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Gold nanoparticles (AuNPs) are promising nanocarriers for therapeutics due to their facile synthesis, ease of functionalization, biocompatibility, and inherent non-toxicity. The unique chemical and physical properties of AuNP monolayers provide versatility in delivery method and tunability of surface properties. Here, we discuss several strategies to utilize the properties of AuNPs for drug delivery.

1. Introduction

Considerable effort has been focused on the creation of drug delivery systems (DDSs) for improving cancer chemotherapy, with the goals of enhancing therapeutic selectivity and efficacy.[1] One area where DDSs have proved highly beneficial is in targeted delivery, where marked improvements in chemotherapy have been achieved through passive and/or active targeting approaches with nanocarriers including liposomes,[2] polymer micelles and vesicles,[3] dendrimers,[4] and metal nanoparticles.[5]

Gold nanoparticles (AuNPs) have recently emerged as highly promising DDSs.[6] AuNPs have multiple aspects that make them well-suited for use in delivery applications. First, AuNPs of many different sizes (1–150 nm) can be fabricated in a controlled fashion with control over size dispersity,[7] whereas polymer-based carriers can be heterogeneous in structure due to high polydispersity.[5] Furthermore, AuNPs have high surface-area-to-volume ratios, and, as a result, a high density of ligands can be appended for targeting or drug-loading purposes. For example, a 2 nm diameter core AuNP can load ~100 ligands per particle.[8] Secondly, functional diversity can be readily achieved by creation of multifunctional monolayers using techniques such as ligand place-exchange reactions,[9] allowing multiple functional moieties such as drugs and targeting agents to be placed onto the particle surface with fewer synthetic challenges than many other delivery vehicles.[5] Finally, the gold core is essentially inert, non-toxic, and biocompatible, making it an ideal starting point for carrier construction.[10]

The ease of functionalization of AuNPs has enabled their use in a variety of delivery strategies Scheme 1). In one approach, prodrugs can be covalently conjugated to AuNPs *via* cleavable linkers. Alternatively, hydrophobic drugs can be non-covalently loaded onto AuNPs, allowing conjugation without structural modification of the drug payload. Once loaded, AuNP payloads can be released by either internal (*e.g.* glutathione)[6a] or external (*e.g.* light)[11] stimuli. The versatility of the AuNP monolayer is central to both release mechanisms, providing tunability for endogenous release mechanisms and a functional platform for external release strategies.

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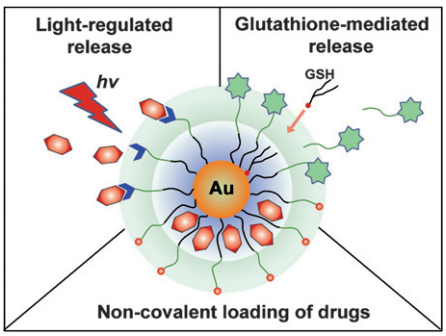


Scheme 1 AuNPs as a multimodal drug delivery system.

Several reviews regarding Au NPs for biomedical applications have recently been published, generally describing biosensing, diagnostics, and therapy.[12] In this review we will discuss various strategies which have been employed in creating AuNPs as DDS platforms, focusing on the role of the monolayer structure on DDS function.

2. Synthesis of gold nanoparticles

One of the key advantages of AuNPs is our ability to fabricate these nanomaterials with a wide variety of core sizes. Some general synthetic procedures of core–shell AuNPs are summarized in Table 1. The one-pot protocol developed by Brust and Schiffrin *et al.* in 1994 (Scheme 2) is of particular interest.[13] A wide variety of monolayer-protected AuNPs can be formed rapidly and in a scalable fashion using this approach. In this method, an $AuCl_4^-$ salt is reduced with $NaBH_4$ in presence of the desired ligands. The core size of these particles can be varied from 1.5 nm to ~6 nm by varying the ligand–gold stoichiometry. Larger particles can be fabricated through either ripening approaches[14] or *via* citrate reduction of gold salts.[15] To enhance the functional versatility of AuNPs, mixed monolayer-protected AuNPs can be synthesized directly with proper ligands or through post-functionalization. The most commonly used method for creation of mixed monolayer-protected AuNPs is through use of the place-exchange reaction first introduced by Murray (Scheme 2).[9] In this protocol, external thiols displace the existing ligands of AuNPs in an equilibrium process. Several research groups have fabricated delivery systems based on

