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STUDYING MEMBRANE TRANSPORT PROTEIN DYNAMICS WITH TRYPTOPHAN PHOSPHORESCENCE SPECROSCOPY

Jaap Broos[1], Giovanni B. Strambini[2], Margherita Gonelli[2], Gea Schuurman Wolters[1], Erwin P.P. Vos[1], George T. Robillard[1]: [1]University of Groningen, Biochemistry, Nyenborgh 4, Groningen, 9747AG Netherlands, [2]CNR, biofisica, via Alfieri 1, Pisa, 56010 Italy

A detailed description of the mechanism of membrane-bound transporters requires the characterization of the conformations of the protein involved in the different stages of solute translocation. However these changes are either are not detected by conventional biophysical methods or, as in the case of fluorescence, are not readily ascribed to specific structural alterations. In the past decade, the phosphorescence emission of tryptophan residues was shown to provide an exquisitely sensitive monitor of the local protein structure. We implemented this technique for membrane proteins as demonstrated for the mannitol transporter, EII mtl , from E. coli. This protein is responsible for the transport of mannitol across the inner membrane and its concomitant phosphorylation to mannitol 1-phosphate. Phosphorescence decays in buffer revealed large variations of the triplet lifetimes of the wild-type protein and six single-tryptophan-containing mutants. Mannitol binding induces a more ordered- and homogeneous structure near the mannitol binding site. In contrast, enzyme phosphorylation induces a large relaxation of the protein structure at the reporter sites. The implications of these structural changes on the coupling mechanism between the transport and the phosphorylation activity of EII mtl are discussed.