THEORY & BIO-SYSTEMS

Research in the Department of Theory & Bio-Systems

"The good thing about science is that it's true whether or not you believe in it."
Neil deGrasse Tyson

The main objective of our research activities is to understand the hidden dimensions of self-organization and pattern formation in biomimetic and biological systems. The molecular building blocks of these systems join "by themselves" and form a variety of supermolecular assemblies, which then interact to produce even larger structures and networks.

Reinhard Lipowsky 11.11.1953
1978: Diploma, Physics, (University of Heidelberg)
1982: PhD (Dr. rer. nat.), Physics (University of Munich)
1979-1984: Teaching Associate (University of Munich)
1984-1986: Research Associate (Cornell University)
1986-1988: Group leader (FZ Jülich)
1987: Habilitation, Theoretical Physics (University of Munich)
Thesis: Critical behavior of interfaces: Wetting, surface melting and related phenomena
1989-1990: Associate Professorship (University of Munich)
1990-1993: Full Professorship (University of Cologne), Director of the Division "Theory II" (FZ Jülich)
Since Nov 1993: Director (Max Planck Institute of Colloids and Interfaces, Potsdam)

The associates of the department form several research groups. At present, the research group leaders and topics are (in alphabetic order):

- Rumiana Dimova: Biophysics Lab;
- Andrea Grafmüller: Multiscale Simulations;
- Roland Knorr: Dynamics of Biomembranes (since 2016);
- Hans Riegler: Solid-Air Interfaces;
- Tom Robinson: Biomicrofluidic Systems (since 2016);
- Sophia Rudorf: Biomolecular Processes;
- Mark Santer: Carbohydrates and Polysaccharides;
- Angelo Valleriani: Stochastic Processes;
- Ana Vila Verde: Soft Matter Simulations;
- Thomas Weikl: Proteins and Membranes.

The experimental group of Tom Robinson is an independent junior group, funded by the Max Planck Research Network in Synthetic Biology (MaxSynBio).

The main results of these research groups are described in separate reports on the following pages. These reports are ordered in a bottom-up manner, i.e., from small to large length scales, and related to four research areas: Biopolymers, Biomolecular Processes, Membranes and Vesicles as well as Interfacial Phenomena. In this introductory overview, the reports of these research groups will be briefly summarized and a few additional aspects will be highlighted.

Biopolymers

The three research groups of Andrea Grafmüller, Mark Santer, and Ana Vila Verde study the behavior of biopolymers using atomistic and coarsegrained molecular dynamics simulations. The Vila Verde group investigated the water dynamics in electrolyte solutions as well as the interactions of ions with proteins and dendrimers. Other projects of the Vila Verde group address the mechanical response of single helices and coiled coils under tension. The Santer group has worked on force field modularization for carbohydrate compounds (glycans). The modular force fields were applied to the recognition of lipopolysaccharides by proteins, to the compaction of DNA by azo-containing peptidomimetic molecules, and to GPI-anchors in lipid membranes. The Grafmüller group studied the solubility of different mono- and oligosaccharides and introduced an improved force field that describes the concentration dependence of the osmotic pressure in a reliable manner. Based on the improved force field, a coarse-grained model was developed and used to show that the water-uptake of linear and branched polysaccharides is rather different.

Biomolecular Processes

The two research groups of Sophia Rudorf and Angelo Valleriani use stochastic modelling to study protein synthesis and post-translational gene expression. The Rudorf group determined the dependence of protein translation on EF-Tu concentration and developed a new algorithm for codon optimization. Two doctoral projects of the Rudorf group address the entry of the nascent peptide chain into the ribosomal exit tunnel and the co-translational assembly of dimeric proteins. The Valleriani group studied the influence of degradation of mRNA, ribosomal drop-off, as well as protein ageing and degradation on gene expression.

One topic that is not covered in the following reports is the cargo transport by molecular motors. Some years ago, we introduced a stochastic model for the bidirectional cargo transport by two antagonistic motor teams such as kinesin and dynein. [M. I. J. Müller et al, PNAS, 2008] This theoretical model, which has received a fair amount of attention, is based on a simplified description for the force balance underlying the tug-of-war between the two motor teams. In order to improve this theory, we have recently considered two antagonistic motor teams that are elastically coupled to the cargo. We first studied the simplest case of one kinesin against one dynein, see **Fig. 1,** and found that the elastic interactions forces between the two motors depend rather strongly (i) on the unbinding rate for the single motors and (ii) on the strength of the elastic coupling between the motors. We now extended our theory to more than 1+1 motors, which revealed how the elastic interaction forces are shared among all motors from the same team.

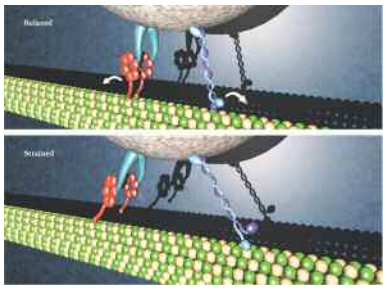

Fig. 1: Tug-of-war between one dynein motor (left, blue-red) and one kinesin motor (right, blue-purple) that step along a microtubule (green-yellow) and pull a cargo (grey) into opposite directions: In the upper panel, the two elastic linkers between the motors and the cargo are relaxed and the motors do not exert elastic forces onto each other. When one of the motors performs a discrete forward step (white arrows), the stalks become stretched and the motor proteins become strained as shown in the lower panel. [M. Ucar and R. Lipowsky, Soft Matter 13, 328 (2017)]

Membranes and Vesicles

The behavior of biomembranes and giant vesicles has been addressed by the three experimental research groups of Rumiana Dimova, Roland Knorr, and Tom Robinson as well as by the theoretical research group of Thomas Weikl. The Weikl group has elucidated the binding of membrane-anchored proteins and the conformational changes during protein binding. This group also continued its studies on the wrapping of nanoparticles by membranes.

One project that involved the concerted efforts of theory, simulation, and experiment addressed the intimate relation between the asymmetry of bilayer membranes and the spontaneous tubulation of giant vesicles. We have now developed three different methods to deduce the spontaneous curvature of membranes from the morphology of giant vesicles with nanotubes. These quantitative methods are based on the detailed image analysis of spontaneously tubulated vesicles, on the application of local forces that pull additional tubes from these vesicles, and on the initial aspiration of such vesicles by micropipettes. Giant vesicles with membrane nanotubes have unusual mechanical properties because the tubes provide a large area reservoir for the mother vesicles. Therefore, these vesicles can adapt to strong mechanical perturbations by exchanging membrane area with the tubes. As a consequence, tubulated vesicles behave, to a large extent, like liquid droplets with constant volume and variable surface area.

Jaime Agudo-Canaleijo and myself have developed a rather detailed analytical theory for the interactions of nanoparticles with membranes and vesicles. We have shown that the spontaneous curvature of the membranes provides a key parameter for the engulfment process which leads to four different engulfment regimes for a single nanoparticle. When exposed to a finite concentration of dispersed nanoparticles, a vesicle membrane exhibits distinct engulfment patterns consisting of up to three different membrane segments. Partially engulfed nanoparticles experience curvature-induced forces that bias the diffusion of these particles along the membrane. As a consequence, the probability to find such a particle at a certain membrane position depends on the local mean curvarture of the membrane. The corresponding distributions are shown in **Fig. 2** for Janus particles with one adhesive and one non-adhesive surface domain. As illustrated in this figure, any shape transformation of the vesicle implies a concomitant transformation of the particle distribution and, thus, a strong change in the associated color pattern.

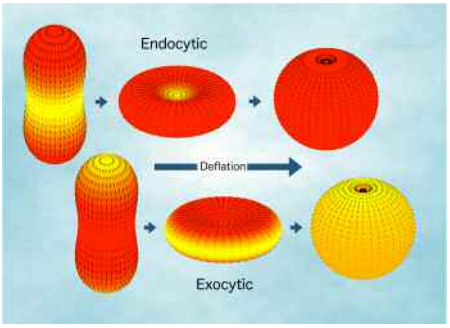

Fig. 2: When osmotically deflated, the prolate vesicle on the left is first transformed into a discocyte and subsequently into a stomatocyte (the inner segment of the stomatocyte is masked by its outer segment). When Janus particles are partially engulfed by the membranes of these vesicles, the probability to find such a particle is high for the yellow membrane segments and low for the red ones, reflecting the curvature-induced forces acting on the particles. When the particles are attached to the outer and inner membrane leaflet, corresponding to endocytic and exocytic engulfment, the particles prefer to stay at membrane positions with large negative and large positive curvature, respectively. [J. Agudo-Canalejo and R. Lipowsky, Soft Matter (2017), in press]

The Dimova group studied the effects of bilayer asymmetry and tension on lipid phase separation, the polymorphism and adhesion of giant vesicles, the spontaneous and force-induced formation of membrane nanotubes, and the shaping of vesicles by electric fields, light and proteins. The ongoing projects include curvature generation by ions, STED microscopy of nanotubes, light-controlled shape transformations, and the behavior of giant plasma membrane vesicles, so-called blebs. The Robinson group developed assays to localize membrane fusion to intramembrane domains formed by liquid-disordered or liquid-ordered lipid phases as well as new microfluidic tools for the handling and trapping of vesicles. These tools will now be used to construct multi-compartment vesicles systems ("vesicles in vesicles") and to encapsulate enzymatic reactions. The Knorr group studied shape transformations of double-membrane vesicles, which are relevant for autophagy, the reconstitution of protein cascades at membranes, and the interaction of membrane-enclosed organelles with membrane-less organelles. The latter organelles behave like liquid droplets and undergo wetting transitions at membranes and vesicles. We have identified several control parameters for these transitions which lead to a complete redistribution of the molecules that are enriched in the droplet-like organelles. Furthermore, because a membrane segment in contact with such an organelle acquires a spontaneous curvature, the wetting transitions can be used to locally control this curvature.

Interfacial Phenomena

The group of Hans Riegler continued their investigations of phase transitions and transport phenomena at solid-air interfaces. Of particular interest were drop-drop coalescence, interfacial flow and drop evaporation, melting and solidification of nano-structures as well as patterned growth induced by heterogeneous nucleation.

Biannual Series of Symposia

We continued our biannual series of topical symposia and organized a symposium on 'Multiscale Motility of Biomolecular Machines' in 2015 as well as another 'Biomembrane Days' in 2016.

International Max Planck Research School

The department of Theory & Bio-Systems was in charge of the International Max Planck Research School on "Multiscale Biosystems", which started in July 2013 and will operate at least until 2019. We recently organized an on-site evalutaion of our School, with a very positive outcome.

For additional information about research at the Department of Theory & Bio-Systems, see the subsequent reports and www.mpikg.mpg.de/th/.

Reinhard Lipowsky
Head, Department of Theory & Bio-Systems

From Ionic Solutions to Interacting Proteins

Ana Vila Verde 20.08.1976
1994-1999: Undergraduate degree (5 years) in Teaching of Physics and Chemistry, University of Minho, Braga, Portugal
1999-2000: Teacher of physics and chemistry (António Feijó junior high school, Ponte de Lima, Portugal)
2000-2001: Teacher of physics and chemistry (Alcaides de Faria high school, Barcelos, Portugal)
2001-2005: Doctoral thesis: Optimization of minimal invasive dental laser ablation by mesoscopic modeling (Department of Physics, University of Minho, Braga, Portugal)
2005-2007: Post-doctoral Researcher, (Pennsylvania State University, Pennsylvania, USA)
2007-2010: Post-doctoral Researcher, (FOM Institute AMOLF, Amsterdam, The Netherlands)
2010-2011: Post-doctoral Researcher, (University of Amsterdam, The Netherlands)
2012-02/2014: Post-doctoral Researcher, Department of Theory & Bio-Systems (Max Planck Institute of Colloids and Interfaces)
Since 03/2014: Research Group Leader, Department of Theory & Bio-Systems (Max Planck Institute of Colloids and Interfaces)

We use molecular simulations and classical, atomistic models to investigate various systems relevant for biology. The systems chosen – ranging from simple solutions to full size proteins – reflect a general approach: we first focus on simple systems, and then apply the knowledge obtained with them to the study of more complex ones.

Our studies of *electrolyte solutions* demonstrated the strong connection between the ion-pair structure of the solution and the emergence of non-additive effects in the stiffness of the water hydrogen bond network. This connection is likely important for protein function. To enable the study of biological systems in the presence of ions, *we developed optimized parameters (force fields) for monoatomic and polyatomic* ions in water. We go beyond state-of-the-art parameterization approaches, which prove insufficient for these systems.

Experimental studies of fluorinated proteins have demonstrated the potential of fluorination to tune protein properties, but the mechanisms underlying the observed changes remain unknown. Our initial studies showed the need to follow a non-standard approach to parameterize fluorinated amino acids. Using the force field we created, we have clarified the molecular mechanisms behind changes in hydration free energies upon fluorination. This property is key to understand how proteins respond to fluorination.

Our studies of *dimeric and trimeric coiled coils* under tension are revealing how their mechanical response emerges from that of α-helices. We investigate these systems at low pulling speeds, which requires particularly long simulation times. Our results show that previous effects mentioned in the literature are not biologically relevant because they arise at high pulling speeds only.

Interactions between Water and Ions

The effects of ions on the properties of water (e.g., the strength of the hydrogen bond network), or the properties of other solutes in water (e.g., solubility of proteins) are commonly thought to be additive: the impact of a given salt on a given property is interpreted as the sum of the impact of the anions and cations.

Experiments probing water rotational dynamics – which indirectly reports on the stiffness of the water hydrogen bond network – however, have challenged this view. To gain insight into this issue, we use polarizable models to investigate the dynamics of rotation of water in aqueous solutions containing $MgSO_4$, for which the largest supra-additive effect was observed in experiment **[1]**. We parameterize these models to reproduce both the free energy of hydration of single ions and the solution activity derivative at high concentration. The models are thus appropriate to gain insight into water dynamics in a wide range of concentrations, necessary for comparison with experiment. We find that $MgSO_4$ greatly slows down water dynamics, in agreement with experiment. To understand the mechanisms behind this slow down, we investigate water dynamics for different water subpopulations near static ion pairs. We find that large, supra-additive slowdown occurs only for water molecules directly bridging the $Mg^{2+}\cdots SO_4^{2-}$ ions. Non-intuitively, supra-additive slowdown is not a purely electrostatic effect, as **Fig. 1** illustrates: water reorientation times larger than 8 ps – for which supra-additive slowdown exists – are uncorrelated with more intense local electric fields. Instead, supra-additive slowdown seems to result from a change in the free energy landscape associated with hydrogen bond breakage and formation events, for water molecules that are hydrogen-bonded to SO_4^{2-} and which simultaneously belong to the first hydration layer of Mg^{2+}.

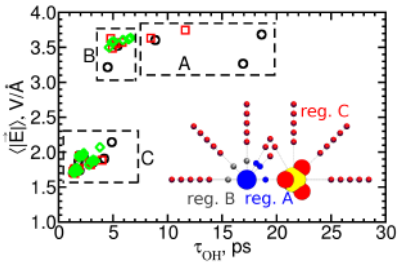

*Figure 1: Local electric field vs. water reorientation time for water molecules in each subpopulation (indicated by the small spheres in the inset) around $Mg^{2+}\cdots SO_4^{2-}$ ion pairs. Supra-additive slowdown is only observed for water subpopulations in region A **[1]**.*

The coupling between solution structure – the proportion of different types of ion pairs – and the stiffness of the water hydrogen bond network, demonstrated by the above-mentioned results, highlights the general importance of both ion-ion and ion-water coupling to understand ion-specific effects in biology. To allow the investigation of these effects, we have recently developed an *optimized force field for the alkali, alkali earth and halide ions and the TIP5P water model, based on experimental data* **[2]**. This force field is desirable for simulations of saccharides. Our results show that the state-of-the-art approach often used to parameterize anion-cation interactions is insufficient, and that existing force-fields often over-estimate the number of ions in direct contact in solution.

Interactions between Ions and Proteins

Molecular scale studies of ion-specific effects which arise from interactions between mono- or polyatomic ions and proteins, have been hindered by the absence of classical force fields that are compatible with existing force fields for proteins. To address this need, we have developed a set of parameters for the SO_4^{2-}, SO_3^{2-}, HPO_4^{2-}, $H_2PO_4^-$ ions, the methylated versions of these anions, and for CH_3COO^- **[3]**. Their interactions with positively charged amino acids and with the physiologically relevant Na^+ cation are explicitly parameterized. Our results show that existing force fields greatly overestimate interactions between negative amino acids and Na^+, as well as the strength of salt bridges in proteins. Our newly developed force field will be applied to study interactions between selectins – cationic proteins involved in cancer metastasis and in inflammatory response – and anionic polymeric inhibitors, which are being experimentally studied in the Haag group at the Free University of Berlin together with Peter Fratzl at this institute.

