Supporting Information

Orotic acid-capped Tb(III)-doped Calcium Sulphate Nanorods for the Selective Detection of Tryptophan

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1. Experimental Section:

1.1. Materials:

Calcium nitrate ($Ca(NO_3)_2$)·$4H_2O$ and Nitric Acid (HNO_3 (65%)) were purchased from Merck. Orotic acid ($C_5H_4N_2O_4$), terbium oxide (Tb_4O_7) (99.99%), ammonium fluoride (NH_4F) (99%), L-tryptophan ($C_{11}H_{12}N_2O_2$), L-tyrosine ($C_9H_{11}NO_3$), L-glutamine ($C_5H_{10}N_2O_3$), L-arginine ($C_6H_{14}N_4O_2$), L-valine ($C_5H_{11}NO_2$), L-cysteine ($C_3H_7NO_2S$), L-histidine ($C_6H_9N_3O_2$), L-lysine ($C_6H_{14}N_2O_2$), L-serine ($C_3H_7NO_3$), L-leucine ($C_6H_{13}NO_2$), L-threonine ($C_4H_9NO_3$), L-asparagine ($C_4H_8N_2O_3$), zinc nitrate ($Zn(NO_3)_2$·$6H_2O$), manganese nitrate ($Mn(NO_3)_2$·$4H_2O$), cobalt nitrate ($Co(NO_3)_2$·$6H_2O$), lead nitrate ($Pb(NO_3)_2$), cadmium nitrate ($Cd(NO_3)_2$·$4H_2O$), copper nitrate ($Cu(NO_3)_2$·$3H_2O$), mercury nitrate ($Hg(NO_3)_2$·H_2O), ferrous chloride ($FeCl_2$·$4H_2O$), nickel sulphate ($NiSO_4$·$6H_2O$), EDTA were obtained from Sigma Aldrich and used without further purification. All other chemicals utilized in this experiment were of analytical reagent grade. Dimethyl sulfoxide (DMSO) and double-deionized water were used during the synthesis.

1.2. Synthesis of orotic acid capped $CaSO_4$:Tb^{3+} (3%) nanorods:

OA-capped terbium-doped $CaSO_4$ nanorods were prepared using the microwave synthesis method. At first, Tb_4O_7 in the stoichiometric amount was dissolved in 1 N nitric acid, and nitration was performed to form its corresponding metal nitrates. Subsequently, 0.97 mmol of $Ca(NO_3)_2$·$4H_2O$ and 0.03 mmol of $Tb(NO_3)_3$ were taken into a glass beaker (100 ml) and completely dissolved in 10 ml DMSO. A 1 mmol of OA was placed in another beaker and mixed with 10 ml of DMSO. Further, the OA solution was mixed with the nitrate solution and stirred continuously for 30 minutes on a magnetic stir. Next, 5 ml of a 4 mmol NH_4F aqueous solution was added dropwise to the precursor mixture, followed by an additional 30 minutes of stirring. The resulting colloidal dispersion, appearing white in colour, was transferred to a Teflon-capped glass vial and tightly sealed. The system was then heated for 15 minutes at 210°C using microwave irradiation. Upon completion of the reaction, the resulting white precipitate was centrifuged at 9000 rpm and washed twice with ethanol. Finally, the products were dried at 50°C in a hot air oven and stored in a vial at room temperature for further studies.

1.3. Physical Characterization of the material:

The XRD patterns were collected using the Rigaku-Miniflex diffractometer, which was equipped with a D/tex ultra-detector and a Cu-Kα source operating at 40 kV, was used. The scan range was set between 20° - 80° (2θ) at a step size of 0.020, and a count time of 2 seconds was used. Prior to analysis, the samples were thoroughly powdered, dried, and uniformly spread on a quartz slide. Ultra-high-resolution field-emission gun transmission electron microscopy **(**FEG-TEM) with a 200 kV electron source (JEOL JEM 2100F) was utilized for the TEM analysis. The nanocrystal dispersion was drop-casted onto a 300 mesh Cu grid coated with carbon and left to dry overnight. Field-emission scanning electron microscopy (FESEM) images were captured using a SUPRA 55-VP JSM equipped with patented GEMINI column technology. Before introducing the samples into the chamber, a thin layer of gold was coated to prevent any charging effects.[51] The Perkin Elmer Spectrometer RX1 spectrophotometer with KBr disk technique was utilized to record the FTIR spectra over the range of 4000 - 400 cm^{-1}. To make the pellets, the dried nanorods were mixed with KBr in a 1:10 ratio.[52] For recording ultraviolet-visible (UV-Vis) Spectroscopy data, a Perkin-Elmer UV-Vis spectrophotometer was used to measure all the absorbance spectra over a range of 200-800 nm. To record the room temperature photoluminescence spectra, a Horiba Fluoromax-3 spectrophotometer was utilized. This instrument was equipped with a 150 W Xe lamp as a light source for exciting the nanorods. The excitation and emission light were separated and dispersed by a Czerny-Turner monochromator, which had an optical resolution of 1 nm. The emitted photons were then detected using a Hamamatsu R928 detector, with the output signal being captured by a computer. Prior to measurements, all nanorods were dispersed in DMSO at a concentration of 1mg/10ml and sonicated thoroughly. It was then stored as a stock solution for further procedure.

