TECHNISCHE
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29.12.2003

Subject: application for the for the tenure-track faculty position at the Biocomplexity Institute

Dear Members of the Biocomplexity Faculty Search Committee,

I am writing you to express my interest in the tenure-track faculty position at the Biocomplexity Institute of the Indiana University.

My area of expertise is theoretical biophysics. Please find further information about me in the enclosed documents and in my homepage: www.physik.tu-muenchen.de/~aboulbit.

I arranged for letters of references which will soon arrive separately.

In February 1-6, 2004 I am going to take part in a conference and then to visit few Universities in US. This opportunity could be used, if you chose to invite me for the interview. In this case my visit to the Biocomplexity Institute could be arranged either before or after the conference.

Thank you for your consideration and I look forward to hearing from you soon.

Sincerely yours,

Alexei Boulbitch

Dr. A. Boulbitch

STATEMENT OF RESEARCH PLANS

In my research I focus on new aspects of mechanical behavior of such biological objects as biomembranes, biogels, single biomolecules, etc. which result from (i) their **meso- or nanoscales** and (ii) from the **interplay between their mechanics and biochemistry**.

My **research proposal** (see below) is divided into two parts.

The first part involves development of a theory of **dynamics of spontaneous adhesion** and enforced unbinding of biomembranes. In this project **a theory will be developed accounting for the interplay between micromechanical and biochemical degrees of freedom** of biomembranes. This will enable me to describe kinetics of bioadhesion.

The second part deals with a theory of the behavior of **complex fluids during microrheological experiments**. A **new type of resistive force** will be introduced. The latter arises due to the mesoscopic size of a probe bead used in the microrheological measurements and cannot show up on the macroscopic scale.

These problems raise important issues of **mechanics of nano- and mesoscopic** systems that are not dealt with in current theories. In each case I will develop a theory combining analytical, numerical approaches and molecular dynamic simulations.

Training and Supervision

These projects allows to train 3 to 4 PhD students and 2 postdoc. The second project will include a lot of numeric calculation. This allows to train several diploma students.

I. Theory of dynamics of adhesion of biomembranes and its control by mobility, affinity and clusterization of ligand-receptor pairs, and by mechanical stress.

Abstract

The objective of the present project is to develop a theory of the kinetics of adhesion of biomembranes mediated by specific interactions, its dependence on (i) the mobility of the ligand-receptor pairs and repeller molecules, (ii) lateral phase separation of receptors possibly followed by an amplification of the affinity between receptors and ligands, (iii) competitive inhibition of ligand-receptor binding in the presence of antibodies in the bulk. I first focus on the description of a membrane adhered onto substrate through mobile ligand-receptor pairs and study kinetics of its spontaneous binding and enforced unbinding. I will then develop a theoretical model describing control of adhesion kinetics by a variation of the affinity between receptors and ligands due to the lateral phase separation of receptors. I will further study kinetics of the adhesion and unbinding mediated by competitive chemical reactions. Here I plan to concentrate on two cases: (i) unbinding mediated by competitive binding of antibodies (which are in the bulk of solution) to the receptors residing in the membrane and (ii) switching between the adhesion states due to a competition of two kinds of the ligand-receptor pairs. I will finally develop a theory of unbinding of a membrane from a pinning center formed by a cluster of receptors.

Current State of Research

Introduction

Though adhesion of cells represents an essential stage in various biological processes (Alberts and others 1994) its physical basis is still poorly understood. The importance of this area is demonstrated by the fact that nearly 21000 publications on adhesion appeared during last few years (Bruinsma and others 1999). Most of the papers concerned with the role of specific bonds. But since the pioneering theory of Bell (Bell 1978) it is known that adhesion is controlled by osmotic pressure of ligands, receptors and ligand-receptor pairs (Bell and others 1984). To study the physical basics of adhesion it is essential to establish simplified experimental and theoretical models which contain main components of cell membranes and "catch" main feathers of bioadhesion.

In the most simple case of non-specifically interacting membranes adhesion can be described in terms of an inter-membrane, or membrane-substrate potential arising as the result of the superposition of generic forces of the system (such as electrostatic, van der Waals and hydration interaction). In addition adhesion of a soft shell is strongly affected by Helfrich undulation force. Adhesion of one-component elastic shells mediated by generic interaction has been extensively studied theoretically in recent years by Lipowsky and Seifert (Lipowsky and Seifert 1991; Seifert 1991; Seifert 1997; Seifert and Lipowsky 1990). Predictions of these theoretical models were verified experimentally by studies of the adhesion of giant vesicles on supported membranes in the applicants laboratory.

Adhesion may become an origin of a lateral phase separation. This aspect has been studied in detail for the case of the adhesion mediated by generic forces both experimentally (Marx and others 2002; Nardi and others 1998; Nardi and others 1997) and theoretically (Bruinsma and others 1999; Komura and Andelman 2000; Komura and Andelman 2002). On the other hand, it is shown experimentally that the receptors of the integrin family gives rise to adhesive contacts provided it forms clusters (Dustin and others 1996). This is in line with a recent observation of a lateral attraction between the bound receptor molecules (Maier et. al., 2001). So far no theoretical explanation of these facts exists. It will be addressed in the present project.

Despite of the biological relevance few theoretical approaches have been proposed recently to describe specific interactions between biomembranes. Adhesion of membranes mediated by stickers has been studied by R. Lipowsky, who have shown that two membranes adhere to one another, if the concentration of the stickers exceeds a threshold (Lipowsky 1996). A theory of the adhesion based on chemical reactions of ligand-receptor pairs

$$L + R \rightarrow LR \quad \Leftrightarrow \quad \frac{\partial C_{LR}}{\partial t} = k_+ C_L C_R - k_- C_{LR} \tag{1}$$

has been proposed by Bell et. al. (Bell and others 1984; Dembo and others 1988). Here L stays for ligands, R for receptors and LR for the ligand-receptor pairs. C_{LR}, C_L and C_R are the corresponding concentrations, k_+ and k_- are the forward and the backward reaction rates.

Rupture of multiple parallel ligand-receptor bonds (a phenomenon closely related to that of cell adhesion) has been studied showing that the rupture force depends on the number of bonds linearly, like a square root, or logarithmically (Seifert 2000). The effect of repeller molecules was considered in a combined theoretical and experimental study. It was shown that a tight adhesion may be possible at low receptor densities by first order adhesion transition accompanied by receptor segregation and adhesion plaque formation. A correlation between adhesion of soft shells and wetting transition was established more recently (Bruinsma and others 1999). It was shown that for the case of stickers-mediated adhesion a generic repulsion between the membrane and the substrate gives rise to a lateral phase separation (Weikl and others 2002a). Adhesion dynamics in a system containing stickers and repellers has been studied in the paper (Weikl and others 2002b). It has been shown that in the case of a high barrier adhesion takes place via formation and growth of a single nucleus, while in the case of a low barrier one should expect multiple nucleation.

If ligand-receptor pairs are mobile, motion of the adhesion rim will give rise to the diffusive motion of ligand-receptor pairs in its front. If $V > (D/t)^{1/2}$, where V is the rim velocity, D is the diffusion coefficient of the ligand-receptor pairs and t is time, the ligand-receptor pairs inside the adhered area do not have enough time to diffuse away from the moving rim. This gives rise to a lag force, which is osmotic pressure effect and resists the rim motion (Brochard-Wyart and De Gennes 2002). The approach used in (Brochard-Wyart and De Gennes 2002) however, cannot account for the binding-unbinding reactions between ligands and receptors and does not describe therefore, the membrane unbinding. The description of the membrane unbinding accounting for the mobility of ligand-receptor pairs can be made using my previous results (see Eq. (2)-(4) below) and will be addressed in this proposal.

Experimental and theoretical background for the proposal

Interactions between cells and between a cell and a tissue surface takes place via an interplay of specific and non-specific forces. Various types of ligands and receptors may be involved in the adhesion process, some of the ligand-receptor pairs are mobile while others are immobile, so that the adhesion of cells involves a redistribution of molecules. To study basic physical properties of specific adhesion one needs a simplified model experimental system which is still able to catch them. Recently such an experimental model system, simple but versatile, has been developed (Albersdörfer and others 1998; Albersdorfer and others 1997; Guttenberg 2000; Kloboucek and others 1999). It consists of a giant vesicle with reconstituted lipid coupled ligands (referred to as lipo-ligands) which acts as a test cell. In addition, lipo-polymers (such as the polyethyleneglycol covalently attached to the lipid head-group mimicking the repulsive effect of the glycocalix) are reconstituted into the vesicle. To mimic the target cell, either a supported membrane with reconstituted conjugated receptors or a substrate coated by them is used. With this new system kinetics of the spontaneous adhesion and enforced unbinding has been studied and revealed new properties. Theoretical description of this system is addressed in the present proposal.

1. Determination of adhesion energies from membrane shapes

In the previous work (Guttenberg and others 2001) we reported an observation of a discontinuous transition between adhesion states in system described above. The system exhibits a weakly bound state characterized by a large (~150÷200 nm) substrate-membrane distance (Schematically shown in Fig. 1 a) and a tightly bound state exhibiting a distance about few manometers (Fig. 1 c). We have shown that the first state is controlled by gravitation, while the tightly adhered state is

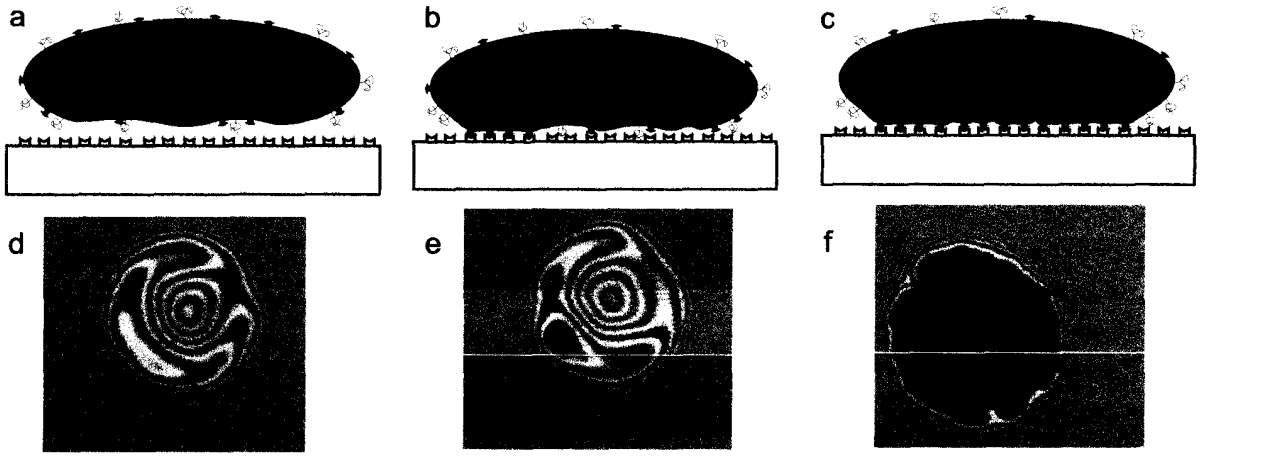

Fig. 1. Kinetics of the adhesion. (a)-(c) Schematic view of the adhesion kinetics, (d)-(f) shows the corresponding experimental images of the adhesion area. (a) and (d) The vesicle with reconstituted ligands and repeller molecules hovering over the substrate. (b) and (e) Nucleation of the region of the tight adhesion in which ligands are bound to the receptors (seen as the black area in (e)). After nucleation the region of the tight adhesion grows until it occupies the whole adhesion area (c) and (f).

due to specific ligand-receptor interaction. Our measurements showed that the previously established model (Bruinsma 1996) describing the shape of the membrane in the vicinity of the adhesion rim does not give even a qualitative agreement with our data. We extended the previous model so that in addition to the local bending it accounts for stretching of the membrane at the rim of the adhesion disc as well as for the gravitational force. With this excellent agreement between the measured and calculated vesicle profiles has been achieved. This theory enabled us to extract for the first time the energy of adhesion mediated by the interaction of a single type of the ligand-receptor pair from the experimental data. We also have calculated the energy of adhesion in the spirit of the theory (Bell and others 1984), but extended in such a way that it accounts for the contribution of the repeller molecules. These arguments show that this energy is controlled by the lateral osmotic pressure of the repeller molecules, which explains the coincidence of these two values noticed in our experiment.

