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COMMUNICATION

Metal-Organic Framework MIL-101 Enhanced Fluorescence Anisotropy for Sensitive Detection of DNA

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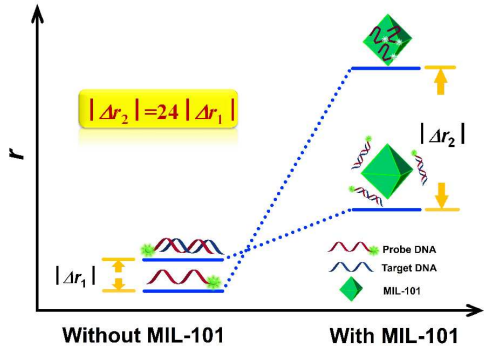
The metal-organic framework (MOF) was first utilized as an amplification platform for fluorescence anisotropy assay, which was identified to be effective for highly sensitive detection of DNA.

Fluorescence anisotropy (FA) has been developed for the measurements of the rotational motion-related factors of a fluorophore or fluorophore-labeled complex.[1] FA has two advantages in homogeneous and high throughput assays. One is that FA was applicable in such case that the rotation of the fluorophore is changed and no separation of the excessive reagent from the reacting media during the experimental process.[2] The other is that the quantitative FA measurements are not significantly affected by the variations of fluorescence intensity due to photobleaching or other effects.[3] Therefore, FA technique has been widely applied in those fields such as clinical diagnosis,[4] food analysis[5] and environmental areas.[6] Theoretically, if the environmental factors such as solution temperature and viscosity are constant, FA is directly proportional to the molecular volume and molecular mass,[1] and thus high FA values are expected if the fluorophore is associated with a large molecule. Hence, to improve the sensitivity of FA assay, proteins,[7] molecular imprinted polymers,[8] and gold nanoparticles (AuNPs)[9] have been employed as mass amplifiers. For example, thrombin was designed as the anisotropy amplifier for detection of ATP in complex biological samples,[2] and AuNPs could be employed to enhance FA and homogeneous detection of single nucleotide polymorphism (SNP) was achieved.[10] To identify the size effect in the FA chemistry, Our group employed graphene oxide (GO) to enhance the FA of fluorophore and further constructed a label-free assay of metal ions.[11] In this work, a more apparent FA enhancement was provided for the larger molecular masses of GO and without extra covalent linkage with it, which was simple and cost-effective. Later on, GO was also used as FA amplifier for the detection of ATP.[12] These reports suggested that nanomaterial with larger molecular mass may exhibit a better amplification effect.

Metal-organic frameworks (MOFs), a relatively new class of crystalline porous materials that combine metal ions with rigid organic ligands,[13] usually have large molecular masses and volumes, supplying the potential possibility of higher enhancement efficiency of FA. Over the past years, MOFs have been widely used in gas storage,[14] sensing,[15] catalysis[16] and drug delivery.[17] Additionally, Yan *et al*. explored a series of analytical applications of MOFs from sampling to chromatographic separation.[18] Our group has also established several novel strategies with MOFs for detection of glucose, cytidine triphosphate and adenosine 5′-triphosphate,[19] respectively. Recently, to further broaden the application of MOFs acting as sensing platforms for the recognition of biomolecules, Chen *et al*. first utilized MOF as a fluorescence quenching platform for assay of DNA and thrombin analysis.[20] These reports showed that MOFs are of high promise either as absorber of small molecules or analytical platform.

In terms of the structures of MOFs, the ligands usually contain a conjugated π-electron system, allowing to bind ssDNA through π-π interactions, which mechanism is similar to the interaction between GO and biomolecules. Chromium-benzenedicarboxylates (MIL-101), one of the cationic MOFs, contains the ligand of terephthalic acid, supplying conjugated π-electron system for the binding of ssDNA.[20a] Hence, MIL-101 should have strong affinity to negatively charged ssDNA through π-π stacking and electrostatic interaction. Additionally, MIL-101 displays superb hydrothermal stability compared with other benzenedicarboxylate based MOFs.[21] Herein, we first introduced MIL-101 as a FA amplifier for detection of DNA. We expect that the rotation of the fluorophore labelled on ssDNA molecules could be confined by MIL-101, resulting in enhanced FA values.



Scheme 1 The concept and the principle of MIL-101 amplified fluorescence anisotropy strategy for HIV-DNA detection.

In this contribution, MIL-101 was synthesized according to published literatures.[22] The scanning electron microscope (SEM) and powder X-ray diffraction (XRD) (displayed in Fig. S1 and Fig. S2, ESI†) showed that the synthesized MIL-101 was regarding the same as the published literatures. MIL-101 was employed as FA amplification platform for DNA detection, in which a 23-mer synthetic oligonucleotide from the HIV-1 U5 long terminal repeat (LTR) sequence was involved as an ssDNA

model. Probe DNA (P) is complementary with HIV-1 DNA with the sequence of 5′-AGT CAG TGT GGA AAA TCT CTA GC-3′. P was 6-carboxyfluorescein (FAM)-labelled at 5'-terminal, and thus the hybridization of P and target DNA (T) can monitored by measuring the changes of FA values.

