SCIENCE CHINA
Life Sciences

SPECIAL TOPIC
• REVIEW •

October 2012 Vol.55 No.10: 872–883
doi: 10.1007/s11427-012-4389-5

Au nanostructures: an emerging prospect in cancer theranostics

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Received June 10, 2012; accepted September 19, 2012

Au nanoparticles have been used in biomedical applications since ancient times. However, the rapid development of nanotechnology over the past century has led to recognition of the great potential of Au nanoparticles in a wide range of applications. Advanced fabrication techniques allow us to synthesize a variety of Au nanostructures possessing physiochemical properties that can be exploited for different purposes. Functionalization of the surface of Au nanoparticles further eases their application in various roles. These advantages of Au nanoparticles make them particularly suited for cancer treatment and diagnosis. The small size of Au particles enables them to preferentially accumulate at tumor sites to achieve *in vivo* targeting after systemic administration. Efficient light absorption followed by rapid heat conversion makes them very promising in photothermal therapy. The facile surface chemistry of Au nanoparticles eases delivery of drugs, ligands or imaging contrast agents *in vivo*. In this review, we summarize recent development of Au nanoparticles in cancer theranostics including imaging-based detection, photothermal therapy, chemical therapy and drug delivery. The multifunctional nature of Au nanoparticles means they hold great promise as novel anti-cancer therapeutics.

Au nanoparticle, anti-tumor activity, photothermal therapy, drug delivery, surface plasmon resonance, diagnostics

Citation: Nie X, Chen C Y. Au nanostructures: an emerging prospect in cancer theranostics. Sci China Life Sci, 2012, 55: 872−883, doi: 10.1007/s11427-012-4389-5

Cancer is a major public health problem globally. According to the American Cancer Society, over 1.6 million new cancer cases and 0.6 million deaths from cancer were projected to occur in the United States alone in 2011 [1]. Thus there is an urgent need for novel anti-cancer therapeutics. Nanoparticles, as an extension of nanotechnology, have shown potential in multiple applications including cancer treatment [2]. Gold (Au) nanoparticles are one of the most promising candidates for cancer treatment because of their unique physiochemical properties. This review will focus on recent advances of the use of Au nanoparticles in cancer theranostics including imaging-based detection, photothermal therapy, chemical therapy and drug delivery.

1 Au nanoparticles—a brief history and their biocompatibility

Colloidal Au nanoparticles were discovered by Faraday in 1857, when he observed a range of colors originating from some unidentified particles in a gold chloride solution after adding reducing and stabilizing agents [3]. In 1908, Mie explained the intense colors in Faraday's gold solution by solving Maxwell's electromagnetic equation, concluding the phenomenon was related to the absorption and scattering of light by gold nanospheres present in the solution [4]. With the advent of the electron microscope in 1932 [5], different nanostructures formed under varying synthetic conditions could be studied systematically, resulting in the rapid development of methodology to prepare nanostructures. Thereafter, a range of Au nanostructures (Figure 1) were synthe-

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sized, and chemical conditions were optimized [6–12]. The size, shape and surface chemistry of Au nanoparticles were also controlled to produce nanoparticles suitable for different purposes [13].

Biocompatibility is essential and is systemically evaluated before chemical structures are used in clinical experiments. It is well known that current anti-cancer drugs produce severe side effects because of their strong cytotoxicity and poor targeting. In contrast, Au nanoparticles are considered to be relatively safe, a view supported by most *in vitro* studies to date. Elemental Au is highly inert, so direct chemical interaction with biomolecules can be minimized. However, potent catalytic activity arising from the large relative surface area of small particles (several nm) [14,15], and additional agents present in Au nanoparticles [16–18] are reported to be hazardous in some cases. Recently, Qiu and co-workers [19] from our group have demonstrated coating molecule-dependent cytotoxicity using Au nanorods. Different coating agents, cetyltrimethylammonium bromide (CTAB), polystyrene sulfonate (PSS) and polydiallyldimethyl ammonium chloride (PDDAC), were systemically studied. It was found that CTAB-coated Au nanorods induced a cytotoxic effect in human breast cancer cell line MCF-7 (Figure 2), resulting in apoptosis and cell cycle arrest, but PSS and PDDAC-coated ones did not. Later, Wang *et al*. [20] from our group further showed that the intracellular presence of free CTAB molecules could break lysosome membrane integrity and lead to accumulation of Au nanorods released from lysosomes in mitochondria, which eventually caused mitochondrial dysfunction-mediated cell death. These results stress the importance of the surfactant CTAB in Au nanorod-induced cytotoxicity. However, such undesired side effects can be avoided simply by changing the capping agent. The flexible synthetic conditions of Au nanoparticles mean that materials suitable for biomedical applications are a realistic possibility.

2 Au nanoparticles in cancer diagnosis—imaging-based detection

Au has been expensive since historic times because of its rarity, stability and brilliant colors. In the past, people made use of the brilliant colors of Au in stained glass and decorative artworks. In modern times, people learned the reason for these brilliant colors and developed novel strategies to exploit the unique optical properties of Au in biomedical applications including biosensing and imaging.

Faraday was the first to attribute the bright colors of some Au solutions to colloidal Au [3], and Mie [4] expounded the origin of this phenomenon by solving Maxwell's electromagnetic equation for the interaction of light with spherical particles. For a spherical nanoparticle much smaller than the wavelength of light (diameter $d \ll \lambda$), an electromagnetic field at a certain frequency (v) induces a resonant oscillation of the metal-free electrons across the nanoparticle. This oscillation is known as surface plasmon resonance (SPR) [21–23]. The surface plasmon oscillation of the metal electrons results in a strong enhancement of absorption and scattering of electromagnetic radiation in

