Lateral flow nucleic acid biosensor for Cu^{2+} detection in aqueous solution with high sensitivity and selectivity†

Zhiyuan Fang,‡[a] Jing Huang,‡[ab] Puchang Lie,[a] Zhuo Xiao,[a] Chuanyan Ouyang,[a] Qing Wu,[a] Yixing Wu,[c] Guodong Liu*[d] and Lingwen Zeng*[a]

Received 24th July 2010, Accepted 15th October 2010
DOI: 10.1039/c0cc02782k

A lateral flow nucleic acid biosensor based on copper-dependent DNA-cleaving DNAzyme and gold nanoparticles has been developed for the visual detection of copper ions (Cu^{2+}) in an aqueous solution with a detection limit of 10 nM.

Copper (Cu^{2+}) is an essential element required as a cofactor and/or structural component of numerous enzymes and other proteins needed in metabolic processes. However, high levels of copper have adverse health consequences, such as stomach and intestinal distress, liver or kidney damage, and loss of cognition in the elderly and individuals with Wilson's disease.[1,2] The U.S. Environmental Protection Agency (EPA) set 1.25 ppm (20 μM) as the maximum contamination concentration for Cu^{2+} in drinking water.[3]

Various strategies and technologies have been developed to detect Cu^{2+}. Conventional instrumental analysis methods, such as inductively coupled plasma (ICP)[4] and graphite furnace atomic absorption spectroscopy,[5] have been used as standard procedures. The advent of new methods and technologies, including electrochemical sensors,[6] surface plasmon resonance (SPR),[7] and scanning electrochemical microscopy (SECM) combined with SPR,[8] quantum dots-based assay,[9] fluorescence anisotropy assay,[10] and Cu^{2+}-specific-DNAzyme based fluorescence sensors,[11] offer sensitive and selective tools for detecting Cu^{2+}; however, high equipment cost and the requirement of highly trained personnel prevent their application in the routine detection of Cu^{2+}. Recently Au-nanoparticle (Au-NP)-based colorimetric assays, which don't require instruments and complex operation, have attracted considerable interest for the detection of various analytes, including heavy metal ions.[12,13] Particularly, DNAzyme-based colorimetric assays have been applied to the detection of Pb^{2+} and Cu^{2+}.[14,15] A significant challenge for colorimetric assays is the difficulty of distinguishing the color change at extremely low concentrations (for example, nanomolar level) of metal ions with the naked eye. Furthermore, the detection condition should be tightly controlled because the cleavage reaction in the presence of DNAzyme is vulnerable to salt concentration.

Recently we developed lateral flow nucleic acid biosensors (LFNABs) for the detection of nucleic acids,[16] proteins,[17] and cancer cells.[18] Lu's group reported "dipstick" tests for the detection of cocaine[19] and lead.[20] In this communication we first presented a LFNAB for detecting Cu^{2+} with Cu^{2+}-specific-DNAzyme and Au-NPs. The sensor has the following features: (1) high sensitivity to detect a minimum of 10 nM Cu^{2+}, (2) high selectivity, and (3) no requirement for complex and expensive instrumentation.

Based on the sequences of Cu^{2+}-dependent DNA-cleaving DNAzymes that catalyze cleavage of a ribonucleotide linkage,[21,22] we designed a new substrate for the detection of Cu^{2+} with the LFNAB. The substrate is elongated at the 3′-end to 48 nt without changes of the original DNAzyme; the sequence of the new substrate is shown in Fig. 1A (left). In the presence of Cu^{2+}, the substrate is irreversibly cleaved at the cleavage site (the guanine in red). The cleaved piece (in green

