nature portfolio

Peer Review File

Cryo-EM structure of Alzheimer's disease tau filaments with PET ligand MK-6240

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this study, Shahmoradian and coworkers presented the cryo-electron microscopy (cryo-EM) structure of an ex vivo tau paired helical filament (PHF) and the bound positron emission tomography (PET) ligand MK-6240 at a resolution of 2.31 Å. Second-generation PET ligands such as [18F]MK-6240 are explored as diagnostic tools for patients suffering from Alzheimer's. The extracted tau filaments used in this study were from a patient with advanced Alzheimer's disease. After incubation of the extracted tau fibrils with the MK-6240 ligand, a reconstruction was performed that revealed an additional density that was assigned to the PET ligand. Their assignment is supported by their control, an additional reconstructed (unbound) fibril (3.0 Å) incubated with DMSO instead of the ligand and lacking this additional density. Local determination of the ligand then allows conclusions to be drawn about the interactions with tau protein. Probable interactions of amino acids Q351 and I360 as well as the possible hydrogen bond K353 are listed due to their close proximity to the ligand. The side view of the reconstructed map shows the 4.8 Å distance of the stacked MK-6240 ligand and the significance for the halogen and pi-pi aromatic interactions of MK-6240. The results help in the development of new and improved PET ligands that can be better used in diagnostics. The study seems technically sound overall (there is important information missing however). Still, it is now the fourth study on this subjects and cryo-EM structures of three similar ligands have been published (https://doi.org/10.1007/s00401-021-02294-3, https://doi.org/10.1038/s41467-023-38537-y, https://doi.org/10.1016/j.jmb.2023.168025). While the current study deviates to some extend from these previous investigations there is not enough important novelty and the manuscript should be sent to a more specialized journal. One of the other studies was published in JMB, for instance.

Reviewer #2 (Remarks to the Author):

This manuscript by Kunach et al. describes the high-resolution cryoEM structure of tau paired helical filaments purified from AD postmortem brain in complex with the small molecule PET tracer, MK-6240. There is strong interest in the development of improved PET radioligands for the diagnosis of AD and other tauopathies through the in vivo detention of tau aggregates and tangles in patients. However, small molecule binding to the different disease forms of tau amyloids is relatively under characterized given the filaments, which adopt different disease conformations, currently must be purified directly from brain tissue. Additionally the relatively shallow repeating surfaces of amyloids present challenges to conventual computational small molecule modeling methods. The structure presented here identifies MK-6240 binds stably, with high occupancy to the C-shaped cavity of tau PHFs, as evidenced by the well-resolved density in the cleft that is not present in their unliganded structure. Based on modeling they identify the compound binds in a slanted, stacked arrangement along the filament axis with a 4.8 angstrom spacing similar to the rungs of the PHF. With this arrangement they identify MK-6240 ligand-ligand interactions buries more surface area than the ligand-protein interface, indicating the pi-pi aromatic interaction between the molecules plays an important role in binding. Thus, this work provides an important contribution to understanding the mechanism of binding of the MK-6240 ligand to AD PHFs. The cryo-EM structures and modeling analyses are of high quality and are well-described. Interestingly, the authors also perform MK-6240 PET imaging data of rat brains following injection of AD patient brain material to track propagation of tau filaments in living animals, although there does not appear to be a strong response. Notably, and as discussed by the authors, a previously published study identified by cryoEM that the PET tracer GTP-1 also binds the same pocket in a similar stacked arrangement. The significance of the work presented here is therefore somewhat diminished by these previous findings. Nonetheless, identification that another small molecule ligand binds in this same unusual manner is an important advance to the field, thus this work is expected to provide key data for future computational modeling and small molecule development

studies for targeting disease amyloids. As presented in this short format, this work is quite suitable for Nature Communications.

Reviewer Concerns:
• One major concern is the absence of discussion of straight filaments, which are present in their data (Ext. data 3b). Was (or could) a SF structure be determined and does it contain bound MK-6240? It is mentioned in the very last sentence that this would be the topic for future studies. However, it is surprising that SFs are not addressed in the work presented given that these should come up in the classification as they do for other published studies. Analysis of the SFs would add additional novelty given that they were also not addressed in the GTP-1 study.

