JOC*Perspective*

Supramolecular Analytical Chemistry

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A large fraction of the field of supramolecular chemistry has focused in previous decades upon the study and use of synthetic receptors as a means of mimicking natural receptors. Recently, the demand for synthetic receptors is rapidly increasing within the analytical sciences. These classes of receptors are finding uses in simple indicator chemistry, cellular imaging, and enantiomeric excess analysis, while also being involved in various truly practical assays of bodily fluids. Moreover, one of the most promising areas for the use of synthetic receptors is in the arena of differential sensing. Although many synthetic receptors have been shown to yield exquisite selectivities, in general, this class of receptor suffers from cross-reactivities. Yet, cross-reactivity is an attribute that is crucial to the success of differential sensing schemes. Therefore, both selective and nonselective synthetic receptors are finding uses in analytical applications. Hence, a field of chemistry that herein is entitled "Supramolecular Analytical Chemistry" is emerging, and is predicted to undergo increasingly rapid growth in the near future.

I. Introduction

This perspective discusses a relatively new cross-disciplinary field of chemistry, which is herein called "Supramolecular Analytical Chemistry". Previous examples of the use of supramolecular chemistry in the analytical sciences are given as a historical backdrop, followed by several examples of the author's own work in this regard. Necessarily, the article is not a review of the author's work, nor is it meant to be comprehensive in regard to the impact of supramolecular chemistry on analytical sciences. Instead, this article describes those studies in the field of supramolecular chemistry that inspired, and continue to inspire, our group's work. In the act of doing this, a few anticdotes associated with the author's career are given to relay how our group's ideas were derived. Of paramount importance to our group's current work are two topics: indicator-displacement assays (IDAs)[3] as a signaling protocol and the use of differential receptors. Hence, this article is focused on these main topics following the introduction. The take home lesson is that supramolecular chemistry is ripe for exploiting in the context of analytical chemistry.

(A) Sensors. Supramolecular chemistry has a focus "beyond the molecule".[1] It is not a hybrid of the common chemical disciplines, such as bioanalytical, organometallic, or biophysical chemistry. Instead, it describes a field of chemistry that encompasses all of the disciplines and subdisciplines involving intermolecular interactions. Although the term covers many fields, it is primarily associated with chemists working on synthetic organic and/or synthetic inorganic structures.

In recent years, many research groups around the world have shown that supramolecular chemistry can play a uniquely powerful role in analytical sciences. The field involves analytical chemistry applications of synthetic organic and inorganic chemical structures that undergo molecular recognition and self-assembly. The descriptor proposed for this burgeoning field is "Supramolecular Analytical Chemistry".

In the most common embodiment of supramolecular analytical chemistry one creates a sensor. This word deserves some scrutiny. Webster's dictionary defines a sensor as "a mechanical device sensitive to light, temperature, radiation level, or the like, that transmits a signal to a measuring or control instrument".[2] Similarly, my colleague Allen Bard—a "card carrying" analytical chemist—defines a sensor as a device that makes a measurement. For example, the combination of a pH electrode with the pH meter is a sensor. However, the term sensor has a different meaning to a supramolecular chemist. Here, the author defines a sensor as "a receptor that interacts with an analyte producing a detectable change in a signal". To the purists, the sensors discussed herein are simply indicators.

Before highlighting the work of others, one article needs to be mentioned as partially inspiring the field. It was published in 1995 by Anthony Czarnik and was entitled "Desperately Seeking Sensors".[3] This title was intentionally meant to invoke

the movie "Desperately Seeking Susan" starring Madonna, and so this article was of immediate interest. Although I read the paper with only minor interest at the time, the ideas presented in it continued to percolate and grow in my mind.

(B) What is Supramolecular Analytical Chemistry?. When is a chemical interaction supramolecular? In Jean-Marie Lehn's book entitled *Supramolecular Chemistry*, his definition focuses upon the use of intermolecular interactions for the creation of assemblies.[1] A problem arises in defining an intermolecular interaction. For example, many of the sensors described below exploit the dynamic exchange of covalent bonds. Strictly speaking, this is *not* a supramolecular interchange. Further, dynamic combinatorial chemistry exploits such reactions: imine exchange,[4] disulfide exchange,[5] and Diels−Alder reactions.[6] Admittedly, the interchange of these reversible covalent bonds is templated by reversible intermolecular interactions that are clearly supramolecular in nature. But, should the reversible covalent bonding also be considered supramolecular? The best answer is "no". But, as a last perplexing example, what about metal ligation and metal coordination chemistry—when should it be considered supramolecular?

One way to answer these questions is to define a cutoff threshold for the strength of an interaction, or the rates associated with establishing equilibrium. Maybe interactions worth 30 kcal/mol or less and that are dynamic enough to thoroughly exchange in 24 h should be considered supramolecular. While such arguments have previously been made, they are limiting and arbitrary and would end up including the dissociation of weak covalent bonds into radicals.

In the opinion of the author, establishing rigid definitions is useful, but can be limiting. Hence, in this article the term supramolecular is going to be extended. Upon consideration that supramolecular chemists are primarily focused upon the use of synthetic receptors,[7] that many receptors utilize the exchange of covalent bonds, and as a means of encompassing the breadth of the field, the author suggests the following: "Supramolecular Analytical Chemistry" exploits the dynamic exchange of synthetic chemical structures that create assemblies which result in signal modulations upon addition of analytes. These assemblies can be as simple as 1:1 host/guest structures created from noncovalent or covalent bonds as well as higher order assemblies. The reactions should be dynamic on whatever time scale is necessary for the application. Clearly, not all chemists will accept this definition, but it suits the content of this article.

II. Approaches to Single Analyte Sensing

(A) Complexone Agents as Indicators. pH indicators are likely the oldest use of organic chemical structures in analytical applications. In 1664, Robert Boyle completed his work, *The Experimental History of Colours*.[8] In his work, he showed that particular plant extracts could be utilized as indicators. Boyle showed how treatment with acid or base caused the material to change color. Boyle used Brazil wood in his experiments, which at the time, was extensively used as a dyestuff for staining or coloring fabrics and in manufacturing textiles like velvet. Much later, in 1957, Bitskei and Moritz built on Boyle's work,[8] providing a range of color results using Brazil wood.

Even before the term supramolecular chemistry came into existence, organic chemists were using the notion of molecular recognition with pH indicators for sensing purposes. For example, the "complexone" agents derive from the 1950s.[11] In

these structures metal chelating groups are covalently tethered to pH indicators. Binding of the metals to the chelating groups in proximity to the ionizable acidic sites lead to differing protonation states of the pH indicator with and without bound metal. Two such examples are compounds **1** and **2**, which colorimetrically signal Ln(III) and Ca(II), respectively. Importantly, as described below, complexone agents are still of great use in the analysis of metals and have recently found new uses in the context of indicator-displacement assays.
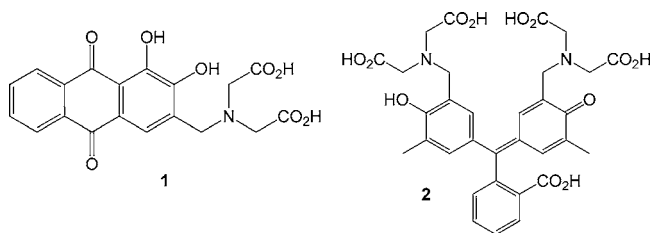
Coming much more up to date, the field of supramolecular chemistry started with crown ethers, cryptands, and carcerands.[9] Interestingly, such structures quickly found use in analytical chemistry by also appending pH indicators. Compound **3**, created by Cram, is an example that signals Na^+ in the presence of K^+ and can be used in an analytical assay.[10a] Further, crownlike structures are now commercially available. One example is compound **4**,[10b] which is sold by Molecular Probes for the cellular imaging of Na^+. This entity has seen great commercial success, a testament to the use of supramolecular design in analytical chemistry. With hindsight, we can consider the complexone agents as chemical ancestors of the crown-type structures **3** and **4**, while also considering all crown-type structures with appended indicators simply as updated complexone agents.

CoroNa Green, AM
Na+ sensor in vivo
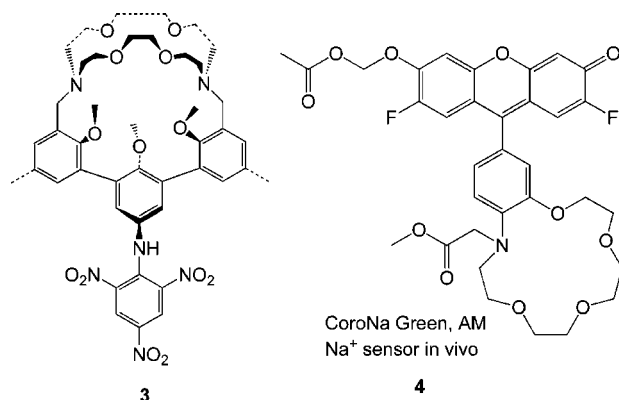
(B) The Use of the Receptor−Spacer−Reporter Paradigm and PET. The complexone agents are good examples of the receptor−spacer−reporter paradigm for creating sensing agents. In this approach, the receptor is covalently tethered to a reporter, albeit a chromophore/fluorophore or an electrochemically active entity.[12] One must incorporate a mechanism that translates analyte binding into some kind of modulation of the reporter, thereby altering optical or electrochemical properties. In the case of modulating optical properties, the incorporation of a photo-induced electron transfer (PET) mechanism stands out as the most popular.

The PET mechanism involves the reduction of the ^1S excited state of a fluorophore by a neighboring high-lying filled orbital.[13] The electron transfer occurs after excitation and before emission
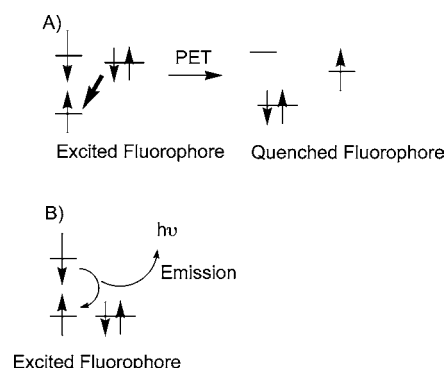
**FIGURE 1.** Electron-transfer mechanism associated with PET.

and results in quenching of the fluorescence (Figure 1). Upon binding of the analyte, the energy level of the donor orbital is lowered, thereby diminishing the ability to transfer an electron to the excited-state acceptor, and fluorescence is restored. Hence, an advantage of this method is that it results in an off-to-on signal.

