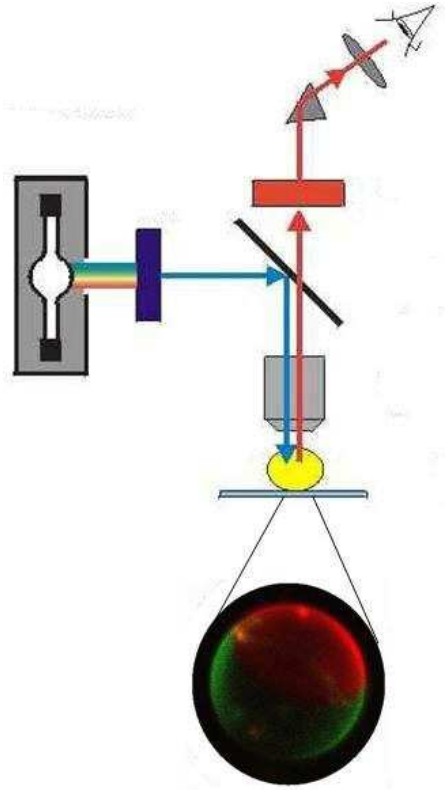
Fluorescence Microscopy as a Tool to Investigate the Membrane-binding Behavior of Lipidated Ras Proteins



Confocal fluorescence microscopy is a widely used tool to study the phase behavior of model membranes in so called giant unilamellar vesicles (GUVs). Depending on the composition of the lipid systems, these model membranes exhibit phase segregation into liquid-disordered (l_d) and liquid-ordered (l_o, "raft-like") domains. To visualize phase coexistence and study the membrane partitioning behavior of labeled, membrane-associated signaling proteins, such as Ras, the different lipid domains can be selectively labeled. Besides the lateral organization, the same setup also allows to determine the translational dynamics of the system, i.e., diffusion coefficients of the labeled lipids and the incorporated proteins, by fluorescence correlation spectroscopy (FCS). FCS enables to study diffusion modes on the single molecule level and relies on the autocorrelation of time-dependent fluorescence intensity fluctuations.

The aim of the course will be to study the partitioning behavior of fluorescently labeled Ras proteins into heterogeneous model raft membranes with confocal laser scanning microscopy (CLSM). In addition, FCS will be used to determine the corresponding lateral diffusion coefficient in bulk solution and after membrane association. Preparation and imaging of labeled GUVs and FCS-measurements in solution will be done at the first day. Determination of the dynamics of membrane associated Ras, image processing and data analysis are scheduled for the second day.

Please register via email to christopher.rosin@tu-dortmund.de until 15.09.2012!

Time frame:	01.10.2012 – 14.12.2012 (exact date after consultation)
Duration:	2 days (10:00 am – 4:00 pm)
Max. participants:	5
Venue:	Physical Chemistry I, TU Dortmund, Otto-Hahn-Str. 6, 44227 Dortmund Room: C1-01-102
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