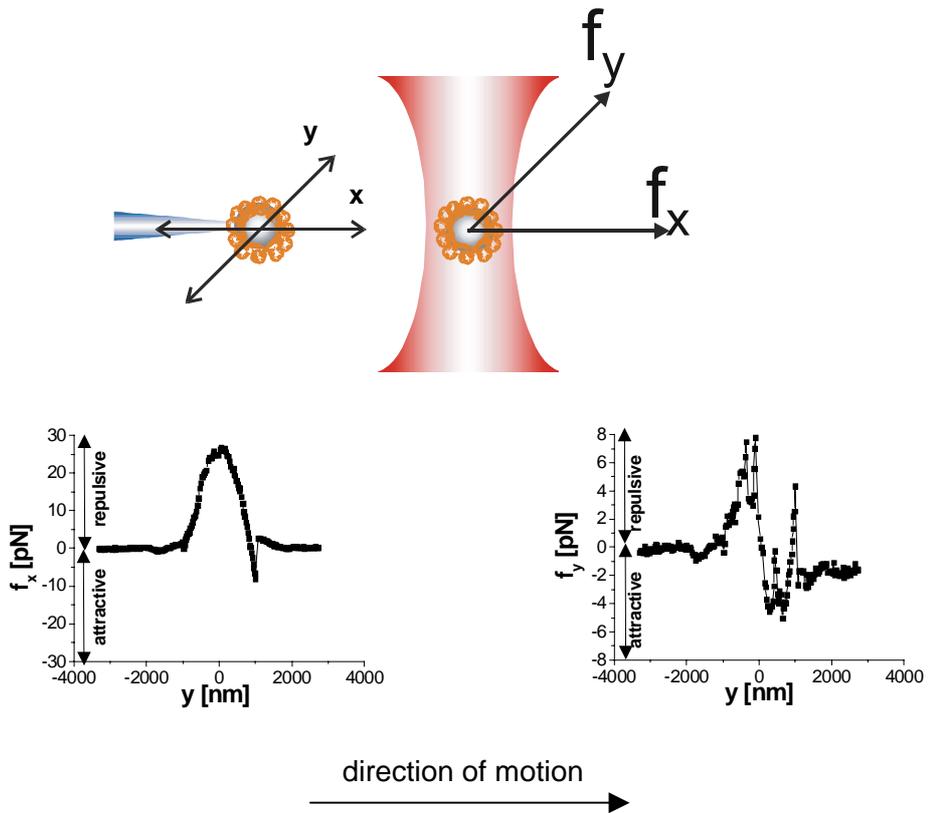


UNIVERSITY OF LEIPZIG
Faculty of Physics and Earth Sciences
Department of Experimental Physics I
GROUP "PHYSIK ANISOTROPER FLUIDE"



Annual Report 2004

Cover picture: Upper part: Scheme of the set-up to measure the interaction between *single* DNA-grafted colloids (diameter: $2.08\ \mu\text{m}$). The left colloid is hold with a micropipette, the right with optical tweezers. In close contact (lower part) of the DNA-brushes at a separation of $64 \pm 2\ \text{nm}$ between the solid colloidal surfaces repulsive forces are measured in x-direction while in y-direction stick-slip behaviour is observed due to interdigitation of the DNA-brushes.

The year 2004

In the year 2004 the consolidation of our group continued after the changes in 2003. In the traditional research topics (Broadband Dielectric Spectroscopy and time-resolved Fourier Transform Infrared Spectroscopy) novel and detailed insights were gained into the dynamics of thin (~ 10 nm) polymer layers and in the response of (nematic) liquid crystalline elastomers to external mechanical fields. In the experiments with optical tweezers for the first time it could be shown that the force-elongation dependence of *single* DNA-chains of varying length shows systematic deviations from the predictions of the wormlike chain model. Furthermore refined experiments on the interaction between DNA-grafted colloids were carried out and - depending on the direction with respect to the axis between the colloids – the forces parallel and perpendicular to it could be separated and analysed. This opens completely novel and exiting perspectives.

F. Kremer

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1. Organization of the group

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Dipl.-Phys. Kati Kegler
Dipl.-Biochem. Mathias Salomo
Dipl.-Phys. Anatoli Serghei
M. Sc. Michael Tammer

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Dipl.-Phys. Wiktor Skokow
Dipl.-Phys. Uwe Weber

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Prof. Dr. Siegbert Grande

2. Projects

2.1 Molecular dynamics in thin films of polymers with special architecture

A.Serghei, F.Kremer*

This project aims to investigate confinement effects on the dynamic glass transition of polymers having a special architecture. Thin films of hyper-branched polymers (fig. 1a), grafted polymers and star-branched polymers are prepared and their molecular dynamics is investigated in dependence on the confinement size (film thickness). Two questions are addressed. The first one, what role plays the architecture of the macromolecular systems in the deviations of the dynamic glass transition from the bulk behaviour observed in thin films. For example, thin layers of hyper-branched polyesters show an increase of the average alpha relaxation rate with decreasing films thickness. This effect appears at much larger thicknesses (fig. 1b) than those reported for linear polymers and therefore must be assigned to the special architecture of these dendritic macromolecules.

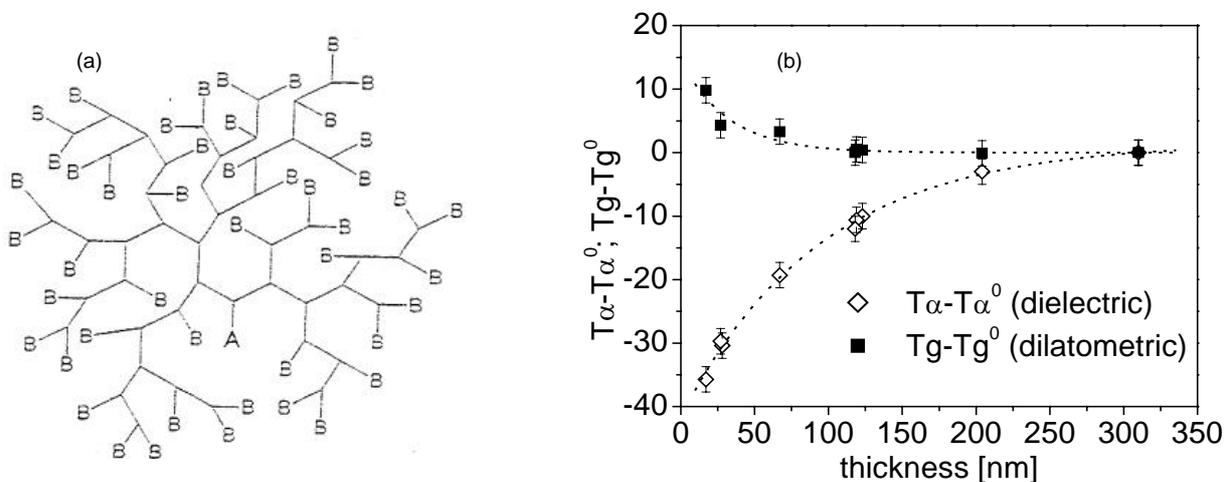


Fig.1 a) scheme showing the architecture of hyper-branched polymers b) the maximum temperature position of the alpha relaxation peak (by Broadband Dielectric Spectroscopy, at 0.6 Hz) and the glass transition temperature (by capacitive dilatometry), both normalized in respect to the values corresponding to 310 nm, as a function of film thickness.

