Electronic Supplementary Material (ESI) for Analyst.

Supplementary material

A microfluidic chip based ratiometric aptasensor for antibiotic detection in foods using stir bar assisted sorptive extraction and rolling circle amplification

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Content

Table.S1. The sequence oligonucleotides used in this strategy.

Name	Sequence(5'—3')
Kana apt	SH-TTT AGA TGG GGG TTG AGG CTA AGC CGA TCA CTA
Ligation probe	CCA CGA CGG GAG TGA CGC AGG TGT AAT TGG AGC CGA CGG ATC GGC TTA GCC TC
Padlock probe	CTG CGT CAC TCC CGT CGT GGG CTG AAT CCG TTA GCC AGC AGT CAC TTA TTC AGG CGT AGC ACC AGT CCG TCG GCT CCA ATT ACAC
Primer	TAG TGA TCG GCT TAG CTG GTG CTA CGC CTG AAT AAG TGA

Table. S2. Voltage program applied for sample injection and MCE separation. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir.

Procedure	Time(s)	Voltage applied into the reservoirs (V)			
		S	B	SW	BW
sample injection	50	280	320	510	0
MCE separation	185	250	0	250	1000

Figure. S1. The structure of the MultiNA 202 system.

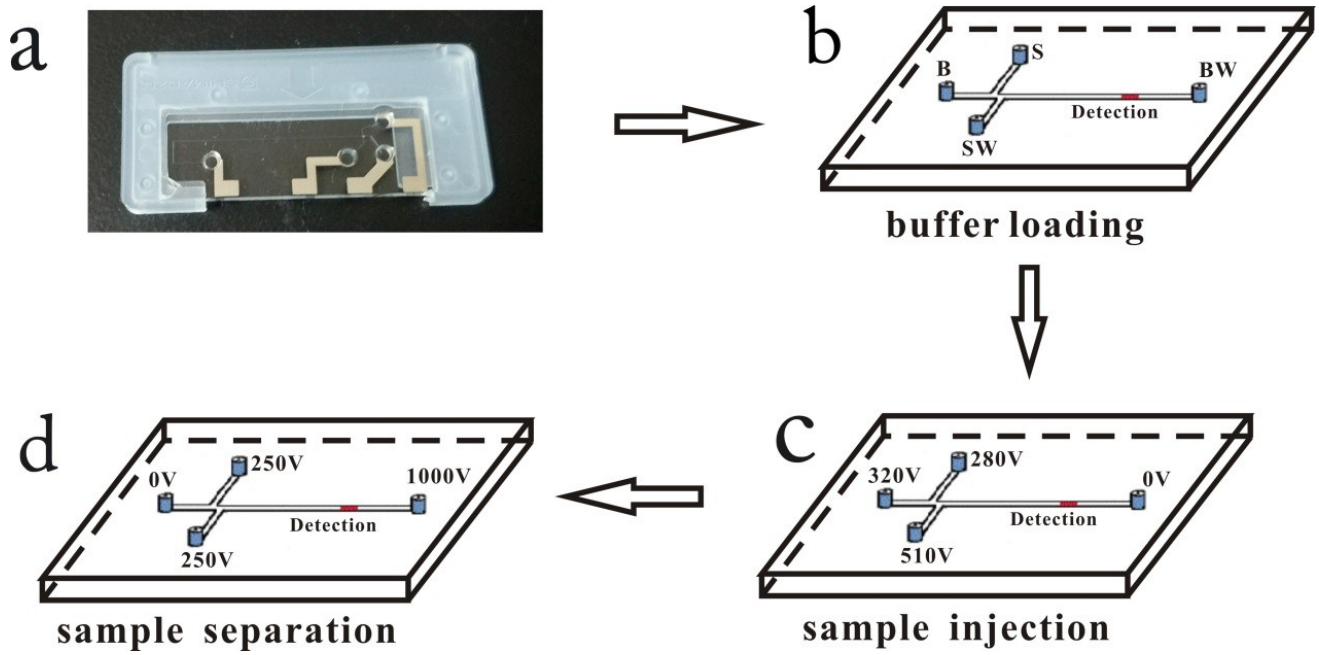


Figure. S2. Scheme illustration of sample analysis based on MCE with (a) the image of microchip; (b) buffer loading; (c) sample injection; (d) sample separation.

