Electronic Supplementary Material (ESI) for Nanoscale
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# Electronic Supplementary Information

# Visible Light Powered Self-Disinfecting Coatings for Influenza Viruses

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## 1. Materials

Washed pooled chicken red blood cells (10% in Alsever's solution) were purchased from Lampire Biological Laboratories. Glass cover slips were purchased from Ted Pella, Inc. P25 $TiO_2$ nanoparticles were purchased from Evonik Degussa GmbH. All other chemicals were purchased from Sigma-Aldrich unless otherwise noted and were used as received.

## 2. Instruments

Solar Cell & Module Test Equipment from PV Measurements, INC. was utilized as Solar light simulator with a UV filter (HOYA HMC 58mm UV(0)) to block UV light, which light intensity is 53 mW/cm$^2$ without UV filter and 51 mW/cm$^2$ with UV filter measuring by a Gigahertz-Optik X97 Irradiance Meter. UV-Visible spectra were acquired with a GeneSys 6 spectrometer (Thermo Scientific). Fluorescence spectra were obtained with a QuantaMaster Spectrofluorimeter (Photon Technology International). Zeta potential and particle size distribution were measured with a Malvern particle sizer Nano-ZS. SEM-EDS were acquired with JSM-6701F Scanning Electron Microscope (JOEL) and the Sapphire Si(Li) Detecting Unit (EDAX). Fluorescent images of cells were obtained with a Leica DM IRB microscope. Transmission electron microscope images were observed with a PHILIPS CM120 TEM. Atomic force microscope data were acquired with a Veeco Multimode Atomic Force Microscope.

## 3. Methods

### 3.1 Disinfection of influenza A virus by $TiO_2$ (P25) with UV irradiation as reference control:

Glass substrates were drop coated by P25 $TiO_2$ nanoparticles with the surface concentration of 5 ug/mm$^2$ and dried under room temperature. Then 300 uL of A/PR/8/34 H1N1 influenza virus (PR8) with the original titer of $6.81\times10^5$ $TCID_{50}$/mL (50% Tissue Culture Infectious Dose per mL) was added onto the slides and exposed to the light source with same light intensity as previous experiments for 1 hr without UV filter applied for the light group while dark group was wrapped by aluminum foil. The remaining infectivity of PR8 was plotted in Figure S2. As UV lights were not blocked, the virus only group in light condition already lost 63% of their infectivity due to UV lights irradiation, while the $TiO_2$ treated group lost total 92% of their infectivity. By deducting the donation from directly exposing to UV lights, here we could make the conclusion that P25 $TiO_2$ could disinfect only 29% of PR8's infectivity.

### 3.2 Photo inactivation of influenza A virus envelope proteins.

For hemagglutinin (HA) assay, washed pooled chicken red blood cells (10% in Alsever's solution, Lampire Biological Laboratories) were diluted to 1% using PBS buffer solution. After 2 hr treatment by 20 ug/mm$^2$ of CIZS coated slips, 100 uL PR8 solution, which titer was $3.57\times10^7$ $TCID_{50}$/mL on MDCK cell line, was added into the first column of a Corning V-bottom 96-well plate, which was 2-fold serially diluted across the 96 well plate with PBS. The final 50uL from each well of the last column was disposed. Then 50uL freshly PBS-diluted chicken red blood cells (1%) was added to each well and mixed by gently tapping the plate. The plate was incubated for 30 min at 4 °C and HA Unit was observed directly from end point of agglutination phenomena.

Neuraminidase (NA) assays were carried out in a 48-well plate. CIZS-coated slips were immersed in 250 uL neuraminidase solution (10 UN/mL, Type V, Clostridium perfringens) on ice and illuminated for 1hr. Then 200 uL solution was taken out and mixed with 80 uL of 5 mM 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate aqueous solution, 550 uL of 100 mM sodium acetate buffer with 2 mM calcium chloride (pH 5.0), and incubated at 37°C

with vigorous stirring. After 30 min, 170 uL of 200 mM glycine buffer (pH 10.7) was added and the fluorescence signal from 400 to 500 nm was collected using a QuantaMaster Spectrofluorimeter (Photon Technology International) with an exciting wavelength of 365 nm. The emission intensity at 450 nm was selected to compare the activity of neuraminidase.

**3.3 Photo inactivation of trypsin:**

First of all, CIZS nanocrystals were prepared to be sustainable in aqueous solution by ligand exchange. Typically, 1 g of cysteamine was dispersed in 5 mL water and 1 mL ethanol, then 200 uL as-prepared CIZS solution was added and vigorous stir was applied. The vial was covered by aluminum foil to prevent illumination. After 12 hours, the solution was mixed with 20 mL ethanol, centrifuged and the solid was re-dispersed in 4mL of water. Typical zeta potential was around 40 mV and the yield was about 90%.

For trypsin inactivation, 2 mg of trypsin, 0.4 mg of CIZS and 2 mL of 50 mM pH 8 Tris-HCl buffer were mixed and stirred vigorously at 0°C. 30 uL of sample solution was taken out every hour and diluted to 195uL using the same buffer. After adding 5 uL of 50 mM N-benzoyl-DL-arginine 4-nitroanilide dimethylsulphoxide solution, adsorption at wavelength 410 nm was selected to quantify the degradation kinetics, which was summarized in Figure 4C. It clearly showed that with visible light exposure, trypsin's activity dropped significantly to 68%, 37%, 21% and 15% after 1, 2, 3 and 4 hr treatment, respectively, while no significant difference was observed in dark controls.