2. Unbinding of a bead from a biomembrane

One often finds situations in which a biomembrane adheres onto a curved, rather than onto a plane surface. An example is a microbead adhered on the membrane (Vetvicka and Fornusek 1987). Another example is the cantilever tip of the atomic force microscope functionalized in such a way as to be attached to a cell surface (Benoit and others 2000). In a recent paper (Boulbitch 2002) the enforced unbinding of a bead from (i) a supported membrane, and (ii) from a cell surface has been studied theoretically. The adhesion mediated by generic forces has been considered. In contrast to the situation of the adhesion mediated by specific forces, unbinding is only possible if the external force exceeds a threshold value. The threshold unbinding force has been calculated. Estimates show that it is within the range accessible in micromechanical experiments.

3. Spontaneous adhesion dominated by specific interactions

In a recent paper (Boulbitch and others 2001) we reported the first measurement of the kinetics of adhesion of a single giant vesicle controlled by the competition between the membrane-substrate interaction mediated by ligand-receptor interactions, the effects of gravitation and Helfrich repulsion. The system was analogous to that reported in (Guttenberg and others 2001). In the beginning of the experiments vesicles were hovering over the substrate in the weakly adhered state

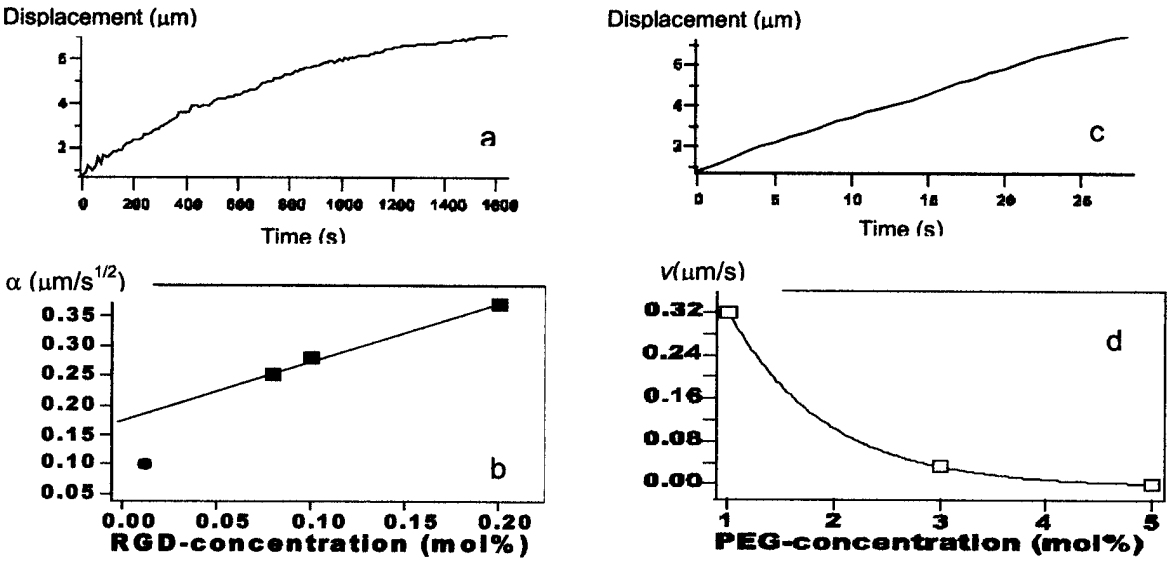

Fig. 2. Displacement of the adhesion rim versus time exhibits a square-root dependence at small concentrations of ligands (a). The coefficient α exhibits the linear dependence on the concentration of ligands (b) according to the prediction of our theory (solid line). Blue squares show our data and the red one is taken from the paper (Park, at. al. 1990) where kinetics of adhesion of platelets on fibrinogen has been measured. At high concentration of ligands the rim displacement linearly depends on time (c) and its velocity exponentially decreases with the concentration of repeller molecules (d) according to the theoretical prediction (solid line)

mediated by the competition of the gravitation and undulation repulsion (see (Boulbitch and others 2001)) This is schematically illustrated in Fig. 1 a while Fig. 1 d shows the reflection interference contrast microscopy (RICM) image in this state. After a while a nucleation of a small area of the tight adhesion (mediated by the ligand-receptor interaction) was observed (Fig. 1 b and e). This tight adhesion area then grew until it occupied the whole interface of the vesicle adhesion (Fig.1 c and f). At small ligand concentrations we observed the displacement, x, of the rim of the domain of tight adhesion to follow the square root law $x \sim t^{1/2}$, while at high concentrations we found a linear law $x \sim t$.

In order to analyze these measurements I assumed that the adhesion rim is narrow and deduced a condition which must be fulfilled at the rim:

$$\frac{b}{C_{LR}}\frac{\partial C_{LR}}{\partial t} = \frac{\partial x}{\partial t} \qquad (2)$$

where b is the width of the reaction zone, x is the displacement of the rim, t is the time. For the regime of the low ligand concentration this equation yields the boundary condition on the moving boundary for the diffusion equation describing the

distribution of ligands. This enabled us to reduce the problem to the so-called Stefan's problem whose solution for the rim displacement yields $x = \alpha\sqrt{t}$ which agrees with the observations (as shown in Fig. 2 a).

By calculating α I predicted its linear dependence on the concentration of ligands which fits to experimental results (Fig. 2 b). We further gave qualitative arguments showing that in the case of a high concentration of ligands the rim displacement depends linearly on time, $x = vt$. We demonstrated that for the velocity v of the adhesion rim decreases exponentially with increasing of the repeller concentration. This result perfectly describes observations in the regime of high ligand concentration (Fig. 2 c and d).

4. Enforced unbinding of a membrane whose adhesion to the substrate is mediated by specific interactions

Recently, the vesicles with reconstituted lipo-ligands (recognized by the integrin receptors of the endothelial cell) were used to study the enforced unbinding of vesicles from living endothelial cells (Prechtl and others 2002). A vesicle was sucked into a micropipette and brought in contact with an endothelial cell until their mutual adhesion takes place. After that, the unbinding experiments were performed at constant force rates yielding the dependence of the unbinding force F_* as the function of the loading rate $\dot{F}$ in the form $F_* \sim \dot{F}^{\beta}$ with $\beta \approx 0.4$.

A theoretical description of the dynamics of unbinding of a membrane from the substrate under application of external force was proposed in my paper (Boulbitch 2002). This work is based on two ideas. The first of them is that the rim motion must be determined by the dissociation constant K_d of the chemical reaction Eq. (1). This enabled me to deduce equation of motion of the adhesion rim:

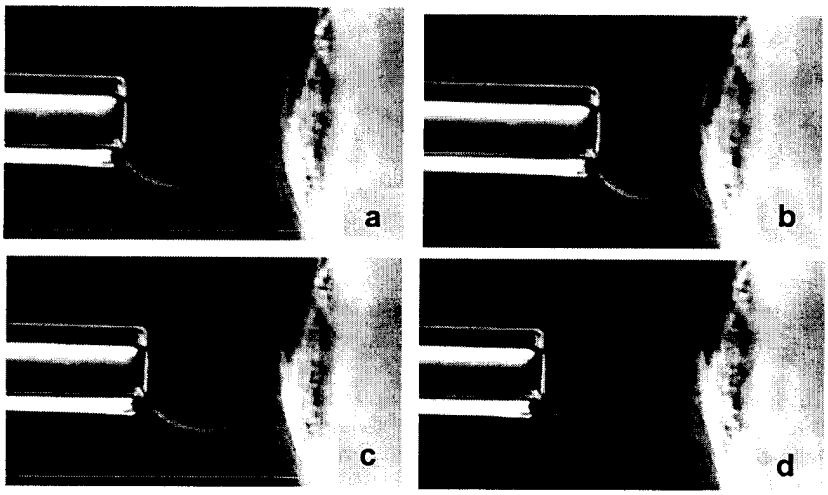

Fig. 3. Unbinding of a vesicle from the endothelial cell. The vesicle with the reconstituted ligands and repellers is sucked into the micropipette. The ligands of the vesicle are recognized by the receptors of the endothelial cell. (a) The vesicle approaches to the surface of the endothelial cell. (b) The vesicle is brought in contact with the endothelial cell which results in its specific adhesion. (c) The stage of retraction. One can see the vesicle deformation. (d) The image is taken during the breaking of the bond between the vesicle and the cell. One can simultaneously see two shapes of the vesicle: right before and right after the unbinding. (With kind permission of Prof. R. Merkel)

$$\frac{\partial x}{\partial t} = bk_{-}\left(1 - \frac{K_d}{K_d^{(0)}}\right) \tag{3}$$

Here $K_d^{(0)}$ is the dissociation constant of ligand-receptor pairs in the rest state, while K_d is the dissociation constant in a situation when the membrane is subjected to a mechanical load.

The second idea of this work is that if the vesicle is subjected to a mechanical load, mechanical energy of the vesicle deformation is stored in the softest part of the system, which is the curved piece of the membrane at the rim. When the adhesion rim propagates this energy is released and breaks the ligand-receptor bonds. This leads to a modification of the dissociation constant of the binding-unbinding reaction between ligands and receptors, if they are reconstituted into a membrane. The dissociation constant depends in this case on the mechanical torque M at the rim per unit length:

$$\frac{K_d}{K_d^{(0)}} = \exp\left(\frac{M^2 a}{2\kappa k_B T}\right) \tag{4}$$

Here κ is the bending elasticity of the membrane, T is the temperature, k_B is the Boltzman's constant and a is the area per receptor molecule.

With the dissociation constant determined in such a way the equation of motion of the adhesion rim (3) can be solved exactly. This solution predicts that the rim motion has an initial slow phase followed by a phase of a rapid motion. This ends up with the breakage of adhesion. The solution predicts a power law $F_* \sim \dot{F}^{\beta}$ for the dependence of the unbinding force on the force rate with $\beta = 1/2$ for small and $\beta = 1/3$ for large applied forces.

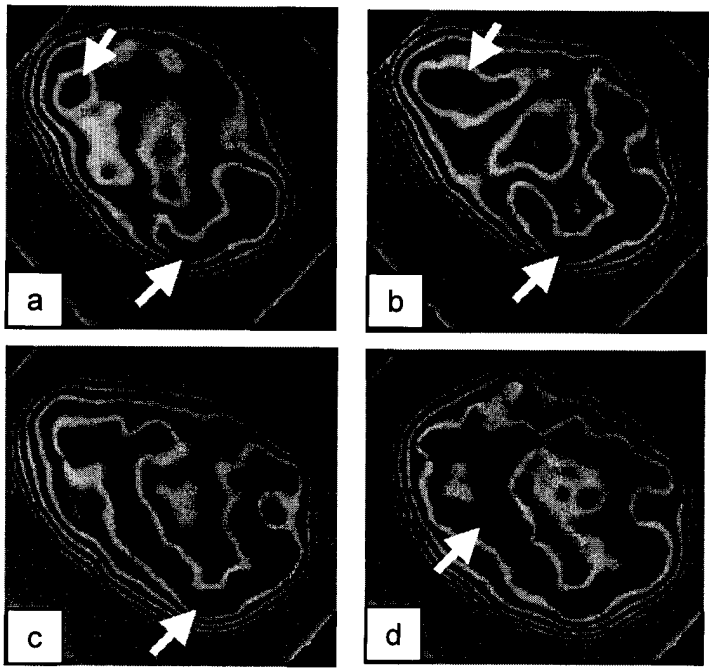

Fig. 4. Sequence of successive images showing the evolution of the adhesion area of a vesicle on the substrate. Adhesion is mediated by the ligand-receptor pair represented by Sialyl-LewisX-selectin at ultra-low concentrations of ligands. The images resemble a random walk process. Arrows show the tightly adhered area.

