Scientific Description

The project was focussed on construction of a stable high-affinity binding protein against protein A from *Staphylococcus aureus.* The monomeric binder shows high affinity against protein A, whereas disulphide linked tail-to-tail dimer of the same binder demonstrates even higher affinity. On the other hand, a head-to-tail dimer of same binder binds to protein A with low affinity. The project involved construction of series of dimers of the head-to-tail binder by inserting different length peptide linkers between the two identical copies of Affibody®. The tail-to-tail dimer has high affinity for Protein A but it is not stable in prokaryotic expression system. The necessity to construct head-to-tail dimer with peptide linker was to obtain a single polypeptide chain with certain degree of conformational mobility, that can stay intact in a Prokaryotic expression system having reducing atmosphere (with no disulphides). In order to achieve conformational mobility between the monomers within a dimer, peptide linkers of different length and properties were selected. Short flexible, long α-helical, and long flexible peptide linkers were chosen to be introduced respectively and test for binding to Protein A. The project involved cloning, expression and purification in prokaryotic expression system and analysis of the binding affinities to protein A using Biacore. Later upon analysis of sequencing data, a sequence was confirmed to contain 'Glycine-Glycine-Glycine' insert but followed by some extra undesired sequence as well as one mutation at sixth nucleotide position of first copy of Affibody®. Many attempts were made in order to introduce insert between monomers but upon analysis of sequencing data it was confirmed that there is no insertion of any peptide linkers between the affibody copies. The project did not worked perhaps because of following possible reasons: temperature chosen in PCR program wasn't favorable for mutation, enzymes *Taq/Pfu* used in PCR mixture were not suitable for the reaction, designed primers were complementary to the Affibody® dimer and got annealed at more than one place thereby initiating polymerization. It was possible to choose other cloning strategies, but since insertion of 'GGG' linker was observed in early trials, we hoped that it will be possible to perform insertion by mutagenesis PCR.