

Color Transitions in Monolayers of a Polymerizable Single-Chain Diacetylenic Lipid

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We report on the polymerization kinetics of floating and supported monolayers of the lipid ethyl morpholine pentacosadiynoic amide. The in situ absorption measurements on a floating monolayer at the air/water interface enabled us to study the reaction kinetics of the diacetylenic UV-triggered photopolymerization. The rate constants for a first-order consecutive reaction $M \rightarrow B \rightarrow R$ (monomer to blue polymer to red polymer) were determined. The mechanical action of the atomic force microscope (AFM) tip had an immense impact on the scanned regions of the deposited film, changing locally the properties of the monolayer toward the red, fluorescing moiety. Distinct levels of fluorescence were observed which were contributed to distinct levels of interaction of the AFM tip with the monolayer. The results suggest that the spectral shift arises from a slight rearrangement in the packing of the side chains in the polymeric backbone.

Introduction

The quest for better and modern biosensors has long since drawn its attention to ultrathin films of polydiacetylenes. This class of substances features several unique physical exploitable properties such as high third-order nonlinear response, strong dichroism, photoconductivity because of the extended conjugation of the polydiacetylene backbone, low dimensionality, and intense intrinsic fluorescence with high anisotropy.^{1–14} Particularly interesting is the property of many of these materials to display a large, reversible or nonreversible spectral shift depending on their chemical, thermal, or mechanical treatment (chemo-, thermo-, and mechano-chromism).^{3,5,6,15–28}

Mixtures of headgroup-modified polydiacetylenes have been used in biomembranes and for reversible immobilization of proteins in a biocompatible way.^{29–34} Even the adsorption of viral fractions to an adequate film has been shown to lead to a spectral shift of the absorption maxima (biochromism).^{24,31–33} Monomolecular films of polydiacetylenes have, e.g., proven to withstand the mechanical stress resulting from contact scanning by atomic force microscopy (AFM) on a molecular level.^{35–38} In the following study we report on the kinetics of the spectral shift of floating and supported monolayers of the polymerizable lipid ethyl morpholine pentacosadiynoic amide (EMPDA) and support the results of Lio et al.³³ in explaining the nature of the spectral shift by a slight rearrangement in the packing of the polymer crystal.

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Materials and Methods

Chemicals. The polymerizable lipid EMPDA was a kind gift of the Bicocircuits Corp., Sunnyvale, CA. Its basic properties as a monolayer forming lipid are published in detail elsewhere.³⁹ All chemicals were purchased by Sigma unless otherwise stated. Solvents were of HPLC grade.

Spectral in Situ Measurements. The in situ spectral data of a monolayer floating at the air/water interface were obtained at a surface pressure of 25 mN/m, leading to a crystalline packing of approximately 25 Å² per molecule on a thermostated Langmuir trough with dimensions of the free subphase surface of 450 × 150 mm² (KSV 5000, KSV-Instruments Ltd., Helsinki, Finland) in a class 100 cleanroom hood. The absorption measurements were carried out with a glass-fiber-equipped spectrophotometer (Photal MCPD-100, Otsuka electronic, Osaka, Japan) and a halogen lamp (Otsuka MC 963) driven with 30 V and 7.5 A. The ends of the optical fibers were mounted to the dipper device of the Langmuir–Blodgett (LB) setup and carefully driven to a close distance from the water surface. They were mounted in a way that the light penetrated the air/water interface at an angle of 45°. The light beam was reflected by an aluminized mirror at the bottom of the trough and collected by another glass fiber, which carried it to the input slit of the spectrophotometer. The spectra were taken every 0.5 s with typical sampling times of 25 ms and averaged out of two samples. The polymerization was induced by a 30-W Hg low-pressure lamp which had the main emission peak centered at 252 nm. The lamp was switched on at least 10 min before polymerization and kept at a 0.1-m distance from the air/water interface. Absorption spectra were digitized, and the peak values normalized and further analyzed with a computer.

