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Application of super resolution microscopy to visualise immune activated interactions between lipid droplets and STAT proteins

Abbey J Milligan[1], Ashley M Rozario[1], Ebony A Monson[2], Jay L Laws[2], Karla J Helbig[2] & Donna R Whelan[1]

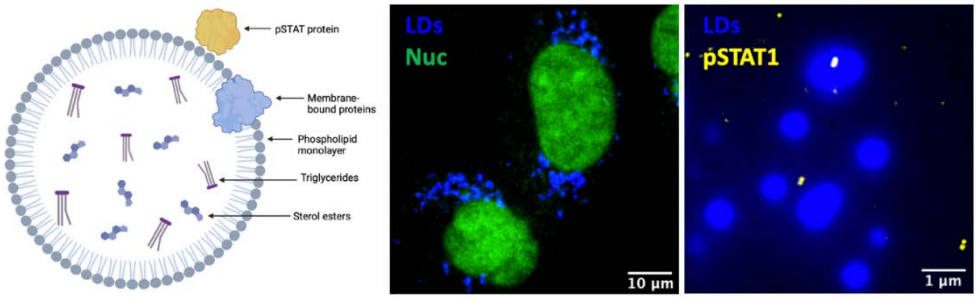
[1] *La Trobe Institute for Molecular Science, La Trobe University, Bendigo, Victoria, 3552*
[2] *La Trobe School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Victoria, 3086*

During viral infection, cells produce and secrete antiviral signaling proteins called interferons (IFNs). IFNs activate the JAK-STAT pathway, which is essential for the subsequent expression of IFN stimulated genes and are integral for viral clearance. Lipid droplets (LDs) are cellular organelles with known roles in intracellular signaling, inter-organelle interactions and transient protein sequestration. Work by our group more recently discovered that LDs increase in number, size and motility following viral infection and are key in stimulating IFN in the antiviral response [1]. However, the mechanistic role of LDs in viral clearance and particularly their interaction with the JAK-STAT pathway remains unknown.

In this study, we aim to characterise the role of LDs in the cellular antiviral response by interrogating LD interaction with the signaling protein signal transducer and activator of transcription (STAT1) and its phosphorylated state pSTAT1, having undergone phosphorylation at the tyrosine 701 [2]. We apply single molecule localisation microscopy (SMLM) [3] to image LDs and signaling proteins achieving single molecule sensitivity and resolutions of ~20-50 nm. The resolution gain over diffraction-limited fluorescence methods (eg. confocal microscopy limited to ~200 nm resolutions) provides improved visualisation and quantification of LD and STAT1 co-localisation events. STAT proteins were detected using conventional immunofluorescence, while LDs were labelled with the fluorescent lipid dye BODIPY, which we optimised for binding-based SMLM imaging.

For virally infected cell models, we transfected immortalised astrocyte cells with double stranded RNA (dsRNA) to stimulate the antiviral response and subsequent production of LDs. Figure 1 (left) depicts a diagram of LD composition with a STAT protein co-localisation event and (middle) a widefield fluorescence micrograph of fixed human astrocyte cells after 24h transfection with dsRNA; LDs (blue) stained with BODIPY, and nuclei (green) stained with DAPI. (Right) depicts a pSTAT-LD colocalization event of dsRNA transfected cells with LDs (blue) imaged using widefield microscopy together with pSTAT protein (yellow) immunolabelled with Alexa Fluor 647 and imaged using SMLM. Super resolution microscopy revealed increased LD-STAT co-localisation events following dsRNA transfection or direct IFN stimulation. This suggests the potential role for LDs as signaling and/ or trafficking platforms during the antiviral response. This interactive role of LDs and STAT proteins within the immune response could provide a novel target in the design and development of antiviral drugs and the treatment of viral infection in humans.

Figure 1.



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