

Spatial and temporal control of the alkyne–azide cycloaddition by photoinitiated Cu(II) reduction

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The click reaction paradigm is focused on the development and implementation of reactions that are simple to perform while being robust and providing exquisite control of the reaction and its products. Arguably the most prolific and powerful of these reactions, the copper-catalysed alkyne–azide reaction (CuAAC) is highly efficient and ubiquitous in an ever increasing number of synthetic methodologies and applications, including bioconjugation, labelling, surface functionalization, dendrimer synthesis, polymer synthesis and polymer modification. Unfortunately, as the Cu(I) catalyst is typically generated by the chemical reduction of Cu(II) to Cu(I), or added as a Cu(I) salt, temporal and spatial control of the CuAAC reaction is not readily achieved. Here, we demonstrate catalysis of the CuAAC reaction via the photochemical reduction of Cu(II) to Cu(I), affording comprehensive spatial and temporal control of the CuAAC reaction using standard photolithographic techniques. Results reveal the diverse capability of this technique in small molecule synthesis, patterned material fabrication and patterned chemical modification.

The diversity in the implementation of the CuAAC reaction is owed to its simplicity, high yield, fast reaction kinetics, orthogonal reactivity and tolerance of a wide variety of solvent conditions¹. The CuAAC reaction is therefore valuable across a vast library of materials applications^{2–6} and chemical modifications^{7–10}. However, the conventional generation of the catalytic Cu(I) species lacks spatial and temporal control. Although this deficit may seem trivial from a purely synthetic point of view, spatial control is necessary in the creation of numerous functional materials^{11,12}, such as inks, coatings, adhesives, metamaterials, contact lenses, dental materials and photoresists, and is used in techniques such as parallel protein synthesis¹³, cell encapsulation¹⁴, tissue engineering¹⁵ and three-dimensional prototyping^{16,17}. Temporal control enables workers to initiate a reaction on demand, which is critical when materials must be spin-coated or otherwise prepared after mixing of the components, but before a reaction occurs. The ability to spatially and temporally control the CuAAC reaction would extend the CuAAC reaction capabilities, improve its ease of use, and increase the number of materials that can be patterned readily.

The critical need for complete spatial control of the CuAAC reaction is demonstrated by the extent to which researchers have gone to facilitate partial control of this reaction. Dip-pen lithography using a Cu(I) inked tip or a copper tip^{18,19} has been used to trace a pattern on the substrate and catalyse the CuAAC reaction between a functionalized surface and the alternative click reagent in the bulk. This technique has produced features with dimensions as small as 50 nm, and micrometre-scale features require half a second to complete. Similarly, microcontact printing makes use of an elastomeric stamp inked with a solution of reagent to promote spatial control^{2,20}. Here, the catalytic Cu(I) is either included in the solution, or is generated from a copper metal layer coating the surface of the stamp. The stamp is brought into contact with a functionalized surface for 15 min to an hour. Features on the order of tens of micrometres are readily fabricated using this technique. Electroclick chemistry involves the application of an electric

potential across a pair of electrodes^{21,22}. At the negative electrode, Cu(II) is reduced to Cu(I), and the CuAAC reaction is subsequently catalysed, allowing the production of features as small as 10 μm . These techniques are not without their drawbacks. Microcontact printing uses inexpensive elastomeric stamps than can rapidly reproduce images. However, the master for the stamps must be fabricated using another technique that is capable of directly writing the master. Electroclick chemistry shares this drawback. Dip-pen lithography is capable of directly writing high-fidelity images, but is therefore ill-suited to the reproduction of large images and features. Photolithography uses masked or focused light to irradiate a specific area, and induces chemical reactions that change the solubility of the photosensitive material. An image is then developed by immersion in a solvent. Photolithography can both directly write images and reproduce images, even using masks produced by inkjet printers²³. Unlike all the techniques currently used in patterning a CuAAC reaction, photolithography can also be used to produce three-dimensional images and reactions^{16,17}, to write images within a material⁴, and to functionalize a material anywhere throughout it.

