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Supporting Information

FRET and PET paired Dual Mechanistic Carbon Dots Approach for Tyrosinase Sensing

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Experimental Procedure:

Chemicals and Instruments: 1,8-Naphthalic anhydride, bovine serum albumin (HBA), human serum albumin (HSA) and phosphate buffer saline (PBS) were purchased from Sigma-Aldrich. Hydrazine hydrate, glutaric anhydride, citric acid, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide sodium salt and 4-dimethylaminopyridine (DMAP) were obtained from the Avra synthesis. Ethanol was purchased from Hayman Ltd. Milli-Q water (MΩ 18) was used in all experiments.

^{1}H NMR operated at 400 and ^{13}C NMR operated at 100 MHz were recorded on a Jeol instrument. UV-vis absorption and emission properties were recorded using Shimadzu UV-2400 spectrophotometer and PerkinElmer LS 55 fluorescence spectrophotometer respectively. Photoluminescence quantum yield and fluorescence decay time were recorded on FluoTime 300 High-Performance Fluorescence Lifetime Spectrometer using a time-correlated single photon counting (TCSPC) technique. LUDOX was used to record the response function of the instrument. Hitachi (H-7500) instrument was employed to record the TEM images of the CDs. The hydrodynamic particle diameter was measured using DLS with an external probe of Metrohm Microtrac Ultra Nanotrac particle size analyzer. IR spectra of the solid samples were recorded by using a solid cell technique on a Bruker Tensor 27 spectrophotometer.

Synthesis of CDs: Amino functionalized CDs were synthesized as per literature reports with slight modification.[1] Briefly, 0.3 g of citric acid dissolved in purified water and 3 mL of ethylene diamine was added into it. The reaction mixture was stirred at room temperature for 15 min and afterward transferred to Teflon lined autoclave. The autoclave was placed in preheated oven at 160 °C. After 6 h of heating, the autoclave was cooled to room temperature and reaction mixture was dialyzed against water for 48 h using dialysis membrane (MW = 3500 DA) to remove unreacted organic residues. CDs were freeze-dried and stored at 4 °C for further use.

Syntheis of L1: Tyramine hydrochloride (224 mg, 1.3 mmol) and three drop of triethyl amine was added into the ethanol and stirred for 15 min. 4-Bromonaphthalic anhydride (275 mg, 1 mmol) was added into reaction mixture and refluxed for 6 h. Light yellowish precipitaes were filtered off after cooling and dried under vaccum to get product L1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.51 (t, J = 8.4 Hz, 2H), 8.28 (d, J = 7.9 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.98 – 7.93 (m, 1H), 7.01 (d, J = 8.4 Hz, 2H), 6.64 (d, J = 8.4 Hz, 2H), 4.15 – 4.10 (m, 2H), 2.79 – 2.73 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.25, 156.34, 133.16,

132.10, 131.91, 131.48, 130.30, 130.05, 129.69, 129.36, 129.20, 128.77, 123.24, 122.47, 115.79, 42.00, 33.12.

Synthesis of L2: L1 (198 mg, 0.5 mmol) was dissolved in ethanol and and hydrazine hydrate (2 mL) was added dropwise into it. Reaction mixture was heated at 60 °C. After 8 h mixture was cooled down at 0 °C and orange precipiates were collected and dried in vaccum. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 7.3 Hz, 1H), 8.37 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.3 Hz, 1H), 7.94 (s, 1H), 7.53 – 7.45 (m, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 4.22 – 4.15 (m, 2H), 2.81 – 2.74 (m, 2H).

Synthesis of L3: Compound L2 (100 mg, 0.29 mmol) was dissolved in THF and two drops of triethyl amine was added into it. Solution of glutaric anhydride (33 mg, 0.29 mmol) in THF was added dropwise to the mixture. The reaction mixture was stirrered at room temprature for 12 h. Afterward the reaction mixture was diluted with dichloromethane, washed with brine two times and passed over $MgSO_4$. Solvent was evaporated under vaccum and dried to get pure compound L3. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.55 (s, 1H), 8.64 (d, J = 8.5 Hz, 1H), 8.43 (d, J = 7.1 Hz, 1H), 8.26 (d, J = 8.5 Hz, 1H), 7.77 – 7.68 (m, 1H), 7.00 (d, J = 6.5 Hz, 2H), 6.84 (d, J = 8.4 Hz, 1H), 6.64 (d, J = 8.4 Hz, 2H), 4.15 – 4.07 (m, 2H), 2.78 – 2.70 (m, 2H), 2.35 – 2.25 (m, 4H), 1.84 – 1.77 (m, 2H). ^{13}C NMR (101 MHz, DMSO-D_6) δ 174.76, 172.24, 164.06, 163.32, 156.27, 151.22, 134.17, 131.38, 130.03, 129.52, 128.95, 127.79, 125.57, 122.53, 119.33, 115.75, 111.05, 105.37, 41.60, 33.60, 33.36, 32.94, 21.00; HR-ESI-MS: m/z calcd for probe L3 ($C_{25}H_{23}N_3O_6$, [M+H]), 462.1665; found, 462.1662 [M+H].

Synthesis of Naph-CDs Sensor Probe: The ligand **L3** (20 mg) was dissolved in a 3 mL of DMSO under nitrogen atmosphere in dark conditions. 4-Dimethylaminopyridine (DMAP, 5.25 mg) and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 6.77 mg) was added into the reaction mixture and stirred for 1 h at room temperature. 2 mL solution of CDs (10 mg) in 50% DMSO in water was added dropwise to above reaction mixture and stirred the reaction mixture for 48 h at room temperature in dark condition. The reaction products was dialyzed against water for 48 h and freeze-dried. Freeze-dried product was stored in the dark at 0 °C for further use.

