The Golden Age: Shining the Light on Theragnostics

Yamin Yang* and Hongjun Wang*

Gold nanoparticles have attracted unprecedented attention as one of the leading nanomaterials in the field of biomedicine. Of particular interest are their inherent and geometrically tunable optical properties upon the interaction with light, facilitating breakthroughs in light-mediated diagnosis and therapeutics. Herein, an up-to-date overview of the current research in utilizing versatile gold-based nanosystems with distinctive optical features for optical imaging and phototherapy is provided. The recent advances in optical biosensing, surface-enhanced Raman scattering-based detection, photoacoustic imaging, optical coherence tomography, photothermal therapy, light-triggered drug release, photodynamic therapy, and optomodulation of neural functions using the novel and elegant gold nanostructures of unique design are summarized. In recognition of the burgeoning popularity of integrating multiple diagnostic and therapeutic functions in one system, recent developments of various multifunctional gold nanoplatforms for the light-enabled concomitant diagnosis and therapy are also highlighted. In the end, the current limitations and perspectives in clinical translation of gold nanomaterial-assisted photodiagnosis and phototherapy are discussed along with the concluding remarks.

1. Introduction

Nanotechnology has been considered as a game-changing revolution capable of providing innovative solutions to address some unmet clinical challenges. Nanomaterials have been extensively investigated as delivery vehicles to enhance the drug payloads, prolong the drug circulation, and control the drug release profiles. Nanosized materials can preferentially accumulate within tumors via the enhanced permeability and retention (EPR) effect, as a result of the inherently leaky and highly disorganized tumor vasculature. The great surface-to-volume ratio of nanomaterials allows for surface modification with multiple functional groups to achieve both specific targeting and imaging modality.

Among various nanomaterials, gold nanoparticles (AuNPs) have attracted unprecedented attention in the field of biomedicine for their superiority and versatility in diagnostic and therapeutic applications, which can be attributed to several unique characteristics, including 1) excellent biocompatibility and stability in biological systems, 2) ease of synthesis and generation of a variety of shapes and surface modifications via simple gold-thiol (Au–S) bonding chemistry, and 3) tunable optical and electronic properties.

The primary focus of this concise Review is to provide the readers with an update of the recent progress on the development of innovative gold nanomaterials with versatile structures and distinctive optical features for light-mediated diagnosis and therapy. We first briefly introduce the optical properties of plasmonic gold nanomaterials and discuss the fundamentals of their interactions with light. We then highlight the state-of-the-art advances in using various gold nanostructures for biomedical applications, covering optical biosensing, surface-enhanced Raman scattering (SERS)-based detection, photoacoustic imaging (PAI), optical coherence tomography (OCT), photothermal therapy (PTT), light-triggered drug release, photodynamic therapy (PDT), and optogenetics (Figure 1). In recognition of the exponentially increasing interest in combining more than one diagnostic and therapeutic modality within the same nanomaterials, we will also discuss the potentials of multifunctional and multimodal gold nanostructures for their use in theragnostic and/or combinatorial therapy. We conclude in the end by outlining the ongoing challenges and discuss the future directions for clinical translation of gold nanomaterials from bench-top to bedside.

2. Optical Properties of Gold Nanomaterials

The unique optical characteristic that distinguishes gold nanomaterials from many other nanomaterials is known as the localized surface plasmon resonance (LSPR). When the gold materials at the nanoscale are illuminated with light, the free
electrons along the gold lattices are excited. The collective coherent oscillations of these electrons occur when the frequency of the incident light matches the oscillation frequency of the conduction band electrons within the gold nanostructures, which is termed LSPR.[1]

Due to the LSPR, highly amplified and localized electromagnetic (EM) fields are generated in the vicinity of gold nanomaterial surface upon irradiation with appropriate light. The photonic energy absorbed by gold nanomaterials would decay radiatively by scattering the emitting photons with the same frequency as the incident light and at the same time, undergo nonradiatively relaxation, resulting in significant local heat. In addition, as gold nanomaterials are capable of confining the resonant photons, the spatially restricted electron motions under LSPR lead to further enhancement of both light absorption and scattering, affecting surrounding molecules and environment. Consequently, compared with other small molecules, gold nanomaterials render orders of magnitude higher optical absorption and/or scattering cross sections, which enable the use of gold nanomaterials for various bioimaging and optical sensing applications beyond light-assisted therapeutics.

As LSPR is closely regulated by the factors influencing the density and motion of electrons on the particle surface, the discrete size and unique shape of gold nanomaterials are directly correlated with their plasmonic absorption and scattering properties. Gold nanostructures are typically synthesized via the reduction of gold salt, and adapted reaction procedures have been extensively explored to obtain various shapes and sizes, which subsequently fine tune the corresponding optical properties. Some representative gold nanostructures via precise synthesis are shown in Figure 2. Besides the colloidal spherical AuNPs, gold nanorods (AuNRs), core–shell nanostructures with a silica core, nanoshells, nanocages, or nanorings with a hollow interior have been extensively investigated as potential candidates for phototherapy and drug delivery.

In the context of biomedical applications, the near-infrared (NIR) light between 750 and 1700 nm is more favorable for deep-tissue penetration due to minimal tissue scattering or water absorption. In particular, the light within the second NIR window (NIR-II: 1000–1350 nm) can penetrate even deeper (>2 cm) than the first NIR window (NIR-I: 750–1000 nm) (≈1 cm) (Figure 2). However, so far, very limited biocompatible probes are available for in vivo use in the NIR window. In this regard, the possibility of shifting the light absorption and the SPR band of gold nanomaterials from visible to NIR region by manipulating their sizes and shapes makes them promising alternatives for in vivo imaging and phototherapy.[2] Recently, the recognition of possible rendering of LSPR within the favorable NIR region from anisotropic shapes of gold nanostructures has inspired the syntheses of various novel gold nanostructures such as gold nanoprisms, gold nanostars, gold nanoplates, gold nanourchins (AuNUs), gold nanoframeworks (AuNFs), and gold nanoclusters. Oftentimes, an increasingly complex level of surface decorations is the prerequisite for imparting new properties to gold nanostructures or advancing their unique functions. Among many, some typical biomolecules or functional groups (see Figure 2) are incorporated onto the aforementioned gold nanostructures to achieve the desirable multifunctionality.

Figure 1. The utility of gold nanomaterials in light-mediated diagnosis and therapy employing their distinctive optical features.

Figure 2. Some representative gold nanostructures via precise synthesis.
3. Gold Nanomaterials for Optical Detection and Single-Mode Imaging

3.1. Optical Biosensing

The strong absorption and scattering of AuNPs can be easily detected in the visible light region. That is, any subtle alteration around AuNPs (such as changes in the refractive index of the surrounding media and particle aggregation) can induce the variation of the LSPR extinction maxima of AuNPs. Thus, they can be used as sensitive optical probes to detect the target analyte-induced changes. Due to their high sensitivity, low cost, ease of surface functionalization, and convenience in point-of-care detection, colloidal gold-based colorimetric assays or immunoassays have been widely adopted for simple diagnosis of pathogens.[3–5] Jansa and Huo published a critical review on the AuNP-enabled biological and chemical sensing and analysis based on their optical properties with the highlight of their high sensitivity and multiplex capabilities.[6]

The ongoing Coronavirus Disease 2019 (COVID-19) pandemic challenge affirms the necessity of developing cost-effective and convenient platforms for on-site and rapid viral detection.[7–9] As a matter of fact, gold nanomaterials have demonstrated their potentials in virus detection and coronavirus diagnostics based on optical changes. For example, in an early study reported by Li and Rothberg, a simple colorimetric hybridization assay was designed to detect severe acute respiratory syndrome-associated coronavirus (SARS-CoV) with unmodified AuNPs.[10] Given the difference in electrostatic properties, the formation of double-stranded DNA (dsDNA) molecules upon reaction with the target viral RNAs interfered with the interactions between single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA) and citrate ions on the surface of AuNPs, causing color changes associated with aggregation. The colorimetric detection confirmed the formation of dsDNA within 5 min and the easily detectable amount as low as 60 fmol. Kim et al. also developed a colorimetric assay using AuNPs to detect the Middle East respiratory syndrome coronavirus (MERS-CoV) down to 1 pmol μL−1 of 30 bp MERS-CoV. In this colorimetric assay (Figure 3a), two thiol-modified complementary base pairs of viral DNA were conjugated with AuNPs via the high Au–S affinity. The salt-induced particle aggregation in the presence of a positive electrolyte was prevented upon the formation of self-assembled complexes between modified dsDNA and target virus, leading to detectable optical transition.[11] In a recent study (Figure 3b), Moitra et al. reported a colorimetric bioassay for rapid diagnosis of positive COVID-19 cases from the isolated RNA samples utilizing the anisotropic plasmonic properties of AuNPs. The AuNPs were capped with thiol-modified antisense oligonucleotides (ASOs) specific for N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2, and the functionalized AuNPs selectively agglomerated in the presence of its target RNA sequence of SARS-CoV-2, resulting in its SPR signal change. Furthermore, addition of RNaseH cleaved the RNA strand from the composite hybrid of RNA and Au-ASOmix, leading to a visually detectable precipitate in the solution.[12]

