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Fluorogenic Polydiacetylene Supramolecules: Immobilization, Micropatterning, and Application to Label-Free Chemosensors

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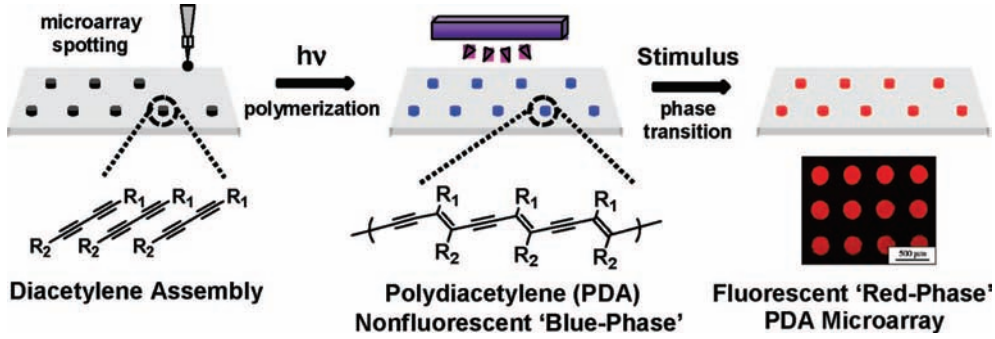
CONSPECTUS



This Account describes a new strategy for the preparation of label-free sensor systems based on the fluorogenic properties of the conjugated polymer, polydiacetylene (PDA). PDA has been extensively investigated as a sensor matrix, owing to a brilliant blue-to-red color transition that takes place in response to environmental perturbations. It has been known for some time that "blue-phase" PDAs are nonfluorescent while their "red-phase" counterparts fluoresce. For the most part, however, the significance of the different fluorogenic properties of PDAs has been ignored in the context of sensor applications. In the course of developing PDA-based sensors, we discovered that PDA vesicles can be readily immobilized on solid substrates. This is an attractive property of PDAs since it leads to the combined advantages of the vesicle sensors (which have three-dimensional interactions between sensor and target molecules) and film sensors (which are applicable to a two-dimensional array or chip format). Stable blue-phase immobilized PDAs can be prepared by employing one of three strategies involving the formation of covalent adducts, biotin–avidin complexes, or complexes formed through nonspecific physical adsorption. A procedure for generating well-patterned fluorescence images is necessary for the immobilized PDAs to function in chip-based sensor systems. Patterned fluorescence images are readily constructed by employing (1) the photolithographic technique, (2) the micromolding in capillaries (MIMIC) method, or (3) an array spotting system. Heat treatment of the patterned "blue-phase" PDA vesicles transforms the nonfluorescent images into their fluorescent red forms. The observation that finely resolved fluorescence patterns can be generated by heat treatment of microarrayed PDAs is highly significant in that it indicates that fluorescence signals might be produced by specific molecular recognition events. Indeed, red fluorescence emission is observed when immobilized PDAs are subjected to specific molecular recognition events, such as ligand−cyclodextrin or protein–protein interactions. The facile immobilization of PDA vesicles on solid substrates and the affinity-induced fluorescence emission combine to make this system applicable to the fabrication of label-free PDA sensors. Since in theory any molecular recognition event that promotes the blue-to-red color transition of PDAs should result in the generation of fluorescence, it should be possible to reformat a variety of previously described colorimetric PDA sensors into fluorescence-based sensor systems. The fluorescence properties of PDAs, when combined with modern methods for the fabrication of microarrays, should stimulate the development of a number of new label-free chemosensor systems.

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Introduction

The development of efficient chemosensors based on conjugated polymers continues to be an important goal of scientists working in both fundamental and applied research areas.[1] Conjugated polymer systems are highly attractive for this purpose because changes in their absorption, emission, and redox properties can often be promoted by environmental perturbations. A major advantage of using conjugated polymer-based chemosensors, in comparison to small molecule-based conventional sensors, lies in the potential for signal amplification when subjected to external stimuli. As a result, a wide variety of conjugated polymers, including polythiophenes, polyanilines, polypyrroles, and polyphenylene, as well as poly(phenylene ethynylenes), polyacetylenes, and polydiacetylenes have been investigated as sensing matrices.[2–7]

Among the conjugated polymers reported to date, polydiacetylene (PDA)-based chemosensors are unique from the perspective of method of preparation, molecular structure, and output signal. Unlike other conjugated polymers, functionalized PDAs are generally prepared by using photopolymerization of self-assembled diacetylene monomers.[8–26] Closely packed and properly ordered diacetylene lipids undergo polymerization via a 1,4-addition reaction to form alternating ene−yne polymer chains upon irradiation with 254 nm light (in the case of thin films and vesicle solutions) or with γ-irradiation (in the case of solid powders). Since no chemical initiators or catalysts are required for the polymerization process, the polymers are not contaminated with impurities, and consequently, purification steps are not required. PDAs generated under optimized photochemical conditions have an intense blue color. One unique property of nanostructured PDAs that has fostered their application in sensing systems is the occurrence of a blue-to-red color change that takes place in response to heat (thermochromism),[27–31] organic solvents (solvatochromism),[12,32,33] mechanical stress (mechanochromism),[8a,34,35] and ligand–receptor interaction (affinochromism or biochromism)[36–48] (Figure 1).

It has been known for some time that "blue-phase" PDAs are nonfluorescent, while their "red-phase" counterparts fluoresce.[49] Despite having this unique property, little effort has been given to exploiting the fluorescence signaling features of PDA sensors.[50–52] During the course of our investigations,[31,32,46–48,52–58] targeted at the development of PDA-based sensors, we discovered that PDA vesicles can be readily immobilized on solid substrates.[52–54] In addition, we observed that the immobilized PDAs, when heated or subjected to specific molecular recognition events, emit red fluorescence. The stimulation-induced fluorogenic property of the immobilized PDAs enables the generation of patterned fluorescence images as well as the construction of label-free sensors. In this Account, the PDA-based chemosensors reported to date are briefly reviewed, and the results of our recent studies directed at the development of fluorescence-based PDA sensor systems are discussed in detail.

