ProCS15: A DFT-based chemical shift predictor for backbone and Cβ atoms in proteins
(#2015:08:6439:0:0:REVIEW)

Response to editor and reviewer comments

Editor
Both reviewers have some minor corrects to make and the second reviewer raises a point of skepticism about QM-based vs empirical estimators. A discussion addressing this would likely be of benefit to the field.

Our general response it that in the case of amide protons our previous work (Christensen et al., 2013) has shown that the accuracy of a QM-based estimator can be made to rival that of empirical predictors by making relatively small changes to the protein structure that actually improved the structure. The extension of ProCS to other nuclei, as described here, is the first step in testing whether this is true for other atom types. We feel that all these points have already been made in the manuscript so we have only made minor changes as described below.

Reviewer 1
1. “In the Introduction section, “RMSD observed for QM-based chemical shift predictions may, at least in part, be due to relatively small errors in the protein structures used for the predictions, and not a deficiency in the underlying method.” I agree with the first half of the statement, however, the limitation of current density functionals also contributes to the discrepancy between experiment and DFT calculations, especially for the 15N chemical shift prediction.”

Response: We have now changed “underlying method” to “choice of functional and basis set”

2. The first AF-QM/MM work is highly recommended to be cited in the paper,
He X., Wang B. and Merz K.M., Protein NMR Chemical Shift Calculations Based on the Automated Fragmentation QM/MM Approach. J. Phys. Chem. B 113, 10380 (2009)

Response: we have added the reference

Reviewer 2
“The performance is comparable to other quantum based predictors but is worse than current empirical predictors. Because of this, I am still skeptical about all quantum-based predictors. Without solid cross-validation, it is very hard to argue that quantum predictors can capture subtle effect better than empirical predictors. It is true they respond more sensitively to minor structural change, but not necessary in a correct way.”

Response: As we wrote in the Introduction

“Some of us recently showed (Christensen et al., 2013) that protein refinement using a DFT-based backbone amide proton chemical shift predictor (ProCS) yielded more accurate hydrogen-bond geometries and $^{3h}J_{NC'}$ coupling constants involving backbone amide groups than corresponding refinement with CamShift.”

So, in the case of amide protons this has been established with solid cross-validation. We believe we are careful to stress that we don’t know whether this observation also be true for other nuclei (emphasis added here clarification):

“This suggests that the larger RMSD observed for QM-based chemical shift predictions **may, at least in part**, be due to relatively small errors in the protein structures used for the predictions, and not a deficiency in the underlying method. However, **in order to test whether this is true in general** we need to include the effect of more than one type of chemical shift in the structural refinement.”

2. “All Ubiquitin NMR structures cited in this work are generated specifically to be a more realistic presentation of protein ensemble in solutions, except 1D3Z. 1D3Z is a traditional NMR structure model, where NMR conformer “bundle” should not be confused with a dynamic ensemble representation of the protein. In these types of NMR models, the spread of atomic positions merely provides information about the uncertainties of the atomic positions with respect to the average structure and has no direct physical meaning. The author may need to provide more comments on this in their last section titled “Comparison to experimental chemical shifts using NMR-derived ensembles”.”

Response: “We have now added the following text: “Indeed, all but one of the ensembles used here were generated specifically to be a more realistic presentation of protein ensemble in solutions. The exception is 1D3Z, which is a traditional NMR structural model where the conformational diversity is mainly an expression of lack of structural constraints.”