Supplementary materials for Analytical Chemistry

Two-Photon Graphene Oxide/Aptamer Nanosensing Conjugate for *In Vitro* or *In Vivo* Molecular Probing

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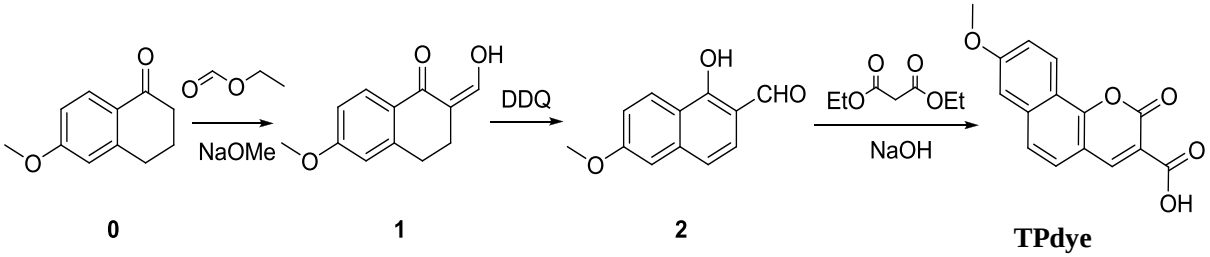
1. Synthesis of the two-photon dye (TPdye).



Scheme S1. Synthetic routs for the TPdye.

***Preparation of 1*:** To a mixture of sodium methoxide (3.29 g, 60 mmol) and ethyl formate (2.25 mL, 27.8 mmol) in dry benzene (30 mL) at room temperature was added dropwise a solution of 6-methoxy-1-tetralone (1.76 g, 10 mmol) in dry benzene

(30 mL). After addition, the reaction mixture was stirred at room temperature for 4 h. Et_2O (50 mL) was added, and the resulting mixture was washed with water, pH 12. The pH of the aqueous layer was adjusted to 4.0 and extracted with Et_2O. The organic layer was washed with water and brine, then dried over $MgSO_4$, and the solvent was evaporated under reduced pressure to give crude solid. Which was purified by column chromatography on silica gel using petrol ether : ethyl acetate (20:1) as the mobile phase to afford brownish solid **1** (1.932 g, 95% yield). H^1 NMR (400 MHz, $CDCl_3$): δ 2.54 (m, 2H), 2.86 (m, 2H), 3.94 (s, 3H), 6.61(d, 1H), 6.82 (dd, 1H), 7.92 (d, 1H), 9.88 (s, 1H), 12.28 (br, 1H); EI-MS m/z: $\mathbf{1}^+$ calc. for, 204.08; found, 204.1

***Preparation of 2*:** A mixture of **1** (1.5 g, 20.74 mmol) and dichloro-2,3-dicyano-5,6-benzoquinone (DDQ) (1.95 g, 8.8 mmol) in dioxane (40 mL) was stirred at room temperature for 1 h. The hydroquinone was filtered off, and the solvent was removed under reduced pressure. The remaining product was purified by flash chromatography (PE : CH_2Cl_2 =1 : 9) to yield **2** as an yellow powder (1.46 g, 98 %). H^1 NMR (400 MHz, CDCl3): δ 3.91 (s, 3H), 2.86 (m, 2H), 7.01(d, 1H), 7.13 (dd, 1H), 7.17 (d, 1H), 7.36 (d, 1H), 9.84 (s, 1H), 12.67 (br, 1H); EI-MS m/z: $\mathbf{2}^+$ calc. for, 202.06; found, 202.1

