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An amine-functionalized metal–organic framework as a sensing platform for DNA detection†

Hao-Tian Zhang,^a Jian-Wei Zhang,^{ab} Gang Huang,^a Zi-Yi Du^b and Hai-Long Jiang^{*a}

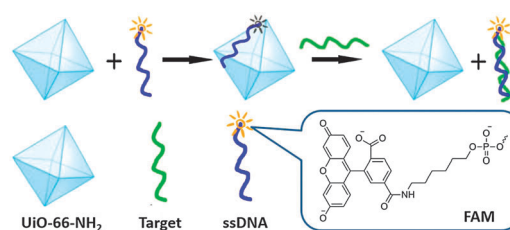
An amine-functionalized metal–organic framework (MOF) has been employed as an effective fluorescent sensing platform for DNA detection and is capable of distinguishing complementary and mismatched target sequences with high sensitivity and selectivity.

The detection of DNA sequences is of particular interest and importance in genetics, pathology, criminology, pharmacogenetics, food safety, and so on.¹ The polymerase chain reaction (PCR) method suffers from high cost, risk of contamination, and false-negative results, although it is well-known for DNA amplification and sequencing and has extensive applications in modern biological and medical sciences.² Another mature technique involving gene chips is widely employed for high-throughput DNA detection; however, it requires high-cost instrumentation for fluorescence signal readout and sophisticated numerical algorithms for data explanation.³ Therefore, it is necessary to develop simple, rapid, sensitive and cost-effective approaches for this purpose. In recent years, many endeavors have been devoted to developing homogeneous fluorescence assays, most of which are based on fluorescence resonance energy transfer (FRET) *via* fluorophore–quencher pairs for the detection of DNA sequences, including carbon nanostructures with different forms, Au nanoparticles (NPs) and other nanomaterials.⁴ Although they have been proven to be effective fluorescent platforms, the respective drawbacks limit their practical use; the preparation of the detection agent in many systems is time-consuming, tedious or labor-intensive, and some of them cannot be prepared on a large scale or suffer from stability issues.^{4a–c,5}

On the other hand, metal–organic frameworks (MOFs), constructed by metal ions/clusters and organic linkers, are a

class of crystalline porous materials.⁶ In recent two decades, MOFs have captured widespread research interest due to their intriguing structural topologies and potential applications as functional materials in a wide range of fields.^{7–10} Particularly, MOFs have been demonstrated to be fluorescent sensors for the detection and recognition of various cations, anions, vapors and small molecules based on their fluorescence response.¹⁰ However, to the best of our knowledge, very rare MOFs have been studied for the detection of DNA or biomolecules.¹¹

The organic linkers involved in MOFs usually have a conjugated π -electron system and offer a source for possible hydrogen bonds that allow suitable interaction between MOFs and single-stranded DNA (ssDNA). Therefore, MOFs could be reasonably able to recognize DNA molecules *via* fluorescence changes, similar to previously reported detection agents. In this work, we have developed an amine-functionalized MOF, UiO-66-NH₂ (UiO = University of Oslo), as an efficient biosensor for the detection of DNA with high selectivity. The principle of this assay is proposed in Scheme 1. The free ssDNA with a fluorophore (FAM) at its 5' end has strong fluorescence emission at 518 nm ($\lambda_{\text{ex}} = 480$ nm). Electrostatic attractions such as $\pi \cdots \pi$ stacking or hydrogen bond interactions between aromatic nucleotide bases in the ssDNA and UiO-66-NH₂ allow them to attach or come in close proximity to each other, which results in the substantial fluorescence quenching of FAM (off-state), possibly due to the photoinduced electron transfer.¹² The absorption spectrum of MOF dispersed in Tris-HCl buffer (pH: 7.42) exhibits two absorption peaks at 217 nm



Scheme 1 Proposed principle for the fluorophore-labeled DNA detection by a MOF, UiO-66-NH₂, as a sensing platform.

^a Hefei National Laboratory for Physical Sciences at the Microscale, Collaborative Innovation Center of Suzhou Nano Science and Technology, Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, P.R. China. E-mail: jianglab@ustc.edu.cn; Fax: +86-551-63607861; Tel: +86-551-63607861

^b College of Chemistry and Chemical Engineering, Gannan Normal University, Ganzhou 341000, P.R. China

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and 328 nm (Fig. S3, ESI[†]), suggesting that there is no spectral overlap and thus no FRET occurs between the MOF and ssDNA. Upon the introduction of target ssDNA (tDNA) into the system, double-stranded DNA (dsDNA) detaches from UiO-66-NH₂. As a result, the energy transfer process is inhibited and the fluorescence of ssDNA is recovered (turn-on state). It is proposed that hydrogen bond interaction between ssDNA and the amino group in UiO-66-NH₂ could play a critical role in the DNA detection.

