Biotinylation of recombinant proteins by co-expression with BirA in a range of different cell hosts

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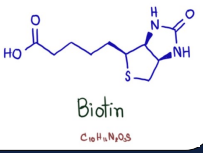
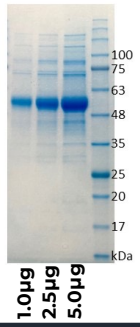
- Biotin is added to a handful of proteins naturally (interacts strongly with Avidin/Streptavidin) and this Biotin-Avidin interaction can be used for protein biology (sensitive protein detection, stable protein immobilisation – e.g., SPR).
- Biotin can be added to proteins site specifically at introduced Avi tags using BirA. This can be *in vitro* as part of a purification scheme but can also be via co-expression of BirA.
- Co-expression offers several advantages (reduced handling time – cost and ease, increased efficiency).
- This route also offers 2 chances at achieving biotinylation (as proteins can still be biotinylated *in vitro* if needed).

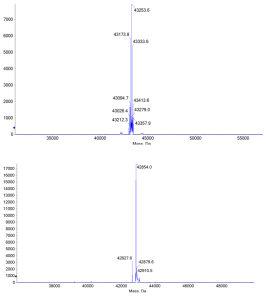


1 Insect (production of a biotinylated transcription factor)

Expression & purification



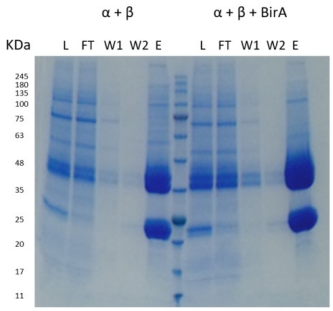
Intact mass analysis



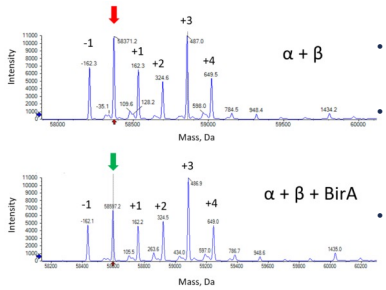
- A biotinylated version of a highly disordered protein was required for a high-throughput screening assay. Sf21 cells were co-infected with two viruses expressing the genes encoding the target protein and BirA. Cells were supplemented with biotin at infection.
- Biotinylated protein was purified using a combination of IMAC, ion exchange and SEC.
- Mass spec analysis was complicated by a series of post translational modifications (multiple phosphoforms and acetylation) in addition to the biotinylation. The predicted mass was 42628.1Da. The measured mass after phosphatase treatment was increased by 226Da consistent with biotinylation or 268Da consistent with biotinylation and acetylation. This demonstrated almost complete biotinylation of the protein.

2 Mammalian (production of a biotinylated heterotrimeric protein)

Expression & purification



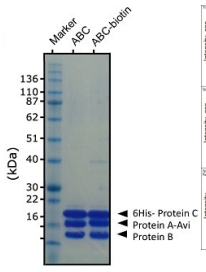
Intact mass analysis



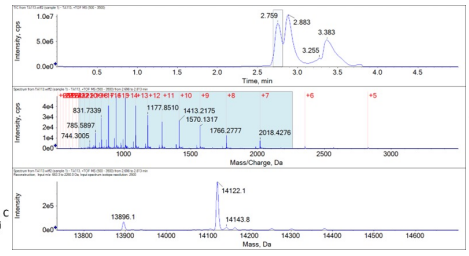
- HEK293 cells were co-transfected with 3 plasmids expressing proteins with secretory signals: two encoding a covalent heterodimeric protein (α + β) and BirA. Cells were grown supplemented with biotin.
- Supernatant was purified by nickel affinity chromatography, showing two clear bands in the nickel eluate on a reducing gel.
- The size was predicted to be 57,004 (without reduction of the interchain disulphide) based on the mass of proteins α and β but was anticipated to be higher due to glycosylation. Multiple glycan species are shown all increasing by a single saccharide unit (162 Da).
- Each glycan species was 226 Da larger following co-expression with BirA. Consistent with a single biotin addition. Furthermore, the shift in size was 100%, indicating we had achieved complete biotinylation of all the expressed protein.

3 *E. coli* (production of a biotinylated heterotrimeric complex)

Expression & purification



Intact mass analysis



- *E. coli* cells were co-transformed to produce a biotinylated heterotrimeric complex. Proteins A (with an Avi Tag sequence at the C-terminus) and B were cloned into a pCDFduet-1 vector and transformed into *E.coli*. Then a pETDuet vector containing Protein C (which contained an N-terminal 6His-TEV tag) and BirA was transformed into these same *E.coli* cells. Expression was induced by IPTG and biotin added.
- The complex was captured using the 6-His tag on Protein C, with all three proteins seen on SDS PAGE after purification. The intact mass data of Protein A shows masses correct for unbiotinylated protein and with one biotin added.
- From the peak heights its estimated that 80% of the complex has been biotinylated.

Summary

- **Biotinylation of recombinant proteins can be carried out by co-expression of a target protein along with BirA and this can be done across different expression systems for both intracellular and secreted proteins.**
- **We regularly use this technology to biotinylate proteins for a range of uses including compound screening and biophysical assays. This offers savings in time and material costs.**