

UC Merced

UC Merced Previously Published Works

Title

Measuring and Suppressing the Oxidative Damage to DNA During Cu(I)-Catalyzed Azide-Alkyne Cycloaddition

Permalink

<https://escholarship.org/uc/item/80s9j0r1>

Journal

Bioconjugate Chemistry, 27(3)

ISSN

1043-1802

Authors

Abel, Gary R
Calabrese, Zachary A
Ayco, Jeffrey
[et al.](#)

Publication Date

2016-03-16

DOI

10.1021/acs.bioconjchem.5b00665

Peer reviewed

Measuring and suppressing the oxidative damage to DNA during Cu(I)-catalyzed azide-alkyne cycloaddition

Gary R. Abel, Jr.,[†] Zachary A. Calabrese,[†] Jeffrey Ayco,[†] Jason E. Hein,^{†,‡,*} and Tao Ye^{†,*}

[†] Chemistry & Chemical Biology, School of Natural Sciences, University of California, Merced, 5200 N Lake Rd., Merced, CA 95343, USA

[‡] Department of Chemistry, UBC Faculty of Science, The University of British Columbia, 2036 Main Mall, Vancouver, BC Canada V6T 1Z1.

ABSTRACT: We have used quantitative polymerase chain reaction (qPCR) to measure the extent of oxidative DNA damage under varying reaction conditions used for copper(I)-catalyzed click chemistry. We systematically studied how the damage depends on a number of key reaction parameters, including the amounts of copper, ascorbate, and ligand used, and found that the damage is significant under nearly all conditions tested, including those commonly used for bioconjugation. Furthermore, we discovered that the addition of dimethylsulfoxide, a known radical scavenger, into the aqueous mixture dramatically suppresses DNA damage during the reaction. We also measured the efficiency of crosslinking two short synthetic oligonucleotides via click chemistry, and found that the reaction could proceed reasonably efficiently even with DMSO present. This approach for screening both DNA damage and reactivity under a range of reaction conditions will be valuable for improving the biocompatibility of click chemistry, and should help to extend this powerful synthetic tool for both *in vitro* and *in vivo* applications.

Due to its biocompatibility and facile reaction kinetics, the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) has become widely adopted as a robust means of functionalizing biomolecules.¹⁻³ However, the toxicity of the copper catalyst has been a persistent impediment to carrying out bioconjugation in settings that are susceptible to oxidative damage.⁴⁻⁸ The most commonly utilized reaction conditions involve generation of the catalytically active Cu(I) species via *in situ* reduction of Cu(II) by ascorbate, which occurs with the concomitant generation of reactive oxygen species (ROS) that are damaging to biomolecules.⁹ Significant efforts have been focused on the discovery and optimization of Cu(I)-stabilizing ligands that can improve reactivity and suppress oxidative damage. Finn,^{6,10} Wu⁷ and Pezacki¹¹ and coworkers have developed water-soluble ligands that can stabilize the Cu(I) catalyst and also function as sacrificial reagents that intercept ROS. However, even with protective ligands, the damage is often extensive enough to affect cell viability.^{6,7,11} More recently, azides that chelate with copper¹² have been found to accelerate the reaction and allow the use of lower copper concentrations to reduce oxidative damage.^{8,13} Despite the progress made so far, the concern of toxicity in CuAAC-based bioconjugation has not been eliminated. As a result, copper free alternatives such as strain-promoted azide-alkyne cycloaddition, which have slower kinetics and lower specificity, remain preferred over CuAAC in applications that are more sensitive to oxidative damage.^{3,14-16}

Here we focus on a gap in the development of effective CuAAC protocols: the methods used to monitor oxidative damage during CuAAC are not sufficiently sensitive for applications that are highly susceptible to damage. While ROS generated during CuAAC are detrimental to a variety of biological

molecules, of particular concern is the oxidative damage to DNA, including base modifications and scission of the phosphodiester backbone in one or both strands.¹⁷⁻²⁰ Such damage can have deleterious genotoxic and mutagenic consequences for living organisms,^{19,21,22} particularly when the high levels of oxidative stress overwhelm cellular DNA repair mechanisms.^{23,24} In relation to health, oxidative DNA damage has been implicated in cancer and other aging-related diseases.^{17,19,25,26} Anticipating this issue, many CuAAC bioconjugation studies have evaluated cell viability or proliferation.^{6,7,11,27} However, these assays do not provide chemical insight into the kinetics of oxidative damage, which is needed for rationally minimizing the cytotoxicity of CuAAC. In addition, a large portion of genetic mutations do not observably impact cell viability,²⁸ and thus it is likely that much of the damage goes undetected by these methods. Some other studies have measured oxidation kinetics of proteins²⁹ as well as proxy molecules, such as histidine and short oligonucleotides, using HPLC and gel electrophoresis.¹⁰ However, because of their relatively low sensitivity, these methods cannot detect very low levels of damage that, while sporadic, are detrimental for most *in vivo* chemical biology applications. Such damage may also be a concern for a number of methods that use click chemistry to prepare DNA bioconjugates³⁰ for sensing,^{31,32} diagnostics,³³ sequencing,³⁴ and gene synthesis.³⁵⁻³⁷ Therefore, owing to the biological and technological importance of maintaining genomic integrity, a highly sensitive method for directly measuring the damage to DNA would be valuable for improving the biocompatibility of CuAAC chemistry.

In this work we have used a qPCR-based analytical method to study the oxidative damage to a long double-stranded DNA molecule (3.5 kbp) under varying conditions used for CuAAC

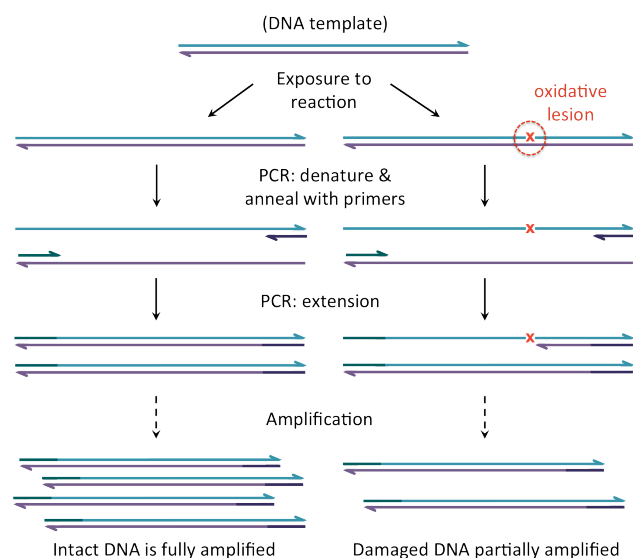


Figure 1. Illustration of the use of qPCR to determine the fraction of DNA damaged during a reaction. First, the DNA template strand is exposed to the reaction for a predetermined amount of time before being quenched in TAE (Tris-Acetate-EDTA) buffer on ice. Next, the DNA is subjected to PCR amplification. During the extension step of PCR, oxidative lesions in either strand of the template obstruct the progress of the polymerase enzyme, preventing it from generating a full complement of that strand. Thus, as the cycle is repeated only intact strands contribute to the exponential amplification of the DNA template. By monitoring the fluorescence of an intercalating dye and comparing to an untreated control sample, the relative fraction of intact DNA present in the initial sample can be quantitatively determined.

bioconjugation. This method is capable of measuring the frequency of oxidatively induced strand lesions (base modifications or strand scission) in double-stranded DNA as low as 1 per 10^5 nucleotides. The order-of-magnitude higher sensitivity has afforded us new insight into how the damage rate is influenced by key reaction parameters, including the type of Cu(I)-stabilizing ligand used and the concentrations of copper, ascorbate, and the ligand, at a relevant time scale. This improved measurement capability has led us to discover that the addition to the aqueous reaction of up to 10% dimethylsulfoxide (DMSO), a commonly used component for bioconjugation solvent mixtures, reduces the rate of oxidative damage by as much as two orders of magnitude. Moreover, our measurements of the efficiency of CuAAC in crosslinking two short synthetic oligonucleotides showed only a relatively modest reduction in reaction rate attributed to the inclusion of DMSO. The strategy presented here is complementary to the existing efforts in improving the biocompatibility of CuAAC through engineering the ligand and reactants,^{6-8,10,11} and allows for rationally minimizing the oxidative damage of CuAAC for both *in vitro* and *in vivo* bioconjugation.

