

Mitochondrial Membrane Fusion: Insights from a Model Membrane Platform

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INTRODUCTION

Mitochondrial fusion and fission are essential for maintaining normal architecture of the highly dynamic mitochondrial network. A proper equilibrium between the two process ensures mixing of mt DNA, metabolites and is dependent on metabolite state, therefore is essential for the function of the organelle, including respiration, calcium signaling and cell death induction[1]. Here we will focus on mitochondrial membrane fusion.

At molecular level, mitochondrial membrane fusion are regulated by a group of membrane proteins (Fig. 2) [2] . Till now, the molecular mechanism of mitochondrial fusion are regulated in response to cellular needs is largely unknown.

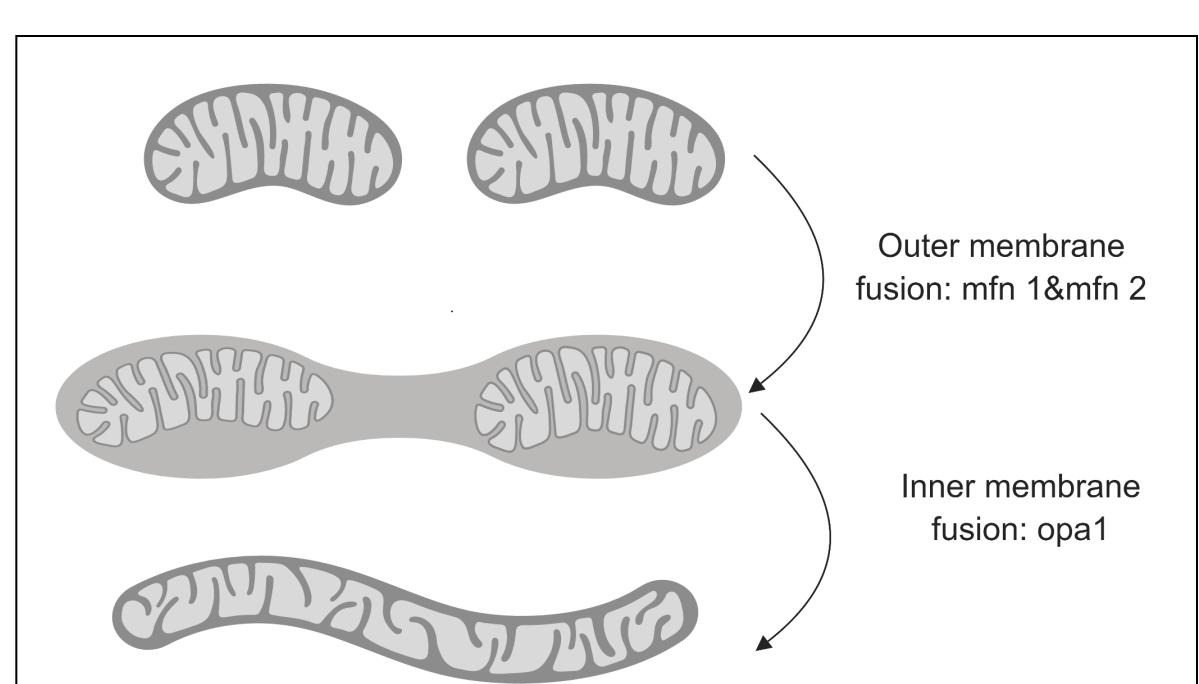
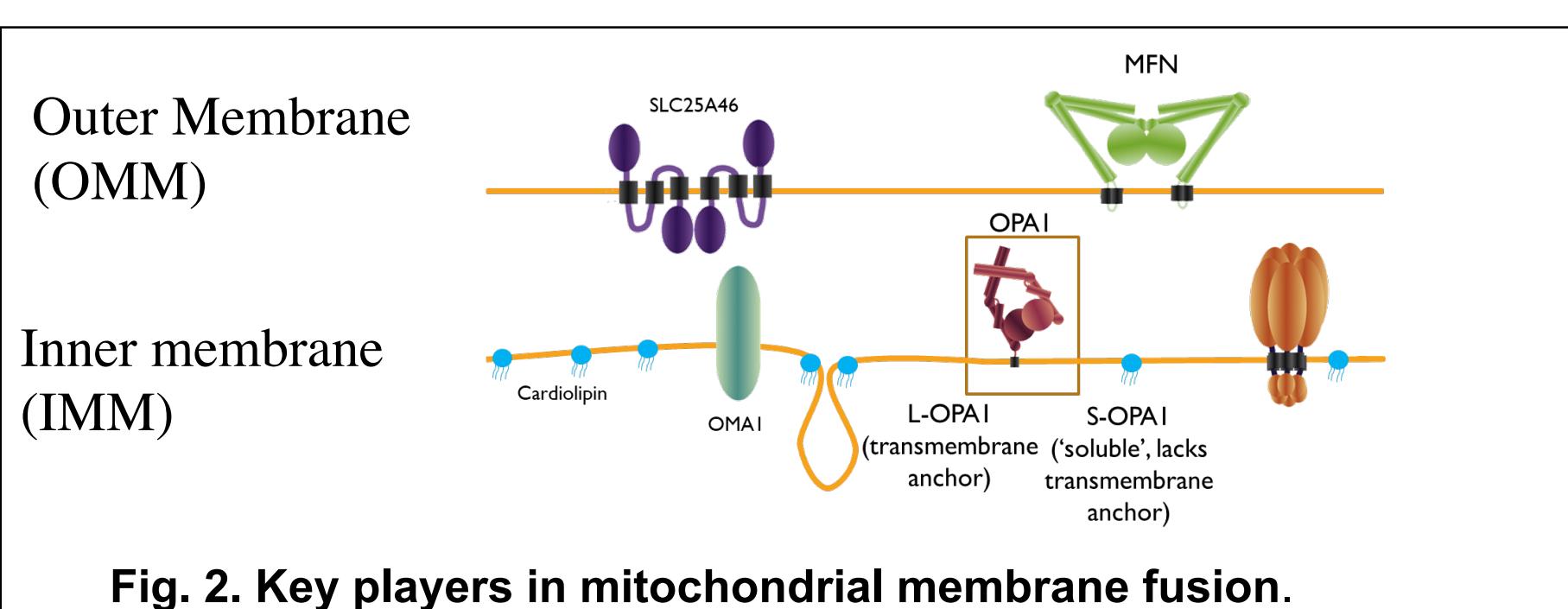


