OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jentashapir Journal of Cellular and Molecular Biology
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Peer Review Report for "In Silico Study of Pacific oyster Antiviral Polypeptides as Potential Inhibitory Compounds for SARS-CoV-2 Main Protease"

Author(s): Mehrdad Mohammadi, Sajjad Rajabi, Ahmad Piroozmand, Seyed Ali Mirhosseini

Review Timeline:

Submit Date:	1 Sep 2020
Revised Date:	2 Nov 2020
Accepted Date:	3 Nov 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0)

10 Sep 2020

Dear AE,
Thanks for this valuable manuscript, but the writing needs to be revised.
1- There are some grammatical problems. Some of your sentences unclear or hard to follow, and Some of your sentences, possibly wordy sentences, and too many non-content words may indicate wordiness. Please consider rephrasing.
2- The default of Molegro software is five choices for cavities. Why did you choose four cavities? Please state the basis of your choice.
3- How was processed PDB file (PDB ID: 6LU7) to obtain the pure Mpro without additional ligands and sequences? (main protease (Mpro) protein of SARS-CoV2 in complex with an inhibitor N3 in PDB ID: 6LU7). In other words, do you perform any manipulate in the PDB file or directory add PDB file into Molegro tools?
4- Why the author only selected the pose to belong to the wider and biggest cavity? It seems to be a better examination of all the best poses for the five cavities.
5- Comparing the obtained numbers from HBond, Torsions, Total interaction energy, Rerank Score, MolDock Score, and Similarity Score were only written in the discussion section and the results. But in the discussion, except for HBond, no explanation has been given as to the reason for these comparisons and how they are superior. Please write the comparative

role and value of each of the above factors in the discussion section.
Best regards

Fakher Rahim: Reviewer | **Revision (0)** 14 Oct 2020

Dear AE,
There are some comments which should be addressed by the author:
- Short/Running title: “Pacific oyster compounds docking in SARS-CoV-2”, is an abbreviated form of the main title. This title is usually displayed at the top of each page of the article or left-hand text pages. It serves to guide readers while scanning through a journal or toggling between multiple papers. I suggest “Pacific oyster docking against SARS-CoV-2”
Abstract: Method:
1. The methods section is usually the second-longest section in the abstract. It should contain enough information to enable the reader to understand what was done, and how. There are important questions to which the methods section should provide brief answers. As the authors used in silico method, therefore, refer to methods or prediction using computational approaches. So I was expecting more detail of computerized method. I suggest to have stress on method, instead of having two paragraphs on background and objective.
2. Moreover, move “Of the two screened Pacific oyster polypeptides, Leu-Leu-Glu-Tyr-Ser-Ileu ligand have been gained more scores in comparison with Remdesivir” to result section.
3. Conclusion: This part is totally should replace by main point of study and future recommendation such as “Pacific oyster compounds may have the potency to be evolved as an anti-COVID-19 main protease drug to fight against the novel coronavirus but before that, it must go through under the proper preclinical and clinical trials for further experimental and/or clinical scientific validation”
Methods:
1. Change “Target receptor preparation” and “Ligands preparation” to “Receptor and ligand preparation” and merge two sections.
2. In “was retrieved from RCSB Protein Data Bank (PDB ID: 6LU7).” add the resolution of PDB selected file.
3. A very important technical point is that “The optimization firstly should be done by adding hydrogen atoms”, because most macromolecular structure data don't contain hydrogen atoms in their corresponding PDB files. In addition, the ionization state of the protein residues is often in question, which is why it is necessary to include both of the points in the optimization process. In addition, the definition of at least the polar hydrogens allows the establishment of hydrogen bonds that may be present between the macromolecule and the compounds tested. The docking algorithms contemplate these forces of intermolecular union, and their non-definition, would not allow to contemplate them when calculating the binding energy of the compounds to the target, making a mistake by omission.
4. The authors stated that “Since the water molecules have not participated in the ligand-receptor binding process; it is generally more desirable to discharge them before the molecular docking”, but the correct reason for removing water molecules before docking is that “They are therefore deleted to make computations easier and clear the binding pocket of possible water molecules that would distort the pose search. Remember that in docking you typically search for molecules that can create multiple favorable contacts to the protein,

