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Teaching Old Indicators New Tricks

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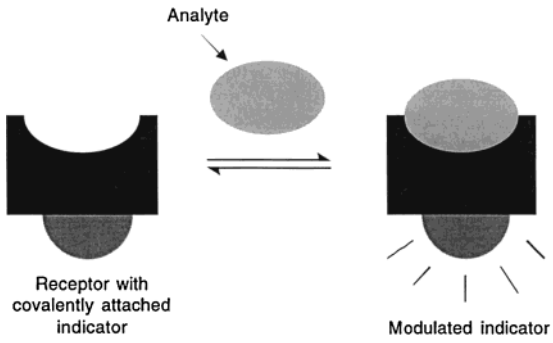
ABSTRACT

Most synthetic sensors are designed with covalent attachment between a receptor and a reporter moiety. In this report, we describe the current progress of our use of noncovalently attached indicators to signal binding of analytes. With these systems, analyte binding leads to indicator displacement from the binding cavity, which in turn yields an optical signal modulation. We include previous examples, the strategies involved in our development, and the advantages as well as disadvantages of this method. Finally, our latest research in this field is briefly presented.

Introduction

A significant thrust in chemical research has recently focused on the notion that molecular design can lead to practical sensors. To make a useful chemosensor, a compound must contain a "binding site" and a "signaling site", such as a chromophore, fluorophore, or redox active center. Further, a mechanism to communicate between them must exist.[1] Traditionally, when an analyte associates with the binding site, a microenvironment modulation occurs that perturbs the properties of the signaling site. From changes in the spectroscopic or redox properties, binding constants and stoichiometries can be obtained.[2] With organic structures, absorbance or fluorescence changes are commonly observed. A change in signal upon binding can result from photoinduced electron transfer (PET),[3] charge transfer, fluorescence resonance energy transfer (FRET), or simple microenvironment changes such as those that arise from changes in local ionic strength or pH. For the most part, these synthetic sensors possess covalent links between the fluorophore or chromophore and the binding site. In this Account, we would like to show that the use of noncovalently attached indicators can be conveniently used to signal binding. This signaling mechanism is well precedented and works in a manner similar to that of many antibody-based biosensors in competitive immunoassays.[4] A solution containing the unlabeled antigen is added to the antibody receptor, which is associated with a tagged antigen. Upon displacement of the tagged antigen, a signal modulation is observed. Although the method is easy and convenient, it has seen relatively little incorporation in the molecular recognition/supramolecular community. Herein, we summarize our work in this area, general design principles, and the advantages and disadvantages of the method.

Scheme 1



Indicator−Covalent Attachment

In the development of chemosensors, one important feature is the mechanism through which the receptor signals the binding of the analyte. Many analytes of interest do not incorporate their own chromophore or fluorophore, and development of a sensor that could detect these without chemical modification is preferred. A colorimetric or fluorescent indicator needs to be associated with the receptor, and the most common way is to covalently attach the indicator. Upon binding the analyte, a change is induced in the sensor to indicate that binding has occurred. This can be seen in Scheme 1, where upon addition of an analyte the chromophore is "switched on" giving a signal change. A common example of an indicator is one used to detect changes in pH such as phenolphthalein. Another commonly used signaling technique involves PET. This generally entails a fluorophore−spacer−receptor system, where the fluorescence can be "switched" on or off upon binding. A popular example of this utilizes the fluorophore, anthracene, attached to an amine built into the binding site. In the absence of an analyte, the lone pair of the amine quenches the fluorescence of the fluorophore through PET. Upon binding of an analyte, the lone pair is no longer available for electron transfer because of association with the

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Dr. Hassan Ait-Haddou, born in 1961 in Oulmès, Morocco, received his B.Sc. in 1984 from the University of Paris XI in France and his Ph.D. in 1988 from the same university under the direction of Professor Jean-Yves Nedelec. He was appointed as a CNRS Research Engineer in Professor Gilbert Balavoine's group in Paris and Toulouse, France. In Professor Anslyn's group, he is working on the design and syntheses of new organometallic hosts for the molecular recognition of biorelevant analytes.

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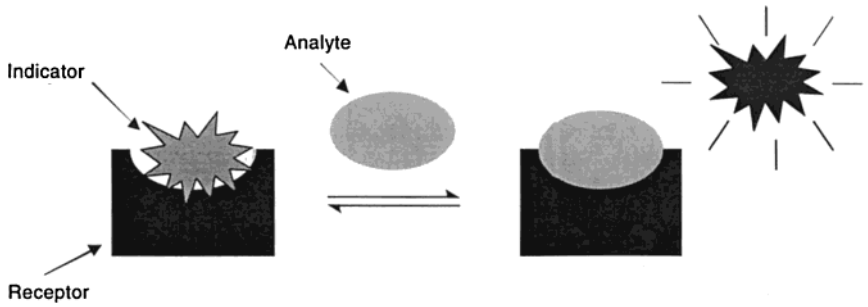
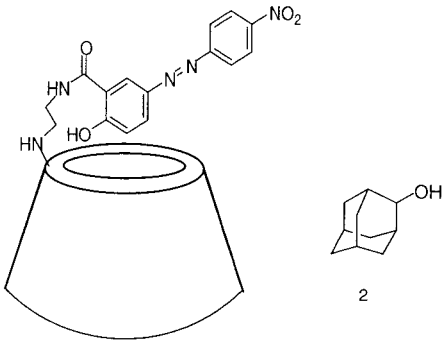
Scheme 2



analyte, and thus, the fluorescence is regenerated. A large number of sensors have been constructed on the basis of this principle.[5]