• The phrase: "Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain…" is an overstatement given there are several previously determined PHF structures with small molecules.

• Additional minor comment: reference to figures 2C and 2D in the main text seem to be incorrect (there is no figure 2D).

Reviewer #3 (Remarks to the Author):

NCOMM MK binding cryoEM

I was asked to comment on the relevance of the manuscript from the imaging perspective.

The manuscript describes characterization of the structure of PHF from a patient with advanced AD neuropathological change bound in vitro to MK-6240 (I will refer to as 'MK') using cryoEM. The authors find a primary binding pocket similar to other tau PET ligands, identify key amino acid interactions, and the binding orientation.

The manuscript does not have much detail in setting up prior knowledge and future directions. It would be useful to know prior information about known binding pockets on PHF tau in the introduction, for example. The ABC categorization could use some context for those not familiar, that tau filaments were derived from a patient with the most advanced neuropathological staging. The statements on impact of the finding are fairly generic. It would be better if the authors could discuss more how the specific information in this work and recent similar works can be used for future drug development. Does the binding interaction/alignment suggest opportunities for improvement for another generation of tracers?

There are several places where the document could be shortened to allow for better context:
1. The rodent experiments are relevant but the methods could be moved to supplement.
2. Paragraph starting, 'It has been well documented': The authors seem to be going beyond the data at hand. the MK compound was previously known to bind PHFs, and while that aspect was further investigated here, the ability to state that it is 'useful' depends on features well beyond binding. The last 2 sentences of this paragraph are not warranted here (and the rest of the paragraph doesn't really have a place either).

There is a paragraph on the differences in PHF structure between bound and unbound states (starting with 'To further understand Mk-6240-induced side chain…'). The concluding statement is a bit of a letdown and also entirely obvious. I'm not familiar with expectations for RSMD, so context of whether these observed deviations are small or large would be helpful. It also seems that MK induces more deviation than GTP-1 (1.1-1.6 angstroms for MK and 0.6 angstroms for GTP relative to unbound); is that correct and is it meaningful?

There's a limitation in using the unpublished SymDOCK model.

Reference 48 is cited as Liu et al in the text but it is Tao et al. in the references. It also would probably be better to reference the primary works for each ligand. it is interesting to compare the

binding modes of the various ligands, however it is not clear what lesson we learn from doing so. Does this account for some known pharmacokinetic differences between ligands?

Is there cryoEM for flortaucipir?

A figure 2D panel is referenced in the manuscript but it is not in the figure. Based on text it is not clear whether the diagrams in B represents the cryoEM result or some other prediction. Also Panels B and C should have amino acids labeled, particularly since K353 is specifically discussed in the legend.

'SF' isn't used enough to warrant an acronym. The sentence starting 'To this end…' needs editing for grammar.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this study, Shahmoradian and coworkers presented the cryo-electron microscopy (cryo-EM) structure of an ex vivo tau paired helical filament (PHF) and the bound positron emission tomography (PET) ligand MK-6240 at a resolution of 2.31 Å. Second-generation PET ligands such as [18F]MK-6240 are explored as diagnostic tools for patients suffering from Alzheimer's. The extracted tau filaments used in this study were from a patient with advanced Alzheimer's disease. After incubation of the extracted tau fibrils with the MK-6240 ligand, a reconstruction was performed that revealed an additional density that was assigned to the PET ligand. Their assignment is supported by their control, an additional reconstructed (unbound) fibril (3.0 Å) incubated with DMSO instead of the ligand and lacking this additional density. Local determination of the ligand then allows conclusions to be drawn about the interactions with tau protein. Probable interactions of amino acids Q351 and I360 as well as the possible hydrogen bond K353 are listed due to their close proximity to the ligand. The side view of the reconstructed map shows the 4.8 Å distance of the stacked MK-6240 ligand and the significance for the halogen and pi-pi aromatic interactions of MK-6240. The results help in the development of new and improved PET ligands that can be better used in diagnostics.

