Gold Nanoclusters: Imaging, Therapy, and Theranostic Roles in Biomedical Applications

Sanne M. van de Looij, Erik R. Hebels, Martina Viola, Mathew Hembury, Sabrina Oliveira, and Tina Vermonden*

ABSTRACT: For the past two decades, atomic gold nanoclusters (AuNCs, ultrasmall clusters of several to 100 gold atoms, having a total diameter of <2 nm) have emerged as promising agents in the diagnosis and treatment of cancer. Owing to their small size, significant quantization occurs to their conduction band, which leads to emergent photonic properties and the disappearance of the plasmonic responses observed in larger gold nanoparticles. For example, AuNCs exhibit native luminescent properties, which have been well-explored in the literature. Using proteins, peptides, or other biomolecules as structural scaffolds or capping ligands, required for the stabilization of AuNCs, improves their biocompatibility, while retaining their distinct optical properties. This paved the way for the use of AuNCs in fluorescent bioimaging, which later developed into multimodal imaging combined with computer tomography and magnetic resonance imaging as examples. The development of AuNC-based systems for diagnostic applications in cancer treatment was then made possible by employing active or passive tumor targeting strategies. Finally, the potential therapeutic applications of AuNCs are extensive, having been used as light-activated and radiotherapy agents, as well as nanocarriers for chemotherapeutic drugs, which can be bound to the capping ligand or directly to the AuNCs via different mechanisms. In this review, we present an overview of the diverse biomedical applications of AuNCs in terms of cancer imaging, therapy, and combinations thereof, as well as highlighting some additional applications relevant to biomedical research.

1. INTRODUCTION

For the past few decades, cancer has been a major public health concern, being the second leading cause of mortality worldwide.1 It has become clear that upon earlier detection of the tumor, the 5-year survival rates of patients are improved.2,3 Detection and identification of the disease before metastasis are thus critical when treating cancer. Therefore, while priority must be given to providing treatment options for these patients, designing new or improving already existing cancer detection methods is crucial to improve treatment success.

Recently, gold nanoclusters (AuNCs) have emerged as a promising detection approach in biomedical imaging, due to their unique molecule-like properties and good biocompatibility.1 Gold, as an element in particular, is an attractive inert noble metal with good biocompatibility and substantial history in biomedical applications.5 AuNCs are ultrasmall clusters of several to 100 gold atoms, having a total diameter of less than 2 nm.6 As opposed to gold nanoparticles with a diameter larger than 2 nm and having a continuous band of electronic energy, distinct electron excitation levels start to appear when the size of the gold core becomes comparable to the Fermi wavelength of an electron (~0.5 nm).6,7,8,9 Because of the quantum confinement effects,10 significant quantization of the conduction band occurs,6 and the AuNC can be energetically considered as a molecule.1 This leads to unique optical properties such as fluorescence, which is caused by electron transitions between these electron energy levels upon light activation.4 Considering that AuNCs absorb light in the near-infrared (NIR) range between 650 and 900 nm,6 they are especially useful in a biological window of cancer diagnostics. NIR light has a tissue penetration between that of optical light and X-rays and is relatively harmless to healthy cells, in contrast to wavelengths that are currently used for medicinal purposes.11 Besides that, fluorescent probes that emit light in the NIR range have the advantage of having minimum interference from background fluorescence and light scattering in biological systems.7,12 In addition to their photoluminescence, AuNC-based systems generally also are biocompatible, more photostable than commonly used organic dyes, and possess a large Stokes shift and a long luminescence lifetime.1,8 By utilizing surface protecting ligands, also known as capping

Received: September 30, 2021
Revised: November 25, 2021
Published: December 12, 2021
ligands, the AuNCs can be stabilized to prevent coalescence. Also, with the help of these ligands, the specific photoluminescent wavelengths can be tuned by adapting either the size or the surface-chemistry of the metal core. By performing reduction of gold in solution in the presence of thiols or macromolecular templating agents, nanocluster and ligands can be covalently bound.

AuNCs are not only applicable as a tool for in vivo bioimaging. By conjugating drugs to either the capping ligands or the AuNCs directly, the system can aid in therapy too. Additionally, because of the inherent properties of AuNCs, such as a good photothermal conversion, other types of triggered therapy are also within reach.

Herein, a review regarding cancer nanomedicines with AuNCs is given, a platform where medical imaging and cancer cell-targeted therapy can be integrated. The development of AuNCs over the past few years will be discussed in terms of imaging, therapy, and theranostic applications to provide an overview of research that has been executed thus far. A theranostic system is defined as a material that combines therapy and diagnostic imaging in one platform. They deliver therapeutic drugs or aid in another form of therapy, while also acting as or delivering a diagnostic imaging agent at the same time. Moreover, the potential applications of AuNCs in neurological disorders, antibiotics, and vaccine development will be discussed briefly (Scheme 1), followed by a discussion to determine the knowledge gap and future perspectives.

2. AUNCS IN FLUORESCENT BIOIMAGING

Most of the research conducted has focused on using thiols, bovine serum albumin, or glutathione as capping ligands to obtain stabilized fluorescent AuNCs. However, many different capping ligands have been used in the synthesis to improve the quantum yield of the luminescent emission (QY) or pharmacokinetic factors. The QY of the ligand-protected AuNCs is an essential parameter for the characterization of their light emission properties, where a higher QY is an indication of increased photoluminescent signal. It is the ratio of the amount of photons that are emitted to the amount of photons that are absorbed.

One of the current challenges in the development of AuNCs for bioimaging techniques is that the ligand-protected AuNCs often have a very low QY of less than 1%, limiting their in vivo applications. Alongside that, the colloidal stability of the protected AuNCs is usually not optimal either, nor are the biodistribution or clearance and accumulation in target organs. Nevertheless, multiple research groups have aimed to figure out how to adapt the surface-chemistry of the AuNCs to solve all these problems. Wu et al. (2010) found that the most effective strategy to enhance fluorescence from AuNCs is to employ ligands with electron-rich atoms (e.g., N, O, S, P) and groups (e.g., carboxylic acid, amines). AuNCs are often developed with polymers as capping ligands or endogenous biomolecules, such as peptides, proteins, and DNA in order to increase their biocompatibility. Table 1 and Table 2 give an overview of some relevant articles for fluorescent bioimaging systems employing AuNCs and additional fluorescent properties of AuNCs, respectively.

**Fluorescence of AuNCs with Different Capping Ligands.** Well-explored are the bovine serum albumin (BSA)-AuNCs, which were first reported by Xie et al. in 2009. They synthesized red emitting BSA-AuNCs ($\lambda_{em max}$ =...
640 nm) with QY of approximately 6%. However, later research found an unsatisfying colloidal stability and biodistribution,17,19 providing challenges for in vivo applications. Yet, BSA-templated AuNCs have been extensively studied in the context of targeting and drug delivery,13 considering their ability to be functionalized with targeting molecules such as the monoclonal antibody Herceptin or the synthetic vitamin folic acid. BSA is not the only protein that can be attributed to that variable. On several occasions, it was shown that the proteins kept their endogenous functions even after the AuNC synthesis.26,27

By making use of zwitterionic and bidentate thiol molecules as capping ligands, Chen et al. (2017) have reported short wavelength infrared (SWIR, \(\lambda = 1–2 \mu m\)) emitting AuNCs with a QY of 0.6% to 3.8% for emission wavelengths between 1000 and 900 nm, respectively.30

DNA-templated AuNC synthesis has also been performed.31 The emission color of the AuNCs is mainly dependent on the degree of metal reduction, while the DNA sequence and chain lifetimes.29

It was found that protein templates with many cysteine residues cause a shift in the fluorescent emission to higher wavelengths (red shift). They also appear to cause shorter fluorescent lifetimes.29

The tripeptide glutathione (GSH) has been commonly used as a AuNC surface ligand because of its limited interaction and affinity to cellular proteins.3 It has been found that glutathione reduces the accumulation of AuNCs in the liver and the spleen, while improving renal clearance, leading to at least 50% of GSH-coated AuNCs to be effectively removed from the body via the urinary systems within 24 h after IV-injection.23 Zhou et al. reported a QY of 3.5% and a photoluminescence in the NIR range of approximately 560 nm.25 In addition to this, the findings of Luo et al. in 201233 showed that in the context of

### Table 1. Overview of Key Properties of the AuNC-Based Systems from the Studies Mentioned in the AuNCs in Fluorescent Bioimaging Section

| reference | capping ligand | additional functionality | excitation/emission | quantum yield | CT/MRI/PET/PAI |
|-----------|----------------|--------------------------|---------------------|--------------|----------------|
| Hembury, 2018 | PEG–PNIPAM | - | 550/720 nm | 3.6% | - |
| Xie, 2009 | BSA | - | 480/640 nm | 6% | - |
| Wang, 2013 | Transferrin | - | 380/710 nm | 7.7% | - |
| Chen, 2012 | Lysozyme type VI | - | 380/455 nm | 56% | - |
| Wei, 2010 | Lysozyme | - | 360/657 nm | 7% | - |
| West, 2014 | Dnase-1 | - | 460/640 nm | Not reported | - |
| Wen, 2011 | Horseradish peroxidase | - | 365/650 nm | Not reported | - |
| Li, 2020 | Keratin-Ag | - | 470/710 nm | 10.5% | - |
| Kawasaki, 2011 | Pepsin | - | 360/670 nm | 3.50% | - |
| Liu, 2011 | Insulin | - | 400/670 nm | 7% | - |
| Kong, 2013 | RNase-A | - | 470/682 nm | 12.1% | - |
| Chen, 2019 | Zwitterionic/bidentate thiol molecules | - | 532/1000–900 nm | 0.6–3.8% | - |
| Lopez, 2015 | DNA | - | 560/764 nm | 3% | - |
| Zhou, 2011 | GSH | - | 420/560 nm | 3.5% | - |
| Han, 2019 | HSA | Tumor-targeting peptide luteinizing hormone release hormone, iodine 124 | Not reported | Not reported | PET |
| Zheng, 2004 | PAMAM dendrimer | - | 340/380 nm to 760/880 nm | 70–10% | - |
| Santiago González, 2010 | PVP | - | 240/350 nm | 12.5% | - |
| Duan, 2007 | PEI | - | 353/445 nm | 10–20% | - |
| Hussain, 2005 | Thiol-terminated PMMA | - | Not reported | Not reported | - |
| Adnan, 2017 | OEGMA-AcSEMAs | - | 440/640–660 nm | 0.24% and 0.17% | - |
| Al Zaki, 2014 | Polymeric micelle | - | Not reported | Not reported | CT |
| Chen, 2013 | Polymeric micelle | - | Not reported | Not reported | - |
| Liu, 2013 | Trypsin | Folic acid | 520/690 nm | 6.5% | - |
| Zhang, 2014 | BSA | Folic acid or Hyaluronic acid | 370 or 470/600 nm | ~15% | - |
| Liu, 2016 | Lysozyme | Folic acid | 550/690 nm | 19.61% | CT |
| Hu, 2013 | None | Gadolinium ions | 435–480/600–800 nm | Not reported | CT, MRI |
| Hembury, 2015 | Mesoporous silica shells | Gold nanoparticles | 680/800–860 nm | 0.02% | MRI, PAI |
| Liu, 2019 | GSH | Doped nanocluster | 808/1300–1700 nm | 0.67% (Cu-doped) | - |
### Table 2. Overview of Key Properties of the AuNC-Based Systems from the Studies Mentioned in the Additional Fluorescence-Based Applications of AuNCs Section

