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PAPER

Fenton's reagent-tuned DNA-templated fluorescent silver nanoclusters as a versatile fluorescence probe and logic device†

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A label-free strategy based on the Fenton reaction with DNA-templated silver nanoclusters (DNA-Ag NCs) as a probe is demonstrated for the sequential detection of Cu^{2+}, ascorbic acid (AA) and H_2O_2. Cu^{2+} causes a structural change of the DNA template in DNA-Ag NCs to resist the environmental quenching and emit stronger fluorescence. The addition of AA in the presence of Cu^{2+} results in a further fluorescence increase of the DNA-Ag NCs. Interestingly, an even higher fluorescence enhancement is recorded by introducing Cu^{2+} into the DNA-Ag NCs–AA probing system. The fluorescence turn-on probe offers detection limits of 3 nM for Cu^{2+} and 7 nM for AA. Thereafter, the addition of H_2O_2 generates hydroxyl radicals from the Fenton reaction, which induces cleavage of the DNA template, leading to fluorescence quenching of the DNA-Ag NCs. This facilitates H_2O_2 detection. Moreover, based on the DNA-templated fluorescent silver nanoclusters and Fenton reaction, a multiple logic gate system, including AND and a three-input logic gate, is constructed, with Cu^{2+}, AA and H_2O_2 as inputs, and the fluorescence intensity of the DNA-Ag NCs probe as output.

Introduction

The Fenton reaction has been recognized as the source of the aging process and a variety of diseases.[1–3] It shows that some metal cations, *e.g.*, Cu^{2+} and Fe^{2+}, have special oxygen transfer properties for improving the generation of highly reactive hydroxyl free radicals by hydrogen peroxide in the presence of biological reducing reagents, such as ascorbic acid. Hydroxyl free radicals are one of the most powerful oxidants that can attack biomolecules, including DNA. In biological systems, copper and iron are both essential for electron-transfer reactions, however, Cu^{2+} induces significantly more DNA damage in the presence of H_2O_2 than Fe^{2+} and other biologically relevant transition metals.[4,5] Therefore, there is increasing interest in exploiting the ability of copper cations and biological reducing reagents for damaging DNA *in vivo*.[6] It has been demonstrated that DNA damage induced by copper in the presence of H_2O_2 occurs site-specifically at the sites of DNA-associated copper.[7] Most studies have been focused on elucidating the reaction mechanism, while few have been employed for sensory purposes based on the Fenton reaction.[8] The pH change induced by the Fenton reaction could be a novel approach for naked-eye and turn-on fluorescent detection of Cu^{2+}.[9] Ascorbic acid sensing was facilitated by a label-free strategy based on the Fenton reaction with unmodified gold nanoparticles as the probe.[10] Herein, we demonstrate a novel approach for the sequential detection of multi-analytes, *i.e.*, Cu^{2+}, ascorbic acid and H_2O_2, based on a single Fenton reaction and a suitable nano-probe.

Nucleic acids have recently become an extremely favorable tool in nanotechnology and materials science owing to their remarkable molecular recognition properties and self-assembly capability.[11,12] DNA has been exploited for the fabrication of metallic and semi-conducting nanowires.[13,14] In particular, fluorescent silver nanoclusters with DNA as a template have attracted special attention.[15,16] With respect to quantum dots or dye molecules, DNA-Ag NCs exhibit low toxicity and good biocompatibility, which facilitate their applications as environmentally-friendly and biocompatible fluorescence probes in biological or chemical sensing.[17] Up to now, the analytical applications of fluorescent DNA-Ag NCs have mainly been based on the fluorescence quenching or increasing effect of DNA-Ag NCs through their interaction with metal cations (Hg^{2+}, Cu^{2+}) and biothiols.[18–21] It is known that the fluorescence of DNA-Ag NCs correlates with the surface ligands or scaffolds.[17] Thus the rational design of fluorescent DNA-Ag NCs with functional ligands or scaffolds for more extensive applications deserves further exploration. Therefore, in the present paper we report the label-free sequential detection of Cu^{2+}, ascorbic acid and H_2O_2 based on the use of DNA-stabilized Ag nanoclusters and Fenton's reagent (Cu^{2+}, ascorbic acid, H_2O_2), by taking advantage of the specific relationship of DNA and the Fenton reaction.

