Supplementary information for

Superquenching acridinium ester chemiluminescence by gold nanoparticles for DNA sequence detection

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Experimental section

Materials

Sodium citrate, HEPES, and sodium hydroxide were purchased from sigma (St. Louis, MO). Sodium phosphates (mono- and dibasic-) were purchased from Fluka (Buchs, Switzerland). The $HAuCl_4$ and 30% H_2O_2 were bought from Sinopharm Chemical Reagent Co. (China). Lysine was purchased from Wako Pure Chemical Industries (Osaka, Japan). 10-methyl-9-(phenoxycarbonyl)acridiniumfluorosulfonate (AE), 4-(2-Succinimidyl-oxycarbonylethyl)phenyl-10-acridinium-9-carboxylate trifluoromethyl sulfonate (AE-NHS), were purchased from Cayman Chemical (MI, USA). Other chemicals were of analytical-reagent grade or better. Ultrapure water with a resistivity of 18.2 MΩ cm was produced by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA).

All oligonucleotide with different sequences were synthesized and HPLC purified by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. The sequences of the oligonucleotide used in this work are as follows:

(1) 5'-NH_2-TGC ATC CAG GTC ATG TTA-3' (probe);

(2) 5'-AGA AGA TAT TTG GAA TAA CAT GAC CTG GAT GCA-3'(complement target);

(3) 5'-TTG GCT TTC AGT TAT ATG GAT GAT GTG TCT GTA-3' (non-complement target);

(4) 5'-AGA AGA TAT TTG GAA TAA CAT GAC TTG GAT GCA-3' (single base mismatched target).

Instruments

All chemiluminescence (CL) measurements were made with a MPI-B flow injection-chemiluminescence system (Xi'an Remex Electronic and Technological Co., China). The fluorescence spectra were recorded on a RF5301 fluorometer (Shimadzu, Japan). Absorption spectra were recorded on a UV-2550 spectrometer (Shimadzu, Japan). The centrifugation was performed in a Sorvall Legend Micro 17R centrifuge (Thermo Scientific).

Synthesis of gold nanoparticles (AuNPs)

AuNPs of ~13nm in diameter were prepared according to a literature method.[1] Briefly, a sodium citrate solution (38.8 mM, 10 mL) was rapidly added to a boiled $HAuCl_4$ solution (1 mM, 100 mL) under vigorous stirring. The mixed solution was boiled for 10 min and further stirred for 15 min. The solution was cooled to room temperature and filtered

through a 0.22 μm membrane filter, which was stored in a 4 °C refrigerator before use. The concentration of Au-NPs was calculated by extinction spectra based on an extinction coefficient of 2.7×10^8 $M^{-1} \cdot cm^{-1}$ at $\lambda = 520$ nm for 13-nm particles.[2]

Covalent linkage of AE to oligonucleotide probe

The oligonucleotide probe was labeled with AE-NHS according to reference with some modification.[3] The probe oligonucleotide (19.3 nmol) was dissolved in labeling buffer (50μL, HEPES 0.125 M, pH 8.0). And then a solution of the AE-NHS in DMSO (10 μL, 25 mM) was added to the above solution. The solution was gently mixed and incubated for 30 min at 37°C. Then, the excess AE-NHS was deactivated by addition of lysine (5 μL, 0.125 M in labeling buffer). By adding sequentially aqueous sodium acetate (30 μL, 3 M), water (210 μL) and chilled (-20°C) ethanol (640 μL), the precipitate reaction was carried out in refrigerator at -20°C for 30 min. After centrifuging the solution in 4°C at 13000 rpm for 20 min, the supernatant was carefully removed and the precipitate was rinsed with cold 70% ethanol twice. The precipitate was re-dissolved in 200 μL of 0.1 M sodium acetate (pH 5.2) and stored under -20°C.

