Peer Review File

Manuscript Title: Structures and Mechanisms of tRNA Methylation by METTL1-WDR4

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors describe their structure-function studies of the tRNA methyltransferase that catalyses the m7G46 methylation. This is required for stability of the tRNA and mutations in the METTL1-WDR4 complex that is involved in the methylation reaction has important phenotypes in yeast, flies, mice and man. The complex structure was previously described, it is also known that the complex is important for activity. This work sheds important molecular insights into the mechanism of action of the METTL1-WDR4 complex that methylates a specific position on some tRNAs.
Main findings: A pre-formed RNA-binding surface is formed between METTL1-WDR4 to bind the tRNA. Like with many other RNA methyltransferases, an “activity loop” becomes ordered in the complex containing the RNA substrate. The flexible N-term of METTL1 is found to be part of the catalytic center, as it is visible in the protein complex with tRNA and SAM. Phosphorylation of this N-term is previously shown to inactivate the enzyme.
Overall, this is a beautiful structure-function analysis of a tRNA methylase complex. The claims are supported and should be of interest to a broad audience.

Minor:
1. Will modifications of other positions on the tRNA (at least that is facing the complex) influence the methylation by METTL1-WDR4?

Referee #2 (Remarks to the Author):

In this manuscript, the authors present structures of the METTL1-WDR4 complex, which carries out methylation at N7 of G46 in tRNAs. The authors determined cryo-EM structures of a human METTL1-WDR4-tRNA complex as well as a METTL1-WDR4-tRNA-SAM complex. In addition, they present crystal structures of a binary METTL1-WDR4 complex and METTL1 with SAM or SAH bound. From analysis and comparison of these structures, the authors propose a mechanism for tRNA recognition and methylation by the METTL1-WDR4 complex. As I am not an expert on METTL1-WDR4 in particular or tRNA modification in general, my review of this manuscript will largely focus on the structural analysis.

The cryo-EM analysis of high quality and follows state-of-the-art practices. The resulting maps are very good, and the model derived by the authors is in good agreement with the experimental data. I would like to complement the authors for their very well-executed presentation of the structural

and functional data, in particular for the use of clear color coding which makes the figures very easy to understand at first glance. I have only a couple of suggestions and comments:

Major points
- The most speculative part of the structural analysis is in my opinion the placement of the C-terminal helix of WDR4 near the tRNA substrate. While this seems generally supported by the unsharpened maps, I wonder whether the authors have attempted focussed classification and refinement with a map around the aminoacyl-arm of the tRNA, which could improve the density?
- I strongly encourage the authors to upload all maps that are necessary to recapitulate the modeling done in this paper to the EMDB, if not already the case (unsharpenend half-maps, post-processed maps, focussed refinement maps and masks used).
- Extended Data Figures 2 and 6: Please show viewing angle distribution plots and directional FSC plots (see Aiyer et al., Methods Mol Biol 2021).

Minor points
- Page 4: The resolutions achieved are not “atomic”. Please rephrase (for example to “near-atomic”).
- Extended Data Figure 1b: Please also indicate the RNA concentration, either here or in methods.
- Extended Data Figure 1c,d: What parts of the structures were aligned? (METTL1 only?) Please indicate.
- Figure 1g: Which map is shown, sharpened or unsharpenend? Please indicate.
- Figure 2b: Please indicate how “residues in protein contact” is defined (e.g. within xx Å distance?)
- Figure 3d: Please also indicate RNA concentration.
- Extended Data 4: Please indicate and cite what algorithm was used for alignment and visualization, if any.
- Figure 4: Please state what the dashed lines are supposed to indicate.
- Page 7: The text says the structures align well with other structures in the PDB, but these are neither mentioned nor shown in Extended Data Figure 5.
- Page 13: Please give more details or reference for purification of RNAs.
- Please indicate the number of replicates from which mean and SD are derived for all in vitro methylation assay results.