3.2. SERS-Based Detection and Imaging

SERS involves drastic amplification (106–107 times) of the Raman scattering from molecules (Raman reporters) adsorbed on or in close vicinity to the surface of a nanostructured metal. In general, the magnified EM field of the metallic surface plasmon upon incident light excitation is responsible for the enhancement of Raman scattering. Due to the remarkable intrinsic SPR properties, gold nanomaterials have been used as effective SERS substrates to amplify Raman signals for decades. Both computational and experimental studies have demonstrated that...
the SERS enhancement factors of AuNPs can be upward of $10^8$.\cite{13,14} As such, AuNP-based SPR biosensors have been widely used to detect biomolecules for clinical diagnoses.\cite{15,16} Provided we have the vibrational signatures of the Raman reporters, detailed spectroscopic information can be translated into imaging signals. Thus, Raman spectroscopy has recently emerged as a desirable modality for in vivo imaging (Raman imaging) with high sensitivity and molecular structure selectivity.\cite{17}

Applications of various gold nanostructures as contrast agents for in vivo or ex vivo biomedical SERS imaging have been comprehensively reviewed recently.\cite{18}

In an early attempt, Nie’s group reported their study on the conjugation of tumor-targeting ligand single-chain variable fragment (ScFv) antibodies onto PEGylated AuNPs for active tumor targeting and SERS detection. It was found that the PEGylated SERS nanoparticles were 4200 times brighter than semiconductor quantum dots with light emission in the NIR window. Attributed to the large optical enhancements of AuNPs, spectroscopic detection of small tumors (0.03 cm$^3$) could be achieved with a penetration depth of 1–2 cm in the animal xenograft tumor models.\cite{19} By simply changing the adsorbed Raman active tags on the AuNP surface, each SERS nanoparticle can produce a distinct Raman spectrum, allowing for multiplexed imaging. Zavaleta et al. reported the utility of Raman spectroscopy in conjunction with ten types of SERS nanoparticles with unparalleled spectral fingerprints for multiplexed in vivo imaging. Upon injection into nude mice, colocalization of different SERS nanoparticles within deep tissues could be simultaneously imaged, offering the opportunity to detect multiple biomarkers in one single experiment.\cite{20}

Shape dependence of SPR affords a viable approach to tune the SERS activity of gold nanostructures by controlling their morphology.\cite{21} Gold nanostructures with various shapes such as spheres, rods, cubes, and stars have been extensively used as SERS probes. Recently, a new class of gold nanostructures with rough surfaces (i.e., the presence of “hot spots”) exhibits additional enhancement of the EM field at the sharp apexes and edges. Xie et al. synthesized the flower-like gold nanocrystals with abundant tips on the nanostructure surface for substantial enhancement of the local EM field. By packaging Rhodamine

![Figure 3.](image-url)
B-capped gold nanoflowers with denatured bovine serum albumin, the particle nanocomposite exhibited strong SERS effects and could be used as a stable Raman-active tag for living cells.\textsuperscript{[22]}

Intense EM field is typically found at the interstices of aggregated or assembled AuNPs. To achieve controllable assembly of gold nanostructures, Kang et al. introduced a specific spacer sequence of oligonucleotides to the core DNA-modified AuNPs to trigger the formation of narrow nanogaps between AuNPs. Placing Raman-active molecules in the narrow nanogap showed a strong and uniform Raman intensity with the magnitude increased 7.7 times, which enabled high-speed and high-resolution live cell Raman images.\textsuperscript{[23]}

### 3.3. Photoacoustic Imaging (PAI)

PAI is a noninvasive medical imaging modality, which effectively hybridizes the advantages of optical imaging with excellent spatial resolution and acoustic imaging with good tissue penetrability (up to 5–6 cm). In PAI, tissue is irradiated with a pulsed laser and the light absorption produces transient heating of the absorbing species. The temperature rise triggers thermoelastic expansion and subsequent propagation of the acoustic waves, which can be detected using an ultrasonic transducer for ultrasound imaging. The information obtained from PAI is therefore related to the optical properties of different tissues, including those endogenous chromophores such as melanin and hemoglobin. Pulsed excitation of plasmonic AuNPs leads to periodic heating, which induces thermoregulated expansion and contraction of the particles and the surrounding medium. AuNPs can thus be used as exogenous contrast agents to further boost the sensitivity of PAI by offering better tissue contrast.\textsuperscript{[24–27]}

Among various PAI contrast agents, the key advantages of gold nanostructures are their high optical absorption cross-section and high photothermal efficiency, which afford much stronger photoacoustic (PA) signals (several orders of magnitude higher than that of small organic dyes).\textsuperscript{[28]} In a recent report by Sun et al., glycol-chitosan-coated AuNPs showed preferable accumulation in the breast cancer cells with strong PA signals, which originated from the plasmon coupling effect of AuNPs under pulsed laser light irradiation.\textsuperscript{[29]} In another study, a pH-responsive active AuNP nanoprobe was designed by Li et al. for PAI, aiming to target the acidic extracellular microenvironment of the tumor. The nanoprobe could smartly aggregate in response to the acidic tumor conditions after intravenous injection to the U87MG tumor-bearing nude mice and yielded an approximately threefold increase of PA imaging contrasts.\textsuperscript{[28]}

In view of the geometrically induced optical properties, various gold nanostructures (such as rods, shells, prisms, cages, stars, and vesicles) with tunable SPR absorption in the tissue optical window have recently received growing interests in the PAI visualization of target tissue at a high penetration depth.\textsuperscript{[30]}

For example, AuNRs with tunable absorption behavior in the NIR-II window by increasing the aspect ratio (length to width) have demonstrated the potential as contrast agents for PAI. Chen et al. reported the synthesis of miniaturized AuNRs (length = 8 nm) with the absorption at 1064 nm, which generated 3.5 times stronger photoacoustic signals under the nanosecond pulsed laser illumination and 4.5 times greater PA contrast in the in vivo PAI as compared with larger AuNRs (length = 18 nm) with the same aspect ratio.\textsuperscript{[31]} Moon et al. reported the amplified PA performance of reduced graphene oxide (rGO)-coated AuNRs for sensitive PAI. The rGO layer allowed highly efficient heat transfer, which endowed the nanocomposites with a 4 times higher magnitude of enhanced EM field and an extremely high PA amplitude in the 4–11 MHz operating frequency of ultrasonic transducers.\textsuperscript{[32]}

Edges and tips presented on the nanostructure surface and the “hotspots” between gold nanostructures are considered beneficial to further enhance the PA signals. The plasmonic absorption peak of these gold nanostructures can achieve a red shift to tissue optical windows by precisely controlling the synthesis process. Such novel gold nanostructures are favorable for PAI, offering both exceptional contrast and imaging depth. For example, Nie et al. demonstrated that cyclic Arg-Gly-Asp (RGD) peptide-conjugated plasmonic gold nanostars with superior biostability were able to render 4.3-fold PA signal enhancement at 6 h after the administration in mice, where tumor angiogenesis could be clearly imaged with a volumetric homogeneous spatial resolution of $\approx 200 \mu$m.\textsuperscript{[33]}

### 3.4. Optical Coherence Tomography (OCT)

OCT is an established clinical modality for retinal imaging in ophthalmology. OCT applies a low-coherence light and measures the interference from the resultant reflection backscattered by the tissue, which can generate cross-sectional images of tissues at a micrometer-level resolution. Distinct scattering properties between retina cells and coronary arteries can generate exogenous contrast for OCT, whereas other tissues require exogenous agents to enhance the contrast. Due to the significant scattering of normal tissues, the imaging depth of current OCT is limited to $\approx 2$ mm. Gold nanomaterials have been developed as the contrast agents to expand the utility of OCT as a result of the high level of scattering enhancement.\textsuperscript{[34]} Gold nanomaterials provide distinctive reflective signals beyond the native tissues, and their highly tunable SPR effects can further improve the intensity of backscattered light and thereafter result in strong OCT signals.\textsuperscript{[35–37]}

The efficacy of using gold nanomaterials as the contrast agents for OCT has been demonstrated with animals. de la Zerda and coworkers conducted a series of studies using various gold nanostructures to enhance ophthalmic imaging. In their early study (Figure 4a), they found injection of a low dose of 30 nM of AuNRs (13 nm in diameter and 45 nm in length) to the anterior chamber of the mouse’s eyes increased OCT contrast by almost 50 folds as compared with the control group.\textsuperscript{[38]} Taking advantage of the ease of matching the LSPR peak of gold nanostructures to the deep-penetrating OCT source wavelength, an increased signal-to-noise ratio and depth of penetration can be achieved. The earlier research group recently demonstrated that gold nanoprism with strong scattering in the NIR-II window could be used as the contrast agents for deep-tissue OCT. After intravenous injection of PEGylated gold nanoprism, significant improvement of the contrast in OCT angiograms was achieved, allowing for in vivo melanoma tumor detection based on the tumor microvasculature characterization.\textsuperscript{[39]} In another recent study from Nguyen et al., gold nanostars functionalized with
PTT has been rapidly developing as a promising light-mediated treatment paradigm that uses laser irradiation of the photothermal transduction agents to generate hyperthermia. By offering a few orders of magnitude of light absorbance of most photoabsorbing dye molecules, plasmonic AuNPs can convert the absorbed photonic energy into nonradiative heat at high efficiency, ensuring effective therapeutic outcomes at relatively low laser dosages. In addition, AuNPs can selectively accumulate in the targeted tissues via active or passive routes, which confine the temperature increase at the specific site to avoid unwanted thermal damage to surrounding healthy tissues. Together with the control of external light dosage, the spatiotemporal specificity can be achieved on top of the effective photothermal effects.

