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Ultrafast Au(III)-Mediated Arylation of Cysteine

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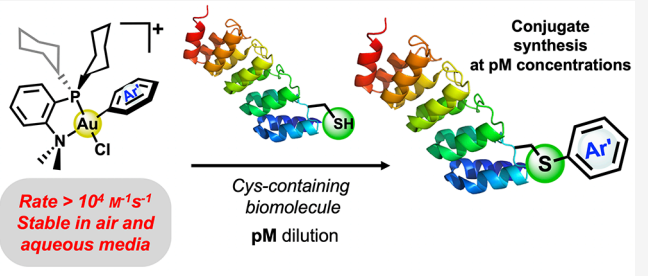
ABSTRACT: Through mechanistic work and rational design, we have developed the fastest organometallic abiotic Cys bioconjugation. As a result, the developed organometallic Au(III) bioconjugation reagents enable selective labeling of Cys moieties down to picomolar concentrations and allow for the rapid construction of complex heterostructures from peptides, proteins, and oligonucleotides. This work showcases how organometallic chemistry can be interfaced with biomolecules and lead to a range of reactivities that are largely unmatched by classical organic chemistry tools.



INTRODUCTION

Cysteine (Cys) bioconjugation ranks among the cornerstone methods to access highly tailored biomolecules widely applied in the development of therapeutics, diagnostic methods, and research tools in chemistry, biology, and materials science.[1−10] Effective Cys bioconjugation reagents, and bioconjugation reagents generally, must perform under mild reaction conditions compatible with biomolecules, modify desired targets with a high degree of selectivity, and display efficient reaction kinetics (Figure 1A).[11−25] For Cys bioconjugation reagents, the reaction kinetics are often a readily modulated parameter, which has led to the development of reagents that display "fast Cys bioconjugation", with second-order rate constants greater than 100 $M^{-1}s^{-1}$.[26] Previously developed reagents for Cys modifications cover a broad kinetic spectrum of reactivity (10^{-1}−10^{2} $M^{-1}s^{-1}$) and include commonly used organic species such as alkyl halides, maleimides, and heteroaromatic nitriles (Figure 1B).[1,6,9,10,12,20,26,27] The unifying mechanistic theme of these reagents centers on their ability to selectively undergo nucleophilic substitution or addition of a thiol or thiolate, depending on the pH of the media. In the past decade, chemists have developed new variants of organic reagents that have significantly improved kinetics of the Cys bioconjugation with second-order rate constants as high as 5.5×10^{3} $M^{-1}s^{-1}$.[28,29] After our present study was posted on a preprint server,[30a] an elegant strategy utilizing sulfone-activated pyridinium salts for Cys arylation displaying second-order rate constants up to 8.7×10^{5} $M^{-1}s^{-1}$ was reported. The fastest of these reagents, however, have a limited half-life (5 min −1 h) under aqueous conditions and have not been shown to perform bioconjugations at submicromolar concentrations.[30b]

Several classes of organometallic compounds have recently emerged as highly selective reagents that can produce stable Cys bioconjugates featuring S−C_{sp^2}, S−C_{sp^3}, and S−B bonds in peptides and proteins.[31−44] These transformations can exhibit fast reaction kinetics, reaching completion in seconds to minutes at micromolar concentrations of biomolecules. Among the existing pool of organometallic reagents, there are now several examples of group 10 or 11 metal-based compounds capable of transferring an aryl group to a Cys residue in biomolecules. Originally inspired by metal-catalyzed cross-coupling chemistry,[45−47] the *ex situ* synthesis of an oxidative addition (OA) complex as a stoichiometric arylation reagent positions these organometallic species outside of their conventional usage in catalytic contexts (Figure 1C). By decoupling the slow OA from the significantly faster subsequent elementary steps, many of the challenges associated with the compatibility of biological systems and transition metal-mediated catalytic processes (e.g., the use of organic solvents, elevated temperatures, rigorously anaerobic and/or moisture-free conditions, high catalyst loading, and careful selection of ligands) are simplified or eliminated.[46−52] Mechanistically, the overall Cys arylation process facilitated by organometallic OA complexes is hypothesized to rely on the combination of two elementary steps: bioconjugation via Cys thiol coordination to the metal center and subsequent