Fig. 1. Steps of mitochondrial membrane fusion.



Experimental Approach

Supported-planar lipid bilayer + lipid vesicles:

- Reconstitute transmembrane protein in a near-native state.
- Strictly control lipid, protein, nucleotide environment.
- Distinguish membrane steps (docking, tethering, lipid demixing and content release).

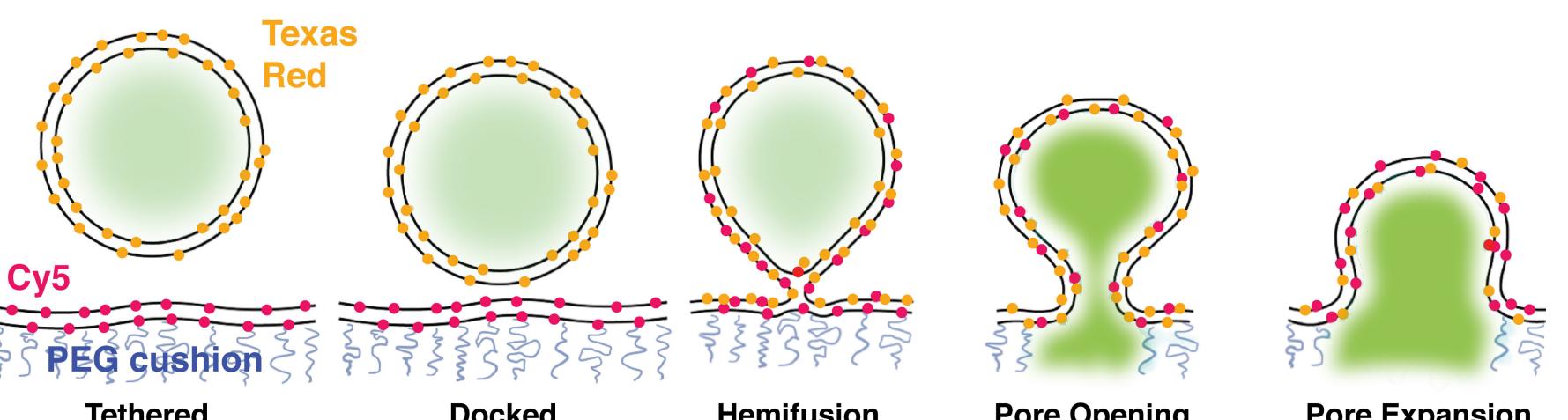


Fig. 3 Using IMM mimicking model membrane system to reconstituted inner membrane fusion.