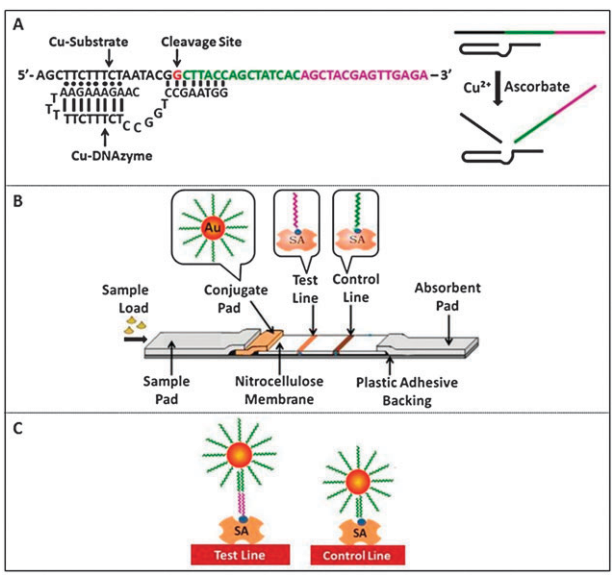

Fig. 1 (A) Structure of the Cu^{2+}-specific-DNAzyme (left) and cleavage of the substrate in the presence of Cu^{2+} and ascorbate (right). The cleavage site is indicated by an arrow at the emphasized red "G". (B) Schematic illustration of lateral flow nucleic acid biosensor for detecting the cleaved DNA strands. (C) Visual detection of Cu^{2+} by capturing Au-NPs on the test and control zones of the lateral flow nucleic acid biosensor. SA: streptavidin.

[a] *South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510663, China. E-mail: zeng6@yahoo.com*
[b] *University of Science and Technology of China, Hefei, 230022, China*
[c] *Department of Occupational Diseases, Guangzhou No.12 Hospital, Guangzhou, 510620, China*
[d] *Department of Chemistry and Biochemistry, North Dakota StateUniversity, Fargo, ND 58015, USA. E-mail: guodong.liu@ndsu.edu*

† Electronic supplementary information (ESI) available: Reagents and materials, instruments, experimental procedures and conditions. See DOI: 10.1039/c0cc02782k

‡ Joint first authors: These two authors contributed equally to this work.

and purple) at the 3′-end of the substrate is released due to decreased affinity to the enzyme (Fig. 1A, right). The cleaved product is then applied to the LFNAB, in which three DNA probes are immobilized (Fig. 1B). The LFNAB consists of a sample pad, a conjugate pad, a nitrocellulose membrane and an absorbent pad. A 5′-thiol-modified 15-mer-DNA probe 1 (in green), which is complementary with part of the cleaved piece (in green at the 5′ end), is conjugated with Au-NP (15 nm in diameter), and the conjugates are dispensed on the conjugate pad. Another 15-mer 3′-biotin- modified DNA probe 2 (in purple) complementary with the part of the cleaved piece at the 3′-end is immobilized on the test zone of nitrocellulose membrane through a streptavidin(SA)-biotin interaction. Control DNA probe 3 (in green), complementary with DNA probe 1, is immobilized on the control zone of the nitrocellulose membrane. The reaction mixture is applied to the sample pad of the LFNAB; the solution passes the conjugate pad and rehydrates the Au-NP-DNA probe 1 conjugates. The cleaved DNA pieces (in green and purple) hybridize with DNA probe 1 of the Au-NP-DNA probe 1 conjugates to form the complex and continue to migrate along the strip. The hybrids are captured on the test zone by the second hybridization reactions between the cleaved DNA piece (purple part) and the immobilized DNA probe 2 (Fig. 1C). The accumulation of Au-NPs in the test zone of the nitrocellulose membrane is visualized as a characteristic red band. The excess Au-NP-DNA probe 1 conjugates continue to migrate and pass the control zone. Then, the excess Au-NP-DNA probe 1 conjugates are captured by the hybridization events between DNA probe 1 and DNA probe 3, thus forming the second red band (Fig. 1C). In the absence of Cu^{2+}, there is no cleavage of the DNAzyme substrate, and no red band is observed on the test zone. In this case, a red band on the control zone shows that the biosensor is working properly. Qualitative analysis is performed by observing the color change of the test zone, and quantitative detection can be realized by recording the optical density of the test band with a portable "strip reader".