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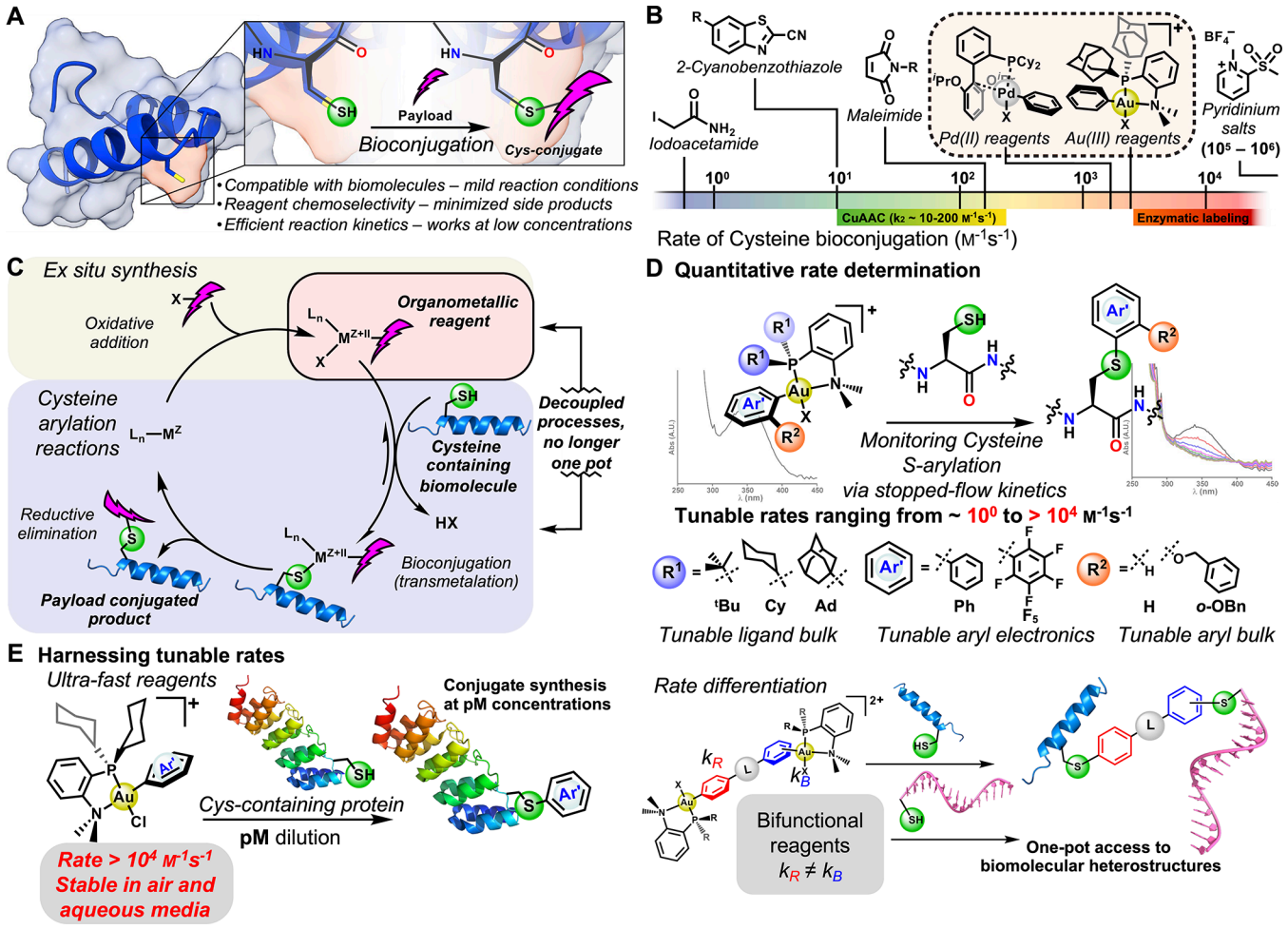

Figure 1. Overview of Cys arylation and the development of kinetically tunable Au(III) Cys arylation reagents. (A) Cartoon of the Cys bioconjugation process and key parameters for designing effective reagents. (B) Kinetic rate spectrum of several key classes of organic reagents commonly used for Cys bioconjugation and the relative placement of organometallic Cys arylation reagents on that spectrum. Copper-catalyzed azaide-alkyne cycloaddition (CuAAC) and enzymatic labeling rates are included for benchmark purposes. (C) Decoupling concept of the catalytic cycle using stoichiometric organometallic Cys arylation reagents. The *ex situ* synthesis of the organometallic Cys arylation reagents effectively decouples the slow oxidative addition step from the subsequent fast transmetalation and reductive elimination components. The cyclic representation does not imply that these reactions occur in one pot like a typical catalytic reaction. (D) Overview of the use of stopped-flow kinetic experiments to quantitatively measure rates of Cys arylation kinetics and leading to the development of kinetically tunable Au(III) reagents. (E) Applications of kinetically tunable Au(III) reagents to synthesize ultrafast reagents and construct hybrid biomolecular conjugates.

reductive elimination to produce highly stable S−C_{aryl} bonds. Together, these two mechanistic steps are generally assumed to be fast and effectively irreversible under the reaction conditions required for Cys modification.[46,48,53−55] Importantly, the mechanistic landscape of these organometallic arylation reagents is distinct from their organic counterparts, which often rely on S_NAr, S_N2, or Michael additions, which can be kinetically slow, reversible, or both.[6,20,26,56]

Previously developed Pd(II) and Au(III) organometallic OA reagents can selectively transfer aryl groups onto Cys residues in peptides and proteins under mild, aqueous conditions, reach reaction completion within minutes, and display high chemoselectivity for Cys compared with other nucleophilic residues, even when used in excess.[31,32,34,40−42,44,57] These reagents can be synthesized in one step, are air-stable, and show little to no decrease in reactivity after months of storage on the bench.[31,34] Despite their facile reactivity, only rudimentary investigations of the kinetic profiles of these systems have been performed. For example, competition experiments suggest that the Au(III) reagents display rates of Cys arylation above 10^3 $M^{-1}s^{-1}$, with later studies hinting that a perturbation within the ligand structure could produce an enhancement in the rate of arylation.[31,39] With a focus on the Au(III) system, we sought to clearly delineate the design principles that lead to kinetically faster Cys arylation reagents through quantitative kinetic analysis of the Cys arylation reactions via stopped-flow kinetic experiments (Figure 1D). As a result of our studies, we developed a kinetic model deconvoluting the influence of the bioconjugation (Cys thiol coordination) and arylation (reductive elimination) steps in the context of overall Cys arylation. This in turn led to the discovery of a Cys arylation reagent, which exhibits a rapid rate of organometallic modification on a Cys (1.7×10^4 $M^{-1}s^{-1}$). Furthermore, by understanding what factors result in kinetically faster or slower arylations, we developed a toolbox of reagents with tunable bioconjugation rates covering 10^0−10^4 $M^{-1}s^{-1}$, based on the initial coordination of the two-step mechanism. Using this toolbox, organometallic bifunctional linkers were synthesized that enabled the one-pot construction of multiple complex biomolecular heterostructures (Figure 1E).[58]

