SHORT RESEARCH ARTICLE

Protein structure quality assessment based on the distance profiles of consecutive backbone C α atoms [version 1; peer review: 2 approved]

Sandeep Chakraborty¹, Ravindra Venkatramani², Basuthkar J. Rao¹, Bjarni Asgeirsson³, Abhaya M. Dandekar⁴

¹Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, 400 005, India

²Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, 400 005, India

³Science Institute, Department of Biochemistry, University of Iceland, Reykjavik, IS-107, Iceland

⁴Plant Sciences Department, University of California, Davis, CA 95616, USA

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Abstract

Predicting the three dimensional native state structure of a protein from its primary sequence is an unsolved grand challenge in molecular biology. Two main computational approaches have evolved to obtain the structure from the protein sequence - *ab initio/de novo* methods and template-based modeling - both of which typically generate multiple possible native state structures. Model quality assessment programs (MQAP) validate these predicted structures in order to identify the correct native state structure. Here, we propose a MQAP for assessing the quality of protein structures based on the distances of consecutive C α atoms. We hypothesize that the root-mean-square deviation of the distance of consecutive C α (RDCC) atoms from the ideal value of 3.8 Å, derived from a statistical analysis of high quality protein structures (top100H database), is minimized in native structures. Based on tests with the top100H set, we propose a RDCC cutoff value of 0.012 Å, above which a structure can be filtered out as a non-native structure. We applied the RDCC discriminator on decoy sets from the Decoys 'R' Us database to show that the native structures in all decoy sets tested have RDCC below the 0.012 Å cutoff. While most decoy sets were either indistinguishable using this discriminator or had very few violations, all the decoy structures in the fisa decoy set were discriminated by applying the RDCC criterion. This highlights the physical non-viability of the fisa decoy set, and possible issues in benchmarking other methods using this set. The source code and manual is made available at <https://github.com/sanchak/mqap> and permanently available on [10.5281/zenodo.7134](https://doi.org/10.5281/zenodo.7134).

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1. **Bairong Shen**, Soochow University, Suzhou, China

2. **Simon Lovell**, University of Manchester, Manchester, UK

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Keywords

Computational biology ; protein structure prediction ; Model quality assessment programs ; Boltzmann distribution ; Annsen's thermodynamic hypothesis ; statistical potentials ; protein backbone ; decoy sets ;

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Corresponding author: Sandeep Chakraborty (sanchak@gmail.com)

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Introduction

The structure of a protein is a veritable source of information about its physiological relevance in the cellular context¹. In spite of rapid technical advances in crystallization techniques, the number of protein sequences known far exceeds the known structures. There are essentially two different computational approaches to predict protein structures from its primary sequence: 1) Template based methods (TBM) which are based on features obtained from the database of known protein structures²⁻⁴ and 2) *ab initio* or *de novo* methods which are based on the intrinsic laws governing atomic interactions and are applicable in the absence of a template structure with significant sequence homology^{5,6}. While at present TBM methods fare much better than the *de novo* approaches, the requirement of a known template protein can sometimes be a constraining factor. Both these methods typically generate multiple possibilities for the native structure of a given sequence. Selecting the best candidate from the set of putative structures is an essential aspect that is performed by model quality assessment programs (MQAP).

MQAPs can be classified as energy based, consensus based or knowledge based. The refinement of structures based on modeling of atomic interactions in energy based methods, such as molecular dynamic simulations, are subject to limited sampling of possible conformations due to large run times, and force field inaccuracies due to the approximations involved in describing the dynamics of large multi-atomic systems⁷⁻¹⁰. Consensus methods are based on the principle that sub-structures of the native structure are likely to feature frequently in a set of near-native structures¹¹⁻¹⁴. These methods are currently the best performing amongst MQAPs¹³, but are prone to be computationally intensive due to structure-to-structure comparison of all models¹⁴, and are of limited use when the number of possible structures is small¹⁵. Knowledge based methods rely on the assignment of an empirical potential (also known as statistical potential) from the frequency of residue contacts in the known structures of native proteins^{16,17}. In statistical physics, for a system in thermodynamic equilibrium, the accessible states are populated with a frequency which depends on the free energy of the state and is given by the Boltzmann distribution. The Boltzmann hypothesis states that if the database of known native protein structures is assumed to be a statistical system in thermodynamic equilibrium, specific structural features would be populated based on the free energy of the protein conformational state. Sippl argued using a converse logic that the frequencies of occurrence of structural features such as interatomic distances in the database of known protein structures could determine a free energy (potential of mean force) for a given protein conformation, and thus be used to discriminate the native structure^{18,19}. A crucial aspect in applying statistical potentials is the proper characterization of the reference state²⁰. The application of such empirical energy functions to predict and assess protein structures, while quite popular, are vigorously debated^{21,22}, and several approaches for using statistical potentials for protein structure prediction have been described to date^{20,23-26}.

