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Fluorescence biosensor for the H_5N_1 antibody based on a metal–organic framework platform†

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Based on the adsorption of a metal–organic framework (MOF) to DNA, a fluorescent biosensor for H_5N_1 antibodies has been developed. In this system, a short oligonucleotide was modified with the fluorescent dye 5′6-FAM (5′6-carboxyfluorescein) as the fluorescent DNA probe. With the introduction of a MOF aqueous solution, the DNA probe can be adsorbed by the MOF and the fluorescence of the dye will be quenched by the MOF. Subsequently, exonuclease I (Exo I) is employed to specifically hydrolyze the DNA probe at the 3′-terminus and the fluorescent dye FAM is released from the MOF, which results in recovery of the fluorescence. If the 3′-ends of the DNA probe are linked to H_5N_1 antigens, which can specifically recognize the H_5N_1 antibody, the hydrolysis of Exo I would be inhibited, so the fluorescence of the system would not recover. The fluorescence is related to the logarithm of the H_5N_1 antibody concentration in the range 1.0×10^{-6}–5.0×10^{-9} mol L^{-1} with a detection limit of 1.6×10^{-9} mol L^{-1} ($S/N = 3$). The proposed method has been applied to detect the H_5N_1 antibody in serum samples with satisfactory results.

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Introduction

Influenza is an aperiodically occurring epidemic that causes millions of deaths around the world. The influenza virus comprises several types and subtypes, and the H_5N_1 virus has been deemed to be one of the world's most prevalent influenza-like illnesses because of its high infection rate and lethality.[1–5] Hence, sensitive, rapid and reliable pathogen detection is extremely important for exploring precautionary measures and as a diagnostic approach. So far, several diagnostic technologies have been developed for detecting human or animal influenza viruses. Conventional methods include cell culture,[6] serological tests,[7] polymerase chain reaction (PCR),[8,9] reverse transcription (RT)-PCR,[10,11] enzyme-linked immunoassays (ELISAs),[12,13] immunoblotting,[14] fluorescence resonance energy transfer (FRET) assays,[15] electro-chemical sequence specific genetic detection[16,17] and surface plasmon resonance (SPR).[18] Most of these approaches are limited in that they are time-consuming, use exorbitant reagents and instruments or require intricate manipulation. Except for some cellular or genetic methods, the aforementioned methods are mainly dependent on the sensing of viral antigens or antibodies to indirectly detect the virus as the clinical point-of-care. This is due to the demanding growth environment of some pathogens, with long cultivation periods and so on, which restricts direct clinical monitoring as a method of diagnosis. Consequently, the detection of viral antigens or antibodies can be difficult to achieve due to the above defects.

Carbon nanotubes[19–23] and graphene oxide (GO)[24] have been pervasively used in biomolecular sensors. For instance, carbon nanotubes and single-stranded DNA (ssDNA) assemblies have been used by Tan and co-workers for the homogeneous detection of biomolecules.[25–27] And the analogous application of GO has already been employed by Chen and co-workers.[24] Nonetheless, in their systems, the ssDNA is desorbed from the carbon nanotubes or GO platforms through conjugation with the complementary DNA. This process of DNA hybridization is time-consuming and limited by the efficiency of DNA crossing. Recently, the topic of metal–organic frameworks (MOFs) has been extensively covered in various areas. MOFs are one class of emerging functional materials with ultrahigh porosity, exceptional tunability, tremendous internal surface areas, structural diversity, and robust thermal stability. They have been utilized for plenty of applications, such as gas storage,[28,29] separation[30] and heterogeneous catalysis.[31] Besides, for spare sensing applications, they mainly concentrate upon quartz crystal microbalance (QCM) equipment,[32,33] stress-induced chemical detection,[34] chemical luminescent sensor to detect cations and anions,[35–37] small molecules,[38] gases or vapors[39] and so forth.

In our early research, a fluorescent biosensor based on the terminal protection of small-molecule-linked DNA has been applied to detect folate receptors, with favorable repeatability

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and selectivity.[40] In this study, the specificity of the DNA probe with terminal protection and the porosity of the MOF material are integrated as an innovative concept for our biosensor. The FAM-ssDNA was adsorbed by the MOF and the fluorescence of FAM can be quenched effectively by the MOF. In the presence of Exo I, the ssDNA can be hydrolysed by Exo I at the 3′ terminal and FAM will disassemble from the MOF, and the fluorescence of the system will recover.[41,42] Since the presence of macromolecules can inhibit hydrolysis, the H_5N_1 antigen was linked with FAM-ssDNA which can then conjugate to H_5N_1 antibody macromolecules, so the fluorescence of the system cannot recover. Based on this system, a sensitive and selective biosensor for the H_5N_1 antibody can be developed. This route circuitously bypasses the procedure of DNA hybridization.

Experimental

Materials and apparatus

H_5N_1 M2 fragment peptide (H_5N_1 antigen, the molecular weight is about 2600 Dalton and the sequences SLLTEVETPIRNEWGCRCNDSSD 2-24/97), anti-matrix protein 2 (H_5N_1 antibody), H_7N_2 antibody, H_3N_2 antibody and H_1N_1 antibody were purchased from Biosynthesis Ltd (Beijing, China), and their stock solutions were prepared in phosphate buffered solution (PBS, pH 7.4) and stored at −20 °C for later use. Exonuclease I (Exo I) was obtained from Sangon Inc. (Shanghai, China). $CuSO_4 \cdot 5H_2O$ crystals were purchased from Xilong chemical factory (Guangdong, China). Dithiooxamide was obtained from International Laboratory USA corporation. Double-distilled water was used throughout the whole process. All other chemicals were of analytical reagent grade and used without further purification.

The DNA sequences used herein were synthesized and purified by Sangon Inc. (Shanghai, China), they were dissolved in tris–HCl (pH 7.4) buffer solution and stored at 4 °C for later use. The 3′-end amino-modified single-strand oligomer is a 35-mer oligonucleotide with the 5′6-FAM labeled at the 5′-end and the sequence is shown below:

5′-FAM-TCTCTCAGTCCGTGGTAGGGCAGGTTGGGGTGACT-NH_2-3′ (FAM-DNA).

A Cary Eclipse Fluorescence Spectrophotometer (Varian Corporation, USA) was used for the measurements. The apparatus parameters were set as follows: $\lambda_{ex} = 480$ nm (slit 10 nm), $\lambda_{em} = 490$–600 nm (slit 10 nm).

Synthesis of the H_2dtoaCu-MOF

H_2dtoaCu-MOF was prepared according to the published procedure.[43–45] The as-prepared synthetic MOF was ground to uniformity using an agate mortar and pestle. 1.0 mg MOF powder was dispersed in 1.0 mL double-distilled water with oscillation and ultrasonication to form 1.0 mg mL^{-1} MOF aqueous solution and then stored at 4 °C for later use.

Preparation of the MOF-based biosensor

Firstly, the FAM-DNA was diluted to 1.0 μmol L^{-1} with double-distilled water and stored at 4 °C in a lucifugal container for later use. Meanwhile, the H_5N_1 antigen was also diluted to 1.0 μmol L^{-1} in phosphate buffered solution (PBS, pH 7.4). Subsequently, we incubated 10 μL (1.0 μmol L^{-1}) FAM-DNA with the equivalent H_5N_1 antigen solution for 3 h at 37 °C in the presence of the cross-linking reagent (2.0 μL 5% glutaraldehyde), forming the DNA-3′-small molecule chimeras. Afterwards, different concentrations of 10 μL H_5N_1 antibody and 180 μL tris–HCl (pH 7.4) were added into the mixture for sufficient incubation at 37 °C for 1 h. Then, 20 μL (1.0 mg mL^{-1}) MOF aqueous solution was added into the reaction system, quenching the fluorescence. Ultimately, 1.0 μL Exo I (20 U mL^{-1}) had been added into the above solution, and the DNA chimeras adsorbed on MOF were hydrolyzed absolutely about 1 h into the enzymolysis. So the FAM dye was released from the MOF, leading to recovery of the fluorescence signal.

