Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Click chemistry-mediated catalytic hairpin self-assembly for amplified and sensitive fluorescent detection of Cu^{2+} in human serum

Daxiu Li, Wenjiao Zhou, Yaqin Chai, Ruo Yuan and Yun Xiang*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China; Fax: +86-23-68252277; Tel: +86-23-68253172; E-mail: yunatswu@swu.edu.cn (Y. Xiang).

Experimental Section

Apparatus: Fluorescence measurements were performed on a RF-5301PC spectrophotometer (Shimadzu, Tokyo, Japan) at room temperature with a 150W Xenon lamp (Ushio Inc., Japan) as the excitation source. The emission spectra were collected from 500 nm to 600 nm with excitation wavelength of 490 nm, and the fluorescence intensity was measured at 520 nm. Both the excitation and emission slits are 5 nm.

Materials and reagents: $CuSO_4 \cdot 5H_2O$, [tris(hydroxymethyl)aminomethane] (tris) and sodium ascorbate were obtained from Kelong Chemical Inc. (Chengdu, China). Healthy human serum samples were provided by the 9th People's Hospital of Chongqing (Chongqing, China). All DNA sequences used in the experiments were synthesized and HPLC purified by Shanghai Sangon Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of the DNAs were listed as follows:

alkynyl-DNA: 5'-GACGGGAAGC-CH≡CH-3';

azido-DNA: 5'-N_3-CACGAGACAC-3';

MB1:5'-(DABCYL)-CCTCTCCGTGTCTCGTGGCTTCCCGTCAGAGAGGATG-(FAM)-3';

MB2:5'-(FAM)-CATCCTCTCTGACGGGAAGCCACGAGACACGGAGAGA-(DABCYL)-3'

All reagents were analytical grade and were used without further purification. The solutions were prepared using ultrapure water (18.25 MΩ-cm).

Assay procedures: All the oligonucleotides used in this work were dissolved to final concentration of 100 μM by ultrapure water, and stored at -20°C for further use. Prior to experiments, MB1 and MB2 were separately heated at 90°C for 5 min and cooled down to room temperature for 1 hour. Next, alkynyl-DNA (0.1 μM), azido-DNA (0.1 μM), MB1 (0.2 μM) and MB2 (0.2 μM) were mixed with Tris-HCl buffer (20 mM Tris, 0.1 M NaCl, 5 mM $MgCl_2$, PH 7.4). Then, Cu^{2+} at different concentrations and sodium ascorbate at five times the concentration of Cu^{2+} were added to the mixture to a total volume of 50 μL. The mixture was incubated at room temperature for 90 min. After the incubation, the resulting solutions were diluted with Tris-HCl buffer to a total volume 200 μL. Finally, the fluorescence intensity of the solution was measured.

Native Polyacrylamide Gel Electrophoresis (PAGE): The sample solutions loaded with 6×loading buffer were transferred to the freshly prepared 16% native polyacrylamide gel for analysis. The electrophoresis was carried out in 1×TBE (pH 8.3) at a 100 V constant voltage for 2 h. After staining with ethidium bromide for 10 min, the gel was illuminated under UV light and imaged with a digital camera.

Supplementary Figure:

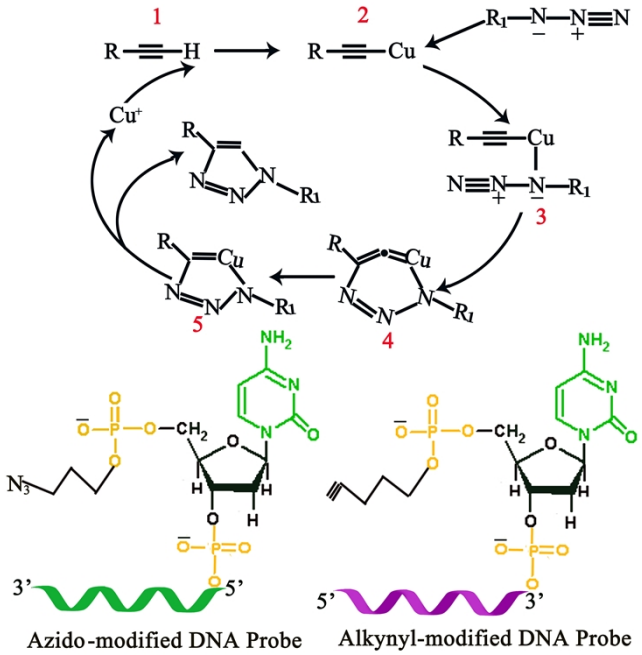

Fig. S1 The molecular mechanism for Cu^{+}-mediated click ligation of the alkynyl- and azido-DNA.

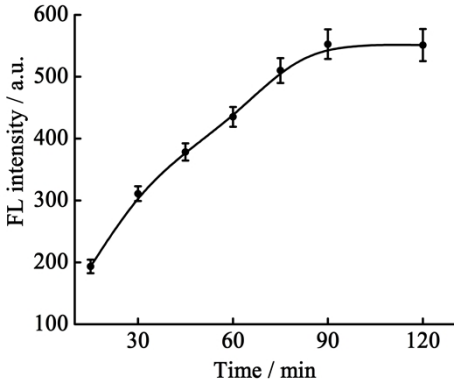

Fig. S2 The Effect of reaction time on the fluorescence intensity of the probe solutions with the presence of Cu^{2+} at 0.5 μM.

In order to achieve the best analytical performance for Cu^{2+} detection by using the proposed method, the reaction time was optimized at room temperature. For this purpose,

the effect of the reaction time on the signal output of the proposed method was investigated by monitoring the fluorescence emission intensity of the probe solution with the presence of Cu^{2+} (0.5 μM) at a time interval of 15 min from 15 to 120 min. As shown in Fig. S1, the fluorescence intensity increases rapidly with increasing reaction time from 15 min to 90 min and reaches a plateau after 90 min. Therefore, 90 min is chosen as the optimal reaction time for subsequent experiments.

Table S1 Comparison of different methods for Cu^{2+} detection

Cu^{2+} sensors	Detection method	Detection limit	Assay time	Reference
Chemodosimeters	fluorescence	20 nM	20 min	1
Chemodosimeters	fluorescence	100 nM	10 min	2
Chemodosimeters	fluorescence	0.74 nM	10 min	3
Click chemistry	fluorescence	290 nM	2 h	4
Click chemistry	Colorimetry	5.9 nM	2 h	5
Click chemistry	fluorescence	65 nM	3 h	6
Click chemistry	fluorescence	0.2 nM	90 min	This work

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