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Biomass-derived dual crosslinked DNA-Nanoparticle hydrogel for visible light induced photodynamic bacterial inactivation

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Supporting Information

Text S1: Instrumentations

Powder XRD (pXRD) analysis of the DNA-NP was performed on an Empyrean, Malvern Panalytical device using a Cu-K (λ= 1.54 A°) X-ray source at a scanning rate of 2° per minute and a voltage of 10 kV in the 2 range of 4° to 70°. TEM images were captured using a JEM-F200 (Jeol, Japan) operating at 100 kV voltage with samples deposited on carbon-coated copper grid. Fourier transform–infrared spectroscopy (FTIR) experiments were performed with Parkin Elmer Spectrum-400 and a KBr pellet in the 400–4000 cm^{-1} range. Using a modular compact rheometer (Anton Paar, MCR 302, Austria) and a constant strain of 0.1% at room temperature, rheological tests of the hydrogel were conducted. Rheological measurements of the hydrogel were performed using a modular compact rheometer (Anton Paar, MCR 302, Austria) sweeping the frequency from 0.1 to 100 rad/s, at a constant strain of 0.1% at room temperature. UV-Visible absorption and steady-state fluorescence spectra were recorded using UV-2550 spectrophotometer (Shimadzu, Japan) and Fluoromax-400 spectrofluorometer (Horiba, Japan). Field emission scanning electron microscopy (FESEM) images were taken using a GeminiSEM 500 microscope (ZEISS) placing lyophilized samples on a carbon tap followed by coating with Au. The zeta potential and dynamic light scattering experiments were performed using an Anton Paar (Lightsizer 500) instrument. Digital images were obtained on a Canon D60 digital camera.

Text S2: SEM sample preparation of bacteria

Cells of *E. coli* strain MTCC 433 were harvested before and after photodynamic treatment for 1 h with 2 mg/ml DNA-NP. The cells were then collected by centrifugation at 5000 rpm for 5 min and washed twice with PBS followed by air drying. The 2.5 % glutaraldehyde was added and kept at 4°C for overnight for fixation on a silicon wafer (10×10mm). Then the fixed slides

were washed with PBS for one more time followed by dehydration using ethanol with increasing percentages i.e. 10, 30, 50, 70, 90% in step gradient for 15 min each (including adding ethanol) and air-drying for 15 min. After dehydration, the specimen in slides were gold-coated using standard procedure and visualized under Scanning Electron Microscope under 5 kV voltage.

Text S3: Determination of Quantum Yield of DNA-NP

Quantum Yield was determined by using a solution of quinine sulphate (QY = 0.55) in 0.5 M as standard. Quantum yield was calculated using the following equation,

$QY_x = QY_s \times (A_s/A_x) \times (I_x/I_s) \times (n_x^2/n_s^2)$

Here, subscripts x and s stand for the sample and the reference material respectively, QY stands for the quantum yield; A refers to the absorbance at the excitation wavelength, I to the integrated fluorescence intensity and n to the refractive index of the solvent.

