In silico prediction of stability, thermal resistance and solubility changes upon mutations. Uses experimentally resolved or modeled 3D structures. Reduces time and expensive experimental practices.

Description

Our goal is to control modification of specific physical, chemical and biological features, such as stability, thermal resistance and solubility, without affecting the protein's activity. We exploit for that purpose newly developed energy functions derived from known wild type and mutant protein structures.

Upon completion, our software predicts the most stabilizing, destabilizing or neutral mutations. It is very fast and allows dealing with all possible mutations in a medium size protein in less than a minute. It is moreover user-friendly implemented and particularly easy to exploit.

Rational protein design can be achieved using our software. An original functionality is the estimation of the optimality of each amino acid in the sequence with respect to the stability. Clusters of non-optimal residues represent particularly interesting sites for introducing targeted mutations.

Outcome

• High scores: the predictive power of our method is shown to be significantly higher than that of other programs described in the literature. This drastically limits the number of experimental tests.

• Large applicability: the prediction score is only marginally reduced with usage of modeled rather than experimental structures.

• Rational protein design: our software has the unique functionality of allowing systematic in silico screening for mutations that improve the stability and the thermal resistance.

• Unusual proteins: proteins that are difficult to express can be investigated using this method (i.e. proteins with solubility issues, receptors...).

• Expert proposition: Moreover, the database-derived energy functions approach provides an out of the box thinking and sometimes reveals a mutation that could have been firstly rejected on the basis of an intuitive approach (e.g. switching a surface residue with another being strongly hydrophobic).

VALUE PROPOSITION

As your partner in protein in silico optimization, we provide access to our software suite and a range of services including advisory, experimental validation and mutant production according to your needs.
APPLICATIONS

The rational design of modified proteins with controlled stability, through amino acid substitutions, is of extreme importance in a whole range of applications, notably in the biotechnological, industrial and environmental areas where proteins are used for their catalytic or other functional activities, sometimes under unusual conditions.

• Conformational stabilization.
  In this case, we had to find mutations stabilizing the active form of a protein and destabilizing its polymerized form, which causes a range of disease. The polymerization time was used as indicator of the quality of identified mutations. Negative values suggest stabilization. After the experimental validation, four of the five predictions were in agreement with the predictions.

• Solubility improvement.
  Our objective was to increase the thermodynamic stability and the solubility of TEV protease, a protein that is frequently used to remove affinity tags from purified proteins. All the single-site mutations were introduced in silico in the protein and their stability change was evaluated. We selected surface mutations that stabilize the protease, assuming that they stabilize both the enzyme and the interactions with the solvent, and thus increase the solubility. We identified two mutations that present a solubility of about 6 mg/mL, whereas that of the wild-type protein is 1 mg/mL. The double mutant presents a solubility of 40 mg/mL. Based on these good results, we are now promoting the development of a dedicated predictor of solubility changes upon mutations.

• Industrial process improvement.
  The objective was to hyper-thermo-stabilize an enzyme used in agro-food industry. Our software identified two relevant mutations that were tested individually and simultaneously. The experimental validation of these three mutant proteins shows that each mutant improves enzymatic activity in Erlenmeyer (cf. results of table 1). The same observation was obtained in real process condition.

CONCLUSION

Our software is highly useful for identifying very rapidly a list of possibly relevant mutations with the desired stability properties, on which subsequent experimental studies can be focused. It can also be used to detect sequence regions corresponding to structural weaknesses, which could be functionally important or structurally delicate regions, with obvious applications in rational protein design.

References: