Supplementary Information

ZrO_2 decorated Pluronic F-127 modified gCN nanosheets for IFE-driven detection of Tetracycline

Arushi Sharma [a], Aashima Sharma [a], Neena Mehta [b], Ramesh Kataria [a], S.K. Mehta* [a]

a Department of Chemistry and Centre of Advanced Studies in Chemistry, Panjab University, Sector-14, Chandigarh 160014, India

b Department of Biochemistry, Rayat Bahra University, Sahauran, Mohali, 140104, India

***Corresponding author: skmehta@pu.ac.in**

S1. Materials and Methods

S1.1 Chemicals and Reagents

All chemicals employed were of analytical grade and used directly without additional purification. L-Arginine, Glutamic acid, Glycine, Histidine, D-Fructose, L-Dopa, Valine, Sodium hydroxide pellets (NaOH), Pluronic F-127 and Melamine were acquired from Loba Chemie (Mumbai, India). Sodium Nitrite ($NaNO_2$), Sodium Fluoride (NaF), Sodium Iodide (NaI), Sodium Bromide (NaBr), Copper (II) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$), Sodium sulfate anhydrous (Na_2SO_4), Calcium chloride anhydrous, Sodium phosphate dibasic heptahydrate ($Na_2HPO_4.7H_2O$), and Sodium Chloride (NaCl), were procured from Qualigens-Thermo Fischer Scientific. Trypsin, Mannose, Glucose, Caffeine, and Sucrose were obtained from HiMedia. Iron (III) nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$, 98%), Citric acid, Aluminium nitrate nonahydrate ($Al(NO_3)_3 \cdot 9H_2O$, 98%), and Potassium nitrate (KNO_3, 99%), were received from Avra. Cobalt (II) nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$, ≥98%), L-Lysine, Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, L-Cysteine, Phenylalanine, Ascorbic acid, Hydrochloric acid were purchased from Sigma Aldrich. Norfloxacin, Paracetamol, Tetracycline, Ciprofloxacin, Levofloxacin, Ofloxacin, and Oxytetracycline Hydrochloride were procured from Tokyo Chemical Industries (TCI). All aqueous solutions were prepared using deionized water.

S1.2 Instrumentation

X-ray photoelectron spectroscopy (XPS) studies were carried out with Thermo Scientific NEXA operating with an Al Kα X-ray source. Powder X-ray diffraction (PXRD) patterns of synthesized materials were obtained on a Shimadzu Maxima-7000 using Cu-target in 2θ range of 5-80°. Hitachi-H-7500 transmission electron microscope (HRTEM) equipped with energy dispersive X-ray spectroscopy (EDX) was utilized for particle size and morphological analysis of samples. UV-*vis* spectra were recorded on Evolution 201 UV-visible spectrophotometer and UV-vis diffuse reflectance spectrum was obtained by JEOL double beam spectrophotometer V-750. The FTIR spectrum was recorded by a PerkinElmer Spectrum Two instrument. Thermogravimetric Analysis (TGA) was assessed *via* SDT-Q-600 (TA instruments New Castle, DE) in the temperature range of 25-800 °C. The surface area of the samples was analysed using Anton Paar Autosorp-iQ-XR. Centrifugation was performed using Eppendorf Centrifuge 5425. Cyclic Voltammetry measurements were conducted using an Origalys Origaflex OGF-500 electrochemical workstation in a three-electrode setup with Phosphate buffer solution as an electrolyte. A catalyst (5 mg) was

dispersed with deionized water and Nafion (binder), sonicated, and dropcasted onto glassy carbon electrode (2.5 µL). The Fluorescence spectra were recorded on a Hitachi (F-7000) fluorescence spectrophotometer and fluorescence lifetime measurements were analysed on DeltaFlex TCSPC Lifetime Fluorimeter.

S2. Fluorometric methodology and Sample Pretreatment

S2.1 Fluorescence sensing strategies for detection of tetracycline

Fluorescence measurements were performed in deionized water under the following instrumental conditions: photomultiplier tube (PMT) voltage of 500 V, excitation and emission slit widths of 10 nm, a scanning speed of 1200 nm/min, and an excitation wavelength of 370 nm. The ZrO_2/P-gCN fluorescent probe (0.5 mg/mL) was first dispersed in deionized water to ensure a uniform suspension and then ultrasonicated for 30 min at 30 °C. For each measurement, 300 µL of the probe suspension was transferred into a quartz cuvette, after which the required amount of analyte was added. The mixture was subsequently diluted with deionized water to obtain a final volume of 2000 µL. To evaluate the sensing response, different volumes of TC and OTC were introduced into the probe suspension, yielding final concentrations of 0-75 µM and 0-50 µM, respectively. Selectivity experiments were conducted using the same procedure, where potential interfering substances such as antibiotics, amino acids, carbohydrates, essential metals, electrolytes, and various anions were examined.