The second question, to what extent different methods to investigate the glass transition provide comparable results when applied to thin films. For that, complementary measurements by Broadband Dielectric Spectroscopy, capacitive dilatometry and AC – calorimetry are employed. In the case of hyper-branched polyesters, the dielectric measurements show a faster dynamic glass transition with decreasing film thickness while simultaneous dilatometric determinations reveal a slight increase of T_g . Such an apparent controversy, reported for linear polymers, too, indicates that dielectric relaxation spectroscopy and dilatometry do not sense the same component of the molecular dynamics in thin polymer films. A combinative approach for the investigation of the dynamic glass transition might help one to get new insights into the mechanism underlying the confinement effects reported since one decade in the literature.

References:

- [1] Y. H. Kim, J. Polym. Sci. (A) 36, 1685 (1998).
- [2] A. Serghei et al., submitted to Eur. Phys. J. E (2005).

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2.2 Novel developments in the preparation of thin polymer films

A. Serghei, F. Kremer

This work attempts to develop novel methods for the preparation of thin polymer films which could allow one to investigate the molecular dynamics of polymers in a one dimensional confinement down to a geometrical constraint comparable with size of the polymer coil (i.e. 10 nm). The method should additionally allow a relative easy adjustment of the interfacial interactions (by treating the surface of the solid electrodes) in order to investigate their role in the shifts of the dynamic glass transition observed in confinement. It should enable also a reliable quantitative determination of the relaxation time distribution

in dependence on the confinement size which may facilitate a better understanding of the mechanism underlying the confinement effects. Our current approach is to use ultra flat conductive silicon wafers as hard electrodes, while nanostructures of SiO_x are going to serve as spacers (fig. 1a). After cleaning, contacting and annealing, the empty condenser, consisting of two parallel electrodes separated by a matrix of isolating nano-spacers, is filled by capillarity with the material under investigation. The determination of the sample capacity before and after filling provides an additional way to check the thickness of the polymer film. First measurements using nano-structures of 200 nm were proven successfully (fig. 1b), a second sample of 10 nm thickness is currently in preparation.

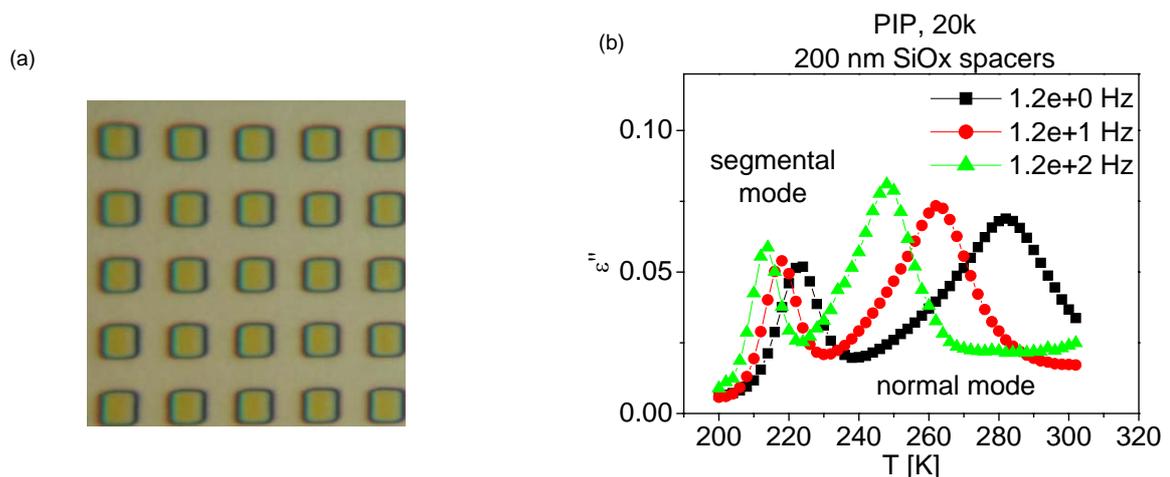


Fig. 1 a): matrix of nano-spacers (height of 200 nm, width of 5 μm) on the surface of a flat silicon wafer; b) Dielectric loss ϵ'' vs. temperature T at different frequencies, as indicated, showing the segmental and the normal mode for a 200 nm thin film of poly-isoprene ($M_w=20$ Kg/mol).

Collaborators:

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2.3 Shifts of the dynamic glass transition in thin polymer films: a possible mechanism

A.Serghei, F.Kremer

The origin of the confinement effects on the dynamic glass transition in thin polymer films observed since one decade in numerous studies is still not elucidated. Moreover, the overview emerging from the data reported in the literature is pretty controversial, since there are many studies indicating no shifts of the glass transition temperature in the confinement of thin films. Here, a possible cause of the Tg deviations is thoroughly investigated: as well-known, a change of the molecular weight distribution, possibly by chain breaking or cross-linking in the presence of oxygen, has definitely an impact on the dynamic glass transition. Since in many studies revealing Tg shifts the sample annealing or the measurements are done in a moderate vacuum or even in ambient air, this possibility cannot be excluded. The first indications of a chain breaking effect which causes above 414 K a reduction of the glass temperature in ambient air (fig. 1) were found in a study investigating a pattern formation in thin films of polystyrene: samples kept in a pure nitrogen atmosphere remains stable while in ambient air they exhibit an enhanced mobility and undergo changes ending up with the formation of a characteristic morphology. Measurements by Broadband Dielectric Spectroscopy, capacitive dilatometry and AC-calorimetry reveal indeed the Tg reductions in ambient air, while the proof of a chain scission effect was provided by IR-spectroscopic measurements. To what extent the results indicating a faster dynamics in thin PS films are caused by chain scissions in the presence of oxygen and especially whether there is a thickness dependence of this effect is currently investigated.

