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Fluorescence Sensing

Polyfluorophores on a DNA Backbone: Sensors of Small Molecules in the Vapor Phase**

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Vapors of small molecules are under widespread study in natural signaling, environmental monitoring, industrial quality control, and in medicine.[1–3] In the last two decades, advances in chemistry and materials science have enabled the development of chemical sensors of such volatile organic compounds,[4–7] whereby conjugated polymers and cross-reactive chemical sensor arrays are the most widespread sensor types. Among them, optical vapor-sensing arrays based on luminescence (including fluorescence) and absorbance have become essential.[8–13] In the recent past, the colorimetric detection of volatile organic compounds with cross-reactive chemical sensors has been demonstrated in multiple applications.[14–16]

Although absorbance-based methods for vapor detection have shown good success, fluorescence-based methods may offer some advantages, such as high sensitivity and low background. For example, the detection of vapors of nitroaromatic compounds with conjugated polymers and biopolymers has showed very high sensitivity.[17–23] However, much of the power of fluorescence-based detection remains unharnessed, since most current approaches involve the use of only one or a few types of sensor molecules; therefore, limited sensing diversity is possible (i.e., quenching).[19,20] Additionally, in most current approaches, the difficulty of the synthesis of a set of sensor molecules and the lack of flexibility in their conjugation to supports can restrict their general utility.

We envisioned that sensor molecules built by assembling multiple fluorophores on a DNA backbone could address some of these limitations. Such DNA-like oligomers can be synthesized rapidly in widely varied sequences and lengths on an automated instrument, and the iterative synthesis makes the preparation of large combinatorial libraries straightforward.[24,25] Furthermore, the resulting oligomers are water-soluble and are readily conjugated both to small molecules and to solid supports. Most importantly for sensing, such oligodeoxyfluorosides (ODFs) can participate in multiple forms of electronic interactions among the closely spaced chromophores; thus, complex fluorescence emission properties result that are quite distinct from those of the component monomers.[26–28] We considered the possibility that interaction with a small-molecule analyte might well alter such electronic interactions selectively and result in changes in emission, including quenching,[27,29] but also possibly enhancement and/or shifts in wavelength.

To test this possibility, we constructed a library of 2401 tetramer ODFs conjugated to poly(ethylene glycol) (PEG)–polystyrene (PS) beads by a stable amide linkage. Standard split-and-pool methods yielded a library in which each bead contained one specific tetramer structure. The sensor candidates were constructed in all possible sequences of seven monomer deoxyribosides (Scheme 1). The monomers included four fluorophores (Y, E, B, K),[28] a potential quencher (I), an abasic spacer (S), and a spacer/hydrogen-bonding element (dihydrothymidine, H). Sequences were encoded by the tagging method developed by Still and coworkers and were readable on the single-bead level by electron-capture GC.[30]

Our initial qualitative tests of the sensing capabilities of the library members were carried out on vapors of four distinct small molecules. Acrolein (an unsaturated aldehyde), mesitylene (an electron-rich aromatic compound), propionic acid (an aliphatic carboxylic acid), and nitrobenzene (an electron-poor aromatic compound) were selected for their diverse structural and electronic properties. A closed chamber was designed by using a quartz fluorescence cell in which beads from the library were placed onto a small microscope slide. The beads were exposed to one adjacent drop (4 μL) of analyte inside the cell, which was then capped. Fluorescence was monitored under an epifluorescence microscope with excitation at 340–380 nm, and all visible emission was observed (long-pass filter, > 400 nm). Images were taken before and after exposure for 2, 7, and 30 min to the vapor in the chamber at 23 °C.

