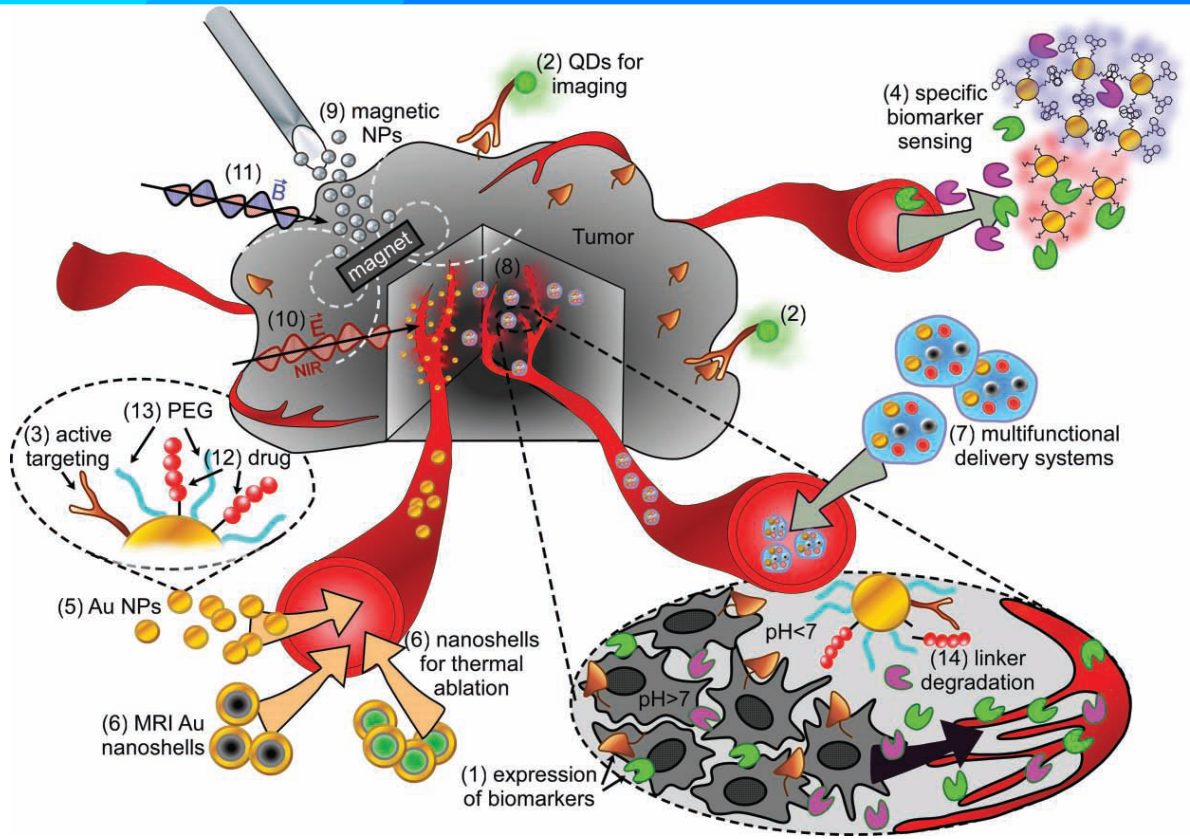
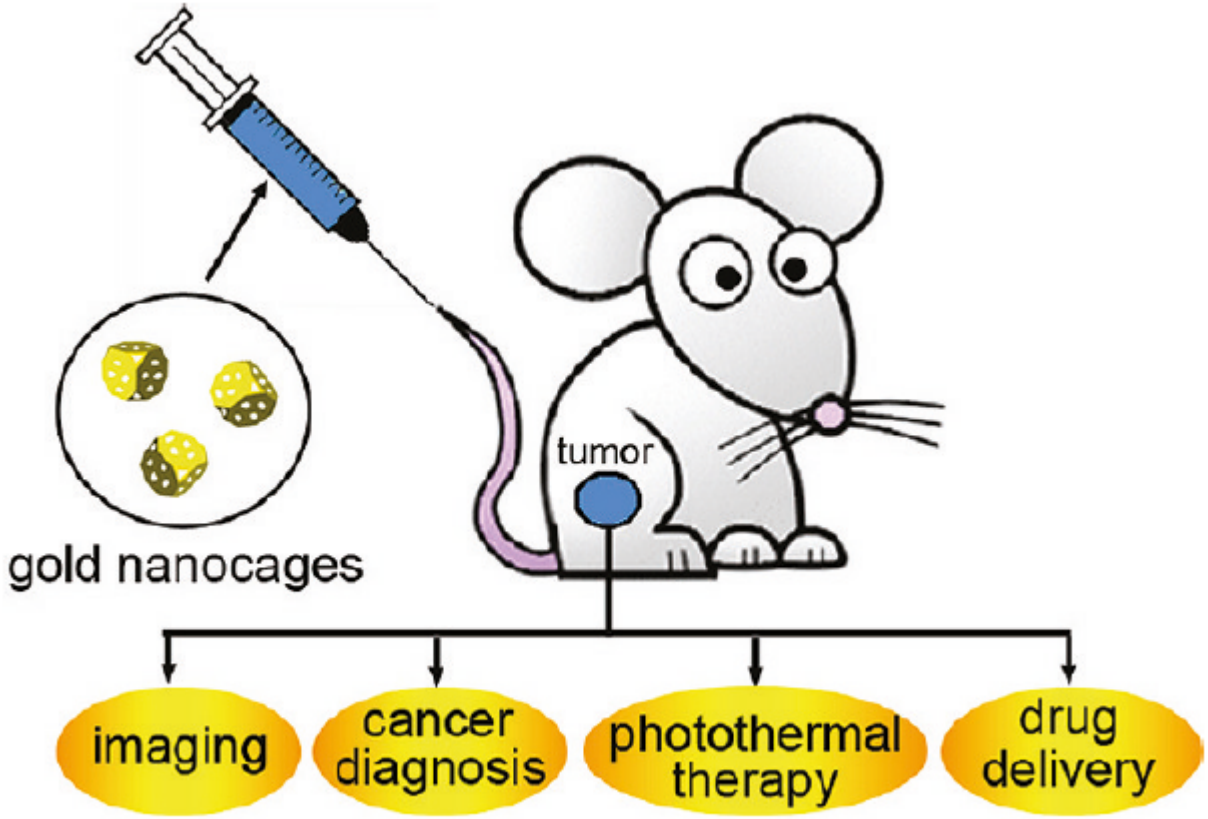
nanomaterials for diagnosis, imaging, and therapy



C. Minelli, S. B. Lowe, M. M. Stevens *Small* **2010**, *6*, 2336-2357.

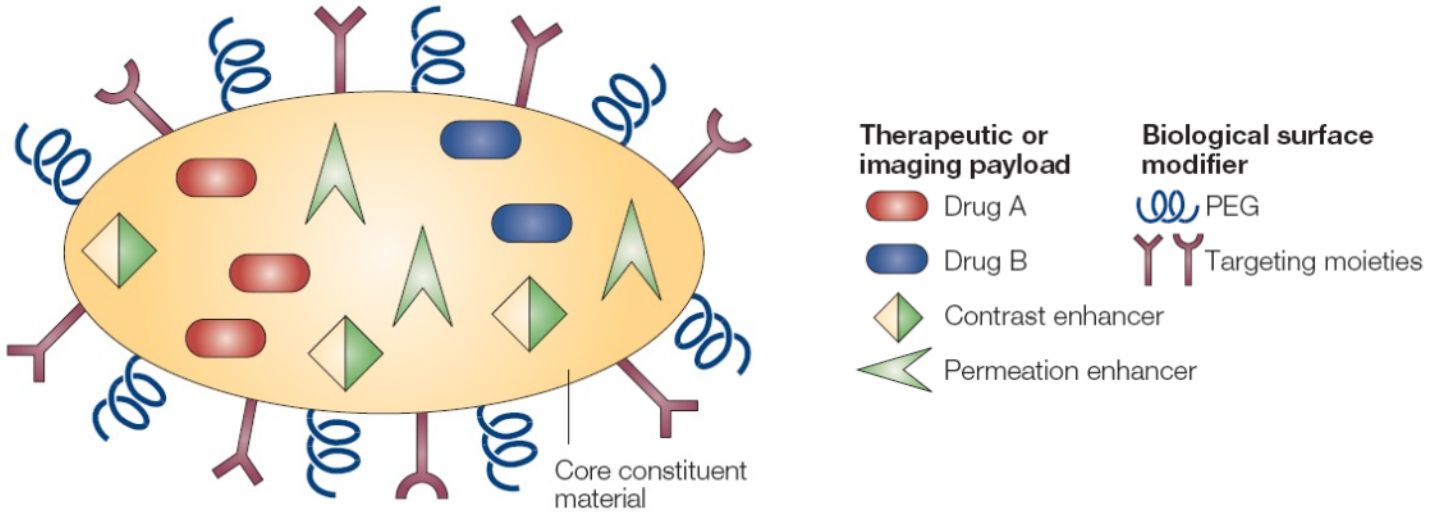
- improved delivery of poorly water-soluble drugs
- targeted delivery of drugs in a cell- or tissue-specific manner
- transcytosis of drugs across tight epithelial and endothelial barriers
- delivery of large macromolecule drugs to intracellular sites of action
- co-delivery of two or more drugs or therapeutic modality for combination therapy
- visualization of sites of drug delivery by combining therapeutic agents with imaging modalities
- real-time read on the *in vivo* efficacy of a therapeutic agent

