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 100 uM reaction you will need:
100 nmoles fluorophore, 10 ul of a 10 mM stock
-make for fluorescein maleimide (Fmal) and tetrametyl rhodamine maleimide (TMRmal)
-dissolve in dry DMSO
20 nmoles (80 ug) GxTX peptide with spinster cysteine,
-20 ul of a 100 uM solution in 50% ACN + 1 mM EDTA pH 5 on ice.
20 ul 200 mM Tris, 20 mM EDTA pH 6.8
50 ul 20% ACN, 0.1% TFA
2 clear 1.5 ml tubes

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 + Fmal
Tube 2: GxTx + TMRmal
Tube 3: no GxTX + Fmal (20 ul 50% ACN + 1 mM EDTA instead of GxTX)
Tube 4: no GxTX + TMRmal (20 ul 50% ACN + 1 mM EDTA instead of GxTX)

Protocol:
Place 20ul of dissolved peptide in 1.5ul tube
Add 20 ul of 200 mM Tris, 20 mM EDTA pH 6.8
Add 10 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 0: 20% ACN
time 1: 20% ACN
time 2: 30% ACN
time 22: 35% ACN
time 24: 95% ACN
time 25: 95% ACN
time 26: 20% ACN
time 30: 20% ACN, end, ready for next run
Quantify A280 and absorbance of fluorophore, record peptide concentration and degree of labeling.