Peer-Review
Viswanathan, Anya. 2025. “Identifying High Efficacy Potential Targets in Alzheimer’s Disease by a Mechanistic Comparison with Prion Diseases.” *Journal of High School Science* 9 (4): 129–51. https://doi.org/10.64336/001c.146596

This is a well written paper that appears on the cusp of answering the obvious question - so what? -, but never answers it. I would like to see this review attempt to answer this question. What are the implications of these diseases being similar ? Is this just semantics or can we envision real alternative (or even subtly different) methods of treatment for AD based on its similarities with PD? AD has been proposed to be a ‘double’ PD. Should we focus treatment on just one; either amyloidbeta or hyperphostau? Which is more similar in structure/damage causing pathways to PrPc ? Why ?

Why is PrPC infectious but not AD (although see below). Does the premise of being more infectious equate to greater pharmacological futility? If yes, how can we make AD less infectious ? Change aggregation pathway with small molecule ligands to soluble precursors? Instead of inhibiting BASE outright, should we make subtle changes to its catalytic or allostearic sites to redirect the aggregation pathway?

PrPc is anti-apoptotic but amyloid beta is pro-apoptotic (extremely generalized). Is there a way to make amyloid beta anti-apoptotic ? How ? By changing its structure ? (see above) Implications? “No form of PrP fulfills Koch’s postulates for infection”. The evidence that PrP is a result of virus or gum/gut baterial infection has been largely neglected. Assuming that a virus is implicated in the causative chain of misfolded proteins (protective neuroinflammation), can research identify the bacteria/viruses that scrappie or encephalopathy animals were exposed to more often than their healthy counterparts? Can vaccines be made and tested in animals, and then eventually in humans? Should individuals who have had serious infections be prescribed NSAIDS for life to prevent late onset neuroinflammation and AD ?

Do PrP and amyloid beta/hyperphostau have similar intrinsic disordered regions (IDRs) in their quaternary structures? And if so, if PrP aggregation can be inhibited by LLPS to fibrils inhibition, then so can beta-amyloid.

Please include another section called “implications” or something similar. Including this content will add significant value to your manuscript.

References
https://doi.org/10.1038/s41591-023-02729-2
https://doi.org/10.1002/jcb.21090
https://doi.org/10.1016/j.neuron.2022.12.029
https://doi.org/10.1016/j.jbc.2024.107310

In my previous manuscript, I mainly focused on cross-examining prion diseases and Alzheimer’s, and answering whether Alzheimer’s should or shouldn’t be considered a prion disease. However, in my revisions, I tried to steer the manuscript to answer the question “So what?”.

To give a brief summary of my changes, I first focused on tweaking the cross examination of prion diseases and Alzheimer’s disease, to not only give information but to contextualize what similarities and differences are important to note and why. This is mainly seen in how I frame the differences in seeding behavior for each protein in the cross-examination. It is also shown in the ‘Implications’

section, where I detail how the seeding behavior has led many to jump to the conclusion that Alzheimer's is a prion disease, and which protein is more similar to PrPsc in seeding behavior. Furthermore, when I reached the conclusion that the seeding behavior was too different between each disease to become a common therapeutic target—at least with respect to amyloid-β and PrPsc—I did more research on other commonalities that could be exploited for a common therapeutic approach. This led me to focus on the intracellular interactions of amyloid-β, hyperphosphorylated tau, and PrPsc. Common usage of caspase activation pathways, ER stress, oxidative stress, and the role of the Bcl protein family were all explored and discussed at length as part of edits to the 'Mechanisms of Neurodegeneration' section and the end of the 'Implications' section, which talks at length about therapies used only for prion or Alzheimer's involving these elements that could be applied to both diseases.

