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Gold Nanoclusters Development History in the Sphere of Smart Theranostics: Passive Targeting Mechanisms and Transformation into Microenvironment-Responsive Activation

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Abstract

Due to the numerous advantages of gold nanoparticles, such as ultra-small size, tunable fluorescence, enzyme-like catalysis, and renal clearance capability, the field of nanotheranostics has emerged as a research hotspot. However, the balance between circulatory stability and efficient activation at lesion sites still limits its clinical translation. Following the trajectory of "from passive to active, then to intelligent, and finally to external synergy," this article systematically reviews the development of AuNCs from 1.0 in a passive targeting state, 2.0 in active targeting, 3.0 in microenvironment-responsive behavior, to 4.0 in multimodal energy field synergy.

Keywords

nanoclusters of gold, smart theranostics, response of tumour microenvironment, active targeting, passive targeting, external energy fields, clinical translation

1. Introduction: The Need for Intelligent Integration of Theranostic Nanotools

1.1 Dilemmas in Clinical Diagnosis and Treatment of Malignant Tumors and Immune Diseases

Malignant tumors are one of the leading challenges faced in clinical diagnosis on a global scale. The deadly properties of these tumors are unregulated cell proliferation and lack of programmed cell death, and this results in unchecked growth of these abnormal cells. Conventional surgery, radiotherapy, and chemotherapy have considerable drawbacks: surgery cannot easily distinguish micrometastases, radiotherapy is strongly destructive, nonspecific, and chemotherapy is susceptible to off-target toxicity as well as multi-drug resistance. Thus, there is an urgent need for more targeted and non-invasive methods of tumor diagnosis and treatment [1].

The development of nanotechnology has led to the use of multifunctional nanotechnologies in cancer therapy and tissue repair. They are less invasive, highly specific, and minimally invasive compared to conventional methods. The increased surface area improves their catalytic and drug-loading, and the capacity

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to change surface chemical properties gives extensive room for target modification and intelligent responsive design [2].

1.2 Unique Advantages of Gold Nanoclusters as Theranostic Platforms

Nanoclusters of gold (several to hundreds of gold atoms) usually have a diameter below 3 nm. They have wide perspectives of application in sensing, imaging, and catalysis due to their ultra-small size, strong fluorescence characteristics, high biocompatibility, and photostability [3]. The advantages they have are:

First, ultra-small size and renal clearability. Au NCs are significantly smaller than the glomerular filtration limit of about 5.5 nm and can be excreted through metabolism and not stored in high-MPS organs like the liver and spleen over a long time. It is known that GSH-protected Au25 (about 2.4 nm) has an effective renal clearance, while BSA-protected Au25 (about 6 nm) does not have any rapid clearance and leads to liver damage, which demonstrates the critical role of size control in biosafety [2].

Second, tunable fluorescence. Quantum confinement effects allow Au NCs to have molecule-like discrete energy levels and the fluorescence wavelength can be accurately controlled by the amount of gold atoms and surface ligands. Centimeter penetration depth and better resolution, as well as reduced background noise with near-infrared second window (NIR-II) fluorescence Au NCs (1000-1700 nm) can be achieved, providing novel imaging modalities to highly sensitive and deep in vivo imaging [4].

Third, excellent catalytic performance. Au NCs have free electrons and a large specific surface area, allowing them to react with numerous reactive oxygen species present in the tumor cell microenvironment, thus interfering with cellular redox balance. They may be used to catalyse the breakdown of hydrogen peroxide to improve the effectiveness of photodynamic therapy (PDT), and other materials have also shown peroxidase and superoxide catalytic capabilities, which introduce new approaches to chemodynamic therapy (CDT) [1,5].

Fourth, surface modifiability with multiple molecules and groups. Not only can modified surface molecules enhance the water solubility and stability of AuNCs, they can also enable targeting. At this moment, reductive glutathione, thiols small molecules, bovine serum albumin, cyclodextrin, and so on are widely applied as modifying molecules. The regulation of the ratios of these molecules to metal atoms allows controlling the fluorescence wavelength, and certain functional molecules can provide highly sensitive NIR-II fluorescence imaging [4].

1.3 Core Contradiction: Evolution from “Possessing” to “Optimal”

Despite the numerous benefits of Au NCs, an essential conflict exists: the equilibrium between circulatory stability and effective activation in lesion areas. The ultra-small size allows renal clearance, but reduces the circulation time in blood, which decreases the selectivity of lesions and the retention of nanoparticles. Citrate-stabilized Au NCs have a half-life of merely 0.7 hours, and more than 70% of the dose is eliminated through the kidneys after 1 hour [6]. Circulation times can be extended by using PEGylation or other methods to minimize reticuloendothelial system uptake, but they will lower the uptake and activation efficiency of tumor cells.

The main problem with this contradiction is that it must be ensured that precise theranostics are attained in complex biological systems. The creation of AuNCs as a process of constant optimization and resolution of this central contradiction has been through the pursuit of nanotheranostic capabilities to the pursuit of optimal intelligent responsive theranostics.

