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A quinacridone derivative with intensive emission in both solution and solid state via a facile preparation for cell imaging application

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Materials and general instrumentation

All chemical reagents including di-tert-butyl dicarbonate ($tBOC_2O$), N, N'-dimethylaminopyridine (DMAP), were purchased from Sigma-Aldrich and used as received unless otherwise noted. Quinacridone (QA) was obtained from Tokyo Chemical Industry (Japan). All anhydrous solvents including tetrahydrofuran (THF) were also provided by Sigma-Aldrich and used directly. All the other solvents were analytical grade. C4-QA was synthetized according to literature.[1] ^1H NMR spectra were measured on the AVANCEIIIHD 500 MHz spectrometer (USA) with tetramthylsilane as the internal standard. Elemental analyses were performed on a flash EA 1112 spectrometer (Germany). The optical characteristics of materials were investigated by UV−vis absorption spectra (Lambda-35 UV−vis spectrophotometer, PerkinElmer, MA, USA). The absolute fluorescence quantum yields of solutions and films were measured on Edinburgh FLS920. The fluorescence images of cells were acquired using optical imaging system for small animals (IVIS Lumina Series III). The size distribution and Zeta-potential of resulting micelles was determined by dynamic light scattering (DLS) using a BI-200SM (Brookhaven, USA). The TEM images were collected on a field emission high-resolution 2100F transmission electron microscope (JEOL, Japan) operating at an acceleration voltage of 200 kV. All reactions were carried out using Schlenk techniques under a nitrogen atmosphere.

Theoretical Calculations

The ground state geometries were fully optimized by the DFT method with the Becke three-parameter hybrid exchange and the Lee-Yang-Parr correlation functional (B3LYP) and 6-31G* basis set using the Gaussian 03 software package.

Synthesis of TBOC-QA

N,N-Bis(tert-butyloxycarbonyl)-quinacridone (TBOC-QA): Quinacridone (1.0 g, 3.2 mmol) was dispersed in dichloromethane (200 mL) at room temperature under continuous stirring. Then $tBOC_2O$ (1.7 g, 7.8 mmol) and DMAP (0.6 g, 4.8 mmol) were

added and stirred continuously for 1 day. The reaction mixtures were filtrated and concentrated almost to dryness. The crude product was purified by column chromatography using silica gel with dichloromethane and hexane as the eluents to obtain the yellow solid. Yield: 1.1 g (66.5%). ^{1}H NMR: 1.68 (s, 18 H, H-6, 6'), 7.33 (m, 2 H, H-2, 2'), 7.67 (m, 2 H, H-3, 3'), 7.78 (m, 2 H, H-4, 4'), 8.35 (m, 2 H, H-1, 1'), 8.67 (s, 2 H, H-5, 5'). Elemental analysis calcd: C 70.30%, H 5.51%, N 5.47%; found: C 70.28%, H 5.53%, N 5.46%.

Preparation of TBOC-QA/F127 NPs

A mixture of 2 mg of TBOC-QA and 30 mg of Pluronic 127 (F127) was completely dissolved in 1 mL of THF for 2 h. Then 10 mL of deionized-water was quickly injected into the mixture under vigorous stirring at room temperature. After being stirred for 5 min, the dispersion was dialyzed against deionized-water by 3.5 KDa dialysis membranes for 48 h to remove THF. The TBOC-QA/F127 aqueous solutions were separated by centrifugation at 10000 rpm for 5 min to remove unencapsulated surfactant and then redispersed in deionized-water before characterization and cell study.

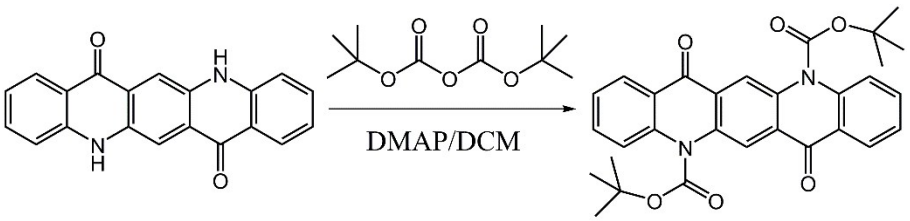
Cell labelling

HeLa cells were regularly cultured and passaged using DMEM medium supplemented with 10% FBS and 1% penicillin−streptomycin at 37 °C with 5% CO_2 in a humidified incubator for 24 h. Subsequently, the cells were treated with TBOC-QA/F127 NPs (10 μg mL^{-1}) and continued to incubate for another 4 h, washed three times using PBS buffer and then fixed using 4% of paraformaldehyde (20 min). The nucleus were then stained with DAPI (0.4 μg mL^{-1}, 10 min) in order to track intracellular absorption.

