

## A NOVEL SITE FOR 6-PHOSPHOFRUCTO-1-KINASE ASSOCIATION WITHIN ERYTHROCYTES MEMBRANE CYTOSKELETON

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Erythrocytes are an important cellular type that has a unique cytoskeleton attached to the inner part of the bi-layer membrane anchored by membrane proteins. F-actin is one of the most important structural protein of cells cytoskeleton. The erythrocyte membrane protein Band 3 is described to be a site for association of glycolytic enzymes, such as 6-phosphofructo-1-kinase (PFK), presenting inhibitory properties which is the hitherto site described for glycolytic enzymes. The present study investigates the association of PFK with F-actin present on erythrocyte membrane cytoskeleton complex. Fluorescence microscopy experiments demonstrate the co-localization of PFK and F-actin within the inner side of membranes of intact erythrocytes, as well as in experiments using purified erythrocytes membrane and PFK. Co-sedimentation experiments followed by Western Blotting analysis confirmed these results revealing that PFK co-sediments with F-actin in fractionated erythrocytes extracts. Additionally, fluorescent resonance energy transference (FRET) demonstrated that FITC-labeled PFK binds to phalloidin-TRITC-labeled erythrocytes membrane, which was not mimicked using FITC-labeled glyceraldehyde-3-phosphate dehydrogenase (an enzyme that binds to Band-3). Altogether, these results support evidences to propose a novel site for PFK binding within erythrocytes membrane cytoskeleton with potential regulatory properties.

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