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Identification of optimal protein binders through the use of large genetically encoded display libraries

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The use of large genetically encoded binder libraries in co-operation with display technologies has matured over the past 25 years, and is now one of the primary methods used for selection of protein binders. Display technology has proven to be a robust and versatile method for generating binders to almost any antigen of interest. The evolution of this technology beyond antibody phage display has opened up new aspects for the concept of designer biologics. The ability to construct large populations of eukaryotic cells, including mammalian cells, where each cell expresses an individual antibody, peptide or engineered protein has added great value in identifying binders with desired properties. Here we review the evolution of display technology and highlight how it is being used today to generate binders with exquisite specificity, selectivity, affinity and developability characteristics.

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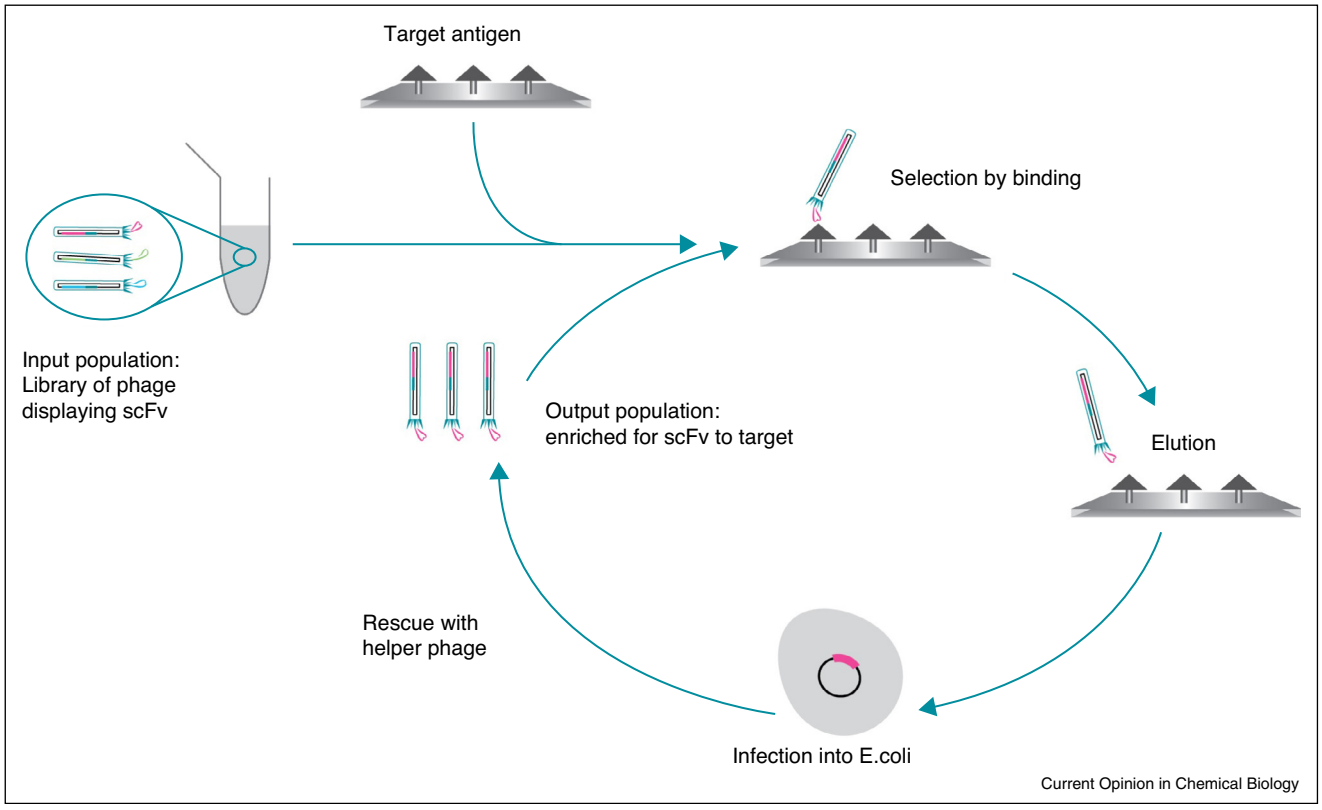
Introduction

