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Applications Note

Structural bioinformatics

pyFoldX: enabling biomolecular analysis and engineering along structural ensembles

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Abstract

Summary: Recent years have seen an increase in the number of structures available, not only for new proteins but also for the same protein crystallized with different molecules and proteins. While protein design software has proven to be successful in designing and modifying proteins, they can also be overly sensitive to small conformational differences between structures of the same protein. To cope with this, we introduce here pyFoldX, a python library that allows the integrative analysis of structures of the same protein using FoldX, an established forcefield and modelling software. The library offers new functionalities for handling different structures of the same protein, an improved molecular parametrization module and an easy integration with the data analysis ecosystem of the python programming language.

Availability and implementation: pyFoldX rely on the FoldX software for energy calculations and modelling, which can be downloaded upon registration in http://foldxsuite.crg.eu/ and its licence is free of charge for academics. The pyFoldX library is open-source. Full details on installation, tutorials covering the library functionality and the scripts used to generate the data and figures presented in this paper are available at https://github.com/leandroradusky/pyFoldX.

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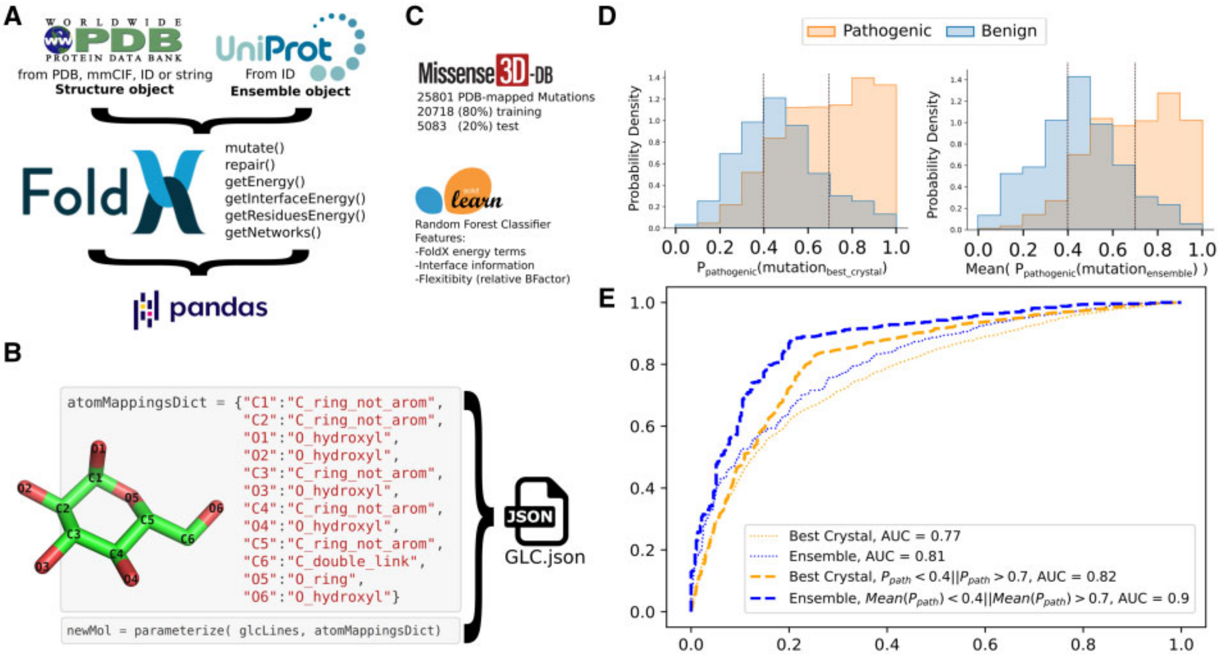
Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Outstanding advances in the experimentally determinable biomolecular space have been recently made (Berman *et al.*, 2020); also, the last CASP competition (Senior *et al.*, 2020) revealed striking improvements in the field of *in silico* biomolecular modelling. It is now increasingly likely that a protein of interest has several structures, alone or in complex with other biomolecules and ligands; however, protein design software users/researchers creating structural datasets commonly only select one of the available structures (normally the one with highest resolution and/or the highest sequence coverage) and disregard the rest. This loses the potential power of using different structures of the same protein and can result in significant differences in the analyses due to small conformational differences (Delgado Blanco *et al.*, 2020) or the presence of different molecular partners along available structures (Kiel and Serrano, 2014).