As show in scheme 1, without MIL-101, only small FA change occurred for the little molecular mass variation before and after the hybridization of P with T. However, with the involvement of MIL-101, P was absorbed and twined onto the surface of MIL-101. As a result, the rotation of fluorophore was restricted manifestly, exhibiting a large FA value. If P hybridized with T first, the formed dsDNA of P/T kept away from MIL-101, resulting in lower FA value. Thus, a larger FA change was observed before and after the hybridization. Therefore, the MIL-101-amplified FA strategy can be established to assay of DNA.

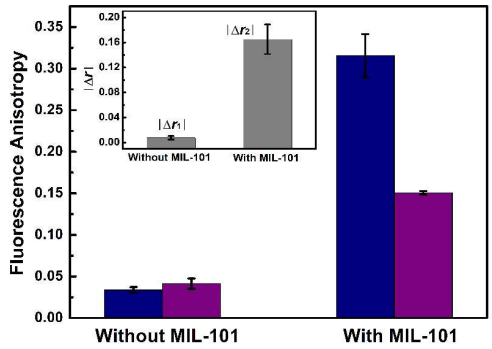


Fig. 1 Fluorescence anisotropy of P in the absence (navy columns) and presence (purple columns) of T with/without subsequently incubation with MIL-101. Concentrations: P, 20 nM; T, 8 nM; MIL-101, 45 μg/mL; pH, 7.2; λ_{ex}, 480 nm.

Originally, we investigated the FA changes responding to the process of DNA hybridization both in the absence and presence of MIL-101. The change of FA value was expressed as $|\Delta r|$ ($\Delta r = r_1 - r_2$), wherein r_1 and r_2 stand for the FA values of the P in the absence and presence of T, respectively. The anisotropy (r), which sensitive to the rotational motion changes of the fluorophore-linked object, can be described by the flowing equation (For details, see the eq3 and eq4, ESI†).[11, 23]

$$\frac{1}{r} = \frac{1}{r_0} + \frac{\tau RT}{r_0 \eta V} \qquad (1)$$

Where r is the measured anisotropy, r_0 is the fundamental anisotropy in the absence of rotational diffusion, τ is excited state lifetime, T is the temperature in Kelvin, R is the gas constant, η is the viscosity of the solution and V is the effective volume of the rotating unit. The anisotropy of a fluorophore is proportional to its rotational relaxation time, which in turn depends on its molecular volume. Therefore, a smaller molecule rotates faster, exhibiting smaller FA value, while larger molecules have larger FA value due to their confined motion.

As shown in Fig.1, in the absence of MIL-101, the FA value changed from 0.034 to 0.041 with the $|\Delta r_1|$ of 0.007 before and after hybridization. However, if P incubated with MIL-101, FA value increased to 0.32 which is due to the rotation of fluorophore labelled at the end of P was confined by MIL-101 (eq1). And once P was hybridized with T at first and form a stable double stranded P/T structure, which can't conjugate with MIL-101, the FA value of P/T was only about 0.15. In other words, in the presence of MIL-101 the FA value decreased from 0.32 of the P-MIL-101 structure to 0.15 with hybridization of P/T, resulting in an enhanced value ($|\Delta r_2|$) of 0.17, which is 24-fold higher than that without MIL-101 (the inset of Fig. 1).

Agarose gel electrophoresis was carried out (Fig. S3, ESI†) to confirm that ssDNA and dsDNA exhibit different affinities toward MIL-101. Under UV excitation, P displayed one light band with a strong fluorescence (lane 2). However, no light band was observed after P was incubated with MIL-101 (lane 3), suggesting that P was integrated with MIL-101 and the P/MIL-101 ensemble was too large to pass through the gel.[12] Once T was introduced, the hybridization products of P and T exhibited two light bands (lane 4) in the absence of MIL-101. The brighter one represents the dsDNA of P/T which possesses higher molecular weight and moved slower, while the darker one represents the remaining unhybridized P which possesses lower molecular weight and moved faster. However, only one light band (lane 5) was visible if MIL-101 was employed after the hybridization of P with T, which attributed to that the dsDNA of P/T can penetrate into the gel.[24] This experiment certificated that MIL-101 can distinguish ssDNA from dsDNA and verified the proposed mechanism effectively. The mass variations of P brought by MIL-101 and T provided a basis for FA-based detection of DNA.

To obtain the best quantification result, several factors including the dosage of MIL-101, pH value, incubation time and ionic strength were optimized. Firstly, the amount of MIL-101 plays a very important role on the amplification effect. Low content of MIL-101 would cause incomplete integration of MIL-101 with P, resulting in lower background FA signal. However, excessive MIL-101 would inhibit the release of P/T complex, bring about lower Δr value. Additionally, zeta potential measurement showed that MIL-101 was positively charged and DNA was negatively charged, indicating that the possible occurrence of electrostatic interaction between them. Therefore, pH and ionic strength of the environment would have great effect on FA value. Experimental showed that the optimal conditions were 45 μg/mL of MIL-101 at pH 7.2 incubated with 20 nM of P for 50 min with 150 mM NaCl (Fig. S4a, S4b, S4c, S4d) at 25 °C.