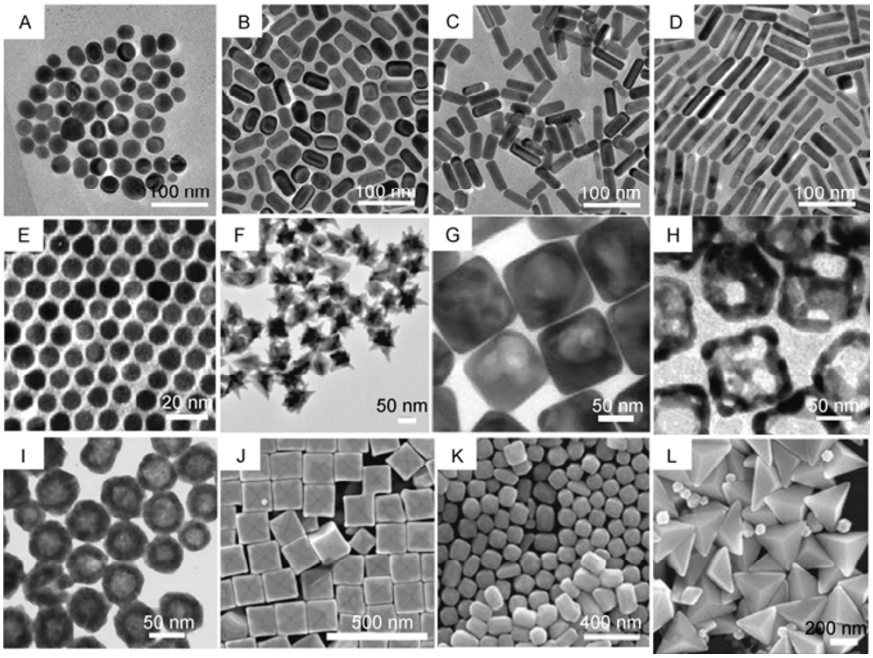

Figure 1 Au nanoparticles of various size and shape with potential applications in biomedicine. A–D, Au nanorods with different aspect ratios. E, Au nanospheres. F, Au nanostars. G, Au nanoboxes. H, Au nanocages. I, Hollow Au nanospheres. J, Au nanocubes. K, Au nanocrystals. L, Au obtuse triangular bipyramids. Adapted from ref. [8–12,19] with permission from the American Chemical Society and Elsevier.

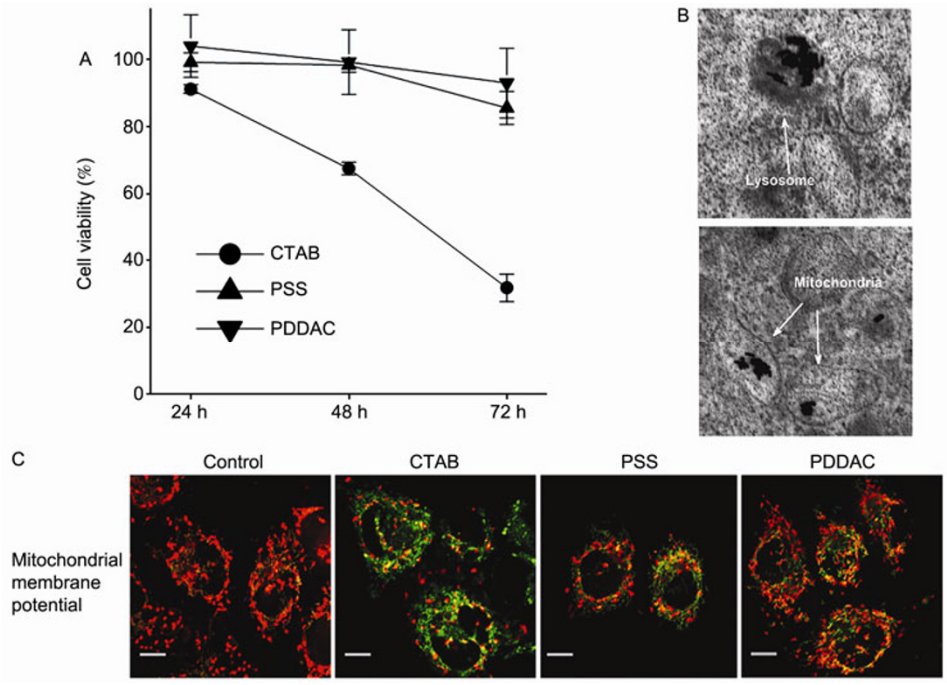

Figure 2 Cytotoxicity studies using a human breast cancer line MCF-7 treated with Au nanoparticles coated with CTAB, PSS and PDDAC. A, CCK-8 assays illustrating cell viability after 24, 48, or 72 h of treatment with Au nanoparticles with different coatings. B, TEM images of cells incubated with CTAB-coated Au nanoparticles. Most Au nanoparticles appeared to be within the lysosome initially, whereas some were observed in the mitochondria thereafter. C, Mitochondrial damage caused by Au nanoparticles coated with CTAB. The probe molecule JC-1 emits red fluorescence when the mitochondrial membrane potential is normal and turns green as the reactive oxygen species (ROS) level increases through oxidative stress caused by mitochondria damage. Scale bar, 10 mm. Reproduced with permission from ref. [19].

resonance with the SPR frequency of the Au nanoparticles, giving them intense colors and interesting optical properties [24]. The frequency and cross-section of SPR absorption and scattering is dependent on the metal composition [25], nanoparticle size and shape [26], dielectric properties of the medium/substrate [27] and presence of interparticle interactions [28]. These factors provide much room for Au nanoparticles to adjust their SPR performance to suit the requirements of different applications. Meanwhile, any variation in SPR performance reveals corresponding information related to changes in the Au nanoparticles, thus enabling them to be developed as sensors that can detect the local refractive index/dielectric constant of the environment surrounding the nanoparticles [29]. The SPR of nanospheres shifts to longer wavelength as the refractive index of the medium increases [25]. This phenomenon has been exploited in the sensing of biomolecular analytes by monitoring the changes in the SPR wavelength following adsorption/binding events at the surface of Au nanoparticles [30]. The SPR of Au nanoparticles can also be red-shifted by the self-assembly or aggregation of particles [31].

Besides showing great potential as biosensors, the application of Au nanoparticles in cancer diagnosis has mainly focused on cancer cell imaging-based detection. There are four major types of imaging using Au nanoparticles, including light scattering imaging, two-photon fluorescence (TPF) imaging, photoacoustic imaging and X-ray computed tomography (CT).

