Electronic Supplementary Information

A molecular recognition assisted colorimetric aptasensor for tetracycline

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Supplementary figures

Supplementary figure 1

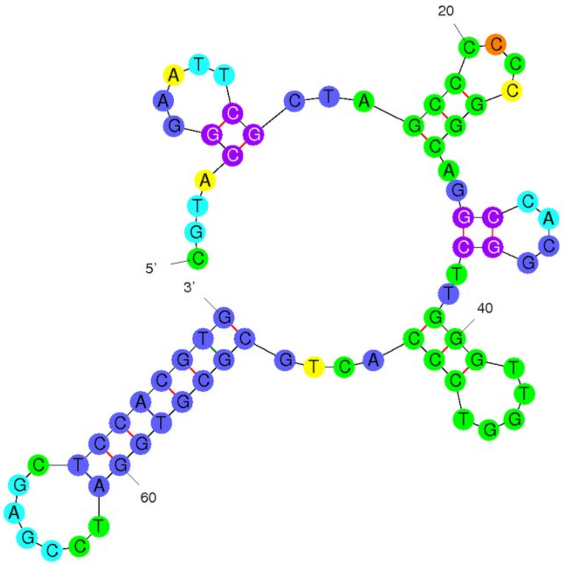

Fig. S1 Predicted secondary structure of the 76 mer aptamer [1,2].

The single-strand 76 mer DNA aptamer was custom synthesized with 3' -end biotinylated modification (Mw. (23747.43), Kd=63.6 nM) by Sangon Biotech Co., Ltd. (Shanghai, China), with the following sequence[2]: 5'-CGTACGGAATTCGCTAGCCCCCCGGCAG GCCACGGC TTGGGTTGGTCCCACTGCGCGTGGATCCGAGCTCCACGTG-3'.

Supplementary figure 2

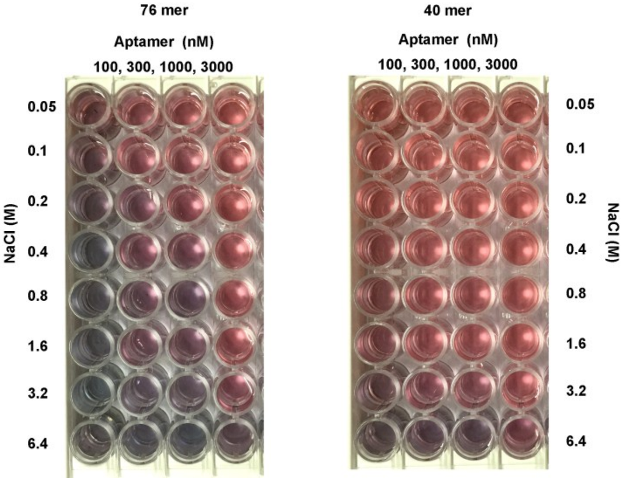

Fig. S2 Primary evaluation of the 76 mer aptamer and 40 mer aptamer in AuNPs-based aptasensor. 100, 300, 1000, 3000 nM of aptamers were used, and different concentrations of NaCl were applied to trigger the aggregation of the AuNPs.

We applied different concentrations of NaCl to evaluate different concentrations of the two aptamers. The assay protocol was designed as following: The citrate-coated AuNPs were modified with the 40 mer/76 mer aptamer for 15 min at room temperature with mild shaking. Then different concentrations of NaCl were added into different cuvettes, and visual results were observed.

As is shown, to protect the AuNPs from aggregation, higher concentration of the 76 mer was needed than the 40 mer aptamer. As the aptamer is the recognition element in the detection system, high concentration of the aptamers will result in low sensitivity of the system. Thus the 76 mer aptamer was not chosen as the recognition element in the present AuNPs-based aptasensor.

