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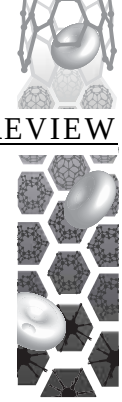

Biomedical applications of plasmon resonant metal nanoparticles

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The strong optical absorption and scattering of noble metal nanoparticles is due to an effect called localized surface plasmon resonance, which enables the development of novel biomedical applications. The resonant extinction, which can be tuned to the near-infrared, allows the nanoparticles to act as molecular contrast agents in a spectral region where tissue is relatively transparent. The localized heating due to resonant absorption, also tunable into the near-infared, enables new thermal ablation therapies and drug delivery mechanisms. The sensitivity of these resonances to their environment leads to simple affinity sensors for the detection of low-level molecular analytes. Coupled with their general lack of toxicity, these applications suggest that noble metal nanoparticles are a highly promising class of nanomaterials for new biomedical applications.

Keywords:
bionanotechnology, biosensing, nanoparticle, nanophotonics, optical coherence tomography, optoacoustic tomography, photothermal therapy, plasmonics, plasmon resonance, thermal ablation therapy, thermolytic therapy

future medicine

An intensive research effort over the past 20 years has yielded nanoparticles of controlled size and structure that are composed of a variety of materials, including semiconductors, metals, oxides, fullerenes and organics. These nanomaterials often exhibit novel, tunable physical properties not found in their molecular or bulk precursors. When coupled to surface ligands for a controlled interface with their environment, the nanoparticle properties enable the development of new biomedical technologies for the detection and treatment of disease [1–5]. Common themes include their use as molecular imaging contrast agents, sensors for disease markers, vehicles for drug delivery and therapeutic agents based on their novel properties. Each class of nanomaterial has a unique set of properties that can be exploited in different ways. In this review, we will outline several biomedical applications of noble metal nanoparticles (hereafter referred to simply as 'nanoparticles') that are made possible owing to localized surface plasmon resonance (LSPR).

Localized surface plasmon resonance

Localized surface plasmon resonance occurs when an electromagnetic field drives the collective oscillations of a nanoparticle's free electrons into resonance. This essentially classical effect was described theoretically by Mie in 1908 by solving Maxwell's equations for a metal sphere surrounded by a dielectric medium using the dielectric function of the bulk metal [6]. The dielectric function of gold and silver yields resonances at visible wavelengths for spherical nanoparticles [7]. The collective electron oscillations cause extinction of incident light by two mechanisms: Rayleigh scattering of the light into other directions and absorption of the light to generate heat. Therefore, gold and silver nanoparticle solutions exhibit strong peaks in their extinction spectra at visible wavelengths. Nanoparticles with diameters less than approximately 1/10 the radiation wavelength are excited as simple dipole oscillators. Dipole-limited nanoparticles are not strong scatterers, thus their extinction is dominated by absorption with resonance linewidths of less than 100 nm [8,9]. As the nanoparticle diameter increases beyond the dipole limit, higher-order excitations become significant, which greatly increase the scattering efficiency and broaden the resonance since they peak at longer wavelengths.

Although the basic LSPR phenomenon has been long understood, the field has experienced significant growth recently owing to advances in the synthesis of nanoparticles with more complex shapes and structures [10]. As aforementioned, spherical nanoparticles are only tunable by increasing their size beyond the dipole limit. For example, gold nanoparticles of 20 nm in diameter have a resonance at a wavelength of 520 nm in water, which red shifts to 600 nm owing to broadening as the nanoparticle diameter increases to 100 nm. Beyond this size, the resonance continues to shift but becomes significantly broadened [11]. Silver nanoparticle solutions are known to exhibit multicolored plasmon resonances, although the varied wavelengths are actually due to nonspherical shapes that exist in typical silver colloid preparations [8]. The key to achieving broad tunability without sacrificing