Tyrosinase Sensing: Assay of tyrosinase was performed in PBS at pH 7.4. To a 2.5 mL solution (10 μg/mL) of probe in PBS (pH 7.4), an appopriate amount of TYR solution was added in different tubes and mixed gently for 5 min. The final volume of solution was make upto 10 ml with PBS and incubated for 120 min at 37 °C. Fluorescence spectra of the solution

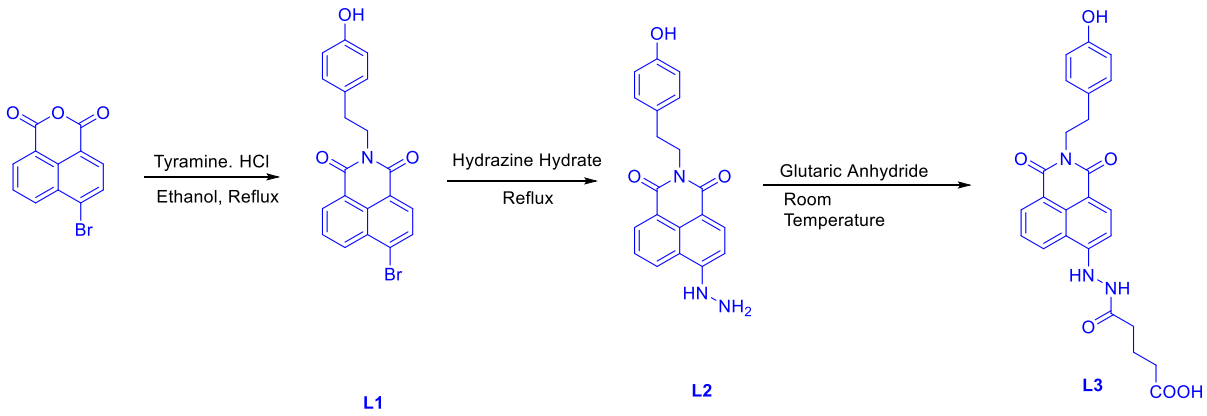
was recorded using quartz cuvette having 1 cm path length. Excitation and emission slit width were adjusted to 5 nm.

Selectivity Study: Selectivity study was performed by incubating the sensor probe solution (3 μg/mL) with various analytes for 15 minutes and enzymes. All the experiments were performed at physiological condition and similar to tyrosinase sensing procedure.

Cytotoxicity Assay: cytotoxicity of prepared sensor probe was evaluated against MCF-7 and HeLa cells. Both cells were cultured in DMEM media containing 10% fetal bovine serum at 37 °C for 24 h. Culture medium was removed and washed with PBS. Cells were treated with new medium containing a different concentration of sensor probe for 24 h. Cytotoxicity of probe was analyzed by MTT assay.

Cellular imaging: B16 cells were cultured in RPMI media having 10% FBS and seeded in 6 well plates containing cell culturing glass slides. The cells were incubated at 37 °C for 24 h in the presence of 5% CO_2. Afterward old media was replaced with new media containing 10 μg/mL solution of sensor probe and incubated for 15 min. Cells were three times washed with PBS to remove the extracellular probe and further incubated for 3 h. Cell images were collected under confocal laser scanning microscope (Nikon Eclipse Ti-U inverted microscope) after 15 min and 3 h of incubation.

Quantum yield and Fluorescence life Time Measurement: The quantum yield of CDs and sensor probe was calculated by an absolute method with slit width 5 nm. Phosphate buffer was used as a blank sample in a 10 mm quartz cuvette. The accuracy of the instrument wasevaluated using 2-aminopyridine and rhodamine 101 as a reference standard. Fluorescence life time was measured using an excitation wavelength 380 nm with slit width 5 nm. Emission band of CDs at 460 nm was considered for lifetime measurement.



Scheme S1: Synthesis of Probe L3 to prepare the nanosensor probe

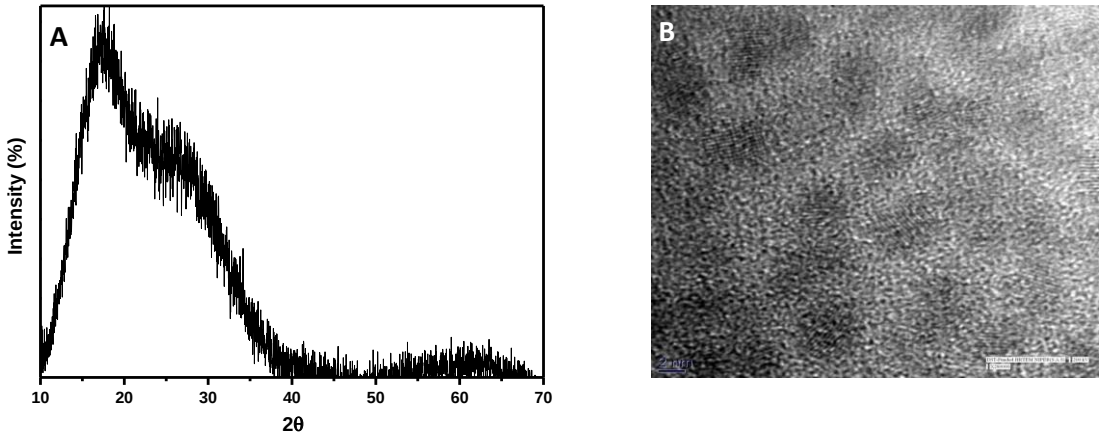


Fig. S1: (A) Powder XRD pattern of CDs (B) HRTEM image of CDs

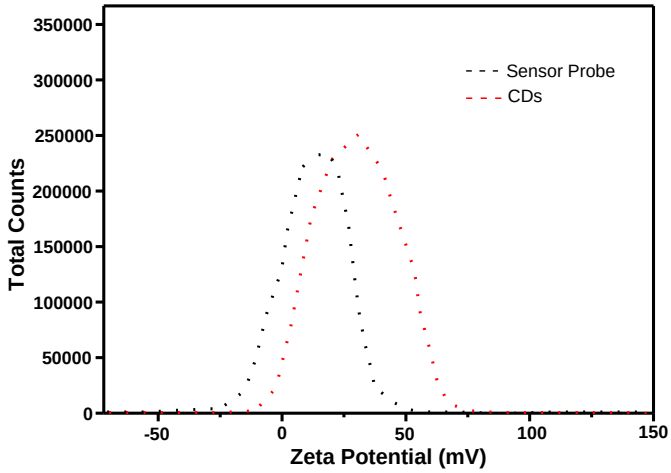


Fig. S3: Zeta potential graph of CDs (25 mV) and S-Tyr-CD (6 mV). This decrease in zeta potential suggested the consumption of free amine group of CDs.

