Click Chemistry

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Copper-Free Azide–Alkyne Cycloadditions: New Insights and Perspectives**

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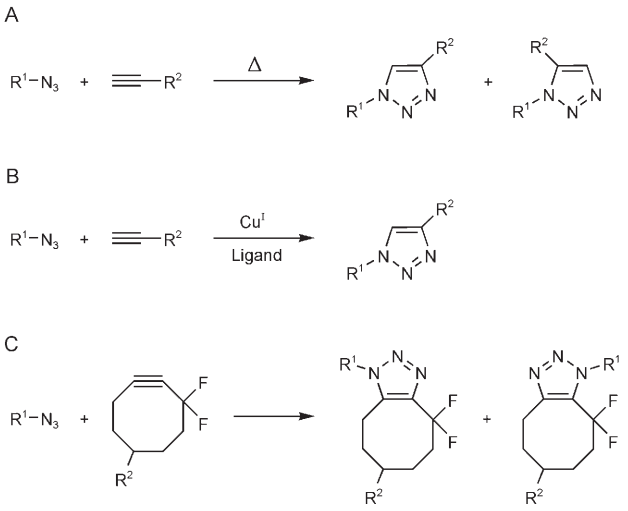
The "click" concept, proposed by Sharpless, Kolb, and Finn in 2001, is undeniably one of the most noticeable synthetic trends in this new century.[1] The catchy term "click" refers to energetically favored, specific, and versatile chemical transformations, which lead to a single reaction product. In other words, the essence of "click" chemistry is simplicity and efficiency.[2,3] This tantalizing concept seems to answer perfectly the needs of modern scientists working in areas of research as diverse as molecular biology, drug-design, biotechnology, macromolecular chemistry, or materials science.[3,4] It is indeed noteworthy that over recent years, complicated reactions requiring either complex apparatus, harsh experimental conditions, or high-purification techniques, have been less frequently studied than in the last century and gradually replaced by simpler tools. In this context, the straightforward "click" reactions have become tremendously popular in both academic and industrial research.

Reactions of the "click" type are rather rare. Yet, the last few years saw the emergence of a rudimentary "click" toolbox, which includes, for example Diels–Alder cycloadditions, thiol–ene additions, oxime formation, and copper-catalyzed Huisgen azide–alkyne cycloadditions (CuAAC).[5] However, in recent literature, the term "click chemistry" has been used almost exclusively to denote the latter reactions. The synthesis of 1,2,3-triazoles by 1,3-dipolar cycloaddition of azides and alkynes was discovered by Arthur Michael at the end of the 19th century and significantly developed by Rolf Huisgen in the 1960s.[6,7] In the absence of a transition-metal catalyst, these reactions are not regioselective, relatively slow, and require high temperatures to reach acceptable yields (Scheme 1 A). In early 2002, Meldal and co-workers reported that the use of catalytic amounts of copper(I), which can bind to terminal alkynes, leads to fast, highly efficient, and regioselective azide–alkyne cycloadditions at room temperature in organic medium (Scheme 1 B).[8] Shortly after,



Scheme 1. Different types of azide–alkyne cycloaddition: A) standard thermal cycloaddition,[7] B) copper(I)-catalyzed cycloaddition,[8,9,11] C) strain-promoted and fluorine-activated cycloaddition.[12]

Sharpless and Fokin demonstrated that CuAAC can be successfully performed in polar media, such as *tert*-butyl alcohol, ethanol or pure water.[9] These two important breakthroughs led to a remarkable renaissance of Huisgen cycloadditions in synthetic chemistry. Hence, research on CuAAC has increased exponentially in the last few years in organic synthesis, inorganic chemistry, polymer chemistry, and biochemistry. Numerous authors collectively demonstrated that CuAAC is a true example of efficient and versatile "click" chemistry.[10]

