Analysis of nanoscale compartmentalization of PI(4,5)P2 in living cells and model membranes through fluorescence spectroscopy and FRET imaging

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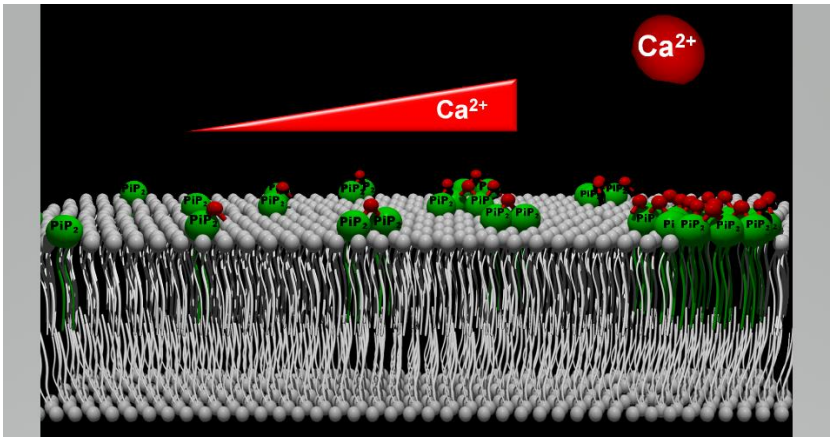
$PI(4,5)P_2$ is an essential membrane component involved in a large number of cellular functions, including membrane trafficking and cytoskeleton organization. The variation of its local availability, in time and space, has been claimed to be responsible for the spatiotemporal recruitment of proteins with diverse functions, such as endocytosis and cytoskeleton adhesion to the membrane. Among other factors, the presence of cholesterol-enriched domains, elevated concentrations of divalent cations and the cytoskeleton itself have been suggested to be involved in determining $PI(4,5)P_2$ organization and clustering. Using a combination of fluorescence spectroscopy and microscopy techniques, we show that both the number and position of phosphorylations in the inositol ring of phosphoinositides are crucial for defining the extent of PIP clustering and relative cluster size[1,2]. Additionally, we show that formation of the liquid ordered phase strongly promotes formation of PIP clusters in model membranes[2]. Evidence for the formation of $PI(4,5)P_2$ enriched nanodomains in the plasma membrane of living cells was obtained through FRET microscopy of pleckstrin homology (PH) domains tagged with fluorescent proteins. Disruption of the cytoskeleton in HeLa cells decreased significantly the compartmentalization of $PI(4,5)P_2$, proving that distinct pools of compartmentalized $PI(4,5)P_2$ are present, of which one is dependent and other independent of actin cytoskeleton-membrane adhesions. On the other hand, $PI(4,5)P_2$ compartmentalization is shown to be independent of cholesterol concentration in the plasma membrane.



References:

(1) Sarmento et al. *Biochim. Biophys. Acta - Biomembr.* **2014**, *1838* (3), 822–830.
(2) Sarmento, et al. *Langmuir* **2017** (in press).

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