

The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel

Tomaž Trobec

DOI: 10.1113/JP281785

Corresponding author(s): Tomaž Trobec (tomaz.trobec@vf.uni-lj.si)

The referees have opted to remain anonymous.

Review Timeline:

Submission Date:	29-Apr-2021
Editorial Decision:	17-May-2021
Revision Received:	26-May-2021
Editorial Decision:	27-May-2021
Revision Received:	03-Jun-2021
Accepted:	03-Jun-2021

Senior Editor: Ian Forsythe

Reviewing Editor: Peying Fong

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Mr Trobec,

Re: JP-JC-2021-281785X "The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel" by Tomaž Trobec

Thank you for resubmitting your revised Journal Club to The Journal of Physiology. It has been assessed by the original Reviewing Editor and Referees and has been well received. Some final revisions have been requested.

Please advise your co-authors of this decision as soon as possible.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

I hope you will find the comments helpful and have no difficulty in revising your manuscript within 2 weeks.

Your revised article should be submitted online using the links in Author Tasks Link Not Available. This link is to the Corresponding Author's own account, if this will cause any problems when submitting the revised version please contact us.

Your revised submission should include:

- A Word file of the article file
- No more than 5 references
- A copy of the manuscript with the changes highlighted.

To create your 'Response to Referees' copy all the reports, including any comments from the Reviewing Editor into a Word, or similar, file and respond to each point in colour or CAPITALS and upload this when you submit your revision.

I look forward to receiving your revised submission.

Yours sincerely,

Ian D. Forsythe
Deputy Editor-in-Chief
The Journal of Physiology
<https://jp.msubmit.net>
<http://jp.physoc.org>
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane

London, EC1R 3AW
UK
<http://www.physoc.org>
<http://journals.physoc.org>

EDITOR COMMENTS

Reviewing Editor:

The Author offers a synopsis of a recent paper by Cabrera-Garcia and colleagues published in The Journal of Physiology. It is presented within four densely written sections encompassing 1) a brief introduction, 2) a detailed report of the manuscript's Results section, 3) a consideration of the study's implications, placed within the context of the existing E protein literature, and 4) a brief concluding paragraph. Sections 2 and 3 are the densest and require the greatest attention, as they both comprise many different sets of results (section 2) and multiple, emergent questions (section 3). As noted by the Referee, these make the manuscript difficult to unpack.

In addition to the Referee's comments regarding paragraph length management, some sections might be reorganized to avoid misinterpretation, confusion, and redundancy.

For example, in section/paragraph 2, some potential for misinterpretation/confusion appears within the section describing selectivity. Here, the Author indicates that the channel does not discriminate between sodium and potassium, but does not carry chloride. This is followed by a statement that N-methyl-D-glucamine (noted with a "+" as a cation), did not permeate. Yet in the very next sentence, "This confirms that the SARS-CoV-2-E protein forms a non-selective cation channel" appears. This seemingly contradicts the immediately preceding statement regarding lack of N-methyl-D-glucamine permeation; this is, after all, a cation.

On redundancy: The matter of cation permeability is reiterated in section/paragraph 3, in slightly greater detail: "The SARS-CoV-2 E protein forms a nonselective cation channel that is permeable to Na⁺ and K⁺. The permeability is 3-fold higher for K⁺ than for Na⁺. On the other hand, Cl⁻ had difficulty passing through the ion channel..." Is it really necessary to say this again? And what happened to N-methyl-D-glucamine?

Senior Editor:

Thank you for this this revision. Although it is much improved, there are two issues which need further effort. First the overall structure needs improvement, as pointed out by the editor. Second the sentence construction needs further assistance. I note you acknowledge the help of your supervisors and American Journal experts, but I think you also need to ask a native English speaker to read through your text as a final polish to the the English and sentence construction.

REFEREE COMMENTS

Referee #1:

This contribution has been extensively revised and is improved over the original version. The reference to Gourab et al., is missing.

Many of the paragraphs are quite long, which makes the article dense and somewhat difficult to read. Using shorter paragraphs can help to break up long technical papers in Physiology and can mark the boundaries between one idea and the next, just a small suggestion for the future!

