

WEBLEM 4

Introduction to Validation server – SAVES server

Homology model, threading method and ab-initio method of tertiary structure prediction all have to be evaluated to make sure that the structural features of the model are consistent with the physicochemical rules. This involves checking anomalies in ϕ - ψ angles, bond lengths, close contacts, and so on. Another way of checking the quality of a protein model is to implicitly take these stereochemical properties into account. This is a method that detects errors by compiling statistical profiles of spatial features and interaction energy from experimentally determined structures. By comparing the statistical parameters with the constructed model, the method reveals which regions of a sequence appear to be folded normally and which regions do not. If structural irregularities are found, the region is considered to have errors and has to be further refined.

SAVES server:

SAVES server contains various tools for structure validation that are all integrated in one single server. The tool available are:

ERRAT:

A novel method for differentiating between correctly and incorrectly determined regions of protein structures based on characteristic atomic interaction is described. Different types of atoms are distributed nonrandomly with respect to each other in proteins. Errors in model building lead to more randomized distributions of the different atom types, which can be distinguished from correct distributions by statistical methods. Atoms are classified in one of three categories: carbon (C), nitrogen (N), and oxygen (O). This leads to six different combinations of pairwise noncovalently bonded interactions (CC, CN, CO, NN, NO, and OO). A quadratic error function is used to characterize the set of pairwise interactions from nine-residue sliding windows in a database of 96 reliable protein structures. Regions of candidate protein structures that are mistraced or misregistered can then be identified by analysis of the pattern of nonbonded interactions from each window.

Errat is a program for verifying protein structures determined by crystallography. Error values are plotted as a function of the position of a sliding 9-residue window. The error function is based on the statistics of non-bonded atom-atom interactions in the reported structure (compared to a database of reliable high-resolution structures).

A plot of an initial model and a final model is retrieved. Regions of the structure that can be rejected at the 95% confidence level are yellow; 5% of a good protein structure is expected to have an error value above this level. Regions that can be rejected at the 99% level are shown in red. Generally speaking, the method is sensitive to smaller errors than 3-D Profile analysis.

Verify3D:

It is another server using the statistical approach. It uses a precomputed database containing eighteen environmental profiles based on secondary structures and solvent exposure, compiled from high-resolution protein structures. To assess the quality of a protein model, the secondary structure and solvent exposure propensity of each residue are calculated. It determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc) and comparing the results to good structures. If the parameters of a residue fall within one of the profiles, it receives a high score, otherwise a low score. The result is a two-dimensional graph illustrating the folding quality of each residue of the protein structure. The threshold value is normally set at zero. Residues with scores below zero are considered to have an unfavourable environment.

PROVES:

It calculates the volumes of atoms in macromolecules using an algorithm which treats the atoms like hard spheres and calculates a statistical Z-score deviation for the model from highly resolved (2.0 Å or better) and refined (R-factor of 0.2 or better) PDB-deposited structures.

Standard ranges of atomic and residue volumes are computed in 64 highly resolved and well-refined protein crystal structures using the classical Voronoi procedure. Deviations of the atomic volumes from the standard values, evaluated as the volume Z-scores, are used to assess the quality of protein crystal structures. To score a structure globally, we compute the volume Z-score root mean square deviation (Z-score rms), which measures the average magnitude of the volume irregularities in the structure. We find that the Z-score rms decreases as the resolution and R-factor improve, consistent with the fact that these improvements generally reflect more accurate models. From the Z-score rms distribution in structures with a given resolution or R-factor, we determine the normal limits in Z-score rms values for structures solved at that resolution or R-factor. Structures whose Z-score rms exceeds these limits are considered as outliers. Such structures also exhibit unusual stereochemistry, as revealed by other analyses. Absolute Z-scores of individual atoms are used to identify problems in specific regions within a protein model. These Z-scores correlate fairly well with the atomic B-factors, and atoms having absolute Z-scores > 3, occur at or near regions in the model where programs such as PROCHECK identify unusual stereochemistry. Atomic volumes, themselves not directly restrained in crystallographic refinement, can thus provide an independent, rather sensitive, measure of the quality of a protein structure.

WHAT_CHECK:

Derived from a subset of protein verification tools from the WHAT IF program, this does extensive checking of many stereochemical parameters of the residues in the model. WHAT IF is a comprehensive protein analysis server that validates a protein model for chemical correctness. It has many functions, including checking of planarity, collisions with symmetry axes (close contacts), proline puckering, anomalous bond angles, and bond lengths. It also allows the generation of Ramachandran plots as an assessment of the quality of the model.

PROCHECK:

It is a UNIX program that is able to check general physicochemical parameters such as ϕ - ψ angles, chirality, bond lengths, bond angles, and so on. It checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry. The parameters of the model are used to compare with those compiled from well-defined, high-resolution structures. If the program detects unusual features, it highlights the regions that should be checked or refined further.

CRYST:

This program searches the Protein Data Bank for entries that have a unit cell similar to your input file. CRYST1 record required. Use the standalone CRYST server for more options.

The assessment results can be different using different verification programs. Because no single method is clearly superior to any other, a good strategy is to use multiple verification methods and identify the consensus between them. It is also important to keep in mind that the evaluation tests performed by these programs only check the stereochemical correctness, regardless of the accuracy of the model, which may or may not have any biological meaning. Thus, SAVES server is an excellent platform that provides various validation methods to accurately validate the structures.

REFERENCES:

1. SAVESv6.0 - Structure Validation Server. (n.d.-b). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/>
2. ERRAT – UCLA-DOE Institute. (n.d.). Retrieved March 8, 2022, from <https://www.doembi.ucla.edu/errat/>

3. Chris Colovos; Todd O. Yeates (1993). Verification of protein structures: Patterns of nonbonded atomic interactions. , 2(9), 1511–1519. doi:10.1002/pro.5560020916
4. Xiong, J. (2008).Tertiary structure prediction. Essential bioinformatics. Cambridge: Cambridge University Press. 220-222.
5. Joan Pontius; Jean Richelle; Shoshana J. Wodak (1996). Deviations from Standard Atomic Volumes as a Quality Measure for Protein Crystal Structures. , 264(1), 0–136. doi:10.1006/jmbi.1996.0628

WEBLEM 4a

SAVES server

(URL: <https://saves.mbi.ucla.edu/>)**AIM:**

To validate structure qseq.B99990005 generated from modeller.

Introduction:

qseq.B99990005 is the structure predicted using homology modelling using modeller. The structure has to be evaluated to make sure that the structural features of the model are consistent with the physicochemical rules. This can be done using SAVES server.

SAVES is a structure validation server that has various tools like Errat, Verify3D, Prove, Whatcheck, Procheck and Cryst integrated in one single platform. This involves checking anomalies in ϕ - ψ angles, bond lengths, close contacts, and so on. Another way of checking the quality of a protein model is to implicitly take these stereochemical properties into account. This is a method that detects errors by compiling statistical profiles of spatial features and interaction energy from experimentally determined structures. By comparing the statistical parameters with the constructed model, the method reveals which regions of a sequence appear to be folded normally and which regions do not. If structural irregularities are found, the region is considered to have errors and has to be further refined.

METHODOLOGY:

1. Open homepage for SAVES server. (URL: <https://saves.mbi.ucla.edu/>)
2. Upload structure retrieved from Modeller in PDB format.
3. Obtain results for Errat, Verify3D, Prove, Whatcheck and Procheck.
4. Observe and interpret the results.