The principle of operation for the novel nano-switch is illustrated in Scheme 1. Cu^{2+} is reported to attach mainly to

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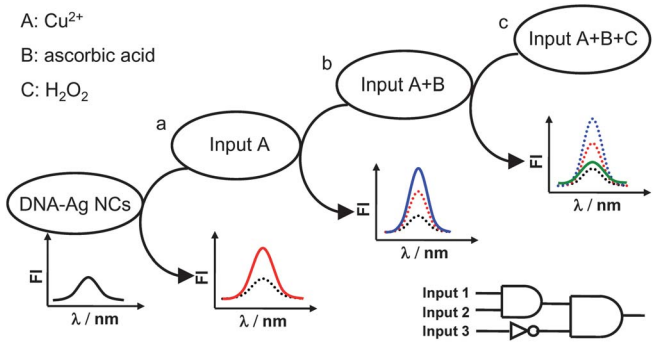


Scheme 1 A schematic illustration of the strategy for the detection of Fenton's reagent (Cu^{2+}, AA, H_2O_2) using DNA-Ag NCs and molecular logic gate operation.

phosphate in nucleic acids and interact with nucleobases,[22] uridine and its analogues.[23] In this sense, it is conceivable that the introduction of Cu^{2+} into DNA-Ag NCs would cause a change in the structure of the DNA template to resist the environmental quenching and emit stronger fluorescence[19] (Scheme 1a). Considering that copper nanostructures can be achieved through association of Cu^{2+} with the DNA template and further reduced with ascorbic acid,[24] the presence of ascorbic acid would turn on the fluorescence of the DNA-Ag/Cu NCs probe (Scheme 1b). It is also known that the Fenton's reagent generates hydroxyl free radicals (˙OH) to randomly cut the ssDNA into different sequence fragments.[25] The oxygen-derived free radicals attack DNA molecules, resulting in DNA oxidative damage.[26] Thus, upon addition of H_2O_2, the ˙OH generated from the Fenton reaction could induce damage of the DNA template, leading to the quenching of fluorescence of DNA-Ag NCs (Scheme 1c). As expected, these hypotheses were experimentally demonstrated and a new strategy for the sequential detection of Cu^{2+}, ascorbic acid and H_2O_2 was developed in a single operation run based on the DNA-Ag NCs nano-probe and Fenton reaction. In addition, multiple logic gates were constructed.

Experimental

Materials

All chemicals were at least of analytical reagent grade and used directly without further purification. Oligonucleotides with a specific sequence (5′–CCCTTAATCCCC–3′) were purchased from Sangon Inc. (Shanghai, China). Silver nitrates ($AgNO_3$), sodium tetrahydroborate ($NaBH_4$), hydrogen peroxide (H_2O_2), copper nitrate ($CuNO_3$) were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Ascorbic acid was obtained from Tianjin Damao Chemicals (Tianjin, China). DI water of 18 MΩ cm is used throughout.

Instrumentation

Fluorescence spectra were recorded on an F-7000 fluorescence spectrophotometer (Hitachi Ltd, Japan) under excitation at 470 nm with a 700 V xenon discharge lamp as light source. The slot widths for both the excitation and emission were set at 10 nm. Photoluminescence decay was measured on a FluoroMax-4 spectrofluorometer (Horiba Ltd, France). Circular dichroism spectra were recorded on a MOS-450 spectrometer/polarimeter (Biologic Science Instrument, France) and were acquired every 0.5 nm with a bandwidth setting of 1 nm at a speed of 100 nm min^{-1} under nitrogen protection.

Preparation of DNA-Ag NCs

The DNA-templated silver nanoclusters (DNA-Ag NCs) were prepared using a slight modification based on a reported procedure.[27] Briefly, 80 μL of DNA solution (50 μM, prepared in 20 mM PBS buffer, pH 7) and 260 μL of $AgNO_3$ solution (1 mM) were sequentially added into a 1.5 mL centrifugal tube. The reaction mixture was incubated for 15 min at room temperature. Afterwards, 60 μL of $NaBH_4$ solution (2 mM, prepared in ice water) was added and the reaction mixture was incubated at room temperature in dark for 60 min. For simplicity, we denote the concentration of the as-prepared DNA-Ag NCs as "1*X*". Following reduction of Ag^+ ions, highly fluorescent DNA-Ag NCs were produced with fluorescence at 564 nm.

DNA-Ag NCs probe for Fenton's reagent

Cu^{2+}, AA and H_2O_2 aqueous solution with different concentrations were freshly prepared before use. Afterwards, 40 μL of 0.3*X* DNA-Ag NCs was incubated for 15 min with Cu^{2+} (0–50 μM), or 40 μL of Cu^{2+} (10 μM) + AA (0–20 μM), or 40 μL of AA (10 μM) + Cu^{2+} (0–30 μM), or 40 μL of Cu^{2+} (10 μM) + 40 μL AA (10 μM) + H_2O_2 (0–10 mM). The solutions were then diluted to 400 μL with 0.2 mol L^{-1} PBS buffer at pH 7, and the fluorescence was recorded within 490–650 nm.

