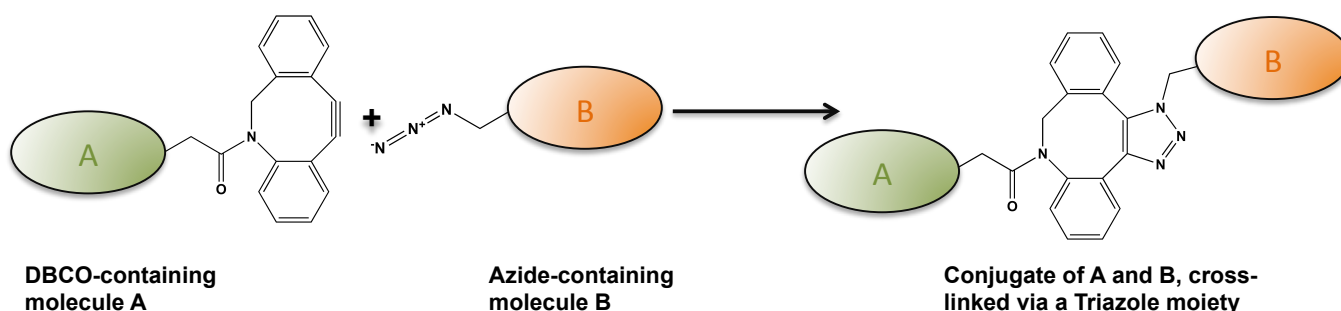


# Copper-Free Click Reactions

## Background Information

### Introduction

The strain-promoted or Cu(I)-free [2+3] cycloaddition strategy relies on the use of strained cyclooctynes. Their use decreases the activation energy for the cycloaddition click reaction, enabling it to be carried out without the need for catalysis at low temperatures with an efficiency greater than that of the Cu(I)-catalyzed ligation.



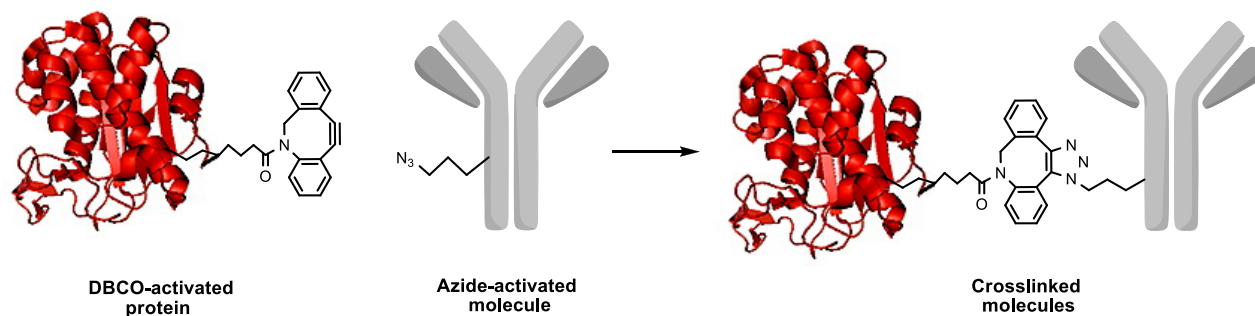
Diarylcyclooctynes are thermally stable compounds with very narrow and specific reactivity toward azides. The ligation reaction is very fast and results in almost quantitative yield of stable triazoles.

The strain-promoted Click reaction and the so called Staudinger ligation (phosphine-azide) are competing technologies for chemoselective ligation. Both reactions are chemoselective and do not require copper, so both do not damage biomolecules. However, the rate of Staudinger ligation is about 100fold lower than the rate of the DBCO cycloaddition, which makes the Staudinger ligation hardly useful for studying dynamic biological systems. Only in cases where the speed of ligation is irrelevant, both reactions can be used with about equal efficiency.

### Crosslinking Biomolecules using Click Reactions

Our conjugation chemistry is based on the reaction of a dibenzylcyclooctyne (DBCO) linker with an azide linker to form a stable triazole. This „click reaction“ is very fast at room temperature, does not require a cytotoxic Cu(II) catalyst and creates stable triazoles. This unique covalent bond is created when DBCO, incorporated into one type of biomolecule, reacts with an azide linker, incorporated into a second biomolecule.

Unlike many conjugation reagents DBCO and azide are long term stable when attached to biomolecules. DBCO - azide conjugation chemistry is complementary and thus they react only with each other.



# Copper-Free Click Reactions

## Background Information

### ***This method requires a three-step reaction:***

- Step 1: Activation of biomolecule #1 with DBCO
- Step 2: Activation of biomolecule #2 with azide
- Step 3: Mixing the two activated biomolecules to form a conjugate
- Step 4 (optional): Removing excess of azide or DBCO activated biomolecule with DBCO or azide scavenger

### ***Product Features and Benefits:***

- Stable – forms a triazole
- Biocompatible – no catalyst required (e.g. Cu(I))
- Specific – azide reacts only with DBCO, even in presence of -NH<sub>2</sub>, -SH, -COOH or other protein functionalities
- The reactive moieties do not interact with functionalities on biomolecules
- All reactions are carried out in aqueous buffered media, yielding high conjugation efficiency.

This three step process is better than previous methods as it does not form homo-polymers and allows for more controllable formation of the desired conjugate. The DBCO and the azide linkers are available in various lengths and may be chosen to react with either an amine, thiol or carboxyl group on biomolecules. To get started, simply two reagents are required (DBCO and azide).

These crosslinkers are the most efficient and quantitative linkers available and produce high quality, easily reproducible conjugates for better performance in your assays.