Classic examples of PET-based sensing strategies come from the Czarnik, deSilva, and Shinkai laboratories. Compounds **5**, **6**, and **7** incorporate the ubiquitous amine and anthracene reporter unit duet covalently attached to receptors for pyrophosphate,[14] Na$^+$,[15] and fructose,[16] respectively. With sensors **5** and **6**, the amine donor is integral to the receptor, and hence, the reporter and receptor units overlap in identity. The mechanism by which the donor energy levels modulate upon binding when using of *o*-aminomethyl phenylboronic acids is under debate. The manner in which the structure of these receptors is drawn, with a water inserted between the amine and the boronic acid, derives from a postulate put forth by Wang[17] and experimental support from our own group.[18]
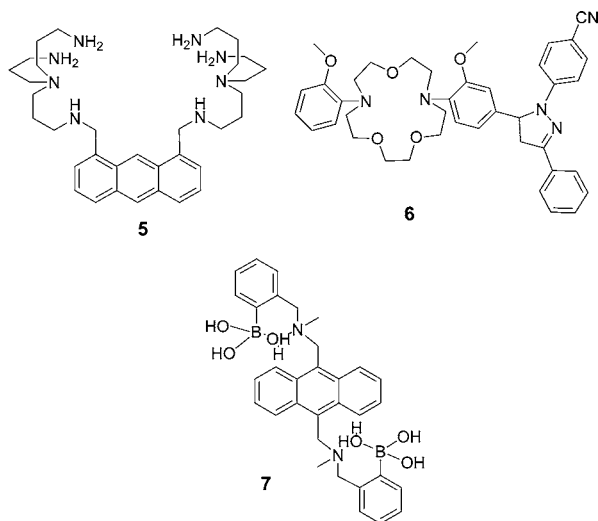

One example of a fluorescent sensor from our group which functions in part due to a PET mechanism is for the detection and quantitation of heparin in blood.[19] Compound **8** selectively binds heparin with nanomolar affinities. The fluorescence of the core scaffold is quenched upon heparin binding, likely due to the proximity of numerous carboxylate and sulfate negative charges in heparin. The high-lying filled orbitals associated with these negative charges act as donors to quench the excited state of the fluorophore core. To our knowledge, sensor **8** is the only

synthetic system that can be used to quantitate this important anticoagulant in crude biological media.
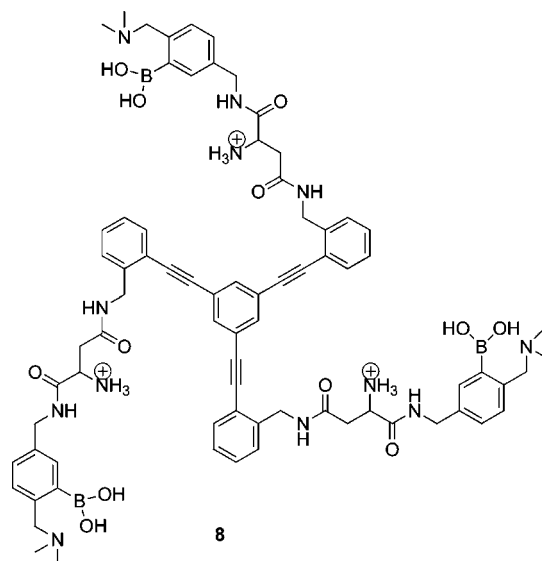

(C) Whole Cell Imaging. Tsien took the complexone concept and extended it to cellular imaging agents for Ca(II) called INDO-1 and FURA-2.[20] This work has inspired many chemists to devise imaging agents using the receptor-spacer-reporter paradigm. Recently, a large number of cellular imaging agents are being created, many of which rely on multiple mechanisms to give optical signal modulations.[21a]

The creation of imaging agents is likely the area with the most growth potential for supramolecular chemists. The recent work of Tetsuo Nagano,[21b] Christoph Fahrni,[21c] and Young-Tae Chang[21d] is of particular note for the directions our group is moving. The mechanistic insights and combinatorial methods that these individuals are putting forth for sensor design will be far reaching.
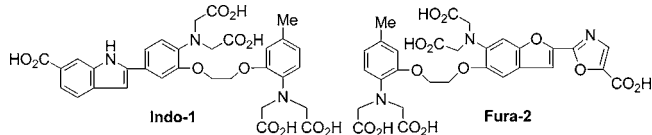

(D) Chemical Indicators as Sensing Entities. Gerhard Mohr is popularizing the use of trifluoromethyl ketones, tricyanovinyl groups, and boronic acids within the context of azo compounds as signaling entities for alcohols, amines, and sugars, respectively (**9**, **10**, and **11**, respectively).[22] These structures were part of the inspiration for the work of Timothy Glass concerned with the fluorescence detection of amines using compound **12**.[23] In an extension of Glass's work, our group has studied compound **13** for the detection of sarin/soman analogues.[24] The mechanism of detection exploits PET quenching of the coumarin by the oximate anion, which is diminished upon phosphorylation, leading to an "off-to-on" detection method.

(E) Indicator-Displacement Assays (IDAs). Likely the signaling method most closely associated with our group's work is the "Indicator-Displacement Assay" (IDA). We did not invent this method, but we have popularized it to such an extent that it is now widely used and recognized as a standard method for the creation of sensors.[26] In the context of supramolecular chemistry, we trace its origin back to studies by Inoue and

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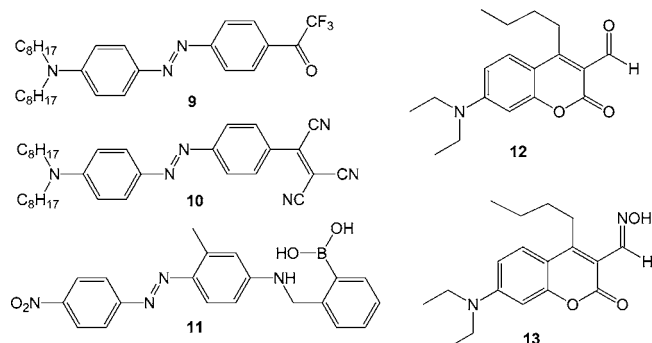
Shinkai for making an optical detection method for acetylcholine.[25] In these studies, the guest was labeled with an indicator and bound to a calixarene. Upon addition of the unlabeled analyte a displacement of the labeled analyte occurred leading to a detection method.

At the heart of an IDA is a colorimetric or fluorescent indicator (I) that changes optical or electrochemical properties when bound to a receptor/host (H) relative to being free in the bulk medium (eq 1). The medium can be the solvent, a polymer

$$H{:}I + G \rightleftharpoons H{:}G + I \qquad (1)$$
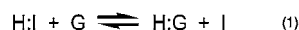
matrix, a surface, or another phase. The indicators can be solvatochromic, ionic strength dependent, or, most commonly, pH indicators. The indicators can undergo fluorescence resonance energy transfer or photoinduced electron transfer with the receptor, which will be modulated when the indicator is released from the host. Displacement of the indicator occurs upon addition of the analyte/guest (G). We call the combination of the host and the indicator the sensing ensemble.

I received the idea for this simple concept as a postdoctoral fellow with Dr. Ronald Breslow at Columbia. The Breslow group would commonly use the binding of dansyl to cyclodextrin and follow the displacement of dansyl using fluorescence spectroscopy upon addition of various guests, simply as a means of measuring the binding constants of the guests.[27] It was obvious that the method could also be used for signaling, although it was more than a decade later before my group used the idea.

The first use of an indicator-displacement assay by our group was for measuring citrate in soda pop.[28] The idea originated due to a vial of receptor 14 sitting on my desk while I was drinking a Fresca. Compound 14 was a byproduct of a reaction that the group was running for the creation of a phosphate−ester hydrolysis catalyts.[29] A major ingredient of Fresca is citrate, and it was immediately obvious that 14 would be a good receptor for citrate. Combining this idea with the notion of displacing an indicator led to our first practical example of using a synthetic receptor with an indicator to measure an analyte in various commercial beverages. The indicator we used was 5-carboxyfluorescein (CF). Buffers are a necessary evil in these studies to eliminate any pH response. The buffers maintain the pH, but by virtue of the fact that all buffers are ionic, they lower any electrostatic driven molecular recognition via competitive ion pairing with the hosts and guests.

A series of advantages of an IDA over the receptor−spacer−reporter paradigm have been published in the literature. The advantages of IDAs stem from the independence of the receptor from the indicator. We showed that signaling can be tuned to respond to a more narrow concentration range than is capable

with a standard 1:1 binding stoichiometry.[30] Further, our group continually exploits the ability to rapidly screen indicators with the same receptor to discover the indicator that gives the largest optical response, or an optical response with a color desired for the assay. Another advantage was noted by Fabbrizzi.[31] He published a study that demonstrated excellent selectivity in the discrimination of two analytes, in which good discrimination was achieved by choosing an indicator whose binding affinity with the host was between that of the two analytes. Hence, only one of the two analytes could efficiently displace the indicator and be detected.


