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Modulating the Chemical Reactivity of Gold Complexes in Living Systems: From Concept to Biomedical Applications

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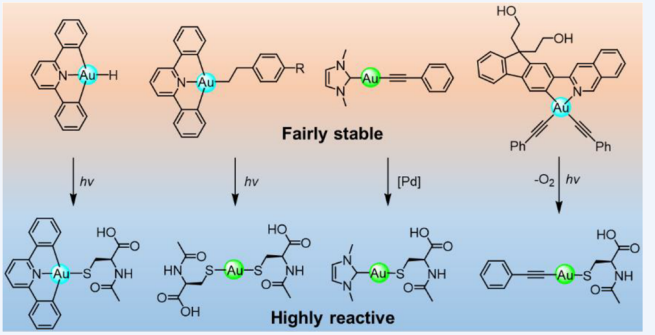
CONSPECTUS: Over the past few decades, research on the chemistry of gold has progressed rapidly, encompassing topics like catalysis, supramolecular chemistry, molecular recognition, *etc.* These chemical properties are of great value in developing therapeutics or orthogonal catalysts in biology. However, the presence of concentrated nucleophiles and reductants, particularly thiol-containing serum albumin in blood and glutathione (GSH) inside cells that can strongly bind and quench the active gold species, makes it difficult to translate the chemistry of gold from test tubes into living systems. In this regard, modulating the chemical reactivity of gold complexes to conquer nonspecific interactions with thiols and meanwhile to controllably activate their reactivity in a spatiotemporal manner is of pivotal importance to develop gold complexes for biomedical applications. In this account, we aim to highlight the concept of developing stimuli-activatable gold complexes with masked chemical properties, the bioactivity of which can be spatiotemporally activated at the target site by leveraging approaches from classic structure design to recently emerged photo- and bioorthogonal-activation.



A straightforward approach to tuning the reactivity of gold complexes is based on structure modification. This is achieved by introducing strong carbon donor ligands, such as N-heterocyclic carbene, alkynyl, and diphosphine, to improve the stability of gold(I) complexes against off-target thiols. Likewise, GSH-responsive gold(III) prodrug and supramolecular Au(I)−Au(I) interaction have been harnessed to keep a reasonable stability against serum albumin and confer tumor-targeted cytotoxicity by inhibiting thiol- and selenol-containing thioredoxin reductase (TrxR) for potent cancer treatment *in vivo*. To achieve better spatiotemporal controllability, photoactivatable prodrugs are developed. These complexes are equipped with cyclometalated pincer-type ligands and carbanion or hydride as ancillary ligands, rendering high thiol-stability in the dark, but upon photoirradiation, the complexes can undergo unprecedented photoinduced ligand substitution, β-hydride elimination, and/or reduction to release active gold species for TrxR inhibition at the diseased tissue. To further improve the therapeutic activity, an oxygen-dependent conditional photoreactivity of gold(III) complexes by evolving from photodynamic into photoactivated chemotherapy has been achieved, resulting in highly potent antitumor efficacy in tumor-bearing mice. Of equal importance is harnessing the bioorthogonal activation approach by chemical inducers, as exemplified by a palladium-triggered transmetalation reaction to selectively activate the chemical reactivities of gold including its TrxR inhibition and catalytic activity in living cells and zebrafish. Collectively, strategies to modulate gold chemistry *in vitro* and *in vivo* are emerging, and it is hoped that this Account will spur the creation of better approaches to advance gold complexes closer to clinical application.

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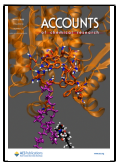

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1. INTRODUCTION

Gold is among the very first metals known to early civilizations and has long been used as coinage or in decoration.[5] Metallic gold is stable against biological degradation and erosion, making its chemistry rapidly grown only in the last decades. At +I oxidation state, gold displays a soft Lewis acidity with a high binding affinity toward electron rich sulfur and unsaturated bonds, making it possible for enzyme inhibition or metal catalysis. Of significance is the tendency of mononuclear gold(I) complexes to form short Au(I)···Au(I) distances, which has recently been recognized by Che and co-workers that such interaction is caused by ligand−ligand dispersion and electrostatic interaction that counterbalance the strong Au(I)−Au(I) Pauli repulsion.[6] Aurophilicity also leads to unique photophysical properties originated from ^{3} [5dσ*-6pσ] excited state-solvent exciplex.[7] The luminescence of gold(III) complexes can be endowed as well, provided that strong σ-donor ligands are introduced to destabilize the antibonding 5dσ* ($5d_{x^2-y^2}$), giving a long-lived triplet excited state.[8] These properties render gold complexes with tremendously useful applications in biological systems.[9]

The biomedical application of gold dates back to ancient Chinese and Arabic medicine.[10] In the late 19th century, $[Au(CN)_2]^-$ was discovered to be effective in treating tuberculosis. Later, gold−thiolates were noticed with anti-rheumatic activity. In 1985, auranofin, a gold-phosphine-thiolate complex, was approved by the FDA for oral treatment of rheumatoid arthritis. In the meantime, auranofin was found to exhibit antiproliferative properties toward a panel of cancer cells.[11] This has raised a surge of research interests in developing anticancer gold(I) complexes containing different ligands. On the other hand, the discovery of cisplatin in the 1960s has spurred research in studying the isoelectronic gold(III) complexes. Indeed, gold(III) complexes are able to form covalent adducts with nucleobases or act as DNA intercalator.[10] However, due to the high oxidation potential (Au^{3+}/Au^+ 1.41 V, Au^{3+}/Au^0 1.50 V), strong donor ligands are necessary to stabilize the + III oxidative state. Ligand modification makes gold(I) and gold(III) complexes with distinct kinetic and thermodynamic profiles and hence different mechanisms of action *in vitro* and *in vivo*.[12]

Besides the promising anticancer efficacy in Petri dishes, auranofin could increase the lifespan of leukemia-bearing mice and inhibit tumor growth in liver, lung, and other cancer models.[11,13,14] But a high dosage was usually required, possibly due to the interference from albumins in the blood. Gold distribution in auranofin-treated rats indicated that gold retention beyond 48 h is principally in the kidneys, which leads to renal toxicity.[15] Within its working condition for rheumatoid arthritis, the off-target toxicity is often less severe, related to gastrointestinal diarrhea, skin rashes, and eye-related events. For other synthesized gold complexes, the *in vivo* efficacy and toxicity have been well-documented in other reviews.[12,16−23]

The strong binding affinity of gold toward soft atoms such as sulfur leads to potent inhibition toward thiol-/selenol-containing enzymes.[12,24] The most notable example is thioredoxin reductase (TrxR), a critical enzyme that is overexpressed in many types of cancers and has been widely considered as a key anticancer molecular target of gold with inhibition down to low nanomolar or picomolar level.[25] Other thiol-enzymes also play important mechanistic roles, exemplified by glutathione reductase, cysteine protease, protein tyrosine phosphatases, glutathione peroxidase, iodothyronine deiodinase, aquaporin 3, deubiquitinase, IκB kinase *etc.*[12] In addition, gold complexes have been identified to engage nonthiol enzyme targets such as heat shock protein 60, cyclophilin 3, proteasome, HMG-CoA reductase, PARP-1, topoisomerase I, and DNA G-quadruplex, *etc.*[12,26−32] Binding to these molecular targets could result in distinct anticancer pathways, and certain gold(I) complexes have recently been shown to induce immunogenic cell death (ICD), a death type that causes anticancer immunity mainly by the endoplasmic reticulum (ER) stress-related release of pro-inflammatory signals. Several gold complexes were reported to elicit potent ICD activities, possibly due to their ability to boost intracellular ROS levels and subsequent ER stress.[33,34] As mentioned above, while high reactivities are necessary to potently target these enzymes in solution and *in vitro*, the highly reactive gold complexes also tend to bind off-target thiols, particularly serum albumin which contains a free cysteine thiol (Cys34) with a low pK_a (∼5) and presents at a rather high concentration (∼600 μM) in blood. This will significantly decrease the bioavailability of gold *in vivo* and cause unwanted side effects. To this end, strategies to tune the thiol reactivity of gold is of paramount importance to conquer nonspecific interactions. This Account discusses the concept of modulating the chemical reactivity of gold in living systems and highlights several examples developed in our group with such strategies for biomedical applications. It has been noted that recently several excellent reviews related to gold-based anticancer complexes with emphasizes on mechanism and specific ligands have been described elsewhere.[16−23]

