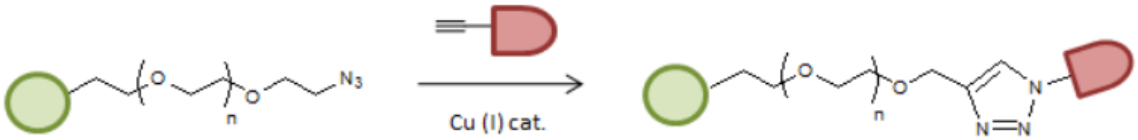
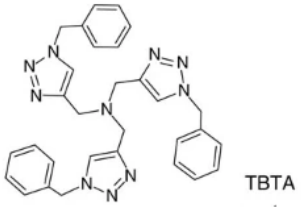
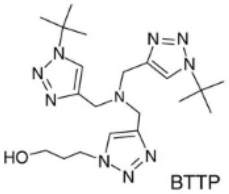
Azide-Alkyne Click Chemistry

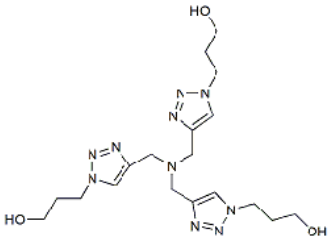
Introduction

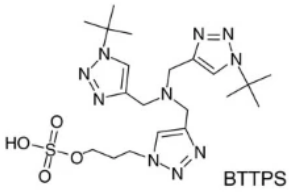
Classic Click Chemistry uses copper, Cu(I), to catalyze the 1,3-dipolar cycloaddition of an alkyne with an azide to form a 1,2,3-triazole.[1,2] Sources of Cu(I) include copper(I) iodide, copper(I) bromide, copper turnings, or copper (II) sulfate ($CuSO_4$) and a reducing agent.[1] However, the thermodynamic instability of Cu(I), which readily oxidizes to inactive Cu (II) usually requires the copper catalyst to be prepared with an appropriate chelating ligand.




TBTA


BTTP


THPTA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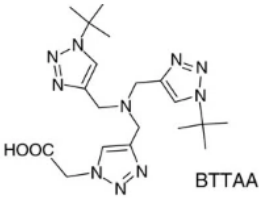
BTTAA

An improvement in Click Chemistry uses the in-situ preparation of Cu(I) from the reduction of $CuSO_4$ with sodium ascorbate and a Cu(I) stabilizing ligand, tris-(benzyltriazolylmethyl)amine (TBTA). This leads to a more reliable click reaction by avoiding the oxidation of catalytic Cu(I) by dissolved oxygen. In a typical reaction, copper sulfate is pre-complexed with TBTA. This complexed catalyst is mixed with an alkyne and an azide, followed by the addition of sodium ascorbate to initiate the click reaction.

TBTA covers some of the practical applications for Click Chemistry except for aqueous conjugation reactions. The water-soluble tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) click ligand further simplifies Click Chemistry by allowing the entire reaction to be run in water, affording biological compatibility for Click reactions.[4] The THPTA ligand binds Cu(I), blocking the bioavailability of Cu(I) and ameliorating the potential toxic effects while maintaining the catalytic effectiveness in click conjugations. The THPTA ligand was effectively used to label live cells with high efficiency while maintaining cell viability.[5]

In our hands, we have found THPTA to be a highly efficient ligand for click chemistry, in partially in completely aqueous reactions. Labeling is complete in as little as 15-30 minutes at room temperature. The ligand $CuSO_4$ complex exhibits no loss of activity when frozen for at least a month.

Example protocols

Labeling of oligonucleotides and DNA

1. Prepare the following stock solutions
   - 200 mM THPTA ligand in water
   - 100 mM $CuSO_4$ in water
   - alkyne labeled oligo in water
   - 100 mM sodium ascorbate in water
   - 10 mM azide in DMSO/t-BuOH or water

2. Incubate $CuSO_4$ with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.

3. To the oligo/DNA solution, add an excess of azide (4-50 eq).

4. Add 25 equivalents of THPTA/$CuSO_4$.

5. Add 40 equivalents of sodium ascorbate.

6. Let the reaction stand at room temperature for 30-60 minutes.

7. Ethanol-precipitate the oligo or purify.

Labeling of cell lysate

1. Prepare the following click solutions:
   - 100 mM THPTA ligand in aqueous buffer or water
   - 20 mM $CuSO_4$ in water
   - 300 mM sodium ascorbate in water
   - 2.5 mM alkyne or azide labeling reagent in water or DMSO

2. For each azide or alkyne- modified protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
   - 50 µL protein lysate (1-5 mg/mL) in protein extraction buffer
   - 90 µL PBS buffer
   - 20 µL of 2.5 mM corresponding azide (or alkyne) detection reagent in DMSO or water

3. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.

