

Electronic Supplementary Information

for

Metal-organic framework MIL-101 enhanced fluorescence anisotropy for sensitive detection of DNA

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EXPERIMENTAL SECTION

1. Apparatus

An S-4800 scanning electron microscope (SEM) (Hitachi, Japan) was used to scan the SEM images. A XD-3 X-ray diffractometer with Cu K_{α} radiation ($\lambda = 1.5406 \text{ \AA}$) was used to collect powder X-ray diffraction (PXRD) patterns at a scan rate of 2.00 min^{-1} (Purkinje, China). Fluorescence anisotropy was measured with an F-2500 fluorescence spectrophotometer equipped with a polarization filter (Hitachi, Tokyo, Japan). Oakton pH 510 meter (Singapore) was employed to adjust the pH values. Vortex mixer QL-901 (Haimen, china) was employed to blend the solution. A constant-temperature water-base boiler (Jiangsu, China) was employed to control the hybridization temperature. Millipore water from Milli-Q filtration system (Millipore, USA) was employed to prepare all the solutions.

2. Materials

Cr (NO₃)₃ · 9H₂O (99%), fluorhydric acid (HF) (48%) and terephthalic acid (H₂BDC) (99%) were purchased from Aladdin chemistry Co., Ltd. (Shanghai, China). The human immunodeficiency virus (HIV)-1 U5 long terminal repeats (LTR) DNA and other ssDNA were synthesized and purified by Sangon Biotechnology Co. Ltd (shanghai, china), and all DNAs were used without further purification. The sequence of the probe DNA (P) was 5'-AGT CAG TGT GGA AAA TCT CTA GC-3', with 6-carboxyfluorescein (FAM)-labeled at 5'-terminal. The sequence of the complementary target DNA (T) was 5'- GCT AGA GAT TTT CCA CAC TGA

CT-3'. Mismatched oligomers were employed to confirm the specificity of our strategy, including, one-base-mismatched oligomer (M1), 5'- GCT AGA GAT TTT ACA CAC TGA CT-3'; two-base-mismatched oligomer (M2), 5'- GCT AGA GAT TTT ACA CAC TAA CT-3'; three-base-mismatched oligomer (M3), 5'- GCT ATA GAT TTT ACA CAC TAA CT-3'.

3. Preparation of MIL-101

MIL-101 was synthesized according to the published procedures with a few modifications¹. Briefly, Cr(NO₃)₃·9H₂O (2.00 g, 5.0 mM), HF (48 wt%, 5.0 mM), terephthalic acid (0.82 g, 5.0 mM) and 24 mL of deionized water were added into a hydrothermal bomb and then put in an autoclave held at 220°C for 8 h. Then, cooled it to room temperature and filtered the solution with a large pore fritted glass filter to remove the large amount of recrystallized terephthalic acid. After that, the MIL-101 powder was separated from the solution using a small pores filter and washed with deionized water and ethanol. Subsequently, soaked the MIL-101 in ethanol (95% ethanol with 5% water) at 80°C for 24 h and washed thoroughly with hot ethanol. Then, MIL-101 powder was refluxed for 24 h in 1 M NH₄F aqueous solution and washed with hot water. Finally, the obtained solid was dried overnight at 150°C under vacuum and kept in a desiccator for the following experiments. The SEM images and powder XRD spectra of the as-synthesized MIL-101 were showed in Fig. S1 and S2, which was consistent with reported in the literatures¹.

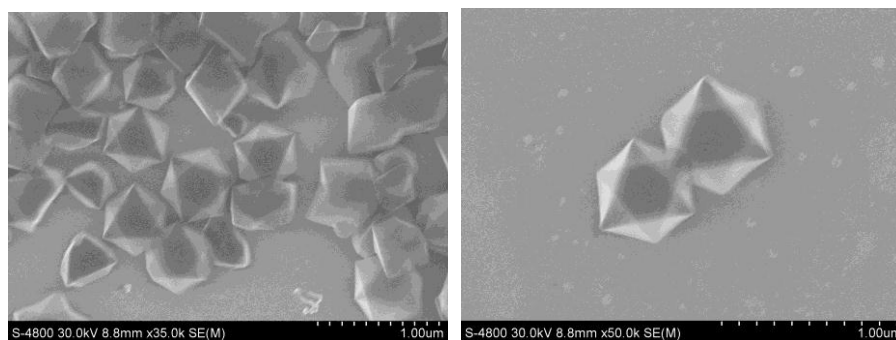


Fig. S1 The SEM images of as-synthesized MIL-101.

