**COVER LETTER**

**Submission ID**: 17-0790

**Title**: Assessing the Accessibility of Protein Tunnels using Motion Planning

**Date**: December 9th, 2017

We would like to thank the anonymous reviewers for their helpful comments. We attempted to address all remarks in the revised version of the paper. In this document, we give detailed responses to all reviewer comments and describe the corresponding changes in our manuscript.

**Summary of all reviews**

1. The ligand conformation library is critical to the contribution of this method, as it relates to prior work. However, there are little to no details given as to how this library is generated/selected, what is the diversity of this library, and how is it stored during RRT construction?

TODO

1. The biophysical realism of the RRT solution is not explained. For example, the scaling is not justified biophysically. Perhaps some scaling (as suggested by Reviewer 2) can be understood, however, it is never proven to be realistic. A justification, that does not just cite prior work, needs to be provided.

TODO

1. Disappointingly, no direct comparison is made. Rather, the paper mentions that the result is in agreement. A quantitative result should be demonstrated, and the parameters of the MD simulation should be provided. This AE also suggests that the MD be quantitatively compared to the different scaling runs, in order to identify a single scaling value that generally holds and allows the method to validate against MD runs. However, this will require MD runs on additional ligand/receptor pairs.

TODO

1. The authors have a prior workshop paper on this same topic. A clarification between that work and the submitted work should be provided, even if it is noted that the workshop is non-archival or extensions have been made.

TODO

**Reviewer 1**

1. Scaling of ligand (up to half its size) is problematic and not adequately supported, need better defense that this compensates for keeping the protein static, otherwise this is nonsensical from a motion planning perspective.

TODO

1. No experimental validation, absolutely necessary for this application domain; existing results are not clarified in terms of goals/objectives, observations, and inferences.

TODO

1. Needs support for using a method declared as inadequate because it only uses a spherical probe as the basis for this method, in other words, why are tunnels defined by spherical probes appropriate as a basis for narrowing the search for a flexible ligand?

There are already dozens of research publications, published in top biochemical and biological journals, describing the usage of computational tools detecting tunnels using a spherical probe, thus confirming the biochemical relevance of this approach. Of course, the tunnels detected by the spherical probe describe the actual void space only very roughly and they detect only those tunnels which can accept the bounding sphere of the desired ligand. So the first impression would be that these methods omit many feasible paths which can be taken by the actual shape of the ligand. However, in practice, the size of the spherical probe is usually much smaller than the actual bounding box of the ligand because when the ligand traverses the tunnel, it influences the amino acids in the interaction distance and causes their shift. Therefore, the spherical probe based methods provide the biochemists with feasible results. However, when studying the tunnel behavior over time, these methods still have many limitations and other methods, such as other proposed one, should be subsequently used to verify the tunnel shape stability and permeability over time.

The advantage of our approach is that this method can be easily replace by some other, when available.

Just as a side note, narrowing the search space helps to significantly reduce the computational time and resources, traversing the entire void space inside protein would lead to inacceptable costs and in such case, we believe that molecular docking would be already a better option for protein void space exploration.

1. Study of which modifications yield which performance gains is needed (atom-based distance metric, selecting conformations of various packing properties, region restriction, etc).

TODO

1. Why is energy never considered? This is often critical in these applications as it is not a purely geometric problem.

We completely agree with the reviewer that energy and forces are a factor which would shift the relevance of the detected tunnels to another level. However, including these into the calculation causes significant increase of computational complexity. Additionally, the purely geometric solutions proved to provide the users with sufficiently precise results when studying the overall protein void space and the detected tunnels mostly correspond to the actual paths taken by the ligands. Therefore, we still believe that our improved geometric solution represents an interesting tradeoff between the Voronoi-based geometric tunnel detection approaches and robust and costly solutions taking into account the energies (such as molecular docking).