For anti-interference tests, the detection of TC and OTC was carried out in the presence of

300 µM solutions of commonly used pharmaceuticals and antibiotics, including Paracetamol, Ofloxacin, Norfloxacin, Ciprofloxacin, and Levofloxacin. In addition, amino acids such as L-arginine, Glutamic acid, Histidine, Glycine, Phenylalanine, L-valine, L-lysine, Cysteine, and L-DOPA (each at 1 mM) were carried out, along with carbohydrates and related biomolecules including Ascorbic Acid, Glucose, Mannose, Fructose, Sucrose, Trypsin, Citric Acid, and Caffeine (each at 1 mM). To probe the effect of competing ionic species, essential metal ions and common electrolytes/anion species such as Zn^{2+}, Al^{3+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Na^{+}, K^{+}, Fe^{3+}, F^{-}, Cl^{-}, PO_4^{3-}, and SO_4^{2-} (each at 500 µM) were examined under identical conditions. The role of ionic strength was further assessed by adding NaCl solutions of varying concentrations (0.2-5 M) to the probe suspension. For pH-dependent studies, phosphate buffer solutions were used, and pH adjustments were made using dilute NaOH and HCl solutions.

S2.2 Pre-analytical treatment of samples

For the quantitative analysis of TC and OTC, water samples were collected from three different sources to represent diverse environmental conditions. Tap and industrial wastewater samples were obtained from Panchkula, Haryana, India, and groundwater samples were collected from Ambala, Haryana, India. To maintain sample integrity and ensure realistic assessment, all samples were analyzed in their original form without any pretreatment.

The known concentrations of TC and OTC were subsequently spiked into each sample to evaluate the performance of sensor under actual conditions, following the same experimental protocol used for standard solutions. The quantification of TC and OTC was carried out using the standard addition method to minimize matrix effects and enhance analytical accuracy. The recovery percentage was calculated according to equation (1) [1]:

$$\text{Recovery}\ (\%) = (C_{found} / C_{added}) \times 100 \qquad \text{(S1)}$$

where C_{found} is the concentration measured after spiking, C_{added} is the initial concentration of TC and OTC spiked.

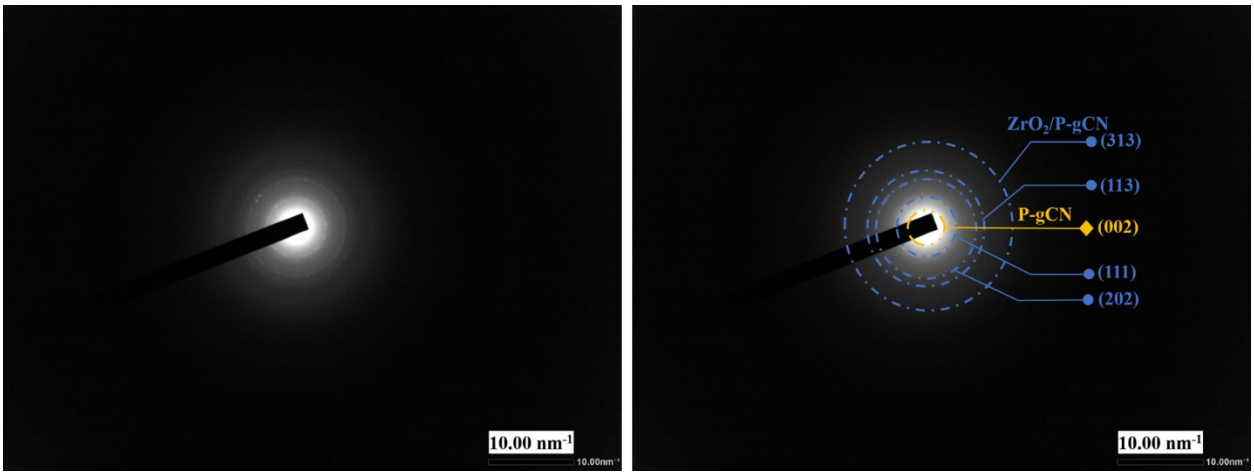

Figure S1. SAED pattern of ZrO_2/P-gCN nanocomposite.