2. CONCEPT IN GOLD DRUG DESIGN

2.1. Ligand Design

The stability and reactivity of gold(I) complexes are strongly associated with the coordinated ligands. Sadler carefully examined the thermodynamic stability of gold(I) complexes with different ligands by NMR,[10] following the order:

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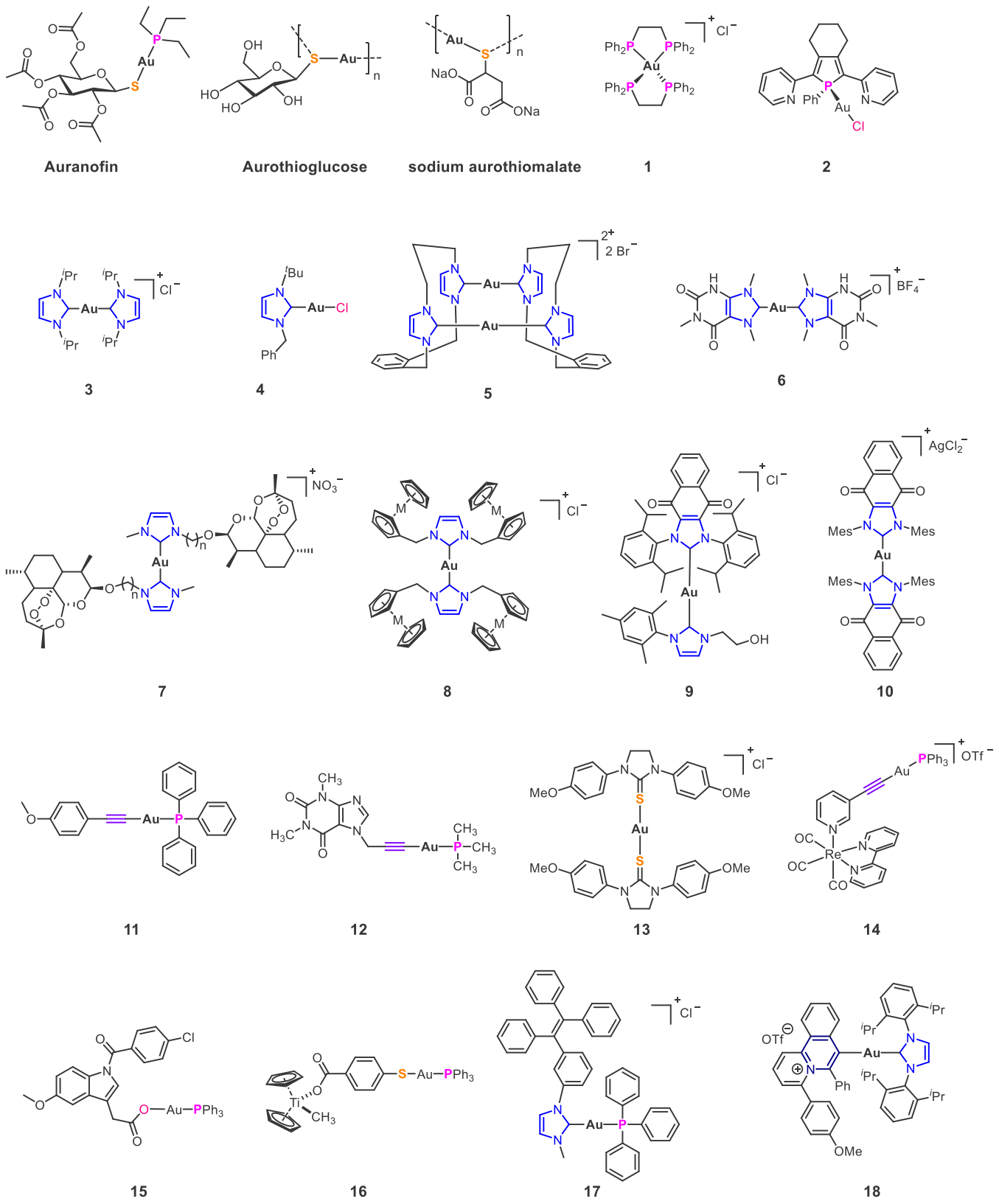

Figure 1. Examples of anticancer active gold(I) complexes containing S (thiolate, thiourea), P (phosphine), C (NHC, alkynyl, C_{sp2}), O (carboxylate), and Cl ligands.

$CN^- \sim CysS^- \sim PR_3 \gg$ Met-S-$CH_3 \sim$ His(=N-) > Cl $\gg COO^-$

Accordingly, ligands of phosphine and thiolate have been widely utilized to construct gold(I) complexes for more than 50 years. While potent TrxR inhibition and good cytotoxicity have been found in many Au(I)-S/P complexes, gold(I) compounds with such ligands may still be easily replaced by thiols in serum albumin and/or glutathione. In the recent two decades, strong σ-donors particularly the carbon donors have

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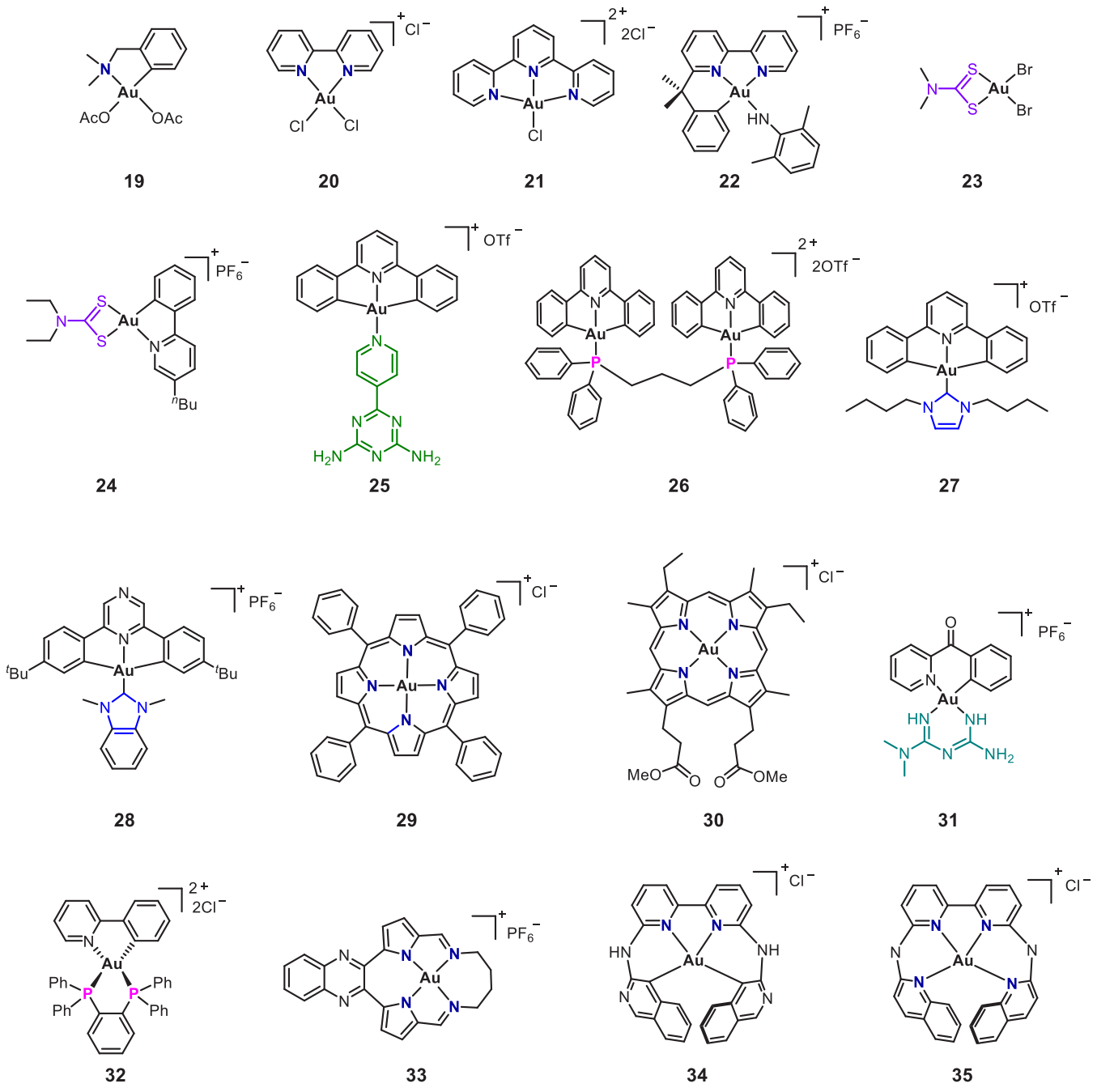

