

Supporting Information

Nitrogen doped carbon quantum dots for bioimaging and detection of norfloxacin residues in food samples

S. Sivaselvam^a, R.S. Anjana^a, Dhujana N.S^a, Marina Victor^a and R.S. Jayasree^{a,*}

^aDivision of Biophotonics and Imaging, Department of Biomaterial Sciences and Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum 695012, India.

*Corresponding author Email: jayasree@sctimst.ac.in

S1.1. Quantum Yield calculation

The quantum yield of prepared Ch-QDs were calculated using the following formula¹

$$\Phi = \Phi_R X \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{n^2}{n_R^2} \quad \text{equation (1)}$$

Where, Φ is the quantum yield, I is the measured integrated emission intensity, n represents the refractive index, A represents the absorbance measured in UV-visible spectroscopy and the subscript r refers the standard. The quinine sulphate in 0.1 M of sulfuric acid (H_2SO_4) with the quantum yield of 54% at the excitation wavelength of 350 nm was taken as standard.

S1.2. Effect of Ch-QDs on lifespan of nematodes

For the lifespan analysis, nearly 20-30 worms were treated with Ch-QDs at different concentration (0-500 $\mu\text{g}/\text{mL}$) and incubated at 22°C. After 52 h, the worms were transferred to fresh NGM plate that contains 5-fluoro-2'-deoxyuridine (0.05 M) to restrict the growth of progeny. Every three days, the worms were moved into a fresh NGM plate to avoid starvation and the numbers of deceased and surviving worms were tallied. The worm exhibiting creeping movement and the mechanical death were considered as censored. The assay was continued until the last worm reached the censored state. Each experiment was replicated at least 3 times under similar conditions.

S1.3. Effect of Ch-QDs on growth and reproduction behavior of nematodes

The age synchronized L1 larva were placed in the NGM plate containing *E. coli* OP50 and Ch-QDs at different concentration (0-500 $\mu\text{g}/\text{mL}$). At the L4 stage, 50 randomly chosen worms/treatment were mounted on 1.5% agar pad and anesthetized with 30 mM of NaN_3 and analyzed using inverted microscope (Olympus CKX53 microscope with 40X objective). In the reproduction assay, age synchronized L1 worms were treated with Ch-QDs. At the L4 stage, 30 randomly chosen worms were individually moved to new NGM plate each day until the cessation of the worm's egg-laying capability. The progeny out from the eggs were counted and noted at the L3 stage using stereo microscope (Almicro, DSZ-77). Each experiment was replicated at least 3 times under similar condition.

S1.4. Effect of Ch-QDs on locomotion and defecation behavior

The locomotion behavior in nematodes were evaluated using the end point of head thrash and body bends. Briefly, nematodes treated with Ch-QDs (n=30 worms/experiment) were washed and transferred to the agar padded slide containing 50 μL of M9 buffer. The worms were touched using a platinum wire under stereo microscope and the locomotion behavior was counted according to the method described.² For analyzing the defecation cycle length, we recorded the duration between two consecutive posterior muscle movements using an inverted microscope. Each experiment was replicated at least 3 times under similar condition.