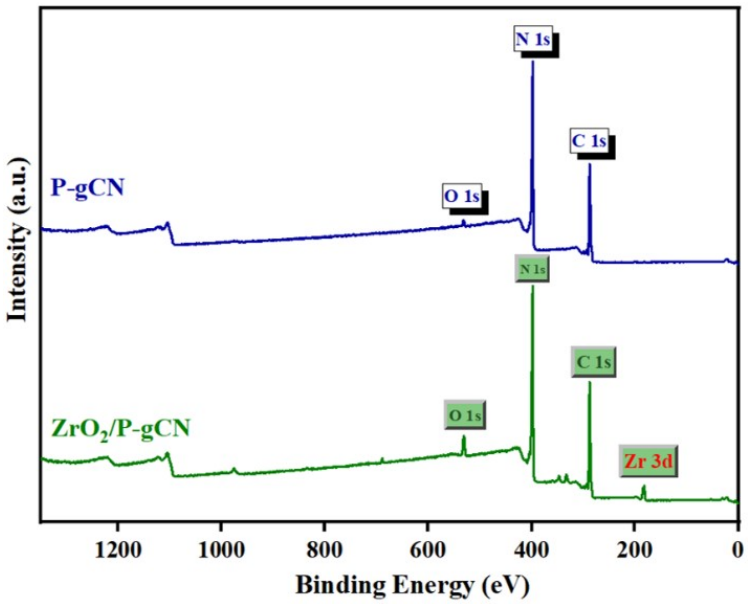

Figure S2. Survey spectrum of P-gCN and nanocomposite ZrO_2/P-gCN.

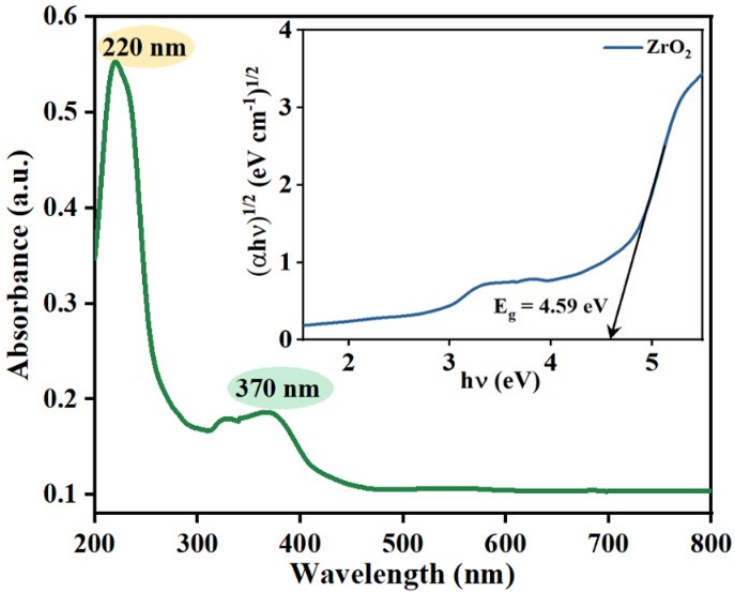

Figure S3. UV-DRS (inset: Tauc Plot) of pure ZrO_2 nanoparticles.

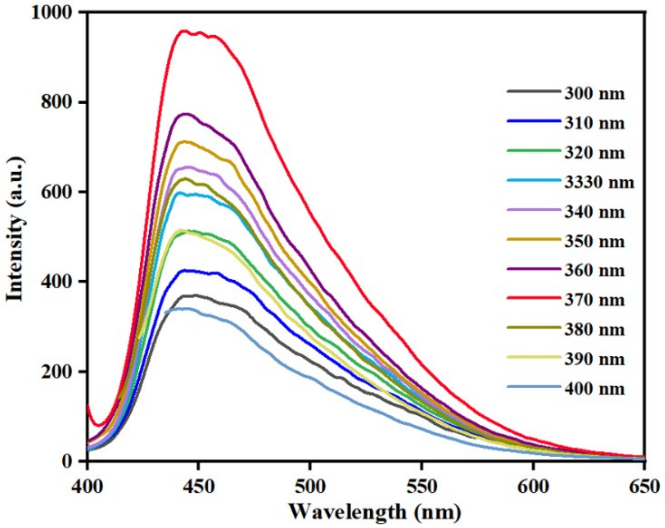

Figure S4. Wavelength studies from 300 to 400 nm of ZrO_2/P-gCN.