Supplementary figure 3

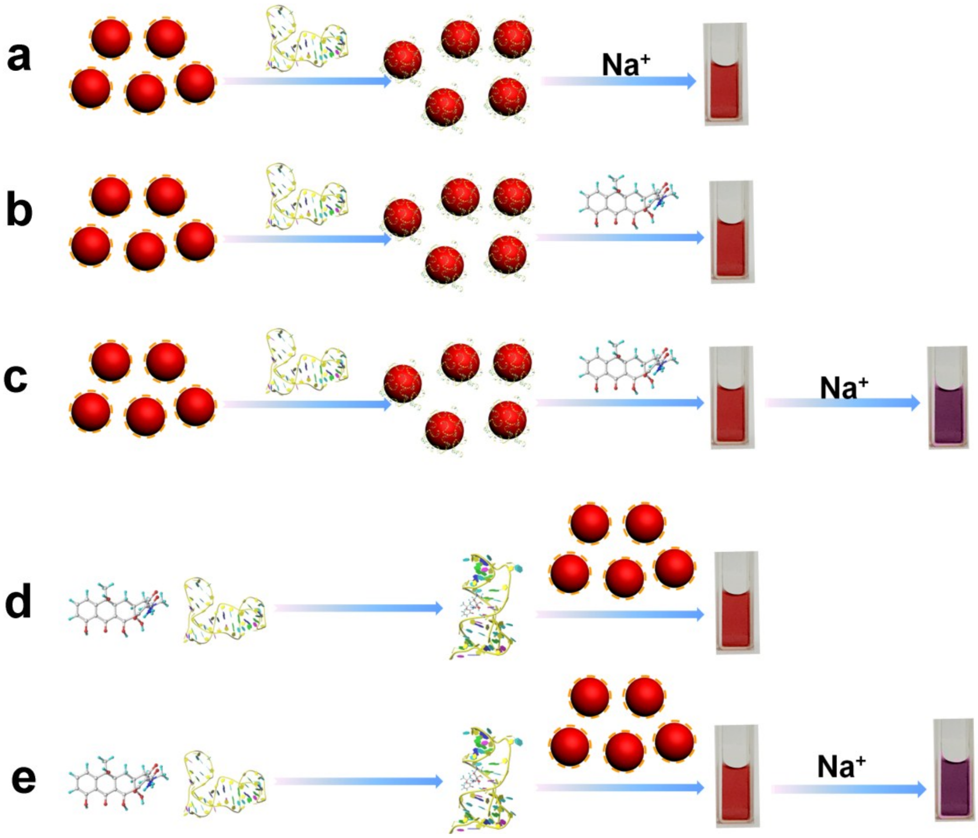

Fig. S3 Schemes of the confirmation assays of the adsorption and desorption of the 40 mer aptamer. (a) The citrate-coated AuNPs were modified with the 40 mer aptamer for 15 min at room temperature with mild shaking. Then 0.2 M of NaCl was added into the mixed solutions. (b) The citrate-coated AuNPs were modified with the 40 mer aptamer for 15 min at room temperature with mild shaking. Then different concentrations of TC were added to react with the aptamer-coated AuNPs for 10 min in different cuvettes. (c) The citrate-coated AuNPs were modified with the 40 mer aptamer for 15 min at room temperature with mild shaking. Different concentrations of TC were added to react with the aptamer-coated AuNPs for 10 min. Then 0.2 M of NaCl was added into the mixed solutions. (d) 300 nM of aptames were mixed with different concentrations of TC for 10 min at room temperature with mild shaking. Then the mixture was added into AuNPs solutions. (e) 300 nM of aptamers was mixed with different concentrations of TC for 10 min at room temperature with mild shaking. Then the mixture was added into AuNPs solutions and 0.2 M of NaCl was added. The gradient concentrations of TC was 20, 40, 80, 160, 320 ng/mL.

To confirm the scheme in the manuscript, herein we designed several experiments to prove the label-free aptamer can be adsorbed onto the surface of AuNPs firstly, and will be desorbed from the surface of AuNPs after the addition of TC. The adsorption can be proved easily as shown in **Fig. S3a**. For the desorption (detachment) of aptamers after the addition of TC, as the concentration of aptamer used in the detection system was not high, it is not suitable to measure the DNA concentration to prove the detachment of aptamer. Thus, in the present study, we proved the easy detachment of aptamer with the comparison results of several assays (**Fig. S3b-e**). And the results are shown in **Fig. S4**.