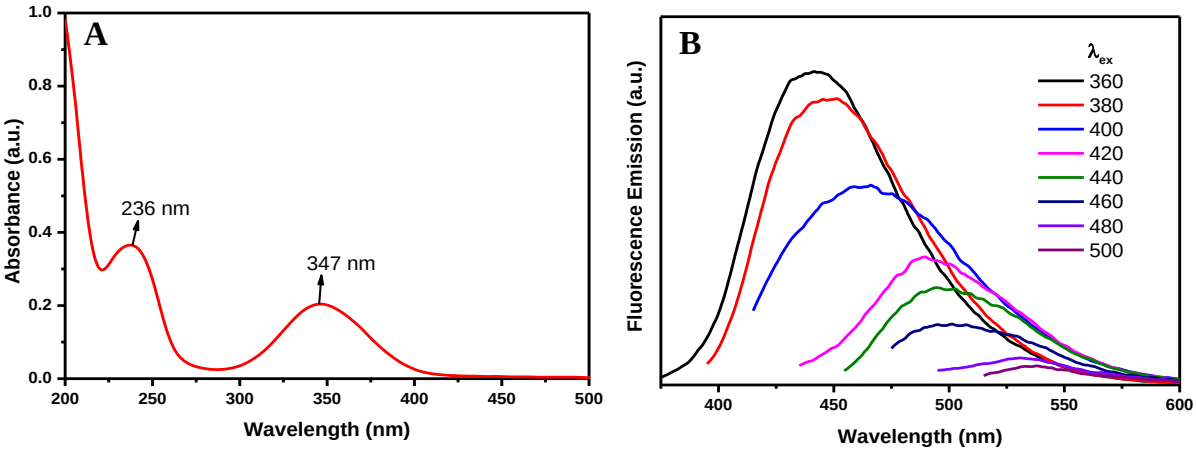


Fig. S3: (**A**) UV-vis absorption spectra of CDs exhibiting absorption maxima at 347 and 236 nm. (**B**) Fluorescence emission spectra of CDs showing excitation-dependent emission behavior

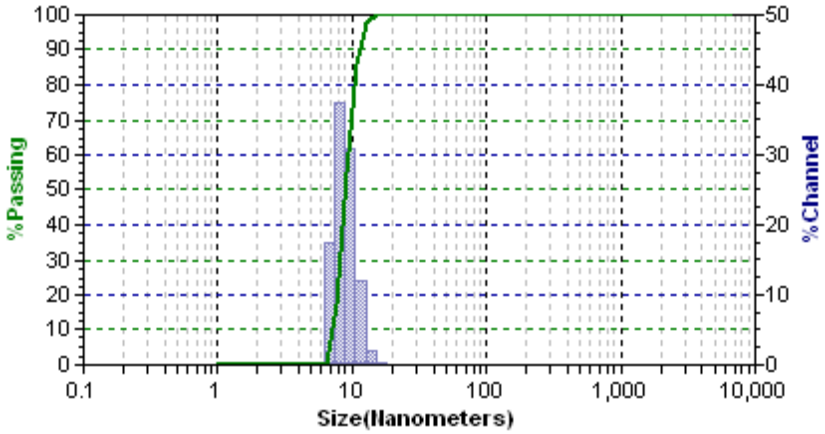


Fig. S4: DLS histogram of S-Tyr-CD showing the particle diameter of ca. 8 nm

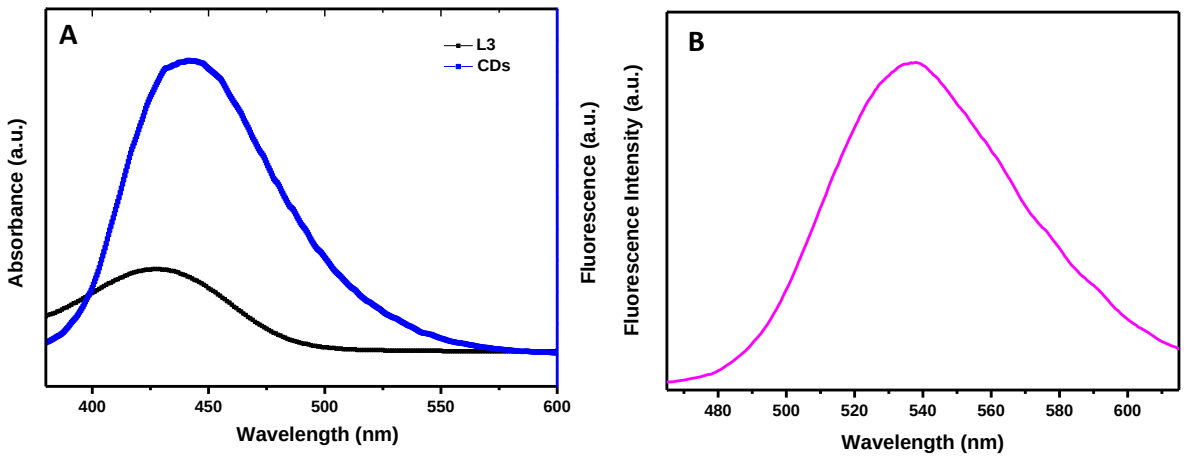


Fig. S5: (A) Spectral overlap between emission spectra of CDs and absorption spectra of energy acceptor L3 in PBS buffer at pH 7.4 (B) Fluorescence emission spectra of L3 when excited at 440 nm