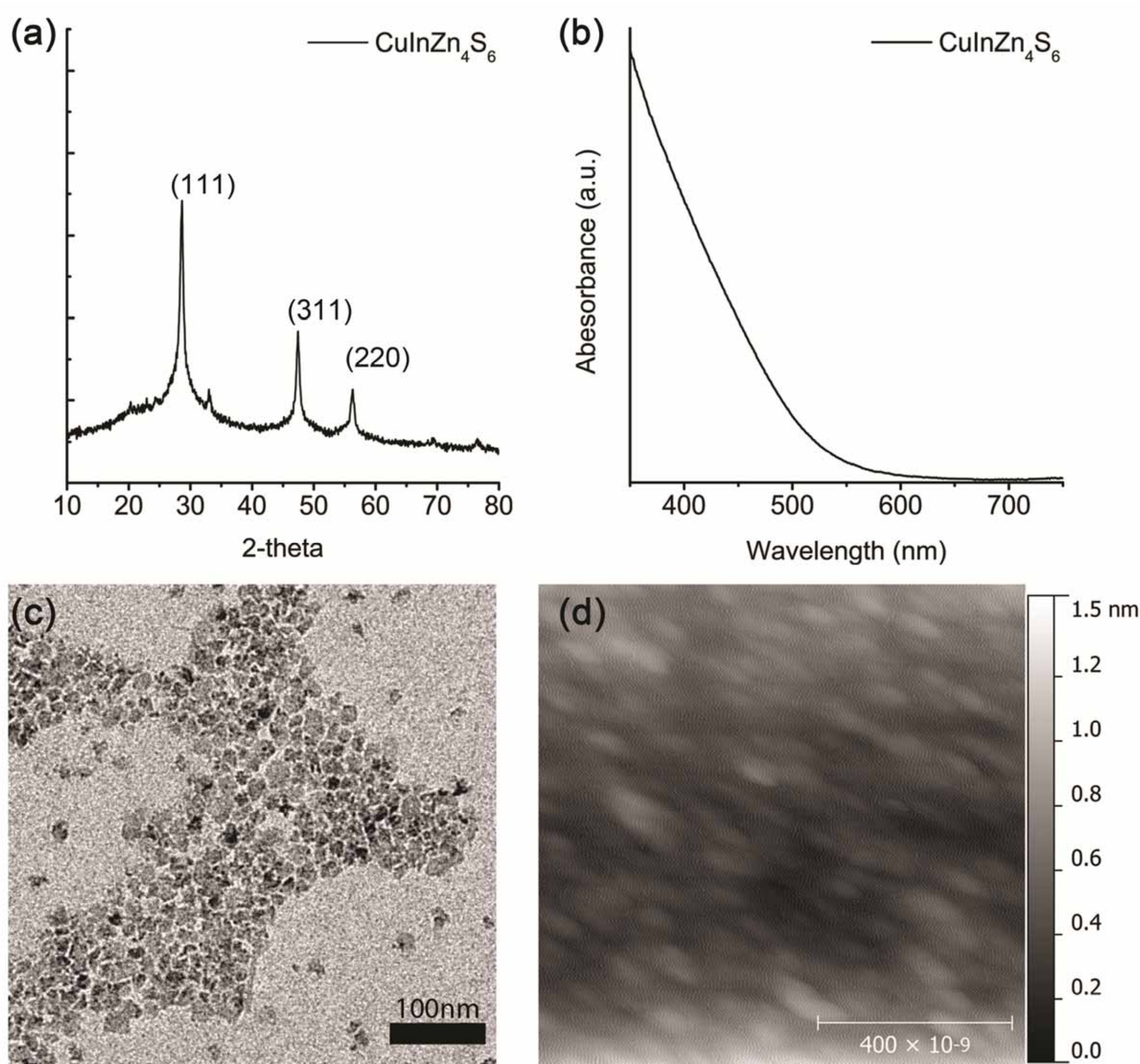


**Figure S1** (a) XRD pattern of as-prepared $CuInZn_4S_6$ nanoparticles, which suggested ~20 nm crystal size calculated from Debye-Sherre Equation. (b) Absorbtion spectrum of as-prepared $CuInZn_4S_6$ nanoparticles. (c) TEM image of NCs shows a particle size around 20 nm by direct observation. (d) AFM image of the NC-coated surface suggest the formation of uniformed self-disinfecting coatings.

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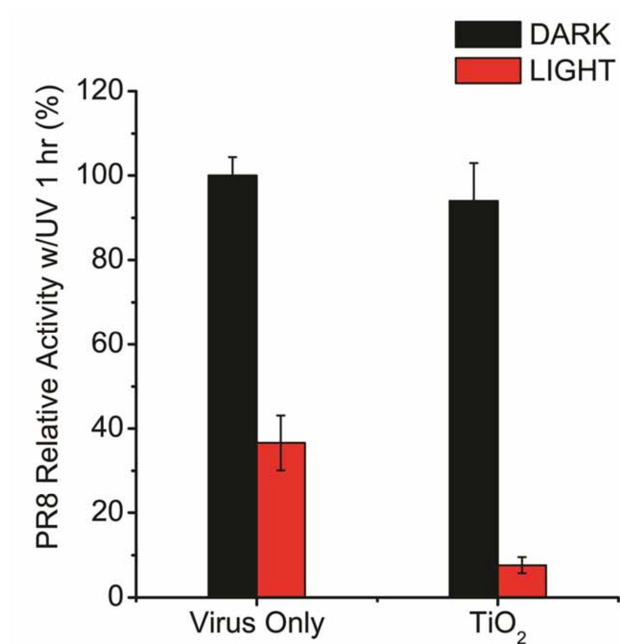


**Figure S2** Disinfection efficiency of P25-coated surfaces with and without illumination for 1 hr. UV lights were not blocked, thus $TiO_2$ just contribute 29% of total disinfection on PR8.