

Supporting Information

Autocatalytic Cycles in a Copper-Catalyzed Azide-Alkyne Cycloaddition Reaction

Sergey N. Semenov,^{a‡} Lee Belding,^a Brian J. Cafferty,^a Maral P.S. Mousavi,^a Anastasiia M. Finogenova,^a
Ricardo S. Cruz,^a Ekaterina V. Skorb,^{a†} and George M. Whitesides^{a,b,c*}

^a *Department of Chemistry and Chemical Biology, Harvard University*

12 Oxford Street, MA 02138

^b *Kavli Institute for Bionano Inspired Science and Technology, School of Engineering and Applied Sciences, Harvard University*

29 Oxford Street, MA 02138

^c *Wyss Institute for Biologically Inspired Engineering, Harvard University*

60 Oxford Street, MA 02138

Experimental

Synthesis

General:

Unless otherwise stated, all starting materials were obtained commercially and were used without further purification. *WARNING: azides are explosive—especially in the presence of transition metal ions—and should be handled in small amounts, and with great caution.* Tetraethylene glycol diazide was synthesized accordingly to a literature procedure.¹ Nuclear Magnetic Resonance (NMR) spectra were measured on a *Varian INOVA D-600* spectrometer at 600 MHz for ¹H, a *Varian INOVA I-500* spectrometer at 500 MHz for ¹H and 125.8 MHz for ¹³C(¹H), or on a *Varian INOVA I-400* spectrometer at 400 MHz for ¹H. The chemical shifts for ¹H and ¹³C are given in parts per million (ppm) relative to TMS, and calibrated using the residual ¹H peak of the solvent; $\delta = 7.26$ for CDCl₃ and $\delta = 4.79$ for D₂O in ¹H NMR, and $\delta = 77.2$ for CDCl₃ in ¹³C NMR.

2-Azidoethanol:

Sodium azide (10 g, 154 mmol) was added to a stirred solution of 2-bromoethanol (4.00 g, 37 mmol) in 30 mL of dry DMF at 0 °C under an argon atmosphere. The mixture was left at room temperature overnight. DMF (20 mL) was removed *in vacuo* and 20 mL water was added to the residual mixture. The resulting solution was extracted with diethyl ether (10 x 30 ml). The ether phase was concentrated to the total volume of 50 ml, washed by water (10 x 1 ml), dried over Na₂SO₄, and concentrated on the rotary evaporator to give 2-azidoethanol as transparent liquid in 62 % yield (2 g, 23 mmol). ¹H NMR: (500 MHz, CDCl₃) $\delta = 2.87$ (t, ³J_{H-H} = 7.4 Hz, 2H, CH₂), 1.25 (t, ³J_{H-H} = 7.4 Hz, 2H, CH₂).

Benzyl azide:

Sodium azide (1.4 g, 22 mmol) was added to a stirred solution of benzyl bromide (2.00 g, 12 mmol) in 8 mL of dry DMF at room temperature under an argon atmosphere. The mixture was left at room temperature overnight. DMF was removed *in vacuo* and 20 mL of water was added to the residual mixture. The resulting solution was extracted with diethyl ether (3 x 20 mL). The ether phase was washed

by water (2 x 20 mL), dried over Na₂SO₄, and concentrated on the rotary evaporator to provide a transparent liquid in 85 % yield (1.36 g, 10 mmol). ¹H NMR: (500 MHz, CDCl₃) δ = 2.87 (t, ³J_{H-H} = 7.4 Hz, 2H, CH₂), 1.25 (t, ³J_{H-H} = 7.4 Hz, 2H, CH₂).

***tris*-(Hydroxyethyltriazoilylmethyl)amine (N(C₃N₃)₃):**

Tripropargylamine (91.3 mg, 0.68 mmol) in MeOH (0.5 mL) was mixed with a solution of 2-azidoethanol (200 mg, 2.3 mmol) and CuSO₄ 5H₂O (5 mg, 0.02 mmol) in 1 mL of water. Ascorbic acid (5 mg, 0.028 mmol) in 0.1 mL of water was added to the mixture under an argon atmosphere. The reaction mixture was left at room temperature for three days. Solvents were removed *in vacuo* and the residue was dissolved in 1 mL of water. Separation on a C18 column (H₂O/CH₃CN gradient 0-10% CH₃CN) afforded the desired compound as a colorless oil in 41 % yield (110 mg, 0.28 mmol). To prevent broadening of the peaks because of small admixtures of Cu(II), we added 5 mg of KCN to the NMR sample. ¹H NMR: (500 MHz, D₂O) δ = 7.95 (s, 3H, CH_{triazole}), 4.51 (t, ³J_{H-H} = 5.1 Hz, 6H, CH₂OH), 3.96 (t, ³J_{H-H} = 4.9 Hz, 6H, CH₂N_{triazole}), 3.80 (s, 6H, CH₂N).

