Author's response to reviews

Title: Modeling of chemical inhibition from amyloid protein aggregation kinetics.

Authors:

Jose A. Vázquez (jvazquez@iim.csic.es)

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Author's response to reviews:

Revision notes (response to reviewers)

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Manuscript title: Modeling of chemical inhibition from amyloid protein aggregation kinetics.

Dr. José Antonio Vázquez, Corresponding author

The referee’s comments are included before. The response to the comments of the referees is written in red.

Reviewers’ comments

Reviewer: Carlos W Bertoncini

Reviewer’s report:

The paper by Vazquez addresses a relevant and current topic in modern anti-amyloid drug discovery, which is the efficient characterization of the inhibitory action of an anti-amyloid compound. By clever combination of Logistic and Weibull equations Vazquez develops an analytical model for the accurate description of the effect of various anti-amyloid compounds on several amyloidogenic proteins and peptides. The surface-type representation is quite useful for the evaluation of the concentration dependence of the inhibitory effect of these compounds on the kinetics of amyloid fibrillization. The paper builds correctly on previous work published on the subject, and the use of previously published data is well acknowledged.

The manuscript is well written and describes precisely the mathematical methods, some of which unfortunately escape the ability of this reviewer. Hence no consideration has been made on the derivation of the equations, which current programs such as Mathematica may check automatically.

Overall the impression of this reviewer is that the paper is a useful tool that would allow a considerable increment in the knowledge of the mechanisms by which different anti-amyloid compounds work. This may aid the future development of anti-amyloid drugs. This reviewer has, however a few concerns that if addressed properly may help to strengthen the applicability of the method.
Minor Essential Revisions:

a) Data analyzed in the manuscript arises from two methods generally used to probe amyloid formation in vitro, the increment in light scattering of the protein solution due to insolubilization, and the increase in ThT fluorescence due to amyloid binding. These two methods report on quite distinct phenomena; while the first is sensitive to the early steps in protein aggregation and does not differentiates between amorphous and amyloid aggregation, the second is insensitive to only amyloid species. In addition, light scattering usually saturates quite early in the reaction coordinate, while ThT fluorescence takes several hours and even days to saturate. It could be good to discuss these features a little more, and perhaps find some datasets that compare these two methods head to head.

Response: Two little sentences (in M&M and Conclusion sections) have been now inserted according to your comment. On the other hand, I have revised again the literature but I have not found a complete datasets (with enough data of chemicals concentrations and times of reaction) that compare both methods. If you know those datasets (comparing head to head both methods) they could be evaluated by the model proposed.

b) A serious drawback in aggregation kinetics is the intrinsic variability of the experimental data that demands the usage of several replicates. In drug discovery platforms these assays are run in plate readers, with at least triplicates or quintuplicates of the samples. It could be good to know how the analytical method can be implemented efficiently in the case of global fittings.

Response: Among the nine datasets selected for the present work only in three cases the error bars are shown. We did not show them to avoid confusion between points, bars and lines in the theoretical response surfaces. The global fittings of datasets were done with the mean of the experimental values and not with the replicates. Such replicates are a measure of the error associated to the experimental analysis of samples and the methods of determining protein aggregation. Our modelling and numerical analysis are implemented for the global fittings of concentration-time data using experimental mean values but not for the sum of replicates (any approach uses them for modelling).

c) This reviewer is concerned with the implementation of the method. How does the author propose to distribute this body of work and make it accessible to the scientific community? Could a MS EXCEL plugin/spreadsheet or a web-based server be useful? Would any of the data be distributed?

Response: The proposed model is not difficult to write in Excel, Mathematica, Matlab, Datafit, Statistica or any other mathematical software. Below you can see a screenshot of the Excel employees in the present work. Honestly, I had not though about Excel spreadsheet distribution but if other authors are interested I can provide it.
Review: Modeling of chemical inhibition from amyloid protein aggregation kinetics

1. Is the question posed by the authors well defined? Yes it is well stated and an important question.
2. Are the methods appropriate and well described? Yes.
3. Are the data sound? Yes, the data is taken from previously published works.
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes.
5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes, for the most part they are. I also make some comments about this below.
6. Are limitations of the work clearly stated? No. This is not clearly mentioned in the discussion.
7. Do the authors clearly acknowledge any work up on which they are building, both published and unpublished? Yes.
8. Do the title and abstract accurately convey what has been found? Yes.
9. Is the writing acceptable? Yes. There are a few minor errors which I point out below.

Specific Comments
• Compulsory Revisions:
  1. There is no discussion about previous attempts to fit the data.
  Since the experimental data are taken from the literature, I imagine there must have been some attempts to conduct some analysis on them.
  Response: No attempts to fit the data were needed for the present work. I had previous experience about the ability of proposed equation to model similar
profiles of chemical inhibition on bacterial and microalgae growths. Some datasets of protein fibrillation observed in the literature followed the same trends when they are affected by chemicals. Thus, after reviewing the literature looking for datasets that had a sufficient number of concentrations and times for statistical viability (enough degrees of freedom, complete sigmoid profiles), only nine cases were valid and therefore selected for modeling. Those experimental data were directly evaluated by bivariate equation (without any modification) and the results are shown in the present document.