We appreciate the reviewers' comments here. They summarize the key findings accurately and concisely.

The study seems technically sound overall (there is important information missing however). Still, it is now the fourth study on this subjects and cryo-EM structures of three similar ligands have been published (https://doi.org/10.1007/s00401-021-02294-3, https://doi.org/10.1038/s41467-023-38537-y, https://doi.org/10.1016/j.jmb.2023.168025). While the current study deviates to some extend from these previous investigations there is not enough important novelty and the manuscript should be sent to a more specialized journal. One of the other studies was published in JMB, for instance.

We recognize this the need in highlighting the novelty in our first submission and would like to address this critique by emphasizing that this manuscript describes only the second instance of stacked binding arrangements of a PET ligand. As it pertains to a high-affinity tau tracer used in clinical trials, we believe it retains novelty and relevance to the structural pharmacology field. It reinforces the notion that the interactions between adjacent molecules may be an important characteristic shared by high affinity ligands. Furthermore, we would like to highlight that although cryo-EM structures with PET ligands have been previously reported, our study focuses on the PET ligand MK-6240, never before structurally described by cryo-EM. This ligand is distinguished by its high specificity and sensitivity to early-stage changes in the brains of living Alzheimer's Disease patients.

Reviewer #2 (Remarks to the Author):

This manuscript by Kunach et al. describes the high-resolution cryoEM structure of tau paired helical filaments purified from AD postmortem brain in complex with the small molecule PET tracer, MK-6240. There is strong interest in the development of improved PET radioligands for the diagnosis of AD and other tauopathies through the in vivo detention of tau aggregates and tangles in patients. However, small molecule binding to the different disease forms of tau amyloids is relatively under characterized

given the filaments, which adopt different disease conformations, currently must be purified directly from brain tissue. Additionally the relatively shallow repeating surfaces of amyloids present challenges to conventual computational small molecule modeling methods. The structure presented here identifies MK-6240 binds stably, with high occupancy to the C-shaped cavity of tau PHFs, as evidenced by the well-resolved density in the cleft that is not present in their unliganded structure. Based on modeling they identify the compound binds in a slanted, stacked arrangement along the filament axis with a 4.8 angstrom spacing similar to the rungs of the PHF. With this arrangement they identify MK-6240 ligand-ligand interactions buries more surface area than the ligand-protein interface, indicating the pi-pi aromatic interaction between the molecules plays an important role in binding. Thus, this work provides an important contribution to understanding the mechanism of binding of the MK-6240 ligand to AD PHFs. The cryo-EM structures and modeling analyses are of high quality and are well-described. Interestingly, the authors also perform MK-6240 PET imaging data of rat brains following injection of AD patient brain material to track propagation of tau filaments in living animals, although there does not appear to be a strong response. Notably, and as discussed by the authors, a previously published study identified by cryoEM that the PET tracer GTP-1 also binds the same pocket in a similar stacked arrangement. The significance of the work presented here is therefore somewhat diminished by these previous findings. Nonetheless, identification that another small molecule ligand binds in this same unusual manner is an important advance to the field, thus this work is expected to provide key data for future computational modeling and small molecule development studies for targeting disease amyloids. As presented in this short format, this work is quite suitable for Nature Communications.

We appreciate the reviewers' supportive commentary.

Reviewer Concerns:

• One major concern is the absence of discussion of straight filaments, which are present in their data (Ext. data 3b).

This is an important observation, although we suspect the presence of straight filaments in some of the micrographs from this study we were unable to extract the 2D class average associated with this filament type. This seems to be a common observation. At the inception of this study, we aimed to determine the binding properties of MK-6240 and Thioflavin T, with the addition of a control structure where only DMSO was present. As such, three datasets were collected from the same fibril extraction. Of the three datasets, reconstructions of straight filaments were only possible in the sample incubated with Thioflavin T. This example and other datasets collected (our own, GTP-1 study, and EGCG study) suggests that the number of SF's extracted from AD-brain are a minority of filaments and the ability to obtain a 3D reconstruction is unpredicatble.

Was (or could) a SF structure be determined and does it contain bound MK-6240?

