Electronic Supplementary Material (ESI) for Materials Advances.


Supporting Information of

# Peroxidase-like behavior and photothermal effect of chitosan coated Prussian-blue nanoparticles: dual-modality antibacterial action with enhanced bioaffinity

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## 1. Characterization

### 1.1 Thermal gravimetric Analysis

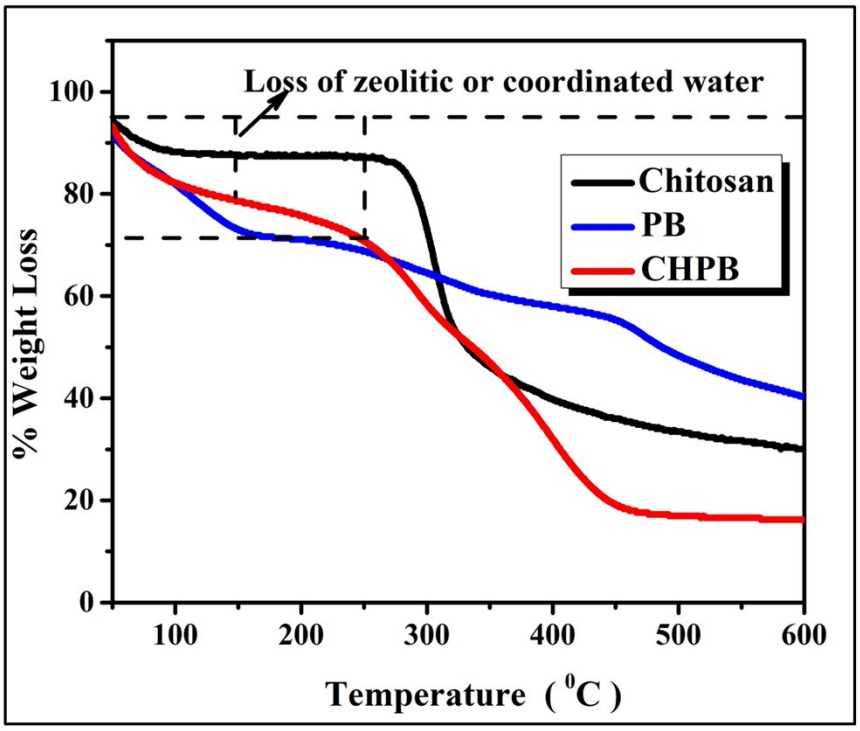


**Figure S1: Thermal degradation curve of free Chitosan, PB NPs and CHPB NPs.**

## 1.2 Energy Dispersive X-ray (EDX) Spectroscopy

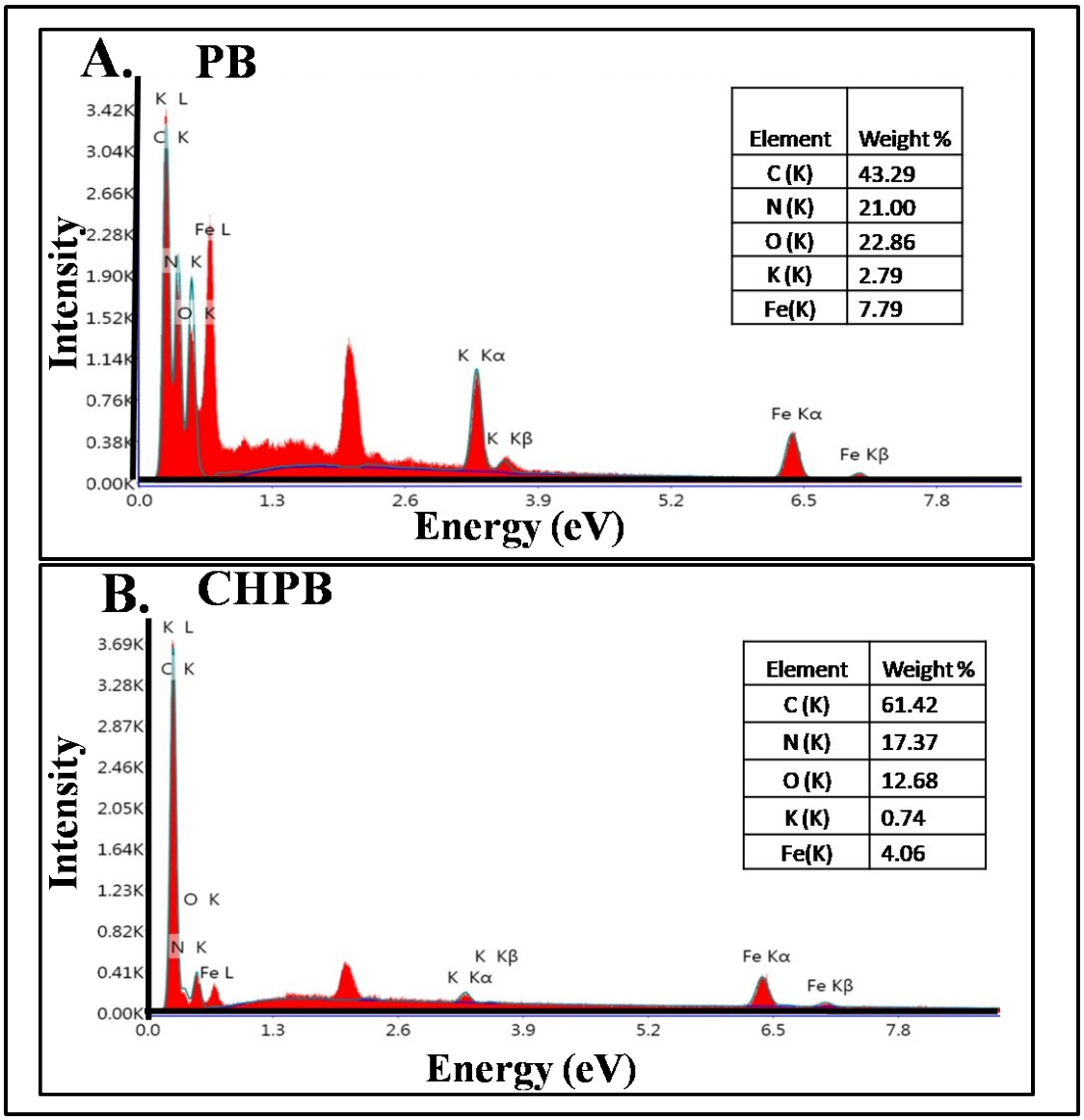


**Figure S2: EDX spectra of (A) PB, and (B) CHPB NPs.**

### 1.3 Atomic Force Microscopy (AFM)

Atomic force microscopy is a widely used tool to study the three dimensional visualization of the nanoparticles. The tool is extensively explored for imaging and for studying the size, morphology, roughness and surface texture. The change in the surface morphology of the CHPB and PB NPs were investigated and we further recorded the three dimensional images with non-contact mode and scanned through semi-contact mode. The AFM height images of samples were used to calculate the root-mean-square ($R_{rms}$) roughness of the nanoparticle surface using Eq. 1.

$$R_{rms} = \sqrt{\frac{\sum (Zi - Zavg)^2}{Np}}$$

Eq.1

Where, $Z_i$ is the current Z value $Z_{avg}$ is average of the z values of the area selected for analysis and $N_p$ is the number of point within the given area.