4. Add 10 µL of 20 mM $CuSO_4$ solution, vortex briefly to mix.

5. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.

6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.

7. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Labeling live cells by copper-catalyzed alkyne-azide click chemistry

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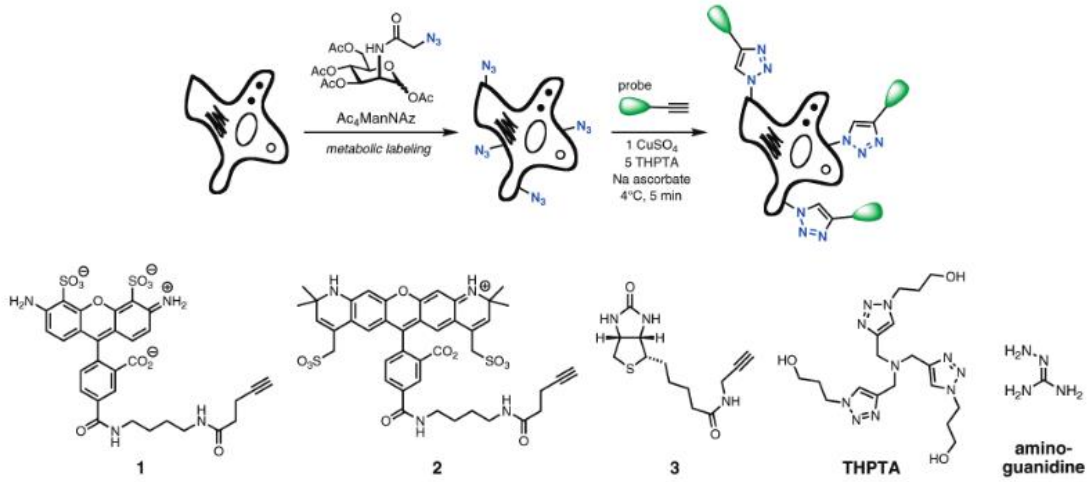

Figure 1. (Top) Cell labeling steps. (Bottom) Alkynyl probe reagents and catalyst additives.

Cell-surface labeling of azido glycans on HeLa and CHO cells and imaging by confocal microscopy

Cells were seeded at 1 × 10^5 cells/mL on glass-bottom Petri dishes (35 mm) and grown overnight at 37 °C and 5% CO_2 in growth medium (MEM medium containing 10% fetal calf serum, 1% glutamine, and 1% penstrep) with or without 50 µM Ac4ManNAz for 2 days. The medium was gently aspirated, and the cells were washed two times with 1 mL of DPBS. In an eppendorf tube, $CuSO_4$ and THPTA in a 1:5 molar ratio was added to DPBS at 4 °C containing dye-alkynes 1 or 2 (final conc. 25 µM) and aminoguanidine (final conc. 1 mM). A freshly prepared stock solution of sodium ascorbate (100 mM) was added to establish a final ascorbate concentration of 2.5 mM. This reaction mixture was incubated on ice for 10 min at 4 °C before adding to the cells. After incubation at 4 °C for 1or 5 min, the cells were washed and fixed with a mixture of 3% paraformaldehyde, 0.3% glutaraldehyde, and 1 mM MgCl2 in DBPS for 10 min at room temperature. Cell nuclei were stained by adding 4′,6-diamidino-2-phenylindole (DAPI). Between each step, the slides were rinsed three times with DPBS. Slides were mounted using Vecta Shield mounting medium (Vector Laboratories, Burlingame, CA). Sections were imaged using a Bio-Rad 2100 confocal microscope with a 60× oil objective. Data were analyzed and images were created using ImageJ (http://rsbweb.nih.gov/ij/). For dual labeling studies, the cells were washed twice with 1 mL of growth medium after the labeling reaction and returned to medium containing 50 µM Ac4ManNAz for another 20 h. Optimized conditions for cell-surface labeling were 25 µM alkyne-488, CuSO4 (50 µM), THPTA (250 µM), aminoguanidine (1 mM), and sodium ascorbate (2.5 mM) for 1 to 5 min in medium at 4 °C.

References

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