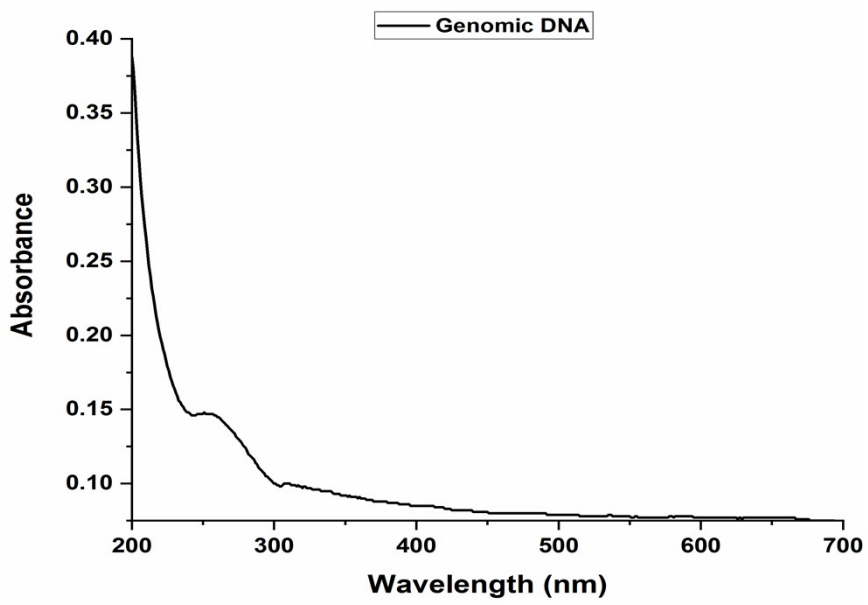

Figure S1: UV-Vis Spectrum of biomass DNA extracted from onion

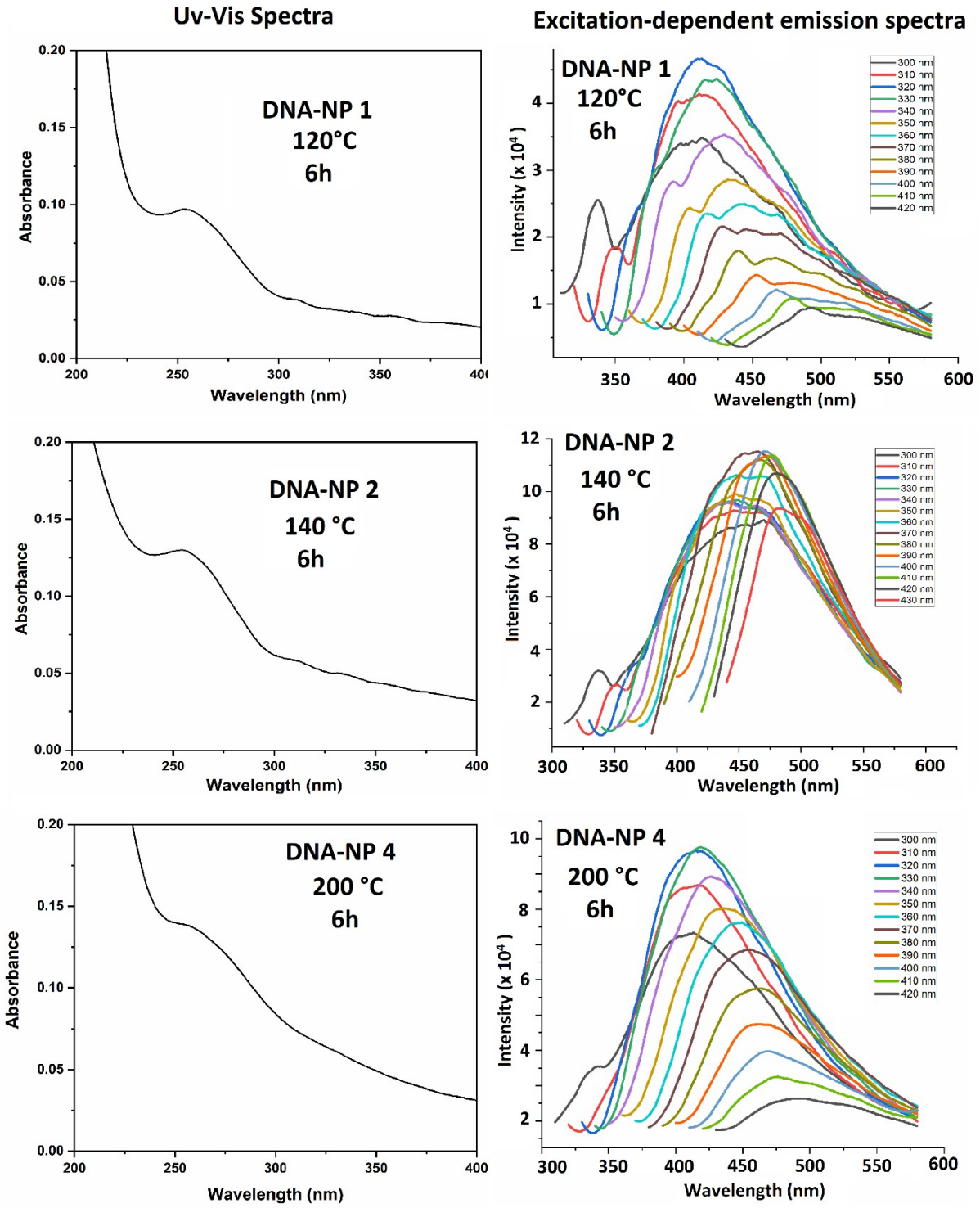

Fig. S2: Excitation dependent emission spectra and UV-Vis Spectra of DNA-NPs synthesised at different conditions (DNA-NP 3 or DNA-NP, the optimized candidate not shown here, demonstrated in figure 2, main manuscript)

