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The Gold Standard: Gold Nanoparticle Libraries To Understand the Nano–Bio Interface

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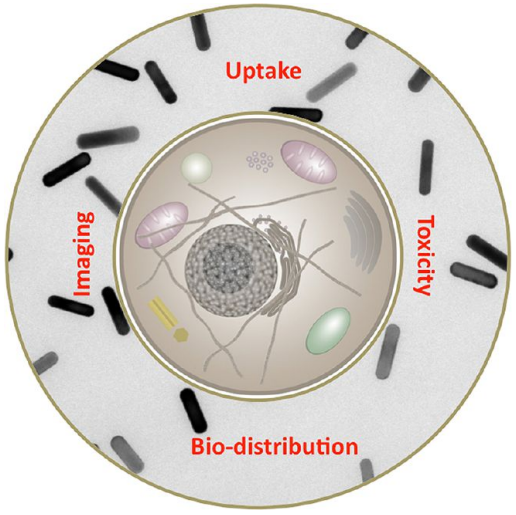
CONSPECTUS

Since the late 1980s, researchers have prepared inorganic nanoparticles of many types—including elemental metals, metal oxides, metal sulfides, metal selenides, and metal tellurides—with excellent control over size and shape. Originally many researchers were primarily interested in exploring the quantum size effects predicted for such materials. Applications of inorganic nanomaterials initially centered on physics, optics, and engineering but have expanded to include biology. Many current nanomaterials can serve as biochemical sensors, contrast agents in cellular or tissue imaging, drug delivery vehicles, or even as therapeutics.



In this Account we emphasize that the understanding of how nanomaterials will function in a biological system relies on the knowledge of the interface between biological systems and nanomaterials, the nano-bio interface. Gold nanoparticles can serve as excellent standards to understand more general features of the nano-bio interface because of its many advantages over other inorganic materials. The bulk material is chemically inert, and well-established synthetic methods allow researchers to control its size, shape, and surface chemistry. Gold's background concentration in biological systems is low, which makes it relatively easy to measure it at the part-per-billion level or lower in water. In addition, the large electron density of gold enables relatively simple electron microscopic experiments to localize it within thin sections of cells or tissue. Finally, gold's brilliant optical properties at the nanoscale are tunable with size, shape, and aggregation state and enable many of the promising chemical sensing, imaging, and therapeutic applications.

Basic experiments with gold nanoparticles and cells include measuring the toxicity of the particles to cells in in vitro experiments. The species other than gold in the nanoparticle solution can be responsible for the apparent toxicity at a particular dose. Once the identity of the toxic agent in nanoparticle solutions is known, researchers can employ strategies to mitigate toxicity. For example, the surfactant used at high concentration in the synthesis (0.1 M) of gold nanorods remains on their surface in the form of a bilayer and can be toxic to certain cells at 200 nM concentrations. Several strategies can alleviate the toxic response. Polyelectrolyte layer-by-layer wrapping can cover up the surfactant bilayer, or researchers can exchange the surfactant with chemically similar molecules. Researchers can also replace the surfactant with a biocompatible thiol or use a polymerizable surfactant that can be "stitched" onto the nanorods and reduce its lability. In all these cases, however, proteins or other molecules from the cellular media cover the engineered surface of the nanoparticles, which can drastically change the charges and functional groups on the nanoparticle surface.

Introduction

Nanotechnology is an emerging field that has attracted tremendous academic and industrial interest over the past decade. Gold nanoparticles are of great interest due to their fascinating optical properties and their promising applications.[1−6] While the first chemical preparation of

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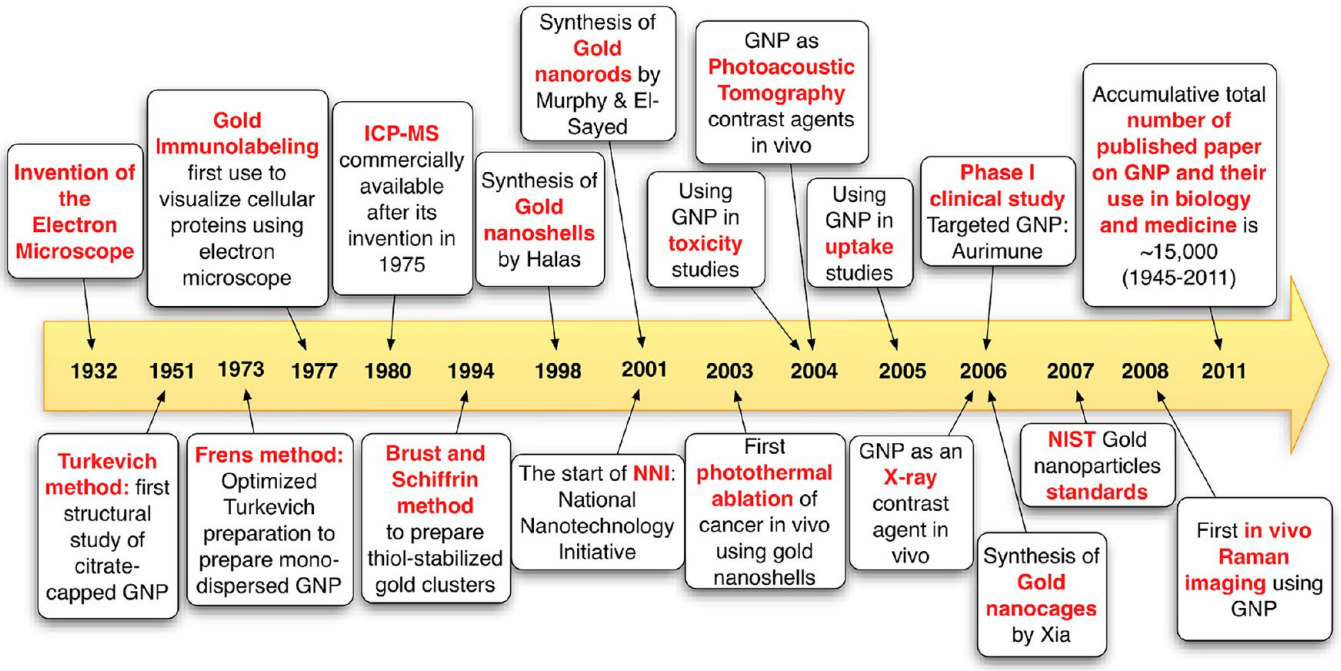

FIGURE 1. The golden timeline. Major events/discoveries that sparked the evolution of our ability to prepare GNPs with size/shape control, understand their structural and optical properties, and employ them in various biomedical applications.

