

Electronic Supplementary Information
***In vivo* target bio-imaging of Alzheimer's**
disease by fluorescent zinc oxide nanoclusters

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Materials and Methods

***In vivo* bio-imaging study**

All the chemicals used in the experiments were purchased from Sigma Company. Milli-Q water (Millipore M-Q purification system, 18.2 MΩ cm) was used in all experiments. Experimental animal mice including normal control group of mice and different month old Alzheimer's model mice. Three-month-old (AD-1) and six-month-old (AD-2) APP/PS1 male mice were bought from Beijing Xiehe animal centers and normal control group of nude male mice (NOR) were purchased from Nanjing animal centers. All animals were maintained in clean facilities with a 12-hour light/dark cycle and received water and food through a semi-barrier system. All experiments involving mice were approved by the National Institute of Biological Science and Animal Care Research Advisory Committee of Southeast University, and experiments were conducted following the guidelines of the Animal Research Ethics Board of Southeast University.

The mice were randomly assigned to groups for experiments (i.e., 4 mice per

group). The blank control group of mice (CON), normal control group of mice (NOR), three-month-old (AD-1) and six-month-old Alzheimer's model mice (AD-2) were utilized as the experimental models, where the CON was not given zinc gluconate, and the NOR, AD-1 and AD-2 were given different concentrations of zinc gluconate solution prepared in ultrapure H₂O via tail-vein injection. During experiments, these groups of mice were anesthetized first and fur of their whole bodies was removed for better imaging by using Perkin Elmer *in vivo* imaging system (IVIS Lumina XRMS Series III). For bio-imaging of the relevant diseased area in the brain location, the mice were fully anesthetized by inhalation of a mixture of oxygen with 5% isoflurane and fluorescence imaging of all the models was observed at times of post-injection for 1 H (1 hour), 6 H (6 hours), 18 H (18 hours), 24 H (24 hours), 30 H (30 hours) etc.

The *in vivo* bio-images were acquired on Perkin Elmer animal imaging system (IVIS Lumina XRMS Series III, with excitation wavelength of 420 nm or 480nm and emission wavelength of 620 nm or 670 nm). The ROI (regions of interest) analysis was measured under the assistance of Perkin Elmer Image software. These animal studies were approved by the National Institute of Biological Science and Animal Care Research Advisory Committee of Southeast University, while experiments conducted following the guidelines of the Animal Research Ethics Board of Southeast University.

Histopathologic analyses of tissues

After injection of zinc gluconate solution via tail-vein injection for 30H, the relevant organs and tissues samples were harvested from mice post-injection. Afterwards, mice were sacrificed. The brain, livers, spleens and kidneys were extracted from the control mice and experimental Alzheimer's model mice, and then fixed in a 4% paraformaldehyde solution. The organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The histological sections were observed under an optical microscope. The images were obtained by using a BX53 microscopy system (Olympus, Tokyo, Japan) that was equipped with a color CCD (DMK 41BU02, Sony Co., Tokyo, Japan).

Characterization of in situ biosynthesized zinc oxide nanoclusters

Zinc oxide nanoclusters were biosynthesized *in situ* through tail-vein injection of 0.3mL 5mmol/L zinc gluconate solution in the affected sites of Alzheimer's brain. Then the presence of biosynthesized zinc oxide nanoclusters which was extracted from AD's brain by a repetitive freeze thaw method were characterized by X-ray photoelectron spectroscopy (XPS), XPS-element mapping, fluorescence spectroscopy (FL), transmission electron microscope (TEM). Contrastively, no formation of zinc oxide nanoclusters was observed in the brain of normal control group of mice injected with the same amount zinc gluconate.

The valence state of zinc and element mapping of zinc in the biosynthesized zinc oxide nanoclusters *in vivo* in the AD's brain was investigated by a PHI Quantera II X-ray photoelectron spectrometer (XPS). Fluorescence spectroscopy (FL) was detected by using a fluorescence spectrometer (PerkinElmer, LS-55). A JEM-2100 transmission electron microscope (TEM) was used to characterize the size and size distribution of *in vivo* biosynthesized zinc oxide nanoclusters.