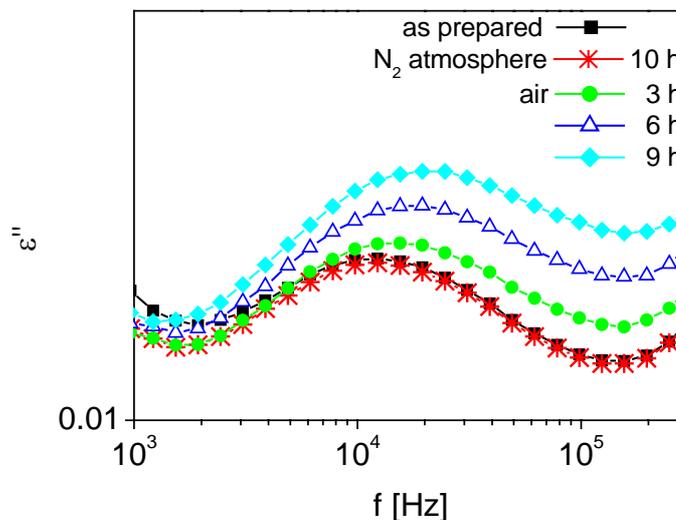


Fig. 1 Dielectric loss ϵ'' vs. frequency f [Hz], showing the dynamic glass transition of a 86 nm thin PS film at a constant temperature of 414 K, as prepared, in a pure nitrogen atmosphere and in air as a function of time, as indicated.

References:

- [1] J. A Forrest, Eur. Phys. J. E 8, 261 (2002).
- [2] A. Serghei et al., submitted to Phys. Rev. E (2005).

Collaborators:

C. Schick (Rostock University)

2.4 Molecular dynamics in semifluorinated side-chain Polyesters as studied by Broadband Dielectric Spectroscopy

J.Tsuwi, F.Kremer

Structural segments consisting of alkyl and perfluoroalkyl groups covalently linked by a C-C bond are well known for their microphase separation resulting in highly ordered bulk structures. The use of such materials for surface modification is numerous because of the resulting low surface free energy. We are employing Broadband Dielectric Spectroscopy to study the molecular dynamics in fluorinated side-chain polyesters.

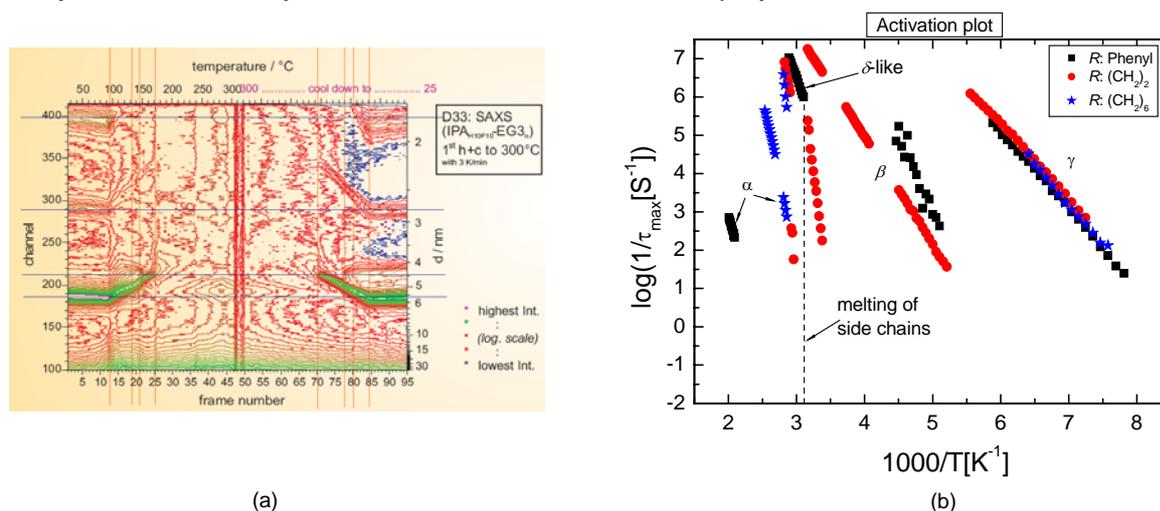


Fig. 1: (a) Temperature dependent small angle X-ray (*t*-SAXS) (b) Activation plot showing four relaxation processes γ , β , δ and α in order of their mean relaxation times.

It is observed that the fluorinated side-chain exhibits two relaxation processes γ and δ -like. The γ process is assigned to librational fluctuations at the terminal position of the side chain while the δ -like process reflects a cooperative motion of the side chain as a whole. Two more processes, β and α , are observed, which are associated with motion of the main chain. The β process is assigned to fluctuations of the dielectrically active carbonyl groups together with the phenyl ring whereas the α relaxation corresponds to the dynamic glass transition process of the polymers. With respect to the flexibility of the main chain, an interesting behavior is seen in the dynamics of the backbone when compared to side chain motion. Based on the analysis of the activation plot, it is concluded that a flexible main chain exhibits faster mobility in direct contrast to its side chain motion. On the other hand, the less flexible backbones show the reverse trend. The dielectric results are analyzed in the context of micro-phase separated layered structures, supported by differential scanning calorimetry (DSC) and *t*-SAXS studies (Fig. 1a).

References:

- [1] Gottwald A, Pospiech D, *et al* (2002) *Macromol. Chem. Phys.* 203 (5-6): 854
- [2] Tsuwi J, Appelhans D, Zschoche S, Friedel P, Kremer F (2004) *Macrom.* 37:6050
- [3] Tsuwi J, Appelhans D, *et al.* (Colloid&Polym., *in Press*)
- [4] Tsuwi J, Pospiech D, *et al.* (J.Polym. Sci. B: Polym. Phys., *in preparation*)

Collaborator:

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2.5 High frequency dielectric studies of microwave (MW) enhanced chemical reactions

J.Tsuwi, F.Kremer

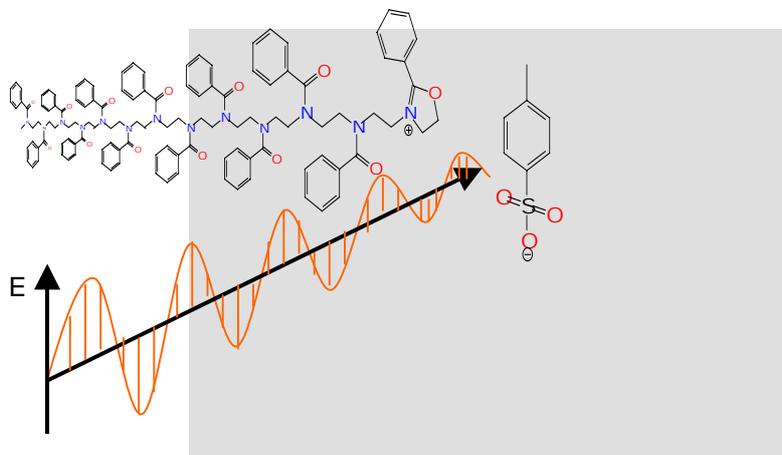


Fig. 1: Scheme of activation of the reactive site by MW irradiation (Illustration adapted from Macrom. Rapid Comm.2005, 26, 160)

Microwave-assisted chemical reactions (Fig.1) are rapidly gaining interest from polymer chemists. This is because of the fact that it has been proved to greatly increase reaction rates and thereby reducing reaction times from hours to just a few minutes. With MW irradiation, high polymerization yields have been realized in short times and the advantage of non-contact heating compared to convectional heating is highly appreciated.

