Electronic Supporting Information

for

Target analyte induced fluorescence band shift of piperazine modified carbon quantum dots: a specific visual detection method for oxytetracycline

Lei Yang, Haitong Zhao, Ning Liu, Wei Wang*

MOE Key Laboratory of Pollution Processes and Environmental Criteria,

Tianjin Key Laboratory of Environmental Remediation and Pollution Control,

College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

*E-mail address: nkwangwei@126.com

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1. Reagents and instruments

Citric acid, urea, ethylene glycol, N-Hydroxysuccinimide (NHS), 1-boc-piperazine (Boc-piperazine), ascorbic acid, tetracycline hydrochloride, phenol, hydroquinone, $NaHCO_3$, NaOH, NaCl, KCl, Na_2HPO_4 and KH_2PO_4 were purchased from Sanjiang Chemical Technology Co., Ltd (Tianjin, China). Piperazine,1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Energy Chemical Technology Co., Ltd (Shanghai, China), and 2-(N-Morpholino)-ethanesulfonic acid (MES), oxytetracycline were purchased from Dingguo Co., Ltd (Tianjin, China). Tryptophan, lysine, acetamiprid and ciprofloxacin were purchased from Solomon Biotechnology Co., Ltd (Tianjin, China). All of the reagents were analytical grade and received without further purification.

The fluorescence spectra and three-dimensional excitation emission fluorescence spectroscopy spectra were recorded on F-7000 fluorescence spectrophotometer (Tokyo, Japan) with excitation slit set at 2.5nm and emission slit set at 5.0nm (except for Figure S12 and Figure S13, in which the excitation slit and emission slit were all set at 5.0nm). PMT voltage was set at 700V. UV/Vis absorption spectra were obtained using Shimadzu UV-2600 spectrophotometer (Tokyo, Japan). Transmission electron microscopy (TEM) images were measured by JEM-2800 microscopy (JEOl, Japan) at 200kV. Fourier transform infrared (FTIR) spectra were recorded on Tensor-37 FTIR spectrophotometer (Bruker, Germany) within a range of 40 – 4000 cm^{-1}. The hydration size and zeta potential

were measured on a Zetasizer Nano ZS90 dynamic light scattering (DLS) instrument (Malvern Instruments Co., UK) at a constant temperature of 25 ºC. The time-resolved decay curves were recorded on FLS980 fluorescence spectrophotometer (Edinburgh, UK).

2. Synthesis of CQDs

Typically, 0.615g citric acid and 0.385g urea were dissolved into 30ml ethylene glycol. After stirring for 2h, the mixture was placed into 50 ml Teflon-lined stainless-steel autoclave and heated at 180 ºC for 7h. After cooling to room temperature, the black product was filtered through 0.22μm filter membrane to remove large particle impurities. Subsequently, the product was purified by dialyzing against distilled water with a dialysis membrane (1000 MWCO) for 48 h, and the outer distilled water was changed three times a day. Then, CQDs powder was obtained through vacuum freeze-drying. A certain amount of CQDs powder was dissolved into distilled water to obtained standard solution of CQDs with the concentration of 1mg/ml and stored in 4 ºC for further application.

3. Synthesis of P-CQDs

Typically, 25mg CQDs powder was dispersed in 25ml MES buffer liquid (pH=6) under ultrasonic vibration for 20min. This system was deaerated with nitrogen before adding 100mg EDC and 50mg NHS, and then the mixture was incubated in ice-bath for 12 hours in a nitrogen atmosphere under magnetic

stirring. Next, 0.08g 1-Boc- piperazine was added to the above solution and the pH was adjusted to 7.4 by addition of 2ml NaOH (1M) dropwise under the ice-bath and nitrogen protection condition. After stirring for 24h, CQDs with Boc-piperazine connecting onto their surface were formed. Subsequently, many bubbles appeared when adding 10ml 1M HCl in the solution and stirring for 3h at room temperature, which can be ascribe to the transformation from Boc-piperazine to piperazine on the surface of CQDs. Then the solution was adjusted to pH 6 by adding $NaHCO_3$ and dialyzed against distilled water(1000ml) for 48h under magnetic stirring to remove impurities. Finally, P-CQDs were obtained after vacuum freeze-drying. A certain amount of P-CQDs powder was dissolved into distilled water to obtained standard solution of P-CQDs with the concentration of 1mg/ml and stored in 4 ºC for further application.

4. OTC detection procedure

Firstly, 1ml of P-CQDs solution (diluted to 0.05mg/ml) and 1ml of OTC at various concentrations (10μM, 40μM, 60μM, 80μM, 100μM) were successively pipetted into 10ml vial, and then 8ml of $NaHCO_3$-NaOH buffer (0.025M, pH=9.8) was added to the mixture. After incubating for 1h at room temperature, the mixture was measured by fluorescence spectrophotometer at an excitation wavelength of 370nm. To investigate the selectivity of P-CQDs, other substances (tetracycline hydrochloride, tryptophan, lysine, acetamiprid, ciprofloxacin, etc) were detected with the same method.

