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Gold compounds for catalysis and metal-mediated transformations in biological systems

Sophie R. Thomas[1] and Angela Casini[1,2]

Abstract

One of the challenges of modern inorganic chemistry is translating the potential of metal catalysts to living systems to achieve controlled non-natural transformations. This field poses numerous issues associated with the metal compounds biocompatibility, stability, and reactivity in complex aqueous environment. Moreover, it should be noted that although referring to 'metal catalysis', turnover has not yet been fully demonstrated in most of the examples within living systems. Nevertheless, transition metal catalysts offer an opportunity of modulating bioprocesses through reactions that are complementary to enzymes. In this context, gold complexes, both coordination and organometallic, have emerged as promising tools for bio-orthogonal transformations, endowed with excellent reactivity and selectivity, compatibility within aqueous reaction medium, fast kinetics of ligand exchange reactions, and mild reaction conditions. Thus, a number of examples of gold-templated reactions in a biologically relevant context will be presented and discussed here in relation to their potential applications in biological and medicinal chemistry.

Addresses

[1] School of Chemistry, Cardiff University, Main Building, Park Place, CF10 3AT, Cardiff, United Kingdom

[2] Chair of Medicinal and Bioinorganic Chemistry, Department of Chemistry, Technical University of Munich, Lichtenbergstr. 4, 85748, Garching, Germany

Corresponding author: Casini, Angela (angela.casini@tum.de)

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Gold complexes, Catalysis, Cross-coupling reactions, Bio-orthogonal transformations, Therapy.

Introduction

The use of metal complex catalysts within living biological systems is an area of research that has recently gained significant attention [1–6]. Metal-mediated reactions have potential use for biological sensing, imaging, and caging applications, as well as for therapy. Certainly, the peculiar reactivity and selectivity of metal-based complexes significantly broadens the scope of the chemical reaction toolbox for biomolecule modification. Current examples of metal-catalysed reactions in biological settings and of therapeutic relevance include redox-active transition metal complexes that can be used to promote the formation of reactive oxygen species or metal-bound oxygen species, responsible for subsequent oxidative damage to biological targets [7–9], and that are regenerated through electron transfer from intracellular reducing biomolecules [10]. In this category of reactive oxygen species–generating compounds, metal complexes producing singlet oxygen inside cells upon irradiation with light (photodynamic therapy, PDT) are relevant, with some examples already close to clinical application [11].

In addition to redox processes, the chemistry of transition metal complexes has been rapidly expanding to other types of transformations in living conditions, including cross-coupling reactions [12–14], cycloadditions [15], hydrogenation and transfer hydrogenation reactions [16,17], or functional group deprotection (uncaging) reactions [18–21]. It is worth mentioning that the past decade witnessed new ways of tagging proteins with fluorophores or other probes based on metal-templated mechanisms including the Suzuki–Miyaura, Mizoroki–Heck and Sonogashira cross-coupling reactions [4,5,22]. In this context, palladium compounds occupy a pivotal role [5]. Although the field is dominated by homogenous metal-based compounds, heterogeneous Pd catalysts hold promise [23,24], with potential for *in vivo* application. Recent work in the metal promoted C–H functionalization of nucleobases in aqueous media might also lead to future biological applications [25]. All these processes can be exploited for therapy, both to deactivate relevant pharmacological targets and to induce intracellular redox damage, or for prodrug activation *in situ*, as well as for bio-orthogonal modifications of biomolecules in physiological environment enabling studies of biochemical interactions on the single-molecule level.

Recently, some examples have appeared concerning the use of gold compounds for selective modifications of

biomolecules *via* C–C or C-X (X = heteroatom) bond formation for different applications in biological systems. This is not surprising because the power of gold catalysis also stems from the ability of gold cationic complexes to coordinate and activate unsaturated bonds in a chemoselective manner [26–28]. In this review, we will critically present various examples of gold-templated reactions, illustrating the compounds' design concept and proposed mechanisms, as well as the advantages with respect to other metal-mediated approaches. Reports on the selective sensing of 'free' gold ions in cells will be included, in which the ability of Au^{III}/Au^{I} ions to promote cyclization and fluorescence activation of organic molecules containing alkyne groups is exploited. The use of gold-mediated cross-coupling reactions for selective modification of proteins will also be discussed. Furthermore, examples of catalytic gold complexes and their potential therapeutic applications will be highlighted.