14

CF
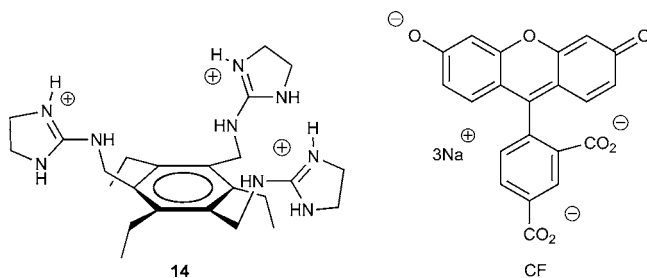
(F) IDAs for Common Natural Product Anions. After the study for citrate, our group has published IDAs for a variety of anions: tartrate,[32] phosphate,[33] IP$_3$,[34] nitrate,[35] heparin,[36] and 2,3-bisphosphoglycerate.[37] The anions were targeted with a variety of host structures and types and quickly converted to optical sensors using IDAs. IDAs are particularly useful for anion sensing because many indicators themselves are anions and, hence, have a natural affinity for receptors that are designed to bind other anions.[38]

The phosphate anion has been an important target for the molecular recognition community for decades, but no receptors with good affinities and high selectivity for phosphate over sulfate in water at neutral pH had been developed. In 2002, we introduced a C_3v symmetric design for phosphate (15).[33] This receptor was highly selective for phosphate over other tetrahedral, trigonal planar, and spherical anions; only arsenate was a major competitor to phosphate binding. Hence, in analyses that are arsenate free, the receptor essentially only binds phosphate. With an IDA involving CF, we were able to accurately quantitate phosphate in human blood and saliva, as well as horse blood.[39]

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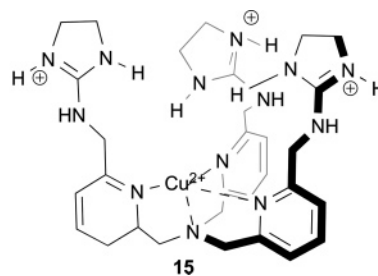
Our most recent example using an IDA is the detection of glucoronic acid in blood, which is the product of oxidation of glucose-by-glucose oxidase.[40] We found that 16 has no detectable affinity for glucose, but it binds gluconic acid in 3:1 methanol/water with an affinity constant of 5.6×10^6 M^{-1}.[41] This has led to a colorimetric method for glucose detection in blood. We add glucose oxidase to blood samples, and a small aliquot of these crude samples are then added to an ensemble of 16 and pyrocatechol violet (PV). The resulting color gives

an accurate measurement of the original glucose concentration in the blood.[42]



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pyrocatechol violet (PV)
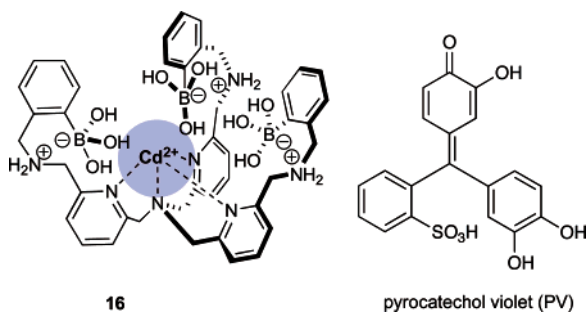
(G) Choosing a Solvent for an IDA. In any binding-based assay, it is useful if the binding of the analyte to the receptor is near the dissociation constant of the analyte to that receptor. If the receptor's concentration is near that of the dissociation constant (K_d), a concentration of the analyte much above the K_d saturates the receptor and the assay is not sensitive. A concentration far below the K_d gives little binding and little sensitivity. Hence, the assay needs to be tuned to operate near the K_d. With synthetic receptors this is particularly easy to achieve because one can change the solvent to dial in the dissociation constant. A lower dielectric constant consistently enhances binding that is ion-pairing or hydrogen-bonding driven.

Even just a decade ago, the field of synthetic receptors was criticized because many of the receptors did not function well in water. Instead, many of the receptors were used in chloroform,[43] where competitive inhibition by the solvent to binding was minimized. In hindsight, the ability to work in media other than water is a strength of synthetic receptors, especially in analytical protocols. In an analysis on chips, in a cuvette, or a 96-well plate reader, it does not matter what the bulk solvent for the assay needs to be. Even if the target analytes are in water to start, as long as the solvent system is miscible with water these analytes can be studied. Hence, our group has used a variety of solvent systems in our indicator-displacement assay protocols.

In most analytical assays, one has an estimate of the concentration of the target analytes. Therefore, in our IDA methods we adjust the solvent system until we tune a K_d near the concentration we are estimating for the analyte of interest. In many of the anions we have studied, a mixture of water and methanol, ranging from pure water and pure methanol, has sufficed. When a lower dielectric is required, we mix in acetonitrile, DMSO, or THF. Because the basis of an IDA is a protonation change of the indicator when bound versus free in solution, we have found that any solvent which can be buffered and has a pH scale defined can be used.[44] Hence, one can envision using pyridine, DMF, or acetic acid as a means to tune the dissociation constants to the range needed in any particular assay.

(H) Enantioselective IDAs. One of the most attractive areas in which to exploit an IDA for the purposes of having naked-eye color changes is in the sensing of chirality. Our group envisions a future where different colors for enantiomers of simple functional groups can be seen in both a qualitative litmus-paper like test strip and a quantitative assay in an UV/vis cuvette or a 96-well plate reader. There is little work in this area in the literature, but we note chiral receptors that give optical modulations from Lin Pu,[45] Yuji Kubo,[46] and Kyo Han Ahn,[47] which

inspired us to pursue this area of research (**17**, **18**, and **19**, respectively). Another pioneer in this area is Matt Shair. He uses kinetic resolutions in array formats to quantitate enantiomeric excesses of unknown samples.[48] These pioneers in the area of chiral sensing gave us the confidence that we could make a contribution by applying IDAs to chiral recognition.

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X=
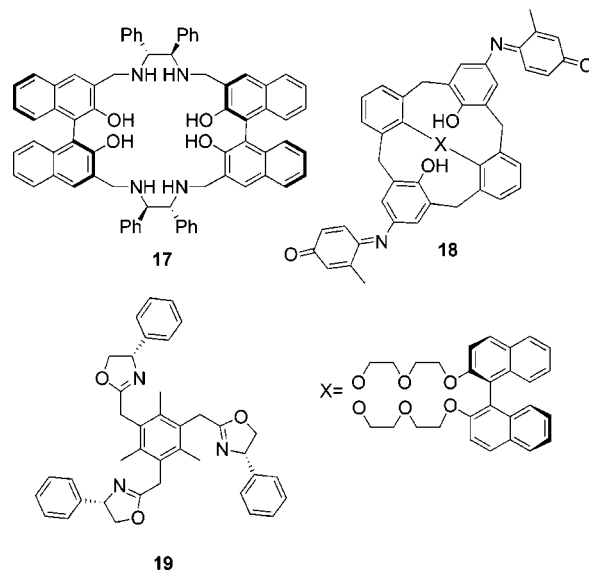
Receptors **20** and **21** bind the enantiomers of α-hydroxy acids[49] and α-amino acids,[50] respectively, with chiral discrimination. Either can be paired with a variety of indicators to give different colors for the enantiomers of these two analyte classes. When having a mixture of the two enantiomers of these analytes, one chiral receptor, and one indicator, extensive algebra allows one to derive a polynomial that relates enantiomeric excess to absorbance values.[49]


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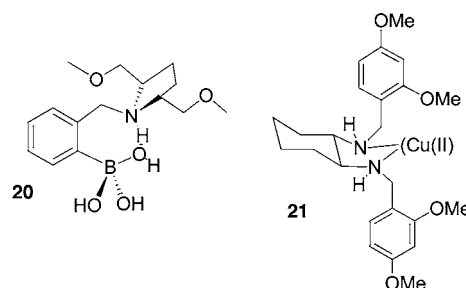
When the assays were first performed, we achieved errors on the ee determinations between 4 and 13% depending upon the chiral analyte. Admittedly, a 13% error for ee is large, and for many applications, it is unacceptable. However, even errors as large as this in a quick screening protocol are acceptable.[51] Our technique can consistently identify the solution with the highest ee relative to other solutions. This means that when screening catalysts for asymmetric induction one can find the catalyst giving the best ee values, even if the error on those values is large. Further, we can routinely improve the error in the ee determination by screening indicators to discover the indicator that gives the largest dynamic range in absorbance or emission modulations. In this way, we have improved errors from the 13% range to near 6%.

(I) Receptors Derived from Combinatorial Chemistry. Historically, our group has followed the classic paradigm for

the field of molecular recognition for creating synthetic receptors—design, synthesis, and testing of the design. Such a protocol led us to the receptors for citrate and phosphate discussed above, as well as many others. However, it is well recognized that screening a combinatorial library of receptors as a means of discovery can be quite fruitful. The field of DNA and RNA aptamers,[52] as well as phage-display of proteins,[53] has taught the supramolecular chemistry community that winnowing down a large collection of structures to a few winners will routinely lead to exceptionally selective and high affinity receptors—simply by virtue of serendipity.

Within the field of synthetic receptors, we took our lead from Still,[54] Liskamp,[55] and Kilburn.[56] Clark Still was a pioneer in using macrocyclic receptors to screen libraries of peptides to discover "hits" which had the highest affinity for the host. Liskamp continued in this tradition using molecular clefts.

In our one and only study where we pulled a single receptor out of a library of structures to quantitate the concentration of a single analyte, we screened library **22** for binding to ATP.[57] This library incorporated a hexasubstituted benzene scaffold,[58] along with guanidinium groups. These groups were used because we knew that they would ensure that each and every member of the library would have a basal level of affinity for the triphosphate linkage of ATP. We found that in the library of **22**, Ser-Tyr-Ser yielded a very selective receptor for ATP.[57] This receptor could signal the presence of ATP, but not AMP or GTP. Unfortunately, as is so often the case with the screening of libraries, we have achieved success, but we still have no idea as to why Ser-Tyr-Ser in the context of structure **22** leads to a selective and high affinity assay.

22
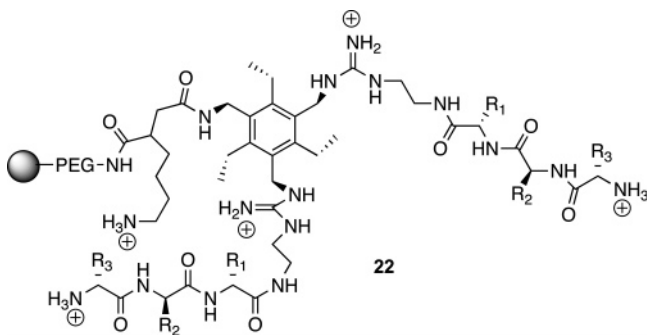
It was at approximately the time that we were screening library **22** and creating our assays for citrate that our group took a major turn due to a lunch that I had with my colleague John McDevitt. That revelation, now described below, has dramatically affected most of the sensing protocols our group has pursued ever since.

