Sensitive and selective detection of alcohols via fluorescence modulation

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ABSTRACT
Reported herein is the selective detection of aliphatic alcohols using cyclodextrin-promoted, proximity-induced fluorescence modulation of a high-quantum yield fluorophore. This fluorescence modulation occurred when the analyte was held in close proximity to the fluorophore via non-covalent cyclodextrin–analyte–fluorophore interactions, and led to unique modulation responses for each analyte, fluorophore and cyclodextrin investigated. These changes in fluorescence were used for the generation of an array using linear discriminant analysis that successfully generated unique pattern identifiers for 99% of the investigated analytes, and could detect alcohols at micromolar concentrations. These results represent a fundamentally new detection approach for these challenging analytes, and have significant potential in the development of novel detection schemes.

Introduction
The sensitive and selective detection of alcohols is an important research objective with implications in public health and environmental remediation, as many of these alcohols are known or suspected toxicants (1–3). Selectivity in distinguishing aliphatic alcohols from similar analogues is particularly crucial, as structurally similar analogues often have widely disparate toxicities. These aliphatic alcohols are found in fuels and fuel additives (4), foods and beverages (5), and other commercial products (6, 7). Current methods for aliphatic alcohol detection overwhelmingly rely on mass spectrometry (8, 9); however, the high sensitivity associated with mass spectrometry often leads to extraneous signals, which increases the analysis time and limits the ability to conduct high-throughput assays (10, 11). Some examples of non-mass spectrometry detection methods have been reported, most of which require a derivatization step to enable accurate detection (12, 13).

A January 2014 chemical spill in Elk River, West Virginia of more than 7500 gallons of 4-methylcyclohexanemethanol (4-MCHM) (14, 15) highlighted a key problem with the use of mass spectrometry-based detection in complex environments: the need for a priori knowledge of the toxicant of interest to enable high-throughput detection of contaminated samples (16, 17). Because every compound generates a mass spectral signal, first responders needed to know the identity of the contaminant prior to detection and clean up. In the case of the Elk River spill, this knowledge was not disclosed by the offending company in a timely fashion, significantly hampering remediation efforts (18, 19).

We recently developed a fundamentally new approach for small-molecule detection using γ-cyclodextrin as a supramolecular scaffold to promote proximity-induced energy transfer from aromatic toxicants and toxicant metabolites to high-quantum yield fluorophores (Figure 1) (20–23). This energy transfer operates successfully in multiple complex...
not allow for the detection of non-aromatic small molecule analytes such as aliphatic alcohols. Although these alcohols do not participate in proximity-induced energy transfer, they nonetheless are known to bind in or near the cyclodextrin cavity \(^{(31)}\). This binding, in turn, can lead to significant fluorescence modulation of a high-quantum yield fluorophore held in close proximity by the cyclodextrin scaffold (Figure 2).

This modulation relies on favourable intermolecular interactions between the analyte, fluorophore and cyclodextrin, including hydrogen bonding, hydrophobic interactions, CH-π interactions and a variety of other intermolecular forces \(^{(32–34)}\). Although all of these forces are well-known in the chemical literature, to the best of our knowledge, they have not been applied to the detection of this highly relevant class of analytes to date. Reported herein is the successful realisation of such proximity-induced, cyclodextrin-promoted fluorescence modulation that uses a multitude of intermolecular forces to achieve the sensitive and selective detection of a variety of alcohols, including aliphatic, aromatic and benzylic alcohols.

All previous work on cyclodextrin-based detection relied on the ability of the toxicants to act as energy donors to high quantum yield fluorophores \(^{(29, 30)}\), which does not allow for the detection of non-aromatic small molecule analytes such as aliphatic alcohols. Although these alcohols do not participate in proximity-induced energy transfer, they nonetheless are known to bind in or near the cyclodextrin cavity \(^{(31)}\). This binding, in turn, can lead to significant fluorescence modulation of a high-quantum yield fluorophore held in close proximity by the cyclodextrin scaffold (Figure 2).

