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# The influence of gold(I) on the mechanism of thiolate, disulfide exchange†

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**The mechanism of gold(I)-thiolate, disulfide exchange was investigated by using initial-rate kinetic studies, 2D ($^1$H–$^1$H) ROESY NMR spectroscopy, and electrochemical/chemical techniques. The rate law for exchange is overall second order, first order in gold(I)-thiolate and disulfide. 2D NMR experiments show evidence of association between gold(I)-thiolate and disulfide. Electrochemical/chemical investigations do not show evidence of free thiolate and are consistent with a mechanism involving formation of a [Au–S, S–S], four-centered metallacycle intermediate during gold(I)-thiolate, disulfide exchange.**

Thiol-disulfide exchange is an essential feature of structural and functional modifications that occur in many proteins. Formation of mixed disulfides between proteins and glutathione or cysteine is thought to play a role in cell signalling and regulatory pathways that are important in oxidative stress, apoptosis and aging.[1–4] For example, S-glutathionylation, which protects proteins against irreversible oxidation, can occur *via* exchange of glutathione disulfide with protein sulfhydryls to create a mixed protein-glutathione disulfide.[5] The mechanism of thiol-disulfide exchange is generally accepted as consisting of deprotonation of thiol, followed by $S_N2$ attack of thiolate on disulfide, and proceeds through a transition state with a linear orientation of the three sulfur atoms.[6–8]

While it has been noted that metals, such as $Hg^{2+}$ exert deleterious effects by directly inducing oxidative stress,[9] other authors have pointed out that metals may impart protection against oxidation through their interaction with cysteine thiolate. This has led us to the interesting question: are metals capable of inserting into the thiol-disulfide exchange reaction, and if so, what is the effect on the mechanism of the reaction?

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† Electronic supplementary information (ESI) available: Additional experimental details are provided for initial rate experiments, free thiolate trapping studies, 2D ($^1$H–$^1$H) NOESY and ROESY NMR spectroscopy, and DFT calculations. See DOI: 10.1039/c6dt01400c

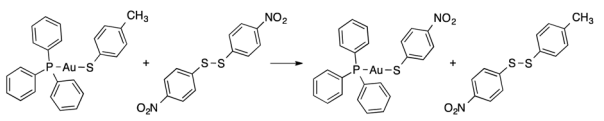
We and others have shown that the thiophilic metal ions, $Zn^{2+}$ and $Au^{+}$ can participate in thiolate-disulfide exchange.[10–14] We have been investigating metal-mediated thiol-disulfide exchange reactions to understand the mechanism, rate, redox potential and other electronic properties (*e.g.* dielectric effects of solvents).[15–17] Our goal is to assess the likelihood that $Zn^{2+}$ and $Au^{+}$ can influence cellular redox processes involving thiol-disulfide exchange.

Zinc is an essential transition metal that plays an important role in cellular antioxidant defense.[18,19] Although $Zn^{2+}$ is not a redox active metal ion, coordination environments with cysteine thiol ligands permit reversible redox reactions leading to release and binding of $Zn^{2+}$.[20–25] For example, release of $Zn^{2+}$ from metallothionein is proposed to occur *via* interactions of the protein with glutathione disulfide, which increases in concentration during oxidative stress.[26,27] Such a mechanism is supported by studies on small molecule zinc thiolate complexes, which undergo thiolate-disulfide exchange.[10,28,29] In addition, calculational results suggest a role for $Zn^{2+}$ in protein-thiolate, disulfide exchange.[30]

Gold(I) has been used for more than 100 years in the treatment of rheumatoid arthritis (RA) and is currently a focus of research aimed at developing new approaches for treatment of cancer and other diseases.[31–39] Although there have been numerous studies on the biological activity of gold drugs, their mechanism of action is not fully understood. Gold(I) has a high affinity for sulfur and selenium and cellular targets for gold compounds primarily include cysteine and/or selenocysteine-containing proteins.[31,32,34] Early studies demonstrated that gold accumulates in inflamed synovial tissues,[32,40] and similarly to other heavy metals, $Au^{+}$ ions lead to expression of thionein.[41] Studies also indicate that bio-released gold(I) ions and the orally active RA drug, auranofin, exhibit anti-inflammatory activity in mouse models for brain injuries.[42,43] More recent studies have demonstrated that $Au^{+}$ triggers release of $Zn^{2+}$ from a zinc finger protein.[44] In addition, thioredoxin reductase, a selenoenzyme in the thioredoxin system that is integral to cellular redox regulation, has been identified as a target for gold compounds.[32,34,45–47]