Following irradiation, photochemical reactions occur either by direct excitation of chromophoric species, as in the case of [2 + 2] cycloadditions, or by the generation of an active species that initiates multiple reactions, as is typical of photopolymerizations. Photochemical 1,3-cycloadditions are symmetry-forbidden²⁴, and the photopatterning of azides and alkynes has been limited to the photochemical decarbonylation of propenones to dibenzocyclooctynes, which subsequently undergo a thermal 1,3-dipolar cycloaddition (copper-free azide–alkyne click reaction)²⁵. Despite the success of this innovative approach for labelling cells²⁵ and functionalizing surfaces²⁶, it is limited by the complexity and scarcity of materials with the requisite cyclopropenone functional group, the synthesis of which is non-trivial. Furthermore, this mechanism requires large irradiation doses to obtain high conversions, as each absorbed photon leads to a maximum of one bimolecular coupling event. In contrast, unprotected, reactive azides are readily

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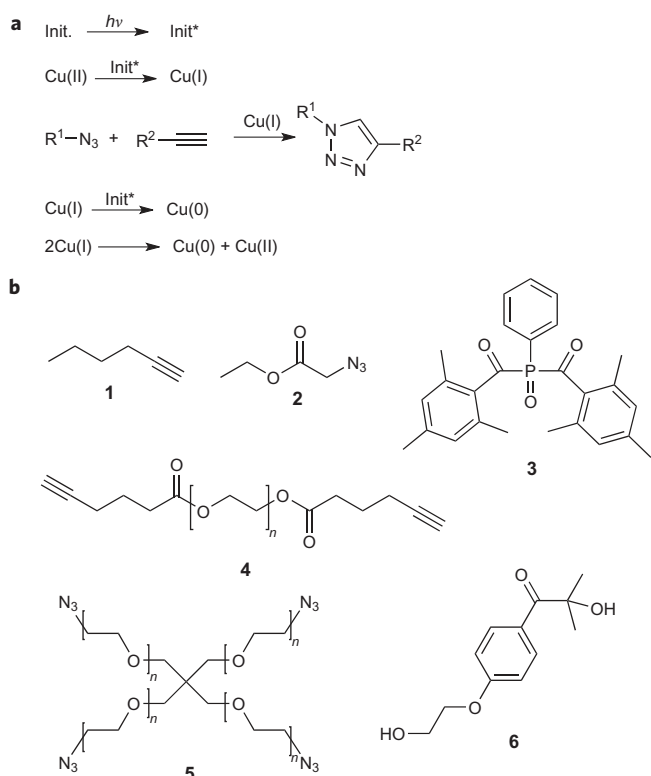


Figure 1 | General scheme for a photocatalysed CuAAC reaction. **a**, A photoinitiator is first used to generate radicals, which reduce Cu(II) to Cu(I). The transiently generated Cu(I) then catalyses the 1,3-dipolar cycloaddition. The Cu(I) is ultimately either reduced to copper metal or disproportionates to form Cu(0) and Cu(II). Reactions of Cu(I) and oxygen are also possible (not shown). **b**, 1-Hexyne (**1**), ethyl azidoacetate (**2**), and Irgacure 819 (**3**) were used in the ¹H-NMR and FTIR experiments, and a 3K PEG-dialkyne (**4**), a 10K PEG-tetraazide (**5**) and Irgacure 2959 (**6**) were used for hydrogel synthesis.

synthesized from acyl halides, and typical photoinitiated polymerizations follow a chain reaction mechanism, in which a single absorbed photon leads to a reaction cascade that ultimately consumes many reactant molecules per absorbed photon.

Although the 1,3-dipolar cycloaddition is not influenced by conventional photogenerated active species, such as carbocations and radicals, the reaction rate is increased by a factor of $\sim 10^7$ by catalytic amounts of Cu(I) (ref. 27). This catalytic behaviour permits the photochemical generation of Cu(I) to be used in a manner analogous to the generation of radical or carbocation species in traditional photopolymerization processes, and would enable spatial and temporal control of the CuAAC reaction. Furthermore, photochemical generation of Cu(0) from Cu(II) was previously demonstrated in the synthesis of three-dimensional nanoparticles by photoinduced radical generation, where the radicals reduce Cu(II) to Cu(0) (ref. 28). Here, we demonstrate that the Cu(I) transiently generated in the course of such a process is able to catalyse the Huisgen 1,3-dipolar cycloaddition, enabling a photoinducible azide-alkyne cycloaddition (pCuAAC) reaction (Fig. 1a).