Referee #3 (Remarks to the Author):

Structures and mechanisms of tRNA methylation by METTL1-WDR4
In this paper, the authors report the crystal structures of METTL1-WDR4, METTL1-SAM, METTL1-SAH and the cryoEM structure of METTL1-WDR4-tRNALys and METTL1D163A-WDR4-tRNALys-SAM. These structures reveal how METTL1 and WDR4 interact globally with tRNAs, show that SAM cofactor binding promotes a conformational change in the METTL1 catalytic loop to accomplish the m7G46 modification, and provide information on how AKT phosphorylation of a serine in the N-terminus of METTL1 renders METTL1 inactive. I think the analysis of the structures could be improved and a structure of METTL1 wild-type-WDR4-tRNA-SAH is lacking. It would have shown the conformation of the catalytic loop with the cofactor and tRNA bound without perturbing it with a mutation and perhapps they would have been able to observe the flipping of G47 in the active site. I would like to

recommend the publication of this paper after they have resolved this structure and improved the analysis of the structures.

Specific comments:

1) The authors state that METTL1-WDR4 recognizes tRNA by shape complementation and there is not much information about specific interactions, interactions with conserved residues... I think the interaction should be discussed a bit more. A sequence alignment of the two proteins in extended data could also help the reader.
They also say that there is heterogeneity in the structure of the tRNA, near the variable loop, so the shape complementarity they observe may be due to the data not allowing them to see conformational changes?
Are they not seeing specific interactions because the resolution is too poor and/or the side chains are not defined?
It might be interesting to add the density on some figures to show the side chains of the residues to give an idea of the quality of the density maps.

2) About the choice of tRNALys3: "because its structural stability ... is established", the authors refer to a crystal structure of tRNALys3 that is fully modified and not transcribed in vitro. Therefore, I am not sure that the argument is correct. It is also not clear to me what "known to be sensitive to METTL1-WDR4 levels" means?

3) The authors should explain a bit more how they assess the affinity of WR4 for tRNA at 40 nM. They only show one gel and no quantification, no curve fitting...

4) It is difficult to see the WRD4 rotating to contact tRNA and wrapping around the D loop in Fig 1h. Could they add a zoom, show the side chains... at least improve this figure?

5) A figure with the contacts between METTL1 and SAM or SAH residues should be added.

6) "The catalytic centre of the enzyme is probably formed after RNA binding", but the SAM cofactor is not present and they show that the catalytic loop changes conformation upon SAM binding. This sentence is therefore misleading.

7) The authors use a catalytically inactive METTL1D163A mutant to solve the structure of the complex, but the mutation is in the catalytic loop and probably changes the structure of that loop. I think this explains why they do not observe the flipping of G46 in the catalytic centre of METTL1. This should be taken into account at the end of the paragraph "Insight into the catalytic mechanism...".
A solution would have been to use METTL1 WT and add SAH instead of SAM.

Author Rebuttals to Initial Comments:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors describe their structure-function studies of the tRNA methyltransferase that catalyses the m7G46 methylation. This is required for stability of the tRNA and mutations in the METTL1-WDR4 complex that is involved in the methylation reaction has important phenotypes in yeast, flies, mice and man. The complex structure was previously described, it is also known that the complex is important for activity. This work sheds important molecular insights into the mechanism of action of the METTL1-WDR4 complex that methylates a specific position on some tRNAs.
Main findings: A pre-formed RNA-binding surface is formed between METTL1-WDR4 to bind the tRNA. Like with many other RNA methyltransferases, an “activity loop” becomes ordered in the complex containing the RNA substrate. The flexible N-term of METTL1 is found to be part of the catalytic center, as it is visible in the protein complex with tRNA and SAM. Phosphorylation of this N-term is previously shown to inactivate the enzyme.
Overall, this is a beautiful structure-function analysis of a tRNA methylase complex. The claims are supported and should be of interest to a broad audience.
We thank the reviewer very much for the positive feedback!

Minor:
1. Will modifications of other positions on the tRNA (at least that is facing the complex) influence the methylation by METTL1-WDR4?

We have tested mutations in the variable loop sequence as shown in Figure 4. Among all the nucleobases in our structures, there are 5 that come in close enough contact with the proteins (defined as within 4 Å) outside the variable loop (Extended Data Fig 6c). To preserve the tRNA structure and function, we tested methylation of known tRNA sequences (Fig 4i) rather than introducing mutations that may alter the tRNA structure and generate misleading results. We have added these remarks in the text.
Furthermore, with the reviewer’s encouragement, we also added more mutagenesis data to show that the variable loop sequence is not sufficient to make a tRNA a good target, by introducing mutations into the variable loop of the Gln tRNA (Extended Data Fig. 6d).