Au Nanocages for Theranostic applications



Y. Xia et al. Acc. Chem. Res. 2011, 44, 914.

Multifunctional nanoparticle



an ideal nanovector should possess:

- the ability to carry one or more therapeutic agents;
- biomolecular targeting through one or more recognition agents;
- imaging elements;
- biobarrier avoidance.

M. Ferrari *Nat. Rev. Cancer* **2005**, *5*, 161-171.

Gold NPs in Clinical trials

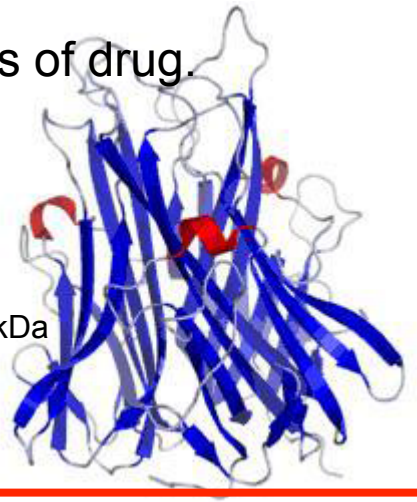
Phase I and Pharmacokinetic of CYT-6091

L. Tamarkin et al. *Clin. Cancer Res*. **2010**, *16*, 6139.

CYT-6091 – which is comprised of recombinant human tumor necrosis factor alpha (rh
Bound to the surface of PEGylated 27 nm colloidal gold particles
85% complete remission rates are routinely observed.
PEGylation of GNPs

- avoids immediate uptake by the reticuloendothelial reticulum (RES)

-Reduces the toxicity of rhTNF
-Allows the nanomedicine to sequester in solid tumors
maximal antitumor responses were achieved at lower doses of drug.



TNF-alpha is a homotrimer with a subunit molecular mass of 17 kDa

Gold NPs in Clinical trials

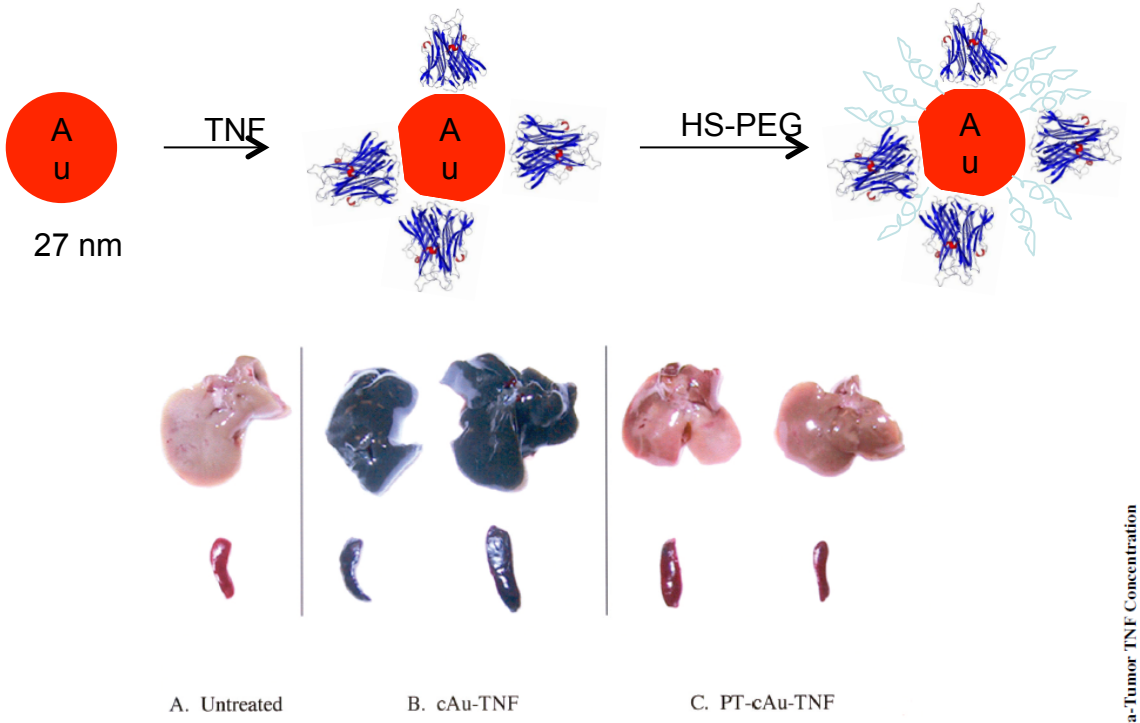


FIG. 2. Hepatic and splenic uptake of the cAu-TNF formulation and the PEG-THIOL cAu-TNF formulation (PT-cAu-TNF) compared to an untreated mouse. Two MC-38 tumor-burdened C57/BL6 mice were injected intravenously with the cAu-TNF vector, and another two tumor-burdened C57/BL6 mice were injected with the PT-cAu-TNF formulation. An untreated mouse and the four treated mice were sacrificed 5 hr after injection of 15 μg of the two different cAu formulations, and all five animals were perfused with heparinized saline. The livers and spleens from these animals were collected and photographed using a digitial camera. (a) Liver and spleen from an untreated mouse. (b) Livers and spleens from mice receiving the cAu-TNF vector. (c) Liver and spleen from mice receiving the PT-cAu-TNF vector.

