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Magn. Reson. Discuss., referee comment RC1
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Comment on mr-2022-6

Anonymous Referee #1

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-RC1, 2022

The present article argues that the Abl ligand imatinib binds to the ATP, not allosteric, site and that this leads to disassembly of the regulatory core of Abl. The authors show NMR titration data that indicate tight binding of the ligand at a protein concentration of 79 µM. The data are presented in response to a paragraph in a recent article by Xie et al. (2022), who report not having found any evidence of a shift in conformational equilibrium when imatinib binds to the catalytic pocket. There appears to be a clear discrepancy in the interpretation of data regarding the Abl-imatinib complex, which the authors of the present article attribute to different protein constructs used. Imatinib is an approved anti-cancer drug (Gleevec), i.e. the questions raised are important.

The binding curves of Figure 1B are characteristic of slow exchange on the chemical shift time scale, indicative of a dissociation constant at least 100-fold smaller than the protein concentration (reported to be 79 µM). Still, a more detailed argument would help convince the reader that an exchange rate < 200 s^{-1} is expected for nanomolar affinities and not compatible with micromolar affinities (line 176).

Arguably, the spectral data shown in the present article do not, by themselves, identify the site of binding, as ligand binding can cause changes in chemical shifts all over the place, if the target protein is an allosterically active, flexible protein. Can the argument for binding to the ATP site be strengthened? Previous research seems to have clearly identified the catalytic site as the site of tight binding and, to my understanding, the publication by Xie et al. (2022) does not claim anything else.

Granted that binding is in the ATP site, it is still difficult to follow the authors' conclusion that 'the imatinib-induced opening of Abl's regulatory core is caused by imatinib binding to the ATP site'. What exactly is the evidence of opening of the structure? This deserves better explanation.

Section 3.3 reads like a referee report, which would be unusual in a regular article. I am certain that the technical queries can easily be answered by the authors of the article by Xie et al. (2022). In this case, I suggest to remove the entire section prior to publication of the article in Magn. Reson. If the queries remain unanswered, the section should still be condensed to a few sentences to simply and concisely state the open questions.

One may wonder why the communication between the authors of the articles is so difficult?

In general, it is a regrettable trend that many high-profile articles present some of the data with incomplete descriptions, which prevents others from reproducing the results with reasonable effort. Referees and authors should do better.

In this vein, the authors of the present article could be encouraged to show the complete TROSY spectra as supporting information, for the benefit of readers who do not wish to access the data deposited in Zenodo but are still interested to get an impression of their quality, as the construct used was very big (452 residues) and the protein, which was apparently only labelled with ^{15}N, relatively low in concentration (79 µM).

Minor suggestions:

Line 14: remove 'some'

Line 15: should it be 'Although' instead of 'Albeit'?

Lines 11 and 19: conventional style would be to abbreviate journal names to Proc. Natl. Acad. Sci. and J. Am. Chem. Soc. and omit issue numbers.

Line 35: P1 for the article by Xie et al. is an unfortunate abbreviation, as P1 is later used to refer to a protein state (Fig. 1D). The different articles may be better referred to as A1, A2 etc.

Line 37: what is 1b numbering - a reference might help.

Line 54: why 'now', if no new information has been provided by the authors of P1 since publication of the P1 article?

Line 58: The way the sentence starting with ‘However’ reads, it sounds as if the reference to a past article is sold as an experimental result. It may be less confusing for the reader to start with ‘We regret that the authors of P1 …’

Fig. 1: use uniform font sizes for the figure titles, axis labels and residue numberings in the structure.

Line 80: no deuteration?

Thoughout the text, legibility would improve if variables like K_{DA} were written in italics style (as in Fig. 1D).

Line 116: it appears to me that the argumentation regarding binding to the allosteric site at concentrations above 100 µM is correct. Would it be correct to say ‘While high concentrations can lead to binding of imatinib to the allosteric site, in the following we discuss and provide evidence that binding of imatinib to the catalytic site is sufficient to disassemble the regulatory core.’

Line 119: instead of starting a sentence with ‘However’, it often is better style to start with ‘In contrast’.

Line 131: explain ‘DFG out’

Line 158: ‘when’ instead of ‘since’ would be clearer.

Line 160: ‘reported’ instead of ‘have observed’

Line 162: ‘broadened beyond detection’ instead of ‘bleached out’

Line 174: for all these residues