

# Color and Chromism of Polydiacetylene Vesicles

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## Introduction

Biosensor design research attempts to couple Nature's "lock and key" interactions with cleverly engineered signal transduction mechanisms.<sup>1</sup> These lock and key (molecular recognition) interactions take on many forms: between enzyme and substrate, between receptor and ligand, or between antibody and antigen. All of these motifs can be incorporated into the design of biosensors. The way in which these capture molecules are coated onto or integrated with a transducer surface is itself the subject of numerous research efforts, and is often referred to as "interfacial design" or "surface modification".<sup>2</sup> When considering interfacial design motifs, it is often fruitful to borrow from Nature's most elegantly designed interface—the cell membrane. The biological cell membrane is a highly evolved self-assembled nanostructure that *integrates* molecular recognition and signal transduction functions. The surface of the membrane is heavily functionalized with recognition molecules, primarily in the form of glycosylated lipids and proteins (Figure 1). These recognition sites often serve as "antenna" for specifically recognizing other molecules or other cell surfaces, result-

ing in a cascade of events such as the opening of ion channels, activation of cellular enzymes, or altering the rate of transport, secretion, or oxidative metabolism. While there are several examples of whole cell biosensors in the literature,<sup>3–5</sup> we will focus our attention on simpler synthetic systems that mimic, to a certain degree, the self-organization and functionalization of the cell membrane. These "membrane mimetic" systems<sup>6</sup> have been used in a number of biosensor design strategies, primarily as the molecular recognition function of the biosensor. For example, self-assembled membrane-like films can be coated on a variety of device surfaces that provide signalling. These include measurement of conductivity for electrode-modified surfaces, or measurement of mass for quartz crystal microbalance (QCM) or surface acoustic wave (SAW) surfaces.

In this Account, we zero-in on the application of conjugated polymer vesicles, based on polydiacetylene (PDA-vesicles), for biosensing. The surface of the lipid-polymer material is chemically modified by bio-recognition molecules, and the  $\pi$ -conjugation of the polymer's backbone signals analyte binding by undergoing a color transition. Although much simpler and more crude, the ligand-modified PDA-vesicles are analogous to the cell membrane in that molecular recognition is directly linked to signal transduction within a single supra-molecular assembly.

First, we will briefly review the general area of polymerized vesicles, and the importance of these materials to emerging technologies. We then shift to our own work, describing the synthesis, vesicle formation, and physical properties of a palette of diacetylenic lipids. Finally, the chromic properties of these materials are examined with an eye toward their ultimate use as one-step colorimetric biosensors. In particular, it is possible to relate the thermochromic response of the PDA-vesicles to their observed biochromic transitions.

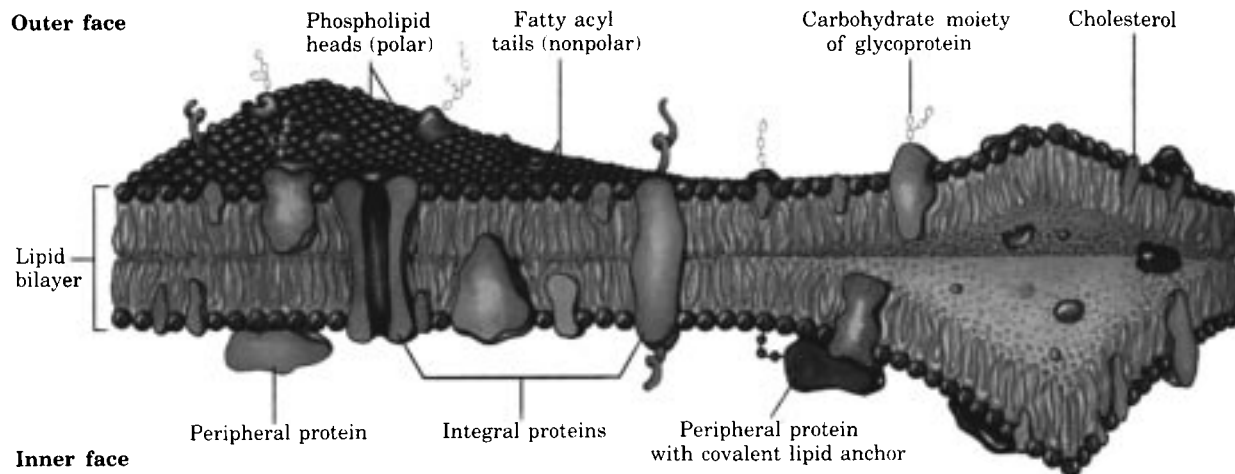
## Polymerized Vesicles

Vesicles (or liposomes) are broadly defined as any structure composed of amphiphilic molecular bilayers that enclose a volume.<sup>7–9</sup> Although phospholipids are the most common constituent, the lipid need not be of natural origin. Many authors reserve the term "liposome" for vesicles composed of phospholipids. In 1977, Kunitaka and Okahata<sup>10</sup> discovered that synthetic surfactants also form bilayer membrane structures, leading to the more generic term "vesicle". However, the distinctions are often blurred in the case of synthetic polymerizable phospholipid analogues or mixed systems composed of both natural and synthetic amphiphiles.<sup>11,12</sup> Irrespective of terminology, both systems share the common attribute of forming membrane-like structures in aqueous solution, and are reminiscent of native cell membrane architecture. Interestingly, it is hypothesized that the first living cells closely resembled liposomes, since these materials successfully isolate their aqueous interior from the surround-

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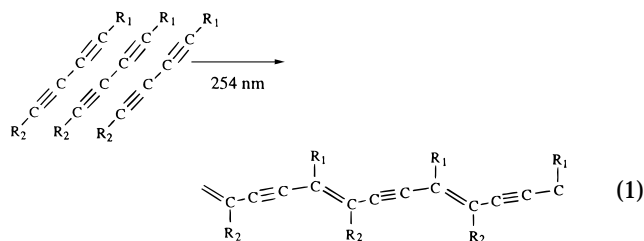
**FIGURE 1.** The fluid mosaic model for biomembrane structure. The carbohydrate groups attached to proteins and lipids are presented at the membrane surface and are often the site of specific molecular recognition interactions that are transduced into cellular messages. (From Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of Biochemistry*, 2nd ed.; Worth Publishers: New York, 1993. Reprinted with permission.)