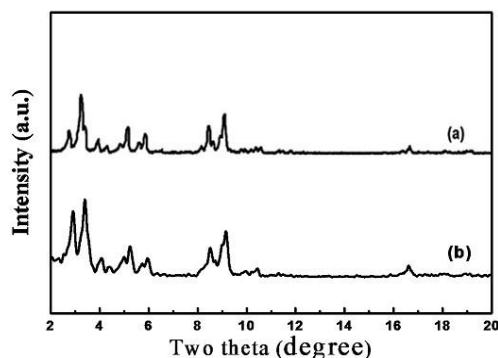


Fig. S2 The powder XRD spectra of MIL-101: (a) simulated, (b) the as-synthesized.

4. Fluorescence anisotropy measurements

Briefly, 50 μL of Tris-HCl buffer (pH 7.2), 25 μL of NaCl solution (3 M), 100 μL of FAM-labeled probe DNA (0.1 μM) and a certain volume of target DNA (0.1 μM) were sequentially added into a 1.5 mL tube, and kept at room temperature (25°C) for 60 min. Later on, 75 μL of MIL-101 solution (0.3 mg/mL) was added into the mixture and further diluted with ultrapure water to 500 μL . After 50 min incubation, fluorescence anisotropy measurements were carried out on the F-2500 fluorescence spectrophotometer with an excitation wavelength of 480 nm and emission intensity at 520 nm was recorded.

The anisotropy, r , of the test solution was calculated by

$$r = \frac{I_{VV} - G \times I_{VH}}{I_{VV} + 2G \times I_{VH}} \quad (1)$$

and

$$G = \frac{I_{HV}}{I_{HH}} \quad (2)$$

Where I represents the intensity of the fluorescence signal and the subscripts define the orientation H for horizontal and V for vertical of the excitation and emission polarizers, respectively. G is the grating factor of the fluorescence spectrophotometer, which is used to correct for the wavelength response to polarization of the emission optics and detectors².

It is known that the anisotropy value, r , is sensitive to the rotational motion changes of the fluorophore-linked object. It can be described by the Perrin equation³:

$$r = \frac{r_0}{1 + (\tau/\theta)} \quad (3)$$

$$\theta = \eta V / RT \quad (4)$$

So

$$\frac{1}{r} = \frac{1}{r_0} + \frac{\tau RT}{r_0 \eta V} \quad (5)$$

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 (molecular mass). Therefore, a smaller molecule rotates faster, exhibiting smaller FA value, while larger molecules have larger FA value due to their confined motion.

5. Gel electrophoresis

10 μL of different reaction products were mixed with 2 μL 6 \times loading buffer, and then dropped into 2% agarose gel containing an appropriate amount of Gold View dye. The gel electrophoresis was run at 100 V for about 50 min in 1 \times TBE buffer, and finally photographed with a digital camera under the irradiation of the UV lamp (365 nm)⁴.

