

**P044** Generation and Characterisation of PtdIns(3,4)P<sub>2</sub> Transgenic Bioprobe to Explore Spatio-temporal Regulation of PI(3,4)P<sub>2</sub> Accumulation In T Lymphocytes.

**Richard V Parry<sup>1</sup>, Linda Sinclair<sup>2</sup>, Doreen A Cantrell<sup>2</sup> and Stephen G Ward<sup>1</sup>.**

*<sup>1</sup>University of Bath, Dept of Pharmacy and Pharmacology, Claverton Down, Bath BA2 7AY U.K. <sup>2</sup>University of Dundee, Division of Cell Biology and Immunology, Nethergate, Dundee, DD1 4HN U.K.*

Phosphoinositide 3-kinases (PI3Ks) have been implicated in a wide range of cellular functions by pharmacologic or genetic experiments that oppose lipid kinase catalytic function. These experiments however, do not distinguish between the individual lipid products of PI3Ks. The major lipid product of PI 3-kinases is PtdIns(3,4,5)P<sub>3</sub> however, levels of PtdIns(3,4)P<sub>2</sub> also rise within T lymphocytes following cellular activation. Pleckstrin homology (PH) domains of some proteins can bind these lipids separately, for example the tandem PH domain protein (TAPP)1 binds PtdIns(3,4)P<sub>2</sub> but not PtdIns(3,4,5)P<sub>3</sub>. To explore the biology of PtdIns(3,4)P<sub>2</sub> we have fused the TAPP1 PH domain to fluorescent reporters such as GFP and RFP to create a lipid specific bioprobes. We have expressed RFP-TAPP in T cell lines and human primary CD4<sup>+</sup> T cells to image PtdIns(3,4)P<sub>2</sub> within cells, responding to different stimuli. Further we have placed (G)/RFP-TAPP under the control of the mouse Vav promoter to create transgenic mice expressing G/RFP-TAPP in their T cell compartment. These studies should enable non-invasive imaging of PtdIns(3,4)P<sub>2</sub> in live cells responding to physiological stimuli.