In specific as to where one can find my edits, the main additions in content are found in the second paragraph of the 'Protein Seeding and Structure' section, the second and third paragraphs of the 'Mechanisms of Neurodegeneration' section, and the sixth, seventh, eighth, ninth, tenth, and eleventh paragraphs of the 'Implications' section. My 'Implications' section also includes what used to be called the 'Changed Understanding and Diagnosis' section in my old manuscript, and I cut the small mention of PrPc being active in Alzheimer's Disease because I didn't find that added much to the manuscript in terms of finding common therapeutics, since PrPc in Alzheimer's acts differently than it does/how its isoforms act in prion diseases. Myconclusion and abstract were also edited to better fit the new manuscript and reflect a more refined and targeted conclusion from this literature review.

In making these edits, I hope to better steer the reader in how they can use this information to inform their framework of thinking when it comes to Alzheimer's, prion diseases, and their overlap. These similarities and endless debates often seem like semantics because there isn't always much context as to how to exploit the similarities and differences, but I truly do think that understanding the comparisons between these diseases can lead to real change, and I hope my manuscript shows that, as well.

Please list each comment/concern by the reviewer verbatim, followed by your response to that comment/concern (how and where you have addressed that concern in the manuscript). Submit this as a separate file.

This is a well written paper that appears on the cusp of answering the obvious question - so what? -, but never answers it. I would like to see this review attempt to answer this question. What are the implications of these diseases being similar ? Is this just semantics or can we envision real alternative (or even subtly different) methods of treatment for AD based on its similarities with PD?"

Response in manuscript:
I treated the "so-what" as my main guiding question when editing this paper. The biggest change I made was adding an entire 'Implications' section in my paper, mostly talking about different methods for treatment of Alzheimer's and prion diseases based on their similarities to each other (I also changed my focus to understanding how Alzheimer's might help develop better treatments for prion diseases as well, which you see reflected in my introduction and abstract!) Furthermore, I made pointed modifications in the earlier cross examinations of prion diseases and Alzheimer's Disease, especially in how I describe the protein seeding in the 'Protein Structure and Seeding' section and the inclusions of intracellular interactions of each protein that lead to neurodegeneration

in the ‘Mechanisms of Neurodegeneration’ section to sharpen this literature review to a point – that common treatments for Alzheimer’s and prion diseases need to be looked at form the point of view of intracellular mechanisms leading to apoptosis rather than seeding and aggregation based therapies. The questions and answers below should give more specific examples on how I modified this paper, but I do think that in general it has become more shaped to have an actual perspective that is unique and looks at the information from a specific lens rather than just putting out information with little synthesis.

Question/Concern #2: “AD has been proposed to be a ‘double’ PD. Should wefocus treatment on just one; either amyloidbeta or hyperphostau? Which is moresimilar in structure/damage causing pathways to PrPc ? Why ?”

Response in manuscript:
Both questions, “Should we focus treatment on just one: either amyloidbeta or hyperphostau?”, and “Which is more similar in structure/damage causing pathways to PrPc? Why?” are primarily addressed in the 5th and 6th paragraphs of the Implications section. In short, I argue that tau is more similar to PrPsc in how they utilize seeding to convert benign proteins and incite damage. Thus, any anti-aggregation treatments developed for tau or PrPsc should be applied to the other, as opposed to being applied to amyloid beta. However, amyloid beta and PrPsc share many commonalities in their intracellular interactions which incite apoptosis. Moreover, amyloid beta creation, aggregation, and intracellular interactions are upstream of tau phosphorylation. Thus, treatment should primarily focus on amyloid beta because it could prevent tau hyperphosphorylation, and because it’s more similar to PrPsc in how it causes apoptosis – which many scientists are starting to gravitate towards as the main source of neuronal loss.

Question/Concern #3: “Why is PrPC infectious but not AD (although see below). Does the premise of being more infectious equate to greater pharmacological futility? If yes, how can we make AD less infectious ? Change aggregation pathway with small molecule ligands to soluble precursors? Instead of inhibitingBASE outright, should we make subtle changes to its catalytic or allostearic sites to redirect the aggregation pathway?”