5. Supporting figures

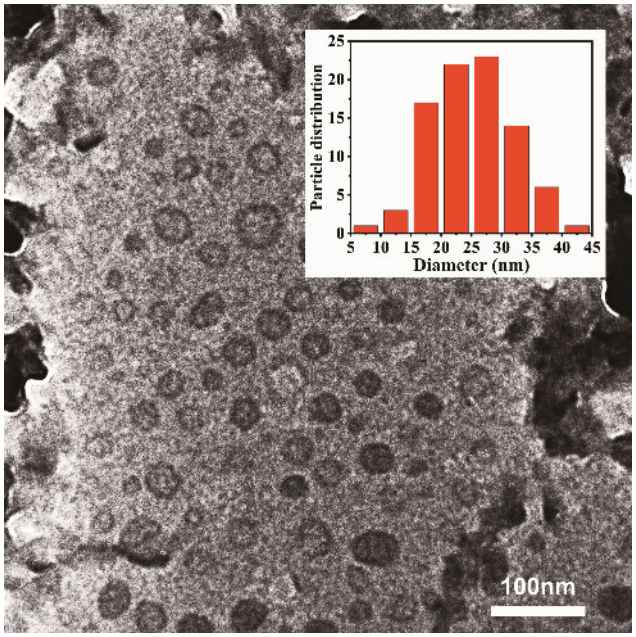

Figure S1.TEM image and size distribution of P-CQDs.

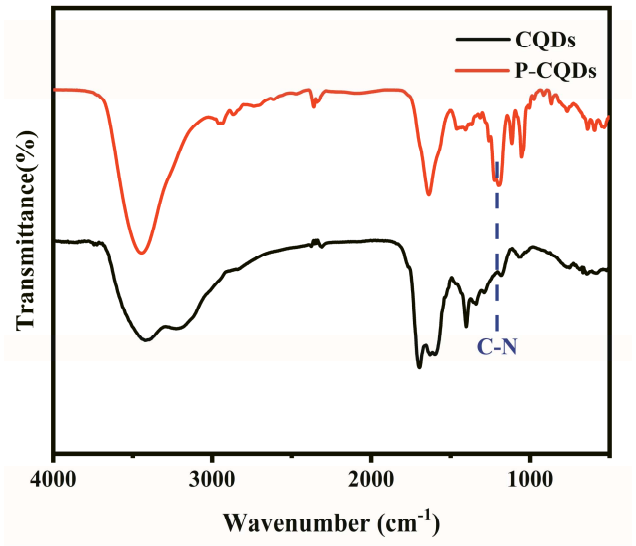

Figure S2. FTIR spectrum of CQDs and P-CQDs.

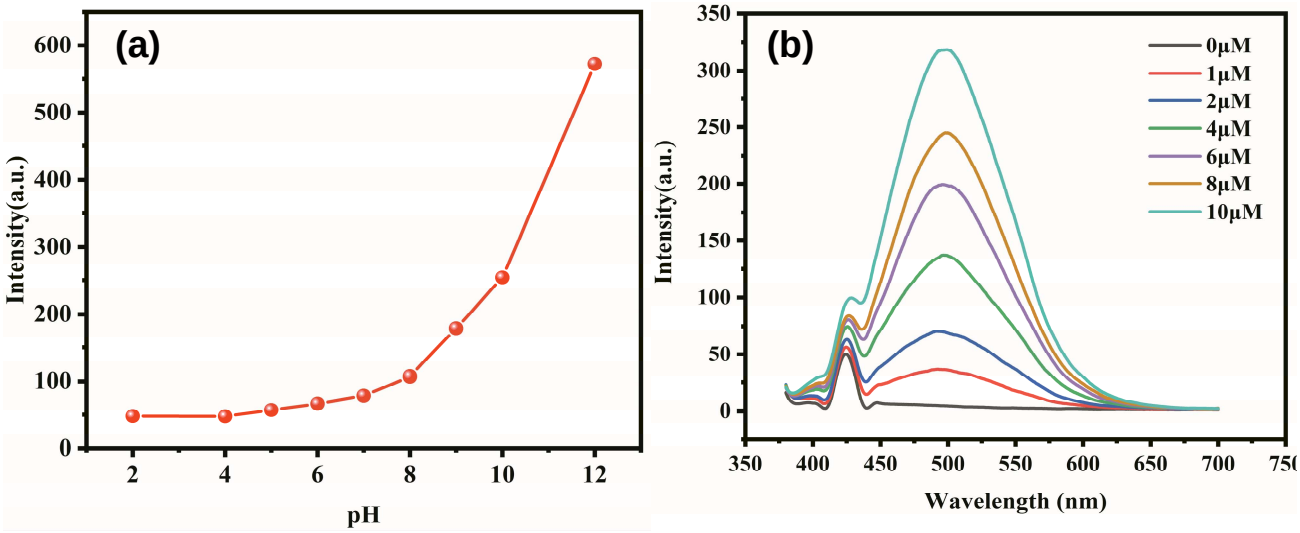

Figure S3. (a) Fluorescence intensity of 10μM OTC under different pH conditions (excitation wavelength: 370nm, emission wavelength: 495nm), (b) Fluorescence emission spectra of different concentrations of OTC in 0.025M $NaHCO_3$-NaOH buffer (excitation wavelength: 370nm, pH=9.8).