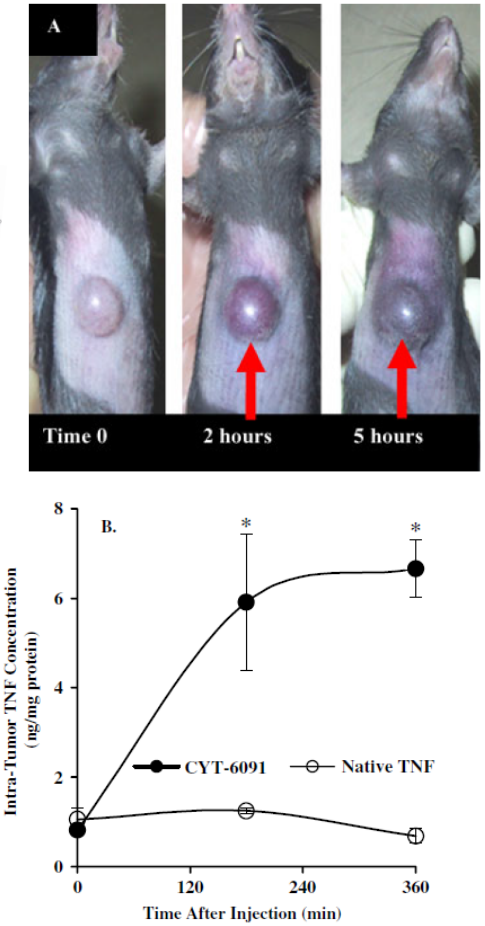
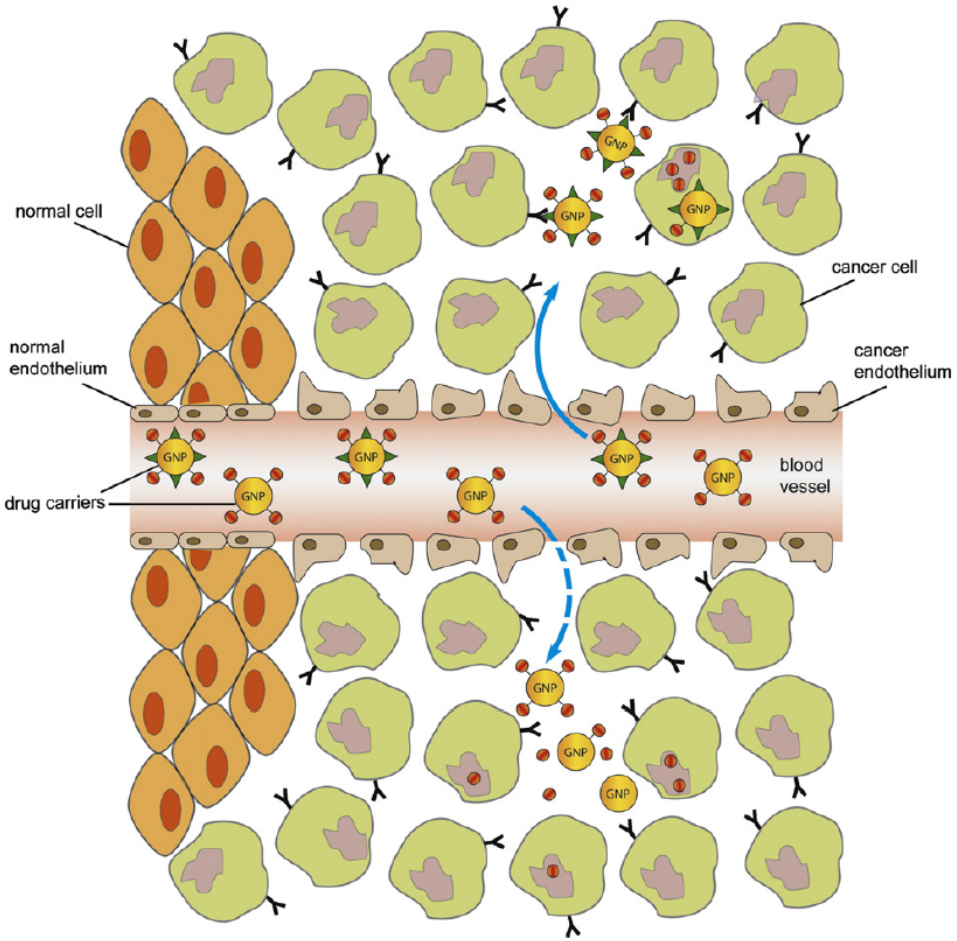


Fig. 3. **A:** Visual documentation of the accumulation of CYT-6091 (red arrows indicate location of the particle drug) in MC-38 tumors. **B:** Sequestration of CYT-6091 and TNF in MC-38-tumors. MC-38 tumor-burdened C57/BL6 mice were injected with 15µg of either native TNF or CYT-6091. TNF actively accumulates in the MC-38 tumors ($^*P<0.05$ vs. native TNF Treatment and the time 0 point for CYT-6091 treatment). Reproduced from Paciotti et al., [2004], with permission of the publisher.

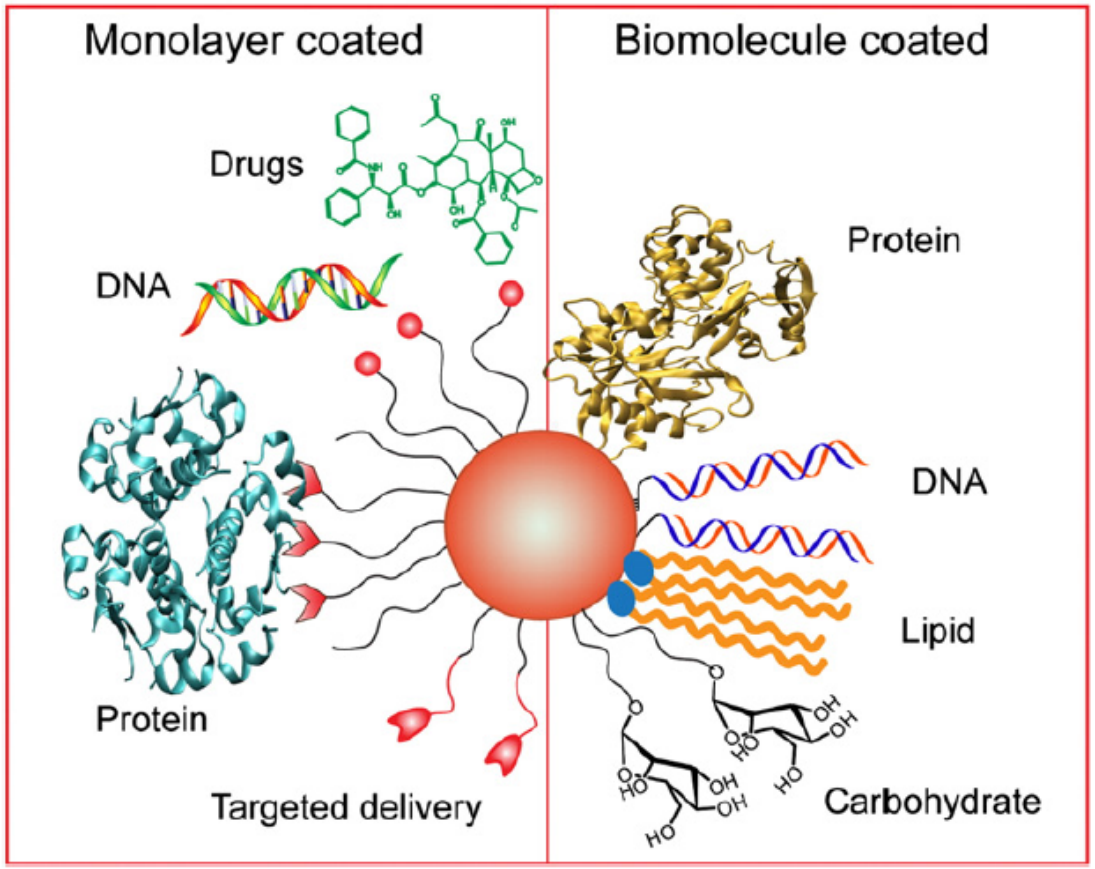
CytImmune is now collaborating with AstraZeneca to add a chemotherapeutic drug to further improve the killing power of CYT-6091.

Delivery

drug delivery via ‘active’ and ‘passive’ targeting



Delivery



Schematic presentation of the two AuNP surface structures commonly employed in delivery applications.