| ref | capping-ligand | other functionality | excitation/ emission | QY | application | result |
|-----|----------------|---------------------|----------------------|----|-------------|--------|
| Wang, 2018<sup>19</sup> | Keratin | Ag ions | 400/725 nm | 10.7% | Sensing mercury(II) | Lower limit of detection of 2.31 nM |
| Liu, 2013<sup>47</sup> | Trypsin | Folic acid | 520/690 nm | 6.5% | Sensing heparin | Lower limit of detection 0.05 μg/mL, sense heparin in human serum with a linear range between 0.1 and 4.0 μg/mL |
| Zhang, 2019<sup>53</sup> | GSH | - | 430/610 nm | Not reported | Temperature sensing | Temperature resolution of 0.73 °C in hepatic stellate cells between 35 and 43 °C |
| Yang, 2019<sup>34</sup> | PAMAM dendrimer | - | 390/453 nm | 18% | Temperature sensing, sensing dichromate | Distinguish temperature in the range of 15 to 80 °C. Lower limit of detection of 1.9 μM |
| Shang, 2013<sup>55</sup> | Lipoic acid | - | 580/710 nm | Not reported | Temperature sensing | Temperature resolution between 0.3 and 0.5 °C in HeLa cells between 15 and 45 °C. |
| Selvaprakash, 2014<sup>58</sup> | Chicken egg ovalbumin | - | 370/640 nm | 6.6% | Sensing ATP and pyrophosphate | Lower limit of detection ATP 19 μM, lower limit of detection pyrophosphate 5 μM |
| Xu, 2013<sup>59</sup> | Glucose-oxidase | - | 507/650 nm | ~7% | Sensing of Glucose | Lower limit of detection of 0.7 μM within a linear range between 2.0 and 140 nM |
| Peng, 2012<sup>50</sup> | Calcium carbonate | Horseradish peroxidase/ antibody conjugates | Not reported/ 605 nm | Not reported | Sensing neuron-specific enolase | Lower limit of detection 2.0 pg/mL via fluorescent detection. |
| Govindaraju, 2017<sup>71</sup> | BSA | - | 450/650 nm | ~8% | Sensing dopamine in the cerebrospinal fluid | Lower limit of detection 0.830 nM in cerebrospinal fluid. Linear range between 0 and 10 nM |
| Yang, 2013<sup>62</sup> | Poly diallyldimethyl ammonium chloride/Boron nitride | - | 405/625 nm | Not reported | Sensing interleukin-6 | Lower limit of detection 0.03 ng/mL, with a logarithmic range between 0.1 and 500 ng/mL |
| Liu, 2017<sup>63</sup> | Peptide (NH₂-CCYLRRASLG-COOH) | - | 330/405 nm | Not reported | Sensing activity protein kinase A | Lower limit of detection 0.02 U/mL. Activity of protein kinase A can be detected in the range between 0.05 and 1.6 U/mL activity |
GSH-Au complexes, a lower ratio of thiol-to-gold (1.5:1 instead of 2:1) and controlled aggregation by solvent mixing in the synthesis of AuNCs can lead to a higher QY of around 15%. Metal nanocluster aggregation induced emission is a now a promising and well-recognized phenomenon, providing efficient syntheses of highly luminescent nanoclusters. For a detailed review of aggregation induced emission of metal nanoclusters, the reader is referred to Bera et al. In 2004, Zheng et al. discovered a synthesis for highly fluorescent, water-soluble, and size-tunable AuNCs using poly(amidoamine) (PAMAM) dendrimers. They found a way to encapsulate the AuNCs with different sizes with PAMAM dendrimers to obtain a new platform for in vivo applications. The nanoclusters were reported with well-defined excitation and emission spectra ranging from UV to NIR, with QYs between 70% and 10%, respectively, depending on the size of the gold clusters. Since then, more research has been done with polymers or dendrimers as templates for AuNCs, using poly(N-vinylpyrrolidone) (PVP), polyethylenimine (PEI), or thiol-terminated PMMA (poly(methyl methacrylate)) polymers. More recently, an approach was described using copolymers comprising oligo(ethylene glycol) methyl ether methacrylate (OEGMA) and 2-(acetyloxy)ethyl methacrylate (AcSEMA) monomers. Hembury et al. combined AuNCs and thermosensitive diblock copolymers consisting of poly(ethylene glycol) (PEG) and poly(N-isopropylacrylamide) (PNIPAM) and obtained a QY of 3.6% at a maximum emission wavelength of 720 nm.

Not only polymers but also polymeric micelles have been explored as possible encapsulation scaffolds for AuNCs. Because of their ability to encapsulate other compounds besides the AuNCs, polymeric micelles are usually investigated as theranostic systems. In terms of imaging, Al Zaki et al. investigated gold-loaded polymeric micelles for computed tomography (CT) imaging. They synthesized polymeric micelles consisting of the amphiphilic diblock polymer poly(ethylene glycol)-b-poly(ɛ-caprolactone). The AuNCs were encapsulated within the hydrophobic core of these micelles. Whereas the CT imaging capabilities of the micelles were investigated, the fluorescent properties were not investigated at all. This is in contrast to the research of Chen et al. in 2013, where the fluorescence of the amphiphilic gold-loaded polymeric micelles was investigated (λ_em = 610 nm).

Imaging in the near-infrared II (NIR-II) region of between 1100 and 1700 nm is attracting wide interest due to reduced tissue scattering as compared to the NIR-I region (750–900 nm). In line with this, Liu et al. in 2019 synthesized atomically precise GSH capped AuNS clusters that emit fluorescence between 1100 and 1350 nm by charge transfer between GSH and the gold core. Metal doping of these AuNCs, with copper for example, increases the QY up to 5-fold. In vivo imaging of primary and even metastatic tumors following IV injection of these AuNCs could be performed in mice with excitation at 808 nm and emission of 1300–1700 nm. Cerebral blood vessel imaging was possible as well due to the long wavelength emission in this NIR-II region which allows penetration of the skull. The applicability of AuNCs and hybrid materials including AuNCs as tumor imaging agents has been investigated in vivo involving techniques such as X-ray computed tomography, NIR fluorescent imaging, positron emission tomography (PET), and magnetic resonance imaging (MRI). Each imaging technique has its own advantages and disadvantages. Multimodal imaging is a combination of multiple imaging techniques, combining the best features of each. Having a single imaging agent that could be used for multiple imaging techniques therefore gives advantages over imaging with one technique alone. When imaging for cancer diagnostics, the amount of imaging agent that accumulates in the tumor should be high compared to other organs. The platform should, therefore, target the tumor, via either passive or active targeting. Passive targeting is done by exploiting the enhanced permeability and retention (EPR) effect, a process that occurs in regions of the body with a high degree of hypoxia and/or inflammation. Both are typical for the tumor microenvironment. The tumor vasculature has several abnormalities due to their rapid and disordered growth, resulting in gaps in the endothelium, which provide an opportunity for AuNC containing nanoparticle platforms ranging from 10 to 500 nm in the blood serum to extravasate into the tumor tissue.

Active targeting can be achieved by conjugating targeting ligands to the nanoclusters, to obtain cellular uptake via receptor mediated endocytosis as illustrated in Scheme 2.

**Scheme 2. Schematic Illustration of Receptor Mediated Endocytosis of Active Targeted AuNCs**

Examples of targeting ligands are folic acid (FA), hyaluronic acid (HA), methionine, or cyclic RGD. By employing active targeting, there is an altered biodistribution with a possible higher tumor uptake, causing a strong fluorescent signal for tumor sites in vivo. This was demonstrated by several studies, such as that by Liu et al., who showed that folic acid functionalized, trypsin-protected AuNCs (FA-try-AuNCs) could be used for in vivo imaging in mice. The FA-try-AuNCs were injected intratumorally in nude mice bearing HeLa tumors of 8 mm. The NIR fluorescent signal in the tumors was detectable immediately from injection and up to 12 hours after injection. In subcutaneously injected healthy control mice, the fluorescent signal could be seen spread over the entire body 5 min post-injection, which disappeared slowly after 12 hours, indicating metabolism and degradation of the AuNCs. A final experiment showed that upon subcutaneous injection, the tumor site was visible after 30 min, although it was less clear than when injection happened intratumorally (Figure 1). BSA-stabilized AuNCs coated with FA or HA showed similar fluorescent properties in vivo and accumulated in either...
The shortwave infrared emitting AuNCs that Chen et al. synthesized showed great potential for in vivo imaging with a higher contrast than conventional NIR imaging, while also allowing for PET-scans. Additionally, by coupling iodine-124 to a peptide protected AuNC, as done by Han et al. in 2019, the obtained system could be used for PET and fluorescent dual-imaging in lung cancer. They reported the production of these AuNCs by conjugating the tumor-targeting peptide luteinizing hormone releasing hormone to human serum albumin (HSA), and using this as a template for AuNC synthesis.

The lysozyme-capped AuNCs that were designed by Liu et al. in 2016 show great promise for diagnostic purposes. By making use of bimodal bioimaging consisting of NIR fluorescence and CT imaging in vivo, cancer tissue and healthy tissues can be distinguished more easily. They found that by adding folic acid as a targeting agent to the lysozyme-capped AuNCs, they accumulated in the tumor site of HeLa tumorbearing mice following IV administration. When not using the folic acid modification, the fluorescence of the AuNCs did not appear at the tumor site. When injecting the AuNCs for the purpose of CT imaging, positive signal enhancements could be seen in the liver and kidney one hour after injection, meaning that the AuNCs mainly accumulate in these organs in the absence of tumor tissue.

In 2013, Hu et al. even managed to develop gold-gadolinium nanoclusters for high-performance triple-modal imaging with NIR fluorescence, CT, and MR imaging in a single agent in vivo. While doing in vitro research, they found that at a concentration as low as 2.1 μM, the hybrid nanoclusters exhibited remarkable signals for NIR fluorescence, CT, and MRI. This triple-modal contrast agent capability was further tested in MCF-7 tumor-bearing mice, where the in vitro results were confirmed. The gold–gadolinium nanoclusters also showed a high tumor accumulation and quick renal clearance in vivo. Another triple-modal imaging platform was described in 2015 by Hembury et al. utilizing the aforementioned gold-silica quantum rattles. In this case, NIR, MR, and photoacoustic imaging (PAI) could be performed using this single agent in in vitro and in vivo settings.

Additional Fluorescence-Based Applications of AuNCs. The photoluminescent properties of AuNCs have been researched not only in the context of NIR, CT, and MR imaging, but also in diagnostics via nanothermometry or biosensing of heavy metals, small biomolecules, proteins, and cancer biomarkers.

The intracellular temperature is an important parameter in most cellular activities, including gene expression, cell division, and metabolism. When abnormal processes occur within the cell, such as cancer cell growth or inflammation, this may result in intracellular temperature changes. AuNCs can be used as intracellular nanothermometers because of the high temperature-sensitivity of their fluorescence lifetime and emission intensity. It was found that both of these factors change drastically within a physiologically relevant temperature range of 15 to 45 °C. By making use of fluorescence lifetime imaging microscopy (FLIM), the thermometric properties of AuNCs were tested on several occasions. In 2013, Shang et al. used lipoic-acid protected AuNCs to show that the fluorescent emission intensity and fluorescence lifetime both have a negative linear relationship with

![Figure 1. In vivo time-dependent tumor imaging by NIR fluorescence imaging. The FA-try-AuNCs were injected (A) intratumorally in HeLa tumor-bearing mice, and subcutaneously into the left forelimb region of (B) normal nude (control) mice and (C) tumor-bearing mice. The red circle and green circle indicate the tumor site and injection site, respectively. Reproduced from ref 47. Copyright (2013), American Chemical Society.](https://doi.org/10.1021/acs.bioconjchem.1c00475)

