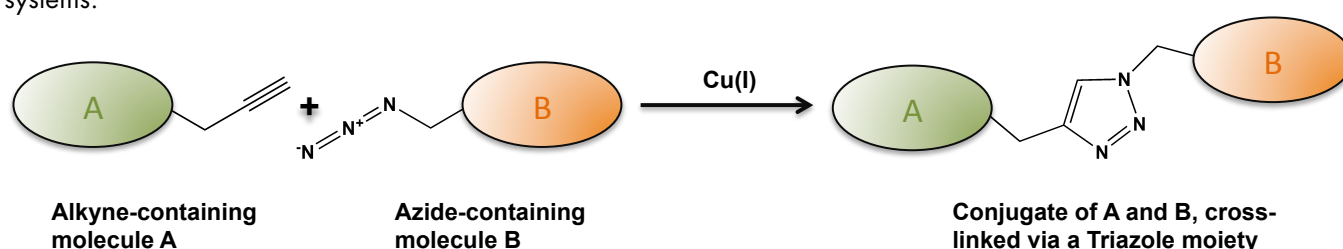


Copper(I) Catalyzed Click Reactions

Background Information and Protocols

Introduction

The Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) is the most prominent example of a group of reactions named click reactions. According to Sharpless' definition, these reactions are characterized by high yields, mild reaction conditions, and by their tolerance of a broad range of functional groups^[1]. Typically, the reactions require simple or no workup or purification of the product. The most important characteristic of the CuAAC reaction is its unique bioorthogonality, as neither azide nor terminal alkyne functional groups are generally present in natural systems.



The use of this method for DNA modification has been somewhat delayed by the fact that copper ions damage DNA, typically yielding strand breaks^[2]. As these problems have now been overcome by the use of copper(I)-stabilizing ligands (e.g. tris(benzyltriazolylmethyl)amine, TBTA^[3]), Carell *et al.* and Seela *et al.* discovered that the CuAAC reaction can be used to functionalize alkyne-modified DNA nucleobases with extremely high efficiency^[4,5].

Click Chemistry represents an easy to use technology and has enhanced research in fields such as:

- **DNA and RNA labeling** – incorporation of alkyne-phosphoramidites in oligos and labeling with marker azides (single- or multi-labeling)
- **PCR assays, PCR primers and labeling of large fragments** – use of alkyne-triphosphates in the nucleotide mixture and labeling of the resulting PCR-fragments with marker azides, or use of oligonucleotides as multi-labeled primers for *pre-* or *post-*synthetic modification
- **FISH probes and FISH experiments** – use of alkyne-modified oligonucleotides and labeling with marker azides (*pre-* or *post-*hybridization)
- **PEGylation** – introduction of PEG-tags via click chemistry with outstanding yields and modularity
- **Flow cytometry, cell feeding, cell tracking and cell-based assays** – feeding of cells with Ethynyl-dU (EdU) and subsequent detection of newly synthesized DNA by labeling with marker azides in Cell Proliferation or TUNEL assays
- **Microarrays** – use of phosphoramidites, triphosphates or oligonucleotides to set up microarrays
- **Nanoparticles, Bioconjugation** and many other applications.

Copper(I) Catalyzed Click Reactions

Background Information and Protocols

		DNA/RNA labelling	PCR-assays	FISH Probes	Micro-arrays	PEG-ylation	Cell-feeding	Nano-particles
Phosphoramidites	C8-Alkyne	•			•			
	Silyl-C8	•			•			
Triphosphates	C8-dUTP		•	•				
	C8-dCTP		•	•				
Free Nucleosides	EdU						•	
Fluorescent Dye Azides		•	•	•	•		•	•
Non-Fluorescent Azides	Quenchers	•	•	•	•			•
	Affinity Markers	•	•	•	•		•	•
PEG- & Linker Azides	PEGs	•	•	•	•	•		•
	Other linkers	•	•	•	•			•
Amino Acid Azides		•	•	•	•			•
Miscellaneous	CuBr/TBTA	•	•	•	•	•		•
	CuSO ₄ /Ascorbate	•	•	•	•	•	•	•
Custom Oligonucleotides	Alkyne-oligos	•	•	•	•	•		•
	Labeled-oligos	•	•	•	•			•

Selected References:

- [1] Tornøe *et al.* (2002) *J. Org. Chem.* **67**:3057.
 Rostovtsev *et al.* (2002) *Angew. Chem.* **114**:2708.
 Rostovtsev *et al.* (2002) *Angew. Chem. Int. Ed.* **41**:2596.
- [2] Burrows *et al.* (1998) *Chem. Rev.* **98**:1109.
- [3] Chan *et al.* (2004) *Org. Lett.* **6**:2853.
- [4] Gierlich *et al.* (2006) *Org. Lett.* **8**:3639.
 Seela *et al.* (2006) *Chem. Biodiversity* **3**:509.
- [5] Gramlich *et al.* (2008) *Angew. Chem.* **120**:3491.
 Gramlich *et al.* (2008) *Angew. Chem. Int. Ed.* **47**:3442.

Copper(I) Catalyzed Click Reactions

Background Information and Protocols

BaseClick™ Kits – Basics and Labeling Strategies

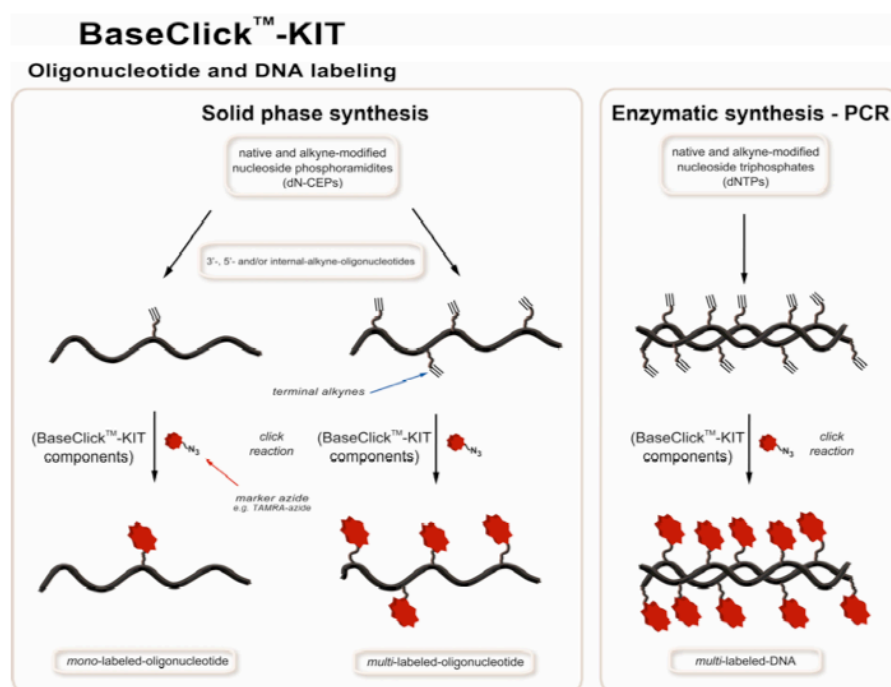
BaseClick™-Kits contain all necessary reagents to label your alkyne-modified oligonucleotides and alkyne-modified PCR-products with marker azides of your choice via Cu-catalyzed azide-alkyne cycloaddition reaction.

Alkyne-modified oligos and alkyne-modified PCR-products can be prepared by solid phase synthesis (use our Alkyne-containing Phosphoramidites) or enzymatic synthesis (PCR; use our Alkyne-containing Nucleotides). Additionally, alkyne-modified oligonucleotides can also be purchased from Jena Bioscience, please see our Primers and Oligonucleotides catalog section for more information.

The complete selection of our Azide-containing Reagents can also be found at our website.

Advantages of Click Chemistry labeling

- Labeling by simple, efficient reactions
- High labeling yield (nearly quantitative)
- Allows introduction of sensitive labels (mild conditions)
- Highly modular system (combinatorial approach)
- Orthogonal to conventional methods
- Bioorthogonal reaction (no azides or alkynes in natural systems)
- Labeling of all bases possible (G, A, C, T, U)
- Broad range of marker- and dye-azides available
- Also adaptable to linking beads, microarrays, nanoparticles, peptides etc.