Fluorinated Amino Acids and Proteins

Fluorination – replacing C-H bonds by C-F bonds – of the side chains of hydrophobic residues in proteins often improves the protein's thermal stability. Despite the fact that the intrinsic physicochemical properties of fluorine are well understood, understanding and predicting how fluorination affects protein properties is not yet possible. Experimental reports on fluorinated proteins led to a number of questions regarding the *mechanisms* by which fluorination alters, e.g., the hydrophobicity of amino acids. To investigate these issues we developed a force field for fluorinated amino acids. The force field is fully compatible with the widely used AMBER force fields for proteins, but relied on a non-standard parameterization of the amino acid charges because the standard procedure proved insufficient. Our initial work focused on understanding how CH_3-to-CF_3 substitutions affect the hydration free energy of amino acids **[4]**. We find that CH_3-to-CF_3 substitutions increase the amino acid hydrophobicity, i.e., they result in less negative hydration free energies. This result is consistent with experiment. Surprisingly, however, even for a system as simple as a single amino acid, the *magnitude of the change* in hydration free energy upon a single CH_3-to-CF_3 substitution can vary between 0.5 and 1.5 kcal/mol, depending on the identity of the amino acid and the position in which fluorination occurs. These differences cannot be completely explained in terms of differences in the solvent-accessible apolar surface area between the various amino acids, as is commonly believed. Our results demonstrate that fluorination changes the free energy of hydration largely by altering the number of backbone-water hydrogen bonds, an effect that was not previously demonstrated. These results suggest that different fluorinated amino acid isomers may lead to different changes in protein structural stability, an effect which will be explored by the Koksch group at the Free University of Berlin.

Mechanical Response of Single Helices and Coiled Coils under Tension

Coiled-coils (CC; **Fig. 2**) are ubiquitous structural motifs in many proteins: e.g., they are present in the cytoskeleton and the extracellular matrix of cells.

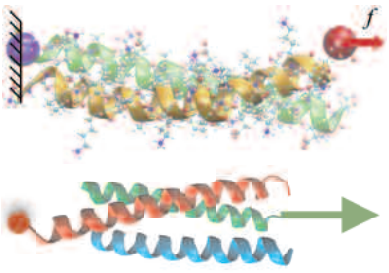

Fig. 2: Dimeric and trimeric coiled coil under a pulling force f.

Coiled-coils are thought to be necessary for chemical functions as well as for mechanical or chemomechanical ones. Our aim is to understand the *mechanical* response of coiled coils: how this response emerges from the properties of the single α-helices that compose them and how it is affected by the multimerization state (dimeric or trimeric) of the coiled coil. Clarifying these issues is critical to understand the role of coiled coils with different multimerization states in biology. We perform pulling simulations where one end of an α-helix is kept fixed, and another one is pulled (**Fig. 2**). These simulations are analogous to atomic force microscopy (AFM) experiments. We find that single α-helices and dimeric coiled coils have a very different force-velocity dependence, with α-helices being equally stiff at all speeds whereas the coiled-coil clearly becomes stiffer as the pulling speed increases (**Fig. 3**). Despite having a different force-velocity dependence, both systems behave as constant-force springs and have similar force plateaus (circa 50 pN) at the lowest pulling speed. This low velocity regime is the closest that simulations can currently get to physiologically relevant conditions and also to AFM experiments.

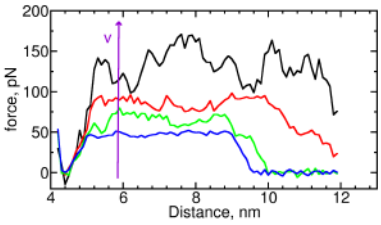

*Fig. 3: Force vs. extension for a dimeric coiled coil. The different colors indicate different pulling speeds (v), ranging from 10^6 nm/ps to 10^3 nm/ps **[6]**.*

The similarity in the force plateaus of single α-helices and of dimeric coiled coils is puzzling, because the plateau in the single α-helix is clearly related with the unfolding of that helix, but pulling of the dimeric coiled coil results only in sliding of one helix relative to the other, without net unfolding. A deeper look into our results offers a possible explanation for the similarity of the force plateaus: sliding occurs via transient opening and closing of the α-helices composing the coiled coil **[5, 6]**. Future work will include AFM experiments (in the Blank group at this institute) and simulations with mutated sequences to determine which of these trends are general and which are system-specific.

Ana Vila Verde, Ana Bergues Pupo, Chuanfu Luo, Sadra Kashef Ol Gheta, João Robalo
ana.vilaverde@mpikg.mpg.de

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As Glycans Grow Rich

Mark Santer 27.10.1971
2003: PhD in Physics (University of Freiburg)
2003-2006: Postdoc, Group leader Nanofluidics (Institute of Microsystems Engineering, University of Freiburg)
2006-2009: Employee (Fraunhofer Institute of Mechanics of Materials (IWM), Freiburg)
2010: Postdoc at the Theory Department, MPI for Colloids and Interfaces, Potsdam
Since 2010: Group leader Carbohydrates and Polysaccharides in the department of Theory and Biosystems.

In virtually all organisms carbohydrate compounds *(glycans)* are involved to modify or enhance the function of other biomolecules. The glycosylation of proteins and lipids in the extracellular matrix is important for initiating cell recognition, fine tuning inter-cell communication or establishing protective barriers. These different functionalities reflect the underlying diversity in glycan composition and conformational flexibility. Developing reliable force fields for computer simulations of these biomolecules is the main theme of our work. The current research activities are centered around the question how modeling is to be pursued when glycan containing biomolecules consist of an increasing number of components. How can we improve modularity of the force fields? How then do we interpret diverging predictions of different force fields?

Recognition of Lipopolysaccharides by Proteins.
The latter problem emerged in the context of the question how phages can infect Gram-negative bacteria, which protect themselves from invasion with a dense lipopolysaccharide (LPS) coat.

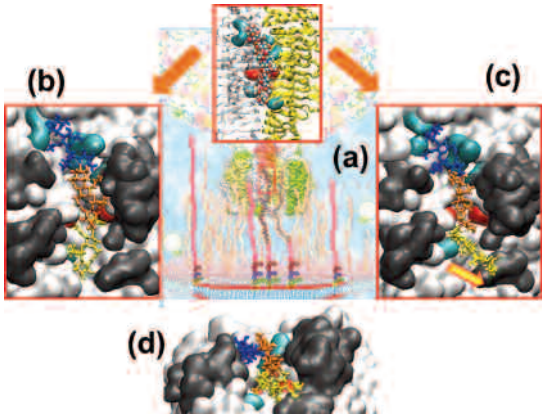

Fig. 1. (a) Schematic view of a phage approaching the LPS coat of the bacterium Shigella flexneri. The successive enhancements show how the phage grasps a fragment of the long LPS side chain. These polysaccharides consist of repeating units of tetrasaccharides, and are docked to the groove between two of the three monomers forming a tail spike protein below the capsid. Binding (b,c) is mediated mostly by hydrogen bonding residues (cyan) interacting with the first repeat unit (RU1: blue), while the trailing units (RU2: orange and RU3: yellow) sterically adapt to the side walls formed by unstructured loops (gray), see view along groove (d). Red: catalytically active sites E366/D399. (b) shows a stable pose in the GLYCAM case; (c) indicates the frequent excursions (arrow) found for CHARMM.

It is well known that recognition of certain epitopes followed by enzymatic cleavage of the polysaccharide O-Antigens is the key to this process **[1]**, see **Fig. 1**. Supporting an extensive body of experimental evidence, we were able to comprehensively characterize the recognition of a two-repeat unit epitope of Serotype Y polysaccharide to the tail spike proteins (TSP) of phage Sf6 **[2]**. Both force fields employed, GLYCAM and CHARMM, agreed in the description of the binding mode as a concerted action of hydrogen bonding, loop flexibility and conformational selection. They are at variance in predicting the placement of longer fragments, such as the 3RU dodecamer shown in (b,c). This divergence is quite useful. It directly points to the questions of how infection proceeds on time scales far longer than the atomistic, and the unspecific interaction of LPS with TPS beyond the epitope. Are LPS degraded processively, with strong anchoring within the LPS coat, or does the phage only randomly attempt to break through?

Force Field Modularization.
The great diversity of carbohydrate compounds requires a modular organization of the force fields, where complex molecules can be built up from smaller, invariant building blocks.

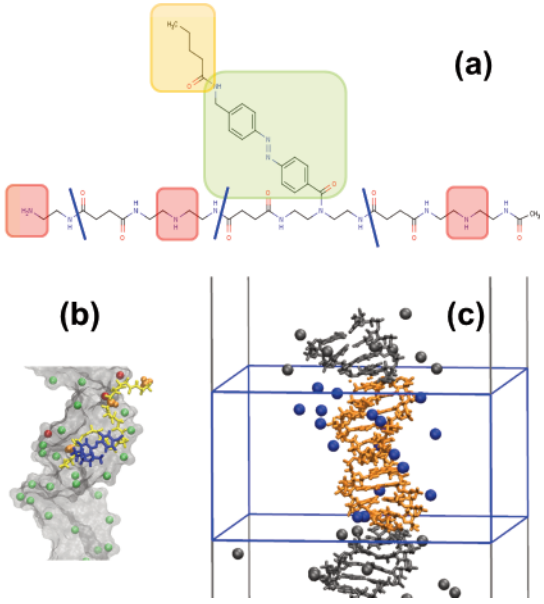

Fig. 2. (a) structure of the azo-containing peptidomimetic (Azo-PM) compacting agent. Red: protonated amine groups; green: azobenzene containing side chain; yellow: trailing hydrophopic moiety. (b) interaction of trans Azo-PM with a 22bp segment of B-DNA; green: phosphates; orange: protonated amines; read: proximal phosphates. (c) Snapshot of Na+ atmosphere around the segment, with the simulation box as indicated.

In modeling the interaction of LPS core oligosaccharides with lung surfactant proteins **[3]**, we have tested a procedure by which a carbohydrate building block is created with respect to how it is embedded into the neighboring molecular environment. It turned out that this approach can conveniently be applied to other complex biopolymers as well, such as the light sensitive, DNA compacting agent Azo-PM shown in **Fig. 2**. The blue solid lines indicate the decomposition of the molecule into different building blocks. Partial atomic charges are determined by considering combinations of building blocks and defining suitable overlap regions. The resulting model for Azo-PM was successfully used to study its interaction with a DNA strand **[4]**. The *cis*- isomer of azo moiety leads to an overall weaker interaction of the protonated amine groups with the negatively charged phosphates. In the experiment, this difference triggers decompaction/compaction of single DNA molecules under photo(UV-)induced *cis-trans* isomerization.

Conclusions and Current Developments.

How to deal with force field dependent outcomes of a computer simulation will certainly become an interesting aspect of our future work, in particular if available experimental evidence cannot favor one over the other. In this respect, complementary simulation techniques can prove valuable. In the example of dodecamer accomodation at the TSP binding groove one might obtain further information from estimating how efficiently the polysaccharide can be cleaved depending on its conformation in the binding site. Currently, we are exploring hybrid quantum mechanical/molecular mechanical (QM/MM) techniques in order to model the corresponding process of enzymatic hydrolysis explicitly. As a starting point, however, we have first returned to azobenzene derivatives. Their isomerization process is genuinely quantum mechanical, the kinetics of which is greatly influenced by an environment that can be kept at the classical molecular mechanical level. This also facilitates the use of path sampling techniques, by which we can access the dynamics of reaction mechanisms **[5]**.

M. Santer, S. Kunstmann, A. Muzdalo, P. Banerjee, S. Schimka
mark.santer@mpikg.mpg.de

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Modelling Aqueous Saccharides: How Sticky are these Sugars?

Andrea Grafmüller 18.08.1980
2000-2004: Master in Science (MSci), Physics, Queen Mary University of London. Thesis: Computer Simulation of Kinetic Ising Systems with Glauber Dynamics
2004-2007: Doctoral Thesis: The Fusion of Membranes and Vesicles (Max Planck Institute for Colloids and Interfaces)
2007-2009: Postdoctoral Scientist Department of Theory and Bio systems, Max Planck Institute of Colloids and Interfaces.
2009-2010: Postdoctoral Scientist, University of Utah
2010-2011: Postdoctoral Scientist, University of Chicago
2011-2012: Research Scientist, Department of Theory and Bio systems, Max Planck Institute of Colloids and Interfaces.
Since 2012: Research Group Leader, Department of Theory and Bio systems, Max Planck Institute of Colloids and Interfaces

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Carbohydrates are abundant in nature and natural materials. Their function ranges from structural stability and energy storage to functions in the glycocalyx, the extracellular matrix, cell signaling and the molecular recognition of pathogens. In order to understand these diverse functions, increased efforts have been made recently to model these carbohydrates in order to elucidate their molecular properties.

All-atom molecular dynamics (MD) simulations are a useful method to study biomolecular systems **[1–5],** and several force fields have been developed specifically for carbohydrates. A comparison of several such force-fields have shown that the best agreement with the sparse experimental data, both for solution properties and hydration free energies, could be obtained using the GLYCAM06 force-field with the TIP5P water model **[6]**.

While models with atomistic resolution give a detailed picture, they often cannot reach the length and time scales required to sample larger polysaccharides. Strategies to overcome these difficulties involve the use of simplified coarse-grained models, with fewer degrees of freedom **[7–10]**. Here we describe the application these modelling strategies to hemicellulose polysaccharide systems. The simulations described below were motivated by an attempt to explain the molecular origin of the actuated motion performed by plant cell wall materials in response to hydration, e.g. in the opening of pine cones and many other processes.

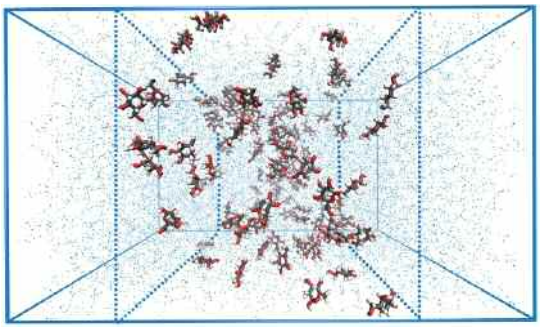

*Fig. 1: Simulation setup to measure the Osmotic Pressure **[11].***

Osmotic Pressure in Carbohydrate Solutions

A key property to quantify the aggregation of solutes is the osmotic pressure π of a solution. An intuitive method to obtain π directly in MD simulations is the use of virtual, semi-permeable membranes, which confine the solute molecules to a central region in the simulation box, as shown in **Fig. 1**. The pressure π can then be calculated from the wall force acting on the solute atoms **[11]**.

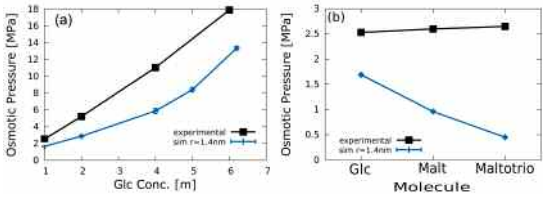

*Fig. 2: Osmotic pressure calculated as a function of concentration (a) and degree of polymerization (b) **[11].***

Fig. 2a shows π as a function of concentration for β-D-Glucose solutions, in comparison to experimental data. Clearly, the simulated results systematically underestimate π by approximately a factor of two. The low osmotic pressure indicates that, although no aggregation is directly observed in the simulated systems, the sugar-sugar interactions are overrepresented by the force-field. As expected from the previous force field comparison, other common force fields perform even worse.

Even more severe is the observation **(Fig. 2b)** that π decreases for larger molecules, i.e. a Maltose dimer and a Malto-triose, whereas experimental data shows a slight increase of π with the degree of polymerization.

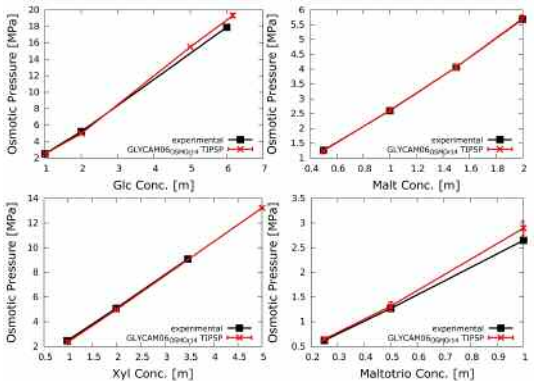

*Fig. 3: Osmotic pressure calculated using the new force field parameters $GLYCAM_{06SMOr14}$.TIP5P **[11].***

Considering these shortcomings, improved force field parameters are required to obtain any predictive power about the factors affecting the water sorption capacities. Since aggregation was found to be driven by the Lennard Jones interactions, and as the charges of the force field have been optimized specifically for carbohydrate conformations, we focus on adjusting the Lennard Jones parameters ε. The data shown in **Fig. 3**, illustrates, that the optimized parameters lead to excellent agreement with experimental data, both for molecules used in the reparametrization process (Glucose, Maltose), and for test molecules (Xylose, Maltotriose).

