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Entrapment of Hydrophobic Drugs in Nanoparticle Monolayers with Efficient Release into Cancer Cells

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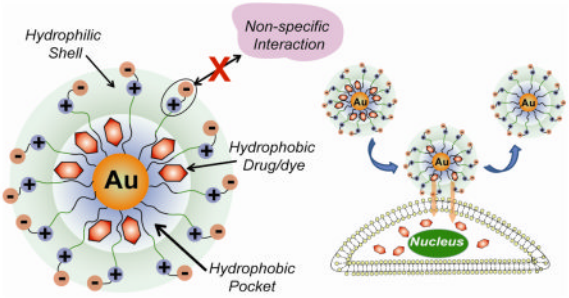
Abstract



Gold nanoparticles functionalized with water-soluble zwitterionic ligands form kinetically stable complexes with hydrophobic drugs and dyes. These drugs and dyes are efficiently released into cells, as demonstrated through fluorescence microscopy and cytotoxicity assays. Significantly, there is little or no cellular uptake of particle, making these low toxicity particles promising for delivery applications.

Drug delivery systems (DDSs) provide an important tool for increasing efficacy of pharmaceuticals through improved pharmacokinetics and biodistribution.[1] A wide variety of nanoscale materials such as liposomes, polymeric micelles, and dendrimers, have been employed as drug carriers. [2] Both covalent and non-covalent approaches can be applied to the conjugation of drugs into/onto these DDSs.[3] Non-covalent approaches have the capability of employing active drugs, whereas covalent attachment generally requires chemical modification which can cause reduced efficiency of drug release or incomplete intracellular processing of a prodrug.[4]

Recently, gold nanoparticle (AuNP) based drug/gene delivery systems have attracted attention due to their functional versatility,[5] biocompatibility,[6] and low toxicity,[7] Recent studies have demonstrated controlled release of payload by intracellular thiols.[8] However, controlled dissociation of drugs in active form from covalent AuNP-drug conjugates remains a challenge for clinical applications.[2]

Noncovalent incorporation of drugs into AuNP monolayers provides an alternative delivery strategy with the potential for avoiding drug release and prodrug processing issues. The structure of commonly used water-soluble AuNPs is similar to that of unimolecular micelles

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such as dendrimers, featuring a hydrophobic interior and a hydrophilic exterior.[9] The alkanethiol monolayer of the nanoparticle coupled with the radial nature of the ligands[10] creates "hydrophobic pockets" inside monolayer of AuNP where organic solutes can be partitioned, as demonstrated by Lucarini and Pasquato.[11] We report here the use of these pockets to encapsulate drugs and deliver them with high efficiency to cells.

The biocompatible AuNPs used in this study features two functional domains: a hydrophobic alkanethiol interior and a hydrophilic shell composed of a tetraethylene glycol (TEG) unit terminated with a zwitterionic headgroup. Particles with this general structure have been shown to minimize non-specific binding with biomacromolecules.[12]

We chose three different hydrophobic guest compounds: 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**Bodipy**) as a fluorescent probe,[13] and the highly hydrophobic therapeutics tamoxifen (**TAF**) and β-lapachone (**LAP**) as drugs (Figure 1). The nanoparticle-payload conjugates (**AuNPZwit-Bodipy**, **TAF**, and **LAP**) were prepared by solvent displacement method.[14] First, **AuNPZwit** (Au core: 2.5 ± 0.4 nm) and guest were dissolved in an acetone/water and the solvent slowly evaporated. The bulk of the excess guest precipitated out and was removed by filtration; the particles were further purified by multiple filtrations through a molecular weight cutoff filter until no free guest was observed, followed by dialysis against buffer. The number of entrapped guest molecules per particle was determined from ^{1}H NMR spectrum and NaCN-induced decomposition experiments (see Supporting Information) and varied depending on size, hydrophobicity (logP), and molecular structure of hydrophobic molecules (Figure 1). The particle/guest complexes are stable in buffer for >1 month and to extended dialysis, a level of kinetic entrapment greater than that observed with dendrimers.[4]

The ability of the delivery systems to release their payload was first explored *in vitro* using **AuNPZwit-Bodipy** in a two-phase dichloromethane (DCM)-water system, [15] where the dye is quenched by the AuNP, and photoluminescence (PL) only observed upon dye release. In these studies a rapid increase in PL intensity is observed along with transfer of **Bodipy** into the DCM layer (Figure 1c). Significantly, since no release is observed in monophasic aqueous conditions and no particle was observed in the DCM layer, payload release occurs interfacially.