Delivery

Au NPs for transfection of DNA

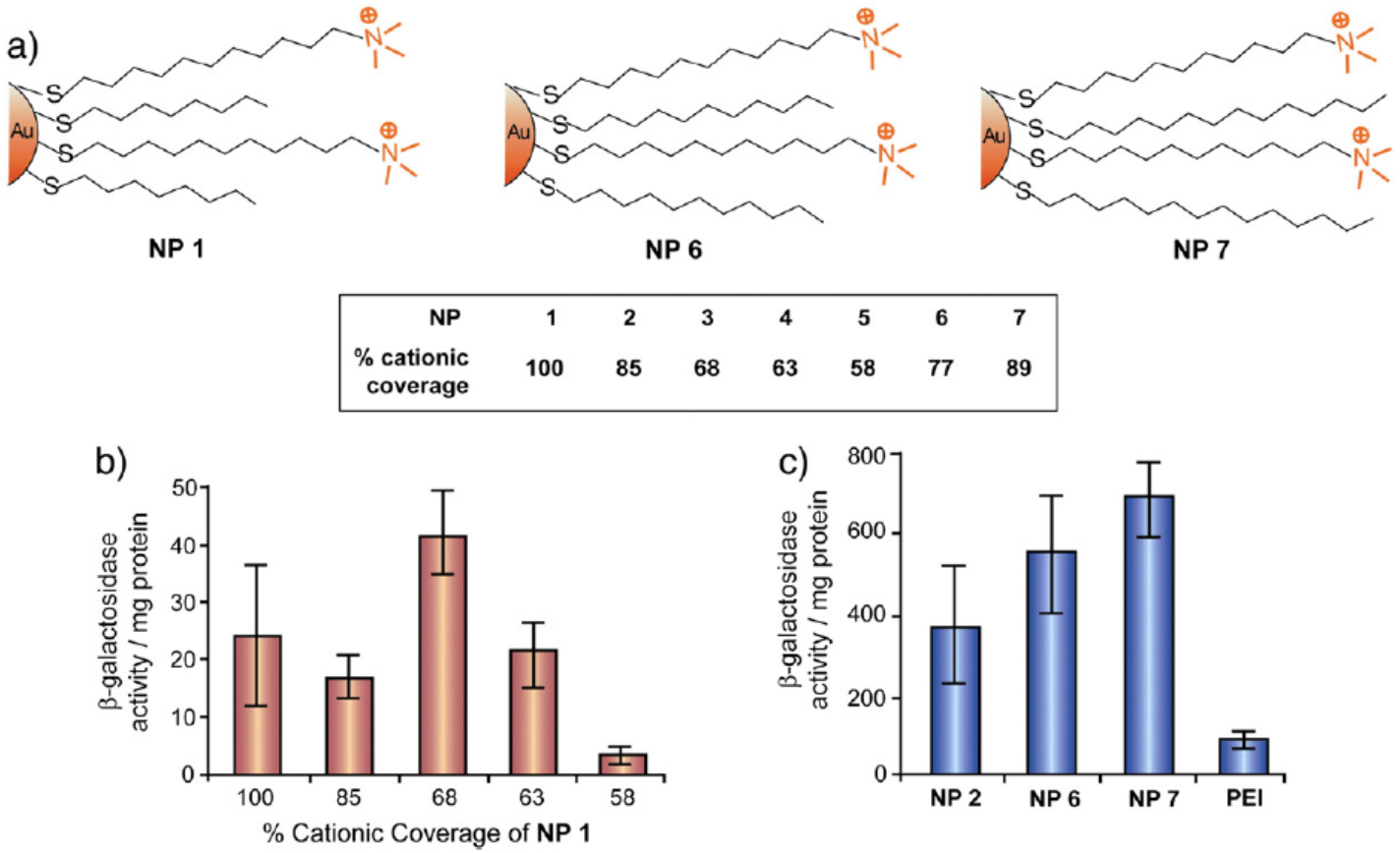
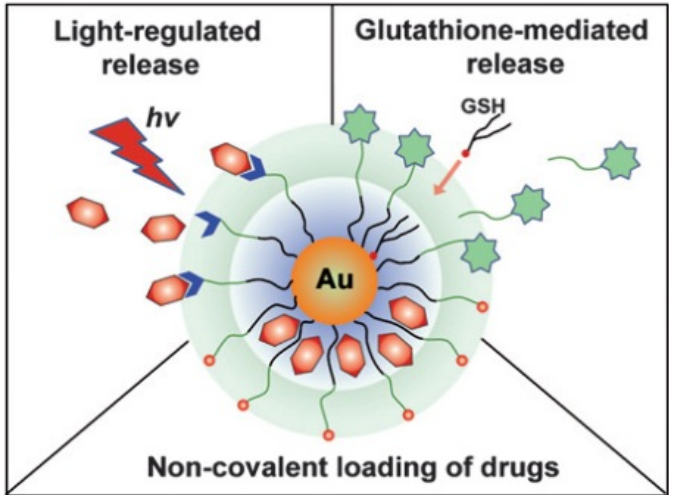


Fig. 3. (a) Structures of cationic NPs varied with amount of cationic ligands (NP 1–5) and alkyl chain length (NP 6–7) used for transfection of DNA. (b) Transfection of β-galactosidase using NP-DNA complexes (at 2200:1 ratio), measured by β-gal activity. (c) Transfection efficiency of NPs 2, 6, 7 (2200:1 NP/DNA ratio) and commercially available PEI (60 kDa).

V. M. Rotello et al. Bioconjug. Chem. 2002, 13, 3–6.

multimodal drug delivery gold nanoparticles

- Prodrugs can be convalently conjugated to AuNPs via cleavable linkers.
- Hydrophobic drugs can be loaded onto AuNPs monolayer exploiting hydrophobic interactions, without structural modification of the drug payload.
- The release can be trigger by either internal (e.g. glutathione) or external (e.g. light) stimuli.

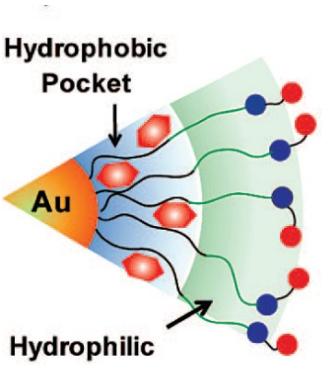


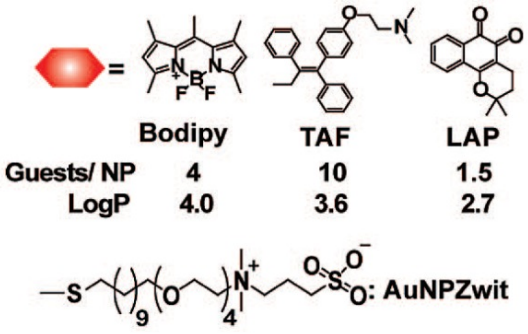
intracellurar GSH concentration
1-10 mM

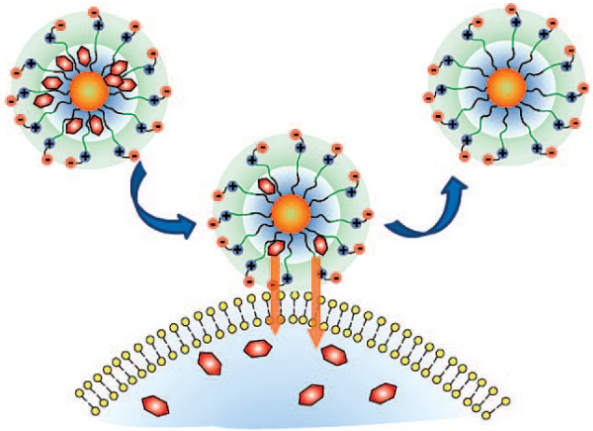
Encapsulation or conjugation of therapeutic agents with NPs has proven to be a succesful approach

- to improving therapeutic's water solubility
- circulation time in the body
- uptake, in turn reducing drug payloads and associated toxic effects.

gold nanoparticles

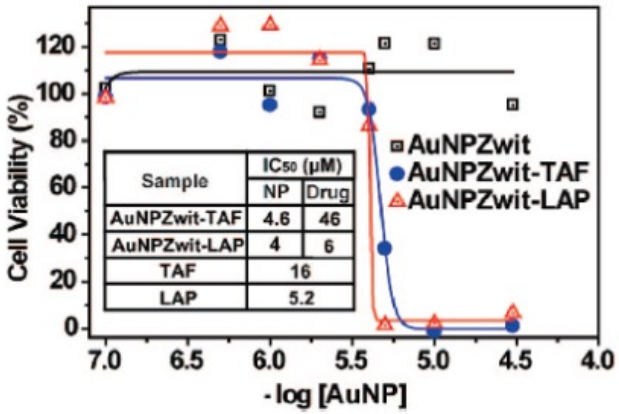






TAF = tamoxifen
LAP = β-lapachone

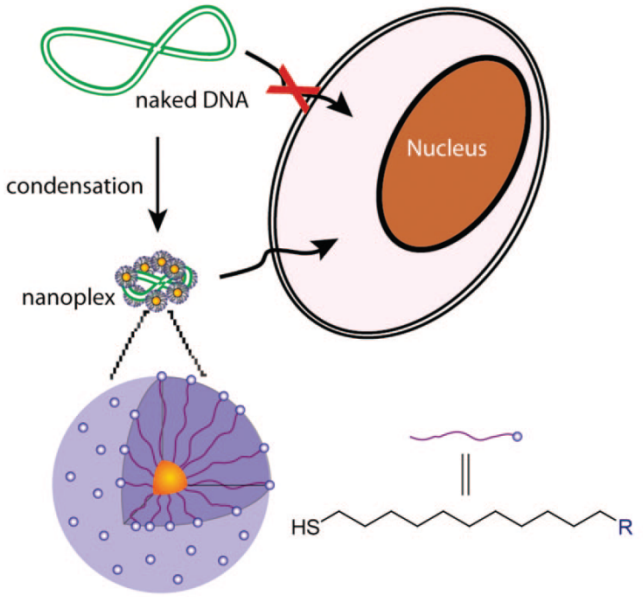
passive targeting



Cytotoxicity of **AuNPZwit** complexes measured by Alamar blue assay after 24 h incubation with MCF-7 cells. IC50 of AuNP (NP), equivalent drugs (Drug), and free drugs are shown in table.

