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Cell

# Clinical and preclinical utility of click chemistry

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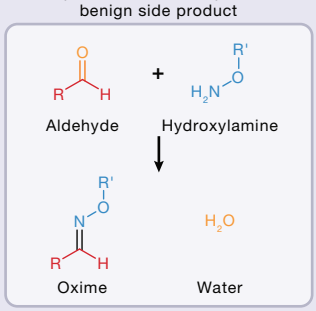
**Bioorthogonal click reaction:** a chemical transformation that irreversibly joins two components in a modular fashion at room or body temperature, producing no or benign side products and with no or minimal reactivity with other parts of a biological system, in aqueous media.

## Reactions

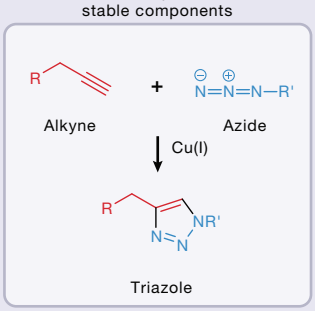
### Oxime ligation

Simple and stable components, benign side product



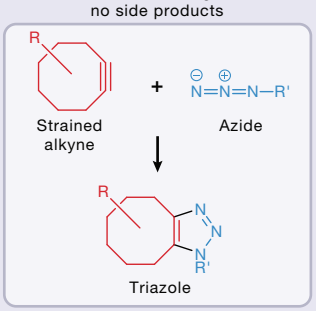

### Copper-catalyzed azide-alkyne cycloaddition (CuAAC)

Fast, simple, and stable components



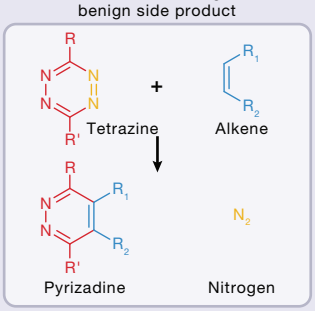

### Strain-promoted azide-alkyne cycloaddition (SPAAC)

Close to bioorthogonal, no side products



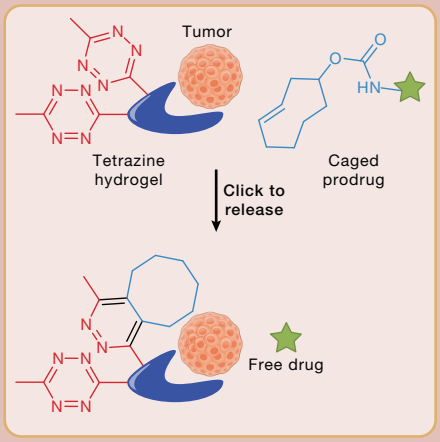

### Inverse electron demand Diels-Alder (IEDDA)

Can be fast, biorthogonal, benign side product

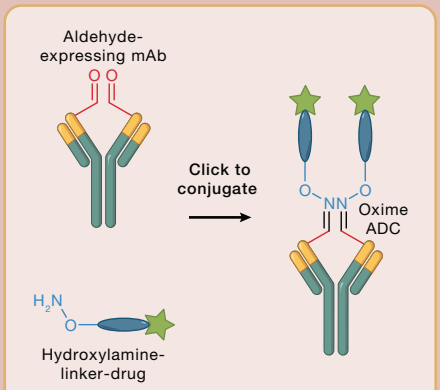



## Clinical developments

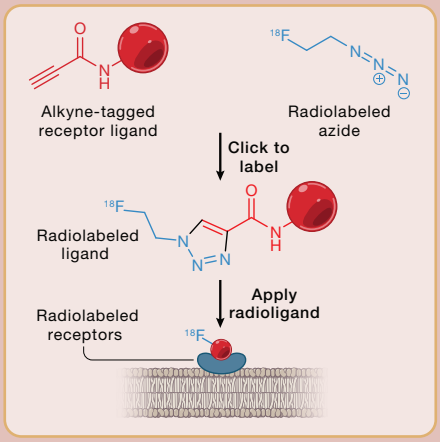
### Drug release




### Antibody-drug conjugates (ADCs)

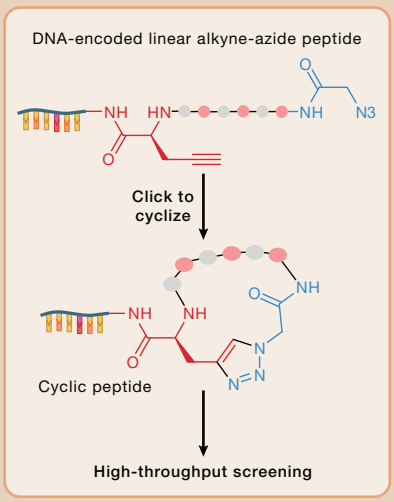



### Radiolabeling

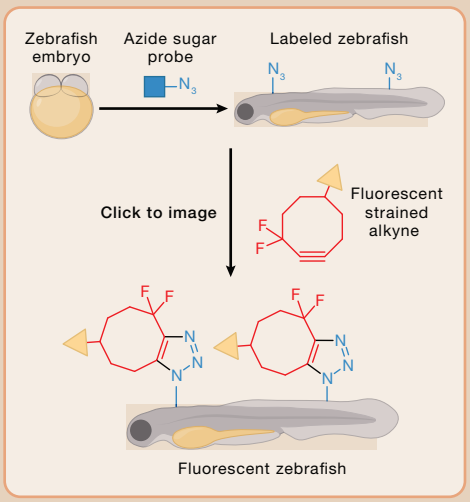



## Pre-clinical technologies

### High-throughput screening

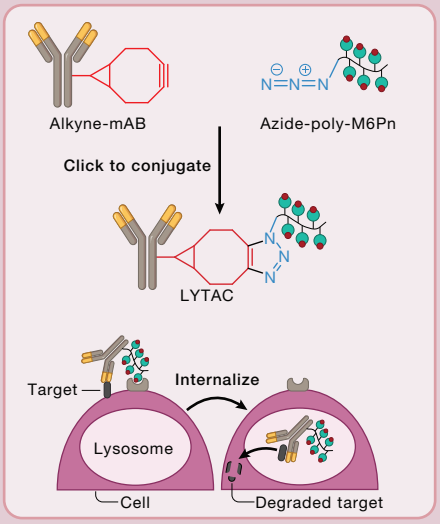



### Click-enabled molecular biology techniques




## Near-clinical developments

### Lysosomal-targeting chimeras (LYTACs)

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## Defining click and bioorthogonal chemistry