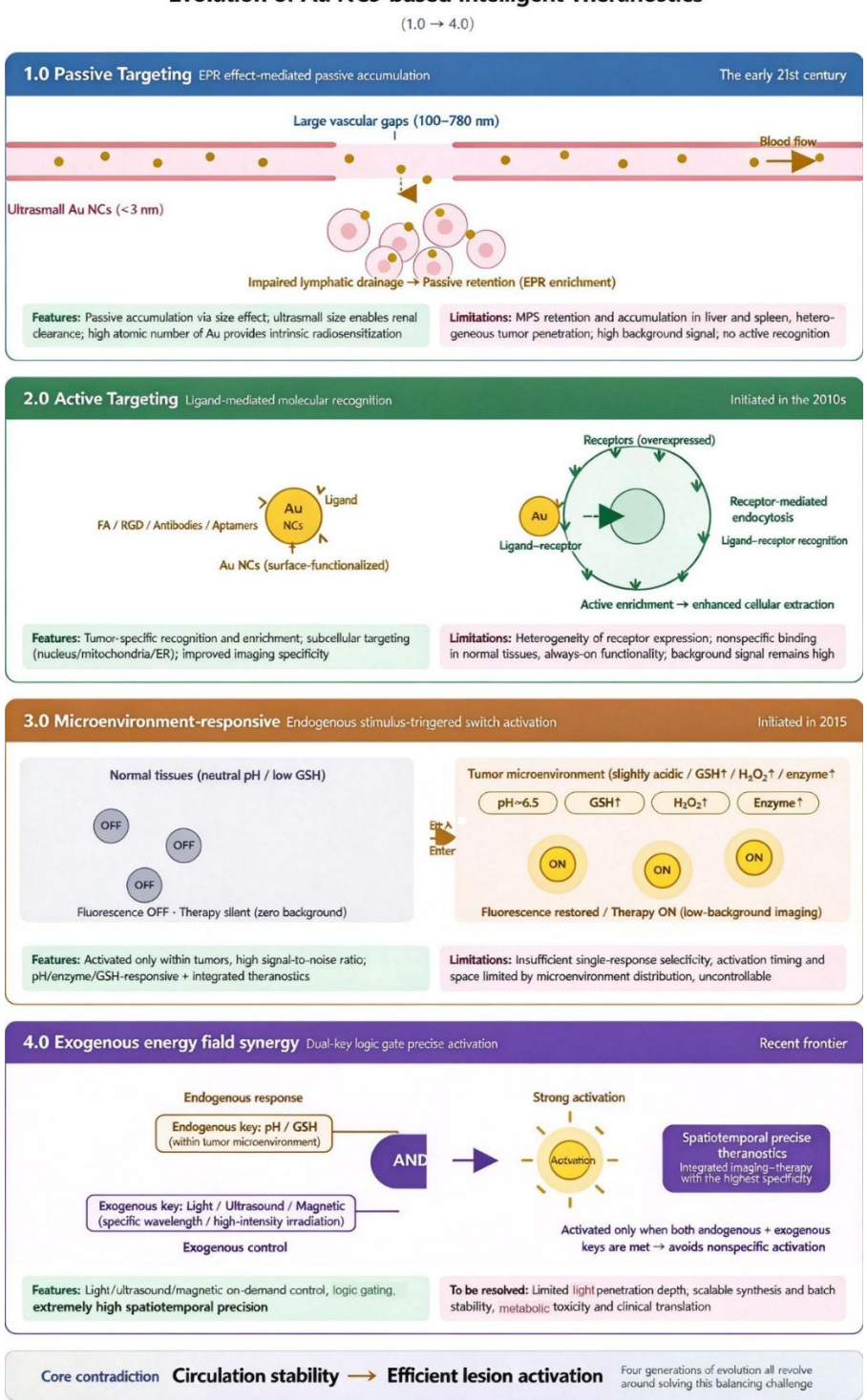
1.4 Review Framework: From Passive to Active, Then to Intelligent, Finally to Exogenous Synergy

The article considers the balance between stability of circulation and effective activation at lesion sites as the fundamental contradiction and conducts a systematic overview of the developmental history of gold nanoclusters (Au NCs) in the area of smart theranostics. To begin with, the clinical context of tumor theranostics and the peculiarities of Au NCs as a combined theranostic system are detailed. The main body is divided into four phases in order: 1.0 passive targeting, 2.0 active targeting, 3.0 microenvironment response, and 4.0 exogenous energy field synergy. Every phase presents its central mechanism, its representative works, and its natural constraints, and the reaction of the next phase to the constraint of the preceding phase represents

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the evolutionary thread, which proceeds in layers. In the last chapter, the discussion begins with bottlenecks like scalable synthesis, batch-to-batch stability, and safety evaluation, and the clinical translation opportunities of AuNCs smart theranostic systems are outlined. This paper is a systematic staged review of the field that clarifies the major advances of each stage and the key weaknesses that were present in each of them, and provides clear historical indications and the list of unsolved issues that the further research should consider. At the same time, the article states that scalable preparation, multi-key logic-gated design and deep integration of multiple therapies are the main directions along which the future development will occur, and it is intended to serve as a reference framework and direction guide to the in-depth studies on similar topics. The evolution of gold nanoclusters over four generations is summarized in Figure 1.

Figure 1: Evolution of Au NCs-based Intelligent Theranostics (1.0- 4.0)



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2. The 1.0 Era: Initiation and Limitations of Passive Targeting (Early 21St Century)

At this point, the most important thing was to employ the distinctive benefits of the ultra-small size of Au NCs in an effort to address the problem of tumors. Researchers found out at the beginning of the 21st century that ultra-small Au NCs can be actively enriched using the specific physiological properties of tumor tissues, leading to the dawn of the era of the so-called passive homing of Au NCs tumor theranostics.

2.1 EPR Effect: Theoretical Foundation and the Golden Age of "Passive Homing"

The basis of passive targeting is the enhanced permeability and retention (EPR) effect. This principle was originally proposed by Maeda and Matsumura in 1986: solid tumors create structurally defective and immature new vasculature to support high-speed growth, which has large gaps between endothelial cells (100~780 nm) and poorly developed lymphatics, enabling nanomaterials of particular sizes to easily pass out of the vessel wall and become captured as a result of poor lymphatic recirculation, and thus achieve passive enrichment [1].

The ultra-small dimension is what makes AuNCs stand out as an additional benefit of EPR-based passive targeting. They are capable of penetrating the tumor vessel wall into the interstitium and, at the same time, converting light into heat in the absorption spectrum to cause a local photothermal ablation, so that AuNCs cannot be left out in early passive imaging.

2.2 Milestone Works: Passive Tumor Imaging and X-ray Sensitization of Early Bare Gold Nanoclusters

AuNCs without the targeting ligand modification were the common subject of research during the passive targeting era. Scientists used the EPR phenomenon to inject ultra-small AuNCs intravenously, and later performed imaging and therapeutic experiments upon passive enrichment into tumor tissues.

In tumor imaging, since AuNCs are found to have superior NIR-II fluorescence and CT enhancement signal, they have come to be used as an important diagnostic tool and have been used in vivo imaging of the gastrointestinal tract and the vascular system [4]. As an illustration, sodium alginate-stabilized SA-AuNCs (about 4.2 nm) enriched significantly in KB tumors (10.4% ID/g) 24 hours following intravenous administration.

As a high-atomic-number element (79), Au has significant X-ray attenuation properties in radiotherapy and is an inherent radiosensitizer. Xie et al. created two kinds of Au25 NCs with GSH and BSA as ligands, which both have the radiotherapy-enhancing property of Au. Nevertheless, GSH-protected Au25 (about 2.4 nm) was efficiently cleared by the kidneys and did not appear toxic, whereas BSA-protected Au25 (about 6 nm) was not quickly excreted and induced injury to the liver. Zang et al. prepared exactly organized Au8 NCs with levonorgestrel as a ligand that produced high levels of ROS at low-dose X-ray exposure, which suppressed the growth of tumors and minimized side effects [2].