Supplementary Figures

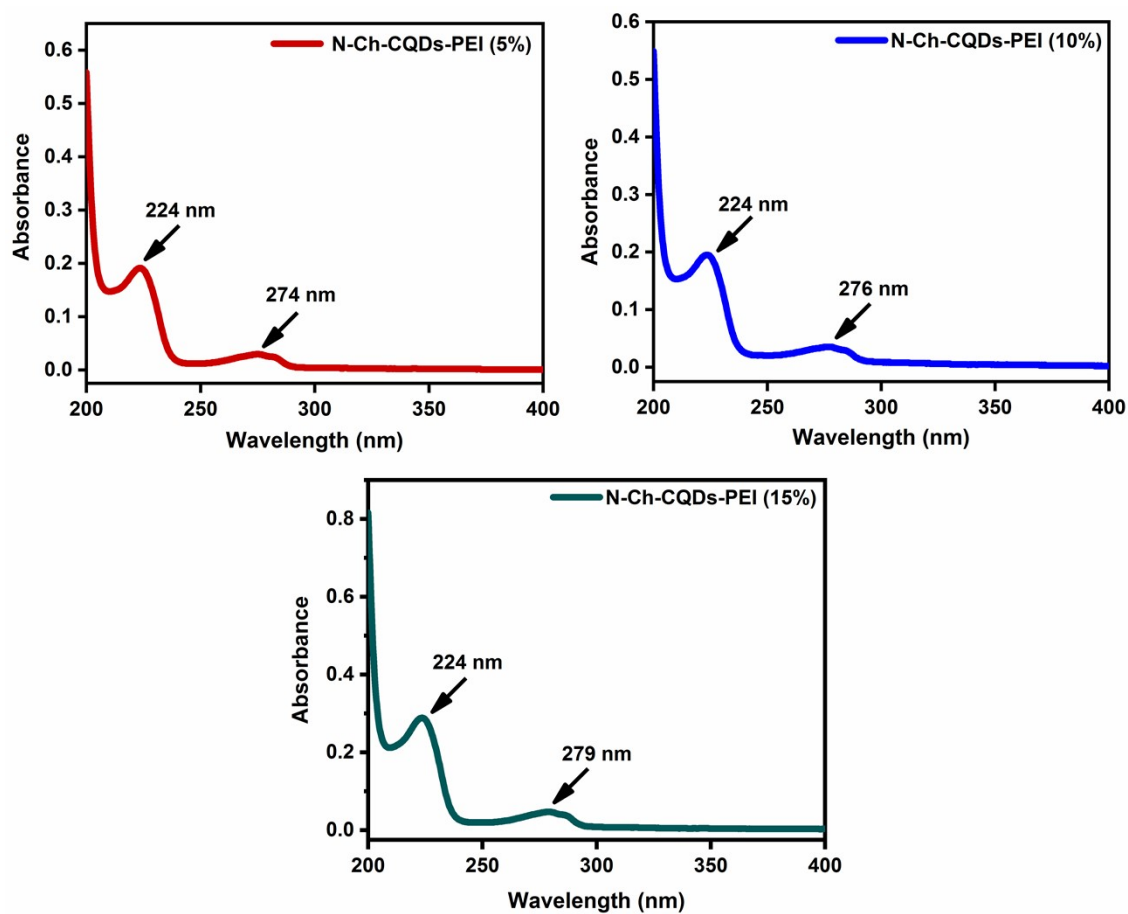


Fig. S1. UV-Visible spectra of N-Ch-CQDs with different concentration of PEI

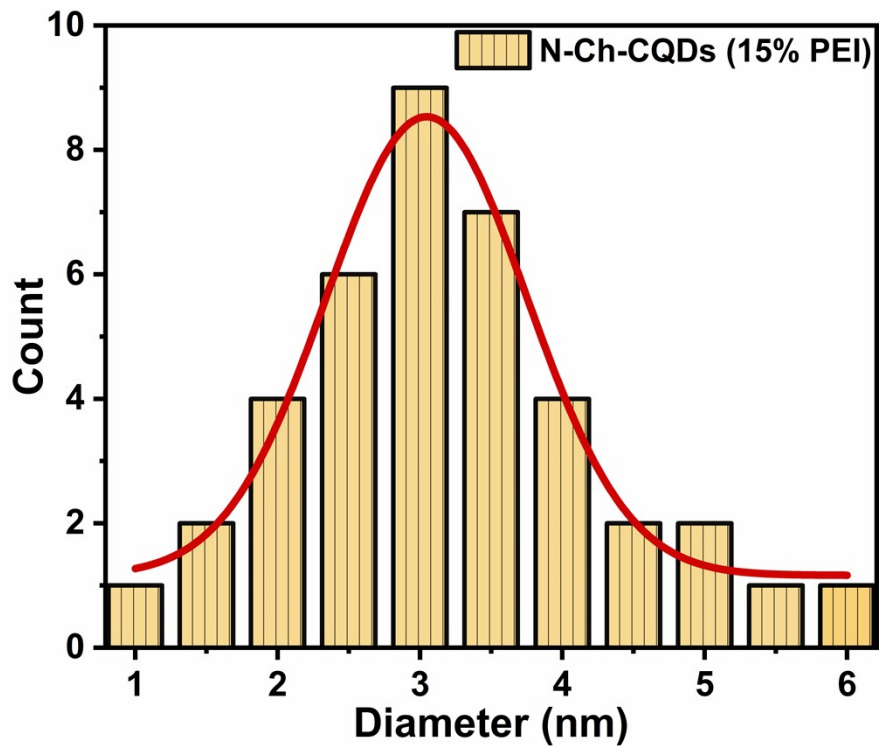


Fig. S2. Average size distribution of N-Ch-CQDs (15% PEI) from TEM image

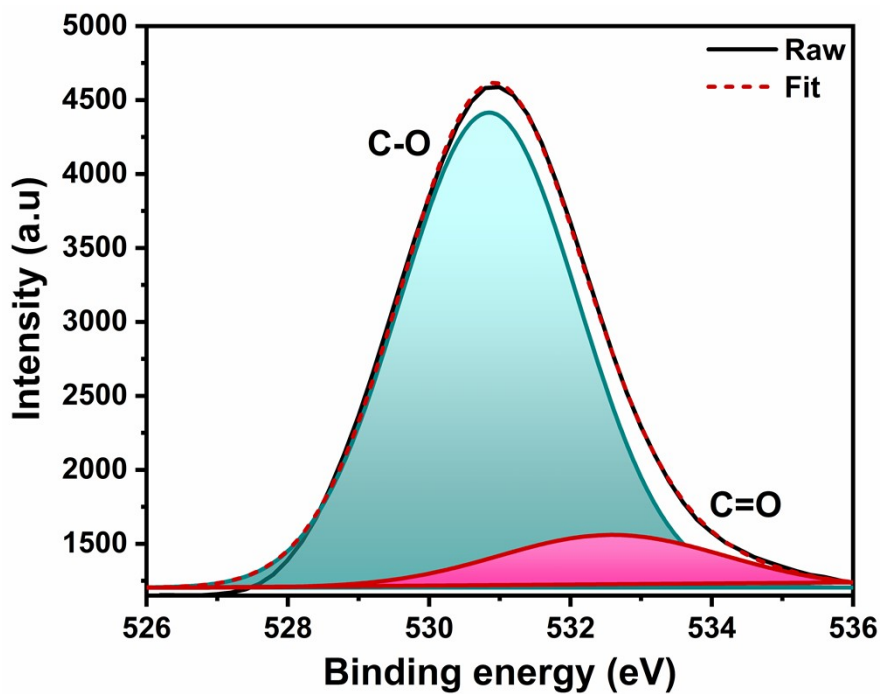


Fig. S3. Deconvoluted O 1s XPS spectra of N-Ch-CQDs

