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Pitfalls to avoid when using phage display for snake toxins

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ABSTRACT

Antivenoms against bites and stings from snakes, spiders, and scorpions are associated with immunological side effects and high cost of production, since these therapies are still derived from the serum of hyper-immunized production animals. Biotechnological innovations within envenoming therapies are thus warranted, and phage display technology may be a promising avenue for bringing antivenoms into the modern era of biologics. Although phage display technology represents a robust and high-throughput approach for the discovery of antibody-based antitoxins, several pitfalls may present themselves when animal toxins are used as targets for phage display selection. Here, we report selected critical challenges from our own phage display experiments associated with biotinylation of antigens, clone picking, and the presence of amber codons within antibody fragment structures in some phage display libraries. These challenges may be detrimental to the outcome of phage display experiments, and we aim to help other researchers avoiding these pitfalls by presenting their solutions.

1. Introduction

Envenomings from snakes, scorpions, and spiders represent a serious neglected health issue in large parts of the developing world, causing pain and suffering to millions of victims with severe cases resulting in amputation (for snakebite) or even death (Warrell et al., 2007; Williams et al., 2011). The cornerstone of envenoming therapies still consists of animal-derived antisera, which remain the only effective treatment options against snakebites, spider bites, and scorpion stings (Chippaux, 2012; Rodríguez Rodríguez et al., 2015; Gutiérrez et al., 2011). However, since antisera suffer from drawbacks including immunogenicity due to their heterologous nature, complex production processes due to dependence on venoms and the immune systems of production animals, and batch-to-batch variation, an increasing amount of research is being focused on alternative approaches based on monoclonal antibodies and recombinant DNA technology (Laustsen et al., 2016a, 2016c; Richard et al., 2013; Rodríguez-Rodríguez et al., 2016; Roncolato et al., 2015). These novel approaches may hold the promise of delivering biotechnology-based therapies with improved efficacy, higher safety, and potentially lower cost of production (Laustsen et al., 2016a, 2016b; Rodríguez-Rodríguez et al., 2016).

One approach that has gained increasing attention within development of novel antivenoms is the use of phage display technology for discovery of antibodies and antibody fragments (Roncolato et al., 2015). Phage display technology exploits the linkage between antibody genotype and phenotype obtained by incorporating antibody genes, typically single-chain variable fragments (scFvs) or single-domain antibody fragments (V_HHs or Nanobodies®) (Fig. 1) into the DNA of bacteriophages displaying the antibody peptide sequence on its outer coat (Laustsen, 2016a) (Fig. 2). In addition to phage display technology, several other approaches for development of novel antivenoms and antitoxins have been investigated, such as the use of small molecules (Lewin et al., 2016), the use of DNA epitopes strings (Harrison, 2004; Wagstaff et al., 2006), or the use of hybdridoma technology (Castro et al., 2014; Frauches et al., 2013). However, it is beyond the scope of this article to discuss these approaches in detail (see (Laustsen et al., 2016a, 2016d; Roncolato et al., 2015) for comprehensive reviews of these topics).

In phage display experiments, the M13 bacteriophage is often

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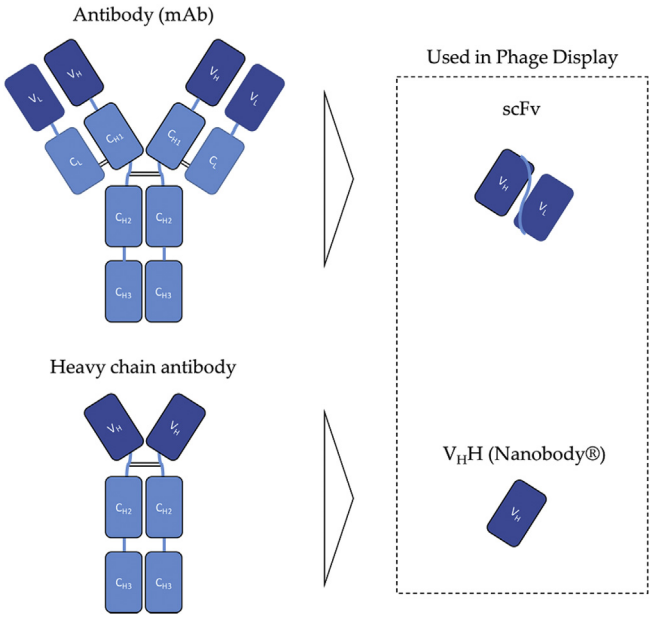

Fig. 1. Overview of the different antibody formats employed to construct phage display antibody libraries and how these formats relate to human (scFv) and camelid (V_HH) antibodies, respectively.

