jz-2024-023327.R1

Name: Peer Review Information for "Prediction of Threonine-Tyrosine Kinase Receptor-Ligand Unbinding Kinetics with Multiscale Milestoning and Metadynamics"

First Round of Reviewer Comments

Reviewer: 1

Comments to the Author

This is a technically sound study where the method SEEKR is demonstrated to estimate binding and unbinding kinetics for the TTK system using eight small molecules with residence times from seconds to hours.

The overall work is a nice example that may be used by others to guide additional applications.

The only outliner is the compound TC-Mps1-12, which is just off. The authors have clearly menitioned that the reason for this odd result is still unclear, and I fully appreciate to have failures included, which is realistic. However, I was wondering whether by now the author have realized (or at least can offer some possible explanations/speculations on) why such a negative result was obtained for this particular compound... ?

Overall, this is a nice piece of work.

Reviewer: 2

Comments to the Author

This manuscript of Votapka et al. describes the extentions of the

Simulation-Enabled Estimation of Kinetics Rates (SEEKR) method by

incorporating metadynamics molecular dynamics simulations. The authors

compare the metadynamics with steered molecular dynamics for

generating starting structures in SEEKR simulations. They demonstrate

that metadynamics provides superior results in predicting accurate and rank-ordered ligand residence times and binding free energies for the threonine-tyrosine kinase (TTK) receptor and its lon-residence-time inhibitors.

I have a few suggestions for manuscript improvement.

TOC graphic is not informative, needs improvement. Figure 3 is better suited for TOC.

The CV abbreviation is not described. The same for MMVT. For a reader not familiar with MMVT it is difficult to guess.

Figure captions are too long. The authors' comments and discussion of data presented in the figures should be moved to the text.

Figure 1 - label the amino acids in the protein structure instead of naming them in the caption

Figure 2 - change colors, the shades of blue are not clearly visible and not distinguishable.

All the methods descriptions are in SI which makes understanding the article difficult as the authors emphasize the importance of proper system preparation, force field parameter selection, and convergence analysis in achieving accurate results. This is one of the reasons why I believe this manuscript is more suitable for a full article than a letter.

The manuscript does not provide detailed information on how metadynamics parameters (such as Gaussian height and width) were chosen or optimized, which is crucial for reproducibility.

Some statements on While the generalizability of the method to other protein-ligand systems could be includes in Conclusion.

Reviewer: 3

Comments to the Author

Review of manuscript jz-2024-023327

The authors present a carefully conducted methodological study that combines the SEEKR milestoning method for estimating binding/unbinding kinetic rates with metadynamics simulations to attain more accurate predictions of small molecule drug residence times. The new protocol avoids reliance on steered molecular dynamics (SMD) to generate starting structures for SEEKR. Instead, it uses GPU-accelerated well-tempered metadynamics (MTD) to markedly improve the sampling of protein unbinding for complex ligands with large, flexible structures. By rigorous analysis of eight long-residence-time inhibitors of threonine-tyrosine kinase the authors show that the new method produces superior starting structures compared to SMD. It prevents forced tearing and local unfolding of the protein during the unbinding process. Overall, I think this will be a worthy contribution to the Journal of Physical Chemistry Letters. The topic is important and would be of interest to many physical chemists concerned with computational prediction of binding kinetics. The molecular dynamics simulations were performed with appropriate and well-established protocols. The computational methods were appropriate and suitably described. The authors were careful in explaining that not only the sampling method (MTD), but many additional factors affect the accuracy of the computed rates, including the choice and preparation of the receptor/ligand systems, the quality of the experimental structures, matching of the experimental conditions in the simulation and the careful consideration of protonation states among others.

Author's Response to Peer Review Comments:

Review Responses for Prediction of Threonine-Tyrosine Kinase Receptor-Ligand Unbinding Kinetics with Multiscale Milestoning and Metadynamics

Reviewer(s)' Comments to Author:

Reviewer: 1

Recommendation: This paper is publishable subject to minor revisions noted. Further review is not needed.

Comments:
This is a technically sound study where the method SEEKR is demonstrated to estimate binding and unbinding kinetics for the TTK system using eight small molecules with residence times from seconds to hours.

The overall work is a nice example that may be used by others to guide additional applications. The only outliner is the compound TC-Mps1-12, which is just off. The authors have clearly mentioned that the reason for this odd result is still unclear, and I fully appreciate to have failures included, which is realistic. However, I was wondering whether by now the authors have realized (or at least can offer some possible explanations/speculations on) why such a negative result was obtained for this particular compound. Overall, this is a nice piece of work.

