Electronic Supplementary Material (ESI) for Nanoscale.

Mesoporous Persistent Nanophosphors for In vivo Optical Bioimaging and Drug-delivery

*Thomas Maldiney,[a] Benoit Ballet,[a] Michel Bessodes,[a] Daniel Scherman,[a] Cyrille Richard,[a,]**

EXPERIMENTAL PROCEDURES.

Chemicals. Doxorubicin hydrochloride (>98%), Cetyltrimethylammonium bromide (>98%) and Tetraethyl ortosilicate (TEOS, >98%) were obtained from Sigma-Aldrich. Zinc nitrate hexahydrate (>99%) was purchased from Fluka. Gallium oxide (99.999%) and chromium (III) nitrate nonahydrate (99.99%) were purchased from Alfa Aesar. Dimethylformamide (>99.9%) was purchased from SDS.

Preparation of ZGO@SiO_2 nanoparticles. $ZnGa_{1,995}Cr_{0.005}O_4$ (ZGO) nanoparticles were synthesized by a hydrothermal method and low-temperature sintering in air. First, gallium nitrate was formed by reacting 8.94 mmol of gallium oxide with 10 mL concentrated nitric acid (35 wt%) under hydrothermal condition at 150°C overnight. Then, a mixture of 0.04 mmol of chromium nitrate and 8.97 mmol of zinc nitrate in 10 mL of water was added to the previous solution of gallium nitrate under vigorous stirring. The resulting solution was adjusted to pH 7.5 with an ammonia solution (30 wt%), stirred for 3 hours at room temperature, and transferred into a 25 mL Teflon-lined stainless steel autoclave for 24h heat treatment at 120°C. The resulting compound was washed several times with water and ethanol before drying at 60°C for 2 hours. The dry white powder was finally sintered in air at 750°C for 5 hours.

Hydroxylation was performed by basic wet grinding of the powder (500 mg) for 15 minutes, with a mortar and pestle in 50 mL of 5 mM NaOH solution, and overnight vigorous stirring of the resulting suspension at room temperature. Nanoparticles with a diameter of 85 nm were selected from the whole polydisperse colloidal suspension by centrifugation on a SANYO MSE Mistral 1000 at 4500 rpm for 5 minutes. They were located in the supernatant (assessed by Dynamic Light Scattering). The supernatants were gathered and concentrated to a final 5 mg/mL suspension.

The core-shell structured ZGO@SiO_2 nanoparticles were elaborated via a modified Stöber sol-gel process followed by a hydrothermal treatment. In a typical procedure, 20 mg of ZGO nanoparticles and 80 mg of CTAB were dispersed in 20 mL of 5 mM NaOH solution under ultrasonication and placed under magnetic stirring at 45°C. The silica shell formation was initiated by adding 0.6 mL of a 10 % v/v solution of TEOS in methanol dropwise to the ZGO suspension. After 90 minutes, 0.6 mL of the same 10 % v/v solution of TEOS in methanol was further added to the reaction mixture. The suspension was stirred for an additional 90 minutes, and transferred into a 25 mL Teflon-lined stainless steel autoclave for 24h heat treatment at 120°C. The resulting compound was washed several times with water and ethanol. The structure-directing agent (CTAB) was subsequently removed by several extraction steps in a 1% NaCl/methanol solution. Briefly, 10 mg of the above CTAB-containing product were dispersed in 10 mL of a 1% NaCl/methanol solution and stirred for 3 hours at room temperature. The extraction was repeated four times, until the template was completely removed.

Nanoparticles characterization. Persistent luminescence decay curves were recorded after 2 minutes excitation under either a UV light (6W mercury discharge 254 nm lamp) or an orange/red LEDs source (Bridgelux). Signal acquisition was carried out using a photon-counting system based on a cooled GaAs intensified charge-coupled device (ICCD) camera (Photon-Imager, Biospace, Paris, France).

ZGO@SiO_2 nanoparticles were characterized using transmission electron microscopy (JEOL JEM-100S), dynamic light scattering and zeta potential measurements in 20 mM NaCl, performed on a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA) equipped with a 632.8 nm helium neon laser and 5-mW power, with a detection angle at 173° (non-invasive backscattering).

The N_2 adsorption/desorption isotherms were obtained on a BELSORP-max gas adsorption instrument. The cryogenic temperature of 77 K was controlled by liquid nitrogen. The initial outgassing process for the sample was carried out under a high vacuum (less than 10^{-6} mbar) at 120°C for 15 hours. The degassed sample and sample tube were weighed precisely and transferred to the analyzer. The pore size distribution was calculated from the adsorption branch of the N_2 adsorption/desorption isotherm and the Barret-Joyner-Halenda (BJH) method. The Brunauer-Emmett-Teller (BET) surface areas were determined using the data between 0.05 and 0.375 just before the capillary condensation.

