Dynamics of conformational change in model lipid monolayers and bilayers as a probe for membrane-peptide interaction

Supervisors: Christoph Nowak (NTU/AIT), Jonathan Hobley (IMRE) and Erik Reimhult (BOKU)

Synopsis:

Artificial bio-mimetic membranes in the form of lipid monolayers and bilayers, with or without incorporated membrane proteins, are of interest both from a fundamental and an applied point of view. In the first case they present us with model systems through which we can observe dynamics of phase transitions, pressure transduction and deformation, as well as the possibility to study the collective behavior of reductionist interaction processes. In the second case they provide us with cell membrane like scaffolds to construct sensor devices using biological transduction mechanisms. The proposed work will study the kinetics of peptide interactions with lipid monolayer and bilayer systems using a novel set of complementary measurement and analysis techniques. To gain mechanistic understanding of such systems is extremely important for drug-screening, novel peptide based antimicrobials and further biosensing applications.
Structural mapping of the association of peptides with membranes, which are highly organized systems but only a few nm thick, requires an approach collecting data using multiple complementary techniques. The proposed project team shares a common interest in peptide-membrane interactions and spectroscopic techniques to address the same, but they contribute different methodology and expertise. Additionally they have considerable experience in developing surface-based biomimetic membranes. The proposed set of surface analytical techniques that will be used include Dual Polarization Interferometry (DPI)[1], Dynamic FTIR[2-4] and nanosecond-laser Brewster Angle Microscopy (BAM)[5]. Through this project we will further develop these time-resolved techniques for the study processes in biomimetic membranes. The goal is to understand changes in membrane morphology, from initial molecular level changes in response to physical interactions with peptides or membrane active drugs, to the final collective changes in the membrane. In particular mapping the intermediate steps from first association of peptides to insertion and induced phase change and disruption in membranes mimicking different types of cell membranes of pharmacological relevance is the objective.

Model systems

Although tremendously important in various parts of biology such as toxicology, viral entry, signaling and cell internalization as well as for antimicrobial, drug delivery and transfection technologies, the detailed interaction of small peptides with lipid membranes is more or less unknown except for less than a handful of model peptides. This is the case despite a large number of proposed interaction models for different classes of peptides mentioned in the literature. The unique set of complementary measurements that our project team proposes to combine would be uniquely suited to develop new experimental methodology and insights into peptide membrane interactions. We have already developed and published a large set of protocols for assembling lipid membranes of various complexity, mimicking both eukaryote and prokaryote membranes on substrates compatible with the measurement techniques presented in this proposal, both tethered of monolayer character and completely fluid lipid bilayers formed by liposome fusion [6-12]. We propose to first verify the readout on such model membranes on the different platforms to develop a comprehensive view of the expected membrane properties (parameters) that are to be monitored with high time resolution. The core part of the project will then be performed through investigating the interaction of a select set of peptides with primarily supported lipid bilayers, but also for control, supported lipid monolayers of suitable lipid composition to model aspects of the natural target membranes. As a first set of peptides we propose to investigate eukaryote model membrane interaction with melittin for which the mechanism of interaction has recently been described in a time and concentration resolved manner and the HIV derived Tat peptide which has been increasingly used for penetrating cargo through cell membranes, but for which the mechanism of entry is still under debate. The main goal is to create a platform of techniques and analysis methods which in the future can be used for joint investigations of cell penetrating peptides of use in novel nanoscale drug delivery systems. The same generic platform would also be highly useful to test the interaction of novel antimicrobial peptide systems by exchanging the eukaryote type supported lipid membrane systems with supported bacterial membrane models also previously demonstrated by us.

The key potential of this collaboration is a unique combination of spectroscopic molecular level information from FTIR, being coupled with structural information of high accuracy from DPI as well as with imaging information and molecular level information from BAM. Our laboratories also possess a wide range of other standard techniques used to verify the assembly of membrane systems, adsorption of molecules at interfaces and membrane integrity which will be used when required within the project. These include quartz crystal microbalance with dissipation monitoring (QCM-D), electrochemical impedance spectroscopy (EIS), surface plasmon resonance spectroscopy (SPR), confocal fluorescence microscopy (CLSM), fluorescence recovery after photobleaching (FRAP) and atomic force microscopy (AFM).

