Electronic Supplementary Information

Label-free fluorescence DNA walker for protein analysis based on terminal protection and dual-enzyme-assisted cleavage induced G-quadruplex/berberine conformation

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Table S1. Sequence of the DNA

note	*sequence*
Swing arm probe:	5′-SH-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAT CTCTTCTCCGAGCCGGTCGAAATAGTTGGTTTTTTTTTTTT-biotion-3′;
Report probe:	5′-TTAGGGTTAGGGTTAGGGTTAGGGTTTACCAACTAT*r*AGGAAGAGAT GTTTTTTTT-SH-3′

Table S2. Comparison of detection limit for STA based on different methods

Technique	Output signal	Detection limit	Ref.
Polymerase-powered DNA walker	Electrochemistry	16 pM	1
Steric hindrance hybridization based on CdSe quantum dots	Electrochemistry	0.01 pM	2
Biotin-streptavidin recognition and metallic labels	Localized surface plasmon resonance	5 nM	3
Target-protecting dumbbell molecular probe	Fluorescence	12.7 pM	4
Microfluidic biosensor	Fluorescence	1 pM	5
Terminal protection and dual-enzyme-assisted DNA walker	Fluorescence	20 pM	This work

Table S3. Determination of stability using the proposed sensor.

Sample number	*Added (nM)*	*Time*	*FL signal*	*Found (nM)*	*RSD (%)*
1	0.5	1 h	110.94±6.72	0.478±0.043	9.08
		2 h	112.45±5.82	0.487±0.038	7.71
		5 h	111.42±4.73	0.481±0.031	6.35
		8 h	106.58±6.73	0.449±0.043	9.67
		RSD (%)	2.35	3.53	
2	1	1 h	186.77±11.07	0.967±0.071	7.39
		2 h	187.48±8.97	0.971±0.058	5.96
		5 h	189.81±7.54	0.986±0.049	4.93
		8 h	180.50±10.04	0.926±0.070	6.99
		RSD (%)	2.14	2.67	

Table S4. Determination of reproducibility using the proposed sensor.

	Added (nM)	*0.2*		*0.8*	
	Sample	*FL signal*	*Found (nM)*	*FL signal*	*Found (nM)*
A	*1*	75.22	0.247	164.64	0.824
	2	73.03	0.233	158.23	0.783
	3	69.09	0.208	169.18	0.853
	Average	72.44	0.229	164.02	0.820
	SD	3.10	0.020	5.50	0.035
	RSD (%)	4.29	8.74	3.35	4.33
B	*1*	77.31	0.261	157.44	0.778
	2	76.23	0.254	155.81	0.767
	3	72.88	0.232	160.12	0.795
	Average	75.47	0.249	157.79	0.780
	SD	2.31	0.015	2.18	0.014
	RSD (%)	3.06	5.99	1.38	1.80
C	*1*	66.73	0.198	170.33	0.861
	2	64.21	0.176	158.92	0.787
	3	69.82	0.212	154.15	0.756
	Average	66.92	0.194	161.13	0.801
	SD	2.81	1.81	8.31	0.054
	RSD (%)	4.20	9.37	5.16	6.69
	Average	72.58	0.230	158.65	0.785
	SD	2.83	0.018	4.99	0.032
	RSD (%)	3.90	7.93	3.14	4.10

Table S5. Determination of streptavidin in the diluted fetal calf serum sample using the proposed sensor.

Sample number	*Added (nM)*	*Found (nM)*	*Recovery (%)*
1	0.1	0.095±0.040	95.0
2	0.5	0.437±0.039	87.4
3	0.8	0.757±0.039	94.6