Eq. (2)-(4) are the important new findings enabling one to advance the understanding of the adhesion kinetics

Recent experiments: motivation for a theoretical studies

1. **Adhesion at low ligand concentration**

Recently a novel model system was established and studied (Lorz and Sackmann 2003) which consists of selectin

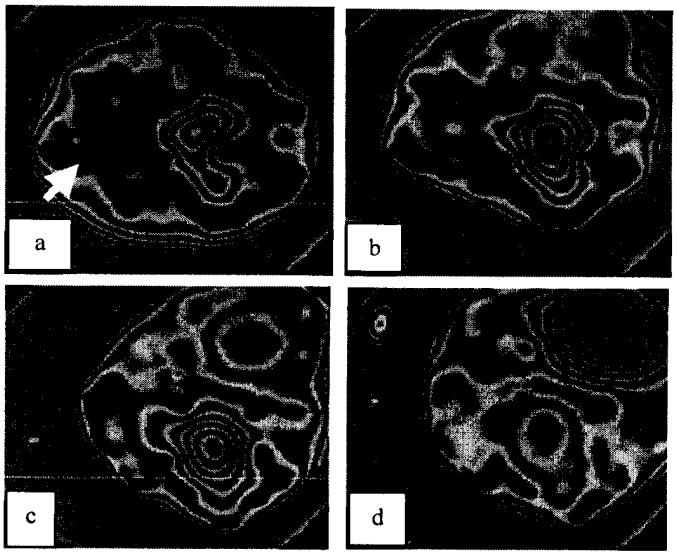

Fig. 5. Sequence of successive images showing the unbinding of the membrane whose adhesion is due to the Sialyl-LewisX-selectin pair. (a) Shows the adhesion area of the membrane. In the black regions (arrow) it is bound to the substrate. The image is taken in the moment when the selectin antibodies are added to the system. (b-d) Show the evolution of the adhesion area during the antibodies-mediated unbinding.

receptors immobilized on solid substrate and giant vesicle with reconstituted Sialyl-LewisX oligosaccharides recognizing by selectin receptors and playing a central role in immunological response. In addition the polyethyleneglycol repellers are reconstituted into the membrane. A major difference with respect to the model system with integrin is that the Flory radius

of the mushroom formed by the grafted polyethyleneglycol molecule is smaller than the length of the ligand. To prevent an immediate binding a very low ligand concentration has been chosen. Evolution of the adhesion area in this case clearly resembles a random walk process (Fig. 4) which qualitatively correspond to the prediction of Weikl an co-authors (Weikl and others 2002b) concerning a multiple nucleation. However, in addition to the multiple nucleation of the adhered states on the background of the unbound state observations show also nucleation of an unbound state on the background of the adhered state, the whole process is highly dynamic. This phenomenon is not understood so far.

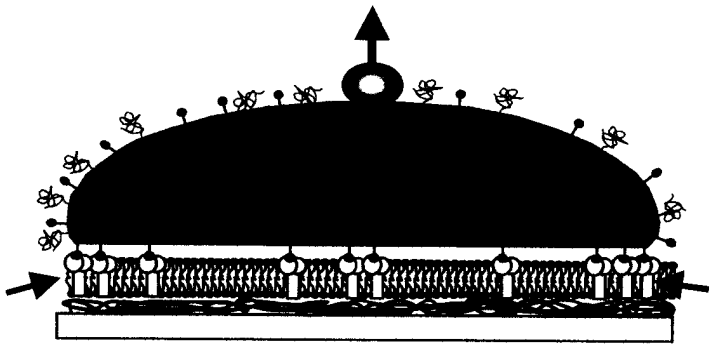

Fig. 6. Schematic view of the vesicle with reconstituted ligands and repellers over the supported lipid bilayer membrane separated from solid by a polymeric cushion. The latter carries mobile receptors (the transmembrane proteins integrins). Clustering of the receptors (red arrows) results in the membrane pinning. Enforced unbinding of the membrane is achieved by application of a force with the help of the magnetic bead (the top of the figure). The unbinding process takes place by two mutually dependent processes: (i) lateral motion of the ligand-receptor pairs and (ii) unbinding of ligands.

2. Unbinding of the membrane by a competitive inhibition

In the same set of experiments (Lorz and Sackmann 2003) the membrane unbinding mediated by competitive inhibition of receptor-ligand binding by antibodies to selectin receptors has been observed (Fig. 5). The antibodies for selectin have the higher affinity to the selectin receptors than the Sialyl-LewisX ligands. Therefore, they replace the receptors which results in unbinding of the membrane. This process is not understood so far. This problem I address theoretically in the present proposal.

Recent progress in synthesis of artificial ligands such as arginine-glycine-asparate acid recognized by natural receptors of the integrin family is expected to develop a new strategy of treatment of several diseases (among them the osteoporosis, vascular restenosis, cancer (Carron and others 1998; Lin and others 1998) and autoimmune diseases (Falcioni and others 1999)). This strategy is based on the competitive inhibition of the adhesion involved in the disease development.

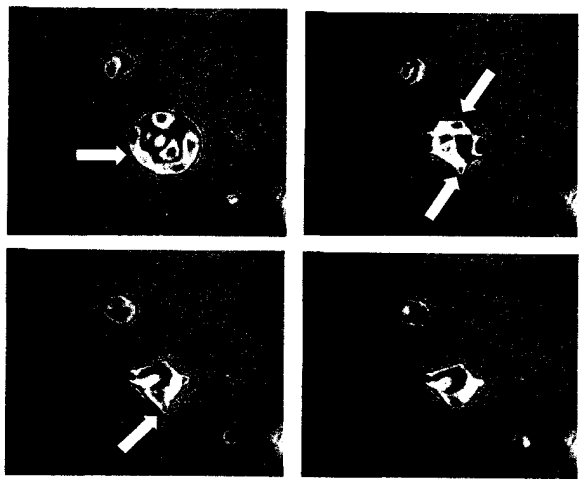

Fig. 7. Experimental image showing successive stages of the unbinding of the vesicle schematically shown in Fig. 6. (a) The adhesion area of the vesicle before the unbinding started. The vesicle under study is shown by the arrow. (b) Application of the force (see Fig. 6) gives rise to unbinding. The pinning centers show up. Two pinning centers are indicated by the arrows. (c) Further unbinding results in the sharpening of the corner made by the adhesion rim at the pinning center (arrow). (d) The adhesion area after unbinding of the pinning center

4. Effect of membrane pinning on the unbinding

In another series of experiments (Gönnewein and Sackmann 2003) a supported membrane is separated from the solid support by a polymer cushion composed by cellulose. The receptors are reconstituted into a supported lipid bilayer (Fig. 6). Under this condition the receptors are mobile and therefore, may form clusters. During the unbinding experiments such type of clustering followed by the membrane pinning has been observed (Fig. 7). Observation show that the pinning centers are the main sites of membrane attachment to the substrate and that unbinding of the pinning center was followed by a rapid decreasing of the adhesion area. The latter lasts until new pinning centers show up and arrest the motion of the adhesion rim. Adhesion energy measured in these experiments $\varepsilon_{adh}\sim 10^{-4}$J/m^2 is much higher than that $\sim 10^{-6}$ to 10^{-5}J/m^2 in the case of physisorbed integrins measured in previous experiments.

A theoretical interpretation of this phenomenon as well as of the kinetics of the unbinding of the pinning center is still missing. I address this problem in the proposal.

What is challenging?

The experimental data reviewed above show the possibility to manipulate adhesion in several ways. Adhesion can be controlled by

- variation of the repeller concentration
- variation of antibodies in the bulk solution
- influencing the receptor aggregation and
- mechanical loading of the membrane

Manipulating the adhesion state requires theoretical predictions of controlled adhesion kinetics.

No such theory exists by now, and I will develop it in the present project.

Goals and Working Plan

Goals

The major goal of the present project will be to describe the control of the dynamics of adhesion and unbinding of biomembranes by the formation of ligand-receptor bonds. In particular I will put major emphasis on

- the unbinding dynamics of a membrane whose adhesion is mediated by mobile ligand-receptor pairs and its control by mechanical loading,
- the dynamics of the membrane unbinding due to interaction with molecules (such as antibodies) which competitively bind to receptors and control of this process by the concentration of these molecules and membrane mechanical state.
- the effect of a lateral phase separation of receptors on their affinity to ligands. In this section we will study dynamics of adhesion induced by lateral phase separation of receptors.

The following questions will be addressed:

- **What is the relation between the force applied to membrane and the unbinding rate if the unbinding of ligand-receptor pairs is accompanied by their lateral movement?**
- **How does the dissociation constant of ligand-receptor pairs depend on the lateral phase separation of receptors mediated by their lateral attraction? How is adhesion dynamics in this case controlled by the lateral phase separation?**
- **How is adhesion and unbinding controlled by the presence of molecules inhibiting the ligand-receptor binding?**
- **How is dynamics of adhesion affected by competition of two types of ligand-receptor pairs?**
- **What is the mechanism of membrane unbinding at a pinning centers?**

Working Plan

1. The enforced unbinding of a biomembrane adhering through mobile ligand-receptor pairs: interplay of lateral diffusion of ligands/receptors and unbinding reaction

The displacement x of the rim can be represented as $x = x_d + x_r$. The first term, x_d, is the displacement due to the inward diffusion of the ligand-receptor pairs, while the second, x_r, is due to their unbinding. In a recent paper by (Brochard-Wyart and De Gennes 2002) the case of the membrane dynamics giving rise to inward diffusion of the ligand-receptor pairs with no unbinding ($x = x_d$) has been considered. This case does not cover the unbinding process, since it does not account for the dissociation of ligand-receptor pairs.

I will study the interplay between dissociation of ligand-receptor pairs and their unbinding-induced diffusion into the adhesion area. As it has been shown in (Brochard-Wyart and De Gennes 2002), such a motion of the ligand-receptor pairs gives rise to a force impeding the rim motion (referred to as osmotic force). I will concentrate on the most important case in which the adhesion process is determined by a joint motion of the rim and a cloud of the ligand-receptor pairs accompanied by unbinding. I will calculate the dissociation constant making use of Eq. (4). In this case however, it depends self-consistently upon the lag force predicted by Brochard-Wyart and De Gennes. Establishing a self-consistent equation of motion will allow me to describe the joint motion of adhesion rim and a cloud of the ligand-receptor pairs. Possible instabilities of the joint motion should be analyzed.

The aim is to predict the dependence of the unbinding rate in such a system on both the applied load and the mobility of ligand-receptor pairs

2. Control of the adhesion state by the amount of repeller molecules

I will study a system consisting of mobile ligands and repellers reconstituted into a biomembrane adhered on a substrate covered with receptors. I will first examine the effect of the repellers on the equilibrium state of this system and calculate the phase diagram of the unbinding transition. I expect to describe the phase diagram reported in (Guttenberg and others 2001). I will further deduce the kinetic equation (analogous to Eq. 1 and 2) which, however, takes into account the effect of repeller molecules. I will finally study possible regimes of the adhesion dynamics depending on the relative amounts of ligands, receptors and repellers.

The aim of this part is to understand the experimentally measured adhesion phase diagram and to be able to predict possible adhesion states depending of types of molecules reconstituted into the membrane

3. The lateral phase separation of receptors during adhesion

I intend to show that lateral phase separation of receptors (due to their lateral attraction) result in a decreasing of the dissociation constant of the ligand-receptor pairs. Since the kinetics of the rim crucially depends on the dissociation constant (as it follows from Eq. (3) and (4)) this makes a strong effect on the adhesion kinetics. The influence of the lateral phase separation on the adhesion kinetics (and vice versa) will be studied in this part of the project.

Two particularly interesting situations to be studied are the following.

(i) If the affinity of receptors and ligands in the low-concentration phase is too small to mediate any adhesion, and if the adhesion occurs only in the high-concentration phase, one can expect that the process will be dominated by the phase separation. I will study the effect of the kinetics of the phase separation on the kinetics of adhesion.

(ii) The lateral attraction between the receptors may arise due to their binding to ligands, while the unbound receptors do not attract one another. I will study how in this case adhesion triggers either the phase separation process of receptors or their aggregation in clusters.

Within this projects equations of the adhesion kinetics (3), (4) will be solved together with kinetic equations describing the phase separation.

