

**P025** A fluorescent probe to visualize PKB/Akt activity in living cells  
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Phosphoinositide 3-kinase (PI3K) and its downstream targets contribute to the control of cell growth and proliferation<sup>1</sup>. Protein kinase B (PKB/Akt) is a key element in these signalling pathways, but can currently not be easily measured in living cells.

Here, we describe a fluorescence resonance energy transfer (FRET)-based assay aiming to monitor PKB/Akt activity through phosphorylation of artificial substrates. The over-expressed fusion proteins serving as substrates consist of a cyan fluorescent protein (CFP) followed by a PKB/Akt substrate region and an adjacent yellow fluorescent protein (YFP). The substrate region was varied and harbours a truncated 14-3-3 $\zeta$  flanked by PKB phosphorylation sites derived from the forkhead transcription factor FKHL-1 (P1). A second series of substrates integrated amino acids 27-34 or 247-254 from FKHL-1 only (P2). 14-3-3 proteins bind to phosphorylated serine and threonine side chains in a sequence specific manner. Thus, they retain FKHL-1 cytosolic, when phosphorylated by PKB/Akt at Thr32, Ser 253 and Ser 319.

The artificial, fluorescent substrates were strongly phosphorylated in COS7 cells when PKB/Akt was active, and the PI3K-inhibitor wortmannin prevented this phosphorylation. That the expressed substrates were phosphorylated at the predicted sites was verified using Ser/Thr to Ala mutations of the various constructs. Cellular redistribution and technical issues are currently being optimized.

<sup>1</sup>Wymann et al. 2003, *Trends Pharmacol. Sci.* 24:366