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Monitoring autophagy in live cells with a fluorescent light-up probe for G-quadruplex structures

Hongbo Chen,[a,b] Hongxia Sun,[,a] Suge Zhang,[a,b] Wenpeng Yan,[c] Qian Li,[a] Aijiao Guan,[a] Junfeng Xiang,[d] Meirong Liu,[d] Yalin Tang[*,a,b]*

[a]Beijing National Laboratory for Molecular Sciences (BNLMS), State Key Laboratory for Structural Chemistry of Unstable and Stable Species, CAS Research/Education Center for Excellence in Molecular Sciences, Institute of Chemistry Chinese Academy of Sciences, Beijing, P R China

[b]University of Chinese Academy of Sciences, Beijing, PR China

[c]Technical Institute of Physics and Chemistry, Chinese Academy of Sciences. Beijing, PR China.

[d]Center for Physiochemical Analysis & Measurement, Institute of Chemistry Chinese Academy of Sciences, Beijing, PR China.

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Experiments

Materials. All DNA oligonucleotides were obtained from Invitrogen (Beijing, China), purified by PAGE. RNA oligonucleotides were purchased from RiboBio Co., Ltd. (Guangzhou, China), purified by HPLC. The stock solution of the oligonucleotides (sequence information in Table S1) was prepared by directly dissolving the oligonucleotides in 10 mM Tris-HCl buffer (pH 7.4) and annealing in a thermocycler (heating at 90 ℃ for 5 min and cooling down to room temperature slowly). Metal salts, methanol and Tris(hydroxymethyl)aminomethane were all analytical grade, being purchased from Beijing Chemical Company. Various amino acids, reduced glutathione, glucose, vitamin C, thrombin, LAMP1, LAMP2, cathepsin B, cathepsin D, acid phosphatase, LysoTracker Red DND-99, Lyso Brite NIR, and Mito Tracker Deep Red were obtained from Thermo Fisher Scientific (China). RHPS4 (NO.B6186) and BRACO-19 was purchased from APExBIO (USA) and SIGMA (USA), respectively. The cyanine dye DMOTY, FMOTY1 and FMOTY2 were synthesized according to Hamer and Badger's methods,[1,2] and the purity was proved by mass spectrometry and nuclear magnetic resonance (NMR). Ultrapure water, prepared by Milli-Q Gradient ultrapure water system (Millipore), was used in all experiments.

Instruments. ^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded at 500 MHz and ^{19}F-NMR spectra were recorded at 600 MHz, respectively, on a Bruker Model Advance Spectrometer with deuterated methanol as the internal standard. Confocal imaging was done using an OLYMPUS FV1000IX81 confocal laser-scanning microscope (Olympus Corporation, Japan). The absorption spectra were all recorded on Agilent 8453 UV-visible spectrophotometer at the wavelength range 190-1100 nm using a 1 cm quartz cuvette. Fluorescence spectra were recorded on a HITACHI F-4600 Fluorescence Spectrophotometer (Hitachi Limited, Japan). CD spectra were collected from 200 to 360 nm on a Jasco-815 spectropolarimeter in a 1-cm path-length quartz cell.

Cell culture. HeLa (cervical carcinoma cell line) and MCF-7 (human breast adenocarcinoma cell line) were obtained from National Infrastructure of Cell Line Resource (Chinese Academy of Medical Sciences, Beijing, China). Cells were cultured in high glucose DMEM (Dulbecco's Modified Eadle's Medium) containing 10% fetal bovine serum (FBS) and 1% 100 U/mL double antibody in 5% CO_2 at 37 °C for 48 h.

Cytotoxicity assay. Cytotoxicity assays were carried out using HeLa and MCF-7 cells. Cell viability was determined using MTT assay. 5000−6000 cells per well were seeded in a 96-well plate and incubated for 16 h in a cell incubator for adherence. DMOTY dissolved in methanol was added to cells at the final concentration of 0.1, 1, 5, 10, and 40 μM and incubated for 24 h. MTT diluted by DMEM medium (10%) was added to each well after the removal of culture media and incubated for 4 h. Following that, the absorbance was measured at 492 nm on a plate reader after the removal of culture media and adding DMSO. Cell viability was determined as $VR = (A-A_0)/(As-A_0) \times 100\%$, where A is the absorbance of the experimental group, As is the absorbance of the control group, and A_0 is the absorbance of the blank group (no cells).

Localization experiment of DMOTY in cells. HeLa and MCF-7 cells were incubated with DMOTY (10 μM) for 4 h, followed by washing three times with PBS (pH 7.4). The adherent cells were then incubated with MitoTracker Deep Red (50 nM) and LysoTracker Red DND-99 (50 nM) for 10 min, washed three times with PBS (pH 7.4) to remove the excess. The cells were then imaged under CLSM equipped with an oil immersion 100× objective lens. The fluorescence of LysoTracker Red DND-99 was excited with a 559 nm laser and the emission was collected at 570−630 nm; the fluorescence of Mito Tracker Deep Red was excited with a 633 nm laser and the emission was collected at 650−750 nm; the fluorescence of DMOTY was excited with a 405 nm laser and the emission was collected at 450−550 nm.

Oligonucleotide incubation. Different concentrations of mt377, s-myc and ds22 were added to the serum medium at 37 °C for 2 h. Then, the cells were incubated with DMOTY at 37 °C for 4 h. Prior to

imaging, all the cells were washed three times by PBS and stained with Lyso Tracker Red DND-99 (50 nM) for 10 min. For mt377-Cy3 treatment, mt377-Cy3 (1 μM) was added in cells with serum medium for 6 h at 37 °C. Then, cells were incubated with Lyso Brite NIR (50 nM) for 10 min and washed three times with PBS (pH 7.4) to remove the excess. The excitation of Cy3 and Lyso Brite NIR was 559 nm and 633 nm, respectively. The fluorescence of Cy3 and Lyso Brite NIR was collected at 570−620 nm and 650−750 nm, respectively.

Dexamethasone treatment. HeLa and MCF-7 cells in serum medium were incubated with DMOTY (10 μM) for 4 h, and then different concentrations of (5, 10 μM) dexamethasone were added to the medium for 30 min. Then adherent cells were then incubated with Lyso Tracker Red DND-99 (50 nM) for 10 min. Before imaging, all the cells were washed three times by PBS and replaced in serum-free medium containing dexamethasone.

Imaging of autophagy in cells. For starvation treatment, DMOTY (10 μM) was first added to HeLa and MCF-7 cells with complete medium. The complete medium was then replaced with serum-free medium containing an equal amount of DMOTY at different time. For Rapamycin treatment, cells were incubated with DMOTY (10 μM) and different concentrations of Rapamycin in serum medium for 4 h at 37 °C. For Bafilomycin A1 treatment, cells were incubated with DMOTY (10 μM) and different concentrations of Bafilomycin A1 in serum-free medium for 4 h at 37 °C. Prior to imaging, cells were incubated with Lyso Tracker Red DND-99 (50 nM) for 10 min and washed three times with PBS (pH 7.4) to remove the excess. The fluorescence of LysoTracker Red DND-99 was excited with a 559 nm laser with emission collected at 570−630 nm; the fluorescence of DMOTY was excited with a 405 nm laser with emission collected at 450−550 nm.

Quantification of the fluorescence intensity in the confocal images. The exact quantification of the fluorescence intensity in the confocal images was analyzed using Olympus FluoView Ver.3.1 Viewer software and ImageJ software (National Institutes of Health, USA). First, the cell area was selected by using freeform selection tools, and then the fluorescence intensity of DOMTY was calculated by measuring the mean gray value of the selected area. The relative fluorescence intensity in each sample was normalized to the fluorescence intensity in the control.

Synthesis and characterization of probes

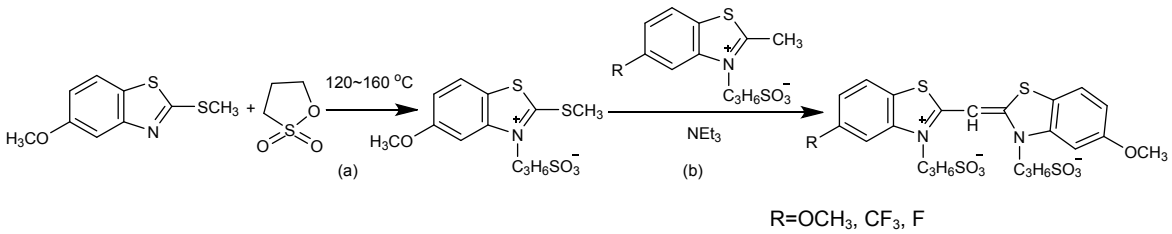


Figure S1. Synthesis of DMOTY, FMOTY1 and FMOTY2.

Synthesis of 5-methoxyl-2-methylthio-3-γ-propyl sulfonate) benzothiazole (a): A mixture of 5-methoxy-2-methinoyl benzothiazole and 1'3-propane sultone was added for melting reaction (120~160 °C, 10 h). Then recrystallization to obtain off-white powder (yield 57.2%). The calculated value of N, C, H is 3.99, 41.01, 4.88, and the measured values of the elemental analysis is 4.01, 40.58, and 4.40, respectively. The ν_{max} data of infrared spectra are 3434 (OH), 3008 (CH_3), 1598, 1412, 1352, 1232 (Ar-O-CH_3), 1188, 1036 (S=O), 866, 824 and 790 cm^{-1}.

Synthesis of DMOTY, DMOTY1 and DMOTY2 (b): A mixture of 5-methoxyl-2-methylthio-3- (γ-propyl sulfonate) benzothiazole and 5-R-2-methyl-3- (γ-propyl sulfonate) benzothiazole (1:1) was stirred with water-alcohol (1:2) solvent at 100 °C for 1~2 h, using triethylamine as a catalyzer, to produce a yellow crude product. The final product was obtained after recrystallization.

DMOTY

^{1}H NMR (500 MHz, MeOD) δ 7.72 (s, 1H), 7.71 (s, 1H), 7.25 (d, J = 2.1 Hz, 2H), 6.90 (s, 1H), 6.88 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 4.76 – 4.66 (m, 4H), 3.86 (s, 6H), 3.22 (q, J = 7.3 Hz, 6H), 3.13 – 3.11 (m, 4H), 2.38 – 2.31 (m, 4H), 1.32 (t, J = 7.3 Hz, 9H).

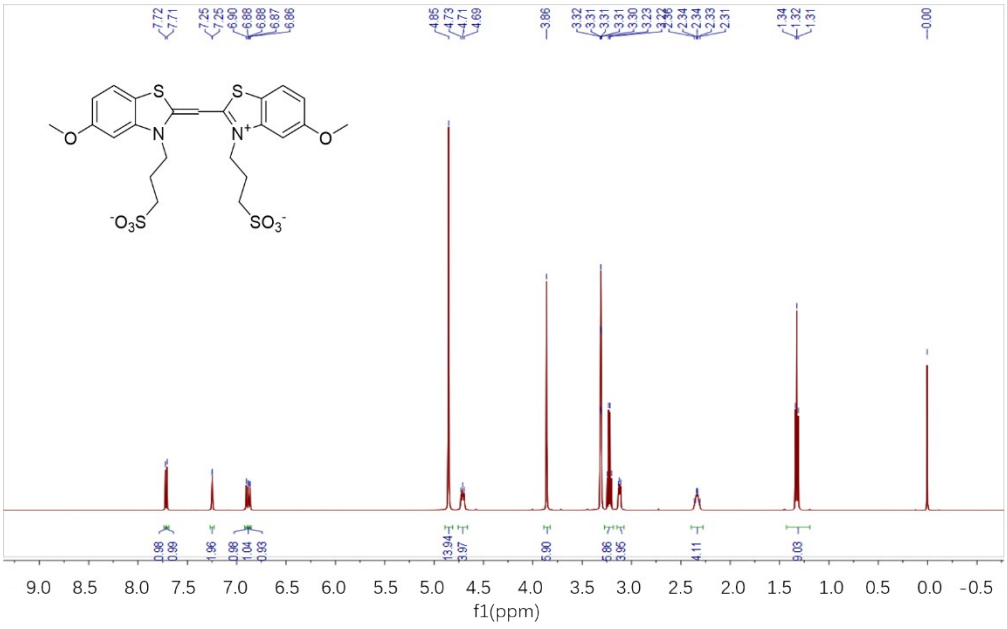


Figure S2. ^{1}H-NMR spectra of DMOTY.

^{13}C NMR (126 MHz, MeOD) δ 163.62, 162.49, 142.74, 124.42, 117.41, 114.83, 98.97, 83.71, 56.73, 47.95, 46.43, 23.89, 9.24.

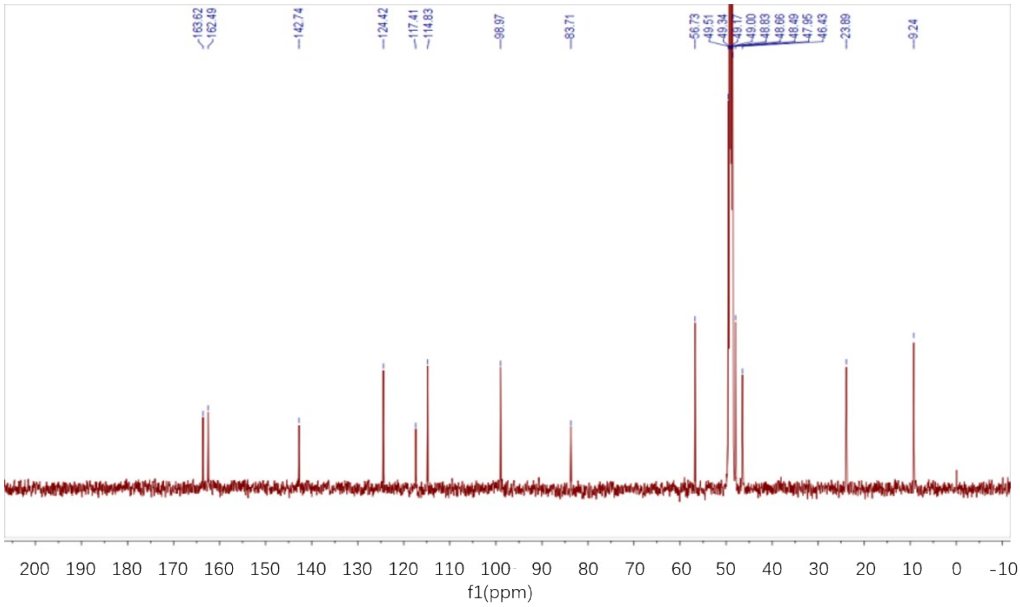


Figure S3. ^{13}C-NMR spectra of DMOTY.