Time resolved BAM [5, 13, 14]

This method can observe subtle optical changes in a molecular layer on a dielectric despite the fact that a monolayer is optically very thin. It relies on the fact that p-polarized light incident at an interface is all refracted at Brewster's angle. However the presence of a thin film or monolayer can cause some light to be reflected and changes in the refractive index of the monolayer attenuate the amount of reflected light. In this way monolayers become visible in a BAM seen in Fig. 1a.

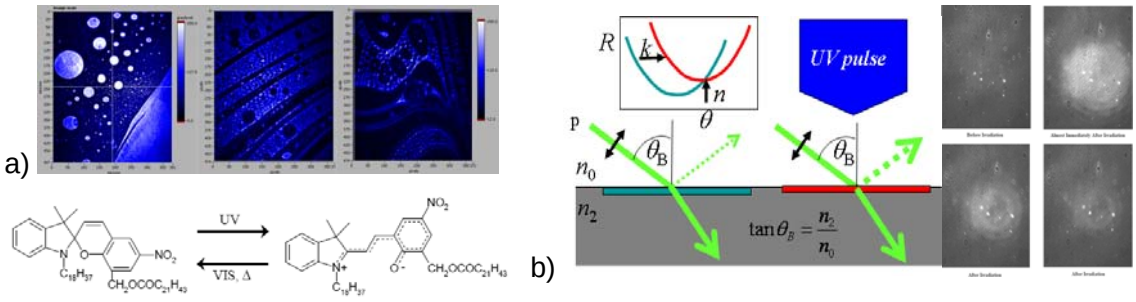

Fig 1. a) A spiropyran monolayer viewed with BAM b) Optical changes in a photoresponsive spiropyran monolayer after laser excitation – clockwise from top left; before and after irradiation at various times, measured using nanosecond time-resolved BAM. Reflectivity changes as real and imaginary parts of refractive index change

The novelty of our approach is twofold. Firstly we can monitor changes in both real and imaginary parts of the refractive index of a monolayer as shown in Fig. 2b. This can also be done with nanosecond time resolution. Secondly we can apply nanosecond imaging techniques to observe rapidly evolving morphology changes induced in a monolayer as shown in Fig. 2 [5, 13].

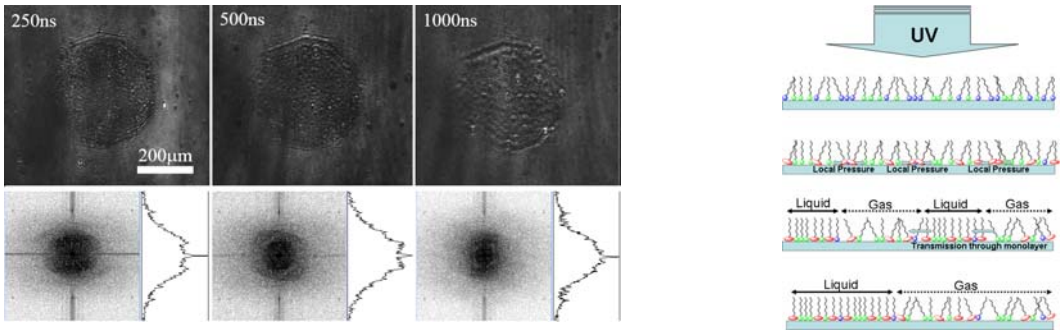

Fig 2. Morphological changes in a photoresponsive monolayer of spiropyran after laser excitation, measured using nanosecond time-resolved BAM [14].

Time-resolved IR spectroscopy

Time-resolved IR is a powerful tool to investigate conformational changes of biological or synthetic macromolecules [2, 15]. Here one distinguishes between the Rapid-Scan (RS) mode and the Step-Scan mode (SS). In RS mode one can achieve time resolved spectra in the sub millisecond regime. For faster time resolved measurements one uses the SS mode to achieve time resolution up to nanosecond regime.

