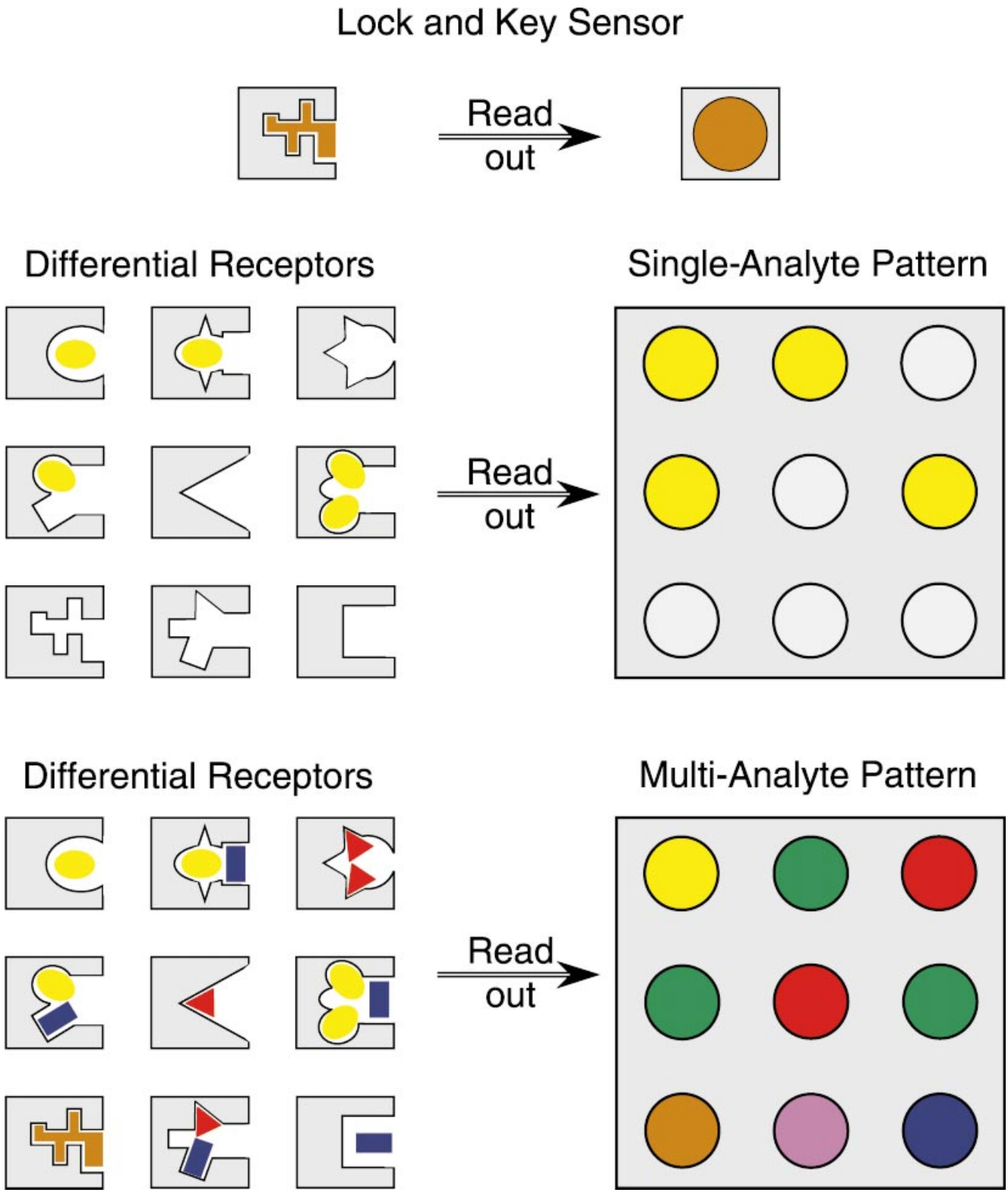


Sensing A Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors

John J. Lavigne and Eric V. Anslyn*

Molecular recognition has evolved from a science designed to understand biological systems into a much more diverse area of research. While work continues to elucidate "nature's tricks" with respect to intermolecular interactions, much attention has turned to the perspective that molecular recognition, by design, can lead to new technologies. Applications ranging from molecular sensing to information storage and even working molecular machines have been envisioned. This review will highlight a few historical hallmarks of molecular recognition oriented at studying the basic science of intermolecular interactions, but then detail recent advances in molecular recognition aimed towards applications in the field of molecular sensing. Rational design can be used to create synthetic receptors with a good deal of predictability and selectivity, and many signal transduction mechanisms exist for converting these receptors into sensors. This is the first topic discussed. The concept of "differential" or "generalized" sensing is then presented, where one uses an array of sensors that do not necessarily conform to the "lock and key" principle. This approach to sensing is inspired by the mammalian senses of taste and smell, which we briefly describe. To mimic senses of taste and smell, one is naturally led to the use of combinatorial libraries, a direction of research that has seen continued growth over the past few years. We summarize the current state of the art in synthetic combinatorial receptors/sensors, and then predict a future direction that the field of molecular recognition will possibly take. The review is not meant for the specialist, but instead for a general audience. It does not present a highly detailed analysis of each individual topic: synthetic receptors, sensors, olfaction/gustation, and combinatorial receptors/sensors. Instead, this review shows how all these fields complement each other and fit together to create sensing devices. Our conclusion is that specific analyte sensing, differential sensing, and combinatorial chemistry can and will be combined to create sensor arrays, and give the subfield of molecular recognition that uses synthetic systems a bright future in this type of sensing scenario.

Keywords: analytical methods · combinatorial chemistry · molecular recognition · receptors · sensors

1. Introduction

The field of molecular recognition has reached a stage where one can confidently design and synthesize a receptor with a good degree of predictability and selectivity for many kinds of small to medium-sized molecules. The quest to obtain this goal has been primarily driven by a desire to understand and mimic nature's exquisitely specific binding interactions that are exemplified by the "lock and key" analogy (Figure 1 A).[1] Yet, there is an aspect to nature's methods of molecular recognition that has largely been unexplored by synthetic chemists, and only recently has begun to be examined, that of "differential" binding. By differential, rather than specific or selective, we refer to receptors that have different binding characteristics, none of which are

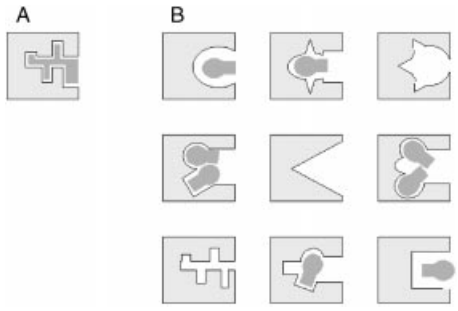


Figure 1. Schematic representation of host–guest interactions. A) A specific binding event (the lock and key paradigm), which exhibits a large degree of complimentarity between the host and guest. B) An array of generalized receptors interacting with one analyte. The identification results from the pattern of responses obtained from the entire array.

[*] Prof. Dr. E. V. Anslyn, Dr. J. J. Lavigne
Department of Chemistry and Biochemistry
The University of Texas at Austin
Austin, TX 78712 (USA)
Fax: (+1) 512-471-7791
E-mail: anslyn@ccwf.cc.utexas.edu

  1433-7851/01/4017-3119 $ 17.50+.50/0

necessarily specific or even very selective. These receptors are "generalized" rather than "specialized". This approach requires that an array of sensors be created (Figure 1 B) and the composite signal evaluated and interpreted by pattern-recognition protocols. This is the binding scenario used in the mammalian senses of taste and smell. In the following sections, selective synthetic receptors will be briefly reviewed, with an emphasis on representative successes, and then contrasted to this new area of differential receptors. Applications of synthetic chemistry to the creation of differential receptors are discussed, especially focusing upon combinatorial chemistry. Correlations between the specific and differential approaches to sensors are made, which leads us to conclude that there is a very bright future for the field of molecular recognition in arrayed sensors.

