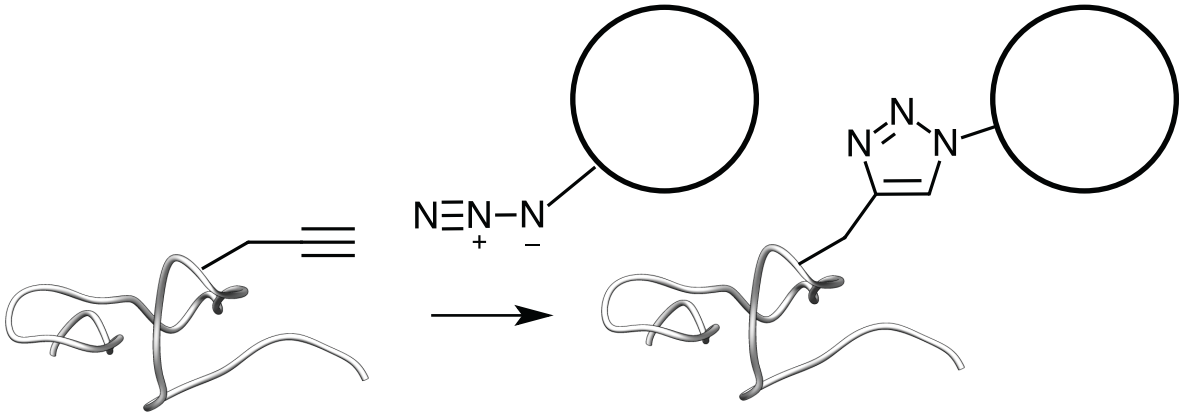
Copper-click Alkyne / Azide Condensation Reaction: peptide to tentagel beads



Materials
alkyne: Guangxitoxin peptide, amphipathic, 36aa, 3 disulfide bonds, with propargylglycine residue inserted at various locations
azide: azide functionalized tentagel (0.32 umol/mg) or ~~AM sure (0.7 umol/mg)~~ beads from Kit Lam

*Final Concentrations (*adapted from Besanceney-Weber 2011 Angew Chem 50: 8051-8056)
CuSO4: 100 µM
Ligand: 600 uM BTTAA, 6:1 ligand:Cu+ ratio is best
Sodium Ascorbate: 5 mM
Azido fluor: 1.5 mM
Propargyl peptide: 100 µM
Buffer: 0.1 M sodium phosphate, pH 7.0
DMSO: 15%, DMSO has a subtle effect on the chelating effect of the ligand).

Stock Solutions:
Propargyl GxTX: 2.0 mM (8 mg/ml) in water
Alkyne fluor: 10 mM in DMSO
CuSO4: 10 mM in water
Ligand: BTTAA 20 mM in water
Sodium Ascorbate: 50 mM in water, prepare fresh
Buffer: 0.5 M sodium phosphate, pH 7.0

prepare beads fresh for each reaction.
weigh out ~ 10 mg beads per tube (not impotant ot be accurate, just record how much)
add 800ul DMF (dimethylformamide) rotate beads for at least 1 hour
transfer to bio-rad microbio spin column on vacuum flask
start washing: wash = add 800 ul wait at least 1 second, vacuum away
wash 3X 50% DMF 50% water, 3X water, 3X 0.5 M sodium phosphate, pH 7.0
leave soaking in 0.5 M sodium phosphate, pH 7.0
then immediately before use drain solution and
resuspend with # of tubes * 125 ul 0.5 M sodium phosphate, pH 7.0 (500 ul for this protocol)

Materials
for 1 reaction:
????

tube 1: tentagel GxTX Pra13
tube 2: tentagel GxTX Pra17
tube 3: tentagel alkyne545

Procedure for 200 µL reaction:
Add reagents to polypropylene tube in following order, vortex 5 seconds after each step

I. 122 µL of beads in 0.5 M phosphate buffer, pH 7.0
use p1000 tips

II. 20 µl of 2 mM Ser13Pra (propargyl peptide, 80 nmol) (tube 1), 20ul of 200uM of Ser13Pra (tube 2) or 20ul water (tube 3)

III. 8 µl Cu:BTTAA 6:1 premix ([Cu] = 2.5 mM, [BTTAA] = 15 mM)

a. 24 ul total volume: 18uL of BTTAA(20mM stock), 6ul of Copper (10mM stock)

IV. 30 µl of DMSO (tubes 1&2) or 10 mM fluorophore in DMSO (tube 3)

V. vortex 5 sec

VI. take 20ul "pre-ascorbate" aliquot for analytical HPLC, freeze @ -80

VII. 20 µl of 50 mM sodium ascorbate, vortex 5 sec, slowly rotate in dark for 2 hour at room temperature.
ascorbate starts the reaction reduces Cu2+ to Cu+ which is active form

VIII. spin at <1000g for >5 sec to pellet beads

IX. remove supernatant "2-hour" for analytical HPLC, freeze @ -80

X. rinse beads: rinse = add 1ml, spin and remove supernatant.
1X water, 1X 50% DMF, 1X DMF, rotate 5 minutes, 1X 50% DMF, 3X water

XI. store in 70% EtOH in refrigerator in black box for a maxiumum period of 2-3days.