Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information for

Deep-red fluorogenic cyanine dyes carrying amino group-terminated side chain for the improved RNA detection and nucleolar RNA imaging

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Reagents: Calf thymus DNA and *E. coli* total RNA were purchased from Sigma-Aldrich (Darmstadt, Germany) and Thermo Fisher Scientific (Tokyo, Japan), respectively. The other reagents were commercially available and of analytical grade. NMR spectra were recorded on a Bruker Avance III 500 spectrometer. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-T100CS or JMS-T100GCV instrument. The concentrations of calf thymus DNA and *E. coli* total RNA were determined from the absorbance at 260 nm at 25°C using a molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ and 9250 $M^{-1}cm^{-1}$ (M = mole/L per nucleotide), respectively.(S1) Water was deionized (≥18.0 MΩ cm specific resistance) by an Elix 5 UV water purification system and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA, USA), followed by filtration through a BioPak filter (Millipore Corp., Bedford, MA, USA) in order to remove RNase.

Unless otherwise mentioned, BIQ derivatives were dissolved in DMSO to obtain the stock solutions. The stock solutions were kept at 4°C in the dark before their use. Final DMSO concentration in sample solutions containing the probes was below 0.5 % (v/v).

UV-visible and fluorescence spectra measurements. Absorption and fluorescence spectra were measured using a JASCO model V-570 UV–vis spectrophotometer and FP-6500 spectrofluorophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan), respectively. Both instruments were equipped with thermoelectrically temperature-controlled cell holders. Measurements of absorption and fluorescence spectra were done using a 2 × 10 mm quartz cuvette (optical path length: 10 mm) and a 3 × 3 mm quartz cuvette, respectively. Fluorescence quantum yield (ϕ) was determined relative to cresyl violet (Sigma-Aldrich) in MeOH.(S2)

Fluorescence imaging in live cells. MCF-7 cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin at 37°C in a 5% CO_2 incubator. For the fluorescence imaging experiments, MCF-7 cells were seeded in a 8-chambered glass plate (Iwaki, Tokyo, Japan) at a density of about 5.0×10^3 cells/well and maintained for 24 h. The cells were incubated in media containing 5 μM BIQ derivatives for 20 min at 37˚C in a 5% CO_2 incubator. After washing with HBSS buffer twice, the cells were imaged in a HBSS buffer using a Deltavision Elite microscopy system (GE Healthcare Japan, Tokyo, Japan). In the co-staining experiments with Hoechst 33342, the cells stained with BIQ derivatives were incubated in media containing 100 nM Hoechst 33342 for 20 min according to the manufacturer's protocol. The following filter sets were used: DAPI filter set (Ex 390/18; Em 435/48) for Hoechst 33342; Cy5 filter set (Ex 632/22; Em 679/34) for BIQ derivatives. The obtained images were processed with the softWoRx software.

Fluorescence imaging in the fixed and permeabilized cells. All measurements were done according to our previous report (Ref. 4 in the main text).

Synthesis of BIQ-N^+Me_3 and BIQ-NH_2:

4-methyl-1-(3-(trimethylammonio)propyl)quinolin-1-ium (2):[S3] 4-Methylquinoline (0.74 mL, 5.6 mmol) and (3-bromopropyl)trimethylammonium bromide (0.78 g, 30 mmol) were dissolved in CH_3CN (10 mL). The reaction mixture was stirred at 85°C for 24 h. After cooling to room temperature, the solvent was removed. This affords the crude **2**, verified by HRMS (ESI).

