

P022 The N-terminus of the class II phosphoinositide 3-kinase-C2 β regulates lipid kinase activity and its binding to clathrin
Matthew Wheeler and Jan Domin
*Renal Section, Faculty of Medicine Imperial College London,
Hammersmith Hospital, Du Cane Road, London W12 0NN, UK*

Our data shows that elevating the expression of PI3K-C2 β and EGFR increases the association of both enzymes *in vivo* following ligand activation. Deletion of the PI3K-C2 β N-terminus attenuates this interaction, confirming our previous *in vitro* findings. Immunoprecipitation of PI3K-C2 β N-terminal mutants revealed that deletion of proline rich motifs failed to alter specific activity. However, purification of each preparation to homogeneity demonstrated that deletion of residues 1-136 abolished enzyme activity while deletion of residues 1-148 and 1-261 restored and increased kinase activity respectively. This raised the possibility that proteins associating with this region of PI3K-C2 β regulate enzyme activity. To identify candidate proteins, residues 1-273 of PI3K-C2 β were expressed as a recombinant fusion protein to affinity purify proteins from A431 cell lysates. A candidate protein with an apparent molecular mass of 190kDa was identified as clathrin heavy chain. Association of clathrin with PI3K-C2 β was confirmed by co-immunoprecipitation from cell lysates while confocal microscopy revealed their intracellular co-localisation.