Drug storage/release study. Doxorubicin was used as a model drug to assess the drug storage and controlled release behavior of ZGO@SiO_2 nanoparticles. Typically, 1 mg of the ZGO@SiO_2 nanoparticles were dispersed in 1 mL of doxorubicin solution in PBS (1 mg/mL). The mixture was stirred for 24 h at room temperature to reach the equilibrium state. The ZGO@SiO_2-Dox nanoparticles were then centrifuged and washed several times with PBS. The amount of doxorubicin adsorbed in ZGO@SiO_2 nanoparticles was determined by measuring the absorbance of the doxorubicin solution at 480 nm on a UV-vis spectrophotometer before and after the adsorption step in PBS.

For the in vitro drug release study, 1 mg of ZGO@SiO_2-Dox nanoparticles was dispersed into 0.5 mL of PBS. The colloidal solution was transferred into a dialysis bag (cut off molecular weight 10 kDa), then the bag was immersed into 50 mL of either PBS (pH = 7.4) or slightly acidic aqueous solution (pH = 5) at room temperature with magnetic stirring. An amount of 1 mL of solution was withdrawn at a given time interval for the reading at 480 nm, and put back into the dialysis solution.

Cell Culture. Human glioblastoma-astrocytoma, epithelial-like cell line, referred to as U87MG cells, were purchased from the ATCC and cultured in Dubecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum and antibiotics (100 µg/ml penicillin and 100 U/ml streptomycin). Cells were grown at 37°C in 5% CO_2 humidified atmosphere.

Cytotoxicity assay. U87MG cells were seeded in flat bottom 96-well at a density of 10^5 cells/well and grown overnight in 100 µL medium. For the MTT assay, either doxorubicin alone, ZGO@SiO_2 or ZGO@SiO_2-Dox nanoparticles were incubated at different concentrations for 24 hours at 37°C in DMEM culture medium. At the end of the incubation period, MTT solution (20 µL, diluted in culture medium to a final concentration of 0.5 mg/mL) was added. After incubation at 37°C in the dark for 2.5 h, 100 µL of acidified lysis buffer containing isopropanol was added to each well, and the absorbance was monitored with a microplate reader at a wavelength of 562 nm.

In vivo systemic injections. Five weeks old female BALB/c mice (Janvier, Le Genest St. Isle, France) were anesthetized by i.p. injection of a mixture of ketamine (85.8 mg/kg, Centravet, Plancoët, France) and xylazine (3.1 mg/kg, Bayer, Leverkusen, Germany) diluted in 150 mM NaCl. Systemic injections of

10^{13} ZGO@SiO_2-Dox nanoparticles, dispersed in 5% sterile glucose solution were then realized to perform imaging studies.

Imaging. Signal acquisition was carried out using a photon-counting system based on a cooled GaAs intensified charge-coupled device (ICCD) camera (Photon-Imager, Biospace, Paris, France). The ICCD aperture time was set to three minutes. A suspension of 2 mg of ZGO@SiO_2 nanoparticles in 5% glucose sterile solution, which corresponds to approximately 10^{13} nanoparticles, was first excited *ex vivo* for 2 minutes under UV light (6W mercury discharge 254 nm lamp) before the injection to mice via the caudal vein. Animals were then placed on their back under the photon-counting device, and the signal acquisitions were performed. After a 24 hours period and complete persistent luminescence extinction, the orange/red LEDs source was shined on the animal for two minutes to re-activate the persistent luminescence from ZGO nanoparticles, and the signal acquisition was resumed. Semi-quantization was achieved through the use of Biospace developed software, PhotoVision+. Experiments were conducted in agreement with a regional ethic committee for animal experimentation.

SUPPORTING INFORMATION FIGURES.