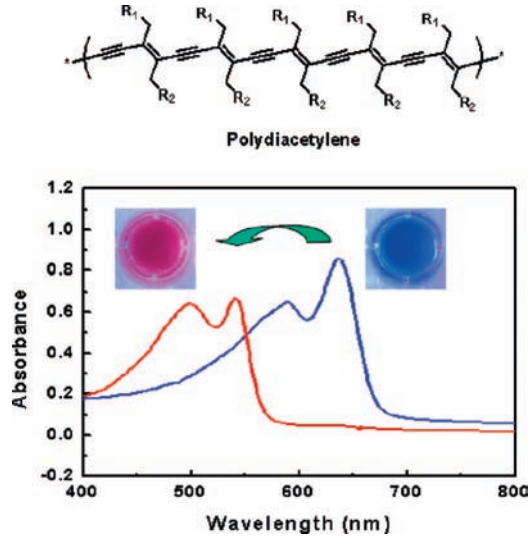

FIGURE 1. Absorption spectral and color changes of a PDA solution that take place upon stimulation.

Polydiacetylene Chemosensors

Wegner, Ringsdorf, and Charych carried out pioneering work in the field of PDA sensors. Wegner[9] discovered PDAs while Ringsdorf[8d,10] developed strategies for the preparation of PDA vesicles and films. Based on this knowledge, Charych demonstrated that PDAs serve as fascinating sensor matrices for the detection of biologically interesting target molecules.[36] In an exceptionally interesting early experiment, Charych and her co-workers prepared a PDA Langmuir–Blodgett (LB) film, functionalized with sialic acid, and showed that the film undergoes a blue-to-red color change when exposed to an influenza virus.

Since the time of the discovery of the influenza virus detecting system, a variety of colorimetric PDA-based chemo/biosensors have been investigated. A schematic representation of PDA sensor systems reported to date is shown in Figure 2. PDA films and vesicles, functionalized with carbohydrates, have proven to be effective biosensors for the detection of the influenza virus,[37] cholera toxin,[38] and *Escherichia coli.*[39] PDA vesicles immobilized with probe DNA molecules undergo the blue-to-red colorimetric transition upon binding with complementary strands of DNA, enabling them to be used as colori-

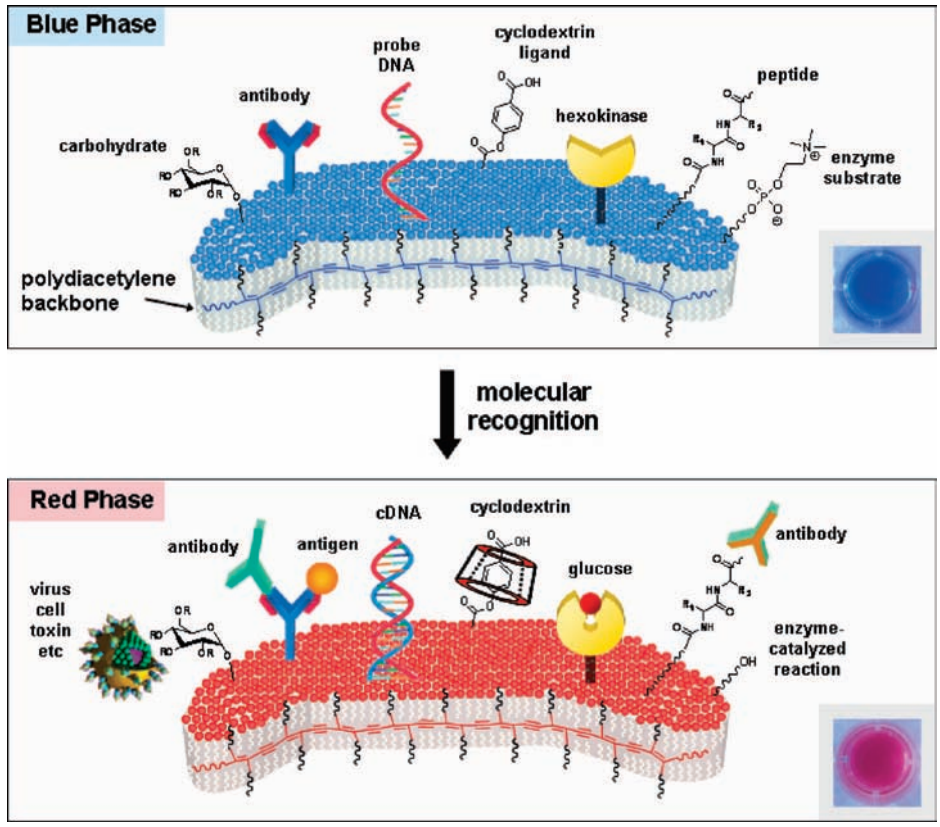

FIGURE 2. A schematic representation of surface ligands and their interactions with target molecules in colorimetric PDA sensors.

