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ORIGINAL PAPER

A ratiometric fluorescent nanoprobe consisting of ssDNA-templated silver nanoclusters for detection of histidine/cysteine, and the construction of combinatorial logic circuits

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Abstract

A ratiometric fluorescent nanoprobe consisting of ssDNA-templated silver nanoclusters (DNA-AgNCs) as dual fluorophore has been constructed for highly sensitive and selective detection of cystein (Cys) and histidine (His). The DNA-AgNCs displays dual emission in that photoexcitation at 470 nm results in weak-green fluorescence peaking at 560 nm, while excitation at 550 nm gives strong red fluorescence with a peak at 595 nm. It is found that copper ions (Cu^{2+}) enhance the green fluorescence but quenche the red fluorescence. A ratiometric nanoprobe for Cys (or His) is designed that is based on the competitive interaction of Cys (or His), Cu^{2+} and DNA-AgNCs. Cys can be distinguished from His by adding Ni^{2+} as the masking agent, andr His can be distinguished from Cys by adding N-ethylmaleimide (NEM) as the masking agent, respectively. The limits of detection are 5.1 and 4.5 nM for Cys and His, respectively. Furthermore, the ratiometric fluorescent nanoprobe is used for constructing combinatorial logic circuits in parallel, including OR//NOR and INHIBIT//IMPLICATION (INH//IMP).

Keywords Ratiometric nanoprobe · Amino acid · DNA-AgNCs · Fluorometric biosensor · Logic gates

Introduction

Cysteine (Cys) and histidine (His) play crucial roles in many biological functions and systems [1–3]. It has been proven that abnormal level of Cys or His would seriously threaten the health of human being [4]. Excessive or Deficient intake of Cys does harm to human health including the neurotoxicity, liver damage, slowed growth and so forth [5–7]. Furthermore, abnormal His levels are also associated with some diseases, such as Friedreich ataxia, Parkinson's disease and inflammation of intoxication [8, 9]. Thus, high sensitive detection of Cys and His is of significant importance for human health. Great efforts have been devoted to develop highly sensitive and selective strategy for Cys and His detection [10–12]. Among detection assays, fluorescence sensors have emerged as the subject of considerable research owing to the highly sensitive and cost-effective features. While, it is noted that most of the sensing systems for amino acid detection are based on single-channel output signal with either signal-on or signal-off strategy, which might cause false-negative or false-positive result [13, 14]. Different from single-channel biosensors, a ratiometric fluorescent biosensor reports output signal according to the responses of at least two fluorescent probes triggered [15]. This presents advantages of reducing environment effects and eliminating false results over single-channel biosensors [16]. It is worthy of noting, however, most of the ratiometric biosensors are related to two or more fluorophores, which usually requires chemical modification or pre-conjugated with sophisticated procedures in the detection process [17, 18]. A single fluorophore with dual emission would

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be an instructive way for constructing cost-effective ratiometric sensing system.

DNA-templated silver nanocluster (DNA-AgNCs) is a significant new class of fluorescence emitters and presents advantages of excellent photostability, low cost, biocompatibility, low-toxicity, and tunable photoemission [19, 20]. Interestingly, DNA-AgNCs presents diverse fluorescence signals with different emission wavelength, which is related to the DNA sequence, pH value or the used reduction reagent [21]. The outstanding properties endow DNA-AgNCs as promising alternative to conventional organic dyes and quantum dots for biosensing and bioimaging [22, 23]. While, most of the reported DNA-AgNCs presents fluorescence with single emission. In the present investigation, a single-strand DNA template silver nanoclusters with dual fluorescent emission is successfully synthesized. It is found that Cu^{2+} enhances one of the two fluorescent emissions while quench another one. The effect can be restored by the addition of His/Cys due to the strong interaction between His/Cys and Cu^{2+} [24]. Thereafter, a DNA-AgNCs-based ratiometric fluorescent nanoprobe is constructed to detect His and Cys. To individually distinguish His or Cys, Ni^{2+} and N-ethylmaleinide (NEM) are used as mask reagent to eliminate the interference of His to Cys, or Cys to His.