C. K. Kim, P. Ghosh, C. Pagliuca, Z.-J. Zhu, S. Menichetti, V. M. Rotello, *J. Am. Chem. Soc.*, **2009**, *131*, 1360–1361.

gold nanoparticles for gene delivery



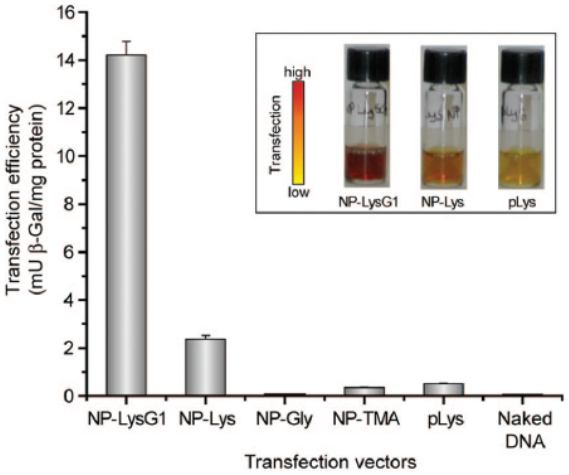
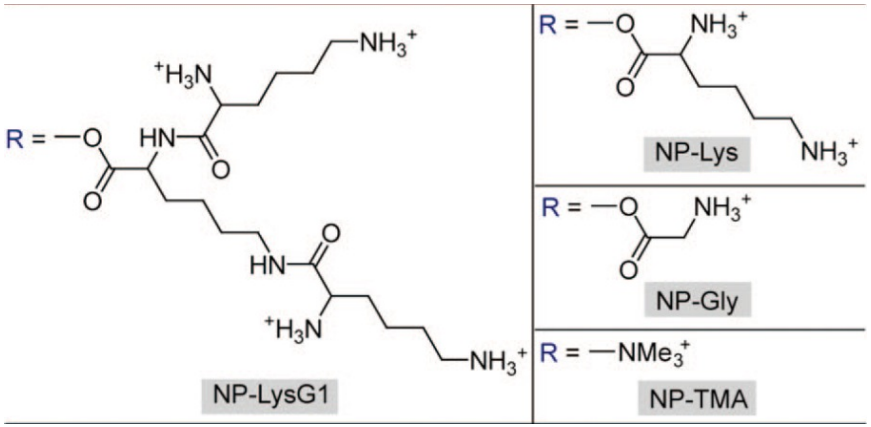
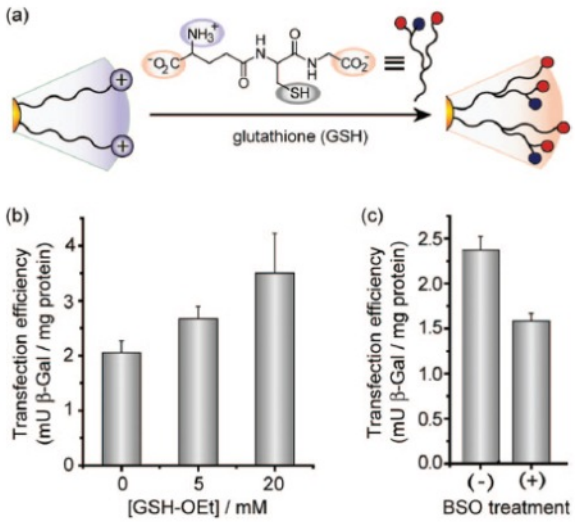


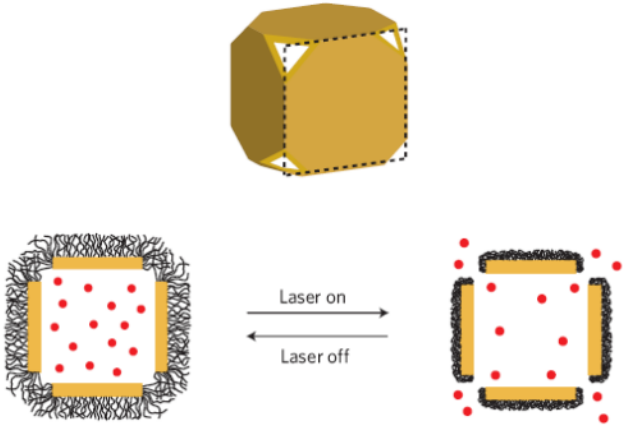
Figure 4. Enhanced transfection using NP−LysG1 and NP−Lys relative to positive controls, NP−TMA, and poly-lysine (pLys). No appreciable enzyme activity was observed in the absence of vectors. Inset shows solution colors during β-Gal activity assay performed after transfection. The color changes from yellow (substrate) to red (product) in the presence of active enzyme.

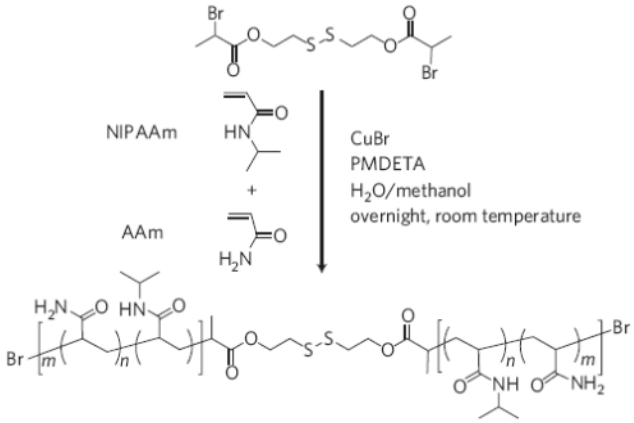


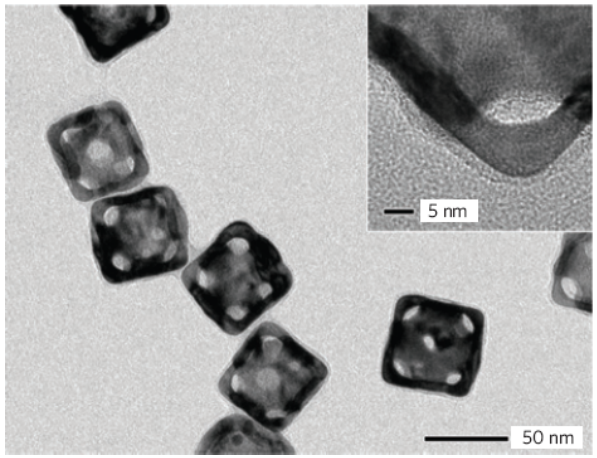


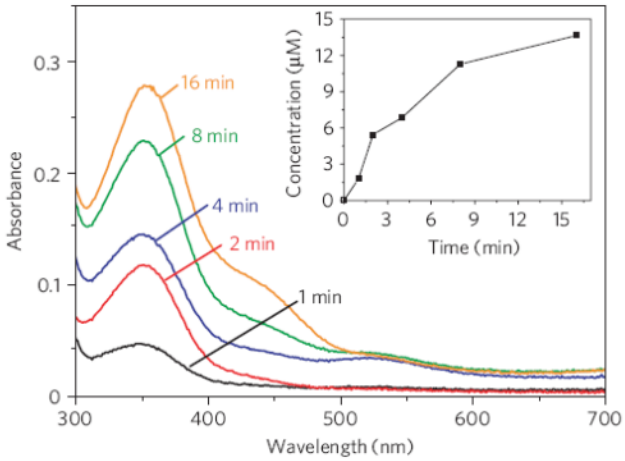
P. S. Ghosh, C.-Kyu Kim, G. Han, N. S. Forbes, V. M. Rotello
ACS Nano **2008**, *2*, 2213-2218.

gold nanocages for drug delivery trigger by light



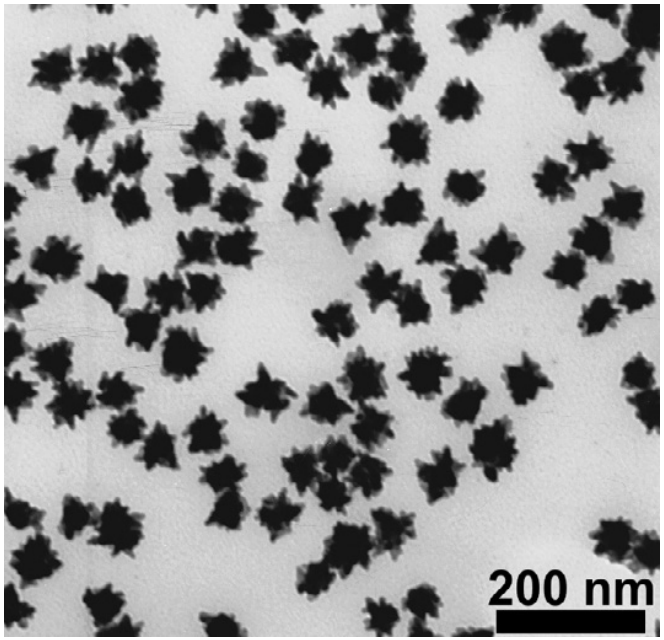






M. S. Yavuz, Y. Cheng, J. Chen, C. M. Cobley, Q. Zhang, M. Rycenga,
J. Xie, C. Kim, K. H. Song, A. G. Schwartz, L. V..Wang, Y. Xia *Nat. Mater.* **2009**, *8*, 935-939.

