Electronic Supplementary Material (ESI) for Chemical Communications

A novel DNA-templated click chemistry strategy for fluorescent detection of copper (II) ions

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Supporting Information

Experimental section

Materials and Measurements

Klenow fragment (3'-5' exo-), dNTPs and streptavidin-coated 1 μm magnetic microparticles (SA-MMPs) were purchased from New England Biolabs (NEB) Inc. 10000 × SYBR Green I in anhydrous DMSO was obtained from Invitrogen (Carlsbad, CA, USA). All chemical reagents were of analytical grade and used without further purification. All solutions were prepared with ultra-pure water (18.25 MΩ cm) from a Millipore system. Fluoresence spectra were obtained on a Hitachi F-4500 fluorometer (Hitachi Co. Ltd., Japan). All the oligonucleotides used in this work were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of the synthesized oligonucleotides are as following.

Synthesized Oligonucleotides Used in the Experiments

A: 5'-Biotin-GCT AGC GTG TAG-Alkyne-3'

B: 5'-azide-CTA CAC GCT AGC CTT CAG CAG TCG-3'

C: 5'- CGACTGCTGAAGGCTAGCGTGTAG-3'

D: 5'-azide-TGACCGACCGTAGGAATTCCGA-3'

E: 5'-CG ACT GCT GAA G-3'

A-OH: 5'-GCT AGC GTG TAG-3'

B-OH: 5'-CTA CAC GCT AGC CTT CAG CAG TCG -3'

Synthesis of Tris-(hydroxypropyltriazolylmethyl)amine (THPTA)

The Tris-(hydroxypropyltriazolylmethyl)amine (THPTA), a water soluble Cu^{+} binding ligand, was

synthesized according to published literature.[1] Tripropargylamine (1.3 g, 10 mmol) in an acetonitrile/ methanol solution (20 mL) was treated sequentially with 3-azido-1-propanol (4.0 g, 40 mmol) (prepared according to a literature procedure[2]), 2,6-lutidine (1.1 g, 10 mmol), and $Cu(MeCN)_4PF_6$ (3 mol % with respect to total alkyne units). Upon addition of the copper salt, the reaction mixture was cooled in an ice bath. After the mixture was stirred at room temperature for 3 days, the reaction mixture was evaporated and then dissolved in methanol. The crude product was precipitated in acetonitrile. Yield: 81%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 7.83 (s, 3H), 4.39 (t, 6H, J = 6.8 Hz), 3.71 (s, 6H), 3.46 (t, 6H, J=6.0 Hz), 2.01 (m, 6H). ^{13}C NMR (D_2O) δ (ppm): 143.16, 125.32, 58.12, 47.50, 47.22, 31.84.

Denaturing PAGE analysis of click ligation of oligonucleotides

Double-stranded DNA hybrid A and B (1 μM), THPTA (1 mM) and sodium ascorbate (1 mM) were added to 4.5 μL of PBS buffer (10 mM sodium phosphate, 0.2 M NaCl, pH 9.0) sequentially. Then 0.5 μL solution of Cu^{2+} with different concentration was added, and the reaction mixture was incubated at 30 °C for 2 h.

The other control experiments were conducted as the above procedure, except for adding different DNA instead of A and B to the reaction solution.

The reaction mixture was then diluted into 1 x PAGE loading buffer containing 7 M urea. The reaction mixture was separated by denaturing PAGE on a 15% TBE/urea polyacrylamide gel. The electrophoresis was carried in 1× tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 82 V for 2.5 h. The gels were stained by SYBR Green II.

Typical experimental process for Cu^{2+} detection

Double-stranded DNA hybrid A and B (1 μM), THPTA (1 mM) and sodium ascorbate (1 mM) were added to 4.5 μL of PBS buffer (10 mM sodium phosphate, 0.2 M NaCl, pH 9.0) sequentially. Then 0.5 μL solution of Cu^{2+} with different concentration was added, and the reaction mixture was incubated at 30 °C for 2 h.