Referee #2 (Remarks to the Author):

In this manuscript, the authors present structures of the METTL1-WDR4 complex, which carries out methylation at N7 of G46 in tRNAs. The authors determined cryo-EM structures of a human METTL1-WDR4-tRNA complex as well as a METTL1-WDR4-tRNA-SAM complex. In addition, they present crystal structures of a binary METTL1-WDR4 complex and METTL1 with SAM or SAH bound. From analysis and comparison of these structures, the authors propose a mechanism for tRNA recognition and methylation by the METTL1-WDR4 complex. As I am not an expert on METTL1-WDR4 in particular or tRNA modification in general, my review of this manuscript will largely focus on the structural analysis.

The cryo-EM analysis of high quality and follows state-of-the-art practices. The resulting maps are very good, and the model derived by the authors is in good agreement with the experimental data. I would like to complement the authors for their very well-executed presentation of the structural and functional data, in particular for the use of clear color coding which makes the figures very easy to understand at first glance. I have only a couple of suggestions and comments:

We did our best to perform high-quality structural analysis. We are sincerely grateful for the positive feedback.

Major points

- The most speculative part of the structural analysis is in my opinion the placement of the C-terminal helix of WDR4 near the tRNA substrate. While this seems generally supported by the unsharpened maps, I wonder whether the authors have attempted focussed classification and refinement with a map around the aminoacyl-arm of the tRNA, which could improve the density?

We have tried focused 3D classification with several masks near the region, but we have not been able to obtain better maps for the C-terminal helix of WDR4 with the cryo-EM data for the apo or SAM-bound complexes. However, our newly added cryo-EM data for the SAH-bound state provides higher-resolution maps that have similarly strong signal for the C-terminal helix as the rest of the complex. In fact, no focused refinement was necessary to visualize the entire tRNA or the WDR4 Cterminal helix for the SAH-bound complex. Using the better data for the SAH-bound complex, we were able to provide a better model for how the C-terminal helix binds the RNA and even interact with the N-terminal extension of METTL1 (Fig. 5). We agree with the reviewer that the map is weak for the helix in the apo complex. Since the helix can still

be included with better data from the SAH-bound complex, we have now removed it from the apo structure to keep the data interpretation more parsimonious.

- I strongly encourage the authors to upload all maps that are necessary to recapitulate the modeling done in this paper to the EMDB, if not already the case (unsharpenend half-maps, post-processed maps, focussed refinement maps and masks used).
All relevant maps including sharpened maps and masks are deposited in the EMDB.

- Extended Data Figures 2 and 6: Please show viewing angle distribution plots and directional FSC plots (see Aiyer et al., Methods Mol Biol 2021).
We have added the viewing angle distribution plots and directional FSC plots (Extended Data Fig. 2-4), and we have also added the reference. We thank the reviewer for the suggestion to present the data quality more thoroughly.

Minor points
- Page 4: The resolutions achieved are not “atomic”. Please rephrase (for example to “near-atomic”).
We have changed it to “near-atomic”.

- Extended Data Figure 1b: Please also indicate the RNA concentration, either here or in methods.
We have included the tRNA concentration information in the Methods section.

- Extended Data Figure 1c,d: What parts of the structures were aligned? (METTL1 only?) Please indicate.
The structural comparison was performed by aligning METTL1 to Trm8. We have revised the legend to clarify how the structure comparisons were made.

- Figure 1g: Which map is shown, sharpened or unsharpenend? Please indicate.
The sharpened cryo-EM map is shown. We have included the information in the figure legends.

- Figure 2b: Please indicate how “residues in protein contact” is defined (e.g. within xx Å distance?)
Given the resolution, we have defined contact as residues or nucleotides within 4 Å distance. We have included the definition wherever relevant in figure legends.

- Figure 3d: Please also indicate RNA concentration.
We have included the RNA concentration information in the Methods section.