To analyze possible changes in emission in response to analytes, we first constructed graphical 50 % gray-based difference maps of the beads by inverting color/intensity of the image before exposure (i.e. making a photonegative) and merging it with the image taken after 30 min of exposure using 50 % transparency (see Table 1 and details in the Supporting Information). This difference image enables an easy visualization of the effect of the small-molecule vapor on the library of tetramers. Any part of the image that is 50 % gray (including the background and beads) indicates no change, whereas beads that are darker than the 50 % gray background reveal quenching, brighter beads show emission enhancement, and colors reflect a combination of the original ODF emission color and any wavelength shifts that occur on sensing. Furthermore, for selected sequences we determined a quantitative color-change profile, a three-dimensional vector

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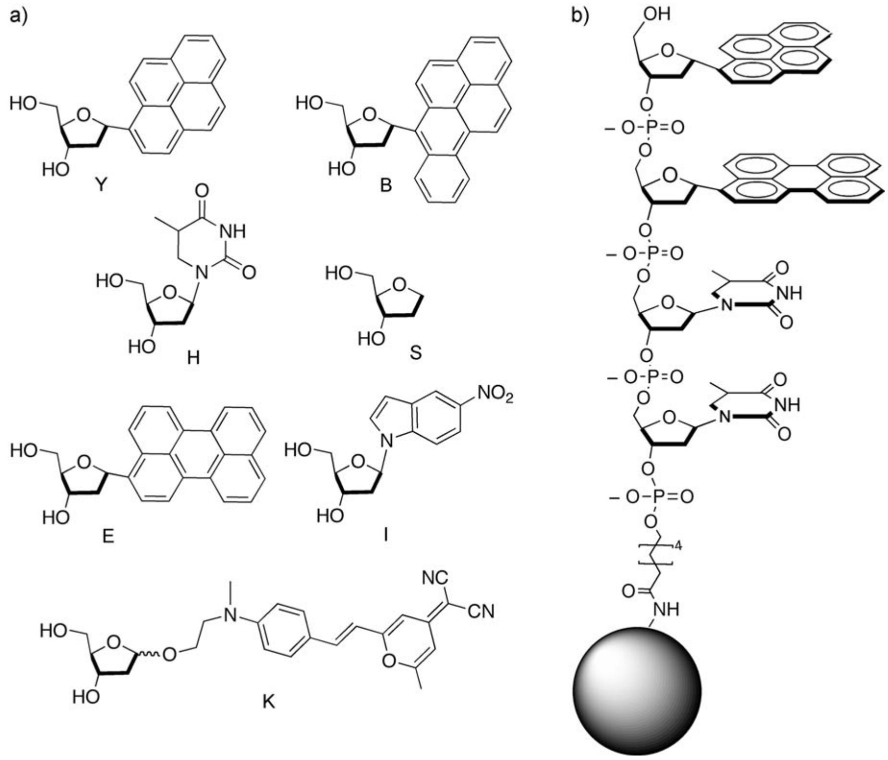

Scheme 1. a) Fluorescent and nonfluorescent monomers employed as components of the library. b) Example of a sensor (5′-Y-E-H-H-3′) attached by an amide linkage to a PEG–PS bead.

Table 1: Summary of cross-screening results: ODF sequences and their qualitative responses to analyte vapors (actual blended difference images of beads with attached sensor molecules are shown).

Sensor sequence	AC[a]	MS[b]	PA[c]	NB[d]
5′-H-I-E-H (**1**)				
5′-Y-E-H-H (**2**)				
5′-S-S-Y-E (**3**)				
5′-Y-Y-S-B (**4**)				
5′-S-H-E-S (**5**)				
5′-B-K-H-H (**6**)				
5′-Y-S-E-S (**7**)				
5′-Y-Y-E-K (**8**)				

[a] Acrolein. [b] Mesitylene. [c] Propionic acid. [d] Nitrobenzene.

(ΔR, ΔG, ΔB), by subtracting the RGB values of the image before exposure from the values of the image after exposure (R is red, G is green, B is blue). The color-change maps were then analyzed by chemometric and statistical methods.[31]

During screening, we observed marked fluorescence responses for many of the ODF library members upon exposure to analytes, as indicated by color-shifted beads in the difference map (see Figures 2–5 in the Supporting Information). Two strongly responding examples were selected for each analyte, resynthesized, and characterized by MALDI MS. Their absorption and emission spectra were measured, and the compounds were then retested for their sensing response on beads. All beads reproduced the trends observed during the screening process (see Table 2 in the Supporting Information) with the exception of sequence **2**; in this case it is possible that we mistakenly picked up a different bead from the one observed in the difference image. However, even this sequence showed good sensing properties that were distinct from those of the other sensor ODFs (Table 1).