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Figure 4. Fluorescence emission spectra of fluorophores in β-cyclodextrin in the presence of compound 1, compound 2, and compound 8. (A) Fluorophore 14; (B) fluorophore 15; and (C) fluorophore 16. (λ<sub>e</sub> compound 14 = 460 nm; compound 15 = 420 nm; compound 16 = 490 nm)

Figure 5. Fluorescence emission spectra of fluorophores in 2-hydroxypropyl-β-cyclodextrin in the presence of compound 6, compound 7, water and with no analyte. (A) Fluorophore 14; (B) fluorophore 15; and (C) fluorophore 16. (λ<sub>e</sub> compound 14 = 460 nm; compound 15 = 420 nm; compound 16 = 490 nm)

Figure 6. Generated array for the selective identification of alcohols in all cyclodextrins and in phosphate-buffered saline (PBS).
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14. About 20 μL of an analyte solution (1 mg/mL) was added to the cuvette, and fluorescence measurements were repeated at 460 nm. The experiments were repeated for fluorophore 15 and 16 at excitation wavelengths of 420 nm and 490 nm, respectively. The fluorescence spectra were integrated vs. wavenumber on the X-axis, and fluorescence modulation was determined using Equation (1).

Fluorophore Ratio = \( \frac{F_{\text{alcohol}}}{F_{\text{blank}}} \)  

where \( F_{\text{alcohol}} \) is the fluorescence emission of the fluorophore in the presence of the alcohol, and \( F_{\text{blank}} \) is the fluorescence emission of the fluorophore in the absence of the alcohol. Each experiment was repeated four times. The reported values are the average of the results.

Experimental details for array generation experiments

Array analysis was performed using SYSTAT 13 statistical computing software with the following settings:

Figure 7. (Colour online) Array using mixtures of analytes 2 and 8 using all CDs and fluorophore 14.
detection (LoD), defined as the lowest concentration of analyte at which a signal can be detected, and the limit of quantification, defined as the lowest concentration of analyte that can be accurately quantified.

Results and discussion

Selection of alcohols

The alcohol analytes were selected to include a wide range of analytes (Chart 1), including those that are structurally similar;

- Classical Discriminant Analysis
- Grouping Variable: Analytes
- Predictors: Bodipy, Rhodamine 6G, Coumarin 6
- Long-Range Statistics: Mahal

Experimental details for limit of detection experiments

These experiments were conducted following literature-reported procedures (36), to determine the limit of detection (LOD), defined as the lowest concentration of analyte at which a signal can be detected, and the limit of quantification, defined as the lowest concentration of analyte that can be accurately quantified.

Results and discussion

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The alcohol analytes were selected to include a wide range of analytes (Chart 1), including those that are structurally similar.
Cyclodextrins were chosen as supramolecular hosts for this proximity-induced fluorescence modulation, as a result of their high aqueous solubility and known ability to promote non-covalent intermolecular interactions (42). Most of these interactions rely on the ability of cyclodextrin to bind hydrophobic guests in the hydrophobic interior cavity (43); an alternate mode of complexation involves hydrogen bonding between the cyclodextrin hydroxyl groups (hydrogen bond donors) and hydrogen bond acceptor moieties on small molecule guests (44). The alcohol analytes are expected to associate through hydrogen bond formation involving the free hydroxyl groups, as well as hydrophobically driven inclusion involving the hydrocarbon segments of the alcohol structures. We selected four commercially available cyclodextrin hosts: α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), randomly methylated β-cyclodextrin (Me-β-CD, with an average degree of methylation of 1.8 methyl groups per glucose unit) (45) and 2-hydroxypropyl-β-cyclodextrin (2-HpCD) (46).