As NIR laser is highly desirable for deep-tissue penetration, gold nanostructures with tunable resonance wavelength at the NIR biowindows have emerged as the promising agents for PTT. The use of core–shell AuNPs with NIR–SPR wavelength for PTT was first reported by Halas and coworkers. As a continuum of the experimental success, AuroLase therapy, a silica–gold nanoshell-mediated PTT developed by Nanospectra Biosciences, Inc., is now under clinical trials for prostate cancer ablation. Xia’s group also tailored the structures of gold nanocages to achieve strong NIR absorption and then used them as effective PTT agents for cancer treatment. Similarly, Huang et al. explored the possible AuNR-based NIR-PTT, wherein the longitudinal SPR peak of the AuNRs could be shifted to the NIR range. The AuNRs conjugated with the antipidermal growth factor receptor (EGFR) could target malignant oral epithelial cell lines (HOC 313 clone 8 and HSC 3) that resulted in selective thermal destruction of tumor cells at low energy (80 mW–10 W/cm²) upon exposure to the NIR laser at 800 nm.

4.2. Light-Triggered Drug Release

It is well established that nanoparticulate systems for drug delivery can protect the active compounds from systemic destruction and increase the efficacy and bioavailability of therapeutic payload by prolonging the release and selective accumulation and therefore improve the therapeutic index. Compared with the conventional drug release systems achieved using simple and functionalized liposomes, micelles, dendrimers, and mesoporous polymeric particles, stimuli-responsive drug release provides several foreseeable advantages such as reduced systemic toxicity, better-controlled dosage, and low unwanted side effects. Light is particularly preferred to trigger the drug release in a biological system with the high spatial and temporal precision. In this regard, light-induced release offers a very promising mechanism to deliver chemotherapeutic agents, where photodissociable linkers or thermosensitive nanogel-crosslinked hydrogels are usually involved in the design of such light-responsiveness. In either strategy, gold nanostructures with significant light absorption and fast light-to-thermal conversion can be entrapped or incorporated along with drug molecules.

For instance, the transient localized heating from photothermal AuNPs is able to trigger the volume-associated phase transition of poly(N-isopropylacrylamide) (PNIPAM), a well-known thermosensitive copolymer, from hydrophilic state (swelling) to hydrophobic state (deswelling), which consequently leads to the release of encapsulated payloads. Jiang et al. reported their utility of the AuNR-embedded PNIPAM hydrogels for NIR-triggered dual drug release, in which the temperature increase from NIR light-irradiated AuNRs caused the contraction of the hydrogels to on-demand release the encapsulated doxorubicin (DOX) and curcumin (CUR) (Figure 5a). Similarly, Kang et al. synthesized AuNPs modified with mercaptosuccinic acid
(MSA) as the host carriers for DOX. In vivo fluorescence imaging demonstrated that light irradiation of the MSA-modified AuNPs induced local heating thereon enough to trigger the release of the encapsulated DOX.\(^{[50]}\)

Other than thermosensitive materials, photoresponsive linkers are often used to formulate the gold nanocomposite for drug loading. As shown in Figure 5b, Hernandez-Montoto et al. developed an NIR light-triggered drug delivery system based on gold nanostars coated with a mesoporous silica shell and capped with a photo-labile polyethylene glycol (PEG) derivative containing a 2-nitrobenzyl (N2) linker. NIR light (875 nm) triggered the release of DOX followed by the photocleavage of N2 linker.\(^{[51]}\) Upon NIR light irradiation, the detachment of bulky PEG derivatives from the surface of nanoparticles enabled the subsequent release of DOX entrapped in the mesoporous shell, which caused a remarkable reduction of cell viability as a result of the synergistic chemotherapy and PTT.
The light-triggered drug release from gold nanomaterial-based systems is highly dependent on the light source and the drug-laden host molecules. Halas’ group recently studied the effects of continuous wave (CW) and pulsed NIR light to induce the release of drugs from two AuNS-based complexes with distinctive mechanisms. Two breast cancer drugs, docetaxel and HER2-targeted lapatinib, were attached onto the AuNSs functionalized with thiolated dsDNA and human serum albumin, respectively. CW irradiation induced the bulk temperature increase above the thiolated dsDNA dehybridization temperature, triggering docetaxel release, whereas the femtosecond pulsed irradiation led to the Au–S bond breakage to release lapatinib from the dsDNA-drug complex with no measurable bulk temperature change.

4.3. Photodynamic Therapy (PDT)

PDT is a minimally invasive modality where photosensitizers (PS) and light are used to induce irreversible cell destruction and tissue damage. During the photodynamic reaction, organic PS molecules absorb and transfer incident photonic energy to the surrounding tissue oxygen and generate highly cytotoxic reactive oxygen species (ROS), such as singlet oxygen (1\textsuperscript{O}2) and other free radicals. The efficacy of PDT greatly relies on the light absorption of PS and its triplet yield upon activation. However, conventional PS is generally hampering the clinical use of PDT for deep tumor treatment because of the relatively small absorption cross sections with deep-tissue-penetrating light.

Similar to other nanomaterial-based drug delivery systems, gold nanostructures have also been used as a delivery vehicle for hydrophobic PS. In addition to an increased accumulation of PS in the tumor, gold nanostructures can further elevate the triplet yield of encapsulated or conjugated PS by coupling with surface plasmons upon light irradiation to further enhance the treatment efficiency. A recent review from Russell et al. has overviewed the PS–AuNP conjugates for PDT and highlighted the enhanced efficacy and selectivity of traditional PSs by versatile AuNPs in both in vitro and in vivo PDT.[53]

Ferreira et al. reported their study on the hybrid system consisting of AuNRs and PS porphyrin, showing far more efficiency (about one order of magnitude) than individual components in 1\textsuperscript{O}2 generation during PDT, which is most likely due to the rapid energy transfer from irradiated AuNRs to PS.[54] In our early studies, we also investigated the size-dependent and surface charge-dependent enhancement of PS accumulation and ROS formation enabled by spherical solid AuNPs.[55,56] We compared the SPR properties of colloidal AuNPs and gold nanorings to determine shape-dependent cell killing efficiency during NIR-assisted PDT in breast cancer cell line MDA-MB-231.[57,58] Notably, as shown in Figure 6a, layer-by-layer (LbL) assembly strategy was adopted to incorporate more PS Al(III) phthalocyanine chloride tetrasulfonic acid (AlPcS4) onto plasmonic gold nanorings, resulting in an eightfold increase in tumor cell killing as compared with PS alone in NIR-PDT.[58] To better reveal the underlying mechanism, we also investigated the intricate correlation between enhanced 1\textsuperscript{O}2 generation and excited plasmonic...

Figure 6. Layer-by-layer assembly of PS AlPcS4 onto NIR-adsorbing gold nanorings led to ≈85% cell killing after PDT with NIR irradiation. Reproduced with permission.[58] Copyright 2020, American Chemical Society.
gold nanostructures. As noted, the energy transfer between gold nanorings and PS is not only dependent on the LSPR of the nanostructure itself but also the proximity distance between PS and nanostructures.

In recognition of the essential functions of some subcellular components, such as mitochondria and nucleus, and the intrinsic oxygen-dependent nature of PDT, the state-of-the-art design of gold nanomaterial-mediated PDT has involved intracellular targeting and/or oxygen-replenishing strategies. We previously reviewed the developed strategies in the design of AuNPs for intracellular targeting and selective delivery of PS to subcellular compartments for enhanced PDT. We also demonstrated that mitochondria-targeting AuNPs conjugated with triphenylphosphonium groups could render selective delivery of PS 5-aminolevulinic acid (5-ALA) to the mitochondria of human breast cancer cell line (MDA-MB-231) and the elevated mitochondrial ROS burst indeed significantly enhanced the therapeutic efficacy of PDT upon light irradiation.

Recently, gold nanomaterials have also been recognized for their capabilities of enhancing the photodynamic activity by either directly sensitizing the formation of $^{1}\text{O}_2$ or increasing the triplet yield of PS upon photoexcitation. Irradiation of gold nanomaterials with short laser pulses, CW light, or two-photon excitation is able to generate $^{1}\text{O}_2$ via electron transfer. With outstanding photostability and high absorption cross-sections, gold nanomaterials can thus be considered as a promising PS for PDT, exhibiting much higher $^{1}\text{O}_2$ generation efficiency than traditional PS such as rose bengal and indocyanine green (ICG). As cell nucleus is another desirable target of PDT, Vankayala et al. functionalized gold nanoclusters with nucleus-targeting TAT peptide, which serves as DNA delivery cargo for gene therapy and as PS for PDT without the need of organic PS. The TAT peptide—gold nanoclusters achieved impressively high cellular uptake and gene transfection efficiency in HeLa cells, while exerting effective nucleus-targeting photodynamic effects with photo-induced DNA damage upon NIR photoexcitation. In addition, the red fluorescence emitted by gold nanoclusters allowed to track the particle distribution and simultaneously monitored the gene delivery and therapeutic process via fluorescence imaging.