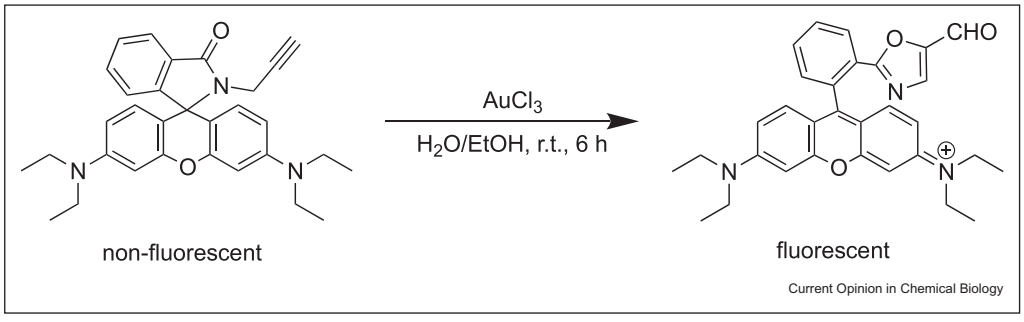
Gold compounds for metal-mediated bioorthogonal modifications and catalysis in cells

To the best of our knowledge, the first report of gold compounds as catalysts in biological conditions was published by Jou et al. in 2009, [29**] when a rhodamine–alkyne derivative was investigated as a selective fluorescence sensor — 'chemodosimeter' — for Au^{III} ions. In detail, the Au^{III} ions mediated the intramolecular cyclization of the propargylamide moiety on the rhodamine derivative to an oxazolecarbaldehyde moiety (Figure 1, Table 1). This instigated over 100-fold enhancement in the substrate fluorescence and induced a colorimetric change to pink from colourless [29**]. This effect was also previously observed in the presence of Pd^{2+} salts; however, addition of stoichiometric amount of an oxidant was required in this latter case [30]. The selectivity of the sensor was evaluated in the presence of a variety of metal ions, including divalent cations of the transition metal series and alkaline and alkaline earth ions; however, fluorescence was produced only in the presence of Au^{III} ions, illustrating its exceptional selectivity [29**]. To elucidate the applicability of the rhodamine–alkyne probe in living systems, the reaction was investigated by fluorescence live cell imaging using HaCaT cells (human keratinocyte cell line). The cells were first incubated with the non-fluorescent probe (20 μM) for 2 h and then with $AuCl_3$ (10 μM) for 1 h. Confocal microscopy images showed intracellular localization of the fluorescence, demonstrating the potential of the probe for applications in biological systems [29**]. In the same year, Yang et al. [31] published a similar work describing the use of a slightly different rhodamine derivative that could act as a probe for Au^{III} ions (Table 1). As in the previous example, the irreversible Au^{III}-promoted cyclization of the nonfluorescent rhodamine amide tethered with an alkyne to a highly fluorescent compound was observed by fluorescence spectroscopy and microscopy in cells [31].

An alternative Au^{III} ion sensor was published one year later by Do et al. [34**] based on an apo-coumarin scaffold rather than the propargylamide-derived rhodamine previously reported [[29**], [31]]. The latent apo-coumarin fluorophore contained a dialkylamino group in para position to the Michael acceptor, favouring the Au^{III}-mediated hydroarylation [34**]. The cyclization reaction *via* C–C bond formation led to a marked increase in fluorescence which could be observed also in HaCaT cells (Table 1) [34**].

After a similar carbocyclization approach, in 2018 Vidal et al. [41**] attempted the Au^{I}-mediated hydroarylation of a pro-coumarin substrate, resulting in a highly fluorescent coumarin product (Table 1). In this case, coordination complexes of the type $[Au^{I}(L)Cl]$ (L = phosphane ligand) were used. Thus, the complex [Au(PTA)Cl] (PTA = 1,3,5-triaza-7-phosphaadamantane) (5 mol%) was tested and the reaction, performed in water/acetonitrile (20% vol) at 37 °C, resulted in excellent yields [41**]. Afterwards,

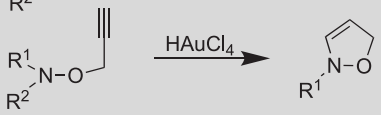
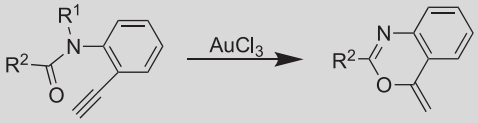
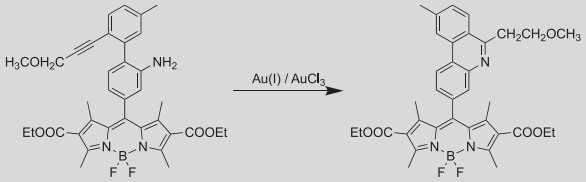
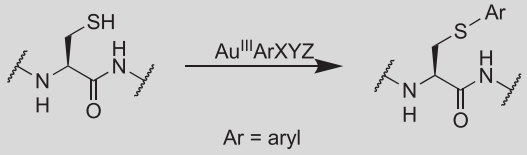
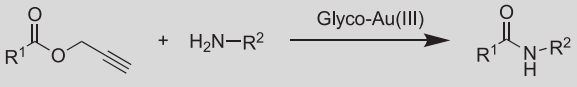
Figure 1



Au^{III}-induced cyclization from propargylamide to oxazolecarbaldehyde [29**].

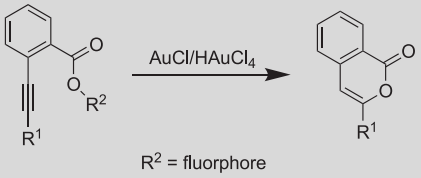
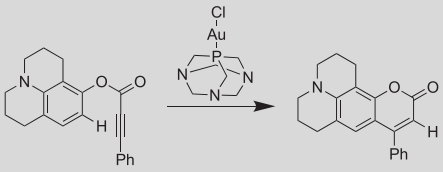
Table 1

Gold complex–mediated reactions in biological systems.

Transformation	Catalyst	Biological system	Year	Reference
Heterocyclization	**Au^{III}**			
oxa-cyclizations				
		HaCaT (cells)	2009	[29**]
		HeLa (cells)	2009	[31]
		N2A (cells)	2014	[32*]
hydroamination				
		HeLa (cells)	2012	[33]
Carbocyclization				
hydroarylation				
		HaCaT (cells)	2010	[34**]
Cysteine Arylation				
		BSA and HSA (protein)	2014	[35**]
		DARPin and FGF2 (protein)	2018	[36**]
		ZF-NCp7 (peptide)	2018	[37*]
		ZF-Cys_2His_2 (peptide)	2019	[38**]
Ester Amidation				
		Mouse (animal)	2017	[39**]

(*continued on next page*)

Table 1 (*continued*)

Transformation	Catalyst	Biological system	Year	Reference
Cyclization *oxa-cyclizations*  R^2 = fluorphore	**Au^I**	A549 (cells)	2012	[40*]
hydroarylation 		HeLa (cells)	2018	[41**]

BSA, bovine serum albumin; HSA, human serum albumin; FGF2, fibroblast growth factor 2; ZF, zinc finger; DARPin, designed ankyrin repeat protein.

the performance of other water soluble Au^I complexes with different phosphane ligands were investigated, also producing the carbocyclization product in very high yields [41**]. The mechanistic hypothesis evidenced the importance of the hydrolysis of the chlorido ligands to foster the desired catalytic reactivity. In this study, Au^{III} complexes or salts were only scarcely reactive. Fluorescent microscopy images were then obtained for HeLa (cervical cancer) cells treated with [Au(PTA)Cl] (50 μM, 30 min) and the pro-coumarin substrate (100 μM, 6 h), resulting in the fluorescent coumarin product accumulating in lysosomes after 6 h incubation [41**].