Procedure for CL detection

The schematic diagram of the flow system employed for the AE CL detection is shown in Figure S1. A peristaltic pump was used to deliver flow, and PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. In a typical measurement, the NaOH solution mixed firstly with H_2O_2 in 3 mM HCl solution. The acidic conditions were used to stabilize the H_2O_2 solution. Then, the sample solution (AE) was injected into the carrier stream (water) using an eight-way injection valve equipped with a 75 μL sample

loop, and the mixture of AE sample and alkaline H_2O_2 solution finally reached detector to produce CL signals.

Quenching AE CL by AuNPs

For the experiment of AuNPs quenching AE CL, the various amounts of AuNPs were added to the AE solution (1×10^{-9} M, in 5 mM PB, pH 5.2). The flow rate was 6.8 mL/min for all lines. The mixture was introduced to the flow injection system, and the quenched CL signal was recorded.

In the CL reaction solution the unmodified AuNPs tend to aggregate in a time-dependent manner (Fig. S2A, B). To overcome this limitation, the fast flow rate (6.8 mL/min) was required for the rapid mixing and detection. The detection completed in 2-3 s (Fig. S2C inset), and the quenching efficiency of AE by AuNPs was reproducible (Fig. S2C). Furthermore, low concentration of PB buffer (5 mM) was chosen since the quenching behavior was not found to follow a "Stern-Volumer" relationship in 10 mM PB (Fig. S2D), probably due to the aggregation induced by the adsorption of AE on AuNPs.

To explore the quenching mechanism, the CL emission was measured in different conditions: solution I, 5×10^{-8} M AE; solution II, 5×10^{-8} M AE + AuNPs (10 μL, 12 nM); and solution III, the supernatant of the solution II (centrifuged at 13000 rpm for 15 min). For fluorescence measurement, 200 μL solution (I, II, or III) was mixed with H_2O_2 in 3 mM HCl (5 mM, 200 μL), the mixed with NaOH solution (0.1 M, 200 μL). The addition of AuNPs (2 μL, 12 nM) to reacted solution I was used as the control experiment. Fluorescence spectra were measured with excitation at 380 nm and emission range from 400 to 600 nm.

DNA detection based on quenching AE CL by AuNPs

The AE labeled oligonucleotide probes were hybridized with targets in 10 mM phosphate buffer solution (pH 5.2) with 0.3 M NaCl for 5 min at room temperature. An aliquot of the trial hybridization solution (10 μL) is added to 100 μL of 5 nM AuNPs diluted with PB (10 mM, pH 5.2) and an additional 100 μL of the PB solution (10 mM, pH 5.2, 0.2 M NaCl) is added immediately. After incubating 60 s, the CL emission of this mixture is measured using the flow injection system. The flow rate was 2.5 mL/min for all lines.

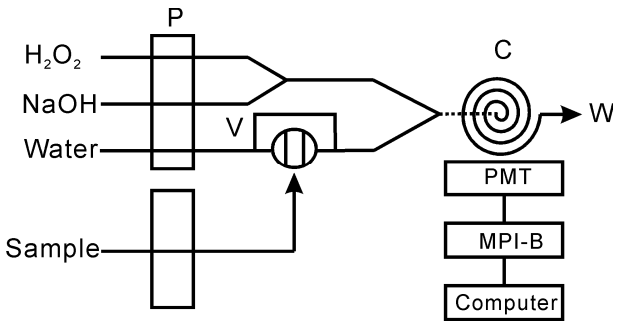

Figure S1 Schematic diagram of flow injection chemiluminescence (CL) detection system. P, peristaltic pump; V, injection valve; C, flow cell; PMT, photomultiplier tube; MPI-B, luminescence analyzer; W, waste.