Thus, there is a need for bioinformatics tools that allow all available structures for a target protein to be considered when trying to diagnose mutational effects or to engineer new properties. These tools should take into account all structural information resulting from the formation of complexes and binding modes, as well as the distinct conformations arising from the crystallization conditions.

Here, we introduce pyFoldX, a python library powered by the FoldX suite (Delgado *et al.*, 2019; Schymkowitz *et al.*, 2005). pyFoldX enables full integration of standardized data analysis tools within the python programming language. The pyFoldX library mainly comprises two packages: (i) the **structure** package, which contains classes to handle single structures or ensembles of structures (Fig. 1A). It is fully integrated with FoldX functionality (energy measurements, mutational modelling, etc.) and stores the results of energetic calculations in pandas data frames (McKinney, 2015); and (ii) the **paramx** package, which improves a functionality introduced in the latest version of FoldX, thus allowing for user-friendly parameterization of new molecules (Fig. 1B) based on standard atom types. Using these new functionalities, we created a comprehensive database of repaired PDB structures with minimized energy and improved structural quality. Our results from analysing a comprehensive set of benign and pathogenic mutations mapped into protein structures underscored that considering the ensemble of available



```
atomMappingsDict = {"C1":"C_ring_not_arom",
                    "C2":"C_ring_not_arom",
                    "O1":"O_hydroxyl",
                    "O2":"O_hydroxyl",
                    "C3":"C_ring_not_arom",
                    "O3":"O_hydroxyl",
                    "C4":"C_ring_not_arom",
                    "O4":"O_hydroxyl",
                    "C5":"C_ring_not_arom",
                    "C6":"C_double_link",
                    "O5":"O_ring",
                    "O6":"O_hydroxyl"}
```

```
newMol = parameterize( glcLines, atomMappingsDict)
```

Fig. 1. (**A**) pyFoldx structure-handling capabilities. Single structures can be instantiated from different formats, while ensembles of structures of the same protein can be instantiated from the protein's UniProt accession. FoldX commands can be executed into structures and ensembles, returning pandas dataframes with energies and, if applicable, objects with the transformed structures. (**B**) Example of parametrization of a glucose molecule with the pyFoldX paramx package. (**C**) Analysed mutations dataset description. To train a random forest classifier, 80% of the Missense3D-DB mutations were used in order to estimate the probability of belonging to the 'pathogenic' category. The remaining 20% were used for testing and analysed by using the indicated structure in the database and the ensemble of good resolution structures for these proteins. (**D**) Histogram of probability of belonging to the 'pathogenic' category given by the created classifier for mutations mapped into their best structure by Missense3D-DB (left) and the mean of the probabilities for all crystals of good resolution along its ensemble (right). (**E**) ROC curve of mutation class prediction by the generated classifier taking into account best crystal (orange lines) or mean predictions for crystals along ensemble (blue lines). Thin lines: classifying mutations as pathogenic ($P_{pathogenic} > 0.5$) or benign ($P_{pathogenic} \leq 0.5$). Thick lines: mutations with no clear prediction are discarded ($0.4 > P_{pathogenic} > 0.7$). Overall, predictions are better when ensembles are considered and high accuracy is achieved (AUC = 0.9) when no clear predictions are discarded from the analysis

structures can improve the FoldX diagnosis. We have made the pyFoldX code open to the public and encourage the community to extend the library and/or request new specific features.

2 Functionality and results

2.1 Molecule parameterization

A key feature that was introduced in the last version of FoldX was the possibility to parameterize small molecules not previously recognized by the software; this feature was effectively used on the parameterization of RNA bases. We have now improved it in pyFoldX by allowing the auto parameterization of molecules through the simple definition of template atoms (Supplementary Table S1). This feature will permit users to introduce new organic molecules in the FoldX force field using atoms that fit into the template atom set provided. A complete tutorial that demonstrates the parameterization of a glucose molecule complexed with a lectin is available online (Tutorials section of project's GitHub).