Results and discussion

UV irradiation of an aqueous solution of monofunctional azide and alkyne species (1-hexyne **1** and ethyl azidoacetate **2**) in the presence of a cleavage-type photoinitiator (Irgacure 819, **3**) and copper sulfate pentahydrate results in the generation of radicals that subsequently reduce Cu(II) to Cu(I) (structures are shown in Fig. 1b). ¹H nuclear magnetic resonance (NMR) spectroscopy reveals complete

conversion (98% yield) of azide and alkyne functional groups and the production of the expected triazole species without side products (see Supplementary Information). The use of TiO₂ as a radical source produced similar results, but due to the heterogeneous nature of TiO₂, the system was not readily amenable to further experimental analysis. The performance of the CuAAC reaction depends strongly on the amplified character of this process, as each photoinitiated radical leads to Cu(I) species that subsequently catalyse many reactions. Kinetic aspects of the reaction were explored by monitoring the concentration of ethyl azidoacetate via Fourier transform infrared spectroscopy (FTIR). These experiments had a maximum rate of $9 \times 10^{-2} \text{ mM s}^{-1}$, which falls within the range of the two sodium ascorbate and copper sulfate systems measured by Kasuga and colleagues²⁹. Control experiments with the absence of light, initiator or copper indicated that each component was necessary, therefore supporting our proposed mechanism (Fig. 2). Off-stoichiometric experiments further verified the absence of side reactions such as Eglinton coupling.

The persistence of catalytic Cu(I) was explored by monitoring the reaction behaviour following irradiation cessation (in other words, turning off the light), and before complete reactant consumption, revealing that once initiated, the reaction persists for extended times in the dark (Fig. 2). This continuing reaction suggests that Cu(I) is not rapidly consumed during the dark reaction (after irradiation has ceased), suggesting that the disproportionation of Cu(I) to Cu(II) and Cu(0) occurs over a timescale longer than that of the CuAAC reaction (as radicals are no longer available for reduction). This persistent behaviour of Cu(I) enables a complete reaction to be achieved with a minimal amount of required irradiation. For example, a sample irradiated for 5 min reaches 20% conversion during irradiation, but proceeds to 80% conversion after 160 min in the dark. Accordingly, in a continuously irradiated sample, each photon absorbed results in the reaction of ~ 20 azides and alkynes, and a sample irradiated for 5 min produces at least 130 reaction events per absorbed photon.

Although temporal control of material-forming reactions increases their ease of use, much of the interest in the CuAAC reaction is associated with its high conversion and functional group tolerance, promoting efficient orthogonal synthesis as well as the facile modification of materials such as surfaces and polymers³⁰. The spatial control enabled by photopolymerization should extend the capability of the CuAAC reaction to creating patterned devices, materials and structures. Hence, we explored the synthesis

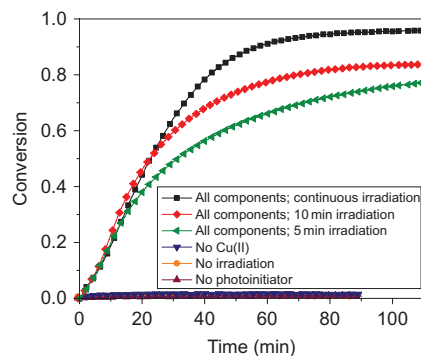


Figure 2 | Photo-CuAAC reaction kinetics. Complete conversion of the azide species to form triazole **7** is shown to occur in ~ 60 min for a dimethylformamide solution with 200 mM 1-hexyne (**1**), 200 mM ethyl azidoacetate (**2**), 10 mM copper sulfate and 10 mM Irgacure 819 (**3**) irradiated with 10 mW cm^{-2} 400–500 nm light. Also shown is the azide conversion for mixtures lacking Cu(I), irradiation or photoinitiator. No significant reaction is noted for any of these control samples and all three lines overlie one another. Conversion is also shown when irradiation was ceased after 5 or 10 min during the course of the reaction.

