**Organizations**
Umeå University
Department of Chemistry, Umeå, SE
&
Charles University in Prague,
Faculty of Science, Prague, CZ

**Document name**
Doctoral thesis

**Date of issue**
February 2012

**Author**
Radek Šachl

**Title**
Localisation of Fluorescent Probes and the Estimation of Lipid Nanodomain Sizes by Modern Fluorescence Techniques

**Abstract**
The thesis is divided into two major parts. The first part focuses on the localisation of probes in lipid/polymeric bilayers and in $G_{M1}$ micelles. Included in this thesis is a new approach based on electronic energy transfer/migration (FRET/DDEM), which efficiently determines transversal positions of fluorescent molecules in lipid bilayers. This approach has been used to locate newly synthesized lipid probes in DOPC bilayers. The label was introduced at the end of *sn*-2 acyl chains of variable length.
Analytical models accounting for FRET exist for a limited number of basic geometries. Here, a combination of FRET and Monte Carlo simulations enables the localisation of probes in bicelles and in bilayers containing pores, *i.e.* in lipid systems with variable curvature, or in non-homogenous lipid systems. This approach has been used to test whether conical-like fluorescence probes have an increased affinity to highly curved regions, which would enable preferential labelling of membrane pores.
A simplified FRET model has been applied to localize 2-pyridones, a class of potential drugs, in $G_{M1}$ micelles. Since the localisation of drugs within nanoparticles might influence the release kinetics and loading efficiency, knowledge about the drug location is highly relevant. It turned out that all derivatives were localised at the core-shell interface of $G_{M1}$ micelles.
The second part of the thesis focuses mainly on the estimation of lipid nanodomain size by means of FRET, which still remains the most powerful method in this field. Limitations of FRET in the determination of domain size have been explored. We showed that the limitations of FRET are mainly caused by a low probes affinity to either the liquid-ordered or liquid-disordered phase. In the continuing work we provided a detailed dynamic and structural study of crosslinker-triggered formation of nanodomains. Here, two different domains have been revealed, *i.e.* *i*) domains whose size grows with increasing amount of added cholera toxin (CTxB), and to which CTxB binds tightly; *ii*) domains formed in membranes containing a slightly increased amount of sphingomyelin (as compared to *i*) whose size does not change during titration by additional CTxB and to which CTxB binds less tightly.

**Keywords**
Electronic energy transfer/migration, FRET, FCS, Monte Carlo simulations, fluorescence, solvent relaxation, lipid bilayer, rafts, micelles, BODIPY, phase diagram.

**Language:** English **ISBN:** 978-91-7459-390-7 **Number of pages:** 46 + 7 papers