Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A Sensitive and Low background Fluorescent Sensing Strategy Based on g-C_3N_4-MnO_2 Sandwich Nanocomposite and Liposome Amplification for Ricin Detection

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Supplementary Discussion

Calculation of the number of enzyme-encapsulated liposomes

The lipid molecules (N_{tot}) number in a GOD-liposome was calculated according to equation (1):

$$N_{tot} = \frac{[4\pi\left(\frac{d}{2}\right)^2 + 4\pi\left[\frac{d}{2} - h\right]^2]}{\alpha_L} \quad (1)$$

Where d is the hydrodynamic diameter, h is the bilayer thickness, and α_L is the average head group surface area per lipid. The lipid bilayer thickness was assumed to be 4 nm and α_L value for phosphatidylcholine, phosphoethanolamine and cholesterol were 0.65 ± 0.01 nm^2, 0.52 ± 0.01 nm^2, and 0.41 nm^2, respectively. Rely on those values and the molar fraction of each component, the α_L value obtained for our produced liposomes was 0.6 nm^2/lipid. The N_{tot} was calculated to be 2.7 ×10^5 lipid molecules per liposome. The number of liposomes per milliliter (N_{lipo}) can be derived from the lipid concentration with Avogadro's number as shown in equation (2):

$$N_{lipo} = \frac{M_{lipid} \times N_A}{N_{tot} \times 1000} \quad (2)$$

Where M_{lipid} is the molar concentration of lipid and N_A is Avogadro's number. The number of GOD-encapsulated liposome was calculated based on the number of lipid molecules in a liposome and the lipid concentration used to compose the liposome solution. Given that the total concentration of lipid used to compose the liposomes is 13 mM, the number of liposomes N_{lipo} was calculated to be 2.9×10^{13} liposomes per mL.

During the fabrication process, the liposome solution lost during extrusion and dialysis step was estimated to be around 10 ±3% and 40.8±2%, respectively. The data were evaluated preparing similar liposomes with fluorescent phosphatidylcholine lipids and analyzed with UV-vis spectrometry. Thus, liposome recovery number after preparation is calculated to be 1.2×10^{12} liposomes per mL.

The number of glucose oxidase molecules encapsulated in one liposome was calculated according to equation (3):

$$N_{GoD} = \frac{M \times N_A}{M_r \times N_{lipo}} \quad (3)$$

Where M is enzyme mass, and Mr is the relative molecular mass according to the aforementioned equation. Therefore, the loading capacity was obtained to be 1184±7 molecules/liposome.

Calculation of the encapsulation efficiency in the liposome

Encapsulation efficiency (EE) was evaluated through three steps. First, a standard calibration curve of free GOD was established (Fig. S3a) and linear absorption equation: A=0.9997 c+ 0.9993 was obtained; second, 30 μL of liposomes was broken by 1×PBST (1×PBS and 0.05% Tween-20, pH 7.4) and release the encapsulated GOD, which can produce certain absorption (A=6.90). According to the linear equation, we can deduce the concentration of GOD as 5.9 mg/mL. Therefore, the total encapsulated GOD mass in liposome is calculated to be 11.8 mg (5.9 mg/mL×2 mL). Third, 59% of encapsulation efficiency was acquired by using total encapsulated GOD mass (11.8 mg) divide the added mass (20 mg).

The enzyme activity is directly related to the sensitivity of the proposed sensing assay. The influence of Triton X-100 on GOD activity was investigated. The detailed procedure is: 1% Triton X-100, 0.075 mg/mL GOD, 1.5 mM glucose and 40 μg/mL g-C_3N_4-MnO_2 nanocomposite were added together and kept at 55 ℃ for 10 minutes. PBS solution was used to replace Triton X-100 as a control. The fluorescence intensity signal is negligible change when adding Triton X-100 (Fig. S3d). It was confirmed that Triton X-100 has no influence on GOD activity.

