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SUPPLEMENTARY INFORMATION

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The functional universe of membrane contact sites

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Box1. The challenge of visualizing and quantifying MCSs.

Many MCS studies assume that any region at which two organelles come within a certain distance, often 30 nm or less, is a contact site; we have used this criterium in a study[1]. However, this assumption may not be correct. A definitive demonstration that a membrane contact site (MCS) has formed requires not just showing that two membranes are close to another but also proof that the contact alters the properties of the organelles. This proof involves demonstrating that proteins or lipids are enriched at the MCS or that enzymes are activated (or inhibited) there.

Visualizing potential MCSs is challenging because the size of most MSCs is below the limit of resolution of light microscopy, including most super resolution microscopes. Therefore, electron microscopy (EM) remains the best way to visualize contact sites. However, there are some drawbacks to EM: it cannot be performed on live cells and localizing proteins by immunogold labeling or other techniques can be challenging. Moreover, only a 3 dimensional reconstruction allows assessment of the whole interface at which two organelles are in contact.

Various techniques have been used to visualize MCSs by light microscopy. One approach is to use a fluorescent reporter that has affinity for two organelles, which enriches the reporter at regions where the organelles are in close apposition[2,3]. Other techniques use reporters localized in two different organelles that only fluoresce when the reporters interact with each other at contact sites. One example is seen with bimolecular fluorescence complementation (also known as BiFC), in which two halves of GFP are fused to proteins in different organelles and only form a functional GFP when they interact[4]. One caveat of this approach and related approaches is that the reporters can themselves drive contact formation; for BiFC, the two halves of GFP have high affinity for one another[5] and may stabilize and expand contacts. To avoid this problem, some techniques for visualizing MCSs use approaches that do not promote contact. One technique, called proximity ligation, identifies proteins close to one another at MCSs by determining regions where antibodies against the proteins can be crosslinked[6]. However, this technique requires cell fixation and cannot be used on live cells. Another method is to use dimerization-dependent fluorescent proteins that only fluoresce when the pairs are in close proximity[7]. An alternative method for assessing organelle proximity in live cells without perturbing them or fostering contact is to use Förster resonance energy transfer (FRET)- fluorescence lifetime imaging microscopy (FLIM) between two endogenously tagged fluorescent proteins that are localized in different organelles[8]. However, the limited sensitivity of this technique in live cells makes it challenging to use, particularly for detecting subtle changes in MCSs over time.

Quantifying MCSs remains another important challenge for the field. Addressing this challenge is particularly important because many studies on MCSs hinge on correlating changes in contact size or number with alterations in organelle function, dynamics, composition, or signaling. How MCS size and number are quantifed varies between studies. Thus, there is a

pressing need for the development of algorithms to quantify contacts from imaging data, particularly 3D data.

Quantifying protein enrichment at MCSs can also be challenging, at least for proteins that are not highly enriched at these sites. Cell fractionation is widely employed to quantify proteins and particularly to estimate protein enrichment at ER–mitochondria contacts. A fraction called mitochondrial associated membranes (MAMs), which is isolated by density-gradient centrifugation, is commonly used to determine protein enrichment at ER–mitochondria contacts. However, some proteins that are highly enriched in MAMs are less enriched at ER–mitochondria contacts when visualized by light microscopy; for example, the ER oxidoreductase Ero1α[9]. Therefore, the accurate quantification of protein enrichment at contacts sites may require more than one method.

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