N, S co-doped graphene quantum dots from a single source precursor used for photodynamic cancer therapy under two-photon excitation

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Part S1. Synthesis and characterization of PTPD

The synthesis routes of PTPD are based upon the reported method (M. Lan, et al., *J. Am. Chem. Soc.*, 2012, **134**, 6685-6694), as exhibited in Fig. S1a. The according characterizations of PTPD are provided as below. As illustrated in Fig. S1b, the ^{1}H-NMR (400 MHz, $CDCl_3$, TMS, ppm): δ 0.86-0.89 (t, 3H), 1.23-1.33 (m, 18H), 1.80 (m, 2H), 3.31 (s, 6H), 3.51-3.56 (m, 2H), 5.14 (s, 2H), 7.35-7.36 (d, J = 5 Hz, 1H), 7.39-7.41 (d, J = 8 Hz, 1H), 7.49 (s, 1H), 7.61-7.63 (d, J = 8 Hz, 2H), 7.69-7.70 (d, J = 8 Hz, 2H). The ^{13}C-NMR (100 MHz, $CDCl_3$, TMS, ppm): δ 14.1, 22.7, 23.0, 26.4, 29.3, 29.5, 29.6, 31.9, 49.6, 63.8, 67.2, 121.7, 126.0, 126.8, 126.9, 133.9, 137.9, 140.8. The MALDI-TOF Mass spectrum m/z: Calculated: 386.29; Found: 386.13. DTPD (*yield:* 50.0%) GPC: M_n = 6.655 × 10^4 (PDI = 1.161). ^{1}H-NMR (400 Mz, CD_3CN-D_2O (v/v = 1/1), TMS, ppm) δ 0.67-0.69 (br), 1.06 (s,br), 1.60 (s, br), 2.85 (s, br), 3.00 (s, br), 4.56 (s, br), 6.81 (s, br), 7.35-7.37 (dbr), 7.48-7.50 (dbr).

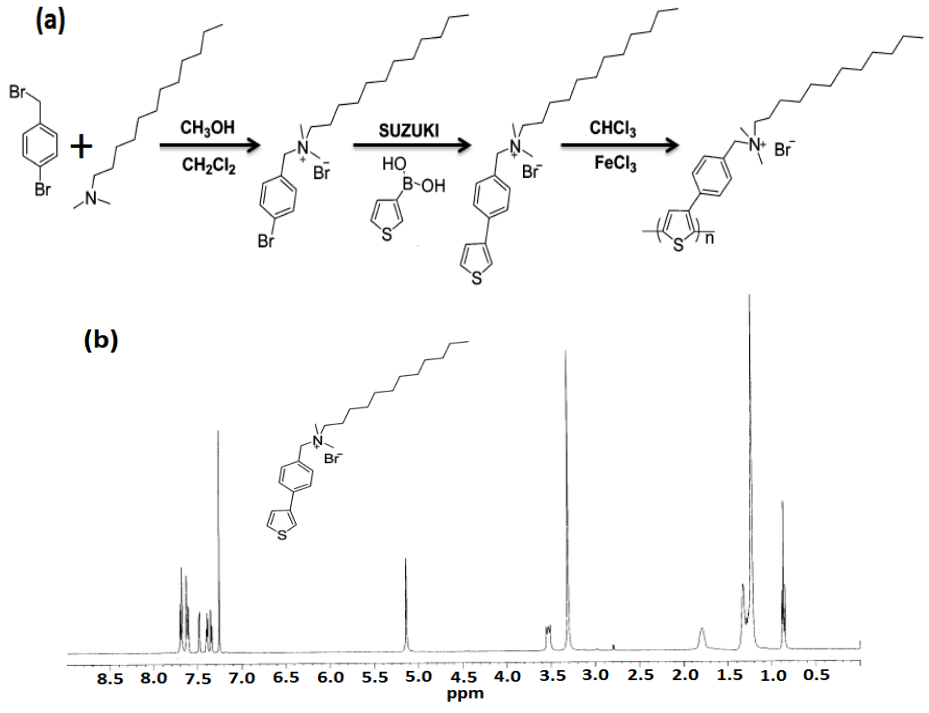

Fig. S1 (a) The synthetic route and (b) according ^{1}H-NMR spectra of polythiophene derivative (PTPD).

