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Review

Engineered affinity proteins—Generation and applications

Caroline Grönwall, Stefan Ståhl*

Division of Molecular Biotechnology, School of Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), SE-106 91 Stockholm, Sweden

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ABSTRACT

The use of combinatorial protein engineering to design proteins with novel binding specificities and desired properties has evolved into a powerful technology, resulting in the recent advances in protein library selection strategies and the emerge of a variety of new engineered affinity proteins. The need for different protein library selection methods is due to that each target protein pose different challenges in terms of its availability and inherent properties. At present, alternative engineered affinity proteins are starting to complement and even challenge the classical immunoglobulins in different applications in biotechnology and potentially also for *in vivo* use as imaging agents or as biotherapeutics. This review article covers the generation and use of affinity proteins generated through combinatorial protein engineering. The most commonly used selection techniques for isolation of desired variants from large protein libraries are described. Different antibody derivatives, as well as a variety of the most validated engineered protein scaffolds, are discussed. In addition, we provide an overview of some of the major present and future applications for these engineered affinity proteins in biotechnology and medicine.

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* Corresponding author. Tel.: +46 8 5537 8329; fax: +46 8 5537 8481.
E-mail address: stefan.stahl@biotech.kth.se (S. Ståhl).

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All processes in life progress through complex networks of interactions mediated by proteins. Thus, many proteins possess a remarkable capability of molecular recognition and their biological function is dependent on specific interactions with other biomolecules. The proteins ability of selectively recognize molecules have been intensively explored through applications in biotechnology and medicine. Affinity proteins generated to specifically and with high affinity bind to other molecules have become an invaluable tool in molecular biology research and biotechnology. They can be employed as reagents in a wide range of applications, e.g. for bioseparations, detection, and proteomics analysis. Furthermore, proteins binding highly specifically to defined targets in the body can be used (as) for *in vivo* diagnostics applications, and the use of such proteins also in medical applications, i.e. as targeted therapeutic drugs, is rapidly growing. Although affinity proteins have traditionally been dominated by immunoglobulins, recently a new generation of engineered affinity proteins based on so-called scaffold proteins has arisen and rapidly gained increased interest.

Antibodies are naturally evolved affinity proteins, designed by nature to specifically bind to other molecules. They are the most extensively used affinity proteins and there are today approximately 10,000 antibodies commercially available, employed in research and diagnostics (Borrebaeck, 2000; Michaud et al., 2003). Furthermore, more than 20 antibody-based products have been approved as biopharmaceuticals and at least 150 antibody-based drug candidates are today in clinical development (Carter, 2006). However, molecular recognition and specific binding in nature are not at all unique for antibodies and there exist many other naturally designed binding proteins that can be used as starting point for developing of new affinity ligands. Increased protein structural data and advances in the field of protein engineering have given scientists tools to develop proteins with novel binding properties through construction of combinatorial protein libraries. When generating a combinatorial protein library, a number of mutations are combined to create a large pool of protein variants, i.e. a library, from which a protein with desired functions can be isolated using methods mimicking the natural process of evolution. This is in contrast to the rational protein engineering approach in which collected data and knowledge about the proteins structure and function contribute to a prediction about the result of a given alteration of the amino acid sequence. Due to the complex nature of proteins, rational design of proteins is often very complicated, e.g. in terms of creating a modified protein with desired activity or binding specificity. Combinatorial protein engineering has indeed proven to be a powerful tool in design of new affinity proteins and has lead to the development of a number of alternative affinity proteins based on non-immunoglobulin scaffold proteins (Binz et al., 2005). From protein libraries containing billions of molecules with the same constant framework and randomized variable regions, affinity protein targeting virtually any molecule can be isolated. These new affinity ligands have demonstrated great potential as affinity reagents in various biotechnology applications and recently also for therapeutic approaches.

1. Generation of novel affinity proteins by selection from protein libraries

Evolution has generated the great diversity of proteins with all different features required for the processes of life primarily by using the 20 amino acids available in nature. Despite all our efforts to fully understand the nature of proteins, we are still far from being able to create completely new proteins with desired structure and function merely by rational design. By utilizing state-of-the-art *in vitro* evolution technology it is possible to generate protein variants with new features. However, it is a challenge to predict what combination of changes in the different amino acid residues that is needed to design a protein with novel functionality but with retained structure. The desired function could for example be improved stability or solubility, or modified substrate specificity or improved properties of an enzyme. Hereinafter the focus in this report will be on the generation of proteins with novel binding specificity, i.e. affinity proteins. When developing affinity proteins with new binding specificities, proteins can be selected for the capability to bind to a target molecule. There are a variety of different strategies, commonly termed protein selection systems, for isolating proteins with new target affinity from a combinatorial protein library.

Since sequencing of proteins is difficult, all successful affinity protein selection systems are all based on linkage between the genotype and phenotype of the affinity protein. This enables easy identification and amplifications of the selected polypeptides via the nucleic acids, being DNA or RNA. The selection procedure can be summarized in the steps: diversification, selection and amplification. This includes construction of a protein or peptide library, screening for binding to a defined target molecule, amplification of selected molecules and identification of binding clones. The selection process is typically repeated a number of times, i.e. in cycles, to enrich molecules with desired binding properties. After selection and identification, the selected novel protein is typically recombinantly produced and characterized in more detail. The need for different selection systems, all with their advantages and limitations, are due to a number of factors, e.g. availability and inherent properties of the target protein, and therefore a number of different selection methods has evolved. The success of a selection strategy depends to high extent on the diversity and quality of the constructed library. However, methods for construction of affinity protein libraries will not be discussed in detail in this review.

The different selection systems, described below, can be divided into three different categories: cell-dependent display systems, cell-free display systems and non-display systems (see Table 1). In the cell-dependent display systems, the affinity proteins are displayed on the surface of phage particles or cells, or expressed in a cellular compartment. The most utilized system in this group is phage display (Barbas et al., 2001) utilizing bacteriophage for display of foreign proteins. In addition, a large number of strategies for expressing affinity proteins from libraries on the surface of different cell types have been investigated. The major advantage with cell surface display systems is the possibility of using fluorescence labeling and powerful flow cytometric sorting for the screening, enabling affinity discrimination in the selection without the need for elution from the target protein. However, there are some lim-

Table 1
Examples of different selection systems employed in combinatorial protein engineering.

Selection system	Illustrative references
Cell-dependent systems	
Phage display	Barbas et al. (2001)
E. coli surface display	Daugherty et al. (1998)
Staphylococcal surface display	Löfblom et al. (2005)
APEx *E. coli* display	Harvey et al. (2004)
Yeast display	Boder and Wittrup (1997)
Cell-free systems	
Ribosomal display	Hanes and Plückthun (1997)
mRNA display	Nemoto et al. (1997); Roberts and Szostak (1997)
CIS display	Odegrip et al. (2004)
DNA display	Tabuchi et al. (2001)
Covalent DNA display	Bertschinger et al. (2007)
Microbead display	Sepp et al. (2002); Nord et al. (2003)
Non-display systems	
Yeast-two-hybrid	Parrish et al. (2006)
PCA	Koch et al. (2006)