What are best practices and the protocol for peptide biotinylation?

For proteins >1000 Da, we recommend using a 1:1 molar coupling ratio (MCR) of biotin reagent to protein for protein biotinylation. This procedure results in controlled biotinylation and yields proteins that can be immobilized onto Streptavidin (SA) biosensors through the formation of a biotin-streptavidin bond (Please see Technical Note 28: Biotinylation of Protein for Immobilization onto Streptavidin Biosensors for more information on protein biotinylation). Since small peptides are difficult to buffer exchange with a desalting column or through dialysis, a 3-5 peptide to 1 biotin MCR should be used to ensure complete incorporation of a single biotin to each peptide. After an hour of biotinylation reaction, the mixture can be used immediately since only the 1:1 biotinylated peptide will be immobilized on an SA Biosensor and the excess free peptide will remain in solution. The free peptide should not bind to the SA Biosensor.

Example protocol for peptide biotinylation (using Thermo 21902BID No-Weigh biotin-PEG2 or Thermo 21329 No-Weight NHS-PO4-Biotin):

1. Prepare a fresh 1 mg/mL peptide solution in PBS, pH 7.4. A volume of 0.5 to 1.0 mL should be plenty.
2. Add 170 µL of PBS to one tube of biotin-PEG2 and mix well. This makes a 20 mM biotin stock solution.
3. Calculate the volume of the biotin stock to add to your peptide to generate a 3-5:1 MCR of peptide:biotin, and add this amount of biotin stock to your peptide solution. If the volume calculated using the 20 mM stock is less than 1 µL, make a 1:10 dilution of the 20 mM biotin stock with PBS, and use 10 times the original calculated volume.
4. Incubate the mixture for 1-2 hours at room temperature.
5. Use the mixture in your BLI experiments directly without desalting. Use the biotin-peptide conjugate at a 50-200 nM concentration for initial scouting experiments.

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