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A Highly Efficient Single-Chain Metal–Organic Nanoparticle Catalyst for Alkyne–Azide "Click" Reactions in Water and in Cells

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Supporting Information

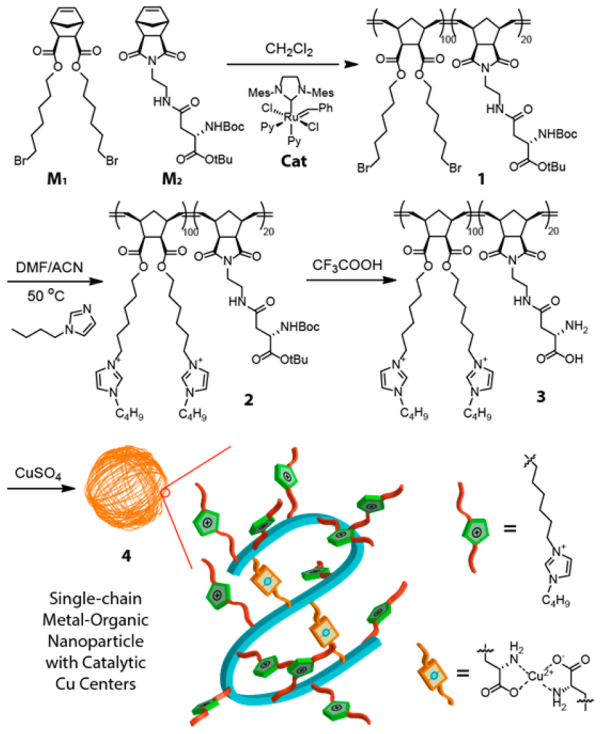
ABSTRACT: We show that copper-containing metal–organic nanoparticles (MONPs) are readily synthesized via Cu(II)-mediated intramolecular cross-linking of aspartate-containing polyolefins in water. In situ reduction with sodium ascorbate yields Cu(I)-containing MONPs that serve as highly efficient supramolecular catalysts for alkyne–azide "click chemistry" reactions, yielding the desired 1,4-adducts at low parts per million catalyst levels. The nanoparticles have low toxicity and low metal loadings, making them convenient, green catalysts for alkyne–azide "click" reactions in water. The Cu-MONPs enter cells and perform efficient, biocompatible click chemistry, thus acting as intracellular nanoscale molecular synthesizers.

The key role played by transition metal catalysts in chemical reactions[1,2] has led to their integration into a wide array of more complex structures. Indeed, several types of macromolecular metal catalysts have been developed to increase catalyst selectivity, stability, and recyclability. For example, metal–organic frameworks (MOFs) are heterogeneous lattices in which the metal is integral to the three-dimensional structure.[3] As catalysts, MOFs' highly organized structures represent one end of the spectrum,[4] whereas at the other are homogeneous or heterogeneous polymer-supported catalysts that are much less defined structurally, with one or more metal complexes attached to the side chain or the end of a random-coil polymer.[5] A new macromolecular motif was reported recently by Lemcoff and co-workers, which they named organometallic nanoparticles (OMNPs).[6−8] The OMNPs are single-chain linear polymers (SCNPs)[9] that are controllably "folded" by the formation of carbon–metal bonds into denser structures that contain metal centers and cavities, resembling metalloenzymes. Indeed, both Rh(I) and Ir(I) OMNPs showed catalytic activity in organic solvents. Pomposo and co-workers described copper containing SCNPs that catalyze coupling reactions and polymerizations, including in water.[10,11]

We recently reported several types of covalently cross-linked polymers, termed organic nanoparticles (ONPs), that present an array of functional groups and enter cells in a controllable manner.[12−14] Here we describe water-soluble, copper-cross-linked single-chain metal–organic nanoparticles (MONPs) as nanoscale catalysts. As with the Lemcoff and Pomposo systems, our Cu-containing MONPs were designed to share some structural similarity with metalloenzymes and, in our case, to promote the copper-catalyzed alkyne–azide cycloaddition (CuAAC). The CuAAC "click" reaction was pioneered by Sharpless,[15] with significantly improved catalysts reported later.[16] We show herein that the easily synthesized Cu-MONPs have low toxicity and promote the regioselective ligation of terminal alkynes and azides with high efficiency at low parts per million levels in water.

