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# Lanthanide ($Gd^{3+}$ and $Yb^{3+}$) functionalized gold nanoparticles for *in vivo* imaging and therapy

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ABSTRACT

Nanoparticles are regularly used as contrast agents in bioimaging. Unlike other agents such as composite materials, nanoparticles can also be used for treating as well as imaging disease. Here we synthesized lanthanide functionalized gold nanoparticles that can be used for both imaging and therapy *in vivo*. That is a multifunctional nanoplatform was developed based on a simple and versatile method, by incorporating 10-nm gold nanoparticles and lanthanide ions ($Gd^{3+}$ and $Yb^{3+}$), denoted as LnAu nanoparticles hereby. The LnAu nanoparticles were then surface-modified using a PEGylated amphiphilic polymer ($C_{18}$MH-mPEG), and the resulting PEG modified LnAu nanoparticles (PEG-LnAu) display good monodispersion in water and good solubility in biological media. Due to the low toxicity *in vitro* and *in vivo* (as determined by a cell viability assay and histological and serum biochemistry analysis), the PEG-LnAu nanoparticles can be successfully applied to *in vivo* magnetic resonance imaging (MRI), *in vivo* computed tomography (CT) imaging and photothermal therapy (PTT) for tumor-bearing mice. Therefore, the present work developed an easy yet powerful strategy to combine lanthanide ions and gold nanoparticles to a unified nanoplatform for integrating bioimaging and therapy.



## 1. Introduction

The field of nanotechnology has witnessed a rapid development in biology and medicine, specifically as drug delivery vehicles and as contrast agents for imaging [1–7]. Among the various kinds of imaging technologies, computed tomography (CT) excels by providing high resolution 3D information on the anatomy of tissue based on its differential X-ray absorption capability. Magnetic resonance imaging (MRI), in turn, provides superior 3D details and tomographic information of soft tissue with good spatial resolution and high sensitivity. In photothermal therapy (PTT), absorbed light is converted into localized heat to ablate cancer cells [8–13]. Each of these techniques has advantages and drawbacks in terms of sensitivity, resolution, data, acquisition time and complexity [14,15]. Thus, the combination of multimode applications may offer more comprehensive and accurate diagnostic or therapy.

To date, much attention has been paid to combine multimode applications into a single system for integration with CT/MRI, PTT, or other applications. Nanoparticles (NPs) made from either gold, photon upconversion nanomaterials (UCNMs), magnetite ($Fe_3O_4$), gadolinium chelates, polydopamine or graphene have been used as contrast agents, photothermal sensitizers, and photodynamic agents. However, multifunctional nanomaterials have so far always been composed of two or more agents. This usually results in large particle size or relatively complicated synthesis, examples being materials such as Au@$Fe_3O_4$ and $Fe_3O_4$@$Cu_{2-x}S$ core-shell structure [3,16], Au-$Cu_9S_5$ and UCNPs-CuS satellite structure [12,17–19].

Obviously, the increasing number of functionalities increases particle size and complicates the method of preparation. The size of the particles is, however, a key factor to realize bio-applications, and sub-10 nm sizes are clearly preferred due to the respective large specific surface areas and small sizes, the ease of clearance

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from the body, and their low toxicity which allows for higher dosages [20,21]. Therefore, single NPs that fulfill diagnostic and therapeutic function in parallel (such as MRI/CT/PTT) are essential in order to overcome the inherent shortcomings of composite materials.

In the present study, we demonstrate a simple yet powerful strategy to combine lanthanide ion ($Gd^{3+}$, $Yb^{3+}$) and gold nanoparticle based technologies into a single nanoplatform for multimodal bioimaging and photothermal therapy. $Gd^{3+}$ has an isotropic electronic state ($^8S_{7/2}$) and a half-filled f-orbital (with seven electrons), rendering Gd-based materials potential MRI contrast agents [22–24]. Both gold and $Yb^{3+}$ have high atomic number and atomic mass, satisfying an important prerequisite in terms of CT imaging. With respect to the photothermal effect, the gold NPs combined with lanthanide ions (LnAu NPs) were surface-modified using a PEGylated amphiphilic polymer in order to warrant biocompatibility. The resulting nanoparticles (referred to as PEG-LnAu NPs) were successfully applied to *in vivo* MRI, *in vivo* CT imaging and PTT of tumor-bearing mice. To the best of our knowledge, such multifunctional nanoplatform for uses in *in vivo* MRI, CT imaging and PTT has never been reported to date.

## 2. Experiment

### 2.1. Materials

All the chemicals were used as received without further purification. Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ACS reagent, ≥49.0% Au basis), gadolinium(III) chloride hexahydrate ($GdCl_3 \cdot 6H_2O$, 99%), ytterbium(III) chloride hexahydrate ($YbCl_3 \cdot 6H_2O$, 99.9%), oleylamine (technical grade, 70%), poly(-maleic anhydride-alt-1-octadecene) (average Mn 30,000–50,000), and methoxypolyethylene glycol amine (Mn = 2000) were purchased from Sigma Aldrich (USA).

