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Reply on RC1

Amber Y. S. Balazs et al.

Author 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-AC1, 2021

Q: 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.

A: We use DMSO in routine application of this approach to ensure consistency across ligands, since solubility issues will often arise in pure water. Our aim is always to balance computational and experimental insight – we simulate in the biorelevant solvent, but do not forgo experimental insights by insisting on a single solvent system. This combination of water models with DMSO-derived data reflects the real world application of the workflow.

Q: 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.

A: High rotational barriers will be subject to hysteresis and sampling effects and it is not possible to assign rigorous values to those barriers. The profiles are indicative of barrier height, but we rely on NMR data to rationalize these.

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

A: The minima lie at the same energy but sampling has been limited, as discussed further in Fig 5.

Q: 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.

A: Yes, the matched pair exemplar supports the complementarity of the computation and experiment for use as a platform to guide design, while keeping within the highest practicality for our current scope.

- *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.*
- In REST-MD the temperature is only a surrogate value, since the Hamiltonian is actually modified rather than the temperature. Other groups use the terminology Hamiltonian replica exchange to distinguish this from standard temperature replica exchange. We show this very high surrogate temperature to demonstrate that sampling of high torsional barriers can be limited, even when the Hamiltonian is modified to mimic such a high temperature.
- *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?*
- In a case where we see no issues with sampling of hindered rotations, as discussed in Fig 5, we use Boltzmann counting to assign simulation populations, after clustering using full-molecule RMSD. This would correspond to the circular histograms.
- *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?*
- In principle the MD trajectory should equilibrate to Boltzmann-weighted populations, consistent with the free energy of each conformational state. We use the computed torsion barriers to guide analysis of NMR data and look for consistency between barrier heights and NMR signals. As noted above, it is not guaranteed that an unbiased MD trajectory will be able to explore all the conformers and cross all barriers to establish an equilibrium population. The most effective approach that we have found is to look at both experimental and computational data and to rely on models only when we have robust evidence that we are seeing trends consistent with experiment.
- *Could references be provided that support the statements that NMR provides information on permeability and bioavailability etc.?*

ie: p.12 lines 288-289: In addition, NMR provides design teams with information on the presence of intramolecular hydrogen bonds (IMHB), and the combined influences on properties such as potency, permeability and oral bioavailability

- The first four references to follow this reply are to ePSA, as a measure of masked polarity through indirect detection of intra-molecular hydrogen bonds (IMHB), where ePSA is used as a surrogate for permeability measurements. The fifth reference is for Anmr as a measure of IMHB by NMR. We're suggesting (manuscript in preparation) the calculated hydrogen bond donor (HBD) count on a 2D structure can be greater than the "effective" number of HBDs. Using NMR to measure IMHB's and subtracting that from the 2D calculated HBDs gives the number of "effective" HBDs, and fewer HBDs, via IMHB masking, correlates with improved passive permeability which correlates with improved bioavailability.

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