Table 1 Synthetic methods and capping agents for AuNPs of varying core sizes

Core size (*d*)	Synthetic methods	Capping agents	References
1–2 nm	Reduction of $AuCl(PPh_3)$ with diborane or sodium borohydride	Phosphine	7
1.5–5 nm	Biphasic reduction of $HAuCl_4$ by sodium borohydride in the presence of thiol capping agents	Alkanethiol	13
3.5–10 nm	Heat-induced size ripening method	Alkanethiol	14
10–150 nm	Reduction of $HAuCl_4$ with sodium citrate in water	Citrate	15

AuNPs bearing functional moieties that are anchored with thiol linkers.[6] Control of ligand structure can be used to make the particles more suitable for delivery applications, *e.g.* through use of biocompatible oligo(ethylene glycol) (OEG) and poly(ethylene glycol) (PEG) moieties.[16]

3. Cellular uptake of gold nanoparticles

The first requirement for developing a DDS is an understanding of the interaction of the carrier with cells. Cellular uptake and intracellular fate of AuNPs depend on surface functionality and size.[17] Charge is also an important determinant,[18] Rotello and Vachet have demonstrated that cellular uptake of functionalized AuNPs is dependent on both charge and hydrophobicity.[19] Stellacci *et al.* have reported that cell membrane penetration by NPs was also dependent on ligand shell morphology,[20] with "striped" (structured ligand shell) NPs apparently capable of passing directly through the plasma membrane of the cells without creating pores on the cell membrane that can cause cytotoxicity (Fig. 1). Furthermore, organelle-specific localization of particles can be easily achieved by decorating the surface with a targeting moiety. For example, Feldheim *et al.* have demonstrated nuclear targeting of particles modified with a nuclear localization sequence (NLS).[21] Recently, Brust *et al.* have used transmission electron microscopy[22] to show that specific cellular targets such as the nucleus and other organelles can be targeted by modifying the particle surface with cell penetrating peptides (CPPs) [*e.g.* the oligopeptides TAT (AGRKKRRQRRR) and Pntn (GRQIKIWFQNRRMKWKK)] and the nuclear localization sequence (NLS: GGFSTSLRARKA) (Fig. 2).

Particle size provides the second key determinant of particle uptake. Chan *et al.* have demonstrated that cellular uptake depends on the size of nanoparticles.[23] AuNPs (2–100 nm) coated with Herceptin were fabricated and tested for ErbB2 receptor-mediated internalization in breast cells. They found that most efficient cellular uptake occurred with the 20–50 nm particles and programmed cell death (apoptosis) was enhanced by particles in the 40–50 nm range. The authors also have investigated the effect of AuNP size (10–100 nm) on passive targeting of tumors *in vivo*.[24] The larger nanoparticles remained near the vasculature, whereas smaller nanoparticles rapidly diffused from blood vessels to the tumor matrix (Fig. 3).

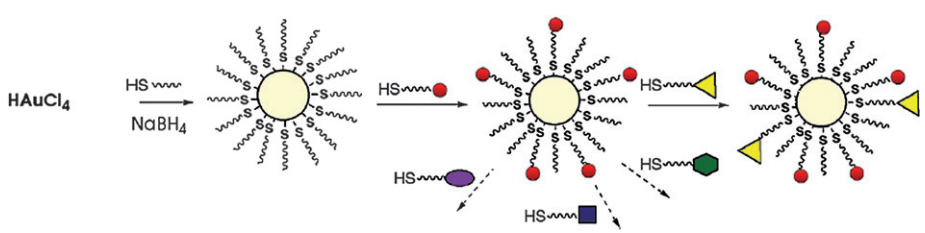
4. Drug attachment and release strategies using gold nanoparticles

Both the transport and release of drugs play critical roles in providing effective delivery systems. In general drugs can be loaded onto nanocarriers by either covalent conjugation or non-covalent interactions.[25] The non-covalent approach employs active drugs while the covalent attachment generally requires intercellular processing of a prodrug.[26] The ease of controlling the functionality and structure of AuNP monolayers makes them excellent platforms for DDS creation.

4.1 Glutathione-mediated release in covalent and non-covalent conjugation strategies

DDS systems based on glutathione (GSH)-mediated payload release exploit the dramatic higher intracellular GSH concentration (1–10 mM)[27] relative to extracellular thiol levels (GSH 2 μM, cysteine 8 μM). [28] The high levels of intracellular GSH can be used to release prodrugs (payloads) on AuNPs through either place-exchange reactions at the core or *via* disulfide exchange. Importantly, the monolayer of nanoparticles can provide steric shielding against exchange with surface cysteines of proteins in the bloodstream,[29] enabling their potential use *in vivo*.