(2-Hydroxyethyltriazoilylmethyl)dipropargylamine (N(C₃)₂(C₃N₃)) and *bis*-(2-Hydroxyethyltriazoilylmethyl)propargylamine (N(C₃)(C₃N₃)₂):

Tripropargylamine (113 mg, 0.86 mmol) in MeOH (0.5 mL) was mixed with a solution of 2-azidoethanol (175 mg, 2 mmol) and CuSO₄ 5H₂O (5 mg, 0.02 mmol) in 1 mL of water. Ascorbic acid (5 mg, 0.028 mmol) in 0.1 mL of water was added to the mixture under an argon atmosphere. The reaction mixture was left at room temperature for three days. Solvents were removed *in vacuo*, and the residue was dissolved in 1 mL of water. Separation on a C18 column (H₂O/CH₃CN gradient 0-10% CH₃CN) afforded the N(C₃)₂(C₃N₃) in 25 % yield (47 mg, 0.22 mmol) and N(C₃)(C₃N₃)₂ in 12 % yield (31 mg, 0.1 mmol). To prevent broadening of the peaks because of small admixtures of Cu(II), we added 5 mg of KCN to the NMR samples. ¹H NMR (N(C₃)₂(C₃N₃)): (500 MHz, D₂O) δ = 7.77 (s, 1H, CH_{triazole}), 4.51 (t, ³J_{H-H} = 5.1 Hz, 2H, CH₂OH), 3.96 (t, ³J_{H-H} = 4.9 Hz, 2H, CH₂N_{triazole}), 3.74 (s, 2H, CH₂N) 3.28 (s, 4H, CH₂CCH). ¹H NMR (N(C₃)(C₃N₃)₂): (500 MHz, D₂O) δ = 7.84 (s, 2H, CH_{triazole}), 4.51 (t, ³J_{H-H} = 5.1 Hz, 4H, CH₂OH),

3.96 (t, $^3J_{\text{H-H}} = 4.9$ Hz, 4H, $\text{CH}_2\text{N}_{\text{triazole}}$), 3.73 (s, 4H, CH_2N), 3.15 (s, 2H, CH_2CCH). The alkyne proton exchanges very quickly to deuterium in the basic solution of KCN and is invisible in these NMR spectra.

Tripropargylamine- d_3 (1-d_3)²:

Tripropargylamine (262.3 mg, 2.0 mmol), acetonitrile (5 mL) and K_2CO_3 (1.15 g, 4.5 mmol) were mixed in a flame-dried round bottom flask, backfilled with argon gas, and stirred at 50 °C for 2 hours. The reaction was allowed to cool to room temperature, at which point D_2O (1.8 mL, 100 mmol) was added and the reaction was stirred for an additional 1 hour. Afterwards, dichloromethane (1 mL) was added and the reaction mixture was transferred to a separatory funnel. The organic phase was dried over MgSO_4 and concentrated *in vacuo* to afford a yellow oil in >95% yield (261.0 mg, 1.94 mmol). ^1H NMR: (500 MHz, CDCl_3) $\delta = 3.50$ (s, 2H), 2.28 (t, $^3J_{\text{H-H}} = 2.28$ Hz, 0.05H).

Kinetic Experiments

General:

The kinetics experiments were performed at 25 °C in a $\text{D}_2\text{O}:\text{CD}_3\text{OD}$ mixture (9:4; v:v) by monitoring the change in the ^1H NMR spectra. We calculated the progress of the cycloaddition reaction between the azide and alkyne by integrating the ^1H NMR signal of the alkyne proton. The concentrations of compounds were calculated relative to an internal standard of *t*-BuOH. UV-Vis spectroscopy was also used to monitor kinetics in a quartz cuvette with a 1-mm light path using a Cary 60 UV-Vis spectrophotometer. The electrochemical measurements were performed on Lawsun Labs EMF16 potentiometer and CHI600C Potentiostat (CH Instruments, TX). ESI-MS data were obtained on Bruker micrOTOF-QII mass spectrometer.

NMR Kinetics of the Reaction between Tripropargylamine, Azidoethanol, and CuSO_4 .

Azidoethanol (15 μL) was added to a solution of $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (7 mg, 43 mM) in 0.415 mL of D_2O . A solution of tripropargylamine (10 μL) in 0.2 mL of CD_3OD was then added to the first solution. The final solution was filtered into an NMR tube, and we recorded the ^1H NMR spectrum every two minutes (132 s time intervals, including the 12 s of acquisition time).

NMR Kinetics of the Reaction Between Tripropargylamine and Azidoethanol with Reduced Copper.