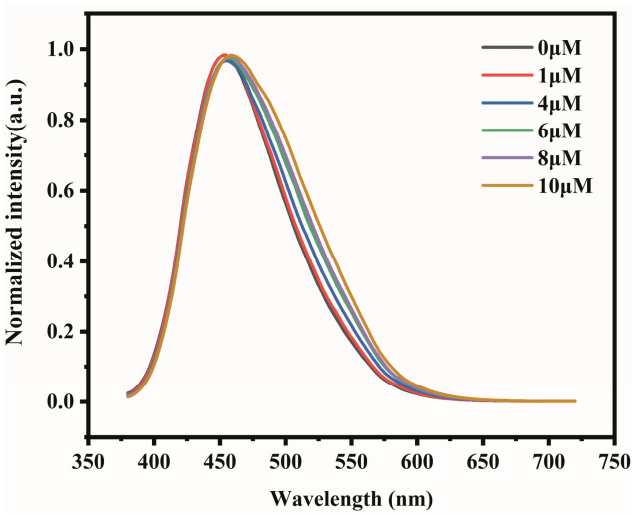

Figure S4. Fluorescence emission spectra of CQDs in the presence of different concentrations of OTC in 0.025M $NaHCO_3$-NaOH buffer (CQDs concentration: 5mg/L, excitation wavelength: 370nm, pH=9.8).

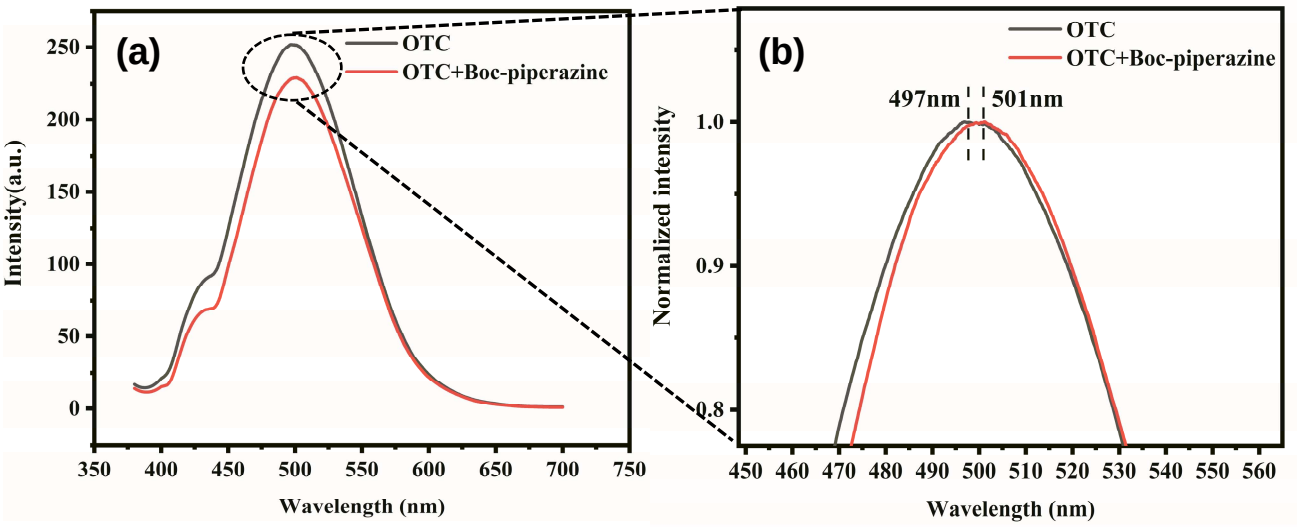

Figure S5. (a) Fluorescence emission spectra of 10 μM OTC and mixture of 10μM OTC and 10μM Boc-piperazine (excitation wavelength: 370nm, pH=9.8), (b) The enlarged view of figure (a).

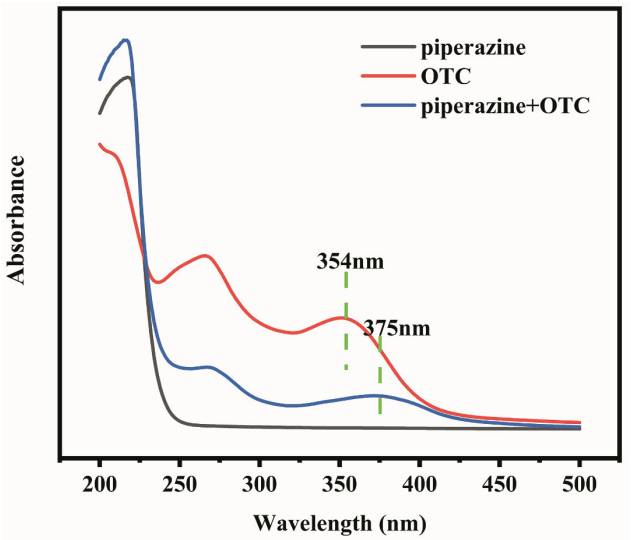

Figure S6. UV/Vis absorption spectra of piperazine (0.02M), OTC (10μM) and mixture of piperazine (0.01M) and OTC (2μM).