ings.<sup>13</sup> Vesicles form upon hydration of the appropriate amphiphile in water or buffer using a number of methods such as probe or bath sonication, solvent injection, or simple shaking.<sup>8</sup> Vesicles can either be made from naturally-derived amphiphiles such as phosphatidylcholine or phosphatidylethanolamine, or from synthetic amphiphiles. Increasingly, these “synthetic membranes” are finding their way into industrial, pharmaceutical, and diagnostic applications.<sup>7,14–19</sup>

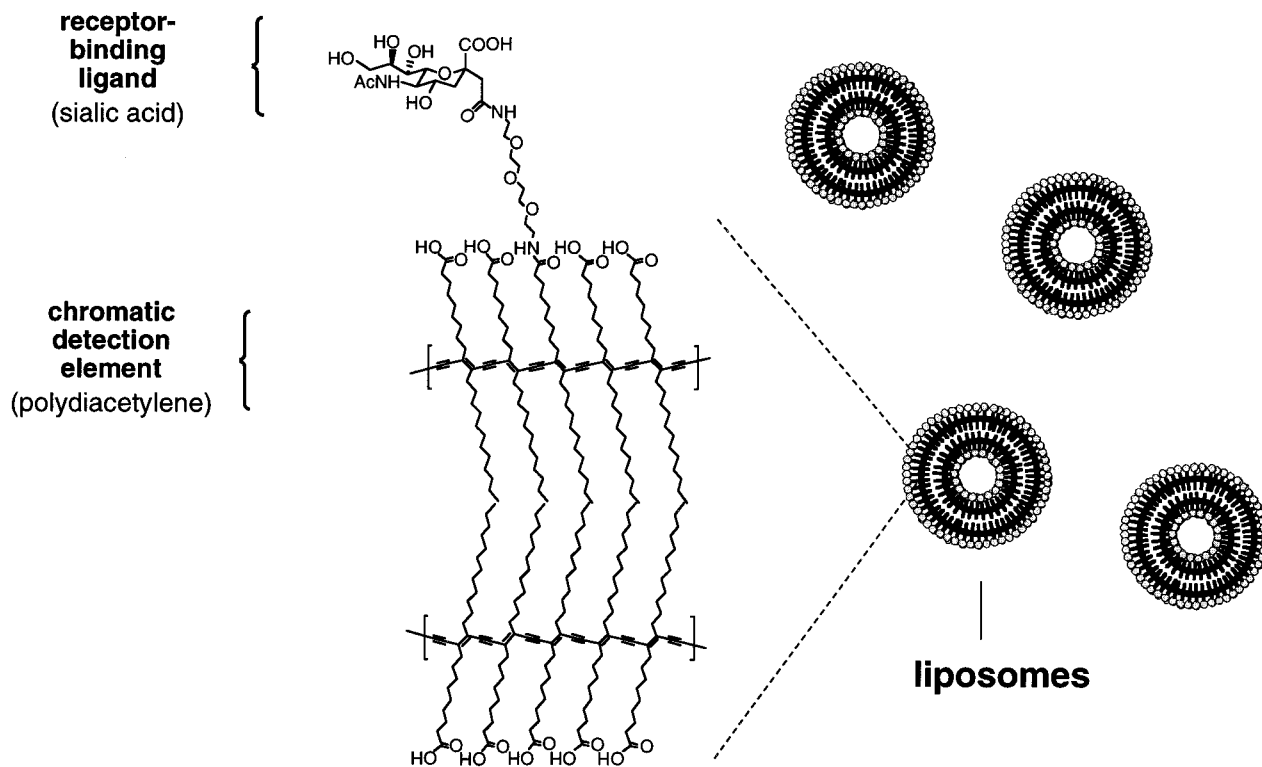
While the majority of the literature on liposomes revolves around naturally-derived monomeric lipids, an increasing number of studies are focusing on polymerized vesicles.<sup>11,12,20–23</sup> These materials are assembled from amphiphilic monomers containing a polymerizable group somewhere along the molecular unit. Polymerized vesicles offer the possibility of engineering various properties such as stability, biocompatibility, enhanced in vivo circulation, or enhanced optical characteristics. Natural cell membranes are highly stable, yet flexible enough to allow transmembrane transport. The stability and flexibility of cell membranes typically arises from the coupling between the lipid membrane and a filamentous network of proteins known as the cytoskeleton. In some sense, therefore, the polymerized lipids mimic the role of the cytoskeleton by stabilizing the synthetic membrane structure.<sup>24</sup> Ringsdorf,<sup>25,26</sup> Regen,<sup>27</sup> Chapman,<sup>28</sup> and O'Brien<sup>29</sup> pioneered the study of polymerized vesicles in the late 1970's and early 1980's by synthesizing a variety of lipids with polymerizable groups located at various points along the molecule. Reactive groups such as butadiene,<sup>30</sup> diacetylene,<sup>30</sup> vinyl,<sup>31</sup> or methacryloyl<sup>27</sup> have been realized. The reactive groups on the lipid may be “stitched” in the headgroup region, the hydrocarbon core, or at the hydrocarbon termini. These stabilized model membranes have been used to study lectin–carbohydrate interactions<sup>32–35</sup> and protein molecular recognition,<sup>36</sup> and have even found their way into the membrane of bacterial cells. As an example of the latter, Leaver et al.<sup>37</sup> showed that the bacteria *Acholeplasma laidlawii* incorporate up

to 90% diacetylenic fatty acids into their membranes. The diacetylene lipid polymerized normally upon UV irradiation, indicating phase separation of the diacetylene lipids from the native lipids in the membrane. More recently, Ravoo et al.<sup>38</sup> used  $\beta$ -nitrostyrene to cross-link only the inner leaflet of the vesicle bilayer, creating nanostructures that may be applied to the study of hemifusion events. Polymerized vesicles encapsulating  $\text{Fe}_3\text{O}_4$  particles allow tracking and intestinal retention, by application of an external magnetic field.<sup>22</sup> On the other hand, circulating paramagnetic polymerized vesicles offer potentially new magnetic resonance contrast reagents.<sup>39</sup> In this case, polymerized vesicles modified with a contrast ion were injected into rats and shown to have a half-life for elimination from the blood pool of 19 h—longer than their nonpolymerized counterparts. The polymerized vesicles also appeared to be nontoxic to the rats.

