Electronic Supplementary Information:

A new label-free and turn-on fluorescence probe for hydrogen peroxide and glucose detection based on DNA–silver nanoclusters

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1. Experimental Section

Reagents and materials

All oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences are listed in Table S1. Glucose, glucose oxidase (GOx), silver nitrate ($AgNO_3$), ascorbic acid (vitamin C) and N-ethylmaleimide (NEM) were supplied by Sigma Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2), sodium borohydride ($NaBH_4$) and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ethylene diamine tetraacetic acid (EDTA) was obtained from Xilong Chemical Co., Ltd. (Guangdong, China). 5× TBE buffer was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). 100 mM PB buffer (pH 7.4, 100 mM NaH_2PO4-Na_2HPO4 containing 15 mM EDTA), 100 mM PB buffer (pH 7.0) and 100 mM PB buffer (pH 7.4) made in-house, was used as the medium for the detection process. All aqueous solutions were prepared and diluted using ultrapure water, which was purified with a Millipore Milli-Q water purification system (Billerica, MA, USA), and had an electric resistance > 18.3 MΩ. All other chemicals were at least analytical grade and were used without further purification or treatment.

Apparatus

All fluorescence measurements were performed using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon, Inc., NJ, USA). Both Ex and Em slits were set at 5.0 nm with a 950 V PMT voltage. The emission spectra of each system were collected at the excitation wavelength of 570 nm and emission range of 590–750 nm.

All fluorescence measurements were recorded at room temperature unless otherwise stated.

Synthesis of DNA- A g NCs

The synthesis of DNA-Ag NCs was based on a previous literature report with minor modifications.[S1] Briefly, DNA solution (10 μL, 100 μM) and $AgNO_3$ solution (20 μL, 300 μM) were sequentially mixed with 100 mM phosphate buffer (pH 7.0) and ultrapure water, this provided an Ag+ to DNA molar ratio of 6:1, which enabled the preparation of DNA-Ag NCs at their most fluorescence, followed by vigorous shaking for 1 min. After cooling on ice for 20 min, $NaBH_4$ aqueous solution (20 μL, 300 μM, freshly prepared) was added quickly with vigorous shaking for 2 min. The reduction condition of the mixture was 12 h in the dark at 4 ℃. The final concentrations of DNA, $AgNO_3$, and $NaBH_4$ were 10 μM, 60 μM, and 60 μM.

Gel electrophoresis analysis

Gel electrophoresis analysis was carried out on 5 % (w/w) agarose gels containing 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide. It was conducted in 0.5× TBE buffer with loading of 20 μL of each sample into the lanes at a constant voltage of 100 V for 1.5 h at room temperature. After electrophoresis, the gel was visualized via a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology Company, China).

Fluorescence Detection of H_2O_2

To detect H_2O_2, different concentrations of H_2O_2 were added to a mixture of B-DNA (5 μL, 10 μM) and $FeSO4{\cdot}7H_2O$ (10 μL, 1.5 mM) in 100 mM PB buffer (pH

7.4, containing 15 mM EDTA), followed by added suitable ultrapure water to a final volume of 50 μL.[S2] The solution was firstly incubated at 25 ℃ for 10 min to allow chemical reactions which related to cleavage B-DNA to proceed sufficiently, the cleavage reaction was ended by heating at 90 ℃ for 20 min. Then G-DNA (5 μL, 10 μM), PB buffer (PH 7.0) and an appropriate volume of ultrapure water were introduced to the mixture solution. The above mixture was heated at 80 ℃ for 20 min, and gradually cooled down to room temperature. Finally, 5 μL of the as-prepared Ag NCs solution was added into the system and the fluorescence was measured after the mixture was incubated at 25 ℃ for 90 min.