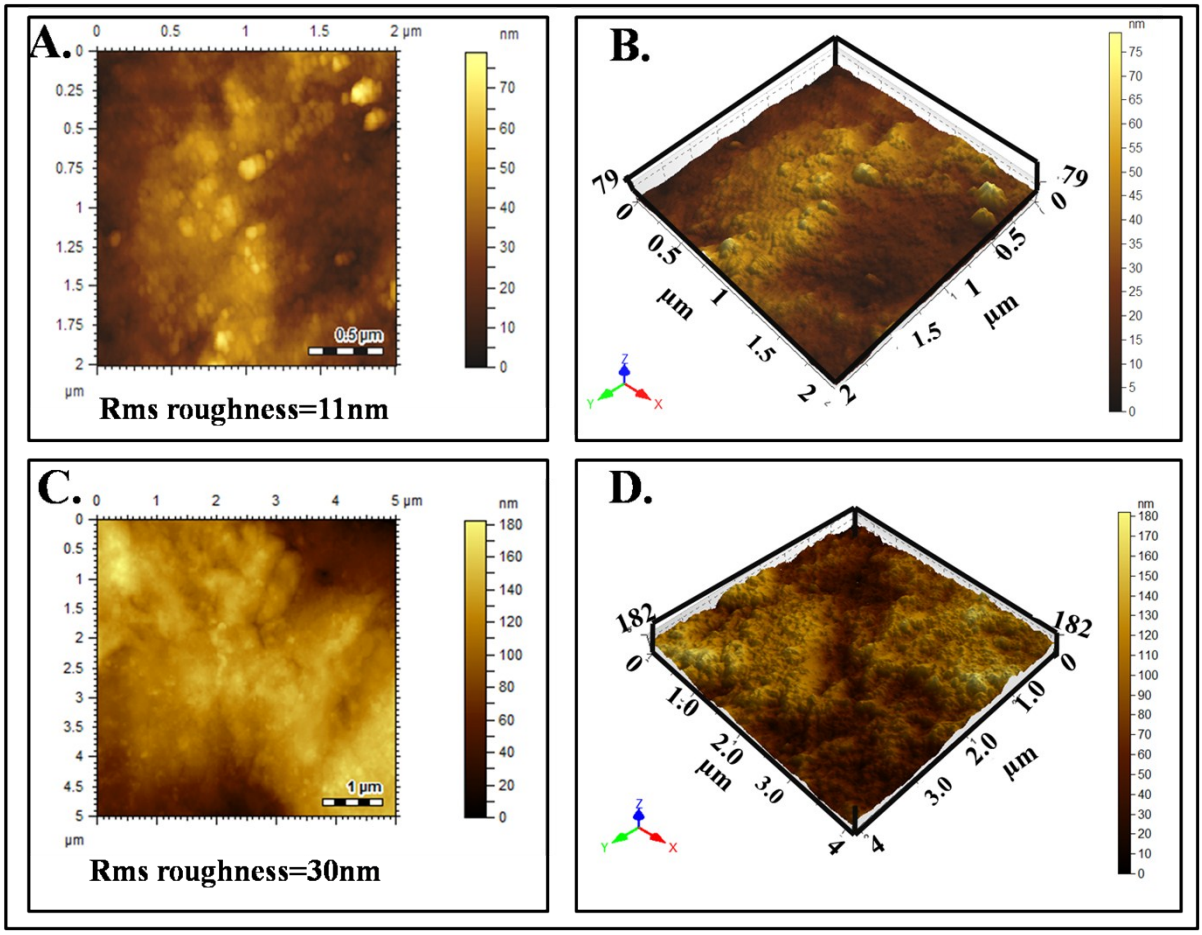


**Figure S3: AFM (A) 2D image, and (B) same field in 3D image of CHPB NPs.**

**AFM (C) 2D image, and (D) same field in 3D image of PB NPs.**

## 2. Kinetic study of Peroxidase-like activity of PB and CHPB NPs

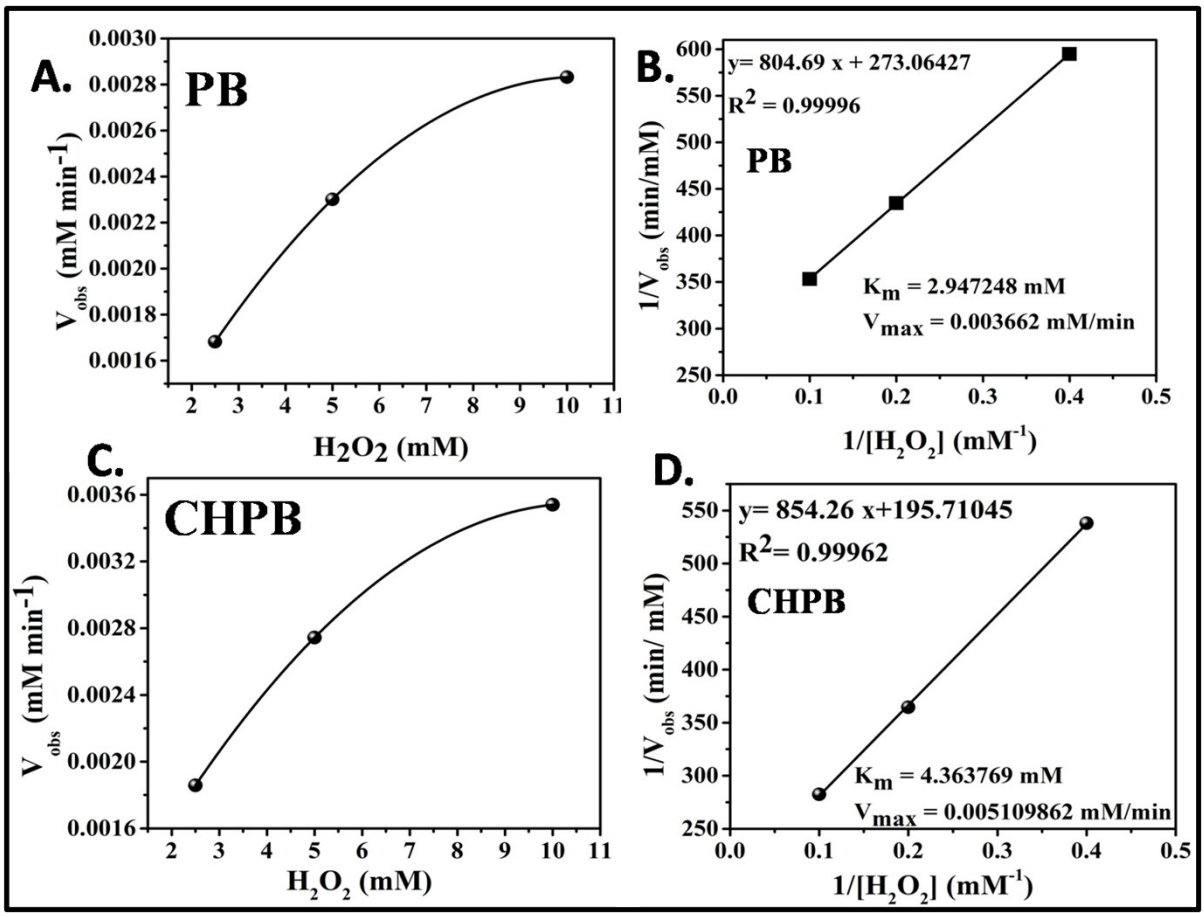


**Figure S4: Kinetic analysis Plot of CHPB and PB NPs. (A) Michaelis-Menten type plot, and (B) Lineweaver-Burk plot for determination of apparent $V_{max}$ and $K_m$ values for PB NPs. (C) Michaelis- Menten plot, and (D) Lineweaver-Burk Plot for determination of apparent $V_{max}$ and Km values for CHPB NPs.**