Figure 2. Examples of anticancer active gold(III) complexes containing bidentate (C^N, N^N), tridentate (N^N^N, C^N^N, C^N^C), and tetradentate (N_4 or N_2C_2) ligands.

been harnessed to increase the stability of gold(I) complexes (Figure 1). Among them, N-heterocyclic carbene (NHC) has been demonstrated to be particularly useful since this ligand can be easily modified to tune lipophilicity, steric hindrance, and hence reactivities with thiols.[17,35] Berners-Price and co-workers have carefully examined the ligand exchanges between $[Au(NHC)_2]^+$ and Cys or Sec, and a stepwise release of NHC ligand was observed together with a 20- to 80-fold higher reaction rate for Sec than Cys.[36] This study has laid the foundation to develop a surge of NHC-gold(I) complexes with different functionalities that can inhibit both thiol-enzymes and nonthiol targets.[29,37−40] Another useful type of carbon donor ligand is alkynyl. For example, Ott and co-workers prepared alkynyl gold(I) complexes which showed increased stability, selective inhibition toward TrxR, and antiangiogenisis activity in zebrafish models.[41] A more recent study demonstrated a multitarget nature of the alkynyl phosphane gold(I) complex by disrupting mitochondrial bioenergetics and glycolysis.[42] Endeavors of using other donor ligands with dual or synergistic activities also emerged recently.[43−46]

Unlike gold(I) complexes, gold(III) complexes are four-coordinated. Multidentate ligands are necessary to stabilize the Au^{3+} ions (Figure 2). Useful ligands include bidentate S^S (e.g., dithiocarbamate),[47,48] N^N (e.g., 2,2′-bipyridine),[49] C^N (e.g., 2-phenylpyridine, *N*,*N*-dimethyl-1-phenylmethanamine),[50,51] tridendate N^N^N (e.g., 2,2′:6′,2′′-terpyridine),[52] C^N^N (e.g., 6-(2-phenylpropan-2-yl)-2,2′-bipyridine),[20] C^N^C (e.g., 2,6-diphenylpyridine),[18,53,54] and tetradentate

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porphyrin and other macrocycle ligands.[28,55,56] Of note, gold(III) complexes with tridentate ligands containing C-deprotonated carbanion and tetradentate ligand display high stability against reduction. Complexes with these ligands are usually highly potent to suppress cancer proliferation in association with a number of distinct mechanisms-of-action.

2.2. Privileged Chemical Scaffolds

The stability and reactivity of gold can be efficiently tuned by varying chemical structures. For example, Sadler, Berners-Price and co-workers introduced dppe (bis(diphenylphosphino)-ethane) to construct a four-coordinated $[Au^{I}(dppe)_2]Cl$ complex. This complex displays excellent stability toward GSH and exhibits significant *in vivo* antitumor activities in different mouse models.[57] The lipophilicity and thiol-reactivity of $[Au^{I}(dppe)_2]Cl$ were further tuned by replacing dppe with dppp or d2pypp ligands, giving better TrxR inhibition and lower side effects.[17] Another useful strategy is to harness the aurophilic interaction to increase the stability, as exemplified by the dinuclear gold(I) complexes containing two bridging bis(NHC) ligands. The dinuclear gold(I) complexes are more stable than the mononuclear counterparts, and their unique luminescent property is useful as cell imaging agents.[58] For gold(III) complexes, cyclometalated tridentate C^N^N and C^N^C ligands are particularly useful in stabilizing Au^{3+}. For instance, Che and co-workers have well demonstrated that $[Au^{III}(C^N^C)L]$ is highly stable against reduction, and combination with different auxiliary ligands (e.g., NHC, phosphine) could generate divergent chemical reactivities and target selectivity. The tetradentate porphyrin developed by Che and co-workers is among the most notable ligands, and the gold(III)-porphyrin totally did not react with GSH. Particularly, gold(III) meso-tetraphenylporphyrin, named gold-**1a** (**29**, Figure 2) shows very promising antitumor activities in a series of tumor models, and it is being tested at various stages of preclinical trials.[55] Last but not least, both gold(I) and gold(III) complexes have the tendency to form supramolecular polymers due to either aurophilicity or π stacking interactions, which has been demonstrated to be useful for bioorthogonal photodynamic therapy and for drug delivery to minimize side effects.[45,53,59]

2.3. Useful Chemical Transformations

As mentioned before, gold complexes have rich chemistry in solution and can be considered as prodrugs due to their various chemical transformations under physiological conditions. The linear two-coordinate gold(I) complexes are vulnerable to stepwise ligand exchange with physiological thiols via a three-coordinate intermediate/transition state (Figure 3a). Ligand exchange also occurs in gold(III) complexes stabilized by cyclometalated ligands (Figure 3b). While previous studies showed that gold(I) may be oxidized into gold(III) such as by the myeloperoxidase system of white blood cells *in vivo*, more recent studies revealed that gold(III) complexes tend to undergo reduction into gold(I) and release the coordinated ligands (Figure 3c).[10,12] On the other hand, the Lewis acid property is useful to access extraordinary structures or to detect active gold species in cells. For example, arylboronic acids can form stable Au(III)/Au(I)-aryl bonds by trans-metalation reaction.[9] Alternatively, the Au(I)-C(sp^2) bond can be generated by direct activation of the alkyne[34] or by a spontaneous cycloaddition reaction between gold(I)-azide and alkyne.[60] These organometallic gold complexes display potent cytotoxicity against cisplatin-resistant cancer cells and strong