Increasing attention has been focused on combining analyte detection with construction of molecular logic gate in that the implementation of Boolean operations at molecular level can improve the performance of methods and stimulate new advances in the fields of chemical sensing, diagnostics, and functional biochemistry [25, 26]. For example, Huang et al. demonstrated that a biosensing system for Hg^{2+} detection can be used to construct an INHIBIT logic gate with Hg^{2+} and Cys as the two inputs [27]. Freeman et al. reported CdSe/ZnS quantum dot-based Hg^{2+} and Ag^{+} detection and their subsequent application in AND and OR logic gate operations [28]. With increasing computing complex, it is critical to construct advanced logic gates on a simple platform [29]. In a ratiometric sensing system, one target can simultaneously cause inverse change of two signals, which can be a charming platform for construction of combinational logic circuits in parallel.

Experimental section

Materials and chemicals

Oligonucleotides (5'-CCCTTAATCCCCTTTTTTTTTTTTTTCCCTAACTCCCC-3′, HPLC) were bought from Sangon Biological Engineering Technology Co., Ltd., (Shanghai, China, https://www.sangon.com/). Silver nitrate was purchased from Tianjin Tiangan chemical technology development Co., Ltd., (Tianjin, China, http://www.tjtghg.com/). Sodium borohydride was obtained from Alfa-Aesar chemicals Co., Ltd., (Tianjin, China, https://www.alfa.com/). His, Cys, Asn, Phe, Ala, Val, Lys, Glu, Thr, Gly, Pro, Ser, Tyr, Arg, Asp, $Cu(NO_3)_2$, $NiCl_2$, Na_2HPO_4 and NaH_2PO_4 were purchased from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China, https://www.sinoreagent.com/). N-ethylmaleimide (NEM) was obtained from Sigma-Aldrich. (St. Louis, MO, USA, https://www.sigmaaldrich.com/). Ultrapure water (18.2 MΩ•cm) used for preparing solution was obtained via Milli-Q purification system.

Instruments

Transmission electron microscopy (TEM) images of DNA-AgNCs were obtained with a 2010FEF microscope (JEOL, Japan). Fluorescence spectra were recorded with a LUMINA Fluorescence Spectrometer (Thermo, USA). Oligonucleotide DNA concentration was quantified by measuring the absorbance at 260 nm with Cary 500 Scan UV/vis Spectrophotometer (Varian, USA).

Preparation of DNA-AgNCs

The DNA-AgNCs were synthesized according to the previous reports [30]. Typically, $AgNO_3$ solution (600 μM, 4.0 μL) and oligonucleotides solution (20 μM, 10 μL) were added into phosphate buffer (PB; pH 7.4, 20 mM, 182 μL). The mixture was then incubated at 4 °C for 30 min, followed by rapid addition of $NaBH_4$ (600 μM, 4.0 μL) solution. After vigorously shaking for 1 min, the resultant mixture was kept in the dark at 4 °C overnight.

Protocol for Cys and His detection

An optimized concentration of Cu^{2+} was obtained by detecting the fluorescence changes of DNA-AgNCs at 560 nm and 595 nm. For Cys detection, the Cys with different concentration (10 μL) was incubated with Cu^{2+} (20 μM, 10 μL) and DNA-AgNCs (2.0 μM, 100 μL) for 1 h. The final volume of detected sample was 200 μL for fluorometric measurements in PB (pH 7.4, 20 mM). The final concentrations of both Cu^{2+} and DNA-AgNCs were 1.0 μM. The fluorescence of DNA-AgNCs at 560 nm was recorded by setting excitation at 470 nm. The fluorescence of DNA-AgNCs at 595 nm was recorded by setting excitation at 550 nm. To distinguish His and Cys from each other, NEM was used as mask reagent to inhibit the interference of Cys on His detection. Ni^{2+} was used as mask reagent to inhibit the interference of His on Cys detection. The masking agent of Ni^{2+} (100 μM, 10 μL) or NEM (500 μM, 10 μL) was premixed with amino acid sample before the addition of Cu^{2+}. The following procedure was the same as described above.

