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#### Supplementary Materials

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# Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions

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Copper(I)-catalyzed azide-alkyne cycloaddition has become a commonly employed method for the synthesis of complex molecular architectures under challenging conditions. Despite the widespread use of copper-catalyzed cycloaddition reactions, the mechanism of these processes has remained difficult to establish due to the involvement of multiple equilibria between several reactive intermediates. Real-time monitoring of a representative cycloaddition process via heat-flow reaction calorimetry revealed that monomeric copper acetylide complexes are not reactive toward organic azides unless an exogenous copper catalyst is added. Furthermore, crossover experiments with an isotopically enriched exogenous copper source illustrated the stepwise nature of the carbon–nitrogen bond-forming events and the equivalence of the two copper atoms within the cycloaddition steps.

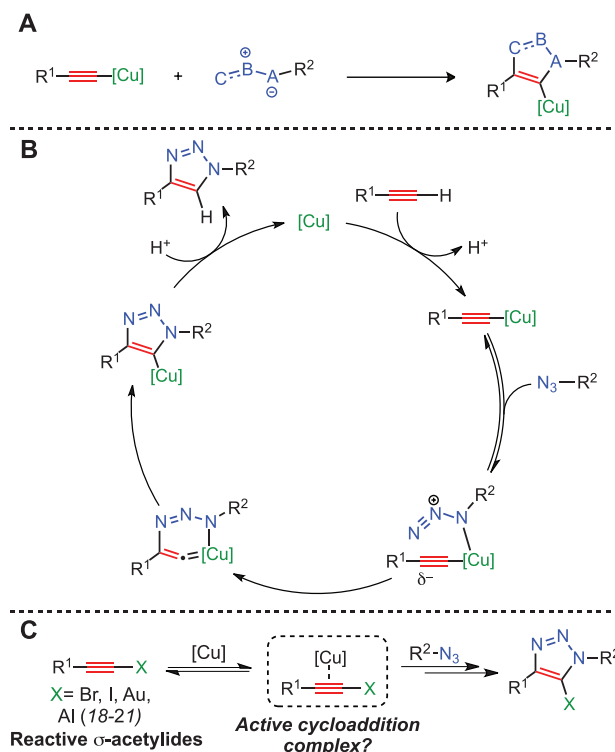
**R**igorous examination of catalytic reaction mechanisms remains a formidable but necessary pursuit for organic chemists. In-depth reaction analysis can aid in the optimization of reaction parameters, spur the development of new catalytic processes, and enhance understanding of fundamental reactivity. The status quo for analysis of catalytic systems usually relies on one or a combination of the following methods: computational studies; isolation and reintroduction of an intermediate from the reaction mixture as proof of its involvement within the catalytic cycle; analysis of stereoelectronic effects of substituents present in the reactive intermediates; isotope crossover and kinetic isotope effects; and, most commonly, initial rate studies. Although these methods have shaped our current understanding of reaction mechanisms, they seldom provide a complete picture of complex catalytic reactions.

Among transition-metal-catalyzed processes, reactions involving copper remain particularly difficult for rigorous mechanistic investigation due to the low reduction potential of copper [+0.159 V from  $\text{Cu}^{2+}$  to  $\text{Cu}^+$  and +0.520 V from  $\text{Cu}^+$  to  $\text{Cu}^0$  (1)], the propensity for  $\text{Cu}^+$  species to disproportionate in solution (2), the generally poor ability of copper to backbond to ligands (3), and the well-documented tendency of copper complexes to aggregate (4). Despite these complications, copper catalysis has been em-

ployed successfully for decades in a variety of methods, including carbon–heteroatom bond formation (5); C–C bond formation (6); oxidative processes (7, 8); and, most recently, dipolar cycloadditions (Fig. 1A) (9–12). The latter of these includes the copper(I)-catalyzed azide-alkyne cy-

cloaddition (CuAAC), which has garnered widespread attention since its debut in 2001 as a facile and robust method for the creation of covalent linkages in a variety of environments. However, due to the aforementioned challenges in the direct study of copper catalysis, a mechanism of this reaction has yet to be fully elucidated; specifically, the identity and nuclearity of the copper species involved in catalysis has not been established (Fig. 1B). Herein, we propose from simple, deductive experiments a mechanism that necessitates two copper atoms within the active cycloaddition complex, and we describe their relation to one another.