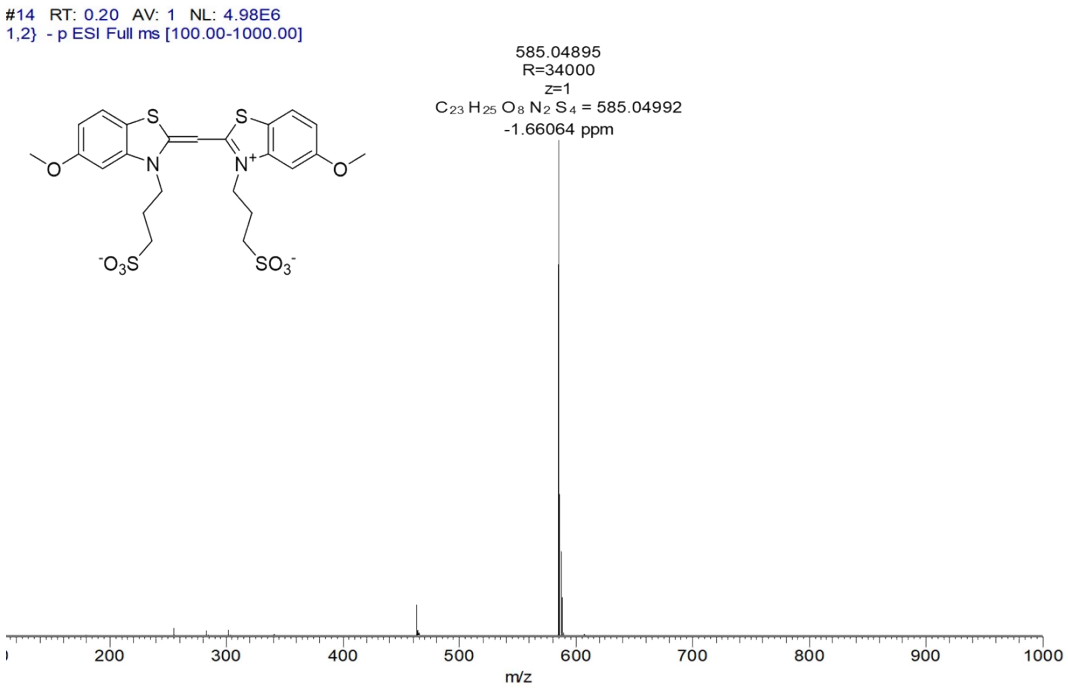


Figure S4. HRMS of DMOTY.

FMOTY1

^{1}H NMR (500 MHz, MeOD) δ 7.95 (dd, *J* = 8.7, 5.0 Hz, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.70 (dd, *J* = 9.6, 2.2 Hz, 1H), 7.52 (d, *J* = 2.2 Hz, 1H), 7.18 (td, *J* = 8.7, 2.2 Hz, 1H), 7.08 (s, 1H), 7.01 (dd, *J* = 8.8, 2.2 Hz, 1H), 4.89–4.83 (m, 2H), 4.77 – 4.74 (m, 2H), 3.92 (s, 3H), 3.22 (q, *J* = 7.3 Hz, 6H), 3.14 – 3.11 (m, 2H), 3.09 – 3.08 (m, 2H), 2.39 – 2.32 (m, 4H), 1.32 (t, *J* = 7.3 Hz, 9H).

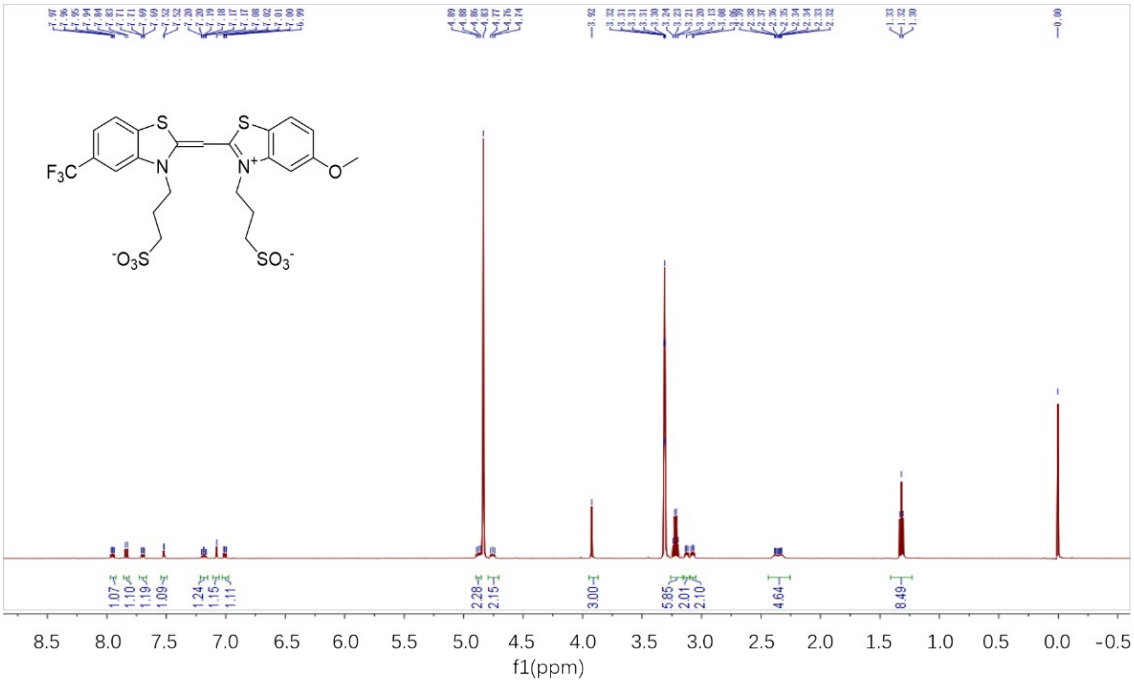


Figure S5. ^{1}H-NMR spectra of FMOTY1.

^{13}C NMR (126 MHz, Acetic) δ 165.69, 164.87, 164.18, 163.73, 162.78, 143.09, 125.55, 125.47, 124.51, 121.93, 117.80, 115.61, 113.64 (d, *J* = 24.8 Hz), 102.33 (d, *J* = 29.4 Hz), 99.32, 84.22, 56.92, 47.96, 46.71, 46.61, 23.98, 23.79, 9.23.

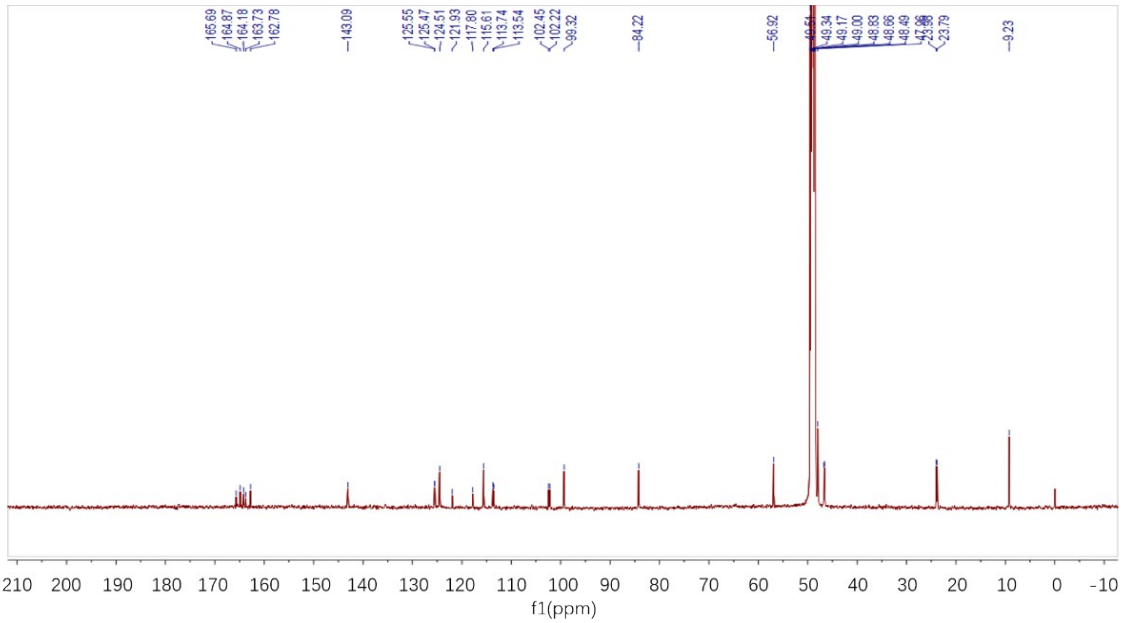


Figure S6. ^{13}C-NMR spectra of FMOTY1.

^{19}F NMR (565 MHz, MeOD) δ -63.22 (s, 3F).

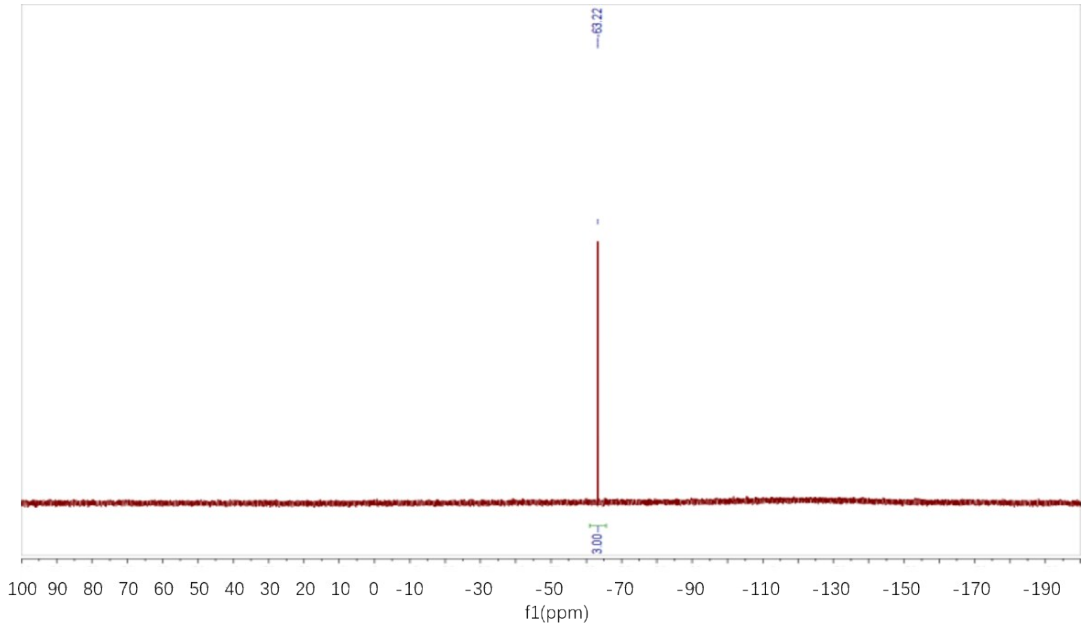


Figure S7. ^{19}F-NMR spectra of FMOTY1.

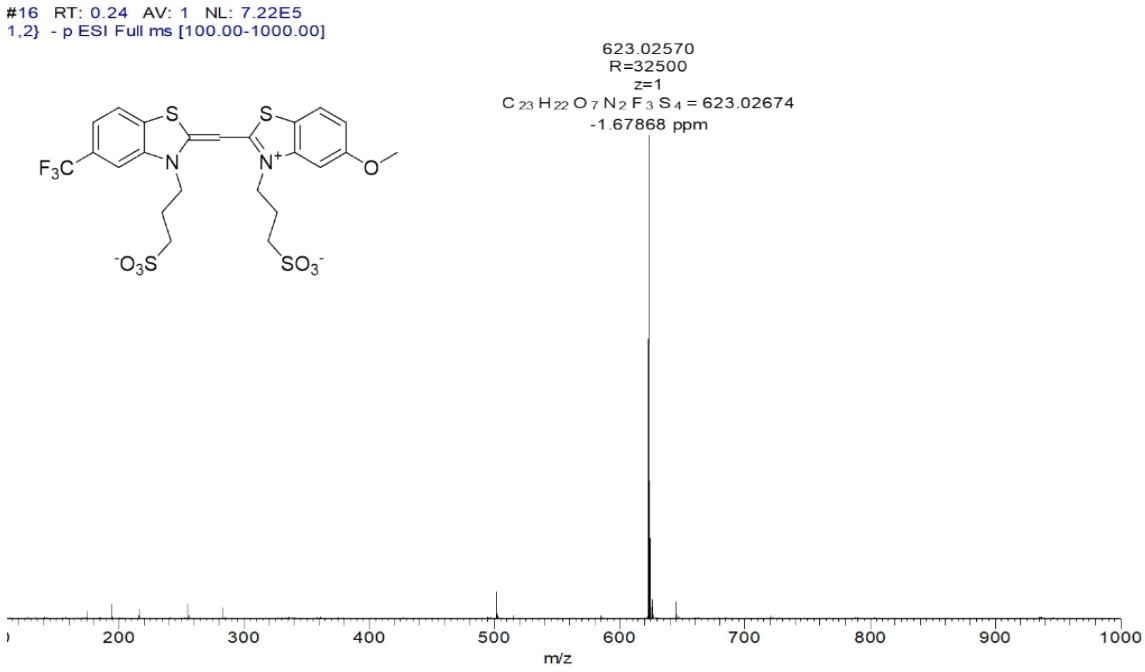


Figure S8. HRMS spectra of FMOTY1.

FMOTY2

^{1}H NMR (500 MHz, MeOD) δ 8.03 (d, *J* = 7.4 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 2.0 Hz, 1H), 7.03 (s, 1H), 6.86 (dd, *J* = 8.8, 2.1 Hz, 1H), 4.83 – 4.75 (m, 4H), 3.79 (s, 3H), 3.23 (q, *J* = 7.3 Hz, 6H), 3.25 – 3.21 (m, 2H), 3.17 – 3.10 (m, 2H), 2.38 – 2.33(m, 4H), 1.33 (t, *J* = 7.3 Hz, 9H).