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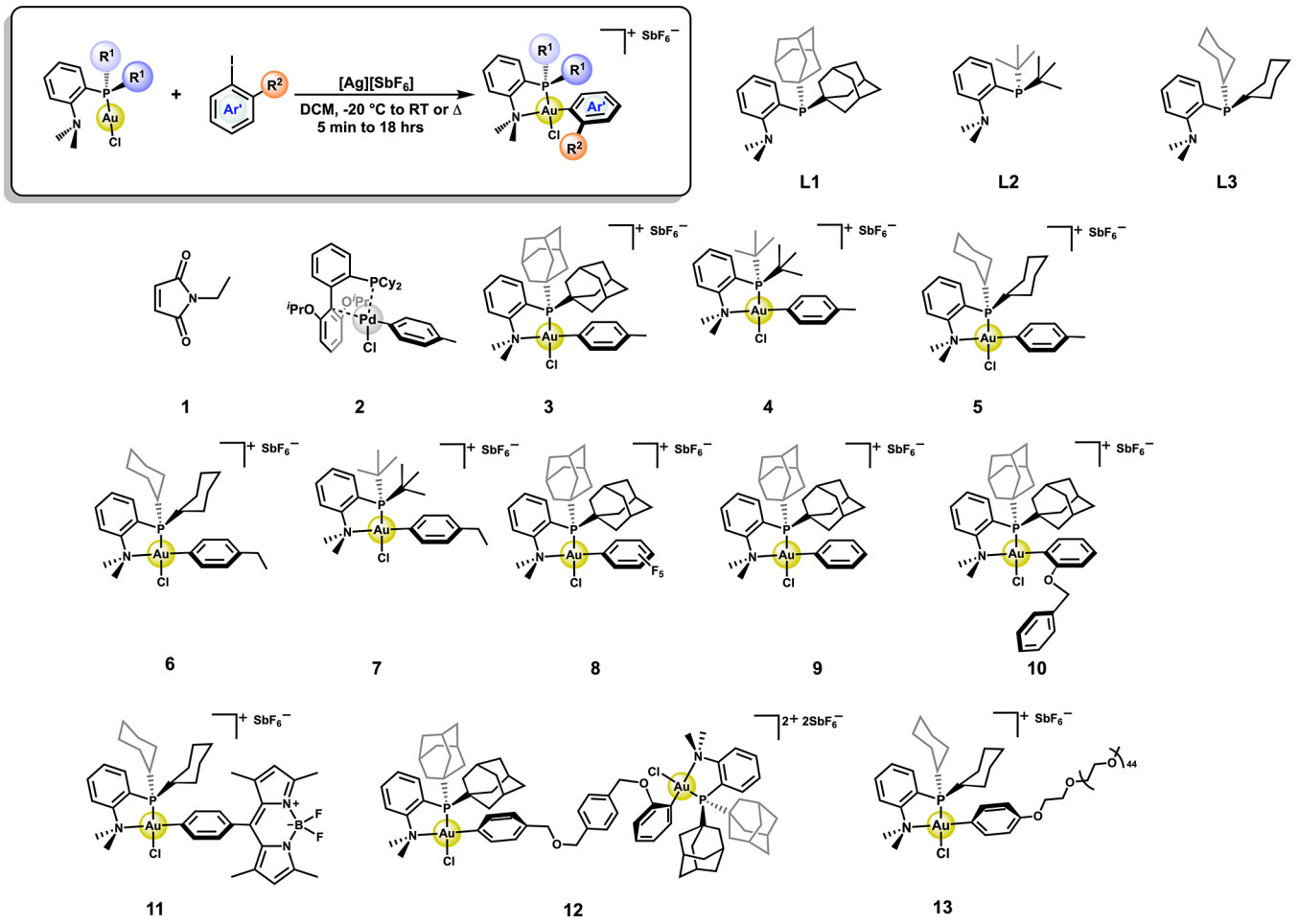

Figure 2. Synthetic scheme and compounds used in this study. (A) General scheme for the synthesis of Au(III) organometallic Cys arylation reagents. (B) Three ligands used to synthesize the Au(III) organometallic complexes. (C) Bioconjugation and Cys arylation reagents used in this study.

RESULTS AND DISCUSSION

Kinetic and Mechanistic Investigations via Stopped-Flow Experiments. In a series of competition experiments, we and others demonstrated that both Pd(II)-tolyl, **2**, and Au(III)-tolyl, **3**, reagents (see Figure 2 and SI section S2.2 for chemical structures and synthetic details) outcompete *N*-ethylmaleimide, **1**, in conjugation experiments.[31,44] However, a quantitative mechanistic understanding of the observed phenomena remains unclear. We therefore commenced our investigations by devising an approach that could shed light on the mechanistic nature of Cys arylation with organometallic reagents. In the past, stopped-flow kinetics experiments have been applied to biochemical processes that are kinetically fast or can be difficult to study via other methods[59−61] and have been employed in the assignment of key intermediates proposed in the mechanisms of metallic cofactors;[62] however, this approach has never been employed for nonenzymatic organometallic bioconjugation reagents. Starting with the conjugation of **GSH** with **1** under pseudo-first-order conditions, stopped-flow experiments were performed. By following the disappearance of the absorbance at 300 nm, which corresponds to the formation of the **1**-**GSH** conjugate (Figure 3A) (see SI section S3), the second-order rate constant of the reaction of **1** with **GSH** was measured as 99.3 ± 6.1 $M^{-1}s^{-1}$. This value of the second-order rate constant with **1** is consistent with literature reports of *n*-alkyl maleimides and validates the use of stopped-flow for determining the rate of Cys bioconjugation in this context.[20] Looking next at the model Pd(II) and Au(III) organometallic reagents **2** and **3**, conjugation experiments were performed under similar conditions to those of the experiments with **1**. Monitoring the absorbances of these reactions over the course of 120 and 2 s, respectively, revealed a nonlinear relationship between the observed rate constants and **GSH** concentration (Figure 3A). This nonlinearity is a characteristic of reactions that feature multistep mechanisms.[63,64] Monitoring the same reactions of **2** and **3** with **GSH** over a shorter time scale of less than 0.2 s revealed a well-defined reaction event, which we postulate is the bioconjugation process featuring coordination of the Cys sulfur ligand to the metal center (Figure 3B).