Supplementary Tables

Table S1 Sequences of Oligonucleotides[1, 2]

Name	Sequences (5' to 3')
aptamer	ACACCCACCGCAGGCAGACGCAACGCCTCGGAGACT TTTTT-biotin
blocker	CCTGCGGTGGGTGTTTTTTTTTTTTTTTTTTTTT-cholesterol
poly-T	TTTTT-biotin

Table S2 Characterizations of GOD-free liposome and GOD-liposome

Sample	mean diameter(nm)[a]	Polydispersiy index[a]	Zeta potential(mV)[a]
GOD-free Liposome	142±10.3	0.250±0.020	-26.5±0.9
GOD-liposome	165±15.5	0.287±0.032	-27.4±1.5

[a]Mean ± standard deviation, n=3; Mean diameter is the hydrodynamic diameter; Polydispersity index indicates the quantification of dispersity; Zeta potential indicates the average surface charge. Mean diameter, polydipersity index and surface charge were obtained by a Malvern Zetasizer instrument.

Table S3 The fluorescence lifetime of g-C_3N_4 nanosheet and g-C_3N_4-MnO_2 nanocomposite

Sample	Fluorescence lifetime (ns)
g-C_3N_4 nanosheet	7.80
g-C_3N_4-MnO_2 nanocomposite	2.96

Table S4 Comparison of different methods for RTB detection

Method	LOD (ng/mL)	Assay time	Real sample analysis	Ref
Fluoroimmunoassay	1000	12 h	Not given	[3]
Aptamer arrays biosensor	320	6 h	Not given	[4]
SPR biosensor	200	20 min	Not given	[5]
Fluorescence strategy	190	90 min	Caster beans	This work

Supplementary Figures

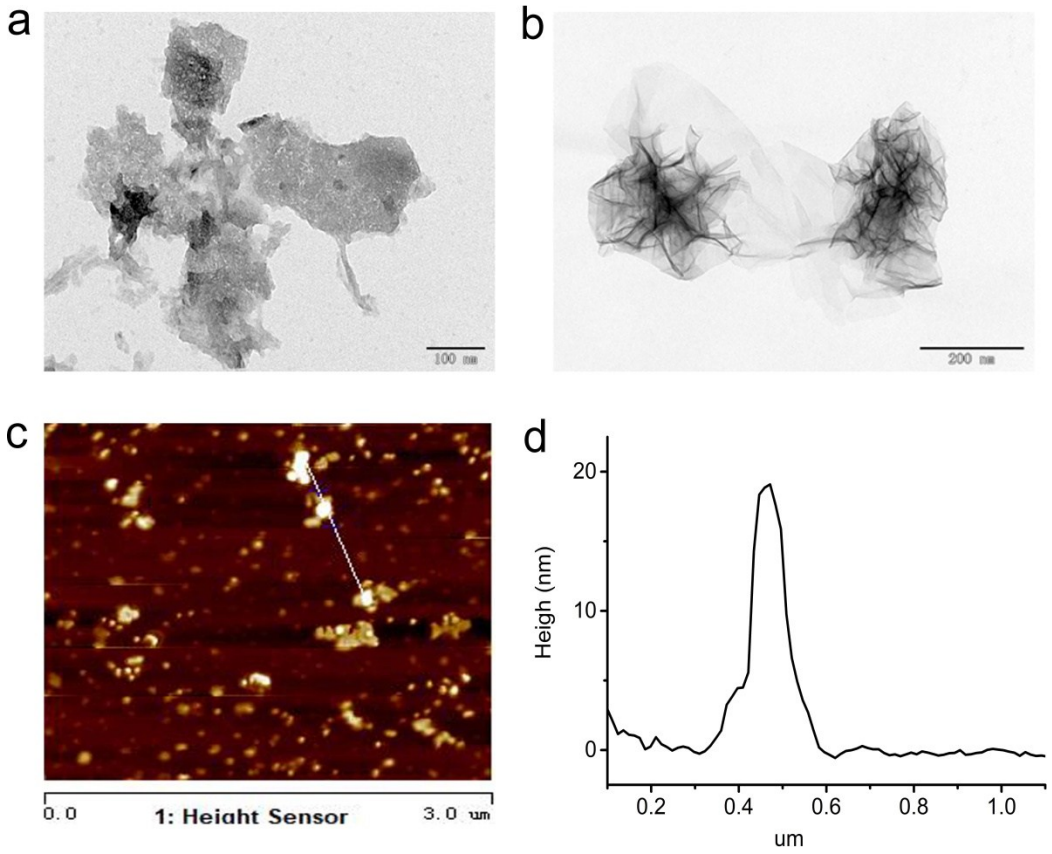

Fig. S1 TEM picture of g-C_3N_4 nanosheet (a) and g-C_3N_4-MnO_2 nanocomposite (b), AFM image of g-C_3N_4-MnO_2 nanocomposite (c & d).

