Dear Dr Grigorieff

I was fascinated to hear from Chris Miller that you are currently completing a low-resolution structure of the Shaker K+ channel. I understand that you might be ready to submit this for publication in the near future and I wanted to let you know that Nature would be very interested in considering the manuscript.

Obviously, the choice of journal to which you submit your work is entirely up to you, but I hope that by expressing an interest in the work I might help you in that decision. If you decide to submit your work to another journal (or have already done so) I would be very grateful if you could let me know when it is due to appear so that we could cover the work in our News and Views section.

I would be more than happy to chat to you over the phone if you have any uncertainties about review and publication in Nature; please don't hesitate to call me on the number below.

I look forward to seeing Shaker in all its glory!

With kind regards

Associate Editor
Nature
Porters South
4-6 Crinan Street
London N1 9XW

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Dear Niko

Many thanks for the abstract; it sounds great and I can't wait to see the structure! I'll start lining up referees straight away however it would be helpful if you could you let me know exactly what technique you have used so that I can ask the appropriate people.

It would also be helpful if you could drop me a quick e-mail after you submit the manuscript so that I can keep my eye out for it.

Look forward to it!

Best wishes

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Authors' replies to referees' comments
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The most important points of criticism are raised by Referee 2: a) the assignment of the cytoplasmic density to the T1 domain is based on poor evidence (also raised by Referee 3), and b) the discussion of the structure at the 10 angstrom level is outside the resolution of this study.

a) The authors disagree that the assignment in the original manuscript is based on poor evidence. However, the evidence from work performed by the authors and others was not presented in the best possible way. As pointed out at the end of the

original manuscript, Kobertz & Miller recently showed that the T1 domain forms a tetramer in the fully assembled channel. These data are central to our interpretation of the structure, and in the revised manuscript we make this clear (top of page 4): The fourfold symmetry of the KcsA and T1 structures demands that both must be positioned on the symmetry axis of the channel. The KcsA channel is too large to fit into the small domain and thus must be placed in the large domain. Therefore, this domain must represent the membrane-intrinsic part of Shaker. With KcsA placed in the large domain, the only other density sufficiently large to accommodate the T1 crystal structure along the fourfold axis is the small domain. The T1 crystal structure accounts for most of the volume of the small domain. The authors therefore identify the smaller domain to be the cytoplasmic mass of Shaker containing T1. The orientation of the T1 tetramer has been chosen to agree with the crystal structure of the isolated T1-ß-subunit complex. As Referee 2 points out, some of the density of the small domain may contain part of the C-terminus, and the authors discuss this possibility in the revised manuscript in light of cross-linking experiments between the C-terminus and T1 (end of page 4).
b) The authors disagree that a discussion at the 10 angstrom level is outside the resolution of this study. Referee 2 makes this point him/herself by noting that the T1 crystal structure “does not fit well into the density at all”, thereby referring to the unaccounted density in the small domain which varies in dimension between 5 and 10 angstroms. The authors have now performed a more rigorous analysis of the window size and clearly stated the assumption this is based on (last paragraph in Methods). The authors assume that the domains and connectors have a well-defined surface, i.e. no loops or disordered protein producing weak density extends into the windows. This appears reasonable in light of the X-ray structure of T1 and recent studies of the “cytoplasmic assembly” containing T1 and the ß subunit (Gulbis et al. Science 289, 123-127 (2000)). The smallest window size can then be inferred from the density profile across the window at 25 angstrom resolution. As the referee points out, two densities (here: connectors in the EM structure) separated by 8 angstroms would not be resolved at 25 angstrom resolution. However, the density profile across the window crossing two connectors (long axis) does show a clear minimum and, therefore, the connectors must be further apart than 8 angstroms. The authors had estimated the separation of the connectors at 15 angstroms in the original manuscript. The authors performed model calculations which show that the connectors must be at least 20 angstroms apart to produce such a minimum at 25 angstrom resolution. The density profile across the windows parallel to the channel symmetry axis (short axis) is different because the two domains limiting the top and bottom of the windows are much larger than the thin connectors. A density minimum will be visible even if the two large domains are closer together than 20 angstroms. The situation is more like that of a single density (here: density minimum) in an otherwise homogeneous background. The depth of the minimum can be analysed in terms of the width of the gap between the domains. This analysis showed that the gap has to be at least 10 angstroms to account for the observed density profile. A discussion of these points has been included in the Methods section. However, the discussion of the 10 angstroms of density of KcsA extending beyond that of the EM structure has been omitted.

