Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Determination of binding constant of specific interaction and binding target concentration simultaneously using a general chemiluminescence method

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S1. Binding fraction of TNT aptamer for TNT

The relationship between CL intensity and TNT concentration is shown in Figure S1. The CL intensity decreased rapidly with low TNT concentration from 0.1 nM to 0.03 μM. The decreasing rate became slow when TNT concentration was over 0.03 μM. The CL intensity almost kept instant when TNT concentration was over 0.3 μM, implying that the binding of TNT reached saturation. In order to estimate the binding fraction of TNT by TNT aptamer, we assumed that the interaction between TNT and TNT aptamer was strong enough to capture most of the TNT added into the system. The amount of the forming complexes was approximately equal to the amount of the TNT added into the system. Based on the assumption, when the CL intensity reached stability after 0.3 μM, all of the TNT aptamer had bound with TNT (see Figure S1). Thus, the amount of the TNT aptamer was approximately equal to 0.3 μM, and the plot of the fraction of TNT binding as functions of TNT concentration is also shown in Figure S1 (inset). The estimate of the binding fraction of TNT aptamer for TNT indicated that the binding fraction below 7 nM TNT was less than 2.3% and could thus be ignored. The curve from 1.0×10^{-8} to 7.0×10^{-7} M was applied to measure binding constant.

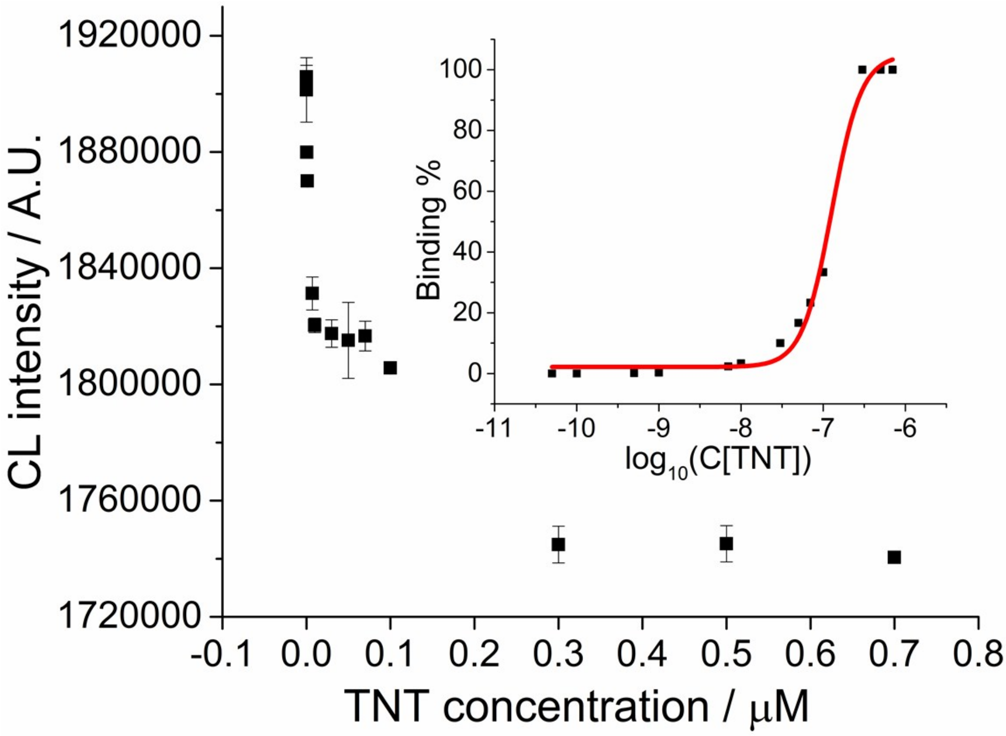

Fig. S1 Relationship between CL intensity and TNT concentration. Inset: Plot of the fraction of TNT binding as functions of TNT concentration. Reaction condition: 100 μL pH 13 0.15 M H_2O_2.

S2. A comparison of the proposed CL method with previously reported methods for the determination of various targets.

Table S1 A comparison of the proposed CL method with previously reported methods for the determination of various targets

