Atomically precise gold nanoclusters for healthcare applications

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Competing interests
The authors declare no conflicts of interest.

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Abbreviations
NPs, nanoparticles; GNPs, gold nanoparticles; NCs, nanoclusters; GNcs, gold nanoclusters; APGNCs, atomically precise gold nanoclusters; NIR-I, Near-infrared I; NIR-II, Near-infrared II; FGGC, N-terminal Phe-Gly-Gly-Cys peptide; CB, cucurbituril; QY, quantum yield; MDR, bacteria-multidrug resistant; ROS, reactive oxygen species; MHA, 6-mercaptopentanoic acid; 4MMP, 4-mercapto-4-methyl-2-pentanol; MRSA, methicillin-resistant Staphylococcus aureus; PAH, poly (allylamine hydrochloride); RA, retinoic acid; MEL, Melitin; Capt, Captopril; PTT, photothermal therapy; PDT, photodynamic therapy; ICG, indocyanine green; cSCC, cutaneous squamous cell carcinoma.

Citation

Abstract
The potential application of gold nanoparticles (GNPs) in biomedicine has been extensively reported. However, there is still too much puzzle about their real face and potential health risks in comparison with the commercial drug molecules. The emergence of atomically precise gold nanoclusters (APGNCs) provides the opportunity to address the puzzle due to their ultrasmall size, defined molecular formula, editable surface engineering, available structures and unique physicochemical properties including excellent biocompatibility, strong luminescence, enzyme-like activity and efficient renal clearance, et al. Recently, these advantages of APGNCs also endow them promising performances in healthcare such as bioimaging, drug delivery, antibacterial and cancer therapy. Especially, their clear composition and structures like the commercial drug molecules facilitate the study of their functions and the structure-activity relationship in healthcare, which is essential for the guided design of APGNC nanomedicine. Therefore, this review will focus the advantages and recent progress of APGNCs in health care and envision their prospects for the future.

Keywords: atomically precise gold nanoclusters; biological imaging; antibacterial; therapy
Background

Gold, as an inert noble metal with good biocompatibility, has a substantial history in biomedicine applications [1, 2]. As the typical cases of gold, the earliest research of gold nanoparticles (GNPs) can be dated to 16th Europe, when drinking gold consisting of GNPs was used to treat some mental diseases [3]. Up to now, GNPs as a versatile nanomaterial have received worldwide concerns and display vast potential in biomedicine, like the diagnosis and therapy of cancer [4]. However, some challenging issues on GNPs began to get scientist’s attention [5]. First, the polydispersity issue remains unsolved, although relative monodisperse GNPs have been made. Exactly speaking, no two GNPs are the same. Thus, the synthesis of exactly uniform GNPs at the ultimate atomic level is a major dream of scientists. Secondly, the precise surface information of GNPs, including organic stabilizers and inorganic-organic interface, is still unclear. Thirdly, their relatively large size (> 50 nm) or the formation of large aggregates often leads to the inability to escape the reticuloendothelial system (RES) and the accumulation in some organs [4, 6–8].

With the development of nanotechnology, these issues ushered in brightness. A type of ultrasmall GNPs, also called gold nanoclusters (GNCs) protected by different ligands, are reported [9]. Different from the traditional GNPs, the GNCs generally consist of a few to hundreds of gold atoms, which corresponds to GNPs smaller than 2 nm metal core diameter and gets close to the electronic formi wavelength. Therefore, the electronic states of GNCs are discrete, similar to those of the molecules. As a result, the GNCs displayed unique physicochemical properties, including multi-band absorption, high catalytic performance, molecular chirality, magnetism, low toxicity, and so on [10]. Based on these properties, they have shown vast potential in nanoprobes, catalysis, bioimaging, drug delivery and therapy, et al [11–17].

Taking the thiolated GNCs as an example, the GNCs can be defined as Auₙ(SR)ₘ, where n and m represent the number of Au atoms and thiolate ligands, respectively. Nevertheless, the values of n and m are not precisely identified due to the limitation of the previous separation and characterization techniques [18]. Since the last decade, a large number of GNCs with precise numbers of gold atoms and defined ligands called atomically precise GNCs (APGNCs for short) were identified with the rapid development of separation and characterization techniques, such as the Auₙ₋₁(SR)ₘ₋₁, Auₙ₋₂(SR)ₘ₋₂, Auₙ₋₃(SR)ₘ₋₃, Auₙ₋₄(SR)ₘ₋₄ et al. [19–22]. Compared with the GNCs without defined molecular formula, APGNCs have great expectations, as they usually have identical size, the same and known composition, uniform physicochemical properties, et al. [23]. Especially, APGNCs with well-defined structures have attracted intensive attention, which are highly close to commercial drug molecules and facilitate the study of their functions and the structure-activity relationship [24]. It is noted that herein, APGNCs include GNCs with defined molecular formulas and GNCs with defined molecular formulas and structures. The study of APGNCs in the field of nanoscience has become one of the exciting new directions in light of excellent biocompatibility, rich and editable ligand engineering, and various properties. As expected, APGNCs have also shown a flourishing and prosperous panorama in biological imaging, drug delivery, and antimicrobial therapy. In this review, we will emphasize the recent advancements of APGNCs in bioimaging, antibacterial, and cancer treatment (Figure 1) and provide insights into the bright future of APGNCs.