While the selected sequences were able to detect the analytes they were chosen for quite clearly, we wished to see whether there was selectivity: did a given ODF respond in the same way to its selected analyte as to the other analytes? To answer this question, we performed a cross-screening study of all eight ODF sequences against the four small molecules. The qualitative results are shown in Table 1. Importantly, most of the sensors showed widely varied responses to the four vapors. This observation established that differences in the sequence and monomer composition affect the responses markedly. Interestingly, sequences selected for their responses to individual analytes demonstrated (in the majority of cases) fluorescence responses to the other three analytes as well. For the eight different sequences, there were two or three distinct response patterns to the four analytes, as exemplified by sequences **2**, **4**, and **7.** For example, 5′-Y-E-H-H (**2**) gave a blue shift for acrolein and a bright-maroon difference image for nitrobenzene (Table 1). In contrast, 5′-Y-Y-S-B (**4**) gave a marked red difference response and a light-brown response for these two analytes, respectively. Notably, the data showed multiple clear cases of wavelength shifts in emission rather than simple quenching. Examples include 5′-Y-E-H-H (**2**), whose green emission shifted to turquoise upon exposure to acrolein (see Table 2 in the Supporting Information), and 5′-S-H-E-S (**5**), the emission of which switched from green to cyan in the presence of propionic acid.

The responses and reproducibility were further investigated quantitatively on the basis of the RGB color-change profiles. Color-change plots (Figure 1; see also the Supporting Information) reflected multiple strong responses, including those of the sequences **2**, **4**, and **7**. Indeed, recognition patterns were observed even for sequences that differed by only one component. Thus, 5′-Y-E-H-H (**2**) and 5′-Y-S-E-S (**7**) exhibited a strong red and a slight green quenching upon

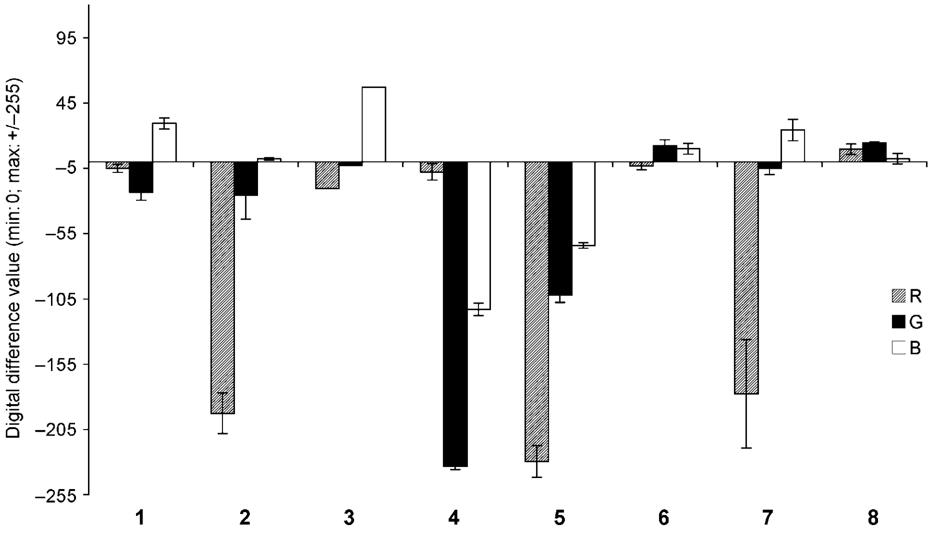

Figure 1. Quantitative color-change profile of sensor sequences upon exposure to acrolein. Profiles for other analytes can be found in the Supporting Information.

exposure to acrolein, whereas the blue wavelengths remained unchanged or increased slightly. Notably, these sequences contain the same two chromophores and differ only in nonfluorescent components. Sequence 5′-S-H-E-S (**5**) showed a decrease in all three RGB values with acrolein, whereas 5′-Y-Y-S-B (**4**) exhibited mainly a green and blue quenching. The same tetramers showed a slight quenching in response to mesitylene vapor, but with a different pattern, and a stronger general quenching in presence of nitrobenzene vapor. Interestingly, in the presence of propionic acid vapors, an increase in the blue and green channels was observed for **1**, **3**, and **6**, and an increase in red and green for **8**; thus, "light up" responses were demonstrated in these cases.