A SF structure could be determined using another AD-brain fibril extraction, bearing in mind the points raised above. It has been reported that SF's are the predominant polymorph in Primary Age Related Tauopathy (PART) brain. Efficiently answering MK-6240's ability to bind SF's would be done using a fibril extraction starting from PART brain material, which would be the basis of a separate manuscript.

It is mentioned in the very last sentence that this would be the topic for future studies. However, it is surprising that SFs are not addressed in the work presented given that these should come up in the classification as they do for other published studies. Analysis of the SFs would add additional novelty given that they were also not addressed in the GTP-1 study.

We appreciate this comment and agree that the analysis of SF structure in the presence of MK-6240 would be a novel contribution. However, due to the limitations of using AD brain material, we would recommend starting with and aim to pursue this experiment with PART brain material, as a separate manuscript.

• The phrase: "Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain…" is an overstatement given there are several previously determined PHF structures with small molecules.

*Our aim with this sentence was not to mislead, instead it was simply to draw attention to the importance of MK-6240 in our study. The sentence in its entirety is the following: "***Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain, complexed with MK-6240, a second-generation, high avidity tau-PET ligand**."

• Additional minor comment: reference to figures 2C and 2D in the main text seem to be incorrect (there is no figure 2D).

We appreciate Reviewer 2 and 3 attention to detail; this was an oversight. We have corrected this in the manuscript.

Reviewer #3 (Remarks to the Author):

NCOMM MK binding cryoEM

I was asked to comment on the relevance of the manuscript from the imaging perspective.

The manuscript describes characterization of the structure of PHF from a patient with advanced AD neuropathological change bound in vitro to MK-6240 (I will refer to as 'MK') using cryoEM. The authors find a primary binding pocket similar to other tau PET ligands, identify key amino acid interactions, and the binding orientation.

The manuscript does not have much detail in setting up prior knowledge and future directions. It would be useful to know **prior information about known binding pockets on PHF tau in the introduction**, for example.

We thank the reviewer for their astute feedback. We have added such prior information to the manuscript, focusing on the cryo-EM structures resolved to the same binding pocket and relevant competition experiments, in lines 121-131 (below):

"Previous work using cryo-EM has resolved the interaction between PM-PBB3 (APN-1607) and PHFs/SFs, showing a parallel binding mode between the ligand and receptor, where a ligand-to-tau monomer ratio of 1:6 was observed (29). Surprisingly, the same group could not resolve an interaction between T-807 and PHFs or SFs from AD or PART (26). Recently, GTP-1, a second-generation tau ligand, has been resolved with PHFs using cryo-EM (31). The cryo-EM structures resolved for APN-1607 and GTP-1 show occupancy of a common binding site including amino acids Q351, K353, and I360. In vitro studies have shown that GTP-1 and T-807 (44) and MK-6240 and T807 (24, 27), compete for the same binding site. Therefore, the absence of a structure between T-807 and tau fibrils from AD-patient brain is perplexing. However, based on this data, we hypothesized that MK-6240 could be resolved within this common binding site."

The **ABC categorization could use some context** for those not familiar, that tau filaments were derived from a patient with the most advanced neuropathological staging.

We appreciate this comment and have revised the manuscript to include a statement to contextualize the brain material used for fibril extraction (Lines 137-139). Sentence is provided below:

"This combination of A, B, and C scores constitutes a high degree of AD neuropathologic change, which characterizes the most advanced neuropathological stage (Montine et al., 2012)."

The statements on impact of the finding are fairly generic. It would be better if the authors could discuss more **how the specific information in this work and recent similar works can be used for future drug development**. Does the binding interaction/alignment suggest opportunities for improvement for another generation of tracers?

We appreciate this comment and have revised the manuscript to include a statement to clarify our predictions based on our work and recent work for leveraging this information for the development of novel PET ligands (line 240-247). The sentence amended below:

"With this in mind, we suspect that the development of libraries of chemical scaffolds capable of adopting stacked binding modes facilitated by pi-pi interactions will serve as a useful starting point. Therefore, structure-activity relationship studies can focus on adjusting these scaffolds to contain functional groups capable of complexing with unique specific amino acids on the amyloid surface. In the case of MK-6240 and GTP-1, leveraging the formation of salt bridge interactions may also be a promising starting point. We have begun implementing these ideas in pursuing novel binders."