UiO-66-NH₂ was constructed from Zr^{IV} and 2-amino-1,4-benzenedicarboxylic acid (NH₂-BDC) exhibiting great chemical and thermal stability. The framework built up from Zr₆O₄(OH)₄ oxoclusters linked together by 12 NH₂-BDC ligands, formulated as Zr₆O₄(OH)₄(BDC-NH₂)₆, features a 3D network involving tetrahedral and octahedral cages of 6 and 11 Å, respectively, accessible through microporous windows (4–6 Å) (Fig. 1A–C). Powder X-ray diffraction (XRD) profiles demonstrate its phase purity and great water stability. The scanning electron microscopy (SEM) image indicates that the sizes of UiO-66-NH₂ particles are in the range of 50–100 nm (Fig. 1D), suitable for dispersion in the aqueous solution. N₂ sorption isotherms confirm their permanent porosity and the BET surface area is 615 m² g⁻¹ (Fig. S2, ESI[†]).

To demonstrate the feasibility of employing UiO-66-NH₂ as a fluorescent sensing platform for DNA detection, an oligonucleotide sequence associated with human immunodeficiency virus (HIV) was employed as a model system. As displayed in Fig. 2, the FAM-ssDNA probe (P_{HIV}) exhibits fairly strong fluorescence emission due to the presence of a fluorescein-based dye, FAM. The introduction of UiO-66-NH₂ leads to around 56% drop in the fluorescence intensity, suggesting that the MOF has interaction with ssDNA and quenches the fluorescence effectively. Significantly, the MOF-P_{HIV} composite exhibits remarkable fluorescence enhancement with a recovery of up to 70% upon its incubation with complementary target T₁ (tDNA), revealing the release of ssDNA due to its binding to tDNA. Actually, the fluorescence of P_{HIV} is nearly not affected by the tDNA without MOF and the MOF itself has no fluorescence contribution in the investigated wavelength range.

The influence of the amount of MOF introduced in the system shows that the fluorescence quenching efficiency increases while the recovery efficiency decreases along with more MOFs used ranging from 0–50 μL. More MOFs nearly do not affect both efficiencies (Fig. 3A). It is understandable because more MOFs would result in more efficient adsorption of ssDNA and thus maximize the fluorescence quenching to some extent. In this case, some tDNA may also be adsorbed on MOFs and the

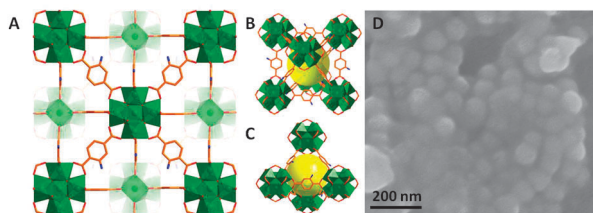


Fig. 1 (A) The 3D structure of UiO-66-NH₂ and it contains (B) large octahedral and (C) small tetrahedral cages. The Zr, C, O and N atoms are represented by olive, orange, red and blue, respectively, and Zr₆O₄(OH)₄ clusters are shaded in olive polyhedra. (D) A SEM image of UiO-66-NH₂.

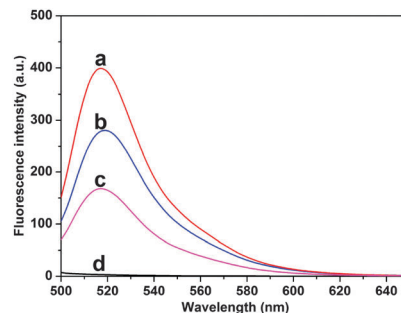


Fig. 2 Fluorescence spectra of P_{HIV} (50 nM) in different systems ($\lambda_{\text{ex}} = 480$ nm): (a) P_{HIV}; (b) P_{HIV} + UiO-66-NH₂ + T₁ (150 nM); (c) P_{HIV} + UiO-66-NH₂; (d) UiO-66-NH₂ in the absence of P_{HIV}.

binding between ssDNA and tDNA is suppressed, thus reducing the release of ssDNA and the fluorescence recovery. Therefore, 20 μL of the MOF as an optimized volume was used in this study, otherwise specified. In addition, the amount of tDNA has a positive influence on the fluorescence recovery of MOF-ssDNA. The fluorescence recovery efficiency increases when more tDNA, ranging from 10–150 nM, is introduced into the system, while more tDNA would not cause the change any more (Fig. 3B).

To further understand the kinetics of the fluorescence quenching and recovery, the time-dependent fluorescence intensity of P_{HIV} by UiO-66-NH₂ and the MOF-P_{HIV} composite with tDNA as a function of incubation time was examined (Fig. 3C). In the absence of tDNA, the curve shows a rapid decrease in the first 5 min and reaches an equilibrium in around 20 min, suggesting that UiO-66-NH₂ can adsorb ssDNA effectively and quickly. Moreover, it seems to be a trend that more MOFs make the system reach equilibrium more quickly (Fig. S4, ESI[†]). The introduction of tDNA allows the