"colloidal gold" appeared well before 1900,[7] recent advances in chemistry, physics, and microscopy have led to better control of particle size and shape distribution, a deeper understanding of how gold nanoparticles interact with light, and more details on their atomic structure (Figure 1).

Modern applications of gold nanoparticles (GNPs) are tremendous, ranging from chemical sensing to imaging to cancer treatment and drug delivery.[1−6] However, putting high-surface-area nanomaterials in biological systems can lead to unanticipated or detrimental effects on living cells or organisms, highlighting the need to understand the "nano−bio interface". This Account will focus on the use of GNPs both as probes to understand the interface of nanoparticles with biological systems and as models to study the impact of nanoparticle parameters on biological systems (Figure 2).

The use of GNPs as probes and reference materials is well-established in many other contexts; GNPs are commercially available standards for high-magnification calibration of electron microscopes. Antibody-functionalized GNPs (Immunogold) have been used since the late 1970s as stains to visualize cellular compartments, proteins, and receptors with electron microscopy.[8] GNPs are used as biological contrast agents in photoacoustic, DIC (differential interference contrast), CT (computed tomography), Raman, and dark field imaging platforms.[9]

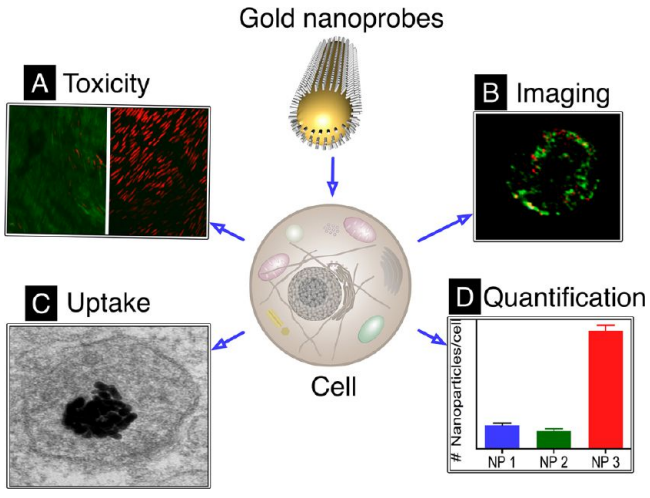

FIGURE 2. Measurement modes for nanoparticle−cell interactions. (A) Confocal fluorescence image with live/dead cell staining showing dead cells (red) and live cells (green) upon exposure to various GNPs. (B) Dark field microscope image of GNPs attached to colon cancer cells. (C) Transmission electron microscope image of gold nanorods inside an endocytic vesicle in the cytoplasm of an endothelial cell (no staining required). (D) Cellular uptake quantification of different GNP formulations using inductively coupled plasma mass spectrometry.

Reasons for a "Gold" Standard

GNPs are ideal nanomaterials to serve as probes for investigating the influence of nanoparticle size, shape, and surface chemistry on their biological interactions.[1−4,15−19] GNPs are generally more chemically stable than other NP