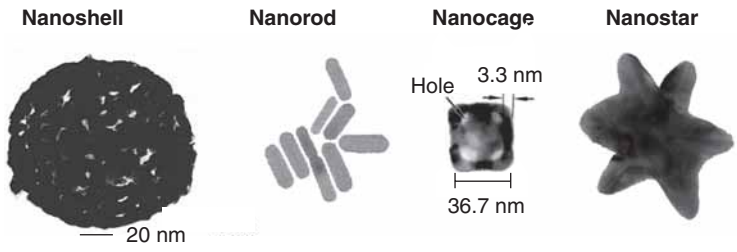
the linewidth of the resonance is to create nanoparticles with controlled, nonspherical shapes. Nanoparticles whose resonances are tuned into the near-infrared (NIR) spectral region where tissue is relatively transparent are especially valued for biomedical applications [12]. One path to NIR plasmon resonances is simply to aggregate the spherical nanoparticles [13], however aggregates are difficult to fabricate reproducibly. The syntheses of several well defined, monodisperse nanoparticles have been developed, whose resonant wavelength can be tuned through the visible and NIR spectrum (Figure 1). Metallodielectric gold nanoshells were used for initial demonstrations of several of the NIR applications described here [14]. More recently, surfactant-directed synthesis has enabled the high-yield production of gold nanorods [15,16], which are also tunable [17]. Silver nanoparticles can be synthesized with controlled shape and optical properties guided by optical illumination [18]. More complex architectures have also been described, including gold nanocages [19–21], elongated core–shell geometries [22] and gold nanostars [23–28].

Biomedical imaging contrast

The plasmon resonant scattering from a single gold or silver nanoparticle is many orders of magnitude brighter than the signal from single fluorophores, fluorescent beads or quantum dots in microscopic imaging applications [29,30]. In addition, the scattering signal does not photobleach or blink and polarization reveals the local orientation for nonspherical nanoparticles [31]. These effects have generated interest in the use of gold and silver nanoparticles as microscopic imaging labels [32]. However, the large nanoparticle size relative to fluorophores and quantum dots may limit intracellular imaging applications. Resonant scattering applications are therefore more likely to focus on particle tracking experiments and molecular contrast in tissues [33]. For example, the two-photon luminescence from NIR resonant gold nanorods has been used to monitor microscopic blood flow *in vivo* (Figure 2) [34]. Single particle imaging has also been exploited in a release assay to sense the functional activity of a biomolecular target, as opposed to simply measuring its concentration [35].

Fiber-based endoscopic imaging modalities provide high resolution *in vivo* imaging of tissue microanatomy with contrast based on changes in the refractive index [36]. Potential imaging mechanisms include optical coherence tomography (OCT), reflectance confocal microscopy (RCM) and optoacoustic tomography [37]. While these 'optical biopsies' achieve a resolution sufficient for the early diagnosis of disease based on cellular anatomy, their contrast is not ideally suited for imaging specific molecular changes. Imaging contrast and molecular specificity can be greatly enhanced by plasmon resonant scattering or absorption from targeted noble metal nanoparticles. Gold nanoparticles conjugated to anti-epidermal growth factor receptor (EGFR) have been used to target epithelial precancers specifically and provide imaging contrast sensitive to the EGFR expression level due to particle agglutination [38]. Nonspherical nanoparticles have also been pursued as contrast agents. Their NIR resonances could enable investigation deeper into tissue and even the possibility of diagnostic imaging with exogenous illumination and detection. Bioconjugated gold nanoshells [39–41], gold nanocages [19,21] and gold nanorods [42] are being developed for this purpose. These nanoparticles have been conjugated to specifically target cancer cells *in vitro*, where their specificity has been confirmed by dark-field optical microscopy (Figure 3). Nanoshells and nanocages have also been demonstrated to provide enhanced contrast OCT *in vitro* [19,41] and gold nanospheres and nanorods have enhanced optoacoustic tomography [37].

Figure 1. Transmission electron micrographs of goldnanoparticles with localized surface plasmon resonance that can be tuned through the visible and near-infrared.

