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Gold Nanocages for Biomedical Applications**

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Abstract

¶ Nanostructured materials provide a promising platform for early cancer detection and treatment. Here we highlight recent advances in the synthesis and use of Au nanocages for such biomedical applications. Gold nanocages represent a novel class of nanostructures, which can be prepared via a remarkably simple route based on the galvanic replacement reaction between Ag nanocubes and HAuCl₄. The Au nanocages have a tunable surface plasmon resonance peak that extends into the near-infrared, where the optical attenuation caused by blood and soft tissue is essentially negligible. They are also biocompatible and present a well-established surface for easy functionalization. We have tailored the scattering and absorption cross-sections of Au nanocages for use in optical coherence tomography and photothermal treatment, respectively. Our preliminary studies show greatly improved spectroscopic image contrast for tissue phantoms containing Au nanocages. Our most recent results also demonstrate the photothermal destruction of breast cancer cells in vitro by using immuno-targeted Au nanocages as an effective photo-thermal transducer. These experiments suggest that Au nanocages may be a new class of nanometer-sized agents for cancer diagnosis and therapy.

1. Introduction

Metal nanostructures have garnered significant research attention because of their unique and tunable surface plasmon resonance (SPR) properties.[1] SPR is an optical phenomenon arising from the collective oscillation of conduction electrons, which is also responsible for the brilliant colors of metal colloids, such as those first prepared by Michael Faraday in 1856 from the chemical reduction of gold chloride with phosphorus.[2] At the resonant frequency, the incident photons can either be scattered (at the same frequency but in all directions) or absorbed and subsequently converted into phonons (i.e., vibrations of the lattice). Therefore, the SPR peak of a metal nanostructure typically includes both scattering and absorption components.

Interestingly, the resonant frequency and the cross-sections of both scattering and absorption components are dependent on the size, shape, and chemical composition of the nanostructure.

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[3] This observation allows one to tune the SPR features of metal nanostructures, making such materials potentially very useful. For example, with their SPR peaks in the visible region, Au colloids have been demonstrated for colorimetric sensing in the selective detection of heavy metal ions and various biomolecules.[4] Additionally, given the biocompatibility and ease of surface modification of Au, biomedical applications have been explored that utilize the scattering and/or absorption properties of Au nanostructures.[5] To this end, Au nanostructures can serve as a class of agents for imaging contrast enhancement or photothermal therapy if their SPR peaks can be tuned into the near-infrared region (particularly, 700 to 900 nm, where blood and soft tissue are relatively transparent).[6]

In 1908, Mie derived the formula for calculating the wavelength-dependent scattering and absorption cross-sections of solid spherical particles with dimensions less than the wavelength of light.[7] It was predicted that Au nanospheres 40 nm in diameter would have an extinction peak centered at 520 nm, whereas smaller Au spheres would be slightly blue-shifted and larger Au spheres would be slightly red-shifted (e.g., Au colloids 140 nm in diameter would have an SPR peak around 650 nm). These predictions have been validated experimentally, demonstrating the utility of Mie's formula for predicting the SPR spectra of spherical particles. Such calculations, however, also indicate that a different type of nanostructure must be fabricated in order to produce Au nanostructures with their SPR peaks tuned to the near-infrared.

To this end, researchers have considered the following Au nanostructures: i) aggregates of spherical nanoparticles;[8] ii) nanorods;[9] and iii) composite or hollow nanostructures.[6c–g,10] The third option is particularly interesting as different surfaces and/or hollow interiors provide a platform for multi-functionalization and encapsulation. In 1989, Neeves and Birnboim calculated the SPR spectra for composite spherical particles (i.e., those consisting of a metal shell and dielectric core such as silica or air) and found that they would give rise to SPR modes extending into the near-infrared region.[11] It has, however, been difficult to verify these predictions experimentally. Halas and coworkers were among the first to prepare such materials and validate the calculations of Neeves and Birnboim.[12] Halas' approach was to coat silica or polymer beads with Au shells of variable thickness via a direct deposition/chemical reduction method.[13] While this approach has been used as a general synthetic route to dielectric/metallic core-shell particles, the preparation of such composite structures is nontrivial, since most metals do not sufficiently wet the surface of an oxide or polymeric material.[14] In addition, it is very difficult to obtain a smooth surface coating, together with accurate control over the shell thickness on the scale of a few nanometers. Alternatively, we have recently demonstrated the capability and feasibility to prepare Au nanostructures with hollow interiors via a galvanic replacement reaction with Ag nanostructures as a sacrificial template.[15] Using this approach, hollow and porous Au nanostructures can be routinely synthesized from Ag nanostructures of any morphology. Here, we review recent advances in both the preparation and potential use of Au nanocages for biomedical applications.