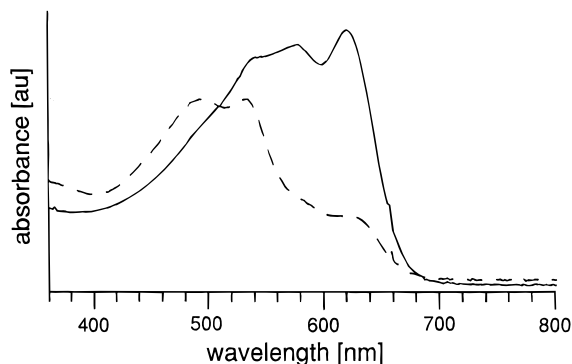
In our laboratory, we have studied polymerized vesicles based on polydiacetylene (PDA) because of their unique optical transitions. The polydiacetylene polymer is formed by 1,4 addition of the diacetylenic monomers, initiated by UV irradiation (eq 1). The resulting polymer is



intensely colored, typically a deep blue. If the R groups of the diacetylenic monomers are designed to impart amphiphilic character to the molecule, the reactive monomers can be self-assembled into thin films or vesicles, forming chromic molecular assemblies. For example, PDA-vesicles decorated with virus-specific or toxin-specific ligands undergo dramatic color changes from blue to red in direct response to pathogen binding at the



**FIGURE 2.** Schematic structure of the PDA-vesicles modified with a carbohydrate capture molecule, sialic acid in this case. This sugar binds to the influenza virus lectin, hemagglutinin. A variety of capture molecules may be co-assembled with or grafted onto the PDA-vesicle.



**FIGURE 3.** Colorimetric detection of influenza virus by PDA-vesicles (5% sialic acid lipid **13**). Visible absorption spectra of blue liposome solution without virus (solid line) and after incubation with 60 HAU of influenza virus (dashed line). The concentration of the vesicle solution in PBS buffer was 0.13 mM.




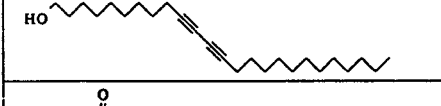
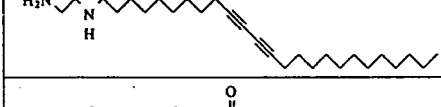
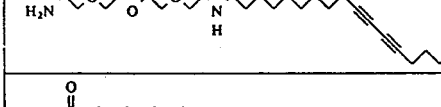
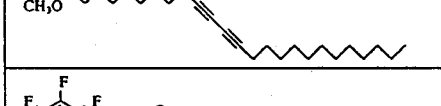
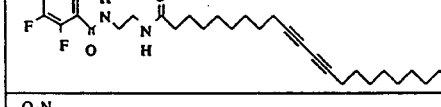
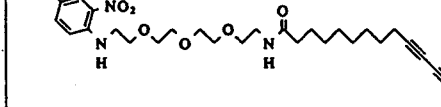
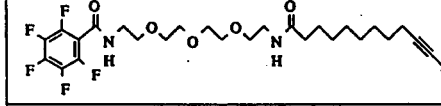
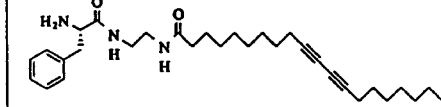
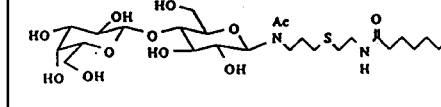
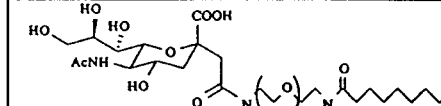
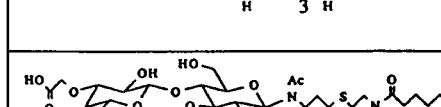
interface.<sup>40–43</sup> The color change arises from the conjugated ene-yne polymer backbone that intensely absorbs visible light wavelengths, and acts as a “reporter” of molecular recognition interactions at the membrane interface (Figure 2). The supramolecular assembly therefore integrates both molecular recognition and signal transduction functions. Figure 3 shows the observed spectral changes upon exposure of sialic acid-modified PDA-vesicles to influenza virus. Initially, the unbound vesicle solution yields a spectrum indicative of the blue-phase PDA with an absorption maximum at  $\approx 630$  nm. When influenza virus is added to the cuvette, the blue phase decreases with a concomitant increase of the red-phase peak ( $\approx 490$  nm), and the solution appears pink/orange. Similar results are obtained for protein toxins that

bind to ganglioside-modified thin films and vesicles.<sup>41,42</sup> More recently, Stevens and Cheng<sup>44</sup> demonstrated a novel colorimetric detection of glucose using hexokinase-modified polydiacetylene thin films. All of these “biochromic” effects are a new addition to the previously known thermochromic and mechanochromic induction of the blue to red color change in polydiacetylene-based materials. Biochromism arises from (1) multipoint interactions of the receptor at the PDA-vesicle surface, disrupting the ordered membrane structure, and/or (2) insertion of viral membrane or toxin hydrophobic domains into the PDA membrane.