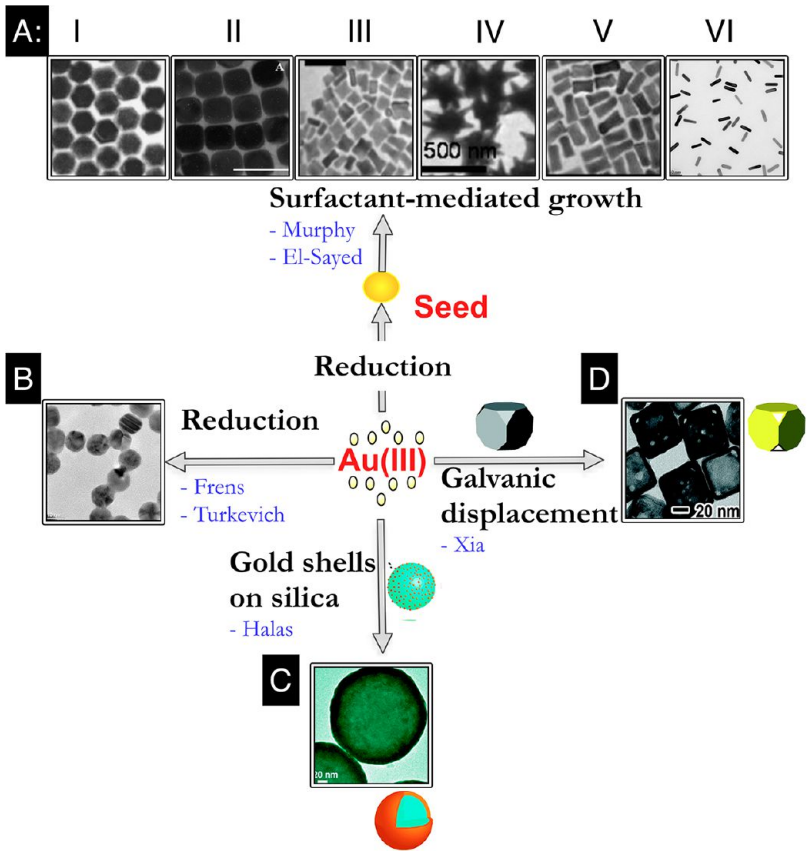

FIGURE 3. Various synthetic methods to prepare GNPs with controllable size and shape. (A) Surfactant-mediated wet chemical approach to prepare GNPs with hexagonal (A, I), cubic (A, II), rectangular (A, III), star (A, IV), dog bone (A, V), and rod (A, VI) shapes. (B) Direct reduction of gold ions to prepare spherical nanoparticles. (C) Gold shell formation at the surface of silica nanoparticles to prepare gold–silica core–shell nanoparticles. (D) Galvanic replacement reaction on the surface of silver nanocubes to prepare porous/hollow gold nanocages. Images in A and D are reprinted with permission from references 16 and 4 respectively; Copyright (2004, 2011, respectively) American Chemical Society. Images in C is reprinted with permission from Wang et al. *Acc. Chem. Res.* **2007**, *40*, 53–62; Copyright (2007) American Chemical Society.

core materials.[1–4,20] GNPs possess properties (intense visible light scattering/absorption; large electron density; low background concentrations in natural aqueous environments) that make them relatively easy to quantify, locate, and image in biological systems. Finally, simple synthetic routes are now available that permit the synthesis of GNPs with precisely controlled size and shape (Figure 3).[10–19]

Gold Nanoparticle Libraries

The synthesis of GNPs with precisely controlled sizes (less than 5% standard deviation in diameter) and shapes is now routine. As a consequence, GNP libraries with systematically varied dimensions and surface chemistry can be obtained commercially or prepared in-house. Spherical GNPs may be prepared using direct reduction of a metal salt in the presence of a capping agent (Turkevich, Frens, Brust preparations; Figure 3) or *via* seeded growth approaches, depending on the desired size and surface chemistry.[6,14,15] These syntheses provide GNPs with core diameters (d_{core}) between 0.8 and 200.0 nm. Anisotropic (nonspherical) GNPs (including gold nanorods [GNRs], stars, and cubes) can also be readily prepared, using seeded growth techniques (Figure 3).[16,17] The seeded growth approach is used to produce GNRs with aspect ratios (length/diameter) between 2.0 and 18.0.[2,18] Gold nanocages (hollow gold cubes with varying thickness) are prepared using galvanic replacement reactions.[19] This synthetic control over GNP dimensions translates to precise control over their size-dependent optical properties. Spherical GNPs possess an intense plasmon absorption that can be tuned over a limited spectral range (520–580 nm; Figure 4). More complex shapes possess multiple plasmonic absorptions. For instance, GNR solutions possess two plasmon bands, a transverse plasmon band corresponding to light absorption and scattering along the short axis of the material ($\lambda_{max} \sim 520$ nm), and an aspect ratio-dependent longitudinal plasmon band ($\lambda_{max} \sim 600$–1800 nm; Figure 4).[18,20]