### 2.2. Synthesis of lanthanide ($Gd^{3+}$, $Yb^{3+}$) functionalized gold nanoparticles (named as LnAu NPs)

60 μL $GdCl_3$ (1 M) solution, 30 μL $YbCl_3$ (1 M) solution, 5 mL oleylamine were mixed in a 50 mL flask. Under argon atmosphere, the mixture was heated to 150 °C and maintained for 30 min with magnetic stirring to form a homogeneous transparent and light yellow solution. Then, 0.24 mmol $HAuCl_4 \cdot 3H_2O$ in 1 mL of oleylamine was injected into the hot mixture quickly and the solution were allowed to stir for 90 min. Then, the solution was cooled to room temperature, and the obtained mixture was precipitated by adding ethanol (11,000 rpm, 5 min), washed with chloroform (2000 rpm, 5 min). The centrifugation and washing process repeated twice, and the final product was redispersed in 10 mL chloroform (LnAu NPs).

For the control experiment, the pure gold nanoparticles were synthesized, and the process was similar with that of LnAu NPs except for no $GdCl_3$ and $YbCl_3$ solution addition.

### 2.3. Synthesis of PEG-modified LnAu nanoparticles (PEG-LnAu NPs)

The synthesis of $C_{18}$PMH-PEG was carried out according to previous reported method [25]. 2 mL above LnAu NPs chloroform solution and 2 mL chloroform solution containing 15 mg of $C_{18}$PMH-PEG were mixed. The mixture was stirred at room temperature to evaporate the chloroform. Then, 10 mL water was added and sonicated for 10 min. To remove excess polymer, the solution was repetitively centrifugated at 15,000 rpm for 10 min and washed with water. The obtained PEG-LnAu NPs were re-dispersed in water and passed through a 0.22 μm syringe filter.

For the control experiment, the PEG-modified gold nanoparticles (denoted as PEG-Au NPs) were synthesized, and the process was similar with that of PEG-LnAu NPs, except that LnAu NPs was replaced by pure gold nanoparticles.

### 2.4. Characterization

The size and morphology of the nanoparticles were characterized on a JEM-2010F low-to-high resolution transmission electron microscope (TEM) operated at 120 kV. X-ray diffraction (XRD) measurement was performed on an 18 KW D/MAX2200 V PC diffractometer using Cu kα radiation (60 kV, 80 mA) at a step width of $8^\circ \cdot min^{-1}$. Fourier transform infrared spectroscopy (FT-IR) spectra were acquired in the spectral range from 4000 to 400 $cm^{-1}$ on an Avatar 370 instrument using the pressed KBr pellet technique. Inductively coupled plasma mass spectrometry (ICP-MS) was employed to detect the content of Au, Gd in nanoparticles.

The extended X-ray absorption fine structure (EXAFS) spectra at Au $L_3$-edge ($E_0$ = 11,919 eV), Gd $L_3$-edge ($E_0$ = 7243 eV) and Yb $L_3$-edge ($E_0$ = 8944 eV) were performed at BL14W1 beam line of Shanghai Synchrotron Radiation Facility (SSRF) operated at 3.5 GeV under "top-up" mode with a constant current of 240 mA. The measured sample solution (solvent: cyclohexane) was sealed in a liquid cell with the Kapton windows for the X-ray path. The EXAFS data were recorded under transmission mode with high-flux ion chambers for Au $L_3$ edge or under fluorescence mode with Lytle-type ion chamber for Gd $L_3$-edge and Yb $L_3$-edge. For Au $L_3$-edge, the energy was calibrated accordingly to the absorption edge of pure Au foil. Athena and Artemis codes were used to extract the data and fit the profiles. The Fourier transformed (FT) data in R space were analyzed by applying metallic Au model for the Au-Au shell or first shell approximation for Gd–N/Yb–N, Gd–O/Yb–O and Gd–Au/Yb–Au contributions.

### 2.5. Cell viability assay

The HeLa cell (human epithelial cervical cancer) was obtained from the Cell Bank of Chinese Academy of Sciences in Shanghai, China. The HeLa cells were grown in DMEM medium (Evergreen Bio-Engineering Materials Co. Ltd., China) with 10% (v/v) fetal bovine serum (Zhejiang Tianhang Bio-Engineering Co. Ltd., China) The cells were cultured in a humidified atmosphere with 5% $CO_2$ and 95% air at 37 °C.

The cell viability was evaluated by a WST-8 cell counting kit assay (CCK-8, Dojindo Molecular Technologies Inc., Japan). Hela cells (5000 per well) were seeded into 96-well plates and grew overnight. Then, culture media containing PEG-LnAu in different concentrations (0, 50, 100, 200, 300, 400 μg $mL^{-1}$) were added to the cells and cultured for 24 h. After that, the culture medium was replaced by CCK-8 solution (20 μL CCK-8 in 200 μL medium) and incubated for 2 h at 37 °C. The optical density (OD) of each well was recorded at 450 nm on the microplate reader (Thermo, Varioskan Flash, Boston, MA USA).

### 2.6. Measurement of photothermal performance of PEG-LnAu NPs

To measure the photothermal performances of the as-synthesized PEG-LnAu NPs, the NPs were dispersed in solution and exposed to an 808 nm laser irradiation at different concentration of PEG-LnAu for 5 min. The output power was independently calibrated by using the optical power meter. A thermocouple thermometer with an accuracy of 0.1 °C was employed to measure the temperature every 20 s. The thermometer was inserted into the NPs solutions perpendicular to the laser path to avoid the direct light irradiation on the probe.