Labeling of Oligonucleotides

Single labeling:

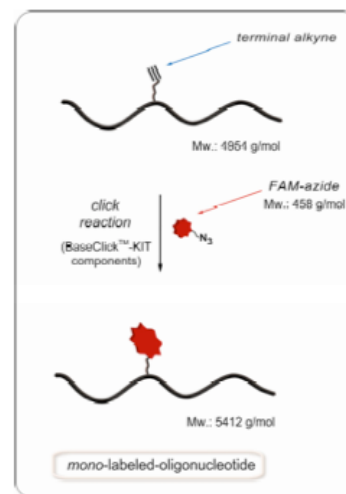
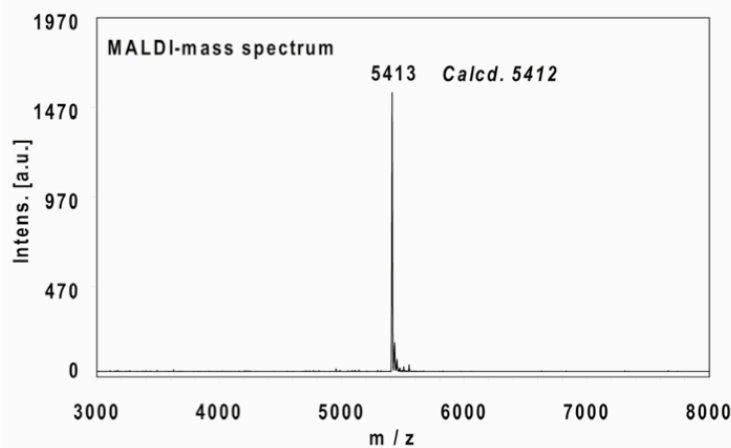
Purified oligonucleotides bearing a single alkyne moiety are usually modified with 2-5 equivalents of the corresponding marker-azide (e.g. fluorescent-dye azides). After the addition of precomplexed Cu(I), complete conversion to the labeled oligo is observed in a time span between 30 min and 4 hours. After a simple precipitation step, labeled oligonucleotides can be recovered in near quantitative yields. Below an

Copper(I) Catalyzed Click Reactions

Background Information and Protocols

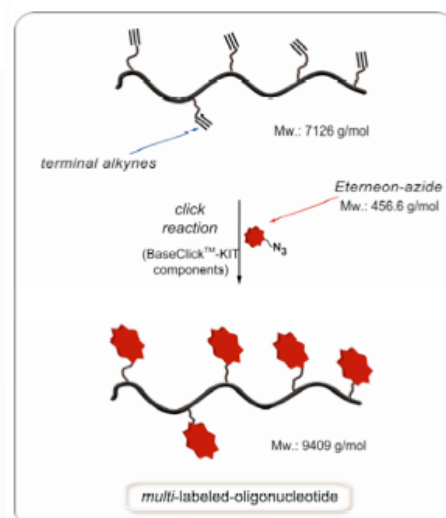
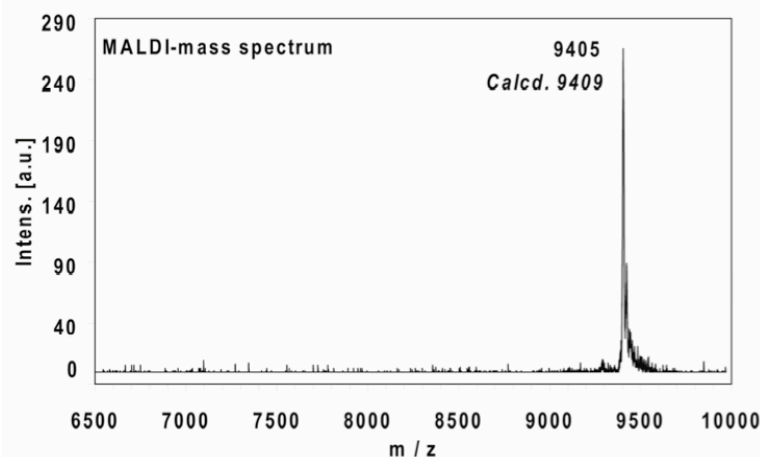
example of MALDI-mass spectrum measured directly after the click reaction and the precipitation step, without further purifications.

16mer (Molecular Weight (Mw) = 4954 g/mol), internal alkyne reacted with 2 equivalents Fluorescein-Azide (FAM-Azide, Mw = 458 g/mol), 3 h at 37 °C. Ethanol precipitation with 99% recovery of the labeled oligo. MALDI-mass analysis of the crude product → 100% oligo-dye conjugate (Calcd. 5412; Found 5413).