**Fluorescence modulation experiments**

These experiments measure the ability of the analyte to induce measurable and unique changes in the fluorescence emission spectra of a high-quantum yield fluorophore in the presence of a cyclodextrin host. The experiments were conducted by adding small amounts of a concentrated solution of the fluorophore in tetrahydrofuran to a 10 mM cyclodextrin solution in aqueous buffer, which led to a 91% aqueous/9% organic final solvent composition (volume/volume). Similar but have widely disparate toxicities. Ethylene glycol 5 was chosen due to its widespread availability and known toxicity (37), as well as the desire to assay the ability of the cyclodextrin-based system to identify diol motifs via fluorescence modulation. 1-Hexanol (compound 1) and cyclohexanol (compound 2) were selected to challenge the ability of the fluorescence modulation system to distinguish between analytes with identical molecular weights, which are difficult to distinguish via high-throughput mass spectral analysis (although such compounds have unique fragmentation patterns in the mass spectrometer) (38). 4-MCHM (compound 8) was selected due to its prevalence in the 2014 Elk River contamination event (14), as well as its structural similarity to cyclohexanol 2. Alcohols 9–12 were chosen to assay the ability of the fluorescence modulation technique to detect propargylic alcohol, aromatic alcohols and highly hindered tertiary alcohols, and to distinguish between these analytes and simple primary aliphatic alcohols 1, 3 and 4. Control analytes 6 and 7, which lack free hydroxyl moieties, were also included in these investigations, as was tetrahydrofuran (13), the solvent used to dissolve the fluorophores.

**Selection of cyclodextrins**

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**Table 1. Fluorophore ratios of analytes 1–13 in combination with fluorophore 14.**

| Analyte | α-CD | β-CD | Me-β-CD | 2-HpCD |
|---------|------|------|---------|--------|
| 1       | 1.07 ± 0.04 | 1.07 ± 0.02 | 1.15 ± 0.01 | 1.22 ± 0.01 |
| 2       | 1.02 ± 0.03 | 1.04 ± 0.01 | 1.05 ± 0.01 | 1.07 ± 0.01 |
| 3       | 1.03 ± 0.04 | 1.00 ± 0.01 | 1.07 ± 0.01 | 1.03 ± 0.00 |
| 4       | 1.04 ± 0.03 | 1.00 ± 0.01 | 1.04 ± 0.01 | 1.04 ± 0.01 |
| 5       | 1.01 ± 0.03 | 0.92 ± 0.04 | 1.02 ± 0.01 | 1.05 ± 0.02 |
| 6       | 1.10 ± 0.02 | 1.12 ± 0.02 | 0.99 ± 0.01 | 1.13 ± 0.06 |
| 7       | 1.15 ± 0.03 | 1.17 ± 0.02 | 0.98 ± 0.00 | 1.08 ± 0.00 |
| 8       | 1.08 ± 0.04 | 1.11 ± 0.04 | 0.98 ± 0.01 | 1.06 ± 0.01 |
| 9       | 0.93 ± 0.01 | 1.16 ± 0.02 | 0.95 ± 0.01 | 1.10 ± 0.02 |
| 10      | 1.18 ± 0.01 | 1.23 ± 0.01 | 1.09 ± 0.02 | 1.31 ± 0.01 |
| 11      | 0.99 ± 0.01 | 1.12 ± 0.01 | 0.94 ± 0.00 | 1.06 ± 0.01 |
| 12      | 1.14 ± 0.03 | 1.14 ± 0.04 | 0.96 ± 0.01 | 1.13 ± 0.03 |
| 13      | 1.05 ± 0.02 | 1.06 ± 0.02 | 0.99 ± 0.00 | 1.07 ± 0.01 |

*All values were calculated according to Equation (1) and represent the average of four trials.

**Table 2. Fluorophore ratios of analyte 8 in β-cyclodextrin.**

| Analyte       | Fluorophore 14 | Fluorophore 15 | Fluorophore 16 |
|---------------|----------------|----------------|----------------|
| 1 M NaCl      | 1.02 ± 0.01    | 0.97 ± 0.01    | 0.99 ± 0.00    |
| Ultrapure water| 1.02 ± 0.01    | 0.89 ± 0.00    | 0.96 ± 0.00    |
| Seawater      | 1.06 ± 0.01    | 1.03 ± 0.01    | 0.98 ± 0.00    |
| PBS           | 1.06 ± 0.01    | 1.03 ± 0.01    | 0.96 ± 0.00    |

*All values were calculated according to Equation (1) and represent the average of four trials.