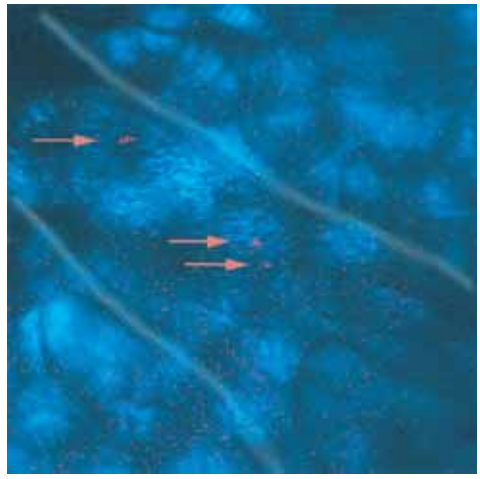


The nanoparticles are all shown to the same scale.
Adapted from [14] (nanoshell), [19] (nanocage) and [26] (nanostar). The nanorod image is supplied by the authors.

Drug delivery & thermal ablation therapy

Plasmon resonant absorption of light by noble metal nanoparticles provides a nanoscopic heat source and enables novel therapeutic strategies for the treatment of disease. In a manner similar to photodynamic therapy [43], plasmon resonant

Figure 2. *In vivo* gold nanorod particle tracking.



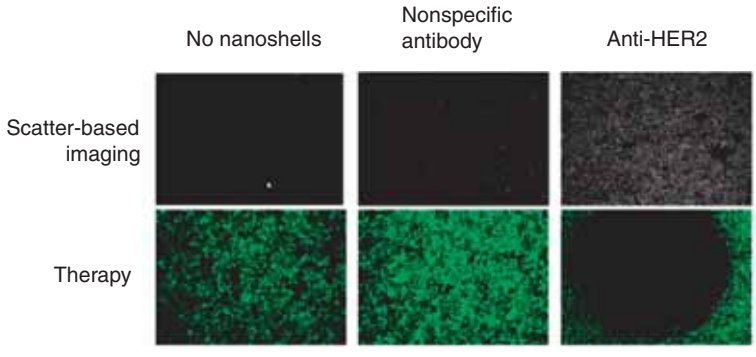
This composite figure consists of a transmission optical image of a mouse ear blood vessel (blue), with the two-photon luminescence signal from single gold nanorods overlaid in red (highlighted by arrows).
Reproduced with permission from [34].

nanoparticles can be delivered systemically and activated locally by exposure to resonant illumination. The local temperature increase can deliver drugs bound with the nanoparticle or have a direct photothermal or thermolytic therapeutic effect. For example, gold nanoshell–hydrogel composite materials have been loaded with protein and then illuminated at the plasmon resonance to stimulate release by shrinking the hydrogel [44]. The nanoshells caused enhanced drug release and enabled multiple bursts of protein by modulated heating. Such devices could have significant drug delivery applications, especially if the stimulant radiation can be administered exogenously to NIR resonant nanoparticles. A similar strategy has been pursued based on hollow polymer capsules filled with the substances to be delivered [45–47]. The capsule walls were impregnated with gold or silver nanoparticles so that absorption of light damaged the capsules, thus releasing their contents. Delivery has also been accomplished by associating the drug directly with the nanoparticle. DNA has been bound to lipid-stabilized gold nanorods. Upon resonant illumination, the nanorods transformed into spheres and the DNA was released without significant structural degradation, based on gel electrophoresis [48]. However, the function of the released genetic material has not yet been confirmed.

Thermal ablation treatments for cancer rely on the local application of heat to destroy diseased tissue selectively. Thermal therapies are simple and minimally invasive relative to conventional surgical treatments, although their effectiveness is limited by the ability to locally and specifically apply heat so as not to destroy healthy tissue. Plasmon resonant nanoparticles are highly effective enhancers of thermal ablation therapies since they can heat tissue locally owing to resonant absorption of radiation and can be targeted to tumors. Gold nanoshells photothermally destroyed breast carcinoma cells *in vitro* with a continuous wave (CW) NIR laser radiation dose that did not harm cells in the absence of the nanoparticles (Figure 3) [40]. Similar results were reported recently using conjugated gold nanorods [42]. An *in vivo* study in mice relied on the relatively high permeability of tumor vasculature to target the nanoparticles. Gold nanoshells were administered intravenously and deposited at the tumor site. Exogenous radiation of the tumor with NIR resonant radiation caused abatement of the tumor with excellent survival of the treated mice [49].