Multiple labeling:

22mer (Mw. = 7126 g/mol), five internal alkynes reacted with 5 equivalents Eterneon™-(350/430)-Azide (Mw. = 456.6 g/mol), 4 h at 37 °C. Ethanol precipitation with 85% recovery of the labeled oligo. MALDI-mass analysis of the crude product → 100% oligo-dye conjugate (Calcd. 9409; Found 9405).



Copper(I) Catalyzed Click Reactions

Background Information and Protocols

Example Protocols

This section contains some published examples of click reactions. These protocols may be used as a starting point for optimization of your particular click chemistry procedures.

Preparation of the "Click Solution"

- The "click solution" (0.1 M CuBr / 0.1 M TBTA 1:2 in DMSO/*t*BuOH 3:1) must always be freshly prepared prior to use!
- Dissolve 1 mg CuBr in 70 μ l DMSO/*t*BuOH 3:1 to obtain a 0.1 M solution. This solution must be freshly prepared and cannot be stored.
- Dissolve 54 mg TBTA in 1 ml DMSO/*t*BuOH 3:1 for a 0.1 M solution. This solution can be stored at -20 °C.
- Add 1 volume of the 0.1 M CuBr solution quickly to 2 volumes of the 0.1 M TBTA solution to obtain "click solution", ready to use.

Click Procedure for Short DNA Oligos

Procedure using CuBr: To 5 μ l of a 2 mM DNA solution (10 nmol) in water, 2 μ l of an azide solution (50 mM, 50 nmol, 5 eq. in DMSO or in 3:1 DMSO/*t*BuOH), 3 μ l of a freshly prepared solution containing 0.1 M CuBr and 0.1 M TBTA ligand in a 1:2 ratio in 3:1 DMSO/*t*BuOH is added. The mixture is thoroughly mixed and shaken at 25 °C for 3 h. The reaction is subsequently diluted with 0.3 M NaOAc (100 μ l) and the DNA is precipitated using 1 ml cold EtOH. The supernatant is then removed and the residue is washed twice with 1 ml cold EtOH. The washed residue is re-dissolved in pure water (20 μ l) and can be used without further purification.

Considerations for the CuBr method:

- The labelling reaction works more efficiently with concentrated solutions of alkynes (oligo) and azides (label).
- The best way to carry out the click reaction is to mix the oligo and the azide-label in a minimal amount of solvent.
- Alkyne / Azide ratio: from 1:2 to 1:10 for high-density labelling reactions (e.g. 10 alkynes in a row).
- The click reaction is normally accelerated by elevated temperature and can be ready in less than 30 min when the reaction temperature is around 40 - 45 °C.
- The reaction time depends on: a) concentration of azide and oligo in the solution; b) reaction temperature; c) stirring and/or mixing of the solution.
- The work-up of the reaction is normally carried out by precipitation of the labeled oligo (addition of a salt solution, e.g. 0.3 M NaOAc followed by addition of cold abs EtOH).

Click Procedure using alternative Cu(I) Sources

Procedure using TCEP: To 25 μ l of a 0.5 mM DNA solution (12.5 nmol) in water, 6.25 μ l of an azide solution (0.1 N, 625 nmol) and 10 μ l of a solution containing Cu(II)-salt (CuSO₄) and TBTA ligand in a 1:2 ratio in 4:3:1 water/DMSO/*t*BuOH is added (0.05 N, 250 nmol). The mixture is vortexed and 5 μ l of a freshly prepared tris-(2-carboxyethyl)-phosphine (TCEP) solution in water is added (0.1 N, 500 nmol). The solution is shaken at 15 °C over night and subsequently diluted with water (200 μ l) and used for gel electrophoresis without further purification. Instead of TCEP, also sodium-ascorbate can be used.

Copper(I) Catalyzed Click Reactions

Background Information and Protocols

Click Procedure for a 300 bp PCR Product

To 10 μ l DNA solution (1-4 pmol DNA, 10 mM Tris), 10 μ l fluorescein azide solution (5 mM, diluted with 10 mM Tris with 5 % t -BuOH from a stock of 0.1 N in DMSO) and 10 μ l pre-complexed Cu(I) was added (10 mM; 1 mg CuBr (99.99%) dissolved in 700 μ l of 10 mM TBTA ligand in t -BuOH/DMSO 1:3) The sample is shaken at 37 °C for 2 h. Then formamide buffer is added and the samples are analyzed using a 5 % PAGE gel. Control experiments show that the reaction is completed in less than 30 min.