Factors affecting the Osmotic Pressure

We can now apply the optimized force field to gain some insight into the factors which affect π, and thus the water sorption capability of carbohydrate molecules. First, we compare the influence of the chemical structure of the monomer building blocks. While changes in the geometric configura-

tion of the atoms have no appreciable effect – Glucose, Mannose and Galactose give indistinguishable results – the removal of an OH group (e.g. Xylose) or substitution of an apolar group (e.g. 06-acetyl-β-D-galactose) both decreases the osmotic coefficient of the solution.

Then, we consider the effect of xyloglucan structure on π. To that effect, short structures with monomeric and dimeric branches are compared to linear saccharides made from the same monomers. In all cases, π is higher for the branched structure. The magnitude of the difference increased with the number of side chains, reaching ~40% for three monomer side chains. The location of the side chains has a comparatively small influence. The branching point has no appreciable effect for monomer side chains, whereas for a longer (dimeric) sidechain a small influence of the side chain location on π emerges.

A Coarse- Grained Sugar Model

The osmotic pressure calculations for linear saccharides are limited to 7 monomers. Equilibration of longer polymers could not be achieved, which illustrates the limitations of all-atom MD simulations. As many natural polysaccharides are much longer, a reliable coarse-grained representation is required to study such systems.

Therefore, we develop a procedure to generate a coarse-grained model based on the sampling at the atomistic scale, which employs Boltzmann Inversion, to obtain parameters for the bonded interactions within one molecule, and the Force-Matching method for non-bonded interactions between different molecules (and all sites not interacting by bonded interactions). This hybrid model reproduces the structural data from the atomistic system quite well, provided the solute-solute interactions and the interactions involving solvent are treated separately **[10]**.

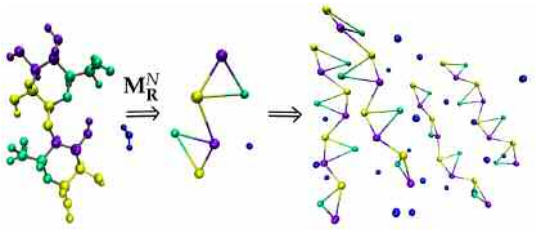

*Fig. 4: Mapping between the atomistic and coarse-grained Model **[10]**.*

To be useful for the simulation of polymer systems, it is crucial, that the developed model is transferable to different polymer lengths or concentrations. Tests have shown that the models can be transferred to different lengths and to higher concentrations, and perform with a similar accuracy as the models derived specifically at that concentration/polymer length. However, care has to be taken when the method is applied at low concentrations, where first the native, and then the transferred model fail to capture the correct aggregation behavior of the molecules. This is related to small perturbations in the long range interactions, which gain more importance at lower concentrations.

The aggregation at low concentration can be corrected by applying a small cut-off to the long range interactions. This cut-off has no appreciable effect on the system at higher concentrations. Finally, the same procedure can be used to generate implicit solvent models, which have the highest efficiency, where specific water interactions are not important.

Application

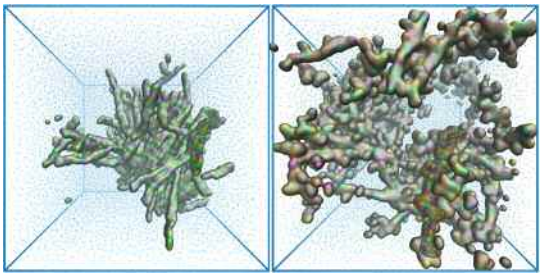

*Fig. 5: Representative snapshots from the coarse-grained system of linear (left) and branched (right) polysaccharides **[10].***

As a first application of the coarse-grained model, the water-uptake by clusters of linear or branched polysaccharide was studied and compared. The linear polysaccharides remain in tight aggregates, while the branched molecules absorb water, until their network spans the entire simulation box, as shown by the representative snapshots in **Fig. 5.**

Because the coarse-graining procedure applied to develop the model relies on reproducing the forces present in the atomistic system, and because the osmotic pressure π is calculated from the wall force acting on the sugar molecules, it is also possible, to measure π of the coarse-grained system, and excellent agreement between π measured in the atomistic and the coarse-grained systems is achieved. The computational speedup of this procedure will allow to measure π of much larger molecules and longer branches, to gain a concise understanding of the factors tuning the water sorption of polysaccharide gels, such as the hemicellulose matrix.

What about Interactions with other Molecules?

In most biological systems, carbohydrates do not act by themselves, but are in contact with other biomolecules such as lipids and proteins.

Even the simple addition of salt to the system turned out to be problematic, as no optimized ion parameters exist to be used with the TIP5P water model. Tests show, that parameters optimized for other water models do not give satisfactory results, so that a new set of LJ parameters for alkali and halide ions has been developed to reproduce the hydration free energy as well as the activity derivative, a_{cc}, and coordination numbers of chosen salts. In the process, we have shown that matching a_{cc} alone is not sufficient, because a_{cc} as a function of the interaction strength often reaches a plateau. This means that parameters leading to equally good agreement with experiment can yield very different solution structures **[12].**

A. Grafmüller, A. Quaas, V. Satarifard, J.Sauter, A. Singhal, S. Wagle
gmueller@mpikg.mpg.de

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Protein Synthesis in the Cell

Sophia Rudorf 12.02.1985
2004-2009: Diploma, Physics
Thesis: Investigation of the Self Assembly of Monomolecular DNA-Lipid Complexes (University of Potsdam/LMU Munich)
2010-2015: PhD, Physics,
Thesis: Protein Synthesis by Ribosomes (MPI of Colloids and Interfaces)
Since 2015: Research Group Leader, Department of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces

The synthesis of proteins is a fundamental task of all living cells because almost every cellular process is governed by proteins. Every protein consists of at least one chain of amino acids. The concatenation of individual amino acids into peptide chains is achieved by molecular machines called ribosomes. To synthesize a protein, a ribosome uses the genetic information stored in the corresponding messenger RNA (mRNA). A mRNA consists of a sequence of codons, each of which codes for a specific tRNA and, thus, for a specific amino acid. Each amino acid is carried by a transfer RNA (tRNA) molecule. An aminoacylated tRNA and an elongation factor EF-Tu form a ternary complex that reaches the ribosome by diffusive motion. The ribosome reads the mRNA codon by codon and takes up the corresponding ternary complexes. This process is called translation. Our group studies translation at different levels from individual biochemical kinetic rates to cell-wide protein synthesis.

Ultrasensitive Dependence of Protein Synthesis on EF-Tu Concentration

The bacterial doc-phd toxin-antitoxin system has a strong influence on the rate of cell growth. The toxic protein Doc suppresses the growth rate by inhibiting the elongation factor EF-Tu, which is crucial for bacterial translation, see **Fig. 1**. Given that EF-Tu is one of the most abundant proteins in bacteria, it is astonishing that Doc is such an effective toxin. To find the origin of the high Doc efficiency, we study the effect of EF-Tu inhibition on protein synthesis within a recently established theoretical framework for bacterial translation **[1, 2].** Surprisingly, we find a very sensitive dependence of the overall translation rate on EF-Tu abundance: a small decrease in EF-Tu concentration leads to a strong suppression of overall protein synthesis, despite the extremely high cellular abundance of the elongation factor **[3].** We show that this ultrasensitivity is caused by imbalances in the interplay of different codons and tRNAs and can be observed for complex *in-vivo* protein synthesis as well as in simple artificial translation systems based on only two codons and their cognate tRNAs. Thus, the abundance of EF-Tu is a highly effective control variable for bacterial protein synthesis whereby the growth-inhibiting effect of Doc is strongly amplified.

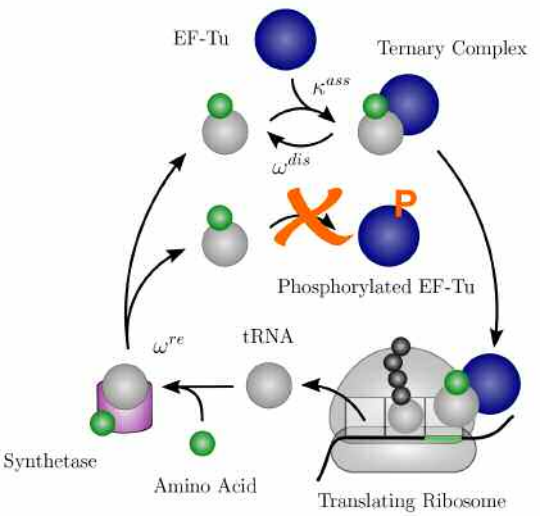

Fig. 1: Ternary Complex Formation. After a tRNA is released from a ribosome, it binds to an aminoacyl tRNA synthetase that recharges the tRNA with its cognate amino acid. The recharged tRNA binds to elongation factor EF-Tu to form a ternary complex that delivers its amino acid to a translating ribosome (upper pathway). If an EF-Tu molecule gets phosphorylated by the toxin Doc, it is no longer able to bind aminoacylated tRNAs (lower pathway).

Optimizing the Dynamics of Protein Translation

Optimizing protein translation for synthetic gene expression is a complex task. Conventionally, improvement of protein synthesis was approached by replacing rarely used codons by the target organism's preferred codons. However, this strategy does not always yield the best results. In contrast to these conventional approaches, we predict optimal codon usage based on translation speed and accuracy combined with further relevant covariates and confirm our optimization approach with proteome data from widely used prokaryotic, eukaryotic, and human expression systems **[4].** We optimized and tested heterologous expression of two genes, *manA* and *ova* in *Salmonella Enterica serovar Typhimurium,* which showed a threefold increase in protein yield compared both to wild type and commercially optimized sequences. Our multi-parameter algorithm cannot only be used for protein yield optimization but also encompasses fine-tuning protein expression, including deoptimization, e.g. for synthetic attenuated virus engineering.

Studying the Nascent Peptide Chain in the Ribosomal Exit Tunnel

Our collaborators Prof. Dr. Marina Rodnina and Dr. Wolf Holtkamp from the Max Planck Institute for Biophysical Chemistry use a stopped-flow instrument to study translation as shown in **Fig. 2**: One syringe of the instrument is filled with a solution containing the ribosomes and mRNAs, whereas the other holds the corresponding tRNAs. Translation starts

as soon as the two solutions are mixed together. During translation, the elongating nascent peptide chains traverse the ribosomes' exit tunnels. We monitor this co-translational movement via fluorescent probes attached to the N-termini of the nascent chains. Due to fluorophore quenching, the time-dependent fluorescence signal emitted by an individual peptide is determined by co-translational events, such as secondary structure formation and peptide-tunnel interactions. To obtain information on these individual events, the measured total fluorescence signal has to be decomposed into position-dependent intensities. To this end, we describe mRNA translation as a Markov process and assign a specific fluorescence intensity to each ribosomal state. **Fig. 3** shows the measured time-dependent total fluorescence intensity for poly-phenylalanine peptides and numerical results obtained by the evaluation of our model. Our theoretical description provides a good representation of the biological process. We find that the N-terminus of poly-phenylalanine experiences major environmental changes, which occur primarily during translation of the first eight amino acids.

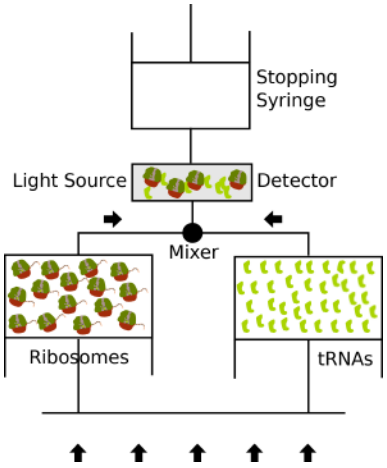

Fig. 2: Schematic drawing of a stopped-flow instrument to study translation by fluorescent probes. The syringe on the left is filled with a mixture of ribosomes and mRNAs, whereas the right syringe contains the tRNAs. Translation begins when both solutions are mixed. Progression of translation is monitored by a fluorescent signal emitted by fluorophores attached to the N-termini of the elongating peptides.

Co-Translational Assembly of Protein Subunits

Protein assembly from multiple subunits inside the crowded cell environment is subject of ongoing research. In contrast to the general thinking of protein assembly as a post-translational process, recent experiments show that protein complexes can also assemble co-translationally, i.e., subunits may assemble *before* translation has finished (Yu-Wei Shieh et al., Science 2015). Our collaborator Roy Bar-Ziv and his group from the Weizmann Institute of Science in Israel performed *in-vitro* translation experiments to assess the role of

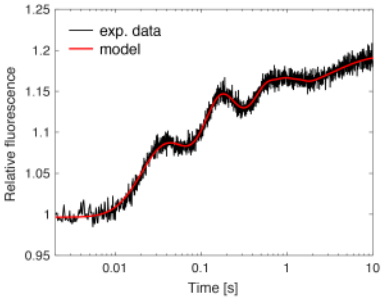

Fig. 3: Fluorescence signal of poly-phenylalanine translation (black line) obtained by a stopped-flow experiment and signal as predicted from our translation model (red line).

the spatial distance between the translation sites of different protein subunits. As a first step, we modeled the synthesis and spread-out of one type of proteins in one dimension as a Markov process (**Fig. 4**) in good agreement with the experimental data (**Fig. 5**). As a next step, we will study post- and co-translational interactions of multiple protein subunits by Gillespie simulations to understand the dynamics of protein assembly processes.

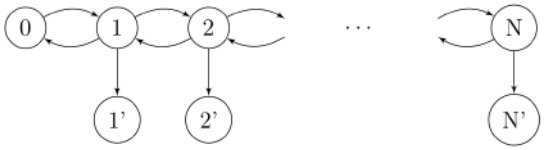

Fig. 4: Markov model for the spread-out of one type of protein in one dimension.

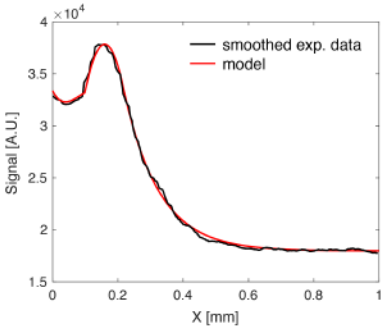

Fig. 5: Fluorescence signal of a protein from an in-vitro translation experiment (black line) and signal as predicted from the Markov model (red line).

S. Rudorf, S. Förste, N. Haase
sophia.rudorf@mpikg.mpg.de

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Post-transcriptional Regulation of Gene Expression

Angelo Valleriani 14.03.1966
1992: Diploma, Physics (University of Bologna)
Thesis: Conformal Invariance, Renormalization Group and Integrable Models in Two-Dimensional Quantum Field Theories
1996: PhD, High-Energy Physics (SISSA-ISAS, Trieste)
Thesis: Form Factors and Correlation Functions
1996-1998: Postdoc, (Max Planck Institute for the Physics of Complex Systems, Dresden)
1998-2000: Postdoc (Max Planck Institute of Colloids and Interfaces, Potsdam)
Since 2000: Group Leader and IMPRS Coordinator, (Max Planck Institute of Colloids and Interfaces, Potsdam)

Research in this group is concerned with stochastic processes in complex and biological systems. Our study includes both formal and mathematical aspects of such processes **[1],** some formulations applied to models of molecular motors **[2],** and to the understanding of stochastic processes with application to data analysis **[3],** to cell cycle dynamics **[4],** to computational neuroscience **[5],** and to population genetics **[6].**

The main research focus of the group in the years covered by this report is concerned with various processes related to the post-transcriptional regulation of gene expression. Gene expression is a generic term that is commonly related to what is known as the central dogma of molecular biology. Accordingly, genes found in the DNA are first transcribed into RNA molecules. The majority of the total RNA molecules present in each cell plays a key role in the production of proteins. Some species of RNA molecules become part of ribosomes. Some RNAs become transfer RNA, called also tRNA. Some RNAs are found in the form of small or micro RNA and finally a prominent role in gene expression is played by messenger RNA molecules (mRNA). Protein synthesis is the final product of gene expression: in this process, the ribosomes read the information encoded in the mRNA and synthesize the proteins using the amino-acid delivered by the tRNAs. This process is called translation. The particular way in which the ribosome reads the mRNA is the basis of what we know as the genetic code. The ribosome reads the nucleotide sequence of the mRNA one triplet per step. To each triplet, called codon, corresponds one amino-acid that will be incorporated to the nascent protein. The amount of proteins corresponding to a given gene present in the cell will thus depend on several factors. The first factor is the amount of mRNAs of that gene: this is determined by the balance between the synthesis rate of the mRNA (transcription rate) and the degradation rate of the mRNA. The second factor is the amount of ribosomes translating each mRNA molecules, which eventually determines the protein synthesis rate. The final factor is the degradation rate of the proteins.