The qPCR technique is a facile, parallel and highly sensitive method for quantifying the frequency of lesions in DNA.^{38,39} As illustrated in Figure 1, the 3.5 kbp DNA strand is first exposed to the CuAAC reaction mixture for a predetermined amount of time before being quenched by dilution in TAE buffer on ice. Next, the DNA is used as a template for PCR amplification. During the extension step of PCR, lesions in either strand of the template inhibit the polymerase from generating a full complement of that strand, and thus only intact

strands contribute to the exponential amplification of the product DNA. By monitoring the total amount of DNA using an intercalating dye and comparing to an untreated control, the relative fraction of intact DNA present in the sample can be quantitatively determined. In order to calculate the DNA damage frequency (lesions per base) from the intact DNA fraction ϕ , we assumed that the damage occurs in a random and sequence-independent fashion, an approximation that is often used for long genomic DNA.³⁹ Then the lesions can be described by a Poisson distribution $f(k, \lambda)$, such that the probability P of a DNA strand containing k lesions is given by the following equation:

$$P(k) = f(k, \lambda) = \frac{\lambda^k e^{-\lambda}}{k!}$$

where λ is the mean number of lesions per single strand. Then, equating the probability $P(0)$ of $k = 0$ lesions with the intact DNA fraction ϕ gives the following:

$$\phi = P(0) = \frac{\lambda^0 e^{-\lambda}}{0!} = e^{-\lambda}$$

which can be rearranged to give the mean number of lesions per DNA strand:

$$\lambda = -\ln(\phi)$$

Finally, dividing λ by the number of bases n in each strand gives the mean number of lesions per base:

$$\text{damage frequency (lesions per base)} = \frac{\lambda}{n} = \frac{-\ln(\phi)}{n}$$

We used this equation to determine the frequency of damage to a 3.5 kbp DNA strand after exposure to varying catalytic conditions, with the goal of understanding the factors affecting the extent of damage. For most bioconjugation applications, the catalyst consists of three key components: the copper source, typically a Cu(II) salt; a reducing agent such as ascorbate, which is needed to generate and maintain the catalytically active Cu(I) species; and finally a protective ligand, which is used to stabilize copper in the +1 oxidation state. For this study we chose to use Cu(II) sulfate and sodium ascorbate, along with the commonly used tris-(3-hydroxypropyl)triazolylmethylamine (THPTA) ligand,¹⁰ to serve as a model catalytic system. In order to investigate the effects of the catalyst composition on the DNA damage, we systematically varied the concentration of copper, the ascorbate:copper ratio (Asc:Cu), and the ligand:copper ratio (L:Cu) during the reaction, and measured both the fraction of DNA remaining intact and the corresponding DNA damage frequency after a timed exposure to the catalyst, as compared to an untreated control.

The results of our DNA damage measurements are shown in Figure 2. When the Cu concentration was increased while the Asc:Cu and L:Cu ratios were held constant, we observed a decrease in the fraction of DNA remaining intact, which indicates an increasing frequency of damage (Figure 2a). The degradation was extremely rapid for Cu concentrations of 50 μM or higher, with less than 1 in 5,000 DNA molecules remaining intact after only 2 minutes of reaction time. Transition metals are known to generate ROS in the presence of ascorbate via Fenton chemistry:

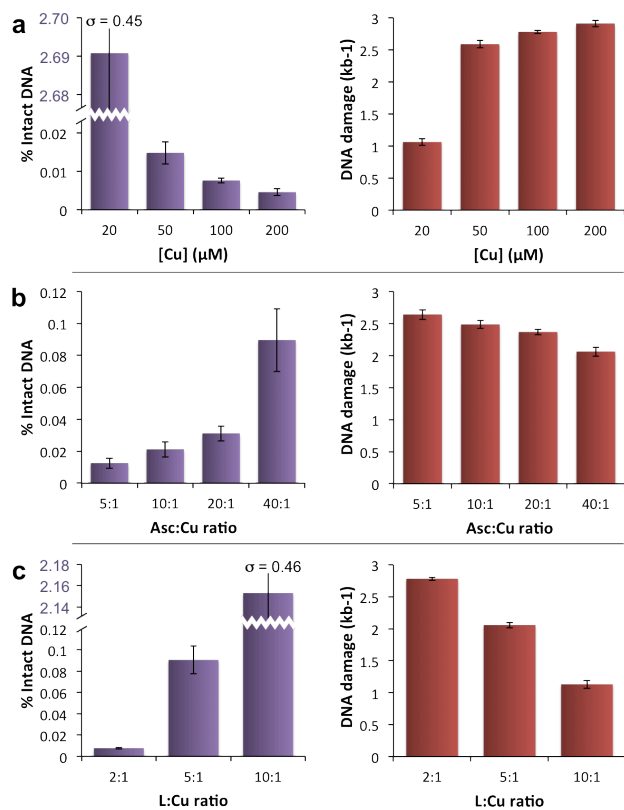
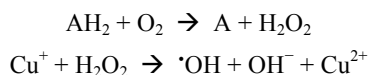


Figure 2. Results from systematically varying the reaction conditions and measuring the percentage of intact DNA and the DNA damage frequency (lesions per kilobase) after a 2-minute reaction time. (a) Percentage of intact DNA (purple) and corresponding DNA damage frequency (red) as a function of copper concentration ([Cu]). The ratios of ascorbate to copper (Asc:Cu) and THPTA ligand to copper (L:Cu) were held constant at 10:1 and 2:1, respectively. (b) Intact DNA percentage and damage frequency as a function of Asc:Cu, for constant [Cu] = 100 μM and L:Cu = 2:1. (c) Intact DNA percentage and damage frequency as a function of L:Cu, for constant [Cu] = 100 μM and Asc:Cu = 10:1. The error bars represent \pm one standard deviation (σ) calculated from at least four identical qPCR replicates.



where AH_2 is ascorbic acid, A is dehydroascorbic acid, and $\cdot\text{OH}$ is the hydroxyl radical.⁴⁰ Thus increasing the copper concentration increases the rate of ROS production. Our results corroborate previous *in vitro* and *in vivo* studies that showed more extensive oxidative damage with increasing copper concentration during CuAAC.^{6,7} The role of ascorbate, however, is more complex. From the above chemical equations, it is apparent that increasing the ascorbate concentration also accelerates ROS generation, and this has been observed experimentally.⁴¹ However, ascorbate is also known to scavenge oxygen radicals, meaning that it can function as both an antioxidant and a pro-oxidant in the same system.^{9,42} Note that when the reaction is performed under ambient conditions that oxidize Cu(I), excess ascorbate is needed to maintain the copper in the +1 oxidation state. When we varied the ratio of ascorbate to copper in the catalyst, we generally observed a decrease in the damage with increasing Asc:Cu, particularly

for very high concentrations of ascorbate (Figure 2b). This data suggests that for the conditions used here, the antioxidant activity of ascorbate more than compensates for its ability to generate ROS. A similar trend was observed in plasmid relaxation studies of DNA damage by ascorbate and copper.⁹ Finally, we tested the effect of varying the ratio of the THPTA ligand to copper. In addition to stabilizing the catalytically active Cu(I) species, such ligands can also act as sacrificial agents that intercept and react with the ROS generated,⁴³ and previous reports have found that using excess ligand relative to copper can have a protective effect on molecules both *in vitro*¹⁰ and inside cells.⁶ Indeed, we observed that increasing the L:Cu ratio from 2:1 to 10:1 reduces the damage frequency by more than a factor of two (Figure 2c). However, even in the presence of tenfold excess ligand, damage to the relatively long DNA is substantial—after 2 minutes, roughly 98% of the DNA has been damaged. The extensive DNA damage observed in our qPCR experiments is consistent with previous studies that found a decrease in cell viability or proliferation rate after exposure to the Cu/Asc/THPTA catalyst.^{7,11} This problem is not specific to the THPTA ligand, as we observed similarly high levels of damage when we tested alternative Cu-binding ligands (Figure S4). More generally, we found that the frequency of damage is a complex function of both the catalyst composition (copper, ascorbate and ligand) and the reaction time, and cannot be easily predicted. This highlights the importance of directly measuring the oxidative damage under the conditions being used, in order to optimize the reactivity while minimizing the damage.