The aim of this subproject is to understand the phase separation-assisted adhesion and to explore mechanisms of its control

4. Adhesion and unbinding of a membrane due to the competition of chemical reactions.

4.1. Unbinding by a competitive inhibition.

A very important biological situation is the impeding of adhesion by antibodies to receptors. Experiments with the model system (Lorz and Sackmann 2003) show that the adhesion process is very sensitively affected by antibodies in the bulk phase. Addition of small quantities of antibodies can lead to unbinding. This is an important result, since it yields a tool to control the adhesion state by variation of the antibodies concentration. Within this part of the project a mathematical model will be developed which will explain the observed weakening of adhesion depending on the concentration of antibodies in the bulk.

The aim is to interpret recent experiments on impediment of adhesion by the antibodies.

4.2. Adhesion in the presence of two competing ligand-receptor pairs.

A usual situation in bioadhesion is that two types of ligand-receptor pairs compete. I therefore, study a membrane-substrate system carrying two types of ligand-receptor pairs possessing different values (i) of the lateral diffusion coefficient, (ii) of the dissociation constants, (iii) of the reaction rates and (iv) of the ligand-receptor distances. I expect that such a system will exhibit different regimes of the adhesion kinetics.

The aim is to predict the possible kinetic regimes of adhesion mediated by two types of ligand-receptor couples.

5. Effect of pinning center on resistance to unbinding

Formation of clusters consisting of a group of receptors is usually followed by local membrane pinning. Experiments are designed in the applicants laboratory which allow to study formation and unbinding of a pinning center and to measure line forces at the adhesion rim which balance the pinning center (see Section 2.1.2.7) (Gönnewein and Sackmann 2003). I intend to describe kinetics of unbinding of a single pinning center and of a pinned membrane and its control by a configuration of pinning centers and by the number of ligand-receptor pairs constituting the pinning center.

The aim is to understand the process of unbinding of a pinned membrane.

Outlook

One way to expand the ideas of the present proposal is to study dynamics of adhesion of a cell membrane supported by the actin cortex.

Another way is related to the point of view in which bioadhesion can be considered as an example of plasticity in biology in 2 dimensions. Biological plasticity in 3 dimensions is represented by the mechanical behavior of a biological gel containing specific cross-linkers. (A biologically relevant example is the cytoskeleton - the major stress-bearing component of animal cells). In this case the degree of cross-linking which determines the character of its vicoelasticity must nonlinearly depend on the stressed state of the gel. A theory of behavior of such a gel subjected to a mechanical stress is a challenging direction of a further work.

Further important way is to study the binding-unbinding process accompanied by structural changes leading to mechanical motion **on the level of a single molecule**. This group of problems include also such enzymes which possess domains exhibiting mechanical motion associated with the enzymatic reaction. Molecular motors (such as those belonging to the myosin family and many others) are examples of such enzymes. The challenging problem in this case is to study the influence of mechanical loads on biochemical reactions.

References

1. Recent theoretical publications of the applicant related to the project

Arnoldi, M., M. Fritz, E. Bäuerlein, M. Radmacher, E. Sackmann, and A. Boulbitch. 2000. Bacterial turgor pressure can be measured by atomic force microscopy. *Phys. Rev. E.* 62:1034-1044

Bausch, A. R., F. Ziemann, A. A. Boulbitch, K. Jacobson, and E. Sackmann. 1998. Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophys. J.* 75:2038-49.

Boulbitch, A. A. 1998. Deflection of a cell membrane under application of a local force. *Phys. Rev. E.* 57:2123-8.

Boulbitch, A. A. 1999a. Equations of heterophase equilibrium of a biomembrane. *Arch. Appl. Mech.* 69:83-93.

Boulbitch, A. A. 1999b. Strain of a biomembrane caused by a local tangential force: Application to magnetic tweezers measurements. *Phys. Rev. E.* 59:3402-7

Boulbitch, A. A. 2000. Deformation of the envelope of a spherical Gram-negative bacterium during the atomic force microscopic measurements. *J. Electron. Microscopy.* 49:459-462.

Boulbitch, A., B. Quinn, and D. Pink. 2000a. Elasticity of Rod-Shaped Gram-Negative Eubacteria. *Phys. Rev. Lett.* 85: 5246-9

Boulbitch, A., R. Simson, D. Simson, R. Merkel, W. Häckl, M. Bärmann, and E. Sackmann. 2000b. Shape Instability of a Biomembrane Driven by a Local Softening of the Underlying Actin Cortex. *Phys. Rev. E* 62: 3974-85

Guttenberg, Z., B. Lorz, E. Sackmann, and A. Boulbitch. 2001. First order transition between adhesion states in a system mimicking cell-tissue interaction. *Europhys. Lett.* 54:826-832.

Boulbitch, A., Z. Guttenberg, and E. Sackmann. 2001. Kinetics of membrane adhesion mediated by ligand-receptor interaction studied with a biomimetic system. *Biophysical Journal.* 81:2743-51.

Boulbitch, A. 2002a. Enforced unbinding of a bead adhered to a biomembrane by generic forces. *Europhys. Lett.* 59:910-915.

Boulbitch, A. 2003. Enforced unbinding of biomembranes whose mutual adhesion is mediated by a specific interaction. *Eur. Biophys. Lett.* 31: 637-642

2. References cited in the proposal

Albersdörfer A, Bruinsma R, Sackmann E. 1998. Force spectroscopy on adhesive vesicles. Europhys. Lett. 42(2):227-231.

Albersdorfer A, Feder T, Sackmann E. 1997. Adhesion-induced domain formation by interplay of long-range repulsion and short-range attraction force: A model membrane study. 73(1):245-257.

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. 1994. Molecular Biology of the Cell. New York, London: Garland Publishing Inc.

Bell GI. 1978. Models for the specific adhesion of cells to cells. Science 200:618-627.

Bell GI, Dembo M, Bongrand P. 1984. Cell adhesion. Competition between nonspecific repulsion and specific bonding. Biophys. J. 45(6):1051-64.

Benoit M, Gabriel D, Gerisch G, Gaub HE. 2000. Discrete interactions in cell adhesion measured by single-molecule force spectroscopy. Nat Cell Biol 2(6):313-7.

Boulbitch A. 2002. Enforced unbinding of a bead adhered to a biomembrane by generic forces. Europhys. Lett. 59(6):910-915.

Boulbitch A, Guttenberg Z, Sackmann E. 2001. Kinetics of membrane adhesion mediated by ligand-receptor interaction studied with a biomimetic system. Biophysical Journal 81(5):2743-51.

Brochard-Wyart F, De Gennes PG. 2002. Adhesion induced by mobile binders: Dynamics. Proc Natl Acad Sci U S A 99(12):7854-9.

Bruinsma R. 1996. Adhesion and rolling of leukocytes: a physical model. In: D.Sherrington ETRa, editor. NATO ASI Series. Proc. Of NATO Advanced Institute on Physics of Biomaterials, Geilo, 1995. Kluwer, Dordrecht. p 61.

Bruinsma R, Behrisch A, Sackmann E. 1999. Adhesive switching of membranes: experiment and theory. Phys. Rev. E 61(4):4253-4267.

Carron CP, Meyer DM, Pegg JA, Engleman VW, Nickols MA, Settle SL, Westlin WF, Ruminski PG, Nickols GA. 1998. A peptidomimetic antagonist of the integrin alpha(v)beta3 inhibits Leydig cell tumor growth and the development of hypercalcemia of malignancy. Cancer Res 58(9):1930-5.

Dembo M, Torney DC, Saxman K, Hammer D. 1988. The reaction-limited kinetics of membrane-to-surface adhesion and detachment. Proc R Soc Lond B Biol Sci 234(1274):55-83.

Dustin ML, Ferguson LM, Chan PY, Springer TA, Golan DE. 1996. Visualization of CD2 interaction with LFA-3 and determination of the two-dimensional dissociation constant for adhesion receptors in a contact area. J. Cell. Biol. 132(3):465-477.

Falcioni F, Ito K, Vidovic D, Belunis C, Campbell R, Berthel SJ, Bolin DR, Gillespie PB, Huby N, Olson GL and others. 1999. Peptidomimetic compounds that inhibit antigen presentation by autoimmune disease-associated class II major histocompatibility molecules. Nat Biotechnol 17(6):562-7.

Gönnewein S, Sackmann E. 2003. Enforced unbinding of a specifically adhered membrane with pinning centers using the magnetic tweezers setup - private communication.

Guttenberg Z. 2000. Die Untersuchungen der RGD-Integrin Bindung als Modellsystem fur die Zelladhesion [Doctorarbeit]. München: Technische Universität München.

Guttenberg Z, Lorz B, Sackmann E, Boulbitch A. 2001. First order transition between adhesion states in a system mimicking cell-tissue interaction. Europhys. Lett. 54(6):826-832.

Kloboucek A, Behrisch A, Faix J, Sackmann E. 1999. Adhesion-induced receptor segregation and adhesion plaque formation: A model membrane study. Biophys J 77(4):2311-28.

Komura S, Andelman D. 2000. Adhesion-induced lateral phase separation in membranes. Eur. Phys. J. E 3:259-271.

Komura S, Andelman D. 2002. The unbinding transition of mixed fluid membranes. arXiv:cond-mat/0205144v2; Europhys. Lett. - submitted.

Lin EC, Carron CP, Meyer DM, Smith JW. 1998. A series of function blocking antibodies against the alpha v beta 3 integrin bind allosteric to the ligand binding site and induce ligand dissociation. Cell Adhes Commun 6(6):451-64.

Lipowsky R. 1996. Adhesion of membranes via anchored stickers. Phys. Rev. Lett. 77(8):1652-1655.

Lipowsky R, Seifert U. 1991. Adhesion of vesicles and membranes. Mol.Cryst.Liq.Cryst. 202:17-25.

Lorz B, Sackmann E. 2003. Unbinding of a specifically adhered membrane by adding of antibodies for receptors to the bulk solution - private communication.

Marx S, Schilling J, Sackmann E, Bruinsma R. 2002. Helfrich repulsion and dynamical phase separation of multicomponent lipid bilayers. Phys. Rev. Lett. 88(13):138102-1-138102-4.

Nardi J, Bruinsma R, Sackmann E. 1998. Adhesion-induced reorganization of charged fluid membranes. Physical Review E 58(5):6340-54.

Nardi J, Feder T, Bruinsma R, Sackmann E. 1997. Electrostatic adhesion between fluid membranes: phase separation and blistering. Europhys.Lett. 37(5):371-376.

Prechtl K, Bausch AR, Marchi-Artzner V, Kantlehner M, Kessler H, Merkel R. 2002. Dynamic force spectroscopy to probe adhesion strength of living cells. Phys. Rev. Lett. 89(2):art. no. 028101.

Seifert U. 1991. Adhesion of vesicles in two dimensions. Phys. Rev. A 43(12):6803-6814.

Seifert U. 1997. Configurations of fluid membranes and vesicles. Advances in Physics 46(1):13-17.

Seifert U. 2000. Rupture of multiple parallel molecular bonds under dynamic loading. Phys. Rev. Lett. 84(12):2750-2753.

Seifert U, Lipowsky R. 1990. Adhesion of vesicles. Phys. Rev. A 42(8):4768-4771.

Vetvicka V, Fornusek L. 1987. Polymer microbeads in immunology. Biomaterials 8:341-345.

Weikl TR, Andelman D, Komura S, Lipowsky R. 2002a. Adhesion of membranes with competing specific and generic interactions. Eur. Phys. J. E 8(1):59-66.

Weikl TR, Groves JT, Lipowsky R. 2002b. Pattern formation during adhesion of multicomponent membranes. Europhys. Lett. 59(6):916-922.

II. Mathematical modeling of microrheological experiments in a complex fluid with self-diffusion

Abstract

Within this project I will study the enforced motion of a bead through a complex fluid possessing a component moving by the way of a self-diffusion. I intend to introduce a new type of force resisting the motion of the bead and giving rise to an unusual mechanical response. In collaboration with D. Pink (Canada) we will perform a molecular dynamics simulation of the bead motion in the entangled actin gel. New regimes of the bead motion recently discovered in micromechanical experiments will be studied. We will further study the effect of the gel density, pH and concentration of divalent cataions on the micromechanical properties. Both by application an analytical theory of motion in a random potential and by the molecular dynamics simulations we will analyze the enforced motion of a single actin filament through a (spatially random) actin gel. We will finally simulate equation of state of the actin gel.

