Thank you for the suggestions that improved the manuscript. We revised the manuscript throughout to address the comments. We included more subdivisions and NOTES for clarity. We revised the grammar and increased the number of the subdivisions; we extended the glossary and included a new supplementary file with three figures.

Comment: Title: 3D Modeling of dendritic spines

Answer: 3D Modeling of Dendritic Spines with Synaptic Plasticity. We changed the title but maintained the synaptic plasticity.

Comment: Please reword

Answer: Computational Modeling of diffusion and reaction of chemical species in a three dimensional (3D) geometry is a fundamental method to understand the mechanisms of synaptic plasticity in dendritic spines.

Comment: Citation?
Answer: The citation has been inserted: The AMPAR synapses are located on the top of small volume structures called dendritic spines[3]

3. Buonarati, O.R., Hammes, E.A., Watson, J.F., Greger, I.H., Hell, J.W. Mechanisms of postsynaptic localization of AMPA-type glutamate receptors and their regulation during long-term potentiation. *Science Signaling*. 12 (562), doi: 10.1126/scisignal.aar6889 (2019).

Comment:

Presently the protocol sounds like a manual/tutorial which lists all the features of the software used. Please show this as a tool and how you used this to answer your specific research question. This can be done using a specific example to show how you are creating dendritic spine/s, and how is this being used to model synaptic plasticity.

Presently there is a missing link from one section to other. Please bring out clarity on this.

There is a 10 page limit for the protocol section (including headings and spacings) and there is a 2.75 page limit for the highlighted section (including headings and spacings). Presently both the limits are exceeding. Some of the shoter steps can be combined to 2-3 actions per step. Please see section 1 and 2 and reformat the other sections accordingly.
Please remove the redundancy from the protocol section.
Please define all abbreviations during the first time use. E.g., LTD, LTP, PSD, etc.

Answer:
We presented the protocol using an example as suggested. It created a link between the sections. We defined the abbreviations during the first time that they appear. We reduced the manuscript for the protocol section and reduced the highlighted section.

Comment:
Is it ok to add this here?
All these softwares are open access and does not require reprint permission to be used.
Answer:
OK. All these software are open access and does not require reprint permission to be used.

Comment:
This step can be further divided to in subsections: creating spine head, making the head flat, creating extrusions to match the dendrites etc. to bring out clarity.
Answer:
Done. We included more subsections for clarity

Comment:
This is not an action step so moved here. Also notes cannot be filmed so removed the highlights.
Answer:
OK. We removed the highlights.

Comment:
What will happen once you do this?
Answer:
We removed this sentence, since it was not necessary.

Comment:
Is this ok to include to bring out clarity?
Zoom in and out can also be performed using the scroll button of the mouse (**Figure 1C**).

Answer:
OK.

Comment:

So before this the object(dendrite spine) is created. Now you are editing it, right?
Answer:
Correct. Now I am editing it.

Comment:
How and in which step was the mesh selected previously? Why do you need to make the mesh transparent at this stage
Answer:
The mesh is automatically selected when it has been created. It needs to be transparent for better visualization of the editing parts.

Comment:
Reowrded to bring out clarity.
Answer:
OK.

Comment:
What is being done here with respect to creating the spine head? Please bring out clarity.
Answer: We included new subdivisions for clarity.

Comment:
Added here please check.
to seal the top with the vertices still selected. Move the blue arrow down to align to the top of the spine head
Answer: OK.

Comment:
What is being done here and why?
Select **Tool and Knife**. Cut a circle with the knife around the center of the top (**Figure 3B**). Select **Tool** and **Loop Cut and Slide**. Repeat this step four times to create 4 concentric circles around the center of the top (**Figure 3C**).
Answer: We added a NOTE to clarify it.

Comment:
Moved here please check. Why any citations? Please expand the PSD region.
NOTE: The top of the sphere is made flat to approach the PSD region of the spine head.

Answer: We included new references.

Comment: What is being done here with respect to dendritic spine creation?
Press *b* and select the bottom of the mesh (Figure 4C).
Answer: The creation of the spine neck. More subdivisions were added for clarity.

Comment:
Why this number?
Press *e* and *z*, *-0.45* to create an extrusion (Figure 4D).

Answer: This is the z axis position of the extrusion (-0.45). We made it clear in the text.

Comment:
What is being done here with respect to dendritic spine creation?
Press *crtl+t* to triangulate the mesh.
Answer: We clarified it in the text. It is making the mesh compatible with MCell.

Comment:
When did you create duplicates ?
Answer: Doubles can be created accidentally during the process of mesh editing. It means the creation of superposed vertices. We included a NOTE for clarity.