## 3. Antibacterial activity of PB and CHPB nanoparticles

### 3.1 Growth curves

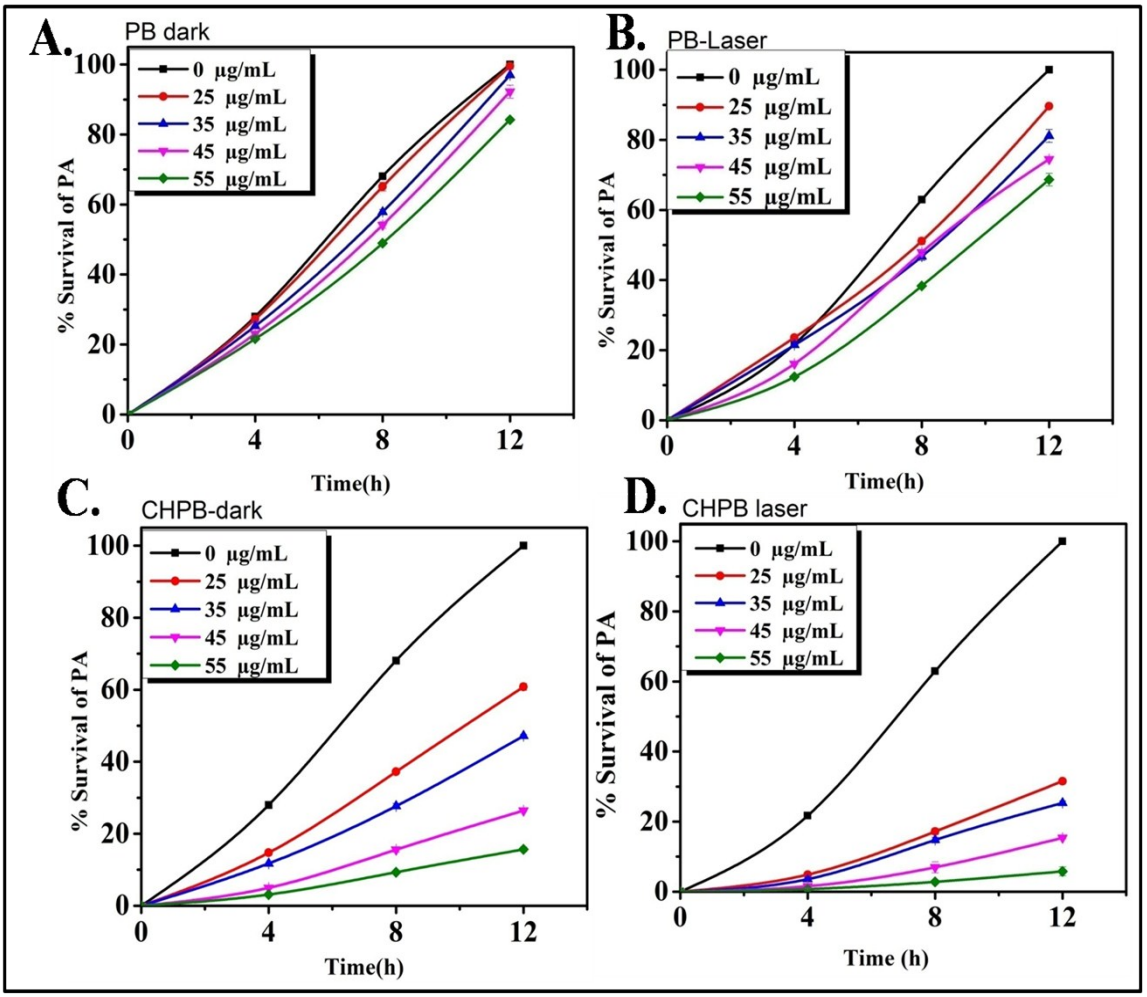


**Figure S5a: Growth kinetics of Gram-negative bacteria P. aeruginosa (PA), treated with different concentrations of (A) PB NPs without laser irradiation (dark), (B) PB NPs with light irradiation (laser), (C) CHPB NPs without laser irradiation (dark), and (D) CHPB NPs with light irradiation (laser).**