2. Synthesis and Optical Properties of Au Nanocages

In the presence of Ag solid, HAuCl₄ can be reduced to generate Au atoms via the galvanic replacement reaction in aqueous solution:

Silver nanostructures of controlled morphology provide a template surface for Au nucleation and growth, imparting their shape to the resultant Au nanostructures. Hollow structures are produced as a result of the template effect and the reaction stoichiometry. In this way, Au

nanotubes are derived from Ag nanowires.[15e] Similarly, as the focus of this review, Au nanoboxes (hollow, nonporous walls) and nanocages (hollow, porous walls) are prepared from templates based on Ag nanocubes, which can be produced in large quantities using polyol reduction.

2.1. Ag Nanocubes via the Polyol Synthesis

Polyol reduction provides a simple and convenient method for generating Ag nanostructures with controlled morphologies.[16] For this technique, ethylene glycol serves as both the solvent and reducing agent. In a typical synthesis, AgNO_3 is reduced by ethylene glycol to generate Ag atoms and then nanocrystals, or seeds. Subsequent addition of Ag atoms to the seeds produces nanostructures. In the presence of poly(vinyl pyrrolidone) (PVP), a polymer that selectively binds to {100} facets, addition of Ag atoms can be directed to other crystal faces to generate Ag nanostructures with well-defined and controllable shapes. For example, in the presence of PVP, pentagonal Ag nanorods enclosed by {100} side faces and {111} ends can be transformed into nanowires tens of micrometers in length as the {100} facets are preferentially passivated by PVP, facilitating addition of Ag to the {111} facets.[15] Similarly, when PVP is present, preferential addition of Ag atoms to the {111} facets of single-crystal, cuboctahedral seeds produces nanocubes with sharp corners, like those shown in Figure 1A.

The key to producing a particular Ag nanostructure in high yields is to control the crystallinity of the initial seed. Under typical reaction conditions, single-crystals are produced as well as twinned seeds; however, recent work in our group has shown that twinned seeds can be selectively etched by O_2 /chloride, leaving behind only single-crystals in solution.[17] The single-crystal seeds can then grow to produce Ag nanocubes. More recently, we have also been able to effectively limit twinned seed formation by enhancing the reduction rate of Ag^+ . [18] This was achieved by adding a trace amount (on the ppm level) of sodium sulfide to the reaction medium, producing Ag_2S nanocrystallites that can catalyze the reduction of additional Ag^+ . This method is particularly advantageous as the overall reaction time can be significantly shortened from ~24 hours to less than 10 minutes. Additionally, a more monodispersed sample can be produced by limiting the process to a single nucleation event. Electron diffraction indicates that the Ag nanocubes are single crystals. This method is becoming the most commonly used procedure for producing Ag nanocubes in large quantities.

2.2. Au Nanocages via the Galvanic Replacement Reaction

The Ag nanostructures can be transformed into Au nanostructures with hollow interiors via the galvanic replacement reaction shown in Equation 1. The mechanism involved in this process has been systematically investigated using both electron microscopy and spectroscopy methods. Figure 1, A–C, shows SEM and TEM images of samples derived from Ag nanocubes with sharp corners.[19] This work shows that the replacement proceeds through the following steps: i) initiation of Ag dissolution by pitting at a specific site on the surface of a Ag nanocube; ii) formation of a pinhole-free nanobox consisting of thin, uniform walls through a combination of galvanic replacement and Au–Ag alloying; and iii) generation of pores in the wall through a dealloying process. A schematic of this mechanism is shown in Figure 2A.