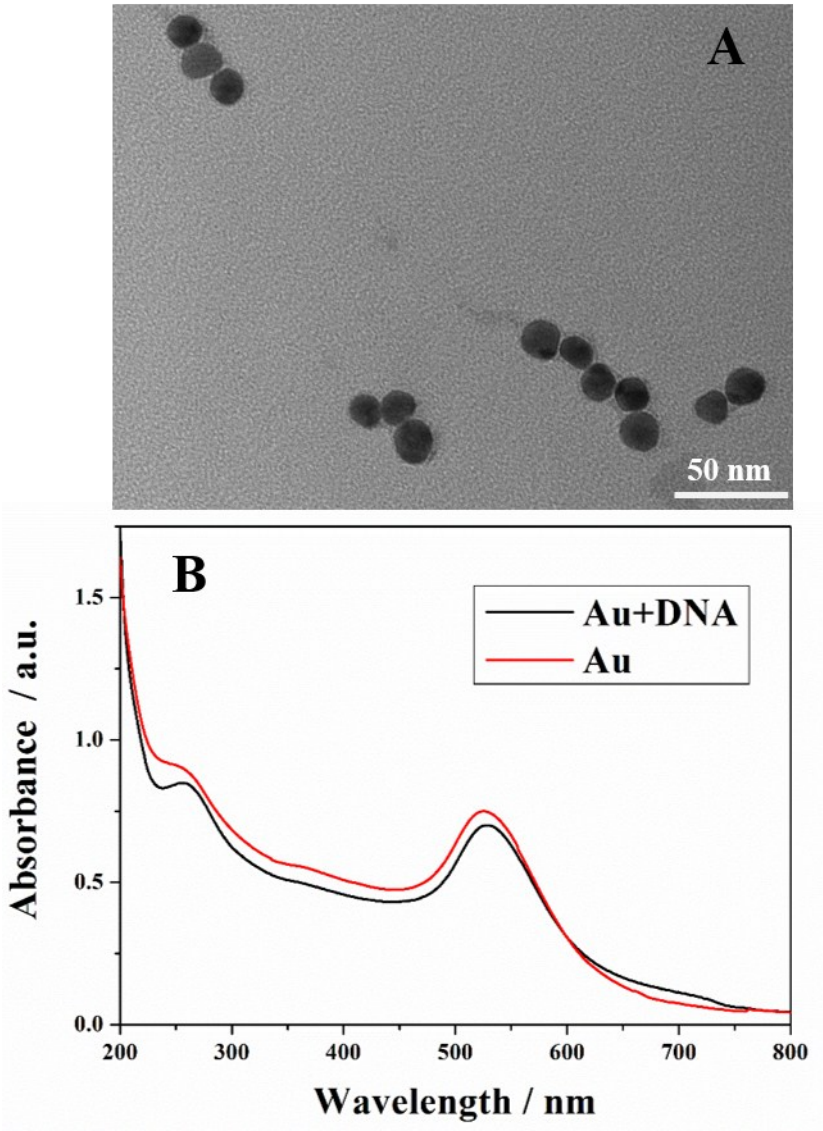

Fig. S1 (A) TEM images of 13 nm AuNPs. Scale bars: 50 nm. (B) UV-vis spectra of AuNPs and Au+DNA.

