Assessing the impact of Orai1 channel cross-linking on store operated ER refilling: Insights from a mathematical model

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Motivation

Store operated Ca^{2+} entry (SOCE) is the influx of Ca^{2+} through store operated channels (i.e. Orai channels) in response to depletion of ER Ca stores and is crucial for cell function, regulating processes such as gene expression.

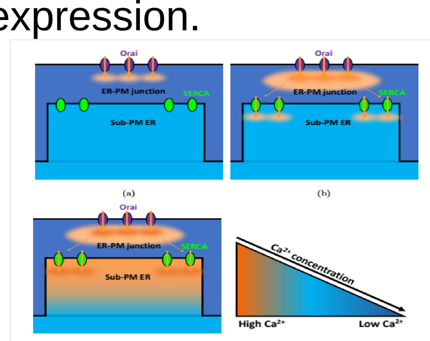

Fig. 1. Illustration of SOCE. Orai chanels open upon store depletion leading to Ca influx and ER refilling via SERCA pumps.

Zhou *et al.* observed that Orai1 channels can be cross-linked by STIM1 to form tight lattices within ER-PM junctions. This leads to higher frequency IP_3 receptor mediated Ca^{2+} oscillations than non cross-linked Orai1 channels. They proposed that cross-linked Orai1 enhance store operated ER refilling resulting in higher frequency Ca^{2+} oscillations.

We have developed a three dimensional spatio-temporal model of Ca^{2+} dynamics within ER-PM junctions as the small size of ER-PM junctions precludes direct measurements of the Ca^{2+} concentration. We use this model to assess the impact of cross-linking of Orai1 on ER refilling by addressing the following questions:

1. How does Orai1 cross-linking affect the spatial Ca^{2+} signature?
2. What impact does Orai1 cross-linking have on SERCA pump activity?
3. Is clustering sufficient to enhance store operated ER refilling to affect IP_3 receptor mediated Ca oscillations?

Mathematical model

Diffusion in the ER-PM junction is described by,

$$\frac{\partial C}{\partial t} = D\nabla^2 C,$$

where C represents the Ca^{2+} concentration within the ER-PM junction or sub-PM ER. We approximate the ER-PM junction and sub-PM ER as cuboids of width 300nm and height 15nm and 500nm, respectively, as shown in **Fig. 1**.

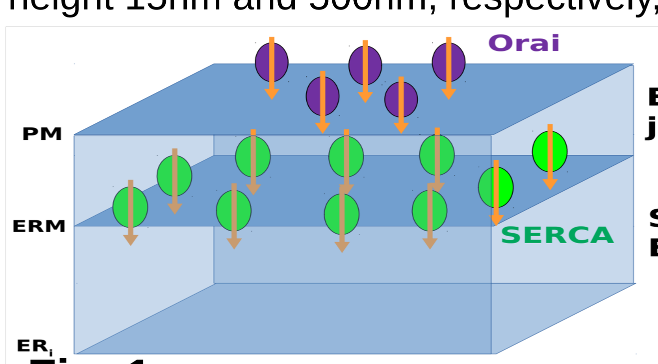

Fig. 1. Illustration of mathematical approximation to ER-PM junction and sub-PM ER

We impose no flux boundary conditions along the membranes so Ca^{2+} cannot diffuse across the membrane and fix the Ca^{2+} concentration at the edge of the ER-PM junction, as illustrated in **Fig. 2**.

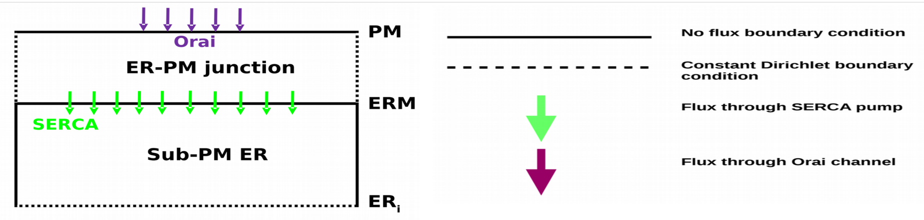

Fig. 2. Illustration of the boundary conditions imposed.