Glucose sensing and antioxidants experiments

In a typical experiment, For the sensing of glucose , to a 200 μL centrifugal tube were sequentially added 100 mM PB buffer (pH 7.4, containing 15 mM EDTA), 10 μL B-DNA (10 μM), different concentrations of glucose, 6 μL $FeSO4{\cdot}7H_2O$ (0.625 mM), 6 μL GOx (250 μg/mL) and then diluted with ultrapure water to a final volume of 50 μL.[S3-S5] The mixture solution was heated at 37 ℃ for 4.5 h to allow chemical reactions which related to cleavage B-DNA to proceed sufficiently, the cleavage reaction was ended by heating at 90 ℃ for 15 min. Then G-DNA (10 μL, 10 μM), PB buffer (PH 7.0) and an appropriate volume of ultrapure water was added into the above mixture and incubated at 80 ℃ for 20 min, and gradually cooled down to room temperature, followed by the addition of 10 μL of the as-prepared Ag NCs solution. The fluorescence was measured after the above mixture was incubated at 25 ℃ for 90 min. To test the practicability, glucose levels in urine samples (spiked with

89 glucose) were detected by the proposed method.

90 For the antioxidants experiments, all procedures were the same as the
91 aforementioned assay of glucose activity except that the different concentrations of
92 ascorbic acid (vitamin C) were added before GOx.

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2. Supplementary Tables and Figures

Table S1 Names and sequences of the oligonucleotides.

Name	Sequence
B-DNA	5′-CCCCACCCCACCCCACCCAGCACATCTGATAGTTC-3′
G-DNA	5′-GAACTATCAGATGTGCTGGGTGGGGTGGGGTGGGG-3′
Ag-DNA	5′-CCCTTAATCCCCAGCACATCTGATAGTTC-3′

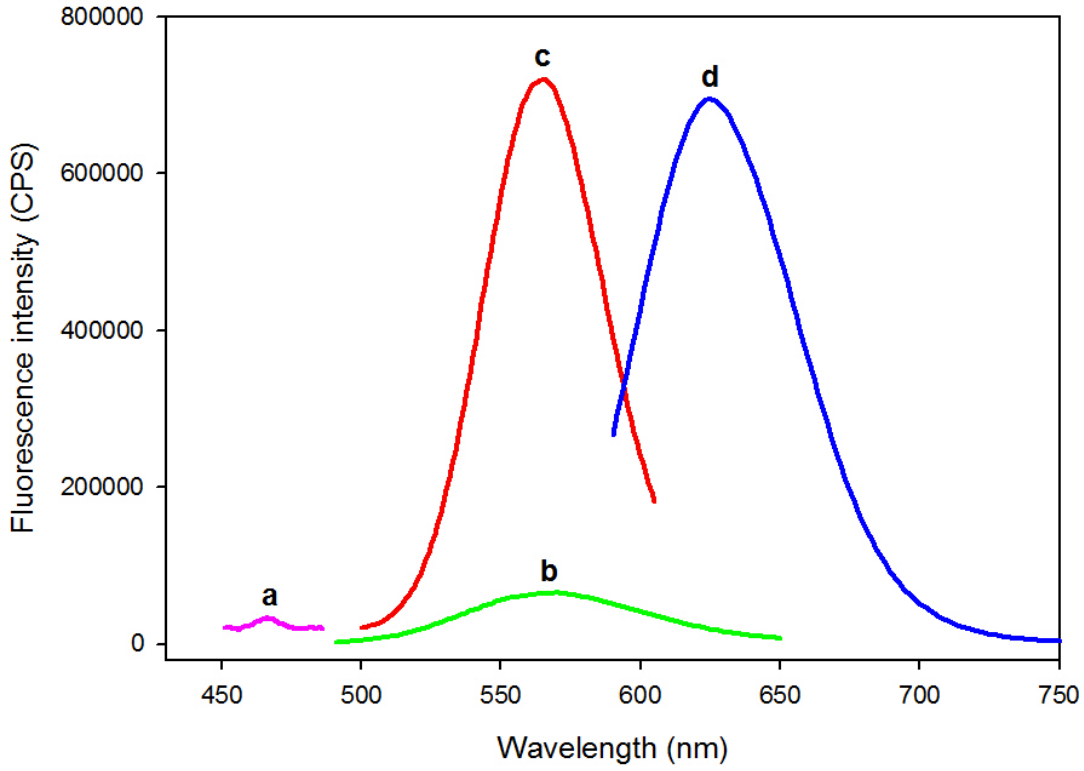
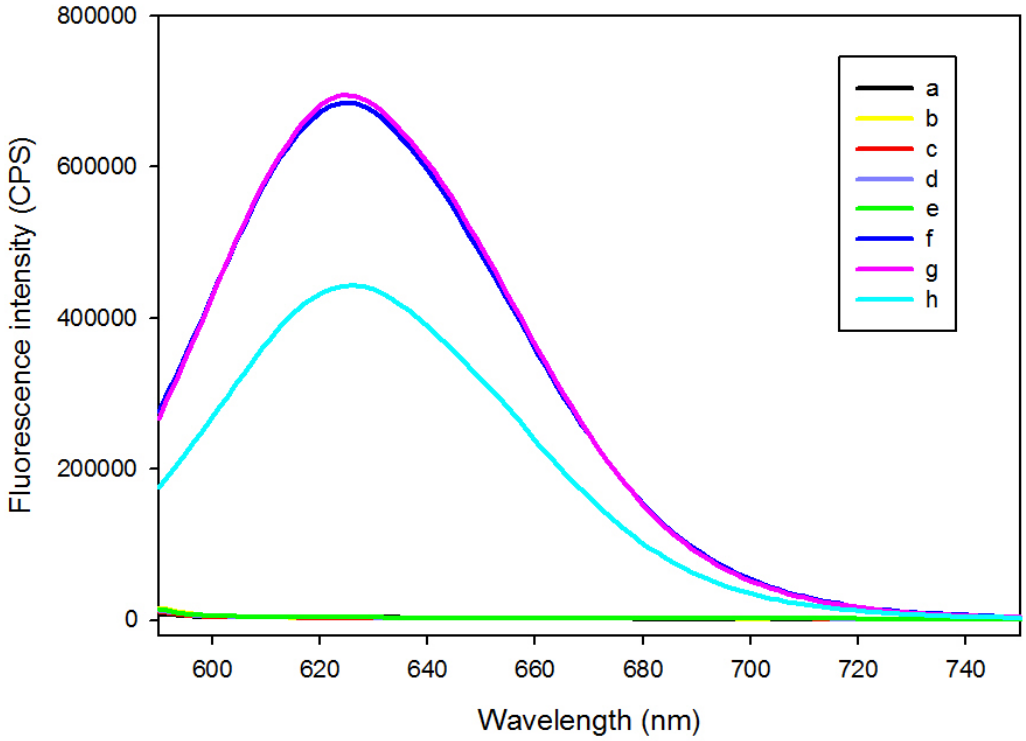