An example of a sensor that uses a different mechanism to modulate absorbance upon complexation with a guest is **1**, an alizarin yellow-modified β-cyclodextrin (β-CD).[6] The cyclodextrin receptor is known to form inclusion complexes with organic guests in aqueous media.[7] To create a chemosensor for such entities, alizarin yellow, a pH indicator,[8] was covalently attached to a β-CD through an ethylenediamine linkage. In solution, the indicator is partially incorporated within the CD cavity, protecting it from the aqueous environment. Upon inclusion of a guest such as 1-adamantanol (**2**), the indicator is displaced from the cavity, and the pK_a values are shifted. This causes a change in the protonation state of the alizarin yellow and, hence, a change in the absorption spectrum. This example highlights a sensor with a fundamentally different signaling mechanism than that of sensors based upon PET. A displacement occurs that leads to a signal modulation due to a change in protonation state of a pH indicator. In this kind of general scheme, one may ask "why have any covalent attachment between the receptor and the reporter?" By eliminating several synthetic steps, the creation of chemosensors would then be more facile.



1

2

Indicator Displacement

An alternative method to a covalently attached chromophore or fluorophore is a competition between the indicator and the analyte for the binding pocket. An indicator is displaced from the binding pocket upon addition of an analyte, causing a signal modulation (Scheme 2). We would like to show that a competition assay can be applied to most synthetic receptors. There are several advantages to this method of signaling: (1) because the receptor is not covalently attached to the indicator, it is possible to change indictors at will, (2) no extra covalent bond architecture is required in the synthesis, allowing one to focus on the design of the host first and choose an indicator later, and (3) it works well in both aqueous and organic solvents, and therefore, one can tune the solvent system to obtain the desired K_a values of the indicator and analyte. The major disadvantage of this technique is that it is not amenable to imaging tissue or whole cells, because the indicator is present everywhere in solution, not just isolated to the receptor.

Prior to our exploitation of this technique, few examples were found in the literature. One example was reported by Inouye[9] for the detection of acetylcholine (**3**) (Scheme 3). The resorcinal-based calixarene **4** forms inclusion complexes with alkylammonium cations in an alkaline media through electrostatic and/or cation$-\pi$ interactions. The indicator chosen was a pyrene-modified *N*-alkylpyridinium cation (**5**). When **5** was bound in the cavity of **4**, its orange fluorescence was quenched through PET from the anionic oxygen of **4**. Upon addition of **3** to the solution, a competition for the binding cavity occurred that led to the release of the fluorophore and the regeneration of fluorescence.

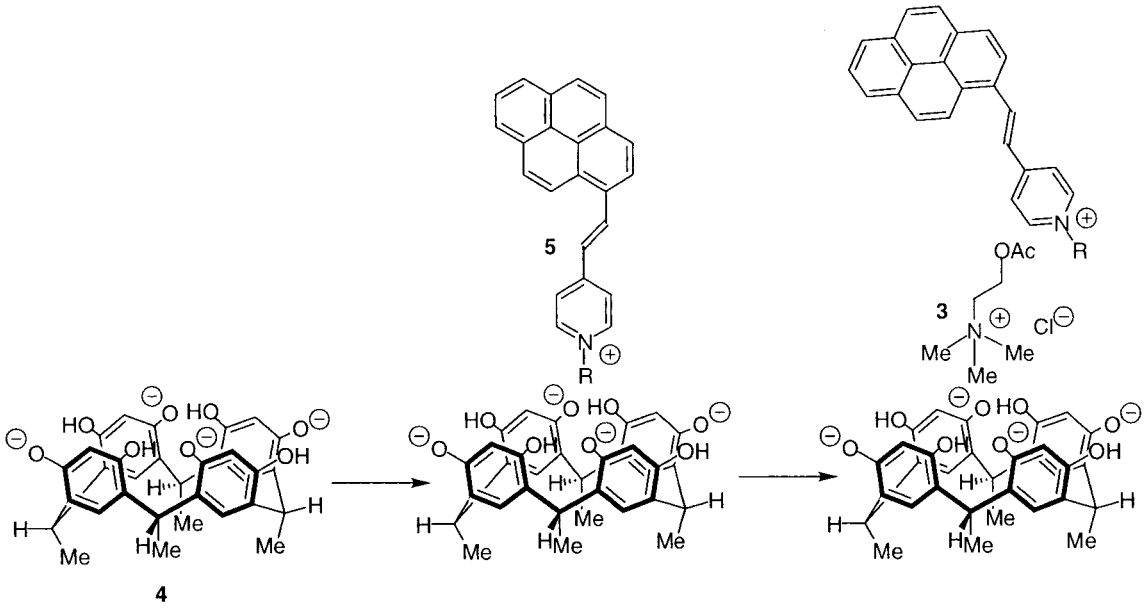
Inouye's system required strongly basic conditions to deprotonate four of the hydroxy groups of **4**, which caused problems such as nucleophilic attack on the pyridinium and degradation of the acetylcholine. This prompted Shinkai[10] to investigate a way to monitor the presence of acetylcholine in a neutral environment. Calix[*n*]arene-*p*-sulfonates ($n = 4$ or 6) were chosen as the receptors because they have a lower pK_a value, making them, therefore, anionic at neutral pH, and they also form inclusion complexes with cationic guests through electrostatic and/or cation$-\pi$ interactions. The fluorescence of **5** was quenched upon inclusion in this cavity and was regenerated upon addition of **3**.