Results and discussion

DNA-Ag NCs probe for Cu^{2+}

We first conducted proof-of-concept experiments by measuring the fluorescence of the DNA-Ag NCs in the presence of Cu^{2+}. It is obvious that a trace amount of Cu^{2+} induced an obvious fluorescence enhancement at 564 nm (Fig. 1a and b). This fact makes it possible to develop a silver nanocluster-based fluorescence sensing system for Cu^{2+}. Further investigations on the

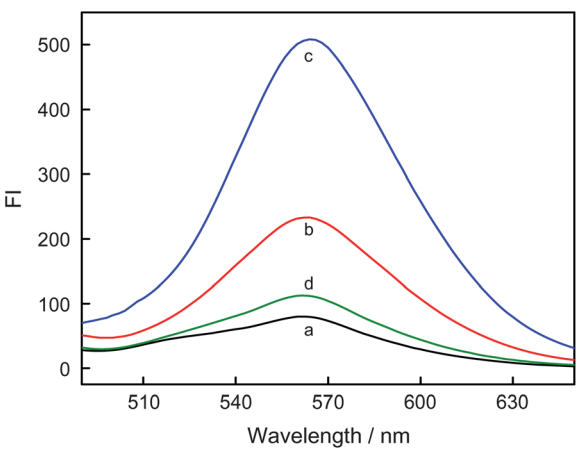


Fig. 1 Fluorescence spectra of (a) DNA-Ag NCs; (b) DNA-Ag NCs + Cu^{2+}; (c) DNA-Ag NCs + Cu^{2+} + AA; (d) DNA-Ag NCs + Cu^{2+} + AA + H_2O_2. DNA-Ag NCs: 0.03*X*; Cu^{2+}: 1 μM; AA: 1 μM; H_2O_2: 500 μM; pH 7.

variation of fluorescence intensity with pH value of the reaction system indicated that a maximum fluorescence signal was achieved at pH 7. In practice, the formation of some other copper species at pH > 7, *e.g.*, $Cu(OH)_2$, tends to affect the fluorescence enhancement of the DNA-Ag NCs.[28] On the other hand, at a lower pH the Cu^{2+} cation has greater difficulty accessing the surface of the DNA-Ag NCs due to the decrease in the negative charge density of the DNA fragments.[19] The dependence of fluorescence intensity of the DNA-Ag NCs on the incubation time was further investigated, showing that in the presence of Cu^{2+} the enhancement effect remains for a longer time (>15 min), which leaves sufficient time for the detection of Cu^{2+}. Fig. 2 illustrated the relationship between the fluorescence enhancement of the DNA-Ag NCs and Cu^{2+} concentration, giving rise to a linear range of 75–1000 nM. A linear regression equation of $[F - F_0 = 9.5729 + 208.262C_{cu}]$ is achieved along with a detection limit ($S/N = 3$) of 23 nM (F and F_0 are the fluorescence intensities of DNA-Ag NCs in the presence or absence of Cu^{2+}). A RSD value of 4.1% is obtained at 1 μM Cu^{2+}. It is obvious that in the present case the DNA-Ag NCs provide a limited sensitivity for Cu^{2+} detection. A further improvement on the sensitivity of Cu^{2+} for this system will be described in the following section.

DNA-Ag NCs probe for ascorbic acid

Ascorbic acid is an extremely important biological molecule with many roles, including enzyme cofactor, antioxidant, involvement in neurotransmitter-related enzymes and essential nutritional factor.[29–31] It is interesting to see that in the DNA-Ag NCs-Cu^{2+} system, the addition of a trace amount of AA causes a significant fluorescence enhancement (Fig. 1a and c and Fig. 3a). As a control experiment, it is demonstrated that in the absence of Cu^{2+}, AA itself results in no obvious change of the fluorescence of DNA-Ag NCs. This is a clear indication that the DNA-Ag NCs-Cu^{2+} is a promising probe for the assay of AA. Fig. 3b illustrates that when fixing the acidity at pH 7 and a reaction time of 10 min, a linear relationship between AA concentration and the fluorescence enhancement of the DNA-Ag NCs-Cu^{2+} system (1 μM Cu^{2+}) was achieved within 20–500 nM. The regression

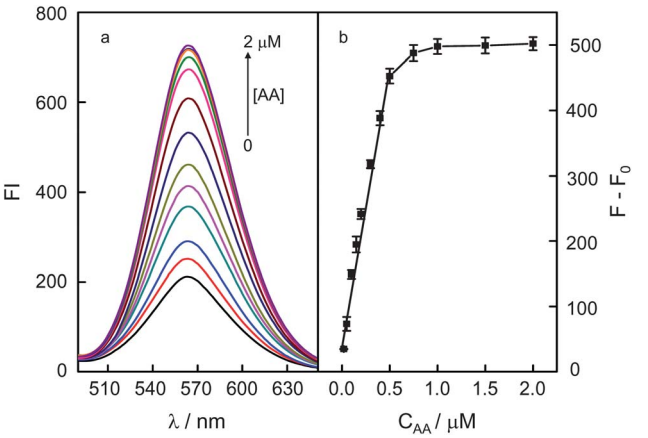


Fig. 3 (a) Fluorescence emission spectra of DNA-Ag NCs in the presence of 1 μM Cu^{2+} and ascorbic acid within a concentration range of 0–2 μM. (b) Fluorescence intensity at 564 nm as a function of AA concentration. DNA-Ag NCs: 0.03X; pH 7.