For maximum utility, MONPs should have three key properties: water solubility, catalytically active metal centers, and the ability to bind hydrophobic substrates. To install these properties, ring-opening metathesis polymerization[17] of monomers $\mathbf{M}_1$ and $\mathbf{M}_2$ (Scheme 1) was performed using Grubbs third-

Scheme 1. Schematic Illustration of the Synthesis of the Copper-Containing MONPs and a Cartoon Showing the Internal Structure of the Cross-Linkage



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generation catalyst[18] with a M_1:M_2:Cat ratio of 100:20:1 to give **1** (Scheme 1). Gel-permeation chromatography (GPC) indicated a molecular weight close to the calculated value and a low polydispersity index (1.04) for the parent linear polymer (see Figure S2). The polymer was quaternized with *N*-butylimidazole to give **2**, which was purified by precipitation. The imidazolium groups in **2** provide water solubility,[19] whereas the alkyl chains provide a hydrophobic interior to the MONP for substrate binding.

α-Amino acids form stable coordination complexes with Cu(II)[20] and Cu(I),[21,22] so **2** was prepared with a few aspartate-bearing comonomers M_2, allowing a limited number of copper-ion-mediated intrachain cross-links. Polymer **2** was deprotected with trifluoroacetic acid to give linear polymer **3**, which was intramolecularly cross-linked at high dilution in water using 0.5 equiv of $CuSO_4$ per amino acid residue, yielding Cu-MONPs **4**. Dynamic light scattering showed the products to have slightly smaller radii of gyration (R_g) (Figure S6) with no evidence of interchain cross-linking. The minimally reduced particle size is consistent with a small contraction from a few intrachain cross-links on a hydrophobically collapsed random-coil polynorbornene.

Atomic force microscopy (AFM) was used to visualize and probe the mechanical properties of polymer **3** and Cu-MONPs **4**. The un-cross-linked polymers appear larger and less homogeneous than the Cu-MONPs (Figure 1a), similar to what was

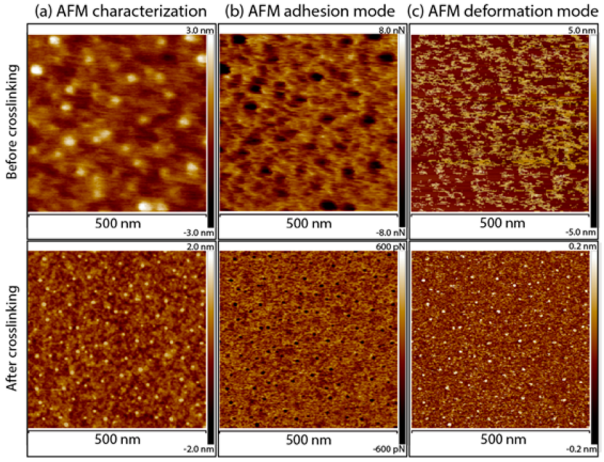
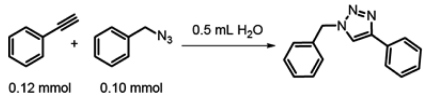


Figure 1. AFM characterization of (bottom) the Cu-MONP and (top) its parent linear polymer in (a) normal (height) mode, (b) adhesion mode, and (c) deformation mode.

observed with ONPs.[12] The relatively narrow size distribution of the MONPs is consistent with intramolecular cross-linking. Similar results were observed in adhesion mode (Figure 1b). Importantly, the adhesion force from the Cu-MONPs was much smaller than found with the linear polymers. For un-cross-linked linear polymers with random-coil structures, the number of conformations available during macromolecular extension is high, leading to a greater adhesive force. In contrast, Cu-MONPs have more fixed conformations with a smaller entropic elasticity.[23] The difference in AFM adhesion-mode further supports the formation of intramolecular cross-links in the MONP synthesis. In AFM deformation-mode, the Cu-MONPs show significantly higher rigidity and resistance to deformation (Figure 1c), consistent with the observation of previously reported dendritic ONPs,[24] providing additional evidence of cross-linking mediated by Cu(II). Likewise, there is a small increase in particle height (z) despite the significant diameter reduction observed in the xy plane after cross-linking (Figure S8).[24]

To compare the activity of the Cu-MONPs to those of polymeric copper catalysts reported previously for the Cu(I)-catalyzed regioselective Huisgen ligation,[16,25] i.e., the "click" reaction, the cycloaddition between phenylacetylene and benzyl azide was investigated as a model system. Sodium ascorbate was used to reduce Cu(II) to Cu(I) in the MONPs *in situ*,[26] and the reaction was performed in pure water. Phenylacetylene was added in slight excess, and the yield of the reaction was calculated by comparison of the integrated intensity of the ^{1}H NMR signal of the methylene group in benzyl azide with that in the product. In the presence of 2.5–10 ppm Cu-MONPs with respect to benzyl azide at 50 °C, reactions went to completion in 24 h, with the 1,4-triazole as the only product. The 1,4-triazole was obtained in 58% yield with just 1 ppm Cu-MONPs (Table 1). Without

Table 1. "Click" Reactions of Benzyl Azide and Phenylacetylene with Cu-MONPs or $CuSO_4$



Cu-MONP level (ppm)	Cu level (ppm)	NaAsc[a]	t (h)	T (°C)	yield[b] (%)
1.0	10	+	24	50	58[c]
2.5	25	+	24	50	>99
5.0	50	+	24	50	>99
10	100	+	24	50	>99
2.5	25	+	72	25	>99
0	25[d]	+	336	25	9[e]
2.5	25	–	336	25	9[e]
0	0	–	24	50	6[e]
0	25[d]	+	24	50	6[e]

[a]Reactions marked "+" contained 2.5 mg of sodium L-(+)-ascorbate (NaAsc). [b]By NMR integration (for the procedure, see the Supporting Information). [c]The reaction was performed in 0.25 mL of water. [d]Because no Cu-MONPs were used, the corresponding copper level was reached by adding $CuSO_4$ solution. [e]NMR yield of the 1,4-isomer. The crude product also contained approximately the same amount of the 1,5-isomer for these entries.

Cu(I) or with $CuSO_4$, only a low conversion of the starting material to product was observed, with a mixture of 1,4- and 1,5-triazoles, indicative of an uncatalyzed reaction.

To validate the generality of Cu-MONP catalysis, the cycloaddition reactions of additional substrate pairs were investigated. As can be seen in Table 2, seven different terminal alkynes and five different azides underwent the Cu-MONP-catalyzed click reaction, with isolated yields ranging from 83% to 98% at 5–30 ppm Cu-MONPs after 24 h at 50 °C. For entries 2 and 3, chloroform was added to improve the reaction kinetics (see Table S1 and the discussion in the Supporting Information). Preliminary data suggest that Cu-MONPs are significantly less effective with highly hydrophilic substrates at preparative scale, such as propargyl alcohol and 6-azidohexanoic acid (see the Supporting Information for additional details). These results support the importance of hydrophobic binding, but further studies are needed to reach a firm conclusion.

For the examples shown, the catalyst loadings needed for Cu-MONPs were significantly lower than those in most previous reports and comparable to the lowest catalyst loadings reported

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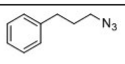
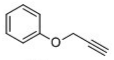
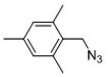
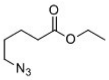
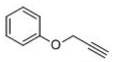
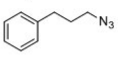
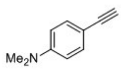
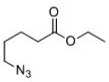
Table 2. "Click" Reactions between Various Terminal Alkynes and Azides Using Cu-MONPs as the Catalyst[a]

	Alkyne	Azide	Cu-MONP (ppm)	NMR Yield[b] (%)	Isolated Yield (%)
1			5	93	91
2[c]			7.5	97	94
3[c]			5	95	94
4			10	>99	95
5			10	93	93
6			7.5	92	90
7			15	87	84
8			5	>99	96
9			5	>99	97
10			5	88	83
11			30	>99	98

[a]Unless otherwise noted, all reactions were carried out with 0.10 mmol of azide, 0.12 mmol of alkyne, 2.5 mg of sodium ascorbate in 0.5 mL of water at 50 °C for 24 h. Dosages of Cu-MONPs are listed in the table for each reaction. [b]By NMR integration (see the Supporting Information). [c]$CDCl_3$ (50 μL) was added to the reaction mixture to delay the crystallization of the product and improve the kinetics.

in water, e.g., by Astruc[16b] and Yamada and Uozumi.[16d] The fate of the Cu ion during and after the reaction was not determined. For example, it was beyond the scope of this study to determine whether Cu was lost from the MONPs. Even if metal loss occurs, it would represent a very low level of contamination. For the reaction catalyzed by 2.5 ppm Cu-MONPs, the 0.5 mL of water used as the solvent contained only 2.5 nmol of Cu, equivalent to 0.32 ppm by weight, which is lower than the maximum contaminant level goal (MCLG) for Cu in drinking water (1.3 ppm) defined by the U.S. Environmental Protection Agency.

In addition to their potential utility in chemical synthesis, the low Cu concentrations needed for a highly reactive catalyst suggest the possibility of performing "click" reactions under biologically relevant conditions. Successful intracellular "click" reactions were pioneered by Ting and co-workers using copper-chelating azides for biomolecular labeling,[27] and such intracellular reactions have also been found to be useful for the measurement of pH inside *Escherichia coli*.[28] Both studies used >100 μM concentrations of ligands analogous to tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) developed by Sharpless and Fokin[16a] and Cu concentrations of at least 40 μM.