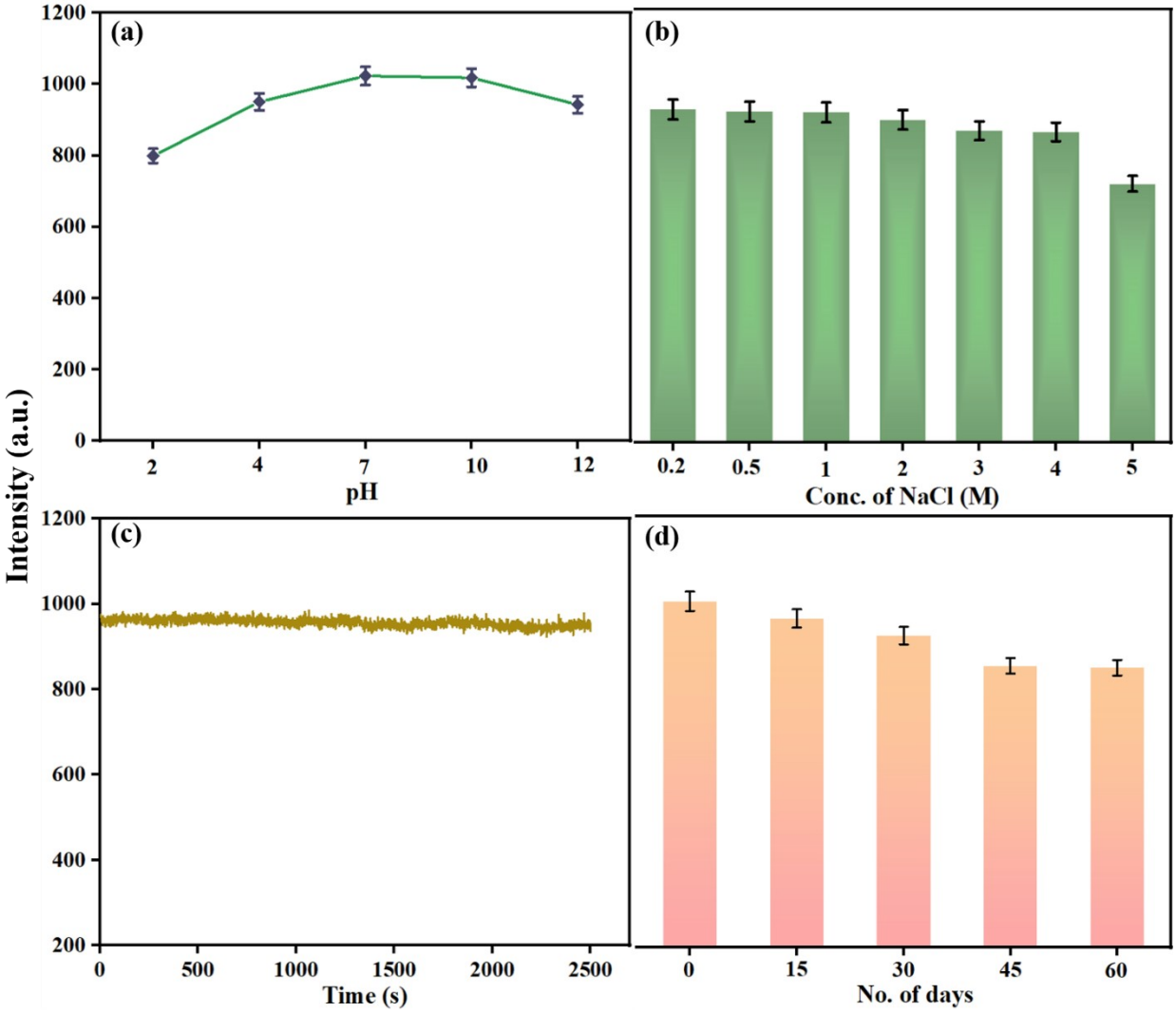

Figure S5. (a) pH studies (b) ionic strength (c) time study (d) storage stability studies of ZrO_2/P-gCN.

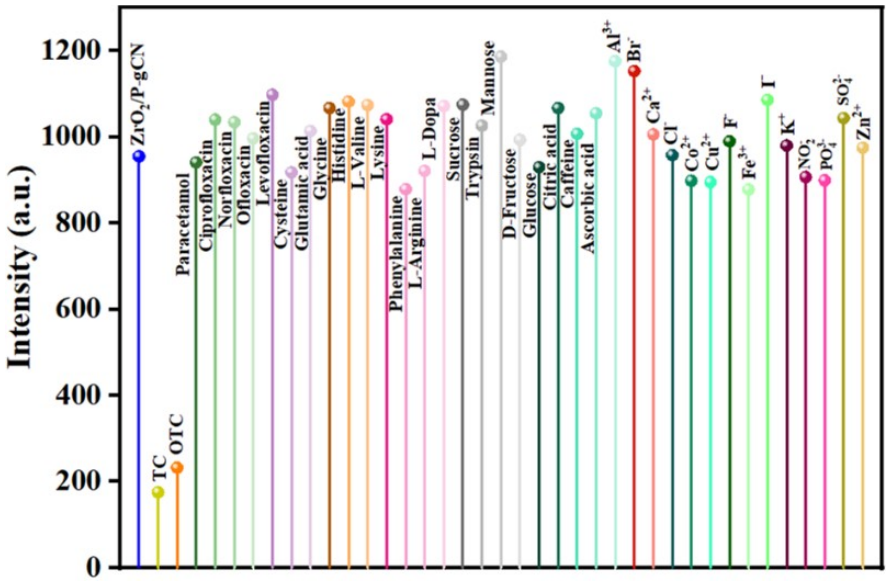

Figure S6. Selectivity assessment of ZrO_2/P-gCN nanocomposite for TC and OTC detection in comparison with various antibiotics, amino acids, carbohydrates, electrolytes essential metal ions and anions.

