NANOBODY'S GUIDELINE

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A short compilation of the background, techniques and steps needed to create the specific nanobody for your target

1. Introduction

Antibodies are widely used as binding agents for experimental, diagnostic and clinical uses. There are three types of antibodies: polyclonal antibodies, monoclonal antibodies and recombinant antibodies. The first ones are mixtures of several monoclonal antibodies that can be obtained from the immune response of animals, but its heterogeneity hinders its use in some applications.

Monoclonal antibodies are produced from a single B lymphocyte clone using hybridoma technology (Kohler & Milstein 1975). Generally, the conventional IgG antibodies show high affinity and specificity of binding to its antigen (Correia 2010). This allows for the use of many different species of animals and easier scale up during production. However, production of monoclonal antibodies is in general not possible in bacteria and yeast. This raises the price and difficulty of production.

Recombinant antibodies are produced or improved through molecular biology techniques. One example of these antibodies are the single-chain variable fragments (scVF). They are the smallest unit of an immunoglobulin that maintains binding capability. It is composed of the variable regions of the heavy and light chain of a monoclonal antibody which have been joined by a flexible peptide linker (Marty et al 2001). scVF maintain the specificity of the source antibody, but also allow for easier engineering and alteration of affinity and specificity through directed evolution, biopanning and other techniques that will be explored in this page. scVF are relevant to Nanoflex, as it is reasonable to assume that they can be used in our system for developing cellular biosensors to many relevant targets with known monoclonal antibodies.

2. Our choice

However, the most important type of antibodies for us during the summer were the natural single-domain antibodies (sdAb). Nanobodies belong to this category. sdAb show several major improvements for production and development in comparison with full-size humanized antibodies.

The benefits are numerous: smaller size, which simplifies their genetic engineering, production in bacteria, lowers variability between batches and easiest large-scale production (Leow et al 2018). In particular, the most relevant sbAb to us are the variable heavy-chain domains from camelids (VHH) (Huang et al 2010), such the anti-caffeine sdAb used in the proof-of-concept section in this wiki, or the model anti-HSP 16.3 (M. tuberculosis biomarker), the heavy-chain new antigen receptor domains in cartilaginous fish such as sharks (VNAR) (Diaz et al 1998), and variable lymphocyte receptors (VLR) found in lampreys (Pancer et al 2004).

These proteins have shown that modular expression and fusion to signalling systems is possible while maintaining binding characteristics but in order to use the full range of possibilities available sometimes it is necessary to develop new sdAb not found in nature.

In order to broaden the potential targets, it is necessary to introduce variability into the binding regions of sdAb This can be achieved through randomized libraries of mutagenic primers (Liu et al 2007), error-prone DNA polymerases (Brakmann et al 2004) or other methods such as gene shuffling (Stemmer 1994).

Libraries can be selected by binding affinity in a process known as *biopanning*. Proteins are displayed over a vector capable of coding for a particular protein. The displayed peptides are later selected by its binding affinity and specificity to an antigen. The most used method is *phage display*, where a phage expresses the sdAb on its capsid proteins (Jostock et al 2004). M13 filamentous phage (Smith 1985) and T7 lytic phage (Rosenberg et al 1998) are commonly used vectors for biopanning. Recently developed methods for biopanning such as bacterial display, yeast display and mammalian cell surface display (Tsuruta & Moro 2017) widen the possible methods that can be used to engineer useful sdAb for Nanoflex!

3. How to make new binding modules for Nanoflex

Here after we describe step by step the procedure to generate nanobodies recognizing your specific target.

1. Select a target. Try to identify a biomarker or protein that will provide the desired information.
2. Find a sdAb that binds to your target. There are several options to this, each one a bit more complicated than the next one. Be careful when designing the fusion protein, if the binding region is located on the N-terminal region the fusion protein might interfere. Remember that the DBD protein must dimerize for activation, so choose your antibodies with this in mind.
    a. Look for an already known sdAb in the literature. They are harder to find than expected!
    b. If no sdAb is found, look for known monoclonal antibodies for your target. Engineering scVF is the next best choice for attaining high binding affinity and specificity, but it might be necessary to characterize the newly developed scAb.
    c. If none are available, developing new ones is the only option! Build your library, choose your display method and be ready for several rounds of directed evolution until a potential sdAb is found. Characterizing these new proteins is a priority before using it with Nanoflex.
3. Assemble your DBD-sdAb fusion protein. Prove that the protein is being expressed on the membrane and develop an assay to test the correct folding and binding parameters of the sdAb. You cannot assume that the sd-Ab is going to fold properly *a priori*.

Express the protein, add the target to the media and try to induce reporter expression. The priority is to find the Level of Detection, the lowest concentration that triggers induction on the pCadBA operon. You have just created a cellular biosensor, congratulations!

4. References

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