maximum absorbance ratio was selected as the optimal concentration.

A5-b concentration. Different concentrations of A5-b (0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140 nM) were individually incubated with the optimal PDDA concentration for 20 minutes. Then, 1.62 nM AuNPs were added to each well and incubated for 15 minutes. Finally, the absorption spectrum ranging from 400 to 800 nm was scanned, and the absorbance ratio (A_{670}/A_{520}) was calculated. The A5-b concentration corresponding to the minimum absorbance ratio was chosen as the optimal concentration.

pH. Under optimal conditions, PDDA and A5-b were incubated at different pH (2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5) MOPS buffers for 20 minutes, respectively. Then, add 1.62 nM AuNPs to each well and incubate for 15 minutes. Finally, scan the absorption spectrum from 400 to 800 nm and calculate the absorbance ratio (A_{670}/A_{520}). The pH corresponding to the minimum absorbance ratio was selected as the optimal concentration.

Temperatures. Under optimal conditions, PDDA and A5-b were incubated at different temperatures (5, 10, 15, 20, 25, 30 °C) for 20 minutes respectively. Then, add 1.62 nM AuNPs to each well and incubate for 15 minutes. Finally, scan the absorption spectrum from 400 to 800 nm and calculate the absorbance ratio (A_{670}/A_{520}). The temperature corresponding to the minimum absorbance ratio was selected as the optimal concentration.

2. Supplementary figures

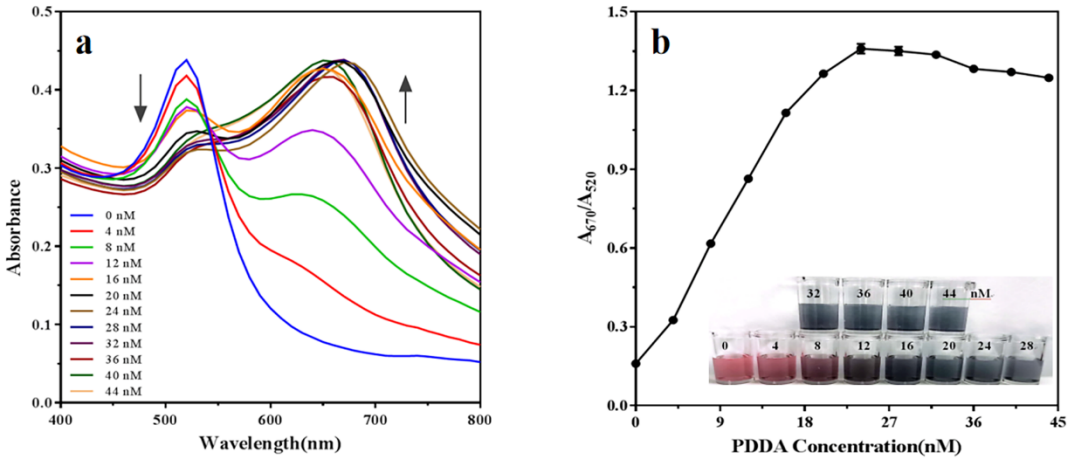

Fig.S1 (a) Absorption spectra and (b) A_{670}/A_{520} ratio saturation curve of AuNPs at various PDDA concentrations

