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Click Chemistry background information

Introduction

Click Chemistry[1] describes pairs of functional groups that rapidly and selectively react ("click") with each other in mild, aqueous conditions. The concept of Click Chemistry has been transformed into convenient, versatile and reliable two-step coupling procedures of two molecules A and B[1-5], that are widely used in biosciences[6-8], drug discovery[9] and material science[10].

Principle of Click Chemistry

1. Activation of molecule A and B
Compatible CLICK-functional groups are introduced via CLICK Reagents

2. CLICK-coupling of molecule A and B
The CLICK-activated molecules A and B form a stable conjugate

Advantages of Click Chemistry

- **Highly selective, low background labeling:** CLICK-functional groups are inert to naturally occurring functional groups ("bioorthogonal") such as amines
- **Rapid and quantitative labeling**
- **Allows non-radioactive analysis of enzymatic activities both *in vitro* and *in vivo*:** Small-sized CLICK-functional groups possess excellent substrate properties

Especially biomolecule labeling requires reaction procedures that can be performed under physiological conditions (neutral pH, aqueous solution, ambient temperature) with low reactant concentrations to ensure non-toxic, low background labeling at reasonable time scales while still preserving biological function. Among the plethora of possible reactions only a few generally fit the necessary reactivity, selectivity and biocompatibility criteria (Fig. 1):

1. **Cu(I)- catalyzed Azide - Alkyne Click Chemistry reaction (CuAAC)**
2. **Strain-promoted Azide - Alkyne Click Chemistry reaction (SPAAC)**
3. **Inverse electron demand Diels-Alder ligation (IEDDA)**

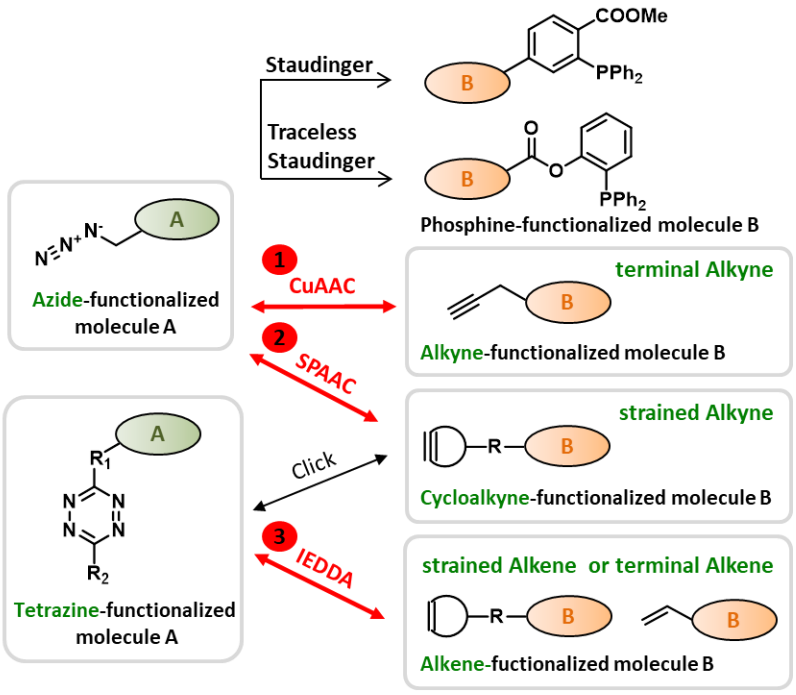

Figure 1: Overview of the most common Click Chemistry reactions.
Click Chemistry reactions can be categorized into two separate groups: Copper (Cu(I))-catalyzed and Copper-free. The Cu(I)- catalyzed Azide - Alkyne Click Chemistry reaction (CuAAC) (1.) relies on the presence of Cu(I) ions whereas the Copper-free strain-promoted Azide - Alkyne Click Chemistry reaction (SPAAC) (2.) and Inverse electron demand Diels-Alder ligation (IEDDA) (3.) efficiently proceed without metal catalysis. The well-known Copper-free Azide-Phosphine reaction (Staudinger and traceless Staudinger ligation) is hampered by the instability of phosphines and slow reaction kinetics. Recent focus therefore shifted towards strain-promoted reactions with cyclooctynes and Inverse electron demand Diels-Alder ligation, respectively.