Here, we propose a new statistical potential based MQAP for assessing the quality of protein structures based on the distances of consecutive $C\alpha$ atoms - Protein structure quality assessing based on Distance profile of backbone atoms (PROQUAD). We first

propose a statistical potential based on the distance of consecutive $C\alpha$ distances. In a set of high quality protein structures (top100H²⁷), we demonstrate that the distance between consecutive $C\alpha$ atoms are distributed normally with a mean of 3.8 Å and standard deviation of 0.04 Å. Based on this observation, the reference state for our statistical potential calculations is defined as one where all consecutive $C\alpha$ atoms are 3.8 Å apart. We propose a scoring function which measures the deviation of consecutive $C\alpha$ atoms from 3.8 Å, and hypothesize that this score is minimized in native structures. Based on the top100H database, we chose a cutoff of 0.012 Å for this scoring function to identify non-native states. We show that all the decoy structures from the fisa decoy set taken from the Decoys 'R' Us database²⁸ are distinguished using this discriminator. It has been previously proposed that native structures have constrained interatomic distances²⁹. Interatomic distances, and other metrics, have been combined in several such methods - Molprobit (http://molprobit.biochem.duke.edu/), PROSA (https://prosa.services.came.sbg.ac.at/prosa.php) and the WHATIF server (http://swift.cmbi.ru.nl/whatif)³⁰⁻³². These identify possible anomalies in a given protein structure. While Molprobit and WHATIF identified steric clashes in the decoy structure in fisa, distance checks between consecutive $C\alpha$ are not part of checks in these methods, and they failed to detect the consecutive $C\alpha$ atoms anomaly in the fisa decoy set.

Thus, we propose a simple and fast discriminator for protein structure quality based on the distance profiles of consecutive backbone $C\alpha$ atoms that identifies decoy structures that are physically nonviable.

Results and discussion

The frequency distribution of the distance of consecutive $C\alpha$ atoms in ~100 proteins in the top100H database (a database consisting of high quality structures)²⁷ shows that the distance between consecutive $C\alpha$ atoms are distributed normally with a mean of 3.8 Å and standard deviation of 0.04 Å (Figure 1a). Out of 16,162 pairs of consecutive $C\alpha$ atom distances, 14,281 (88%) were spaced 3.8 Å apart, 1297 (8%) were spaced 3.9 Å apart and 553 (3%) were spaced 3.7 Å apart. Only 31 (0.1%) pairs of consecutive $C\alpha$ atom distances had values different than these (highest being 4 Å and the lowest being 2.9 Å). It would be interesting to correlate these distance deviant residue pairs to structural or functional aspects of the protein - *It is well worth examining every outlier and either correcting it if possible, giving up gracefully if it really cant be improved (more often true at low resolution), or celebrating the significance of why it is being held in an unfavorable conformation*³³.

Figure 1b plots the root-mean-square deviation of the distance of consecutive $C\alpha$ (RDCC) for these ~100 proteins. All structures in the top100H database have low RDCC values, barring three proteins (PDBids: 2ER7, 1XSO and 4PTP), which had multiple conformations for some residues, and were excluded from the processing. This validates our hypothesis that RDCC is minimized in native structures. Hence, structures that have a RDCC value more than a user specified threshold can be pruned out as structures with low quality or non-native structures.