water molecules might confound this procedure". Please correct this clarification.
5. Please move the paragraph "The MVD docking software was beginning and the main protease structure along with all the selected ligands was picked out for docking procedures. The selective and best binding site constraints were fixed to comprise the largest detected cavity on the main protease protein structure and its sizes were reduced so that decline the simulation treating time and raise accuracy. After searching the best docking cavities of the receptor and prepare an excellent docking grid with a radius of 10 (Å) and coordinates of X: -10.76, Y: 12.64 and Z: 68.81. The five poses of each ligand with 10 runs have been docked." after "Ligands preparation" section.
6. I suggest authors to add more detail of docking, including active site selection, virtual molecular docking, molecular dynamics simulation, and drug-likeness property. With all my respect to the authors, they put the stress on the first part of ligand and receptor selection, but the most important part that I have mentioned missing.
7. Another methodological missing point is the calculation of RMSD. The root-mean-square deviation of atomic positions (or simply root-mean-square deviation, RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. In the study of globular protein conformations, one customarily measures the similarity in three-dimensional structure by the RMSD of the Cα atomic coordinates after optimal rigid body superposition. I think authors should add this value and its calculation in both method and result sections.
Results:
1. I know this is a theoretical method, but the results section is very week to support your novel idea. I think the following findings are necessary to add:
• A figure illustrating docking of the native ligand has shown that it binds to the same site of the Covid-19 main protease (Mpro) as the co-crystal ligand binds.
• A table showing the receptor-ligand interactions and indicated that all the compounds used for molecular docking have a substantial binding affinity towards the receptor as compared to the native ligand, with details of Compound name, Score, Kd(μM), No. of H-bond All, No. of H-bond Ligand Atom, No. of H-bond Receptor Atom.
• Figures and text on "Root mean square deviation (RMSD) analysis"
• A table including Interaction energy analysis, with details of Van der Waals, electrostatic, polar solvation, SASA, and binding energy for the docked compounds into Mpro inhibition site.
• The important missing data is "The ΔG binding energy of all the ligands binding to the COVID −19 main protease with respect to different time points has been calculated "
• Last but not least, did the analysis has passed the Modern drug data report (MDDR Like Rule)?
Discussion:
Generally, this section is more like introduction, authors started with introducing the importance of designing potent and safe drug candidates against COVID19, The Pacific oyster, MolDock and its advantages, etc…So, it was expected to start with virtual molecular docking findings and its comparison with others, RMSD results compared to others, interaction analyses, free energy calculation, Drug-likeness property compared to available compounds, the probable mechanism of action, and a revised final conclusion.
General comment
- Lots of typographic and grammatical errors there. I advise respected authors to go through

all manuscript and correct these errors. For example:

Abstract

- Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of a global healthcare crisis in the field of pulmonary diseases and there are various efforts to find effective drugs to manage its infection.
- Objectives: The Molecular docking analysis was used to test whether the human immunodeficiency virusevirus 1 (HIV-1) protease inhibitory peptides. These marine polypeptides were isolated from the hydrolysate of Pacific oyster had the potential for main protease (Mpro) protein of SARS-CoV2 (ID 6LU7.)
- Methods: Then The molecular docking process was done by Molegro Virtual Docker software. The Remdesivir drug was used as a positive control in our docking project. Of the two screened Pacific oyster polypeptides, Leu-Leu-Glu-Tyr-Ser-Ileu ligand have hase been gained more scores in comparison with Remdesivir.
- Results: The results of docking procedures were evaluated based on several bioinformatics docking scores including MolDock Score, Rerank Score, Total interaction energy [between energy), and Hydrogen Bond (H-bond). The Hydrogen bondH-bond of Remdesivir was -6.03673 and Leu-Leu-Glu-Tyr-Ser-Ileu polypeptide was -6.44185. The Rerank Score of Remdesivir was -98.9254 and for Leu-Leu-Glu-Tyr-Ser-Ileu polypeptide was -107.821.
- Conclusions: Designed drugs based on Leu-Leu-Glu-Tyr-Ser-Ileu ligand can be able to control and inhibit SARS-CoV-2 infection in silico analysis.

Thanks.

Niloofar Neisi: Associate Editor | **Revision (0)** 14 Oct 2020

Dear EIC,

A major revision is recommended by reviewers.

Thanks.

Seyyed Ali Mard: EIC | **Revision (0)** 14 Oct 2020

Dear Author,

The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.

Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Response Letter

Dear Chief Editor of Jentashapir Journal of Cellular and Molecular Biology, Dr. Seyyed Ali Mard

We are grateful to the editors and reviewers for their time and constructive comments on our manuscript. We have implemented their comments and suggestions and wish to submit a revised version of the manuscript for further consideration in the journal. Changes in the initial version of the manuscript are either highlighted for added sentences or strikethrough for deleted sentences in the revised version. Below, we also provide a point-by-point response explaining how we have addressed each of the editors or reviewers' comments. We look forward to the outcome of your assessment.

Yours sincerely,

On behalf of the co-authors

Mehrdad Mohammadi, PhD student of medical Bacteriology

Comments to the author (if any):
Comment from **Jentashapir Journal of Cellular and Molecular Biology** and our responses:

Reviewer 1:
Dear authors

Thanks for your valuable manuscript, but the writing needs to be reviewed.