**Selection of fluorophores**

These compounds include three of the major classes of high-quantum yield organic fluorophores: BODIPY, Rhodamine, and Coumarin. They were either commercially available or were synthesised following literature-reported procedures (35). The efficacy of these fluorophores in energy transfer-based detection systems has already been established by us (20) and others (39–41).
volume). These solvent conditions were designed to maximise hydrophobic association between the cyclodextrin hosts and small molecule guests (47), while ensuring solubility of all organic components. The fluorescence emission spectrum of the fluorophore under these conditions was measured and compared to the fluorescence emission spectrum of the fluorophore obtained after introduction of 20 μL of the analyte of interest under the same conditions. The difference between these two fluorophore emission spectra was defined as the degree of fluorescence modulation, and quantified according to Equation (1) (above).

Selected results of the fluorophore modulation experiments are summarised in Table 1 and in Figures 3–5. Several aspects of these results merit further discussion.

The introduction of an alcohol to a fluorophore–cyclodextrin solution leads to marked changes in the observed fluorophore emission signal. In the case of fluorophore 14, these changes generally show up as an increase in the fluorophore emission signal in the presence of the alcohol (indicated by a ratio higher than 1.00). Aliphatic alcohols have been shown to bind to cyclodextrins under a variety of conditions (48–50), although in most instances the alcohol addition causes a decrease in the observed fluorophore emission signal. In our system, the observed fluorescence increases are a result of multiple, analyte-specific factors:

1. Linear alcohol analytes (1, 3 and 4) assist in the formation of cyclodextrin–fluorophore–alcohol complexes, leading to higher fluorophore emission signals (51, 52). Ethanol 4, in particular, has been shown to form ternary complexes in 2-HPCD and to stabilize complex formation in Me-β-CD (53). The addition of these alcohols also increases the cyclodextrin solubility, although the small amount of alcohol added (20 μL in a 2.5 mL solution) means that the effect on cyclodextrin solubility is likely to be negligible (54, 55). The two exceptions to this trend are analytes 3 and 4 in the presence of β-cyclodextrin, which cause no measurable change in the fluorophore emission signal. β-Cyclodextrin is the least water soluble host investigated (56), which may be a factor in establishing its limited response to these analytes.

2. Control analytes 6 and 7 generally lead to increases in the fluorophore emission signal, with the exception of the methyl-β-cyclodextrin solutions which demonstrate no significant change. Analyte 6 is likely effecting these increases through acting as a hydrogen bond acceptor with the cyclodextrin hydroxyl groups, in line with analogous literature reports (57). Analyte 7, in contrast, is known to organise into hydrophobic aggregates in a highly polar, aqueous solvent system; these hydrophobic regions, in turn, are attractive regions for localising a hydrophobic fluorophore and facilitating moderate fluorescence increases (58).

3. Diol 5 shows limited changes in the fluorophore emission signal, with the exception of a significant fluorescence decrease observed in the presence of β-cyclodextrin. Ethylene glycol is known to hydrogen bond to cyclodextrins and lead to the self-assembly of cyclodextrin monomers into supramolecular aggregates (59, 60); to the extent that these cyclodextrin aggregates cause fluorophore aggregation, that would explain the observed fluorescence decreases (as many types of fluorophore aggregates are also less fluorescent than their monomeric counterparts) (61).

4. Analytes 2 and 8 exhibit variable changes in the fluorophore emission signal, with no clearly observable trends. Interestingly, the structural differences between these two analytes are relatively minor, as they both contain free hydroxyl moieties and a cyclohexane ring structure. The minor differences (primary vs. secondary alcohol, presence of additional methyl group) are sufficient to lead to clear differences in the fluorophore emission ratios of compound 14 in

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**Table 3.** Selected LODs for analytes 1–13 using the fluorescence modulation of fluorophore 14 in the presence of varying cyclodextrin hosts.

| Analyte | α-CD | β-CD | Me-β-CD | 2-HPCD |
|---------|------|------|---------|--------|
| 1       | 102 μM | a    | a       | 109 μM |
| 2       | 18 μM  | 30 μM| 402 μM  | 22 μM  |
| 3       | a     | a    | 2298 μM | 352 μM |
| 4       | 178 μM| 4 μM | 115 μM  | 183 μM |
| 8       | 64 μM | 6 μM | a       | 165 μM |
| 9       | a     | 27 μM| 47 μM   | a      |
| 10      | 109 μM| 63 μM| 41 μM   | 94 μM  |
| 11      | 0.2 μM| 24 μM| 534 μM  | 64 μM  |
| 12      | 124 μM| a    | 29 μM   | a      |

*aNegative values were obtained for these analyte–cyclodextrin combinations; current efforts are focused on understanding the reason for these values.*
cycloxdextrin solutions with the introduction of these two analytes.