equation is $F - F_0 = 15.3112 + 978.5441C_{AA}$ (F and F_0 are the fluorescence intensities of DNA-Ag NCs-Cu^{2+} in the presence or absence of AA), along with a detection limit of 7 nM and a RSD value of 3.3% at 0.1 μM AA. Further investigations indicate that the LOD value of the present system is at least comparable or better than those reported by some other fluorescence detection approaches.[32–34] The DNA-Ag NCs-Cu^{2+} system also offers promising selectivity for AA detection. As illustrated in Fig. 4 amino acids cause negligible interferences on the assay of AA except for L-cysteine. This might be due to the fact that the affinity of thiol compounds to silver deteriorates the interaction between DNA fragments and the metal clusters, leading to thiol-induced fluorescence quenching of the DNA-Ag NCs.[35] As an irreversible inhibitor for cysteine peptidase, *N*-ethylmaleimide (NEM) can be used for eliminating the interferences from thiol-containing compounds by blocking the biothiols.[36] Our experiments showed that the interferences from L-cysteine could be eliminated by adding 20 μM NEM into the solution of DNA-Ag NCs with 20 μM L-cysteine. As a comparison, Fig. 4 indicates

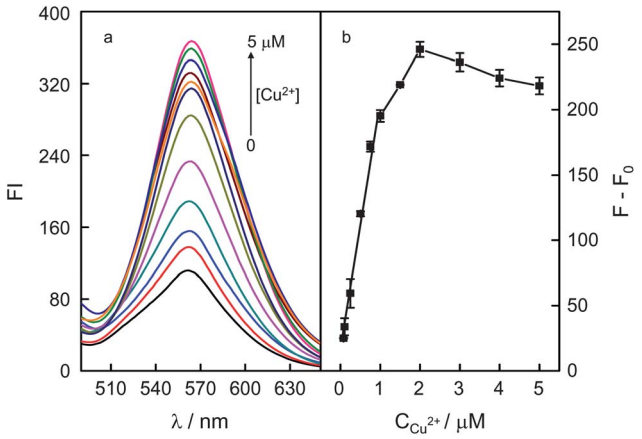


Fig. 2 (a) Fluorescence emission spectra of DNA-Ag NCs in the presence of 0–5 μM Cu^{2+}. (b) Fluorescence intensity at 564 nm as a function of Cu^{2+} concentration, showing a linear range of 75–1000 nM. DNA-Ag NCs: 0.03X; pH 7.

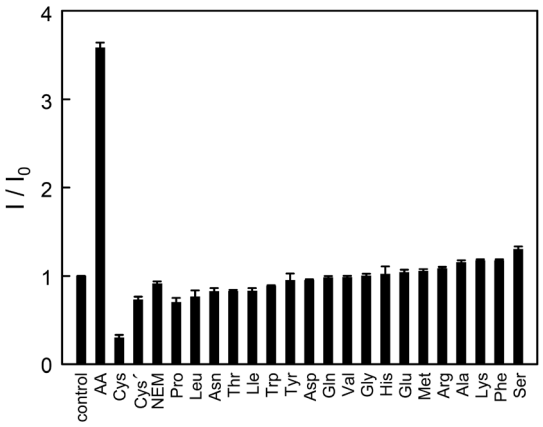


Fig. 4 The effect of amino acids on the fluorescence emission of DNA-Ag NCs in the presence of Cu^{2+} at 564 nm. Cys′: fluorescence emission of DNA-Ag NCs in the presence of L-Cys and NEM. DNA-Ag NCs: 0.03X; Cu^{2+}: 1 μM; AA: 1 μM; amino acids: 20 μM; NEM: 20 μM; pH 7.

that NEM itself causes no change in the fluorescence of the DNA-Ag NCs system.