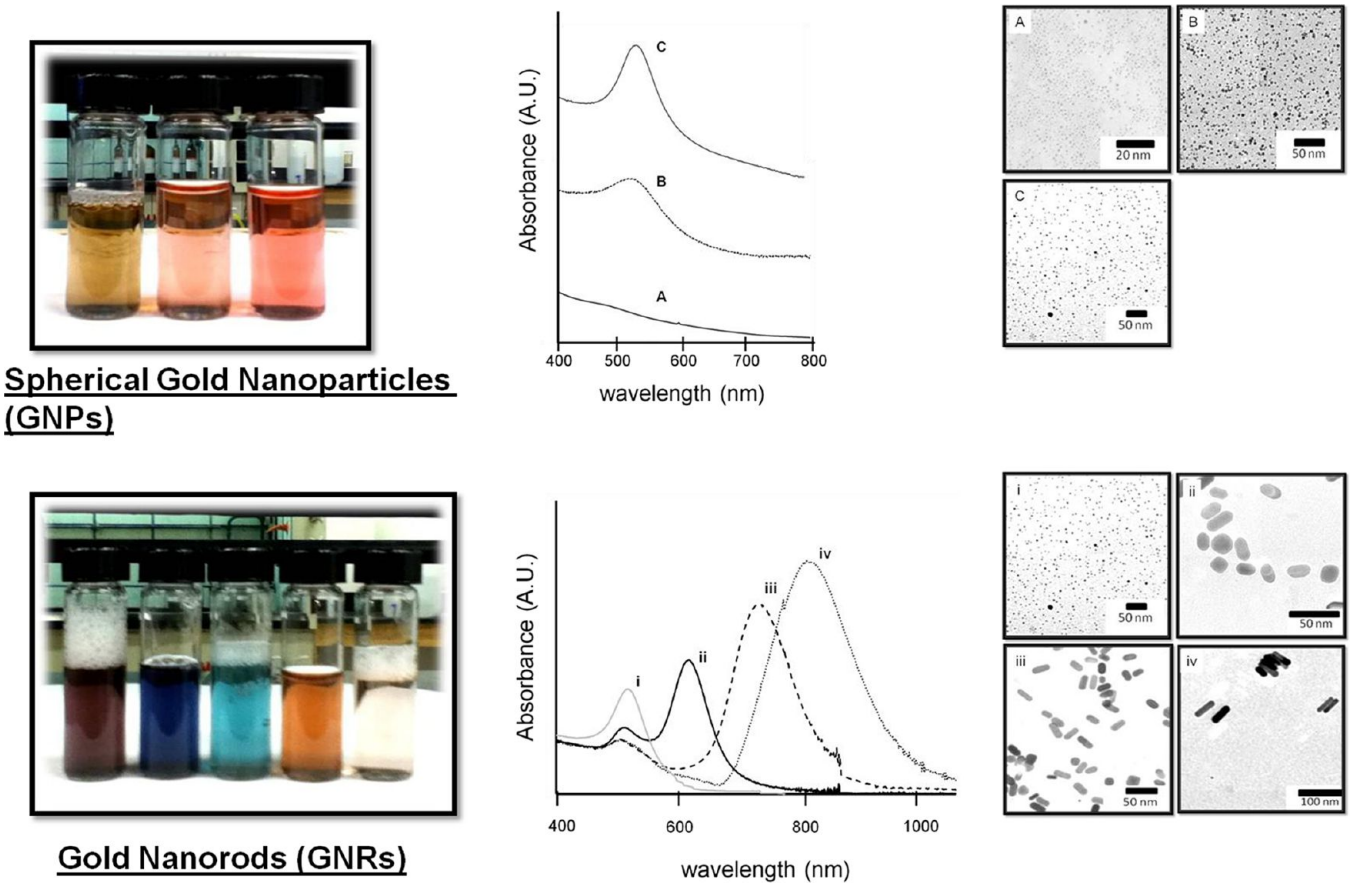

FIGURE 4. Gold nanoparticles possess size- and shape-dependent optical properties. (Top panel): Spherical gold nanoparticles (GNPs) of diameter 3 nm (A) are too small to support a plasmon band; GNPs of 5 (B) and 20 nm (C) possess a single plasmon absorption band. (Bottom panel): Gold nanorods (GNRs) possess two plasmonic absorption bands, corresponding to the transverse and longitudinal dimensions of the rod. The longitudinal plasmon absorbance band is aspect ratio-dependent (i–iv).

A versatile array of postsynthetic surface modifications has been developed to produce GNPs that display functionalized ligands, antibodies, and biomacromolecules on their surfaces.[14,21] These postsynthetic surface modification strategies include electrostatic adsorption or covalent binding of biomolecules, antibodies, polymers, and polyelectrolytes, as well as ligand exchange with functionalized thiols, phosphines, and surfactants (Figure 5).[14,22–25] These synthetic strategies can control the surface charge, modify the biocompatibility, and improve the colloidal stability of GNPs. Our research group has frequently made use of the polyelectrolyte layer-by-layer wrapping procedure to control the surface charge of GNRs or provide attachment points to covalently attach antibodies or targeting molecules (Figure 5).

Cellular Uptake

Many investigations regarding GNPs behavior in vitro have focused on how GNP size, shape, and surface charge affect their cellular uptake and toxicity using cell cultures.[13] The results from recent studies are summarized in Table 1. Typically, GNPs are delivered to cells as a suspension of GNPs in cell culture media, and GNP uptake is quantified using a combination of ultraviolet–visible absorbance spectroscopy (UV–vis) and inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is a very sensitive analytical technique for gold, capable of detecting 60 parts per trillion gold atoms, which translates to femtomolar to attomolar concentrations of nanoparticle, depending on their size.[1] Transmission electron microscopy (TEM) visualizes GNRs within the cell following uptake. Cytotoxicity is typically assessed using cell viability assays such as the widely used MTT assay, which measures the ability of mitochondria to properly metabolize a dye as a function of agent concentration.[26]

GNP libraries with systematically varied size and surface chemistry have been deployed to investigate the effect of these properties on cellular uptake. Chithrani et al. have explored the effect of GNPs size and shape on cellular

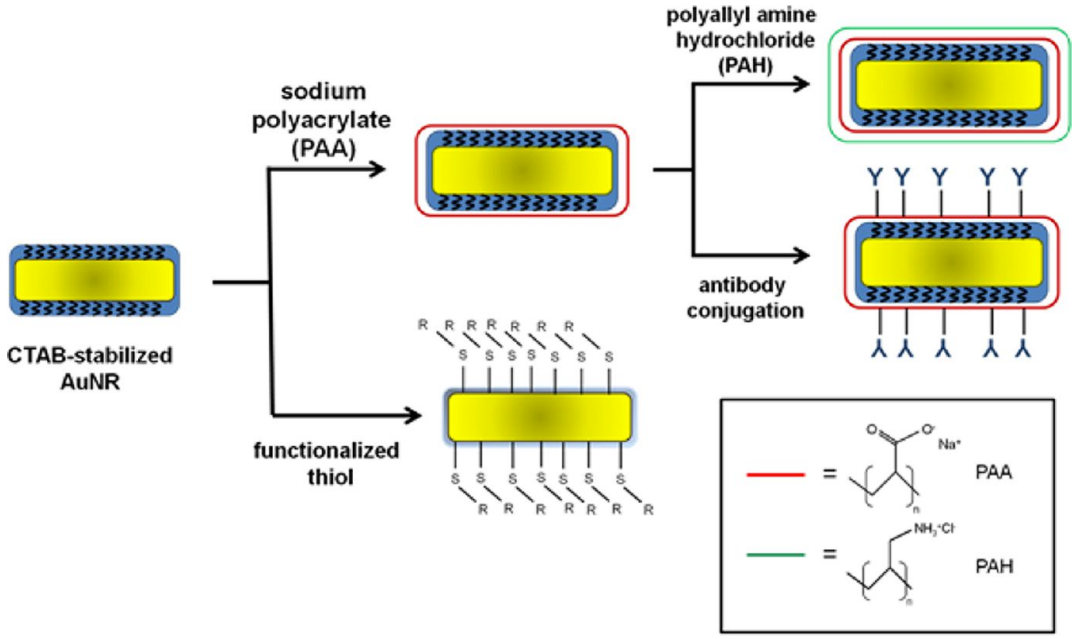

FIGURE 5. Functionalized GNRs can be prepared using a variety of postsynthetic modification strategies. Two strategies are generally employed: layer-by-layer wrapping using polyelectrolytes or thiol exchange. In the layer-by-layer approach, cationic CTAB GNRs are overcoated with anionic polyacrylate (PAA) at the proper pH, which can then be further overcoated with another cationic polyelectrolyte, such as polyallylamine hydrochloride (PAH). The functional groups of these polymers also provide an opportunity to covalently bind antibodies or other proteins to the GNR surface. As an alternative, the CTAB can be exchanged for functionalized thiols, yielding thiol-stabilized GNRs, which may display a variety of ω-functionalities.

