Reversible Color Switching and Unusual Solution Polymerization of Hydrazide-Modified Diacetylene Lipids

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Abstract: Layer systems composed of polydiacetylene lipids are known to undergo an irreversible colorimetric transition from blue to red upon exposure to an external trigger (e.g., heat, pH change, specific ligand−target interaction). These optical transitions offer numerous possibilities toward the development of molecule-based sensory materials. Polymerization of the diacetylene systems usually occurs only in a highly ordered phase as a topochemical reaction, if the diacetylenic units are packed in an optimal orientation. A novel system based on hydrazide derivatives of single-chain diacetylene lipids is described. These materials show an unusual aggregation and polymerization behavior in organic solution, in contrast to the parent carboxylic acids. In addition, these hydrazide lipids undergo an unprecedented *reversible* color change (blue/red) in polymerized vesicles when the pH of the surrounding aqueous medium is cycled between acidic and basic conditions. This unusual behavior is attributed to the unique hydrogen-bonding pattern of the hydrazide headgroup, and was investigated by ^{1}H NMR, FTIR, UV−vis spectroscopy, and transmission electron microscopy. From the experimental data an ab initio computer model was developed to relate the observed colorimetric properties to intermolecular interactions.

Introduction

The irreversible blue to red color transition observed in polymeric diacetylene layer systems (vesicles and Langmuir−Blodgett films) has been used as a platform for the development of biological sensors.[1−5] In these conjugated systems, the color change is induced through binding of a target from the surrounding medium to a specific ligand which is attached at the surface of the polydiacetylene layer. The polymer itself is formed by UV irradiation after the monomeric diacetylene lipids have self-assembled into ordered layer structures. The mechanism of diacetylene polymerization is quite different from that of other polymer systems that usually polymerize in solution or in the liquid state. Diacetylene polymerization occurs only when the material is in a highly ordered state (i.e., topochemical or solid-state polymerization).[6−8] It requires an optimal packing of the diacetylenic units to allow propagation of the linear chain polymerization through the ordered phase. The packing of monomers in a crystalline lattice is determined by the side groups attached to the diacetylene units and hence can be tailored by choosing the appropriate substituents or headgroups, in the case of lipids.

The polymeric backbone formed is composed of alternating double and triple bonds, and the extent of conjugation depends on its conformation.[9−11] In an optimal conformation, which is substantially controlled by the side chains, the conjugated polymer absorbs light at approximately 650 nm, giving it a blue appearance that is easily perceived by the eye. If the effective conjugation length is reduced due to strain and torsion imposed onto the backbone, the absorption maximum is shifted to about 550 nm corresponding to a bright red color. The backbone distortion can be induced by order−disorder transitions in the side chains through exposure to heat,[12−14] organic solvents,[15,16] pH and salt changes,[17,18] or mechanical stress,[19−22] or, in the

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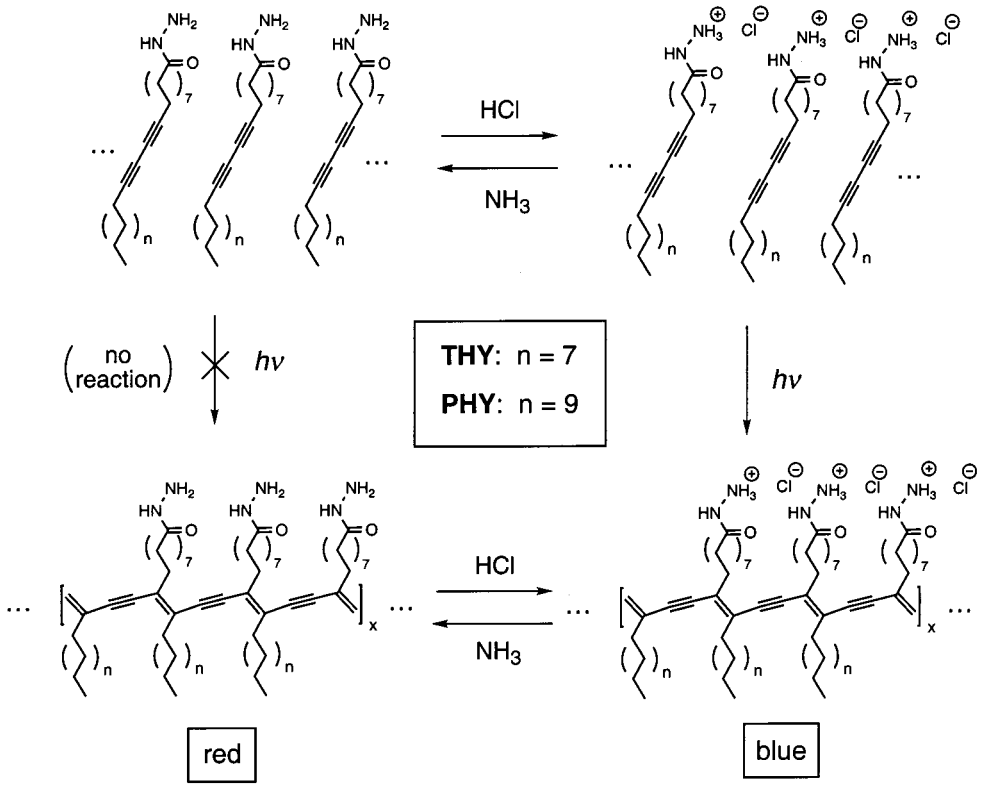
Scheme 1. Schematic Representation of the Polymerization Behavior and Colorimetric Changes of the Diacetylene Hydrazides PHY and THY in the Presence of HCl or NH_3[a]



[a]Only the HCl salt can be photopolymerized to yield a blue polymer, which reversibly converts to the red polymer upon base treatment.

case of biosensors, by the specific interaction between a surface bound ligand and its complementary target.[1−5] Another mechanism for the thermally induced color change is the transition between two ordered states, as shown for Langmuir−Blodgett films by surface force microscopic experiments.[23,24] In this case the parallelogrammatic (distorted rectangular symmetry) packing of the lipid monomers is locked into place by polymerization to yield a metastable blue phase. Upon heating, the lipid side chains rearrange into an energetically more favorable hexagonal packing for the polymeric layer, with reduced effective conjugation length and a red appearance.[25,26] The colorimetric shift of polydiacetylene lipid systems from blue to red is usually irreversible, and reflects a transition from a thermodynamically metastable blue form to a lower energy red form.

