Store operated calcium entry, Orai channels and microdomains: Modelling the spatial signature of local Ca^{2+} profiles

Emma McIvor, Steve Coombes, Rüdiger Thul, University of Nottingham, emma.mcivor@nottingham.ac.uk

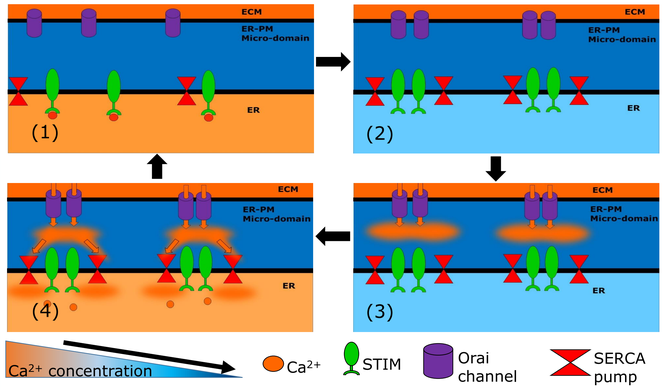
The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Introduction

Ca^{2+} microdomains form in response to local Ca^{2+} influx through open channels. Ca^{2+} microdomains allow rapid and selective activation of signalling pathways according to the spatial profile of the Ca^{2+} signal[1].

Store operated calcium entry (SOCE) couples depletion of the endoplasmic reticulum (ER) to Ca^{2+} influx across the plasma membrane (PM) into the cytoplasm[1,2]. SOCE occurs in a Ca^{2+} microdomain and refills the ER Ca^{2+} store, but local Ca^{2+} influx through Orai channels, a type of SOC channel, is involved in cellular functions such as gene expression[3].

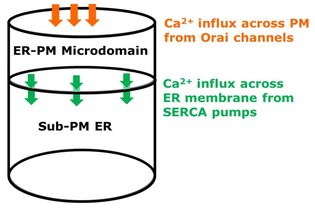
It is difficult to measure the local Ca^{2+} concentration directly[1] so mathematical models could provide insights into the signalling dynamics occurring in microdomains[2].



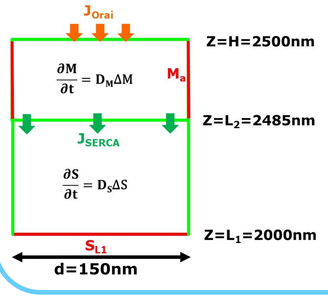
(1) Cell with full Ca^{2+} stores (2) Depletion of ER Ca^{2+} store signals STIM and Orai to cluster and co-localise on PM and ER, respectively (3) Orai channels open and allow Ca^{2+} influx from extracellular matrix (ECM) into ER-PM micro-domain (4) SERCA pumps in the ER membrane are activated and transport Ca^{2+} into ER. Once the ER is refilled we return to the distribution shown in (1).

Mathematical Model

SOCE occurs in hundreds of Ca^{2+} microdomains in the cell. We focus on a single Ca^{2+} microdomain and the associated sub-PM ER. This is the area enclosed by the yellow dashed line.



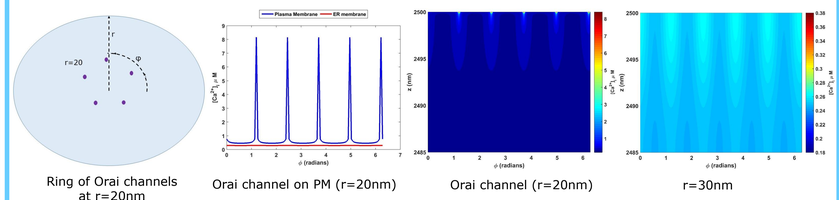
The ER-PM microdomain is a Ca^{2+} microdomain containing Orai channels on the PM. We allow Ca^{2+} to enter the microdomain as fluxes across the PM.
The sub-PM ER is the junctional ER closely apposed to the PM and we allow Ca^{2+} flux through SERCA pumps on the ER membrane.