uptake by preparing libraries of GNPs with d_{core} 14–100 nm and GNRs with aspect ratios 1.5–5.0.[27] They found that 50 nm GNPs were more rapidly taken up in cell cultures than other GNPs. Stellacci and others have examined how the surface chemistry of GNPs influences their cellular uptake and interactions with proteins.[3,8,28] Several of these studies have suggested that relatively small variations in surface chemistry (e.g., changes in ligand arrangement and orientation on the GNPs surface) as well as gross changes in NP surface charge can significantly influence the rate of cellular GNP uptake.[28] It appears that positively charged GNPs are taken up more rapidly than other GNPs. An obvious rationalization, which is subject to debate, is that positively charged GNPs associate more quickly with negatively charged cell membranes, resulting in a faster rate of uptake.[12,29]

We have examined the cellular uptake of GNRs with different aspect ratios and surface chemistries at sublethal doses (<0.2 nM in particles). Our library consisted of GNRs with various aspect ratios between 1.5 and 4.0, functionalized with three different ligands: cetyltrimethylammonium bromide (CTAB), sodium polyacrylate (PAA), and polyallylamine hydrochloride (PAH). CTAB- and PAH-functionalized GNRs are cationic whereas PAA-functionalized GNRs are anionic at pH 7. We found that GNRs functionalized with PAH were more rapidly taken up into cells than CTAB- or PAA-functionalized GNRs with the same aspect ratio (~2200 PAH-coated GNRs per cell vs 250 PAA-GNR vs 50 CTAB-GNR, at the same time point, as quantified by ICP-MS).[30] However, our study also revealed that the initial surface charge of GNRs is a poor predictor of "true" surface charge, because GNRs exposed to cellular media adopt an identical surface charge due to the adsorption of serum proteins (see below).[30]

Cellular Toxicity

GNPs have been employed as model systems to understand the potential toxicity of nanomaterials. GNP libraries with a variety of core diameters (0.8–100.0 nm) were used to study the effect of core size on nanoparticle acute toxicity, either when administered to cell cultures directly or when injected into whole organisms.[13,31] As early as 2007, there was significant concern that small GNPs ($d_{core} < 2.0$ nm), which are more redox-reactive than larger GNPs, might prove to have significant toxicity following studies of GNPs in fibroblasts, epithelial cells, and macrophages.[32,33] However, subsequent studies, both *in vitro* and *in vivo*, have indicated that GNPs are not acutely toxic, regardless of core diameter.[34]

In our own work, we tested the toxicity of GNRs with aspect ratios between 1.5 and 4.0 functionalized with CTAB, PAA, and PAH. We found that cell viability is independent of

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*).

GNR aspect ratio and that the surface chemistry effect was predominant over nanoparticle's size. While CTAB-GNRs reduced cell viability to 30% at a concentration of 0.4 nM (in gold nanorods), PAA and PAH-GNRs were nontoxic at the same doses.[30] The molecular origin of cellular toxicity is discussed in the next section. Related studies, at various doses, are summarized in Table 1.

While cytotoxicity and uptake of GNP probes are still being extensively investigated *in vitro*, the underlying methodology behind these studies must be carefully examined to ensure that the results across laboratories are comparable.[35] Cho et al. recently demonstrated that some of the trends previously observed in cellular uptake might be attributable to the experimental setup used in the uptake rate studies. They used a library of functionalized GNPs with different sizes, shapes, and surface groups to show that if cell cultures were dipped into and suspended in the NP-bearing media, rather than grown on the bottom of the media dish, GNPs were taken up into cells at the same rate, regardless of size or surface charge.[36] Their study indicated that previous results showing size dependence in NP uptake may be attributable to differences in sedimentation rates in NP of different sizes or solubilities, resulting in an artificially increased concentration of GNPs at the bottom of the media dish. Additional methodological concerns revolve around the need to develop standardized dosages (per particle?, per mass?, per surface area?), impurity profiles, and polydispersity requirements so that toxicology studies using similar nanoprobe libraries can be accurately compared. One implication of Cho's study is that most of the previous work in the literature might need to be redone to know the true concentration of nanoparticles from the cell's point of view! We note that a new journal, *Nanotoxicology*, is entirely devoted to the study of nanoparticle toxicity and that the ACS journal *Chemical Research in Toxicology* has an increasing number of papers on nanoparticle toxicity.

Uncovering the Molecular Origin of Apparent Toxicity with GNPs Models

Nanoparticle solutions are far more complex than molecular solutions. Nanoparticles in aqueous solution bear a halo of ions, capping agents, or biomolecular ligands that may have been deliberately placed there, or may be physisorbed from the environment (Figure 6A). In a typical toxicity evaluation, a nanoparticle solution is injected into culture media at a certain concentration for a certain time. Cultured cells are then exposed to this nanoparticle solution—and to any other "free" organic/inorganic compounds in the