Response in manuscript:
The questions “Why is PrPC infectious but not AD?” and “Does the premise of being more infectious equate to greater pharmacological futility? If yes, how can we make AD less infectious?” are responded to in the 10th paragraph of the Implications section. In short summary, prion diseases are considered infectious because of how transmissible prions are between animals, where they spread rapidly through bodily fluids and close contact, which give rise to more strains and mutations that could potentially end up infecting humans. Acquired prion diseases in humans are somewhat rare, but definitely have more occurrence than acquired Alzheimer’s, which has only been recorded to happen once and to very few people compared to how many were exposed. Furthermore, because prion diseases are both human and animal diseases while Alzheimer’s only occurs in humans, there is little to no propensity of Alzheimer’s strains popping up and gaining greater transmissibility or potential for driving neurodegeneration through mutations. Furthermore, given that more infectious potential can often lead to more strains, treating prion diseases or vaccinating for scrapie prion can be incredibly difficult. Unlike a regular viral infection, prions aren’t treated through anti-virals but through competitive inhibitors that either block PrPc from binding with strains of scrapie prion or block the scrapie prion strains from binding with PrPc – since prions are native to the body and hard to seek out as a foreign body. Thus, the larger number of strains has made prion vaccine production very hard in terms of blocking all the possible different types of scrapie prion thatcould bind to PrPc. Moreover, most labs have pivoted to inhibit the binding sites on the native PrPc, but even that is hard because each person’s PrPc is slightly

different. Since Alzheimer's doesn't seem to have propensity of developing new strains, there's only ever been one incident of it spreading through a medical procedure – which has been long discontinued – and amyloid beta seeds don't actually seem to contribute that much to neurodegeneration as general production of amyloid beta monomers, making Alzheimer's 'less infectious' hasn't been a concern of most researchers tackling Alzheimer's treatment. In regards to the questions, "... how can we make AD less infectious ? Change aggregation pathway with small molecule ligands to soluble precursors? Instead of inhibiting BASE outright, should we make subtle changes to its catalytic or allostearic sites to redirect the aggregation pathway?": as mentioned previously, making Alzheimer's 'less infectious' hasn't been a primary concern of most researching Alzheimer's because of its low propensity to jump from organism to organism or form strains. However, in the context of a protein 'infecting' another protein – or in this case, driving soluble monomers to form aggregates – there have been efforts to remove amyloid aggregate seeds before they spur too much excess aggregation with native soluble amyloid beta monomers. Notably, the Alzheimer's vaccine, as I talk about in paragraph 10 of the Implications section as well, uses the peripheral sink method to flush out aggregates through the blood in efforts to lessen the development of amyloid beta aggregates and to keep the soluble monomers as soluble monomers. However, it is worth noting that even this hasn't proven to be enough in clinical trials to fully reverse or prevent neurodegeneration in Alzheimer's, because the soluble monomers and oligomers have been proven to still exert neurotoxic effects. Thus, treating Alzheimer's is likely less of a question of making it less infection/minimizing interaction between amyloid beta seeds and monomers like in prion diseases, and more of a question of preventing apoptosis and excessive action from the amyloid beta monomers in the brain.

Question/Concern #4: "PrPc is anti-apoptotic but amyloid beta is pro-apoptotic (extremely generalized). Is there a way to make amyloid beta anti-apoptotic ? How ? By changing its structure ? (see above) Implications?"