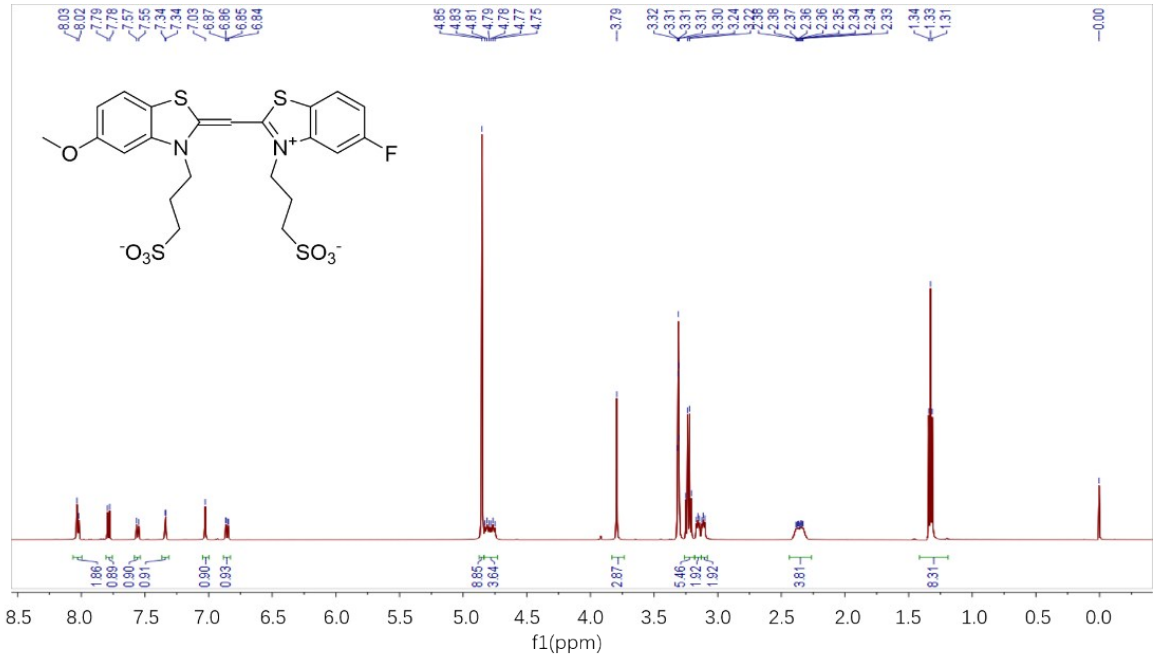


Figure S9. ^{1}H-NMR spectra of FMOTY2.

^{13}C NMR (126 MHz, MeOD) δ 165.12, 162.88, 162.63, 142.82, 141.71, 132.02 (d, *J* = 32.9 Hz), 130.24, 125.06, 124.53, 122.28, 117.56, 115.90, 111.32, 99.16, 84.52, 47.95, 46.88, 46.47, 24.17, 23.96, 9.25.

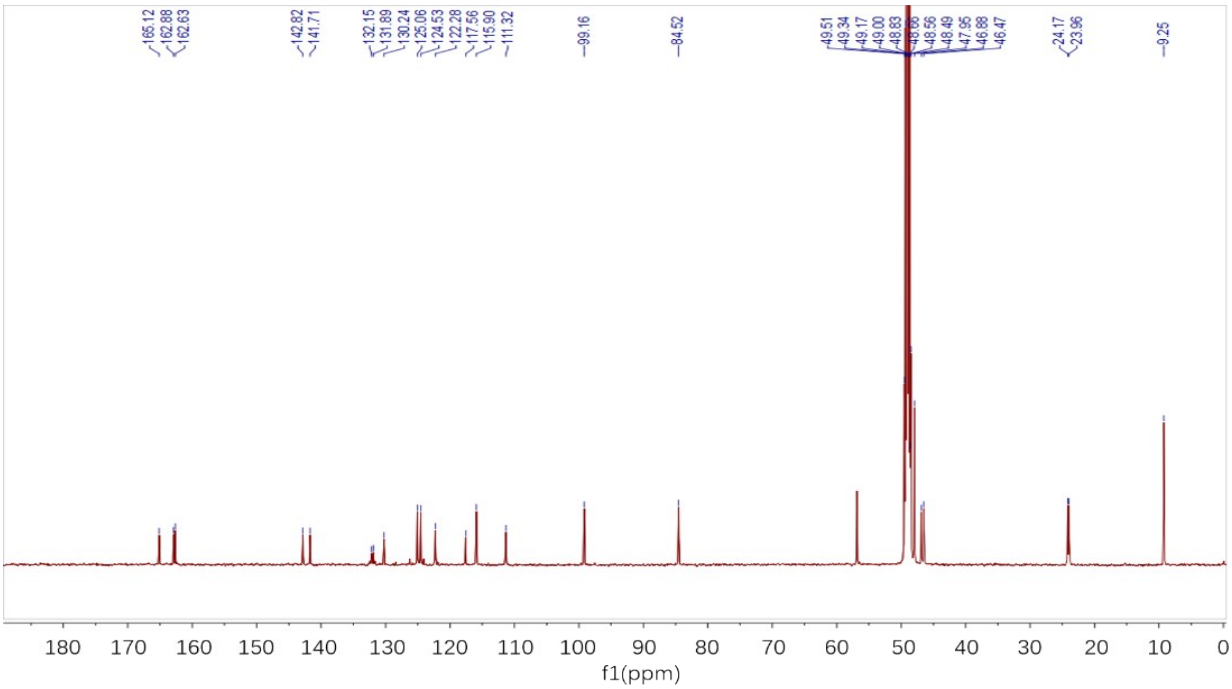


Figure S10. ^{13}C-NMR spectra of FMOTY2.

^{19}F NMR (565 MHz, MeOD) δ -112.64 (s, F).

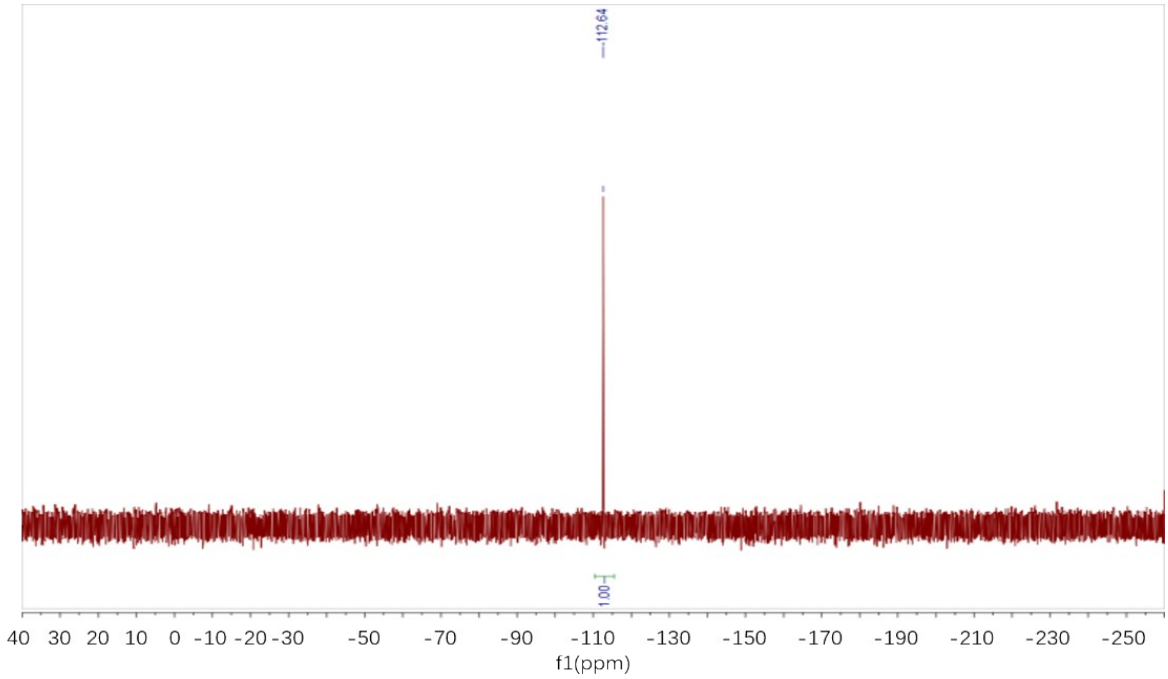


Figure S11. ^{19}F-NMR spectra of FMOTY2.

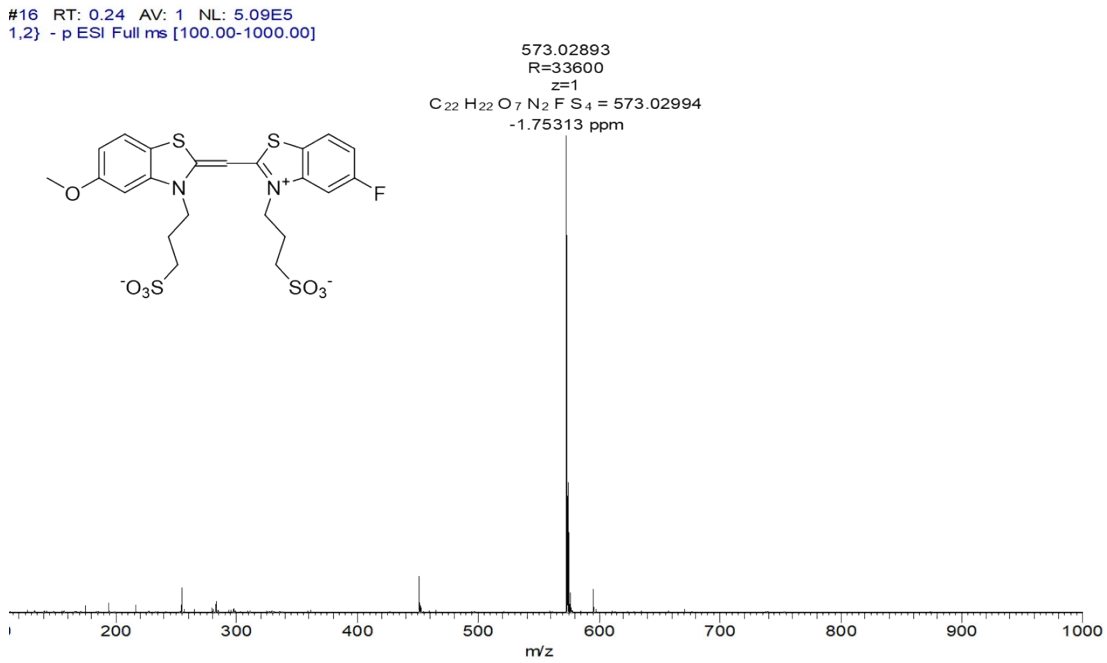


Figure S12. HRMS of FMOTY2.

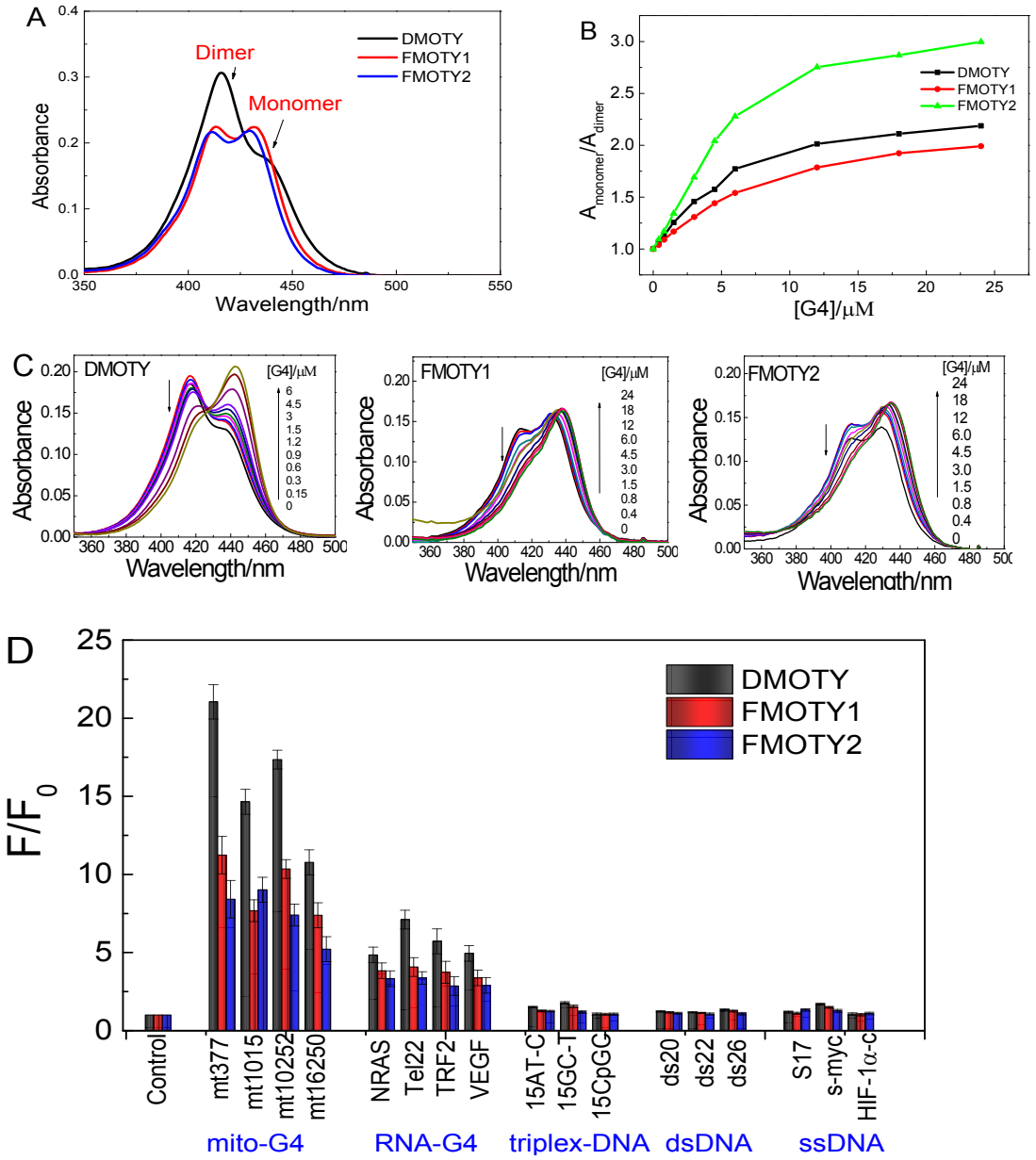


Figure S13. (A) The absorption spectra of the three probes (5 μM) in aqueous solution. (B) Changes in absorbance ratio of $A_{monomer}/A_{dimer}$ with increasing amounts of mt1015 G-quadruplexes (G4s). (C) The absorption spectra of the three probes (3 μM) with increasing amounts of mt1015 G-quadruplexes. (D) Dependence of the three probes (2 μM) fluorescence intensity at 480 nm on various nucleotide samples (2 μM). All these samples in B−D were measured in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM). λex= 430 nm

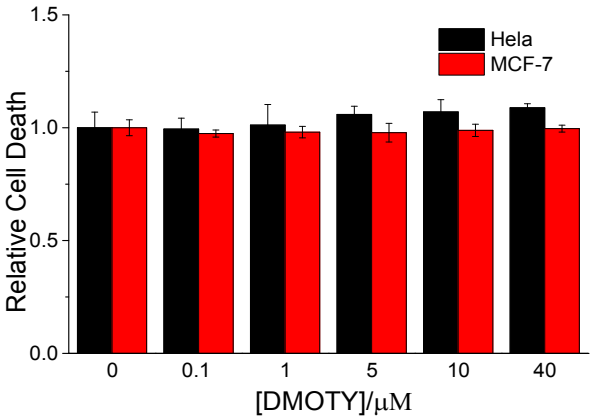


Figure S14. Cell viability of HeLa and MCF-7 cell in the presence of different concentrations of DMOTY (0−40 μM) after 24 h incubation. The viability of the cells without DMOTY is defined as 1. The results are expressed as the mean ± standard derivation of three separate measurement.