A ubiquitous strategy used by biological systems to reduce

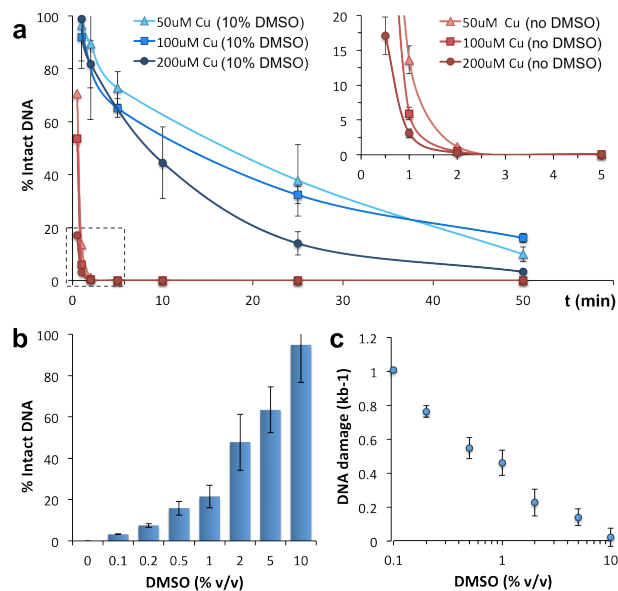


Figure 3. Suppression of oxidative DNA damage by DMSO during CuAAC. (a) Time dependence of the degradation of DNA for a range of Cu concentrations, both with (blue) and without (red) 10% DMSO included in the reaction. Inset shows early time points, scaled for clarity. (b) Percentage of intact DNA remaining after a 5-minute reaction as a function of DMSO volume percentage. (c) DNA damage frequency as a function of DMSO volume percentage, as calculated from the data in (b). For reference, the damage without DMSO is ~ 2.5 kb⁻¹. For all reactions, Asc:Cu = 10:1 and L:Cu = 2:1. Error bars represent \pm one standard deviation.

and mediate oxidative stress is the production of cellular anti-oxidants.⁴⁴ However, a general challenge in adopting this strategy for CuAAC is that many antioxidants, such as TCEP (tris(2-carboxyethyl)phosphine, a sacrificial reducing agent) and catalase (an enzymatic catalyst for H₂O₂ decomposition) are likely to inhibit the reaction by adversely affecting the concentration or coordination environment of the catalytically active Cu(I) ions.¹⁰ Our trials with small molecule ROS scavengers, including trolox⁴⁵ and histidine,¹¹ found that although the damage was suppressed, the inhibition of CuAAC reaction was similarly too excessive for these additives to be useful.

In order to improve the biocompatibility of CuAAC, we sought an alternative antioxidant that could suppress the oxidative damage without significantly inhibiting the reaction. DMSO is a promising candidate, based on its established use as a solvent for CuAAC and its known capacity as an oxygen radical scavenger.^{46,47} To test how DMSO would affect the kinetics of DNA damage during CuAAC, we measured the time-dependence of degradation for three different copper concentrations, in both aqueous buffer and 10% DMSO. As shown in Figure 3a, without DMSO the DNA was rapidly damaged, with less than 0.1% of the DNA remaining intact after just 5 minutes for all three copper concentrations. In contrast, with the addition of 10% DMSO, the majority of the DNA was still intact after 5 minutes, and even after 25 minutes as much as one third of the DNA remained undamaged. We also varied the amount of DMSO in the reaction mixture (Figure 3b), and found it to be inversely correlated to the measured damage frequency (Figure 3c). This suggests that DMSO can indeed scavenge ROS that are produced during the reaction. DMSO is known to react with [•]OH, a key mediator of DNA damage that is generated during CuAAC, to produce the methyl radical,^{48,49} which can dimerize to form ethane, thus avoiding the otherwise damaging effects of [•]OH on DNA. What is notable is that the addition of DMSO leads to a much more pronounced reduction in the damage rate (up to one hundred fold, Figure 3) than varying other reaction parameters, such as copper concentration or the L:Cu ratio, within commonly used ranges (Figure 2).

For an additive to be useful in CuAAC bioconjugation, it must suppress the oxidative damage without strongly inhibiting the reaction itself. To determine the impact of DMSO on CuAAC bioconjugation, we compared the yield of crosslinking two short synthetic oligonucleotides (24 bases) in both water and 10% DMSO. As illustrated in Figure 4a, the 24-base strand bearing an azide group was hybridized with a complimentary 16-base strand modified with an octadecynyl-deoxyuracil base. After exposure to the catalyst mixture, the reactions were quenched and analyzed by denaturing polyacrylamide gel electrophoresis (d-PAGE), which separates the crosslinked products from the unreacted DNA.^{50,51} As shown in Figure 4b, we found that DMSO did modestly reduce the rate of crosslinking, possibly due to weak coordination to the copper center;⁵² however, even with 10% DMSO, the reaction proceeded reasonably efficiently, reaching nearly the same yield after 50 minutes. From a practical standpoint, it would make sense to compare the amount of damage measured at times that give a comparable reaction yield with and without DMSO. For example, reactions with the same copper concentration reach a comparable yield after 25 minutes with the DMSO/water mixture, as compared to 5 minutes in the aqueous solution, but they have 10²-10³ times more intact DNA than their aqueous counterparts. This corresponds to a five- to tenfold decrease in

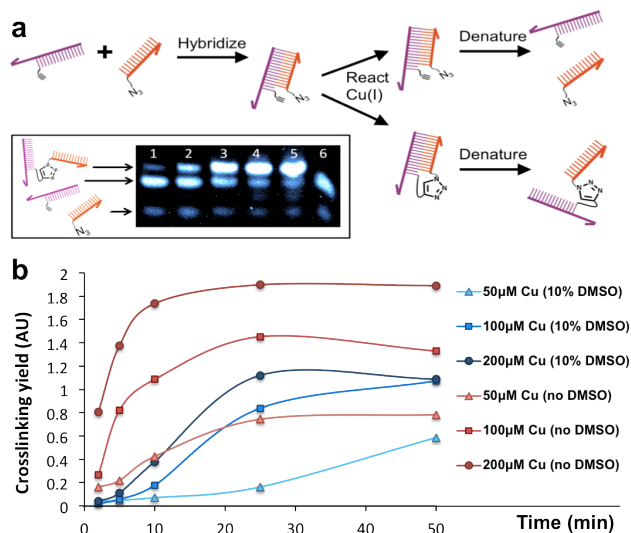


Figure 4. Measuring the time-dependent reaction yield for the crosslinking of two short DNA oligonucleotides by CuAAC. (a) Schematic illustration of the method used to determine the yield of the DNA crosslinking reaction. Inset shows an example denaturing PAGE image, where the uppermost band corresponds to the product. Lanes 1-5 are increasing time points for the reaction, while lane 6 is a control sample that did not contain any Cu catalyst. (b) The time-dependent normalized reaction yield of the DNA-templated CuAAC crosslinking reaction for a range of Cu concentrations, shown both without (red) and with (blue) 10% DMSO. Trend lines are added for clarity.

the frequency of DNA damage at comparable yield. Thus the damage suppression by DMSO more than offsets the reduction in rate of the bioconjugation reaction, and represents a significant improvement over the standard CuAAC bioconjugation protocols.

In addition to solution phase reactions, we have also adapted this approach for CuAAC-based surface immobilization of DNA,⁵³ during which the extent of oxidative damage is unknown. We previously described a method for sequence-specific and covalent tethering of long DNA to a solid surface using click chemistry.⁵¹ This method uses short DNA ‘anchor’ strands that are attached to a self-assembled monolayer surface to capture much longer DNA target strands with a complementary sequence; then CuAAC is used to crosslink the anchor strand and the target DNA. In the present work, we used qPCR to measure the amount of oxidative damage to surface-bound DNA during the CuAAC reaction. A major challenge to adapting qPCR analysis to quantify damage on surface-bound DNA is the requirement for a control sample with an identical initial DNA quantity in order to carry out relative quantification. In practice, sample-to-sample variation in surface coverage is difficult to avoid. To account for variation in the total amount of DNA on the surface, we have normalized the DNA quantity to an internal standard, a shorter 200 bp product that is amplified from a segment within the same template strand (see Supporting Information). This allows the shorter product to serve as a reference that, due to its much smaller footprint, is unlikely to be cleaved except in severely damaging conditions.^{39,54} To assess the damage sustained by surface-bound DNA, we first hybridized the long DNA template strands with short, single-stranded anchor strands that were attached to an alkanethiol self-assembled monolayer on gold. Next, the sur-