We thank the reviewer for the positive feedback, and we are glad to hear that the reviewer agrees that our research is technically sound, significant, novel, and urgent. During review and revisions, we looked very closely at the reasons for why the k_{on} (and, by extension the free energy of binding) of TC-Mps1-12 was estimated so incorrectly. We believe we know the reason and have replaced the unclear reasons with the following text: "Predictions within a single order of magnitude of experiment were found for all TTK system k_{on} estimates except TC-Mps1-12 (Figure 2B). We examined the reasons for the large deviation and believe it is due to the reparametrized partial charges of the ligand in the bound state using QMrebind, which caused the charges to become highly polarized. The k_{on} calculation depends on an accurate description of the unbound state, where molecular charges are likely to be less polarized. Applying the bound state QMrebind charges to the unbound state of compound TC-Mps1-12, as done in this study, a large desolvation penalty is incurred, significantly slowing the binding rate. Preliminary studies (data not shown) support this hypothesis, though further careful analysis is needed to fully validate it."

Reviewer: 2

Recommendation: Reconsider as an article in The Journal of Physical Chemistry A/B/C.

Comments:
This manuscript by Votapka et al. describes the extentions of the Simulation-Enabled Estimation of Kinetics Rates (SEEKR) method by incorporating metadynamics molecular dynamics simulations. The authors compare the metadynamics with steered molecular dynamics for generating starting structures in SEEKR simulations. They demonstrate that metadynamics provides superior results in predicting accurate and rank-ordered ligand residence times and binding free energies for the threonine-tyrosine kinase (TTK) receptor and its lon-residence-time Inhibitors. I have a few suggestions for manuscript improvement.

The TOC graphic is not informative and needs improvement. Figure 3 is better suited for TOC.

Thank you for the suggestion. We have replaced the TOC graphic with a more informative image.

The CV abbreviation is not described, and the same is true for MMVT. For a reader not familiar with MMVT, it is difficult to guess.

We thank the reviewer for pointing out this oversight. We have corrected this problem by defining these acronyms in the first place they appear.

The figure captions are too long. The authors' comments and discussion of the data presented in the figures should be moved to the text.

We have significantly shortened the figure captions within the manuscript by moving much of the caption to the text.

Figure 1 - Label the amino acids in the protein structure instead of naming them in the caption.

We have modified this figure as suggested.

Figure 2 - Change colors, the shades of blue are not clearly visible and not distinguishable.

Thank you. We have adjusted the colors within the figure to make it easier to visualize and distinguish the shades.

All the methods descriptions are in SI, which makes understanding the article difficult as the authors emphasize the importance of proper system preparation, force field parameter selection, and convergence analysis in achieving accurate results. This is one of the reasons why I believe this manuscript is more suitable for a full article than a Letter.

We can appreciate the reviewer's point; however, due to the article's brevity, area of interest, and urgency, we submitted the manuscript as a Journal of Physical Chemistry Letter. The instructions for JPC Letters allow one to place one's materials and methods in the SI. We have placed a sentence at the bottom of page 4 that orients the readers to the SI for the complete Computational Methods section: "Details of the computational methods, benchmarks, and simulation costs are in the SI." Unfortunately, due to the word count constraints of a JPC Letter, we have placed the (fairly lengthy) Computational Methods section into the SI. Indeed, we would not be able to place the Computational Methods section within the main article and keep it under the word count required by JPC Letters - which we believe is the publication avenue best suited to this manuscript.

The manuscript does not provide detailed information on how metadynamics parameters (such as Gaussian height and width) were chosen or optimized, which is crucial for reproducibility.

The reviewer makes a good point, and we have added the following sentence and references to the Computational Methods to explain how the metaD parameters were chosen:

"MetaD parameters, such as Gaussian heights, widths, bias factors, and deposition intervals, were chosen by performing a literature review on several similar applications of metaD (see citations below) and choosing characteristic values for each of them, and then refined based on visual assessment of the quality of generated starting structures, and their ability to generate accurate results with SEEKR." Citations:

Ghosh, S.; Jana, K.; Ganguly, B. Revealing the Mechanistic Pathway of Cholinergic Inhibition of Alzheimer's Disease by Donepezil: A Metadynamics Simulation Study. *Phys. Chem. Chem. Phys.* **2019**, *21* (25), 13578–13589. https://doi.org/10.1039/C9CP02613D.

Brandt, A. A. M. L.; Rodrigues-da-Silva, R. N.; Lima-Junior, J. C.; Alves, C. R.; de Souza-Silva, F. Combining Well-Tempered Metadynamics Simulation and SPR Assays to Characterize the Binding Mechanism of the Universal T-Lymphocyte Tetanus Toxin Epitope TT830-843. *BioMed Res. Int.* **2021**, *2021* (1), 5568980. https://doi.org/10.1155/2021/5568980.

Wakchaure, P. D.; Ganguly, B. Deciphering the Mechanism of Action of 5FDQD and the Design of New Neutral Analogues for the FMN Riboswitch: A Well-Tempered Metadynamics Simulation Study. *Phys. Chem. Chem. Phys.* **2022**, *24* (2), 817–828. https://doi.org/10.1039/D1CP01348C.