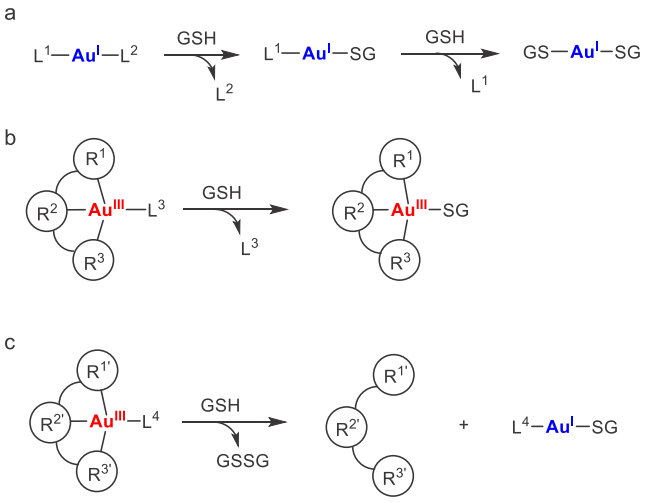

Figure 3. Typical chemical transformation of gold complexes by GSH in living cells. (a,b) Ligand exchange reactions for gold(I) and gold(III) complexes. (c) Reduction of gold(III) complexes into gold(I) species. SG, the deprotonated form of GSH.

activity in inducing ICD.[34] In addition to drug synthesis in solution, the catalytic activity of gold has also been demonstrated to be useful *in vitro* and *in vivo* in developing gold-based fluorescent probes or in *in situ* drug synthesis.[61,62]

3. STIMULI-RESPONSIVE GOLD PRODRUGS

3.1. Tunable Thiol Reactivity in Gold-Based Anticancer Complexes

Since thiols are prevalent in the body, reactive gold complexes cannot accumulate selectively in tumors. In addition, serum albumin strongly binds and blocks the blood-circulating gold compounds, exemplified by the clinical drug auranofin, of which over 80% of the molecules in the blood were observed bound to albumin.[10,12] Therefore, regulating the thiol reactivity of gold drugs through ligand design is necessary to reduce off-target binding. In 2013, Che and co-workers reported a series of gold(III) compounds containing NHC and N^N^N (H_2N^N^N = 2,6-bis(imidazol-2-yl)pyridine or 2,6-bis-(benzimidazol-2-yl)pyridine) ligands.[63] On account of the not-so-strong ability of N^N^N ligands to stabling Au^{3+}, these gold complexes (such as **Au-1**) can be readily reduced to anticancer active Au(I)-NHC species by GSH, meanwhile releasing the emissive N^N^N ligands (Figure 4a). Importantly, thiol-free cellular reducing agent, ascorbic acid and albumin are less effective than GSH in the reduction.

Supramolecular Au(I)−Au(I) interaction has been utilized to modulate the thiol reactivity of Au(I) anticancer agents. In 2014, Che and co-workers synthesized binuclear gold(I) complexes with diphosphine and/or bis(NHC) bridging ligands to tune the thiol reactivity and evaluated their bioactivity (Figure 4b).[64] The bidentate ligands could produce supramolecular Au(I)−Au(I) interaction, where the bis(NHC) further improves the stability, whereas the diphosphine ligand retains the ligand-exchange reactivity with thiols. Hence, the binuclear gold(I) complex **Au-2** with two bis(NHC) ligands was found too stable to react with thiols, resulting in low antiproliferative activity. However, **Au-3** containing mixed diphosphine and bis(NHC) ligands displayed good cytotoxicity against a panel of cancer cells. In addition, experiment results showed that the cytotoxicity of **Au-3** against HCT116

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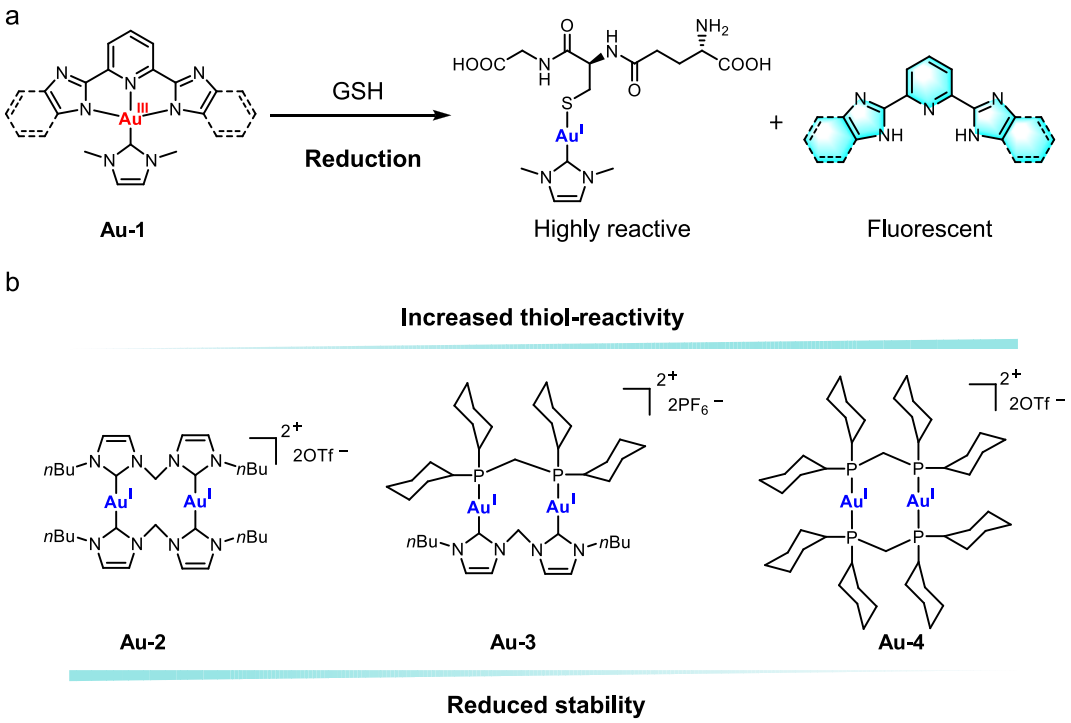

Figure 4. (a) Gold(III) complex **Au-1** that can be selectively reduced by GSH in tumor cells, generating Au(I)-NHC species and releasing fluorescent $H_2N^N^N$ ligand. (b) Reactivity and stability of dinuclear gold(I) complexes containing bis(NHC) or diphosphine ligands.