Membrane Docking and Tethering

When l-Opa1 is present in supported lipid-bilayers and liposomes, tethering occurs. In the absence of cardiolipin, addition of GTP does not change the number of tethered particles. In contrast, with cardiolipin-containing liposomes and bilayers, homotypic l-Opa1:l-Opa1 tethering is enhanced by GTP (Fig. 4A) . Non- hydrolysable analog, as well as GDP, disrupt membrane tethering (Fig. 4B) .

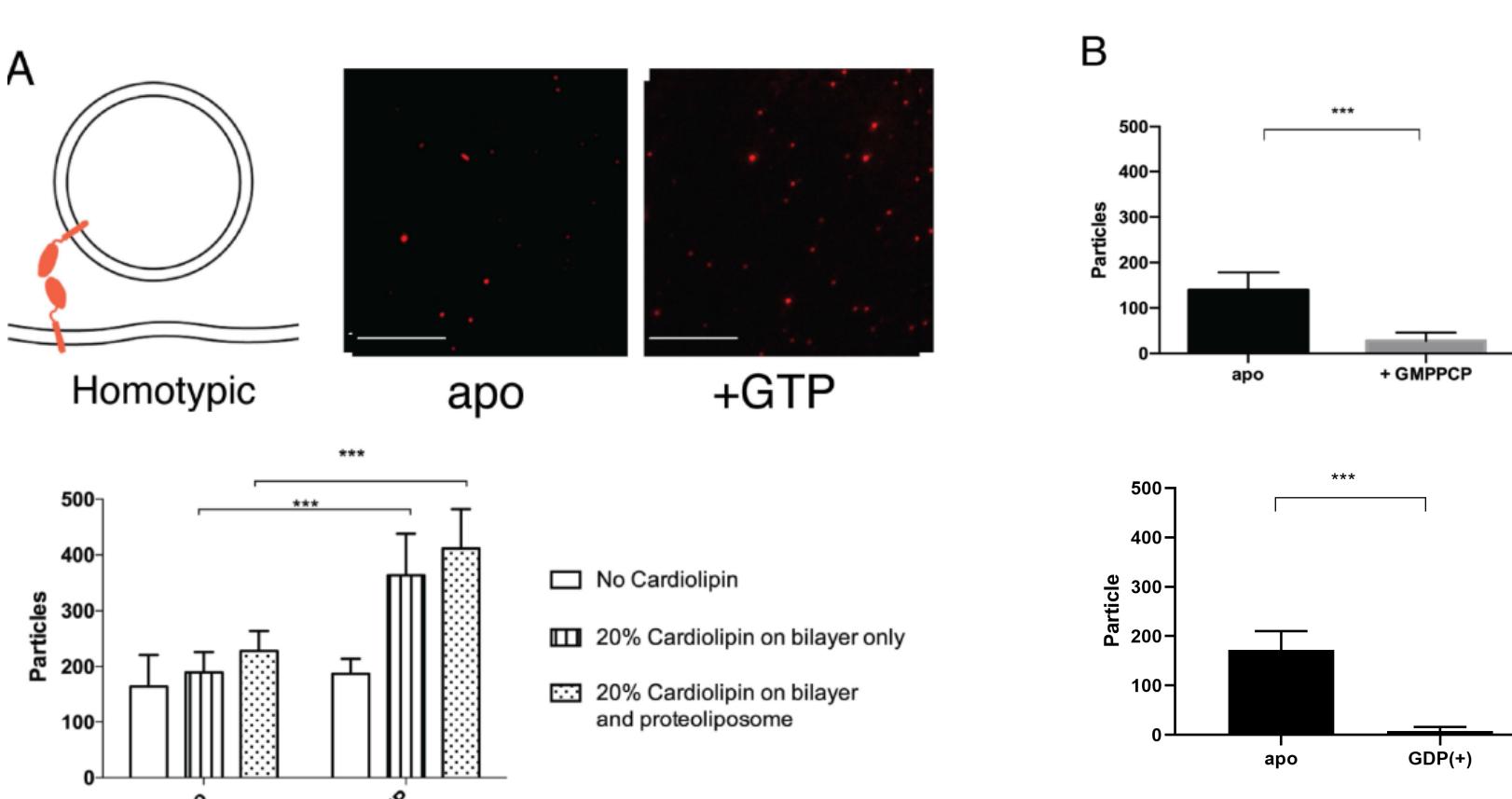


Fig. 4. I-opa1 mediated homotypical tethering is GTP and cardiolipin dependent.

