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# Supplementary document

## Hypochlorite scavenging activity of cerium oxide nanoparticles

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# Experimental Section

## 1. Nanoparticles preparation and characterization

Synthesis of the nanoparticles were described in our previous publications. Briefly, CNP1 was synthesized using precipitation method. Cerium nitrate hexahydrate was dissolved in distilled water and equimolar mixture $NH_4OH$ was used as oxidizer[1]. The solution was the stirred for 4 hours and particles were washed with distilled water for three times. CNP2 was prepared using hydrothermal method. Cerium nitrate hexahydrate was dissolve in water and then NaOH was added to adjust pH to 10. This solution were mixed properly in a Teflon bottle and subjected to hydrothermal treatment at 80° C for 6 h[2]. Then particles were washed with distilled water for three times. CNP3 was also prepared using precipitation method. Cerium nitrate hexahydrate was dissolved in distilled water and excess amount of $NH_4OH$ was added. Then solution was stirred for 4 hours and washed with distilled water for three times. Pellet was resuspended in water and 1N $HNO_3$ was added to decrease the pH to 4 for better stability[3]. Lastly, CNP4 was prepared using wet chemical method, where equimolar $H_2O_2$ was used as oxidizer. 1N $HNO_3$ was used to decrease the pH to 4 for better stability[1].

These four nanoparticles were then thoroughly characterized. Size and morphology of the nanoparticles were analyzed using High Resolution Transmission Electron Microscopy (HRTEM). Surface charge and hydrodynamic radius of the nanoparticles were estimated using Zetasizer (Nano-ZS from Malvern Instruments, Houston, TX). X-ray photoelectron spectroscopy (5400 PHI ESCA; Mg KR X-ray irradiation (1253.6 eV) and 350 W) was used to determine the surface chemistry of the nanoparticles.

## 2. UV-Vis $ClO^-$ absorption study

The interaction of CNP3 with $ClO^-$ was revealed by UV-vis absorption spectrometry. A 10 mM stock solution of hypochlorite ($ClO^-$) was prepared immediately before the experiment. The reaction mixture contained, in a final volume of 1.5 ml, $ClO^-$ (5 mM) and increasing concentrations (from 1 to 2.5 mM) of each CNPs (CNP3 and CNP2). After mixing both, the nanoparticles were centrifuged at 23000 g during 5 min and carefully washed three times with distilled water, in order to avoid the presence of free $ClO^-$. Finally, the absorption spectra (220-750 nm) were recorded using a Perkin Elmer Lambda 750S instrument.

## 3. Oxygen evolution measurements

Oxygen evolution was measured at 25°C under stirring (0.1 g) with a Clark-type oxygen electrode (Hansatech, Kings Lynn, UK). Firstly, 2 ml of distilled water were added to the chamber and dissolved oxygen was displaced with argon gas. Then, the chamber cap was opened and CNP3 or CNP4 at final concentration of 1 mM was added. Finally, the reaction mixture was completed when $ClO^-$ (5 mM) was added. Oxygen evolution was tracked during 30 min. There was no evidence of oxygen release when nanoparticle or $ClO^-$ alone was added (see supplementary figure S3).

## 4. $ClO^-$ measurement in RAW cells

$30 \times 10^3$ RAW cells/cover slips were seeded and then cultured overnight. Cells were then treated with two different concentrations of CNP3 (1 and 10 µM) and incubated for 6 h. After the incubation, 10 µM and 100 µM NaOCl were added to the culture and incubated 30 min for at 37 °C. Following washing with sterile saline solution, cells were treated with 2 µl of 10 mM HCS (HSC was provided by Dr. Shu-pao Wu) dissolved in DMSO and was incubated for 30 min at 37 °C. Finally, cells were washed with sterile saline and immediately, imaged under a confocal (Carl Zeiss confocal microscope with Volocity image processing software) microscope using 20x water immersion lenses and ten different regions were randomly imaged. Image J was used to get semi-quantitative data from the confocal images.

## References

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2. T. Sakthivel, S. Das, A. Kumar, D. L. Reid, A. Gupta, D. C. Sayle and S. Seal, *ChemPlusChem*, 2013, **78**, 1446-1455.
3. J. M. Dowding, T. Dosani, A. Kumar, S. Seal and W. T. Self, *Chemical communications*, 2012, **48**, 4896-4898.

