Structural bioinformatics

Thermometer: a webserver to predict protein thermal stability

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Abstract

Motivation: Thermal properties of proteins are of great importance for a number of theoretical and practical implications. Predicting the thermal stability of a protein is a difficult and still scarcely addressed task.

Results: Here, we introduce Thermometer, a webserver to assess the thermal stability of a protein using structural information. Thermometer is implemented as a publicly available, user-friendly interface.

Availability and implementation: Our server can be found at the following link (all major browser supported): http://service.tartaglia.lab.com/new_submission/thermometer_file.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The thermal behavior of a protein is influenced by environmental factors such as the pH of the solution, presence of ions, excipients or cofactors and internal characteristics, such as the amino acids’ composition of the protein sequence and their disposition in the 3D structure (Kumar et al., 2000; Miotto et al., 2019). In particular, specific amino acids contribute to thermal stability (Vijayabaskar and Vishveshwara, 2010) and salt bridges in hyperthermostable proteins have stronger electrostatic interactions than their mesostable counterparts (Lee et al., 2014). Importantly, proteins from thermophilic organisms display a more interwired network of interactions with respect to mesophile counterparts (Miotto et al., 2020). A lower number of cavities and a higher average relative contact order have been observed when comparing thermostable proteins with mesostable paralogs and orthologs (Robinson-Rechavi and Godzik, 2005). Based on these observations, we previously proposed an innovative approach of representing proteins as energy-weighted graphs and comparing them using ensembles of random interaction networks (Miotto et al., 2019). Our parameter-free network descriptor allows us to distinguish thermostable and mesostable proteins with an accuracy of 73% ± 2% area under the receiver operating characteristic curve of 78% (see Section 2).

2 Materials and methods

The Thermometer webserver assesses protein thermal stability from a given structure in PDB format. At its basis there are four steps:

(i) each protein structure is represented as a residue interaction network (RIN), where protein residues correspond to graph nodes while Coulombic and van der Waals energetic interactions between couples of nodes correspond to the network link weights;

(ii) starting from the calculated RIN, an ensemble of randomized networks is built in which the nodes and architecture are preserved but each link weight is randomly sampled from an energy distribution typical of mesostable proteins. (iii) The Ts descriptor is defined as $T_s = <S > - ( < S > , < S > , < S > )$ where $< S >$ and $< S >$ are the mean strengths (sum of all the weights of a node) over the protein nodes, for the real network and the random ensemble, respectively. $< S >$ is the square root of the strength variance over the random ensemble. Finally, (iv) a clustering analysis is performed considering for each protein the set of three global $T_s$ descriptors obtained using total energy, Coulombic energy and Lennard–Jones interactions as network links. The clusters are discriminated by the Euclidean distance in the three-dimensional space of the $T_s$ descriptors using the Ward method. The thermal properties of the given protein can thus be compared with those of a large dataset of proteins with known melting temperatures. The dataset has been manually collected starting from the most recent version of the proTherm database (Nikam et al., 2021) and imposing the same set of constraint used in Miotto et al. (2019) (pH range, known $T_{m}$, absence of ions, ligands, mutations, and/or missing residues, etc.). We obtained 15 additional protein structures to be added to the dataset proposed in Miotto et al. (2019). The ratio of found thermostable proteins is one over three, in accordance with literature. The complete dataset is reported in the Supporting Information.
As one can see from the cluster shown in Figure 1, we retrieve a good two-group separation (thermostable proteins are colored in red, while mesostable in blue) performing a clustering analysis on the 99 proteins of the assembled dataset using the three $T_s$ descriptors. More in detail, the ‘mesostable’ cluster (prevalence of blue labels) is composed of 46 proteins, with 39 mesostable ones (85%). The ‘thermostable’ cluster is instead composed of 53 proteins, 33 of which are thermostable (62%). The overall performance is $73\pm2\%$ (the error is obtained assuming to swap two proteins between the clusters) in classifying 99 proteins with an area under the receiver operating characteristic curve of 0.78.

4 Conclusion

The Thermometer web server is fast and for a 50-residue-long protein, results are available to the user in about 4 minutes, while for a protein of average size (250 residues) the waiting time is of nearly 20 minutes. Moreover, the webserver is user-friendly and can be run without any a priori knowledge on theoretical or computational biology. We believe Thermometer can contribute to better understanding thermal stability and we hope that it could be useful in a number of practical applications.

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Conflict of Interest

None declared.

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