END OF COMMENTS

Tomaž Trobec, PhD student
Institute of Preclinical Sciences
Veterinary Faculty, University of Ljubljana
Gerbičeva 60, 1000 Ljubljana
Slovenia

Professor Ian D. Forsythe
The Journal of Physiology

May 27, 2021

The Journal of Physiology
Special Case Resubmission for JP-JC-2021-281785X

Title: The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel

Author: Tomaž Trobec

Dear Editor,

Thank you very much for your comments, advice and suggestions on my Journal Club article entitled: "The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel". I appreciate the valid points raised by the reviewers and the opportunity given to address each of these issues and resubmit the manuscript within 14 days. I have corrected the manuscript according to the suggestions of the referee and the editors. In addition, the manuscript was also reviewed by a native English speaker Dr. Christopher Berrie.

I sincerely hope that now the revised Journal Club article will be suitable for publication in your journal.

Yours sincerely,

Tomaž Trobec

The reviewer's comments are addressed as follows:

Reviewing Editor:

Comments to the Author

The Author offers a synopsis of a recent paper by Cabrera-Garcia and colleagues published in The Journal of Physiology. It is presented within four densely written sections encompassing 1) a brief introduction, 2) a detailed report of the manuscript's Results section, 3) a consideration of the study's implications, placed within the context of the existing E protein literature, and 4) a brief concluding paragraph. Sections 2 and 3 are the densest and require the greatest attention, as they both comprise many different sets of results (section 2) and multiple, emergent questions (section 3). As noted by the Referee, these make the manuscript difficult to unpack.

In addition to the Referee's comments regarding paragraph length management, some sections might be reorganized to avoid misinterpretation, confusion, and redundancy.

For example, in section/paragraph 2, some potential for misinterpretation/confusion appears within the section describing selectivity. Here, the Author indicates that the channel does not discriminate between sodium and potassium, but does not carry chloride. This is followed by a statement that N-methyl-D-glucamine (noted with a "+" as a cation), did not permeate. Yet in the very next sentence, "This confirms that the SARS-CoV-2-E protein forms a non-selective cation channel" appears. This seemingly contradicts the immediately preceding statement regarding lack of N-methyl-D-glucamine permeation; this is, after all, a cation.

Response: thank you for pointing out the discrepancy in the statements. I have corrected this discrepancy as follows:

Before: Changes in internal or external solutions facilitated the study of ion selectivity of ion channels formed by E proteins. Na^+ and K^+ can pass through this ion channel, while Cl^- have difficulty penetrating the ion channel. Cations such as N-methyl-D-glucamine⁺ could not permeate through the pore. This confirms that the SARS-CoV-2-E protein forms a non-selective cation channel.

Corrected: Changes in the intracellular and extracellular solutions facilitated the study of the selectivity of the ion channels formed by E protein. Na^+ and K^+ passed through these ion channels, while Cl^- showed little ion channel permeation. Although the bulky N-methyl-D-glucamine⁺ cation did not permeate through the channel, they confirmed that E protein forms a channel for monovalent cations, including Na^+ and K^+ .

On redundancy: The matter of cation permeability is reiterated in section/paragraph 3, in slightly greater detail: "The SARS-CoV-2 E protein forms a nonselective cation channel that is permeable to Na^+ and K^+ . The permeability is 3-fold higher for K^+ than for Na^+ . On the

other hand, Cl⁻ had difficulty passing through the ion channel..." Is it really necessary to say this again? And what happened to N-methyl-D-glucamine?

Answer: indeed, it was an unnecessary repetition of statements already made. I have corrected this statement as follows:

Before: The SARS-CoV-2 E protein forms a nonselective ion channel that is permeable to Na⁺ and K⁺. The permeability is 3-fold higher for K⁺ than for Na⁺. On the other hand, Cl⁻ had difficulty passing through the ion channel (Cabrera-Garcia et al., 2021).

Corrected: E protein forms cation channels that are permeable to Na⁺ and K⁺ (Cabrera-Garcia et al., 2021).

Senior Editor:

Thank you for this this revision. Although it is much improved, there are two issues which need further effort. First the overall structure needs improvement, as pointed out by the editor. Second the sentence construction needs further assistance. I note you acknowledge the help of your supervisors and American Journal experts, but I think you also need to ask a native English speaker to read through your text as a final polish to the English and sentence construction.

Response: thank you for the critical comments and suggestions. I have corrected the manuscript according to your advice. In addition, I have kindly asked for the help Dr. Christopher Berrie (a native English speaker), who gave me advice and assistance in correcting the sentence structure and in the final proofreading of the manuscript.

REFEREE COMMENTS

Referee #1:

This contribution has been extensively revised and is improved over the original version. The reference to Gourab et al., is missing.