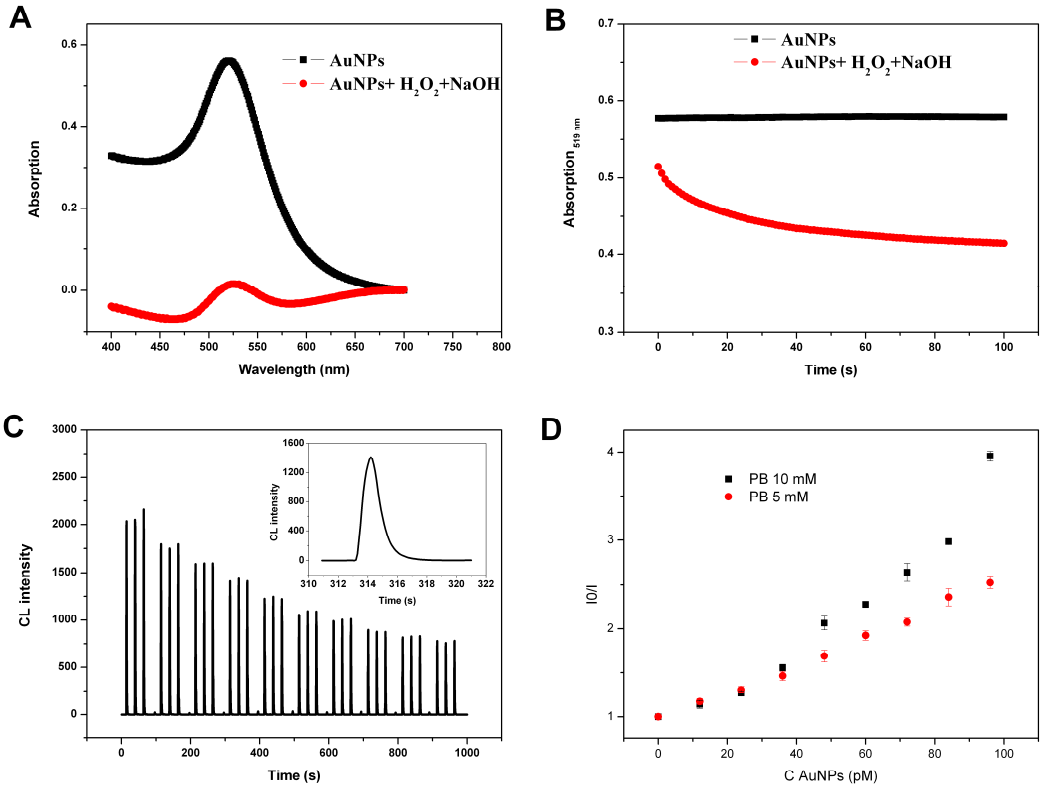

Figure S2 Absorption spectra (A) and plot of the absorption *vs.* time (B) of AuNPs in PB buffer (■) (2 nM) and in mixture of H_2O_2 and NaOH (●) (6 nM AuNPs 1.0 mL + 3 mM H_2O_2 in HCl 1.0 mL + 0.2 M NaOH 1.0mL); (C) CL profiles of 1 nM AE at different concentrations of AuNPs: 0, 12, 24, 36, 48, 60, 72, 84, 96, 108 pM (detection was repeated three times); Inset: the CL profile with addition of 36 pM of AuNPs; (D) The relationship between I_0/I and the concentration of AuNPs in different concentrations of PB buffer: 5 mM (■) and 10 mM (●).