There are several places where the document could be shortened to allow for better context:
1. The **rodent experiments** are relevant, but the **methods could be moved to supplement**.

We appreciate this suggestion and have moved the rodent experimental methods section to the extended data methods section.

2. Paragraph starting, 'It has been well documented': The authors seem to be going beyond the data at hand.

We appreciate the comment and understand the skepticism. However, based on the observations derived from structural characterization of tau PHFs in different forms of neurodegenerative conditions, we believe that this inference/prediction is valuable and aims to introduce the specificity of PET-ligand binding outcomes in vivo from generalized terms such as "Tau burden" or "NFTs", to more structurally concrete terms such as PHF burden, which is justified given the body of data available.

"the MK compound was previously known to bind PHFs,"

Actually, it may have been suspected to bind PHF's and SF's based off of increased binding in crude AD brain homogenates and/or sarkosyl insoluble preparations. However, direct interaction with PHF's is an observation derived from this manuscript.

"and while that aspect was further investigated here, the ability to state that it is 'useful' depends on features well beyond binding."

We agree with this point from the reviewer regarding the utility of the MK-6240 binding data presented being extrapolated to binding in vivo. However, this remains a good estimate considering that most tracers used to study AD have been resolved using Cryo-EM. An argument against this would be understanding T-807's ability to bind in AD tissue, where a cryo-EM structure could not be resolved.

The last 2 sentences of this paragraph are not warranted here (and the rest of the paragraph doesn't really have a place either).

The last two sentences are the following: Our data suggests that MK-6240 is a useful tool for measuring PHFs. Therefore, we predict that the utility of MK-6240 in vivo is contingent on the prevalence of the PHF polymorph in the respective condition.

This paragraph is designed to setup a prediction based off of the Cryo-EM data of the MM-6240 binding pocket. We predict that diseases where the presence of PHF's have been shown, MK-6240 binding may be a useful tool for imaging, a point explicitly mentioned in the manuscript. We are also setting the stage for the specificity of the ligand, as future studies continue to pursue its ability to bind other amyloids, it would be useful for the field to narrow their terminology to reflect the outcome measure as PHF burden and not a blanket tau burden as is currently the case in the imaging field.

There is a paragraph on the differences in PHF structure between bound and unbound states (starting with 'To further understand Mk-6240-induced side chain…'). **The concluding statement is a bit of a letdown and also entirely obvious.**

This statement technically explains our results without embellishment and is not meant as a concluding statement. If the reviewer has suggestions on alternate placement or phrasing of this text, please inform.

I'm not familiar with **expectations for RSMD, so context of whether these observed deviations are small or large would be helpful**. It also seems that MK induces more deviation than GTP-1 (1.1-1.6 angstroms for MK and 0.6 angstroms for GTP relative to unbound); **is that correct and is it meaningful?**

We have rewritten this paragraph to read:

We observed an MK-6240-induced amino acid rearrangement at the level of the cryo-EM map. To show this, we generated a difference map by subtracting the unbound PHF map from the bound PHF+MK-6240 map. We overlaid the difference map (Salmon density, Extended Data Fig. 6B) onto the unbound PHF map (Grey density, Extended Data Fig. 6B). To validate the observation, we aligned the bound PHF+MK-6240 model to three unbound PHF models using the amino acids outside the region we observed the rearrangement (i.e., 306-339 and 356-374). We show the alignment between our PHF+MK-6240 map and our unbound model (Extended Data Fig. 6C). Next, we calculated the α-carbon root-mean-square deviations (RMSDs) of residues 340-355 between the MK-6240 bound model and our unbound PHF model with two additional published PHF models to account for differences in model building. We found that the RMSDs of residues 340-355 were 1.1 Å, 1.6 Å, and 1.2 Å for the unbound PHF models 5o3l (32), 6HRE (51), and our vehicle control model, respectively. To contextualize these findings, we compared the α-carbon RMSD between our control structure and unbound PHF models 5o3l and 6HRE which was 0.65 Å each, indicating highly similar unbound structures (Extended Data Fig. 9A). Lastly, Merz et al., (31) observed a subtle side-chain rearrangement with the binding of GTP-1 when compared to the unbound model 5o3l. We used the GTP-1 bound model and compared it to our unbound model and found that the α-carbon RMSD of residues 340-355 RMSD values of 0.56 Å. The comparison of α-carbon RMSD from the GTP-1 model and PHF+MK-6240 model was 1.65 Å (Extended Data Fig. 9B). This contrasts the effects of a nanomolar binder (GTP-1) and a sub-nanomolar binder (MK-6240) in its ability to induce backbone changes at the binding interface, with MK-6240 eliciting more significant conformational alterations as evidenced by the higher RMSD values.