Results and discussion

Principle of the MOF-based biosensor

The principle of the MOF-based biosensor for the H_5N_1 antibody is shown in Fig. 1. Firstly, the FAM-DNA fluorescent probe and the H_5N_1 antigen were incubated with 5% glutaraldehyde to form the DNA-3′-small molecule chimera. In the absence of the H_5N_1 antibody, because the MOF has ultrahigh porosity and a tremendous internal surface area, the DNA chimera would be bound to the MOF and the fluorescence of FAM quenched sharply by photoinduced electron transfer (PET).[46] With the addition of Exo I, FAM-DNA was hydrolyzed from the 3′-end of the ssDNA and FAM at the 5′-end was liberated from the MOF surface. Therefore the fluorescence of the system recovered. Conversely, in the presence of the H_5N_1 antibody, it would specifically conjugate to the H_5N_1 antigen-modified DNA. The macromolecular H_5N_1 antibody protected the ssDNA chimera from degradation by Exo I. In this case the fluorescence of the system doesn't change. The amplitude of the recovered fluorescence intensity is related to the H_5N_1 antibody concentration.

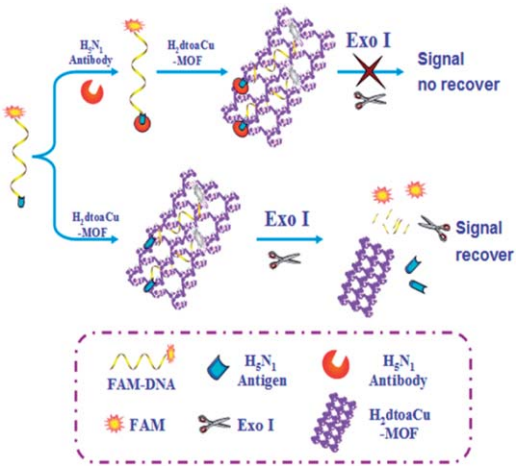

Fig. 1 Mechanism of the proposed fluorescence biosensor for the H_5N_1 antibody based on a H_2dtoaCu-MOF detection platform.

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A simple assistant test has been conducted to verify our assumption. The fluorescence signals of the H_5N_1 antigen linked FAM-DNA chimera with/without H_5N_1 antibodies under three conditions ((a) the fluorescence probe, (b) the fluorescence probe + MOF, and (c) the fluorescence probe + MOF + Exo I) were detected respectively. As shown in Fig. 2, the fluorescence intensities of the H_5N_1 antigen linked FAM-DNA chimera with/without H_5N_1 antibodies were nearly the same (see line a′ and line a), which indicates that the fluorescence intensity of FAM-DNA is unaffected by conjugation of the H_5N_1 antibody. After the addition of the MOF into the solution, the fluorescence signals were quenched (see line b′ and line b). With the addition of Exo I, the chimera with the H_5N_1 antibody was protected from degradation of the H_5N_1 antibody macromolecule, and the fluorescence of the system increased by a small amount (see line c′). The reason lies in that not all of the FAM-DNA chimera had been linked with antibodies, so some free FAM-DNA chimera still existed, and it would be digested by the Exo I, resulting in the fluorescence signal increasing. Otherwise, the chimera without the H_5N_1 antibodies was hydrolyzed by Exo I to mononucleotides and FAM, which returned into the aqueous solution, so the fluorescence of the system increased greatly (see line c). Herein, the regained fluorescence intensity is lower than the fluorescence signal of the unquenched solution. Due to the porous structure of the MOF, FAM released by the hydrolysis of Exo I can freely immigrate or emigrate. Thus, segmental FAM remained inside the MOF without fluorescence signal excitation. These results demonstrate the feasibility of our proposal.

As shown in Fig. 3, if Dnase I and Exo III at concentrations of 5.0 U μL^{-1} had been added into the solution, the fluorescence intensity ΔI would increase a little bit (ΔI is the fluorescence recovery against the fluorescence intensity after MOF quenching). However, a remarkable fluorescence recovery emerged after the addition of 1.0 U μL^{-1} Exo I. This consequence definitely certifies that the enzymolysis of Exo I selectively occurred for FAM-DNA without the interference of other enzymes.