We report here kinetic and mechanistic studies for the exchange reaction of $Ph_3PAuSC_6H_4R$ (R = $CH_3$, $NO_2$) and $O_2NC_6H_4SSC_6H_4NO_2$ which provide insight into the influence of gold(I) on the mechanism of thiolate, disulfide exchange.

The method of initial rates[48] was used to study the reaction of $Ph_3PAuSC_6H_4CH_3$ with $O_2NC_6H_4SSC_6H_4NO_2$ (eqn (1)) in acetonitrile. The choice of acetonitrile comes from other preliminary work, which examined the influence of solvent on the reaction of phosphine gold thiolates and disulfides. In DMSO solution, a side reaction occurs that produces $Ph_3P{=}O$.[17] In contrast, $Ph_3P{=}O$ does not form in acetonitrile and the reaction proceeds cleanly to the product gold(I)-thiolate and mixed disulfide shown in eqn (1) at early stages of the reaction, while at longer reaction times the symmetric disulfide, $CH_3C_6H_4SSC_6H_4CH_3$, forms.[15] The reaction was monitored by $^1$H NMR array spectroscopy for the first 10 minutes and rates were calculated using the initial formal concentrations of reactants at $t = 0$. The rate of disappearance of the gold(I)-thiolate reactant, which was measured relative to an internal standard, mirrors the rate of appearance of mixed disulfide. The data are shown in Table S1 and Fig. S1 (see ESI†). Analysis of the results reveals a rate law that is overall second order, first order in $[Ph_3PAuSC_6H_4CH_3]$ and $[O_2NC_6H_4SSC_6H_4NO_2]$ with $k_{obs} = 0.422 \pm 0.014\ M^{-1}\ s^{-1}$ (eqn (2)).




(1)

$$\frac{-\mathrm{d}[Ph_3PAuSC_6H_4CH_3]}{\mathrm{d}t} = k_{obs}[Ph_3PAuSC_6H_4CH_3][(O_2NC_6H_4S)_2] \quad (2)$$

Since the mechanism of metal-free thiol-disulfide exchange involves attack of thiolate on disulfide in the rate determining step, we considered the possibility that free thiolate, which might form *via* dissociation from the gold(I) complex, is responsible for the reaction with disulfide. To examine this possibility, we conducted several lines of investigation: (1) chemical thiolate trapping, (2) electrochemical studies, (3) kinetics studies in the presence and absence of air and (4) kinetic studies in the presence of added thiolate.

## Chemical thiolate trapping studies

We selected trimethylphosphate [$(CH_3O)_3PO$, TMP] as a chemical thiolate trap, since TMP has been shown to react with free thiolate.[49,50] In a control experiment, $CD_3CN$ solutions of 0.25 mM TMP and 0.05 mM $[Me_4N][SC_6H_4CH_3]$, prepared in an Ar-filled drybox, showed clear evidence in the $^1$H NMR of formation of dimethylphosphate (DMP; doublet at 3.36 ppm) within 15 minutes. Additional aliquots of $[Me_4N][SC_6H_4CH_3]$ (to a concentration of 0.55 mM) and longer reaction times (24 hours) were required to detect a significant amount of the methylated sulfur compound, $CH_3SC_6H_4CH_3$ (singlets at 2.44 and 2.28 ppm, respectively) (eqn (3)).

$$(CH_3O)_3PO + CH_3C_6H_4S^- \rightarrow CH_3SC_6H_4CH_3 + (CH_3O)_2PO_2^- \quad (3)$$