Azidoethanol (15 μL) and ascorbic acid (0.25 mg) was added to a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3 mg, 0.2 mM) in 0.415 mL of D_2O . A solution of tripropargylamine (10 μL) in 0.2 mL of CD_3OD was then added to the first solution. The final solution was filtered into an NMR tube, and we recorded the ^1H NMR spectrum every two minutes to monitor the disappearance of tripropargylamine, and the formation of the triazolyl ligands.

NMR Kinetics of the Reaction Between Tripropargylamine, Azidoethanol, and CuSO_4 in the Presence of Ammonia.

Azidoethanol (15 μL) was added to a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (7 mg, 43 mM), in 0.415 mL of D_2O , and the resulting solution filtered. To the filtered solution was added NH_4Cl (15 mg), and NH_3 (15 μL of a 28% solution). This solution was placed into an NMR tube, and a solution of tripropargylamine (10 μL) in 0.2 mL of CD_3OD was added. ^1H NMR spectrum were recorded every two minutes.

Electrochemical Measurements.

We recorded the cyclic voltammogram (scan rate 100 mV/s) with a CHI600C Potentiostat (CH Instruments, TX). We used a three-electrode cell with a 2.0 mm-diameter glassy carbon electrode (CH Instruments), a Pt wire (99.998%, Alfa Aesar, MA) as counter electrode, and an Ag/AgCl reference electrode (CH Instruments, reference solution: 1.0 M KCl). Prior to each measurement, we polished the working electrode on Microcloth polishing pads containing 0.3 μm Micropolish II deagglomerated alumina (CH Instruments). After polishing, we rinsed the electrode with deionized water and then with ethanol, sonicated the electrode in ethanol for five minutes, and dried the electrode under a stream of nitrogen. We prepared the solution of CuSO_4 with deionized water. The solution contained 5 mM CuSO_4 , 50 mM Na_2SO_4 (as supporting electrolyte), and 10 mM of the ligand $\text{N}(\text{C}_3\text{N}_3)_3$. We performed the open-circuit measurements with a two-electrode cell using a Pt wire (99.998%, Alfa Aesar, MA) as working electrode and Ag/AgCl reference electrode (CH Instruments, reference solution: 1.0 M KCl). We

recorded the open-circuit potential with an EMF 16 channel potentiometer (Lawson Labs, Malvern, PA) controlled with EMF Suite 1.02 software (Lawson Labs).

***In situ* Potentiometric Measurements of the Autocatalytic CuAAC.**

A solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (14 mg) and azidoethanol (30 μL) in 900 μL of H_2O was placed into an eppendorf tube equipped with stirring bar, Pt-wire working electrode, and $\text{Ag}|\text{AgCl}$ reference electrode. A solution of tripropargylamine (20 μL) in 400 μL of CH_3OH was then added. The reaction was initiated by the addition of 1% (w:w, with respect to $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) of products of the complete reaction, which contained *tris*-(2-hydroxyethyltriazolylmethyl)amine ($\text{N}(\text{C}_3\text{N}_3)_3$) and complexes of $\text{N}(\text{C}_3\text{N}_3)_3$ with copper. The potential of the Pt electrode vs a $\text{Ag}|\text{AgCl}$ reference electrode was recorded for 3 hours.

ESI-MS Study of the Autocatalytic CuAAC.

A solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (7 mg) and azidoethanol (15 μL) in 450 μL of H_2O was placed into a glass vial (2 mL) equipped with a stir bar, followed by the addition of a solution of tripropargylamine (10 μL) in 200 μL of CH_3OH . The reaction was initiated by the addition of 1% of products of the complete reaction. Aliquots (10 μL) were taken during the course of the reaction, diluted 100 times with H_2O , and analyzed by ESI-MS.

Kinetics of the Autocatalytic CuAAC Reaction by NMR by Quenching Aliquots with KCN.

A solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (21 mg) and azidoethanol (45 μL) in 1350 μL of D_2O was placed into the glass vial (2 ml) equipped with stirring bar, followed by the addition of a solution of tripropargylamine (30 μL) in 600 μL of CD_3OD . Aliquots (100 μL) were taken during the course of the reaction, quenched with 40 μL of a solution of KCN in D_2O (20 mg/mL), and analyzed by ^1H NMR with *t*-BuOH as an internal standard.

pH of the reaction of initial mixture of Tripropargylamine and 2-Azidoethanol in the Presence of CuSO_4

We measured the pH of the initial mixture of tripropargylamine **1** (109 mM), 2-azidoethanol **2** (309 mM) and CuSO_4 (43 mM) in a water:methanol mixture (9:4; v:v), at room temperature. The pH (or pD) of the initial mixture was 4.70 – a value that is primarily determined by the reaction of Cu(II) ions with water, and falls above the $\text{p}K_a$ of compound **1**. We measured the $\text{p}K_a$ of the conjugate acid of the amine **1** by pH titration of **1** in $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ (1:1, v:v) with 1M aqueous HCl (Fig. S1). The conjugate acid of compound **1** had a remarkably low $\text{p}K_a$ of 2.65 ± 0.04 (albeit measured in a 1:1 (v:v) mixture of H_2O and CH_3OH , and not pure H_2O).