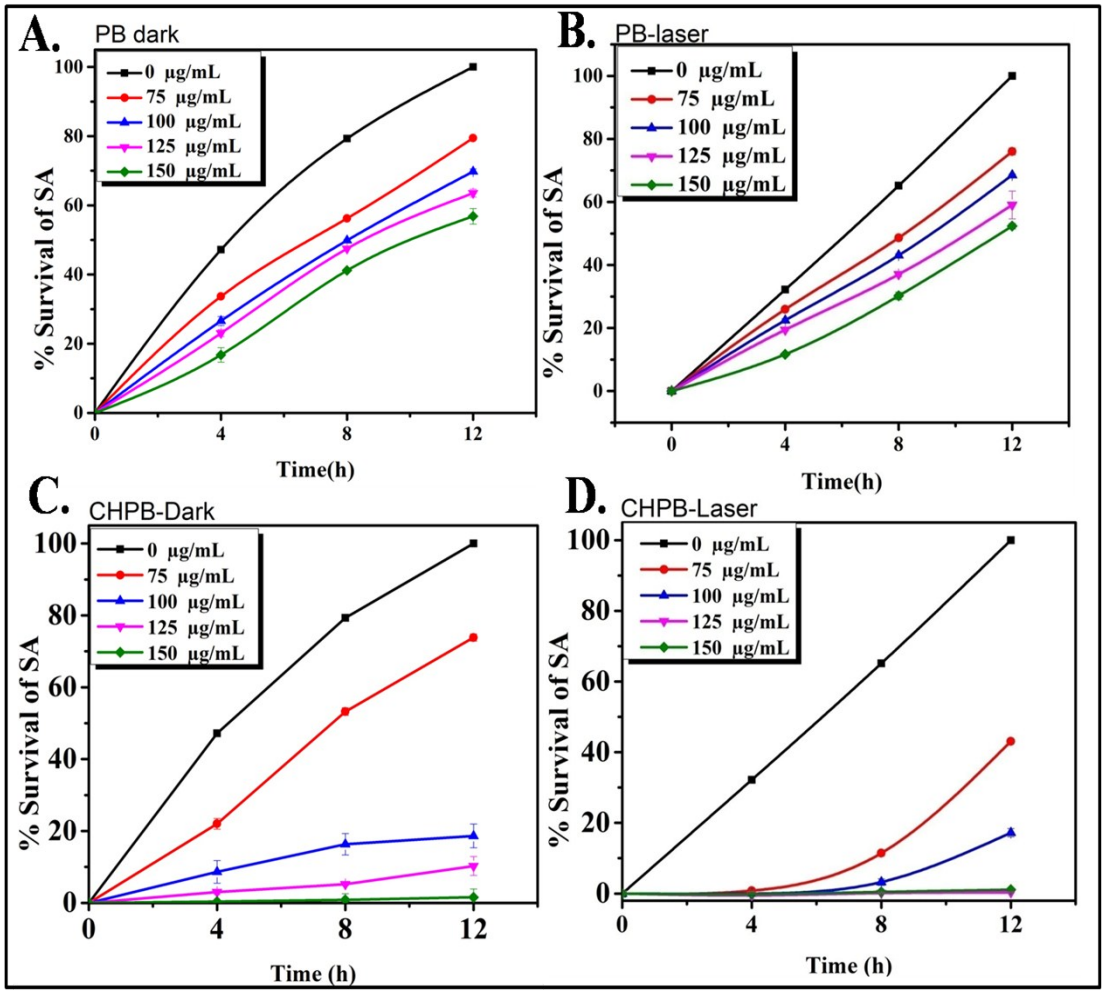


**Figure S5b: Growth kinetics of Gram-positive bacteria *S. aureus* (SA), treated with different concentrations of (A) PB NPs without laser irradiation (dark), (B) PB NPs with light irradiation (laser), (C) CHPB NPs without laser irradiation (dark), and (D) CHPB NPs with light irradiation (laser).**

### 3.2 Colony count assay for PB and CHPB NP

The Gram-negative bacteria *P. aeruginosa* (PA) bacteria strains were grown in Mueller Hinton Broth (MHB) medium. These strains were further treated with the concentrations corresponding to respective MIC