

## **P010** Proteomic Analysis of Intracellular Signalling

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Bristol University's Proteomics Facility is involved in several projects aimed at identifying components of specific signalling pathways. The gel-based approach we are taking involves the use of 2D gel electrophoresis coupled to western blotting with antibodies that detect proteins which have been phosphorylated by specific kinases, such as PKA, PKB and PKC isoforms. Proteins that cross-react with the antibody of interest are picked from the 2D gel and identified using mass spectrometry. We are also developing a gel-free approach based on a modified Isotope-Coded Affinity Tagging (ICAT) technique. In this approach, we use a  $\beta$ -elimination and Michael addition reaction to replace the phosphate group on phosphoserine and phosphothreonine residues with ethane dithiol. The modified peptides from two different conditions can be captured and labelled in a single step using thiol-reactive solid-phase reagents containing either light or heavy (deuterated) stable isotope tags. The captured peptides are then pooled, released from the solid phase support by UV photo-cleavage and analysed by capillary liquid chromatography and tandem mass spectrometry, allowing identification of the phosphoprotein and the site of phosphorylation. Signals for peptides present in both samples will appear as doublets separated by the mass of the tag, with the ratio of the two signals in the doublet reflecting the amount of the particular phosphoprotein in each sample.