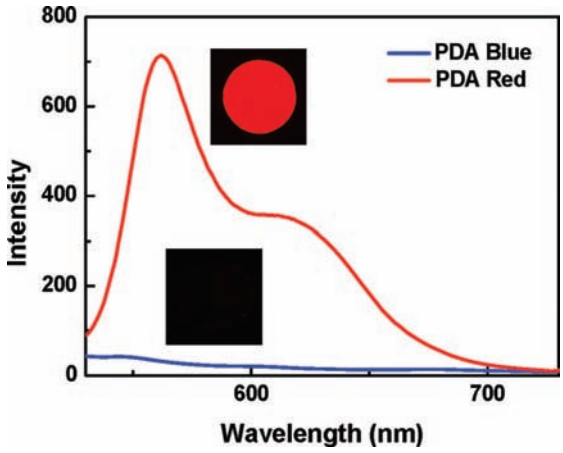

FIGURE 3. Fluorescence properties of PDAs in blue and red phases.

metric DNA sensors.[40] Colorimetric detection of glucose, based on ligand-induced conformational changes of hexokinase immobilized on a PDA monolayer, represents another elegant application of PDA-based biosensing.[41] A system for selective detection of metal ions, formed by embedding an ionophore into a PDA liposome, also has been reported.[42] A PDA-based enzyme detecting sensor, driven by a hydrophilic-to-hydrophobic transformation of an enzyme–substrate, has been described recently.[43] In this system, hydrophobic products of enzyme-catalyzed reactions of hydrophilic substrates perturb the ordered structures of PDAs thus causing the color transition. Very recently, colorimetric PDA sensor systems based on specific antibody–antigen (epitope) interactions have been developed.[44–46] Finally, we have observed cyclodextrin-induced color changes of PDA vesicles[47] and polymerized diacetylene Langmuir–Schaefer (LS) films.[48]

Fluorogenic Properties of Polydiacetylenes

Owing to the readily detectable color change that PDAs undergo upon environmental perturbation, the significance of the different fluorogenic properties of PDAs has been nearly ignored from the perspective of sensor applications.[50–52] However, close inspection of the fluorogenic properties of PDA reveals several very important and attractive features. First, as shown in Figure 3 and described in the Introduction section, a fluorescence signal is generated when PDAs undergo a phase transition from blue to red. In general, for sensor applications, it is desirable to have the system generate a fluorescence signal (i.e., turn-on type) rather than experience a loss or a decrease of fluorescence (i.e., turn-off type) when the background fluorescence signal prior to stimulation is negligible. Thus, the stress-induced nonfluorescence-to-fluorescence transition of PDAs should be applicable to the construction of sensitive PDA-based chemosensors. The exact

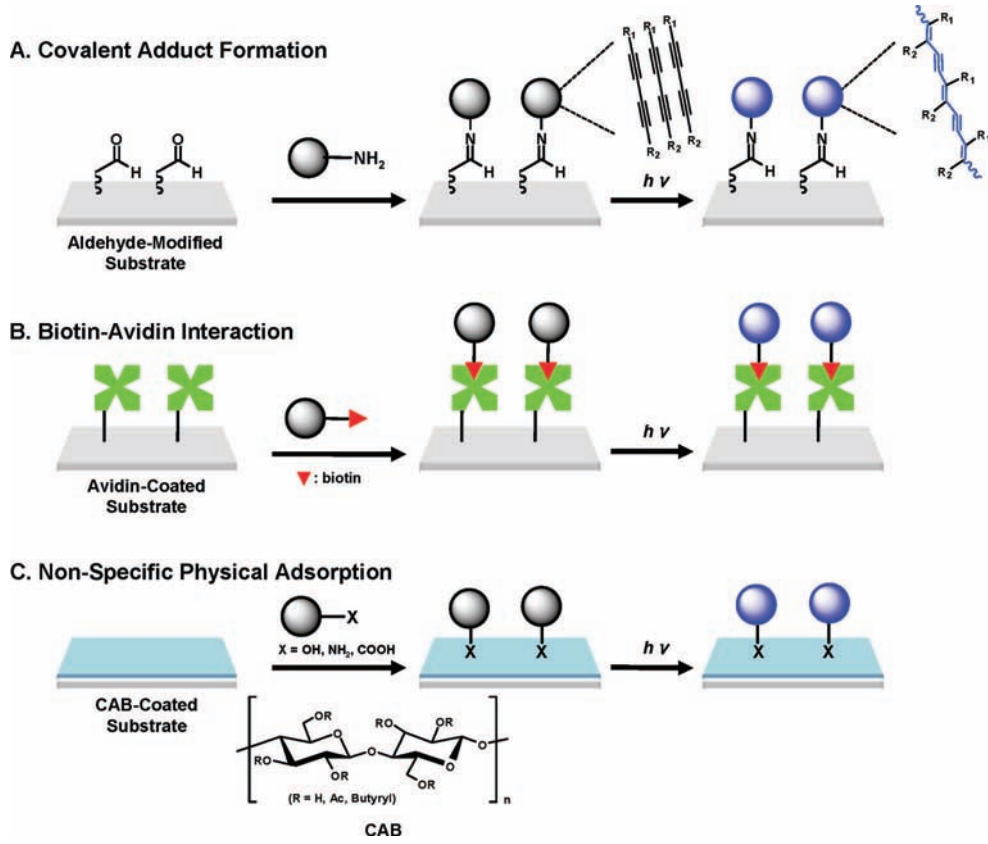

FIGURE 4. A schematic representation of three strategies employed for the fabrication of immobilized "blue-phase" PDAs.

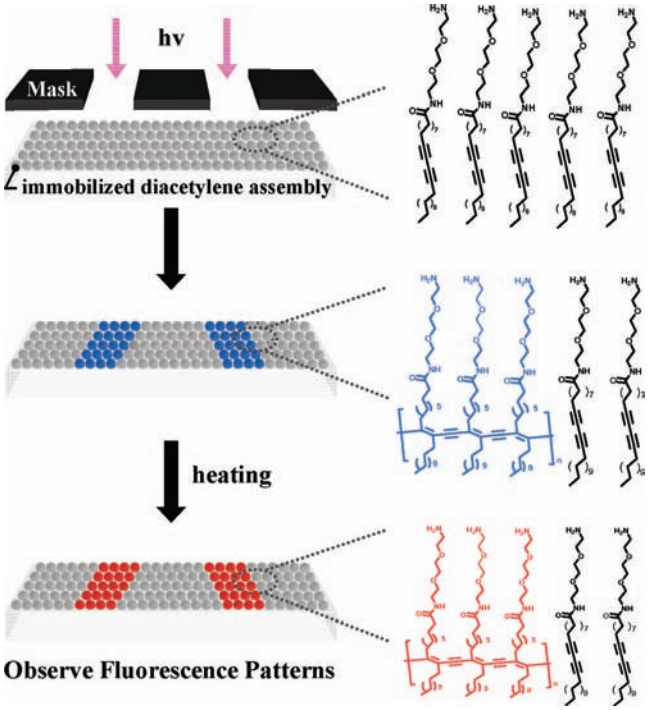

FIGURE 5. Procedure for the generation of patterned fluorescence images with immobilized diacetylene supramolecules on the solid substrate.