The cytotoxicity of DMOTY using a standard methyl thiazolyl tetrazolium (MTT) assay. After incubation of HeLa and MCF-7 cells with different concentrations of DMOTY for 24 h, no obvious cytotoxicity was observed even when the concentration of DMOTY was as high as 40 Mm, indicating that the toxicity of DMOTY is negligible.

Figure S15. Confocal laser scanning microscopy showing the fluorescence of DMOTY (10 μM, Ex 405 nm) in zebra fish. Scale bars, 500 μm. Zebrafish, 3-5 days old, were selected and maintained in 10 μM DMOTY for 48 hours. We confirm that we have read the policies of RSC journal on human and animal testing. We have taken great efforts to reduce the number of animal used in this study and also taken effort to reduce animal suffering from pain and discomfort. By exposing zebrafish to DMOTY for 48 h, we found no behavioral changes in the fish, further demonstrating the low acute toxicity of DMOTY.

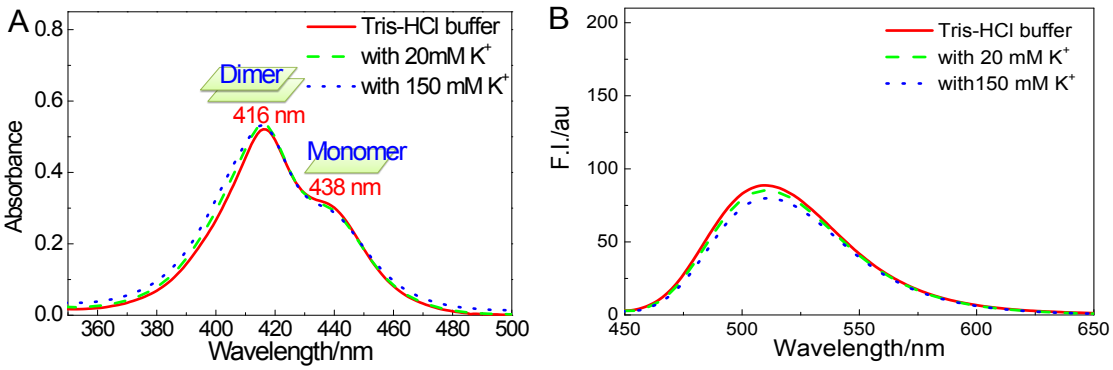


Figure S16. UV-vis absorption (A) and fluorescence spectra (B) of DMOTY (8 μM) in Tris-HCl buffer solution (pH 7.4) in the absence and presence of KCl. λex= 430 nm.

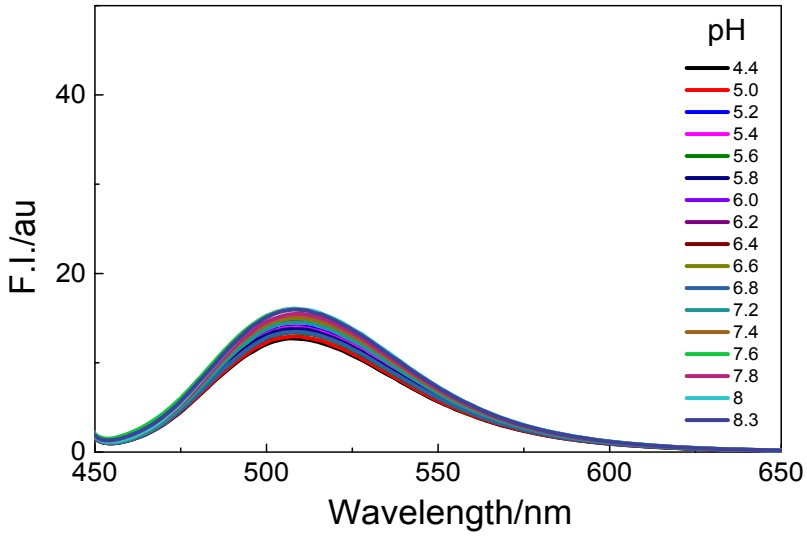


Figure S17. Fluorescence spectra of DMOTY (2 μM) with pH value ranging from 4.4 to 8.3. λex= 430 nm

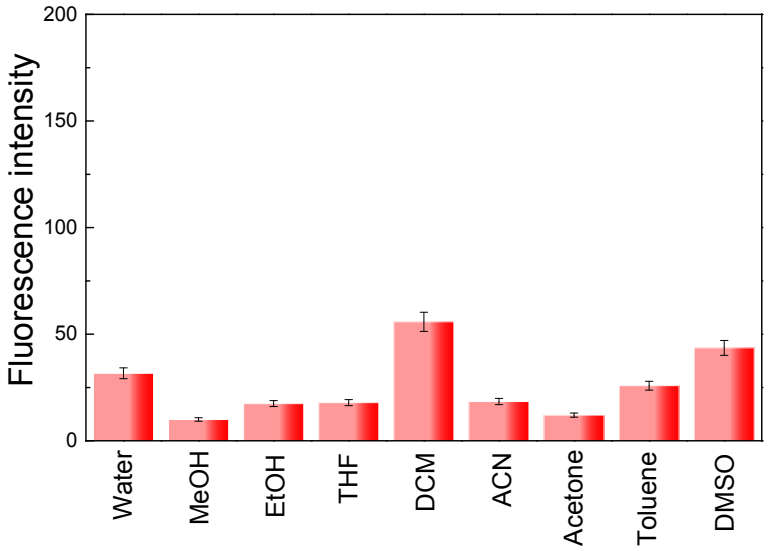


Figure S18. Fluorescence intensity of DMOTY (2 μM) at 509nm in response to solvents of different dielectric constant values. Error bars represent SD of three replicates. λex= 430 nm

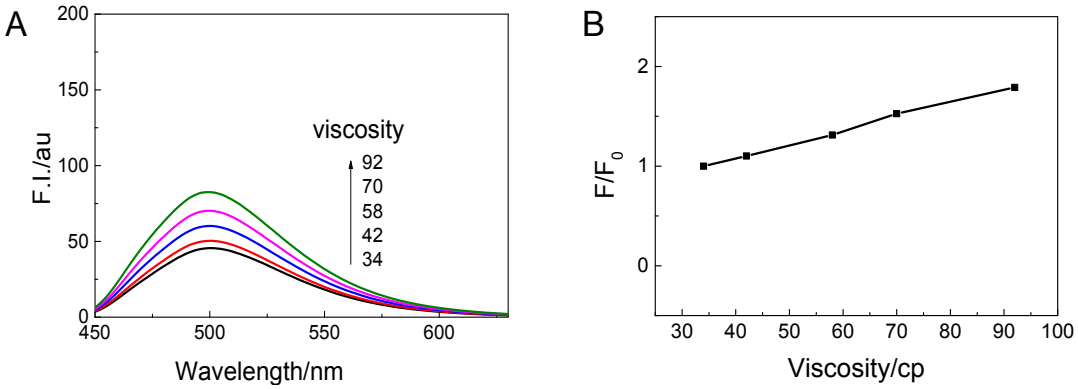


Figure S19. (A) Changes of fluorescent spectra of DMOTY (2 μM) with the variation of solution viscosity (water-glycerol system). (B) Relationship of the F/F0 ratio with solution viscosity. λex= 430 nm. The average viscosity of lysosomes is ~65 cp,[3] which increases by about 20 cp when autophagy occurs.[4] Within this viscosity range, the fluorescence enhancement of DMOTY is less than 1 fold indicating that DMOTY will not be disturbed by viscosity in potential applications of autophagy detection.

Table S1. Sequences of the oligonucleotides used in this study and their structures in the buffer solution with 150 mM K^+ and 12 mM Na^+.

Name	Sequence (from5' to 3')	Structure
DNA-Quadruplex		
mt377	GGGGGGGGGGGGTTTGATGTGGGTTGGG	Mito Hybird G4
mt1015	GGGCTTGATGTGGGGAGGGGTGTTTAAGGG	Mito Hybird G4
mt10252	GGGTGGGAGTAGTTCCCTGCTAAGGGAGGG	Mito Parallel G4
mt16250	GAAGCGGGGGAGGGGGGGTTTGGTGGAAAT	Mito Hybird G4
RNA-Quadruplex		
VEGF-RNA	GGAGGAGGGGGAGGAGGA	Parallel G4
Tel22-RNA	AGGGUUAGGGUUAGGGUUAGGG	Parallel G4
TRF2-RNA	CGGGAGGGCGGGGAGGGC	Parallel G4
NRAS-RNA	GGAGGGGCGGGUCUGGG	Parallel G4
ss-DNA		
HIF-1α-c	CCCGCCCCCTCTCCCTCCCAAA	Single strand
S17	CCAGTTCGTAGTAACCC	Single strand
s-myc	CCTTCCCCACCCTCCCCACCCTCCCCA	Single strand
ds-DNA		
ds20	CGAATTCGTCTCCGAATTCG	Duplex
ds22	TTCGCGCGCGTTTTCGCGCGCG	Duplex
ds26	CAATCGGATCGAATTCGATCCGATTG	Duplex
Triplex DNA		
15GC-T	GAAAAAAAAAAAAAGTTTTCTTTTTTTTTTT TTCTTTTCTTTTTTTTTTTTC	Intramolecular triplex
15AT-C	AAAAAAAAAAAAAAACCCCTTTTTTTTTTTT TTTCCCCTTTTTTTTTTTTTT	Intramolecular triplex
15CpGC	CTTTTTTCTCTCTCC GAAAAAAGAGAGAGG CCTCTCTCTTTTTTC	Intermolecular triplex

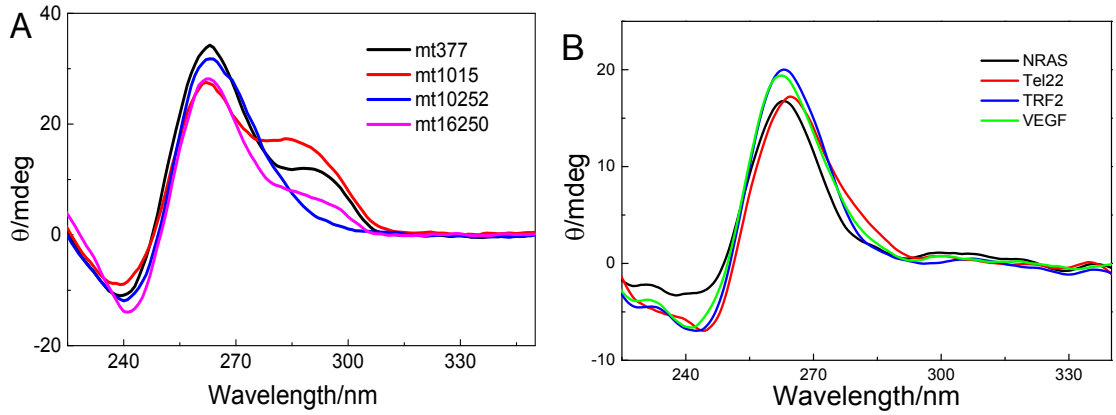
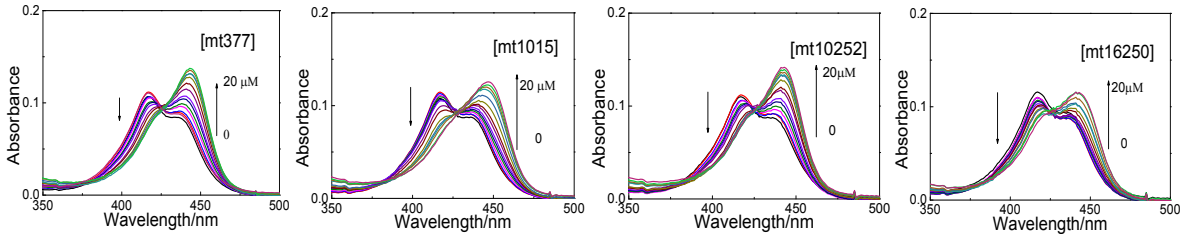


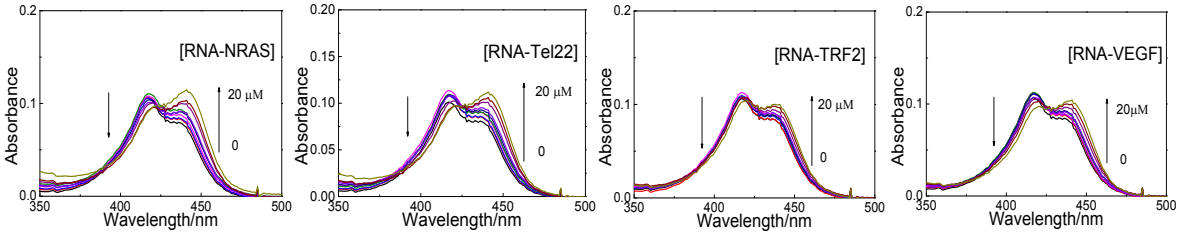
Figure S20. CD spectra of different DNA (A) and RNA (B) oligonucleotides (4 μM) in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM).

The CD profiles with a positive peak at around 265 nm and a significant negative peak at around 240 nm were typical signatures of parallel G-quadruplex structures.[5] The CD profiles with a significant negative peak at around 240 nm, a positive peak at around 265 nm, and a positive shoulder at around 295 nm were typical feature of hybrid-type G-quadruplex structures.

