Supporting Information

Photoactivated Histidine-Modified ZnO Nanostructures as Esterase Mimics: Calcination-Driven Surface Engineering for Hydrolytic Cleavage of Bioactive Esters

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1. EXPERIMENTAL SECTION

1.1 General: All chemicals (reagents, solvents and chemicals) were bought from best-known local chemical suppliers, such as Sigma Aldrich (https://www.sigmaaldrich.com), Spectrochem (https://spectrochem.in), Avra (https://www.avrasynthesis.com), etc and used without further purification. FTIR spectra were recorded on a Perkin-Elmer (https://www.perkinelmer.com) FTIR Spectrum BX system and were reported in wave numbers (cm^{-1}). Mass spectra were recorded on a Micromass Q-TOF Micro TM spectrometer.

1.2 Spectroscopic Studies: The UV-vis spectroscopic studies were recorded on a JASCO (model V-650) UV-vis spectrophotometer. The slit width for the experiment was kept at 5 nm using standard quartz cuvettes (path length = 1 cm). Fluorescence measurements were carried out using a FluoroLog-TM (Horiba Scientific) spectrofluorometer, with slit widths set at 5 nm using standard quartz cuvettes (path length = 1 cm).

1.3 Reactor Geometry & Stirring: 50 mL double-walled glass reactor and the stirring rate 500 rpm using a magnetic stir bar of fixed size to ensure reproducibility.

1.4 Mass Loading: A stock solution of p-nitrophenyl acetate (p-NPA, 0.5 mM) was first prepared in 5 mL PBS buffer solution (10 mM, pH 7.4). The catalyst loading was maintained at 0.5 mg $mL^{-1,}$ and reactions were performed under continuous magnetic stirring (500 rpm) to ensure uniform dispersion of the catalyst particles.

1.5 Electrolyte/Feed Composition: The reaction medium (feed composition) consisted of phosphate-buffered saline (PBS, 10 mM, pH 7.4), which provided a controlled ionic environment for hydrolysis. The substrate, p-nitrophenyl acetate (PNPA, AR grade 99% pure), was used at a concentration of 0.5 mM. In comparative studies, p-nitrophenyl dodecanoate (PNPD, AR grade, 99% pure) and other ester substrates were introduced under identical conditions. The ZnO-based catalyst was dispersed uniformly in this medium without the use of additional cosolvents or supporting electrolytes.

1.6 Temperature Control: All experiments were carried out at controlled room temperature (25 ± 5 °C).

1.7 UV light Irradiance: For photo-assisted studies, a UV light source ($\lambda \approx 365$ nm Xenon lamp) was employed, and the distance between the light source and reaction system was kept 1 cm, constant throughout all experiments to maintain uniform irradiation conditions. No optical filters were applied. Control experiments under ambient daylight were performed under identical conditions without UV illumination.

1.8 Scanning Electron Microscopy (SEM): Powder Samples for SEM analysis were loaded onto a carbon tape. Then it was subsequently sputter-coated using a Leica Ultra Microtome EM UC7. The prepared stubs were then imaged at 1 μm magnification using an FEI Apreo LoVac.

1.9 X-ray photoelectron spectra (XPS) analysis: The surface composition, elemental oxidation state and bonding were explicitly studied by X-ray photoelectron spectra (XPS) recorded on a Thermo Scientific K-Alpha surface-analysis spectrometer housing Al Kα as the X-ray source (1486.6 eV). The instrument was operated at 72 W. The base pressure in the analysis chamber was maintained at 5×10^{-9} mbar. The data profiles were subjected to nonlinear least-squares curve fitting using a Gaussian–Lorentzian production function and processed in Avantage software. The binding energy (B.E.) of all XPS data was calibrated versus the standard C 1s peak at 284.85 eV.

