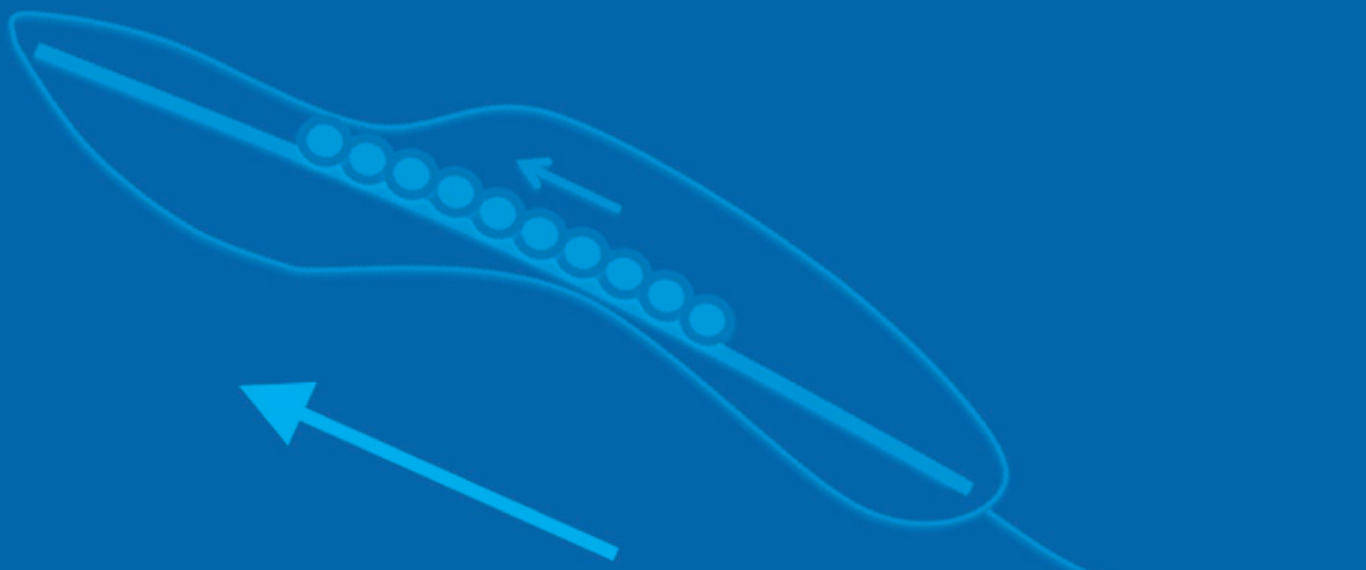
→ Biopolymers
→ Biomolecular Processes
→ Membranes and Vesicles
→ Interfacial Phenomena
→ Complex Systems

THEORY & BIO-SYSTEMS



Research in the Department of Theory & Bio-Systems

„Das Ganze ist mehr als die Summe seiner Teile" Aristoteles

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.

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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 alphabetical order):

- Rumiana Dimova: Biophysics Lab;
- Andrea Grafmüller: Multiscale Simulations;
- Stefan Klumpp: Regulation of Bioprocesses;
- Hans Riegler: Solid-Air Interfaces (since 2014);
- Sophia Rudorf: Biomolecular Processes (since 2015);
- Mark Santer: Carbohydrates and Polysaccharides;
- Angelo Valleriani: Stochastic Processes;
- Ana Vila Verde: Soft Matter Simulations (since 2014);
- Thomas Weikl: Proteins and Membranes.

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 five research areas: Biopolymers, Biomolecular Processes, Membranes and Vesicles, Interfacial Phenomena, and Complex Systems. In the following, the projects of these research groups will be briefly summarized and some additional projects will be highlighted.

Biopolymers

The smallest `bio-systems' are aqueous solution as studied by *A. Vila Verde* who elucidated the effect of ions on the dynamics of water molecules. More recently, her group has started to address water dynamics near hydrophobic interfaces such as fluorinated amino acids and the interactions of anionic polymers with proteins. The group of *M. Santer* continued its studies of polysaccharides and glycans by molecular dynamics simulations. Two topics that have been investigated in some detail were the development of force fields for hybrid molecules such as the GPI-anchor involving lipids, oligsaccharides, and proteins as well as the cleavage of oligosaccharides by protein complexes of bacteriophages. *A. Grafmüller* and her group determined the effective interactions for coarse-grained descriptions of polysaccharides in order to address their materials properties. One important target is hemicellulose, which typically consists of more than 500 monomeric building blocks. The group of *T. Weikl* further elucidated the different pathways for the binding of protein molecules, induced-fit versus conformational selection, which differ in the temporal ordering of binding steps and conformational changes.

Biomolecular Processes

We continued our study of the multi-scale motility of molecular motors, which involves the chemomechanical coupling of single motors, cargo transport by teams of motors, and motor traffic of many cargo-motor complexes. The allosteric coupling between different subdomains of kinesin's motor domain has been investigated by atomistic molecular dynamics simulations **(Fig. 1)** and, was found to be strongly affected by the presence of tubulin. A new topic was protein synthesis by ribosomes for which a detailed Markov model has been developed and used to predict the transition rates *in vivo* from the measured *in-vitro* values (report of *S. Rudorf* and **Fig. 2**).

Membranes and Vesicles

The adhesion of membranes via specific receptor-ligand bonds is strongly affected by the membrane's nanoroughness (report of *T. Weikl).* A new experimental method has been developed to prepare multi-component membranes with a well-defined composition (report of *R. Dimova*). This method is important in order to determine the phase behavior of these membranes in a quantitative manner. Additional topics included the spontaneous and stable tubulation of vesicle membranes **(Fig. 3),** bilayer asymmetry arising from the adsorption of small molecules onto the two leaflets of the membranes **(Fig. 4),** and the strong influence of spontaneous curvature on the engulfment of nanoparticles by membranes (Fig. 5). The latter figure explains the instability of the completely engulfed state for particle radii that are smaller than the threshold value

$$\left[\frac{1}{R_W} + M'_{\rm ms} - 2m\right]^{-1}$$

which depends on the adhesion length R_W, on the segment curvature M'_{ms} of the mother membrane, and on the spontaneous curvature of the membrane, m, which can be positive or negative.

Interfacial Phenomena

The group of *H. Riegler* continued its investigations of phase transitions and transport phenomena at solid-air interfaces. Two phenomena of interest were the melting of terraces of long chain alkanes at SiO_2-air interfaces and the heterogeneous nucleation on planar solid substrates with a regular array of nano-indents.

Complex Systems

A. Valleriani and coworkers applied stochastic modelling methods to the degradation of mRNA and studied the relations between the network structure of the Markov process and the distribution of first-passage times. The independent research group of *S. Klumpp* addressed the interplay of physical constraints and functional requirements in living systems. Two major topics were the interplay of gene expression and cell growth as well as bacterial motility. In the latter context, both the pili-based motility along surfaces and the movements of magnetotactic bacteria have been studied.

Biannual Series of Symposia
We continued our biannual series of topical symposia and organized a three-day symposium on `Multiscale Motility of Molecular Motors' in 2013 as well as another three-day symposium, the `Biomembrane Days 2014'.

International Max Planck Research Schools
The department of Theory & Bio-Systems was in charge of the new IMPRS on "Multiscale Biosystems", which started its operation in July 2013.

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

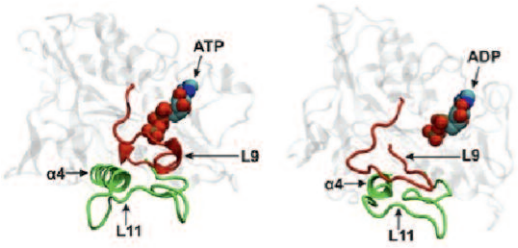

*Fig. 1: Molecular Dynamics simulations of kinesin's motor domain attached to tubulin. Allosteric coupling between the nucleotide binding pocket (red) and the microtubule binding domain (green): (left) state with bound ATP; and (right) state after hydrolysis and phosphate release with bound ADP. During the release step, the L9 loop (red) undergoes a conformational change and rotates the α4 helix (green). [A. Krukau et al, PCCP **16,** 6189 (2014)]*

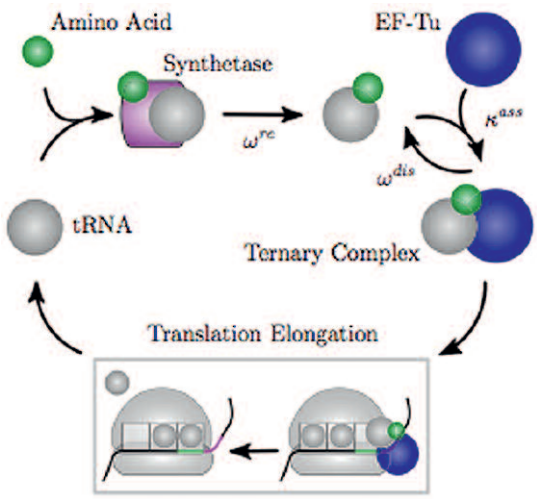

Fig. 2: Protein synthesis by ribosomes: Extended Markov process for translation elongation and its coupling to recharging of deacylated tRNA (upper left) and to ternary complex formation (upper right). This extended scheme is important in order to identify the different subpopulations of tRNA molecules in vivo. [S. Rudorf and R. Lipowsky, PLoS ONE in press]

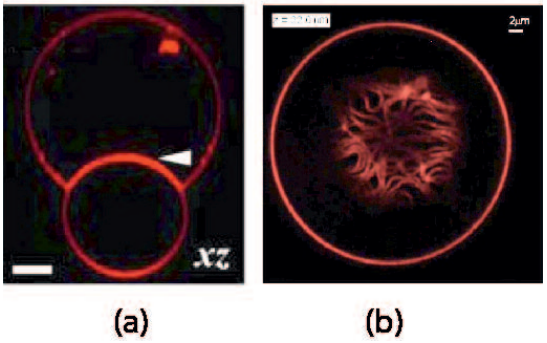

Fig. 3: Formation of many stable membrane nanotubes protruding into the interior of a vesicle enclosing two aqueous phase-separated droplets. The image in (b) corresponds to a confocal scan at the height of the white arrowhead in (a). [Y. Liu et al, to be submitted]

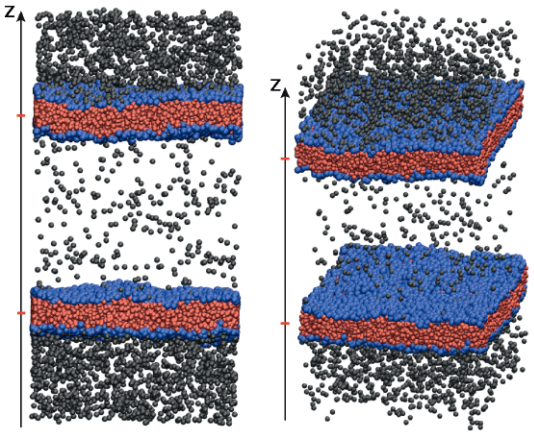

*Fig. 4: Side view (left) and oblique view (right) of two lipid bilayers (blue-red), which separate two aqueous compartments. Both compartments contain adsorbate particles (gray) but with different concentrations. The adsorption of these particles onto the head group layers (blue) leads to asymmetric bilayers with a spontaneous curvature that can be as large as 1/(20 nm). [B. Rozycki and R. Lipowsky, J. Chem. Phys. **142**, 054101 (2015)]*