Supplementary figure 4

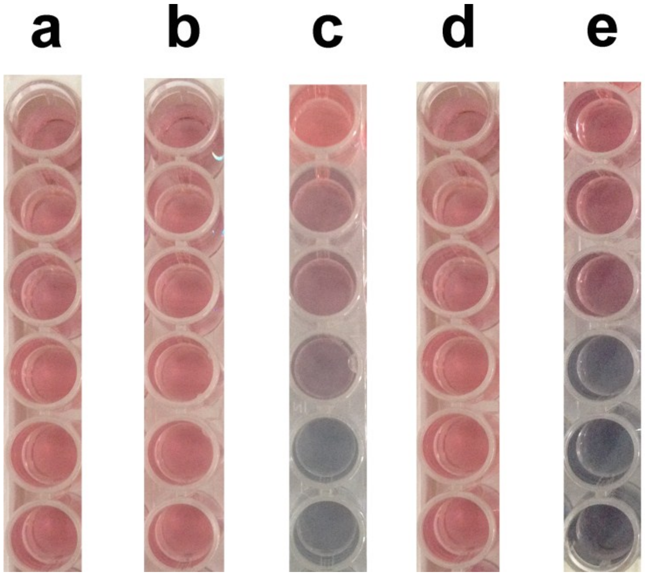

Fig. S4 Colorimetric results of confirmation assays according to schemes in **Fig. S3**. Results are corresponding to the schemes in **Fig. S3**. (a) Citrate-coated AuNPs + Aptamers + NaCl. (b) Citrate-coated AuNPs + Aptamers +TC. (c) Citrate-coated AuNPs + Aptamers +TC + NaCl. (d) Aptamer-TC mixture + Citrate-coated AuNPs. (e) Aptamer-TC mixture + Citrate-coated AuNPs + NaCl. The gradient concentrations of TC was 20, 40, 80, 160, 320 ng/mL, from top to bottom.

As is shown in **Fig. S4**, the adsorption of aptamers on the surface of AuNPs can be proved by Fig. 4a (please refer to the scheme in **Fig. S3a**). The adsorbed aptamers will protect AuNPs from aggregation. To prove the detachment of aptamers after addition of TC, we carried out experiments and analyzed the results as following: If the aggregation of AuNPs is caused by anchoring of aptamers on multiple AuNPs to one tetracycline molecule, then after addition of TC into the aptamer-coated AuNPs (please refer to the scheme in **Fig. S3b**), the microwell with high concentration of TC should change color from red to blue. However, the results in **Fig. S4b** demonstrate that there was no obvious aggregation after adding TC into aptamer-coated AuNPs, and there is obvious aggregation only after the addition of NaCl (**Fig. S4c**, please refer to the scheme in **Fig. S3c**). With the results of these three groups, we can prove that the aptamers are detached from the surface of AuNPs by TC, because if the aptamers are adsorbed on the surface of AuNPs, there will be no aggregation after the addition of NaCl (please refer to the protective effect of aptamer as shown in **Fig. S3a and Fig. S4a**), but our results showed there is aggregation after the addition of TC and NaCl (as shown in **Fig. S3c and Fig. S4c**). And if the aggregation is caused by anchoring of aptamers on multiple AuNPs to one tetracycline molecule, there will be obvious aggregation after adding enough TC (as shown in **Fig. S3b and Fig. S4b**), while there is no obvious aggregation after adding TC and the aggregation will occur only after addition of NaCl (**Fig. S3c and Fig. S4c**).

In addition, to further prove the folded aptamers cannot be adsorbed onto the surface of citrate-coated AuNPs, we designed two other experiments (please refer to the scheme in **Fig. S3d** and **Fig. S3e**). Aptamers were pre-incubated with different concentrations of TC for 10 min at room temperature with mild shaking. Then the mixture was added into citrate-coated AuNPs solution. There is no obvious aggregation of AuNPs after the addition of TC (as shown in **Fig. S3d** and **Fig. S4d**), and there is obvious aggregation after the addition of NaCl (as shown in **Fig. S3e** and **Fig. S4e**), which means that aptamers in the aptamer-TC mixture tended to fold into specific secondary structure with high rigidity, hidding the positively charged bases [3], and these structures cannot be adsorbed onto the surface of AuNPs. Thus after addition of NaCl, there is obvious aggregation. Otherwise, if the aptamers can still be adsorbed onto the surface of AuNPs, the adsorbed aptamers will protect the AuNPs from aggregation.

References

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