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Magn. Reson. Discuss., author comment AC3
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Reply on CC1

Nestor Kamdem et al.

Author comment on "Small-molecule inhibitors of the PDZ domain of Dishevelled proteins interrupt Wnt signalling" by Nestor Kamdem et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-20-AC3, 2021

Most importantly, Ji Zheng requested an NMR binding assay. We have done the requested NMR binding assay with Dvl-3, which indicated binding in the three-digit micromolar range. Employing an eight-fold excess of ligand (as commonly in this study), we observed only very small chemical shift changes, with a maximum change of still below 0.1 ppm for only one signal. Table S1 lists similar chemical shift changes (but above 0.1 ppm as an average over three signals) for ligands that bind in the three-digit micromolar range (eg. compound 2 and 6). Those three shifting signals are indicated in Fig. S2, and comparison of S2 and S8 displays a considerable difference. In S2, the highest ligand concentration was 8-fold excess. Our best binding compounds show shift changes larger 0.3 ppm as an average over three signals. The compound was checked by mass spectrometry. We will include the NMR binding assay employing 8-fold excess of the compound into Figure S8 and reword the discussion related to this compound, see reply to Minye Zhang: 'Based on our NMR and ITC studies, the binding affinity of CBC-322338/3289-8625 to DVL-3 seems to be less than 50 micromolar, comparing CSPs from the NMR assay with those of our other compounds listed in Table S1 and considering the weak heat development in our ITC assay, which was larger than the originally reported value (10.6 +/- 1.7) (Grandy 2009) that was OBTAINED WITH A DIFFERENT METHOD.'

We would like to mention that the ITC and NMR assays above were performed by different persons in different labs here on the campus in Berlin Buch, and the compound was obtained from the EU-OPENSCREEN (an EU facility) and FMP compound library that is handled under professional conditions. The compound was obtained from the facility already dissolved in DMSO and this solution was quality-controlled by LC-MS. Independently, Hori et al. found a similar binding characteristic, also in a direct binding assay.

With respect to the inclusion of the ITC and NMR results into Table 1 we made sure that the values for Dvl-1 and Dvl-3 are determined with the same method. We would like to mention that we do not have both values in all of the cases, especially as many values are associated with compounds synthesized at intermediate stages of the project, and one value was sufficient for finding the right path. It was important to us to provide values determined by ITC for the important compounds 18-21 since it may be considered the superior method in such investigations. However, we have reported chemical shift changes

(DCSP) for most of the compounds in the supplementary information (Table S1 on page 14).