III. Differential Receptors

This section covers the concept of differential sensing arrays. Much of the impetus for the current studies in our group is a sense on our part that the field of molecular recognition (MR) is undergoing a paradigm shift.[59] Currently, MR is driven by the goal of synthesizing receptors for various guests as a means of mimicking Mother Nature's methods, hence achieving an understanding of nature. For specific binding purposes, however, due to their simplicity, synthetic receptors often suffer interference from similar analytes. The use of synthetic receptors cannot compete with antibodies and aptamers for specificity of binding medium and large complex analytes. As now described, the lack
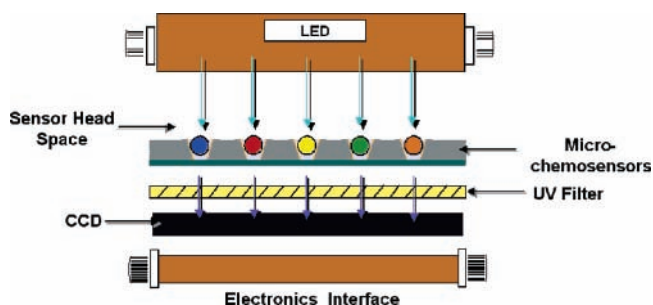
FIGURE 2. Schematic representation of the U.T. Taste Chip. Thanks to the John T. McDevitt group for supplying a similar graphical picture.

of selectivity associated with synthetic receptors is a virtue in the context of array sensing. In a manner of speaking, the use of synthetic receptors in this context is "creating lemonade from lemons", as a reviewer of one of our grants recently noted.

(A) The "Lock and Key" Paradigm. In the studies described above, our group and others used one unifying design theme—the "lock and key" analogy. Over 100 years ago, Emil Fischer proposed this analogy as a means of understanding the binding of natural receptors.[60] The receptor is the lock and the analyte is the key. Pairwise interactions are created between the receptor and analyte using the standard molecular recognition binding forces: hydrogen bonding, ion pairing, dipole alignment, solvophobic interactions, etc. The notion leads to the concept of one key that is complementary to each lock, thereby opening up only one lock. However, as now described, this is not the approach used by the senses of taste and smell.

(B) The History of the U.T. Taste Chip. When having lunch in 1995 with my friend and colleague John T. McDevitt, a professor of analytical and inorganic chemistry in our department, he told me about the field of electronic noses. Electronic noses are devices used by analytical chemists to study multiple analytes in the vapor phase. John was interested in creating an "electronic tongue", which was a term he used to describe the potential analysis of multiple analytes in solution-phase rather than in the gas phase. His idea was to involve another colleague, Dean Neikirk, a professor of electrical and computer engineering, to create silicon wafers to immobilize receptors that could be simultaneously analyzed with a charge-coupled device (CCD) to create color or emission changes at each receptor spot. Together we devised the idea of using beads to immobilize the receptors, and in consultation with Dean Neikirk, we devised the idea of using etched pits to hold the beads.[61] A few months later, Dr. Jason Shear joined our team to perform enzyme assays in the array. In this manner, one of the very first electronic tongues was born, which became known as the U.T. Electronic Tongue (now called the U.T. Taste Chip). The device attracted considerable attention from the popular press, being highlighted on CNN, ABC Nightly News, and the BBC. John McDevitt has taken the collaboration in the direction of using antibodies on the beads for detecting various antigens and interfacing the bead array with a membrane for detecting bacteria and viruses.[62] Our group has taken the collaboration in the direction of using arrays of synthetic receptors in tandem to give fingerprints that can be used to interrogate complex mixtures.

The U.T. Taste Chip is another in the myriad of arrays involved in genomics, proteomics, and glycomics.[63] Our array uses 200 μm beads in a 10 × 10 or smaller array of pits that is about the size of a dime (Figure 2). Arrays created by other researchers use automated spotting techniques or microlithog-

raphy. Further, arrays are becoming smaller and smaller; even arrays of single molecules are being contemplated.[64]

Hence, after nearly a decade, we should reflect: "What is the strength and uniqueness of the U.T. Taste Chip?" In our group's opinion there are three essential aspects of the U.T. Taste Chip: (1) It is simple to create and place the beads into. (2) It is mesoscopic in size and therefore easily integrated with standard HPLC machines and optical microscopes. (3) The solution passes through the beads. For example, one simply drops the beads into known positions on the chip. This means that the bead identity does not have to be coded as in other systems.[65] This is made possible by the fact that the array is the size of millimeters. The device can be easily manipulated by hand, and the naked eye can see the beads. Each bead is large enough to have enough path length that averaging over a series of beads is not needed, as with other bead-based arrays.[65] Most important, the solution of analytes passes through the beads and can be cycled hundreds of times. This allows the beads to act as a sponge and soak up analyte during each pass, making the device potentially the most sensitive array yet produced.[66] In all other array devices, the spots are on a surface and the analytes become bound via passive diffusion rather than due to active flow of multiple passes as with the U.T. Taste Chip.

The idea of electronic noses and tongues dramatically affected the manner in which our group now approaches sensing protocols, not only because of our continued use of the U.T. Electronic Taste Chip, but because it inspired our research group to delve into the mechanisms used by the mammalian senses of taste and smell.

(C) The Senses of Taste and Smell. Mammals have five senses: vision, touch, hearing, taste, and smell. The latter two are targeted to the detection of chemicals. Hence, in the spirit of biomimetic chemistry, supramolecular chemists should take their inspiration for creating chemical sensors by analyzing the senses of taste and smell. Children learn at a very early age that the senses of taste are limited to sweet, sour, salty, and bitter. It is now recognized that a fifth taste exists called umamé—a savory or delicious sensation—and that the original four senses of taste have multiple aspects to them.[67] However, it is still true that there are a limited number of taste sensations that lead to our ability to decipher and recognize thousands of flavors. How is this possible? First, one must recognize that the sense of taste is highly dependent upon the sense of smell.

The human sense of smell is made up of around 1000 different protein receptors that are cross reactive, while the sense of smell in dogs involves nearly a million different receptors.[68] Except for pheromones, these receptors are not very specific for individual molecules, but rather are targeted to a class of molecules and are cross reactive within that class. For example, nasal receptors for aldehydes from rats have been characterized and were found to bind a variety of different aldehydes.[69] The composite response of all the receptors to the multiple different analytes in a mixture creates a pattern that the mind records and recalls as a particular odor or flavor. If we limit our consideration of the sense of taste to sweet, sour, salty, and bitter, then the concept is clear. Our sweet receptors are biased to polyhydroxylated structures, while the bitter receptors are biased to hydrophobic heterocycles. The magnitude of response of each of the taste sensations is the pattern for a particular flavor. We can imagine bar graphs for each of the flavors giving a fingerprint of the solution. Of course, as alluded to above, the sense of taste is clearly more complex than this, but a simple bar graph notion goes a long way in depicting the mechanism of taste.

(D) Electronic Tongue and Nose Inspirations. Persaud can be considered the father of electronic nose technology.[70] His work, as well as the vast majority of the electronic nose technology,[71] utilizes an array of different materials that undergo modulations of their properties upon exposure to a vapor containing a mixture of entities. Simply by virtue of the fact that the materials are different, the response of each changes differently. A Birmingham-based company called Aromascan and a California-based company called Cyrano market such devices.[72] Interestingly, most electronic noses involve little to no thought given to molecular recognition or supramolecular chemistry.[72]

In solution analyses, when interacting with biological structures such as proteins, peptides, and carbohydrates in the biological milieu, we believe that design imparted to the receptors using molecular recognition principles will be advantageous. After all, our senses of taste and smell are indeed "designed", meaning they have evolved to have a bias toward the class of structures that the animals are going to encounter. Molecular recognition is important to the senses of taste and smell because it allows animals to have a limited number of taste and smell receptors targeted to analyte classes, rather than needing a nearly limitless number of receptors to cover the diversity of tastants and odorants that an animal is likely to encounter throughout its life span.

One of the pioneers that inspired our own work is that of Dr. Toko at the University of Kyushu.[73] His group studies electrochemical sensors in small array formats. He uses semipermeable membranes to analyze entities that give a sweet, sour, salty, and bitter human response. Using membranes on electrodes responsive to only these four sensations he can create patterns that correctly predict the response of human test panels to the taste of various beverages. This remarkable work goes a long way to verify the conclusion that only sweet, sour, salty, and bitter are our primary tastes. Hence, imparting a bias of the receptors to the analytes of interest created a powerful mimic of the human taste response.

Two other pioneers in the mimicry of taste and smell are Drs. Ken Suslick and David Walt at the University of Illinois and Tufts University, respectively. Suslick published a significant advance in the field in 2000 in *Nature*.[74] Using a series of porphyrins (**23**), he could fingerprint various vapors for small organics. This work showed that the use of a series of receptors that are known to have affinities to various gases could fingerprint mixtures of those gases. The molecular recognition derives from known ligation events at the porphyrin metal centers.

R =

$M = Sn^{4+}, Co^{3+}, Cr^{3+},$
$Mn^{3+}, Fe^{3+}, Co^{2+},$
$Cu^{2+}, Ru^{2+}, Zn^{2+},$
$Ag^{2+}, 2H^+$

23
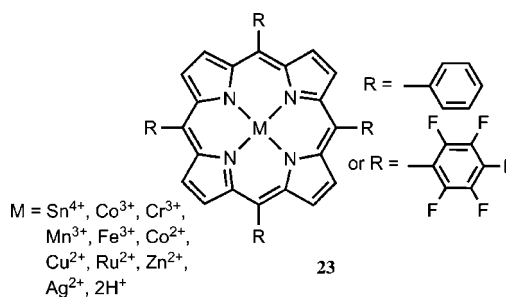
Dr. Walt is a pioneer in device creation. In the early 1990s, he published the use of polymer deposits on the tips of optical fibers for creating patterns that could analyze gases.[75] He deposited polymers containing enzymes, antibodies, and other natural receptors on the optical fibers. These devices rely upon selective molecular recognition responses. In truly brilliant fashion, Dr. Walt has also created self-assembled bead-based arrays on the tips of optical fibers, which are the basis of a company called Illumina involved in genomic analysis.[76] The beads are only 5 μm in size and self-assemble themselves into divots on the optical fibers. Importantly, the DNA interactions are highly selective, accentuating the necessity of having highly specific molecular recognition events in certain applications. In genomics, even a single base mismatch cannot be tolerated.