Fig.S1 Excitation and emission spectra of the fluorescent Ag NCs obtained before (curves a and b) and after (curves c and d) addition of G-DNA. The concentration of all DNA used here was 1 μM.



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119 **Fig.S2** The fluorescence emission spectra of DNA–Ag NCs were recorded in the

120 presence of (a) no added reagent, (b) B-DNA + G-DNA, (c) GOx + Fe^{2+} + B-DNA +

121 G-DNA, (d) glucose + GOx + B-DNA + G-DNA, (e) glucose + Fe^{2+} + B-DNA + G-

122 DNA, (f) glucose + GOx + Fe^{2+} + G-DNA, (g) G-DNA, (h) glucose + GOx + Fe^{2+} +

123 B-DNA + G-DNA. The concentration of all DNA used here was 1 μM. The

124 concentration of glucose was 3.75 mM.

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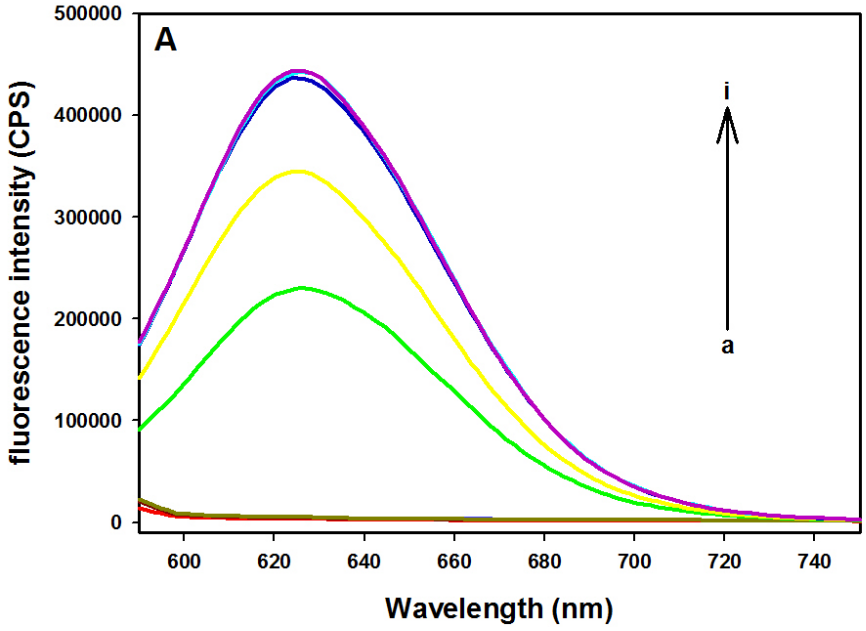