We evaluated the results using the measures of specificity (the ability of a test to identify negative results) which is defined as:

Figure 1. Root-mean-square deviation of the distance of consecutive $C\alpha$ (RDCC) atoms from the ideal value of 3.8 Å. (a) Probability distribution ($P(x)$) for the distance of consecutive $C\alpha$ in ~100 proteins in the top100H database. **(b)** RDCC in ~100 high quality structures from the top100H database. **(c)** Variation in specificity based on the cutoff value. We choose 0.012 Å as the cutoff for filtering out non-native structures.

$$specificity = \frac{TN}{FP + TN} \quad (1)$$

(TN = true negatives, FP = false positives). The specificity variation with the cutoff chosen is shown in Figure 1c. We choose 0.012 Å as the cutoff value for RDCC, which has a specificity of 1. We have applied this cutoff on decoy sets from the Decoys 'R' Us database²⁸. The first protein (the native structure) in all decoy sets has RDCC below the 0.012 Å cutoff (Figure 1c). Figure 2 shows the RDCC for the hg_structural and fisa decoy sets from the Decoys 'R' Us database. All 500 decoy structures in each protein structure in the fisa decoy set are discriminated by applying the RDCC criterion. Figure 3 shows the superimposition of the native structure and the first decoy structure (AXPROA00-MIN) for a protein (PDBid: 1FC2) taken from the fisa decoy set. The distance between Ile12/ $C\alpha$ and Leu13/ $C\alpha$ atoms is 3.8 Å and 4.1 Å in the native and the decoy structures, respectively. According to our hypothesis, a 4.1 Å distance between consecutive $C\alpha$ atoms is typically unfeasible in protein structures, and their occurrence should be relatively rare.

Figure 2. Root-mean-square deviation (RMSD) of the distance of consecutive $C\alpha$ (RDCC) atoms from the ideal value of 3.8 Å in decoy sets. The hg_structural and misfold decoy sets are indistinguishable using the distance discriminator, unlike the fisa decoy set. We have shown ~25 decoy structures from the fisa set, but the values apply to all the decoys (more than 500). The first protein (the native structure) in each set has RDCC below the 0.012 Å cutoff.

Figure 3. Superimposition of the native structure and a decoy structure (AXPROA00-MIN) for a protein (PDBid: 1FC2) taken from the fisa decoy set. The native structure is in red, and the decoy structure is in green. The structures are superimposed using MUSTANG⁵³. The distance between Ile12/CA and Leu13/CA atoms is 3.8 Å and 4.1 Å in the native and the decoy structures, respectively.

The presence of such deviations throughout the protein structures categorizes it as a non-native structure. MolProbity³⁰ and ProSA³¹ are two programs used as a pre-processing step for structures used in CASP³⁴. MolProbity was able to discriminate the decoy structure (AXPROA00-MIN) from the native structure (PDBid: 1FC2) using a metric called the ClashScore (the number of serious steric overlaps) and the C β deviations³⁵. PROSA was unable to discriminate between the decoy and the native structures, reporting equivalent Zscores of -4.12 and -5.28, respectively. The WHATIF server report also reports steric clashes in the decoy structures (Data File 1). None of the above mentioned methods use a metric similar to the RDCC proposed in this paper, and thus did not report the abnormal distance between consecutive C α atoms in the decoy structure.

The hg_structal and misfold decoy sets are indistinguishable using this distance discriminator, while only a few decoy structures failed in the 4state_reduced decoy set. This relationship between RDCC and proteins structure quality is therefore not an equivalence relationship. In propositional calculus, a relationship is equivalent if 'A' implies 'B' and 'B' implies 'A'. A high RDCC implies a low quality structure, but a low quality structure does not necessitate a high RDCC. We therefore suggest the usage of the RDCC measure as a first pass to rule out the non-native contacts prior to applying other discriminators.

The model quality assessment program (MQAP) used to choose the best structure from the multiple closely related structures generated by structure prediction programs is of critical importance. We have in the past used electrostatic congruence to detect a promiscuous serine protease scaffold in alkaline phosphatases³⁶ and a phosphoinositide-specific phospholipase C from *Bacillus cereus*³⁷, and a scaffold recognizing a β -lactam (imipenem) in a cold-active *Vibrio* alkaline phosphatase^{38,39}. However, continuum models⁴⁰ that compute potential differences and pK_a values from charge interactions in proteins⁴¹ are sensitive to the spatial arrangement of the atoms in the structure. Thus, an incorrect model will generate an inaccurate electrostatic

profile of the peptide⁴². It is thus possible to functionally characterize a protein from its sequence by applying such *in silico* tools subsequent to the protein structure prediction and MQAPs tools⁴³.

