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Magn. Reson. Discuss., referee comment RC5
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Comment on mr-2022-6

Anonymous Referee #3

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-RC5, 2022

The manuscript by Grzesiek and coworkers is a response to a recent publication by the Kalodimos lab (Xie et al JMB 2022) who concluded that imatinib, a well-established nanomolar ATP-site inhibitor of Abl kinase, can also bind to the allosteric myristoyl site, although with 10 uM affinity. Xie et al concluded that binding to the myristoyl and not the ATP site may be responsible for the imatinib-induced opening of the Abl regulatory core, which would suggest an alternative mechanisms and might rationalize the effect of imatinib on ATP-site resistance mutations.

The response by Stephan Grzesiek and coworkers, presents a number of clear and convincing argumentsm why the conclusions put forward by Xie et al are not supported by data presented and likely are linked to the use of truncated protein constructs. The manuscript presented here provides additional support for their previous work, which had shown that imabinib binding to the ATP-site indeed induces opening of the regulatory core.

There are key arguments and data presented that suggest that several aspects of the work by Xie et al are flawed, and that reconfirm the previous work by Grzesiek and Jahnke groups. Key points made are:

- Xie et al do not show key results using the full regulatory core kinase construct. Rather, evidence is based on a crystal structure that shows binding of imatinib to the allosteric site, however, using a truncated fragment of the protein, which in fact deletes parts of helix alpha1 that regulates the myristoyl site. This is suggests a crystallization artifact.
- No evidence was shown using solution techniques with the full regulatory core by Xie et al. Grzesiek et al now show NMR titrations that are fully consistent with binding of imatinib to the ATP-site and inconsistent with significant binding occupancy of the myristoyl site in solution.
- This is also consistent with mass-action analysis of binding site occupancies of imatinib

with known nM Kds to the ATP-site and the 10 uM Kd reported by Xie et al, which shows that the higher affinity binding site for the ATP sites is dominant.

- Previous competition experiments using a 19F labeled reporter (Skora/Jahnke,2017) with the full regulatory core has established that imatinib does not compete with a 43 uM binding reporter to the allosteric site.

Additional arguments and data presented and missing experimental details and incorrect labeling in the paper published by Xie et al, indicate that the conclusions made by Xie et al likely are linked to the observation of a binding site in a crystal structure with a truncated kinase fragment and lack of rigor and consistency in analyzing the experimental data.

It is very unfortunate and dissappointing, that apparently neither JMB editors nor the corresponding author of the manuscript published in JMB, have responded to the arguments presented here. An editorial response and an offer to present the arguments in a public letter to JMB would be the correct way to address this situation.

In any case, the arguments presented are fully convincing and should be published as soon as possible to make the scientific community aware of the issues with the conclusions presented by Xie et al.