mechanistic reason for the change in PDA fluorescence properties is not fully understood. It has been suggested that fluorescence in these systems is due to radiative decay from the lowest excited B_u state.[8a,59,60] The blue-phase PDA is believed to have A_g symmetry, and consequently, emission from its singlet excited state is associated with a dipole-forbidden transition.

Another important feature of the fluorogenic properties of PDAs was demonstrated by our observation that readily detectable fluorescence signals arise from stress-exposed PDA vesicles that are immobilized on solid substrates (*vide infra*). This is an important finding since fluorescence intensities of most conjugated polymers or small molecules tend to decrease drastically or are completely quenched when they are part of solid aggregates.[61] The results suggest that fluorescence quenching by intermolecular energy or electron transfer processes, a common occurrence for other fluorescent molecules, is not significant in PDAs found within isolated vesicular structures unless quencher molecules are embedded in the vicinity of the PDA backbone. Recently, Cheng and co-workers reported an elegant fluorescence sensor system based on intermolecular energy transfer between PDA backbones and BODIPY dyes embedded in the interior of the PDA vesicles.[25] The energy or electron transfer process was found to be negligible when the quencher dyes are

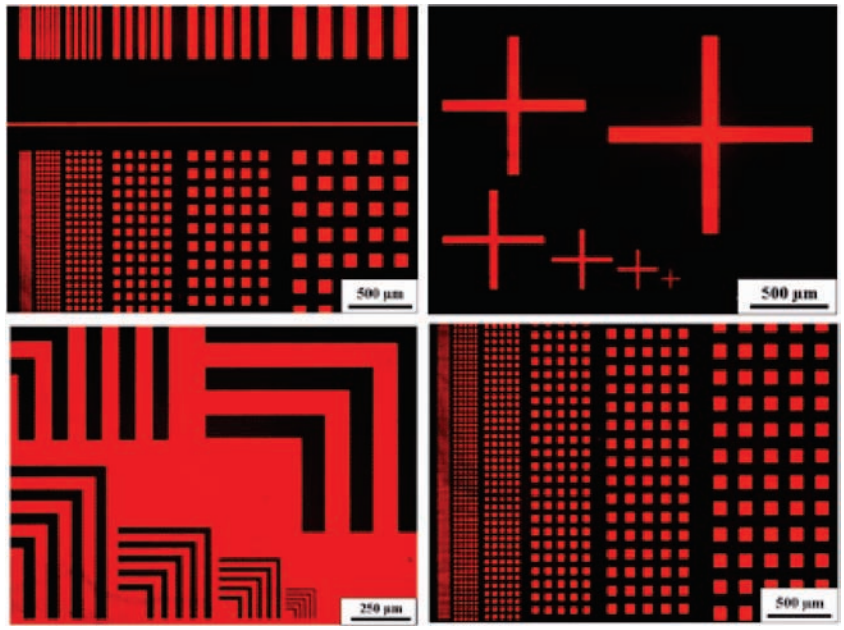

FIGURE 6. Patterned fluorescent images obtained from photomasked irradiation of immobilized diacetylene vesicles on the aldehyde-modified glass substrate. Reproduced from ref 52. Copyright 2005 American Chemical Society.