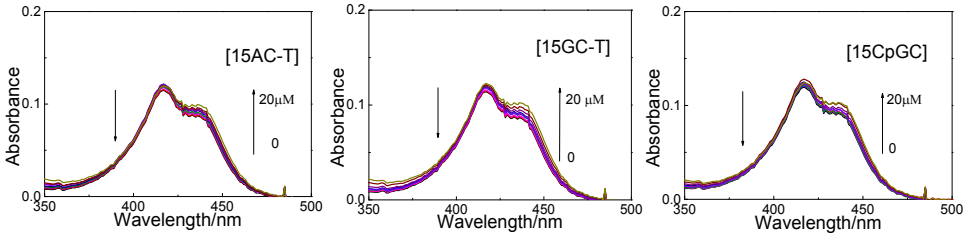
DNA G-quadruplexes



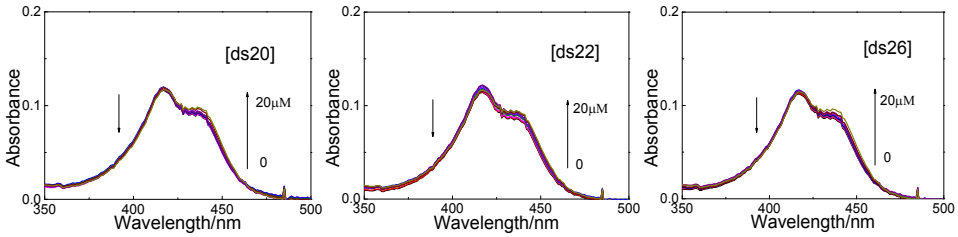
RNA G-quadruplexes



Triplex DNA



Duplex DNA



Single-stranded DNA

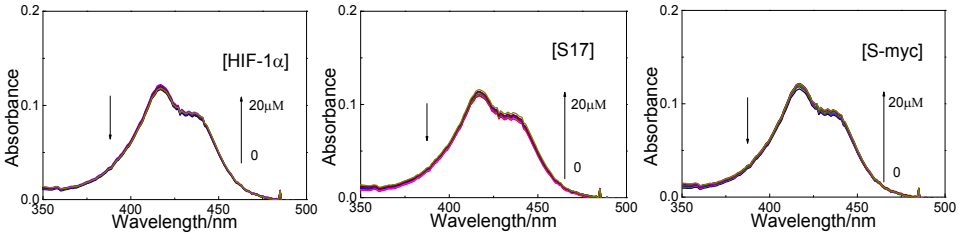
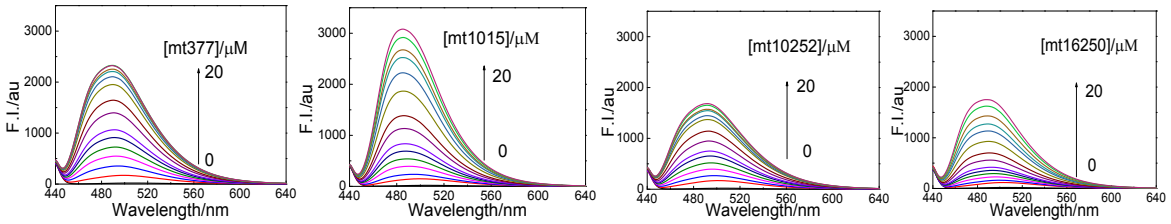
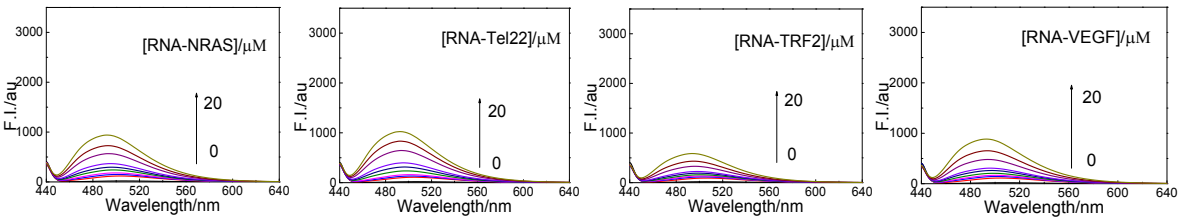


Figure S21. UV-vis absorption spectra of DMOTY (2 μM) with increasing concentrations of DNA and RNA G-quadruplexes, single-stranded, duplex and triplex DNA in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM).

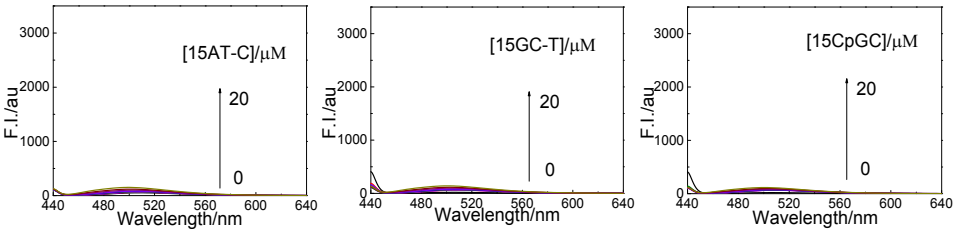
DNA G-quadruplexes



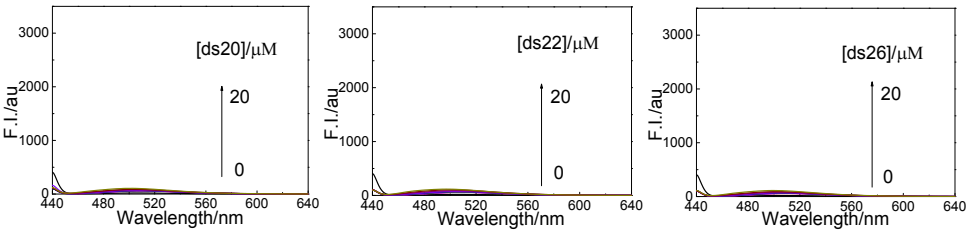
RNA G-quadruplexes



Triplex DNA



Duplex DNA



Single-stranded DNA

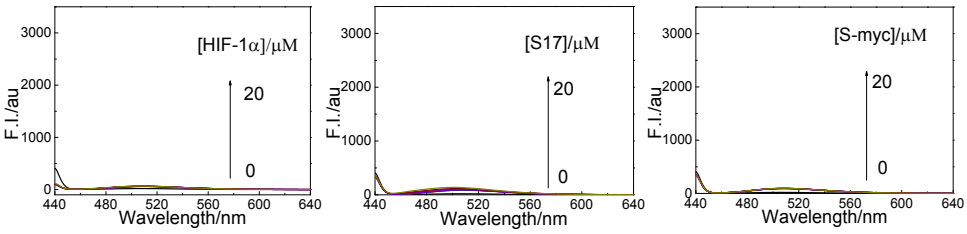


Figure S22. Fluorescence spectra of DMOTY (2 μM) with increasing concentrations of DNA and RNA G-quadruplexes, single-stranded, duplex and triplex DNA in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM). λex= 430 nm.

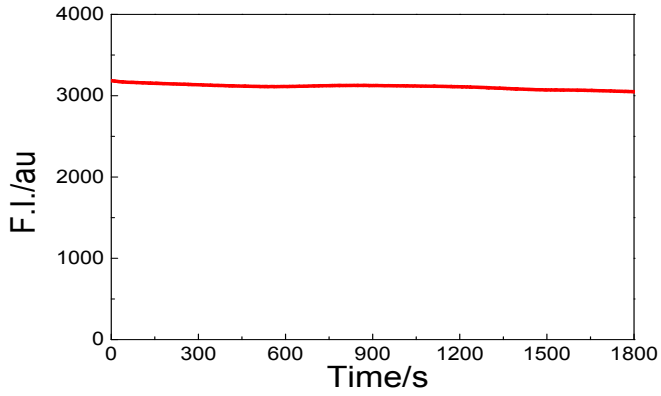


Figure S23. The fluorescence intensity at 490 nm of 4 μM DMOTY with 8 μM mt1015 under continuous excitation at 430 nm. All the experiments were conducted in 10 mM Tris–HCl buffer, 150 mM KCl, pH 7.2. The fluorescence intensity of DMOTY remained unchanged for 30 minutes. This indicated that the DMOTY was photostable. This indicates that the DMOTY –quadruplex complex is photostable.

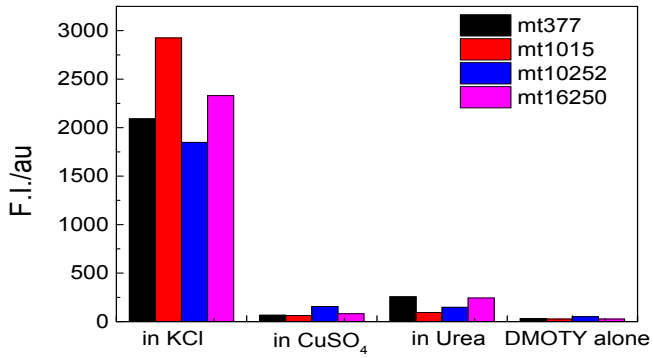


Figure S24. The fluorescence intensity at 490 nm of 4 μM DMOTY with 8 μM different G-quadruplexes under KCl or quadruplex-unfolding conditions. Incubation of DMOTY with G-quadruplexes in quadruplex-unfolding conditions (1 mM $CuSO_4$ or 2 M Urea)[6-8] dramatically decreased the fluorescence intensity. This result indicates that DMOTY preferentially recognizes G-quadruplex structures.

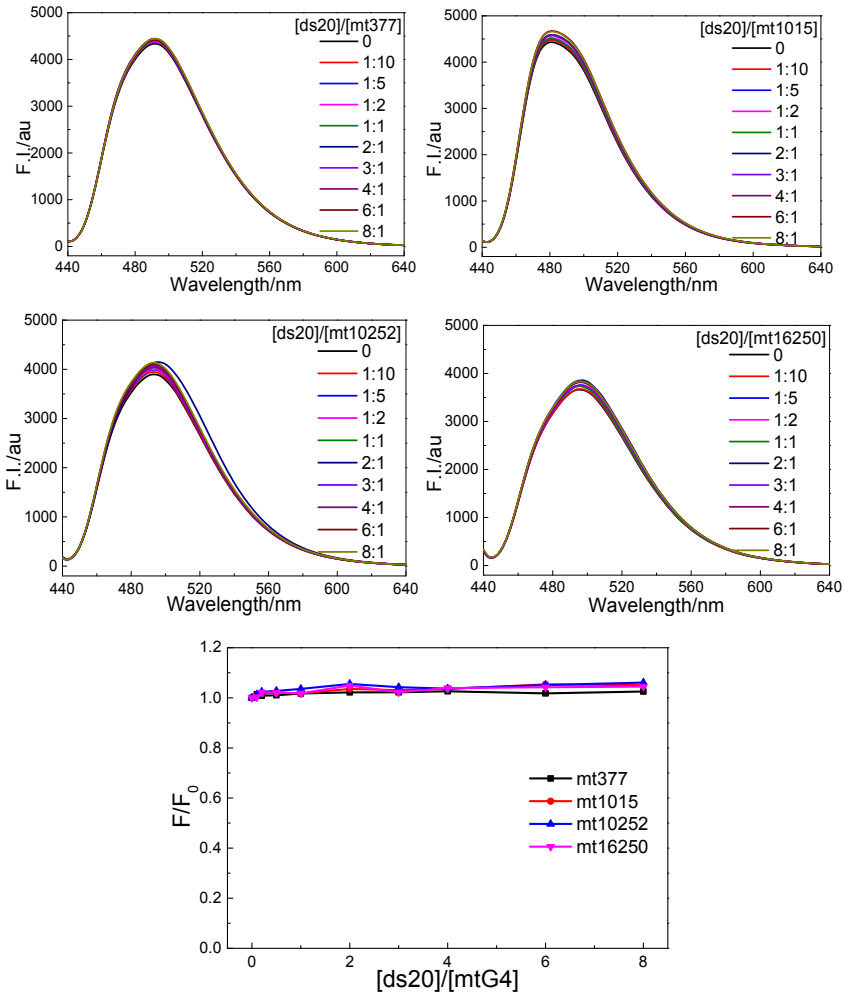


Figure S25. Competitive fluorescence titrations of DMOTY (4 μM) and different G-quadruplex (8 μM) complexes with increasing amounts of duplex DNA ds20. All the samples were measured in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM). λex= 430 nm. The fluorescence of the DMOTY and G-quadruplex complexes was slightly affected by the presence of 8 molar equivalents of duplex-DNA, further supporting the better selectivity of DMOTY to quadruplex structures.

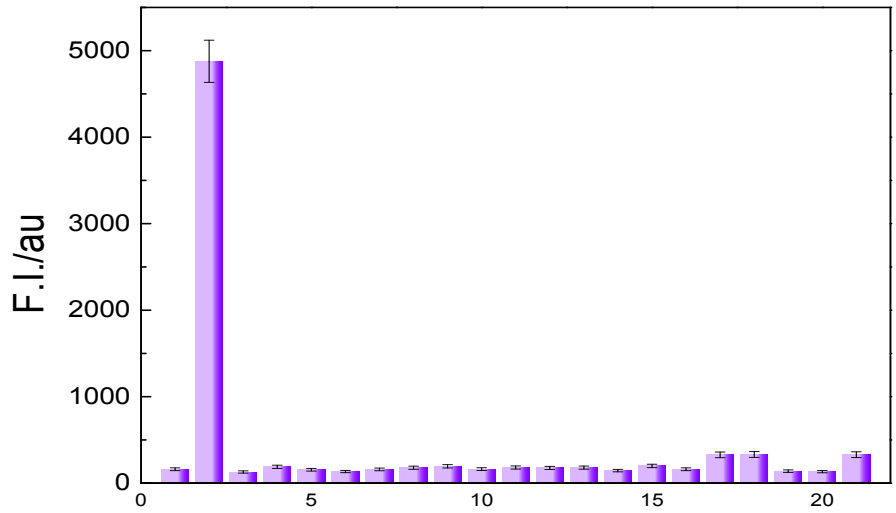


Figure S26. Specificity of DMOTY (4 μM) to G-quadruplex structures. (1) Blank, (2) mt377 (10 μM), (3) Glutamate (1 mM), (4) arginine (1 mM), (5) serine (1 mM), (6) alanine (1 mM), (7) aspartate (1 mM), (8) cysteine (1 mM), (9) reduced glutathione (5 mM), (10) glucose (1 mM), (11) Mg^{2+} (2 mM), (12) Ca^{2+} (2 mM), (13) Zn^{2+} (100 μM), (14) Cu^{2+}(100 μM), (15) vitamin C (1 mM), (16) thrombin (20 nM), (17) LAMP1 (0.025 μM), (18) LAMP2 (0.025 μM), (19) cathepsin B (1 mM), (20) cathepsin D (0.2 U), (21) acid phosphatase (0.2 U). All the samples were measured in Tris-HCl buffer solution (10 mM, pH 7.2) containing K^+ (150 mM) and Na^+ (12 mM). λex= 430 nm.