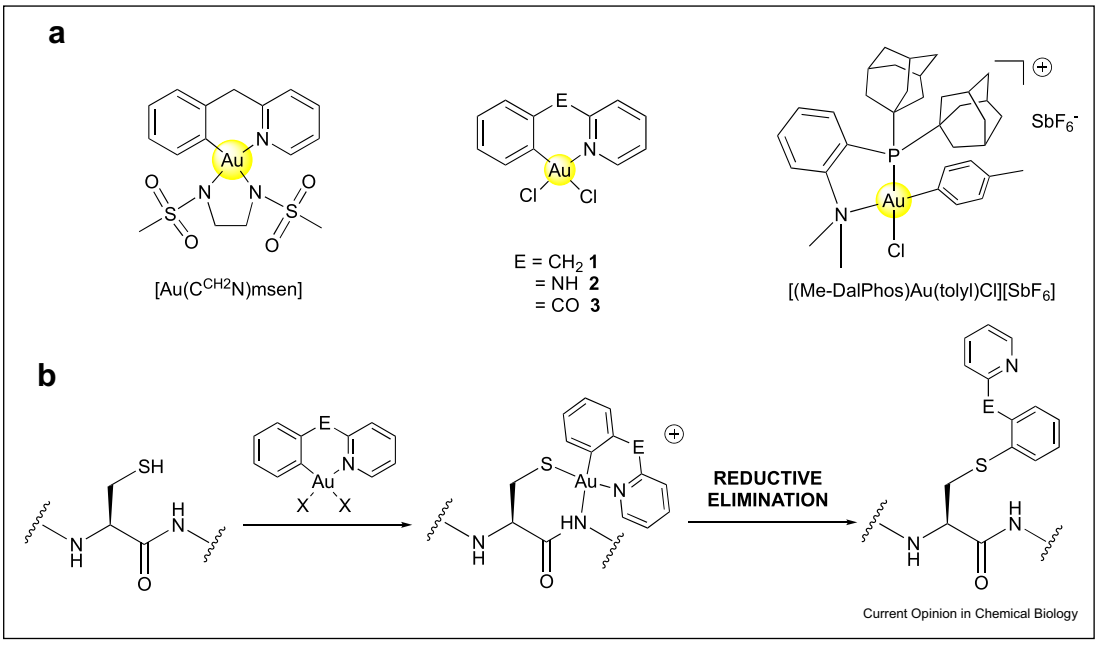
Unfortunately, the presence of excess thiols such as glutathione and cysteine, typically found in physiological conditions, led to a marked inhibition of the catalytic ability of [Au(PTA)Cl], most likely due to the competitive binding of the thiols to the gold [41**]. Therefore, these types of complexes may have scarce chances to be catalytically active *in vivo* unless properly targeted to cancer tissues and protected from speciation. Of note, this work also included a concurrent Ru-mediated deallylation reaction alongside the Au-mediated hydroarylation, with the same substrates [41**]. Infrared fluorescence was observed for the deallylation product, whereas the hydroarylation product produced green and blue emission as seen previously. This is the first example of concurrent and orthogonal Au/Ru-mediated intracellular reactions, providing an initial step towards the production of artificial metabolic networks mediated by metals [41**].

Over the years, different probes for gold ions' sensing in cells were developed, including molecules which underwent heterocyclization reactions (with Au^{III} ions) within a fluorescence resonance energy transfer system [32*], as well as intramolecular hydroamination (for both Au^I and Au^{III} sensing) (Table 1) [33]. In 2012, a different approach was reported by Patil et al. [40*] involving the 'anchoring and unanchoring' of a fluorophore for gold sensing (Table 1), whereby, in a first step, upon addition of a fluorescent molecule to an organic substrate, masking of the fluorescence was observed. In the presence of Au^I ions (and Au^{III}, although at a slower rate), the fluorescent probe is released producing an intense fluorescence signal. When A549 cells (lung cancer cell line) were incubated with the sensor and Au^I ions, green fluorescence appeared intracellularly [40*].