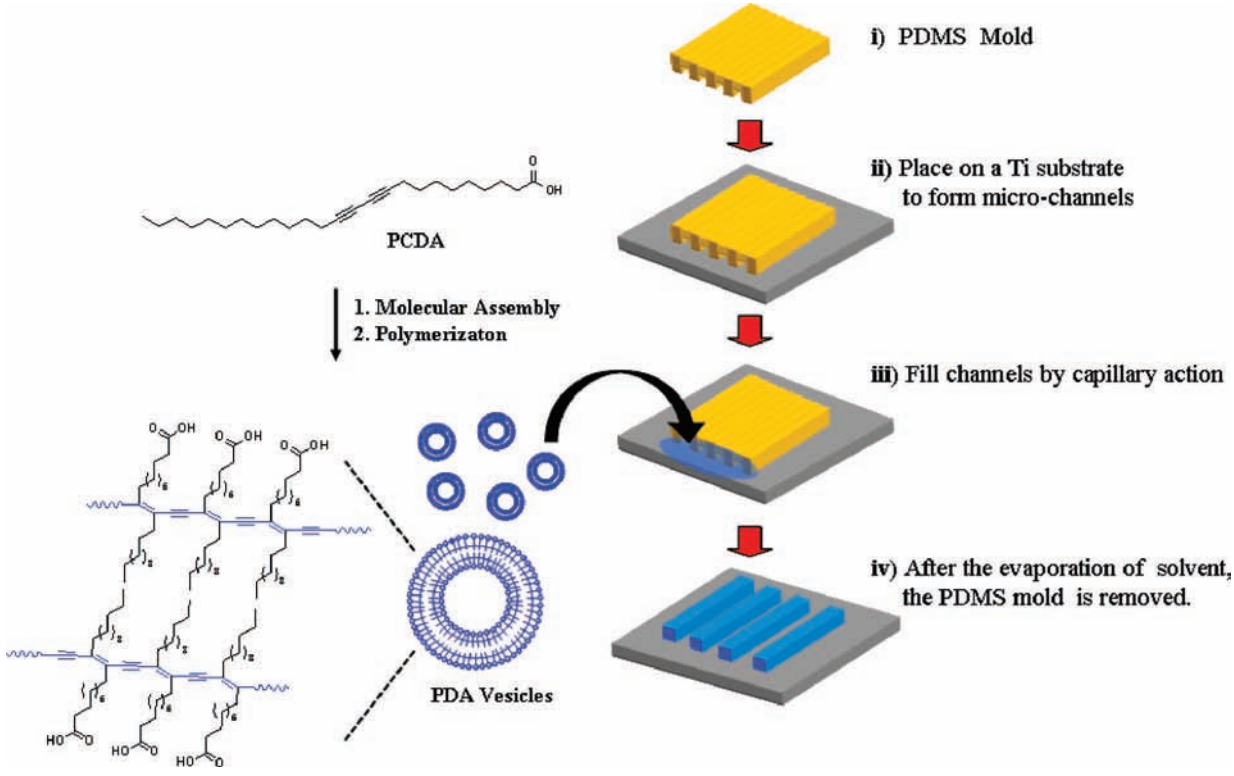

FIGURE 7. A schematic illustration of the procedure employed for PDA patterns on a substrate using the MIMIC method. Adapted from ref 65.

located away from the PDA backbone. For example, we have attempted unsuccessfully to quench the fluorescence of negatively charged red-phase PDAs by the addition of a positively charged excellent fluorescence quencher, methyl viologen. The positively charged quencher molecules on the surface of PDA vesicles are not accessible to the PDA backbones due to the hydrophobic alkyl shells. These are unique features of PDA supramolecules and should be contrasted with those obtained in studies with other fluorescent-conjugated polymers, which experience almost complete fluorescence quenching in the

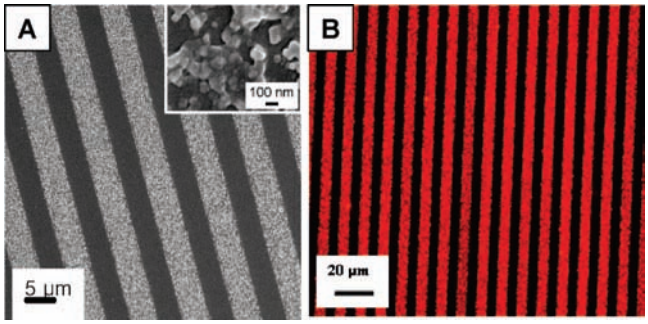

FIGURE 8. SEM (A) and fluorescence (B) images of PDA patterns fabricated on Ti substrates by MIMIC. Inset shows a magnified image of the immobilized PDA region. The fluorescence image was obtained after heat treatment (100 °C, 1 min) of the immobilized PDAs. Adapted from ref 65.

presence of singlet quenchers such as cyanine dyes or heme-containing proteins.[62]

The fluorescence quantum yield of the PDAs in the red form is measured to be ca. 2×10^{-2} using cresyl violet and rhodamine 6G as standards.[49b] The background fluorescence emission from the blue-phase PDAs is almost negligible due to the extremely low quantum yield ($<1 \times 10^{-4}$). Although the fluorescence quantum yield of the red-phase PDA is relatively low, the stress-stimulated nonfluorescence-to-fluorescence transition is readily detectable under a fluorescence microscope (*vide infra*). Very recently, a significant increase (ca. 20 times) of fluorescence intensity of the red-phase PDAs was observed by employing the fluorescence resonance energy transfer (FRET) concept.[51] Thus, the facile immobilization on solid substrates and the readily detectable fluorescence signal make PDAs ideal materials for chip-based label-free detection systems.

Immobilization of Polydiacetylene Vesicles on Solid Substrates

Immobilization of PDA vesicles on solid substrates is very attractive since it can combine advantages of the vesicle sensor (having three-dimensional interactions between sensor and the target molecules) and film sensor (applicable to two-dimensional array or chip format). Since one of the most important aspects of the PDA-based chemosensors is their unique blue-to-red/nonfluorescence-to-fluorescence transition that takes place upon stimulation, it is essential to prepare the sensor system in its "blue phase" before the environmental perturbation is applied. Preparation of stable, blue-phase, immobilized PDAs can be executed by employing one of three strategies involving the formation of covalent adducts, biotin–avidin complexes, or complexes formed through nonspecific physical adsorption (Figure 4). In methodologies involving covalent adduct formation, incubation of an aldehyde-modified glass substrate in an aqueous solution containing diacetylene vesicles having terminal amino groups results in covalent attachment of the diacetylene vesicles via imine linkages.[53] UV irradiation of the diacetylene-immobilized solid substrate should induce photopolymerization of the diacetylenes to give the immobilized, "blue-phase" PDAs (Figure 4A). Although direct immobilization of polymerized PDA vesicles is possible, it is observed that this method induces significant blue-to-red phase transition during the immobilization process. This is likely due to stress imposed on the PDA vesicles during the condensation reaction linking the surface aldehyde groups with the amine residues in the PDA vesicles. The typical blue-colored PDAs, in general, have maximum absorption wavelength at around 640 nm. The immobilized PDA vesicles formed by this method often have higher absorbance at 550 nm than at 640 nm, indicating that the colorimetric transition has occurred during the immobilization process. In contrast, when photopolymerization is carried out after immobilization, the PDA vesicles are generated in their blue phase on the solid substrates.

Alternatively, specific ligand–receptor interactions can be utilized for the fabrication of immobilized PDA sensor systems (Figure 4B).[63] For example, diacetylene vesicles possessing biotin moieties can be immobilized on avidin-coated solid substrates by way of biotin–avidin complex formation. Photopolymerization of the resulting immobilized diacetylenes produces the desired blue-colored PDAs. A major advantage of this methodology is its flexibility. For example, diacetylene vesicles that are prepared from monomers that contain both biotin- and amine-terminated diacetylenes can be subjected to further modification after or before immobilization since the amine groups do not interact with avidin molecules present on the surface of the solid substrate.