Table S2. Fluorescence quantum yields (Φ_F) of DMOTY (10 μM) with or without 20 μM DNAs and RNAs.

DNA/RNA	Φ_F	Relative Φ_F	Structure in K^+ Solution
none	0.028	1	
mt377	0.399	14.2	Mito Hybird G4
mt1015	0.506	18.1	Mito Hybird G4
mt10252	0.284	10.1	Mito Parallel G4
mt16250	0.353	12.6	Mito Hybird G4
KRAS-RNA	0.184	6.6	Parallel G4 RNA
TRF2-RNA	0.169	6.0	Parallel G4 RNA
Tel22-RNA	0.142	5.1	Parallel G4 RNA
VEGF-RNA	0.103	3.7	Parallel G4 RNA
15GC-T	0.048	1.7	Triplex
15AT-C	0.053	1.9	Triplex
22CpGC	0.048	1.7	Triplex
ds20	0.054	1.9	Duplex
ds22	0.048	1.7	Duplex
ds26	0.057	2.0	Duplex
HIF-1α-c	0.056	2.0	Single-strand
S17	0.043	1.5	Single-strand
s-myc	0.047	1.7	Single-strand

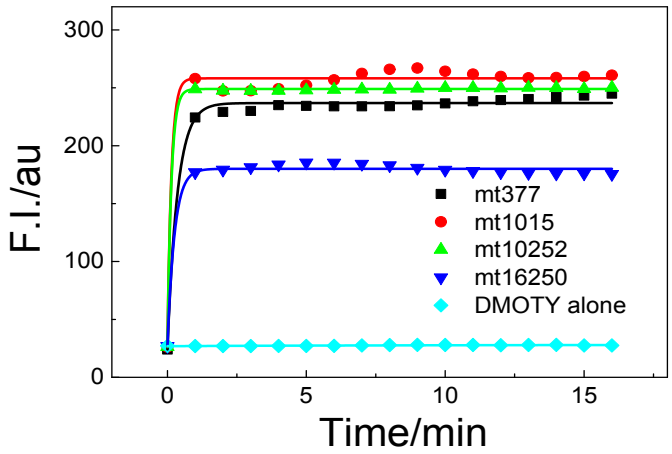


Figure S27. Plots of fluorescence intensity at 490 nm versus time for DMOTY interacting with G-quadruplexes. The sample of DMOTY (2 μM) and G-quadruplex DNA (2 μM) was measured immediately after adding the G-quadruplex DNA in Tris-HCl buffer solution (10 mM, pH 7.2) with 150 mM K^+ and 12 mM Na^+. λex= 430 nm.

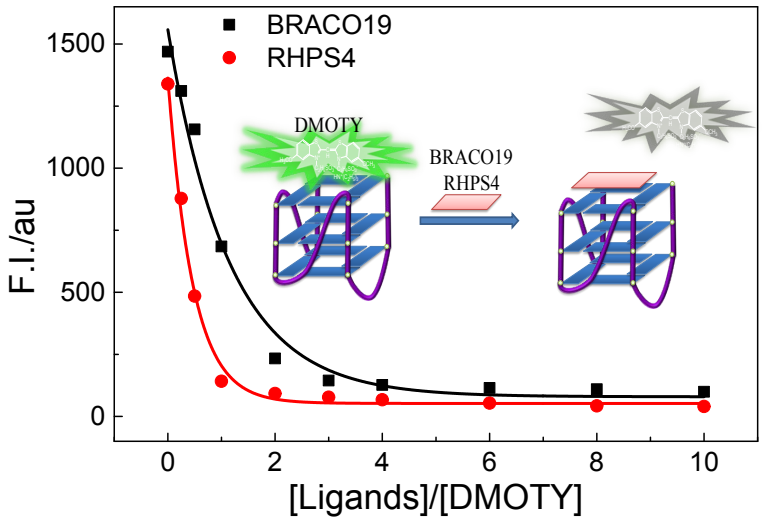


Figure S28. Change in fluorescence intensity at 480 nm of DMOTY (4 μM) with the mt1015 G-quadruplexes (4 μM) upon addition of BRACO19 and RHPS4 measured in Tris-HCl (10 mM, pH 7.2) with 150 mM K^+ and 12 mM Na^+.λex= 430 nm.

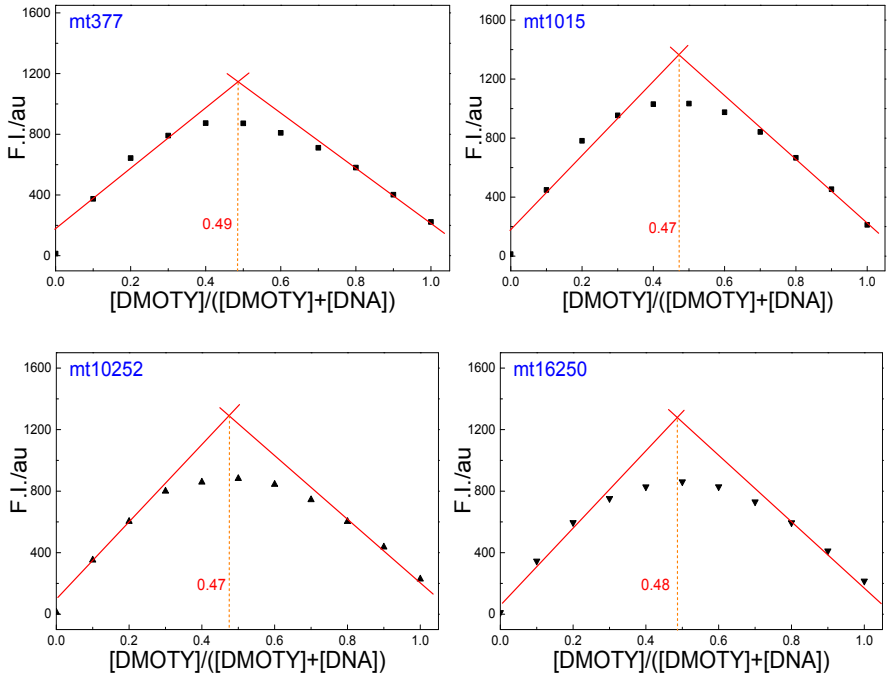


Figure S29. Job plot analysis for the binding stoichiometry of DMOTY to the mtDNA G-quadruplex structures. The total concentration of DNA and DMOTY is 2 μM, and all samples were prepared in Tris-HCl (10 mM, pH 7.2) with 150 mM K^+ and 12 mM Na^+. Excitation was set at 430 nm and emission was measured at 490 nm. The point of intersection for the Job plot is near 0.48, showing a 1:1 stoichiometry of DMOTY binding to these Gquadruplexes.

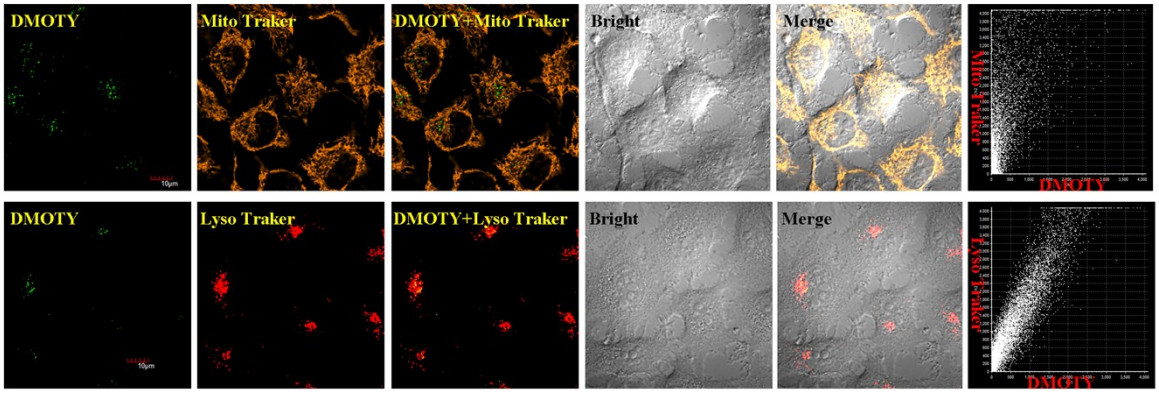


Figure S30. CLSM images of live MCF-7 cells stained with DMOTY (10 μM, Ex 405 nm), MitoTracker Deep Red (50 nM, Ex 633 nm) and LysoTracker Red DND-99 (50 nM, Ex 559 nm). Scale bar, 10 μm. For clarity, the images were presented in pseudocolors of red (LysoTracker Red), orange (MitoTracker Deep Red), and green (DMOTY). Plots in the two diagrams on the right side represent the intensity correlation plot of DMOTY and commercial probes.

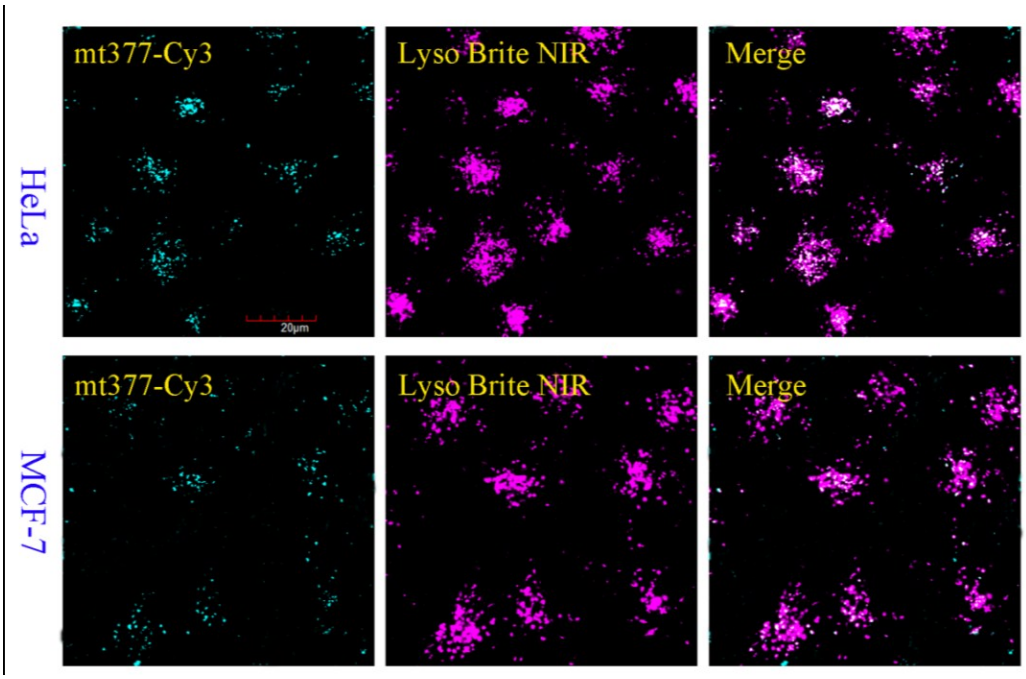


Figure S31. CLSM images of live HeLa and MCF-7 cells after 6 h incubation of cells with mt377-Cy3 (1 μM, Ex 559 nm). Lysosomes were stained with Lyso Brite NIR (50 nM, Ex 633 nm). Scale bar, 20 μm.

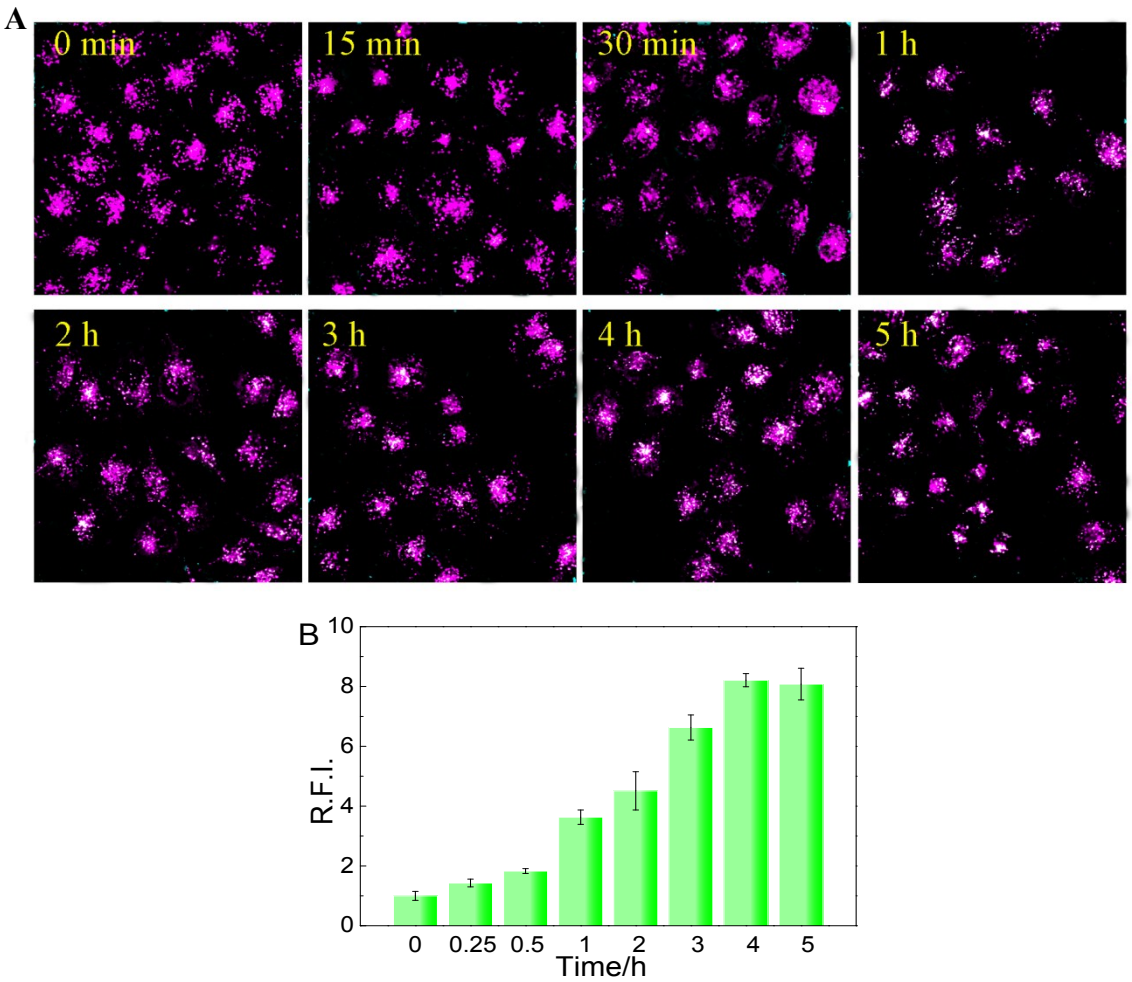


Figure S32. (A) Real-time confocal imaging of live HeLa cells incubated with mt377-Cy3 (1 μM, Ex 559 nm). Lysosomes were stained with Lyso Brite NIR (50 nM, Ex 633 nm). (B) Quantification of the mt377-Cy3 fluorescence intensity for A. For each sample, approximately 300 cells were measured.