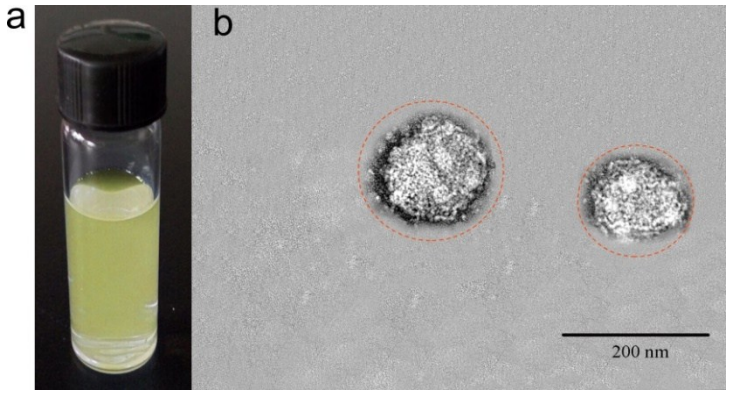

Fig. S2 The picture (a) and TEM image (b) of the glucose oxidase-encapsulated liposome (GOD-L).

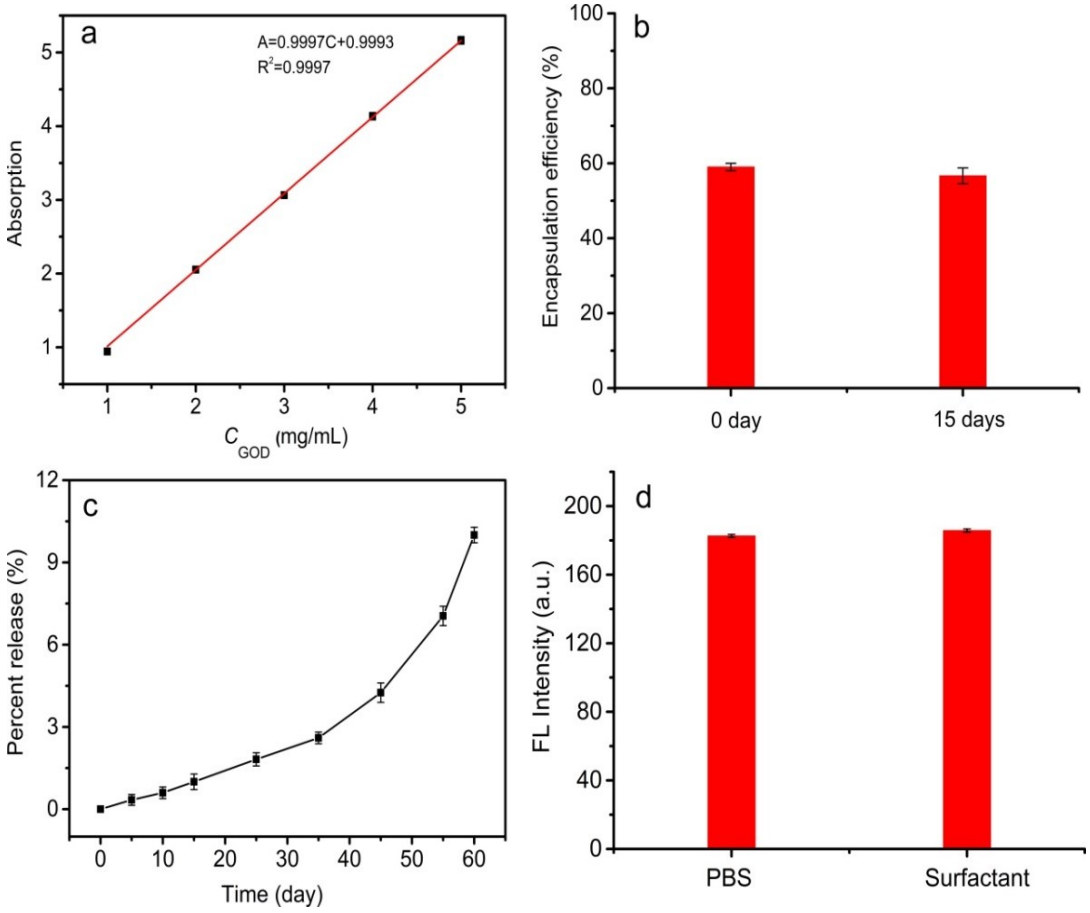

Fig. S3 (a) UV absorbance of different free GOD concentrations. (b) Encapsulation efficiency of liposomes at 0 day and 15 days. (c) Leakage percent of liposomes at different storage day. (d) Effect of surfactant on enzyme activity.