OBSERVATION:

UCLA-DOE LAB — SAVES v6.0

**To run any or all programs:
upload your structure, in PDB format only**

Choose File No file chosen

Run programs

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

PROCHECK

- PROCHECK source information
- Result analysis

Fig1. Homepage for SAVES server

To run any or all programs:
upload your structure, in PDB format only

qseq.B99990005.pdb

Customize job name:

qseq.B99990005.pdb

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

Fig2. Structure from Modeller for validation

Job 937164 has been created

job #937164: qseq.B99990005.pdb [\[job link\]](#) [\[3D Viewer\]](#)

<p>ERRAT Complete</p> <p><u>Overall Quality Factor</u></p> <p>16.1491</p> <p><input type="button" value="Results"/></p>	<p>VERIFY Complete</p> <p>51.33% of the residues have averaged 3D-1D score >= 0.2</p> <p>Fail</p> <p>Fewer than 80% of the amino acids have scored >= 0.2 in the 3D/1D profile.</p> <p><input type="button" value="Results"/></p>	<p>PROVE Complete</p> <p>Buried outlier protein atoms total from 1 Model: 11.2%</p> <p>fail</p> <p><input type="button" value="Results"/></p>																																																																	
<p>WHATCHECK Complete</p> <table border="1"> <tr><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td><td>13</td></tr> <tr><td>14</td><td>15</td><td>16</td><td>17</td><td>18</td><td>19</td><td>20</td><td>21</td><td>22</td><td>23</td><td></td><td></td><td></td></tr> <tr><td>24</td><td>25</td><td>26</td><td>27</td><td>28</td><td>29</td><td>30</td><td>31</td><td>32</td><td>33</td><td></td><td></td><td></td></tr> <tr><td>34</td><td>35</td><td>36</td><td>37</td><td>38</td><td>39</td><td>40</td><td>41</td><td>42</td><td>43</td><td></td><td></td><td></td></tr> <tr><td>44</td><td>45</td><td>46</td><td>47</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr> </table> <p><input type="button" value="Results"/></p>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23				24	25	26	27	28	29	30	31	32	33				34	35	36	37	38	39	40	41	42	43				44	45	46	47										<p>PROCHECK Complete</p> <p>Out of 8 evaluations</p> <ul style="list-style-type: none"> • Errors: 5 • Warning: 1 • Pass: 2 <p><input type="button" value="Results"/></p>	<p>Almost ready, check back soon</p>
1	2	3	4	5	6	7	8	9	10	11	12	13																																																							
14	15	16	17	18	19	20	21	22	23																																																										
24	25	26	27	28	29	30	31	32	33																																																										
34	35	36	37	38	39	40	41	42	43																																																										
44	45	46	47																																																																