2.0 Photocatalytic hydrolysis of PNPA: Catalysis studies were carried out using a cylindrical annular batch photoreactor fitted with a Xenon lamp of 125 W, which consisted of a double-walled borosilicate immersion well for water circulation around the lamp to maintain the reaction at room temperature. All photocatalytic reactions were performed under ambient atmospheric conditions (air). Radical scavenger experiments were conducted using isopropanol (IPA) as a hydroxyl radical (•OH) scavenger, fluorescent probe used to detect hydroxyl radicals (•OH) generated during photocatalysis.

2.1 Kinetics Calculation: To make comparisons among the various morphologies, k was normalized by catalyst loading (km, in $g^{-1}min^{-1}$) and by surface area (ks, in $m^{-2}min^{-1}$) according to the following equations:

$$k_m = \frac{k}{m_{cat}} \quad \text{.......................................} (1)$$

$$k_s = \frac{k}{m_{cat} SA_{cat}} \quad \text{.......................................} (2)$$

Where, k_m= mass loading of ZnO nanocatalyst (in g)
SA_{cat} = surface area of ZnO nanocatalyst (in m^2/g)

2.2 Raman Analysis: The Raman measurements were performed by a UniDRON-A micro-Raman mapping spectrometer equipped with 457, 532, and 632.8 nm laser excitation sources with a spot size of ~1 μm^2, using a 50× objective with a numerical aperture of 0.55. For 1.75 mW power from a 532 nm laser, the final power density to the sample was calculated to be ~2.2 × 105 W/cm^2