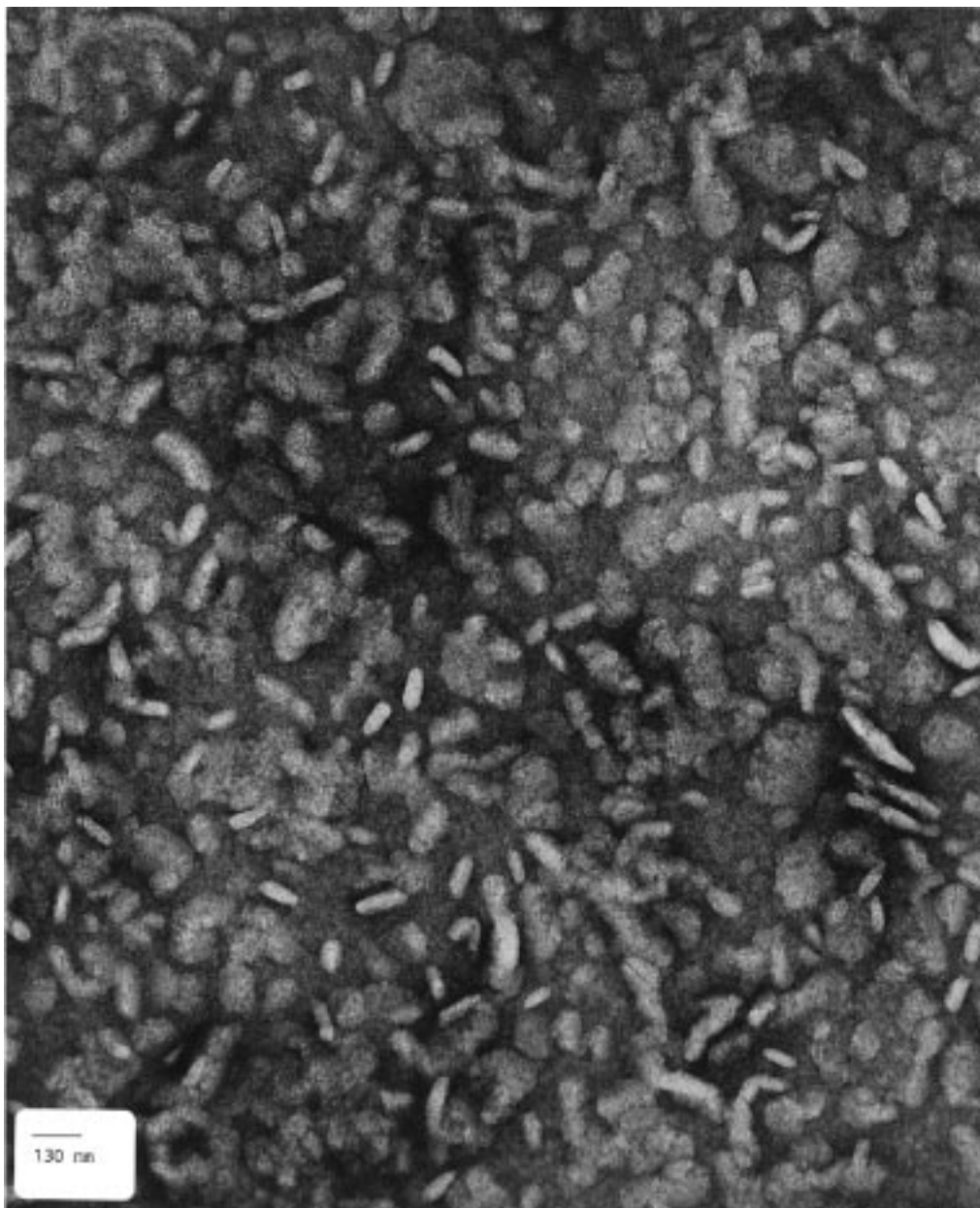
## Vesicle Formation and Polymerizability

Many amphiphilic compounds hydrate to form vesicles. Double-chain phospholipids are perhaps the most common material for vesicle formation, although many single-chained amphiphiles are also capable of forming multibilayer vesicle structures (as opposed to simple micellar structures). In the case of polydiacetylene vesicle formation, the lipids must hydrate and also organize into the correct packing and orientation to undergo the 1,4 addition to the conjugated polymer. Table 1 lists a sampling of polymerizable lipids slated for use in PDA-vesicle biosensors. The ability to hydrate into vesicles and polymerize is indicated in the table, along with qualitative optical characteristics. Vesicle formation can readily be determined by transmission electron microscopy (TEM) or by observation of a cooperative melting transition,  $T_m$  (see next section). The suspension must be cooled below the  $T_m$  to allow polymer formation. Therefore, the po-

Table 1. Vesicle Formation of Amphiphilic Diacetylenic Derivatives in Water<sup>a</sup>

Diacetylene Lipid Structure	#	Hydration	Polymer Formation
	1	Y	Blue
	2	Y	Blue
	3	Y	Purple
	4	Y	Orange
	5	Y	Purple
	6	Y	Purple
	7	Y	Red
	8	Y	N
	9	Y	Black
	10	Y	Red
	11	N	N
	12	Y	N
	13	Y	N
	14	Y	N

<sup>a</sup> Hydration refers to the formation of unpolymerized vesicles. If polymerization occurs, the color of the vesicle suspension is indicated. "N" indicates no polymer formation.



**FIGURE 4.** TEM image of polymerized vesicles composed of 10% sialic acid PDA (**13**) and 90% **3**. The average particle size is approximately 100 nm. The vesicles were negatively stained with 2% uranyl acetate.

lymerization reaction itself (and the optical detection of the blue color) provides indirect evidence of an ordered lipid assembly. Polymerization of the suspension was directly correlated to vesicle formation (as evidenced by TEM) using a number of samples; therefore, formation of the colored polymer can be used as a quick “benchtop” test of vesicle formation. However, the converse is not always true. While all of the compounds in Table 1 hydrate, not all undergo polymerization. For those that do polymerize, a variety of different colors result, ranging from blue to orange. The differences in color likely arise from the differences in packing efficiencies between the monomers. Poor packing results in shorter oligomers that absorb shorter wavelengths. The most intensely blue solutions typically result from strongly hydrogen-bonded headgroups such as the amino acid headgroup glycine (**2**) or other modifications incorporating amide functionalities.

Efficient packing of the chains alone does not necessarily indicate formation of long conjugated lengths as evidenced by the orange color of vesicles of alcohol derivative **4**. Similarly, larger headgroups such as those containing carbohydrates (**11–14**, for example) hinder the solid-state polymerization due to the increased distance between diacetylene units. As expected, these materials form liquid-like expanded layers at the air–water interface as measured by the Langmuir film balance apparatus. Limiting areas range from 70 to 90 Å<sup>2</sup>/molecule compared to 22 to 28 Å<sup>2</sup>/molecule for the compounds that polymerize.

### Physical Properties

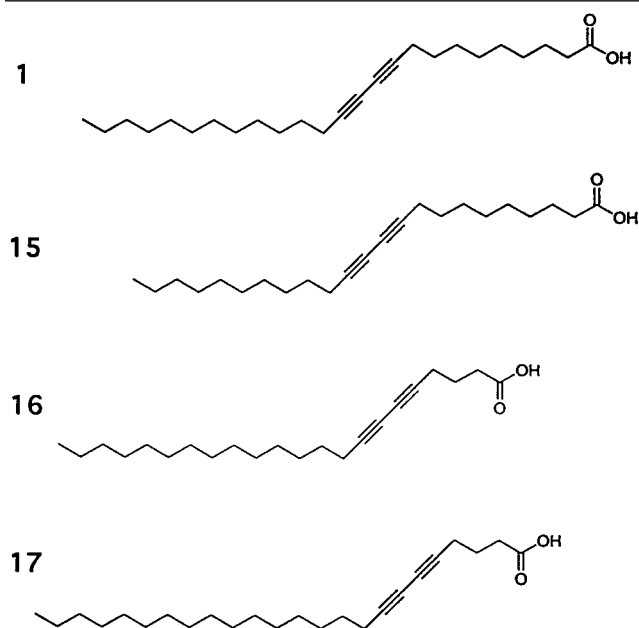
Although the polymerization reaction is commonly associated with the presence of a vesicle morphology, additional evidence comes from transmission electron

