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Construction and selection of phage display libraries based on leucine-rich repeats

Abstract

Traditionally, monoclonal antibodies have been used for most applications where a specific protein binding with high affinity to its target was required. Nevertheless, certain limitations have appeared for this class of molecule including large size, glycosylation and a complex architecture with several polypeptide chains, which complicates recombinant production and manufacturing. Consequently, there is an emerging need for antibody alternatives with improved features. So far, approximately 50 protein scaffolds have been proposed, mainly in an academic setting. The earliest examples of successfully engineered binding proteins include the peptide aptamers, Affibodies, Adnectins, and Anticalins. Interesting protein family, are so-colled repeat proteins, such as leucine-rich repeat polypeptides. Adaptive immunity in jawless vertebrates is mediated by variable antigen receptors (VLRs) that consist just of leucine-rich repeat modules. Relatively low molecular weight makes VLRs an interesting alternative to antibodies in biotechnological applications.

Using consensus approach we designed a novel VLR protein (called dVLR) containing six LRR repeats based on a sea lamprey receptor sequence. The designed protein was expressed in *Escherichia coli* in a soluble, native form and showed very favorable biophysical properties. Recombinant dVLR is monomeric in solution and preserves its secondary structure within the pH range 3.0 to 11.0 and tertiary structure between pH 4.0 and 10.0. It undergoes reversible thermal denaturation in a broad pH range (4.0 to 10.0). The maximal denaturation temperature of 73.9 °C is observed at pH 6.0, 0.3 M NaCl. Chemical denaturation of dVLR at pH 7.5 is a cooperative two-state process with a midpoint at 3.3 M GdmCl and a very high free energy change of unfolding in the absence of denaturant equal to 14.1 kcal/mol. The biophysical properties of dVLR make it highly suitable for biotechnological applications such as generation of specific ligand-binding molecules.

In the secend part of the project two different types of phage display libraries based on the proposed scaffold were constructed (P16 and Sm11S). The polypeptide were presented monovalently on the surface of M13 phage. Next step involved the selection of dVLR binders specific to four antigens: S100A7, extracelluled domain of HER2, hen egg white lysozyme and antigen of human blood group system Lewis^{y}. Multiple selection protocols were tested, both on plastic surface and in solution, the concentration of antigen, time of its incubation with phages presenting on its surface dVLR protein variants, the intensity of washing and the way of elution of binding phages. Positive results were obtained for phage display selection against lysozyme and S100A7 proteins.