2. ADDITIONAL DATA

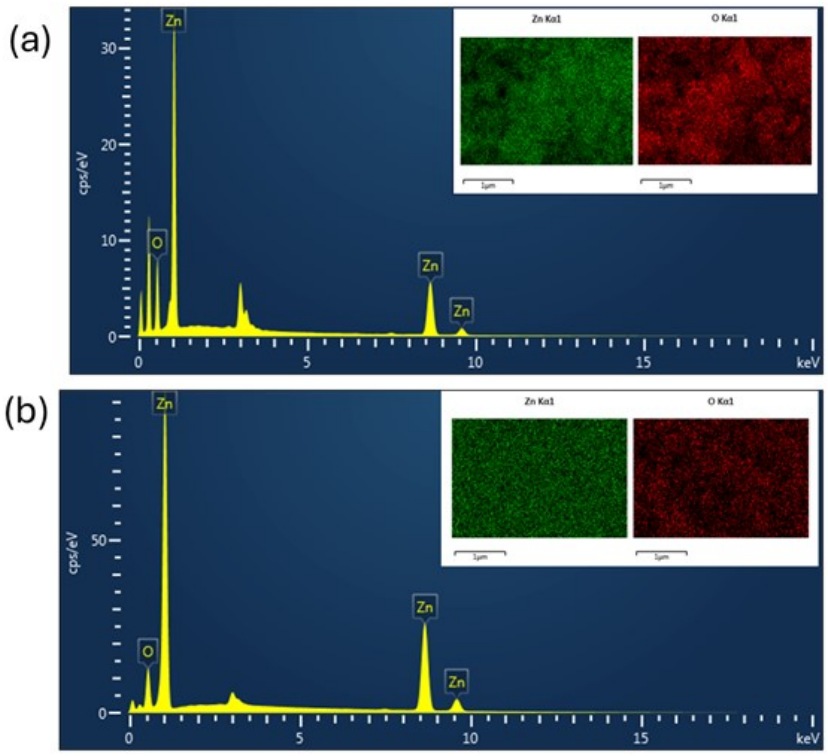

Figure S1. EDAX mapping image of (a) ZnO-300 (b) ZnO-700

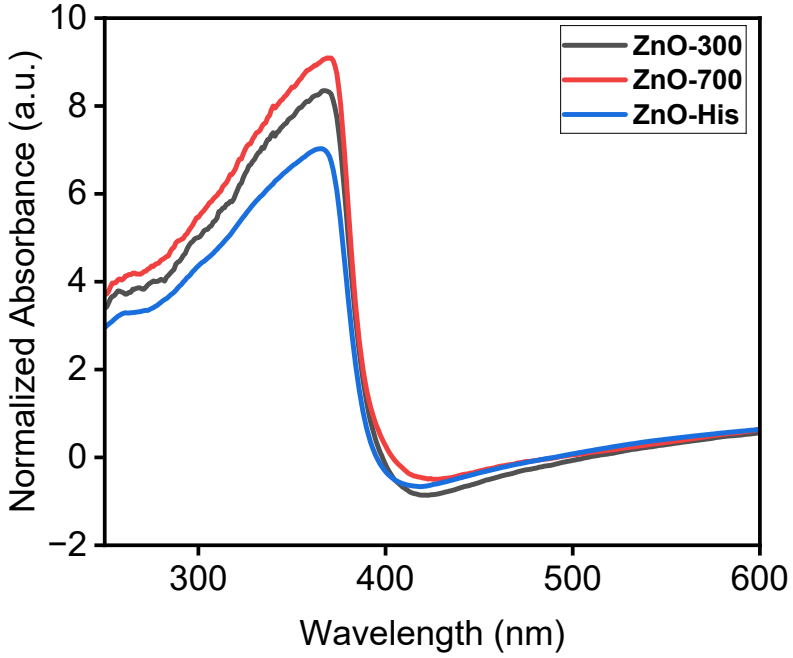

Figure S2. UV-visible spectra of ZnO-300, ZnO-700 and ZnO-Histidine.

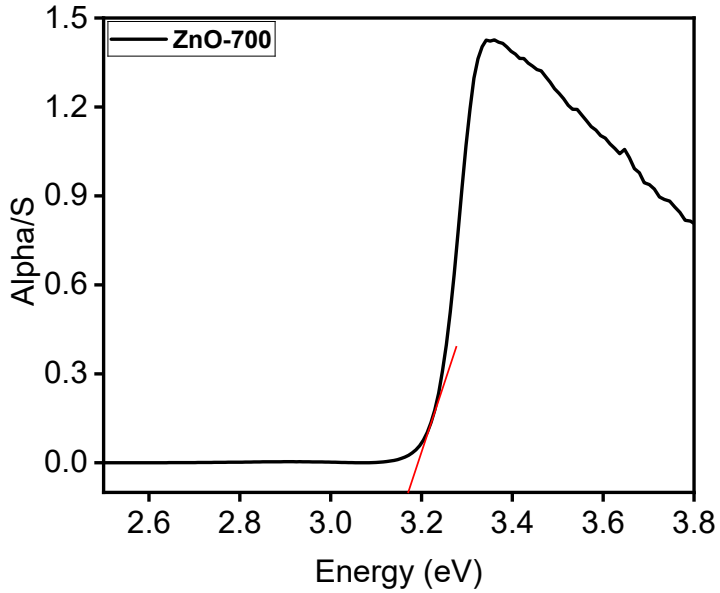

Figure S3. Band gap elucidation of ZnO-700.