2. Lock and Key Inspired Synthetic Receptors

Hallmarks of specific binding from nature are enzyme–substrate, antigen–antibody, and complementary DNA annealing. Historical hallmarks for synthetic receptors include cryptand–ion,[2] cyclophane–aromatic,[3] boronic acid–sugar,[4] and guanidinium–carboxylate interactions,[5] while more recent molecular recognition studies target much broader chemical structures.[6] Scheme 1 shows a few representative synthetic hosts that have been studied to elucidate their selectivity for various target guests over other similar guests. In general, the design of such receptors involved an identification of the proper recognition entities to be incorporated into the receptor in order to bind each particular epitope on the guest. Following this, identification of a proper spacer to pre-organize the recognition entities to complement the display of epitopes on the guest provides the final host design. Molecular-recognition forces including solvophobic effects, hydrogen bonding, and ion pairing can all be exploited with such synthetic systems. Significant rational design and computer modeling, as well as trial and error testing, take place to optimize the ability of the receptor to recognize the guest. Through this process it is becoming more and more routine to produce synthetic receptors with good selectivity for many classes of small and medium-sized guests, especially those which possess a reasonable number of sites for binding interactions.

2.1. Combinatorial Approach to Receptors

Another approach for targeting small and medium-sized molecules is the creation of a combinatorial library of receptors. This process allows for the creation of several different compounds through the combination of rapid parallel and combinatorial syntheses.[11] The subsequent screening of the new materials can provide compounds for many applications. Typically, combinatorial chemistry has been used to develop the substrate rather than the host, and is best exemplified with respect to drug discovery, where the final product is often an inhibitor of a biological pathway.

This is not to say that combinatorial approaches have not been used to create effective host compounds. These have usually been formed from oligomeric structures, such as peptides[12] and nucleotides (aptamers).[13] Recently unnatural biopolymers formed from linking groups such as ureas,[14] peptoids,[15] and guanidiniums[16] have been created from which useful receptors may be expected quite soon. Encoding schemes can be employed to decipher the structure of a successful receptor derived from an oligomer.[17]

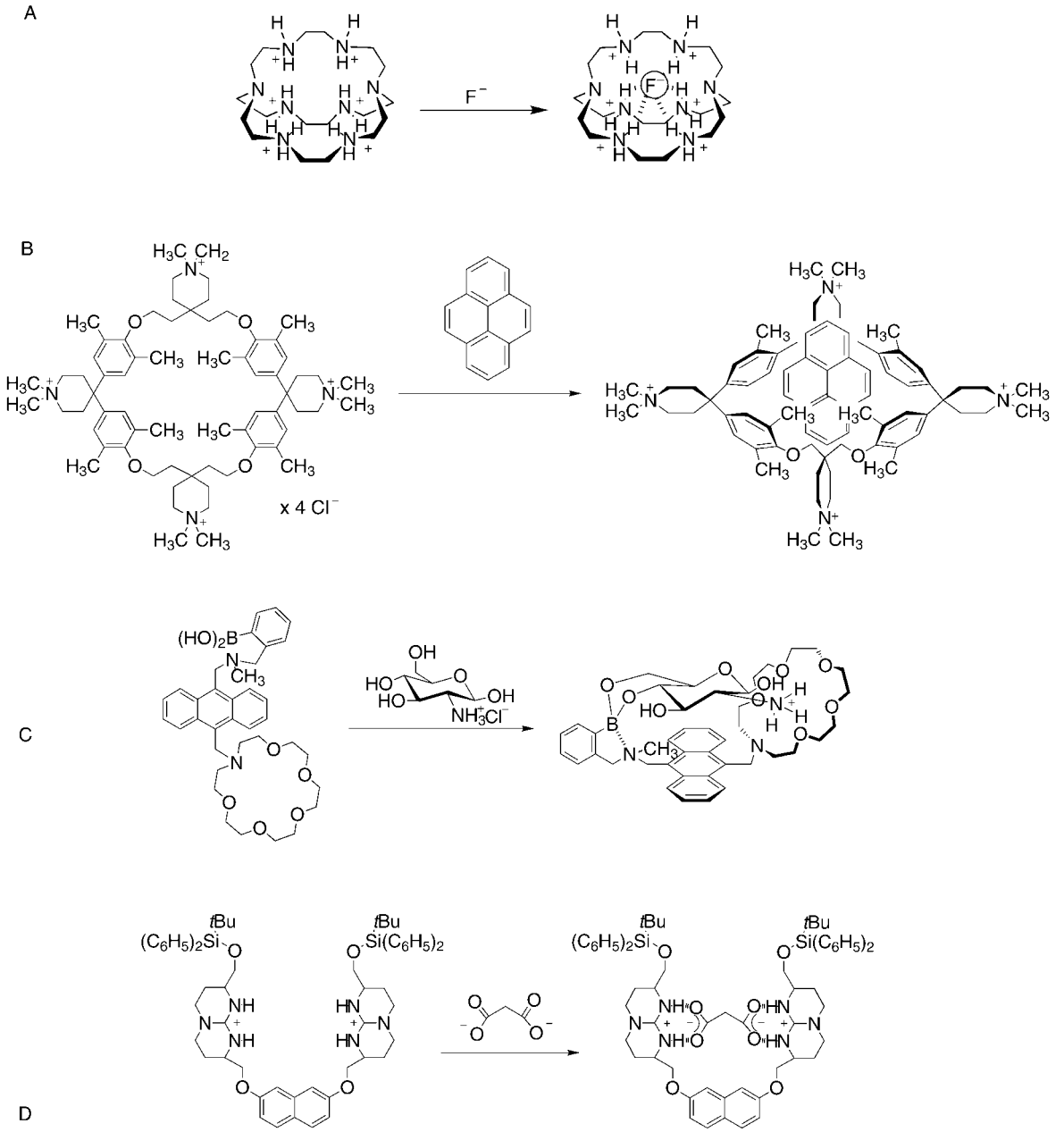
For example, Still and co-workers have taken this approach to create a library of peptidosteroidal receptors (Scheme 2 A).[18] The driving force for this work was to mimic nature's ability to bind large oligomeric substrates with high selectivity (for example, antibodies). The library consisted of a conserved steroidal core derivatized with two different tripeptide arms on rings A and B (aa1-aa2-aa3, aa4-aa5-aa6) to generate approximately 10^4 different compounds. The resin-bound library was screened against a series of labeled

Eric V. Anslyn was born in 1960, and received his BS from California State University Northridge in 1983. He received his Ph.D. in 1987 from the California Institute of Technology by performing experimental and theoretical studies of olefin metathesis mechanisms under the direction of Dr. Robert Grubbs and Dr. William Goddard. After a 1.5 year post-doctoral position at Columbia University, where he worked on RNA hydrolysis mechanisms under the direction of Dr. Ronald Breslow, he moved to The University of Texas at Austin. Two years ago he was promoted to Full Professor.

John J. Lavigne was born in 1971. He received his BS from St. Lawrence University in 1993 and his Masters of Education from St. Lawrence University in 1995. He received is Ph.D. from the University of Texas at Austin in 2000 for developing single-analyte and multi-analyte sensing systems under the direction of Dr. Eric Anslyn. Dr. Lavigne is currently a Senior Scientist at Labnetics, an array sensor firm.