- Extended Data 4: Please indicate and cite what algorithm was used for alignment and visualization, if any.
We have included the algorithm (clustal omega) and the visualization software (Geneious) in the figure legends.

- Figure 4: Please state what the dashed lines are supposed to indicate.
We have included in the figure legend that the dashed lines represent hydrogen bonds.

- Page 7: The text says the structures align well with other structures in the PDB, but these are neither mentioned nor shown in Extended Data Figure 5.
We have added a figure to show the superimposed structures of METTL1 (Extended Data Fig. 1f).

- Page 13: Please give more details or reference for purification of RNAs.
We added more details and a reference for RNA purification.

- Please indicate the number of replicates from which mean and SD are derived for all in vitro methylation assay results.
Wherever omitted by mistake, we have added the replicate information in the figure legends.

*** We sincerely thank the reviewer for their help with making our manuscript more complete, through the above suggestions.

Referee #3 (Remarks to the Author):

Structures and mechanisms of tRNA methylation by METTL1-WDR4
In this paper, the authors report the crystal structures of METTL1-WDR4, METTL1-SAM, METTL1-SAH and the cryoEM structure of METTL1-WDR4-tRNALys and METTL1D163A-WDR4-tRNALys-SAM. These structures reveal how METTL1 and WDR4 interact globally with tRNAs, show that SAM cofactor binding promotes a conformational change in the METTL1 catalytic loop to accomplish the m7G46 modification, and provide information on how AKT phosphorylation of a serine in the

N-terminus of METTL1 renders METTL1 inactive. I think the analysis of the structures could be improved and a structure of METTL1 wild-type-WDR4-tRNA-SAH is lacking. It would have shown the conformation of the catalytic loop with the cofactor and tRNA bound without perturbing it with a mutation and perhapps they would have been able to observe the flipping of G47 in the active site. I would like to recommend the publication of this paper after they have resolved this structure and improved the analysis of the structures.

We thank the reviewer for the suggestion to add the METTL1^{WT}-WDR4-tRNA-SAH structure and expand the analysis. We have added the new cryo-EM structure to the manuscript, which reveals the precatalytic state with G46 flipped into the active site. Furthermore, with higher resolution data we have expanded the structure description. We are happy to see the improvements in our manuscript with the reviewer's recommendations, and we hope that the manuscript is now acceptable for publication.

Specific comments:

1) The authors state that METTL1-WDR4 recognizes tRNA by shape complementation and there is not much information about specific interactions, interactions with conserved residues... I think the interaction should be discussed a bit more. A sequence alignment of the two proteins in extended data could also help the reader.
We have reorganized the manuscript to present all three structures together, and we devoted the entire Fig. 3 to discuss the protein-RNA shape recognition. With the reviewer's suggestion, we have also included a view of the structure colored by sequence conservation (Extended Data Fig. 5) and added sequence aligments of METTL1 and WDR4 (Extended Data Fig. 7-8).

They also say that there is heterogeneity in the structure of the tRNA, near the variable loop, so the shape complementarity they observe may be due to the data not allowing them to see conformational changes? Are they not seeing specific interactions because the resolution is too poor and/or the side chains are not defined? It might be interesting to add the density on some figures to show the side chains of the residues to give an idea of the quality of the density maps.
Most of shape recognition is through the structured tRNA Elbow region, rather than the variable loop. In this protein-RNA contact that is conserved in all three structures, we do not observe structural heterogeneity and the map is well defined to the indicated resolution (Extended Fig. 2c, 3c, 4c). Thankfully, with the reviewer's suggestion, we

were able to determine an even higher resolution structure of the SAH-bound state with more extensive protein-RNA interactions. Nevertheless, due to the structure of the tRNA and how it interacts with the protein, most bases are not exposed enough to interact with protein in a base-specific manner. Thus, overall, tRNA recognition seems to mostly driven by shape recognition. We agree with the reviewer that there is always a chance for us to miss some base-specific contacts due to limited resolution. We have thus expanded the discussion on the sequence variance among tRNAs and how it does not seem to affect the methylation activities dramatically, except for the variable loop sequence. Furthermore, we agree with the reviewer that the quality of the density maps is important. We have added zoomed-in density figures in Extended Data Fig. 6, in addition to the global maps provided in Fig 2. Finally, we have also uploaded all the maps and pdb files for all of the structures we present in the manuscript to help the reviewers evaluate our models.

