Production of a Nanobody-based affinity resin for the purification of GFP-tagged proteins

Paul White[1], Niklas Amthor[2], Chi Tung Wong[1], Sandeep Talapatra[1]

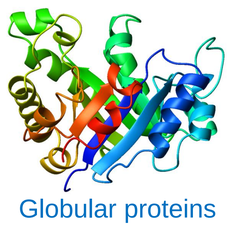
1. GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY
2. University of Leeds, Woodhouse Lane, Leeds, LS2 9JT

Introduction to the Protein Science and Innovation Group at GSK

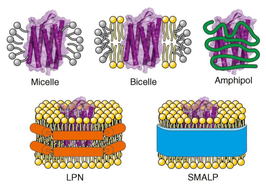
The Protein Science team is responsible for devising purification strategies and carrying them out to enable the purification of cytoplasmic, secreted and membrane proteins for downstream applications. Our team handles the initial screening for suitable conditions to isolate proteins of interest as well as the large-scale expression, purification and quality checks. We are also responsible for carrying out protein modifications including biotinylation and fluorescent labelling. A large part of our remit is the development and utilisation novel technologies and capabilities to increase efficiency and probability of success.

Proteins we support

Fibrous proteins

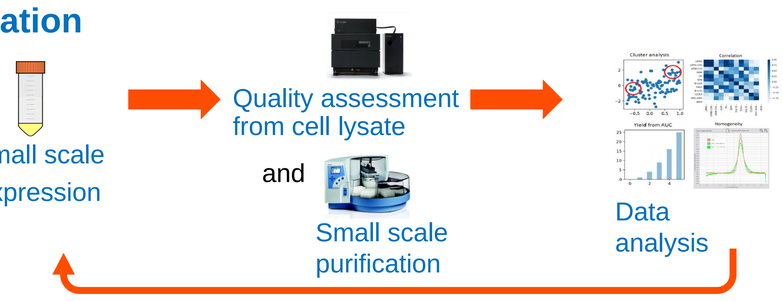


Globular proteins

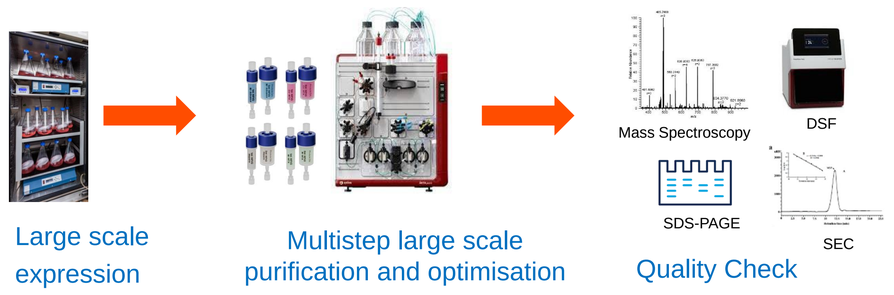


Membrane proteins

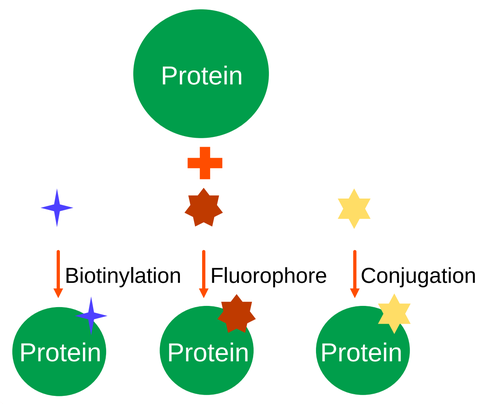
Microgram (μg) scale preliminary screen & protein purification



Milligram (mg) scale expression & purification



Recombinant protein modifications



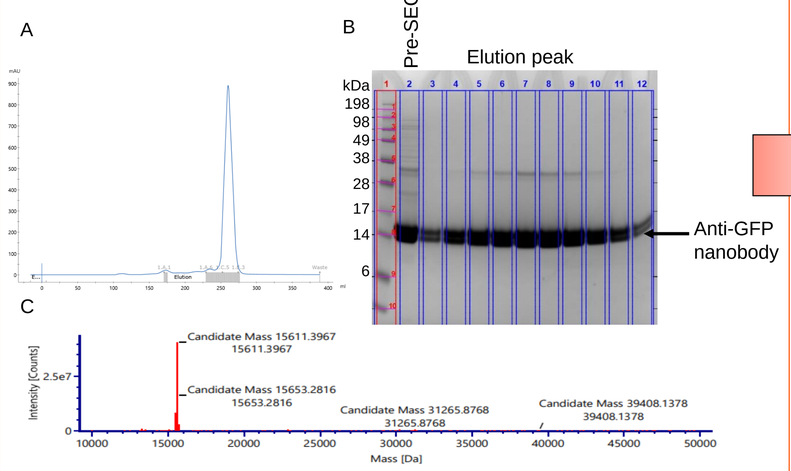
Developing novel protein expression and purification technologies

Production of an anti-GFP nanobody resin

Background: Nanobodies are single variable domain fragments of camelid heavy chain antibodies (V_HH) and have been exploited in the development of novel protein affinity purification technologies[1]. Here we purify a recombinant GFP-specific nanobody described in recent literature[2,3] and covalently conjugate it to NHS-activated agarose thereby producing an in-house anti-GFP affinity resin. The affinity resin can bind purified EGFP and EYFP as well as capture EGFP-tagged proteins from cell lysates. This proof-of-concept work sets the foundations for the generation of a repertoire of nanobody-based affinity resins specific for a range of protein tags commonly used in our existing high-throughput expression (HTX) platform. This concept can be expanded to magnetic resins for use in the automated purification screening platforms currently in development within the Protein Science Group at GSK.