Recently, we developed a new strategy for the immobilization of PDA vesicles based on the finding that cellulose acetate butyrate (CAB) is an efficient matrix polymer for the immobilization of PDA sensors (Figure 4C).[64] Diacetylene vesicles were found to efficiently bind with the commercially available and spin-castable polymer matrix on glass substrates via physical adsorption, regardless of the types of functional groups they possess. Thus, alcohol, amine, or carboxylic acid-terminated diacetylene vesicles can be immobilized on the CAB-coated solid substrates without the need for covalent bonding between PDAs and the glass.

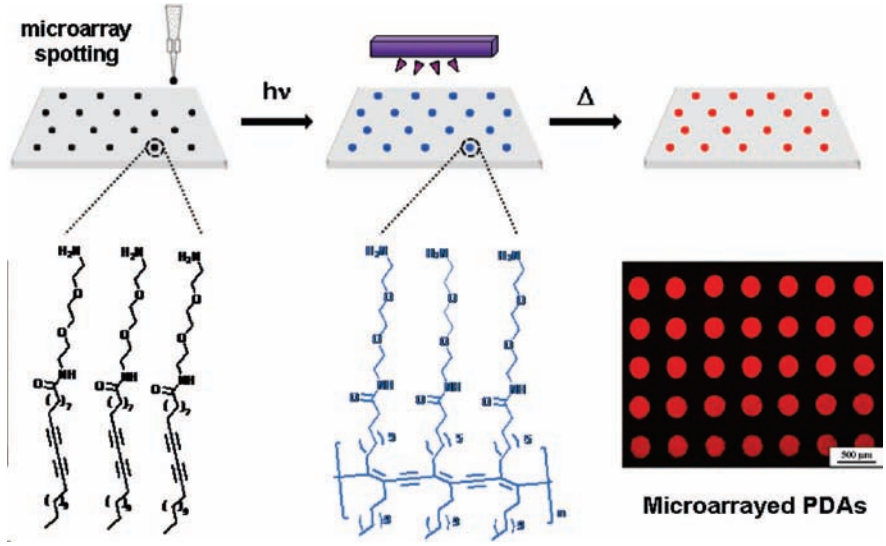

FIGURE 9. Schematic representation and fluorescence images of the microarrayed PDAs using a conventional microarray spotter.

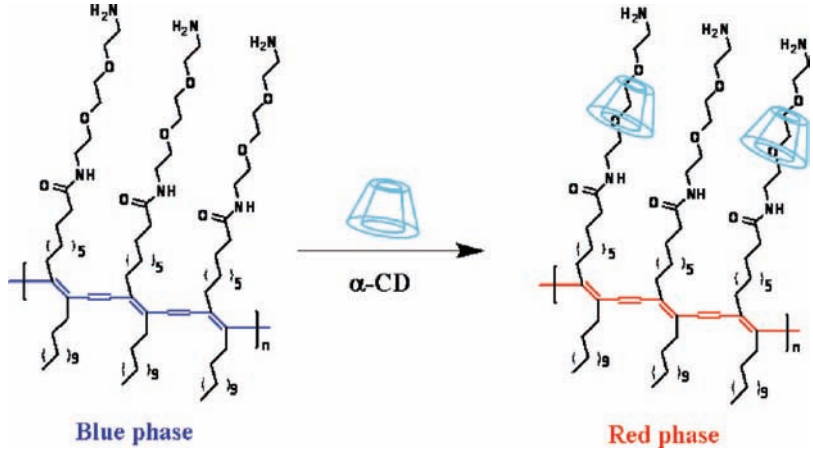

FIGURE 10. A schematic representation of interaction between PDA and CD.

Patterned Fluorescence Images

Although PDA immobilization processes can be monitored by using visible spectroscopy and SEM analysis, evaluation of the patterned images formed in this manner is best carried out by using fluorescence spectroscopy. In order for the immobilized PDAs to be applicable to chip-based sensor systems, a procedure for generating well-patterned fluorescence images is required. A facile method to accomplish fabrication of high-quality images uses photolithography (Figure 5).[52] In this methodology, an aldehyde-modified glass substrate is immobilized with unpolymerized self-assembled diacetylene vesicles. The diacetylene-immobilized glass substrate is then irradiated through a photomask with 254 nm UV light (1 mW/cm^2) leading to photopolymerization of the immobilized diacetylene vesicles only in exposed areas. The glass substrate is then heated at 100 °C for 10 s to induce the blue-to-red phase transition of the polymer. Since "red-phase" PDAs are fluorescent and polymerization does not occur thermally, patterned fluorescence images are generated after the thermal treatment.

In Figure 6 are displayed patterned fluorescence images observed by using fluorescent microscopy (red, bright areas correspond to areas exposed to UV light). The clear images obtained by employing this methodology demonstrate the successful immobilization of PDA vesicles. By using this method, we were able to create patterned fluorescence images of PDAs having resolutions that approach the limit of fluorescence microscopy (<5 μm).

We recently discovered a new, straightforward strategy for the fabrication of patterned fluorescent PDA images that is based on the micromolding in capillaries (MIMIC) technology.[65] A schematic representation of the MIMIC[66] procedure employed for this purpose is given in Figure 7. A polydimethylsiloxane (PDMS) mold was treated with UV/ozone (UVO) to

