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# A novel aggregation-induced emission fluorescent probe for nucleic acid detection and its applications in cell imaging

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ABSTRACT

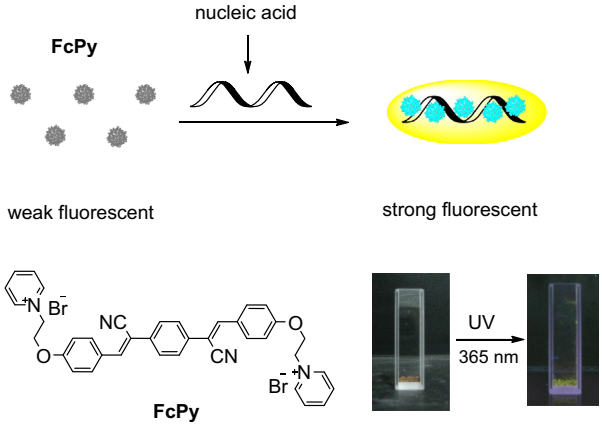
A new kind of aggregation-induced emission compound was synthesized and used as the probe of nucleic acid. The characterization of this compound was studied. Both the RNA and DNA were detected by using this probe. And the detection scope of DNA and RNA was different. We researched the selectivity of our probe in double and single strand DNA sequences. The visualization of gel electrophoresis and the cell nucleus imaging were researched as well. Compared with the traditional nucleus dye Hoechst 33258, our probe also has the potential to be nucleus dye. And the cell toxicity was well performed by MTT assays.



It is well known that the detection of nucleic acid is very important in genetic engineering, forensics, and bioinformatics.[1–3] Therefore, many nucleic acid probes have been designed and synthesized based on the structures of nucleic acids. It has been reported that ethidium bromide (EB), Hoechst dyes, acridizinium salts, cyanine derivatives, and ruthenium complexes which based on fluorescent enhancement have been developed as nucleic acid probes.[4–15] There into, EB is cheap and widely used in molecular biology laboratories as a nucleic acid stain. However, EB is thought to be a strong mutagen or carcinogen. Currently, some alternatives to EB such as SYBR-based dyes are found to be less carcinogen.[14] So it is desirable to develop more fluorescent probes which could be safer for detection of nucleic acid in aqueous solution.

Some fluorescent dyes can aggregate in aqueous buffer or be bound to biomacromolecules.[16–18] The fluorescent signals fade when the dyes aggregate. This aggregation-caused quenching (ACQ) effect is a major problem in the development of bioprobes and biosensors.[19–21]

Herein we designed and synthesized a derivative of *p*-phenylenediacetonitrile for the detection of nucleic acid. And we named the compound as **FcPy**. **FcPy** has strong fluorescence emission under UV light ($\lambda_{ex}$ = 365 nm) (Scheme 1). This phenomenon of aggregation-induced emission (AIE) that the fluorescence enhancement attributed not only to the spatial confinement effect but also to the formation of specific supramolecular stacking architecture is the opposite of ACQ effect and has been widely studied recently.[1–3,22] We studied the AIE characteristic of **FcPy** by using the traditional methods (Fig. S8).




**Scheme 1.** Schematic illustration of the fluorescence 'Turn-On' system for detection of nucleic acid with **FcPy**.

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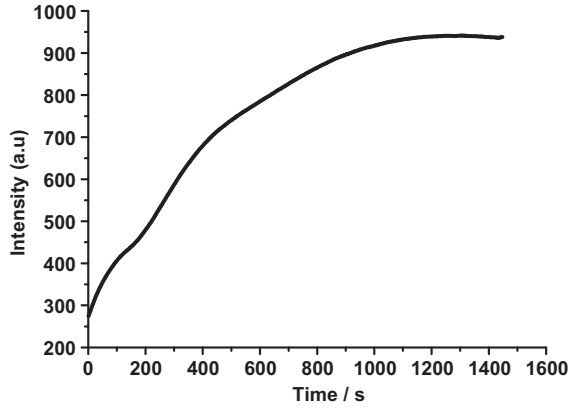


**Figure 1.** Real-time emission intensity changes of **FcPy** (100 μM) at 505 nm upon single-strand DNA (1 μM, 75 nt). $\lambda_{ex}$ = 396 nm.