1.4. Sample preparation for analyte detection

To investigate the sensitivity of nanorods toward analytes, the nanocrystal powder was dispersed in DMSO at a concentration of 1 mg per 10 ml. Photoluminescence measurements were collected, with excitation at 291 nm, and observed characteristic emissions at 542 nm, 488, 585, and 620 nm. The analytes were prepared with an initial stock concentration of 10 $mmolL^{-1}$ in water. Lower concentrations were made by further diluting the stock concentrations with deionized water. To perform the photoluminescence (PL) detection experiments, 2 ml of the NCs dispersion was placed in a quartz cuvette, and the luminescence spectrum from a blank sample (i.e., only the colloidal dispersion of NCs) was collected. Next,

1.9 ml of the dispersion was mixed with 100 µL of different analytes, including all the amino acids and other metal ions, stock solutions using an appropriate micro-pipette to achieve the desired final concentration. Luminescence signals were recorded 30 seconds after adding analytes to the nanocrystal dispersion for the detection measurements.

1.5. Procedure for luminescent determination of tryptophan:

A stock solution of OA@$CaSO_4$:Tb^{3+} was prepared by dissolving 1mg of the product in 10 ml of DMSO. In addition, various concentrations of a tryptophan solution were prepared. Next, 100 µl of the desired concentration (ranging from 5µM to 50 mM) of the tryptophan solution was added to the above mixture, stirred, and subsequently left at ambient temperature for 30 seconds before measurement. The resulting dispersion was then transferred to a 3 ml quartz cuvette followed by photoluminescence analysis (λ_{ex} = 291 nm; λ_{Em} = 542 nm; slit width = 5 nm).

2.6. Procedure for biological sample analysis

2 ml of human blood was collected in EDTA-coated vacutainer tubes. The blood sample was centrifuged at 3000 rpm, at 25 ^oC for 10 minutes, and plasma was isolated.[53] To determine free tryptophan concentration, the plasma was deproteinated using a ratio of 1:2 of DMSO through centrifugation.[54] The deproteinated plasma was again separated and stored at 4^oC until further analysis. To perform luminescence measurements, a solution containing 0.1 wt% (1 mg/ml) of OA@$CaSO_4$:Tb^{3+} NCs dispersed in 2 ml DMSO was prepared. 100 µl of pure plasma and deproteinated plasma were added separately to detect the concentration of tryptophan. Urine and saliva samples were used directly, without deproteination, to detect the tryptophan concentration, following the same protocol as used for the plasma samples. This study was approved by the institutional ethics committee of the Indian Institute of Science Education and Research (IISER)- Kolkata. The luminescence detection method described in the previous section was subsequently employed.

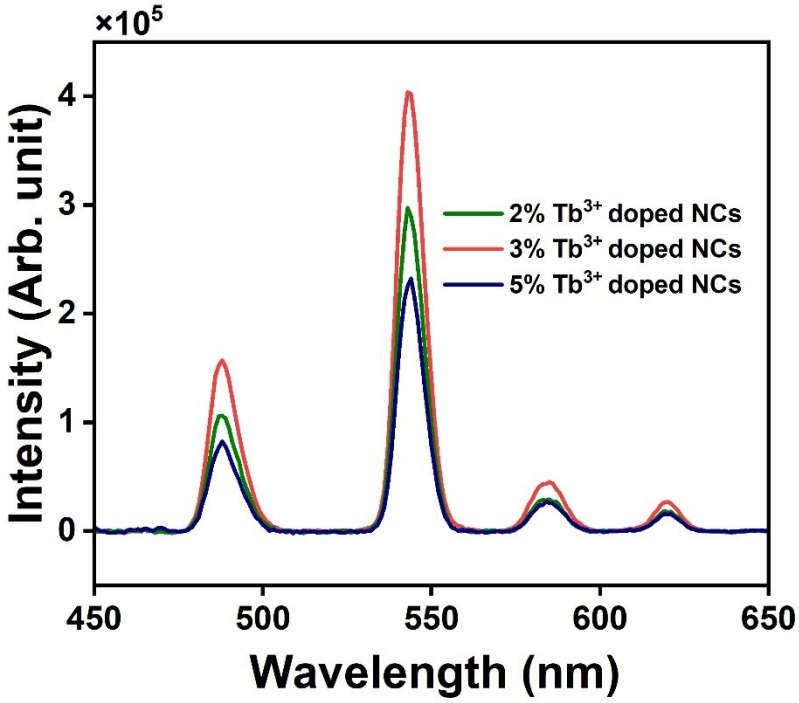

Figure S1: PL spectra: Emission spectra of OA@$CaSO_4$:Tb^{3+} NRs, doped at 2%, 3% and 5% concentrations of Tb^{3+}.