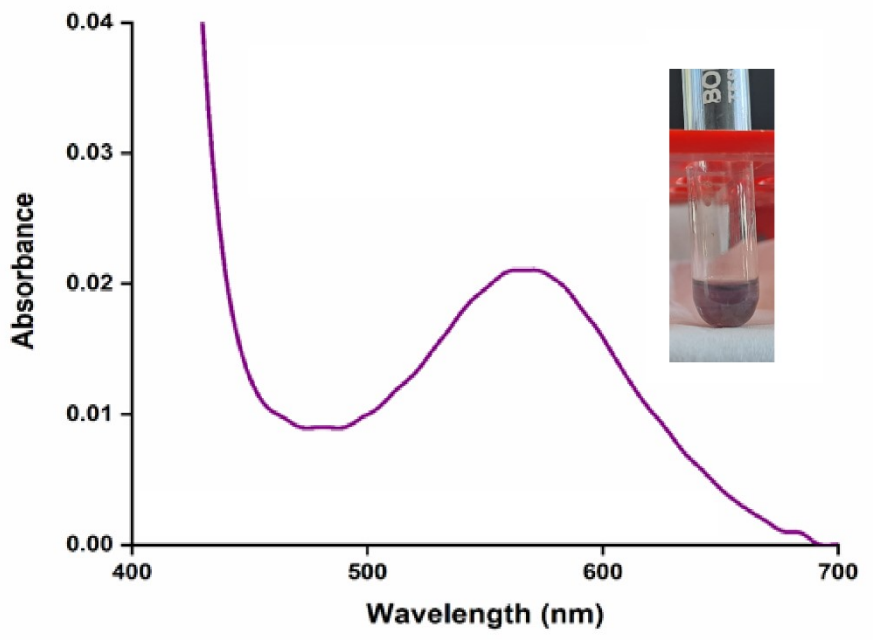

Figure S3: Formation of Ruhemman's purple (λ_{max} = 570 nm) by DNA-NP

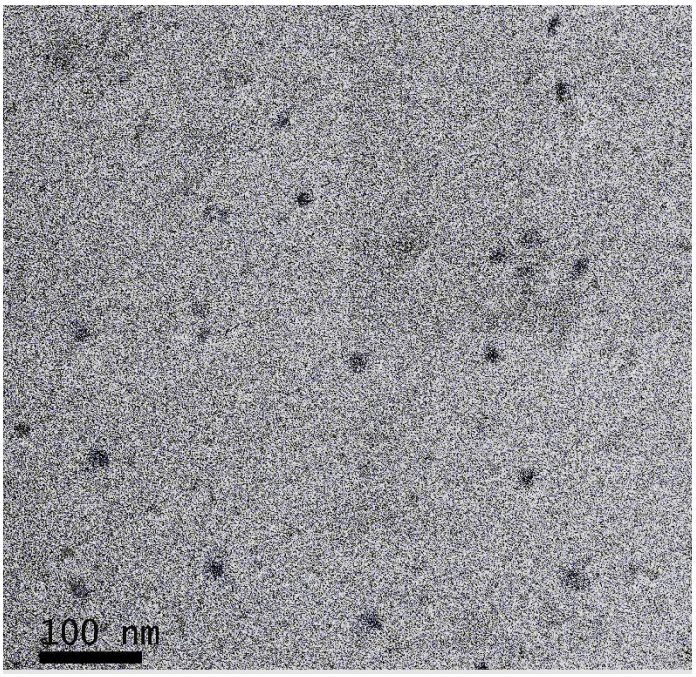

Figure S4: TEM image of DNA NP at lower resolution

