

## Detection of fluorescently labeled proteins by electron microscopy

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### Abstract

Localising the position of proteins in the cell at high-resolution is critical for determining cellular function. As GFP revolutionized the detection of proteins by light microscopy, similarly new genetic tags for electron microscopy (EM) have great potential for EM visualization of proteins in cells. We have developed a modular system for enzyme-based protein tagging that facilitates the detection of fluorescently-tagged proteins in the electron microscope by employing a modified soybean ascorbate peroxidase system termed APEX [1]. This system allows for efficient analysis of subcellular protein distributions using existing GFP- and mCherry-tagged protein libraries. We demonstrate we can target APEX to any GFP- or mCherry-tagged protein of interest by engineering and genetically encoding a specific nanobody/binding peptide (BP) fused directly to the APEX-tag. We show that this system is robust, rapid and can be used in animal models. Moreover, this method compatible with correlative light and electron microscopy and can be coupled with sensitive methods for detecting protein-protein interactions through the use of split-GFP. The application of this method to a number of cell biological questions will be addressed in the talk.

- 1 Lam, S.S. et al. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nature Methods* (2014).

### Bio

Dr Ariotti began his research career at the University of Queensland where he completed a PhD in Cell and Membrane Biology with Prof Rob Parton at the Institute for Molecular Biosciences. During this time, he used diverse TEM-based techniques to understand the structure, function and composition of specialized plasma membrane microdomains termed caveolae. Since then, Dr Ariotti's research has focused on developing novel correlative light and electron microscopy techniques to understand how proteins and lipids are organised at the cell surface. In early 2017 he took up an Associate Director role at the Electron Microscope Unit at The University of New South Wales. He has published 23 peer reviewed articles in journals including *Cell*, *Nature Cell Biology*, *Current Biology*, *The Journal of Cell Biology* and *Developmental Cell*.