Electronic Supplementary Information (ESI)

Photostable AIE Fluorogens for Accurate and Sensitive Detection of S-Phase DNA Synthesis and Cell Proliferation

Yueyue Zhao,[ab] Chris Y. Y. Yu,[ab] Ryan T. K. Kwok,[ab] Yilong Chen,[ab] Sijie Chen,[ab] Jacky W. Y. Lam[ab] and Ben Zhong Tang[abc]*

[a] Department of Chemistry, Institute for Advanced Study, Division of Biomedical Engineering, Division of Life Science, State Key Laboratory of Molecular Neuroscience, Institute of Molecular Functional Materials, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

[b] Guangdong Innovative Research Team, SCUT-HKUST Joint Research Laboratory, State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, China

[c] HKUST Shenzhen Research Institute, No. 9 Yuexing First RD, South Area, Hi-tech Park, Nanshan, Shenzhen 518057, China

Table of Contents

Experimental Section

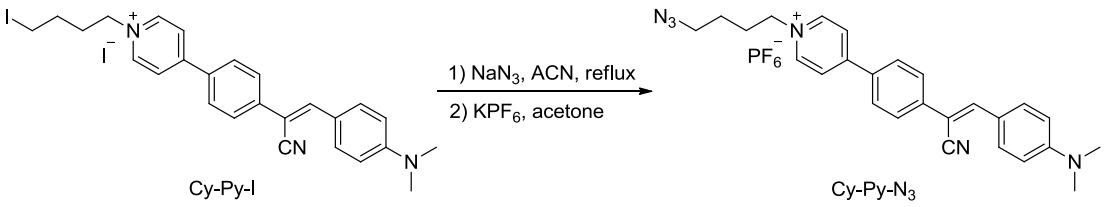
Materials

Sodium azide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), formaldehyde, Triton X-100, dimethylsulfoxide (DMSO), copper (II) sulfate ($CuSO_4$), ascorbic acid, bovine serum albumin (BSA) were all purchased from Sigma-Aldrich. Modified essential medium (MEM), 5-ethynyl-2'-deoxyuridine (EdU) and Alexa647-azide were obtained from Invitrogen. Fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Life Technologies. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation , United States).

Instruments

^{1}H NMR spectra were measured on a Bruker AV 400 NMR spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS; δ = 0) was chosen as an internal reference. The UV spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. High-resolution mass spectra (HRMS) were obtained on a GCT Premier CAB 048 mass spectrometer operated in MALDI-TOF mode.

Scheme S1. Synthetic route to Cy-Py-N_3.



Synthesis of Cy-Py-N_3

Into a 100 mL two-necked round bottom flask equipped a condenser was added an acetonitrile solution (5 mL) of Cy-Py-I (50.0 mg, 0.08 mmol) and sodium azide (20.5 mg, 0.32 mmol). After reflux for 8 h, the mixture was cooled to room temperature poured into diethyl ether. The precipitates formed were collected by suction filtration and re-dissolved in acetone with 5 mL of saturated KPF_6 solution. The resulting mixture was stirred for 1 h. After solvent evaporation, dark red precipitates were formed. The precipitates were washed with water three times and obtained in a yield of 95%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.09–9.07 (d, 2H, J = 6.8 Hz), 8.57–8.55 (d, 2H, J = 6.8 Hz), 8.20–8.18 (d, 2H, J = 8.4 Hz), 8.05 (s, 1H), 7.93–7.91 (m, 4H,), 6.84–6.82 (d, 2H, J = 8.8 Hz), 4.60–4.57 (t, 2H, J = 7.2 Hz), 3.40–3.33 (t, 2H, J = 6.4 Hz), 3.04 (s, 6H), 2.02–1.95 (m, 2H), 1.59–1.52 (m, 2H). HRMS (MALDI-TOF): m/z 423.2243 (M^+, calcd. 423.5322).

Cell culture

Human cervical cancer cell (HeLa) line was provided by American Type Culture Collection. The HeLa cells were cultured in MEM containing 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin (Thermo Scientific), and maintained at 37 ℃ in a humidified incubator with 5% CO_2. Before experiment, the cells were pre-cultured until confluence was reached.