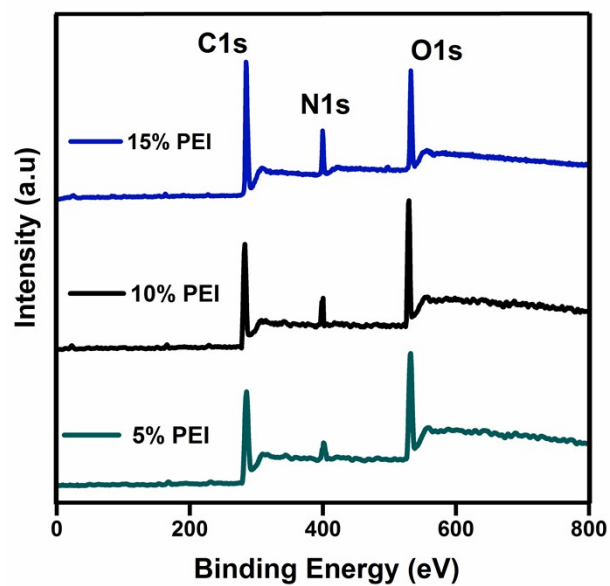


Fig. S4. XPS spectra of N-Ch-CQDs prepared with different concentration of PEI

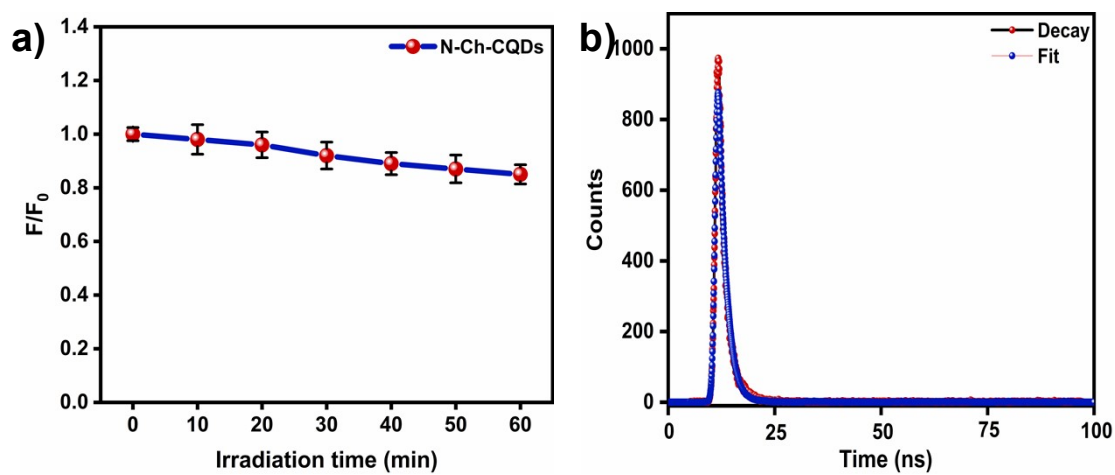


Fig. S5. Fluorescence intensity ratio of N-Ch-CQDs at different UV-irradiation time (0-60 min). Time-resolved fluorescence decay profiles of N-Ch-CQDs. The error bar indicates the SD of 3 independent experiments.