References

Fig3. Result page for structure validation for various servers

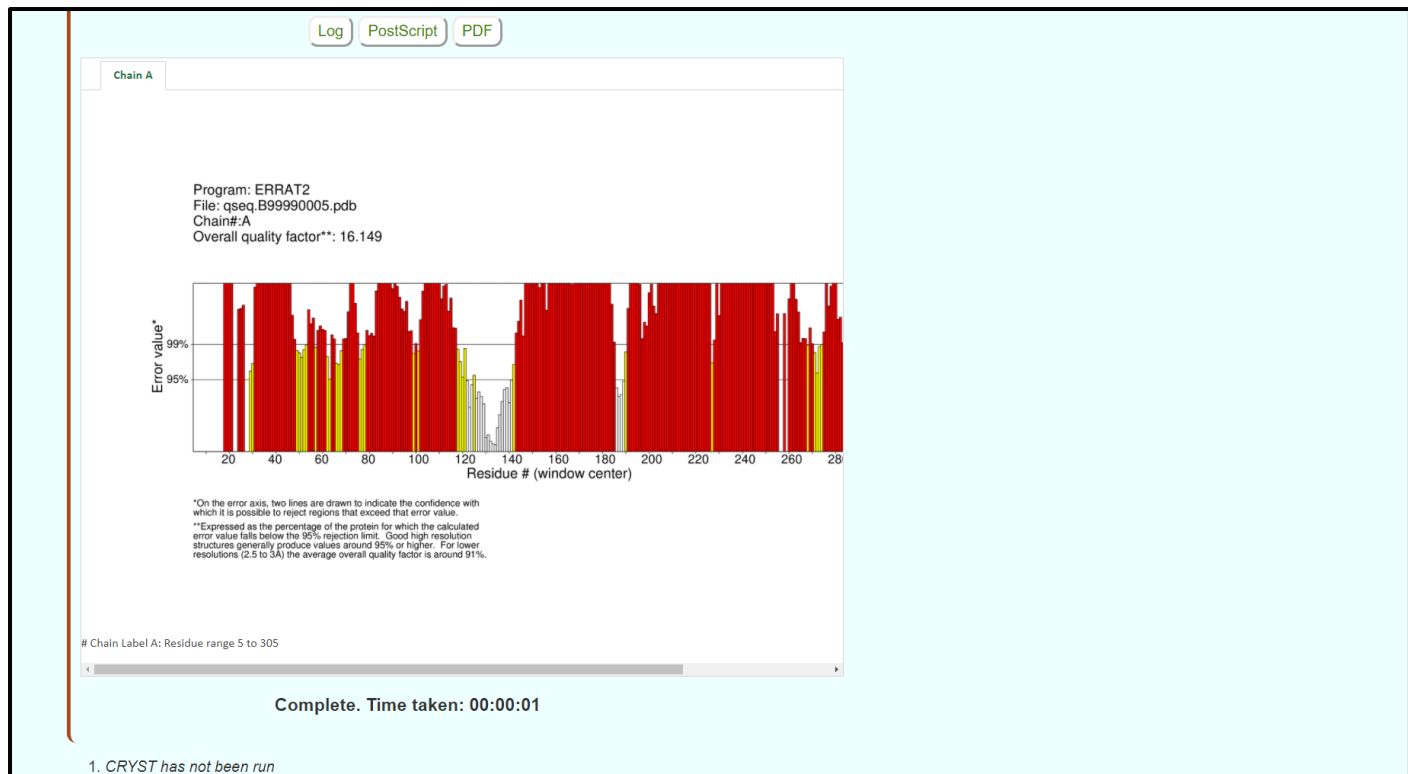


Fig4. Result page for ERRAT

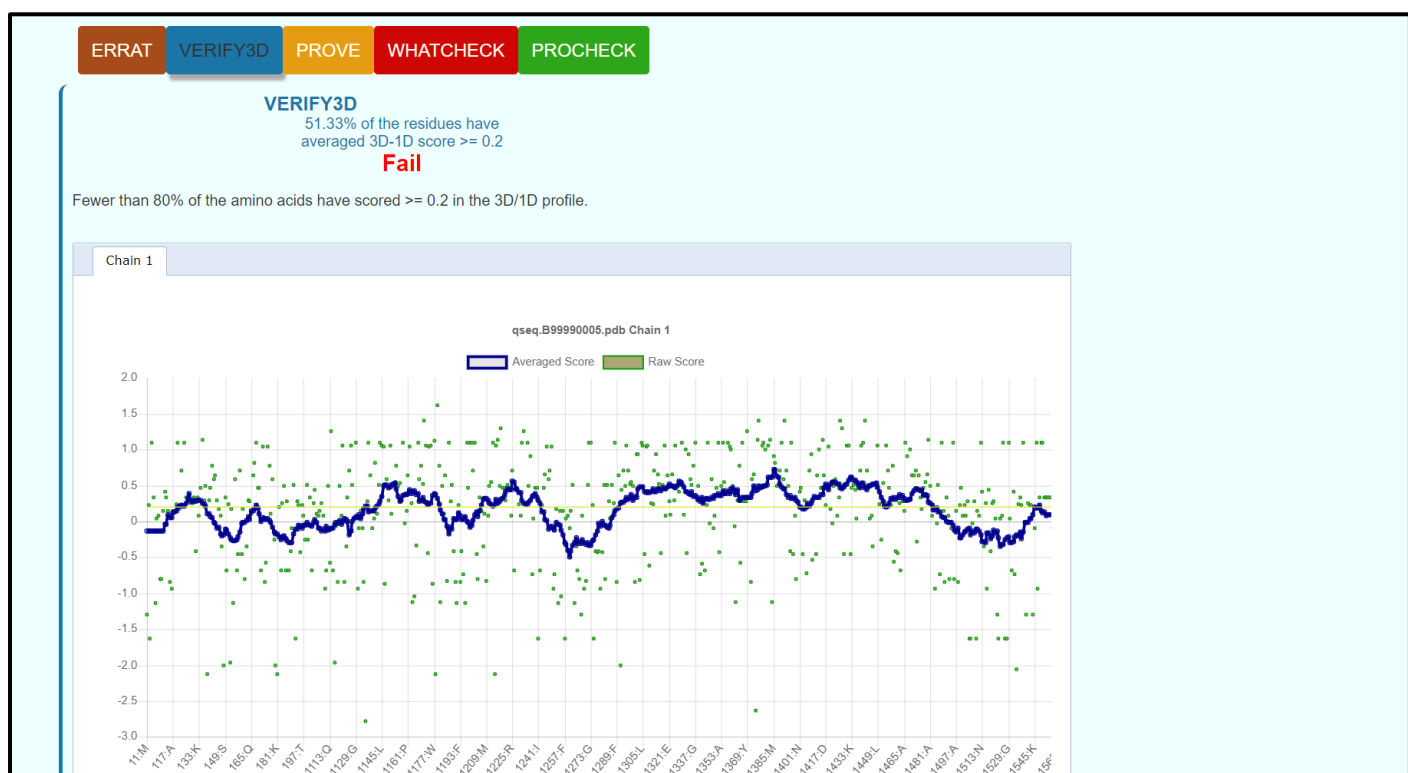


Fig5. Result page for Verify3D

ERRAT

VERIFY3D

PROVE

WHATCHECK

PROCHECK

PROVE

Original plot files: PDF PostScript

Page 1

Plotted data (CIF)

Calculations

Job run log

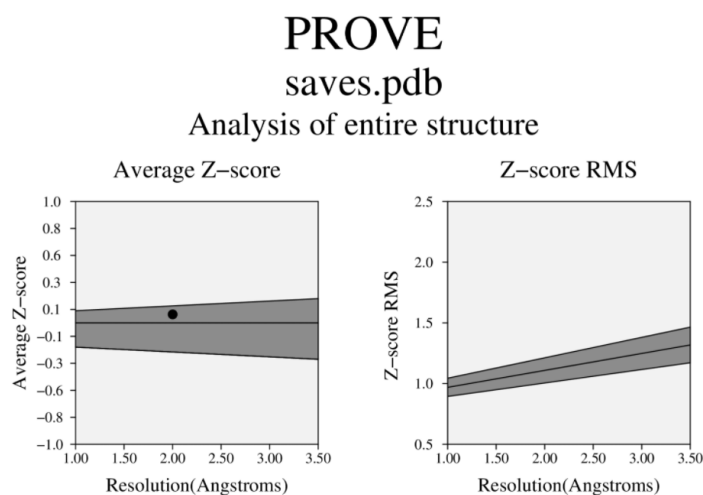


Fig6. Result page for Prove

ERRAT

VERIFY3D

PROVE

WHATCHECK

PROCHECK

WHATCHECK

Headings

1 2 3 4 5
6 7 8 9 10
11 12 13 14
15 16 17 18
19 20 21 22
23 24 25 26
27 28 29 30
31 32 33 34
35 36 37 38
39 40 41 42
43 44 45 46
47

#33. Error: Abnormally short interatomic distances

Error: Abnormally short interatomic distances
The pairs of atoms listed in the table below have an unusually short distance.

The contact distances of all atom pairs have been checked. Two atoms are said to 'bump' if they are closer than the sum of their Van der Waals radii minus 0.40 Angstrom. For hydrogen bonded pairs a tolerance of 0.55 Angstrom is used. The first number in the table tells you how much shorter that specific contact is than the acceptable limit. The second distance is the distance between the centers of the two atoms.

The last text-item on each line represents the status of the atom pair. The text 'INTRA' means that the bump is between atoms that are explicitly listed in the PDB file. 'INTER' means it is an inter-symmetry bump. If a line contains the text 'HB', the bump criterium was relaxed because there could be a hydrogen bond. If the text 'BF' is present, the sum of the B-factors of the atoms is higher than 80, which makes the appearance of the bump somewhat less severe because the atoms probably aren't there anyway.

Bumps between atoms for which the sum of their occupancies is lower than one are not reported. In any case, each bump is listed in only one direction.

212	THR	(212)	A	N	--	220	LYS	(220)	A	N	0.443	2.557	INTRA	BF
212	THR	(212)	A	N	--	221	LEU	(221)	A	N	0.404	2.596	INTRA	BF
394	ALA	(394)	A	C	--	398	LYS	(398)	A	CD	0.389	2.811	INTRA	BF
249	PHE	(249)	A	CA	--	386	ILE	(386)	A	CG2	0.380	2.820	INTRA	BF
397	GLU	(397)	A	N	--	398	LYS	(398)	A	CG	0.378	2.722	INTRA	BF
395	ARG	(395)	A	N	--	398	LYS	(398)	A	CB	0.373	2.727	INTRA	BF
395	ARG	(395)	A	C	--	398	LYS	(398)	A	CG	0.360	2.840	INTRA	BF
214	LYS	(214)	A	CD	--	216	TYR	(216)	A	C	0.354	2.846	INTRA	BF
211	ALA	(211)	A	C	--	219	LYS	(219)	A	C	0.351	2.849	INTRA	BF
123	CYS	(123)	A	SG	--	124	SER	(124)	A	N	0.349	2.951	INTRA	BF
247	SER	(247)	A	N	--	419	PHE	(419)	A	CB	0.345	2.755	INTRA	BF
212	THR	(212)	A	C	--	220	LYS	(220)	A	N	0.345	2.755	INTRA	BF
214	LYS	(214)	A	CD	--	218	CYS	(218)	A	N	0.335	2.765	INTRA	BF
396	GLY	(396)	A	N	--	398	LYS	(398)	A	CG	0.333	2.767	INTRA	BF
267	MET	(267)	A	SD	--	268	THR	(268)	A	CG2	0.331	3.069	INTRA	BF

Fig7. Result page for Whatcheck

PROCHECK

Out of 8 evaluations

- Errors: 5
- Warning: 1
- Pass: 2

The evaluations are the '+' (Warning) and '*' (Error) in the summary. The categories on the left do not always correspond in number due to PROCHECK output documents.

Summary
Ramachandran plot Error
All Ramachandrans Error
Chi1-chi2 plots Pass
Main-chain params
Side-chain params Error
Residue properties Pass
Bond len/angle Pass
M/c bond lengths
M/c bond angles
Planar groups Pass
Program output

```

+-----<<< P R O C H E C K   S U M M A R Y   >>>-----+
| /var/www/SAVES/3jobs/937164/saves.pdb  1.5              563 residues |
| * Ramachandran plot:  79.6% core  14.5% allow  4.1% gener  1.8% disall |
| * All Ramachandrans:  50 labelled residues (out of 561) |
| * Chi1-chi2 plots:    11 labelled residues (out of 362) |
| * Side-chain params:   5 better    0 inside    0 worse   |
| * Residue properties: Max.deviation:   6.4          Bad contacts:  32 |
| *                   Bond len/angle:  13.2        Morris et al class: 1 1 2 |
| + G-factors          Dihedrals:  -0.17 Covalent:  -0.73 Overall:  -0.36 |
| Planar groups:      100.0% within limits  0.0% highlighted |
+-----+
| + May be worth investigating further.  * Worth investigating further. |

```

Summary file

Complete. Time taken: 00:00:21

Fig8. Result page for procheck

Ramachandran plot Error
All Ramachandrans Error
Chi1-chi2 plots Pass
Main-chain params
Side-chain params Error
Residue properties Pass
Bond len/angle Pass
M/c bond lengths
M/c bond angles
Planar groups Pass
Program output

