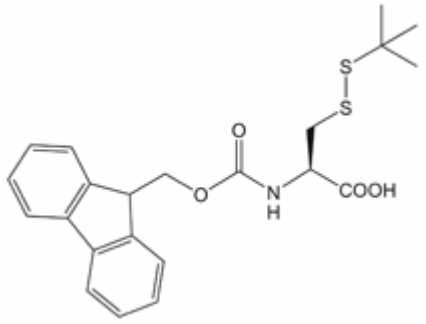
This protocol will enable labeling of a PNA chain on-resin with a fluorophore using a free sulfhydryl. The resulting linkage will produce a thioether bond (iodoacetamide or maleimide).

General Considerations

- All solvents should be kept cold (in fridge or freezer) when not in use
- Be wary of photo and thermal bleaching of fluorophore – never expose it to heat, and minimize exposure to light
- The fluorophore may contain either iodoacetamide or maleimide handle – this reaction scheme works well for both

Step 1: PNA Synthesis

- Create desired PNA sequence using SPPS – include one protected cysteine monomer (N-α-Fmoc-S-t.-butylthio-L-cysteine, No. 04-12-1041, Novabiochem)



Cys(tbuthio)-OH
MW: 431.6

- Cap with aliphatic tail, resuspend resin in DCM
- Using plastic transfer pipette, carefully collect all beads that will not be labeled. Place unused beads in microcentrifuge tube, speed vac to dryness, store dry at -80ºC indefinitely

Step 2: Deprotection

- Change solvent from DCM to DMF in the resin-filled reactor vessel. Note the approximate weight of resin to be labeled
- Dry the resin
- Place 1-2 ml of 0.1 M dithiothreitol (DTT) dissolved in 2:1 v:v DMF/0.2 M NH_4CO_3. The solution will have a fishy smell (from the DTT)
- Incubate, while rocking, for 120 minutes
- Upon completion, capture the filtrate, save for HPLC analysis

** The filtrate should smell strongly of thiol, which should mask the fishy DTT smell. If this is not the case, the deprotection did not work**

- Wash resin with DMF, DCM, DMF, DCM
- Resuspend resin in DCM

- Using transfer pipette, carefully transfer all of the remaining beads to a microcentrifuge tube. Speed vac to dryness

Step 3: Fluorophore Labeling

- Reconstitute dried fluorophore in DMF containing at least 3 eq. DIPEA, keeping total solvent volume to a minimum (use "Fluorophore labeling" worksheet to determine amount of fluorophore needed for reaction)

** It is important to minimize volume used to keep the [fluorophore] as high as possible. [Fluorophore] should be several mM, and should be present at 1-5x excess compared to theoretical resin loading (for complete coupling)**

- Introduce fluor/DMF solution into microcentrifuge tube containing dry beads
- Incubate for 120-180 minutes while vigorously mixing using the vortex.

Shield reaction mixture from all light using copious amounts of aluminum foil

Step 4: Fluor-PNAA cleavage

- Transfer reaction mix to clean, foil-shielded reactor vessel
- Capture reaction eluent, save for HPLC analysis

** Do not reuse unreacted fluorophore. I have had bad results trying this!**

- Wash beads several times with DMF, DCM

At this point, the beads should be a bright color (from fluor linkage), and may display some aggregation, based on which solvent was used to wash. This is normal.

- Cleave PNAA with 4:1 TFA:m-cresol mixture for 120 minutes with no rocking
- Upon completion, capture eluent, perform HPLC purification

Step 5: HPLC Purification

Analytical

- Ensure proper analytical HPLC setup (buffers, column, flow direction, detector flow cell, waste line)
- Ensure proper software setup (gradient, detector settings)
- During cleavage, degas buffers with He, equilibrate column and lines with starting buffer. Prime if necessary
- Using analytical column, introduce 50-75 μl of cleavage cocktail
- Using fraction collector, collect all product peaks in separate microcentrifuge tubes
- Speed vac products to dryness, save for MALDI analysis
- Upon completion, wash and store analytical column in 100% acetonitrile

Preparative

- Switch equipment for preparative HPLC

Ensure that you have ample amount of buffers, as flowrate is 10-20ml/min

- Introduce entire cleavage cocktail (~2 ml) into HPLC, immediately start the fraction collector

It is a good idea to collect the entire run in 30 sec increments to ensure product capture

- Save vials containing products, place liquid in 50 ml centrifuge tubes, freeze, and lyophilize

You now have fluorophore-labeled PNAA!

The PNA-fluorophore labeling technique was adapted from these sources:

1. Liu, Xiaohai and Balasubramanian, Shankar. Strategies for the synthesis of fluorescently labelled PNA. Tetrahedron Letters, 2000. 41: p. 6153-6156
2. Mayfield, Lynn D. and Corey, David R. Enhancing solid phase synthesis by a noncovalent protection strategy-efficient coupling of rhodamine to resin-bound peptide nucleic acids. Bioorganic & Medicinal Chemistry Letters, 1999. 9: p. 1419-1422.