. Overall Recommendation
High Borderline
2. Is this submission appropriate for its type (Long Format vs. Blue Skies Format)?
Agreement accepted
3. Is this submission best suited for an oral presentation or an interactive/panel presentation?
Oral
4. Comments for the Author(s)
Summary:
This work uses motion planning to measure how traversable a protein tunnel is for a ligand when reaching/exiting an active site. Like other work in this field, it models the ligand as a flexible movable object and the protein as a static obstacle it must navigate through. To reduce the search space, it only allows ligand conformations from a dictionary of predefined values called rotamers. It then biases RRT growth from the active site along the tunnel to the outside surface of the protein. They quantify how traversable this tunnel is by conducting many RRT searches and measuring how many reach different parts of the tunnel. They also allow the method to scale the ligand atoms down as needed to avoid collisions.

Comments:

- Motivating application - The motivating application here is well thought out and has potential to impact the community. It is an interesting application of motion planning, and fits with the large body of work already in this area of molecular modeling through sampling based motion planning techniques.

- Validation - Unfortunately, there is no experimental validation of this method. The intent is to provide a tool to identify which ligands from a library are more likely to access a given binding site along a given tunnel. The paper shows how different metrics may be computed for different ligands in a tunnel, and the various visualizations are great. However, there is no validation as a predictive accessibility tool as this work was advertised. For example, given two ligands and the same active site, this method should be able to identify which is more likely to bind and then compare this to known binding affinities. This kind of validation (or something like it) will need to be done before biochemists will trust and use the approach. This limits their current impact. With thorough validation, this paper would be well-suited for a top computational biology conference such as RECOMB or ISMB.

- Technical questions -
* Scaling of the ligand seems problematic and not fully supported. What value do paths have with ligands scaled down? This will never happen in the physical world. Is it due to approximations made by the tunnel detection method? Or to approximations from the rotamer library? These issues should be better explained. Otherwise, it is difficult to see the applicability of scaled down ligands to predicting actual tunnel accessibility. This is a critical issue to resolve/explain as the work stems from the motion planning community. (A follow question is how is local planning performed between ligand conformations with different scale factors?)
* How does this compare to MD simulations in the tunnel to estimate accessibility.
* Instead of scaling the ligand, it might be interesting to look into scaling the tunnel (to help model tunnel flexibility).

- Missing citations - Some citations are missing and some relation to cited work is downplayed. Many of the ideas of biasing RRT growth along a region space have been done by several groups (some of them cited in the paper) and should be give more credit here as contributing to the ideas in this work.

Some of the areas of missing citations are:
* C-space definition
* original sampling based motion planning work (only surveys/textbooks cited which misplaces credit of the original authors)
* a similar comment holds for motion planning for protein folding work
* motion planning for ligand binding work not cited
* little to no work cited on RRTs for narrow passages

Minor Comments:
- At the top of each page reads "Authors/Title Suppressed Due to Excessive Length" which should be fixed.
- The conclusion would be better expanded.
REVIEWER #2

REVIEW QUESTIONS

1. Overall Recommendation
Definitely Reject
2. Is this submission appropriate for its type (Long Format vs. Blue Skies Format)?
Yes
3. Is this submission best suited for an oral presentation or an interactive/panel presentation?
Oral
4. Comments for the Author(s)
The authors present an RRT technique for planning motion through traversable tunnels in receptor proteins from the perspective of a flexible ligand.

The work overall appears to be straightforward application of RRTs with some basic modifications for the ligand tunnel problem. Some of these modifications seem under-informed or at least they are insufficiently defended in the text.

1) It is not clear how ligand conformations are generated. The authors correctly state that a rotamer library can be used to generate ligand conformations, but this applies only to amino acid. The ligands used by the authors were 1-chlorpropan and 1-chlorbutan, which are not peptides. The authors state that energy functions can be assessed in order to determine acceptable conformations, so we assume that such a function was used to determine the acceptable conformations, but no details were given. Also, there is the question of what energy was deemed acceptable, and why, and whether all acceptable conformations of the ligand are actually accessible in all states of the plan - the ligand obviously cannot magically "snap" to a different low energy conformation half way through the tunnel, especially if tunnel clearance doesn't allow the transition. None of that is discussed or explained.

