

## Supplementary Material

### A Rapid and Sensitive Aptasensor for Cyromazine Detection in Raw Milk Based on the Nanogold Probe and G-Quadruplex Formation

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## Experimental

### Materials and apparatus

A ssDNA was designed to form a G-quadruplex-CYR complex with cyromazine self-assembly, and some ssDNA with different sequences were also used as a control. All the sequences are as follows:

Tcyr1 5'-GGTTTGGTTGGTTTGGTT-3' (18 bp)

Apt1 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3' (31 bp)

Apt2 5'-GGGTAGGGCGGGTTGGGG-3' (18 bp)

Tcy2 5'-FAM-GGTTGGTTGGTTGGTTT-3' (18 bp)<sup>1</sup>

RandomDNA 5'-TCATGATACGCGATGAGC-3' (18 bp)

All these aptamers above were synthesized by Sangon Biotechnology Co, Ltd. (Shanghai, China).

Chloroauric acid, trisodium citrate, potassium chloride and sodium chloride were bought from Shanghai Chemical Reagent Company (Shanghai, China). Cyromazine and Poly (diallyldimethylammonium chloride) (PDDA) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). The raw milk was purchased from a local cattle farm nearby. All reagents were of analytical grade. A J-815 CD spectrometer (Jasco, Japan) was employed to characterize the structural changes in the oligonucleotides. The scans (100 nm min<sup>-1</sup>) from 200 to 320 nm were taken three times at 1 nm intervals, then accumulated and averaged<sup>2,3</sup>. Absorption spectra was performed on a UV-2410 PC UV-vis Spectrophotometer (SHIMADZU). The pH measurements were recorded on DELTA 320 pH meter (METTLER TOLEDO).

### **Preparation of AuNPs**

The trisodium citrate reduction method was used to prepare AuNPs. 100 mL of 0.01% (w/w) HAuCl<sub>4</sub> solution was heated to boiling for 2 min, and 3.5 mL of 1% sodium citrate solution was added quickly into the boiling HAuCl<sub>4</sub> solution with vigorous stirring. The color of the mixed solution immediately changed from pale yellow to purple, then to bright red in the end. After the heating stopping, the solution was stirred continuously about 15 min until it cooled to room temperature. Finally, the cooled solution was diluted with 100 mL of water and stored in the refrigerator at 4 °C.

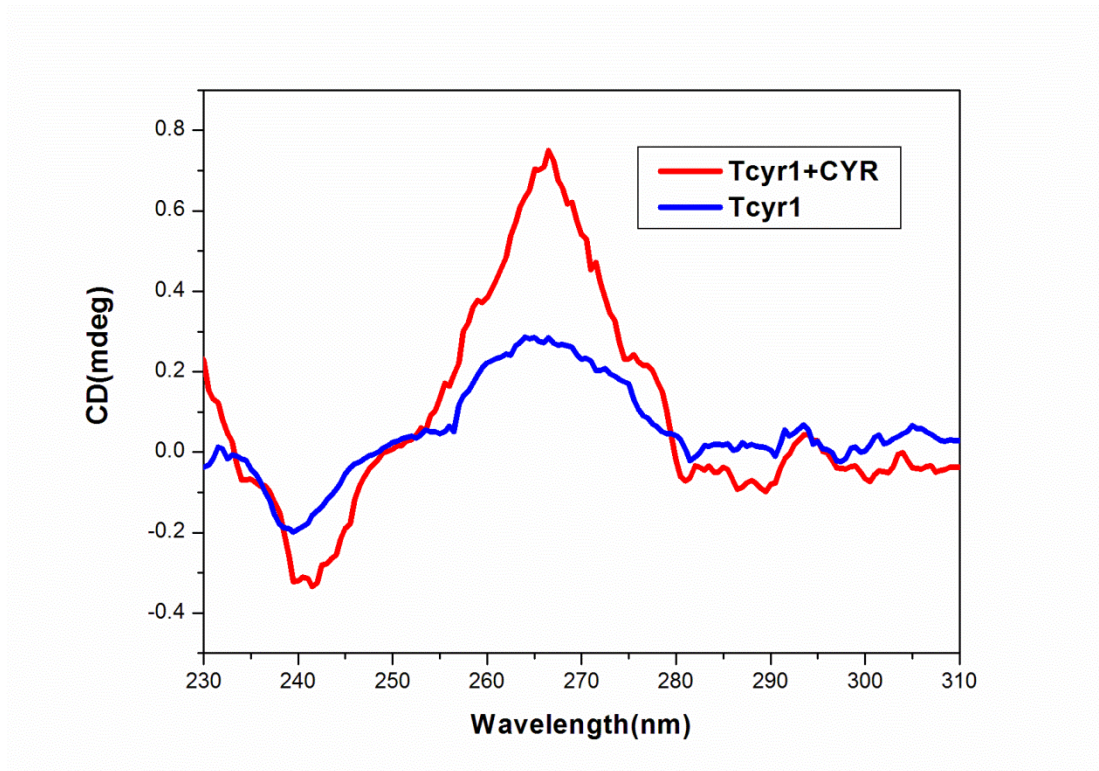
### **Preparation of biosensor solution for cyromazine detection**