Determination of Cys and His in serum sample

After being centrifuged for 10 min (10,000 rpm), the fetal bovine serum (100 μL) was separated and then diluted with PB (p5.0 mL). The treated serum sample (50 μL) was then added into the sensing system containing DNA-AgNCs (2.0 μM, 100 μL), Cu^{2+} (20 μM, 10 μL), and PB (40 μL). For Cys detection in serum, Cys was spiked into the treated serum sample to reach final concentration (0.5 μM, 2.0 μM, and 5.0 μM). For the detection of His, His was spiked into the treated serum sample to reach final concentration (0.2 μM, 2.0 μM, and 4.0 μM). To distinguish Cys from His (or His from Cys), mask reagent of Ni^{2+} (200 μM, 5.0 μL) or NEM (1.0 mM, 5.0 μL) was added in the sensing system. After incubation for 1 h, detection of Cys or His was carried out according to the above mentioned procedure.

Results and discussion

Detection mechanism of the ratiometric assay

The ratiometric fluorescence strategy for His and Cys detection based on Cu^{2+}/DNA-AgNCs ensemble is outlined in Fig. 1a. The DNA-AgNCs shows dual fluorescence emissions at 560 nm and 595 nm under the two excitations at 470 nm and around 550 nm, respectively (see Fig. S1 in Electronic Supporting Material (ESM)). Cu^{2+} enhances the fluorescence at 560 nm, while quenches the fluorescence at 595 nm. The further added His or Cys can restore the fluorescence of DNA-AgNCs by the interaction between Cu^{2+} and Cys (or His). The TEM image shows that the DNA-AgNCs is very uniform and distributed within diameter size of 1.6 ± 0.4 nm (Fig. S2 in ESM), steady-state fluorescence experiments are conducted to serve as a proof of concept to evaluate the feasibility of the strategy. The DNA-AgNCs emits dual fluorescent signals (Fig. 1b), a dim fluorescence at 560 nm (curve 1) and a distinguished fluorescence response at 595 nm (curve 2). The added Cys has no influence on the two fluorescent emissions of DNA-AgNCs (curve 3, 4). Interestingly, fluorescence signal at 560 nm is augmented (curve 5), while the fluorescence signal at 595 nm is quenched (curve 6) once Cu^{2+} is introduced, which might be ascribed to the microenvironment change of DNA-AgNCs. As well known, Cys can strongly coordinate with Cu^{2+} via its thiol group [21], which resulted in the fluorescence quenching at 560 nm (curve 7) and the fluorescence recovery at 595 nm (curve 8). The results indicate that the binding ability between Cu^{2+} and Cys is stronger than the interaction between Cu^{2+} and DNA-AgNCs. Similar phenomenon is found if His is added into the Cu^{2+}/DNA-AgNCs system since His containing imidazole group can coordinate with Cu^{2+} to form a strong-binding complex (Fig. S3 in ESM) [31]. Therefore, a ratiometric nanosensor for Cys and His detection can be constructed with the ratio of F_{595}/F_{560} as readout, where, F_{595} and F_{560} are the fluorescent intensity of DNA-AgNCs at 595 nm and 560 nm, respectively. To distinguish His and Cys from each other, mask agents are selected to eliminate the influence of His on Cys or Cys on His which were systematically investigated in the following experiments.

The specific influence of Cu^{2+} on the sensing system

In the ratiometric sensing strategy, Cu^{2+} is used as mediator to modulate the fluorescence of DNA-AgNCs for His or Cys detection. To guarantee the detection accurate, here, the interference of other metal ions on the fluorescence response of the sensing system is also evaluated, Fig. 2. In the initiate state, a high fluorescent signal at 595 nm and a low fluorescent signal at 560 nm are detected, resulting in a high ratio of F_{595}/F_{560} (blank). The addition of Cu^{2+} (5.0 μM) causes a weak fluorescence at 595 nm and a remarkable fluorescence at 560 nm. Thus, F_{595}/F_{560} with a low ratio value is obtained for providing a quite low signal background for further Cys (or His) detection. Differently, no obvious change is found from