In particular, azide–alkyne cycloadditions have been shown to be highly relevant for biological applications. Indeed, such reactions can be performed under experimental conditions, which are compatible with biological environments (e.g. aqueous medium and body temperature). Moreover, azide and alkyne functions are, respectively, absent or relatively rare in the biological world. Thus, azide–alkyne chemistry constitutes a very interesting chemoselective platform for the functionalization or ligation of biological systems. For instance, CuAAC has been recently investigated for designing a wide range of biomaterials, such as stationary phases for bioseparation, site-specific modified proteins or viruses, drug- or gene- delivery carriers, protein or oligonucleotide microarrays, and functionalized cell surfaces.[13,14]

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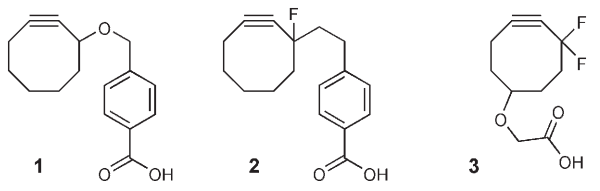
However, in some particular cases, the presence of transition-metal catalysts may be a problem. Some examples of in vitro copper-induced degradation of viruses or oligonucleotide strands have been reported.[14] Additionally, the use of CuAAC for in vivo applications is limited by the fact that, if present in more than trace quantities, copper ions are potentially toxic for living organisms. In this context, the development of metal-free "click" strategies is particularly relevant. Researchers at Scripps demonstrated that copper-free azide–alkyne cycloadditions are appropriate reactions for the target-guided synthesis of enzyme inhibitors.[15] In this case, the sluggishness of standard Huisgen cycloadditions at 37 °C was elegantly turned into an advantage, as slow kinetics are required in this application. Moreover, in this strategy, the regiospecificity of the "click" reaction is not induced by an added catalyst but by confinement in the binding pocket of the enzyme. However, such an approach is rather specific and cannot be extended to more standard ligation situations.

Cornelissen et al. investigated an elegant metal-free strategy for preparing 1,2,3-triazole linkages.[16] This method does not rely on substituted alkynes but on oxanorbornadienes, which react with organic azides in a tandem [3+2] cycloaddition-retro-Diels–Alder reaction. It was demonstrated that this particular reaction is faster at room temperature than standard Huisgen azide–alkyne cycloadditions. Hence, this approach was successfully used for modifying model peptides and proteins. However, one drawback of this method is the formation of a relatively toxic byproduct (i.e. furan) during the retro-Diels–Alder step.

An interesting copper-free azide–alkyne cycloaddition strategy has been recently proposed by Bertozzi and co-workers and relies on the use of strained cycloalkynes (Scheme 1 C).[12,17–19] The roots of this approach go back to the late works of Georg Wittig who described the exothermic cycloaddition of cyclooctyne with phenyl azide leading to the corresponding triazole in good yields.[20] Such high reactivity is a consequence of the geometrical deformation of the alkyne bond arising from ring strain.

In the emerging context of "click" chemistry, the Bertozzi group investigated novel substituted cyclooctynes (Scheme 2) and their reaction with azido derivatives. In a first report, it was shown that compound **1** successfully undergoes [3+2] cycloaddition with various low-molecular-weight compounds, such as 2-azido ethanol, benzyl azide, or *N*-butyl α-azidoacetamide.[17] Furthermore, the validity of this copper-free approach for the chemoselective modification of biomolecules and living cells was demonstrated. For instance, azide-modified human leukemic T cells could be functionalized with a biotin derivative of **1** without any apparent cytotoxicity.



Scheme 2. Cyclooctynes studied in strain-promoted cycloadditions with functional azides.[12,17,18]

However, in comparison to CuAAC, these cycloalkynes gave rather slow cycloaddition kinetics.