EdU labeling of HeLa cells and cell fixation

HeLa cells were grown on glass coverslips in MEM supplemented with 10% FBS, penicillin and streptomycin. EdU was added to the culture medium with concentration ranging from 10 μM to 200 μM, for durations of time between 1 and 24 h. After EdU labeling, the cells were fixed by using 4% formaldehyde in PBS for 20 min.

Fluorescent dye staining

After fixation, cells were rinsed once with 3% BSA in PBS followed by adding 0.5% Triton X-100 for 30 min. The cells were incubated in 1–200 μM fluorescent dyes (from 10–100 mM stock solution in DMSO), 100 mM Tris buffer (pH = 8.5), 1 mM $CuSO_4$ and 50–100 mM ascorbic acid for 30 min. The staining mixture was freshly

prepared each time and was used for staining cells immediately after addition of ascorbate.

Cell imaging

The fluorescent labelled cells were mounted in standard mounting media and imaged by fluorescence microscopy (BX 41 Microscope). Conditions: excitation wavelength = 330–385 nm, dichroic mirror = 400 nm and emission long pass filter = 420 nm (TPE-Py-N_3);, excitation filter = 540–580 nm, dichroic mirror = 600 nm and emission long pass filter = 610 nm (Cy-Py-N_3).

Photostability test

The fluorescent labelled cells were imaged by stimulated emission depletion (STED) super resolution laser scanning microscope. Excitation wavelength: 442 nm (TPE-Py-N_3), 458 nm (Cy-Py-N_3) and 633 nm (Alexa647-azide); emission filter: 450–750 nm (TPE-Py-N_3), 480–750 nm (Cy-Py-N_3) and 640–750 nm (Alexa647-azide). Laser power was unified as 0.3 μW.

Flow cytometry study

The HeLa cells were precultured in a 35 mm petri dish to achieve desired confluence and incubated with EdU for the designated time. After incubation, the HeLa cells were treated with trypsin, washed with PBS twice. Flow cytometry analysis were conducted using Becton Dickinson FACSAria IIIu flow cytometer. The mean fluorescence was determined by counting 10,000 events.

Cytotoxicity studies

MTT assays were used to evaluate the cytotoxicity of EdU. The cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 5×10^3 cells/mL. After 24 h incubation, the cells were exposed to a series of doses of EdU in culture medium at 37 ℃. After 24 h incubation, 10 μL of freshly prepared MTT solution (5 mg/mL in PBS) was added into each well. After 4 h incubation, 100 μL of solubilization solution containing 10% SDS and 0.01 M HCl was added to dissolve the purple crystals. After 4 h incubation, the absorbance of MTT at 595 nm was monitored using a Perkin-Elmer Victor plate reader. Cell viability was expressed by the ratio of absorbance of the cells incubated with EdU to that of the cells incubated with culture medium only. Each of the experiments was performed at least three times.