microscopy (TEM). A representative example is shown in Figure 4 for vesicles composed of 10% sialic acid-modified PDA (**13**) and 90% **3**. In this case, the polymerized vesicles are elliptical with reasonably uniform size distribution. The vesicles are stable over a period of years and show no evidence of fusion to form larger aggregates once polymerized. The increased stability of the polymerized vesicles are advantageous for biosensor or drug delivery applications. The diameters for the vesicles are in the range of 15–300 nm, depending on the lipid and preparation conditions. Those shown in Figure 4 are approximately 100 nm. Although TEM is an excellent tool for examining polymerized vesicle preparations, it relies on observing a collapsed structure under high vacuum. Independent verification of vesicle formation in a non-destructive manner can be achieved with dynamic light scattering (DLS). The simplest method for vesicles is to use a particle sizing apparatus, and assume that a uniform Gaussian distribution of diameters exists. The assumption of spherical particle shapes makes the DLS results a bit less reliable compared to TEM. However, the technique yielded particle diameters between 50 and 350 nm at 30° scattering angle, in reasonable agreement with the TEM results. Finally, differential scanning calorimetry (DSC) is used to measure the main phase transition ( $T_m$ ). The existence of a phase transition also provides additional evidence for the presence of ordered structures. The  $T_m$  represents the transition between a phase with closely packed (all trans) conformation of the lipid chains to a more disordered phase with the lipid chains rotating out of the all-trans conformation. The diacetylenic lipids that formed vesicles exhibited a  $T_m$  in the range of 30–70 °C. After heating the lipid suspension above  $T_m$ , the vesicle solution must be cooled to allow formation of the solid-analogous phase and subsequent polymerization.

## Thermochromism of Polydiacetylene

One of the more fascinating aspects of polydiacetylene chemistry is the color and chromism of the materials. The energy of electronic excitations, and therefore the color of the material, can be dependent upon many factors such as the original packing state of the monomers and the exposure of the polymeric material to environmental perturbations such as heat (thermochromism),<sup>45–59</sup> mechanical stress (mechanochromism),<sup>60–65</sup> or solvent (solvatochromism).<sup>52,55,66,67</sup> Of the various chromic effects, the thermochromic transition of polydiacetylene has been the most exhaustively studied, and much of the PDA literature is devoted to developing an understanding of the thermochromic blue to red color transition. Some basic threads emerge from this work: (1) the blue to red transition is associated with a conformational change of the PDA backbone from planar to nonplanar and (2) side-chain conformation appears to play a critical role in the planar to nonplanar conformational change of the enyne backbone. However, teasing apart the exact molecular interactions between the side chains and their effect on the backbone remains an active area of research.

**Table 2. Compounds Used for Thermochromic Analysis<sup>a</sup>**



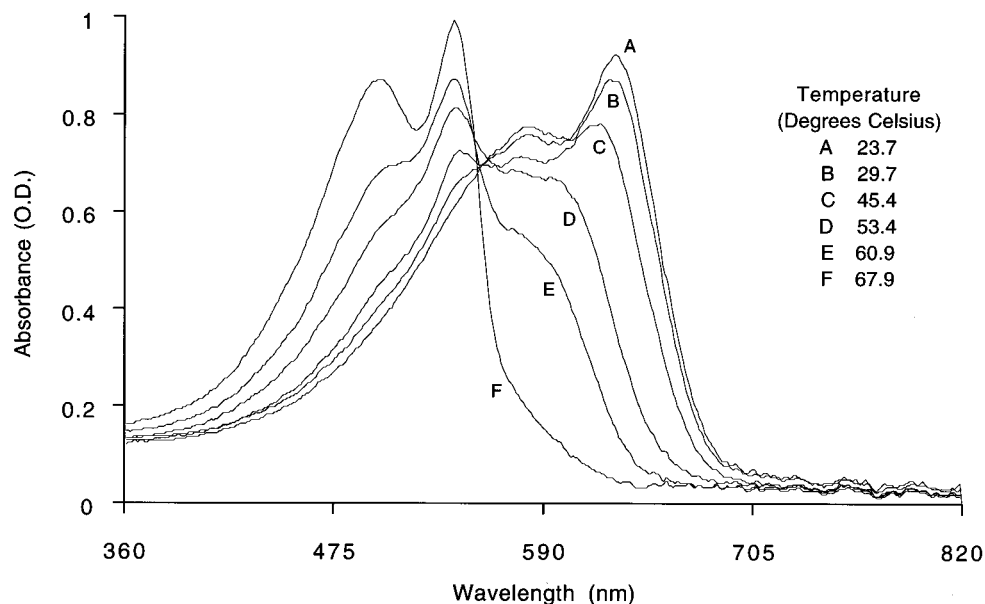
<sup>a</sup> The alkyl chain lengths and the diacetylene group positions are varied.

In one of the first studies of PDA thermochromism, it was proposed that the acetylenic backbone converts to the butatrienic form:<sup>58</sup>



However, subsequent NMR and laser Raman spectroscopic analysis of PDA single crystals have shown that a stable butatriene form is absent in the red-phase PDA.<sup>50,51</sup> Other reports have suggested that, in the case of strongly hydrogen-bonded substituents, heating above the thermochromic transition serves to break the hydrogen bonding, changing the side-chain conformation and straining the backbone from a planar to a nonplanar form.<sup>56</sup> Later studies of single-crystal poly(ETCD) ( $\text{R} = (\text{CH}_2)_4\text{-O-C(O)-NH-C}_2\text{H}_5$ ) have shown that the side chains actually remain hydrogen bonded while undergoing a gauche-trans conformational transition.<sup>51,68</sup> The conformational transition of the side chains places a strain on the conjugated polymer backbone that produces the nonplanar, less conjugated backbone.