Gold compounds have also been explored as catalysts for selective bio-orthogonal modifications of proteins in living cells. This stemmed from the success of Pd^{II} complexes, including the studies by Vinogradova et al. [42] concerning the arylation of cysteine residues, which are, nevertheless, still hampered by unwanted interactions with endogenous functional groups in cells. This lead to interest in gold complexes owing to their compatibility with catalysis in aqueous environment, mild reaction conditions, chemoselectivity and fast kinetics of ligand exchange reactions [43,44].

In 2014, Kung et al. [35**] were the first to publish on the use of cyclometalated Au^{III} complexes for the arylation of cysteine *via* C–S bond forming reductive elimination (Table 1, Figure 2). This approach was proposed as an alternative to the prevalent *N*-methylmaleimide cysteine ligation, which suffered with lack of stability in physiological conditions [22]. In detail, the modular synthesis of four cyclometalated Au^{III} C^N complexes was reported, including [Au($C^{CH2}N$)msen] ($C^{CH2}N$ = 2-benzylpyridine; msen = *N,N′*-

Figure 2



Gold templated C-S cross-coupling reaction with peptides. (a) Representative organometallic Au^{III} complexes studied for Cys-arylation. **(b)** Mechanisms of Cys-arylation *via* reductive elimination mediated by cyclometalated Au^{III} C^N complexes with Cys residues [35**–38**].

bis(methanesulfonyl)ethylenediamine) (Figure 2), which mediated cysteine arylation in good yields *via* reductive elimination when treated with different cysteine containing peptides in phosphate buffered saline (PBS, pH 7.4): DMSO (9:1) solution at 25 °C for 2 h, as confirmed by liquid chromatography-mass spectrometry (LC-MS).[35**]. The [Au(C^{CH2}N)Cl_2] (1, Figure 2) derivative was also studied, and showed poor cysteine chemoselectivity, evidencing the need for the 'soft' chelating msen ligand to be displaced by 'soft' thiol groups of cysteine forming an initial coordination adduct before reductive elimination. The [Au(phepy) msen] (phepy = 2-phenyl-pyridinate) derivative was not able to give C–S cross-coupling with Cys residues but could only form [Au(phepy)-Cys] adducts [35**]. Furthermore, a cyclometalated Au^{III} C^N dansyl-functionalised cyclometalated compound was incubated with bovine serum albumin and human serum albumin in PBS:DMSO (9:1) at 37 °C for 24 h [35**]. Selective arylation of the single, surface-exposed cysteine in both proteins was successful, as shown by liquid chromatography-tandem mass spectrometry (LC-MS/MS), demonstrating the possibility of protein modifications in biological systems [35**].

In 2018, Messina et al. [36**] were next to report on Au^{III}-mediated cysteine arylation. In this work, 3 equivalent of an oxidative addition complex [(Me-DalPhos) Au(tolyl)Cl][SbF_6] (Me-DalPhos = adamantyl$_2$P(*o*-C_6H_4)NMe_2, Figure 2) showed quantitative arylation of glutathione at 25 °C in H_2O: MeCN (80:20) after a few minutes. The gold-mediated reaction was compatible with a large pH range, several buffers, the disulfide reducing agent tris(2-carboxyethyl)phosphine and the protein denaturing agent (guanidine•HCl) [36**]. Derivatives of the lead compound able to perform C–S cross-coupling reaction with complex peptide substrates were also prepared by replacing the tolyl group with biorelevant moieties such as heterocycles, an affinity label fluorescent tag, a drug molecule and a poly(-ethylene glycol) polymer [36**].

The biocompatibility of the organometallic Au^{III} system was demonstrated further by cysteine arylation of designed ankyrin repeat protein and fibroblast growth factor 2, as observed by LC-MS analysis [36**]. Afterwards, the Au^{III} compound was challenged against a comparable Pd^{II} complex reported for cysteine arylation [42], with respect to GSH arylation [36**]. The results showed ca. 92% conversion to the Au-mediated conjugate, indicating that substrate arylation occurred at a quicker rate than the palladium-mediated arylation [36**].

