COMBINING PHOTOACTIVATED LOCALIZATION MICROSCOPY AND SPATIOTEMPORAL IMAGE CORRELATION SPECTROSCOPY TO CHARACTERIZE THE SPATIAL-TEMPORAL DYNAMICS OF AQUAPORIN-2

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ABSTRACT

Aquaporin proteins are homo-tetrameric proteins which transport water and are crucial to the functioning of most organs in the body, especially the kidney. Here, we studied the nanoscale dynamics and organization of the water channel aquaporin-2 (AQP2) in kidney collecting duct cells (MDCK cells) using single particle tracking photoactivated localization microscopy (sptPALM). AQP2 functions in the kidney to help concentrate urine. In kidney cells, AQP2 normally resides in intra-cellular vesicles in the cell cytoplasm, but upon phosphorylation the protein is sent to the membrane, where it exerts its function in transporting water. To characterize both the diffusion coefficient and velocity vectors of AQP2 molecules on the plasma membrane of kidney cells, we acquired sptPALM datasets and then analyzed them with spatiotemporal imaging correlation spectroscopy (STICS). STICs uses a filtering mechanism to remove frequencies associated with immobile components, allowing measurements of protein dynamics even in the presence of a large fraction of immobile species. Using sptPALM, STICS and classical MSD trajectory analysis, we acquired trajectories and spatial maps of AQP2 molecules in the plasma membrane in kidney cells. Our results provide the first look at the broad, nanoscale organization and dynamics of AQP2 in kidney cells, opening the door for a deeper understanding of AQP2 function in urine concentration.