Zhou, X.; Shi, M.; Wang, X.; Xu, D. Exploring the Binding Mechanism of a Supramolecular Tweezer CLR01 to 14-3-3σ Protein via Well-Tempered Metadynamics. *Front. Chem.* **2022**, *10*. https://doi.org/10.3389/fchem.2022.921695.

Some statements on the generalizability of the method to other protein-ligand systems could be included in the Conclusion.

To address the generalizability of the SEEKR method to other protein-ligand systems, we have added the following sentences to the conclusion: "The combined use of metaD for initial structure generation and quantum-mechanically reparametrized ligands significantly improves SEEKR calculations. We believe this protocol, which integrates SEEKR and metaD to predict binding and unbinding kinetics and thermodynamics, will be broadly useful for various ligand-receptor systems, provided each system is described with sufficient physical accuracy. However, challenges remain for systems with heavy halogens due to sigma holes, systems where protonation states or partial charges change significantly along the unbinding pathway, or systems lacking suitable molecular mechanics force fields. Additionally, increasingly large or complex ligands may pose significant challenges. Efforts to address these and other unforeseen issues are ongoing and will be resolved in future research iterations."

Reviewer: 3

Recommendation: This paper represents a significant new contribution and should be published as is.

Comments:

The authors present a carefully conducted methodological study that combines the SEEKR milestoning method for estimating binding/unbinding kinetic rates with metadynamics simulations to attain more accurate predictions of small molecule drug residence times. The new protocol avoids reliance on steered molecular dynamics (SMD) to generate starting structures for SEEKR. Instead, it uses GPU-accelerated, well-tempered metadynamics (MTD) to markedly improve the sampling of protein unbinding for complex ligands with large, flexible structures. By rigorous analysis of eight long-residence-time inhibitors of threonine-tyrosine kinase, the authors show that the new method produces superior starting structures compared to SMD. It prevents forced tearing and local unfolding of the protein during the unbinding process. Overall, I think this will be a worthy contribution to the Journal of Physical Chemistry Letters. The topic is important and would be of interest to many physical chemists concerned with computational prediction of binding kinetics. The molecular dynamics simulations were performed with appropriate and well-established protocols. The computational methods were appropriate and suitably described. The authors were careful in explaining that not only the sampling method (MTD) but many additional factors affect the accuracy of the computed rates, including the choice and preparation of the receptor/ligand systems, the quality of the experimental structures, matching the experimental conditions in the simulation and the careful consideration of protonation states among others.

We thank the reviewer for the positive feedback and are very pleased to hear that the reviewer recognized the high value of our work.

Other comments for the manuscript:

You recently received a Revision Request from Alessandra Magistrato. In addition to addressing the Editor's concerns and the requests of the reviewers, we request your assistance with the following issues so that we may process your manuscript as quickly as possible:

1. Please provide full contact information for all authors in the manuscript file: institution, city, state, postal code, and country for each affiliation (states are required for United States addresses only). Postal codes are also required for addresses outside the U.S., for countries with them. Each separate affiliation requires its address information; they cannot be combined.

We have added the postal code for author Naoya Asada, who is based in Osaka. We have also added the new affiliation of one of the authors and the old one.

2. Please remove the section headings from your manuscript file, e.g., Introduction, Results and Discussion, Conclusion. Experimental section headings as well as paragraph headings, are okay.

We have removed the “Introduction” and “Background” section headings from the manuscript. All section heading should now be removed.

3. If the manuscript is accompanied by any Supporting Information for Publication, the manuscript should contain a brief, nonsentence description of the actual contents of each Supporting Information file. This description should be labeled Supporting Information and appear directly before the Acknowledgment and Reference sections. The appropriate format is as follows: Supporting Information. A brief statement in nonsentence format of the contents of the material supplied as Supporting Information.

We have reformatted the Supporting Information section as requested and placed it before the Acknowledgment and Reference sections.

4. Incomplete references:

Please include the journal name and at least the first page number for the following incomplete journal references: 11, 15, 21. Please include the section name, if provided, DOI or URL (accessed YYYY-MM-DD) for the following incomplete preprint reference: 14.

Thank you. We have corrected these references as requested and replaced preprint reference 14 with the final publication, which has been released now.

5. The TOC graphic should fit in an area no larger than 3.25 in. × 1.75 in. (approx. 8.25 cm × 4.45 cm) should have adequate resolution and clarity. Confirm that all text is legible at this size.

A TOC graphic of the correct size and resolution is provided in the manuscript.

6. Your Supporting Information for Publication file should include a manuscript title, list of authors, and their affiliations that match exactly the title, author list, and affiliations in the manuscript file.

We have updated the SI with a full author list and affiliations and corrected the mismatch between the manuscript title and the SI title.