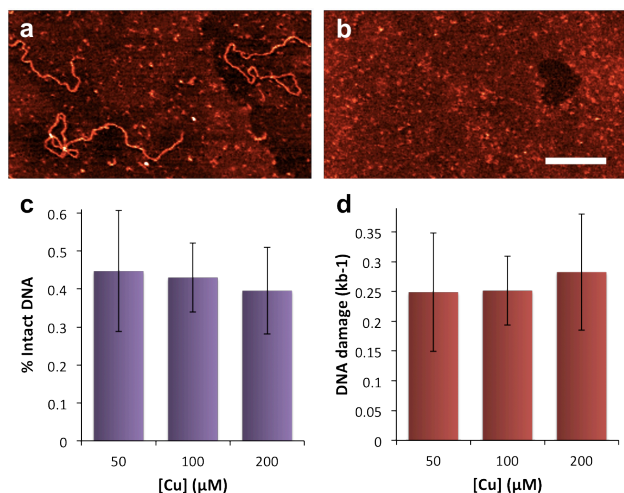


Figure 5. Measuring the oxidative damage to DNA during the CuAAC surface-coupling reaction. (a) Atomic force microscopy (AFM) image of the 3.5 kbp DNA template after hybridization with short anchor DNA strands on a self-assembled monolayer surface and exposure to the catalyst. (b) AFM image of the same surface after denaturation and collection of the DNA for qPCR analysis. Scale bar is 200nm. (c) Measured percentage of DNA remaining intact after the surface reaction as a function of copper concentration. (d) DNA damage frequency as a function of [Cu], as calculated from the data in (c). Reaction times were 5 minutes, and all reactions included 10% DMSO, along with Asc:Cu = 10:1 and L:Cu = 2:1. Error bars represent \pm one standard deviation.

face-bound DNA was exposed to the CuAAC reaction mixture, after which the DNA was released from the surface by rinsing with a denaturing alkaline buffer, collected, and analyzed by qPCR. In order to permit collection of the DNA by chemical denaturation, we omitted the azide and alkyne groups that are normally used to crosslink the DNA to the surface. Atomic force microscopy (AFM) imaging was used to monitor hybridization of the DNA template to the surface anchor strands, as well as to confirm the release of over 99% of the DNA after denaturation. AFM images of the surface acquired after the reaction and denaturation steps are shown in Figures 5a and 5b, respectively. Dual amplification qPCR was then used to determine the fraction of intact DNA (Figure 5c) and corresponding damage frequency (Figure 5d) after a 5-minute reaction with varying concentrations of copper in 10% DMSO. Notably, the damage frequency on the surface is two to three times higher than that measured for the same reaction conditions in solution. These results show that the kinetics of oxidative damage may diverge from that in the solution phase, which may be due to a possible increase in the local concentration of copper ions near the surface carboxylate groups. Additionally, the weak dependence of the damage on copper concentration also suggests that the copper ions binding to the surface may mediate the damage, and that the surface coverage of copper ions depends only weakly on bulk concentration; however, further studies are needed to elucidate this surface effect. Unfortunately the damage rate of the surface DNA in the absence of DMSO was too high to permit accurate quantification using this method, which requires that the 200 bp reference be mostly intact. Despite this difficulty, the results confirm that the damage during the surface reaction is also considerably suppressed by the inclusion of DMSO.

We have used qPCR to measure the extent of oxidative damage to a 3.5 kbp DNA molecule under varying catalytic reaction conditions for CuAAC. Combining high-throughput and direct quantification of DNA damage with measurement of coupling kinetics, our approach will broaden the utility of CuAAC for bioconjugation in both solution phase and on solid surfaces.^{51,55} For example, CuAAC bioconjugation has been utilized in a number of sensing and diagnostic applications,^{31-33,53} and the results presented here may lead to increased specificity by reducing the extent of unintended modification of the probe molecules. Because the qPCR-based assay is also applicable to live cells and organisms,^{38,39} our approach to optimizing CuAAC, which is informed by quantitative information concerning DNA damage, may help to improve the biocompatibility of CuAAC for *in vivo* applications. Our discovery that DMSO can suppress the rate of oxidative damage by two orders of magnitude has practical implications for *in vitro* bioconjugation with CuAAC, given that DMSO/water mixtures are popular solvents for *in vitro* bioconjugation. In addition, as DMSO has previously been shown to have low toxicity^{49,56} and protect cells from radical-mediated damage,⁵⁷⁻⁵⁹ it may prove to be a viable strategy for protecting live cells during CuAAC bioconjugation as well.

ASSOCIATED CONTENT

Supporting Information

Additional qPCR results, details on experimental procedures, and a list of DNA sequences used. The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

* E-mail: jhein@chem.ubc.ca, tao.ye@ucmerced.edu

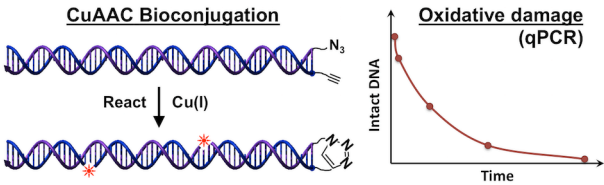
ACKNOWLEDGMENTS

The authors acknowledge the support by NSF, NASA and UC Cancer Research Coordinating Committee. GRA acknowledges support by a UC Merced Graduate Dean's dissertation year fellowship. We thank Prof. Michael Cleary's group and Dr. Glenda Pollack for their assistance with qPCR. We also thank Diana Yu and Matthew Berry for their help with ligand synthesis.

REFERENCES

- Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A. and Bertozzi, C. R. (2006) A comparative study of bioorthogonal reactions with azides. *ACS Chem. Biol.* **1**, 644-648.
- Baskin, J. M. and Bertozzi, C. R. (2007) Bioorthogonal click chemistry: Covalent labeling in living systems. *QSAR Comb. Sci.* **26**, 1211-1219.
- Baskin, J. M., Prescher, J. A., Laughlin, S. T., Agard, N. J., Chang, P. V., Miller, I. A., Lo, A., Codelli, J. A. and Bertozzi, C. R. (2007) Copper-free click chemistry for dynamic *in vivo* imaging. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 16793-16797.
- Speers, A. E. and Cravatt, B. F. (2004) Profiling enzyme activities *in vivo* using click chemistry methods. *Chem. Biol.* **11**, 535-546.
- Salic, A. and Mitchison, T. J. (2008) A chemical method for fast and sensitive detection of DNA synthesis *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 2415-2420.
- Hong, V., Steinmetz, N. F., Manchester, M. and Finn, M. (2010) Labeling live cells by copper-catalyzed alkyne-azide click chemistry. *Bioconjugate Chem.* **21**, 1912-1916.
- Besanceney-Webler, C., Jiang, H., Zheng, T., Feng, L., Soriano del Amo, D., Wang, W., Klivansky, L. M., Marlow, F. L., Liu, Y. and Wu, P. (2011) Increasing the efficacy of bioorthogonal click reactions

TOC graph



Measuring and suppressing the oxidative damage to DNA during Cu(I)-catalyzed azide-alkyne cycloaddition

Gary R. Abel, Jr.,[†] Zachary A. Calabrese,[†] Jeffrey Ayco,[†] Jason E. Hein,^{‡,*} and Tao Ye^{†,*}

[†] Chemistry & Chemical Biology, School of Natural Sciences, University of California, Merced, 5200 N Lake Rd., Merced, CA 95343, USA

[‡] Department of Chemistry, UBC Faculty of Science, The University of British Columbia, 2036 Main Mall, Vancouver, BC Canada V6T 1Z1

* E-mail: jhein@chem.ubc.ca, tao.ye@ucmerced.edu

Table of contents

Materials and methods.....	S2
Table of DNA sequences used.....	S2
Preparation of DNA template strands.....	S2
Figure S1: PCR generation of the double-stranded DNA.....	S3
Preparation of Cu-binding ligands.....	S4
Figure S2: Molecular structures of Cu(I)-binding ligands.....	S4
Measuring DNA damage during CuAAC reaction.....	S4
Measuring DNA damage during the surface-coupling reaction.....	S5
Figure S3: Schematic illustration of dual-amplification qPCR.....	S7
AFM imaging.....	S8
Testing the DNA-templated crosslinking reaction.....	S8
Additional qPCR results.....	S10
Figure S4: Results from qPCR measurements with HLTA ligand.....	S10
References.....	S10

Materials and methods

The following synthetic oligonucleotides were used in this study:

Name	DNA Sequence & Modifications	Purpose
DCD-05	catttgaggggattcaatg	forward primer (qPCR)
DCD-02	ttgaaataccgaccgtgtga	reverse primer (3.5kbp product)
DCD-16	taccacattcaactaatgcag	reverse primer (225bp product)
SF-78	cgtactgactgctcacgaggtagc/(C3)/tctgaactgttaagcatttgaggg	forward primer with ssDNA tail
SF-3.6k	tccttgaaaacatagc gatagcttag	reverse primer (3.6kbp product)
SF-88	(Az)/cgtactgactgctcacgaggtagc	azide-modified oligo
SF-89	gtgagcagtcagtagc/(Oct-dU)	alkyne-modified oligo
SF-17	gctacctcgtgagcagtcagtagcttttt/(C11-SS)	disulfide-DNA anchor strand

Abbreviations for DNA modifications: (Oct-dU) = octadiynyl deoxyuracil; (Az) = azide; (C3) = propyl spacer; (C11-SS) = undecyl disulfide;

The undecyldisulfide-DNA (SF-17) was purchased from Biosearch technologies (Petaluma, CA, USA), and all other synthetic DNA oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, USA). The purity of the custom ordered oligonucleotides was verified by the manufacturers using mass spectrometry, and the DNA was used without further purification. DNA was stored long-term at -20°C and short-term at 4°C in phosphate buffer (PB, 20 mM sodium phosphate, pH 7).