Stability of mRNA

The RNA molecules that become part of the ribosome are called rRNA. Both rRNA and tRNA are very stable. Their function is to provide the machinery of the process of translation, independently of what has to be translated. The mRNA molecules instead are typically not so stable and their lifetime is regulated by some internal cellular mechanisms. Indeed, when the cell needs to change the kind of proteins to be synthesized, due for instance to some stressful condition, it can do so by changing the composition of the cell mRNA population **[7].** Beside the important role played by the regulation of transcription, one way to tune the amount of mRNA is to activate or deactivate specific degradation mechanisms. In eukaryotic organisms, one such degradation mechanism is driven by short and specific RNA sequences called miRNA. From the molecular biology viewpoint, it is often very important to know which factors and in which temporal sequence they affect a specific biochemical process. In the case of miRNA it was known that this RNA first forms a complex called miRISC and then it acts by recruiting the target mRNA and other protein complexes called NOT1 and PAN3. Despite very insightful experiments, it was not clear if miRISC first recruits the mRNA and then the proteins or *vice versa*. In a recent study **[8],** we have analysed the experimental data and shown that they are only compatible with miRISC first binding to NOT1 and/or PAN3 and then recruiting the mRNA (**Fig 1**). In the analysis of the data we have employed a hierarchical approach and modelled the single-molecule degradation as a continuous time Markov chain. As a side product, we have found that there must be another degradation mechanism for the targeted mRNA that accounts for about 20% of the degradation events.

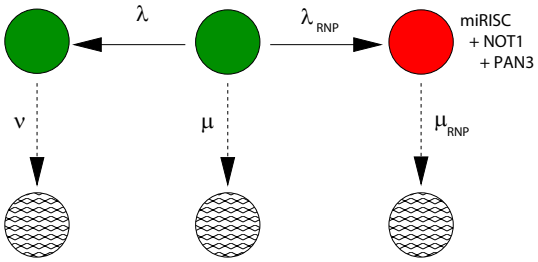

Fig. 1.The experimental data for the degradation of a specific mRNA in drosophila can be explained by means of this most parsimonious model in which the miRISC factor first recruits NOT1 and/or PAN3 and then binds to the target mRNA (red state). As a by-product, we find that another alternative pathway (left green state) is also necessary ***[8].***

Drop-off of Ribosomes

Another way to modulate the amount of proteins synthesized per mRNA is through what is known as translational control. One of the ways to see if translational control is at work is by monitoring the change in the amount of ribosomes per mRNA for all genes in the cell **[7].** The experimental technique mostly used in recent times for this analysis is called riboseq, which consists in blocking the ribosomes during the process of translation and analysing the short strings of mRNA found inside the ribosome body. After aligning those short strings (called "reads") with the DNA, it is possible to infer the spatial distribution of ribosomes along each mRNA species and the increase or decrease of the ribosome density per mRNA after certain stress conditions **[7].** Another process known to happen during translation is when ribosomes abort the synthesis of proteins and abandon the mRNA. This process is called ribosome drop-off (**Fig 2**).

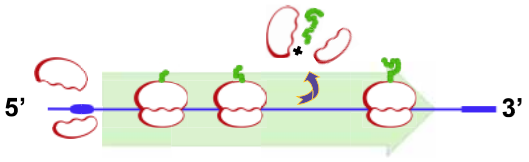

Fig. 2. In the process of translation, the ribosome starts at the 5' (left) end of the mRNA and proceed towards the 3' (right) end thereby synthesizing the protein. When drop-off occurs, the ribosome has a certain probability to stop translation at any point and thus leave the mRNA and release the nascent protein. Under the condition of very small initiation rate, the drop-off leads to decreasing ribosome density along the mRNA ***[9].***

Although some specific RNA sequences may be responsible for drop-off at certain mRNA species, it has also been postulated that ribosomes drop-off as a consequence of unspecific processivity errors. While in the late 70's experimental research found evidence of ribosome drop-off in *Escherichia coli*, recent research based on riboseq data found no trace of ribosome drop-off in this organism. This lack of evidence seemed to us quite strange because this organism possesses a set of enzymes and special RNA molecules specifically devoted to take care of the toxic effects of ribosome drop-off. We thus decided to analyse a large set of riboseq data from different labs by developing and applying more advanced and sensible data analysis techniques **[9].** Finally, we found out that across all data collected under normal growth conditions there is clear quantitative evidence of ribosome drop-off at a rate consistent with the rate found experimentally in the late 70's. Furthermore, we could see that several acute stress conditions have the effect of increasing the rate of ribosome drop-off, thus indicating that ribosome drop-off may be one of the first reaction modes of *E. coli* under acute stress.

Protein Ageing and Degradation

Further down in the chain of processes that regulate gene expression we have protein degradation. In prokaryotic cells, proteins have an average lifetime typically longer than the cell division time. This makes the detection of their degradation difficult because its rate is much smaller than the dilution rate due to cell division. In eukaryotic cells, instead, there are many proteins whose lifetime is shorter than the cell cycle, thus rendering the measurement of their decay experimentally accessible. One key technique to detect protein decay is to first pulse the cells with labelled amino acids and then chase the labelled proteins and measure their decaying amount over time. The data resulting from these pulse-chase experiments are traditionally analysed by assuming an exponential decay. However, if proteins age during their lifetime then a more complex data analysis approach is necessary and was developed in our group **[10].**

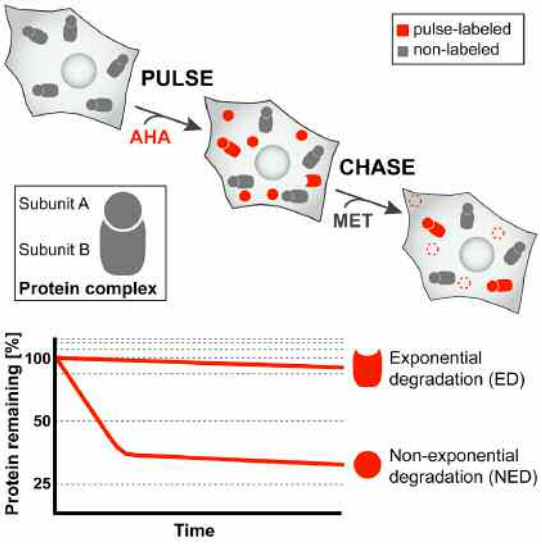

Fig. 3. Pulse and chase experimental technique combined with mass spec analysis allows to monitor the decaying amount of proteins over time with a minimal disturbance of the normal cell functioning. When proteins decay exponentially, their decay pattern is a straight line in a log-linear plot ***[10].*** *It is likely that isolated single subunits of protein complexes decay exponentially but become more stable once the complex is formed* ***[11].***

In fact, this advanced approach (**Fig 3**) is a tool to detect ageing from decay data. In a collaborative project with the group of Matthias Selbach at the MDC in Buch, protein decay from mice cells was measured by means of mass spec data. We found that at least 15% of all proteins have a non-exponential decay, which means that these proteins age during their lifetime **[11].** Ageing, in fact, means that the probability to be degraded per unit of time changes with the age of the molecule. In the specific case of the measured decay patterns, we found that for those ageing proteins their degradation rate decreased with age. Although the ultimate reason for this increase in stability with age is not clear for each single protein species, control experiments showed that some proteins normally found in complexes have a first phase in their life in which they are still not incorporated in the complex and thus very unstable. The increase in stability occurs when they are finally incorporated into the protein complex.

A. Valleriani, M. Bühning, J. Kochan
angelo.valleriani@mpikg.mpg.de

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Protein Binding and Membrane Adhesion

Thomas Weikl 01.04.1970
1996: Diploma, Physics
(Freie Universität Berlin)
Thesis: Interactions of rigid membrane inclusions
1999: PhD, Physics
(Max Planck Institute of Colloids and Interfaces, Potsdam)
Thesis: Adhesion of multicomponent membranes
2000-2002: Postdoc
(University of California, San Francisco)
Since 2002: Group Leader
(Max Planck Institute of Colloids and Interfaces, Potsdam)
2008: Habilitation, Physics
(University Potsdam)
Thesis: Transition states and loop-closure principles in protein folding

Binding of Membrane-anchored Proteins

Cell adhesion processes and the adhesion of vesicles to the membranes of cells or organelles depend sensitively on the binding constant and binding kinetics of the membrane-anchored receptor and ligand molecules that mediate adhesion. Since the binding equilibrium constant K_{2D} and the on- and off-rate con-stants of these receptor and ligand molecules are difficult to measure in their natural two-dimensional (2D) membrane environment, a central question is how they are related to the binding equilibrium constant K_{3D} and the on- and off-rate constants of soluble variants of the receptors and ligands that lack the membrane anchors and are free to diffuse in three dimensions (3D). The binding constant K_{3D} and on- and off-rate constants of these soluble receptors and ligands can be quantified with standard experimental methods **[1, 2, 3].**

A membrane-anchored receptor can only bind to an apposing membrane-anchored ligand if the local membrane separation l at the site of the receptor and ligand is within an appropriate range. This local separation l of the membranes varies – along the membranes, and in time – because of thermally excited membrane shape fluctuations. Experiments that probe the binding equilibrium constant K_{2D} imply averages in space and time over membrane adhesion regions and measurement durations. Our recent simulations and theories indicate that these averages can be expressed as **[1]**

$$K_{2D} = \int K_{2D}(l) P(l)\, dl$$

where $K_{2D}(l)$ is the binding equilibrium constant as a function of the local membrane separation l, and P(l) is the distribution of local membrane separations that reflects the spatial and temporal variations of l. The function $K_{2D}(l)$ is maximal at a preferred local separation of the receptors and ligands, and asymmetric around this maximum because the complexes can tilt at smaller separations but need to stretch at larger separations (**see Fig. 1c**). Our simulations show that the distribution P(l) of the local separation is well approximated by a Gaussian function in situations in which the adhesion is mediated by a single type of receptors and ligands. The two key membrane properties that emerge from this general theory are the average separation and relative roughness of the membranes, which are the mean and standard deviation of P(l).

The binding constants K_{2D} and K_{3D} of membrane-anchored and soluble receptors and ligands can be calculated from the translational and rotational entropy loss upon binding **[1, 3]**. As a function of the local membrane separation l, the binding constant K_{2D} has the general form **[1]**

$$K_{2D}(l) = \sqrt{8\pi} K_{3D} \frac{A_b}{V_b} \frac{\Omega_{RL}(l)}{\Omega_R \Omega_L}$$

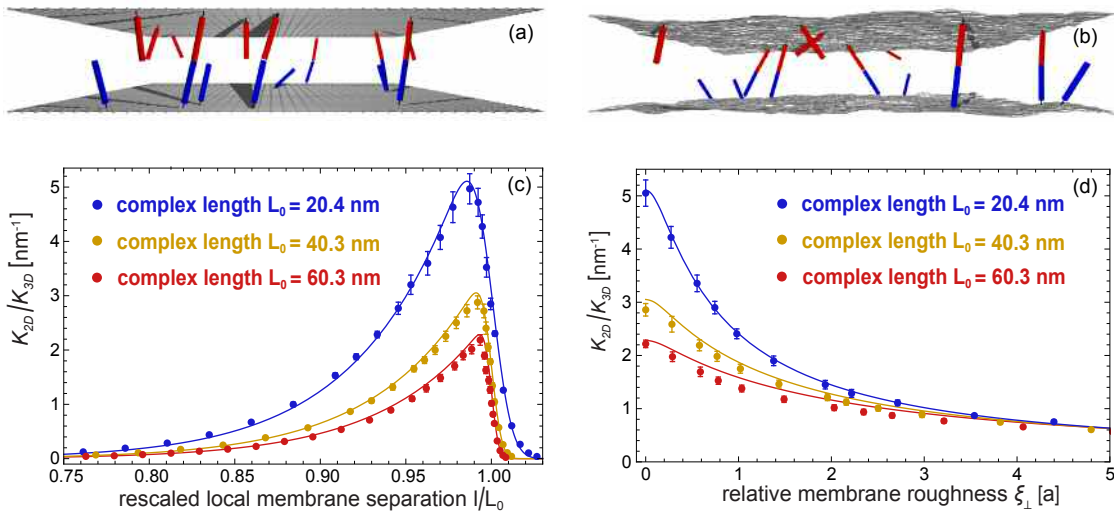

*Fig. 1: (a) Snapshot from a Monte Carlo (MC) simulation with receptors and ligands anchored to parallel and planar membranes. The receptors and ligands diffuse along the membranes and rotate around their anchoring points. (b) Snap-shot from a MC simulation with flexible membranes that exhibit thermally excited shape fluctuations. (c) Ratio K_{2D}/K_{3D} of the binding constants of membrane-anchored and soluble receptors and ligands versus local membrane separation l for different lengths L_0 of the receptor-ligand complexes. The binding constant K_{3D} of soluble variants of the receptors and ligand is determined by the binding potential of the receptors and ligands and does not depend on the complex length L_0. (d) Ratio K_{2D}/K_{3D} of binding constants versus relative membrane roughness of two thermally fluctuating membranes at their preferred average separation. The binding constant K_{2D} strongly decreases with the relative membrane roughness. The data points in (c) and (d) represent MC data, and the lines theoretical results without data fitting (from Ref. **[1]**).*

where Ω_R, Ω_L, and $\Omega_{RL}(l)$ are the rotational phase space volumes of the unbound receptor R, unbound ligand L, and bound receptor-ligand complex RL relative to the membranes, and A_b and V_b are the translational phase space area and translational phase space volume of the bound ligand relative to the receptor in 2D and 3D. Our theory for the ratio of K_{2D} and K_{3D} agrees with data from Monte Carlo simulations without fit parameters (**see Fig. 1**), and can be extended to the on- and off-rate constants of the receptors and ligands **[2]**.

Conformational Changes during Protein Binding

The function of proteins is affected by their conformational dynamics, i.e. by transitions between lower-energy ground-state conformations and higher-energy excited-state confirmations of the proteins. Advanced nuclear magnetic resonance and single-molecule experiments indicate that higher-energy conformations in the unbound state of proteins can be similar to ground-state conformations in the bound state, and vice versa. These experiments illustrate that the conformational change of a protein during binding may occur before a binding event, rather than being induced by this binding event. However, determining the temporal order of conformational transitions and binding events typically requires additional information from chemical relaxation experiments that probe the relaxation kinetics of a mixture of proteins and ligands into binding equilibrium. These chemical relaxation experiments are usually performed and analysed at ligand concentrations that are much larger than the protein concentrations. At such high ligand concentrations, the temporal order of conformational transitions and binding events can only be inferred in special cases.

We have derived general equations that describe the dominant chemical relaxation kinetics at all protein and ligand concentrations **[4]**. Our general equations allow to clearly infer from relaxation data whether a conformational transition occurs prior to a binding event ('conformational selection'), or after the binding event ('induced fit'), see **Fig. 2**.

Wrapping of Nanoparticles by Membranes

Nanoparticles are wrapped spontaneously by biomembranes if the adhesive interactions between the particles and membranes compensate for the cost of membrane bending **[5, 6, 7].** In previous simulations and elasticity calculations, we have observed the cooperative wrapping of spherical nanoparticles in membrane tubules. For spherical nanoparticles, the stability of the particle-filled membrane tubules strongly depends on the range of the adhesive particle-membrane interactions. Our recent elasticity calculations show that elongated and patchy particles are wrapped cooperatively in membrane tubules that are highly stable for all ranges of the particle-membrane interactions, compared to the individual wrapping of the particles **[6]**. The cooperative wrapping of linear chains of elongated or patchy particles in membrane tubules may thus provide an efficient route to induce membrane tubulation, or to store such particles in membranes. In addition, we have investigated how the wrapping process of spherical nanoparticles depends on the initial curvature of the membrane **[7]**.

