THE BIO-MANUFACTURE OF SINGLE-DOMAIN ANTIBODIES

BIO-MANUFACTURING | BINDING OF TARGET | STABILITY AND USE

Introduction

Single-domain antibodies (sdAbs), also known as Nanobodies®, are antibody fragments derived from *Camelidae* heavy-chain IgG antibodies. These antibody fragments have superior qualities when compared to monoclonal antibodies and are increasingly being utilized because of their small size (~14kDa), simplicity, high-binding affinity, and incredible stability across temperatures and pH. Their overall shape is slightly convex which allows them to bind into more difficult pockets of targets with high affinity as compared to the 10x larger full-sized antibodies (~150kDa). The simplicity and small size also makes sdAbs easier to design with new AI models that rapidly create novel sequences that theoretically bind to a target structure. As is the case with all proteins, however, sdAbs need to be bio-manufactured for further testing and use.

NanoExpress

At Nanovitics, Inc. we custom bio-manufacture sdAbs using a simple and efficient leaf-based bio-production system termed NanoExpress. The result is highly functional sdAbs that can be used in a wide variety of experiments and products that showcase their incredible properties. In addition, the sdAbs from Nanovitics are endotoxin free, bioreactor free, single-use plastic free, and always free of animal, human, an bacterial components.

Experimental Method

Single-domain antibodies that bind to fluorescent proteins mCherry and Green Fluorescent Protein were bio-manufactured in the NanoExpress system using our proprietary vector and method. The purified fully-functional sdAbs were stored at room temperature in 1x phosphate buffered saline (PBS). The sdAbs were then immobilized on Pierce NHS-activated agarose (Thermo Fisher Scientific) via primary amines and stored at room temperature. Fluorescent proteins mCherry and GFP were also bio-

manufactured in the NanoExpress system, purified, stored in PBS at room temperature, and used in binding assays to their respective single-domain antibody.

Results

Bio-manufacturing

Single-domain antibodies were bio-manufactured using the NanoExpress system resulting in pure protein stored at 1mg/ml in PBS. To illustrate purity of the protein, 5ug of the sdAb was loaded on an SDS-PAGE gel (Figure 1) showing correct size and purity. Recombinant mCherry and GFP were also bio-manufactured in NanoExpress and purified to 1mg/ml and stored in PBS.

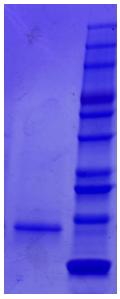

Figure 1: SDS-PAGE of single-domain antibody expressed in NanoExpress system.

Binding Assay

Single-domain antibody (1mg) was immobilized on NHS-activated agarose and recombinant mCherry (1mg in 1mL) and GFP (1mg in 1mL) was passed through the agarose and visualized on resin (Figure 2). Control agarose was also prepared that contained a control single-domain antibody immobilized on the agarose.

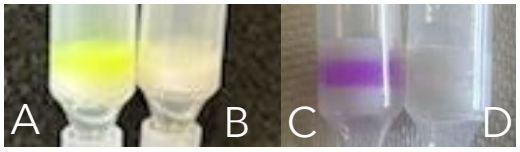

Figure 2: Single-domain antibody to GFP (A) and mCherry (C) immobilized on NHS-activated agarose. Control antibodies immobilized on (B) and (D).

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). Interestingly, the column containing the target specific antibody fragment bound nearly the entirety of the respective GFP or mCherry sample in just a few seconds that the sample was in contact with the sdAb fixed on the agarose. The control sdAb agarose did not bind target recombinant GFP or mCherry as nearly all of the sample flowed through the agarose and was measured in the flow-through fraction.

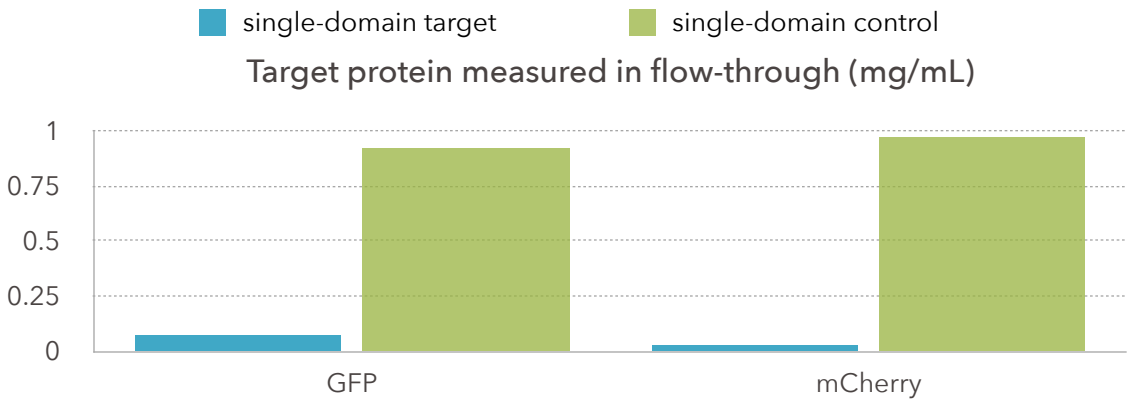

Figure 3: Single-domain antibody to GFP and mCherry were immobilized on NHS-activated agarose and 1mg/mL of GFP or mCherry recombinant protein was passed through the column by gravity and then a short centrifugation. The amount of protein was measured in the flow-through fractions using a nanodrop at 280nm.

Conclusion

Single-domain antibodies were manufactured and utilized in a binding assay. The affinity of sdAbs to target was high and immediate as only a small percentage of target protein was found in the flow-through after a single pass through the agarose resin by gravity and a short centrifugation. The sdAbs were efficient to manufacture in a cost effective manner and stable at room temperature which could be beneficial in several industries where cold storage is not practical or necessary such as point of need diagnostics.

The diverse 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 incredible potential.