Controversial discussions have however emerged as to whether there are non-thermal microwave effects during MW-enhanced polymerization or purely thermal processes. In view of this, we carry out high frequency (>10MHz) dielectric studies on different solvent/monomer/initiator combinations in order to investigate the underlying molecular mechanisms.

References:

- [1] Sinnwell S, Ritter H (2005) Macrom. Rapid Comm. 26, 160-163
- [2] Iannelli M, Ritter H (2005) Macrom. Chem.Phys. 206, 349-353
- [3] Wiesbrock F, Hoogenboom R, Schubert US (2004) Macrom. Rapid Comm.25, 1739
- [4] Wiesbrock F, Hoogenboom R, Abeln CH, Schubert US (2004) Macrom. Rapid Comm.25, 1895

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2.6 Time resolved FTIR-spectroscopy on segmental reorientation of nematic elastomers under external mechanical fields

J. Li, M. Tammer, F. Kremer

Fourier transform infrared (FTIR) spectroscopy [1-3] is an especially suitable method for obtaining molecular level information for liquid crystalline networks. Time resolved FTIR spectroscopy with polarized light is employed to study the segmental orientation and the order parameters of nematic liquid crystalline elastomers (NLCEs, Fig. 1) with a monodomain structure in response to an external mechanical field [4,5]. Detailed results about the reorientation of the mesogens, the spacer molecules and the main chain are obtained due to the specificity of the FTIR measurements.

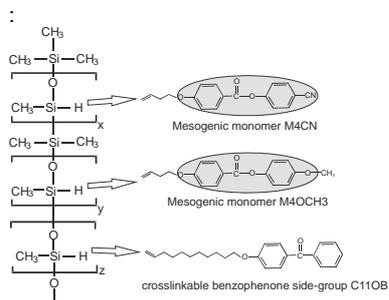


Fig. 1: NLCE-material

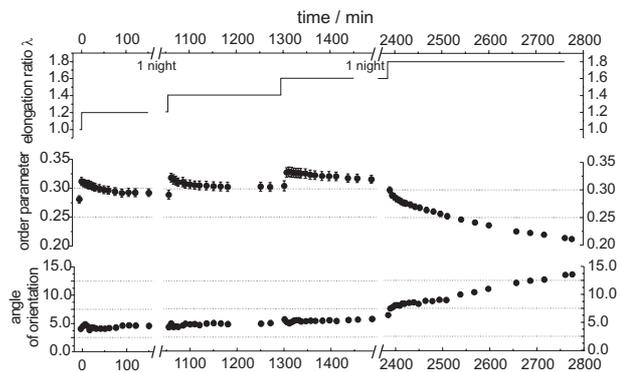


Fig. 2: Orientation and order parameters for the CC group (mesogens) at perpendicular strain

Mechanical strain is applied to thin NLCE films parallel and perpendicular to the initial mesogen orientation and the evolution of the orientation and the molecular order parameter with time is obtained for the different molecular moieties after each increase of the elongation ratio. While at parallel strain neither a reorientation nor a significant change of the order parameters takes place for all groups, the molecular units react differently to a strain applied perpendicular to the initial mesogen orientation (Fig. 2). Below a threshold value of the elongation ratio the orientation and order parameters remain nearly constant. Above this value a continuous reorientation is found for all molecular segments while the order parameters decrease. The network is not stable anymore and flows until the film breaks.

References:

- [1] H. W. Siesler, K. Holland-Moritz, *Infrared and Raman Spectroscopy of Polymers*, Practical Spectroscopy Volume 4; Marcel Dekker: New York, **1980**.
- [2] S. V. Shilov, S. Okretic, H. W. Siesler, M. A. Czarnecki, *Appl. Spectrosc. Rev.* **1996**, 31, 125.
- [3] H. Skupin, F. Kremer, S. V. Shilov, P. Stein, H. Finkelmann, *Macromolecules* **1999**, 32, 3746.
- [4] M. Tammer, J. Li, A. Komp, H. Finkelmann, F. Kremer, **2005** *Macromol. Chem. Phys.* accepted
- [5] J. Li, M. Tammer, A. Komp, H. Finkelmann, F. Kremer, **2005** *Eur. Phys. J. E* to be submitted

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2.7 Dynamic stress strain response in thin films of nematic liquid crystal elastomers under external mechanical fields

M. Tammer, J. Li, F. Kremer

The macroscopic response of thin films of nematic liquid crystalline elastomers (NLCEs) to deformations under a mechanical field is highly anisotropic due to the ordered structure of these materials. Next to the effects like segmental reorientation and change of molecular order parameters investigated by Fourier transform infrared (FTIR) spectroscopy, the stress – strain dependency and its phase relation is of high interest in order to analyze the response of the network. The most remarkable property of NLCEs is the phenomenon of soft elasticity. First experimental results for this theoretical concept were found by K upfer and Finkelmann [1]. Soft elasticity is defined by a non-linear soft response over a full range of strain and described by the theory of nematic-rubber elasticity developed by Warner and Olmsted [2,3].

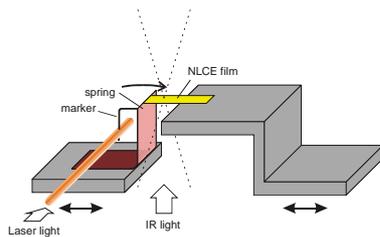


Figure 1: scheme of the force detection setup

In order to measure the force applied to the NLCE sample at the same time as the polarized IR spectra, one end of the film is fixed on a metal spring (Fig. 1). The deformation of this calibrated spring is determined by image processing. A detection by optical sensors is planned for dynamic measurements. A first example of a simultaneous FTIR and stress – strain experiment is shown in Fig. 2. The angle of orientation and the molecular order parameter of the mesogens are given together with the force affecting the sample.

