BP 31: Posters: Membranes and Vesicles

Time: Thursday 17:15–20:00 Location: Poster B1

BP 31.1 Thu 17:15 Poster B1

Investigation of Erythrocytes Cell-Cell Adhesion using Holographic Optical Tweezers — •Patrick Steffen and Christian Wagner — Universität des Sarlandes, Saarbrücken

In the classical model, the role of red blood cells (erythrocytes) in blood clot formation is thought to be passive. It is supposed that they get caught into a fibrin-network, generated in the clotting process, just for reasons of geometrical restrictions. Additionally, it is commonly believed that there exist no adhesion forces among the cells. The main part in clot formation take activated platelets. Lysophosphatidic acid (LPA) is a messenger released from these activated platelets. Treating red blood cells (RBC) with LPA leads to a Ca2+ influx into the cells. The consecutive rise of internal calcium level activates the Scramblase protein whereby the negatively charged Phosphatidylserine (PS) gets to the outer leaflet of the cell membrane. Thus the objective is to investigate the contribution of red blood cells in blood clot formation. In order to test this hypothesis we built up an integrated microfluidic holographic optical tweezers setup to study this cell adhesion. Measurements with LPA and the calcium Ionophor A23187 showed that by this increased intracellular calcium level an adhesion of the cells among each other occurs. Thus, we postulate that the response of RBCs on LPA reveals a direct and active participation of these cells in blood clot formation.

BP 31.2 Thu 17:15 Poster B1

Near membrane particle fluctuations and single receptor bindings — •Tim Meyer[1], Holger Kress[2], and Alexander Rohrbach[1] — [1]Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany — [2]Yale University, New Haven, USA

The usage of optical traps combined with arbitrary microscopy methods has a decisive advantage especially in biology: rare events can be turned into frequent events by bringing e.g. interaction partners into close proximity to each other. New insights especially in cell biology are enabled by recording the relevant processes in a small volume at ultra-high speed and with nanometer precision. Here we investigate phagocytosis, which is the process by which bacteria are internalized into macrophages. This process, which is a central mechanism in the immune system, was so far mainly investigated by conventional light and electron microscopies. However, its mechanical properties were barely known up to now. The motion of an optically trapped bead was tracked interferometrically in 3D with nanometer precision at a microsecond timescale. The measurement of the thermal bead fluctuations during the binding to the cell membrane enabled the observation of individual receptor-ligand bond formation. Comparison with Brownian Dynamic Simulations confirm the feasibility of several new types of experiments, which enable fast and precise images of local interactions - information which is not accessible with current light microscopy techniques!

BP 31.3 Thu 17:15 Poster B1

Oscillations in the lateral pressure of lipid monolayers induced by the second messengers MARCKS and Protein Kinase C — •Sergio Alonso[1], Markus Bär[1], Undine Dietrich[2], and Josef A. Käs[2] — [1]Physikalisch-Technische Bundesanstalt, Berlin, Germany — [2]University of Leipzig, Leipzig, Germany

The binding dynamics of the peptide MARCKS to the lipid PIP2 modulated by protein kinase C leads to damped oscillations in lateral pressure of a lipid monolayer. These periodic dynamics can be attributed to changes in the crystalline lipid domain size. We elaborate a mathematical model to explain the observations based on the changes in the physical structure of the monolayer by the translocation of MARCKS peptides. The model equations are numerically integrated and reproduce the experimental observations.

BP 31.4 Thu 17:15 Poster B1

Multi-Bilayer Substrates for Cellular Mechanosensing Assays — •Philipp Rauch[1], Daniel Minner[2], Lydia Woiterski[1], Josef Käs[1], and Christoph Naumann[2] — [1]Universität Leipzig, Germany — [2]Indiana University, Indianapolis, USA

