Summary

In the past decade redesigning proteins has become an effective technique in examining the structure-function correlation of proteins. This method is often used to improve enzyme stability, to change an enzyme's substrate specificity, to generate a protein with a new activity, to increase a protein's fitness, etc.

Often the activity of a wild-type enzyme is too low to be of commercial or therapeutic use. Efficient application in industry of proteins produced by recombinant DNA techniques requires a stringent optimization of their properties in order to fulfill the requirements imposed by the market, or by the control institutes. Sometimes process conditions require also an optimization of the proteins property in order to be capable of producing a higher amount of protein or enzyme.

Rational protein design requires the availability of the structure of the enzyme and both the relation between the structure and the protein's function. A large public database of crystal structures of proteins and DNA sequences are available for scientists. Since the human genetic code has been revealed, structure-based enzyme redesign has become a new tool in optimizing human enzyme properties.

With the help of different software by *in silico* molecular modeling we can predict the relations between structure and function of protein's. In order to create a new property, an enhanced activity or sensitivity, to improve some characteristics, or to stabilize an enzyme, we can easily generate mutant proteins based on their crystal structures or predicted models using the public databases and bioinformatics tools.

There are two possible approaches to plan a mutagenesis: one is to introduce a new functional group into a protein's structure by site-directed mutagenesis, than the effect of the amino acid replacement will be investigated. This method requires a very stable protein, so that the mutagenesis does not have any effect on the other functions nor the structure of the protein. Enhanced Green Fluorescent Protein (EGFP) proved to be a very stable protein, and experiments lead to the conclusion that by changing amino acids into histidines, we can increase the sensibility for fluorescence quenching when it comes in contact with copper ions.

This way the modified, enhanced protein can be used as a biosensor, being able to detect a very low concentration of metal ions from water or blood (under 1 μM).
Another approach in demonstrating the role of bioinformatics in directed evolution or rational protein design is eliminating a functional group of an amino acid from the structure of a very problematic protein. Granzyme H is a human enzyme, which role in the apoptosis is not yet clarified. This enzyme is almost impossible to be expressed by heterologous expression systems in a soluble form. After producing a big quantity of insoluble protein, it needs to be denaturized and refolded for activity measurements. The correct disulphide bond forming is essential while refolding, so with the help of site-directed mutagenesis we eliminated an –SH functional group from the gene encoding Granzyme H thus prohibiting an incorrect disulfide bond formation to increase the refolding ratio. The mutant version of Granzyme H could be more easily refolded.
This study aimed to present the tools and exact steps of rational protein design from *in silico* modeling, gene isolation, designing different vector constructs for protein production, several purification and refolding techniques to experiments on the protein's properties which will demonstrate the success of the study: fluorescent quenching measurements in the case of the modified Enhanced Green Fluorescent Protein, and the ration of refolding in the case of Granzyme H.