Detection of IFITM3 clusters on the plasma membrane by single-molecule localization microscopy

Jacqueline Sung, Vicky Kliemke, Qian Liu, Chen Liang

Hôpital général juif
Jewish General Hospital
Institut Lady Davis | Lady Davis Institute

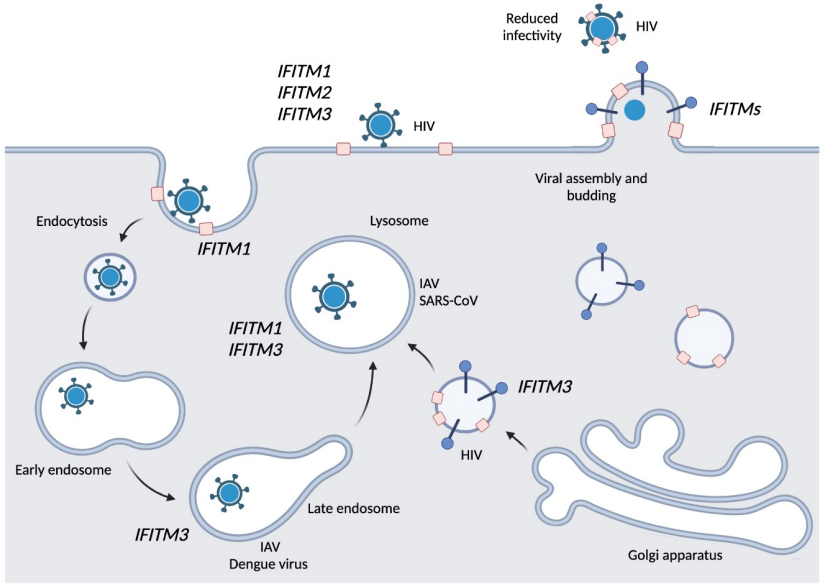
McGill

IFITMs in viral infections

- Interferon-induced membrane (IFITM) proteins inhibit a wide range of enveloped viruses such as Influenza A virus, Dengue virus, and human immunodeficiency virus (HIV-1)
- Knocking-out ifitm3 in mice upon influenza A virus results in severe morbidity and high mortality
- Single nucleotide polymorphisms in IFITM3 have been associated with rapid progression of HIV-1 infection

IFITMs and the Membrane

- IFITM3 alter membrane properties, halting the fusion between viral and cellular membrane
- It was thought that IFITM3 localized to lipid rafts from their palmitoylated residues
- A recent study demonstrated the localization of IFITM3 to the non-raft region in artificial liposomes



IFITMs in viral infections

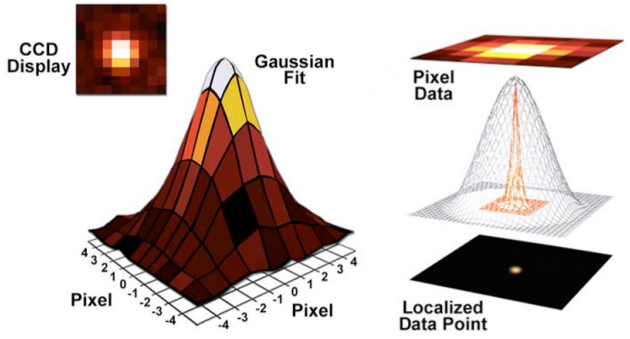
This figure demonstrates the diversity of IFITMs in virus restriction. In the case of HIV, IFITMs can inhibit membrane fusion, preventing viral entry. IFITM-containing HIV virions have also been demonstrated to have reduced infectivity. Additionally, IFITM3 can antagonize envelope protein and target the latter for degradation.

Ren et al., Front Immunol, 2020; Everitt et al., Nature, 2012; Zhang et al., AIDS, 2015; Guo et al., ACS Nano, 2021; Diamond et al., Nat Rev Immunol, 2012; Marziali et al., Cells, 2021; Ahi et al., mBio, 2020; Yu et al., Cell Rep, 2015. Figure created by Biorender.