The observation of two resolvable reaction events within the stopped-flow data provides unprecedented insight into the mechanism of bioconjugation by an organometallic Cys arylation reagent. Using this technique, the presence of two observable and distinguishable mechanistic events provides direct experimental substantiation to the assumption that the mechanism of Cys arylation by an organometallic reagent parallels the elementary steps proposed in a traditional cross-coupling catalytic cycle.[54] Contextualizing these steps within Cys arylation, we postulate sulfur coordination to the metal center followed by reductive elimination to be the elementary

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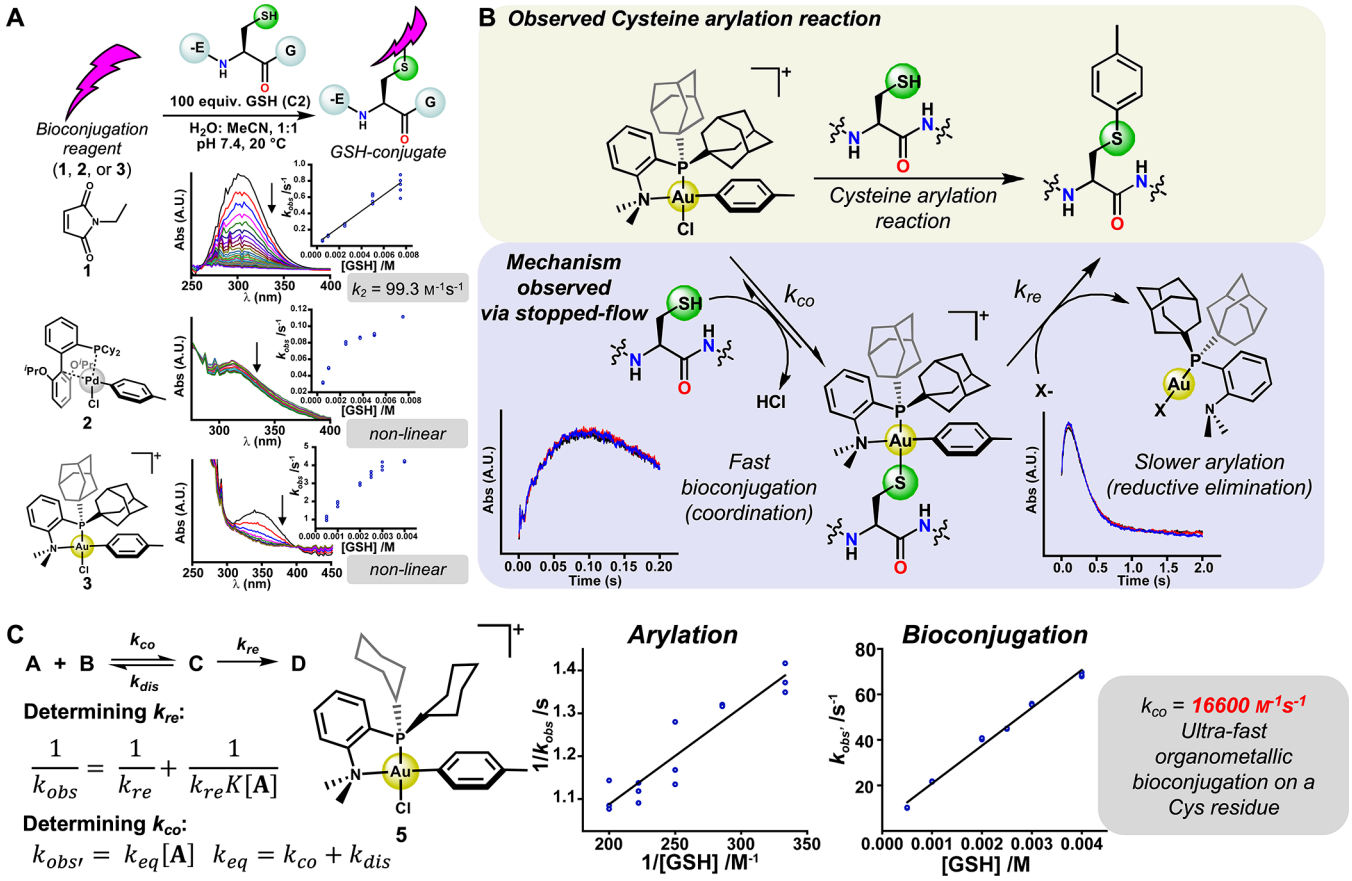

Figure 3. Stopped-flow kinetic analysis of bioconjugation reagents and the development of a mechanistic model. (A) Initial stopped-flow kinetic analyses of a model bioconjugation reaction with **GSH** as the substrate and the reagents **1**, **2**, and **3** performing either the bioconjugation or arylation; the arrow in each absorbance trace indicates a decrease in absorbance over time. (B) Overall observed Cys arylation reaction with the Au(III) organometallic reagent **4** and the proposed two mechanistic steps, bioconjugation and arylation, observed via stopped-flow kinetic analysis. (C) Kinetic data obtained for the organometallic Au(III) reagent **5**.

steps of organometallic Cys arylation (Figure 3B). Additionally, the observation of these two individual steps enables the investigation of their respective kinetic identities and structure−function relationship in the context of tuning the overall reactivity of the arylation reagents.