2. It is essential to include discuss the possible short comings of this work.
Response: We have now included a paragraph about short comings:

“The bivariate equation was validated using data obtained by two methods based on different chemical phenomena. Although this model can not define the mechanisms of action of chemical inhibitors on protein aggregation, it provides a consistent tool for the comparison of the ability of such compounds in the inhibition of the protein fibrillation process, regardless of the method used for its determination, and is a first step for the optimization of the in vitro application of them. Further experiments and corresponding modeling should be done to establish its validity for in vivo applications of anti-aggregation chemicals.”

• Minor Essential Revisions:
1. Page 3, line 76-80: This sentence is far too long and unclear. This sentence needs to be restated.
Response: This sentence was re-written as follows:

“So remarkable, two new and similar proposal: “Ockham's razor”/minimalistic and Crystallization-like Model, have been recently developed. Both have solid biophysical basis and they were successfully applied to describe and explain the experimental data of different amyloid protein aggregation [25-28].”

2. Page 4, line 85: ‘underlying in’ should be changed to ‘underlie’.
Response: Modified.

3. Page 4, line 87: What does the author mean by the term ‘geometrical’?
Response: Geometrical was deleted to avoid confusion.

4. Page 4, line s 89-91: this sentence is grammatically incorrect. Please state this sentence simply. Page 4: line 93: not sure what is meant by ‘capacity of #t’.
Response: They were rewritten as follows:

“Nonetheless, that equation is always formulated without the parameters (fibrillation rate and lag phase) in an explicit form hindering the estimation of their statistical error.”

Apologise for the mistake, “capacity of fit” was changed by:

“…the capability of fit and experimental data predictability…”
The model developed to simulate the process of aggregation and hence insulin fibrillation was defined by a bivariate equation. Such model is based on the combination of Weibull function as chemical-concentration model [35,36] modifying the most important parameters of the reparameterized logistic equation [37] used for aggregation description.

What does the author mean by ‘flexible’ equation?

Flexible was deleted to avoid confusion.

Move these lines right after the equation. It is best to define the parameters right after the equation.

These lines were moved following your comment.

What does the author mean by the ‘overall aggregation process rate’? Even though this is shown in figure 1, it needs to be stated clearly whether the slope is being averaged between the entire second phase of the aggregation graph or in the ‘linear’ portion of this phase.

‘Overall aggregation process rate’ was deleted to avoid confusion.

The labels #m#, Xm#, vm# need to be clarified.

They have been improved as follows:

“Right, Simulations of the most common profiles for the parameters (Xm#, v m#), affected by chemical concentration, using the Weibull equations (A.15).”

Reviewer's report

Title: Modeling of chemical inhibition from amyloid protein aggregation kinetics.

Review: Bertrand MOREL

Reviewer's report: Modeling of chemical inhibition from amyloid protein aggregation kinetics. Jose A. Vazquez

BMC Pharmacology and Toxicology Research article

1. Is the question posed by the authors well defined? Yes
2. Are the methods appropriate and well described? Yes
3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes
In this work, the author describes a mathematical model to predict chemical inhibition from amyloid protein aggregation kinetics. Protein aggregation has been commonly associated with numerous degenerative disorders and also biotechnological applications. For this reason the development of theoretical models for data interpretation becomes important in this investigation field. Here, the author presents an accurate and effective model to investigate the inhibition of chemicals on amyloid protein aggregation and applied this methodology to experimental results already published. This model is based on a general bivariate model that combines the logistic equation for the description of kinetics and the Weibull equation for the chemical concentration effect. The manuscript is clearly written and the simulations robustly carried out. I strongly recommend this manuscript for publication in “BMC pharmacology and toxicology”.

However, there are some minor points to be changed.

Minor Essential Revisions:

1) In Background, Page 3 line 66: The author states “Human A# 42 amyloid protein is a peptide of 39-42 residues and the major isoform…”. I would rather say that Human amyloid peptides (A#) are peptides of rather 39-42 residues. A#42 is a 42 residues polypeptide chain and A#40 contains 40 amino acids.

Response: We have modified the text according with your comment:

“Human amyloid proteins (A#) are peptides of rather 39-42 residues. A#40 contains 40 amino acids and A#42 is the major isoform in the A##peptides with 42 residues polypeptide chain and it is the responsible of amyloid plaques generated in Alzheimer´s disorder [15,16].”


Response: It was added as reference 22.


Response: It was added as reference 23.

4) In “Inhibitory effect of di-C7-PC and methylglyoxal on insulin aggregation” page 11 line 264; “…but a much longer time was required to obtain this parametric value (133 h)”. In Table 3, the actual value is 150.68 hours.

Response: I agree with your comment, the correct value is 150.68 h. The error in the text was amended.
5) Some references are not in the correct format. Specially the abbreviated journal titles. This should be checked and corrected.
Response: References have been revised and corrected.

6) In figure captions, page 23 line 619, Figure 3. Write “Insulin fibrillation kinetics…” and not “Insuline Fibrillation kinetics….”
Response: Apologies for the misprint, it was corrected.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.