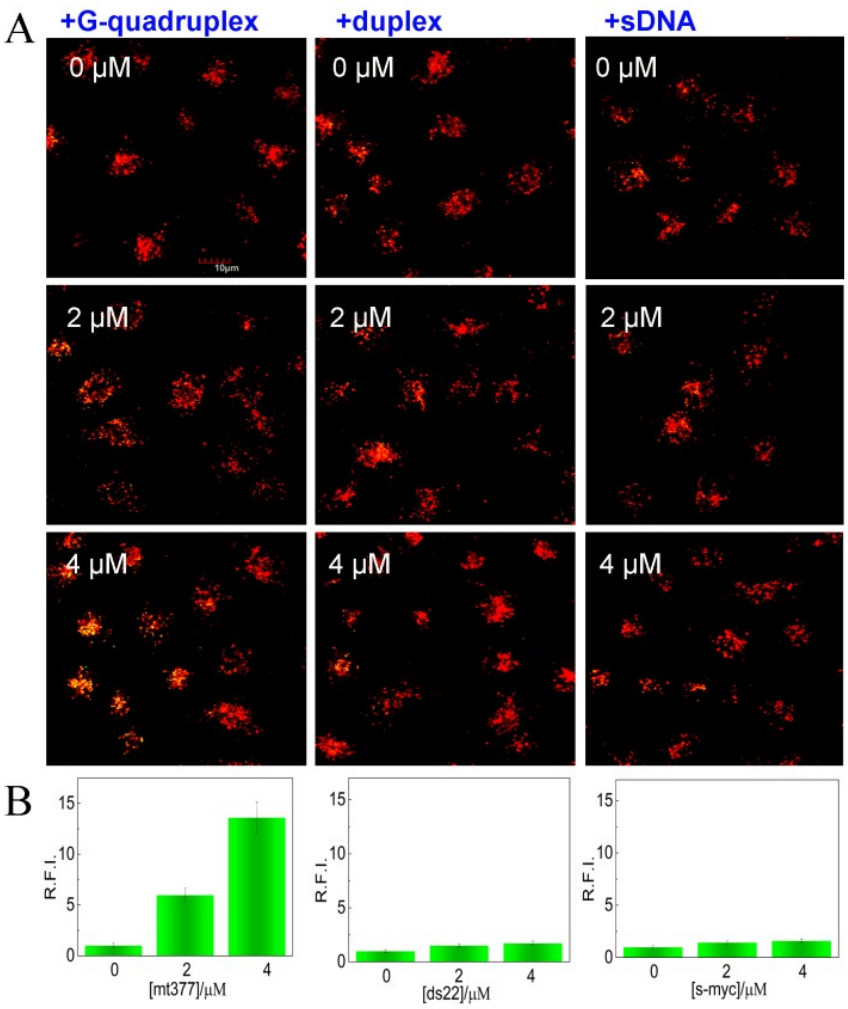


Figure S33. (A) CLSM images of live HeLa cells stained with DMOTY (5 μM, Ex 405 nm) and then incubated with G-quadruplex DNA (mt377), single-stranded DNA (s-myc), duplex DNA (ds22), respectively. Lysosomes were labeled as red with LysoTracker Red DND-99 (50 nM, Ex 559 nm). Scale bar, 10 μm. (B) Quantification of the DMOTY fluorescence intensity for A. For each sample, approximately 300 cells were measured.

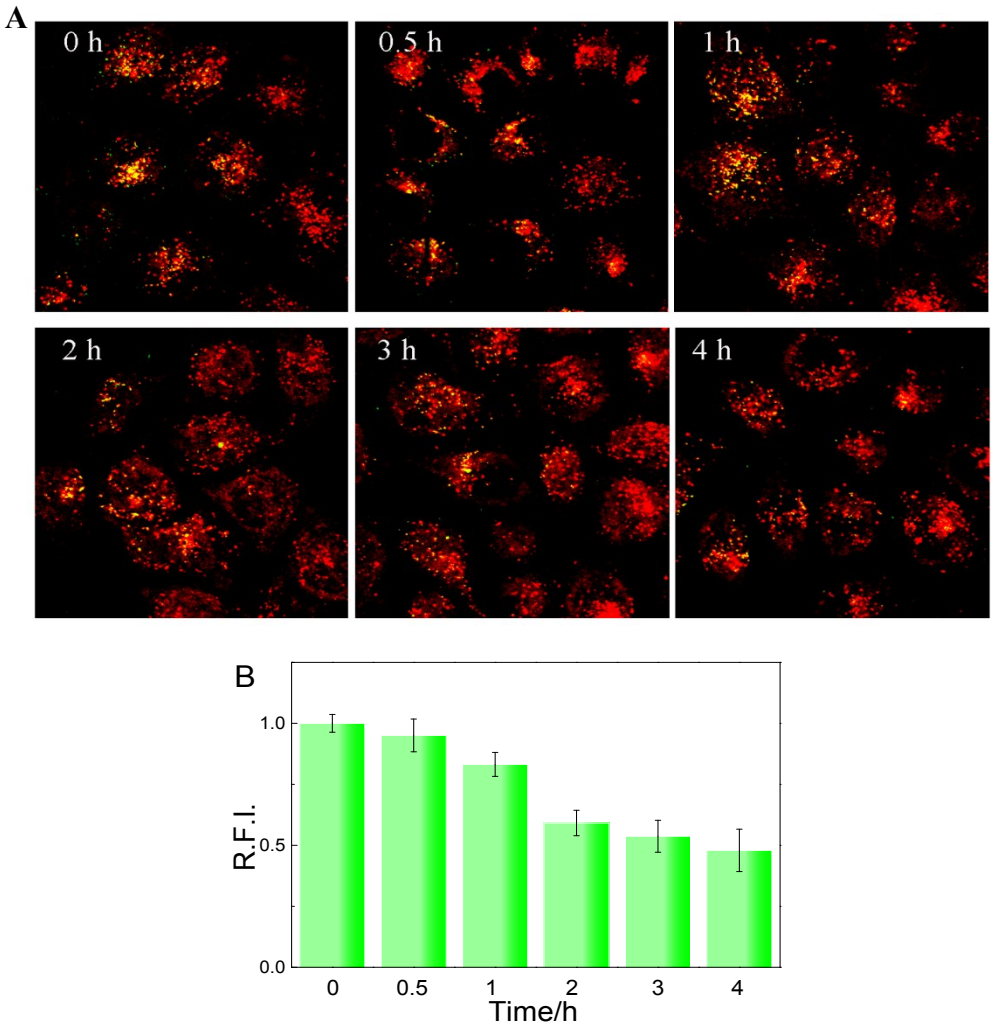


Figure S34. (A) Real-time confocal imaging of live HeLa cells incubated with unlabeled mt377 G-quadruplexes (2 μM). Live HeLa cells were first cultured in the medium containing mt377 G-quadruplexes (2 μM) for 2 h, then DMOTY (10 μM) was added to the medium for 4 h, after which mt377 was washed away with a blank medium containing DMOTY (10 μM). Cell images were collected at different points of time. Prior to imaging, lysosomes were stained with Lyso Tracker Red DND-99 (50 nM, Ex 559 nm) for 10 min. (B) Quantification of the DMOTY fluorescence intensity for A. For each sample, approximately 300 cells were measured.

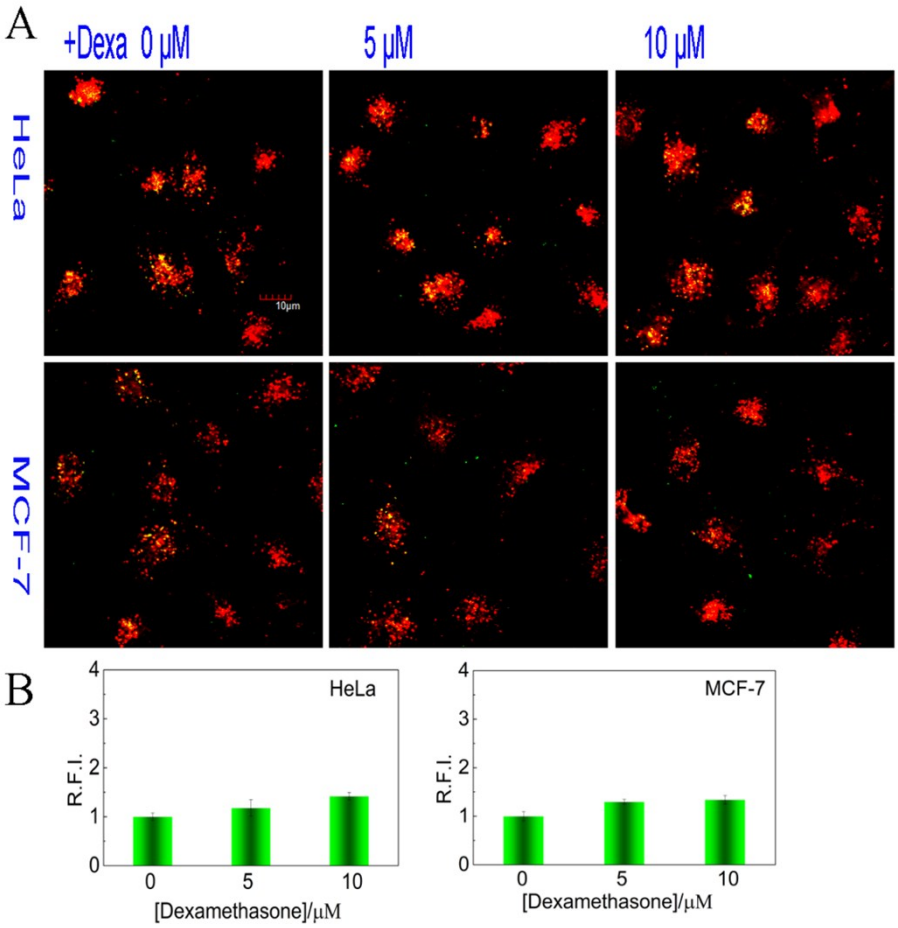


Figure S35. (A) CLSM images of live HeLa and MCF-7 cells after 0.5 h incubation of cells with dexamethasone (5, 10 μM) and DMOTY (10 μM, Ex 405 nm). Lysosomes were stained with Lyso Tracker Red DND-99 (50 nM, Ex 559 nm). Scale bar, 10 μm. (B) Quantification of the DMOTY fluorescence intensity for A. For each sample, approximately 300 cells were measured.

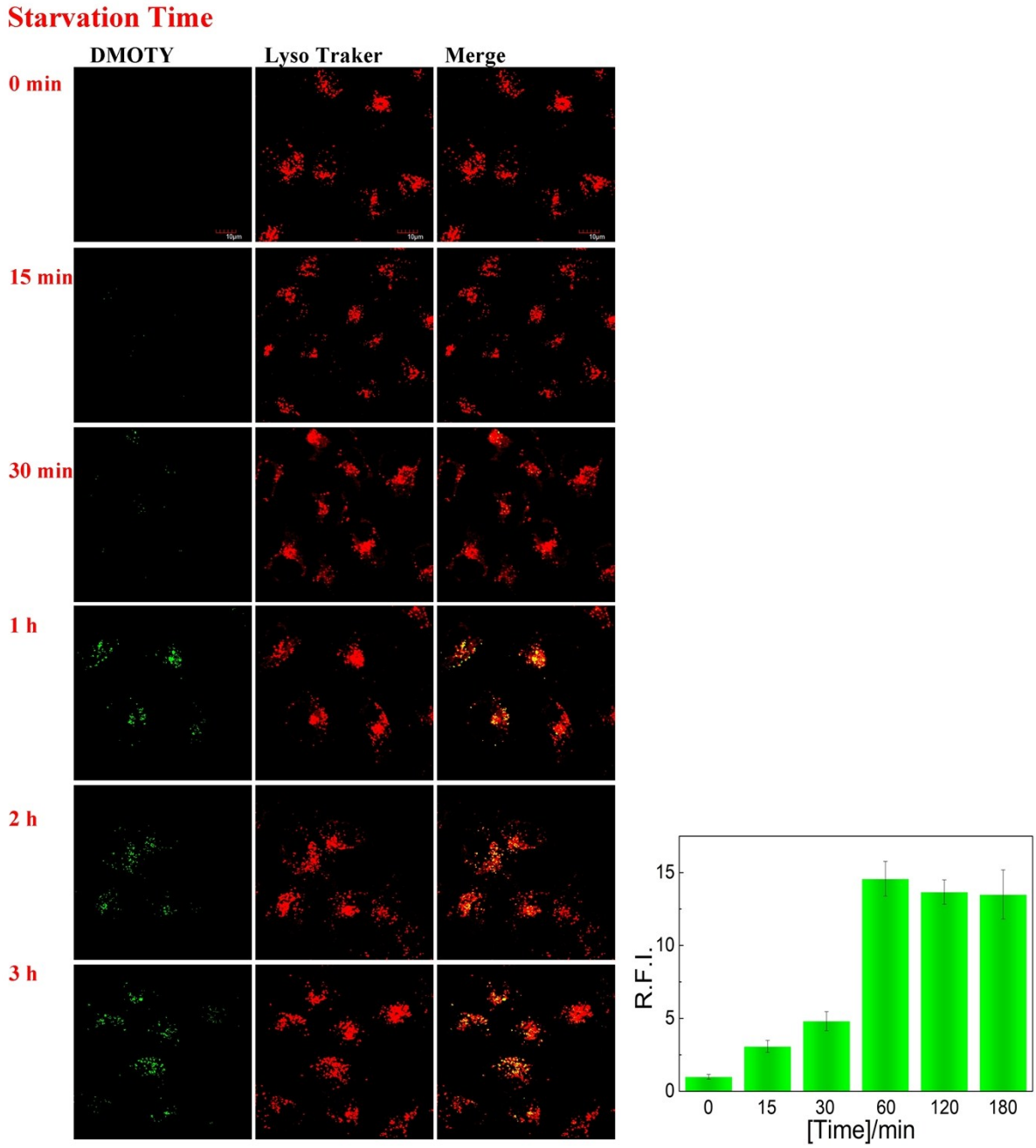


Figure S36. Confocal images of MCF-7 cells incubated with DMOTY (10 μM, Ex 405 nm) in serum-free medium. Lysosomes were stained with Lyso Tracker Red DND-99 (50 nM, Ex 559 nm). The histogram is quantification of the DMOTY fluorescence intensity. For each sample, approximately 300 cells were measured.