APGNCs for healthcare applications

Bioimaging

Bioimaging is an important research tool to understand the tissue
structure of organisms and elucidate various physiological functions of organisms [25]. In recent years, with the development of optical imaging technology, especially the introduction of digital imaging technology and computer image analysis technology, bioimaging technology has become an indispensable method in biological research and medical diagnosis [26]. A wide range of bioimaging reagents has been developed for biomedical applications, including organic fluorescence dyes, carbon nanomaterials, quantum dots, up-conversion nanomaterials, et al. [27]. However, the above materials have several or all of their disadvantages, such as small Stokes displacement, cytotoxicity, and the tendency to accumulate in cells, easy of oxidation, and poor biocompatibility. In contrast, APGNCs have become a promising bioimaging material due to their tunable photoluminescence, large Stokes shift, low photobleaching, and good biocompatibility [28–30].

Thiol-containing ligands, such as glutathione (GSH) and cysteine, are common ligands on Au NC surfaces, and such APGNCs have been synthesized and used for bioimaging in cells and in vivo [31–33]. In 2009, Muhammed et al. synthesized three Near-infrared I (NIR-I) emitting Au32 NCs by using single-phase etching of [Au32(SG)32] (Figure 2A) [34]. The as-obtained Au32 NCs can be used as bioimaging reagents because of their relatively high quantum yield (QY: 1.3%), excellent photostability, and low cytotoxicity. Importantly, Au32 NCs were selectively conjugated with streptavidin for specific labeling of cells. After conjugation, the inherent fluorescence of Au32 was used for imaging human hepatoma cells by employing the avidin–biotin interaction (Figure 2B–2D).

Gan et al. also reported three visible fluorescent Au32(SR)20 NCs (R = C6H5Ph, CH3Ph, or CH3C6H4Bu) (Figure 2E) [35]. The three NCs adopt similar structures that feature a bitetrahedral Au6 kernel protected by four tetrameric Au6(SR)6 motifs. The three Au32 NCs exhibit bright red photoluminescence, and the fluorescence intensity increases with an increase in the electron-donor strength of the ligand. After the exchange ligand by bovine serum albumin (BSA), the clusters can be successfully applied in macrophage labeling (Figure 2F–2I).

Although the above-mentioned GNCs can be considered as a potential imaging material, the relatively low luminescence yield in solutions is still a perplexing issue to be resolved. Jiang et al. developed a supramolecular self-assembly method to enhance the emission intensity of GNCs by immobilizing cucurbiturils (CBs) on the surface of Au32(FGGC)18 (FGGC = N-terminal Phe-Gly-Cys peptide) (Figure 3A) [36]. Specifically, Au32(FGGC)18 was firstly synthesized by a one-pot method under alkaline conditions, and then a certain proportion of CB[n] was added to the aqueous solution to assemble into CB/FGGC-Au NCs. In aqueous solutions, CB/FGGC-Au NCs presented an enhanced red phosphorescence emission with a quantum yield (QY) of 51% for CB[7] and 39% for CB[8] (Figure 3B–3E). CB[7]/FGGC-Au NCs showed good performances in luminescence imaging of A549 cancer cells.

Compared with visible and NIR-I imaging, Near-infrared II (NIR-II) imaging has received much more attention because NIR-II emission has a wavelength characteristic to penetrate deep biological tissues, thereby improving the spatial resolution of imaging in vivo. Liu et al. reported a water-soluble and photostable GNC (Au62(CB)30) as an efficient NIR-II fluorophore, which can penetrate deep tissue and can be applied in imaging of cancer metastasis in vivo [15]. High-resolution imaging of cancer metastasis allows for the identification of the primary tumor, blood vessel, and lymphatic

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metastasis (Figure 4), indicating that these clusters can serve as an NIR-II dye in biological imaging and potential medical diagnosis.

The photoluminescence of APGNCs is a significant feature that makes them promising agents for biological imaging and medical diagnosis. In particular, imaging in NIR-II can better penetrate into deep biological tissues and improve the resolution. However, the multi-mode imaging technology based on APGNCs will be the important research direction due to the limited function of the present single-mode imaging in clinical diagnostics.

Antibacterial

Antibiotic development lags far behind the pace of bacterial evolution. The dependence and abuse of fixed antibiotics lead to the resistance of pathogenic bacteria to multiple antimicrobial agents [37]. Thus, a new group of bacteria-multidrug resistant (MDR) bacteria, also known as super bacteria, has emerged. Infections caused by MDR bacteria are difficult to treat [38]. The emergence and spread of antibiotic resistance is one of the biggest challenges, which has become a major global public health problem [39]. Therefore, there is an urgent need to develop new antibacterial materials against the occurrence of MDR bacteria [40].

In recent decades, many new antibacterial materials have been developed, such as silver ions, silver NPs, GNPs, metal oxidation, graphene, graphene oxide, and polymer compounds containing amino groups et al. [41, 42]. Among them, GNPs have become a promising wide-spectrum antibacterial agent. GNPs show several antibacterial actions, such as delivering antibiotics, producing reactive oxygen species (ROS), offering photothermal treatments, and avoiding drug resistance of bacteria [43]. Zheng et al. found that the size of GNPs decreases to close to 1 nm or sub-nanometer dimensions, showing an unexpected antimicrobial activity (Figure 5A) [44]. They synthesized Au50(MHA)15 NCs (MHA: 6-mercaptohexanoic acid) for antimicrobial and studied the difference in antibacterial properties of Au50(MHA)15 NCs and GNPs. It is possible to confer antimicrobial activity to GNPs through precise control of their size down to the NC dimension (typically less than 3 nm). Au50(MHA)15 NCs could kill both gram-positive and gram-negative bacteria and show the potential as a wide-spectrum bactericide. In particular, these sub-2 nm sized Au50(MHA)15 showed the antimicrobial effect that was absent from their larger counterpart, GNPs (> 3 nm) (Figure 5B). The antibacterial
Figure 4 NIR-II imaging of tumor metastasis in NIR-IIa window taken in the range of 1.3-1.7 µm under an excitation of 808 nm at 140 mW/cm² with an exposure time of 300 ms. (A) Schematic diagram of tumor metastasis. (B–F) Dynamic tumor metastasis imaging and principal component analysis images with gold clusters. (G) NIR-II signal of the primary tumor from the left leg and healthy tissue from the right leg, respectively. (H–J) High-resolution imaging of tumor metastasis, showing significant dynamic progress in the left leg. (K–M) Principal component analysis overlaid images with vessel (red) and metastasis tumor tissue (green). Reproduced with permission. Liu H, Hong G, Luo Z, et al. Atomic-precision gold clusters for NIR-II imaging. Adv Mater 2019;31:1901015. Copyright 2019 Wiley.