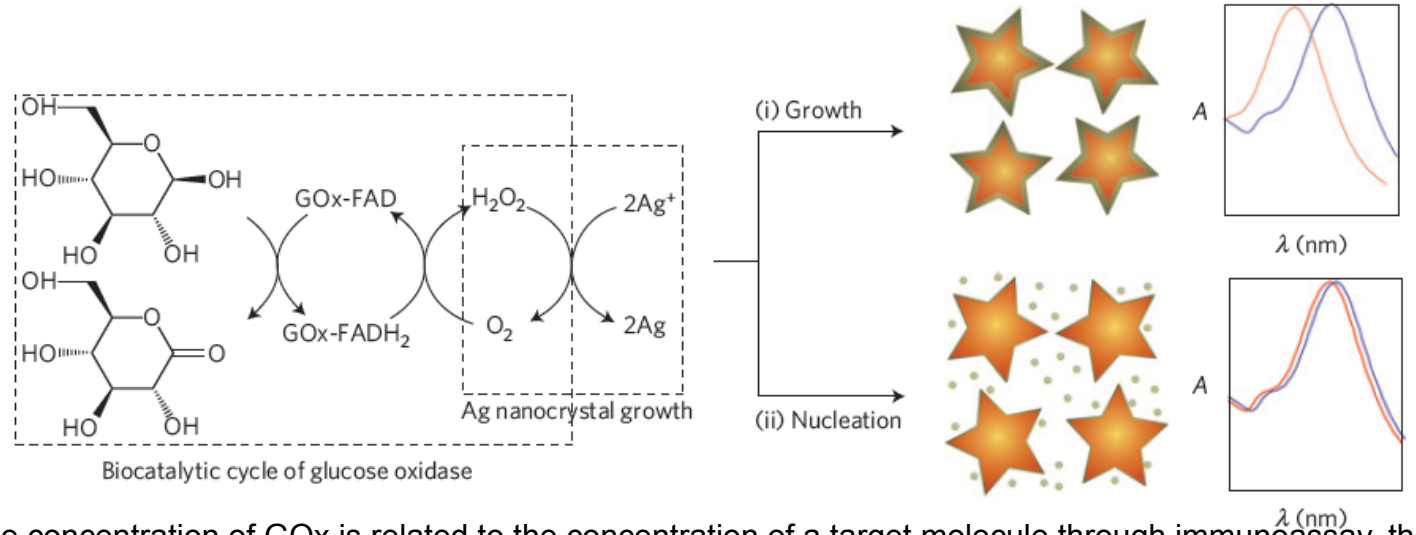
Gold nanostars



TEM image of Au nanostars synthesized through reduction of $HAuCl_4$ in a PVP/DMF mixture, in the presence of preformed Au seeds, using 10 mM PVP (Mw = 24 000).

Nature Materials **2012**, *11*, 604.

Gold nanostars



When the concentration of GOx is related to the concentration of a target molecule through immunoassay, this signal-generation step induces inverse sensitivity because condition (i) is fulfilled at low concentrations of analyte. FAD and FADH2 are the oxidized and reduced forms of flavin adenine dinucleotide.

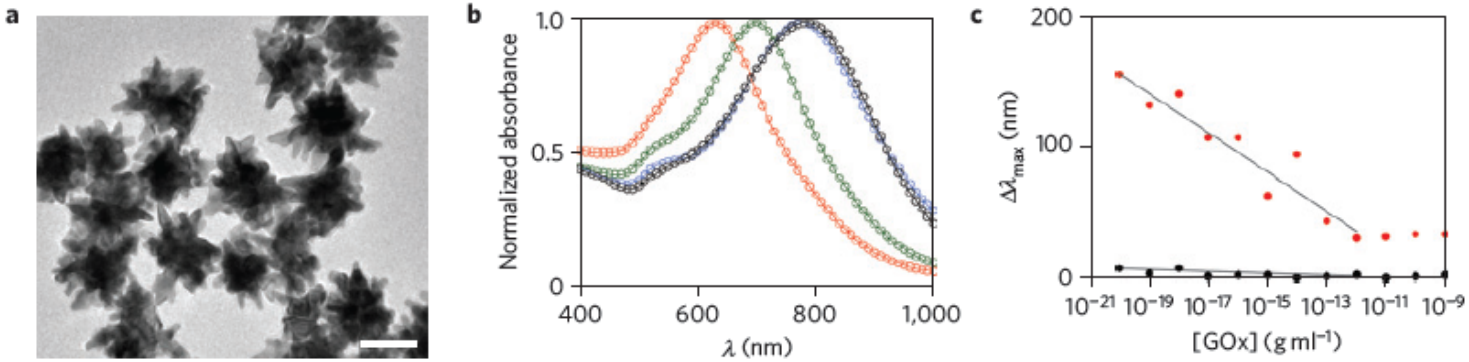
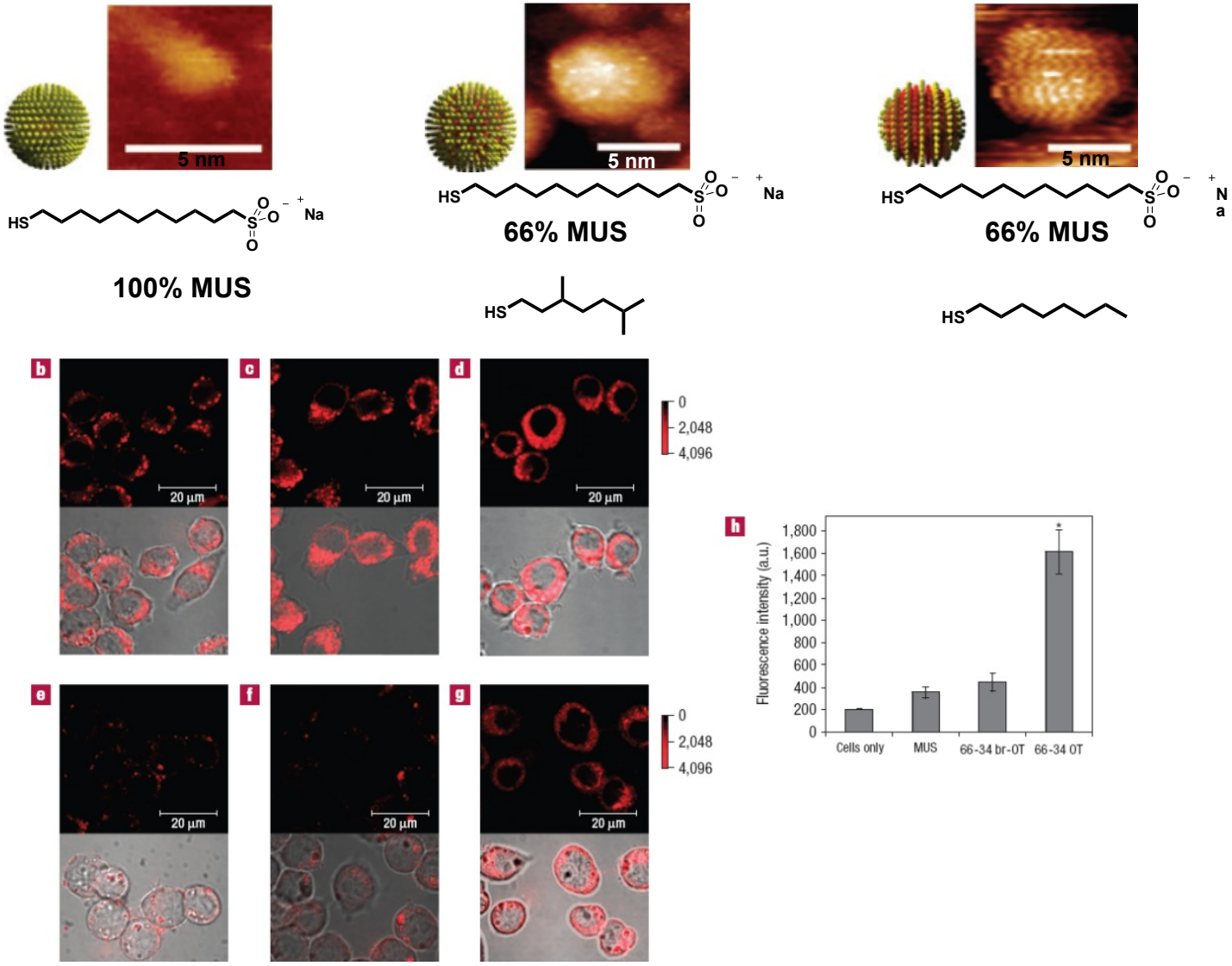


