Chemical crosslinking

A: Reversible method

Reagent:

DSP (dithiobis[succinimidylpropionate], crosslinking reagent)

Stop solution (1M tris)

Step:

1. Dissolve DSP 5mg into 500ul DMSO freshly. (DSP is moisture sensitive. **Do not open DSP container before pre-worm it to room temperate)**
2. Add 1/10 volume of DSP solution into protein sample. ie. 450ul protein sample should add in 50ul DSP solution.
   !! DSP will react with NH group, be sure protein sample do not contain Tris, Glycin
3. Mix well, incubate on ice 2hr reaction or room temperature 30min.
4. Add the stop solution at a final concentration of 20-50mM, incubate 15min.
5. If cleavage of crosslink is required, add DTT at final concentration of 50mM, incubate 37°C 30min.

B: Non-reversible method

Reagent:

BMB, (1,4-bis(maleimido)butane, crosslinking reagent)

Stop solution (1M cysteine)

Step:

1. Dissolve BMB 1mg into 400ul DMSO freshly. (BMB does not dissolve DMSO easily, shake for long time for dissolve)
2. Add 1/10 volume of BMB solution into protein sample. ie. 450ul protein sample should add in 50ul BMB solution. Make sure the protein sample pH value is within 6.5-7.5.
3. Mix well, incubate on ice 2hr reaction or room temperature 1hr. (BMB corsslinking efficiency is lower the DSP, longer time may required)
4. Add the stop solution at a final concentration of 20-50mM, incubate 15min.