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A novel bispyrene compound was synthesized to selectively detect RNA through excimer emission “turn-on” in aqueous solution at physiological pH (7.4). The compound was used to successfully image RNA in HeLa cells.

Since their discovery in 1868,1 ribonucleic acids (RNAs) have attracted great interest due to their critical roles in biological processes. RNA is known to perform active roles in the coding, regulation, and expression of genes, among other functions.2 Consequently, the development of RNA detection and recognition technology is of great importance. Although there have been many reports regarding fluorescent detection of DNA,3 G-Quadruplex DNA,4 and nucleotides5-6, relatively few examples can be found of RNA detection7-9. These probes for RNA detection often require complicated synthetic steps to link the oligonucleotides to fluorophores. In addition, the selectivity for RNA over other nucleic acids is often insufficient. Recently, Chang et al.3 and Kim et al.8 reported the development of RNA-selective probes based on small positively charged molecules. Chang and co-workers reported two small molecules for RNA detection; however, relatively small differences in affinity were observed between RNA and DNA.7 On the other hand, Kim et al. recently reported small cyclophane-containing imidazolium moieties which can selectively detect RNA over DNA in living cells.8 As delineated above, selective fluorescent detection of RNA is still a challenging task. Here we report a neutral bispyrene probe with high selectivity and sensitivity for RNA in aqueous solution at physiological pH, 7.4, which can be used for fluorescence imaging of RNA in live cells.

For the synthesis of probe 1, 1-hydroxyxyprene-2-carbaldehyde (compound 2) was first prepared from 1-bromopyrene in 3 steps following a previously reported procedure.9 Compound 2 was then reacted with diethylenetriamine to give 1 as a red solid in 80% yield (Scheme 1). Probe 1 was fully characterized by 1H NMR, 13C NMR and HRMS (FAB).

Fluorescence properties were examined in 99.8% Tris-HCl buffer (and 0.2% DMSO) at pH 7.4. The emission spectra are presented in Fig. 1. Upon the addition of 1.75 eq. of ssRNA, ctDNA, UTP, TTP, poly (dA-dT) or poly (dG-dC) to probe 1, no change, including any quenching effect, was observed in the emission of probe 1. On the other hand, when 1.75 eq. of total RNA was added, a selective fluorescence enhancement was observed at 470 nm, the excimer emission of pyrene fluorophores10. Fig. 2 presents the fluorescence titrations of probe 1 with total RNA. As shown in Fig. 2(b), the peak at 470 nm arose from excimer emission of pyrene enhanced by increasing amounts of RNA, which may indicate that the two pyrenes in probe 1 are mostly π-stacked in RNA. Total cellular RNA consists of ssRNA, tRNA, rRNA and microRNA. As shown in Fig. 1, excimer peak enhancement was not observed for ssRNA or tRNA, unlike with total RNA. Thus, one can propose that rRNA, accounting for ~80% of total RNA, induces the excimer peak enhancement. Fig. 2 presents the 1H NMR spectra of probe 1 with total RNA. The aromatic protons of the pyrene moieties were assigned based on TOCSY and ROESY 2D NMR experiments as shown in Fig. S4. After adding 1 equiv. of total RNA, aliphatic protons

**Scheme 1.** Synthesis of fluorescent probe 1.

![Scheme 1](http://example.com/scheme1.png)

**Fig. 1.** (a) Fluorescence changes of 1 (20 µM) upon the addition of RNA, tRNA, ssRNA, ctDNA, UTP, TTP, poly (dA-dT), poly (dG-dC) (1.75 equiv. each) in 0.05 M Tris-HCl (pH 7.4). (b) Fluorescence titrations of 1 (20 µM) with total RNA (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 equiv.) (Δλx = 348 nm).