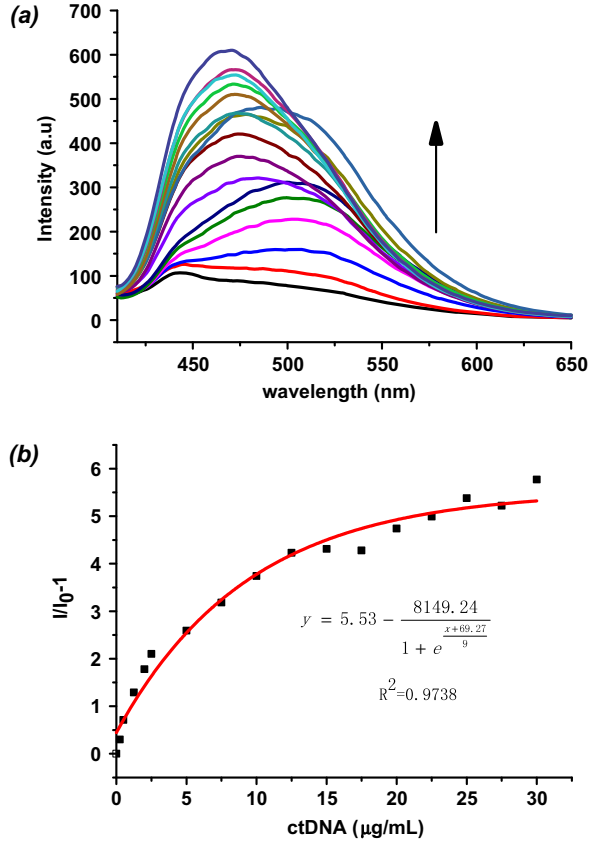


**Figure 2.** (a) Changes in the emission spectrum of **FcPy** (5 μM) upon the different concentrations (0–30 μg) of ctDNA in PBS. $\lambda_{ex}$ = 396 nm, $\lambda_{em}$ = 420–600 nm. (b) Plot of $I/I_0 - 1$ at 475 nm versus the ctDNA concentration. $I_0$ = emission intensity in the absence of ctDNA.

Firstly, we investigated the interaction of **FcPy** with nucleic acid and single strand DNA sequences were chosen as the model. We found that the fluorescence emission was brighter with increasing length of the DNA sequences (Fig. S1). This was consistent with AIE effect. As **FcPy** can be cations in aqueous media and DNA sequences have negative charges, the longer sequences have more negative charges to be adhered with more **FcPy**.

Then we attempted to explore whether **FcPy** has an ability of selectivity in the detection of double strand DNA and single strand DNA. We tested the fluorescence emission of a single strand DNA and its double strand DNA (Fig. S2) after interaction with **FcPy**.

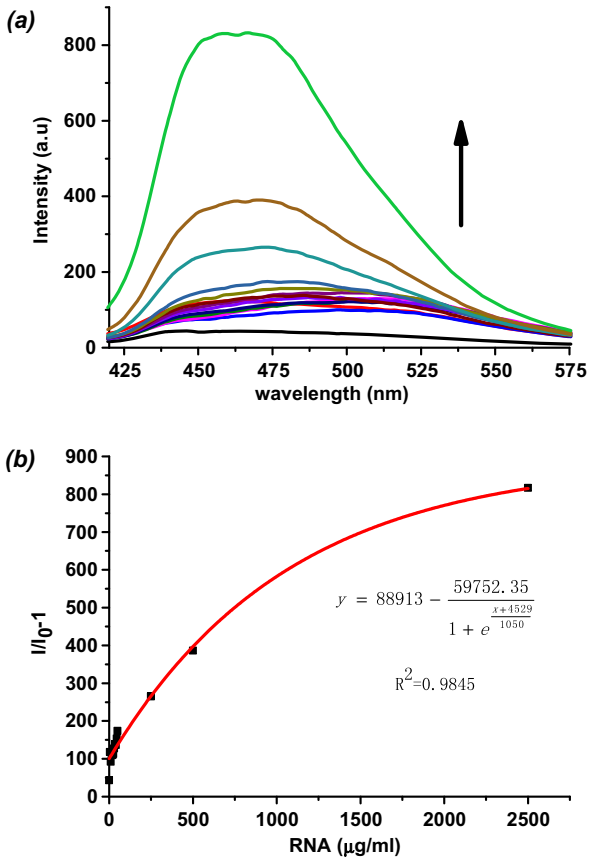


**Figure 3.** (a) Changes in the emission spectrum of **FcPy** (5 μM) upon the different concentrations (0–2.5 mg) of RNA in PBS. $\lambda_{ex}$ = 396 nm, $\lambda_{em}$ = 420–575 nm. (b) Plot of $I/I_0 - 1$ at 470 nm versus the RNA concentration. $I_0$ = emission intensity in the absence of RNA.

The results indicated that **FcPy** had almost no selectivity between single and double strand DNA as the intensity of double strand DNA was almost double of single strand DNA when interacted with **FcPy**. Next, we used S1 nuclease to digest the DNA sequence and recorded the fluorescence emission. From Figure S4 we can see that the fluorescence intensity was nearly the same as control after digesting with S1 nuclease. This is because that when the DNA sequence is digested into nucleotides, the aggregated fluorescent probes adhered to DNA sequence is also dispersed into the aqueous solution. This also proved that **FcPy** has the property of AIE effect.

In order to study the kinetics of FcPy and nucleic acid interaction, we recorded the real-time emission intensity of **FcPy** (100 μM) at 505 nm upon single-strand DNA (1 μM, 75 nt) with excitation at 396 nm. The fluorescence intensity went to plateau at about 20 min (Fig. 1).