Proteoliposomes containing l-Opa1 will tether to a cardiolipin-containing bilayer lacking any protein binding partner. This process is GTP dependent.

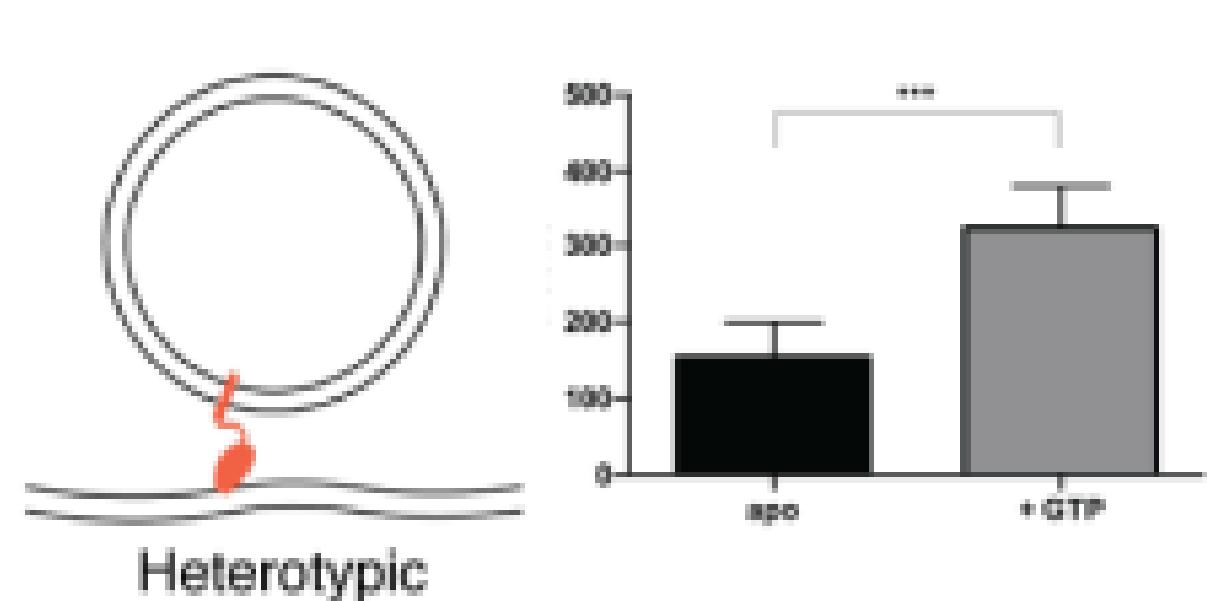


Fig. 5. I-opa1 induces heterotypic membrane tethering.

s-Opa1 (added at a protein:lipid ratio of 1:5000) can tether cardiolipin liposomes to a cardiolipin-containing planar bilayer. Indeed, we observe that this s-Opa1 is enhanced by the presence of GTP (Figure 3D). Planar bilayer retains integrity after GTP addition and no evidence of tubulation.