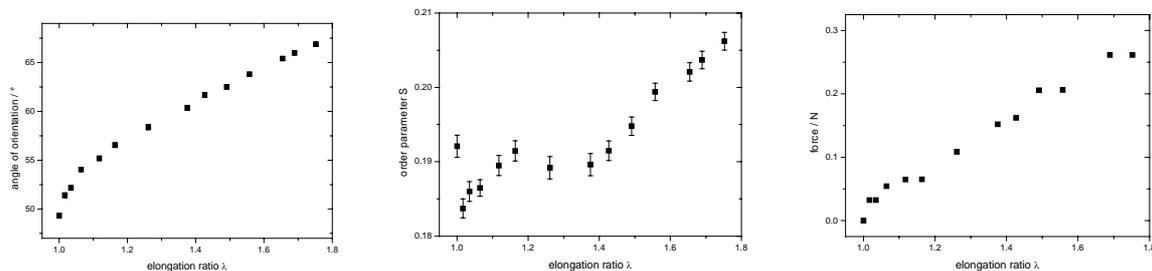


Figure 2: angle of orientation (left) and order parameter (middle) of the CC group (mesogens) at mechanical strain obliquely to the original mesogen orientation; right: force vs. elongation ratio for this NLCE film

References:

- [1] J. K upfer, H. Finkelmann, *Macromol. Chem. Phys.* **1994**, *195*, 1353
- [2] M. Warner, P. Bladon, E. M. Terentjev, *J. Phys. II* **1994**, *4*, 91
- [3] P. D. Olmsted, *J Phys. II* **1994**, *4*, 2215

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2.8 Liquid crystalline physical gels: functional materials with controlled microphase-separated structures and memory states

J. Li, F. Kremer

Self-assembly and micrometer scale phase-segregates of the two discrete components, i.e. hydrogen-bonded gelators and conventional LC molecules, results in the generation of LC physical gels. In contrast to chemical gels, the gelation is thermo-reversible. LC physical gels are very promising functional materials in application for their unique features, such as the reversibly memorized states and controlled microphase-separated structures [1].

We study the electro-optic characteristics of these LC physical gels in freely suspended films by means of polarizing microscopy. It is found that during the gelation an anisotropic network of thin birefringent strands phase-separates from the surrounding LC material. The network creates a memory of the 'frozen-in' texture and influences the switching behavior of the ferroelectric phase considerably. Under application of electric fields, the sample orientation can be switched in the LC microdomains, and after removal of the electric field, the elastic forces restore the memorized local alignment [2].

We demonstrate that proton NMR spectra can provide direct information about order and orientation of the director in the LC physical gels. The results show that small percentages of gel network are sufficient to stabilize the director even in the strong external magnetic field (several Tesla). The critical amount of the gelling agent that is necessary to fix the director orientations is above 3.0 wt%. At lower concentrations, the 2.34 T magnetic field distorts the LC director field. From the NMR spectra, there are no observable differences between the nematic and smectic gel states in 8CB. The order parameter of the 8CB in the gel is not changed significantly from that of the pure mesogen [3].

We show that the Laser Intensity Modulation Method (LIMM) spectra of the hydrogen-bonded FLC gels is able to exhibit spatially resolved polarization distributions and LC director orientation in sandwich cells. Under electric fields, contribution to the resulting distribution caused by the induced polarization due to unwinding the FLC helix has been detected. The influence of hydrogen-bonded network on the polarization distribution is also found when the gel former is increased up to 5.0 wt%. Therein the shape of the measured pyrospectra is completely different to that of other gel samples with lower gel former concentration. Its maximum distribution still locates at the surface of FLC layer which is comparable to the field-free state. These results indicate that the helical structure and director of FLC can be stabilized effectively by the gel network [4].

References:

- [1] Prigann, J., Ch. Tolksdorf, H. Skupin, R. Zentel and F. Kremer, *Macromolecules* 35, 4150 (2002)
- [2] Kato T., *Science*, 295, 2414 (2002)
- [3] Li, J., Stannarius, R., Tolksdorf, C., Zentel, R., *Phys. Chem. Chem. Phys.*, 5, 916 (2003)
- [4] Li, J., Geschke, D., Stannarius, R., *Liq. Cryst.*, 31, 21 (2004)
- [5] Li, J., Geschke, D., *Polym. Adv. Technol.*, 16, 11 (2005)

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2.9 Optical tweezers as a tool to investigate the elastic properties of single DNA molecules with different length

M. Salomo, K. Kegler, M. Struhalla, J. Reinmuth, V. Skokow, F. Kremer

Optical tweezers are commonly used to manipulate microscopic particles, with applications in cell manipulation, colloid research, manipulation of micromachines and studies of the properties of light beams. With their extraordinary resolution in space (~ 2 nm) and force (~ 0.050 pN) they became an irreplaceable tool for such purposes.

In our project we want to use them to study the elastic properties of single DNA molecules. Therefore we developed an easy and reproducible procedure for the immobilization of single double stranded DNA molecules 1000 to 6000 bp in length obtained by PCR between two micro particles supplying a general approach to address this problem.

A fully reversible elastic behaviour is found for the force-extension dependence of ds-DNA for relative elongations up to 50 % as described in the corresponding literature. It is not possible to describe the data for DNA >2000 bp with the wormlike chain model. This might be caused by the development of an underlying superstructure of the DNA chains.

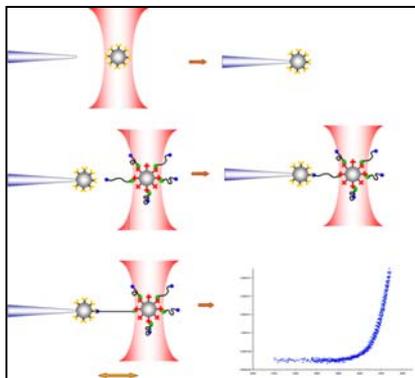


Fig. 1: Assembly of an "DNA"-bridge: A polystyrene bead modified with A-DIG antibodies was trapped in the optical tweezers (a) and then fixed at a glass micropipette. After this first step a SA labelled particle with immobilized DNA on its surface was trapped and brought closely to the particle that was fixed at the femtotip so that binding between the digoxigenine labelled end of the DNA and the antibodies on the surface of the particle could take place. The single DNA molecule immobilized by this procedure could now be stretched and the occurring forces were documented in a force extension plot.

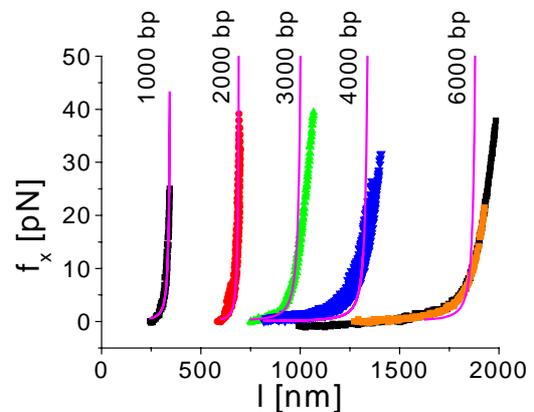


Fig. 2: Results of the Force extension measurements: DNAs of lengths between 1000 and 6000 bp were stretched and relaxed several times and the results were recorded in a force extension plot. The curves of the 1000 and 2000 bp DNA could be fitted with the wormlike chain model. Longer DNAs showed deviations from this fit. The reason for this is at the moment not clear.

References:

- [1] Allemand JF, Bensimon D, Croquette C; *Current Opinion in Structural Biology* **13**: 266-274 (2003).
- [2] Ashkin A; *PNAS* **94**: 4853-4860 (1997).
- [3] Baumann CG, Bloomfield VA, Smith SB, Bustamante C, Wang MD, Block SM; *Biophysical Journal* **78**: 1965-1978 (2000).
- [4] Baumann CG, Smith SB, Bloomfield VA, Bustamante C; *PNAS* **94**: 6185-6190 (1997).