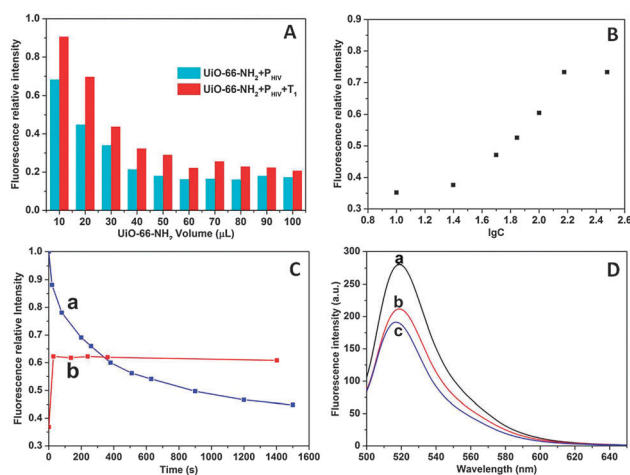


Fig. 3 Influence of the amount of (A) UiO-66-NH₂ or (B) T₁ on the fluorescence quenching efficiency (P_{HIV}: 50 nM). (C) Kinetic behavior study: (a) fluorescence quenching of P_{HIV} (50 nM) by UiO-66-NH₂ and (b) fluorescence recovery of P_{HIV}-UiO-66-NH₂ by T₁ (150 nM) as a function of incubation time. (D) Fluorescence intensity of P_{HIV} + UiO-66-NH₂ under $\lambda_{\text{ex}} = 480$ nm upon introducing different targets: (a) T₁; (b) single-base mismatched target T₂ to ssDNA; (c) mismatched target T₃ to ssDNA. All measurements were done in Tris-HCl buffer with 5 mM Mg²⁺ (pH: 7.42).

fluorescence recovery in a faster kinetics. Upon the addition of tDNA, the fluorescence intensity increases rapidly and reaches equilibrium in around 3 min, suggesting that T₁ can detach ssDNA from UiO-66-NH₂ effectively in a very short time. Rapid adsorption and detachment of ssDNA make UiO-66-NH₂ promising for fluorescent sensing of DNA molecules.

In addition to the complementary target T₁, mismatched sequences (single-base mismatched T₂ and mismatched T₃ to ssDNA) were introduced into the system to investigate the discrimination ability of the sensing platform (Fig. 3D). It was observed that the fluorescence intensity of the system in the presence of T₁, T₂ and T₃ exhibits 67%, 26%, and 14% enhancement, respectively, compared to that in the absence of the target. The fluorescence intensity is clearly associated with the matching level of base pairs between ssDNA and the target. These results indicate that the sensing platform is able to distinguish between the complementary and unmatched target sequences, no matter whether only single-base or total mismatching exists.

To understand the role of the amino group in UiO-66-NH₂ for the sensing property, another MOF bearing the same structure but without an amino group, UiO-66, was examined. Unexpectedly, although the introduction of UiO-66 leads to around 42% drop in the fluorescence intensity, a bit less than that observed with UiO-66-NH₂ (56%), the fluorescence intensity of the system in the presence of T₁ and T₂, respectively, exhibits 58% and 56% enhancement compared to that observed in the absence of the target, indicating that UiO-66 cannot distinguish the complementary and the single-base mismatching targets (Fig. S5, ESI[†]). Given the negatively charged backbone of ssDNA,¹³ the zeta potentials of UiO-66 and UiO-66-NH₂ of +1.28 and -5.54 mV, respectively, indicate that UiO-66 has weak electrostatic interactions with ssDNA while a bit of electrostatic repulsion between UiO-66-NH₂ and ssDNA could exist. Meanwhile, UiO-66 has a similar structure and a conjugated π -electron system to UiO-66-NH₂. In this context, what interaction is able to offset the electrostatic repulsion and enables the successful adsorption of ssDNA on the surface of UiO-66-NH₂? It is well known that a DNA double helix is formed depending on the hydrogen bonds between amino groups of bases on two single-stranded DNAs. Accordingly, we may infer that there are hydrogen bonds between the amino groups in UiO-66-NH₂ and bases in ssDNA, resulting in the adsorption of ssDNA on UiO-66-NH₂. In contrast, UiO-66 without an amino group does not form hydrogen bonds with ssDNA, which could be responsible for its indistinguishability for different target DNAs. The infrared (IR) absorption spectrum of the MOF-P_{HIV} composite shows the N-H stretching vibration at 3460 and 3376 cm⁻¹ (Fig. S6, ESI[†]), which exhibits a blue shift compared to the characteristic peaks of the unbounded NH₂ group at 3500 and 3386 cm⁻¹ in pristine UiO-66-NH₂.¹⁴ The peak shift further supports the existence of hydrogen bond interaction between the amino group in UiO-66-NH₂ and ssDNA bases.¹⁵

In conclusion, our results indicate that an amine-functionalized MOF, UiO-66-NH₂, can afford an effective fluorescent sensing platform for DNA detection. The sensing system can distinguish complementary and mismatched DNA sequences down to single-base mismatch with high selectivity and good reproducibility. In contrast,

UiO-66 with a similar structure in the absence of an amino group cannot realize such a function. For the first time, the hydrogen bond interaction between MOFs and ssDNA has been proposed to account for DNA detection. Given the high stability and the facile and scalable synthesis using cheap reactants, the UiO-66-NH₂-based assay holds great promise for practical applications in clinical sample analysis.

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