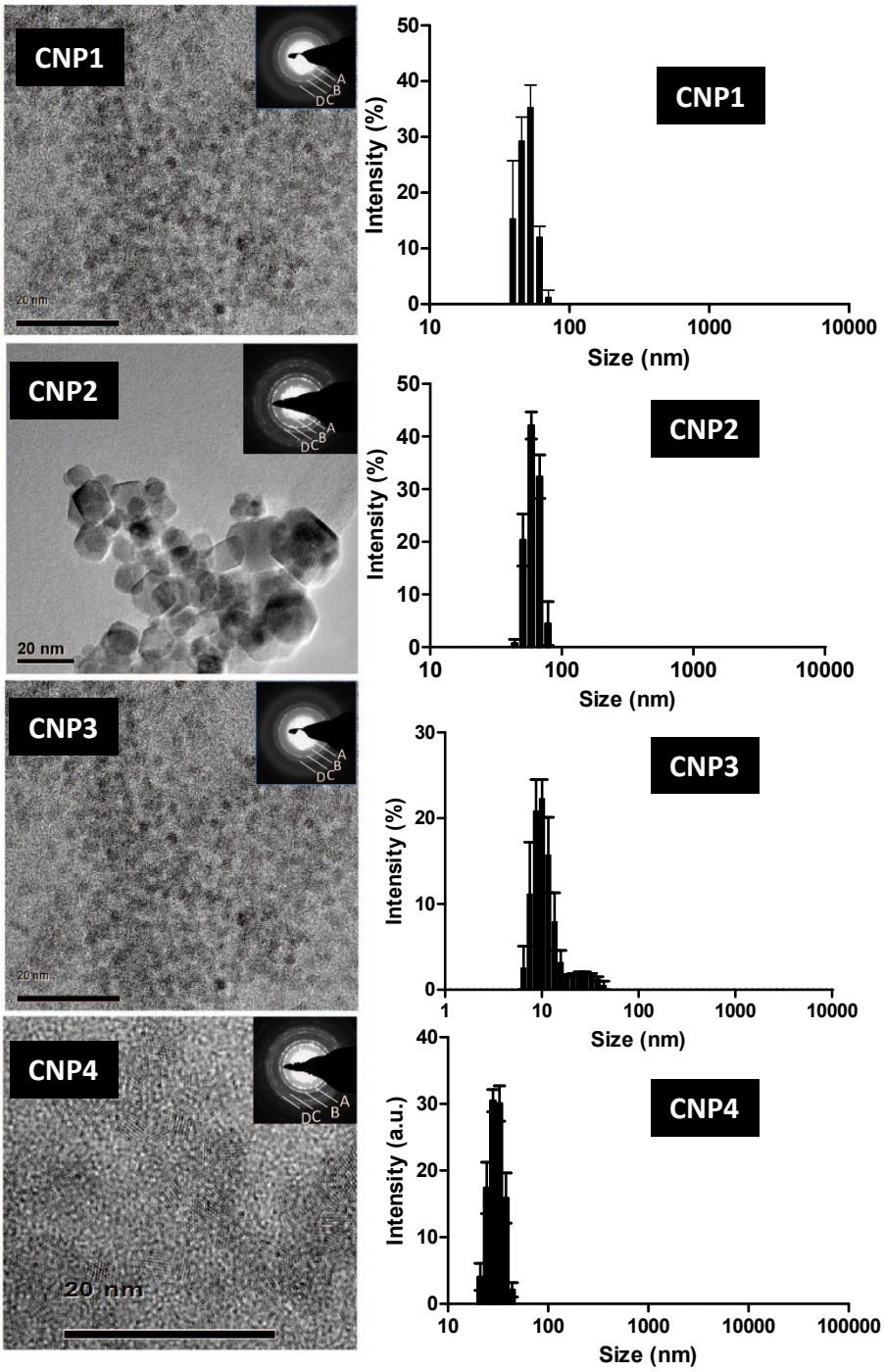


**Figure S1.** HRTEM images of CNPs show nanoparticles morphology (left panel) and the size distribution histogram using dynamic light scattering (DLS) (Left panel). The nominal sizes of the particles are as follows: CNP1 5-8 nm, CNP2 15-20 nm, CNP3 5-8 nm, CNP4 3-5 nm. Selected area electron diffraction patterns (SAED) of the particles are shown in the inset. SAED patterns confirm the crystalline nature and A(111), B(200), C(220) and D(311) are different lattice planes of CNPs fluorite crystal structure. Scale bars 20 nm. DLS data showed size of the different nanoparticles as follows: CNP1 – 50.8 ± 0.9 nm; CNP1 – 58.8 ± 0.8nm; CNP3 – 9.4 ± 2.1 nm; CNP4 – 30.8 ± 2.8 nm. The increase in the size of the nanoparticles is due to partial agglomeration of the nanoparticles.