Introduction

1. Microrheology of complex fluids

Microbeads are widely used in biology: in micro agglutination techniques, in adjuvant immunization, for drug delivery, in surface markers detection technique, in solid- and liquid-phase separation of cells (Vetvicka and Fornusek 1987) in the magnetic separation technique (Olsvik and others 1994) and in the studies of phagocytosis (Vetvicka and Fornusek 1987; Wetzel and others 1969).

In addition one can use microbeads as a tool to probe locally micromechanical properties of biological materials (Amblard and others 1996; Bausch and others 1999; Bausch and others 1998; Glogauer and others 1997; Heilbronn 1922; Lee and others 1992; Sato and others 1984; Schmidt and others 1996a; Valberg and Butler 1987; Valberg and Feldman 1987; Wang and others 1993; Zaner and Valberg 1989; Ziemann and others 1994a).

Passive and active approaches are formally distinguished in microrheological measurements. During passive measurements thermal fluctuations of beads are observed (MacKintosh and Schmidt 1999a; Mukhopadhyay and Granick 2001; Solomon and Lu 2001), while during active microrheological experiments a force is applied to the bead by a laser beam (optical tweezers) (Ashkin 1991; Kuo 2001) or by magnetic field (magnetic tweezers) (Glogauer and Ferrier 1998), (MacKintosh and Schmidt 1999a) and the displacement of the bead is measured (Amblard and others 1996; Bausch and others 1999; Bausch and others 1998; Schmidt and others 1996a; Ziemann and others 1994a). This method enables one to establish local viscoelastic properties of biological materials.

Interpretation of the microrheological experiments requires a model of the system under study and such models have been proposed for the purposes of interpretation of micromechanical measurements. (Duszuk and others 1989; Fung 1988; MacKintosh and Schmidt 1999a; Solomon and Lu 2001; Theret and others 1988; Valberg and Butler 1987), (Arnoldi and others 2000; Boulbitch 1998; Boulbitch 1999; Boulbitch 2000; Boulbitch 2002; Boulbitch 2003; Merkel and others 2000). It is generally accepted that the microrheological measurements can be completely understood on the basis of the assumption that the medium exhibits a purely viscoelastic response (MacKintosh and Schmidt 1999a; Mukhopadhyay and Granick 2001; Solomon and Lu 2001). In this proposal I will show that in interpretation of the microrheological measurements one needs to account for an osmotic resistive force in addition to the viscoelastic effects.

2. Entangled actin networks.

In the second part of the present project I will apply properties of the osmotic resistance outlined in the first part of the present project in order to describe microrheological measurements in actin networks.

The filamentous protein, F-actin, is a component of the cytoskeleton which makes it possible for cells to bear and respond mechanical loads. The cytoskeleton represents a gel composed of several types of filamentous proteins. The major protein of the cytoskeleton is F-actin. This determines its extraordinary role in the cell mechanics and the interest to its mechanical properties (Mackintosh and Janmey 1997; MacKintosh and Schmidt 1999b).

Semi-dilute solutions of F-actin exhibit viscoelasticity even without cross-linking (Janmey and others 2001). Mechanical behavior of actin filaments in solution was studied experimentally by several techniques, such as dynamic light scattering (Götter and others 1996), enforced oscillations (Riveline and others 1998), macrorheology (Hinner and others 1998) and magnetic tweezers (Dichtl and Sackmann 1999; Dichtl and Sackmann 2002; Schmidt 1999; Schmidt and others 2000; Schmidt and others 1996b; Ziemann and others 1994b). The main challenge of these experiments is to study viscoelastic properties of actin gels determined by its frequency-dependent shear modulus $G=G(\omega)$. Several theoretical models have been proposed to describe the viscoelastic behavior of the semi-dilute actin solutions (see recent reviews (MacKintosh and Schmidt 1999a; Solomon and Lu 2001). The high-frequency dependence of the shear modulus $G(\omega)\sim\omega^{3/4}$ predicted in (Gittes and MacKintosh 1998) accurately describes the actually observed behavior (Gittes and others 1997). The well-known reptation tube approximation (Morse 1998a; Morse 1998b; Morse 1998c; Morse 1998d; Morse 1999) was used to account for its low-frequency viscoelastic behavior. However, recent experiments with a newly established colloidal probe technique (Dichtl and Sackmann 1999) and the macro-rheometry within the frequency range 0.1 to 10Hz (Schmidt and others 2000) revealed that the measured viscosity differs by one to two orders of magnitude from that predicted by the reptation tube model. In this project I will address this problem. I intend to show that the discrepancy is due to the spatially inhomogeneous structure of the reptation tube.

Up to now it was generally accepted that the properties of the actin gel can be described in terms of viscous and elastic properties of its components on either microscopic level (Morse 1998a; Morse 1998b; Morse 1998c; Morse 1998d; Morse 1999) or phenomenologically (Levine and Lubensky 2000; Levine and Lubensky 2001). Recent experimental observations

(see the discussion below) show that the viscoelasticity alone is not enough to describe the microrheology in the actin gel within the interval 1 to 50s. Description of this regime of the bead motion is addressed in the present proposal.

It is important to note that cytoplasm of cells and especially of bacteria is filled with a dense solution of proteins. In addition, animal cells possess a cytoskeleton that penetrates through the whole cytoplasm. In this situation the hydrodynamic modes of the cytoplasmic motion may be hindered and (passive) motion of proteins of the cytoplasm may take place by the way of self-diffusion.

In this project I will study dense solutions of proteins in which at least one component exhibits a self-diffusional mode of motion. I will study forces arising in microrheological experiments in such systems. I will apply the results for explanation of experiments with actin gel.

Experimental background

1. Enforced motion of a bead through the actin gel.

It is important to understand the microscopic viscoelastic properties of actin gel, i.e. its response to a mechanical

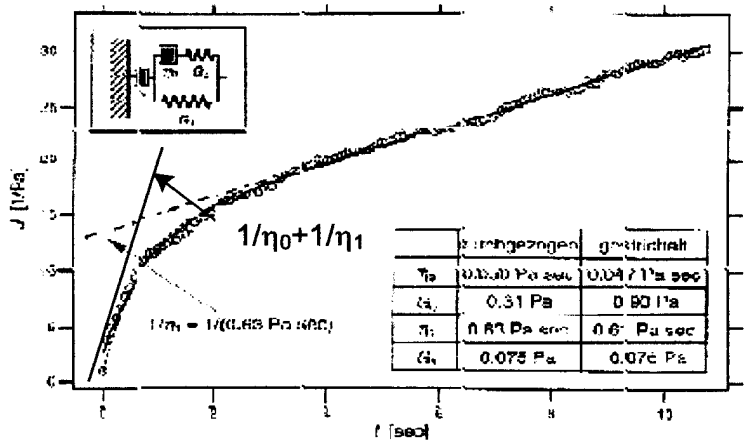

Fig. 8. Experimental data showing the typical response of the bead embedded into actin gel to which a rectangular force pulse has been applied. The response function J is the bead displacement normalized by the force: J(t)=x(t)/F. The data is interpreted on the basis of a viscoelastic model represented by the equivalent mechanical circuit consisting of dash-pots and springs shown in the insert.

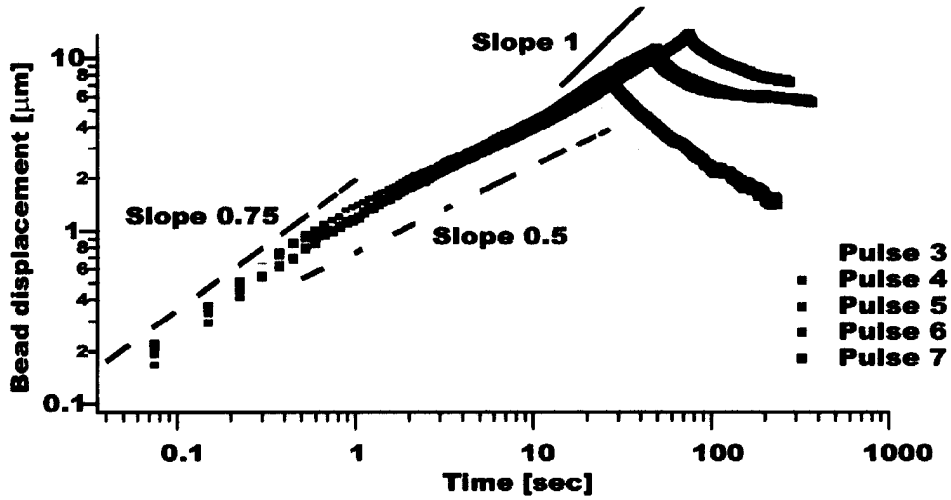

Fig. 9. The double logarithmic plot showing the displacement of the bead in the actin gel versus time in response to rectangular force pulses. The duration of the force pulses is up to 100s. Different colors correspond to different force pulses. One can distinguish three regimes: (i) the initial regime (t<1s) with $x \sim t^{0.75}$ (the slope is shown by the dashed, black line, (ii) the square-root regime at 1s<t<50÷80s with $x \sim t^{0.5}$ (the slope is shown by the dotted and dashed, black line) and (iii) the crossover to the viscous regime t>80s with x~t (the slope is shown by solid, black line). With kind permission of W. Fenneberg, J. Uhde and E. Sackmann

load localized within few micrometers, since this is the scale on which the mechanical loads are usually applied to cells.

This problem has been studied with the help of the so-called "magnetic tweezers" set up (Dichtl and Sackmann 1999; Dichtl and Sackmann 2002; Hinner and others 1998; Schmidt 1999; Schmidt and others 1996b; Ziemann and others 1994b). Within this method a paramagnetic bead is embedded into the gel and subjected to an inhomogeneous magnetic field **B** giving rise to a force $\mathbf{F}\sim(\mathbf{B}\cdot\nabla)\mathbf{B}$ acting on the bead. The force **F**, the displacement of the bead $x=x(t)$ and the strain field of the gel can be measured. The time resolution of the set up is 50ms. The set up is described in details in the paper (Ziemann and others 1994b).

A typical experimental data obtained during the creep experiments (Schmidt 1999) is shown in Fig. 8 and exhibits the displacement of the bead in response to a rectangular force pulse. These data have been interpreted on the basis of a viscoelastic model of entangled actin solution. This can be done in terms of a so-called equivalent mechanical circuit consisting of interconnected dash-pots and springs (shown in the insert in Fig.8).

A characteristic feature of the equivalent circuit shown in Fig. 8 is that it assumes a final tilt of the response curve at $t=0$. This means that in the beginning of the force pulse the bead displacement linearly depends on time: $x \sim \left(\eta_0^{-1} + \eta_1^{-1}\right)t$, where η_0 and η_1 are the viscosities of the dash-pots shown in Fig.8. These viscosities are phenomenological parameters. In order to understand their origin one needs to establish a model describing this the microrheology in actin gel and predicting such a behavior. So far such a model does not exist.

Recently the experimental set up has been modified which enabled one to improve the time resolution up to 6ms. This revealed an unexpected behavior of the response curve in the very beginning of the force pulse (Fig. 9) (W. Fenneberg, J. Uhde and E. Sackmann – unpublished). The data plotted in a double logarithmic scale over four decades exhibit three regimes with the power law behavior:

$$x(t) \sim t^{a} \tag{5}$$

(i) During the first regime ($t<1$s) the data can be fitted by $a\approx 0.75$ (the slope is shown by the black, dashed line in Fig. 9). This is in accord with the high frequency dependence of the shear modulus $G(\omega)\sim\omega^{3/4}$ (Gittes and MacKintosh 1998). (ii) During the intermediate regime $1\text{s}<t<80\text{s}$ one can fit the data with the square-root law $a\approx 0.5$. The slope is shown by the dotted and dashed line in Fig. 9. One can see that square-root regime takes place over at least two decades. (iii) At $t>80$s the slope increases again, which probably indicates a crossover to the viscous regime with $a=1$ (shown by black solid line Fig. 9).

Note that the square-root regime cannot be described by any equivalent mechanical circuit (in other words, by any visco-elastic model). It requires introduction of a new, non-viscoelastic circuit element. This problem will be addressed in the present project.