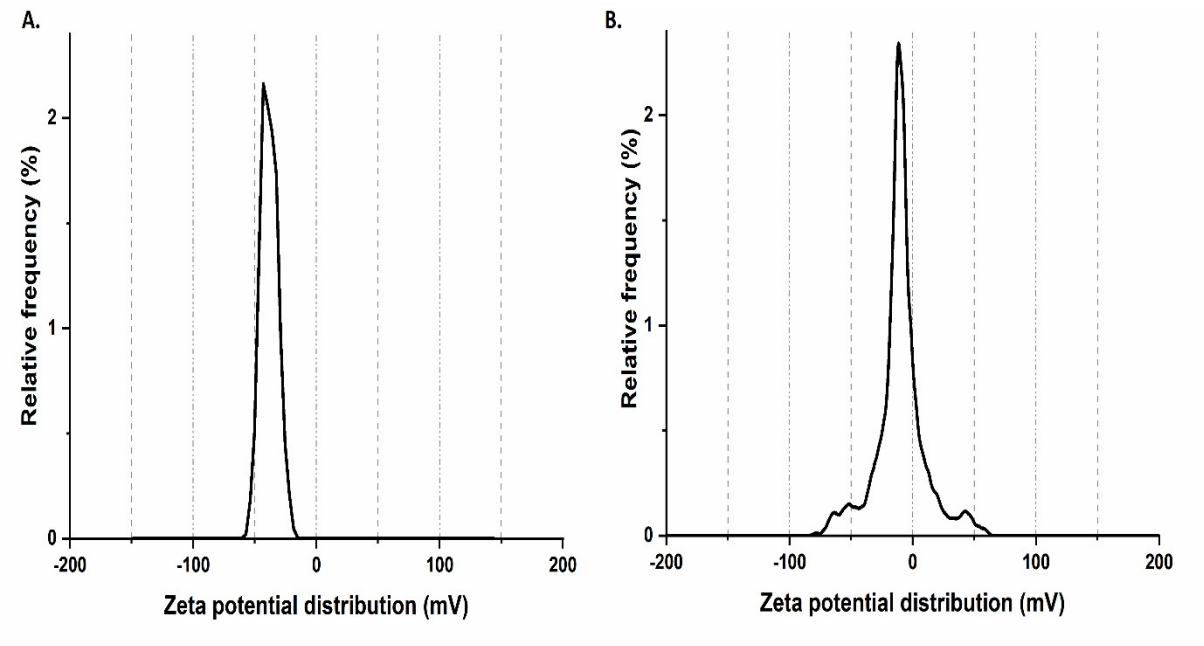

Fig S5: Zeta potential of (A) DNA and (B) DNA-NP

Fig. S6: Demonstration of self-healing property of DNA-NP hydrogel

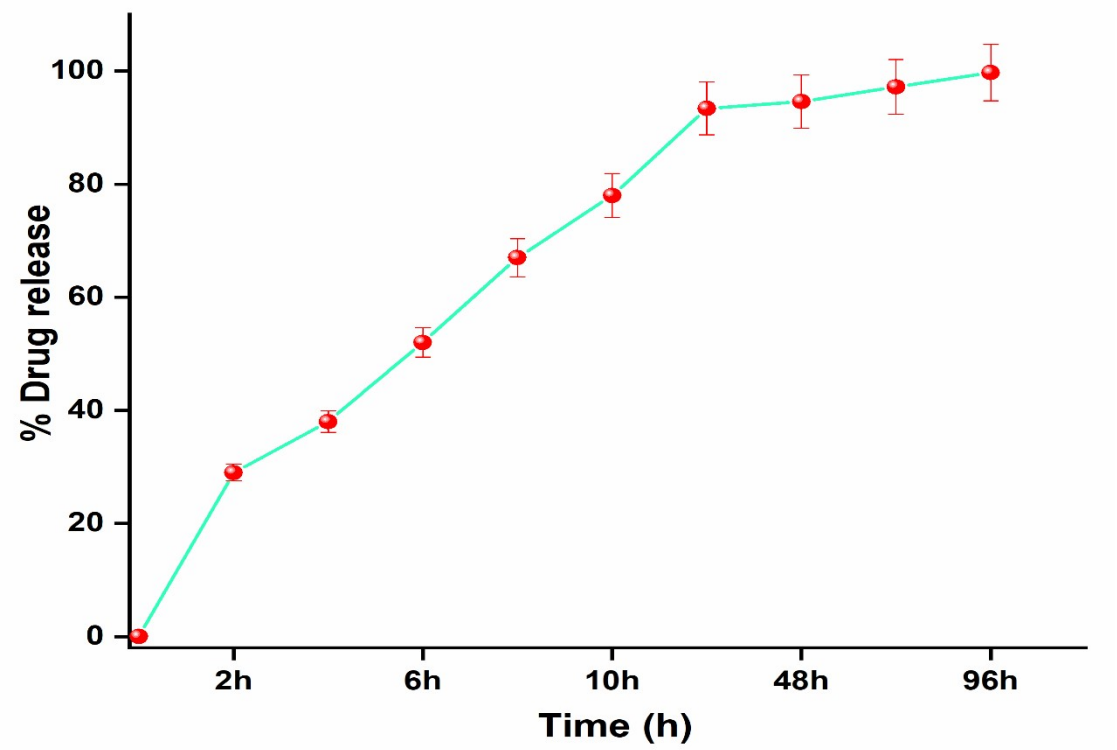