To test the Cu-MONPs for activity at low substrate and catalyst concentrations, 3-azido-7-hydroxycoumarin (**5**) and 7-ethynylcoumarin (**6**) were used as well-known fluorogenic coumarin derivatives that "light up" upon alkyne–azide cycloaddition. Results for two pairs of compounds are given in Figure S9. The click efficiencies for the two pairs of compounds were similar, leading to ≥90% conversion in 60 min at 1 μM Cu-MONPs. The potential for intracellular Cu-MONP catalysis of the reaction between **5** and **7** was tested in live cells using confocal microscopy. NCI-H460 (human non-small cell lung carcinoma) and MDA-MB-231 (human breast cancer) cells were preincubated with phosphate-buffered saline (PBS) or Cu-MONPs (0.5 μM in PBS) for 1 h. The PBS or Cu-MONPs was removed, and the cells were washed three times. The substrates (100 μM) were added along with sodium ascorbate (2 mM) to effect Cu(II) reduction. As shown in Figure 2b, only weak

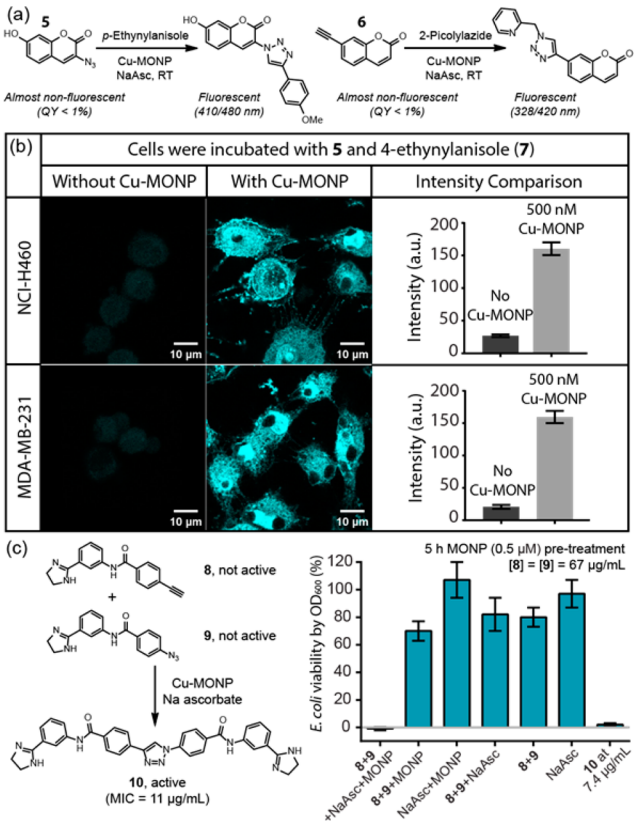


Figure 2. (a) Fluorogenic click compounds **5** and **6**. (b) Confocal microscopy study of intracellular catalysis of the "click" reaction between **5** (100 μM) and **7** (100 μM) by **4** (0 or 500 nM). (c) Model study of the intracellular synthesis of antimicrobial bisamidine **10** from **8** and **9** in *E. coli* catalyzed by **4**. NaAsc = sodium L-ascorbate.

fluorescence, likely from unreacted azidocoumarin, could be observed in the absence of Cu-MONPs, whereas cells were intensely fluorescent in the presence of the catalyst. Cytotoxicity, hemolytic, and Amplex-Red/horseradish peroxidase[29] assays were performed and revealed acceptable biocompatibility for the Cu-MONPs (Figures S10–S12).

The apparent intracellular click reaction described above suggests the potential utility of MONPs in the intracellular synthesis of more complex molecules. As a proof of concept, the modular assembly of an antimicrobial agent inside *E. coli* was tested. This experiment used amidine-containing alkyne **8** and azide **9**, each of which showed minimal antimicrobial activity against *E. coli* (MIC > 300 μg/mL). The click product of the two, bisamidine **10**, showed much higher activity (MIC = 11 μg/mL).[30] Thus, *E. coli* with or without MONP **4** pretreatment and washing were treated with various combinations of **8**, **9**, and

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sodium ascorbate. Strong inhibition of bacterial cell growth was observed in the experiment containing MONPs, **8**, **9**, and sodium ascorbate, whereas all other treatment combinations showed little inhibitory effect (Figure 2c). These data suggest the intracellular formation of **10** mediated by the Cu-MONPs and subsequent growth inhibition.

In summary, we developed Cu-containing organic nanoparticles that catalyze the CuAAC click reaction with high efficiency in water. Beyond their utility in chemical synthesis in water, our results demonstrate that Cu-MONPs can effect intracellular click reactions inside both bacteria and mammalian cells. The intracellular examples featured simple azides and alkynes, but more complicated modular assembly of complex molecules may be possible using appropriate MONPs and bioorthogonal reaction partners. Indeed, it may be possible to selectively synthesize inside cells compounds that would otherwise be difficult to deliver.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04477.

Detailed synthetic procedures and characterization data of the MONPs, "click" substrates, and products (PDF)

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Notes

The authors declare no competing financial interest.

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