We selected the best performing CLICK reactions in terms of selectivity, reactivity, biocompatibility and stability!

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1. Cu(I)-catalyzed Azide-Alkyne Click Chemistry (CuAAC) reaction

Clearly the most prominent example of click chemistry is the Cu(I)-catalyzed Azide-Alkyne Click Chemistry (CuAAC) reaction[1]. An Azide-functionalized molecule A reacts with a terminal Alkyne-functionalized molecule B thereby forming a stable conjugate A-B via a Triazole moiety (Fig. 2).

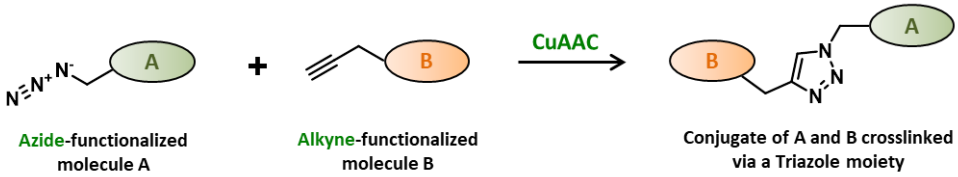

Figure 2: Principle of Cu(I)-catalyzed Azide-Alkyne Click Chemistry(CuAAC).

Since terminal Alkynes are fairly unreactive towards Azides, the efficiency of a CuAAC reaction strongly depends on the presence of a metal catalyst such as copper. Different copper sources and reduction reagents are available however, the Cu(II) salt $CuSO_4$ as copper source in combination with ascorbate as a reduction reagent has been recommended for most biomolecule labeling applications[11,12].

The use of CuAAC reactions in live cells is hampered by the toxicity of Cu(I) ions. This problem has been partially overcome by the use of Cu(I) chelating ligands such as THPTA, TBTA, BTTES, BTTPS and BTTAA, that serve a dual purpose: 1) Acceleration of the CuAAC reaction by maintaining the Cu(I) oxidation state and 2) Protection of the biomolecule from oxidative damage. BTTAA showed the highest activity in accelerating CuAAC reactions[13]. BTTAA and BTTPS, two water soluble TBTA analogues, exhibited the highest reactivity in catalyzing CuAAC reaction *in vivo* protein labelling process [14]. The utility of a copper-chelating organic azide such as picolyl azide, a ligand BTTAA and low Cu concentrations represent the fastest and most biocompatible version of CuAAC to date[15].

Presolski *et. al.*[11] and Hong *et. al.*[12] provide a general protocol for CuAAC reactions that may be used as a starting point for the set up and optimization of individual assays.

Features:

- Small-sized azides and alkynes possess excellent substrate properties
- Optimization of assay conditions required (type & concentration of Copper source, reduction reagent and Copper ligand)
- Suitable if potential copper toxicity does not matter (not recommended for *in vivo* or live cell labeling)
- Slowest reaction speed compared to 2. and 3.

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2. Strain-promoted Azide-Alkyne Click Chemistry (SPAAC) reaction

The requirement of a cytotoxic copper catalyst often limits the usage of CuAAC reactions (see 2.) A Copper free and thus non-toxic labeling method of Azides is the **S**train-**P**romoted **A**zide - **A**lkyne **C**lick Chemistry (**SPAAC**) reaction[3]. SPAAC reactions rely on the use of strained cyclooctynes that possess a remarkably decreased activation energy in contrast to terminal Alkynes and thus do not require an exogenous catalyst[16].