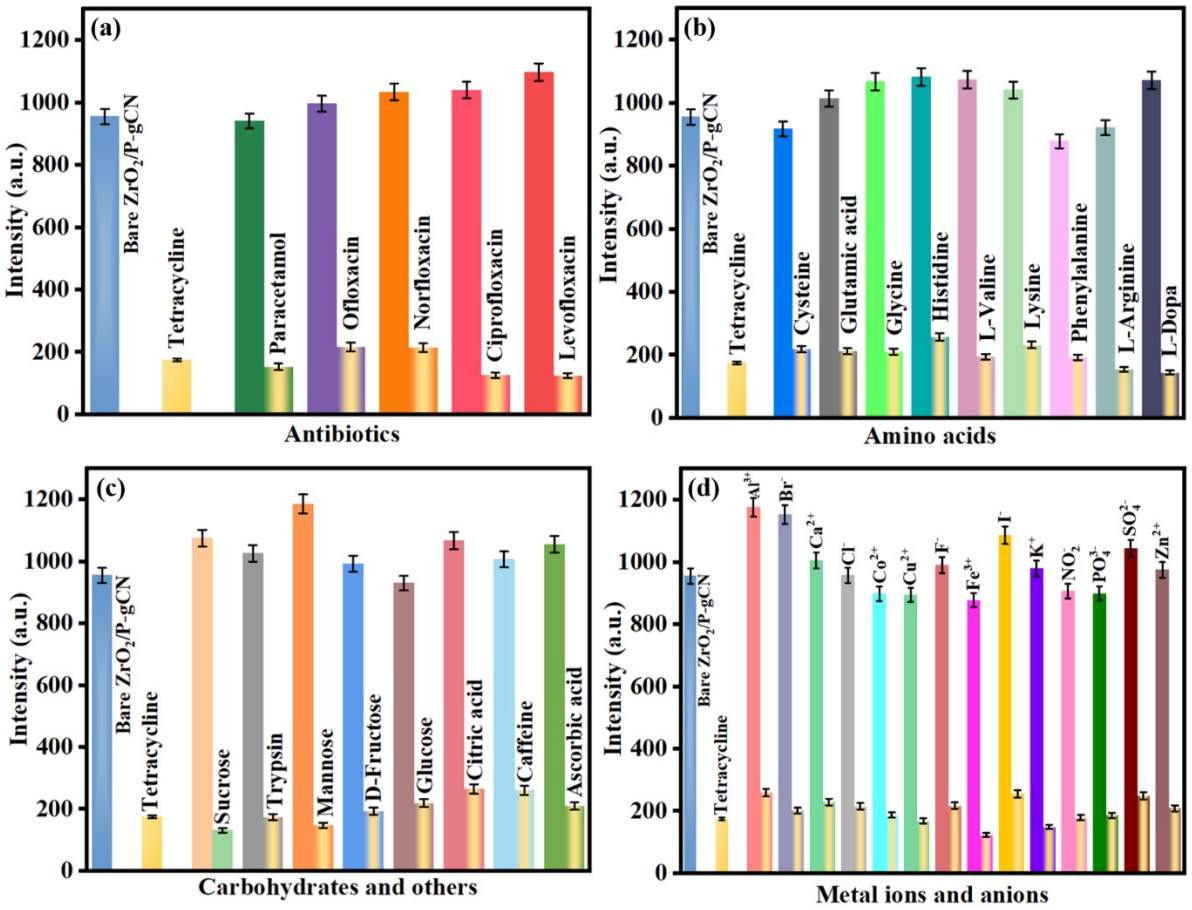

Figure S7. FL response and selectivity evaluation of the ZrO_2/P-gCN nanocomposite toward Tetracycline (TC) in the presence of various potentially interfering substances. Comparative fluorescence intensity of the probe in the presence of (a) different antibiotics (Paracetamol, Ofloxacin, Norfloxacin, Ciprofloxacin and Levofloxacin), (b) amino acids (Cysteine, Glutamic acid, Glycine, Histidine, L-Valine, L-Lysine, Phenylalanine, l-Arginine, and L-Dopa), (c) Carbohydrates and other organic compounds (Sucrose, Trypsin, Mannose, D-Fructose, Glucose, Citric acid, Caffeine and Ascorbic acid), (d) various metals and anions (Al^{3+}, Br^-, Ca^{2+}, Cl^-, Co^{2+}, Cu^{2+}, F^-, Fe^{3+}, I^-, K^+, NO_2^-, PO_4^{3-}, SO_4^{2-}, and Zn^{2+}). A significant quenching response is observed specifically for TC, whereas negligible changes in fluorescence intensity are obtained for other coexisting species, showing the high selectivity and specificity of the ZrO_2/P-gCN nanocomposite toward TC detection.