Part S2. Synthesis procedures of NS-GQD

The synthesis of NS-GQD was performed by a direct hydrothermal treatment of PTPD in basic aqueous solution. Typically, 20 mg of PTPD was added into 25 mL of NaOH aqueous solution (0.5 mM) to produce homogeneous mixture solution. Under ultrasonication, the mixture solution was treated for 30 min and then was transferred into an autoclave, followed by heating at 170 °C for 24 h. After that, the reaction mixture was cooled to room temperature. The resulting products were collected by filtering to remove the produced larger particles, using 0.22 μm of membranes. The obtained solution after filtration treatment was dialyzed against distilled water several times (frequently, in a period of 24 h) to remove residual NaOH. After these purified procedures, the final sediments (*i.e.* purposed products, NS-GQD) were dispersed in distilled water for further characterizations and uses in subsequent experiments.

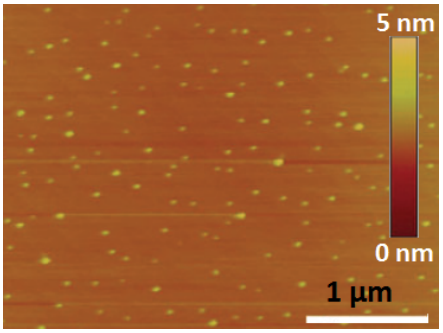

Fig. S2 Atomic force microscope (AFM) images of the as-prepared NS-GQD.

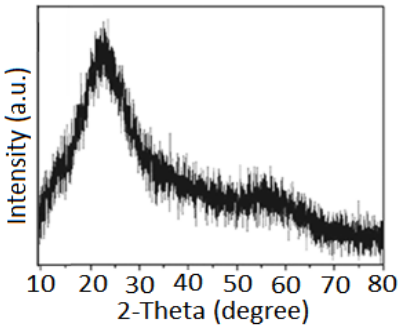

Fig. S3 X-ray diffraction (XRD) patterns of the as-prepared NS-GQD.

According to the typical XRD patterns of NS-GQD mentioned above, a sharp diffraction peak appears at 22.5°. Based upon the results from XRD patterns and Raman spectra (Fig. 1c), the sp^2 configuration of NS-GQD could be further confirmed (L. Liu, et al., *J. Am. Chem. Soc.*, 2011, **133**, 15221. T. López-Ríos, et al., *Phys. Rev. Lett.*, 1996, **76**, 4935).

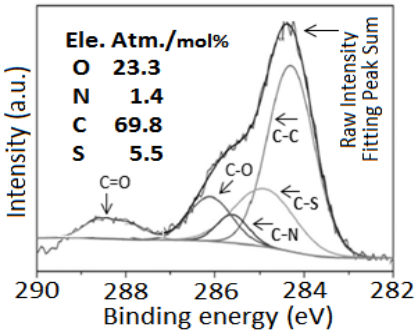

Fig. S4 The de-convolution of high-resolution C1s XPS spectra.

The mentioned above high-resolution C1s XPS spectra of NS-GQD indicate five obvious peaks. Based on the de-convolution treatment, there are peaks at 284.4, 285.0, 285.6 and 286.1 eV, which are assigned to the C-C, C-S, C-N and C-O bonding, respectively. The contents of N and S elements were respectively evaluated to be 1.4% and 5.5%, implying the incorporation of N and S into NS-GQD.

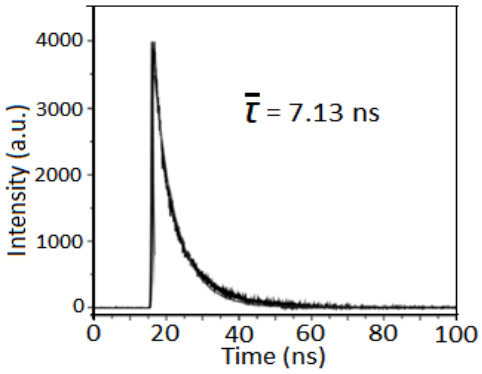

Fig. S5 Fluorescence decay curve of NS-GQD, recorded at 675 nm with an excitation of 498 nm.

Table S1 Comparison of fluorescence lifetimes and quantum yields of NS-GQD in different atmospheres.

Atmosphere	[a] Lifetime (ns)								[b] Quantum yield (%)
	τ_1 /ns	a_1 /%	τ_2 /ns	a_2 /%	τ_3 /ns	a_3 /%	τ_{ave} /ns	χ^2	
O_2	0.31	26	1.19	37	7.97	47	7.126	1.012	4.7 %
N_2	0.43	27	1.28	34	8.04	49	7.188	1.129	6.1 %
Air	0.44	25	1.30	37	8.07	48	7.153	1.181	5.8 %