E. V. Anslyn


J. J. Lavigne

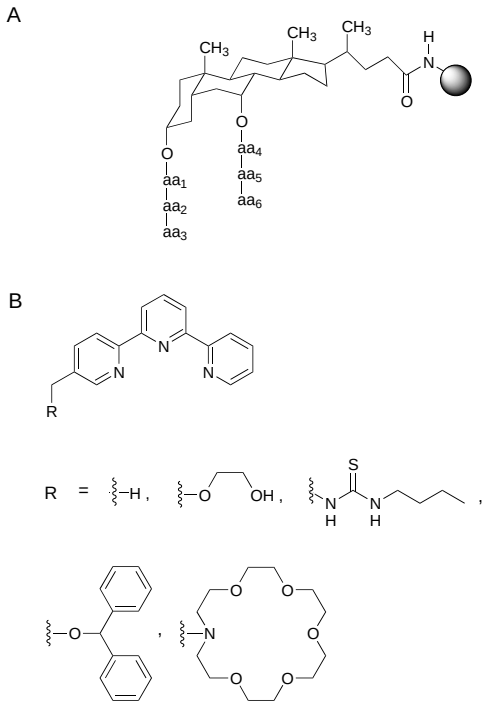


Scheme 1. Representative hallmarks in molecular recognition: A) cryptand–anion recognition,[7] B) cyclophane–aromatic complexation,[8] C) interaction of boronic acid with a polyol,[9] and D) guanidinium–carboxylate interactions.[10]

pentapeptides. Differentiation of oligomers varying by only one amino acid was accomplished, thus demonstrating the feasibility of the approach. Similarly, Hamilton and co-workers have taken a more targeted approach to create a small molecule library (15 compounds). The compounds were again based on an antibody structure and incorporated a constant template region (a terpyridine) with a variable binding region (Scheme 2 B).[19] The recognition capabilities of the compounds followed expected trends, with cationic binding groups recognizing anions and so on.

A unique approach results in polymeric receptors generated from combinatorial molecular imprinting techniques.[20] A semi-automatic system has been developed to combinatorially generate libraries of molecular imprinted polymers (MIP) and screen these compounds for selectivity and affinity towards the original templating species. Receptors for triazine herbicides were prepared by using varying ratios of two functional monomers (methacrylic acid and 2-(trifluoromethyl)acrylic acid). The results suggest that different herbicides prefer different monomer ratios.

One of the most recent advances in the field of combinatorial chemistry is the use of dynamic combinatorial libraries for the creation of receptors.[21] Herein, a series of monomers are used to create libraries of aggregated structures that are in equilibrium with one another, each of which could act as a host. Introduction of a guest shifts the equilibrium to the host that binds that guest. Covalent capture of the assembled aggregate leads to receptors complementary to the added



Scheme 2. Molecular receptors derived from combinatorial libraries: A) the generic structure for a receptor based on a peptidosteroidal core which were found to be able to differentiate between single amino acid differences in pentapeptides, B) the terpyridine backbone (constant region) and the variable sidearms attached to create a library of dimeric compounds.

guest.[22] This very ingenious method for the creation of receptors is definitely applicable to the array sensor technologies discussed in Section 2.2.

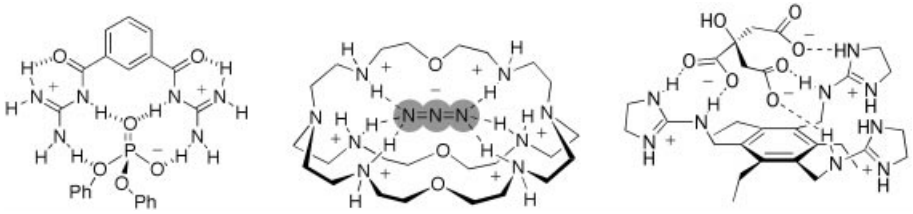
Recently, combinatorial chemistry has been exploited for the creation of libraries of compounds intended to act as new catalysts.[23] The size of the library can be readily increased by simply varying process and reaction conditions, such as solvent, temperature, and additives, which are held constant in traditional assays to eliminate assay variability. Materials exhibiting vastly different characteristics have also been generated with combinatorial approaches.[24] Certainly the use of chemical libraries for creating receptors from synthetic compounds will be seeing an explosion of growth in the near future, and as we explore herein, this method is ideal for the creation of differential or generalized receptors.

2.2. Single-Analyte Chemosensors

Although much of the driving force for the development of the synthetic receptors discussed above derives from a desire to mimic and understand natural systems, these receptors can also be used for practical applications. Sensing is one area where the synthetic systems have found great utility and promise. For example, it is more practical to sense small cations such as sodium,[25] potassium,[25] calcium,[26] mercury,[27] and lead[28] using a synthetic system (a chemosensor) rather than an antibody or aptamer. Similarly, receptors for small anions, such as phosphate[29] and phosphodiesters,[30] azide,[31] and even medium-sized anions, such as citrate,[32] are readily developed using a synthetic system rather than a large biopolymer (Scheme 3). In addition, synthetic receptors can be successfully used in devices, such as chemically modified field-effect transistors.[33]

One of the first analytes to be measured using a synthetic sensor was probably the hydronium ion. Derivatization of these pH reporters with chelating arms analogous to those found in ethylenediaminotetraacetic acid (EDTA) leads to metal ion sensors commonly referred to as the complexones.[34] These derivatives display a response to pH much like their precursors, however, there is a change in the pK_a value of the indicator in the presence of certain ions such that the color change occurs at a different pH value (Scheme 4 A). Crown ethers have undergone a similar transformation. Sensors have been developed from these rather simple structures by covalently attaching a chromophore or fluorophore into or onto the ring skeleton (Scheme 4 B).[35]

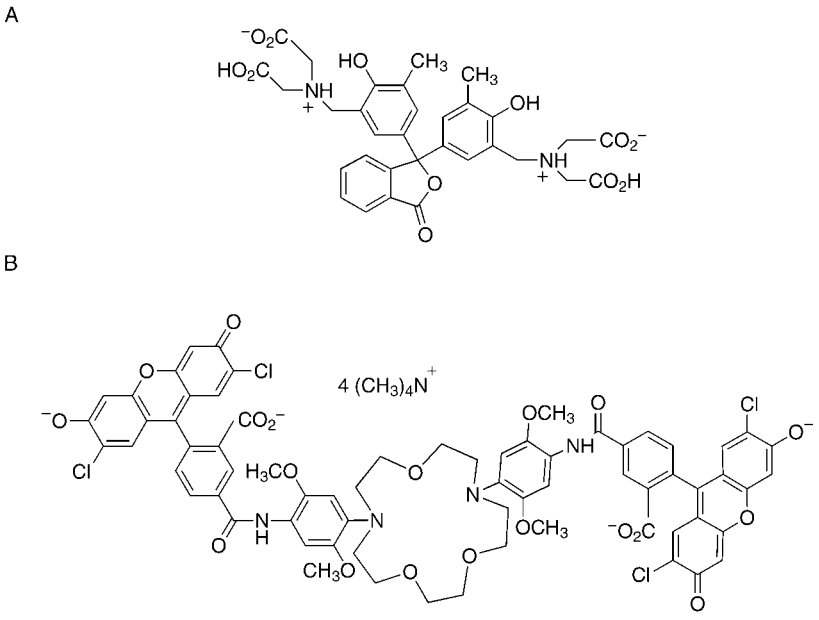
Several more intricate molecular sensors have been developed recently, again with a focus upon selective binding coupled with signal modulation. Most of these sensors have a chromophore or fluorophore covalently attached to the recognition unit. The microenvironment of the sensor is perturbed sufficiently upon binding of analyte to modify the optical signal. Fluorescence resonance energy transfer



Scheme 3. Receptors for small and medium-sized anions.