DNA-Ag NCs–AA probe for highly sensitive detection of Cu^{2+}

As discussed in the previous section, the low sensitivity for Cu^{2+} detection by the DNA-Ag NCs probe makes it not practical for real sample analysis. For improving the sensitivity of Cu^{2+} detection, AA is firstly used to interact with DNA-Ag NCs and form the DNA-Ag NCs–AA probe. Afterwards, the introduction of a trace amount of Cu^{2+} results in a much greater improvement on the fluorescence than that observed in Fig. 2, when Cu^{2+} is used to enhance the fluorescence of DNA-Ag NCs. Fig. 5 illustrates a linear relationship between the fluorescence enhancement and Cu^{2+} concentration within 10–300 nM, with a linear regression equation of $F - F_0 = 6.8350 + 2087.3473C_{Cu}$ (F and F_0 are the fluorescence intensities of DNA-Ag NCs-AA in the presence or absence of Cu^{2+}). The present label-free probe offers a much lower detection limit of 3 nM, which is better than those of the DNA-Ag NCs–Cu^{2+} system and other complicated fluorescent chemosensors for Cu^{2+} based on organic dye molecules. Those sensing systems rarely provide sensitivity at nanomolar levels due to the fact that copper sensing is based on fluorescence quenching rather than fluorescence enhancement.[37,38] The precision of 3.4% RSD at 0.1 μM showed that the reproducibility of the sensing system is suitable for practical assays. The selectivity of the DNA-Ag NCs–AA probing system for Cu^{2+} is illustrated in Fig. 6, where no obvious interfering effects were observed for the detection of 1 μM Cu^{2+} from 20 μM of Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}. Hg^{2+} is found to be an exception, however, it should be noted that the concentration of Hg^{2+} in most environmental samples is well below that of Cu^{2+},[19] thus its interfering effects should be less pronounced in practical applications.

AND logic gate

Molecular logic gates enable the development of molecular-scale computers and autonomously regulated chemical systems.[39] Based on the observation that Cu^{2+} and AA induce different

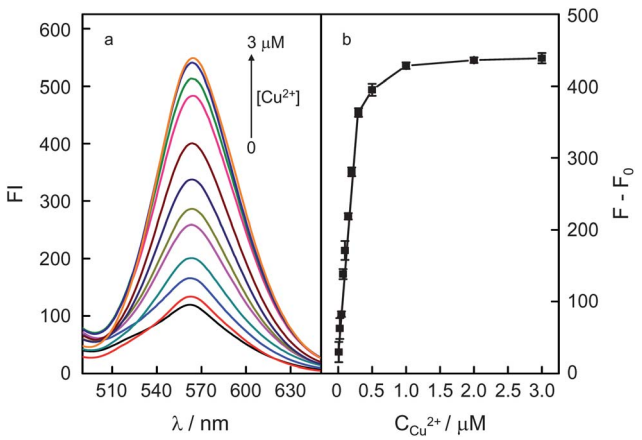


Fig. 5 (a) Fluorescence emission spectra of DNA-Ag NCs in the presence of 1 μM AA and Cu^{2+} within 0–3 μM. (b) Fluorescence intensity at 564 nm as a function of Cu^{2+} concentration within 0–3 μM. DNA-Ag NCs: 0.03X; pH 7.

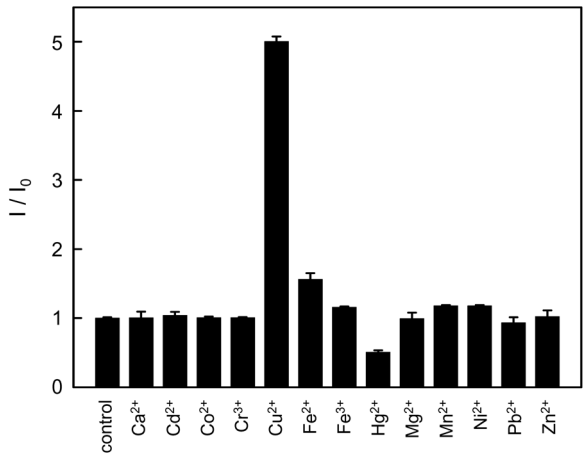


Fig. 6 The effect of some metal cations on the fluorescence emission of the DNA-Ag NCs–AA probe by introducing AA. DNA-Ag NCs: 0.03X; AA: 1 μM; Cu^{2+}: 1 μM; metal cations: 20 μM; pH 7.

fluorescence enhancement for DNA-Ag NCs, we designed a two-analyte AND logic gate[40] with the concentration of Cu^{2+} and AA as inputs and the fluorescence intensity of DNA-Ag NCs as output. For input, the presence of Cu^{2+} or AA was defined as 1 and their absence as 0. The fluorescence intensity at 564 nm serves as the output (1 or 0), with a threshold of *ca.* three times above the background of DNA-Ag NCs (Fig. 7A and C). The absence of both inputs (0/0) or in the presence of either input (1/0, 0/1) gives an output signal of "0" (Fig. 7A, a–c). Only the presence of both inputs (1/1) causes a significant fluorescence enhancement, giving an output signal of "1" (Fig. 7A, d). A logic operation could be realized by controlling the concentration of Cu^{2+} and AA to generate a truth table (Fig. 7B) and a schematic representation of this logic gate was given in Fig. 7D.