Excitation with pulsed lasers further enhances the degree to which nanoparticles locally heat the target tissue since less time is available for the heat to diffuse from the nanoparticle. Gold nanoparticles of 30 nm in diameter have been conjugated to $CD8^+$ T lymphocytes and exposed to 20 ns, 565 nm pulses *in vitro*, resulting in selective loss of viability in the targeted cell population [50]. Pulsed excitation has also been explored

Figure 3. Combined nanoshell imaging and therapy on SKBr3 breast cancer cells *in vitro*.



Dark-field microscopy images (top row) highlight the gold nanoshells targeted to the HER2 receptor. Calcein stain for viability (bottom row) after laser exposure demonstrates the locality and selectivity of the photothermal therapy.
Reproduced with permission from [40].

to selectively purge leukemic cells from a cell suspension. Gold nanoparticles of 30 nm in diameter were targeted to the leukemic cells and made to form clusters through the use of primary and secondary monoclonal antibodies. Upon pulsed laser excitation, these clusters form microbubbles owing to rapid heating at their surface, locally destroying the target cells thermolytically [51]. These experiments demonstrate that pulsed laser excitation can localize thermal ablation therapies to the cellular level.

Biosensing applications

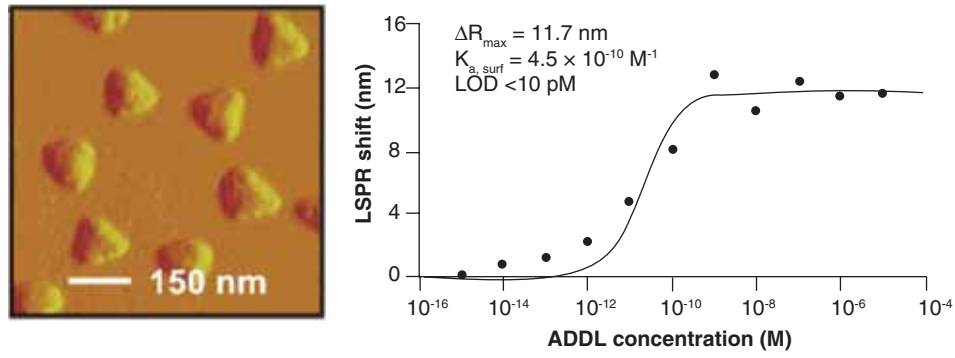
The plasmon resonances of noble metal nanoparticles are sensitive to the dielectric properties of their local environment. This effect has been exploited for a range of sensing strategies in which the presence of the molecule to be detected alters the extinction spectrum. In fact, one of the earliest demonstrations of nanoparticle biosensing relied on this effect [52,53]. Gold nanoparticles were functionalized with two-probe oligonucleotide sequences and exposed to a target oligonucleotide sequence, whose two ends were complementary to the probe sequences. When mixed, the target and probes hybridized, causing aggregation of the gold nanoparticles. The unhybridized gold nanoparticle solutions were red in color, typical of gold nanoparticles whose plasmon resonant peak absorption is 520 nm. Upon aggregation, the plasmon resonances became severely damped, resulting in a blue color and eventually precipitation of the colloid. This dramatic change in spectral properties can be detected colorimetrically, yielding an extremely simple means of sensing specific oligonucleotide sequences, an application of considerable biomedical interest. Since this initial demonstration, several significant extensions of the technique have been developed to detect single oligonucleotide base pair mismatches [54], sequence amplified DNA [55] and reduce the limits of detection drastically [56]. These advances are the result of chemical properties of the gold nanoparticles, such as their high density of DNA coverage and their ability to reduce silver to enhance the signals [57].