(FRET) and photoinduced electron transfer (PET) are common mechanisms for transduction. Examples where these processes are utilized include indicators for the recognition of organic cations, anions, and even neutral molecules.[38] An example illustrating this chemistry is a chemosensor for pyrophosphate (Scheme 5 A).[39] This host displays a thousandfold selectivity for pyrophosphate over phosphate ions. The signaling event is PET based[38] and is facilitated by a change in the protonation state of the host upon binding the analyte. The PET effect has also been used to signal the presence of sugars. In particular, a bisboronic acid host that exhibits selectivity for glucose has been reported (Scheme 5 B).[40] Metal-containing receptors have also been efficiently used for optical and electrochemical sensing techniques.[41] All these approaches involve the attachment of the signaling moiety to the recognition unit.

Competition assays using synthetic receptors are also currently being exploited. This methodology takes advantage



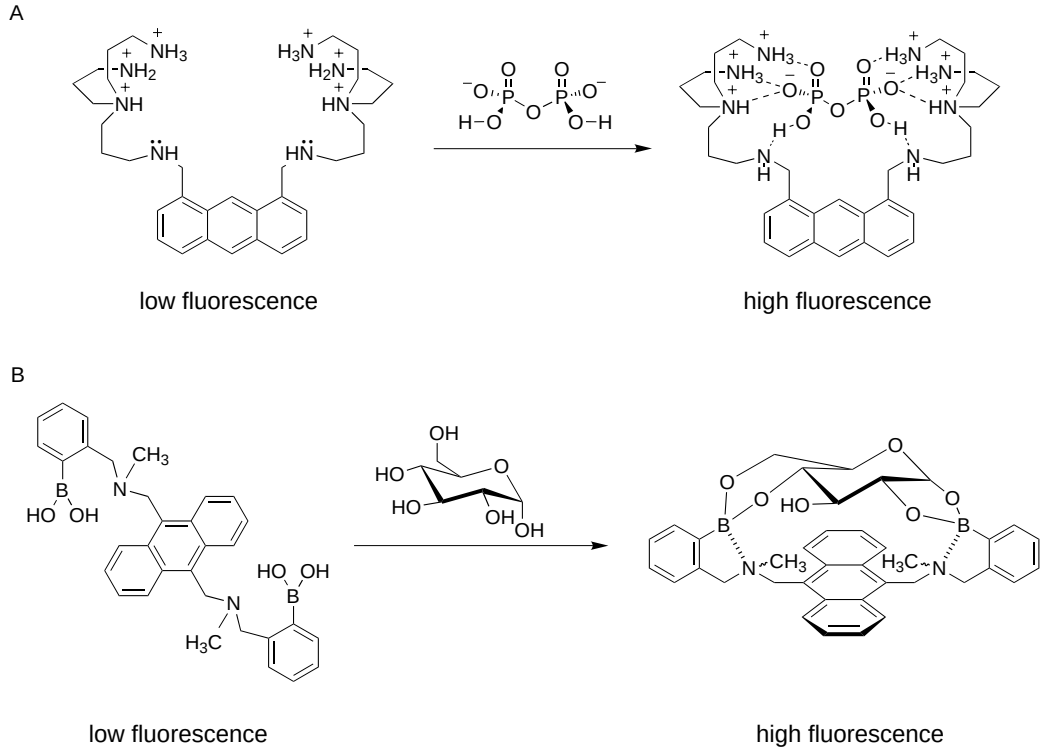
Scheme 4. Complexone and crown-type indicators: A) *o*-cresolphthalein complexone,[36] an EDTA-type cation chelator that incorporates a colorimetric transducer, and B) "sodium green",[37] a commercial indicator which can be used to signal the presence of and quantify sodium ions in vivo.

of an optical modulation of a dye molecule being displaced from the binding cavity of a host by an analyte having a higher binding affinity. In this technique, the indicator is used to identify an analyte that it was not originally designed to signal.[42] The advantage of these approaches is that less covalent bond architecture is required. The disadvantages are the inability to use the system for applications that require full imaging of a surface or volume, such as whole cell imaging,[43] and they are single-use systems.

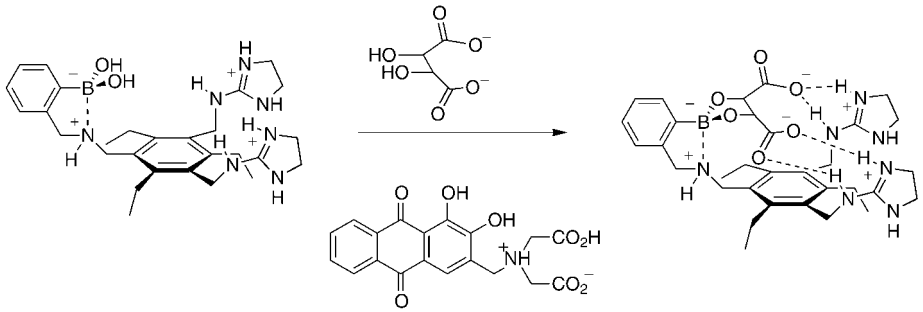
The sensing of hydrophobic analytes using cyclodextrins (CDs) and naphthalene sulfonates is a common example of a competition assay.[44] The naphthalene sulfonate unit binds within the CD cavity, but is displaced from the binding pocket upon exposure of this ensemble to a hydrophobic guest. The naphthalene sulfonate moiety exhibits solvatochromism by displaying a modulation of its fluorescence intensity in bulk water relative to that in the hydrophobic cavity of the CD.

We have shown that even more elaborate receptors can be coupled with a signaling molecule to create a sensing ensemble. For example, tartrate can be readily quantified in common beverages by using a synthetic receptor with selectivity for tartrate, together with alizarin complexone as the signaling molecule (Scheme 6).[45] This competition technique has been used by other research groups and expanded by us by using common indicators to encompass chemosensory systems for acetyl choline,[46] nitrate,[47] citrate[48] and inositol trisphosphate (IP_3).[49]

In summary, it is clear that the "lock and key" inspiration can lead to the creation of synthetic receptors, by design or combinatorial techniques, for simple complexation or sensing applications. Molecular recognition is now sophisticated



Scheme 5. Molecular recognition of pyrophosphate and glucose: A) a 1,8-polyamino-disubstituted anthranyl host displays a 2000-fold selectivity for pyrophosphate over phosphate ions, B) glucose is bound by a 9-10-bisboronic acid anthranyl host with 6 – 1000-fold selectivity over other monosaccharides.



Scheme 6. A synthetic host for tartrate. The recognition takes advantage of the guanidinium – carboxylate and boronic acid – diol interactions. A competition between tartrate and alizarin complexone to bind to the host results in a colorimetric assay for tartrate.

enough that selectivity in the recognition process is a common outcome. However, lock and key type selectivity is not the only method used by nature for the development of receptors and sensors. The second method is that used in the senses of taste and smell, where a series of generalized receptors is used. In keeping with the spirit that one goal of the field of molecular recognition is to mimic nature's "tricks", many chemists are beginning to turn their attention in this direction.

3. Differential Receptors: Multi-Analyte Sensing

Interestingly, the sensors used in arrays may be designed to exhibit highly selective association or they may be random, and lack any targeted design. One of the strengths of multicomponent arrays is the fingerprints or pattern generated from the response of all of the sensor elements, which may display specific or nonspecific binding of an analyte. The interpretation of this pattern allows for the analysis of complex mixtures. To this end much work has been done to mimic the senses of smell and taste, thereby leading to vapor-phase (noses) and solution-phase (tongues) sensors.