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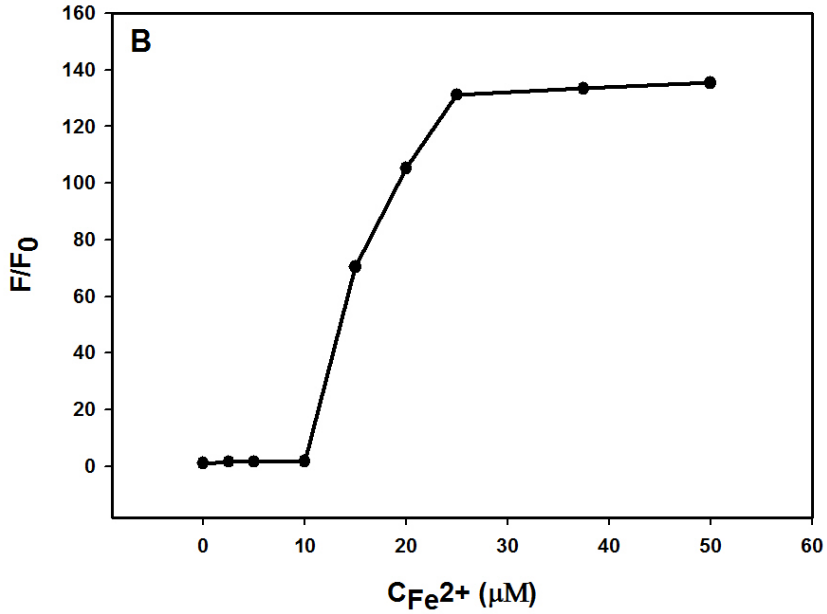

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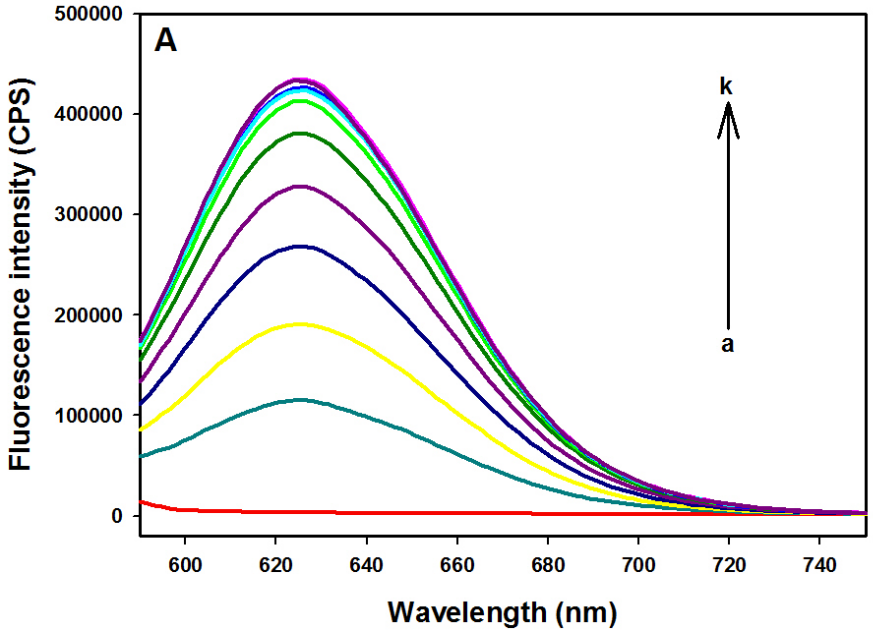
128 **Fig.S3** (A) The fluorescence emission spectra of the sensing system at different