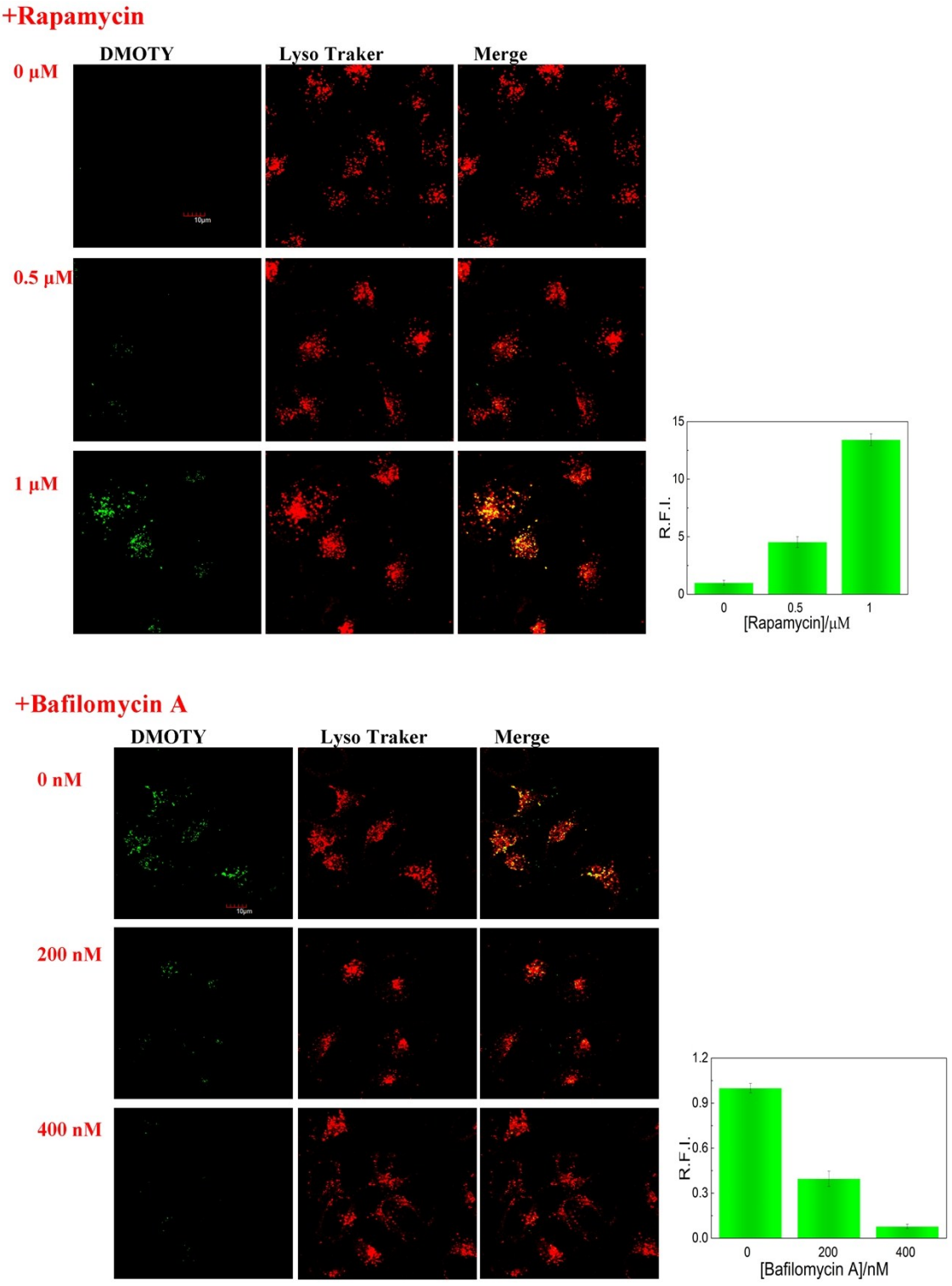


Figure S37. Confocal images of MCF-7 cells incubated with DMOTY (10 μM, Ex 405 nm) in complete medium with different concentrations of Rapamycin (0, 0.5 μM, 1 μM) and in serum-free medium with different concentrations of Bafilomycin A_1 (0, 200 nM, 400 nM). Lysosomes were stained with Lyso Tracker Red DND-99 (50 nM, Ex 559 nm). The histogram is quantification of the DMOTY fluorescence intensity. For each sample, approximately 300 cells were measured.

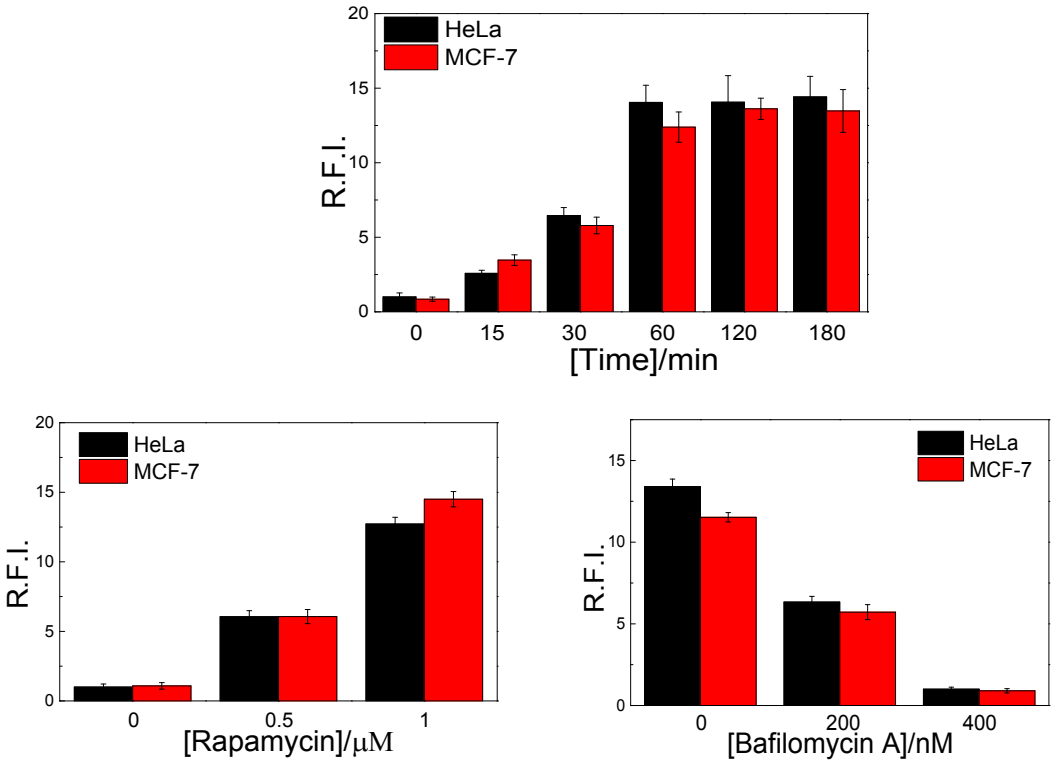


Figure S38. The relative fluorescence intensity of DMOTY in HeLa and MCF-7 cells with the starvation treatment and drug stimulation. The fluorescence intensity of DMOTY in untreated HeLa cells was set to 1.

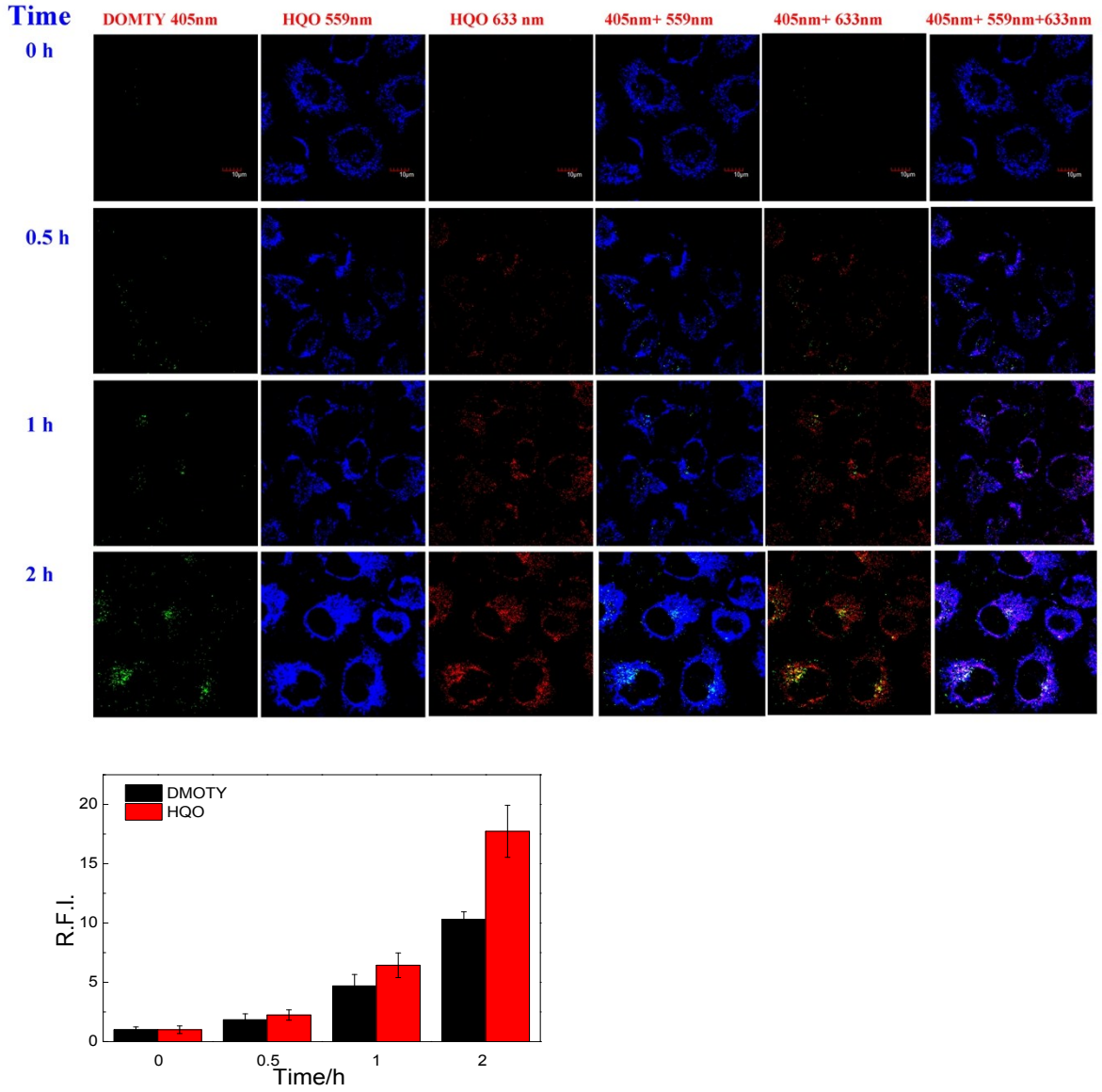


Figure S39. Fluorescence imaging of MCF-7 cells costained with DMOTY (10 μM, Ex 405 nm) and HQO (20 μM, Ex 559 nm & 633 nm) in serum-free medium. The histogram is quantification of the DMOTY fluorescence intensity. For each sample, approximately 300 cells were measured. The fluorescence intensity measured at 0 h was set to 1.

References

(1) F. M. Hamer in *The cyanine dyes and related compounds* (Eds.: A. Weissberger), Interscience Publishers, New York-London, **1964**.

(2) G. E. Ficken in *Cyanine Dyes* (Eds.: K. Venkataraman), Academic Press, **1971**.

(3) L. Wang, Y. Xiao, W. Tian, L. Deng, *J. Am. Chem. Soc.* **2013**, *135*, 2903−2906.

(4) L. Hou, P. Ning, Y. Feng, Y. Ding, L. Bai, L. Li, H. Yu, X. Meng, *Anal. Chem.* **2018**, *90*, 7122−7126.

(5) Karsisiotis, A. I.; Hessari, N. M.; Novellino, E.; Spada, G. P.; Randazzo, A.; Webba da Silva, M. Topological characterization of nucleic acid G-quadruplexes by UV absorption and circular dichroism, *Angew. Chem. Int. Ed.* **2011**, *50*, 10645–10648.

(6) Dey, S.; Jaschke, A. Tuning the stereoselectivity of a DNA-catalyzed michael addition through covalent modification. *Angew. Chem. Int. Ed.* **2015**, *54*, 11279–11282.

(7) Priyakumar, U. D.; Hyeon, C.; Thirumalai, D.; MacKerell, A. D. Urea destabilizes RNA by forming stacking interactions and multiple hydrogen bonds with nucleic acid bases. *J. Am. Chem. Soc.* **2009**, 131, 17759–17761.

(8) Ueda,Y. M.; Zouzumi, Y. K.; Maruyama, A.; Nakano, S. I.; Sugimoto, N.; Miyoshi, D. Effects of trimethylamine N-oxide and urea on DNA duplex and G-quadruplex. *Sci. Technol. Adv. Mat.* **2016**, *17*, 753–759.

(9) Rachwal, P. A.; Fox, K. R. Quadruplex melting. *Methods* **2007**, *43*, 291–301.

(10) Green, J. J.; Ying, L.; Klenerman, D.; Balasubramanian, S. Kinetics of unfolding the human telomeric DNA quadruplex using a PNA trap. *J. Am. Chem. Soc.* **2003**, *125*, 3763–3767.

(11) Paramasivan, S.; Rujan, I.; Bolton, P. H. Circular dichroism of quadruplex DNAs: Applications to structure, cation effects and ligand binding. *Methods* **2007**, *43*, 324–331.