***Preparation of the TPdye*:** A solution of **2** (0.76 g, 3.7 mmol) and diethyl malonate (9.6 g, 0.06 mol) in EtOH (40 mL) was treated with piperidine (0.5 mL) and glacial acetic acid (7 drops) and refluxed for 12 h. To the reaction mixture was added 100 mL of H_2O, and the mixture was cooled to 0 °C. The crystalline solid was filtered and washed by 50% cold ethanol (20 mL). Recrystallization from 50% EtOH gave colorless crystals **TP-COOEt** (1.1 g, 71 %). To the solution of **TP-COOEt** (0.5 g, 1.68 mmol) in EtOH (15 mL) was added 15 mL of 10 % NaOH and heated under reflux for 1 h. Acidification to pH 2.0 using concentrated hydrochloric acid and cooling to 0 °C gave a yellow crystalline deposit **TPdye** (386 mg, 85 %). H^1 NMR (400 MHz, DMSO-d_6): δ 3.95 (s, 3H), 7.36(dd, 1H), 7.51 (d, 1H), 7.75-7.83 (m, 2H), 8.30 (d, 1H), 8.87 (s, 1H), 13.15 (br, 1H). C^{13} NMR (400 MHz, DMSO-d_6): δ 56.02, 107.67, 112.47, 115.69, 116.81, 120.04, 123.93, 124.30, 126.13, 138.25, 150.20, 153.18, 157.30, 160.80, 164.37. EI-MS m/z: $\mathbf{TPdye}^+$ calc. for, 270.05; found, 270.1

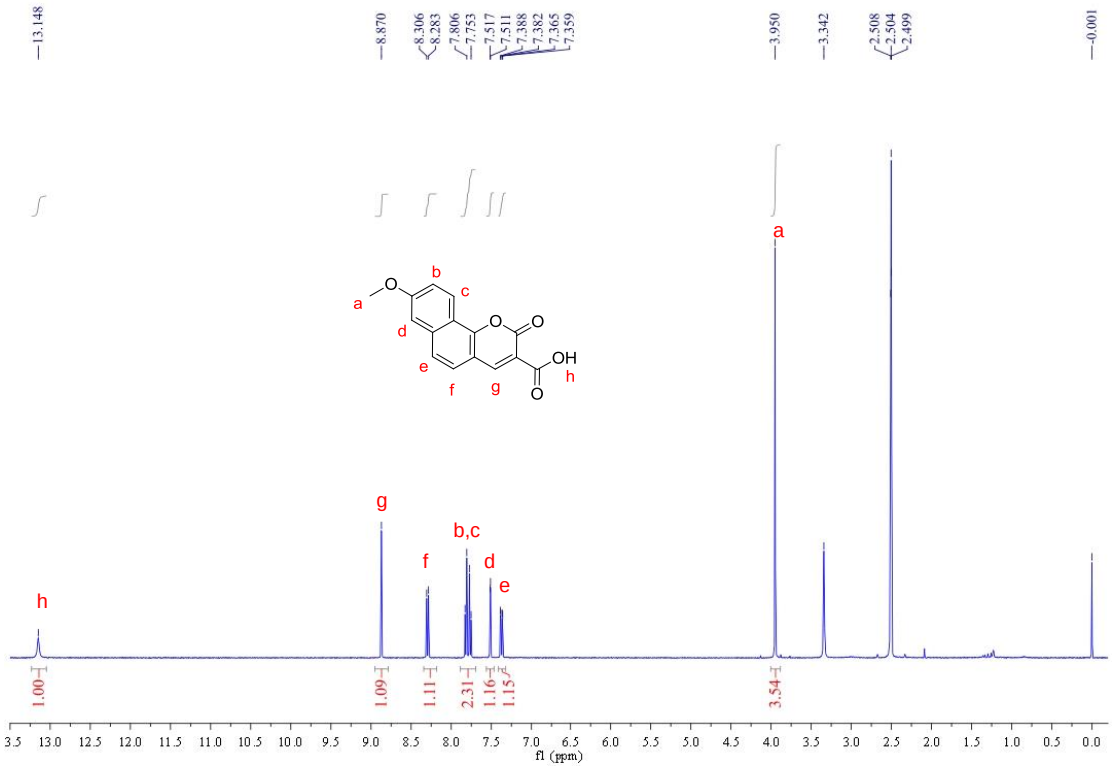
Reference:

[1] Dax, C.; Coincon, M.; Sygusch, J.; Blonski, C Biochemistry 2005, 44, 5430-5443.