Comment:
Please use imperative tense if this step needs to be filmed. If not this can be converted to a note instead.
2.36 The analysis will print the information in the **Mesh Analysis Panel** and it should be **watertight**, **manifold** and **outward-facing normals**. This step is required to ensure that the created mesh will work on MCell. Otherwise, probably there is a missed a step. In this case, *delete* the mesh and start from step 2.1 again.

Answer: It has been converted to NOTE.

Comment:

Again this section can be divided into creating multiple spines (refer to the top section for this), creating ectrusions for each, joining each to create a dendrite with multiple spines, etc to bring out clarity for the first time readers.
3. Creating a Dendrite with Multiple Spines.

Answer:

We added new sections for clarity.

Comment:

Added here to bring out clarity.

3.1 Generate spine head as described previously in step 2.1-2.4. Press *a* to deselect the spine. Type *shift+c* to centralize the cursor.

Answer:

OK.

Comment:

How did you come up with tis radius and depth.

3.2. Press *shift+a* to open the mesh palette. Select **Mesh** and then **Cylinder**. Change the parameters on the **Add Cylinder** menu: **Radius** *0.3*, **Depth** *2*. Press *enter*.

Answer:

Those are suggested general values for an ordinary dendrite compartment. We included a NOTE for clarity.

Comment:

Where di you type this?

Type *r* and type *90* to rotate the cylinder 90° (Figure 5A). Use the blue arrow to drag the cylinder down to the bottom of the spine. Press *3* on the keypad to have a front view of the cylinder.

Answer: We meant press instead of type. R is the short for rotate and 90 rotates the mesh 90 degrees. I included the shortcut in the glossary. I changed to press r. After that one need to type a number that is 90 in this case.

Comment:

What is being done here and why? Needs clarity.

3.14 Select **Object spine**.

Answer:

Those are steps to insert a spine in the dendrite. I have created a subtitle to indicate it.

Comment:

3.16 Press *a* to deselect the meshes.

From here on to the end of the protocol, please bring clarity as to what is being done and why. Please refer to my previous comments and work accordingly

Answer:

Done.

Comment:

Please move this note right after the step whis action is being performed.
NOTE: In step 3.15, the new mesh will be the join dendrite and spine. The isolated dendrite disappears when the different meshes are combined, but the isolated spine remains overlapping with the new mesh and is used to generate multiple copies of the same spine. Delete all the isolated spines after finishing the mesh. It is critical to have a complete overlap between the spine neck and the dendrite; otherwise, the mesh will not be watertight.

Answer: We moved it.

Comment:
Press *tab* to change to **edit mode**.

The previous section ends with save the file. Before this step please include a step to show that you reopen the file dendrite_with_spines in the xxx software to perform these actions.

Answer: Done.

Comment:
Press *a* to select the whole mesh.
Of what? Where?

Answer:
Of the dendrite with spines. We clarified it.

Comment:
4.4 Press *t* to hide the **CellBlender panel** and visualize the whole mesh in detail.

Where is the whole mesh?

Answer:
The whole mesh is in the main panel. We clarified it.

Comment:
Use + and – on the keypad to zoom in and out or scroll with the mouse.

Why do you need to do this at this stage?
Answer:
It is required to visualize the surface regions.

Comment:
Why this? Does this have any relationship to AMPAR? Citations if any?
Answer:
It is the creation of the AMPARs. This is the diffusion constant of AMPARs in the membrane. We included a citation for the value (Antunes et al., 2019).

Comment:
Citation to support this value?
Answer:
We included a citation for the value (Antunes et al., 2019).

Comment:
5.1, 5.2, 5.3 are same. Please bring out significance
Answer:
They are all part of the procedure of creating molecules. We included more subdivisions for clarity.

Comment:
What is anchor_LTP? Somewhere in the introduction, please define with citations what are anchors, how these are bound to AMPARs, what are anchor_LTP, anchor LTD, etc. why do you need to create all these. This will bring out clarity in the protocol.

Answer:
We included an explanation in the introduction.

Comment:
Please refer to my comments above and reformat accordingly to bring out clarity.

Answer: We did it.

Comment:

Please refer to my comments above and reformat accordingly to bring out clarity

Answer:
Done. We created more subtitles for clarity.

Comment:
Please refer to my comments above and reformat accordingly to bring out clarity.

Answer: Done.

Comment:
Please refer to my comments above and reformat accordingly to bring out clarity.
Answer: Done. We included more subdivisions and NOTES to address it.

Comment:
How do you generate graphs presented in this figure 8 and how do you link it to synaptic plasticity. This is still not clearly explained.

Answer:
We included more Notes to explain it in the protocol.

Comment:
Legend of Figure 8.
How is this measured? Please explain and detail these observations somewhere in the result.

Answer:
It is measured by the observation of the opposite effect at the neighbor spines of the homosynaptic plasticity at a single spine. For instance, homosynaptic LTP induction at a single spine will create a heterosynaptic LTD effect at the neighbor spines. We included this information in the results.