Many processes in cell motility, morphogenesis and differentiation are influenced by mechanical signals and responses from the cellular environment. Similar mechanisms can be found in different types of cells, amongst others in neuroblasts moving to their final location in the brain, in fibroblasts responding to mechanical properties of the surrounding or in mesenchymal stem cells during differentiaton. The development of in vitro systems to investigate the underlying mechanisms has been in focus of many research groups in different fields. Most present assays are based on functionalized hydrogels or polymeric substrates with variable elasticity which mimic the extracellular matrix. A viscous or viscoelastic environment, however, as it is found in organic tissue and especially in cell-cell contacts cannot be implemented with these methods. In order to overcome these limitations we developed and tested multi-stacked tethered lipid bilayer substrates with adjustable viscosity suitable for cell adhesion. The lateral mobility of lipid molecules in these layers can be varied from values close to the reduced diffusion in a typical fibroblast plasma membrane to that of a free floating bilayer. As we could show, different cell types respond to subtle changes within that range through altered morphology and growth behavior. Details of the composition and manufacturing process as well as the physical properties of the novel system will be presented.

BP 31.5 Thu 17:15 Poster B1

Microarray device for local electric recording of planar lipid bilayers — •Theresa Kaufeld and Christoph F. Schmidt — Drittes Physikalisches Institut, Georg-August-Universität Göttingen

Mechanosensitive ion channels play an important role in cell function. They are involved in cell communication and act as emergency release valves to regulate osmotic pressure in cells. In order to understand their function and gating behaviour they can be reconstituted in artificial lipid bilayers and examined with electrophysiological and optical techniques such as single-channel recording and light microscopy.

We have here designed a device for simultaneous electrical recording, fluorescence microscopy and optical trapping experiments to stimulate and characterize the opening of mechanosensitive channels. We form phospholipid bilayers on microfabricated porous silicon substrates because they combine the stability of solid supported membranes and the accessibility to both sides of the bilayer, which is necessary for electrical recordings. We produced a microchip for electrical recording using standard cleanroom techniques.

Apertures of micrometer size were etched into a silicon substrate forming porous microarrays. To electrically isolate the substrate, an oxide layer was grown by thermal oxidation. Integrated Ag/AgCl electrodes surrounding each microarray were fabricated by vapour deposition to make them individually addressable for electrical recordings and to be able to switch between the microarrays during the measurement.

BP 31.6 Thu 17:15 Poster B1

An in-house x-ray scattering study of membrane fusion intermediates: Sample environment and the effect of cholesterol — •Tobias Reusch, Sebastian Aeffner, Britta Weinhausen, and Tim Salditt — Institute for X-ray Physics, Göttingen, Germany

We have developed an x-ray scattering setup which allows to study membrane fusion intermediates or other nonlamellar lipid mesophases by laboratory-scale x-ray sources at quasi arbitrary degrees of hydration.

We report results of a study of pure lipid bilayers and phospholipid/cholesterol binary mixtures. Stalks, putative intermediate structures occurring during the membrane fusion process, can clearly be identified from reconstructed electron density maps. The choice of phases corresponding to the observed diffraction peaks can be narrowed down substantially by the application of the swelling method.

Phase diagrams of the lyotropic phase behavior of DOPC/cholesterol and DPhPC/cholesterol samples are presented. If cholesterol is present in moderate concentrations, it can substantially promote the formation of stalks at higher degrees of hydration or lower osmotic pressure respectively.

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Monte Carlo simulation of two-component membranes: Phase separation dynamics and anomalous diffusion — •Jens Ehrig, Eugene P. Petrov, and Petra Schwille — Biophysics, BIOTEC, TU Dresden, Tatzberg 47/49, 01307 Dresden

Anomalous subdiffusion is an intriguing phenomenon frequently observed in cell membranes, e.g. in SPT, FCS, and FRAP experiments. It is usually ascribed to the presence of membrane heterogeneities with dimensions below the optical resolution limit. In order to understand how the sub-micrometer-scale phase separation in the cell membrane can affect the lipid diffusion and manifest itself experimentally, we carry out dynamic Monte Carlo simulations of a two-component lipid membrane (DMPC/DSPC) with the size on the micrometer scale over time intervals of order of a second. Our model correctly reproduces the thermodynamic properties, as well as the phase diagram of the lipid mixture. Upon an abrupt temperature quench of the system into the two-phase coexistence region of the phase diagram, a power-law domain growth is observed, as predicted theoretically and observed experimentally. For certain ranges of the membrane compositions and temperatures it is found that the Brownian motion of lipid molecules shows strong deviations from the normal diffusion law. In cases where the membrane shows critical fluctuations, results of simulated single particle tracking and fluorescence correlation spectroscopy experiments show transient subdiffusion behavior spanning several orders of magnitude in time.