Figure 5 (A) The antimicrobial activity of Au NCs and Au NPs. (B) Au NCs showed high killing efficiency to S. aureus, which was absent when the cells were treated with Au NPs and Au (I)-MHA complexes (Au (I) complex). (C) Au NCs induced intracellular ROS production. (D) Differential expression of genes related with cell metabolism, substrate transport, membrane integrity, and cell transcriptomic process were greatly affected following the Au NC treatment. Reproduced with permission. Zheng KY, Setyawati MI, Leong DT, et al. Antimicrobial gold nanoclusters. ACS Nano 2017;11:6904-6910. Copyright © 2017 American Chemical Society.
mechanism is related to the metabolic imbalance of the bacterial cells after the internalization of Au25(MHA)46, leading to the increased production of intracellular ROS, thus killing the bacteria (Figure 5C). Moreover, Au25(MHA)46 could cause damage to bacterial membranes and also influence the genes involved in transcription and translation (Figure 5D). More importantly, Au25(MHA)46 NCs at the same concentration as for the bacteria did not elicit cytotoxicity for human cells. Further experiments were validated by using the human intestinal mucosa epithelial cell line (NCM460) and human microvascular endothelial cells (HMEC). Increasing the cluster dose to 5-fold higher than the initial dose also did not cause any significant toxic effect on the two cell classes, indicating they would have potential utility in clinical treatment.

Ligands have a large effect on the antibacterial properties of GNCs. To verify the effect of the surface ligands of the GNCs on the antimicrobial activity, Zheng et al. synthesized a series of clusters of Au25 NC with different ligands (Figure 6A-6C) (45). The surface charge of the cluster was changed by adjusting the type and proportion of the surface ligands on the cluster. To analyze the killing effect of each type of Au25 NC, a commonly used gram-positive bacterium, Staphylococcus aureus (S. aureus), was selected as the bacterial model. S. aureus was treated with the same concentration of the same Au25 NC. The results showed that Au25(MHA)46 was the best bactericidal and could kill 95% of S. aureus. Au25(MBA)46 could kill 93% of S. aureus. Au25(Cys)46 has the worst antibacterial properties and can only kill 15% of S. aureus, the reason for which could be related to their different functional groups. Both Au25(MHA)46 and Au25(MBA)46 carry a large number of -COOH, whereas Au25(Cys)46 carry more -NH2 and less -COOH. As positively charged -NH2 could quench singlet oxygen, their presence on the surface would deplete the produced ROS. In contrast to the positively charged -NH2, the negative -COOH could better preserve the generated ROS, resulting in Au25 NCs protected with these groups having good antimicrobial performance (Figure 6D-6G).

However, another work demonstrated that the positively charged clusters with amphiphilicity can lyse cell membranes to kill viral bacteria. Pang et al. synthesized a pyridinium-zwitterionic dual-ligand

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functionalized Au_{8}(SR)_{1}Au_{8} to address MDR bacteria-induced infections [46]. This cluster has good antibacterial activity and low cytotoxicity. The combination of GNCs with conventional antibiotics can significantly improve the bactericidal capability of MDR. The binding of the positive charge on the GNCs to the bacterial cell membrane can induce the membrane potential dissipation, leading to cell death. This result is in sharp contrast to the above conclusion, indicating the complexity of nanobiotic interactions.

Lindkater et al. share an insight into the antimicrobial action of APGNCs based on their ability to passively translocate across the bacterial membrane [47]. They synthesized two Au_{8} NCs protected by different ligands, namely Au_{8} NC-ZwBuR protected by a hydrophilic modified-bidentate sulfobetaine zwitterionic molecule and Au_{8} NC-MHA protected by a more hydrophobic monodentate-thiolate, mercaptohexanoic acid. The two Au_{8} NCs were lethal to both gram-negative Pseudomonas aeruginosa and gram-positive Staphylococcus aureus bacteria (Figure 6H-6L). The bactericidal efficiency is related to the strain, time, concentration, and the entry of NCs into the cytoplasm.

The main obstacles to wound healing are bacterial infection and dehydration. During wound healing, infection is mostly prevented by keeping local cleanliness and oral antibiotics. Zheng et al. found that Au_{8}(Captop)_{8} can be used as an effective phototherapeutic agent to eliminate bacterial infections [48]. Au_{8}(Captop)_{8} was embedded into carrageenan to build an antimicrobial platform for Au_{8}(Captop)_{8} hydrogel (Figure 6J). In vivo wound infection treatment, Au_{8}(Captop)_{8} hydrogel showed a strong wound-healing ability under NIR irradiation (Figure 6K-6L). Besides, Au_{8}(Captop)_{8} hydrogel has good water retention, hemostasis, and air permeability, accelerating wound healing.