2.1 Light scattering imaging

Light scattering imaging originates from the significant SPR-based light scattering capability of Au nanoparticles at their plasmon wavelengths [32]. Compared with emission from fluorescent dye molecules, the scattering cross-sections of Au nanoparticles could provide stronger photon intensity by more than five orders of magnitude [33]. Thus, high-contrast images are attainable as long as the Au nanoparticles accumulate only within cancer cells. To date, most studies used immune-targeting to localize Au nanoparticles in the desired region. Sokolov and co-workers [34] conjugated anti-epidermal growth factor receptor (EGFR) antibodies to colloidal Au nanoparticles with a diameter of 12 nm via noncovalent electrostatic adsorption. Emission from the nanoparticles was observed inside cervical cancer cell line SiHa, which overexpressed EGFR at the cell membrane, under dark-field monochromatic light illumination. In contrast, Au nanoparticles conjugated with bovine serum albumin molecules were not observed on the dark background, which illustrated the necessity of antibody-mediated targeting for cancer cell imaging. Another representative work was conducted by Huang *et al*. [35]; they used white light as an excitation source and observed strong light scattering from Au nanorods in the near-infrared (NIR) region. After

conjugation with anti-EGFR antibodies, Au nanorods selectively accumulated in two malignant epithelial cell lines (human oral squamous carcinomas, HSC and HOC) but not in a benign cell line (HaCaT). The Au nanorods in HSC and HOC cells were visualized on a dark background (Figure 3). Currently, dark-field imaging based on the light scattering properties of Au nanoparticles is widely used for cancer imaging through functionalized nanoparticle-receptor binding to cell surface biomarkers [36,37].

2.2 Two-photon fluorescence (TPF) imaging

TPF is another advantage of Au nanoparticles (especially Au nanorods) that can be used to visualize target cells or the particles themselves. TPF, also called two-photon luminescence, refers to the stronger luminescence emitted by nanoscale materials than their bulk form [38] (e.g., the quantum yield of emission of Au nanorod is 6–7 orders of magnitude larger than that of bulk gold [39]). Under femtosecond NIR laser excitation, enhanced TPF is able to produce very strong signals, even allowing single nanoparticles to be detected [39]. Durr and co-workers [40] explored the capability of TPF imaging in a 3D tissue model using Au nanorods conjugated with antibodies to target cancer cells embedded in the collagen matrices. The TPF signals of Au nanorods are three orders of magnitude stronger than the autofluorescence from the surrounding tissues, so in cancer cell imaging with a spatial resolution of 75 μm was attained. Tong *et al*. [41] tracked the cellular uptake of Au nanorods conjugated with a folate ligand in KB cancer cells, which overexpress folate receptors, and NIH-3T3 normal fibroblasts using TPF. The localization of Au nanorods after different periods was sharply visualized, illustrating ligand-receptor binding-mediated endocytosis.

2.3 Photoacoustic imaging

Photoacoustic imaging is a new combinational imaging method. It uses a laser as an excitation source and acoustic waves as the detection target, and requires the application of a specific material as a linker. This material needs to be able to absorb light and convert it into heat, which leads to thermal expansion of the air surrounding the material, creating acoustic waves for imaging. Au nanoparticles are ideal candidates for photoacoustic imaging because of their SPR-based light absorption. SPR leads to both light absorption and scattering enhancement [24], so relatively large nanoparticles are preferred because they possess higher absorption/scattering ratios [42]. Li and co-workers [43] demonstrated photoacoustic imaging *in vivo* using Au nanorods conjugated with antibodies targeting cancer cells. The contour of the tumor region was sharply visualized in fusion images acquired from photoacoustic and ultrasound signals. Antibody-mediated targeting was found to be essential for such *in vivo* tumor visualization because images were not obtained at the tumor sites of mice injected with Au nanorods alone. The intensity of the photoacoustic signal was time dependent and reached a maximum at 7 h post injection.

2.4 X-ray computed tomography

Au nanorods also readily absorb X-rays. Based on this property, Bahatia *et al*. [44] developed a three-dimensionally reconstructed tumor xenograft model using X-ray CT and the contrast from tumor-accumulated Au nanorods (Figure 4A). Under the guidance of this model, they achieved complete tumor regression in mice treated with PEGylated Au nanorods following computationally tailored irradiation (810 nm, 2 W cm^{-2}, 5 min) (Figure 4B).

3 Au nanoparticles in cancer therapy

3.1 Photothermal contrast agents

Plasmonic photothermal therapy (PPTT) is the most promising application of Au nanoparticles in cancer treatment [44–48]. The ability of Au nanoparticles to efficiently absorb light followed by rapid heat conversion makes them a suitable candidate for cancer hyperthermia treatment. In addition, Au nanoparticles are not susceptible to photobleaching [49], and their optical absorption can be easily tuned by changing their size and shape [21,33]. As a result,

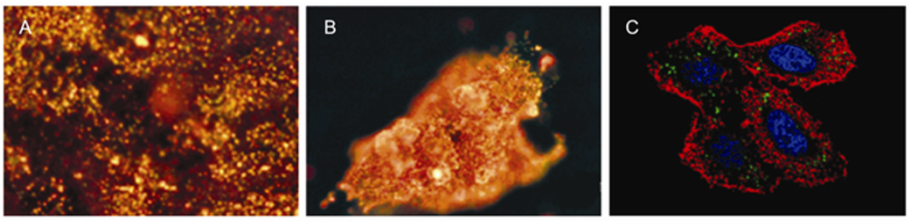

Figure 3 Light scattering and TPF images of different cells. A and B, Dark-field images of Au nanorods conjugated with anti-EGFR antibodies. For cancer cell line HOC (B), which overexpresses EGFR, the cell morphology was clearly visualized because of the accumulation of Au nanorods, whereas the cell morphology of normal cell line HaCaT (A) is not recognized because of nonspecific binding. C, Confocal image of HeLa cells in the presence of Au nanorods (blue, nuclei; red, actin cytoskeleton; green, Au nanorods). This image was taken by two-photon microscopy. Reproduced with permission from ref. [35,39].