A specific and useful property of diacetylene lipids is their ability to form self-assembled structures composed of molecular layers such as vesicles or Langmuir−Blodgett (LB) films. Such layer systems are ideal for sensor applications since the surface-to-volume ratio is very large, and binding of a potential target occurs only at the surface of the polydiacetylene layers. The molecular structure of the lipid can determine both the ability to form stable films and vesicles and the polymerization and chromic behavior. The key structural factors for single-chain lipids include the total chain length, position of the diacetylenic unit within the chain, and chemical nature of the headgroup. Interactions between neighboring lipid molecules via their headgroups are especially important for the propagation of an induced point distortion across the layer (which is significant for signal amplification in sensors) and can be tailored by synthesis. The hydrazide lipids described here are particularly interesting in two major aspects: (1) the hydrazide function may be linked to electrophiles, such as aldehydes and ketones, to decorate the polydiacetylene layer surface with specific ligands and other functionalities, and (2) the hydrazide group has the intrinsic ability to form strong hydrogen bonds to neighboring hydrazide moieties which can be altered by protonation of the terminal nitrogen. The synthesis of the hydrazides was achieved in two steps by converting the diacetylene carboxylic acid into the hydroxysuccinimide ester with carbodiimide as a condensation reagent. The activated ester was then transformed into the hydrazide by treatment with hydrazine hydrate.

Like their parent carboxylic acids, the synthesized hydrazide lipids are capable of forming vesicles or LB films. In contrast to other diacetylene lipids with acid or amide headgroups, the hydrazides show an unusual monomer gelation and polymerization behavior in CH_2Cl_2 and $CHCl_3$ solution. This indicates that ordered aggregates exist in organic solution that are stabilized by specific attractive interactions between the hydrazide headgroups. Furthermore, the polymerization behavior in hydrazide vesicles and films depends strongly on the pH of the surrounding aqueous medium in that photopolymerization only occurs at low pH. The color of the polymeric vesicles also depends on the pH and can be reversibly switched between the blue and red forms by the addition of acid or base, respectively. This pH-controlled reversibility of the colorimetric transition is very unusual and is not shown by other polydiacetylene vesicle systems investigated so far.[17] The correlation among pH, polymerization efficiency, and color is illustrated in Scheme 1. The lipids were investigated by various spectroscopic methods in organic solution, solid films, and vesicles to explore the

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specific role of the hydrazide headgroup interactions. In addition, the intermolecular interactions were modeled at the RHF/3-21G ab initio level for the neutral hydrazide and its HCl salt.

Experimental Section

Materials and Synthesis. All solvents and chemicals were reagent grade and used as received. The diacetylenic acids 10,12-tricosadiynoic acid (TRCDA) and 10,12-pentacosadiynoic acid (PCDA) were purchased from Farchan Laboratory, Inc. (Ganesville, FL). The water used for synthesis and vesicle preparation was deionized and passed through a filter train composed of a Polygard 5 μm particle filter, a carbon cartridge Super-C filter, and a Milli-Q UF Plus system from Millipore Intertech (Marlborough, MA) to yield water of 18.2 MΩ cm resistivity. The stock solutions of the lipids usually had a concentration of 1−2 mM and were made by dissolving an appropriate, dry sample of the light-sensitive diacetylenes in CH_2Cl_2 followed by filtration through a 0.2 μm Teflon filter to remove any traces of polymeric residues.

10,12-Tricosadiynohydrazide (THY). To a solution of 10,12-tricosadiynoic acid (400 mg, 1.15 mmol) and *N*-hydroxysuccinimide (172 mg, 1.5 mmol) in 20 mL of CH_2Cl_2 was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (287 mg, 1.5 mmol), and the resulting solution was stirred overnight. The solution was concentrated, taken up in ethyl acetate, and extracted with water. The organic phase was dried with Na_2SO_4 and rotoevaporated down to a white solid. The solid was redissolved in 10 mL of CH_2Cl_2 and mixed with 1 mL of hydrazine hydrate (∼55%), and the resulting solution was stirred overnight. After being washed with water and dried over Na_2SO_4, the organic solution was filtered through a 0.2 μm Teflon syringe filter to remove polymeric residues and rotoevaporated to dryness to yield a white, light-sensitive powder of THY (331 mg, 92% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.86 (t, J = 6.8 Hz, 3 H, $-CH_3$), 1.2−1.7 (br m, 28 H, $-CH_2-$), 2.13 (t, J = 8 Hz, 2 H, α-CH_2-), 2.22 (t, J = 7 Hz, 4 H, $-CH_2-C\equiv$), 3.78 (br s, 2 H, $-NH_2$), 6.86 (br s, 1 H, $-NH-$). ^{13}C {^{1}H} NMR (50 MHz, $CDCl_3$): δ (ppm) 14.1, 19.2, 22.6, 25.4, 28.2, 28.3, 28.7, 28.8, 29.1, 29.2, 29.3, 29.4, 29.5, 31.9, 34.5, 65.2, 65.3, 77.4, 173.9. IR (neat film on Si) $\tilde{\nu}$ (cm^{-1}) 3305, 2952, 2918, 2850, 1645, 1605, 1533, 1471, 1420, 716. HRMS (FAB$^+$) calcd for $C_{23}H_{41}N_2O$ [MH$^+$] m/z 361.321 889, found 361.321 366.

10,12-Pentacosadiynohydrazide (PHY). The procedure for the synthesis is analogous to that for THY with 10,12-pentacosadiynoic acid (400 mg, 1.07 mmol) as the starting material. *N*-Hydroxysuccinimide (160 mg, 1.4 mmol) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (266 mg, 1.5 mmol) were used. The product PHY was obtained as a white powder (light sensitive) in 89% yield (370 mg). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.87 (t, J = 6.7 Hz, 3 H, $-CH_3$), 1.2−1.7 (br m, 32 H, $-CH_2-$), 2.14 (t, J = 7.9 Hz, 2 H, α-CH_2-), 2.27 (t, J = 6.8 Hz, 4 H, $-CH_2-C\equiv$), 3.15 (br s, 2 H, $-NH_2$), 6.75 (br s, 1 H, $-NH-$). ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ (ppm) 14.08, 19.14, 19.17, 22.65, 25.41, 28.24, 28.32, 28.69, 28.83, 28.85, 29.07, 29.15, 29.31, 29.44, 29.57, 29.59, 29.61, 31.88, 34.50, 65.18, 65.28, 77.37, 77.58, 173.94. IR (neat film on Si) $\tilde{\nu}$ (cm^{-1}) 3310, 2951, 2918, 2851, 1645, 1606, 1534, 1471, 1422, 716. HRMS (FAB$^+$) calcd for $C_{25}H_{45}N_2O$ [MH$^+$] m/z 389.353 189, found 389.352 580.

