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The GPI-anchored Urokinase Receptor (uPAR) dynamics on the cells surface followed by fluorescence fluctuation spectroscopy.

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Abstract

The Urokinase receptor (uPAR) is a GPI-anchored protein and all of its functions depend on its interactions with proteins and lipids present in the membrane and in the pericellular environment. Although there is much known about the uPAR system using molecular biology and biochemical techniques, nothing is known about the distribution and mobility of the receptor on the cell surface, the alterations induced locally by pericellular stimuli (e.g. uPA or vitronectin) and by cleavage of the D1 domain, which strongly affects the biological activity of uPAR. To investigate the mobility of the uPAR in living cells, we have produced clones of HEK293 stably expressing the uPAR/EGFP/GPI chimera.

2-Photon-imaging combined to fluorescence fluctuation spectroscopy measurements clearly demonstrate the heterogeneous distribution of uPAR all over the cell membrane, as expected for GPI-anchored proteins.

The dominant diffusion time component obtained by autocorrelation functions is in the range of tenth of milliseconds (D » 0.5 mm2/sec) as usually found for membrane protein diffusion. However, fluorescence traces acquired over 300 sec also show a number of fluorescence spikes of variable duration and amplitude that cannot be resolved by autocorrelation of the fluorescence signal. A close analysis of these spikes reveals a slow diffusion of ~ 0.01 mm2/sec or less. Interestingly, the motility of the receptor in the cell matrix is much more confined in distinct low and high density areas. Freely diffusing uPAR/EGFP/GPI is detected only in low density receptor domains, whereas in the high density domains the receptor is immobile. We use Photon Counting Histograms to ...
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