B- FITTER

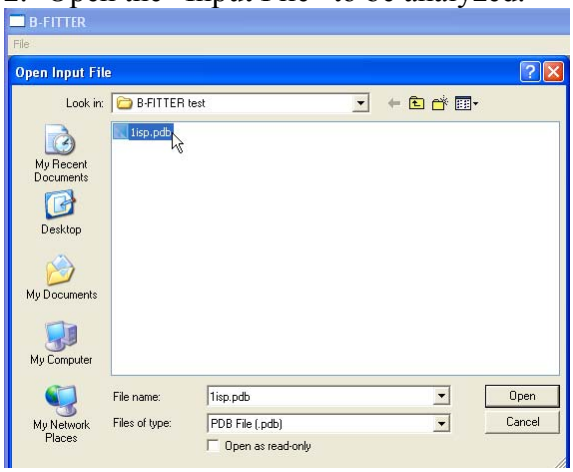
B-FITTER is a user-friendly computer aid when applying the B-FIT method in the quest to enhance the thermal robustness of proteins, which can be related to an increase in kinetic or thermodynamic stability or thermo-resistance to undesired aggregation/precipitation. B-FIT is an embodiment of Iterative Saturation Mutagenesis (ISM), one of the most useful methods in directed evolution. An early review can be found in: M. T. Reetz, J. D. Carballeira, *Nature Protoc*. **2007**, 2, 891-903.

This program calculates the "amino acid B-factor" as an average of the B-factors of all of the atoms of an amino acid in a given protein excluding hydrogen. It works using .pdb files from the Protein Data Bank. It is able to recognize the existence of non-resolved atoms and takes them into account for the calculation of the average amino acid B-factors.

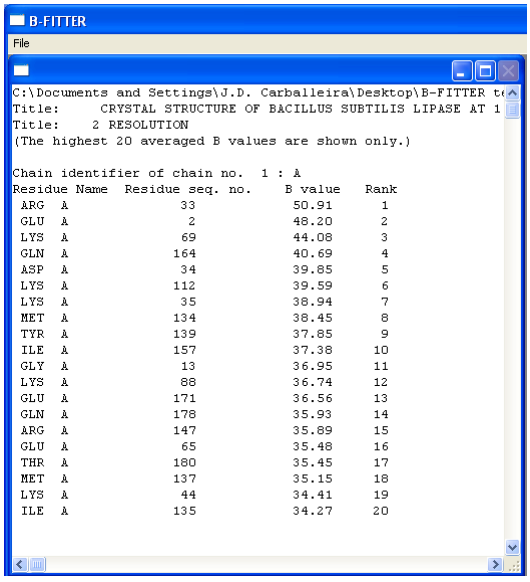
Instructions

1.- Run B-FITTER.exe

2.- Open the "Input File" to be analyzed.



When the program B-FITTER.exe is executed, the user has to open an input file by browsing to the location of the pdb file to be analyzed. As soon as the file is selected, the program is executed and a window opens, showing the ranking of the 20 amino acid residues with the highest B-values.



Automatically, B-FITTER generates an output file (.out), saved in the same folder where the .pdb file is and with the same name. This file contains a ranked classification of all the residues of the protein according to the B-factors.

This output file can be easily opened using any text editor available (e.g., Notepad, Wordpad, etc).

Appendix I. Using data from different .pdb files of the same protein.

In order to calculate average values for each amino acid taking into account information coming from different .pdb files, it is neccesary to standardize the values first as the B-factors scales differ from file to file.

The amino acid B-factor data obtained by B-FITTER from each X-ray should be converted in a standardize data set using the formula.

$$Z = \frac{x - \mu}{\sigma}$$

Z is the standardized value of the amino acid B-factor
x is a raw score to be standardized (amino acid B-factor)
μ is the mean of the amino acid B-factors;
σ is the standard deviation of the amino acid B-factors.

In this way each data set results in a standardized distribution characterized by a mean of the population equal to 0 and standard deviation equal to 1. Distributions of same mean and standard deviation are directly comparable.

When more than one X-ray structure is available, the user of B-FITTER should pick the one having the highest resolution. Another option is to consider all structures, obtaining

the B-FITTER output file .out from each. Then it is possible to calculate the desired B-factor of each amino acid as an average of the B-factor value displayed by that specific amino acid in the different structures. These average calculations have to be performed standardizing the data of each structure previously.