A number of structurally varied cyclooctyne derivatives (e.g. DIFO, BCN, DIBAC, DIBO, ADIBO, OCT, DIMAC, MOFO, PYRROC, Sondheimer, TMDIBO, COMBO, BARAC) have been developed that strongly differ in terms of reaction kinetics and hydrophility[17] Our SPAAC conjugation chemistry is based on the reaction of **A**za**d**i**b**enzyl**c**yclo**o**ctyne (**ADIBO = DBCO = DIBAC**) (Fig. 3).

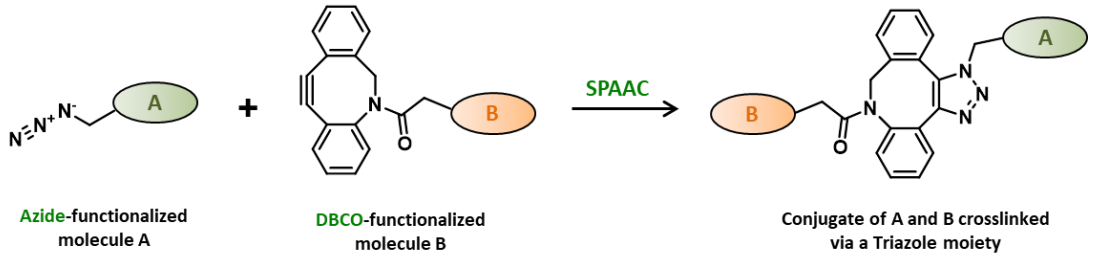

Figure 3: Principle of Strain-Promoted Azide-Alkyne Click Chemistry (SPAAC). DBCO = ADIBO = DIBAC

Azadibenzocyclooctyne (ADIBO=DBCO)-based reagents combine high reactivity with sufficient hydrophility[18,19] and thus allow low background labeling of Azide-functionalized molecules[20] with even greater efficiency than CuAAC reactions. Azide-DBCO reactions are furthermore highly selective and therefore ideally suited for dual labeling approaches with Tetrazine - *trans*-Cyclooctene Ligation (see 3.)[21].

Features:

- Faster detection of small-sized Azides compared to CuAAC reactions (see 2.)
- Copper free and thus non-toxic
- No catalyst or accessory reagents and thus no extensive optimization of assay conditions required
- Suitable for dual-labeling approaches in combination with Tetrazine - *trans*-Cyclooctene Ligation

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3. Tetrazine-Alkene Ligation

The Tetrazine - Alkene Ligation constitutes a non-toxic biomolecule labeling method of unparalleled speed that is ideally suited for *in vivo* cell labeling and low concentration applications. A Tetrazine - functionalized molecule A reacts with a terminal or strained Alkene - functionalized molecule B thereby forming a stable conjugate A-B via a Dihydropyrazine moiety (Fig. 4).