In contrast, $CH_3SC_6H_4CH_3$ was not detected in the $^1$H NMR of a mixture of a 10-fold excess of TMP (50 mM) $Ph_3PAuSC_6H_4CH_3$ (5 mM) and $O_2NC_6H_4SSC_6H_4NO_2$, (5 mM) even after 40 hours. (We note that within 15 hours, the gold(I) thiolate complex has completely reacted *via* thiolate disulfide exchange, *i.e.* eqn (1).) Similarly, the self-exchange reaction of $Ph_3PAuSC_6H_4NO_2$ (5 mM) and $O_2NC_6H_4SSC_6H_4NO_2$ (5 mM), was monitored in the presence of TMP (50 mM) with no evidence of formation of $CH_3SC_6H_4NO_2$ up to 40 hours. Finally, the rate of gold-thiolate disulfide exchange for 1.27 mM $[Ph_3PAuSC_6H_4CH_3]$ and 1.17 mM $[O_2NC_6H_4SSC_6H_4NO_2]$ was measured in the absence and in the presence of 4 mM TMP. Results indicate that the rate of exchange is unaffected by the addition of TMP. However, interpretation of these results is somewhat limited by the slow kinetics of the reaction of TMP with free thiolate. Therefore we turned to electrochemical techniques for the detection of thiolate.

## Electrochemical studies

Cyclic voltammetry (CV) experiments were conducted first to determine the potentials at which free thiolate *vs.* gold(I)-thiolate oxidizes. Oxidation of free thiolate has been shown to occur at significantly lower potentials than when it is coordinated to metals.[51–53] For example, Savéant *et al.*,[53] measured the formal potentials of aryl thiolates ($ArS^{\bullet}/ArS^-$) using fast microelectrode cyclic voltammetry in acetonitrile and determined that the formal potential for the oxidation of *p*-methylbenzenethiolate occurs at +0.04 V (*vs.* SCE). In comparison, oxidation of the coordinated thiolate ligand in $Ph_3PAuSC_6H_4CH_3$ occurs at >+0.55 V.[51,52] We note that due to the irreversible nature of these sulfur-based redox couples, the peak oxidation potentials observed in cyclic voltammetry studies vary depending on scan rate (*e.g.* 20–500 mV $s^{-1}$), working electrode (Pt, GC), solvent ($CH_3CN$, $CH_2Cl_2$) and electrode adsorption effects.[51,52] Accordingly, when making a comparison of the redox properties of sulfur-containing compounds, it is useful to employ the same experimental conditions. For example, Fig. 1 shows the CV scans of $Ph_3PAuSC_6H_4CH_3$ and $[Me_4N][SC_6H_4CH_3]$, both at concentrations of 1.9 mM in 0.1 M TBAH/$CH_3CN$ solutions at 250 mV $s^{-1}$. Oxidation of free thiolate (−0.003 V *vs.* Ag/AgCl; Fig. 1b), occurs 750 mV lower in potential than the metal-coordinated thiolate (+0.76 V *vs.* Ag/AgCl) (Fig. 1a).

A similar comparison of CVs of 1.9 mM $Ph_3PAuSC_6H_4NO_2$ and 1.9 mM $[Me_4N][SC_6H_4NO_2]$ is shown in Fig. S2 (see ESI†). Oxidation of free thiolate ($[^-SC_6H_4NO_2]$: +0.42 V *vs.* Ag/AgCl; Fig. S2b†), occurs 720 mV lower than the metal-thiolate ($Ph_3PAuSC_6H_4NO_2$: +1.14 V *vs.* Ag/AgCl; Fig. S2a†).

Constant potential electrolysis experiments were conducted to determine whether there was a significant amount of free thiolate in solutions of $Ph_3PAuSC_6H_4CH_3$. Fig. S3† shows

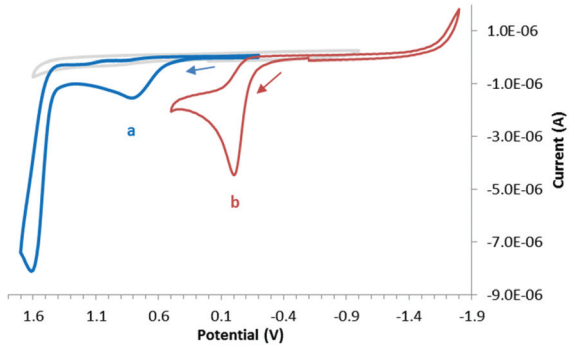



**Fig. 1** Cyclic voltammograms of (a) 1.9 mM $Ph_3PAuSC_6H_4CH_3$ and (b) 1.9 mM $[Me_4N][SC_6H_4CH_3]$ in 0.1 M TBAH/$CH_3CN$ in a two-compartment cell. Pt *vs.* Ag/AgCl|Pt, 250 mV $s^{-1}$. The background current is light gray.