![Figure 2. In vitro fluorescent imaging of AuNCs by making use of fluorescence lifetime imaging. Left: Average lifetime histograms of intracellular gold nanoclusters at varying temperatures. Right: FLIM images of HeLa cells with internalized AuNCs at varying temperatures. Adapted with permission from ref 55. Copyright (2013), John Wiley and Sons.](https://doi.org/10.1021/acsbio.2100647)
| Reference | Capping ligand | Targeting ligand | Disease type | Function/AuNCs | Emission/absorption wavelength | Drug-delivery | Linker/Interaction | Specifics | Results |
|-----------|----------------|-----------------|--------------|----------------|--------------------------|-------------|-----------------|----------|---------|
| Liu, 2017<sup>1</sup> | Captofyl | Cutaneous squamous cell carcinoma | Photothermal therapy and Photodynamic therapy | NIR 808 nm | Photothermal conversion of 41% | | | | |
| Liu, 2017<sup>2</sup> | PAMAM-N-H<sub>4</sub> | Lung cancer | Ablative hypoxic for Photothermal therapy | 932 nm laser, power density 200 mW/cm<sup>2</sup> with photosensitizer | | | | Reduced hypoxic conditions in tumor tissue improved photothermal therapy efficacy with statistical significance |
| Ghimenes, Ross, 2017<sup>3</sup> | BSA | Si - maleimide | Photothermal and radiotherapy | 6 minutes, 138% 1.8 Gy magnetic field | | | | | |
| Salahbakhshi, 2017<sup>4</sup> | BSA | A549 cell line – alpinotin | Breast cancer | Magneto-optical radiation therapy | 0.2, 4, 6 Gy gamma rays emitted | | | Factor 2.7 enhanced radiation therapy |
| Zheng, 2017<sup>5</sup> | GSH | Cervical cancer | Radiotherapy | 5 Gy gamma-rays from 170Co (photon energy 662 keV) with an activity of 360 Ci | | | | Enhanced radiobiology of 30%, possibly due to higher cell uptake, after 20 days 30% smaller tumor volume compared to radiation alone |
| Zheng, 2017<sup>6</sup> | BSA | Cervical cancer | Radiotherapy | 5 Gy gamma-rays from 170Co (photon energy 662 keV) with an activity of 360 Ci | | | | Enhanced radiobiology of 21%, no significant tumor volume reduction compared to radiation alone |
| El-Maggad, 2020<sup>7</sup> | D-penicillamine | Cancer | Drug delivery | D-penicillamine | Interactions: Au 0/SN, hydrogen bonds, and electrostatic bond | | | Calculated in silico | Calculated the interactions and binding energies between D-penicillamine and gold nanoparticles |
| Lemaire, 2019<sup>8</sup> | BSA | Breast and pancreatic cancer | Drug delivery | DOX and SN3M | pH - or redox-sensitive | Conjugation to IBA by disulfide and maleimide linkers | | | |
| Gondimara, 2019<sup>9</sup> | BSA | Lung cancer | Drug delivery | 550/650 nm | Kaempferol | Physical interactions | | No cytotoxicity in healthy kidney cells, cytotoxic in lung cancer cells |
| Lati, 2019<sup>10</sup> | BSA | Lung cancer | Drug delivery | Fluorescence not tested in cells | Quercetin | Au-OH interaction | Good cellular uptake and biocompatibility properties, High cytotoxicity in lung cancer cells, minimal death in normal fibroblast cells |
| Lai, 2019<sup>11</sup> | GSH | Pancreatic cancer | Improving stability and circulation time | 430/596 nm | NGF p75RNA | Adsorbing via electrostatic interaction | Vein loading capacity of 226 pmol p75RNA per 60G | Target tumor neuron interaction by silencing NGF gene successfully |
| Hebbel, 2021<sup>12</sup> | PEG-PIR-PMAM cor-crosslinked polymer/ meso-polymeric | Breast Cancer | Triggered drug delivery by NIR light activation | 550/720 nm | DOX-SH | Au-S bonds | Highly localized cytotoxicity on MDA-MB-231 cells upon 850 nm light activation |
| Chen, 2021<sup>13</sup> | BSA-GPIS | Folic acid | Cervical cancer | Fluorescent imaging | 686/620 nm | Camptothecin | Encapsulated | Empty nanocarriers had mild cytotoxicity in HeLa cells |
| Cossrot, 2021<sup>14</sup> | BSA | Ovarian and breast cancer | Fluorescent imaging | 77/645 nm | Gamitobolin and doxorubicin | pH sensitive | Menopausal silica nanoparticles | Accumulated in kidney and liver |
| Muthus, 2015<sup>15</sup> | TPPG mouse | Breast cancer | Fluorescent imaging | 365/620 nm | Doxetaxel | Encapsulated in liposomolm | | Targeted tumor neuron interaction by silencing NGF gene successfully |
| Jiang, 2020<sup>16</sup> | GSH | Breast cancer | Photothermal therapy | | | | | | |
| Chen, 2020<sup>17</sup> | L-Resorcinol | Cyclo-RGD and A549 cell line | Malignant gliomas | Drug delivery | Duocarmycin | Covalent bond between amine DOX activated carbazole group of histidine | Indocyanine green-NIR dye | Near-infrared fluorescent dye imaging | The AuNPs managed to completely eradicate tumor xenografts |
| Jiang, 2020<sup>18</sup> | GSH | Breast cancer | Drug delivery, enhance photothermal therapy, improve stability and biocompatibility | 76/285 (KCI) | Indocyanine green | Covalent bond via NH2-NH2 coupling between GSH and RGD | In vivo 808 nm NIR laser, 0.8 W/cm<sup>2</sup> power density for 8 minutes | 16 days after PTT, breast cancer tumors disappeared for mice treated with ICG–GSH–Free KCI and PBS in combination with PTT showed to therapeutic efficacy |
| Luo, 2019<sup>19</sup> | CT | PSM4 | Prostate cancer | Fluorescent imaging, CT imaging and Radiotherapy | 490/790 nm | | | Functional in PSA positive and PSA negative tumors |
| Ali, 2019<sup>20</sup> | CT | Polymeric micelle | Fibrosarcoma | Fluorescent imaging and CT imaging and radiotherapy | | | | 6 Gy X-ray radiation |
| Wang, 2020<sup>21</sup> | PTT | Liver cancer | Fluorescent imaging and Act as a 3-F drug | 490 nm | | | | In situ biosynthesis after injection of HuaK24 and PTT in DNA |
| Gondimara, 2020<sup>22</sup> | BSA and curcumin | Cervical cancer | Fluorescent imaging and drug delivery | 550/650 nm | Curcumin Capping ligand | | | No cytotoxicity in mortal cell lines, high lethality in human cervical cancer cells |
| Fu, 2018<sup>23</sup> | BSA | Neuroblastoma | Fluorescent imaging and drug delivery | 510/634 nm | Curcumin | Encapsulated | Higher intratumor efficacy compared to free curcumin or BSA–AuNCs alone |
| Zhu, 2018<sup>24</sup> | BSA | Folic acid | Metastasized breast cancer | 415/820 nm | Cleptatin | Radiosensitive | | Did not affect normal healthy organs |
| Khodadadi, 2017<sup>25</sup> | BSA | Cervical cancer | Fluorescent imaging and drug delivery | 505/655 nm | Doxorubicin | Electrostatic interactions and hydrogen bonding | Encapsulation efficiency 83.0% | Lower cytotoxicity than free DOX, possibly due to incomplete release |
| Kumar, 2018<sup>26</sup> | Thio - mesoporous silica | Tamarind resistant breast cancer | Fluorescent imaging and drug delivery | 530/510 nm | Resovist and Vancoutre | Adsorption bound to Au, RGD and encapsulated silica | Inhibition of EGFR, VEGFR and AKT pathway | Tumor growth was slowed down |
| Wang, 2019<sup>27</sup> | PEG | Liver cancer | Fluorescent imaging, act as a drug | Confirmed in vivo | | | | In situ biosynthesis after injection of HuaK24 after 30 days reduced tumor growth, reducing signals in PEG–NPX-pathway |
| Chen, 2018<sup>28</sup> | PEG-DOX- Ovalbumin | Breast cancer | Fluorescent imaging and Photodynamic therapy | 300/650–650 nm | | | | Due to the CAT functionality, hypoxia was alleviated, PTT to possible |
| Zhang, 2019<sup>29</sup> | GSH | Liposomal | Lung cancer | Fluorescent imaging and Photodynamic therapy | 58/S/57 nm | S-Fluorescein | Light/energy triggered | Near-infrared light radiation for 20 minutes | Conjugation to graphene, also PTT at 618 nm |
| Liang, 2019<sup>30</sup> | RGD peptide | RGD-peptide | Breast cancer | Fluorescent imaging, CT imaging and Radiotherapy | 688/600 nm | | | 6 Gy X-ray radiation at 100 kVp |
| Li, 2020<sup>31</sup> | Keratin | Breast cancer | Fluorescent imaging, MRI, drug delivery | 52/470 nm | Doxorubicin | Radiosensitive | Gadolinium and silver ions for MRI and enhanced Fluorescence | Significant reduction of tumor growth, enhanced fluorescent intensity, biocompatibility and colloidal stability, NIR Fluorescence |
| Fernandez, 2019<sup>32</sup> | Zwitterionic ligand | Vaccines | DC maturation | | | | | | Zwitterionic AuNPs cause T helper 1 regulatory cell responses in the form of cytokines, while not leading to proliferation of DC cells and T cell |
| Tao, 2018<sup>33</sup> | Gal-ODN-Ovalbumin | Vaccines | Fluorescent imaging, delivery of antigen and adjuvant | 490/595 nm | | | | Secretion of TGF-α and IL-4,6-kinase, gold nanomaterials cause maturation of APCs. Mice developed enhanced anti-ovalbumin IgG response |
| Tao, 2018<sup>34</sup> | Thiolated Gal-ODN-Ovalbumin peptide | Vaccines | Fluorescent imaging, delivery of antigen and adjuvant | 500/400 nm | | | | Gal-ODN-AuNPs can promote cross-presentatin by simultaneous delivery of antigen and adjuvant |
| Wang, 2018<sup>35</sup> | HEV | Vaccines | Fluorescent imaging, improve immune response and safety profile | 365/410 nm | | | | Improved safety profile and immunotherapeutic efficacy in vivo by influencing a PTL7/N2 response |
temperature between 15 and 45 °C. They showed a temperature resolution between 0.1 and 0.3 °C in phosphate-buffered saline (PBS), and between 0.3 and 0.5 °C in HeLa cells (Figure 2). Even temperature differences between subcellular locations could be identified. Similar results were reported for glutathione-capped AuNCs by Zhang et al. in 2019, who showed a temperature resolution of 0.73 °C in hepatic stellate cells within a temperature range from 35 to 43 °C. Also, PAMAM-protected AuNCs demonstrated possible use as nanothermometers. The described AuNC-based temperature probes compare well to already existing fluorescence-based nanothermometers, which present temperature resolutions between 0.1 and 2 °C, with a few exceptions between 0.001 and 0.01 °C. Of these, only a few temperature probes that employ NIR fluorescence to limit interference from autofluorescence of biological samples have been investigated. Green fluorescent protein (GFP) can also serve as a temperature probe and was used to accurately determine the temperature in GFP-transfected HeLa cells with a resolution of 0.4 °C.7

The PAMAM-protected AuNCs could also be used for the intracellular sensing of Cr2O7^2− (dichromate), owing to the fluorescence quenching effects at room temperature in the presence of trace amounts of this ion, with a limit of detection (LOD) of 1.9 μM. AuNCs have been studied quite often for the application of sensitive probes for biosensing. Keratin-Ag-AuNCs have a sensing ability for the heavy metal mercury(II). The LOD was found to be 2.31 nM, showing that these keratin-protected, silver-modified AuNCs are sensitive enough for the detection of mercury in tap water.19 In addition, complex samples the fluorescence quenching effects with increasing mercury concentration were difficult to measure. Yet, it was eventually manageable in fish samples.13 GSH-capped AuNCs turned out to be sensitive but not selective to mercury, lead, and copper ions. Besides heavy metals and inorganic ions, the concentrations of small biomolecules and proteins can also be determined by making use of the fluorescence properties of AuNCs. Chicken egg ovalbumin-based AuNCs were employed for biosensing phosphate-containing biomolecules, such as ATP and pyrophosphate. Glucose-oxidase-functionalized AuNCs were able to sense glucose with a lower detection limit of 0.7 μM.19 Trypsin-stabilized AuNCs, as described by Liu et al. in 2013,47 were able to sense heparin in human serum samples with a linear range between 0.1 and 4.0 μg/mL and a LOD of 0.05 μg/mL. AuNCs can also be used for the detection and quantification of cancer biomarkers such as neuron-specific enolase, dopamine in the cerebrospinal fluid, interleukin-6, and protein kinase A, among others.13 In an additional application, Colombé et al. investigated whether AuNCs could be used for image-guided surgery in mice. In cancers such as head and neck squamous cell carcinoma (HNSCC), tumor resection is difficult due to many complex structures in this area that should not be damaged, such as nerves, tendons, and small muscles. By making use of real-time image-guided surgery, these structures may be preserved while allowing for complete tumor excision with good margins. NIR fluorescence image-guided surgery is a method that has been validated in mice. Because NIR fluorescence provides good optical contrast between healthy and cancer tissue in the case of tumor-targeting fluorescent probes, the probability of efficient tumor resection is improved. Currently, NIR image-guided surgery with the help of fluorophores is under investigation in clinical trials. However, the use of AuNCs as an imaging agent is still in the preclinical phase. It was found that NIR image-guided surgery using zwitterionic or pegylated moieties as capping
ligands on AuNCs increases the survival time compared to control animals without image-guidance, as well as the number of mice without any local recurrent tumors due to better detection of tumor residues.44

3. AUNCS AS A TOOL IN THERAPY

The unique properties of AuNCs are not only useful for bioimaging and diagnostics, but they can also be employed in advanced therapeutic strategies against cancer. AuNCs can assist in radiotherapy or thermal therapy, or facilitate drug delivery.78−80 Drugs may be encapsulated together with the AuNCs in protein or polymeric scaffolds or can be covalently bound to the capping ligand itself. The therapeutic uses of AuNCs will be discussed in the following section including photothermal and photodynamic therapies, electromagnetic and radiotherapy, and drug delivery. The relevant published studies that employ AuNCs for the purpose of therapy are summarized in Table 3.