Fig. 2 presents the typical photo images and corresponding optical responses of the LFNABs in the presence of different concentrations of Cu^{2+}. The sample solutions with seven concentrations (100 μM, 10 μM, 1 μM, 100 nM, 50 nM, 10 nM, 0 nM) were prepared by dissolving $CuCl_2$ in ultrapure water. For each test, 100 μL of sample solution was mixed with 100 μL of working solution (100 nM substrate and 500 nM DNAzyme in 4× saline–sodium citrate (SSC)). After 30 min incubation, the mixture was applied to the sample pad of the LFNAB. One can see that there was no red band observed on the test zone of the LFNAB in the absence of Cu^{2+} (negative control, bottom); red bands were observed on the test zones in the presence of Cu^{2+} at different concentration levels. The intensities of the bands on the test zones (peak areas), which were recorded by a handheld strip reader (Fig. 2, right), increased with the increment of Cu^{2+} concentrations. The test zone band is quite visible even at 10 nM Cu^{2+} which can be used as the threshold for the visual detection of Cu^{2+} (based on a signal to noise ratio threshold of 3). The sensitivity is comparable with that of the fluorescence-based DNAzyme catalytic beacon Cu^{2+} sensor (35 nM).[11] The LFNAB has a dynamic range up to 100 μM,

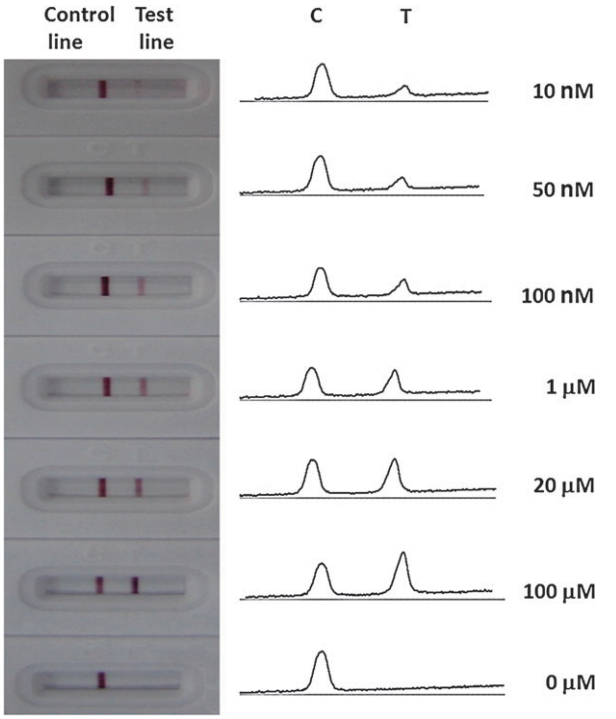

Fig. 2 Typical photo images (left) and corresponding intensities (right) of the LFNABs in the presence of different concentrations of Cu^{2+}. The intensities of the bands were recorded by a handheld strip reader.

which is useful for detecting Cu^{2+} in drinking water because the U.S. EPA has defined a maximum contamination level of 20 μM.[3]

To test the specificity of the assay, sample solutions containing different metal ions, including Hg^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Ba^{2+}, Ca^{2+}, Ni^{2+}, and Co^{2+}, were applied to LFNAB under the same experimental conditions. The images (left) of LFNAB and corresponding optical responses (right) from the strip reader are shown in Fig. 3. Except where weak responses were observed with 20 μM Pb^{2+} and Co^{2+}, there was no red band observed on the test zones of the LFNABs in the presence of other 20 μM metal ions, indicating the high specificity of the detection of Cu^{2+}.