Preparation of DNA template strands

DNA templates for qPCR: DNA template strands were prepared by conventional PCR amplification from the M13 bacteriophage genome. To serve as a template for PCR, M13mp18 RF I DNA (New England Biolabs Inc, Massachusetts, USA) was linearized using the EcoRI restriction enzyme (New England Biolabs) and purified by agarose gel electrophoresis using a QIAquick gel extraction kit (QIAGEN, Limburg, Netherlands). For the PCR reaction, 50 pg of linearized M13 DNA was combined with Taq DNA polymerase master mix (Bioexpress, Utah, USA), along with

200nM each of the forward (SF-08) and reverse (SF-3.6k) primers in a 250 μ L PCR tube. Ultrapure water produced by a Barnstead Nanopure Diamond water purification system (Thermo Scientific, North Carolina, USA) was used to bring the solution up to a volume of 50 μ L. Solutions were incubated through the following program on an thermal cycler (Eppendorf, Hamburg, Germany): an initial melting step of 94°C for 2 min, followed by 30 cycles of melting at 94°C for 30 sec, annealing at 49°C for 45 sec, and extension at 70°C for 120 sec, followed by a final cycle with an extension of 5 minutes. Remaining primers and enzymes were removed using the QIAquick PCR Purification Kit (QIAGEN). Purified DNA templates were kept in TAE buffer at 4°C for short-term, and -20°C for long-term storage (1X TAE = 40 mM Tris(hydroxymethyl)aminomethane acetate, 1 mM EDTA, pH 8.3).

DNA templates with single-stranded tail for surface hybridization: In order to hybridize with the surface anchor strands, the double-stranded DNA targets contain a terminal 24-base single-stranded DNA tail segment that is complementary to the anchor strand sequence. The target strands are generated via polymerase chain reaction (PCR) with a forward primer that is connected to the 24-base tail by a propyl spacer group, which prevents the polymerase enzyme from copying over the tail region. Therefore, PCR can produce a double-stranded product with a short, single-stranded tail (see Figure S1). PCR was carried out with the same protocol that was described in the previous step (see DNA templates, above), using the SF-78/ SF-3.6K primer pair.

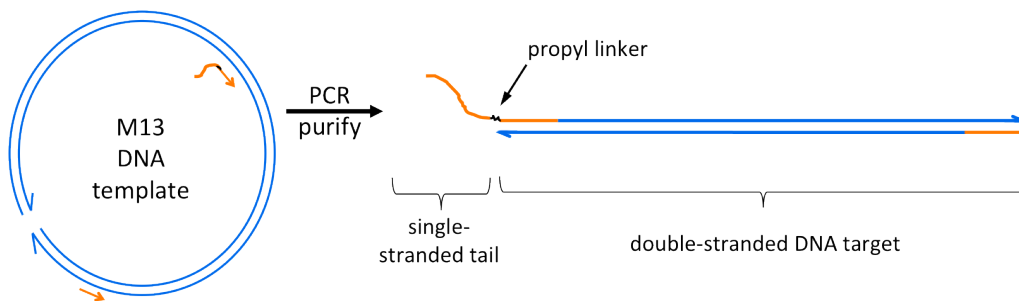


Figure S1: Generation of the double-stranded DNA targets via PCR with modified primers (primers in orange).

Preparation of the Cu-binding ligands

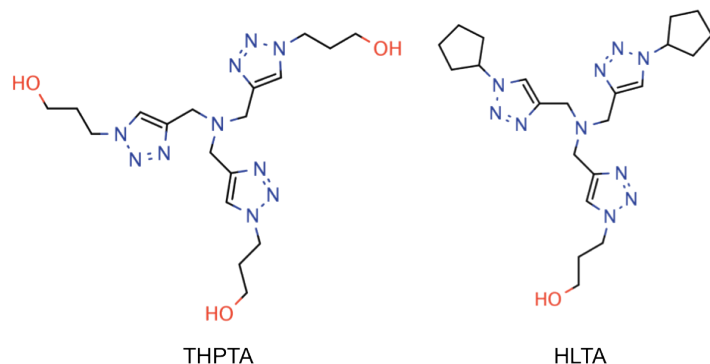


Figure S2: Molecular structures of the Cu(I)-binding ligands used in this study.

THPTA: The tris(3-hydroxypropyltriazolylmethyl)amine ligand was purchased as a solid from Sigma Aldrich (St. Louis, MO, USA).

HLTA: The 3-(4-((bis((1-cyclopentyl-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol ligand was synthesized according to a previously published protocol.¹

Preparation of catalyst solutions: The copper/ligand catalyst solutions were prepared at 2X working concentration by dissolving Cu(II) sulfate in water and mixing with a 2:1 molar ratio of the appropriate ligand (THPTA or HLTA) in either water or a water/DMSO mixture containing 40mM phosphate buffer (pH 7) and 400 mM NaCl. Cu(II)/ligand solutions were stored at 4°C until use.

Measuring DNA damage during CuAAC reaction

DNA exposure to the Cu catalyst: The method for quantifying single-stranded DNA lesions that occurred during the CuAAC reaction was as follows. First, 10µL of the 2X Cu(II)/ligand solution was combined with 5.0µL of the DNA template (4ng/uL stock) in a 200µL PCR tube. Next, 5.0µL of a 4X-concentrated solution of sodium ascorbate was added to initiate the reaction, and the solution was mixed and immediately capped. After the desired reaction time, a 2.0µL aliquot of the reaction was quenched by diluting in 1.0mL in 0.1X TAE buffer (4mM Tris acetate, 0.1mM EDTA,

pH ~8) on ice. Note that no effort was made to exclude oxygen during the reaction step, aside from capping the tube.

Quantitative polymerase chain reaction (qPCR): All qPCR reactions were prepared with at least 3 technical replicates. Each 20uL reaction contained the following: 1X Genemate Taq DNA polymerase Master Mix (BioExpress, Utah, USA), 200nM each of the forward (DCD-05) and reverse (DCD-02) primers, 0.15X Sybr Green I fluorescent dye (Life Technologies, New York, USA), and 5.0μL of the diluted, quenched DNA/Cu reaction (see DNA exposure step, above). Reactions were prepared on ice in 0.1mL clear plastic PCR tube strips (Qiagen). Quantitative PCR was carried out on a Rotorgene Q real-time PCR cycler (Qiagen), using a minimum of 32 cycles of the following temperature profile: denature at 95°C for 30 sec, anneal at 55°C for 20 sec, and extend at 71°C for 120 sec. Following completion of cycling, a melt analysis was performed by slowly ramping the temperature up to 95°C and continuously monitoring the fluorescence.

qPCR data analysis: For each qPCR reaction a threshold quantification cycle (C_q) was defined as the fractional cycle at which the measured fluorescence intensity crosses a threshold value, which is chosen to correspond to a region in which the PCR is in the exponential amplification phase. For each sample, the relative DNA quantity φ , as compared to the reference sample, was determined by evaluating the following equation:

$$\varphi = \frac{[DNA]}{[DNA]_{ref}} = 2^{-\Delta C_q} = 2^{-(C_q - C_{q.ref})}$$

where C_q and $C_{q.ref}$ correspond to the sample and reference quantification cycles, respectively. The values reported are average values of at least three identical replicates for each sample.

Measuring DNA damage during the surface-coupling reaction

Preparation of DNA anchor surface: Carboxyl-terminated alkanethiol self-assembled monolayers containing short, thiolated DNA anchor strands (SF17) were prepared on a single crystal gold substrate, as described in a previous publication.²

Hybridization with the target DNA: The purified target DNA PCR product was diluted to approximately 1 ng/μL in the hybridization buffer (HB, 200mM NaCl, 40mM Tris acetate, 1mM

EDTA, 1.0mM sodium dodecyl sulfate, pH 8.3), and was incubated with the DNA anchor surface for 20-30 minutes. After hybridization, the surface was rinsed repeatedly with HB to remove any unbound DNA.

DNA surface exposure to the Cu catalyst: The Cu(II)/ligand catalyst solution was prepared as described earlier (see Preparation of catalyst solutions, above). Prior to the surface reaction, the surface was rinsed with PBS to thoroughly remove Tris and EDTA, both of which inhibit the reaction.³ Then the sodium ascorbate reducing agent was added to the Cu(II)/ligand catalyst solution, and then the surface was immediately exposed to the resulting mixture. After the desired reaction time, the surface was rinsed repeatedly with saline TAE buffer (STAE, 200mM NaCl, 40mM Tris acetate, 5mM EDTA, pH 8.3). Note that no effort was made to exclude oxygen during this step.