Another key observation in [1] was that the non cross-linked Orai1 channels are activated to a lesser extent. We incorporate this into the model by reducing the Ca^{2+} flux through the non cross-linked channels to 12.5% of the cross-linked channel flux, as proposed in [1].

Results

Cross-linking of Orai1 channels creates distinct spatial Ca profiles

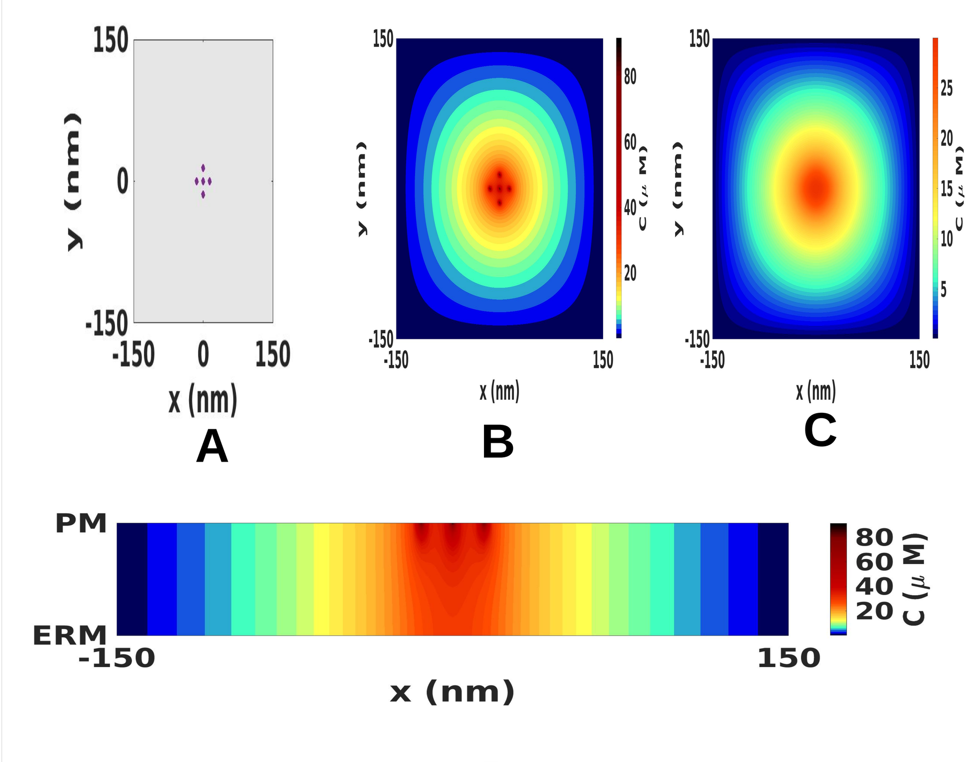

Fig. 4
A Illustration the showing the placement of cross-linked Orai1 channels in ER-PM junction. **B, C** Ca^{2+} profile along PM and ER membrane, respectively. **D** Ca^{2+} profile inside ER-PM junction