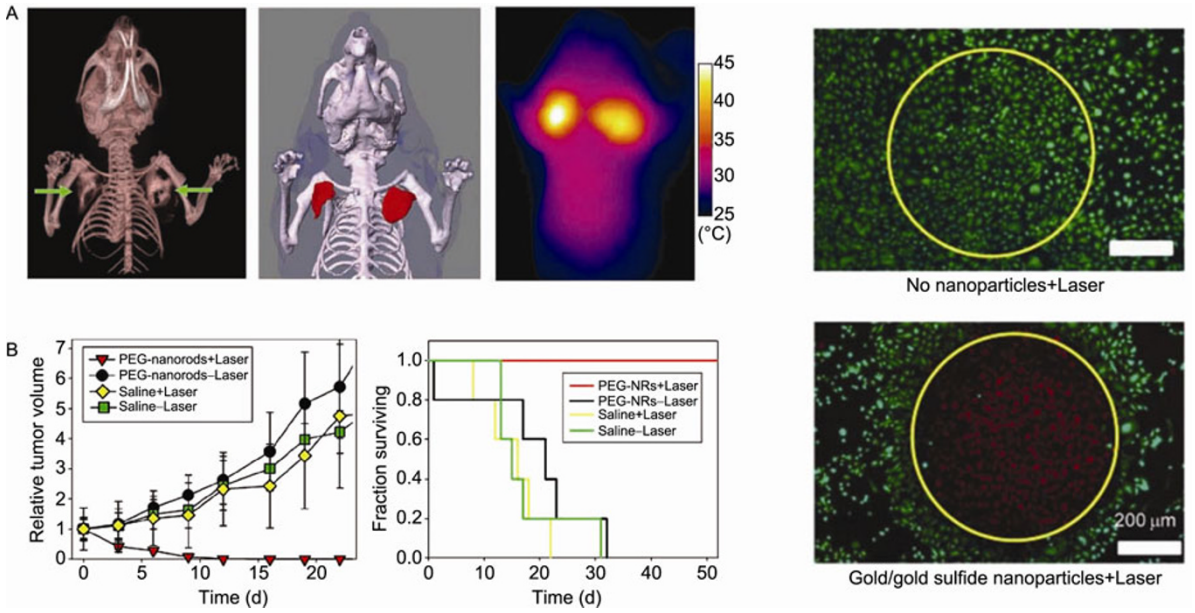

Figure 4 *In vivo* and *in vitro* plasmonic photothermal therapy (PPTT) using Au nanoparticles. A, PEGylated Au nanorods were added intratumorally to mice with bilateral MDA-MB-435 tumors and imaged using X-ray CT to visualize the three-dimensional distribution of nanoparticles in the tumors (left). A three-dimensional solid model of the complete geometry was rapidly reconstructed by image processing for use with computational photothermal modeling (middle; red, PEGylated Au nanorods). Experimental thermographic surveillance of NIR irradiation after X-ray CT (0.75 W cm^{-2}, 1 min; right). B, Mice harboring MDA-MB-435 human tumors were injected with either saline or PEGylated Au nanorods. 72 h post injection, the flank of each mouse was exposed to a computationally designed irradiation regimen (810 nm, 2 W cm^{-2}, 5 min). Plots of tumor volume (left) and long-term survival (right) over time after irradiation are also shown. C, Cellular ablation achieved using bare Au/Au sulfide nanoparticles with laser irradiation. The yellow circle indicates the laser spot; live/dead stain for viability shows dead cells as red while viable cells appear green. Reproduced with permission from references [44,60].

the electromagnetic spectrum of Au nanoparticles can be red-shifted to the NIR region, which allows high-depth PPTT in tissues [50]. Jain *et al*. [33] used Mie theory and the discrete dipole approximation method to calculate the absorption and scattering efficiencies and optical resonance wavelengths of three typical classes of nanoparticles: Au nanospheres, silica-Au nanoshells, and Au nanorods. Au nanoshells were found to have optical cross-sections comparable to or higher than nanospheres. The optical resonance of the nanorods could be linearly tuned across the NIR region by changing either their effective size or aspect ratio. Au nanorods show per micron absorption and scattering coefficients that are an order of magnitude higher than those of nanoshells and nanospheres. While nanorods with a higher aspect ratio and smaller effective radius are the best photoabsorbing nanoparticles, the highest scattering contrast for imaging applications is obtained from nanorods with a high aspect ratio and a larger effective radius [33].

Compared with conventional agents used in laser photothermal tumor therapy (e.g., indocyanine green, and naphthalocyanines), there are several distinct advantages of Au nanoparticles: (i) higher absorption cross-section because of their enhanced SPR effect (five orders of magnitude larger than indocyanine green for nanospheres with a diameter of 40 nm and an absorption band around 530 nm) [49,51]; (ii) deep tissue penetration of up to a few centimeters after the "NIR window" shift using the tunable optical extinction of Au nanoparticles; (iii) facile bioconjugation with various molecules (e.g., peptides, proteins and DNA) [52,53], enabling multi-targeting in a single treatment.

PPTT using Au nanoparticles was first demonstrated by Lin and co-workers in 2003 [54]. This method exploits the unique ability of noble metal nanoparticles (e.g., Au, Ag, and Cu nanoparticles) to efficiently absorb photons through SPR [55], which distinguishes them from traditional photoabsorbing agents such as chromophores and dye molecules. Subsequently, a number of Au nanostructures including nanorods, Au-silica nanoshells, nanocages, hollow nanoshells and Au-Au sulfide nanoparticles have been synthesized and used in both *in vitro* and *in vivo* cancer hyperthermia research [54–62].

The first gold nanostructure employed for PPTT was spherical Au nanoparticles. Following conjugation to IgG, which targets the CD8 receptor on peripheral blood lymphocyte cells, Lin *et al*. [54] found 95% of cells containing as few as ca. 500 nanoparticles per cell were killed (versus 5%–8% in a control treatment) after exposure to a nanosecond pulsed visible laser. Later, researchers from the El-Sayed group [56] demonstrated selective PPTT using spherical Au nanoparticles with a diameter of 40 nm conjugated to EGFR IgG. Cancer cell-targeting mediated by EGFR antibody allowed Au nanoparticles to specifically accumulate around/within HSC and HOC human oral squamous cell lines after 30 min incubation compared with

in a benign cell line (HaCaT). Intense SPR scattering of Au nanoparticles under white light excitation on a dark background confirmed the difference of accumulation in the various types of cells. Using a continues wave argon ion laser at 514 nm, the two cancer cell lines suffered severe photothermal damage within 4 min at laser energy thresholds of 19 and 25 W cm^{-2}, which were less than half that of the benign cells (57 W cm^{-2}). This selective killing of cancerous cells combined with the high absorption cross-section of the Au nanoparticles eases photothermal cancer therapy at sufficiently low laser energy that the benign cells remain undamaged.

The *in vitro* success of cancer therapy using visible light-absorbing Au nanoparticles can be extended to skin or surface cancer. However, deep tissue penetration is not possible with visible light [50], so tuning the SPR absorption band of Au nanoparticles to the "NIR window" is required to extend their use to cancer in deep tissues.