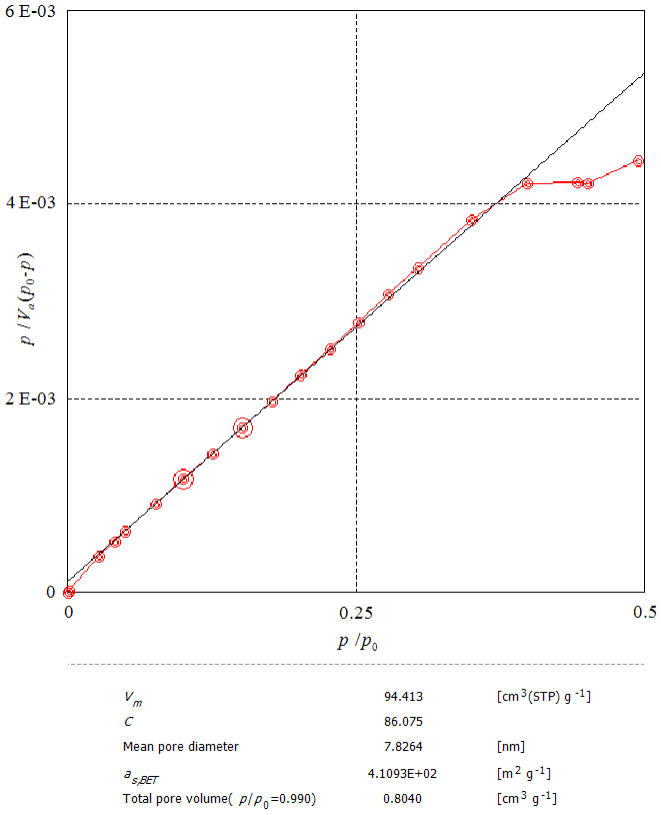

Figure S1. BET plot curve.

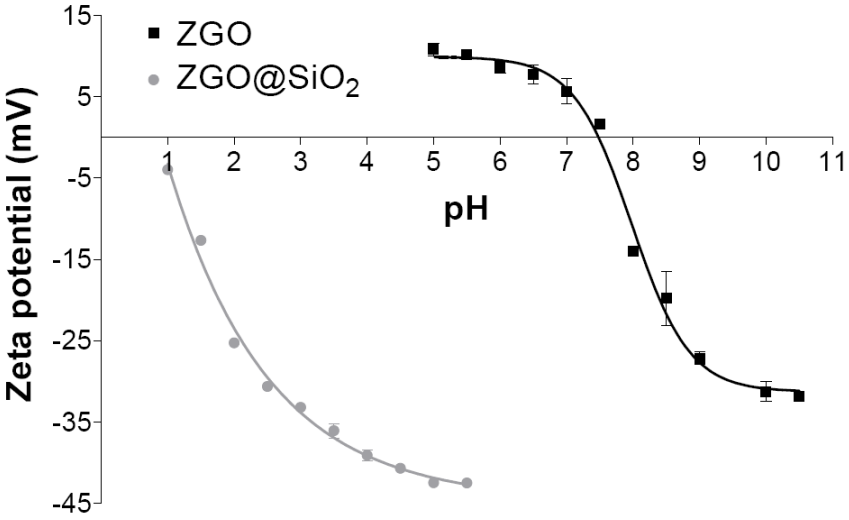

Figure S2. Evolution of ZGO and ZGO@SiO_2 zeta potential as a function of pH.

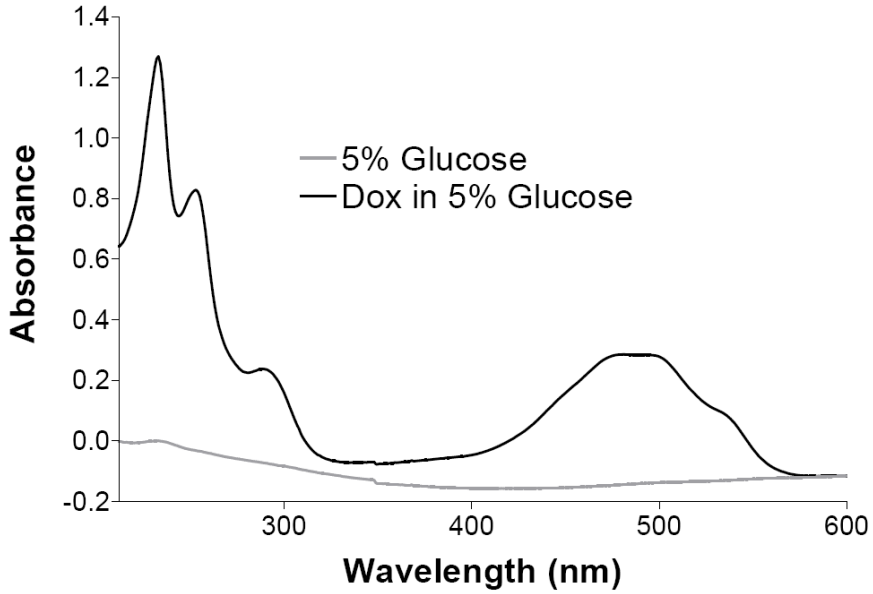

Figure S3. UV-Vis absorption spectrum of Doxorubicin in 5% Glucose solution.

