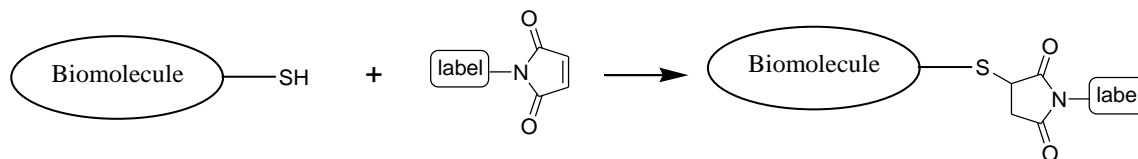


Protocol: Maleimide labeling of proteins and other thiolated biomolecules



The reaction of maleimides with thiols is widely used for bioconjugation and labeling of biomolecules such as proteins and peptides. Maleimides are electrophilic compounds which show high selectivity towards thiols. Maleimides and thiols are encountered in proteins and peptides as cysteine residues. Although natural DNA does not contain thiols, biomolecules with thiol groups can be easily synthesized.

Since thiols are prone to oxidative dimerization with the formation of disulfide bonds. Cysteine residues thus form cystine bridges, which stabilize protein tertiary structures. Disulfides do not react with maleimides. It is necessary to reduce disulfides prior to the conjugation to exclude oxygen from the reaction.

The following protocol is recommended for the conjugation of maleimides with proteins, peptides, and other thiolated biomolecules.

1. Dissolve the protein or other biomolecules containing thiol in degassed buffer (PBS, Tris, HEPES are good buffers) at pH 7-7.5. Buffer can be degassed by applying vacuum for several minutes, or by bubbling through inert gas (nitrogen, argon, or helium). For proteins, concentration between 1-10mg/mL is preferred.
2. Add an excess of TCEP (tris-carboxyethylphosphine) reagent to reduce disulfide bonds, flush with inert gas, and close. 100x molar excess of TCEP is fine. Keep the mixture at room temperature for 20 minutes.
3. Dissolve maleimide in DMSO or fresh DMF (1-10mg in 100uL).
4. Add dye solution to thiol solution (20x fold excess of dye), flush vial with inert gas, and close tightly.
5. Mix thoroughly and keep at room temperature or 4°C overnight.
6. Purify by gel filtration, HPLC, FPLC, or electrophoresis.

For maleimides with poor hydrophilicity, co-solvent (DMF or DMSO) is recommended. Maleimides with good hydrophilicity (such as sulfo-Cy maleimides) can be dissolved in water. If precipitation occurs, add more organic co-solvent to the mixture to achieve better labeling.

Dialysis is recommended as a means of purification only for maleimides with good aqueous solubility.