2) About the choice of tRNALys3: "because its structural stability ... is established", the authors refer to a crystal structure of tRNALys3 that is fully modified and not transcribed in vitro. Therefore, I am not sure that the argument is correct. It is also not clear to me what "known to be sensitive to METTL1-WDR4 levels" means?

We chose tRNA-Lys-TTT for the wealth of structural information available. The structure of a fully modified, isolated tRNA and another protein-bound unmodified tRNA structure superimpose well (RMSD ~ 0.9 Å), even with some engineering mutations to aid with crystallization (Extended Data Fig. 5). These observations suggest that the tRNA structure is stable with and without modifications, and the available structures can serve as a good starting model to build and refine our complex structures. In METTL1 KO cells, m^7G level changes were seen for many tRNAs, including tRNA-Lys-TTT. Furthermore, we observe robust methylation activity of METTL1/WDR4 with tRNA-Lys-TTT and binding affinity, making it a good candidate for structural studies. We have revised the text to clarify the choice of the tRNA, including more references.

3) The authors should explain a bit more how they assess the affinity of WR4 for tRNA at 40 nM. They only show one gel and no quantification, no curve fitting...

We estimated the apparent affinity by titrating the protein, and we show the curve-fitting analysis for the wild-type complex in Extended Data Fig. 1, as the reviewer requested. The binding curve for the wild-type complex is from 3 independent replicated experiments. Given some smears in gels (unavoidable with some of the tRNAs despite a lot of effort to improve the experiment conditions) and other factors

that might affect the apparent affinity, our intent was not to dwell on the absolute affinity but rather compare the relative affinities. To emphasize the purpose of the EMSA experiments, we have modified the text to state low nanomolar range given the noise in the gels.

4) It is difficult to see the WRD4 rotating to contact tRNA and wrapping around the D loop in Fig 1h. Could they add a zoom, show the side chains... at least improve this figure?
We thank the reviewer for this suggestion. We have included a superimposition figure to depict how the C-terminal tail of WDR4 is drastically different in the crystal structure vs in the cryo-EM structure (Extended Fig. 5d). We have also expanded the figure showing the WDR4-tRNA interactions using more side chains as the reviewer requested (Fig. 3d).

5) A figure with the contacts between METTL1 and SAM or SAH residues should be added.
The figure with the contacts between METTL1 and SAH is shown in Fig. 1g, using the crystal structure at 1.9 Å resolution. The figure to show the SAH-binding site in the cryo-EM structure is also shown in Fig. 4f.

6) "The catalytic centre of the enzyme is probably formed after RNA binding", but the SAM cofactor is not present and they show that the catalytic loop changes conformation upon SAM binding. This sentence is therefore misleading.
What we intended to say is that SAM binding and RNA binding are both required to form an active conformation of the protein, because RNA binding is required to order the catalytic loop (disordered in all the crystal structures). We have removed this sentence, and we present a more complete mechanistic model describing all the conformational changes taking place using all three states, including the SAH-bound structure newly added to this manuscript.

7) The authors use a catalytically inactive METTL1D163A mutant to solve the structure of the complex, but the mutation is in the catalytic loop and probably changes the structure of that loop. I think this explains why they do not observe the flipping of G46 in the catalytic centre of METTL1. This should be taken into account at the end of the paragraph "Insight into the catalytic mechanism...".
A solution would have been to use METTL1 WT and add SAH instead of SAM.

We thank the reviewer for the suggestion. We have determined a structure of the METTL1-WDR4-SAH complex by cryo-EM. This combination produces a more stable conformation that captures the active state of the enzyme with the modified Gua46 in the catalytic pocket, along with the accompanying conformational changes in the tRNA and WDR4. With the new data, we have reorganized the entire manuscript to present the available structures in a more logical fashion. We thank the reviewer for this suggestion as the new structure has improved our manuscript.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have revised the manuscript to my satisfaction.