The estimation of the model quality by MQAPs is achieved by formalizing a scoring function⁴⁴, referred to as a knowledge-based or statistical potential, constructed from the database of known structures, assuming that the distribution of the structural features obtained from these structures follows the Boltzmann distribution^{20,23,24,26}. The validity of statistical potential and the method to choose a proper reference frame in such models are still widely debated^{21,22}. Methods that use consensus values from numerous models outperform other MQAP methods^{11–14}, and are 'very useful for structural meta-predictors'⁴⁵. It has been shown that many of the MQAP programs perform considerably better when different statistical metrics are combined^{46–48}. The state of the art methods for predicting structures⁴⁹ and MQAPs^{34,45,50} are evaluated by researchers every two years.

Here, we propose a discriminator (RDCC) based on the distance of consecutive C α atoms in the peptide structure. The discriminator is independent of the database of structures⁵¹, and is thus an absolute discriminator. Our proposed RDCC criterion is satisfied in high quality protein structures taken from the top100H database. As a specific application, we show that all decoy structures in the fisa decoy set from the Decoys 'R' Us database C α atoms do not satisfy this criterion. It has been previously shown that the fisa decoy set violates the van der Waals term⁵². We propose a fast complementary method to identify this transgression. It is also an interesting fact that most consensus methods will fare poorly in the fisa decoy set, since the majority of sub-structures are incorrect in all the decoy sets. Therefore, the fisa decoy set consists of physically nonviable structures and one should exercise caution when benchmarking other methods using this decoy set.

Data File 1: WHATIF server report

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.813320>

Materials and methods

The set of proteins Φ_{proteins} consists of the native structure P_1 and M-1 decoys structures (Equation 2). We ignore the first $x=\text{IgnoreNTerm}$ and last $y=\text{IgnoreCTerm}$ pairs of residues in the protein structure to exclude the terminals (Equation 3). For every consecutive pair of residues in the structure we calculate the distance between the consecutive C α atoms ($\text{Res}_n(\text{C}\alpha)$ and $\text{Res}_{n+1}(\text{C}\alpha)$), and its deviation from the ideal value of 3.8 Å. The square of the summation of these deviations is then normalized based on the number of pairs processed, and results in the CADistScore . We hypothesize that CADistScore^{P_1} is minimum in a native structure (Equation 4). Algorithm 1 shows the pseudocode for the function that generates the CADistScore .

$$\Phi_{\text{proteins}} = \{P_1, P_2 \dots P_M\} \quad (2)$$

Algorithm 1: AssessCADist()

Input: P_i : Protein under consideration
Input: *IgnoreNTerm*: Ignore this number of residues in the N Terminal
Input: *IgnoreCTerm*: Ignore this number of residues in the C Terminal
Output: *CADistScore*: Score indicating deviation of successive C α atoms from 3.8 Å
begin
 CADistScore = 0 ; *NumberCompared* = 0 ; *N* = *NumberOfResidues*(P_i);
 for $p \leftarrow \text{IgnoreNTerm}$ **to** $N - \text{IgnoreCTerm}$ **do**
 $q = p + 1$;
 CADist = *Distance*($p, q, C\alpha, C\alpha$)
 NumberCompared = *NumberCompared* + 1 ;
 $\text{diff} = \text{absolute}(\text{CADist} - 3.8 \text{ \AA})$;
 CADistScore = *CADistScore* + $\text{diff} * \text{diff}$;
 end
 /* Normalize */
 CADistScore = $\text{sqrt}(\text{CADistScore} / (\text{NumberCompared} * \text{NumberCompared}))$;
 return (*CADistScore*);
end

$$CADistScore^{P_i} = \sqrt{\frac{\sum_{n=1+x}^{N-y-1} (\text{dist}(\text{Res}_n(C\alpha), \text{Res}_{n+1}(C\alpha)) - 3.8)^2}{(N - y - x - 2)^2}} \quad (3)$$

$$[\forall i = 2 \dots M](CADistScore^{P_1} < CADistScore^{P_i}) \quad (4)$$

In order to validate our hypothesis on known structures, we applied our discriminator to the top100H database (a database consisting

of high quality structures)²⁷ - <http://kinemage.biochem.duke.edu/databases/top100.php>. In order to benchmark model quality assessment programs, we used decoy sets from the Decoys 'R' Us database²⁸ - <http://dd.compbio.washington.edu/>. Each set has several structures that are supposed to be ranked worse than the native structure.