The photothermal conversion properties of Au25(SG)18 NCs are extremely high at 808 nm laser irradiation; therefore, Li et al. used Au25(SG)18 NCs in PTT studies on human breast cancer MDA-MB-231 cells for the first time in 2017. In 2020, Zheng et al. modified indocyanine green by coupling it to Au25(SG)18 surface to create ICG4-GS-Au25, which was more effective in converting to photothermal and targeting tumors with little power of laser irradiation to almost completely ablate a tumor. [2]

2.3 Era Limitations: MPS Retention and Tumor Penetration Heterogeneity

Even though passive targeting is developing over time, its inadequacies have come to be more apparent, especially in these two areas:

The first step is that mononuclear phagocyte system (MPS) stores Au NCs. Conventional metal nanoparticles (8~500 nm) tend to be deposited in the liver and spleen and are not easily excreted. Even ultra-small Au NCs, with improper surface ligand selection (e.g. BSA increased the hydrodynamic size to 6nm) cannot be efficiently removed by the kidneys and are eventually phagocytized by liver and spleen macrophages, leading to chronic cumulative toxicity. Radiotherapy may also cause non-specific non-carcinogenic tissue damage through the accidental non-targeted accumulation of non-targeted particles.

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Then, invasion of tumors is not homogeneous. The physiological environment has a complicated effect on the EPR phenomenon. There are great differences in vascular permeability and interstitial pressure across various types of tumors and their sites, as well as between the center and the outer part of the same tumor. Tumor interstitial fluid pressure (IFP) inhibits deep penetration by nanoparticles. Besides, the EPR effect is much less evident in human tumors compared to mouse models, which is a significant reason why numerous nanomedicines with great animal experimental outcomes do not have good clinical efficacy [7].

The main disadvantage of passive targeting is that it requires a high degree of reliance on the natural physiology of the tumor and its anatomy, does not have the ability to identify the tumor cells, cannot differentiate between tumor and normal cells, and has a hard time regulating treatment intensity and duration. Such drawbacks raised the requirement of targeting initiatives that led scientific advancement towards the endowment of Au NCs with active targeting abilities.

3. The 2.0 Era: Precise Navigation of Active Targeting (Beginning in the 2010S)

At this point, the most important step was the accurate localization of Au NCs to tumor cells. To overcome the limitations of passive targeting, scientists applied different targeting ligands to the surface of Au NCs by surface functionalization, allowing particular recognition of overexpressed receptors or markers on tumor cell surfaces, thus reaching active targeting enrichment and accurate imaging-guided therapy.

3.1 Surface Functionalization Revolution: Biomolecular Modification Conferring Specific Recognition Capability

Active targeting is based on surface modification of Au NCs with biomolecules. The surface-modifying molecules of NIR-II Au NCs may not only enhance the water solubility and stability but also provide the targeted molecule capability. Through changing the targeting molecules (specifically) on the surface and engineering intelligent response mechanisms, tumor-targeted fluorescence imaging based on Au NCs can be realized.

Surface modification can be classified into two types: covalent conjugation and non-covalent adsorption. Covalent conjugation is obtained through gold-sulfur bond formation or carboxyl-amino condensation (EDC/NHS chemistry), whereas non-covalent adsorption depends on electrostatic, hydrogen bond formation, host-guest recognition, and other supramolecular interactions [1]. As an illustration, Yang Huanghao et al. have applied thio-cyclodextrin as a modifier molecule to prepare NIR-II Au NCs with a central emission of 1050 nm by one-step reduction and conjugate the anti-CD326 antibodies by host-guest recognition, thereby leading to breast tumor-targeted NIR-II fluorescence imaging [4].

Anti-CD326 antibodies were labelled on the surface of NIR-II AuNCs by the Cui Daxiang team, which accurately localized MCF-7 and MGC-803 tumors and provided extremely sensitive fluorescence imaging and treatment. The team of Cheng Zhen found out that the carboxyl GSH molecule selectively binds to bone matrix hydroxyapatite, thereby giving GSH-modified NIR-II AuNCs the ability to target bones, thus allowing high-resolution NIR-II fluorescence imaging of mouse ribs and thoracic vertebrae [4].

3.2 Targeting Ligand Library: From Single Targeting to Dual and Multi-Targeting Strategies

The development of active targeting studies has seen the variety of ligands capable of being modified on AuNCs grow to incorporate small molecules, macromolecules and nucleic acid aptamers and have evolved into dual and multi-targeting to enhance the accuracy of tumor localization and targeting efficacy even more.

The two most frequently utilized small molecule targeting ligands are folate acid (FA) and RGD peptide. Folate receptors are very abundant on the surface of different types of tumor cells but absent in normal tissues. Specific uptake by receptor-mediated endocytosis may be achieved with folate-modified probes; Pyo et al. functionalized folic acid to the surface of Au22, and flow cytometry experiments revealed that HeLa and KB cells that over-expressed folate receptors had significantly higher uptake [8]. Integrin αvβ3 on tumor neovascular endothelial cells is recognized by RGD peptide, which enables tumor vascular targeting imaging and therapy [1].

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A variety of antibodies and their fragments (Fab, scFv) and nucleic acid aptamers can often be found in the list of macromolecular targeting ligands. Antibodies like anti-CD326, anti-EGFR, anti-HER2, and others have very specific antigen recognition and are extensively employed to modify AuNCs targeting tumors. Nucleic acid aptamers are single-stranded DNA or RNA selected through SELEX technology with the benefits of simplicity of synthesis, low immunogenicity, and high tissue penetrability, and are known as chemical antibodies.

In order to improve the targeting efficiency due to a single receptor, dual targeting and multi-targeting have also been developed. Gold nanoclusters (GNCs) with dual-ligand or multi-ligand caps were created by the team of Jiang Xingyu, which made the delivery of chemotherapeutic drugs (methotrexate) into the target lymph nodes more efficient and visually transported. Pang et al. synthesized dual/multi-ligand multifunctional Au NCs that can be used to perform NIR fluorescence imaging along with in vivo lymphatic cancer metastasis treatment, which leads to a significant increase in methotrexate delivery efficiency and decrease in toxicity [4].

The special metabolism of tumors is also targeted in active targeting. Cancer cells use glucose in aerobic glycolysis despite the presence of oxygen, according to the Warburg effect. By combining glucose to the surface of gold nanoclusters, probes can be targeted specifically to tumors due to the over-consumption of glucose by cancer cells [9].