Cytotoxicity of TBOC-QA/F127 NPs

HeLa cells were seeded in a 96-well plate, preincubated for 12 h. And then incubated with TBOC-QA/F127 for 24 h and 48 h at concentrations ranging from 0 to 40.0 μg mL^{-1}. Then DMEM medium was replaced with 20 μl 0.5 mg/ml MTT and after 4 h the MTT solution was replaced with 150 μl DMSO solution. Cell viability was measured

at 490 nm by colorimetric assay (Rayto RT-2100C, Shenzhen, China). Cells without treatment in medium were used as control.



QA TBOC-QA

Scheme S1. The synthetic route of TBOC-QA.

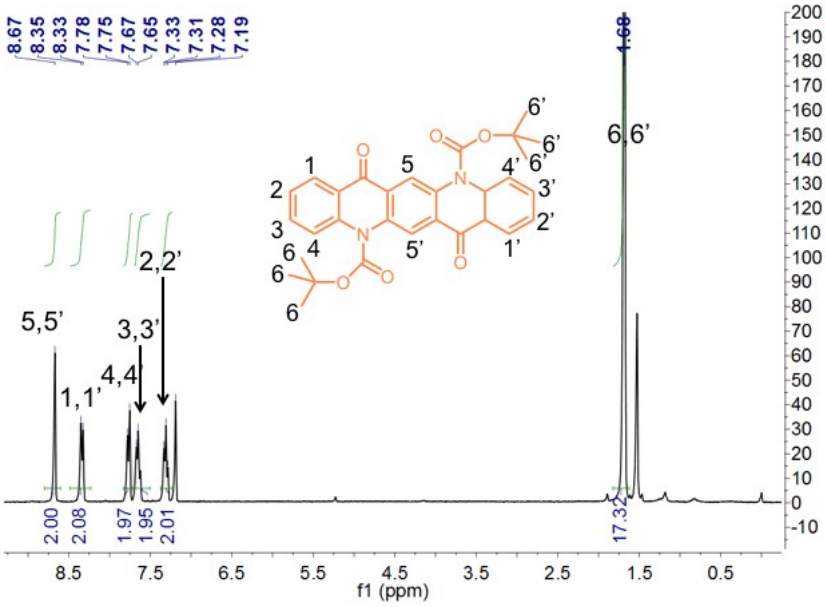


Figure S1. ^{1}H NMR spectrum of TBOC-QA in $CDCl_3$.

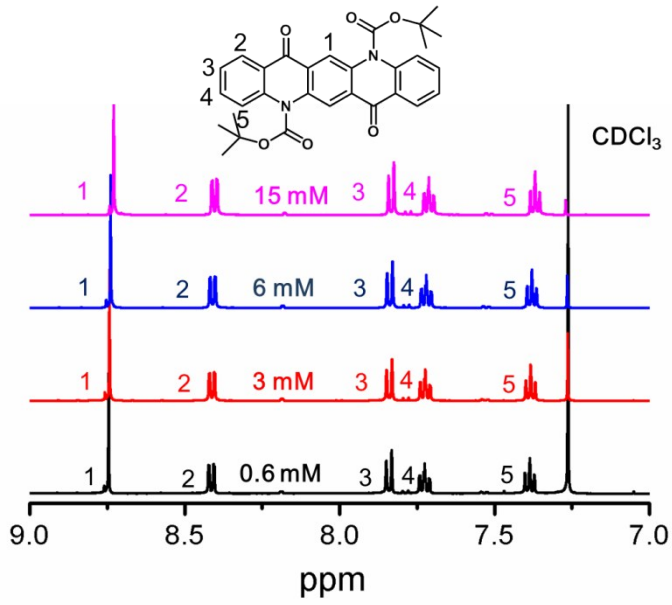


Figure S2. Concentration-dependent ^{1}H NMR spectra of TBOC-QA in $CDCl_3$ at 25℃.