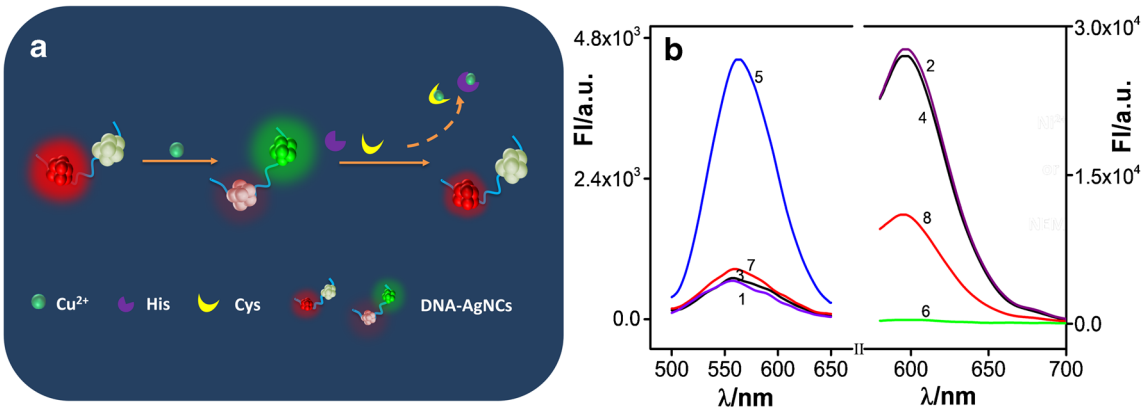


Fig. 1 **a** Schematic diagram of principle for individual detection of His and Cys. **b** The fluorescence spectra of the DNA-AgNCs at 560 nm (1) and 595 nm (2), fluorescence response of DNA-AgNCs in the presence of Cys (3, 4), Cu^{2+} (5, 6), and Cu^{2+} + Cys (7, 8)

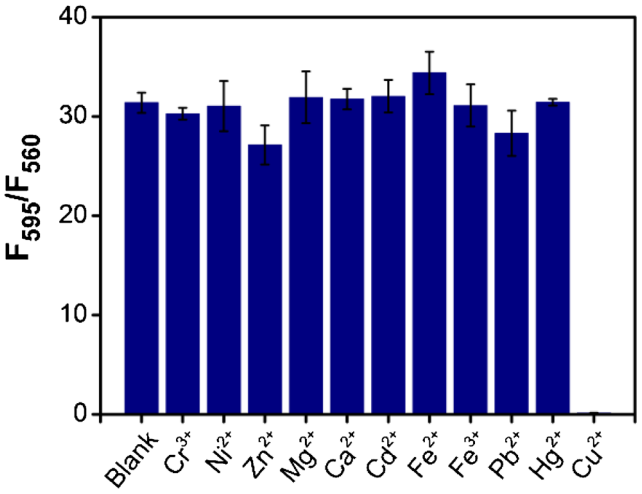


Fig. 2 The F_{595}/F_{560} of DNA-AgNCs in buffer (blank) and in the presence of metal ions. From left to right: Cr^{3+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Cu^{2+}

F_{595}/F_{560} in the addition of other metal ions. Therefore, only Cu^{2+} is qualified to be an excellent mediator for development of ratiometric fluorescent Cys (or His) nanoprobe.

To explore the possible reason for the influence, the absorption spectra and excitation spectra of DNA-AgNCs were investigated in the absence and presence of Cu^{2+}. Two absorption peaks at 440 nm and 550 nm were found in absorption spectra. After being treated with Cu^{2+} (2.0 μM), the absorbance peak at 440 is enhanced while the peak at 550 nm is decreased (Fig. 3), indicating that Cu^{2+} disturbs the microenvironment of DNA-AgNCs. In addition, the fluorescent excitation of DNA-AgNCs is influenced by Cu^{2+}. The excited fluorescence intensity is decreased at 550 nm while is increased at 470 nm with increasing concentration of Cu^{2+} from 0 to 10 μM, which might be ascribed to competitive coordination of DNA-AgNCs and Cu/DNA-AgNCs with the DNA [32]. In addition, it is found Cu^{2+} can quench the fluorescence of DNA-AgNCs due to the static quenching effect (Fig. S4 in ESM).