BP 31.8 Thu 17:15 Poster B1

Conformation of DNA molecules adsorbed on free-standing cationic lipid membranes — •Christoph Herold, Eugene P. Petrov, and Petra Schwille — Biophysics, BIOTEC, TU Dresden, Tatzberg 47/49, 01307 Dresden

We study ds-DNA fragments (5, 10, 20, and 48 kbp) electrostatically adsorbed on free-standing lipid membranes consisting of zwitterionic DOPC with added fractions of cationic DOTAP (1–10 %). The free-standing bilayers are modeled using giant unilamellar vesicles of sizes > 100 μm. We found that DNA molecules are initially adsorbed on the cationic membrane in a coil conformation (gyration radius of ca. 2 μm for 48 kbp DNA) and then collapse into globules with a size below the optical resolution limit (gyration radius of ca. 0.3 μm). The fraction of collapsed DNA globules depends on the cationic lipid concentration and the DNA fragment length. DNA collapse is favored for high DOTAP concentration and long DNA fragments. At low DOTAP concentration and for shorter DNA fragments a coexistence of DNA molecules in coil and globule conformation is observed. We present results of a systematic study of this phenomenon using fluorescence video microscopy with single particle tracking.

BP 31.9 Thu 17:15 Poster B1

Acyl chain correlation at the lamellar-to-rhombohedral phase transition in phospholipid membranes — •Britta Weinhausen, Sebastian Aeffner, and Tim Salditt — Institute for X-ray Physics, Göttingen, Germany

The poster has been withdrawn.

BP 31.10 Thu 17:15 Poster B1

Panta Rhei – Flow Behaviour in Phospholipid Membranes — •Sebastian Busch[1], Christoph Smuda[2], Luis Carlos Pardo[3], and Tobias Unruh[1] — [1]Physik Department E13 and Forschungsneutronenquelle Heinz Maier-Leibnitz (FRM II), Technische Universität München, Lichtenbergstraße 1, 85748 Garching bei München — [2]Institut für Pharmazeutische Wissenschaften, ETH Zürich, CH-8093 Zürich — [3]Grup de Caracterització de Materials, ETSEIB, Universitat Politècnica de Catalunya, E-08028 Barcelona

The long-range motion of phospholipid molecules in the membrane has been of major interest for many years not only because of its importance for processes in the cell membrane but also the puzzling fact that short- and long-time techniques observed vastly different mobilities: Neutron scattering experiments observed much faster motions on the picosecond time scale than macroscopic techniques.

We show that our new high-precision quasielastic neutron scattering experiments are compatible with recent molecular dynamics simulations which propose a flow-like motion of the phospholipid molecules on short times. The difference of observed mobilities can be explained by the transition from this ballistic regime to normal diffusive behaviour.

The influence of additives on the phospholipid mobility, measured on the same time scale, will also be briefly addressed.

BP 31.11 Thu 17:15 Poster B1

Lipid bilayers interacting with polymer chains: A Monte Carlo study — •Marco Werner[1,2] and Jens-Uwe Sommer[1,2] — [1]Leibniz-Institut für Polymerforschung Dresden, Germany — [2]Technische Universität Dresden - Institute for Theoretical Physics

We consider the interaction of amphiphile bilayers and polymer chains by a lattice based Monte Carlo method using the Bond Fluctuation Model [I. Carmesin and K. Kremer, 1988, *Macromol.* 21:2819]. To take advantage of the efficiency of this coarse graining method, we introduce explicit solvent to mediate the amphiphilic interactions. Therewith we observe stable bilayers spanned over the perdiodic boundary, which are formed spontaneously from a random initial state. We also obtain self-organized vesicles appearing in non-periodic boxes. By variation of bending stiffness for the hydrophobic tails we test the model to reproduce the crystalline phase as observed experimentally. We investigate the interactions between lipid bilayers and polymer chains for various chain lengths, chain densities and chain-solvent interactions. Our simulations show that hydrophobic chains are trapped within the hydrophobic layer of the membrane by changing conformations from dense globules into quasi 2D swollen coils. Manipulations of lipid bilayers using polymer chains can have interesting applications for drug delivery systems.

