Protein Science - *In vitro* Biotinylation of Avi-Tagged Proteins

Purpose

Occasionally, Avi-tagged proteins co-expressed in *E. coli* are not fully biotinylated. Also, the analogous co-expression system in insect cells is not yet available, and thus, all proteins from insect cells must be biotinylated *in vitro*.

Scope

This protocol details the biotinylation of Avi-tagged proteins *in vitro* using purified His6-BirA and purified target proteins. This protocol is routinely used to generate ~1–40 mg of biotinylated proteins. The reaction is robust and, often, the procedure is used to take an in-process pool from a purification (i.e., not a final purified protein) for biotinylation (but it can start with a purified protein). Thus, the protocol is designed to keep the relative ratios of the reaction components the same over a range of protein concentrations.

Definitions

ATP: adenosine triphosphate

IMAC: immobilized, metal-ion, affinity chromatography

MWCO: molecular weight cut-off

POI: protein of interest

SDS-PAGE: sodium dodecyl sulphate–polyacrylamide gel electrophoresis

Materials and Equipment

- POI: For example, Avi-KRAS4b(1-169). A range of concentrations can be used, and this protocol has worked for KRAS4b samples in the range of 13–150 µM.
- His6-BirA protein, 180 µM stock
- ATP, 50 mM stock
- Biotin, 50 mM stock
- 1.5 mL tubes (USA Scientific, Inc., 1615-5500)
- 15 mL Corning tubes (Sigma-Aldrich, CLS430790)
- 1× buffer matching the buffer of the POI

The following supplies may need to be customized for your particular protein, and thus, specific protocols are not provided, only general guidelines.

- IMAC column capable of binding the His6-BirA
- Chromatography buffers
- Equipment buffers and reagents for SDS-PAGE analysis (not included in this protocol)
- Final buffer (specific for the POI)
- Dialysis tubing, of appropriate MWCO
- Low protein-binding membrane, centrifugal concentrator of appropriate MWCO (if desired)
- Access to mass spectrometry for quality control

Safety Precautions

Use standard laboratory personal protective equipment.

Procedures

A. Reaction Setup

The example below uses a 6 mL reaction with 5 mL POI at 88.3 µM. The molar ratio between the reaction components is 1 BirA : 7.5 POI : 20 biotin : 300 ATP.

1. Thaw the protein on ice.
2. Pool the thawed POI in an appropriately sized tube (here, a 15 mL conical tube) and add:
   - 5 mL POI (74 µm)
   - 327 µL His6-BirA (10 µM)
   - 353 µL ATP (2.9 mM)
   - 24 µL biotin (196 µM)
   - 296 µL 1× buffer

B. Incubation

1. Mix the reaction components gently, cap the tube, and place the tube on an orbital shaker for gentle mixing at room temperature for 2 hours.
2. The reaction can be stored at 4°C overnight (or frozen) for future purification.

The following information is provided as a guideline only because other proteins may require specific protocols:

- Biotinylated protein can be purified away from the reaction components by using POI-specific

chromatography. We routinely use IMAC (BirA is His6-tagged), and the biotinylated POI will be in the column flow through with His6-BirA binding to the column. SEC can be used to remove small molecular weight components (biotin, ATP, buffer components from the POI if the sample has been taken from an in-process protein purification pool).

- SDS-PAGE can be used to identify fractions from the chromatography that are positive for the POI.
- Intact mass spectrometric analysis can be used to confirm biotinylation and calculate the efficiency of labeling.
- The final protein is typically assayed for protein concentration, concentrated or diluted as necessary for final storage, dispensed in $\leq$ 250 µL aliquots in 1.5 mL tubes, snap-frozen in liquid nitrogen, and stored at –80°C.

Final Considerations

Occasionally, a protein will be resistant to being fully biotinylated. There is no known correlation that helps to explain this observation, although it does appear to be protein-specific. In these rare occasions, the utility of the partially biotinylated protein depends on the application. For instance, chip-based technology like surface plasmon resonance or bead-based technology that binds the biotin will selectively use only properly labeled proteins.