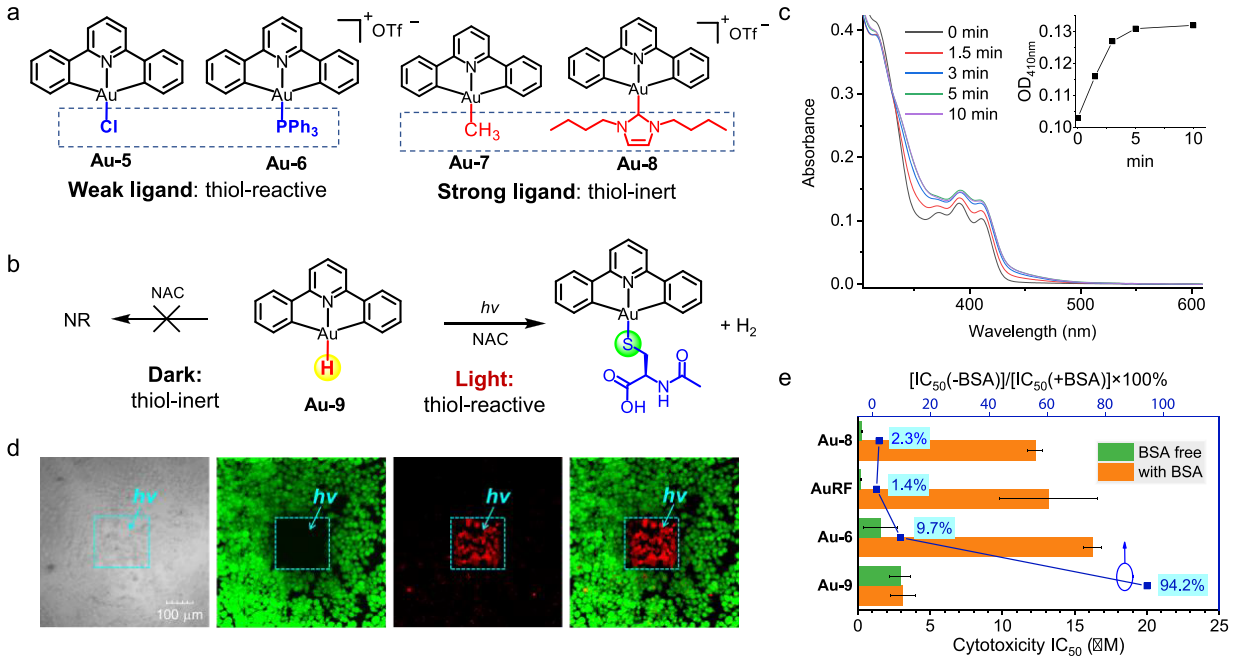

Figure 5. Photoactivatable gold(III)-hydride complexes. (a) Reactivity of cyclometalated gold(III) complexes containing different auxiliary ligands. (b) Reaction of **Au-9** with NAC under dark or light conditions. (c) UV/vis absorption change of **Au-9** in the presence of NAC and under light irradiation. (d) Living/dead cell costaining assay for **Au-9** treated cancer cells. (e) Cytotoxicity of different gold complexes in the presence and absence of BSA. Reproduced with permission from ref 1. Copyright 2020 Wiley-VCH GmbH.

was less affected in the presence of BSA compared to that of compound **Au-4** having two diphosphine ligands. Of note, **Au-3** is effective in suppressing tumor growth in two independent mouse tumor models even at a dosage of 0.6 mg/kg.

3.2. Photoactivatable Gold(III) Prodrugs

3.2.1. Photoactivatable Gold(III) Complexes Containing Tridentate C^N^C Ligands. Although tremendous efforts have been made previously in tuning the thiol reactivity of gold complexes via ligand and/or structure design, it still appears rather difficult to make the trade-off between lack of reactivity to serum albumin on the one hand and high reactivity to inhibit TrxR on the other hand. Therefore, it is important to exploit a new strategy for gold drugs to achieve the switch-on/off reactivity with thiols.

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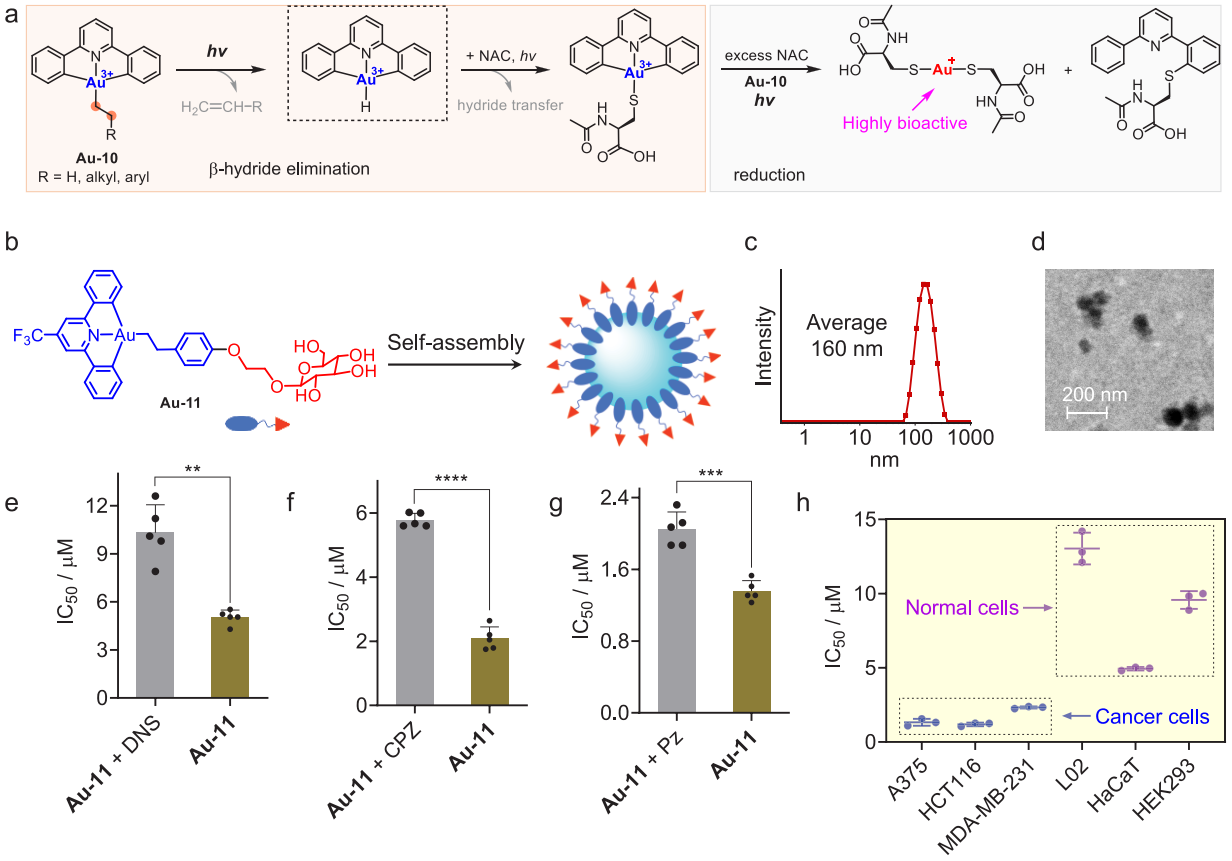

Figure 6. Photoactivated alkylgold(III) complexes. (a) Photoreactivity of **Au-10** (R = H) in the presence of NAC and light irradiation, showing β-H elimination followed by photocatalytic reduction. (b–d) Self-assembly of **Au-11** in solution based on dynamic light scattering and TEM analysis. (e–g) Cytotoxicity of **Au-11** in the presence and absence of dynamin-dependent endocytosis inhibitor dynasore (DNS), clathrin-mediated endocytosis blocker chlorpromazine (CPZ) and glucose transporter inhibitor phlorizin (Pz). (h) Cytotoxicity of **Au-11** toward different cancer and normal cells. Reproduced with permission from ref 2. Copyright 2022 Wiley-VCH GmbH.

Photoactivatable metalloprodrug or photoactivated chemotherapy (PACT) can solve many of the off-target binding issues.[65] Notable examples include Pt(IV) and Ru(II) prodrugs that can be controllably activated by light in the desired tissue.[66] However, it was not easy to construct gold-based PACT complexes because most of the donor (such as N or S) ligands that can stabilize Pt or Ru cannot generate gold complexes that are stable against thiols. Therefore, strong ligands were introduced into gold species to maintain their stability. The ideal gold(III)-stabilizing property of the cyclometalated tridentate C^N^C ligand made it particularly suitable here, in which the additional auxiliary ligand plays a critical role in controlling the thiol-reactivity. For example, while weak donors such Cl^- or PPh_3 lead to high thiol reactivity, the strong carbon donor ligands (such as NHC, alkyl, and alkynyl) can result in high thiol stability (Figure 5a). In view of the fact that hydride is known to be a strong ligand with donor strength comparable to carbon ligands and metal-hydride complexes have well-known photoreactivities,[67] we introduced hydride as auxiliary ligand into the Au(III)-(C^N^C) complex (**Au-9**) to achieve photocontrollable thiol-reactivities.[1] **Au-9** confers higher stability in the presence of 10-fold amount of *N*-acetyl cysteine (NAC). Once irradiated under 365 or 420 nm light, **Au-9** efficiently reacts with NAC, generating [Au^{III}(C^N^C)(NAC)] and H_2 (Figure 5b,c).

