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Comment

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Reply to "Comment on 'A Tensor-Free Method for the Structural and Dynamic Refinement of Proteins using Residual Dipolar Couplings'"

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Wirz and Allison observe that in order to compare the results of molecular dynamics simulations with nuclear magnetic resonance (NMR) spectroscopy measurements it is important to average over the conformational degrees of freedom of protein molecules. We fully agree with this observation,[1−3] and indeed, this idea has been our main motivation in developing the tensor-free "ϑ method" for using residual dipolar couplings (RDCs)[4,5] as structural restraints in replica-averaged molecular dynamics simulations.[6]

Wirz and Allison then suggest that the ϑ method does not consider the averaging over the conformational degrees of freedom of protein molecules. We note that this comment applies only to a very specific application of the ϑ method, which we discussed as an example in our original paper[6]—the RDC-based structural refinement of a single protein conformation. In this case, the degrees of freedom of the protein are considered fixed by hypothesis, since the goal is to determine a single structure, and the dynamics are explicitly ignored. This approximation is relatively accurate in the case of rigid native states, such as that of the protein ubiquitin,[6] but not suitable when sizable structural fluctuations are present.

Leaving aside the particular case of rigid structures, however, the ϑ method, as we noted,[6] is more general, since it can be implemented using replica-averaged restraints to determine also the dynamics of proteins,[1−3] a procedure that represents an implementation of the maximum entropy principle for interpreting experimental measurements.[2,7−9] In this way, the averaging over both the internal and external degrees of freedom of the protein can be performed using the ϑ method.[6] To illustrate this point here, we present the results of calculations in which we considered a rhombic alignment, which is the situation referred to by Wirz and Allison. A rhombic alignment is present when there are dynamics in the external degrees of freedom that result in different interchanging orientations of the protein. In these calculations, we used the PALES method[10] to generate artificial RDCs from an X-ray structure of ubiquitin (PDB 1UBQ), assuming an alignment tensor with a very high rhombicity value (0.60). A calculation in which we used these artificial RDCs as structural restraints and only one replica resulted in a structure in good agreement with the RDCs ($Q \sim 0.2$) but with rhombicity = 0.13. This result shows that the lack of averaging over the external degrees of freedom is not negligible in this case and, even if the internal degrees of freedom are rather well represented, for the external degrees of freedom there is a failure in reproducing the multiple orientations of the protein molecule. When, however, the calculation was repeated using two and eight replicas, respectively, the rhombicity was correctly recovered at 0.60 (Figure 1).

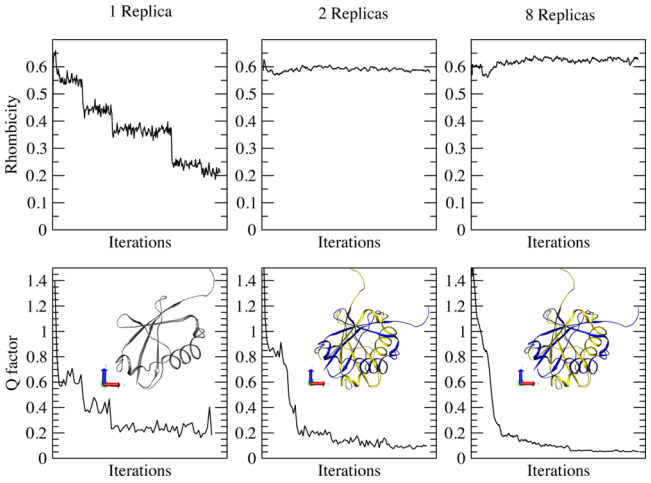

Figure 1. Comparison of the results of the tensor-free "ϑ method" calculations using one, two, and eight replicas in the case of the protein ubiquitin in a rhombic alignment. These results show that the multiple orientations of ubiquitin are recovered using more than one replica.

An additional calculation using two replicas in which the two replicas were forced to maintain the same internal degrees of freedom also correctly recovered a rhombicity of 0.60, indicating explicitly that the averaging over the external degrees of freedom can be carried out appropriately even in the absence of internal dynamics. We note that this calculation is equivalent, in terms of external degrees of freedom, to using a rhombic alignment tensor. However, in implementing methods based on the use of alignment tensors, it may be challenging to perform a correct averaging over the internal degrees of freedom.

Representative structures resulting from the above simulations are shown in the insets of Figure 1; in the case of one replica, the orientation with respect to the z axis is in between the two correct orientations, which were recovered using two and eight replicas. We also note that it has been recently shown that it is possible to generalize the ϑ method using multiple alignment media.[11]

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These results indicate that the use of the ϑ method within a replica-averaging approach[1−3,6] enables the explicit averaging of both internal and external degrees of freedom of protein molecules, without any hypothesis on the relationship between them, to be performed correctly in a tensor-free manner.[12]

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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