Several studies toward elucidation of the mechanism of the CuAAC with terminal alkynes have been reported (13, 14), and experimental evidence of the possible involvement of polynuclear copper(I) intermediates (14–16) has been supported by theoretical studies (17, 18). Furthermore, recent advances in dipolar cycloadditions of 1-halo- and 1-metalloalkynes (formally internal alkynes) with organic azides have prompted additional investigation of the mechanism (19–22). In the case of 1-iodoalkynes, scission of the terminal alkyne-halogen bond to form a copper(I)-acetylide is not necessary for the cycloaddition



**Fig. 1. CuAAC.** (A) Generic form of the copper-catalyzed dipolar cycloaddition reaction. (B) First proposed mechanism of the CuAAC (10). (C) Copper-catalyzed cycloaddition of terminal and 1-iodoalkynes.

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to occur, suggesting that the copper catalyst effects the cycloaddition purely through weak  $\pi$ -interactions with the formally internal alkyne (Fig. 1C). Thus, we hypothesized that the classical CuAAC (with terminal alkynes) also proceeds through a similar reaction manifold, via: (i) in situ formation of the  $\sigma$ -bound copper acetylide; (ii) recruitment of a second  $\pi$ -bound copper atom, forming the catalytically active complex; (iii) reversible coordination of the organic azide to the  $\pi$ -bound copper complex; and (iv) the stepwise annulation events. Obtaining experimental support for this pathway would lead to a common mechanistic model involving activation of  $\sigma$ -acetylides (both terminal and formally internal) by weak and reversible  $\pi$ -interaction with a copper center (Fig. 1C).

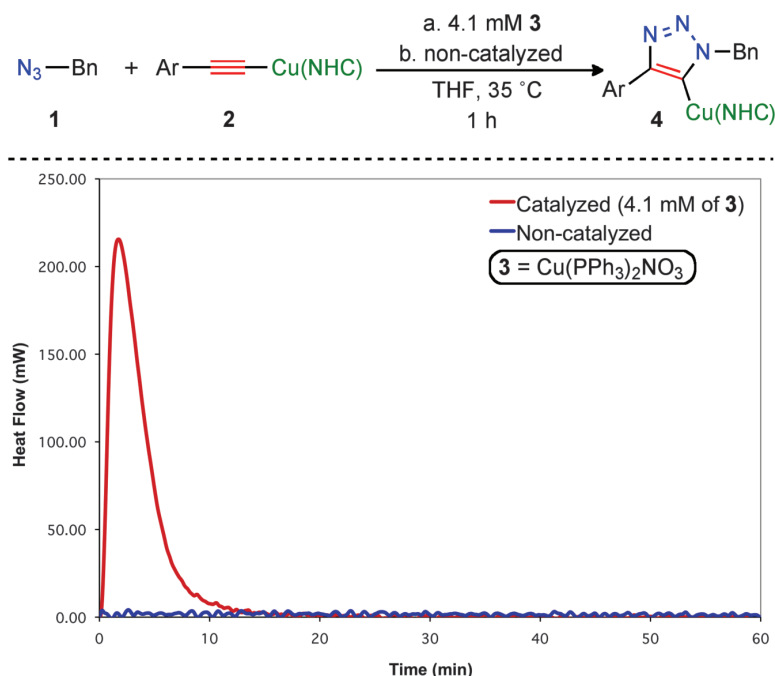
Despite the multiple equilibria of unstable, non-isolable, and highly reactive intermediates within the catalytic cycle of the CuAAC, the copper(I)-acetylide and -triazolide intermediates can be reliably isolated and characterized (fig. S1) (23). Therefore, to investigate the mechanistic model proposed above, we envisioned a simple experiment in which a preformed, stable, mononuclear  $\sigma$ -bound copper(I)-acetylide is treated with a stoichiometric amount of an organic azide in the presence or absence of an added copper(I) catalyst, allowing us to directly verify the requirement of the putative  $\pi$ -bound copper intermediate. To this end, the  $\sigma$ -bound copper(I)-acetylide complex **2** was synthesized on a large scale (>20 g) in five overall steps (fig. S2), following the procedure reported by Nolte *et al.* (23). Subsequently, azide **1** was treated with acetylide **2** [0.090 and 0.082 M in tetrahydrofuran (THF), respectively] under two sets of conditions—with and without soluble exogenous copper catalyst **3** [ $\text{Cu}(\text{PPh}_3)_2\text{NO}_3$ , 0.0041 M; Ph, phenyl]—and reaction progress was tracked by real-time heat-flow reaction calorimetry (Fig. 2) (24). The contrast in rate was drastic: The catalyzed cycloaddition rapidly reached completion, producing copper triazolide **4** within 20 min (tables S2 and S3), whereas the noncatalyzed reaction showed no appreciable conversion to the triazolide (table S4). Lowering the catalyst concentration resulted in a corresponding decrease in the maximum rate, showing a positive-order dependence on the exogenous copper species (fig. S3). Moreover, multiple soluble exogenous copper sources exhibited similar catalytic efficacy (fig. S4), and organic azides bearing sterically encumbered or aliphatic functional groups showed comparable rate acceleration in the presence of an added copper catalyst (fig. S5 and table S5) (25). Similarly, a marked difference in the rates of the catalyzed and noncatalyzed reactions initially shown in THF was observed in other solvents, chloroform and dimethylformamide (25) (fig. S6).