1.0 mL 164 nM ssDNA and 4.0 mL 55.9 µg/mL AuNPs solutions were added into a 5 mL centrifuge tube, mixed well, and then heated in water bath for 20 min at 30°C. Then, 100 µL 20 nM PDDA was added into the mixture. The final concentration of AuNPs was calculated as 44.7 µg/mL, the final concentration of aptamer was calculated as 32.8 nM, as the final concentration of PDDA was 0.4 nM.

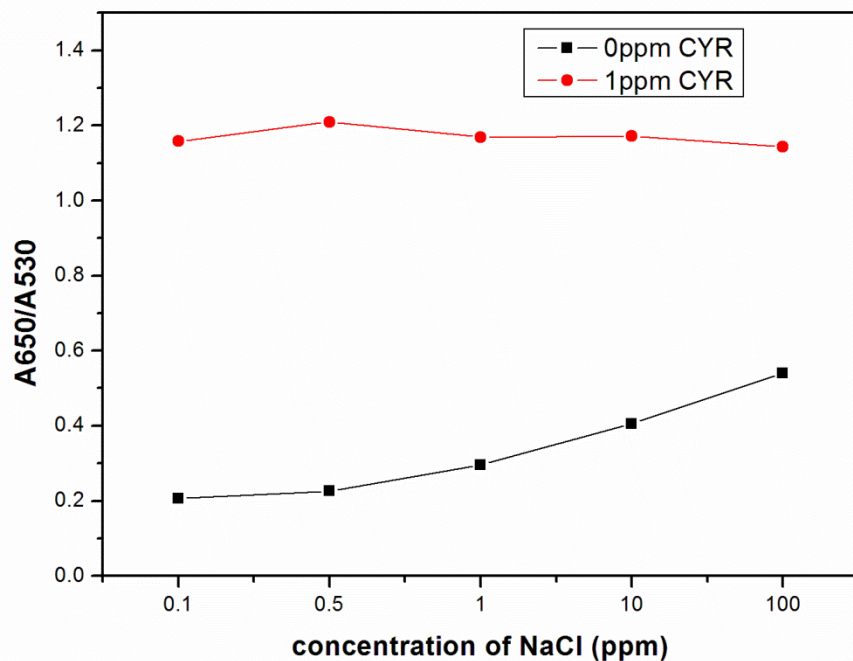
### **Procedure of cyromazine determination**

Varying concentrations of cyromazine were mixed with the biosensor solution (volume ratio 1:1), color and spectra changes were observed by naked eyes and

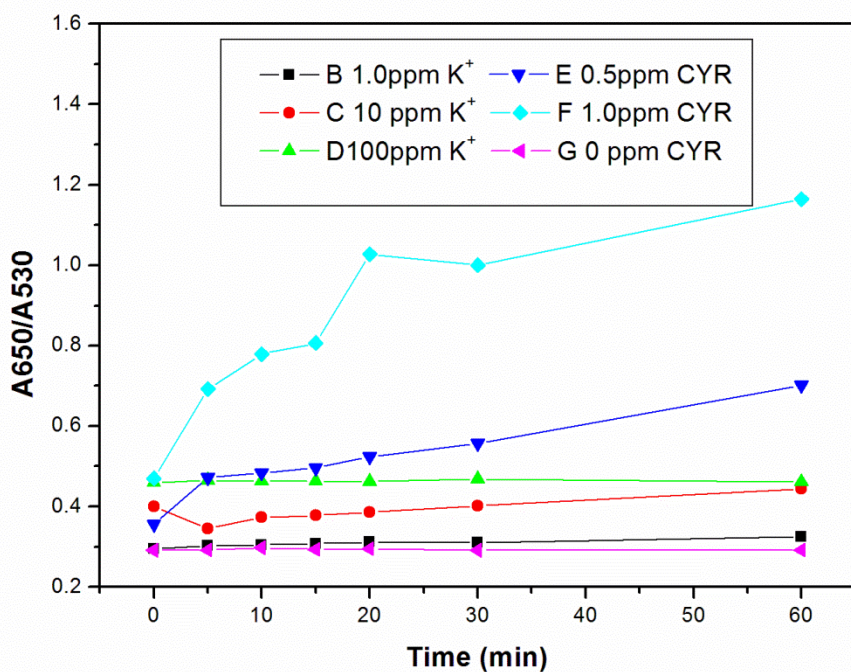
UV-vis. The sample ratios of A650/A530 were recorded to indicate the aggregation of AuNPs. After the optimization of reaction, a series of samples with different concentrations for the biosensor's sensitivity and selectivity research were prepared in the raw milk, and the ratios of A650/A530 were also plotted.