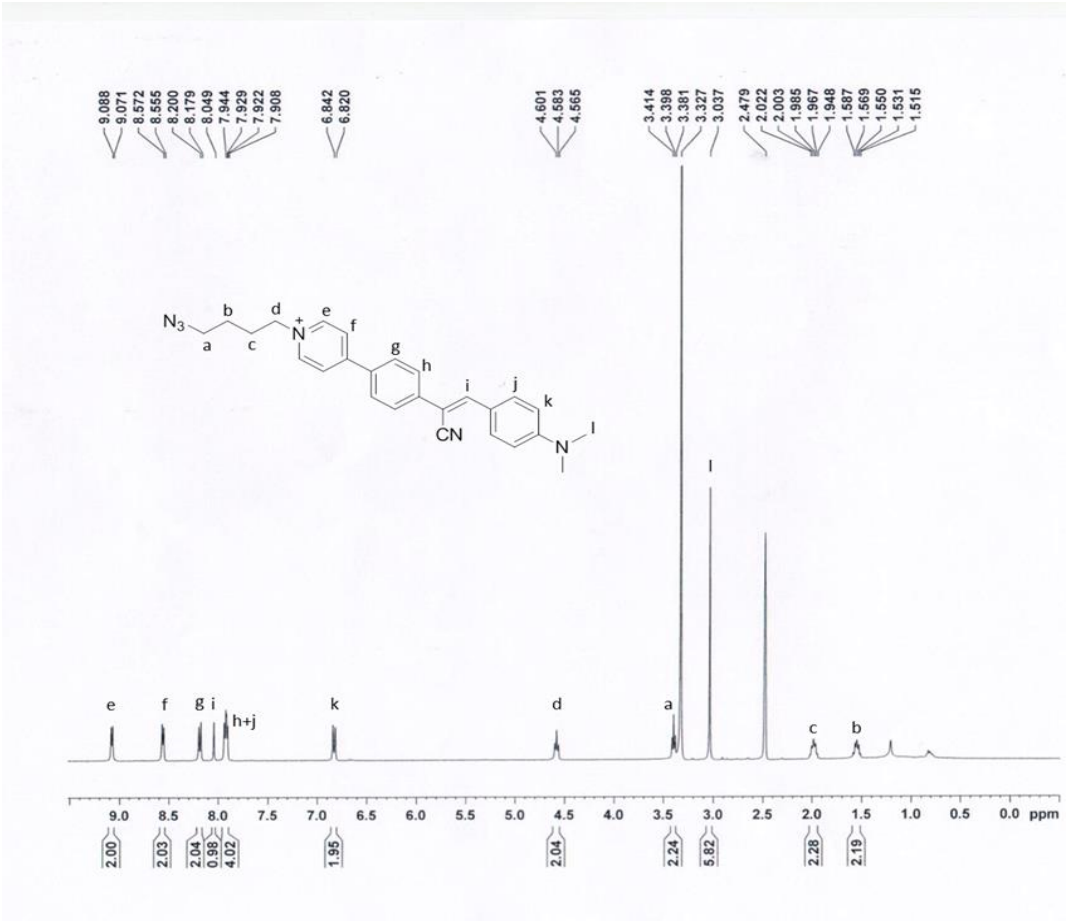


Figure S1. ^{1}H NMR spectrum of Cy-Py-N_3 in $CDCl_3$.

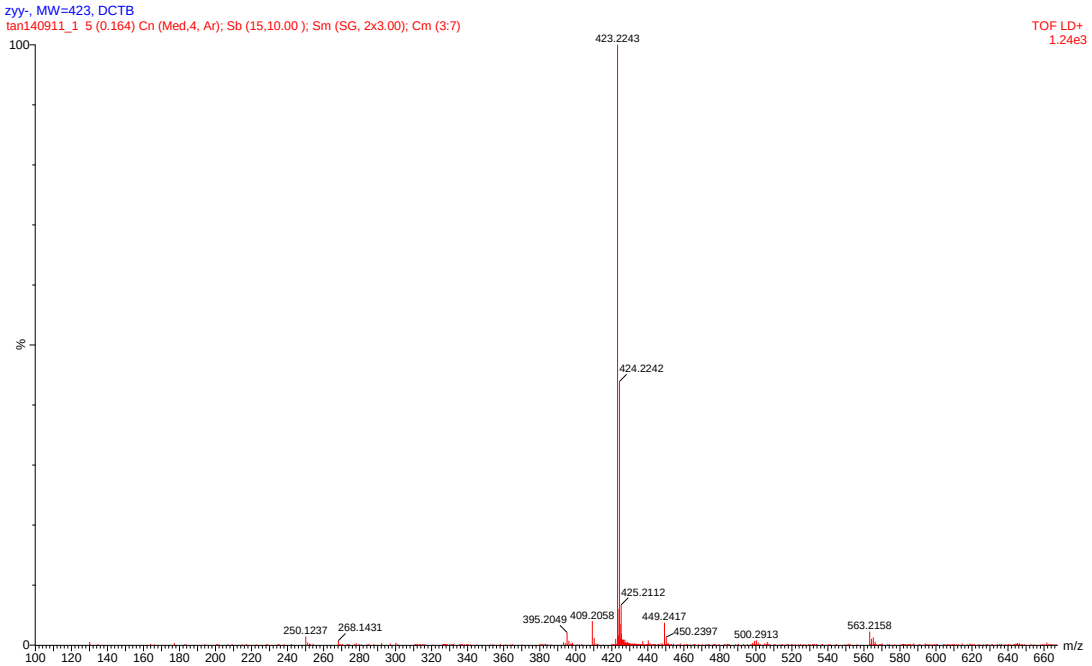


Figure S2. High resolution mass spectrum of Cy-Py-N_3.

