19th November 2007

Dear Dr Grigorieff,

Your manuscript entitled "Near-atomic resolution using electron cryo-microscopy and single particle reconstruction" has now been seen by three referees, whose comments are attached below. While the reviewers find your work of potential interest, they have raised technical points, as well as concerns about the limited novel biological insight that can be gained from the study. All of the reviewers feel that the work be better presented in the context of the upcoming X-ray structure. I am sorry to say that in the light of this advice we have decided that we cannot offer to publish your manuscript in Nature.

I am sorry that we cannot be more positive on this occasion but hope that you will find our referees' comments helpful when preparing your paper for submission elsewhere.

Yours sincerely

Senior Editor, Nature

Reviewers' comments:

Referee #1 (Remarks to the Author):

This manuscript describes the determination of the structure of rotavirus - an important human pathogen - at a resolution almost reaching 5Å - by using single particle reconstruction from cryo-electron microscopy images. The resulting reconstruction is of very high quality, especially in the region corresponding to the second layer, which has a T=13 symmetry, which allows 760-fold averaging within each individual particle image. The authors present interesting technical data of the correlation of the individual images on the grid with the corresponding projection of the final recostruction, and the dependence with the type of support used, the location on the grid, etc., providing selection criteria to eliminate damaged particles. This step was extremely important for improving the ultimate resolution of the final reconstruction, and can provide important guidance when dealing with very fragile particles, for instance those of icosahedral enveloped viruses. The author use an X-ray crystallographic 3.8Å map of the particle as a control and comparison, and find that their reconstruction is similar in clarity. However, in spite of the obvious quality of the data reported here, this reviewer regrets that the most interesting biological data resulting from this work seem to be coming in a manuscript "in preparation" by a subset of the authors of this paper. As a result, this manuscript becomes too technical, and will interest only those who are specialists in the field and not a wider audience. For this reason, I'm not enthusiastic about publishing it in Nature, but in a more specialized journal.

Referee #2 (Remarks to the Author):

This paper reports the main features of a cryo-EM reconstruction of rotavirus-related particles at relatively high resolution. The main message is the claim that cryo-EM reconstructions may now be performed, by a protocol that includes use of the FREALIGN alignment program, at resolutions high enough to allow tracing of polypeptide chains. This claim is based on the purportedly good agreement of this structure with a structure that some of the authors have determined of the same particle by X-ray crystallography at a nominal 3.8 Å resolution. This reviewer accepts that the authors have obtained a good reconstruction but is not convinced that the resolution is as high as is claimed nor that this approach can be expected to be effective in the general case. It has been known for 10 years that cryo-EM reconstructions in the 7 Å resolution range can allow chain traces of icosahedral virus capsid proteins. This paper represents an incremental advance because the

rotavirus proteins, unlike HBV, are not all alpha-helical, but it brings no new biological information and the FREALIGN method has been published before. For these reasons and because the claim that this map is of comparable quality to the X-ray one is entirely anecdotal, I do not believe that this paper warrants publication in Nature. With appropriate clarifications (see below), a revised paper might be suitable for publication in Nature Methods.

1) The claim that this map is comparable in quality to a 3.8 Å map from X-ray crystallography is simply anecdotal. No evidence is presented in support of this claim. I suggest that the authors defer publication of this work until they have published the X-ray structure, at which time they should show side-by-side comparisons between the same regions of both maps. It should also include the same regions of density from cryo-EM maps limited to lower resolutions of 5.8 Å and 6.5 Å (see below).

It is noteworthy that they have decided to base their structure determination of the DLP on the X-ray map, not the EM map.

2) To make this comparison quantitative, they should present an FSC curve calculated between the EM map and the X-ray map.

3) The resolution is 5.3 Å by the FSC curve with a threshold of 0.142. This number is unrealistically optimistic both because of the exaggerating effect of correlated noise (see Yang et al, J Struct Biol. 2003 144:162) and because the threshold of 0.142 is so low. Most such comparisons use a threshold of 0.5, which gives a resolution of about 6.5 Å here or 0.3 (about 5.8 Å).

An argument has been given for the 0.142 threshold is that it compensates for the fact that it refers to only half-sets of data. It would enhance the paper to include an FSC curve for two independent reconstructions from half-data sets, not half sets separated after all the data had been refined together. Here 0.142 might be justified.

4) The bimodal distribution of correlation coefficients is an interesting observation but I doubt if it occurs in most cryo-EM analyses. The basis for this partition is obscure. It is assigned to the particle "batch" but what does that mean? Were different isolation procedures used? Was one batch frozen for storage or transportation, i.e. before vitrification? Was one batch just much older, i.e. a longer time between preparation and observation?

Referee #3 (Remarks to the Author):

This manuscript reports single-particle cryo-EM reconstruction of rotavirus double-layer particle (DLP) at sufficient resolution to recognize secondary structure elements and some large side chains. This is a significant technical achievement and would be appropriate for Nature. However, the manuscript reads very weak in its current form (for example, there is only 1 page for results, and 2 pages each for introduction, discussion and methods). It has essentially no discussions about the features and functional implications of the reconstructed image. It is probably due to the fact that the X-ray structure of this DLP has also been determined, and such descriptions will be given there instead. In this regard, I believe it may be better if this manuscript is published as a (short) companion paper to the X-ray structure paper.

The authors need to define the effective resolution of their reconstructed image. 'Similar in clarity to a 3.8 Å resolution map obtained from x-ray crystallography' (abstract) is not sufficient. The authors have the luxury of comparing to an X-ray map in this case, but there has to be an independent way of assessing the effective resolution of the cryo-EM image, for example Fourier shell correlation or other indicators. Similarly, 'The map compares well with ...' (top of

p. 6) is not sufficient. More detailed and complete analysis should be given here, in addition to simply showing a few regions of the image in Figs. 1-2. Maybe a plot of the real space R factor of all the residues for the X-ray map and the cryo-EM image based on the refined X-ray model?

How much did the 13-fold averaging of VP6 improve the image? The real-space R factors could be used for this analysis.

The term 'high-resolution technique' is used in the abstract, but the resulting image is actually in the 'low-resolution' range for crystallography. A more appropriate term should be used.

'Substantial during' in the abstract. A word is missing here.

Fig. 2d: Consistent labels should be in both views.