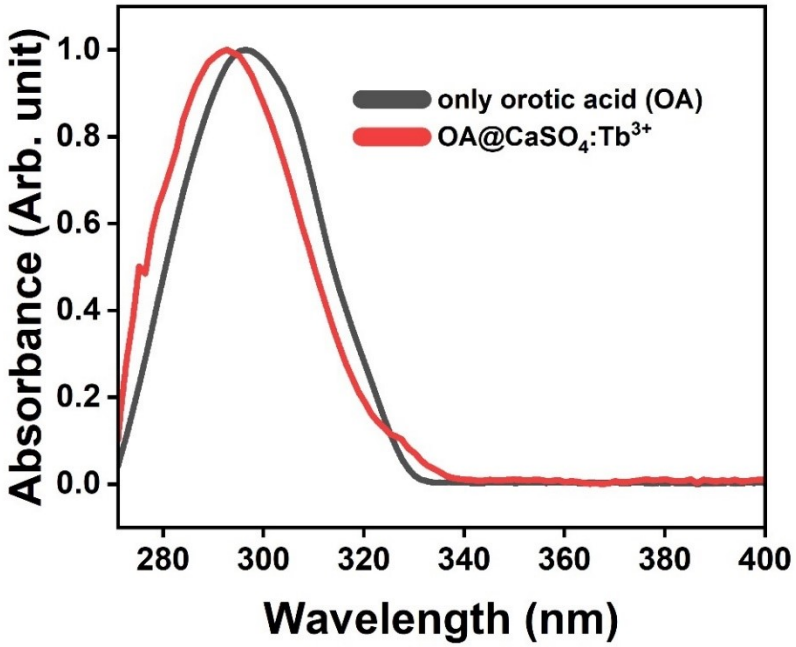

Figure S2: UV-Vis absorption spectra of the pure OA and OA-capped $CaSO_4$:Tb^{3+} NRs.

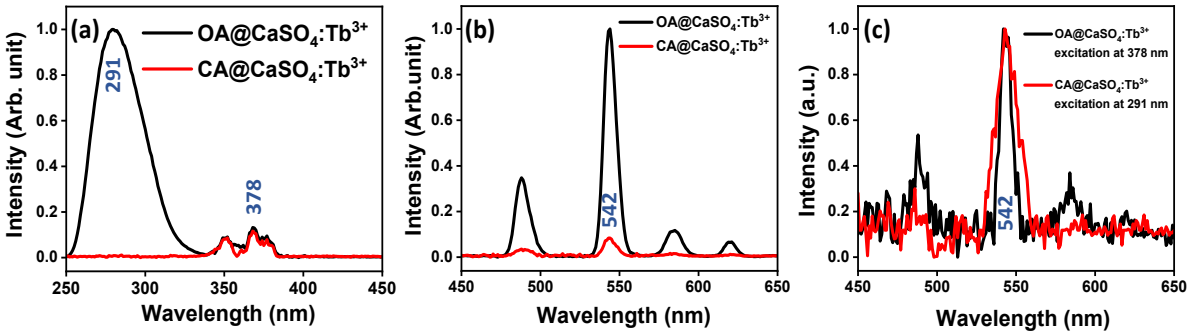

Figure S3: PL spectra: (a) Excitation spectra of $OA@CaSO_4:Tb^{3+}$ and $CA@CaSO_4:Tb^{3+}$ when excited at λ_{em}=542 nm, (b) Emission spectra of $OA@CaSO_4:Tb^{3+}$ and $CA@CaSO_4:Tb^{3+}$ at λ_{ex}=291 nm, (c) Emission spectrum of $CA@CaSO_4:Tb^{3+}$ at λ_{ex}=291 nm, compared with the direct excitation spectrum of Tb^{3+} ion at λ_{ex}=378 nm of $OA@CaSO_4:Tb^{3+}$.