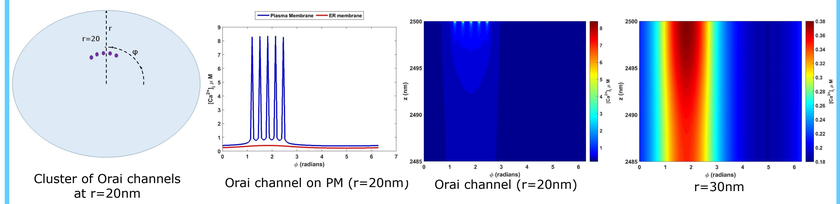
Z=H=2500nm
Z=L_2=2485nm
Z=L_1=2000nm

We have created a spatiotemporal mathematical model using a partial differential equation (PDE) framework to describe diffusion within each domain. The use of PDEs allows us to investigate the importance of spatial effects on the system; such as the effect of clustering of Orai channels and SERCA pump localisation.

Steep gradients and interactions between Orai channels in the microdomain



The Orai channels are equally distributed in the microdomain with a distance of 23.5nm between neighbours. At this spacing the plumes around the channel mouths do not interact.

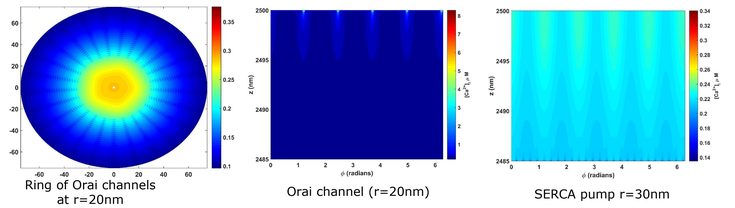


The Orai channels are clustered in the microdomain with a distance of 6.3nm between neighbours. At this spacing the plumes around the channel mouths do interact and raise the Ca^{2+} concentrations near the channel mouths.

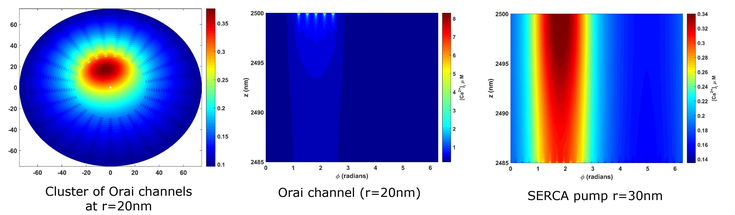
There are very steep Ca^{2+} gradients in the ER-PM microdomain around the open Orai channel mouth. The distance between channels influences the Ca^{2+} peak strength and shape of the Ca^{2+} profile because neighbouring channels feel the effect of the nearby increased Ca^{2+} concentrations.

The arrangement of the Orai channels significantly affects the spatial Ca^{2+} profile generated in the microdomain.

Interactions between Orai channels and SERCA pumps create distinct Ca^{2+} profiles



The channels equally spaced on the ring at r=20nm do not interact and at the SERCA pumps the Ca^{2+} concentration is low so the SERCA pumps are less active.

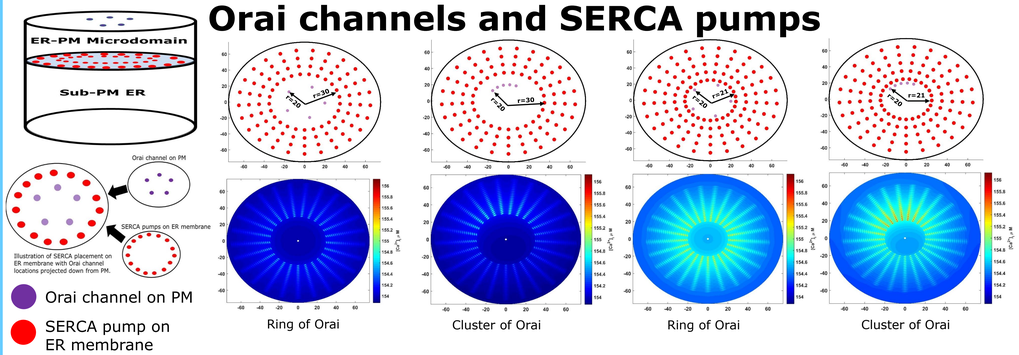


The neighbouring channels clustered on the ring at r=20nm do interact creating a locally high Ca^{2+} concentration so the SERCA pumps are more active.