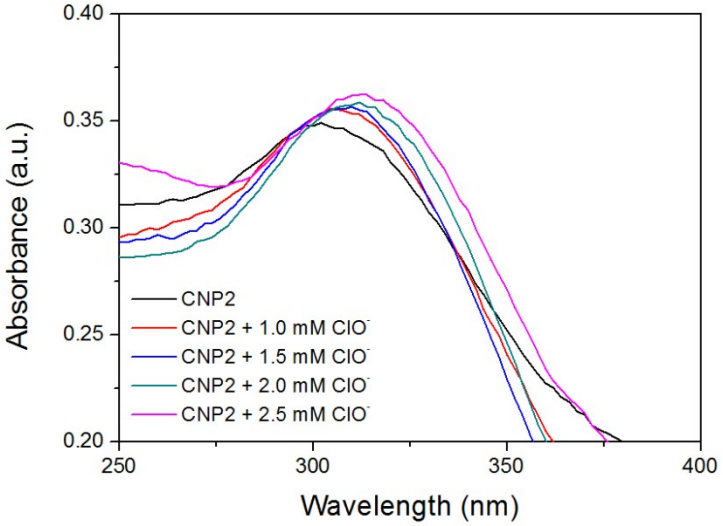


**Figure S2.** UV-vis spectra showing the interaction of CNP2 and $ClO^-$.

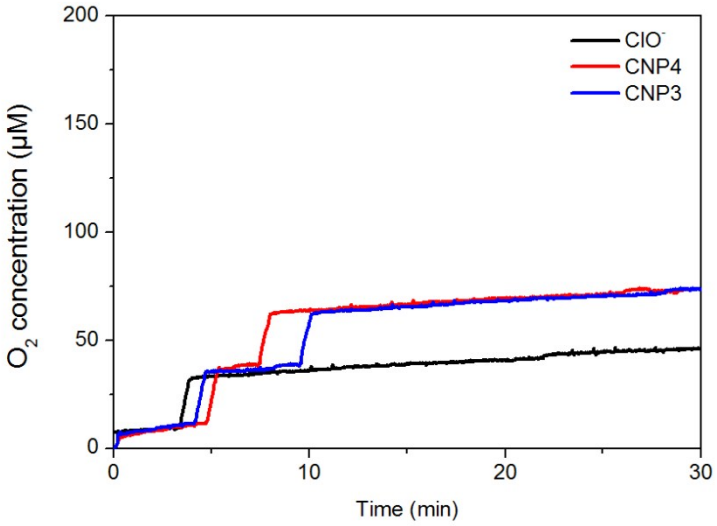


**Figure S3.** Oxygen evolution for CNP3, CNP4 and $ClO^-$ alone, indicating that there was no evidence of oxygen release when nanoparticle or $ClO^-$ alone was added.

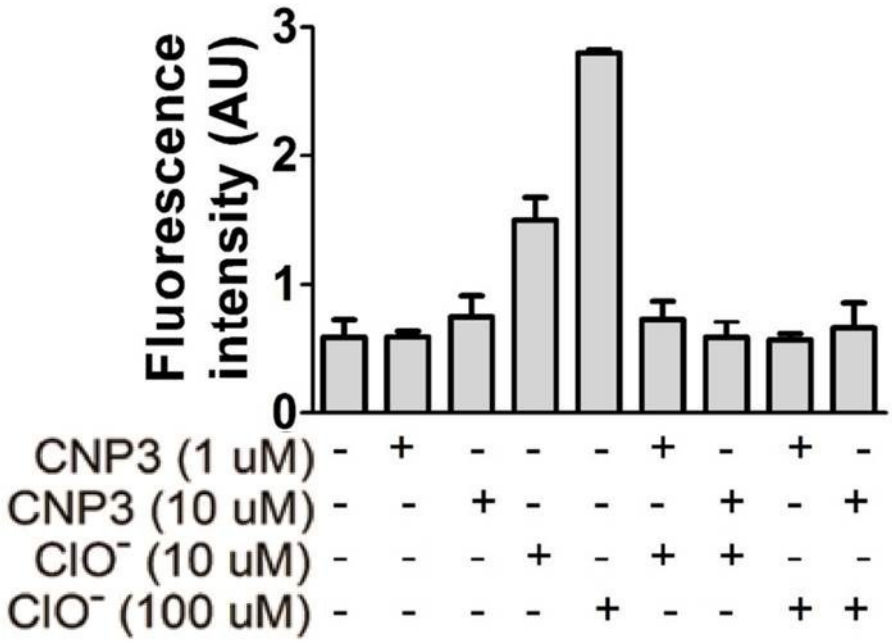


**Figure S4.** Semi-quantitative fluorescence intensity data of $ClO^-$. Data indicated $ClO^-$ in CNP3 pretreated samples were comparable to control. CNP3 itself did not induce any $ClO^-$ generation in RAW cells.