(E) The Definition of Differential Receptors. As just discussed, the first electronic noses involved the use of materials as receptors with no thought given to their molecular recognition properties. Instead, the important aspect is that the materials are different and cross-reactive. Yet, one of the most modern array devices is for genomic analysis using an optical fiber where the specificity of binding is of paramount importance. In 1999, our group coined the term "differential" to accentuate the unifying aspect needed in any array.[32] Simply stated, the individual receptors all interact differently. Of course, this is true of an array of DNA strands or antibodies, in that each is highly selective and different. Protein and gene chips therefore use differential receptors. However, differential also includes receptors that are cross-reactive. Each receptor may bind a number of analytes, but each receptor binds the analytes differently than every other receptor. In this case, the signals of all the receptors are interpreted by a pattern recognition protocol, and the result is a fingerprint.

Our group predicts that the power of an array of synthetic receptors, when coupled with pattern recognition protocols, will be unsurmounted for multianalyte sensors. This is because of the inherent lack of specificity possessed by synthetic receptors. They are naturally cross-reactive, which is the exact attribute desired in many array settings.[77] As stated, within such an array, cross-reactivity is desired, because one wants the receptors to interact with multiple analytes, with natural interferants, and even with analytes whose structures are unknown. The composite pattern of the targeted analytes, the interferants, and the unknown structures, creates the desired fingerprint of the complex solution. These attributes are inherent to synthetic receptors and are not easily achieved with antibodies and aptamers. The use of synthetic receptors in an array format is a research area that we predict will become increasingly important in the coming years within the molecular recognition and supramolecular community.

Importantly, the use of combinatorial chemistry in the creation of synthetic receptors compliments the requirement of differential binding and cross reactivity. In a scenario where the solution contains chemicals whose structures are not known, or genetic differences lead to slightly different structural variations that cannot be predicted, only a combinatorial approach seems feasible. This is the power that we envision synthetic receptors imparting to differential sensing schemes.

(F) The Necessity of Pattern Recognition. If supramolecular chemists are to achieve the dream of using synthetic receptors for analytical chemistry purposes in differential sensing schemes, they must become familiar with pattern recognition protocols. Pattern recognition is a standard talent for analytical chemists,

and the field of chemometrics covers all the various mathematical protocols used in analytical chemistry to extract patterns from data.[78] These methods are approachable by anyone willing to immerse themselves in the various mathematical methods and approaches; knowledge of linear algebra is particularly useful.

There are three methods that our group routinely uses, as do others that are now also working in this field. The most commonly used method is Principle Component Analysis (PCA).[79] This is a method that uses linear algebra to find orthogonal axes that express variance in the data. The mathematical protocol is an Eigenvector problem, and hence, the math is analogous to that used to solve for molecular orbitals under the assumption of the variational principle. The first principle component expresses the most variance in the data, meaning that this axis shows a plot of the greatest extent to which the data collected for the unknown samples differ from one another. The second principle component expresses the second greatest extent of variance, and so on and so forth. We use this method when attempting to qualitatively differentiate one sample from another, with several examples shown below.

Another common method is Hierarchical Cluster Theory (HCT).[80a] This method groups unknowns to visualize similarities between the unknowns. HCT was used by Suslick to distinguish various functional groups from one another in solution, and then within individual classes of functional groups to classify the unknowns as aliphatic or aromatic.[80b] The study used porphyrins **23**, as well as a solvatochromic dye **24** and a pH indicator **25**.

24

25
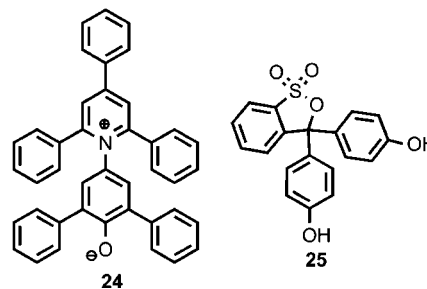
The other method that we routinely use is Artificial Neural Networks (ANN).[81] Whitesides reviewed the use of these methods in chemistry several years ago.[82] The method is the most complex and sophisticated of the techniques. We use the method when quantitative analysis is desired. In this technique, weights and nonlinear functions are applied to each set of training data to correlate their values to the output. The weighted outputs of each function derived from the training set data are combined to calculate a value for what is called a hidden layer (Figure 3). The number of hidden layers can be set by the operator or determined by the program. Subsequently, the values of the hidden layers are used in other nonlinear functions with new applied weights. The outputs from these hidden layers are combined to predict the desired values for the unknowns. ANNs can be both overtrained and undertrained with data, meaning that predictions for the values of the unknowns can be inaccurate due to a lack of proper training data or inaccurate due to too much data that leads to redundancies. While our group uses this method with caution, we have had considerable success, with amazing accuracy, in our sensing methods.

(G) Other Research Groups Combining Supramolecular Chemistry with Pattern Recognition. Before reviewing the studies from our group that combine supramolecular chemistry
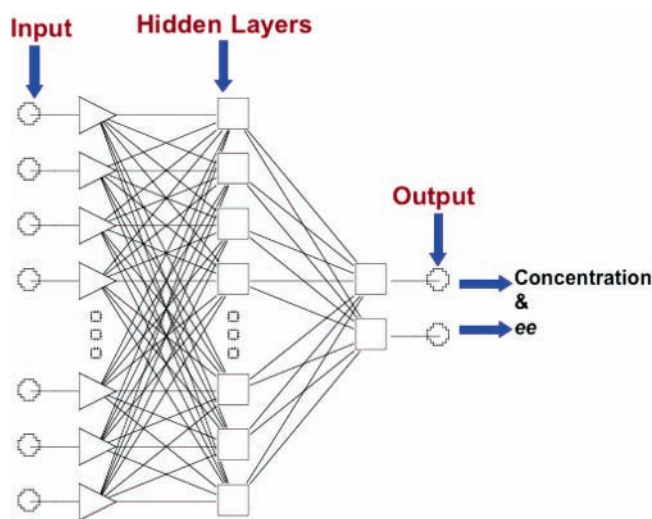
FIGURE 3. Schematic representation of the structure of an ANN, which shows input, hidden, and output layers. We routinely use such methods for concentration and ee determination.
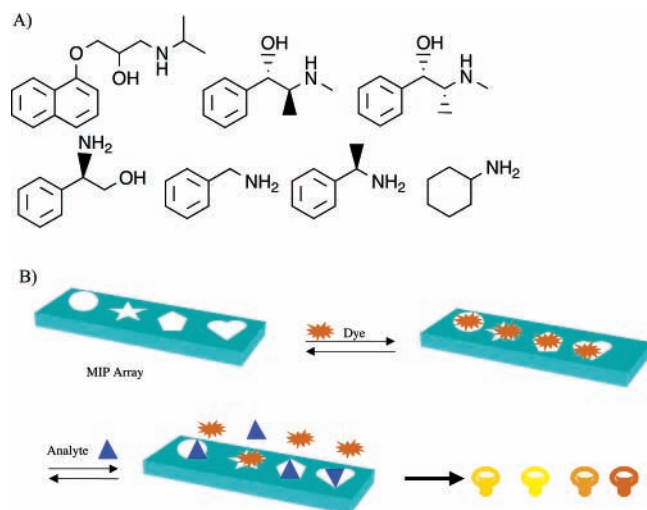

FIGURE 4. (A) Amines that Shimizu could fingerprint. (B) An array of MIPs with IDAs gives a patter.

with pattern recognition, it is important to discuss the work of others that have also ventured into this field, and that continue to influence our own work. Some of the most inspiring work comes from the Ken Shimizu group.[83] Shimizu has used molecularly imprinted polymers (MIPs) to differentiate between complex amines (Figure 4) and has applied the indicator-displacement assay concept for signaling. The work is particularly novel because the creation of MIPs is facile and could be automated, leading to the rapid creation of multiple differential sensing arrays for many complex mixtures. In our estimation, the Shimizu group has one of the most powerful approaches now under development.

Another researcher in this area making novel contributions is Kay Severin. Our group has historically used inorganic coordination complexes in sensing schemes with indicator-displacement assays,[84] but Severin was the first to use organo-metallic complexes.[85] By using a single organometallic complex **26**, a single indicator in an IDA, and a variety of pHs to create an array in a micro-titer plate, he was able to distinguish the 20 natural amino acids using PCA.
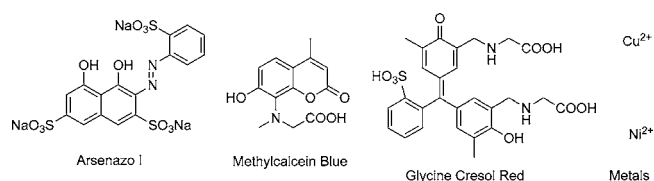

FIGURE 5. Indicators and metals used by Severin to fingerprint amino acids.

In an important advance, in 2005, Severin demonstrated the use of three indicators with two metals for distinguishing amino acids via PCA (Figure 5).[86] He applied the term "dynamic combinatorial library" to the exchange of more than one indicator with more than one host, in an analogous fashion to our 1999 study for simultaneous tartrate/malate detection (see below). Although four to five components constitute a small library, the application of this terminology is an important conceptual advance on Severin's part. It ties together the field of dynamic combinatorial libraries with the newly emerging field of supramolecular analytical chemistry. This tie should have immediate consequences because other research groups will be encouraged to exploit their own dynamic combinatorial libraries for sensing purposes in differential formats.
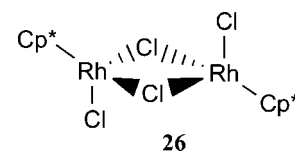
(H) Using IDAs in Differential Sensing Methods. Note that an IDA was used by both Shimizu and Severin in their differential sensing protocols and in our own studies described below. An IDA is a perfect match for differential sensing because it is modular. One of the advantages of an IDA is the ability to change the indicator to change the colors observed and to change the affinity of the indicator relative to the analyte. The indicator becomes a variable that adds diversity to the array. When examining solutions with analytes of different affinities to the receptors, the use of several indicators is advantageous because the affinities of the indicators vary. The use of several receptor/indicator ensembles further leads to cross-reactivity in the array. This is a strength of paramount importance for IDAs in differential sensing schemes.

