

**GLUT4 VESICLE TRANSPORT AND THE INSULIN SIGNALLING
CASCADE – A PROTEOMIC INVESTIGATION BASED ON AFFINITY
PURIFICATION AND 2D LC-MS/MS**

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A powerful combination of the selectivity of 2D LC-MS/MS with affinity based “subsetting” of proteins from cell fractions has been used to investigate GLUT4 Vesicle transport. This pathway is not well understood and is likely to be important in type II diabetes. Insulin stimulates glucose transport in muscle and fat cells by triggering the exocytosis of intracellular GLUT4 storage vesicles (GSVs). The major aim of this project was to map the protein complement of GSVs in order to better understand GLUT4 vesicle translocation, especially regarding the link between the insulin signalling cascade and GLUT4 vesicle translocation. In this study we have identified proteins associated with GLUT4 vesicles from 3T3-L1 adipocytes and rat primary adipocytes using a proteomics approach which combines affinity purification with 2D-LC-MS/MS. 52 proteins were identified in GLUT4 vesicles from 3T3-L1 adipocytes and 19 proteins in GLUT4 vesicles from rat adipocytes, with 18 of these proteins being found in both cell types. One of the most intriguing findings was that the Rab-GAP AS160 associates with GLUT4 vesicles. This presentation will focus on the bioanalytical strategies developed in this project – in particular finding the best combination of sampling approach, separation method and tandem mass spectrometric techniques. For example, one challenge that was addressed was balancing the affinity system to be sensitive to target proteins but not dominated by non-specific interactions.