current *vs.* time traces during constant potential electrolyses experiments performed near 0 V *vs.* Ag/AgCl (in Ar-purged, stirred $CH_3CN$ solutions). Fig. S3a† shows the current as a function of time for a 1.9 mM $Ph_3PAuSC_6H_4CH_3$ solution held at 0.0 V, while Fig. S3b† shows the significant anodic current observed for oxidation of 1.9 mM $[Me_4N][SC_6H_4CH_3]$ at +0.05 V. The trace for the gold compound registers essentially what is observed without metal complex and demonstrates that there is not an appreciable amount of free thiolate in solutions of $Ph_3PAuSC_6H_4CH_3$. However, this procedure does not allow us to detect trace amounts of free thiolate (≤10 μM).

Differential pulse voltammetry (DPV) was used to detect lower levels of free thiolate in solutions of $Ph_3PAuSC_6H_4CH_3$. A detection limit of approximately 1.5 μM for free thiolate (at −0.1 V *vs.* Ag/AgCl) was estimated from a series of experiments on different concentrations of $[Me_4N][SC_6H_4CH_3]$. In experiments under anaerobic conditions, there was no evidence of the presence of free thiolate in 2.0, 3.0 or 4.0 mM solutions of $Ph_3PAuSC_6H_4CH_3$. DPV experiments were also conducted to qualitatively determine the effect of $O_2$ on solutions of thiolate. A 100 μM solution of $[Me_4N][SC_6H_4CH_3]$, prepared under argon, showed a persistent DPV signal that when subsequently exposed to $O_2$, decreased and completely disappeared within 15 minutes.

## Kinetics studies in the presence and absence of air

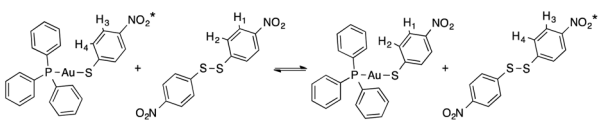
The DPV experiment suggests that if free thiolate forms in acetonitrile solutions of $Ph_3PAuSC_6H_4CH_3$, the thiolate would oxidize, resulting in decomposition of the gold complex. We have observed that $CD_3CN$ solutions of $Ph_3PAuSC_6H_4CH_3$ prepared in air are stable for at least 24 hours, as evidenced by $^1H$ NMR; *i.e.* no decomposition is observed. Further, kinetic experiments were conducted to measure the rate of exchange under anaerobic conditions (2.53 mM $Ph_3PAuSC_6H_4CH_3$ and 2.59 mM $O_2NC_6H_4SSC_6H_4NO_2$ solutions) *vs.* aerobic conditions (2.61 mM $Ph_3PAuSC_6H_4CH_3$ and 2.50 mM $O_2NC_6H_4SSC_6H_4NO_2$ solutions). The rate constants of a set of two anaerobic and three air experiments, show no significant differences in the calculated rate constants: 0.41 $M^{-1}$ $s^{-1}$ *vs.* 0.42 $M^{-1}$ $s^{-1}$, respectively.

## Kinetic studies with added thiolate

The effect of micromolar amounts of added thiolate on the gold(I) thiolate disulfide exchange reaction was also determined. Reaction kinetics were measured for solutions of 1.21 mM $Ph_3PAuSC_6H_4CH_3$ and 1.23 mM $O_2NC_6H_4SSC_6H_4NO_2$ with 5, 10, 20, 40 and 70 μM $[Me_4N][SC_6H_4CH_3]$. At the lowest levels of added thiolate (5, 10 and 20 μM) the reaction rate is essentially unchanged, while at higher levels there is a slight increase in rate (*e.g.* 2× at 70 μM). Since the rate constant for free thiolate disulfide exchange is estimated to be $10^4$ $M^{-1}$ $s^{-1}$ in acetonitrile,[54] if free thiolate was mechanistically the reactive species, addition of free thiolate would be predicted to lead to dramatic increases in the rate of exchange, which is not observed. Preliminary evidence by NMR suggests that the addition of free thiolate results in exchange of the gold bound thiolate and may lead to the formation of a new gold complex. Further experiments are required to understand this process.