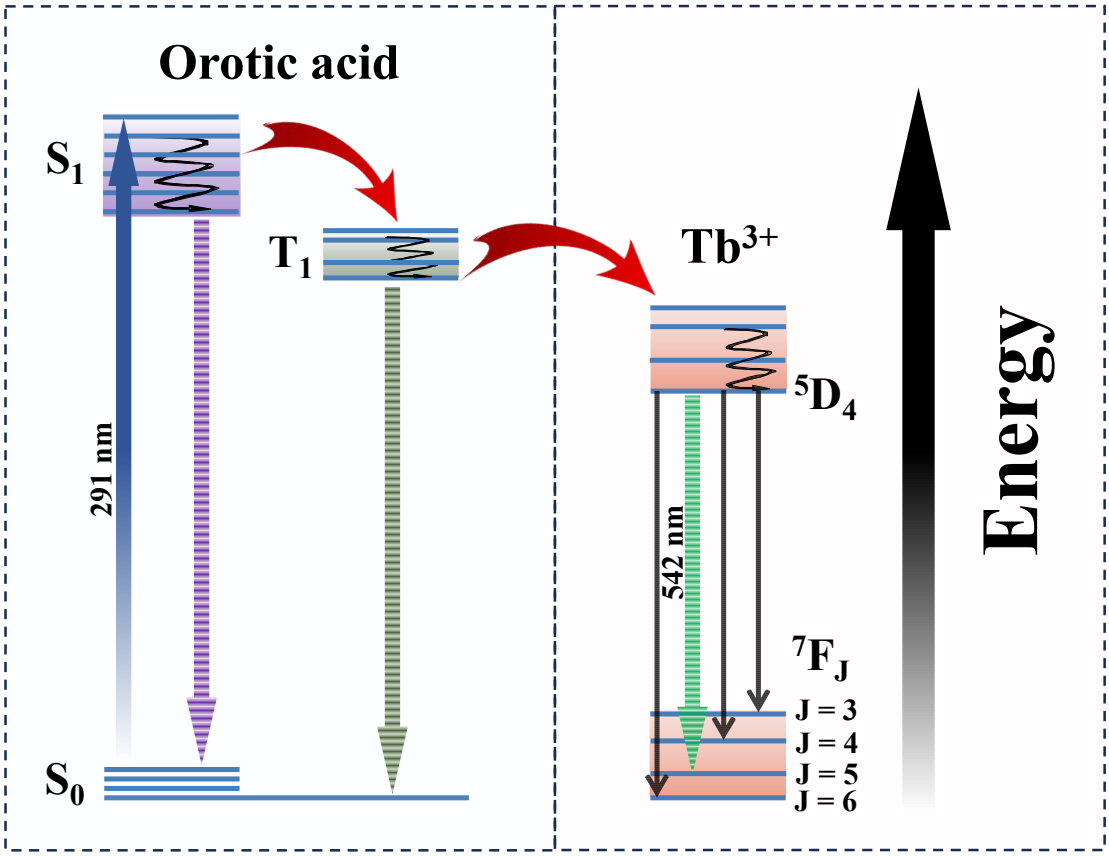

Figure S4: Schematic representation of the proposed energy transfer processes between OA and Tb^{3+} ion in $OA@CaSO_4:Tb^{3+}$ NRs.

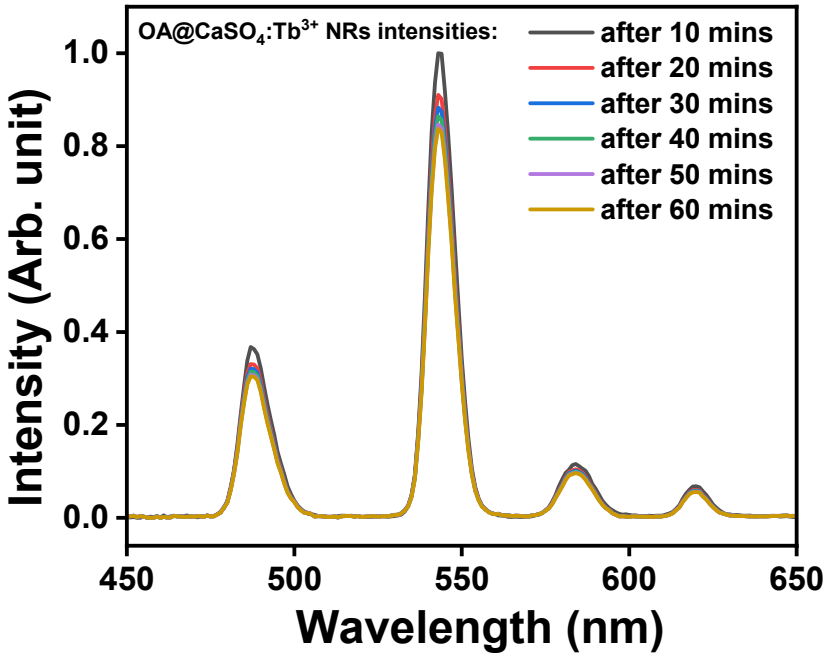

Figure S5: Time-dependent PL spectra indicating the colloidal stability of OA@$CaSO_4$:Tb^{3+} NRs.