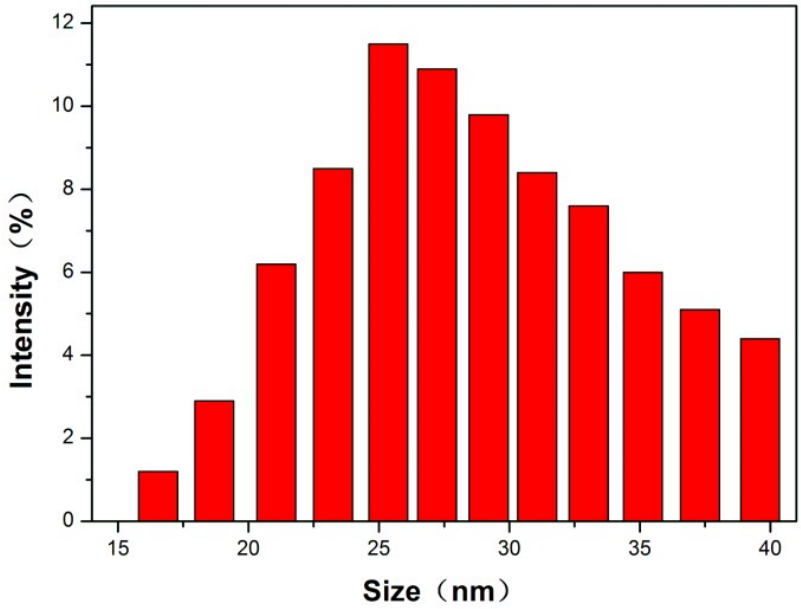


Figure. S3. The DLS images of AuNPs.

Preparation of gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) were prepared by the modified citrate reduction method. In brief, a mixture of water (198 mL) and $HAuCl_4$ (2 mL, 1 wt %) were transferred to a clean beaker and then heated to boil under stirring in an oil bath pan. When the mixture was boiling at about 130 °C, 5 mL of a 1% sodium citrate solution was added to the system quickly, with vigorous stirring until the color turned claret red. The mixture was maintained heated for 15 min before removing the heating source. Finally, the solution was cooled to room temperature and then stored at 4 °C before use.

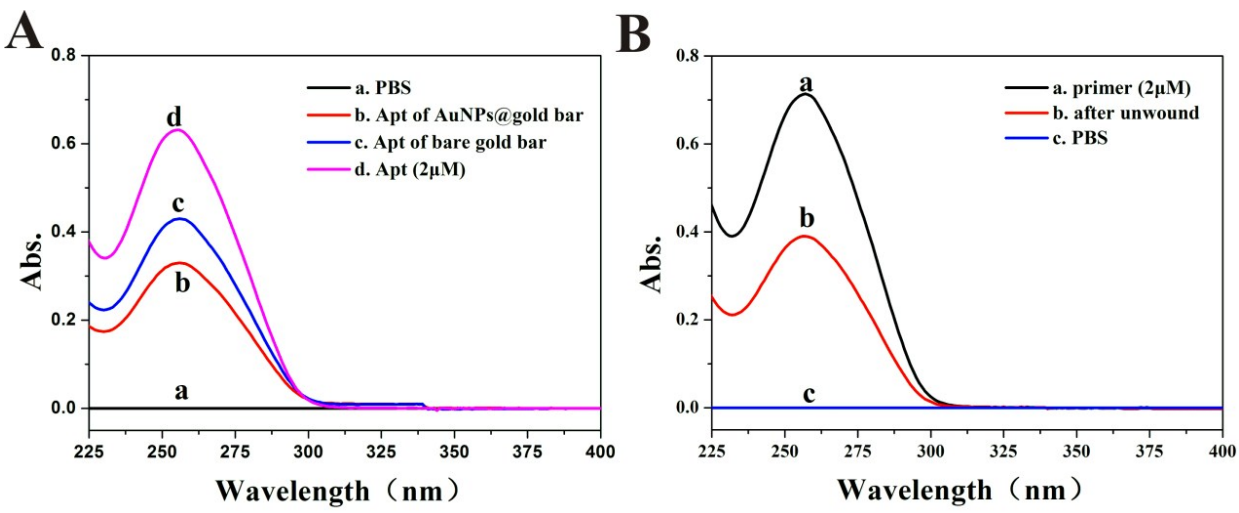
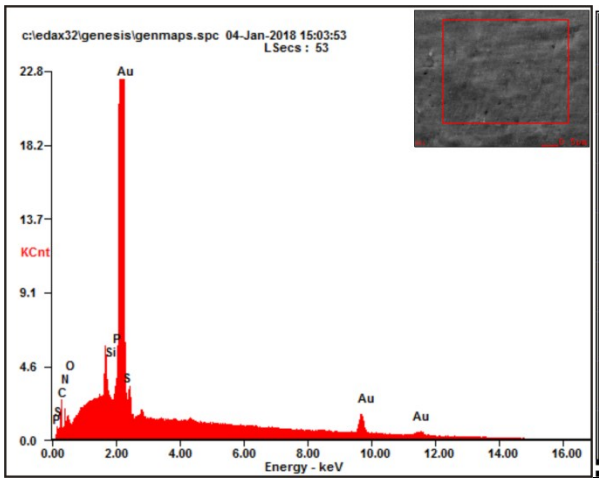