We appreciate this comment as it has resulted in a much clearer message being communicated to the reader.

There's a limitation in using the unpublished SymDOCK model.

This has now been published, and the reference has been updated on line 210.

Reference 48 is cited as Liu et al in the text but it is Tao et al. in the references. It also would probably be better to reference the primary works for each ligand. it is interesting to compare the binding modes of the various ligands, however it is not clear what lesson we learn from doing so. Does this account for some known pharmacokinetic differences between ligands?

Reference to article 48 has been corrected to reflect the accurate name of the first author.

It remains unclear whether this accounts for pharmacokinetic differences, this was not examined on our work. We aimed to address the utility of binding arrangements in the previous comment from this reviewer, i.e. our suggestion would be to explore scaffolds that are known to form stacked binding

orientations via pi-pi interactions.

Is there cryoEM for flortaucipir?

There is and no binding was observed. This was also clarified in a previous comment to this reviewer, hopefully providing additional context to the work being done.

A figure 2D panel is referenced in the manuscript but it is not in the figure.

We appreciate the attention to detail, this has been corrected in the manuscript.

Based on text it is not clear whether the diagrams in B represents the cryoEM result or some other prediction.

We have added the following text to the description for clarity (lines 438-439): (illustrated within the protein chain modelled to the cryo-EM structure, key amino acids are highlighted).

Also Panels B and C should have amino acids labeled, particularly since K353 is specifically discussed in the legend.

We have added labels and arrows to figure 2 to address this comment.

'SF' isn't used enough to warrant an acronym.

We have adjusted this acronym on line 256, during the discussion, where it is separated from the introduction of SF. We appreciate this comment and hope this change results in more clarity for the readers.

The sentence starting 'To this end…' needs editing for grammar.

We have edited that paragraph for improved grammar and clarity in line 225-232:

To this end, MK-6240 has few interactions with its receptor compared to what is typical for such high-affinity ligands. The surface area involved in ligand-ligand interface (243 $Å^2$) is greater than that observed in the ligand-protein interface (208 $Å^2$), resulting in 46% of the solvent-accessible surface area (SASA) of an MK-6240 monomer being buried by the protein, or 69% of the SASA of an MK-6240 monomer bound to protein within a stack of MK-6240 molecules. These values are similar to what has been previously calculated for GTP-1 bound to tau fibrils (30).

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

In their revised manuscript Kunach et al. primarily focus on changes to their manuscript based on reviewer 3's comments and have made appropriate adjustments. For this 2-figure communication this seems generally sufficient. However, it is worth noting that reviewer 1's concerns regarding the minimal overall significance were not fully addressed. The response that "our study focuses on the PET ligand MK-6240, never before structurally described by cryo-EM" does not demonstrate sufficient novelty given that this work does not address its clinical use, and that there are many other promising 2nd generation PET ligands currently under investigation. Why is it important to structurally characterize MK-6240 relative to other ligands? Additionally, for reviewer 2 it was suggested that to increase significance of the study the authors should pursue a structure of the AD straight filaments and determine whether MK-6240 binds to this conformer. However, the authors indicate that SFs were unable to be classified in their data, which is somewhat surprising given other studies, and that this would be better addressed in a separate publication by looking at AD filaments from another purification or filaments from PART brain material (although that alone would be of minimal impact). Nonetheless, addressing this question in light of Reviewer 1's concern seems important.