BIQ-N^+Me_3: The crude compound **2** (0.16 g, 0.61 mmol) and the crude **3** (0.20 g, 1-methylbenzo[c,d]indol-2(1H)-thione) (Ref. 4 in the main text) were dissolved in CH_3CN (12 mL) with TEA (0.5 mL). The mixture was refluxed for 1 h. After cooling to room temperature, diethyl ether was added. The resultant precipitate was collected by filtration. After the obtained solid was dissolved in methanol, the addition of diethyl ether yielded a blue viscous solid. This solid was suspended in a 1:2 (v/v) mixture of EtOH:diethylether (7.5 mL) and stirred at room temperature for 1 h. The solid was collected by filtration and washed with diethylether. This gave BIQ-N^+Me_3 as a blue solid (0.03 g, 0.061 mmol, 10%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.88 (d, J = 6.1 Hz, 1H), 8.80 (d, J = 7.9 Hz, 1H), 8.61 (d, J = 24.4 Hz, 1H), 8.44 (d, J = 8.8 Hz, 1H), 8.16-8.21 (m, 1H), 8.09 (d, J = 7.6 Hz, 1H), 7.92 (t, J = 7.8 Hz, 1H), 7.64 (d, J = 16.5 Hz, 1H), 7.57 (s, 2H), 7.29 (s, 1H), 4.82 (d, J = 24.4 Hz, 2H), 3.69-3.80 (m, 2H), 3.56-3.59 (m, 2H), 3.11 (s, 9H), 2.86 (s, 3H); HRMS (FD) for $C_{28}H_{31}N_3Br^+$: calcd, 488.1696; found, 488.1685.

1-(3-((ethoxycarbonyl)amino)propyl)-4-methylquinolin-1-ium (4): This compound was synthesized according to the literature.[S4]

(E)-2-((1-(3-((ethoxycarbonyl)amino)propyl)quinolin-4(1H)-ylidene)methyl)-1-methylbenzo[cd]indol-1-ium (5): Compound **4** (50 mg, 0.13 mmol), the crude **3** (45 mg) and TEA (0.13 mL) were dissolved in CH_3CN (3 mL). The mixture was refluxed for 1 h. After cooling to room temperature, the solvent was evaporated. The residue was purified by silica chromatography (solvent: $CHCl_3$/MeOH =1:1 (v/v)) to give **5** as a blue solid (30 mg, 0.055 mmol).

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.77 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 8.8 Hz, 1H), 8.14 (t, J = 7.8 Hz, 1H), 7.90 (t, J = 7.8 Hz, 1H), 7.55 (d, J = 5.8 Hz, 2H), 7.25 (d, J = 7.6 Hz, 1H), 7.07-7.09 (m, 1H), 4.81 (t, J = 7.5 Hz, 2H), 3.50 (s, 1H), 3.11 (d, J = 6.7 Hz, 2H), 2.36 (s, 1H); HRMS (ESI) for $C_{30}H_{32}N_3O_2$: calcd, 466.2489; found, 466.2867.

BIQ-NH$_2$: The compound **5** was dissolved in 1,4-dioxane (3.0 mL) and HCl (4M, 1.5 mL), and stirred at room temperature for 1 h. After the solvent was evaporated, the solid was dissolved in $CHCl_3$ (4 mL). The addition of diethylether yielded the precipitate. The obtained solid was washed with diethylether and dried under reduced pressure to give BIQ-NH$_2$ as a blue solid (0.67 mg, 0.0015 mmol, 3.6%)

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.78 (d, J = 8.2 Hz, 1H), 8.45 (d, J = 8.5 Hz, 1H), 8.16 (t, J = 7.8 Hz, 4H), 8.08 (d, J = 7.6 Hz, 1H), 7.91 (t, J = 7.8 Hz, 1H), 7.56 (s, 2H), 7.26 (d, J = 6.4 Hz, 1H), 4.93 (t, J = 7.6 Hz, 2H), 3.50 (s, 2H), 2.24-2.30 (m, 2H), 1.23 (s, 3H); HRMS (ESI) for $C_{25}H_{24}N_3$ ($[M]^+$): calcd, 366.1965; found, 366.2221.

