Cell-derived plasma membrane vesicles as minimal cells for analysing transmembrane signalling

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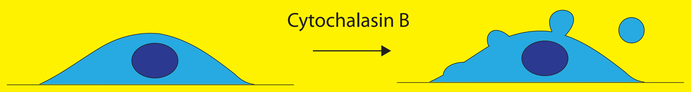
Abstract

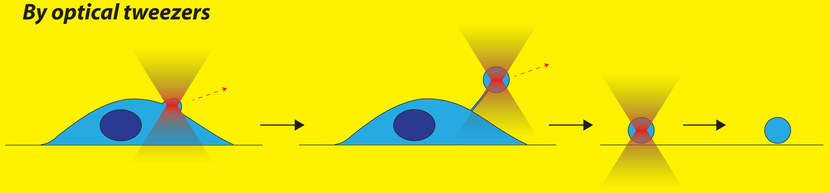
Cellular signalling is classically investigated by measuring optical or electrical properties of single or populations of living cells. Here we show how cell-derived vesicles can be used for anlaysing transmembrane signalling. The vesicles are derived from live mammalian cells by using either chemicals, or by optical tweezers and they comprise parts of the plasma membrane and cytosol of the mother cell. We measured in vesicles derived from individual cells with single molecule sensitivity the different steps of G protein-coupled receptor mediated signalling like ligand binding to receptors, subsequent G protein activation and finally receptor deactivation by interaction with arrestin. Cell-derived plasma membrane vesicles represent the smallest autonomous containers capable of performing cellular signaling reactions thus functioning like minimal cells. Observing cellular signalling reactions in individual vesicles opens the door for downscaling bioanalysis of cellular functions to the attoliter range, multiplexing single cell analysis and investigating receptor mediated signalling in multiarray format.

Vesicles production

By chemical treatment



By optical tweezers

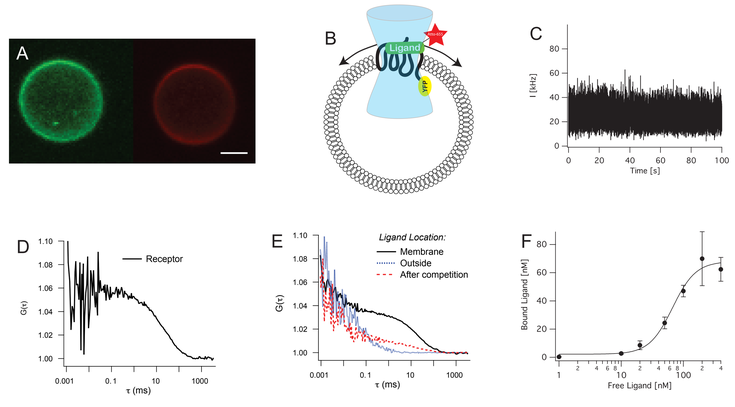


Cell-derived vesicles are small containers composed of the lipid bilayer and the cytosol of the cell whose they are shed.

They can be produced from any eukaryotic cell type by chemical treatment or by optical tweezers.

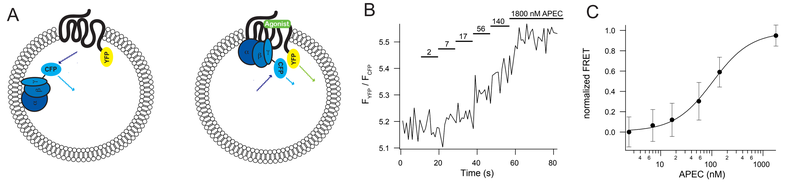
They are in many aspects similar to living cells, with important functional characteristics such as the native membrane lipid composition, the presence of integral membrane receptors and intracellular component essential to perform cellular signalling.