T. Weikl, F. Bonazzi, B. Kav, P. R. Pandey, F. Paul, K. Ziolkowska
weikl@mpikg.mpg.de

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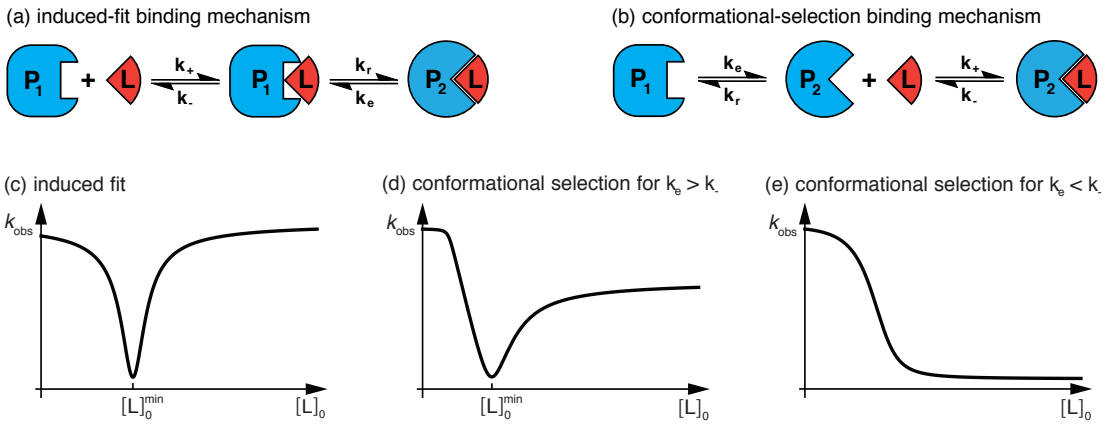

*Fig. 2: Characteristic chemical relaxation of induced-fit and conformational-selection binding (from Ref. **[4]**). (a) In induced-fit binding, the change between the conformations P_1 and P_2 of a protein occurs after binding of the ligand L. (b) In conformational-selection binding, the conformational change of the protein occurs prior to ligand binding. (c) As a function of the total ligand concentration $[L]_0$, the dominant, smallest chemical relaxation rate k_{obs} of induced-fit binding is symmetric with respect to a minimum located at $[L]_0^{min} = [P]_0 - K_d$ where $[P]_0$ is the total protein concentration and K_d the overall dissociation constant. (d) The dominant relaxation rate k_{obs} of conformational-selection binding has a characteristic minimum for $k_e > k_-$, but is not symmetric with respect to this minimum. (e) For $k_e < k_-$, the dominant rate k_{obs} of conformational-selection binding decreases monotonically with $[L]_0$.*

Bilayer Asymmetry and Spontaneous Tubulation

Reinhard Lipowsky 11.11.1953
1978: Diploma, Physics, (University of Heidelberg)
1982: PhD (Dr. rer. nat.), Physics (University of Munich)
1979-1984: Teaching Associate (University of Munich)
1984-1986: Research Associate (Cornell University)
1986-1988: Group leader (FZ Jülich)
1987: Habilitation, Theoretical Physics (University of Munich)
Thesis: Critical behavior of interfaces: Wetting, surface melting and related phenomena
1989-1990: Associate Professorship (University of Munich)
1990-1993: Full Professorship (University of Cologne), Director of the Division "Theory II" (FZ Jülich)
Since Nov 1993: Director (Max Planck Institute of Colloids and Interfaces, Potsdam)

Biological and biomimetic membranes consist of molecular bilayers with two monolayers or leaflets. These leaflets can differ in their composition or be exposed to different aqueous solutions. Because of these bilayer asymmetries, the membranes prefer to attain a certain curvature. In the past, this preferred or spontaneous curvature, which can be positive or negative, was typically treated as a phenomenological parameter, and very few attempts have been made to estimate its magnitude.

What we have achieved within the last couple of years is to develop new and general methods by which one can determine the spontaneous curvature in a quantitative manner. Our results show that the magnitude of this curvature can vary over several orders of magnitude, from 1/(20 nm) to 1/(50 μm).

On the molecular scale, one can distinguish a variety of mechanisms for the local generation of membrane curvature. As described below, these mechanisms include the adsorption and depletion of small solutes, the binding of flexible polymers, and the insertion of glycolipids with large head groups. All of these mechanisms can generate large spontaneous curvatures to which the vesicle membranes adapt by the formation of small buds and thin nanotubes.**[1]** These membrane protrusions involve thin membrane necks, which play an essential role in many biological processes such as endocytosis and cytokinesis.

Mechanisms of Local Curvature Generation

Adsorption and desorption of small solutes. The two leaflets of a bilayer membrane are typically exposed to two aqueous solutions that differ in their solute composition. Let us first consider solutes such as ions or monosaccharides that are smaller than the membrane thickness, which has a typical value between 4 and 5 nm. Attractive interactions between the solutes and the membrane lead to adsorption layers adjacent to the two leaflets **[2]**, repulsive interactions to depletion layers **[3]**. Both types of layers are illustrated in **Fig. 1**. If the aqueous solutions have different solute compositions, the two leaflets of the bilayer experience different molecular interactions and the asymmetric membrane acquires a certain preferred or spontaneous curvature. It is important to realize that both attractive and repulsive membrane-solute interactions generate a preferred curvature. Furthermore, the curvature generated by depletion layers has the opposite sign and a different magnitude compared to the one generated by adsorption layers, see **Fig. 1**.

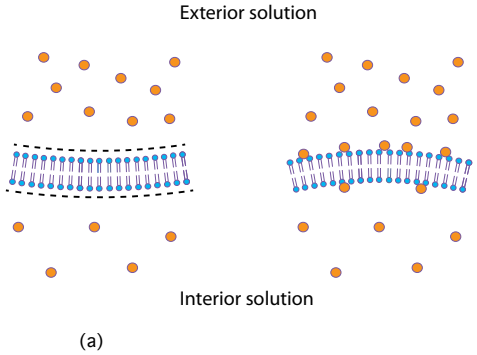

Fig. 1: Segments of lipid bilayers (blue) exposed to small solutes or 'particles' (orange): (a) The particles experience repulsive interactions with the membrane and form two depletion layers (broken lines) adjacent to the two bilayer leaflets. The bilayer then prefers to bulge towards the interior solution and acquires a negative spontaneous curvature; and (b) The particles experience attractive interactions with the membrane and form two adsorption layers adjacent to the two membrane leaflets. The bilayer now prefers to bulge towards the exterior solution and acquires a positive spontaneous curvature.

The spontaneous curvature generated by the adsorption or depletion of small solutes has been recently elucidated by analytical theory and molecular simulations. **[2, 3]** Both for adsorption and for depletion, the spontaneous curvature is found to vary linearly with the concentration difference between the exterior and interior solution. For adsorption, spontaneous curvature values up to 1/(24 nm) were observed in the molecular simulations. These large values can be used to control the budding of relatively small vesicles (project of Rikhia Ghosh).

Binding of flexible polymers. Local membrane curvature can also be generated by the binding of flexible polymers. In general, one should distinguish between hetero-polymers with a few specific anchor groups that bind to the membrane and homo-polymers for which all monomers are attracted by the membrane. One example for the latter case is provided by the adsorption of polyethylene glycol (PEG) chains onto ternary lipid bilayers with different compositions corresponding to liquid-disordered and liquid-ordered phases. This process has been elucidated by atomistic molecular dynamics simulations as illustrated in **Fig. 2. [4]** The PEG molecules are only weakly bound to the membranes, with relatively short contact segments (or 'trains'), and relatively long loops in between. The two terminal OH groups of the PEG molecule were observed to be frequently bound to the membrane via hydrogen bonds. The curvature generated by these adsorbed polymers was not determined in the simulations but was deduced from the spontaneous tubulation of giant vesicles. The m-value obtained by three different methods of image analysis was -1/(125 nm) for the liquid-disordered and -1/(590 nm) for the liquid-ordered membranes. **[4]**

Insertion of glycolipids with large head groups. Cellular membranes often contain glycolipids with large head groups. Because of the mutual exclusion of these head groups, the membranes should prefer to bulge towards the leaflet with the higher ganglioside concentration. This expectation has been confirmed for membranes with a few mole percent of

the ganglioside GM1, as studied by two different experimental methods based on tubulation **[5]** and on initial micropipette aspiration **[6]** as well as by atomistic and coarse-grained molecular simulations (projects of Markus Miettinen and Aparna Sreekumari). In the two experimental studies, the spontaneous curvature was found to vary between -1/(130nm) and -1/(260nm) depending on the overall GM1 concentration.

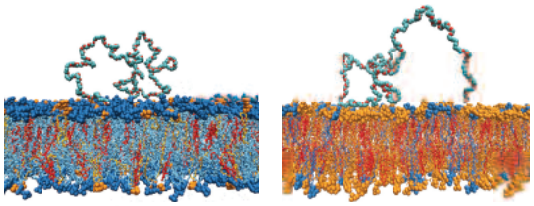

*Fig. 2: Polyethylen glycol (PEG) chains adsorbed onto (a) a liquid-disordered and (b) a liquid-ordered bilayer as observed in atomistic molecular dynamics simulations with explicit water. **[4]** The PEG chains consist of 180 monomers. The bilayers are composed of DOPC (blue), DPPC (orange), and cholesterol (red).*

Spontaneous Tubulation of Giant Vesicles

Giant vesicles often attain a spherical shape even if their membranes have a large spontaneous curvature. When such a vesicle is deflated osmotically, an increasing fraction of the vesicle membrane can adapt to the spontaneous curvature by forming small buds and nanotubes. The nucleation and growth of these membrane protrusions proceeds as follows. **[4]** Initial deflation leads to the formation of a small spherical bud that is connected to the mother vesicle by a thin membrane neck. For negative spontaneous curvature, the bud protrudes into the vesicle interior as shown in **Fig. 3**. Upon further deflation, the vesicle can follow two kinetic pathways which lead (i) to the extension of existing buds into necklace-like tubes and (ii) to the formation of new buds, see red and black arrows in Fig. 3. These competing pathways generate many different morphologies as experimentally observed for PEG adsorption **[4],** see **Fig. 4**, and for asymmetric ganglioside bilayers **[5, 6]**.

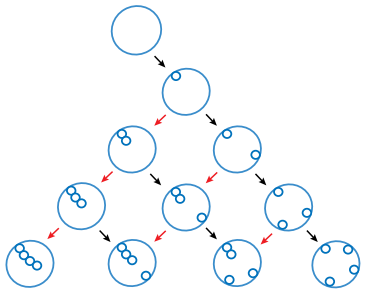

Fig. 3: Polymorphism arising from the osmotic deflation of a GUV in the presence of a large negative spontaneous curvature: Initial deflation of the spherical vesicle at the top leads to the formation of a small spherical in-bud. Further deflation steps can lead to the formation of additional in-buds (black arrows) or to the extension of existing in-buds into extended necklace-like in-tubes (red arrows). As a result of these two kinetic pathways, the vesicle can attain a large variety of shapes as illustrated here for four successive deflation steps.

When the length of a necklace-like tube reaches a certain critical value, the tube changes its morphology and transforms into a cylindrical one. **[4]** This necklace-to-cylinder transformation is disfavored by the end-caps of the cylinder but favored by the reduced volume of the cylindrical tube. The volume reduction implies a free energy contribution that is proportional to the tube length whereas the bending energy of the end- caps is independent of this length. Therefore, if a vesicle membrane forms several tubes, the shorter ones will be necklace-like whereas the longer ones will be cylindrical as observed experimentally, see the example in **Fig. 4(b)**.

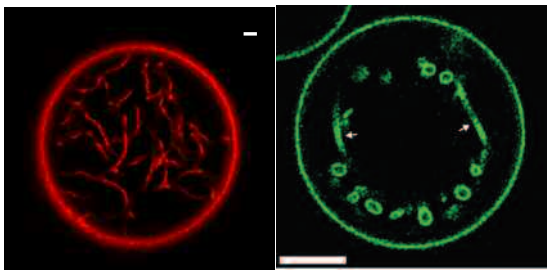

*Fig. 4: Giant vesicles with many nanotubes protruding into the vesicle interior: (a) Nanotubes formed by a liquid-disordered membrane (red) with a spontaneous curvature of -1/(125nm); and (b) Necklace-like tubes coexisting with two cylindrical tubes (white arrows) formed by a liquid-ordered membrane (green) with a spontaneous curvature of -1/(590 nm). **[4]** The scale bar is 2 μm in (a) and 10 μm in (b). The spontaneous curvature is generated by the adsorption of PEG chains as in **Fig. 2**.*

Mechanical Robustness of Tubulated Vesicles

Giant vesicles with membrane nanotubes have unusual mechanical properties because the tubes provide a large area reservoir for the mother vesicles. Therefore, these vesicles can adapt to strong mechanical perturbations by exchanging membrane area with the tubes. The vesicle membranes then experience a small mechanical tension that remains essentially constant until all nanotubes have been retracted. **[1]**

In order to elucidate this behavior, we used giant vesicles composed of POPC and a few mole percent of the ganglioside GM1. These vesicle form stable nanotubes protruding into the vesicle interior **[5, 6]** Micropipette aspiration can then be used to expose the vesicles to adjustable mechanical stresses and to retract the tubes in a controlled and reversible manner. **[6]** The mechanical robustness of the tubulated vesicles is demonstrated by their complete and reversible aspiration into the micropipettes, thereby mimicking the passage of such vesicles through small blood vessels (capillaries).

R. Lipowsky, J. Agudo-Canalejo, T. Bhatia, R. Dimova, R. Ghosh, A. Grafmüller, M. Miettinen, A. Sreekumari
reinhard.lipowsky@mpikg.mpg.de

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Nanoparticles Interacting with Membranes and Vesicles

Jaime Agudo-Canalejo 20.11.1989
2007-2012: Diploma, Physics ("Licenciatura en Física", Complutense University of Madrid)
2012-2016: Doctoral Thesis: Effects of Bilayer Asymmetry on Nanotube Formation and Particle Engulfment by Biomembranes and Vesicles ("summa cum laude", Technische Universität Berlin, Fellow of the International Graduate Research Training Group 1524 "Self-Assembled Soft Matter Non-Structures at Interfaces")
Advisor: Prof. Dr. Reinhard Lipowsky
2012-2016: Doctoral Researcher, Department of Theory & Bio-Systems, Max Planck Institute of Colloids and Interfaces
Since 02/2016: Postdoctoral Scientist, Department of Theory & Bio-Systems, Max Planck Institute of Colloids and Interfaces

In order to enter a cell, a nanoparticle must first cross the outer cell membrane. This entry process, known as endocytosis, begins with the adhesion of the nanoparticle to the cell membrane, followed by the engulfment of the particle by the membrane. The adhesion and engulfment steps of endocytosis can be mimicked in model systems consisting of nanoparticles in contact with lipid or polymer vesicles. Therefore, these steps are governed by an interplay between membrane-nanoparticle adhesion and membrane bending and do not require the coupling to chemical reactions such as nucleotide hydrolysis. Previous theoretical studies focused on the simplest case of nanoparticles interacting with planar and symmetric bilayer membranes. However, biological membranes are neither planar nor symmetric. In fact, they often display complex shapes with non-uniform curvature, and compositional asymmetry between the two leaflets of the bilayer is a hallmark of all cellular membranes. Extending the theoretical framework of curvature elasticity, we have recently shown that both spontaneous curvature, which provides a quantitative measure for the bilayer asymmetry, and membrane curvature have a rather strong effect on the engulfment process.

Engulfment Regimes for a Single Nanoparticle

Depending on the coverage of the particle surface by the membrane, we can distinguish three particle states (**Fig. 1**): (i) free (F) states in which the membrane does not spread over the particle surface at all, in spite of the attractive membrane-particle interactions; (ii) partially engulfed (P) states with a partial coverage of the particle surface by the membrane; and (iii) completely engulfed (C) states with full coverage of the particle by the membrane. In the latter case, the membrane forms a narrow neck that connects the particle-bound membrane to the unbound mother membrane. Combining numerical calculations with theoretical considerations, we have discovered exact analytical conditions for the energetic stability of free and completely engulfed states. **[1]** The completely engulfed state is stable provided the radius of the particle R_{pa} exceeds a certain critical radius R_{ce}, which depends on the particle-membrane adhesiveness, the bending rigidity and the spontaneous curvature of the membrane, and the local mean curvature of the mother membrane at the position of the narrow neck. On the other hand, the free state is stable only if the radius of the particle is smaller than a second critical radius R_{fr}, which again depends on the particle-membrane adhesiveness, the bending rigidity of the membrane and the local mean curvature of the membrane at the point of contact with the particle, but turns out to be independent of the spontaneous curvature. Combining these two stability conditions, we obtain four distinct engulfment regimes according to the stability of the free and completely engulfed states: the free regime (F stable, C unstable), the completely engulfed regime (F unstable, C stable), the bistable regime (both F and C stable, separated by an energy barrier) and the partially engulfed regime (both F and C unstable).