The success of gold-mediated cysteine arylation by [Au^{III}(C^{CH2}N)Cl_2] (1, Figure 2) was confirmed by de Paiva et al. [37*], observing the reaction in zinc finger (ZF) protein domains by MS. Of note, in this case, reductive elimination was observed only in reaction conditions far from the physiological ones and after 48 h

incubation [37*]. Moreover, classical Au^{III} complexes with bidentate N-donor ligands were ineffective with respect to Cys arylation, although able to form Au-ZF adducts.

Inspired by these results, our group recently expanded the library of Au^{III} cyclometalated (CˆN) complexes capable of cysteine arylation, including **1**, complex $Au(C^{NH}N)Cl_2$ (**2**, $C^{NH}N$ = N-phenylpyridin-2-amine) and $Au(C^{CO}N)Cl_2$ (**3**, $C^{CO}N$ = 2-benzoylpyridine) (Figure 2) [38**]. Each compound was incubated with a model ZF peptide of the Cys_2His_2 type in $(NH_4)_2CO_3$ buffer (pH 7.4) at 37 °C for 10 min and 24 h, samples were then analysed by high-resolution LC electrospray ionization MS [38**]. All the compounds initially formed classical apo-ZF-[Au^{III}(CˆN)] adducts following chlorido ligands' hydrolysis, as previously demonstrated with different ZFs [45], but the reaction eventually progressed, although at different rates, towards the formation of apo-ZF-[CˆN] adducts, indicative of C–S cross-coupling *via* reductive elimination [38**].

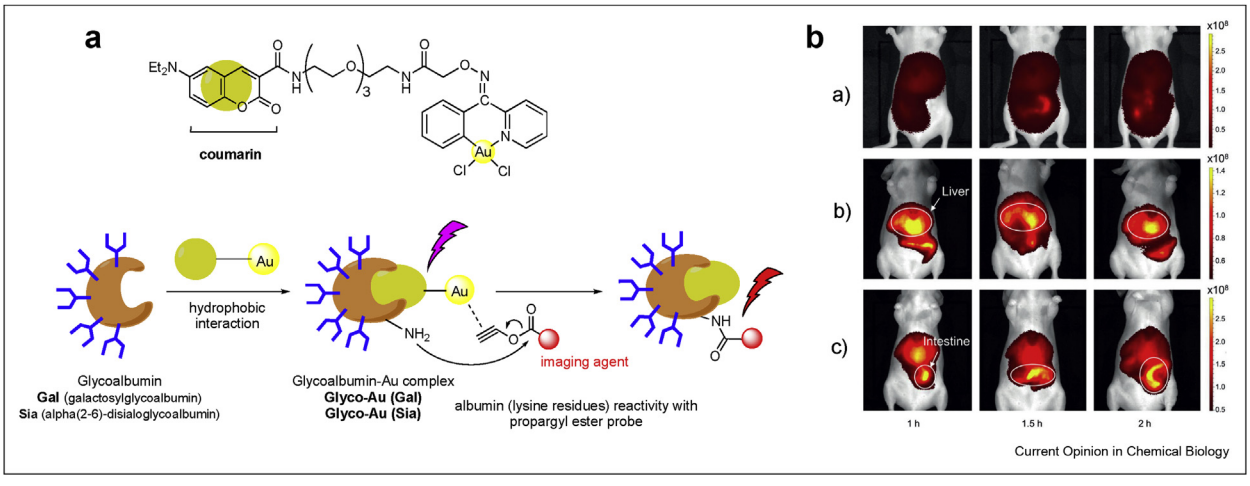
To elucidate the mechanism leading to reductive elimination and to draw initial structure activity relationships, DFT (density functional theory) calculations were performed on the reaction of the complexes with two cysteinate ligands used as models [38**]. Thus, a reaction mechanism was proposed, whereby the first cysteinate binds *trans* to the N and the second one is needed to favour the bond breakage between the nitrogen and the Au^{III} centre forming the intermediate [Au^{III}(CˆN)$(Cys)_2Cl]^-$. This enables rotation of the aryl group, allowing reductive elimination to take place forming the Cys-arylated product and the $[CysAu^{I}Cl]^-$ side complex. The calculated relative standard free Gibbs energy values provided a possible explanation for the increased ability of $Au(C^{CO}N)Cl_2$ to perform reductive elimination compared with $Au(C^{CH2}N)Cl_2$ and even more so $Au(C^{NH}N)Cl_2$. In detail, the activation barrier for the C–S coupling increases in the order **3** < **1** < **2**, following the same trend as the experimental reductive elimination reaction rate [38**].