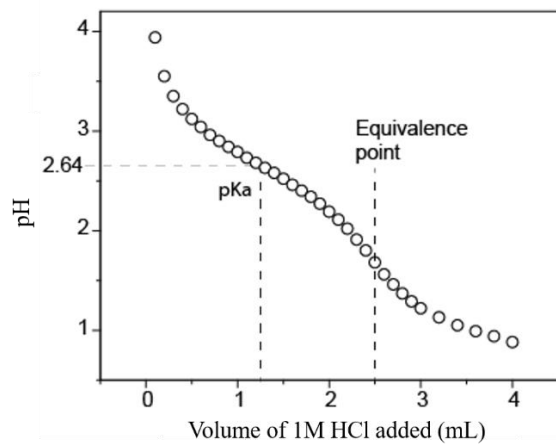


Figure S1. pH titration of tripropargylamine. Titration of a solution of tripropargylamine (500 mM) in H₂O:MeOH (1:1 v:v) (5 mL) with a 1M aqueous solution of HCl, at room temperature. Our measured value of pK_a was supported by the ACDLabs program³, which predicted a pK_a value of 2.86 ± 0.5 (in water).

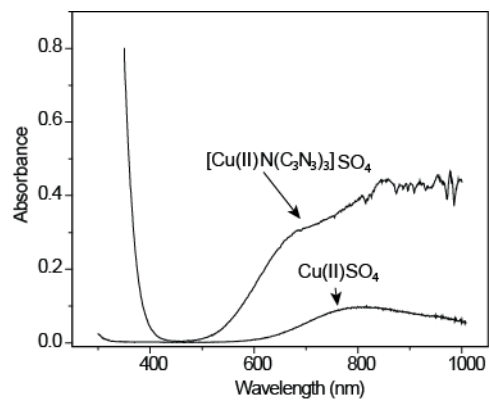


Figure S2. UV-Vis absorption spectra of CuSO₄ (5 mM) and CuSO₄ (5 mM) with N(C₃N₃)₃ ligand (10 mM) in water.

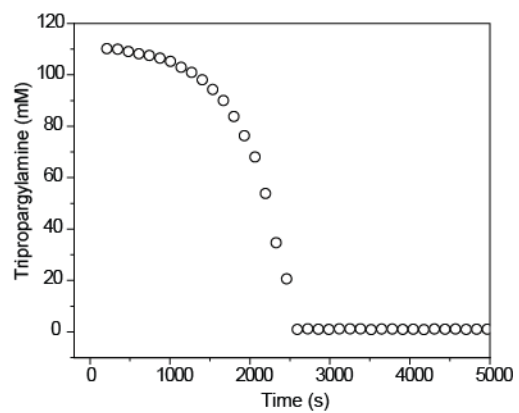


Figure S3. ^1H NMR kinetics study of the reaction between **1** (109 mM), **2** (309 mM), and CuSO_4 (43 mM) in acetate buffer (340 mM, pH 4.7, $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ (9:4, v:v)). Concentration of tripropargylamine was calculated by integrating the alkyne proton against a *tert*-butanol internal standard.