Main Ramachandran plot

- Page 1
- PDF
- PostScript

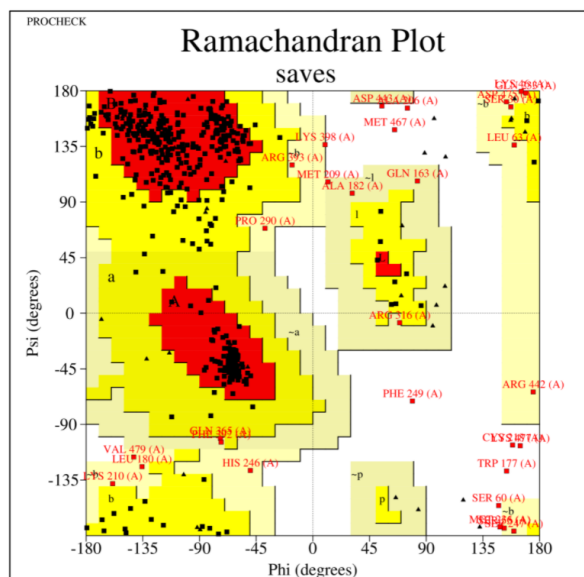


Fig9. Result page for Ramachandran plot

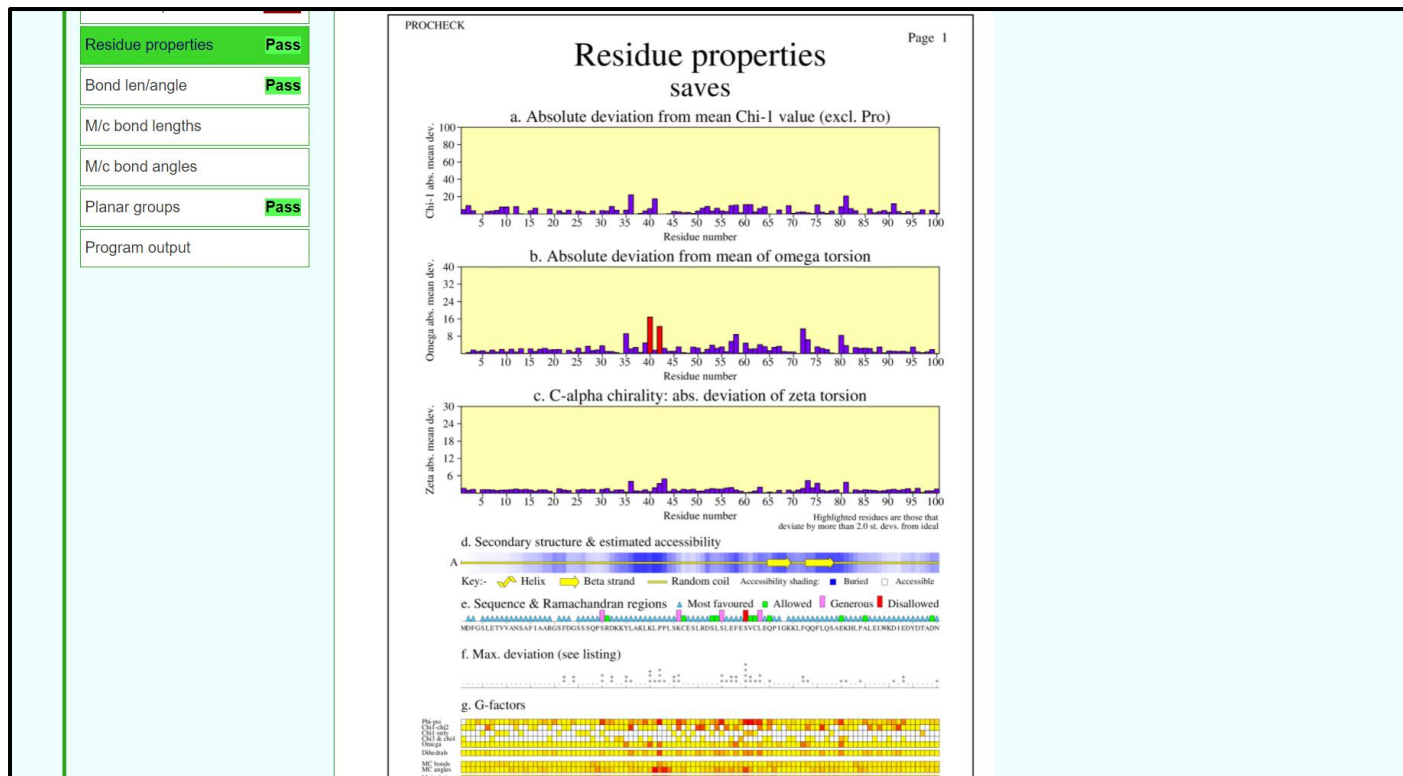


Fig10. Result page for residue properties

RESULT:

The structure predicted for enzyme kinase by homology modelling using modeller was validated using SAVES server.

CONCLUSION:

SAVES is an integrated server containing various tools on a single platform that can be used for tertiary structure validation. The predicted structure for rhodopsin by modeller failed the validation thus, I-TASSER based on threading approach will be used to predict a better structure and will be validated again using SAVES server.

REFERENCES:

1. Xiong, J. (2008). Tertiary structure prediction. Essential bioinformatics. Cambridge: Cambridge University Press. 220-222.
2. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/>
3. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/?job=924086>

WEBLEM 4b

SAVES server

(URL:<https://saves.mbi.ucla.edu/>)

AIM:

To validate structure model1 generated from I-TASSER server.

INTRODUCTION:

Model1 is the structure predicted using threading approach using I-TASSER. The structure has to be evaluated to make sure that the structural features of the model are consistent with the physicochemical rules. This can be done using SAVES server.

SAVES is a structure validation server that has various tools like Errat, Verify3D, Prove, Whatcheck, Procheck, and Cryst integrated in one single platform. This involves checking anomalies in ϕ - ψ angles, bond lengths, close contacts, and so on. Another way of checking the quality of a protein model is to implicitly take these stereochemical properties into account. This is a method that detects errors by compiling statistical profiles of spatial features and interaction energy from experimentally determined structures. By comparing the statistical parameters with the constructed model, the method reveals which regions of a sequence appear to be folded normally and which regions do not. If structural irregularities are found, the region is considered to have errors and has to be further refined.

METHODOLOGY:

1. Open homepage for SAVES server. (URL: <https://saves.mbi.ucla.edu/>)
2. Upload structure retrieved from I-TASSER in PDB format.
3. Obtain results for Errat, Verify3D, Prove, Whatcheck and Procheck.
4. Observe and interpret the results.

OBSERVATION:

UCLA-DOE LAB — SAVES v6.0

**To run any or all programs:
upload your structure, in PDB format only**

Choose File No file chosen

Run programs

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

PROCHECK

- PROCHECK source information
- Result analysis

Fig1. Homepage for SAVES server

UCLA-DOE LAB — SAVES v6.0

UCLA

To run any or all programs:
upload your structure, in PDB format only

Choose File model1.pdb

Customize job name:

model1.pdb

Run programs

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

Fig2. Structure from Modeller for validation

UCLA-DOE LAB — SAVES v6.0

UCLA

Job 937181 has been created

New Job

job #937181: model1.pdb [job link] [3D Viewer]

ERRAT Complete

Overall Quality Factor

97.0588

Results

VERIFY Complete

75.86% of the residues have averaged 3D-1D score ≥ 0.2

Fail

Fewer than 80% of the amino acids have scored ≥ 0.2 in the 3D/1D profile.

Results

PROVE Complete

Buried outlier protein atoms total from 1 Model: 4.2%

warning

Results

WHATCHECK Complete

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23			
24	25	26	27	28	29	30	31	32	33			
34	35	36	37	38	39	40	41	42	43			
44	45	46	47									