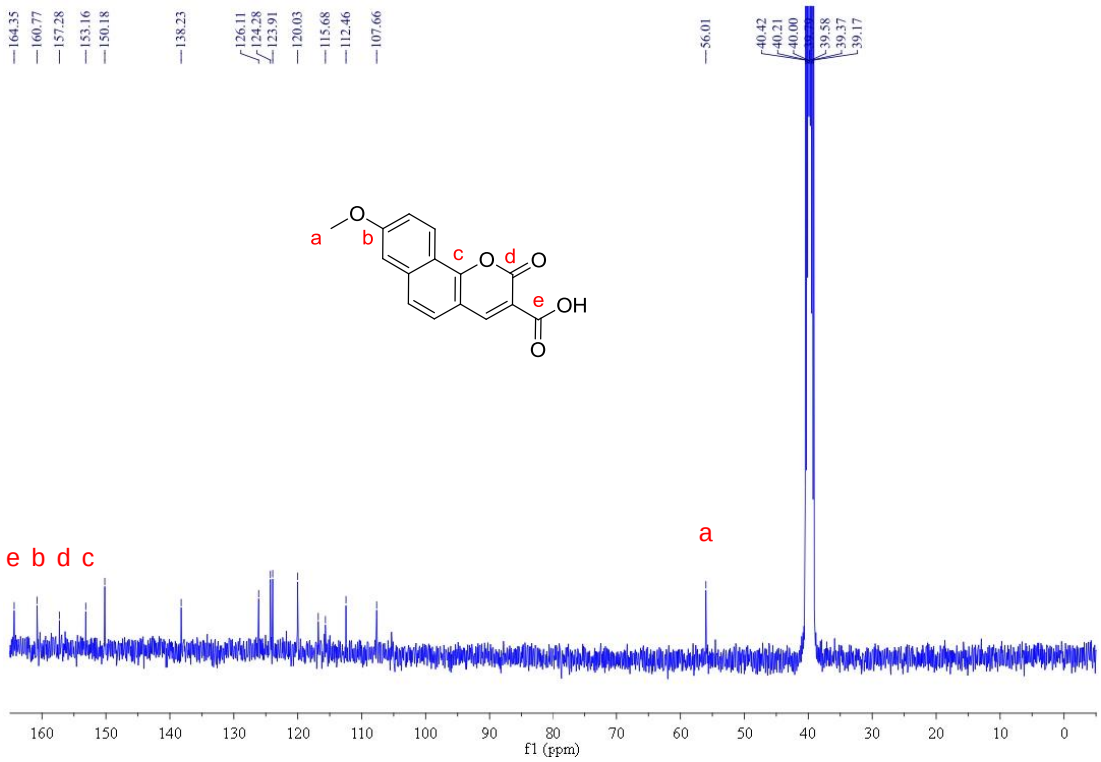
[2] Ma, Y. M.; Luo, W.; Quinn, P. J.; Liu, Z. D.; Hider, R. C. J. Med. Chem. 2004, 47, 6349-6362.