Structural superimposition has been done using MUSTANG⁵³. Protein structures were rendered by Py-Mol (<http://www.pymol.org/>). The source code and manual is made available at <https://github.com/sanchak/mqap> and permanently available from 10.5281/zenodo.7134.

Author contributions

Conceived and performed the experiments: SC. Analyzed the data, and improved experiments: SC BA AMD BJR RV. Wrote the manuscript: SC BA AMD BJR RV.

Competing interests

No competing interests were disclosed.

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References

- Wise EL, Rayment I: Understanding the importance of protein structure to nature's routes for divergent evolution in TIM barrel enzymes. *Acc Chem Res*. 2004; **37**(3): 149–158.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Soding J: Protein homology detection by HMM-HMM comparison. *Bioinformatics*. 2005; **21**(7): 951–960.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Peng J, Xu J: RaptorX: exploiting structure information for protein alignment by statistical inference. *Proteins*. 2011; **79**(Suppl 10): 161–171.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zhang Y: Template-based modeling and free modeling by I-TASSER in CASP7. *Proteins*. 2007; **69**(Suppl 8): 108–117.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wu S, Skolnick J, Zhang Y: Ab initio modeling of small proteins by iterative TASSER simulations. *BMC Biol*. 2007; **5**: 17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rohl CA, Strauss CE, Misura KM, et al.: Protein structure prediction using Rosetta. *Methods Enzymol*. 2004; **383**: 66–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Chen J, Brooks CL: Can molecular dynamics simulations provide high-resolution refinement of protein structure? *Proteins*. 2007; **67**(4): 922–930.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zhu J, Fan H, Periole X, et al.: Refining homology models by combining replica-exchange molecular dynamics and statistical potentials. *Proteins*. 2008; **72**(4): 1171–1188.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Raval A, Piana S, Eastwood MP, et al.: Refinement of protein structure homology models via long, all-atom molecular dynamics simulations. *Proteins*. 2012; **80**(8): 2071–2079.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lee MR, Tsai J, Baker D, et al.: Molecular dynamics in the endgame of protein structure prediction. *J Mol Biol*. 2001; **313**(2): 417–430.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ginalski K, Elofsson A, Fischer D, et al.: 3D-Jury: a simple approach to improve protein structure predictions. *Bioinformatics*. 2003; **19**(8): 1015–1018.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Terashi G, Oosawa M, Nakamura Y, et al.: United3D: a protein model quality assessment program that uses two consensus based methods. *Chem Pharm Bull (Tokyo)*. 2012; **60**(11): 1359–1365.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wallner B, Elofsson A: Prediction of global and local model quality in CASP7 using Pcons and ProQ. *Proteins*. 2007; **69**(Suppl 8): 184–193.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Adamczak R, Pillardy J, Vallat BK, et al.: Fast geometric consensus approach for protein model quality assessment. *J Comput Biol*. 2011; **18**(12): 1807–1818.
[PubMed Abstract](#) | [Publisher Full Text](#)