Data analysis

Data were expressed as the means \pm SD (standard deviation) from at least three independent experiments. One-tailed unpaired Student's t-test was used for significance testing, and $p < 0.05$ was considered significant.

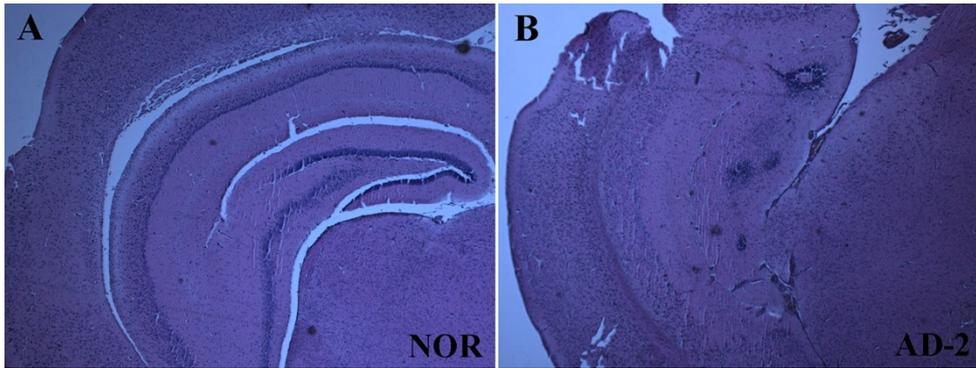


Figure S1 (A, B) Histopathologic analyses of H&E-stained brain sections of the NOR and AD-2.

In Figure S1 (A, B), histopathologic analyses of H&E-stained brain sections of the normal control group of mice (NOR) and six-month-old Alzheimer's model mice (AD-2) demonstrate that no senile plaque was observed in cerebral hippocampal sections of the NOR (Figure S1(A)). To compare, some senile plaques in different size and shapes could be clearly found to distribute in the hippocampus of the AD-2 (Figure S1(B)). Therefore, the relevant AD model mice used in this study already displayed some of the characteristic features of Alzheimer's disease.

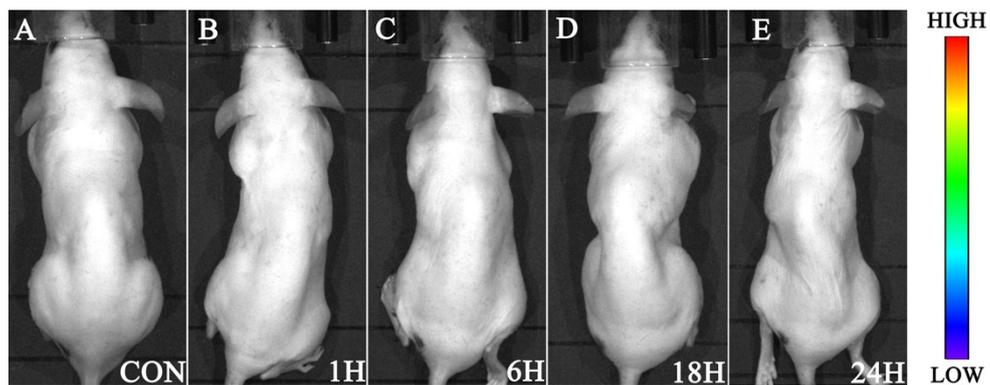


Figure S2 A: Fluorescence imaging of the blank normal control group of mice (NOR). B-E: Fluorescence imaging of the normal control group of mice at different time points via tail-vein injection of 0.3 mL 5 mmol/L zinc gluconate solution (1 H, 6 H, 18 H, 24 H). It demonstrated that there appeared almost no fluorescence for the normal control group of mice at different time points via tail-vein injection of zinc gluconate solution.