Recently, Pang et al. adjusted the ratio of CS (a thiolated zwitterionic ligand) and 4MMP (4-mercapto-4-methyl-2-pentanol) ligands to convert the GNCs conformation from Au_{8}(SR)_{8} to Au_{8}(SR)_{4}. Surprisingly, the as-obtained Au_{8}(SR)_{4} NCs showed excellent antimicrobial potency with a 1024-fold reduction in minimum inhibitory concentration (Figure 7A) [49]. In addition, it also showed high efficacy in the treatment of MRSA-induced (MRSA: methicillin-resistant Staphylococcus aureus) keratitis in mice, followed by the corneal recovery rate being greatly accelerated (about 9 times). Au_{8}(SR)_{4} can effectively kill bacteria without causing membrane rupture and leakage of bacterial contents, avoiding the excessive inflammation and excessive reaction of the immune system. Mostly, the superior antimicrobial potency of vancomycin was verified in vitro and in vivo keratitis models without significant toxic effects (Figure 7B-7D).

Overall, GNCs are now extensively investigated in antimicrobial applications. The antimicrobial mechanism involves the cellular response induction, the damage of the cell membrane and the generation of an amount of ROS species.

**Cancer treatment**

Over the past few decades, cancer has been a major public health problem and the second leading cause of death worldwide [9]. Approximately 10 million cancer patients die every year, accounting for more than 15% of all deaths worldwide [50]. Commonly seen cancer treatments include surgery, chemotherapy, radiotherapy, etc. [51].

**Radiosensitizers.** Radiation therapy for tumors is a method of localized radiation therapy. About 70% of cancer patients use radiation therapy to treat their cancer, and about 40% of cancers can be cured. Radiation therapy plays an increasingly prominent role in the treatment of cancer and has become one of the main means to treat malignant tumors [52]. In radiotherapy, the radiation will certainly have some adverse effects on the normal tissues of the human body, such as radiation reactions and injuries. Therefore, how to effectively eliminate the tumor and avoid damage to normal tissue is a thorny problem in clinical tumor radiotherapy. Radiosensitizers is a kind of drug that can change the response of tumor cells to radiation and increase the killing effect on tumor cells when it is used together with radiotherapy. Therefore, the use of relatively safe radiosensitizers to improve the efficiency of tumor treatment is a feasible way to solve this problem. It is important to boost the radiosensitivity of cancer cells to improve the effect of radiotherapy. GNCs are promising radiation sensitizers due to their strong ability to adsorb, scatter, and re-emit radiation. Usually - the GNPs larger than 50 nm cannot pass through the external barrier of the reticuloendothelial system but form large aggregates during blood circulation. Therefore, the size of the radiosensitizer must be suitable to minimize toxic side effects in vivo. Zhang et al. reported two kinds of radiosensitizers, Au_{8}2(SG)_{8} and Au_{8}2(BSA)_{8} (~1.5 nm) [53]. Their cell viabilities were respectively close to 85% and 70%, even at a high dosage of 0.2 mg/mL Au, indicating their low cytotoxicity (Figure 8A, 8B). Their radiation enhancement effects were measured by the colony formation assay using the HeLa cells. The sensitization enhancement ratios were 1.30 and 1.21 for Au_{8}2(SG)_{8} and Au_{8}2(BSA)_{8} NCs, respectively. The obvious enhancement could be attributed to the enhanced DNA

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damage induced by the photoelectric effect and Compton Scattering of the heavy atom. NCs were rarely found in most of the organs except the liver and bladder, indicating that the NCs have sufficient transit time in the systemic circulation for the deposition in tumors (Figure 8C). In addition, the experiments in vivo further confirmed their strong radiation enhancement effect. Compared with the mice treated by radiation only, the tumor volume of mice treated with Au32(5G)16 plus radiation ($P < 0.05$) and Au32(BSA)16 plus radiation ($P < 0.1$) decreased by 35% and 10%, respectively. Similarly, the tumor weight also decreased by 53% and 39% (Figure 8D, 8E). This work also provides us an insight that introducing biomolecules as ligands is a good approach to improve the biocompatibility and therapy effect of GNCs.

Previously, most GNC radiosensitizers did not obtain precise atomic structures, which makes it difficult to understand the relationship between structure and activity. Jia et al. synthesized an APGNC, Au4(C6H4O)x (Au4 NC), through a one-pot method (Figure 8F) and unveiled its structures [54]. The Au4 NC has bright luminescence (QY: 58.7%), good biocompatibility, and can be used as a superior radiosensitizer for cancer therapy (Figure 8G). The cell colony formation assay indicated that the surviving fraction of cells treated with radiotherapy plus Au4 NC was obviously smaller than that only treated with radiation (Figure 8H-8J). Au4 NC also weakens the migration ability of tumor cells under X-ray irradiation (Figure 8K, 8L). The mechanism of radiation enhancement is related to the burst of ROS in cancer cells after X-ray irradiation, which can lead to the destruction of cells. Moreover, tumor formation assay in vivo demonstrated that tumors treated with Au4 NC + 4 Gy showed 74.2% inhibition compared with those treated with x-ray irradiation alone. Au4 NC as a radiosensitizer not only brings down the x-ray dose but also eliminates the side effects of radiation on normal tissues.

