



DOCTORAL THESIS IN  
STOCKHOLM, SWEDEN 1999

# Affibodies

KARIN NORD

*Academic Dissertation which, with due permission of the KTH Royal Institute of Technology, is submitted for public defence for the on Thursday the 20th May 1999, at 12:00 a.m. in*

## Abstract

The introduction of recombinant DNA technology-based methods for the overproduction of either wild type or engineered proteins in various host cells has had an enormous impact on the availability of proteins for various therapeutic, diagnostic and research applications, which also has created new demands on efficient production and purification methods to provide the desired material. Affinity chromatography has proven to be an outstanding technique to obtain a single-step increase in purity and concentration. However, for affinity chromatography to be attractive for large-scale manufacture of proteins, the affinity ligands have to meet industrial demands on stability and selectivity. The rather high cost and limited stability of many affinity adsorbents available today have undoubtedly contributed to the rather slow uptake in the use of affinity chromatography for protein manufacture, which calls for new methodology for ligand development.

This thesis describes attempts to use combinatorial protein engineering principles to construct large protein collections based on the staphylococcal protein A (SPA) derived  $\alpha$ -helical three-helical-bundle Z domain, from which novel or optimized ligands can be identified using the powerful *in vitro* selection technology "phage display". Combinatorial methods are attractive because they allow systematic and rigorous screening (evaluation) of a large number of related compounds, in the search for target-specific molecules.

Two combinatorial libraries of  $4.5 \times 10^7$  members each were constructed, where 13 surface residues on the Z domain simultaneously were randomized using either NN(G/T) or (C/A/G)NN degeneracy codons. Using the phage display technology, binding proteins (denoted affibodies) with micromolar affinities ( $K_D$ ) were selected against a wide range of targets of different size and origin, including human insulin, human apolipoprotein A-1<sub>M</sub>, *Thermus aquaticus* DNA polymerase, and human coagulation factor VIII. It was also shown that these affibodies could conveniently be used as selective and robust ligands in authentic affinity chromatography applications. For some investigated affibody ligands, a high stability against column sanitation procedures involving repeated exposures to 0.5 M NaOH was demonstrated. Affibody ligands were shown to retain their binding function when further engineered into multimeric constructs or after fusion to other protein domains. Improved ligands were also obtained using affinity maturation strategies, resulting in second generation binders having 15 to 20 fold increased affinities, with  $K_D$  values in the range of 10–25 nM.

In conclusion, it was shown that libraries of the Z domain have the potential to be used as general sources of affinity ligands to a wide range of targets. Z domain variants with completely altered specificities but with affinities in parity to that between the parental wild type Z domain and its natural target IgG have been selected. Affibody ligands should be interesting alternatives

to monoclonal antibodies in the purification of proteins from complex backgrounds. The potential use of affibodies as new biotechnological tools also in other applications is discussed.

Key words: affibody, affinity chromatography, affinity ligand, bacterial receptor, combinatorial library, phage display, selection, staphylococcal protein A, Z domain

© Karin Nord, 1999