With respect to Langmuir–Blodgett films of PDA, many studies have focused on 10,12-pentacosadiynoic acid (PCDA), **1**, Table 1. Results based on FTIR spectroscopy, DSC, and visible absorption spectroscopy have suggested that the thermochromic transition arises from an order–disorder transition of the alkyl side chains.<sup>49</sup> In our own laboratory, however, we have studied the molecular-level morphology of PDA Langmuir–Schaefer films using atomic force microscopy in combination with FTIR spectroscopy. It appears from these data that the side chains actually become more ordered in the red phase and assume a nearly perfect hexagonal packing.<sup>69</sup> The data agree with



**FIGURE 5.** Thermochromism of PDA-vesicles. Visible absorption spectra of vesicles made with **15** are indicated as a function of temperature. The vesicle solution was equilibrated for 10 min after each temperature increase before the spectrum was recorded. The vesicles were polymerized with UV light (0.8 J/cm<sup>2</sup>) prior to the thermochromic experiment.

a conformational transition of the alkyl side chains and maintenance of hydrogen bonding in the headgroup region, analogous to the results obtained for poly(ETCD) single crystals. The motion strains the conjugated polymer backbone to produce the red form.

### Chromic Properties of PDA-Vesicles

Despite the extensive literature on the thermochromic properties of PDA single crystals, solutions, cast films, and Langmuir–Blodgett layers, relatively little information exists regarding the thermochromic properties of PDA-vesicles. Biosensors based on these materials fundamentally rely on the blue to red transition induced by receptor recognition at the interface. Therefore, we have begun to examine the chromic properties of PDA-vesicles as a springboard to future biosensing applications. The effect of chemical structure, UV irradiation dose, and ligand incorporation on the chromic properties of PDA vesicles was investigated, and related to the observed biochromic responses.

Table 2 indicates the compounds that were formed into vesicles, and investigated for their thermochromic properties. Two derivatives carry the diacetylene group at the 10,12 position of the chain (10,12-pentacosadiynoic acid, PCDA, **1**, and 10,12-tricosadiynoic acid, TRCDA, **15**), while the other two carry the reporter group at the 5,7 position (5,7-docosadiynoic acid, DCDA, **16**, and 5,7-tetracosadiynoic acid, TCDA, **17**). The color transitions of the polymerized vesicles were monitored by visible absorption spectroscopy. Peaks within the range of 620–640 nm (PDA blue form) and 490–540 nm (PDA red form) were followed as a function of time at a given temperature of 50 °C. To evaluate the color changes, we define the colorimetric response as the relative change in “percent blue” for the vesicle preparation. The initial percent blue,

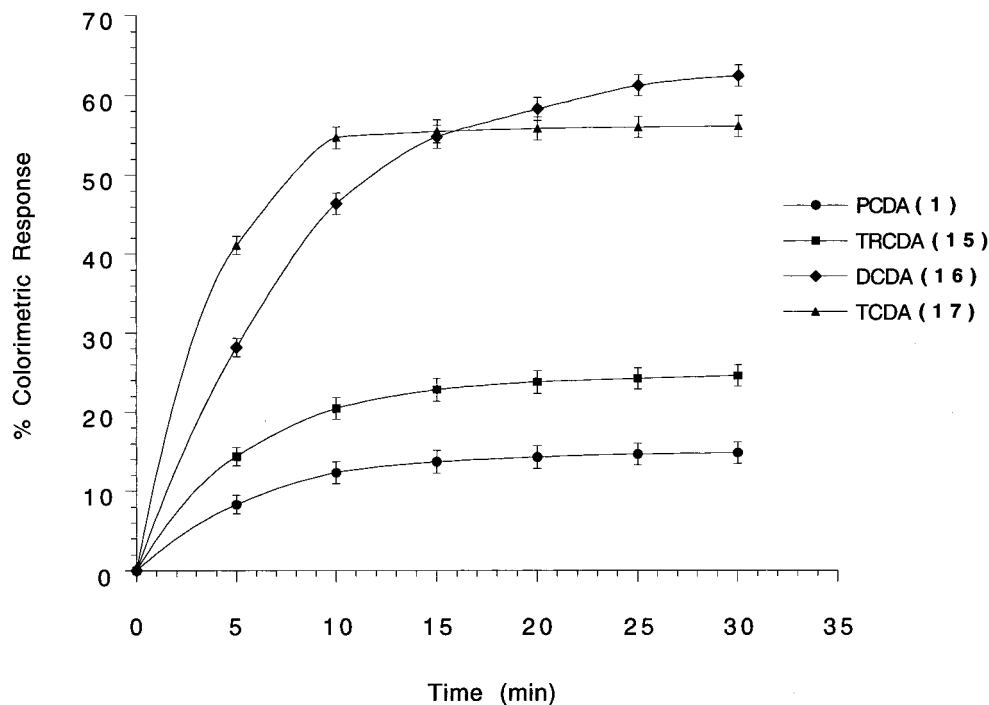
PB<sub>0</sub>, is defined as

$$PB_0 = A_{\text{blue}} / [A_{\text{blue}} + A_{\text{red}}] \times 100\%$$

where *A* is the absorbance at the wavelength of either the blue or red form. (Note: “blue” and “red” refer to the visual appearance of the material, not its relative absorbance.) The colorimetric response characterizes the percent conversion to the red phase at a given temperature for a given time:

$$CR = (PB_0 - PB_f) / PB_0 \times 100\%$$

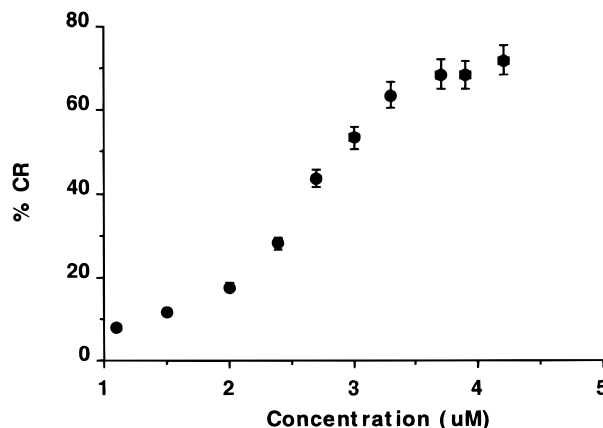
where PB<sub>f</sub> is the final percent blue after the thermochromic transition. Thus, for a completely converted film, PB<sub>f</sub> = 0% and the CR = 100%. The spectral changes of vesicles made with compound **15** as a function of temperature are shown in Figure 5 as an example. The vesicles completely convert to the red phase at a temperature between 61 and 68 °C. Spectrum F would have a CR = 100%. At various points in between, the material takes on a purple to pink color compared to the initial deep royal blue. The final color is a distinct orange. Figure 6 shows the colorimetric response for the four vesicle preparations tested (Table 2) as a function of time at 50 °C. The data are indicative of the relative stability of the blue-phase polymer at this temperature. The trend that emerges is a clear dependency on the positioning of the PDA ene-yne “reporter” group along the chain (i.e., the length of the chain between the headgroup and the backbone) as the CR for the 5,7 derivatives is nearly six times greater than their corresponding 10,12 derivatives. The overall chain length of the molecule also appears to have an effect on the CR although a lesser one. The effect of diacetylene group position may be understood in terms of the cohesive energies of the alkyl chain between the diacetylene and the headgroup. The cohesive energy for a C-3 alkyl chain