Monitoring receptor diffusion and ligand binding



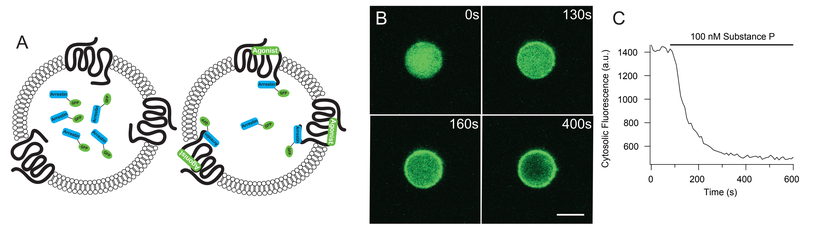
(A) Confocal micrograph of a native vesicle showing the fluorescence of the YFP-A2AR (green, left) and the fluorescence of a receptor-bound antagonists XAC-Atto655 (red, right); scale bar: 1 µm. FCS measured autocorrelation curve of (D) YFP-A2AR (diffusion time t = 22.4 ms) and (E) XAC-Atto655 (t = 32.3 ms). Competition with non-fluorescent XAC confirmed specific binding of XAC-Atto655. (E) Receptor binding of XAC-Atto655 at different concentrations yielded a dissociation constant of Kd = 67±11 nM.

Monitoring G protein activation



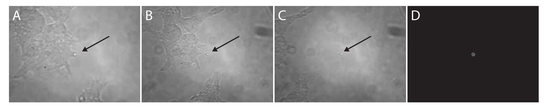
(A) FRET was measured between CFP-tagged Gγ subunits and YFP-tagged receptors. Upon agonist binding to the GPCR the heterotrimeric G protein forms a complex with the GPCR leading to FRET between CFP and YFP. (B) Change in FRET depends on the agonist concentration added to the bulk solution (concentrations are indicated at the bars in nM). (C) A dose-dependent increase of the FRET yields IC50 = 104±51 nM.

Monitoring receptor silencing

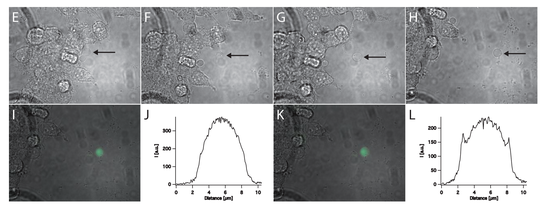


(A) Binding of agonists to GPCRs initiates receptor phosphorylation (by GRKs, PKA or PKC), which in turn leads to binding of arrestin to the GPCRs preventing further activation of G proteins. Here we monitor in an individual native vesicle interaction between GFP-arrestin (in vesicle lumen) and Neurokinin-1 receptor (NK1R, in vesicle membrane) by confocal microscopy. (B) After adding the agonist Substance P to the bulk, it binds to the NK1R and induces rapid recruitment of GFP-arrestin at the vesicle membrane (scale bar: 2µm) as shown in (C) as a time course of the cytosolic fluorescence of GFP-arrestins.

Vesicles production by optical tweezers



(A-C) Optical micrographs showing the production of a vesicle (arrow) which is pulled off from the plasma membrane of HEK expressing GFP-tagged BK1 receptor with an optical tweezer. (D) Fluorescence micrograph of the vesicle obtained in (C) exhibiting the plasma membrane via the fluorescent receptor.



(E-H) Sequence of optical micrographs showing the isolation of a preformed vesicle (arrow) selected with an optical tweezer from HEK cells expressing arrestin-GFP and NK1R. Fluorescence micrographs of the vesicle before (I) and after (K) the perfusion with the agonist SP; (J) and (L) are the respective fluorescence profiles of the vesicles.

Conclusion

We show that cell-derived vesicles function as autonomous containers (minimal cell) capable of performing cellular signalling reactions. We measured the different steps of G protein-coupled receptor mediated signalling in individual vesicles. The observation of cellular signalling reactions in individual (sub)micrometer sized vesicles opens the door to downscale the analysis of cellular functions to the attotoliter range for multiplexing single cell analysis or investigating receptor mediated signalling in multiarray format.