Super-Resolution Characterization of Microtubule Architecture in Cells Expressing Lyssavirus Phosphoprotein

Rozario, A.[1], Bell, T.[1], Brice, A.[2] and Moseley, G.[2]

[1] School of Chemistry, Monash University, Australia, [2] Faculty of Medicine, Nursing & Health Sciences, Monash University, Australia

Viruses are biological agents usually several hundred nanometers in size which cause fatal epidemics throughout human history. Upon infection, viral proteins form extensive interactions with host proteins and organelles to evade immune responses and induce pathogenesis. Lyssavirus phosphoproteins (P1-P5) antagonize interferon-mediated antiviral responses through binding and inhibiting nuclear import of transcription factor STAT1 [1]. Previously, we observed rabies lyssavirus (RABV) P3 binds to MTs and causes bundling *in vivo*. Working with several mutant-P3 RABV strains, we also showed the MT bundling ability of P3 correlated with the level of pathogenicity and fatality in mouse models [2]. From confocal imaging, STAT1 was also detected within the P3-induced bundles. While the P3-STAT1 viral-host interface is essential for immune evasion, the purpose for forming such large MT bundles and its role in disease progression is unclear.

To study bundle structure, we have developed assays for a range of correlative and complementary state-of-the-art imaging techniques including atomic force microscopy (AFM), expansion microscopy (ExM) and super-resolution fluorescence microscopy (SRFM) using our home-built widefield microscope setups [3, 4]. We are also optimizing multi-colour SRFM to simultaneously visualize each component in P3-induced bundles with sub-diffraction resolution. Traditional diffraction-limited fluorescence microscopy lacks sufficient spatial resolution to distinguish individual MT strands (30 nm wide) from bundles containing tens of MTs (60 - 300 nm wide). Single molecule localization microscopy (SMLM) circumvents diffraction and allows for 3D super-resolution imaging with spatial resolution as good as 20 nm. We have used SMLM to determine that P3 of different lyssaviruses have varying effects on host MT architecture (Figure 1) suggesting P3-induce bundle formation is not a conserved mechanism for lyssavirus pathogenesis. We are using SMLM and proteomics to investigate the other P isoforms (P1-P5) to determine key amino acid residues for MT binding and bundling.

Expansion microscopy [5] is a recently developed technique that physically expands the sample embedded and cross-linked in a swellable hydrogel matrix. Using this approach we have been able to expand MT bundles by a factor of ~4 allowing us to interrogate the bundling structure in more detail using conventional confocal scanning microscopy. We are currently attempting to combine ExM with SMLM to theoretically enhance imaging resolution to 5 nm, achieving single protein resolution. By merging advanced imaging techniques with biochemistry assays, we hope to elucidate the mechanism and function of MT bundling in viral disease.

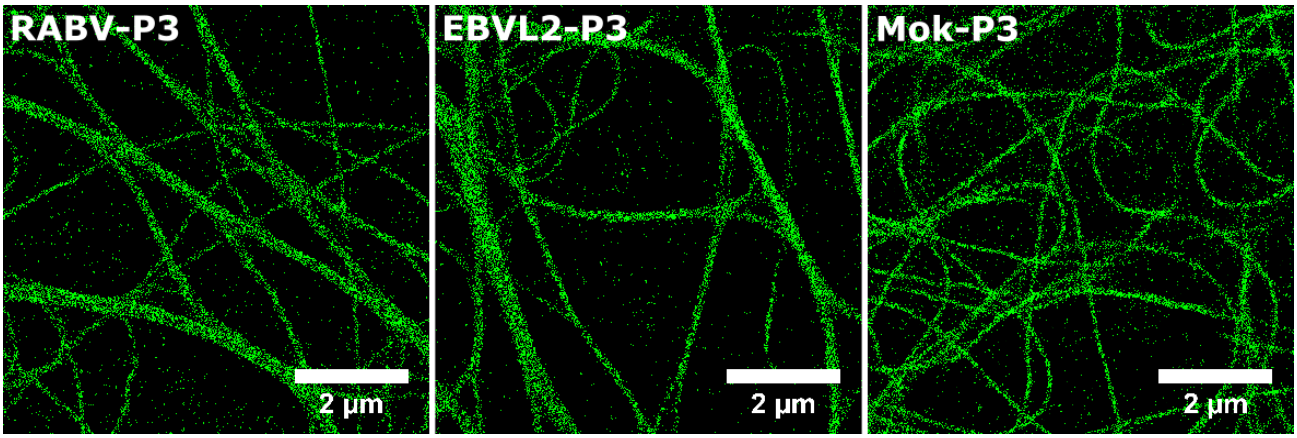

Figure 1. Microtubule bundling is not a conserved effect among lyssavirus P3. Single molecule super-resolution images of COS-7 microtubules labelled with Alexa Fluor 647 and transfected with P3 of Rabies virus (left), European bat virus (middle), and Mokola virus (right).

References

1. Wiltzer, L., et al., *Conservation of a Unique Mechanism of Immune Evasion across the Lyssavirus Genus.* Journal of Virology, 2012. **86**(18): p. 10194-10199.
2. Brice, A., et al., *Quantitative Analysis of the Microtubule Interaction of Rabies Virus P3 Protein: Roles in Immune Evasion and Pathogenesis.* Scientific Reports, 2016. **6**: p. 33493.
3. Proppert, S., et al., *Cubic B-spline calibration for 3D super-resolution measurements using astigmatic imaging.* Optics Express, 2014. **22**(9): p. 10304-10316.
4. Whelan, D.R., et al., *Focus on Super-Resolution Imaging with Direct Stochastic Optical Reconstruction Microscopy (dSTORM).* Australian Journal of Chemistry, 2014. **67**(2): p. 179-183.
5. Chozinski, T.J., et al., *Expansion microscopy with conventional antibodies and fluorescent proteins.* Nat Meth, 2016. **13**(6): p. 485-488.