2. Enforced motion of a filament through the actin gel

Recently an enforced motion of a actin filament through the actin gel has been studied (Dichtl and Sackmann 2002). The paramagnetic bead has been biochemically attached to the actin

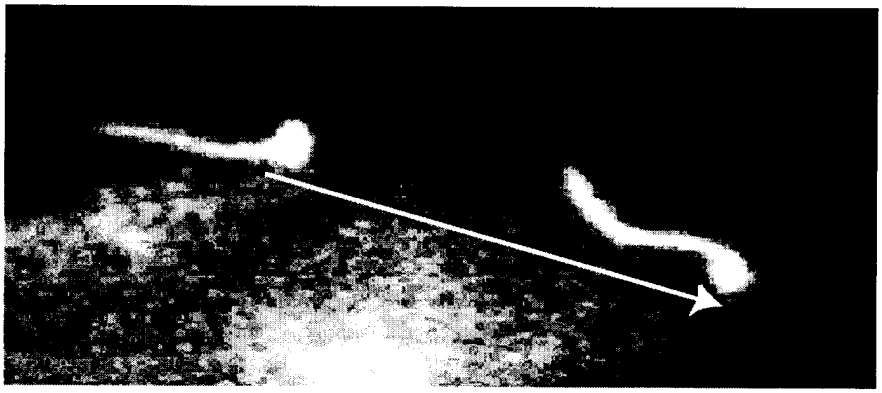

Fig. 10. Enforced reptation of the fluorescently labeled actin filament through the actin gel. The filament with the paramagnetic bead attached to its end can be seen before and after the force pulse (Dichtle, 2002)

filament. The latter has been fluorescently labeled. The labeled filaments (referred to as "the filaments of response") were then embedded into the actin gel. This enabled one to apply the force pulses to the bead, to pull it through the gel. In these experiments the force applied to the bead, the bead displacement as the function of time and the shape of the filament are measured (Fig. 10).

The creep experiments with the beads whose diameter was 4.5μm have exhibited response curves analogous to those of single bead in the actin solution (Fig.8) (Dichtl and Sackmann 2002). However, new recent measurements using beads that were smaller than the mesh size of the gel (J. Uhde and E. Sackmann - unpublished) revealed a purely viscous motion of the bead-filament system. The viscous friction appears to depend on the filament length. It can be interpreted in such a way that in the case of the large beads (4.5μm) the resistance arises mainly from the bead. In contrast, if the beads are smaller than the mesh size, the resistance is determined by the filament itself.

So far there is no theoretical explanation of the observed dependence of the friction on the filament length. This problem will be addressed in my project.

Theoretical prerequisites for the proposal

1. A medium with a constant self-diffusion coefficient: estimates

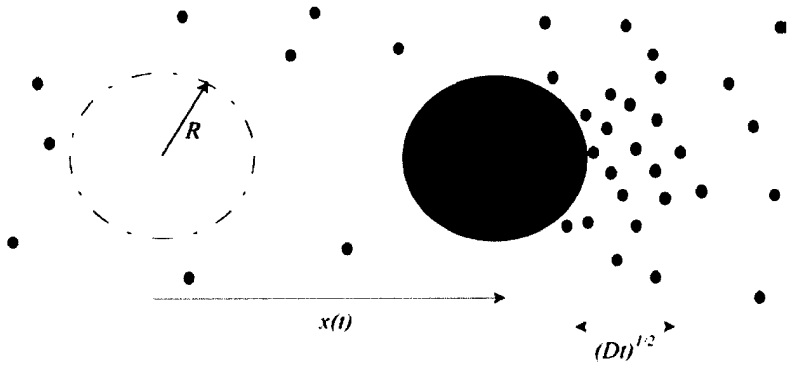

Fig. 11. Schematic view of the motion of a bead (shown in red) immersed into a dense protein solution. During the displacement by x(t) the beads rakes the proteins (blue circles) in front of itself. By diffusion the proteins are redistributed over distance ~$(Dt)^{1/2}$.

Consider motion of a bead (radius R) in a dense solution of proteins with the concentration c (Fig. 11). Assume that during the time t the bead displacement is $x=x(t)$. During its motion the bead rakes up the protein molecules in front of itself (Fig. 11). The region left behind the bead during its motion is free of proteins (Fig. 11). Therefore, an osmotic pressure acts on the front surface of the bead giving rise to a force acting on the bead and resisting its motion which can be estimated as:

$$F_{osm} \sim \frac{ck_BTR^2}{\sqrt{D}}\frac{x(t)}{\sqrt{t}} \tag{6}$$

In microrheological experiments the bead is usually subjected to rectangular force pulses (see for example, (Bausch and others 1998)). If such a pulse with the amplitude F is applied to the bead and is balanced by the osmotic force $F=F_{osm}$ one finds an estimate for the equation of motion of the bead in the following form:

$$x(t) \sim \frac{F\sqrt{D}}{ck_BTR^2}\sqrt{t} \tag{7}$$

Therefore, under the application of the rectangular force pulse one should expect a regime in which the bead displacement is proportional to the square root of time $x \sim t^{1/2}$ in correspondence with the experimental observation shown in Fig. 9.

2. Preliminary results of the numeric approach with finite element method and molecular dynamic simulations.

First results of numeric calculation of a bead moving in a medium with the self-diffusion are shown in Fig. 12. They

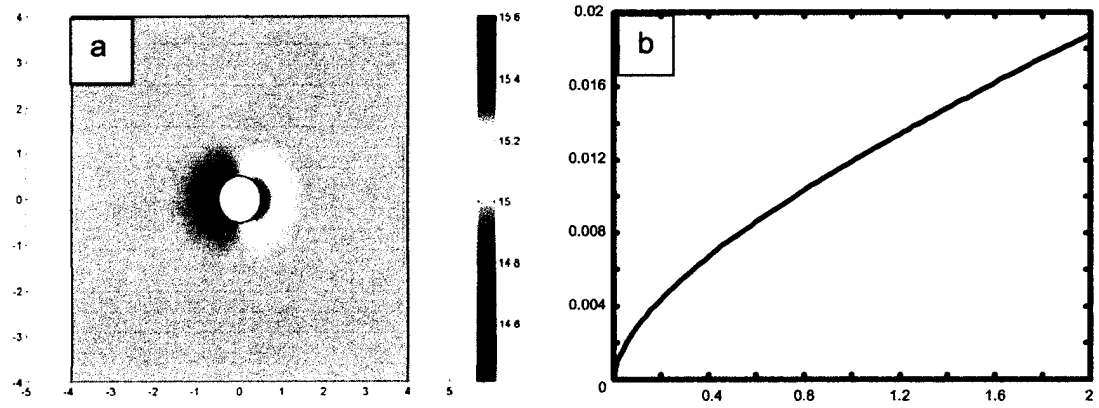

Fig. 12. Results of a numeric solution of equation describing enforced motion of a bead in a self-diffusing medium. (a) The bead (shown as a white disc) moving in the medium of diffusing particles from left to right. Color indicates the concentration of the particles. High concentration is shown in red, and low in blue. (b) The bead displacement (arbitrary units) versus time calculated within the osmotic model shows the square-root regime $x \sim t^{1/2}$ during the initial stage of the motion and a linear regime in the later stage.

qualitatively confirm the above estimates and show the existence of the square-root regime of the bead motion.

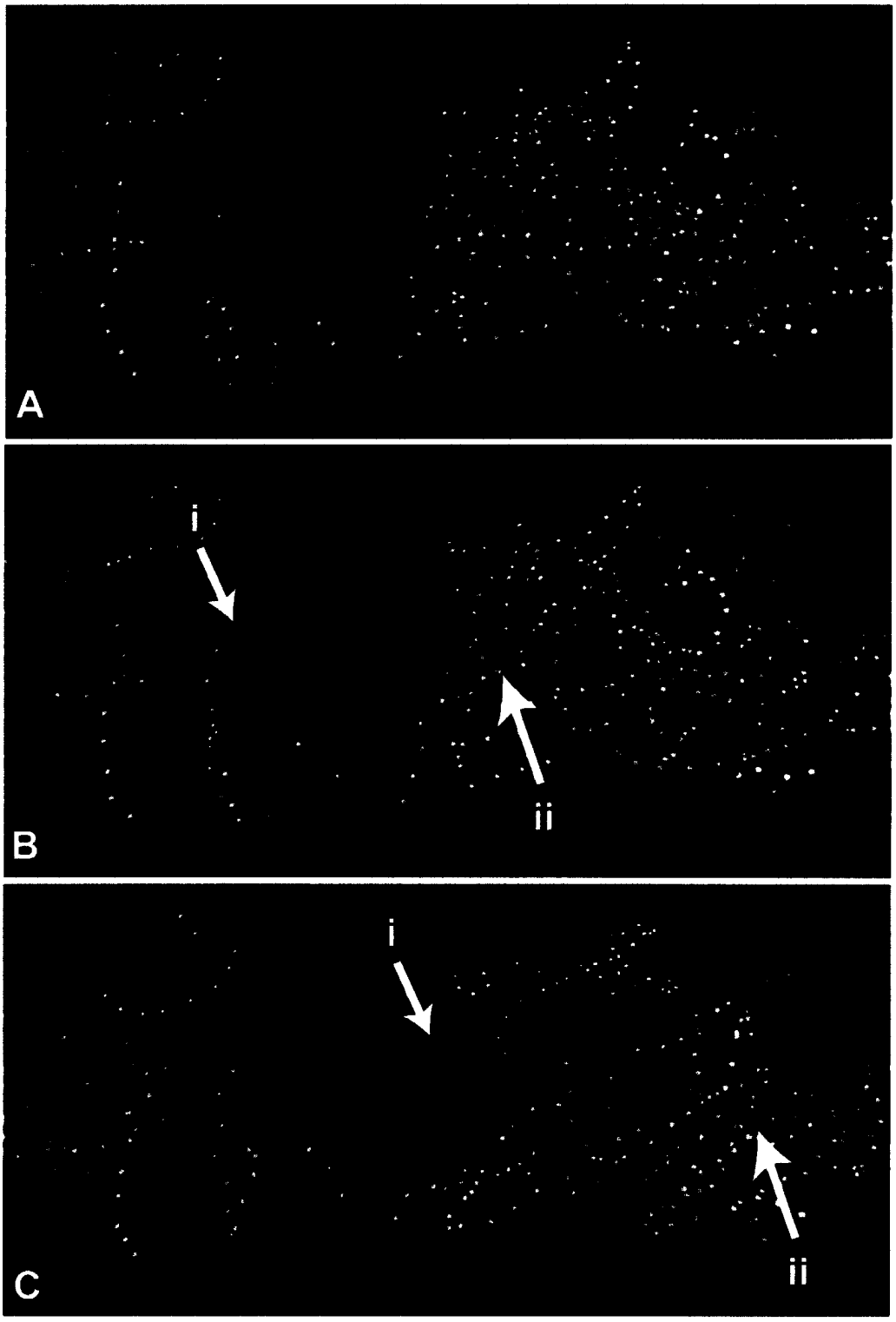

Fig. 13. Preliminary results of the molecular dynamics simulation of the bead moving in the solution of semiflexible polymers. The polymers are modeled as chains consisting of spheres interacting with one another via a harmonic potential (small spheres in the picture). Different colors show spheres belonging to the different polymers. The polymers are only shown in the stripe along the path of the motion of the magnetic bead, other polymers are not shown for the sake of visualization. (A) The magnetic bead in the rest state. (B) and (C) The bead is subjected to a constant force (directed to the right). (B) shows the bead and polymers in the beginning of the bead motion. (C) shows a later stage of the bead motion. (i) The region with the decreased and (ii) with the increased density of the polymers.

In collaboration with professor D. Pink (St. Xavier University, Antigonish, Canada) we developed a program to make a molecular dynamic simulation of the system. The program have been successfully tested. The preliminary results showing the motion of the magnetic bead in the solution of semiflexible filaments obtained with this program are shown in Fig. 13.