(5) Alcohol 9 is the only analyte that led to a decrease in the fluorescence of compound 14 in the presence of an α-cycloxdextrin host, which correlates well with the reported ability of the alkyne moiety to bind in the sterically constrained α-cycloxdextrin cavity (62), and highlights the fact that the fluorescence modulation results are intimately related to key structural features of the analytes. In the presence of the other cyclodextrin hosts, analyte 9 behaved similarly to most of the other investigated analytes, with an increase in fluorescence in the presence of β-cycloxdextrin and 2-hydroxypropyl-β-cycloxdextrin, and a moderate fluorescence decrease in the presence of methyl-β-cycloxdextrin, which is likely driven by the strong association of fluorophore 14 with the methyl-β-cycloxdextrin host.

(6) Alcohol 10, the only example of an aromatic alcohol, led to increases in fluorescence for all hosts investigated, which is qualitatively similar to the results obtained with aliphatic alcohols 1 and 2. However, the magnitude of the fluorescence changes were markedly higher for alcohol 10 compared to the other investigated analytes, and indicates that aromatic alcohols demonstrate different intermolecular interactions that translate into easily observable differences in fluorescence modulation behaviours, in line with literature reports of aromatic guest binding in the cyclodextrin cavities (63, 64).

(7) The behaviour of fluorophore 14-cycloxdextrin complexes with the introduction of alcohol 11 resembles that obtained with introduction of alcohol 9, with the exception of α-cycloxdextrin solutions which demonstrated significant changes in the presence of alcohol 9, but virtually no changes in the presence of alcohol 11. These results highlight that the alkyne-α-cycloxdextrin intermolecular interactions lead to unique photophysical read-out signals.

(8) Alcohol 12 demonstrates behaviours that are qualitatively similar to analyte 8, with moderate fluorescence increases observed in the presence of α-cycloxdextrin, β-cycloxdextrin, and 2-hydroxypropyl-β-cycloxdextrin, and slight fluorescence decreases observed in the presence of methyl-β-cycloxdextrin. Interestingly, the behaviour of these analytes is also similar to that of control analytes 6 and 7, which indicates that the fluorescence modulation is driven by the hydrocarbon segments of the molecules and the resultant effects of these segments on cycloxdextrin inclusion complexes, and less affected by the free hydroxyl groups in these cases.

(9) The introduction of control analyte 13 led to limited fluorescence changes in all hosts investigated, with no change observed in the methyl-β-cycloxdextrin solution and limited fluorescence increases observed in other cases. These results indicate that the fluorescence changes observed in the other cases are related to specific analyte-cycloxdextrin-fluorophore intermolecular interactions rather than being solely a result of the addition of organic solvent.

The results for fluorophores 15 and 16 are markedly different from those obtained using fluorophore 14, and reflect the fact that intermolecular interactions that lead to fluorescence modulation are exquisitely sensitive to the molecular structures of each component (see ESI for full tables with these fluorophores). For example, in the presence of methyl-β-cycloxdextrin, fluorophore 15 undergoes substantial fluorescence decreases upon introduction of all analytes. These decreases are likely a result of the fact that fluorophore 15 binds strongly in the methyl-β-cycloxdextrin cavity in the absence of any added alcohol; the addition of alcohols then disrupts the strong fluorophore-cycloxdextrin complex and lowers the observed fluorescence. In another example, the fluorescence emission of fluorophore 16 changes very little with the introduction of analytes 1–13 to the cycloxdextrin solution for all analytes in α-cycloxdextrin and β-cycloxdextrin, which indicates a limited interaction between the analytes and the fluorophore in these cases. Steric incompatibilities may prevent the larger fluorophore 16 from binding in the cycloxdextrin cavity; as a result, the alcohol-cycloxdextrin non-covalent association complexes have no effect on the photophysical properties of fluorophore 16.