After incubation, for sequential strand displacement by polymerase extension of primer, DNA primer E (0.5 μM), dNTPs (0.5 mM), 2.5 units of Klenow fragment (3'-5' exo-) and 5 μL of 1 × buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, and 1 mM DTT, pH 8.0) were added to the resulting solution

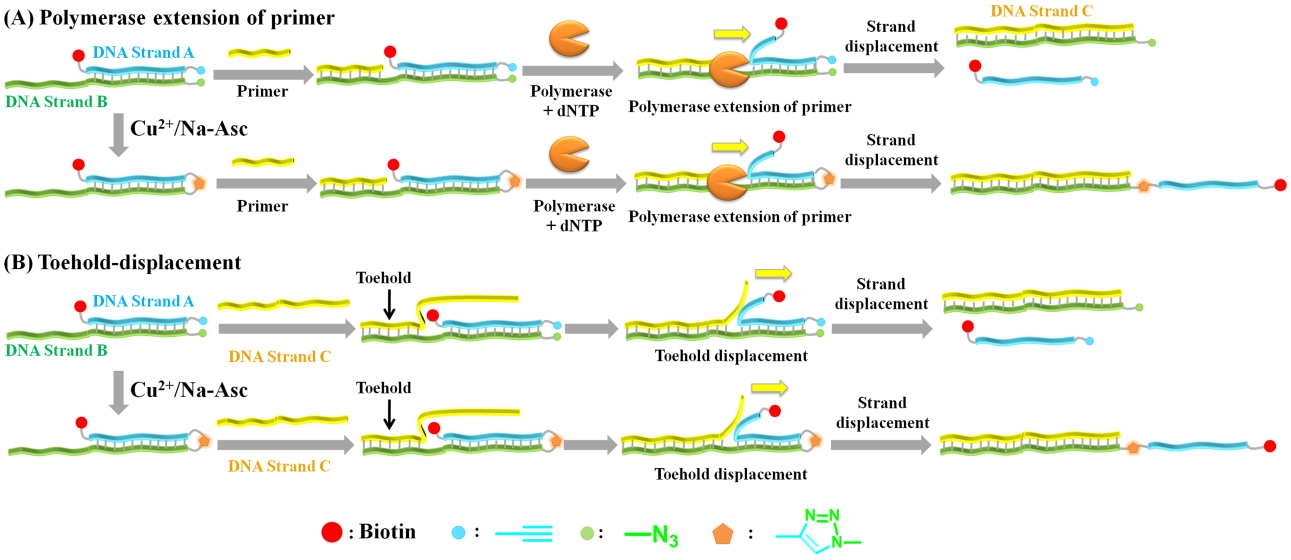
and the mixture were incubated at 37 °C for 30 min. For sequential strand displacement by toehold-displacement, 5 μL of C (0.5 μM) was added to the resulting solution and the mixture was incubated at 30 °C for 30 min.

Magnetic enrichment and separation of biotinylated DNA by SA-MMPs were conducted after strand displacement. Two microlitres of SA-MMPs (80 μg / mL) and 38 μL binding & washing (B & W) buffer (20 mM Tris-HCl, 0.5 M NaCl, and 1 mM EDTA, pH 7.5) were added and incubated at room temperature for 30 min. The MMPs were deposited and separated from the solution with the assistance of magnet. The deposited MMPs were then washed with B & W buffer three times (3 × 100 μL) and finally were dissolved in 94 μL of B & W buffer. Then 6 μL of 20 × SYBR Green I was added and incubated for 20 min at room temperature. The fluorescence was then measured with Hitachi F-4500 fluorometer. The excitation wavelength was 490 nm, and the emission wavelengths were in the range from 510 to 640 nm with both excitation and emission slites of 5 nm.

References

(1) T. R., Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Org. Lett.*, 2004, **6**, 2853-2855.