Halas and co-workers [57] used Au nanoshells (silica core with a diameter of 55 nm, and a 10 nm-thick Au shell) in NIR PPTT. By varying the relative core and shell thickness of the Au nanoshells, their SPR absorption was varied across a broad range of wavelengths that extended from the visible to the NIR region. Breast carcinoma cells labeled with PEGylated Au nanoshells that showed SPR centered at 800 nm suffered notable photothermal damage after exposure to NIR laser light (820 nm, 4 W cm^{-2}, 7 min). Subsequently, systemic administration of these Au nanoshells to a colon cancer model was conducted by the same group to evaluate their efficacy in NIR PPTT *in vivo* [57]. PEGylated Au nanoshells were intravenously injected and tumors received NIR laser radiation 6 h post passive accumulation (4 W cm^{-2}, 3 min). Tumors in mice treated with Au nanoshells exhibited complete regression after a single PPTT treatment, and the animals were tumor free after 90 d. In contrast, tumors in the control group continued to grow, which led to a mortality of almost 50% after just 10 d. El-Sayed and co-workers later demonstrated the effectiveness of Au nanorods in NIR PPTT both *in vitro* [35] and *in vivo* [58]. Unlike Au nanoshells, Au nanorods are smaller, so the "NIR window" shift can be easily obtained based on the plasmon oscillation of electrons along the longitudinal axis of the nanorods [59]. In a tumor-bearing mice model, PEGylated Au nanorods accumulated efficiently and exhibited enhanced NIR absorption at the tumor site following intravenous administration. After a single NIR laser radiation (2 W cm^{-2}, 10 min), 25% of the tumors in treated mice were completely resorbed at day 13 [58]. West *et al*. [60] realized NIR PPTT using Au-Au sulfide nanoparticles. The biggest advantage of this nanostructure is that its absorption/scattering ratio (98%–2%) is higher than those of other nanostructures like Au-silica nanoshells (70%–30%). In their experiment, human prostate cancer cell line PC3 was efficiently ablated *in vitro* after laser irradiation (Figure 4C) and more than 80% of mice that received PPTT remained alive 8 weeks after treatment.

Overall, the unique SPR effect of Au nanoparticles makes them highly potent in photothermal cancer therapy. In addition, the easy modification of Au nanostructures means their intrinsic properties can readily be adapted to suit different applications.

3.2 Antineoplastic activity

The SPR-based optical properties of Au nanoparticles [61] have directed much attention toward their development as novel contrast agents in photothermal cancer therapy [48,62]. However, another typical characteristic of Au nanoparticles is that they are of comparable size to biomolecules, ranging from several to hundreds of nanometers. This resemblance provides great opportunities for them to directly interact with biomolecules such as proteins, lipids, and nucleic acids, so they can be used to regulate cellular behaviors.

Malignant cells display several characteristics including reliance on mitogenic signals and abnormal energy metabolism [63] that are different from normal cells, which make them quite vulnerable to particular treatments like hyperthermia. Recently, we demonstrated a cancer cell-specific inhibitory effect using Au nanorods coated with serum protein [20]. The cell viability of human pulmonary carcinoma cell line A549 was found to be severely inhibited (Figure 5A), while normal bronchial epithelial cell line 16HBE and primary adult stem cell MSC exhibited little cytotoxicity after incubation with Au nanorods at the same concentration. Intracellular trafficking of Au nanorods was investigated using TEM. A marked dissimilarity was observed in the distribution patterns in A549 and 16HBE/MSC. Au nanorods selectively entered the mitochondria in cancer cell line A549 but not in 16HBE and MSC cells, although most of the Au nanorods appeared to be trapped in the lysosomes initially in all three types of cells. The assembly of Au nanorods in mitochondria further led to a series of pathological changes to this organelle including swollen morphology and partial disappearance of crista. In the end, the shape of the A549 cells was changed significantly (Figure 5B), and apoptosis-like characteristics were observed. The mechanism of Au nanorod translocation from lysosome to mitochondria specifically in the A549 cells was thought to be related to an interaction between Au nanorods and lysosome membrane that resulted in leakage of the nanorods (Figure 5C), and finally determined the fate of A549 cells. This work introduced a novel anti-cancer strategy using Au nanorods as a mitochondrial targeting agent to selectively kill cancer cells because of the different responses of the lysosome membranes in cancer and normal cells to Au nanorods (Figures 5C and D).

Besides a mitochondrial targeting-induced cytotoxic effect, it has been reported that Au nanoparticles can be used