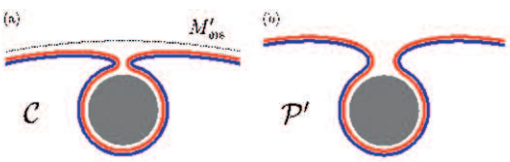

*Fig. 5: Engulfment of nanoparticle (gray) by asymmetric bilayer membrane (blue/red): The completely engulfed state of the nanoparticle (left panel) is unstable and the membrane neck starts to open (right panel) if the particle size is smaller than a certain threshold size. [J. Agudo-Canalejo and R. Lipowsky, ACS Nano **9**, 3704 (2015)]*

From Ionic Solutions to Interacting Proteins

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2001-2005: Doctoral thesis: Optimization of minimal invasive dental laser ablation by mesoscopic modeling (Department of Physics, University of Minho, Braga, Portugal)
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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)

The ability of biopolymers such as proteins to fulfil their biological function is determined by the balance between their intramolecular interactions, their interactions with natural or artificial ligands, water, free ions and other molecules in solution. Seemingly small changes to the protein or its environment – e.g., fluorinating a single protein alkyl group or sulphating a few amino acids in a ligand - often lead to large alterations in protein properties. In this group we use molecular simulations to investigate interactions relevant for protein structure and function: we gain fundamental knowledge by investigating small model systems: 1) interactions between water and ions; 2) water at hydrophobic interfaces (with the experimental groups of R. Kramer Campen at the Fritz Haber Institute, Berlin, and Beate Koksch at the Free University (FU), Berlin). We apply this knowledge in the study of biologically important protein systems: 3) interactions between anionic polymers and proteins (with the experimental groups of R. Haag, FU, and Peter Fratzl at this institute).

Interactions between Water and Ions

Reports from ultrafast pump-probe spectroscopy experiments suggest that densely charged ions such as magnesium and sulphate have a long-range effect on water dynamics: together, they slow down water rotation beyond what would be expected from an additive model. Similar experiments also indicate that even in 1:1 salts, monovalent cations affect the rotational dynamics of the water shell of their counterions. These claims defy evidence from other experiments and simulations, which suggest that the effect of anions and cations on water dynamics is limited to their first hydration shell and is largely additive. We address this on-going controversy by using atomistic molecular dynamics simulations and polarizable models to investigate the dynamics of rotation of hydroxyl groups in aqueous solutions containing ions with either high or low charge density: $MgSO_4$ and CsCl. These models are parameterized by us to reproduce both the free energy of hydration of single ions and the solution activity derivative at high concentration, thus being appropriate to gain insight into water dynamics at both low and high salt concentrations. Using these models, we calculate the average water reorientation autocorrelation function in solutions of CsCl or $MgSO_4$ at various concentrations. We find that $MgSO_4$ greatly slows down water dynamics whereas water is only minimally affected by the presence of CsCl. These trends qualitatively reproduce the experimental ones, confirming the soundness of the models. Examining the water rotational dynamics in subpopulations near static contact- or solvent-shared ion pairs, from separate simulations of isolated ion pairs, indicates that water rotation may be slower than, equal to or larger than that predicted by a simple additive model, for both $Cs^+\ldots Cl^-$ and $Mg^{2+}\ldots SO_4^{2-}$ ion pairs. Large, supra-additive, slowdown, is observed only for water molecules directly bridging $Mg^{2+}\ldots SO_4^{2-}$ solvent-shared ion pairs **(Fig. 1).**

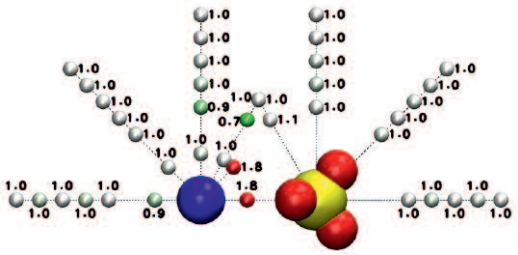

Fig. 1: Supra-additive factor for different water subpopulations around a $MgSO_4$ solvent-shared ion pair. Values >1 indicate supra-additive slowdown of water rotation.

To verify that the supra-additive slowdown observed in simulations of static ion pairs is not an artefact we build an analytical model to predict the average rotational dynamics in salt solutions, where the ions are free to move, from the contributions of water around static ion pairs. We find that the average water rotational dynamics in concentrated $MgSO_4$ solutions can indeed be predicted from the contributions of water near static ion pairs. On the other hand, at $MgSO_4$ concentrations close to the solubility limit, the supra-additive slowdown brought by ion pairs is insufficient to explain the extremely slow water dynamics in these solutions. Our results show that this extremely slow dynamics is associated with multi-ion clusters. Because non-additive effects are small for pairs of low charge density ions and these pairs have lifetimes comparable to the rotational dynamics of water, the standard additive picture holds for CsCl. These results clarify the molecular scale mechanism by which static properties of an electrolyte solution – number and type of ion pairs – will influence the solution dynamics, and show that supra-additive slowdown of solution dynamics at high concentrations may be expected when ions preferentially form solvent-shared ion pairs with life-times larger than the water reorientation dynamics **[1].** Our results have direct implications for studies with proteins: they suggest that the dynamics of the water of hydration of proteins, thought to influence protein conformational dynamics and activity, may be modulated by the formation of solvent-shared ion pairs between the protein and densely charged ions.

Recent experimental studies probing the interface of aqueous salt solutions have suggested that anion-cation association at the interface is greatly altered relative to the bulk. The exact nature and magnitude of these changes is still unclear, though, largely because most experiments are sensitive to the interfacial water, but not to the ions. In the next stage of this project, we will use our models to investigate these outstanding questions.

Water at Hydrophobic Interfaces

Interfaces between water and non-polar functional groups are ubiquitous in biopolymers. These interfaces are at the origin of the hydrophobic effect which largely drives the self-

assembly of biopolymers into stable structures. While the thermodynamic signature of hydrophobic interfaces is well known, the molecular details of water at hydrophobic interfaces and the molecular origin of the hydrophobic effect are still under study. The air/water interface is a useful model for this study because of its simplicity. Using atomistic molecular dynamics simulations, we characterize the orientation and dynamics of two subpopulations of OH groups belonging to water molecules at the air/water interface: those OH groups that donate a hydrogen bond (called "bonded") and those that do not (called "free") **[2-3].** We found that free interfacial OH groups reorient in two distinct regimes: a fast regime from 0 to 1 ps and a slow regime thereafter. Qualitatively similar behavior was reported by others for free OH groups near extended hydrophobic surfaces. Our results clarify that the free OH groups are structurally and dynamically heterogeneous: longer lived free OH groups tend to point closer to the surface normal, have a narrower orientation distribution, and are closer to the vapor phase. Existing descriptions of extended hydrophobic interfaces focus on one of two aspects of these interfaces: the presence of free OH groups and of large density fluctuations. The connection between these two aspects is not yet clear. Our work shows that the net reorientation of bonded interfacial OH groups occurs at a rate similar to that of bulk water, which suggests that the molecular origin of the density fluctuations that are characteristic of extended hydrophobic interfaces lies with the free OH groups.

The knowledge gained on the air/water interface serves as the foundation for our on-going study of hydrophobic interfaces containing fluorinated alkyl groups. Inserting these groups into proteins is known to alter their properties, and those of their water of hydration, but at present no experimental or computational studies have directly investigated how the water of hydration of fluorinated alkyl groups differs from that at typical hydrophobic interfaces. A single experimental study has *indirectly* probed water dynamics near a fluorinated amino acid, with unusual results: water near fluorinated groups appears to have unusually slow dynamics. This work, which started in September 2014, will be done using all-atom models of small fluorinated analogues of alkanes and aliphatic amino acid side chains. We are in the process of developing those models.

Interactions between Anionic Polymers and Proteins

Selectins are well known for their role in the adhesion of leukocytes and platelets to the endothelium that takes place, e.g., during inflammation. Because of the important biological role played by selectins, much effort has been put into finding artificial ligands that effectively compete with the natural ones. The Haag group investigated the potential of dendrimeric polyglycerol (dPG) polymers functionalized with various anionic groups as possible inhibitors of the interaction between L-selectin **(Fig. 2)** and its natural ligands.

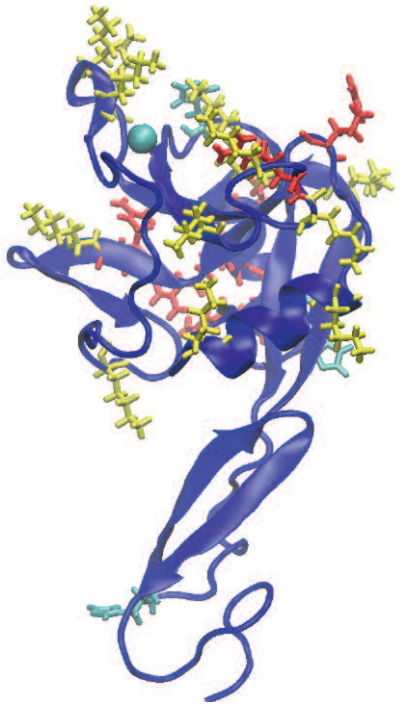

Fig. 2: L-selectin. The cationic amino acids are shown in stick representation.

They found that the affinity of dendrimers for selectins depends strongly on the nature of the anionic group, increasing in the order carboxylate < phosphate < phosphonate ≈ sulfonate < bisphosphonate <<< sulfate. This anionic series cannot be understood in terms of simple considerations based on electrostatic interactions or the acidity of the anionic groups, but instead suggests that complex and competitive microscopic intermolecular and intramolecular interactions might play a role. The first stage of this project, which started in July 2014, consists of characterizing the *intrinsic* interactions between various anionic functional groups and positively charged amino acids, using small molecule analogues (e.g. methylphosphate or methylamine). This work is done using classical all-atom models and explicit water. Results from these simulations will be compared against those from ab *initio* calculations, to ensure the reliability of the classical models. In the next stage, we will use classical, atomistic models to investigate the interaction between selectin and triglycerol sulfate. Our results will be compared with experiments done in the Haag group, thus yielding both a stringent test of the quality of the models and molecular scale insight into multivalent interactions between a small, rigid, ligand and selectin.

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Glycans under the Computational Microscope

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2003-2006: Postdoc, Group leader Nanofluidics (Institute of Microsystems Engineering, University of Freiburg)
2006-2009: Employee (Fraunhofer Institute of Mechanics of Materials (IWM), Freiburg)
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In virtually all organisms carbohydrate compounds *(glycans)* are involved to modify or enhance the function of many biomolecules **[1].** 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. This heterogeneous functionality is provided by diversity in glycan composition and conformational flexibility **[2].** Experimental insight into the interplay of these aspects at the molecular level is rather limited, and atomistic simulation techniques appear as a promising complementary approach. In our group we pursue two long-term case studies highlighting the essential aspects of the mutual interaction of carbohydrates, proteins and lipids. One of them, carried out in close collaboration with S. Barbirz (U. Potsdam), is related to the infection of the Gram-negative bacterium *Shigella flexneri* by phage Sf6, and clearly teaches us that understanding structure alone is not enough.

How do Phages Penetrate the Protective LPS Coat of Gram-Negative Bacteria?