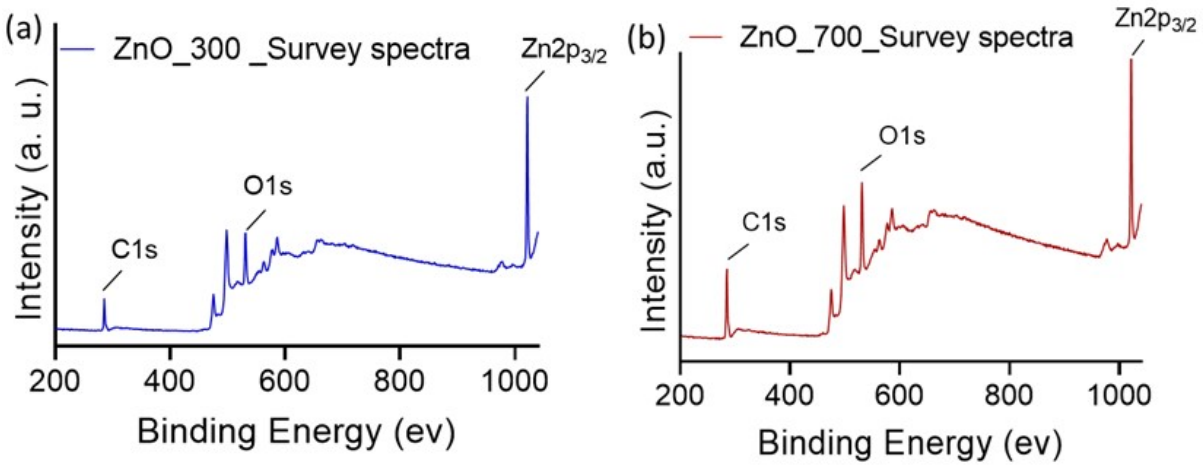

Figure S4. XPS survey spectra of (a) ZnO-300 (b) ZnO-700.

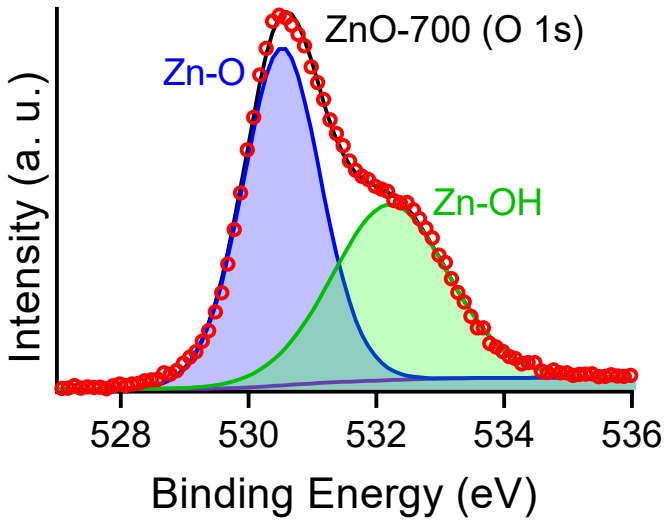

Figure S5. O 1s core level XPS spectra of ZnO-700.

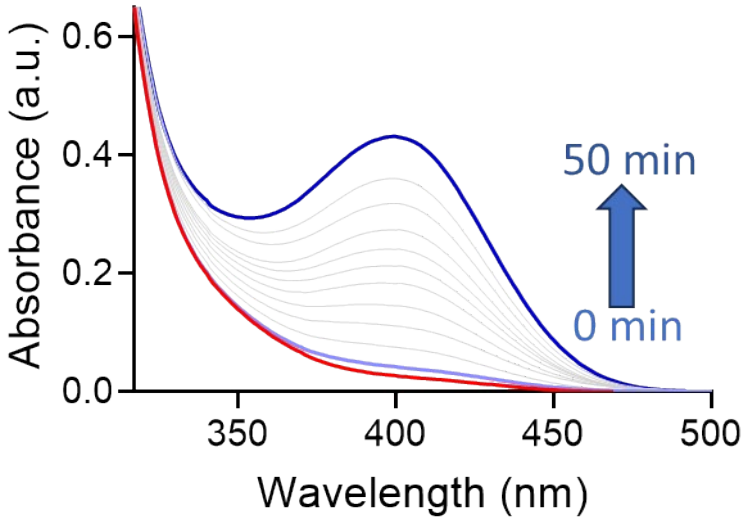

Figure S6. Time-dependent changes in UV-visible spectra of PNPA (0.5 mM) in the presence of ZnO-700 (0.5 mg/mL) at pH 7.4 in buffered medium.