Methodology

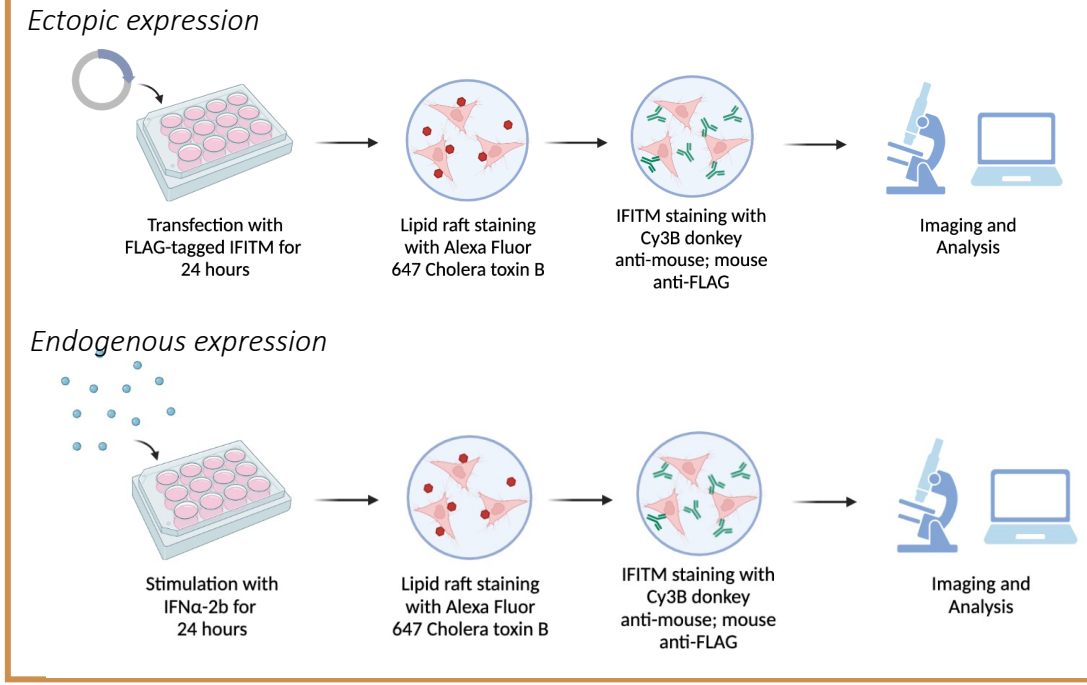
To determine the distribution of IFITM3 on the plasma membrane of intact cells with reference to lipid rafts, we used the single-molecule localization microscopy that allows the direct observation of IFITM3 organization at a 20 nm resolution.

Single-Molecule Localization Microscopy



- Due to the diffraction limit of light, resolution of conventional light microscopy is restrained
- In SMLM, the locations of detected emitters are determined
- Each location is fitted into a Gaussian curve to determine a more precise and localized signal

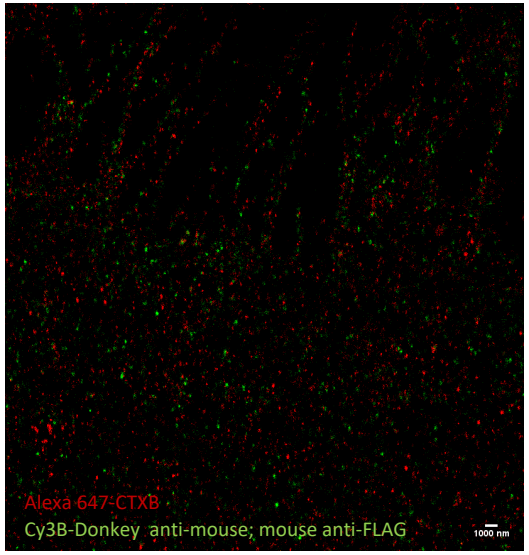
Experimental Methods



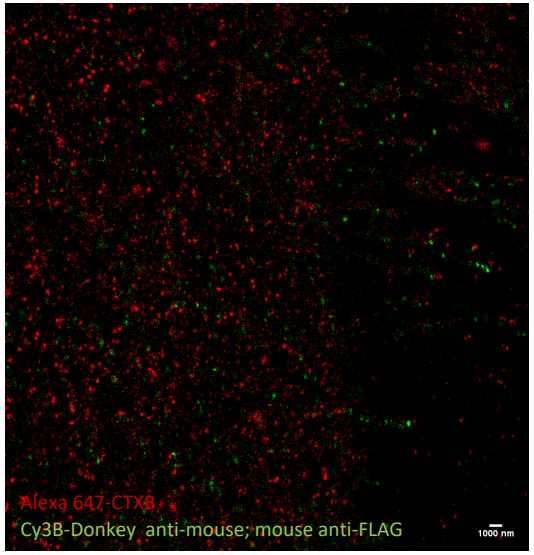
Allen et al., J Opt, 2013. Experimental Methods figure created by Biorender.