Note: [a] Fluorescence lifetime of NS-GQD was measured at 675 nm under an excitation of 498 nm. Average lifetime ($\boldsymbol{\tau}_{ave}$) was calculated *via* the following equation (M. J. Rueda-Rama, et al., *Chem. Commun.*, 2011, **47**, 2898; *Analyst*, 2012, **137**, 1500) as below; $\boldsymbol{\tau}_{ave} = \sum a_i \cdot (\boldsymbol{\tau}_i)^2 / \sum a_i \cdot \boldsymbol{\tau}_i$ (i = 1, 2, 3). The fluorescence decay curves are treated using a standard fitting formula of triplet-exponential decay as below;

$\boldsymbol{Y} = \boldsymbol{A} + \boldsymbol{B}_1 \cdot exp(-x/\boldsymbol{\tau}_1) + \boldsymbol{B}_2 \cdot exp(-x/\boldsymbol{\tau}_2) + \boldsymbol{B}_3 \cdot exp(-x/\boldsymbol{\tau}_3)$

[b] Fluorescence quantum yields of NS-GQD were calculated through comparing the integrated emission of NS-GQD in solution with a fluorescence dye with the identical optical density at excitation wavelength (W. Zhang, et al., *Inorg. Chem.*, 2009, **48**, 9723). Quantum yield of rhodamine 6G (R6G in ethanol) is 95%. Standard calculation of fluorescence quantum yield of NS-GQD was performed by the equation as below;

$\boldsymbol{\Phi}_s = \boldsymbol{\Phi}'_f (\boldsymbol{I}_s/\boldsymbol{I}'_f) \cdot (\boldsymbol{A}'_f/\boldsymbol{A}_s) \cdot (\boldsymbol{n}_s/\boldsymbol{n}'_f)$, where $\boldsymbol{\Phi}_s$, $\boldsymbol{I}_s$, $\boldsymbol{A}_s$ and $\boldsymbol{n}_s$ represent the quantum yield, emission peak area, integrated absorption intensity and refractive indices of NS-GQD, respectively, while $\boldsymbol{\Phi}'_f$, $\boldsymbol{I}'_f$, $\boldsymbol{A}'_f$, and $\boldsymbol{n}'_f$ respectively stand for the corresponding parameters of R6G.

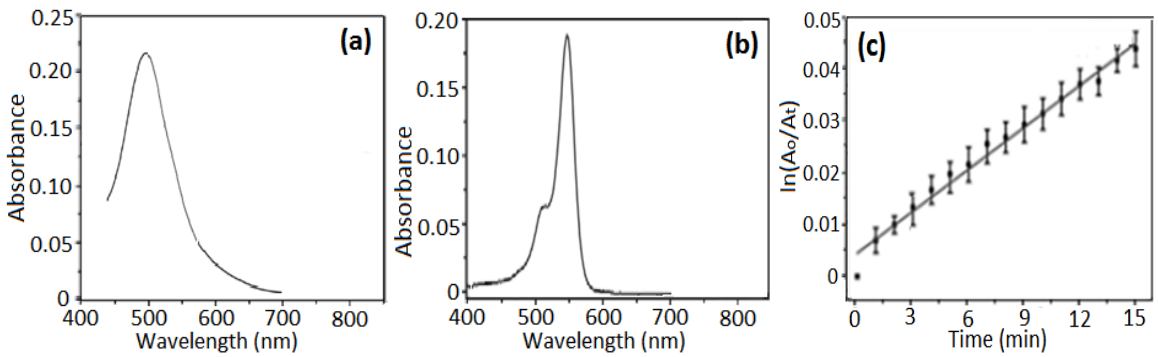

Fig. S6 Absorption spectra of NS-GQD (a) and RB (b) in aqueous solution, and (c) the reduction in absorption of ADPA at 378 nm in the presence of RB. The ln (A_0/A_t) was plotted as a function of TPE time (with an 800 nm fs laser, 6 mW).

Part S3. Calculation method of $^1O_2$ quantum yield

The decomposition rate constants (***K***) of ADPA in the presence of NS-GQD and RB were respectively determined to be 0.0144 ($K_{NS\text{-}GQD}$, Fig. 3b) and 0.0028 (K_{RB}, Fig. S5c). The integral areas (***A***, ranging from 440 nm to 700 nm) of the absorption spectra of NS-GQD and RB were respectively calculated to be 25.4 ($A_{NS\text{-}GQD}$, Fig. S5a) and 8.3 (A_{RB}, Fig. S5b). In addition, RB as the standard photosensitizer possesses 0.75 of $^1O_2$ quantum yield (Φ_{RB} = 0.75) in water (L. Xiao, et al., *ACS Nano*, 2011, **5**, 3651). The $^1O_2$ quantum yield of NS-GQD ($\Phi_{NS\text{-}GQD}$) can be calculated using the following formula as below;

$$\Phi_{NS\text{-}GQD} = (\Phi_{RB} * K_{NS\text{-}GQD} * A_{RB}) / (K_{RB} * A_{NS\text{-}GQD})$$

According to this formula, the $\Phi_{NS\text{-}GQD}$ can be calculated to be 1.26.