Results

PROCHECK Complete

Out of 8 evaluations

- Errors: 6
- Warning: 0
- Pass: 2

Results

Almost ready, check back soon

References

Fig3. Result page for structure validation for various servers

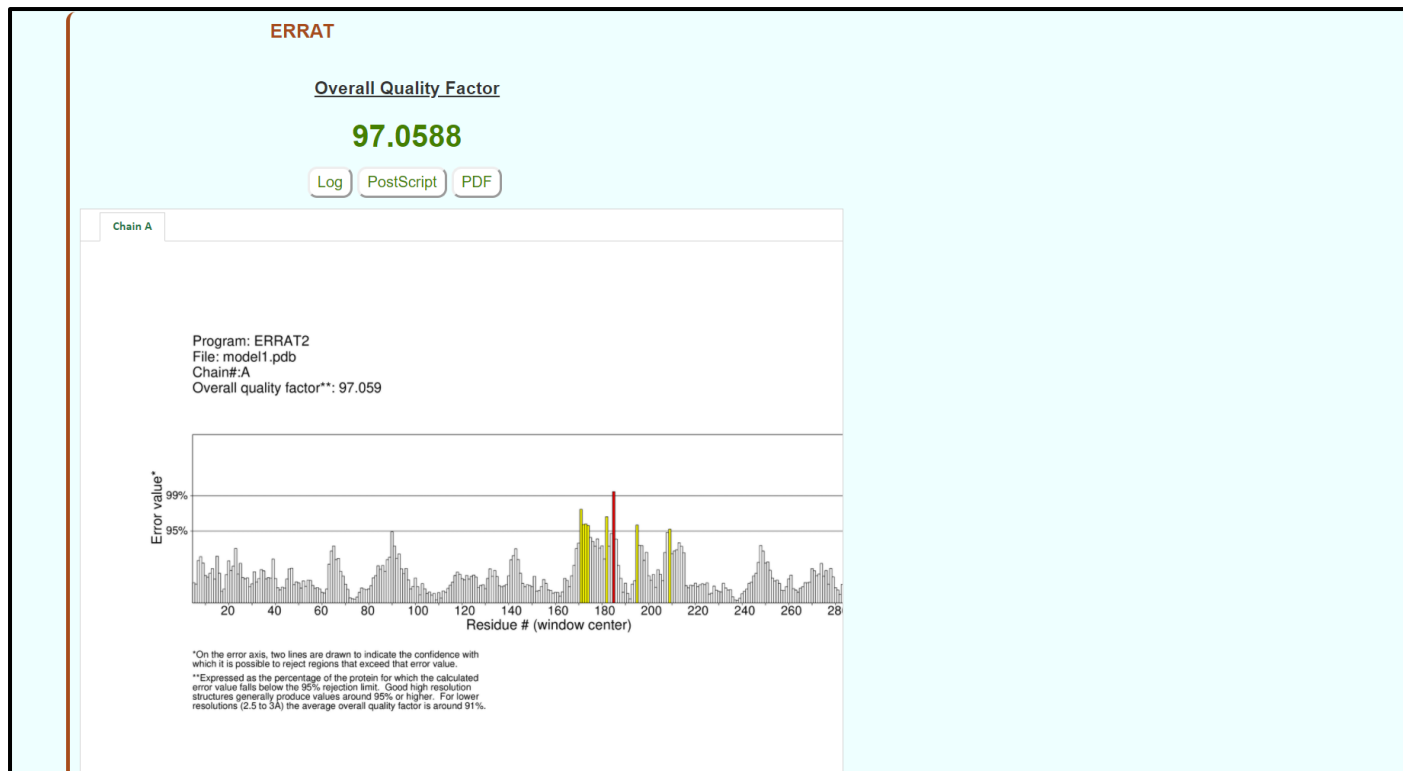


Fig4. Result page for Errat

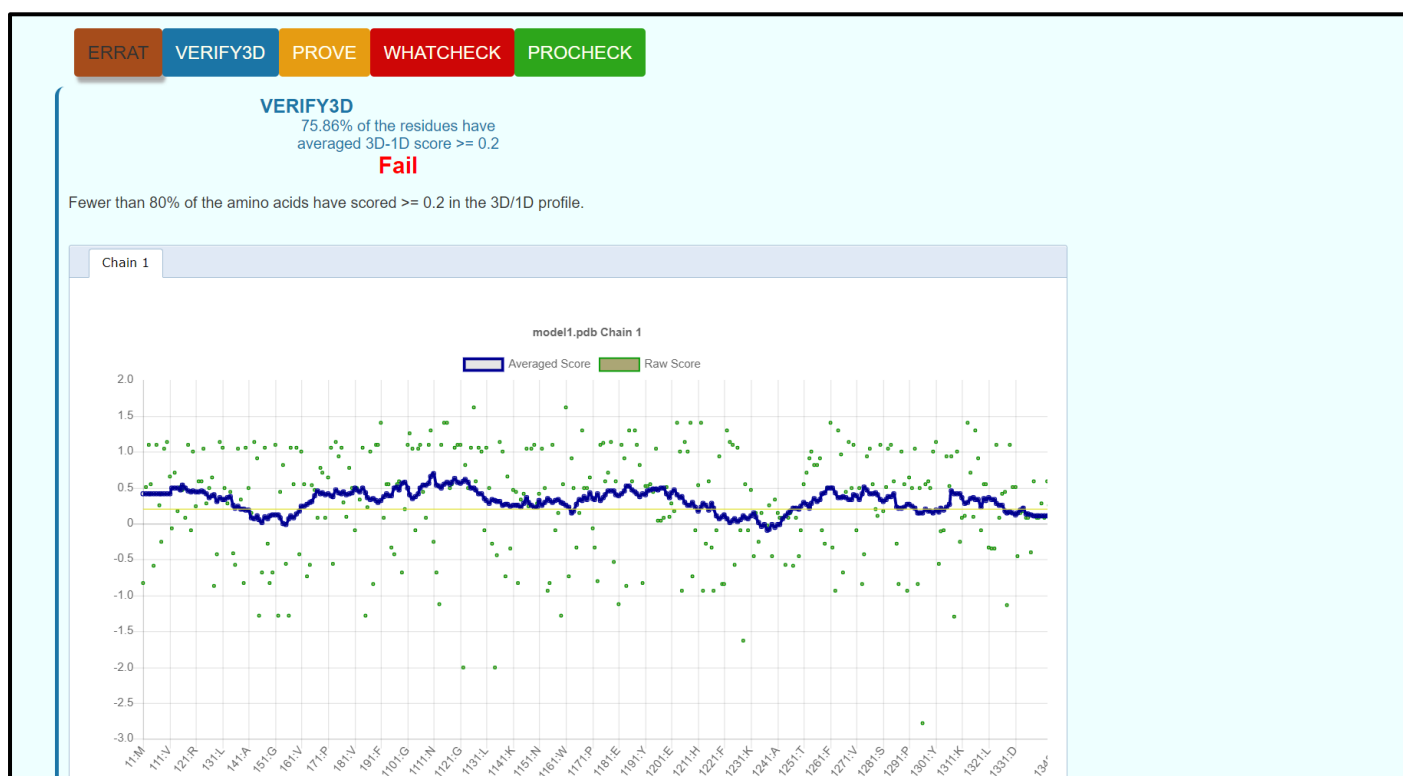


Fig5. Result page for Verify3D

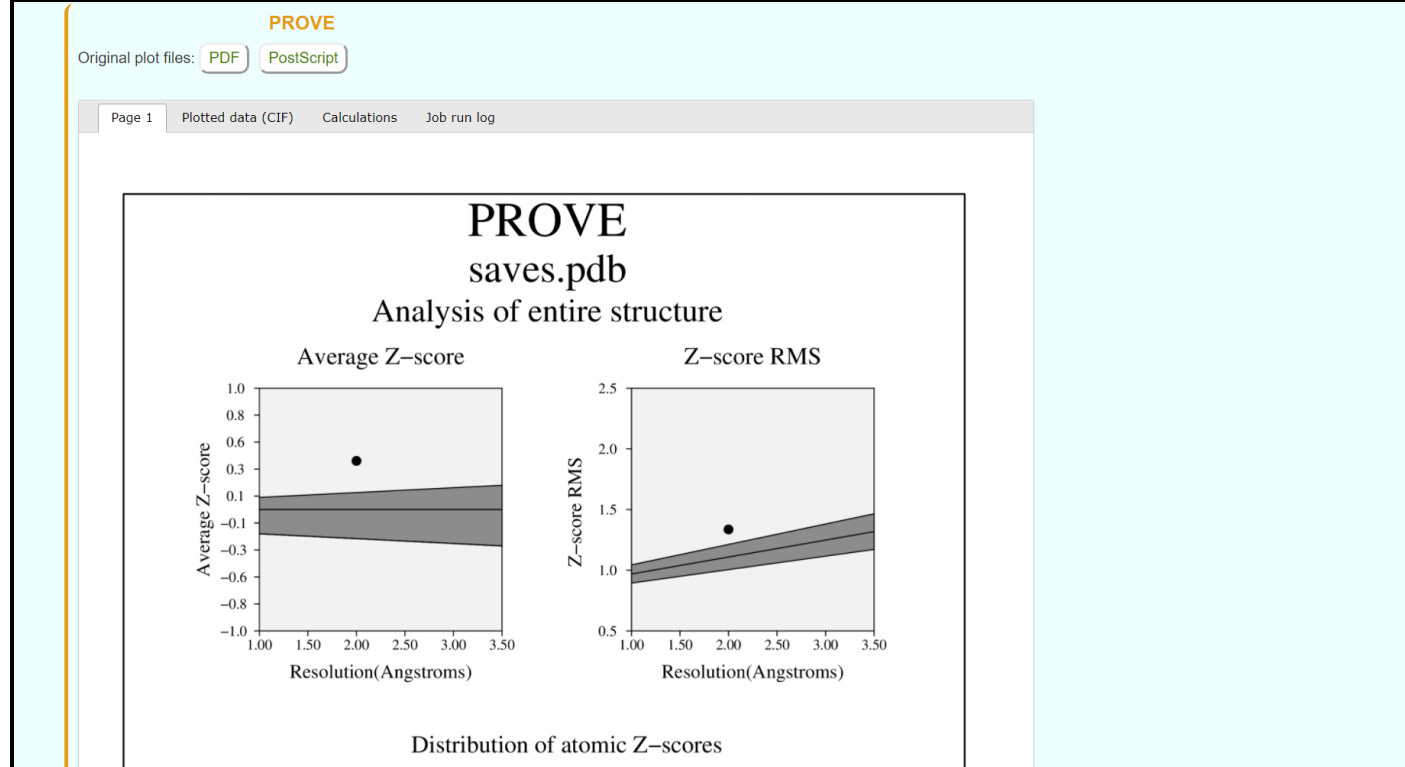


Fig6. Result page for Prove

ERRAT | VERIFY3D | **PROVE** | WHATCHECK | PROCHECK

WHATCHECK

Headings

#33. Error: Abnormally short interatomic distances

Error: Abnormally short interatomic distances
The pairs of atoms listed in the table below have an unusually short distance.

The contact distances of all atom pairs have been checked. Two atoms are said to 'bump' if they are closer than the sum of their Van der Waals radii minus 0.40 Angstrom. For hydrogen bonded pairs a tolerance of 0.55 Angstrom is used. The first number in the table tells you how much shorter that specific contact is than the acceptable limit. The second distance is the distance between the centers of the two atoms.

The last text-item on each line represents the status of the atom pair. The text 'INTRA' means that the bump is between atoms that are explicitly listed in the PDB file. 'INTER' means it is an inter-symmetry bump. If a line contains the text 'HB', the bump criterion was relaxed because there could be a hydrogen bond. If the text 'BF' is present, the sum of the B-factors of the atoms is higher than 80, which makes the appearance of the bump somewhat less severe because the atoms probably aren't there anyway.

Bumps between atoms for which the sum of their occupancies is lower than one are not reported. In any case, each bump is listed in only one direction.

103	PHE	(103)	A	CE2	--	187	CYS	(187)	A	SG	0.117	3.283	INTRA
132	ALA	(132)	A	O	--	222	CYS	(222)	A	SG	0.062	2.938	INTRA