Detection sensitivity of the ratiometirc assay

The concentration of Cu^{2+} is first optimized by implementing fluorescence titration experiments (Fig. S5 in ESM). Under the optimal condition, the detection sensitivity of the ratiometirc assay is investigated by testing with various concentrations of amino acids. The Cys detection is first implemented according to the competition among Cys, Cu^{2+} and DNA-AgNCs. it is worth noting that Cu^{2+} is preferentially bond with Cys compared with DNA-AgNCs due to the stronger chelation between Cys and Cu^{2+}. As illustrated in Fig. 4a, the fluorescence responses of the biosensing system at 595 nm gradually increases while the fluorescence responses of the biosensing system at 560 nm gradually decreases with the increasing of Cys concentration. Herein, the fluorescence ratio of F_{595}/F_{560} is used as output signal of the biosensing system. Two linear relationships are found within the concentration range from 10 nM to 1.0 μM with a regress factor of 0.993, and from 1.0 μM to 8.0 μM with a regress factor of 0.987, respectively (Fig. 4b). By calculating the three times ratio of signal-to-noise (3σ), a limit of detection (LOD) is obtained as low as 5.1 nM. His detection is further conducted according to the same strategy. All the procedures are the same to that of Cys detection. Similar to the phenomenon of Cys detection, the F_{595} of the biosensing system gradually increases, while the F_{560} gradually decreases with the increasing of His concentration (Fig. 4c). The fluorescence ratio of F_{595}/F_{560} linearly relates on the His concentrations from 10 nM to 0.5 μM with a regress factor of 0.995 and from 0.5 μM to 5.0 μM with a regress factor of 0.994 (Fig. 4d). The LOD of His detection reaches as low as 4.5 nM according to the calculation of three times ratio of signal-to-noise. The detection limitations of His and Cys are comparable with previous reports (Table S1 and Table S2 in ESM), confirming the excellent sensitivity of the assay approach. It is worthy of noting that signal probes labels are required to generate ratiometric fluorescence signal in previous reports [33]. In our investigation, the presented ratiometric nanoprobe is labelfree and realizes just based on the single-strand DNA synthesized DNAAgNCs, which simplifies the experimental operation and reducesexperimental cost.

Detection specificity of the ratiometric strategy

The detection selectivity of the strategy is further evaluated by comparing the fluorescence response of the Cu^{2+}/DNA-AgNCs sensing system to various amino acids. Except for

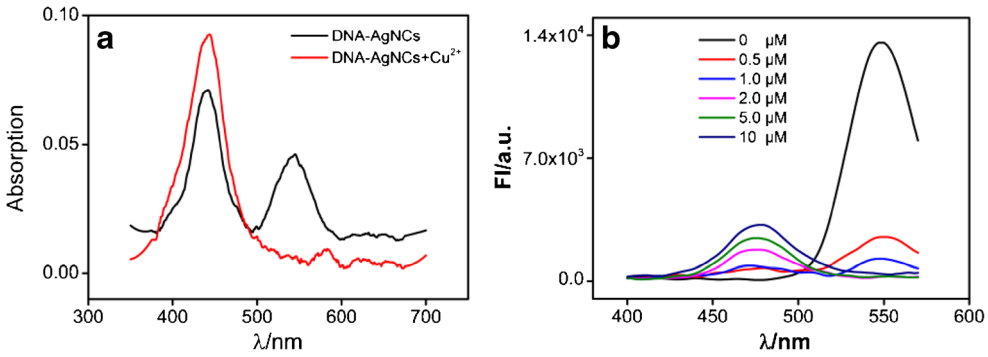


Fig. 3 **a** UV-Vis absorption spectra of the DNA-AgNCs in the absence (black curve) and presence of Cu^{2+} (red curve). **b** Excitation spectra of the DNA-AgNCs upon addition of Cu^{2+} from 0 to 10 μM by setting emission at 595 nm