With the necessity for a second copper atom within the active cycloaddition complex established, we sought to determine the respective roles

of each copper atom. Given the well-documented stability of the copper-carbene complexes (26) and the robust nature of acetylide **2**, we hypothesized that the two copper atoms act in discrete and specialized roles, with copper in acetylide **2** acting purely as a strongly  $\sigma$ -bound ligand, whereas the second copper atom operates exclusively through weak  $\pi$ -complexation (Fig. 1C, where X = Cu). To probe this hypothesis, we prepared an isotopically pure  $^{63}\text{Cu}$  exogenous catalyst to differentiate it from the naturally abundant distribution of copper isotopes ( $^{63}\text{Cu}$ : $^{65}\text{Cu}$  ratio of

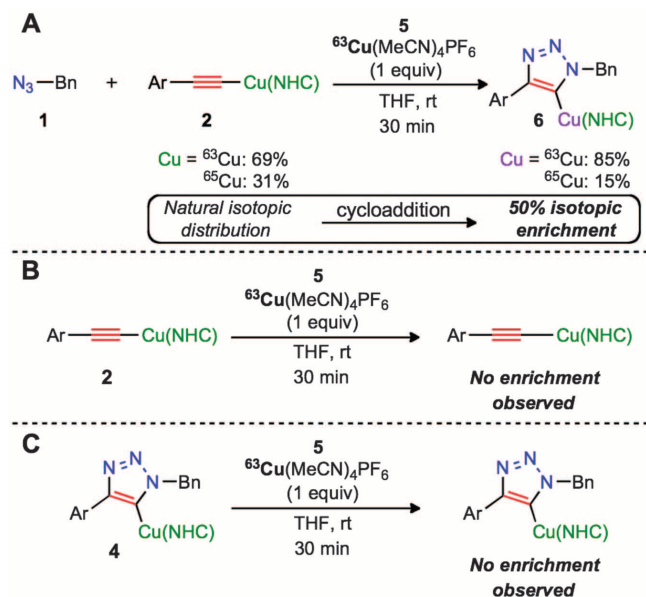
69:31) contained in the preformed copper(I)-acetylide. The copper(I) coordination complex  $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$  **5** (Me, methyl) (27), which is an air-stable, crystalline solid, was selected for the stoichiometric crossover studies and synthesized in short order from the isotopically pure, commercially available copper(II) oxide (99.9%  $^{63}\text{Cu}$ ).

As both the acetylide reactant **2** and the copper triazolide product **4** contain a copper atom, we designed a stoichiometric crossover experiment in which the absence of isotopic



**Fig. 2. Reaction progress.** Global heat (mW, milliwatts) output graph for the catalyzed and uncatalyzed formation of triazolide **4**. Bn, benzyl; Ar, *p*-<sup>t</sup>BuPh; <sup>t</sup>Bu, *tert*-butyl; NHC, 1,3-bis(2,6-diisopropyl)phenyl-4,5-dihydroimidazol-2-ylidene.

**Fig. 3. Isotope crossover studies.** (A) Observed isotopic enrichment of triazolide **6**. (B) No isotopic enrichment of acetylide **2** observed. (C) No isotopic enrichment of triazolide **4** observed. rt, room temperature.



enrichment of the metal center of the resulting triazolidine would support the hypothesized independent roles of the two copper species. Thus, we combined azide **1**, acetylide **2** (natural isotopic ratio of  $^{63/65}\text{Cu}$ ), and isotopically enriched copper complex **5** (isotopically pure  $^{63}\text{Cu}$ ) and used direct-injection time-of-flight mass spectrometry (TOF-MS) to analyze the resultant triazolidine (Fig. 3A). To our surprise, we observed statistical enrichment of the resulting triazolidine, yielding complex **6** (with the  $^{63}\text{Cu}/^{65}\text{Cu}$  ratio of 85:15) and disproving the discrete bonding hypothesis of the two copper atoms postulated above (fig. S9). This enrichment event would require the migration of the N-heterocyclic carbene (NHC) from one copper atom to another; given the established strength of the copper–NHC bond [energetically on the order of a C–C alkane bond (26)], this is an unexpected result.