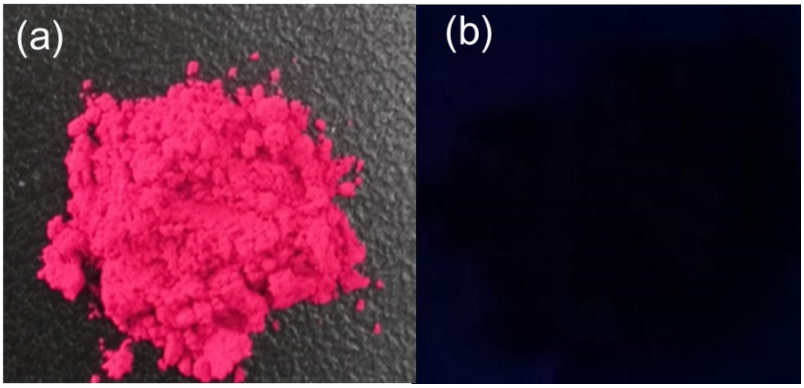


Figure S3. The digital images of QA under fluorescent lamp (a) and UV lamp (λ = 365 nm) (b).

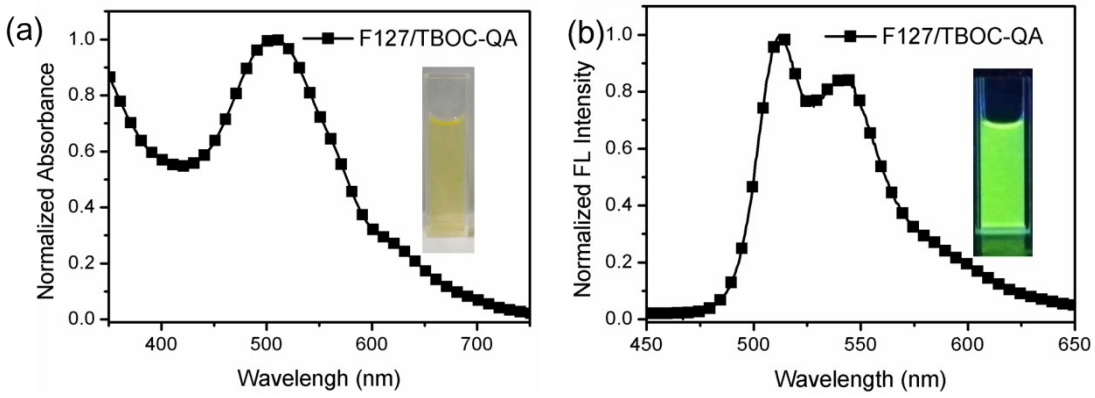


Figure S4. The UV-Vis absorbtion (a), FL (b) spectrum and digital image of TBOC-QA/F127 NPs.

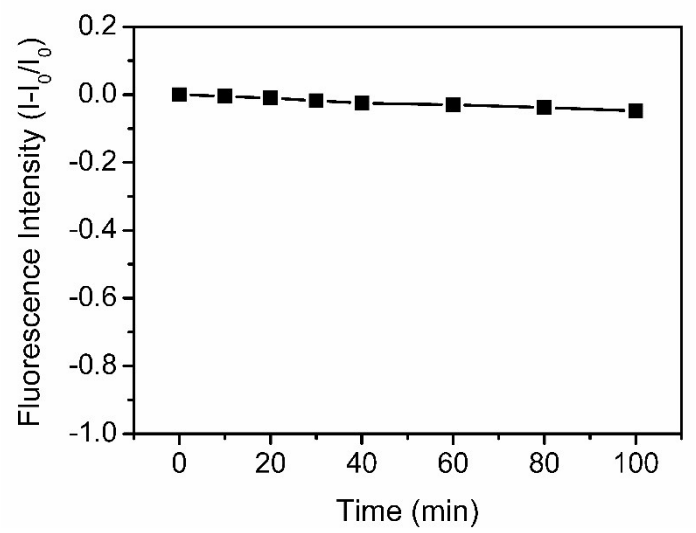


Figure S5. Fluorescence intensity of TBOC-QA/F127 NPs at 498 nm upon irradiation with blue LED light (20 W) in aqueous solution. I is fluorescence intensity after blue LED light (20 W) irradiation and I_0 is fluorescence intensity without LED irradiation.