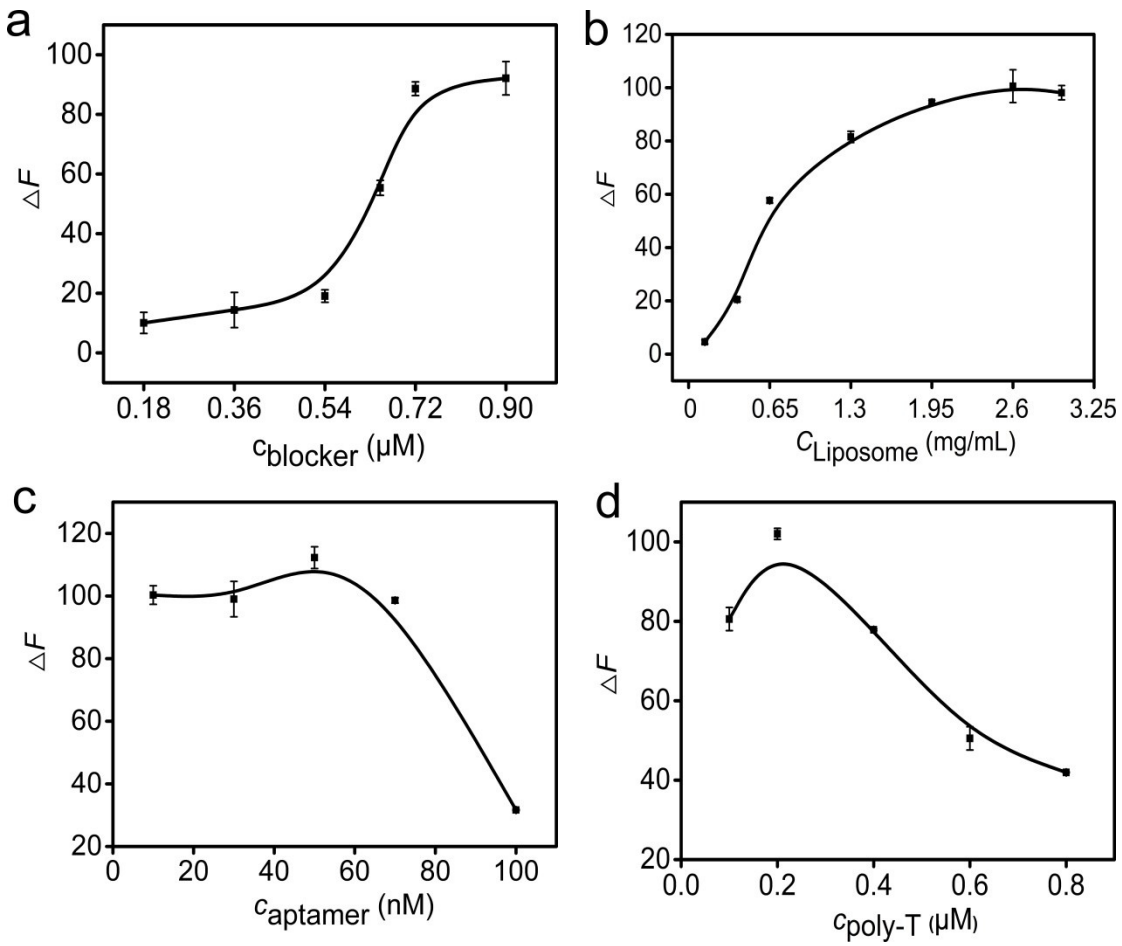

Fig. S4 Effect of the concentrations of blocker (a), liposome (b), aptamer (c), and poly-T (d) on the detection of RTB. Conditions: a: 2.6 mg/mL liposome, 50 nM aptamer, 0.2 μM poly-T; b: 0.72 μM blocker, 50 nM aptamer, 0.2 μM poly-T; c: 0.72 μM blocker, 2.6 mg/mL liposome, 0.2 μM poly-T; d: 0.72 μM blocker, 2.6 mg/mL liposome, 50 nM aptamer. All of the concentrations of RTB in a-d were 30 μg/mL, 0.5 mg/mL MBs, 50 min RTB reaction time, 40 min enzyme reaction time. The error bars showed the standard deviation of two replicate determinations.

