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Applications Note

Structural bioinformatics

MAESTROweb: a web server for structure-based protein stability prediction

Josef Laimer[1,2], Julia Hiebl-Flach[2], Daniel Lengauer[2] and Peter Lackner[1,*]

[1]Department of Molecular Biology, University of Salzburg, Salzburg 5020 and [2]School of Informatics, Communications and Media, University of Applied Sciences Upper Austria, Hagenberg 4232, Austria

*To whom correspondence should be addressed.
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Abstract

Summary: The prediction of change in stability upon point mutations in proteins has many applications in protein analysis and engineering. We recently adjoined a new structure-based method called MAESTRO, which is distributed as command line program. We now provide access to the most important features of MAESTRO by an easy to use web service. MAESTROweb allows the prediction of change in stability for user-defined mutations, provides a scan functionality for the most (de)stabilizing n-point mutations for a maximum of $n = 5$, creates mutation sensitivity profiles and evaluates potential disulfide bonds. MAESTROweb operates on monomers, multimers and biological assemblies as defined by PDB.

Availability and implementation: MAESTROweb is freely available for non-commercial use at https://biwww.che.sbg.ac.at/maestro/web.

Contact: peter.lackner@sbg.ac.at

1 Introduction

Point mutation can have a strong impact on protein stability. While in biomedical research functional consequences are of particular interest, in protein engineering often targeted stability changes are intended. Different computational approaches to predict the change in stability upon point mutations have been developed. Methods using the 3D structure of the wild type protein, e.g. I-Mutant2.0 (Capriotti *et al.*, 2005), PoPMuSiC (Dehouck *et al.*, 2009) or DUET (Pires *et al.*, 2014), turned out to perform better than purely sequence-based approaches (Khan and Vihinen, 2010) in general. However, the new sequence-based method INPS (Fariselli *et al.*, 2015) closes this performance gap for some datasets and is also able to complement and improve structure-based approaches. We recently introduced a novel structure-based method called MAESTRO which has a similar or better accuracy than the competitor methods (Laimer *et al.*, 2015). MAESTRO offers additional benefits which are (i) report of a prediction confidence value, (ii) operation on multimeric proteins, (iii) a scan mode for the most (de)stabilizing n-point mutations and (iv) evaluation of potential disulfide bonds.

Here, we present MAESTROweb, which provides an easy to use web interface to MAESTRO for the most frequent application scenarios.

2 Features

MAESTROweb provides four experiment types. First, the investigation of user-specified mutations. The mutations sites and types can be selected through menus or by flexible mutation syntax.

The second type of experiment is a scan for the most (de)stabilizing n-point mutation ($n \leq 5$), which is useful for protein engineering tasks. For $n = 1$, an optimal search is applied. For $n > 1$, a greedy search or evolutionary algorithm (EA) is utilized.

Third, MAESTROweb offers the calculation of mutation sensitivity profiles, where the impact of each mutation at each possible position is visualized, similar to the PoPMuSiC web server (Dehouck *et al.*, 2011). This allows the identification of cold or hot spot sites, respectively, which are resistant or sensitive to mutations.

Table 1. Run-time statistics

PDB-ID	Size	Specific	Scan gr.	Scan EA	Profile	SS-bonds
1CRN	46	≤1	20	185	9	≤1
4BCT	201	2	140	283	47	4
3TSH	490	3	507	571	192	16

Run time statistics for the four provided experiments: evaluation of 10 user-specified mutations (Specific), scan for the most stabilizing 3-point mutations utilizing a greedy (Scan gr.) or an EA algorithm (Scan EA), calculation of a mutation sensitivity profile (Profile) and the evaluation of potential disulfide bonds (SS-bonds). The values represent the median run time in seconds of 10 experiment repeats.