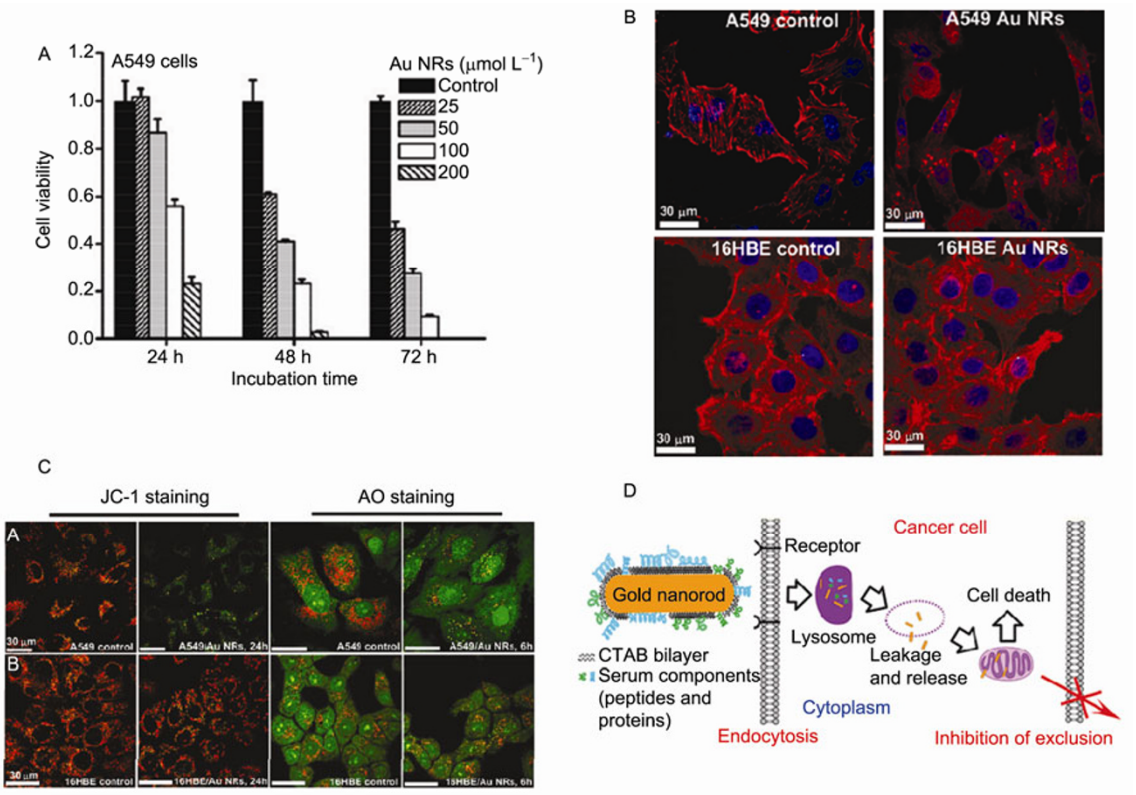

Figure 5 CTAB-coated Au nanorods were able to selectively kill cancer cells. A, Cell viability in A549 determined by CCK-8 after exposure to Au nanorods. B, Shrinkage in cytoskeleton (stained by rhodamine-labeled phalloidin) was observed in A549, but not in 16HBE cells treated with Au nanorods for 24 h. C, Mitochondrial membrane potentials and the integrity of lysosomal membrane were severely damaged by Au nanorods only in A549 cells (as indicated by varied fluorescent colors in JC-1 probe and decreasing red fluorescence of AO probe). D, Possible mechanism of cell damage. Reproduced with permission from ref. [20].

to treat neoplasia. The underlying mechanisms of this treatment involve cell cycle regulation [64], DNA-targeted damage [66], anti-angiogenesis [65,67] and oxidative stress [68].

New blood vessel formation (also called angiogenesis) is required for cancer to develop from cells into tissue. Thus anti-angiogenesis is considered to be an effective method to treat neoplasia. Mukhopadhyay and co-workers [65] investigated the influence of spherical Au nanoparticles with a diameter of 5 nm on human umbilical vascular endothelial cells (HUVEC). The Au nanoparticles significantly inhibited the VEGF-165-induced cell proliferation of HUVEC by directly binding to the heparin-binding domain of VEGF-165 via sulfur and/or nitrogen bonds. This interaction severely hindered the normal biological process involving association of VEGF-165 with cell membrane receptors, which is necessary for self-proliferation and angiogenic signaling transduction. Subsequently, they showed the binding interaction was highly specific because the Au nanoparticles did not affect the signaling/proliferation associated with another non-heparin-binding growth factor, VEGF-121. The group later investigated the anti-angiogenic effect of Au nanoparticles in an ovarian tumor-bearing nude mice model and achieved results consistent with *in vitro* findings. This research is intuitively associated with an antibody-based regimen (e.g., avastin, an antibody that binds specifically to vascular endothelial growth factor receptors (VEGFRs) in a clinical setting because they possess similar functions. More nanostructures like this need to be designed and developed to offer some alternatives in antibody-mediated cancer therapy.

Interference with mitogenesis is another strategy in which Au nanoparticles can be exploited in anti-cancer therapies. Xing *et al*. [64] examined the regulatory effects of glucose-capped Au nanoparticles with a diameter of 10.8 nm in the cell cycle in human prostate carcinoma cell line DU-145. The glucose-Au nanoparticles arrested the cell cycle of DU-145 cells in the G2/M phase through upregulation of the expression of cyclin E, a promoter that modulates the transition from the G1 to S phase and accelerates cell cycle progression through the G1 phase. Malignant cells blocked in the G2/M phase are more vulnerable to radiation, which implies the G2/M phase retardation of cancer cells regulated by glucose-Au nanoparticles would be killed effectively during subsequent radiotherapy. Because cancer cells are more sensitive to cell cycle disruption and DNA damage than healthy ones, Au nanoparticles that induce such effects can be selectively delivered to malignant cells

to help destroy them.

Although most Au nanoparticles are preferentially translocated into the endosome/lysosome because of their larger relative sizes, Au nanoparticles can also be conjugated to specific peptide sequences to actively target different organelles or the nucleus in the cell. The El-Sayed group [66] developed novel Au nanospheres conjugated to two different peptides, arginine-glycine-aspartic acid (RGD) and nuclear localization sequence (NLS), which recognize alpha V beta integrins on the cancer cell surface and cytoplasmic importins located near the nuclear pore complex, respectively. Using time-resolved dark-field scattering microscopy, they observed distinct cytokinesis arrest in squamous carcinoma cell line HSC-3 after 24 h incubation with the Au nanosphere conjugates. Neither non-malignant cell line HaCaT or nanoparticles conjugated to the RGD peptide only (without NLS) induced a similar phenomenon, indicating the specific pathological process as a result of Au nanospheres accumulating in the nucleus of the cancer cells. Subsequently, the group studied the cancer cells that underwent cytokinesis arrest by immunofluorescence microscopy and found a significant number of breaks in the double-stranded DNA. Flow cytometry also confirmed 20% more apoptosis in malignant cells than non-malignant ones when both were treated with RGD/NLS particles. Thus, the selective and anti-proliferative properties of targeted nanoparticle conjugates could, in the future, serve in anti-cancer treatments functionally analogous to current radiotherapy and chemotherapy methods.

3.3 Au nanoparticles as drug delivery vehicles

Like most nanocarriers, Au nanoparticles possess several advantages when serving as drug delivery vehicles. The enhanced permeability and retention effect [69] of Au nanoparticles allows them to preferentially accumulate at tumor sites because tumors possess leaky blood vessels. Facile bioconjugation can be realized at the particle surface because of the strong binding affinity of Au nanoparticles for thiols, which allows Au nanoparticles to be easily functionalized with antibodies and biomolecules such as folate to achieve active targeting. In addition, the unique optical properties of Au nanoparticles favor them as latent photothermal agents in most cases, indicating the possibility of combinational therapeutics in one treatment.