Gram-negative bacteria protect themselves from phage invasion with a lipopolysaccharide (LPS) brush, see **Fig. 1.**

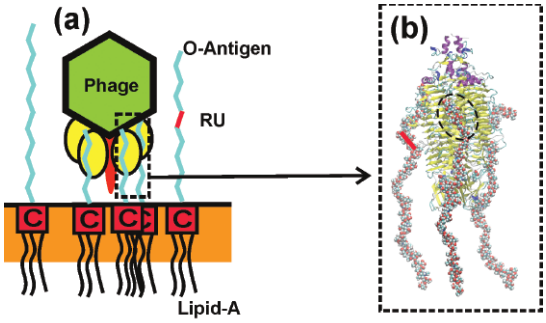

Fig. 1: (a) Schematic representation of a phage penetrating the outer LPS coat of a bacterium. The O-Antigen consists of tetrasaccharide repeat units (RU) (b) MD simulation snapshot where three O-Antigens of Shigella flexneri Serotype Y simultaneously attach to TSP Sf6. The dashed ellipse marks the location of a binding site that can host a 2RU (octasaccharide) fragment.

The so-called O-Antigen polysaccharide side chains normally form a repelling barrier, yet the phage's tail spike proteins (TSP) repeatedly target a specific 2RU fragment at a rather shallow binding site **(Fig. 1b),** cleave the chains by hydrolysis, and pave the way for DNA injection. We have recently carried out a numerical study of octasaccharides bound to Sf6 TSP, supported by detailed experimental evidence from X-ray diffraction (U. Gohlke, Max Delbrück Center Berlin), see **Fig. 2,** and NMR data (G. Widmalm, University of Stockholm) **[3].**

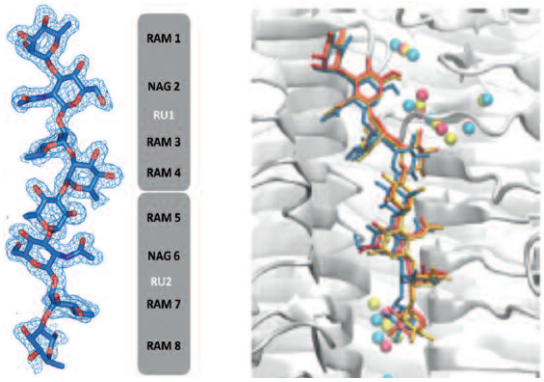

Fig. 2: Left: Stick representation of an O-Antigen octasaccharide placed within an iso-surface of the electron density map from X-ray analysis. Repeat units RU1 and RU2 consist of rhamnoses (RAM) and N-Acetylglucosamines (NAG). Right: superposition of the crystal structure (blue) and average structures of the Wild Type (red) and the E366A/D399A mutant (orange) inferred from simulations. Saturation transfer difference (STD) NMR data in solution support the binding mode shown.

The binding mode resembles the dominating solution conformation of the octasaccharide, although further numerical analysis shows that free fragments with more repeat units cannot be directly accommodated. As conveyed by *Fig 1b,* distortion, dynamics and conformational selection are essential aspects of the recognition process.

Our second case study, conducted in collaboration with D. Varón-Silva (Department of Biomolecular Systems, MPIKG) involves a special class of glycolipids. Here, the question in how far we can extrapolate the behavior of a larger glycan from a set of smaller fragments reappears.

The Nature of GPI Anchors

Many proteins are attached to the outer leaflet of a cell membrane by Glycosylphosphatidylinositol (GPI) anchors **[1],** see also **Fig. 3(a).** The carbohydrate part of the GPI, a linear pseudo-pentasaccharide backbone, is an invariant part of every GPI anchored protein. This short anchor segment determines how the protein interacts with the outer leaflet of the cell membrane. With a computational study, we can give insight into whether the backbone should be pictured as a spacer or rather establishes a close contact with the lipid headgroup region. It is important to realize that a GPI anchored protein consists of three different classes of biomolecules, the specificities of which usually are taken care of in independent lines of force field development. However, using corresponding force fields from the same family, we can create a reasonable hybrid representation, compare the scheme in **Fig. 3(b) [5].**

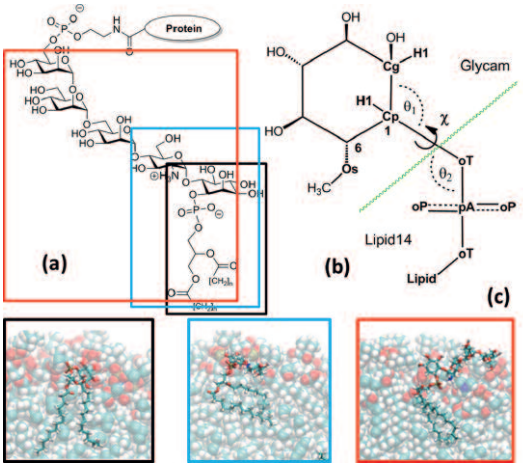

Fig. 3: (a) Chemical structure of a general GPI anchored protein; (b) scheme of the hybrid representation of the GPI anchor, the green wavy line indicates the transition from the GLYCAM (pertaining to carbohydrates) to the Lipid14 force field (optimized for lipids) of the Amber family, along with a few parameters requiring re-paramerization. (c) Simulation snapshots of the GPI fragments indicated in (a) with the hybrid model. Here, we chose palmitoyl lipid tails embedded in a POPC lipid bilayer (only one leaflet shown).

Although our previous studies of the isolated GPI backbone have suggested that it should be rather rigid (favoring the notion of a spacer), **Fig. 3** indicates that this does not exclude almost full embedding in the membrane headgroup region. The conformational preferences of the GPI backbone here are almost indistinguishable from those in isolation.

Conclusions.

Although continuous and important progress in the experimental setup and analysis of purified GPI systems has been made using, e.g., Langmuir monolayers **[6],** or glycan fragments as tagged molecules suitable for advanced NMR analysis **[7],** the *computational microscope* will remain an important device to visualize, e.g., a GPI anchor in a more natural environment. The same applies to the interaction of TSP with long O-Antigen chains. Numerically, however, we are facing a series of problems that require unconventional ideas. For instance, adequate conformational sampling of large glycans can be established in a hierarchical fashion, also including coarse-grain models. This allows us to treat rather long O-Antigen fragments **[4].** In a similar way, employing a "grafting from" approach, the binding modes of O-Antigens at the TSP surface can characterized **[3].** The bound or embedded epitope is systematically grown into a larger structure, a strategy also successfully applied in the binding of multivalent glyco-oligomers to lectins **[8].** The hybrid modeling of GPI anchors finally suggests a systematic "tuning" of the force field. Instead of relying on a postulated "best" set of parameters, we might be interested in how persistent simulation results are under admissible parameter changes, given the approximate nature of bio-molecular force fields in general.

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Multi-Scale Modelling of Natural Polysaccharides

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Polysaccharides are among the most abundant polymers in nature and natural materials. In living organisms, the most common forms are related to structural stability or energy storage related. However, saccharides play many other important roles for instance as part of the glycocalyx, the extracellular matrix and the molecular recognition of pathogens, the extent of which has only just begun to emerge.

The basic building blocks of natural polysaccharides are simple sugars linked by glycosidic bonds to linear or branched chains, as shown in **Fig. 1,** which lead to a large variety of material properties. The divers properties derive from the structure and hierarchical spatial organization in natural materials achieved by controlled self-assembly. The intrinsically multi-scale nature of these structures makes polysaccharides, like many biomolecules and biomaterials, challenging systems to model. While models with atomistic resolution can give a detailed picture of the molecular interactions **[1]** they often cannot reach the length and timescales required to sample larger biomolecules. Strategies to overcome these difficulties involve the use of simplified coarse-grained (CG) models, with fewer degrees of freedom as used in **[2-4]** to study membrane fusion, or enhanced sampling methods such as umbrella sampling **[5, 6],** or a combination of both **[7].** Here we describe the application these modelling strategies to two different natural polysaccharide systems.

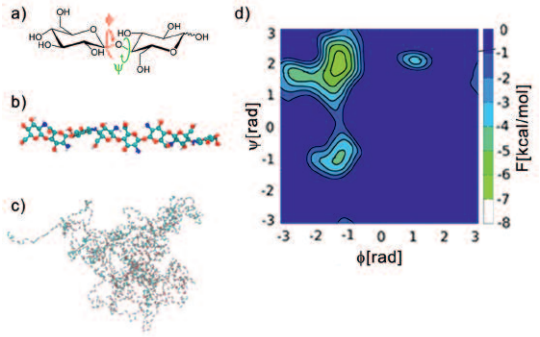

Fig.1: (a) Chemical structure of cellobiose as a typical disaccharide. The flexible glycosidic torsion angles ϕ and ψ are highlighted; (b) all atom representation of a chitosan octamere; (c) coarse-grained representation of a chitosan polysaccharide with 1250 monomers and (d) free energy map for the conformations of the glycosidic angles in cellobiose, shown together with snapshots of the corresponding molecular conformations.

Hemicellulose Building Blocks

In many plant tissues, arrangement of crystalline cellulose fibrils embedded in a matrix of hemicelluloses polysaccharides enables the generation of reliable actuated movement **[8],** induced by the exceptional propensity for swelling of the hemicellulose matrix.

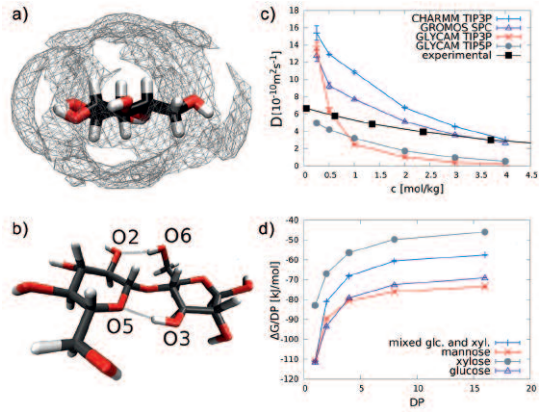

Fig.2: Water interaction of Hemicellulose building blocks; (a) spatial distribution of water molecules around a glucose monomer. (b) intramolecular H-bond formation in cellobiose; (c) comparison of the concentration dependent diffusion coefficients for four common carbohydrate FFs with experimental data; (d) hydration free energy per monomer for short linear oligosaccharides.

All atom molecular dynamics (MD) simulations provide the means of gaining a concise understanding of local interactions of small polysaccharides with water, which are a key factor for the molecular origin of hemicellulose swelling. MD simulations rely on the accurate parametrization of the force field (FF). Since carbohydrate FFs are comparatively new and little experimental data is available, the performance of four common FFs with respect to their ability to reproduce the solution properties has been evaluated **[9].** These properties include the aggregation numbers, diffusion coefficients, bulk density and the free energy of hydration of the saccharides in water.

All FFs except GLYCAM with TIP3P water, for which aggregation sets in at non-physically low concentrations of 0.5 mol kg^{-1}, show the correct trends for the aggregation and diffusion (**Fig. 2c**). As a result of the aggregation, GLYCAM-TIP3P does not lead to a reasonable dependence of the diffusion coefficients on the concentration of the sugar monomers. Inversely, at high concentrations, no FF except GLYCAM TIP3P shows aggregation. The best quantitative agreement with experimental data was found for the GLYCAM FF with TIP5P water.