Next, we validated its spatiotemporal cytotoxicity, controlled by light. Using the live/dead fluorescence staining dyes with the **Au-9**-treated HepG2 cells, we were able to observe a selective death signal (red) in the light irradiated region over nonirradiated cells (green, Figure 5d). Such a result is consistent with the ICP-MS experiments showing 3.8-fold increase of protein-bound gold and >12-fold increase of TrxR inhibition after photoactivation, during which process singlet oxygen and hydroxyl radical are not noticeably involved. To verify if our photoactivation can overcome albumin binding, the cytotoxicity with or without BSA was performed. As expected, auranofin and other [Au(C^N^C)L] compounds are all vulnerable to albumin, with cytotoxicity decreased by >90% in the BSA-containing condition (Figure 5e). In contrast, the photocytotoxicity remains constant in the case of **Au-9**, suggesting its expected stability in blood.

While **Au-9** displays a good photoactivated anticancer activity, the two-electron hydride ligand in **Au-9** curbs further modification. To avoid such limitations, we sought to use alkyl ligands to replace hydride.[2] First, we tested the methyl group as the auxiliary ligand, discovering that it could only be rarely activated by light. Interestingly, compounds containing an alkyl ligand with β-hydrogen (such as ethyl) showed a potent light activation feature. After irradiation, a DMSO-coordinated active gold compound was identified in association with the generation of one molecule of ethylene, suggesting a β-hydride elimination process (Figure 6a). In the literature, β-hydride

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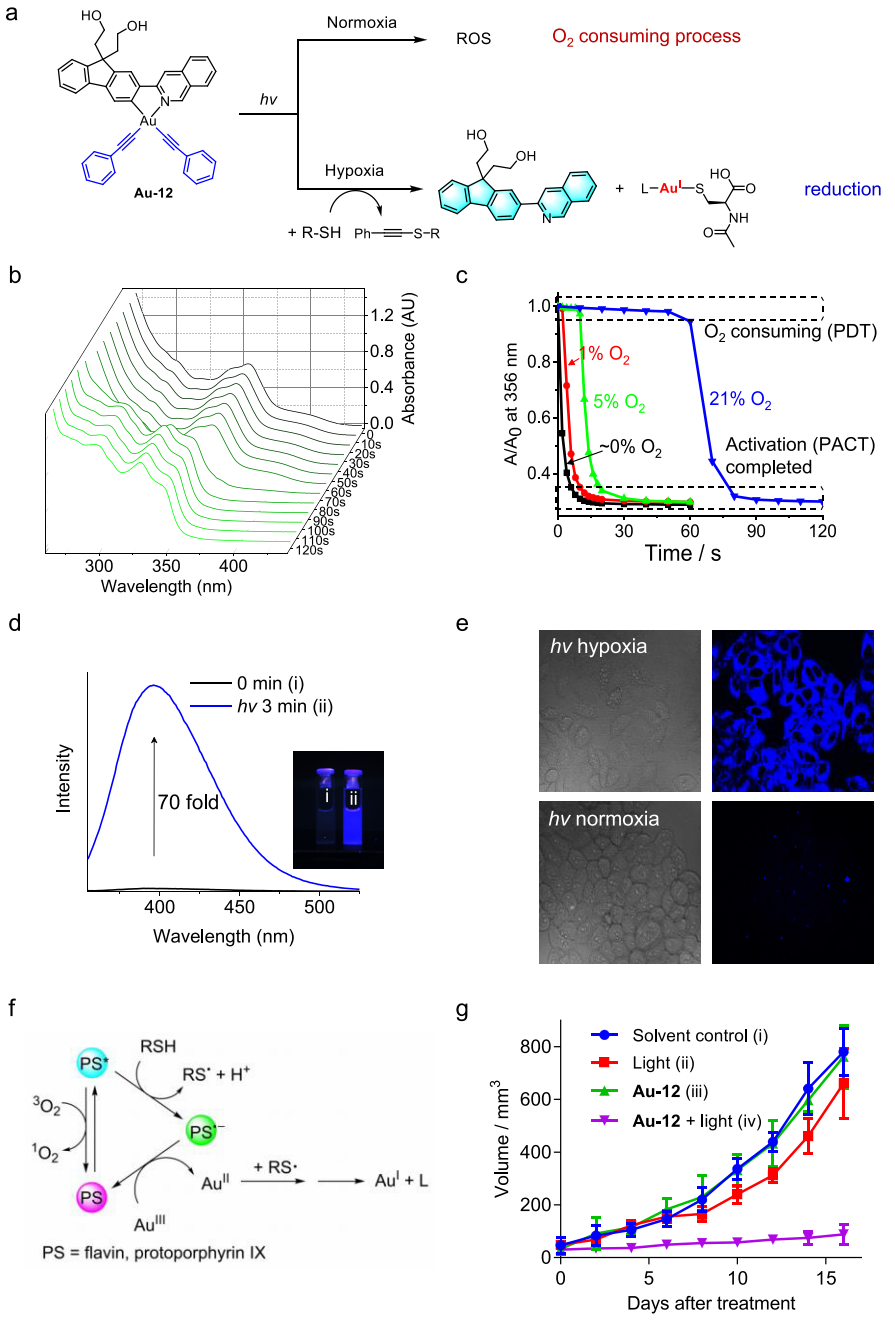

Figure 7. (a) Oxygen-dependent photoactivity of **Au-12**. (b,c) Change of UV/vis absorption (b) of **Au-12** in the presence of NAC in DMSO and under photoirradiation in air or plot of A/A_0 at 356 nm (c) under different O_2 conditions. (d) Emission spectra of **Au-12** in the presence of NAC in DMSO and under photoirradiation in air. (e) Fluorescence images of A375 cells treated by **Au-12** and under light irradiation in normoxia or hypoxia conditions. (f) Mechanistic description of the PDT-to-PACT evolving process induced by external photosensitizers. (g) Inhibition of mouse bearing A375 xenograft by **Au-12** under 465 nm light irradiation. Reproduced with permission from ref 3. Copyright 2020 Wiley-VCH GmbH.

elimination can only happen in metal complexes with open coordination sites. However, in this case all four coordination sites of gold are occupied. To understand the possible mechanism, we performed DFT/TDDFT calculations.[2] First, the electron−hole distribution analysis showed that the gold ion is highly electrophilic in the T_1 excited state. In the meantime, the geometry of **Au-10** (R = H) at T_1 is distorted, showing out-of-plane bending from the square geometry. Such results are reminiscent of coordination expansion, as in the case of the isoelectronic Ni(II) and Pt(II) complexes,[68,69] which renders a possibility to promote β-hydride elimination. Following elimination, we also observed the generation of $[Au^{III}(C^N^C)(NAC)]$ in the presence of NAC. Of interest is the possibility that this intermediate could further be transformed to highly active Au(I) species.