Citrate Receptor Sensing Ensemble

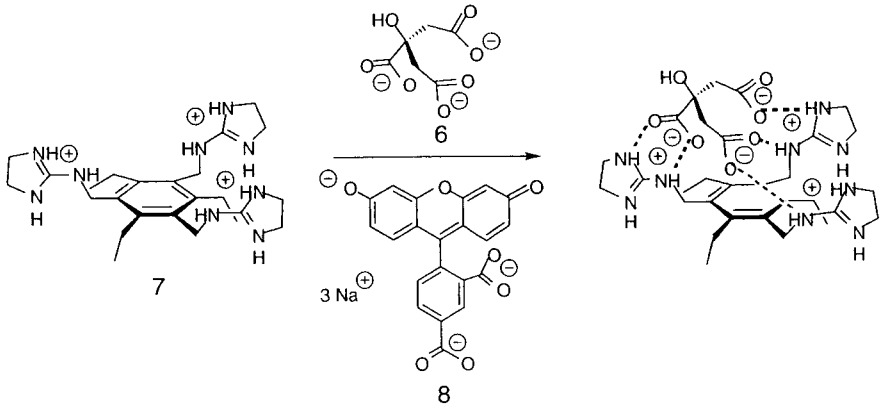
Using the idea of an indicator displacement assay, we developed a chemosensor for citrate (**6**) (Scheme 4). The sensor consisted of an ensemble of host molecule **7** and the indicator 5-carboxyfluorescein (**8**).[11] It was anticipated that this competition assay would be able to signal the presence of citrate in highly competitive media.

The design of **7** focused on a 1,3,5-trisubstituted-2,4,6-triethylbenzene scaffold incorporating three guanidinium

Scheme 3



Scheme 4



recognition units. The six substituents point alternately up and down around the ring, thereby preorganizing the guanidinium binding sites on one face of the aromatic ring.[12,13] When the binding of citrate to a host lacking the ethyl groups was compared to that of **7**, the binding affinity dropped by a factor of 2.[14] Further, because ammonium and guanidinium functionalities are commonly used for binding anions in aqueous media,[15] the two were contrasted to determine selectivity. The tris-guanidinium receptor was nearly 3-fold better than the tris-ammonium receptor for binding citrate in water, as determined by ^{1}H NMR.

Fluorescent indicator **8** was chosen because of its similar characteristics to citrate; it is tris-anionic and a pH indicator.[16] Because the absorbance and fluorescence intensities of **8** are sensitive to changes in pH, it was expected that small microenvironment differences such as the binding cavity of **7** would induce local pH changes. Upon addition of **7** to a solution of **8** in a solvent mixture of methanol and water, the absorbance increased at λ_{max} 498 nm (Figure 1A) as more of the indicator became bound, inducing a microenvironment change. This was the expected modulation. The indicator is more highly ionized when bound in the cavity of **7**, which is associated with an increase in absorbance and emission intensity. When **6** was added to the solution of **7** and **8**, the absorbance decreased (Figure 1B) as **8** was displaced from the binding pocket. A binding constant of 2.9×10^5 M^{-1} was determined for citrate by UV−vis spectroscopy.[2]

Upon testing different solvent systems, a ratio of 3:1 methanol in water at a pH of 7.4 (HEPES buffer) was chosen. A stipulation in picking a buffer is that it should not inhibit the binding of the analyte. HEPES was the buffer of choice because of its low association with guanidiniums. When the buffer was changed from one containing sulfonate groups to one containing phosphate groups, the complexation between **6** and **7** was inhibited because of buffer interference. Methanol was used in the solutions to increase the host's affinity for both **6** and **8**. By changing the solvent system, the association constants of the indicator or analyte to host can be tuned to be able to work in the desired concentration range of the analyte. When the solvent was changed from water to 3:1 methanol−water, the binding constant increased approximately an order of magnitude. Finally, the pH was adjusted within

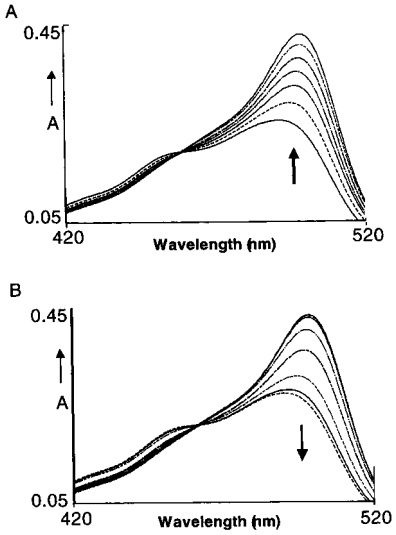


FIGURE 1. UV−vis spectra of **8** (25% water in methanol (v/v), 5 mM HEPES, pH 7.4). (A) Absorbance was measured as **7** was added to a solution of **8** at constant concentration. (B) Absorbance was measured as citrate was added to a solution of **7** and **8** at constant concentrations.

range of the pK_a of **8** to increase the sensitivity to microenvironment changes.

Calibration curves were generated for citrate under the same solution conditions discussed previously. Figure 2A shows a decrease in absorbance at 498 nm as the concentration of citrate is increased. Figure 2B displays the selectivity of **7** for citrate by examination of emission calibration curves at 525 nm. Addition of di- and monocarboxylates such as succinate and acetate, respectively, results in little or no fluorescence change. The same result is achieved when the analytes are simple salts or sugars.

Once the selectivity of the chemosensor was determined, its ability to detect citrate in a highly competitive media was tested. The sensing ensemble **7**−**8** was used to assay the concentration of citrate in beverages such as soft drinks and sports drinks. Table 1 depicts these results from colorimetric and fluorescent analysis as compared to NMR titrations as a control. Regardless of the highly competitive media, the assay performed with good agreement across the three methods.

