

Abstract

Protein-protein interactions play an important role in nearly all processes of the living cells and the function of many proteins is dependent on their specific interactions with other biomolecules. A reliable tool to modulate these interactions would be invaluable for the development of molecules suitable for diagnostics, medicine, and biotechnology. In this work, we aimed to study the specificity of interactions in the model system of Interferon gamma receptor 1 (IFN γ R1) and its natural ligand Interferon gamma (IFN γ), important in innate immunity.

We searched for mutations within the interferon receptor molecule IFN γ R1 to modulate (increase as well as decrease) its affinity to IFN γ by *in silico* analysis of the existing crystal structures of the complex between IFN γ R1 and IFN γ . We modeled amino acid substitutions and gauged how they influenced the interaction using empirical force field implemented in software FoldX. All selected promising IFN γ R1 variants were expressed in *Escherichia coli*, purified to homogeneity, characterized, and kinetics of their interactions with IFN γ was measured by Surface Plasmon Resonance (SPR).

The first set of IFN γ R1 variants included mutations on the interface of the IFN γ /IFN γ R1 complex. According to our SPR measurements, the affinity of most of these receptor variants had virtually the same affinity as the wild-type receptor, a few had affinity slightly decreased, but a few variants bound IFN γ with significantly higher affinity. The second, less orthodox approach comprised single mutations within the cavities of the IFN γ R1 molecule. The results of these calculations suggested that they influenced the receptor affinity to IFN γ very little. However, two cavity mutations increased the IFN γ R1 affinity significantly in combination with the interface mutations.

Our results demonstrated that the combination of a computer-aided design using a relatively simple and accessible computational protocol together with experimental approaches was capable of predicting IFN γ R1 variants with significantly increased affinity to IFN γ . These new high-affinity binders help in better understanding of forces governing protein-protein interactions and could be developed into a new diagnostic tool.