These differential responses between analytes in the presence of different cycloxdextrin hosts can be seen more clearly in Figures 3–5. As illustrated in Figure 3, in the presence of methyl-β-cycloxdextrin, compounds 1–4 effect noticeably different changes in the fluorophore emission spectra of compounds 14–16. Compounds 3 and 4 lead to markedly higher fluorescence emissions of compound 14 compared to compounds 1 and 2 (Figure 3(A)). Fluorophore 15, in contrast, demonstrates relative insensitivity to the presence of the aliphatic alcohols (Figure 3(B)), with minimal differences in the fluorophore emission spectra observed. Finally, fluorophore 16 demonstrates higher emission signals in the presence of compounds 3 and 1, with relatively lower signals observed for compounds 2 and 4 (Figure 3(C)).
Figure 4 illustrates the differential responses of fluorophores 14–16 to the introduction of analytes 1, 2 and 8 in the presence of β-cyclodextrin host. Here, noticeably different response patterns are observed for each analyte, which is striking given the structural similarities between the analytes. Furthermore, it would be difficult to distinguish analytes 1 and 2 via mass spectral analysis in a rapid time frame, due to their identical molecular weights (despite known differences in their fragmentation patterns) (65), which makes the differential fluorescence response patterns even more significant.

Finally, Figure 5 illustrates the limited degree of fluorescence differentiation observed in the presence of analytes 6 and 7. Relatively little changes in the fluorophore emission signals are observed upon introduction of these analytes, or with the introduction of 20 μL of water (instead of an organic analyte). The one exception to this is the marked decrease observed in the fluorescence emission of fluorophore 16 in the presence of 20 μL of analyte 6 (Figure 5(C)), and current efforts are focused on elucidating the reason for this aberrant behaviour.

Overall, substantially different fluorescence response patterns were observed for fluorophores 14–16 in the presence of cyclodextrin hosts with the introduction of analytes 1–13, which enables the development of selective array-based detection systems. Array-based detection has been well-established as a tool for selective analyte detection (66), using statistical analysis such as linear discriminant analysis (LDA) and principal component analysis (PCA) to identify unique response patterns for a variety of target analytes (67–69). For the fluorescence modulation system reported herein, the integrated fluorophore emission in the presence of the target analyte \( F_{\text{Alcohol}} \) (see Equation (1)) was used as the input data for the arrays. These modulation experiments generated well separated signals for 99% of the analyte-cyclodextrin combinations investigated (Figure 6). Interestingly, the signals for cyclohexanol and 4-MCHM (analytes 2 and 8) are grouped near each other for each cyclodextrin host, but still generate well-separated signals in every case, highlighting their structural similarities that lead to similar response patterns in combination with cyclodextrin hosts and high-quantum yield fluorophores. Similarly, ethanol and ethylene glycol (analytes 4 and 5) generate signals that are close to, but well separated from, each other for each cyclodextrin host. Finally, the signals for methanol and ethanol (analytes 3 and 4) are found far away from each other, indicating that despite their apparent structural similarities, their interactions with cyclodextrin hosts are in fact quite different.

Analytes 9–11 displayed signals that were relatively near each other and clearly distinct from signals generated by analytes 1–8, highlighting that their unique structural features contribute to different intermolecular interactions with the fluorophores and cyclodextrin hosts, leading in turn to unique spectroscopic signatures. Tertiary alcohol 12, in contrast, generated response patterns that were similar to many of the other aliphatic alcohols investigated, which indicates that the substitution around the free hydroxyl group is less important than the other structural features of the molecule such as the presence or absence of an aromatic ring or alkene group that lead to grouping of the analytes in similar regions in the LDA-generated plots.