Finally, given the previous studies of filaments bound to PET ligands there was concern about the following sentence being misleading: "Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain, complexed with MK-6240, a second-generation, high avidity tau-PET ligand". While the authors respond that their intent was not to mislead, they opted to not change the sentence. The confusion here is based on the comma use. The sentence appears misleading because the reference to MK-6240 is set off by two commas and thus not essential to the meaning of the sentence. Thereby, this gives the impression that this is the first study looking at molecular binding interfaces of PHFs, which is not true. Simply change the sentence to: "Our study represents the first examination of the molecular binding interface between AD PHFs and MK-6240, a second-generation, high avidity tau-PET ligand". Or something similar. Overall, the experimental work here is of high quality, in particular the structure determination and docking of MK-6240.

Reviewer #3 (Remarks to the Author):

I appreciate the authors' changes to the manuscript. I have a few minor additional comments to hopefully improve clarity for readers:

The paragraph, 'It has been well documented…' (Ln 163). Consider:
"It has been well documented that the PHF is the predominant tau polymorph in Alzheimer's disease (4,5), however, it has also been observed in various other neurodegenerative conditions, such as Familial British Dementia, Familial Danish Dementia, and PrP Cerebral Amyloid Angiopathy (6). Our data suggests that MK-6240 is a useful tool for measuring PHFs. Therefore, MK-6240 may have in vivo utility in these respective conditions, contingent on the presence of sufficient PHF polymorph."

Based on comment from another reviewer, the authors rewrote a sentence:
"Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain, complexed with MK-6240, a second-generation, high avidity tau-PET ligand. "
This must be corrected to remove a comma:
"Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain complexed with MK-6240, a second-generation, high avidity tau-PET ligand.

Line 178: this paragraph is the closest to a 'limitations' paragraph. Following another reviewer's

comments, the authors could add a sentence about how straight filaments were not evaluated, so contribution to binding can not be commented. Also they may wish to introduce the relative prevalence of PHF vs SF when they are introduced in the 1st paragraph.

Please refer to ’T807’ as ‘flortaucipir’ throughout; authors could introduce it as ‘flortaucipir (previously called T-807 and AV-1451)’. This will allow readers to better connect this to the abundant literature.

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

In their revised manuscript Kunach et al. primarily focus on changes to their manuscript based on reviewer 3's comments and have made appropriate adjustments. For this 2-figure communication this seems generally sufficient. However, it is worth noting that reviewer 1's concerns regarding the minimal overall significance were not fully addressed. The response that "our study focuses on the PET ligand MK-6240, never before structurally described by cryo-EM" does not demonstrate sufficient novelty given that this work does not address its clinical use, and that there are many other promising 2nd generation PET ligands currently under investigation. Why is it important to structurally characterize MK-6240 relative to other ligands?

We appreciate the reviewer's commitment to this point. We have clarified the reason why studying MK-6240 binding was more important than other promising second generation PET ligands. Amendment to line 133:

Our study focuses on MK-6240, which is the only second-generation tau-PET ligand with subnanomolar EC_{50} for tau pathology in AD (24, 25, 27, 28) and increasingly used in clinical studies (23) and whether its structure in complex with AD-derived tau fibrils using cryo-EM could reveal unique binding features related to its characteristics.

Additionally, for reviewer 2 it was suggested that to increase significance of the study the authors should pursue a structure of the AD straight filaments and determine whether MK-6240 binds to this conformer. However, the authors indicate that SFs were unable to be classified in their data, which is somewhat surprising given other studies, and that this would be better addressed in a separate publication by looking at AD filaments from another purification or filaments from PART brain material (although that alone would be of minimal impact). Nonetheless, addressing this question in light of Reviewer 1's concern seems important.

We appreciate Reviewer 2's suggestion to also determine the structure of AD straight filaments (SFs), which aligns with expanding interest in understanding the diverse morphological variants of tau filaments in neurodegenerative diseases.