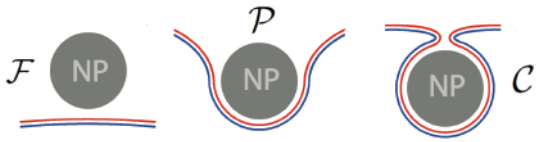

Fig. 1: A nanoparticle (NP, grey) in contact with a membrane with bilayer asymmetry (red and blue leaflets) can attain a free (F), partially engulfed (P) or completely engulfed (C) state. In the latter state, the membrane forms a narrow neck between the particle-bound and unbound membrane segments.

Engulfment Patterns of Nonspherical Vesicles Exposed to Many Nanoparticles

The two stability conditions that define the four engulfment regimes depend on the local curvature of the membrane. Therefore, when a nonspherical vesicle with nonuniform curvature is exposed to many nanoparticles, the vesicle membrane consists, in general, of several membrane segments that belong to different engulfment regimes. As a consequence, nonspherical vesicles can exhibit distinct engulfment patterns. **[2]** Examples for such patterns are displayed in **Fig. 2,** for the particular case of a prolate vesicle. It is important to note that not all combinations of engulfment regimes can be present on the surface of a single vesicle. In fact, our theory predicts that only 10 distinct engulfment patterns are possible.

Curvature-Induced Forces Acting on Uniform and Janus-like Nanoparticles

Going beyond the stability analysis of F and C states, we have developed an analytical theory for the case in which the particle size is small compared to the vesicle size. This theory provides the full energy landscapes of the membrane-particle systems, including the height of the energy barriers for the bistable regimes and the binding energies of partially engulfed particles. **[3]** Our theory predicts that the energy of partially engulfed particles depends on the local mean curvature of the vesicle membrane. As a consequence, partially engulfed nanoparticles experience curvature-induced forces that act to displace the particles towards membrane segments of lower or higher mean curvature, depending on whether the particles originate from the outside or inside of the vesicle, respectively. The partial engulfment of nanoparticles with a chemically uniform surface requires fine tuning of particle size and adhesiveness with respect to the properties of the membrane. In contrast, Janus particles with one strongly adhesive and one non-adhesive surface domain are always partially engulfed. Therefore, the curvature-induced forces are directly accessible to experimental studies when the vesicles are exposed to such Janus particles **(Fig. 3).**

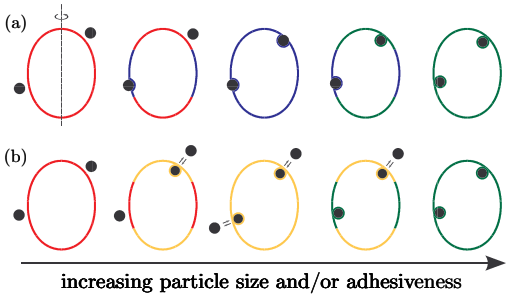

Fig. 2: Engulfment patterns of nanoparticles (black) on a prolate vesicle. The spontaneous curvature of the membrane is positive in (a) and negative in (b). The patterns involve four types of membrane segments: free segments with no engulfment (red) and bistable segments with energy barriers (orange) as well as segments decorated by partially engulfed (blue) and completely engulfed (green) particles. A change in particle size or adhesiveness leads to continuous morphological transitions between these patterns.

Endocytosis via Adhesion-Induced Segregation of Membrane-Anchored Receptor Molecules

So far, we have considered membranes with a uniform lateral composition. In order to model the more complex process of endocytosis in real cells, we have investigated the possibility of adhesion-induced segregation of membrane components, resulting in particle-bound and unbound membrane segments that differ in their bending rigidities and spontaneous curvatures. **[1]** In this way, we could explain experimental data for clathrin-mediated endocytosis of gold nanoparticles by HeLa cells. These data show a non-monotonic dependence of the particle uptake on the particle size with a maximum at a particle diameter of about 50 nm.

Stabilization of Narrow Membrane Necks by Adhesive Surfaces and Constriction Forces

As mentioned before, a completely engulfed particle implies a narrow membrane neck, see **Fig. 1.** It is important to note that such narrow necks arise in many other membrane processes. Important examples are the budding and tubulation of supported lipid bilayers, the formation of extracellular and outer membrane vesicles by eukaryotic and prokaryotic cells, cytokinesis during cell division, or the collective engulfment of many particles into necklace-like tubes, see **Fig. 4.** Furthermore, in cells, the formation of narrow necks is often assisted by constriction forces directly applied to the membrane neck by proteins such as dynamin in endocytosis, or actomyosin in cytokinesis. In order to account for these different situations, we have extended our stability analysis of narrow necks to different geometries and included constriction forces acting at the neck. **[4]** As a result, we obtained relatively simple stability conditions that are directly applicable to many systems of experimental interest and provide bounds on the material parameters of the systems.

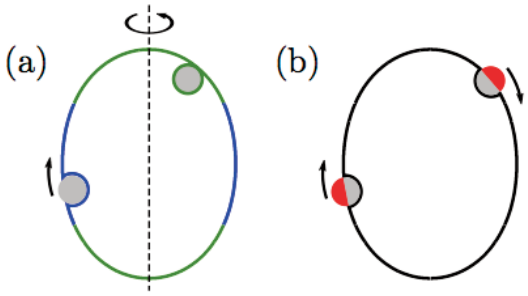

Fig. 3: (a) Prolate vesicle (green-blue) in contact with uniform adhesive nanoparticles (grey). In this example, particles are completely engulfed at the strongly curved poles (green) and partially engulfed at the weakly curved equatorial region (blue). In the endocytic case shown here, partially engulfed particles experience a curvature-induced force towards regions of lower membrane curvature, whereas completely engulfed particles experience no such force. (b) For the same vesicle, Janus particles with one strongly adhesive (grey) and one non-adhesive (red) surface domain are partially engulfed everywhere on the membrane, and therefore always experience curvature-induced forces towards regions of lower membrane curvature.

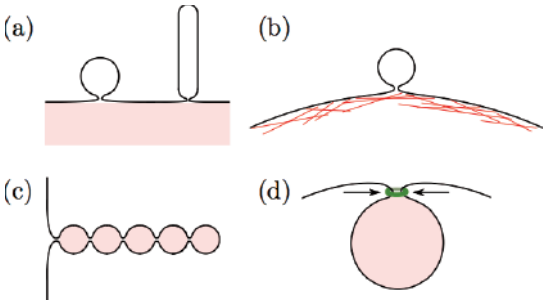

Fig. 4: Different systems in which narrow membrane necks form in the presence of adhesive surfaces (pink) or constriction forces. (a) Budding or tubulation of a supported lipid bilayer; (b) Formation of giant plasma membrane vesicles originating from the outer cell membrane in the presence of the adhesive actin cortex; (c) Engulfment of many nanoparticles into necklace-like tubes; and (d) Engulfment assisted by a contractile ring (green), representing endocytosis-associated protein machinery.

J. Agudo-Canalejo, R. Lipowsky
jaime.agudo@mpikg.mpg.de

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The Colors and Shapes of Vesicles

Rumiana Dimova 06.04.1971
1991–1995: Master of Science in Theoretical Chemistry and Chemical Physics (Sofia University, Bulgaria)
1996–1997: Second Master of Science in Separation Processes in Industry and Environmental Protection (Sofia University, Bulgaria)
1997-1999: PhD with Doctoral Thesis: Hydrodynamic properties of model lipid membranes: study by means of optical trapping manipulation of particles. (Bordeaux University, Centre de Recherche Paul Pascal, Bordeaux, France)
2000-2001: Postdoctoral Scientist, Theory Department, Max Planck Institute of Colloids and Interfaces
Since 2001: Research Group Leader, Department of Theory and BioSystems, Max Planck Institute of Colloids and Interfaces
2012: Habilitation in Biophysics at Potsdam University
2014: Privatdozent in Biophysics at Potsdam University
2014: Emmy Noether distinction for women in physics (European Physical Society)
2016: Elected as Scientific Representative of Max Planck Institute of Colloids and Interfaces
2016/2017: Chair of the Membrane Structure and Assembly Subgroup of the Biophysical Society

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Giant unilamellar vesicles (GUVs) are tiny membrane compartments filled with aqueous solution. One needs a microscope to see them, but the view is often spectacular and reveals many important aspects of membrane behavior. For example, by employing suitable fluorescent labels, the vesicles appear colored and one can resolve membrane heterogeneities inherent to biological membranes (**see Fig. 1c**). External perturbations, such as the presence of added molecules, applied flows or electric fields, will set the picture under the microscope in motion as they lead to dynamic behavior that can be monitored from microseconds to hours. The resulting changes of the vesicle shape can tell us much about the membrane mechanical properties. This report will discuss phase separation in membranes as can be observed from vesicle images obtained with fluorescence microscopy and membrane shapes and morphological changes induced by external factors.

Imaging of GUVs is not always simple. Because of convection, they can be displaced, thus hampering long-term observations. We recently developed a method to immobilize the vesicles. The approach is based on building a cage of agarose around the GUVs **[1],** without compromising the mechanical properties of their membrane as is the case of vesicles encapsulating this polysaccharide **[2].** Our immobilization strategy allows us to trap and hold the vesicle for high-resolution pictures and long-term observations, see **Fig. 1.**

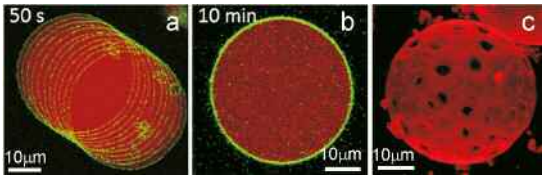

*Fig. 1: Immobilizing GUVs in agarose allows for long-term observations **[1]**. (a) Superimposed time scans over 50 seconds of the equatorial section of one GUV exhibiting a small drift; measurements requiring long acquisition times will be compromised. The vesicle membrane is labeled in green and the encapsulated solution in red. (b) Superimposed time scans over 10 min of a vesicle immobilized in agarose. The vesicle is trapped by the agarose cage around it and does not drift. (c) High-resolution image of a multicomponent GUV with domains. The vesicle is immobilized in agarose.*

Phase Separation in Membranes

For many years, the prevailing view of the cell membrane structure has been the fluid mosaic model proposed by Singer and Nicolson. More recently, it has been proposed that cell membranes may contain lipid domains of liquid-ordered (Lo) and liquid-disordered (Ld) phases and that the functionality of proteins can be influenced by the phase state of the lipids around them. GUVs can be employed to visualize phase separation in membranes made of only a few components. At constant temperature, the phase diagram of a ternary lipid mixture is given by the Gibbs triangle as in **Fig. 2.** In this example, the mixture consists of DOPG, a charged unsaturated lipid, egg sphingomyelin (eSM), and cholesterol (Chol). Each point in the Gibbs triangle represents a certain membrane composition. The membrane can exhibit Lo, Ld or solid (S) phases as well as phase coexistence (e.g. the vesicle in **Fig. 1c** exhibits Ld/Lo phase coexistence). The phase state of the membrane can be assessed from the domain shapes and mobility. Domains are visualized by incorporating a small fraction (<0.5 mol%) of fluorophores, which preferentially partition into a certain phase.

Two-component membranes can also exhibit coexistence of fluid and solid (or gel) phases. We recently found out that, when added to the lipid POPC, even small fractions of the glycolipid GM1 (a few mol %) are sufficient to induce micron-sized gel-like domains attributing facets to GUVs **[3, 4].** Being enriched in neuronal membranes, GM1 concentration fluctuations will easily shape the membrane morphology, fluidity and stiffness in cells.

Inspired by the asymmetric environment of the plasma membrane, we investigated vesicles with asymmetry of the solutions across their membrane. The solution exchange around the vesicles was performed with microfluidic devices, see report by Tom Robinson. We found that the bilayer phase state is affected by solution asymmetry and presence of salt **[5]** as illustrated with the example in **Fig. 2.** These results have direct implications for protein adsorption onto these membranes and for the repartitioning of proteins within membrane domains.

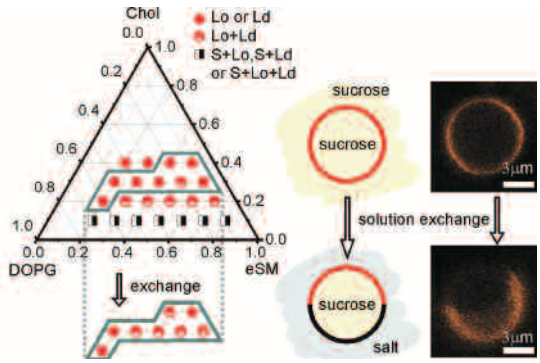

*Fig. 2: Effect of trans-membrane solution asymmetry on the phase behavior of DOPG/eSM/Chol membranes at room temperature: filled circles correspond to homogeneous membranes; half-filled symbols to vesicles with domains. The phase diagram for GUVs with symmetric sucrose/sucrose (in/out) conditions changes after imposing asymmetric sucrose/salt conditions via exchange of the vesicle external solution with high-salinity buffer (see changes in the region delineated by the polygon showing the vesicle compositions which we have examined experimentally). The cartoons and confocal images on the right illustrate the solution conditions and the dominant domain pattern within the delineated section **[5].***

Are Vesicles Always Spheres?

Researchers new to vesicles, whether giant or not, intuitively expect them to be spherical. This is not necessarily so. If a vesicle membrane is fluid and under low tension, it will undulate when exposed to the Brownian motion of water. Membrane flexibility is characterized by their bending rigidity, which depends not only on membrane composition and the presence of inclusions **[3],** but also on molecules and ions in the bathing media **[6].** Typically, the bending rigidity is of the order of 10 k_BT, which is why the membrane of vesicles under low tension can exhibit thermal fluctuations. Upon deflation, vesicles may adopt a variety of shapes depending, among others, on their area-to-volume ratio. Furthermore, GUVs are easy to deform when exposed to perturbations such as electric fields or adhesion as discussed below.

Membrane Nanotubes

When exposed to bilayer asymmetry, the membrane will develop spontaneous curvature (see report of Reinhard Lipowsky) which can be directly seen in GUVs. For example, even the weak adsorption of poly(ethylene glycol) (PEG), a molecule that is generally considered not to interact with membranes, can generate spontaneous curvature sufficient to drive the formation of cylindrical or pearl-like membrane nanotubes in GUVs **[7, 8],** see **Fig. 3a.** Asymmetrically anchored GM1 can also drive tubulation (**Fig. 3b**) as a result of the generated spontaneous curvature **[4].** This membrane property can be measured by pulling inward or outward tubes from GUVs using optical tweezers **[9],** see **Fig. 3c, d.**

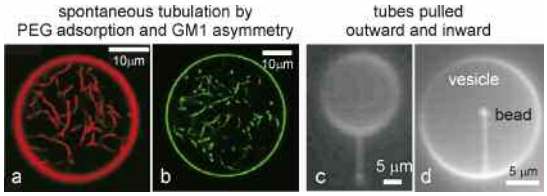

*Fig. 3: Membrane nanotubes in vesicles. Tubes can be spontaneously generated by (a) asymmetric PEG adsorption **[7, 8]** or (b) GM1 asymmetrically anchored in the membrane **[4].** One can also mechanically pull (c) outward or (d) inward tubes via manipulating a membrane-attached bead with optical tweezers **[9].***

Shaping Vesicles with Electric Fields, Light and Proteins

The overall vesicle shape is also easy to modulate. Application of electric fields offers one way of shaping vesicles. Strong DC pulses can induce short-lived prolate deformations **[10, 11],** while weak DC fields can be employed to reversibly adhere and press charged vesicles onto an electrode, **Fig. 4a [12, 13],** a process similar to electrowetting. Another approach, employed in our group, for changing the vesicles morphology relies on the light-induced isomerization of a tetrafluorazobenzene derivative (F-azo). Inserted into the membrane, F-azo increases the vesicle area upon *trans-cis* isomerization under UV light and the vesicles can expel outward buds **[14].** The process is completely reversed under blue light, **Fig. 4b.**

Buds generated in GUVs can also point towards the vesicle interior. ESCRT proteins can induce inward buds in GUVs and even detach these buds inside the mother vesicle via scission. Membrane scission or fission is a step which also occurs after the closure of the phagophore cup and the formation of the autophagosome during the process of autophagy **[15, 16],** see report of Roland Knorr.