Other comments:

Referee 1

Comment: If the C-terminus were disordered, the contour level would be too low.
Reply: Sentence added at the end of page 4.

Comment: A second image of a cross-section of Shaker, without the superimposed structure of KcsA, would show more detail of the structure.
Reply: In view of the comments of referee 2, the discussion of the density of KcsA extending beyond the density of the EM structure has been removed. The authors agree with referee 2 that the discussion of the EM structure cannot include details at the 10 angstrom level (except for the window size, see later). Therefore, the authors feel that a second image of a cross-section without KcsA would not reveal any further useful information but would only lengthen the manuscript. Therefore, a second figure has not been included.

Comment: At the end of page 4, it should read “20 kDa per subunit”.
Reply: Added to the end of page 4.

Referee 3

Comment: The work is not sufficiently significant to be of general interest.
Reply: The work presents the first three-dimensional structure of an entire voltage-gated ion channel. This is a break-through in the field of ion channels where this data, as presented at a prominent meeting, already had a large impact on the interpretation of experiments probing channel gating and general channel assembly. Therefore, the authors feel this is a significant contribution of more general interest.

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August 18, 2000

Rejection by Nature with the following comments from the referees:

Referee 1

I've looked over the reviews, response, and revised manuscript from Niko Grigorieff. I remain convinced, in spite of the comments of the other reviewers, that this paper is sound and that it contains qualitatively new information about the structure, as weil as a substantial gee-whiz factor. Let me know if you need a more formal review.

Referee 2

The authors did not address the main point raised in my review. The study does not compare in quality to other electron microscopiC studies at the same resolution (look for yourself). In the absence of support labelling data I have my doubts.

Referee 3

I am still worried about the lack of density for the C-terminal domain, which is bigger than T1! The authors have softened their strong assertions fram the first submission and included a few sentences to explain the lack of density attributable to the C-terminal part. I would agree with referee 2 that gold labelling would be an excellent experiment to do, as I remain somewhat unconvinced.

As for significance, I stand by my original statement that the insights gained from the work are incremental.

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Manuscript was passed on to Nature Strutural Biology:

Nature Structural Biology MS# NS*****

September 12, 2000

Dear Dr. Grigorieff:

Thank you for submitting your manuscript, "Three-dimensional structure of a voltage-gated potassium channel at 2.5 nm resolution". I apologize for the delay in responding, which resulted from the tight production deadline of the October issue. We have carefully considered your revised manuscript and the comments from Nature's referees. Sadly, we have decided that we cannot offer to publish your study in Nature Structural Biology, and we are returning the manuscript to you. I am sorry we could not be more positive on this occasion.

Sincerely,

Associate Editor
Nature Structural Biology

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Nature Structural Biology MS# NS*****

September 22, 2000

Dear Dr. Grigorieff:

Thank you for your letter concerning your manuscript "Three-dimensional structure of a voltage-gated potassium channel at 2.5 nm resolution". We have now had a chance to discuss the points you raise in detail. While we understand your disappointment at our decision not to consider your paper further, I am afraid we do not feel that we can reverse that original decision.

While we find your report interesting, we agree with Nature's referees that the locations of both the T1 domain and the C-terminal region of the channel should be firmly established to represent a significant advance. Please note that we are indeed editorially independent and we do not follow the recommendation from Nature. In this case, because we would have chosen the same group of experts as the Nature referees to review your paper, we do not believe that our decision would have been different had we sent it out ourselves.

We do wish you luck with a more suitable avenue of publication.

Sincerely,

Associate Editor
Nature Structural Biology