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# Reversible Modulation of Fluorescence Signals of Conjugated Polydiacetylene Supramolecules in a Microfluidic Sensor Chip

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Owing to the extensively delocalized π-electrons and the unique optical properties, polydiacetylenes (PDAs),[1-3] a family of conjugated polymers, have received substantial attention. In general, PDAs are prepared from self-assembled diacetylene monomers. In order for the polymerization to occur, the diacetylenic units in the monomer should be closely located each other (*ca*. 0.5 nm). Thus, it is extremely difficult (almost impossible) to obtain PDAs with extended conjugation length from conventional solution-based chemical approaches that are typically employed for the preparation of conjugated polymers. Since only UV light is required to promote the polymerization, the resulting polymers are not contaminated with unwanted by-products such as catalysts or initiators. One very attractive feature of the PDA is that the polymer can be readily prepared in aqueous solution, allowing utilization of the polymer as an effective sensor matrix for biomolecule detection. For most importantly, as displayed in Figure 1, the polymer undergoes color (blue-to-red) and fluorescence (non-to-red) changes upon environmental perturbations, such as temperature and ligand-receptor interactions.[4-7]

PDAs can be prepared in a variety of different forms such as vesicles in aqueous solutions,[5] Langmuir-Blodgett (LB)/Langmuir-Schaefer (LS) films,[4] immobilized vesicles in/on solid supports[8] as well as microarray chips[9] and PDA-embedded electrospun fiber sensors.[10] Recently, we developed a conceptually new and interesting PDA sensor system.[11,12] Without exception, the PDA sensor systems described before our report have been created in batch-type processes and are designed for single use. More importantly, the majority of PDA sensors reported to date use color change (or absorption spectral change) as a sensing signal, requiring relatively large amounts of test samples. In order to overcome the limitations associated with batch-type and color-based PDA sensors, we have designed a continuously flowing microfluidic PDA sensor system that employs fluorescence microscopy for monitoring. The microfluidic sensors would have several advantages such as consumption of nascent samples and reagents, large interfacial area and short molecular diffusion distance.[13-17] We were able to monitor a fast nonfluorescent-to-fluorescent phase transition of PDA upon interaction with analyte molecules. The PDA-based microfluidic sensor reported previously,[11,12] however, was an irreversible 'off-on' type in terms of fluorescence signal. We were curious if a reversibly operating off-on-off-on type microfluidic sensor could be designed. A reversibly operating microfluidic chip should be more flexible in terms of practical application of the PDA sensor. Thus, the main aim of current investigation is the feasibility test of repeated PDA-phase-transition in a microfluidic chip. Controlling the phase transition in a very short microchannel is a very challenging task. In order to test this possibility, we have employed a colorimetrically reversible PDA system. The diacetylene monomer PCDA-mBzA shown in Figure 2 is known to display heat- or pH-induced reversible chromism when it is transformed to PDAs.[18] It is expected that PDAs derived from PCDA-mBzA undergo a non-fluorescence-to-fluorescence transition when they are exposed to a high pH environment. A reverse transition should be possible by lowering the pH of the medium.

Figure 3 shows a schematic of the microfluidic sensor chip designed for the monitoring of the fluorescence signal emitted from PDAs upon exposure to a NaOH or HCl solution. The chip with five inlet channels was fabricated with a standard photolithography and molding techniques.[11,16] Microchannel height and width are 50 and 100 μm, respectively. Sample and sheath flow rates are 0.3 and 0.1 mL/h, respectively, that are