Fig S7: Release profile of Amp from DNA-NP hydrogel at physiological conditions (37°C, pH~7.4)

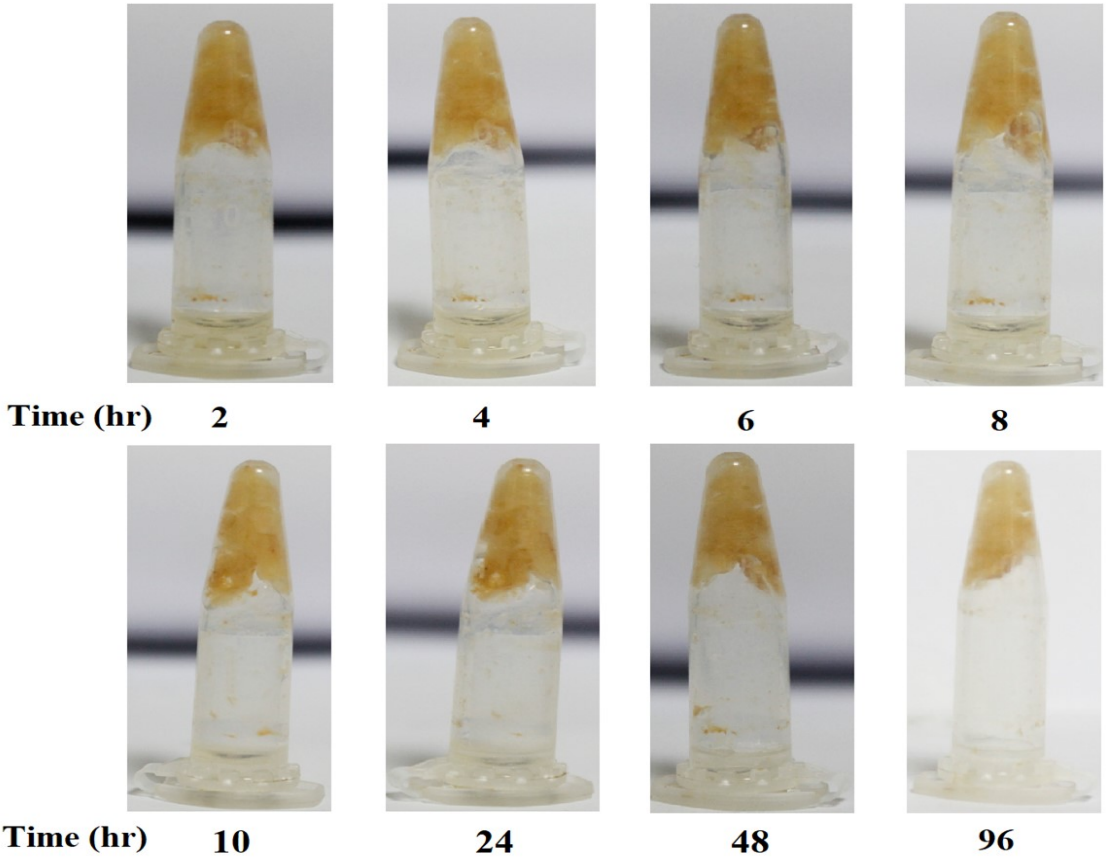

Fig S8: Digital images of DNA-NP hydrogel during drug release taken at different time interval.