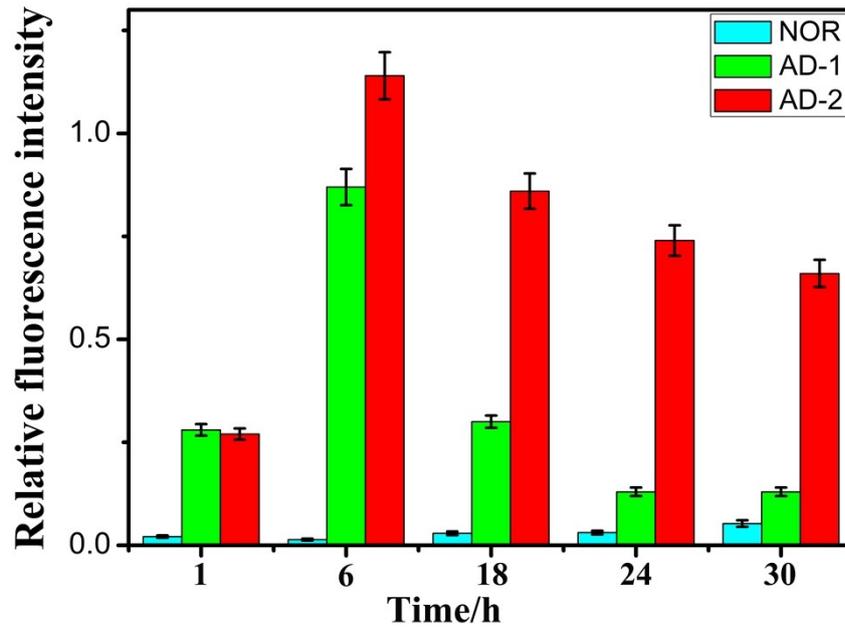


Figure S3 The variations of mean fluorescence intensity of Alzheimer's model mice (AD-1, AD-2) and normal control group of mice (NOR) at various time points (1 H, 6 H, 18 H, 24 H, 30 H) via tail-vein injection of 0.3 mL 5 mmol/L zinc gluconate solution.

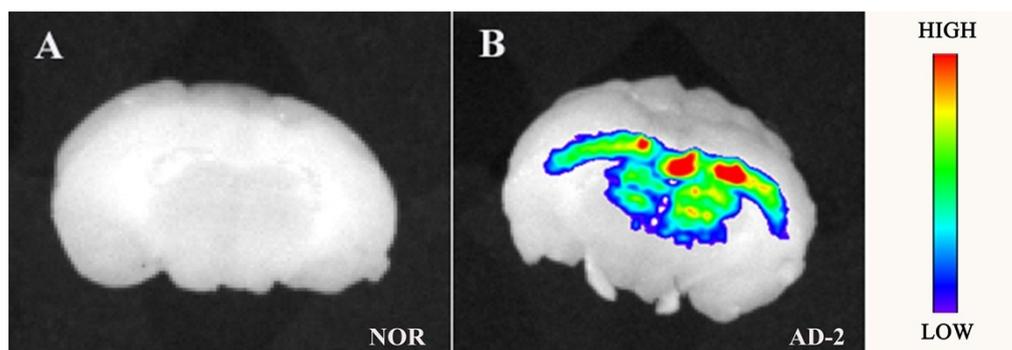


Figure S4 (A, B) Fluorescence imaging of the transverse section of the normal control mice's brain (**NOR**) and the AD-2's brain (**AD-2**) via tail-vein injection of 5 mmol/L zinc gluconate solution at 30 h post-injection which were cut into two parts near the hippocampus area. It showed that the biosynthesized ZnO NCs could realize labeling the affected hippocampus regions of the brain of Alzheimer's model mice through intravenous injection of zinc gluconate solution.