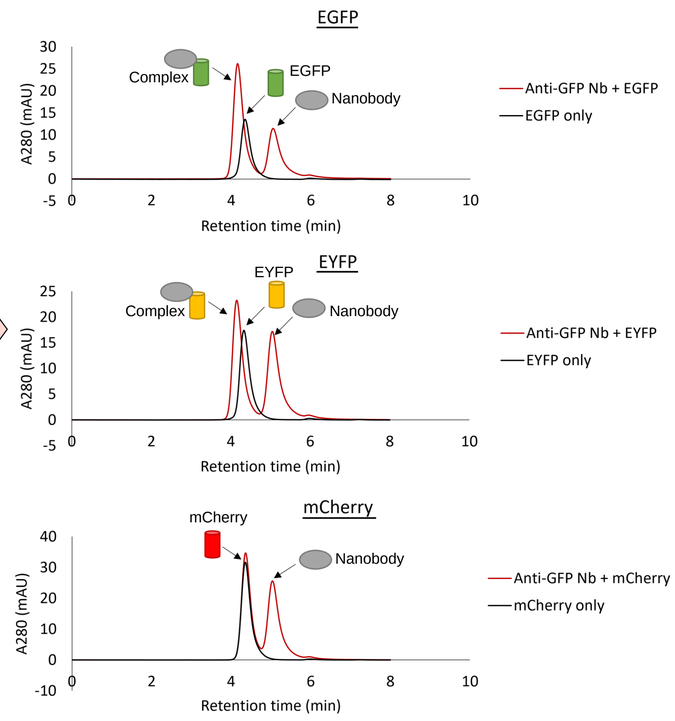
Purification of an anti-GFP nanobody

Expression in *E. coli* Origami™ B (DE3) cells → Ni-affinity purification → SEC purification → QC



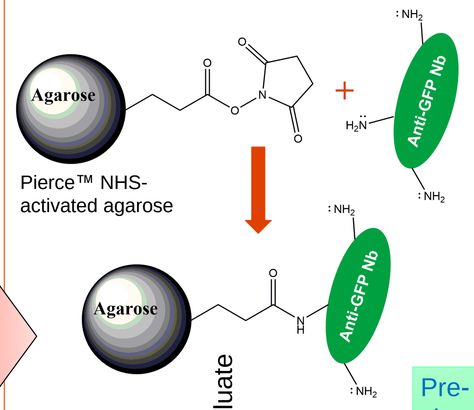
A. SEC elution profile shows single symmetric peak. **B**. SDS-PAGE analysis of SEC elution peak. **C**. Confirmation of expected mass by LC-MS. Resulting protein was highly pure with a yield of ~30 mg from 6 L culture.

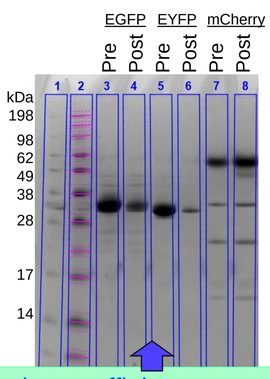
Verification of binding



Binding to EGFP and EYFP confirmed by ASEC. No binding to mCherry

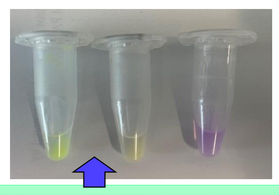
Generation and testing of affinity resin





Pre- and post-affinity capture showing EGFP and EYFP, but not mCherry bind to the resin

Elution of a partially-purified GFP-tagged protein captured from a cell lysate



EGFP and EYFP localised to the resin pellet

Conclusions

- Anti-GFP nanobody was successfully purified from *E. coli* with a yield of ~30 mg (6 L culture)
- Purified nanobody was able to bind EGFP and EYFP, but not mCherry
- The nanobody was successfully conjugated to NHS-activated agarose
- The binding capacity of the resin was determined to be 1.2 mg purified GFP per 1 ml resin, consistent with commercially available resins
- The resin was able to capture and partially purify a GFP-tagged protein from a cell lysate
- GFP-tagged proteins can be eluted by tag cleavage or low pH elution

References and Acknowledgements

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2. Zhang, Z., Wang, Y., Ding, Y. et al. Structure-based engineering of anti-GFP nanobody tandems as ultra-high-affinity reagents for purification. Sci Rep 10, 6239 (2020). https://doi.org/10.1038/s41598-020-62606-7
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GSK

Ahead Together