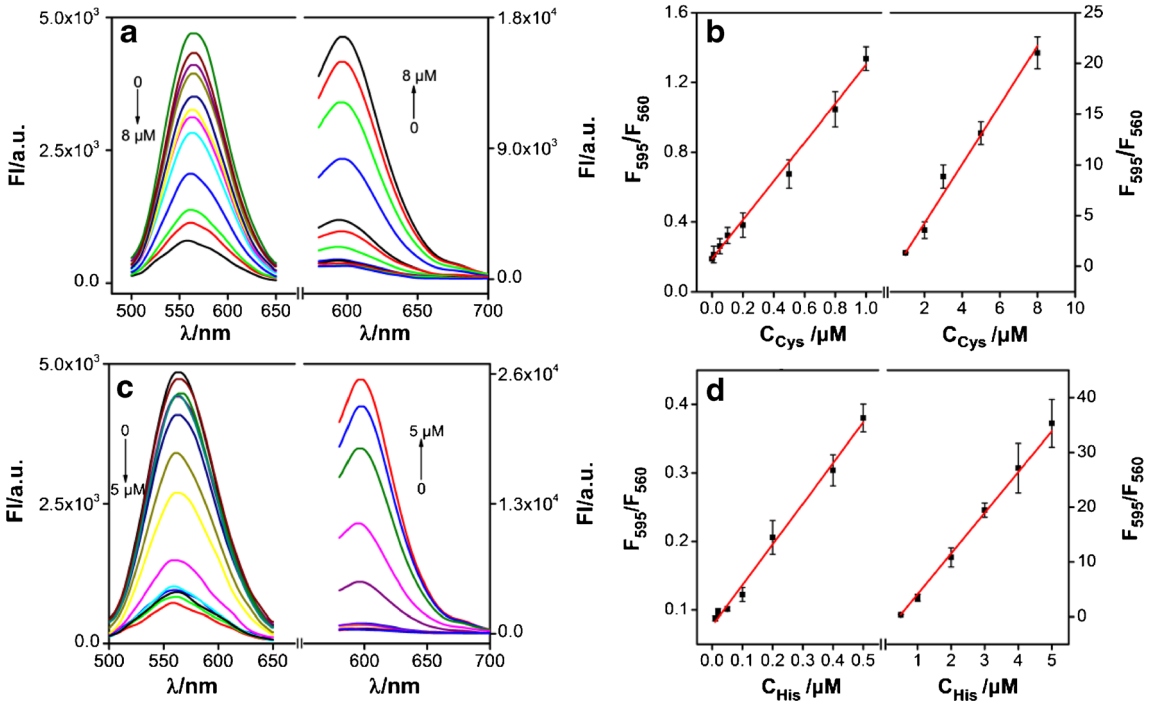


Fig. 4 Fluorescence emission spectra (**a**) and the ratio F_{595}/F_{560} (**b**) of the Cu^{2+}/DNA-AgNCs in the presence of Cys with different concentrations (0, 0.01, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, 3.0, 5.0, 8.0 μM). Fluorescence emission spectra (**c**) and the ratio F_{595}/F_{560} (d) of the Cu^{2+}/DNA-AgNCs in the presence of His with different concentrations (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 μM). The error bar represents the standard deviation of three independent measurements

His and Cys, the other amino acids can not cause any obvious fluorescence change of the sensing system as shown in Fig. 5a. Here, it should be noted that either His or Cys can inhibit the influence of Cu^{2+} on fluorescence of DNA-AgNCs. It means that His and Cys would interfere with each other according to the assay discussed above. Considering that amino acids might be coexistence in a real sample, it would be critical to find solution to distinguish His and Cys from each other. As known that Ni^{2+} has strong affinity with His [34], which may act as mask agent to block the interference of His on Cys detection. As shown in Fig. 5a, Ni^{2+} has no influence on the sensing system of Cu^{2+}/DNA-AgNCs. In the coexistence of His and Ni^{2+}, the product of His/Ni^{2+} blocks the interaction between His and Cu^{2+} and thus would not cause any obvious signal change of the Cu^{2+}/DNA-AgNCs sensing system. To validate that Cys can be individually detected under the assistance of Ni^{2+}, the detection of Cys in the presence of His is conducted following the same experiment procedure. As illustrated in Fig. 5b, the dual fluorescence emissions of DNA-AgNCs present similar changing tendency as that in the absence of His. The F_{560} decreases while the F_{595} increases with increasing concentration of Cys. The fluorescence ratio of F_{595}/F_{560} linearly relates with the concentration of Cys from 10 nM to 1.0 μM and also from 1.0 μM to 8.0 μM (Fig. 5c). By calculating the 3σ, a LOD is found at 5.6 nM, which is similar to the results of Cys detection in the absence of His. The results confirm the excellent selectivity of the strategy. The amount of His can be obtained by calculating the fluorescence signal difference before and after adding Ni^{2+}. Additionally, It is reported that NEM has strong affinity with Cys [35], which may act as mask agents to eliminate the interference of Cys on His detection (Fig. S6 in ESM). Therefore, the His can be detected in the presence of Cys under assistant of mask agent NEM (Fig. S7 in ESM). In addition, the ratiometric strategy can detect His and Cys in real sample of serum. To mimic a biological sample, serum is diluted 200-fold with PB in final assay system. The recoveries of His and Cys at different concentrations within the linear range are executed by a standard spiking method. The recoveries distribute within range from 94 to 110%, indicating the reliability and practicality of the assay strategy (Table S3 in ESM).