Glucose-6-phosphate Sensing Ensemble

Using the same basic principle, a sensing ensemble was designed to signal the presence of glucose-6-phosphate (**9**). To achieve selectivity, a receptor needed to be designed that incorporated binding sites with an affinity for diols and anions in water. Boronic acids are known to rapidly and reversibly form boronate esters with 1,2- and 1,3-diols in basic aqueous media.[17] Thus, glucose-6-phosphate receptor **10** incorporates three *m*-aminomethyl benzene boronic acids as the binding sites on the 1,3,5-trisubstituted-2,4,6-triethylbenzene scaffold.[18] The boronic acids are in a position to form cyclic boronate esters with the hydroxyls of glucose-6-phosphate, while the ammoniums were incorporated to coordinate with the phosphate through charge pairing interactions. It is worth noting here that the kinetics of the boronate ester formation is fast in a basic environment when the boron is tetrahedral. These studies were done near neutral pH

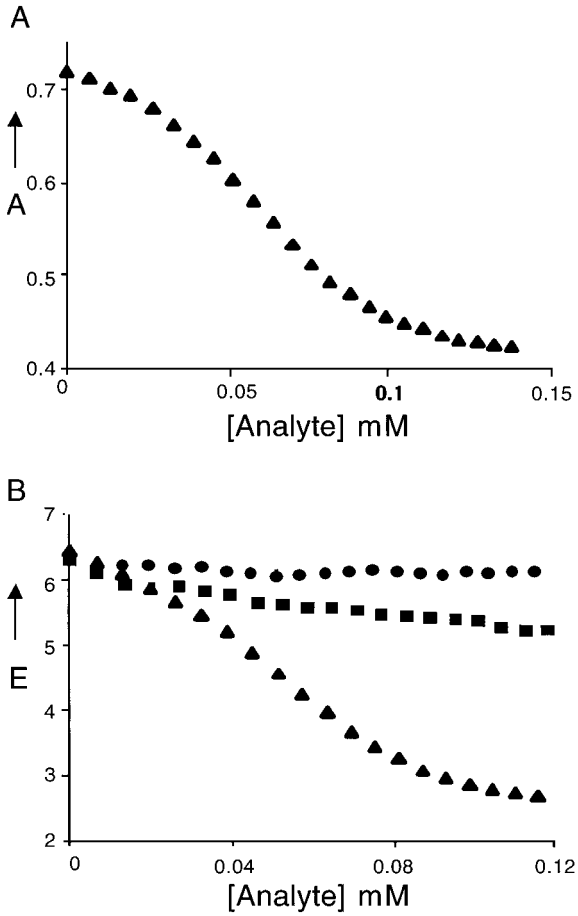
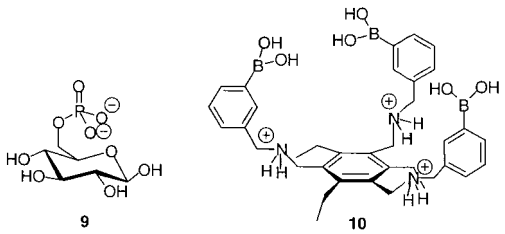


FIGURE 2. Calibration curves (25% water in methanol (v/v), 5 mM HEPES, pH 7.4). (A) UV−vis calibration curve of citrate at $\lambda = 498$ nm. (B) Fluorescence emission calibration curve for citrate (▲), succinate (■), and acetate (●). Excitation at $\lambda = 490$ nm and emission at $\lambda = 525$ nm.

Table 1. Analysis of Citrate Concentration (mM) in Beverages Determined by NMR and Competition Assay 7−8 by Absorbance and Fluorescence

	by NMR	**7** plus **8**, absorbance [mM]	**7** plus **8**, emission [mM]
citrate model solution		30.3	29.9
orange juice	43.1	44.1	44.7
Gatorade	16.0	15.1	15.1
Powerade	12.4	11.1	11.3
All Sport	7.4	7.1	8.1
Mountain Dew	8.0	5.5	5.4
tonic water	21.0	21.2	20.8
Coca Cola	0	0	<0.5
Diet Coke	<0.2	<0.4	<0.7



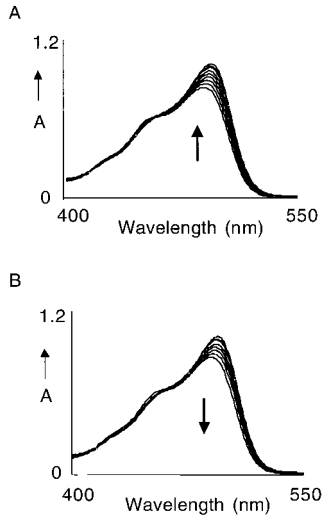


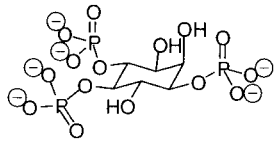
FIGURE 3. UV−vis spectra of **8** (70% methanol in water (v/v), 40 mM HEPES, pH 7.4). (A) Absorbance increase as **10** was added to a solution of **8** at constant concentration. (B) Absorbance decrease as **9** was added to a solution of **8** and **10** at constant concentration.

where the boron is planar (sp^2). Even though the assay was not under optimal conditions, with a slow rate of exchange, binding was still feasible.