Phase Sensitive Detection (PSD) is an additional tool which acts as a software log-in amplifier. With PSD one can discriminate between signals changing with the excitation (signals from the sample) and signals which are not changing regularly with the applied excitation (noise) [16, 17]. With that powerful tool the user can subtract the noise

from the sample spectrum. Additional to the PSD method one can use time-resolved 2D IR to deconvolute the achieved IR spectra as shown in Fig 3.

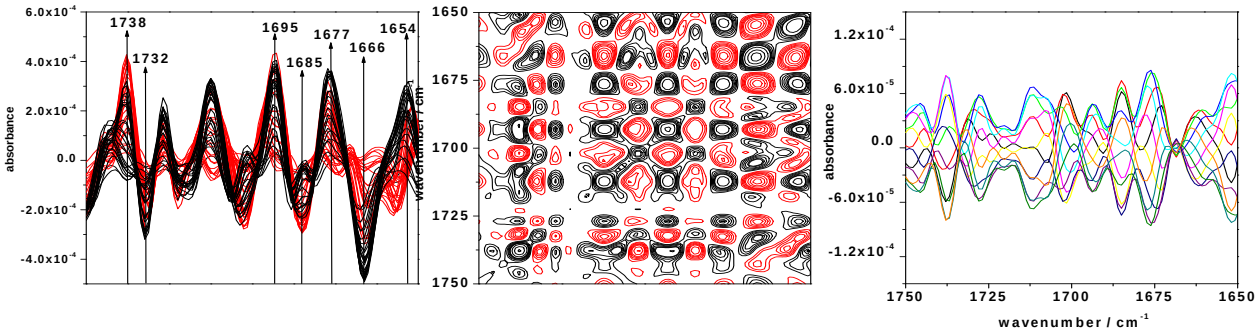

Fig 3. A detail of a time-resolved IR spectra of Cytochrome c Oxidase (example of a biological macromolecule) combined with the according 2D IR(middle) and PSD (right) spectra.

Dual polarization Interferometry

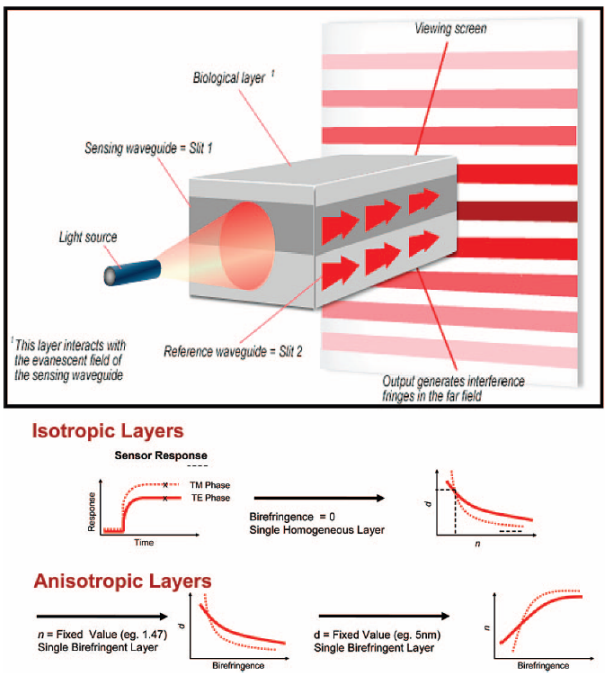

Figure 4. Schematics of a dual polarization interferometry sensor and the basis for its analysis. Using information from both the absolute and relative changes in optical path length for propagation of two waveguide modes, the thickness and refractive index of an adsorbed layer can be determined; or if one of them is known the degree of anisotropy determined.

Dual polarization interferometry (DPI) [1] is a new, commercialized waveguide biosensor technique, which is targeted to and mainly has had an impact on lipid membrane sensing. The use of two linearly polarized waveguide light modes at an angle makes it possible to resolve optical path length differences as molecules are adsorbed on the waveguide surface, and from these determine e.g. adsorbed layer thickness and refractive index (mass) with sub-second (down to ms resolution possible but typically not practiced). The specific waveguide construction and phase sensitive readout gives the instrument unsurpassed sensitivity compared to other available waveguide techniques.