Construction of multiple combinatorial logic gates

Cu^{2+}/DNA-AgNCs is defined as the logic platform to implement multiple combinatorial logic gates. With Cys and His as the two inputs, the dim fluorescence of DNA-AgNCs at 595 nm can be restored in the presence of either Cys or His or both of them, which is the feature of OR logic gate. A NOR logic gate is realized with fluorescence of DNA-AgNCs at 560 nm as the output since the light fluorescence of the DNA-AgNCs can be quenched by the inputs, Fig. 6a. Thus, an OR and a NOR logic gate functions are realized in parallel (OR//NOR), which is illustrated in truth table

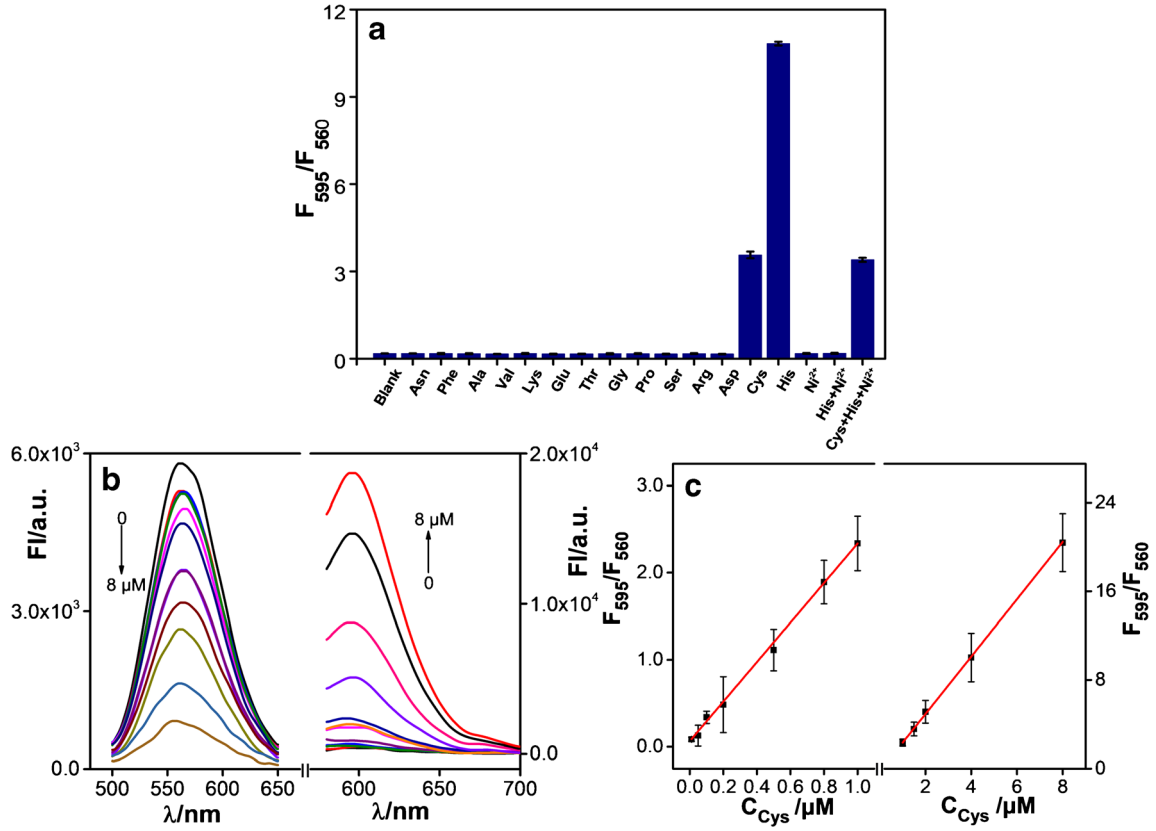
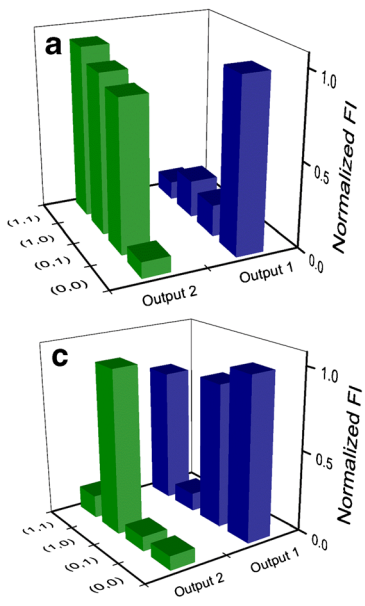