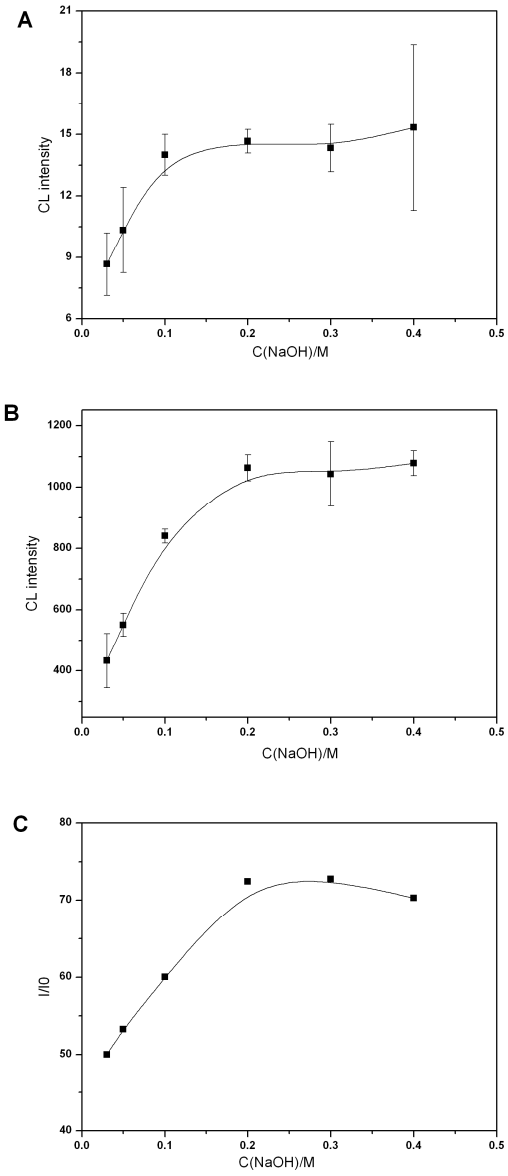

Figure S3 Effects of the concentration of NaOH on DNA sequence detection: CL intensity in absence (A) and presence (B) of target DNA; (C) CL intensity ratio in presence of target DNA.

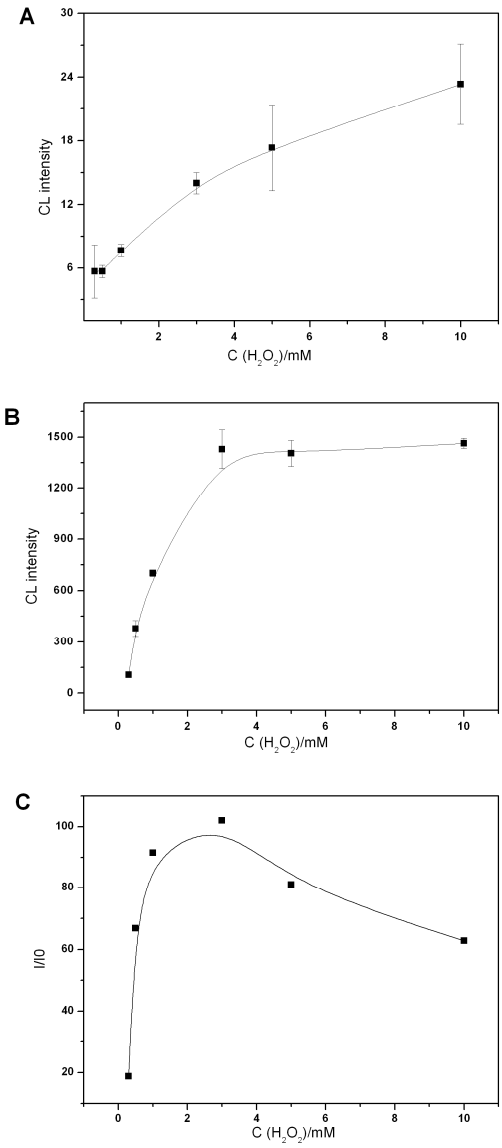

Figure S4 Effects of the concentration of H_2O_2 on DNA sequence detection: CL intensity in absence (A) and presence (B) of target DNA; (C) CL intensity ratio in presence of target DNA.