As an oxygen-dependent process, the hypoxia of tumor microenvironment would significantly deteriorate the PDT efficacy. As such, extensive attempts have been made to incorporate oxygen-replenishing mechanisms by taking full advantage of well-designed gold nanostructures. In one of our recent studies, for example, we developed a new bimetallic and biphasic gold core—rhodium shell nanosystem, which was able to effectively catalyze endogenous hydrogen peroxide into oxygen so as to relieve the hypoxic tumor microenvironment during traditional PDT. This nanostructure was further coated with the tumor cell membrane for tumor targeting while loading PS ICG in the mesopores of the rhodium shell for PDT. Both in vitro and in vivo observations demonstrated that this novel nanocomposite could effectively alleviate the tumor hypoxia and consequently yield increased therapeutic efficacy upon laser irradiation of tumors. We also noticed that, due to the presence of nanogold and its photothermal effect, the mild temperature increase under light irradiation was also able to promote cellular uptake of ICG and subsequently enhance $^{1}\text{O}_2$ generation.

### 4.4. Optomodulation of Neural Functions

While a majority of current efforts in photodiagnosis and phototherapy have been focused on cancer, the use of light to modulate the electrical activity of neurons offers a new horizon toward the understanding of brain functions and brain disorder therapies, with immediately noticeable advantages such as noninvasiveness and superior spatiotemporal resolution. One recently established approach is optogenetics, wherein light triggers the opening of photon-sensitive ion channels expressed on the genetically modified neurons and leads to subsequent cell depolarization. Furthermore, it has also been recognized that direct optical stimulation of unmodified neurons with infrared light can either excite or inhibit neural cells. While the underlying mechanism remains elusive, the photothermal effect of infrared light can lead to a transient temperature gradient on the neuron membrane, which further mediates the capacitive currents or activates temperature-gated ion channels such as members of transient receptor potential (TRP) channel family. To reduce the off-target delivery of light energy, photothermal transducers capable of photon-to-heat conversion can be targeted to specific sites, allowing for in vivo neural modulations at a high precision. In this regard, the large optical absorption cross section of AuNPs implies their suitability as the exogenous light absorbers and efficient heat transducers. Upon light excitation, the heat generated through oscillation and collision of electrons on the AuNP surface is dissipated to the surroundings within a typical time scale of 100 ps to 10 ns via thermal conduction. As a consequence, AuNP-assisted photothermal modulation provides a temporally precise stimulation of the neuronal activity, allowing for the specific neural response with a higher stimulation efficiency and a lower stimulation threshold.

Carvalho-de-Souza’s group has conducted a series of studies to tune the photoactivities of neurons with cell-targeted AuNPs based on the light-induced, temperature-dependent alteration of membrane capacitance. It was found that photoexcitation of the membrane-tethered AuNPs under millisecond light pulses could induce action potentials (APs) in dorsal root ganglion (DRG) neurons (Figure 7a). As the AuNP—streptavidin conjugates bound directly to the membrane proteins of the voltage-gated sodium channels, the modest local heating from the photoexcited AuNPs can robustly increase the transient depolarizing capacitive current to generate APs. Instead of targeting the channel proteins, the same group also used cholesterol moiety-modified AuNPs to attach to the extracellular leaflet of the plasma membrane of neurons for rapid and slight increase of the membrane temperature upon photoexcitation of AuNPs, which facilitated timely generation of APs by the DRG neurons.

In another example as shown in Figure 7b, de Boer et al. demonstrated that the energy absorption of AuNPs mediated by the second-order nonlinear low-power optical excitation could also evoke neuronal activity in mouse cortical neurons with great spatial accuracy and low photodamage. The AuNP-targeted stimulation could further stimulate individual epitheliomuscular cells and trigger body contractions in Hydra Vulgaris.

In addition to single-neuron modulation, the AuNP-assisted optical stimulation could also be used to investigate the neural circuit functions and synapse signaling by controlling $\text{Ca}^{2+}$ dynamics.
during neurotransmitter uncaging. Taking advantage of the plasmonic excitation of AuNPs and their small size, Lavoie-Cardinal et al. demonstrated with the combined Ca$^{2+}$ imaging and whole-cell patch clamping that hippocampal neurons decorated with AuNPs could drive local Ca$^{2+}$ transients and Ca$^{2+}$ signaling in dendritic domains under 800 nm NIR stimulation.[70] By appropriately choosing the nanoparticle geometry and ligand modification, the combination of AuNPs and infrared simulation allows optical neural modulation with improved efficiency while increasing the penetration depth. Yong et al. first confirmed that AuNRs illuminated with a 780 nm laser can activate rat primary auditory neurons.[71] Nakatsuji et al. further revealed that the photothermal heat generated by plasma-membrane-targeted AuNRs induced Ca$^{2+}$ influx in DRG neurons by activating the thermosensitive cation transient receptor potential vanilloid 1 (TRPV1) channel.[72] Different from the excitation effects on triggering AP, AuNP-based neuromodulation was also used for neural suppression through the mode of neural inhibition. Yoo et al. reported the photothermal inhibition of neural activity with NIR-sensitive AuNRs. Membrane-bound AuNRs which activated the thermosensitive potassium channels (TREK-1) would suppress the electrical activity of neurons upon NIR irradiation, exhibiting a close correlation with the laser intensity.[73]

5. Multifunctional Gold Nanomaterials for Light-Mediated Theragnostics

Theragnostics is a term derived from a combination of therapeutics and diagnostics, which brings about a union of detection and identification, simultaneous or sequential drug delivery, real-time tracking of drug biodistribution, imaging-guided therapy, and continuous monitoring of therapeutic responses. Recently, gold nanomaterials have been implemented widely as one of the leading nanomaterials for theragnostic purposes.[74] In particular, thanks to the unique optical properties and the large surface-to-volume ratios, gold nanomaterials allow for the integration of multiple diagnostic and therapeutic functions within the same platform. With the burgeoning interest in multimodal utilization, exponential growth in developing multifunctional gold nanomaterials is therefore expected. In the following sections, we particularly discussed the light-enabled concomitant diagnosis and therapy using various gold nanostructures. In Table 1, we summarized some representative multifunctional gold materials with theragnostic functions, where the irradiation light wavelength and targeted cells/tissues are shown for better comparison.

5.1. SERS in Combination with Phototherapy

Both SERS and the photothermal effects of AuNPs share the same fundamental mechanism, whereby plasmon excitation can amplify the localized EM fields concurrently for SERS and heat. This enables the AuNP-based system as an effective platform for in situ precise identification of the diseased area, subsequent accurate phototherapy, and monitoring of light-mediated therapeutic responses.[75] Versatile gold nanostructures are accordingly developed for SERS optical diagnosis in conjunction with PTT. In an early work reported by Bhatia’s group in 2009, SERS-coded AuNRs were exploited for densely multiplexed Raman detection and remotely controlled photothermal heating.[76] Three AuNR formulations were designed by coating PEGylated AuNRs with visible- and NIR-absorbing molecules (such as IR-792 perchlorate) and these SERS-coded AuNRs were readily detected in vivo following subcutaneous or intratumoral injection into the tumor-bearing athymic mice while photothermally heating the tumor to reach the ablative temperature. Li et al. conjugated a Raman reporter 4-mercaptobenzoic acid (4-MBA) to Eu$^{3+}$-doped CaMoO$_4$@AuNR hybrid nanoparticles

![Figure 7. a) AuNPs were perfused over a patch-clamped DRG cell, and AP in neurons in response to electrical stimuli can be stimulated by 532 nm light with the presence of AuNPs. Reproduced with permission.[67] Copyright 2015, Elsevier Inc. b) In vivo nonlinear photoactivation of cortical neurons with streptavidin-coated AuNPs in response to optical excitation. Extracellular patch-clamp recording shows that the intrinsic firing rate of the cell drastically increases as a result of the pulsed NIR excitation. Reproduced under the terms and conditions of the Creative Commons Attribution 4.0 International License.[69] Copyright 2018, The Authors, published by Springer Nature.](image-url)
with SPR at 790 nm, which yielded a strong SERS signal (enhancement factor: $5.0 \times 10^5$) along with a good heat conversion efficiency of 25.6% for hyperthermia.\[77\] He et al. also conjugated gold–silver nanoshells (AuAgNSs) with a Raman tag 3,3'-diethylthiatricarbocyanine iodide (DTTC), enabling SERS imaging with an extremely high sensitivity (down to 300 CFU mL\(^{-1}\) for a typical multidrug-resistant bacteria strain). Simultaneously, the AuAgNSs exhibited an efficient photothermal effect during laser irradiation to trigger the silver-ion releases for preventing bacterial infection during wound healing.\[78\]