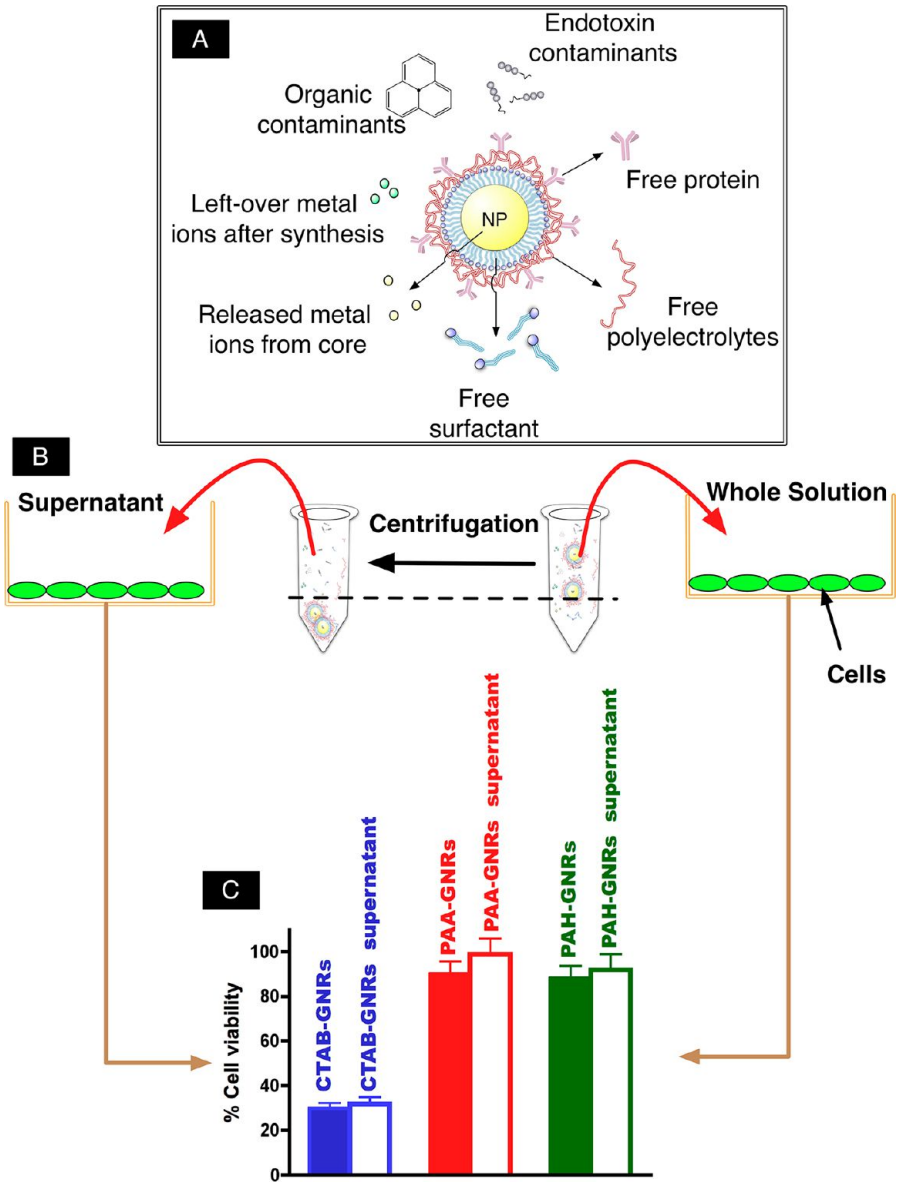

FIGURE 6. Possible impurities in nanoparticle solution include the following: free ions, proteins, polymers, surfactants, and organic molecules. The "supernatant control" in B is the supernatant of the original GNP solution after centrifugation, to study the contribution of these impurities in toxicity 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). Data in part C taken from ref 30.

nanoparticle solution. Therefore, the toxic contribution of the nanoparticles themselves should be resolved from the contribution of other components in the solution. The ease of separating GNPs from their supernatants is advantageous to study the contribution of other components in nanoparticle solutions. Gold is dense, and GNPs can be separated by simple centrifugation for a short time (<5 min) without significant destruction to the nanoparticles or their surface composition. Moreover, quantification of GNPs that do remain in the supernatant solution is simple using UV–vis spectroscopy, thanks to the extremely high molar extinction coefficients of $\sim 10^9\ cm^{-1}\ M^{-1}$.[37] Therefore, we recommend that a "supernatant control" be performed alongside every cellular toxicity experiment with nanoparticles: the difference in effects that a nanoparticle solution and its supernatant solution have on cellular viability and behavior can then be tied to either the nanoparticles or the "impurities" more clearly.[13]

For example, consider the case of CTAB-capped gold nanorods.[38] CTAB acts both as a shape directing agent and as a stabilizing agent against aggregation.[39] As a surfactant itself, it is known to be toxic to cells at submicromolar

concentrations, but it is used at 0.1 M concentrations in the synthesis of the nanorods. CTAB-capped gold nanorods (CTAB-GNRs) are cationic and can be overcoated with anionic polyelectrolytes (e.g., poly(acrylic acid), PAA-GNRs) at pH 7, which allowed us to test the effect of particle surface charge on the cellular toxicity of GNRs.[30] Using colon cancer cell lines and MTT cell viability assays, we found that cationic CTAB-GNRs were much more toxic to cells than anionic PAA-GNRs at identical doses.[30] We initially thought that the positively charged nanoparticles were more toxic due to their ability to disrupt the negatively charged cellular membrane, as is commonly postulated.[29] To check for free CTAB, however, we did the supernatant control experiment (Figure 6B).[30] Interestingly, the measured amount of cell death from the original gold nanorod solutions and their corresponding supernatants was identical (Figure 6C) and similar to that measured for a 200 nM aqueous solution of CTAB alone (a level of free CTAB in GNRs solution as quantified using liquid chromatography–mass spectrometry).[30] Our results quantitatively indicated that the GNR solution toxicity is due to the presence of free CTAB molecules and not to the cationic GNRs themselves. This finding was further supported by overcoating PAA-GNRs with a positively charged polyelectrolyte coat to prepare again cationic nanorods. The later cationic nanorods had the exact dose–response toxicity curves to anionic PAA-GNRs.[30] Our results clearly showed that GNRs (anionic or cationic) have similar toxicity profiles and the toxicity for CTAB-GNRs originates only from the free capping agent (CTAB). These findings highlight the importance of the "supernatant control" as a method to assess the toxic effects of free impurities in nanoparticle solutions (Figure 6).