values of PB and CHPB NPs, with and without irradiation in a 24 well-plate overnight at 37$^{o}$C and kept in an incubator shaker at 180 rpm. Following day, the strains were diluted serially to $10^{-4}$ in fresh MHB media and 100µL of this dilution was plated on MHB agar plate.

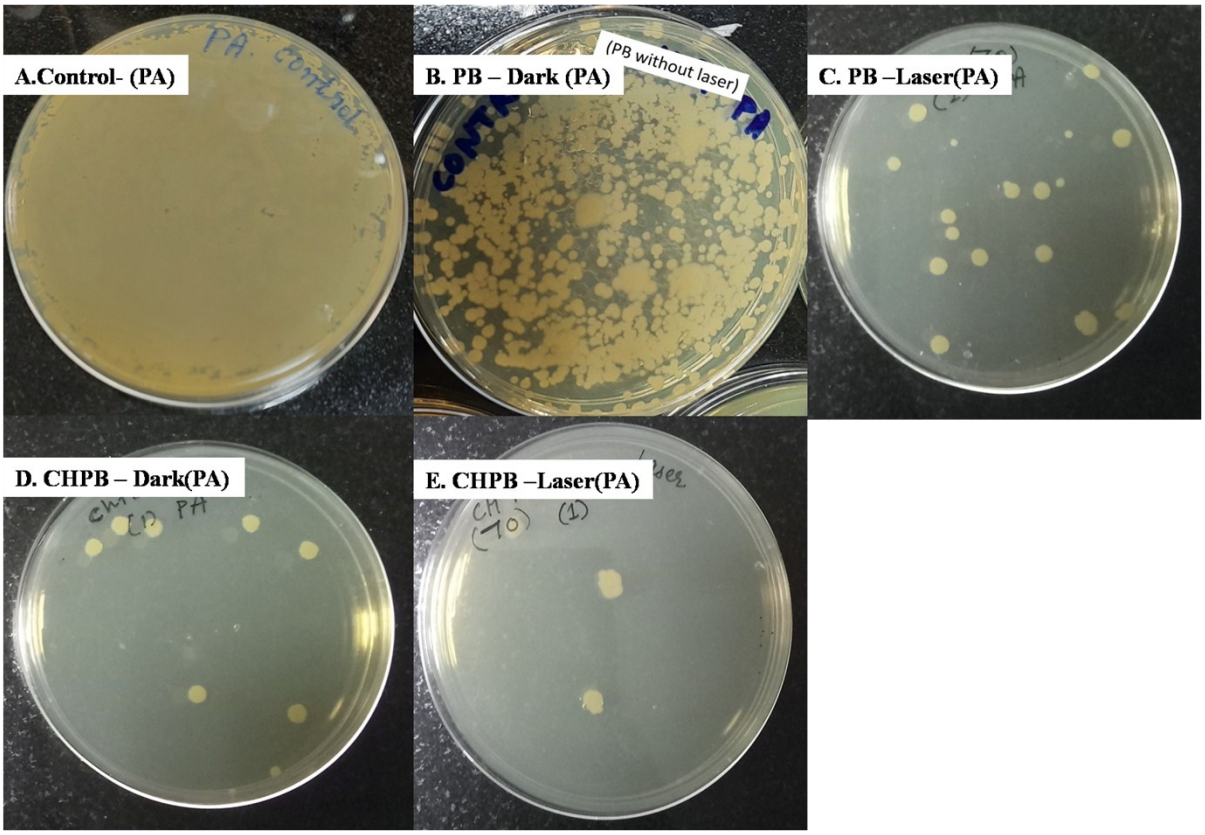


**Figure S6: Colony forming units assay on *P. aeruginosa* (PA), (A) without treatment, (B) treated with MIC value of PB NPs without laser irradiation (dark), (C) treated with MIC value of PB NPs laser irradiation, (D) treated with MIC value of CHPB NPs without laser**