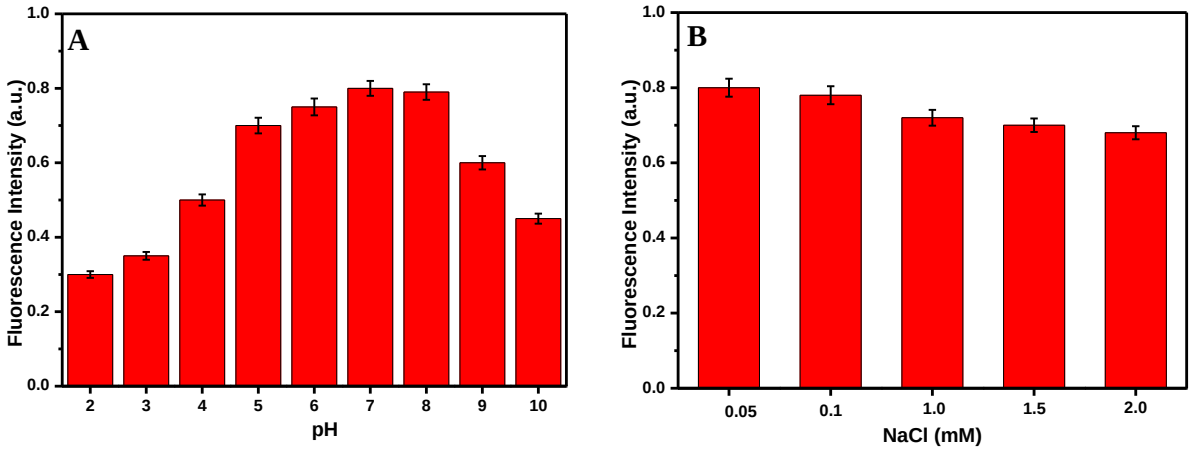


Fig. S6: Fluorescence Stability study of the sensor probe. (A) Effect of pH over (F_{540}/F_{440}) of sensor probe. (B) Fluorescence intensity ratio (F_{540}/F_{440}) of the probe in the presence of different concentration of NaCl.

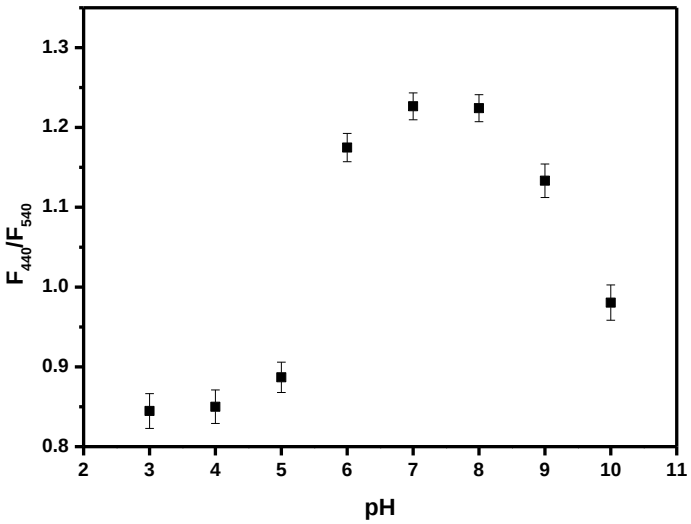


Fig. S7: Effect of pH over fluorescence intensity of sensor probe in the presence of TYR (200 U mL^{-1}) at a 37 °C after 3h of incubation

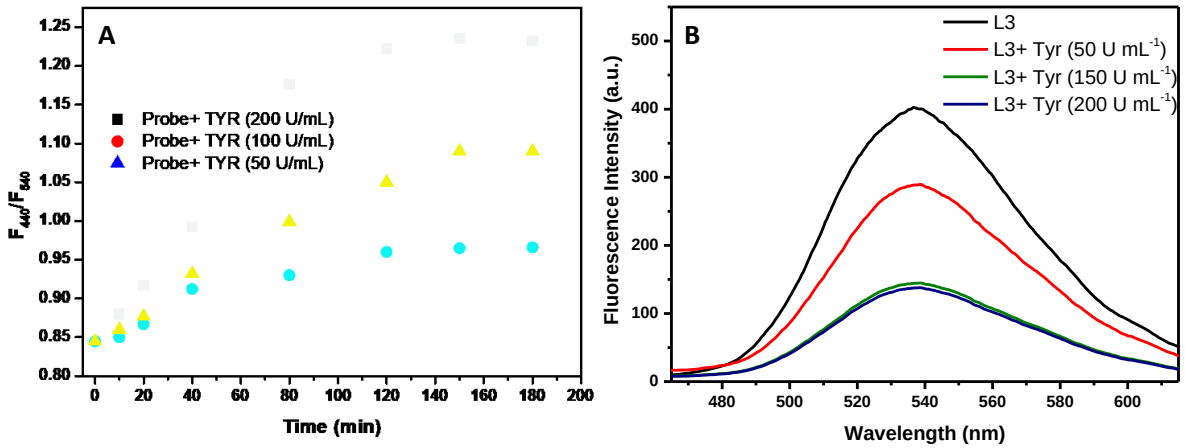


Fig. S8: (A) Fluorescence response of probe (3 µg/mL) with different concentrations of tyrosinase (50 - 200 U/mL). (B) Fluorescence emission record of L-3(10 µM) on incubation with TYR at different concentration. The decrease in fluorescence intensity confirmed the existence of PET phenomenon of enzyme-catalyzed product with L3-fluorophore. The measurements were performed at 37 °C in PBS (pH 7.4).