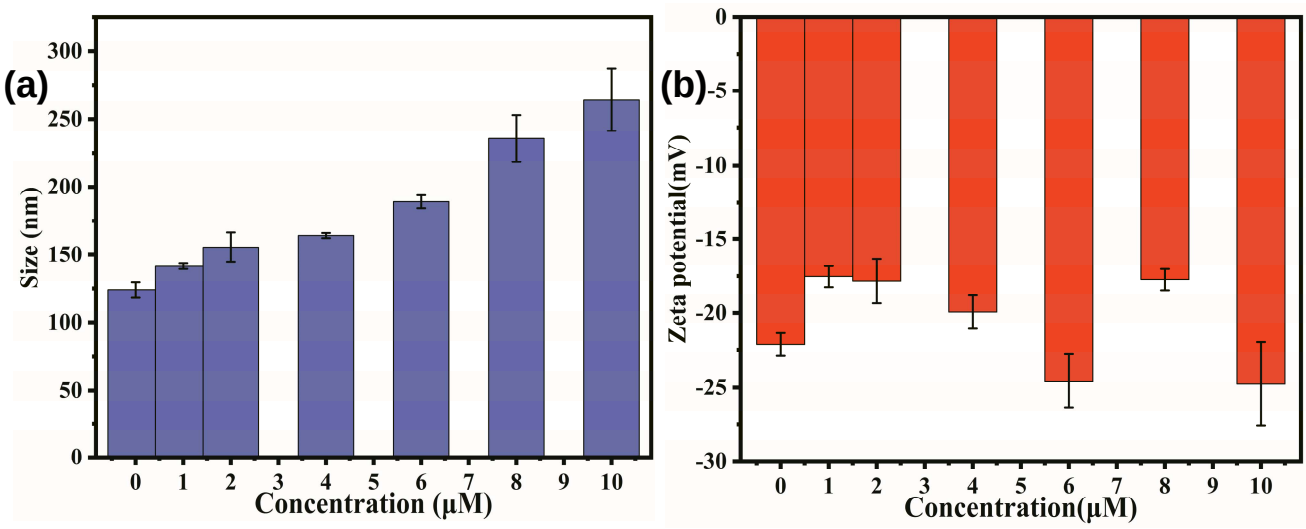

Figure S7. The particle size (a) and zeta potential (b) of P-CQDs in the presence of different concentrations of OTC (P-CQDs concentrations: 5mg/L, pH=9.8, the number of parallel samples was three).

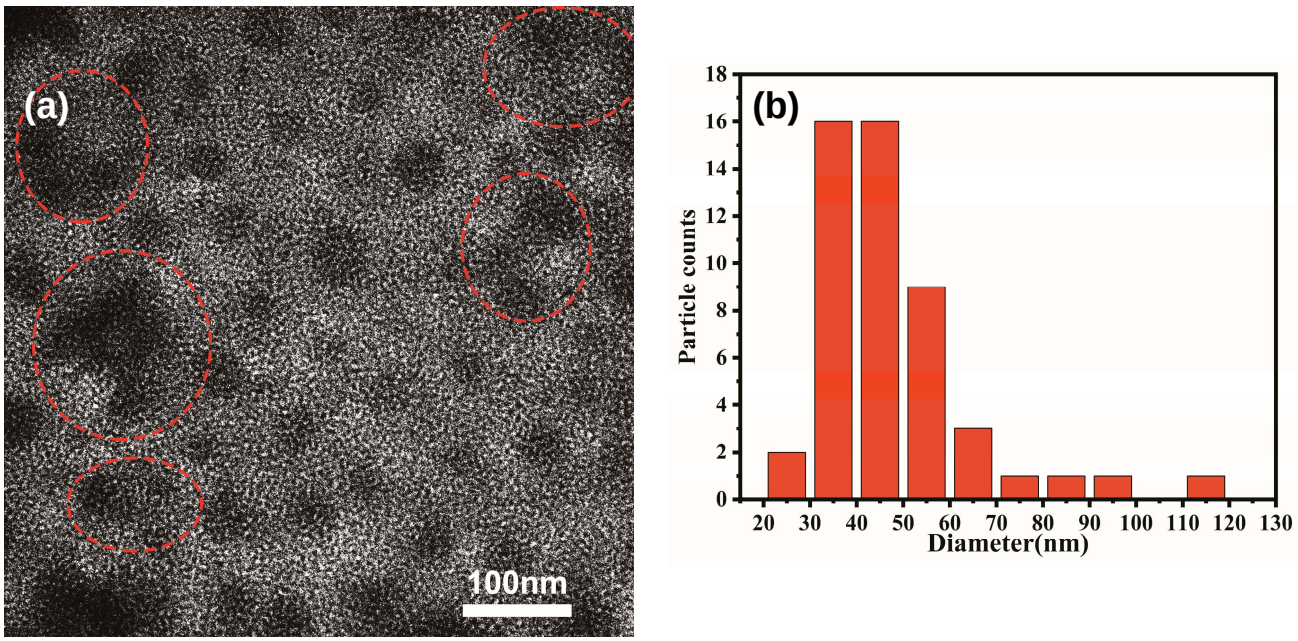

Figure S8.TEM image (a) and size distribution (b) of P-CQDs in the presence of OTC (OTC: 10μM, the particle size distribution was calculated by measuring 50 particles).

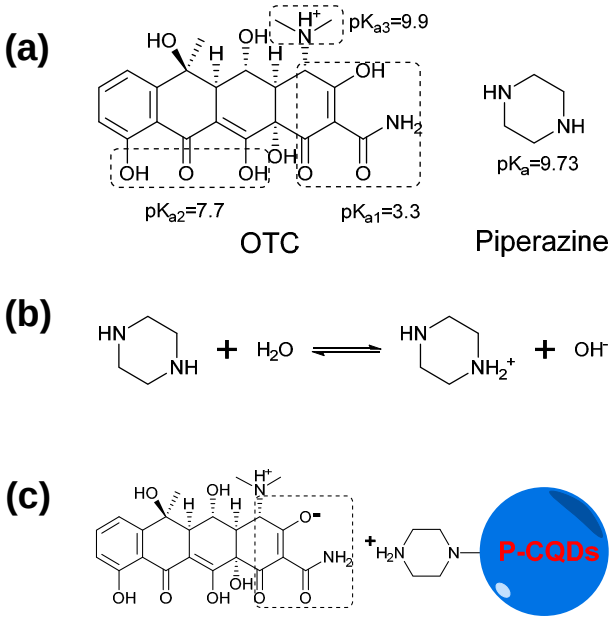

Figure S9. (a) The structure and pKa of OTC and piperazine molecule, (b) the dissociation equation of piperazine when pH is 9.8, and (c) the possible electrostatic interaction between P-CQDs and OTC.

