

Science\_comments.txt

Date: July 22, 2010 3:47:19 PM EDT  
To: <harrison@crystal.harvard.edu>  
Subject: Decision on your Science Manuscript \*\*\*\*\*

22 July 2010

Dr. Stephen C. Harrison  
Children's Hospital Boston  
3 Blackfan Circle  
Boston MA 02115  
USA

Dear Dr. Harrison:

Manuscript number: \*\*\*\*\*

Thank you for submitting your manuscript "Atomic model of an infectious rotavirus particle" to Science. We have now received the detailed reviews of your paper. Unfortunately they are not positive enough to support publication of the paper in Science. Although we recognize that you could likely address many of these specific criticisms in a revised manuscript, the overall nature of the reviews is such that the paper would not be able to compete for our limited space. We hope that you find the comments helpful in preparing the manuscript for submission to another journal.

We are grateful that you gave Science the opportunity to consider your work.

Sincerely,

Senior Editor

MSID:\*\*\*\*\*

Title Atomic model of an infectious rotavirus particle

Author Name:

Settembre, Ethan C

## Review

In this manuscript, Settembre et al. report a near-atomic resolution structure of rotavirus strain RRV determined by cryoEM and single-particle image analysis. The structure allows each of the three VP4 monomers to be unambiguously placed in the structure and resolves a controversy in the field about the arrangement of VP4 molecules in rotavirus particles. This new structural information allowed the authors to formulate a more complete model of the conformational changes in VP4 required for membrane penetration by rotavirus. Most remarkable is the 180 degree rotation of the VP4 beta-barrel domain from the pre-penetration to post-penetration conformer of the protein. The structure reported in this study now sets the stage for hypothesis-driven queries to precisely define the biophysical mechanisms by which rotavirus penetrates cell membranes.

I offer a few specific comments for the authors' consideration:

1)  The sentence in paragraph five that begins, "Second, the beta-barrel domain..." reports a key finding of the study and should be unpacked to improve clarity.

2)  Is it known whether VP4 forms trimers prior to assembly onto DLPs? Does the structure suggest that VP4 trimerization can only occur during assembly? Is it possible that complexes of VP4 and VP7 might form prior to assembly onto DLPs?

3)  Why might the particular asymmetric conformer of VP4 observed in the cryoEM maps be observed? In other words, why should this particular arrangement of VP4 molecules be captured in the structure?

4)  The authors indicate that the density for the lectin domain derived from the C chain is missing. Does the high-resolution structural information gathered in this study provide a clue about how portions from only one subunit of the trimer are lost by trypsin-mediated cleavage? Could this finding be related to the conditions used for preparation of the virus particles for cryoEM (e.g., the amount of trypsin used to digest the particles)?

5)  Is it thought that virion exposure to alkaline pH is in some way physiologically linked to the conformational alterations in VP4 required for membrane penetration? Or is alkaline pH used to perturb the particle in a way to mimic conformational changes that are triggered under different (presumably physiologic) circumstances?

6)  It would be helpful to show in Figure 4C both models of membrane penetration by VP4. The distinction between the two models in the text is not entirely clear.

7)  The study would be enhanced by at least one set of functional experiments to discriminate between the two proposed models of VP4-mediated membrane penetration.

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## Review

This paper reports a nice structural study of rhesus rotavirus by electron cryomicroscopy at a nominal resolution of 3.8 Å. The focus is on the structure of the VP4 spike that binds to the host cell during infection. In the virion, as has long been known, VP4 has an unusual mixed symmetry. The outer part is 2-fold symmetric (dimeric) and the inner part is 3-fold symmetric (trimeric). The main observation of the present study is that the beta-barrel domain of the third subunit is the main component of the stalk connecting the "heads" with the 3-fold "foot" and appears to have the same fold as the other two copies but an entirely non-equivalent position and set of interactions. Some other details, such as a 3-stranded coiled coil formed by the N-terminal parts are also reported. This represents incremental progress towards understanding a complex molecular system but gaps remain. Where is the third lectin domain? If it was proteolytically removed, as suggested, this should be demonstrated by SDS-PAGE. Also, the explanation given for the absence of VP4 trimers from the other (unoccupied) site in the VP7 shell is not clear to this reviewer: is the underlying VP6 site not also quasi-threefold? Also big questions about VP4 remain. Are there other intermediates? What is the pre-cleavage structure – is it totally disordered? In summary, the authors are performing microscopy at the state of the art and the work is technically impressive (but see comments below) but I have some reservations about whether the return of insightful information. Although care has clearly gone into describing these complex structures, the text is still difficult to follow for non-specialist readers.

Specific points:

1) Since much is made of the specimen's infectivity (title), at least the infectivity (particles/pfu) should be given, preferably as measured on the sample analyzed.

2) The evidence for the claim that VP4 has been visualized at 3.8Å resolution is not strong and the "atomic structure" of the title looks to be an overstatement. By an atomic structure I would expect all or almost all of the sidechains to be clearly resolved and it is not clear from one one piece of density shown (presumably, not the worst) hat that is the case. It seems likely that much idechain information comes from docking in or refining against crystal structures. Note: the previous paper (ref.14) claimed "near atomic" and the present paper "atomic" although the only density shown here (Fig S2) looks to be of lower quality than what is shown in ref. 14.

3) Compared to the FSC curves given in refs 5 and 14, the FSC curves in Fig S1 have different slopes with unusual peaks around 6.5Å to 4.5Å. This could be an artifact of defocus refinement. Also, the use of the 0.143 FSC threshold may be overly optimistic (most other authors seem to use 0.5).

4) What is a "structured-based mutant" and a "coherent mechanism" (abstract)?

5) While I understand that the authors do not wish to make Figure 1 too busy, some further labeling would help readers.

6) Did not find the maps at the stated locations.

7) Yellow and orange are not well differentiated in Figure 4.

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