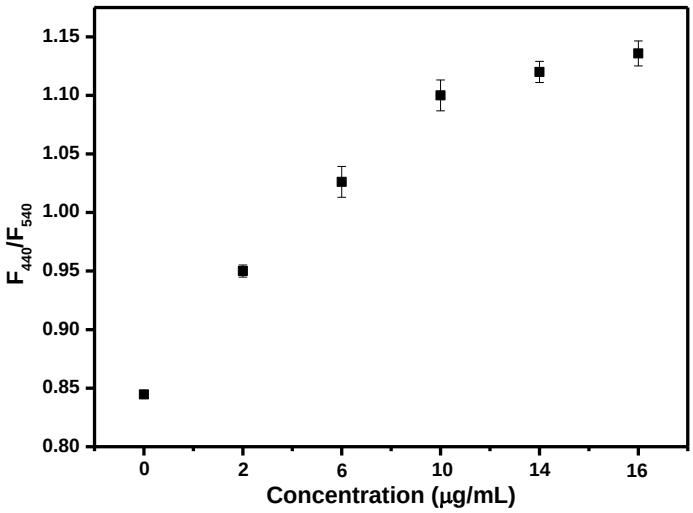


Fig. S9: Fluorescence emission ratio of probe at different concentration (0, 2, 6, 10, 14 and 16 μg/mL) in response to TYR (200 U/mL). At each concentration, probe was incubated with TYR for 40 min.

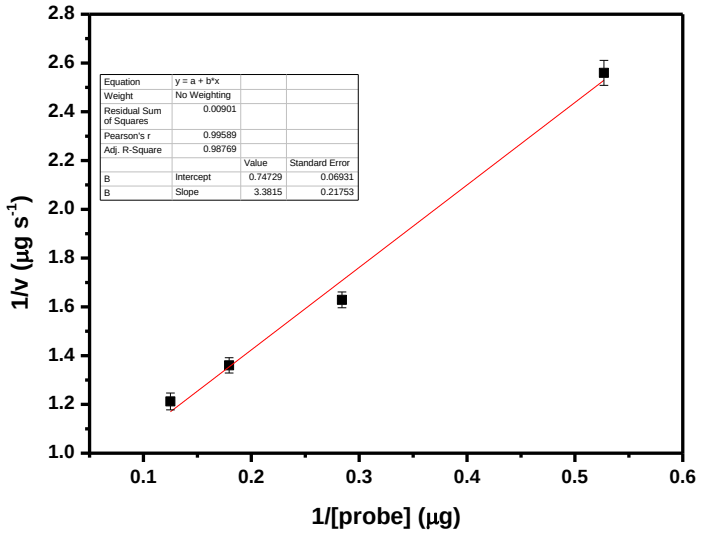


Fig. S10: Lineweaver-Burk plot for Kinetics parameter study of sensor probe with tyrosinase

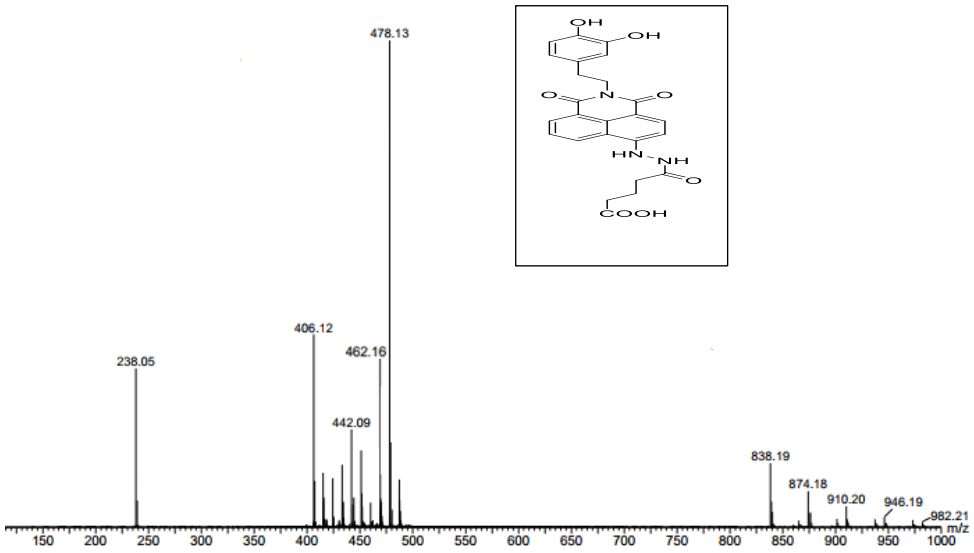


Fig. S11: Mass spectra of L3 in the presence of TYR confirmed the synthesis of dihydroxy derivative of L3 and corresponding m/z was appeared at 478.13.

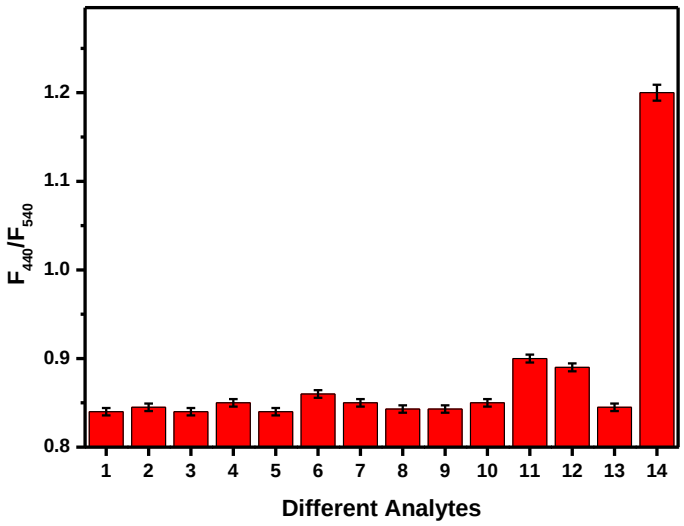


Fig. S12: Fluorescence response of sensor probe in the presence of different interfering analytes. (1) Blank (2) $FeCl_2$ (100 µM) (3) Glutamic acid (1 mM) (4) Glutathione (1 mM) (5) ALP (6) BSA (7) MAO-A (1 µg/mL) (8) creatinine (1 mM) (9) AchE (1 µg/mL) (10) $ONOO^-$ (1 µM) (11) $^{\cdot}OH$ (1 µM) (12) H_2O_2 (1 µM) (13) $^1O_2$ (1 µM) (14) Tyrosinase (200 U mL^{-1})