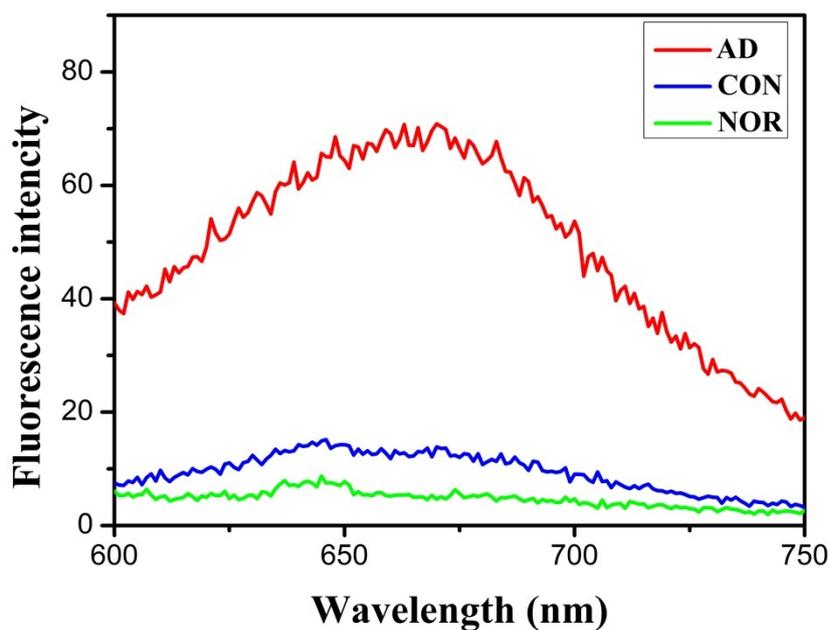


Figure S5 Emission spectra of the NOR, CON and AD's brain tissue which was grinded and then diluted by ultrapure water (Excitation wavelength: 420 nm).

As shown in Figure S5, fluorescence (FL) emission spectra of the NOR, CON and AD's brain tissue which was grinded and then diluted by ultrapure water immediately before these spectroscopic studies, demonstrating stronger fluorescence in AD's than that in the NOR and CON. It is noted that the AD's has a strong FL emission peak at ca. 669 nm, while that of the CON has a much lower FL emission peak at ca. 650 nm and the NOR's has a quite weaker fluorescence emission peak at ca. 645 nm when they were detected in the same concentration and under the identical experimental conditions, respectively.

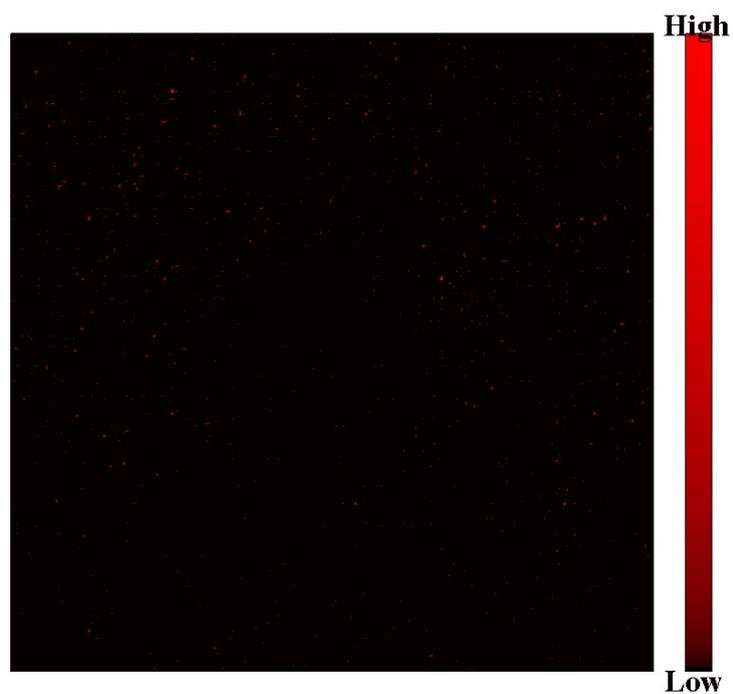


Figure S6 XPS-element mapping of the brain tissues of the blank control group of AD mice (CON) without injection of 5 mmol/L zinc gluconate solution, indicating that there was very few ZnO NCs found in the CON's brain areas compared with that of AD's brain tissues (AD) shown in Figure 4(K).