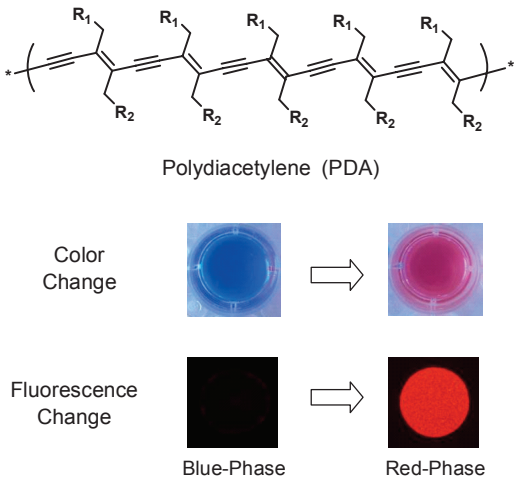


**Figure 1.** Structure of polydiacetylene (PDA) and its typical color/fluorescence changes upon stimulation.

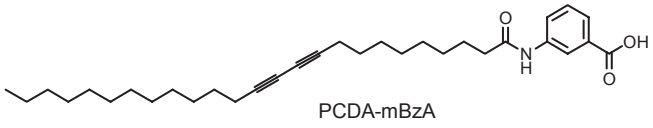


**Figure 2.** Structure of diacetylene monomer PCDA-mBzA.

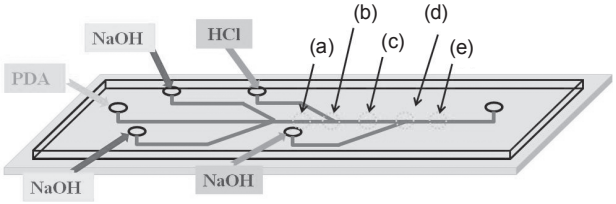


**Figure 3.** Schematic of the PDA-based microfluidic chip.

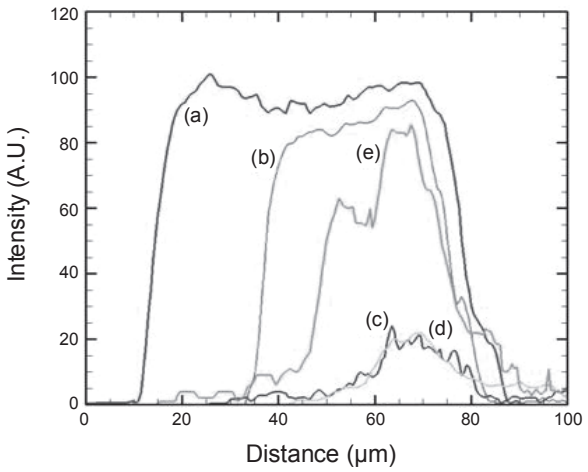


**Figure 4.** Fluorescence intensity profiles across the microchannel monitored at five different positions as described in Figure 3.

precisely controlled using syringe pumps. An aqueous suspension (*ca.* 1 mM) of polymerized PCDA-mBzA, prepared as described in the literature,[18] is injected into the center inlet channel while NaOH solutions are injected into the sheath channels. HCl and additional NaOH solutions are injected into the main channel with a 10 mm interval from junctions. Fluorescence intensity across the main channel was measured at five different points; (a) after introducing first NaOH solutions, (b) at the junction of HCl exposure, (c) between HCl and second NaOH introduction, d) at the junction of second NaOH exposure, e) after second NaOH exposure.

Figure 4 displays the fluorescence intensity profiles of PDAs monitored across the microchannel upon exposure to NaOH and HCl solutions. It appears that PDAs emit fluorescence when the polymer and 10 mM NaOH (pH 14) flows encounter (Figure 4a). This confirms that PDAs undergo a blue-to-red phase transition. The fluorescence intensity of the PDAs decreases immediately at the junction of HCl (50 mM) (pH 1) introduction (Figure 4b). Note that the HCl solution is inserted from only one side of the main channel, and the fluorescence intensity profile appears to collapse on the HCl exposed side only. As the PDA and HCl solutions are further mixed along the main channel the fluorescence intensity of the polymer is substantially decreased (Figure 4c). This indicates that the red phase PDAs experience a phase transition from red to blue. The feasibility of the reverse blue-to-red phase transition of the polymer was tested by introduction of a 100 mM NaOH solution. No meaningful increase of the fluorescence intensity was observed at the junction (Figure 4d). The fluorescence intensity, however, increases significantly along the microchannel, and more than 80% of the original intensity is recovered (Figure 4e). It is expected that the fluorescence intensity would be recovered more efficiently if NaOH solutions are introduced from both sides of the main channel.

In conclusion, we have demonstrated, for the first time, that fluorescence property of the PDA supramolecules could be manipulated reversibly in a microfluidic sensor chip. Thus, the introduction of aqueous NaOH solutions to the PDA flow allows emission of the polymer fluorescence, which was readily monitored using a fluorescence microscope. Upon exposure to a flow of aqueous HCl solution, the fluorescence of the polymer was significantly quenched, confirming a red-to-blue phase transition of the PDA molecules. More importantly, introduction of a flow of aqueous NaOH solution afforded substantial recovery of the fluorescence intensity, demonstrating successful recovery of the red-phase PDAs.

The results described above are important since an on-off-on switching function of PDA fluorescence is possible in a microfluidic sensor chip. Although we have investigated the reversible fluorescence modulation by manipulating the pH of the PDA medium, it is reasonable to expect that similar results could be obtainable using specific ligand-receptor interactions. Thus, we believe that strategy described above should be an important addition to the ever increasing PDA-based sensors.

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