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Magn. Reson. Discuss., referee comment RC1
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Comment on mr-2021-27

Anonymous Referee #1

Referee comment on "Nuclear magnetic resonance free ligand conformations and atomic resolution dynamics" by Amber Y. S. Balazs et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-27-RC1, 2021

General comments

Balazs et al. present an addition to their NAMFIS approach which now includes characterization of populations of alternate conformations and dynamics of the molecules under study. With the replica exchange approach, simulations times are virtually extended such that slow conformational exchanges can be accessed. The refined approach supports prospective ranking of design ideas in medicinal chemistry in a very efficient manner and sheds light onto alternative conformations and dynamics. Further, REST-MD indicates where potentially interesting alternate conformations and dynamics may occur which should be tested by NMR experiments, thereby much simplifying and reducing the NMR analysis in a targeted manner.

Specific comments

Why were NMR experiments carried out in DMSO? This is somewhat puzzling since the MD simulations were carried out in explicit water with NaCl. I could well imagine that alternative conformations are populated differently in DMSO than in aqueous buffer. Also, activity assays used to identify the bioactive conformation are carried out in aqueous

buffer.

The MD protocol seems to yield very nice results for those torsion angles with an energy barrier < 10 kcal/mol. However, there are some questions about the rotation with the 25 kcal/mol barrier in the top right diagram in Figure 3b: Why aren't the two rotational energy barriers of identical height? The one at -170° seems to agree with the experimental value, while the one at 10° seems significantly higher.

Figure 3b, top right diagram: the profile seems to imply lower energy for the +100° rotation angle, yet the populations are opposite.

The molecule presented is a very nice example of hindered rotation. However, if would be very instructive in order to assess the method, if more than just one example would be shown. But I guess that this is not possible due to trade secrets.

In order to reproduce such simulations, what would be your recommendation for the maximal temperature of the simulation? 3300 K looks a bit harsh, but seems to be required to compensate for short simulation times, even with replica exchange.

For prioritization of design ideas, relative populations of alternative conformations are important. The populations in Figure 5 however don't seem to correlate with the actual populations observed by NMR. In your experience, which parameter of the simulation should be used to derive relative populations? Populations during MD (blue histograms), fragment based energy landscape (solid blue lines) or a combination of these parameters?

A very similar question comes up for how the dynamics in the MD correlate with the NMR experiment: Should the calculated energy barrier of rotation be used or how often an alternate conformation was visited in the MD?

Could references be provided that support the statements that NMR provides information on permeability and bioavailability etc.?