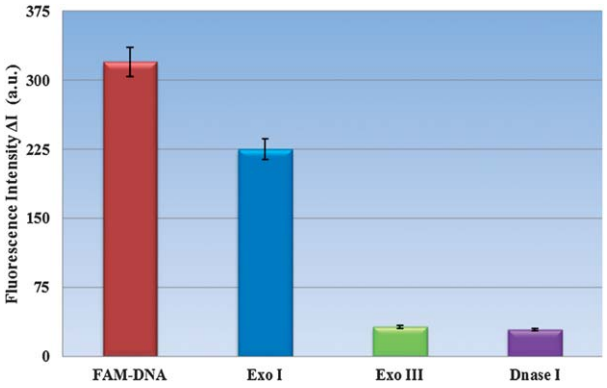

Fig. 3 Fluorescence intensity ΔI changed by Exo I, Exo III and Dnase I. [FAM-DNA] = 1.0 × 10^{-6} mol L^{-1}, [antigen] = 1.0 × 10^{-6} mol L^{-1}, [MOF] = 1.0 mg mL^{-1}, [Exo I] = 1.0 U μL^{-1}, [Exo III] = 5.0 U μL^{-1}, [Dnase I] = 5.0 U μL^{-1}. The error bars were calculated from three independent experiments.

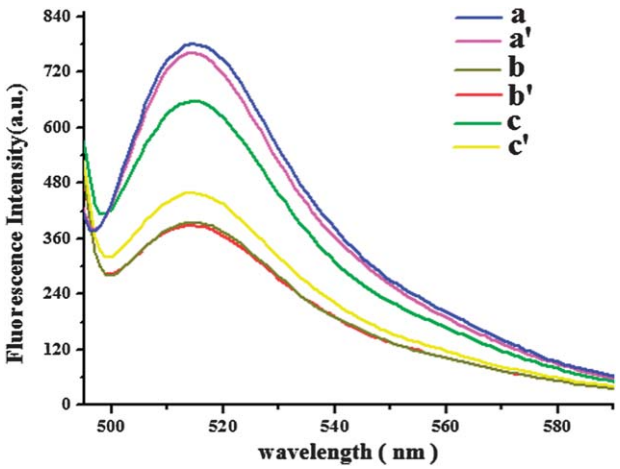

Fig. 2 Fluorescence spectra in different conditions. (a) H_5N_1 antigen linked FAM-DNA; (a′) H_5N_1 antigen linked FAM-DNA + H_5N_1 antibody; (b) H_5N_1 antigen linked FAM-DNA + MOF; (b′) H_5N_1 antigen linked FAM-DNA + H_5N_1 antibody + MOF; (c) H_5N_1 antigen linked FAM-DNA + MOF + Exo I; and (c′) H_5N_1 antigen linked FAM-DNA + H_5N_1 antibody + MOF + Exo I. [FAM-DNA] = 1.0 × 10^{-6} mol L^{-1}, [antigen] = 1.0 × 10^{-6} mol L^{-1}, [antibody] = 5.0 × 10^{-6} mol L^{-1}, [MOF] = 1.0 mg mL^{-1}, [Exo I] = 1.0 U μL^{-1}.