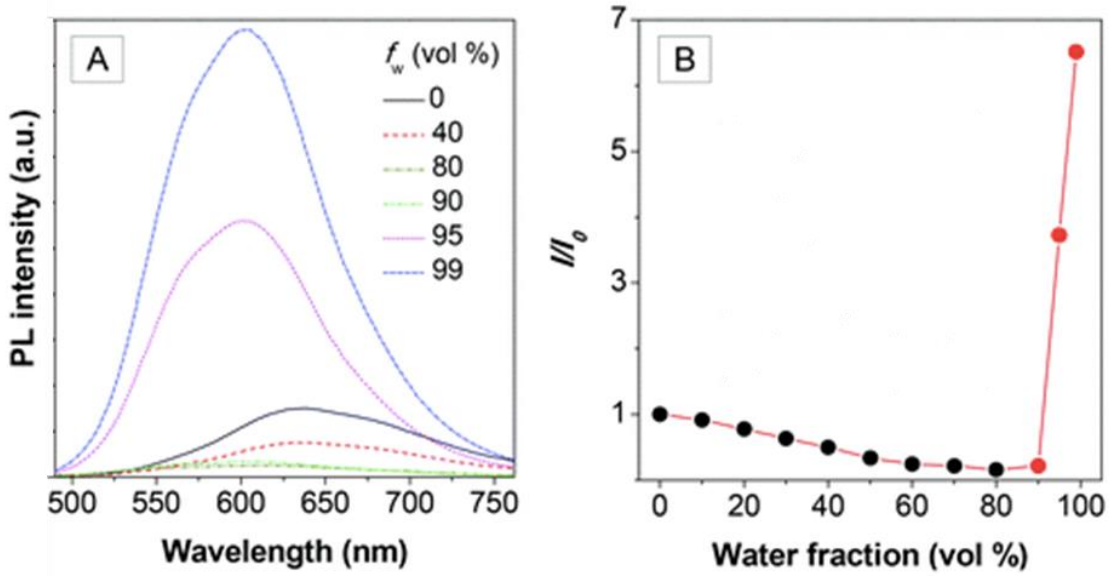


Figure S3 (A) PL spectra of TPE-Py-N_3 in DMSO/water mixtures with different water fractions (f_w). (B) Plot of (I/I_0) values *versus* the compositions of the aqueous mixtures of TPE-Py-N_3. I_0 = PL intensity in pure DMSO solution. [TPE-Py-N_3] = 10 μM; excitation wavelength = 405 nm.

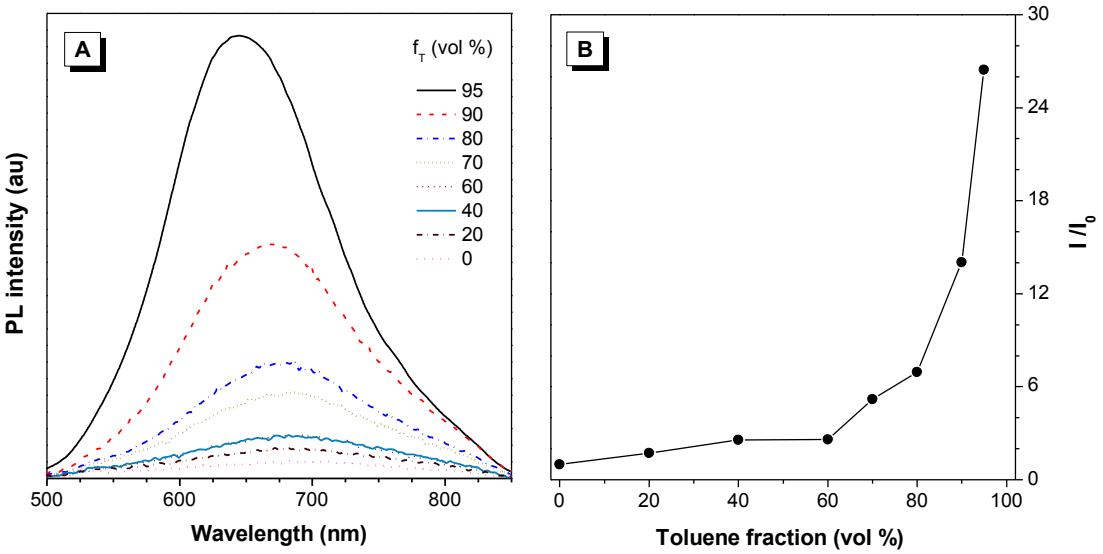


Figure S4. (A) PL spectra of Cy-Py-N_3 in DMSO/toluene mixtures with different toluene fractions (f_T). (B) Plot of (I/I_0) values *versus* the compositions of the aqueous mixtures of Cy-Py-N_3. I_0 = PL intensity in pure DMSO solution. [Cy-Py-N_3] = 10 μM; excitation wavelength = 460 nm.