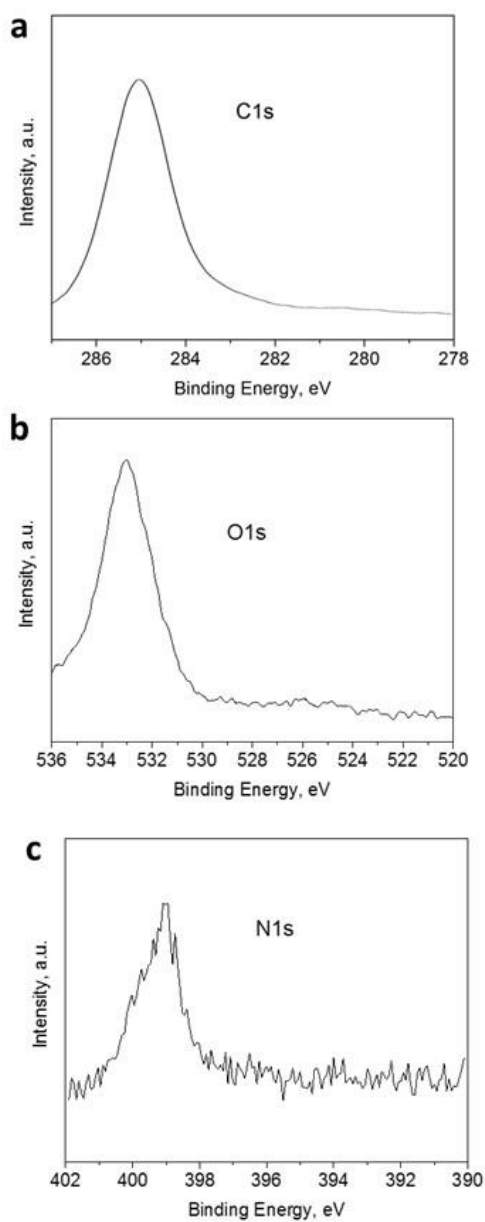


Figure S4. XPS data showing the presence of carbon, nitrogen, and oxygen in the precipitate formed in the reaction of **1** (109 mM) and CuSO₄ (43 mM) in a D₂O/CD₃OD (9:4, v:v) mixture.

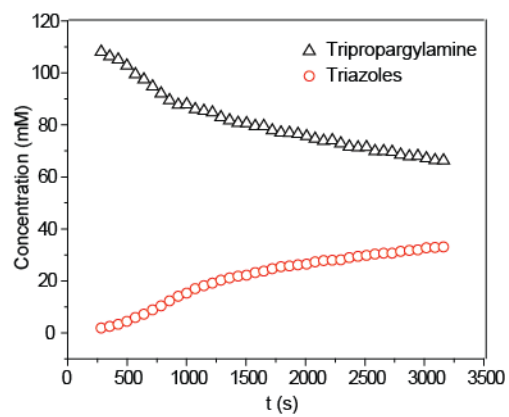


Figure S5. ^1H NMR kinetic experiment for the reaction between tripropargylamine (109 mM), azidoethanol (309 mM), CuSO_4 (2 mM), and ascorbic acid (2 mM) in a mixture of $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ (9:4, v:v) at 25 $^\circ\text{C}$.