Clustering the Orai channels creates local regions of high Ca^{2+} concentration which in turn increase the activity of the SERCA pumps. The different arrangement of CRAC channels in the microdomain result in different interactions with the SERCA pumps creating distinct spatial profiles. These distinct spatial profiles could allow selective activation of downstream Ca^{2+} signalling targets within the microdomain.

Ca^{2+} signals in the microdomain are shaped by interactions between Orai channels and SERCA pumps.

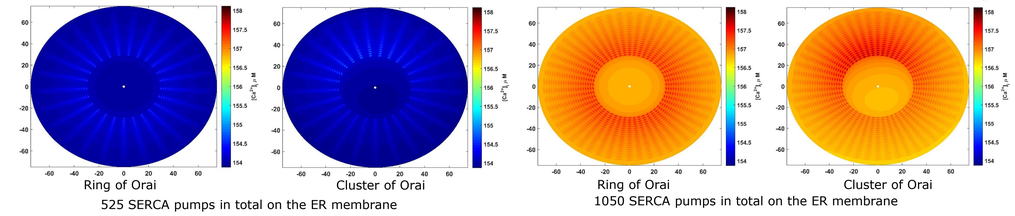
SERCA activity depends on the distance between the Orai channels and SERCA pumps



Clustering results in a group of highly activated SERCA pumps near the Orai channels and equal distribution of Orai channels results in equal activation of pumps on the inner ring. The level of SERCA activation decreases further from the Orai channels. Placing the SERCA pumps closer to the Orai channels allows the SERCA pumps to interact with the local plumes of high Ca^{2+} resulting in stronger activation of the SERCA pumps leading to faster refilling of the ER Ca^{2+} stores.

The location of Orai channels and SERCA pumps influences the level of SERCA activation and ER refilling. This suggests there is an optimum arrangement of Orai channels and SERCA pumps leading to most efficient refilling of the ER.

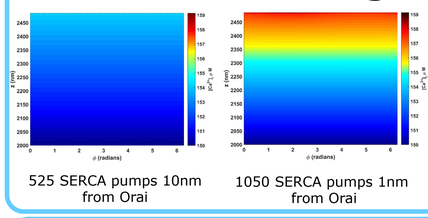
How does the number of SERCA pumps affect refilling?



Increasing the total number of SERCA pumps present on the ER membrane from 525 pumps to 1050 pumps increases the Ca^{2+} concentration by about 2μM. This suggests that 525 SERCA pumps are not able to capture all the Ca^{2+} entering the system.

Investigating how the refilling changes with SERCA number could provide insight into how many SERCA pumps are required for efficient refilling of the ER.

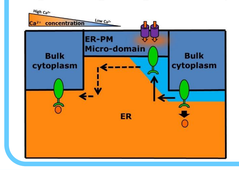
Effective refilling of the ER is a multidimensional problem



Refilling of the ER depends on the level of SERCA activation; this is affected by the number of SERCA pumps, the Orai channel distribution and the distance between the Orai channels and SERCA pumps. The flux strength of the SERCA pumps will also affect the level at which the ER is refilled during SOCE.

Conclusions and Future work

The distribution of Orai channels and SERCA pumps generates distinct Ca^{2+} profiles in the microdomain and controls the activation of SERCA pumps and refilling of the ER.
The Ca^{2+} patterns generated could encourage rapid and selective activation of downstream calcium signalling targets so spatial modelling could provide insights into the Ca^{2+} dynamics of the microdomain.



We will continue to test and refine our model to further investigate the effect of Orai channel and SERCA pump location on the microdomain dynamics and refilling properties.
We will extend the model to a four domain system, as shown, to compare the global and local dynamics of the system.

References:
1. Hogan, Patrick G. "The STIM1-ORAI1 microdomain." *Cell calcium* 58 4 (2015): 357-67.
2. Parekh, Anant B. "Ca^{2+} Microdomains near Plasma Membrane Ca^{2+} Channels: Impact on Cell Function." *The Journal of Physiology* 586.Pt 13 (2008): 3043–3054.
3. Samanta, Krishna et al. "Ca^{2+} Channel Re-Localization to Plasma-Membrane Microdomains Strengthens Activation of Ca^{2+}-Dependent Nuclear Gene Expression." *Cell Reports* 12.2 (2015): 203–216.

Acknowledgements
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