129 concentrations of Fe^{2+} (from a to i): 0, 2.5, 5, 10, 15, 20, 25, 37.5, 50 μM. (B)

130 Fluorescence responses of the sensing system to various concentrations of Fe^{2+}. F and

131 F_0 are the fluorescence intensity of the sensing system in the presence and absence of

132 Fe^{2+}.



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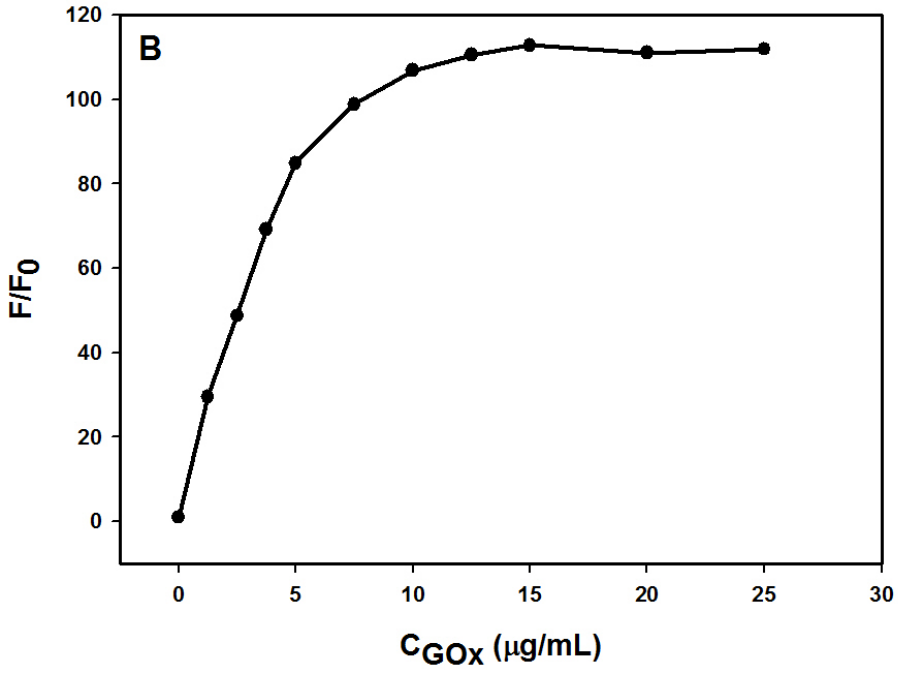

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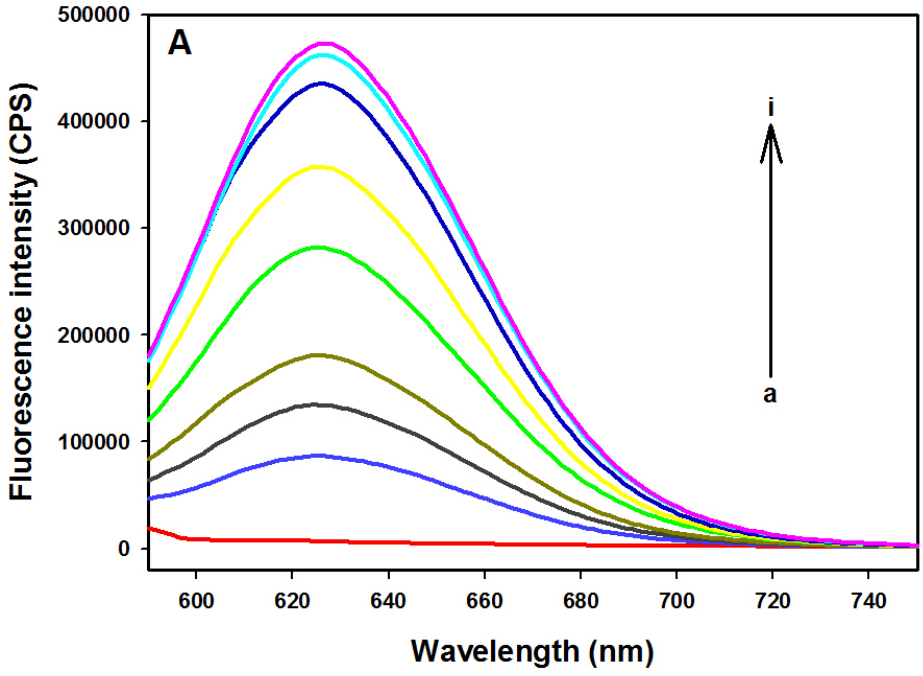
135 **Fig.S4** (A) Fluorescence spectra of the sensing system upon addition of various
136 concentrations of GOx (from a to k): 0, 1.25, 2.5, 3.75, 5, 7.5, 10, 12.5, 15, 20, 25
137 μg/mL. (B) Fluorescence enhancement of the sensing system at different
138 concentrations of GOx. F and F_0 are the fluorescence intensity of the sensing system
139 in the presence and absence of GOx.