To probe the kinetics of the fast coordination and the reductive elimination steps of our proposed mechanism, we applied a kinetic model that considers the fast coordination of a Cys thiol to the organometallic center as an initial pre-equilibrium step (Figure 3C). This allowed for the assumption that the slower changes in absorbances observed in the stopped-flow experiments of **2** and **3** were due to the reductive elimination of the conjugated products from the metal centers. Using this model and applying the assumption that the initial coordination has reached equilibrium before reductive elimination occurs, we were able to extract values for the rate of reductive elimination, k_{re}, for both organometallic reagents **2** and **3** of 0.112 and 7.92 s^{-1}, respectively. These first-order rate constants demonstrate the half-life of the metal−sulfur complex to be 6.19 s for **2** and 0.0875 s for **3**. Additionally, we were able to calculate values for the equilibrium constant, K, of the fast coordination of a Cys thiol to **2** and **3** of 701 and 306 M^{-1}, respectively, suggesting that the equilibrium is significantly shifted toward the S-coordinated metal species.

Continuing our analysis, we examined the very fast initial changes in absorbance that are due to the coordination of the thiol to the organometallic center. Neither the rate constants of coordination, k_{co}, nor dissociation, k_{dis}, can be directly measured due to the reversibility of the process.[64] Thus, the second-order rate constant measured experimentally (k_{eq}, calculated from the pseudo-first-order rate constant $k_{obs'}$) describes the system reaching pre-equilibrium. From k_{eq} and K, k_{co} can be calculated for this initial coordination step. The observed second-order rate constants of thiol coordination, k_{co}, for **2** and **3** were found to be 2160 and 2560 $M^{-1}s^{-1}$, respectively. Correlating these results with the observations from previous competition experiments and considering the kinetic profiles of the two mechanistic steps in totality, we note that the initial fast coordination event can be defined as the bioconjugation step in this process. Under the conditions where K is significantly biased toward the S-coordinated metal species, k_{co} represents a key kinetic metric for the bioconjugation process. Furthermore, while the reductive elimination is considered to be the rate-limiting step for the overall Cys arylation process (but not bioconjugation), under these circumstances and when the k_{re} is sufficiently fast, the product of the overall Cys arylation process is ultimately dictated by the rate of the fast coordination event, k_{co}.

To further evaluate how k_{co} can influence the overall Cys arylation process, we investigated possible methods to modulate the rate of coordination of Cys thiol to the Au(III) metal center. As a first step, we evaluated several ligand frameworks containing phosphine moieties with varied steric

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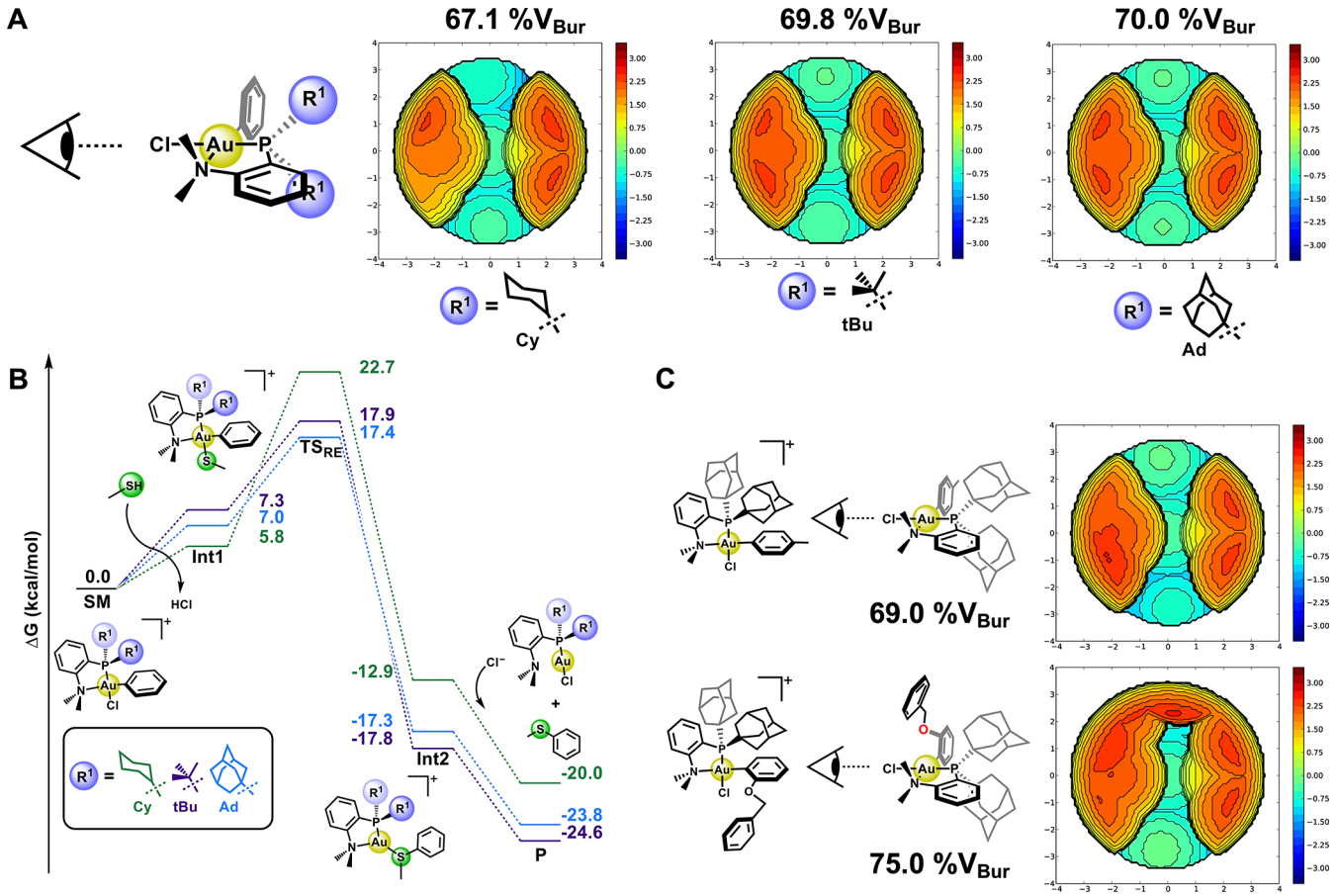