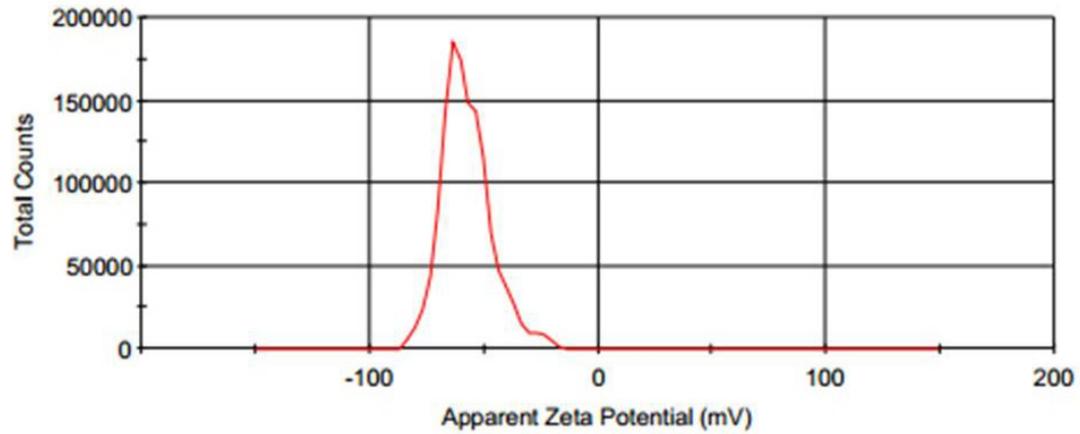


Figure S7 The mean zeta potential of the biosynthesized Zn NCs in the brain of AD mice is -57.5 mv via tail-vein injection of 0.3 mL 5 mmol/L zinc gluconate solution. It illustrates that the biosynthesized ZnO NCs is stable and the surface of the ZnO NCs is packaged with the negatively charged proteins.