2.10 Investigating DNA-binding proteins with optical tweezers

M. Salomo, K. Kegler, M. Struhalla, J. Reinmuth, V. Skokow, F. Kremer

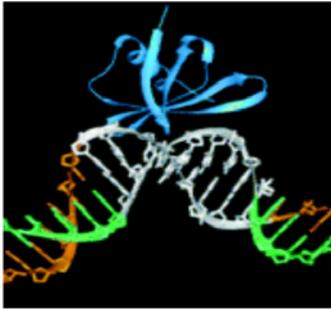


Fig. 1: Model of the binding mechanism of the Sac7d protein (blue) to a DNA helix.

ongs to a class of small chromosomal proteins in the hyperthermophilic archaeon *Sulfolobus riu*s. It is extremely strongly to the minor groove of ing a stable to heat, acid and chemical agents and) kinking of the DNA helix leading to a shortening of (Figure 1). Our project has the aim to investigate the of this DNA-binding protein on a DNA-double helix.) use the optical tweezers to measure the dimension rtening. Theoretically the protein binds to the DNA every 3 bp leading to a theoretical compaction ratio of ~1.2. For the experiment a single DNA molecule was immobilized as described previously.

The molecule was than stretched and relaxed several times and force extension plots (Figure 2) were recorded (black curve). Afterwards the Sac7e protein was injected into the cell. During the injection the stretching and relaxing cycles were continued (orange curve). When the protein reached the immobilized DNA the force/extension behaviour of the DNA changed rapidly (green curve). In the moment the protein began to bind the curve released from the plot were no protein was present what has to be interpreted as a shortening of the DNA because of the kinking induced by Sac7e. The achieved shortening of the DNA ranges between 120 and 170 nm that corresponds to a compaction ratio of 1,1-1,17. At higher forces the curve than passes into the plot without protein. That indicates that either the protein is not longer able to stand the applied forces at this moment and releases the DNA from its binding side but remains bound to the DNA or the protein is disrupted from the DNA and binds immediately again when the DNA becomes relaxed.

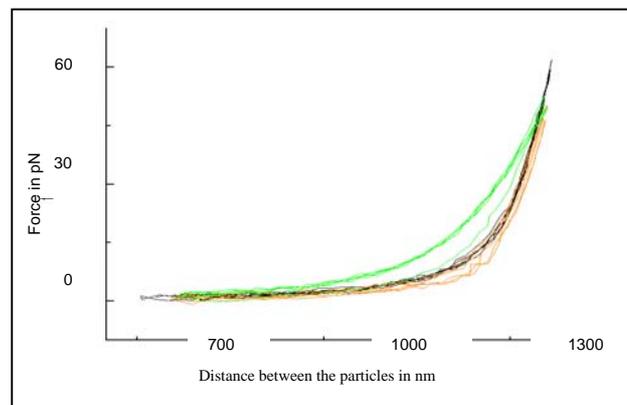


Fig. 2: Result of the binding experiment of Sac7e to a 4000 bp long DNA molecule

References:

- [1] H. Robinson et al.; Nature **392**, 202-205 (1998)
- [2] J.G. McAfee et al. ; Bioch. **34**, 10063- 10077 (1995)
- [3] D. Kulms et al.; Biol. Chem. **378**, 545-551 (1997)

2.11 Archaeal Nucleosome Formation by HMfB protein Studied using Optical Tweezers

F.Kremer, S. R. SethuNarayanan, M.Salomo, J.Reinmuth, W.Skokow

HMfB (Fig.1) belongs to a class histone proteins identified in the hyperthermophilic archaeon, *Methanothermus fervidus*. It is extremely stable to heat and acid, and binds strongly to the DNA to form a tetramer complex of nucleosome known as archael nucleosome in contrast to the octamer complex formed by eukaryotic core histones. The aim of this project is to investigate the archaeal nucleosome formation and stability under different salt and buffer conditions to discern the possible *in vivo* mechanisms of nucleosome assembly and dynamics. Plasmid vector carrying HMfB gene was overexpressed in *E.coli* and the protein was purified using affinity (Heparin columns) and gel filtration chromatography. This highly purified HMfB protein was subsequently used to study the nucleosome formation with a double-stranded 4000-bp DNA labelled with biotin and digoxigenin at their 5' end. The DNA was captured between 2 colloids using the previously well-established protocols in the lab. Briefly, anti-digoxigenin antibody coated 2.1 μ m polystyrene bead was held by using a glass micropipette while the streptavidin coated bead with 100-200 molecules of DNA was held by using the photonic potential. Force-extension data were obtained by image capturing using a CCD video camera and subsequent image analysis using an IDL program. Repeated measurements were carried out for the DNA with and without HMfB protein. The typical force-extension curve obtained with and without HMfB is shown in Fig. 2. The results suggest that the binding of HMfB to DNA is weak and future experiments will further precisely quantify the strength of this interaction between DNA and HMfB in the piconewton range.

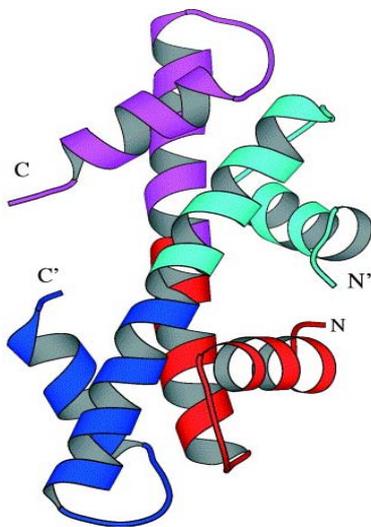


Fig.1: Ribbon structure of HMfB (69 amino acids long). One monomer is colored cyan (N-terminal, 1 to 35 residues) to blue (C-terminal, residues 36 to 69), and the other monomer is colored orange (N-terminal) to magenta (C-terminal).

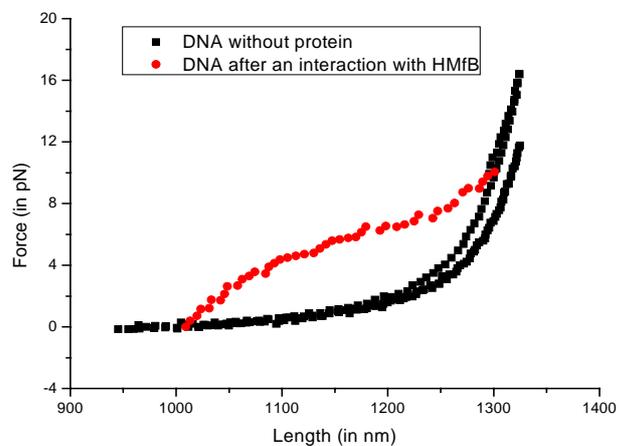


Fig.2: Force-extension curve of 4000bp dsDNA with (red) and without HMfB protein (black).