This situation was dramatically improved by introducing electron-withdrawing substituents on the α position of the triple bond (Scheme 2, **2** and **3**).[12,18] For instance, fluoro substituents were selected as they are relatively inert in a biological environment. It was very recently reported that compound **3** lead to very fast and efficient azide–alkyne cycloadditions.[12] These reactions are not regioselective but exhibit some "click" features in the sense that they are chemoselective and readily applicable in physiological conditions. Moreover, strain-promoted and fluorine-activated cycloadditions exhibit reaction kinetics comparable to those of CuAAC. For instance, the surface of mammalian cells could be functionalized with fluorescent dyes within minutes using this approach (Figure 1). Boons and co-workers have since reported another example of rapid copper-free azide–alkyne bioconjugation.[21] Their approach makes use of substituted dibenzocyclooctynes and allows efficient labeling of living cells.

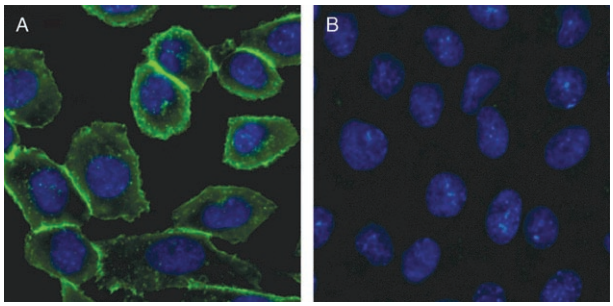


Figure 1. Fluorescent micrographs of Chinese hamster ovary cells after 1 minute of reaction with a fluorescent derivative of **3**: A) azide-functionalized cells, B) control cells without azide groups.[12] Images appear courtesy of Professor Carolyn Bertozzi (UC Berkeley).

These novel results of Bertozzi, Boons, and their co-workers constitute a significant step-forward for the selective functionalization and ligation of biological entities. Thus, cyclooctyne-based ligations could become important tools in chemical biology but also in some specific areas of materials science, where the use of transition-metal catalysts is problematic. However, it seems improbable that these reactions will fully replace CuAAC in a near future, considering that synthesis of substituted cyclooctynes is relatively demanding and the non-regioselectivity of strain-promoted cycloadditions might be an issue in some applications (e.g. drug design, peptidomimetics). Nevertheless, these novel reactions appear as appealing "biologically friendly" complementary tools in the growing set of straightforward "click" reactions.

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[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056 – 2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004 – 2021.
[2] a) C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Aust. J. Chem.* **2007**, *60*, 381 – 383; b) C. J. Hawker, K. L. Wooley, *Science* **2005**, *309*, 1200 – 1205.

[3] J.-F. Lutz, *Angew. Chem.* **2007**, *119*, 1036 – 1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018 – 1025.

[4] a) W. H. Binder, C. Kluger, *Curr. Org. Chem.* **2006**, *10*, 1791 – 1815; b) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51 – 68; c) Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674 – 1689; d) A. Dondoni, *Chem. Asian J.* **2007**, *2*, 700 – 708; e) M. V. Gil, M. J. Arevalo, O. Lopez, *Synthesis* **2007**, 1589 – 1620; f) P. Wu, V. V. Fokin, *Aldrichimica Acta* **2007**, *40*, 7 – 17.

[5] J.-F. Lutz, H. Schlaad, *Polymer* **2008**, *49*, 817 – 824.

[6] A. Michael, *J. Prakt. Chem.* **1893**, *48*, 94.

[7] a) R. Huisgen, *Angew. Chem.* **1963**, *75*, 604 – 637; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 565 – 598; b) R. Huisgen, *Angew. Chem.* **1963**, *75*, 742 – 754; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 633 – 645.

[8] C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057 – 3064.

[9] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708 – 2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596 – 2599.

[10] a) J. P. Collman, N. K. Devaraj, C. E. D. Chidsey, *Langmuir* **2004**, *20*, 1051 – 1053; b) D. D. Díaz, S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin, M. G. Finn, *J. Polym. Sci. Part A* **2004**, *42*, 4392 – 4403; c) B. Helms, J. L. Mynar, C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2004**, *126*, 15020 – 15021; d) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Fréchet, K. B. Sharpless, V. V. Fokin, *Angew. Chem.* **2004**, *116*, 4018 – 4022; *Angew. Chem. Int. Ed.* **2004**, *43*, 3928 – 3932; e) J.-F. Lutz, H. G. Börner, K. Weichenhan, *Macromol. Rapid Commun.* **2005**, *26*, 514 – 518; f) N. V. Tsarevsky, B. S. Sumerlin, K. Matyjaszewski, *Macromolecules* **2005**, *38*, 3558 – 3561.