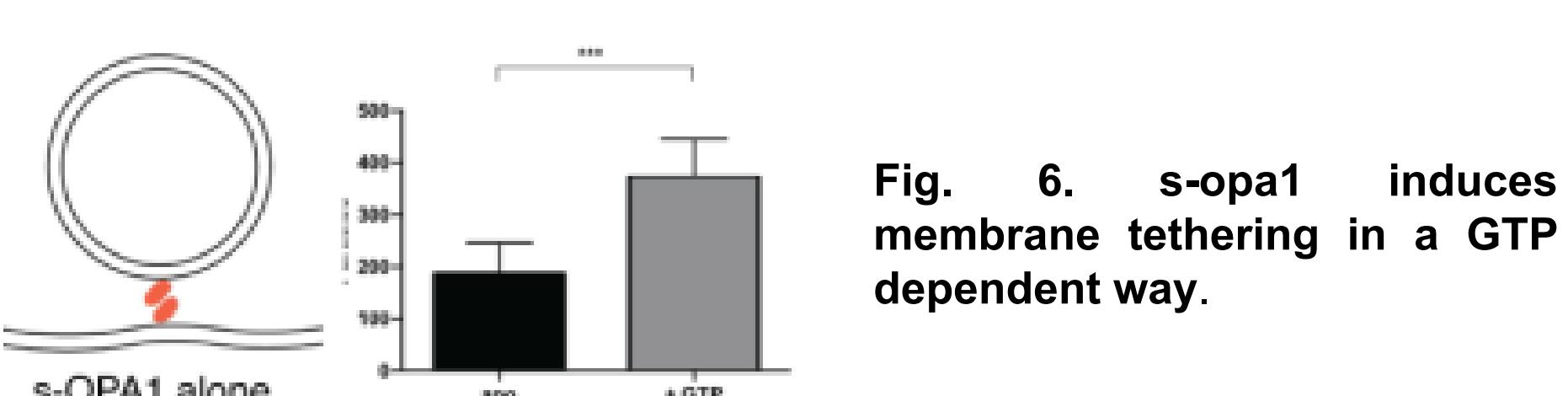


Fig. 6. s-opa1 induces membrane tethering in a GTP dependent way.

All the previously described tethering conditions exhibits differences in the membrane proximity using a FRET assay.



Fig. 7. Estimate membrane docking/tethering using FRET signal.

Hemi fusion and Pore opening (Full fusion)

l-Opa1, when present only on one bilayer, in a heterotypic format, can mediate membrane docking in a GTP-dependent manner , with no evidence of pore opening. Additionally, l-Opa1 can induce hemifusion and homotypic l-Opa1:l-Opa1 tethering is enhanced by GTP (Fig. 4A) . Non- hydrolysable analog, as well as GDP, disrupt membrane tethering (Fig. 4B) .