Fig. S7. Effect of N-Ch-CQDs on the lifespan of wild-type *C. elegans*. Survival curves of wild-type worms. The representative Kaplan–Meier survival curves was generated from three independent biological trials.

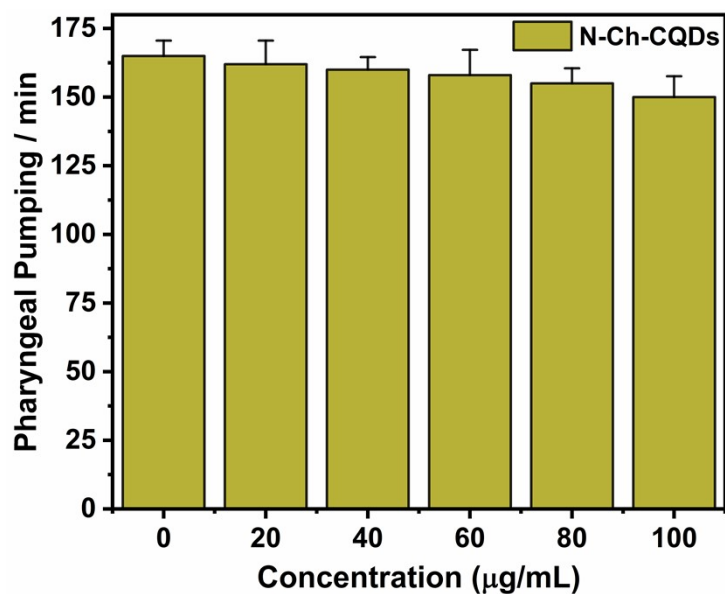


Fig. S8. Effect of N-Ch-CQDs on pharyngeal pumping rate of wild-type N2 nematodes. Error bars indicates the SD of 3 experiments.

