*Notes:*
Keep peptide solutions as cold as possible. Freeze at -80C ASAP. Whenever possible, purge oxygen from solutions, centrifugation at max speed for a minute removes dissolved gas. To minimize O2 blowing off with N2 or Argon is a good thing.
Keep maleimide solutions as dry as possible. Do not open tubes when cold, to avoid condensation. Aliquot and freeze in prechilled dessicant at -20C. To thaw remove tube from frozen dessicant and place in room temp dessicant in the dark.
Keep fluorophore solutions in the dark whenever practical, dim lights when fluorophore is exposed
Spin down peptide solution, note any precipitate; record A280.

For each 50 ul reaction you will need:
2 clear 1.5 ml tubes

50 nmoles fluorophore, 5 ul of a 10 mM stock
-make for dylight 550, and Dylight 680. (see reagent calculations sheet)
-dissolve in dry DMSO
6 nmoles (80 ug) GxTX peptide with spinster cysteine,
-20 ul of a 300 uM solution in 50% ACN + 1 mM EDTA pH 5 on ice.
25 ul 200 mM Tris, 20 mM EDTA pH 6.8

for
50 ul 20% ACN, 0.1% TFA

ice bucket
a dark place
1.5 ml centrifuge, preferably chilled to 4°C
spectrophotometer, nanodrop is fine

*Setup Controls as follows:*
Tube 1: GxTx + Dylight550
Tube 2: GxTx + Dylight680
Tube 3: no GxTX + Dylight550 (0.1X dylight, 20 ul 50% ACN + 1 mM EDTA instead of GxTX)
Tube 4: no GxTX + Dylight680 (0.1X dylight, 20 ul 50% ACN + 1 mM EDTA instead of GxTX)

*Protocol:*
Place 20ul of dissolved peptide in 1.5ul tube
Add 25 ul of 200 mM Tris, 20 mM EDTA pH 6.8
Add 5 ul of (10 mM solution of maleimide fluorophore in DMSO), pipet slowly until well mixed, avoid mixing air in.
Centrifuge 1 min max speed in centrifuge, note any precipitate.
React overnight at 4C.
Dilute with 50 uL 20% ACN, 0.1% TFA, mix well, spin-down, remove supernatant, spin again.
Quantify A280 and absorbance of fluorophore
Inject ~99 ul supernatant onto HPLC (save a tiny drop for mass spec, followed by 150uL of 20% ACN, and .1% TFA.
run HPLC protocol collect 1 ml fractions from tubes 1 and 2, take 5 ul sample of interesting fractions for mass spec and freeze remainder at -80C.
HPLC protocol:
time 1: 20% ACN
time 41: 40% ACN
time 43: 95% ACN
time 45: 95% ACN
(time 47: 20% ACN optional step if preparing for next run)
Quantify A280 and absorbance of fluorophore, record peptide concentration in the appropriate solvent B percentage and calculate degree of labeling.

jon 2/6/12 3:56 PM
**Comment [1]:** for next peptide stock, make more concentrated- try for 2 mM