Version 2

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Prediction of ligand binding using FunFOLD2 V.2

In 1 collection

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ABSTRACT

Clues to functionality can be gleaned from comparing unknown or predicted structures with previously characterized structures of known function and characteristics. These scans can be biased against novel proteins or proteins with similar structures but distinct functions, but for initial guesses can be powerful. These methods align the structure and/or amino acid sequence to a database of structures with known ligand binding sites and look for structures with the similarity in amino acid composition, position, and overall 3-D similarity. The idea being that similar structures lead to similar functions. This protocol describes the use of the FunFOLD2 server which predicts ligand identity and binding site from the amino acid sequence.

PROTOCOL CITATION

Chris Berndsen 2021. Prediction of ligand binding using FunFOLD2. **protocols.io** https://protocols.io/view/prediction-of-ligand-binding-using-funfold2-bw9vph66 Version created by Chris Berndsen

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Roche, D. B., Buenavista, M. T., and McGuffin, L. J. (2013) The FunFOLD2 server for the prediction of protein-ligand interactions. Nucleic Acids Res. 41, W303–7.

COLLECTIONS

Biochemistry I methods

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52245

PARENT PROTOCOLS

Part of collection
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MATERIALS TEXT

A protein sequence in one letter code
Internet connection
Molecular visualization program

Uploading sequence

1 Navigate to the FunFOLD2 server submission form.

2 Paste in your sequence in single letter amino acid code.

3 Provide an email address and a short name for your protein. Record the short name as a note in this step.

4 Press Predict to submit your sequence.

FunFOLD takes 24 to 72 hours to respond. You should record the results link as a note in this step in case you don't get the email.

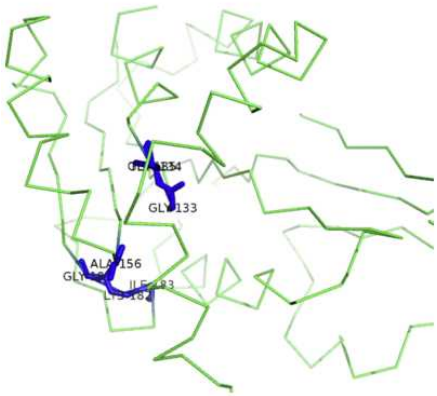
Analyzing data

5 When the results are ready there will be four areas of results.

6 At top is the predicted binding site in a homology model. The binding site is shown and labeled to be the amino acid numbers in the submitted sequence.

Download the structure and view it in YASARA.

PyMOL generated image of ligand binding residues prediction for test



Click here to download PDB file of this model with the superposition of all identified ligands.

6.1 Save your downloaded file as

```
[date]_[sequencename]_[groupname]_FunFOLD2.pdb
```

Replace **[Group_name]** with your name/group name without the brackets. Replace **[sequence_name]** with the name of the sequence.

6.2 Indicate your file location as a link within a note on this step.

7 Below this is information on the ligand identity and the binding site. The ligand identity is abbreviated as it would be in a crystal structure.

Predicted ligand binding residues are shown as blue sticks in the image above.

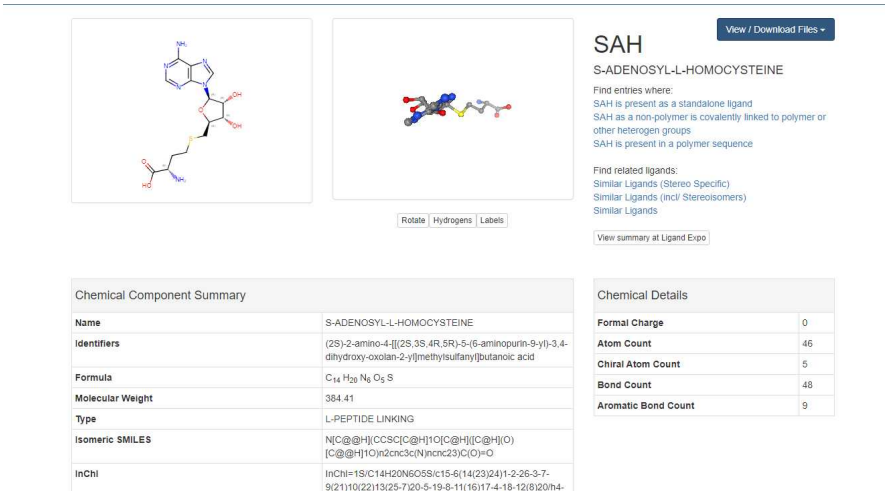
Binding site: 133, 134, 135, 156, 181, 182, 183
Most likely ligand (Type): SAH
Centroid ligand (Type): SAH
All ligands clustered at site (Type-Number): SAH-6

If there are multiple potential ligands, you will need to search each individually.

