Decoding the AQP-Ezrin connection: a structural and functional insight into their interactions

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All cells in our body relies on exact connections between the plasma membrane and the actin cytoskeleton to maintain their functionality. Key linkers of this system are the family of proteins Ezrin, Radixin and Moesin (ERM) which are responsible for coordinating, protein localization, signaling, and membrane organization (1). Among their partners are human aquaporins (AQPs), whose positioning at the plasma membrane is thought to depend on interactions between their C-terminal regions and the ezrin FERM-domain (2).

In our published work **Structural Basis for the Interaction between the Ezrin FERM-Domain and Human Aquaporins**, we managed to uncover that this interaction occurs in AQP2 and AQP5. Utilizing microscale thermophoresis (MST), we observed that both the full-length AQPs and their corresponding C-terminal peptides binds to the ezrin FERM-domain within a micromolar affinity. We then proceeded with structural modeling in ColabFold, which revealed an unexpected image: instead of a single contact point, the AQP C-terminal appeared to engage two distinct binding sites on the FERM-domain simultaneously (3). These interaction sites reflect known FERM-binding motifs at each individual site; however, the combined binding has previously only been seen in self-inhibition of Moesin. Our results therefore point to a previously unrecognized binding mechanism, suggesting that AQP2 and AQP5 may associate with ERM proteins in a different manner than previously believed (3).

To further understand the interaction between Ezrin and AQP we want to investigate how AQP2 phosphorylation affects the AQP2-FERM binding affinity. We are currently working on generating five phospho-mimicking mutants of AQP2 in which the phosphorylation sites of serine and threonine are substituted with glutamic acid. Binding studies utilizing the MST between the different AQP2 mutants and FERM-domain will then be conducted to see if the interaction between the two is controlled by phosphorylation of AQP2. By studying the stoichiometry of the AQP2-FERM-complex using Mass photometry and by performing structural analysis with Cryogenic Electron microscopy (CryoEM) we will potentially have a detailed overview of the exact interactions and trafficking mechanisms of AQP2 and Ezrin.

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2) Chivasso, C., et al. Ezrin Is a Novel Protein Partner of Aquaporin-5 in Human Salivary Glands and Shows Altered Expression and Cellular Localization in Sjögren's Syndrome. Int. J. Mol. Sci. 2021, 22, 9213. https://doi.org/10.3390/ijms22179213
3) Strandberg, H., et al. Int. J. Mol. Sci. 2024, 25, 7672. https://doi.org/10.3390/ijms25147672