Taken together, the trapping experiments involving TMP, the electrochemical studies and kinetic experiments with added thiolate indicate that free thiolate is not detectable in any significant quantity either from solutions of gold(I)-thiolate, or during the gold(I)-mediated thiolate, disulfide exchange reactions in acetonitrile. We note that other similar studies such as zinc(II)-thiolate, disulfide exchange in DMSO,[10] and gold(I)-thiolate, disulfide exchange in $CH_2Cl_2$,[11] have arrived at similar conclusions.

To gain additional mechanistic insight, the self-exchange reaction shown in eqn (4) was studied using 2D ($^1H$–$^1H$) ROESY NMR spectroscopy carried out in solutions of 5.0 mM $Ph_3PAuSC_6H_4NO_2$ and 5.0 mM $NO_2C_6H_4SSC_6H_4NO_2$ in $CD_3CN$. A star is used in eqn (4) to label the self-exchange reaction. Numbered hydrogen atoms on the thiolate and disulfide moieties are used to discuss the 2D NMR spectra.




There are two different colors of off-diagonal cross-peaks in the 2D ($^1H$–$^1H$)-ROESY NMR spectrum (Fig. 2A). The blue peaks are indicative of association while the red peaks indicate exchange. The blue cross-peaks B1 and B2 at the intersections of H2–H3 and H1–H4 indicate intermolecular NOE's caused by association between gold(I)-thiolate and disulfide. The blue cross-peaks B3 and B4 at the intersection of H1–H2 and H3–H4 are also caused by association, but these occur *via* intramolecular NOE's between adjacent aromatic protons. (Note: the H4 protons are partially obscured by aromatic protons on

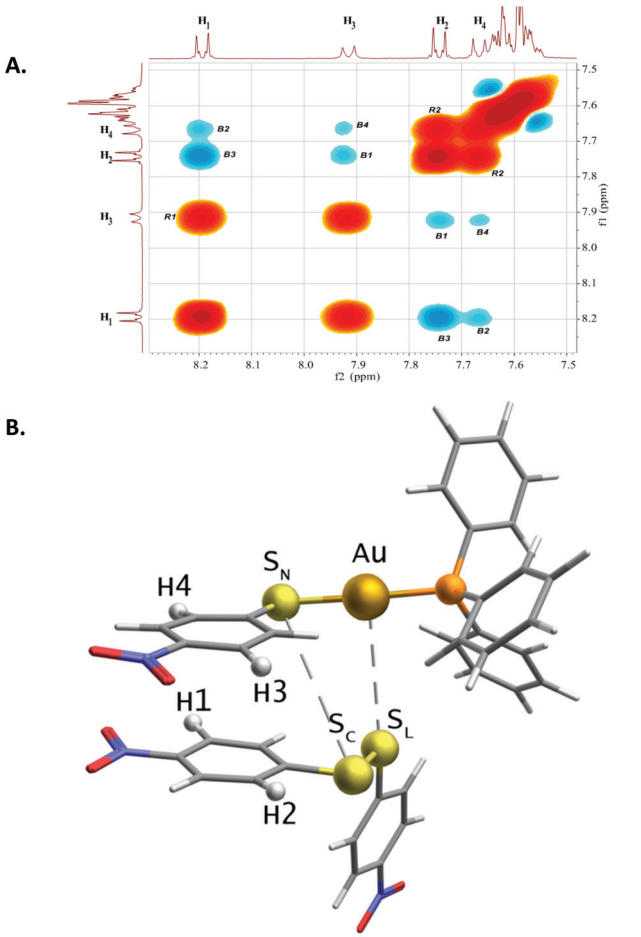



**Fig. 2** A. 2D ($^1$H–$^1$H)-ROESY NMR spectrum of involving $Ph_3PAuSC_6H_4NO_2$ and $NO_2C_6H_4SSC_6H_4NO_2$. B. A cartoon to help visualize the intermolecular association (dashed lines) between $Ph_3PAuSC_6H_4NO_2$ and $NO_2C_6H_4SSC_6H_4NO_2$, generated from a minimized DFT calculation by tethering H1–H4 and H2–H3 distances at 3.1 Å (suggested from 2D ($^1$H–$^1$H)-ROESY NMR results).