DNA-Ag NCs probe for H_2O_2

The interaction of reactive oxygen species (ROS) with DNA is of considerable interest for the potential pathobiological significance of ROS-induced DNA damage. Studies have shown that the reduction of H_2O_2 by copper produces highly reactive DNA-damaging species.[41] Although the nature of DNA-oxidizing species produced by the interaction of the DNA–Cu complex with H_2O_2 remains uncertain, exposure of DNA to copper has been reported to result in single- and double-strand breaks, modified bases.[42,43] Based on these facts, it is expected that fluorescent assay of H_2O_2 is feasible by damaging the DNA template in the DNA-Ag NCs and quenching the fluorescence through the Fenton reaction. The proof-of-concept experiments by adding H_2O_2 into the DNA-Ag NCs–Cu^{2+}–AA sensing system (with 1 μM Cu^{2+} and 1 μM AA) indicated a significant decrease of fluorescence (Fig. 8a). We further demonstrated that H_2O_2 itself or Cu^{2+} + H_2O_2 or AA + H_2O_2 (1–200 μM H_2O_2) caused no obvious changes in the fluorescence of DNA-Ag NCs. As shown in Fig. 8b, concentration dependence of the fluorescence intensity of the DNA-Ag NCs follows a Langmuir-type binding isotherm, *i.e.*, $C/F = 1/(BF_{max}) + (1/F_{max}) \times C$, where C is the concentration of H_2O_2, F is the fluorescence at that H_2O_2 concentration, F_{max} is the maximum fluorescence, B is the binding constant. After Langmuir fitting, a quenching efficiency $(I_{F_0} - I_F)/I_{F_0}$ (I_F and I_{F_0} are the

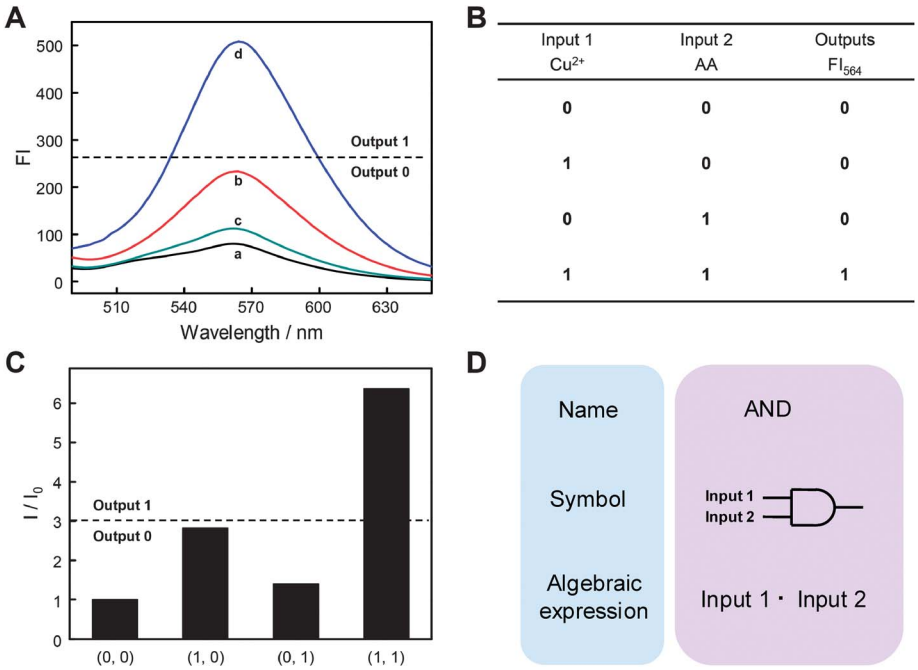


Fig. 7 Operation of the AND logic gate system of DNA-Ag NCs with Cu^{2+} or AA as inputs and fluorescence as output. (A): Fluorescence spectra of the DNA-Ag NCs in the presence of different inputs. (a): 0 μM Cu^{2+} + 0 μM AA; (b): 1 μM Cu^{2+} + 0 μM AA; (c): 0 μM Cu^{2+} + 1 μM AA; (d): 1 μM Cu^{2+} + 1 μM AA. (B): Truth table for the AND logic gate. (C): Fluorescence intensities of DNA-Ag NCs at 564 nm in the presence of different inputs. (D): The name, symbol, and algebraic expression of the logic gate.