Strategies To Mitigate Cytotoxicity Using Surface Chemistry: Examples with Gold Nanoparticles

When the origin of nanoparticle toxicity is unraveled by the proper experimental design, the next step is to "detoxify" the particles and make them safer. In this section we will summarize the strategies one can use, with CTAB-coated GNRs as our example.

Since free CTAB molecules are the toxic agent in CTAB-GNR solutions, mitigation strategies include (i) overcoating the CTAB on the rods; (ii) zipping up the CTAB on the particle surface so it cannot desorb, and (iii) replacing the CTAB once the rods are made with a more biocompatible ligand. We and others have shown that coating CTAB-GNRs with polyelectrolytes decreases their toxicity to cells by retarding the desorption of CTAB molecules from the surface of GNRs (Figure 7A, I).[30,40] A "zipping" approach can be realized by synthesizing a polymerizable version of CTAB and performing the polymerization reaction on the particle to "stitch" it on the surface of GNRs (Figure 7A, II).[22,41] After on-particle polymerization, the biocompatibility of GNRs was enhanced significantly (Figure 7B and C), which was correlated to a decrease in surfactant desorption from the GNR surface as measured by mass spectrometry.[22,41]

Displacing the CTAB molecules from the surface of GNPs is another effective approach to improve their biocompatibility. At least three methods have been reported to displace CTAB: extraction with organic solvent followed by capping GNPs with "safer" ligands,[42] cationic exchange for CTAB molecules by competing cationic ligands that bear similar quaternary ammonium headgroups,[22] and displacement using thiols[43] (Figure 7A, IV). The latter method relies on excess thiol to displace chemisorbed CTAB molecules on the surface of gold and form (presumably) strong gold–thiol bonds. Qualitatively, it has been found that incubating CTAB GNP's with phospholipids or thiolated polyethylene glycol enhances colloidal stability and decreases the toxicity of GNPs significantly.[22,42,43]

Biodistribution of Nanoparticles in Whole Organisms

The ease of visualizing GNPs in biological compartments by TEM, and the sensitivity of standard quantification methods for gold, such as ICP-MS, make them excellent probes to understand the distribution of nanomaterials in whole organisms. GNPs have been used to understand the effect of nanoparticle size and surface chemistry on their accumulation inside the cytoplasm, lysosomes, or the nucleus in cultured cells and to evaluate the pharmacokinetic parameters of nanomaterials following systemic injections.[44] Targeted GNPs (that is, initially surface-modified with a ligand to recognize certain cells) were used as tracers to evaluate their accumulation in tumors *in vivo* compared to nontargeted nanoparticles as an attempt to re-examine the known phenomena of "enhanced permeation and retention effect" (EPR effect).[45] This EPR effect is a boon for medicinal uses of nanoscale objects: the hypothesis is that rapidly growing tumors lay down their vasculature supply lines imperfectly, rendering them leaky to nanosized objects, so ~10–100 nm particles can simply accumulate passively in tumors without targeting.[45] Quantification of how well the EPR effect works in practice, however, is still ongoing.[45] Many other examples of GNP biodistribution in whole organisms have been reported, some of which are

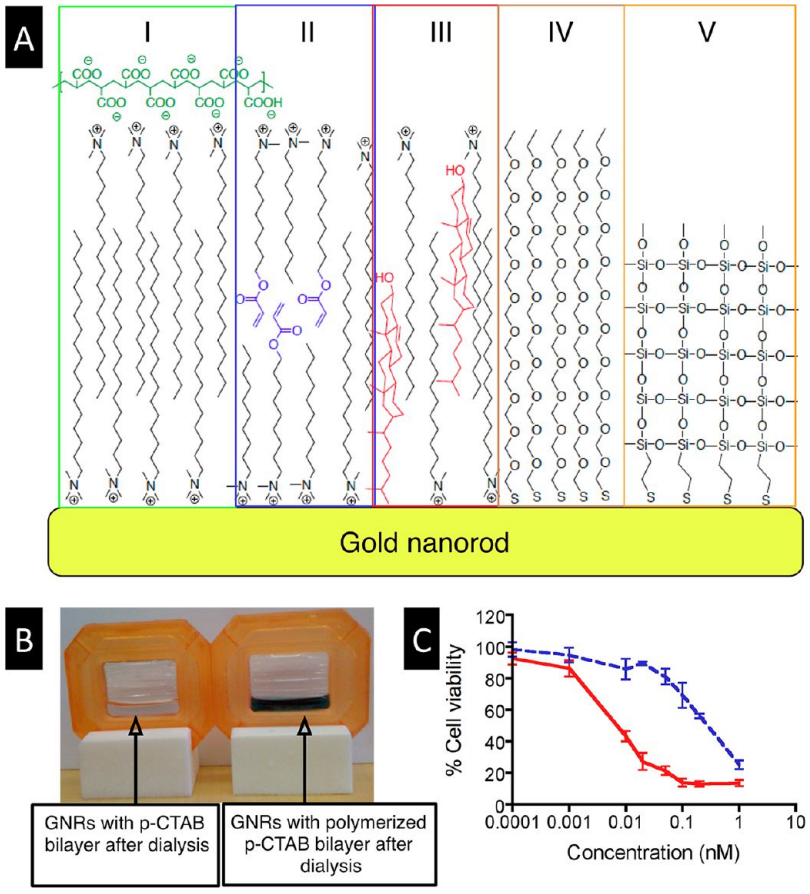

FIGURE 7. (A) Various approaches to detoxify GNRs and enhance their colloidal stability by surface modification: (I) electrostatic coating, (II) on-particle polymerization to fix on the surfactant, (III) insertion of hydrophobic molecules to retard the desorption of surfactant molecules from the bilayer, (IV) displacement of the surfactant by thiols, (V) silica coating. (B) Photograph of GNR solutions in dialysis cassettes after dialysis for 24 h (left: gold nanorods with surfactant bilayer aggregated and precipitated out from solution and therefore not visible; right: gold nanorods with fixed surfactant bilayer by polymerization are stable and do not aggregate in response to dialysis). P-CTAB: polymerizable version of CTAB.[22] (C) Dose–response viability of colon cancer cells exposed to GNRs with a polymerizable surfactant bilayer before polymerization (red solid line) or gold nanorods with a polymerizable surfactant bilayer after polymerization (blue line).[22]

summarized in a recent review of ours,[13] and results on the important issue of nanoscale object clearance through the kidneys are also appearing.[46]