2. Characterization of the synthetic compounds.

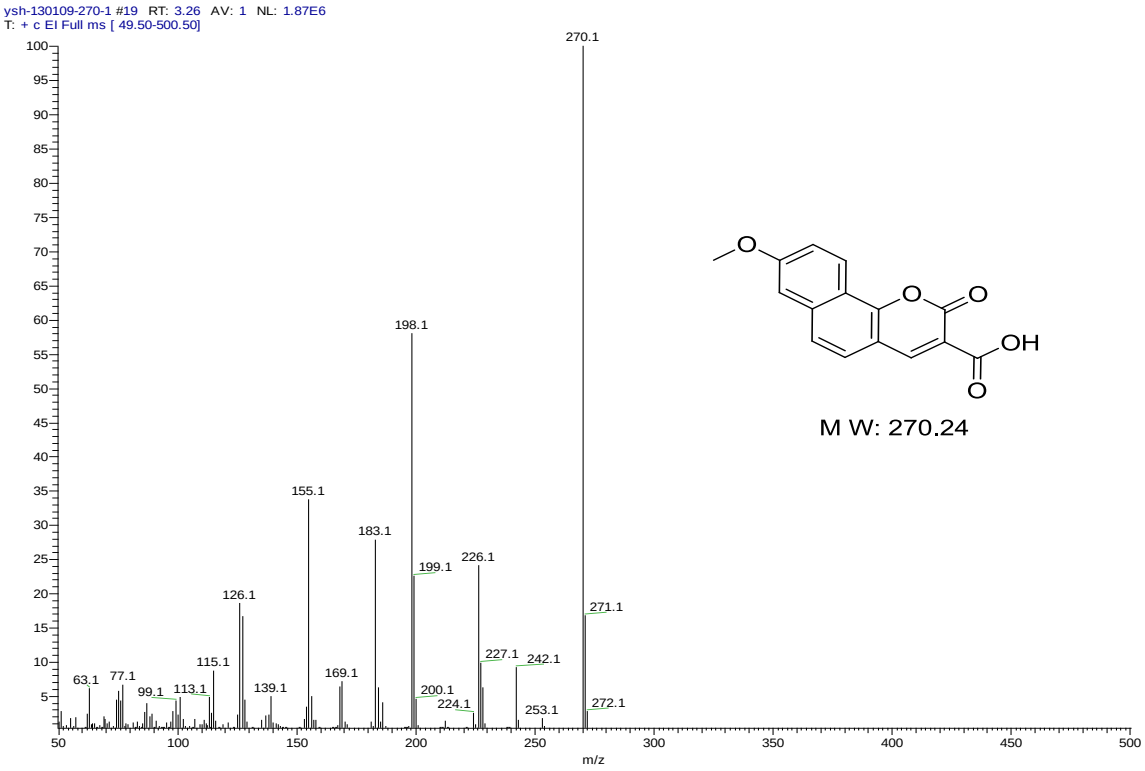
^{1}H NMR of **TPdye** in DMSO-d_6



^{13}C NMR of **TPdye** in DMSO-d_6



EI-MS of **TPdye**



3. Spectroscopic data.

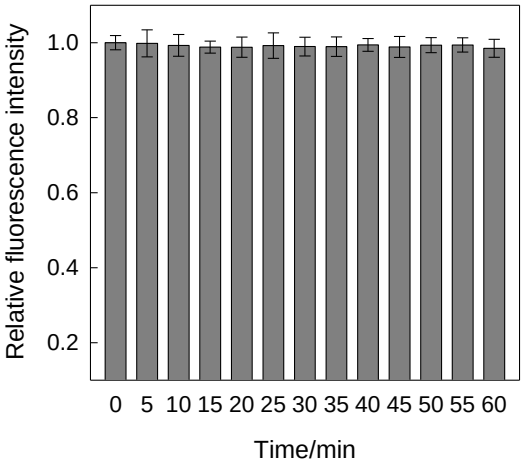


Figure S1. Fluorescence emission intensity changes of TPdye (1 μM) as a function of time in buffer solution under xenon lamp as an excitation. All error bars were obtained through the detection of three parallel samples. λ_{ex} /λ_{em} = 370 nm/460 nm

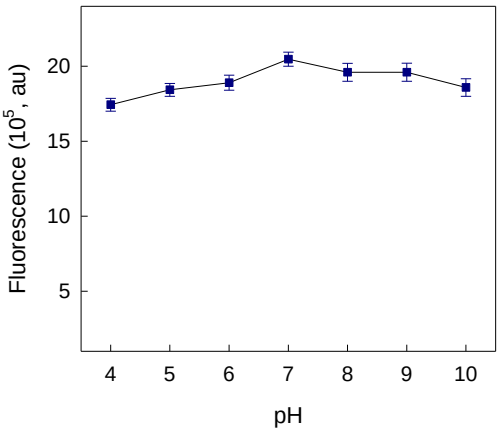


Figure S2. Effect of pH on the one-photon excited fluorescence intensity of TPdye (1 μM) in HEPES buffer solution. All error bars were obtained through the detection of three parallel samples. λ_{ex} /λ_{em} = 370 nm/460 nm.