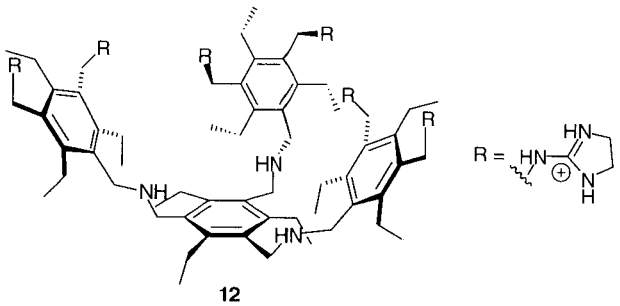
Binding studies were performed using ^{31}P NMR and UV−vis spectroscopy in a solvent system of 70% methanol in water. A 1:1 binding stoichiometry was determined using ^{31}P NMR. Using these data, the host was found to have a binding constant for glucose-6-phosphate of $1.6 \times 10^3\ M^{-1}$. With UV−vis spectroscopy, a competition assay was employed to signal binding. Once again, 5-carboxyfluorescein (**8**) was chosen as the indicator. Because the indicator's absorbance and fluorescence are sensitive to small pH changes, upon binding to **10**, it was expected to behave in a fashion similar to that of the previous sensing ensemble. Indeed, upon addition of **10** to a solution of **8**, the absorbance increases at 494 nm (Figure 3A) as more of the indicator is bound to the host. As **9** is added to a solution of **8** and **10**, the absorbance decreases (Figure 3B) as more of the indicator is displaced from the binding pocket by **9**. Using these data, the binding constant between **9** and **10** was determined to be $2.2 \times 10^3\ M^{-1}$ (by UV−vis), similar to the value determined using ^{31}P NMR. When testing similar analytes, there was no detectable change in the sensing ensemble's absorbance upon addition of glucose or sodium phosphate. However, the overall spectral response is relatively small and is not likely to be practical. Yet, we have discovered ways to improve upon low signal response, as shown in a sensing ensemble for IP_3.

IP_3 Sensing Ensemble

The next receptor was designed because of an interest in being able to detect inositol-1,4,5-trisphosphate (IP_3) (**11**), a polyanionic secondary messenger, by utilizing capillary electrophoresis (CE) during cellular processes.[19] In this regard, a sensor needed to be developed that can signal the presence of IP_3. In the development of a sensor with an affinity constant appropriate for such an application, the binding of anions in aqueous media needed to be further analyzed. Guanidiniums were chosen as binding sites because of their high affinity for not only carboxylates, but phosphates as well.[20] Further, several guanidiniums were required and needed to be preorganized to compliment IP_3. As a result, the cleftlike receptor **12**[21] consists of four units of the 1,3,5-trisubstituted-2,4,6-triethylbenzenes with one acting as the base and the other three surrounding it as substituents linked via amines. The six guanidinium binding sites were expected to be oriented toward the center of the cavity through steric gearing.



11



12

A 1:1 binding stoichiometry was determined for **11** with **12** using ^1H NMR. Fluorescence spectroscopy was chosen for the binding studies of nanomolar concentrations of IP_3 because of the sensitivity of the technique. The signaling motif again employed a competition assay with 5-carboxyfluorescein (**8**) as the indicator. In water, as with the previous glucose-6-phosphate studies, little intensity and wavelength shift upon indicator binding was observed. A switch between fluorescent and nonfluorescent forms upon binding would increase the sensitivity of the sensor. To achieve this, binding studies were done in methanol, where **8** preferred the nonfluorescent lactonized form, which is generated when the carboxylate undergoes an intramolecular conjugate addition to the quinoid structure, thereby disrupting the conjugation. It was expected that the positive microenvironment of the host would cause the ring to reopen, thereby returning **8** to the fluorescent form. Indeed, Figure 4A shows the regeneration of 530 nm fluorescence upon addition of **12** to the solution as more of the indicator becomes bound to the host. When **11** is incrementally added to an ensemble of **8** and **12**, the fluorescence decreases (Figure 4B) as the indicator is displaced from the binding cavity and the cyclized form dominates. The binding constant between IP_3 and **12** was determined to be $1.0 \times 10^8\ M^{-1}$. It was found that IP_3 in methanol could be detected at the 2 nM range with sensing ensemble **8** and **12** in the absence of any competing analytes. At this detection level, it is feasible that the intracellular concentration of IP_3 can be determined with the assistance of CE.

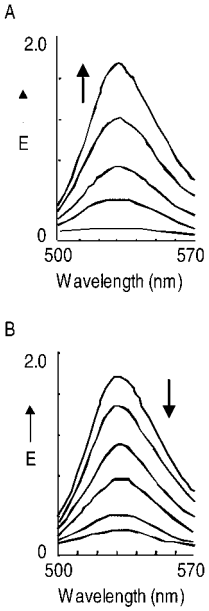


FIGURE 4. Fluorescence spectra of **8** (100% methanol, 10 mM HEPES buffer, pH 7.4). (A) Increase in fluorescence intensity as incremental amounts of **12** are added to a solution of **8**. (B) Decrease in fluorescence intensity as **11** is incrementally added to a solution of **12** and **8**. Excitation is at 450 nm.