Solid Supports and Monolayer Transfer. Quartz microscope slides and silicon wafers with a thermally grown oxide layer of 180 nm thickness (a kind gift of Wacker Chemitronic, Burghausen) were thoroughly cleaned prior to use following the procedure given by Sullivan et al.³⁹ The samples were used either as hydrophilic substrates for Langmuir–Blodgett transfer (vertical dipping) of the monolayers (upstroke only, 50 μm/s) or hydrophobized as needed for the Langmuir–Schäfer transfer (horizontal dipping).³⁶ The hydrophobization was carried out by dipping the clean substrates for 10 s into a mixture of 80% *n*-hexadecane, 20% chloroform and 0.1% octadecyltrichlorosilane (OTS) followed by rinsing in pure chloroform. The prepared glass slides were horizontally pushed through the air/water interface including the floating monolayer, carefully kept under water, and mounted as a second window into a flow chamber equipped with a Pt 100 thermosensor. For all solid supported monolayers the film balance was a custom-built, thermostated system with an accessible area of 415 × 90 mm².³⁸ Transfer parameters were 25 mN/m surface pressure and 12 °C subphase temperature.

Ex Situ Optical Measurements. Absorption measurements on solid supported monolayers either in air (after LB transfer) or buffer (after Langmuir–Schäfer (LS) transfer) were carried out in a stationary spectrophotometer (Lambda 15, Perkin-Elmer, FRG). The absorption spectrum for the monomeric film was taken for background correction. The polymerization reaction was induced by illuminating the films on the solid support with a handheld Hg lamp (emission at 254 nm, PenRay 6035, LOT-Oriel, FRG), which was kept 0.1 m from the sample. The irradiation times used were so short that the warming up of the sample was not considered as a problem. The buffer of the flow chamber (150 μL volume) was continuously exchanged by a peristaltic pump (Melles Griillot, France) with a rate of 500 μL/min. The buffer reservoir was either continuously heated and the temperature within the flow chamber monitored or equipped with subsequent buffer media, which had been proven to be successful on the film balance³⁸ and been monitored for pH values by a Radiometer pH-meter.

Polymeric solid supported films were investigated by a custom-built fluorescence spectrometer attached to a sensitive video microscope setup as described elsewhere.³¹ The employed dichroic mirror filter set (Zeiss IV Fl, Oberkochen, FRG) serves regularly for imaging TexasRed-fluorescence (Molecular Probes) and uses

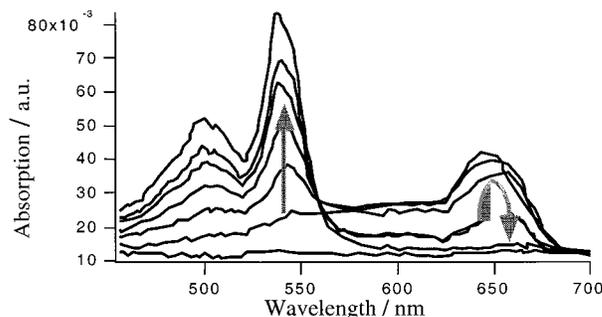
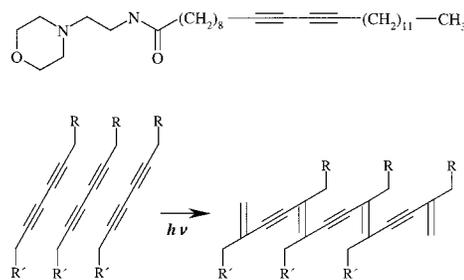


Figure 1. Consecutive in situ absorption spectra of the photopolymerization of a monolayer of EMPDA at the air/water interface at a subphase being thermostated to 5 °C. The absorption curves were taken at illumination times of 0, 2, 4, 7, 15, and 25 s and 3 min. Quantitative data may be found in Figure 2. The inset shows a schematic drawing of the molecule EMPDA and its photoinduced polymerization reaction.

a 530–585 nm passband filter for the excitation and a 620 nm cutoff filter for the emission detection. A 50-W Hg lamp (HBO 50, Osram, FRG) served as the light source.

Atomic Force Microscopy. AFM measurements were conducted on LB-transferred monolayers on silicon-oxide wafers. A Nanoscope III AFM (Digital Instruments, Santa Barbara, CA) with a 150 μm piezo scanner and standard pyramidal Si₃N₄ tips (NP, Digital Instruments) was used. Since all the experiments were carried out under ambient conditions in air, the loading force was quite high (typically 20 nN). The samples were subsequently investigated by the forementioned fluorescence video microscope.