3.3 Crossing Physiological Barriers: Precise Delivery at the Subcellular Level

The scientists did not limit themselves to active targeting of the cellular level but have gone on to pursue targeted delivery at the subcellular level, specifically in individual organelles including the nucleus, mitochondria, and endoplasmic reticulum. Targeting at the subcellular level could be greatly beneficial to the therapeutic efficiency and minimizing the side effects due to the fact that the targets of gene therapy, photodynamic therapy, and chemotherapy are usually found inside specific organelles [10].

To achieve particular nuclear targeting in nuclear targeting, the Pang Daiwen research team modified Au NCs with TAT peptide. The nucleus is an important target of gene therapy and radiotherapy. Introduction of Au NCs into nucleus may play a significant role in increasing the DNA damage efficacy of radiotherapy and enhancing the gene therapy transfection efficacy [8].

Triphenylphosphine (TPP) has been the most popular group in mitochondrial targeting since it can target Au NCs to mitochondria by targeting the mitochondrial matrix with the help of the mitochondrial membrane potential (-150~-180 mV). Thiol-containing triphenylphosphine ligands were incorporated into the synthesis of the Zhu Manzhou research group which converted lysosome targeting Au NCs to mitochondria targeting [8].

The group of Cui et al. synthesized gold-silver doped-nanoclusters that could be used in endoplasmic reticulum targeting due to the chemical similarity of the ligand components to sulfonylureas, and thus, bind to endoplasmic reticulum membrane potassium channel receptors, as do sulfonylureas; likewise, researchers in the Yangang Wang lab group have attached endoplasmic reticulum-targeting sulfonylurea compounds to the surface of Au NCs to target the endoplasmic reticulum membrane [11].

3.4 Development and Reflection: Inherent Deficiencies of Active Targeting and the Need for "Conditional Activation"

Active targeting is highly efficient and specific in tumor targeting by Au NCs, but as research progresses, there are still deficiencies and shortcomings that need to be considered.

First, even targeting ligand modification is not able to accurately differentiate between normal and tumor cells. Most of the tumor-associated receptors are to some degree present in normal tissues as well, which causes non-specific uptake of actively targeted probes by normal tissues and increases the background signal and side effects of therapy. As an illustration, folate receptors can also be expressed by the renal proximal tubular epithelial cells, and folate-modified particles might cause non-specific enrichment within the kidneys.

Second, active targeting depends on the receptor itself and has individual variations. The expression level of the same receptor can differ significantly across various patients and different stages of tumors, which makes it difficult to predict the efficiency of targeting, in combination with the fact that the EPR phenomenon is

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affected by the conditions of the physiological environment, the exact targeting in vivo remains very challenging.

Third, once the actively targeted probes are inside the body, they do not turn on their imaging and therapeutic features in response to the tumor microenvironment, which decreases the signal-to-noise ratio and increases side effects. The Au NCs@ew-Hg^{2+} system developed by Tian Lu et al. is based on using high intracellular GSH levels in tumor cells to initiate fluorescence restoration. The fluorescence recovered and increased with time in HepG2 cells, whereas the fluorescence did not recover in normal HT22 cells, offering a novel way of overcoming the problem of always-on deficiency [12].

The aforementioned drawbacks demonstrate that it is no longer possible to satisfy the demands of accurate theranostics with passive targeting ligands alone. It is highly important to create a concept of conditional activation in which AuNCs would only be activated in particular conditions of the tumor microenvironment, in order to deliver imaging signals and treatment and thus minimize side effects and bring nanotheranostics to the age of microenvironment-responsive smart theranostics.

4. The 3.0 Era: Microenvironment-Responsive SMart Theranostics (Beginning In 2015)

In order to obtain a high signal-to-noise ratio, conditional activation of the tumor microenvironment is necessary. Tumor microenvironment (TME) has its own properties that are not found in normal tissues, such as acidic pH, high GSH content, high H_2O_2 content, special enzyme overexpression, and hypoxia. The main reason behind using these endogenous stimuli in the conditional activation of AuNCs is the smart theranostic logic in the 3.0 era.

4.1 Core Logic: Achieving “Zero Background” Signals via Tumor Intrinsic Characteristics

The concept of microenvironment responsiveness can be defined as the utilization of the internal features of the tumors to commence imaging and treatment procedures with the aim of obtaining the so-called zero-background signals. In contrast to the constant activity of active targeting, microenvironment-responsive AuNCs are inactive in their normal physiological conditions. When they enter the tumor microenvironment, they experience either structural or property changes due to endogenous stimulation, which results in the activation of fluorescence signal and therapeutic effect and is much more useful than the original to increase the imaging signal-to-noise ratio and therapeutic accuracy.

Modifying molecules, environment-responsive units, along with various drugs used in treatment are introduced into a single carrier using the tumor microenvironment to realize smart drug localization and release, which in turn, realizes combined multi-therapy effects [13]. In response to the competition between tumor uptake and renal clearance, numerous groups have developed smart responsive nanomaterials, which, when activated by high levels of sialic acid and glutathione in the microenvironment, change their size, surface charge, or ligand structure wisely to enhance the efficiency of tumor uptake processes [6].

4.2 Endogenous Stimulus-Responsive Mechanisms

4.2.1 pH Response

A typical tumor has a lower pH compared to normal tissue, where the interstitial pH is about 6.5-7.0 and lysosomal pH is even lower at approximately 4.5-5.5, which could provide reasonable conditions for pH-responsive AuNCs. Examples of pH-responsive mechanisms are: surface charge reversal due to protonation/deprotonation in acidic medium, acid-sensitive chemical bond cleavage (e.g., acetal bonds, hydrazone bonds), and acid hydrolysis of metal-ligand coordination bond release.

Xiao Yan’s team incorporated PDA and methylene blue (MB) into NIR-II Au NCs (AuNCs@PDA-MB) to respond to the change of gastric acid pH and were able to monitor gastric acid secretion in vivo [4]. Wang et al. described NIR-II sensitive hollow magnetite nanoclusters which disintegrate within the acidic conditions of a tumor and induce pH-sensitive release of chemotherapy drugs and are capable of absorbing NIR-II radiation to produce local heat (photothermal conversion efficiency 36.3%) to promote chemo-photothermal synergy [11].