To better understand the relationships reflected by the data, we performed principal component analysis (PCA) on the full cross-screening data (Figure 2; see also the Supporting Information). The first component, F1, accounts for 65.7 % of variance, and the second component, F2, accounts for 30.6 %. The sum of these variances is 96.3 %. Two main clusters of sensor behavior were revealed; one is centered along the first-component axis and the other along the second-component axis. Sequences within a cluster act

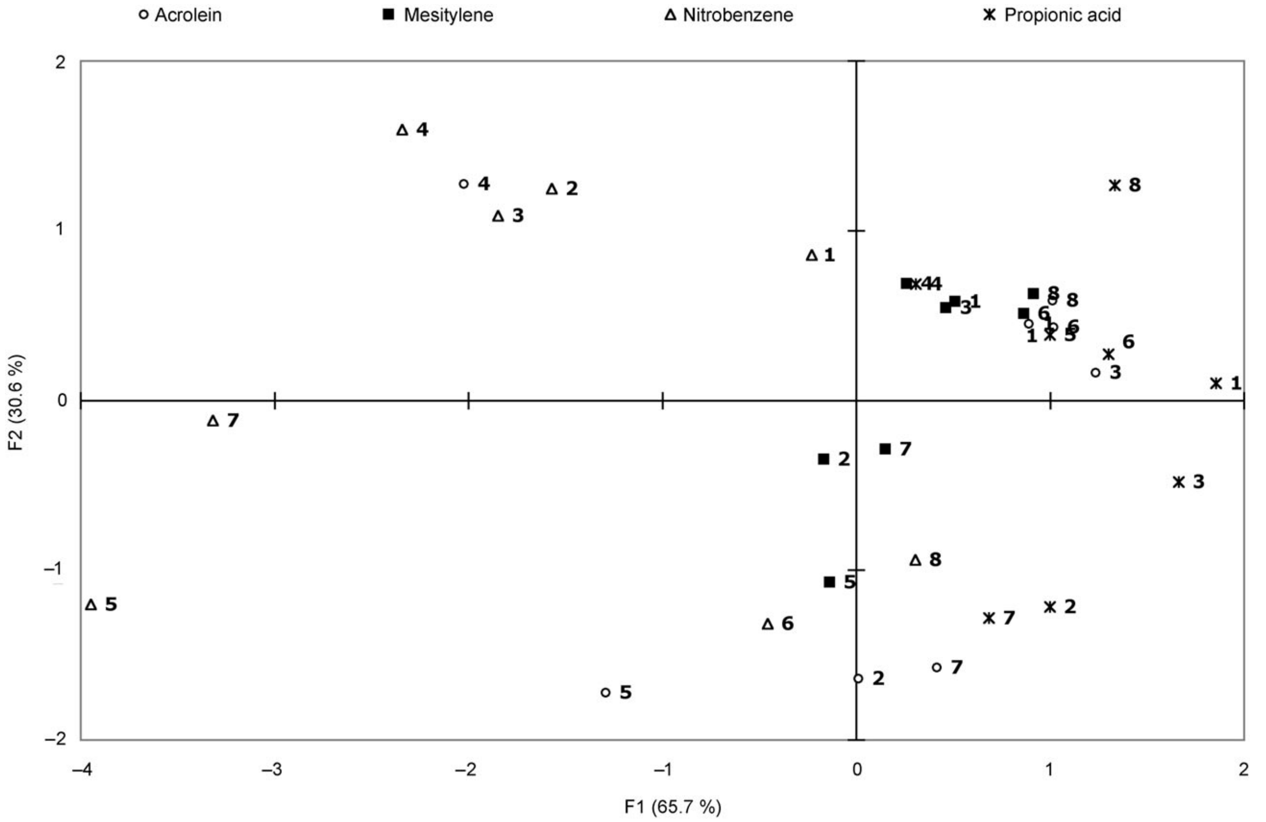

Figure 2. Principal component analysis plot of ODF-sensor responses upon exposure to the four analytes. The sequences of sensors **1**–**8** are listed in Table 1. For each data point, the analyte is indicated next to the sensor number by a shape, according to the key above the plot. Each data point represents an average from 5 or 6 sensor beads.

similarly upon exposure to the analytes. Interestingly, the data for acrolein and nitrobenzene vapors show the largest spread in component values. For example, the responses of 5′-S-H-E-S (**5**), 5′-Y-Y-S-B (**4**), and 5′-Y-Y-E-K (**8**) are very well separated in the presence of nitrobenzene vapor. The plot of the results upon the exposure of those sequences to acrolein and mesitylene also produced a good separation. Their response patterns in the presence of propionic acid are similar to one another but very distinct from the patterns for the other three analytes. Notably, the difference images (Table 1) and color-change profiles (see Figure 6 in the Supporting Information) showed that even a single sensor could be used to distinguish between the four analytes (sensor **5**, for example).

We also performed agglomerative hierarchical clustering (AHC) analysis by the single-linkage method, which provided clusters of sensor responses (see Figures 9 and 10 and Table 3 in the Supporting Information). The clustering scheme is based on the squared Euclidean distance between the centroids of the clusters of sensor response. The data generated a dendrogram in which classes of tetramers can be identified on the basis of their response patterns. The quantitative PCA and AHC methods together enabled us to select a small set of distinct sensors that could be used in a pattern response for these analytes. Importantly, by using these analytical methods, a combination of three sensor molecules, 5′-S-H-E-S (**5**), 5′-Y-Y-S-B (**4**), and 5′-Y-Y-E-K (**8**), was shown to clearly discriminate the four analyte vapors with greater certainty than a single sensor.