In 2017, the first *in vivo* study on a gold-templated reaction was published by Tsubokura et al. [39**], whereby a Au^{III} cyclometalated compound conjugated to coumarin was capable of activating propargyl esters featuring another fluorophore (Table 1, Figure 3). In this case, Au^{III}-mediated amide bond formation occurs between the propargyl ester probes and nearby protein-surface amines. The work exploited the use of organ-targeting glycoalbumins which acted as carriers for the Au-coumarin catalyst owing to the strong binding affinity between the hydrophobic coumarin and the binding pocket of albumin [[39**], [46]]. The compound enabled localization in specific organs in mice by fluorescence imaging (Figure 3), providing the proof of concept for a more therapeutically viable Au catalyst [39**].

Summary and perspectives

The use of transition metal catalysis within living systems is nontrivial, due to stability, efficiency and

Figure 3



In vivo gold catalyzed reaction. (a) Structure of the Au-coumarin conjugate and schematic representation of the Glyco-Au (Gal) and Glyco-Au (Sia) complexes and mechanism of reactivity. **(b)** Time-course imaging of liver- and intestine-selective fluorescence labelling of BALB/c nude mice (abdominal view) treated with **(a)** glycoalbumin (Sia) followed by Cy7.5-OProp as a control, **(b)** Glyco-Au (Sia) followed by Cy7.5-OProp, and **(c)** Glyco-Au (Gal) followed by Cy7.5-OProp. The Glyco-Au complexes (3.4 nmol) or glycoalbumin control were injected into 8–10 week old BALB/cAJcl-nu/nu mice *via* the tail vein (N = 6). After 30 min, Cy7.5-OProp or TAMRA-OProp (5.0 nmol) was injected into the mice. They were then anesthetized with pentobarbital or isoflurane and placed in a fluorescence imager, where images were taken at 30-min intervals. After 2 h, the mice were sacrificed and perfused with 4% paraformaldehyde solution, and the fluorescence intensity in the liver was subsequently measured. Adapted with permission from a study by Tsubokura et al. [39**].

potential poisoning of the catalysts. Nevertheless, this field has gained importance over the past few years and provided a number of promising examples. In this context, gold-promoted reactions represent a significant addition to the toolbox of life compatible transformations. As discussed here, recent reports explore gold compounds for a number of metal-mediated bioorthogonal transformations in living systems, specifically aiming at producing novel chemical tools and therapeutic agents. Although until now the few available examples suffer from the side reactivity of the Au^{I}/Au^{III} complexes with biomolecules and intracellular reducing agents which prevent their catalytic action, in the near future this could be overcome by fine-tuning the compounds' redox and nucleophilic properties *via* judicious choice of the ligand system. For example, different families of organometallic gold complexes, endowed with improved stability and robustness of functionalization, could be explored, including Au^{I} N-heterocyclic carbenes and alkynyl complexes, as well as cyclometalated Au^{III} compounds [47,48]. This strategy has already been exploited in classical medicinal inorganic chemistry, where organometallic gold compounds have shown promising anticancer properties *in vitro* and *in vivo* [48]. Furthermore, the incorporation of the gold catalysts into engineered nanometric scaffold (to achieve so-called 'nanozymes') could be envisaged, with the aim of enhancing their water solubility and to provide protection from speciation *in vivo* [49*].

Conflict of interest statement

Nothing declared.

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** of outstanding interest

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