**Reviewer’s Comments**

Dear Editors,

Thank you very much for the editorial and reviewer comments made on our manuscript. We hope the changes we have made in response to the comments are sufficient and that our manuscript will continue to be considered for publication in the Journal of Visualized Experiments. All editorial and reviewer modifications to the manuscript were considered and changes made to improve the manuscript. We would like to comment on each editorial or reviewer comment separately.

**Editorial comments:  
1) Protocol section is beyond JoVEs 3 page guideline, please highlight less than 3 pages of text to identify which portions of the protocol are most important to film; i.e. which steps should be visualized to best supplement the written section of the protocol. Please see JoVEs instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.**

We have carefully gone over the steps in the protocol and only steps we feel are necessary for the effective use of the webtool were retained. This left approximately 2.5 pages of highlighted text. Hopefully this has been reduced to a level appropriate for the journal.  
  
**Reviewer #1:   
This manuscript describes a protocol for protein design. It does a nice job in describing the force field for sequence selection, and the structure prediction for validation of design. However, the description lacks a key step on how the sequences are designed, i.e. the move sets for the sequence mutations and searching. Are they generated by randomly mutating the native sequence or from some sort of Monte Carlo/genetic algorithm searching? In either approach, the procedure for sequence space searching needs to be described clearly so that the readers/users can have a complete idea how the sequences were designed.  
  
The paper is generally well-written and highlighted with several successfully designed protein examples.**

This comment concerns the clarity of the document in conveying how the sequences are designed in the first stage of the method. The purpose of an optimization method of sequence selection is to make sure that one is not required to search sequence space through random mutation or genetic algorithm based method. Even for proteins of reasonable size this type of search can be computationally prohibitive and there is no guarantee that the sequence one finds is the global minimum in energy. We introduce a deterministic global optimization method which does not rely on random mutations and is theoretically guaranteed to search the complete sequence space and determine a global solution. This is a major advantage of our approach compared to all other existing approaches. We have added the following paragraphs describing what we state above:

“This global optimization method does not rely on random mutations and is theoretically guaranteed to search the complete sequence space and determine a global solution. This is a major advantage of our approach compared to all other existing approaches.” (page 7)

“Any of the above formulated Integer Linear Programming (ILP) problems15-17 can be solved rigorously using branch-and-bound techniques.28-30 Such techniques guarantee consistent and reliable convergence to the global minimum energy sequence.” (page 9)

We recognize that this method may not have been explained in enough detail in the manuscript and hope that the changes made are enough to improve clarity.  
  
**Reviewer #2:   
*Summary:*   
The authors describe their software workbench for computational protein design. The method has been validated on several protein:ligand complexes and detailed background has already been published. The present article is a nice overview that illustrates the possibilities of the method and will allow people to use the tools themselves.  
  
*Major Concerns:*  
No major concerns.  
  
*Minor Concerns:*  
Several figures are non-essential.  
  
*Additional Comments to Authors:*  
none.**

No changes were deemed necessary from Reviewer #2’s comments.

Thank you very much for the comments and we hope the changes we have made are sufficient.

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