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Dear Alisha DSouza,

Thank you for providing editorial comments and the set of four reviewer comments for our recent manuscript submission, “Interactive Molecular Model Assembly with 3D Printing”. Having been our first submittion to JoVE, it was encouraging to see the positive responses from all those involved.

Here, we address both the Editorial staff comments and reviewer requests. In general, there can be summarized as a desire for some additional details, citations, and context. We highlight the manuscript changes in this response document, with excerpts from the revised manuscript. We hope these edits meet both reviewer and editorial expectations for a successful and impactful JoVE publication.

With best regards,

Christopher Fennell, PhD
Department of Chemistry
Oklahoma State University

CC: Elham Fazelpour

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

We have made an effort to address potential spelling and grammar issues with this revision.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
1) Section 1: Please specify all button clicks and menu selections for all software steps.
2) 3.3: Mention sand paper grit and buffer wheel speed.

Further protocol details have been provided, this with a focus on using the Simplify3D 4.1.2 software. While we did not strongly highlight the use of this specific software in the manuscript text, we agree that a very specific case in the manuscript could be beneficial to readers. We also provide more specific protocol details for activities that we originally deemed general, this with some descriptions of motivations. Highlighted excerpts of several of these changes are as follows, though there are more such changes in the revised manuscript. They hopefully provide even clearer guidance to readers/ viewers:

"…with specific considerations given for representative models shown with the Simplify3D 4.1.2 software and MakerGear M2 FDM 3D printers. Dedicated manufacturer directions…"

"1.2 Import one of the C_atom_sp3, H_atom, or C-C_bond files into your slicer program. Use millimeter format for the units if an option is available. In Simplify3D, either click the "Import" button of the "Models" panel of the main window or select the "Import Models" command from the under the "File" pulldown menu. Select the appropriate model file from the resulting file browser."

"1.3 Scale the imported model to the desired size. In Simplify3D, double-click either the graphical model in the main display or by double-click the listed model in the "Models" panel of the main window. This action opens a model editing panel that enables translation, rotation, and scaling of the target model. Representative models are presented for 50%, 100%, 200%, and 320% scale for all interconnecting parts."

"1.4 Duplicate models to generate an array of models as desired by selecting the "Duplicate Models" option from the "Edit" menu of Simplify3D and entering the number of model parts in the resulting dialog box. Arrange the model(s) near the center of the

build platform by clicking the "Center and Arrange" button in the "Models" panel of the main window, or by selecting the "Center and Arrange" option under the "Edit" pulldown menu. See **Figure 1** for an example printed arrangement of six C_atom_sp3 models printed with PLA (polylactic acid)."

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

We have identified and corrected deviations from the JoVE instructions for protocol numbering and display. This included correcting a missing blank space between two protocol items and a refinement of numbering categorization for specific protocol actions.

• **Protocol Highlight:** The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

For the protocol, we have highlighted the main numbered steps and taken care to ensure that the protocol presentation will be cohesive from a video recorded standpoint with an example 3D print. Such highlighting has been extended to include the main protocol section numbers/headers as recommended in the Instructions for Authors. Given JoVE's complementary manuscript + video communication. We have highlighted 3 sections of the Representative Results to emphasize the interactivity of the resulting models. While not part of the Protocol section, showing video of such interaction with resulting models will enhance the narrative by drawing connections to molecular conformer sampling and the accessible phase space of the molecules.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We have enhanced the Discussion to better draw recommended connections with the protocol.