7.1 To identify the ligand, in a new broswer window go to the RCSB (www.rcsb.org) and in the search bar, look for you ligand name and wait for the search options to drop down (do not press enter, just input the code and wait).

Select in Chemical IDs to search the ligands database.

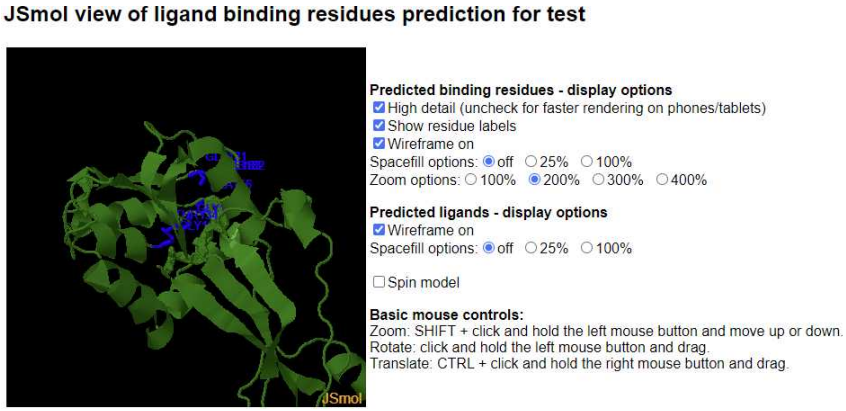
7.2 The search should take you to an information page on the ligand lots of information and the name.



7.3 Record the ligand binding residues and other statistics as well as the ligand identity. Add rows as needed.

Ligand binding residue(s)	
Ligand code(s)	
Ligand identity(ies)	

8 Below the ligand information is a JSmol window for viewing the ligand and binding site in the browser. This is convienient for when you do not have a molecular visualization software available.



9 The last section contains the quality of the fit.

FunFOLDQA scores:

BDTalign = 0.33695015486337826
Identity = 0.19236092
Rescaled BLOSUM62 = 0.2044577
Equivalent Residue Ligand Distance = 0.31665778
Model Quality = 0.86413
Predicted BDT score = 0.5674837
Predicted MCC score = 0.4127715

9.1 Explanation of scores:

A	B
BDTalign	Comparison of the structural match in 3-D between known binding site and modeled binding site. 1 indicates a perfect prediction, while 0 is associated with a random and unreliable prediction.
Identity	Number of amino acids in the binding site that are equivalent to the known binding site. 1 is a perfect match, 0 is imperfect match
Rescaled BLOSUM62	An additional measure of binding site sequence match using the BLOSUM62 algorithm. 1 is a perfect match, 0 is an imperfect match.
Equivalent Residue Ligand Distance	For the amino acids that are equivalent, this measure looks at how well the distances match. 1 is a perfect match, 0 is an imperfect match.
Model Quality	Score based on the ModFOLDclust2 algorithm to give a sense of the overall match of the model. 1 is a perfect match, 0 is an imperfect match.
Predicted BDT score	Binding site distance test indicates how far a predicted binding site residue is from the observed binding residue location. 1 indicates a perfect prediction, while 0 is associated with a random and unreliable prediction.
Predicted MCC score	Matthews Correlation Coefficient score is a statistic for looking at accuracy of predicted vs. observed binding site amino acids. 1 indicates a perfect prediction, while 0 is associated with a random and unreliable prediction.

Information from Roche, D. B., Buenavista, M. T., and McGuffin, L. J. (2012) FunFOLDQA: a quality assessment tool for protein-ligand binding site residue predictions. *PLoS One7*, e38219.

9.2 Record the scores of the prediction below:

BDTalign	
Identity	
Rescaled BLOSUM62	
Equivalent Residue Ligand Distance	
Model Quality	
Predicted BDT score	
Predicted MCC score	

10 If you have homology models from other methods (Phyre2, SWISS-Model, etc.) and refined any of those models, it is worth aligning the model with the ligand bound from go to step #6 to all of these models to compare the

predicted binding site.

Different programs may predict different conformations for the binding site and by comparing the different models, the reliability of the prediction can be further assessed. If there are distinct binding site shapes, then the prediction may be less reliable.

11 Export this protocol as a PDF and save it in your project folder.