Very recently, we developed novel mesoporous silica-coated Au nanorods (Au@SiO_2) as a multifunctional therapeutic platform [70]. The outer mesoporous SiO_2 shell provided a large surface area for doxorubicin (DOX) payload, which made up for the limited capacity of the Au nanorods as drug carriers (Figure 6A). Notably, the silica shell did not alter the SPR maximum of the Au nanorods, so a tissue-penetrating NIR laser could be employed for irradiation. *In vitro* drug release experiments showed that 50% of loaded DOX escaped from Au@SiO_2 within 12 h following laser irradiation (250 mW, 780 nm), whereas less than 5% was released in the control experiment (Figure 6B). Unexpectedly, 5–10-fold increases in the uptake of DOX when loaded into Au@SiO_2 compared with that of free DOX was observed in A549 (Figure 6C). Inductively coupled plasma mass spectrometry experiments also confirmed effective time/dose-dependent accumulation of Au@SiO_2 within the cells (unpublished data). The positive zeta potential of the Au@SiO_2-DOX mechanism might partly account for this phenomenon. Subsequently, the cytotoxicity of Au@SiO_2-DOX in A549 was assessed. As expected, Au@SiO_2-DOX was obviously less toxic than free DOX in the absence of laser irradiation because of limited drug release. Two-photon confocal microscopy further confirmed the localization of most nanoparticles in the endosome/lysosome, thus hyperthermia-induced drug release and lysosome destruction were expected to promote complete drug distribution within the cell. Following laser irradiation (3 min, 24 and 48 W cm^{-2}), both lysosomal membrane disruption and cell death were observed at high power density (48 W cm^{-2}) but not at 24 W cm^{-2}. As a result, the lower power density (24 W cm^{-2}) was chosen to explore the chemotherapeutic potential of Au@SiO_2-DOX and avoid hyperthermia-induced cell death. Interestingly, although no cell death was observed in all groups immediately (3 min) after laser irradiation, Au@SiO_2-DOX-treated cells showed slight/severe cytotoxic effects 12/24 h post irradiation, whereas Au@SiO_2-treated cells still maintained full viability (Figure 6D). This result correlates well with the process of drug release rather than hyperthermia-induced cell death because the latter would only lead to acute cell killing via instant denaturation of biomolecules (proteins, lipids, and nucleotides) caused by rapid temperature elevation. Overall, a successful drug delivery system was developed using Au nanorods coated with mesoporous silica. High drug payload, light-activated controlled release and hyperthermia are three distinct advantages of this novel silica-Au nanocomplex.

To date, the diverse functional possibilities of Au nanoparticles have inspired a variety of approaches for drug delivery system design. Because elemental Au nanostructures differ in many aspects (e.g., size, shape, and surface chemistry) compared with classical organic drug carriers, developing novel drug loading/releasing strategies is essential for their application as drug delivery vehicles. As summarized in an excellent review by Dreaden *et al*. [45], the loading pattern applied to Au nanoparticles has been categorized into five classes as follows: (i) Loading by partitioning. Use the mono/bilayer structure capping the Au nanoparticle surface (e.g., CTAB) as an organic solvent to partition hydrophobic drugs from the surrounding medium. (ii) Loading by surface coordination. Employ the binding affinity of thiols and amines with the Au surface to attach drugs containing such groups via the formation of Au-S or Au-N bonds. (iii) Loading by attachment to capping agent. Couple drugs to the terminal functional group of capping agents at the

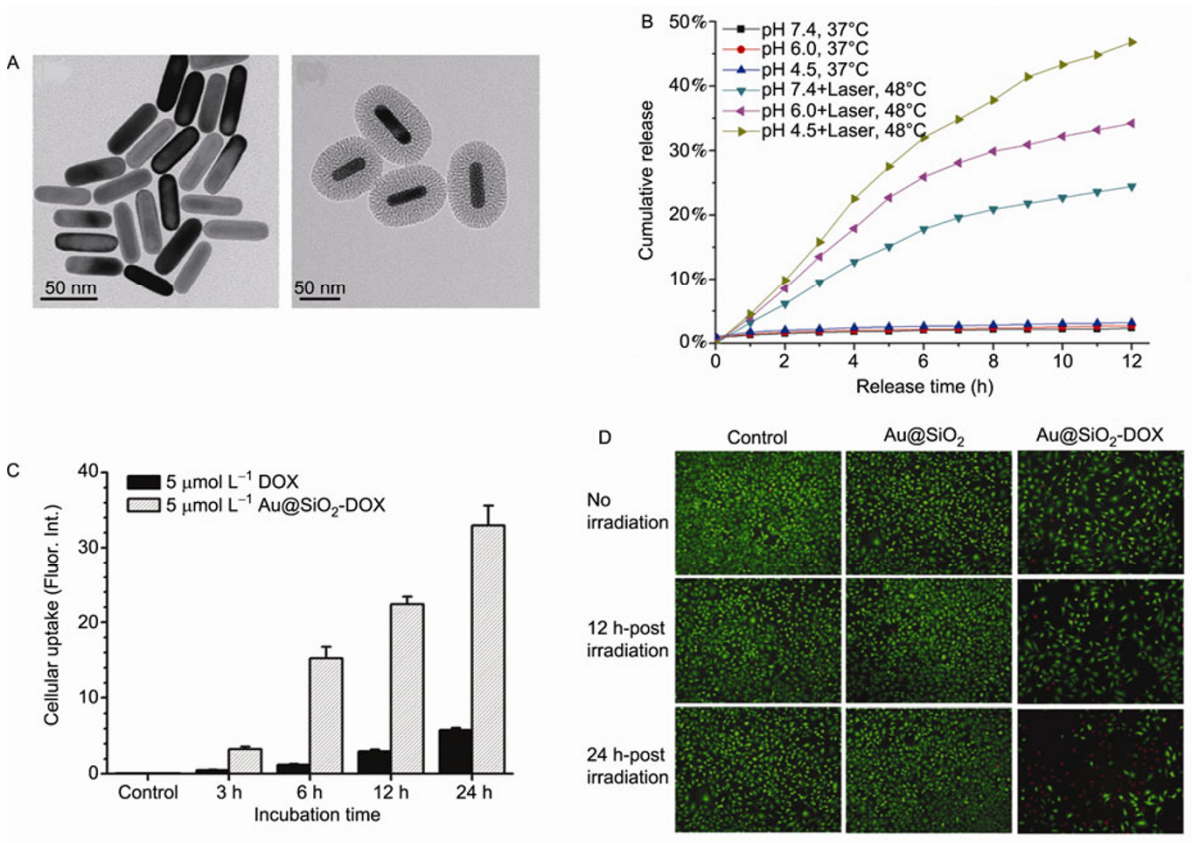

Figure 6 Mesoporous silica-coated Au nanorods (Au@SiO_2) developed as novel drug carriers for the delivery of DOX *in vitro*. A, TEM images of Au nanorods (left), and Au@SiO_2 (right). B, DOX release profiles from Au@SiO_2-DOX triggered by NIR laser irradiation at different pH. C, Cellular uptake of DOX and Au@SiO_2-DOX quantified by flow cytometry. D, Live-dead staining of A549 cells 12 and 24 h post laser irradiation. A549 cells were treated with Au@SiO_2 or Au@SiO_2-DOX for 12 h and then radiated for 3 min using a NIR laser with a power density of 24 W cm^{-2}. Reproduced with permission from ref. [70].

