HIGH-RESOLUTION LOCALIZATION AND FUNCTION OF AUXIN CARRIERS WITHIN THE PLASMA MEMBRANE LANDSCAPE

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Although a numerous developmental roles of PINFORMED (PIN) auxin efflux carriers are well established, there is still very limited knowledge on mechanisms defining their distribution and function within the plasma membrane (PM). In our recent work, we have followed PIN nanodomain organization and dynamics within the PM using tobacco BY-2 cultured cells by confocal laser scanning microscopy (CLSM) and spinning disk microscopy (SD). We use our previously established approach [1], combining fluorescence recovery after photobleaching (FRAP) and raster image correlation spectroscopy (RICS) to show that the dynamics of individual auxin carriers differs. Super-resolution microscopy approach using total internal reflection fluorescence (TIRF) followed by the application of super-resolution radial fluctuations (SRRF) algorithm (Figure 1, upper row) and Airyscan CLSM (Figure 1, bottom row) allowed us to unravel variable appearance of *Nt*PIN2-GFP and *Nt*PIN11-GFP in PM nanodomains. Image analysis-based quantification of these distributions showed that *Nt*PIN11 nanodomains were less dynamic and less clustered in comparison with *Nt*PIN2 nanodomains, suggesting variable, protein-specific dynamics between individual nanodomains. Importantly, we show by auxin transport assays that this differential mobility might affect auxin efflux function. Our results suggest novel regulation of function for members of PIN family, based on their differential stabilization within PM. Altogether, high-spatial resolution imaging within the PM opens a truly new horizons, helping significantly to define the functional importance of nanodomain presence for individual plant PM proteins.

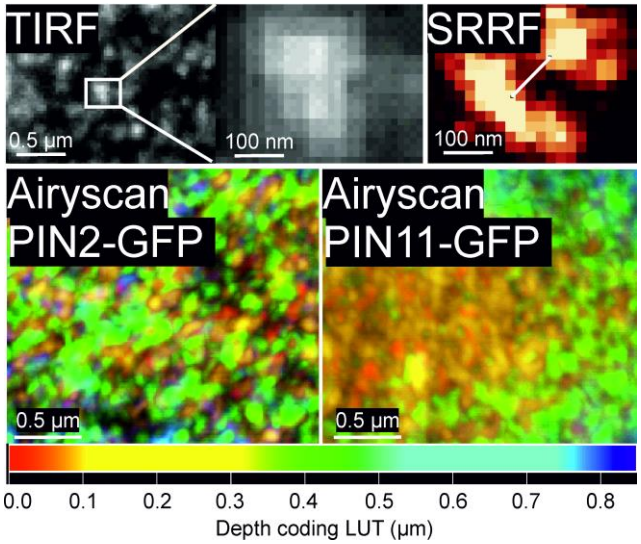

Figure 1: Spatial resolution improvements for the imaging of PIN proteins in nanodomains. Upper row, TIRF-SRRF xy surface imaging in PIN1-GFP cells, 100x, NA 1.45. Bottom row, Airyscan imaging, Z stack from 5 sections (170 nm each), 100x, NA 1.45 in PIN3-GFP and PIN11-GFP cells.

[1] M. Laňková, J. Humpolíčková, S. Vosolsobě, Z. Cit, J. Lacek, M. Čovan, M. Čovanová, M. Hof, J., and J. Petrášek, "Determination of Dynamics of Plant Plasma Membrane Proteins with Fluorescence Recovery and Raster Image Correlation Spectroscopy" *Microsc Microanal*, **22**: 290/299 (2016).