(I) A Simple Venture into "Tasting" with a Synthetic Receptor: Scotch. Just as our sense of sweet responds to many polyhydroxylated structures and our sense of bitter responds to many hydrophobic heterocyclic structures, one of our earliest
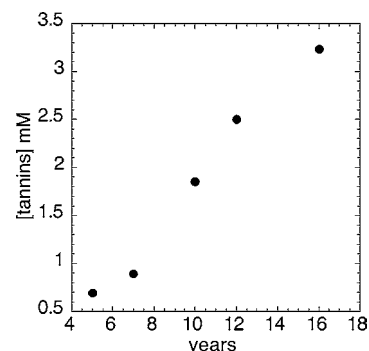

FIGURE 6. Extent of color change ([tannic acids]) as a function of the age of whiskey.

ventures into mimicking these senses was to study a single receptor that had nearly equal response to many compounds of the same class. This is a case where we wanted the receptor to be selective only for a class of compounds, but otherwise be nonselective.

The study we undertook was of scotch whiskey.[87] During the aging of whiskey, the tannins in the oak barrels gradually hydrolyze, releasing tannic acids into the beverage.[88] There are dozens of different tannic acids, whose structures depend upon the oak used. The unifying structural features of these compounds are catechols and a carboxylic acid. To target this class of structures we designed host 27, possessing two boronic acids and a single guanidinium. We tested four tannic acids using an IDA involving pyrocatechol violet (PV), and indeed, the assay was nearly equally responsive to all four tannic acids. Next, five scotch whiskeys were tested, and we found a nearly linear correlation between the tannic acid level, as indicated by the extent of color change in our IDA, with the age of the whiskey (Figure 6). If we examined individual tannic acids, no such correlation was evident. This was an early example of the use of a nonselective receptor, but one that was biased to a class of structures.

27   **28**
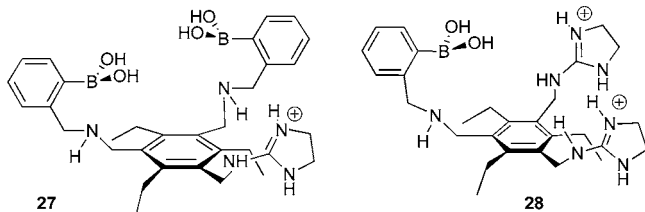
(J) Tartrate and Malate Analysis. In 1999, we had published the use of receptor 28 for quantitating tartrate in wines.[89] Receptor 28 had been designed considering the lock and key principle and in an attempt to create a structure that was highly selective for tartrate. Our original design pictured tartrate binding in the manner shown in structure 29, but after extensive thermodynamic analysis of the preferences boronic acids show toward various functional groups,[90] and our study into the B−N bond,[18] we have concluded that the dominant complex in solution is 30. This leaves one hydroxyl group dangling. Hence, at the time of the original study, we had discovered that 28 binds malate with an affinity similar to that of tartrate, and the reason is now clear. We realized that the lack of selectivity between tartrate and malate could be exploited when receptor 28 is used as a differential receptor.


29   **30**
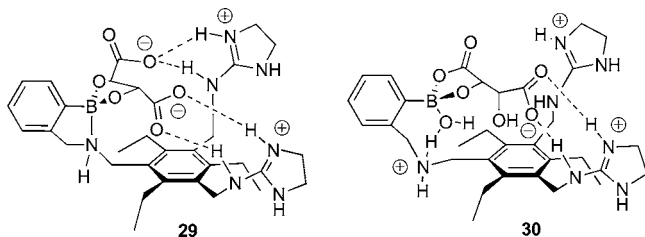
Using a dynamic combinatorial library (the term applied by Severin for a mixture of hosts and indicators), we used two hosts (27 and 28) and two indicators in a single cuvette.[91a] This four-component ensemble gave large UV/vis spectral changes across approximately 300 nms when mixtures of tartrate and malate were added. A series of UV/vis spectra as a function of varying concentrations of both tartrate and malate in mixtures

were used as training set data for an artificial neural network (ANN). We then challenged the ANN to report the concentrations of unknowns that were not part of the original training set, and an amazing accuracy of 2% error was the result. Hence, by recording a single UV/vis spectra of the four-component ensemble after addition of an unknown mixture of only tartrate and malate, the ANN accurately reported their concentrations. This study was one of the first to highlight the power of cross-reactive receptors for solution analysis and concentration determination.

Interestingly, most wines have very little malate. Malate is often removed in a secondary fermentation process because of the fruity sensation it imparts to wines. Hence, our original assay using just receptor 28 did indeed measure primarily tartrate in the wines we studied. However, when we added malate to wine and attempted to use the aforementioned four-component ensemble with the trained ANN, the concentrations we found for malate and tartrate were always too high. We reasoned that there must be other structures in the wine that were also giving a response and therefore adding to the concentrations found. Rather than optimize the wine analysis, we turned to another beverage to expand our techniques: flavored vodkas.

(K) Citrate and Ca(II) in Flavored Vodkas. As discussed earlier, our very first sensor using an IDA was for citrate in soda pops. More recently, we have used host 14 with a cross-reactive indicator xylenol orange (XO) to quantitate both citrate and Ca(II).[91b] In this case, it is the indicator that has differential binding properties. This indicator binds host 14 in both 1:1 and 2:1 stoichiometries, as well as Ca(II) in these stoichiometries. This binding leads to color changes. Further, the indicator is displaced from the host by citrate, and citrate binds Ca(II) releasing the Ca(II) from the indicator. Taken together, there are multiple equilibria established simultaneously in the reaction vessel. When we record UV/vis spectra of XO at varying concentrations and ratios of citrate and Ca(II), and then used these spectra as training sets for an ANN, we were able to quantitate citrate and Ca(II) in five flavored vodkas. Only in the case of green apple vodka did the method fail to accurately report the citrate/Ca(II) concentrations.

XO
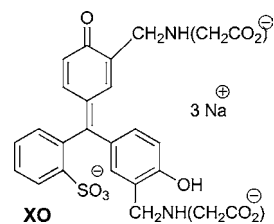
The assay only worked in flavored vodka if unflavored vodka was used as the medium for generating the training set. In other words, when we doped unflavored vodka with citrate and Ca-(II) and then generated the training set data the assay was successful with the flavored vodkas. If we used pure solutions of citrate and Ca(II) as the training sets as we had done for tartrate and malate, then the ANN procedure was not accurate. This shows the necessity of using the correct matrix for collecting the training set data.

(L) Nucleoside Triphosphates. It has already been discussed that by screening combinatorial library 22 we were able to discover that Ser-Tyr-Ser is a selective bead-based receptor for ATP. Although the study of 22 was successful, the screening approach was tedious. Hence, we sought to achieve a different

goal using combinatorial libraries of receptors. Our group postulated that it would be quicker, and just as selective, to create patterns from random members of library **22** for distinguishing nucleoside triphosphates.

To test this concept, we randomly chose 30 beads of library **22** and placed them into the U.T. Taste Chip system.[92] The beads were loaded with fluorescein, and an IDA was used to create patterns. The beads start yellow and go clear upon addition of the triphosphate. The kinetics of the IDA were different for ATP, GTP, and AMP, leading to clear differentiation of these species using PCA.

To some level we are doing differential anion-exchange chromatography. Each bead is its own different anion-exchange resin, and it is known that displacement of anions from different resins occurs with different rates and thermodynamics.[93] Hence, this study used differential anion-exchange resins to create the patterns, where each bead is a different exchange resin. This analogy is only correct to a first approximation because not all anions displace the fluorescein from the beads. Hence, there are also selective molecular recognition events involved.

Interestingly, only six of the 30 beads were important for generating the PCA pattern that differentiated the nucleoside triphosphates. In hindsight, this makes sense. Not all library members of **22** are likely to bind ATP and GTP differently, and in this case, only six of the 30 beads had this property. The sequences of the six beads were also interesting. The amino acids Ser and Thr were present in four of the six, and sometimes were present twice. Position 2 of the tripeptide was predominately hydrophobic. This is consistent with the finding that Ser-Tyr-Ser in the context of **22** was an excellent ATP host. However, although the sequences are homologous, they are not very informative. We still do not understand why these amino acids lead to a differentiation of nucleoside triphosphates.

(M) Patterns for Proteins. If we can use patterns from random beads to distinguish nucleoside triphosphates, we wondered how complex the analytes could be? To answer this question, we turned our attention to proteins. Hamilton had already shown that libraries of porphyrin derivatives gave different patterns with proteins[94] but had not used PCA.[95] The simplest question we posed was whether glycoproteins could be differentiated from normal proteins using patterns. To probe this issue, we designed library **31** possessing boronic acids and random peptides.[96] The boronic acids were used to bind the carbohydrate portions of the glycoproteins. To visualize the patterns we turned to an indicator uptake assay, not an IDA. After exposing the beads to the proteins, a small aliquot of a staining agent was added (BPR). The beads took up the stain in inverse relationship to the amount of protein they had taken up. The kinetics of stain uptake led to patterns in PCA along axes 1 and 2 that easily grouped the glycoproteins from the normal proteins; however, considerable overlap existed between the individual sets. Yet, upon analysis of the third principle component the normal proteins were separated, while the glycoproteins still overlapped. Irrespective of the mixed success from this study, it did show that a series of random structures with bias toward the analytes of interest could lead to pattern recognition-derived differentiation of proteins.

(N) Patterns for Peptides. After having success with proteins, our group felt that such structures are not really that challenging for pattern recognition derived differentiation. Proteins present large surface areas for binding. They present mixtures of hydrophobic and highly charged patches, often


BPR (bromopyrogallol red)
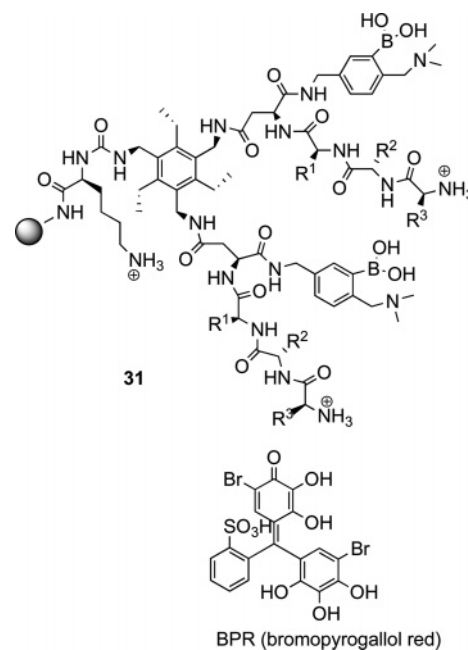
referred to as hot spots.[97] It should not be too surprising that random structures created from charges and hydrophobic groups will find niches and crevices for differential interactions.