**irradiation (dark) and, (E) treated with MIC value of CHPB NPs with laser irradiation .**

The plates were incubated at 37°C for 48h. After counting the colonies manually, it was used in the following equation.

$$Number\ of\ colony\ forming\ units = \frac{Number\ of\ colonies\ counted}{volume\ of\ diluted\ plate \times dilution\ factor}$$

The result of colony forming units (CFU) is summarized in Table 1 below:

| **Nanoparticle** | **Bacteria** | **Condition** | **CFU in original sample (X $10^6$)/mL** |
|---|---|---|---|
| CHPB | *P. aeruginosa* | Untreated | 1390 |
| | | Without laser | 80 |
| | | With Laser | 30 |
| PB | *P. aeruginosa* | Untreated | 1390 |
| | | Without laser | 149 |
| | | With Laser | 115 |

The results show that CHPB NPs have higher anti-bacterial activity. The plates show that at MIC concentrations, the bacterial growth was restricted and further the antibacterial property enhanced with laser irradiation (635 nm). The antibacterial activity with PB NPs was also observed at MIC concentrations with laser irradiation, validating the enhancement in antibacterial efficacy is due to photothermal PB core.

### 3.3 Antibacterial activity of chitosan

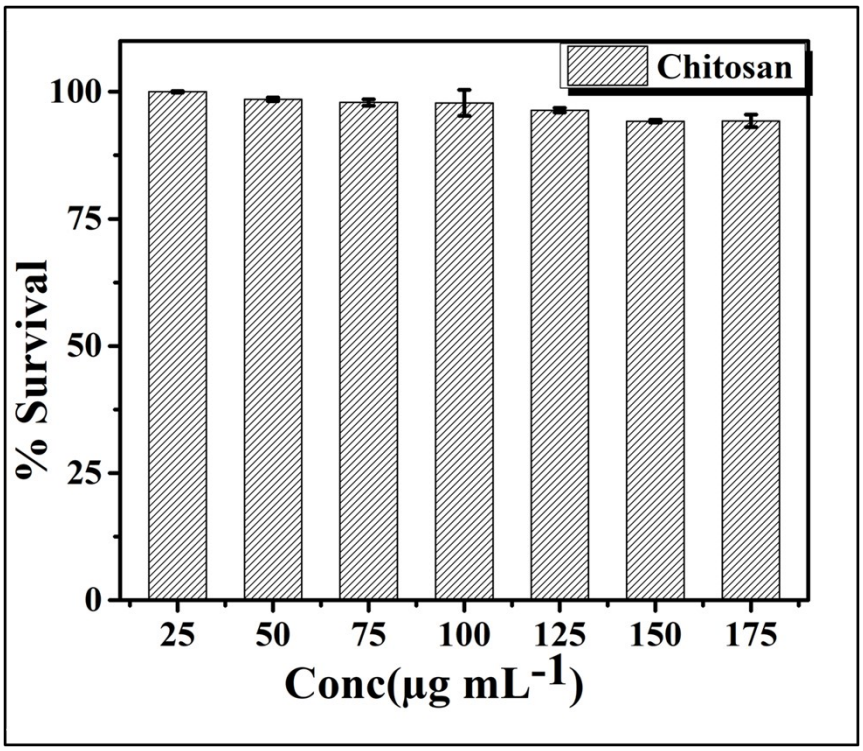


**Figure S7: Microbroth dilution assay for *P. aeruginosa* treated with chitosan.**

**4. Transmission electron microscopy (TEM) analysis nanoparticle-bacteria interaction**

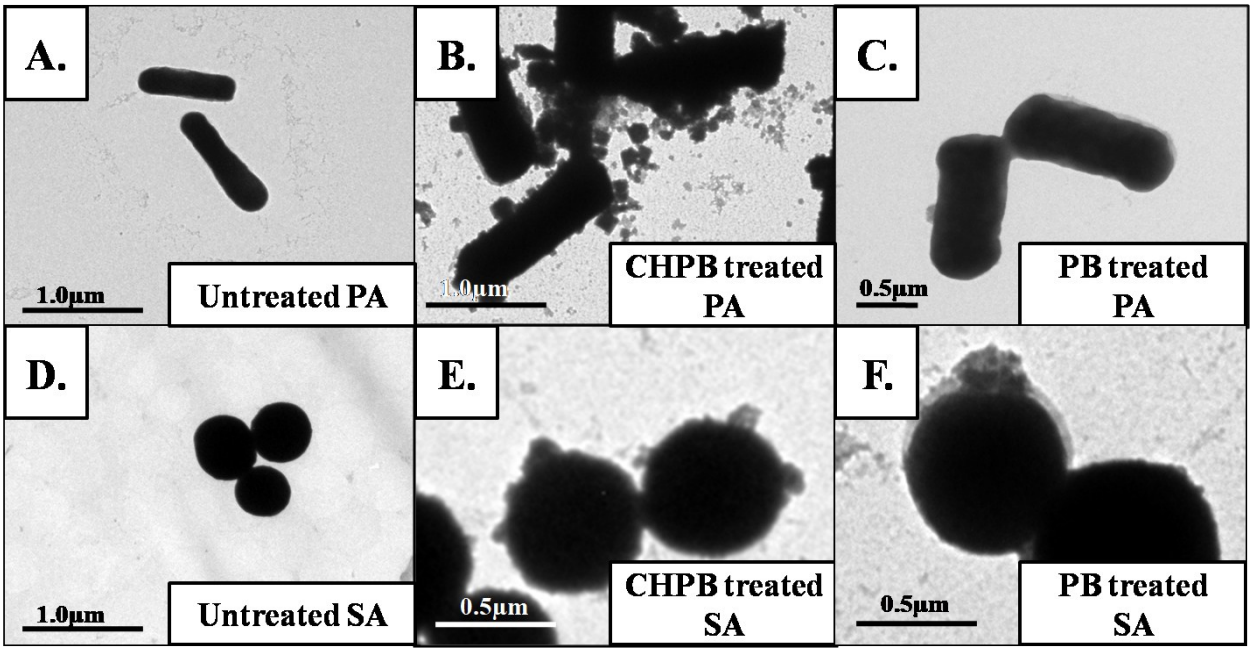


**Figure S8: Surface attachment of nanoparticles with bacteria: (A-C) *P. aeruginosa* (A) Untreated in PBS buffer, (B) CHPB NP treated, and (C) PB NP treated. (D-F) *S. aureus* (D) Untreated in PBS buffer, (E) CHPB NP treated, and (F) PB NP treated.**

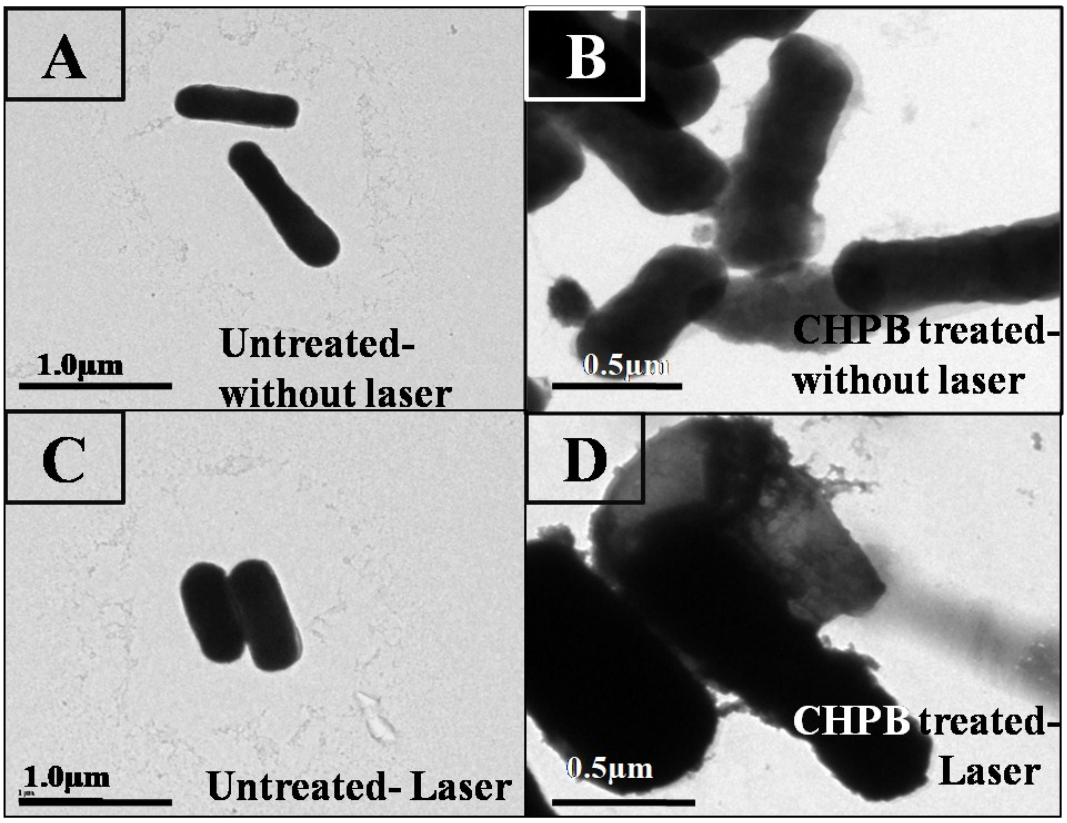


**Figure S9: TEM analysis of *P. aeruginosa*. (A, C) untreated cells (A) without(dark), and (C) treated with laser. (B, D) Cells treated with CHPB NPs at MIC values, (B) without(dark), and (D) with laser treatment for 60 mins.**