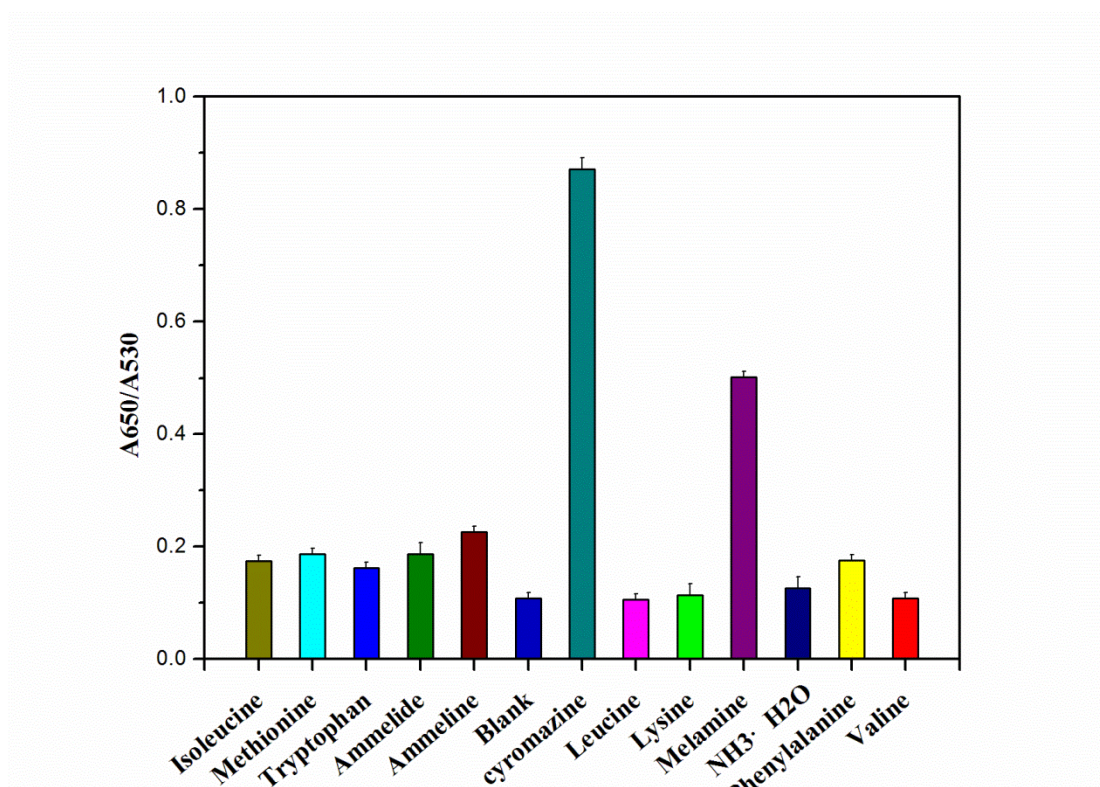
**Fig. S1** CD spectra of 1  $\mu$ M Tcy1 in the absence and in the presence of 1 ppm cyromazine. The cyromazine treatment reaction was performed for 5 min at room temperature



**Fig. S2** The effect of different ionic strengths on cyromazine detection. The concentrations of aptamer and PDDA were 32.8 nM and 0.4 nM. The reaction time was 20 min.



**Fig. S3** Kinetics curves of 32.8 nM Tcyr1 in the absence and in the presence of cyromazine (0.5 ppm, 1.0 ppm)



**Fig. S4** Selectivity of the assay for cyromazine detection over other compounds concentrations: cyromazine (500 ppb); others (500 ppb). The concentration of Tcyr1 is 32.8 nM.

## References

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- 3 DW Jr, AT Showler, MW Donahue, BE Vinson, WL Osbrink, Lethal Effects of the Insect Growth Regulator Cyromazine Against Three Species of Filth Flies, *Musca domestica*, *Stomoxys calcitrans*, and *Fannia canicularis* (Diptera: Muscidae) in Cattle, Swine, and Chicken Manure, *Journal of Economic Entomology* , 2017 , 110 (2) :776-782.