The enrichment of triazolidine **4** must occur in one of the following three steps: (i) via the acetylide intermediate **2**, (ii) via the triazolidine intermediate **4**, or (iii) within the cycloaddition steps. Due to the polynuclear nature of copper(I)-acetylides in solution and multiple equilibria between the various aggregates (28), acetylide **2** could be a candidate for the facile exchange of the embedded copper atom. Hence, we treated acetylide **2** with a stoichiometric amount of the enriched copper complex **5** (tracked by TOF-MS), from which we did not observe isotopic enrichment of the acetylide (Fig. 3B and fig. S10). Although this control experiment showed no

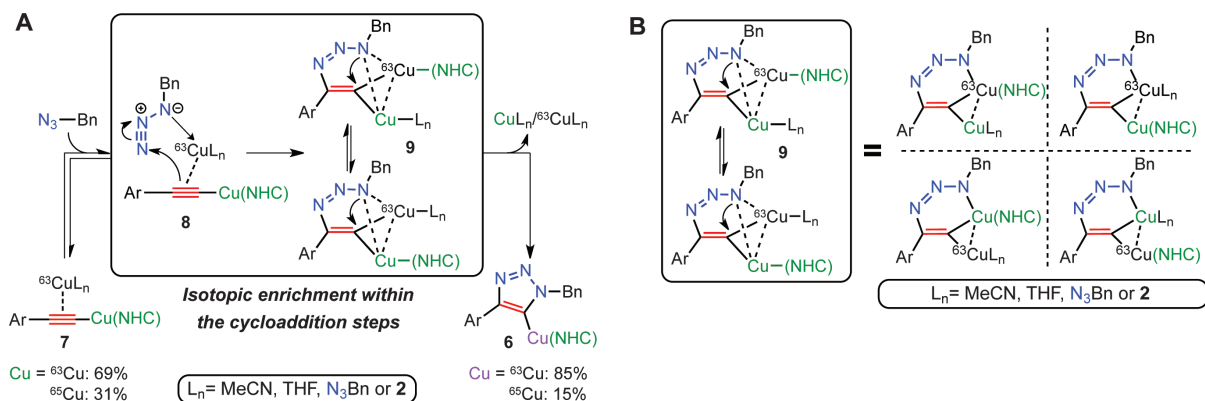
crossover, azide **1** could theoretically act as a Lewis base and mediate the enrichment observed in the initial experiment. To address this possibility, we combined acetylide **2**, isotopically enriched copper complex **5**, and azide **1** (in analogy to the initial crossover experiment) and analyzed the reaction mixture by TOF-MS. Again, no enrichment of acetylide **2** in the presence of azide **1** was noted (figs. S11 and S12). Thus, although organic azides could act as ligands for the copper(I) complex, evidently this interaction is not sufficient to mediate the observed enrichment event.

With acetylide **2** excluded as a possible intermediate for exchange, we next examined whether the isotopic enrichment occurred after the formation of triazolidine **4**. Accordingly, we combined azide **1**, acetylide **2**, and a catalytic amount of  $[\text{Cu}(\text{MeCN})_4]\text{PF}_6$  (natural isotopic distribution, 5 mole percent) in THF to fully form the nonenriched triazolidine **4**. We added a stoichiometric equivalent of the enriched copper complex **5** to this solution (and tracked the results by TOF-MS), from which no enrichment of triazolidine **4** was observed (Fig. 3C and fig. S13), excluding it as a venue for the observed enrichment.

By process of elimination, the observed 50% isotopic enrichment must occur within the cycloaddition steps (boxed intermediates in Fig. 4A). We propose the following mechanistic interpretation to account for these results (Fig. 4A): First, the  $\sigma$ -bound copper acetylide bearing the  $\pi$ -bound enriched copper atom **7** reversibly