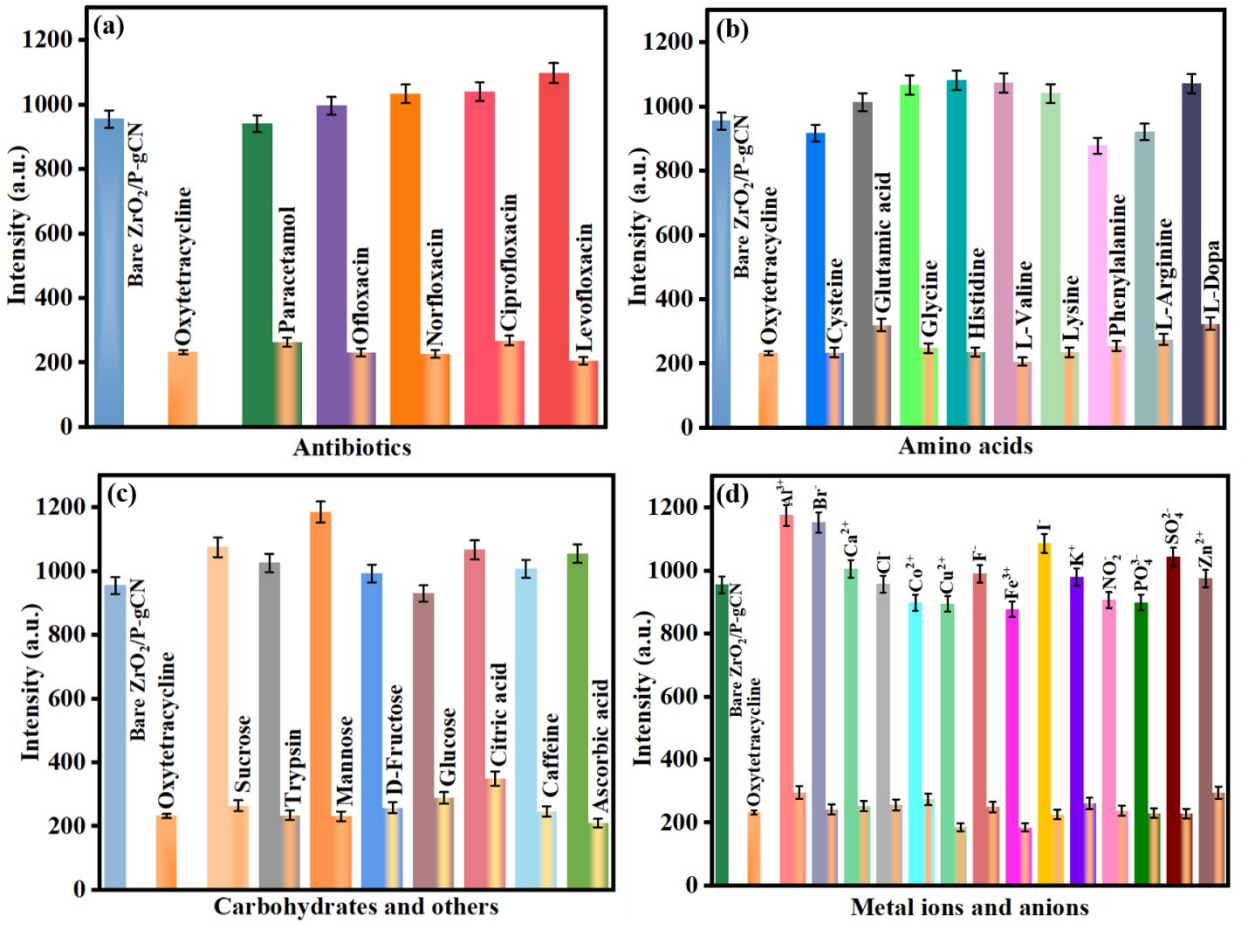

Figure S8. FL response and selectivity evaluation of the ZrO_2/P-gCN nanocomposite toward Oxytetracycline Hydrochloride (OTC) in the presence of various potentially interfering substances. Comparative fluorescence intensity of the probe in the presence of (a) different antibiotics (Paracetamol, Ofloxacin, Norfloxacin, Ciprofloxacin and Levofloxacin), (b) amino acids (Cysteine, Glutamic acid, Glycine, Histidine, L-Valine, L-Lysine, Phenylalanine, L-Arginine, and L-Dopa), (c) Carbohydrates and other organic compounds (Sucrose, Trypsin, Mannose, D-Fructose, Glucose, Citric acid, Caffeine and Ascorbic acid), (d) various metals and anions (Al^{3+}, Br^-, Ca^{2+}, Cl^-, Co^{2+}, Cu^{2+}, F^-, Fe^{3+}, I^-, K^+, NO_2^-, PO_4^{3-}, SO_4^{2-}, and Zn^{2+}). A significant quenching response is observed specifically for OTC, whereas negligible changes in fluorescence intensity are obtained for other coexisting species, showing the high selectivity and specificity of the ZrO_2/P-gCN nanocomposite toward TC detection.

Table S1. Comparison of LOD with different available sensors in literature

Technique	Sensor	Antibiotic	LOD (µM)	Linear Range (µmol/L)	Limit of Quantification	Response Time (min)	Application to Real Sample	Ref.
Fluorescence	S, N-CQDs	TC	0.560	1.88-60	-	3	Milk, Honey, Tap water.	[2]