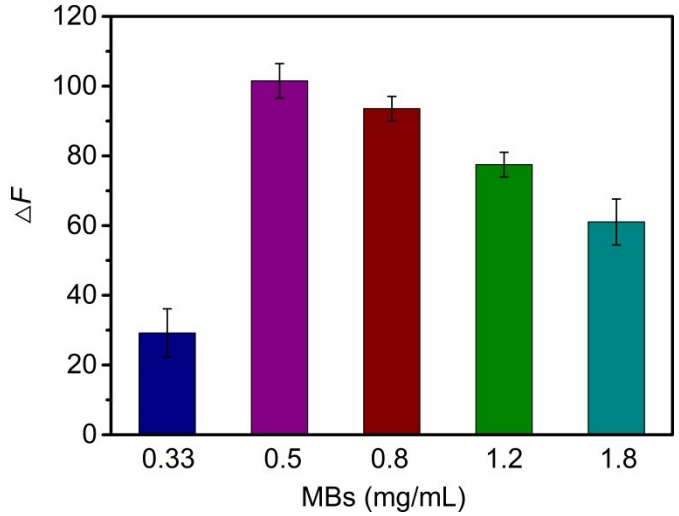

Fig. S5 Effect of different magnetic beads concentrations on the fluorescence signal. Experimental conditions: 0.72 μM blocker, 2.6 mg/mL liposome, 50 nM aptamer, 0.2 μM poly-T, 50 min RTB reaction time, 40 min enzyme reaction time, the concentration of RTB is 30 μg/mL. The error bars showed the standard deviation of two replicate determinations.

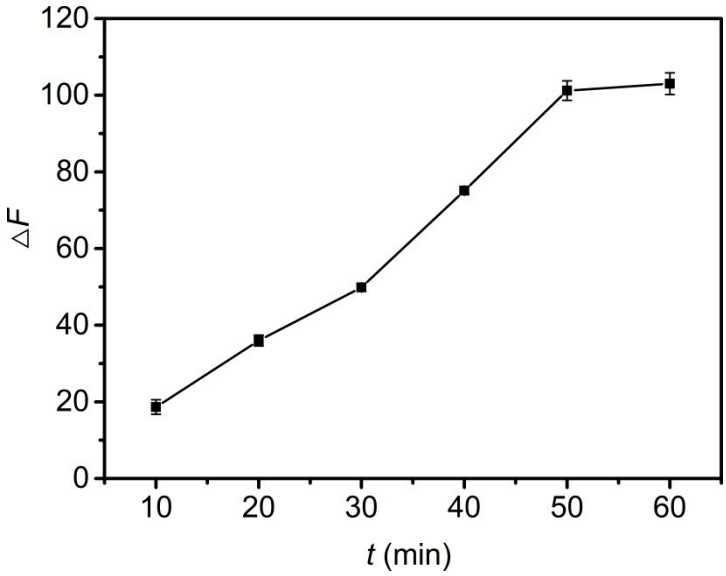

Fig. S6 Effect of RTB reaction time on the fluorescence signal. Experimental conditions: 0.72 μM blocker, 2.6 mg/mL liposome, 50 nM aptamer, 0.2 μM poly-T, 0.5 mg/mL MBs, 40 min enzyme reaction time, the concentration of RTB is 30

μg/mL. The error bars showed the standard deviation of two replicate determinations.