Of the five primary mammalian senses, namely, vision, hearing, touch, smell, and taste, gustation and olfaction have been designated as the "lower senses" because of the minimal influence they exhibit on behavior relative to auditory, visual, and other sensory stimuli.[50] However, these senses form the basis of our response to chemical stimuli through chemoreception, and thus are quite amenable to chemists for mimicry.

3.1. Olfaction

The mechanism by which smell functions is still somewhat debated, however, a dominating theme does exist. As odorants enter the nose through the nostrils, they reach the mucus of the olfactory mucosa. These odorous molecules enter the mucus according to the partition coefficient of their molecular type. Once dissolved in the mucus, the analytes encounter the olfactory cells, which are nerve cells. It is generally assumed that the primary process of chemoreception takes place at the cell membrane of these sensory neurons. This process involves physical contact between the stimulant and potential or actual receptor sites which could either be specialists, which react with only one structural class, or generalists, which would react with a multitude of structural classes.[51] The odor quality of a compound is defined intrinsically by the chemical structure, its solubility, and its diffusion rate. The simultaneous analysis of the multiple components, by potentially thousands of different sensory neurons, leads to a pattern for the odor that is stored in the brain.

Though the understanding of the sense of smell is still somewhat uncertain, it is understood well enough to allow chemists to mimic it using a variety of different transduction schemes.[52] This area has recently been very thoroughly reviewed by Lewis, Walt, and co-workers.[53] The methods described include functional sensors based on surface acoustic wave (SAW) crystals,[54] quartz-crystal microbalances (QCM),[55] tin oxide sensors,[56] and carbon black composites.[57] One example that takes advantage of nonspecific recognition has been called the "electronic nose" and is commercially available as AromaScan.[58] The technology is designed to identify volatile chemicals based on the adsorption of the odors onto an array of semiconducting organic polymers (Figure 2), much like the

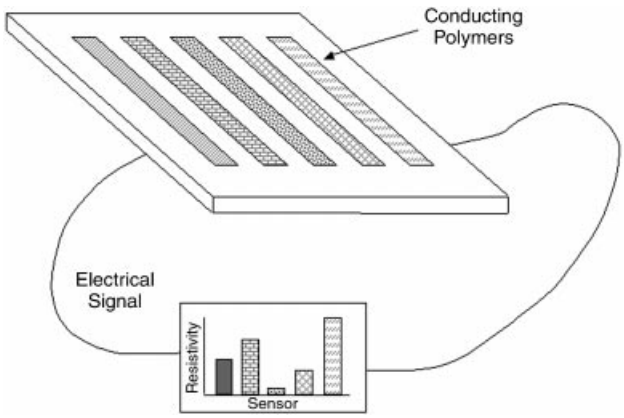


Figure 2. Schematic representation of an electronic nose based on semiconducting organic polymers. The resistance of each polymer is effected by absorption of volatiles into the polymer matrix.

partitioning into olfactory mucosa. The adsorption is dynamic, reversible, and is a function of the size of the voids within the individual polymer sensors. It takes only a few seconds for the system to reach an equilibrium, at which time the changes in electrical resistance across each polymer are measured and a fingerprint pattern created for the particular aroma. This pattern can be stored in a computer and used to identify and quantify further exposures of the array to complex mixtures.

An advantage of this system is that very little chemical design need go into the creation of the conducting polymers. For example, there are no designed receptors appended to the polymers—natural or otherwise—and there are no individual specific binding sites designed into the polymers. The lock and

key principle has little if any influence on the choice of the conducting polymers. The method simply relies on the natural voids within the individual polymers.

The important point is that the polymers are differential, and respond differently to the various analytes in the vapor. Each polymer responds to a multitude of different vapors, similar to the generalized olfactory receptors in the mammalian olfactory mucosa. The identification of the vapor by the electronic nose comes from a composite pattern of the change in resistivity of all the conducting polymers responding simultaneously.

3.2. Gustation

Taste, as part of the chemical senses, is used for food selection and evaluation, including avoidance of potentially harmful substances such as bitter tasting poisons. The surface of the tongue is the major focus of taste transduction in mammals. Clusters of taste buds are located in various regions of the tongue. Taste buds contain taste cells and are located in small depressions in the tongue called taste pores. Some foods are acted upon by enzymes in the saliva which results in components that may be recognized at the cell surface. The taste pores are thought to prevent the diffusion of taste stimuli into the extracellular fluids and restrict the site of interaction to the taste cells.

Similar to the sense of smell, the mechanism of taste is still not clear. Cell surface receptors for particular taste stimuli have not yet been isolated. Traditionally, taste has been divided into four fundamental classes: sweet, salty, sour, and bitter, with each taste bud responding to only one class. Recent findings, however, suggest that most nerve fibers respond to more than one taste stimulus, with only a few responding to stimuli representing one basic taste. In fact most of the nerve fibers react to stimuli from two or three modalities.[59] This observation has led to the development of additional taste classifications. The Japanese, for example, distinguish a fifth taste, umami (savory or delicious taste), which is elicited by monosodium glutamate (MSG), disodium inosinate, and disodium guanylate.[59] Further, there is electrophysiological, biochemical, and behavioral evidence for the presence of at least two different sweet receptors. Therefore, in actuality, taste cells respond to a myriad of chemical stimuli including those that lead to the perception of classical taste qualities. It appears from the available data that these taste cells use several signal transduction mechanisms, even within a particular taste class.[59] Importantly, although there are specific taste sensations, they are elicited from a variety of different tastants. Hence, the taste receptors themselves are not specific, but instead are generalized and differential, similar to the sensors used with smell.

Just as with the sense of smell, the development of human-made arrays to mimic the sense of taste has drawn much attention of late. These approaches include acoustic-wave devices,[60] arrays based on polyaniline,[61] polymerized crystalline colloidal arrays (PCCA),[62] and microelectrodes.[63] Work by Toko et al. focuses on an array composed of lipid membranes that can detect tastes in a manner similar to the human gustation sensation (Figure 3).[64] The output of the sensor is not the amount of taste substances present, but the taste quality and magnitude. The output patterns obtained for different classes of tastants are different, while those obtained

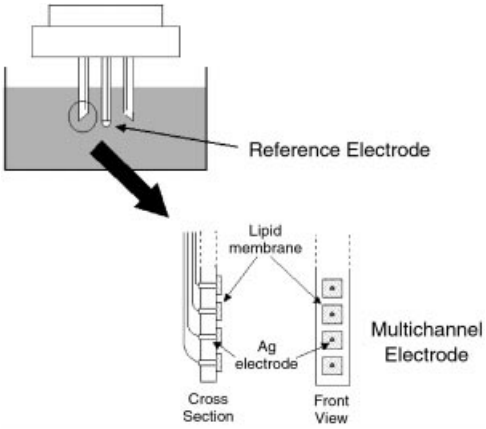


Figure 3. Schematic representation of a taste sensor. This sensor array is based on a multichannel electrode where each individual electrode contact is covered by a lipid membrane. The membranes are designed to function much like the cell surfaces of taste cells.

from analytes within the same class are similar, for example, sodium chloride, potassium chloride, potassium bromide give similar patterns for saltiness. The array was not based on the concept of targeting selective binding to detect specific chemical substances, nor was the design to measure the amount of each chemical substance present. Rather it was designed to measure the taste itself and express it quantitatively.

Though this method has had great success in mimicking the human sense of taste, it seems beneficial to be able to quantitate the amount of analyte in a liquid sample and to be able to assay for specific chemical species, which one may not what to taste, such as toxins, poisons, and bacteria.

