

Supporting Information

Rapid and Ultra-Sensitive Testosterone Detection via Aptamer- functional gold nanoparticles

MATERIALS AND METHODS

Materials and Instruments. All organic solvents were obtained from Beijing Chemical Works and used as received. Dexamethasone, salbutamol, and ractopamine were purchased from ehrenstorfte. Glucose, fructose, salidroside, tetrachloroauric(III) acid trihydrate, and sodium citrate were purchased from J&K Scientific Ltd. All chemicals were used without further purification. All of the oligonucleotides used in this paper were synthesized by Sangon Biotechnology Inc. (Shanghai, China). The aptamer sequence of Testosterone was listed as follow: 5'-

TAGGGAAGAGAAGGACATATGATTGCGTGGGTAGGAAGGGGCGGT
GTGATCTGAATCGTTCGATTGACTAGTACATGACCACTTGA-3'. The UV-Vis absorption spectra were recorded via a Thermo Scientific Varioskan LUX. The dynamic light scattering (DLS) was recorded with Malvern Zetasizer Nano ZS90. Hitachi HT7700 transmission electron microscopy (TEM) was employed to determine the morphology of AuNPs before and after aggregation.

Optimization of NaCl Concentration. 20 μL milli-Q water was mixed with 70 μL AuNPs. Then, different concentration of NaCl solution at the volume of 10 μL was added. After co-incubated for 5 min in 96-well plate, the A_{650}/A_{520} ratio in absorption spectrum was recorded.

Optimization of aptamer during dope detection. 10 μL mill-Q was pre-mixed with different concentration of aptamer at a volume of 10 μL . Then, 70 μL AuNPs were added into solution, and co-incubated for 15 min. Finally, NaCl (10

μL) were added into each well and co-incubated for another 5 min. According to the results of A_{650}/A_{520} ratio from absorption spectrum, the minimum concentration of aptamers to prevent the aggregation of AuNPs was the optimal salt concentration.

Procedures of detection of Tes during dope detection. Aptamer (10 μL) and Tes (10 μL) were mixed and incubated for 20 min. Then, 70 μL AuNPs were added into supernatant. Finally, NaCl was added to mixtures, following the monitoring using UV/Vis spectrometer.

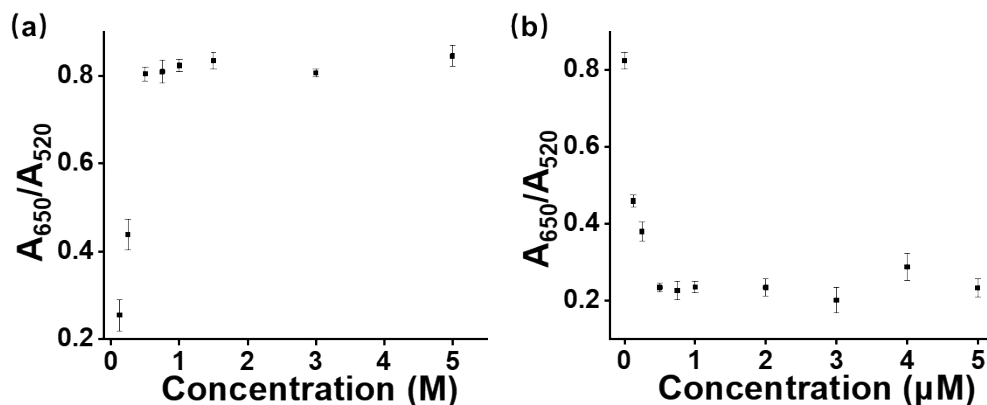


Figure S1. The A_{650}/A_{520} nm of AuNPs under various concentrations of NaCl (a) and Aptamers (b).

Method	LOD [nM]	Linear range [nM]	Ref.
LC-MS/MS	0.17	0.69-27.736	[1]
UPLC-MS	14.68	14.68-137.57	[2]
Enzyme immunoassays (EIA)	23.57	23.57-41.60	[3]

Table S1. Comparison with other reported methods for the detection of TES

References

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2. Escobar-Wilches, D.C.; Ventura-Bahena, A.; de Lourdes Lopez-Gonzalez, M.; Torres-Sanchez, L.; Figueroa, M.; Sierra-Santoyo, A. Analysis of testosterone-hydroxylated metabolites in human urine by ultra high performance liquid chromatography-Mass Spectrometry. *Analytical biochemistry* **2020**, *597*, 113670, doi:10.1016/j.ab.2020.113670.
3. Moyano, H.B.; Santos, R.; Pinilla, M.P.R. Validation of an enzyme immunoassay for the quantification of testosterone in green iguana males (Iguana iguana). *General and comparative endocrinology* **2020**, *287*, 113343, doi:10.1016/j.ygcen.2019.113343.