Results and Discussion

The in situ absorption measurements at the air/water-interface on a floating monolayer of EMPDA showed the well-known characteristics of the diacetylenic UV-triggered photopolymerization^{15,17} (insert in Figure 1). After the onset of the illumination, the absorption spectrum first showed the appearance of a prominent maximum at approximately 650 nm. During continued illumination, this peak decreased in intensity and synchronously a peak at 540 nm developed (Figure 1). The plot of the absorption spectra as a function of irradiation time showed the existence of an isosbestic point at ca. 555 nm (Figure 1), in agreement with earlier reports.^{15,17,40} This corroborates the direct transition within the polymer formation from a blue looking (absorbance maximum at approximately 650 nm) to a reddish appearing moiety (absorbance maximum at approximately 540 nm).^{15,17,18,40}

It was reasonable to use the chronological development of the absorption maxima to determine the reaction kinetics of the UV-induced polymerization of this particular diacetylene lipid as a floating monolayer. Figure 2 shows the progress of the reaction at different subphase temperatures, separated into the development of the two

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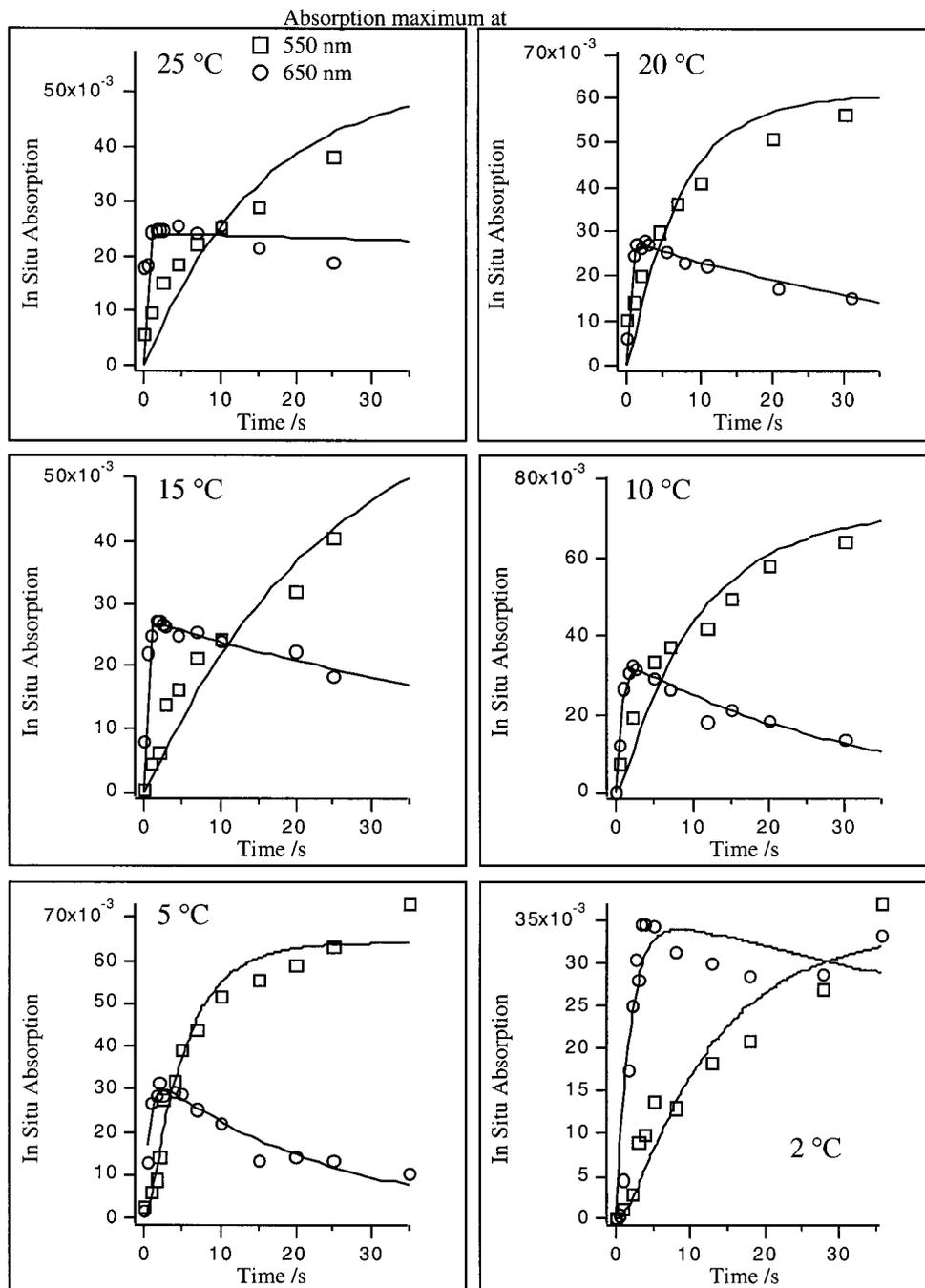