Figure 2 | Inverse sensitivity in plasmonic nanosensors. a, TEM image of gold nanostars (scale bar, 50 nm). **b**, Visible/near-infrared spectra of the nanosensors (black), modified with 10^{-14} g ml^{-1} GOx (green), 10^{-20} g ml^{-1} GOx (red) and without GOx (blue) after the signal-generation step. **c**, Blueshift of the LSPR absorbance band ($\Delta\lambda$max) as a function of the concentration of GOx in the immobilization solution when the signal-generation step is performed in the absence (black) or in the presence (red) of the enzyme-substrate glucose (semilogarithmic scale). The spectral shift was calculated with respect to the control experiment in the absence of GOx (blue curve in **b**).

cellular uptake

morphology of the monolayer



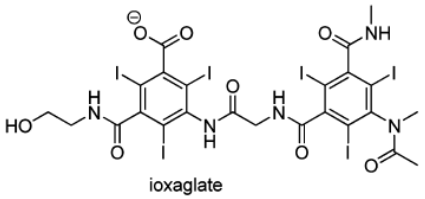
A. Verma, O. Uzun, Y. Hu, Y. Hu, H.-S. Han, N. Watson, S. Chen, D. J. Irvine and F. Stellacci, *Nat. Mater.*, **2008**, *7*, 588–595.

Imaging

X-ray based computed tomography (CT) is among the most convenient imaging/diagnostic tools in hospitals today in terms of availability, efficiency, and cost.

Today, an effective diagnostic dose of a contrast agent for imaging is in the molar concentration range.

for a patient (weighing approximately 75 kg) undergoing a selective coronary arteriography with left ventriculography will be injected intravenously with ~45 mL of Hexabrix a common clinically approved iodinated CT imaging agent solution, in a single dose, containing 24 g of ioxaglate (an equivalent to **14.4 g of iodine**). The total administered amount of Hexabrix solution over the course of the procedure may reach up to ~150 mL, totaling 80 g of ioxaglate (an equivalent of 48 g of iodine).



ioxaglate

An ideal CA should:

- improuve visualization of the target tissue
- have retention time of CA (2-4 h)
- the CA should localize or target the tissue of interest and posses favorable biodistribution and pharmacokinetic profiles.
- CA should be readily soluble or form stable suspensions at aqueous physiological conditions

Imaging

targeting is important

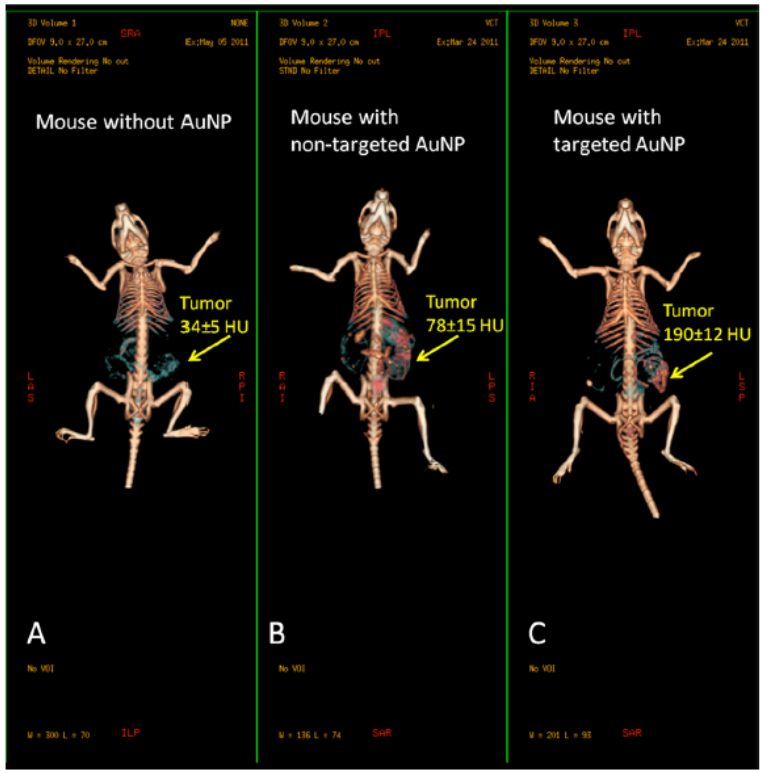
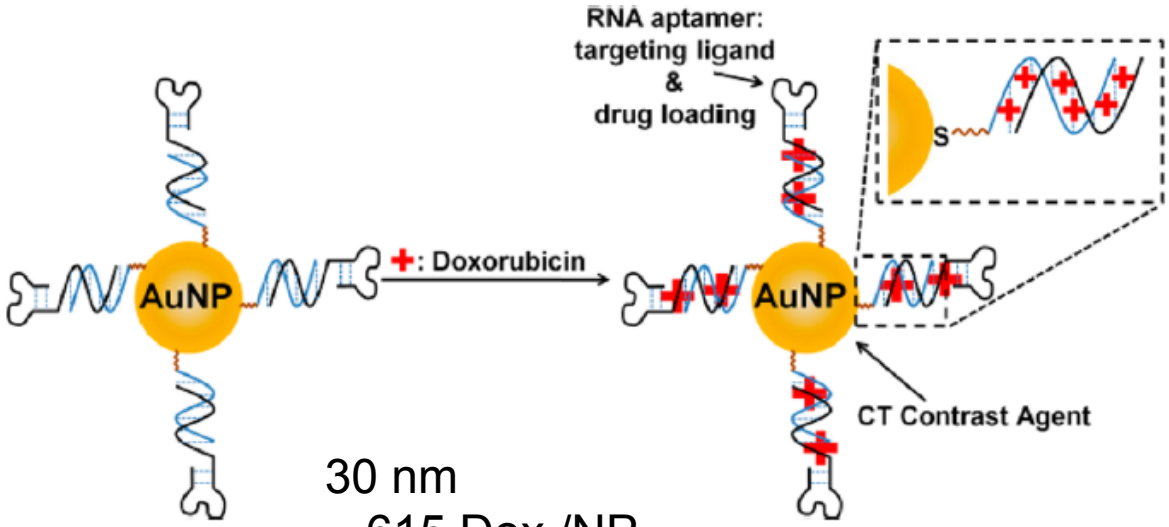


Figure 18. In vivo X-ray computed tomography (CT) volume-rendered images of (A) mouse before injection of gold nanoparticles (AuNPs), (B) mouse 6 h postinjection of nonspecific immunoglobulin-G AuNPs as a passive targeting experiment, and (C) mouse 6 h postinjection of antiepidermal growth factor receptor (EGFR)-coated AuNPs that specifically targeted the squamous cell carcinoma head and neck tumor. Anti-EGFR-targeted AuNPs show clear contrast enhancement of the tumor (C, yellow arrow), which was undetectable without the AuNPs contrast agents (A, yellow arrow). CT numbers represent the average Hounsfield units (HU) of the whole tumor area. All scans were performed using a clinical CT at 80 kVp, 500 mAs, collimation 0.625 × 64 mm, and 0.521 pitch size (64 detector CT scanner, LightSpeed VCT; GE Healthcare, Little Chalfont, U.K.). (Images courtesy of Dr. Rachela Popovtzer, Bar-Ilan University, Israel.)