Photothermal and Photodynamic Therapy. Photothermal therapy (PTT) is a form of phototherapy that is often applied in the treatment of cancer when tumors cannot be removed by surgery.71,72 In PTT, the destruction of cancer cells is achieved via the induction of hyperthermia, by raising the tumor temperature to 41−47 °C for tens of minutes.71 By denaturing intracellular proteins and destroying cellular membranes, the tumor cells are killed via apoptosis or necrosis.73 PTT involves a photothermal agent that is injected in the body either locally or by IV administration. Upon excitation of the photothermal agent, typically by NIR light,74 photoenergy is converted into thermal energy within the cells that have taken up the photothermal agent.72 Tumor cells, particularly in the center of the tumor, are more susceptible to heat than healthy cells,75 improving the selectivity of photothermal therapy. As opposed to bioimaging agents, the ideal photothermal agent has a low fluorescence QY, to obtain an optimal conversion of radiation into heat instead of fluorescence emission.76

Photodynamic therapy (PDT) is a process that uses a photosensitizer, typically with visible light activation, instead of a photothermal agent. Upon activation, the photosensitizers start generating reactive oxygen species (ROS), thereby eliciting phototoxicity.77 Advantages that both PTT and PDT have are that they are relatively selective for cancer tissue because of the locally applied light. However, when using either strategy on its own, there are some disadvantages. In PDT there is limited tissue penetration of visible light. Furthermore, singlet oxygen (1O2), a reactive oxygen species among others that is released upon activation of the photosensitizers, is not always lethal for a whole tumor, owing in part to limited diffusion.76,78 It is therefore important that the PDT platform is sufficiently small to ensure complete coverage of the tumor. Since the tumor microenvironment is often hypoxic and oxygen is crucial for the effectiveness of PDT, this also creates challenges.79 In PTT, there is the problem of limited photothermal conversion efficiency80 and the development of thermotolerance.71 Because of this, the therapies often need to be combined in order to reduce the risk of relapse or recurring cancer.

Since PTT and PDT work via a different mechanism, combined therapy needs both visible and NIR light activation and different drugs to obtain the desired results.72 This combination adds complexity and reduces its usability in the clinic. However, AuNCs may provide an opportunity to combine PTT and PDT in one platform, only requiring a single wavelength for light activation. This was shown by Liu and colleagues in 2019, who reported captopril-stabilized AuNCs that could be used for combined PDT and PTT with near-infrared light activation at a wavelength of 808 nm.72 They showed a photothermal conversion of 41.1%, laying a strong foundation for promoting the use of AuNCs for PTT. Furthermore, these AuNCs were able to generate enough singlet oxygen for efficient PDT. These results were confirmed in vivo by treating cutaneous squamous cell carcinoma tumor-bearing mice with either intratumoral injection of captopril-capped AuNCs combined with light treatment or light treatment alone. It was found that the temperature within the tumor increased by 28.1 ± 6.8 °C in the mice treated with AuNCs compared to 8.4 ± 2.1 °C in mice receiving laser treatment only.72 Whereas the tumor volumes for all control mice increased, the tumor volumes for the mice that received AuNC treatment decreased significantly. The contributions of PDT and PTT in killing tumor cells in vivo was estimated (by use of an ROS scavenger to quench the effect of PDT) to be around 71% and 29%, respectively.72

In the literature, there have also been reports on AuNCs designed to reduce hypoxia. An example of this is the reported amine terminated PAMAM dendrimer-encapsulated AuNCs (NH2−PAMAM−AuNCs), which have the intrinsic ability to produce O2 for PDT via catalase-like activity over a broad pH range (see Scheme 3).30 Because of the extra oxygen present in tumor tissue, the enhanced PDT efficacy was statistically significant. However, the AuNCs themselves were not used for PDT. Instead, an established photosensitizer (protoporphyrin IX) was used. Still, the notion that NH2−PAMAM−AuNCs

Scheme 3. Schematic Illustration of (A) the Enzyme-Like Activities of NH2−PAMAM−AuNCs, Which Can Catalyze H2O2 to Produce O2 via Their Catalase-Like Activity and (B) a Simple Strategy of Conventional PDT Combined with Self-Supplied O2 via the Catalase-Like Activity of NH2−PAMAM−AuNCs, Resulting in an Increase of 'O2 and O2− Generation"
have the ability to alleviate hypoxic conditions could be interesting for future research.80

Radiation and Electromagnetic Therapy. Nowadays, one of the leading therapeutic options for treating cancer is radiotherapy.81,82 Radiotherapy kills tumor cells via treatment with high energy radiation, typically megavolt X-ray or gamma ray radiation with a dose between 3 and 6 Gy.81,83 While it is generally very effective, one of the main setbacks of this treatment option is that it can also cause serious damage to the healthy tissues surrounding the tumor site.81 When using a radiosensitizer, the efficacy of a radiation dose is increased.81 This way, a lower radiation dose can be used for the therapy, one that is relatively safe to healthy cells that have not taken up the radiosensitizer. Radiosensitizers also enhance the outcome of radiation therapy, even when tumor cells are radioresistant (e.g., hypoxic).84 When a radiosensitizer is irradiated with X-rays, secondary effects are generated, for example, scattered photons, electrons, electron–positron pairs, or fluorescence. These secondary effects can then aid in destroying cells.84 Gold is an especially good radiosensitizer, considering its large atomic number and its therefore high absorbance of radiation, which leads to an enhancement of radiotherapy of up to a 100-fold.85 While it is generally very effective, one of the main setbacks of this treatment option is that it can also cause serious damage to the healthy tissues surrounding the tumor site.81 When using a radiosensitizer, the efficacy of a radiation dose is increased.81

AuNCs, however, had no significant reduction in tumor growth after treatment compared to their control, showing a difference with radiation alone of around 10%.83 Ghahremani et al. investigated BSA-AuNCs for the purpose of megavoltage radiation therapy of breast cancer cells.86 Using an AS1411 aptamer moiety conjugated to the BSA-AuNCs as a targeting agent for nucleolin, they were able to efficiently target these cancer cells. In vitro it was found that the combination of the Aptamer-BSA-AuNCs with megavoltage radiation therapy (between 6 and 25 MV) led to efficient cancer cell death, enhanced by the AuNCs with a factor of 2.7 compared to controls.86 Besides that, Cifuentes-Rius et al. found that BSA-AuNCs can also be applied in electromagnetic radiation therapy.87 Upon 8 min light activation with 15 W microwaves (1 GHz electromagnetic fields), cell viability decreased in six types of mammalian cell lines. At a gold concentration of 50 μg/mL, approximately 50% of the B-lymphocytes, 68% of prostate cancer cells, and 28% of neuroblastoma cells died via induction of apoptosis and necrosis.87

AuNCs as a Platform for Drug Delivery. AuNCs have been reported to act as a tool for drug delivery itself, primarily without light activation. For example, the natural flavonoid kaempferol was conjugated to BSA-protected AuNCs via physical interactions and tested for its anticancer properties in lung cancer cells.88 Flavonoids are well-known for their antioxidant activity and can possibly be used for the treatment of cancer,89–91 microbial infection, and angiogenesis, among others.92 While showing little to no cytotoxicity in healthy human kidney cells, kaempferol-BSA-AuNCs were able to kill over 50% of the cancer cells at a concentration of 2.5 μg/mL in vitro. Additionally, the kaempferol-BSA-AuNCs were shown to slow down the migration rate of HeLa cells. Although the purpose of the AuNCs within the drug delivery system was to provide imaging possibilities (λex/λem = 550/650 nm), no cell imaging experiments have been reported.92

In a similar manner, Lakshmi et al. synthesized a flavonoid based drug delivery system, using quercetin as drug conjugate.93 The quercetin was bound to BSA-AuNCs via Au–OH interactions and showed good cellular uptake. The intention of the AuNCs was to use them for their fluorescent bioimaging properties (λex/λem = 360/568 nm), but since these results were not reported on cellular experiments, the quercetin-BSA-AuNCs are not considered theranostic here. They did show high cytotoxicity in lung cancer cells, whereas minimal cell death occurred in healthy fibroblasts.91

There has also been research for a drug carrier using AuNCs that focuses on controlled drug release. Latorre et al. published an article in 2019 where BSA-AuNCs were investigated as nanocarriers for combined chemotherapy against cancer, targeting mainly cancer stem cells.92 To this end, they functionalized the BSA-AuNCs with both doxorubicin (DOX) and a camptothecin analogue SN38 to inhibit topoisomerase II and I in target cells. Herein, the AuNCs serve only as a structural scaffold. Although the results in clinical trials for the combination of free DOX and SN38 have not been satisfactory, it has previously been shown to be one of the most synergistic combinations of chemotherapy when injected as a polymer–drug conjugate.93 Thiols were introduced to the BSA by reacting the BSA-AuNCs with 2-iminothiolane. SN38 was then coupled to BSA with a redox-sensitive linker that is cleaved in a reducing environment, which contains, for example, a relatively high concentration of glutathione. DOX was modified with a pH-sensitive linker that breaks in a slightly acidic environment, as is the case in endosomes and lysosomes. The disulfide bond and maleimide in the linkers enabled conjugation to the thiols of BSA. A schematic overview of the synthesis and the modified chemotherapeutics is depicted in Scheme 4.92 In vitro toxicity studies in MCF7, MDA-MB-231, and Panc-1 cells showed that the BSA-AuNCs with both chemotherapeutics exhibited enhanced cytotoxicity compared to BSA-AuNCs with only one of the drugs. These bifunctionalized AuNCs were shown to induce highly efficient DNA damage, allowing their effective use against cancer stem cells by significantly reducing the size and number of mammospheres at concentrations as low as 0.08 μM.92

AuNCs have not only been investigated as a drug carrier in vitro and in vivo. El-Mageed et al. found in silico that AuNCs have the ability to act as a drug delivery system for D-penicillamine in cancer treatment.94 This research focused mostly on modeling the potential interactions and bonding energies between D-penicillamine and gold. It was found that the drug would be coupled to the gold core mainly via physical interactions. Examples of these are the Au–O/S/N bonds, hydrogen bonds, and electrostatic bonds.94

Recently, Hebels et al. reported a first-in-class platform employing AuNCs for light induced tumor cell killing.95 The AuNCs were formed into a stabilized core-cross-linked micelle
Scheme 4. (A) Schematic Overview of Synthesis of DOX and Camptothecin SN38 Functionalized BSA-AuNCs (Depicted as Coils with Small Yellow Circles in the Center). (B) DOX (red) Modified with pH-Sensitive Linker (Green) and SN38 (Blue) Modified with a Redox-Sensitive Linker (Pink). 

**Reproduced with permission from ref 92. Copyright (2019), Multidisciplinary Digital Publishing Institute.**

A mild cytotoxicity in HeLa cells, which was significantly enhanced when the nanocarriers were loaded with camptothecin by encapsulation. Imaging was performed in vitro by confocal scanning microscopy on HeLa cells (λ<sub>ex</sub> = 496 nm). 

A good example of a theranostic approach in drug delivery and cancer bioimaging, where AuNCs are solely used for their fluorescent properties, was developed by Muthu et al. in 2015. They designed a vitamin E tocopheryl polyethylene glycol 1000 succinate (TPGS) micelle conjugated with transferring for transferring-targeted codelivery of the drug docetaxel and fluorescent AuNCs. Docetaxel and the AuNCs were encapsulated in the lipophilic core of the micelle. The system exhibited cytotoxic properties in transferring receptor overexpressing breast cancer cells, and the micelles emitted fluorescence (λ<sub>ex</sub>/λ<sub>em</sub> = 365/620 nm) in vitro. The biodistribution proved to be satisfactory with a good clearance, where the transferring-targeted micelles reached an IC50 value 72-fold lower than that of the FDA-approved docetaxel formulation. 

Croissant et al. managed to develop a nanocarrier system that encapsulated both gemcitabine and DOX in a mesoporous silica nanoparticle containing BSA-AuNCs for the treatment of ovarian and breast cancers. In this theranostic system, the AuNCs were not involved in the encapsulation of the drugs. Gemcitabine and DOX were both immobilized with an acidi-sensitive linker and released with a pH trigger that resulted in almost complete killing of cancer cells. The biodistribution of the entire system was investigated by imaging (λ<sub>em</sub> = 600 nm) in vivo, showing good tumor targeting efficiency. 

