MS5-7

Studying association of influenza virus HA with membrane-rafts using fluorescence-resonance-energy transfer (FRET)

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Assembly of influenza virus requires enrichment of its glycoprotein hemagglutinin (HA) in membrane-rafts, cholesterol- and sphigomyelin-rich subdomains of the plasma membrane. Our project aims at verifying this model using fluorescence-resonance-energy transfer (FRET) between HA and markers for rafts inside living cells. Cerulean was linked to the cytoplasmic tail of two different HA-proteins, a wild type form and a mutant without palmitoylation sites, which are necessary for raft incoporation. As an established raft-marker we used a double acylated protein consisting of 20 N-terminal amino acids of the lyn-kinase fused to the yellow-fluorescent-protein (YFP). In transfected cells FRET was analyzed with two methods, acceptor-photobleaching and Fluorescence Lifetime Imaging Microscopy (FLIM). The lifetime of HA-Cerulean is strongly reduced after coexpression with the raft marker compared to HA-Cerulean alone. Bleaching the fluorescence of the acceptor leads to an increase in the intensity of the fluorescence of HA-Cerulean. This reveals a strong association between HA and the raft-marker, but the sites of interaction were unequally distributed over the plasma membrane. The effect of dissolving rafts by extraction of cholesterol and of removal of the palmitoylation sites of HA on the FRET-efficiency is currently under investigation.

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MS5-8

The use of nanoparticles to study and manipulate the polarity of plant cells

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Bleaching is often the limiting factor to the spatiotemporal resolution of fluorescence microscopy. CdSe-based nanoparticles with their excellent photostability have the desired properties to make them intriguing tools for their application in cell biology. However, there are two major obstacles to overcome before they are ready to use. The particles have to be bioconjugated to proteins of interest in such a way that the protein retains its biofunctionality, and to make them applicable for in vivo studies, and they have to be inserted into the target cell while preserving its viability. In this work we explored the bioconjugation of primary antibodies against tubulin to nanocrystals, and we can show that direct immunofluorescence via nanocrystals is possible. To address the task of non-destructive insertion, we investigated whether Trojan Peptoids, that can pass the plasmamembrane fast and independently of endocytosis, are a feasible carrier system for the insertion of a cargo into tobacco BY-2 cells. In the present study we focused on uptake kinetics compared to endocytosis, and the intracellular localization of the peptoids. As the delivery of the test cargo fluorescein was fast and efficient, Trojan Peptoids should be a viable option for the insertion of nanoparticles into target cells.

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MS5-9

Opening of calcium channels by noninvasive stimulation on silicon chips to control the intracellular Ca2+ level

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Hybrid systems of neuronal networks and microelectronic chips may be used to elucidate network processes like learning and memory. A prerequisite is a well defined and controlled outgrowth of the neurons that constitute the network. We want to implement this control electrically from a silicon chip: opening of voltage dependent Ca2+ channels (VDCCs) by extracellular capacitive stimulation is our tool to manipulate the intracellular Ca2+ level that is known to play a decisive role in neuronal outgrowth. The basic requirement for this approach is the feasibility of capacitive opening of VDCCs. As a model system, we used HEK293 cells transfected with L-type Ca2+ channel Cav1.2. Capacitive gating of Cav1.2 channels was studied by whole cell voltage clamp and current clamp recordings. In addition we detected the incoming Ca2+ ions by Fura-2 fluorescence microscopy and showed that the intracellular Ca2+ concentration of the cells was greatly enhanced by capacitive chip stimulation. The results provide the basis for further work on neurons.

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