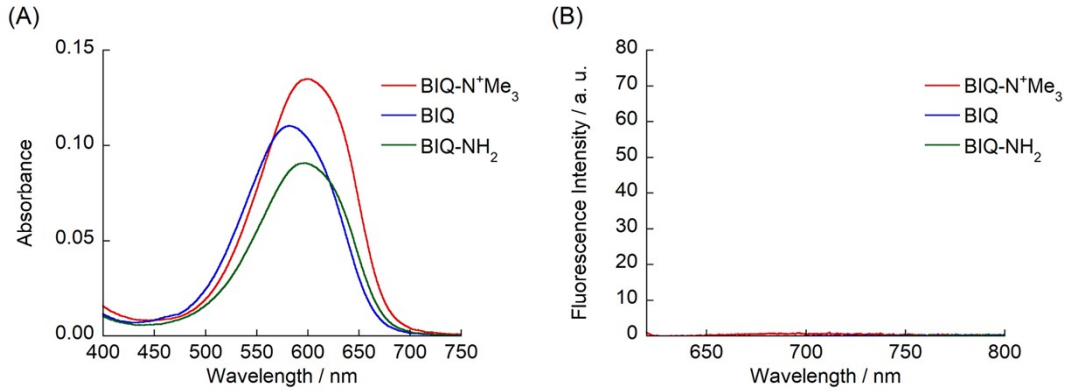
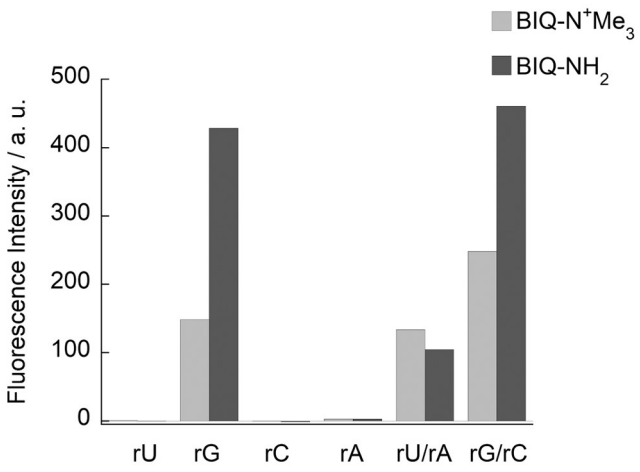


Fig. S1 (A) Absorption and (B) fluorescence spectra of the probes (5.0 μM). Other solution conditions were the same as those given in Fig. 2 in the main text. As for the fluorescence spectra, excitation wavelength for BIQ-N^+Me_3, BIQ-NH_2 and BIQ was set at 597 nm, 607 nm, and 595 nm, respectively. Temperature, 25°C.



rU: 5’ -r(UUU UUU UUU UUU U)-3’
rG: 5’ -r(GGG GGG GGG GGG G)-3’
rC: 5’ -r(CCC CCC CCC CCC C)-3’
rA: 5’ -r(AAA AAA AAA AAA A)-3’

Fig. S2 Fluorescence response of the probes (5.0 μM: BIQ-N^+Me_3 and BIQ-NH_2) for synthetic nucleic acids (20 μM). Excitation: BIQ-N^+Me_3; 597 nm, BIQ-NH_2; 607 nm. Analysis: BIQ-N^+Me_3; 665.5 nm, BIQ-NH_2; 665 nm. RNA sequences used here were also shown.

We observed much larger light-up response of the probes for double-stranded rU/rA and rG/rC compared to single-stranded rU, rC and rA. It is thus highly likely that these probes bind to RNAs through intercalation into the base pairs of RNAs, as observed for the parent BIQ (ref. 4 in the main text). On the other hand, the strong response for single-stranded rG was observed for both probes. This would be due to the binding of G-quadruplex structure that rG can potentially adopt (ref. 4 in the main text).

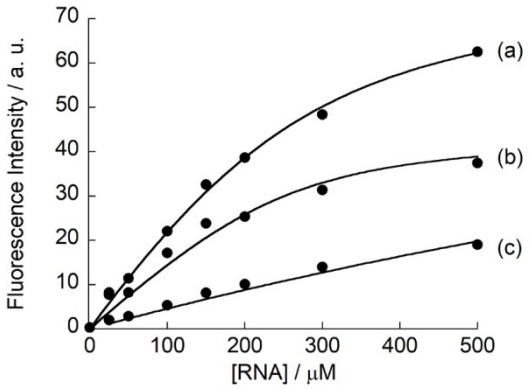


Fig. S3 Fluorescence titration curves for the binding of the probes ((a) BIQ-N^+Me_3, (b) BIQ-NH_2 and (c) BIQ) for *E. coli* total RNA. [probe] = 5.0 μM. [RNA] = 0-500 μM. Other solution conditions were the same as those given in Fig. 2 in the main text. Excitation: (a) 597 nm, (b) 607 nm, (c) 595 nm. Analysis: (a) 665.5 nm, (b) 665 nm, (c) 645 nm. Temperature, 25°C. The apparent dissociation constants (K_d) was determined by the analysis of the titration curve based on a 1:1 binding model.(S5)