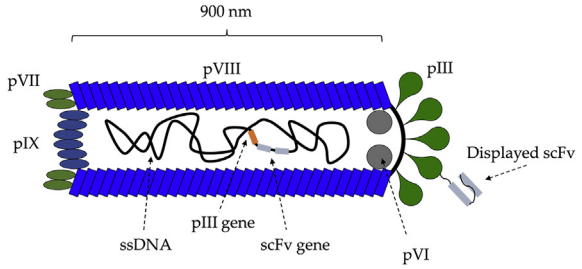

Fig. 2. Schematic representation of the M13 bacteriophage containing single-stranded DNA (ssDNA) and displaying an scFv on the pIII protein.

employed to create a library, where antibody fragments are displayed on the pIII coat protein of the M13 phage virion, while the antibody fragment encoding gene is incorporated into the DNA of the phage virion (Hoogenboom et al., 1998; Rodi and Makowski, 1999; Sidhu, 2000), see Fig. 2. The phage display library is then used for *in vitro* selection by attaching the target antigen to a plate well or bead, and panning the phage virions onto the target (Parmley and Smith, 1988). Non-binding phages are then washed away after sufficient incubation time, and binding phages can be eluted, amplified in *E. coli* TG1, and either subjected to additional rounds of panning or analysis (typically ELISA or gel electrophoresis), see Fig. 3. After a few cycles of panning, monoclonal phage virions can be isolated and their DNA sequenced in order to reveal the sequence of the displayed antibody fragment (Laustsen, 2016a). Several factors influence the outcome of a phage display experiment, including the introduction of deselection steps to remove unwanted antibody fragments, the affinity of the displayed antibody fragments, the level of antibody display, antigen immobilization and presentation, and clonal variation. Clonal variation may further affect antibody fragment translation, folding, transport, and stability of the fusion, which may further create amplification biases towards phage virions displaying undesired antibody fragments (Barbas et al., 1991; Bass et al., 1990; Garrard et al., 1991; McCafferty, 1996; Lowman et al., 1991).

Since McCafferty et al. reported the development of the first scFv phage display library in 1990 (McCafferty et al., 1990), a wealth of antibodies and antibody fragments have successfully reached the clinic for a wide range of indications (Nelson and Reichert, 2009; Schofield et al., 2007). In the field of antivenom development, phage display technology was already introduced in 1995 when Meng et al. isolated the first murine scFv against different Mojave rattlesnake toxins (Meng et al., 1995). Since then, other researchers have reported the discovery of both human scFvs and camelid V_HHs against phospholipases A_2 and neurotoxins from both vipers and elapids (Chavanayarn et al., 2012; Kulkeaw et al., 2009; Richard et al., 2013; Roncolato et al., 2013; Stewart et al., 2007; Tamarozzi et al., 2006). Yet, antivenoms have still not entered the modern era of biopharmaceuticals, where protein-based therapies are produced recombinantly (Laustsen, 2016a; Laustsen et al., 2016a, 2016d). Part of the reason that this transition from serum-based therapies to recombinant antivenoms has not yet occurred is due to the difficulty of

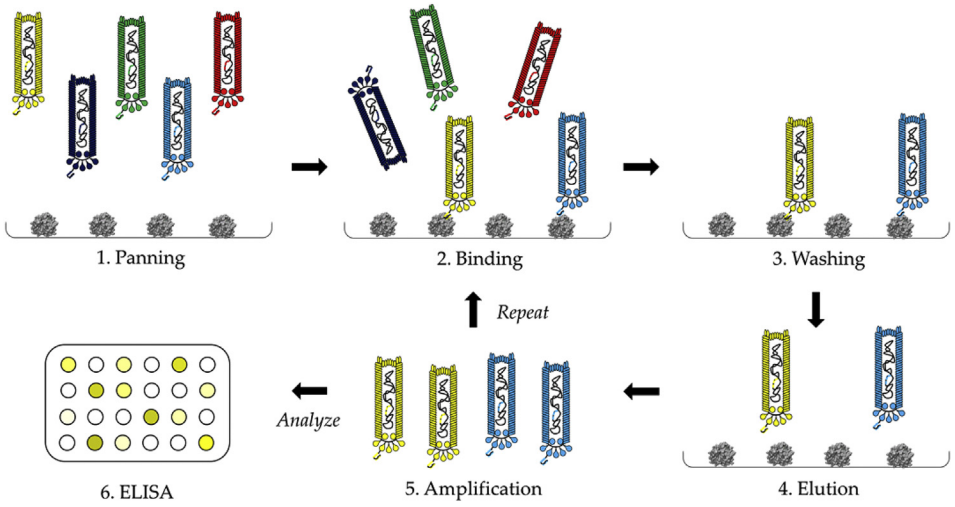

Fig. 3. Schematic representation of a phage display selection experiment. **(1)** First the scFv displaying phage library is panned against the target toxin, which is bound to a well. **(2)** Non-binding phage particles are washed away. **(3)** Binding phage particles are eluted. **(4)** Phage particles are amplified and either submitted to another round of selection or **(5)** analyzed by polyclonal ELISA.