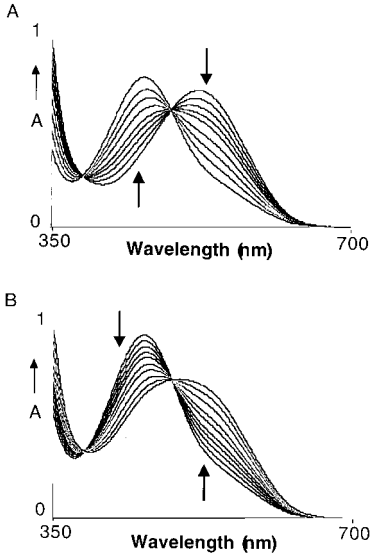


FIGURE 5. UV−vis spectra of **15** (75% methanol in water (v/v), 10 mM HEPES, pH 7.4). (A) Absorbance decrease at $\lambda = 525$ nm and increase at $\lambda = 450$ nm as **14** is added to a solution of **15** at constant concentration. (B) Absorbance decrease at $\lambda = 450$ nm and increase at $\lambda = 525$ nm as **13** is added to a solution of **14** and **15** at constant concentration.

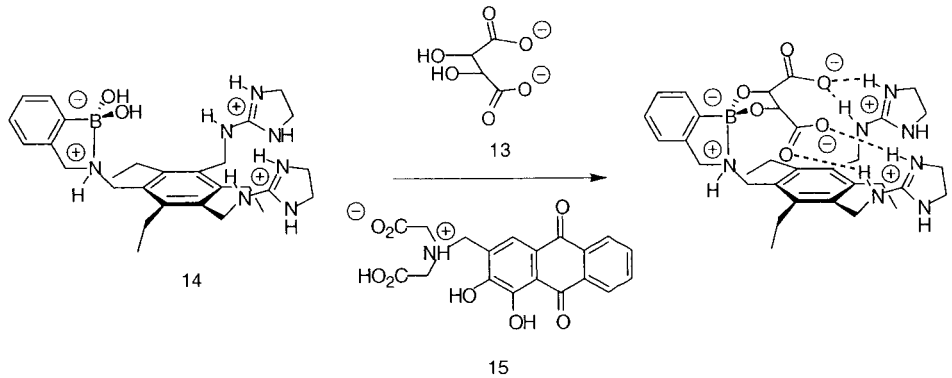
Tartrate Sensing Ensemble

All of the assays presented this far have used 5-carboxyfluorescein as the indicator where either fluorescence or absorbance intensity modulations can be monitored in response to the presence of analyte. In this manner, the indicator is used to signal analytes it was never designed to signal. We wanted to increase the utility of our displacement assay by demonstrating that other indicators can be used and that large color changes can be obtained.

The assay for tartrate (**13**) was developed using a colorimetric indicator (Scheme 5). The host was designed to bind tartrate, which is a common natural product found in grape-derived beverages such as wine and juice.[22] Because tartrate comprises two carboxylates and a diol functionality, the binding sites needed to be chosen accordingly. From what we learned in the use of the previously described hosts, two guanidiniums and a boronic acid were chosen as the recognition moieties in host **14**, and the same hexasubstituted benzene scaffold was used. The boronic acid coordination chemistry used here is different from that of **10**, because the amino methyl group is now ortho to the boronic acid instead of meta. We previously noted that the kinetics of the boronate ester formation is fast when the boronic acid is tetrahedral as in basic media. Because it is not always preferable to work at high pH, it was determined that an amine adjacent to a boronic acid allowed boronate ester formation at neutral pH.[23] The amine's lone pair donates into the empty orbital on the boron, forming the tetrahedral species (Scheme 5). This forms a zwitterionic complex at neutral pH.

The chromophore chosen, alizarin complexone (**15**), possesses similar functionalities to tartrate and is used as an indicator for the determination of pH, fluoride ions, and some rare-earth metals.[24] It was expected that **15** bound to **14** would have a different "protonation state" than **15** free in solution. Indeed, Figure 5A shows that upon incremental addition of **14** to a solution of **15** in a methanol−water mixture the absorbance at 525 nm decreases as the absorbance at 450 nm increases. When **13** is added to a solution of **14** and **15** under the same

Scheme 5



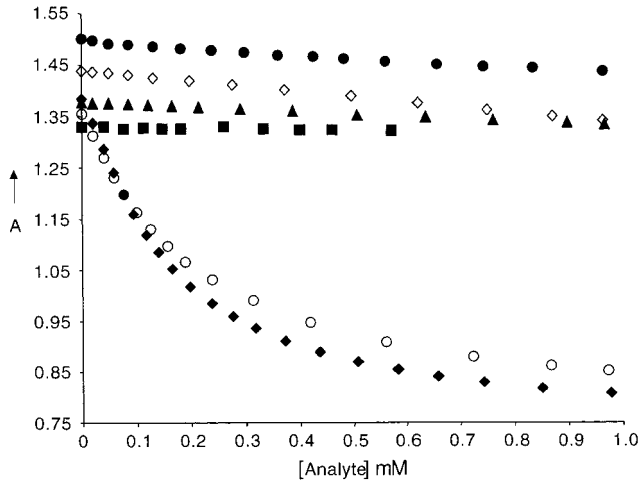


FIGURE 6. UV−vis calibration curves at 450 nm for **14** and **15** upon addition of the analytes: tartrate (◆), malate (○), ascorbate (◇), succinate (▲), glucose (■), and lactate (●) (75% methanol in water (v/v), 10 mM HEPES, pH 7.4).

Table 2. Tartrate Plus Malate Concentrations in Grape-Derived Beverages Determined by Both NMR and Colorimetric Assay

	NMR [mM]	**14** + **15** UV−vis [mM]
tartaric acid model solution	51.2	50.2
Ernest & Julio Gallo sauvignon blanc	35.6	32.9
Ste. Genevieve chardonnay	34.1	36.3
Henri Marchant spumante	26.5	24.9
Talus merlot	19.5	20.3
Santa Cruz organic white grape juice	43.7	42.3
Welch's grape juice	69.4	71.3

conditions, the absorbance change is reversed with an increase at 525 nm and decrease at 450 nm (Figure 5B). A binding constant of 5.5×10^4 M^{-1} between **13** and **14** was determined by UV−vis spectroscopy.