In early studies, Rotello *et al.* developed a nanoparticle-based delivery system featuring glutathione release.[6a] The particles (core diameter: ~2 nm) featured a mixed monolayer composed of cationic ligands (TTMA) and fluorogenic ligands (HSBDP) (Fig. 4). The cationic surface of the nanoparticles facilitated their penetration through cell membranes, and the payload release was triggered by intracellular glutathione (GSH). In this delivery system, the Bodipy moiety of the HSDBP ligand was non-fluorescent when attached to the particle due to fluorescence quenching by the Au core.[30] The fluorescence was recovered upon GSH treatment in a cuvette, or with intracellular thiols in human liver cells (Hep G2). GSH-controlled release of the dye



Scheme 2 Formation of AuNPs using the Schiffrin reaction and a mixed monolayer of AuNPs using the Murray place-exchange reaction.

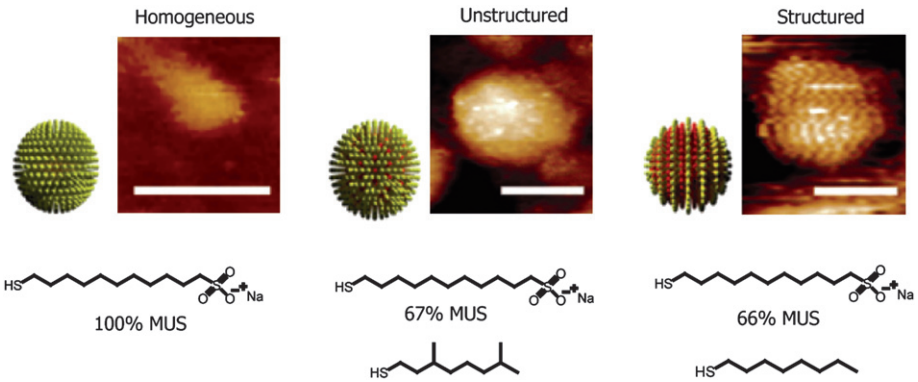


Fig. 1 Schematic diagrams of the ligand shell structure of the nanoparticles and representative STM images (scale bars 5 nm). Reprinted with permission from ref. 20 (Copyright 2008 Nature Publishing Group).

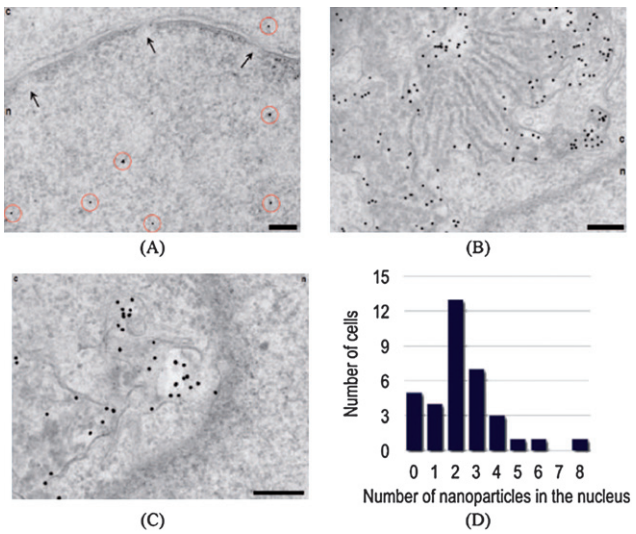


Fig. 2 Nuclear targeting (A) by PEG-modified nanoparticles functionalized with a combination of CPPs (2% TAT and 2% Pntn) and 2% NLS. Nanoparticles are highlighted by red circles. The nuclear envelope with nuclear pores (arrows) is clearly shown in this image. The nucleus is denoted 'n', and the cytosol 'c'. Unusual perinuclear membranous structures (B and C) that are highly loaded with nanoparticles are typically also observed under these conditions. Nuclear targeting is enhanced in comparison with experiments in the absence of CPPs (D). Scale bars are 200 nm. Reprinted with permission from ref. 22 (Copyright 2008 The American Chemical Society).

was verified by treating mouse embryonic fibroblast cells (MEF, having >50% lower intracellular GSH levels than HepG2) with varying concentration of glutathione monoester (GSH-OEt), transiently increasing the glutathione level inside the cells. In these studies, a dose-dependent increase in fluorescence was observed with increasing GSH-OEt concentration. In a similar fashion, AuNP-mediated DNA transfection efficiency was regulated by controlling the intracellular glutathione level.[31]

The ligand-displacement rate by intracellular thiols (*e.g.* DHLA and GSH) depends strongly on the monolayer structure and surface charge,[32] allowing controlled release of payload. As an example, Kotov *et al.* have demonstrated that AuNPs bearing 6-mercaptopurine-9-β-D-ribofuranoside significantly enhanced the anti-proliferative effect against K-562 leukemia cells compared to the same drug in free form.[33] This improvement was attributed to enhanced intracellular transport followed by the subsequent GSH-mediated release in cytoplasm and lysosomes.