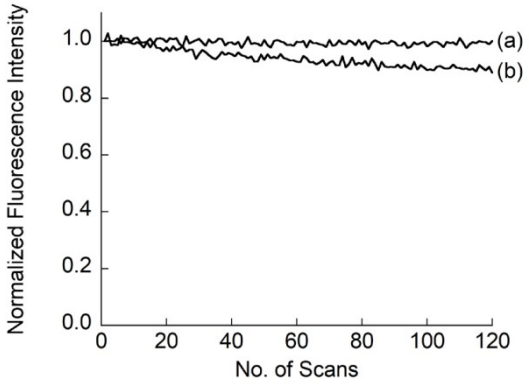


Fig. S4 Decrease in the fluorescence intensity of the probes (5.0 μM: (a) BIQ-NH_2 and (b) BIQ-N^+Me_3) in the bound state with E. coli total RNA (500 μM) under continuous irradiation for 120 scans. Other solution conditions were the same as those given in Fig. 2 in the main text. Excitation: (a) 607 nm, (b) 597 nm. Analysis: (a) 665 nm, (b) 666.5 nm. Temperature, 25°C.

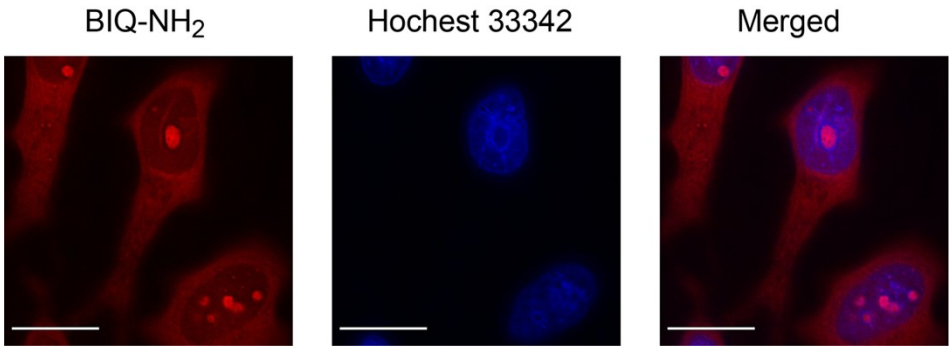


Fig. S5 Fluorescence images of living MCF7 cells stained by BIQ-NH_2 (5.0 μM) with nuclear counterstaining Hoechst 33342 (100 nM). Scale bar: 15 μm.

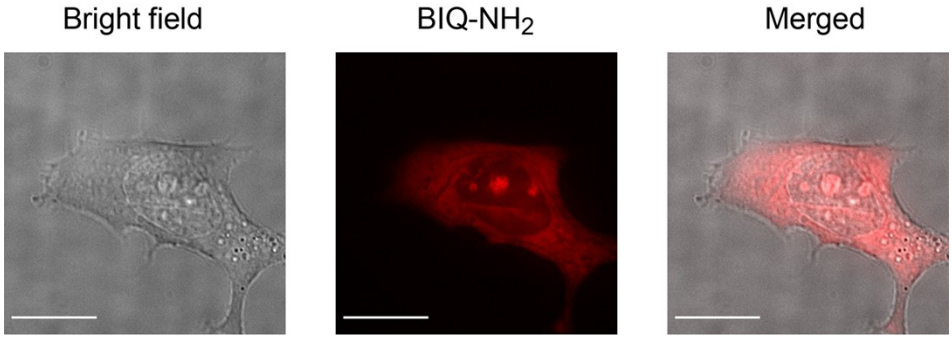


Fig. S6 Images of living MCF7 cells incubated with 5.0 μM BIQ-NH_2 for 24h. Scale bar: 15 μm.