Hence, once again, we decided to challenge our methods by considering what would be an even more difficult set of analytes. Short peptides were our choice because they do not have large areas for finding complementary surfaces to the receptor library members nor are they preorganized into set globular structures.

The tripeptides we targeted possess histidine so that we could bias our library to this class of peptides. Based upon previous studies from our group, we knew that a terpyridine-type ligand in a Cu(II) complex had high affinity for histidine.[98] We had also previously reported that compound **32** had reasonable, but not outstanding, selectivity for binding the tripeptide His-Lys-Lys.[99] We expanded upon this motif by creating library **33**. Using 30 random members of this bead-based library in the U.T. Taste Chip along with a staining assay for visualization and PCA for pattern creation, we were able to successfully group the four different tripeptides as well as binary mixtures of these tripeptides. Therefore, even peptides in water can be differentiated with a series of cross-reactive receptors.[100]

Outlook to the Future

The focus of this perspective was on the power of supramolecular chemistry in creating single analyte sensors and differential receptor arrays. Synthetic receptors can now be developed for single analytes using design and synthesis that yield remarkable selectivity, and thereby have considerable practical use and market potential. However, the very active field of creating and exploiting electronic noses and tongues highlights another utility for synthetic receptors, where the mammalian chemical sensing systems are being mimicked. By virtue of their simplicity, synthetic receptors are naturally not as selective as enzymes and antibodies, and therefore possess a degree of cross-reactivity. In other words, they are perfect for use in differential sensing scheme. Researchers around the world are now realizing this power of synthetic systems. As recently demonstrated, even analytes as complex as peptides and proteins can be distinguished using the differential sensing paradigm.

32

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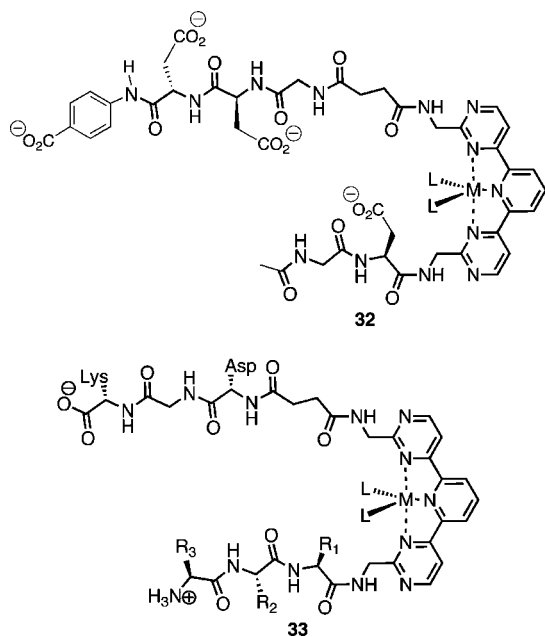
The future of using synthetic receptors as sensors is in the interrogation of complex mixtures of bioanalytes (blood, urine, saliva), cellular imaging, environmental analytes (streams, industrial wastes), and quality control (manufacturing, process). Essentially any solution whose contents need to be tested or monitored for variations represents a possible application of differential arrays of synthetic receptors. The wide versatility and chemical diversity possible in synthetic receptors is far beyond that of peptides, nucleotides, and oligosaccharides, Therefore, the ingenuity of the chemist is the only limitation. This opens up completely new molecular designs and approaches for the use of synthetic receptors in analytical sciences, giving supramolecular chemists new and fertile ground to explore that has truly practical and useful ramifications. Supramolecular analytical chemistry is destined for a bright future.

References

(1) Lehn, J.-M. *Supramolecular Chemistry*; VCH: New York, 1995.
(2) *Random House Webster's Unabridged Dictionary*, 2nd ed.; Random House: New York, 1998.
(3) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423−428.
(4) Goral, V.; Nelen, M. I.; Eliseev, A. V.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1347−1352. Sijbren, O.; Ricardo, L. E.; Sanders, J. K. M. *Drug Dis. Today* **2002**, *7*, 117−125. Polyakov, V. A.; Nelen, M. I.; Nazarpack-Kandlousy, N.; Ryabov, A. D.; Eliseev, A. V. *J. Phys. Org. Chem.* **1999**, *12*, 357−363.
(5) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2000**, *122* (48), 12063−12064. West, K. R.; Bake, K. D.; Otto, S. *Org. Lett.* **2005**, *7* (13), 2615−2618.
(6) Boul, P. J.; Reutenauer, P.; Lehn, J.-M. *Org. Lett.* **2005**, *7* (1), 15−18.
(7) Schrader, T.; Hamilton, A. *Functional Synthetic Receptors*; John Wiley & Sons: New York, 2005.
(8) Madsen-Rancke, E. In *Indicators*; Bishop, E., Ed.; International Series of Monographs In Analytical Chemistry; Pergamon Press: Oxford, UK, 1972; Vol. 51, pp 1−12. Boltz von Rufach, V. *Illuminirbuch künstlich alle Farben zu machen und bereyten* (1566). Citations in this chapter from the Danish translation, "En Ny oc Illuminer-Bog", Kiøbenhaffn, 1648. Boyle, R. *Experimental History of Colours*; London, 1664; quoted in *The Works of the Hounerable Robert Boyle*, London, 1744; Vol. II.
(9) Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004**, *104*, 2723−2750.
(10) (a) Cram, D. J.; Carmack, R. A.; Helgeson, R. C. *J. Am. Chem. Soc.* **1988**, *110*, 571−577. (b) Molecular Probes catalog no. C36676.
(11) Dyatlova, N. M.; Temikina, V. Y.; Popov, K. I. Present-day Aspects of Complexone Coordination Chemistry. In *Complex Formation and Stereochemistry of Coordination Compounds*; Buslaev, Y., Ed.; Nova Science: Hauppauge, NY, 1996; pp 1−48.
(12) de Silva, A. P.; McCaughan, B.; McKinney, B. O. F.; Querol, M. *Dalton Trans.* **2003**, *10*, 1902−1913.
(13) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97* (5), 1515−1566.
(14) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397−9398.
(15) de Silva, A.P.; Gunaratne, H. Q. N.; Gunnalugsson, T.; Nieuwenhuizen, M. *Chem. Commun.* **1996**, 1967−1968.
(16) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159−200.
(17) Ni, W.; Kaur, G.; Springsteen, G.; Wang, B.; Franzen, S. *Bioorg. Chem.* **2004**, *32*, 571−581.
(18) Zhu, L.; Shabbir, S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128*, 1222−1232.
(19) Wright, A.; Zhong, Z.; Anslyn, E. V. *Angew. Chem.* **2005**, *44*, 5679−5682.
(20) Grynkiewcz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260* (6), 3440−3450.
(21) (a) Bornhop, D. J.; Licha, K. *Biomedical Photonics Handbook*; CRC Press LLC: Boca Raton, FL, 2003. (b) Yang, L.; McRae, R.; Henary, M. M.; Patel, R.; Lai, B.; Stefan, V.; Fahrni, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11179−11180. (c) Ueno, T.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 10640−10641. (d) Wang, S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2006**, *128*, 10380−10381.
(22) Mohr. G. J. *Chem. Eur. J.* **2004**, *10*, 1082−1090.
(23) Feuster, E. K.; Glass, T. E. *J. Am. Chem. Soc.* **2003**, *125* (52), 16174−16175.
(24) Wallace, K.; Anslyn, E. *Chem Commun.* **2006**, 3886−3887.
(25) Inouye, M.; Hashimoto, K.; Isagawa, K. *J. Am. Chem. Soc.* **1994**, *116*, 5517−5518. Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 755−758.
(26) Wiskur, S.; Ait-Haddou, H.; Lavigne, J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963−972.
(27) Szejtli, J. *Cyclodextrin Technology*; Kluver Academic Publishers: Dordrecht, Boston, 1988; 450 pp.
(28) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 862−865.
(29) Perreault, D. M.; Cabell, L. A.; Anslyn, E. V. *Bioorg. Med. Chem.* **1997**, *36* (5), 433−450.
(30) Piatek, A. M.; Bomble, Y. J.; Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 6072−6077.
(31) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem., Int. Ed.* **2002**, *41* (20), 3811−3814.
(32) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38* (24), 3666−3669.
(33) Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125* (14), 4026−4027. Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125* (48), 14807−14815.
(34) Niikura, K.; Anslyn, E. V. *J. Chem. Soc. Perkin Trans. 2: Phys. Org. Chem.* **1999**, *12*, 2769−2775. Niikura, K.; Anslyn, E. V. *J. Org. Chem.* **2003**, *68* (26), 10156−10157. Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120* (33), 8533−8534.
(35) Niikura, K.; Bisson, A. P.; Anslyn, E. V. *J. Chem. Soc., Perkins Trans. 2* **1999**, 111−1114.
(36) Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2002**, *124*, 9014−9015.
(37) Zhong, Z.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2003**, *42* (26), 3005−3008.
(38) Nguyen, B.; Anslyn, E. V. *Coor. Chem. Rev.* **2006**, *250*, 3118−3127.
(39) Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5* (12), 2029−2031.
(40) See ref 39.

(41) Zhang, T.; Anslyn, E. V. *Org. Lett.* **2006**, *8* (8), 1649−1652.

(42) Pereira, C. M.; Oliveira, J. M.; Silva, R. M.; Silva, F. *Anal. Chem.* **2004**, *76*, 5547−5551. Heller, A. *Annu. Rev. Biomed. Eng.* **1999**, *01*, 153−175.

(43) Melendez, R. E.; Carr, A. J.; Linton, B. R.; Hamilton, A. D. *Struct. Bonding (Berlin)* **2000**, *96*, 31−61.

(44) Kucharsky, J.; Safarik, L. *Titrations in Non-Aqueous Solvents*; Elsevier: New York, 1965.