Target	Analytical method	Sensing platform	Detection range (mol/L)	Detection limit (mol/L)	Reference
TNT	Electro chemiluminescence	GO and AuNPs	4.4×10^{-7}~ 4.4×10^{-11}	1.6×10^{-11}	[1]
	Fluorescence	fibre-optic	1.0×10^{-5}~ 1.0×10^{-10}	1.0×10^{-12}	[2]
	chemiluminescence	ABEI-Au colloid	1.0×10^{-8}~ 1.0×10^{-10}	8.0×10^{-11}	This work
Dopamine	Square Wave Voltammetry	GO–polyaniline	9.0×10^{-8}~ 7.0×10^{-12}	2.0×10^{-12}	[3]
	electrochemical	Methylene blue	1.5×10^{-7}~ 5.0×10^{-9}	1.0×10^{-9}	[4]
	chemiluminescence	ABEI-Au colloid	1.0×10^{-9}~ 1.0×10^{-12}	9.0×10^{-13}	This work
Tetracycline	electrochemical	MWCNT	5.0×10^{-5}~ 1.0×10^{-8}	5.0×10^{-9}	[5]
	electrochemical	AuNPs	1.0×10^{-3}~ 1.0×10^{-10}	4.2×10^{-12}	[6]
	colorimetric	AuNPs	1.0×10^{-8}~ 3.0×10^{-10}	2.7×10^{-10}	[7]
	chemiluminescence	ABEI-Au colloid	1.0×10^{-9}~ 1.0×10^{-12}	2.0×10^{-13}	This work
IgG	Electro chemiluminescence	ABEI-GO	6.7×10^{-11}~ 6.7×10^{-16}	3.3×10^{-16}	[8]
	Photoelectrochemical	CdS Quantum Dot	6.7×10^{-10}~ 6.7×10^{-14}	5.3×10^{-14}	[9]
	Electro chemiluminescence	GO and AuNPs	6.7×10^{-10}~ 1.3×10^{-13}	8.7×10^{-15}	[10]
	chemiluminescence	ABEI-Au colloid	5.0×10^{-9}~ 1.0×10^{-13}	9.2×10^{-14}	This work
DNA	Cyclic voltammetry	Nickel Hexacyanoferrates Nanoparticles	5.0×10^{-8}~ 1.0×10^{-11}	6.3×10^{-12}	[11]
	Electro chemiluminescence	$Ru(bpy)_3^{2+}$-PtNPs	5.0×10^{-8}~ 5.0×10^{-12}	1.7×10^{-12}	[12]
	Fluorescence	Crystal violet	2.0×10^{-9}~ 2.0×10^{-11}	8×10^{-12}	[13]
	Electro chemiluminescence	luminol-AuNPs	5.0×10^{-8}~ 5.0×10^{-12}	1.7×10^{-12}	[14]
	chemiluminescence	ABEI-Au colloid	1.0×10^{-12}~ 1.0×10^{-14}	4.1×10^{-15}	This work

References:

1. Yu, Y., et al., *A novel homogeneous label-free aptasensor for 2,4,6-trinitrotoluene detection based on an assembly strategy of electrochemiluminescent graphene oxide with gold nanoparticles and aptamer.* Biosensors and Bioelectronics, 2013. **43**(0): p. 137-142.
2. Ehrentreich-Förster, E., et al., *Biosensor-based on-site explosives detection using aptamers as recognition elements.* Analytical and Bioanalytical Chemistry, 2008. **391**(5): p. 1793-1800.
3. Liu, S., et al., *A novel label-free electrochemical aptasensor based on graphene–polyaniline composite film for dopamine determination.* Biosensors and Bioelectronics, 2012. **36**(1): p. 186-191.
4. Zhou, J., et al., *A simple label-free electrochemical aptasensor for dopamine detection.* RSC Advances, 2014. **4**(94): p. 52250-52255.
5. Zhou, L., et al., *Electrochemical aptasensor for the detection of tetracycline with multi-walled carbon nanotubes amplification.* Sensors and Actuators B: Chemical, 2012. **162**(1): p. 201-208.
6. Guo, Y., X. Wang, and X. Sun, *A label-free Electrochemical Aptasensor Based on Electrodeposited Gold Nanoparticles and Methylene Blue for Tetracycline Detection.* Int. J. Electrochem. Sci, 2015. **10**: p. 3668-3679.
7. Ramezani, M., et al., *A novel colorimetric triple-helix molecular switch aptasensor for ultrasensitive detection of tetracycline.* Biosensors and Bioelectronics, 2015. **70**: p. 181-187.
8. Shu, J., W. Shen, and H. Cui, *Ultrasensitive label-free electrochemiluminescence immunosensor based on N-(4-aminobutyl)-N-ethylisoluminol-functionalized graphene composite.* Science China Chemistry, 2015. **58**(3): p. 425-432.
9. Wang, G.-L., et al., *A Label-Free Photoelectrochemical Immunosensor Based on Water-Soluble CdS Quantum Dots.* The Journal of Physical Chemistry C, 2009. **113**(25): p. 11142-11148.
10. Peng, S., G. Zou, and X. Zhang, *Nanocomposite of electrochemically reduced graphene oxide and gold nanoparticles enhanced electrochemilunescence of*

peroxydisulfate and its immunosensing abililty towards human IgG. Journal of Electroanalytical Chemistry, 2012. **686**: p. 25-31.

11. Bai, L., et al., *Highly sensitive electrochemical label-free aptasensor based on dual electrocatalytic amplification of Pt-AuNPs and HRP.* Analyst, 2011. **136**(9): p. 1840-1845.
12. Liao, Y., et al., *Electrochemiluminescence quenching via capture of ferrocene-labeled ligand-bound aptamer molecular beacon for ultrasensitive detection of thrombin.* Sensors and Actuators, B: Chemical, 2011. **158**(1): p. 393-399.
13. Jin, Y., J. Bai, and H. Li, *Label-free protein recognition using aptamer-based fluorescence assay.* Analyst, 2010. **135**(7): p. 1731-1735.
14. Li, F. and H. Cui, *A label-free electrochemiluminescence aptasensor for thrombin based on novel assembly strategy of oligonucleotide and luminol functionalized gold nanoparticles.* Biosensors and Bioelectronics, 2013. **39**(1): p. 261-267.