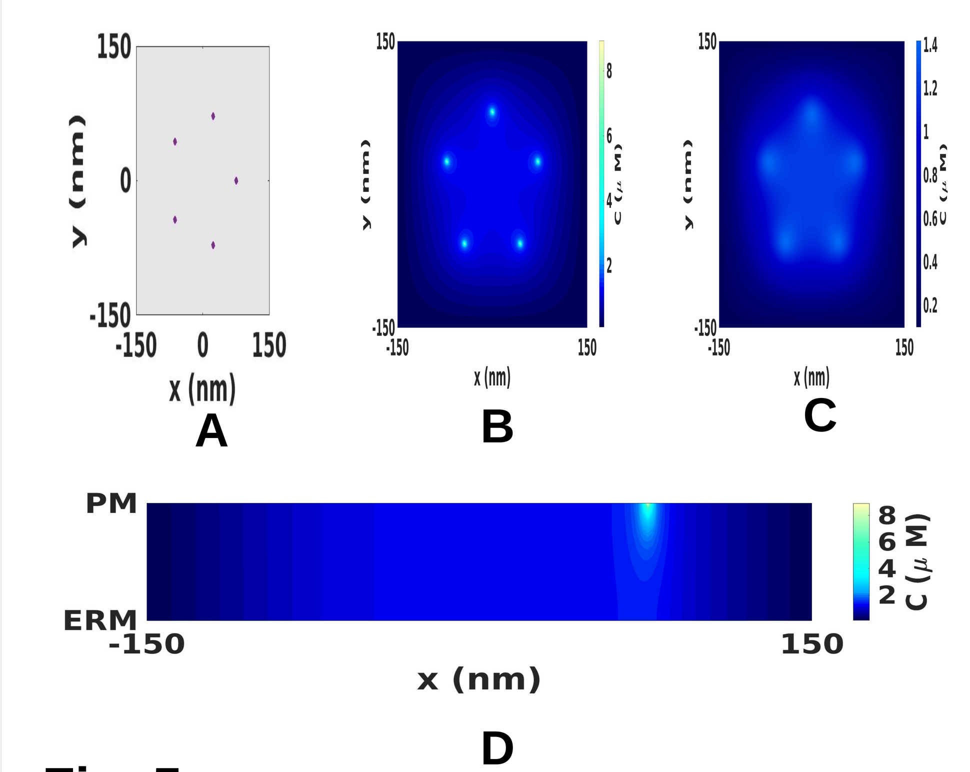

Fig. 5
A Illustration showing the placement of non cross-linked Orai1 channels in ER-PM junction. **B, C** Ca^{2+} profile along PM and ER membrane, respectively. **D** Ca^{2+} profile inside ER-PM junction

Cross-linking of Orai1 channels causes the channel microdomains to overlap creating one large microdomain within the ER-PM junction, as seen in **Fig. 4**. The microdomains of non cross-linked Orai1 channels overlap to a lesser degree and form a star shaped region of increased amplitude but the individual channel microdomains can still be distinguished, **Fig 5**. Cross-linking of Orai channels creates spatially distinct Ca^{2+} profiles within the ER-PM junction.

SERCA pump placement is a key factor determining the level of store operated ER refilling

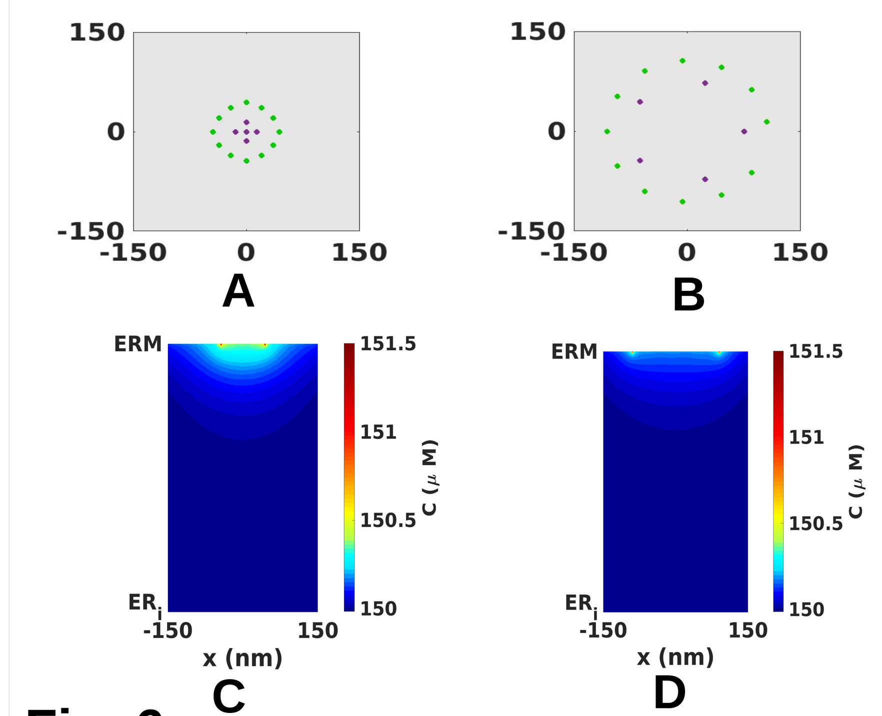

Fig. 6
A, B Illustrations showing the placement of cross-linked and non cross-linked Orai1 channels (purple) and SERCA pumps (green) in the ER-PM junction. **C, D** Ca^{2+} profile in sub-PM ER generated in response to the Orai-SERCA arrangements after 1ms.