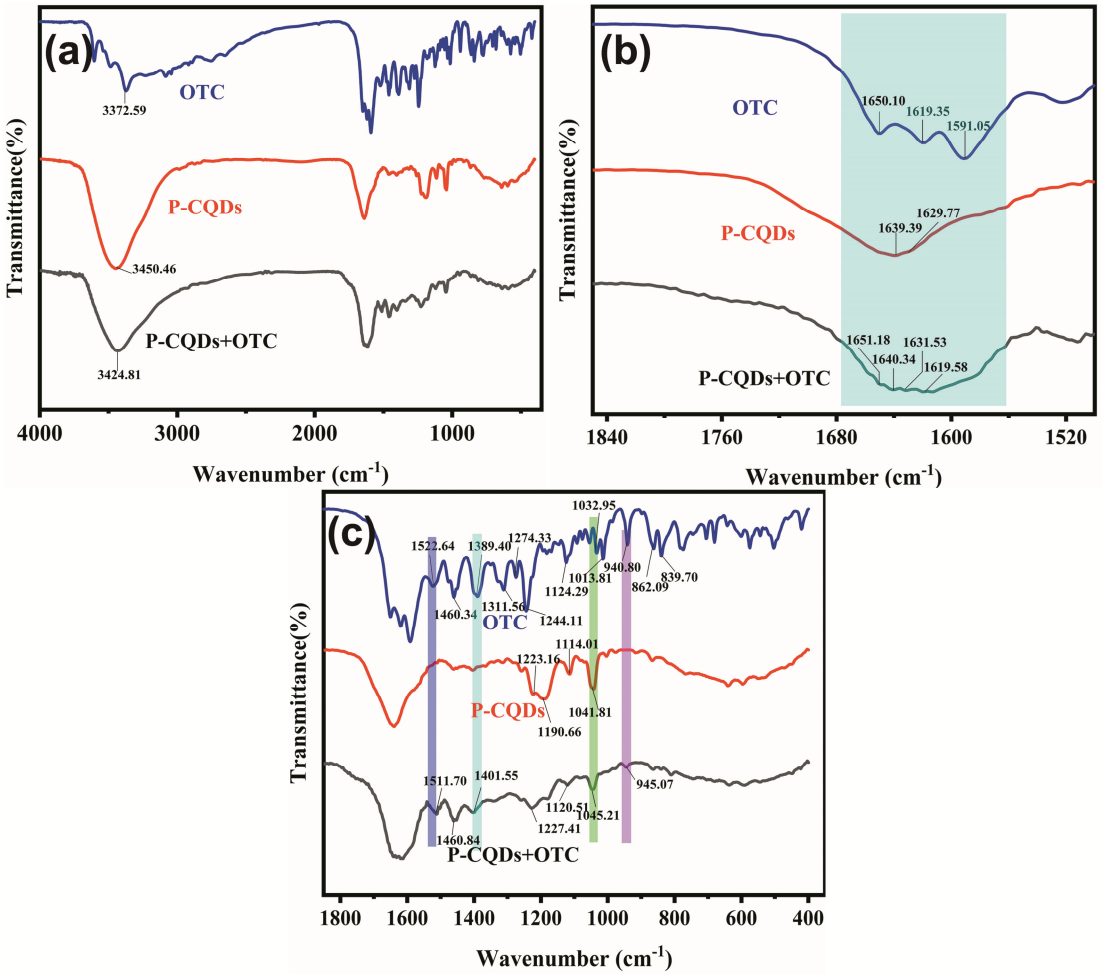

Figure S10. The FTIR of OTC, P-CQDs and mixture of OTC and P-CQDs (all samples were dissolved with KBr in mixture of ethanol and water (1:1) in advance, the products were measured using KBr tablet method after freeze-drying for 24h, the mass ratio of P-CQDs and OTC was 2).