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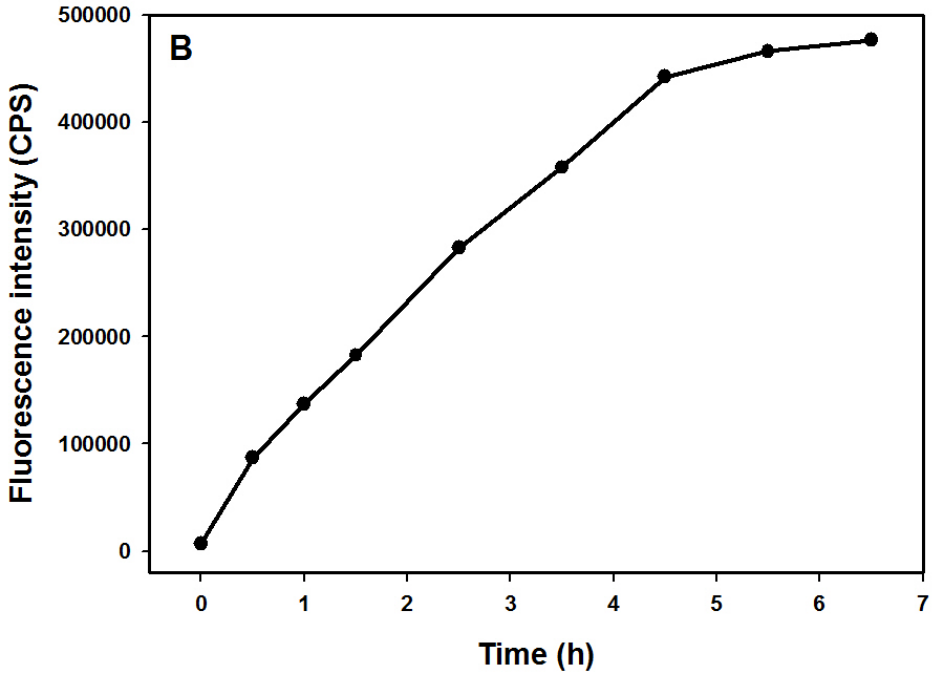