In one example the system functions both in the gaseous[65] and liquid phases.[66] This work uses specific indicators either covalently linked to or trapped within a permeable polymer matrix on the distal end of a fiber-optic bundle (Figure 4 A). The sensor is fabricated by selective photodeposition[67] of the

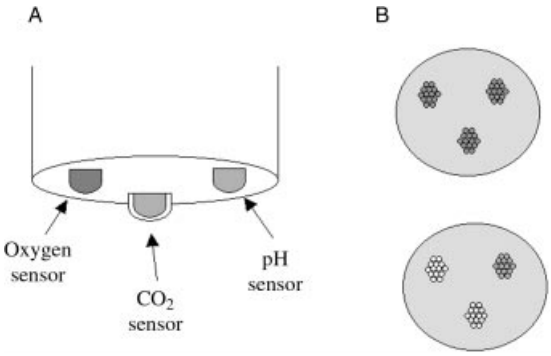


Figure 4. Schematic representation of a fiber-optic-based multi-analyte sensor array. A) This array utilizes indicators incorporated into or trapped within a polymer on the distal end of fiber-optic bundle to assay various analytes in solution. B) The image obtained from fluorescent indicators at the proximal end of the fiber creates the "fingerprint".

analyte-sensitive polymer matrices. By selectively illuminating only small regions of the bundle at one time, sensors with different specificity may all be placed on one bundle. The signal observed is that transmitted up the fiber bundle from a fluorescent sensing element upon responding to the analyte (Figure 4B). The response is fast, efficient, reversible, and the pattern created may be deconvoluted using standard multivariant algorithms.[68] In the early version of this system the receptors used were specific for particular analytes, yet this design is clearly amenable to the utilization of generalized receptors.

An alternate approach is based on an array consisting of fifteen phenolic homopolymers and copolymers.[69] These sensors are generated using a peroxidase-catalyzed oxidative polymerization of five phenolic monomers. The sensory mechanism is based on the change in the intrinsic fluorescence of the polyphenol upon the addition of metal ions. The array was used to assay for iron, copper, cobalt, and nickel ions as well as various mixtures. The array produced distinct fingerprints for the metal ions that were analyzed. Furthermore, the response from the copolymers varied greatly from that of the homopolymers, which suggests that extreme diversity could be obtained from a limited number of monomers combined in various ratios.

The current method under investigation at the University of Texas attempts to mimic the mammalian sense of taste[70] by placing "synthetic taste buds" in the recesses of a micromachined platform (Figure 5). In nature, the taste buds are

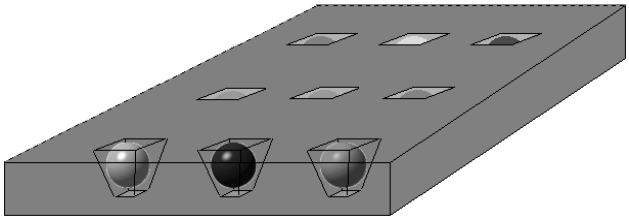


Figure 5. Multicomponent sensor array on a silicon chip. Individual "taste buds" (polyethylene glycol/polystyrene resin beads) are immobilized within cavities on a micromachined silicon "tongue".

located at the bottom of small depressions in the tongue. Analytes are trapped in these recesses and react with receptors on the taste buds. These synthetic taste buds are polyethylene glycol/polystyrene block copolymer resin beads with the desired sensors covalently attached. Simultaneous exposure of the various taste buds to a solution containing multiple analytes causes changes in the optical spectroscopy of the various beads. This change can be fluorescent or colorimetric. Data streams are collected using a three-chip CCD camera, which creates red, green, and blue (RGB) patterns that are distinct and reproducible for their environments.[71] Analysis of this data can identify and potentially quantify the analytes present. This method allows for quick and accurate evaluation.

The advantage that all these approaches provide to solution analysis is that complex mixtures of analytes may be identified and quantified from the mathematical interpretation of the detailed pattern, or fingerprint, created by an array of differentially modified sites. Specific receptors and sensors, such as those discussed in Section 2.2, can be readily used, but adding to the strength of this system is the fact that the sensors do not necessarily need to be specific for the targeted analyte(s). There can be significant "cross-talk" or nonspecific interactions, which actually adds to the success of this method.[53] Synthetic receptors are naturally not as selective as antibodies and aptamers, and therefore compliment this sensing methodology very well. Most importantly, the receptors do not need to be completely rationally designed to target specific analytes. They simply need to be different, with different.

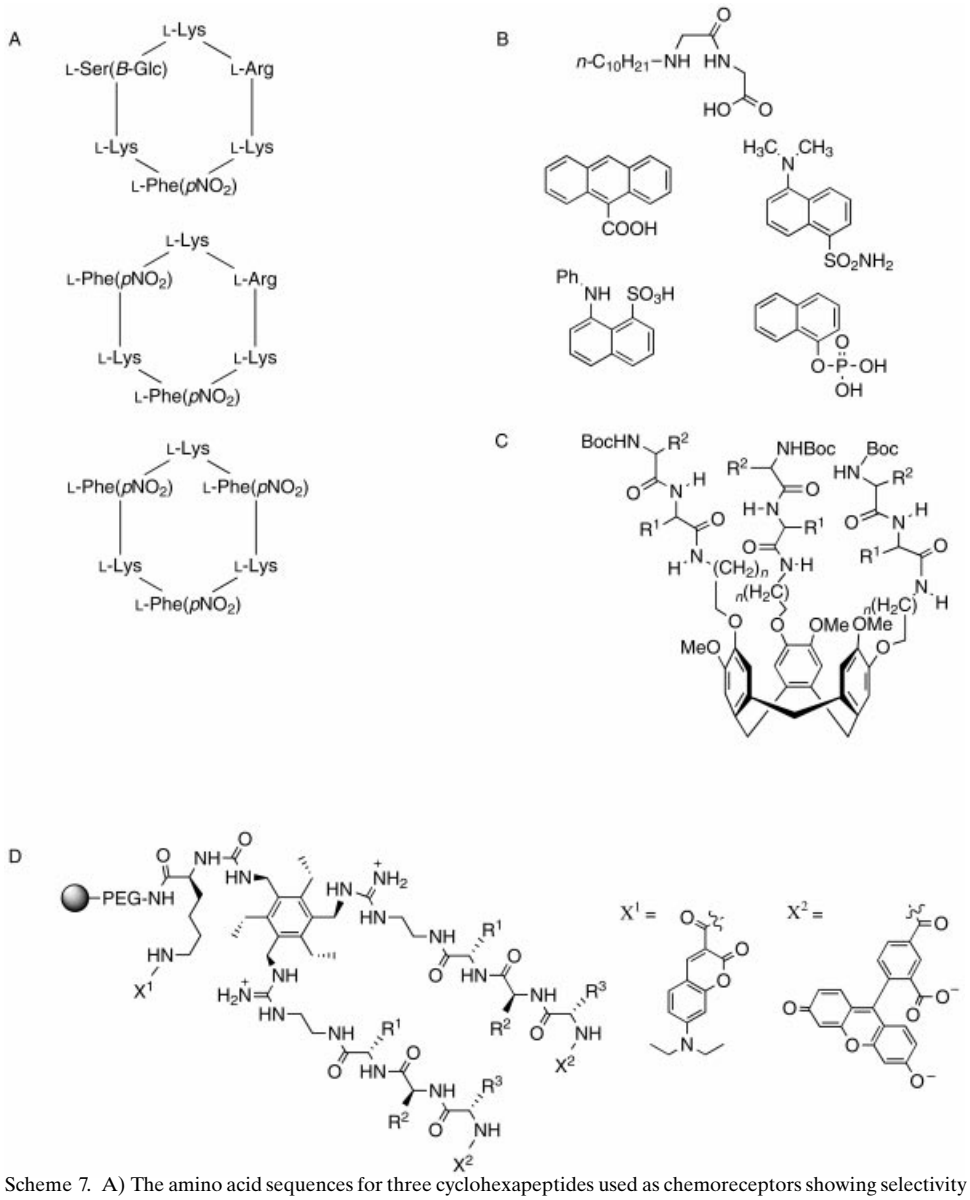
3.3. Combinatorial Approach to Chemosensors

As described in Section 2.1, combinatorial chemistry can create hundreds to thousands of different receptors quickly and efficiently. Hence, it is ideal for the creation of differential receptors. By using the split and pool method[72] each bead has a single receptor type immobilized, yet each bead is different. Thus, incorporating the use of combinatorial chemistry in multi-analyte sensing, where differential/generalized receptors are useful, is a natural direction for the evolution of the sensing field and the practice of molecular recognition.