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Chemodynamic therapy requires a similar response to pH. The authors, Li and Zang et al., prepared accurately formed Cu6 nanoclusters which break down slowly at mild acidic conditions (pH=6.0) and release large quantities of ROS to produce CDT with minimal toxicity to healthy cells as well as concentration-dependent death of tumor cells, such that CDT is triggered only in the tumour acidic environment [2].

4.2.2 Enzyme Response

The expression of different enzymes in the tumor microenvironment is much higher than in normal tissues, including cathepsins and alkaline phosphatase. Based on specific cleavage of specific substrates by enzymes, enzyme-responsive AuNCs can have a design that allows enzyme-induced in situ assembly, fluorescence recovery, or drug release.

The enzyme response mechanism requires initial immobilization of gold nanoclusters on a carrier with a linker, which can be cleaved by a particular enzyme (an enzyme-sensitive linker is a peptide fragment with a sequence GPLGVRG that may be cleaved by matrix metalloproteinases, MMPs). Under healthy conditions, the linker does not break down, the enzyme-responsive state is not activated, and AuNCs are scattered in space, or their fluorescence is suppressed, so that they do not emit any signal. Following penetration into the tumor, the overexpressed enzymes cut the linker and release the AuNCs or allow them to aggregate and assemble in situ, thus extending their time spent at the tumor location. Once aggregated, the clusters will also exhibit aggregation-induced emission (AIE), which will further increase the fluorescence and photothermal effects.

Arginine and 6-aza-2-thiothymine (ATT) protect gold nanoclusters in the process of arginase detection. The hydrogen bonds between the guanidino group of arginine and the cluster fix the vibration of the core and minimize energy loss at the excited state by heat, thus greatly improving the fluorescence. Upon addition of arginase, it breaks down the arginine and the lock is released and the intensity of fluorescence reduces, enabling simple and effective measurement of enzyme concentration to offer a novel tool to detect enzymes that are associated with tumors with high sensitivity [11].

4.2.3 Redox Response (GSH Response)

Tumor cells have an intracellular GSH level of 1-10 mM that is 100~1000 times higher compared to normal cellular levels of GSH, which are only 2~20 μM. The difference in these GSH concentrations has offered a viable responsive environment to AuNCs. The main GSH-responsive mechanisms can be divided into three categories, namely: GSH-induced disulfide bond cleavage, competitive displacement of GSH by surface ligands, and aggregation/deaggregation based on GSH.

Egg white was applied by Tian Lu et al. to synthesize red-fluorescent Au NCs@ew-Hg^{2+} through encapsulating HAuCl4 with microwave-assisted reduction in alkaline conditions. Fluorescence quenching occurs upon addition of Hg^{2+}, which is restored when GSH is added to the quenched Au NCs@ew-Hg^{2+} system. It was found by confocal observation that fluorescence recovered and increased over time in tumor cells (HepG2), whereas it did not recover in normal cells (HT22), indicating that tumor cell recognition is possible based on the redox gradient between high GSH in tumor cells and low GSH in normal cells [12].

The GSH response has an essential part in therapy also. The GSH-responsive MnO_2-based nanozyme described by Chen Xiaoyuan et al. utilizes GSH to reduce cellular oxidative stress, and Mn^{2+} converts H_2O_2 to ·OH to enhance CDT [14]. The group under Yu Zhiqiang applied NIR-II AuNCs that were charged with platinum (AuNCs-Pt). GSH quickly forms GSH-Pt conjugates and gets removed out of the cancer cells, thus inactivating Pt. Platinum transport can be visualized using high-resolution NIR-II imaging [4].

The nanoprobe that was created by Sun Sijia et al., the Mn-Au NCs nanoprobe, responds to GSH and H_2O_2 and is a common example of dual response. In the tumor mildly acidic conditions, Mn2+ reacts with large quantities of H_2O_2 to yield ·OH as CDT, but at the same time producesO_2 to alleviate the tumor hypoxia and increase the efficiency of PDT. The cumulative effect of CDT/PDT of Mn-Au NCs decreased the survival rate of 4T1 cells to 10% under 635 nm laser exposure, in which the tumor completely disappeared and did not recur [14].

4.3 Integration of Theranostics: Response-Triggered Multimodal Synergy

Smart theranostics in the 3.0 era also have the important characteristic of theranostic integration where, on the occurrence of responsive conditions, multimodal therapies such as fluorescence, photothermal, and

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chemotherapy are activated, enabling the smooth integration of both diagnosis and treatment. Au NCs have advantages of an integrated theranostic platform because of their natural fluorescence, photothermal conversion, enzyme-like catalysis, and drug-loading capacity [1].

One common example is imaging-photothermal therapy integration. Sodium alginate-stabilized gold nanoclusters (Au NCs) were constructed by Zhang et al. to intelligently activate NIR photothermal conversion performance when the Au NCs enter the tumor microenvironment and condition response occurs, whereas Au NCs which do not enter the tumor microenvironment do not cause the condition and cannot be used to solve the problem that conventional photothermal therapy is restricted to local therapy.

Imaging-photodynamic therapy integration is another common example. NIR-II imaging-guided photodynamic therapy and photoactivated cancer immunotherapy was created by Yang et al. to provide accurate tumor imaging and PTT/PDT and photoactivated cancer immunotherapy under NIR light. Synthesis of MBA-protected aqueous Au44 nanoclusters capable of executing NIR-II fluorescence imaging and PTT/PDT combined therapy with NIR laser cleavage of linkers via 1 O_2 and coupling with NLG919 to form Au44MBA26-NLG and improved immunotherapy was reported by Yuan et al. [2].

The Mn-Au NCs developed by Sun Sijia et al. are also great magnetic resonance imaging (MRI) contrast agents, because their T1-weighted longitudinal relaxivity (r1) is as high as 31.71 $mM^{-1}s^{-1}$, which means that the tumor location and its borders become much clearer. At the same time, they may use chemodynamic therapy (CDT), i.e.,, when the clusters containing Mn^{2+} catalyze the breakdown of hydrogen peroxide (H_2O_2)-enriched tumors into highly toxic hydroxyl radicals (·OH), which kill tumor cells, releasing oxygen as a by-product. In addition, photodynamic therapy (PDT) at the wavelength of 635 nm (laser power does not exceed 50 mW/cm^2, or a low-power, mild condition) effectively generates singlet oxygen ($^1O_2$, yield up to 95%) to continue killing tumors. Furthermore, the oxygen released during the CDT process is exactly the raw material of PDT, and both of them complement each other. In dual therapy, the animal studies found that all 4T1 mouse breast cancer tumors were completely eliminated and all mice survived the 55 days of monitoring [14].