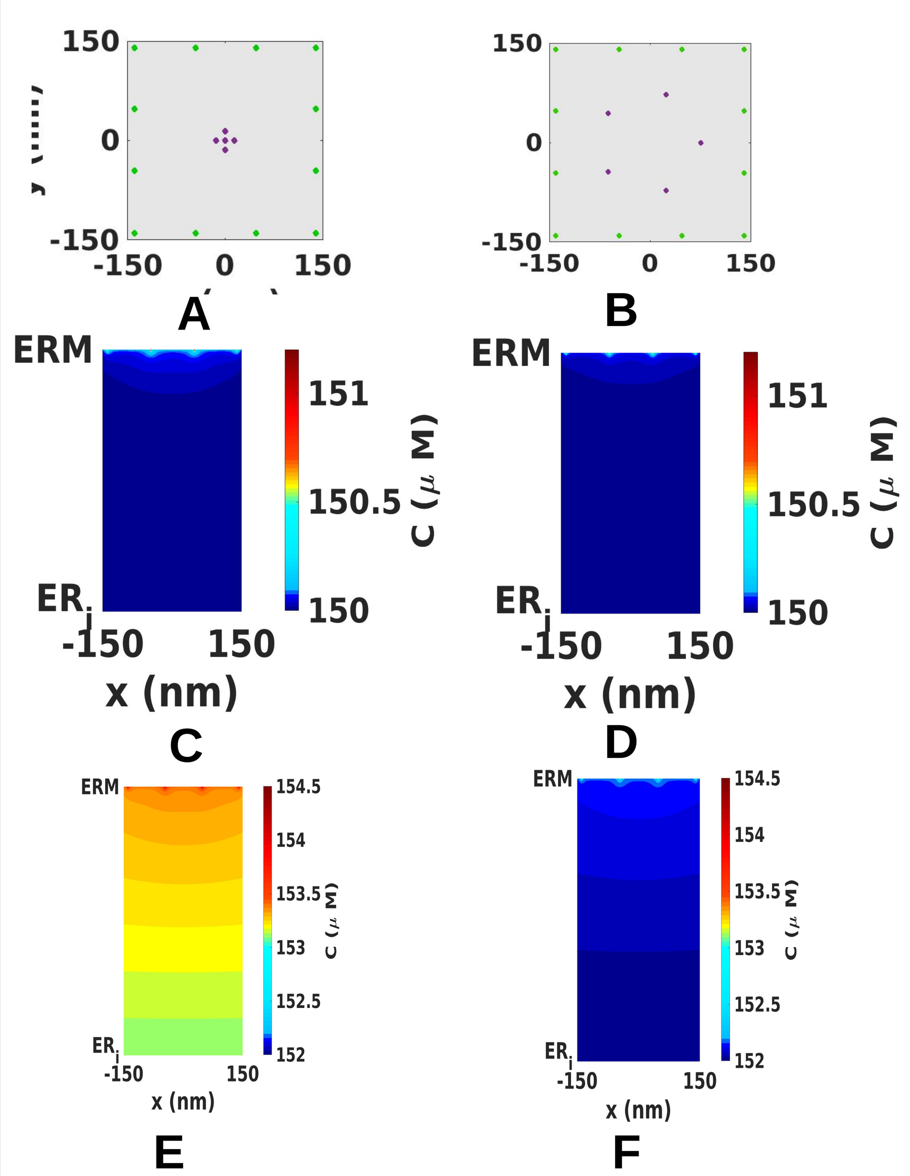

Fig. 7
A, B Illustrations showing the placement of cross-linked and non cross-linked Orai1 channels (purple) and SERCA pumps (green) in the ER-PM junction. **C, D** Ca^{2+} profile in sub-PM ER generated in response to the Orai-SERCA arrangements after 1ms.**C, D** Ca^{2+} profile in sub-PM ER generated in response to the Orai-SERCA arrangements after 10s

Cross-linking does not enhance ER refilling when SERCA pumps are placed close to Orai channels, **Fig. 6**. However, when SERCA pumps are placed at the periphery of the ER-PM junction, **Fig. 7**, store operated ER refilling is 36% less than the refilling occurring in response to the cross-linked Orai channels.