Referee #2 (Remarks to the Author):

The authors have substantially revised this manuscript, and addressed all my comments adequately. Moreover, they have added a large amount of new data in the form of a METTL1-WDR3-tRNA-SAH structure, which reveals previously unresolved details such as the flipping of G46 and thus adds tremendously to this story. I congratulate the authors on a comprehensive structure-function study, which I think is now ready for publication.

I have two more questions which came to my mind during reading, the answers to which the authors may or may not want to add to the manuscript:

1.) Does the comparison of SAM- and SAH-bound structures provide insight as to why SAH leads to base-flipping of G46 while SAM does not? Do the authors think this is due to the catalytically dead mutant?

2.) In the SAH-bound structure, the acceptor nitrogen is around 5 Å from the sulfur atom of SAH. What distance would there be between the methyl group of a SAM modeled in exactly the same position and the acceptor nitrogen in the base? Is this within the range observed in other precatalytic methyltransferase models (e.g. PMID 34489609)?

Minor points:
- Figure 1f: Please indicate number of replicates.
- Figure 1g: Please add to the figure legend that water molecules are shown as red spheres.

Hauke Hillen

Referee #3 (Remarks to the Author):

I am very happy that the suggestion of using SAH and the wild type METTL1 adds so much. I think the article is now acceptable for publication in Nature.
I have just two minor points:
-There is sometimes G46 and sometimes Gua46, I think the one letter code is sufficient and the one letter code is used for residues in the proteins

-There are pi-pi interactions between some Arginines and aromatic residues, for me it is cation-pi interactions. pi-pi interactions are for two aromatic groups

Author Rebuttals to First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):
The authors have revised the manuscript to my satisfaction.
We thank the reviewer for the helpful comments during the revision process.

Referee #2 (Remarks to the Author):
The authors have substantially revised this manuscript, and addressed all my comments adequately. Moreover, they have added a large amount of new data in the form of a METTL1-WDR3-tRNA-SAH structure, which reveals previously unresolved details such as the flipping of G46 and thus adds tremendously to this story. I congratulate the authors on a comprehensive structure-function study, which I think is now ready for publication.
I have two more questions which came to my mind during reading, the answers to which the authors may or may not want to add to the manuscript:
The reviewer's feedback was helpful to ensure that we are thorough in our structure analysis and description. We are grateful for the thoughtful input.

1.) Does the comparison of SAM- and SAH-bound structures provide insight as to why SAH leads to base-flipping of G46 while SAM does not? Do the authors think this is due to the catalytically dead mutant?
We do think that the difference arises from the D163A mutation. The D163A mutation of METTL1 seems to be important for both: prevent catalysis and support the observed ordered conformation of the N-term (Page 10).

2.) In the SAH-bound structure, the acceptor nitrogen is around 5 Å from the sulfur atom of SAH. What distance would there be between the methyl group of a SAM modeled in exactly the same position and the acceptor nitrogen in the base? Is this within the range observed in other precatalytic methyltransferase models (e.g. PMID 34489609)?
When we model SAM using the SAM-bound METTL1 structure, the distance between the methyl group of SAM and the acceptor nitrogen in G46 is 2.9 Å. Given the resolution limit and that we do not have the SAM-bound precatalytic conformation, we think this comes close to the ~2.5 Å reported in PMID 34489609.

Minor points:
- Figure 1f: Please indicate number of replicates.
- Figure 1g: Please add to the figure legend that water molecules are shown as red spheres.
We have corrected the figure legends.

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I am very happy that the suggestion of using SAH and the wild type METTL1 adds so much. I think the article is now acceptable for publication in Nature

We thank the reviewer for the helpful suggestions to improve our manuscript.

I have just two minor points:

-There is sometimes G46 and sometimes Gua46, I think the one letter code is sufficient and the one letter code is used for residues in the proteins

As the reviewer suggested we changed all codes to the one-letter format.

-There are pi-pi interactions between some Arginines and aromatic residues, for me it is cation-pi interactions. pi-pi interactions are for two aromatic groups

We observe parallel stacking of the guanidium pi system in between the aromatic rings, which suggests pi-pi interactions. We agree cation-pi interactions also contribute, and we added it to the text.