The main idea is the transition from single-point response to more accurate logic-gated activation. Though it is possible to get a specific level of conditional activation through single-point response, the distinction between the tumor microenvironment and other inflammatory or normal tissue stimuli might not be large enough to provide absolute selectivity. To address this issue, scientiststs started to design the so-called AND logic-gated systems (e.g., pH AND GSH dual response systems) that need the combined presence of two or more stimuli to activate them, thus forming the basis of the development of dual-key logic gating in the 4.0 era.

5. The 4.0 Era: Spatiotemporal Control via Multimodal Exogenous Energy Field Synergy (Recent Frontier)

At this point, the main focus should be on additional accurate localization using artificial remote manipulation. Although the microenvironment response in the 3.0 generation obtained conditional activation, the time and spatial coverage of activation remain restricted by the natural distribution of the microenvironment. Exogenous energy fields (including light, sound, magnetism, and radiation), along with the photothermal conversion, sonodynamic, magnetothermal conversion, and radiosensitization characteristics of AuNCs, were introduced during the 4.0 era and are used to remotely and precisely control the diagnostic and therapeutic processes.

5.1 Light Control: NIR-II Photothermal Imaging and Precise Photothermal Ablation

Being the most frequently applied exogenous energy field, light has such benefits as low negative impact and good spatial and time control. The Au NCs have a wide range of optical absorptions, which are able to absorb light and convert it into heat in the presence of a laser during photothermal therapy (PTT), and have the ability to image-guide with their NIR-II fluorescence properties, which can be applied to achieve imaging-therapy integration [11].

The NIR-II light (NIR-II, 000~1700 nm) is capable of greater penetration (centimeter-scale penetration depth) and has less background noise, enhancing the signal-to-noise ratio, and therefore can be used as an

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excellent light source in deep tumour light-controlled theranostics. The research team of Yang Ge has synthesized a new NIR-II Au NCs probe by incorporating photosensitizer Cy7 into Au44MBA26 to image-guide cancer therapy with fluorescence and photoacoustic (PA) imaging. The research team of Xie Jianping synthesized NIR-II Au44MBA26 nanoclusters with emission centers at 1080 nm and 1280 nm at room temperature to be used in fluorescence and PA imaging-guided cancer photothermal therapy [4].

Nevertheless, even this type of exogenous light control still has restrictions on depth of penetration, and it is still difficult to apply it to deep tumors like abdominal and brain tumors. Also, the absorption spectra of atomically precise gold nanoclusters have a predominantly step-like behavior, and their absorptivity in the NIR range, especially the NIR-II, is relatively small. The currently used photothermal therapy does not penetrate much deeper than 1 cm into tissue and cannot achieve high photothermal conversion efficiency due to limitations of current laser technology. Hence, there is a need to develop novel synthesis approaches in order to produce CMNCs that can absorb effectively in the NIR-II [2].

5.2 Sound Control: Ultrasound-Triggered Sonodynamic Therapy and Hifu Sensitization

Ultrasound also penetrates more powerfully than light, can penetrate tens of centimeters with no ionizing radiation effects, and is thus a better alternative to light control in terms of deeper tissue penetration. Sonodynamic therapy (SDT) involves the use of ultra-sound-activated sonosensitizers to produce reactive oxygen species (ROS) that kill the tumor cell, and high-intensity focused ultrasound (HIFU) focuses on the tumor location to produce local high temperature [15].

The Au NCs have two major functions in sound control: Firstly, they can be used as sonosensitizers or carriers of sonosensitizers, and when activated by ultrasound, they produce ROS to provide sonodynamic therapy; Secondly, the NIR-II fluorescence and CT contrast abilities of the Au NCs may not only be applied in the surveillance imaging and effectiveness but also act as HIFU sensitisers to enhance the diagnostic and therapeutic efficacy.

Sound control is also an effective means of penetration that can be used to treat a deep tumor, including abdominal or pelvic cavity tumors, precisely and non-invasively, and it has no limitations of light control. Nevertheless, the ROS generation and therapeutic effectiveness of sonodynamic therapy require additional optimization, and the optimization of ultrasound parameters and sonosensitizer design is the focus of current studies.

5.3 Magnetic and Radiation Control: MRI-Guided Magnetothermal Therapy and Radiotherapy Sensitization

Magnetic fields and ionizing radiation are also significant external energy fields. Magnetic metal clusters with MRI contrast and magnetothermal conversion are Gd-doped and Mn-doped AuNCs. These can produce localized heat in an alternating magnetic field to be used in magnetothermal therapy and also use MRI as a means of real-time guidance and control of the therapy process.

Mn-AuNCs created by Sun Sijia et al. can be considered as an ordinary form of MRI-directed therapy with the presence of imaging that could be used to determine the exact location of the tumor and provide a proper course of treatment. Du Guoqing et al. introduced Gd on the surface of AuNCs, which resulted in NIR-II fluorescence and MRI dual-modal imaging and tumor radiotherapy [8].

AuNCs in radiotherapy have good X-ray absorption ability and radiosensitization features because of their large atomic number (Z=79) that increases the local radiation dose and induces the production of ROS when exposed to X-rays, which helps to increase the effectiveness of radiotherapy greatly. Ultra-small AuNCs are more efficient in tumor enrichment and have a higher rate of renal clearance than conventional radiosensitizers, making them more effective and less harmful to normal tissue [1,16].

As an unusual radiation control measure, boron neutron capture therapy (BNCT) is also related to AuNCs. Ma Wenli et al. incorporated icosahedral carborane as a source of boron, and gold nanoclusters as a fluorescent probe scaffold to create a boron delivery agent (AuGSCB-Glu) that integrates targeting, boron loading, and fluorescent imaging. Its imaging specificity was high, and it had strong uptake of boron by MCF-7 cells, and demonstrated BNCT effectiveness on the cellular level [9]. BNCT produces high-energy particles (alpha

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particles and 7 Li) when exposed to neutrons to achieve cell-scale killing, while fluorescence imaging can be used to provide exact directions and assess the efficiency of BNCT.

5.4 Frontier Exploration: Endogenous-Exogenous Dual-Key Logic-Gated Activation System

The latest advancement in the 4.0 era is the integration of endogenous factors (pH, GSH, et al.) and exogenous factors (including NIR-II light) to form a dual-key logical-gated activation system that needs both endogenous stimulation (to be in the tumor microenvironment) and exogenous stimulation (specific wavelength intensity light, sound, or magnetic field irradiation) to be activated, which has significantly increased spatial and temporal accuracy and specificity, and does not cause non-specific activation in healthy tissues.