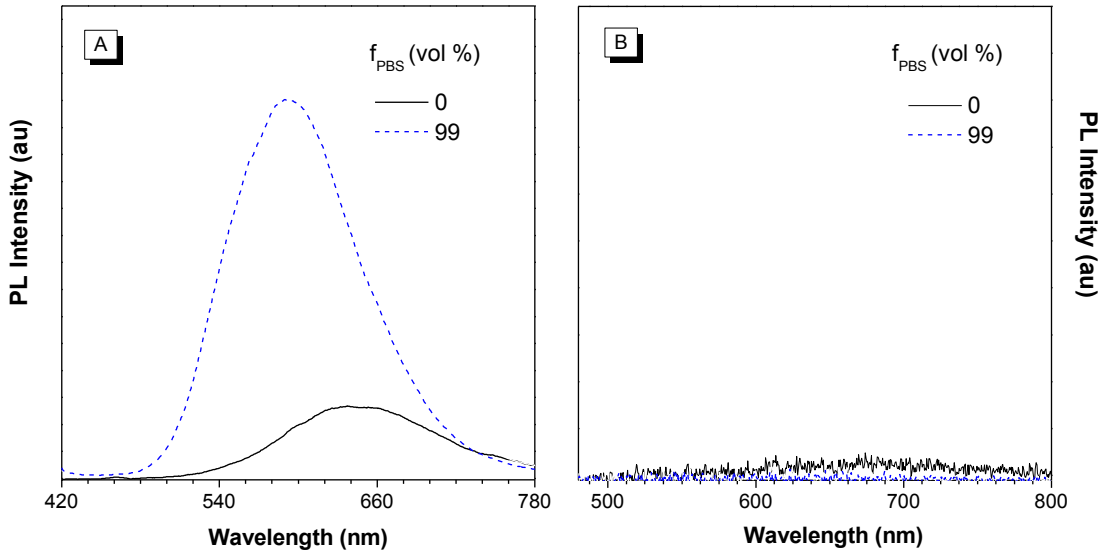


Figure S5. PL spectra of (A) TPE-Py-N_3 and (B) Cy-Py-N_3 in DMSO/PBS mixtures with different PBS fractions (f_{PBS}). [TPE-Py-N_3] = [Cy-Py-N_3] = 10 μM.

Table S1. Summary of emission properties of TPE-Py-N_3 and Cy-Py-N_3.

	λ_{ex} (nm)	λ_{em} (nm)	Φ_F (in DMSO solution)	Φ_F (solid state)
TPE-Py-N_3	405	618	negligible	0.1772
Cy-Py-N_3	470	613	negligible	0.0150