Figure 4. Computational analysis of several Au(III) reagents through $\%V_{Bur}$ and DFT calculations. (A) $\%V_{Bur}$ analysis of the **L1**, **L2**, and **L3** Au(III)-phenyl oxidative addition complexes varying steric bulk used in this study calculated at the ωB97X-D/6-311+G(d,p), SDD, CPCM(Water)//B3LYP-D3/6-31G(d), LANL2DZ, CPCM(Water) level of theory and their corresponding heat maps, looking down the Au−P bond. (B) Free energy diagrams for the model *S*-arylation of methanethiol with Au(III)-phenyl oxidative addition complexes with **L1**, **L2**, and **L3**. (C) $\%V_{Bur}$ analysis of two Au(III) reagents, **3** and **10**, with different steric profiles of their aryl ligands. The steric heat maps are also shown, looking down at the Au−P bond.

bulk (Figure 2C, **3**, **4**, and **5**). When analyzed by stopped-flow experiments, complex **4** (Figure 2C) was determined to have a value for k_{re} of 4.32 s^{-1} and a value for k_{co} of 4220 $M^{-1}s^{-1}$ (see SI section 3.2). Despite the "rate-determining" k_{re} for **4** being almost 50% smaller than k_{re} for **3**, the value for k_{co} is 60% greater than that of **3**. This improvement in k_{co} is directly responsible for the dramatic enhancement in the selectivity of aryl group transfer from **4**, when **3** and **4** are used in competition experiments with **GSH** (see SI section S4.1). For clarity, selectivity is used to describe the preferential formation of one S-aryl product over the other S-aryl product during the competition experiments. These results further highlight the importance of the rate of initial coordination on bioconjugation and corroborate our assumption that the coordination kinetics have a greater influence on determining the selectivity of an aryl group transfer. Strikingly, when the steric profile of the Au(III) ligand framework was further reduced, as in **5**, stopped-flow experiments revealed an ultrafast k_{co} of 16600 $M^{-1}s^{-1}$ when **5** was used in Cys arylation with **GSH**. The observed bimolecular rate constant of 1.7×10^4 $M^{-1}s^{-1}$ in this case represents the fastest bioconjugation rate for an amino acid residue by an organometallic OA reagent reported to date.

The enhanced kinetics and reactivity of the reagents bearing ligand **L3** can raise potential concerns regarding their long-term and operational stability. Despite this, we observe no degradation of reagent **6** bearing **L3** after storage on the benchtop under ambient conditions (>2 weeks at room temperature), as determined by ^{1}H and ^{31}P NMR spectroscopy (see SI Figures S25 and S26). Additionally, a water-soluble reagent, **13**, was monitored by ^{31}P NMR spectroscopy for 20 h and showed no visible degradation (see SI Figure S33). These experiments highlight that the developed Au(III) organometallic reagents are currently the fastest bench-stable Cys modifying bioconjugation tools available to the practitioner.[30b]

Cys Arylation Competition Experiments and Computational Analysis. To corroborate the influence of the phosphine ligand sterics on the mechanistic model proposed by our stopped-flow kinetics experiments, we performed a series of Cys arylation competition experiments with a model unprotected Cys containing peptide, **P1** (see SI section S4.2). The dicyclohexyl phosphino-containing reagents, **5** and **6**, were compared to either the diadamantyl or di-*tert*-butyl phosphino analogues, **3**, **4**, and **7**. In agreement with our kinetic profiles obtained from stopped-flow experiments, **5** and **6** outperformed **3**, **4**, and **7** (see SI Figures S107, S108, and S109), as determined by LCMS analysis of the reaction mixtures. Percent buried volume ($\%V_{Bur}$) calculations (see SI section S5.2) affirm that the steric profile of the phosphine ligand is critical to the coordination of the thiol (Figure 4A). Furthermore, density functional theory (DFT) calculations