Analyte-induced nanoparticle aggregation can also be applied to protein and small molecule sensing, a concept that is certainly not new [58]. However, several advances in nanoparticle synthesis and conjugation have brought new capabilities to aggregation assays with plasmon resonant nanoparticles. Heterobifunctional cross-linkers provide nanoparticle bioconjugates that are more stable under physiological conditions and are more resistant to fouling. For example, a poly(ethylene) glycol linker has been developed with aldehyde distal to the nanoparticle surface. The aldehyde was functionalized with lactose to create a reversible and specific aggregation sensor sensitive to lectins [59]. The advent of plasmon resonant nanoparticles tunable to the NIR has enabled detection in optically turbid media. Using gold nanoshells, immunoglobulins were detected in whole blood below ng/ml concentrations [60]. Recently, aptamers have been applied as the selective binding element rather than antibodies. With gold nanoparticles conjugated to aptamers for platelet-derived growth factor (PDGF), different aptamer-binding strengths to different PDGF isoforms could be distinguished and a competitive assay to detect PDGF receptors was demonstrated [61].

As an alternative to the drastic optical changes due to nanoparticle aggregation, one can monitor the more subtle effect of the binding of a target molecule to the nanoparticle surface [62–66]. The presence of the target molecule alters the local dielectric environment of the nanoparticle, which shifts the LSPR peak wavelength. Since interparticle interactions are not required, the nanoparticles can be supported on a solid substrate to avoid aggregation and, therefore, improve stability of the sensor. When the nanoparticles are coupled to antibodies or aptamers for specificity, LSPR sensors represent a very simple and potentially inexpensive strategy for sensing low-level, label-free analytes in complex media. LSPR sensing with nanoscopic arrays of silver triangles created by nanosphere lithography has proven highly effective [67,68]. With such substrates, amyloid-β-derived diffusible ligands (ADDLs), a biomarker for Alzheimer's disease, have been detected at 10-pM concentrations (Figure 4) [69]. Biomedical applications, including a comparison of LSPR and SPR sensing techniques [70], have been reviewed thoroughly [71,72].

Table 1 reviews the LSPR sensing properties of several gold and silver nanostructures, evaluated by analyzing their spectra in media with different indices of refraction. The sensitivity is reported as 'nm/RIU' or 'eV/RIU', meaning the shift in LSPR peak wavelength or photon energy per unit change of refractive index. The range of observed sensitivity values demonstrates a strong dependence on the nanoparticle shape and composition. It has been highlighted recently that the plasmon resonant linewidth should also be considered in measurements of sensitivity, since the linewidth will affect the ultimate detectivity of an LSPR sensor [73]. A unitless figure of merit (FOM) was therefore calculated, in which the sensitivity values

Figure 4. Localized surface plasmon resonance sensing of amyloid-derived diffusible ligands. The titration demonstrates detectivity down to 10 pM.



The sensor substrate consists of nanoscopic silver triangles.
ΔR_{max}: Maximum shift; ADDL: Amyloid-β-derived diffusible ligands; $K_{a,surf}$: Association constant; LOD: Limit of detection; LSPR: Localized surface plasmon resonance.
Reproduced with permission from [69].

described were divided by the resonance linewidth. According to this FOM, silver nanocubes, silver nanotriangles and gold nanostars are the most highly sensitive nanoparticles. Although the core-shell nanoparticles in Table 1 have the highest nm/RIU shifts, their FOM is low owing to the broad, low energy resonances exhibited by these nanoparticles. However, since grating-based instruments disperse light linear with wavelength and since some applications may be limited by spectral resolution rather than the signal-to-noise ratio, the nm/RIU shift could be the most significant parameter for certain sensing applications. The ideal nanoparticle for LSPR sensing will be strongly application dependent.

Conclusions

LSPR in noble metal nanoparticles results in strong visible and NIR scattering and absorption, which enables significant biomedical applications. Resonant scattering and absorption provide molecular contrast agents for biomedical imaging and resonant absorption enables the development of novel drug delivery methods as well as

Table 1. LSPR properties reported for several gold and silver nanostructures.