References:

- [1] ML. Bennink et al.; Nat Struct Biol. 8(7):606-10 (2001)
- [2] K. Sandman et al.; Meth. Enzymol. 334:116-129 (2001)

2.12 Nano- and microfluidics using optical tweezers with fast single particle tracking

C. Gutsche, S. K. Ayop, F. Kremer

Optical tweezers with fast single particle tracking are microscopic rheometric tools with nanometer resolution in space and subpico-newton resolution in force. The proposed project has two intentions (i) to contribute to basic questions in colloid- and polymer-research and (ii) to address technological problems of micro- and nanofluidics. In detail the following experiments are realised: 1.) Measurements of the force-distance- dependence between two isolated *single* colloids of which one is hold by a micropipette and the other by optical tweezers. 2.) Measurement of the flow profile of homogeneous and heterogeneous liquids in small confining geometries like plates with micrometer separation, microchannels, etc. with and without surface modifications (e.g. hydrophobization).

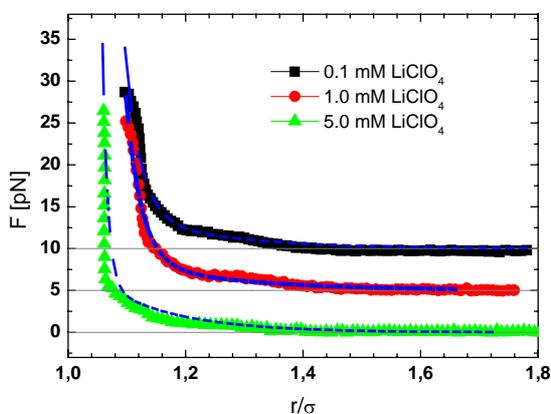


Fig. 1: Force-distance-dependence for two separated blank colloids (diameter σ : 2,23 μm) in differing surrounding medium.

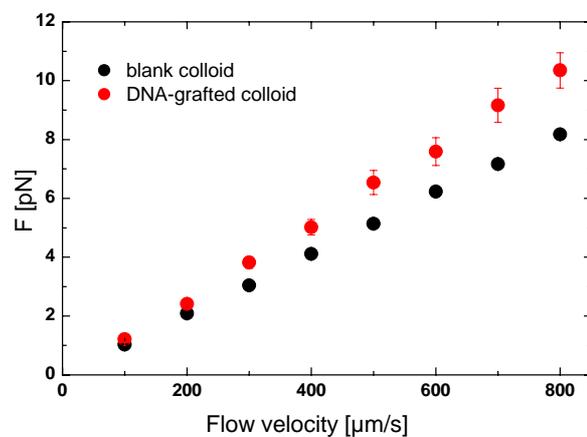


Fig. 2: Flow-resistance vs. velocity of the surrounding medium with respect to the colloid hold by optical tweezers. black: blank colloid of diameter $\sigma = 2,1 \mu\text{m}$. red: DNA-grafted colloids. Typically 1000 DNA-chains with a length of 4000 bp are grafted on one colloid.

Further planned investigations are: 1.) Measurement of the force-distance-dependence between a *single* colloid and a wall in the steady state and in fluid flow for coated and uncoated surfaces. 2.) Measurement of depletion forces between *single* colloids in the steady state and in flow for polymer solutions of varying concentration and for polymers of different topology. 3.) A fluctuation analysis of the Brownian motion of a colloid in an optical trap enables one to deduce the local *tensor* of viscosity. By that inhomogeneous microscopic structures like microchannels or living biological systems can be explored.

This project is funded as part of the DFG-Schwerpunktprogramm "Nano- und Mikrofluide: Von der molekularen Bewegung zur kontinuierlichen Strömung".

References:

- [1] van de Ven, , Colloidal Hydrodynamics in *Colloid Science: A series of monographs*, Academic Press (1989)
- [2] Crocker, J.C., Grier, D.G., Physical Review Letters 77, 1897-1900 (1996)

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2.13 The interaction between *single* colloids with and without grafted DNA as measured by optical tweezers

K. Kegler, F. Kremer

Optical Tweezers (OT) are extraordinary microscopic tools with nanometer resolution in 3-D space and subpico-Newton resolution in force. They are used to measure the interaction potential between single pairs of colloids (blank and grafted with (genetically engineered) DNA of varying length (1000 basepairs (bp), 2000 bp, 3000 bp, 4000 bp).

To measure the interaction potential the following experimental procedure is used: One particle is trapped by the optical tweezers (OT) and fixed at a glass micropipette (by capillary forces) being mounted on a piezoelectric actuator. A second particle is caught with OT and positioned with respect to the other (Fig.1)



Fig. 1 Scheme (a) of the sample cell to measure forces between two separated colloids and scheme (b) of the direction of motion

With this setup we investigate the interaction forces in two dimensions - parallel and perpendicular to the axis between the two colloids (Fig.2). Special emphasis is given to the change of the surrounding media, but as well tribological phenomena will be studied.

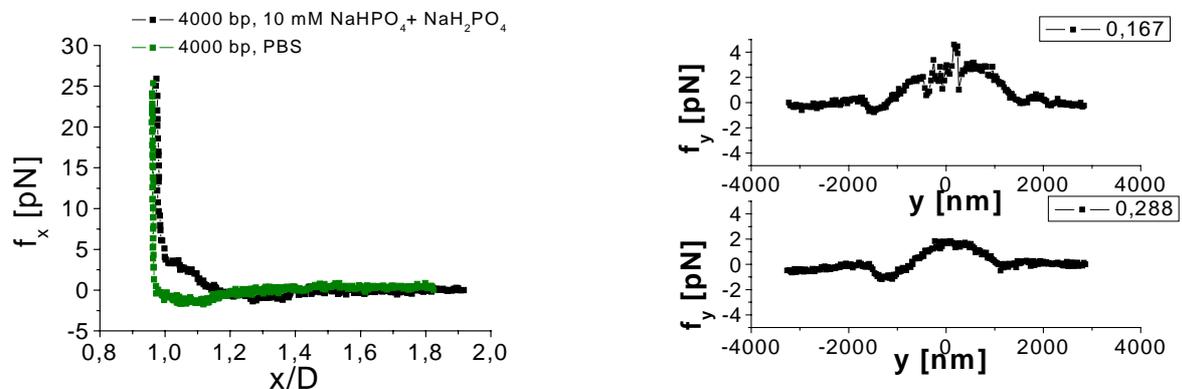


Fig. 2: (a) The interaction forces of DNA-grafted colloids in different buffers (x-direction[s. Fig.1b]), (b) The interaction forces in y-direction (s. Fig.1b) of DNA-grafted colloids without (separation between the colloid surfaces: $l=0,288 \mu\text{m}$) and with ($l=0,167 \mu\text{m}$) contact This project is supported within the DFG-Schwerpunktprogramm "Nano- und Mikrofluidik: Von der molekularen Bewegung zur kontinuierlichen Strömung".