1- There are some grammatical problems. Some of your sentences unclear or hard to follow, and Some of your sentences, possibly wordy sentences, and too many non-content words may indicate wordiness. Please consider rephrasing.
Thanks for your comments. The whole text was read again and edited. Some sentences were also rewritten. These sentences are underlined in the manuscript.

2- The default of Molegro software is five choices for cavities. Why did you choose four cavities? Please state the basis of your choice.

The four cavities were found because the fifth cavities of protein have very similarity with fourth cavity.

3- How was processed PDB file (PDB ID: 6LU7) to obtain the pure Mpro without additional ligands and sequences? (main protease (Mpro) protein of SARS-CoV2 in complex with an inhibitor N3 in PDB ID: 6LU7). In other words, do you perform any manipulate in PDB file or directory add PDB file into Molegro tools? Yes, The main protease (Mpro) protein of SARS-CoV2 in complex with an inhibitor N3 in PDB ID: 6LU7) and need to remove it in preparing of protein in pre-analytical process, therefore we remove this internal ligand from protein.

4- Why the author only selected the pose to belong to the wider and biggest cavity? It seems to be a better examination of all the best poses for the five cavities.

We choice the biggest cavity for our docking process because this cavity is major candidate in previous report for docking process and the major region that a pharmacy substance can be inhibit this protein for coding a major functional structure.

5- Comparing the obtained numbers from HBond, Torsions, Total interaction energy, Rerank Score, MolDock Score, and Similarity Score were only written in the discussion section and the results. But in the discussion, except for HBond, no explanation has been given as to the reason for these comparisons and how they are superior. Please write the comparative role and value of each of the above factors in the discussion section.

Ligand torsion number is related to the flexibility of ligand and generally incorporated as a crucial variable in the thermodynamic function of binding free energy, in according to this issue, the Leu-Leu-Glu-Tyr-Ser-Leu ligand have more torsion in compare with Remdesivir, and we can conclude this substance is more suitable for docking process. The total interaction energy of our compound are very similar and this issue revealed that all of pose and ligand interaction energy are suitable to good interaction in docking process. While the rerank-score in MVD provides an estimate of the strength of the interaction, it is not calibrated in chemical units and it does not take complex contributions (such as entropy) into account [52], the rerank score of Remdisevir in low of our polypeptides that indicate in unrolled gestures there can be a good choice to docking processs and target preparing goals. The Remdesivir MolDock score is -161.419 and in comparison of the above polypeptide is

very near to each over, this score is suitable in controlled status in entropy and near to lab status, therefore in major index in industrial comparing in docking process

Best regards…

Reviewer 2:

Comments

Short/Running title: "Pacific oyster compounds docking in SARS-CoV-2", is an abbreviated form of the main title. This title is usually displayed at the top of each page of the article or left-hand text pages. It serves to guide readers while scanning through a journal or toggling between multiple papers. I suggest "Pacific oyster docking against SARS-CoV-2"

This ruining title wan inserted. " Pacific oyster docking against SARS-CoV-2"

Abstract: Method:

1. The methods section is usually the second-longest section in the abstract. It should contain enough information to enable the reader to understand what was done, and how. There are important questions to which the methods section should provide brief answers. As the authors used in silico method, therefore, refer to methods or prediction using computational approaches. So I was expecting more detail of computerized method. I suggest to have stress on method, instead of having two paragraphs on background and objective.

We extended the methods part and highlighted these parts.

Methods: Then molecular docking process was done by Molegro Virtual Docker software. The protein data bank file of the crystal structure of COVID-19 main protease in complex with an inhibitor N3 (ID 6LU7) was obtained from PubChem data source. After preparing protein and remove od water and internal ligand, the major biggest cavity chooses to dock and the docking process has been done and then the MolDock Score, Rerank Score, Total interaction energy [between energy), and HBond item have been calculated by the software. The Remdesivir drug was used as a positive control in our docking project.

2. Moreover, move "Of the two screened Pacific oyster polypeptides, Leu-Leu-Glu-Tyr-Ser-Ileu ligand have been gained more scores in comparison with Remdesivir" to result section.

This sentences have been moved and highlighted.

3. Conclusion: This part is totally should replace by main point of study and future recommendation such as "Pacific oyster compounds may have the potency to be evolved as an anti-COVID-19 main protease drug to fight against the novel coronavirus but before that, it must go through under the proper preclinical and clinical trials for further experimental and/or clinical scientific validation"

This sentences have been moved and highlighted

Methods:

1. Change "Target receptor preparation" and "Ligands preparation" to "Receptor and ligand preparation" and merge two sections.