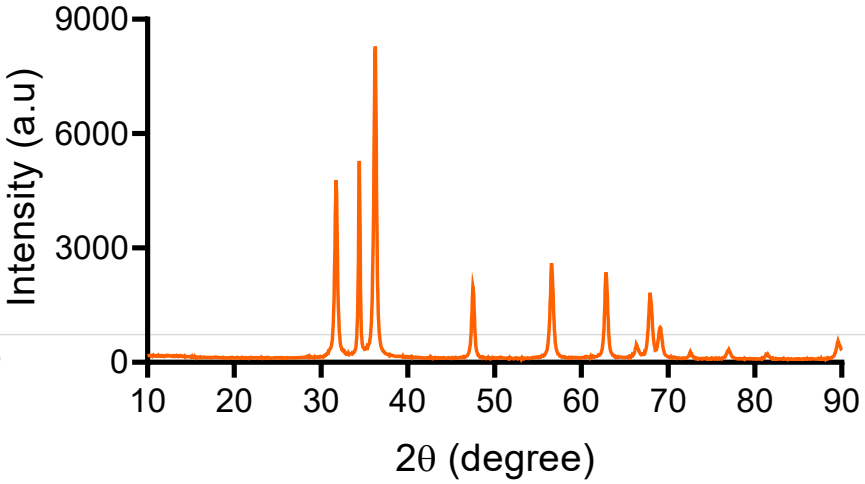

Figure S7. PXRD spectra of ZnO-700.

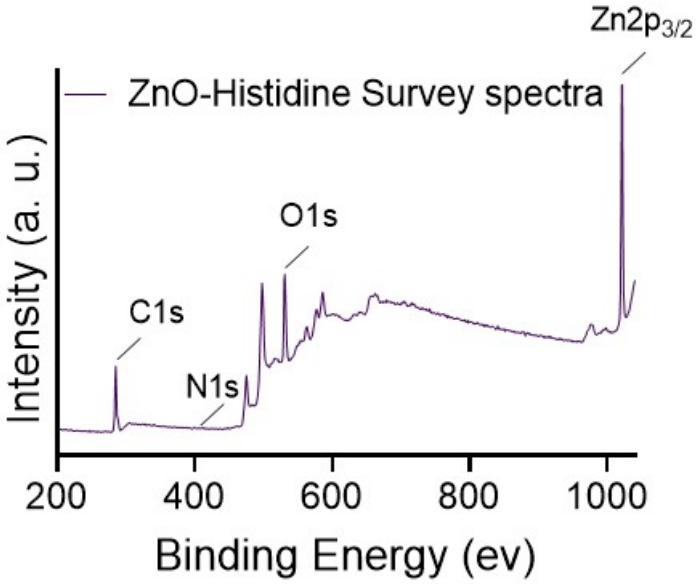

Figure S8. XPS survey spectra ZnO-Histidine.

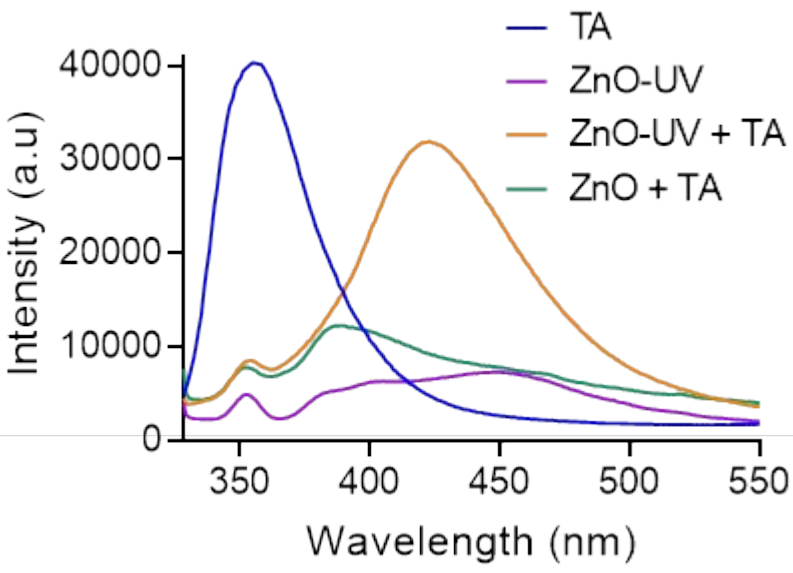

Figure S9. Free radical capture experiment using Terephthalic acid.