One step that must be proven before the use of libraries in arrays is explored is that combinatorial chemistry can lead to receptors with differential responses. Hence, before being incorporated into multicomponent arrays, sensors have been created by a combinatorial approach for the purpose of finding the best sensor for a single analyte, thus demonstrating that receptors with some natural preference for one analyte over another can be created.

Libraries of cyclic hexapeptides have been formed from which selective receptors for amino acids have been isolated.[73] From this library of just under 6000 compounds, three cyclohexapeptides were identified (Scheme 7A) for their selectivity in binding L-arginine over L-lysine in neutral unbuffered aqueous solution. The interaction between the cyclopeptide and the analyte was examined with reflectometric interference spectroscopy (RIS), thus leading to a sensing scenario. Interestingly, when the compounds were tested in buffered media (phosphate-buffered saline, PBS), the selectivity changed such that L-glutamine was most tightly bound, with all three sensors being able to distinguish between L-glutamine and L-asparagine. This result indicates that further selectivities for other amino acids may be investigated by varying the cyclopeptides and the constitution of the buffer solution.

A similar approach has been taken to develop a chemosensor selective for copper(II) ions.[74] The methodology involved creating a system in which a receptor and an indicator would spontaneously assemble without the need for any covalent link between them. To realize this, a known copper(II) sensor and various fluorescent dyes (Scheme 7B) were derivatized with long lipophilic tails. The components self-assemble into micelles in aqueous media. Optical modulation of the dye occurs upon complexation of the metal and results in sub-micromolar detection of copper(II) ions.



Scheme 7. A) The amino acid sequences for three cyclohexapeptides used as chemoreceptors showing selectivity for specific amino acids. B) The recognition unit and fluorophores used in a library to determine a self-assembling sensing ensemble for copper(II) ions. C) The general structure of a library based on a cyclotriveratrylene scaffold. D) The general structure of a library from which a sensor selective for ATP was obtained. Boc = *tert*-butoxycarbonyl.

van Wageningen and Liskamp have also used this targeted approach to create a 40-member library based on substituted cyclotriveratrylene (CVT).[75] The desired receptors were obtained by *o*-alkylation of the CTV triol followed by coupling one or two amino acids to these arms (Scheme 7C). Initial studies showed that library members derivatized with dansyl differentially bound N-acylated dipeptides.

An example where combinatorial synthesis has been employed to create diversity in a multicomponent array comes from Walt and co-workers. They demonstrated that polymerization reactions between different combinations of two starting monomers can lead to the creation of new sensors with differing recognition capabilities.[76] A solvatochromic dye (Nile Red) was entrapped within the polymer matrix to perform vapor-phase sensing. Upon exposure of the sensor to different organic vapors, a fluorescence modulation was observed that varied with the monomer ratio.

A final example utilizes the rational design of a core structure that targets a particular class of compounds, combined with combinatorial chemistry to provide specificity for certain members. To this end, a core containing cationic guanidinium ions was derivatized with two identical tripeptide arms to recognize organic anions (Scheme 7D).[77] Multiple sites were available for indicator attachment, such as the ends of the tripeptide arms as well as the side chain of a lysine moiety used in the linkage of the chemosensor to a resin bead. Attempts to monitor fluorescence resonance energy transfer (FRET) between fluorophores at these two sites proved unfruitful. However, attachment of a fluorophore to the end of the tripeptide arms did provide a signal transduction scheme. From a library of just under 5000 possible members, a sensor for adenosine triphosphate (ATP) was derived which showed selectivity for ATP over GTP and AMP.

While this library was screened with the intent of finding an ATP sensor, the creation of a library on resin beads creates the opportunity to create an array and look at the specific and nonspecific recognition capabilities of several compounds at one time, thereby creating a multicomponent sensor array. The ability to create an array is definitely applicable to the libraries mentioned above for peptide and metal-binding applications, but also a myriad of other classes of analytes. Essentially, all analyte classes are approachable using this technique.

Given these examples of selective sensors derived from combinatorial methods, it is clear that the synthetic libraries themselves inherently possess all the requirements for arrayed sensors. Differential responses from the varying members of the library are present, and significant cross-reactivity is expected. Hence, merging the combinatorial chemistry approach with sensor array technology can be expected to continue for quite some time.

4. Is Design Dead?

Given the concept of arrays of differential receptors, one may ask "Is rational design dead?" within the sensing arena. Our answer is "No". In many settings it will still be necessary to have highly selective host compounds, which can take the form of antibodies, enzymes, aptamers, imprinted polymers, or designed organic receptors. These receptors can target strategically important analytes, whereas the differential receptors can be used to analyze for the rest of the "flotsam and jetsam" of the solution. Furthermore, high affinity is commonly directly related to specificity, and therefore those analytes whose concentrations are exceedingly low are in some cases more easily analyzed for using a highly selective receptor, such as an aptamer or antibody. However, even weak affinity receptors can gain very high avidity as a result of their extremely high effective concentrations when immobilized in matrices such as beads.

Moreover, rational design will facilitate the creation of synthetic combinatorial libraries of functionally differential receptors. The utilization of monomers in the libraries that possess the proper functional groups for binding the analytes of interest is clearly advantageous. One needs to narrow the diversity space to include structures that have a high propensity to lead to the binding interactions desired for a class of analytes. The use of amine and guanidine groups in the combinatorial receptors is the logical choice when targeting multiple carboxylic acid analytes, for example. Similarly, the use of libraries that include boronic acids would be predicted to be successful when analyzing solutions of multiple carbohydrates. Moreover, the creation of cavities is likely to be assisted by monomers that enforce turns and loops. Therefore, incorporation of building blocks with known affinities for certain types of functional groups and that can be used as structural elements, can lead to the creation of differential receptors that target a particular analyte class.[77]

5. Summary and Outlook

In summary, we predict there will be one clear direction in which the field of molecular recognition will be moving in the coming years. It is becoming apparent that the use of large numbers of differential receptors, coupled with pattern-recognition protocols, can and will be used to analyze for single analytes in mixtures of analytes, as well as in the simultaneous analysis of multiple analytes in a mixture. It will not always be necessary to produce highly selective receptors for each and every individual analyte, but instead one can rely on the pattern of responses derived from several differential receptors to identify and quantitate the analytes. Importantly, the guiding principles of molecular recognition will still be at the heart of the process for creating libraries for the various classes of targets. Therefore, the production of both selective receptors and differential receptors for the large number and variety of sensing applications that have societal benefits presents a clear and compelling case for a continued and bright future for the field of molecular recognition in the arena of molecular sensing.