We have recently shown that for lipid membranes assembled on the waveguide surface, which have known dimensions, the recorded information can instead be used to record adsorbed mass and lipid orientation through utilizing the optical refractive index anisotropy of the lipid molecules [9] and to determine the position of membrane-binding peptides relative the membrane surface [18].

This methodology has also recently successfully been employed to study antimicrobial peptide – membrane interaction, for which membrane association, binding, insertion and dissolution could be distinguished in a time-resolved manner for the first time [19]. This opens up the possibility for using DPI to generally investigate peptide interactions with model membranes in a way that also probes mechanisms and not only binding.

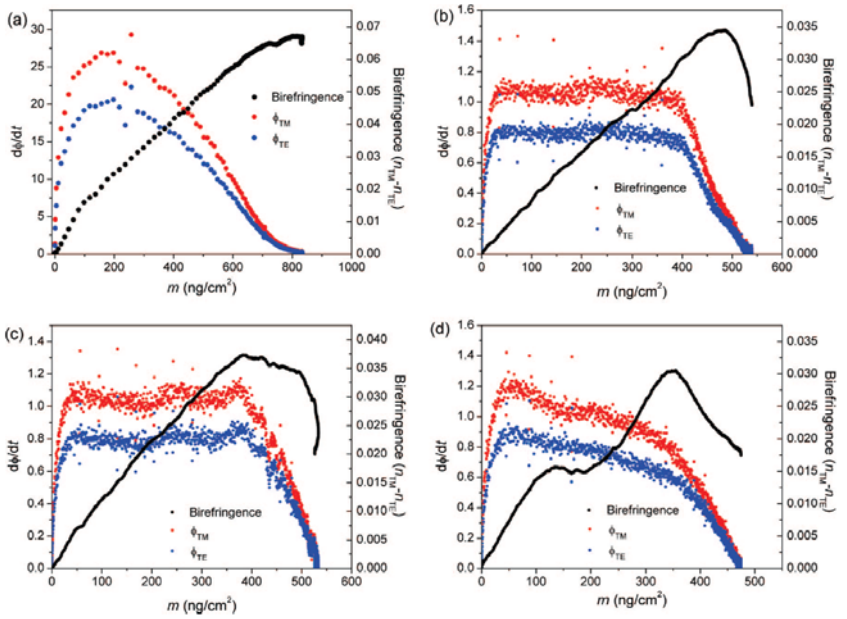

Figure 5. Birefringence and rate of TM and TE phase change, $d\varphi_{TM,TE}/dt$, versus mass, m, measured by DPI for (a) DMPC liposome adsorption at 20 °C, (b) POPC, (c) POPC with Ca^{2+} and (d) POPC:POPS (8:2) with Ca^{2+}. The latter three form complete SLBs. The rate of phase change has the same functional dependence as the rate of mass change. It is clear that all SLB forming systems are easily distinguishable from intact lipsome adsorption in terms of final mass, rate of phase change (infilling behavior) andsmost clearlysin the transition kinetics for the birefringence. The extrapolation of the second phase in (d) for POPC:POPS (8:2) with Ca^{2+} will lead to a mass of about (m_{TM}) 1208 (66, m_{TE}) 1160 (65 ng/cm^2) for the random sequential adsorption jamming limit. This demonstrates the ability of DPI to distinguish different lipid membrane interactions with the sensor surface and a similar methodology will be applied to kinetically investigate the interaction of already formed membranes with peptides.[9]