We have also demonstrated the possibility of producing Au nanocages with pores specifically localized at all the corners.[20] This was achieved by using truncated Ag nanocubes (Fig. 1D) as a template for the galvanic replacement reaction. To prepare truncated Ag nanocubes, the as-produced Ag nanocubes with sharp corners were aged in ethylene glycol to which only a small amount of PVP had been added.[21] As PVP mainly interacts with the {100} facets, the poorly covered corners become truncated and terminated in {111} facets. When added to an aqueous solution of HAuCl_4 , the unprotected corners serve as primary sites for Ag dissolution, leading to the formation of well-defined pores. This reaction was monitored by SEM and TEM

(Figure 1, D–F) and is schematically represented in Figure 2B. Notice that these results contrast greatly with those obtained when Ag nanocubes with sharp corners were used as the template.

Very recently, we have synthesized Au nanocages with sizes smaller than 20 nm.[22] It is generally difficult to prepare Ag nanocubes, and subsequently Au nanocages, with dimensions less than 30 nm. Using Ag multiply twinned particles (MTPs; sizes ~11 and ~14 nm) as a sacrificial template for the galvanic replacement reaction, we have prepared ~12 and ~15 nm polycrystalline hollow Au structures with 2 to 5 nm voids in their interiors. This process was also monitored by TEM (Fig. 1G–I) for the 14 nm MTPs and is schematically represented in Figure 2C. Like the larger Au counterparts, these nanocages also have a tunable SPR peak. For example, the 14 nm Ag MTPs have an SPR peak at ~404 nm, which can be continuously shifted towards the red by adding H_{AuCl}₄ into the suspension. The SPR peak can reach 740 nm before the porous structures eventually collapse into small pieces. Many biomedical applications could benefit from these small nanocages due to enhanced diffusion and delivery rates.

2.3. Optical Properties of Au Nanocages

Recall that the size, shape, and composition of metal nanostructures can all influence the position of their corresponding SPR peak. Thus, the galvanic replacement of Ag nanocubes with H_{AuCl}₄ provides a facile way of tuning the SPR peak of the resultant nanostructure as both the morphology and composition of the material is controllably altered. Figure 3A shows extinction spectra that were obtained by titrating 30 nm Ag nanocubes with different volumes of H_{AuCl}₄ solution (0.2 mM). Significantly, the position of the SPR peak is continuously shifted from the visible (~400 nm for the nanocubes) to the near-infrared as more H_{AuCl}₄ solution is added (~900 nm after 1.4 mL of H_{AuCl}₄ has been added).

Discrete dipole approximation (DDA) calculations indicate that Au nanocages have large scattering and absorption cross-sections. With the DDA method, a particle is approximated by a sufficiently large array of polarizable point dipoles; once the location and polarizability of each individual unit has been specified, the scattering and absorption cross-sections of the particle can be obtained.[23] Figure 3B and C, shows the DDA results for two Au nanocages with different inner edge lengths (30 nm vs. 50 nm); both the wall thickness and corner hole dimensions were held constant. For the 30 nm nanocage, light absorption dominates; whereas, for the 50 nm nanocage, light scattering becomes more significant.

Additional DDA calculations indicate that the SPR peak position is highly sensitive to wall thickness, while the magnitude of both the scattering and absorption cross-sections depends only slightly on wall thickness.[24] The magnitude of both the scattering and absorption cross-sections does, however, depend on the porosity of the nanocages, with both decreasing as the number of holes increase; the SPR peak position does not change with porosity. As these calculations indicate, in addition to being able to finely tune the SPR peak position, the magnitudes of both the absorption and scattering components can be tailored by simply controlling the size and porosity of the nanocages. This flexibility is invaluable when preparing such materials for different biomedical applications, as explained in more detail in Sections 4 and 5.

3. Bioconjugation of Au Nanocages

The surface of Au nanocages has to be derivatized with targeting molecules (such as ligands and antibodies) to achieve cancer cell specificity. Fortunately, Au surfaces can be functionalized with a variety of molecules and ligands using the well-established thiolate-Au monolayer chemistry.[25] Sulfur has a particularly strong affinity for Au (85 to 145 kJ/mol), with organosulfur molecules such as octadecanethiol spontaneously assembling into stable and highly organized layers on Au surfaces. This so-called self-assembled monolayer (or SAM)

binds to a Au surface through the sulfur atoms and form an interface defined primarily by the head group (i.e., in the case of octadecanethiol, a methyl group). As a result, simply varying the thiol molecule used in SAM preparation, the exposed head group can be altered to provide a site for additional functionalization. For example, the carboxyl head groups of SAMs can react with amines, forming an amide bond.[26]

