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Titel des Beitrags:
In vivo detection of membrane protein expression using surface plasmon enhanced fluorescence spectroscopy (SPFS).

Abstract:
Surface plasmon enhanced fluorescence spectroscopy (SPFS) was applied for the detection of expression and functional incorporation of integral membrane proteins into plasma membranes of living cells in real time. A vesicular stomatitis virus (VSV) tagged mutant of photoreceptor bovine rhodopsin was generated for high level expression with the semliki forest virus (SFV) system. Adherent baby hamster kidney (BHK-21) cells were cultivated on fibronectin-coated gold surfaces and infected with genetically engineered virus driving the expression of rhodopsin. Using premixed fluorescently (Alexa Fluor 647) labeled anti-mouse secondary antibody and monoclonal anti-VSV primary antibody, expression of rhodopsin in BHK-21 cells was monitored by SPFS. Fluorescence enhancement by surface plasmons occurs exclusively in the close vicinity of the gold surface. Thus, only the Alexa Fluor 647 labeled antibodies binding to the VSV-tag at rhodopsin molecules exposed on the cell surface experienced fluorescence enhancement, whereas, unbound antibody molecules in the bulk solution were negligibly excited. With this novel technique, we successfully recorded an increase of fluorescence with proceeding rhodopsin expression. Thus, we were able to observe the incorporation of heterologously expressed rhodopsin in the plasma membrane of living cells in real time.
using a relatively simple and rapid method. We confirmed our results by comparison with conventional wide field fluorescence microscopy.