### Table 1. Representative multifunctional gold nanomaterials for light-mediated Theragnostics.

| Gold nanoplatfor | Additional modifications | Light source | Targeted cells/tissues | Diagnostic function | Therapeutic function | Ref. |
|-----------------|--------------------------|--------------|------------------------|---------------------|----------------------|------|
| PEGylated AuNRs | Raman reporter IR-792, DTTC-765, DTDC-655 | 785 nm for SERS; 810 nm for PTT | Hela cells; MDA-MB-435 cancer cells-baring mice | SERS | PTT | \[76\] |
| Eu\(^{3+}\)-doped CaMoO\(_4\)@AuNR | Raman reporter 4-MBA | 785 nm for SERS; 808 nm for PTT | A549 cells | SERS | PTT | \[77\] |
| Gold–silver nanoshells | Raman reporter DTTC | 808 nm | Staphylococcus aureus; Escherichia coli; BALB/c mice | SERS | PTT; preventing bacterial infection | \[78\] |
| Gold nanostars | Raman reporter 4-MBA; RGD molecules for targeting αvβ3 integrin | 785 nm; 1064 nm | A549 human lung adenocarcinoma cells | SERS | PTT | \[79\] |
| Popcorn-shaped AuNPs | Anti-PSMA antibody and A9 RNA aptamers | 670 nm for SERS; 785 nm for PTT | LNCaP human prostate cancer cells | SERS | PTT | \[80\] |
| AuNPs | pH-responsive ligands and Raman probes | 532 nm; 660 nm; 785 nm | B16 F10 mouse melanoma cells | SERS | PTT | \[82\] |
| Silica/polymer-coated AuNR | Raman reporter DTTC; photosensitizer PpIX | 808 nm | Melanoma B16F10 cells | SERS | PDT | \[83\] |
| AuNPs | Palladium (Pd)–pyrolipid | 638 nm | KB carcinoma cells | SERS | PDT | \[84\] |
| AuNPs; Nanoclusters | ICG | 808 nm | Mouse 4T1 breast cancer cells; 4T1 BALB/c mice | PAI | PTT | \[85\] |
| Triangular gold nanoplates | Anti-EGFR peptide | 680 nm for PAI; 660 nm for PTT | Human lung cancer cells (HCC227); HCC827 BALB/c mice | CT/PAI | PTT | \[86\] |
| Gold nanobipyramids | PDA-coated; DOX | 808 nm | Mouse 4T1 breast cancer cells; 4T1 BALB/c mice | PAI | chemo-PTT | \[87\] |
| rGO-coated gold nanostars | Folic acid | 808 nm | Capan-1; HEK293 cells; pancreatic cancer tumor bearing mice | PAI | PTT | \[89\] |
| PEG-coated AuNR | Light-activatable PolyRu; IR1061 | 808 nm | MCF-7 BALB/c mice | PA-fluorescence dual-mode imaging | chemo-PTT | \[90\] |
| Gold nanostars | Perfluorohexane; hollow mesoporous silica nanocapsules; PEG | 808 nm | C6 cell tumor-bearing mice | ultrasonic/CT/PA/thermal imaging | PTT | \[91\] |
| Core—satellite assembly of gold nanobipyramids and AuNPs | miR-21 targeted DNA-chain-modified | 808 nm | MCF-7 BALB/c mice | fluorescence imaging; SERS | PTT | \[92\] |
| AuNUs | Raman reporter 4-MPBA; dopamine; hyaluronic acid; DOX | 808 nm | MDA-MB-231 cells; HUVEC cells; MDA-MB-231 xenograft tumor model | SERS | chemo-PTT | \[93\] |
| AuNFs | Raman reporter 4-MPBA; hyaluronic acid; DOX | 1064 nm | MDA-MB-231 cells; MDA-MB-231 xenograft tumor model | PA-Raman dual imaging | chemo-PTT | \[94\] |
As discussed early, the size, shape, and surface features are the essential factors to determine the SERS performance of AuNPs. Notably, surface roughness, such as tips, corners, and edges on the particle surface, is capable of focusing the EM field at their apexes to significantly enhance the Raman signal intensity. Recently, increasing endeavors have been devoted to designing innovative gold nanostructures with “hot spots” features for simultaneous SERS imaging and image-guided phototherapy. For example, gold nanostars with branched tips are considered as excellent SERS probes and have been incorporated with multiple functionalities for theragnostic applications. Song et al. modified the gold nanostars with 4-MBA as Raman reporters and RGD molecules as ligands for selectively targeting αvβ3 integrin-overexpressed A549 human lung adenocarcinoma cells. The as-prepared gold nanostars demonstrated the capability of SERS mapping and PTT under NIR-I (785 nm) and NIR-II (1064 nm) laser excitation within the targeted cancer cells.\(^\text{79}\) Popcorn-shaped AuNPs were recently fabricated by Lu et al., where the central sphere acted as an electron reservoir and the narrow nanoscaled corners and edges on the particle surface led to a significant enhancement of the Raman signals with several orders of magnitude (2.5 \times 10^4). After coating with monoclonal anti-PSMA antibody and A9 RNA aptamers, the functionalized popcorn-shaped AuNPs selectively accumulated in the LNCPaP human prostate cancer cells while providing highly sensitive detection of cells at a 50-cell level. These particles also induced cellular photothermal damage with the localized heat generation upon NIR irradiation, and this process could be monitored by the change of SERS intensity.\(^\text{80}\)

Based on the established evidence that close interparticle coupling gaps can substantially increase the SERS efficiency of colloidal nanoparticle systems,\(^\text{81}\) gold aggregates with significant EM surface-enhancement factor would hence exhibit favorable Raman imaging performance and photothermal efficacy, and the aggregation process can be triggered in response to a specific environmental condition, such as the mildly acidic environment of cancerous tissues. In a recent study reported by Jung et al., 10 nm AuNPs were modified with pH-responsive ligands and Raman probes. The pH-triggered aggregation of AuNPs in the cancerous environment reached an enhancement factor of 1.3 \times 10^4 as SERS probes. Meanwhile, the “hot spots” from the aggregation also shifted the light absorption to NIR via the coupled plasmon resonances and thus the particles were exploited as therapeutic agents for low-threshold PTT.\(^\text{82}\)

Oftentimes it is also preferred to monitor the PS concentration in the targeted region to deliver sufficient light dose to the region of interest during PDT. Given that a good number of PS are fluorescent, their tissue distribution can be tracked by fluorescence spectroscopy or imaging. However, for those nonfluorescent PS, especially considering the autofluorescence background of tissues, SERS represents an effective alternative imaging modality to trace the PS accumulation in vivo. In a pilot study, Zhang et al. designed a silica/polymer-coated AuNR doped with DTTC Raman reporters and fluorescence agents to achieve in vivo multimodal tumor detection. The photosensitizer protoporphyrin IX (PpIX) was loaded into the multilayered shell for PDT. The as-formulated PDT/SERS composites exhibited the potentials for localized PDT after detection of the tumor by SERS.\(^\text{83}\) Zheng’s group also developed the palladium (Pd)-pyrrolipid AuNPs suitable for simultaneous PDT and SERS molecular imaging. The AuNP-enabled Pd–pyrrolipid acted as both a SERS reporting agent and PS, which was photodynamically active along with the emission of strong SERS signals under the 638 nm laser excitation.\(^\text{84}\)

### 5.2. PAI in Combination with Phototherapy

In the AuNP-assisted PAI process, the PA amplitude is greatly dependent on the temperature gradient in the medium generated by the heat dissipation from irradiated AuNPs. As the therapeutic outcome of PTT is also closely related to the local temperature change, thus PAI can help optimize the treatment parameters (e.g., laser location, irradiation time, and dosage) of PTT while providing real-time monitoring of the therapeutic outcomes. In consideration of the similar underlying mechanism for both PAI and PTT, i.e., relying on the photothermal conversion, gold nanostructures with superior photothermal stability are expected to induce high photoacoustic amplitudes while providing high photothermal conversion efficiency. Various gold nanostructures with combined PAI and PTT have demonstrated their effective imaging guidance to achieve the desired thermal efficacy. Higbee-Dempsey et al. combined the clinically approved ICG and AuNPs, both highly effective in PAI and PTT, into an integrated nanocluster and found a high theragnostic efficacy as compared with individuals. Both in vitro and in vivo evidence indicated that such gold nanoclusters could serve as potent and specific imaging reagents and were highly effective to produce heat, leading to 40% complete remission of the primary tumor and 20% complete disease remission.\(^\text{85}\) Zhao et al. constructed multifunctional triangular gold nanoparticles for computed tomography (CT)/PAI-guided PDT of nonsmall-cell lung cancer. The in vivo CT/PA dual-imaging modality helped visualize the distribution of nanostructures before the treatment and then depict the boundary of tumors for PTT in a real-time fashion. Modification of the nanostructures with anti-EGFR peptide (P7S) significantly increased their tumor accumulation. Meanwhile, the high NIR absorbance and photothermal conversion efficiency of triangular gold nanoparticles rendered a high therapeutic efficacy after laser irradiation.\(^\text{86}\) Strong EM field enhancement and photoluminescence quantum yield were also observed with the gold nanobipyramids. Liu et al. synthesized the polydopamine (PDA)-coated gold nanobipyramids to achieve amplified PAI and enhanced photothermal conversion. Upon loading DOX into the core–shell structure of the nanoprobes, the phototheranostic nanocomposites exhibited synergistic chemotherapy with pH-/photothermal-responsive release of DOX under the low-dose laser irradiation.\(^\text{87}\)

