Supporting Information

Fabrication of activatable hybrid persistent luminescence nanoprobe for background-free bioimaging-guided investigation of food-borne aflatoxin in vivo

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Chemicals and Materials

All chemicals used were at least analytical grade. Ultrapure water (18.2 MΩ cm) obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO) was used throughout. Sodium phosphate dibasic anhydrous (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), 3-Aminopropyltriethoxysilane (APTES, >98%), Tetraethyl ortosilicate (TEOS, >98%), Ascorbic Acid (AA), $Zn(NO_3)_2 \cdot 6H_2O$ (99.99%), $Ga(NO_3)_3 \cdot 9H_2O$ (99.99%), copper nitrate, sodium sulfide, sodium citrate, GeO_2 (99.999%), $Cr(NO_3)_3 \cdot 9H_2O$ (99.99%), $Yb(NO_3)_3 \cdot 5H_2O$ (99.9%), $Er(NO_3)_3 \cdot 5H_2O$ (99.9%) were all purchased from Aladdin (Shanghai, China). Cetyltrimethyl ammonium bromide (CTAB) was purchased from Sinopharm Chemical Reagent (Beijing, China). Polyethyleneimine (PEI, branched, M.W. 10,000, 99%) was from Alfa Aesar (Tianjin, China). Chloroauric acid ($HAuCl_4 \cdot xH_2O$) and nitrite silver ($AgNO_3$) were from Aldrich (Steinheim, Germany). Zearalenone (ZEN), aflatoxin B1 (AFT B1), aflatoxin B2 (AFT B2), aflatoxin M1 (AFT M1), microcystin-leucine-arginine (MC-LR), ochratoxin A (OTA), and vomitoxin (DON) were all purchased from Sigma-Aldrich (USA). All the biomolecules, including the proteins and enzymes, were from Newprobe Biotechnology Co. Ltd. (Beijing, China). Toluene, Oleic acid, ammonium hydroxide, sodium hydroxide and ethanol were all obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). All the glassware was cleaned with aqua regia ($HCl:HNO_3$ = 3:1, v/v) and thoroughly rinsed with ultrapure water before use.

Oligonucleotides information: the 5'-carboxyl-modified AFT B1 binding aptamer (5'-carboxyl -GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCCACA-3') and

the 5'-thiol-modified ssDNA with partial-complementary sequence for AFT B1 aptamer (5'-thiol-TTTTGTGGGCCTAGCGA-3') were obtained from Shanghai Sangon Biotech Co (Shanghai, China).

Instrumentation

Photoluminescence spectra and the persistent luminescence decay curves of the nanoprobes were recorded on an F-4500 spectrofluorometer (Hitachi, Japan). The phosphorescence signals were detected using the Hamamatsu PMT detectors (R928 and R5509-72). All the measurements were carried out at room temperature with the following conditions: photomultiplier tube (PMT) voltage, 700 V; excitation and emission slits, 10 nm; excitation wavelength, 324 nm; quartz cell (1 cm × 1 cm). The ZGGO powder was pre-excited with a 324-nm UV lamp for 5 min before recording the persistent luminescence decay curve.

In vivo fluorescence images of the mice were obtained with a NightOWL LB 983 *in vivo* Imaging System (Berthold, Bad Wildbad, Germany). The excitation filter was set as 530 nm, and the emission filter was set as 700 nm. Phosphorescence images were recorded by the CCD camera with constant exposure time. *In vitro* cytotoxicity of the probe was assessed using the cell counting assay, and cell numbers were counted with a Coulter Particle Size Analyzer (Beckman Coulter, High Wycombe, UK).

XRD patterns were acquired on a D/max-2500 diffractometer (Rigaku, Japan) equipped with Cu Kα radiation (λ = 1.5418 Å). The morphology and microstructure of the prepared nanoparticles was characterized by high-resolution transmission electron micrograph (HRTEM) on a JEM-2100F field emission transmission electron microscope (JEOL, Japan) operating at a 200 kV accelerating voltage. The samples were obtained by drying sample

droplets on a 300-mesh Cu grid coated with a lacey carbon film. The elemental analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500CX).