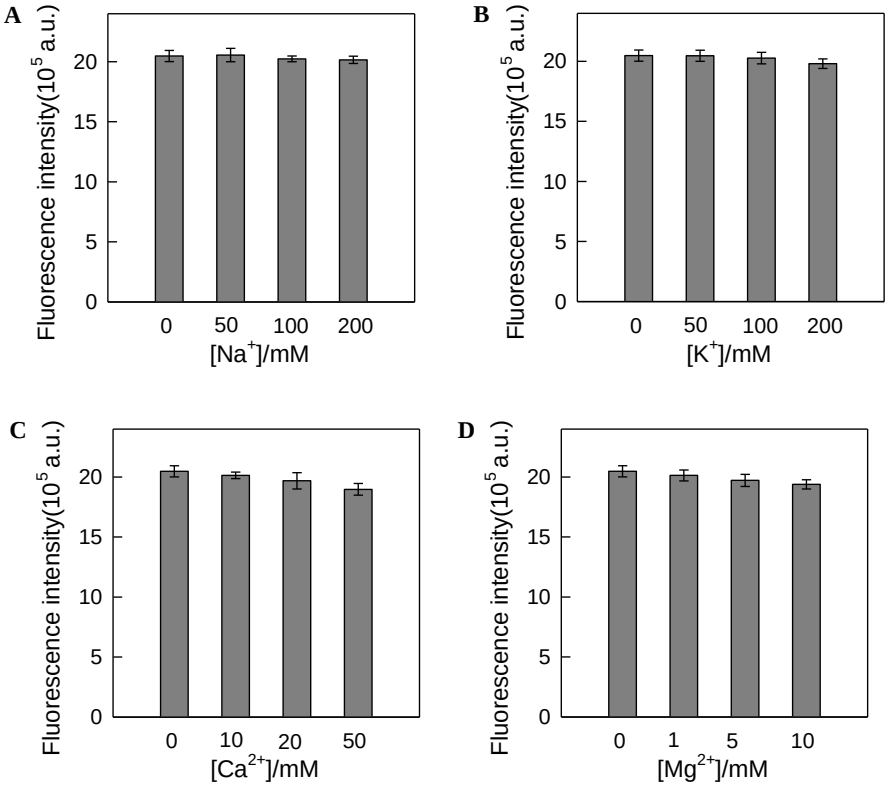


Figure S3. Effect of physiological relevant metal ions including Na^+, K^+, Ca^{2+} and Mg^{2+} on the one-photon excited fluorescence intensity of TPdye (1 μM) in HEPES buffer solution. All error bars were obtained through the detection of three parallel samples. λ_{ex} /λ_{em} = 370 nm/460 nm.

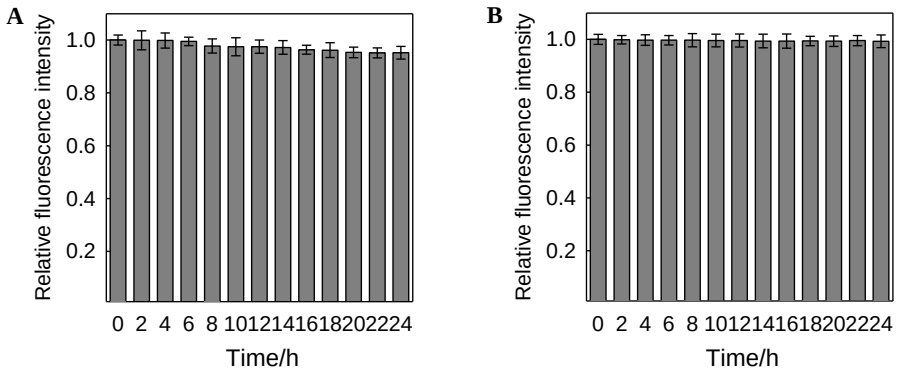


Figure S4. Fluorescence emission intensity changes of TPdye (1 μM) as a function of time in human serum (A) or cell lysate (B). All error bars were obtained through the detection of three parallel samples. λ_{ex} /λ_{em} = 370 nm/460 nm.

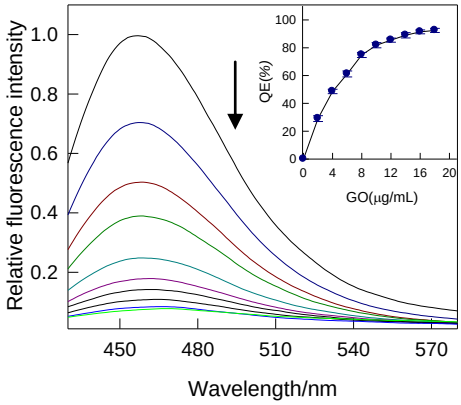


Figure S5. Effect of different amounts of GO on the fluorescence emission spectra of Aptamer-TPdye in Hepes buffer solution (pH 7.4). Inset: Fluorescence quenching efficiency (QE %) as a function of the GO concentration. All error bars were obtained through the detection of three parallel samples. [Aptamer-TPdye] = 50 nM, [GO] = 0-20 μg/mL. λ_{ex} = 370 nm.

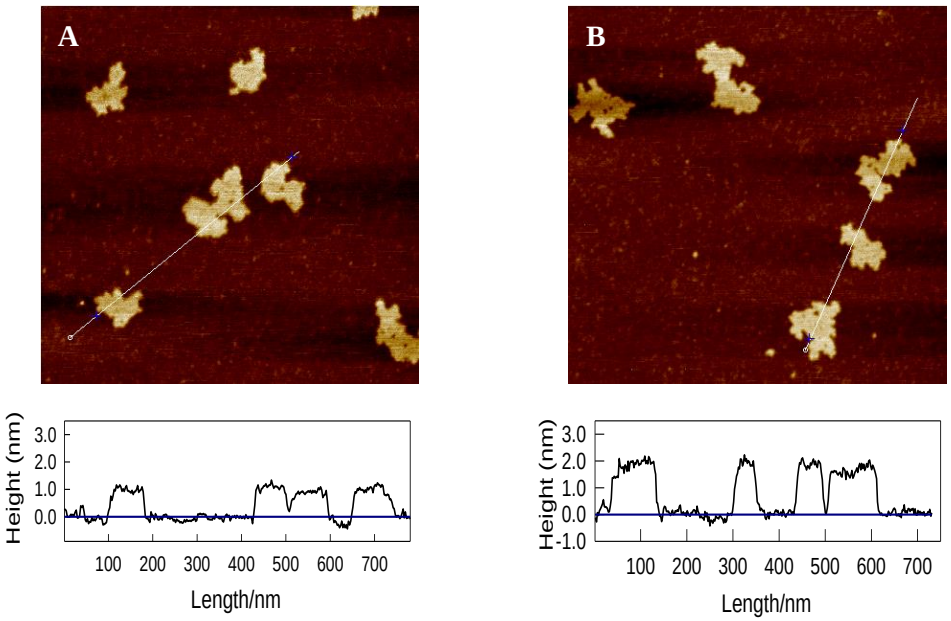
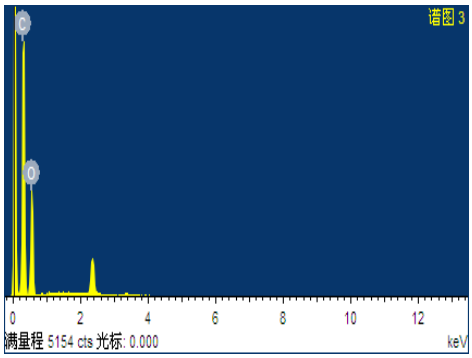


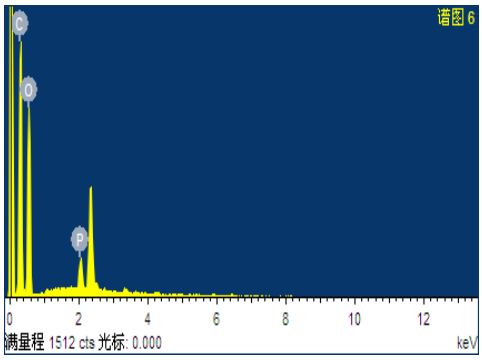
Figure S6. AFM image and profile analysis of GO (A) and GO/Aptamer-TPdye conjugates (B).

A



Element	Weight%	Atom%
C	55.10	62.05
O	44.90	37.95
P	0.00	0.00
Totals	100.00	

B



Element	Weight%	Atom%
C	47.66	55.49
O	49.40	43.18
P	2.94	1.33
Totals	100.00	

Figure S7. Elemental analysis of GO(A) and GO/Aptamer-TPdye conjugates (B).