Coating additional thermal conductive materials onto AuNP surface is more favorable for efficient heat transfer and strong PA signal. For example, silica coating on AuNP surface as a heat-conducting layer imparts better heat-transfer capabilities. Raghavan et al. did demonstrate the in vivo photothermal efficiency of silica-coated gold nanostars in inducing localized hyperthermia to shrink the tumor upon irradiation with 1064 nm CW laser at 0.5 W cm\(^{-2}\).\(^\text{88}\) Moon and coworkers also looked into the use of rGO-coated gold nanostructures, such as AuNRs and gold nanostars, for PAI-guided PTT. As demonstrated, the
In our recent study, we constructed the novel urchin-like gold nanostructures for tumor-targeted chemo-PTT integrated with multimodal imaging. In view of the attractive benefits of NIR-II gold nanostructures, we recently also synthesized novel AuNFs with large mesopores (≈40 nm) via a liposome template-guided route, and the AuNFs could be tuned to have strong absorbance in the NIR-II region. In both systems, gold nanostructures were conjugated with the Raman reporter 4-mercaptophenylboronic acid (4-MPBA), affording SERS detection. In the AuNUs system, as shown in Figure 8, the spiny surface of AuNUs, consisting of tiny gold branches (≈7 nm) in close proximity to each other, conferred the SERS capacity and PAI with high contrast. Following sequential modification with dopamine (DA) and hyaluronic acid (HA), DOX adsorbed onto the AuNUs surface by virtue of π–π stacking could be triggered to release by intracellularly overproduced ATP and HAase in the tumor microenvironment. Through CD44-mediated endocytosis, the AuNUs-based nanocomposites were selectively ingested by MDA-MB-231 cells, which subsequently enabled dual chemo-PTT for suppressing or eradicating tumor xenografts in vivo via controllable DOX release and efficient photothermal effects upon NIR irradiation.

In the AuNFs system (Figure 9), the high photothermal conversion efficiency and significantly amplified Raman imaging were obtained as a result of the presence of high-density hotspots in the framework structure. Functionalization of AuNFs with HA endowed the targeting capability for CD44-overexpressed tumor cells, along with gatekeeping of DOX loaded into the mesopores. Both in vitro and in vivo evaluations demonstrated the high efficacy of such AuNFs-based nanocomposites for PA-Raman dual imaging-guided photochemotherapy of solid tumors in NIR-II, achieving almost complete tumor eradication.

6. Multifunctional Gold Nanomaterials for Combinatorial Phototherapy

Clinically, combinational therapy normally exhibits much stronger therapeutic effects than individual monotherapies. In this regard, gold nanostructures have been explored as suitable

---

**Figure 8.** Raman reporter-bearing AuNUs for multimodal imaging and photo-chemo-tumor ablation. Representative Raman mapping of AuNUs and in vivo PA images of tumors in mice were obtained. Significant in vivo tumor suppression was observed over the photo-chemo treatment period. Reproduced with permission.© 2020, Wiley-VCH.
platforms for multimodality therapy for their fascinating LPSR properties in phototherapy and the easy modification with chemotherapeutic, immunological, or genetic agents.\[95,96\] In a recent review, Xie et al. comprehensively summarized the emerging combination of phototherapy with other cancer therapeutic modalities including chemotherapy, immunotherapy, gene therapy, and radiotherapy in cancer nanomedicine.\[97\] In this section, we rather focused on the utility of recently developed gold nanostructures to obtain the synergic cytotoxic effects by combining light-mediated therapy and other therapeutic techniques. Some representative multifunctional gold nanostructures used for combinatorial phototherapy are shown in Table 2 along with the information on therapeutic modalities and light irradiation conditions.

6.1. PTT in Combination with PDT

Simultaneous PTT and PDT are readily applied with AuNPs as the photothermal agent and the PS delivery carrier.\[98\] Meanwhile, the amplified EM field in the vicinity of irradiated plasmonic AuNPs not only increases the efficiency of PTT but also enhances PDT. In very recent years, a few AuNP-based photosensitive materials have been synthesized with an intention to mutually boost the therapeutic efficacy of PTT and PDT. For example, Kuo et al. demonstrated an additive effect of ICG-conjugated AuNRs in the combined PDT and hyperthermia in comparison with standalone treatment. As an integrated photodynamic and photothermal therapeutic agent, poly(styrenemaleic acid) PSMA-ICG AuNRs were able to extinguish cancer via the improved photodestruction while monitoring intracellular localization in the NIR region.\[99\] Tham et al. reported the synergy of PDT and PTT achieved with functionalized AuNRs, in which the PS ZnPc was anchored on the surface of silica-coated AuNR for elevated $^{1}\text{O}_2$ production along with synergistic photothermal effects.\[100\] Wu et al. developed a dual phototherapeutic system, consisting of Ce6-conjugated gold nanoflowers coated with a polydopamine layer, for synergistic...
PDT and PTT. NIR laser of different wavelengths was respectively used for each modality, i.e., 660 nm for PDT and 808 nm for PTT. Both in vitro and in vivo evaluation confirmed the superior phototoxicity and synergistic effects of this multifunctional nanosystem on killing cancer cells. However, many of the reported PTT-PDT dual therapies simply leverage the combination of gold nanomaterials and PS molecules, still requiring individual light sources for each modality, which cause unnecessary complexity in clinical applications. Ideally, the photosensitive agent for simultaneous PTT and PDT should be photostirced under the same light irradiation. Based on that photoluminescent gold nanoclusters with a long triplet-exciton lifetime could extend the energy transfer to surrounding O$_2$ for cytotoxic O$_2$ generation under appropriate light excitation, Liu et al. reported the use of gold nanoclusters for concurrent PTT and PDT treatment of cutaneous squamous cell carcinoma using the 808 nm NIR laser. Together with the high photothermal conversion efficiency and strong photothermal stability, gold nanoclusters are promising to deliver the integrated PTT and PDT using single-laser irradiation.

6.2. Phototherapy in Combination with Chemotherapy/Gene Therapy/Radiotherapy/Immunotherapy

While conventional PTT is considered as a standalone therapy effective for tumor ablation, the associated high laser energy and elevated temperature usually induce unwanted side effects on healthy tissues. To this end, complementing PTT with secondary therapy is beneficial to overcome the drawbacks of individual treatment with more advanced therapeutic outcomes. Apart from their photothermal attributes, gold nanostructures are effective for selectively delivering therapeutic agents like small-molecule anticancer drugs, genetic agents, immunoadjuvants, and immune checkpoint blockers. Notably, the combination of AuNP-mediated phototherapy with drug delivery is not merely an additive effect but a synergistic enhancement. Off-target toxicity can be further minimized due to the EPR effects and active cellular targeting of gold nanostructures. Moreover, the temperature increase during PTT can facilitate the infiltration of therapeutic agents into tumors with enhanced vascular permeability and promote cellular uptake of drugs by modulating membrane proteins of cancer cells.

In the combined phototherapy and chemotherapy, it is highly desirable for the carrier to be porous for maximum drug encapsulation. In our AuNFs system aforementioned, the framework structure with large mesopores not only served as matrices to load DOX in a high capacity but also provided high-density “hot spots” under light irradiation. Zeng et al. designed the metal-organic-framework-coated AuNRs, where PS molecules were integrated into the metal-organic frameworks and the chemotherapeutic drug camptothecin was loaded in the porous structure with large pore sizes and high surface areas. The synergistic treatment efficiency enabled by such multifunctional nanostructures was evidently demonstrated with in vitro destruction of cancer cells and in vivo inhibition of tumor growth and metastasis. As shown in Figure 10a, Zhang et al. developed the mesoporous silica-coated gold cube-in-cube nanosystem for triple-collaborative targeted cancer therapy and multimodal imaging. The pH-/H$_2$O$_2$-responsive Mn–Cdots were anchored onto gold cube-in-cube-SiO$_2$ nanocomposite, which catalyzed the decomposition of endogenous H$_2$O$_2$ to produce O$_2$ for enhanced PDT. DOX was entrapped in the mesoporous reservoirs and the