Evaluation of the PLNPs-CuS Toxicity

In vitro cytotoxicity of the PLNPs-CuS hybrid nanoprobes were measured by the methyl thiazolyl tetrazolium (MTT) assay. Mouse embryo fibroblast cell lines (Balb/3T3), Human hepatocellular liver carcinoma cell line (HepG2), and human breast carcinoma cell lines (MCF-7) were obtained from China Center for Type Culture Collection (Wuhan, China). The three cell lines were plated at a density of 4×10^4 cells per well in 96-well plate, respectively, and grown for 24 h at 37°C in 5% CO_2. The nanoprobes dispersed in 10 mM PBS solution with a wide concentration range from 50 to 1000 μg mL^{-1} were subsequently added into the cell and incubated for another 24 h under the same conditions as above. Thereafter, MTT solution (10 μL, 3 mg mL^{-1}) was added to each well, and the cells were incubated for another 4 h. After the addition of dimethyl sulfoxide (150 μL per well), the assay plate was allowed to stand at room temperature for 10 min. A Tecan Infinite M200 monochromator-based multifunction microplate reader was used to measure the OD570 (*Abs.* value) of each well with background subtraction at 690 nm. The viability of cell growth (CV) was calculated as the ratio of the treatment group absorption value to the control group absorption value. *In vivo* toxicity of the PLNPs-CuS nanoprobes were evaluated by monitoring the body weight change of the nude mice (normal and tumor-bearing) injected with the PLNPs-CuS nanoprobes (0.4 mL, 1.0 mg mL^{-1}).

Histopathology

The histological changes in the main organs of mice injected with PLNPs-CuS nanoprobes

(0.4 mL, 1.0 mg mL^{-1}) were observed after 7 days of injection. The selected organs (heart, liver, spleen, lung and kidney) were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 μm thick), and stained with hematoxylin and eosin (H&E). The histological sections were observed under an optical microscope.

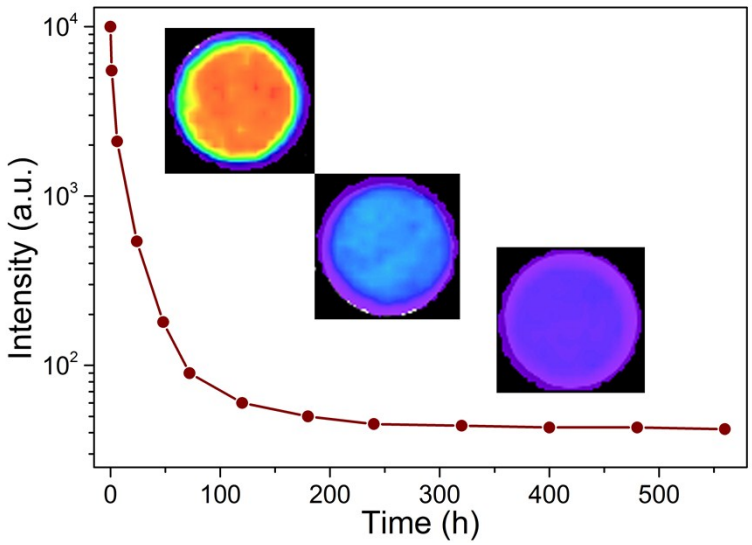

Fig. S1 The long afterglow property of as-prepared PLNPs.