Molecular biology and protein engineering techniques have permitted the creation of large, diverse, genetically encoded populations of related molecules (e.g. antibodies, proteins, peptides) from which individual variants with novel or improved binding or catalytic properties have been isolated. The power and utility of such genetically encoded libraries has best been exemplified through display of antibodies on filamentous bacteriophage (antibody phage display). A number of other display technologies using different cells or viruses have subsequently been described including retroviral display, baculoviral display, bacterial cell display, yeast display and display on higher eukaryotes such as mammalian cells [1–3]. The basic principle of display technology, whether for antibodies or other proteins and peptides, relies on the linkage of the binding molecule displayed on the surface of a cell or virus to the genetic information encoding that molecule (this principle also applies to cell-free systems such as ribosome display where *in vitro* translation is used and the linkage of genetic information and encoded protein is achieved through ribosome stalling causing retention of the encoding mRNA and the nascent protein within the ribosome complex). The binding properties of the displayed binding molecule are used to isolate the gene which encodes it. To achieve this, the binding molecules are presented on the surface of a cell or virus by cloning the gene encoding the binding molecule in-frame with a gene encoding a protein which is normally displayed on the surface of the cell or virus. For example, in the case of filamentous phage display, genes encoding binding molecules (e.g. an antibody) are cloned into a display vector which expresses the binder as a fusion with the minor coat protein encoded by gene 3 of filamentous phage and the construct is transformed into *Escherichia coli*. The vectors also carry a packaging sequence which ensures that the encoding DNA is packaged into phage particles along with the encoded fusion. Generation of bacteriophage from the transformed *E. coli* using standard methods results in the generation of bacteriophage particles displaying an antibody fragment on their surface and encapsulating the encoding antibody gene within the bacteriophage. By introducing a diverse population of different antibody genes (or genetic variants of alternative binding molecules) into the different display systems it becomes possible to generate a 'library', that is a collection of cells (or viruses derived from them), of 'display packages' from which variants with optimal binding properties can be isolated. In practice specific binders and their associated genes are enriched within the population by exposing the 'library' to a target molecule of interest. To allow recovery of a specific 'display package' presenting a binder recognising a target of interest, the target molecule needs to be immobilised onto the surface of a support matrix or needs to be recoverable from solution by secondary reagents (e.g. biotinylated target protein recovered from solution using streptavidin coated magnetic beads). Following incubation of the library with the target molecule, unbound display packages are removed through washing the matrix to which the target is attached. Bound display packages and their associated genes can be recovered, amplified and the process repeated if necessary. Using the approach outlined above it becomes possible to enrich a subset of binder variants capable of binding a target molecule of choice (see Figure 1).

Figure 1



The phage display selection cycle. A selection starts with a library of antibody fragments displayed on the surface of bacteriophage particles and an antigen, here shown immobilised to a solid support. Antibody phage clones are incubated with the antigen and allowed to bind. After repeated washing, any clones bound to the antigen are eluted and the phage recovered is allowed to infect *E. coli* cells to amplify the selected phagemid DNA. Phagemid bearing *E. coli* cells are then infected with helper phage particles which provide the necessary genes to enable packaging of the enriched phagemid DNA and to generate new antibody bearing phage particles for further rounds of selection.

The power of display technology has best been exemplified by display of antibodies on filamentous bacteriophage and this approach will be used to discuss the benefit and power of display technology in general for generation of novel protein-based binding molecules. George Smith first demonstrated phage display technology using short, linear peptides displayed on the surface of filamentous phage which could be recognised by existing antibodies [4]. McCafferty *et al.* (1990) [5] subsequently demonstrated that functional antibody fragments could be displayed on filamentous phage particles allowing significant enrichment of binders from non-binders. This was followed by the isolation of novel antibodies from libraries derived from immunised [6,7] and non-immunised [8,9,10•,11,12] antibody V gene repertoires. As with traditional hybridoma technology, antibody libraries created from immunised sources benefit from the process of *in vivo* affinity maturation within deliberately immunised animals. This improves the affinity range and frequency of antibodies to a desired target within the antibody repertoire. In the case of human antibodies, it is rarely possible to deliberately immunise but material from therapeutically immunised or infected individuals can be used. Libraries built from immune repertoires are already biased towards the immunising antigen and diversities of 10^7–10^8 clones are typically sufficient to generate high affinity binders.

Using approaches based on animal immunisation, antibody diversity is often compromised by immune tolerance or the presence of immunodominant epitopes. Problems can also occur with toxins, non-protein targets such as carbohydrates, lipids and post-translation modifications of proteins, all of which have been difficult to generate via rodent immunizations due to either their inherent toxicity to mammals or being generally poorly immunogenic *in vivo*. The ability to isolate human antibodies from pre-made non-immune libraries has circumvented some of these problems and has greatly extended the power of display technologies. Naïve libraries are typically generated from mRNA derived from peripheral blood lymphocytes and tonsil tissue of donors who have not been specifically immunised. The success of this approach is closely related to the size and diversity of