The results show clearly that ODFs on beads can act as selective fluorescence sensors of a range of chemically distinct organic analytes in the vapor phase. We hypothesize that the varied responses arise from distinct electronic interactions between the analytes and each ODF, which has its own sequence-based electronic interactions initially. The specific mechanisms underlying such interactions are not yet clear, and further studies will be required to clarify the origins of the fluorescence changes. Future work is also needed to evaluate the sensitivity of responses to these analytes in low concentrations, and selectivity among more closely related analytes.

Also of interest but not yet known is the mechanism of association between the analyte molecules and the ODFs. Some but not all of the observed responses were reversed upon opening the chamber to air (data not shown), which implicates weak noncovalent attractions in some cases but possibly stronger bonds in others. Possible noncovalent contributions may include hydrogen bonding to the dihydrothymidine monomer (present in half of the selected sensors), electrostatic attraction to the phosphate anion backbone and its counterion, and van der Waals/stacking interactions with the large aromatic chromophores. It is also possible that the PEG–PS bead itself helps absorb and concentrate the vapor near the sensors; some bead swelling was observed with selected analytes (see Figures 2–4 in the Supporting Information).

Overall, the results of this study are significant on multiple counts. First, they establish that oligomeric fluorophores can behave as vapor sensors with varied responses beyond simple quenching, which has been the main response observed to date in most fluorescent gas-phase sensors. Second, the data show sequence-based responses that are distinct for multiple classes of analytes. Thus, fluorescent vapor sensing can be extended far beyond the detection of simple nitroaromatic compounds. Third, the described library-based synthesis and screening approach enables facile evaluation of thousands of potential sensors for many analytes, in contrast to the previous one-at-a-time approach. Finally, the ODF sensor molecules can be rapidly and easily synthesized in an automated fashion from a small set of monomer components. In future studies on this class of sensors, a broader array of analytes will be tested, and the mechanisms and limits of these sensor molecules will be investigated.

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