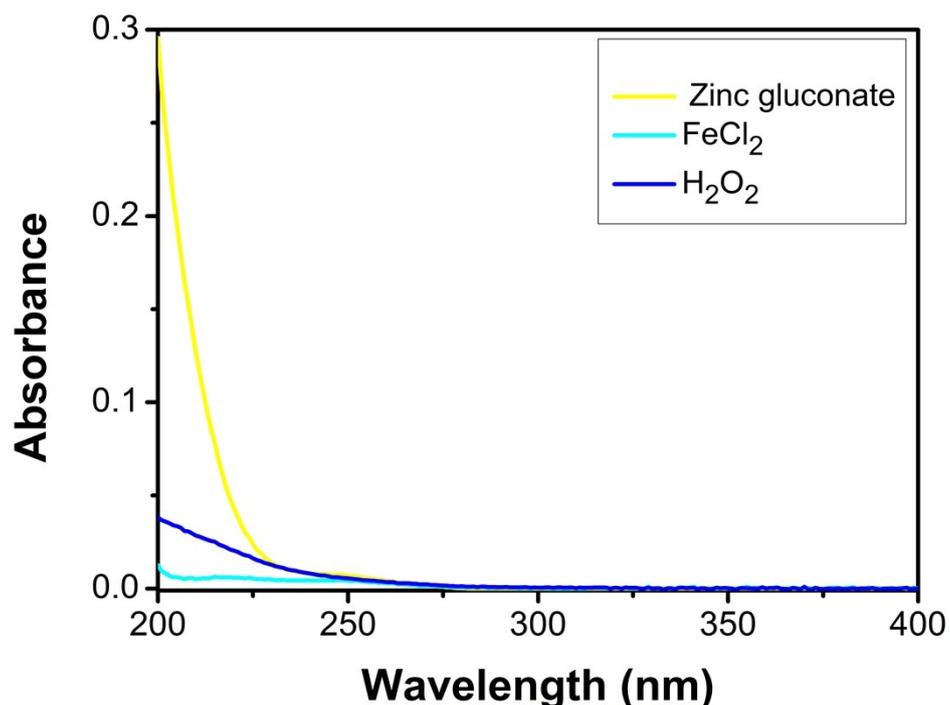


Figure S8 UV-Vis absorption spectrum of 0.5 mmol/L zinc gluconate solution (**yellow curve**), 0.15 mmol/L FeCl₂ solution (**cyan curve**) and the 0.2% H₂O₂ solution (**blue curve**) respectively. The very weak ultraviolet absorption peak of zinc gluconate solution is at 250 nm. And both FeCl₂ solution and H₂O₂ solution have almost no ultraviolet-visible absorption.

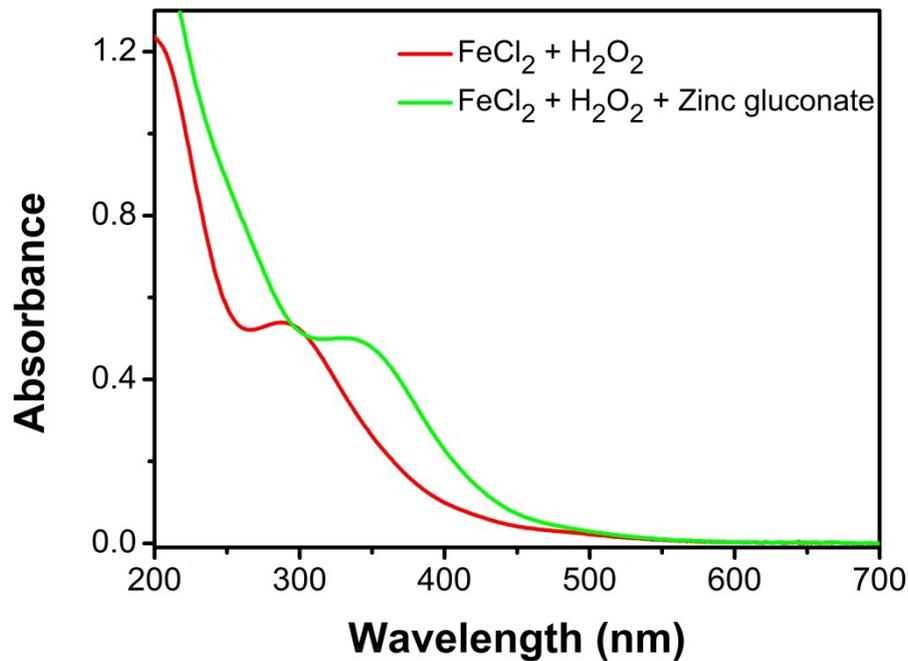


Figure S9 UV-Vis absorption spectrum of the mixture of 0.3 mmol/L FeCl₂ solution and 0.2% H₂O₂ solution when the H₂O₂ solution was just added to FeCl₂ solution (**red curve**). As we can see, there appeared a new peak at about 290 nm of the newly generated Fe³⁺. UV-Vis absorption spectrum of the mixture of 0.3 mmol/L FeCl₂ solution, 0.2% H₂O₂ solution and 0.15 mmol/L zinc gluconate solution when zinc gluconate solution was just added to the last mixture of FeCl₂ solution and H₂O₂ solution (**green curve**). Then there appeared another new peak at about 350 nm of the new product ZnO NCs when zinc gluconate solution was just added to the mixture of H₂O₂ solution and FeCl₂ solution.