(2) X. Liu, A. Thakur, and D. Wang, *Biomacromolecules*, 2007, **8**, 2653-2658.



Scheme S1 Schematic representation of two strategies for sequential strand-displacement. (route A) polymerase extension of primer and (route B) toehold-displacement.

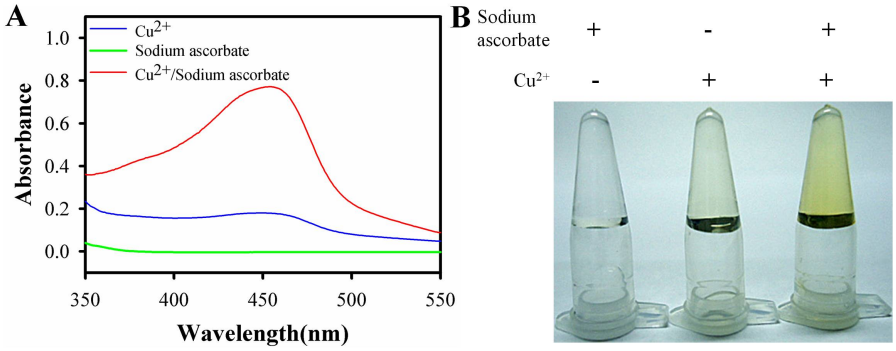


Fig. S1 The absorption spectra (A) and color change (B) of neocuproine in the presence of sodium ascorbate (green), Cu^{2+} (blue), or co-existence of sodium ascorbate and Cu^{2+} (red).

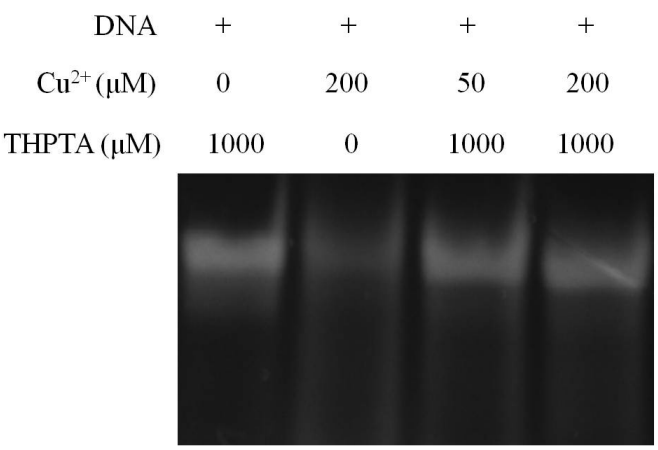


Fig. S2 Non-denaturing PAGE analysis of the protection effect of THPTA on DNA against Cu^{2+}-induced oxidative degradation.