The possibility of alkyl-ligand-based modification inspired us to incorporate a tumor-targeting group into **Au-10**. According to the literature, many cancer cells overexpress glucose transporter GLUT. Hence, we introduced a glucose moiety as the tumor-targeting group. The fine-tuned complex **Au-11**

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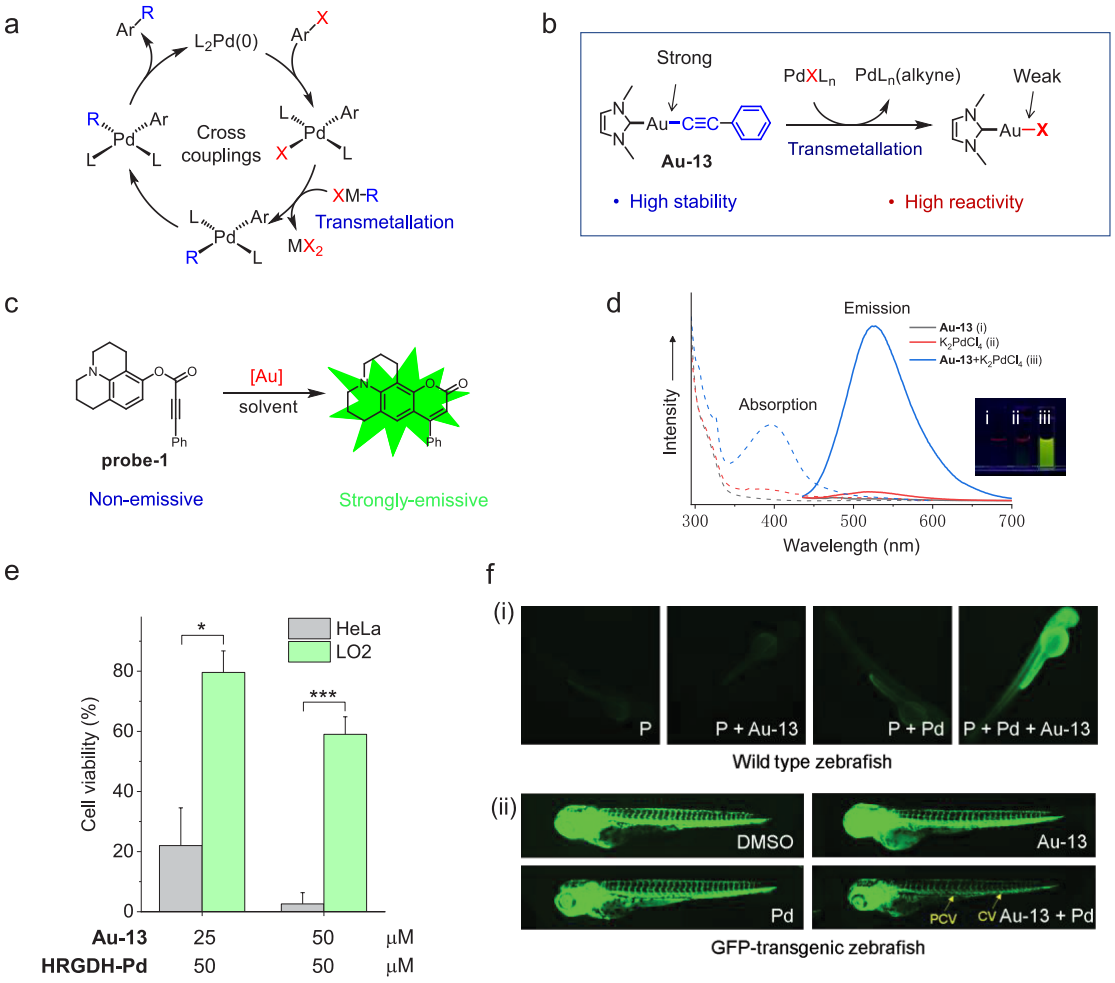

Figure 8. (a) Mechanism of cross-coupling reactions. (b) Pd(II)-mediated transmetalation of **Au-13** into NHC-Au-X species. (c) Pd(II)-activated **Au-13** catalyzed the cyclization of probe-1 to form fluorescent coumarin. (d) Activation of a coumarin probe by **Au-13** and K_2PdCl_4 in water. (e) **HRGDH-Pd** mediated selective cytotoxicity toward HeLa over L02 cells. (f) Activation of **Au-13** by Pd species based on probe-1 (i) and blood vessel inhibition (ii) in zebrafish models. Reproduced with permission from ref 4. Copyright 2020 Wiley-VCH GmbH.

with absorption at visible region, resulting from the $-CF_3$ moiety on the pyridine ring, can self-assemble in solution forming nanoparticles with an average size of 160 nm and entered cancer cells via the GLUT-mediated endocytosis pathway based on the cytotoxicity and ICP-MS studies in the presence of different uptake inhibitors (Figure 6b–g), giving up to 10-fold higher cytotoxicity to cancer cells (IC_{50} = 1.25–2.40 μM) than normal cells (IC_{50} = 5.0–12.8 μM, Figure 6h). Notably, the complex exhibited promising photocontrollable TrxR inhibition and cytotoxicity with a photoindex value (IC_{50} dark versus IC_{50} light) reaching over 400 to HCT116 cells. The *in vivo* light-induced anticancer activity was also confirmed in a nude mouse model bearing A375 xenograft.

3.2.2. Photoactivatable Gold(III) Complexes Containing Bidentate C^N Ligands. Since the cytotoxicity of PACT complexes mainly originates from bioactive ligands or metal centers, PACT complexes with additional PDT activity can enhance the anticancer efficacy.[70] However, PACT and PDT are in fact competing the excited state of metal complexes, where PACT decomposes the photosensitizer and PDT compromises the efficiency of PACT. Recently, we discovered an oxygen-dependent conditional photoactivity that could maximize the photoefficiency in the cyclometalated Au(III)-alkynyl complex (**Au-12**) with a bidentate C^N ligand.[3] Of great interest is the underlying PDT-to-PACT mechanism of this compound with ROS production and active gold species release (Figure 7a). In the photoreaction of **Au-12** with NAC in DMSO and under aerobic condition, a biphasic UV/vis absorption change was found, of which the compound remained stable for the first 60 s (plateau phase) followed by a dramatic change as a second process in ∼10 s (Figure 7b). Then we sought to figure out the determinants of this shift. First, we tested the photoactivation of **Au-12** under different concentrations of O_2. The results showed an oxygen-dependent manner, as the PACT occurred immediately upon the light irradiation when no oxygen is present (Figure 7c). The ROS production was also confirmed as expected using cognate probe in the living cells. Then, owing to the fluorescent property of the bidentate ligand released from the **Au-12** (Figure 7d), we were able to observe that oxygen could block the PACT effects in cells (by PDT instead, Figure 7e). All the above observations pointed to an oxygen-dependent PDT-to-PACT process.

Despite promising light-inducible cytotoxicity, the excitation wavelength of **Au-12** is around 420 nm rendering limited tissue penetration. Since the complex acts as a dual photosensitizer and photocatalytic substrate, we wondered if the PACT process of **Au-12** could be tethered with the PDT effect from

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other photosensitizers with a red-shift wavelength. Riboflavin, an endogenous photosensitizer in the human body, possesses sufficient reduction potential ($[Rf/[Rf^{\bullet -}]$ = −1.25 V vs SHE) to catalyze Au(III) reduction ($[Au^{III}]/[Au^{II}]$ = −0.65 V vs SHE). When using a combined treatment of riboflavin and **Au-12**, the PACT process of **Au-12** was achieved at 460 nm, the excitation wavelength for riboflavin but not **Au-12** (Figure 7f). Another advantage of using riboflavin as the trigger is enhanced cancer selectivity. The riboflavin transporter RFVT is known to be overexpressed in melanoma cancer cells. Correlated to their flavin levels, the melanoma cell A375 was most sensitive to **Au-12** compared to the other cancer cells like HepG2, A549 and HCT116, and additionally introducing riboflavin can selectively increase the cytotoxicity to A375 over normal L02 cells (viability of 18.5% vs 81.5%). Interestingly, no additional riboflavin was required to achieve the 460 nm excitation of **Au-12** in the A375 xenograft mouse model, possibly due to enough enrichment of riboflavin in the tumor tissues (Figure 7g).