1. What happens when the litanies are more elongated (larger deviation in atomic distances)?

In these cases our method is applicable as well, it depends on the shape and number of rotamer configurations taken into account.

1. Missing related work on biasing sampling to known regions of space, all workspace guided methods do this and there are many available, here are just a few:

D. Hsu, T. Jiang, J. Reif, and Z. Sun. Bridge test for sampling narrow passages with proabilistic roadmap planners. In Proc. IEEE Int. Conf. Robot. Autom. (ICRA), pages 4420–4426, 2003.

van den Berg, J.P., Overmars, M.H.: Using workspace information as a guide to non-uniform sampling in probabilistic roadmap planners. Int. J. Robot. Res. 24(12), 1055–1071 (2005)

An efficient retraction-based RRT planner, L. Zhang, D. Manocha, In Proc. Int. Conf. Rob. and Auto. (ICRA), 2008.

A General Region-Based Framework for Collaborative Planning, Jory Denny, Read Sandstrom, Nancy M. Amato, In Proc. Inter. Symp. On Robotics Research (ISRR), pp. 563-579, Genova, Italy, Sep 2015.

TODO

1. Missing related work on other mp techniques for ligand binding and motion:

A.P. Singh, J.C. Latombe, and D.L. Brutlag. A motion planning approach to flexible ligand binding. In 7th Int. Conf. on Intelligent Systems for Molecular Biology (ISMB), pages 252–261, 1999.

Ligand Binding with OBPRM and Haptic User Input, O. Burchan Bayazit, Guang Song, Nancy M. Amato, In Proc. IEEE Int. Conf. Robot. Autom. (ICRA), pp. 954-959, May 2001.

TODO

1. Subsection headings would help clarify transitions from ligand binding problems to motion planning problems.

TODO

1. Disconnect in flow from introduction to related work.

Corrected.

1. Last 2 paragraphs of results would be better as a separate subsection.

TODO

1. Some parameters need better explanation and support for how they are set: R\_init, p\_tunnel, d\_tunnel.

TODO

**Reviewer 2**

1. The MD simulation is not clearly described. What was the force field used? (Which version of AMBER?) What was the MD time step? (maybe 1 fs?) What was the temperature? Were the 100 time frames of MD simulation consecutive time frames, or were they taken at an interval? If the time step was 1 fs and the 100 time frames correspond to 100 fs, are the authors confident that the simulation time was long enough for the protein structure to equilibrate? It is ambiguous whether the authors performed MD for the dynamics of the ligand as it traverses the tunnels or if MD was done only for the protein, in order to understand the tunnel dynamics. It seems from Section V.C that MD was done to test the results for the DCP ligand, but other than stating that MD worked, there is nothing else in the text to substantiate this claim (no results from MD).

TODO

1. While the work has possible applications in the field of biochemistry, where virtual screening of ligands binding to a receptor provides insight on drug discovery, there are still major issues to be resolved before it can be used in biochemistry, as the authors rightly state in the Discussion. The authors justify scaling down ligand atoms' sizes by citing previous works that changed the size of the hard-sphere van der Waals radii of atoms (refs. [6],[8],[12] in the paper, but it is not clear if ref [8] does rescaling explicitly). In refs [6],[12] a reduction of 20% of VDW radii is justified by the collision check: If atomic radii have been reduced to 80% of their size and are still in collision, then this collision truly is unphysical and configurations in collision are prohibited. In other words, if one needs to reduce models below 80% of their size in order to avoid collisions, then the resulting models will be unphysical. So it follows from the same argument given in refs [6],[12], that any reduction below 80% of the original atoms’ VDW radii should be forbidden. This is problematic since the central results of this paper focus on atomic radii reductions of 20% to 50% for the ligands. Have the authors considered reducing not only the ligand’s but the protein’s atoms as well? Since the limit of a 20% reduction is well justified by the previous works, ligand and protein atoms could be reduced by no more than 20% (s\_min in [0.8,1]). If the protein atoms are reduced, then the tunnels could be wider and the ligand might not need to shrink too much. In this scenario, possibly a s\_min=0.8 might lead to more successful results.

TODO

The idea of reducing the size of the protein’s atoms is very interesting and definitely needs to be tested in our future research.

1. The atom-based metric is interesting, but it isn’t clear how it considers ligand shapes. A figure would be helpful. How are rotational constraints imposed? Have the authors considered, instead of taking the shortest Euclidean distance overall between any pair of atoms (atom I in q to any atom j in q’), picking the shortest distance between the same atoms in each ligand (i in q to i’ in q’)? Could this constrain how much a ligand can rotate from one node to another, avoiding large motions?

TODO

1. Why, when computing the initial configurations, is the initial scale set to s\_min instead of 1 (full-size)?

TODO

1. It would be useful to discuss more on ligand conformations, eg. How many are actually chosen by the algorithm in each trajectory?

TODO

1. Adding percent signs (%) to the numbers in the tables would be helpful to the reader.

TODO

1. How many trajectories are shown in Figure 4? Are these all successful trajectories? What was the method used to find these trajectories (A1? A2? MD?). Are the results shown in Figure 4 the same as reported in Table III? As only 8% of the results are successful for DCP using A1 (s\_min=0.8), what do the successful trajectories have in common? Does the success rate depend on the particular conformations that are added to the tree? Does success depend on initial conformations?

TODO

1. Other than the issues stated, the paper would also benefit from an English style and grammar review.

TODO

**Reviewer 4**

1. The paper utilizes a set of ligand configurations (L) but provides no method and no citation as to how these configurations were generated. Sect. IV-D provides no metrics on the variance of the deviations from center. Without any analysis of the diversity contained within the libraries, the sampling strategy employed seems to simplistic. In that ligand configurations with a unique (well separated) deviation from center measurement could be missed and replaced with highly redundant configurations. No cardinality information is given for the protein/ligands listed in table 3.

TODO

1. Although work has been done about ligands exiting proteins with ML-RRT, finding the ligand entrance path is much harder (since the first problem starts with the ligand in a bound state, and thus, you know the ligand and the protein interact). The paper would be strengthen by mentioning that the prior work cannot act as a ligand "filter" (which is one of the goals of this work).

We thank the reviewer for pointing this out. We added the following sentence to the Introduction section:

“Another advantage of our method in comparison with the existing approaches is that we enable the users to trace the ligand path leading from the outside solvent towards the protein active site.”

1. Continuing from the point above, given the relatively quick run time (under 1 hour according to fig 3), analyzing more than 50 ligand configurations seems prudent. How does increasing this size impact the scaling factor and the number of solutions found. This choice of 50 seems to have been made independent of the size of ligand library.

TODO

1. For a pair of connected nodes in the search tree, these nodes could have different ligand configurations (lets call them L1 and L2). Do the authors assume that the ligand can transition from L1 to L2 within this very small space (epsilon = 0.05). Is this reasoning based on the fact that L1 is collision free at its location and L2 is collision free at its location? It would seem that in some cases (where the ligand goes from an elongated form to a compact one) that this assumption may not hold. At a minimum, this concern needs to be addressed in the text.

TODO

1. The end of the introduction indicates the wet lab experimental data was analyzed. Molecular dynamics is not a "wet lab" technique, but rather a computational technique. Thus, this statement is very misleading.

TODO

1. No analysis of the distance traveled from the Qinit configurations to the active site are provided. At a minimum, the experiments outlined in table 2/3 should include the number of spheres considered in each problem. This goes directly towards validating the premise of the planner modifications. This makes the thresholds of 0.5 A for each planner and 2.0 A for each traversability analysis (last sentence of Section V) very difficult to interpret. Further, for the larger ligands, is the final configuration compact of elongated. Is it the center of mass that is measured for proximity to the active site, or just the closest atom in the ligand?

TODO