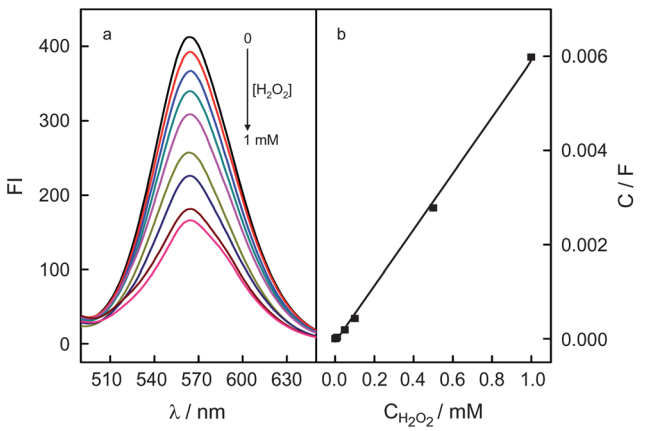


Fig. 8 (a) Fluorescence emission spectra of DNA-Ag NCs in the presence of 1 μM Cu^{2+}, 1 μM AA and H_2O_2 within a concentration range of 0–1 mM. (b) Langmuir binding isotherm showing a linear fit for a function of H_2O_2 concentration within 0–1 mM. DNA-Ag NCs: 0.03*X*; Cu^{2+}: 1 μM; AA: 1 μM; pH 7.

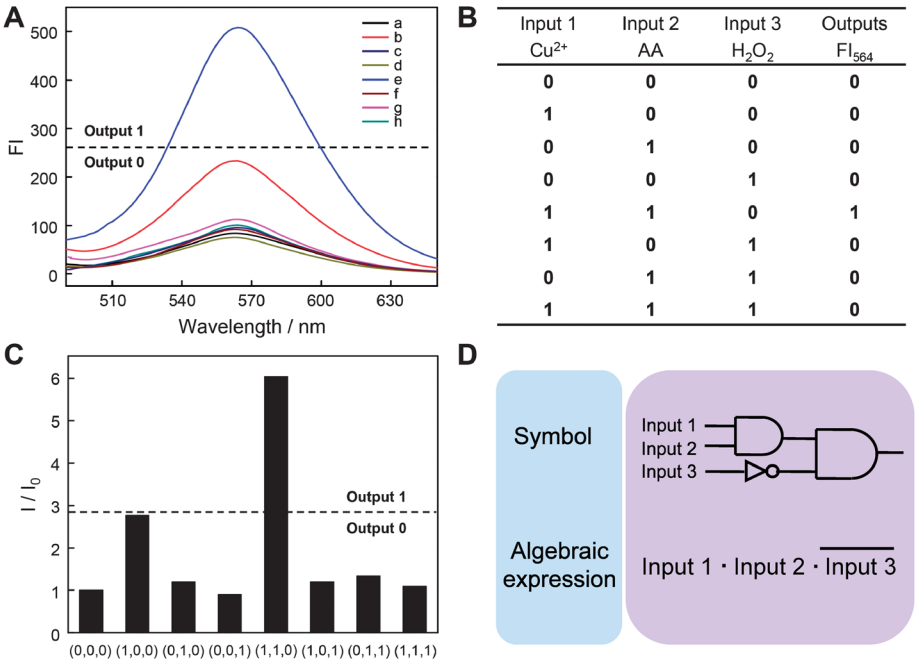
fluorescence intensities of DNA-Ag NCs–Cu^{2+}–AA system in the presence or absence of H_2O_2) of 25.2% was achieved at 100 μM H_2O_2. It can be seen that although this sensing system provides an approach for H_2O_2 assay, the detection sensitivity needs to be further improved for practical applications.

Three-input logic gate

On the basis of the AND logic gate a three-input logic gate was developed by employing H_2O_2 as the third input, which by generating hydroxyl radicals from the Fenton reaction to induce DNA-damaging reactions to quench the fluorescence of DNA-Ag NCs. For input, the presence of H_2O_2 was defined as "on" or "1". For output, the fluorescence intensity at 564 nm served as the output "1" or "0", with a threshold of three times the background of DNA-Ag NCs (Fig. 9A and C). Thus, a three-input logic gate is constructed by controlling the concentrations of Cu^{2+}, AA and H_2O_2. With no input, or with one analyte input (0/0/0, 1/0/0, 0/1/0, 0/0/1), the output is "0" (Fig. 9A, a–d). With Cu^{2+} and AA without H_2O_2 (1/1/0), the fluorescence enhancement of DNA-Ag NCs occurs and corresponds to "1" (Fig. 9A, e). In the presence of both Cu^{2+} and H_2O_2 inputs or AA and H_2O_2 inputs (1/0/1, 0/1/1), the fluorescence intensity of DNA-Ag NCs changes slightly, the outputs are "0" (Fig. 9A, f–g). When the system is subjected to the three inputs together (1/1/1), the hydroxyl radicals (˙OH) generated from the Fenton reaction induce cleavage of the DNA template, leading to effective fluorescence quenching of the DNA-Ag NCs, corresponding to the "0" (Fig. 9A, h). The truth table and schematic representation of the three-input logic gate are presented in Fig. 9B and D.