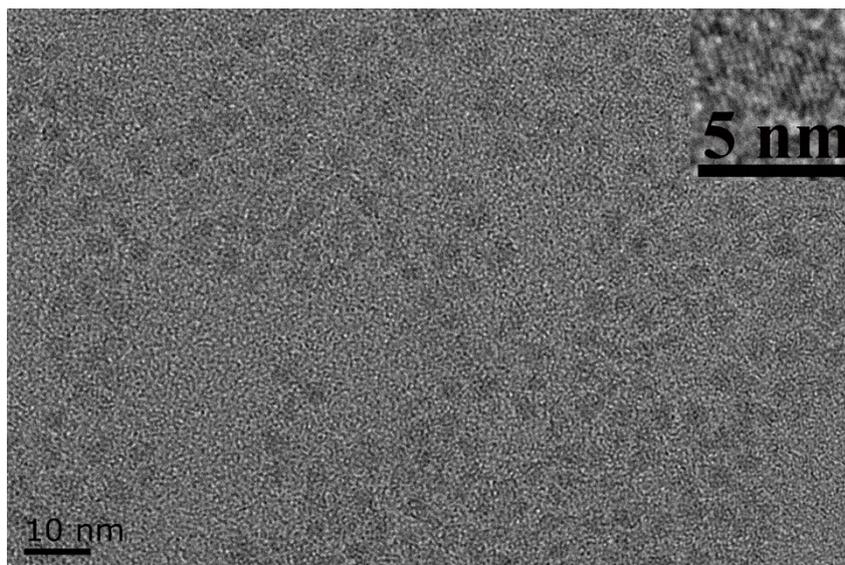


Figure S10 Typical TEM image of the resulting ZnO NCs in the reaction of the mixture of 0.3 mmol/L FeCl_2 solution, 0.2% H_2O_2 solution and 0.15 mmol/L zinc gluconate solution as showed in Figure S9, showing that the ZnO nanoparticles are nearly spherical with no noticeable trend to aggregate. In Figure S10 (**the inset**), the HRTEM image indicates that the ZnO nanoclusters kept their interplanar spacing of ~ 0.19 nm, so the ZnO crystal nucleus grew along the direction of the 102 crystal surface [1,2].

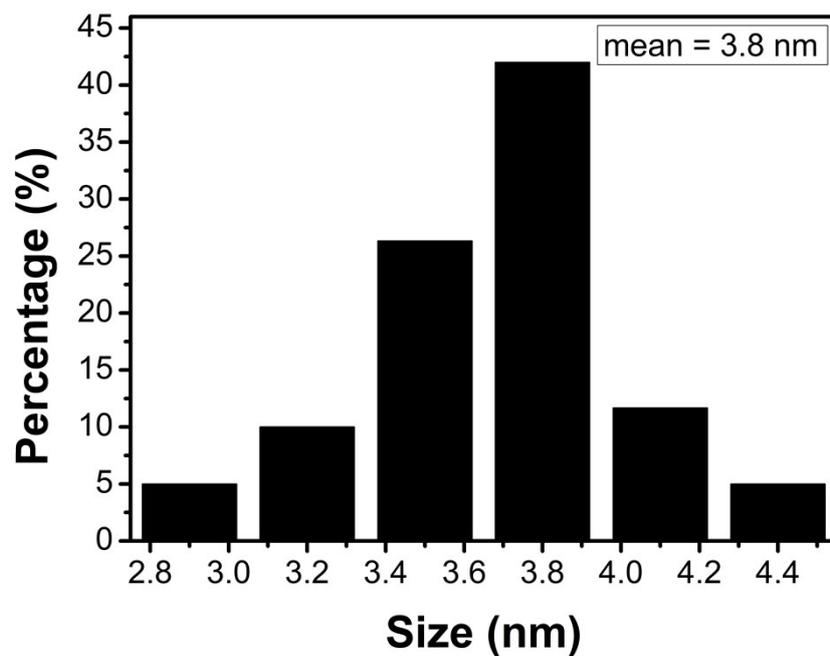


Figure S11 The size distribution of resulting ZnO NCs in Figure S7 showing that 98% of the ZnO NCs ranged between 2.9 to 4.4 nm in diameter with a distribution peak at ca. 3.8 nm.

References

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- 2 J. Antony, X. B. Chen, J. Morrison, L. Bergman, Y. Qiang, D. E. Mccready and M. H. Engelhard, *Appl. Phys. Lett.*, 2005, **87**, 241917.