Denaturation and collection of DNA: After exposure to the catalyst solution, the surface-bound DNA was denatured using alkaline conditions, which disrupt the hydrogen bonds in the base pairs and release the DNA from the surface. The surface was exposed to a 70 μ L aqueous solution of 10mM NaOH and 330 μ M EDTA (pH 12) for 5 minutes, which was then collected and neutralized by diluting 4:1 in TAE and stored at -20°C. It was then rinsed with the same solution to remove any free DNA.

qPCR of surface DNA: The protocol for qPCR with the collected surface DNA is the same as described in an earlier section (see Quantitative polymerase chain reaction, above). The collected, neutralized surface DNA (see previous section) was further diluted 9:1 in water, and was then used as the template for qPCR.

Dual-amplification qPCR: Relative quantification requires comparison of C_q values to an untreated reference containing the same initial DNA quantity; however, the amount of DNA bound to the surface can vary from sample to sample. To compensate for this variation, qPCR was used for each surface DNA sample to separately amplify both the full-length 3.5kbp product (primer pair DCD-05/ DCD-02), and a shorter 200bp product (primer pair DCD-05/ DCD-16) from within the same template. Because of its much smaller footprint, the 200bp region is unlikely to be cleaved under conditions with a modest damage frequency, allowing the shorter product to serve as an internal reference (see Figure S3).^{4,5} After qPCR, the amount of intact, full-length DNA was divided by the total amount of DNA present in the sample, as determined using a standard

calibration curve with the 200bp product. This ratio of intact/total DNA was then normalized by dividing by the corresponding ratio determined for the untreated control sample. This normalization process compensates for variation in surface coverage, as well as factoring out any difference in amplification efficiency between the two product lengths. Thus it allows for direct comparison between different samples and the untreated control. Note that the requirement for modestly damaging conditions prevented accurate quantification of surface DNA under conditions that caused extensive damage. In this case, the total amount of DNA would need to be carefully determined using an alternative method.

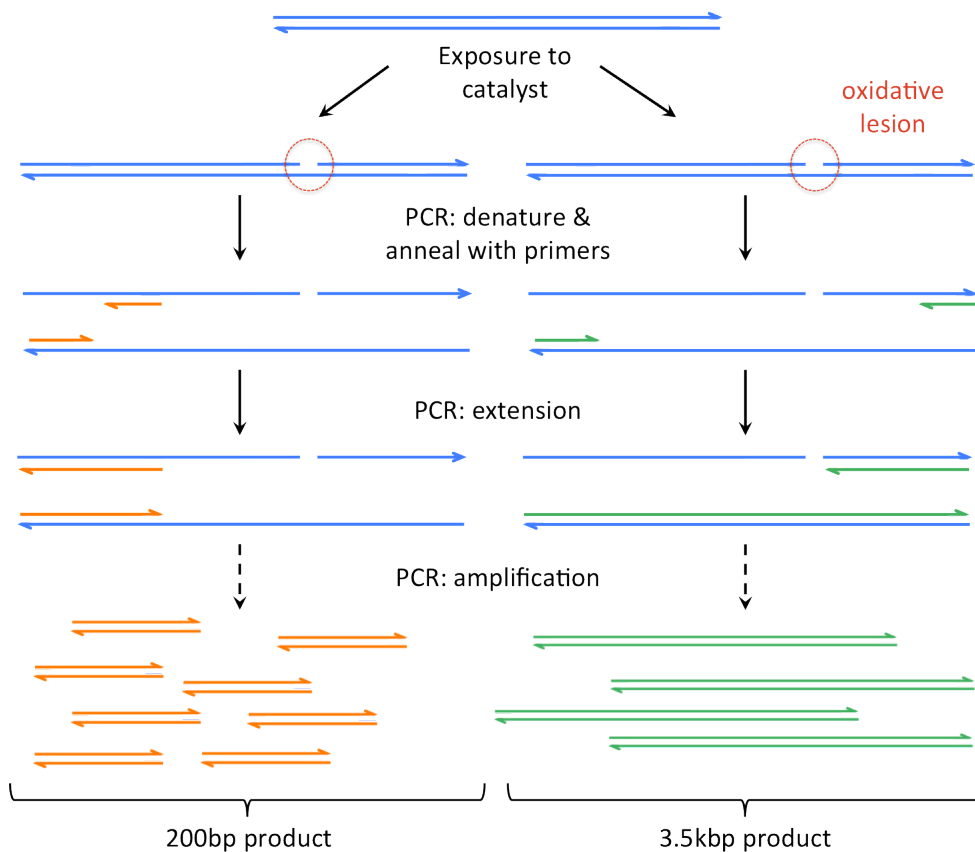


Figure S3: Schematic illustration of dual-amplification qPCR, which accounts for differences in total amount of DNA by amplifying both the long (3.5kbp, green) product, and a short (200bp, orange) region that is unlikely to be damaged.

AFM imaging

All imaging was carried out using an NTEGRA Vita Atomic Force Microscope, manufactured by NT-MDT (Moscow, Russia). Images were acquired while operating in semi-contact (tapping) mode under an aqueous Ni(II) imaging buffer (NB, 5mM Ni(II) acetate, 0.1X TAE), using silicon tips mounted on silicon nitride cantilevers with a nominal spring constant of 0.3 N/m and a resonant frequency of approximately 16 kHz in liquid (model SNL-10, manufactured by Bruker, California, USA).

As described in a previous publication,² the Ni(II) ions are coordinated by the surface carboxylate groups and function as salt bridges that immobilize the anionic DNA molecules. Prior to the hybridization, reaction, or denaturation steps, the surface was rinsed repeatedly with STAE to remove any Ni(II) ions that were bound to the surface.

A note on cleanliness: All glassware, teflon fluid cells, and ceramic tweezers were cleaned in piranha solution and rinsed thoroughly with water before use. (Piranha is 3:1 sulfuric acid: hydrogen peroxide. CAUTION— piranha is highly corrosive and reacts violently with organics).

Testing the DNA-templated crosslinking reaction

The efficiency of the CuAAC reaction for crosslinking two complementary DNA oligonucleotides was measured by carrying out the coupling reaction in solution, quenching the reaction at specified time points, and measuring the yield at each time point with denaturing polyacrylamide gel electrophoresis (D-PAGE), as described below.

Hybridization and reaction with copper: First the single-stranded, azide- and alkyne-modified DNA strands (SF-88 and SF-89, respectively) were hybridized by mixing in a 1:1 molar ratio in 0.8M NaCl, 20 mM PB, pH7, heating briefly to 70°C, and cooling from 65°C to 22°C at a rate of 0.75°C/min in a MJ-Mini thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Then 7.5µL of the hybridized DNA was added to 15µL of the 2X Cu(II)/ligand solution. Finally, 7.5µL of a 4X-concentrated solution of sodium ascorbate was added to initiate the reaction, and the solution was mixed and immediately capped. The final volume was 30µL, and the final DNA concentration was 1.25µM. At specified time points, a 4.0µL aliquot of the reaction mixture was removed and added

to 16 μ L of 1.25X quenching buffer (1X QB = 3:1 formamide:water, 20mM Tris acetate, 2.5 mM EDTA, 0.15X gel loading dye). The quenched reaction aliquot was immediately placed on ice and transferred to the freezer (-20°C) until being denatured and loaded into the gel.

Denaturing polyacrylamide gel electrophoresis: Polyacrylamide gels were prepared with a polyacrylamide/bisacrylamide (29:1) concentration of 12-14%, and contained 8.3M urea in order to denature the DNA during the gel runs. After casting the gel and setting for at least 30 min, the wells were rinsed with 0.5X TBE running buffer (1X = 89mM Tris base, 89mM boric acid, 2mM EDTA, pH 8.3). The gel was then pre-run in 0.5X TBE for at least 40 min at 150V in an Enduro vertical gel electrophoresis system (Labnet International, Inc, New Jersey, USA). Prior to loading the gel, the samples were removed from the freezer and heated > 90°C in QB for 5 min to completely denature the DNA. They were then immediately loaded into the gel and run for 120 minutes at a voltage of 150V. An ice pack was included in the outer buffer chamber to reduce overheating, which causes distortion of the gel bands. After the run, the gels were removed and stained by soaking in 0.5X TBE containing a 1X concentration of Sybr Green II dye (Life Technologies, California, USA) for 15 min under gentle agitation. The gels were visualized and imaged on an Enduro Gel Documentation System (Labnet).