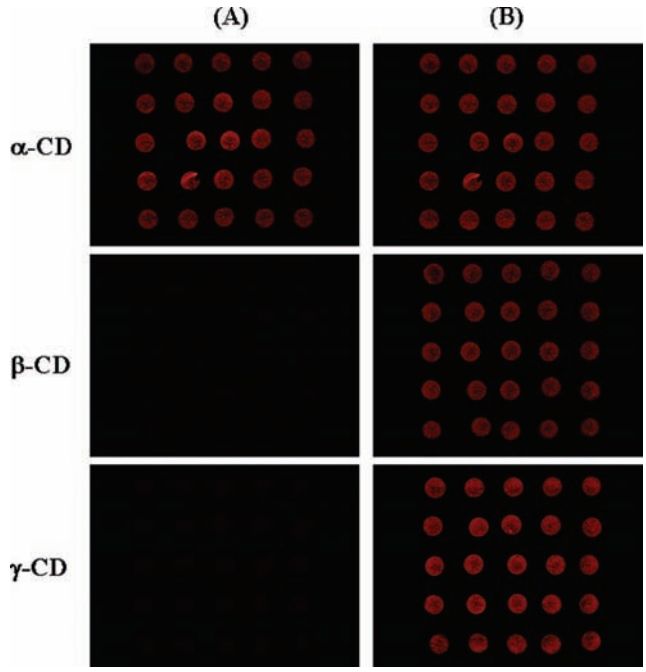

FIGURE 11. Fluorescence microscope images of polymerized PDA immobilized on CAB-coated glass substrates after treatment with 10 mM CDs for 30 min (A) and after heating the glasses in panel A at 100 °C for 2 min (B). Adapted from ref 64.

introduce surface hydroxyl groups. The UVO-treated PDMS mold was then placed on a solid substrate to generate microchannels, which were filled by applying a PDA solution at one end. The PDA solution, derived from 10,12-pentacosadiynoic acid (PCDA), for example, is expected to move into the channels by capillary force. After evaporation of the solvent, the PDMS mold was removed to leave PDA vesicles on the solid substrate.

SEM and fluorescence microscope images of PDA patterns on Ti substrates, fabricated using the MIMIC procedure, are shown in Figure 8. A master bearing 5 μm wide line patterns was used for the micrometer patterning of PDA vesicles. The bright lines shown in Figure 8A are patterned PDA vesicles and the dark lines are bare Ti surfaces. The fluorescence microscopic images shown in Figure 8B are obtained after heat treatment (100 °C, 1 min) of the Ti substrate immobilized with PDAs.

The photolithographic and MIMIC approaches described above are very convenient for the generation of large area fluorescence patterns of PDAs. However, for the construction of chip-sensor systems, it would be more versatile and practical if patterned images could be obtained by using a conventional microarray spotter. Consequently, our attention has also focused on assessing the feasibility of using a microarray spotter to generate patterned arrays of PDA images. In order to assess the viability of this procedure, a diacetylene vesicle solution prepared from an amine-terminated diacetylenic lipid monomer was applied to an aldehyde-modified glass substrate by using a standard array spotter (Figure 9).[52] The glass substrate coated with arrayed diacetylenes was irradiated with UV light to induce photopolymerization. Heat treatment of the resultant blue-phase PDA arrays afforded the red light emitting fluorescence patterns displayed in Figure 9.

Label-Free Sensor Application of Polydiacetylenes

The observation that finely resolved patterned fluorescence images can be generated by heat treatment of microarrayed PDAs is highly significant in that it indicates that fluorescence signals might be produced by specific molecular recognition events. If so, the arrayed PDA system would serve as a label-free chip sensor. In order to test the feasibility of nonthermal affinity-induced fluorescence generation, we have exposed the amine-terminated blue-phase PDAs to cyclodextrin (CD) solutions.[52,64] Previously, we reported that CDs disrupt the ordered structures of PDA supramolecules by forming inclusion complexes and induce the blue-to-red color transition (Figure 10).[47] α-CD was found to be superior to β-CD or γ-CD in its ability to disrupt the PDA assemblies.

In order to determine whether CDs can promote fluorescence generation, PDA microarrays on CAB-coated glass substrates were prepared. The immobilized PDAs then were independently exposed to solutions containing 10 mM α-CD, β-CD, and γ-CD in deionized water for 30 min. In Figure 11 are displayed the fluorescence microscope images of the resulting glass substrates. The polymerized PDA microarray treated with α-CD appears as a red-colored fluorescent image, while those treated with β-CD and γ-CD show only negligible fluorescence (see Figure 11A). The fluorescence spot intensities promoted by α-CD are concentration dependent (ref 52, Supporting Information). To prove that the fluorescence signal observed with α-CD-treated PDA glass substrate is due to specific molecular interactions between PDAs and α-CD, all three CD-treated glass substrates were heated at 100 °C for 2 min. Fluorescence was observed from all of the heat-treated glass substrates (see Figure 11B). The fluorescence patterns shown in Figure 11B indicate that the PDAs remain immobilized even after treatment with β- and γ-CD solutions. Accordingly, the negligible fluorescence signals observed with β- and γ-CD-exposed PDAs displayed in Figure 11A are due to poor or no perturbation of the PDA supramolecules by β- and γ-CD.

We have also probed applications of fluorogenic PDA-based label-free sensor chips for monitoring protein–protein interactions.[63] Diacetylene vesicles containing biotin and acti-