Results

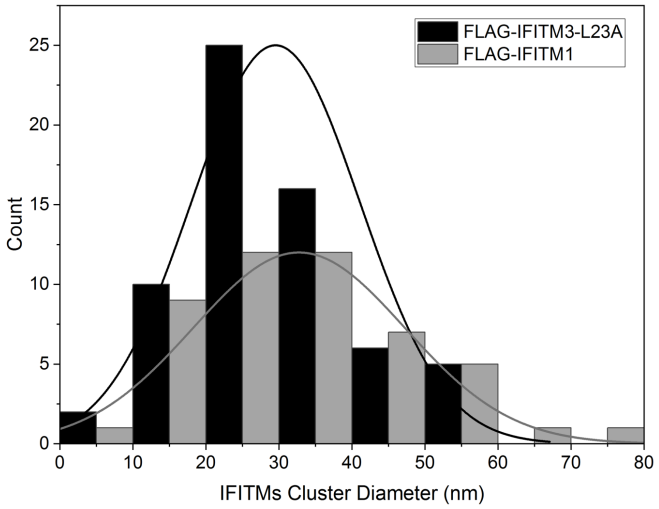
a. FLAG-IFITM3-L23A



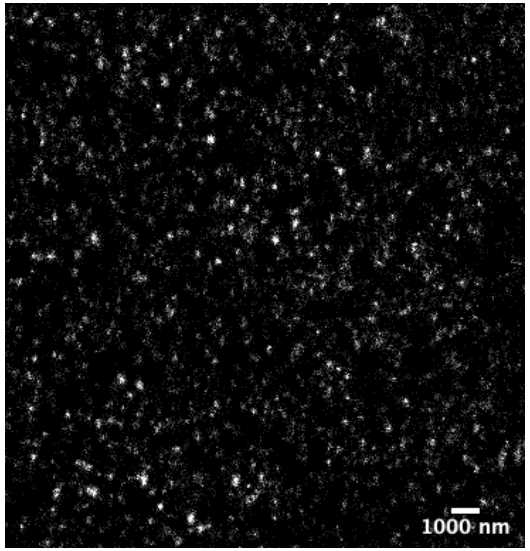
b. FLAG-IFITM1



c.



d. FLAG-IFITM3-L23A

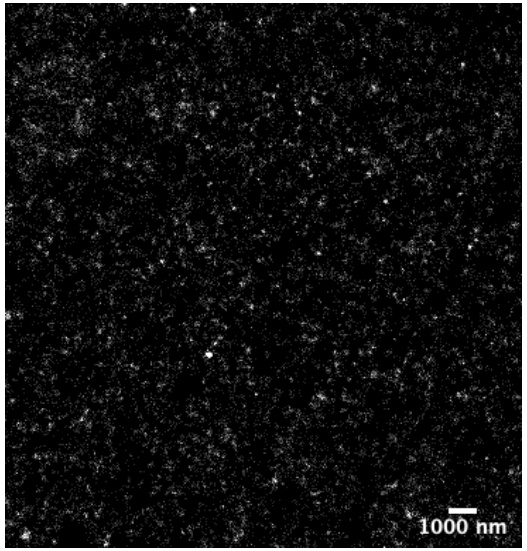


To determine the localization of IFITM3, HeLa cells were transfected with FLAG-tagged IFITM3 containing the L23A mutation which prevents its endocytosis and facilitates cell surface imaging. a) shows the lipid rafts (red) and IFITM3 clusters (green) at the plasma membrane. Clusters of IFITM3 did not overlap with those of lipid rafts.

To investigate the difference in localization between IFITMs, cells were also transfected with FLAG-tagged IFITM1. b) shows that IFITM1 clusters did not localize in lipid rafts; however, further optimization of protein expression is needed for a formal analysis. c) shows no significant difference in cluster size between IFITM3 and IFITM1.