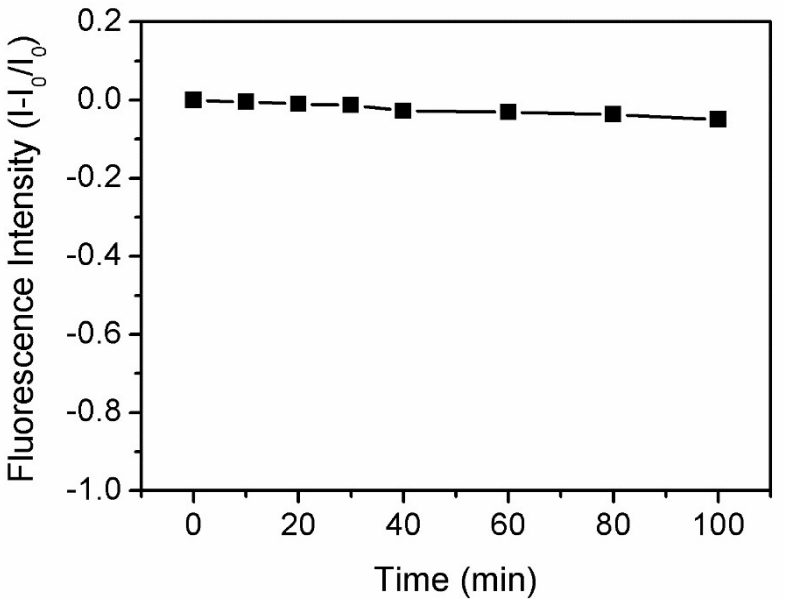


Figure S6. Fluorescence intensity of TBOC-QA/F127 NPs at 498 nm upon irradiation with blue LED light (20 W) in PBS solution. I is fluorescence intensity after blue LED light (20 W) irradiation and I_0 is fluorescence intensity without LED irradiation.

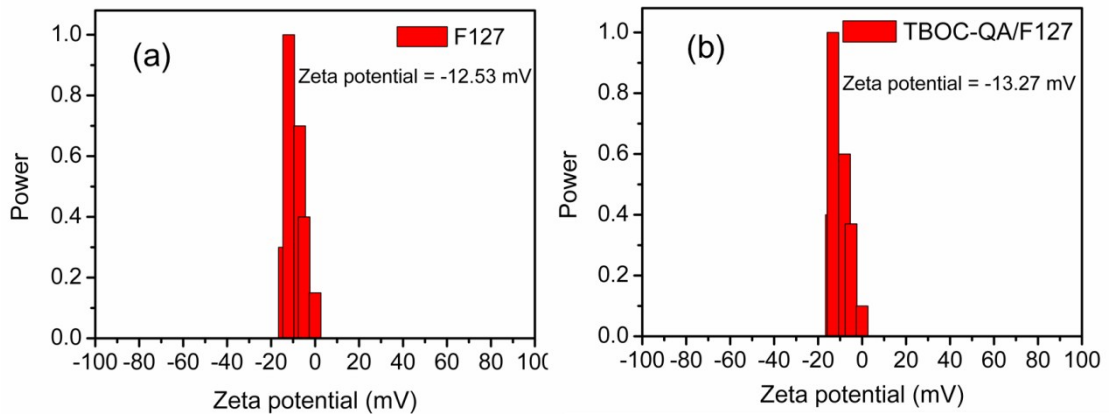


Figure S7. The Zeta potentials of F127 (C_{F127} = 300 μg mL^{-1}) (a) and TBOC-QA/F127 ($C_{TBOC-QA}$ = 20 μg mL^{-1}) (b) aqueous solution.

References:

1. K. Q. Ye, J. Wang, H. Sun, Y. Liu, Z. Mu, F. Li, S. Jiang, J. Zhang, H. Zhang, Y. Wang and C. M. Che, *J. Phys. Chem. B*, 2005, 109, 8008–8016.