The practical utility of this array would be markedly enhanced by its ability to generate well-separated signals for mixtures of analytes, as nearly all instances of analyte identification in complex environments involve toxicant mixtures (70). To that end, an array was generated using mixtures of analytes 2 and 8, and the results are summarised in Figure 7 for fluorophore 14. These results indicate that well-separated signals are generated for all mixtures of analytes 2 and 8 in all cyclodextrin hosts, indicating the high degree of selectivity. Moreover, the signals for the single component mixtures were relatively far away from those of the analyte mixtures, which points to the possibility of some degree of cooperativity between analytes 2 and 8 that is responsible for the observed non-linearity in array response patterns.

The fundamental basis for the observed high levels of differential selectivity in these alcohol–cyclodextrin–fluorophore systems is that non-covalent interactions between the analyte and fluorophore lead to measurable, analyte-specific fluorescence changes. Control experiments using a cyclodextrin-free phosphate-buffered saline solution indicate that the presence of the cyclodextrin hosts affects the fluorescence modulation results in specific, cyclodextrin-dependent ways.

One phenomenon that is facilitated in the presence of cyclodextrin hosts is the binding of the fluorophores in the cyclodextrin cavity, and concomitant increases in the fluorophore emission signals (Figure 8). Fluorophore emission signals in the absence of cyclodextrin and in the presence of α-cyclodextrin are markedly lower compared to the emission signals observed in the presence of β-cyclodextrin, methyl-β-cyclodextrin, and 2-hydroxypropyl-β-cyclodextrin, which indicates that the fluorophores bind in the cavities of larger cyclodextrin hosts (71). This increase occurs both for systems without an alcohol analyte (Figure 8(A–C)) and systems that contain an alcohol additive (compound 1 used as an example; Figure 8(D–F)). This fluorophore–cyclodextrin binding is one of several intermolecular interactions that exist for each unique analyte–fluorophore–cyclodextrin combination and enables excellent differentiation between the target analytes.
The practicality of this modulation was further demonstrated through determining the fluorescence modulation induced by analyte 8 in β-cyclodextrin dissolved in different solvent systems: ultrapure water, phosphate-buffered saline, 1 M NaCl, and crude seawater collected directly from the Narragansett Bay. The resulting ratios demonstrated solvent dependence that was translated into a highly selective array which distinguishes the analyte in different solvent environments with 100% accuracy (Table 2). The ability of the cyclodextrin-promoted fluorescence modulation to operate in crude seawater highlights the practicality of this method in real-world environmental contamination scenarios.

Practical applications of this detection method require the ability to detect alcohols at levels at or below literature-reported levels of concern (72). Limits of detection for all analyte–cyclodextrin combinations were calculated following literature-reported procedures, and selected results are highlighted in Table 3. Micromolar detection limits for these analytes are a promising start towards the development of highly sensitive detection systems. One way to further improve sensitivity would be to introduce specific recognition groups to the fluorophores. Such groups would improve binding ability by promoting stronger intermolecular interactions between the analyte and fluorophore (73). Efforts towards improving the sensitivity of this system are currently underway in our research group.

Conclusions

A variety of high-quantum yield fluorophores demonstrate measurable changes in their fluorescence emission spectra with introduction of small amounts of alcohols. These changes, termed ‘fluorescence modulation’, are highly specific to each analyte–fluorophore–cyclodextrin combination and can be used to develop an array with 99% success in generating unique pattern identifiers for each analyte. Notably, small structural changes in the alcohols lead to markedly different fluorescence response patterns and unique pattern identifiers that can distinguish, for example, cyclohexanol from 1-hexanol, methanol from ethanol, and benzyl alcohol from phenol. More broadly, different classes of alcohols such as aliphatic alcohols display signals in a region of the plot that is unique from signals obtained from propargylic, aromatic and benzylic alcohols, and point to the potential of developing class-specific signatures for different alcohol classes. Finally, the highly selective array demonstrates significant promise in detecting binary mixtures of analytes with high selectivity, a result that significantly broadens the practical applicability of this technology for the development of fluorescence-based alcohol detection systems. Efforts towards the development of such detection systems are currently underway in our laboratory, and results of these and other investigations will be reported in due course.

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Supplemental material

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