According to the results mentioned above, we tried to detect natural DNA and RNA with **FcPy**. We chose ctDNA and RNA from torula yeast as the target nucleic acid since they are cheap and easy to obtain. **FcPy** was dissolved in phosphate-buffered saline (PBS) solution (10 mM, pH 7.4), and the concentration of **FcPy** was 10 mM. The fluorescence intensity recorded at 475 nm is enhanced quickly when the concentration of ctDNA is low, but it is gradually saturated when the concentration of ctDNA becomes higher (Fig. 2a). There is a gradual blueshift of the emission maximum from 505 to 475 nm, this was elucidated by Park and coworkers.[22] This is because that there two forms of π–π overlap of the molecule in different situations. The change of $(I/I_0 - 1)$ versus ctDNA concentration (0–30 μg/mL) was fitted well to the Boltzmann function with an $R^2$ value of 0.9738 as shown in Figure 2b. As the same as

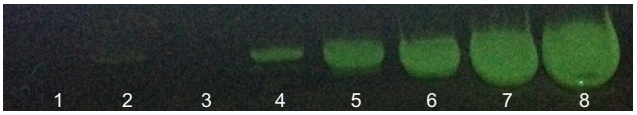


**Figure 4.** Staining of single-strand DNA in PAGE by **FcPy**. Concentrations of DNA (75 nt) in lanes 1–8: 0, 0.25, 0.5, 1.0, 10.0, 25.0 and 50.0 μg (from left to right). Concentration of dyes: 100 μM. Staining time: 20 min.

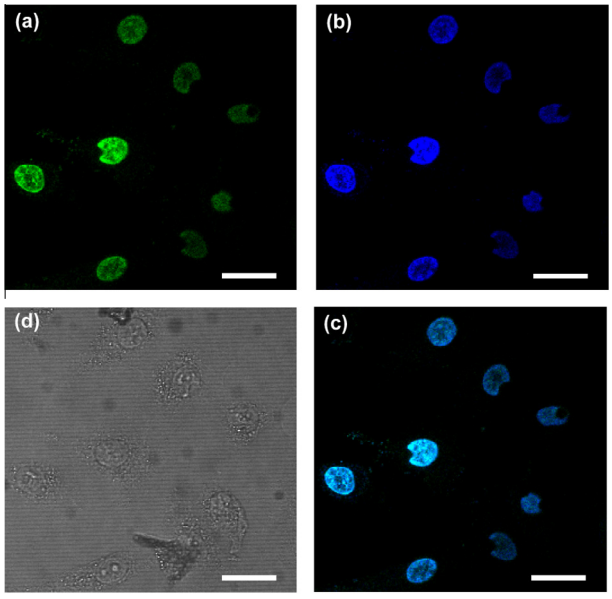


**Figure 5.** Fluorescence images of HeLa cells stained with **FcPy** (a) (10 μM) ($\lambda_{ex}$ = 488 nm) and Hoechst 33258 (b) (5 ng/μL, 10 μM) ($\lambda_{ex}$ = 408 nm). (c) Merged image of (a) and (b). (d) Bright field. Scale bars: 20 μm.

ctDNA, the fluorescence process of **FcPy** could also be trigged by RNA. There was also a gradual blueshift from 505 to 475 nm at the maximum emission (Fig. 3a). However the fluorescence intensity recorded at 475 nm is slightly enhanced when the concentration of RNA is low. But the fluorescence intensity could increase rapidly with higher concentration of RNA. So **FcPy** could detect a wider range of RNA concentration than that of DNA. Even though, we supposed that the fluorescence intensity would still be saturated when the concentration of RNA was too high. So we still fitted the change of $(I/I_0 - 1)$ versus RNA concentration (0–2.5 mg/mL) with Boltzmann function (Fig. 3b). The $R^2$ value is 0.9845, and this is consistent to our hypothesis.

Then we tried to use **FcPy** as the nucleic acid stain for gel electrophoresis. Figure 4 shows the gel image of electrophoresed oligonucleotides after staining with **FcPy** solution for 20 min. The DNA band becomes visible under UV illumination. The colour is green so the maximum emission wavelength of **FcPy** is a relatively long wavelength. The detection limit of **FcPy** in gel staining could be as low as 1.0 μg (lane 4, Fig. 4).

At last, we employed **FcPy** in cell imaging. Before we performed **FcPy** in cells, we tested the cytotoxicity of **FcPy**. Living HeLa cells were incubated with various concentrations of **FcPy** for 48 h, after which the percentages of the viable cells were quantified. We calculated out the $IC_{50}$ of **FcPy** as 26.0 μM (Fig. S5). From Figure 5, the fluorescence image of HeLa cells stained with **FcPy** was significantly bright. Then we stained the cells with Hoechst 33258 which is commercial available, the merged image (Fig. 5c) shows that **FcPy** could successfully stained the nucleus of cell as well as Hoechst 33258.

In summary, we have successfully developed a new kind of probe for the detection of nucleic acid. It could detect double strand, single strand DNA and RNA. And it also can quantitatively analyze the nucleic acids in solution and visualize the DNA bind in gels. With the results of the cell imaging, **FcPy** might be used as the potential nucleus dye.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.071.

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