coordinates an organic azide, forming complex **8**. Following this step, nucleophilic attack at N-3 of the azide by the  $\beta$ -carbon of the acetylide forms the first covalent C–N bond, producing intermediate **9**. The ligand exchange in this intermediate (isomers shown in Fig. 4B) is faster than the formation of the second covalent C–N bond, which results in ring closure, accounting for the statistical (50%) incorporation of  $^{63}\text{Cu}$  into triazolidine **6**. The exclusive formation of triazolidine **6** indicates a thermodynamic preference for the NHC-bound copper triazolidine; however, rapid ligand exchange renders equal the probability for either copper atom (natural or enriched) to be incorporated in the resulting intermediate. We attribute the unprecedented lability and rapid exchange of the NHC ligand between the two copper atoms in intermediate **9** to the weakened copper–carbene backbonding (29–31), as the formal oxidation state of copper increases upon the formation of the first C–N bond. The degenerate, proximal copper centers in **9** facilitate this oxidation event, with the second copper atom acting as a stabilizing donor ligand to the otherwise highly energetic and unstable mononuclear metallacycle intermediate (**13**) (Fig. 1B). Taken together with prior studies of the full catalytic cycle, these experiments support the mechanistic model for the CuAAC, featuring two chemically equivalent copper atoms working in concert for the regioselective formation of 1,4-substituted 1,2,3-triazoles (Fig. 4C and fig. S14).

The in situ reaction calorimetry and metal isotope crossover methods used in this study



**Fig. 4. Mechanistic conclusions.** (A) Mechanistic rationale for the isotopic enrichment of triazolidine **6**. (B) Rapidly interconverting isomers of intermediate **9**. (C) Proposed catalytic model for the CuAAC with two copper atoms.



have permitted us to deduce the involvement of unstable, non-isolable intermediates that have previously eluded more conventional mechanistic studies. Similarly innovative studies that delineate reactivity patterns of more capricious but abundant metals (Cu, Fe, Ni) are needed to further understand and develop new catalytic processes, reducing synthetic reliance on well-behaved but rare and expensive transition metals (Pd, Rh, Ru). Moreover, the mechanistic insights presented in this study, specifically the formation of the active cycloaddition complex with two differentiable copper atoms, imply a unified reactivity of electronically rich  $\sigma$ -acetylides (1-halo- or 1-metallo-) with 1,3-dipoles (e.g., azides, nitrile oxides, and nitrones). As such, we propose a common reactivity trend in which any  $\sigma$ -acetylide that can effectively recruit a  $\pi$ -bound copper atom will undergo annulation with a compatible dipolar partner.

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### Supplementary Materials

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Supplementary Text

Figs. S1 to S14

Tables S1 to S5

NMR Spectra

References (32–34)

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## Observations of Ejecta Clouds Produced by Impacts onto Saturn's Rings

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We report observations of dusty clouds in Saturn's rings, which we interpret as resulting from impacts onto the rings that occurred between 1 and 50 hours before the clouds were observed. The largest of these clouds was observed twice; its brightness and cant angle evolved in a manner consistent with this hypothesis. Several arguments suggest that these clouds cannot be due to the primary impact of one solid meteoroid onto the rings, but rather are due to the impact of a compact stream of Saturn-orbiting material derived from previous breakup of a meteoroid. The responsible interplanetary meteoroids were initially between 1 centimeter and several meters in size, and their influx rate is consistent with the sparse prior knowledge of smaller meteoroids in the outer solar system.

Quantifying the mass flux onto Saturn's rings has been a long-desired goal. Mass infall likely dominates the rings' coloration and their levels of non-ice pollution, as well as influencing angular momentum transport and erosion of particles, all of which in turn have the potential to set limits on the age of the rings (1–3). Impacts may be a trigger mechanism for the “spokes” observed in the B ring (4, 5), and they may be responsible for producing some dust outbursts observed in the F ring (6, 7). Additionally, direct measurement of the mass infall

onto Saturn's rings would constrain the population of interplanetary meteoroids in the outer solar system, which is poorly determined for macroscopic particles (1, 8). However, although impact flashes have been observed on Earth's Moon [e.g., (9)], efforts to detect impact flashes in the outer solar system have been unsuccessful (10, 11).

Here, we report images of ejecta clouds above Saturn's rings (Fig. 1), obtained by Cassini's Imaging Science Subsystem (12, 13). These features are normally not visible owing to low contrast

with the background ring, but there are two particular viewing geometries in which they become visible: (i) during saturnian equinox, at which time the background ring becomes very dark, and (ii) when Cassini views the rings at high resolution and very high phase angle, at which time the dusty features appear very bright (Fig. 2).

The saturnian equinox, which occurs every ~14.5 years and is closely accompanied by edge-on viewing of Saturn's rings from Earth, has led to many scientific discoveries over the centuries since 1612, when the ring system's disappearance from Galileo's view gave the first hint of its disk geometry (14). The 2009 equinox was the first for which an observer (namely, the Cassini spacecraft) was in place at close range and high saturnian latitudes. During a few days surrounding the precise moment of the Sun's passage through the ring plane, the main rings provided an unusually dark background, due to the edge-on illumination, while anything (e.g., an ejecta cloud) extending vertically out of the ring plane

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