Fig. 5 **a** Fluorescence intensity ratios F_{595}/F_{560} of the Cu^{2+}/DNA-AgNCs sensing system in the absence and presence of Asn, Phe, Ala, Val, Lys, Glu, Thr, Gly, Pro, Ser, Arg, Asp, Cys, His, Ni^{2+}, Ni^{2+} + His, Ni^{2+} + His + Cys. The concentration of amino acid was 2.0 μM. The concentration of Ni^{2+} was 5.0 μM. **b** Fluorescence emission spectra of Cu^{2+}/DNA-AgNCs for Cys detection in the presence of His (2.0 μM) and Ni^{2+} (5.0 μM). Concentration of Cys: 0, 0.01, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0, 4.0, 8.0 μM. **c** Linear relationship of F_{595}/F_{560} against Cys concentration in the range from 0.01 μM to 1.0 μM and 1.0 μM to 8.0 μM. The error bar represents the standard deviation of three independent measurements

(Fig. 6b). If Cys and NEM are selected as the two inputs, an INH logic gate is then constructed with fluorescence at 595 nm as the output signal since only Cys can restore the fluorescence. An IMP logic gate can be constructed with fluorescence at 560 nm as the output signal, Fig. 6c. This results in an INH//IMP logic gates in parallel as illustrated in the truth table (Fig. 6d).



b

IN1 (Cys)	IN2 (His)	Output 1 (560 nm)	Output 2 (595 nm)
0	0	1	0
0	1	0	1
1	0	0	1
1	1	0	1

d

IN1 (Cys)	IN2 (NEM)	Output 1 (560 nm)	Output 2 (595 nm)
0	0	1	0
0	1	1	0
1	0	0	1
1	1	1	0

Fig. 6 The column bars of F_{595} and F_{560} against the combinations of Cys and His (**a**) with the corresponding truth table (**b**). The column bars of F_{595} and F_{560} against the combinations of Cys and NEM (**c**) with the corresponding truth table (**d**)

Conclusion

A ratiometric strategy has been constructed for sensitive and selective detection of His and Cys based on DNA-AgNCs as signal probe. In order to fully take into accounts DNA-AgNCs character and sensitive detection of His and Cys, the ratiometric fluorescent nanoprobes are executed in buffer (pH 7.4) and room temperature condition. According to the highly specific competitive interaction among Cys (or His), Cu^{2+}, and DNA-AgNCs, the two fluorescence emissions of DNA-AgNCs are augmented or weakened, which is related to the concentration of Cys (or His). The ratiometric system specifically sensing His and Cys while not other amino acids. Under the assistance of masker agents, NEM for Cys or Ni^{2+} for His, the strategy can furthermore distinguish Cys and His from each other. Limit of detection reaches 5.1 nM for Cys and 4.5 nM for His, respectively, which are compatible with the previous reports. Furthermore, the Cys and His assays are qualified for detecting in serum samples with good accuracy (94%–110% of recoveries), showing potential application detection in real samples. However, it is noted that the developed assay is irreversible and only can be used once. It would be interesting if testing paper is fabricated according to the assay. Taking advantages of ratiometric nanoprobe system, combinatorial logic gates in parallel, OR//NOR and INH/IMP, have been successfully constructed on a universal platform for the first time. The dual emission signal based logic gates provides an instructive prototype for developing combinatorial logic gates.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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