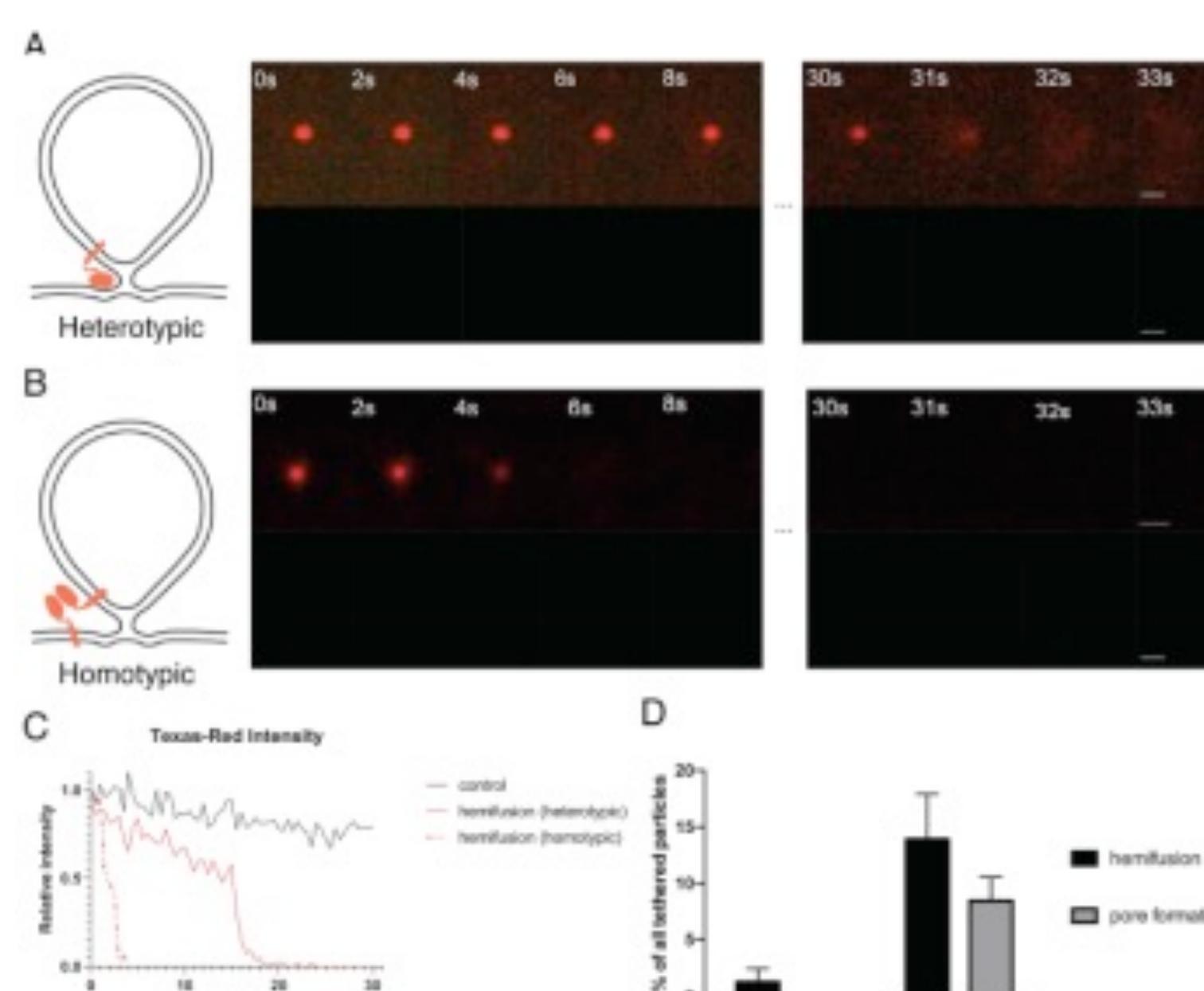


Fig. 8. homotypic and heterotypic hemifusion.

I-Opa1 is sufficient for pore opening. However the fusion efficiency was quite low. Addition of s-Opa1 promotes a faster pore opening kinetics. The ratio between l-and s-OPA1 determines fusion efficiency.