Payload delivery to cells using **AuNPZwit-Bodipy** was determined by confocal laser scanning microscopy (CLSM) using human breast cancer (MCF-7) cells. Efficient delivery of the dye to the cytosol is observed after 2 h incubation with **AuNPZwit-Bodipy (**Figure 2a–c). Cellular uptake of nanoparticle was studied using transmission electron microscopy (TEM), and inductively coupled plasma mass spectrometry (ICP-MS), using the analogous cationic particle/dye conjugate **AuNPTTMA-Bodipy** as a positive control. Little cellular uptake of **AuNPZwit** was observed by either TEM (Figure 2 d,e) or ICP-MS for **AuNPZwit-Bodipy** (31 ng/well at 4h (Figures 2), 71 ng/well at 24h Figure S3, corresponding to uptake of 0.06% and 0.14% of available particle, respectively), whereas substantial particle uptake was observed with **AuNPTTMA-Bodipy** (1750 ng/well (4 h), 2150 ng/well (24 h)) Since no free dye was observed during the 24 h incubation of **AuNPZwit-Bodipy** in medium or serum solution at 37 °C (Figure 1 d), **Bodipy** delivery presumably occurs via a monolayer-membrane transfer process, consistent with our *in vitro* studies[16]

Demonstration of drug delivery to MCF-7 cells through presumably the same mechanism was determined through cytotoxicity studies of free and encapsulated drugs using an Alamar blue assay (Figure 3). Notably, **AuNPZwit** itself was non-toxic at 30 μM. In contrast, IC_{50} values of 4 μM and 4.6 μM were observed using **AuNPZwit-LAP** and **AuNPZwit-TAF**, respectively. The delivery process was quite efficient, with the per drug molecule IC_{50} of **AuNPZwit-TAF** (46 μM) only three-fold higher than that of **TAF** (16 μM), and that of **AuNPZwit-LAP** (6.0 μM) essentially identical to that of **LAP** (5.2 μM).

In conclusion, we have demonstrated that hydrophobic dyes/drugs can be stably entrapped in hydrophobic pocket of AuNPs and released into cell by membrane-mediated diffusion without uptake of the carrier nanoparticle. Importantly, the small size of these nanocarriers coupled with their biocompatible surface functionality should provide long circulation lifetimes and preferential accumulation in tumor tissues by the enhanced permeability and retention (EPR) effect.[17] Additionally, the noninteracting nature of their monolayer should make these systems highly amenable to targeting strategies. We are currently exploring these applications as well as the role of monolayer and guest structure in the encapsulation process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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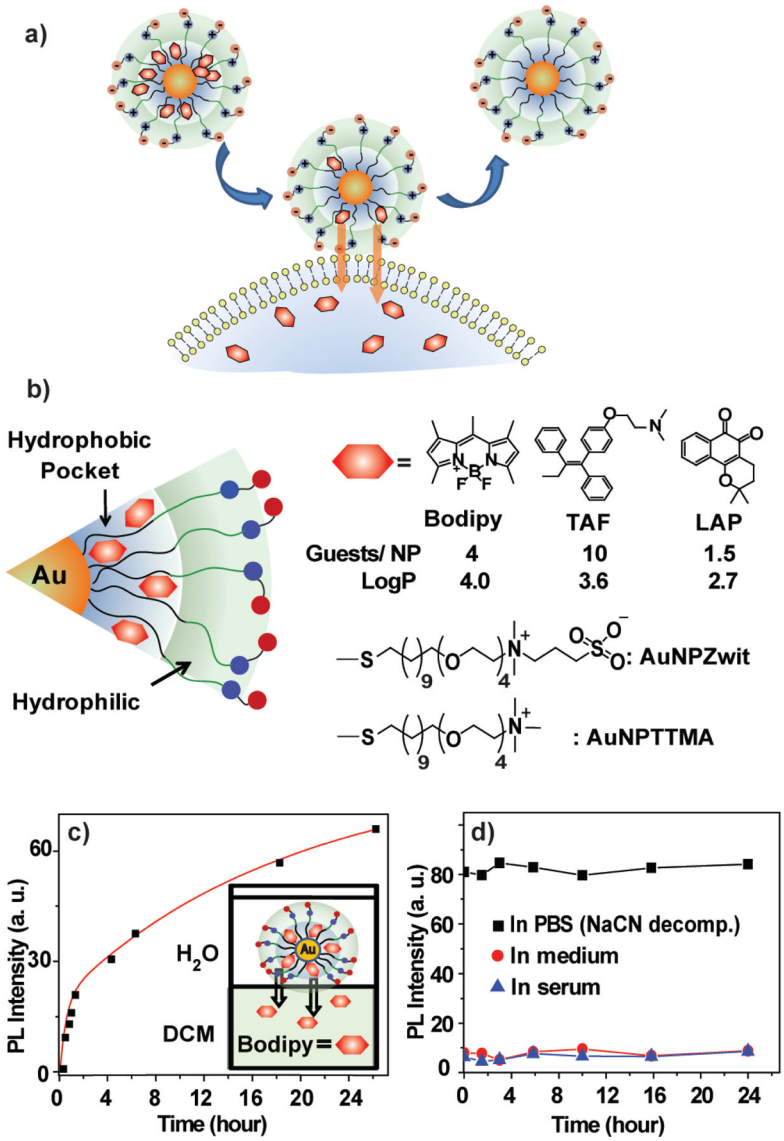

