Opinion on the PhD work of Radek Sachl.

Umeå University has appointed me “opponent“ on the occasion when Radek Sachl defends his thesis in Umeå on March 27, 2012. I was also asked to state in writing my opinions for the sake of Charles University in Prague. This statement follows.

I know Radek Sachl only through his thesis and the seven papers it is based upon. I have also received a statement from him concerning what he is responsible for in the different papers. The following papers are included in the thesis:

2. A comparative study on ganglioside micelles using electronic energy transfer, fluorescence correlation spectroscopy and light scattering techniques PCCP 2009
3. Localisation of BODIPY-labelled phosphatidylcholines in lipid bilayers PCCP 2010
5. Distribution of BODIPY-labelled phosphatidylethanolamines in lipid bilayers exhibiting different curvatures PCCP 2011
6. Limitations of electronic energy transfer in the determination of lipid nanodomain sizes Biophys J Biophys Lett 2012 (accepted)
7. Dynamics and size of crosslinking-induced lipid nanodomains in model membranes manuscript

The investigations are concerned with self-assembled systems, micelles or bilayer-based, which are built by polar lipids or amphiphilic block-copolymers. A number of different methods are used, notably sophisticated time-resolved fluorescence methods, including fluorescence correlation microscopy. The focus is on assessing the potential of various methods and probes to address and answer pertinent questions concerning the properties and organisation of self-assembled structures: polarity and “microviscosity” in different parts of the structures, the preferentially location of guest molecules in different parts of the structures (depending for instance on the curvature), detecting and characterization association of lipids into nano-domains (or “rafts”), localisation of solvated probe molecules in the structures.

The first paper is a continuation of similar studies made earlier by some of the co-authors. Solvent Relaxation Methods are used to seek information about the environment of two fluorescent probes that differ in the length of a hydrophobic anchor group, but with the same fluorescent group. It is clearly shown what limitations such methods have in organized systems. The study is carried out skilfully.

The second paper deals with ganglioside GM1 micelles. A most peculiar behaviour has been observed by Corti, Cantu, and co-workers, namely that the aggregation number of...
the micelles decrease with increasing temperature up to a temperature of about 60°C, but remains at the same value on a decrease in temperature and on subsequent heating. Orthaber and Glatter have contested these findings. The present study takes up these questions again, and confirms the curious behaviour. A second more important aim of the paper is to compare different methods of micelle size determination: Static and dynamic light scattering (as also used previously by Corti’s team) as well as two novel methods, namely Energy Transfer Deactivation (using several DA pairs) and Fluorescence Correlation Spectroscopy. These methods are demanding and have been applied with inventiveness to give results that are in good agreement with each other. The light scattering aggregation number deviates from the ET and FCS results, probably due to the polydispersity of the micelle size.

**Paper 3 and 4** deal with the localization of probes in lipid bilayers and GMI micelles, respectively. A series of PC lipids are labelled with a fluorescent group at different positions in one of the acyl chains. Such probes have been claimed to sense the bilayer at different depths. Here it is convincingly shown, in skilful experiments, that the probes bend back so that their fluorophores in all cases are located close to the hydrophobic/hydrophilic interface. **Paper 4** focuses on a fluorescent probe with properties as a drug. It is shown that it resides close to the interface, where it may undergo slow and restricted tumbling, a finding with some relevance for the drug release characteristics.

In **paper 5** resonance energy transfer experiments were made with the aim to find if two fluorescent lipid probes, one with two acyl tails, the other with only one, differed with respect to their preferences for more or less curved regions of bilayer structures. The experiments are demanding, and were very well carried out. For the interpretation Monte Carlo simulations were performed in order to ascertain how well different preferences could be discriminated. It was shown, unexpectedly, that the probe with one tail had no significant preference for curved regions. The reason for this not clear; an attractive interaction between the probes is a complication.

**Paper 6:** Monte Carlo simulations are employed to investigate if FET experiments can be designed that would allow the detection of nano-sized circular domains of one phase, surrounded by bilayer with different properties. Donor and acceptor probes with assigned preferences for distribution in one or the other phase are placed in the system. The decays and intensities from these DA distributions are compared with what should be observed for DA pairs with equal propensities for both phases. This is a very useful study for the design and critical interpretation of such experiments.

**Paper 7** Domains called rafts have long been claimed to exist in biological bilayer membranes. The rafts are now understood as domains of an ordered phase (often surrounding a peptide, protein or other component) in a liquid disordered environment, and are thought to have important functions. It is still difficult to identify such domains unless they are relatively large and long-lived. In the paper early stages of the phase separation in giant unilamellar vesicles are investigated, in particular as induced by an agent that crosslinks GMI gangliosides. A number of sophisticated fluorescence based methods are used: z-scan fluorescence correlation spectroscopy and FRET, combined with MC-simulations. The results give a clear picture of the onset of clustering, and a determination of sizes of domains as small as about 5 nm radius. It is a very impressive study.
It has been interesting and rewarding to read Radek Sachl’s thesis. I am impressed by the high quality of the measurements, the elegant design of the experiments, and the careful interpretations. The work is not only technically skilful, but it also demonstrates a deep understanding of the physical chemistry behind the behaviour of probes, polymers and lipids in self-assembled structures.

In conclusion, it is a pleasure to recommend that Radek Sachl be awarded with the PhD degree.

Uppsala 2012-02-20

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