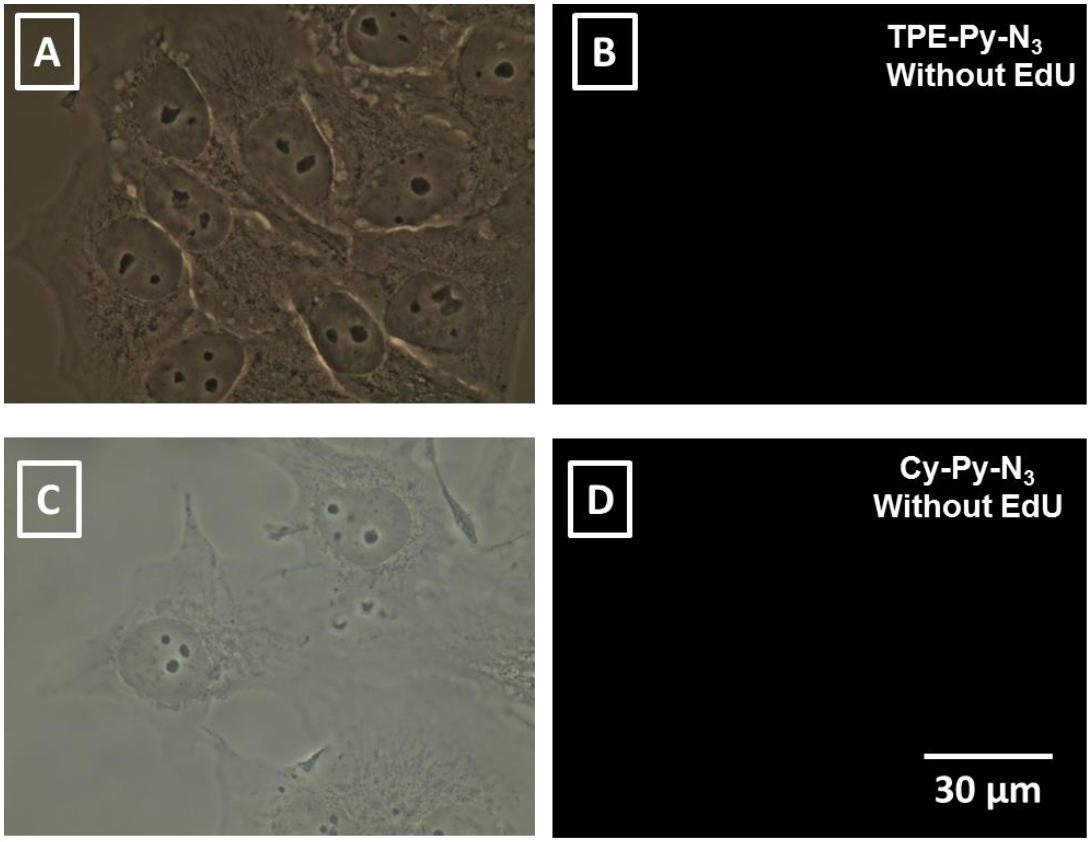


Figure S6. (A and C) Bright field and (B and D) fluorescent images of fixed HeLa cells stained with (A and B) TPE-Py-N_3, and (C and D) Cy-Py-N_3 for 30 min followed by washing with pure DMSO. [TPE-Py-N_3] = [Cy-Py-N_3] = 20 μM. Excitation wavelength: 330–385 nm (TPE-Py-N_3) and 520–560 nm (Cy-Py-N_3). All the images share the same scale bar = 30 μm.