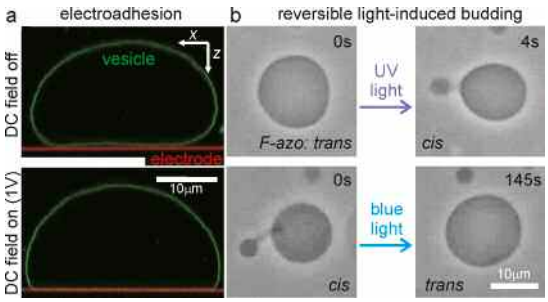

*Fig. 4: Modulating the vesicle morphology. (a) The application of a DC field to a deflated vesicle (green) resting on the electrode surface (red) induces reversible adhesion to the electrode while deforming the vesicle into a truncated sphere **[12, 13].** (b) Under UV or blue light, the reversible isomerization of the light-responsive molecule F-azo incorporated in the membrane can induce reversible vesicle budding **[14]**.*

All in all, giant vesicles are susceptible to all kinds of reshaping, whether induced by adsorbed or anchored molecules, protein scaffolds, (electro)adhesion, or wetting. A beautiful spectrum of responses can be observed under the optical microscope helping us to elucidate underlying mechanisms of membrane behavior and interactions. And this, only by following the shape and color of vesicles.

R. Dimova, Y. Avalos Padilla, T. Bhatia, E. Ewins, V. Georgiev, M. Karimi, B. Kubsch, R. Lira, D. Roy, J. Steinkühler, Z. Zhao
dimova@mpikg.mpg.de

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Dynamics of Bio-Membranes

Roland L. Knorr 1977
from 1997: University studies at the Friedrich-Schiller-University, Jena/ Germany majoring human nutrition
2004: Diploma thesis at the Dept. of Pharmaceutical Biology, Friedrich-Schiller-University, Jena/Germany
2005: Analytical scientist in a biopharmaceutical company (IDT Biologika, Dessau/Germany)
2006: Scientist in a biotech startup (liposomal delivery, Novosom AG Halle/Germany)
2007-2010: Doctoral Thesis: Giant vesicles – influence of phase state, composition and electric pulses (Physical Biochemistry of the University Potsdam and Dept. of Theory and Biosystems, Max Planck Institute of Colloids and Interfaces)
2010/11: DFG Post-doc Fellow
2011-2016: Postdoctoral Scientist (Morphological transitions of biomembranes during autophagy; Dept. of Theory and Biosystems, MPI of Colloids and Interfaces)
Since 10/2016: Research Group Leader, Dept. of Theory and Biosystems, MPI of Colloids and Interfaces

How lipid vesicles and organelles regulate their changes in morphology and topology is of fundamental importance in cell biology. Topological transformations of membranes (membrane fusion and scission), for example, take part in all vesicular transport pathways, as well as during autophagy, viral infection, and cell division as well as in the dynamics of organelles such as mitochondria. Moreover, applications such as liposomal drug delivery or production of monoclonal antibodies (cell-cell fusion) depend on changes in membrane topology.

Autophagy is a complex membrane process within eukaryotic cells and used to digest cytosolic components including organelles. This process involves an extraordinary large number of membrane shape transformations as illustrated in **Fig. 1 [1, 2].** The process is regulated by a large number of proteins which were identified by Yoshinori Ohsumi and coworkers.

In this group, which was established in 2016, we investigate the dynamics of bio-membranes by focusing on four different topics: 1) Morphological transitions of autophagic membranes, 2) Membrane scission during autophagy, 3) Interaction of membrane-bound organelles with non-membrane-bound organelles; and 4) Reconstitution of membrane proteins. All themes deal with understanding the changes of membrane shapes by applying a different set of experimental methods at the interface between biochemistry and biophysics, typically in combination with theoretical approaches as developed in the department. Experimentally, we collaborate with various groups at the MPIKG, very closely with those of R. Dimova and T. Robinson.

Shape Transitions of Autophagic Membranes

Autophagy is regulated by a conserved set of autophagy related proteins (Atgs), many of them seem to be essential for the various steps in **Fig.1.** Atg8 was known to regulate the size of the autophagosomes. By a combination of theory and experiment, we clarified the underlying mechanism of the size regulation, see step 3 in **Fig. 1 [1, 3].** We currently focus on the question how closed autophagosomes can manage to reopen into cup-shaped organelles (dotted arrows and step 6 in **Fig. 1**). Such events can be observed when topological transformations during autophagy do not occur in the correct order (step 5 without step 4, **Fig. 1**) leading to abortion of the process. Such incidents might lead to severe physiological consequences and thus, are important to understand. Some recent insight is shown in **Fig. 2.**

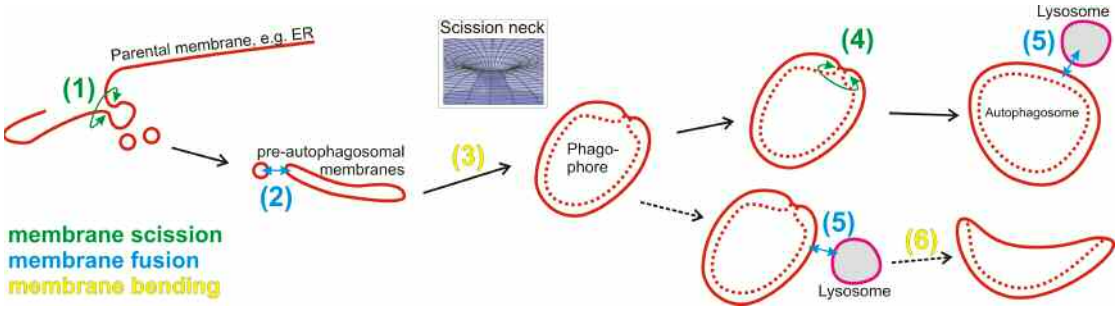

Fig. 1: Shape transformation of membranes during autophagy. Five main steps can be distinguished (continuous arrows): two early and two late topological transitions (membrane fusion and membrane scission) with one major change of membrane morphology in between (membrane bending and autophagosome closure). The case that topological transformations do not occur in the correct order (dotted arrows), for example fusion with the lysosome (step 5) without prior membrane scission (step 4), can impair autophagy severely. Phagophores can reopen (step 6) and thus, the cargo cannot be degraded.

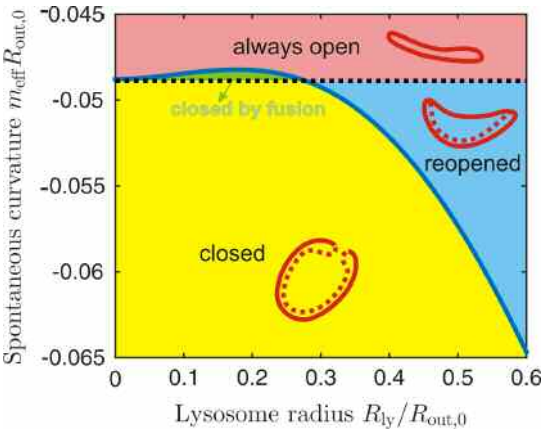

*Fig. 2: Phase diagram for the bending of phagophores. We show that the shape stability of the autophagosomes is critically influenced by alterations of two organelle properties: reduced volume and membrane spontaneous curvature. The membrane spontaneous curvature determines if phagophores were open or close initially (dotted line). The radius of the fusing lysosome (relative to the radius of the autophagosome) and associated changes in spontaneous curvature determine, if autophagosomes were closed or reopen after their fusion with the lysosome **[4]** Under very specific conditions (green area), open phagophores can close by fusion with lysosomes.*

The second membrane scission event during autophagy is still not understood (step 4, **Fig. 1**) **[4].** The timing of this event is critical for successful autophagy (**Fig 2.**). The membrane morphology of the second autophagic scission neck is similar to membrane structures which are cleaved by ESCRT proteins, **Fig. 1,** inset. Therefore, ESCRTs might be involved during autophagy as well, but, so far, there is no experimental evidence for this involvement. One reason is that the correct morphology of the neck is difficult to observe *in vitro*.

Future work will be dedicated to develop reliable protocols to obtain biomimetic models of such scission necks. These model systems will be employed to functionally reconstitute protein cascades which lead to autophagic membrane scission.

Reconstitution of Protein Cascades at Membranes

The importance of membrane proteins is highlighted by the fact that about 30 % of all proteins are membrane proteins and that every second pharmaceutical drug is supposed to target membrane proteins.

An important focus of the group is towards gaining a more fundamental understanding of membrane proteins. Reconstitutions of single proteins or reaction cascades in synthetic model membranes enable us to decipher protein function *in vitro* by studying them in well-defined environments such as giant unilamellar vesicles. Previously, we reconstituted a minimal, ubiquitin-like conjugation machinery and showed that this cascade changes the properties of membranes as predicted theoretically by us **[1, 3].**

Recent work includes contributions to the development of a new method to immobilize model membranes **[5]** (see R. Dimova, Biophysics lab) and to the reconstitution of the copper ATPase CopA. Copper ATPases are vital for activation of essential copper-dependent enzymes and for removal of excess copper from cells. CopA is an integral membrane protein with eight transmembrane domains. We demonstrated a quantitative correlation between ATPase activity and metal transport with a turnover ratio Cu : ATP of one **[6]**.

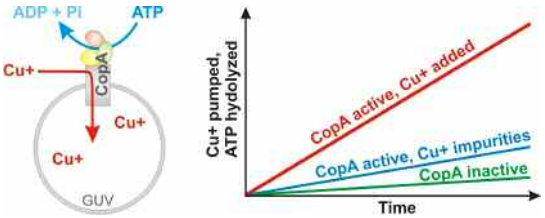

Fig. 3: Fig. 3: Copper (I) transport across membranes of GUVs mediated by the ATPase CopA.

Interaction of Membrane-bound Organelles with Non-membrane-bound Organelles

In living cells, many subcellular structures behave like liquid droplets, examples are stress granules or nucleoli. These non-membrane-bound compartments concentrate certain reactants, which can mediate specific reactions in turn. An active regulation of the interfacial contact area between membrane-bound and non-membrane-bound organelles would have tremendous implication for all signalling pathways transporting information from the outside to the inside of cells, i.e. linking the plasma membrane and the cytoplasm. Our goal therefore is to gain fundamental understanding of the interactions between liquid droplets and bio-membranes. Initial data suggest that liquid droplets can switch between three different states depending on environmental conditions: 1) without contact to membranes (dewetting), 2) spatially restricted interaction with membranes (partial wetting); and 3) full coverage of the membrane by the droplet (complete wetting). These main morphologies are highlighted in **Fig. 4.** By simply altering the salinity of the environment it was possible to reversibly change the size of the interface between reconstituted ribonucleoprotein droplets and bio-membranes.

In the future, our work will shed light on details of the two wetting transitions, will reveal additional factors influencing wetting and thus, enables us to fine-tune wetting states. The fundamental understanding of intracellular wetting processes has important implications for cell biology since it will allow to specifically manipulate signalling pathways linking cyto-/nucleo-plasms, non-membrane-bound organelles and cellular membranes.

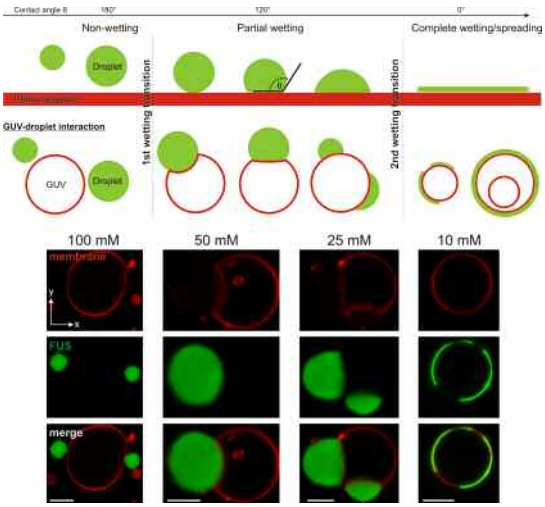

*Fig. 4: Wetting transitions of ribonucleoprotein granules on bio-membranes **[7].** The sketches illustrate wetting transitions which can be observed on planar surfaces and the corresponding shapes which can be expected to occur between droplets and vesicles. The images show confocal cross sections for various ionic strengths. Scale bar, 10 µm.*

R.L. Knorr, C. Ferencz, T. Zeuschner
Knorr@mpikg.mpg.de

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From Membrane Fusion to Multi-compartment Biomimetic Systems

Tom Robinson 17.06.1982
2001-2005: Master of Science in Physics (Imperial College, London, UK)
2006-2007: Master of Research in Chemical Biology (Institute of Chemical Biology, Imperial College, London, UK)
2007-2011: PhD in Chemistry (Departments of Chemistry and Physics, Imperial College, London, UK).
Thesis: The Application of Multi-dimensional Fluorescence Imaging to Microfluidic Systems.
2011-2014: Postdoctoral research fellow (ETH, Zurich, Switzerland)
2014-2015: Postdoctoral research fellow in the group of Dr Rumiana Dimova (Department of Theory & Bio-systems, Max Planck Institute of Colloids and Interfaces)
Since 01/2016: Independent Research Group Leader, funded via the MaxSynBio (Department of Theory & Bio-systems, Max Planck Institute of Colloids and Interfaces)

Compartmentalisation is one of the key features to emerge from the evolution of eukaryotic cells. Their multi-compartment structure, consisting of membrane-bound organelles, ensures vital spatial separation of different cellular functions and metabolic processes. Transport of molecules between these compartments is mediated by membrane proteins but can also proceed via the fusion of two separate membranes. To study these and other cellular processes, an increasingly common technique is to use artificial cells. Our group produces synthetic lipid vesicles and uses them as biomimetic systems. This engineering approach allows us to tune certain components, such as the membrane composition, in a highly controlled manner. We achieve a further level of control by using microfluidic devices to handle these delicate cell-sized objects. Currently, we use this combination of synthetic lipid vesicles and microfluidic technology to study two membrane fusion systems. In the future we will also create biomimetic organelles assembled from multi-compartment lipid vesicles, with the goal of initiating enzymatic reactions within them.

The first section of this report presents results on membrane fusion conducted within Dr. Rumiana Dimova's group. The second section discusses the progress and future aims of the Robinson lab.

Membrane Fusion Systems

The fusion of two biological membranes is essential to processes such as neurotransmission, egg fertilization, exocytosis, and viral infection. Studying this process *in vivo* presents many challenges due to the complexity of cells and the difficulty in controlling environmental factors. Here we use lipid vesicles to control the membrane composition in order to better understand the mechanisms and components necessary for membrane fusion.

Domain-specific Membrane Fusion

Because a variety of different cellular processes rely on membrane fusion, it is vital that cells are able to spatially confine fusion events to specific organelles or sites in the plasma membrane. For this reason we are interested in demonstrating domain-specific fusion in a model cell system using lipid vesicles. Our inspiration comes directly from nature which uses the SNARE protein complex to fuse biological membranes in eukaryotic cells. Membrane fusion is energetically unfavourable as a hydration barrier must first be overcome. To achieve this, different protein domains insert themselves into the two opposing membranes which are then brought together when a zipper-like complex is formed. Once they are in close contact, fusion can proceed, although the precise mechanism is still unknown. Here, we use two SNARE-mimetic systems where the ligand and receptor pairs are based on DNA hybridisation, or coiled-coil peptides (in collaboration with Prof. Janshoff, University of Göttingen). In both cases they are linked to lipids within large unilamellar vesicles (LUVs) or giant unilamellar vesicles (GUVs). GUVs with liquid-liquid phase-separation are grown in a physiologically relevant buffer **[1]** and the lipidated receptor is confined to either the liquid ordered or liquid disordered phase. Domain- or phase-specific fusion is achieved when LUVs with the ligand are introduced and fuse only to the GUV phase with the corresponding receptor (**Fig.1a**) **[2].** Domain-specific docking (**Fig. 1b**), and lipid mixing (**Fig. 1c**) have been proven. Preliminary data using a content mixing assay and microfluidics indicates full fusion events (**Fig. 1c**). Future work will involve combining spatially specific fusion with spatially specific fission in the same GUV system.

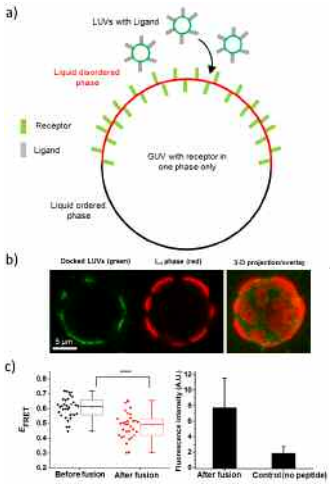

Fig. 1: a) Cartoon of location-specific fusion assay. b) Demonstrating that the LUVs (green) dock to the liquid-ordered phase and not to the liquid-disordered phase (red) on the GUV. c) Left: FRET-based lipid mixing assays proves at least hemi-fusion. Right: content mixing assay indicates full fusion.

