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Magn. Reson. Discuss., referee comment RC3
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Comment on mr-2022-6

Paul Schanda (Referee)

Referee comment on "Imatinib disassembles the regulatory core of Abelson kinase by binding to its ATP site and not by binding to its myristoyl pocket" by Stephan Grzesiek et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2022-6-RC3, 2022

In this manuscript, Grzesiek and co-workers describe new experimental data and use previously published data to critically evaluate a manuscript by the Kalodimos group in JMB. Correcting flawed data in the literature is extremely important for the scientific community and an integral part of our work, and, hence, this manuscript deserves much attention (as well as a critical reading).

It is a pity that the JMB editor(s) have decided not to consider the manuscript on their own pages, but it is also my experience that journals are hesitant to publish critical views of manuscripts earlier reported on their pages.

The matter of debate here is whether imatinib, a small molecule that acts as an ATP-site inhibitor of the Abl kinase, binds to the myristoyl binding site, in addition to the ATP binding site. There are two conflicting models: the Grzesiek group has claimed that binding to the ATP site (nM affinity) leads to opening of the regulatory core. In contrast, the Kalodimos group has proposed that the binding may occur at an allosteric site instead of the orthosteric site, and that this binding may lead to disassembly of the core and thus activation.

These two models (nM-affinity binding to the ATP-site or microM-affinity binding to an allosteric site) shall have very different consequences for NMR titrations. And the authors of the present manuscript go and do exactly this experiment: a titration experiment. The data, shown in Figure 1 A, B, are extremely convincing to me, and they show that binding occurs at the orthosteric site, rather than the allosteric site.

In itself, this experiment clarifies the debate: binding is in agreement with nM affinity binding, but not with uM allosteric binding. This new data is, thus, important to the field.

However, the data do not directly show that the binding occurs at the ATP site. The groups seem to agree that nM binding is necessarily at the ATP site. But could there be any direct NMR evidence for this? I wonder if the NMR chemical shifts during the titration provides hints to the binding site. Has this been done in the previous papers? This should be clarified.

I also agree with the additional problematic points that the authors listed (lines 190 – 225; see some more specific comments below). It would be nice if these points could be clarified with Kalodimos' group before the present manuscript gets out.

My conclusion is, thus, that this manuscript should be published. The ideal place would be JMB, and Kalodimos and co-workers may want to have a chance to reply to the criticism (although the case seems really clear to me). But if JMB editors feel like they are not ethically bound to clarifying/correcting what is on their pages, then Magn. Reson. is a possible place.

The question is whether the current manuscript can be made more easy to understand and read. It would be tremendously helpful if the structure was displayed and the important structural features labeled somewhere. For example, the "A-loop", and "pushing forces" onto that loop, as well as the helix alpha-I are described in the text; likewise, the authors write about the "DFG out" state. But without seeing where these things are located, it is difficult. It would already help to label Figure 1C accordingly, and a scheme like the one of Figure 1A and 4A of reference P1 (the Kalodimos paper); some scheme to show the binding sites and the changes (disassembly, loops being pushed, helix alpha-I kinked etc).

And then the critical question is which observations have lead Kalodimos' group into conclusions which the present data show as incorrect. Now the authors provide some bits of information why the Kalodimos group may have gotten it wrong (e.g. ITC not done on the relevant constructs).

I would find it very helpful if the text was structured as follows:

- Show and discuss the structures/sketch/models that highlight the divergent views of the two groups

- Highlight what the two models imply, e.g. for a titration experiment
- Discuss the new titration experiment
- Discuss the possibly reasons why the Kalodimos group may have come to wrong conclusions (ITC, short constructs,….)
- Discuss the further problematic points, which in my opinion are all good
- "Reply" to the criticism that Kalodimos raised against Grzesiek's work (which is now the first part of 3.1.)

Personally, it would find this much nicer to read and understand. Currently, one needs to read the JMB paper, and possibly read the present manuscript more than once to get the point.

Another important point is that the authors make the new data totally open – something that lacks in Kalodimos' paper. It is nice to see that the titration spectra are all deposited on zenodo. It would be nice to have them additionally also as figures. I would like to see the full spectra, and/or multiple zooms, rather than only four examples.

Below are further points that are comments or suggestions for improvements.

Point 3. on lines 209-211 is valid: the concentrations are an important information. In addition, having looked at the original paper P1, and the Supp Info of the P1 paper by Kalodimos, I could not really identify that peak in the full spectrum, and I would have liked seeing the full spectra of all the states of Figure 4B of P1. It is a poor practice to show only a zoom onto one peak; it does not allow getting a comprehensive picture. The authors may want to make this point.

The same is true for the point 4, which refers to Figure 5. Again, the authors of P1 have decided not to show the full spectra, which is really a pity. It is also noteworthy that the identity of the spectrum shown in orange color in Figure 5 of P1 is not specified.

Line 165: "It also needs to be indicated that the obtained KD solution structures (Xie et al., 2020), on which the argument is based, are of low definition having at most 3 NOE and 2 dihedral (the origin of which is not documented) constraints per residue. Hence, a well-defined A-loop conformation cannot be postulated without additional evidence.» This is indeed an important and compelling argument by the authors. It would be useful to state here explicitly where they have obtained the information about the number of

constraints. I assume they retrieved the constraints in the BMRB/PDB.

On lines 171-173, the titration data are described, and four residues for which a new peak appears are named. I would find it useful to state explicitly which residues, in addition to the named ones, show a second peak. Moreover, please show the full spectra in the Supplementary Information.

On lines 197-200, it is stated that «often» the description in P1 is insufficient. I would recommend to be as specific as possible, because a direct criticisms calls for being very specific. I propose that the authors explicitly name occurrences where information is lacking.

Methods: The protein concentration, specified as “79 uM” appears to be very precise, but I wonder if this precision is real. “Ca. 80 uM” (with some error estimate?) is most likely more realistic.

Figure 6 of P1 contains an interesting y-label in a plot, “Lorem ipsum”. Not a sign of very careful work; but probably nothing that can be addressed here.