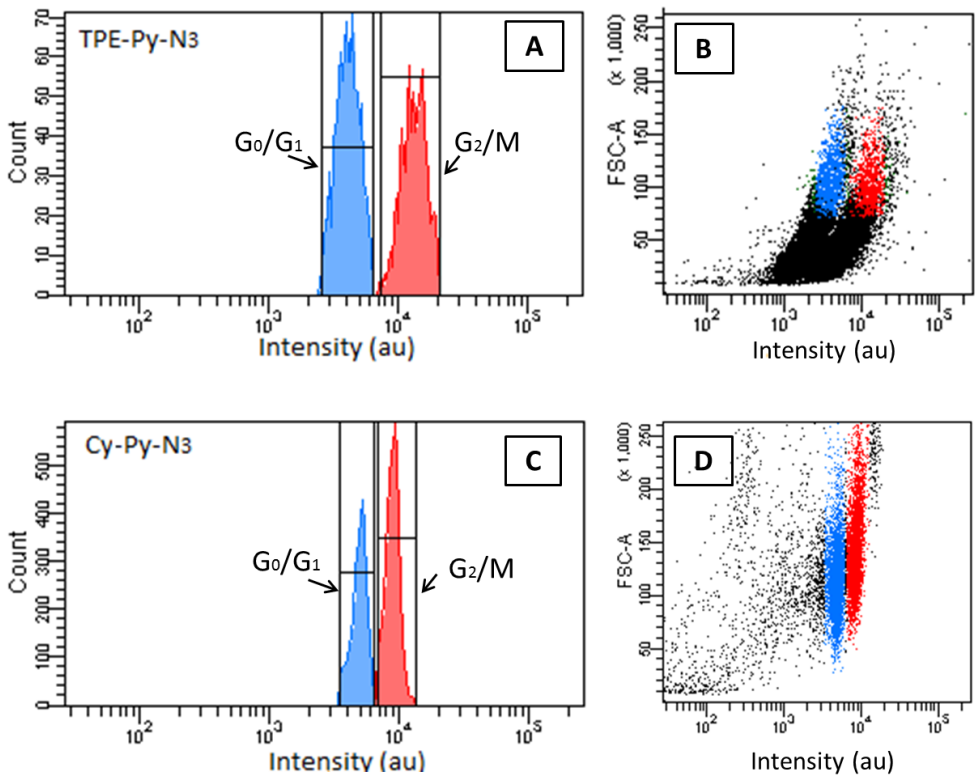


Figure S7. Flow cytograms of HeLa cells labeled with (A and B) TPE-Py-N_3 and (C and D) Cy-Py-N_3. The data was acquired using 405 nm, 488 nm, and 633 nm lasers and analyzed with FACSAria software. The blue peak represents the G_1 phase and the red peak suggests the occurrence of G_2/M phase.

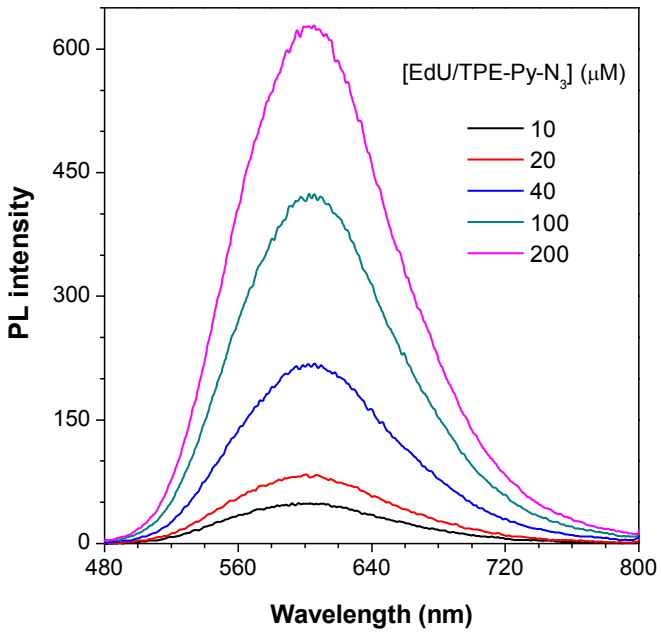


Figure S8. Change in PL from HeLa cells stained with different concentrations of EdU/TPE-Py-N_3 (1:1, M/M). Excitation wavelength = 405 nm.

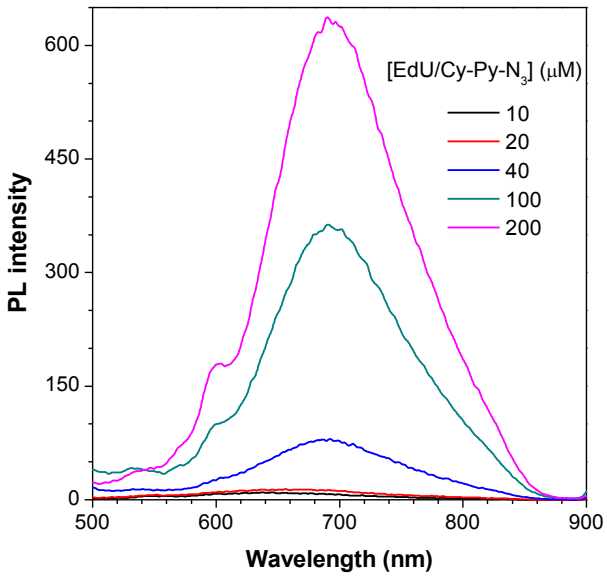


Figure S9. Change in PL from HeLa cells stained with different concentrations of EdU/Cy-Py-N_3 (1:1, M/M). Excitation wavelength = 460 nm.