This monolayer chemistry can be exploited to functionalize Au nanocages with biological molecules (e.g., antibodies, nucleic acids, and small-molecule inhibitors, many of which contain primary amines) that can selectively recognize the specific receptors on tumor cells. This allows for high concentrations of material to localize at sites of interest. To demonstrate this technique, we functionalized Au nanocages with tumor-specific antibodies.[27] Specifically, a breast-cancer cell line SK-BR-3 which over expresses epidermal growth factor receptor 2 (EGFR2 or HER2) was used to test the specificity. In a typical procedure, the primary antibody (monoclonal anti-HER2) was immobilized on the cancer cells by incubating the SK-BR-3 cells in a buffered anti-HER2 antibody solution. A buffered solution containing fluorescence-labeled immunoglobulin G (IgG) functionalized Au nanocages prepared by the protocol shown in Figure 4A was then applied to the anti-HER2-bound SK-BR-3 cells. After extensive washing, a fluorescence image (Figure 4B) of the cells revealed a uniform green color, indicating a relatively homogeneous distribution of the primary anti-HER2 antibody on the cell surface.

4. Au Nanocages as Optical Contrast Agents

Optical coherence tomography (OCT) and spectroscopic optical coherence tomography (SOCT) are emerging as very promising biomedical diagnostic tools for the noninvasive, *in vivo* imaging of biological tissues for early cancer detection.[28] For both OCT and SOCT, image contrast arises mainly from the scattering and absorption of light by tissue, with both the sensitivity and specificity of OCT and SOCT being dependent on the intrinsic optical properties of the tissue sample. To improve image contrast, various light scattering and absorption materials (e.g., air- or oil-filled protein microspheres and silica-Au core/shell nanospheres ~100 nm in size) have been examined as potential contrast agents.[6d,29] Our Au nanocages are attractive contrast agents because of their strong, tunable SPR peaks in the near-infrared and comparatively smaller size.

To demonstrate the feasibility of Au nanocages as contrast enhancement agents, both OCT and SOCT were performed on phantom samples with and without Au nanocages.[6f,27] Specifically, 35 nm Au nanocages were selected, and their SPR peak was tuned to 700 nm, which is on the blue side of the OCT source spectrum. Each phantom was made of gelatin embedded with titania granules to mimic the scattering background of biological tissues. Gold nanocages were added to the phantom at a nM level. Both the OCT and SOCT imaging were conducted using a 7-fs Ti:Sapphire laser with a center wavelength of 825 nm and a bandwidth of 155 nm. As the laser was scanned over the tissue phantom, the interferometric OCT signal was measured as a function of sample depth. Using the SOCT technique,[28c] the depth-dependent back-scattered light spectrum along one-axial scan from the phantom can be obtained as shown in Figure 5A. Note that the blue side of the source spectrum is attenuated more than the red side, presumably because of the greater absorption of light by the Au nanocages at shorter wavelengths. Figure 5B shows a conventional OCT image of the tissue phantom displaying strong light attenuation on the right side that resulted from the strong absorption of the Au nanocages.

The SOCT image was obtained by encoding the spatially resolved centroid wavelength by hue, as shown in Figure 5C. The centroid is red shifted as evident on the right portion of the SOCT image, which corresponds to where Au nanocages were added to the phantom, and

demonstrates the enhanced spectroscopic contrast achieved when Au nanocages are added. Finally, the extinction cross-section of the Au nanocages was quantified from the depth-dependent OCT back-scattered intensity by spectroscopic OCT analysis. For example, at 716 nm, the back-scattering and extinction cross-sections of the 35 nm Au nanocages were determined to be $2.41 \times 10^{-15} \text{ m}^2$ and $1.29 \times 10^{-14} \text{ m}^2$, respectively. From these results, and using the ratio of absorption to the total extinction cross-section predicted by DDA calculation, we were then able to deduce the absorption cross-section to be $1.13 \times 10^{-14} \text{ m}^2$, which is about 5 orders of magnitude greater than that of the conventional dye Indocyanine Green (ICG) which has an absorption cross-section of $2.90 \times 10^{-20} \text{ m}^2$ at 800 nm.[30] These results suggest that Au nanocages represent a new class of absorption contrast agents for OCT. With such a large absorption cross-section, the Au nanocages might also be useful as thermal therapeutic agents.