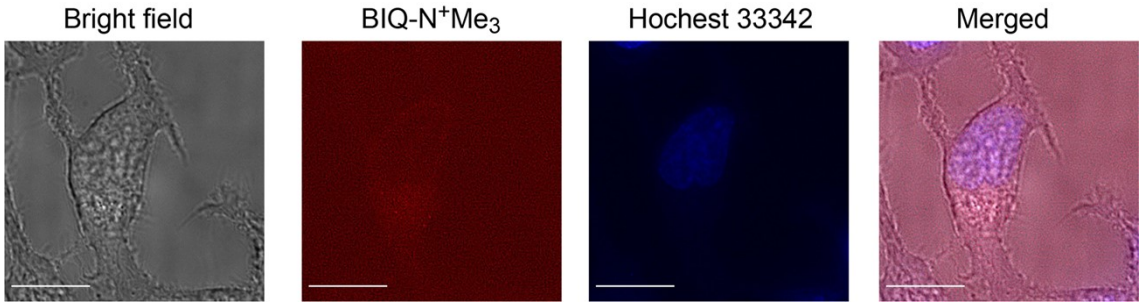


Fig. S7 Images of living MCF7 cells stained by BIQ-N^+Me_3 (5.0 μM), with costaining of nuclear with Hoechst 33342 (100 nM). Scale bar: 15 μm.

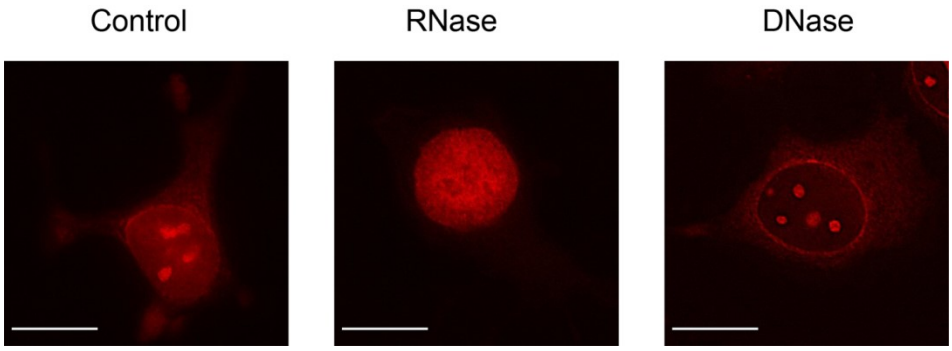


Fig. S8 Fluorescence images of fixed-permeablized MCF7 cells stained by BIQ-N^+Me_3 (5.0 μM) before (control) and after treatment of RNase or DNase. Scale bar: 15 μm.

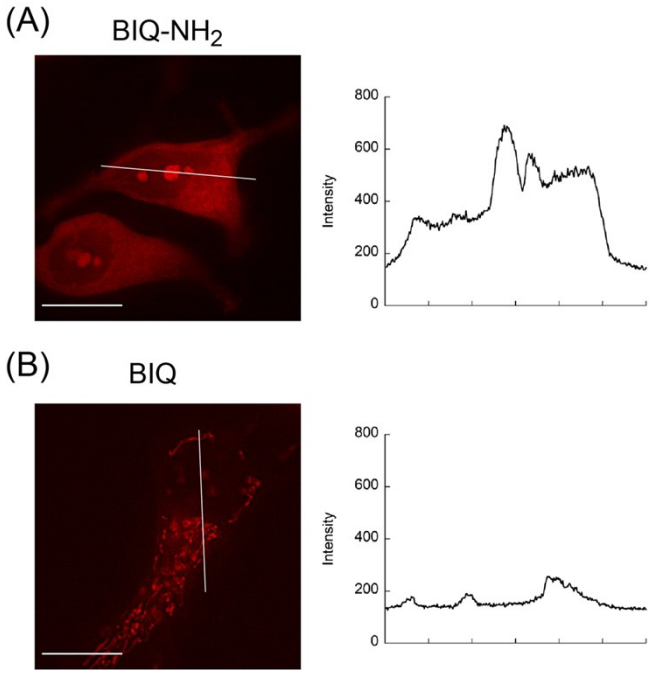


Fig. S9 Fluorescence images of living MCF7 cells stained by the probes (500 nM: (A) BIQ-NH_2 and (B) BIQ). Scale bar: 15 μm. Fluorescence intensity profiles along the white line are also shown.

References

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