Therefore, the GLYCAM TIP5P FF has been chosen to characterize the water interactions, H-bond formation both with water and within the molecule, solution properties, and solvation free energies of short segments of the most abundant hemicellulose backbone components. **Fig. 2d** shows the free energy of hydration per monomer for several such segments. The differences observed for different monomer types are almost entirely determined by the number of free hydroxyl groups, which form highly occupied H-bonds with water, resulting in the high water density regions in **Fig. 2a.** The ability to form intra-molecular H-bonds, such as in **Fig. 2b,** has a negligible effect for the short polysaccharides investigated here, because the occupancy of those H-bonds is much lower.

Development of a Coarse Grained Model

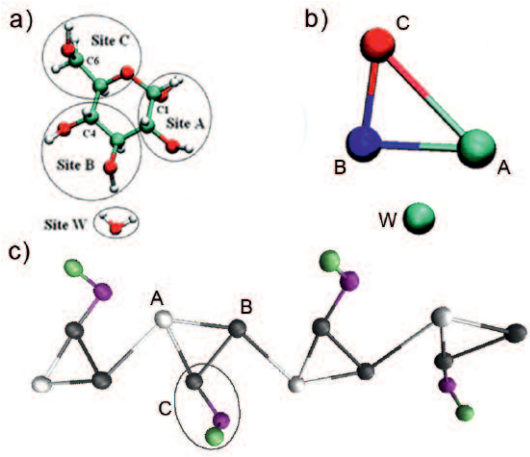

Fig.3: CG mapping of a glucose monomer (a) all atom representation, indicating which atoms group into which CG site; (b) 3-site CG model; (c) 5-site CG model with higher resolution of the former C sites containing the C6 atom (purple & green).

Since a typical hemicellulose polysaccharide consists of more than 500 monomers, simulations with atomistic resolution of even a single molecule in water are not feasible. Therefore, we aim to develop CG models able to reproduce as much of the atomistic conformational features as possible.

After comparing several CG approaches, the best strategy for coarse-graining a solution of small oligosaccharides appears to be a hybrid method using Boltzmann inversion to parametrize bonded interactions while non-bonded interactions are constructed using the force matching procedure, which infers optimal CG interactions directly from the forces in the atomistic simulations. The CG model shown in **Fig. 3** uses three CG sites for each monomer and an explicit 1-site CG water, and is able to reproduce structural features of the saccharide solutions very successfully. The same strategy also works with implicit water for efficient simulations at constant water concentration.

In order to simulate large polymers, it is crucial to introduce transferable monosaccharide potentials from which longer polysaccharides can be constructed. However, the aggregation behavior of the CG system depends sensitively on coarse-graining parameters such as the cutoff radius used in the force matching procedure and the saccharide concentration, which differ for polymers of different length. This lack of transferability currently presents the greatest challenge for an accurate CG description. The most prominent differences in CG potentials are observed for the site containing the C6 atom. Therefore, a promising strategy is to use a higher resolution for the atoms contained in that site. In addition, methods to systematically adapt the force-matching cutoff radius, which determines how long range effects enter into the model, have to be investigated.

Chemical Potential of Water

The excess chemical potential of water μ_{ex} is a key parameter to predict the swelling behavior and water uptake for polysaccharide networks. To determine μ_{ex} a hybrid method combining Widom Test Particle Insertion and Bennet Acceptance Ratio was used. While this method yields reasonable results for all water models in pure water systems, in the saccharide solutions the "nobel" water leads to aggregation which perturbs the results significantly.

An alternative method to measure μ_{ex} directly is to confine the saccharides within a region of the simulation box with a potential mimicking a semipermeable membrane, and measuring the force on these virtual walls throughout the simulation. The latter method leads to a reasonable comparison between experimental values and simulation results for glucose solutions. An additional advantage is that, because it measures forces directly, it is straightforward to apply in the CG model, which was developed to closely mimic the forces in the atomistic model. First results for the glucose monomer solution show that the osmotic forces for the two spatial resolutions differ by at most 15%.

Polysaccharides for Biomimetic Materials

Their ready availability and versatile functionality make polysaccharides promising molecules for the development of functional materials. Many common biologically derived polysaccharides behave as polyelectrolytes and their conformation, charge density, and solubility depend strongly on pH and ionic strength of the system.

To gain a detailed understanding of the factors governing the properties of polysaccharides, we study their conformations based on the free energy landscapes of the glycosidic angles, the most flexible degrees of freedom. The free energy maps, for example shown in **Fig. 1d,** have been recorded using metadynamics MD simulations. In addition, steric repulsion between the stiff rings and, in the case of charged monomers, electrostatic interactions are taken into account. Conformations are sampled using Monte Carlo schemes for polymers. PH and ionic strength of the solution are included via protonation moves for the titratable sites, based on the intrinsic pK values of the monomers and the local electrostatic environment.

Such a relatively simple model is able to accurately predict polymer properties such as the radius of gyration, the persistence length, or the degree of protonation, for large polymers with over 1000 monomers and a large range of physic-chemical conditions.

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Protein Synthesis by Ribosomes

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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, see **Fig. 1.** This process is called translation elongation.

Protein Synthesis Proceeds with Variable Speed

Translation elongation is a nonuniform process with codon-specific elongation rates and error frequencies. This process has to meet conflicting requirements concerning speed and accuracy. On the one hand, protein synthesis must be fast enough to ensure doubling of protein mass within the time scale of cell division. On the other hand, translation must be very precise to avoid erroneous proteins that are often dysfunctional or even harmful to the cell. Therefore, perturbations that hamper or dysregulate protein synthesis can lead to all kinds of cellular defects and even to cell death, a well-known strategy to fight bacterial infections: Antibiotics kill microorganisms by blocking their protein synthesis. The speed and the accuracy of translation have direct influence, for example, on the folding dynamics of peptide chains and the functionality and abundance of the synthesized protein. It is therefore important to elucidate the underlying molecular mechanisms that influence the local and global speed and accuracy of translation. We developed and applied a new theoretical framework for the process of protein synthesis to address this question.

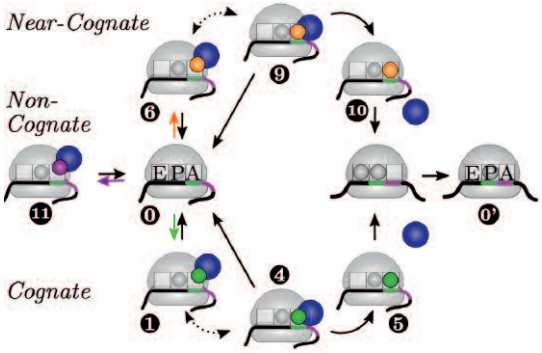

Fig. 1: Translation elongation cycle. The ribosome (gray dome) has three tRNA binding sites, the A, P, and E site. A ribosome that has just arrived at a new (green) codon of a mRNA (black-green-purple-black line) has an empty A site, whereas the P site is occupied by a tRNA (gray sphere) that is cognate or near-cognate to the preceding codon. Elongation factors (EF-Tu, large blue spheres) and tRNAs carrying amino acids (purple, orange, and green spheres) form ternary complexes. Cognate, as well as near-cognate or non-cognate ternary complexes can bind to the ribosome. Since the initial binding is not codon-specific, all ternary complexes unbind again from the internal binding site with the same dissociation rate. Alternatively, a cognate or near-cognate ternary complex can be recognized by the ribosome and is subsequently accommodated in the ribosomal A site. The new A-site tRNA is then further processed and shifted to the P site, while the ribosome translocates to the next (purple) codon. The former P-site tRNA has now reached the E site and dissociates from the ribosome. The numbers correspond to the states of the codon-specific Markov process shown in Fig. 2.

Protein Synthesis as a Markov Process

We describe translation as a Markov process to capture its stochastic nature, see **Fig. 2.** Our theory takes major aspects of in vivo protein synthesis into account: We distinguish between non-cognate, near-cognate and cognate ternary complexes, which compete for binding to the ribosome in a concentration-dependent manner. The concentrations of free ternary complexes are calculated from the total tRNA concentrations by considering the details of a tRNA life cycle. These concentrations determine the codon-specific elongation rates and error frequencies. Like many other cellular processes, translation is a complex multistep process with numerous individual transitions of the molecules involved, see **Figs. 1 and 2.** Our theory incorporates experimental data on the rates of these transitions obtained by Marina V. Rodnina and her co-workers, our collaborators from the Max Planck Institute for Biophysical Chemistry (Göttingen). All transition rates were measured in vitro because so far it is not possible to determine these rates in vivo. To bridge the gap between in vitro measurements and in vivo translation, we developed a method to predict in vivo rates from their in vitro values **[1].** We introduced the kinetic distance, a new measure to quantitatively compare the kinetics of a process in different envi-

ronments, and predicted the in vivo rates by a constrained minimization of this kinetic distance. We found that nine out of twelve in vivo transition rates are similar to the measured in vitro rates, whereas the other three have considerably increased in vivo values. Closer analysis revealed that initial selection of ternary complexes and proofreading by the ribosome are much more reliable in vivo than in vitro, i.e., in vivo translation is less error-prone.

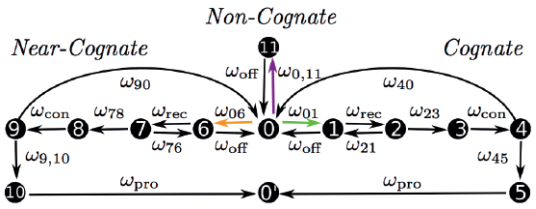

Fig. 2: Representation of translation as a codon-specific Markov process. For each codon c, the translation elongation cycle is represented by twelve states numbered from 0 to 11. The state (cl0) describes a ribosome with its A site at codon c and no initially bound tRNA. The states (cl1) - (cl5) represent the cognate branch for the decoding and full accommodation of a cognate tRNA. The states (cl6) - (cl10) belong to the near-cognate branch for the erroneous decoding of a near-cognate tRNA. The state (cl11) represents an initially bound non-cognate tRNA. An arrow from state i to state j indicates a transition with rate ω_{ij}. All rates of transitions between states are taken to be codon- independent, except for the binding rates of cognate, near-, and non-cognate ternary complexes (green, orange and purple arrows). State (c'l0') is attained by the ribosome after translocation to the next codon c'.

Protein Synthesis in Bacteria

We applied our theory to translation in Escherichia coli (E. coli) and studied various parametric dependencies of the translational speed and accuracy. We found that the overall speed of protein synthesis strongly depends on individual tRNA concentrations, see **Fig.3,** and on the abundance of active ribosomes. Furthermore, the overall elongation rate exhibits a phase transition at low tRNA and at high ribosome concentrations. We also found that codon-specific elongation rates and error frequencies are considerably influenced by the overall codon usage in the cell, see **Fig. 3 [2].** We focused on translation in E. coli because of the extensive database available for these cells. However, our theoretical framework is general and can be applied to all prokaryotic and eukaryotic cells. It can be used, for example, to study protein synthesis in human mitochondria, which are to some extend similar to bacteria, reflecting the endosymbiotic origin of these organelles. Our theoretical framework can also be applied to protein synthesis in eukaryotic cells to predict mutated codon sequences of individual genes that lead to an optimized gene expression in human cells and yeast in the context of protein-based vaccines.