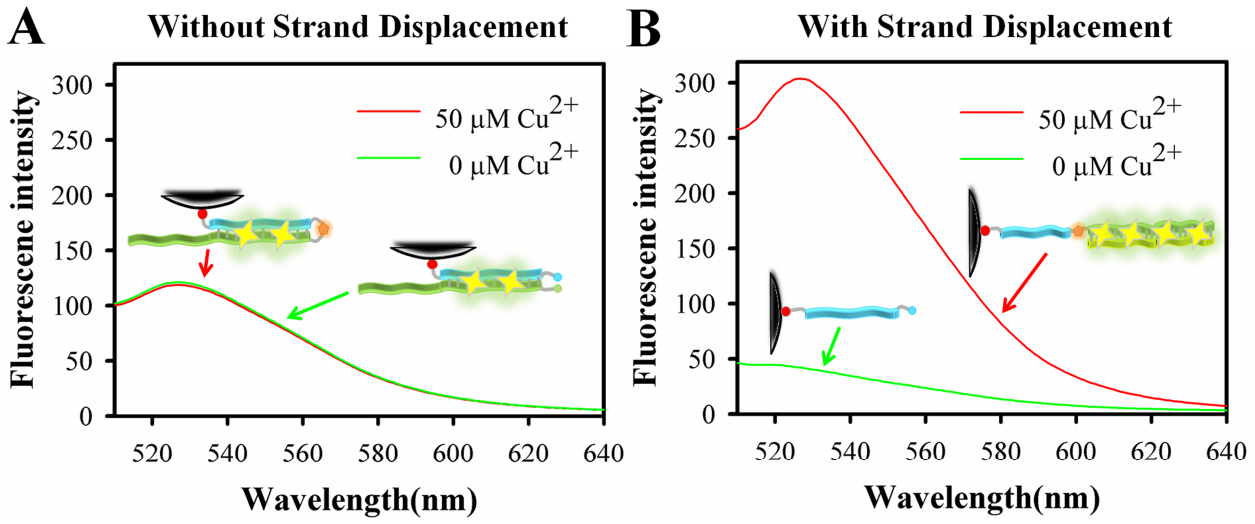


Fig. S3 The effect of strand displacement on the fluorescence response of the biosensor to Cu^{2+}. Fluorescence spectra and graphic illustration of SG 1 stained DNA probe bound by SA-MMPs in the presence (red line) or absence (green line) of 50 μM Cu^{2+} without strand displacement (A) and with strand displacement (B).

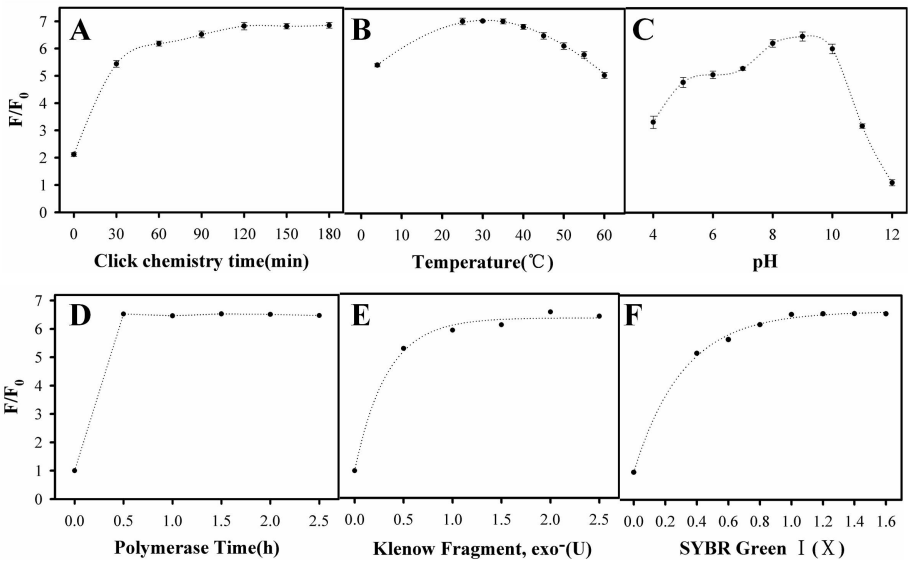


Fig. S4 The effect of some factors of the sensing system on the fluorescence change at 527 nm: (A) the time of click chemistry (0, 30, 60, 90, 120, 150 and 180 min, respectively), (B) the temperature of click chemistry (4, 25, 30, 35, 40, 45, 50, 55 and 60 °C, respectively), (C) the pH value of click chemistry (4, 5, 6, 7, 8, 9, 10, 11 and 12, respectively), (D) the time of polymerase reaction (0, 0.5, 1, 1.5, 2 and 2.5 h, respectively), (E) the concentration of polymerase (0, 0.5, 1, 1.5, 2 and 2.5 U, respectively), and (F) the concentration of SYBR Green I (0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 and 1.6 ×, respectively).