Evidence for the interaction of DNA-Ag NCs and Fenton's reagent

The electronic transition of the bases in DNA fragments exhibits a small circular dichroism (CD) due to the chirality of the riboses, and this spectroscopic technique is sensitive to the arrangement of the bases.[44] For single-stranded DNA (ssDNA) and Ag^+ complex with ssDNA, we observed a significant change in the ellipticity (Fig. S1a and b†), revealing that Ag^+ induces nonplanar and tilted orientations of the bases relative to the helical axis.[45] Upon reduction with $NaBH_4$, a decrease of



Input 1 Cu^{2+}	Input 2 AA	Input 3 H_2O_2	Outputs FI_{564}
0	0	0	0
1	0	0	0
0	1	0	0
0	0	1	0
1	1	0	1
1	0	1	0
0	1	1	0
1	1	1	0

Fig. 9 Operation of the three-input logic gate system of DNA-Ag NCs with Cu^{2+}, AA and H_2O_2 as inputs. (A): Fluorescence spectra of the DNA-Ag NCs in the presence of different inputs. (a): 0 μM Cu^{2+} + 0 μM AA + 0 mM H_2O_2; (b): 1 μM Cu^{2+} + 0 μM AA + 0 mM H_2O_2; (c): 0 μM Cu^{2+} + 1 μM AA + 0 mM H_2O_2; (d): 0 μM Cu^{2+} + 0 μM AA + 1 mM H_2O_2; (e): 1 μM Cu^{2+} + 1 μM AA + 0 mM H_2O_2; (f): 1 μM Cu^{2+} + 0 μM AA + 1 mM H_2O_2; (g): 0 μM Cu^{2+} + 1 μM AA + 1 mM H_2O_2; (h): 1 μM Cu^{2+} + 1 μM AA + 1 mM H_2O_2. (B): Truth table for the three-input logic gate. (C): Fluorescence intensities of the DNA-Ag NCs in the presence of different inputs at 564 nm. (D): The symbolic and algebraic expression describing the action of this logic gate.

the ellipticity at 215 nm and 265 nm was observed due to the structural changes in DNA induced by the silver nanoclusters. (Fig. S1c†). After addition of Cu^{2+} the ellipticity became more negative, demonstrating a rigid structure of the DNA template (Fig. S1d†). In the presence of both Cu^{2+} and ascorbic acid, the ellipticity at 215 nm and 265 nm became more negative than in the absence of Cu^{2+} (Fig. S1e†), showing that the helicity of the DNA template increases and has even more structural changes. Upon addition of Fenton's reagent (Cu^{2+}, ascorbic acid and H_2O_2), the ellipticity at 215 nm was more positive than DNA-Ag NCs and more negative at 265 nm (Fig. S1f†), which may be related to a decrease of DNA helicity,[46,47] suggesting oxidative damage of the ssDNA by Fenton's reagent.

A time-resolved spectroscopic technique was employed to gain insight into the fluorescence change in the absence or presence of the three analytes, since time-resolved fluorescence is sensitive to electron dynamics.[48] The lifetimes of DNA-Ag NCs were listed in Table S1.† The decays follow bi-exponential phenomenon, which was possibly attributed to the differential distribution of complicated luminescent pathways of polynuclear metal–DNA complexes.[49] An average fluorescence lifetime of 0.66 ns is recorded for DNA-Ag NCs. The addition of Cu^{2+} or both Cu^{2+} and AA results in a significant increase of the fluorescence lifetime, *i.e.*, 0.99 ns and 1.21 ns, respectively, indicating that electron charge transfer from metal to DNA scaffolds occurs in the metal–DNA complex. After adding the analytes (AA, H_2O_2, Cu^{2+} + AA, AA + H_2O_2) into DNA-Ag NCs solution, a slight change of the average fluorescence lifetime was observed, which was consistent with the fluorescence intensities. A significant decrease in the average fluorescence lifetime from 0.66 to 0.34 ns was observed after addition of Fenton's reagent, indicating that ·OH generated from Fenton reaction could induce damage of the DNA template to reduce the capacity of DNA as electron acceptors.[50]

Conclusions

By combining the Fenton reaction and DNA-Ag NCs, we demonstrated a novel strategy for label-free sequential detection of Cu^{2+}, ascorbic acid and H_2O_2. The approach provides favorable detection limits for Cu^{2+} and ascorbic acid, while that for H_2O_2 needs to be further improved in practical applications. Based on this sensing system, an AND and a three-input logic gate were constructed with Cu^{2+}, ascorbic acid and H_2O_2 as inputs, while the fluorescence intensity of DNA-Ag NCs was the output. These logic gates may expand the scope of the potential applications of nanomaterials in label-free optical bioassays, molecular-level chemical sensing and environmental science to mimic more complex computing operations.

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