Figure 1.

a) Delivery of payload to cell through monolayer-membrane interactions. b) Structure of particles and guest compounds: **Bodipy**, **TAF**, and **LAP**, the number of encapsulated guests per particle, and logP of the guests. c) Release of **Bodipy** from AuNPZwit-Bodipy in DCM-aqueous solution two-phase systems (λ_{ex} = 499 nm, λ_{em} = 517 nm) d) PL intensity **AuNPZwit-Bodipy** in cell culture medium and 100 % serum, indicating little or no release relative to **AuNPZwit-Bodipy** in PBS after NaCN-induced release of guest molecules ((λ_{ex} = 499 nm, λ_{em} = 510 nm).

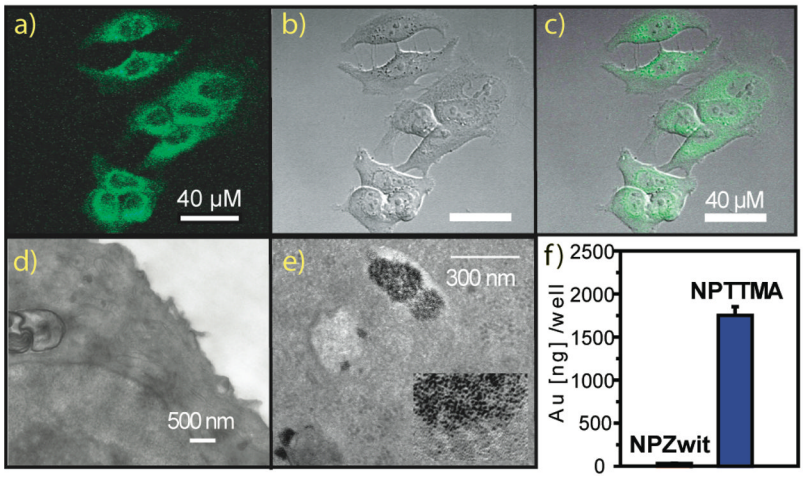

Figure 2.

CLSM images of MCF-7 cell treated with **AuNPZwit-Bodipy** for 2h: a) green channel b) bright field, and c) overlap. TEM images of fixed cell treated with d) **AuNPZwit-Bodipy** and e) **AuNPTTMA** as a positive control, Endosomally trapped AuNPs are marked by arrow. (f) ICP-MS measurement. (200,000 cells/well), indicating low cellular uptake of **AuNPZwit** (31 ng/well after 4 h)

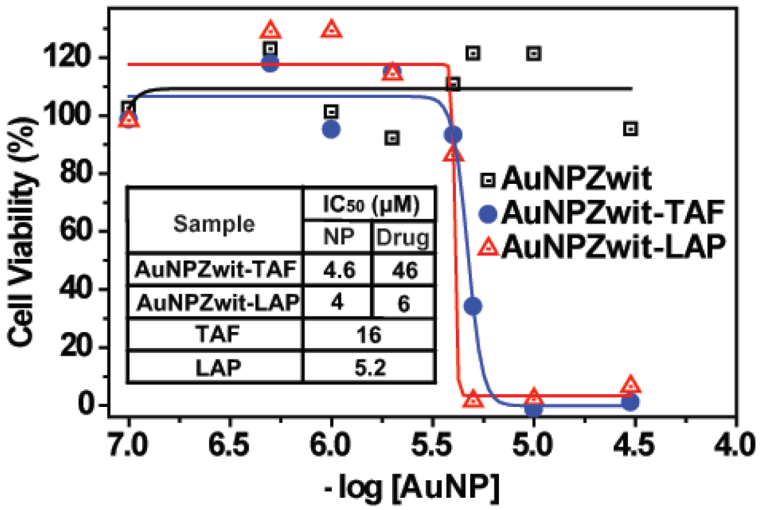

Figure 3.

Cytotoxicity of **AuNPZwit** complexes measured by Alamar blue assay after 24h incubation with MCF-7 cells. IC_{50} of AuNP (NP), equivalent drugs (Drug), and free drugs are shown in table.