Response: the reference was incorrectly given; I have corrected it as follows:

Before: (Gourab, et al., 2021); Das G, Das T, Chowdhury N, Chatterjee D, Bagchi A & Ghosh Z (2021). Repurposed drugs and nutraceuticals targeting envelope protein: A possible therapeutic strategy against COVID-19. *Genomics* **113**, 1129–1140.

Corrected: (Das et al., 2021); Das G, Das T, Chowdhury N, Chatterjee D, Bagchi A & Ghosh Z (2021). Repurposed drugs and nutraceuticals targeting envelope protein: a possible therapeutic strategy against COVID-19. *Genomics* **113**, 1129–1140.

Many of the paragraphs are quite long, which makes the article dense and somewhat difficult to read. Using shorter paragraphs can help to break up long technical papers in Physiology and can mark the boundaries between one idea and the next, just a small suggestion for the future!

Response: many thanks for the valuable suggestion. In correcting this manuscript, I have already followed your advice as far as possible and created shorter paragraphs.

Dear Mr Trobec,

Re: JP-JC-2021-281785XR1 "The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel" by Tomaž Trobec

Thank you for submitting your Journal Club article to The Journal of Physiology. It has been assessed by a Reviewing Editor and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

I hope you will find the comments helpful and have no difficulty in revising your manuscript within 2 weeks.

Your revised article should be submitted online using the links in Author Tasks Link Not Available. This link is to the Corresponding Author's own account, if this will cause any problems when submitting the revised version please contact us.

Your revised submission should include:

- A Word file of the article file
- No more than 5 references
- A copy of the manuscript with the changes highlighted.

To create your 'Response to Referees' copy all the reports, including any comments from the Reviewing Editor into a Word, or similar, file and respond to each point in colour or CAPITALS and upload this when you submit your revision.

I look forward to receiving your revised submission.

Yours sincerely,

Ian D. Forsythe
Deputy Editor-in-Chief
The Journal of Physiology
<https://jp.msubmit.net>
<http://jp.physoc.org>
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London, EC1R 3AW
UK

EDITOR COMMENTS

Reviewing Editor:

Thank you for your responsiveness to my previous critique. There remain several minor details that require your attention. I highlight the most problematic in the following.

1) Suggest starting paragraphs 2 and 3 differently, so that they are not so repetitious (both start with "SARS-CoV-2 E protein...").

Why not start paragraph 3 by restructuring how you present the logical thread: "The E protein of SARS-CoV-1 has properties consistent with ion channel activity, and importantly shares 95% sequence identity with SARS-CoV-2 E. This information, together with functional studies of SARS-CoV-2 E in bacteria and in bilayer recordings, inspired Cabrera-Garcia et al (2021) to propose that..."?

2) The apparent contradiction regarding cation selectivity remains. I am not sure if the Author understood fully all dimensions requiring attention. Let me try again.

The statement "Although the bulky N-methyl-D-glucamine+ cation did not permeate through the channel, they confirmed that E protein forms a channel for monovalent cations, including Na+ and K+" is misleading.

How can lack of NMDG permeation--a monovalent cation--be confirmation, even if one starts the statement out with "Although"?

The observations do not confirm but are simply consistent with the notion that E protein permeates small monovalent cations indiscriminately. The point is that NMDG is not small; potential confusion arises from the fact that NMDG is monovalent.

Also, I presume "they" refers to the data. If so, why not just say "the data"? Alternatively, it could mean Cabrera-Garcia and co-authors. If the latter, then the second clause is better phrased as "these observations led Cabrera-Garcia et al to conclude that E protein..."

END OF COMMENTS

Tomaž Trobec, PhD student
Institute of Preclinical Sciences
Veterinary Faculty, University of Ljubljana
Gerbičeva 60, 1000 Ljubljana
Slovenia

Professor Ian D. Forsythe
The Journal of Physiology

June 3, 2021

The Journal of Physiology
Special Case Resubmission for JP-JC-2021-281785X

Title: The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel

Author: Tomaž Trobec

Dear Editor,

Thank you very much for additional comments and suggestions on my Journal Club article entitled: "The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel". I have corrected the manuscript according to the suggestions of the reviewing editor.

I sincerely hope that now the revised Journal Club article will be suitable for publication in your journal.

Yours sincerely,

Tomaž Trobec

The reviewer's comments are addressed as follows:

Reviewing Editor:

Comments to the Author

Thank you for your responsiveness to my previous critique. There remain several minor details that require your attention. I highlight the most problematic in the following.

1) Suggest starting paragraphs 2 and 3 differently, so that they are not so repetitious (both start with "SARS-CoV-2 E protein...").