Huang Peng team developed a combination of PA/MRI dual-mode imaging and NIR-II photothermal-chemodynamic therapy using manganese dioxide-coated gold nanorods (GNR@SiO_2@MnO_2) in NIR-II. Under these conditions, MnO_2 was reductively decomposed in a weakly acidic and highly concentrated GSH solution to release Mn^{2+} to enhance MRI and CDT, whereas Au nanorods produced photothermal effects with the exposure to the NIR-II light to reach the endogenous (pH/GSH) and exogenous (NIR-II light) dual-stimulus synergistic precise theranostics [4].

The spatial magnetite nanoclusters responsive to the NIR-II are also based on the dual-key design concept as reported by Wang et al. The tumor acidic environment will cause dissolution and trigger pH-responsive drug release, and at the same time, absorb NIR light and turn it into local heat, with photothermal conversion efficiency of 36.3%, which improves chemo-photothermal synergy [11]. It is only in the presence of both the tumor acidic microenvironment (endogenous key) and NIR-II light irradiation (exogenous key) that the optimal drug release and therapeutic effect can be obtained.

Dual-key logic-gated activation system is the ultimate level of AuNCs evolution in smart theranostics, with a perfect combination of the specificity of the endogenous microenvironment response and the spatiotemporal controllability of exogenous energy fields to reach the ultimate goal of precision theranostics, which is to do the right thing at the right time and in the right place, offering essential technical assistance to AuNCs in their transition between fundamental research and clinical use.

6. Challenges and Prospects: Exploring Future Directions Through the Development History

6.1 A Long Journey Ahead: In Vivo Tracking, Batch Stability, and Scalable Synthesis

Even though AuNCs smart medical platforms have advanced greatly over the last 20 years, a lot of work has been done since basic research up to clinical translation, and in vivo tracking of fate, batch stability, and scalable synthesis are three of the most critical bottlenecks.

The tracking of in vivo fate, the biological composition, metabolic pathways, long-term retention, and possible toxicity of AuNCs is also something that requires systematic research. The ultra-small AuNCs are primarily eliminated via the kidneys, but the processes of their interaction with proteins, amino acids, and biological cells are unknown. Experimental works like those conducted by Wang Tianyuan demonstrated that most of the alginate-stabilized AuNC is excreted out of the body through urinary filtration, and about 54 percent of the total dosage administered is removed within 24 hours. Nevertheless, this metabolic process can be associated with slight disturbances in renal function that are reversible, which means that in vivo biosafety should be assessed further.

In respect to batch stability, AuNCs synthesis is very sensitive to the reaction parameters, including gold ion concentration, ligand ratio, reducing agents, temperature, and pH. Minor variations may lead to major changes in the size, structure, and optical properties of the product. At present, only a small number of hydrophilic atomically precise gold nanoclusters have been reported because synthesis procedures are limited, and the products are typically dispersed and hard to isolate and purify [2]. The clinical translation must consider the main concern that the property disparities between batches have the potential to negatively impact the reproducibility of the diagnostic and therapeutic actions.

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The existing NIR-II synthesis techniques are not scalable due to their inefficiency in providing high yield and inability to be prepared on a scale. In laboratory-scale synthesis, the product is usually produced in milligrams which are far much less than the grams or even kilograms needed in clinical trials. Also, quality control, purification processes, and cost control during scale-up should be considered as well, which involves interdisciplinary integration of chemical synthesis, materials engineering, and pharmaceutical engineering [4].

6.2 Integration of New Dimensions: Combination with Immune Regulation, Senescent Cell Clearance, and Cell Therapy

Besides conventional applications in tumor theranostics, the convergence and merging of Au NCs with the developing therapeutic field are introducing new directions of applications, with combined applications in immunomodulation, cell therapy, and senescent cell elimination being especially appealing.

When regulating the immune system, photothermal and photodynamic treatments of Au NCs are capable of directly eliminating tumor cells in addition to releasing tumor-associated antigens and DAMPs through induction of immunogenic cell death (ICD) to promote anti-tumor responses of the immune system [1,17]. The composite probes like those of Yang have been shown to release the IDO inhibitor NLG919 when excited with NIR light, which inhibits the development of both primary and distant tumors [2]. It is obvious that Au NCs could play a significant role in immunotherapy.

The Au NCs are ultra-small-sized and highly biocompatible, and thus, they may be used as cell markers and intracellular delivery vehicles in cell therapy. The properties of NIR-II fluorescence allow tracking and imaging of immune cells (such as T cells, NK cells, and macrophages) in real time and in vivo, visualizing the research on the kinetics of cell therapy and evaluating its effectiveness. They can also be used to deliver drugs or gene editing devices through cell carriers to bypass physiological barriers to nanomedicine delivery.

The quantity of senescent cells has a close relationship with numerous aging-related disorders in senescent cell clearance. NCs have enzymatic catalytic activity and can regulate oxidative stress, which gives a novel treatment strategy of selective clearance at the aging stage. Copper single-atom active sites on Au22 and Cd single-atom active sites on Au25 were developed by the Zhang Xiaodong team through atomic engineering to reduce oxidative stress and inflammatory injury to the kidney and the brain, with applications to real-time imaging and early interventional strategies for AKI and other diseases [4].

6.3 Clinical Translation: Current Stage, Pharmacokinetic and Toxicological Evaluation Systems, and the Next Breakthrough

The use of AuNCs is currently in the preliminary and preclinical research phases in clinical translation. Researchers in 2019 had successfully used FDA-approved indocyanine green (ICG) as the fluorescent probe, which was the first-ever demonstration of highly sensitive fluorescence imaging of small lesions and metastasis in patients with liver cancer. Nevertheless, because of numerous factors, including the intricacy of the synthesis of materials, possible in vivo toxicity, and stringent clinical control, NIR-II fluorescence probes that may be utilized in clinical studies are greatly restricted, and AuNCs have a long way to go before they can achieve their objectives [1].

The pharmacokinetic and toxicological evaluation systems are important connections in clinical translation. Tianyuan et al. Wang analyzed gold nanocluster stabilized by sodium alginate: SA-AuNCs were found to have a blood circulation half-life of 8.2±0.2h which was significantly higher compared to citrate-stabilized AuNCs (0.7h) and tumor uptake efficiency of 10.4%ID/g with major clearance via the kidney, with no significant change in liver function and renal function being substantially within normal range, indicating good biosafety [6]. Nevertheless, the existing pharmacokinetic and toxicology studies are mostly focused on mouse models, and the long-term safety studies of large animals and non-human primates are still not conducted, which is a gap that needs to be addressed to achieve the clinical translation.