Charge-based Membrane Fusion

Although proteins are ultimately responsible for fusion of biomembranes, little is known about the role of the lipids themselves. Considering that biological membranes are charged and SNARE proteins are usually reconstituted in liposomes containing charged lipids, it is not clear whether charge is important for the protein environment itself or as the fusion trigger. Here, we developed a charge based fusion assay using model membranes consisting purely of synthetic lipids (**Fig. 2a**) (in collaboration with the Riske group, Federal University of São Paulo). We show that positively charged LUVs containing the cationic lipid DOTAP and a fluorescent lipid analogue spontaneously fuse to negatively charged GUVs **[3].** The electrostatic interaction initially brings the membranes into close contact and the aromatic rings of the fluorophore are believed to cause membrane destabilisation and subsequent fusion. A FRET-based lipid mixing assay was implemented within a microfluidic device to monitor the fusion dynamics (**Fig. 2b**) and the fusion efficiency was shown to be strongly dependent on the percentage of negatively charged lipids in the GUVs. These results could also be interesting for cell labelling or drug delivery applications.

Biomimetic Vesicles in Microfluidic Systems

Traditional vs. Microfluidic Handling of Vesicles

A core technology in the group is microfluidics. These are small devices with micron-sized fluidic channels containing picolitre volume chambers. The use of microfluidic systems has exploded in the past 10 years with applications ranging

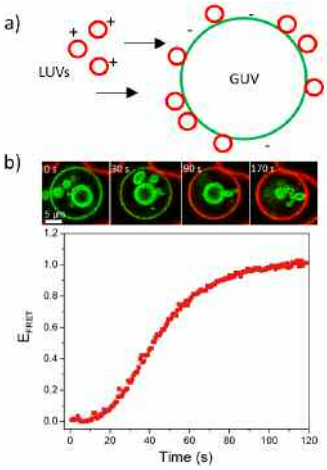

Fig. 2: a) Cartoon of charge-based fusion assay. b) Top: time series of fusion as the outer membrane of a multi-vesicular GUV (green) becomes increasingly saturated with LUVs (red). Bottom: FRET efficiency increase as the lipids of the two membranes mix.

of single-cell analysis to so called 'Lab-on-chip' in the field applications. Here we use the technology for the handling and creation of lipid vesicles. Imaging and studying GUVs is traditionally performed in simple millilitre volume observation chambers. While easy to use, this setup does not allow the rapid and homogeneous delivery of analytes to the GUVs. Moreover, tracking of single GUVs over time is non-trivial. Microfluidic technology, on the other hand, can overcome these challenges. One of our devices contains micrometre sized posts that are engineered to trap and isolate single GUVs (**Fig. 3a**) **[4].** Once captured, the vesicles are stable for hours or days, which allows single vesicle tracking and analysis over time **[1].** Moreover, the fluidic flow control offers the opportunity to rapidly and homogenous exchange the surrounding solution and therefore add or remove solutes which interact with the vesicle's membrane (**Fig. 3b**).

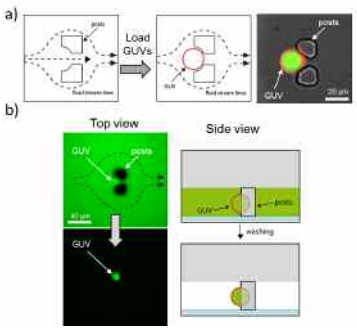

Fig. 3: a) A microfluidic device with micrometre-sized posts to capture a single GUV for analysis. b) Fluidic control allows washing steps to exchange the solution surrounding the GUV ***[4].***

Multi-compartment Vesicle Systems

A key requirement of eukaryotic cells is their ability to compartmentalise different functions within different organelles. The aim of the work here is to study the role of compartmentalisation by creating a multi-compartment vesicle system to mimic cellular organelles (see **Fig. 4**). The challenge here lies in reliably encapsulating smaller vesicles within larger GUVs. Therefore more sophisticated vesicle production methods will need to be explored. One such approach will be to use a microfluidic device to generate water-in-oil droplets that will serve as templates for GUV formation. The advantage here is that the size of the vesicles can be controlled allowing us to inject small GUVs inside larger droplets. Once

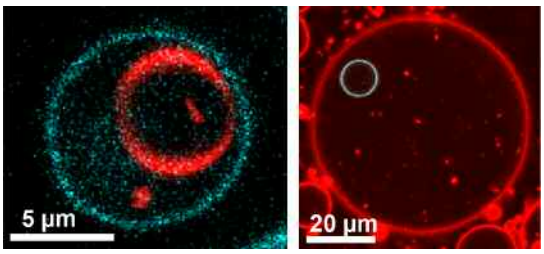

Fig. 4: Confocal images of multi-compartment GUV systems mimicking cell organelles.

these multi-compartment vesicles are created, we will use phase-separated membranes and adhesive moieties for self-organisation and triggering of morphological changes.

Encapsulating Enzymatic Reactions

Many enzymatic pathways are confined to specific organelles or proceed between different organelles. This project aims to dissect the role of compartmentalization of biosynthetic pathways in eukaryotes by studying the first steps of the biosynthesis of the molybdenum cofactor (Moco). While in prokaryotes all steps for Moco biosynthesis are localized in the cytosol, in eukaryotes the first step is localized in the mitochondria and a stable intermediate is transported to the cytosol where all further steps proceed. We are planning to separate the steps using the bacterial proteins for Moco biosynthesis (in collaboration with Prof. Leimkühler, University of Potsdam) and will encapsulate them into vesicles. This will enable studies of the transported intermediate in detail and will give insights into the role of mitochondria for Moco biosynthesis in humans.

Novel Microfluidic Vesicle Trapping Systems

Although the current microfluidic systems have been successfully implemented in a number of different applications involving GUVs **[4–6],** we are continually improving the platform to enable more advanced handling and manipulation of vesicles. To this end, we have developed a device that is able to trap large collections of GUVs to allow better statistics (**Fig. 5**). Moreover, a dense assembly of vesicles could be used to model cells in their natural environment within multicellular organisms.

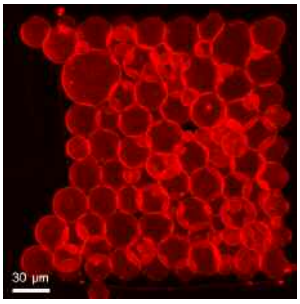

Fig. 5: 3-D confocal microscopy image of tissue-like assemblies of GUVs captured in a novel microfluidic device

T. Robinson, A. Moga, T. Hantke, N. Yandrapalli, T. Kirchhofer
tom.robinson@mpikg.mpg.de

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Phase Transitions and Transport Phenomena at Interfaces

Hans Riegler 29.01.1955
1986: PhD in Physics
(Technical University, Munich)
1986-1988: Postdoc (Bell Laboratories)
1988-1994: Research Group Leader,
Postdoc, Physical Chemistry Department
(University of Mainz)
1995: Habilitation
Since 1994: Research Group Leader,
Department of Interfaces
(Max Planck Institute of Colloids and
Interfaces)

We focus on the impact of interfacial contributions on volume flows (via surface Marangoni flows) and on interfacial energy contributions on the phase behaviour of nano-size systems.

These phenomena are of practical relevance. Phase transition processes of small/confined systems are ubiquitous. Liquid flows induced by surface tension gradients are also widespread in nature and in technology (e.g., ink jet printing).

Our research is strongly motivated by application but clearly focuses on a better fundamental understanding of the phenomena.

Drop-Drop Coalescence, Interfacial Flow and Drop Evaporation.

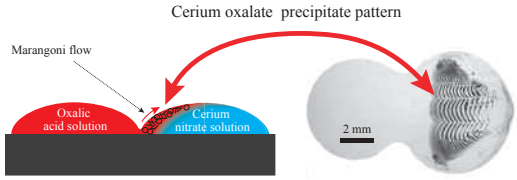

Fig. 1: Patterned precipitation of Cerium-Oxalate appearing during the coalescence of sessile drops with reacting liquids. The stripes consist of different aggregate sizes causing different light scattering ***[1].***

We investigate the coalescence behavior of two sessile drops that contain different chemical reactants (cerium nitrate and oxalic acid) and its impact on the formation of the solid precipitate (cerium oxalate). With different liquids, the surface tension difference in the moment of drop–drop contact can induce a Marangoni flow. This flow can strongly influence the drop–drop coalescence behavior and thus, with reacting liquids, also the reaction and its products (through the liquid mixing). We find three distinctly different coalescence behaviors ("barrier", "intermediate", "noncoalescence"), in contrast to only two behaviors that were observed in the case of nonreacting liquids. The amount of liquid mixing and thus the precipitation rate are very different for the three cases. The "intermediate" case, which exhibits the strongest mixing, has been studied in more detail. For high oxalic acid concentrations, mainly needle-like aggregates, and for low concentrations, mainly flower-like precipitate morphologies are obtained. In a transition range of the oxalic acid concentration, both morphologies can be produced. With the applied coalescence conditions, the different aggregate particles are arranged and fixed in a precipitate raft in a regular, periodic line pattern (**Fig. 1**). The drop-drop coalescence configuration is a convection-reaction-diffusion system, which can have stationary as well as oscillatory behavior depending on the system parameters.

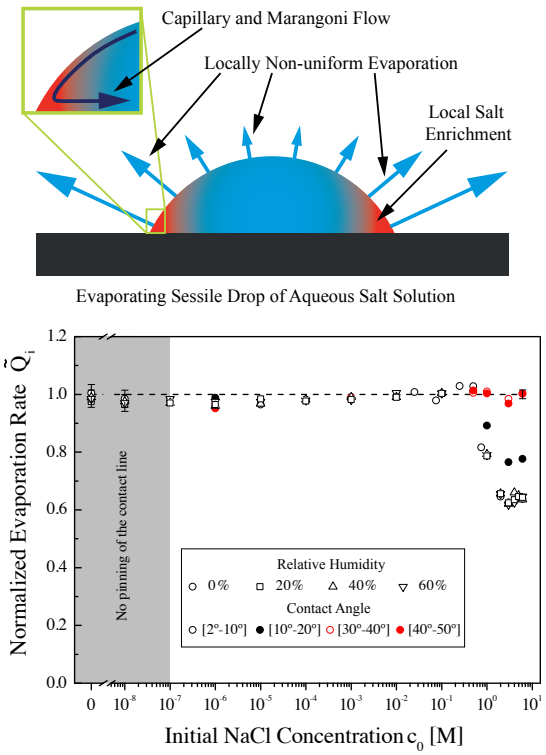

Fig. 2: The evaporation behaviour of sessile drops of salt solutions is governed by the interplay between the locally non-uniform evaporation behaviour inherent to sessile drops and the resulting non-uniform local liquid composition. This can induce a combination of capillary and Marangoni flow near the drop periphery (top). As a result the evaporation behavior of salty sessile drops can be significantly different to sessile drop with uniform composition (lower graph, deviation from dashed line) ***[4].***

In a related project we investigate the evaporation behaviour of sessile drops from mixtures of liquids with nonvolatile components (NaCl, **Fig. 2**). Experiments were performed with seven decades of initial NaCl concentrations, with various droplet sizes and with different contact angles. The investigations reveal that the evaporation depends in a complicated way on the salt concentration and droplet shape. Even if the change of the vapor pressure due to the salt is taken into account the evaporation rate is significantly lower for high salt concentrations and small contact angles than what is expected from the well-accepted diffusion-controlled evaporation scenario for sessile droplets. Particle tracking velocimetry reveals that this modification of the evaporation behavior is caused by Marangoni flows that are induced by surface tension gradients originating from the local evaporative peripheral salt enrichment. In addition it is found that droplets with NaCl concentrations as low as 10^{-6} M are rapidly pinned as soon as evaporation starts, whereas droplets with lower salt concentration do evaporate in a constant contact angle mode. Supposedly, this pinning is caused by deposits of solid salt grains. Such deposits can occur even at

very low salt concentrations due to the peculiar evaporation and flow conditions at the drop periphery. These findings are relevant for a better understanding of the widespread phenomenon of corrosion initiated by sessile droplets.

Melting/Solidification of Nano Size Structures

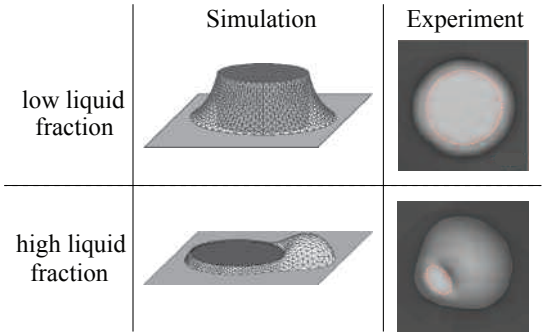

Fig. 3: Melting behaviour of nano size cylinders. Within a certain temeprature range solid and liquid can coexist. Depending on the relative amount of liquid the liquid/vapor interface may form either an axisymmetric rouloid morphology (low liquid fraction) or a bulged morphology (high liquid fraction). Results from simulations are in agreement with experimental data from optical reflection microscopy ***[6].***

Most studies on melting under confinement focus only on contributions from the solid and liquid melt phases and the solid/melt interface. Capillary effects from a second interface (liquid/vapor) are typically neglected. We analyze the melting behavior of small cylindrical aggregates in vapor environment attached to planar surfaces. For the assumed boundary conditions (cylindrical solid with a nonwetting top plane and a wettable side wall), the solid and the liquid phases can coexist within a certain temperature range. Due to capillary instability, the liquid phase can form either an axisymmetric rouloid morphology or, above a certain threshold liquid volume fraction, a bulge that coexists with a rouloid-like section (**Fig. 3**). The melting points of the two morphologies are different. Our theoretical analysis describes the melting behavior of a real system of small aggregates of long chain alkanes on planar substrates as observed by optical microscopy. It also gives qualitative insights into the melting behavior of small aggregates with anisotropic wetting behaviors in general. It reveals in particular how melting points and melting pathways depend on the pathways leading to complete melting.

Patterned Growth Induced by Heterogeneous Nucleation

We investigate experimentally heterogeneous nucleation processes that occur repeatedly/reproducibly at the same location under the same conditions. We investigate in particular the nucleation and growth of aggregates induced by and located at nano size, local „active" sites. Active sites are interfacial locations, where the energetic barrier for heterogeneous nucleation is different (lower) than for heterogeneous nucleation in the neighboring (homogeneous) interfacial environment. Conical pores are for instance active sites for capillary condensation.

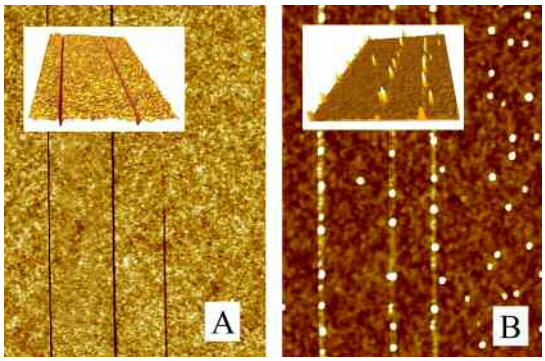

Fig. 4: AFM-image of nano size scratches prior (A) and after deposition of C60 (B). The scratches are about 20nm wide, 1nm deep, and more than µm long ***[7].***

Our active sites are very small „nanoscratches" (dents or groves) in a planar, smooth surface. The nucleation/growth of solute aggregates is induced by exceeding the solute solubility limit in a solute/solvent system as the concentration of a nonvolatile solute increases due to the continuously evaporating solvent. It is found that solute aggregates (C60) grow preferentially at the active sites. We investigate:
1.) How the nanoscratch geometry influences its nucleation „activity" (lowers the nucleation barrier);
2.) How adjacent active sites influence each other (we use arrays of active sites);
3.) How reproducible/repeatable the nucleation sites act (ergodicity); and
4.) How random/stochastic the seemingly smooth environment really is regarding heterogeneous nucleation;

H. Riegler, Guoxiang Chen, Jose Danglad-Flores,
Stephan Eickelmann, Rodrigo Perez-Garcia
Hans.Riegler@mpikg.mpg.de

A great deal of the Marangoni-flow activities are done in collaboration with French research groups (CEA, Saclay and ICSM, Marcoule). Some of the nucleation studies are performed within an international graduate school (funded by DFG) in collaboration with universities in the Berlin area and partners in the US (NC State).

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