3. Osmotic force in a medium with frequency-dependent shear modulus

If the deformation of the medium excites internal degrees of freedom of protein molecules the shear modulus may become frequency-dependent. This case takes place in actin gel where the power-law dependence of the shear modulus on frequency $G(\omega) \sim \omega^{3/4}$ is attributed to the extension of the actin molecules (Gittes and MacKintosh 1998; Gittes and others 1997). Assuming that the Stockes-Einstein relation is valid for the medium under consideration (Solomon and Lu 2001), one finds $x(\omega) \sim F(\omega)/G(\omega)$, where $x(\omega)$ is the Fourrier-image of the bead displacement and $F(\omega)$ is that of the applied force. According to the Einstein relation the diffusion coefficient takes the form $D(\omega) \sim i\omega k_B T/G(\omega)$. Analogous to the previous case, one finds the estimate for the osmotic resistive force in the frequency-dependent regime:

$$F_{osm}(\omega) \sim cR^2 x(\omega)\sqrt{k_B T G(\omega)} \qquad (8)$$

So far no theory exists describing effect of osmotic force on microrheological experiments. The ideas leading to the estimates Eq. (6)-(8) will be used in the present project to describe the effect of the osmotic force on the microrheological experiments.

Goals

The aim of this project is to develop a theoretical framework for the description of microrheological experiments in which components of a complex fluid move by self-diffusion, rather than hydrodynamically. I will develop the model outlined above both analytically and numerically. I will extend it to account for the case of the medium with internal degrees of freedom giving rise to the frequency-dependent shear modulus and diffusion coefficient. I will finally apply this model to the actin solution to explain the experiments described above. In this case, in addition to the analytical and numerical calculations I will analyze the actin gel using molecular dynamics simulations (in collaboration with D. Pink, St. Francis Xavier University, Canada).

In this project I address the following questions:

- How does a bead move through a complex fluid in which at least one of its components exhibits a self-diffusive motion?
- What is the effect of the osmotic force on the bead motion at the early stage of motion (high frequencies), if the complex fluid is characterized by a frequency-dependent shear modulus?
- What is the effect of the osmotic force during the microrheological measurements in actin gels?
- How the variation of concentration of salt or of divalent cataions (such as Ca^{++}) in the actin gels influences the bead motion?
- How does an enforced motion of a single actin filament through the actin gel take place?
- What is equation of state of the actin gel?

Working Plan

1. Enforced motion of the bead through a self-diffusive medium.

I will develop the theory of the osmotic force in a medium in which the motion of its components takes place by the way of self-diffusion. In the one-dimensional case this problem can be solved exactly, while in the 2- and 3-dimensional cases I will look for approximate analytical solutions. I will further solve the 2- and 3-dimensional problem numerically in order to check the approximation. The later will be performed using the finite element method (the software FEMLAB).

2. Osmotic force in a medium with the frequency-dependent shear modulus.

In this project I will study the bead motion inside the medium whose shear modulus is frequency-dependent. I will deduce the osmotic force in this case and analyze regimes of the bead motion. I will apply the results to the case of the actin gel.

Equations of motion of the complex fluid in this case is the so-called two-fluid hydroelastodynamics depending on the concentration of the actin component introduced in the papers of Levine and Lubensky (Levine and Lubensky 2000; Levine and Lubensky 2001). They assumed the concentration to be a constant. In this project I will go beyond this restriction: I will assume that in addition to the two-fluid equations of the hydroelastodynamics, the concentration obeys the diffusion equation. I will solve these equations and find the influence of the diffusive mode on the bead motion.

3. Enforced motion of the bead through the actin gel

Application of the osmotic model to the actin gel assumes that the motion of the filaments in front of the bead is mostly self-diffusive. We need however to check the validity of this assumption and to establish what type of processes occure during the enforced motion of the bead through the actin gel. For this purposes we need to perform molecular dynamic simulations of the enforced motion of the bead through the actin network. A program enabling such a simulation has been recently developed, in collaboration with my group, by professor D. Pink (St. Francis Xavier University, Canada). The program is based on the method of Dissipative Particle Dynamics. The latter is a minimal model enabling one to describe

such a system as the actin filaments embedded into water. The model has the strong advantage of correctly simulating the motion of water through the actin gel.

The program has been written, and the first preliminary results have been obtained which enable us to be sure that it works. The next step will be to run the program in various regimes corresponding to experimental situations and to analyze the results.

The program is designed in such a way that it describes the actin gel and a bead immersed in water. To study the bead motion the calculations will be done several times with different initial configurations of the filaments. Then averaging over the initial configurations will be performed. This procedure will be repeated for different concentrations of the filaments in solution to study dependence on the density.

4. Effect of the salt and divalent ions on creep.

The program accounts for the interaction between the actin molecules via the screened electrostatic interaction, the hydrogen bonding and hard-core repulsion. The variation of the salt concentration can be accounted for through the variation of the Debye radius which will result therefore, in variation of the interaction of filaments and may influence the creep behavior.

Few regimes with realistic parameters will be chosen and simulations of the creep experiments with various values of the Debay radius will be performed. In this way we will find out the effect of salt on the micromechanical response of the gel.

Divalent ions are known to cross-bridge charged filaments under physiological conditions. We expect that this will influence the micromechanical response of the gel.

Accounting for the divalent ions will require some changes in the code. We will run the simulation of the system described above in the presence of the divalent ions of different concentration.

5. Enforced motion of the filament through the actin gel: the analytical approach

I propose to consider the actin filament moving along the reptation tube in the actin gel as a chain moving in a randomly corrugated tube. In this way I will account for the random spatial structure of the gel. As it was shown by simulations (Hunter and others 1999), one should expect that in this case the viscous friction coefficient will depend upon the normal force (i.e. the force that presses the chain against the tube wall). The latter is in this case the undulation force (analogous to the Helfrich repulsion force in biomembranes (Helfrich and Servuss 1984)). This approach will enable me to describe the amplification of the viscous friction of the actin gel and in this way to fill the gap between the previous theoretical predictions and experimental observations (Dichtl and Sackmann 1999).

6. Enforced motion of the filament through the actin gel: the molecular dynamics simulation

By minor changes of the code we can describe an actin filament which is pulled through the gel when a force is applied to its end. By running the simulations with varying concentrations of the filaments in the gel on one hand and the length of the filament of response on the other hand we will understand the behavior of the filament during its enforced reptation.

6. Equation of state of the actin gel

The equation of state of the actin gel can be obtained by variation of the concentration of filaments and the temperature provided the volume is constant and by calculation of the pressure of the actin filaments on the walls.

Outlook

A challenging direction of further research is the study of the micromechanics of a complex fluid in which biochemical reactions take place during bead motion. A microrheology of a cross-linked actin gel is a problem of this class. Some microrheological experiments with cross-linked actin gels have already been done. They can be divided into two classes: those in which the actin filaments are passively cross-linked (by such molecules as for example, α-actinin), or actively cross-linked by molecular motors (such as for example, myoslin V) in the presence of ATP.

References

1. Recent theoretical publications of the applicant related to the project

Arnoldi, M., M. Fritz, E. Bäuerlein, M. Radmacher, E. Sackmann, and A. Boulbitch. 2000. Bacterial turgor pressure can be measured by atomic force microscopy. *Phys. Rev. E.* 62:1034-1044.

Bausch, A. R., F. Ziemann, A. A. Boulbitch, K. Jacobson, and E. Sackmann. 1998. Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophysical Journal.* 75:2038-49.

Boulbitch, A. 1998. Deflection of a cell membrane under application of a local force. *Physical Review E.* 57:2123-8.

Boulbitch, A. 1999. Strain of a biomembrane caused by a local tangential force: Application to magnetic tweezer measurements. *Physical Review E.* 59:3402-7.

Boulbitch, A. 2000. Deformation of the envelope of a spherical Gram-negative bacterium during the atomic force microscopic measurements. *J. Electron. Microscopy.* 49:459-462.

Boulbitch, A. 2002. Enforced unbinding of a bead adhered to a biomembrane by generic forces. *Europhys. Lett.* 59:910-915.

Boulbitch, A. 2003. Enforced unbinding of biomembranes whose mutual adhesion is mediated by a specific interaction. *Eur. Biophys. Lett.* 31:637-642.

Merkel, R., R. Simson, D. A. Simson, M. Hohenadl, A. Boulbitch, E. Wallraff, and E. Sackmann. 2000. A micromechanic study of cell polarity and plasma membrane cell body coupling in dictyostelium. *Biophys J.* 79:707-19.

2. References cited in the proposal

Amblard F, Yurke B, Pargellis A, Leibler S. 1996. A magnetic manipulator for studyind local rheology and micromechanical properties of biological systems. Rev. Sci. Instrum. 67(3):818-827.

Arnoldi M, Fritz M, Bäuerlein E, Radmacher M, Sackmann E, Boulbitch A. 2000. Bacterial turgor pressure can be measured by atomic force microscopy. Phys. Rev. E 62(1):1034-1044.

Ashkin A. 1991. The study of cells by optical trapping and manipulation of living cells using infrared laser beams. ASGSB Bull 4(2):133-46.

Bausch AR, Möller W, Sackmann E. 1999. Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. Biophys. J. 76:573-579.

Bausch AR, Ziemann F, Boulbitch AA, Jacobson K, Sackmann E. 1998. Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. Biophysical Journal 75(4):2038-49.

Boulbitch A. 1998. Deflection of a cell membrane under application of a local force. Physical Review E 57(2):2123-8.

Boulbitch A. 1999. Strain of a biomembrane caused by a local tangential force: Application to magnetic tweezer measurements. Physical Review E 59(3):3402-7.

Boulbitch A. 2000. Deformation of the envelope of a spherical Gram-negative bacterium during the atomic force microscopic measurements. J. Electron. Microscopy 49(3):459-462.

Boulbitch A. 2002. Enforced unbinding of a bead adhered to a biomembrane by generic forces. Europhys. Lett. 59(6):910-915.

Boulbitch A. 2003. Enforced unbinding of biomembranes whose mutual adhesion is mediated by a specific interaction. Eur. Biophys. Lett. 31(8):637-642.

Dichtl MA, Sackmann E. 1999. Colloidal probe study of short time local and long time reptation motion of semi-flexible macromolecules in entangled networks. New J. Phys. 1:18.1/18.11.

Dichtl MA, Sackmann E. 2002. Microrheometry of semiflexible actin networks through enforced single-filament reptation: frictional coupling and heterogeneities in entangled networks. Proc Natl Acad Sci U S A 99(10):6533-6538.

Duszuk M, Schwab B, Zahalak GI, Quian H, Elson E. 1989. Cell poking: Quantitative analysis of indentation of thick viscoelastic layers. Biophis.J. 55:683-690.

Fung YC. 1988. Microrheology and constitutive equation of soft tissue. Biorheology 25(1-2):261-70.

Gittes F, MacKintosh FC. 1998. Dynamic shear modulus of a semiflrxible polymer network. Phys. Rev. E 58(2):R1241-R1244.

Gittes F, Schnurr B, Olmsted PD, MacKintosh FC, Schmidt CF. 1997. Microscopic viscoelasticity: shear modulus of soft materials determined from thermal fluctuations. Phys. Rev. Lett. 79(17):3286-3289.

Glogauer M, Arora P, Yao G, I. S, Ferrier J, McCulloch CAG. 1997. Calcium ions and tyrosine phosphorilation interact coordinately with actin to regulate cytoprotective responses to stretching. J. Cell Sci. 110:11-21.

Glogauer M, Ferrier J. 1998. A new method for application of force to cells via ferric oxide beads. Pflugers Arch 435(2):320-7.

Götter R, Kroy K, Frey E, Bärmann M, Sackmann E. 1996. Dinamic light scattering from semidilute solutions: a study of hydrodynamic screening, filament bending stiffness, and the effect of tropomyosin/troponin-binding. Macromol. 29:30-36.

Heilbronn A. 1922. Eine neue Methode zur Bestimmung der Viskositat lebender Protoplasm. Jahrb. Wiss. Bot. 61:284-338.

Helfrich W, Servuss RM. 1984. Undulations, steric interaction and cohesion of fluid membranes. Nuovo Cimento 3D:137-151.

Hinner B, Tempel M, Sackmann E, Kroy K, Frey E. 1998. Entanglement, elasticity, and viscous relaxation of actin solutions. Phys. Rev. Lett. 81(12):2614-2617.