the $Ph_3P$ ligand.) The red cross peaks, R1 and R2 indicate chemical exchange between H1–H3 and H2–H4 respectively, and arise from "nonproductive" thiolate disulfide exchange between $Ph_3PAuSC_6H_4NO_2$ and $NO_2C_6H_4SSC_6H_4NO_2$ (eqn (4)).

A key feature of the 2D ($^1$H–$^1$H) ROESY spectrum is the set of blue cross peaks, B1 and B2, indicating intermolecular long-range interactions between H2–H3 and H1–H4. The distances between these spatially close nuclei can be estimated,[55] from the relationship: $(r_{ab}/r_{ref}) = (\eta_{ref}/\eta_{ab})^{1/6}$. For our calculation, $r_{ref}$ was chosen as the intramolecular distances between H1 and H2 in the disulfide. The crystal structure of the symmetrical disulfide, $(SC_6H_4NO_2)_2$, is available in the Cambridge Structural Database (CSD Refcode: NIPHSS),[56,57] and the average distance between these protons is 2.365 Å. Using this value and the experimental ROE volumes, the spatial separation between H1–H4 and H2–H3 was estimated to be 3.1 Å.

To help visualize how intermolecular interactions might lead to gold(I)-thiolate, disulfide exchange, we tethered H1–H4 and H2–H3 at a distance of 3.13 Å and performed a DFT calculation to minimize the structure.[58] The result is illustrated in Fig. 2B, which shows a cartoon rendered with Autodesk 3DS MAX 2013. ($S_N$ stands for the nucleophilic sulfur, $S_C$ is the central sulfur, and $S_L$ is the leaving sulfur.) The $S_N$–Au bond length (2.36 Å) is typical of lengths in Au(I)-thiolate complexes. The nucleophilic sulfur ($S_N$) approaches the disulfide sulfur ($S_C$) at a distance 5.34 Å. The $S_C$–$S_L$ bond length is 2.07 Å while the $S_L$···Au distance is 4.20 Å. When the constraints on the H1–H4 and H2–H3 distances are removed and the structure is reoptimized, the disulfide and gold complex move apart, but the orientation is similar to the conformation shown in Fig. 2b (see S4b†). The H2–H3 distance increases from 3.13 Å to 3.59 Å, while the H1–H4 distance increases to 6.28 Å. The $S_N$···$S_C$ and $S_L$···Au distances also increase significantly.

The conformation shown in Fig. 2b was estimated to be approximately 13 kJ mol$^{-1}$ higher in energy than the separated reactants and approximately 9 kJ mol$^{-1}$ higher than the minimized structure without constraints on the H1–H4 and H2–H3 distances. This suggests that the conformation shown in Fig. 2b would be accessible at room temperature. Further, such an association can be imagined to lead to gold(I)-thiolate, disulfide exchange through a (Au–$S_N$, $S_C$–$S_L$) 4-centered metallacycle arrangement. We note that this arrangement is nearly identical to the calculation recently reported for the transition state involving zinc(II)-thioredoxin, glutathione disulfide exchange.[30] The metal-mediated mechanism differs significantly from non-metal thiol-disulfide exchange, which proceeds through a linear arrangement of sulfurs. This difference in mechanism is expected to lead to a different dependence of rates as a function of solvent for metal *vs*. non-metal mediated thiol-disulfide exchange, which is currently being investigated. Finally, based on our results, we suggest that further studies are needed to eliminate the possibility that trace free thiolate at levels below 100 nM may influence gold(I)-thiolate, disulfide exchange in acetontrile.

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