A major question for the nanobiotechnology field is the following: what is the state of the nanoparticle once it is in a real living system? Nanoparticles could aggregate due to the presence of salt or due to the loss of the capping agents; biomolecules could also bridge (in low concentration) or coat (in high concentration) nanoparticles.[47,48] Aggregation and protein adsorption to the surface of GNPs could lead to a dramatic change to the size, shape, surface charge, and hydrophilicity of nanoparticles, which could affect their aggregation state, sedimentation rate, cellular uptake, and toxicity.[47] Even in simple cell culture, proteins from the media adsorb spontaneously to the surface of cationic GNPs and flip their surface charge from positive to negative (Figure 8).[30,49,50] With this in mind, the simple picture of a direct electrostatic interaction of cationic nanocarriers with the negatively charged cellular membrane needs to be revised.[30] The composition of the protein "corona" and its dynamic nature could affect NP cellular uptake.[51]

Probing the protein corona on the surface of GNPs can unravel the complexity of protein adsorption to the surface of nanoparticles.[52] Stauber and co-workers identified 120 proteins associated with GNPs mixed with human plasma with on-particle abundance differing from that in plasma.[53] It is important to note that the composition of the protein corona and its dynamic nature could affect NP cellular uptake.[51]

Biodistribution of Nanoparticles in an Entire Ecosystem

Another plane of complexity is nanoparticle biodistribution in a food web. Following a nanoparticle as it moves between

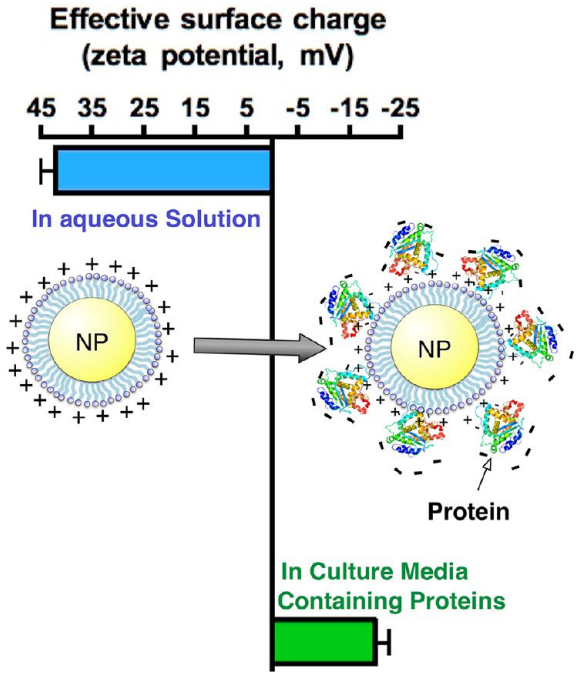

FIGURE 8. Cartoon demonstrating the adsorption of protein from biological media to the surface of initially cationic GNPs, and the resulting alteration of the surface charge in cellular media to a negative value, as measured by ζ potential analysis.

organisms in an entire ecosystem requires that the nanoparticle be easy to measure in an environmental context. Gold again is an excellent model system because of its low background in the environment and its ease of quantitative detection by ICP-MS. We used CTAB-coated GNRs to study nanoparticle partitioning and biodistribution in a model estuarine system containing seawater, sediments, microbial biofilms, and filter feeders such as clams, grazers, and omnivores.[54] The content of GNRs in each component was analyzed using ICP-MS as a function of time.[54] During the course of this two-week study, no animals died. The main point of entry of GNRs into the food web was the microbial biofilms, followed by the filter feeders.[54] Bertsch and coworkers used citrate-capped gold nanospheres to study nanoparticle fate in a soil–earthworm ecosystem.[55] The same group reported the uptake of GNPs (5, 10, 15 nm) by plants (primary producers) and the transfer to hornworms (primary consumers) in a size-dependent manner.[56] In these studies the detection of GNPs in any compartment (seawater, soil, worms, etc.) was performed with ICP-MS, with additional visualization inside organisms using TEM. Using GNPs as stable probes to understand the fate of nanoparticles in different ecosystems is promising, and we expect more research and new understanding in this direction.

Concluding Remarks

We hope that this Account highlights our thesis that gold nanoparticles are excellent nanoparticle models to probe the interaction of nanomaterials with cells, biological media, whole organisms, and ecosystems. The selection of gold nanoparticles is based on the availability of various simple methods to prepare them in libraries, the ease of surface modification, the ability to track and detect them with various analytical tools, and finally their biocompatible core. The U.S. National Institute of Standards and Technology (NIST) released the first GNPs (spheres 10, 30, 60 nm in diameter) in 2007 as nanoparticle reference materials, which are intended to be used by biomedical researchers to evaluate the physiochemical and biological properties of new nanomaterials and to allow comparison of results across different laboratories. The promising use of GNPs as a drug delivery platform and biomedical phototherapeutic agents along with using GNPs in toxicological and ecological studies will enrich our knowledge on how nanomaterials interact and behave in biological and environmental compartments. The study of nanoparticle toxicity both *in vitro* and *in vivo* is a field that has grown substantially in recent years, and this research is constantly producing new results regarding the interaction of nanoparticles with these complex biological systems. We hope that this brief Account stimulates the reader to consult additional sources.[11,15,44,45,57–60]

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FOOTNOTES

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