In the current study, we optimized the length of DNA probes (DNA probe 1 and 2) which were used to capture the cleaved pieces through sandwich-type DNA hybridization events on the LFNAB. It was found that the sensitivity of the test with the probes consisting of 15 nt or more was higher than that of probes with fewer nucleotides (results not shown). Such different sensitivities could be caused by the affinity difference of DNA probes that have different lengths. Therefore, the DNA probes with 15 nt were chosen to synthesize the DNA probes for the preparation of LFNABs. False-positive results can be caused by un-hybridized substrates in the working solution. Despite excess enzyme strands used, there were still free substrates which could hybridize with DNA probe 1 on the Au-NP-DNA probe 1 conjugates to form the duplexes, resulting in Au-NPs that were captured on the test zone of the LFNAB. To overcome this kind of false-positive result, a competitive DNA probe

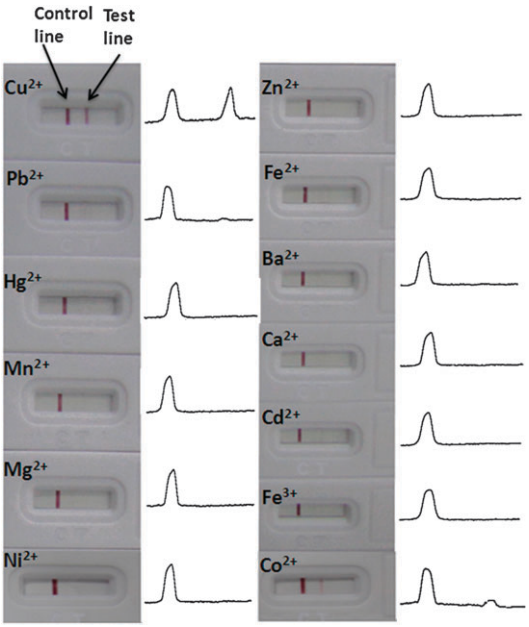

Fig. 3 Photo images (left) and corresponding optical responses (right) of the LFNABs in the presence of different metal ions. The substrate-DNAzyme component was first incubated with these ions for 30 min and then applied to LFNAB. The photo was taken within 20 min after the sample loading. The specificity of the biosensor over other metal ions was tested at a concentration of 20 μM.

(AGCTACGAGTTGAGA), which was complementary with the DNA probe 2 on the test zone, was used to treat the sample pad of the LFNAB. The sample pad was soaked with the competitive DNA probe solution from different concentrations; it was found that 50 nM of competitive DNA probe in 0.5 μg/ml of salmon DNA was sufficient to block the unbounded substrates and eliminated the "false positive" completely (ESI,† Fig. S1). Although this method sacrificed the sensitivity to some extent (the detection limit of 5 nM (S/N = 3) was obtained with the strip reader in the absence of competitive DNA probe), the detection limit of 10 nM in the presence of competitive DNA was still superior to the previous colorimetric sensors.[15]

A running buffer would have a strong effect on the sensitivity of the test. The sample pad was treated with various running buffers which may minimize the non-specific adsorption and increase the sensitivity of the LFNAB. We compared the performances of different running buffers, including phosphate buffer (PB), 4×SSC, and HEPES. It was found that SSC buffer yielded the best results (ESI,† Fig. S2). This may due to the fact that DNAzyme and the substrate were well hybridized in SSC,[16,18] rendering a high cleaving activity and low background.

The shelf life of the LFNAB was tested by storing the biosensors at room temperature. It was found that the responses of LFNABs did not change significantly after one month's storage. The signals of the LFNABs for 20 μM of Cu^{2+} decreased less than 10% of that obtained with the newly prepared biosensors, and the detection limit of LFNAB was the same (10 nM), indicating that the LFNAB has a good shelf life.