**Fig. 2.** (a) Fluorescence intensity spectra of probe 1 with total RNA.

![Fig. 2](http://example.com/fig2.png)
at 3.79 and 3.04 showed small downfield shifts to 3.83 and 3.10, respectively, (Fig. 2). In addition, the H10 resonances of pyrene ring (doublet, 8.29 and 8.32) slightly shifted upfield to 8.29 and 8.32 ppm (Fig. 2). However, the other aromatic protons showed no significant chemical shift changes (Fig. 2). These results can be attributed to the possible hydrogen bonding interaction between the OH group and phosphate backbone of RNA. When the unique conformation of rRNA induces π–π stacking interaction between two pyrenes in probe 1 through intermolecular hydrogen bonding interaction, the probe 1 exhibits fluorescence emission.

**Fig 2.** $^1$H NMR titration of 1 (1 mM) in DMSO-$_d_6$ with 0.0, 0.5, 1.0 equiv. RNA (from bottom to up). RNA was dissolved in D$_2$O.

To test the RNA imaging properties of probe 1, HeLa cells (human epithelial adenocarcinoma) were incubated with probe 1 (20 µM in 0.2% DMSO) in the culture media at 37 °C for 1 h. In the absence of probe 1, the cells exhibited no fluorescence, but the cells incubated with probe 1 exhibited strong fluorescence (Fig. 3). To determine whether probe 1 binds to DNA or RNA, the cells were fixed and treated with 1% FBS and 10% FBS displayed strong fluorescence (Fig. 4). In addition, these data confirm that the live cell membrane is permeable to probe 1.

**Fig 3.** Fluorescence images of probe 1 in HeLa cell. Cells were fixed with 4 % paraformaldehyde and treated with RNase or DNase in the 40 mM Tris-HCl, pH 7.5, 5 mM MgCl$_2$ buffer for 30 min at 37 °C. And the cells were incubated in the absence or presence of 20 µM JEJ-1 for 1 h at 37 °C. DRAQ5 was used as DNA stain. (a) 1 only (b) DRAQ5 (c) merge (d) DIC. Scale bars: 10 µm.

DNase or RNase. When the cells were treated with RNase, no fluorescence was observed, however, cells treated with DNase exhibited fluorescence, as shown in Fig. 3. DRAQ5 stain was used as a DNA staining agent.

Serum (FBS, fetal bovine serum) in the culture media is essential to cellular growth and RNA synthesis. Cells were cultured with 1% FBS in RPMI-1640 media for 24 h, and then a subset thereof were cultured with 10% FBS in RPMI-1640 media for an additional 8 h. After 1 h incubation with 1 and 10 min incubation with DRAQ5, the cell images were captured using a confocal microscopy. (a) 1 only (b) DRAQ5 (c) merge (d) DIC. Scale bars: 10 µm.

**Fig 4.** Fluorescence intensities of probe 1 according to different serum concentrations. The HeLa cells were cultured in 1 % fetal bovine serum, RPMI 1640 media for 24 h and then exchanged 10 % fetal bovine serum, RPMI 1640 media for 8 h. After 1 h incubation with 1 and 10 min incubation with DRAQ5, the cell images were captured using a confocal microscopy. (a) 1 only (b) DRAQ5 (c) merge (d) DIC. Scale bars: 10 µm.

The potential cytotoxic effects of probe 1 were also examined in HeLa cells (Fig. S6). Cells were incubated with each concentration of probe 1 for 4 h and 24 h. Cell death was not detected after 4 h of incubation, but 83% cell death occurred after 24 h incubation with 50 µM probe 1. This result can be attributed to the binding of RNA by probe 1 causing...
inhibition of RNA translation or degradation.

In conclusion, we have shown that a relatively simple bispyrene derivative, probe 1, can serve as a fluorescent probe for selectively detecting RNA within living cells. This probe is suited for biological conditions and is designed to specifically target RNA out of many other biological molecules. Cell imaging of RNA was also successfully performed in HeLa cells. The molecular interaction of probe 1 with specific RNA sequences will be explored in the future.

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Notes and references

A Bispyrene Derivative as a Selective Fluorescent Probe for RNA

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A novel bispyrene compound was synthesized to selectively detect RNA through excimer emission “turn-on” at physiological pH.