As one of the most important geometric properties in nature, chirality plays an indispensable role in various physiological processes [55]. Some important bioorganic molecules that make up life, like DNA, RNA, and proteins, are either completely right-handed or completely left-handed [56, 57]. Due to the homochiral preference for...
most chiral species, it is important to synthesize enantiomer drugs and study the pharmacological activity of each enantiomer. Inspired by this, Jia et al. designed and prepared a pair of enantiomeric alkynyl-protected L/D-Au$_{10}$(C$_9$H$_{17}$O$_2$)$_{10}$ NCs (L/D-Au NCs) as radiosensitizers (Figure 9) [58]. The experiment in vitro indicated that D-Au NC has lower cellular uptake, better monodispersibility and higher efficiency of radiosensitization than those of L-Au NCs. The radio enhancement mechanism was also identified to be ROS burst-mediated DNA damage, cell cycle arrest, and up-regulation of apoptotic protein expression. Compared with the X-ray group, tumor treated with D-Au NCs and X-ray was reduced by 51%, and no pathological tissue damage was observed in major organs, the reason for which could be that D-Au NCs burst more ROS in cells, thus accelerating DNA breaks. The chirality-dependent radiosensitization of L/D-Au NCs not only promulgated the possible role of chirality in radiotherapy but also provided a new approach to designing effective radiosensitizers.

The alloying strategy is an effective method that can adjust the properties of metal clusters [59, 60]. Based on the conception, Hua et al. prepared atomically precise Pt$_{M}$ (M = Au, Ag, Cu) alloy clusters by using levonorgestrel as a ligand and disclosed their crystal structures (Figure 10A) [61]. The investigation of composition-dependent enzyme mimicking activity and radiosensitizing effect revealed that the Pt$_{Au}$ cluster displayed good catalase-like activity and a high radiosensitization enhancement ratio (Figure 10B). Further investigation indicated that Pt$_{Au}$ cluster + x-ray can greatly inhibit the volume of the tumor (Figure 10C-10F). At the same time, no apparent tissue damage was observed in the major organs (heart, liver, spleen, lung, and kidneys) in the therapeutic groups (Figure 10G, 10H). The catalase-like activity of the Pt$_{Au}$ cluster could bring about oxygen ($O_2$) by persistently catalyzing the decomposition of excessive $H_2O_2$ in tumors, thus reducing the tumor radioresistance and achieving a better therapeutic effect. It can be concluded that the Pt$_{Au}$ cluster holds great potential for serving as a relatively safe and effective radiosensitizer to overcome hypoxia-induced therapy tolerance.


**Figure 10** (A) Schematic illustration of the synthesis of desired Pt$_{Au}$, Pt$_{Au}$, and Pt$_{Au}$ clusters. (B) The Pt$_{Au}$ cluster modulates tumor hypoxia for enhanced radiotherapy. (C) HeLa tumor volume curves of mice after various treatments. (D) Statistical results of tumor weights in different groups. (E) Images of dissected tumors in: I) Control, II) 4 Gy, III) L + 4 Gy, IV) Pt$_{Cu}$ + 4 Gy, V) Pt$_{Ag}$ + 4 Gy, and VI) Pt$_{Au}$ + 4 Gy groups. (F) Representative images of mice under various therapeutic conditions at day 14. (G) H&E and (H) TUNEL stained images of tumor sections after various treatments. Reproduced with permission. Hua Y, Huang JH, Shao ZH, et al. Composition-dependent enzyme mimicking activity and radiosensitizing effect of bimetallic clusters to modulate tumor hypoxia for enhanced cancer therapy. Adv Mater 2022;34:2203734. Copyright 2022, Wiley.

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At present, the APGNC radiosensitizer is limited to low nuclear clusters with well-defined structures and high nuclear clusters without well-defined structures. The high nuclear APGNC radiosensitizer with well-defined structures remains challenging. Very recently, Hua et al. successfully designed and prepared high nuclear Au_{10}(S-TPP)$_{18}$ (TPP-SNa = sodium 3-triphenylphosphine) propane-1-thiolate bromide) NC and clarify its structures [62]. Due to its excellent mitochondria-targeting, high ROS production, and significant inhibition of thioredoxin reductase, Au_{10}(S-TPP)$_{18}$ NCs displayed a superior radiosensitivity than that of the previous Au$_{8}$-SG$_{16}$ NCs. This work further verifies the ligand-regulated radiosensitivity and provides guidance for the construction of the highly efficient GPNC radiosensitizers.

**Chemotherapy.** Different from the radiotherapy, Chemotherapy belongs to drug treatment which can kill cancer cells by preventing the proliferation, infiltration, and metastasis of cancer cells with chemical drugs [63]. Chemotherapy drugs can kill fast-growing cancer cells but cause damage to normal cells with the spread of drugs throughout the body. For example, some chemotherapy drugs can damage cells in the heart, kidneys, bladder, lungs, and nervous system. Therefore, the toxicity and side effect of chemotherapy drugs is also an unignorable problem. The ideal chemotherapy drugs should have such features as "low dose, low side effect, and high effect" [64]. Otherwise, targeted drug delivery based on nanomedicine carriers is also considered one of the most effective strategies to solve the problem [65].