5. Gold Nanocages for Photothermal Therapy

When light interacts with Au nanocages, the incident photons are either scattered or absorbed. The absorbed photons are converted into phonons (or vibrations of the lattice), causing an increase in temperature. Significantly, the rise of temperature can be enough to induce melting or surface reconstruction when the nanocages are placed in an environment such as air with poor thermal conductivity. In fact, exposure to a camera flash transforms the Au nanocages (deposited on a carbon-coated TEM grid and surrounded by air) into spherical droplets.[24, 31] In biological systems where water offers greater thermal conductivity, the Au nanocages should not melt but rather produce a local temperature rise that can provide a therapeutic effect on cancer cells that are selectively targeted by bioconjugated Au nanocages. Such photothermal therapy is less invasive than chemotherapy or surgery and holds strong promise as a new form of cancer treatment.[32]

Very recently, we demonstrated the selective photothermal destruction of cancer cells *in vitro* using immuno Au nanocages.[6g] For this study, 45 nm Au nanocages were prepared with their SPR peaks tuned to 810 nm to match the center wavelength of laser irradiation used for photothermal experiments. The nanocages were functionalized with the HER2-antibody to target the EGFR2 receptor, which over-expresses on the surface of SK-BR-3 breast cancer cells. After incubation with the immuno Au nanocages, the SK-BR-3 breast cancer cells were irradiated with a femto-second Ti:Sapphire laser at varying power densities for 5 minutes. To assess the effectiveness of the treatment, the cells were then treated with calcein AM and Ethidium homodimer 1 (EthD-1) following standard staining protocols and subsequently examined by fluorescence microscopy. Calcein AM is non-fluorescent but is enzymatically converted into fluorescent green calcein by living cells. EthD-1, on the other hand, fluoresces red only when it is able to penetrate through irreversibly compromised cell walls and nucleus membranes to stain DNAs, thus indicating cellular death. Figure 6A and B shows the corresponding fluorescence microscopy images for SK-BR-3 cells treated with immuno Au nanocages when a power density of 1.5 W/cm^2 was used. The results from a control experiment in which no Au nanocages were used are shown in Figure 6C and D. When the Au nanocages were incorporated, a well-defined area of cellular death corresponding to the laser spot ($\sim 2 \text{ mm}$ in diameter) was observed. In comparison, little cellular death occurred when no Au nanocages were used. Below a power density of 1.5 W/cm^2 , the SK-BR-3 cells derivatized with Au nanocages remained alive. Note that this threshold for selective cancer cell destruction is substantially lower than those reported for Au nanoshells (35 W/cm^2) and Au nanorods (10 W/cm^2), which can be attributed to the larger absorption cross-section of our Au nanocages and/or a higher density of coverage of cancer cells by the immuno Au nanocages (partially resulting from the compact size of these nanostructures).

6. Concluding Remarks

The synthesis of Au nanocages requires nanoscale templates of silver. With their uniform shape and size, Ag nanocubes seem to be the ideal template for generating Au nanocages via the galvanic replacement reaction. The recent advancement in Ag nanocube synthesis, through the mediation of sodium sulfide, offers a powerful scaling-up approach for producing monodispersed Ag nanocubes in large quantities and in less than 10 minutes. This result in turn enables larger-scale production of Au nanocages. Additionally, by simply adjusting the ratio of Ag to HAuCl_4 , we are able to precisely control the SPR peaks of the resultant nanostructure to any wavelength from 400 to 1200 nm, while DDA calculations indicate that the Au nanocages have large scattering and absorption cross-sections that can be utilized in a variety of applications.

Our preliminary *in vitro* data with both OCT imaging and photothermal therapy demonstrate that the Au nanocages are promising materials for biomedical applications and could be effective tools for *in vivo* optical detection and subsequent treatment of cancers. Indeed, cancer survival rates could be greatly improved through the development of more effective and earlier methods of detection. Typically, tissue biopsy is used to detect cancer, but this method is both invasive and subject to high sampling errors. Optical imaging methods such as OCT provide an attractive alternative. Additionally, more effective methods of cancer treatment are under extensive investigation, but a general approach that has the power of chemotherapy without its harmful side effects is still missing. We believe that our Au nanocages, functionalized with cancer-specific antibodies, could provide a platform for simultaneous cancer detection and site-specific treatment.

