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Click Chemistry: A Universal Ligation Strategy for Biotechnology and Materials Science

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1.1 Introduction

Current advances in our understanding of molecular biology, microelectronics and sensorics have fueled an increased need for tightly defined structural materials and surfaces.[1] The controlled synthesis of such materials, however, imposes major challenges. Moreover, man-made materials have struggled to achieve the superb structural and functional properties of natural macromolecules, such as proteins, DNA or sugars. Recognizing these limitations, researchers in the materials and polymer sciences as well as in biotechnology have been continuously searching for well-defined ligation strategies that can be effectively used in the presence of a wide range of different functional groups typically encountered in these fields. Key requirements for successful ligation strategies include high selectivity, orthogonality to other functional groups, compatibility with water and other protic solvents and, of course, close-to-quantitative yields. To find improved ligation reactions, materials scientists and biotechnologists have increasingly turned towards advanced synthetic organic concepts. In this respect, the most powerful example is undoubtedly the acceptance of the click chemistry concept by the materials science community[2–8] since the first reports on the use of click chemistry for materials science, which appeared as recently as 2004.[9]

In his landmark review published in 2001, Sharpless and coworkers defined click chemistry as a 'set of powerful, highly reliable, and selective reactions for the rapid synthesis of useful new compounds and combinatorial libraries.'[10] Click reactions are driven by a

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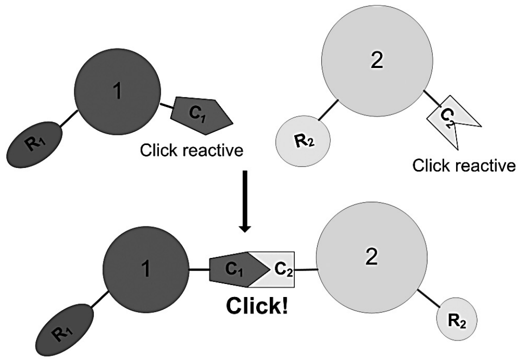
high thermodynamic driving force (>20 kcal mol^{-1}), which is typically associated with the formation of carbon–heteroatom bonds. Click chemistry is not limited to a specific type of reaction, but stands for a synthetic philosophy that comprises of a range of reactions, with different reaction mechanisms but common reaction trajectories. The prime example of a click reaction is the copper-catalyzed Huisgen's 1,3-dipolar cycloaddition of azides and terminal alkynes.[10,11] This reaction is regioselective, forming only 1,4-substituted products, is insensitive to the solvent, and can be performed at room temperature. Moreover, it proceeds with high yields and is about 10^7 times faster than the uncatalyzed reaction. Another important aspect of the success of this reaction pertaining to materials science and biotechnology is that the starting materials, azides and terminal alkynes, are exceptionally stable and can be introduced in a wide range of macromolecules.