Author	Particle	Single/ ensemble	Resonance		Linewidth		Shift/RIU		FOM	Ref.
			nm	eV	nm	meV	nm	meV		
Tam (2004)	Au/SiO_2 shell	Ensemble	770	1.61	350	732	314	657	0.9	[76]
Sun (2002)	Au/AuS shell	Ensemble	700	1.77	400	1012	409	1035	1.0	[77]
Wang (2006)	Au nanorice	Ensemble	1600	0.775	600	291	801	388	1.3	[22]
Underwood (1994)	Au sphere	Ensemble	530	2.34	060	265	090	397	1.5	[78]
Raschke (2004)	Au/AuS shell	Single	660	1.88	077	220	117	333	1.5	[79]
Sherry (2005)	Ag cube	Single	510	2.43	091	433	146	695	1.6	[73]
Malinsky (2001)	Ag triangle	Ensemble	564	2.20	104	405	191	745	1.8	[62]
Nehl (2006)	Au star	Single	675	1.84	125	340	238	649	1.9	[26]
Mock (2003)	Ag sphere	Single	520	2.38	073	335	160	734	2.2	[80]
MacFarland (2003)	Ag particle	Single	585	2.12	49	178	203	736	4.1	[81]
Mock (2003)	Ag triangle	Single	760	1.63	080	172	350	751	4.4	[80]
Nehl (2006)	Au star	Single	770	1.61	124	260	665	1410	5.4	[26]
Sherry (2005)	Ag cube-sub	Single	430	2.88	022	146	118	792	5.4	[73]

To compare the figures of merit, results are only shown for reports that provide either the resonance linewidth or LSPR spectrum.
FOM: Figure of merit; LSPR: Localized surface plasmon resonance; meV: Millielectron volt; RIU: Refractive index unit.

photothermal and thermolytic laser therapies. These applications can be carried out with exogenous illumination with NIR resonant nanoparticles. The sensitivity of plasmon resonances to their local environment enables sensing schemes, which provide simple, label-free mechanisms to transduce molecular binding in specific affinity assays. The broad array of applications already demonstrated, the pace of development in nanoparticle synthesis and the general low toxicity of noble metal nanoparticles suggests that LSPR resonances will have a significant impact on medical applications of nanotechnology [74].

Future perspectives

Many challenges remain before the applications described here can be brought into clinical use. Although new nanoparticle structures are synthesized constantly, the exact mechanisms of their syntheses are rarely understood. New methods of studying complex nanoparticle synthesis reactions are needed to unravel these mechanisms. The use of commercially available heterobifunctional cross-linkers has had, and will continue to have, a positive impact on biological applications of noble metal nanoparticles. However, their effectiveness *in vivo* may be reduced. New targeting strategies, such as one demonstrated recently based on an adenoviral vector, should be pursued actively [75]. Also, standardized, quantitative assays for bioconjugated nanoparticles are required to determine their activity over biological applications. While these open questions affect the general development of biomedical applications of plasmon resonant nanoparticles, it appears likely that some of the demonstrations described here will reach clinical use in the next 10 years.

Executive summary

Plasmon resonant nanoparticles

- Collective excitations of free electrons in gold and silver nanoparticles leads to resonant optical absorption and scattering in the visible and near-infrared (NIR).
- The resonance wavelength can be tuned through the visible and NIR with nanoparticle shape, and resonant linewidths are typically 10s to 100s of nanometers.
- Scattering cross sections are many orders larger than fluorophores and quantum dots, and the signal does not bleach or blink.

Biomedical imaging contrast

- Bioconjugated gold nanoparticles enhance molecular contrast in 'optical biopsies' performed endoscopically.
- NIR resonant nanoparticles demonstrated targeted imaging *in vitro* and show promise for biomedical imaging applications with exogenous illumination.

Drug delivery and thermal ablation therapies

- Resonant absorption creates a local heat source that can be used for the targeted and controlled release of drugs associated with the nanoparticles.
- Resonant absorption can have direct photothermal or thermolytic therapeutic effects on tumors, as demonstrated *in vivo* and at the cellular level.

Biosensing applications

- Sensitivity of the plasmon resonance to the local environment yields simple, label-free mechanisms to transduce molecular binding in affinity assays.

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