Response in Manuscript:
In the 9th paragraph of my Implications section, I address amyloid-beta's pro-apoptotic effects primarily through the lens of how its interactions can often 'set off' apoptotic pathways, and how inhibiting its interactions, such as the one it has with PrPc, could mitigate or completely remove amyloid beta's apoptotic effects, essentially stripping it of its "pro-apoptotic"-ness. I primarily talk about inhibiting interactions through allosteric and competitive inhibitors on amyloid beta or whatever it's binding to rather than changing the structure entirely for two reasons: I wanted to stay true to my point that the intracellular mechanisms between amyloid-beta, like PrPsc, and apoptotic pathways should be targeted more than aggregation and interactions between amyloid-beta monomers/oligomers; I think that it would be practically easier to apply specific inhibitors than to try to change the structure of amyloid-beta without any repercussions, especially in vivo. That isn't to say there aren't issues with inhibitors, as the exact makeup of a binding site often differs, especially on PrPc, between people and can make developing a high-affinity inhibitor hard (I detail a lot of this in the 10th paragraph, when I talk about the PrPsc/PrPc binding siteinhibitors in the prion vaccine). However, I do think it's more realistic and less likely to propagate unforeseen consequences like a new protein structure could.

Question/Concern 5#: "'No form of PrP fulfills Koch's postulates for infection'. Theevidence that PrP is a result of virus or gum/gut baterial infection has been largely neglected. Assuming that a virus is implicated in the causative chain of misfolded proteins (protective neuroinflammation), can research identify the bacteria/viruses that scrappie or encephalopathy animals were exposed to more often than their healthy counterparts? Can vaccines be made and tested in animals, and then eventually in humans? Should individuals who have had serious infections be prescribed NSAIDS for life to prevent late onset neuroinflammation and AD ?"

Response in Manuscript:
The questions and challenges to the Prion Hypothesis are incredibly important to explore in order to understand how exactly prions work. I address the skepticism about the Prion hypothesis and the attempts to prove Koch's postulates for infection in the second paragraph of the Pathology section. In this section, I also address whether we can track down the bacteria or viruses animals with encephalopathy are exposed to by talking about literature reviews and analyses that show a great amount of evidence supporting that prion proteins and PrP is actually a relic of peptide evolution rather than stemming from viruses or being part of a virino, which is a virus that would use prion proteins like an agent of its own agenda. Thus, I talk about the vaccines being used to tackle prion diseasesas a proteinaceous disease driving agent rather than a virus in the 9th paragraph of the Implications section, in which I detail specific challenges the vaccine itself is having with tackling the disease such as mutating strains and an overwhelming immune reaction – something that's harder to modulate in a protein-targeted vaccine than one targeting a virus because the protein is native and mounting an immune reaction against it runs a higher risk of being too extremely little to actually destroy a native body or too much to destroy useful cells.
Even though I assert that prions haven't been shown to come from viruses, I do talk about NSAID usage both in Alzheimer's and in prion diseases when it comes to lessening the neuroinflammation. This is in the 11th paragraph of the implications section, where I basically talk about how NSAID usage in prion diseases hasn't really been tested beyond in vitro studies, in which NSAIDs have been proven to be somewhat useless for preventing extensive neurodegeneration or neuroinflammation as the microglia don't seem to be dependent on prostaglandin signaling. However, prolonged NSAID usage before Alzheimer's diagnosis and throughout treatment has been shown to modestly reduce the risk of extensive neuroinflammation and even lessen the risk of developing Alzheimer's altogether. In this way, the longer progression time of Alzheimer's is a blessing as it gives time for long-term interventions to actually work before the disease progresses too much. Conversely, short-term or intermediate-term usage of NSAIDs are often found to have no effect or a modestly harmful effect on Alzheimer's progression.

Question 6#: Do PrP and amyloid beta/hyperphostau have similar intrinsic disordered regions (IDRs) in their quaternary structures? And if so, if PrP aggregation can be inhibited by LLPS to fibrils inhibition, then so can beta-amyloid.