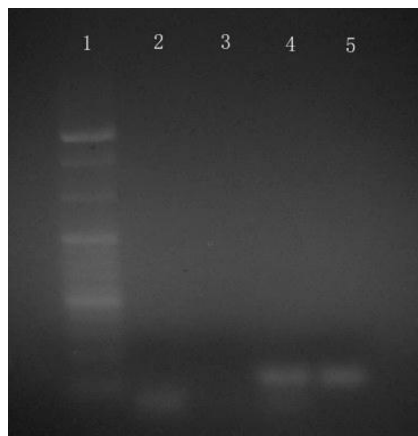
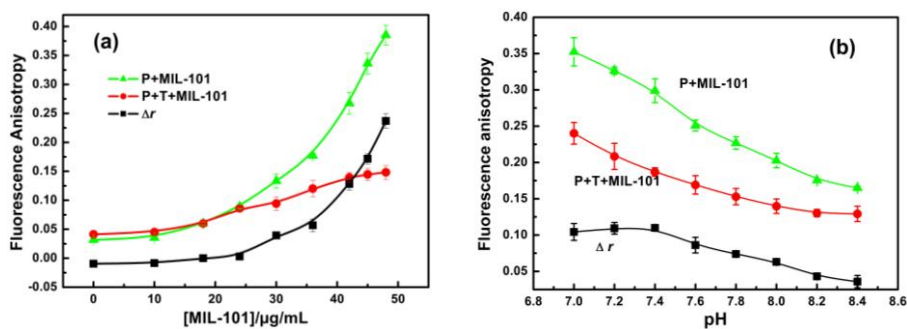


Fig. S3. Gel electrophoresis of the FAM-labeled probe DNA (P) and its binding complexes with MIL-101 and T. From left to right: Lane 1: marker; lane 2: 1.0 μM P; lane 3: P was treated with MIL-101; lane 4: P was hybridized with 0.5 mM T; lane 5: P was hybridized with 0.5 mM T and subsequently treated with MIL-101; pH: 7.2.

6. Optimum conditions for the detection

As shown in Fig. S4(a), with increasing amount of MIL-101, both the FA values of P and P/T were increased. In this situation, 45 $\mu\text{g}/\text{mL}$ of MIL-101 was employed since Δr reached maximum while all of the FA values were less than 0.4. The FA values increased gradually with pH decreasing (b) and the Δr reached a platform at pH 7.2, so Tris-HCl buffer pH 7.2 was chosen for the following experiments. The Δr reached a platform when the incubation time of P react with MIL-101 was 50 min (c) and the concentration of NaCl was 150 mM (d), respectively. So, the incubation time of 50 min and 150 mM NaCl was selected as the optimal conditions.



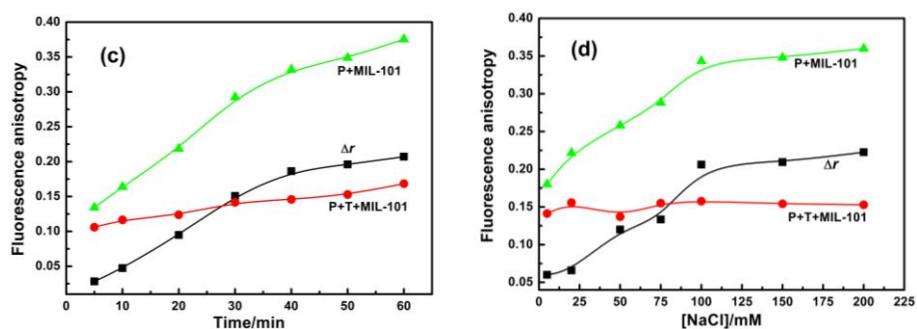


Fig. S4 Dependence of the fluorescence anisotropy on (a) concentrations of MIL-101; (b) pH; (c) incubation time of P react with MIL-101 and (d) concentrations of NaCl in the absence (green line) and presence (red line) of 8 nM T, the dark line: Δr .

(a) Experiments were carried in the presence of 20 nM P and 150 mM NaCl at pH 7.2 for 50 min in different amount MIL-101. (b) 45 $\mu\text{g}/\text{mL}$ of MIL-101, 20 nM P and 150 mM NaCl in different pH for 50 min. (c) 45 $\mu\text{g}/\text{mL}$ of MIL-101, 20 nM P and 150 mM NaCl at pH 7.2 for different incubation time. (d) Various concentrations of NaCl with 45 $\mu\text{g}/\text{mL}$ of MIL-101 and 20 nM P at pH 7.2 for 50min.

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