Reviewer #1:

In the video article, the authors describe some molecular model components which can be printed by a FDM 3D printer and used to create molecular models of saturated hydrocarbons. This method is not new, since it has been reported in many other protocols and publications. Although the novelty is not mandatory for JOVE, in my opinion, the efficacy of the protocol needs to be emphasized especially in sections 3 and 4.
Looking on JOVE website, I found a video that is quite similar to the initial part of the protocol which authors would like to make (chapters 4 and 5).
https://www.jove.com/video/55427/3d-printing-of-biomolecular-models-for-research-and-pedagogy
Said this, even if the "Preparation of model files for 3D printing" section and the "Preparation of the printer for printing of parts" section will be repeated, the third part of the manuscript deals with the assembly of the model structures and the "representative results" will be focused on the visualization of the properties of the cyclohexane structure. These two aspects have not been visualized so far, and I think that a video visualization of the interconversion between cyclohexane boat and chair conformers represents a useful novelty for teaching purpose.
My only suggestion during the realization of the video is to dedicate more emphasis on 3rd and 4th section with respect to sections 1 and 2.

We appreciate the reviewer's emphasis on originality and their efforts to frame the presented work in context with existing literature efforts. We also feel the video the reviewer discovered is relevant to the current effort, which is why that particular JoVE publication was similarly discoverable by looking at the cited references our manuscript. This earlier published effort focused on creating static printable representations from PDB models of biomolecular structure files. We have taken care to focus more on the practical considerations for reliable printing of dynamic and interactive molecular structures, this with commodity 3D printers. This is likely why the reviewer recognizes the value added from the latter sections of our manuscript.

We strenuously agree with the reviewer that highlighting of a dynamic application is a critical aspect of this manuscript. JoVE is one of the few journals that directly integrates video in a scientific report, and this provides an opportunity to communicate motion in ways that are not possible with traditional scientific journals. While we were instructed by the JoVE Editorial Staff to expand on the early portion of the protocol with additional details, we have responded in a way that emphasizes the importance of the dynamical aspects of constructed 3D molecular models.

The text of the manuscript is very clear and no modification is required, I think that the manuscript can be accepted in its form. Some important references are missing:

1. J. Chem. Educ. 2015, 92, 1398-1401.
2. J. Chem. Educ. 2016, 93, 1586-1590.
3. J. Chem. Educ. 2017, 95, 88-96.
4. J. Chem. Educ. 2017, 94, 886-891.
5. J. Chem. Educ. 2017, 94, 964-969.
6. J. Chem. Educ. 2018, 95, 1607-1611
7. Chemistry Teacher International 2019: doi 10.1515/cti-2019-0005

We thank the reviewer for the suggestion of additional references. Some of these are already cited but others do expand on the scope of the field of "molecules and 3D printing". It is better to consider more efforts to highlight relevant context, so we have included many of these references in the revised manuscript.

Reviewer #2:

Manuscript Summary:
The authors present a method for 3D-printing molecular model systems with improved functionality over previously 3d-printed molecular models, in that the models can be adjusted to show various steroisomers. They can also make these models in various sizes, which could be helpful for classroom use (as you could print huge models for everyone to see). The paper is thorough and the figures are well made. The procedure may be of use to some educators who want to bring 3d-printing to their classroom and promote tactile learning strategies, or possibly a researcher who wants to display a molecule of interest. I recommend this manuscript to be published in JoVE, as I believe it would be useful for many people (teachers) unfamiliar with 3D-printing to be able to approach this project and bring it into their classroom.

We appreciate the reviewer's summary and opinion on the relevance of this work. The reviewer has highlighted a key aim of this work, helping encourage researchers and educators to make more regular use of 3D printing. As discussed in response to another reviewers comments, JoVE provides a unique pathway for aiding this effort.

Major Concerns:
Penny et al (Journal of Chemical Education (2017), 94 (9),) published on pretty much this same method, so have several others in the Journal of Chemical Education (Dean et al Journal of Chemical Education (2016), 93 (9),). (Fourches, Denis; Feducia, Jeremiah. Journal of Chemical Education (2019), 96))
This needs to be cited and discussed. Mention the benefits that these researchers found on using 3D-printed models in the classroom. compare your models to theirs, if appropriate.