**FIGURE 6.** Thermochromic colorimetric response of PDA-vesicles composed of the different diacetylenic lipids shown in Table 2. The samples were incubated at 50 °C and the visible absorption spectrum recorded as a function of time. Samples that produce a colorimetric response (CR) between 10 and 20% have a purple appearance, whereas the samples that produced a CR of 50% are pink. The positioning of the diacetylene group produces the largest effect on the CR. The CR is calculated as in the text.

of the 5,7 derivatives is only ~20 kJ/mol compared to ~55 kJ/mol for the C-8 chain of the 10,12 derivatives. Given the strong linkage between the conformation of the alkyl side chains and the polymer's electronic structure, it appears that the longer, more rigid C-8 chain stabilizes the extended conjugation associated with the blue phase.

The thermochromic data of Figure 6 are directly related to the design of colorimetric PDA-vesicle biosensors. The differences in thermochromic transition temperature provide a method of "tuning" the PDA-based biosensors by modulating the stability of the blue phase. For example, vesicles of sialic acid-PDA (**13**) and PCDA or TRCDA readily change color upon interaction with the large (ca. 1000 Å) influenza virus particle. The virus offers several possibilities for perturbing the ordered side-chain structure including multivalent attachment and partial membrane intercalation. Upon moving to smaller bio-analytes such as cholera protein toxin, we find that vesicles based on PCDA and the ganglioside  $G_{M1}$  ligand are significantly less sensitive. (The  $G_{M1}$  ganglioside is found on the surface of intestinal epithelial cells. This ligand is the primary target of the bacterial toxin that causes the debilitating diarrhea of cholera by increasing intracellular cyclic AMP.) However, if the vesicles are based on DCDA, **16**, a rapid color change is observed when cholera toxin is added.<sup>42</sup> The enhanced response is due to the shorter length between the headgroup and the reporter group. Titration of the color change with increasing amounts of cholera toxin results in a sigmoidal binding curve that saturates at a concentration that corresponds, approximately, to the density of  $G_{M1}$  ligand sites (Figure 7). The sigmoid behavior of the curve suggests cooperative effects



**FIGURE 7.** Colorimetric detection of cholera toxin. PDA-vesicles containing 5% ganglioside  $G_{M1}$  and 95% **16** were exposed to successive additions of cholera toxin. The vesicles were incubated with toxin for 2 min after each addition and the spectra recorded.

of the color change upon binding of toxin. The drawback of the enhanced response of compound **16** over that of **1** or **15** is that vesicles based on this material are more susceptible to solutions containing high salts, although they are quite stable in Tris buffers. (Detailed studies on the ionochromic characteristic of conjugated polymers have been previously reported in the literature.<sup>70,71</sup>) Therefore, it is necessary to first purify the toxin from any salts that may be associated with the protein. Taken together, the thermochromic analysis of the PDA-vesicles provides a measure of blue-phase stability that ultimately can be related to the biosensor response.

Destabilization of the blue phase and hence the chromic properties of PDA-vesicles are also a function of



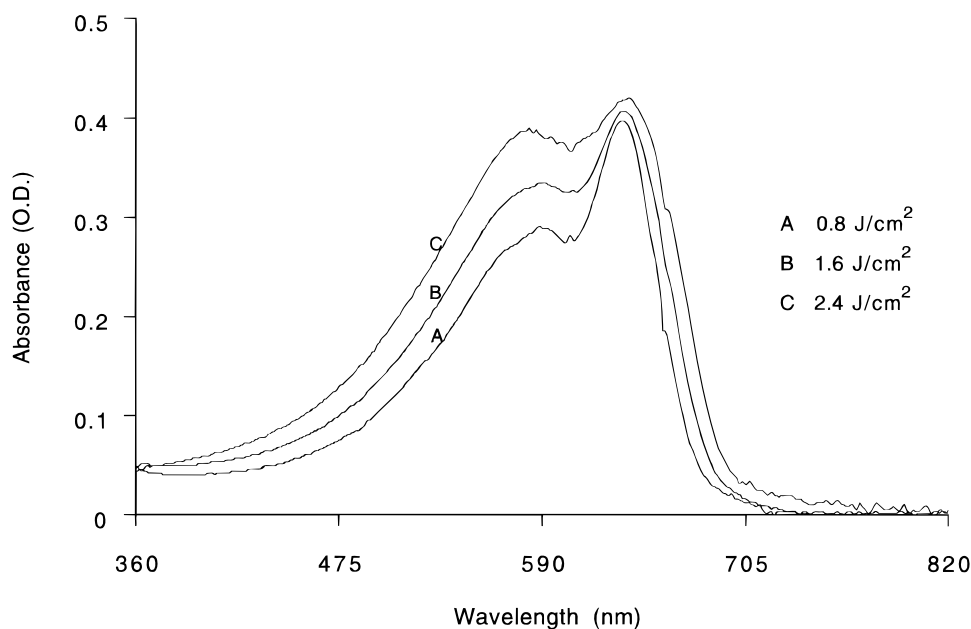


FIGURE 8. Visible absorption spectra of PDA-vesicles of **1** with increasing doses of 254-nm radiation.