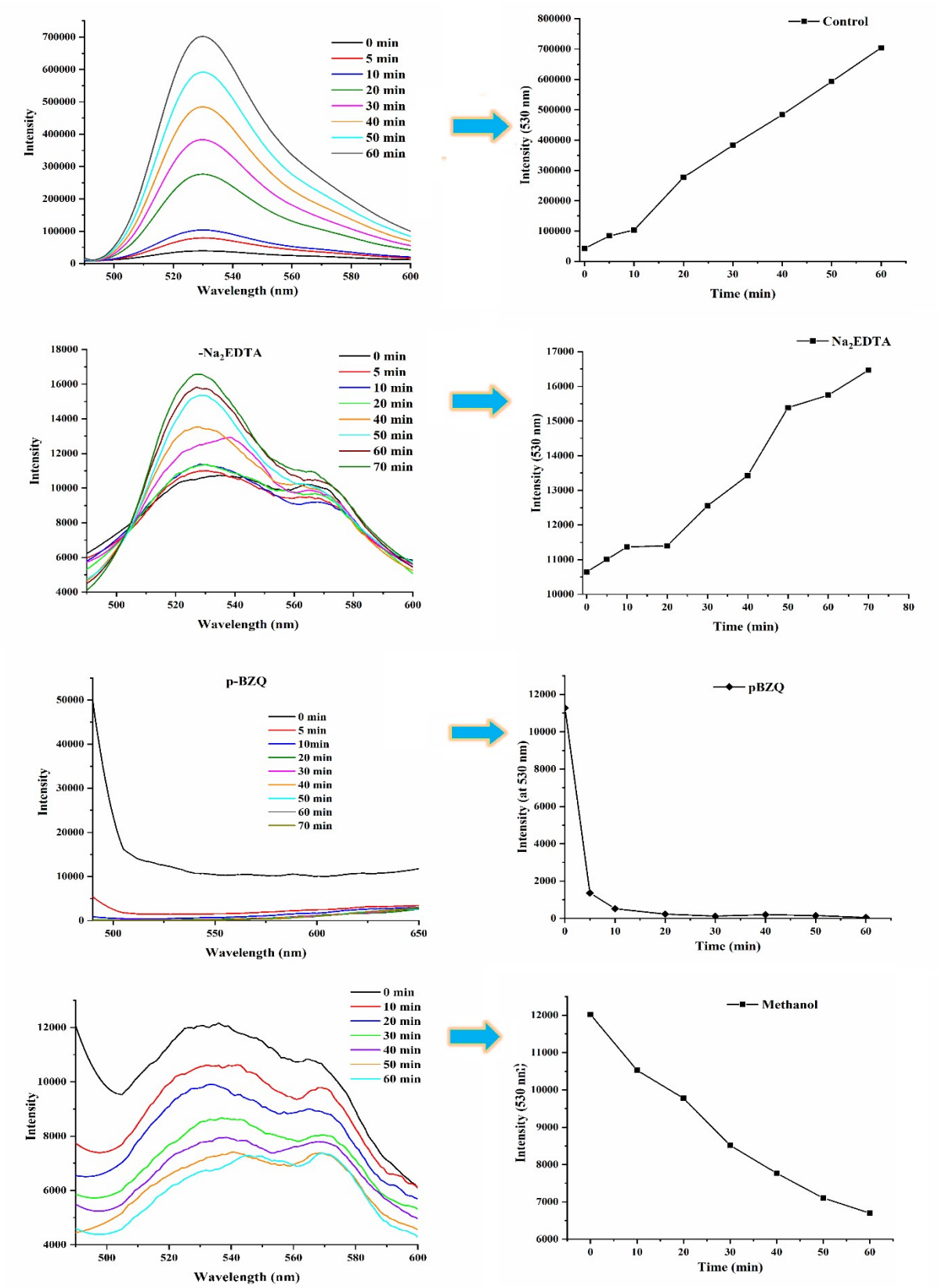

Figure S9: ROS generation of DNA-NP hydrogel in presence of visible light by using various radical scavengers A. control, B. Na_2EDTA, C. p-BZQ, D. Methanol.