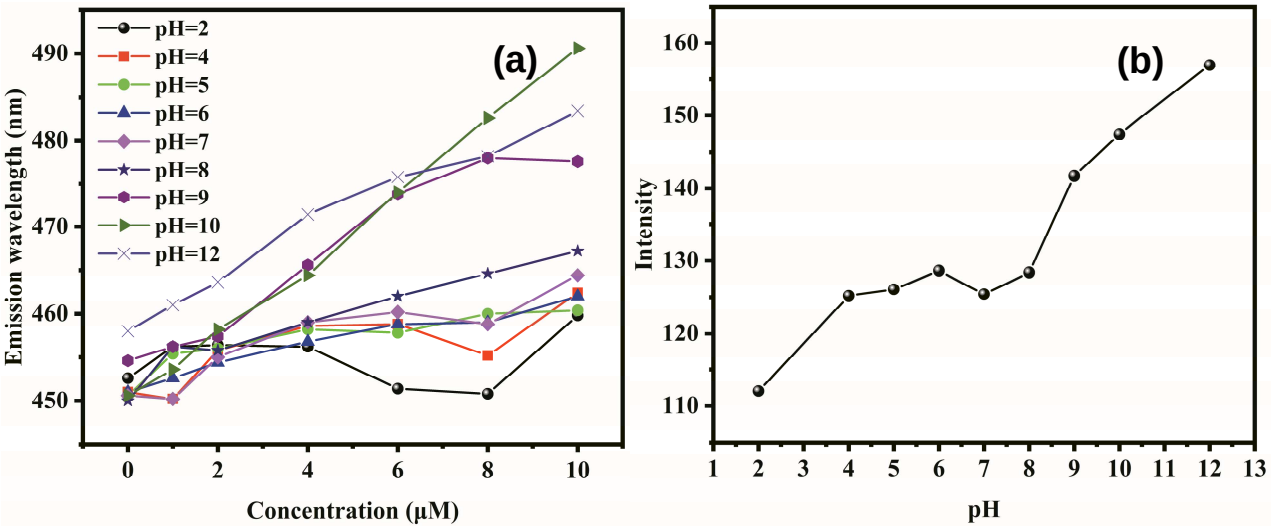

Figure S11. (a) The maximum fluorescence emission wavelength of P-CQDs in the presence of different concentrations of OTC under a wide range of pH conditions, (b) The fluorescence intensity of 1mg/L P-CQDs under different pH conditions (excitation wavelength: 370nm, emission wavelength: 457nm, the pH of solutions was adjusted by dropping a certain volume of 1M HCl or 1M NaOH into PBS buffers).

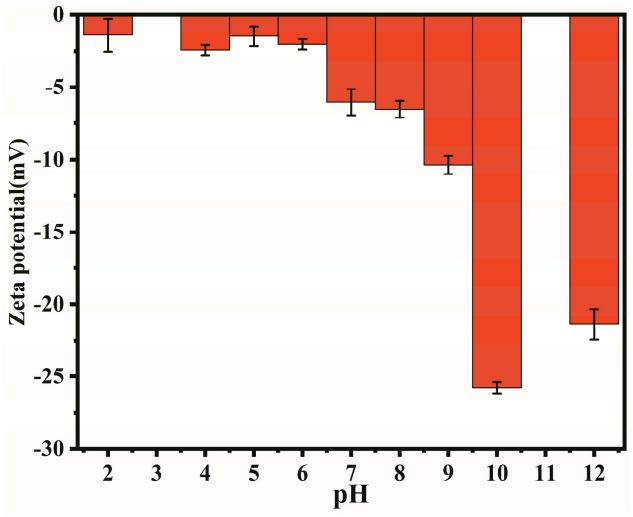

Figure S12. The zeta potential of P-CQDs under different pH conditions (P-CQDs concentrations: 5mg/L, the number of parallel samples was three).

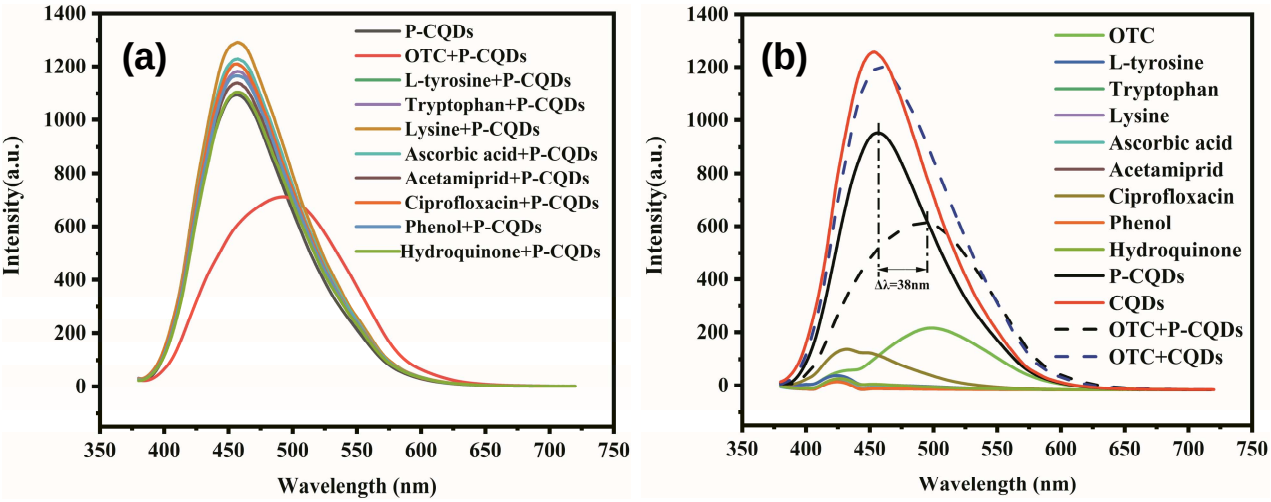

Figure S13. Fluorescence emission spectra of (a) P-CQDs in the presence of OTC or other organic pollutants and (b) P-CQDs, CQDs, the mixture of P-CQDs and OTC, the mixture of CQDs and OTC, and other organic pollutants in 0.025M $NaHCO_3$-NaOH buffer (the concentrations of CQDs and P-CQDs are 5mg/L, the concentrations of L-tyrosine, tryptophan, lysine and ascorbic are 5μM, the concentrations of other substances are 10μM, excitation wavelength: 370nm, pH=9.8).