3.3. Bioorthogonal Activation of Gold(I) Prodrugs

While photoactivation is very effective in controlling the thiol-reactivity of gold complexes, light still suffers from the tissue penetration issue. As an alternative approach, we have developed a bioorthogonal approach that can spatiotemporally activate organogold(I) complexes by external agents.[4] In cross-coupling reactions, transmetalation is a key step for transferring organoligand to the metal catalyst, in which the strong metal−carbon bond is broken down and replaced by a labile ligand (Figure 8a). It was previously reported that aryl-Au(I)-phosphine species could undergo transmetalation to break the gold(I)-aryl bond by Pd(II) species.[71] Inspired by such a reaction, we conceived that transmetalation may be utilized to controllably activate stable Au(I) prodrugs. We came up with using the NHC-Au(I)-alkynyl complex because this compound, due to the presence of two carbon donor ligands, may display a high thiol-stability. Indeed, no obvious change of ^{1}H NMR of **Au-13** was found with excess amounts of NAC after 48 h. Then, we incubated **Au-13** with K_2PdCl_4 to check the transmetalation efficiency (Figure 8b). Surprisingly, **Au-13** completely converted to NHC-Au-Cl as measured by ^{1}H NMR within 10 min.

The efficient transmetalation of **Au-13** encouraged us to further optimize the reaction in physiological-resembling conditions. As we discussed before, the active Au(I) compounds exhibit π-bond activation activity and are capable of catalyzing the maturation of a coumarin precursor, generating a strong fluorescence (Figure 8c). Using this signal as the readout, we built a high-throughput screening strategy to identify Au(I)/Pd(II) combinations with better performance in aqueous solution. Notably, **Au-13** and K_2PdCl_4 worked quite well in water (Figure 8d). To verify the transmetalation in the living systems, we added probe-1 along with cognate gold and palladium compounds to the wild-type zebrafish. We used **Au-13** as the prodrug and a less toxic $Pd(OAc)_2L_2$ (L = tetramethylguanidine) as the transmetalation agent. The bright fluorescence generated in wild-type zebrafish under combined treatment suggests that such a reaction is compatible with the living systems (Figure 8f, panel (i)). Moreover, the same treatment on the zebrafish also showed significant antiangiogenesis (Figure 8f, panel (ii)).

Considering the primary aim of anticancer prodrugs is tumor-selective activation, we next sought to modify the Pd reagents. Since Pd(II) coupled with an RGD peptide could elevate the tumor specificity, we applied **HRGDH-Pd** to activate **Au-13**. As expected, **HRGDH-Pd** induced 3.8- to 12.0-fold higher cytotoxicity of **Au-13** toward integrin-overexpressing HeLa cells than normal L02 cells (Figure 8e).

4. SUMMARY AND OUTLOOK

Early used gold drugs, like auranofin, have shown great potential for utilizing gold to address biological issues, particularly to overcome platinum drug-resistance in view of their distinct mode of action. However, those clinic gold drugs with weak ligands such as thioglucose are labile toward the abundant nucleophiles in the human body, without giving desirable anticancer activities. Encouragingly, the past decades have witnessed big progress at both structure design and mechanism-of-action levels. In particular, various ligands, such as diphosphine, NHC, C^N^C, and alkynyl, have been found capable of not only tuning the stability/reactivity but also achieving dual/synergistic therapeutic effects. Of note, gold(I) complexes containing certain ligands displayed potent capability to induce immunogenic cell death of cancer cells. Nevertheless, compared to the platinum-based drugs, the development of gold-based anticancer drugs was still largely lagging behind. As demonstrated by Che, Berners-Price, Ott, and others, one important reason might be the dilemma of gold drugs which require a high stability against off-target bindings but at the same time a high reactivity toward thiol-enzyme inhibition (Figure 9a). To achieve a trade-off, our group has designed a series of stable gold(III) and gold(I)

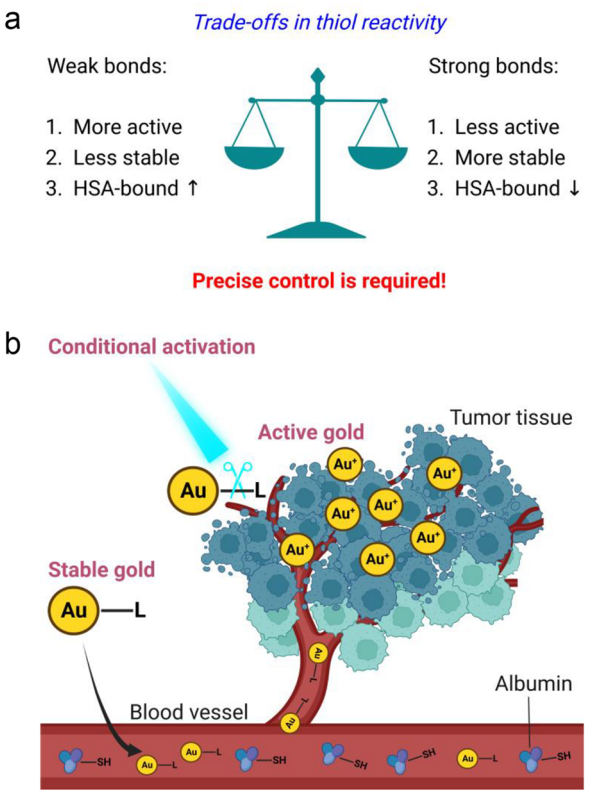

Figure 9. (a) The challenge of tuning thiol reactivity of gold compounds. (b) Stimuli-activatable strategy of gold complexes to minimize the influence of nontarget thiols. Created with permission from Biorender.com.

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complexes (with strong donor ligands) whose thiol-reactivity can only be conditionally activated. Concretely, those complexes are prodrugs which usually remain intact in physiological conditions and will only be uncaged by a certain stimulus such as light or tumor-targeting agents. Those prodrugs successfully combated the off-target interference by serum albumin and resulted in reduced side toxicity and enhanced tumor selectivity (Figure 9b). Importantly, the tactics presented in our works are possibly expanded to other metallodrugs: (1) As a common mechanism in metal-based catalysis, transmetalation carried out for Au(I) species may be implemented in Pt or Ru complexes; (2) photoinduced β-hydride elimination may also occur in the isoelectronic Pt-based drugs; and (3) the oxygen-dependent PDT−PACT process in the gold(III)-alkynyl compound may similarly exist in other photocatalytic prodrug activation processes.

For future development, two questions remain to be addressed: (1) Can the gold-based prodrug be activated by light in the red region together with high efficiency? We have demonstrated that protoporphyrin IX is able to activate gold(III)-alkynyl complexes by 630 nm light, but the activation efficiency is quite low particularly compared to its intrinsic photoreactivity by blue light irradiation. (2) Or if the prodrugs are possibly activated by more biocompatible stimuli such as those endogenous triggers that are specifically expressed or enriched in the tumor tissues? A possible answer to this question in our work is the use of endogenous tumor-enriching riboflavin as a PDT agent to stimulate the PACT process of the gold(III) prodrug. Future development may require more efforts on rational design, followed by large-scale screening. Fluorescence-based screening strategies may realize a high-throughput identification of potential strategies as we did in optimizing suitable palladium reagents for Au(I) activation. Of note, a rapid way to generate a large-scale library of gold-based complexes with expanded chemical diversity will increase the chance of discovering better scaffolds.

In view of the findings and discussions summarized in this Account, we expect that our recent works on modulating the chemical reactivities of gold-based prodrugs in living systems will inspire future advancements of gold-based anticancer drugs. Along with deeper investigations on their modes-of-action, it is hoped that gold-based compounds could function as delicate modulators of biological pathways in more disease models, not limited to cytotoxic agents, and move a step further toward the clinic.

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Notes

The authors declare no competing financial interest.

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