A Fluorescence "Turn-On" Microfluidic Sensor Based on an Acenaphthopyrrolcarbonitrile Derivative

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The design and synthesis for a selective and sensitive fluorescent sensor has been the central focus of recent investigation.[1-4] In general, for sensor applications, it is more 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. In this regard, 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile (APC), a flat and highly electron-deficient heteroaromatic compound, is very attractive. APC is virtually nonfluorescent and displays strong fluorescence upon formation of covalent adducts with certain amine or thiol compounds due to their strong intramolecular charge transfer (ICT) nature (Figure 1).[5,6] This intriguing fluorogenic property of APC has been elegantly applied to the detection of amino acid, cysteine in MeOH.

In this communication, we report an APC-based microfluidic sensor system. In general, a conventional bulk system requires relatively large amounts of test samples, whereas microfluidic sensor systems[7-9] consume minimal samples and reagents. In addition, the large interfacial area and fast molecular diffusion relative to a microchannel dimension would enable a rapid nonfluorescent-to-fluorescent phase transition of APC upon interaction with analyte molecules. Three substrates, butylamine (BA), ethylenediamine (EDA), and 2-aminoethanethiol (AET) shown in Figure 1 are used in current investigation. It would be interesting to investigate the strength of nucleophilicity among three substrates.

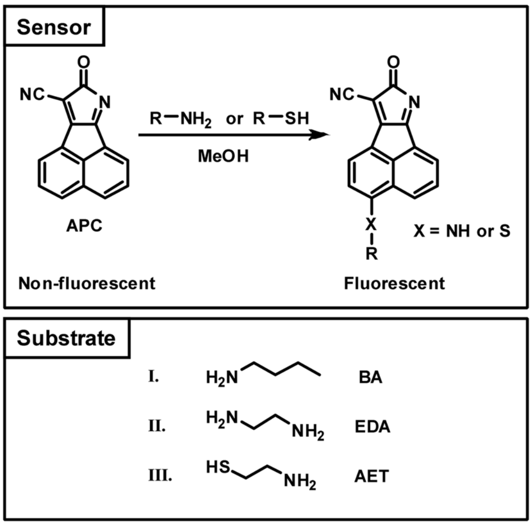

Figure 1. Structures of APC, covalent adduct, and substrates used in this investigation.

APC was prepared according to the literature method.[5,6] Figure 2 shows the color and fluorescence changes of APC solutions (50 μM in MeOH) in the presence of 2 mM of BA (A), EDA (B), and AET (C), respectively. Among tested substrates, AET was found to promote most drastic color and fluorescence changes (Figure 2C, b). Thus, the pale yellow APC solution became pale purple immediately upon interaction with AET and resulted in the shift of the maximum absorption wavelength from 434 nm to 574 nm. In addition, the solution emits a strong fluorescence under 365 nm light (emission maximum: 588 nm when monitored with a fluorescence spectrophotometer). The APC solutions incubated with BA and EDA resulted in no significant color changes along with weak fluorescence signals (Figure 2A and 2B). However, it should be noted that prolonged incubation (> 24 h) of the BA and EDA-containing APC solution also resulted in the development of the purple color and the generation of the fluorescence. The AET-induced prominent color and fluorescence changes of APC solution indicates the thiol group is more efficient in terms of adduct formation.

Since the aminoethanethiol-APC interaction causes very strong fluorescence, we next investigated the feasibility of sensing of aminoethanethiol using a microfluidic sensor system. The key question addressed in current investigations is whether or not the AET-APC interactions will provide a sufficiently energetic driving force to promote formation of fluorescence signals in a continuously flowing microfluidic

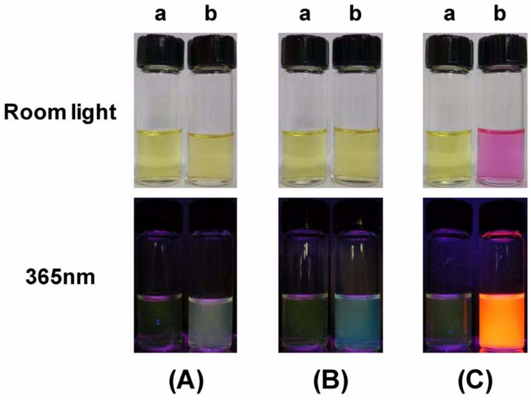

Figure 2. Photographs of APC solutions (50 μM, MeOH) upon interaction with 2 mM of BA (A), EDA (B), and AET (C), respectively. (a: APC solution, b: APC + substrate).