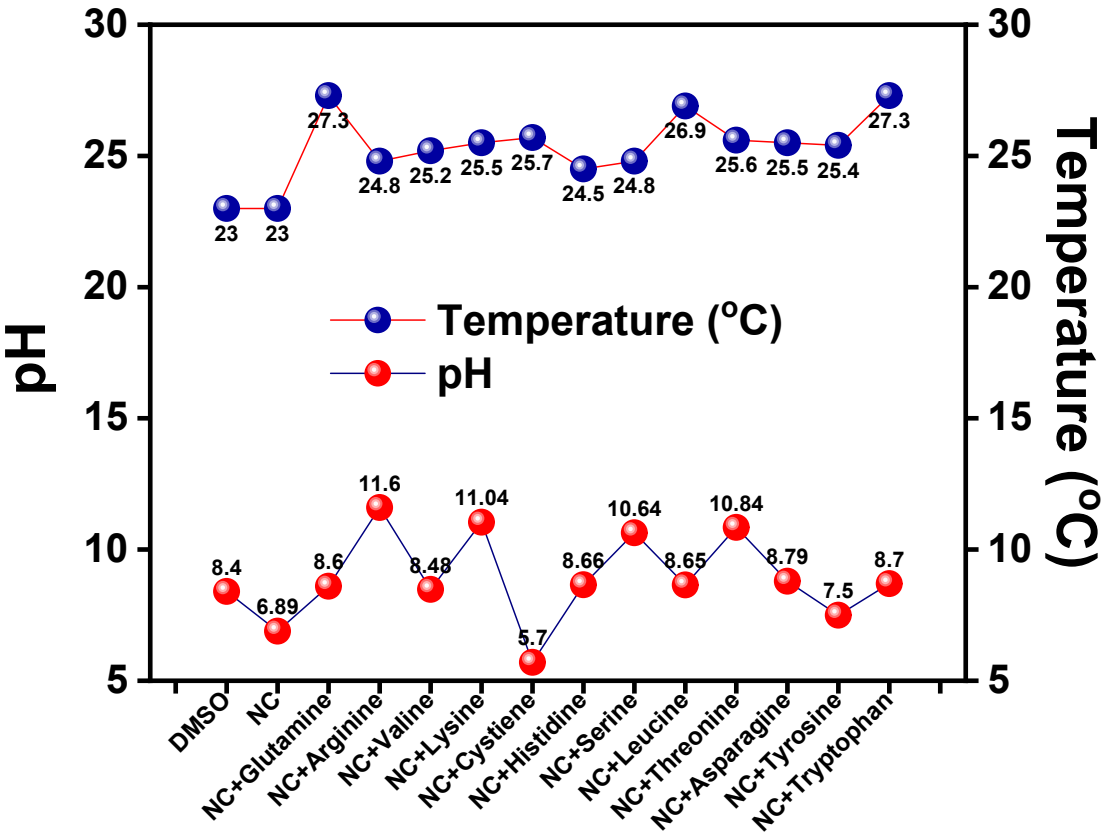

Figure S6: pH and temperature analysis upon treatment of the OA@$CaSO_4$:Tb^{3+} NRs with different amino acids.

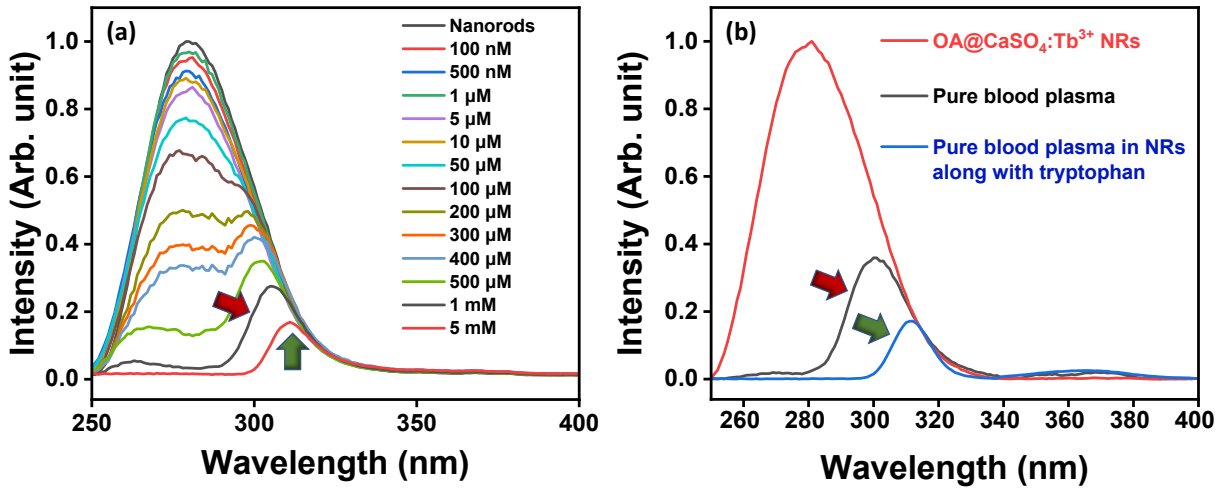

Figure S7: (a) Excitation spectra of OA@$CaSO_4$:Tb^{3+} NRs (a) in the presence of different concentrations of tryptophan and (b) with the addition of pure plasma sample and plasma containing tryptophan.

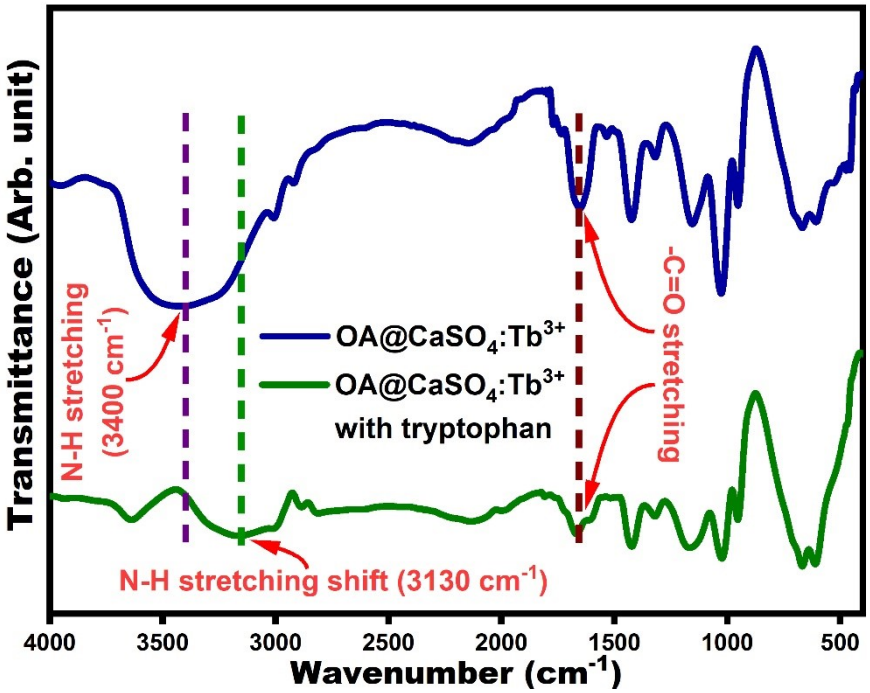

Figure S8: FT-IR spectra of OA@$CaSO_4$:Tb^{3+} NRs before and after the addition of tryptophan. A shift in the N-H stretching towards the lower wavenumber is observed for the tryptophan-attached NRs.