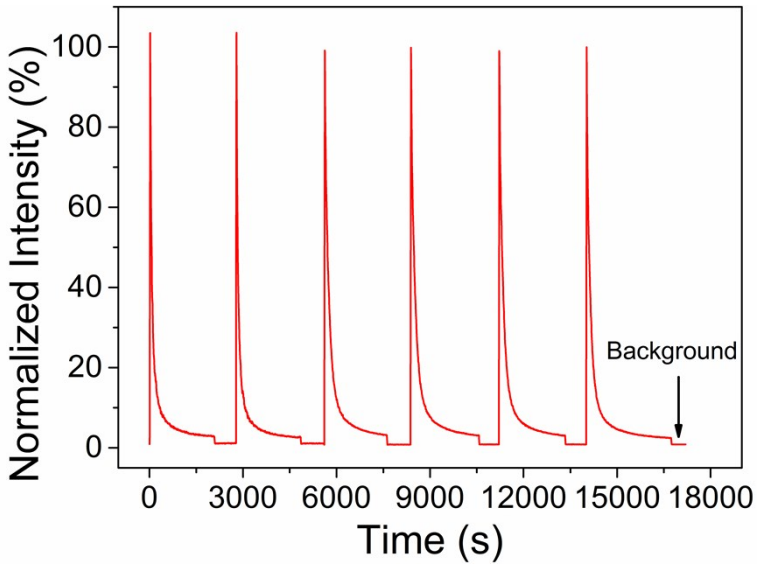

Fig. S2 Repeated decay curves of ZGGO monitored at 700 nm after re-activated with a red LED light (650 ± 10 nm) for 200 s; Sample mass = 100 mg.

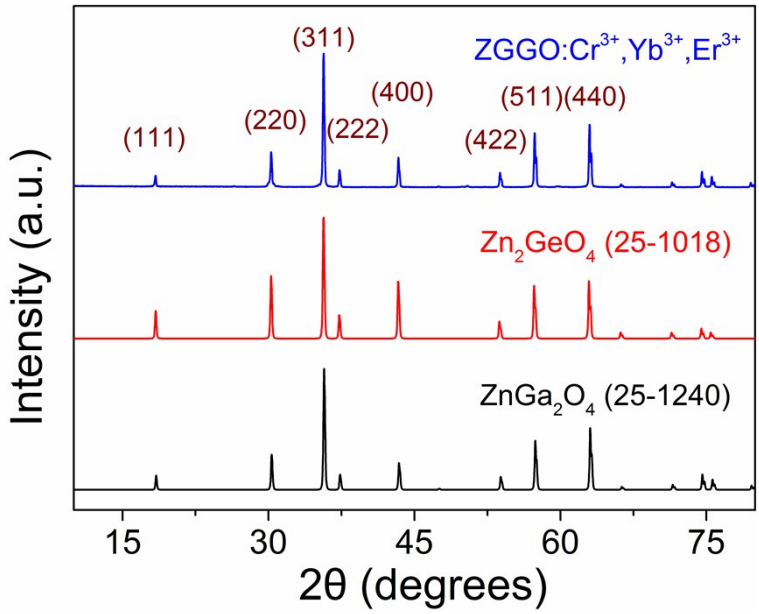

Fig. S3 XRD patterns of the ZGGO powder compared with the reported Zn_2GeO_4 phase of PDF = 25-1018 and $ZnGa_2O_4$ phase of PDF = 25-1240.

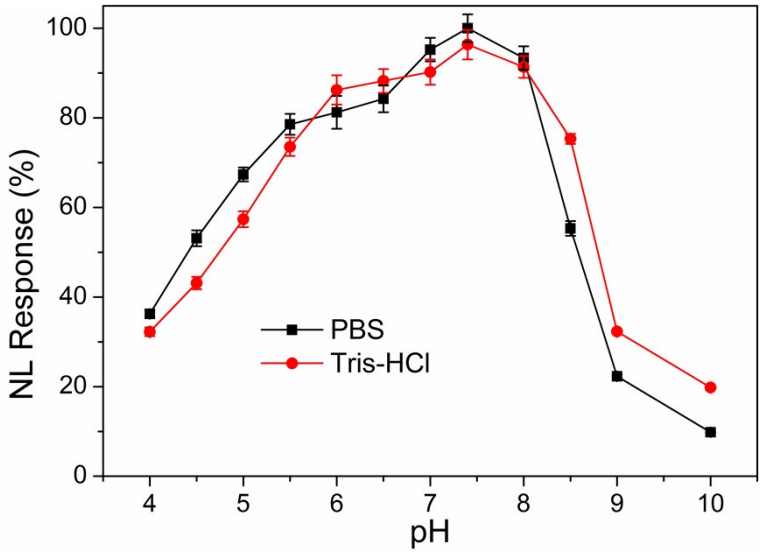

Fig. S4 The pH effect to the PLNPs-CuS response to AFT B1.