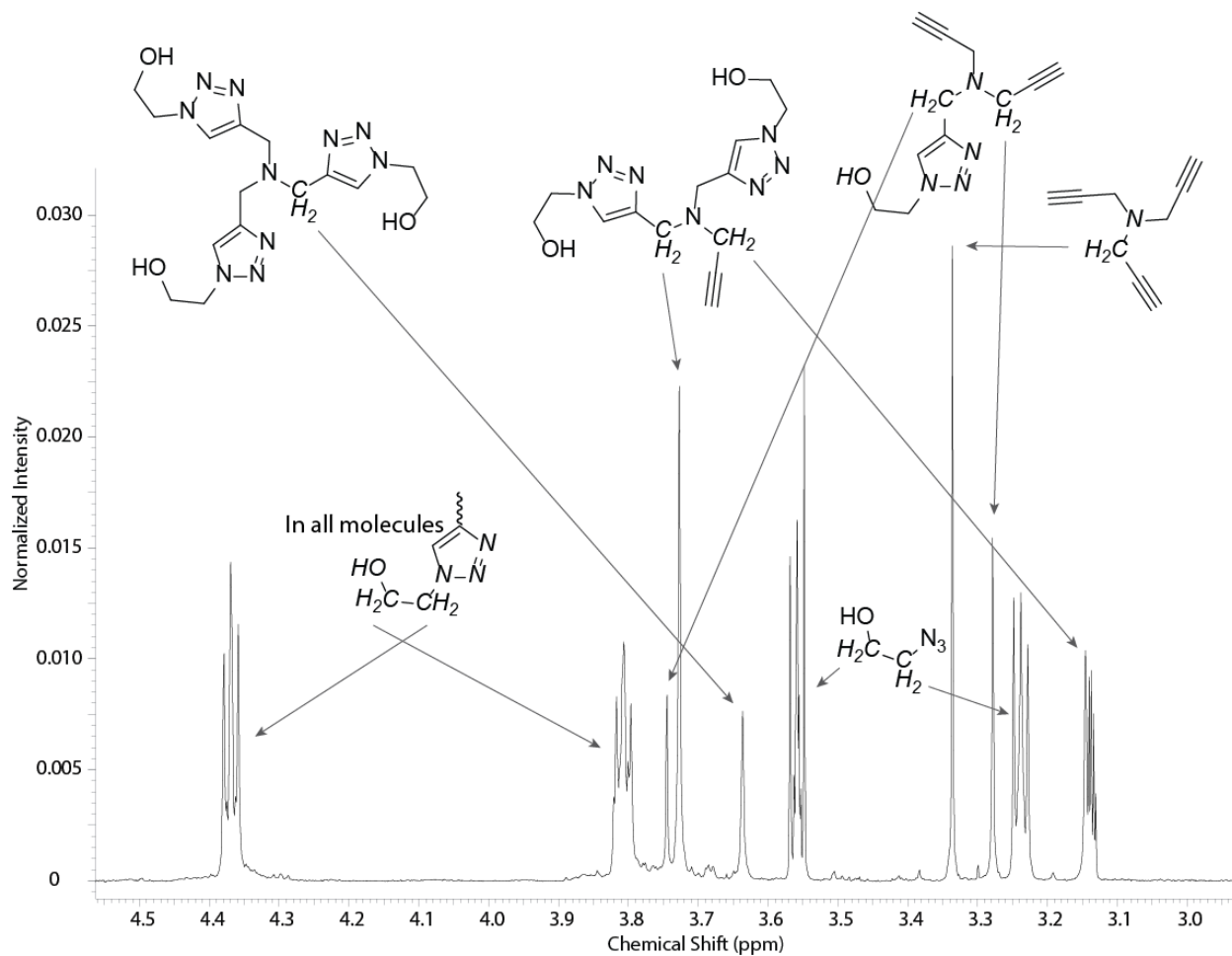


Figure S6. Sample ¹H NMR spectrum obtained by quenching the reaction of **1** (109 mM), **2** (309 mM), and CuSO₄ (43 mM) in a D₂O/CD₃OD (9:4 v:v) mixture at 25 °C, with KCN.

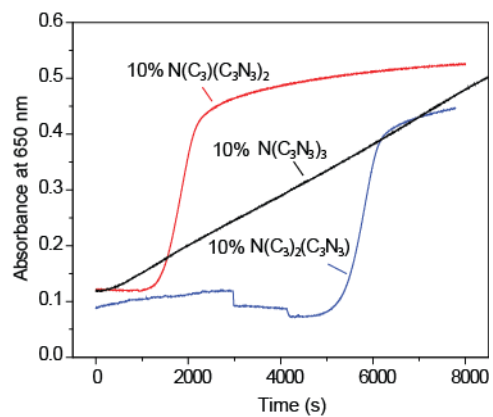


Figure S7. UV-vis analysis of a reaction with 10 mol% starting concentration of $N(C_3)_2(C_3N_3)_2$, $N(C_3)(C_3N_3)_2$, and $N(C_3N_3)_3$. Reaction contained **1** (109 mM), **2** (309 mM), and $CuSO_4$ (43 mM) in a H_2O/CH_3OH (9:4 v:v) mixture at 25 °C.

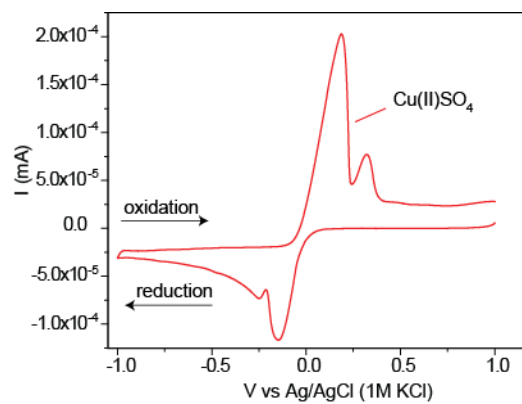
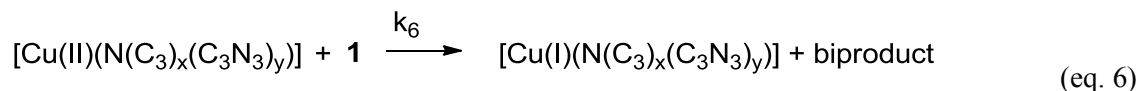
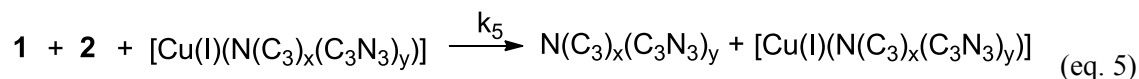
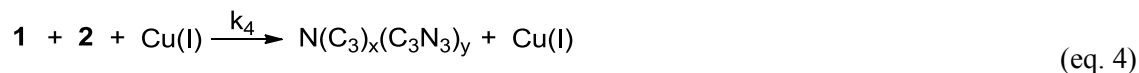
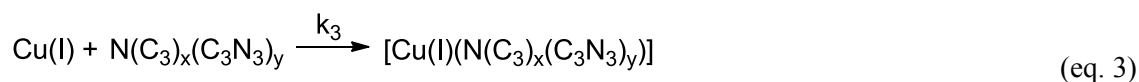
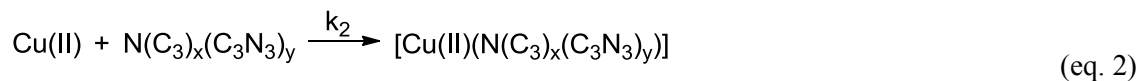


Figure S8. Cyclic voltammograms (scan rate 100 mV/s) of CuSO_4 (5 mM) solution in $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ (9:4 v:v). **b)**

A Model to Describe Autocatalysis.