As per the evolutionary history, it is possible that the next breakthrough points will be in: Firstly, advances in scalable and standardized synthesis technology to atomically precise multifunctional AuNCs; Secondly, continued evolution of endogenous-exogenous dual-key or even multi-key logic-gated systems; Thirdly, extensive integration of AuNCs with the new wave of therapies including immunotherapy, cell therapy, and gene editing; Fourthly, multimodal imaging-directed multimodal combination therapy will be the trend-setting

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approach; Fifthly, creation of wearable and implantable theranostics devices on the basis of AuNCs to monitor a chronic disease and deliver on-demand treatment.

To sum up, the AuNCs smart theranostic platform has developed through 1.0 passive targeting, 2.0 active targeting, 3.0 microenvironment response, to 4.0 multimodal exogenous energy field synergy. Its history of development is exactly the evolutionary process of addressing the fundamental contradiction of the balance between in vivo stability and effective activation at lesion sites, and surely, it will become even more significant in the future of precision medicine.

7. Conclusion

The review is carried out thoroughly and in detail about the four-generation development, starting with 1.0 passive targeting, 2.0 active targeting, 3.0 microenvironment response, and ending with 4.0 multimodal exogenous energy field synergy. The focus of this development has always been on the balance between circulatory stability and efficient activation at lesion sites, beginning with passive EPR enrichment and active ligand recognition, and eventually reaching spatial and temporal specificity in theranostics, which combine endogenous responses with the manipulation of exogenous energy. With its numerous benefits such as ultra-small dimensions, variable fluorescence, enzyme-like catalytic activity, and kidney clearance ability, AuNCs have been developed into an integrated theranostic system with high potential in the field of precision medicine.

Nonetheless, their clinical implementation also has numerous obstacles, such as unclear in vivo behavior and long-term safety, lack of sufficient batch stability, and challenges in scalability synthesis. The emphasis in the future should be on mass production through standardization, multi-key logic gating, and integration with newer modalities of therapy like immunotherapy, alongside enhancement of pharmacokinetic and toxicological evaluation systems to hasten clinical translation.

References

[1] Jia Lv, Ruoping Wang, Linlin Zhu. (2026). Gold nanoclusters-based anticancer therapeutic agents: Current applications and future challenges. Progress in Biochemistry and Biophysics, 53 (3), 623–642.

[2] Tingting Xu, Wenjing Zhang & Yongbo Song. (2024). Research advances of atomic precision coinage metal nanoclusters in tumor therapy. Chinese Journal of Inorganic Chemistry, 40 (12), 2275–2285.

[3] Jiayan Zang, Yuqi Yang, Hao Wang, et al. (2025). Applications of near-infrared II gold nanoclusters in biological fluorescence imaging (Invited). Chinese Journal of Lasers, 52 (21), 2107104.

[4] Siyu Li, Fangzheng Tian, Duyang Gao, et al. (2024). NIR-II fluorescent gold nanoclusters for biomedical photonics: Advances and challenges. Chinese Journal of Lasers, 51 (3), 0307201.

[5] Yuyan Liang, Fangying Sun & Li Shang. (2021). Colorimetric sensing based on enzyme-mimetic activity of gold nanoclusters. Chinese Journal of Analytical Chemistry, 49 (6), 931–940.

[6] Tianyuan Wang, Huazhen Zhang, Pin Zhao, et al. (2025). Renal clearance of sodium alginate stabilized Au nanoclusters. Chemical Journal of Chinese Universities, 46 (1), 182–191.

[7] Jun Huang, Junna Zou, Huanhuan Ren, et al. (2022). Novel strategies for promoting tumor penetration of anticancer nanomedicines. Acta Pharmaceutica Sinica, 57 (6), 1758–1770.

[8] Wencheng Zhong, Wenfeng Guo & Li Shang. (2022). Advances in biological imaging applications of fluorescent gold nanoclusters (Invited). Infrared and Laser Engineering, 51 (11), 20220527.

[9] Wenli Ma, Liyan Wang, Jibo Zong, et al. (2026). Boron cluster modified gold nanoclusters for boron neutron capture therapy. Chinese Journal of Inorganic Chemistry, 42 (6), 1146–1154.

[10] Jai Cai, Hongwu Huang, Bingzong Peng, et al. (2026). Recent advancements in mitochondria-targeted nanoformulations for cancer immunotherapy. Acta Pharmaceutica Sinica, 61 (2), 426–440.

[11] Le Hong, Weinan Dong, Zhennan Wu, et al. (2024). Applications of photoresponsive behavior of metal nanoclusters in biological diagnosis and treatment. Chinese Journal of Applied Chemistry, 41 (9), 1238–1252.

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[12] Lu Tian, Wenjing Zhao, Lin Li. (2016). Application of gold nanoclusters in tumor cell recognition. In Abstracts of the 30th Annual Meeting of the Chinese Chemical Society (Vol. 3: Novel Principles and Methods of Nano-sensing). Chinese Chemical Society.

[13] Yuqi Yang, Fei Gong, Shang Bai, et al. (2021). Progress in tumor microenvironment responsive nano-platforms for cancer theranostics. Acta Pharmaceutica Sinica, 56 (2), 465–475.

[14] Sijia Sun, Di Wu, Jiahe Zhang, et al. (2021). Manganese-doped gold nanoclusters with ultrasmall size and microenvironment-responsive visualized theranostics of tumor. Scientia Sinica Chimica, 51 (9), 1259–1268.

[15] Long Zhu, Yanxiu Zhang, Liping Yuan, et al. (2026). Research progress of nanomaterial-based sonodynamic therapy in tumor treatment. International Journal of Biomedical Engineering, 49 (1), 15–21.

[16] Jinglin Chang, Yumin Zhang & Hui Dong. (2018). Advances in the application of gold nanoparticles in tumor radiotherapy. International Journal of Radiation Medicine and Nuclear Medicine, 42 (3), 260–264.

[17] Xiaoyu Pang, Anjie Dong, Liandong Deng, et al. (2022). Biomedical nanomaterials for the enhancement of immunogenic cell death-based cancer immunotherapy. Chinese Science Bulletin, 67 (13), 1436–1448.

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Conflicts of Interest

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