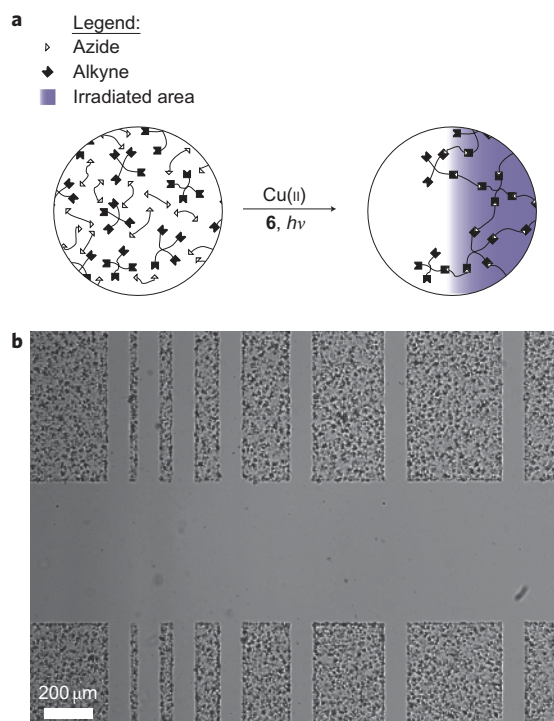


Figure 3 | Hydrogel formation patterned by photo-CuAAC reaction. a, Hydrogels are formed by irradiating a 3K PEG dialkyne (**4**) and a 10K PEG tetraazide (**5**) in the presence of Irgacure 2959 (**6**) and copper sulfate using masked light. The gels form only in the irradiated area. **b,** Bright-field image showing one such dehydrated gel, $\sim 4 \mu\text{m}$ thick. The photomask consists of 25, 50, 100, 200, 300 and 400 μm bars separated by 100 μm spaces.

and *in situ* modification of hydrogels, an important class of polymers that show promise as tissue mimics¹⁴, as well as alkyne-functionalized silica surfaces. Multifunctional alkyne (**4**) and azide (**5**) functionalized polyethylene glycol (PEG) monomers were readily synthesized (see Supplementary Information) and irradiated in the presence of copper sulfate and photoinitiator (Irgacure 2959, **6**), yielding hydrogels that were formed in less than 4 min (as determined by rheometry; see Supplemental Information). The time to gel is comparable with gels formed by similar concentrations of Cu(II) and reduced using sodium ascorbate, and is easily controlled by varying the light intensity. As a consequence of the reduced functional group concentration, the ratio of Cu(II) to functional groups is much higher, and the reaction appears to proceed faster. Uniquely, this photopolymerization proceeds by a step growth mechanism, an attribute of photopolymerizations that is currently shared only with the thiol-ene and thiol-yne photopolymerization reactions³¹. Compared to chain growth polymerizations, this mechanism allows for delayed and readily predicted gel-point conversions. The nearly ideal hydrogels formed by the CuAAC reaction have been shown to have improved mechanical properties compared to networks formed by radical crosslinking of diacrylates³². Furthermore, it has been shown that the cytotoxic Cu(I) from the CuAAC reaction can be removed by ethylenediaminetetraacetic acid (EDTA)³².

The spatial resolution of a photolithographic process is either limited by the ability of the optics to project the desired image, or the capability of the photoinduced process to reproduce the image. The persistence of Cu(I) in the previous experiments suggests that the diffusivity of Cu(I) may be an important consideration, and taking the diffusivity of Cu(I) to be on the order of $10^{-5} \text{ cm}^2 \text{ s}^{-1}$, a characteristic distance of hundreds of micrometres is calculated for relevant experimental timescales³³. However, patterning experiments readily produced 35- μm -wide (4.2- μm -deep) features that

were only 5 μm wider than the mask (see Fig. 3). This illustrative result suggests that translational diffusion of Cu(I) is greatly limited by the nature of the reaction. There are three potential explanations for this observation. First, the binding of Cu(I) to the alkyne-functionalized monomer during the course of the CuAAC reaction results in diffusion being dictated by the dynamics of the telechelic polymer rather than Cu(I). Second, the ligandization of the Cu(I) with the triazole product results in a similar decrease in diffusion of Cu(I). Third, Cu(I) is rapidly consumed once it diffuses out of the irradiated area, probably via reactions with oxygen³⁴. The exact explanation remains an open question at this time. If indeed oxidation of Cu(I) is responsible for the resolution of the system, then oxygen plays a much different role in these systems than it does in other click reactions, such as thiol-ene and thiol-yne reactions. Perhaps, pattern resolution could be improved by the intentional addition of an oxidizer that would decrease the diffusion of Cu(I) outside the irradiated area. Regardless, the resolution of this process is suitable for numerous surface and three-dimensional material patterning applications such as the construction of hydrogel-based microreactors³⁵.