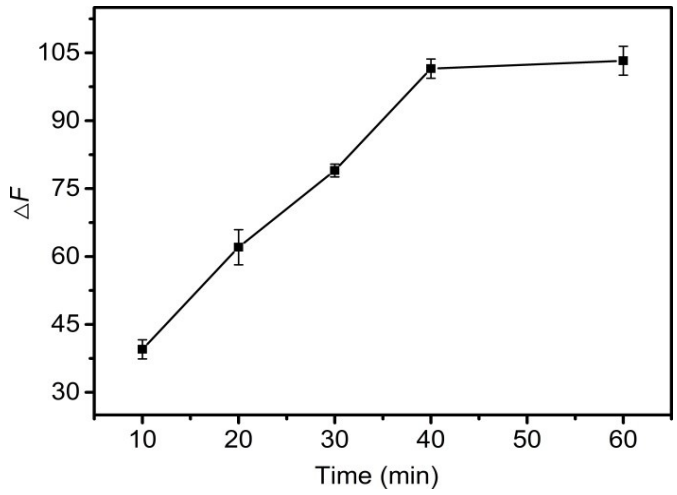

Fig. S7 Effect of enzyme reaction time on the fluorescence signal. Experimental conditions: 0.72 μM blocker, 2.6 mg/mL liposome, 50 nM aptamer, 0.2 μM poly-T, 0.5 mg/mL MBs, 50 min RTB reaction time, the concentration of RTB is 30 μg/mL. The error bars showed the standard deviation of two replicate determinations.

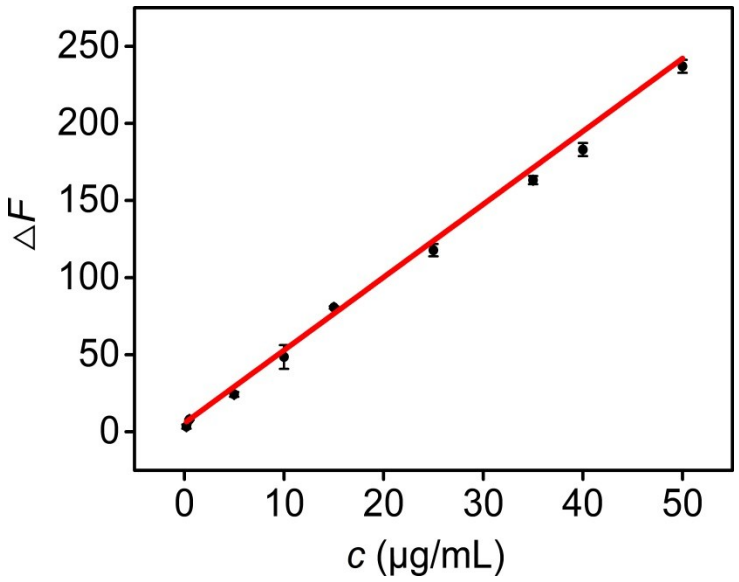

Fig. S8 Linear relationship between the change in fluorescence intensity (ΔF) and RTB concentration. Experimental conditions: RTB, 1-10, 0, 0.2, 0.5, 5, 10, 15, 25, 35,

40, 50 μg/mL; 0.72 μM blocker, 2.6 mg/mL liposome, 50 nM aptamer, 0.2 μM poly-T, 0.5 mg/mL MBs, 50 min RTB reaction time, 40 min enzyme reaction time. Error bars represent the standard deviations of three independent measurements.

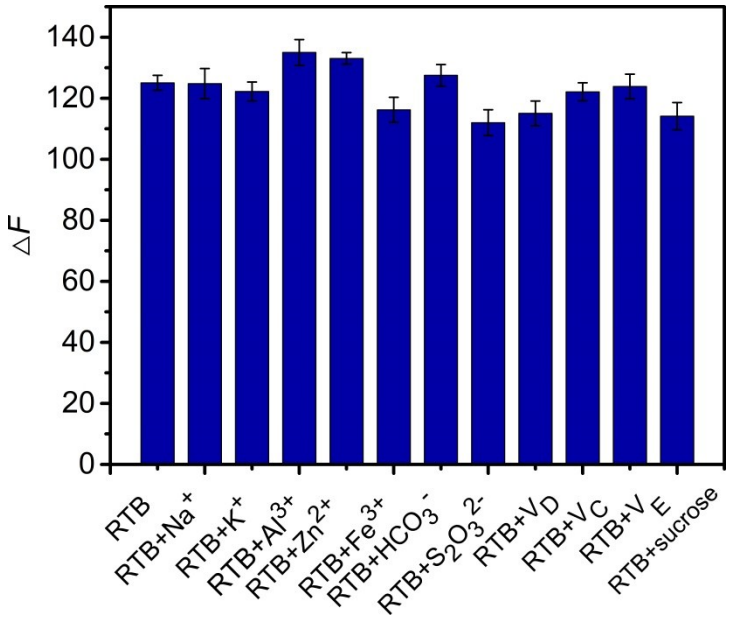

Fig. S9 The effect of RTB in the presence of various interfering substances. Experimental conditions: RTB, 30 μg/mL; Na^+, K^+, Al^{3+}, Zn^{2+}, Fe^{3+}, HCO_3^-, $S_2O_3^{2-}$, V_D, V_C, V_E, sucrose, 300 μg/mL. The error bars showed the standard deviation of two replicate determinations.