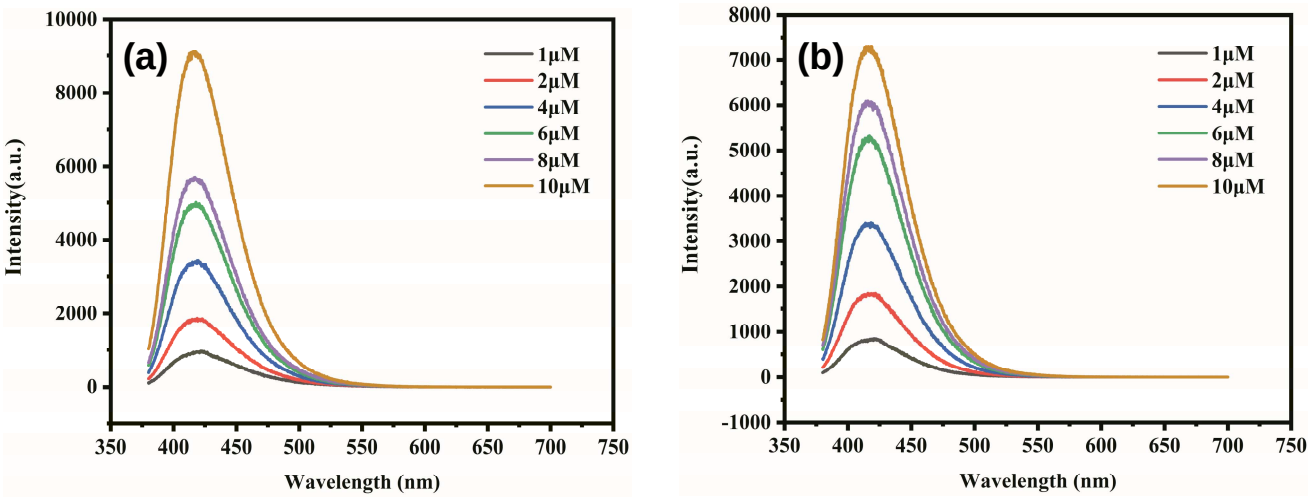

Figure S14. Fluorescence emission spectra of (a) P-CQDs in the presence of different concentrations of CTC, (b) different concentrations of CTC in 0.025M

$NaHCO_3$-NaOH buffer (P-CQDs concentration: 5mg/L, excitation wavelength: 370nm, pH=9.8).

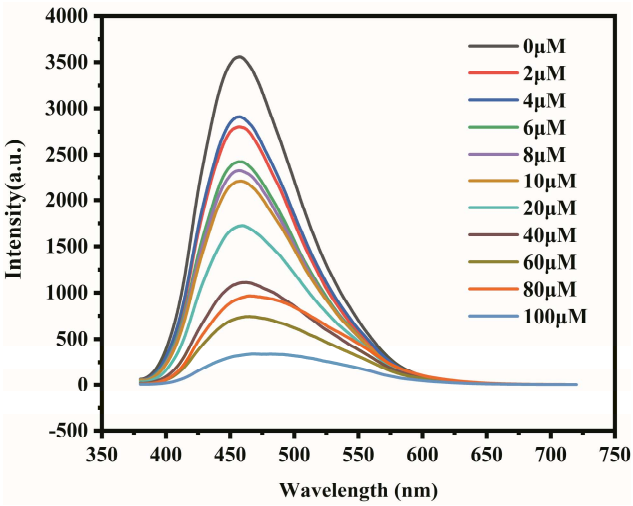

Figure S15. Fluorescence emission spectra of P-CQDs in the presence of different concentrations of TC in 0.025M $NaHCO_3$-NaOH buffer (P-CQDs concentration: 5μg/ml, excitation wavelength: 370nm, pH=9.8).

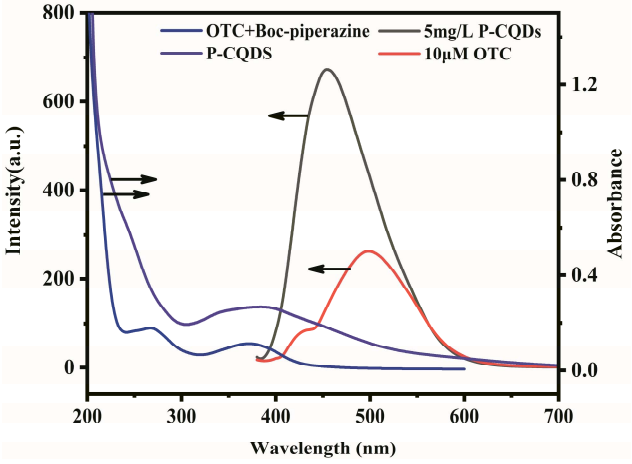

Figure S16. Fluorescence emission intensity of P-CQDs and OTC, and UV-vis absorption spectra of P-CQDs and mixture of OTC and Boc-piperazine (pH=9.8, excitation wavelength: 370nm, the concentration of Boc-piperazine was 10μM).