2.2 Mutational effect diagnosis

Taking advantage of the integration of pyFoldX with pandas, we built a random forest classifier (n_estimators = 500) using the sklearn library (Trappenberg, 2019). This allowed us to assess the accuracy of FoldX energetic features, along with other structural annotations, as well as to discriminate between benign and pathogenic mutations contained in the Missense3D-DB (Khanna *et al.*, 2021). This dataset contains 31 283 structurally mapped non-redundant variants and its publicly available in the Supplementary Material of the cited paper. We trained the classifier with a random set encompassing 80% of the annotated mutations (this procedure was repeated several times for different randomly train and test dataset with no significant differences), with the remaining 20% used for testing (Fig. 1C). We then analysed mutations along the ensemble of good resolution structures (resolution <2.0 Å) for the same set of mutations. Based on the classifier probabilities for a mutation to belong to the 'pathogenic' or 'benign' category ($P_{pathogenic} = 1\text{-}P_{benign}$), we found that mutations classified as benign tend to keep such classification along the ensemble of available structures for their proteins. This allowed us to achieve a better separation in the histogram of probabilities from both types of mutations (Fig. 1D). Notably, we observed a better discriminative power when we analysed structural ensembles than when we used the best structure (e.g. in terms of resolution), giving us a highly accurate classification (AUC = 0.9; Fig. 1E).

Note that the pyFoldX's GitHub webpage include: (i) tutorials on how features that feed the classifier are computed; (ii) the datasets of these features along single structures and ensembles mutations and (iii) scripts showing how the random forest classifier was created and used.

2.3 FoldX-repaired protein DataBank

By combining the PDB_REDO (Joosten *et al.*, 2014) structure refinement method with the FoldX RepairPDB command for side-chain energy minimization, we designed a pipeline using pyFoldX to refine the quality for virtually all the structures in the Protein Data Bank (Berman *et al.*, 2007; Supplementary Fig. S2). We first examined the structures to determine if they contained organic compounds not recognized by FoldX; we then parameterized the most frequent ones. Supplementary Table S2 shows the list of new molecules now parameterized and recognized by FoldX.

In general, the resulting repaired structures presented lower energies when evaluated with independent force fields (Alford *et al.*, 2017; Yang and Zhou, 2008; Supplementary Fig. S2A) as well as improved structural quality features (as measured with WHAT_CHECK; Dunbrack, 2004; Supplementary Fig. S2B). If any of the independent force fields reported higher energies with respect to the original entries, the structures were further analysed to determine potential flaws in FoldX modelling. The predicted high energies were due proline rotamers placed by FoldX but penalized by these force fields (Supplementary Fig. S3). FoldX uses a probabilistic method that depends on the previous phi, psi and chi angles to generate rotamers; therefore, it is possible that some of the generated rotamers have a poor representation in the PDB structures. Note that all the generated structure files are available online for downloading or as object instantiation through pyFoldX structure package.

3 Discussion and perspectives

Here, we present pyFoldX, a novel library fusing FoldX modelling functionalities within the python programming language. pyFoldX enables easy analyses of extensive structural datasets, and in-depth energy analyses and modelling along the ensemble of structures available for a single protein, in a user-friendly manner. Notably, comprehensive analysis using pyFoldX of mispredicted energy changes versus experimentally measured mutations should lead to improvements in the FoldX sidechain modelling routines and force field. Mutational effect diagnosis can now be improved by incorporating other non-structural features, and other machine learning strategies can be investigated to improve the built classifier. The possibility to parameterize novel organic molecules presents opportunities for ligand binding modelling; notably for carbohydrate molecules, given the ability of pyFoldX to handle mmCIF formatted files that correct historic errors on these kinds of interactions (Feng *et al.*, 2021). Overall, we believe that this tool will provide the structural biology community with a more powerful way to access FoldX functionalities, useful for instance for determining effects of mutations or for engineering new properties into proteins/protein–ligand complexes.

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Conflict of Interest: none declared.

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