### Table 2. Representative multifunctional gold nanomaterials for combinatorial phototherapy.

| Gold nanostructures         | Functional groups                  | Light source | Targeted cells/tissues                        | Applications and Remarks | Ref. |
|-----------------------------|-----------------------------------|--------------|----------------------------------------------|--------------------------|------|
| AuNRs                       | ICC; PSMA                          | 808 nm       | A549 cells                                   | PDT + PTT                | [99] |
| AuNRs                       | ZnPc; silica-coated                | 730 nm       | Hela cells; MCF-7 cells                      | PDT + PTT                | [100]|
| Gold nanoflowers            | Ce6; polydopamine-coated           | 660 nm for PDT; 808 nm for PTT | Hela cells; Hela BALB/c mice | PDT + PTT               | [101]|
| Gold nanoclusters           | Captopril-stabilized               | 808 nm       | Cutaneous squamous cell carcinoma; cSCC tumor-bearing SKH-1 mice | PDT + PTT               | [102]|
| AuNRs                       | Porphyric metal-organic-framework-coated; camptothecin | 808 nm       | Mouse 4T1 breast cancer cells; 4T1 BALB/c mice | PDT + PTT | [104]|
| Gold cube-in-cube nanosystem | Mesoporous silica-coated; pH-/H$_2$O$_2$-responsive Mn–Cdots; DOX | 635 nm for PDT; 808 nm for PTT | 4T1 and L929 cell lines; 4T1 BALB/c mice | PDT + Chemo-PDT; Fluorescence imaging and MRI | [105]|
| Gold nanostars              | RGD-modified dendrimer; siRNA      | 808 nm       | U87MG cells; U87MG tumor-bearing mice | PDT + Gene Therapy; CT/thermal imaging | [107]|
| Gold nanoflowers            | USIO nanoparticles; dendrimer-stabilized | 808 nm       | Mouse 4T1 breast cancer cells; 4T1 BALB/c mice | PTT + Radiotherapy; MRI/CT/PA imaging | [108]|
| AuNPs                       | DC-derived vesicles                | 808 nm       | Murine melanoma B16F10 cells; C57BL/6 mice | PTT + immunotherapy | [111]|
| Spiky AuNPs                 | PDA-coated; DOX                    | 808 nm       | CT26 murine colorectal cancer cells; CT26 colon carcinoma-bearing mice | Chemo-PTT; +immunotherapy | [112]|

© 2021 The Authors. Advanced NanoBiomed Research published by Wiley-VCH GmbH
heat generated from gold-nanostructure-induced PTT stimulated the release of DOX. Such a versatile nanosystem also served as a multimodal bioimaging agent of photothermal, fluorescence, and magnetic resonance imaging (MRI) for guided therapy.⁹⁰⁵

Gold nanostructure-based delivery systems can also improve the stability of oligonucleotides and prevent their degradation while providing the light-induced photothermal effect in a single setting. For instance, Liang and coworkers designed sunflower-like self-assembled gold-DNA nanostructures for enhanced and controlled gene therapy.⁹⁰⁶ As shown in Figure 10a, upon NIR irradiation the large-sized nanostructure (200 nm) disassembled at the rising temperature and generated ultrasmall AuNPs (2 nm) modified with c-myc oncogene silencing sequence, which then directly targeted the cell nucleus and interfered with the gene transcription process. The combination of photothermal conversion ability and nuclear permeability of AuNPs enhanced the transfection efficiency to effectively kill cancer cells. Shi’s group reported their series of works regarding the development of versatile gold nanomaterials combining diagnostic and therapeutic functionalities within one single nanoplatform for tumor theranostics. In one of their studies, the gold nanoanel was stabilized with cyclic arginine-glycine-aspartic (Arg-Gly-Asp, RGD) peptide-modified dendrimers and used as the vector to deliver small interfering RNA (siRNA) to cancer cells overexpressing αvβ3 integrin for CT/thermal imaging, PTT, and gene silencing of tumors.⁹⁰⁷ In another study, they embedded ultrasmall iron oxide (USIO) nanoparticles into dendrimer-stabilized gold nanoflowers, affording a high r1 relaxivity, enhanced NIR absorption property, and photothermal conversion efficiency (82.7%). With good colloidal stability, cytocompatibility, and cellular uptake efficiency, the designed nanoconstruct can be used for multimode T1-weighted MRI/CT/PA imaging-guided combination PTT and radiotherapy of tumors.⁹⁰⁸

Phototherapy facilitated-antitumor immunity has been well recognized and we previously provided an overview of various immunotherapeutic approaches to improving the treatment efficiency in concert with PDT.⁹⁰⁹ As a matter of fact, the combination of phototherapy with immunotherapy was clinically favorable, in which efficient inhibition of primary tumor was achieved by the induced hyperthermia, and tumor metastasis and relapse were prevented by provoking systemic antitumor immune responses. In a recent review, Gao et al. timely summarized the recent advances in phototherapy-synergized cancer immunotherapy and highlighted the essence of multifunctional phototheranostic nanomedicine.⁹¹⁰ Immunological AuNPs have been widely adopted for the combinatorial phototherapy and immunotherapy. For example, Zhang et al. tried to develop AuNPs with retained tumor antigens followed by exoyctosis from murine melanoma B16F10 cells. In addition to the photothermal effects, AuNPs were also internalized by dendritic cells (DCs) and secreted as DC-derived vesicles to stimulate systemic immunity for fighting tumor cells and guarding against tumor metastasis and recurrence.⁹¹¹ Nam et al. developed PDA-coated spiky AuNPs with markedly improved photothermal stability and efficiency for chemo-photothermal combinatory therapy. In vivo test on the CT26 colon carcinoma-bearing mice showed that a single round of PTT combined with a subtherapeutic dose of DOX exerted strong therapeutic effects against primary tumors and elicited potent antitumor immunity against untreated, distal tumors to prevent tumor recurrence.⁹¹²

7. Concluding Remarks and Future Directions

Over the past decades, abundant innovative designs of gold nanostructures with diverse optical properties have been developed. Despite the tremendous promise and pronounced advantages of plasmonic gold nanostructures in in vivo biomedical applications, a large discrepancy between research interests and clinical translations has been noticed.

First, reproducible synthesis of uniform nanomaterials toward mass production is always a prerequisite for their clinical applications. Nevertheless, multifunctional gold nanostructures often coexist with complicated fabrication processes especially with the involvement of more than one functional motif. Delicately balancing each factor has thus been a major bottleneck, partially explaining why the multifunctional gold nanostructures have been stuck in the research phase for decades. The fabrication complexity also causes large batch-to-batch structural variation and inconsistent performance reported by different research groups. In this regard, the recent convergence of artificial intelligence (AI) and nanotechnology could be a viable approach to improve the rational design of nanomaterials and prediction of their diagnostic and therapeutic outputs upon interactions with light.⁹¹³ As demonstrated, modern machine learning algorithms could be applied to understand the nanomaterial properties and the influence of relevant parameters on the synthesis outcomes in terms of nanoparticle structure, size, aspect ratio, polydispersity, and surface chemistry. Coupling with AI and automation would clearly aid the reproducibility and large-scale synthesis of multifunctional nanoparticles in the future. Furthermore, optimal nanomedicine formulation and administration parameters together with light dosage in combinatorial therapies could be achieved with better predictions of the interactions of nanostructures with blood, biological fluids and targeted cells, the synergy of multiple functional groups and drug, etc.

Second, to make AuNPs clinically applicable, the other issue that can never be neglected is that their delivery efficiency to targeted sites. Chan et al. reviewed the literatures from the past decades and estimated that only 0.7% of an injected dose of nanoparticles could really end up in the tumor.⁹¹⁴ The surprisingly low delivery efficiency has brought up the importance to comprehensively study the systemic response of nanoparticles, including their physicochemical-dependent intercellular and transcellular (transendothelial) transport routes, interactions with blood components, pharmacokinetics, circulation time, long-term performance, effects of mononuclear phagocytosis and renal systems, etc. While being aware of limited collection of physiologically relevant information from in vitro studies, in vivo animal tests are the crucial avenues to accommodate the physiological complexity for nanomedicine evaluation. Aside from the high cost, ethical issues, and difficulty of scaling, however, it remains elusive about the predictable value that an animal model can bring about, especially considering the intra-tumor heterogeneities and immunological disparity between animals and humans. The adoption of microfluidic culture
Figure 10. a) The mesoporous silica-coated gold cube-in-cube nanosystem to enhance the overall anticancer efficiency of triple combination of photodynamic/photothermal/chemotherapy for a solid tumor. Reproduced with permission.[105] Copyright 2019, American Chemical Society. b) Self-assembled gold-DNA nanosunflowers for enhanced cellular uptake amount, tunable gene-silencing efficacy, and controlled tumor inhibition effect by NIR irradiation. Reproduced from.[106] Copyright 2019, The Authors, some rights reserved; exclusive licensee AAAS. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC).
platforms allows to partially if not fully emulate the in vivo microenvironment while enabling high-throughput analysis, which offers alternative avenues for nanomedicine evaluation toward clinical translation. We recently reviewed the advances of microfluidic-based approaches for assessing nanotherapeutics with a particular emphasis on the on-chip tumor tissue modeling and personalized nanomedicine screening.\cite{115}

Moreover, in the standardization of light-mediated diagnosis and therapy, while recognizing the essence of pharmacokinetics of photoresponsive nanotherapeutics (i.e., AuNPs) and light and therapy, while recognizing the essence of pharmacokinetics and personalized nanomedicine screening.\cite{115}

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81827803 (Y.Y.), 61875085 (Y.Y.), and 81727804 (Y.Y.)).

Conflict of Interest

The authors declare no conflict of interest.