Figure. S4. (A) The UV–vis characterization of the enrichment capacity of the bare gold bar and the AuNPs@ gold bar. Curve a: PBS solution; curve b: aptamer solutions were modified onto the surface of the AuNPs@ gold bar; curve c: aptamer solutions were modified onto the surface of bare gold bar; curve d: aptamer solutions (2 μM). (B) The amount of hybridized primer on gold stir bar after modified with aptamer. Curve a: primer standard solution (2μM); curve b: the supernatant after unwound; curve c: PBS solutions.

As seen in Fig. S4A, a characteristic absorption peak of thiol Kana aptamer (2 μM) appeared at 260 nm (curve d), after the aptamer solutions were modified onto the surface of the AuNPs@ gold bar (b) and bare gold bar (c), two UV absorption peaks at 260 nm of DNA was observed. Compared to c, the amount of aptamers in the supernatant after reaction in c were decreased more (about 2 folds), it can prove that the amount of aptamers immobilized on the surface of the AuNPs@ gold bar are more than the bare gold bar. The main reason was due to the enlarged surface areas increase of gold bar. Furthermore, we calculated the coupling efficiency from the aptamer concentration in sample solutions before and after the immobilizing reaction, which was measured by UV－Vis absorption spectrometry. The results showed that the coupling efficiency was 51% for the AuNPs@ gold bar while the bare gold bar is 25%. After calculation, it can be seen that the amount of aptamer modified on the stir bar is about 200 pmol. In the same way, the amount of hybridized primer on gold stir bar

after modified with aptamer was calculated about 182 pmol which showed in Fig. S4B.



Element	Wt%	At%
CK	02.89	24.67
NK	02.41	17.65
OK	00.51	03.28
SiK	00.38	01.38
PK	01.27	04.19
AuM	92.26	47.96
SK	00.27	00.88
Matrix	Correction	ZAF

Figure. S5. The EDX of stirring bar functionalized with gold-labeled aptamer.

In order to confirm that aptamers were successfully modified on the surface of gold stirring bar, energy dispersive X-ray spectroscopy (EDX) was performed. Peaks corresponding to C, N, O, Si, P, Au and S were observed. The peak corresponding to phosphorus could be attributed to the immobilization of aptamer.

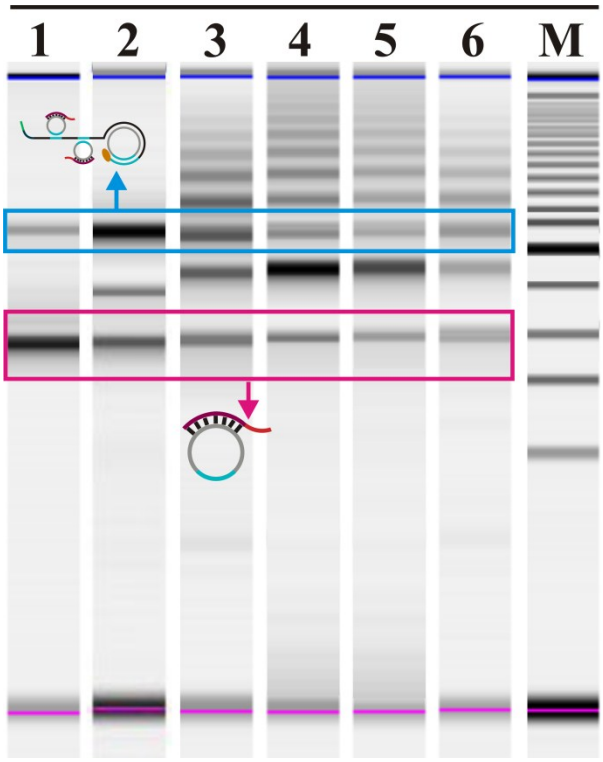


Figure. S6. The different reaction time of RCA (lane 1: 15min; lane 2: 30min; lane 3: 35min; lane 4: 40min; lane 5: 45min; lane 6: 50min; M: marker) corresponds to the Electrophorograms.