In our current study, we focus primarily on the interaction between MK-6240 and paired helical filaments (PHFs) for their pronounced presence and pathological significance in AD brains. The inclusion of SFs in our analysis, while scientifically meritorious, presents several technical challenges. First our sample set didn't present a sufficient quantity of SFs that could be classified with confidence, likely due to their lower abundance or stability under the conditions used in our experiments. This aligns with variability observed in the literature, where the prevalence and detectability of SFs can be highly dependent on the source and treatment of the brain material.

Moreover, the structural elucidation of SFs, while valuable, might not significantly alter the therapeutic implications derived from our study's findings on PHFs.

Therefore, while the study of SFs is undoubtedly important, we would look into this as a separate study using PART patient brain material, for example. we believe that our current focus on PHFs and their interaction with MK-6240 is justified given scope and resources of our current project. We plan to investigate the broader landscape of tau filament structures in future work as new methods and samples come available.

Finally, given the previous studies of filaments bound to PET ligands there was concern about the following sentence being misleading: “Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain, complexed with MK-6240, a second-generation, high avidity tau-PET ligand”. While the authors respond that their intent was not to mislead, they opted to not change the sentence. The confusion here is based on the comma use. The sentence appears misleading because the reference to MK-6240 is set off by two commas and thus not essential to the meaning of the sentence. Thereby, this gives the impression that this is the first study looking at molecular binding interfaces of PHFs, which is not true. Simply change the sentence to: “Our study represents the first examination of the molecular binding interface between AD PHFs and MK-6240, a second-generation, high avidity tau-PET ligand”. Or something similar. Overall, the experimental work here is of high quality, in particular the structure determination and docking of MK-6240.

We have made the appropriate syntactical changes to accurately reflect the meaning of the sentence based on the reviewer’s guidance.

Reviewer #3 (Remarks to the Author):

I appreciate the authors' changes to the manuscript. I have a few minor additional comments to hopefully improve clarity for readers:

The paragraph, ‘It has been well documented…’ (Ln 163). Consider:
“It has been well documented that the PHF is the predominant tau polymorph in Alzheimer's disease (4,5), however, it has also been observed in various other neurodegenerative conditions, such as Familial British Dementia, Familial Danish Dementia, and PrP Cerebral Amyloid Angiopathy (6). Our data suggests that MK-6240 is a useful tool for measuring PHFs. Therefore, MK-6240 may have in vivo utility in these respective conditions, contingent on the presence of sufficient PHF polymorph.”

Line 217/218 has been amended using the reviewer’s wording.

Based on comment from another reviewer, the authors rewrote a sentence:

“Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain, complexed with MK-6240, a second-generation, high avidity tau-PET ligand. “
This must be corrected to remove a comma:
“Our study represents the first examination of the molecular binding interface of PHFs derived from AD brain complexed with MK-6240, a second-generation, high avidity tau-PET ligand.

This comment has been addressed appropriately.

Line 178: this paragraph is the closest to a ‘limitations’ paragraph. Following another reviewer’s comments, the authors could add a sentence about how straight filaments were not evaluated, so contribution to binding can not be commented. Also they may wish to introduce the relative prevalence of PHF vs SF when they are introduced in the 1st paragraph.

Introduction of the abundance in the first paragraph has been amended to read:

Alzheimer's Disease (AD) is characterized by the progressive accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) which central to AD pathogenesis. Neurofibrillary tangles are composed of tau protein fibril polymorphs, notably the paired helical filaments (PHFs) and straight filaments (SFs). Their relative abundance has been previously described as approximately 90% PHF and 10% SF in cortical extractions from AD brain (1-6).

Relating to how SFs were not evaluated, we have amended to read:

Additionally, the structure of MK-6240 complexed with SFs is important to determine because NFTs in AD are composed of both. Additionally, the utility of MK-6240 in PART would hinge on its ability to bind SF, where the SF polymorph is more abundant than the PHF.

Please refer to ’T807’ as ‘flortaucipir’ throughout; authors could introduce it as ‘flortaucipir (previously called T-807 and AV-1451)’. This will allow readers to better connect this to the abundant literature.

All references to T-807 have been changed to Flortaucipir.