

# Caitlin Fischer, Chemistry

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## **Mutations to the caveolin scaffolding domain reduces Caveolin-1 targeting of glycolytic enzymes to lymphocyte membranes**

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Previously, we found caveolin (CAV-1) expressed by transfection in cultured lymphocytes induced caveolae formation and targeted the glycolytic enzyme phosphofructokinase (PFK) to the membrane. We also found CAV-1 targets other glycolytic enzymes such as aldolase (ALD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to the plasma membrane in the CAV-1 transfected lymphocytes. Here we hypothesized that if a mutant CAV-1 (which has essential aromatic residues in the caveolin scaffolding domain (CSD) mutated) is expressed in the lymphocyte then colocalization of the glycolytic enzyme PFK with CAV-1 will be reduced. We tested this hypothesis by comparing the colocalization of CAV-1 with the glycolytic enzymes PFK, ALD and GAPDH in lymphocytes which expressed either a wild type CAV-1 (WT) or a mutant CAV-1 which had either one mutation (SM) or two mutations (DM) in the CSD. Colocalization analysis by confocal microscopy of cells immunoassayed for CAV-1 and ALD was 76.59% in lymphocytes transfected with CAV-1 WT, 23.96% in lymphocytes transfected with CAV-1 SM, and 58.74% in the lymphocytes transfected with CAV-1 DM. Analysis of colocalization of the enzymes PFK, GAPDH, and ALD with CAV-1 averaged 65.17% for the CAV-1 WT cells, 49.29% for the CAV-1 SM cells and 50.81% for the CAV-1 DM cells. The shift in distribution of glycolytic enzymes and CAV-1 in the CAV-1 WT, the CAV-1 SM or DM CAV-1 types indicates that a single mutation to the CSD reduces membrane targeting of glycolytic enzymes, and two mutations in the CSD produces retention of CAV-1 in the cytosol. These results suggest that an intact CSD domain is essential to the CAV-1 targeting of glycolytic enzymes to the membrane.