Effect of the types of adsorption materials

In order to compare the performance of various types of MOFs, MOFs with different metal ions (Cu^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+}) were synthesized.[43–45] Moreover, we changed dithiooxamide to 1,4-benzenedicarboxylate (BDC), and synthesized Fe-BDC, Cr-BDC and Mn-BDC organometallic compounds.[47] As shown in Table S1 (see ESI† for details), H_2dtoaMn, H_2dtoaCo, Fe-BDC, Cr-BDC and Mn-BDC cannot effectively quench the fluorescence. Fortunately, H_2dtoaCu and H_2dtoaNi can quench the fluorescence effectively (over 35%). Then, the fluorescence recovery experiments were performed. The results demonstrate that H_2dtoaCu has a better recovery rate than that of H_2dtoaNi after enzymolysis. The reason for this was also studied. As shown in Table S1,† the addition of $CuSO_4 \cdot 5H_2O$ induced an admirable fluorescence quenching, but the fluorescence was unable to be recovered any more. A small amount of fluorescence quenching was detected on addition of dithiooxamide. Because Cu^{2+} is a paramagnetic ion with a d^9 center, this configuration triggers a demonstrable PET with essentially zero fluorescence.[46] We propose that, critically, the quenching is due to the effects of Cu^{2+} on the FAM-DNA probe. The porous structure of H_2dtoaCu-MOF can ensure FAM-DNA is adsorbed and the Cu^{2+} quenches the FAM fluorescence. After enzymolysis of Exo I, the FAM dye is released from the MOF which results in the fluorescence recovery. So, H_2dtoaCu-MOF was selected as the reaction platform in our approach.

Analogical structural materials, such as GO, SWCNTs and MOFs have been applied as the adsorption materials in this system. Due to the strong noncovalent interactions of GO, SWCNTs and MOFs with nucleobases,[48,49] the fluorescence of the FAM can be quenched (shown in Fig. 4). An eminent fluorescence recovery was achieved with Exo I on the MOF. But the fluorescence can't be recovered using SWCNTs and GO. The reason for this may be that aromatic compounds (such as FAM)

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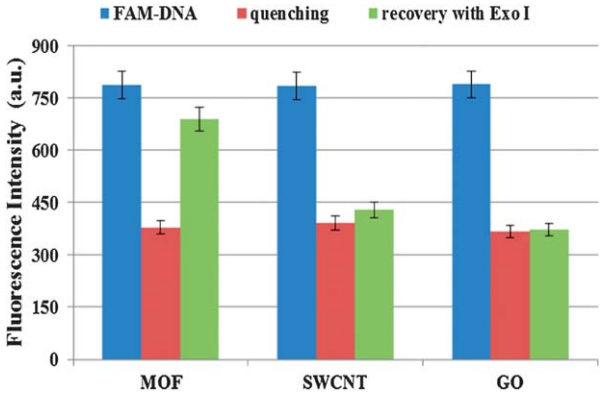

Fig. 4 Histogram of the change in peak fluorescence intensity for MOF, SWCNT and GO without/with Exo I. [FAM-DNA] = 1.0×10^{-6} mol L^{-1}, [antigen] = 1.0×10^{-6} mol L^{-1}, [MOF] = 1.0 mg mL^{-1}, [SWCNT] = 1.0 mg mL^{-1}, [GO] = 1.0 mg mL^{-1}. The error bars were calculated from three independent experiments.

firmly bind to GO and SWCNTs *via* noncovalent van der Waals interactions.[50] This result demonstrates that the MOF platform is more appropriate than GO and SWCNTs in our sensing system.

Optimization of MOF concentration and response time

The effect of the MOF concentration on the fluorescence intensity has been studied. As shown in Fig. 5, the fluorescence intensity decreases with an increase in MOF concentration from 0–0.9 mg mL^{-1}. At higher concentrations, the fluorescence intensity is almost constant. The effect of the reaction time was also studied. We detected fluorescence signals at 0–30 min after mixing with 0.9 mg mL^{-1} MOF. The fluorescence intensity decreased promptly after 5 min oscillation and then reached a plateau. Hence, 0.9 mg mL^{-1} MOF and 5 min oscillation time were chosen as the optimized conditions. In our experiment, up to 50% quenching and instantaneous response was observed.

Dynamic studies of digestion against Exo I

With regard to the recovery procedure of the fluorescence intensity, the digestion against Exo I is a critical factor. We successively detected the influence of the concentration of Exo I and digestion time on the fluorescence intensity. As shown in Fig. 6A, the fluorescence intensity increases with Exo I concentration from 0–1.0 U μL^{-1}. A plateau of the fluorescence intensity is attained after 1.0 U μL^{-1} of Exo I. Then, the fluorescence intensities at different digestion times were measured in the presence of 1.0 U μL^{-1} Exo I. The fluorescence intensity increased progressively until 60 min and then reached a plateau (Fig. 6B). Therefore, 1.0 U μL^{-1} of Exo I and 60 min digestion time were chosen as the optimized conditions.