outermost layer of Au nanoparticles, especially those Au nanoparticles already passivated with various functional groups. (iv) Loading by layer-by-layer assembly. Takes advantage of the highly charged surface of Au nanoparticles caused by the presence of charged capping agents to achieve the attachment of oppositely charged substances (e.g., cationic Au nanoparticles that are positively charged versus DNA or siRNA that are negatively charged). (v) Loading inside the nanoparticles. Design nanostructures that possess internal reservoirs, such as Au nanocages and hollow Au nanoshells, to load drugs.

By employing the strategies listed above, several therapeutics have been successfully delivered by Au nanoparticles in cancer treatment, including receptor-targeted molecules (e.g., TNF-α [71], and tamoxifen [72]), classical cytotoxic agents (e.g., cisplatin [73], oxyplatin [74], DOX [75], paclitaxel [76] and MTX [77]) and photosensitizer molecules [78]. By conjugating TNF-α to spherical Au nanoparticles with a diameter of 26 nm, Paciotti and co-workers [71] observed 7–10-fold greater tumor accumulation of TNF-α in a mouse model administered with the nanoparticle conjugates versus the equivalent mass of protein. The assembly of TNF-α within tumors induced complete tumor regression in 25%–30% of treated mice, which is twice as effective as TNF-α alone. Systemic toxicity was also reduced because no death was observed in THF-α conjugate-treated mice while 15% mortality was observed after treatment with TNF-α alone. Mirkin *et al*. [73] used the same spherical Au nanostructures of smaller size (13 nm in diameter), to load a prodrug form of the widely administered chemotherapeutic, cisplatin. By conjugating the inert Pt^{4+} analog of cisplatin (Pt^{2+}) to Au nanoparticles, efficient delivery of the prodrug into the cytoplasm was achieved in prostate cancer cells without endosome sequestration. Subsequent intracellular reduction of the prodrug caused activation to the cytotoxic Pt^{2+} form, resulting in colocalization of the particles with microtubules and the formation of intrastrand crosslinked nuclear DNA. *In vitro* cytotoxicity was dramatically enhanced over that of the free prodrug, demonstrating that Au nanoparticles are efficient vehicles for the delivery of prodrugs with poor cellular uptake. Targeted delivery of photosensitizer molecules using Au nanoparticles for photodynamic therapy (PDT) is another strategy for the selective treatment of malignant tissues. In PDT, molecules that generate singlet oxygen upon excitation need to be selectively delivered to malignant tissues and activated by exposure to

laser radiation. Burda and co-workers [78] studied the delivery of phthalocyanine photosensitizers to cervical cancer cells *in vitro* using PEGylated Au nanoparticles with a diameter of 5 nm. Interestingly, they found that the efficiency of particle delivery and PDT was superior when using labile amino linkages *versus* stronger thiol bonds between the nanoparticles and phthalocyanine, highlighting the significance of bonding interactions on subsequent treatment efficacy.

As mentioned above, the charged surface of Au nanoparticles is an ideal vehicle for the attachment of therapeutics carrying the opposite charge [44]. Thus, Au nanoparticles are considered to be a perfect candidate for gene delivery. Recently, we evaluated the capacity of different positively charged coating agents, including CTAB, PDDAC and polyethylenimine (PEI), on DNA adsorption and cellular transfection using Au nanorods [79]. Interestingly, the three coating agents exhibited similar DNA adsorption efficiencies ranging from 40%–60%, but varied in transfection performance in HEK293 cells. 48 h post transfection, bright fluorescence was observed from cells treated with PDDAC- and PEI-coated nanorods but not CTAB-coated ones (nanorods were carrying plasmid encoding enhanced green fluorescent protein as an indicator). Notably, the fluorescence intensity from cells treated with PDDAC- and PEI-coated nanorods treated cells was as high as that from commercial transfection agent-PEI treated cells. Subsequent nucleus localization experiments gave similar results. DNA delivered by PDDAC and PEI-coated nanorods was present in the nucleus as early as 2.5 h post transfection, whereas CTAB-coated nanorods produced comparable results after more than 5 h. In this case, Au nanorods coated with positively charged capping agents were an effective means to deliver DNA to both the cytoplasm and nucleus of cells. Novel anti-cancer vaccines could be developed by using Au nanoparticles as tumor-associated antigen carriers.

4 Conclusion and perspective

Au nanoparticles are a large family of nanostructures derived from different chemical conditions. The tremendous diversity of Au nanoparticles has raised a number of possibilities for their design in treating cancer from different aspects. As illustrated in this review, a growing body of evidence has already revealed the huge theranostic potential of Au nanoparticles in serving as (i) photothermal contrast agents, (ii) imaging contrast agents, (iii) drug/gene delivery vehicles and (iv) intrinsic anti-cancer agents. Although the success achieved so far is very encouraging, there are still several important questions that need to be clarified. Biocompatibility is the first and most important issue. To date, the majority of data obtained from published articles suggests Au nanoparticles show low toxicity in experimental systems. However, in most cases toxicity assays were conducted on either cultured cell monolayers or living animals for a short term. Thus, systemic evaluation of toxicity with an additional focus on the long-term effects of Au nanoparticles on human health is critical for their future application in medicine [80]. Another important question is the actual therapeutic efficacy of Au nanoparticles in clinical treatment. In most studies, the experiment model is highly specific (e.g., the selectivity of ligand-conjugated nanoparticles is always tested on a specific cell line with abundant receptors), which means the same results may not be achieved under general conditions. Therefore, despite their vast potential in cancer therapy, further effort is needed to acquire detailed information that will promote the development of Au nanoparticles for clinical applications.

This work was supported by the National Basic Research Program of China (Grant Nos. 2011CB933401 and 2012CB934003), the National Natural Science Foundation of China (Grant No. 31070854), and National Major Scientific Instruments Development Project (Grant No. 2011YQ03013406).

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