We already list and include the Penny et al. work in the manuscript, and we thank the reviewer for mentioning other works that illustrate the impact that 3D printed molecular structures can have in pedagogy. We have included extra references in the manuscript introduction. To draw further distinction of these efforts from our presented work, we further adjusted wording in the discussion to highlight the dynamical and interactive qualities of the models presented in this work.

Reviewer #3:

Manuscript Summary:
The authors describe a procedure for printing 3D molecular models of hydrocarbons. They provide printer files for carbon atoms, hydrogen atoms, and bonds that can be assembled into any saturated alkane. The abstract provides a thorough description of the manuscript. The title could use a few more words describing the resulting models, since the models are limited to sp3-hybridized organic molecules and won't be useful to a reader hoping to use them to teach VSEPR theory, for example.

The authors eloquently situate this work in the historical context of molecular model-building. It is easy to think of molecular models as a tool for beginners. The reminder that they were the basis of such important breakthroughs as Pauling's work and the elucidation of the structure of DNA is worthwhile. I think there is value in pointing this out to our students. The authors are careful to acknowledge that these models are not designed to provide novel structural insight, as is appropriate.

The fact that these models can simulate the variation in barriers between different conformational changes is particularly impressive. Molecular models tend to be either static of floppy. The added tactile feedback the authors report that these models provide should be a valuable addition to the toolkit teachers can give students just beginning to develop their intuition about molecular behavior.

The manuscript lists all of the required materials. The steps are detailed and clear. I have a few suggestions for additions and changes in emphasis that I describe below.

Learning to produce useful 3D prints comes with a steep learning curve. The authors have addressed many of the most important issues that arise. They provide details about how to arrange models on the build plate (orientation can have a large effect on print quality) and what settings to use (infill percentage is a guessing game to beginners). They discuss how the models can be scaled. This is a particulary important issue that I had to learn though much trial and error because carefully calibrated gaps between snap-together parts behave differently at different scalings.

We thank the reviewer for providing this detailed summary. They are simultaneously demonstrating their expertise and highlighting aspects of the manuscript that we were hoping to effectively communicate. 3D printing is a bit of a guessing game when it comes to finding what reproducibly works, so our aim through JoVE is to potentially provide a positive starting point for beginners, as well as some shared experiences for those experienced with the struggles in achieving a quality finalized print.

Minor Concerns:
1.3.2. The authors state that rafts are not necessary at sizes above 100%. This may not be true for all printers. In my experience with a Makerbot Desktop (PLA on unheated bed), a raft is essential for all prints because the thicker layer of resin adheres to the painters tape in a way that the thinner print layers seem incapable of.

We thank the reviewer for this comment. The first layer will make or break a 3D print. We were careful to state that a raft "should not be necessary" as it isn't from our experience, though this requires a well adhered first layer. We have modified the text to emphasize that rafts are generally useful. We also emphasized "First Layer Settings" in the protocol. Excerpts from these changes are below.

"1.3.2 Activate a raft or brim structure for 100% and smaller scale models. Such structures should not be necessary for most larger models as the flat base will have sufficient contact with the bed surface to remain fixed in place. Rafts help provide a well-adhered first layer for a 3D print, so if there are any difficulties in the stability of the first printed layer at any scale, activating a raft structure could lead to more successful prints at the expense of the material needed for the raft structure."

and

"1.5.4 Set the "First Layer Speed" to a value between 25-50% in the "Layer" tab of the process settings. A slowly printed first layer will improve adhesion to the print bed and will result in more successful overall 3D prints."

1.5.6. The authors describe slicing as a separate procedure. The Makerbot software, which many viewers may be using, doesn't emphasize the distinction between slicing and "preparing to print". The authors may want to point out that some all-in-one software packages handle this step internally.

We thank the reviewer for this comment. We have pointed out this distinction as part of the Editorial staff recommendations for specific printing software actions in the protocol.