**Theranostic Systems Employing AuNCs as a Tool for Therapy.** An approach to a dual-targeting theranostic platform was looked into by Chen et al. in 2016. 1-Histidine-capped AuNCs were coupled to cyclic RGD for extracellular targeting, and to the aptamer AS1411 for nuclear targeting. DOX was then immobilized onto the nanocarrier by forming a covalent bond between the primary amine on DOX and the activated carbonyl group of histidine. This formed a drug-delivery system with a high tumor cell affinity. However, a distinct drug release profile has not been reported. Still, cancer cell inhibition occurred in vitro as well as in vivo. Using the quadrupolar anthracene-based near-infrared dye MPA, the complex showed potential toward bioimaging applications. 

AuNCs can also be applied in formulations for the delivery of biological drugs. Lei et al. reported the synthesis of GSH-oligoarginine-capped AuNCs as a nanocarrier for delivery of nerve growth factor (NGF) small interfering RNA (siRNA) in pancreatic cancer. The use of AuNCs to assist in the delivery of siRNA was shown to be beneficial, considering that the gold increased the stability of siRNA in serum, as well as the circulation time, cellular uptake, and tumor accumulation in vivo. With the help of the Cy5 NIR dye, the uptake in cells was visualized by fluorescence. In an in vivo subcutaneous model, the average tumor growth was reduced by 52% compared to saline control. In the orthotopic pancreatic cancer model in Balb/c nude mice, it was shown that the designed formulation decreased tumor sizes compared to saline controls while also showing a low expression level for NGF mRNA and NGF protein. The aim to target tumor–neuron interaction by silencing the NGF gene in pancreatic cancer to inhibit progression was therefore fulfilled. 

Jiang et al. demonstrated that GSH-AuNCs coupled to the fluorescent dye indocyanine green (ICG) via amide coupling could enable a switchable fluorescence and enhance the...
photothermal efficacy of free ICG (see Scheme 5).\textsuperscript{100} When coupled to the GSH-AuNCs, the fluorescence of the ICG was

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{Scheme5.png}
\caption{Schematic Representation of ICG-GSH-AuNC Mediated Photothermal Cancer Therapy and Their In Vivo Clearance Pathways after Dissociation in the Liver\textsuperscript{a}}
\end{scheme}

\textsuperscript{a}Reproduced from ref 100. Copyright (2020), American Chemical Society.

almost completely quenched. However, it was instantaneously recovered once ICG was released (\(\lambda_{\text{ex}}/\lambda_{\text{em}}\text{ ICG} = 760/825\) nm). The gold itself does not show any fluorescence at this excitation wavelength. It was found that the photochemical stability of ICG increased due to conjugation with the GSH-AuNCs. During a 15 min light activation in vitro, a rapid increase in temperature of approximately 20 °C was seen, with minimal decay. By contrast, free ICG and free GSH-AuNCs only exhibited a slight temperature increase, of approximately 10 and 5 °C, respectively. ICG-GSH-AuNCs also showed reduced inherent cytotoxicity compared to free ICG, suggesting that the efficient tumor killing is primarily achieved through PTT. In vivo, mice were irradiated with the laser for 8 min at a power density of 0.8 W/cm\(^2\), showing similar results to in vitro studies. After PTT with ICG-GSH-AuNCs, the breast cancer tumors disappeared within 2 weeks, whereas PTT with free ICG or PBS displayed no therapeutic efficacy. This might partially be because the AuNCs prolong the blood circulation and enhance tumor targeting, as well as increase the photothermal performance compared to free ICG.\textsuperscript{100}

**Theranostic Systems Employing AuNCs for Imaging and Therapy.** AuNCs have often been described for the simultaneous imaging and delivery of chemotherapeutic drugs. An example is described in the study of Zhou et al.\textsuperscript{101} In this work, cisplatin is delivered as a produg conjugated to folic acid functionalized BSA-AuNCs to metastasised breast cancer. The drug release for this chemotherapy was based on a redox sensitive linker that coupled the cisplatin to the AuNCs. The AuNCs were then tested for their fluorescence (\(\lambda_{\text{ex}}/\lambda_{\text{em}} = 415/670\) nm) and biodistribution in vivo. Here, the AuNCs showed good tumor targeting efficiency with inhibition of growth and lung metastasis of 4T1 tumors, while avoiding accumulation in healthy organs.\textsuperscript{101}

Khandela et al. also investigated BSA-capped AuNCs, but for the delivery of DOX with concurrent single-photon or two-photon imaging of cancer cells.\textsuperscript{102} They determined that the emission by two-photon fluorescence falls within the NIR range (\(\lambda_{\text{ex}}/\lambda_{\text{em}} = 505/655\) nm), thereby showing promise for in vivo imaging. They also tested the cytotoxicity of the empty BSA-AuNCs and the release of DOX to human cervical cancer cells in vitro. It was found that the BSA-AuNCs had no inherent cytotoxicity. When DOX was implemented, an IC\(_{50}\) of 6.3 μg/mL of DOX was determined, which is less toxic than free DOX (IC\(_{50}\) = 0.82 μg/mL). This could possibly be explained by incomplete release of DOX over the 36 h incubation time.

By inhibiting the EGFR, VEGFR, and AKT signaling pathways using the dual drugs vandetanib and epigallocatechin gallate (EGCG), Kumar et al. showed a way to circumvent tamoxifen-resistance in breast cancer cells.\textsuperscript{103} They designed a mesoporous silica drug delivery system that encapsulates EGCG. The silica particles are then modified to present thios at their surface, followed by covalent bonding of AuNCs via gold–thiol interactions. Vandetanib was then coupled to the AuNCs to form the complete nanocarrier. Using the fluorescence of the AuNCs (\(\lambda_{\text{ex}}/\lambda_{\text{em}} = 530/670\) nm), the nanocarrier could be localized intracellularly. Tumour growth was delayed in vivo by the inhibition of EGFR, VEGFR, and AKT. Considering that these proteins are overexpressed in tamoxifen-resistant cancer cells, inhibiting them may sensitize the tumor cells to tamoxifen chemotherapy.\textsuperscript{103}

The flavonoid curcumin\textsuperscript{89,90} has been investigated as a drug-conjugate in theranostic systems as well. Curcumin-capped AuNCs were brightly fluorescent (\(\lambda_{\text{ex}}/\lambda_{\text{em}} = 550/650\) nm). Furthermore, curcumin-AuNCs showed almost no toxicity in mortal cell lines (~100% cell survival) compared to a high lethality in human cervical cancer cells (~15% cell survival) at a concentration of 100 μg/mL. The drug delivery system also caused a reduction of the HeLa cell migration rate.\textsuperscript{89} In another study on curcumin-BSA-AuNCs,\textsuperscript{90} the nanocarriers exhibited a higher inhibition efficiency in neuroblastoma tumor cell growth than free curcumin or BSA-AuNCs alone, with an IC\(_{50}\) value of 14.3 nM. In fact, there appeared to be a synergistic induction of cellular apoptosis for this particular nanocarrier system.\textsuperscript{90}

Wang et al. found that when the phosphatase and tensin homologue (PTEN) tumor suppressor gene is conjugated to AuNCs, the complex can be used to inhibit liver tumor growth as well as fluorescent imaging.\textsuperscript{104} Using the low pH and high concentration of reductive substances (e.g., GSH and NADP\(^+\)) in the tumor microenvironment, PTEN-AuNCs were synthesized in situ by injecting the AuNC precursor and PTEN DNA. It was subsequently found that the PTEN-AuNC complexes can inhibit or even prevent liver tumor proliferation, invasion, and metastasis in vitro. Additionally, cancer growth was inhibited significantly in mice, and the imaging possibilities of the complex were also shown.\textsuperscript{104}

Chen et al. investigated HSA and catalase comodified alkyl thiolated AuNCs (AuNC-HSA/CAT) in photodynamic therapy combined with fluorescent imaging (\(\lambda_{\text{ex}}/\lambda_{\text{em}} = 365/600–650\) nm).\textsuperscript{79} Using the NIR-II region for light activation with a wavelength of 1064 nm for 20 min, they showed that PDT utilizing AuNCs is indeed possible at this long wavelength. Because the CAT moiety is also attached to the AuNCs, the problem of hypoxia in the tumors was alleviated. This was illustrated by a significant tumor volume reduction
Radiation therapy was also explored as a modality in theranostic AuNCs by Liang et al. They proposed to use RGD peptide-modified AuNCs as tumor-targeting radiotherapy enhancers (see Scheme 6).\textsuperscript{81} With this particular targeting moiety, the αβ3 integrin-positive cancer cells can be stained and then killed by radiotherapy with a higher efficacy compared to radiation alone. For the mice treated with the RGD peptide-modified AuNCs and radiotherapy, the tumor volume only increased by 30% over a period of 14 days, compared to an increase of over 130% for radiation alone. The RGD peptide-modified AuNCs were shown to have inherent fluorescent properties (λ\textsubscript{ex}/λ\textsubscript{em} = 488/660 nm), as well as CT imaging capabilities.\textsuperscript{81}

In 2019, Luo et al. described cysteine-tyrosine-prostate specific membrane antigen-targeted AuNCs (CY-PSMA-AuNCs) as radiosensitizers for therapy of prostate cancer.\textsuperscript{84} Besides radiosensitization, the CY-PSMA-AuNCs also had in vitro fluorescent (λ\textsubscript{ex}/λ\textsubscript{em} = 490/700 nm) and in vivo CT properties. Fast elimination of the CY-PSMA-AuNCs from the mice via renal clearance was observed during the biodistribution studies. In vivo testing showed that the tumor growth was inhibited much better in mice bearing PSMA over-expressing tumors as compared to negative tumors after IV injection. In both cases, 18 days after being given a radiation dose of 6 Gy, the tumor size increased by only 94% and 311%, respectively. Compared to the control mice with PBS injections plus radiation, who exhibited a tumor growth of respectively. Compared to the control mice with PBS injections plus radiation, who exhibited a tumor growth of 430%, these results show that the AuNCs indeed act as radiosensitizers and by functionalization with PSMA show potential for active targeting applications.\textsuperscript{84}

Al Zaki et al. synthesized polymeric micelles loaded with AuNCs for the application of CT-guided radiation therapy, where the AuNCs act as radiosensitizers.\textsuperscript{40} The micelles, consisting of an amphiphilic diblock polymer poly(ethylene glycol)-b-poly(ε-caprolactone), contained tightly packed 1.9-nm-sized AuNCs (GPMs). It was determined in vivo that following IV injection, the GPMs served as imaging contrast agents for CT imaging, which were used for better visualization of the tumor boundaries. The GPMs exhibited a radiosensitization enhancement ratio of approximately 1.2 in vitro, while a statistically significant improved survival rate was observed in tumor-bearing mice treated with GPMs compared to radiotherapy without GPMs.\textsuperscript{40}

Some theranostic platforms have been developed to incorporate imaging and multiple types of therapy together. An example of this is the AuNC containing vehicle designed by Yang et al. in 2019.\textsuperscript{105} GSH-capped AuNCs conjugated to graphene oxide functionalized with hyaluronic acid as targeting agent were synthesized for fluorescent image-guided synergistic delivery of 5-fluorouracil and phototherapy. Because of the inhibition of the fluorescence of the AuNCs in the presence of graphene oxide, controlled fluorescence turn-on imaging can be realized. Upon cleavage of the glycosidic linkages by hyaluronidase, the HA-GSH-AuNCs were released from the graphene oxide, leading to restoration of the fluorescence (λ\textsubscript{ex}/λ\textsubscript{em} = 580/675 nm). Subsequently, under light activation at 638 nm, photodynamic therapy could occur. Besides that, the light activation caused the loaded 5-fluorouracil to be released quickly and the graphene oxide to exhibit its photothermal properties. This leads to an enzyme and laser-controlled fluorescence, along with chemotherapeutic, photothermal, and photodynamic functionalities. In vitro cytotoxicity studies showed the efficacy of the triple therapy where 84.3% of the lung cancer cells died, which was significantly enhanced compared to the chemotherapy or phototherapy alone.\textsuperscript{105}