For simplicity, this model does not differentiate between $N(C_3)_2(C_3N_3)$, $N(C_3)(C_3N_3)_2$, and $N(C_3N_3)$, and considers each cycloaddition step as the formation of a new triazolylmethylamine unit ($N(C_3)_x(C_3N_3)_y$) with an average rate. It also neglects complexity in the kinetics of the cycloaddition step (we consider the reaction to be first-order in azide, alkyne, and copper).

The model includes six reactions: (i; eq. 1) the reduction of $Cu(II) \cdot 6H_2O$ to $Cu(I)$ by an alkyne; (ii; eq. 2) the complexation of $Cu(II)$ by a triazolylmethylamine ($N(C_3)_x(C_3N_3)_y$) ligand; (iii; eq. 3) the complexation of $Cu(I)$ by $N(C_3)_x(C_3N_3)_y$; (iv; eq. 4) the cycloaddition between an azide and an alkyne, catalyzed by $Cu(I)$; (v; eq. 5) the cycloaddition between an azide and an alkyne, catalyzed by a $[Cu(I) N(C_3)_x(C_3N_3)_y]$ complex; and (vi; eq. 6) the reduction of $[Cu(II) N(C_3)_x(C_3N_3)_y]$ by an alkyne.



We considered the rates of formation of coordination complexes of both $Cu(I)$ and $Cu(II)$ to be fast and irreversible; the rate constants for the other reactions were estimated by fitting experimental data

for the kinetics of formation of the triazolyl groups, in the reaction of **1**, **2**, and CuSO₄, to the model described by equations 1-6. The numerical solution of these equations shows kinetics that resemble the experimental data (Fig. S9).

System of ODEs that describe the kinetics of the reported autocatalytic CuAAC reaction.

$$\begin{aligned} \frac{d[\text{Cu}(I)_{aq}]}{dt} &= k_1[\text{Cu}(II)_{aq}][1] - k_2[\text{Cu}(I)_{aq}][N(C_3)_x(C_3N_3)_y] \\ \frac{d[\text{Cu}(II)_{aq}]}{dt} &= -k_1[\text{Cu}(II)_{aq}][1] - k_3[\text{Cu}(II)_{aq}][N(C_3)_x(C_3N_3)_y] \\ \frac{d[1]}{dt} &= -k_1[\text{Cu}(II)_{aq}][1] - k_4[1][2][\text{Cu}(I)_{aq}] - k_5[1][2][\text{Cu}(I)(N(C_3)_x(C_3N_3)_y)] \\ &\quad - k_6[\text{Cu}(II)(N(C_3)_x(C_3N_3)_y)][1] \\ \frac{d[2]}{dt} &= -k_4[1][2][\text{Cu}(I)_{aq}] - k_5[1][2][\text{Cu}(I)(N(C_3)_x(C_3N_3)_y)] \\ \frac{d[N(C_3)_x(C_3N_3)_y]}{dt} &= k_4[1][2][\text{Cu}(I)_{aq}] + k_5[1][2][\text{Cu}(I)(N(C_3)_x(C_3N_3)_y)] - k_2[\text{Cu}(I)_{aq}][N(C_3)_x(C_3N_3)_y] \\ &\quad - k_3[\text{Cu}(II)_{aq}][N(C_3)_x(C_3N_3)_y] \\ \frac{d[\text{Cu}(I)(N(C_3)_x(C_3N_3)_y)]}{dt} &= k_2[\text{Cu}(I)_{aq}][N(C_3)_x(C_3N_3)_y] + k_6[\text{Cu}(II)(N(C_3)_x(C_3N_3)_y)][1] \\ \frac{d[\text{Cu}(II)(N(C_3)_x(C_3N_3)_y)]}{dt} &= k_3[\text{Cu}(II)_{aq}][N(C_3)_x(C_3N_3)_y] - k_6[\text{Cu}(II)(N(C_3)_x(C_3N_3)_y)][1] \end{aligned}$$