In a strategy that combines glutathione-mediated release with non-covalent loading of drugs (*vide infra*), Kim *et al.* reported cyclodextrin-functionalized AuNPs as carriers of anti-cancer drugs.[34] The AuNPs used the cyclodextrin moieties to encapsulate drugs, anti-epidermal growth factor receptor (anti-EGFR) antibody as a targeting moiety, and poly(ethyleneglycol) (PEG) as an anti-fouling shell (Fig. 5). The anticancer drug β-lapachone, was efficiently encapsulated into the hydrophobic cavity of cyclodextrin on AuNPs and then released by intracellular GSH. The introduction of an anti-EGFR antibody onto the AuNPs was shown to both enhance the uptake of AuNPs and increase the degree of apoptosis.

4.2 Monolayer encapsulation of therapeutics

Non-covalent incorporation of drugs into AuNP monolayers provides an alternative delivery strategy that allows direct use of unmodified drugs, thus avoiding prodrug processing issues. Drug encapsulation with AuNPs relies on the use of ligands that generate a hydrophobic interior to the monolayer. Structurally, the radial nature of the monolayer results in a decrease in ligand density as one goes further from the core of small AuNP cores (< 6 nm).[35] Consequently "hydrophobic pockets" are created inside the monolayer of the AuNP into which hydrophobic materials can be partitioned. Pasquato *et al.* demonstrated the encapsulation of radical probes in AuNP monolayers, using EPR spectroscopy to monitor the partition of lipophilic probes between a monolayer of AuNPs and bulk water.[36] As expected, smaller particles featuring more strongly radial monolayers favor guest encapsulation (Fig. 6).

The concept of monolayer encapsulation has recently been applied to drug delivery. Rotello *et al.* have developed a biocompatible AuNP carrier that employs hydrophobic pockets to encapsulate drugs and deliver them into cancer cells.[37] The particles (~2.5 nm core) featured a hydrophobic alkanethiol interior and a hydrophilic shell composed of a tetra(ethylene glycol) (TEG) unit terminated with a zwitterionic headgroup designed to minimize nonspecific binding with biomacromolecules[38] and other cell surface functionalities (Fig. 7). Hydrophobic payloads were kinetically entrapped in the