Figure 2. Time-resolved development of the in situ absorbance maxima at approximately 650 and 540 nm for 2, 5, 10, 15, 20, and 25 °C subphase temperature. Each data point was obtained by subtracting from the originating peak value the minimal absorbance value of the original spectrum.

distinct absorption maxima. The curves clearly show the described fast onset of the reaction, leading to the formation of the blue moiety and a subsequent delayed formation of the red moiety, while the blue form decays (the absorbance intensity decreases).

Treating the photopolymerization to be of a first-order consecutive reaction $M \rightarrow B \rightarrow R$ (monomer to blue polymer to red polymer),^{6,26,41} the solid lines in Figure 2 show the fit curves of the reactions under the assumption that the monomeric EMPDA monolayer forms the blue moiety with a reaction rate constant k_1 and this automatically transits with the rate constant k_2 to the stable red moiety.⁴²

The obtained rate constants at the accessible temperatures are plotted in a log k vs $1/T$ plot in Figure 3. For the start reaction (formation of the blue polymer) an apparent activation energy of 82 ± 49 kJ/mol was observed. For the subsequent blue-to-red transition the corresponding value was -7 ± 27 kJ/mol. The value for the first reaction is consistent with the published bulk value of 92 kJ/mol.⁵ The relatively large errors arise from the instability and drift of the spectrometer and the detector because of the fact that the single monolayer of a chromophore having a relatively small extinction coefficient gave a weak absorption signal.

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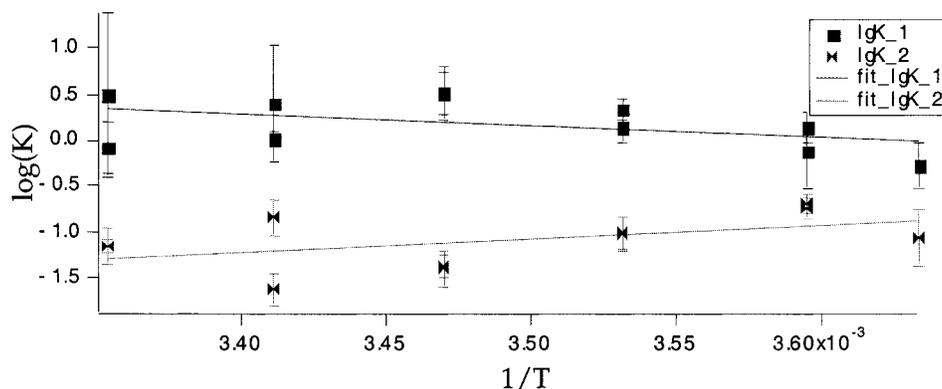


Figure 3. Arrhenius ($\log k$ vs $1/T$) plot of the calculated rate constants. The slopes of the curves give the rate constants for the two consecutive reactions: A monomer to a bluish polymer and a bluish polymer to a reddish polymer.