Methods. Polymerization in Organic Solution. Concentrated stock solutions of PHY and THY in CH_2Cl_2 were stored at 4 °C in the dark. Prior to use the solutions were warmed to room temperature for about 60 min, and then an aliquot sufficient to make a 1 mL sample of 1 mM concentration was dried down to the solid. The material was redissolved in a mixture of 900 μL of CH_2Cl_2 and 100 μL of HCl containing CH_2Cl_2 and then irradiated in a quartz cuvette with 0.3 J cm^{-2} UV light (254 nm wavelength) in a UV cross-linker apparatus FB-UVXL-1000 from Spectronics Corp. (Westbury, NY) to induce polymerization. The stock solution of HCl in CH_2Cl_2 was made by shaking 40 mL of CH_2Cl_2 with 10 mL of concentrated HCl in water (35−38%), separating the organic phase, and drying it over anhydrous Na_2SO_4.

Temperature-Dependent ^{1}H NMR. A dried sample of 26 mg of THY was dissolved in 1.5 mL of $CDCl_3$, and a ^{1}H NMR spectrum (200 MHz) was measured at +25, −15, and −50 °C on a Varian (Palo Alto, CA) XL-200 NMR spectrometer equipped with a liquid nitrogen cooled variable-temperature controller. The sample was kept at each temperature for at least 20 min prior to measurement to allow thermal and morphological equilibration.

Vesicle Preparation. A 2 mL volume of a 1 mM lipid solution (PHY and THY) was rotoevaporated to dryness in a glass test tube, and 2 mL of deionized water was added. The suspension was heated to ∼65 °C with a heat gun and sonicated for 10−30 min at 50 W power with the microtip (1/2 in. diameter) of a vibracell sonicator from Sonics&Materials Inc. (Danbury, CT). The hot clear solution was filtered immediately through a 0.8 μm cellulose acetate syringe filter and then stored at 4 °C for at least 2 h to induce crystallization of the lipid bilayer membranes prior to photopolymerization. To polymerize the hydrazide vesicles, 10 volume parts of the vesicle stock solution (1 mM) was mixed with 1 part of a 1 M aqueous HCl solution, and the resulting solution was incubated at room temperature for 5 min in a quartz cuvette. Then the solution was irradiated with 0.3 J cm^{-2} UV light (254 nm, UV cross-linker) to yield a dark blue colored vesicle solution. The acidified vesicle solution is unstable in contrast to the neutral solution, and the vesicles will precipitate over time (typically <30 min for 1 mM concentration) depending on the lipid concentration. More dilute samples are more stable. The neutral stock solution is very stable when stored at 4 °C (>1 year test period).

UV−Vis Spectroscopy. UV−vis spectra of vesicles in water and lipid solutions in organic solvents were taken in quartz cuvettes with a 10 mm path length on a two-beam Shimadzu (Japan) UV-1601 spectrometer with a solvent-filled cuvette as reference.

FTIR Spectroscopy. FTIR spectra of neat films of PHY and THY were taken with a Perkin-Elmer (Beaconsfield Bucks, England) System 2000 FTIR spectrometer with 16 transients for each measurement. The neat films were prepared on a 0.27 mm thick silicon wafer by evaporation of the lipid solution in CH_2Cl_2. To convert the hydrazides into the corresponding HCl salt and back to the free amine form, the dry films on the silicon wafer were exposed to saturated vapors of HCl (over 35−38% aqueous HCl) or NH_3 (over 28−30% ammonia solution), respectively. Exposure times for the specific experiments are given in Figure 4. After vapor treatment the films were dried in a vacuum for at least 10 min prior to the measurement (until no further change was observed in the IR spectra). Polymerization of the solid films (HCl salt) was achieved by irradiation with 50 mJ cm^{-2} UV light of 254 nm wavelength (UV cross-linker).

Transmission Electron Microscopy. TEM images of PHY and 10,12-tricosadiynoic acid (TRCDA) liposomes were obtained using a JEOL (Japan) 100CX electron microscope operating at 80 kV. Vesicle samples were deposited onto Formvar-coated copper grids (200 mesh) as follows: 5 μL of vesicle solution (1 mM) was applied onto the grid, allowed to stand for 3 min, and then removed by capillary force using filter paper. The same procedure was repeated with negative stain solution (phosphotungstic acid) for 2 min. The red TRCDA vesicles were obtained by mixing a 1 mM solution of blue polymerized vesicles with concentrated ammonia solution (10:1 v/v) just prior to deposition on the grids.

Computation of Molecular Models. The molecular models of the propanohydrazide trimer and its HCl salt were generated on an Apple PowerMacintosh 9600/300 with the ChemOffice Ultra 4.0[27] suite (CambridgeSoft Corp., Cambridge, MA). The models were transferred as Protein Data Bank file format to MacSpartan Plus[28] (Wavefunction, Inc., Irvine, CA) and MacMolPlt/MacGAMESS[29] (ISU Quantum Chemistry Group at Ames Laboratory, Ames, IA) to perform geometry optimization and normal mode vibrational analysis at the ab initio level (RHF) with the 3-21G basis set under vacuum conditions (dielectric constant ϵ = 1, no boundary conditions).[30,31]

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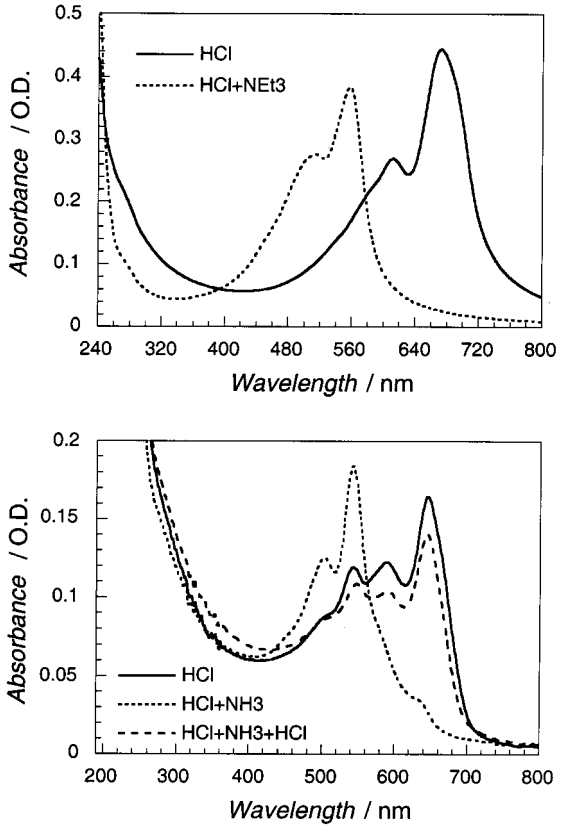

Figure 1. (a, top) UV−vis spectra of a PHY solution (diluted to 0.1 mM before spectroscopic characterization) in CH_2Cl_2 containing HCl and polymerized with 0.3 J cm^{-2} UV light (254 nm), before (solid line) and after (dotted line) addition of 0.01% (v/v) triethylamine. (b, bottom) Reversible color change of 0.1 mM PHY liposomes polymerized (0.3 J cm^{-2}, 254 nm) in 0.1 M aqueous HCl, before (solid line, blue form) and after (dotted line, red form) addition of NH_3, and after subsequent addition of HCl (dashed line, blue form).