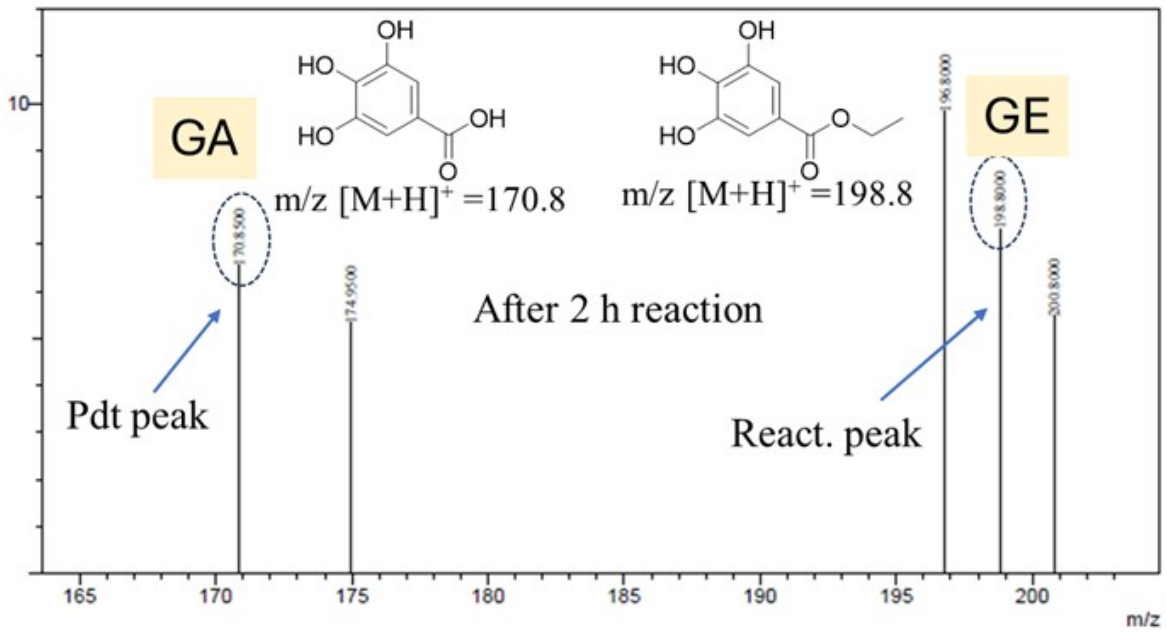

Figure S10. ESI-MS spectra of the Ethyl Gallate hydrolysis product obtained after 120 min in aqueous medium.

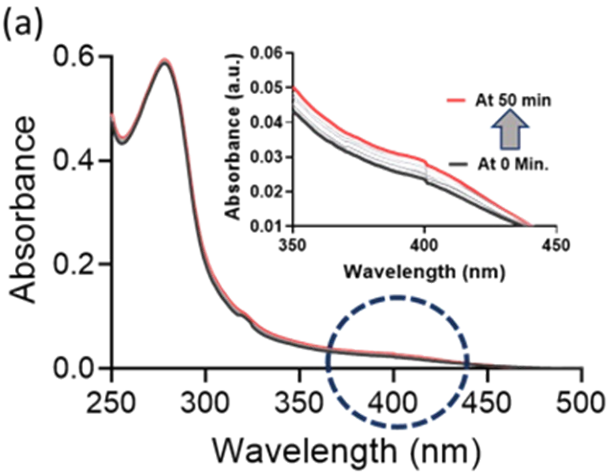

Figure S11. (a) Time-dependent UV-vis spectra of PNPA in UV light irradiation at PBS 7.4 medium (without catalyst).

