

# REVEALING THE HOST-PATHOGEN INTERFACE OF VIRUSES USING SINGLE MOLECULE SUPER-RESOLUTION MICROSCOPY.

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We have applied single molecule localisation microscopy (SMLM) [1,2] to study proteins from rabies and Hendra viruses within the cellular milieu. SMLM vividly reveals that P3 protein of *Nishigahara*, a pathogenic strain of rabies virus, associates with microtubules (MTs) of the cellular cytoskeleton, leading to the formation of bundles containing many MTs. (Fig 1, left). A mutated version of P3 that attenuates viral pathogenesis does not induce bundle formation. Importantly, a single point mutation within P3 is identified, which strongly inhibits both MT bundling, immune evasion, and pathogenesis, indicating key roles of MT bundling in lethal disease.[3] Furthermore, P3 proteins from rabies-related lyssaviruses differ significantly in their relationship with MTs, indicating diverse functional interactions by these lethal pathogens.

We have also studied the important interaction of Hendra virus M protein with subnuclear multifunctional structures called nucleoli, showing that nucleoli can be successfully imaged using SMLM by labelling nucleolar marker proteins. We are now using this approach to map the subnucleolar localization of M protein and its impact on nucleolar ultrastructure.[4] These data reveal detailed information of the host-virus interface at resolution well inside the diffraction limit, improving our understand of the molecular cell biology of two of the most lethal viral pathogens known.

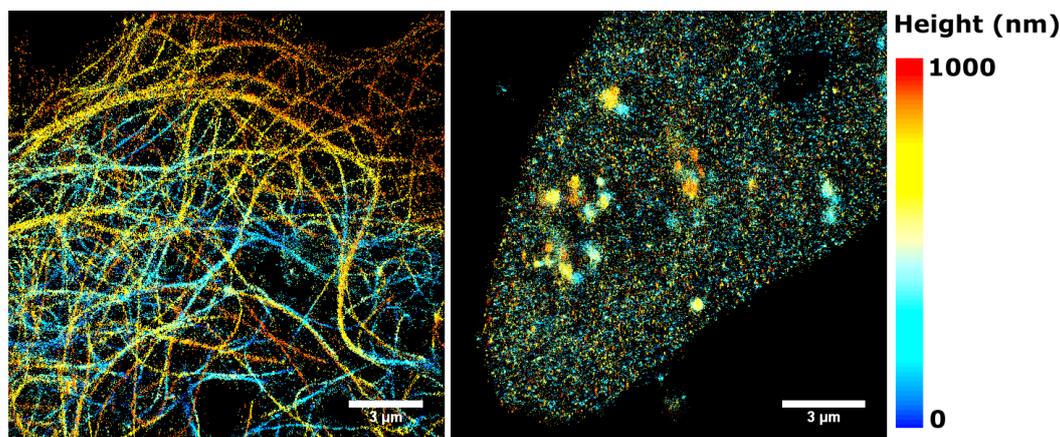


Fig. 1: 3D SMLM images of (left) a COS-7 cell transfected to express RABV P3 proteins and labelled for tubulin with Alexa 647 showing features much larger than expected for a single MT and consistent with MT bundling; and (right) a HeLa cell transfected to express HEV M proteins and labelled for nucleoli marker proteins with Alexa 647 showing 'puncta' located within nucleoli inside the nucleus.

- [1] D. R. Whelan and T. D. M. Bell, Super-Resolution Single-Molecule Localization Microscopy: Tricks of the Trade, *J. Phys. Chem. Lett.*, (2015), doi: 10.1021/jz5019702.
- [2] D. R. Whelan and T. D. M. Bell, Image artifacts in Single Molecule Localization Microscopy: why optimization of sample preparation protocols matters, *Sci. Rep.*, (2015), doi: 10.1038/srep07924.
- [3] A. Brice et al., Quantitative Analysis of the Microtubule Interaction of Rabies Virus P3 Protein: Roles in Immune Evasion and Pathogenesis, *Sci. Rep.*, (2016), doi:10.1038/srep33493.
- [4] S. Rawlinson et al., Viral subversion of Treacle protein: a novel mechanism to regulate ribosomal RNA biogenesis, *submitted*.