Li et al. designed the aforementioned (Table 1) keratin-templated AuNCs functionalized with silver and gadolinium ions.\textsuperscript{23} They showed an enhanced fluorescence intensity, biocompatibility, and colloid stability, and were able to provide in vivo fluorescence imaging (λ\textsubscript{ex}/λ\textsubscript{em} = 488/660 nm) and CT imaging properties. This leads to an enzyme and laser-controlled fluorescence turn-on imaging can be realized. Upon cleavage of the glycosidic linkages by hyaluronidase, the HA-GSH-AuNCs were released from the graphene oxide, leading to restoration of the fluorescence (λ\textsubscript{ex}/λ\textsubscript{em} = 580/675 nm). Subsequently, under light activation at 638 nm, photodynamic therapy could occur. Besides that, the light activation caused the loaded 5-fluorouracil to be released quickly and the graphene oxide to exhibit its photothermal properties. This leads to an enzyme and laser-controlled fluorescence, along with chemotherapeutic, photothermal, and photodynamic functionalities. In vitro cytotoxicity studies showed the efficacy of the triple therapy where 84.3% of the lung cancer cells died, which was significantly enhanced compared to the chemotherapy or phototherapy alone.\textsuperscript{105}
525/710 nm). Subsequently, under light activation at 638 nm, photodynamic therapy could occur. Besides that, by employing a redox-sensitive linker, DOX could be selectively released in cancer cells where the concentration of glutathione is high, at both neutral and low pH. In breast cancer bearing mice, the formulation achieved a significant reduction of tumor growth.23

Besides acting as sensitizers in radiotherapy, as agents in phototherapy, and drug delivery applications, AuNCs themselves can also show therapeutic efficacy. Wang et al. developed a therapy involving in situ biosynthesized AuNCs that could effectively slow down tumor progress by inhibiting the activity of the PI3K-AKT pathway.106 It was found that 24 h after a tail vein injection with HAuCl₄, 2.5-nm-sized AuNCs had formed in the liver tumor, while also showing that the AuNCs were preferentially formed at that site by measuring their intrinsic fluorescence. After 38 days, the tumors of the mice treated with the AuNCs exhibited reduced growth compared to mice treated with PBS injections. By performing RNA-sequence analysis it was determined that the expression of proteins targeted by the PI3K-AKT signaling pathway had decreased. This was further confirmed by real-time PCR and Western blots in vitro. Based on these results, the authors speculated that the inhibition of the PI3K-AKT pathway was the main cause of the observed reduction in tumor growth upon treatment with in situ biosynthesized AuNCs.106

5. AUNCs FOR OTHER THERAPEUTIC APPLICATIONS

AuNCs have not only been investigated for their unique properties in cancer bioimaging, therapy, and theranostic systems, but have also shown promise in other areas of biomedical research such as vaccine development and treatment or prevention of bacterial infection. Recent interest regarding the applicability of AuNCs in central nervous system (CNS) disorders has emerged as well. To provide an overview, the articles mentioned in the following section have also been summarized in Table 3.

AuNCs in Vaccine Development. Several research groups have investigated AuNCs for their immunological properties as well as their potential toward simultaneous fluorescence imaging.107–109 Fernández et al. studied the immunological properties of AuNCs in human dendritic cells (DCs), as well as their cellular uptake.107 Using the fluorescence intensity of the AuNCs, they found that the zwitterionic AuNCs were readily taken up by DCs, which subsequently triggered DC maturation. This was achieved to a lesser extent with PEGylated AuNCs and larger gold nanoparticles. Immunological analysis revealed that the zwitterionic AuNCs cause T helper 1 and T regulatory cell responses, while not leading to proliferation of natural killer cells and cytotoxic T cells. These results encourage the further investigation of AuNCs in vaccines.107

In 2014, Tao et al. had already looked into AuNCs as vaccines using dual-delivery of an antigen and an adjuvant, while the AuNCs simultaneously acted as an imaging agent.108 To this end, they conjugated the adjuvant cytosine-phosphate-guanine (CpG) oligodeoxynucleotides (ODNs) to the antigen ovalbumin and used this as a template for the synthesis of the AuNCs. Based on the secretion of immunostimulatory cytokines TNF-α and IL-6, it was determined that the AuNC containing system induced the maturation of APCs. It was also found that the concurrent delivery of the CpG ODNs and of ovalbumin enhanced cellular immunity. Besides that, the conjugation of CpG ODN and ovalbumin to the gold caused an increased stability and enhanced cellular uptake, further increasing immunostimulatory activities. The notion that the AuNCs could therefore be used as vaccine vehicle was then further confirmed in mice, which developed an enhanced antiovalbumin IgG response.

Wang et al. aimed to develop a vaccine against hepatitis E, by preparing AuNCs in situ within the monomers of the hepatitis E vaccine (HEVA).110 The presence of AuNCs caused a facile synthesis of HEVA aggregates (HEVA/Au), which possess high potency in provoking antibody responses compared to the single monomers. The inherent blue fluorescence of the HEVA/Au solution allowed for tracking of the vaccine aggregates in cells and in vivo (λex/λem = 365/410 nm, QY = 6%). Cell uptake experiments showed that the HEVA/Au was easily taken up by the liver and immune cells, where it was mainly present in the cytosol and lysosomal compartments. In vivo biodistribution studies showed accumulation of HEVA/Au in the liver, heart, kidney, lymph nodes, and spleen. Whereas HEVA was toxic at concentrations above 0.1 mg/mL, HEVA/Au showed no cytotoxicity at a concentration of 1 mg/mL, displaying its improved safety profile. Furthermore, the antibody immune response was enhanced by the HEVA/Au, by influencing the Th1/Th2 immune response in vivo.110

AuNCs in the Prevention and Treatment of Bacterial Infection. Phototherapy is not only useful in treating cancer but can also be applied for dispersing biofilms. In 2020, Xie et al. published a study that investigated the potential of DNase-functionalized AuNCs in eradicating bacteria that are shielded by biofilms.111 They reported that DNase can assist in enzymolysis, thereby breaking down the extracellular polymeric substance matrix, which subsequently exposes the bacteria to the AuNCs. PDT and PTT were then induced by 808 nm light activation. With a photothermal conversion of 6.6% and abundant ROS generation, the combination led to killing of approximately 90% of the biofilm-shielded bacteria. No additional photosensitizer was employed and the O₂ species detected originate from the DNase-functionalized AuNCs themselves.111

AuNCs have also been investigated as a bactericidal agent itself. Zheng et al. developed 6-mercaptohexanoic acid-protected AuNCs that could kill both Gram-positive (S. aureus, S. epidermidis, Bacillus subtilis) and Gram-negative (E. coli, Pseudomonas aeruginosa) bacteria owing to their small size when compared to gold nanoparticles.112 At a concentration of 0.1 mM on the basis of Au atoms, the AuNCs killed more than 90% bacteria within 2 h of incubation. It was found that the AuNCs exhibit an IC₅₀ against S. aureus comparable with widely used antibiotics such as ampicillin and penicillin.112

Jiang et al. also prepared quaternary ammonium-glutathione-capped AuNCs (QA-GSH-AuNCs) for the treatment of multidrug-resistant (MDR) Gram-positive bacteria, such as methicillin-resistant S. aureus (MRSA) and vancomycin-resistant Enterococci (VRE).113 The QA-GSH-AuNCs exhibited bright fluorescence that could be used for bacterial cell counting (λex/λem = 362/592 nm). Because of the positive charge of the capping ligand, the QA-GSH-AuNCs were able to penetrate the bacterial cell wall and damage it. This was followed by ROS formation and disruption of intracellular metabolic pathways, thereby killing the bacteria. By comparing the QA-GSH-AuNCs to commonly used antibiotics, it was found that the time-kill kinetics are similar, and that the dose-
dependent inhibition of *S. aureus* growth was like that of vancomycin. Interestingly, it was shown that the QA-GSH-AuNCs had a broader antibacterial spectrum than any of the tested established antibiotics (ampicillin, oxacillin, linezolid, and vancomycin), including for VRE and MRSA, without inducing drug resistance at sub-inhibitory levels. In vivo, the toxicity to healthy cells and elimination half-life (7.5 ± 2.1 h) was satisfactory. The QA-GSH-AuNCs were able to prevent death of mice infected with MRSA for 16 days at a concentration of 40 mg/kg, which was similar to the effective dose of vancomycin.118

As a follow-up study, Xie et al. aimed to use the QA-GSH-AuNCs for the prevention of oral biofilm formation, also called plaque, to reduce bacterial infection of teeth caused by Invisalign aligners.114 This was done by allowing the QA-GSH-AuNCs to adsorb onto the aligners to make an antibacterial coating against *S. mutans*. The QA-GSH-AuNCs have a minimal inhibitory concentration of 4 μg/mL *in vitro*, killing the bacteria via destruction of the membrane integrity. The QA-GSH-AuNCs showed negligible toxicity and inflammation in mice but were highly efficient in preventing the attachment and biofilm development of *S. mutans*, *S. aureus*, *S. epidermidis*, and their MDR counterparts. It was found that the *S. mutans* biofilms had 85% less biomass and 95% less cell viability on QA-GSH-AuNCs-coated aligners. The antibacterial activity of the coated aligners was shown to remain present for several cycles of use and after storage for three months. This approach could be extended to many other medical devices to reduce bacterial-induced oral diseases.114

Very recently, a modification of AuranoF, an FDA-approved gold(I)-complex with tetraacetylated thioglucose (Ac4GlcSH) and triethylphosphine (PEt3) ligands employed as anti-inflammatory aid in rheumatoid arthritis, was reported for use as a nanoantibiotic.115 Here, AuNCs (instead of gold(I)) were functionalized with mixed phosphine and glycolyl thiol ligands by ligand exchange of PPh3-capped AuNCs. This resulted in improved activity against MDR *P. aeruginosa* (up to 4-fold) while reducing cytotoxicity to human A549 cells (up to 24-fold) when compared to AuranoF, further highlighting the potential of AuNCs in antimicrobial applications.116

**AuNCs in Central Nervous System Disorders.** Another application of AuNCs that has gained recent interest, is their ability to cross the blood-brain-barrier (BBB) owing to their small size.116 Because of this, Xiao et al. explored the potential of dihydroloipic acid-capped AuNCs as probes for therapy in central nervous system (CNS) disorders, such as traumatic brain injury, stroke, Parkinson’s Disease (PD), and Alzheimer’s disease,116 by detecting neuroinflammation. They found that these AuNCs could effectively reduce proinflammatory processes in microglial BV2 cells *in vitro*, indicating that these dihydroloipic acid-capped AuNCs have potential to become a therapeutic agent in CNS disorders.116

Another example of AuNCs acting as a therapeutic agent in CNS disorders was reported by Gao et al in 2019.117 Based on their results, they suspect that N-isobutyryl-L-cysteine (L-NIBC) protected AuNCs can serve as a novel form of therapeutics for the treatment of PD. They found that *in vitro* the L-NIBC-AuNCs prevent the aggregation and fibril formation of α-Synuclein, while having a neuroprotective effect and improving behavioral disorders in a PD mouse model *in vivo* at a dose of 20 mg/kg.117

### 6. DISCUSSION

In the past two decades, the NIR fluorescence of AuNCs, its origin, and how to tune it have been thoroughly studied. Design strategies using proteins, peptides, or other biological molecules as structural scaffolds for the synthesis of AuNCs were developed to preserve the AuNCs’ attractive photoluminescent properties while increasing the biocompatibility. This paved the way for the use of AuNCs in NIR fluorescent bioimaging. AuNCs were also investigated for therapeutic applications, involving drug delivery, phototherapy, and radiotherapy, among others. In particular, a lot of research focused on combining imaging and therapy in a single platform. Although many different definitions exist of what a theranostic platform is, in this review AuNC-containing platforms are considered theranostic when the imaging and therapeutic properties have been tested in cellular experiments or *in vivo*.

Looking at the extensive research done on the topic of AuNCs as theranostic tools, particularly in cancer, numerous approaches have been investigated so far; yet, there has been no report of AuNCs being investigated in clinical trials. There is a chance that this is simply because AuNCs are a relatively new field of research, and not enough preclinical studies have been conducted yet. The earlier developed, larger gold nanoparticles have been under investigation in clinical trials for several years already.118 Considering the current drawbacks of gold nanoparticles, mainly caused by controversial and inconsistent outcomes *in vitro* and *in vivo*, one could argue for the superiority of AuNCs. Whereas some gold nanoparticles can exhibit disadvantages including toxicity, species-specific differences in biodistribution and physiological response, relatively large size, and RES organ accumulation,115 AuNCs presented so far show little to no inherent toxicity, good biocompatibility, satisfactory biodistribution, and renal clearance. Depending on the capping-ligand, the cellular uptake efficiency and clearance are either improved or decreased. In general, AuNCs accumulate well in cells via endocytic pathways.107 A set of experiments comparing the clearance of AuNCs and gold nanoparticles concluded that the size of the AuNCs is an advantage here.84 Using the same capping ligand, targeting agent, and amount of gold, the gold nanoparticles accumulated twice as much in the liver compared to the AuNCs.84 In addition to that, earlier obtained results from Tsvirkun et al. in 2018 stated that they found a reverse correlation between gold nanoparticle size and tumor uptake via CT imaging.119 From these studies, a careful conclusion could be drawn that the AuNCs can be superior to the larger gold nanoparticles for cancer therapy applications *in vivo* with regard to tumor uptake, toxicity, biodistribution, and clearance, which could contribute to improved treatment outcomes.

In other fields, AuNCs and gold nanoparticles compare well. With the appropriate surface modifications, gold nanoparticles too can cross the BBB.118 AuNCs have been found to do this because of their small size.116 They can also both be used for applications in drug delivery, active targeting, photothermal and photodynamic therapy (usually with conjugated photosensitizer), and CT imaging.118

Another feature of AuNCs is that they can be used for various applications such as the sensing of heavy metals, biological molecules, and intracellular temperature. Even in surgery, the AuNCs may be of use, for example, in robot-assisted fluorescence-guided surgery. Furthermore, the use of
AuNCs (among other metal nanoclusters) as bactericidal agents has also gained increasing interest.\textsuperscript{120} However, it is imperative to look at both sides of the coin, as there are limits to the use of AuNCs as diagnostic and therapeutic agents as well. One example is the limitations that arise from the AuNCs’ native fluorescence peaks being most commonly in the NIR range. Deep tissue imaging (imaging with a depth from millimeters to centimeters) requires imaging wavelengths between 650 and 900 nm, because then only little amounts of absorption from water and blood occur.\textsuperscript{121} Nevertheless, the window is not optimal either because of autofluorescence of tissues that cause some background noise. So, even though the tissue penetration of NIR-I and NIR-II light is better than that of visible light and UV-light, the imaging capability is not deeper than 1 or 2 cm.\textsuperscript{122} This is in contrast to other imaging techniques that are currently in use for the purpose of diagnostics. CT, MRI, and PET imaging have unlimited tissue penetration, but they each have their own shortcomings.\textsuperscript{123} Major limitations of CT are that it uses ionizing radiation and that is has a low soft tissue sensitivity. MRI has a high spatial resolution but has the disadvantage that the overall sensitivity is low. PET imaging, on the other hand, has excellent sensitivity, but is very costly and has a low spatial resolution.\textsuperscript{123} When applying NIR-fluorescent imaging alone, not enough functional information can be obtained. However, multimodal fluorescent imaging, where fluorescence imaging is combined with other imaging possibilities, has emerged as a promising tool for imaging with improved sensitivity and accuracy.\textsuperscript{123} Considering that AuNCs have already been researched for multimodal fluorescent imaging, the limitations of NIR light may be overcome.

Currently, indocyanine green is the only fluorescent dye approved by the FDA for clinical use. The IRDye 800CW has entered clinical trials conjugated to antibodies, thus as targeted tracers.\textsuperscript{123,124} These dyes have a slight advantage over NIR-fluorescent AuNCs in the way that they also emit light with a high intensity in the NIR-II window, providing a higher imaging contrast and even deeper tissue penetration.\textsuperscript{125} However, without coupling them to another functionality, they do not have the possibility for multimodal imaging.

Another, perhaps more important, aspect of AuNCs that may hinder their progress to human studies is that GSH-capped AuNCs may cause epigenetic modifications in healthy cells at non-cytotoxic levels.\textsuperscript{126} The notion that they have a direct effect on epigenetic processes could cause unwanted side-effects during diagnosis or treatment. Still, as far as our knowledge goes, it has only been reported once and should thus be investigated more in-depth before drawing conclusions. As illustrated in this review, various biomolecules (including peptides and proteins) as well as various other materials such as polymers may be employed as capping ligands for AuNCs. This provides opportunities for reducing toxicity, improving biocompatibility and targeting, which ultimately make AuNCs an attractive and versatile tool for biomedical applications.\textsuperscript{127,128} Furthermore, the increase in AuNC related publications over the last 15 years for imaging, sensing, and therapy speaks volumes toward the increased interest for AuNCs in biomedical applications, increasing confidence that clinical translation may not be too far off anymore.\textsuperscript{129−132}

7. FUTURE PERSPECTIVES

All in all, while promising research focused on enabling the use of the emergent properties of AuNCs that have been developed, translation of this laboratory knowledge to functional clinical technology will take time. To efficiently image cancer tissue, employing targeting ligands such as folic acid, hyaluronic acid, or the aptamer AS1411 on the surface has been shown to improve uptake in tumor cells by active targeting. By means of fluorescence imaging and CT imaging, tumors can then be diagnosed accurately. With only minor modifications to the surface chemistry, PET and MRI are also within reach. Ideally, the AuNC-containing system would be used for diagnosis first, before locally activating treatment. This would reduce side-effects while providing a highly efficient solution to inhibit tumor growth. In this regard, acid-, enzyme-, or redox-sensitive linkers can help pave the way toward achieving localized release. Drug-release or therapy triggered by external factors, such as local light activation with a (NIR-)laser, could also be a solution for this. Additionally, photothermal, photodynamic, and radiation therapy may also serve as alternative therapies in different combinations. For the application of photothermal therapy, a balance should be found between photoluminescence and photothermal conversion for optimal results. In the end, the most powerful theranostic approach would consist of both multimodal imaging and combination therapy. Perhaps one day, AuNCs will be the new golden standard in the diagnosis and treatment of cancer or other newly emerging fields of application in infectious diseases and neurological disorders.

### AUTHOR INFORMATION

#### Corresponding Author

Tina Vermonden — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Utrecht University, 3508 TB Utrecht, The Netherlands; orcid.org/0000-0002-6047-5900; Email: T.Vermonden@uu.nl

#### Authors

Sanne M. van de Looij — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Utrecht University, 3508 TB Utrecht, The Netherlands

Erik R. Hebels — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Utrecht University, 3508 TB Utrecht, The Netherlands

Martina Viola — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Utrecht University, 3508 TB Utrecht, The Netherlands

Mathew Hembury — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Utrecht University, 3508 TB Utrecht, The Netherlands

Sabrina Oliveira — Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life and Department of Biology, Cell Biology, Neurobiology and Biophysics, Faculty of Science, Utrecht University, 3508 TB Utrecht, The Netherlands; orcid.org/0000-0002-6011-2122

Complete contact information is available at: [https://pubs.acs.org/10.1021/acs.bioconjchem.1c00475](https://pubs.acs.org/10.1021/acs.bioconjchem.1c00475)

#### Author Contributions

*S.N. van de Looij and E.R. Hebels contributed equally to the work*

#### Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

The Netherlands Organization for Scientific Research (NWO/Aспета 015.009.038 and NWO/Industrial Doctorates NWA.ID.17.030) is acknowledged for funding.

ABBREVIATIONS LIST

AcSEMA, 2-(Aethylthio)ethyl methacrylate; APC, Antigen presenting cells; ATP, Adenosine triphosphate; AuNC, Gold nanocluster; BBB, Blood brain barrier; BSA, Bovine serum albumin; CAT, Catalase; CNS, Central nervous system; CGP, Cytosine-phosphate-guanine; CT, Computed tomography; CY, Cysteine; DC, Dendritic cells; DNA, Deoxyribose nucleic acid; DOX, Doxorubicin; DOX-SH, Doxorubicin-iminothiolane; EGCG, Epigallocatechin gallate; EGFPR, Epidermal growth factor receptor; EPR, Enhanced permeability and retention; FA, Folic acid; FDA, Food and Drug Administration; FLIM, Fluorescence lifetime imaging microscopy; GFP, Green fluorescent protein; GPM, Poly(ethylene glycol)-b-polyl(ε-caprolactone) micelles containing AuNCs; GPPS, Sulfated polysaccharide; GSH, Glutathione; HA, Hyaluronic acid; HEVA, Hepatitis E vaccine; HNSSC, Head and neck squamous cell carcinoma; HSA, Human serum albumin; IC50, Inhibiting concentration 50%; ICG, Indocyanine green; IgG, Immunoglobulin G; IL, Interleukin; IV, Intravenous; L-NIBC, N-isobutyryl-1-cysteine; LOD, Limit of detection; Lys, Lysozyme; MDR, Multidrug resistant; MMP2, Matrix metalloproteinase-2; MPA, Quadrupolar anthracene-based dye; MRI, Magnetic resonance imaging; MRS, Methicillin-resistant S. aureus; NADP+, Nicotinamide adenine dinucleotide phosphate; NGF, Nerve growth factor; NIR, Near infrared; NIR-II, Near-Infrared-Light Activatable Nanoparticles for Deep-Tissue-Penetrating Wireless Optogenetics. Adv. Healthcare Mater. 2019, 8 (6), 1801132.

REFERENCES

(1) Li, H.; Li, H.; Wan, A. Luminescence Gold Nanoclusters for Cancer Imaging and Therapy. Anal. Chem. 2012, 84, 2194−2194.
(2) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2019. CA-Cancer J. Clin. 2019, 69 (1), 7−34.
(3) Soper, S. A.; Rasosy, A. Cancer: A Global Concern That Demands New Detection Technologies. Analyst 2016, 141 (2), 367−370.
(4) Zhang, Q.; Yang, M.; Zhu, Y.; Mao, C. Metallic Nanoclusters for Cancer Imaging and Therapy. Curr. Med. Chem. 2018, 25, 1379.
(5) Zheng, Y.; Wu, J.; Jiang, H.; Wang, X. Gold Nanoclusters for Theranostic Applications. Coord. Chem. Rev. 2021, 431, 213689.
(6) Hembury, M.; Beztsinna, N.; Asadi, H.; Van Den Dikkenberg, J. B.; Meeldijk, J. D.; Hennink, W. E.; Vermonden, T. Luminescence Gold Nanocluster-Decorated Polymeric Hybrid Particles with Assembly-Induced Emission. Biomacromolecules 2018, 19 (7), 2841−2848.
(7) Jin, R. Quantum Sized, Thiolate-Protected Gold Nanoclusters. Nanoscale 2010, 2 (3), 343−362.
(8) Cantelli, A.; Battistelli, G.; Guidetti, G.; Manzi, J.; Di Giosia, M.; Montalti, M. Luminescence Gold Nanoclusters as Biocompatible Probes for Optical Imaging and Theranostics. Dyes Pigm. 2016, 135, 64−79.
(9) Yang, T.-Q.; Peng, B.; Shan, B.-Q.; Zong, Y.-X.; Jiang, J.-G.; Wu, P.; Zhang, K. Origin of the Photoluminescence of Metal Nanoclusters: From Metal-Centered Emission to Ligand-Centered Emission. Nanomaterials 2020, 10 (2), 261.
(10) Wu, Z.; Jin, R. On the Ligand’s Role in the Fluorescence of Gold Nanoclusters. Nano Lett. 2010, 10 (7), 2568−2573.
(11) Hebels, E. R.; Najafi, M.; van den Dikkenberg, J.; Beztsinna, N.; van de Looij, S.; Wilbie, D.; Meeldijk, J.; Hembury, M.; Vermonden, T. Luminescence Gold Nanocluster-Decorated Polymeric Hybrid Particles for Laser Guided Therapy. Eur. Polym. J. 2021, 132, 110467.
(12) Yu, N.; Huang, L.; Zhou, Y.; Xue, T.; Chen, Z.; Han, G. Near-Infrared-Light Activatable Nanoparticles for Deep-Tissue-Penetrating Wireless Optogenetics. Adv. Healthcare Mater. 2019, 8 (6), 1801132.
(13) Chen, L.-Y.; Wang, C.-W.; Yuan, Z.; Chang, H.-T. Fluorescent Gold Nanoclusters: Recent Advances in Sensing and Imaging. Anal. Chem. 2015, 87 (1), 216−229.
(14) Kelkar, S. S.; Reineke, T. M. Theranostics: Combining Imaging and Therapy. Bioconjugate Chem. 2011, 22 (10), 1879−1903.
(15) Cheng, Y.; Su, G.; He, Y.; Shen, H.; Zhao, J.; Xia, K.; Gong, Q. Luminescence Quantum Yields of Gold Nanoparticles Varying with Excitation Wavelengths. Nanoscale 2016, 8 (4), 2188−2194.
(16) Chistopulos, T. K.; Diamandis, E. P. Fluorescence Immunoassays. In Immunoassay; Elsevier, 1996; pp 309−335. DOI: 10.1016/B978-012214730-2/50015-7.
(17) Zhang, X. D.; Wu, D.; Shen, X.; Liu, P. X.; Fan, F. Y.; Fan, S. J. In Vivo Renal Clearance, Biodistribution, Toxicity of Gold Nanoclusters. Biomaterials 2012, 33 (18), 4628−4638.
(18) Xie, J.; Zheng, Y.; Ying, J. Y. Protein-Directed Synthesis of Highly Fluorescent Gold Nanoclusters. J. Am. Chem. Soc. 2009, 131 (3), 888−889.
(19) Wang, J.; Ma, S.; Ren, J.; Yang, J.; Qu, Y.; Ding, D.; Zhang, M.; Yang, G. Fluorescence Enhancement of Cysteine-Rich Protein-Templated Gold Nanoclusters Using Silver(I) Ions and Its Sensing Application for Mercury(II). Sens. Actuators, B 2014, 190, 32−330.
(20) Wang, Y.; Chen, J. T.; Yan, X. P. Fabrication of Transferrin Functionalized Gold Nanoclusters/Graphene Oxide Nanocomposite for Turn-on Near-Infrared Fluorescent Bioimaging of Cancer Cells and Small Animals. Anal. Chem. 2013, 85 (4), 2529−2535.
(21) Chen, T.-H.; Tseng, W.-L. (Lysozyme Type VI)-Stabilized Au8 Clusters: Synthesis Mechanism and Application for Sensing of Glutathione in a Single Drop of Blood. Small 2012, 8 (12), 1912−1919.
(22) Wei, H.; Wang, Z.; Yang, L.; Tian, S.; Hou, C.; Lu, Y. Lysozyme-Stabilized Gold Fluorescent Cluster: Synthesis and Application as Hg2+ Sensor. Analyst 2010, 135 (6), 1406.
(23) Li, T.; Cao, Y.; Wei, L.; Wang, J.; Zhang, M.; Yang, X.; Wang, W.; Yang, G. The Assembly of Protein-Templated Gold Nanoclusters for Enhanced Fluorescence Emission and Multifunctional Applications. Acta Biomater. 2020, 101, 436−443.
(24) Kawasaki, H.; Hamaguchi, K.; Osaka, I.; Arakawa, R. Ph-Dependent Synthesis of Pepsin-Mediated Gold Nanoclusters with Blue Green and Red Fluorescent Emission. Adv. Funct. Mater. 2011, 21 (18), 3508−3515.
(25) Liu, C.-L.; Wu, H.-T.; Hsiao, Y.-H.; Lai, C.-W.; Shih, C.-W.; Peng, Y.-K.; Tang, K.-C.; Chang, H.-W.; Chien, Y.-S.; Hsiao, J.-K.; Cheng, J.-T.; Chou, P.-T. Insulin-Directed Synthesis of Fluorescent
Gold Nanoclusters: Preservation of Insulin Bioactivity and Versatility in Cell Imaging. *Angew. Chem., Int. Ed.* 2011, 50 (31), 7056−7060.