Post-synthetic modification of hydrogels was also successfully demonstrated (Fig. 4). Gels were synthesized by the thiol-yne

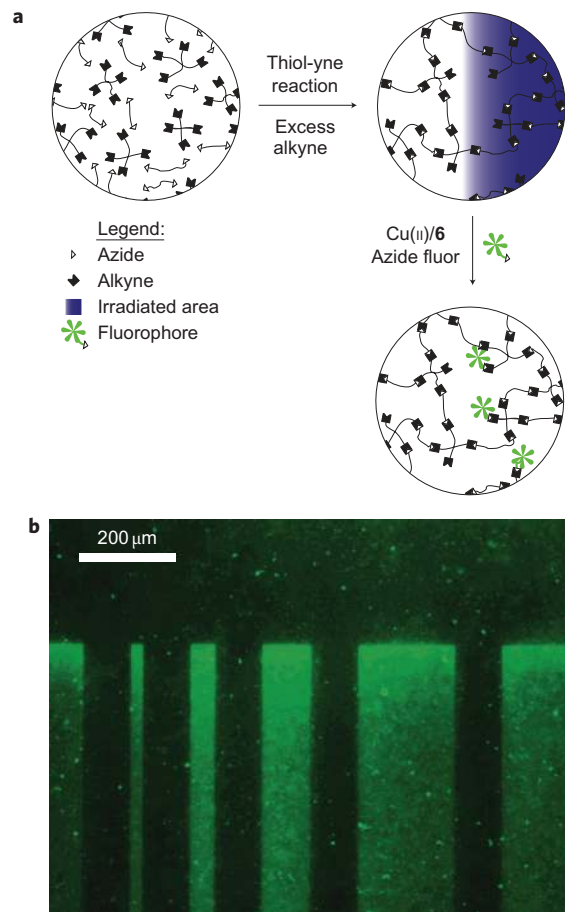


Figure 4 | Fluorescent patterning of a hydrogel by the photo-CuAAC reaction. a, *In situ* patterning of a hydrogel (photograph) was achieved by first forming an alkyne-rich gel via the thiol-yne click reaction of a 10K tetrathiol and 3K dialkyne. A solution of photoinitiator, copper sulfate and an azide-labelled fluorophore was swollen into the gel. Irradiation with a photomask (same as in Fig. 3) resulted in the generation of Cu(I) in the irradiated areas and the subsequent pCuAAC reaction between the pendant alkyne groups and azide-functionalized fluorophore. **b,** After removal of unreacted fluorophore, wide-field microscopy reveals the pattern of the fluorophore.

reaction with a stoichiometric excess of alkynes. A solution of photoinitiator, copper sulfate and a functionalized fluorophore was then swollen into the gel. Upon irradiation, the pCuAAC reaction between the functionalized fluorophore and the pendant alkynes in the polymer network produces a fluorescent pattern within spatially defined regions of the gel. It was determined that 25- μm features could readily be formed and discerned with only 50 s of irradiation. The ability to selectively pattern hydrogels has been used to promote cell adhesion and in the fabrication of high-performance organic electronic devices^{4,36}.

The photochemical generation of chemical species that locally reduce Cu(II) to Cu(I) for catalysis of the CuAAC reaction was studied by a combination of ¹H-NMR spectroscopy, FTIR spectroscopy and dynamic mechanical analysis, while confocal and optical microscopies confirmed the fidelity of the patterned features produced using this technique. This approach is readily adaptable to both aqueous and non-aqueous systems, with the most likely limitation being copper salt solubility. Moreover, a variety of radical generators can be used. TiO₂ and cleavage-type photoinitiators have been demonstrated here, but the abstraction-type photoinitiators frequently used in copper nanoparticle synthesis should work equally well²⁸. Additionally, the uncanny resemblance of this system to reverse atom transfer radical polymerizations (reverse-ATRP³⁷) suggests that systems could be designed to form persistent radicals. Not only would this allow for simultaneous click reactions and living polymerization, but some ATRP polymerizations are capable of using mere parts per million (ppm) levels of copper, suggesting perhaps that this approach could enable a dramatic reduction in copper concentration. Perhaps this reduction could permit the CuAAC reaction to be used in biological systems, as the irradiation conditions and initiators used are cytocompatible³⁸. In conclusion, this approach to catalysis of the CuAAC reaction is broadly applicable, improving the implementation, robustness and control of the CuAAC reaction.

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Author contributions

C.N.B., C.J.K. and B.J.A. developed the concept. B.J.A., Y.T., C.J.K., C.A.D., K.S.A. and C.N.B. designed the experiments. C.A.D. synthesized the materials used. B.J.A., Y.T. and C.A.D. performed the experiments. B.J.A., C.J.K., K.S.A. and C.N.B. composed the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>. Correspondence and requests for materials should be addressed to C.N.B.