The potential energy landscape that affects ligand conformation is profoundly affected by the tunnel around it. The authors never mention this critical interaction, so it's not obvious that the conformations generated are even plausible. What energy function is used? The authors imply that potential energy assessments are time consuming - there is no breakdown on runtime spent measuring potential energies as opposed to path planning.

2) The method is insufficiently tested. The authors have assessed their method on a single protein structure and two ligands. There is no evidence of generality indicated by the results. Moreover, all ligands required having their atomic radii shrunken by 50% in order for even a single path plan to be

discovered. This result is hard to believe. If indeed the tunnel is inaccessible with a rigid receptor, the authors should have simply selected a receptor that is dependably rigid as documented in the experimental literature with sufficient clearance for the ligand. This is a test set - so there need to be examples with ground truth support that indicate that the ligand actually passes along the tunnel, indicating that the method actually predicts real truth. You can't just throw these things together.

3) There is no real motivation for this paper. Why do we even want to discover the pathway taken by the ligand through the receptor? There are no examples of real world applications for this method. Perhaps they exist, but the authors make no effort to support their case.
REVIEWER #3

REVIEW QUESTIONS

1. Overall Recommendation
Low Borderline
2. Is this submission appropriate for its type (Long Format vs. Blue Skies Format)?
Agreement accepted
3. Is this submission best suited for an oral presentation or an interactive/panel presentation?
Oral
4. Comments for the Author(s)
In the paper “Testing Traversability of Protein Tunnels with Flexible Ligands Using Motion Planning”, Vonásek et al. introduce a method to measure the traversability of ligands in protein tunnels using motion planning methods with the goal of revealing tunnel properties that may be useful to biochemists in drug design and protein engineering. To accomplish this, the authors apply a modified RRT method to evaluate ligand trajectories through the protein tunnel. In the modified RRT, configuration space samples are guided along the centerline of a protein tunnel, the radii of ligand atoms can be reduced by a scaling factor, and a library of pre-defined ligand conformations is used to represent flexibility.
Using a biased sample generator scheme and a library of ligand conformations are very novel ideas that can reduce the degrees of freedom of the model and speed up computations. The resultant paths of a modified RRT with these characteristics are indeed interesting for a biophysics community. However, this reviewer is deeply concerned by the dramatic scaling-down of ligands. In the results section, no ligand configuration larger than 60% of the ligand size could successfully traverse the tunnel. This is a very large reduction and a serious limitation of the model. The authors seem aware of this problem, as it is discussed that the paths found should not be understood as “real paths”, but merely as a guide to biochemists/biophysicists. It is still a troubling assumption, and this reviewer fails to see the value for paths of severely scaled-down ligands to the molecular docking community, even as a guide.
Also missing from the results is the effect of flexibility on traversability. While the authors provide a detailed analysis of the success rates and tree sizes for different s_{min}, the analysis of success rates depending on flexibility is lacking. For instance, it would be interesting to see how many different ligand conformations were chosen from the initial library for a given tree. Is there a preferred ligand conformation that appears in most trees? How do conformations change from one another along a path? As the title of the paper mentions ligand flexibility, a more careful analysis of this effect is granted.
Some other comments:
* The explanation of the method is somewhat unclear, and some mathematical parameters were not clearly indicated in the text. However, the figures were well made and helpful to the understanding of the paper.
* The Related Work could be reorganized. Some paragraphs are indeed a discussion of related works, but others are a discussion of the approach used in this paper. The latter should be moved to a Methods section.

* The paper needs a major grammar and spelling review. A careful English review would be recommended prior to submission.
* Some references are missing. For instance, when asserting that “Typical tunnels have bottlenecks smaller than 1.0 angstroms”, the authors need to substantiate their claim.
Another missing reference is a recent paper closely related to this work:
Kingsley, Laura J., and Markus A. Lill. "Including ligand-induced protein flexibility into protein tunnel prediction." Journal of computational chemistry 35.24 (2014): 1748-1756.