Great efforts have been made to design drug delivery systems for anti-cancer therapy. Fortunately, the size of GNCs is smaller than the kidney filtration threshold. Thus, the GNCs can be chosen as drug delivery systems [66]. Xu et al. report a very promising approach by using poly (allylamine hydrochloride) (PAH) as the cross-linking agent. Specifically, solvent-induced crystal transformation of Au$_{10}$([C$_{5}$H$_{5}$O$_{2}$])$_{18}$ (Au$_{10}$ NC) yielded a well-defined Au$_{10}$([C$_{5}$H$_{5}$O$_{2}$])$_{18}$ (Au$_{10}$ NC) (Figure 11A) [67]. Au$_{10}$-NC was combined with PAH to assemble into monodisperse stable NPs (Au$_{10}$-NC-PAH) (Figure 11B). The Au$_{10}$-NC-PAH assemblies were easily modified with peptides or antibodies for biolabeling, drug loading, and release. To judge the drug delivery ability, NG108-15 cells were incubated with Au$_{10}$-NC-PAH-RA (RA: retinoic acid). RA is slowly released from the Au$_{10}$-NC-PAH-RA system, inducing cell differentiation (Figure 11C, 11D).

Qi et al. also reported that Au$_{10}$-MHA$_{18}$ (MHA: 6-mercaptophexanoic acid) was employed as the delivery vehicle of Melittin (MEL, an unstable polypeptide anti-cancer drug) to treat human cervical cancer HeLa cells (Figure 11E) [68]. Au$_{10}$-MHA$_{18}$ NCs can prevent the degradation of MEL and maintain the anti-cancer activity of MEL. Therefore, MEL still has a persistent cytotoxic effect on HeLa cells. The flow cytometry data also supported that the percentage of apoptotic cells in the Au$_{10}$-MHA$_{18}$ group was very close to that of the control group, indicating Au$_{10}$-MHA$_{18}$ NCs had little effect on HeLa cells in vitro. In contrast, the percentage of apoptotic cells increased with the increased concentration of MEL in the MEL-Au$_{10}$-MHA$_{18}$ group. These results also demonstrated that Au$_{10}$-MHA$_{18}$ loaded with MEL has robust anti-cancer activity and can effectively induce the apoptosis of HeLa cells (Figure 11F, 11G). Different ligands might trigger different cellular responses. To understand the ligand effects on cell response, Bhattacharya et al. designed two water-soluble Au$_{10}$-(Capt)$_{18}$ and Au$_{10}$-(GSH)$_{18}$ NCs [69]. Cytotoxicity test demonstrated that the Au$_{10}$-(Capt)$_{18}$ was cytotoxic to HEK293T cells, while Au$_{10}$-(GSH)$_{18}$ and free ligands were completely nontoxic within the used concentration range. In this work, this sharp difference was ascribed to the speculation that the Capt changed its original properties and became a new chemical species after the Capt molecules were clustered around the gold core as the protected ligands. Moreover, the phenomenon is ligand-dependent as the effect of GSH on cellular toxicity is still the same no matter how it exists in the form of NCs or free ligands. Further investigation indicated that Au$_{10}$-(Capt)$_{18}$ inhibited the oxidative phosphorylation of mitochondria and the adenosine triphosphate synthase complex of the electron transport chain, initiating the leakage of electrons into the mitochondrial lumen. Following the increase in both mitochondrial and total cellular ROS, the obvious cellular apoptosis occurred (Figure 12).

![Figure 11](https://www.tmrjournals.com/bmec)

**Figure 11** (A) Schematic illustration of the conversion of Au$_{10}$ NCs and Au$_{10}$NC-PAH. (B) Self-assembly of Au$_{10}$-NC-PAH. (C) High-resolution confocal images of NG108-15 cells incubated with Au$_{10}$-NC-PAH and Au$_{10}$-NC-PAH-Ab. (D) Confocal images of NG108-15 cells incubated with RA loaded Au$_{10}$-NC-PAH assemblies for 7 days (red, actin; blue, nucleus). Reproduced with permission. Xu MM, Jia TT, Li BJ, et al. Tuning the properties of atomically precise gold nanoclusters for biolabeling and drug delivery. Chem Commun 2020; 56: 8766-8769. Copyright 2020 The Royal Society of Chemistry. (E) An illustration of formation of MEL-Au$_{10}$-MHA$_{18}$ complexes and delivery to HeLa cells, (F) Flow cytometric analyses of apoptosis in HeLa cells treated with Au$_{10}$-MHA$_{18}$ nanoclusters and MEL-Au$_{10}$-MHA$_{18}$ complexes. (G) CLSM images of HeLa cells treated with Au$_{10}$-MHA$_{18}$ nanoclusters and MEL-Au$_{10}$-MHA$_{18}$ complexes (Hoechst 33342 for nuclei; DIL for membrane, FITC for MEL). Reproduced with permission. Qi JX, Liu YX, Xu HJ, et al. Anti-cancer effect of melittin-Au$_{10}$-MHA$_{18}$ complexes on the human cervical cancer HeLa cells. Elsevier 2022;68:103078. Copyright 2021, Elsevier.
Figure 12 (A) Effects of AuIICapt_4 and AuIIICapt(GSH)3_4 (0-500 μg/mL) and the corresponding ligands on cell viability measured by the CFTG assay in HEK293T cells after 72 h treatment. (B) Only AuIIICapt(GSH)3_4 induces a higher percentage of cell death in HEK293T cells as determined by the PI staining assay measured by flow cytometry, whereas AuIIICapt(GSH)3_4 and the corresponding ligands are neutral. (C) The left panel represents the apoptotic cell percentage calculated by the flow cytometry based cell cycle assay. Only AuIIICapt(GSH)3_4 induces significant apoptosis. (D) Intracellular ROS levels were measured by flow cytometry using the H2DCFDA staining assay in HeLa cells treated with AuIIICapt(GSH)3_4 and their corresponding ligands. (E) Mitochondrial ROS levels were measured in HeLa cells after treatment with AuIIICapt(GSH)3_4 (500 μg/mL) using MitoSox staining. (F) Bar graph representing the percentage of mitochondrial membrane depolarized cells as a function of time (0, 3, 6, 12, 24, and 36 h) upon AuIIICapt(GSH)3_4 (500 μg/mL) treatment. (G) Confocal images of HeLa cells transiently transfected with mitochondrially targeted EGF (mitoEGFP) and later treated with AuIIICapt(GSH)3_4 to study the colocalization of AuIIICapt(GSH)3_4 into mitochondria. (H) Western blot analysis of Hela cells after treating them with different concentrations of AuIIICapt(GSH)3_4 and AuIIICapt(GSH)3_4 (200, 300, 400, and 500 μg/mL). Reproduced with permission. Bhattacharya SR, Bhattacharya K, Xavier VJ, et al. The atomically precise gold/captopril nanocluster AuIIICapt(GSH)3_4: Gains anticancer activity by inhibiting mitochondrial oxidative phosphorylation. ACS Appl Mater Interfaces 2022;14:29521-29536. Copyright 2023 American Chemical Society.