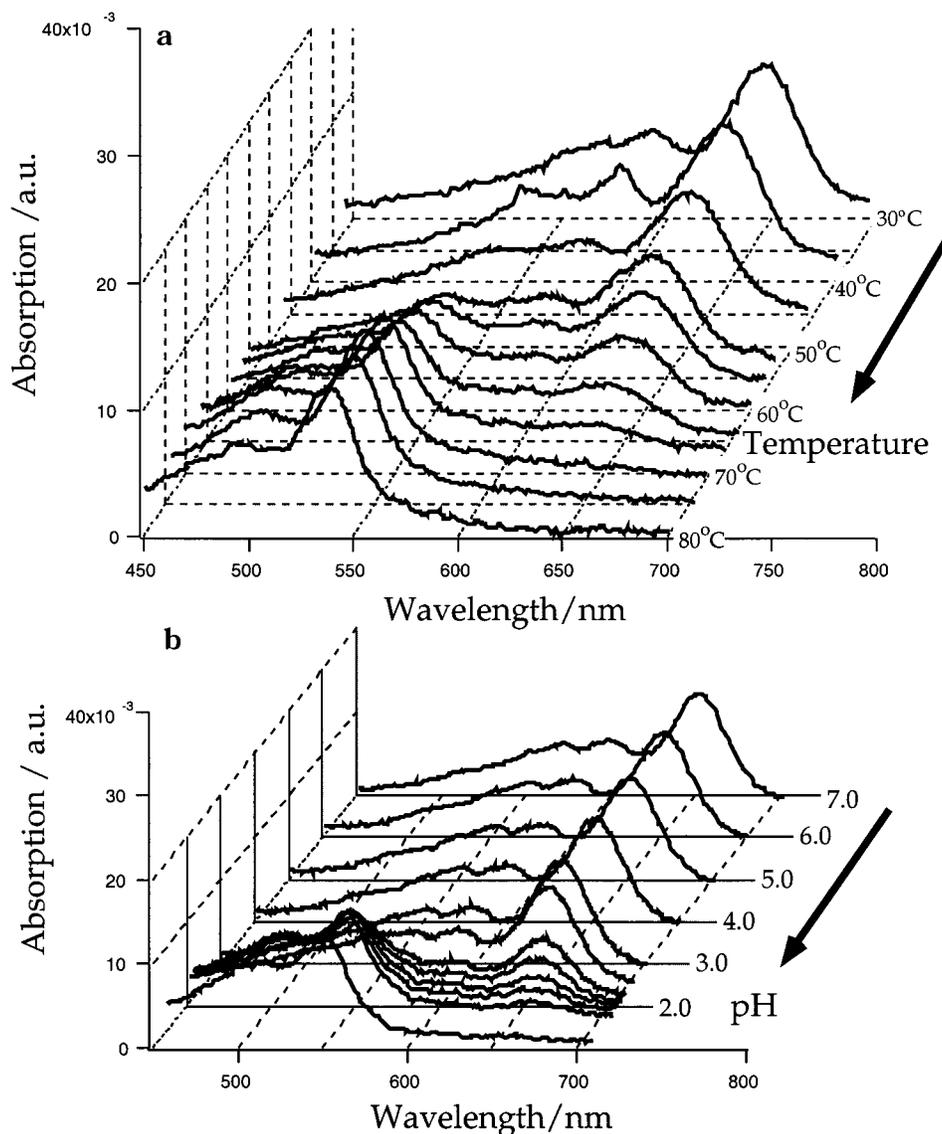


Figure 4. The blue–red transition of a horizontally (LS-) transferred EMPDA monolayer on an octadecyltrichlorosilane (OTS) hydrophobized quartz glass. The monolayer was polymerized in situ by a hand-held Hg lamp. In part a the transition was triggered by slow heating of the surrounding buffer (the heating rate was approximately $1\text{ }^{\circ}\text{C}/\text{min}$). In part b the transition was triggered by a slow drop of the pH of the surrounding buffer from pH 7.0 down to pH 1.5.

Assuming the two maxima to be evidence for two distinct moieties of a polydiacetylene monolayer, the application of the well-known laws of first-order reaction kinetics is reasonable. The resulting values of the rate constants k_1 and k_2 at certain temperatures vary quite substantially,

whereas the overall activation energy for the first polymerization step lies within the boundaries given in the literature. The scattering of the k values and large sigma-value of the activation energy indicates, besides the sources of error of the measurement listed above, that the system