[11] V. O. Rodionov, V. V. Fokin, M. G. Finn, *Angew. Chem.* **2005**, *117*, 2250 – 2255; *Angew. Chem. Int. Ed.* **2005**, *44*, 2210 – 2215.

[12] J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793 – 16797.

[13] a) T. S. Seo, Z. Li, H. Ruparel, J. Ju, *J. Org. Chem.* **2003**, *68*, 609 – 612; b) A. Deiters, T. A. Cropp, D. Summerer, M. Mukherji, P. G. Schultz, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5743 – 5745; c) A. J. Link, M. K. S. Vink, D. A. Tirrell, *J. Am. Chem. Soc.* **2004**, *126*, 10598 – 10602; d) S. Punna, E. Kaltgrad, M. G. Finn, *Bioconjugate Chem.* **2005**, *16*, 1536 – 1541; e) P. C. Lin, S. H. Ueng, M. C. Tseng, J. L. Ko, K. T. Huang, S. C. Yu, A. Kumar Adak, Y. J. Chen, C. C. Lin, *Angew. Chem.* **2006**, *118*, 4392 – 4396; *Angew. Chem. Int. Ed.* **2006**, *45*, 4286 – 4290; f) J.-F. Lutz, H. G. Börner, K. Weichenhan, *Macromolecules* **2006**, *39*, 6376 – 6383; g) X. Jiang, M. C. Lok, W. E. Hennink, *Bioconjugate Chem.* **2007**, *18*, 2077 – 2084; h) J.-F. Lutz, H. G. Börner, *Prog. Polym. Sci.* **2008**, *33*, 1 – 39.

[14] a) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn, *J. Am. Chem. Soc.* **2003**, *125*, 3192 – 3193; b) J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639 – 3642.

[15] a) R. Manetsch, A. Krasinski, Z. Radic, J. Raushel, P. Taylor, K. B. Sharpless, H. C. Kolb, *J. Am. Chem. Soc.* **2004**, *126*, 12809 – 12818; b) V. P. Mocharla, B. Colasson, L. V. Lee, S. Röper, K. B. Sharpless, C.-H. Wong, H. C. Kolb, *Angew. Chem.* **2005**, *117*, 118 – 122; *Angew. Chem. Int. Ed.* **2005**, *44*, 116 – 120; c) M. Whiting, J. Muldoon, Y. C. Lin, S. M. Silverman, W. Lindstrom, A. J. Olson, H. C. Kolb, M. G. Finn, K. B. Sharpless, J. H. Elder, V. V. Fokin, *Angew. Chem.* **2006**, *118*, 1463 – 1467; *Angew. Chem. Int. Ed.* **2006**, *45*, 1435 – 1439.

[16] S. S. van Berkel, A. J. Dirks, M. F. Debets, F. L. van Delft, J. J. L. M. Cornelissen, R. J. M. Nolte, F. P. J. T. Rutjes, *ChemBioChem* **2007**, *8*, 1504 – 1508.

[17] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046 – 15047.

[18] N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *ACS Chem. Biol.* **2006**, *1*, 644 – 648.

[19] J. M. Baskin, C. R. Bertozzi, *QSAR Comb. Sci.* **2007**, *26*, 1211 – 1219.

[20] G. Wittig, A. Krebs, *Chem. Ber.* **1961**, *94*, 3260 – 3275; A. T. Blomquist, L. H. Liu, *J. Am. Chem. Soc.* **1953**, *75*, 2153 – 2154.

[21] X. Ning, J. Guo, M. A. Wolfert, G.-J. Boons, *Angew. Chem.* **2008**, *120*, 2285 – 2287; *Angew. Chem. Int. Ed.* **2008**, DOI: *47*, 2253 – 2255.

