September 3, 2003

Dear Steve

Your manuscript entitled "Three Dimensional Structure of Kv4.2-KChIP2 Channels by Electron Microscopy at 21 Å has now been seen by three referees, whose comments are attached. While they find your work of potential interest, reviewers one and two have raised concerns that in our view are sufficiently important to preclude publication of the work in Nature, at least in its present form.

We very much hope that further experimental data will allow you to address these criticisms, providing convincing evidence for the topology you have proposed. In which case, we would be happy to look at a revised manuscript (unless, of course, something similar has by then been accepted at Nature or appeared elsewhere). In the case of eventual publication, the received date would then be that of the revised paper.

Please use the link below to submit a revised paper.

Please also ensure that your revised manuscript conforms to our format requirements for Letters to Nature. In particular, we would be grateful if you could revise the layout of your figures bearing in mind that they should fit exactly one, or exceptionally two, of our columns in the printed journal.

We hope that you find our referees' comments helpful when revising your manuscript.

Kind regards

Senior Editor
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Reviewers Comments:

Referee #1(Remarks to the Author):

The manuscript by Kim et al. describes the structure of a human potassium channel protein complex at ~20 Angstrom resolution. The work is generally of high quality, and represents a novel and very significant contribution to our understanding of the structural basis of ion channel function. The work is especially important as this particular channel complex is critical to regulating cardiac function, and is also thought to make a major contribution to processing of synaptic inputs in the dendrites of mammalian central neurons. This works represents the first real structural information on an intact human (or any mammalian) ion channel of known molecular composition, and provides important insights relevant to other members of this important protein family. The work is very well done, and in some ways almost amazing in the biochemical expertise needed to generate the high quality material for study. The only criticism of the work is the weakness of the data supporting the membrane topology of the complex, which is critical to interpretation of a great deal of the other results. The authors have fused GFP to a cytoplasmic KChIP subunit of the channel complex, and present data that this leads to an extension of one face of the structure, identifying this as the intracellular face. However, the

resolution of this particular structure is very poor compared to the wild-type channel, and it is difficult to see how this poor structure of the GFP-tagged channel could have led to an unambiguous topological determination. Granted, everything ultimately fits very well into place (e.g. the crystal structures of frequenin, Kv{lower case beta}2, etc) using this topology, but one wonders if it is possible to fit these crystal structures into the EM structure in other configurations. Higher quality data on the structure of the GFP-tagged channel complex, or an explanation of how many other models were rejected on quantitative terms until this was the only one remaining would greatly enhance the ultimate believability of the final model, no matter how well everything seems to fit at the end.

Referee #2(Remarks to the Author):

This manuscript reports preliminary structural analysis of voltage-gated potassium channels assembled from complexes of KChlP2 subunits and pore-forming Kv4.2 subunits. I am not persuaded that any particularly incisive biological insights have emerged from this analysis; further, the technical aspects of the work would require much more depth and rigor if this were to be reported in a specialty structural journal.

The structural analysis of the complex is carried out on negatively stained specimens at room temperature using recombinant KchlP2 and a charybdotoxin-sensitive variant of Kv4.2. The key question of interest is an understanding of the nature of interaction between KChlP2 and Kv4.2. The inference that KchlP2 influences channel function by interaction with the four internal and external columns of the assembly is potentially an interesting finding, but almost nothing that is mechanistically significant can be concluded at the present resolution. The efforts to determine the sidedness by GFP labeling are also at the edge of plausibility, and can be misleading in the absence of proper difference maps, which are not presented. The similarities between the 3D structures with and without GFP in fig. 3c are not readily apparent, especially given the significant differences in regions where GFP is presumed not to be present. Finally, the overlay in figure 5 of the X-ray co-ordinates of T1-KvB2, KvAP and frequenin with the EM structure has primarily artistic value at the present resolution of the EM map.

The purification of the Kv4.2-KChlP2 complex analyzed here is reported in a different companion manuscript. It occurs to me that the data presented in the two manuscripts could have been easily combined. In fact, the last paragraph of the companion manuscript includes an almost verbatim reproduction of the abstract of the present manuscript.

Referee #3(Remarks to the Author):

This paper describes the determination at low resolution of the structure of a potassium channel that is important in the heart and central nervous system. The result is the identification of the modulatory KChIP subunits and where they fit on the intracellular side of the channel. This result of considerable interest to the field as the KChIP subunits are essential in channel trafficking and expression in the heart, and almost no structural information has been available about these or any protein complexes formed with ion channels. An elegant aspect of the work is the use of a GFP-fusion protein to provide a "mass marker" to label the KChIP proteins.

The work is of high quality and is of sufficient broad interest to appear in Nature, but the paper is poorly written, assuming far too much familiarity with the details of the field. As it is, a reader would first have to digest the Nature Neuroscience ms. to be able to understand this one. If some of the introductory information in that ms. were repeated in this one, the paper would become much more accessible. Here is one example: the bottom paragraph on p. 4 assumes that the reader knows that KChIP2 will be found on the intracellular side of the protein.

Some minor questions:
1. p. 4, line 4. How did you determine the CTF for the negative-stain imaging? Specifically, what value for the amplitude contrast did you use and why?