Hunter JE, Seagraves C, Reinhardt WP. 1999. Frictional effects in the simulation of polymers pulled around smooth and corrugated posts. Journal of Chemical Physics 110(12):6066-6078.

Janmey PA, Shah JV, Tang JX, Stossel TP. 2001. Actin filament networks. Results Probl Cell Differ 32:181-99.

Kuo SC. 2001. Using optics to measure biological forces and mechanics. Traffic 2(11):757-63.

Lee B, Litt M, Buchsbaum G. 1992. Rheology of the vitreous body. I: Viscoelasticity of human vitreous. Biorheology 29:521-533.

Levine AJ, Lubensky TC. 2000. One- and two-particle microrheology. Physical Review Letters 85(8):1774-1777.

Levine AJ, Lubensky TC. 2001. Response function of a sphere in a viscoelastic two-fluid medium. Physical Review E 6304(4):art. no.-041510.

Mackintosh FC, Janmey PA. 1997. Actin gels. Current Oppinion in Solid State & Materials Science 2(3):350-357.

MacKintosh FC, Schmidt CF. 1999a. Microrheology. Current Op. Colloid Interface Sci. 4(4):300-307.

MacKintosh FC, Schmidt CF. 1999b. Microrheology. Current Opinion in Colloid & Interface Science 4(4):300-307.

Merkel R, Simson R, Simson DA, Hohenadl M, Boulbitch A, Wallraff E, Sackmann E. 2000. A micromechanic study of cell polarity and plasma membrane cell body coupling in dictyostelium. Biophys J 79(2):707-19.

Morse DC. 1998a. Viscoelasticity of concentrated isotropic solutions of semiflexible polymers. 1. Model and stress tensor. Macromol. 31:7030-7043.

Morse DC. 1998b. Viscoelasticity of concentrated isotropic solutions of semiflexible polymers. 2. Linear response. Macromol. 31(20):7044-7067.

Morse DC. 1998c. Viscoelasticity of concentrated isotropic solutions of semiflexible polymers. 4. Tube diameter and plateau modulus. a manuscript.

Morse DC. 1998d. Viscoelasticity of tightly entangled solutions of semiflexible polymers. Phys. Rev. E 58(2):R1237-R1240.

Morse DC. 1999. Viscoelasticity of concentrated isotropic solutions of semiflexible polymers. 3. Nonlinear rheology. Macromol. 32(18):5934-5943.

Mukhopadhyay A, Granick S. 2001. Micro- and nanorheology. Current Opinion in Colloid & Interface Science 6(5-6):423-429.

Olsvik O, Popovic T, Skjerve E, Cudjoe KS, Hornes E, Ugelstad J, Uhlen M. 1994. Magnetic separation techniques in diagnostic microbiology. Clin. Microbiol. Rev. 7(1):43-54.

Riveline D, Ott A, Julicher F, Winkelmann DA, Cardoso O, Lacapere JJ, Magnusdottir S, Viovy JL, Gorre-Talini L, Prost J. 1998. Acting on actin: the electric motility assay. Eur Biophys J 27(4):403-8.

Sato M, Wong TZ, Brown DT, Allen RD. 1984. Rheological properties of living cytoplasm: a preliminary investigation of squid axoplasm (*Loligo pealei*). Cell Motil. 4:7-23.

Schmidt F. 1999. Mikrorheologie von Netzwerken semiflexibler Biopolymere [PhD Thesis]. München: Technische Universität. 159 p.

Schmidt FG, Hinner B, Sackmann E. 2000. Microrheometry underestimates the values of the viscoelastic moduli in measurements on F-actin solutions compared to macrorheometry. Phys. Rev. E 61(5):5646-5653.

Schmidt FG, Ziemann F, Sackmann E. 1996a. Shear field mapping in actin networks by using magnetic tweezers. European Biophysical Journal Letters 24:348-353.

Schmidt FG, Ziemann F, Sackmann E. 1996b. Shear field mapping in actin networks by using magnetic tweezors. Eur. Biophys. J. Lett. 24:348-353.

Solomon MJ, Lu Q. 2001. Rheology and dynamics of particles in viscoelastic media. Current Opinion in Colloid & Interface Science 6(5-6):430-437.

Theret DP, Levesque MJ, Sato M, Nerem RM, Wheeler LT. 1988. The application of a Homogeneous half-space model in the analysis of endothelial cell micropipette measurements. J.of Biomechanical Engeneering 110:190-199.

Valberg PA, Butler JP. 1987. Magnetic particle motions within living cells. Physical theory and techniques. Biophys. J. 52(4):537-550.

Valberg PA, Feldman HA. 1987. Magnetic particle motion within living cells. Measurements of cytoplasmic viscosity and motile activity. Biophys. J. 52(4):551-561.

Vetvicka V, Fornusek L. 1987. Polymer microbeads in immunology. Biomaterials 8(5):341-5.

Wang N, Butler JP, Ingber DE. 1993. Mechanotransduction accross the cell surface and through the cytoskeleton. Science 260:1124-1127.

Wetzel MG, Korn ED, Weisman RA. 1969. Phagocytosis of latex beads by Acahamoeba castellanii (Neff). 3. Isolation of the phagocytic vesicles and their membranes. Phagocytosis of latex beads by Acanthamoeba. II. Electron microscopic study of the initial events. J. Cell. Biol. 43(1):90-104 issn: 0021-9525.

Zaner KS, Valberg PA. 1989. Viscoelasticity of F-actin measured with magnetic microparticles. J. Cell Biol. 109:2233-2243.

Ziemann F, Rädler J, Sackmann E. 1994a. Local measurement of viscoelastic moduli of entangled actin networks using an oscilating magnetic bead micro-reometer. Biophysical Journal 66:2210-2216.

Ziemann F, Rädler J, Sackmann E. 1994b. Local measurement of viscoelastic moduli of entangled actin networks using an oscilating magnetic bead micro-reometer. Biophys. J. 66:2210-2216.

A. Boulbitch

Statement of Teaching Interests

I like to teach and consider teaching and research as complementary aspects of scientific life. Each step in teaching possesses the significance of being real work with real people. It gives us a unique chance to share our vision of the world with others. I noticed that discussions with my students stimulate my scientific activity.

I have experience in teaching on both undergraduate and graduate levels. Basing on this experience I think that teaching of undergraduates is as important as that of graduates, since their basic knowledge is formed on this stage. In addition during this period the undergraduate students may be involved in the research activities. I believe that the earlier the students are involved in research, the better training they receive.

My students like my lectures. In the course of my lectures at the Technical University Munich I was evaluated by graduate students. Such evaluations in Germany are graded between 1.0 and 6.0, where the best note is 1.0. I received the grade 1.6 with the remark "Taught in this way, theory is a real pleasure". My high standard of teaching was also recognized by the faculty. This was the prerequisite for obtaining my Habilitation in Theoretical Physics in 2001.

I am ready to teach any course for undergraduates offered by the Department of Physics of the Indiana University. However, I would be most efficient in teaching classes related to

- Solid State Physics
- Continuum Mechanics,
- Thermodynamics and Statistical Physics
- Physical Kinetics and
- Biological Physics

which are most close to my experience.

I have also **experience in teaching Mathematics for undergraduates** (see the Appendix below).

Recently I gave several theoretical courses for graduates-experimentalists. These students are well motivated but often lack a necessary knowledge of Mathematics. Therefore, apart from conveying information, my courses had additional intents. One aim was to make the students understand and appreciate existing theoretical approaches, so that they would be able to understand the main ideas contained in research publications. To achieve this, I chose theoretical papers which could be of use in helping them understand their own experiments. For example, I used some aspects of the paper Seifert, Adv. in Physics **46**, 13 (1997) during my classes on vesicles, Brochard and De Gennes, PNAS **99**, 7854 (2002) during the classes on specific adhesion of membranes, a part of the paper Prost et. al., Eur. Phys. J. B **1**, 465 (1998) during the lecture on Helfrich repulsion and the paper Merkel et. al. Nature **397**, 50 (1999) to discuss the enforced unbinding of molecules. I use also other recent papers.

I think that in order to be understood by students, the courses should use the most simple mathematics compatible with the subject. I use dimensional analysis, scaling considerations, and estimates, wherever possible. The courses I gave were oriented towards discussions of applications, rather than towards discussions of theories.

As a method of teaching **I propose to use the "Mathematica" software** wherever possible**.**

First, this is efficient for the purposes of visualization of lecture material.

Second, it is important to teach students to use such a software for solving problems. In a simplest case this can be achieved by making a collection of problems that could be solved from the beginning to the end within the Mathematica. They can be made available for students by placing on the web in a form of the Mathematica files.

The most efficient method would be in combining lectures with "laboratory" classes in which students will solve problems using "Mathematica" software in an interactive mode. Each such a session can be started with a short "talk and chalk" discussion of problems and approaches of their solution using Mathematica, and continued by each student working at his/her terminal with

Mathematica files prepared for this purpose. In order to teach these laboratory classes a classroom equipped with personal computers and a license for Mathematica software ("Mathematica 4.1 for students", $85 per PC) installed on them are necessary.

In addition such an interactive Mathematica course can be placed on the web and utilized for distance education. This will require a so-called "webMathematica" package (webMathematica 2 Pro, $ 9500). Maple can be used instead of Mathematica, if necessary.

This idea is based on my own positive experience with such classes (shortly described in the Appendix).

Courses for graduates I could offer:

I propose to introduce **a course for graduates** entitled "**Modern biological micromechanics**". It will contain introduction to cutting edge results concerning the micromechanical properties of biological objects ranging from the level of a single macromolecule up to the cellular scale.

This course would be based mainly on the material that I have used for courses that I have already given at the Technical University Munich as well as my own scientific work. It will be however, considerably modified by including both the most exciting recent results such as the manipulation of single molecules.

Modern biological micromechanics

- Theoretical background of modern methods used in micromechanics: AFM, optical and magnetic tweezers, micropipettes, passive and active microrheology.
- Manipulations with single molecules. Mechanical properties of a single DNA molecule, enforced unfolding of a protein, enforced unbinding of a ligand receptor bond, manipulations with motor proteins, manipulations with a single semiflexible polymer: filamentous actin molecules and microtubules.
- Multiple molecular bonds. Specific adhesion of biomembranes. Enforced unbinding of multiple bonds.
- Introduction in mechanical properties of vesicles and cells: vesicles and red blood cells, fibroblasts and endothelial cells, bacteria.

Below I give the titles of two other courses for graduates related to my scientific experience which I could also propose:

1. **Introduction to physics of soft matter: liquid crystals, polymers, membranes**
2. **Introduction to phase transitions in solids: superconducting, ferroelectric and ferromagnetic transitions.**

Appendix: Teaching Experience

1. Courses

1998-2003 I taught four 1-semester courses for **graduates** at the Technical University Munich entitled

1. Micromechanics of Biomembrane
2. Gels and biogels
3. Mathematical analysis of enzymatic reactions
4. Basics of Bioadhesion

The characteristic common to these courses is that they are all related to scientific problems on which the Department is focusing. I gave a basic knowledge of the subjects and discussed the most recent results so as to enable the students to appreciate the challenges and the excitement of research at the cutting edge.

1995/1996 I gave a 2-semester course in **Biophysics** accompanied by **Computer classes** for undergraduates at the Department of Physics of Rostov University, Russia. The Computer classes taught the students to perform computer analyses of problems concerned with the course.

1991/1992 I gave a 2-semester course (192 hours) in **Mathematical Analysis and Calculus** for 2nd year undergraduates at the University of Picardie (Amiens, France) during my work there.

1990 I gave a 1-semester course in "**Bifurcations of Nonlinear Equations for Physicists**" at the Department of Physics, Rostov University (Russia) for undergraduates. The aim was to teach students to apply simple methods of analysis of bifurcations of nonlinear equations.

2. Supervision of students

I like to work with students and have the experience of supervision the research of PhD students as well as of involving of undergraduates in my research area.

1996-1993 I was a co-adviser (together with Prof. E. Sackmann) of 4 PhD students-experimentalists (the theses are defended) and the adviser of one PhD student-theorist (the thesis is in preparation)

1988-1991 I was as adviser of the PhD Thesis (defended in 1991, Rostov, Russia)

1986-1990 I supervised the research of 4 diploma students and 3 postgrads.