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Meeting abstract



# Investigation of the PtdIns(4,5)$P_2$ dependence of plasma membrane receptor endocytosis in living cells

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## Background

Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)$P_2$) plays an important role in various cellular processes: not only in calcium signalling as a precursor for the second messenger Ins(1,4,5)$P_3$, but also in the regulation of ion channels, cytoskeletal dynamics and many other events connected to the plasma membrane. Since many of the molecules participating in the process of endocytosis can bind PtdIns(4,5)$P_2$, a role of this lipid in the regulation of the internalization of plasma membrane receptors seemed possible.

## Methods

In this study we focused on the investigation of the lipid dependence of the internalization of plasma membrane receptors, and we used the highly sensitive method of bioluminescence resonance energy transfer (BRET), which allows the detection of molecular closeness between two proteins labeled by bioluminescent and fluorescent markers. By fusing various plasma membrane receptors (e.g. angiotensin II $AT_1$ receptor, serotonin 5-$HT_{2C}$ receptor and EGF receptor) to *Renilla* luciferase and applying YFP-tagged proteins as components of the endocytic machinery (β-arrestin, clathrin, β-adaptin, PM-targeted YFP, Rab proteins) we could follow the process with high temporospatial resolution in HEK cells. To decrease the plasma membrane PtdIns(4,5)$P_2$ level we used the previously developed rapamycin-induced heterodimerization system, in which PtdIns(4,5)$P_2$ depletion was achieved by the recruitment of 5-phosphatase enzymes to the plasma membrane.

## Results

To check whether the PtdIns(4,5)$P_2$ depletion was sufficient we measured the BRET signal between the PH domain of PLC$\delta_1$ - which binds specifically to PtdIns(4,5)$P_2$ - fused to either *Renilla* luciferase or YFP. To follow receptor internalization we measured the BRET ratio between the receptors and plasma membrane-targeted YFP, which decreased upon stimulation with the appropriate agonist. After optimizing our system we were able to show that the internalization of EGF receptor was significantly reduced after depletion of the lipid, and the same was noticed in the case of $AT_1$ and 5-$HT_{2C}$ receptors.

## Conclusion

These data suggest that PtdIns(4,5)$P_2$ level is an important factor in the regulation of plasma membrane receptor endocytosis.