

PhD scholarship project

CSIRO LIVESTOCK INDUSTRIES



Project title: Visualising the structural basis for infection of mammalian cells with Hendra virus

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University affiliation: Monash University **Location:** AAHL, East Geelong

Brief Project description:

This project will investigate the interaction of Hendra virus with the highly conserved mammalian cell surface receptors Ephrin B2 and B3 using state-of-the-art visualisation technology. The combined use of light and electron microscopy will allow correlation of observations from live cell infection experiments with high-resolution structural data, specifically in relation to the interactions of the Hendra virus surface glycoprotein projections with cell surface receptors. Ultimately, cryo- electron tomography will be used to acquire structural data based on initial work with chemically fixed and thus harmless preparations. For the purposes of risk mitigation, it is essential to accurately quantify the numbers of infectious particles. Another pleomorphic virus, avian influenza, will be used as a surrogate for quantitation and technical development under PC3 conditions. This project will provide a basis for the establishment of an electron microscopy research capability within the NCRIS PC4 imaging room, the construction of which is scheduled for completion in early 2011.

In the first 18 months, the student will learn and apply state-of-the-art live cell imaging, biological electron microscopy and fundamental image processing. In the second half of the project, the student will compile comprehensive and systematic visual data concerning the Hendra virus infection cycle. Specifically, the student will acquire a skill base applicable to a broad range of research activities and international demand by undertaking a comprehensive series of experiments directed at:

1. Establishment and testing of quantitative criteria to assess the viability/infectivity of cells/viruses following sample preparation and decontamination steps.
2. Establishment of a functioning system for performing immunocytochemistry on Hendra virus –infected cells.
3. Provision of visual data to interpret Hendra virus interactions with the cell surface and key stages of pathogenesis and virus morphogenesis.

During the 2nd and 3rd years of the project, the student will achieve competency in acquisition and reconstruction of tomographic data. The 2nd year will concentrate on electron tomography of chemically-fixed (and thus attenuated) Hendra virus –infected cells. In the 3rd year, the student will study Hendra virus infection by live cell imaging and correlative light microscopy and electron tomography. The latter can be achieved with a comparatively low risk of failure using conventional sample processing; achievement of this outcome using cryogenic methodology carries a considerably higher risk of failure but will not be critical to basic outcomes. A new picture of infection with Hendra virus will be accompanied by:

1. Knowledge of cell culture and cell biology, specifically via immunolabelling and classical ultrastructure.
2. Expertise in live cell imaging using wide-field epifluorescence microscopy and confocal laser scanning microscopy
3. Expertise in electron tomography including systematic data acquisition, alignment and reconstruction of tilt series, and surface rendering / visualisation.
4. Communication skills via the publication of high quality manuscripts, poster presentations at local virology and microscopy meetings, and ideally, a presentation at an international meeting.

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