Results and Discussion

The hydrazide moiety can be viewed as a hybrid structure, composed of a primary amide function and a terminal amino group. It is the vicinal relationship between these functions with their mutual electronic and steric influence that results in the unique properties of the hydrazides described below. To specify the nitrogen being discussed, it will be referred to as amide or amine group throughout the text.

Polymerization in Organic Solution. Due to the topochemical nature of diacetylene polymerization, common diacetylenic lipids are required to be in the solid state for polymerization to occur. It was thus surprising to find that polymerization occurs in dilute solutions of PHY and THY in CH_2Cl_2 (and $CHCl_3$) upon irradiation with UV light of 254 nm wavelength. Figure 1a shows the absorbance spectrum of a PHY solution in CH_2Cl_2 containing HCl after irradiation with 0.3 J cm^{-2} (diluted to 0.1 mM before spectroscopic characterization). It was observed that polymerization is facilitated by the presence of HCl, but also takes place in the neutral solution at a slower rate. In the presence of triethylamine (0.01% v/v) polymerization is completely inhibited. The absorbance maximum at 672 nm (excitonic band) corresponds to the dark blue color of the conjugated polymer backbone and is reminiscent of the polymer formed in vesicles and crystalline solids of diacetylene lipids. The weaker band at 612 nm results from the vibronic absorption that is generally found in solids and vesicles of polydiacetylenes. When 0.01% (v/v) triethylamine is added to the blue polymer solution, the color changes immediately to a bright red, with an absorbance maximum at 558 nm for the excitonic band and 512 nm for the vibronic band. Adding a large excess of HCl to this solution does not revert the color back to the blue form. In all cases, the solutions of the monomer and polymer were clear, and no precipitation of the polymer occurred over extended periods of time. Irradiation experiments were also conducted in other organic solvents (like toluene, tetrahydrofuran, acetone, methanol, dimethylformamide, and methyl sulfoxide) in the presence and absence of HCl, but no polymerization could be observed in these cases.

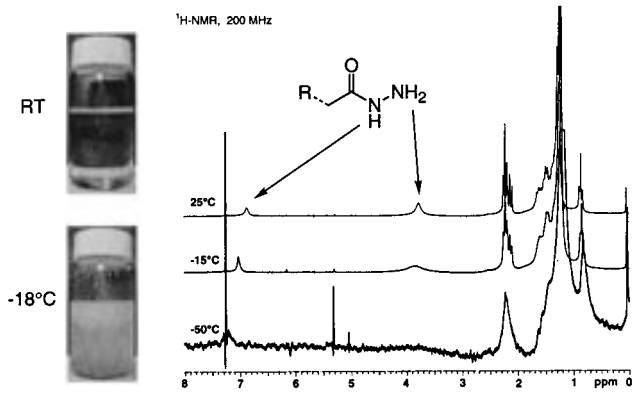

Figure 2. Temperature-dependent ^{1}H NMR (200 MHz) of THY in $CDCl_3$ (26 mg in 1.5 mL). Insets: Photographs of a clear PHY solution (32 mM) at room temperature (RT) and after gelation (white solid) at −18 °C.

When more concentrated solutions (>30 mM) of the hydrazide monomers in CH_2Cl_2 or $CHCl_3$ are cooled below 4 °C, gel formation sets in and the solutions become cloudy to solid, depending on the temperature, as shown in the insets of Figure 2. The temperature-dependent ^{1}H NMR of THY in $CDCl_3$ (72 mM, Figure 2) also reveals the aggregation tendency of the monomeric hydrazide lipids at low temperature as suggested by line broadening and decreased signal-to-noise ratio. These effects are due to the reduced mobility of the molecules in the gel phase. The terminal amine protons (−NH_2 group) shift from 3.75 ppm at room temperature to 3.85 ppm at −50 °C with significant broadening. The amide proton (−CONH−) shifts by a larger amount from 6.85 to 7.2 ppm in the same temperature interval.[32] Here the peak seems to actually become sharper with cooling from room temperature to −15 °C. Only a minor shift of the methylene protons (−CH_2−, about 1 ppm) to higher field is observed between ambient temperature and −50 °C.

The fact that photopolymerization occurs at room temperature in CH_2Cl_2 (and $CHCl_3$) solutions of the diacetylene hydrazide lipids, and that monomer gelation takes place at low temperature, indicates that ordered lipid aggregates exist in these solutions. The aggregates are stabilized by specific interactions between the hydrazide moieties. Other diacetylene lipid derivatives such as carboxylic acids, amides, and alcohols (with identical alkyl chain structure) do not polymerize in solution. Aggregation is improved by salt formation with HCl and is restricted to the chlorinated solvents used, suggesting a sensitive balance between intermolecular and lipid−solvent interactions. This balance can easily be disturbed by trace amounts of triethylamine, which prevents polymerization and induces an irreversible transition of the lipid packing in the soluble polymer. The transition is visualized as a color change from the well-conjugated blue state to a less conjugated red form upon addition of triethylamine. The exact structure of these aggregates in organic solution, and

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the particular role of the chlorinated solvents, is not known at present, and is the subject of further studies. Possible bonding modes of the hydrazide headgroup are discussed below, in the context of computational models that are based on infrared measurements of PHY and THY solid films.

Vesicles. Sonication of hydrazide lipids in water yields vesicles that do not polymerize upon irradiation, in contrast to the analogous carboxylic acids that readily convert to the blue form.[33] The addition of base has no effect, but dilute HCl (10 mM final concentration) induces red polymer formation in the vesicles (1 mM lipid concentration) after irradiation. Increased HCl concentrations (>0.1 M) give dark blue vesicles upon photopolymerization, producing the UV−vis spectrum shown in Figure 1b (after dilution with 0.1 M HCl to a 0.1 mM lipid concentration). The blue polymer is the major component of the vesicles, as evidenced by the excitonic absorption at 645 nm (vibronic 590 nm). In addition, a minor fraction of red polymer is observed, as shown by the absorbance at 542 nm (vibronic 503 nm). Upon neutralization of the HCl with NH_3, the color changes instantaneously to bright red (absorption maximum 542 nm). When the basic hydrazide vesicles are acidified again with HCl, the color reverts almost completely back to the blue form as can be seen in the absorption spectrum of Figure 1b. The reversibility is slightly better in PHY compared to THY, which is shorter by two methylene groups in its terminal alkyl chain. The base-induced color transition from blue to red is also found in polydiacetylene vesicles composed of acid lipids, but it is irreversible in this case. Heating the vesicles always results in an irreversible color change from blue to red, independent of the lipid structure.

Transmission electron microscopy (TEM) was used to characterize the morphology of the different vesicle forms, as presented in Figure 3 for PHY. The freshly prepared hydrazide vesicles in pure water cover a size range of several hundred nanometers and possess irregular shapes with round edges (Figure 3a). After HCl treatment and photopolymerization the blue vesicles acquire more rectangular shapes with internal striations parallel to the polymer backbones (Figure 3b). Such striations are commonly observed in SFM and polarized light microscopy of polydiacetylene films, and can be clearly assigned to the parallel-aligned polymer backbones.[23,24] The transformation into rectangular shapes is most likely caused by HCl-induced crystallization of the bilayer membrane, as required for polymerization. When the vesicles are converted to the red form by exposure to NH_3, the rectangular shapes remain, but the contrast of the striations is increased (Figure 3c). The same morphology is essentially retained upon subsequent reversion to the blue form by excess HCl (Figure 3d). To understand the nature of the reversibility of color change in hydrazides compared to carboxylic acid diacetylenes, TRCDA vesicles were investigated by TEM. The freshly prepared polymeric vesicles are shown in Figure 3e and have a square shape due to membrane crystallization. NH_3 treatment of these vesicles changes their color to red and causes severe fracturing of the vesicle membrane parallel to the polymer backbones, as visible in Figure 3f. Membrane fracturing accompanied by an irreversible blue to red color transition is also observed in all vesicles that were heated above ~70 °C.

The addition of HCl or NH_3, respectively, protonates or deprotonates the amino functionality ($-NH_2$) in the hydrazide headgroup, as shown in Scheme 1 ($pK_a[R-CO-NH-NH_3^+] \approx 2.65$).[34] This alteration of headgroup structure affects the

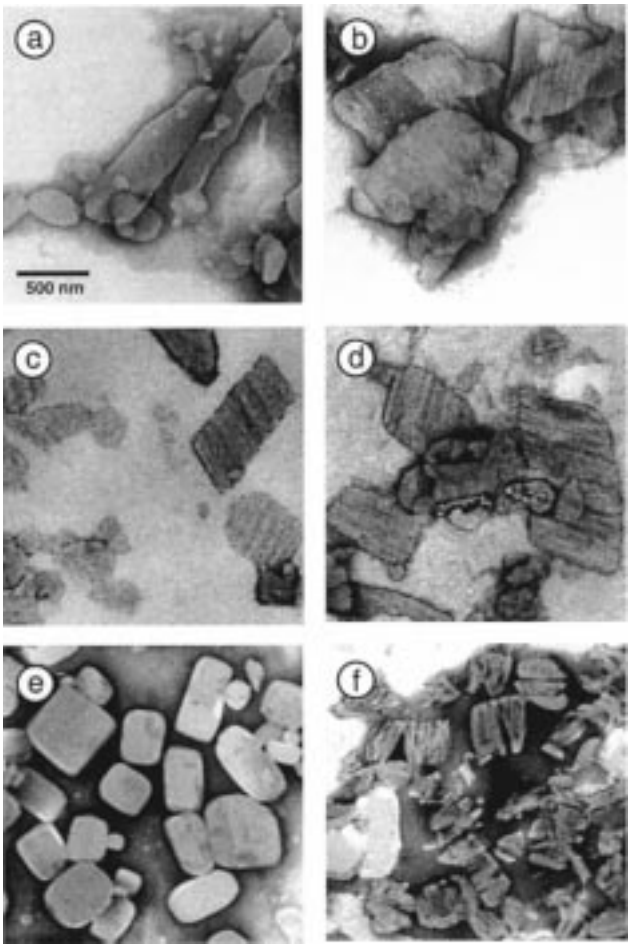

Figure 3. TEM images of diacetylene vesicles under different conditions. The scale bar of 500 nm in (a) applies to all images. (a) Monomeric PHY vesicles freshly prepared. (b) PHY vesicles treated with HCl and polymerized to the blue form. (c) Red PHY vesicles after exposure to NH_3. (d) Reverted blue PHY vesicles upon addition of excess HCl. (e) Freshly prepared and polymerized TRCDA vesicles in the blue form. (f) Irreversibly converted red TRCDA vesicles with fractured membranes after treatment with NH_3.

interaction and packing between neighboring hydrazide moieties, inducing a conformational change in the conjugated polymer backbone, yielding a distinct colorimetric transition. It becomes apparent from the TEM studies and the reversible nature of the color change that protonation and deprotonation do not interfere with the integrity of the dense bilayer (in contrast to TRCDA, which is affected by deprotonation, Figure 3f. In both cases, attractive interactions between the molecules are retained. The terminal side chain packing in the space between polymer backbones and terminal CH_3 groups does not seem to be affected by the structural changes in the hydrazide groups, and helps reversion to the initial conformation of the blue form upon protonation. This is supported by the fact that the effect is more pronounced in the longer PHY molecule, compared to the shorter and less reversible THY. Heat or base treatment (for carboxylic acid lipids) of vesicles significantly disturbs the alkyl chain packing and polymer backbone conformation, leading to the perforation of the vesicle membranes, and a completely irreversible color change. From surface force microscopic studies in thin polydiacetylene Langmuir−Blodgett films, it is known that the heat-induced colorimetric transition from blue to red is accompanied by a change of side chain packing from a contorted

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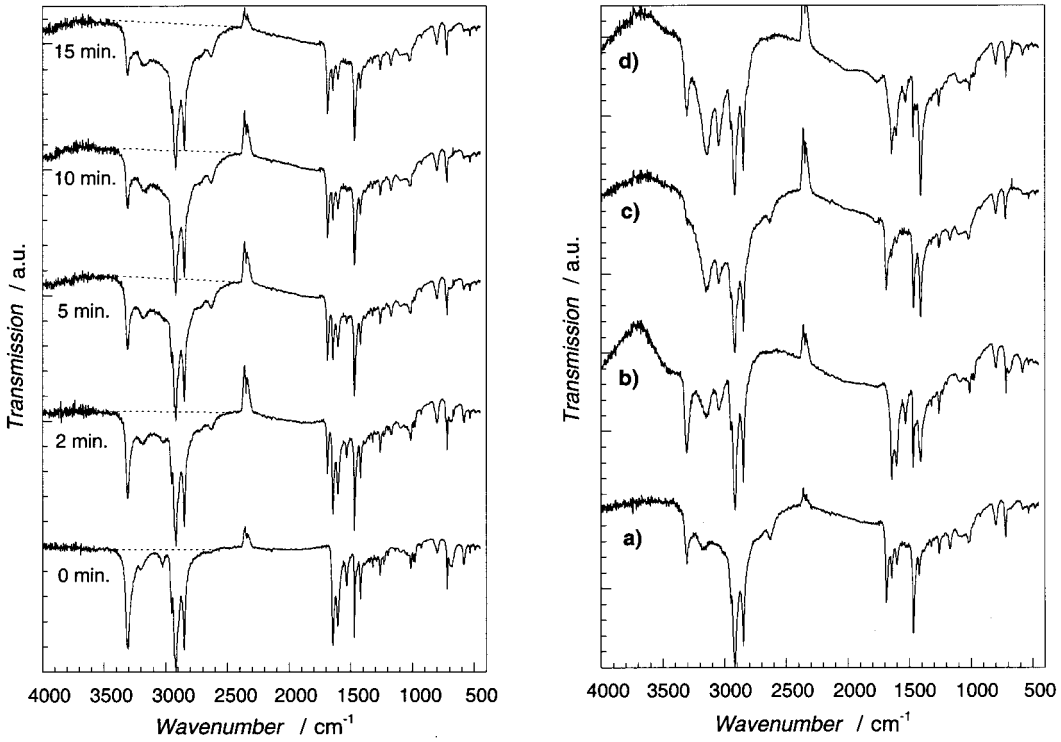

Figure 4. FTIR spectra of a polycrystalline film of THY on a silicon wafer. (a, left) Spectra of the monomeric film exposed to HCl vapor (oversaturated aqueous HCl, ~38%) at different exposure times, revealing substantial changes in the NH and carbonyl stretch regions. (b, right) Polymeric film exposed sequentially to HCl (blue color, spectra a and c) and NH_3 vapor (red, spectra b and d), showing the reversibility of hydrazide salt formation. Spectrum a: blue film after 15 min of HCl exposure and photopolymerization. Spectrum b: film from spectrum a exposed to NH_3 for 15 min. Spectrum c: film from spectrum b exposed to HCl (15 min). Spectrum d: film from spectrum c exposed to NH_3 (15 min) again.

rectangular lattice (termed parallelogrammatic, which is also the monomer packing) to a well-ordered hexagonal structure.[23,24] This lattice transition causes the film to shrink and fracture along the polymer chains in the same way as it is found in heat-treated vesicles. It can be concluded from these observations that the mechanism of pH-induced color change in the hydrazides is different from the base-induced transition in carboxylic acids or the heat-induced transformation generally observed in diacetylene lipids.

Solid Films. The response of solid films of hydrazide diacetylene lipids to HCl and NH_3 vapors was studied by FTIR spectroscopy. In these experiments, the hydrazide diacetylene lipids were deposited from solution onto silicon wafers. This technique allows probing of specific interaction between functional groups of the hydrazide moieties via hydrogen bonding and salt bridging. As with the hydrazide vesicles, it was found that the neutral films did not photopolymerize. However, after exposure to HCl the films were readily converted into the blue polymer upon irradiation. Figure 4a shows the IR spectra of a monomeric THY film exposed to HCl vapor at different times. The assignment of bands to characteristic vibrations is given in Table 1 and is based on data from the literature,[35−39] comparison with authentic samples (alkylamines, alkyl amides, alkyl acids, and combinations thereof), and computational results that are discussed further below. The spectra are substantially different between the neutral and the protonated forms, with the sharp band at 3310 cm^{-1} losing intensity upon extended HCl exposure. This band is assigned to the N−H stretch vibration of the primary amide substructure in the trans conformation, where the amide proton is H-bonded to the neighboring carbonyl oxygen. The N−H stretch of the terminal NH_2 group appears at the same frequency; this was independently confirmed by comparison with IR spectra of primary alkylamines as well as analogous amides, the latter being derived from the parent diacetylene lipid and either glycine (R−CO−NH−CH_2−CH_2−COOH) or ethylenediamine (R−CO−NH−CH_2−CH_2−NH_2). With increasing time, a very broad band appears under the C−H stretch vibrations, centered about 3000 cm^{-1} with features at 2630 and 3170 cm^{-1}. The baseline is approximated by a dashed line. This new band constitutes the N−H stretch of the protonated nitrogen (−NH_3^+) with Cl^- counterions in proximity. The C−H stretch vibrations between 2850 and 2952 cm^{-1} are not affected by the HCl treatment. At

Table 1. Frequencies of Observed IR Absorptions and Corresponding Assignment to Functional Groups[33−37]

$\tilde{\nu}$ (cm^{-1})	neutral	HCl salt	vibrational mode
3310	(s)[a] sharp	attenuated	ν(N−H)$_{CO}$ H-bonded and $\nu(NH_2)$
~3000		(m) very broad	$\nu(NH_3\cdots Cl)$
2850−2952	(s) unaffected		$\nu(CH_{2/3})$
1688		(s) sharp	ν(C═O) in HCl salt
1644	(s) sharp	attenuated	ν(C═O) in neutral form, H-bonded
1605	(s) sharp	attenuated	$\delta(NH_2)$
1533	(w) sharp		combination (C−N−H)
1470	(s) sharp	(s) broadened	$\delta(CH_{2/3})$ and $\delta(NH_3)$ in salt
1421	(m) sharp	(w) reduced	$\delta(CH_{2/3})$

[a] s = strong intensity, m = medium intensity, w = weak intensity.

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1688 cm^{-1} a new peak appears while the three bands of the neutral hydrazide at 1644, 1605, and 1533 cm^{-1} dramatically lose intensity. The new 1688 cm^{-1} absorption is due to the carbonyl stretch in the salt, with the corresponding peak for the neutral hydrazide residing at 1644 cm^{-1} as generally found in H-bonded solids of primary amides (like the glycine and ethylenediamine derivatives). The 1605 cm^{-1} peak arises from the terminal NH_2 deformation vibration and almost vanishes in the protonated form. The C−N−H combination band at 1533 cm^{-1} of the amide substructure (also common in other primary amides) completely disappears in the HCl salt. At 1470 cm^{-1} the $CH_{2/3}$ deformation is broadened, which is most likely due to the appearance of the NH_3^+ deformation at the same frequency. The $CH_{2/3}$ deformation at 1421 cm^{-1} is slightly attenuated in the HCl salt.

The FTIR spectra of the polymerized film, alternatively exposed to HCl (spectra a and c) and NH_3 vapor (spectra b and d), are given in Figure 4b. These spectra clearly show the reversibility of salt formation in accordance with the observed reversible color change. In addition, the characteristic absorptions of NH_4Cl at 3145, 3050, and 1405 cm^{-1} increase with successive salt deposition in the headgroup region.

The observed changes in the IR spectra during salt formation can be understood in terms of changes in headgroup hydrogen bonding, as well as variations in the electronic structure of the hydrazide moiety. A commonly observed feature is the reduction of the stretch vibrational frequency upon hydrogen bonding. The effect of H-bonding on deformation vibrations is more complicated and depends on the specific bonding geometry. It is further known that a higher positive charge in combination with a larger number of coordination partners lowers the stretch vibrational frequency between the central atom and the binding partner.[37] This is the case with protonation of the terminal NH_2 function to yield the cationic NH_3^+ group, shifting the NH_2 stretch from 3310 cm^{-1} to approximately 3000 cm^{-1} in the NH_3^+ group. Broadening of the band is due to coordination with the mobile Cl^- counterions. The increased positive charge also affects the geminal amide NH stretch electronically, and reduces its intensity by the stronger electron-withdrawing inductive (−I) effect of the salt. Interestingly, the carbonyl stretch of the free hydrazide at 1644 cm^{-1} is shifted to higher frequencies (1688 cm^{-1}) in the salt, which would imply, upon first inspection, a loss of carbonyl H-bonding upon protonation. However, from the established bilayer structure, the ability to polymerize, and comparison to analogous amides, it is possible to conclude that the carbonyl oxygen is H-bonded to the amide NH group, even in the salt. The observed shift to higher frequencies is primarily due to electronic effects of the cationic moiety. The same trend of C═O frequency shift upon salt formation with retained H-bonding is also found in the vibrational analysis using the computer models described below. The NH_2 deformation at 1605 cm^{-1} is lowered to 1470 cm^{-1} for NH_3^+ as a consequence of increased positive charge with a larger number of protons, and by H-bonding to the Cl^- ions. The C−N−H combination band at 1533 cm^{-1} is very sensitive to changes in the electronic structure and bonding geometry, and vanishes completely upon salt formation. On the other hand, the stretch and deformation vibrations of the alkyl chains are mostly unaffected by protonation of the hydrazide group, and only the deformation band at 1421 cm^{-1} is slightly reduced in intensity upon HCl exposure. This indicates that substantial changes in the hydrazide headgroup region cause only subtle alterations of side chain packing, and that the integrity of the bilayer structure is retained (allowing the unusal reversibility of the color change). The effect is still sufficient to affect polymerization behavior and conjugation of the polymer backbone as is reflected in the reversible colorimetric transition.

Computational Models. To recognize possible headgroup interactions which account for the reversible colorimetric response, computer models of trimers for the neutral hydrazide, and its HCl salt, were calculated and studied.[39,40] The intention is to obtain a qualitative model with short computation times using a desktop computer, while retaining sufficient accuracy to gain insight into the molecular structure of the hydrazide layer system.[41] An ab initio computation at the RHF/3-21G level was found to yield a reasonably detailed picture of the molecular headgroup interactions, while at the same time providing a practical upper limit of about 150 h for the frequency calculation of the salt trimer. The computer models were based on the known layer geometry, and emphasis was placed on the headgroup structure with the diacetylene side chains being omitted. The general lipid packing in the bilayer is well understood from a vast number of experiments with the alkyl chains aligning parallel in the layer, and the headgroups oriented toward the surface as outlined in Scheme 1. The polymer backbones are embedded parallel to each other inside the lipid layer. Environmental factors such as solvents and neighboring layers or molecules (other than the trimer) were neglected, since the main interest was to map possible bonding sites between the hydrazide groups.

Figure 5a shows the neutral trimer of propanohydrazide after geometry optimization in a vacuum starting with the monomers in a parallel alignment, according to the lipid bilayer structure model. The monomer itself was optimized with the 3-21G basis set in a vacuum before the trimer was composed, which generated the lowest energy conformation of the hydrazide function with the amine hydrogens pointing toward the carbonyl oxygen side (cis conformation).[39,40,42] Besides the expected hydrogen bonding of the amide groups between the carbonyl oxygen and the hydrogen of the amide nitrogen (distance ∼1.8 Å), there is also a H-bonding interaction of the terminal amine hydrogens and their free electron pairs (∼2.1 Å). The amino group acts simultaneously as a hydrogen donor and acceptor, as was previously observed in crystal structures of other hydrazides.[40,42,43] This additional bonding causes the short alkyl chains to fan apart under the conditions of vacuum, in the absence of other neighboring molecules. In the real, condensed lipid layer, the long alkyl chains do not fan apart because the bilayer integrity is retained (see the TEM results of vesicles, Figure 3). However, the interaction between the terminal amine groups sufficiently strains the side chains to slightly disturb the optimal packing, preventing polymerization in vesicles and solid films. On the other hand, the ability to form strong H-bonds allows the lipid molecules to aggregate in organic solution (with CH_2Cl_2 or $CHCl_3$ as solvent) and polymerize.

The amide groups in the HCl salt trimer also form H-bonds (∼1.8 Å), as shown in Figure 5b, but the positively charged ammonium moieties are now bridged by chlorine anions which are coordinated to the hydrogens of the ammonium groups (distance ∼1.9−2.1 Å). The HCl molecule is inserted between two headgroups, increasing the amine−nitrogen distance between two lipid molecules from 3.0 Å (neutral) to 4.9 Å (salt).

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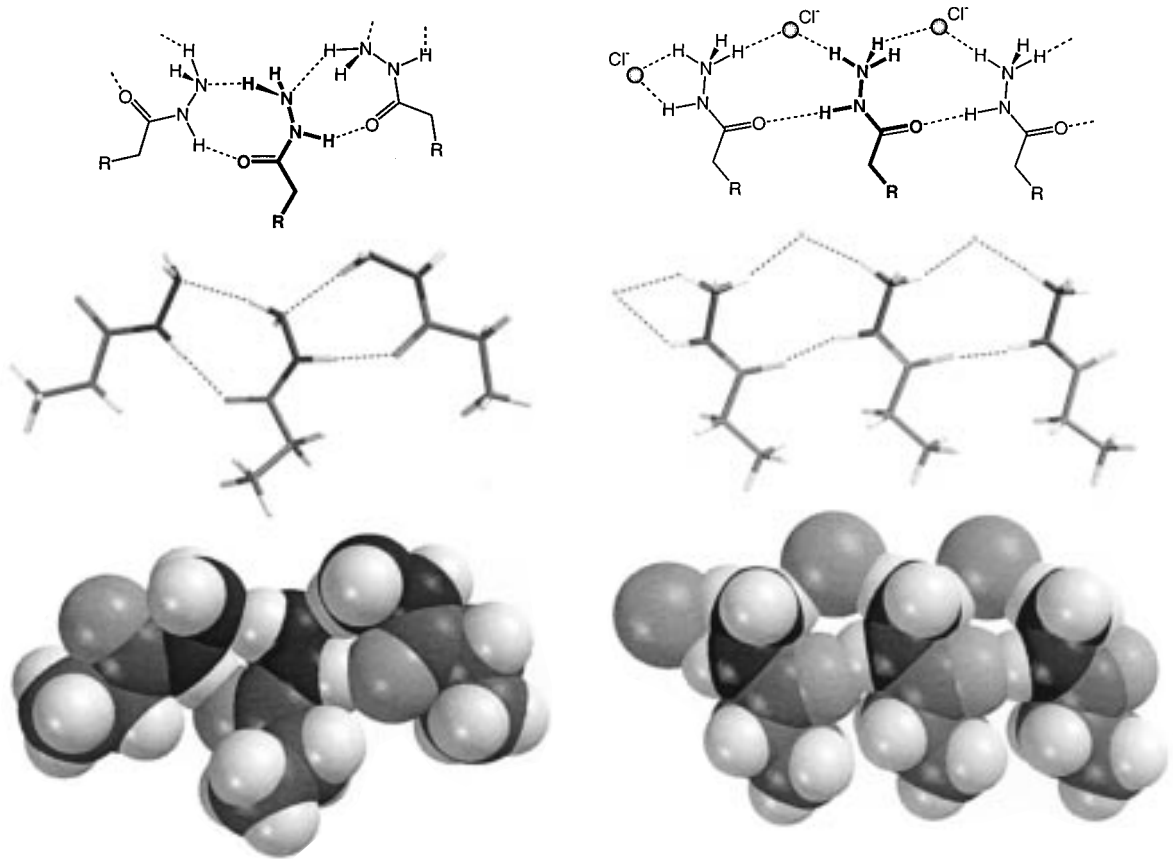

Figure 5. Computer models of geometry-optimized trimers for (a, left) neutral propanohydrazide and (b, right) the HCl salt of propanohydrazide. The ab initio computation was done at the RHF/3-21G level to visualize the H-bonding structure possible between the hydrazide headgroups in the neutral or the salt form and to allow vibrational normal mode analysis in the H-bonded state. In the optimized bonding configuration of the neutral form (a) the alkyl chains fan apart from each other, whereas in the bridged salt structure (b) the chain axes remain in a parallel orientation.

This headgroup bonding motif orients the alkyl side chains in a parallel arrangement to allow polymerization in the layer structure. In the extended bilayer structure, the chlorine ions can also bridge to a third hydrazide molecule located on the side of the current trimer and therefore further stabilize the packing. The calculated amide H-bonding distances agree very well with the crystallographic data of other hydrazides.[44,45]

Besides the study of headgroup interactions, the computer models were also used for normal mode vibrational analysis at the RHF/3-21G level, to calculate IR absorption frequencies. By comparing the calculated frequencies for the monomer and trimer, it is possible to understand the effect of hydrogen bonding on frequency shifts in the hydrazide moieties. In addition, comparison of the neutral trimer with its salt allows exploration of the effect of salt formation on vibrational frequencies, due to changes in headgroup bonding structure. The calculated frequencies were scaled by a factor of 0.89 (based on the CH stretch vibrations) to be comparable with the experimental results, which is a common practice for vibrational analysis on the Hartree−Fock level neglecting electron correlations.[28,41] The amide NH stretch vibration was found to be lowered by about 200 cm^{-1} upon hydrogen bonding, whereas the carbonyl stretch was only reduced by ~40 cm^{-1}. These calculated shifts are in agreement with the experimentally observed changes upon hydrogen bonding, generally reported in the literature. In contrast, the CNH combination band was increased in frequency by ~100 cm^{-1}. The NH stretch of the terminal amine group in the neutral hydrazide was lowered by ~40 cm^{-1} when H-bonded.

These studies have also shown that the amide NH stretch in the neutral trimer at 3235 cm^{-1} is shifted to 3145 cm^{-1} for the salt, upon protonation and salt bridging. Simultaneously, the amine NH_2 stretch at 3281 cm^{-1} of the neutral hydrazide is substantially lowered to the region between 2435 and 2855 cm^{-1} upon salt formation, as experimentally observed in the IR spectra of the solid lipid films. The carbonyl stretch calculated for the neutral trimer at 1632 cm^{-1} was raised to 1664 cm^{-1} for the salt trimer, similar to the shift found in the IR experiments. In both systems the CNH combination band was computed to be at around 1535 cm^{-1}, but this vibration was only observed in the IR spectrum of the neutral form. In general, the calculated frequencies and shifts can be correlated to the measured IR spectra, supporting the assignment of the observed absorptions to the characteristic group vibrations.

Conclusion. By introducing the hydrazide headgroup moiety for single-chain diacetylene lipids, an unusual polymerization behavior and colorimetric response was found. These effects were not previously shown for the parent carboxylic acids or related derivatives. The unusual properties include polymerization in dilute organic solution, the requirement of acid (HCl) to allow polymerization in vesicles and solid films, and the reversible color switching of the polymeric form between blue and red in acidic or basic conditions, respectively. Specific hydrogen bonding of the hydrazide groups in balance with hydrophobic interactions in the alkyl tails is found to be responsible for these unusual properties. The aggregation tendency observed by temperature-dependent ^1H NMR in

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chlorinated organic solvents accounts for the ability to polymerize in solution, and the hydrogen bonding responsible for the aggregation could be investigated by FTIR spectroscopy under acidic and basic conditions. These experiments demonstrated that the bonding structure differs strongly between the neutral hydrazide and the HCl salt, affecting the alkyl chain packing and, consequently, the conformation and color of the polydiacetylene backbone. The lipid membrane integrity was not adversely affected by the pH changes, as shown by TEM analysis of the hydrazide vesicles. Thus, the alterations in headgroup bonding caused only subtle changes in lipid packing, permitting the reversibility of color change. On the basis of this experimental evidence, the hydrazide bonding was modeled to understand the lipid packing on a molecular level, and to obtain a more realistic description beyond that of simple 2D sketches. Calculation of normal mode vibrations in these models agreed well with the observed vibrational spectra, and allowed a conclusive interpretation of the IR data.

The special behavior of the hydrazide moiety, in comparison to other headgroup functionalities, is attributed to the vicinal proximity of the amide and amine functionality, and the ability of the amine group to act simultaneously as hydrogen bond donor and acceptor. This produces the unusual, rigid ladder-type H-bonding of the hydrazide headgroup in layer structures (Figure 5), and controls the alkyl chain packing in a very subtle way. The alkyl chain packing, in turn, dictates directly the position of the diacetylenic units and thus the ability to polymerize. Such steric control is not given in primary amides with linear strand-type hydrogen bonding, or in amides with a flexible spacer between the amide group and a second H-bonding moiety on the same lipid molecule, since such systems have more degrees of freedom to respond to strain. On the other hand, if the whole structure and chain packing of the layer are severely affected by environmental factors (as shown with the base-treated carboxylic acid vesicles or heat-treated vesicles), the system has no driving force to return to the initial ordered state.

In conclusion, the findings from the described experiments provide detailed insight into the mechanism of colorimetric transitions in polydiacetylenes upon changes in the layer packing. The development of these insights is necessary for the ultimate construction of reversible sensors with enhanced sensitivity. By tailoring the headgroup interactions via hydrogen bonding, the colorimetric response can be controlled in a very effective way. In addition, the ability of the hydrazide lipids to polymerize in solution provides new routes for the synthesis and processing of polydiacetylenes beyond that of the solid state.

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