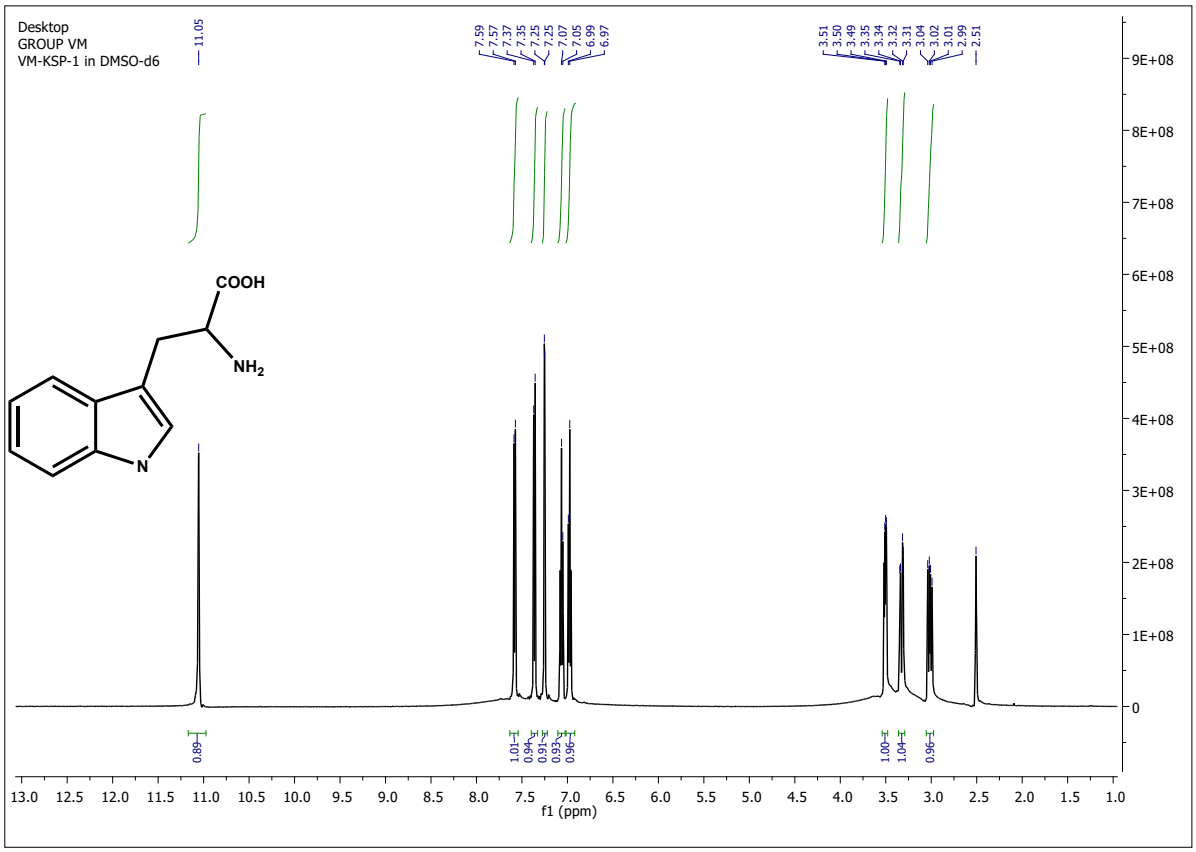

Figure S9. ^{1}H-NMR spectrum of tryptophan.