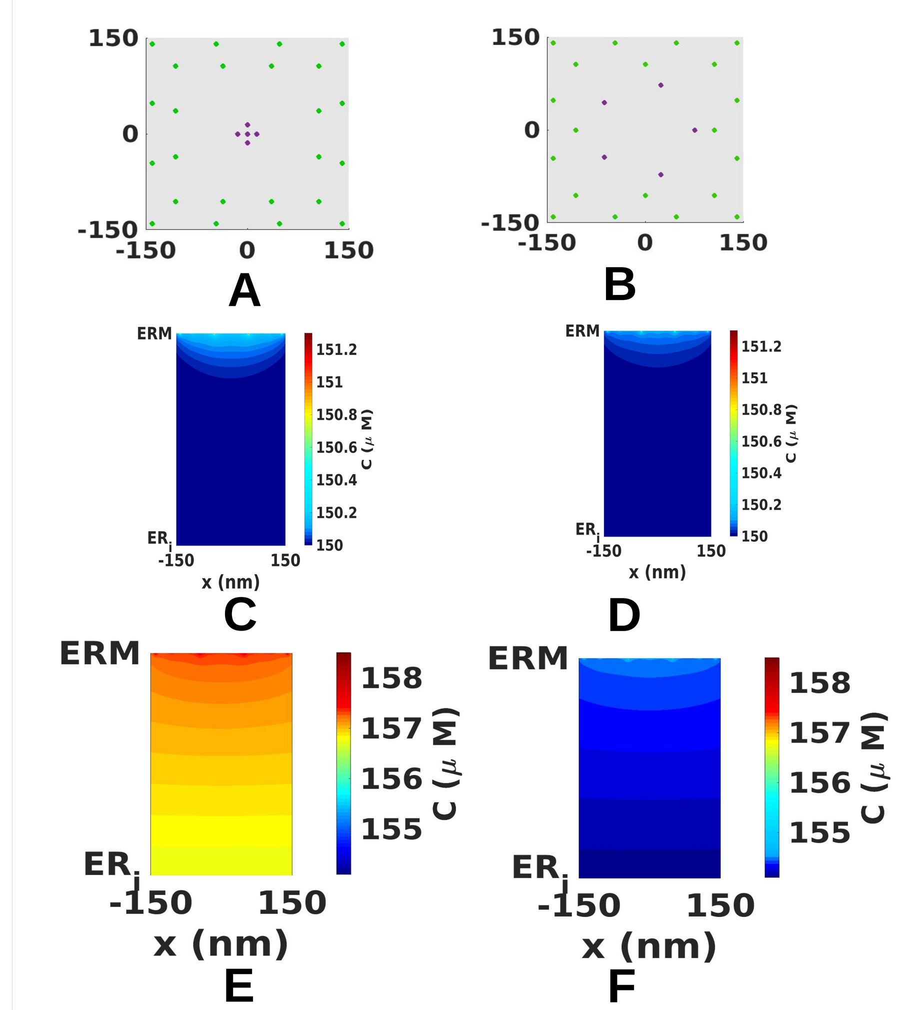

Fig. 8
A, B Illustrations showing the placement of cross-linked and non cross-linked Orai1 channels (purple) and SERCA pumps (green) in the ER-PM junction. **C, D** Ca^{2+} profile in sub-PM ER generated in response to the Orai-SERCA arrangements after 1ms. **E, F** Ca^{2+} profile in sub-PM ER generated in response to the Orai-SERCA arrangements after 10s

ER-PM junctions with cross-linked Orai1 channels create tight clusters of Orai1 thus leaving more space within the ER-PM junction. This could allow greater numbers of SERCA pumps to be present in the ER-PM junction. The non cross-linked Orai1 channel ER-PM junctions contain 20 SERCA pumps and the cross-linked Orai1 channel ER-PM junctions contain 24 SERCA pumps. This leads to 39% less store operated ER refilling with the non cross-linked Orai 1channels as the ER-PM junction contains fewer SERCA pumps. Therefore, cross-linking could act as a method of tuning store operated ER refilling.