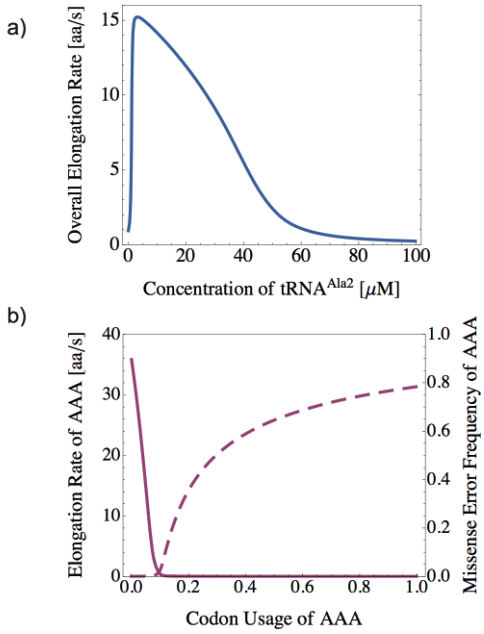

Fig. 3: Speed and accuracy of protein synthesis. (a) Influence of individual tRNA concentration on overall elongation rate. Only the concentration of tRNA^{Ala2} was varied, while leaving the concentrations of all other tRNA species at their in vivo values. (b) Dependence on codon usage (relative frequency) of codon AAA for fixed ratios of the codon usages of all other codons. The concentration of free Lys-tRNA^{Lys} ternary complex decreases when the codon usage of one of its cognate codons, AAA, increases (not shown). This relationship leads to a decreasing elongation rate of codon AAA (solid line, left axis), and an increase in the near-cognate missense error frequency of AAA (dashed line, right axis).

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Protein Binding and Membrane Adhesion

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Binding of Membrane-Anchored Proteins

Biological processes often involve the binding of proteins. These proteins are either soluble, i.e. free to diffuse throughout intracellular compartments or extracellular spaces, or are anchored to membranes that surround cells or cellular compartments. The membrane proteins are central for numerous biological processes but much less understood than soluble proteins since their structure and function is more difficult to assess in experiments. Key biological processes that are mediated by the binding of membrane-anchored proteins are the adhesion of cells and the adhesion of vesicles to cells or organelles in immune responses, tissue formation, cell signaling or intracellular transport. The adhesion processes depend sensitively on the binding constant of the membrane-anchored receptor and ligand proteins that mediate adhesion, but this constant is difficult to measure in experiments.

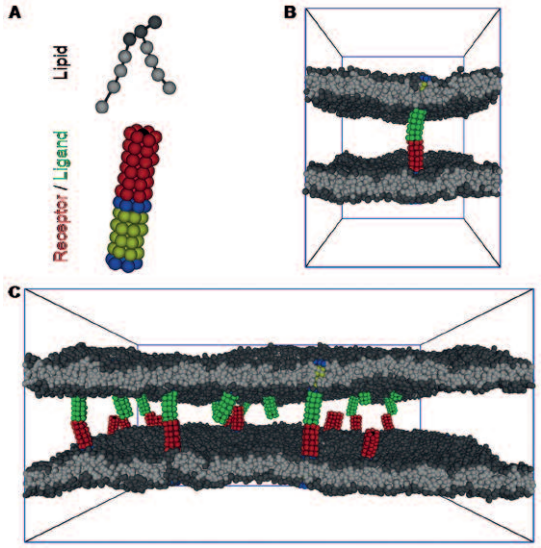

Fig. 1: (A) Coarse-grained structures of a lipid molecule and of a membrane-anchored receptor or ligand. (B) Simulation snapshot of two apposing membrane segments bound together by one receptor-ligand bond. (C) Larger membrane segments bound by many receptor-ligand bonds.

We have investigated the binding of membrane-anchored receptor and ligand proteins with molecular dynamics simulations **(Fig. 1).** These simulations indicate that the binding constant of the anchored proteins strongly decreases with the membrane roughness caused by thermally excited membrane shape fluctuations on nanoscales **(Fig. 2).** In addition, we have derived a theory that explains the roughness dependence of the binding constant for the anchored proteins from membrane confinement and that relates this constant to the binding constant of soluble proteins without membrane anchors. Since the binding constant of soluble proteins is readily accessible in experiments, our results provide a novel route to compute the binding constant of membrane-anchored receptor and ligand proteins **[1].**

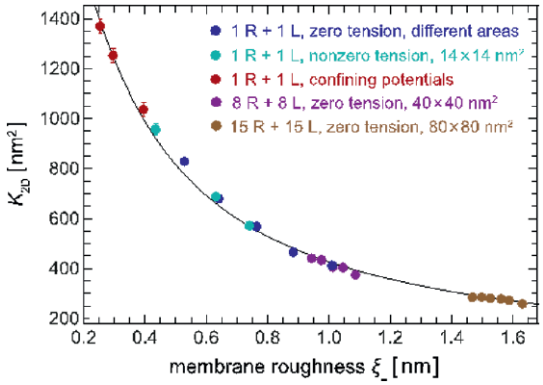

Fig. 2: Binding constant K_{2D} for receptor-ligand binding as a function of membrane roughness of the two apposing membranes caused by thermally excited membrane shape fluctuations. The data points are from molecular dynamics simulations of a variety of membrane systems that differ in the number of receptors R and ligands L, the membrane area, or membrane tension. The red data points are from simulations with confining membrane potentials that strongly suppress shape fluctuations. All simulations are performed at an average separation of the membranes that is close to the optimal separation for binding at which the binding free energy is maximal.

Conformational Changes During Protein Binding

Protein binding often involves conformational changes **[2,3].** Advanced NMR experiments indicate that these conformational changes can occur in the absence of ligand molecules (or with bound ligands), and that the ligands may 'select' protein conformations for binding (or unbinding). We have argued that this conformational selection requires transition times for ligand binding and unbinding that are small compared to the dwell times of proteins in different conformations, which is plausible for small ligand molecules **[2].** Such a separation of timescales leads to a decoupling and temporal ordering of binding/unbinding events and conformational changes. We have proposed that conformational-selection and induced-change processes (such as induced fit) are two sides of the same coin, because the temporal ordering is reversed in binding and unbinding direction **(Fig. 3).** Conformational-selection processes can be characterized by a *conformational excitation* that occurs *prior* to a binding or unbinding event, while induced-change processes exhibit a characteristic *conformational relaxation* that occurs after a binding or unbinding event. The ordering of events can be determined from relaxation rates in mixing experiments, and from the conformational exchange rates measured in advanced NMR or single-molecule experiments **[2].** For larger ligand molecules such as peptides, conformational changes and binding events can be intricately coupled and exhibit aspects of conformational-selection and induced-change processes in both binding and unbinding direction.

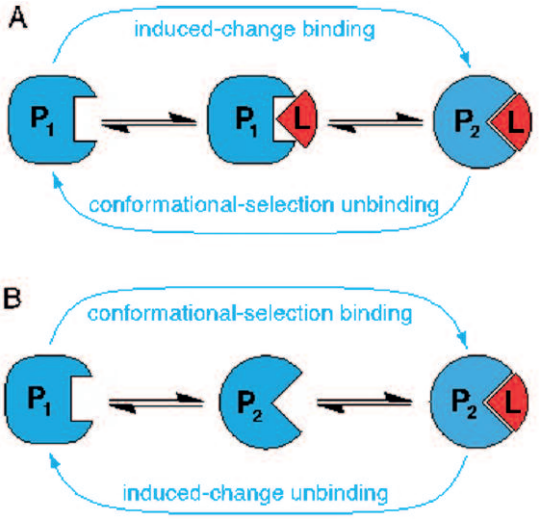

Fig. 3: Two different binding/unbinding pathways that connect the unbound ground state P_1 and bound ground state P_2L of a protein with two conformations 1 and 2. The intermediate states along these pathways are higher-energy, excited states: the state P_1L in (A) is higher in energy than the bound ground state P_2L, and the conformation P_2 in (B) is higher in energy than the unbound ground-state conformation P_1. Along pathway (A), the conformational change occurs after the binding of the ligand molecule L, and is apparently 'induced' by this binding event. In the reverse direction from P_2L to P_1, the conformational change occurs prior to the unbinding of the ligand. The ligand thus appears to 'select' the excited-state conformation P_1L for unbinding. Along pathway (B), the ligand binds via conformational selection, i.e. the conformational change occurs prior to ligand binding. In the reverse direction from P_2L to P_1, the conformational change is induced by the unbinding of the ligand. The conformational transitions of the induced-change processes in (A) and (B) are relaxations into a ground state. The conformational changes of the conformational-selection processes, in contrast, are excitations.

Wrapping of Nanoparticles by Membranes

Advances in nanotechnology have led to an increasing interest in how nanoparticles interact with living organisms. To enter the cells or cell organelles of such organisms, nanoparticles have to cross biomembranes. This crossing requires the wrapping of the particles by the membrane and the subsequent fission of a membrane neck if the particles are larger than the membrane thickness and cannot permeate the membrane directly. In general, both wrapping and fission can either be passive, or can be actively driven or assisted by protein machineries that consume chemical energy. Passive wrapping can occur if the adhesive interaction between the nanoparticles and membranes is sufficiently strong to compensate for the cost of membrane bending **[4].**

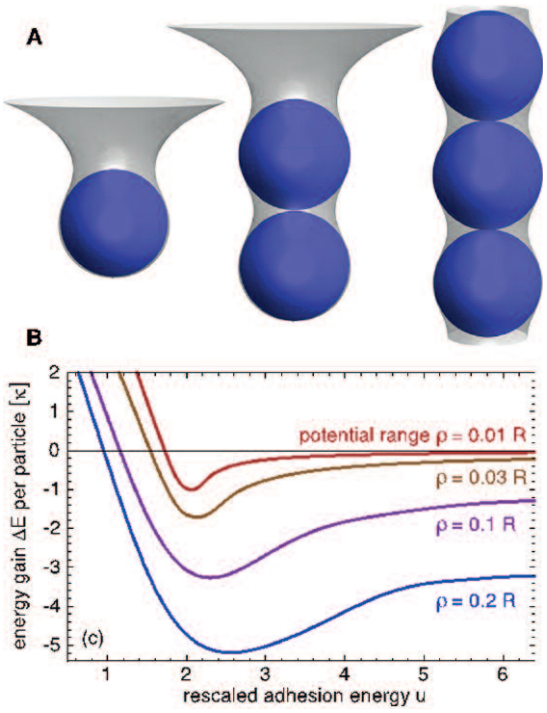

Fig. 4: (A) From left to right: A single particle wrapped by a membrane, two particles wrapped cooperatively in a membrane tube, and three central particles in a long tube. (B) Energy gain ΔE for the cooperative wrapping of particles in a long tube relative to their individual wrapping. The energy gain strongly depends on the range ρ of the particle-membrane adhesion potential, and on the rescaled adhesion energy $u = UR^2/\kappa$ where U is the adhesion energy per area and R is the particle radius. The bending rigidity κ of the membranes is of the order of 10 k_BT.

Our recent Monte Carlo simulations of passive wrapping (reviewed in **[4]**) indicated the cooperative wrapping and internalization of spherical nanoparticles in tubular membrane structures. To understand the formation of these tubular structures, we have systematically investigated the energy gain of this cooperative wrapping by minimizing the energies of the rotationally symmetric shapes of the membrane tubes and of membrane segments wrapping single particles **[5].** We have found that the energy gain for the cooperative wrapping of nanoparticles in membrane tubes relative to their individual wrapping as single particles strongly depends on the ratio ρ/R of the particle radius R and the range ρ of the particle-membrane adhesion potential **(Fig. 4).** For a potential range of the order of one nanometer, the cooperative wrapping in tubes is highly favorable for particles with a radius of tens of nanometers and intermediate adhesion energies, but not for particles that are significantly larger.