In conclusion, we have developed a lateral flow nucleic acid biosensor based on copper-dependent DNA-cleaving DNAzyme and Au-NPs for the visual detection of copper ions (Cu^{2+}) in aqueous solution with high sensitivity and selectivity. Under optimal condition, the LFNAB was capable of detecting a minimum of 10 nM Cu^{2+} in 20 min. Comparing with other reported methods based on copper-dependent DNA-cleaving DNAzymes, the sensitivity of the LFNAB developed here is comparable or higher than that of fluorescence and colorimetric approaches. Moreover, the LFNAB is easy to use, and doesn't require trained personnel and complex and expensive instruments. The LFNAB coupled with a portable strip reader shows great promise for in-field quantitative testing, it can be used in remote areas where diagnostic facilities are limited.

This work was supported by North Dakota Experimental Program to Stimulate Competitive Research (EPSCoR) and new faculty start-up funds of North Dakota State University.

Notes and references

1 G. J. Brewer, *J. Am. Coll. Nutr.*, 2009, **28**, 238–242.
2 P. G. Georgopoulos, A. Roy, M. J. Yonone-Lioy, R. E. Opiekun and P. J. Lioy, *J. Toxicol. Environ. Health, Part B*, 2001, **4**, 341–394.
3 U.S. EPA. Maximum contaminant level goals and national primary drinking water regulations for lead and copper; final rule. Fed Reg 1991, **56**, 26460–26564.
4 T. Kato, S. Nakamur and M. Mirita, *Anal. Sci.*, 1990, **6**, 623–626.
5 M. S. Chan and S. D. Huang, *Talanta*, 2000, **51**, 373–380.
6 W. Yantasee, K. Hongsirikarn, C. L. Warner, D. Choi, T. Sangvanich, M. B. Toloczko, M. G. Warner, G. E. Fryxell, R. S. Addleman and C. Timchalk, *Analyst*, 2008, **133**, 348–355.
7 S. Hong, T. Kang, J. Moon, S. Oh and J. Yi, *Colloids Surf., A*, 2007, **292**(2–3), 264–270.
8 Y. Xin, Y. Gao, J. Guo, Q. Chen, J. Xiang and F. Zhou, *Biosens. Bioelectron.*, 2008, **24**(3), 369–375.
9 K. M. Gattas-Asfura and R. M. Leblanc, *Chem. Commun.*, 2003, **21**, 2684–2685.
10 B. C. Yin, P. Zuo, H. Huo, X. Zhong and B. C. Ye, *Anal. Biochem.*, 2010, **401**(1), 47–52.
11 J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838–9839.
12 M. R. Knecht and M. Sethi, *Anal. Bioanal. Chem.*, 2009, **394**, 33–46.
13 F. Xia, X. Zuo, R. Yang, Y. Xiao, D. Kang, A. Vallée-Bélisle, X. Gong, J. D. Yuen, B. Hsu, A. J. Heeger and K. W. Plaxco, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 10837–10841.
14 H. Wei, B. Li, J. Li, S. Dong and E. Wang, *Nanotechnology*, 2008, **19**, 095501.
15 J. Liu and Y. Lu, *Chem. Commun.*, 2007, 4872–4874.
16 X. Mao, Y. Ma, A. Zhang, L. Zhang, L. Zeng and G. Liu, *Anal. Chem.*, 2009, **81**, 1660–1668.
17 H. Xu, X. Mao, Q. Zeng, S. Wang, A. Kawde and G. Liu, *Anal. Chem.*, 2009, **81**, 669–675.
18 G. Liu, X. Mao, J. A. Phillips, H. Xu, W. Tan and L. Zeng, *Anal. Chem.*, 2009, **81**, 10013–10018.
19 D. Mazumdar, J. Liu, G. Lu, J. Zhou and Y. Lu, *Chem. Commun.*, 2010, **46**, 1416–1418.
20 J. Liu, D. Mazumdar and Y. Lu, *Angew. Chem., Int. Ed.*, 2006, **45**, 7955–7959.
21 N. Carmi and R. R. Breaker, *Bioorg. Med. Chem.*, 2001, **9**, 2589–2600.
22 N. Carmi, S. R. Balkhi and R. R. Breaker, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 2233–2237.