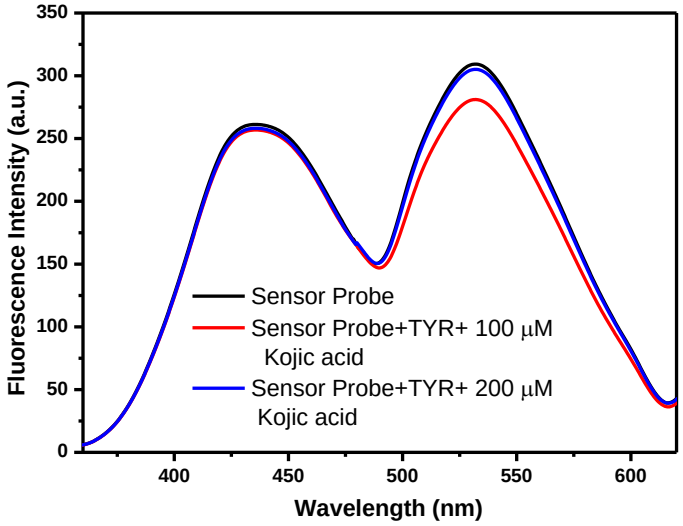


Fig. S13: Fluorescence response of probe in the presence of TYR and different concentration of kojic acid (100 and 200 μM) indicates the selectivity of reaction towards TYR.

Table. S1: Detection of TYR in FBS

	Spiked	Found
Fetal Bovine Serum	0	0.021±0.001
	0.5	0.46±0.003
	1	0.97±0.005

Table. S2: Comparison of different fluorescence sensor for detection of TYR

Probe	Detection Limit	Ref
AuNC	6 U L^{-1}	[2]
NBD		[3]

Cyanine	10 U L^{-1}	4
RF-QDs-DA	10 U L^{-1}	5
Dopa-CQDs	17 U L^{-1}	5
Resofuran	0.04 U mL^{-1}	6
Pdots@Tyr-OMe	1.1 U L^{-1}	5
CDs-Tyr	1.2 U mL^{-1}	This work

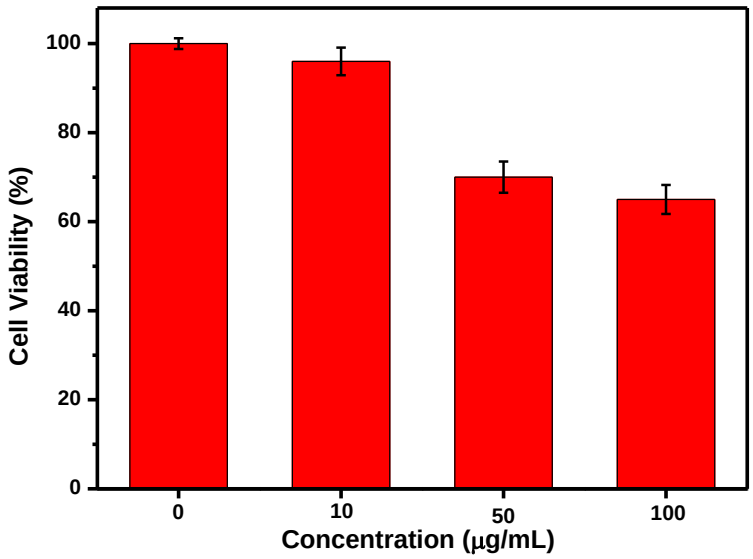


Fig. S14: Cytotoxicity assay of sensor probe against B16 cells

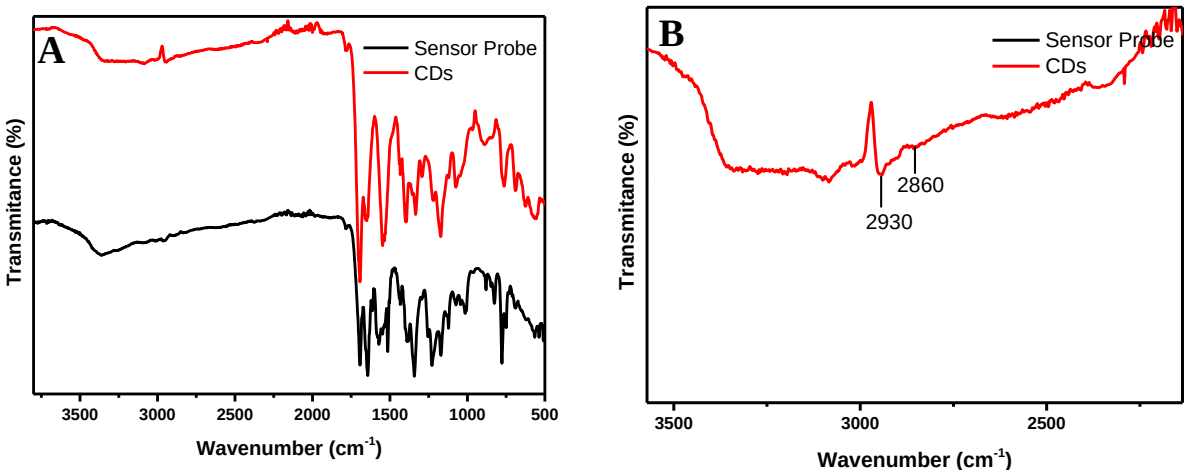


Fig. S15: (A) FTIR spectra of CDs and Sensor Probe (B) Extended spectra of sensor Probe