Other possible competing analytes were tested with the sensing ensemble using UV−vis spectroscopy, including ascorbate, malate, succinate, lactate, and glucose. Figure 6 depicts the calibration curves that were generated in these studies. The sensing ensemble **14**−**15** was selective for tartrate over sugars and mono- and bis-carboxylates, including lactate, with the exception of malate.

With the calibration curves in hand, various beverages were analyzed for their total concentration of tartrate and malate. Wines and grape juices were tested with **14** and **15**, and the values obtained were in good agreement with values independently determined by NMR (Table 2). In summary, the colorimetric sensing ensemble was able to quantitate tartrate and malate in the presence of large concentrations of similar analytes.

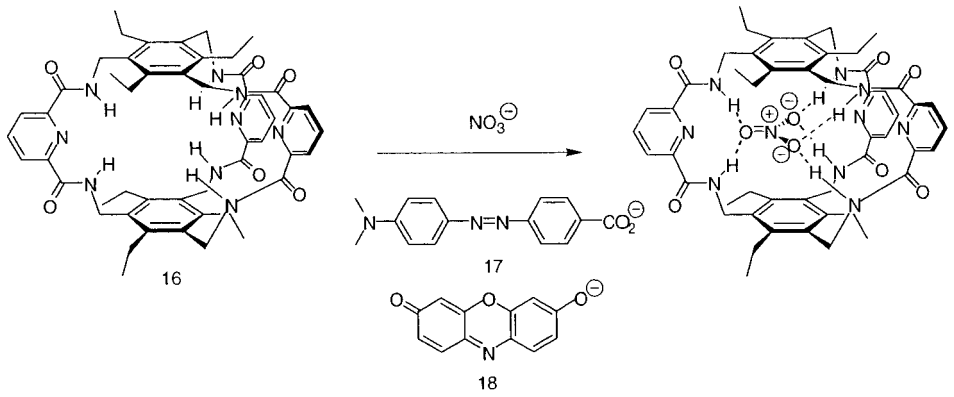
Nitrate Sensing Ensemble

To further generalize our method, we wanted to show that inorganic ions such as nitrate could be targeted as guests. Nature uses amides in proteins to complex anions such as sulfates and phosphates.[25] The receptor designed for the complexation of nitrate was an amide-linked C_3-symmetric bicyclic cyclophane (**16**).[26] It was shown that the amide hydrogens complex as neutral hydrogen bond donors to the anion's π-systems (Scheme 6). Our receptor consists of two molecules of the 1,3,5-tris-aminomethyl-2,4,6-triethylbenzene as the base and the cap. The two are linked by the formation of 2,6-pyridine diamides, where the six amide hydrogens converge into the center of the cavity.

To complete the chemosensor, pH indicators methyl red (**17**) and resorufin (**18**) were chosen.[27] It was expected that the anionic dyes would bind in the cavity causing a change in their absorbance. Upon addition of an appropriate analyte, the dyes would be displaced from the cavity, resulting in a change in absorbance associated with the dye free in solution. Indeed, upon addition of **16** to a solution of **17** or **18**, the absorbance spectrum of the indicators changed. The formation of the complex **16**−**17** resulted in a decrease in absorbance at 575 nm (Figure 7A). An increase in absorbance occurred at 495 nm when the complex **16**−**18** was formed (Figure 7B). When neutral methyl red was tested for complexation with **16**, the spectrum showed no change, indicating that an anion is needed for complexation.

The formation of a complex between **16** and **18** is inhibited in the presence of nitrate. Figure 8A shows the absorbance changes of **18** at 576 nm upon the addition of various analytes. The host **16** shows selectivity for nitrate over other anionic guests such as bromine and perchlorate in 50% (v/v) methanol in dichloromethane. The association constant between **16** and nitrate was determined to be 380 M^{-1} by UV−vis spectroscopy when

Scheme 6



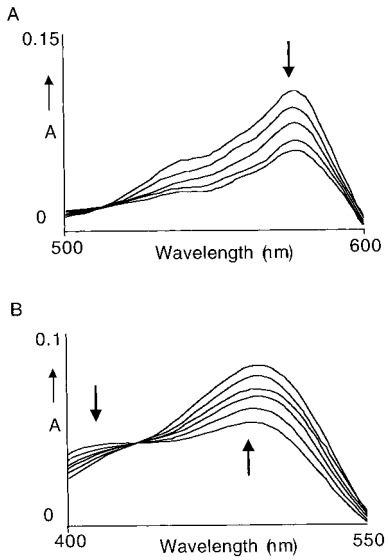


FIGURE 7. UV−vis spectra of (A) resorufin and (B) methyl red at constant concentrations as **16** is added (50% methanol in dichloromethane (v/v)).