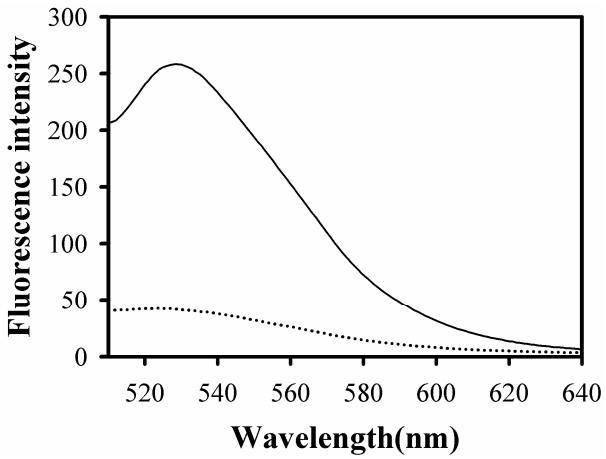


Fig. S5 Fluorescence detection of Cu^{2+} using toehold-displacement. Fluorescence spectra of SG 1 stained DNA probe bound by SA-MMPs with (solid line) or without (dotted line) treatment of 50 μM Cu^{2+}.

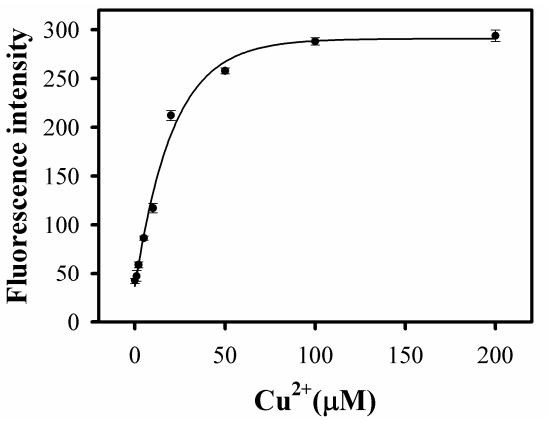


Fig. S6 Fluorescence response at 527 nm of the ligation product through DNA-templated click reaction and toehold-displacement as a function of Cu^{2+} concentration in the presence of complementary (solid line) or unpaired (dotted line) DNA templates.

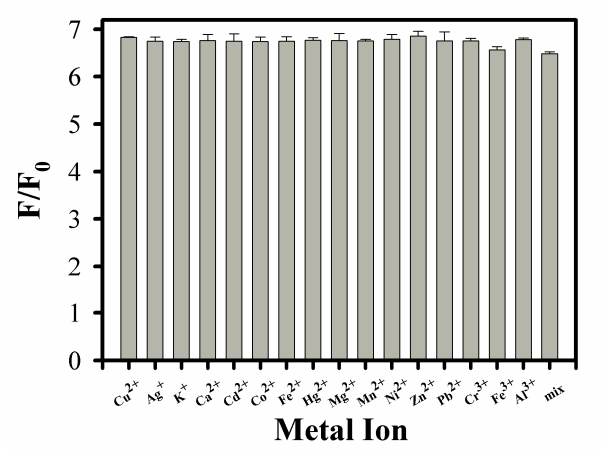


Fig. S7 The Fluorescence response of the sensing system to 50 μM Cu^{2+} in the presence of 50 μM of other metal ion (Ag^{+}, K^{+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+} and Al^{3+}, separately) or a mixture of all these metal ions (50 μM for each metal ion).

Table S1 Determination of Cu^{2+} in real water samples.

Samples	Added (μM)	Mean found[a] (μM)	Mean recovery[b](%)	RSD[c] (%)
1	0.5	0.53	106	0.3
2	1.00	1.02	102	2.4
3	5.00	4.75	95	1.9
4	7.50	7.33	98	0.3

[a] Mean concentration of three replicates.
[b] Mean recovery *(%)* = 100 × ($c_{\text{mean found}}/c_{\text{added}}$).
[c] Relative standard deviation of three determinations