Imaging and Therapy

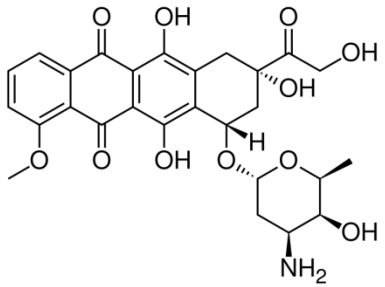
CT Imaging and Therapy of Prostate Cancer

loading of the trifunctional Au NPs with the chemoterapeutic drug Doxorubicin



30 nm
~ 615 Dox /NP

doxorubicin



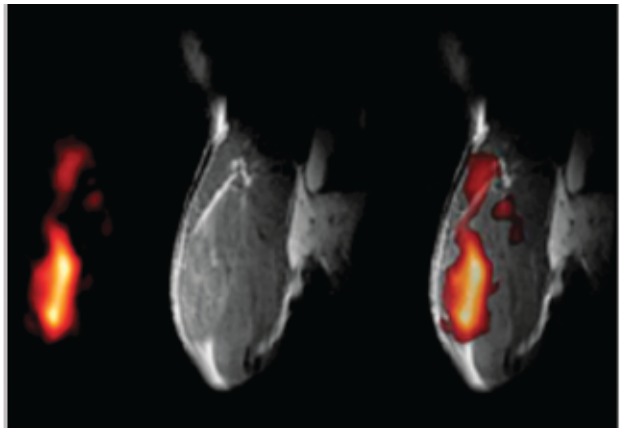
7.5 Dox per aptamer

prostate-specific membrane antigen-specific RNA aptamer target prostate adenocarcinoma LNCaP cells.

the gold center serves as the CT imaging agent, while the RNA aptamer modifications turn the NP into a target drug-delivery vehicle

D. Kim, Y. Y. Jeong, S. Jon *ACS Nano* **2010**, *4*, 3689.

^{19}F MRI



Nat. Biotechnol. **2005** *23*,983.

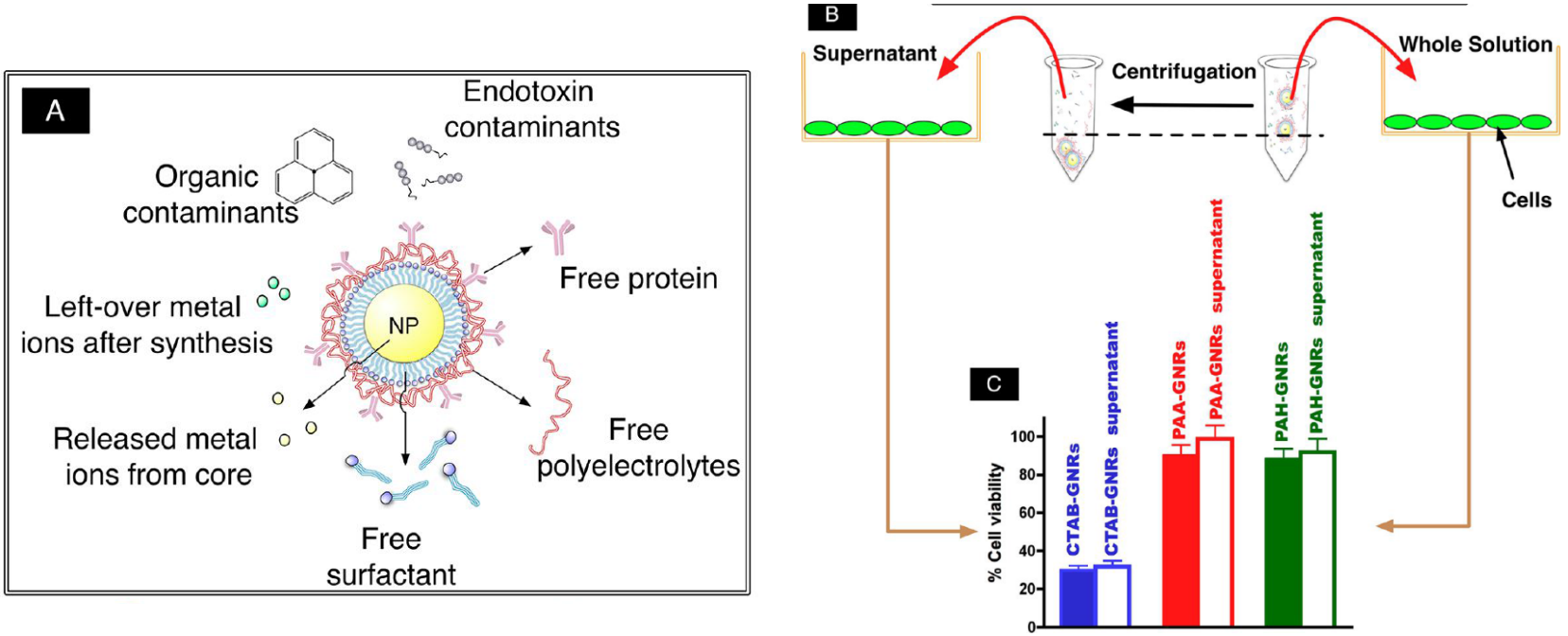
- high relative sensitivity (83% of ^{1}H)
- 100% natural abundance of ^{19}F
- its resonance differs by only 6% from that of ^{1}H
- an extremely broad range (> 200 ppm) which permits unambiguous identification of distinctive ^{19}F-containing compounds, but sensitive to the environment
- tissues have a negligible background ^{19}F signal, quantitative analysis are allowed
- perfluorocarbons (PFCs) have been extensively studied in medicinal applications.
- need of very high ^{19}F concentration.
- Enable quantitative analysis

Ruiz-Cabello, J.; Barnetta, B. P.; Bottomleya, P. A.; Bulte, J. W. M. *NMR Biomed.* **2011**, *24*, 114–129.
Srinivas, M.; Heerschap, A.; Ahrens, E.T.; Figdor, C. G.; de Vries, I. J. M. *Trends Biotech.* **2010**, *28*, 363-370.

Toxicity

Environmental Health and Safety Considerations for Nanotechnology

ACR 2013, 46, issue 3



Possible impurities in nanoparticle solution include the following: free ions, proteins, polymers, surfactants, and organic molecules. The “supernatant control” in B is the supernatant of the original GNP solution after centrifugation, to study the contribution of these impurities in toxicity testing. (C) Toxicities of GNP solutions were similar to those of their corresponding supernatant solutions as measure using the MTT assay on colon cancer cell line (HT-29), highlighting the significant contribution of impurities in the supernatant. CTAB: cetyltrimethylammonium bromide; PAA: poly(acrylic acid, sodium salt); PAH: poly(allylamine hydrochloride).

Toxicity

TABLE 1. AuNP Uptake and Cytotoxicity as a Function of AuNP Size, Shape, and Surface Chemistry

NP sizes/shapes	surface chem	dose/incubation time/cell line	analytical techniques	conclusions
AuNP, 15–118 nm spheres, cages, rods	PEG-ylated, NM[a]	20–120 pM/24 h/HTB-30, SKBR-3, ATCC	UV–vis, ICP-MS	uptake independent of size, shape, surface chem[36]
AuNR, AR 1.0–4.0[b]	CTAB, PAA, PAH	0.001 nM/24 h/HT-29	UV–vis, ICP-MS, MTT	cell uptake PAH-AuNR > PAA-AuNR > CTAB AuNR cytotoxicity observed in CTAB rods (due to free CTAB)[30]
AuNR, AR 4.0	mPEG, targeting peptides	1.0 nM/1–2 h /A549	UV–vis, ICP-MS, optical microscopy	specific targeting affects AuNRs uptake *in vitro* *in vivo* AuNR uptake not strongly influenced by targeting peptides[45]
AuNP, 14–74 nm spheres	transferin-coated AuNPs	6 h/SNB19, HeLa, STO	UV–vis, ICP-MS	50.0 nm transferin-AuNPs taken up most rapidly by endocytosis[27]
AuNP, 20.0 nm spheres	varying ratios of modified peptides	1–2 μM/3 h/A549	UV–vis, TEM, ICP-MS	AuNP uptake rates depend on terminal amino acid residues in the ligand shell[61]
AuNRs, AR 2.0–3.5	CTAB	10 μL rod solution/2 h/MCF-7	UV–vis, MTT	cytotoxicity observed in CTAB rods (due to free CTAB)[62]

[a]NM, not modified; the authors used a variety of GNPs (spheres, rods, cages, each with different capping agents as synthesized), and the GNPs were then PEG-ylated. [b]AR = aspect ratio (l/*d*).