15. McGuffin LJ: **Benchmarking consensus model quality assessment for protein fold recognition.** *BMC Bioinformatics*. 2007; **8**: 345.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Tanaka S, Scheraga HA: **Model of protein folding: inclusion of short-, medium-, and long-range interactions.** *Proc Natl Acad Sci U S A*. 1975; **72**(10): 3802–3806.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Miyazawa S, Jernigan RL: **Estimation of effective interresidue contact energies from protein crystal structures: quasi-chemical approximation.** *Macromolecules*. 1985; **18**(3): 534–552.
[Publisher Full Text](#)
18. Sippl MJ: **Calculation of conformational ensembles from potentials of mean force. An approach to the knowledge-based prediction of local structures in globular proteins.** *J Mol Biol*. 1990; **213**(4): 859–883.
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Sippl MJ: **Knowledge-based potentials for proteins.** *Curr Opin Struct Biol*. 1995; **5**(2): 229–235.
[PubMed Abstract](#) | [Publisher Full Text](#)
20. Zhou H, Zhou Y: **Distance-scaled, finite ideal-gas reference state improves structure-derived potentials of mean force for structure selection and stability prediction.** *Protein Sci*. 2002; **11**(11): 2714–2726.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Thomas PD, Dill KA: **Statistical potentials extracted from protein structures: how accurate are they?** *J Mol Biol*. 1996; **257**(2): 457–469.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Hamelryck T, Borg M, Paluszewski M, *et al.*: **Potentials of mean force for protein structure prediction vindicated, formalized and generalized.** *PLoS ONE*. 2010; **5**(11): e13714.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Samudrala R, Moulton J: **An all-atom distance-dependent conditional probability discriminatory function for protein structure prediction.** *J Mol Biol*. 1998; **275**(5): 895–916.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Shen MY, Sali A: **Statistical potential for assessment and prediction of protein structures.** *Protein Sci*. 2006; **15**(11): 2507–2524.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Rajgaria R, McAllister SR, Floudas CA: **A novel high resolution Calpha-Calpha distance dependent force field based on a high quality decoy set.** *Proteins*. 2006; **65**(3): 726–741.
[PubMed Abstract](#) | [Publisher Full Text](#)
26. Lu H, Skolnick J: **A distance-dependent atomic knowledge-based potential for improved protein structure selection.** *Proteins*. 2001; **44**(3): 223–232.
[PubMed Abstract](#) | [Publisher Full Text](#)
27. Word JM, Lovell SC, LaBean TH, *et al.*: **Visualizing and quantifying molecular goodness-of-fit: small-probe contact dots with explicit hydrogen atoms.** *J Mol Biol*. 1999; **285**(4): 1711–1733.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Samudrala R, Levitt M: **Decoys 'R' Us: a database of incorrect conformations to improve protein structure prediction.** *Protein Sci*. 2000; **9**(7): 1399–1401.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Engh RA, Huber R: **Accurate bond and angle parameters for X-ray protein structure refinement.** *Acta Crystallographica Section A*. 1991; **A47**: 392–400.
[Publisher Full Text](#)
30. Chen VB, Arendall WB, Headd JJ, *et al.*: **MolProbity: all-atom structure validation for macromolecular crystallography.** *Acta Crystallogr D Biol Crystallogr*. 2010; **66**(Pt 1): 12–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Wiederstein M, Sippl MJ: **ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins.** *Nucleic Acids Res*. 2007; **35**(Web Server issue): W407–410.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Vriend G: **WHAT IF: a molecular modeling and drug design program.** *J Mol Graph*. 1990; **8**(1): 52–56.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Richardson J, Richardson D: **The zen of model anomalies – correct most of them. Treasure the meaningful valid few. Live serenely with the rest!** *Advancing Methods for Biomolecular Crystallography*. Springer Netherlands: 2013; 1–10.
[Publisher Full Text](#)
34. Kryshchakovich A, Monastyrskyy B, Fidelis K: **Casp prediction center infrastructure and evaluation measures in casp10 and casp roll.** *Proteins*. 2013.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. Lovell SC, Davis IW, Arendall WB, *et al.*: **Structure validation by Calpha geometry: phi, psi, and Cbeta deviation.** *Proteins*. 2003; **50**(3): 437–450.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Chakraborty S, Minda R, Salaye L, *et al.*: **Active site detection by spatial conformity and electrostatic analysis—unravelling a proteolytic function in shrimp alkaline phosphatase.** *PLoS ONE*. 2011; **6**(12): e28470.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Rendon-Ramirez A, Shukla M, Oda M, *et al.*: **A Computational Module Assembled from Different Protease Family Motifs Identifies PI PLC from Bacillus cereus as a Putative Prolyl Peptidase with a Serine Protease Scaffold.** *PLoS ONE*. 2013; **8**(8): e70923.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Helland R, Larsen RL, Asgerirsson B: **The 1.4 Å crystal structure of the large and cold-active Vibrio. sp. alkaline phosphatase.** *Biochim Biophys Acta*. 2009; **1794**(2): 297–308.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Chakraborty S, Asgerirsson B, Minda R, *et al.*: **Inhibition of a cold-active alkaline phosphatase by imipenem revealed by in silico modeling of metallo-β-lactamase active sites.** *FEBS Lett*. 2012; **586**(20): 3710–3715.
[PubMed Abstract](#) | [Publisher Full Text](#)
40. Honig B, Nicholls A: **Classical electrostatics in biology and chemistry.** *Science*. 1995; **268**(5214): 1144–1149.
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Bashford D, Karplus M: **pKa's of ionizable groups in proteins: atomic detail from a continuum electrostatic model.** *Biochemistry*. 1990; **29**(44): 10219–10225.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Baker NA, Sept D, Joseph S, *et al.*: **Electrostatics of nanosystems: application to microtubules and the ribosome.** *Proc Natl Acad Sci U S A*. 2001; **98**(18): 10037–10041.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Chakraborty S, Rao BJ: **A measure of the promiscuity of proteins and characteristics of residues in the vicinity of the catalytic site that regulate promiscuity.** *PLoS ONE*. 2012; **7**(2): e32011.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Shen MY, Sali A: **Statistical potential for assessment and prediction of protein structures.** *Protein Sci*. 2006; **15**(11): 2507–2524.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Kryshchakovich A, Fidelis K, Tramontano A: **Evaluation of model quality predictions in CASP9.** *Proteins*. 2011; **79**(Suppl 10): 91–106.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
46. Benkert P, Tosatto SC, Schomburg D: **QMEAN: A comprehensive scoring function for model quality assessment.** *Proteins*. 2008; **71**(1): 261–277.
[PubMed Abstract](#) | [Publisher Full Text](#)
47. Tosatto SC: **The victor/FRST function for model quality estimation.** *J Comput Biol*. 2005; **12**(10): 1316–1327.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Bagaria A, Jaravine V, Huang YJ, *et al.*: **Protein structure validation by generalized linear model root-mean-square deviation prediction.** *Protein Sci*. 2012; **21**(2): 229–238.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
49. Moulton J: **A decade of CASP: progress, bottlenecks and prognosis in protein structure prediction.** *Curr Opin Struct Biol*. 2005; **15**(3): 285–289.
[PubMed Abstract](#) | [Publisher Full Text](#)
50. Kryshchakovich A, Barbato A, Fidelis K, *et al.*: **Assessment of the assessment: Evaluation of the model quality estimates in CASP10.** *Proteins*. 2013.
[PubMed Abstract](#) | [Publisher Full Text](#)
51. Benkert P, Biasini M, Schwede T: **Toward the estimation of the absolute quality of individual protein structure models.** *Bioinformatics*. 2011; **27**(3): 343–350.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
52. Handl J, Knowles J, Lovell SC: **Artefacts and biases affecting the evaluation of scoring functions on decoy sets for protein structure prediction.** *Bioinformatics*. 2009; **25**(10): 1271–1279.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
53. Konagurthu AS, Whisstock JC, Stuckey PJ, *et al.*: **MUSTANG: a multiple structural alignment algorithm.** *Proteins*. 2006; **64**(3): 559–574.
[PubMed Abstract](#) | [Publisher Full Text](#)

