PHD STUDENTSHIP

Direct replacement of secondary antibodies by Affimer proteins

Contents

AT A GLANCE

In progress

Award date
January 2021 - March 2024

Grant amount
£90,000

Principal investigator
Dr Christian Tiede

Co-investigator(s)

Overview

Why did we fund this project?

This award aims to replace secondary antibodies produced in mammals, such as goats, sheep and donkeys, with Affimer proteins that can be produced *in vitro*.

Antibodies are used in a wide range of applications, including in preclinical research, to identify specific targets and purify proteins. Primary antibodies can be produced *in vitro* however, secondary antibodies are typically produced in animals, predominantly goats, sheep or donkeys. The animal is immunised and bled so the resultant antibodies can be harvested. This

may be repeated a number of times over an animal's lifetime. Antibodies produced in this manner recognise multiple targets on an antigen of interest, increasing the signal and reducing the background noise in an assay. Dr Christian Tiede and colleagues have previously developed Affimer proteins, which bind to target molecules with a similar specificity and affinity to animal-derived antibodies. These engineered proteins can be produced *in vitro* recombinantly in bacterial cells and have been used to target over 500 different proteins to date.

The student will isolate Affimer proteins to the constant regions of mouse and rabbit antibodies to produce Affimer proteins able to act similarly to secondary antibodies. These will then be fused to either horseradish peroxidase or fluorophores and tested in techniques where antibody use is common such as ELISAs, western blotting and immunohistochemistry. The student will also develop skills in protein production and phage display.

Application abstract

Antibodies are indispensable tools in science, medical research and diagnostics. Sensitive detection of antibody binding to its target molecule generally requires amplification of the detection signal. This commonly involves a two-stage detection process with primary and secondary antibodies. Primary antibodies bind directly to the target antigen, whereas secondary antibodies, typically conjugated to enzymes for detection, bind to the constant regions of primary antibodies. While primary antibodies are typically monoclonal and are produced recombinantly in CHO cell culture, secondary antibodies are usually polyclonal and therefore only produced in mammalians, mostly goat, sheep or donkeys. In

- Dr Darren Tomlinson
- Dr David Lewis
- Professor Philip Quirke

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R
- Reduction
- Replacement

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addition to the ethical issues associated with animal use, cross-reactivity concerns and batch-to-batch variation prevent standardized production required for good manufacture practice. To date only limited attempts have been taken to reduce the secondary antibody production in animals and no alternatives are yet commercially available.

We aim to replace secondary antibodies with *in vitro* selected Affimer proteins that are recombinantly produced in *Escherichia coli*. Affimer proteins are established and versatile binding-protein reagents that can replace antibodies in many applications. Affimer proteins are characterised by short development time (2-4 weeks), high affinity, high stability and high expression yields in *E. coli*. Furthermore, they can be easily manipulated by site-specific labelling with biotin, fluorophores, or genetic-fusion with enzymes, and can be generated as mono- or bispecific Affimer constructs. We have successfully selected primary Affimer proteins, by phage display, against more than 500 protein, peptide or chemicals to date. Their utility has been demonstrated in high impact publications in journals such as eLife, Blood, PNAS, EMBO, Science Signalling, Molecular Cell and Nature Structural & Molecular Biology.

We will apply phage display to isolate Affimer proteins to the constant regions of mouse and rabbit antibodies with counter selection against human fragment crystallizable (Fc). Selected binders will be tested for binding to different epitopes on the constant regions aiming to select as many unique epitope binders as possible. Winning candidates will be fused to a human Fc region to make them bivalent and therefore similar to secondary antibodies. Affimer- Fc fusion proteins will be produced and subsequently labelled with horseradish

peroxidase or fluorophores. Their ability to detect primary antibodies will be tested in direct comparison with commercially available secondary antibodies in enzyme linked immunosorbent assay, western blotting and immunohistochemistry.

Our approach seeks the replacement and reduction of animals used for secondary antibody production. A successful implementation of our proposed secondary reagents could save more than 760 mammalians per year in the UK alone. Affimer proteins provide a true alternative to antibodies. Our previous work has shown that Affimer proteins can directly replace primary antibodies in many applications. This proposal represents a continuation of our efforts made so far and the first attempt to replace secondary antibodies using Affimer technology. Our long-term aim is the implementation of Directive 2010/63/EU, which requires that animals are not be used for scientific purposes where a nonanimal alternative exists.

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