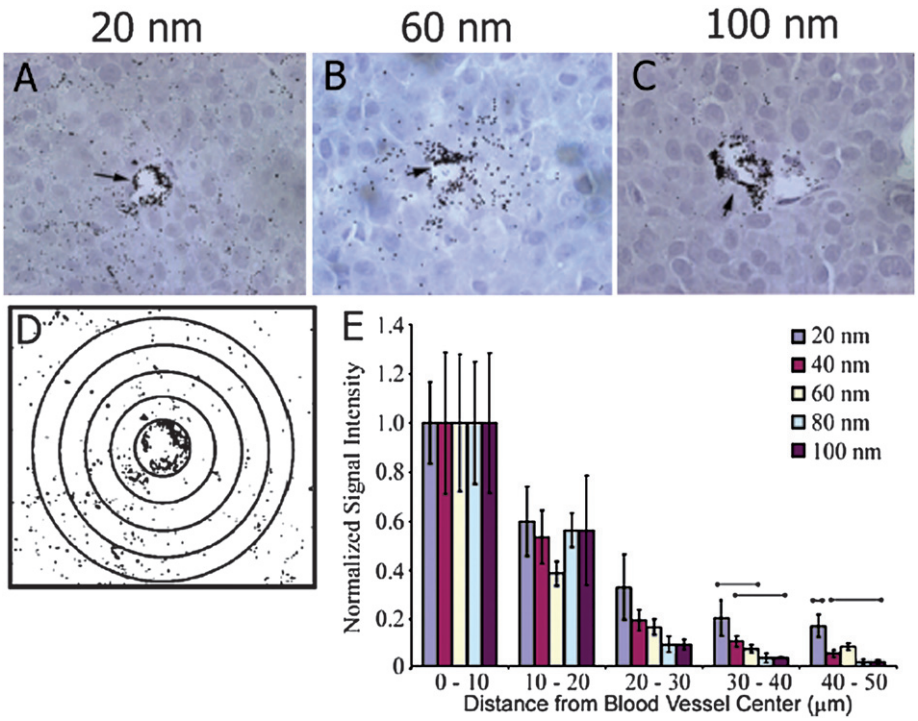


Fig. 3 Particle-size-dependent permeation of the tumor interstitial space. (A–C) Histological samples were obtained for 20, 60, and 100 nm particle sizes at 8 h post-injection (HPI). (D) ImageJ software was used to generate contrast-enhanced images for densitometry analysis. (E) Densitometry signal was quantified at 10 mm distances away from blood vessel centers 8 HPI and was normalized to the signal at 0–10 μm. Reprinted with permission from ref. 24 (Copyright 2009 The American Chemical Society).

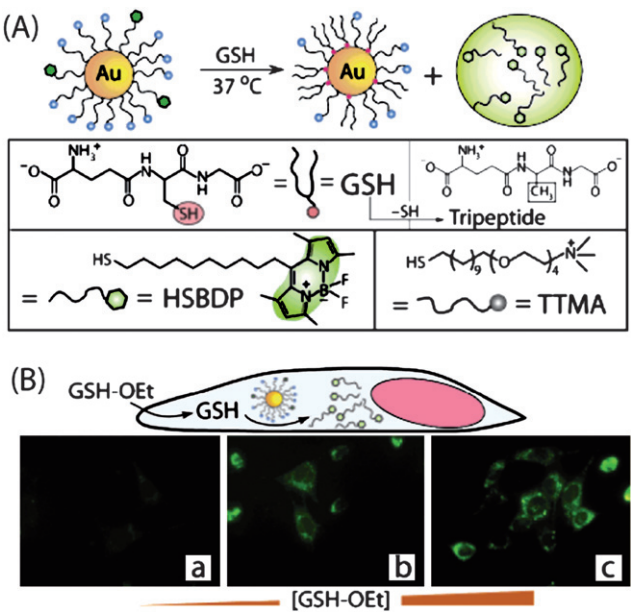


Fig. 4 (A) Schematic illustration of GSH-mediated surface monolayer exchange reaction/payload release. (B) Fluorescence images of MEF cells displaying GSH-controlled release of the fluorophore after incubation with 0, 5, and 20 mM GSH-OEt.

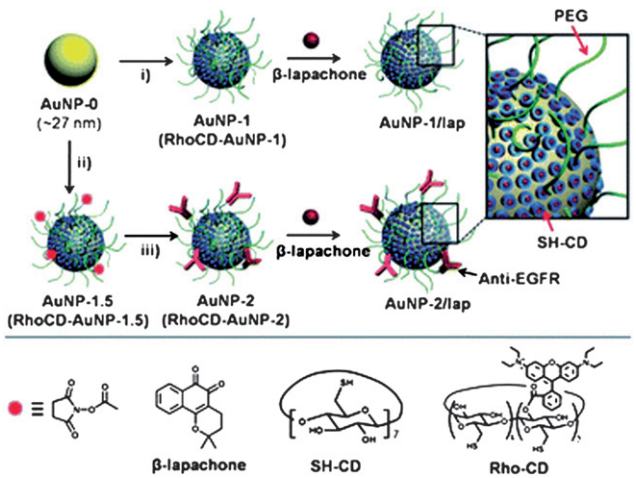


Fig. 5 Schematic illustration of the functionalization of AuNP carriers with β-lapachone, using: i) SH-CD and mPEG-SH for AuNP-1 (RhoCD and mPEG-SH for RhoCD-AuNP-1); ii) SH-CD, mPEG-SH, and NHS-PEG-SH for AuNP-1.5 (RhoCD, mPEG-SH, NHS-PEG-SH for RhoCD-AuNP-1.5) and iii) anti-EGFR. Reprinted with permission from ref. 34 (Copyright 2009 The Royal Society of Chemistry).

monolayer, with the resulting host–guest materials stable in buffer and serum. The entrapped payloads were released into cells by membrane-mediated diffusion, as demonstrated by both fluorescence microscopy (using a fluorophore payload) and through drug efficacy with therapeutic guests. No particle uptake was observed with these systems using ICP-MS, making these systems excellent candidates for passive targeting using the enhanced permeability and retention effect.[39]

Photodynamic therapy (PDT) is a promising strategy that uses reactive oxygen species (ROS) to induce apoptosis or necrosis.