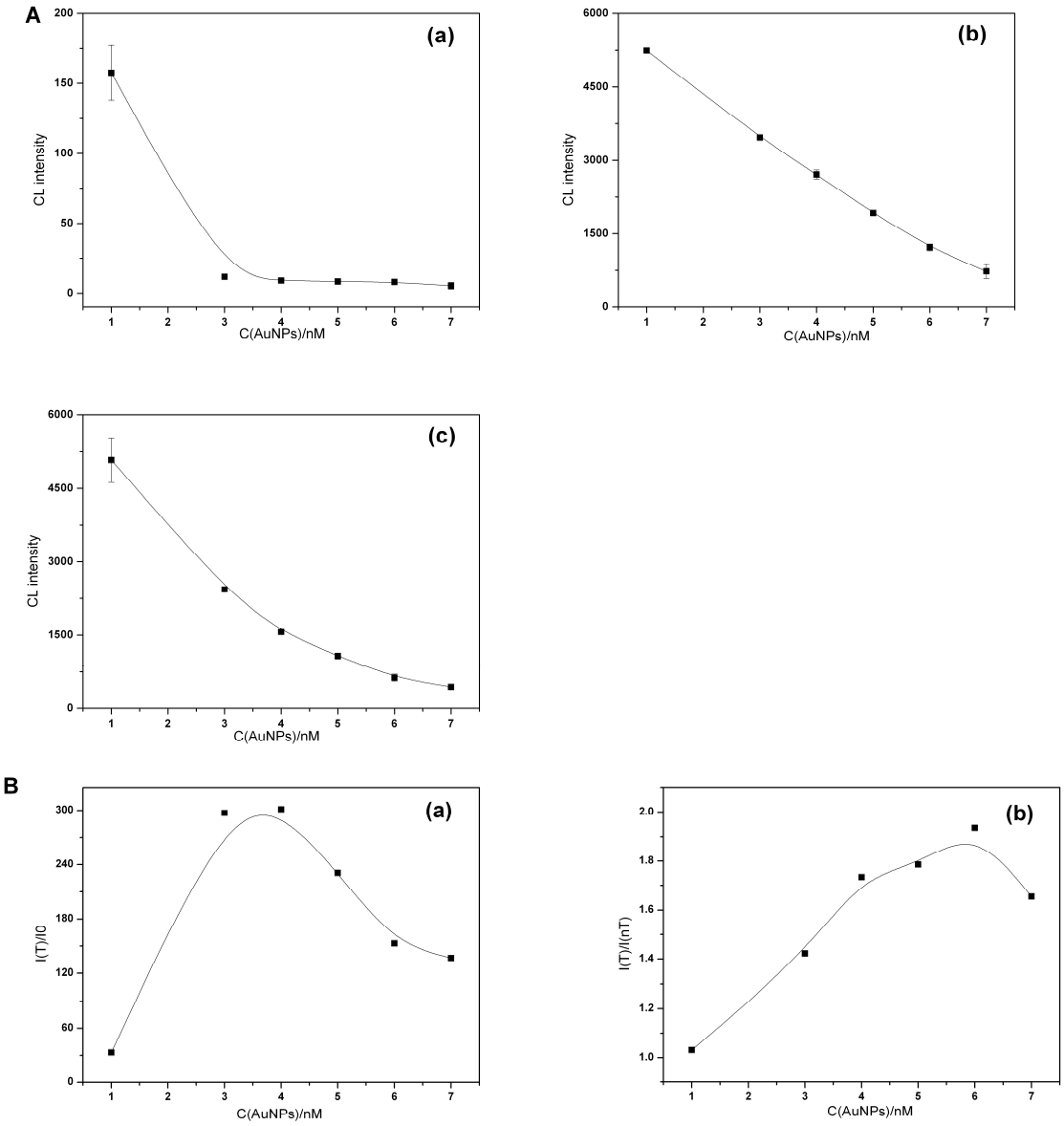

Figure S5 Effects of the concentration of AuNPs on DNA sequence detection. A: CL intensity in absence (a), presence (b) of target DNA and presence of single base mismatch target DNA (c); B: CL intensity ratio in presence of target DNA (a), in presence of single base mismatch target DNA (b).