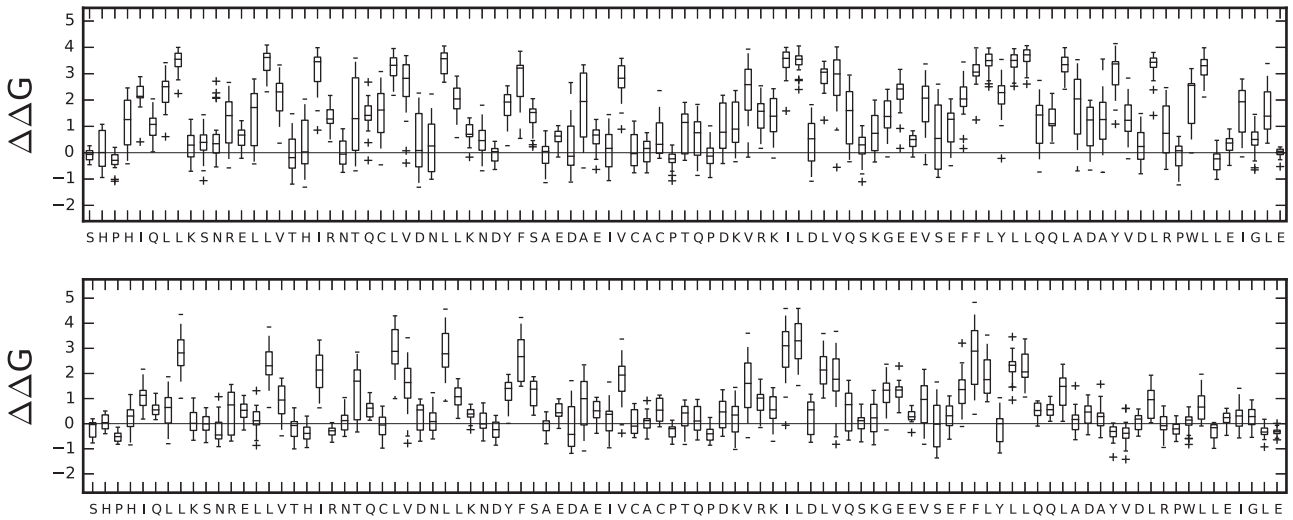

Fig. 1. Mutation sensitivity profile of the biological assembly (top) and the monomer (bottom) of Nod1 caspase activation and recruitment domain 1NFN

The fourth experiment type is the evaluation of potential disulfide bonds. In addition to $\Delta\Delta G$ values, geometric constraints are applied. Both, geometry and $\Delta\Delta G$ are combined to a disulfide bond score.

Depending on the experiment, mutations can be restricted to certain amino acid classes, exposed or buried residues and user-specified regions. Results are stored for a period of 7 days on our server. All tabular results can be downloaded as CSV files.

3 Implementation and run-time statistics

MAESTROweb is mainly implemented in JavaScript. The web server is a node.js instance, the GUI realized with the Jade toolkit and molecular graphics with JSmol (Hanson *et al.*, 2013). The MAESTRO backend is implemented as a C++ library with SWIG generated Python interfaces. Web service and backend are connected through REST-based adapters.

The run time depends on the protein size and on the number of combinatorial mutations. In Table 1, we give the approximate run times of the four provided experiment types on a small, a medium sized and a large protein. The sensitivity profile corresponds to a single-point mutation ($n = 1$) scan.

4 Example application

In order to demonstrate the importance of using multimers, we applied MAESTROweb to the Nod1 caspase activation and recruitment domain (PDB-ID 2NSN, Coussens *et al.*, 2007) using the biological assembly, which is a dimer, and the asymmetric unit, which is a monomer. The last helix undergoes a domain swapping and thus builds a tight interface between the monomers. We first performed a scan for the most stabilizing single-point mutation. The five top scoring mutations in the biological assembly are D42L, H33L, P102K, T32R and A52K, whereas in the monomer these are V98R, S83F, Y97E, S83Y and S83M. We then generated mutation sensitivity profiles, downloaded the corresponding CSV files and generated boxplots with the plotting library matplotlib for direct comparison (Fig. 1). Especially in the region of helix 5 and 6, there are considerable differences. This is the area, where helix 6 swaps the domain and strongly interacts with helix 5 from the other domain. Ignoring the multimeric state would potentially lead to wrong conclusions.

5 Conclusion

We have introduced MAESTROweb, an easy to use web service for the prediction of changes in stability upon point mutations in proteins. It provides several common types of *in silico* mutation experiments. The applicability on multimeric structures and the improved accuracy on multi-point mutations make MAESTROweb outstanding in comparison to other web tools.

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Conflict of Interest: none declared.

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