3.3.3. The authors describe an acetone dip polish, and later mention the problem that arises when dipping parts with gaps. I wonder if the authors have tried an acetone vapor polish instead. It might allow the imperfect parts to be polished. (I have only printed with PLA, and haven't tried it myself, so this just a suggestion.)

The reviewer is correct to point out vapor polishing as a strategy for finishing. It is a bit slower and comes with the same cautions as liquid acetone processing. We have modified the text to include vapor polishing cautions. The reference to it could lead a reader/viewer to explore it if it if they have the means and appropriate environment.

"CAUTION: Acetone is flammable and should be applied sparingly in a fume hood or very well-ventilated area. ABS dissolves in acetone, so parts with layer separation defects due to poor annealing should not be treated with liquid acetone. Acetone will enter models through such defects and dissolve the model infill (**Figure 3C**). Polishing with acetone vapor is a slower process that will result in a similar effect, though safety precautions should be taken given the flammability of acetone."

My last concern is regarding the included print files. Producing models that will print well and accomplish an intended goal presents a very large learning curve to a new practitioner -- much larger than learning to operate the printer. I understand that preparing models is beyond the scope of this submission, but the fact that the set of models can only be used to make saturated hydrocarbons is quite limiting. I also understand that producing models of doubly-bonded molecules that demonstrate the rotation barriers in the way these do is likely to be significantly more difficult. But, I think it would be nice to at least provide for the existence of heteroatoms. At a minimum, the authors could suggest printing carbon atoms in other colors to represent nitrogen, oxygen, and halogens. It would be a nice addition to provide models for those elements with the appropriate valence. Or, as an alternative, they could include a variation of the bond model that only connects on one end, which could be used like the "nubs" that some molecular modelling software uses to represent lone pairs.

We very much agree with the reviewer on the point of the limiting aspect of sp3 hybridized organic compounds. We noted that these parts are part of a larger kit that (yes) has different atom that are sized according to Bondi radii, hybridized structures, and dual-extruder lone-pair structures. These kits are used for demonstrations in beginning General Chemistry courses at Oklahoma State University to aid in teaching of VESPR theory, and we print expanded kits with these structures to give to lab visitors as part of our chemical outreach activities. The presentation of these details are, however, a bit outside the scope of the present study.

To accommodate the reviewer's concerns, we have expanded sections of the text to highlight the expandability of these models and how one can restrict rotation about connection points with atom model adjustments.

"NOTE: Rotation about a connected bond is a desired feature for the provided parts and models. Locking rotation requires an atom model (an sp2 hybridized carbon, for example) with a fixed structure in the connection socket that inserts between the spacings of the tabs on the end of the bond model."

"…More quantitative insight along these lines requires more specific detail than the considerations taken in building these model parts, but these models and recommendations provide a foundation for general interactive physical investigation of molecular systems. These models are an extension of 3D printable model kits we have been producing for research and outreach activities for several years prior to this report, and additional component parts that are compatible with both these models and the described protocol are available from the authors to enable more diverse bonding arrangements and dynamical action."

Reviewer #4:

Manuscript Summary:
This paper reports on the development of a protocol for creating/ assembling molecular models using individual atom/bond pieces built using 3D printers. The manuscript is well written, clear, concise, and is of relevance for Jove and probably a relatively large audience in the research/teaching community. The reviewer appreciated that all technical aspects of the 3D printing process are covered (machines, filaments, temperatures, etc.) as well as the fact that all STL files ensuring the reproducibility/usability of this protocol are available in the Supp Material. Therefore I think this paper could be accepted for publication.

Major Concerns:
None

Minor Concerns:
I would suggest the authors to add one Figure representing their protocol (pages 2-4) as a general workflow/diagram. It would be very helpful for the readers I think.

We thank the reviewer for these comments. To address their concern, we have included references that contain similar flow charts. Additionally, JoVE has an integrated video component that is presented alongside the manuscript, and a flow diagram would strengthen the presentation of this animation/video. We will work to incorporate this in the video.