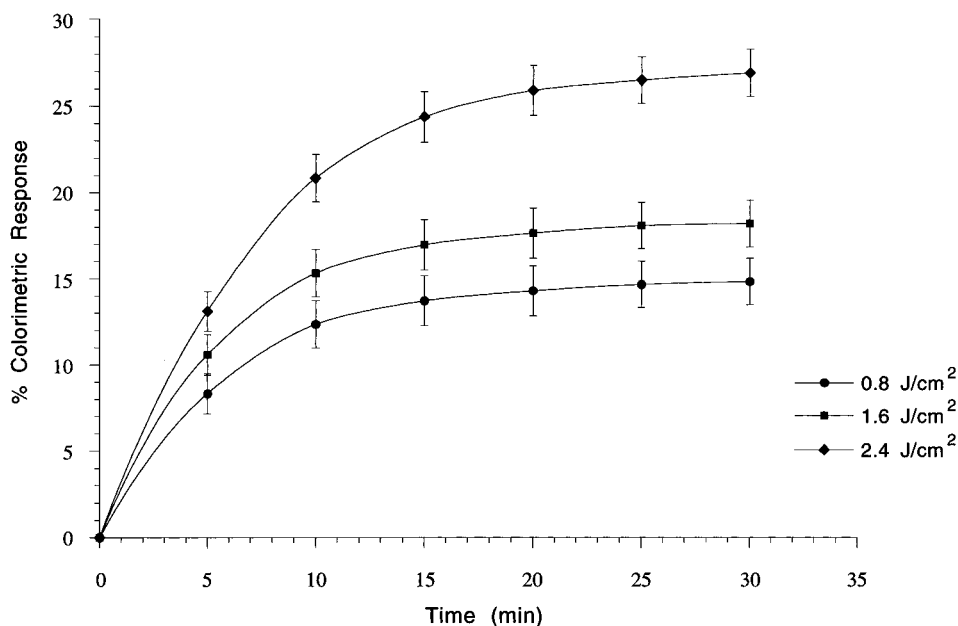


FIGURE 9. Thermochromism of PDA-vesicles made with **1**. The sample was exposed to increasing doses of 254-nm radiation and the CR measured at 50 °C as a function of time.

the polymerization process. Figure 8 shows the visible absorption spectrum of PCDA, **1**, vesicles, polymerized with increasing UV radiation dose. After 0.8 J/cm<sup>2</sup>, it appears that the polymerization reaction is complete. This is based on the fact that subsequent energy doses do not significantly increase the overall absorption density of the material. (The optical density increases as the polymer content increases.) Further irradiation to a total of 1.6 and 2.4 J/cm<sup>2</sup> serves to increase the amount of red-phase polymer as shown by the increased intensity in the region of red-phase absorption. Thermochromic analysis of the vesicles as a function of irradiation dose is shown in Figure 9. The susceptibility of the vesicles to red-phase conversion is clearly enhanced as the material is irradiated

longer. The results suggest a cooperative effect for the blue to red transition and metastability of the blue form. That is, once partial conversion to the red form has taken place by irradiation, the barrier to further conversion is reduced. This result agrees with previous results showing that sialic acid-modified vesicles, incubated with a given quantity of influenza virus, have a greater %CR if they are irradiated longer prior to incubation with the virus.<sup>40</sup>

The incorporation of ligand lipid molecules into the PDA-vesicles also has a destabilizing effect on the blue phase material. Figure 10 shows the thermochromic colorimetric response at 50 °C as a function of mole percentage sialic acid ligand in the vesicles. At 5 mol %, very little effect on the stability of the blue phase is

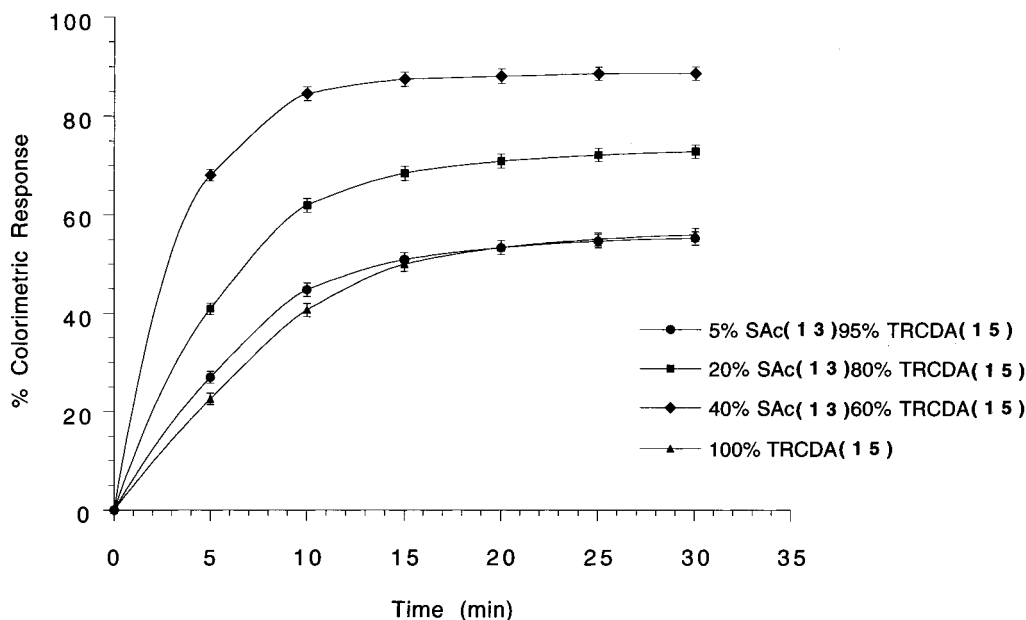


FIGURE 10. Thermochromism of PDA-vesicles containing increasing percentages of carbohydrate ligand **13**. Spectra were recorded at 50 °C as a function of time.

observed. However, at 40% sialic acid, the blue phase is significantly destabilized as shown by a nearly 2-fold increase in the thermochromic colorimetric response. The destabilization again correlates with the biochromic response observed with influenza virus interaction. For example, the %CR = 55 for vesicles composed of 5% sialic acid, compared to %CR = 85 for vesicles containing 40% sialic acid. Therefore, increased ligand doping offers another possibility of modulating or tuning the biochromic response.

## Conclusion and Outlook

Polymerized vesicles are finding their way into a number of pharmaceutical and diagnostic applications such as drug delivery or MRI imaging. In this Account, we have tried to demonstrate that sensors based on conjugated self-assembled polymers such as PDA-vesicles offer an attractive alternative for detecting biological agents. In these materials, the signal transduction mechanism is directly linked to bio-molecular recognition, requiring only the chemistry of the biosensor components themselves. In particular, the color and chromic properties of several diacetylenic lipids were discussed. Depending on the chemical structure of the lipid, a variety of vesicle solution colors are obtained. The thermochromic properties of the PDA-vesicles provide a marker for predicting and modulating the colorimetric response of the material, and reveal the intertwined nature of the various chromic properties. At this juncture, the PDA-vesicle approach has not been developed into practical, commercially available biosensors. However, the properties of these self-assembled chromophoric materials should serve as prototypes for numerous possibilities in the area of molecular biosensing.

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