SINGLE-DOMAIN ANTIBODY FUSIONS RESULT IN BI-DIRECTIONAL FUNCTIONALITY

BIO-MANUFACTURING | FUNCATIONALITY TESTING

Introduction

Single-domain antibodies (sdAbs) have unique properties such as their simplicity, high binding affinity, robust stability, and excellent solubility over a broad range of temperatures and pH. In this technology review we build upon previous research by using the Nanovitics bio-production platform to manufacture a bi-directional sdAb fusion protein that binds to multiple targets.

DNA Construct

The DNA of two sdAbs that bind to mCherry and GFP fluorescent proteins were ligated using a short linker in our proprietary genetics construct and put into the NanoExpress bio-manufacturing platform for protein expression.

Results

A single fusion protein consisting of two sdAbs that bind mCherry and GFP was bio-manufactured in the NanoExpress system, purified, and stored at room temperature in PBS. The fusion protein has a size of 31 kDa with the individual sdAb architecture at each end of the protein structure joined by a short linker. In Figure 1, purity and size of the fusion protein is illustrated in a SDS-PAGE, with 5 ug of fusion protein loaded. Interestingly, there are no truncations of the fusion protein which is sometimes a common occurrence in other bio-production systems and almost absolute purity was attained.

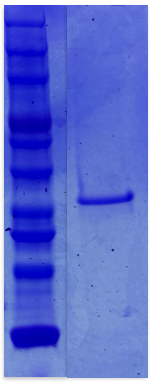

Figure 1: SDS-PAGE of sdAb fusion protein 31 kDa.

Binding Assay

The single-domain antibody fusion protein (100 ug) was immobilized on NHS-activated agarose and 100 ug of recombinant mCherry (1mg in 1mL) or GFP (1mg in 1mL) was passed through the agarose and visualized on the resin (Figure 2 A + B). Control agarose (Figure 2 C) was also prepared that contained 100 ug of a control single-domain antibody fusion immobilized on the agarose and recombinant mCherry and recombinant GFP was passed through the column showing no binding.

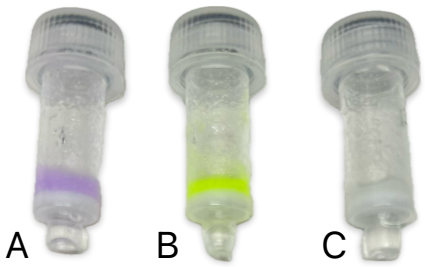

Figure 2: Binding Assay using NHS-activated agarose containing a sdAb fusion protein that binds both mCherry and GFP (A + B) and a control fusion sdAb (C).

After recombinant reporter protein was passed through each column it was briefly centrifuged at 800g for 1 minute and the amount of protein was measured in the flow through using a nanodrop at 280nm wavelength (Figure 3). The fusion sdAb was effective in binding target instantly.

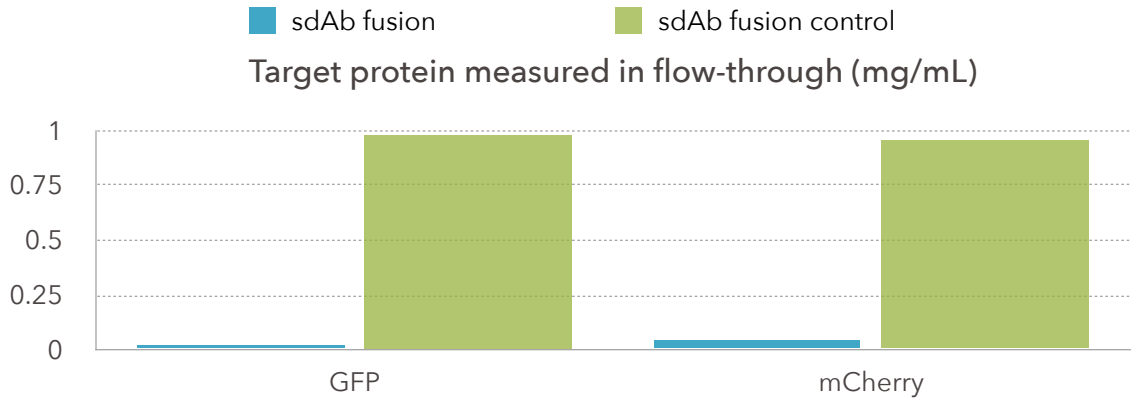

Figure 3: Single-domain antibody fusion protein that binds both GFP and mCherry was immobilized on NHS-activated agarose and GFP or mCherry recombinant protein was passed through the column by gravity and then a short centrifugation. The amount of protein was measured in the flow-through fractions using a nanodrop at 280nm.

Conclusion

A fusion protein was created by linking two sdAbs active against mCherry and GFP fluorescent proteins and bio-manufactured in the NanoExpress protein expression system. The sdAb fusion was fully functional at binding target proteins with high affinity and was efficient to manufacture. These results create an incredibly useful class of bi-directional sdAbs that can bind two targets. The applications of sdAbs due to their innate properties include diagnostics such as lateral flows, therapies with diverse modalities as substitutes for monoclonal Abs, molecular imaging, and drug targeting systems, among many others. The efficient and cost effective bio-manufacturing of sdAbs will be critically important moving forward to realize their excellent potential.