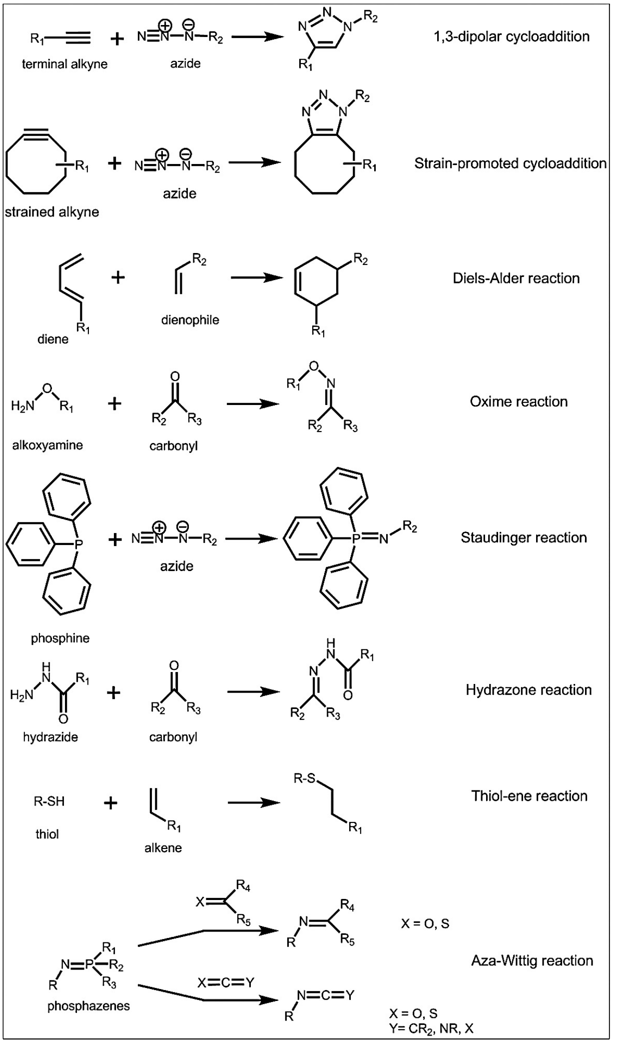
Ever since these initial publications, the area of click chemistry has turned into a highly productive area of research with exponential growth over the last few years. A literature search[12] indicated more than 600 papers and more than 10 000 citations in 2008 that were associated with the term 'click chemistry'. This compares with only about 100 papers and less than 1000 citations in 2005. Interestingly, Sharpless initially introduced the concept of click chemistry with a clear focus on drug discovery. While researchers in the drug discovery field still appear to be somewhat hesitant towards click chemistry,[13] the applicability of click chemistry towards materials science, polymer chemistry and biotechnology has been an astonishing success story, underlining the readiness of these fields for well-defined chemical reactions with the exact reaction profiles developed under the click chemistry framework. In a recent review, Wang and coworkers reported that only 14% of the papers published on click chemistry are actually related to drug discovery, while two-thirds of the click chemistry papers fall into the broad categories of materials science and biotechnology.[13]

1.2 Selected Examples of Click Reactions in Materials Science and Biotechnology

The value of click chemistry for materials synthesis possibly becomes most apparent in the area of polymer chemistry and several recent reviews have described the use of Cu-catalyzed azide–alkyne cycloaddition (CuAAC) for the synthesis of dendritic, branched, linear and cyclic co-polymers.[5–7, 14] Hawker, Sharpless, Fokin and coworkers first introduced CuAAC reaction to polymer chemists for dendrimer synthesis.[9] Triazole-based dendrons were divergently synthesized via CuAAC reaction. These dendrons were then anchored to a variety of polyacetylene cores to generate dendrimers. Since then, the CuAAC reaction has been widely employed to synthesize or modify various dendrimers.[15,16]

The remarkable functional group tolerance of click reactions enables the facile introduction of reactive groups such as hydroxyl and carboxyl through conventional pre-polymerization modification[17,18] or post-polymerization modification.[19] Thus, click reactions have been employed in combination with living polymerization techniques, such as ring-opening polymerization (ROP), ring-opening metathesis polymerization (ROMP), cationic polymerization, nitric oxide-mediated radical polymerization (NMP), atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer polymerization (RAFT).





Scheme 1.1 *List of widely exploited chemical reactions that fall within the framework of click chemistry.*

One of the biggest strengths of click chemistry is its utility in conjunction with one or more of these polymerization methods, thus offering a facile access to a broad range of polymeric materials that would be difficult to prepare otherwise. For example, various functional groups (such as carboxyl, olefin and amine groups) were attached to polymers prepared by ATRP and modified by immobilization of biomolecules.[20–22] Beyond synthesis and modifications of functionalized polymers, click reactions have played an important role in the cross-linking of polymeric materials. One such example is the appearance of cross-linked polymeric adhesives synthesized from polyvalent azide and alkyne building blocks, owing their adhesiveness to the strong affinity of triazoles for metal ions and surfaces.[23] In yet another example, poly(vinyl alcohol)-based hydrogels were synthesized by mixing azido-appended PVA and acetylene-appended PVA in the presence of a Cu(I) catalyst.[24]

The controlled decoration of surfaces and design of biointerfaces are other obvious strongholds of click chemistry. Taking advantage of either Huisgen's 1,3-dipolar cycloaddition or Diels–Alder reaction, several advances were made with respect to the preparation of SAMs containing azido groups on well-defined electrode surfaces and subsequent reaction with ethynylferrocene or propynoneferrocene.[25] Moreover, well-defined surface arrays of acetylene-containing oligonucleotides were immobilized via CuAAC reaction onto azide-functionalized SAMs on gold.[26] This chemistry was unaffected by deactivation due to electrophiles or nucleophiles and showed remarkable stability against hydrolysis. Similarly, CuAAC reaction of acetylenyl-terminated SAMs and azide compounds was used as a versatile tool for tailoring surface functionalities under mild conditions.[27] In a universal surface modification approach, alkyne-containing vapor-deposited polymer coatings were shown to possess remarkable reactivity towards azide-functionalized moieties.[28] These reactive coatings, poly(4-ethynyl-*p*-xylylene-*co*-*p*-xylylene), were applied to a wide range of substrates using chemical vapor deposition and modified by subsequent spatially directed CuAAC reaction.