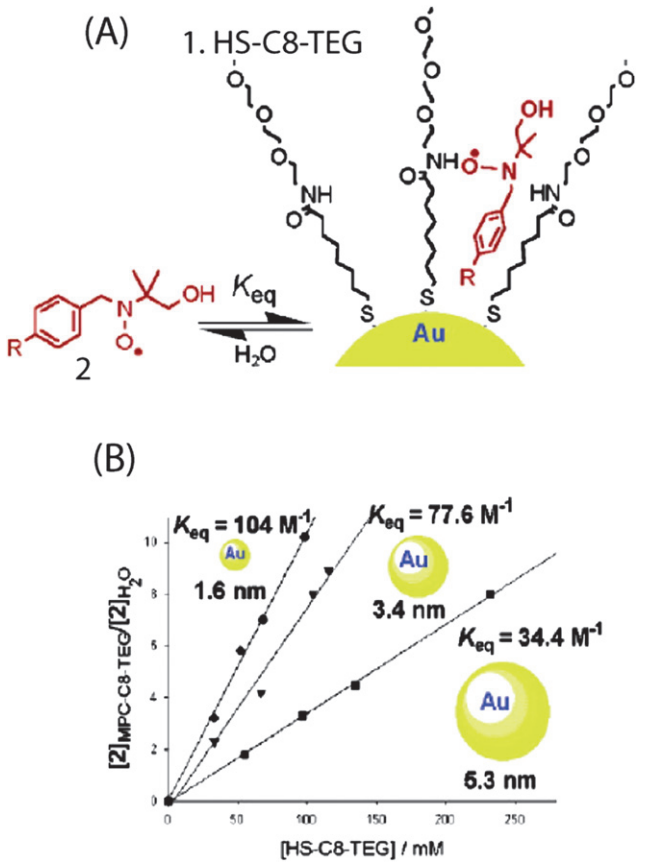


Fig. 6 (A) Schematic representation of AuNP and the nitroxide probe inclusion. (B) Plot of the ratio between the concentration of **2** partitioned in the monolayer and that of the free species (● 16 nm; ▼ 3.4 nm; ■ 5.3 nm) as a function of [HS-C8-TEG] bound to the gold. Reprinted with permission from ref. 36*b* (Copyright 2005 The American Chemical Society).

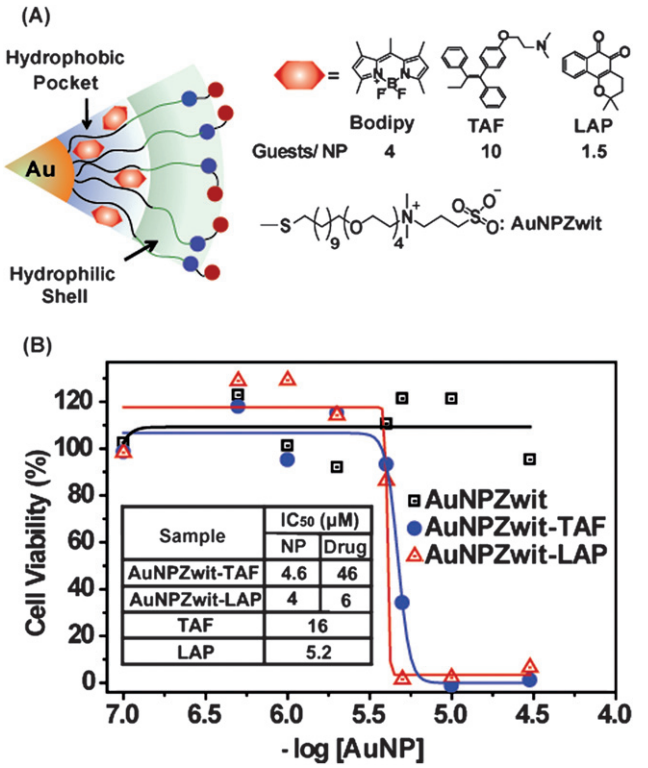


Fig. 7 (A) Structure of particles and guest compounds: Bodipy, TAF, and LAP, the number of encapsulated guests per particle (B) Cytotoxicity of AuNPZwit complexes measured by Alamar blue assay after 24 h incubation with MCF-7 cells. IC_{50} of AuNP (NP), equivalent drugs (Drug), and free drugs are shown in the table.