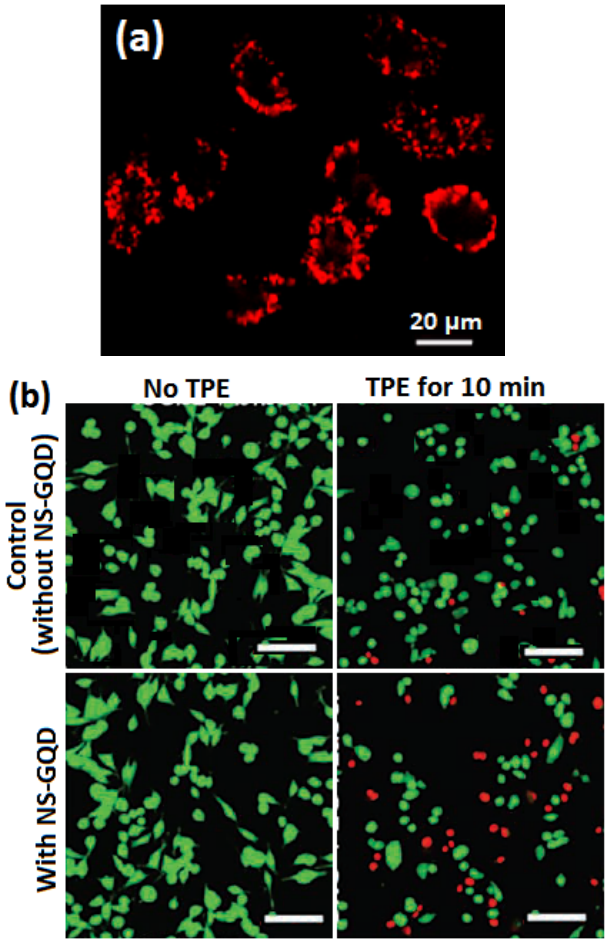

Fig. S7 (a) Fluorescence images of HeLa cells labelled with NS-GQD (1.0 mg mL^{-1}) under TPE (800 nm). (b) Time-dependent fluorescence images of Calcein AM/Ethidium homodimer-1 stained HeLa cells incubated without NS-GQD (as the control) and with NS-GQD (1.0 mg mL^{-1}) after TPE with an 800 nm fs laser (6 mW), irradiated for 0 and 10 min, respectively. Scale bars: 50 μm.

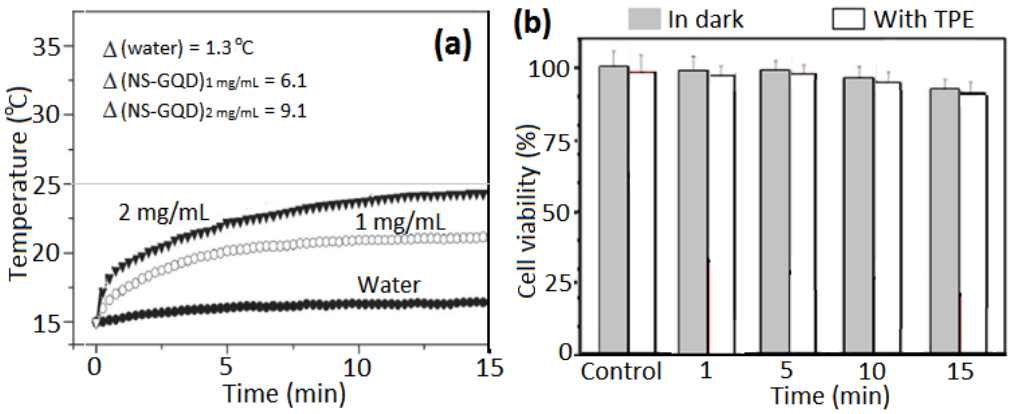

Fig. S8 (a) Temperature elevation of aqueous suspension of NS-GQD with different concentrations (1.0 and 2.0 mg mL^{-1}) and temperature elevation of water without NS-GQD as a function of irradiation time (0~15 min) under TPE with an 800 nm fs laser (6 mW), and temperature changes (Δ) of water and NS-GQD over a period of irradiation time (15 min). These aqueous samples were loaded in a 1 cm × 1 cm cuvette. Under a slight stirring (100 rpm), these samples were continuously irradiated with TPE (800 nm, 6 mW) for 0~15 min. (b) The viabilities of HeLa cells incubating in PBS (10 mM, pH 7.4) without (in the dark) or with the continuous TPE treatment (800 nm, 6 mW) for different times.