Among the more interesting examples is the application of the CuAAC reaction towards chemical functionalization of nanomaterials, such as single-walled carbon nanotubes (SWNT).[29] In this case, alkyne groups introduced onto the surface of the SWNTs offered a route towards highly specific post-modification. This method granted a greater amount of control on the orientation and the density of the polymer attached to the surface of the nanotube, while reducing the risk of side reactions. Similarly, Diels–Alder reactions were used for selective modification of carbon nanotubes. For instance, *o*-quinodimethane was directly coupled to SWNTs with the help of microwave irradiation.[30] These reactions open up possibilities for enhancing the solubility of carbon nanotubes, as needed in several technological applications.

To take the application of click chemistry even a notch higher, click reactions have been recently proposed for covalent labeling in living systems, such as cells or tissue.[31,32] If biomolecules expressed within cells could be fluorescently labeled, their destiny could be tracked in real time. Moreover, important metabolic studies could be conducted *in vitro* or potentially even *in vivo*. For such concepts to become a reality, the reactions used for covalent labeling must not only fulfill the criteria of an efficient click reaction, such as high yields, selectivity and compatibility with an aqueous environment, but also must be bio-orthogonal. The latter refers to the necessity of exploiting reactants that are 'non-interacting towards the functionalities present in biological systems'.[33] Important bio-orthogonal click reactions, including Staudinger ligation[34] and strain-promoted [3 + 2] heterocycloadditions,[35] have been proven to be effective reactions for protein labeling in living biological systems.

From these few selected examples, it is already apparent why click reactions have been so popular and successful for the synthesis and modification of macromolecules. The need to effectively decorate biomaterials and biointerfaces, the ability to drive covalent reactions inside living organisms and the promise of constructing large macromolecules through complimentary junctions present in their constituents will be important technology drivers for decades to come.

1.3 Potential Limitations of Click Chemistry

In spite of the undisputable success of the concept of click chemistry within just a few years, there are still a few limitations associated with the concept. Because of the stringent criteria that are used to identify click reactions, chemical diversity is intrinsically limited. As a matter of fact, the CuAAC reaction is still by far the most widely used click reaction. However, copper is believed to be cytotoxic and demonstrated side effects associated with excessive copper intake include hepatitis, Alzheimer's disease and neurological disorders.[36] For click reactions to be used in contact with living systems, the copper catalyst must be completely removed or alternatives, such as Staudinger ligation or strain-promoted [3 + 2] heterocycloadditions, must be employed.

Azides, among the prime reactants for Huisgen's 1,3-dipolar cycloaddition reaction, are also often associated with potential toxic side effects, and certain azides may bear a very real explosive potential.[37]

Finally, a more practical limitation is that the supply of clickable starting materials often cannot keep up with the demands of the rapidly emerging application space in materials science and biotechnology. Meanwhile, many of the researchers that work in these fields are not synthetic chemists, who can easily synthesize appropriate starting materials, but must rely on commercial sources for obtaining access to these chemicals. However, as the click chemistry philosophy continues to spread through the area of materials science, polymers and biotechnology, more and more clickable building blocks can be expected to become easily available.

1.4 Conclusions

To address the gap between sophisticated function that is required for future advances in bio- and nanotechnology and the limited chemical control offered by many of the currently available synthetic materials' processes, novel synthetic tools are needed. In spite of the evident differences between small molecules and macromolecules, attempts to extend synthetic concepts from organic chemistry into the nano- and meso-scale dimensions have been increasingly popular. It is mainly for this reason that the fields of materials science and biotechnology enthusiastically embraced the concept of click chemistry as a versatile tool for introducing structural control. Ideally, these efforts will offer molecular-level control during the preparation of nanostructured materials. It is likely that this trend will continue and will ultimately result in an increase in the infusion of concepts from synthetic organic chemistry into materials science and biotechnology.[38]

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