Catalyst	Rate constant (k) min^{-1}	K_m (mL mg^{-1} min^{-1})	K_s (min^{-1} m^{-2})
ZnO-300	0.0080	0.016	0.620
ZnO-700	0.0048	0.0096	1.7

Table S1. (a) Kinetics rate constant (at 25 °C) normalized by mass (k_m) and surface area (k_s) of the ZnO nanocatalyst.

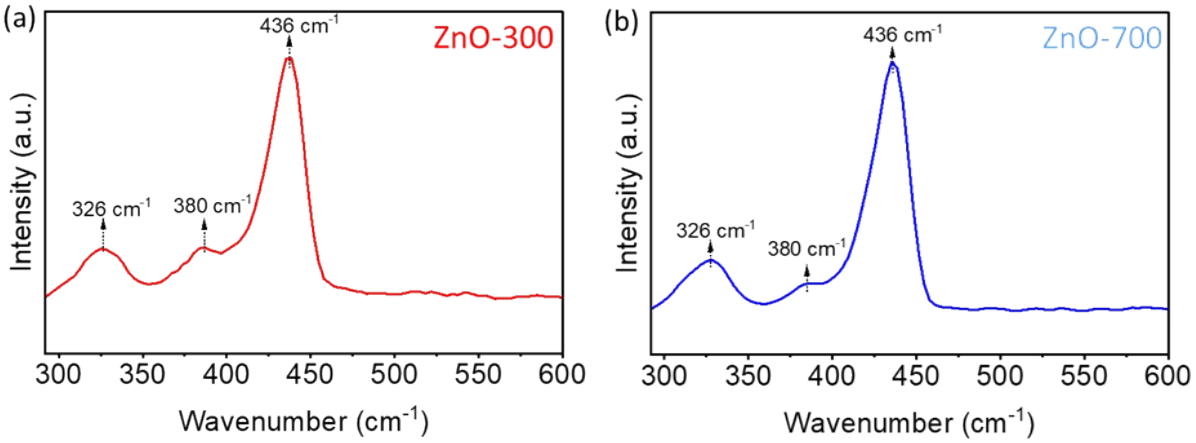

Figure S12. Raman Spectra of (a) ZnO-300 (b) ZnO-700

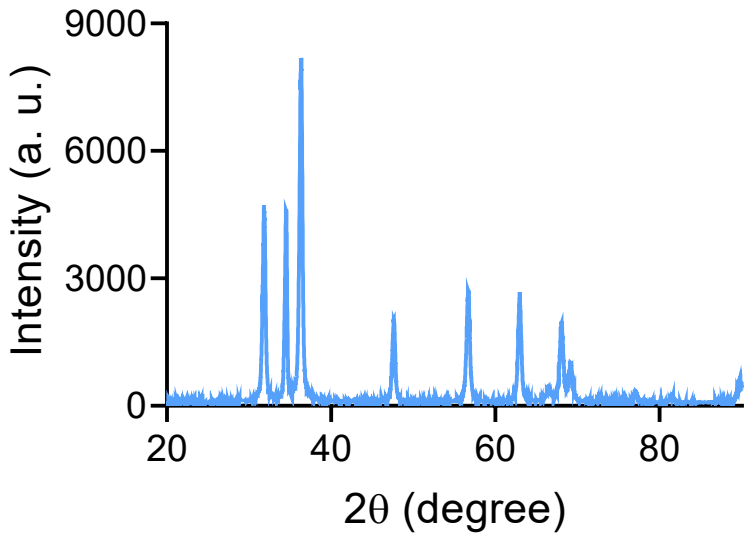

Figure S13. PXRD spectra of ZnO-300 (at low scan rate 0.5 deg./min).