At present, though there are few reports of chemotherapy drugs due to the complex chemotherapy process, APGCNs are becoming one of the most promising materials for cancer chemotherapy. Especially the combination of APGCNs with other anti-cancer drugs is necessary to improve the chemotherapy effect.

Phototherapy. Phototherapy has received extensive concern because of its non-invasive, local selective irradiation and least side effects. Usually, phototherapy can be divided into photothermal therapy (PTT) and photodynamic therapy (PDT). PTT is a treatment method that uses materials with high photothermal conversion efficiency. Thus, materials with high photothermal conversion efficiency are currently being developed. Recently, APGCNs have also been tried for cancer photothermal therapy. For example, Kata et al. investigated the photothermal activity of AuIIICapt(GSH)3_4 in breast cancer cells (MDA-MB-231) [70]. AuIIICapt(GSH)3_4 NCs exhibit excellent photothermal effect and a 100% cell death was obtained at a power of 10 W/cm² for 5 min using an 808 nm laser source (Figure 13).

Jiang et al. reported the binding of AuIIICapt(GSH)3_4 and indocyanine green (ICG) can enhance photosensitivity and tumor targeting for effective photothermal treatment of cancer [71]. After intravenous injection of ICG+GS-AuIIICapt(GSH)3_4, the established tumors were completely ablated under NIR light-induced tumor photothermal therapy (Figure 14A). The power of the NIR laser used in the experiments was 0.8 W/cm². Under the same NIR light irradiation conditions, free ICG could not inhibit tumor growth, but ICG+GS-AuIIICapt(GSH)3_4 completely eradicated the primary tumor. The ICG+GS-AuIIICapt(GSH)3_4 disassociates in the liver by biorisation and is excreted in the urine (Figure 14B-14F).

PDT is a new technology for tumor therapy, which has been rapidly developed since the end of the 1970s. Up to now, this technology has been successfully applied to treat many malignant tumors. PDT requires three basic elements: photosensitizer, light, and molecular oxygen. Photonsensitizer has different half-lives in different tissues and is selectively retained in tumor tissue. Under the excitation of specific wavelength light, singlet oxygen, and other ROS species are produced in the presence of molecular oxygen, leading to tumor cell necrosis and apoptosis. Fakhouri et al. researched the PDT effect of AuIIICapt(GSH)3_4 and AuIIICapt(GSH)3_4, including AuIIICapt(GSH)3_4 and AuIIICapt(GSH)3_4, and AuIIICapt(GSH)3_4 (AcCys: N-acetyl-cysteine) and discussed the effect of the ligand on photoexcitation [72]. Upon single-and two-photon excitation, GNCs rapidly and consistently produce ROS species. However, AuIIICapt(GSH)3_4 can produce ROS with and without photo-excitation, while AuIIICapt(GSH)3_4 produces ROS only under photo-excitation (Figure 15). The result revealed that the surface ligands, composition, and structures of GNCs play a vital role in the generation of ROS. Due to their special physicochemical properties, some GNCs have both photothermal therapy and photodynamic therapy effects. This PTT/PDT synergistic treatment can greatly increase the content of ROS in tumor sites and enhance their anti-tumor effect. Liu et al. reported the captopril-stabilized AuIIICapt(GSH)3_4 for the concurrent PTT and PDT treatment of cutaneous squamous cell carcinoma (cSCC) under a single 808 nm NIR light irradiation (Figure 16) [73]. AuIIICapt(GSH)3_4 has good photothermal stability and a large production of ¹O₂ upon excitation of an 808 nm NIR laser. Furthermore, tumor-infiltrating CD4+ T and CD8+ T cells were found by immunohistochemical examination after PTT and PDT treatment based on AuIIICapt(GSH)3_4 NCs. The anticancer performance of AuIIICapt(GSH)3_4 was also determined by detecting the number of CD4+ helper T cells and CD8+ cytolytic T cells at the tumor location. A large number of activated T cells appeared in tumor sites treated with AuIIICapt(GSH)3_4 + laser group, while no significant activated T cells were found in tumor sites treated with a control group and AuIIICapt(GSH)3_4.
group. This result indicates that Au$_2$($\text{Capt}_1$)$_{1.0}$ mediated phototherapy has a strong specific cellular immunity against eSCC. A combination of PTT and PDT with Au$_2$($\text{Capt}_1$)$_{1.0}$ promotes T cell activation and enhances adaptive anti-tumor immunity, thereby helping to prevent relapsing.

The development of APGNs has brought new chances to cancer treatment, and some significant achievements have come about. However, the number of APGNs reported in the research of cancer treatment is very limited. The therapeutic mechanism of the reported APGNs was not fully understood. To address these challenges, more efforts need to be conducted in the future.