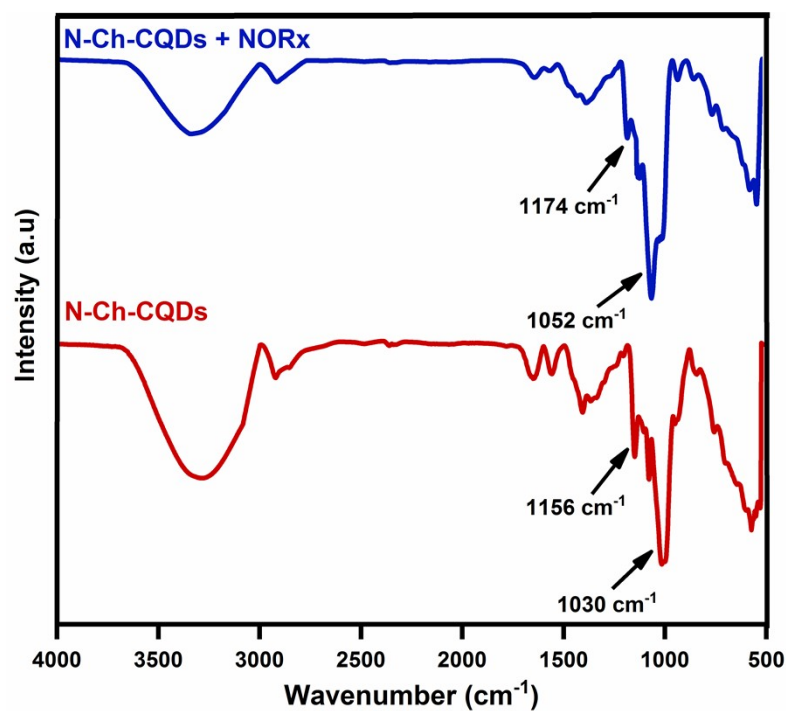


Fig. S9. FTIR spectra of N-Ch-CQDs in the presence and absence of norfloxacin

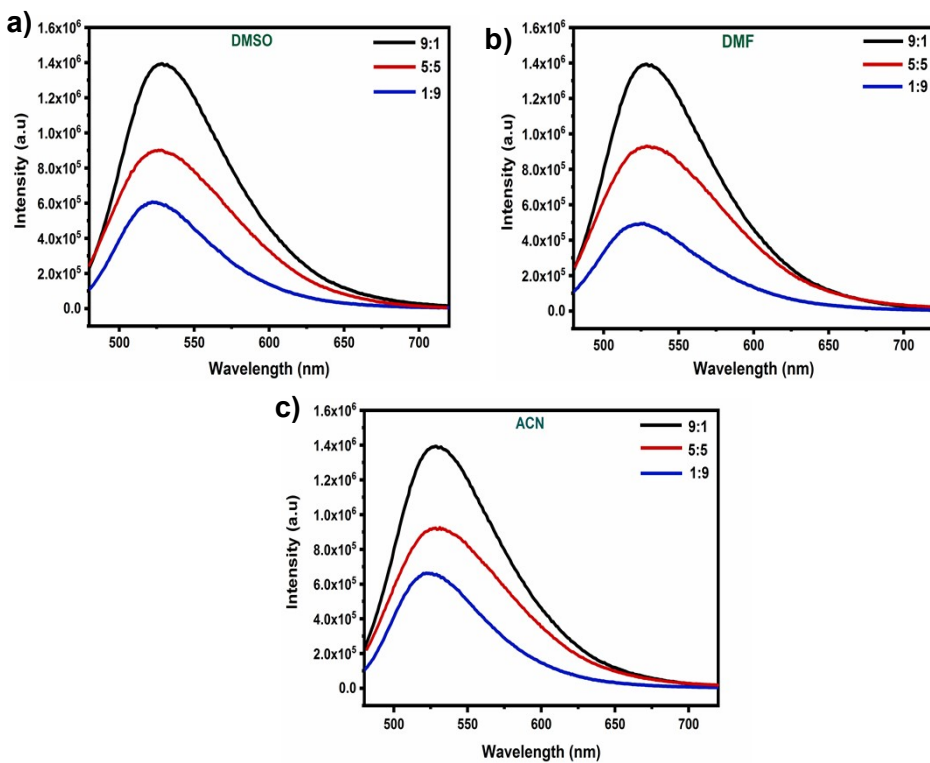


Fig. S10. Effect of N-Ch-CQDs fluorescent intensity in different aprotic solvent a) DMSO, b) DMF and c) CAN

Table. S1. Elemental analysis percentage from XPS spectra of N-Ch-CQDs with different PEI concentration

N-Ch-CQDs	C %	O%	N%	QY%
5% PEI	67.91	28.45	3.64	4.5
10% PEI	68.04	23.83	8.13	9.6
15% PEI	69.57	19.25	11.18	21.5

Table. S2. Survival rate of wild-type *C. elegans* cultured on NGM plates carrying different concentration of N-Ch-CQDs. The mean lifespan of *C. elegans* were calculated using Kaplan Meir survival analysis and significance levels were estimated by long-rank test in Medcalc statistical tool.

Treatment	Mean survival (Mean±SEM)	Maximum lifespan (days)	Sample size (N)	Censored	% Change	<i>p</i> value	
N-Ch-CQDs	Concentration						
	0 µg/mL	16.723±0.387	24	136	13		
	25 µg/mL	16.896±0.405	25	130	7	(+) 1.02	0.7257
	50 µg/mL	16.875±0.432	24	142	15	(+) 0.93	0.7075
	75 µg/mL	16.636±0.399	24	126	9	(-) 0.52	0.3777
	100 µg/mL	16.625±0.399	24	163	11	(-) 0.58	0.3254
	200 µg/mL	16.452±0.399	24	171	10	(-) 1.62	0.3156
	500 µg/mL	13.452±0.484	20	134	12	(-) 19.5	0.0001
	1000 µg/mL	13.210±0.240	19	166	18	(-) 21.00	0.0001

References

- 1 M. Grabolle, M. Spieles, V. Lesnyak, N. Gaponik, A. Eychmüller and U. Resch-Genger, *Analytical Chemistry*, 2009, **81**, 6285–6294.
- 2 S. Sivaselvam, A. Mohankumar, R. Narmadha, R. Selvakumar, P. Sundararaj, C. Viswanathan and N. Ponpandian, *Environmental Pollution*, 2023, **318**, 120933.