Photometry	Ag NPs		13.000	50-5000	-	-	-	[3]
Fluorescence	CdS-QDs		7.780	15-600	-	-	-	[4]
Fluorescence	MQDA-Eu^{3+}		1.700	0-110	-	-	Piped water, Milk, Songhua river water.	[5]
Electrochemical	CB-PS/GCE		1.150	5-120	4.47	-	Tap water, River water, Milk and Commercial medicine.	[6]
Fluorescence	ZrO_2/P-gCN		0.421	0-10	-	-	Tap water, Industrial wastewater, Tap water.	Present work
Fluorescence	UiO-66-NH_2	OTC	0.431	0-7.5	-	-	Milk.	[7]
Fluorescence	MQDA-Eu^{3+}		0.800	0-95	-	-	Piped water, Milk, Songhua river water.	[5]
Fluorescence	BODIPY		0.720	0-42	-	-	Milk, Honey, Pork.	[8]
Fluorescence	CQDs		0.973	2-100	-	-	Tap water, Lake	[9]

						water, Soil samples.	
Fluorescence	ZrO_2/P-gCN	0.420	0-10	-	-	Tap water, Industrial wastewater, Tap water.	Present work

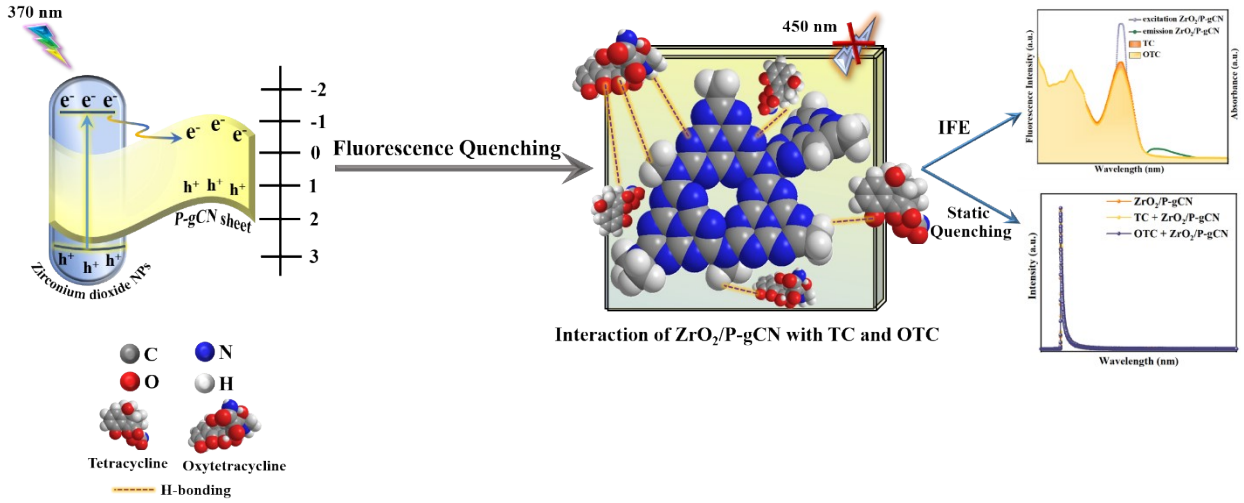

Figure S9. Mechanistic insight for fluorometric sensing of TC using ZrO_2/P-gCN

Table S2. Determination of TC using ZrO_2/P-gCN in water samples

Water Sample	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%) n=3
River water	5.00	4.95	99.00	1.04
	20.00	18.85	94.25	1.31
	40.00	39.05	97.63	4.85
Industrial wastewater	5.00	4.99	99.80	1.95
	20.00	18.73	93.65	0.73
	40.00	42.20	105.50	1.75
Tap water	5.00	5.02	100.40	1.96
	20.00	20.13	100.65	2.94
	40.00	40.02	100.05	2.35