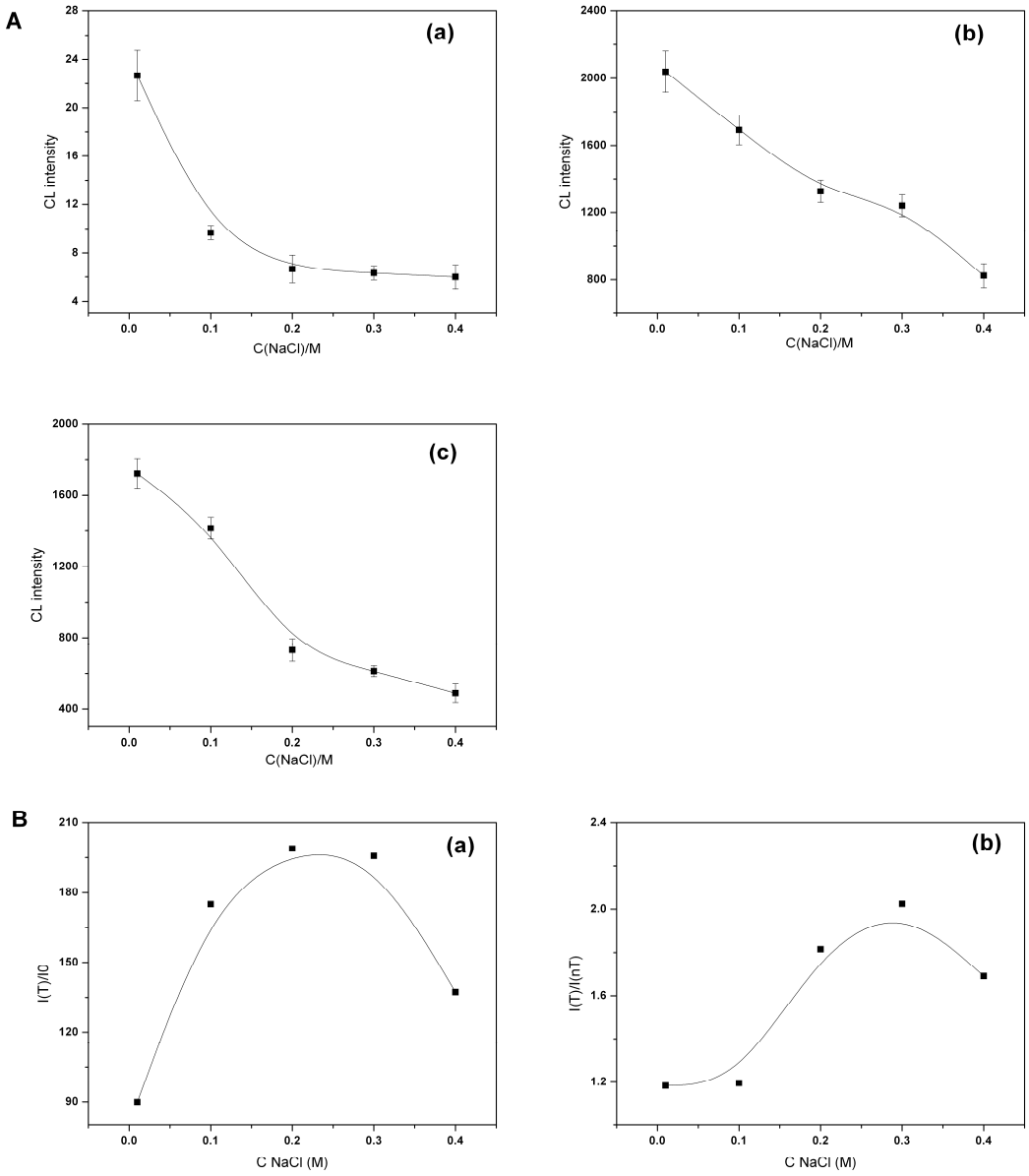

Figure S6 Effects of the concentration of NaCl on DNA sequence detection. A: CL intensity in absence (a), presence (b) of target DNA and presence of single base mismatch target DNA (c); B: CL intensity ratio in presence of target DNA (a), in presence of single base mismatch target DNA (b).

1. K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735-743.
2. L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian and G. Viswanadham, *Anal. Chem.*, 2000, **72**, 5535-5541.
3. R. C. Brown, Z. Li, A. J. Rutter, X. Mu, O. H. Weeks, K. Smith and I. Weeks, *Org. Biomol. Chem.*, 2009, **7**, 386-394.