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Phase Separation in Multicomponent Membranes

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In recent years, the prevailing view of cell membrane structure has gradually evolved from the fluid mosaic model proposed by Singer and Nicolson to a heterogeneous membrane model with domains of lipids in the liquid-ordered (Lo) phase surrounded by lipids in the liquid-disordered (Ld) phase. The Lo domains (also called lipid rafts) are rich in cholesterol and saturated lipids, and are thought to play an important role in regulation of cell processes. To gain insight into the roles of individual membrane components, a variety of model membrane systems have been established, containing the lipid species of interest and exhibiting the richness of phase coexistence as in cell membranes. Giant unilamellar vesicles (GUVs) are a particularly practical biomimetic tool for displaying membrane behavior directly under the optical microscope **[1, 2].** We employed these cell-sized biomimetic systems to study the phase state of membranes composed of ternary lipid mixtures **[3, 4]** and the influence of proteins such as cytochrome c adsorbing onto the membrane **[5, 6].**

Phase Diagrams of Ternary Lipid Mixtures

A typical phase diagram of a ternary lipid mixture is given by the Gibbs triangle in **Fig. 1.** It characterizes membranes composed of DOPC, an unsaturated lipid with a low melting temperature, sphingomyelin (SM), a high melting temperature lipid, and cholesterol (Chol). Each point in the Gibbs triangle represents a certain membrane composition. The corners and the borders of the triangle correspond to single- and two-component membranes, respectively. The membrane can exhibit Lo, Ld or solid (S) phase or their coexistence and the colored areas indicate such coexistence regions. 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 in certain phases.

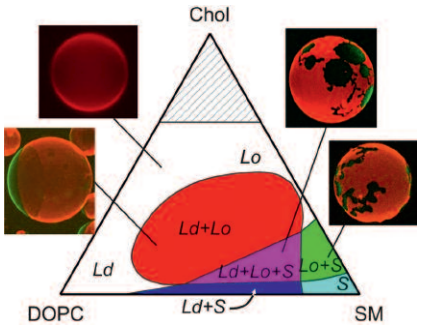

*Fig. 1: Phase diagram of the ternary lipid mixture DOPC/SM/Chol at 23.5°C **[3].** Tentative boundaries of one, two-, and three-phase regions are shown. The hatched area indicates the solubility limit of cholesterol, above which no membrane is formed. The images illustrate vesicles with homogeneous or phase-separated membranes corresponding to certain regions of the phase diagram. The vesicles are around 30 µm in diameter.*

Problems with Multicomponent Vesicles

Phase diagrams as in **Fig. 1** can be determined by preparing and examining GUVs with membrane compositions that vary over the whole Gibbs triangle. However, vesicles from the same batch can have very different compositions depending on their individual history. For example, before observation, a phase-separated vesicle may have budded yielding two daughter vesicles with two compositions that both differ from the composition of the mother vesicle. Thus, vesicles both with and without domains can be detected in the same batch. Deviations in the vesicle composition in a batch can be well demonstrated by the distribution of the area fraction of one of the domain types. If all vesicles had the same composition, they should exhibit the same domain surface area fractions. However, the observed distribution of area fractions is often quite broad, see **Fig. 2**.

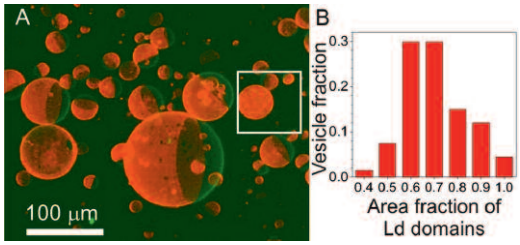

*Fig. 2: Compositional inhomogeneity of vesicles prepared from mixture of 40/40/20 mol% DOPC/SM/Chol observed at 23°C. (A) 3D projections reconstructed from confocal series. Vesicles with this composition exhibit phase separation, as observed for all vesicles on the image except for the framed one. This vesicle has no domains. (B) Distribution of the area fraction of red (Ld) domains over a population of ~70 vesicles from the same batch **[3].***

To overcome this problem, we use an alternative method to obtain a specific vesicle composition: we produce vesicles with domains via electrofusion of two vesicles made of two different fully miscible lipid mixtures. After electrofusion, the lipids in the newly created vesicle redistribute as described by the phase diagram.

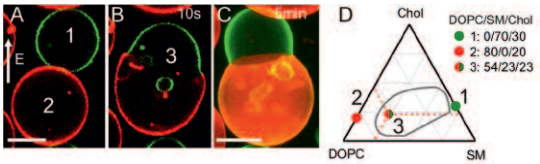

Fig. 3: Electrofusion of single- or two-component vesicles provides a novel protocol to create three-component vesicles with precisely controlled composition. (A–C) Fusion of two vesicles: confocal microscopy cross sections (A and B) and a 3D projection (C). Vesicles 1 and 2 were subjected to an electric pulse (400 kV/m, 150 µs; arrow indicates the field direction) and fused to form vesicle 3 shown in B and C. The time after application of the electric pulse is indicated in the upper-right corners. (D) Compositions of the vesicles in the images. Scale bars correspond to 20 µm.

Tie-Line Determination

Knowing the boundaries of the coexistence regions in the phase diagram is not sufficient to characterize the composition of domains in a multicomponent vesicle. In order to determine the latter, we need to know the tie lines in the coexistence region. Locating these lines is challenging because the coexisting phases in the bilayer membrane cannot be physically isolated and then analyzed for chemical composition. We proposed a new method for locating the tie lines based on microscopy quantification of the domain surface areas in GUVs produced by the electrofusion of two- or single-component vesicles. Contrary to other approaches, this method allows for direct observation of the membrane behavior under the microscope, and easily provides many tie lines.

Domain surface areas obtained from the 3D confocal scans recorded right after electrofusion were used to calculate the composition of the fused vesicle. The method for tie-line determination is based on quantifying the domain areas in the obtained three-component vesicle after equilibration. By applying the lever rule, we can also predict the approximate location of the critical point by extrapolating a curve passing through the midpoints of the found tie lines, see **Fig. 4.**

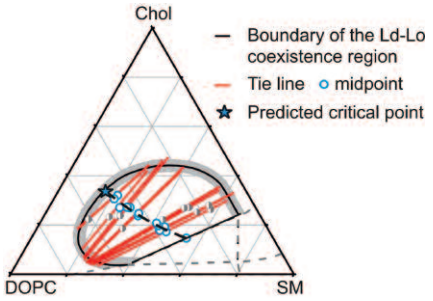

Fig. 4: Tie lines in the phase diagram DOPC/SM/Chol at 23°C ***[3].*** *Half-solid circles in gray indicate the compositions of the fused vesicles whose images were used to locate tie lines (red). The Ld-Lo coexistence region is indicated by the solid black curve shown with ±2 mol % deviation in gray. The blue open circles indicate the midpoints of the found tie lines, and the dashed black curve serves as a guide to the eye to connect them. It is extrapolated to the boundary of the Ld-Lo coexistence region to predict the location of the critical point as indicated by the star.*

Effect of Cytochrome C on the Membrane Phase State

Interactions of water-soluble proteins with membranes play an important role in many biological processes, such as signal transduction and transport processes. A good example for such a protein is cytochrome c (cyt c), a globular heme protein carrying approximately 4 effective positive charges. Upon adsorption to a membrane composed of negatively charged and neutral lipids, a positively charged protein may induce local changes in lipid composition. We used cyt c to address the effect of adsorption of a positively charged protein to negatively charged membranes with several fluid domains. First, we characterized GUVs composed of DOPG, a negatively charged lipid, SM and cholesterol. Confocal microscopy was used to explore more than 70 different membrane compositions in the Gibbs triangle of the DOPG/SM/Chol mixture at room temperature and to locate the Ld-Lo coexistence region in the absence of cyt c, see area shaded in yellow in **Fig. 5.**

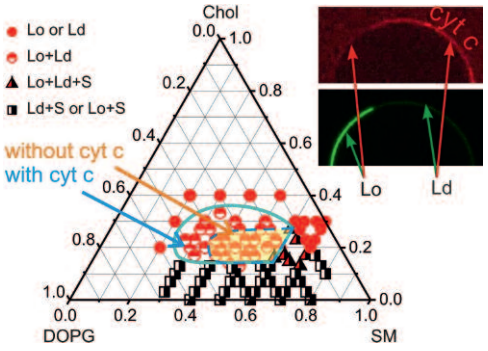

Fig. 5: Cyt expands the Ld-Lo coexistence region in the phase diagram of DOPG/SM/Chol at 23°C. The protein concentration was 0.56 μM. The inset in the upper right corner shows that cyt c preferentially adsorbs to the Ld phase domain as demonstrated by cross section confocal images of a GUV (red and green channel) composed of a 40/40/20 mixture of DOPG/SM/Chol, with fluorescent lipid dye (green) partitioning predominantly in the Lo phase and labeled cyt c (red) adsorbing predominantly to the Ld phase **[5, 6]**

We then studied the influence of cyt c on the membrane phase behavior. Upon the addition of cyt c, the area of Ld domains (rich in charged DOPG) was found to increase. Apparently, the bound protein attracts more charged lipids to the liquid disordered domains where it predominantly binds (see inset in **Fig. 5**). In addition, the protein was found to induce micron-sized domains in membranes belonging to the single-fluid-phase region of the protein-free ternary mixture and, as a result, to expand the Ld-Lo coexistence region, see **Fig. 5.** The protein also induced vesicle leakage even at relatively low concentrations.

In eukaryotic cells under normal physiological conditions, cyt c is localized within the intermembrane space of mitochondria. Thus, during cell apoptosis when cyt c is released into the cytosol and adsorbs to intracellular membranes, it may strongly perturb the lipid distribution within these membranes and induce their leakage, thus, further enhancing the process of apoptosis.

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Phase Transitions and Transport Phenomena at Solid/Air-Interfaces

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We are interested in the impact of interfacial energy contributions on the phase behaviour of nano-size systems and how interfacial contributions affect volume flows (via surface Marangoni-flows).

Both of these phenomena are practically relevant. Phase transition processes of small/confined systems, in particular nucleation phenomena, are ubiquitous (from cloud formation to metallurgy). Liquid flows induced by surface tension gradients are for instance important in ink jet printing.

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

Our research topics and collaborations are motivated by applications. But our research clearly focuses on a better fundamental understanding of the phenomena.

Melting/solidification of Nano Size Structures

We investigate how interfacial contributions affect the nucleation and phase transition behavior of small, confined systems (e.g., small islands on inert surfaces). As experimental systems for this topic, we use terraces (films/multilayers) of long chain alkanes at SiO_2/Air interfaces. The melting is imaged by interference-enhanced microscopy. The findings are analyzed analytically and by simulations.

Our main focus is on how the melting behavior of nano size aggregates is affected by the aggregate geometry (shape, faceting, grooves, edges, etc.) and by their wetting properties. The aim is an improved understanding of the melting process. Very little is known about this process because the emerging liquid phase has no memory of it (in contrast to the reverse solidification process).

First order phase transitions implicate the formation of an interface between the old and the new, emerging phase. This formation process usually causes a nucleation energy barrier for the transition. The nucleation is heterogeneous when the emerging phase is in contact with other (inert) phases in addition to the interface between old and new phase. The latter configuration lowers the energy barrier. If the topography between emerging phase and inert template is non-planar or if the solid is facetted with different interfacial energies, the impact on the nucleation barriers and on the nucleation paths depends on all these properties.