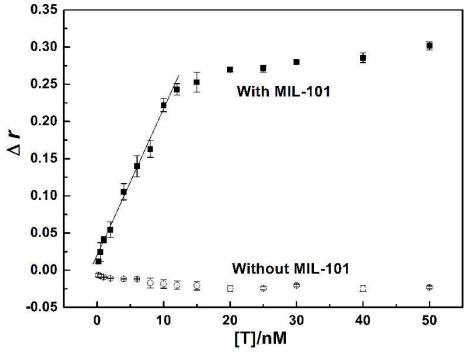


Fig. 2 Δr of P/MIL-101 (filled squares) and P (open circles) with the increasing concentration of T from 0.2 nM to 50 nM. P, 20 nM; MIL-101, 45 μg/ml; pH, 7.2; λ_{ex}: 480 nm.

Fig. 2 displayed the dependence of Δr on the increasing concentration of T. In the presence of MIL-101, there is a linear relationship between Δr and concentration of T in the range of 0.3-12 nM with linear regression equation of $\Delta r = 0.012 + 0.020\ c_T$ and the detection limit of 0.2 nM (S/N = 3). Contrastively, without MIL-101, there is no obvious FA change with the increasing concentration of T under the same conditions. Hence, only the presence of MIL-101 can remarkably enhance Δr, which

is mainly attributed to the slower rotation of fluorophore upon the formation of the complex of P-MIL-101. It confirms that the designed MIL-101 amplified FA strategy can be successfully applied for quantitative detection of HIV-1 DNA.

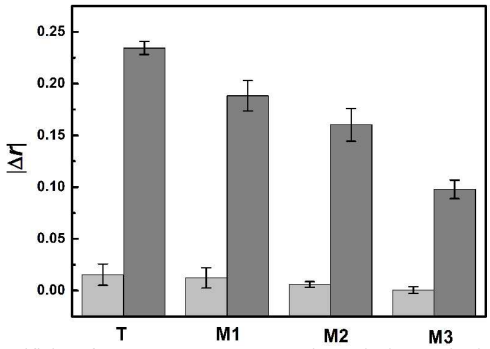


Fig. 3 Specificity of target DNA assay over mismatched DNA in the absence (light gray) and presence (dark gray) of 45 μg/ml MIL-101. Concentrations: P, 20 nM; T, M1, M2 and M3 are all 10 nM; pH, 7.2; λex: 480 nm.

To evaluate the feasibility and sequence specificity, control experiments were carried out by comparing $|\Delta r|$ in the presence of complementary DNA (T), single-base-mismatched DNA (M1), two-base-mismatched DNA (M2) and three-base-mismatched DNA (M3), respectively. As shown in Fig. 3, in the absence of MIL-101, all of the $|\Delta r|$ values of T, M1, M2 and M3 are very small, with negligible difference among them. This can be attributed to the slight molecule masses changes before and after hybridization.[11, 12] However, with the employment of MIL-101, the addition of T leads to significant $|\Delta r|$ increasement, while the mismatched targets M1, M2 and M3 gives relatively lower $|\Delta r|$, exhibiting an excellent specificity to T. The increasement of $|\Delta r|$ is due to the FA amplification effect of MIL-101. In the presence of MIL-101, the formed P-MIL-101 remarkably inhibited the rotation of fluorophore and lead to a high FA background. Furthermore, MIL-101 exhibits different affinity toward ssDNA and dsDNA, so T, M1, M2 and M3 produced distinguishable FA values based on their different hybridization efficiency with P. Therefore, the proposed method using MIL-101 as a FA amplification platform provides high sensitivity and selectivity for DNA detection.

In conclusion, MIL-101 was first utilized as FA amplifier and successfully applied for sensitive detection of DNA based on the different affinities of the ssDNA and dsDNA toward MIL-101. This strategy has several advantages. Firstly, only one end of the probe needs to be fluorescently labelled, without extra covalent linkage with the amplifier, which is simple and cost-effective compared with other strategies that employed nanoparticle as amplifier. Secondly, MIL-101 holds large molecular mass and volume, so the rotation of fluorophore is dramatically inhibited, providing high anisotropy for assay of small molecules. Thirdly, this method can in principle be used to assay different analytes, such as metal ions, by introducing probes that selectively bind to the analytes. These features conduce to the simplicity, efficiency and universality of the platform and expand the applications of MOFs. Besides, it is noteworthy that the various crystal structures and surface morphology of MOFs may contribute to more surprising discoveries in FA or other fields.

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†Electronic Supplementary Information (ESI) available: Experimental details and additional eq1-5 and Fig.S1-S4. See DOI: 10.1039/b000000x/.

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