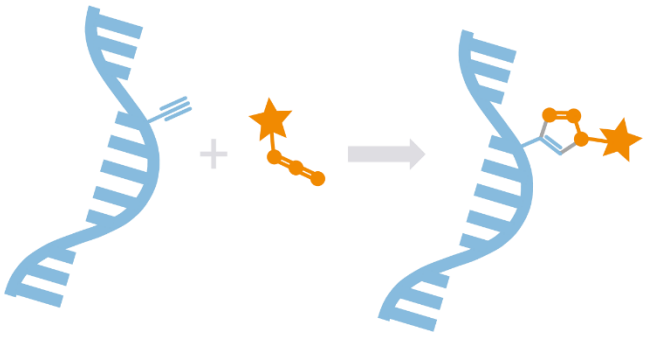
$CuSO_4$-based click reactions



This protocol was tested on DNA oligonucleotides, bearing a clickable alkyne. Aiming for bioconjugation with fluorescent labels and other small molecules in aqueous solution, $CuSO_4$ was used as catalytic reagent to promote the reaction. As click reactions require a Cu(I) source, a reducing agent is needed. We are therefore glad to share with you our preferred protocol. Eventually the reaction mixture is diluted and thus reaction slowed down (reaction partners have to meet each other), so often other catalysts are preferred for highly diluted reactions. Please be aware, that this protocol is only meant as a starting point. For other amounts and reaction partners used, please consider to vary the conditions in order to obtain the optimal reaction outcome.

You will need following reagents and equipment:

- A premixed catalyst solution of 50 mM $CuSO_4$ (BCMI-004) and 250 mM solution of THPTA (BCMI-006) in HPLC grade water. This is important as THPTA is ensuring that no harmful oxidative species could be generated out of Cu.
- 500 mM L-Ascorbic acid sodium salt (BCMI-005) in HPLC grade water to reduce the catalyst to be reactive
- 200 mM solution in DMSO of you preferred label-azide
- 100 mM solution in DMSO of your alkyne-modified oligonucleotide
- Microcentrifuge tubes as reaction tubes (e.g. 1.5 mL vials)
- Table centrifuge
- Thermomixer (also a water bath can be used)
- Purification method (e.g. ethanol precipitation, purification kits, HPLC…)
- Analytical HPLC system (optional to check the reaction outcome)

Click reaction procedure:

1. Mix 3 µL of 100 mM alkyne-modified oligonucleotide and 3 µL of 200 mM solution of label-azide in a 0.2 mL microcentrifuge tube.
2. Next, add 3 µL of the premixed catalyst solution (see above) and 3 µL of 500 mM L-Ascorbic acid sodium solution.
   Attention: upon addition of L-Ascorbic acid sodium salt, the dark blue color of the added premixed solution becomes colorless, corresponding to the reduction of the Cu(II)-THPTA species to the active catalyst Cu(I)-THPTA.

3. Reach a final volume of 25 µL by adding a solvent system which ensures dissolution of all species (e.g. H_2O or different ratios of H_2O/^{t}BuOH or H_2O/DMSO).
4. Let this mixture react for at least 2 hours at 45 °C and 650 rpm in a thermomixer.
5. Purify your dye-labeled oligonucleotide with your method of choice and elute / dissolve then finally your oligo in HPLC grade water for further analyses and usage.
6. Check the quality of your probe e.g. by HPLC measurement.