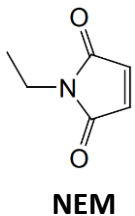


Fig. 1

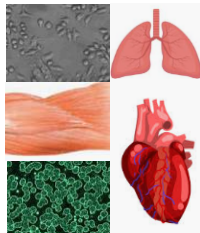
Sample Processing Workflow

Parallel Processing of Samples from Different Conditions

Initial blocking of free thiols with lysis buffer containing NEM

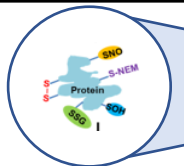


Penetration



! Critical Step
Block Free Thiols

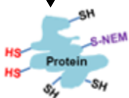
Sample homogenization/cell lysis, protein extraction, precipitation, and resuspension



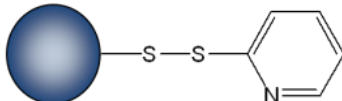
! Critical Step
Mitigate Oxidation
-pH (<7.0)
-temperature

Reduction of oxidative modification of interest (e.g. total oxidation)

DTT Reduction

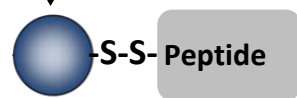


Thiol-affinity enrichment



On-resin tryptic digestion

Trypsin



On-resin multiplex isobaric labeling

TMT



Elute peptides off resin

DTT elute



-Combine
-Alkylation
-SPE cleanup

LC-MS/MS analysis

Protein/peptide level elution



SDS-PAGE, Western Blot

10-Plex TMT Labeling Scheme:

