Pair correlation analysis of Fixed Photoactivatable Analysis of Live PALM applied on the Water Channel Aquaporin -3

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Water transport across the plasma membrane of epithelial cells is mediated by aquaporin (AQP) water channels. In the renal collecting duct, water is reabsorbed from the renal filtrate through apically localized AQP2 and exits the cells through AQP3 and AQP4, which are expressed in the basolateral plasma membrane, where AQP3 is the main exit pathway. The antidiuretic hormone Arginine Vasopressin (AVP) increases urine concentration via an increase in cAMP, leading to insertion of AQP2 containing vesicles into the apical plasma membrane. This instantly increases water reabsorption and urine concentration. We previously found that a short - term increase in cAMP leads to an increase in lateral diffusion of AQP3, revealing short - term regulation. To further study if AQP3 is regulated at the nanoscale level, we first combined single molecule detection using Photoactivatable Localization Microscopy (PALM) imaging of fixed cells with pair correlation (PC). This showed that AQP3 molecules organize in nano - domains smaller than 60 nm and upon stimulation mimicking vasopressin, change organization to 60 – 200 nm sized nano - domains. Thus, PC - PALM revealed regulation at the nanometer resolution. Furthermore, we performed live - PALM of AQP3 upon cAMP stimulation and have initiated analysis by power spectral analysis. The analysis was done by first identifying isolated spots and fitting with a two - dimensional point - spread function. The localization errors were found theoretically and the diffusion coefficient for each trajectory was calculated using a covariance - based estimator. To demonstrate that the identified molecules were indeed freely diffusing with identical diffusion coefficients, we calculated the power - spectrum of each trajectory. The power - spectral values were rescaled with their expected values given theoretically as a function of the averaged diffusion coefficient and the localization errors. Thus, fixed PALM revealed that AQP3 changed nano - organization in the plasma membrane upon stimulation mimicking vasopressin; from an even distribution to an organization in nanoclusters. This indicates short - term hormone regulation of AQP3 at the nanoscale level, which may be important in urine concentration. PC - PALM may be used to reveal previously undetectable protein regulation at the nanoscale. We furthermore did live - PALM and are currently analyzing the data using power spectral analysis.