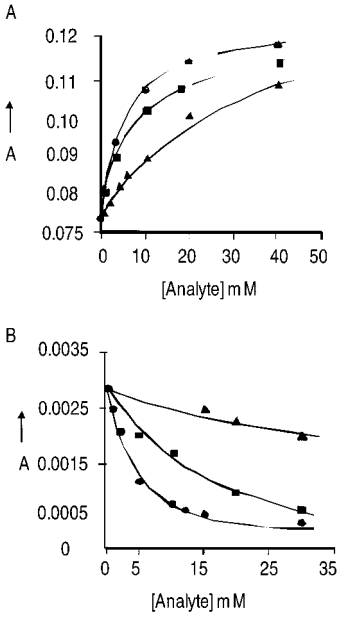


FIGURE 8. UV−vis calibration curves for (A) the sensing ensemble **16** and **18** at 576 nm upon addition of analytes NO_3^- (●), Br^- (■), and ClO_4^- (▲) (50% methanol in dichloromethane (v/v)) and (B) the sensing ensemble **16** and **17** at 423 nm upon addition of analytes NO_3^- (●), Br^- (■), and ClO_4^- (▲) (25% acetonitrile in dichloromethane (v/v)). Countercations are sodium and tetrabutylammonium for the two different solvent systems, respectively.

using **18** as the indicator. When indicator **17** in 75% (v/v) acetonitrile in dichloromethane is used, higher bonding constants were anticipated because of a lower dielectric media and the absence of competing hydrogen bonds. Figure 8B shows the absorbance changes of **17** at 423 nm in the presence of **16** with varying concentrations of anions. Again, **16** is found to be selective for nitrate over other anions with a binding constant for **16** to nitrate of 500 M^{-1}. The development of this chemosensor shows that

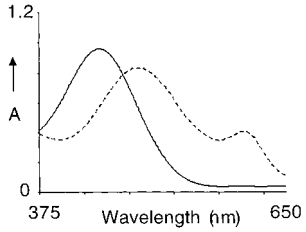
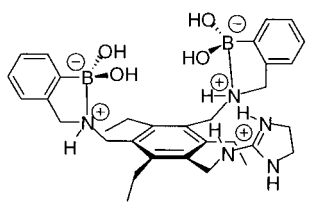


FIGURE 9. UV−vis spectra of a solution of **20** (—) and a solution of **19** and **20** (- - -) (75% methanol in water (v/v), 10 mM HEPES, pH 7.0).

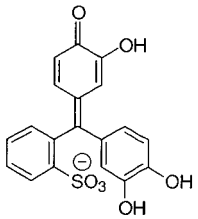
the competition assay is applicable in an organic solvent system, and the sensing ensemble is not limited to one indicator.

Current Sensing Ensembles under Investigation

One of the latest additions to the collection of sensing ensembles is **19**, a bis-boronic acid monoguanidinium receptor.[28] This was developed with the expectation that it will complex analytes that incorporate two diols and a single carboxylate. The indicator chosen was pyrocatechol violet (**20**), an indicator used for the determination of tin and bismuth,[29] which possessed similar traits to our ideal analyte. Upon addition of **19** to a solution of **20** in 25% water in methanol, the λ_{max} shifted from 442 to 488 nm (Figure 9). Current analytes being examined do indeed show a reverse of this wavelength shift.

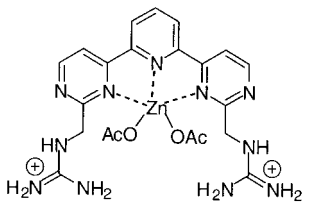


19



20

We are also working on projects aimed at uncovering methods for very large λ_{max} shifts, giving brilliant color changes. The incorporation of metals into our receptors to act as both binding sites and as entities to facilitate color changes seemed attractive. The bis-guanidinium polyaza molecule **21** is being used in one of our first metal containing sensing ensembles.[30] The binding cavity is preorganized through complexation of the pyridine and pyrimidines to zinc. The ideal analyte would possess amines to complex the zinc and carboxylates to pair with the guanidiniums. Pyrocatechol violet was again chosen as the indicator, and upon addition of **21** to a solution of **20** in 50% water in methanol, the λ_{max} shifted from 445 to



21

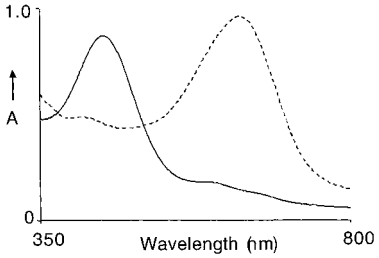


FIGURE 10. UV−vis spectra of a solution of **20** (−) and a solution of **20** and **21** (- - -) (50% methanol in water (v/v), 10 mM HEPES, pH 7.4).

642 nm (Figure 10), nearly a full 200 nm shift. When amino acids are introduced, the equilibrium is reversed as in the previous ensembles.

Conclusion

Synthetic receptors can readily be converted to optical chemosensors using a displacement assay and common fluorophores and chromophores. One focuses first upon design of a host complementary to the analyte of interest, and then one searches the literature for an indicator or dye that possesses similar functional groups to those of the analyte. Studies of the association of the indicator and analyte with the host can then be carried out, manipulating the solvent system to tune the affinity constants to be appropriate for the particular analysis. In every case we have examined thus far, the ensemble formed by host and indicator lead to a method for quantification of the analyte. We feel this is a widely applicable method that can be applied to most synthetic receptors, and we encourage others to adopt the technique for their own use.[31]

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(31) For examples of other research groups that are now starting to exploit this method, please see: Gale, P. A.; Twyman, L. J.; Handlin, C. I.; Sessler, J. L. A Colourimetric calix[4]pyrrole-4-nitrophenolate based anion sensor. *Chem. Commun.* **1999**, 1851−1852. Fabbrizzi, L.; Leone, A.; Taglietti, A. A Chemosensing Ensemble for Selective Carbonate Detection in Water Based on Metal-Ligand Interactions. *Angew. Chem., Int. Ed.* **2001**, *40*, 3066−3069.

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