Determination of reaction yield from gel images: It has been demonstrated previously that the efficiency of DNA crosslinking reactions can be estimated by comparing band intensities from denaturing PAGE.⁶ Due to the urea content of the gel and the elevated temperatures during running, the DNA remains denatured as it runs through the gel.⁷ Any hybrid DNA that has successfully reacted will be crosslinked by the triazole product, and thus will migrate as a single, slower-moving product band, while any DNA that has not reacted will be separated into two different faster-moving bands (see Figure 2a, main text). We estimated the yield of the reaction at different time points using a custom MATLAB script, (The Mathworks, Inc., Massachusetts, USA), as follows. First, each product band was selected by the user and cropped out from the gel image. The average background pixel intensity was subtracted, and the total pixel intensity in the band area was then integrated. Finally, this integrated band intensity was normalized to that of a standard reference band on the same gel. This normalized intensity was plotted as the estimated yield (in arbitrary units) as a function of time, as shown in Figure 2b (main text). It is worth noting that any of the product DNA that has been degraded during the reaction is expected to run faster than the intact

product band due to a smaller size, and thus DNA degradation over time should also lead to a reduction in the apparent yield.

Additional qPCR results

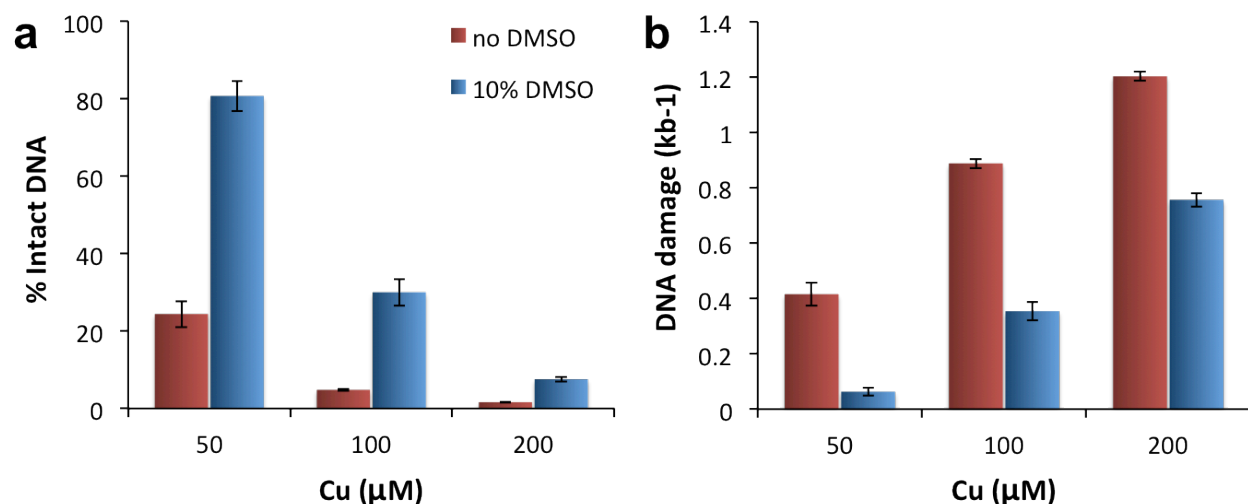


Figure S4: Results from qPCR measurements of oxidative DNA damage during the CuAAC reaction using the HLTA ligand. The percentage of intact DNA (a) and the corresponding DNA damage frequency (b) are plotted as a function of copper concentration, both with (blue) and without (red) 10% DMSO included in the reaction. For all reactions, Asc:Cu = 10:1 and L:Cu = 2:1, and the reaction time was 5 minutes. Error bars represent ± the standard deviation of at least four identical qPCR replicates.

REFERENCES

- (1) Abel, G. R., Cao, B. H., Hein, J. E. and Ye, T. (2014) Covalent, sequence-specific attachment of long DNA molecules to a surface using DNA-templated click chemistry. *Chem. Commun.* 50, 8131-8133.
- (2) Abel Jr., G. R., Josephs, E. A., Luong, N. and Ye, T. (2013) A switchable surface enables visualization of single DNA hybridization events with atomic force microscopy. *J. Am. Chem. Soc.* 135, 6399-6402.
- (3) Hong, V., Presolski, S. I., Ma, C. and Finn, M. G. (2009) Analysis and optimization of copper-catalyzed azide-alkyne cycloaddition for bioconjugation. *Angew. Chem. Int. Ed.* 48, 9879-9883.
- (4) Ayala-Torres, S., Chen, Y., Svoboda, T., Rosenblatt, J. and Van Houten, B. (2000) Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods* 22, 135-147.
- (5) Hunter, S. E., Jung, D., Di Giulio, R. T. and Meyer, J. N. (2010) The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods* 51, 444-451.
- (6) Kocalka, P., El-Sagheer, A. H. and Brown, T. (2008) Rapid and efficient DNA strand cross-linking by click chemistry. *ChemBiochem* 9, 1280-1285.
- (7) Maniatis, T., Jeffrey, A. and van deSande, H. (1975) Chain length determination of small double- and single-stranded DNA molecules by polyacrylamide gel electrophoresis. *Biochemistry* 14, 3787-3794.