33	GLU	(33)	A	CD	--	35	TRP	(35)	A	NE1	0.048	3.052	INTRA
301	TYR	(301)	A	CD1	--	302	ASN	(302)	A	N	0.044	3.056	INTRA
146	PHE	(146)	A	CG	--	147	ARG	(147)	A	N	0.033	3.067	INTRA
331	ASP	(331)	A	CG	--	332	GLU	(332)	A	N	0.031	3.069	INTRA
210	VAL	(210)	A	O	--	215	PRO	(215)	A	CD	0.028	2.772	INTRA
55	ASN	(55)	A	ND2	--	83	ASP	(83)	A	CB	0.014	3.086	INTRA

Complete. Time taken: 00:00:06

Fig7. Result page for Whatcheck

PROCHECK

Out of 8 evaluations

- Errors: 6
- Warning: 0
- Pass: 2

The evaluations are the '+' (Warning) and '*' (Error) in the summary. The categories on the left do not always correspond in number due to PROCHECK output documents.

Summary
Ramachandran plot Error
All Ramachandrans Error
Chi1-chi2 plots Pass
Main-chain params
Side-chain params Error
Residue properties Error
Bond len/angle Error
M/c bond lengths
M/c bond angles
Planar groups Error
Program output

```

-----<<< P R O C H E C K   S U M M A R Y >>>-----
/var/www/SAVES/Jobs/937181/saves.pdb  1.5                348 residues
* Ramachandran plot:  80.9% core  15.5% allow   3.0% gener  0.7% disall
* All Ramachandrans:  21 labelled residues (out of 346)
* Chi1-chi2 plots:    6 labelled residues (out of 195)
Side-chain params:    5 better    0 inside    0 worse
* Residue properties: Max.deviation:  10.6          Bad contacts:    0
                      Bond len/angle:  5.4          Morris et al class: 1 2 1
G-factors             Dihedrals:  -0.42          Covalent:    0.09          Overall:  -0.20
* Planar groups:      87.9% within limits  12.1% highlighted    3 off graph
+ May be worth investigating further.  * Worth investigating further.
  
```

Summary file

Complete. Time taken: 00:00:19

Fig8. Result page for Procheck

Side-chain params Error
Residue properties Error
Bond len/angle Error
M/c bond lengths
M/c bond angles
Planar groups Error
Program output