BP 31.12 Thu 17:15 Poster B1

Hydrodynamic interaction of particles in scanning line optical tweezers — •Benjamin Tränkle[1], Michael Speidel[2], and Alexander Rohrbach[1] — [1]Lab for Bio- and Nano-Photonics, University of Freiburg, Germany — [2]Sick-Stegmann, Donaueschingen, Germany

In living cells, the distance of reaction partners determines whether biological processes take place or not. This is especially the case for the fusion of vesicles. Physical interactions within the cell, i.e. hydrodynamic and entropic forces play a crucial role in this context since the motion of vesicles is confined by the size of compartments inherent to the cell structure. Therefore, we are studying the dynamic interaction of at least 2 particles diffusing within a confined volume by using an optical trapping potential. This model system allows the particles to get in close contact to one another due to Brownian position fluctuations. The system is realized by an oscillating optical trap, with a scanning frequency up to 5 kHz and a lateral extension of about 10μm. The laser power is modulated while scanning. Thereby an elongated optical potential is generated. Artificially created volumes can simulate the cell compartments and the confined motion of particles within these bounding walls is expected to be influenced due to interaction potentials. By scanning the particles, their 3D position is obtained by back focal plane interferometry and recorded with up to 10 kHz. The particle trajectories can now be used to calculate the interaction potential and hydrodynamic coupling.

BP 31.13 Thu 17:15 Poster B1

formation of planar lipid bilayer in a microfluidic chip — •jean-baptiste fleury[1] and ralf seemann[1,2] — [1]Experimental Physics, Saarland University, D-66123 Saarbrücken, Germany — [2]Max Planck Institute for Dynamics and Self-Organization, D-37073 Göttingen, Germany

We propose a new microfluidic approach to produce extremely stable planar lipid bilayer (until days), which can be directly observed with high optical quality. We demonstrate the formation of planar lipid bilayer by producing membrane domains as report in the literature [1]. This method furthermore provides a convenient tool for the analysis of self organization properties of proteins (or other active compounds) which are embedded into planar lipid bilayer, as we will demonstrate explicitly using gold nanoparticles.

[1] S. Mukherjee and all. Annu. Rev. Cell Dev. Biol. 20, 839 866 (2004).

BP 31.14 Thu 17:15 Poster B1

Conformation of adhesion clusters — •Daniel Schmidt[1], Udo Seifert[1], and Ana-Suncana Smith[1,2] — [1]II. Institut für theoretische Physik, Universität Stuttgart — [2]Institut für Theoretische Physik I, Universität Erlangen-Nürnberg

We model domains of ligand-receptor bonds that form when a membrane adheres to a functionalized substrate. The aim is to determine the optimum organization of bonds when the bonds maintain lateral mobility. The bonds are modeled as harmonic springs that are organized on a hexagonal or central hexagonal lattice. The membrane is described by the Helfrich free energy in the Monge representation whereas the nonspecific interaction with a wall is modeled by a harmonic well set at a well defined distance from the wall. The results of our modeling emerge from a variation of the total free energy, and provide both, the optimum membrane conformation and the appropriate spring deformation. We find that depending on both, the stiffness

of the membrane and the springs there are three possible outcomes:

(i) densely packed domains when the bonds try to minimize their distance,

(ii) dilute clusters of bonds where optimum distance between the bonds is found,

(iii) unstable domains.

Such results are in good agreement with recently performed experiments on avidin carrying vesicles that adhere to neutravidin carrying substrates.