Keywords
gold nanoparticles, photoacoustic imaging, photodynamic therapy, photothermal therapy, surface plasmon resonances

Received: December 4, 2020
Revised: February 26, 2021
Published online:

[1] X. Huang, M. A. El-Sayed, J. Adv. Res. 2010, 1, 13.
[2] J. E. Park, M. Kim, J. H. Hwang, J. M. Nam, Small Methods 2017, 1, 1600032.
[3] N. Khebtsov, V. Bogatyrev, L. Dykman, B. Khebtsov, S. Staroverov, Theranostics 2013, 3, 167.
[4] Y. Zhang, W. Chu, A. D. Foroushani, H. Wang, D. Li, J. Liu, C. J. Barrow, X. Wang, W. Yang, Materials (Basel) 2014, 7, 5169.
[5] A. F. Versiani, L. M. Andrade, E. M. N. Martins, S. Salzato, J. M. Geraldo, C. R. Chaves, D. C. Ferreira, M. Ladeira, S. Guatimosim, L. O. Ladeira, F. G. da Fonseca, Future Virol. 2016, 11, 293.
[6] H. Janssa, Q. Huo, Chem. Soc. Rev. 2012, 41, 2849.
[7] Z. Tang, N. Kong, X. Zhang, Y. Liu, P. Hu, S. Mou, P. Liljestöm, J. Shi, W. Tan, S. J. Kim, Y. Cao, R. Langer, K. W. Leong, O. C. Farokhzad, W. Tao, Nat. Rev. Mater. 2020, 5, 847.
[8] R. Medhi, P. Srinoi, N. Ngo, H. Tran, T. R. Lee, ACS Appl. Nano Mater. 2020, 3, 8557.
[9] S. Jindal, P. Gopinath, Nano Express 2020, 1, 22003.
[10] H. Li, L. Rothberg, Proc. Natl. Acad. Sci. U. S. A. 2004, 101, 14036.
[11] H. Kim, M. Park, J. Hwang, J. H. Kim, D. Chung, ACS Sens. 2019, 4, 1306.
[12] P. Moitra, M. Alafeef, K. Dghe, M. B. Frieman, D. Pan, ACS Nano 2020, 14, 7617.
[13] A. Wei, B. Kim, B. Saddler, S. L. Tripp, ChemPhysChem 2001, 2, 743.
[14] G. Yang, J. Nanda, B. Wang, G. Chen, D. T. Hallinan, ACS Appl. Mater. Interfaces 2017, 9, 13457.
[15] T. Lee, M. Mohammadniaei, H. Zhang, J. Yoon, H. K. Choi, S. Guo, Adv. Sci. 2020, 7, 1902477.
[16] E. Akanny, A. Bonhomme, C. Commun, A. Doleans-jordheim, C. Farre, F. Bessuile, S. Bourgeois, J. Raman Spectrosc. 2020, 51, 619.
[17] N. Gandra, S. Singamaneni, Nanomedicine 2013, 8, 317.
[18] M. V. Yigit, Z. Medarova, Am. J. Nucl. Med. Mol. Imaging 2012, 2, 232.
[19] X. Qian, X. Peng, D. O. Ansari, Q. Yin-goen, G. Z. Chen, D. M. Shin, L. Yang, A. N. Young, M. D. Wang, S. Nie, Nat. Biotechnol. 2008, 26, 83.
[20] C. L. Zavaleta, B. R. Smith, I. Walton, W. Doering, G. Davis, B. Shojaei, M. J. Natan, S. S. Gambhir, Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 13511.
[21] F. Tian, F. Bonnier, A. Casey, A. E. Shanahan, H. J. Byrne, Anal. Methods 2014, 6, 9116.
[22] J. Xie, Q. Zhang, J. Y. Lee, D. I. C. Wang, ACS Nano 2008, 2, 2473.
[23] J. W. Kang, P. T. C. So, R. R. Dasari, D. Lim, Nano Lett. 2015, 15, 1766.
[24] P. Dey, N. Stone, Chem. Sci. 2020, 11, 8671.
[25] W. He, X. Wang, X. Gao, Z. Lu, J. Song, JOP Conf. Ser. Mater. Sci. Eng. 2020, 729, 012086.
[26] W. Li, X. Chen, Nanomedicine 2015, 10, 299.
[27] M. Boučeh, J. C. Hsu, Y. C. Dong, J. Kim, T. K. Cormode, Biocovinj. Chem. 2020, 31, 303.
[28] S. Li, K.-H. Lui, T.-H. Tsoi, W.-S. Lo, X. Li, X. Hu, W. C.-S. Tai, C.-H.-L. Hung, G. Yan-Juan, W.-T. Wong, Nanoscale Adv. 2019, 1, 554.
[29] I. Sun, C. Ahn, J. Biomed. Opt. 2020, 24, 121903.
[30] R. García-Álvarez, L. Chen, A. Nedilko, A. Sánchez-iglesias, A. Rix, W. Lederle, V. Pathak, T. Lammers, G. von Plessen, K. Kostarelos et al., ACS Photonics 2020, 7, 646.
[31] Y. Chen, Y. Zhao, S. J. Yoon, S. S. Gambhir, S. Emelianov, Nat. Nanotechnol. 2019, 14, 465.
[32] H. Moon, D. Kumar, H. Kim, C. Sim, J. Chang, J. Kim, H. Kim, D. Lim, B. Engineering. I. Program, et al., ACS Nano 2015, 2711.
[33] L. Nie, S. Wang, X. Wang, P. Rong, A. Bhirde, Small 2013, 10, 1585.
[34] E. V. Zachagyna, M. V. Shirmanova, M. Y. Kirillin, B. N. Khebtsov, A. G. Orlova, I. V. Balalaeva, M. A. Sirotkina, M. L. Bugrova, P. D. Aggra, V. A. Kamensky, Phys. Med. Biol. 2008, 53, 4995.
[35] M. Lapiere-Landry, A. Y. Gordon, J. S. Penn, M. C. Skala, Sci. Rep. 2017, 7, 9228.
[36] Y. Chemla, O. Betzer, A. Markus, N. Farah, M. Motiei, R. Popovtzer, Y. Mandel, Nanomedicine 2019, 14, 1857.
[37] F. Chen, P. Si, A. de la Zerda, J. V. Jokster, D. Myung, Biomater. Sci. 2021, 9, 367.
[38] A. de la Zerda, S. Prabhulkar, V. L. Perez, M. R. MD, A. S. Paranjape, F. Habte, S. S. Gambhir, R. M. Awdeh, Clin. Exp. Ophthalmol. 2015, 43, 358.
[39] P. Si, E. Yuan, O. Liba, Y. Winerutra, S. Yousse, E. D. Sorelle, D. W. Yecies, R. Dutta, A. de la Zerda, ACS Nano 2018, 12, 11986.
[40] V. Nguyen, Y. Li, J. Henry, W. Zhang, M. Aaeborg, S. Jones, T. Qian, X. Wang, Y. M. Paulus, ACS Sens. 2020, 5, 3070.
[41] M. Kim, J. H. Lee, J. M. Nam, Adv. Sci. 2019, 6, 1900471.
[42] R. S. Riley, E. S. Day, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2017, 9, e1449.
[43] R. Riedel, N. Mahr, C. Yao, A. Wu, F. Yang, N. Hampp, Nanoscale 2020, 12, 3007.
[44] H. Park, D. J. Lim, J. B. Vines, J. H. Yoon, N. E. Ryu, Front. Chem. 2019, 7, 167.
[110] D. Gao, X. Guo, X. Zhang, S. Chen, Y. Wang, T. Chen, G. Huang, Y. Gao, Z. Tian, Z. Yang, Mater. Today Bio 2020, 5, 100035.
[111] D. Zhang, T. Wu, X. Qin, Q. Qiao, L. Shang, Q. Song, C. Yang, Z. Zhang, Nano Lett. 2019, 19, 6635.
[112] J. Nam, S. Son, L. J. Ochyl, R. Kuai, A. Schwendeman, J. J. Moon, Nat. Commun. 2018, 9, 1074.
[113] O. Adir, M. Poley, G. Chen, S. Froim, N. Krinsky, J. Shklover, J. Shainsky-Roitman, T. Lammers, A. Schroeder, Adv. Mater. 2020, 32, 1901989.
[114] S. Wilhelm, A. J. Tavares, Q. Dai, S. Ohta, J. Audet, H. F. Dvorak, W. C. W. Chan, Nat. Rev. Mater. 2016, 1, 16014.
[115] Y. Yang, S. Liu, J. Geng, Curr. Pharm. Des. 2019, 25, 2953.

Yamin Yang is an Associate Professor of Biomedical Engineering at Nanjing University of Aeronautics and Astronautics, China. She received her Ph.D. degree in Biomedical Engineering at Stevens Institute of Technology, USA and was a Postdoctoral Research Fellow at the University of Toronto, Canada. Her research interests include nanomedicine, microfluidics, and biophotonics.

Hongjun Wang is a Professor of Biomedical Engineering and Affiliate Professor of Chemistry and Chemical Biology at Stevens Institute of Technology, USA. The research interests of Wang’s lab mainly focus on biomimetic materials design, 3D tissue reconstruction, tissue-on-a-chip and nanomedicine. Prior to joining Stevens, he was a research fellow of the Wellman Center for Photomedicine (Massachusetts General Hospital and Harvard Medical School). Dr. Wang received his 1st Doctorate in Polymer Chemistry & Physics from Nankai University and his 2nd Doctorate in Biomedical Engineering from University of Twente. He is the member of National Academy of Inventors and Sigma Xi.