Click reactions were first defined by Sharpless and colleagues as chemical transformations (a few good reactions) that meet a range of criteria, including modularity, high yields, simple reaction conditions (ability to take place at room temperature, compatibility with aqueous media), and the stability of the product.[1] Bertozzi and coworkers introduced another concept key to chemical reactions in biological systems: bioorthogonality. Components of a bioorthogonal reaction taking place in a biological system should react exclusively with its designated partner.[2] These two concepts have paved the way to performing chemical transformations in biological systems ranging from individual molecules to human beings.

## Bioorthogonal click reactions

Click reactions in a biological setting serve as a way to join two components—for example, a caged prodrug and a decaging agent or a drug and an antibody. The click reaction can take place either *in vivo* to directly exert an effect or *ex vivo* to produce an agent that can be applied *in vivo*. A handful of chemical reactions satisfy the definitions of both click and bioorthogonal chemistry, with at least four having found an application in the clinic. Each one has a unique reaction profile, with different features that might be desirable for particular applications.v

For example, if the aim is to functionalize a biomolecule, it is ideal to use reactions whose components are easy to attach/encode onto biomolecules, e.g., aldehydes for oxime ligations or azides/alkynes for azide-alkyne cycloadditions.[1] However, if the reaction needs to be bioorthogonal *in vivo*, IEDDA (inverse electron demand Diels-Alder), using tetrazines and alkenes might be the best option as components of other reactions mentioned here can cross-react with various biomolecules. For a click reaction to go to completion, it must be sufficiently fast; if that is one of prerequisites for a particular application, IEDDA or CuAAC (copper-catalyzed azide-alkyne cycloaddition)[3] are both good options due to their high rates, with oxime ligation and SPAAC (strain-promoted azide-alkyne cycloaddition)[4] being relatively slower.