References:

- [1] Urbakh, M., Klafter, J., Nature 430,525 (2004)
- [2] Wu, J., Proc. Natl. Acad. Sci. USA 95, 15169 (1998)

3. Publications

- [1] F. Kremer and R. Stannarius
"Molecular Dynamics of Liquids in confinement"
Bookchapter in "Molecules in Interaction with Surfaces and Interfaces", (Eds.: R. Haberlandt, D. Michel, A. Pöpl and R. Stannarius) (Lect. Notes Physics **Vol. 634**)
Springer-Verlag 2004, pp. 275-300, ISBN 3-540-20539-X
- [2] R. Stannarius and F. Kremer
Liquid Crystals in Confining Geometries"
Bookchapter in "Molecules in Interaction with Surfaces and Interfaces", (Eds.: R. Haberlandt, D. Michel, A. Pöpl and R. Stannarius) (Lect. Notes Physics **Vol. 634**)
Springer-Verlag 2004, pp. 301-336, ISBN 3-540-20539-X
- [3] Serghei, A.; L. Hartmann, P. Pouret, L. Léger, F. Kremer
"Molecular dynamics in thin (grafted) polymer layers"
Colloid & Polymer Science **282**, 946-954 (2004)
- [4] Tsui, J.; D. Appelhans, S. Zschoche, P. Friedel and F. Kremer
"Molecular dynamics in poly(ethene-*alt*-*N*-alkylmaleimide)s as studied by Broadband Dielectric Spectroscopy"
Macromolecules **37**, 6050-6054 (2004)
- [5] Ngwa, W.; R. Wannemacher, W. Grill, A. Serghei, F. Kremer and T. Kundu
"Voronoi tessellations in thin polymer blend films"
Macromolecules **37** (5), 1691 (2004)
- [6] Eremin, A.; H. Nádasi, G. Pelzl, S. Diele, H. Kresse, W. Weissflog, S. Grande
"Pараelectric-antiferroelectric transitions in the bent-core liquid-crystalline materials"
Phys. Chem. Chem. Phys. **6**, 1290-1298 (2004)
- [7] Pelzl, G.; M. W. Schröder, U. Dunemann, S. Diele, W. Weissflog, C. Jones, D. Coleman, N. A. Clark, R. Stannarius, J. Li, B. Das, S. Grande
"The first bent-core mesogens exhibiting a dimorphism B₇-SmCP_A"
J. Mater. Chem. **14**, 2492-2498 (2004)
- [8] Weissflog, W.; S. Sokolowski, H. Dehne, B. Das, S. Grande, M. W. Schröder, A. Eremin, S. Diele, G. Pelzl, H. Kresse
"Chiral Ordering in the nematic and an optically isotropic mesophase of bent-core mesogens with a halogen substituent at the central core"
Liquid Crystals **31** (7), 923-933 (2004)
- [9] Wang, R.; H. Schmiedel, B.-R. Paulke
"Isothermal titration calorimetric studies of surfactant interactions with negatively charged, 'hairy' latex nanoparticles"
Colloid Polym. Sci. **283**, 91-97 (2004)
- [10] Li, J.; D. Geschke, R. Stannarius
"Proton NMR investigation of a hydrogen-bonded liquid crystal gel"
Liquid Crystal **31** (1), 21-29 (2004)
- [11] Goede, K.; P. Busch, M. Grundmann
"Binding Specificity of a Peptide on Semiconductor Surfaces"
Nanoletters **4** (11), 2115-2120 (2004)
- [12] Kremer, F.; K. Kegler, C. Gutsche, J. Reinmuth, W. Skokow, M. Salomo, S. Narayanan, M. Struhalla
"Optische Pinzetten zur Untersuchung der Wechselwirkung von Proteinen mit einzelnen DNA-Ketten"
BIOForum (GIT Verlag) **12**, p 47 (2004)

- [13] Li, J.; D. Geschke
"Pyroelectric investigations of a hydrogen bonded ferroelectric liquid crystal gel by LIMM"
Polym. Adv. Technol. **16**, 11-18 (2005)

- in press -

- [14] Tsuwi, J.; D. Appelhans, S. Zschoche, R-C. Zhuang, P. Friedel, L. Häußler, B. Voit, F. Kremer
"Molecular dynamics in fluorinated side-chain MI copolymers as studied by Broadband Dielectric Spectroscopy"
Colloid & Polymer Science 10/2004, in press
- [15] Tammer, M., J. Li, A. Komp, H. Finkelmann, F. Kremer
"FTIR-spectroscopy on segmental reorientation of a nematic elastomer under external mechanical fields"
Macromol. Chemistry Rapid Commun. 12/2004, in press
- [16] Serghei, A.; H. Huth, M. Schellenberger, C. Schick and F. Kremer
"Pattern formation in thin polystyrene films induced by an enhanced mobility in ambient air"
Phys. Rev. E 2004, in press
- [17] Serghei, A.; Y. Mikhailova, K.-J. Eichhorn, B. Voit and F. Kremer
"Molecular dynamics of hyper-branched polyesters in the confinement of thin films"
Eur. Phys. J. E 2004, in press

4. Financial support

Prof. Dr. F. Kremer

Optische Pinzette als mikroskopische Sensoren und Aktuatoren zum Studium der Wechselwirkung zwischen einzelnen Biomolekülen
SMWK-Projekt 7531.50-02-0361-01/11 (2001-2005)

Prof. Dr. F. Kremer

DFG-Teilprojekt im Rahmen des Schwerpunktprogramms "Nano- und Mikrofluidik: Von der molekularen Bewegung zur kontinuierlichen Strömung"
DFG-Schwerpunktprogramm 1164, KR 1138/14-1 (2004-2006)

5. Graduations

Promotions:

Dipl.-Phys. Heidrun Schüring

12.01.2004

"Mechanische und optische Untersuchungen freitragender smektischer Filme"

Dipl.-Phys. Thomas John

21.06.2004

"Experimentelle und theoretische Untersuchungen zur stochastisch getriebenen Elektrokonvektion in nematischen Flüssigkristallen"

Dipl.-Phys. Lutz Hartmann

30.08.2004

"Untersuchung der molekularen Dynamik in dünnen Polymerfilmen mittels dielektrischer Spektroskopie"

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Comtech GmbH

München, Germany

Freudenberg Dichtungs- und Schwingungstechnik KG

Weinheim, Germany

7. Patent application

Deutsches Patentamt, Nr.: 10 03 05 110.4-52

"Verfahren zur Feststellung wenigstens eines Zustandsparameters eines Dichtungssystems sowie Dichtungssystem

Erfinder: Prof. Dr. F. Kremer