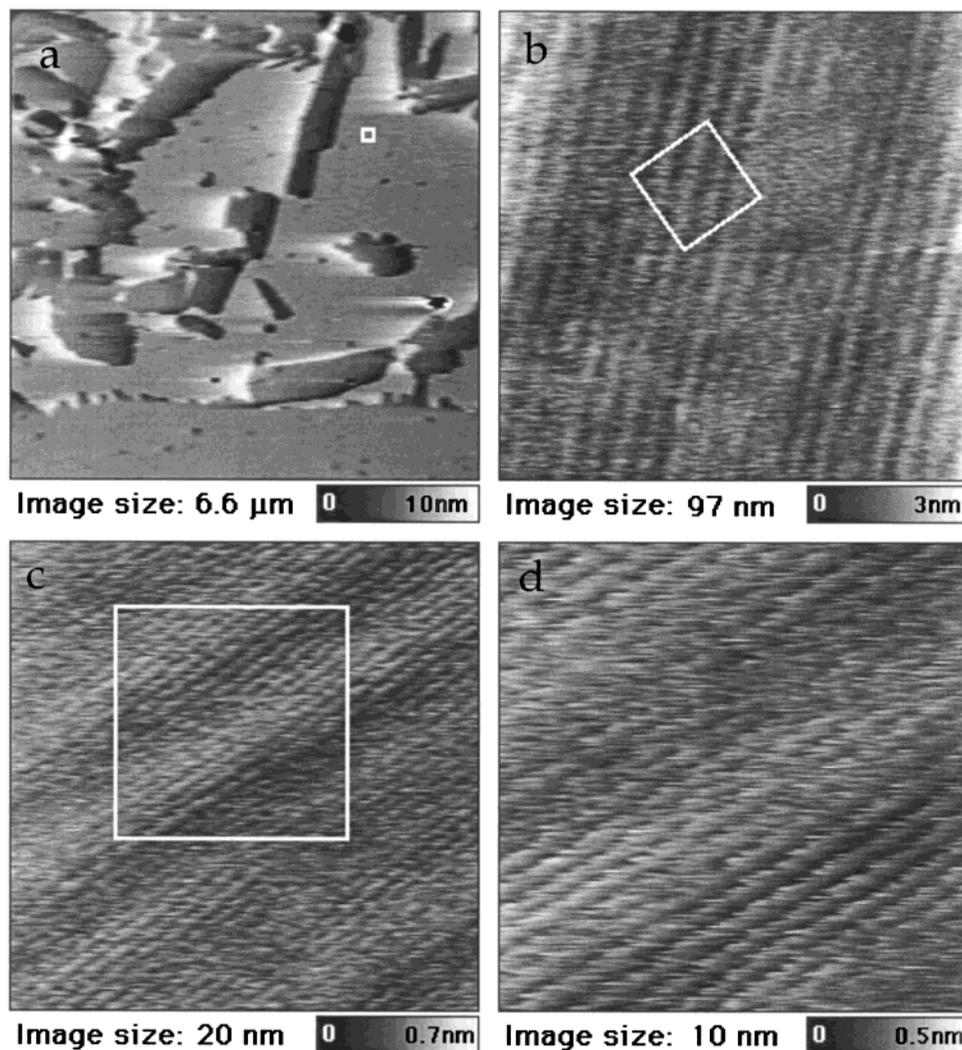


Figure 5. AFM images of a "blue" EMPDA monolayer with increasing resolution. The image sizes are (a) 6.6 μm , (b) 97 nm, (c) 20 nm, and (d) 10 nm. The AFM scans were taken on a blue film, resulting from a horizontally (Langmuir–Schäfer) transferred, unpolymerized but crystalline ($0.26 \text{ nm}^2/\text{molecule}$) EMPDA film which was polymerized by UV irradiation with a PenRay lamp for 3 min.

is thermodynamically very sensitive. In any case, the first appearing blue state seems to be a metastable local minimum of the energy surface of this polymer which easily decays into the more stable red form. This conclusion is supported by the fact that the red state could not be reversed to the blue state either by expansion of the monolayer and lateral pressure drop or through a pH change of the subphase as stated by Mino et al. for different diacetylenic lipid monolayers.^{27,28}

Despite the irreversibility of the blue–red transition, it was possible to selectively preserve the intermediate blue state within an EMPDA monomeric monolayer by first transferring it in its quasi-crystalline state onto a solid support (glass or silicon wafer) and then UV-polymerizing it. The polymerization in the monomeric crystalline state is thought to be topochemical.¹ On a solid support the reaction always led to the formation of the blue state regardless whether it was transferred by the LB technique on a hydrophilic or by the LS technique on a hydrophobic support. The first feature was tested by taking absorption spectra prior to UV polymerization of an LB-transferred, monomeric, quasi-crystalline monolayer on glass (data not shown). The latter was shown by mounting the quartz glass supported, horizontally transferred monolayer as a second window of a flow chamber, taking the absorption spectra of the unilluminated mono-

layer, and then illuminating it through the quartz glass support. Exceptional care was taken not to expose the horizontally transferred monolayer to air, which might lead to a reorganization of the whole film. The UV polymerization of an LS-transferred monolayer was carried out by illuminating it for 3 min through the quartz glass support with the PenRay UV lamp.

The photopolymerization on solid support led to the formation of the blue state (maximum of the absorption at 650 nm). Figure 4 shows the change of the monolayer absorption when the buffer within the flow chamber was either heated or successively exchanged with more acidic buffer. Both treatments resulted in an irreversible shift of the absorption maximum toward the red moiety. The thermochromic shift occurred within a temperature range of 55–70 $^{\circ}\text{C}$, whereas the buffer exchange led to a sharp chemochromic shift at $\text{pH } 2.5 \pm 0.1$. This sharp transition can be attributed to the increasing ratio of protonated to unprotonated morpholine headgroups. LB-transferred and then polymerized monolayers could preserve the blue state for as long as 1 day, when stored under ambient conditions. On exposure to HCl vapor the blue film turned immediately red according to the expected chemochromic effect.