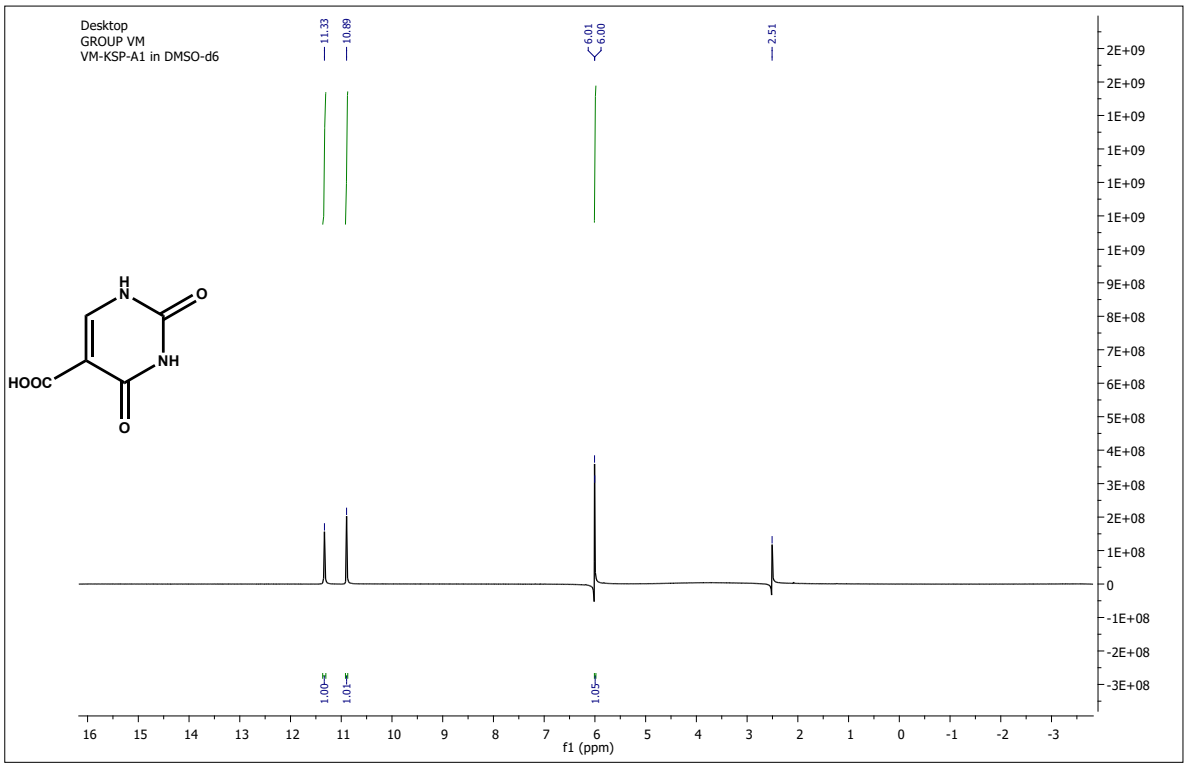

Figure S10. [1]H-NMR spectrum of orotic acid.

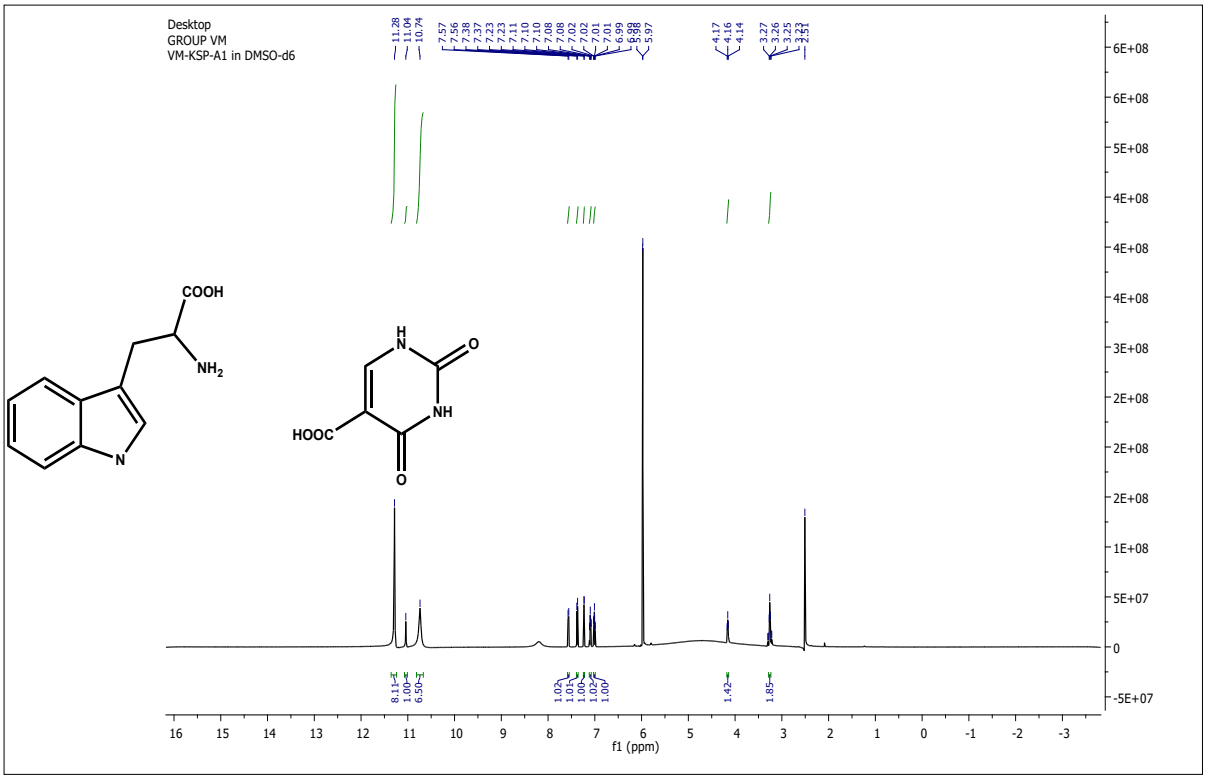

Figure S11. [1]H-NMR spectrum of the mixture of orotic acid and tryptophan.