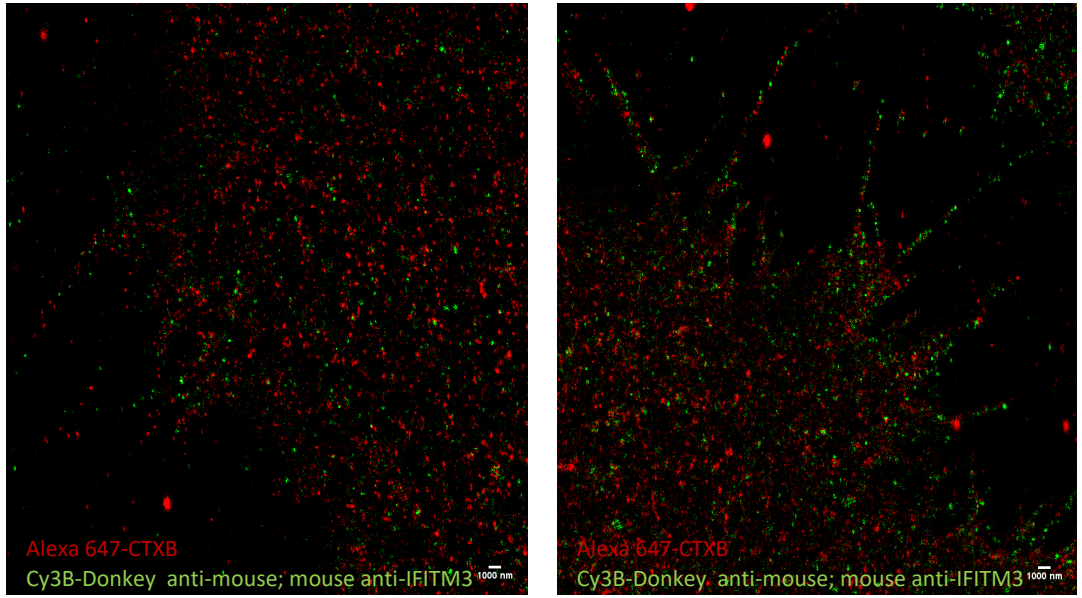
As a negative control, 293T cells were transfected with FLAG-tagged IFITM3-L23A containing a G95L point mutation, which is defective in oligomerization. Indeed, d) demonstrates a dispersion of signals and a reduced number of distinct clusters in the double mutant, confirming that the clustering phenotype observed earlier was not due to artifact of the technology.

FLAG-IFITM3-L23A-G95L

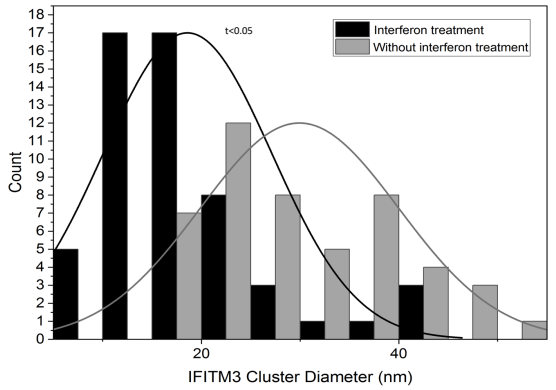


Results

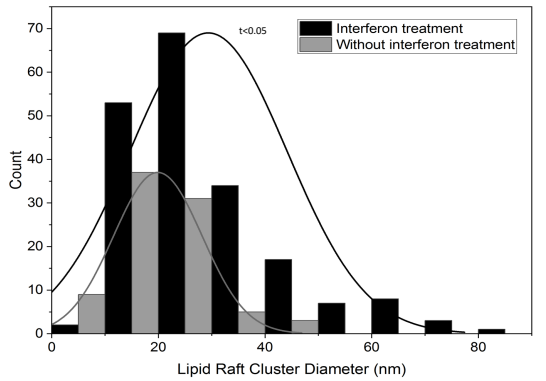
a.