High-resolution AFM studies have revealed a hexagonal molecular packing of the red moiety only. It has been

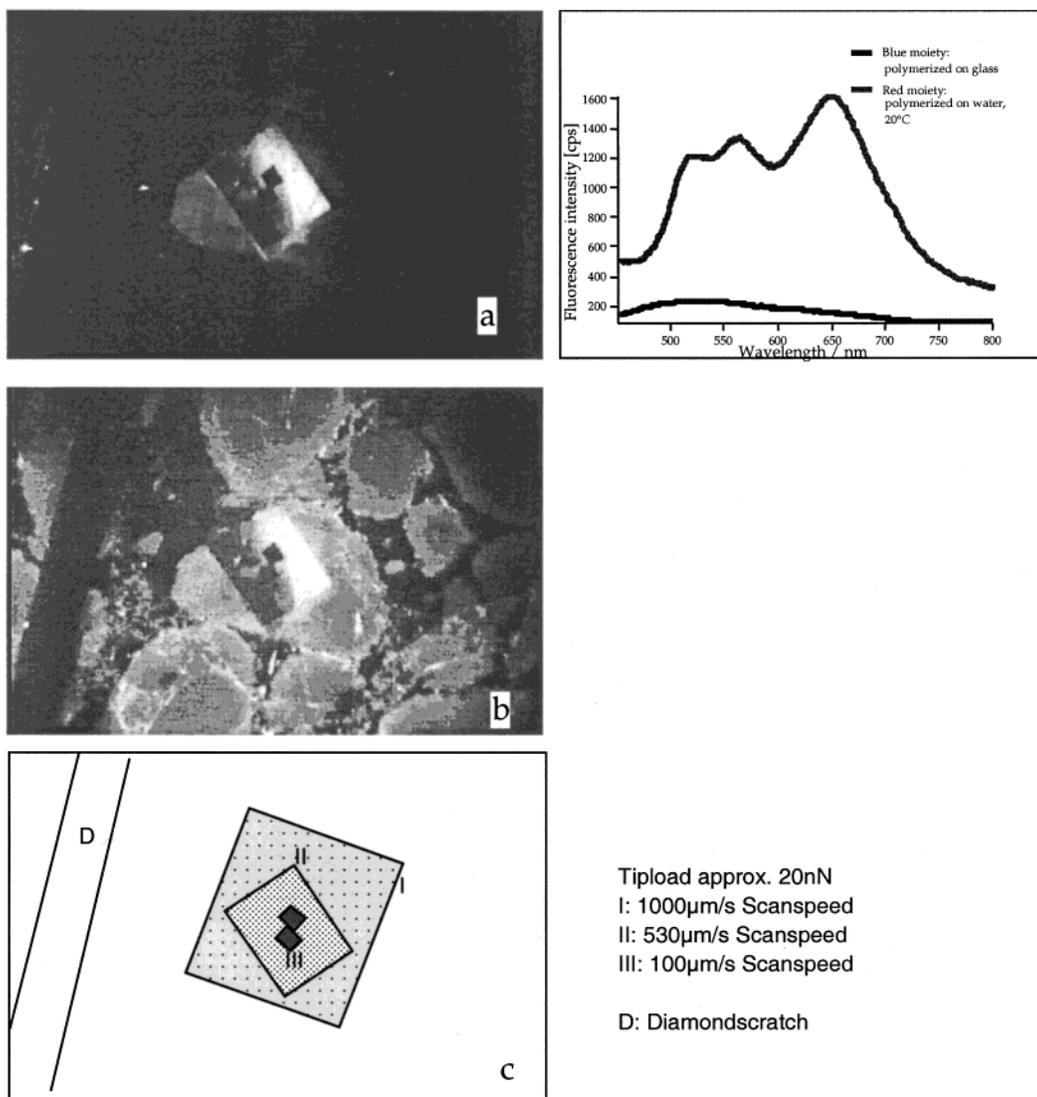


Figure 6. The blue–red transition triggered by scanning a solid supported and there polymerized monolayer of EMPDA with AFM in air (loading force approximately 20 nN, Si₃N₄ tip). The transition is detected through the fluorescence of the red moiety. The inset shows the difference of the fluorescence spectra of an EMPDA monolayer polymerized on the subphase (red moiety, strong intrinsic fluorescence) and on the solid support (blue moiety, almost no intrinsic fluorescence). The partial picture a shows a strongly fluorescent structure as seen by fluorescence microscopy of the prior AFM-imaged area. The partial picture b shows the same area, but after additional exposure to a pH-drop by HCl vapor. The partial picture c shows schematically the distinct rectangular areas of (a) being imaged with different scan speeds: (I) 1000 μ m/s; (II) 530 μ m/s; (III) 100 μ m/s.

reported that the lattice constants and the polymer backbone direction are sensitive to the friction induced by the scanning tip. Even though the blue monolayer appeared flat on a micrometer scale (Figure 5a), it showed a distinct wavy structure in the mesoscopic dimensions (Figure 5b,c). This structure could be traced down to the nanometer scale, but the lattice was resolvable perpendicular to the waves only (Figure 5d). The structure parallel to the waves appeared blurry and unclear. The Fourier transform spectrum of the image revealed a cubic rather than a hexagonal pattern.

For all AFM measurements care was taken to apply an as low as possible loading force to the sample. The mechanical action of the AFM tip had nevertheless an immense impact on the scanned regions: starting with a blue polymeric monolayer on a solid support, which does not show intrinsic fluorescence at wavelengths >620 nm under illumination with filtered (<585 nm) light of a Hg lamp (insert in Figure 6), the scanning action of the tip changed locally the properties of the monolayer toward the red fluorescing moiety (Figure 6a). The monolayer

fluorescence could be activated even more easily by exposing the nonfluorescent blue form to HCl vapor (Figure 6b), which was used as a positive test after each scanning experiment.

These experiments support the results of Lio et al.,³³ stating that diacetylenic color transitions are caused by alkyl side chains straining the conjugated backbone: Exposing the monolayers to HCl vapor protonates at least the morpholine headgroups of the molecules thus forcing the “lower” side chains of the polymer to rearrange laterally, driven by electrostatic repulsion. The scanning mechanism of the AFM tip, however, not