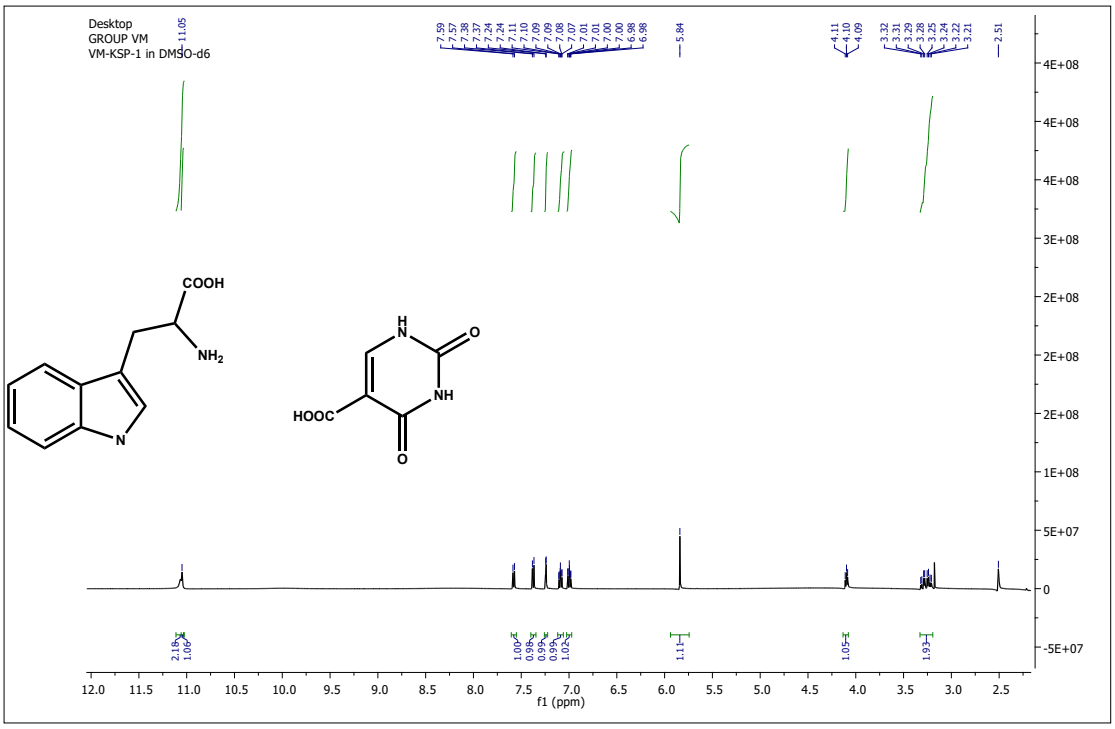

Figure S12. ^{1}H-NMR spectrum of the mixture of 1:1 ratio of orotic acid and tryptophan.

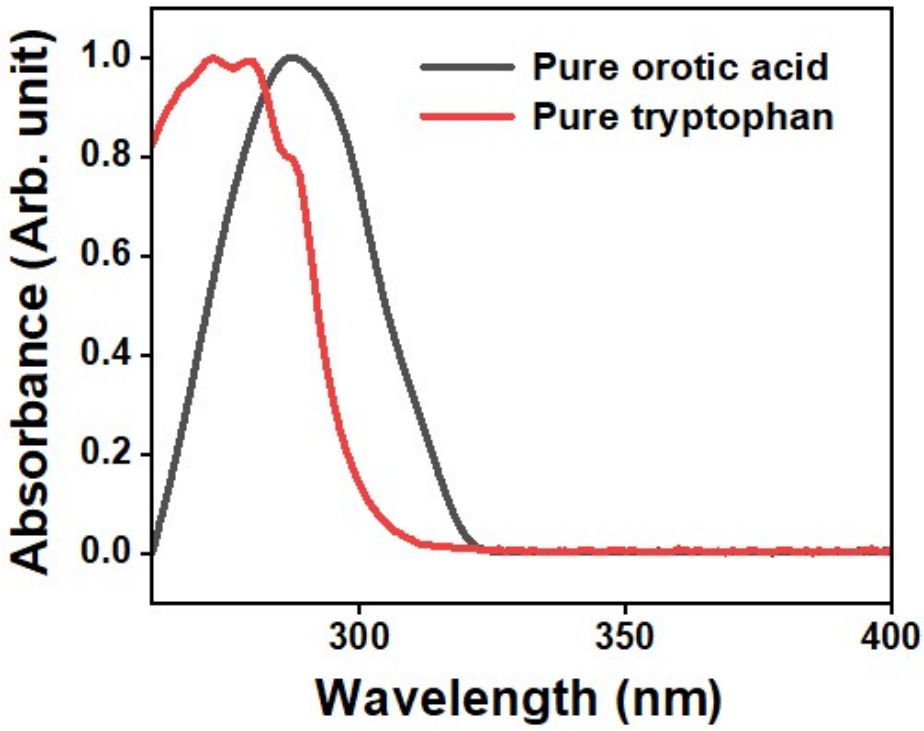

Figure. S13. Excitation spectra of orotic acid, OA@$CaSO_4$:Tb^{3+} NRs, and tryptophan.