Response in Manuscript:
I address this question briefly at the top of the 6th paragraph in my Implications section, in which I state that there are no similar intrinsic disordered regions between amyloid beta, hyperphosphorylated tau, and PrPsc. While they all form beta sheets, there are no similar IDRs that have been found in the countless cross examinations of aggregation and general structure of each protein, which means that LLPS likely cannot inhibit aggregation for amyloid beta. However, I do talk about other anti-fibrillization therapies in the last paragraph of my Implications section. There have been effective anti-fibrillization therapies for Alzheimer's primarily targeting the chaperones in amyloid beta aggregation which efficiently and effectively break the aggregation down. While this may not be extremely beneficial for treating Alzheimer's, as anti-aggregation techniques have not yielded more than modest results for changing disease progression, the same concept could be applied to prion aggregate structures. Since his Prion Hypothesis came out, Dr. Stanley Prusiner has been saying that there is likely some sort of chaperone he calls "Protein X" that is helping PrPsc bind together and stay together as an aggregate. There have been ongoing studies trying to identify what this chaperone could be, and in the case that they find said chaperone, such anti-fibrillization therapies targeting the chaperone could be applied in some sort of way to the prion structures.

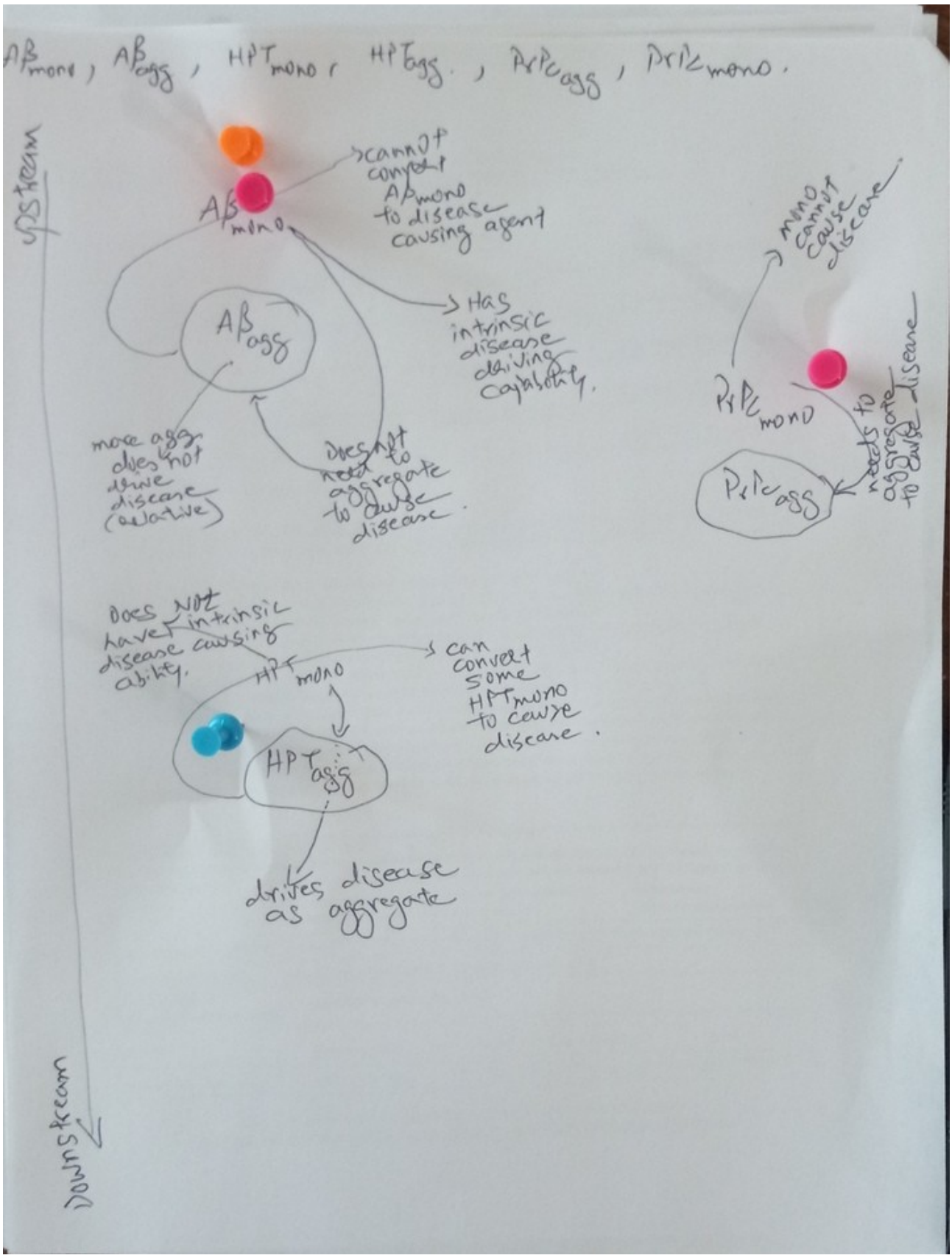
Thank you for addressing my comments. You are obviously knowledgeable about this topic. I hope researchers such as yourself can contain this disease, especially for persons like myself who have the APOE4 mutation. My further comments have to do with formatting.