Quantitative assays of the H_5N_1 antibody

Fig. 7 shows the typical fluorescence responses to different H_5N_1 antibody concentrations. The fluorescence intensities gradually decreased with increasing H_5N_1 antibody concentration. The inset of Fig. 7 is the linear relationship between the fluorescence intensity and the logarithm of H_5N_1 antibody concentration in the range 1.0×10^{-6}–5.0×10^{-9} mol L^{-1}. The detection limit was estimated to be 1.6×10^{-9} mol L^{-1} ($S/N = 3$), which is lower than 2.0×10^{-9} mol L^{-1} by commercial enzyme-linked immunosorbent assay (ELISA). Furthermore, in comparison with virus isolation (VI), a gold standard method for the detection of H_5N_1, this method is cost-efficient and fast in clinical tests.[51]

In addition, we detected the enzymolysis at the same H_5N_1 antibody concentration (5.0×10^{-8} mol L^{-1}) five times. The relative standard deviation (RSD) of the fluorescence signals is 4.76%. This result testifies that the proposed method has a high repeatability.

To verify the selectivity of our biosensor, some other antibodies with relatively high concentrations (the concentrations are 50 times that of the H_5N_1 antibody) were used in place of the H_5N_1 antibody to test the fluorescence recovery. The results showed that they were unable to conjugate with the H_5N_1 antigen-linked FAM-DNA chimera and restrain the digestion from Exo I, so the fluorescence could be recovered (as shown in Fig. 8). Hence, on the basis of the specific combination of the H_5N_1 antibody and the H_5N_1 antigen-linked FAM-DNA chimera, the developed biosensor shows noteworthy selectivity.

To authenticate the feasibility of our approach in complex biological matrixes, a certain amount of H_5N_1 antibody spiked in serum was evaluated. Herein, serum is one part of whole blood separated from the solid fraction with a centrifuge. We extracted standard human serum from the blood of healthy

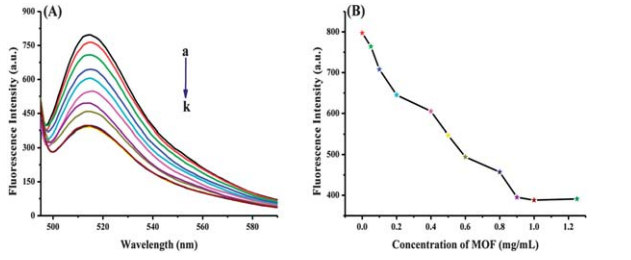

Fig. 5 (A) Fluorescence intensity at different MOF concentrations. Lines (a)–(k): 0, 0.05, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0 and 1.25 mg mL^{-1}; and (B) plot of fluorescence intensity *versus* MOF concentration. [FAM-DNA] = 1.0×10^{-6} mol L^{-1}; [antigen] = 1.0×10^{-6} mol L^{-1}.

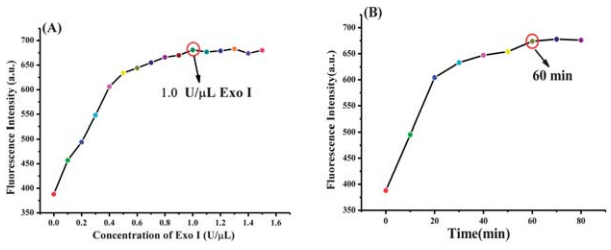

Fig. 6 (A) Fluorescence intensity at different Exo I concentrations (incubated for 2 h); and (B) fluorescence intensity at different enzymolysis time (incubated in 1.0 U μL^{-1} Exo I). Conditions: [FAM-DNA] = 1.0×10^{-6} mol L^{-1}; [antigen] = 1.0×10^{-6} mol L^{-1}; [MOF] = 1.0 mg mL^{-1}; temperature: 37 °C.