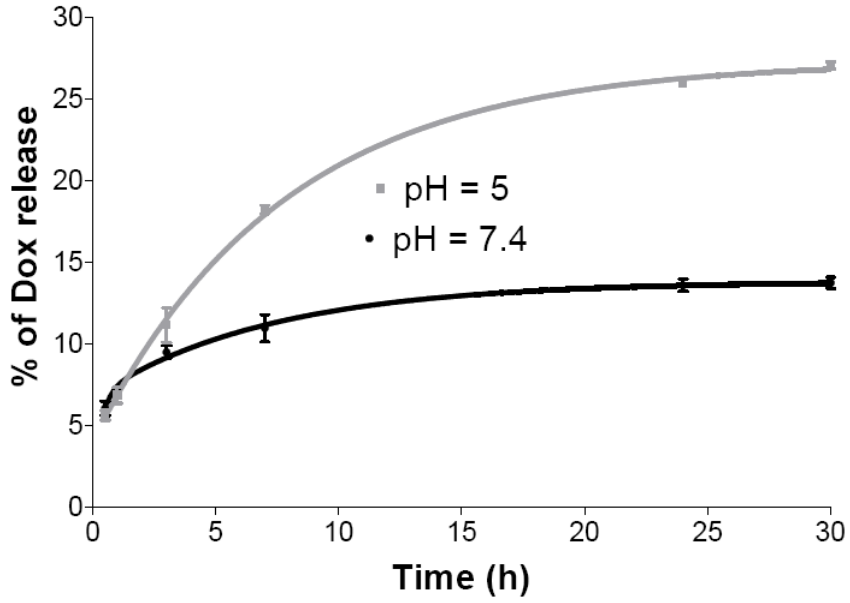

Figure S4. Doxorubicin releasing profile of ZGO@SiO_2-Dox nanoparticles as a function of pH.

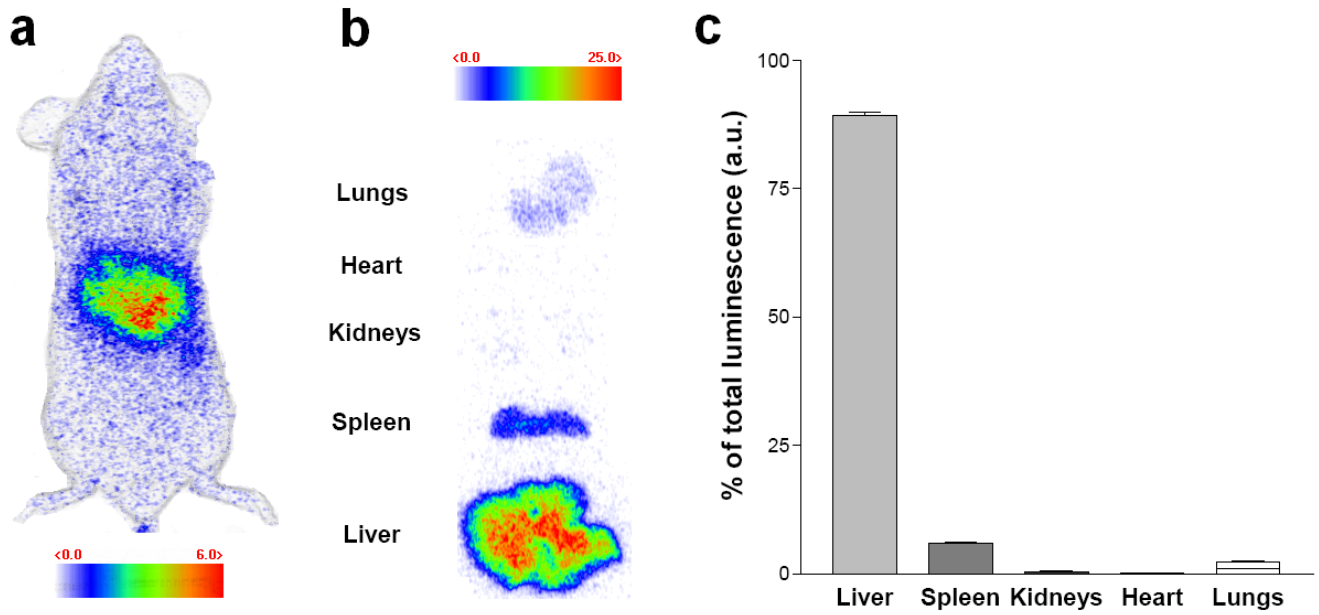

Figure S5. Biodistribution of ZGO@SiO_2-Dox nanoparticles, 24 h after intravenous injection. **a**, Persistent luminescence image of the whole mouse following in situ LEDs activation of persistent luminescence from ZGO@SiO_2-Dox nanoparticles. **b**, *Ex vivo* persistent luminescence image of the harvested organs. **c**, Image-based semi-quantitative *ex vivo* biodistribution.