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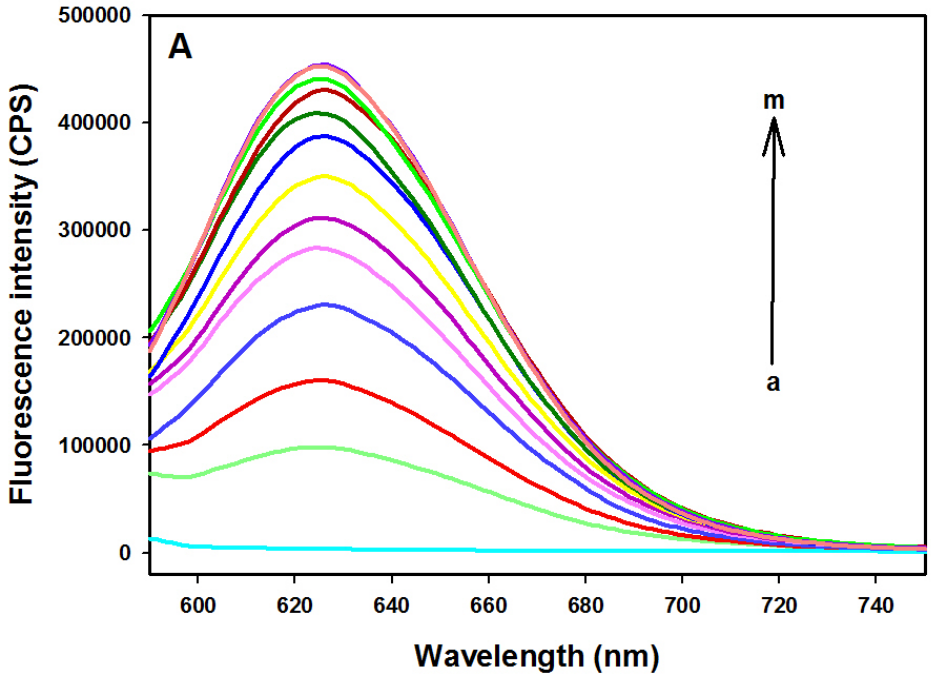
142 **Fig.S5** (A) The fluorescence emission spectra of the sensing system at different
143 reaction time for B-DNA strand scission (from a to i): 0, 0.5, 1, 1.5, 2.5, 3.5, 4.5, 5.5,
144 6.5 h. (B) Effect of reaction time of breaking B-DNA on the fluorescence intensity of
145 the sensing system.



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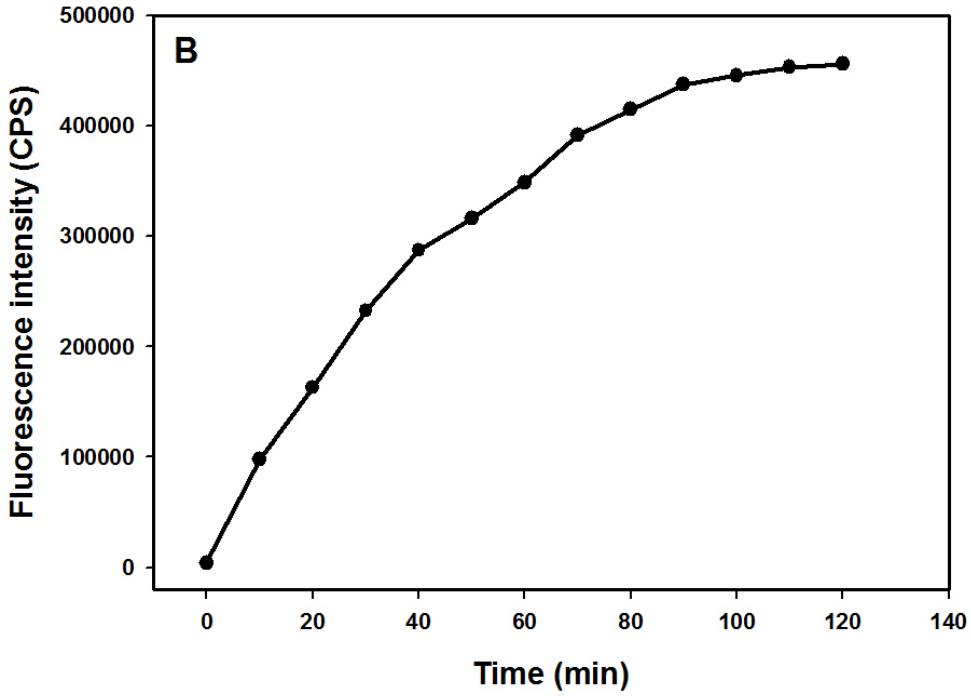

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148 **Fig.S6** (A) Fluorescence spectra of the sensing system at different incubation time for

149 DNA-Ag NCs hybridizing with G-DNA (from a to m): 0, 10, 20, 30, 40, 50, 60, 70,

150 80, 90, 100, 110, 120 min. (B) Fluorescence enhancement of the sensing system at

151 different incubation time for DNA-Ag NCs hybridizing with G-DNA.

3. References

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