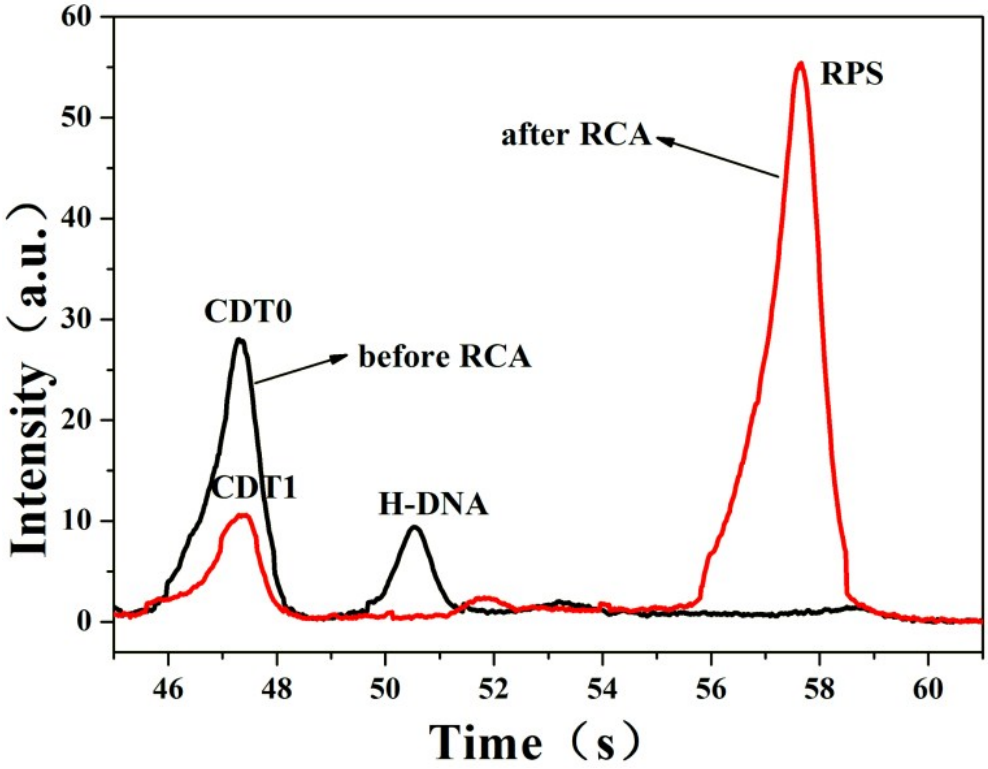


Figure. S7. The signal amplification of the developed method and detection for Kana by MCE. Black curve: before RCA; red curve: after RCA.