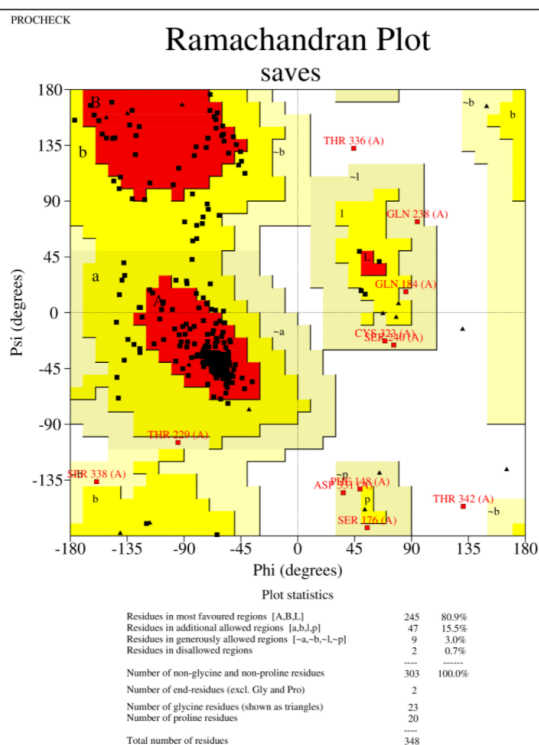


Fig9. Result page for Ramachandran plot

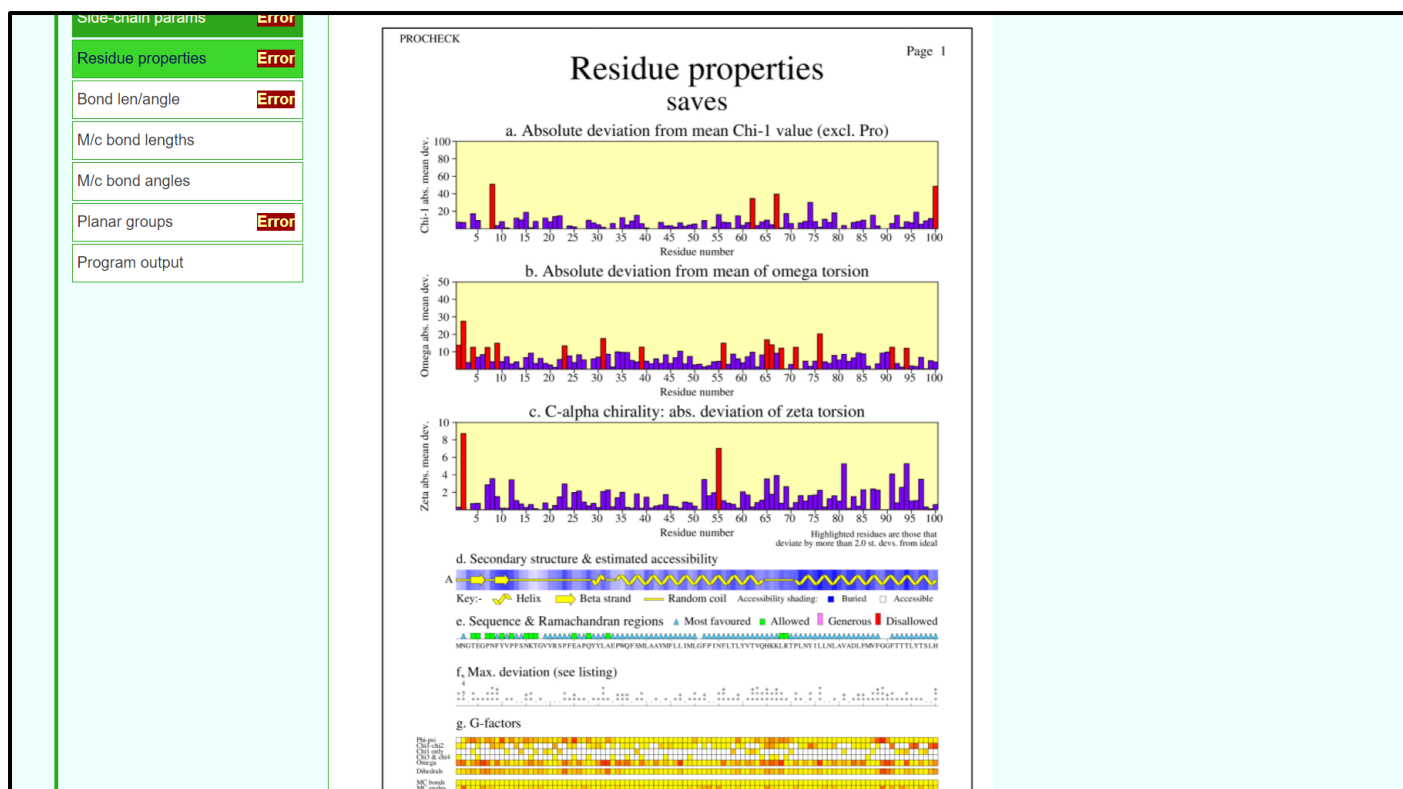


Fig10. Result page for reside properties

RESULT:

The structure predicted for enzyme kinase by threading approach using I-TASSER was validated using SAVES server.

CONCLUSION:

SAVES is an integrated server containing various tools on a single platform that can be used for tertiary structure validation. The predicted structure for kinase by I-TASSER passed only for ERRAT and did not give required results for the rest. Even though I-TASSER gave better predicted structure than modeller, Robetta based on ab-initio approach will be used to predict a better structure and will be validated again using SAVES server.

REFERENCES:

1. Xiong, J. (2008). Tertiary structure prediction. Essential bioinformatics. Cambridge: Cambridge University Press. 220-222.
2. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/>
3. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/?job=924117>

WEBLEM 4c

SAVES server

(URL: <https://saves.mbi.ucla.edu/>)**AIM:**

To validate structure 240040 generated from Robetta server.

INTRODUCTION:

240040 is the structure predicted using Ab-initio approach using Robetta. The structure has to be evaluated to make sure that the structural features of the model are consistent with the physicochemical rules. This can be done using SAVES server.

SAVES is a structure validation server that has various tools like Errat, Verify3D, Prove, Whatcheck, Procheck and Cryst integrated in one single platform. This involves checking anomalies in ϕ - ψ angles, bond lengths, close contacts, and so on. Another way of checking the quality of a protein model is to implicitly take these stereochemical properties into account. This is a method that detects errors by compiling statistical profiles of spatial features and interaction energy from experimentally determined structures. By comparing the statistical parameters with the constructed model, the method reveals which regions of a sequence appear to be folded normally and which regions do not. If structural irregularities are found, the region is considered to have errors and has to be further refined.

METHODOLOGY:

1. Open homepage for SAVES server. (URL: <https://saves.mbi.ucla.edu/>)
2. Upload structure retrieved from Robetta in PDB format.
3. Obtain results for Errat, Verify3D, Prove, Whatcheck and Procheck.
4. Observe and interpret the results.

OBSERVATION:

UCLA-DOE LAB — SAVES v6.0

**To run any or all programs:
upload your structure, in PDB format only**

Choose File No file chosen

Run programs

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

PROCHECK

- PROCHECK source information
- Result analysis

Fig1. Homepage for SAVES server

UCLA-DOE LAB — SAVES v6.0

UCLA

To run any or all programs:
upload your structure, in PDB format only

Choose File robetta_models_240040.pdb

Customize job name:

robetta_models_240040.pdb

Run programs

References

ERRAT

- Reference: Verification of protein structures: patterns of nonbonded atomic interactions, Colovos C and Yeates TO, 1993.
- C++ software

VERIFY 3D

- Profile Search Software [Bowie et al., 1991, Luethy et al., 1992].
- DSSP original and Wikipedia

PROVE

- Reference: Deviations from standard atomic volumes as a quality measure for protein crystal structures, Pontius J, Richelle J, Wodak SJ. 1996

Fig2. Structure from Modeller for validation

UCLA-DOE LAB — SAVES v6.0

UCLA

Job 937186 has been created

New Job

job #937186: robetta_models_240040.pdb [job link] [3D Viewer]

ERRAT Complete Error(s) found. Check full results for more information <u>Overall Quality Factor</u> Results	VERIFY Processing results Error(s) found. Check full results for more information Results	PROVE Complete Buried outlier protein atoms total from 1 Model: 4.6% warning Results
WHATCHECK Complete 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 Results	PROCHECK Complete Out of 9 evaluations • Errors: 3 • Warning: 2 • Pass: 4 Results	Almost ready, check back soon