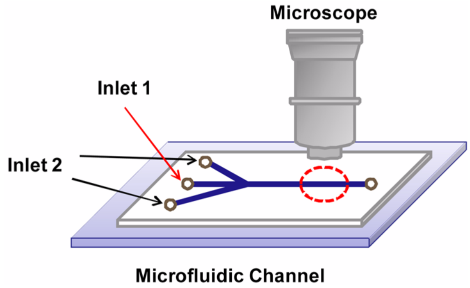

Figure 3. Schematic representation of a microfluidic sensor chip.

sensor system.

In Figure 3 is displayed a schematic of an APC-based microfluidic chip using hydrodynamic focusing. The microfluidic sensor chip was fabricated as described in our previous report.[7] A stream of the APC solution is introduced into the middle of microchannels (inlet 1) and is focused by sheath flows consisting of AET solutions (inlet 2). The flow rates of the APC and AET solutions, controlled by syringe pumps connected to the inlets, are 0.03 mL/h. The main microchannel dimensions are 100 μm in width and 50 μm in depth. Fluorescence microscopic monitoring was carried out with a filtered (510-530 nm) excitation.

Figure 4A contains the fluorescence images when a 100 μM APC flow is in contact with an AET flow (concentrations ranging from 2 to 8 mM) at 10 mm downstream from the junction of the inlet microchannels. Interestingly, the fluorescent intensity of the bands increases with increasing the AET concentration. This observation is a consequence of the fact that a higher concentration of AET molecules causes more rapid diffusion to the APC flow. In Figure 4B is shown the variation of the fluorescent intensity across the microchannel for the three AET concentrations. The results confirm that the fluorescent intensities depend on the AET concentration.

The final phase of current investigation focused on the distance-dependent fluorescence profiles in the main channel, generated from AET-APC interaction. For this purpose, a flow of APC solution (100 μM) was allowed to contact with sheath flows of AET solutions (12 mM), and the fluorescence signal was recorded at several designated points from the junction of the flows to 10 mm downstream of the microchannel. Figure 5 displays the resulting fluorescence profiles. It is clear from the fluorescence images that stronger fluorescence intensities are observed farther downstream. The gradual increase of the fluorescence signals as a function of distance is somewhat expected since there would be more time for APC and AET to interact each other as the distance between the junction and the fluorescence measuring point is longer. It should be noted that two distinct fluorescence bands formed at 3 mm (Figure 5c) begin to merge at 5 mm (Figure 5d).

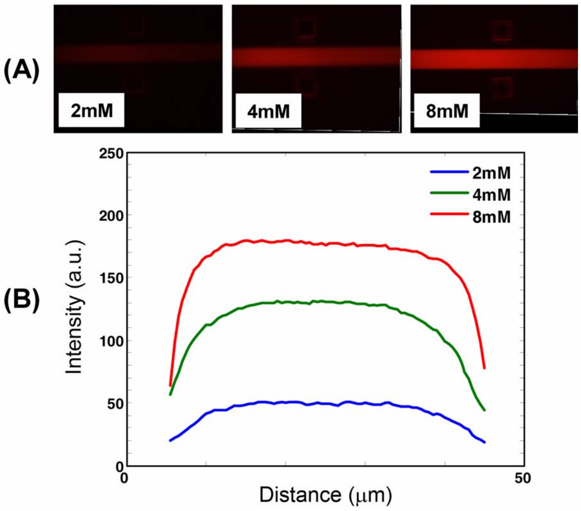

Figure 4. Fluorescence images (A) and intensity profiles across the microchannel (B) of microfluidic APC sensor chips upon interaction with AET.

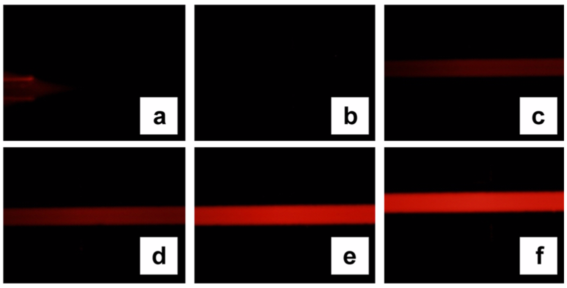

Figure 5. The variation of the fluorescence intensities along the microchannel. (**a** junction, **b** 1 mm, **c** 3 mm, **d** 5 mm, **e** 7 mm, and **f** 10 mm downstream).

In summary, we have explored a microfluidic sensor system with the fluorogenic APC molecule. Relatively strong fluorescence signals are observed when the flows of APC and AET solutions come into contact. In addition, we observed concentration-dependence of the fluorescent signals coming from this interaction, and distance-dependent increase of the fluorescence signal in a microchannel. The methodology described here should be a useful addition to the fluorescence "turn-on" microfluidic sensor systems.

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