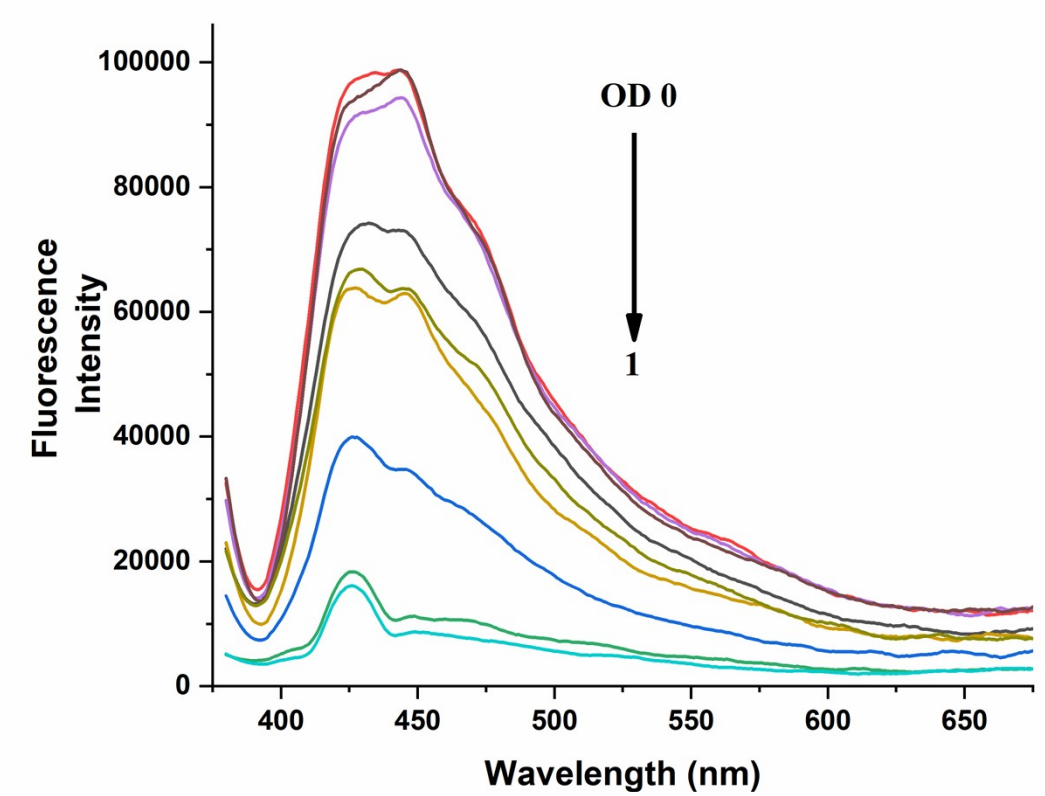

Figure S10: Decreasing fluorescence intensity with increasing absorbance (OD_{600}) of culture media of *E. coli*

DNA-NP Abbreviation	Reaction Condition	Calculated Quantum yield (in %)	Excitation maximum (nm)	Emission maximum (nm)
DNA-NP 1	120°C	0.2	320	410
DNA-NP 2	140°C	1.4	320	428
DNA-NP 3 (DNA-NP)	160°C	**5.33**	320	432
DNA-NP 4	200°C	2.32	320	418

Table 1: Quantum yields and corresponding excitation and emission wavelengths of different synthesised DNA-NPs.

Sample	Lifetime (ns)			T_{avg} (ns)	a^2 (%)	χ^2
	T_1	T_2	T_3			
DNA-NP 3 (DNA-NP) (160^{0}C,6h)	**0.08**	**1.19**	**5.90**	**3.92**	**7.57, 32.60, 59.84**	**0.987**

Table 2: Tri-exponential fitting of Fluorescence Lifetime of DNA-NP