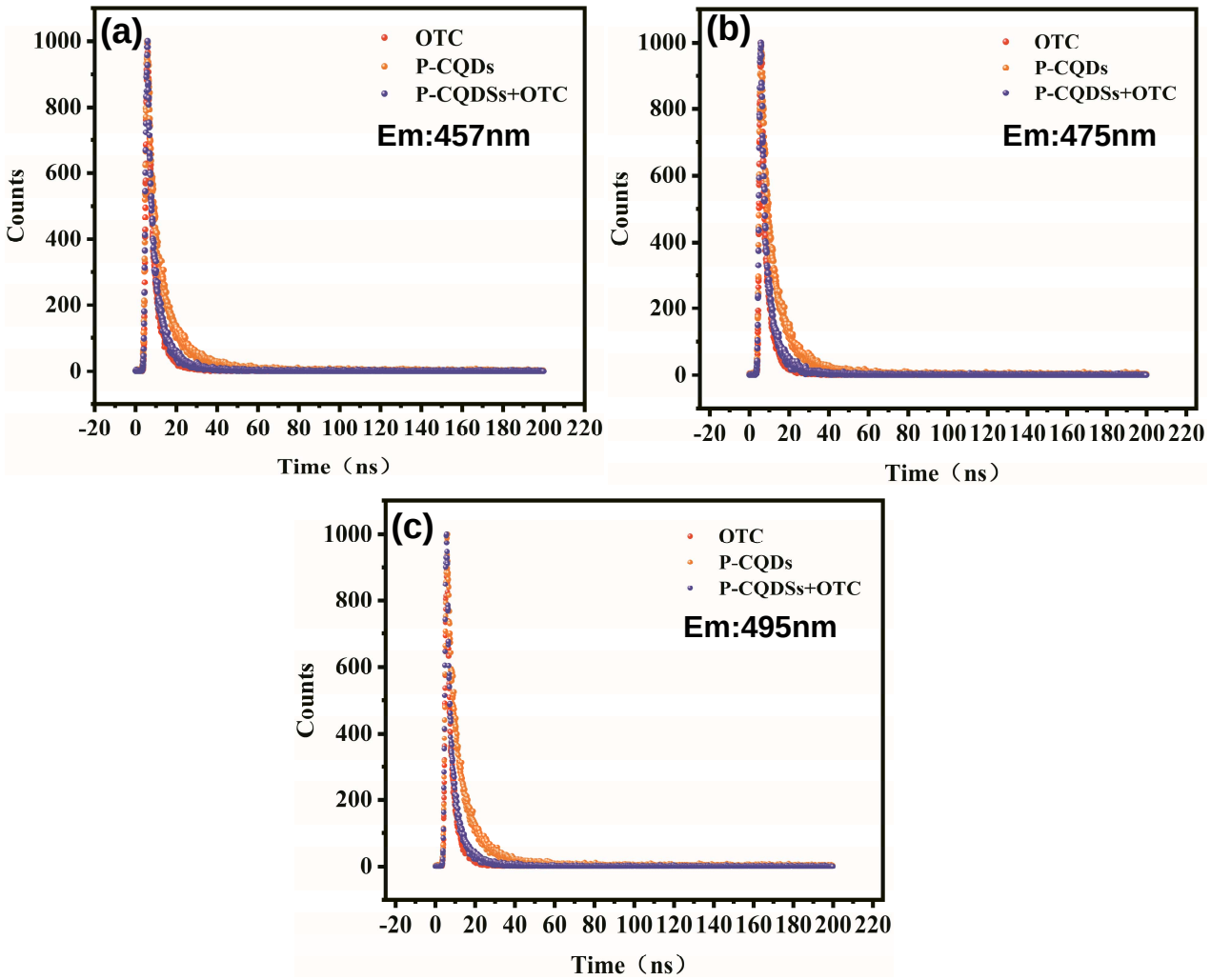

Figure S17. Time-resolved decay curves of OTC, P-CQDs and mixture of P-CQDs and OTC, (a) emission wavelength: 457nm, (b) emission wavelength: 475nm, and (c) emission wavelength: 495nm (excitation wavelength: 370nm, pH=9.8, the concentrations of P-CQDs and OTC was 5mg/L and 10μM, respectively).

Table S1. Fluorescence lifetime of OTC, P-CQDs and mixture of P-CQDs and OTC.

	λ_{ex}/nm	λ_{em}/nm	τ_1(ns)	τ_2(ns)	B_1	B_2	τ_{total}(ns)
OTC	370	457	2.2324	6.2065	705.098	313.103	4.4280
P-CQDs	370	457	3.1927	11.2365	600.759	391.655	8.7949
OTC+P-CQDs	370	457	2.4726	7.7801	655.798	271.458	5.4750
OTC	370	475	1.8216	5.4667	669.179	349.250	4.0463
P-CQDs	370	475	3.3993	10.8803	609.071	413.356	8.5220
OTC+P-CQDs	370	475	2.0730	6.9230	696.958	298.074	4.9257
OTC	370	495	1.5176	5.0887	645.191	314.348	3.7328
P-CQDs	370	495	3.5402	10.7556	524.090	364.620	8.4383
OTC+P-CQDs	370	495	1.7250	6.6921	745.344	279.828	4.6701

Fitting equation: $R(t)=B_1e^{(-t/\tau_1)}+B_2e^{(-t/\tau_2)}$, $\tau_{total}=\frac{B_1\tau_1^2+B_2\tau_2^2}{B_1\tau_1+B_2\tau_2}$

R(t): residual counts, B_1, B_2: fitting coefficients, t: decay time, τ_1, τ_2: fluorescence lifetime, τ_{total}: average fluorescence lifetime.