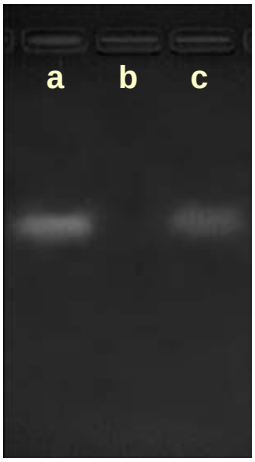


Figure S8. Gel electrophoresis of the aptamer probe and its binding complexes with GO and ATP. From left to right: Lane a, 1.0 μM Aptamer-TPdye; lane b, GO/Aptamer-TPdye conjugates; and lane c, GO/Aptamer-TPdye conjugates with addition of 1.0 mM ATP.

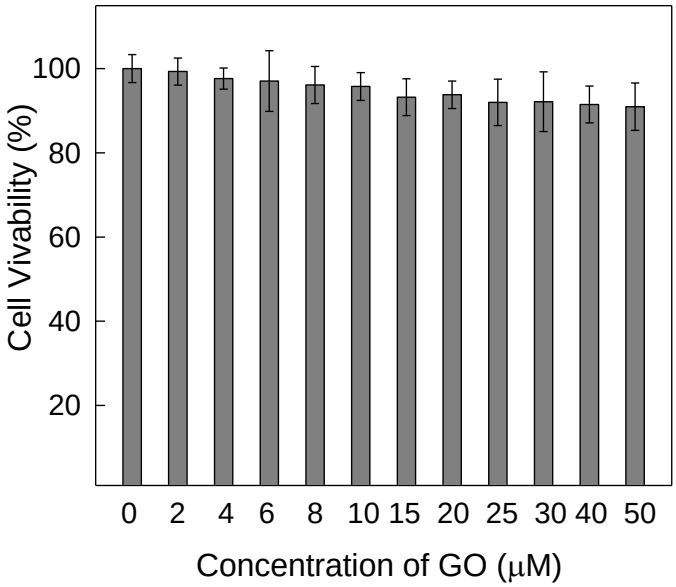


Figure S9. Cell viability of HeLa treated with different concentrations of GO for 24 h in fresh medium. All error bars were obtained through the detection of eight parallel samples.

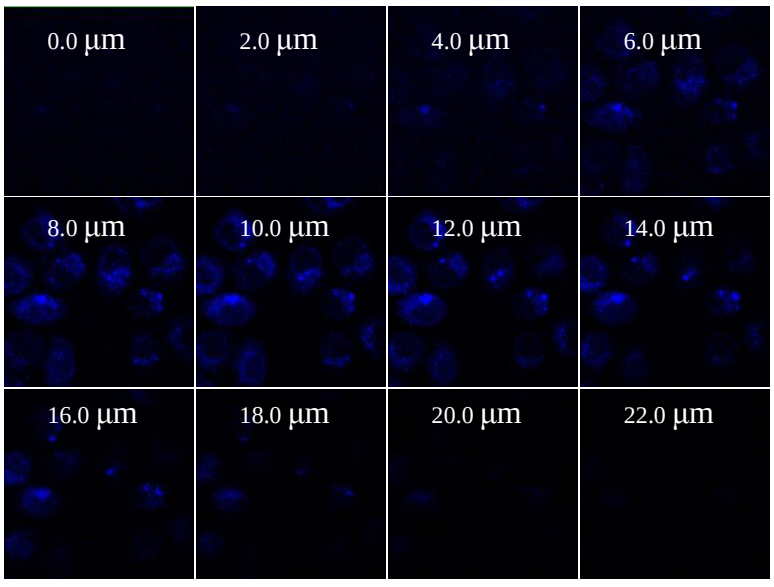


Figure S10. Z-scanning confocal fluorescence microscopy images of HeLa cells incubated with GO/Aptamer-TPdye nanosensing conjugate. [Aptamer-TPdye] = 50 nM, [GO] = 20 μg/mL.