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Magn. Reson. Discuss., author comment AC2
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Reply on RC2

Lilia Milanesi et al.

Author comment on "High-affinity tamoxifen analogues retain extensive positional disorder when bound to calmodulin" by Lilia Milanesi et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-7-AC2, 2021

We would like to thank the reviewer for taking the time to read and comment on our manuscript, and for their positive reaction to it. Regarding the minor issues raised:

We had included the point - that the number and linewidth of the bound idoxifene resonances in Fig S1 require that the reorientation of CaM-bound idoxifene molecules must be fast relative to the overall dissociation rate - in the Discussion section (on line 415) in the original manuscript. Nonetheless, we have amended the manuscript to better emphasise this point.

We agree that the use of a range of mixing times would allow the extent of any spin-diffusion effects to be quantified thoroughly, but unfortunately we are unable to run any more such experiments on a reasonable timescale, as we no longer have access to idoxifene. The conclusion that spin-diffusion effects are not dominant in the bound ligand comes from the absence of NOEs between protons separated by more than 5 Angstroms in idoxifene. We have amended the manuscript to incorporate this point.

On re-reading the Resonance and NOE Assignment part of the Materials and Methods section we see that it had not been finished properly and hence was quite confusing. We have re-written this section more clearly in the revised manuscript, and harmonized it better with the results section.

The numbering system of idoxifene is derived from previous tamoxifen analogues, and every atom is required to be represented uniquely in the topology file for the structure calculation. Only carbon atoms bonded to H are annotated in Fig. 1. Atoms 1-3 are heteroatoms (I, O, N) and carbons 4, 5, 6, 14, 17, 20, and 23 have no directly bonded H atoms.

We have used the 1H signal of TSP in the referencing process and ignored the up to 0.015 ppm potential discrepancy with the 1H signal of DSS. The chemical shift changes reported will be unaffected, and the referencing is all internally consistent.