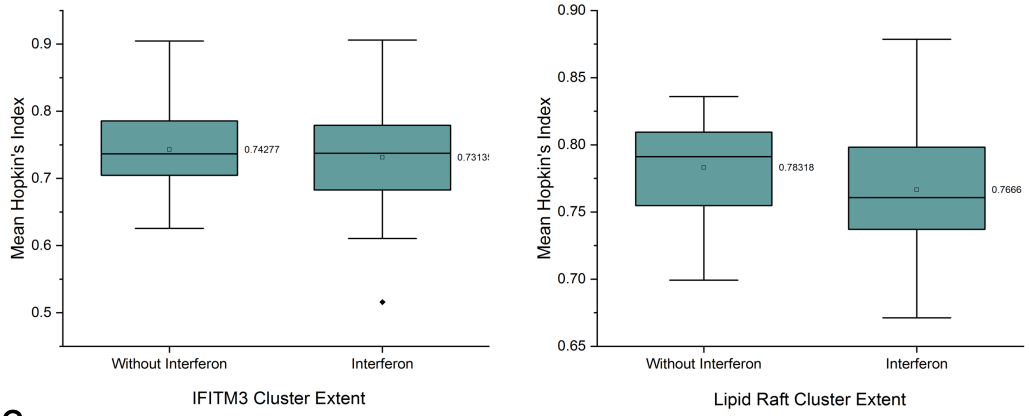
b.



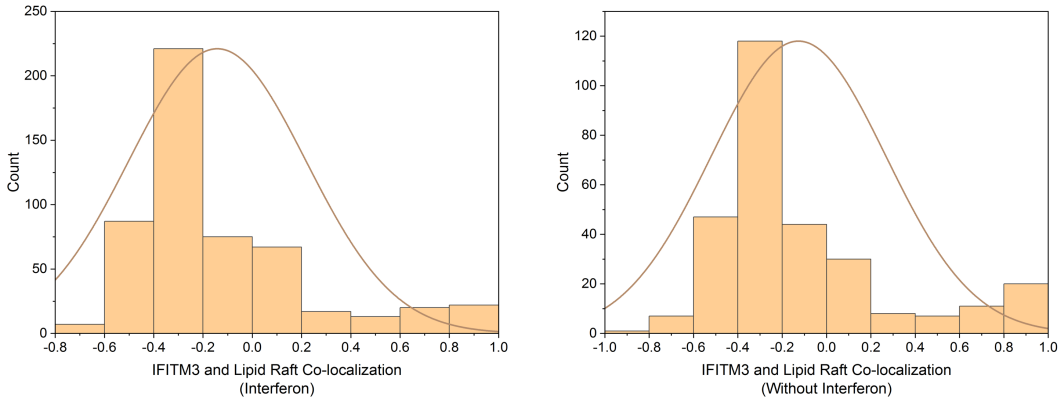
c.



d.



e.



For a more biologically relevant model, interferon treatment (IFNα-2b) was added to induce endogenous IFITM3 expression in HeLa cells. a) shows the lipid rafts (red) and IFITM3 clusters (green) at the cell surface. Endogenous IFITM3 clusters did not localize to lipid raft domains. An increase in IFITM3 cluster number could be observed with interferon treatment; however, formal computational tests are required to confirm this observation. b) and c) shows that IFITM3 cluster diameter decreased whereas lipid raft diameter increased upon stimulation, respectively. The clustering extent of both IFITM3 and lipid rafts showed no significant difference when comparing between conditions (Figure d). Lastly, e) demonstrates that IFITM3 and lipid rafts did not co-localize with one another.

Conclusion

Summary

- IFITM3 is localized to the non-raft domains
- IFITM3 forms clusters of around 20-80 nm in diameter
- Upon interferon stimulation, IFITM3 cluster number increases, cluster size decreases, and lipid raft size increases.

Future Directions

- Investigate the IFITM3 clustering pattern in endosomal and lysosomal membranes
- Determine if IFITM3 clusters affect host receptor and viral protein localization
- Establish a functional assay to assess membrane rigidity to further support our observations

Acknowledgements

Principal Investigator
Dr. Chen Liang

Collaborators
Dr. Vivian Qian Liu
Vicky Kliemke

Hôpital général juif
Jewish General Hospital
Institut Lady Davis | Lady Davis Institute

McGill

Lab members
Dr. Zhen Wang
Qinghua Pan
Myles McLean
Magan Solomon
Ariana Arduini
Frédérique Laprise
Cesar Collazos

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada