Structural insights into membrane protein targeting to multivesicular bodies – characterization of the interaction between LIP5 and AQP2

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The multivesicular sorting machinery is a crucial mechanism for targeting membrane proteins for recycling or degradation. The lysosomal trafficking regulator-interacting protein 5 (LIP5) which coordinates the action of this machinery is also known to bind directly to the membrane protein cargo. In case of aquaporin 2 (AQP2) the binding of LIP5 during the endocytic pathway in kidney collecting duct cells ensures an effective regulation of urine volume [1].

In our group, we have previously studied the role of AQP2 phosphorylation in AQP2-LIP5 interaction [2]. Currently we are focusing on elucidating the structural details of the complex in order to better understand how membrane proteins are delivered to the multivesicular bodies. We have constructed alanine mutants of single residues in the proposed binding sites of both AQP2 and LIP5. Studying the binding affinity of these mutants using fluorescence quenching helps us understand which residues are directly involved in the binding.

Further, AQP2 was successfully incorporated into MSP-based nanodiscs and negative stain electron microscopy confirmed homogeneous state of the particles. We have collected high resolution images on Titan Krios and are currently processing the data.

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[2] J. V. Roche, S. Survery, S. Kreida, V. Nesverova, H. Ampah-Korsah, M. Gourdon, P. M. T. Deen, and S. Törnroth-Horsefield, “Phosphorylation of human aquaporin 2 (AQP2) allosterically controls its interaction with the lysosomal trafficking protein LIP5,” J. Biol. Chem., vol. 292, no. 35, pp. 14636–14648, 2017.