- for bioconjugation: A comparative study. *Angew. Chem. Int. Ed.* 50, 8051-8056.
- (8) Uttamapinant, C., Tangpeerachaiikul, A., Grecian, S., Clarke, S., Singh, U., Slade, P., Gee, K. R. and Ting, A. Y. (2012) Fast, cell-compatible click chemistry with copper-chelating azides for biomolecular labeling. *Angew. Chem.* 124, 5954-5958.
- (9) Liu, P. Y., Jiang, N., Zhang, J., Wei, X., Lin, H. H. and Yu, X. Q. (2006) The oxidative damage of plasmid DNA by ascorbic acid derivatives in vitro: The first research on the relationship between the structure of ascorbic acid and the oxidative damage of plasmid DNA. *Chem. Biodiversity* 3, 958-966.
- (10) Hong, V., Presolski, S. I., Ma, C. and Finn, M. G. (2009) Analysis and optimization of copper-catalyzed azide-alkyne cycloaddition for bioconjugation. *Angew. Chem. Int. Ed.* 48, 9879-9883.
- (11) Kennedy, D. C., McKay, C. S., Legault, M. C., Danielson, D. C., Blake, J. A., Pegoraro, A. F., Stolow, A., Mester, Z. and Pezacki, J. P. (2011) Cellular consequences of copper complexes used to catalyze bioorthogonal click reactions. *J. Am. Chem. Soc.* 133, 17993-18001.
- (12) Brotherton, W. S., Michaels, H. A., Simmons, J. T., Clark, R. J., Dalal, N. S. and Zhu, L. (2009) Apparent copper(II)-accelerated azide-alkyne cycloaddition. *Org. Lett.* 11, 4954-4957.
- (13) Bevilacqua, V., King, M., Chaumontet, M., Nothisen, M., Gabillet, S., Buisson, D., Puente, C., Wagner, A. and Taran, F. (2014) Copper-chelating azides for efficient click conjugation reactions in complex media. *Angew. Chem. Int. Ed.* 53, 5872-5876.
- (14) Saito, F., Noda, H. and Bode, J. W. (2015) Critical evaluation and rate constants of chemoselective ligation reactions for stoichiometric conjugations in water. *ACS Chem. Biol.* 10, 1026-1033.
- (15) Kamber, D. N., Liang, Y., Blizzard, R. J., Liu, F., Mehl, R. A., Houk, K. and Prescher, J. A. (2015) 1,2,4-triazines are versatile bioorthogonal reagents. *J. Am. Chem. Soc.* 137, 8388-8391.
- (16) Ali, A. A., Chetia, M., Saikia, B., Saikia, P. J. and Sarma, D. (2015) AgN(CN)₂/DIPEA/H₂O-EG: A highly efficient catalytic system for synthesis of 1,4-disubstituted-1,2,3 triazoles at room temperature. *Tetrahedron Lett.* 56, 5892-5895.
- (17) Beckman, K. B. and Ames, B. N. (1997) Oxidative decay of DNA. *J. Biol. Chem.* 272, 19633-19636.
- (18) Dizdaroglu, M., Jaruga, P., Birincioglu, M. and Rodriguez, H. (2002) Free radical-induced damage to DNA: Mechanisms and measurement. *Free Radical Biol. Med.* 32, 1102-1115.
- (19) Cooke, M. S., Evans, M. D., Dizdaroglu, M. and Lunec, J. (2003) Oxidative DNA damage: Mechanisms, mutation, and disease. *FASEB J.* 17, 1195-1214.
- (20) Cadet, J., Douki, T., Gasparutto, D. and Ravanat, J. (2003) Oxidative damage to DNA: Formation, measurement and biochemical features. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 531, 5-23.
- (21) Wallace, S. S. (2002) Biological consequences of free radical-damaged DNA bases. *Free Radical Biol. Med.* 33, 1-14.
- (22) Bjelland, S. and Seeberg, E. (2003) Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 531, 37-80.
- (23) Hu, J. J., Dubin, N., Kurland, D., Ma, B. and Roush, G. C. (1995) The effects of hydrogen peroxide on DNA repair activities. *Mutat. Res., DNA Repair* 336, 193-201.
- (24) Eiberger, W., Volkmer, B., Amouroux, R., Dhérin, C., Radicella, J. P. and Epe, B. (2008) Oxidative stress impairs the repair of oxidative DNA base modifications in human skin fibroblasts and melanoma cells. *DNA Repair* 7, 912-921.
- (25) Marnett, L. J. (2000) Oxyradicals and DNA damage. *Carcinogenesis* 21, 361-370.
- (26) Kryston, T. B., Georgiev, A. B., Pissis, P. and Georgakilas, A. G. (2011) Role of oxidative stress and DNA damage in human carcinogenesis. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 711, 193-201.
- (27) Soriano del Amo, D., Wang, W., Jiang, H., Besanceney, C., Yan, A. C., Levy, M., Liu, Y., Marlow, F. L. and Wu, P. (2010) Bio-compatible copper(I) catalysts for in vivo imaging of glycans. *J. Am. Chem. Soc.* 132, 16893-16899.
- (28) Eyre-Walker, A. and Keightley, P. D. (2007) The distribution of fitness effects of new mutations. *Nat. Rev. Genet.* 8, 610-618.
- (29) Nairn, N. W., Bariola, P. A., Graddis, T. J., VanBrunt, M. P., Wang, A., Li, G. and Grabstein, K. (2015) Cysteine as a monothiol reducing agent to prevent copper-mediated oxidation of interferon beta during PEGylation by CuAAC. *Bioconjugate Chem.* 307-313.
- (30) El-Sagheer, A. H. and Brown, T. (2010) Click chemistry with DNA. *Chem. Soc. Rev.* 39, 1388-1405.
- (31) Cañete, S. J. P., Yang, W. and Lai, R. Y. (2009) Folding-based electrochemical DNA sensor fabricated by "click" chemistry. *Chem. Commun.* 4835-4837.
- (32) Cañete, S. J. P. and Lai, R. Y. (2010) Fabrication of an electrochemical DNA sensor array via potential-assisted "click" chemistry. *Chem. Commun.* 46, 3941-3943.
- (33) Mandhare, A., Banerjee, P., Bhutkar, S. and Hirwani, R. (2014) 'Click chemistry' for diagnosis: A patent review on exploitation of its emerging trends. *Expert Opin. Ther. Pat.* 24, 1287-1310.
- (34) Routh, A., Head, S. R., Ordoukhanian, P. and Johnson, J. E. (2015) ClickSeq: Fragmentation-free next-generation sequencing via click ligation of adaptors to stochastically terminated 3'-azido cDNAs. *J. Mol. Biol.* 427, 2610-2616.
- (35) El-Sagheer, A. H., Sanzone, A. P., Gao, R., Tavassoli, A. and Brown, T. (2011) Biocompatible artificial DNA linker that is read through by DNA polymerases and is functional in escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* 108, 11338-11343.
- (36) El-Sagheer, A. H. and Brown, T. (2011) Efficient RNA synthesis by in vitro transcription of a triazole-modified DNA template. *Chem. Commun.* 47, 12057-12058.
- (37) Birts, C. N., Sanzone, A. P., El-Sagheer, A. H., Blaydes, J. P., Brown, T. and Tavassoli, A. (2014) Transcription of click-linked DNA in human cells. *Angew. Chem. Int. Ed.* 53, 2362-2365.
- (38) Yakes, F. M. and Van Houten, B. (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* 94, 514-519.
- (39) Hunter, S. E., Jung, D., Di Giulio, R. T. and Meyer, J. N. (2010) The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods* 51, 444-451.
- (40) Fry, S. (1998) Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochem. J.* 332, 507-515.
- (41) Zhao, M. and Jung, L. (1995) Kinetics of the competitive degradation of deoxyribose and other molecules by hydroxyl radicals produced by the fenton reaction in the presence of ascorbic acid. *Free Radical Res.* 23, 229-243.
- (42) Yen, G., Duh, P. and Tsai, H. (2002) Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chem.* 79, 307-313.
- (43) Wang, Q., Chan, T. R., Hilgraf, R., Fokin, V. R., Sharpless, K. B. and Finn, M. G. (2003) Bioconjugation by copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition. *J. Am. Chem. Soc.* 125, 3192-3193.
- (44) Halliwell, B. (1996) Antioxidants in human health and disease. *Annu. Rev. Nutr.* 16, 33-50.
- (45) Forrest, V. J., Kang, Y., McClain, D. E., Robinson, D. H. and Ramakrishnan, N. (1994) Oxidative stress-induced apoptosis prevented by trolox. *Free Radical Biol. Med.* 16, 675-684.
- (46) Manzl, C., Enrich, J., Ebner, H., Dallinger, R. and Krumschnabel, G. (2004) Copper-induced formation of reactive oxygen species causes cell death and disruption of calcium homeostasis in trout hepatocytes. *Toxicology* 196, 57-64.
- (47) Sanmartín-Suárez, C., Soto-Otero, R., Sánchez-Sellero, I. and Méndez-Álvarez, E. (2011) Antioxidant properties of dimethyl sulfoxide and its viability as a solvent in the evaluation of neuroprotective antioxidants. *J. Pharmacol. Toxicol. Methods* 63, 209-215.
- (48) Eberhardt, M. K. and Colina, R. (1988) The reaction of OH radicals with dimethyl sulfoxide. A comparative study of fenton's reagent and the radiolysis of aqueous dimethyl sulfoxide solutions. *J. Org. Chem.* 53, 1071-1074.
- (49) Steiner, M. G. and Babbs, C. F. (1990) Quantitation of the hydroxyl radical by reaction with dimethyl sulfoxide. *Arch. Biochem. Biophys.* 278, 478-481.

- (50) Kocalka, P., El-Sagheer, A. H. and Brown, T. (2008) Rapid and efficient DNA strand cross-linking by click chemistry. *ChemBiochem* 9, 1280-1285.
- (51) Abel, G. R., Cao, B. H., Hein, J. E. and Ye, T. (2014) Covalent, sequence-specific attachment of long DNA molecules to a surface using DNA-templated click chemistry. *Chem. Commun.* 50, 8131-8133.
- (52) Presolski, S. I., Hong, V., Cho, S. H. and Finn, M. G. (2010) Tailored ligand acceleration of the Cu-catalyzed azide-alkyne cycloaddition reaction: Practical and mechanistic implications. *J. Am. Chem. Soc.* 132, 14570-14576.
- (53) Devaraj, N. K., Miller, G. P., Ebina, W., Kakaradov, B., Collman, J. P., Kool, E. T. and Chidsey, C. E. (2005) Chemoselective covalent coupling of oligonucleotide probes to self-assembled monolayers. *J. Am. Chem. Soc.* 127, 8600-8601.
- (54) Ayala-Torres, S., Chen, Y., Svoboda, T., Rosenblatt, J. and Van Houten, B. (2000) Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. *Methods* 22, 135-147.
- (55) Lutz, J. and Zarafshani, Z. (2008) Efficient construction of therapeutics, bioconjugates, biomaterials and bioactive surfaces using azide-alkyne "click" chemistry. *Adv. Drug Delivery Rev.* 60, 958-970.
- (56) Willson, J., Brown, D. and Timmens, E. (1965) A toxicologic study of dimethyl sulfoxide. *Toxicol. Appl. Pharmacol.* 7, 104-112.
- (57) Brigham, K. L., Meyrick, B., Berry, L. C., Jr and Repine, J. E. (1987) Antioxidants protect cultured bovine lung endothelial cells from injury by endotoxin. *J. Appl. Physiol.* 63, 840-850.
- (58) Bishayee, A., Rao, D. V., Bouchet, L. G., Bolch, W. E. and Howell, R. W. (2000) Protection by DMSO against cell death caused by intracellularly localized iodine-125, iodine-131 and polonium-210. *Radiat. Res.* 153, 416-427.
- (59) Datta, K., Babbar, P., Srivastava, T., Sinha, S. and Chattopadhyay, P. (2002) p53 dependent apoptosis in glioma cell lines in response to hydrogen peroxide induced oxidative stress. *Int. J. Biochem. Cell Biol.* 34, 148-157.