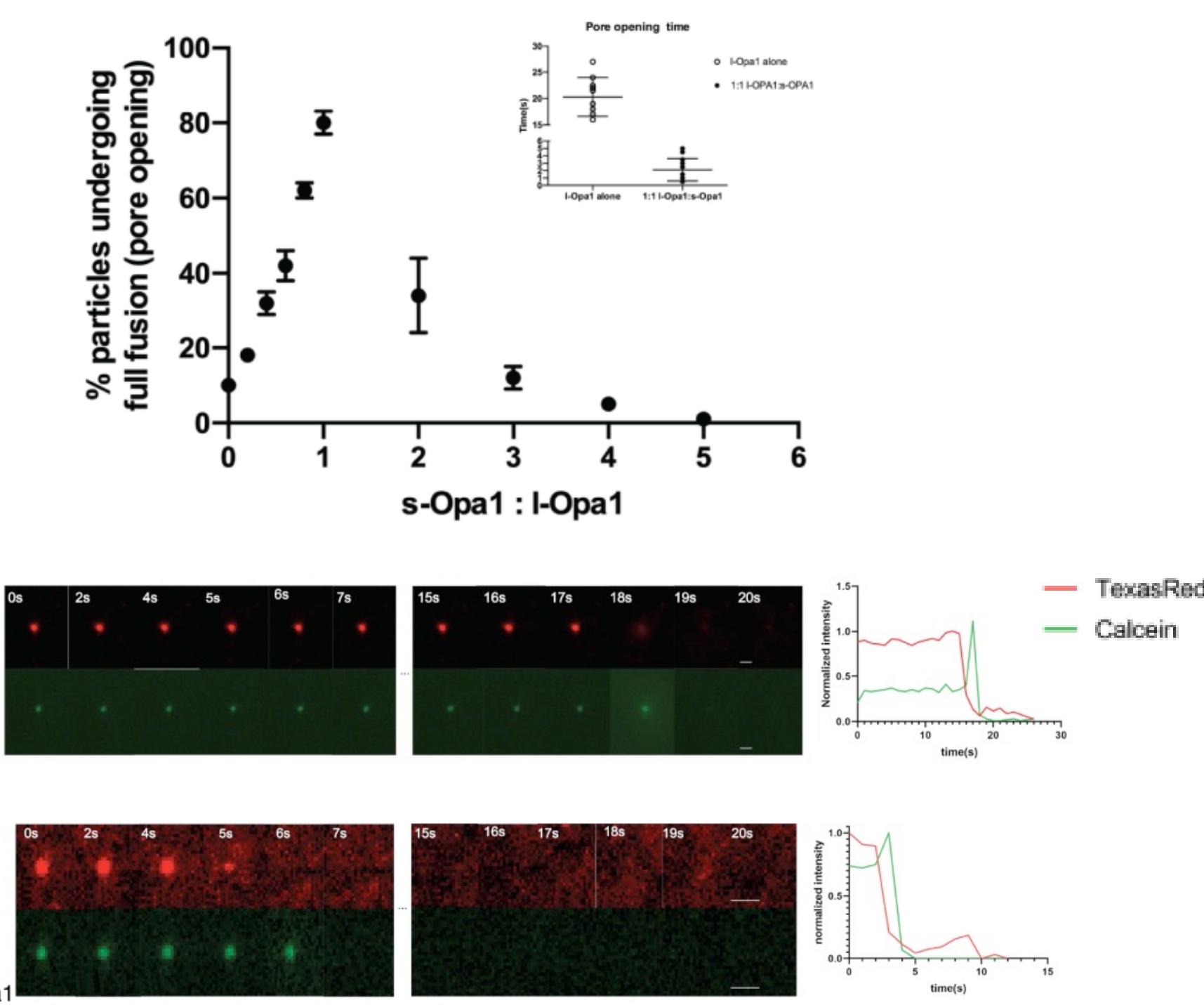


Fig. 9. Effect of s-OPA1 in the process of pore opening.

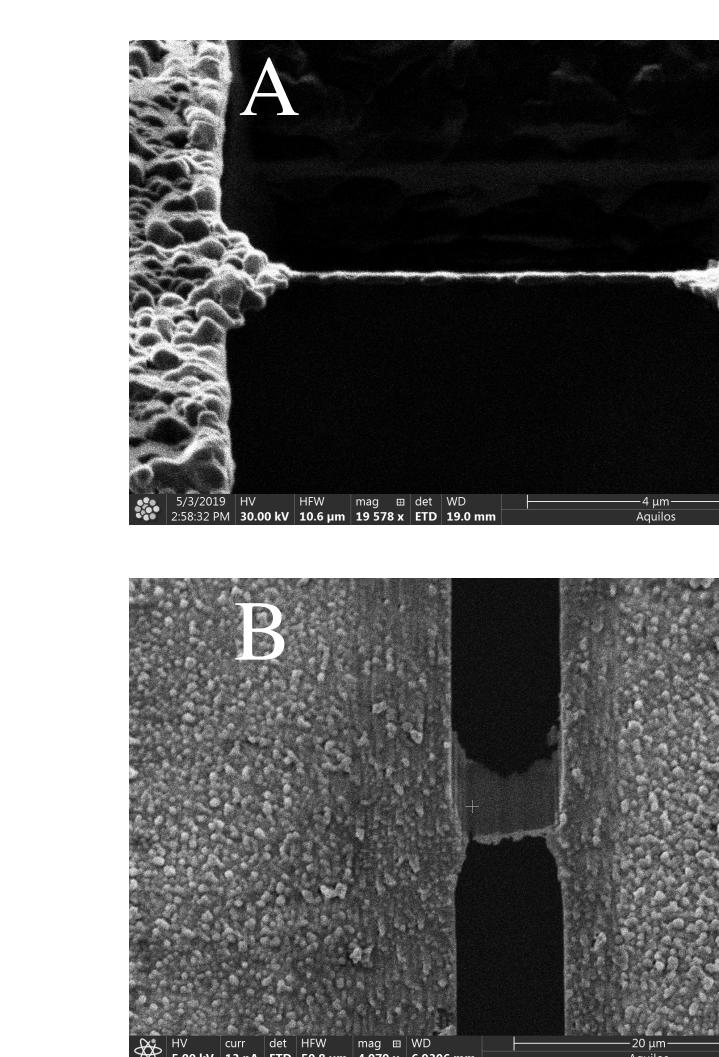
Key References

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Next: visualizing fusion complex *in situ*



Fig.10. TEM image of cell plated on grids .



We plated Opa overexpression MEFs o carbon/gold grids and did FIB to obtain images of mitochondrial. We are working to optimizing the protocols to obtain information of fusion complexes *in situ* using Cyro-ET.

Lamella samples obtained from Thermo Aquilos Cyro- FIB (A&B) and correlative TEM image

Conclusions

Our experiments support the idea that different assembled forms of Opa1 represent functional intermediates along the fusion trajectory, revealing multiple opportunities to regulate mitochondrial inner-membrane fusion. S-Opa1 by it self is not able to induce membrane fusion, but may work as a regulator of IMM fusion. Our titration experiments highlight the importance of understanding the nature of the cooperative interactions in the fusogenic assembly responsible for pore opening.

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