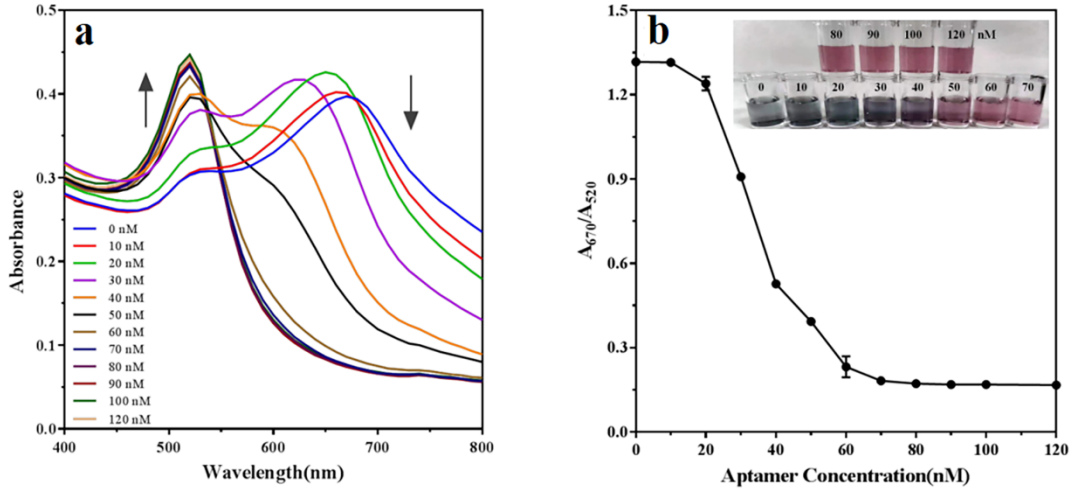

Fig.S2 (a) Absorption spectra and (b) A_{670}/A_{520} ratio saturation curve of AuNPs at different A5-b concentrations

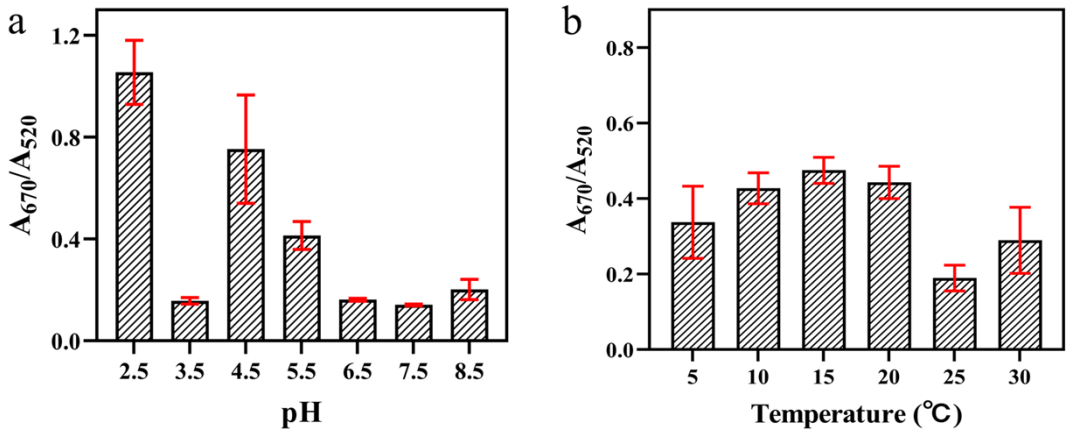

Fig.S3 A_{670}/A_{520} ratio histogram of AuNPs at different pH (a) and temperature (b).

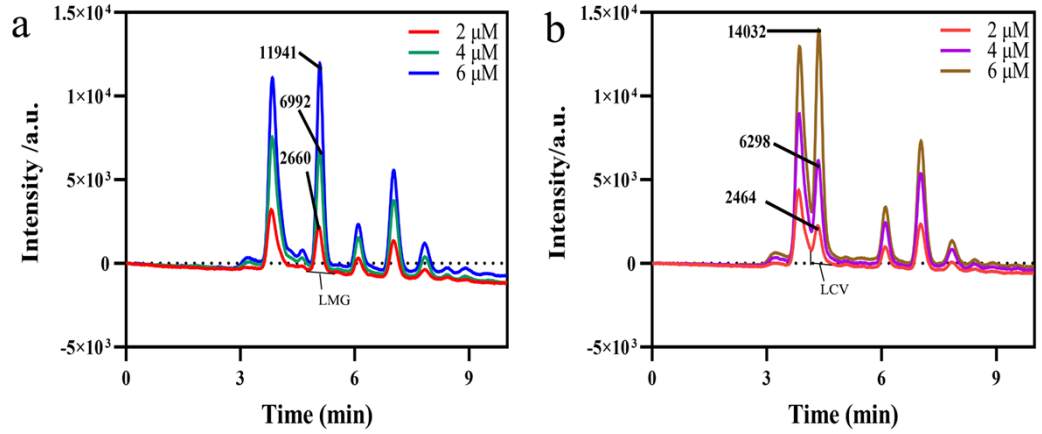

Fig.S4 HPLC of crucian samples spiked with different concentrations of LMG (a) and LCV(b).