## Clinical uses of click chemistry

A prime example of click chemistry in a clinical setting is drug release (decaging). In their seminal paper, Oneto and colleagues describe a strategy to release a cytotoxic drug locally in a tumor environment.[5] In this application, a tetrazine-functionalized hydrogel is applied onto a tumor. Then, a caged prodrug is dosed to the patient. Upon reaching the tumor, the cyclooctene group on the prodrug undergoes a click reaction (IEDDA) with a tetrazine on the hydrogel, which triggers the release of the active drug. Thus, click chemistry enables a method to release a drug specifically in a tumor, without affecting healthy tissues. Currently, a drug decaging system is in phase 2 clinical trials against advanced solid tumors (NCT04106492).

Another clinically relevant field empowered by click chemistry is antibody-drug conjugates (ADCs). The aim of ADCs is to affect cells in a tissue-dependent manner—the drug gets localized in the microenvironment of the antigen targeted by the antibody, e.g., a tumor. Numerous ADCs made via click chemistry have now entered the clinic, with ARX788 being the first one.[6] This ADC utilizes a monoclonal antibody against HER2 engineered to contain two aldehyde moieties. This antibody undergoes an oxime ligation reaction to be conjugated to an auristatin-hydroxylamine, resulting in the active ADC, which triages highly cytotoxic auristatin to cells that express HER2. This antibody is currently in clinical trials against advanced HER2-positive breast cancers (NCT04829604) and advanced cancers with HER2 expression (NCT03255070).

Click chemistry is also being utilized for radiolabeling. For example, a CuAAC-based ligand is developed to visualize tumor lesion.[7] Somatostatin was functionalized with an alkyne moiety, which was then reacted with an azide attached to a radioisotope $^{18}$F. The resulting somatostatin-$^{18}$F conjugate was subjected to patients with gastroenteropancreatic and lung tumors (European Clinical Trials no. 2013-003152-20). In this case, a click reaction allows an easy functionalization of receptor ligands in a modular fashion, with availability of a suitable ligand against the desired receptor being the only hurdle.

## Preclinical utility of click chemistry

Click chemistry enables preclinical technologies, facilitating target and lead discovery. One area where click chemistry is gaining prominence is high-throughput screening. In one example, CuAAC was employed in a strategy to generate a DNA-encoded library of cyclic peptides.[8] The linear precursors of these peptides were functionalized with azide and alkyne handles; thus, treatment with copper would trigger their cyclization via CuAAC. At the time, it was the largest DNA-encoded cyclic peptide library to date, enabled by the ease of click chemistry.

Many modern molecular biology techniques, key to preclinical discovery, also utilize click chemistry. Clickable fluorophores for target visualization and clickable biotins for streptavidin pulldowns have become commonplace tools. Perhaps the key study that demonstrated the power of click chemistry for such applications was *in vivo* surface glycan labeling in zebrafish, carried out by the Bertozzi group.[9] Zebrafish embryos were grown in the presence of azide-containing sugars, leading to incorporation of azide groups into growing glycans. They were functionalized with strained alkyne-fluorophore conjugates using SPAAC, leading to fluorophores getting localized to *de novo* zebrafish surface glycans.

## Future applications of click chemistry in the clinic

Click chemistry has already made quite a few inroads into the clinic with more therapeutic platforms in the making. To highlight one, LYTACs (lysosomal-targeting chimeras) are a class of molecules that induce degradation of extracellular or membrane-associated proteins.[10] They have been made using SPAAC by conjugating an antibody to sugar-peptide. The resulting LYTACs form ternary complexes with a receptor and the target of degradation. They get localized into lysosomes, where the target gets degraded by native proteases.

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