Table S3. Determination of OTC using ZrO_2/P-gCN in water samples

Food Sample	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%) n=3
River water	5.00	4.95	99.00	1.12
	20.00	19.31	96.55	1.45
	40.00	38.84	97.10	2.57

Industrial wastewater	5.00	4.64	92.80	1.98
	20.00	19.33	96.65	3.39
	40.00	40.49	101.22	2.27
Tap water	5.00	4.98	99.60	1.05
	20.00	19.88	99.40	2.29
	40.00	40.33	100.82	0.65

RSD: Relative standard deviation

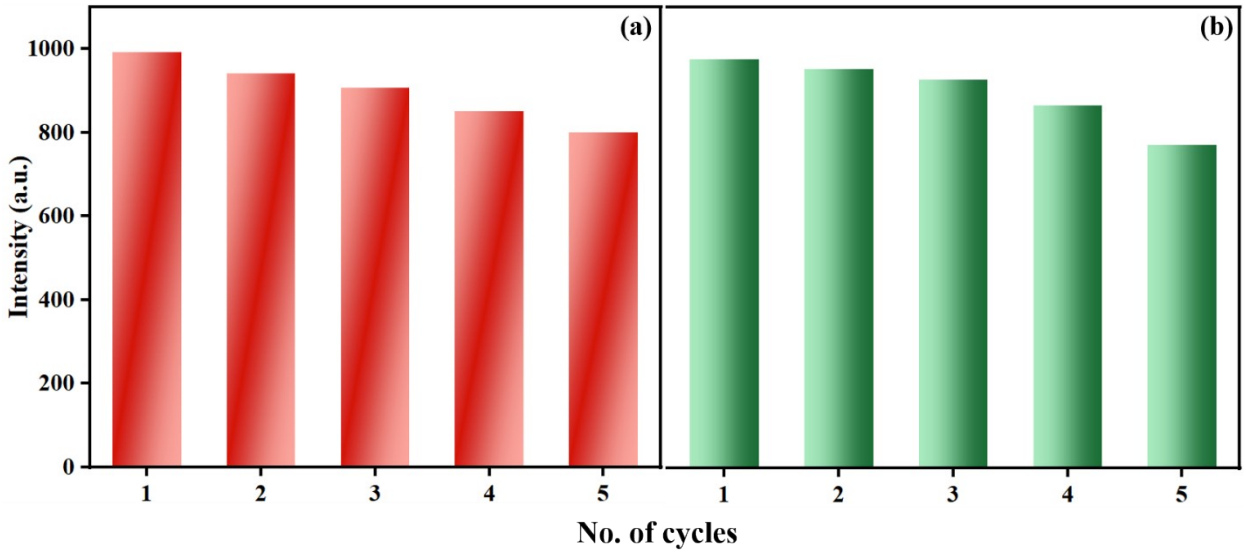

Figure S10. Recyclability study: comparison of fluorescence intensity of ZrO_2/P-gCN nanocomposite for five cycles upon detection of (a) TC and (b) OTC.

References:

1 P. Li, W. Cai and X. Shao, *Journal of Chemometrics*, 2015, **29**, 300–308.

2 Y. Fan, W. Qiao, W. Long, H. Chen, H. Fu, C. Zhou and Y. She, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2022, **274**, 121033.

3 M. Amjadi, J. L. Manzoori and F. Pakpoor, *J Anal Chem*, 2016, **71**, 253–258.

4 S. K. Anand, U. Sivasankaran, A. R. Jose and K. G. Kumar, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2019, **213**, 410–415.

5 J.-M. Ran, L. Yang, C.-T. Liu, Q.-H. Liu, Y.-L. Liu, S.-J. Li, Y. Fu and F. Ye, *Science of The Total Environment*, 2024, **931**, 172866.

6 K. P. Delgado, P. A. Raymundo-Pereira, A. M. Campos, O. N. Oliveira and B. C. Janegitz, *Electroanalysis*, 2018, **30**, 2153–2159.

7 X. Wang and X. Wang, *RSC Adv.*, 2022, **12**, 23427–23436.
8 Z. Xu, X. Yi, Q. Wu, Y. Zhu, M. Ou and X. Xu, *RSC Adv.*, 2016, **6**, 89288–89297.
9 R. Gao, Z. Wu, L. Wang, J. Liu, Y. Deng, Z. Xiao, J. Fang and Y. Liang, *Nanomaterials*, 2020, **10**, 1561.