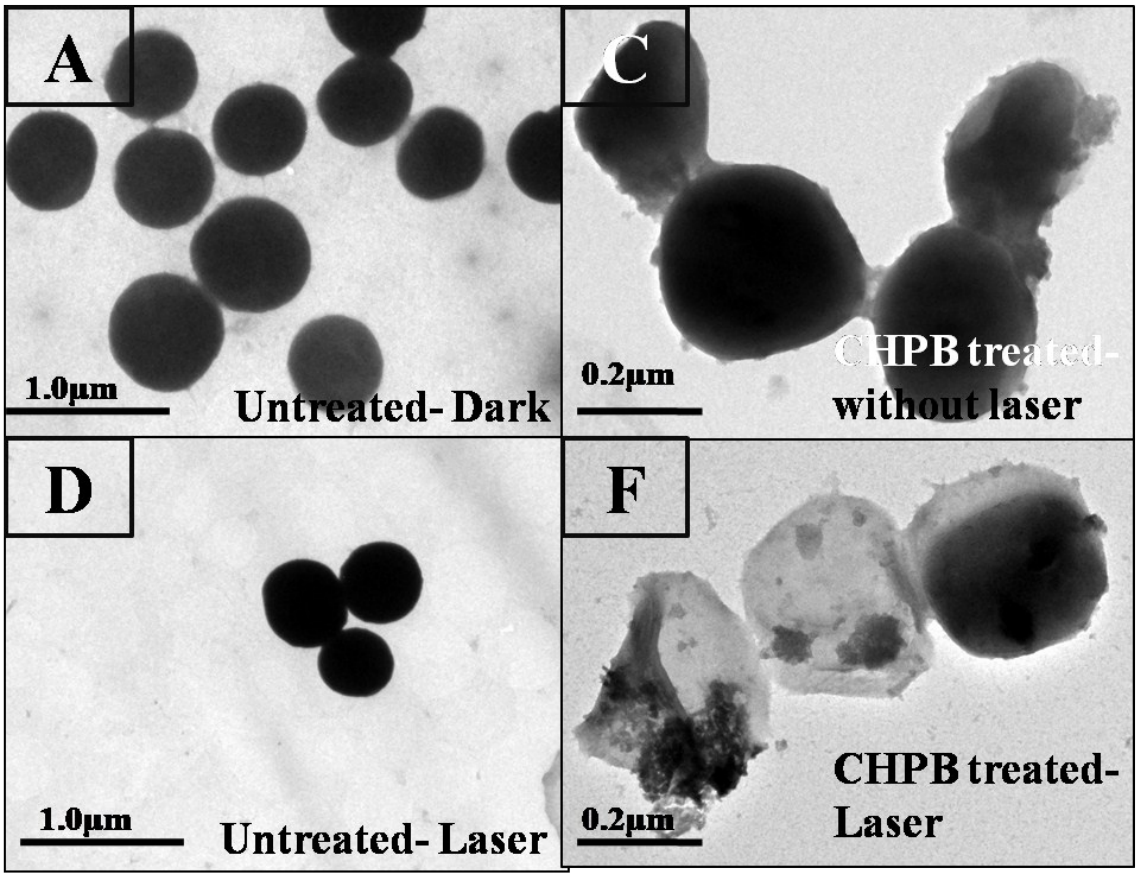


**Figure S10: TEM analysis of *S. aureus*. (A, C) untreated cells (A) without (dark), and (C) treated with laser. (B, D) Cells treated with CHPB NPs at MIC values, (B) without (dark), and (D) with laser treatment for 60 mins.**

**5. Cytotoxicity assay for CHPB NPs**

Apart from the bacterial studies described above, CHPB nanoparticles were also tested for in-vitro toxicity against HEK-293 mammalian cells. The standard methyl thiazolyl tetrazolium (MTT) assay was performed to determine the relative viability of cells treated with increasing concentrations of CHPB NPs. No appreciable toxicity was observed at the concentration range tested (about 90% cell viability even at the highest treatment concentration tested). Thus, CHPB NPs has selective cytotoxicity towards bacterial cells but are in general biocompatible towards mammalian cells.

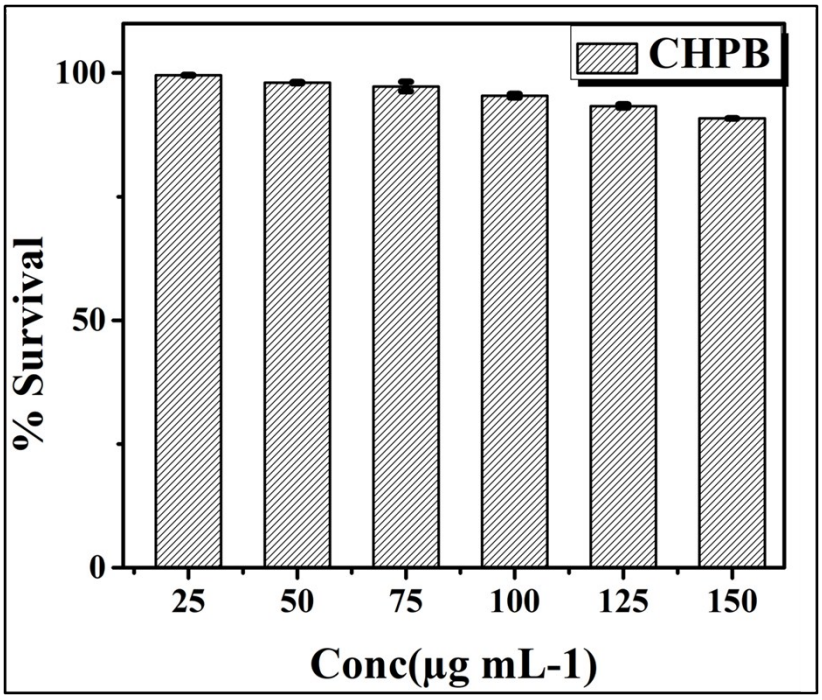


**Figure S11: Cell viability assay (MTT assay) HEK-293 cell line treated with CHPB NPs.**