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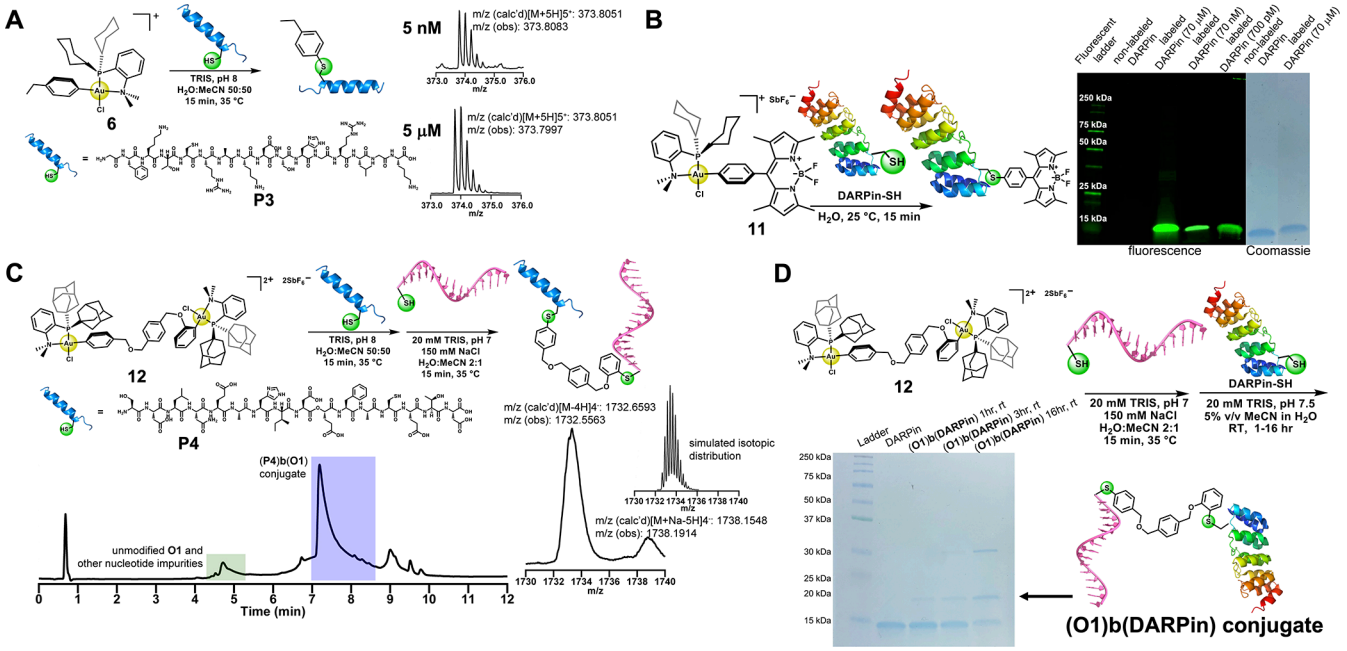

Figure 5. Applications of kinetically tunable Au(III) organometallic Cys arylation reagents. (A) Demonstration of Cys arylation at nM and μM concentrations of both Au(III) reagent and biomolecule. (B) Labeling of a DARPin protein with a fluorescent dye. SDS-PAGE gel demonstrating that labeling of the DARPin protein works down to pM concentrations. (C) Use of a bifunctional Au(III) reagent to construct biomolecular heterostructures. (D) Construction of an oligonucleotide-protein heterostructure using the bifunctional Au(III) reagent; the strong band appearing at ca. 15 kDa represents unlabeled DARPin protein, the band appearing just under 20 kDa represents the (**O1**)**b**(**DARPin**) conjugate product, and the band appearing at ca. 30 kDa corresponds to the linked homodimer of the DARPin protein, a (**DARPin**)**b**(**DARPin**) conjugate, due to an excess of DARPin being used in the conjugation experiment. Estimated conversion to (**O1**)**b**(**DARPin**) was 44%, determined by imageJ quantification, representing a significant improvement over previous one-pot methods.[65]

show that as the size of the phosphine moiety increases, the $\Delta G^{\ddagger}$ of the reductive elimination is lowered, which is consistent with the observed k_{re} values (Figure 4B and SI section S5.4). These experimental and computational observations are consistent with our model, where Cys thiol coordination to the Au(III) center is the selectivity-determining step for the overall arylation process.

We next hypothesized that either the electronic or steric effects of the aryl substituent could also provide an additional source of tunability for the kinetics of the Cys arylation reaction. To probe the effects of electronic changes on the rates of each of the Cys arylation steps, the Au(III) OA complex, **8**, featuring an electronically deficient pentafluorophenyl species was examined via stopped-flow **GSH** arylation experiments. From the observed data, **8** displayed behavior that suggested a rapid pre-equilibrium, and the values for k_{re} and K of 0.047 s^{-1} and 172 M^{-1} were obtained, respectively (see SI Figure S97). The value of k_{co} could not be extracted for **8**, as no appreciable perturbation in absorbance that could be attributed to the initial coordination of a Cys thiol was observed for its reaction with **GSH**. Considering the low values of both k_{re} and K, we conclude that at this extreme, the overall rate of the Cys arylation process is no longer dominated by the rate of Cys thiol coordination, k_{co}. Similar to those performed above, competition experiments comparing the electronically different yet sterically comparable aryl groups of reagents **8** and **9** were performed. We observed greater than 99% selectivity for the phenyl S-aryl product over the pentafluorophenyl product (SI Figure S110). Interestingly, DFT calculations indicate that there is more positive charge on the Au(III) atom of **8** than **9**, which would suggest faster coordination of the nucleophilic sulfur to **8**. Calculations also demonstrate a $\Delta\Delta G^{\ddagger}$ of 1.3 kcal/mol favoring the formation of the phenyl S-aryl product (see SI section S5.4). These experiments suggest that the equilibrium associated with the Cys thiol binding of the bioconjugation step with **8** is not as heavily shifted toward the Cys thiol coordinated species. This is consistent with the stopped-flow kinetics experiments as K for the thiol binding to the Au(III) center is the lowest among all other complexes for which the equilibrium constant could be determined (see above and SI Figure S97). These experiments suggest that while a high degree of selectivity in the Cys arylation reaction can be achieved through tuning the electronics of the aryl substituent, the nature of the coordination process needs to be intricately controlled, and the reversibility in this step can lead to the reduced rate and selectivity of the overall Cys arylation.