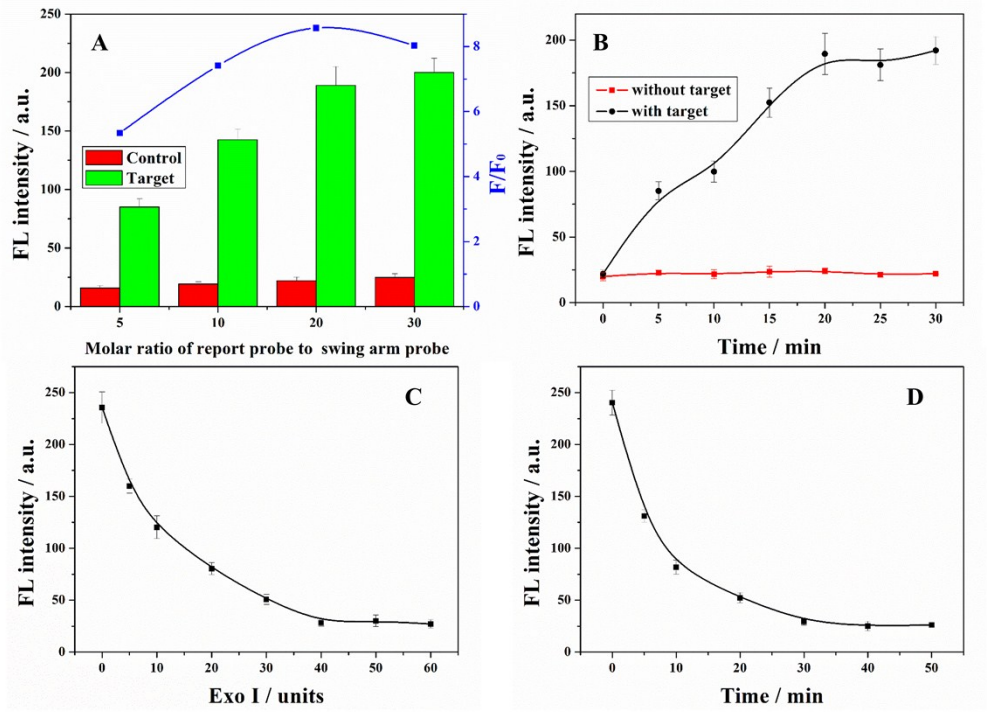

Fig. S2 (A) The effect of molar ratio of report probe to swing arm probe on the fluorescene response of sensing system in the absence (red bar) and presence (green bar) of 1 nM STA. The concentrations of Exo I, Pb^{2+}, K^{+} and berberine were fixed at 40 units, 50 μM, 100 mM and 8 μM, respectively. F and F_0 are the florescence intensity of the sensor in the presence and absence of STA. (B) Optimization of the reaction time between STA and biotin modified DNA report probe in the absence (red line) and presence (black line) of 1 nM of STA. (C) Optimization of Exo I concentration without addition of STA. The reaction time of Exo I was 30 min. (D) Optimization of Exo I reaction time without addition of STA. The concentration of Exo I was 40 units.

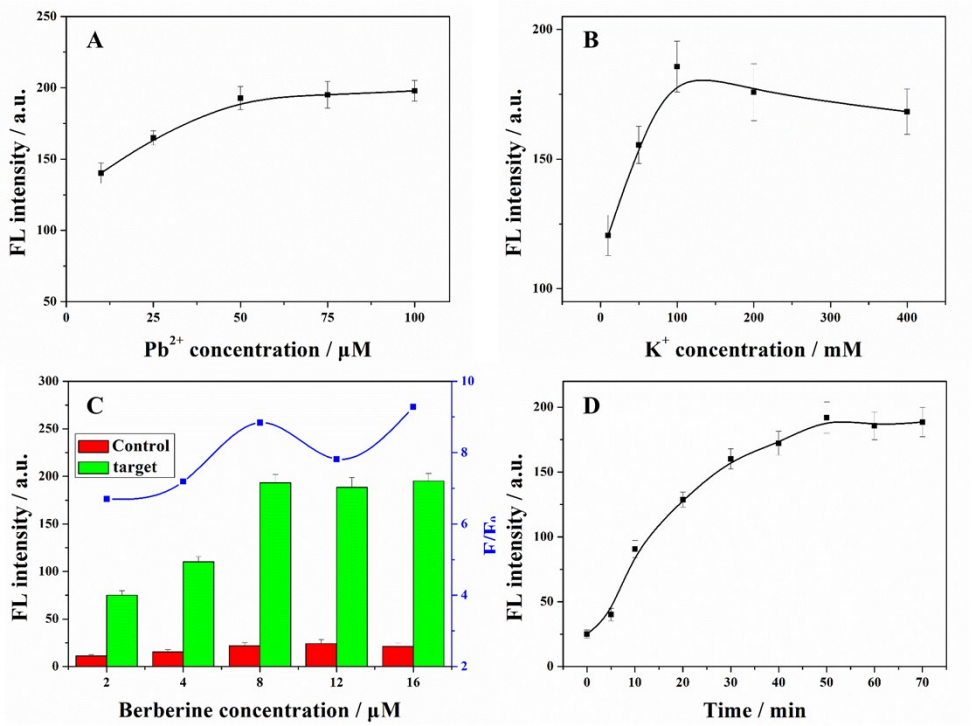

Fig. S3 The effect of (A) Pb^{2+} concentration, (B) K^{+} concentration, (C) berberine and (D) the incubation time between G-quadruplex and berberine on the fluorescence response of the sensing system.

References:

1. Z. Chen, C. Wang, L. Hao, R. Gao, F. Li and S. Liu, *Biosens. Bioelectron.*, 2019, **128**, 104-112.

2. Y. Wei, X. Liu, C. Mao, H. Niu, J. Song and B. Jin, *Biosens. Bioelectron.*, 2018, **103**, 99-103.

3. M. Focsan, A. Campu, A. M. Craciun, M. Potara, C. Leordean, D. Maniu and S. Astilean, *Biosens. Bioelectron.*, 2016, **86**, 728-735.

4. J. Chen, Y. Liu, X. Ji and Z. He, *Biosens. Bioelectron.*, 2016, **83**, 221-228.

5. J. Wang, M. Aki, D. Onoshima, K. Arinaga, N. Kaji, M. Tokeshi, S. Fujita, N. Yokoyama and Y. Baba, *Biosens. Bioelectron.*, 2014, **51**, 280-285.