Burda *et al.* reported the use of PEGylated AuNP-Pc4 (Si-phthalocyanine) conjugates as efficient platforms for PDT (Fig. 8a).[40] The phthalocyanine photosensitizing agent was encapsulated by the PEG ligands through van der Waals interactions, with the PEG ligand inhibiting colloid aggregation and providing significant resistance to non-specific binding with biomacromolecules. The release of Pc4 from AuNPs *in vitro* in a two-phase solution system and *in vivo* in cancer-bearing mice (Fig. 8b) indicated that the delivery process was highly efficient, with Pc4 preferentially accumulated in tumor sites. With the AuNP-Pc 4 conjugates, the drug delivery time required for PDT was reduced from 2 days using the free drug to 2 h, using the conjugate (Fig. 8).

4.3. Light-regulated release

The ability to regulate drug release is an important property for drug delivery systems.[5d] While endogenous approaches such as GSH-mediated release provide very useful strategies for delivery, externally controlled release provides a complementary tool for site- and time-specific control of payload release.[41] Recently, caged drugs have been developed where the activity of the drug was suppressed by attaching it to a blocking element through a photoremovable protecting group.[42] Rotello *et al.* have applied this strategy to AuNP delivery vehicles, utilizing a photo-cleavable *o*-nitrobenzyl ester moiety that dissociates upon light irradiation to alter the surface potential from positive to negative, thereby releasing adsorbed DNA.[11] In more recent studies, the authors demonstrated light-controlled release of anticancer drug (5-fluorouracil) from nanoparticles (Fig. 9).[43] The AuNPs (Au_PCFU, Au core: ~2 nm) featured a mixed monolayer of zwitterionic and photocleavable ligands. The zwitterionic ligand provided solubility and prevented cellular uptake. The photocleavable ligand linked the fluorouracil to the particle through an orthonitrobenzyl group that could be effectively cleaved using near-UV irradiation (365 nm). An IC_{50} value of 0.7 μM was observed upon irradiation for Au_PCFU on a per particle basis, whereas no significant cell death was observed in cells treated with only light or only Au_PCFU.

In related research, Nakanishi *et al.* have reported a photo-responsive nanocarrier of amines, including cell-signaling agents. (Fig. 10).[44] In this approach a carbamate linkage could be dissociated *via* the photocleavage reaction of the 2-nitrobenzyl group upon near-UV irradiation. The caging process was very effective: histamine had no biological activity while it was attached to AuNPs but became active when it was released from the particles upon photo-irradiation.

4.4. Other attachment/release strategies

A variety of additional delivery strategies have been developed using AuNP platforms. Schoenfisch *et al.* have demonstrated that nitric oxide (NO) can be efficiently released at acidic pH from

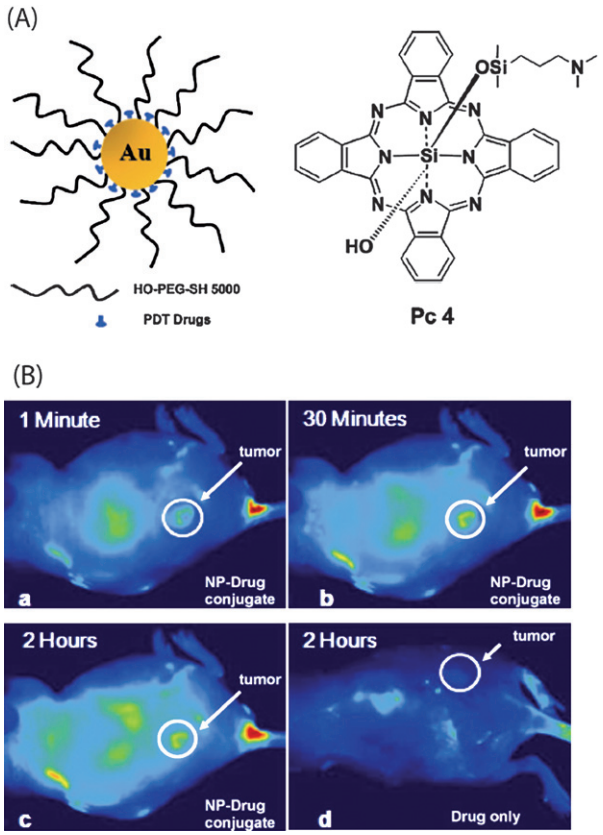


Fig. 8 (A) Structure of the water-soluble AuNPs as a PDT drug delivery agents, Pc 4 structure (B) Fluorescence images of a tumor-bearing mouse after being injected with AuNP-Pc 4 conjugates in normal saline (0.9% NaCl, pH 7.2), (a) 1 min, (b) 30 min, and (c) 120 min after intravenous tail injection. Any bright signal is due to Pc 4 fluorescence. For comparison, a mouse that got only a Pc 4 formulation without the AuNP vector injected is shown in panel (d). No circulation of the drug in the body or into the tumor was detectable 2 h after injection without the AuNPs as drug vector. Reprinted with permission from ref. 40 (Copyright 2008 The American Chemical Society).