(26) Kong, Y.; Chen, J.; Gao, F.; Bryden, R.; Johnson, B.; Heath, G.; Zhang, Y.; Wu, L.; Zhou, D. Near-Infrared Fluorescent Ribonucleoside-A-Encapsulated Gold Nanoclusters: Preparation, Characterization, Cancer Targeting and Imaging. *Nano Lett.* 2013, 5 (3), 1009−1017.

(27) West, A. L.; Griep, M. H.; Cole, D. P.; Karna, S. P. DNase I Retains Endodeoxyribonuclease Activity Following Gold Nanocluster Synthesis. *Anal. Chem.* 2014, 86 (15), 7377−7382.

(28) Wen, F.; Dong, Y.; Feng, L.; Wang, S.; Zhang, S.; Zhang, X. Horseradish Peroxidase Functionalized Fluorescent Gold Nanoclusters for Hydrogen Peroxide Sensing. *Anal. Chem.* 2011, 83 (4), 1193−1196.

(29) Xu, Y.; Sherwood, J.; Qin, Y.; Crowley, D.; Bonizzoni, M.; Bao, Y. The Role of Protein Characteristics in the Formation and Fluorescence of Au Nanoclusters. *Nano Lett.* 2014, 16 (3), 1515−1524.

(30) Chen, Y.; Montana, D. M.; Wei, H.; Cordero, J. M.; Schneider, M.; Le Guével, X.; Chen, O.; Bruns, O. T.; Bawendi, M. G. Shortwave Infrared in Vivo Imaging with Gold Nanoclusters. *Nano Lett.* 2017, 17 (10), 6330−6334.

(31) Lopez, A.; Liu, J. DNA-templated Gold Nanoclusters Reduced by Good’s Buffer: From Blue-Emitting Seeds to Red and Near Infrared Emitters. *Can. J. Chem.* 2015, 93 (6), 615−620.

(32) Zhou, C.; Long, M.; Qin, Y.; Sun, X.; Zheng, J. Luminescent Gold Nanoparticles with Efficient Renal Clearance. *Angew. Chem., Int. Ed.* 2011, 50 (14), 3168−3172.

(33) Luo, Z.; Yuan, X.; Yu, Y.; Zhang, Q.; Leong, D. T.; Lee, J. Y.; Xie, J. From Aggregation-Induced Emission of Au(I)−Thiolate Complexes to Ultraluminescent Au@Au(I)−Thiolate Core−Shell Nanoclusters. *J. Am. Chem. Soc.* 2012, 134 (40), 16662−16670.

(34) Bera, D.; Goswami, N. Driving Forces and Routes for Aggregation-Induced Emission-Based Highly Luminous Metal Nanocluster Assembly. *J. Phys. Chem. Lett.* 2021, 12 (37), 9033−9046.

(35) Zheng, J.; Zhang, C.; Dickson, R. M. Highly Fluorescent, Water-Soluble, Size-Tunable Gold Quantum Dots. *Phys. Rev. Lett.* 2004, 93 (7), 1 DOI: 10.1103/PhysRevLett.93.077402.

(36) Santiago González, B.; Rodríguez, M. J.; Blanco, C.; Rivas, J.; López-Quintela, M. A.; Martinho, J. M. G. One Step Synthesis of the Smallest Photoluminescent and Paramagnetic PVP-Protected Gold Nanoclusters: Preservation of Insulin Bioactivity and Versatility in Cell Imaging. *Angew. Chem., Int. Ed.* 2011, 50 (31), 7056−7060.

(37) Chen, Y.; Montana, D. M.; Wei, H.; Cordero, J. M.; Schneider, M.; Le Guével, X.; Chen, O.; Bruns, O. T.; Bawendi, M. G. Shortwave Infrared in Vivo Imaging with Gold Nanoclusters. *Nano Lett.* 2013, 13 (6), 2107−2111.

(38) Donner, J. S.; Thompson, S. A.; Kreuzer, M. P.; Baffou, G.; Van, H.; Lammers, T. Core-Crosslinked Polymeric Micelles: Principles, Preparation, Biomedical Applications and Clinical Translation. *Nano Today* 2015, 10 (1), 93−117.

(39) Torchilin, V. Tumor Delivery of Macromolecular Drugs Based on the EPR Effect. *Adv. Drug Delivery Rev.* 2011, 63 (3), 131−135.

(40) Porret, E.; Le Guével, X.; Coll, J.-L. Gold Nanoclusters for Biomedical Applications: Toward in Vivo Studies. *J. Mater. Chem. B* 2020, 8 (11), 2216−2232.

(41) Talledi, M.; Barz, M.; Rijcken, C. J. F.; Kiessling, F.; Henkinn, W. E.; Lammers, T. Core-Crosslinked Polymeric Micelles: Principles, Preparation, Biomedical Applications and Clinical Translation. *Nano Today* 2015, 10 (1), 93−117.

(42) Zheng, J.; Yang, X. X.; Wang, Y.; Zhao, N. W.; Xiong, Z. H.; Huang, C. Z. Rapid Synthesis of Highly Luminescent and Stable Au20 Nanoclusters for Active Tumor-Targeted Imaging in Vitro and in Vivo. *Nano Lett.* 2014, 6 (4), 2261−2269.

(43) Hembury, M.; Chiappini, C.; Bertazzo, S.; Kalber, T. L.; Drisko, G. L.; Ogunlade, O.; Walker-Samuel, S.; Krishna, K. S.; Jumeaux, C.; Beard, P.; Kumar, C. S. S. R.; Porter, A. E.; Lythgoe, M. F.; Boissière, C.; Sanchez, C.; Stevens, M. M. Gold−Silica Quantum Rattles for Multimodal Imaging and Therapy. *Proc. Natl. Acad. Sci. U. S. A.* 2015, 112 (7), 1959−1964.

(44) Han, W.; Yang, W.; Gao, F.; Cai, P.; Wang, J.; Wang, S.; Xue, J.; Gao, X.; Liu, Y. Iodine-124 Labeled Gold Nanoclusters for Positron Emission Tomography Imaging in Lung Cancer Model. *J. Nanosci. Nanotechnol.* 2020, 20 (3), 1375−1382.

(45) Liu, Y.; Tian, G. F.; He, X. W.; Li, W. Y.; Zhang, Y. K. Microwave-Assisted One-Step Rapid Synthesis of near-Infrared Gold Nanoclusters for NIR/CT Dual-Modal Bioimaging. *J. Mater. Chem. B* 2016, 4 (7), 1276−1283.

(46) Hu, D. H.; Sheng, Z. H.; Zhang, P. F.; Yang, D. Z.; Liu, S. H.; Gong, P.; Gao, D. Y.; Fang, S. T.; Ma, Y. F.; Cai, L. T. Hybrid Gold-Gadollinium Nanoclusters for Tumor-Targeted NIR/CT/MRI Triple-Modal Imaging in Vivo. *Nano Lett.* 2013, 13 (4), 1624−1628.

(47) Zhang, H.; Han, W.; Cao, X.; Gao, T.; Jia, R.; Liu, M.; Zeng, W. Gold Nanoclusters as a Near-Infrared Fluorescent Nanothermometer for Living Cells. *Microchim. Acta* 2019, 186 (6), 1−6.

(48) Yang, L.; Lou, X.; Yu, F.; Liu, H. Cross-Linking Structure-Induced Strong Blue Emissive Gold Nanoclusters for Intracellular Sensing. *Analyst* 2019, 144 (8), 2765−2772.

(49) Zhang, L.; Stockmar, F.; Azadfar, N.; Nienhaus, G. U. Intracellular Thermometry by Using Fluorescent Gold Nanoclusters. *Angew. Chem., Int. Ed.* 2013, 52 (42), 11154−11157.

(50) Wang, X. D.; Wolfbeis, O. S.; Meier, R. J. Luminescent Probes and Sensors for Temperature. *Chem. Soc. Rev.* 2013, 42 (19), 7834−7869.

(51) Donner, J. S.; Thompson, S. A.; Kreuzer, M. P.; Baffou, G.; Quindant, R. Mapping Intracellular Temperature Using Green Fluorescent Protein. *Nano Lett.* 2012, 12 (4), 2107−2111.

(52) Selvaprakash, K.; Chen, Y. C. Using Protein-Encapsulated Gold Nanoclusters as Photoluminescent Sensing Probes for Biomolecules. *Biosens. Bioelectron.* 2014, 61, 88−94.

(53) Xia, X.; Long, Y.; Wang, J. Glucose Oxidase-Functionalized Fluorescent Gold Nanoclusters as Probes for Glucose. *Anal. Chem.* 2015, 87, 81−86.

(54) Peng, J.; Feng, L.-N.; Zhang, K.; Li, X.-H.; Jiang, L.-P.; Zhu, J.-J. Calcium Carbonate-Gold Nanocluster Hybrid Spheres: Synthesis and Versatile Application in Immunooassays. *Chem. - Eur. J.* 2012, 18 (17), 5261−5268.

(55) Govindaraju, S.; Ankireddy, S. R.; Viswanath, B.; Kim, J.; Yun, K. Fluorescent Gold Nanoclusters for Selective Detection of Dopamine in Cerebrospinal Fluid. *Sci. Rep.* 2017, 7 (1), 1−12.

(56) Yang, G. H.; Shi, J. J.; Wang, S.; Xiong, W. W.; Jiang, L. P.; Burda, C.; Zha, J. J. Fabrication of a Boron Nitride-Gold Nanocluster Composite and Its Versatile Application for Immunooassays. *Chem. Commun.* 2013, 49 (91), 10757−10759.