1.Please review the attached .pdf. I have highlighted text in your Implications section, which I would like you to include in your Conclusion section (as well as in the abstract, after appropriate abbreviation). I have also attached an image which may help the manuscript readers to better understand your argument (you will need to biorender it).

2.Please remove ALL footnotes and instead, include them as References in the References section of the manuscript. Need to be APA style. Please also format the reference numbers in the text (not superscripted) but as in curved brackets.

When you resubmit, please also submit a word doc of your manuscript, in Times New Roman format, 12 font, single column, appropriately formatted for the Journal.
I look forward to accepting your manuscript once you have incorporated these changes.

REVIEW CONTINUES ON NEXT PAGE



Edits and Revisions

The biggest focus from an author standpoint of these edits and revisions was to make the argument clearer from start to end!

Feedback given: “Please review the attached .pdf. I have highlighted text in your Implications section, which I would like you to include in your Conclusion section (as well as in the abstract, after appropriate abbreviation). I have also attached an image which may help the manuscript readers to better understand your argument (you will need to biorender it).

Changes made: The largest change in the manuscript that came from this feedback was the graphic I made in the very end of my article (right before the acknowledgements section), which summarized most of the important information regarding hyperphosphorylated tau behavior, amyloid-β behavior, and PrPc/PrPsc behavior, allowing a much more direct compare and contrast in how their seeding works against each other and how each individual part, including monomers causes disease at what time in the progression of a disease. I also changed my conclusion to include a summary of the crucial information regarding each protein’s behavior to support my core arguments of applying common therapies to the intracellular pro-apoptotic mechanisms between PrPsc and amyloid-β, applying common anti-aggregation therapies to hyperphosphorylated tau and PrPsc, and opting for a competitive or allosteric inhibitor to stop PrPc/amyloid-β binding rather than eradicating PrPc, a therapy that could eventually be modified to stop or hinder some PrPc/PrPsc binding in prion diseases. This information was all mentioned, in slightly less depth for conciseness, in my abstract as well.

Feedback given: “Please remove ALL footnotes and instead, include them as References in the References section of the manuscript. Need to be APA style. Please also format the reference numbers in the text (not superscripted) but as in curved brackets.”

Changes made: In response to this feedback, I went and changed every single citation to APA format and made my references in APA style as well. I cross referenced my reference and citation style with an existing literature review published by the Journal of High School Science to have a visual aid and ensure that my references and citations were in the correct format. I also changed the format of my paper to be in Times New Roman, 12 pt font, and single column to fit other stylistic guidelines of the journal.

Thank you for addressing my comments. Accepted. However, we use a numbered citation system, which means that references must be numbered sequentially in the text, with the corresponding list appearing in the references section of the manuscript. Please resubmit the word doc after these corrections. Also, references need to be in (curved) parentheses separated by commas such as (2,3).