References

Fig3. Result page for structure validation for various servers

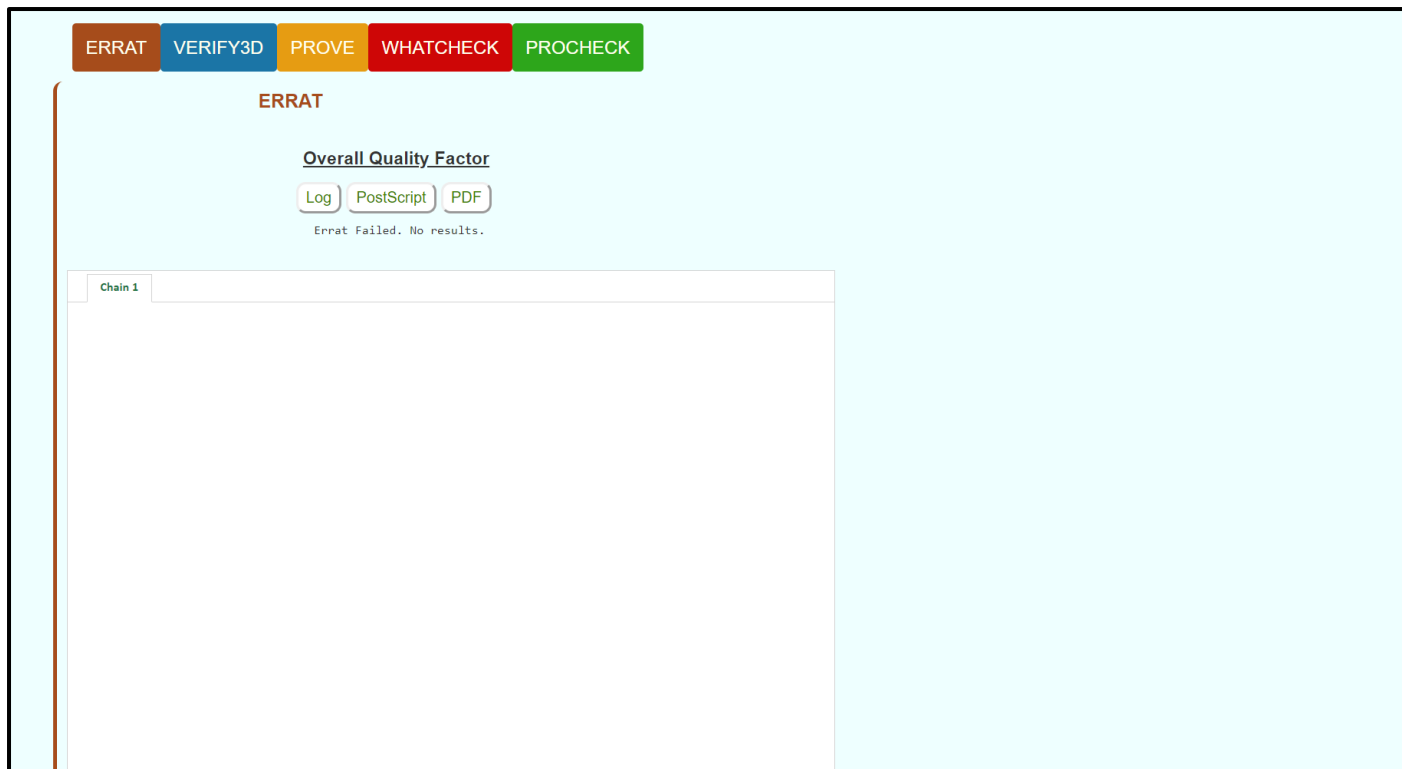


Fig4. Result page for Errat



Fig5. Result page for Verify3D



Fig6. Result page for PROVE

ERRAT VERIFY3D PROVE WHATCHECK PROCHECK

WHATCHECK

Headings

#33. Error: Abnormally short interatomic distances

Error: Abnormally short interatomic distances
The pairs of atoms listed in the table below have an unusually short distance.

The contact distances of all atom pairs have been checked. Two atoms are said to 'bump' if they are closer than the sum of their Van der Waals radii minus 0.40 Angstrom. For hydrogen bonded pairs a tolerance of 0.55 Angstrom is used. The first number in the table tells you how much shorter that specific contact is than the acceptable limit. The second distance is the distance between the centers of the two atoms.

The last text-item on each line represents the status of the atom pair. The text 'INTRA' means that the bump is between atoms that are explicitly listed in the PDB file. 'INTER' means it is an inter-symmetry bump. If a line contains the text 'HB', the bump criterium was relaxed because there could be a hydrogen bond. If the text 'BF' is present, the sum of the B-factors of the atoms is higher than 80, which makes the appearance of the bump somewhat less severe because the atoms probably aren't there anyway.

Bumps between atoms for which the sum of their occupancies is lower than one are not reported. In any case, each bump is listed in only one direction.

392	MET	(44)	A	CE	--	1657	TRP	(265)	A	CG	3.099	0.101	INTRA
377	TYR	(29)	A	CE1	--	1432	LEU	(40)	A	CD2	3.052	0.148	INTRA
415	LYS	(67)	A	CB	--	1535	MET	(143)	A	CE	2.945	0.255	INTRA
2	ASN	(2)	A	N	--	1394	ASN	(2)	A	N	2.920	0.080	INTRA
447	LEU	(99)	A	CD1	--	1483	PHE	(91)	A	CE2	2.916	0.284	INTRA
435	VAL	(87)	A	C	--	1517	LEU	(125)	A	N	2.911	0.189	INTRA
366	GLY	(18)	A	C	--	1422	TYR	(30)	A	CE2	2.892	0.308	INTRA
5	GLU	(5)	A	N	--	1584	TYR	(192)	A	CE2	2.883	0.217	INTRA
435	VAL	(87)	A	CA	--	1517	LEU	(125)	A	CA	2.861	0.339	INTRA
397	MET	(49)	A	SD	--	1694	ASN	(302)	A	C	2.860	0.540	INTRA
195	HIS	(195)	A	CE1	--	538	ASP	(190)	A	C	2.844	0.356	INTRA
437	GLY	(89)	A	N	--	1514	GLU	(122)	A	C	2.828	0.272	INTRA
373	GLU	(25)	A	CG	--	1416	PHE	(24)	A	CE2	2.810	0.390	INTRA
456	THR	(108)	A	CA	--	965	ALA	(269)	A	CB	2.804	0.396	INTRA
395	LEU	(47)	A	CG	--	1653	PHE	(261)	A	C	2.790	0.410	INTRA

Fig7. Result page for Whatcheck

Summary
Ramachandran plot Warning
All Ramachandrans Pass
Chi1-chi2 plots Pass
Main-chain params
Side-chain params Error
Residue properties Pass
Bond len/angle Pass
M/c bond lengths
M/c bond angles
Planar groups Pass
Program output

```

+-----<<< P R O C H E C K   S U M M A R Y >>>-----+
| /var/www/SAVES/3obs/937186/saves.pdb   1.5           348 residues |
| * Ramachandran plot:  89.1% core   9.6% allow   0.3% gener   1.0% disall |
| + All Ramachandrans:  10 labelled residues (out of 346) |
|   Chi1-chi2 plots:    0 labelled residues (out of 195) |
|   Side-chain params:   5 better    0 inside    0 worse   |
| * Residue properties: Max.deviation:  12.4           Bad contacts:   1 |
|   Bond len/angle:     11.8           Morris et al class:  1 1 1 |
| + 1 cis-peptides |
|   G-factors           Dihedrals:  0.33   Covalent:  0.34   Overall:  0.35 |
| Planar groups:  100.0% within limits   0.0% highlighted |
| +-----+ |
| * May be worth investigating further.  * Worth investigating further. |

```

Summary file

Complete. Time taken: 00:00:19

1. CRYST has not been run

saves @2020 - DOE-MBI Services • Usage
Contact: holton at mbi ucla edu for any questions

Fig8. Result page for Procheck

Side-chain params Error
Residue properties Pass
Bond len/angle Pass
M/c bond lengths
M/c bond angles
Planar groups Pass
Program output

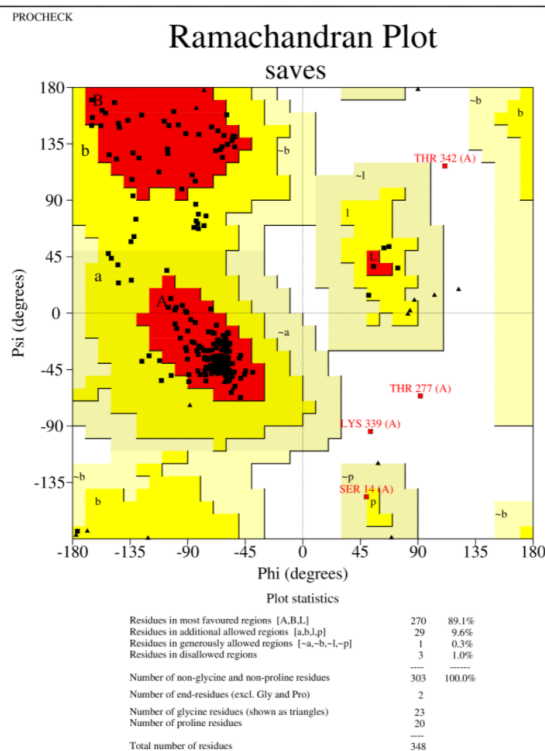


Fig9. Result page for Ramachandran plot



Fig10. Result page for residue properties

RESULT:

The structure predicted for enzyme kinase by abi-initio approach using Robetta was validated using SAVES server.

CONCLUSION:

SAVES is an integrated server containing various tools on a single platform that can be used for tertiary structure validation. The predicted structure for Rhodopsin by Robetta passed maximum requirements of validation. Hence, it can be concluded that the structure predicting by Robetta was the most accurate out of all three methods used for prediction.

REFERENCES:

1. Xiong, J. (2008). Tertiary structure prediction. Essential bioinformatics. Cambridge: Cambridge University Press. 220-222.
2. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/>
3. SAVESv6.0 - Structure Validation Server. (n.d.). Saves.mbi.ucla.edu. Retrieved March 8, 2022, from <https://saves.mbi.ucla.edu/?job=928054>