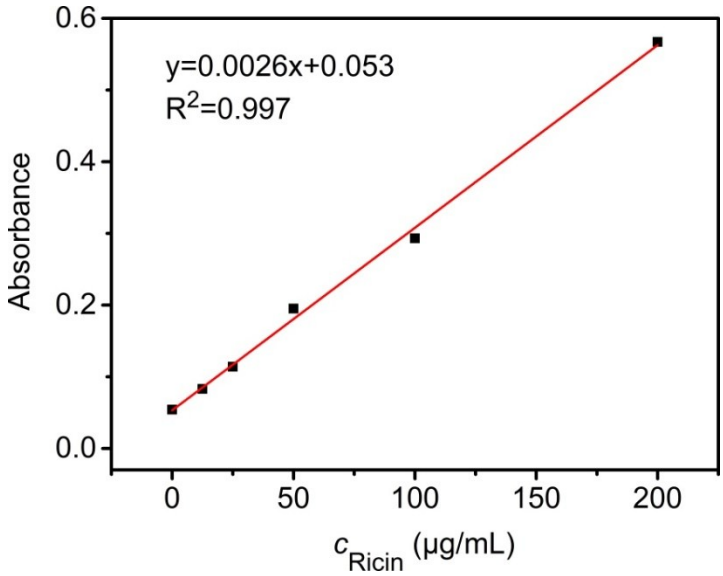

Fig. S10 The standard curve of ricin detection by ELISA kit. The regression equation was $y = 0.0026x + 0.053$ ($R^2 = 0.997$), where y was the absorbance and x was the concentration of ricin (μg/mL). The error bars showed the standard deviation of three replicate determinations.

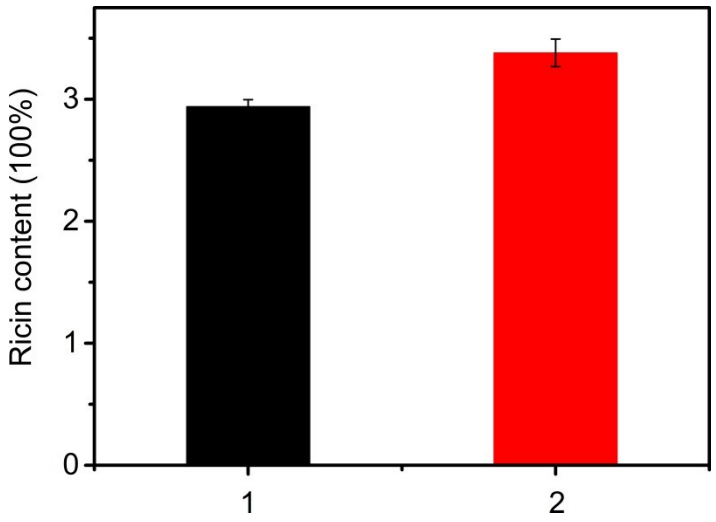

Fig. S11 The ricin content in caster beans. 1, the ricin content tested by ELISA kit, which was 2.94± 0.06%; 2, the ricin content tested by our method, which was 3.38 ± 1.11%. The error bars showed the standard deviation of three replicate determinations.

Supplementary References

1 L. Fetter, J. Richards, J. Daniel, L. Roon, T. J. Rowland and A. J. Bonham, *Chem. Commun.*, 2015, **51**, 15137-15140.

2 X. Xiao, J. Tao, H. Z. Zhang, C. Z. Huang and S. J. Zhen, *Biosens. Bioelectron.*, 2016, **85**, 822-827.

3 G. P. Anderson and N. L. Nerurkar, *J. Immunol. Methods*, 2002, **271**, 17-24.

4 R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y. S. Park, D. P. Neikirk, J. T. McDevitt and A. D. Ellington, *Anal. Chem.*, 2004, **76**, 4066-4075.

5 B. N. Feltis, B. A. Sexton, F. L. Glenn, M. J. Best, M. Wilkins and T. J. Davis, *Biosens. Bioelectron.*, 2008, **23**, 1131-1136.