For melting, empirically in virtually all cases and for virtually all substances, no nucleation barrier is found.

It is commonly assumed that the liquid melt completely wets all solid facets, which would result in a vanishing nucleation barrier for all facets. This assumption has never been proven or tested. To explain the experimental findings it would suffice if melting starts locally i.e., if the nucleation barrier for melting is vanishing only locally or for specific facets/interfaces. Virtually every macroscopic systems has nano size geometrical features, which can serve as such "defects". Therefore nano-size systems with known wettabilites and geometries are much better suited to investigate the melting process.

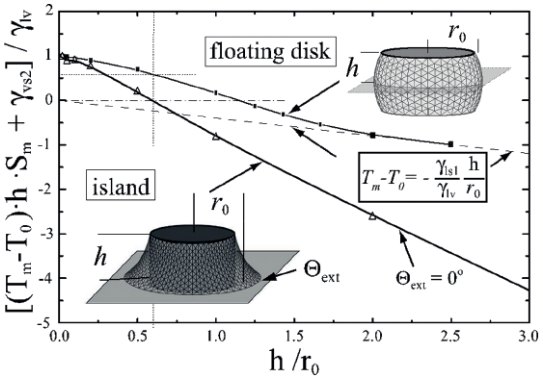

Fig. 1: Scaled shift of the melting temperatures (T_0 = bulk melting temperature) of floating disks and of round islands as a function of the ratio between height and radius, h/r_0 (S_m = melting entropy, γ= interface energies). For comparison also a typical "Gibbs-Thompson"-behavior ($T_m - T_0 \propto 1/h_0$) is shown. Small aggregates can even be overheated.

Our experimental and theoretical studies on small systems reveal details on the melting kinetics. We find for instance, nucleation barriers for the melting of real systems (alkane films) and a melting behavior that is very different from the commonly accepted, single-parameter "Gibbs-Thompson"-behavior **([1], Fig. 1).**

Patterned Growth Induced by Heterogeneous Nucleation

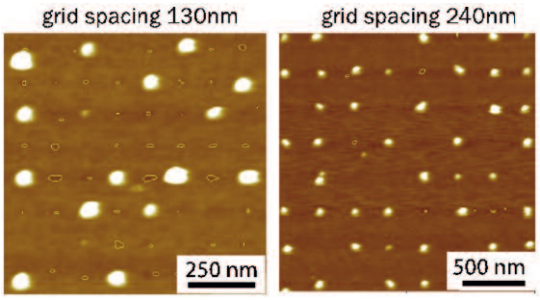

Fig. 2: AFM-image of regular arrangements of C60-aggregates on a regular rectangular 8 x 8 pattern of indents in a smooth planar substrate (SiO_2, surface roughness less than 0.4nm). The indents have diameters of about 20nm. Their depth is less than 1nm.

Heterogeneous nucleation is investigated with planar solid substrates that are pre-structured with an array of nano-indents. Although the indents are very shallow, C60 aggregates that precipitate from solution during spin casting preferentially adsorb within the indents and thus replicate the pattern geometry **(Fig. 2).** This behaviour is not yet understood.

To gain better quantitative insight into the spatio-temporal evolution of the solute/solvent evolution during the film thinning, leading to the phenomena depicted in **Fig 2,** the spin casting process itself is analyzed theoretically **[2]** and experimentally **(Fig. 3).**

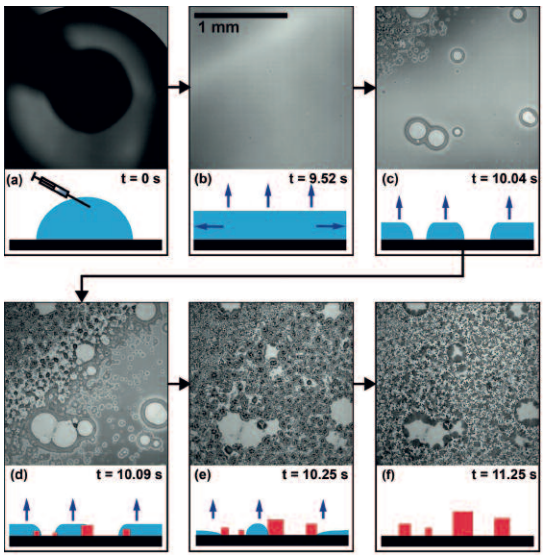

Fig. 3: On-line, time resolved optical imaging of film thinning, precipitation and dewetting during spin casting of mixture of volatile solvent and non-volatile solute (0.5M NaCl in H_2O).

In a related project we also investigate how nano-size interfacial features induce the local formation of gas bubbles within supersaturated solutions. We focus, in particular, on the unknown process how local gas enrichments eventually (trans)form into gas bubbles.

Drop-Drop Coalescence, Interfacial Flow and Drop Evaporation.

The coalescence of sessile droplets is governed by interfacial effects. Capillarity favours fast drop coalescence. With different liquids in both drops however, surface tension gradients may form in the region where the drops connect. The resulting Marangoni flows can unexpectedly cause a long delay of the drop coalescence. The main drop bodies remain separated while connected via a thin neck through which the drops exchange liquid. For simple liquids this phenomenon is meanwhile understood.

Currently we investigate the coalescence behaviour of drops containing different, reacting liquids, which can induce fascinating, self-organized pattern formation **(Fig. 4).**

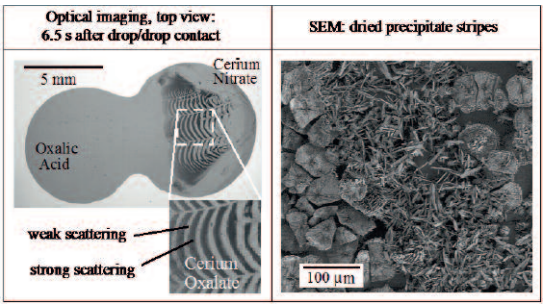

Fig. 4: Patterned precipitation of Cerium-Oxalate appearing during the coalescence of sessile drops with reacting liquids. The SEM images from the dry precipitate show that the stripes consist of different aggregate sizes.

In a related project we also investigate the evaporation behaviour of sessile drops from mixtures of liquids with different volatilities. This behaviour is complicated and not well understood because the locally heterogeneous evaporation flux from sessile drops induces spatio-temporal variations of the liquid composition (e. g., the selective enrichment of components at the drop perimeter) and leads to various interacting transport processes **(Fig. 5).**

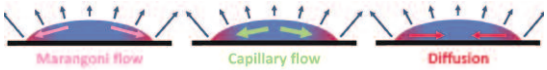

Fig. 5: In the case of complex fluids the locally heterogeneous evaporation flux of sessile drops (indicated by the different lengths of the arrows) can induce spatial constitutional variations and thus various (local) transport processes.

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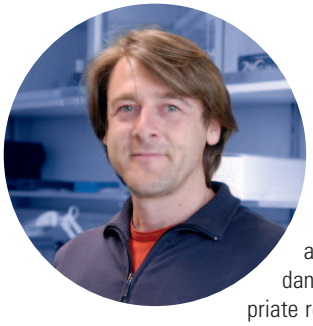
Stochastic Processes in Biological Systems



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Single-Molecule Modeling of mRNA Degradation

The control of the amount of messenger RNA (mRNA) is a key component of the regulatory apparatus of gene expression. Controlled transcription and degradation of mRNA guarantee the timely modulation of mRNA abundance during the lifetime of the cell and an appropriate response to external stimuli. Large experimental research efforts are devoted to uncovering which complex set of proteins and enzymes is responsible for the degradation of mRNA. Most of these studies approach the problem by formulating a single-molecule perspective according to which the mRNA consecutively binds to different complexes until final degradation eventually happens. The experimental studies are thus aimed at defining the interaction network of the target mRNA with these complexes. To achieve this purpose, very ingenious techniques are used to single out the relative strength of various protein complexes on the process of degradation. However, it is still not possible to experimentally monitor single mRNA molecules during the process of degradation. Indeed, all available experimental techniques just allow monitoring the decay pattern of the average amount of mRNA, which is the amount of mRNAs left after a certain time in the cell culture.

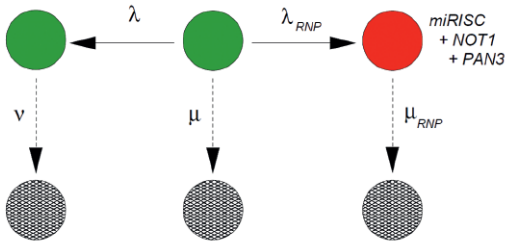

Fig. 1: The network of states responsible for the miRNA-mediated mRNA degradation. The green central dot represents the mRNA before the process of degradation starts. The red dot is the mRNA after having been recruited by miRISC bound to NOT1 and PAN3. According to our analysis ***[3],*** *this is the most parsimonious network supported by the available data.*

Traditionally, decay patterns are either compared qualitatively, to decide which pattern decays faster than another, or are assumed to be exponential functions and fitted to deliver the rate of degradation. In both cases, no useful conclusion can be drawn about the underlying interaction network responsible for the degradation of the mRNA. Moreover, if there is a (complex) interaction network the decay pattern should not be exponential and it should contain some information about the structure of the network.

An extensive analysis of published data of mRNA decay patterns has convinced us that indeed only a fraction of the experimental patterns are exponential. This fact convinced us to develop a mathematical framework to relate the single-molecule viewpoint of mRNA decay with the bulk decay patterns that are experimentally accessible **[1].** This mathematical approach is based on two complementary methods. On the one hand, a very general approach, technically close to survival data analysis, shows that non-exponential decay patterns are related to age-dependent degradation processes. On the other hand, by interpreting the degradation interaction network in terms of Markov chains it is possible to find the structure of the most parsimonious network compatible with data and thus derive both an explicit form of the lifetime distribution and of the age-dependent degradation rate.

Based on the first approach, we have been able to find a mathematical expression of the age-dependent degradation rate when ribosomes shield the mRNA against degradation factors **[2].** The shielding process is known to happen in many organisms. In *E. coli,* in particular, it is easy to see this effect because of the short lifetime of the mRNA. Based on the Markov chain approach, we have been able to reconstruct the backbone of the interaction network with which miRNA mediates the degradation of its target mRNA [3]. In particular, through data analysis of various knock-down experiments in *drosophila,* we found out that the silencing complex miRISC interacts with the proteins NOT1 and PAN3 before recruiting the mRNA for deadenylation. This finding unveils a new aspect of the interaction network of miRNA-mediated degradation that was unknown before (see **Fig. 1**).

Structure and Properties of Complex Stochastic Networks

Many single-molecule processes, including mRNA degradation **[3]** and molecular motor movements **[4],** are successfully modelled as Markov chains on a network of states. In many cases, some of the interesting properties are expressed in terms of first passage time distributions. For instance the step duration of a kinesin molecule, the lifetime of a mRNA molecule, or the time until detachment from a filament are all technically computed as first-passage times or as absorption times. Sometimes, however, the structure of the underlying network is only partially known but first passage time measurements between two chosen states or configurations are available. Which kind of information about the underlying network of states is contained in the distribution of the first passage times? If we had the lifetime distribution of the mRNA, what would this tell us about the network of states underlying its controlled degradation? If we had the distributions of the duration of each kind of molecular motor steps, what would these tell us about the structure of the underlying chemomechanical network? In computer simulations of single-molecule transitions, if we had the first-passage time distributions of the visits to configuration B starting from configuration A, what would this tell us about the structure of the free-energy landscape between A and B?