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Simon Lovell

Faculty of Life Sciences, University of Manchester, Manchester, UK

This is a nice, straightforward analysis of features of protein decoy sets. The authors find that a simple, novel measure of protein geometry is sufficient to distinguish native structures from decoys in the popular fisa decoy set. A rational response to this work would be to include measures of geometry such as that which are used here in the generation of the decoys. This would make the measure less useful, but would improve decoy sets.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Nov 2013

Sandeep Chakraborty, Tata Institute of Fundamental Research, Mumbai, India

Dear Dr Lovell,

Thank you for your encouraging comments on our manuscript. We took a lot of inspiration from your work "Artefacts and biases affecting the evaluation of scoring functions on decoy sets for protein structure prediction. *Bioinformatics*. 2009" (ref 52), while writing our manuscript.

Additionally, we have revised our manuscript adding some additional results we have obtained on the CASP8 I-TASSER decoy set.

Warm regards,

Sandeep

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 October 2013

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Bairong Shen

Center for Systems Biology, Soochow University, Suzhou, China

This paper proposes an absolute discriminator to identify non-native protein structures based on backbone C_α atom distance. Interestingly the authors found that most methods performed poorly in the fisa decoy set from the Decoys "R" Us database and remind researchers to be cautious of using this decoy set since most of the sub-structures in the fisa decoy set are incorrect. This work provides a simple and fast assessment for protein structure quality.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Nov 2013

Sandeep Chakraborty, Tata Institute of Fundamental Research, Mumbai, India

Dear Dr Shen,

Thank you for taking the time to review our manuscript. We appreciate your encouraging comments. We have revised our manuscript with some additional results from the CASP8 I-TASSER decoy set, and also cited some recent manuscripts which had been published since the version 1 went online. We hope you find the revised version improved.

Warm regards,

Sandeep

Competing Interests: No competing interests were disclosed.

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