Both Au nanoshells and rods have already been demonstrated for both cancer detection and treatment; however, our Au nanocages may provide some additional advantages. These features include the easy synthesis of Au nanocages, the ability to precisely tune their SPR peak position, and the convenient variation of their scattering and absorption cross-sections. Additionally, the cage structure of our materials opens up the possibility for the encapsulation of various items including drugs for targeted delivery and controlled release, as well as magnetic nanoparticles for field-directed delivery. To achieve these goals, new methods of Au nanocage generation and modification might be necessary and are underway. For example, we have very recently demonstrated the ability to selectively etch Ag from alloyed Au-Ag nanoboxes.[33] This process decouples the dealloying of silver from the deposition of gold, generating Au nanocages with thinner and more porous walls and further red-shifted SPR peaks than previous methods.

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Figure 1.

SEM and TEM images showing three different stages of the galvanic replacement reaction with different types of Ag nanostructures as the sacrificial template. (A–C) SEM images of Ag nanocubes with sharp corners titrated with 0.1 mM aqueous HAuCl_4 (0, 0.6, 1.6, and 3.0 mL, respectively). (D–F) SEM images of Ag nanocubes with truncated corners titrated with the same volumes of 0.1 mM HAuCl_4 . The insets of A–F give TEM images of each respective sample (scale bar = 100 nm for all TEM images). (G–I) TEM images of 14 nm multiple twinned particles (MTPs) of Ag titrated with 0.5 mM HAuCl_4 (0, 1.2, and 1.8 mL, respectively). Reproduced with permission from [19], [20], and [22]. Copyright 2004, 2006, and 2007, American Chemical Society.

Figure 2. Schematic illustrations detailing all major steps involved in the formation of Au nanocages during the galvanic replacement reaction with three different types of Ag templates: (A) Ag nanocubes with sharp corners; (B) Ag nanocubes with truncated corners; and (C) multiply twinned particles (MTPs) of Ag with an icosahedral shape.

Figure 3.

(A) UV-vis-NIR extinction spectra recorded from aqueous suspensions of Ag nanocubes (30 nm in edge length) after they have been titrated with different volumes of 0.2 mM HAuCl₄. Insert: a TEM image of Au nanocages derived from Ag nanocubes of 30 nm in edge length. (B) Extinction spectra calculated using the DDA method for a Au nanocage of 30 nm in inner edge length, 5 nm in wall thickness, and with 5 nm pores at all corners. (C) Extinction spectra calculated using the DDA method for a Au nanocage 50 nm in inner edge length, 5 nm in wall thickness, and with 5 nm pores at all corners.

Figure 4.

(A) A schematic illustration of the two-step protocol used to conjugate antibodies to the surface of Au nanocages. In the first step, succinimidyl propionyl poly(ethylene glycol) disulfide (NHS-activated PEG, M.W. = 1,109) was reacted with the primary amine of an antibody. In the second step, the PEG-antibody complex was bonded to the Au nanocage by breaking the internal disulfide bond and forming the Au-S linkage. (B) A fluorescence image of SK-BR-3 cells whose surfaces were treated with the anti-HER2 antibodies, followed by incubation with fluorescence-labeled IgG. Reproduced with permission from [27]. Copyright 2005 American Chemical Society).

Figure 5.

(A) Spatially resolved, depth-dependent spectra of the back-scattered light from the phantom. (B) Intensity OCT image of the phantom displaying the strong attenuation of the light back-scattered from the portion which contained Au nanocages. (C) SOCT image illustrating the depth-resolved change in the back-scattered spectral centroid. Reproduced with permission from [6f]. Copyright 2005 American Optical Society Publisher.

Figure 6.

SK-BR-3 breast cancer cells that were treated with immuno Au nanocages and then irradiated by 810 nm laser at a power density of 1.5 W/cm^2 for 5 minutes. A well-defined circular zone of dead cells was revealed by: (A) calcein AM assay, where green fluorescence indicates live cells, and (B) Ethidium homodimer-1 (EthD-1) assay, where red fluorescence indicates dead cells. In a control experiment, cells irradiated under the same conditions but without incubating with immuno Au nanocages maintained viability, as indicated by (C) calcein fluorescence assay, and (D) the lack of intracellular EthD-1 uptake. Reproduced with permission from [6g]. Copyright 2007 American Chemical Society.