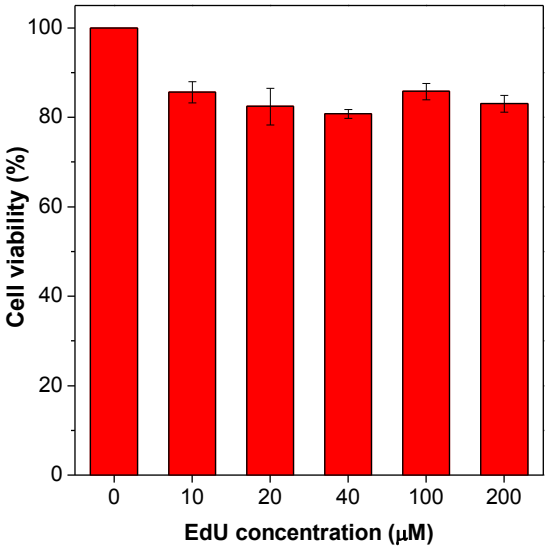


Figure S10. Cell viability of HeLa cells incubated different EdU concentrations.

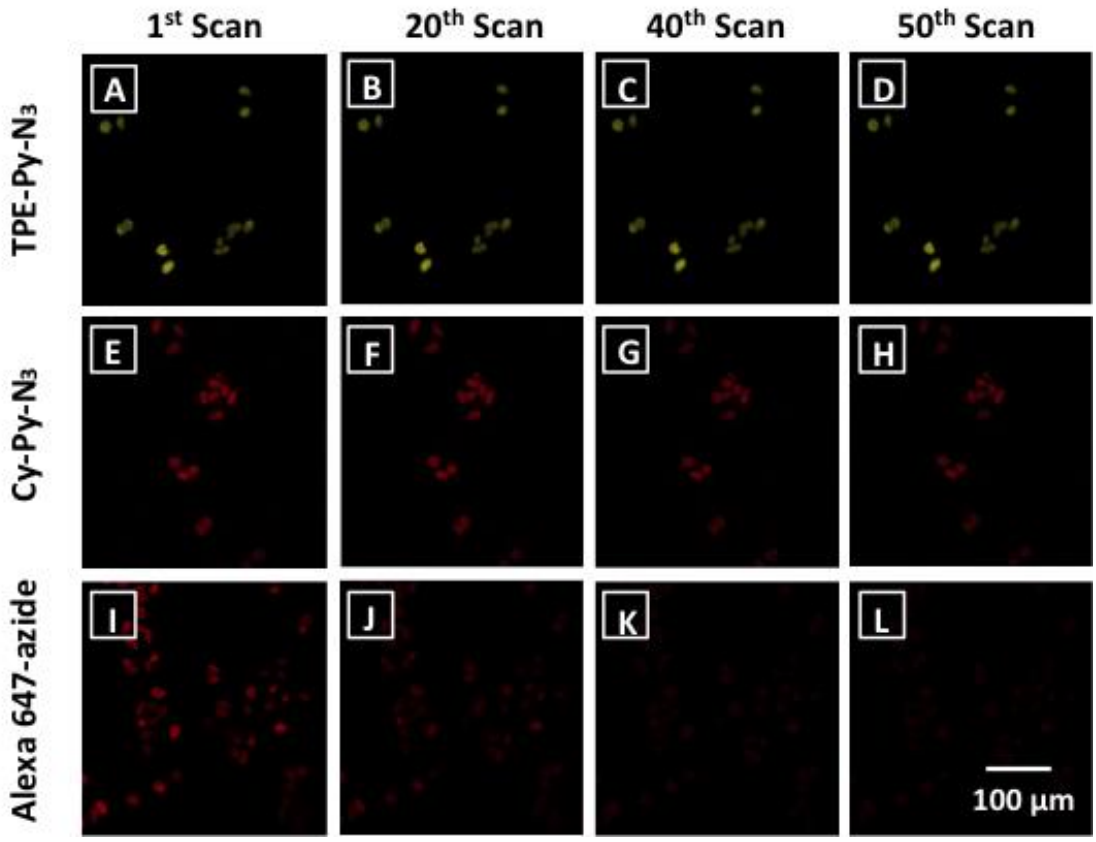


Figure S11. Fluorescent images of dye-labelled fixed HeLa cells obtained at different scans. Excitation wavelength: 442 nm (TPE-Py-N_3) and 458 nm (Cy-Py-N_3) and 633 nm (Alexa647-azide); emission filter: 450–750 nm (TPE-Py-N_3), 480–750 nm (Cy-Py-N_3) and 640–750 nm (Alexa647-azide). Irradiation time: 5.24 s/scan. Laser power: 0.3 μW. All the images share the same scale bar.