Abstract

Scaffolds are proteins with high conformational stability, allowing us to implement multiple mutations into specific parts of the protein. Even with these mutations, the structural integrity of the protein is maintained as well as its physical-chemical properties. These mutations give the specific scaffold new properties. In most cases it is the binding specificity towards previously chosen target. The biggest advantages of scaffolds are their small size, stability, low-cost manufacturing, and easiness of preparation. Scaffold utilized in this thesis is unique for having two binging surfaces designed on which it can be mutated. Each of those two surfaces can be separately mutated to develop a binging site for two different proteins. In our case these mutations led to binding two nonidentical receptors of a human cytokine. Mutations are made with a use of yeast display, one of the methods of directed evolution. The main focus of this thesis is changing an expression system of the binding proteins from the yeast system to a bacterial one, their production and purification followed by characterization of those binding proteins using biophysical methods. These methods were used to evaluate structural and thermal stability, and binding affinity to both receptors of the beforementioned binding proteins. This thesis is a part of a broader project which focus lies on designing, creating and characterizing binding proteins with the ability to specifically bind two cytokine receptors to simulate function of natural cytokine. The binding proteins should be able to bind to the chosen receptor complex and to start signalization that leads to an immune response. These binding proteins have potential diagnostic and therapeutic utilization in treating autoimmune diseases.

Key words: scaffold, cytokine, protein evolution, yeast display, DLS, MST, CD, thermal stability, gel permeation chromatography