2. In "was retrieved from RCSB Protein Data Bank (PDB ID: 6LU7)." add the resolution of PDB selected file.

This action has been done and highlighted.

3. A very important technical point is that "The optimization firstly should be done by adding hydrogen atoms", because most macromolecular structure data don't contain hydrogen atoms in their corresponding PDB files. In addition, the ionization state of the protein residues is often in question, which is why it is necessary to include both of the points in the optimization process. In addition, the definition of at least the polar hydrogens allows the establishment of hydrogen bonds that may be present between the macromolecule and the compounds tested. The docking algorithms contemplate these forces of intermolecular union, and their non-definition, would not allow to contemplate them when calculating the binding energy of the compounds to the target, making a mistake by omission.

This item inserted and highlighted.

4. The authors stated that "Since the water molecules have not participated in the ligand-receptor binding process; it is generally more desirable to discharge them before the molecular docking", but the correct reason for removing water molecules before docking is that "They are therefore deleted to make computations easier and clear the binding pocket of possible water molecules that would distort the pose search. Remember that in docking you typically search for molecules that can create multiple favorable contacts to the protein, water molecules might confound this procedure". Please correct this clarification.

We have been corrected this part and highlighted.

5. Please move the paragraph "The MVD docking software was beginning and the main protease structure along with all the selected ligands was picked out for docking procedures. The selective and best binding site constrains were fixed to comprise the largest detected cavity on the main protease protein structure and its sizes were reduced so that decline the simulation treating time and raise accuracy. After searching the best docking cavities of the receptor and prepare an excellent docking grid with a radius of 10 (Å) and coordinates of X: -10.76, Y: 12.64 and Z: 68.81. The five poses of each ligand with 10 runs have been docked." after "Ligands preparation" section.

We have been moved this part and highlighted.

6. I suggest authors to add more detail of docking, including active site selection, virtual molecular docking, molecular dynamics simulation, and drug likeness property. With all my respect to authors, they put they stress on first part of ligand and receptor selection, but the most important part that I have mentioned missing.

With special respect to our dear reviewer, we represent more details about site selection but in other parts because of this report indicate to pharmacological propose in use of this polypeptides in other reports and in studies, therefore we obstacle to insert more details of dynamic calculation to prevent the confusing of our medical readers of this article.

7. Another methodological missing point is the calculation of RMSD. The root-mean-square deviation of atomic positions (or simply root-mean-square deviation, RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. In the study of globular protein conformations, one customarily measures the similarity in three-dimensional structure by the RMSD of the Cα atomic coordinates after optimal rigid body superposition. I think authors should add this value and its calculation in both method and result sections.

The RMSD of this report under 1 in base of MVD software.

Results:

1. I know this is a theoretical method, but the results section is very week to support your novel idea. I think the following findings are necessary to add:

• A figure illustrating docking of the native ligand has shown that it binds to the same site of the Covid-19 main protease (Mpro) as the co-crystal ligand binds.

• A table showing the receptor-ligand interactions and indicated that all the compounds used for molecular docking have a substantial binding affinity towards the receptor as compared to the native ligand, with details of Compound name, Score, Kd(μM), No. of H-bond All, No. of H-bond Ligand Atom, No. of H-bond Receptor Atom.

• Figures and text on "Root mean square deviation (RMSD) analysis"

• A table including Interaction energy analysis, with details of Van der Waals, electrostatic, polar solvation, SASA and binding energy for the docked compounds into Mpro inhibition site.

• The important missing data is "The ΔG binding energy of all the ligands binding to the COVID −19 main protease with respect to different time points has been calculated "

• Last but not least, did the analysis has passed the Modern drug data report (MDDR Like Rule)?

The requested item in MVD base docking report were not indicated because in Autodock vina in more extended item that you want and therefore we are sorry to present it.

Discussion:

Generally, this section is more like introduction, authors started with introducing the importance of designing potent and safe drug candidates against COVID19, The Pacific oyster, MolDock and its advantages, etc…So, it was expected to start with virtual molecular docking findings and its comparison with others, RMSD results compared to others, interaction analyses, free energy calculation, Drug-likeness property compared to available compounds, the probable mechanism of action, and a revised final conclusion.

Because of no report in this polypeptides and effect of them in COVID-19 we start like this and there is just one report in world that use of them in inhibit of HIV-1 virus that we indicate it I discussion.

With best regards.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) — 2 Nov 2020

Dear AE,
Thanking you in advance. All the corrections made by authors are satisfying, thus, I suggest accepting the paper in a corrected format.
Regards

Niloofar Neisi: Associate Editor | **Revision** (1) — 3 Nov 2020

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

Seyyed Ali Mard: EIC | **Revision** (1) — 3 Nov 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.