BP 31.15 Thu 17:15 Poster B1

The role of diffusion on specific adhesion — •Timo Bihr, Ellen Reister, and Udo Seifert — Uni Stuttgart, II. Institute for Theoretical Physics

We analyse the adhesion of a flexible membrane to a flat substrate. Between the substrate and the membrane acts a confining potential in addition to reactions between receptors and ligands. The ligands in the membrane diffuse freely while the positions of the receptors in the substrate are kept fixed. The backbone of the receptor is modelled as a spring. The membrane fluctuations are described by a Langevin-Equation, which is numerically integrated in our simulation, the diffusion of the ligands is simulated by a simple random walk, and the reaction rate between ligands and receptors depends on the binding energy and the distance between receptor and ligand.

In equilibrium we find that higher binding energies are required to sustain adhesion than in models with fixed ligand positions because of a higher entropy contribution to the free energy. The simulations were run for different ligand concentrations, diffusion constants, reaction rates, binding energies and strain energies of the receptor. The adhesion process depends crucially on the diffusion constant of the ligands. For high diffusion constants bond clusters develop while for low diffusion constants bonds form independently from each other. This effect also has an influence on the average height of the membrane because evenly spread bonds pull the membrane closer to the substrate than bonds that are concentrated in one region.

BP 31.16 Thu 17:15 Poster B1

Observing the growth of lipid droplets in vivo and in vitro — •Mária Hanulová and Matthias Weiss — Cellular Biophysics Group, DKFZ, Im Neuenheimer Feld 280, 69120 Heidelberg

Lipid droplets (LD) are fat deposits of cells. In simple terms, they are balls of triglycerides (TG) and cholesteryl esters (CE) surrounded by a phospholipid monolayer into which proteins are embedded. Lipid droplets store excess fatty acids, release them in case of need, and are linked to many metabolic diseases. As of yet, the biogenesis and growth of LDs is poorly understood. According to the most popular model, TG and CE are synthesized at the endoplasmic reticulum (ER) and self-assemble as a globule between the leaflets of the ER membrane. Most likely, LDs then pinch off and carry away lipids from the ER membrane as a protecting monolayer. The growth of LDs has been hypothesized to rely on two (not mutually exclusive) mechanisms: (i) fusion with other LDs, or (ii) acquisition of newly synthesized lipids. To approach this problem, we observed the growth of lipid droplets in living HeLa cells by time-resolved confocal microscopy. Additionally, we used time-resolved fluorescence correlation spectroscopy in vitro to validate the physical possibility of fusion of lipid droplets. These experimental results are in favorable agreement with large-scale simulations of ours.

BP 31.17 Thu 17:15 Poster B1

Computer simulations of membrane fusion — •Sandra Frank — Universität Göttingen

Fusion of membranes is a universal phenomenon belonging to the basic physiology of higher cells. The process of fusion is essential for a multitude of biological processes like synaptic release, viral infection, and trafficking within cells. We use coarse-grained computer simulations to investigate membrane fusion depending on membrane tension and density of proteins or hydrophobic mismatch between protein and mambrane.

BP 31.18 Thu 17:15 Poster B1

Mechanics of Small Unilamellar Vesicles — •Sai Li, Frederic Eghiaian, and Iwan Schaap — III. Physkalisches Institut, Georg-August-Universität, 37077 Göttingen, Germany

Lysosomes, enveloped viruses, synaptic and secretory vesicles are all examples of natural nano-containers (diameter ˜100 nm) which specifically rely on their lipid bi-layer to protect and exchange their contents with the cell. We have developed methods primarily based on atomic force microscopy that allow precise investigation of the mechanical properties of these vesicles. The mechanical properties of small, spherical vesicles were probed by applying very low forces (0.1-0.3 nN), which led to a maximum 30 % deformation. The effects of lipid composition, temperature, osmotic pressure and the radius of curvature were studied for liposomes with diameters between 30 and 150 nm. In order to extract the lipid bi-layer elastic constants we used finite element methods to model the measured deformations. The elastic constants we found for the lipid bi-layer were in very good agreement with previously reported experiments on micrometer-sized giant vesicles. We will discuss the effects of parameters that increase the stiffness in relation to the high curvature of the vesicles.