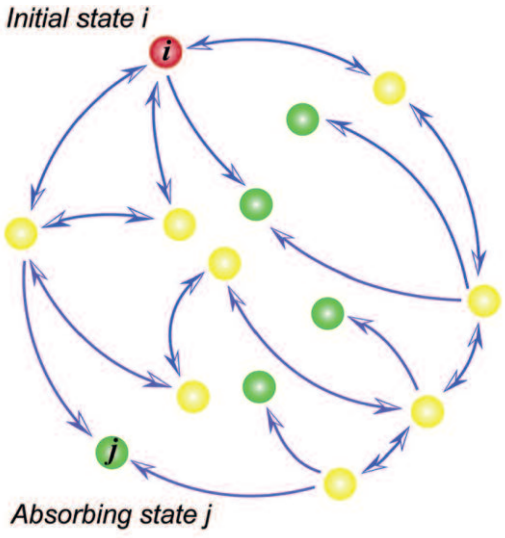

Fig. 2: A generic network of states for a Markov chain. The probability density of the first passage time from state I to state j has a short time behavior that grows linearly in time because the shortest path from I to j contains only one intermediate state ***[5].***

In a work that generalizes an idea of Li and Kolomeisky (2013), we found out that for any general Markov chain on a network of states the first term of the Taylor series (dominating the short time behaviour) of the first-passage time density is necessarily an integer power of time. Using graph theory methods we then showed that this power is a linear function of the number of states along the shortest path(s) from the initial to the final state **[5] (see Fig. 2).** In particular, the power of the first term of the density will be zero only if there is a direct connection between the initial and the final state. Thus, as a tool to discover network structures, the fit of the early time behaviour of experimental first-passage time densities may reveal the number of intermediate states between two accessible states. A natural generalization in this context concerns an extension to semi-Markov chains, where the dwell times on the states can be assumed to be distributed according to a generalized Gamma density. Also in this case, the short time behaviour of the first passage time density reveals the number of intermediate states but some details of the dwell time distributions must also be known **[6].** In complex networks there are usually many paths that join one state to another. Some of these paths are shorter than others but may be slow on average while other paths might be longer but faster on average. Which one of them characterizes the short time behaviour of the first-passage time density? In other terms, if we select transition paths according to which one is the fastest, are we also selecting for the most probable path? Not always. In fact, even if a path is highly improbable but shorter than any other path, there exists a time scale below which almost all realizations will occur through the shortest path **[7].**

When a process is conditioned to first pass through a state before another state is equivalent to a condition in the future outcome. Processes conditioned in their future outcome are very difficult to study because their properties are quite counterintuitive, as the example of the shortest path described above illustrates **[7].** Mathematically, these conditioned processes are related to the original unconditioned process though a Doob-h transform, which essentially completely transforms all the rates of the original process. Among the strange properties produced by this transformation, there are new forms of time duality and balance relationship similar to detailed balance even when the process is not at equilibrium. The structural properties of the network of states that generate such time duality are a topic in current development in the field of Markov chains and their application **[8].**

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Regulation of Bio-Processes

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Biological processes must obey the laws of physics, but are also subject to functional requirements and shaped by the forces of evolution. Our group is interested in how functional requirements are implemented within the given physical constraints. To that end, we develop theoretical tools to describe complex regulatory systems and their coupling to the cellular context. Focusing on bacterial systems, we address these questions in two main areas, *gene regulation and cell growth* as well as *bacterial motility*. In both areas we build on our expertise on *molecular motors and machines*.

Gene Expression and Cell Growth

A long-term interest of the group is the unavoidable coupling of expression of any gene to the physiological state of the whole cell, through (among other things) the sharing of gene expression machinery. Exponentially proliferating bacteria provide a good model system to address these questions, as the physiological state of the cell can to a large extent be characterized by a single parameter, the cell's growth rate. We develop theoretical methods to describe such coupling and include growth-rate dependence into descriptions of gene circuits **[1].** In addition, we study the effects of shared gene expression machinery, i.e. RNA polymerases, ribosomes and their associated factors.

In rapidly growing bacteria, ribosomes are a limiting commodity for the synthesis of proteins and, thus, cell growth. A striking demonstration of the link between ribosomes and growth is the linear relation between ribosome concentration and growth rate **(Fig. 1).** This relation reflects the autocatalytic activity of ribosomes that synthesize ribosomal proteins. We could recently show that the linear relation is consistent with a translation speed that is a function of the growth rate **[2],** reconciling two observations that were previously believed to be in disagreement. The modulation of the translation speed could be explained as resulting from a balance between the cost of making more ribosomes and the cost of making more elongation factors that are needed to speed up the ribosomes. Based on this analysis we concluded that the underlying allocation of resources is close to optimal. Moreover, our theoretical analysis also indicated that the cost associated with the translation speed arises from the slow diffusion of the large elongation factor complexes in the crowded cytoplasm.

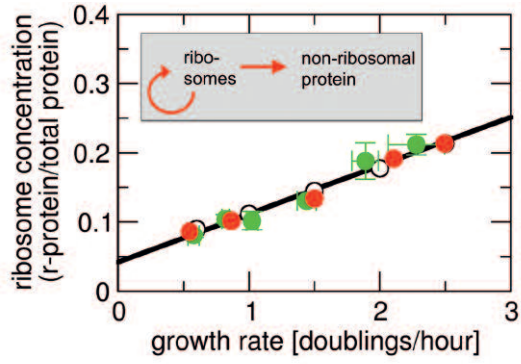

*Fig. 1: A linear relation between cellular ribosome concentration and growth rate, here for the bacterium Escherichia coli, reflects the autocatalytic activity of ribosomes making ribosomal protein. Studying the allocation of ribosomes, toward making ribosomal proteins versus making factors affecting the speed of translation, provides a window into the economic principles of the cell **[2].***

Competition for gene expression machinery is also crucial in the case of sigma factors, proteins associated with RNA polymerases in bacteria. Sigma factors direct RNA polymerases to subclasses of genes and play an important role during the switch of gene expression programs in stress responses. During stress responses, genes under the control of different sigma factors become coupled and exhibit "passive control", i.e. genes become up- or down-regulated indirectly because competing genes are actively regulated in the opposite direction. In particular, we found that type of control can be hypersensitive to changes in RNA polymerase core enzyme concentration, indicating a strong role in stress responses such as the stringent response **[3].**

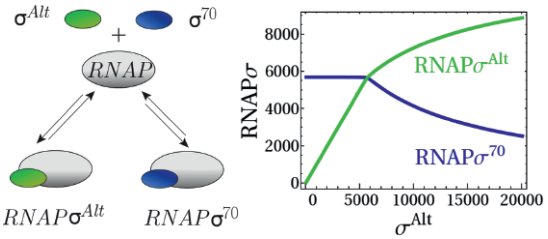

Fig. 2: Sigma factor competition: Two types of sigma factors (σ^{70} and σ^{Alt}) compete for binding to the RNA polymerase (RNAP) core enzyme and direct it to different sets of genes. Beyond a threshold concentration of σ^{Alt}, competition sets in and any further increase in the concentration of σ^{Alt} indirectly inhibits the formation of the RNAP-σ^{70} complex and the expression of the associated genes.

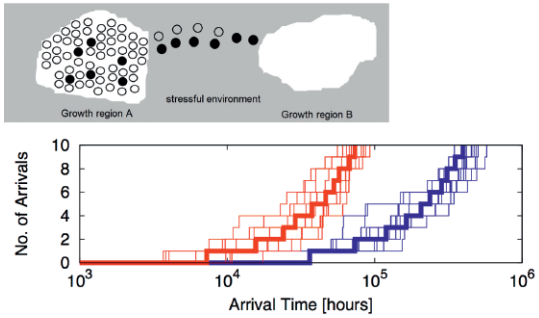

Fig. 3: Crossing of a stressful environment by a heterogeneous population: A population (red trajectories) with a small subpopulation of more tolerant cells (black cells in the sketch) spreads faster in space than a homogeneous population (blue) with only one cell type (white) ***[4].***

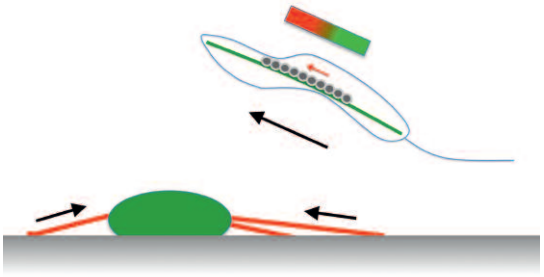

Fig. 4: Bacterial motility: (top) A magnetotactic bacterium swims in the direction defined by an external magnetic field, driven by the rotation of its flagellum. (bottom) The retraction of type IV pili drives the twitching motility of bacteria on surfaces.

In addition, we are interested in understanding the consequences of the coupling of gene expression and growth, in particular for cases where growth mediates (wanted or unwanted) feedback **[1].** We have focused on cases where positive feedback leads to phenotypically heterogeneous populations with subpopulations growing with different growth rates. An important example is bacterial persistence, where a slow-growing subpopulation has increased tolerance towards antibiotics. These examples also allow us to study the population dynamics with phenotypic heterogeneity. Heterogeneity arises as a strategy to survive in varying environmental conditions. We proposed recently that it also provides an advantage for the spreading of a population in spatially structured environments **(Fig. 3) [4].**

Bacterial Motility

The second topic of the group is bacterial motility. Recently, we have studied two systems, both in collaboration with experimental groups: twitching motility and magnetotaxis **(Fig. 4).** In the case of twitching motility (in collaboration with the group of B. Maier, Köln), we have focused on the mechanical coordination of type IV pili that exert forces on each other. The pili are filamentous appendages of these cells that pull the cells forward through cycles of growth, adhesion to a surface and retraction. We have developed a stochastic tug-of-war model integrating the force-dependent dynamics of individual pili to predict the motility pattern (persistent random walks) of the bacteria **[5].** Comparison with experimental bacterial trajectories indicated that a mechanism for directional memory is required to circumvent a limitation due to the two-dimensional geometry of the tug-of-war (pili pulling in random directions on a surface), which can be provided by sufficient stability of the pilus base complex. This idea was confirmed by analyzing the statistics of single pilus retraction experiments (bursts of retractions).

Magnetotatic bacteria align along magnetic field lines with the help of magnetic organelles called magnetosomes. Magnetosomes contain magnetic nanoparticles and are aligned along a cytoskeletal filament to form a cellular "compass needle", called the magnetosome chain. We study both mechanical aspects of the magnetosome chain and the navigation of the bacteria (in collaboration with the group of D. Faivre, Biomaterials department). Specifically, we have studied how magnetic alignment helps the cells to swim in an oxygen concentration gradient towards the preferred micro-oxic zone, a behavior known as magneto-aerotaxis. The magnetic field with a component parallel to the oxygen gradient can provide an axis and/or a direction for motility. A comparison of the magneto-aerotactic behavior of different strains of magnetotactic bacteria shows that many strains use the direction given by the magnetic field instead of sensing an oxygen concentration gradient and that such replacement can occur separately for low-oxygen and high-oxygen conditions **[6].** Other questions we have addressed recently include mechanical properties of the magnetosome chain such as its bending stiffness, its response to magnetic fields as well as aspects of cell division.

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