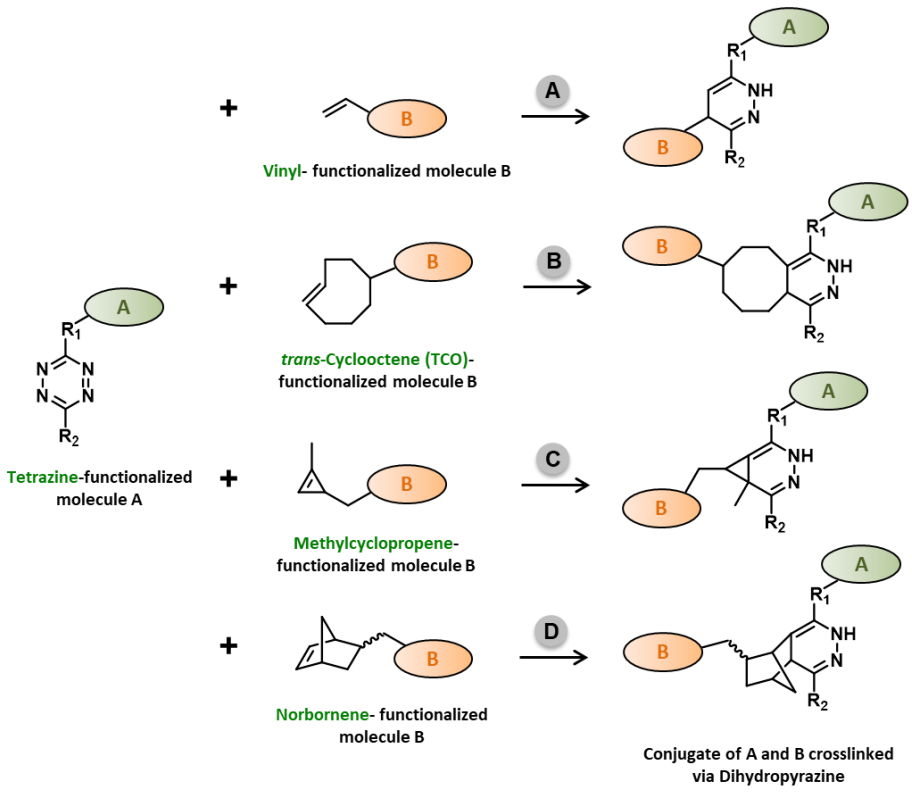

Figure 4: Principle of Tetrazine-Alkene Ligation. A) Tetrazine - Vinyl Ligation. B) Tetrazine - *trans*-Cyclooctene (TCO) Ligation. C) Tetrazine – Methylcyclopropene Ligation. D) Tetrazine-Norbornene ligation. R_1= Phenyl, R_2= H or CH_3

A number of structurally varied alkene and tetrazine derivatives have been developed that strongly differ in terms of reaction kinetics and stability. TCO has been selected (as strained alkene) since it possesses the highest reactivity towards tetrazine[22,23].

Methylcyclopropene (strained alkene)[24-28] and Vinyl[29] (terminal alkene) possess excellent substrate properties for enzymatic applications due to their small size. Recent advances in biorthogonal chemistry demonstrate that strained alkenes, including norbornenes, react rapidly and specifically with tetrazines in inverse electron Diels-Alders cycloaddition reactions to form stable dihydropyrazine products. The reaction is high yielding, selective, and fast in aqueous media[30,31].

The reactivity of the tetrazine derivatives towards TCO is determined by the substituents in the 3 position (Fig. 4, R_1) and 6 position (Fig. 4, R_2). Two Tetrazine versions with different reactivities and stability characteristics have been selected that meet specific application requirements. Tetrazine (R_1=phenyl, R_2=H) reagents are the ideal choice if a rapid reaction kinetic is the key aspect, whereas 6-Methyl-Tetrazine (R_1=phenyl, R_2=CH_3) reagents are ideally suited if an improved chemical stability is required [22].

Features:

- High-speed CLICK reaction that is ideally suited for *in vivo* cell labeling & low concentration applications
- Copper free and thus non-toxic
- No catalyst or accessory reagents and thus no extensive optimization of assay conditions required
- Suitable for dual-labeling approaches in combination with the strain-promoted Azide - DBCO reaction[21]

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Overview of available CLICK Reagents

Overview of available CLICK Reagents	Azides	Alkyne	Dibenzo-cyclooct.	Tetrazine	Trans-cyclooct.	Vinyl	Methyl-cycloprop.	Norbornene
Fluorescent Dyes	✓	✓	✓	✓				
(Desthio)Biotin	✓	✓	✓	✓				
FLAG Tag	✓	✓	✓					
Bifunctional Reagents	✓	✓	✓	✓	✓			
Trifunctional Reagents	✓							
PEGylation Reagents			✓					
Nucleotides	✓	✓	✓		✓	✓		
Nucleosides	✓	✓	✓			✓		
Nucleobases		✓						
Amino Acids	✓	✓						
Monosacharides	✓						✓	✓
Agarose & Magnetic Beads	✓	✓	✓	✓	✓			

You did not find the CLICK Reagent that you are looking for? Please contact us at click@jenabioscience.com

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