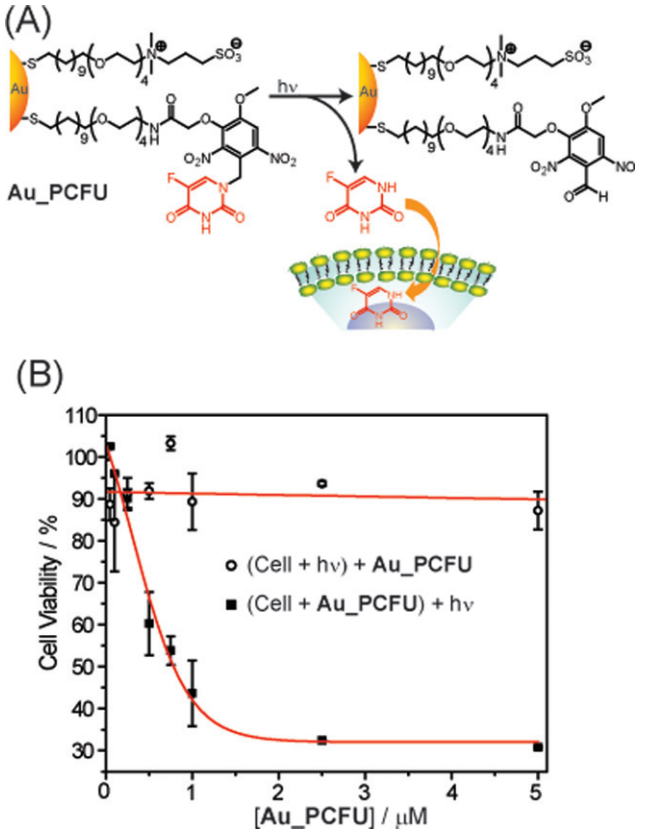


Fig. 9 (A) Photochemical reaction (365 nm) of Au_PCFU and delivery of payload to cell. (B) Cytotoxicity of different concentrations of Au_PCFU under uncaging and control conditions. The IC_{50} value was 0.7 μM per particle, 11.9 μM per drug.

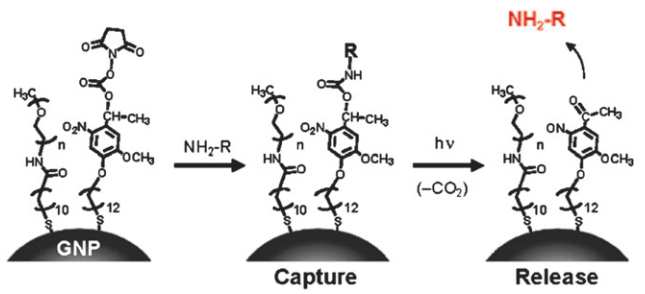


Fig. 10 Capture and release of amines on AuNPs having a photocleavable succinimidyl ester. Reprinted with permission from ref. 44 (Copyright 2009 The American Chemical Society).

AuNPs,[45] providing a potential means of controlling multiple cellular processes including angiogenesis, vasodilation, and the immune response.[46] Hwu *et al.* have used a phosphate linker to conjugate paclitaxel to AuNP and Fe_3O_4 particles.[47] The drug could be released from the particles using phosphodiesterase.

Conclusions and outlook

Gold nanoparticles provide a promising scaffold for drug delivery. The combination of tunable monolayer properties and stability, low inherent toxicity, functional versatility, and controlled release of payloads provides a wealth of options for the design of DDSs. Concurrent with the creation of new vehicles, however, a number of important issues require further examination. These issues include the size and surface dependent cytotoxicity, immune response, and biodistribution/pharmacokinetics of AuNPs. These investigations will serve not only to help in the design of effective DDSs, but will also provide fundamental insight into the interactions of nanomaterials with biological systems.

Acknowledgements

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