We then investigated the steric effects of the aryl substituent. The Au(III) OA complex, **10**, containing the relatively sterically demanding *o*-benzyloxy aryl ligand was synthesized and its kinetic profile was analyzed through stopped-flow **GSH** arylation experiments. Analysis of **10** gave a significantly different kinetic landscape from that of the previously described OA complexes. There was no observable pre-equilibrium from the first fast coordination step, and only a second-order rate constant of 1.97 $M^{-1}s^{-1}$ was obtained for the overall Cys arylation process (see SI Figure S102). $\%V_{Bur}$ calculations indicate that the steric bulk surrounding the substrate is in agreement with the observed kinetic trends (Figure 4C). Performing another set of competition experiments, comparing **9** and the very sterically hindered **10** led to >99% selectivity for the phenyl S-aryl product over the *o*-benzyloxy S-aryl product. These results corroborate the kinetic profiles obtained from the stopped-flow experiments and suggest that sterically demanding aryl substituents can be

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additionally leveraged to modulate the kinetics of the first fast coordination step for the Cys arylation reactions. Combining these results with those obtained for pentafluorophenyl-containing **8**, we observe that the aryl substituents of the Au(III) reagents can have significant effects on the rate of Cys arylation through modulation of the two key mechanistic steps. Finally, combining the kinetic and competition experimental data of our Au(III) reagents, we can now establish a tunable spectrum of reactivity for these reagents that can inform their practical use (SI section S3.3).

Applications at Dilute Reaction Concentrations and in the Construction of Biomolecular Heterostructures. With a working kinetic model for organometallic Cys arylation, we used the developed reagents in reactions that have historically been challenging to accomplish. For example, performing bioconjugations at very dilute regimes can often be challenging for traditional reagents.[26] Considering that **5** exhibits the fastest rate of bioconjugation to an amino acid by an organometallic OA reagent measured to date, we hypothesized that similar reagents bearing ligand **L3** could be developed to modify Cys residues in biomolecules at nanomolar concentrations. To validate this hypothesis, buffered solutions of **P3** were prepared at increasingly dilute concentrations of 10 μM, 100, and 10 nM. Each of these solutions was subjected to the Cys arylation reaction by the addition of an equal volume of an equimolar solution of **6** for final reaction concentrations of 5 μM, 50, and 5 nM (Figure 5A and SI section S2.7.1). For each concentration, the Cys arylation reaction proceeded to quantitative conversion within 30 min at 35 °C, as confirmed by LCMS analysis. An additional experiment was performed to further investigate the performance of these reagents at dilute reaction regimes where an Au(III) reagent bearing a fluorescent dye, **11**, was utilized in the Cys arylation of a thiol-bearing DARPin protein. Protein bioconjugation of **11** and T4 lysozyme V131C was also possible at concentrations as low as 700 nM, which was validated by LCMS (SI Section S2.7.2). To examine subnanomolar concentrations, fluorescent gel studies confirmed the presence of the conjugated protein (DARPin) at 700 pM concentration (Figure 5B and SI section S2.7.2). These studies indicate that the Cys arylation process can successfully occur at subnanomolar concentrations. This reactivity is in contrast to many traditional reagents that require large excesses of reagent and lengthy reaction times when these transformations are conducted even at micromolar concentrations. Additionally, trypsin digest and top-down mass-spectrometry experiments demonstrated that chemoselectivity toward cysteine thiols was maintained (see SI section S2.7.3).

The rapid synthesis of biomolecular heterostructures is challenging; however, we hypothesized that our Au(III) organometallic reagents would be able to perform these transformations with ease. With a method to control the kinetic profile of our reagents through modification of the sterics of the aryl substituents, we designed a bifunctional Au(III) reagent with site-differentiated kinetics. Through the use of a simple organic linker that bears two different steric environments next to aryl iodide sites, we synthesized a bimetallic Cys arylation reagent **12** (see SI section S2.2.2). From our mechanistic experiments, we hypothesized that the Au(III) center in the less sterically demanding environment would undergo Cys arylation significantly faster than the Au(III) center in the more sterically demanding environment. Additionally, utilization of this reagent would provide an opportunity to construct heterostructures in one pot while avoiding potential homoconjugation or ligand scrambling. Indeed, we readily observed the construction of biomolecular heterostructures by using this reagent and strategy. An example of one such heterostructure included the linking of **P4** to **O1** to form a peptide−oligonucleotide heterostructure. To prepare this construct, peptide **P4** to bioconjugation using **12** to yield the **12** conjugate reagent. To a buffered solution of this reagent was added a solution of **O1** to ultimately yield the (**P4**)**g**(**O1**) conjugate heterostructure in 83% conversion (based on **O1**), as determined by LCMS analysis (Figure 5C). Using a similar strategy, we synthesized peptide-small molecule, peptide-saccharide, peptide−peptide, peptide−oligonucleotide, oligonucleotide-small molecule, and protein-oligonucleotide (Figure 5D) conjugate heterostructures (SI sections S2.7.4−S2.7.10). These heterostructures are rapidly synthesized and require little to no purification between conjugation steps, in contrast to existing technologies that often involve isolation and purification between conjugation procedures.[16,66]

CONCLUSION

Through mechanistic work and rational design, we have developed kinetically tunable organometallic Cys bioconjugation reagents. As a result, the developed organometallic Au(III) bioconjugation reagents enable selective labeling of Cys moieties down to pM concentrations and allow for the rapid construction of complex heterostructures from peptides, proteins, and oligonucleotides. This work showcases how organometallic chemistry can be interfaced with biomolecules and lead to a range of reactivities that are unmatched by classical tools.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.3c12170.

Details about instruments, materials, methods, chemical synthesis, ^{1}H and ^{31}P NMR characterization, stopped-flow data, LC-MS characterization, computational information, photophysical characterization, and crystallographic information. (PDF)

Accession Codes

CCDC 2286312 and 2304665 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare the following competing financial interest(s): A.M.S., H.D.M., E.A.D., and H.R.M., are co-inventors on sever-al patent applications from UCLA associated with the Au(III) based bioconjugation technology.

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