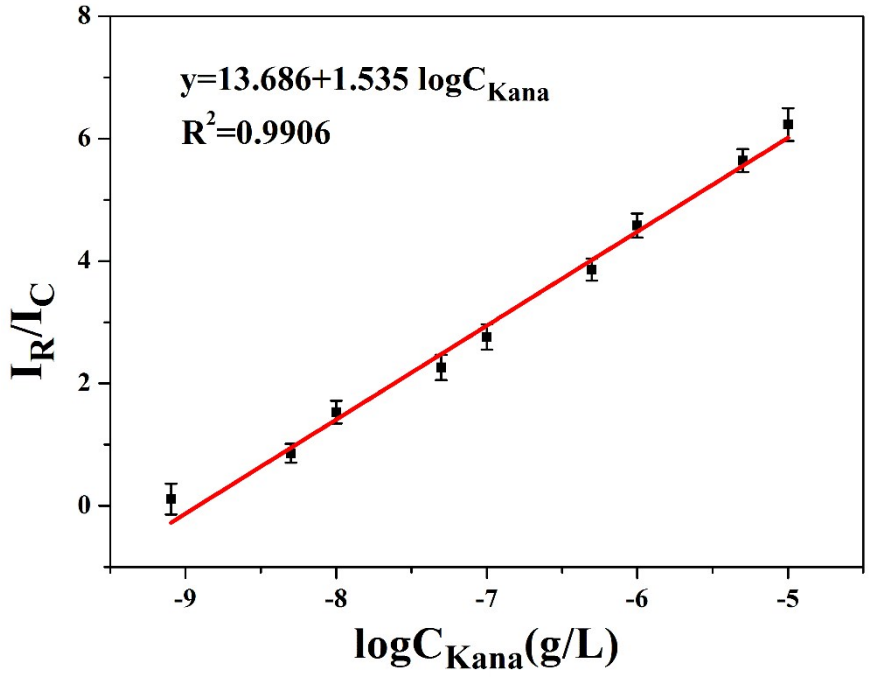


Figure. S8. The corresponding calibration curve for different concentrations of Kana (0.0008–10 ng·mL^{-1}) assay.

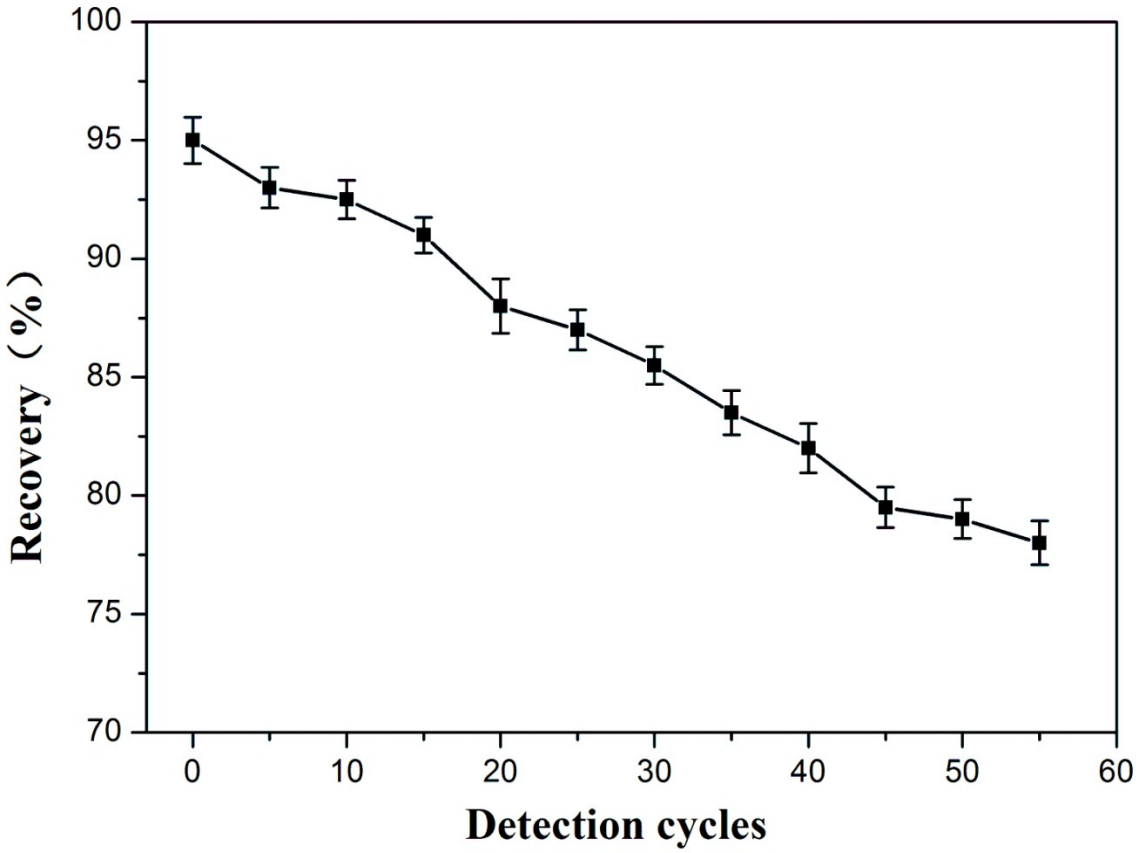


Figure. S9. Reusability of the stir bar functionalized with gold-labeled aptamer (n = 3).

The reusability of the stir bar modified with Kana aptamer was tested in Fig. S9, by performing 55 repetitions of kana detection in foods. The result indicated that the detection recovery had no evident decrease within 40 repetitions. Therefore, it was stable enough to be applied in kana detection at least 40 cycles with a recovery of over 80%.