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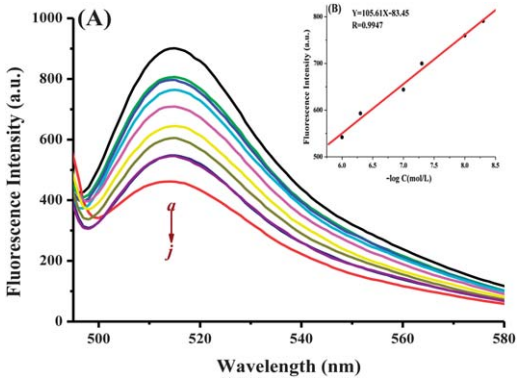

Fig. 7 (A) Fluorescence spectra after incubation with varying concentrations of H_5N_1 antibodies followed by digestion with 1.0 U μL^{-1} Exo I. (a) FAM-DNA; (b) in the absence of H_5N_1 antibody; (c) 5.0×10^{-9} mol L^{-1}; (d) 1.0×10^{-8} mol L^{-1}; (e) 5.0×10^{-8} mol L^{-1}; (f) 1.0×10^{-7} mol L^{-1}; (g) 5.0×10^{-7} mol L^{-1}; (h) 1.0×10^{-6} mol L^{-1}; (i) 5.0×10^{-6} mol L^{-1}; and (j) MOF quenching. (B) Plot of fluorescence intensity *versus* H_5N_1 antibody concentration. [FAM-DNA] = 1.0×10^{-6} mol L^{-1}, [antigen] = 1.0×10^{-6} mol L^{-1}, [MOF] = 1.0 mg mL^{-1}.

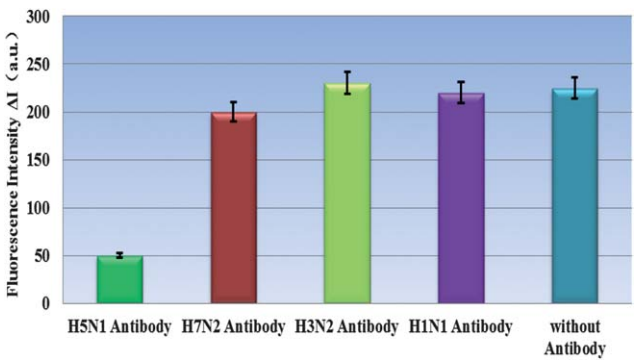

Fig. 8 Selectivity for H_5N_1 antibody over other antibodies. Fluorescence intensity ΔI changed by the digestion from Exo I in H_5N_1 antigen-linked FAM-DNA chimera solutions respectively with H_5N_1, H_7N_2, H_3N_2, and H_1N_1 antibodies. [H_5N_1 antibody] = 1.0×10^{-6} mol L^{-1}, [H_7N_2 antibody] = [H_3N_2 antibody] = [H_1N_1 antibody] = 5.0×10^{-5} mol L^{-1}, [FAM-DNA] = 1.0×10^{-6} mol L^{-1}, [antigen] = 1.0×10^{-6} mol L^{-1}, [MOF] = 1.0 mg mL^{-1}, [Exo I] = 1.0 U μL^{-1}. The error bars were calculated from three independent experiments.

individuals (obtained from Fuzhou University hospital), then diluted them in 1% with tris–HCl buffer and respectively spiked with the H_5N_1 antibody at four concentrations (0, 5, 10 and 100 nM). The recovery values were determined and were acceptable (see Table S2 in ESI†). Consequently, this assay indicates the potential for applications in antibody detection in the biological field.

Conclusions

In summary, we have successfully developed an approach for the detection of biomolecules using fluorescence recovery based on the MOF platform. First, the application of a MOF platform, like GO and carbon nanotubes, is novel for the sensitive and selective detection of biomolecules in a biosensor system. Additionally, our method avoided the DNA hybridization from the enzymolysis of ssDNA for fluorescence recovery, which simplified the experimental process. Furthermore, it is potentially universal because the application of the DNA terminal protection can be easily designed for other specific target pairs such as biotin–avidin and antibody–antigen.

Acknowledgements

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