Why not start paragraph 3 by restructuring how you present the logical thread: "The E protein of SARS-CoV-1 has properties consistent with ion channel activity, and importantly shares 95% sequence identity with SARS-CoV-2 E. This information, together with functional studies of SARS-CoV-2 E in bacteria and in bilayer recordings, inspired Cabrera-Garcia et al (2021) to propose that...?"

[Response: thank you very much for your valuable advice. I have corrected mentioned paragraph in accordance with your very good suggestion to avoid repetition.](#)

Before: SARS-CoV-2 E protein has 95% sequence identity with that of SARS-CoV-1, and the indications are that SARS-CoV-1 E protein has several properties that are consistent with ion channel activity. It has thus been proposed that SARS-CoV-2 E protein can also form a cation channel, as further described by Cabrera-Garcia et al. (2021). This is based not on its 95% sequence identity with SARS-CoV-1 E protein, but more specifically on ion channel functional studies of SARS-CoV-2 E protein in bacteria, and on bilayer recordings.

Corrected: The E protein of SARS-CoV-1 has properties consistent with ion channel activity, and importantly, it shares 95% sequence identity with SARS-CoV-2 E. Together with functional studies of SARS-CoV-2 E in bacteria and in bilayer recordings, this information inspired Cabrera-Garcia et al. (2021) to propose that SARS-CoV-2 E protein forms a cation channel.

2) The apparent contradiction regarding cation selectivity remains. I am not sure if the Author understood fully all dimensions requiring attention. Let me try again.

The statement "Although the bulky N-methyl-D-glucamine⁺ cation did not permeate through the channel, they confirmed that E protein forms a channel for monovalent cations, including Na⁺ and K⁺" is misleading.

How can lack of NMDG permeation--a monovalent cation--be confirmation, even if one starts the statement out with "Although"?

The observations do not confirm but are simply consistent with the notion that E protein permeates small monovalent cations indiscriminately. The point is that NMDG is not small; potential confusion arises from the fact that NMDG is monovalent.

Also, I presume "they" refers to the data. If so, why not just say "the data"? Alternatively, it could mean Cabrera-Garcia and co-authors. If the latter, then the second clause is better phrased as "these observations led Cabrera-Garcia et al to conclude that E protein..."

Response: First of all, I would like to apologize to you because I obviously did not understand your proposal correctly. Now I fully understand your suggestion and I have corrected the contradictory statement. The word "they" was used for Cabrera-Garcia and co-authors, so I have corrected the sentence according to your suggestion.

Before: Changes in the intracellular and extracellular solutions facilitated the study of the selectivity of the ion channels formed by E protein. Na^+ and K^+ passed through these ion channels, while Cl^- showed little ion channel permeation. Although the bulky N-methyl-D-glucamine⁺ cation did not permeate through the channel, they confirmed that E protein forms a channel for monovalent cations, including Na^+ and K^+ .

Corrected: Changes in the intracellular and extracellular solutions facilitated the study of the selectivity of the ion channels formed by E protein. Na^+ and K^+ passed through these ion channels, while Cl^- showed little ion channel permeation, and the bulky N-methyl-D-glucamine⁺ cation did not permeate through the channel. These observations led Cabrera-Garcia et al. (2021) to conclude that E protein forms an ion channel that is permeable to small monovalent cations, including Na^+ and K^+ .

I would like to thank all the editors and reviewers for their comments, advice and suggestions!

Dear Mr Trobec,

Re: JP-JC-2021-281785XR2 "The role of the SARS-CoV-2 envelope protein as a pH-dependent cation channel" by Tomaž Trobec

I am pleased to tell you that your Journal Club article has been accepted for publication in The Journal of Physiology.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

The last Word version of the paper submitted will be used by the Production Editors to prepare your proof. When this is ready you will receive an email containing a link to Wiley's Online Proofing System. The proof should be checked and corrected as quickly as possible.

All queries at proof stage should be sent to tjp@wiley.com

Are you on Twitter? Once your article is online, why not share your achievement with your followers. Please tag The Journal (@jphysiol) in any tweets and we will share with our 22,000+ followers!

Thank you for your contribution to The Journal of Physiology.

Yours sincerely,

Ian D. Forsythe
Deputy Editor-in-Chief
The Journal of Physiology
<https://jpp.msubmit.net>
<http://jpp.physoc.org>
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London, EC1R 3AW
UK
<http://www.physoc.org>
<http://journals.physoc.org>

EDITOR COMMENTS

Reviewing Editor

Thank you for incorporating the suggested changes.

Senior Editor

Thank you for an interesting Journal Club article.