**Figure 13** Photothermal study of Au$_2$(SG)$_{18}$ NCs. MDA-MB-231 cells stained with (A-E) calcine-AM and (F-J) EthD-1 after laser irradiation (power = 10 W/cm$^2$) for a time duration of 1-5 min (time increment, 1 min), respectively. Cell viability histograms: (K) after 5 min laser irradiation at 10 W/cm$^2$ without the addition of Au$_2$(SG)$_{18}$ NCs; (L) with the addition of 0.75 mg/mL of NCs but without the laser treatment; (M) without the addition of NCs and without laser treatment; (N-R) with the addition of 0.75 mg/mL of NCs and laser irradiation for 1-5 min at 5 W/cm$^2$ (N), 6.25 W/cm$^2$ (O), 7.5 W/cm$^2$ (P), 8.75 W/cm$^2$ (Q), and 10 W/cm$^2$ (R), respectively. Reproduced with permission. Katka S K, Zhang J, Castro EA. et al. Atomically precise Au$_2$(SG)$_{18}$ nanoclusters: rapid single-step synthesis and application in photothermal therapy. *ACS Appl Mater Interfaces* 2018;10: 75-82. Copyright 2017 American Chemical Society.

**Figure 14** (A) ICG$_2$-GS-Au$_{25}$-mediated cancer photothermal therapy and its in vivo clearance pathways after dissociation in the liver. (B) 24 h ICG blood pharmacokinetics in mice injected with either ICG$_2$-GS-Au$_{25}$ or an equivalent amount of free ICG. (C) Area under the pharmacokinetics curves (AUCs) of ICG$_2$-GS-Au$_{25}$ and free ICG. (D) Tumor temperature kinetics of mice intravenously injected with ICG$_2$-GS-Au$_{25}$ free ICG, or PBS during 0.8W/cm$^2$-808 nm laser irradiation for 8 min. (E) Representative tumor thermal images of the mice receiving PTT treatment at 8 min of laser irradiation. White arrows indicate the tumors on mice. (F) Representative color images of the tumors on mice at different time points post PTT treatment. (G) Tumor volume kinetics of mice after PTT treatment. Statistical analysis was performed using two-sample equal-variance t test. Reproduced with permission. Jiang XY, Du BJ, Huang YY, et al. Cancer photothermal therapy with ICG-conjugated gold. *Bioconjugate Chem* 2020; 31(5):1522-1528. Copyright 2020 American Chemical Society.
Figure 15 (A) Type I and type II mechanism of ROS generation using photoexcited GNCs (upon one- and two-photon excitation). CT charge transfer, ISC inter system crossing, O$_2^-$, superoxide anion, •OH hydroxyl radical, H$_2$O$_2$, hydrogen peroxide, ^3$O$_2$, triplet state oxygen (molecular oxygen), ^1$O$_2$, singlet oxygen. (B) Representative fluorescence micrographs of ROS level in human microglia (red: CellROX, blue: Nuclei). (C) The average level of ROS per individual cell (white dot) and the average ROS level per condition (black bar ± SD) in microglia treated as in B). (D) Representative fluorescence confocal micrographs of singlet oxygen levels (red, white arrows) in human microglia (blue: Nuclei). (E) The average level of singlet oxygen in individual microglia cells (white dot) treated as in (B). Reproduced with permission. Fakhouri H, Martina PB, Zhang I, et al. Ligand impact on reactive oxygen species generation of Au$_{10}$ and Au$_{25}$ NCs upon one- and two-photon excitation. Commun Chem 2023;6:97. Copyright 2023 Communications Chemistry.

Conclusions and future perspectives

In the past decade, GNCs as a versatile star material have received extensive concerns from different research interests. In particular, the continuous development of synthesis, separation and characterization techniques has enabled GNCs to be produced with molecular purity and defined at precise atom level, which promotes the rapid development of GPNCs in various fields. Due to their unique size range and rich and editable surface ligand engineering, GPNCs have shown vast potential in biomedicine. In this review, we summarize the recent progress of APGNCs in diagnostic and therapeutic applications, including bioimaging, antibacterial, and cancer therapy. At present, the targeted NIR-I and NIR-II single-mode imaging have been achieved no matter how in vitro or in vivo. For antibacterial, APGNCs would adopt different mechanisms based on the difference in composition and structures. The antimicrobial mechanism mainly involves the cellular response induction, the damage of the cell membrane and the generation of an amount of ROS species. In terms of cancer treatment, APGNCs, as a versatile material, play different roles in different therapy methods, such as radiosensitizers, drug delivery systems, photosensitizers, etc. In a word, the reported APGNCs have manifested vast potential in the diagnosis and therapy of diseases like cancer.

Based on the above-mentioned investigation, we envision that APGNCs are increasingly becoming a kind of star biomaterials and promise to be novel drugs in the future. However, some challenges need to be solved and herein some suggestions are given as follows. The reported APGNCs with good biocompatibility are very limited, and their controllable synthesis, precise separation, and identification remain challenging. More attention should be paid to this. Commercial drug molecular ligands and biomolecule ligands such as polymers, proteins, and peptides would play a crucial role in determining the biomedical performances of APGNCs, especially the chiral drugs and biomolecules. Multifunctional APGNCs are welcome to satisfy the requirements of combination therapy. Thus, the modification with bioactive molecules or combination with other materials is very necessary. As an interdisciplinary field, researchers with different backgrounds need to cooperate together and welcome the arrival of the “Golden Era” as soon as possible.

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