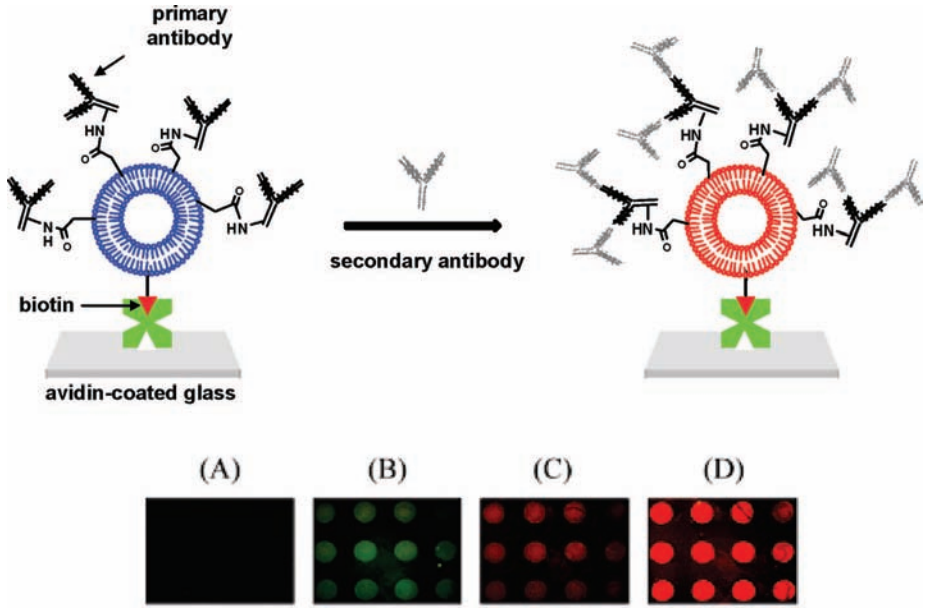

FIGURE 12. A schematic representation (top) of interaction between primary antibody-modified PDA and secondary antibody and fluorescent microscope images (bottom) of microspotted, immobilized, and irradiated PDAs having primary antibody before (A) and after exposure to secondary antibody (150 μg/mL) (B (460−490 nm excitation), C (510−550 nm excitation)) and after heat treatment at 100 °C for 1 min (D). Adapted from ref 63.

vated ester moieties were prepared and reacted with a primary antibody leading to covalent attachment of the antibody to the vesicles. The resultant antibody-modified diacetylene vesicles were immobilized onto an avidin-coated glass substrate in a microarray format. We anticipated that the biotin moieties on the surface of diacetylene vesicles would complex with avidins on the solid substrate. The microarrayed diacetylene vesicles were then irradiated with 254 nm UV light source to promote polymerization. The PDA-immobilized glass substrate was then incubated in a solution of the FITC-conjugated anti-rabbit secondary antibody while monitoring the vesicles using a fluorescence microscope. Because they were labeled with fluorescein, the secondary antibodies were monitored through a green filter at 460−490 nm (Figure 12B). Vesicles in which the secondary antibodies react with primary antibodies were observed through a red filter to become fluorescent (Figure 12C). The images obtained by using both the green and red filters were observed to overlap. This observation suggests that the vesicles undergo a change from nonfluorescent to fluorescent in conjunction with their binding to secondary antibodies.

A control to demonstrate that PDA vesicles were actually present (Figure 12D) involved heating of the glass slide and observing fluorescence from the arrayed spots. Moreover, no fluorescence was observed when PDA vesicles prepared in the absence of the primary antibody were exposed to the secondary antibody. Thus, "turn-on" of the fluorescence is definitely a result of antibody interactions. Finally, the primary antibody attached, microarrayed PDA sensor does not become fluorescent when it is exposed to the anti-mouse secondary antibody. This result confirms the antibody specificity of the PDA sensor system.

Conclusions

One advantage of PDA-based chemosensors over those that employ other conjugated polymers is the color change that takes place in response to environmental perturbations. Owing to this readily detectable blue-to-red chromic transition, most PDA-based chemosensors probed to date have relied heavily on the color change phenomenon. Until the time of our efforts in this area, the fluorogenic properties of the PDA supramolecules received much less attention as a source of the sensing signal. Early on in our investigations, we realized the potential significance of stress-induced fluorescence changes that take place in PDAs. Unlike many other fluorescent small molecule or polymeric materials, almost negligible fluorescence quenching takes place when red-phase PDA vesicles are immobilized on solid substrates. As a result, the stress-induced nonfluorescence-to-fluorescence change of the immobilized PDAs leads to a new strategy for fabrication of label-free sensor systems.

Several methodologies were developed for the immobilization of the PDA vesicles in the initial phase of our efforts in this area. Then, our investigations became focused on photolithographic or MIMIC techniques for generation of patterned

fluorescence images on the solid substrates. The realization that PDA vesicles could be immobilized using conventional microarray spotters led to simple procedures for fabrication of microarrayed PDA sensor systems. Importantly, this finding enabled the construction of microarrayed blue-phase PDAs that become fluorescent in association with the blue-to-red transition upon specific interactions with target molecules. Since in theory any molecular recognition event that promotes the blue-to-red color transition of PDAs should result in the generation of fluorescence, it should be possible to reformat a variety of previously described colorimetric PDA sensors into fluorescence-based sensor systems. The authors believe that the fluorescence properties of PDAs, when combined with modern technology for fabrication of microarrays, will stimulate the development of a number of new label-free chemosensor systems.

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BIOGRAPHICAL INFORMATION

Dong June Ahn received his B.S. and M.S. degrees from Seoul National University, respectively, in 1986 and 1988, and Ph.D. degree from Purdue University under the guidance of Professor Elias I. Franses in the field of interfacial engineering of Langmuir–Blodgett films. Following postdoctoral experiences at Purdue University and Lawrence Berkeley National Laboratory with Dr. Deborah H. Charych, he joined the faculty of the Department of Chemical and Biological Engineering at Korea University in 1995, where he is now a Full Professor. During 2001–2002, he was a visiting professor of the Bioorganic Chemistry Group at the Chiron Research Center. His research interests include nano-to-macroscale molecular and supramolecular assemblies and nanobiotechnology, which lead to applications to rapid on-site detection devices for chemicals of environmental and safety concern and ultrasensitive label-free diagnostic biosensor chips.

Jong-Man Kim received his B.S. degree from Hanyang University (1987) and Ph.D. degree from University of Maryland, College Park (1994), under the guidance of Professor Patrick Mariano in the field of organic and bioorganic chemistry. Following two years of postdoctoral research with Professor Peter Schultz at the University of California, Berkeley, he joined the Korea Institute of Science and Technology as a senior research scientist in 1996. He moved to Department of Chemical Engineering at Hanyang University in 2000 where he is now an Associate Professor. His current research interests focus on the design and synthesis of organic nanomaterials for micropatterned functional images and sensor applications.

FOOTNOTES

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