Future Work

We will continue investigating the relationship between Orai1 cross-linking and IP_3 receptor Ca^{2+} oscillations to highlight the key factors governing this process.

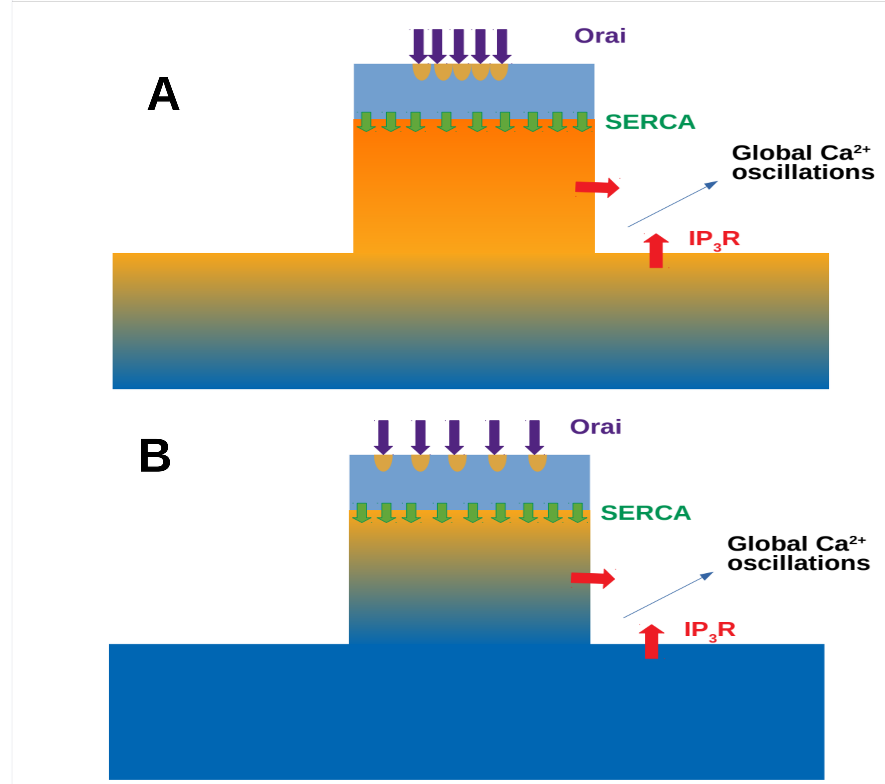

Fig. 9
Illustration of the extended model which includes IP3Rs to further investgate how cross-linked (A) and non cross-linked (B) Orai1 channels affect Ca^{2+} oscillations.

This will include:

1. Extending the mathematical model to include bulk cytoplasmic and bulk ER compartments.
2. Including IP_3 receptors to investigate how Orai1 cross-linking affects IP_3 receptor mediated Ca^{2+} oscillations.
3. Investigating purely numerical mathematical solution techniques, such as finite element methods, to include physically realistic geometries.
4. Incorporating experimental data to validate and refine the mathematical model.

Conclusions

1. Cross-linking of Orai channels increases the amplitude and spread of Ca^{2+} microdomains creating spatially distinct Ca^{2+} profiles.
2. SERCA pump placement regulates store operated ER refilling and is a key factor determining the level of store operated ER refilling.
3. Orai1 cross-linking could act as a mechanism to organise the microenvironment within the ER-PM junction leading to optimal store operated ER refilling conditions.
4. Store operated ER refilling is governed by both Orai1 cross-linking and SERCA placement.

References

[1] Zhou, Yandong, et al. "Cross-linking of Orai1 channels by STIM proteins." Proceedings of the National Academy of Sciences 115.15 (2018): E3398-E3407

[2] McIvor, Emma, Stephen Coombes, and Ruediger Thul. "Three-dimensional spatio-temporal modelling of store operated Ca2+ entry: insights into ER refilling and the spatial signature of Ca2+ signals." Cell Calcium 73 (2018): 11-24.

Code available on GitHub

https://github.com/emmamcivor/matlab_FASEB_Calcium