Fig. S15: ^{1}H NMR of L2

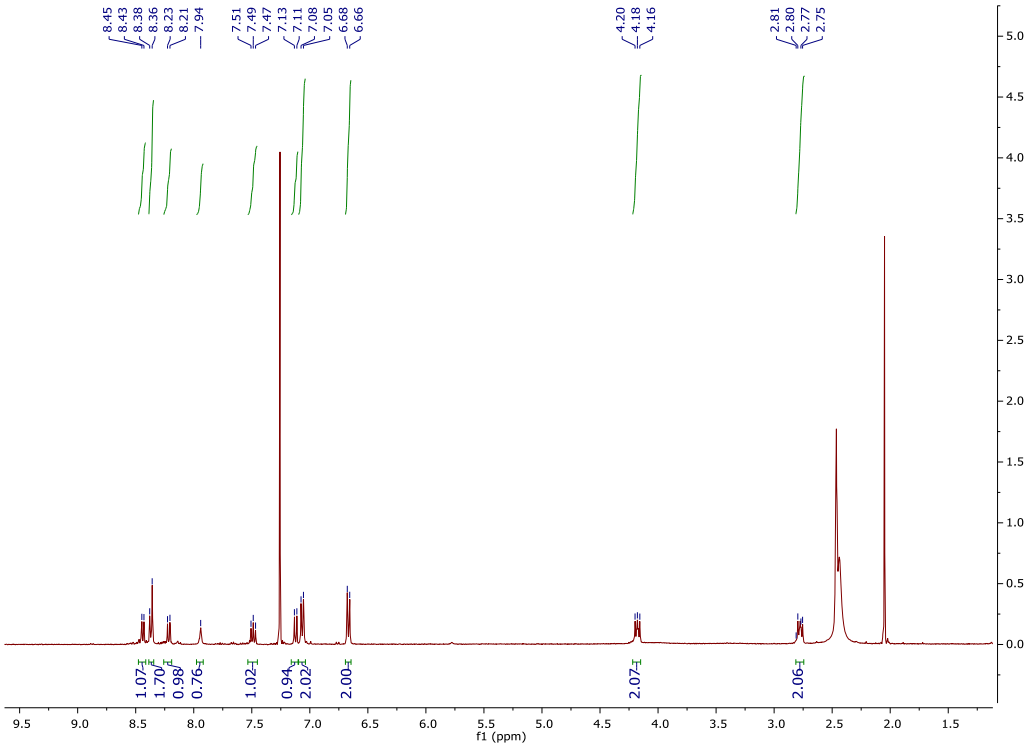


Fig. S16: ^{1}H NMR of L3

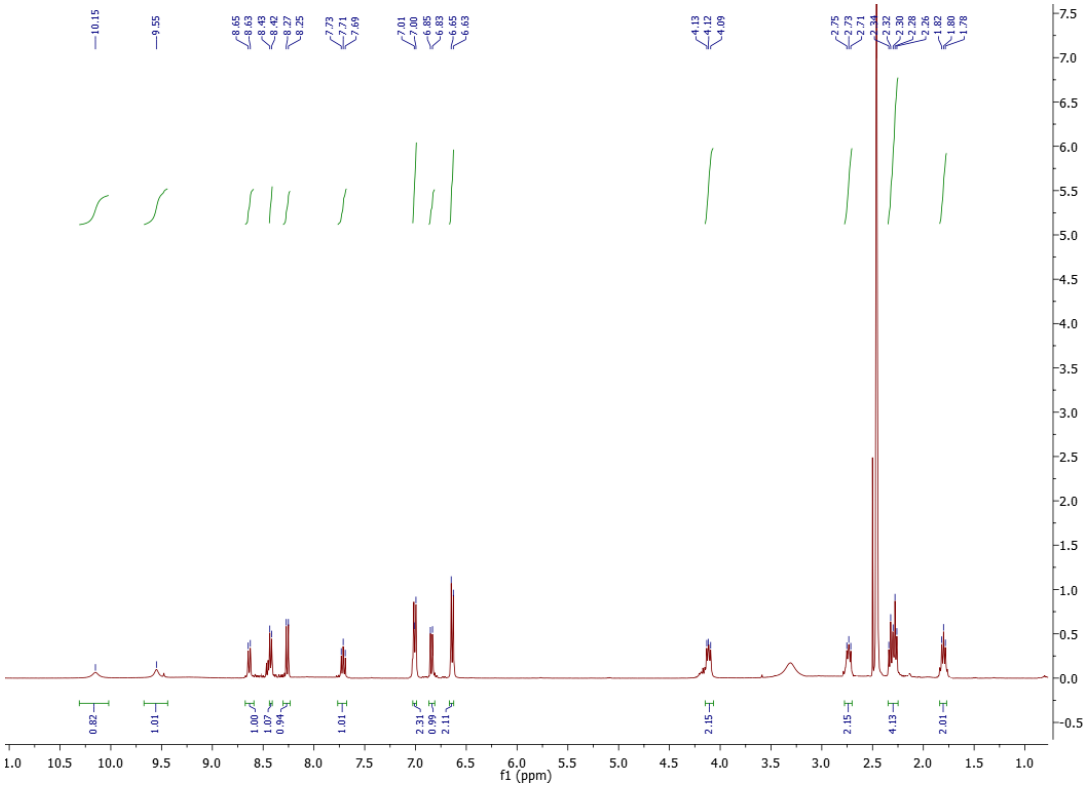


Fig. S17: ^{13}C NMR of L3

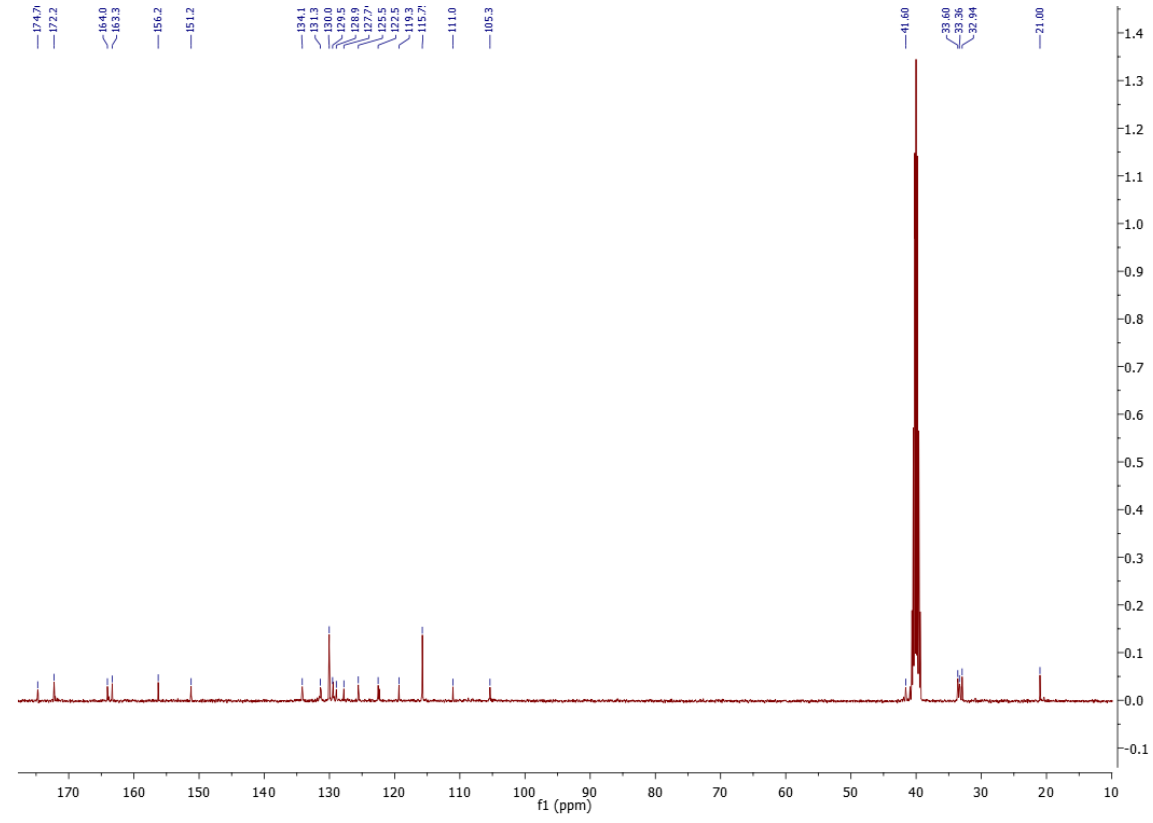
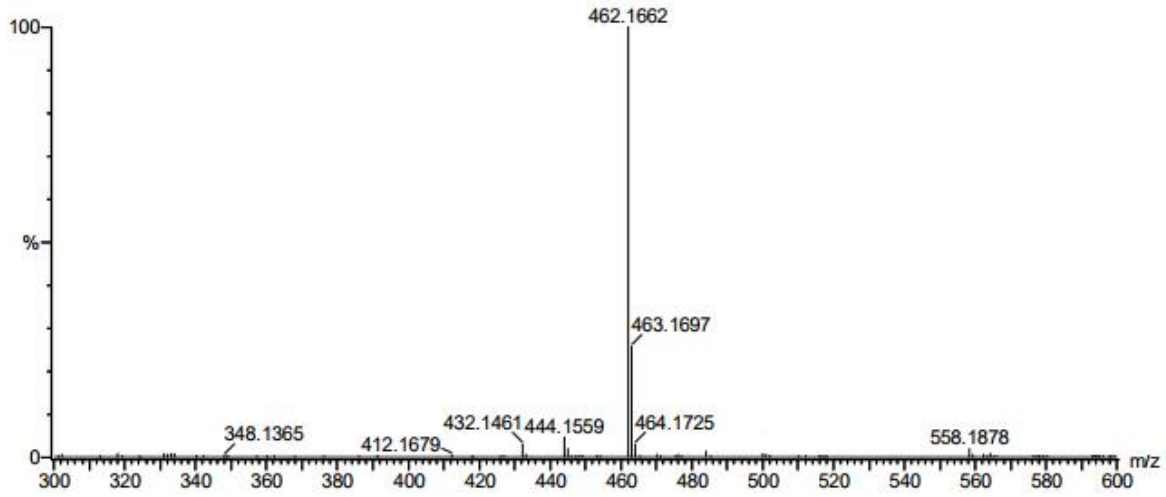


Fig. S18: HRMS of L3



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
462.1662	462.1665	-0.3	-0.6	15.5	378.7	n/a	n/a	C25 H24 N3 O6

References:

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4. Li, X.; Shi, W.; Chen, S.; Jia, J.; Ma, H.; Wolfbeis, O. S. A near-Infrared Fluorescent Probe for Monitoring Tyrosinase Activity. *Chem. Comm.* **2010,** *46*, 2560-2562.
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6. Wu, X.; Li, X.; Li, H.; Shi, W.; Ma, H. A Highly Sensitive and Selective Fluorescence Off-on Probe for the Detection of Intracellular Endogenous Tyrosinase Activity. *Chem. Comm.* **2017,** *53*, 2443-2446.