(45) Li, Z.; Lin, J.; Pu, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1690−1693. Pu, L. *Chem. Rev.* **2004**, *104*, 1687−1716.

(46) Kubo, Y.; Maeda, S.; Tokita, S.; Kubo, M. *Nature* **1996**, *382*, 522−524.

(47) Kim, J.; Kim, S.-G.; Seong, H. R.; Ahn, K. H. *J. Org. Chem.* **2005**, *70*, 7227−7231.

(48) Korbel. G. A.; Lalic, G.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 361−362.

(49) Zhu, L.; Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127* (12), 4260−4269. Zhu, L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126* (12), 3676−3677.

(50) Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127* (22), 7986−7987. Folmer-Andersen, J. F.; Kitamura, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128* (17), 5652−5653.

(51) Gennari, C.; Piarulli, U. *Chem. Rev.* **2003**, *103* (8), 3071−3100. Reetz, M. T. *Angew. Chem., Int. Ed.* **2002**, *41* (8), 1335−1338. Reetz, M. T. *Angew. Chem., Int. Ed.* **2001**, *40* (2), 284−310. Reetz, M. T.; Jaeger, K. E. *Chemistry* **2000**, *6* (3), 407−412.

(52) Jayasena, S. P. *Clin. Chem.* **1999**, *45*, 1628−1680. Bunka, D. H. J.; Stockley, P. G. *Nature Rev. Microbiol.* **2006**, *4*, 588−596. Lee, J. F.; Stovall, G. M.; Ellington, A. P. *Curr. Opin. Chem. Biol.* **2006**, *10*, 282−289.

(53) Sidhu, S. S. *Curr. Opin. Biotechnol.* **2000**, *11* (6), 610−616.

(54) Chen, C.-T.; Wagner, H.; Still W. C. *Science* **1998**, *279*, 851−853. Ryan, K.; Still, W. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2673−2678. Iorio, E. J.; Shao, Y.; Chen, C.-T.; Wagner, H.; Still, W. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1635−1638.

(55) Monnee, M. C. F.; Brouwer, A. J.; Liskamp, R. M. J. *QSAR Combin. Sci.* **2004**, *23* (7), 546−559.

(56) Shepherd, J.; Gale, T.; Jensen, K. B.; Kilburn, J. D. *Chem. Eur. J.* **2006**, *12*, 713−720.

(57) Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122* (3), 542−543.

(58) Hennrich, G.; Lynch, V. M.; Anslyn, E. V. *Chem. Eur. J.* **2002**, *8* (10), 2274−2278.

(59) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118−3130.

(60) Behr, J.-P., Ed. *Perspectives In Supramolecular Chemistry 1*; John Wiley & Sons: Chichester, 1994.

(61) Lavigne, J. J.; Savoy, S.; Clevenger, M. B.; Ritchie, J. E.; McDoniel, B.; Yoo, S.-J.; Anslyn, E. V.; McDevitt, J. T.; Shear, J. B.; Neikirk, D. *J. Am. Chem. Soc.* **1998**, *120*, 6429−6430.

(62) Goodey, A.; Lavigne, J. J.; Savoy, S. M.; Rodriguez, M. D.; Curey, T.; Tsao, A.; Simmons, G.; Wright, J.; Yoo, S.-J.; Sohn, Y.; Anslyn, E. V.; Shear, J. B.; Neikirk, D. P.; McDevitt, J. T. *J. Am. Chem. Soc.* **2001**, *123* (11), 2559−2570.

(63) Vo-Dinh, T.; Askari, M. *Curr. Genom.* **2001**, *2*, 399−415. Jelinek, R.; Kolusheva, S. *Chem. Rev.* **2004**, *104*, 5987−6015.

(64) Gu, J.; Yam, C. M.; Li, S.; Cai, C. *J. Am. Chem. Soc.* **2004**, *126*, 8098−8099.

(65) Monk, D. J.; Walt, D. R. *Anal. Bioanal. Chem.* **2004**, *378*, 931−945. Song, L.; Ahn, S.; Walt, D. R. *Anal. Chem.* **2006**, *78*, 1023−1033.

(66) Christodoulides, N.; Floriano, P. N.; Acosta, S. A.; Ballard, K. L. M.; Weigum, S. E.; Mohanty, S.; Dharshan, P.; Romanovicz, D.; McDevitt, J. T. *Clin. Chem.* **2005**, *51* (12), 2391−2395.

(67) Keverne, E. B. *Chem. Senses* **2002**, *27*, 159−190. Horio, T. *Chem. Senses* **2002**, *27*, 159−190. Taniguchi, M. *Chem. Senses* **2002**, *27*, 159−190. Dalton, P.; Doolittle, N.; Nagata, H.; Breslin, P. A. S. *Nat. Neurosci.* **2000**, *3* (5), 431−432.

(68) Olender, T.; Fuchs, T.; Linhart, C.; Shamir, R.; Adams, M.; Kalush, F.; Khen, M.; Lanceta, D. *Genomics* **2004**, *83*, 361−372.

(69) Firestein, S.; Given, P.; Paredes, D. *Chemistry of Taste: Mechanisms, Behaviors, and Mimics* **2003**, Chapter 9.

(70) Persaud, K.; Dodd, G. *Nature* **1982**, *299*, 352−355.

(71) Hudewenz, V. *Spec. Chem. Mag.* **2005**, *25* (1), 26−27. James, D.; Scott, Simon M.; Ali, Z.; O'Hare, W. T. *Microchim. Acta* **2005**, *149* (1−2), 1−17. Deisingh, A. K.; Stone, D. C.; Thompson, M. *Int. J. Food Sci. Tech.* **2004**, *39* (6), 587−604. Kohl, C.-D. *Nanoelectron. Inf. Technol.* **2003**, 851−864. Turner, A. P.; Magan, N. *Microbology* **2004**, *2* (2), 161−166.

(72) Quotium: http://www.quotium.com. Osmetech: http://www.osmetech-.plc.uk

(73) Toko, K. *Meas. Sci. Technol.* **1998**, *9*, 1919−1936.

(74) Rakow, N. A.; Suslick, K. S. *Nature* **2000**, *406*, 710−713.

(75) Ferguson, J. A.; Healey, B. G.; Bronk, K. S.; Barnard, S. M.; Walt, D. R. *Anal. Chim. Acta* **1997**, *340* (1−3), 123−131.

(76) http://www.illumina.com.

(77) Albert, K. J.; Lewis, N. S.; Schauer, C. L.; Sotzing, G. A.; Stitzel, S. E.; Vaid, T. P.; Walt, D. R. *Chem. Rev.* **2000**, *100* (7), 2595−2626.

(78) Jurs, P. C.; Bakken, G. A.; McClelland, H. E. *Chem. Rev.* **2000**, *100*, 2649−2678.

(79) Jolliffe, I. T. *Principal Component Analysis*, 2nd ed.; Springer-Verlag: New York, 2002.

(80) (a) Haswell, S. J. *Practical Guide to Chemometrics*; Dekker: New York, 1992. (b) Zhang, C.; Suslick, K. S. *J. Am. Chem. Soc.* **2005**, *127*, 11548−11549.

(81) Bishop, C. M. *Neural Networks for Pattern Recognition*; Oxford University Press: Oxford, 1995.

(82) Burns, J. A.; Whitesides, G. M. *Chem. Rev.* **1993**, *93*, 2583−2601.

(83) Greene, N. T.; Shimizu, K. D. *J. Am. Chem. Soc.* **2005**, *127* (15), 5695−5700. Greene, N. T.; Morgan, S. L.; Shimizu, K. D. *Chem. Commun.* **2004**, *10*, 1172−1173.

(84) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123* (45), 11296−11297.

(85) Buryak, A.; Severin, K. *J. Am. Chem. Soc.* **2005**, *127* (11), 3700−3701. Buryak, A.; Severin, K. *Angew. Chem., Int. Ed.* **2004**, *43* (36), 4771−4774.

(86) Buryak, A.; Severin, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7935−7938.

(87) Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123* (41), 10109−10110.

(88) Nishimura, K.; Matsuayama, R. *Science and Technology of Whiskies*; E. Longman Scientific and Technical: Essex, U.K., 1989; p 235.

(89) Lavigne, J. T.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666−3669.

(90) Wiskur, S.; Lavigne, J. T.; Metzger, A.; Tobey, S.; Lynch, V.; Anslyn, E. V. *Chem. Eur. J.* **2004**, *16*, 521−528.

(91) (a) Wiskur, S.; Floriano, P.; Anslyn, E. V.; McDevitt, J. T. *Angew. Chem., Int. Ed.* **2003**, *18*, 2070−2072. (b) McCleskey, S. C.; Floriano, P. N.; Wiskur, S. L.; Anslyn, E. V.; McDevitt, J. T. *Tetrahedron* **2003**, *59*, 10089−10092.

(92) McCleskey, S. C.; Griffin, M. J.; Schneider, S. E.; McDevitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125* (5), 1114−1115.

(93) Janos, P. *J. Chromatogr. A* **1997**, *789* (1+2), 3−19. Heeter, G. A.; Liapis, A. I. *J. Chromatogr., A* **1996**, *743* (1), 3−14.

(94) Baldini, L.; Wilson, A. J.; Hong, J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126* (18), 5656−5657.

(95) Zhou, H.; Baldini, L.; Hong, J.; Wilson, A. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2006**, *128* (7), 2421−2425.

(96) Wright, A. T.; Griffin, M. J.; Zhong, Z.; McCleskey, S. C.; Anslyn, E. V.; McDevitt, J. T. *Angew. Chem., Int. Ed.* **2005**, *44* (39), 6375−6378.

(97) Ciulli, A.; Williams, G.; Smith, A. G.; Blundell, T.; Abell, C. *J. Med. Chem.* **2006**, *49*, 4992−5000.

(98) Folmer-Andersen, J. F.; Ait-Haddou, H.; Lynch, V. M.; Anslyn, E. V. *Inorg. Chem.* **2003**, *42*, 8674−8681.

(99) Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6* (9), 1341−1344.

(100) Wright, A. T.; Anslyn, E. V.; McDevitt, J. T. *J. Am. Chem. Soc.* **2005**, *127* (49), 17405−17411.