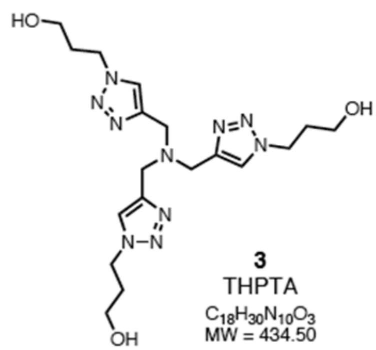
Protocol for CuAAC Coupling of "PEG-N_3" to "Biomolecule-CCH"

From: Hong, V., Presolski, Stanislav I., Ma, C. and Finn, M. G. (2009), Analysis and Optimization of Copper-Catalyzed Azide–Alkyne Cycloaddition for Bioconjugation. Angew. Chem. Int. Ed., 48: 9879–9883. doi: 10.1002/anie.200905087

Note: THPTA Ligand is available from Aldrich (762342)

Stock solutions:
$CuSO_4$: 20 mM (in water)
THPTA Ligand **3**: 50 mM (in water)
Sodium ascorbate: 100 mM (make fresh before using by adding 1 mL of water to 20 mg).
PEG-azide: 10-60mg/mL
Biomolecule-alkyne: 10-20 mg/mL
Buffer: Water or 100 mM phosphate pH 7



3
THPTA
$C_{18}H_{30}N_{10}O_3$
MW = 434.50

Final concentrations:
$CuSO_4$: 0.10 mM (Note: can be adjusted as desired between 50 and 250 µM)
THPTA Ligand **3**: 0.50 mM (*ligand to copper ratio is 5:1*)
Sodium ascorbate: 5 mM
PEG-azide: approx. 2-fold excess with respect to alkyne groups on the biomolecule, down to 20 µM (in other words, if the alkyne concentration is very low, more than two equivalents of azide are needed for fast reaction)
Biomolecule-alkyne: successfully done with 2 µM and higher

Procedure for 0.5 mL reactions: (this example: 200 µM alkyne and 400 µM azide)
In a 2 mL eppendorf tube, *add the reagents in the following order*:
1. 25 µL of Biomolecule-alkyne
2. 407.5 µL of water or 100 mM phosphate buffer pH 7
3. 10 µL of PEG-azide
4. 7.5 µL of premixed $CuSO_4$ and **3** (2.5 µL of $CuSO_4$ and 5.0 µL of **3**).
5. 25 µL of sodium ascorbate.
6. Mix well, close the tube (to prevent more oxygen from diffusing in), and let the reaction go for at least one hour.
7. Workup depends on your application. Copper ions can be removed by washing (dialysis) with EDTA. Copper-adsorbing resins tend to also bind biomolecules. We routinely do no workup, but instead purify the conjugates in such a way as to leave small molecules behind.