References

1. Swann, M.J., et al., *Dual-polarization interferometry: an analytical technique to measure changes in protein structure in real time, to determine the stoichiometry of binding events, and to differentiate between specific and nonspecific interactions.* Analytical Biochemistry, 2004. **329**(2): p. 190-198.
2. C. Nowak, D.S., J.Gebert, M.Grosserüschkamp, R.B.Gennis, S. Ferguson-Miller, W.Knoll, D.Walz, R.Naumann, *Oriented immobilization and electron transfer to the cytochrome c oxidase.* Journal Solid State Electrochemistry, 2010.
3. Nowak, C.L., C.; Schach, D.; Knoll, W.; Naumann, R.L.C., *Electron transfer kinetics of cytochrome c in the sub-ms time regime using time-resolved SEIRAS.* J Phys Chem (C), 2009. **113**: p. 2256-2262.
4. Nowak, C., *A Two-Layer Gold Surface with Improved Surface-Enhancement for Spectro-Electrochemistry using Surface-Enhanced Infrared Absorption Spectroscopy.* Applied Spectroscopy, 2009. **63**(9): p. 1068-1074.
5. Hobley, J., et al., *Time-Resolved Brewster Angle Microscopy for Photochemical and Photothermal Studies on Thin-Films and Monolayers.* Journal of Nanoscience and Nanotechnology, 2009. **9**(1): p. 59-68.
6. Baumann, M.K., et al., *Characterization of supported lipid bilayers incorporating the phosphoinositides phosphatidylinositol 4,5-biphosphate and phosphoinositol-3,4,5-triphosphate by complementary techniques.* Biointerphases, 2010. **5**(4): p. 114-119.
7. Kaufmann, S., et al., *A detailed investigation of the formation kinetics and layer structure of poly(ethylene glycol) tether supported lipid bilayers.* Soft Matter, 2009. **5**(14): p. 2804-2814.
8. Knoll, W., et al., *Polymer-Tethered Bimolecular Lipid Membranes*, in *Polymer Membranes/Biomembranes*, W.P. Meier and W. Knoll, Editors. 2010, Springer-Verlag Berlin: Berlin. p. 87-111.
9. Mashaghi, A., et al., *Optical anisotropy of supported lipid structures probed by waveguide spectroscopy and its application to study of supported lipid bilayer formation kinetics.* Analytical Chemistry, 2008. **80**(10): p. 3666-3676.
10. Merz, C., et al., *Formation of supported bacterial lipid membrane mimics.* Biointerphases, 2008. **3**(2): p. FA41-FA50.

11. Naumann, R.L.C., C. Nowak, and W. Knoll, *Proteins in biomimetic membranes: promises and facts.* Soft Matter, 2011. **7**(20): p. 9535-9548.
12. Reimhult, E., F. Hook, and B. Kasemo, *Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: Influence of surface chemistry, vesicle size, temperature, and osmotic pressure.* Langmuir, 2003. **19**(5): p. 1681-1691.
13. Gorelik, S., et al., *Transient Brewster angle reflectometry of spiropyran monolayers.* Photochemical & Photobiological Sciences, 2010. **9**(2): p. 141-151.
14. Hobley, J., et al., *Cooperative photoinduced two-dimensional condensation in Langmuir films observed using nanosecond pump-probe Brewster angle microscopy.* Biointerphases, 2010. **5**(3): p. FA105-FA109.
15. Nowak, C., *Conformationals Changes of Cytochrome c Oxidase: Varying the Redox State by Electronic Wiring.* Soft Matter, 2010. **submitted**.
16. Baurecht, D., *Methoden der zeitaufgelösten Fourier-Transform-Infrared-(FTIR) Spektroskopie.* Technical report, 1997.
17. Baurecht, D.F., U. P., *Quantitative modulated excitation Fourier transform infrared spectroscopy.* Review of Scientific Instruments, 2001. **72**.
18. Baumann, M.K., et al., *Pleckstrin Homology-Phospholipase C-delta(1) Interaction with Phosphatidylinositol 4,5-Bisphosphate Containing Supported Lipid Bilayers Monitored in Situ with Dual Polarization Interferometry.* Analytical Chemistry, 2011. **83**(16): p. 6267-6274.
19. Lee, T.H., et al., *The membrane insertion of helical antimicrobial peptides from the N-terminus of Helicobacter pylori ribosomal protein L1.* Biochimica Et Biophysica Acta-Biomembranes, 2010. **1798**(3): p. 544-557.