2. p. 6, top paragraph. Is the difference in volume of the extracellular region consistent the 60 kDa of carbohydrate mass? Also, could you perhaps show a vertical section as well as a horizontal section in Fig. 4 for comparing the maps?

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Date: Tue, 14 Oct 2003 08:38:39 -0400
From: Steve Goldstein <steve.goldstein@yale.edu>

Hi ******,

I understand you are in meetings and we will not be able to talk until tonight or tomorrow. Clearly it was an administrative error to have reviewer 2 remain the structural referee given his first emotional review; this is born out by the minor issues raised now (as if they were significant). I am sorry I did not bring this up explicitly in my cover to the revision but I thought that I did not need to since your cover letter seemed to actively ignore the reviewer (by indicating what was needed to make the report NATURE-worthy was "providing convincing evidence for the topology you have proposed" which is now done in spades).

I submit that you must eliminate a reviewer who offers feedback like: "the EM structure has primarily artistic value at the present resolution" or "nothing that is mechanistically significant can be concluded at the present resolution" or "the technical aspects of the work would require much more depth and rigor if this were to be reported in a specialty structural journal" which are all completely absurd...this is remarkable EM work (both the base structure and application of the mass-tag methodology).

This is really not OK. I think this report is FULLY ready for prime time. Send it by email to a real structural biologist for an overnight thumbs up or down (MacKinnon or Miller, etc) but don't base a decision on unreliable feedback like this.

I look forward to talking,
Steve

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Date: Mon, 03 Nov 2003 13:12:22 +0000

Subject: RE: 2003-07-07760C
To: 'Steve Goldstein' <steve.goldstein@yale.edu

Dear Steve

Sorry for the delay in my reply; I was out of the office last Thursday and Friday.

It seems that you must be missing our last piece of correspondence, which I have attached to this e-mail. It was sent to your correct e-mail address last Thursday, so I'm not sure why it hasn't got through and I apologise if the error was to our mail server. Unfortunately, as our letter explains, we remain unable to publish your

manuscript in Nature.

I am sorry that we weren't able to come to a more positive conclusion on this occasion.

With kind regards

Date: Thu, 30 Oct 2003 11:52:16 -0000
From: decisions

To: steve.goldstein@yale.edu

Dear Steve

Thank you for your letters asking us to reconsider our decision on your manuscript entitled "Three Dimensional Structure of Kv4.2-KChIP2 Channels by Electron Microscopy at 21 Å Resolution". Now that I have had chance to solicit comments from a fourth independent referee, and discuss the matter once again with my colleagues, I am sorry to have to tell you that we do not feel able to reverse our original decision.

We do appreciate your point of view and the conclusions that you reach would certainly be of interest to our readership. However, without robust support for these conclusions, publication in Nature would be premature. As the technical concerns raised by referee 2 and 4 are sufficiently important to seriously undermine support for those conclusions, we have decided that we must decline publication of the work. For this reason, we feel that your best course would now be to submit your paper elsewhere so as to avoid further unnecessary delays in publication.

We naturally understand your disappointment with our decision; unfortunately, the number of papers with possible claims on space in Nature vastly exceeds the number that we can publish each week, and we are therefore frequently forced to make difficult decisions.

I am sorry that on this occasion we cannot be more positive.

Kind regards

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Reeferees comments

Referee 4

Presented here are two EM reconstructions from isolated, negatively stained, voltage gated potassium channels. One reconstruction (21A resolution) is of the Kv4.2

channel with the accessory neuronal calcium sensing (NCS) protein KChlP2. The second reconstruction is of the same complex with a GFP attached to identify the KChlp2 (resolution not stated). KChlP2 forms an intracellular fenestrated rotunda around the Kv4.2 hanging gondola. Various x-ray crystal structures and an EM reconstruction of the Shaker channel are used to interpret the reconstruction presented here.

Overall this is a careful piece of work. The new EM reconstructions look good; the GFP difference map identifies the cytoplasmic domain and a reasonable explanation of the large differences between the Kv4.2- KChlP2and Shaker reconstructions is given. Docking of the KChlP2 homologue frequenin is less convincing. The manual docking looks like a reasonable but not unique solution, and it is troubling that the position obtained by automated docking is quite different and partly outside the EM density envelope. (The reviewer's conclusion is that either the program was not used correctly or that the data are not suitable for automated docking procedures.) Published data support the overall location for the accessory protein, but it appears that neither its position nor its orientation can be determined with any certainty.

Although this is a good piece of work, it is really only a preliminary attempt to understand an interesting biological problem. The advance in our understanding of potassium channel structure is incremental, and the manuscript provides no insights into mechanism. The most disappointing aspects of the paper are the minimal comments about the peripheral columns and the voltage sensing paddles (final 2 paragraphs)- two issues that may or may not be related. The accessory protein appears to make direct connections to both the membrane associated domain and to the hanging gondola. One would imagine that this finding is worth exploring more fully. Perhaps these shortcomings of the manuscript should be corrected by additional studies. Given the incremental structural advance and the lack of insight into mechanism, I believe that this manuscript is not suitable for publication in Nature.