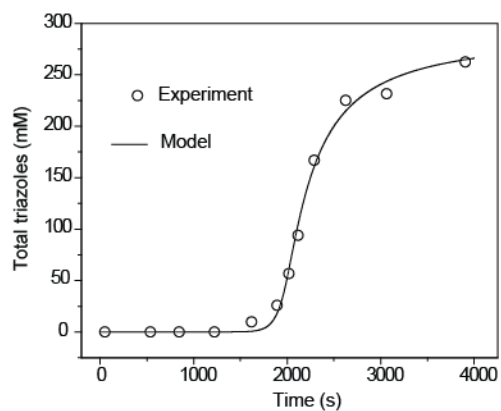


Figure S9. Experimental and simulated kinetics of formation of triazolyl groups. The experimental concentrations of triazolyl groups were calculated from the data of Figure 3e. The parameters of the model were $[1]_0 = 327 \text{ mM}$; $[2]_0 = 309 \text{ mM}$; $[\text{Cu(II)}]_0 = 43 \text{ mM}$; $k_1 = 1.3 \cdot 10^{-7} \text{ M}^{-1}\text{s}^{-1}$; $k_2 = 1 \text{ M}^{-1}\text{s}^{-1}$; $k_3 = 100 \text{ M}^{-1}\text{s}^{-1}$; $k_4 = 1.2 \cdot 10^{-6} \text{ M}^{-2}\text{s}^{-1}$; $k_5 = 0.378 \text{ M}^{-2}\text{s}^{-1}$; $k_6 = 0.0184 \text{ M}^{-1}\text{s}^{-1}$

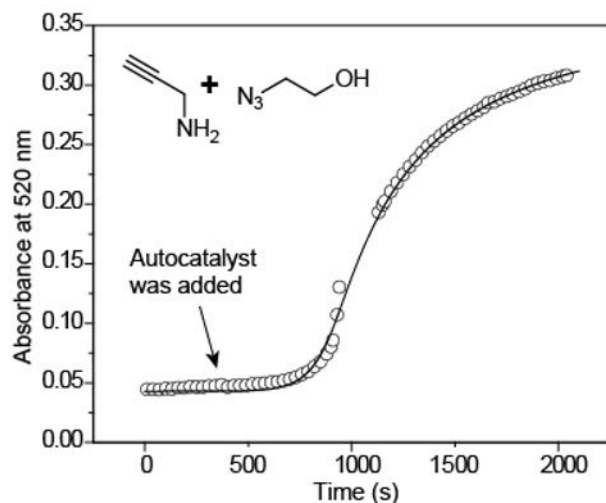
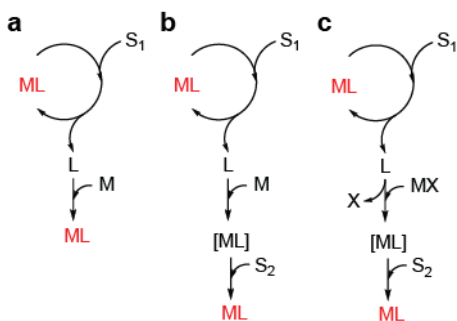


Figure S10. UV-Vis kinetic experiment for the reaction between propargylamine (**6**) (109 mM), **2** (309 mM), CuSO₄ (43 mM), and sodium citrate (60 mM) in a H₂O/CH₃OH (9:4; v:v) mixture at 25 °C. The line is fit with a numerical model, and compensates for the missing points in the transition region. These points are not observed because of scattering by transiently formed particles.

Visually, the reaction in Figure S10 turned spontaneously to a dark blue solution, after an initial lag phase of roughly ten minutes. The formation of precipitates in this reaction, however, prevented us from monitoring their kinetics by NMR or UV spectroscopy. To suppress the formation of these precipitates, in the reaction of **2** with **6**, we added 0.5 equiv (relative to copper) of sodium citrate. Citrate forms a coordination complex with copper, and prevents the formation of copper hydroxides, which are insoluble in water. We then monitored the reaction by UV-Vis spectroscopy (Fig. 6b). The sigmoidal shape of the curve again supported an autocatalytic reaction between **2**, **6**, and CuSO₄.



Scheme S1. Network motifs in the autocatalytic CuAAC. **a)** A motif of the autocatalytic cycle driven by the formation of a ligand (L) for the catalytic metal ion (M) **b)** A motif that requires an extra step to form a catalytically active complex. **c)** A motif that involves ligand exchange. S_1 and S_2 stand for starting materials.

Supplementary Video S1.

Video showing a reaction front driven by the autocatalytic copper catalyzed azide-alkyne cycloaddition. The reaction takes place in a 1 mm thick agarose gel loaded with **1** (125 mM), azidoethanol (320 mM), and CuSO_4 (84 mM). We initiated the reaction at the central point in the gel using a crystal of ascorbic acid. The yellow color comes from the reduced Cu(I) species, the blue color comes from the Cu(II) complex with $\text{N}(\text{C}_3\text{N}_3)_3$ ($\text{Cu}(\text{II}) \text{N}(\text{C}_3\text{N}_3)_3$) and indicates progress of the reaction. The video was accelerated 1024x times, from 1 hour to approximately 3.5 seconds.

References

1. Wang, C.; Abegg, D.; Hoch, D. G.; Adibekian, A., *Angew. Chem. Int. Edit.* **2016**, *55*, 2911-2915.
2. Bew, S. P.; Hiatt-Gipson, G. D.; Lovell, J. A.; Poullain, C., *Org. Lett.* **2012**, *14*, 456-459.
3. *Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2017 ACD/Labs).*