only probes but manipulates the “upper” side chains of the polymer. It induces high shear rates on them thus forcing them to rearrange. Again, this will strain the polymer backbone and change the state of it, thus triggering the blue-to-red transition and switching on of the intrinsic fluorescence. We suggest that it is a disordering process, not a phase transition, that is enough to trigger the spectral shift. There is little reason to believe that the mechanical action of an AFM tip on the monolayer would result in a new,

ordered phase, despite the fact that the properties of the new state resemble those of the hexagonal red polymer film. To unambiguously demonstrate the existence or absence of a phase transition would require additional scattering or diffraction data which we unfortunately lack here.

Different levels of fluorescence as a function of scan speed are demonstrated in Figure 6. In agreement with Rädler et al.,³⁷ we can see three levels of fluorescence caused by different magnitudes of indentation of the tip into the side chain area. Rädler et al. called this phenomenon a "water-skiing effect", since only during the slowest scan (100 $\mu\text{m s}$) the tip was able to dive substantially into the monolayer and thus at most modify the film structure and hence quench the fluorescence. With higher scan speeds, the AFM probe was "skiing" on the layer, thus interacting and rearranging the superficial side chains only.

Conclusions

A monolayer of the single-chain diacetylenic lipid EMPDA in its quasi-crystalline state undergoes a chemical polymerization reaction during UV illumination, which was here monitored by in situ absorption spectroscopy of the floating monolayer. The time-resolved spectra showed the distinct feature of the color change since they exhibited an absorption maximum at about 650 nm within fractures

of the first second which vanished on the favor of an increasing absorption maximum at 540 nm.

The transient blue state could be preserved for a certain time, and the transition of the blue, nonfluorescent EMPDA polymer film to the final and fluorescing red state could be triggered either under water by annealing or pH drop or in air by HCl vapor exposure or microscopic mechanical stress. In the latter case, distinct levels of fluorescence were observed which we contribute to distinct levels of interactions of the AFM tip with the monolayer. In conclusion, our results corroborate the strain of the side chains on the polymeric backbone as the reason for the blue-to-red transition of the diacetylenic polymers. To our best knowledge, this study is the first to support this point of view on a monolayer of polymerized diacetylene lipids.

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