We gratefully acknowledge the National Institutes of Health, the Beckman Foundation, and a MURI grant for support of our array sensor research. We also gratefully acknowledge conversations with Dr. John McDevitt, Dr. Jason Shear, Dr. Brent Iverson, Dr. Andrew Ellington, and Dr. Dean Neikirk which have helped to shape our views of array sensor technology and its impact on the field of supramolecular chemistry.

Received: October 30, 2000 [A 435]

[1] *The Lock and Key Principle. The State of the Art—100 Years On* (Ed.: J.-P. Behr), Wiley, New York, **1994**.
[2] a) E. Grell, R. Warmuth, *Pure Appl. Chem.* **1993**, *65*, 373 – 379; b) J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, New York, **1995**.
[3] F. Diederich, *Angew. Chem.* **1988**, *100*, 372 – 396; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362 – 386.
[4] T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Angew. Chem.* **1996**, *108*, 2038 – 2050; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1910 – 1922.
[5] a) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1997**, *97*, 1609 – 1646; b) B. Linton, A. D. Hamilton, *Tetrahedron* **1999**, *55*, 6027 – 6038.
[6] M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479 – 2493; M. Famulok, G. Mayer, M. Blind, *Acc. Chem. Res.* **2000**, *33*, 591 – 599; J. J. Rebek, Jr., *Chem. Commun.* **2000**, 637 – 643; A. D. Ellington, J. Hesselberth, S. Jhaveri, M. P. Robertson, *Proc. SPIE Int. Soc. Opt. Eng.* **1999**, *3858*, 126 – 134; D. L. Caulder, K. N. Raymond, *Acc. Chem. Res.* **1999**, *32*, 975 – 982; P. D. Beer, P. A. Gale, G. Z. Chen, *J. Chem. Soc. Dalton Trans.* **1999**, 1897 – 1910; G. R. Newkome, *Pure Appl. Chem.* **1998**, *70*, 2337 – 2343; K. Ariga, T. Kunitake, *Acc. Chem. Res.* **1998**, *31*, 371 – 378; T. E. Mallouk, J. A. Gavin, *Acc. Chem. Res.* **1998**, *31*, 209 – 217; M. M. G. Antonisse, D. N. Reinhoudt, *Chem. Commun.* **1998**, 443 – 448.
[7] B. Dietrich, J.-M. Lehn, J. Guilhem, C. Pascard, *Tetrahedron Lett.* **1989**, *30*, 4125 – 4128.
[8] F. Diederich, D. Griebel, *J. Am. Chem. Soc.* **1984**, *106*, 8037 – 8046.
[9] C. R. Cooper, T. D. James, *Chem. Commun.* **1997**, 1419 – 1420.
[10] E. Fan, S. A. Van Arman, S. Kincaid, A. D. Hamilton, *J. Am. Chem. Soc.* **1993**, *115*, 369 – 370.
[11] P. Schuster, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7678 – 7680; J. Drews, *Science* **2000**, *287*, 1960 – 1963; R. A. Houghten, *Annu. Rev. Pharmacol. Toxicol.* **2000**, *40*, 273 – 282; D. R. Liu, P. G. Schultz, *Angew. Chem.* **1999**, *111*, 36 – 56; *Angew. Chem. Int. Ed.* **1999**, *38*, 37 – 54; A. W. Czarnik, *Anal. Chem.* **1998**, *70*, A378 – A386; J. Ellman, B. Stoddard, J. Wells, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2779 – 2782; J. C. J. Hogan, *Nature* **1996**, *384*, 17 – 19; G. L. Verdine, *Nature* **1996**, *384*, 11 – 13; G. Lowe, *Chem. Soc. Rev.* **1995**, *24*, 309 – 317.
[12] a) G. Jung, H. Hofstetter, S. Feiertag, D. Stoll, O. Hofstetter, K.-H. Wiesmüller, V. Schurig, *Angew. Chem.* **1996**, *108*, 2261 – 2263; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2148 – 2150; b) H. Miyake, Y. Kojima, *Coord. Chem. Rev.* **1996**, *148*, 301 – 314.
[13] V. V. Vlassov in *The Lock and Key Principle. The State of the Art—100 Years On* (Ed.: J.-P. Behr), Wiley, New York, **1994**, pp. 89 – 147.
[14] K. Burgess, J. Ibarzo, D. S. Linthicum, D. H. Russell, H. Shin, A. Shitangkoon, R. Totani, A. J. Zhang, *J. Am. Chem. Soc.* **1997**, *119*, 1556 – 1564.
[15] B. C. Hamper, S. A. Kolodziej, A. M. Scates, R. G. Smith, E. Cortez, *J. Org. Chem.* **1998**, *63*, 708 – 718.
[16] S. E. Schneider, P. A. Bishop, M. A. Salazaar, O. A. Bishop, E. V. Anslyn, *Tetrahedron* **1998**, *54*, 15063 – 15086.
[17] a) K. D. Janda, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10779 – 10785; b) L. A. Thompson, J. A. Ellman, *Chem. Rev.* **1996**, *96*, 555 – 600; c) G. Jung, A. G. Beck-Sickinger, *Angew. Chem.* **1992**, *104*, 375 – 394; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 367 – 486.
[18] R. Boyce, G. Li, P. Nestler, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 7955 – 7956.
[19] M. S. Goodman, V. Jubian, B. Linton, A. D. Hamilton, *J. Am. Chem. Soc.* **1995**, *117*, 11610 – 11611.
[20] T. Takeuchi, D. Fukuma, J. Matsui, *Anal. Chem.* **1999**, *71*, 285 – 290; K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495 – 2504.

[21] a) M. C. Calama, P. Timmerman, D. N. Reinhoudt, *Angew. Chem.* **2000**, *112*, 771 – 774; *Angew. Chem. Int. Ed.* **2000**, *39*, 755 – 758; b) A. Star, I. Goldberg, B. Fuchs, *Angew. Chem.* **2000**, *112*, 2797 – 2801; *Angew. Chem. Int. Ed*. **2000**, *39*, 2685 – 2689; c) P. S. Lukeman, J. K. M. Sanders, *Tetrahedron Lett.* **2000**, *41*, 10171 – 10174; d) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106 – 2110; e) G. R. L. Cousins, S. A. Poulsen, J. K. M. Sanders, *Chem. Commun*. **1999**, 1575 – 1576.

[22] F. Cardullo, M. C. Calama, B. H. M. Snellink-Ruel, J. L.Weidmann, A. Bielejewska, *Chem. Commun.* **2000**, 367 – 368.

[23] B. Jandeleit, D. J. Schaefer, T. S. Powers, H. W. Turner, W. H. Weinberg, *Angew. Chem.* **1999**, *111*, 2648 – 2689; *Angew. Chem. Int. Ed.* **1999**, *38*, 2494 – 2532.

[24] K. Sada, K. Yoshikawa, M. Miyata, *Chem. Commun.* **1998**, 1763 – 1764.

[25] a) A. P. de Silva, K. R. A. S. Sandanayake, *Tetrahedron Lett*. **1991**, *32*, 421 – 424; b) H. Yanagi, T. Sakaki, H. Yamamoto, T. Ogata, *Proc. Electrochem. Soc*. **1993**, *93*, 128 – 139.

[26] B. C. R. Sarkar, U. P. S. Chauhan, *Anal. Biochem.* **1967**, *20*, 155 – 166.

[27] M. Y. Chae, A. W. Czarnik, *J. Am. Chem. Soc*. **1992**, *114*, 9704 – 9705.

[28] M. Y. Chae, X. M. Cherian, A. W. Czarnik, *J. Org. Chem*. **1993**, *58*, 5797 – 5801.

[29] R. Gross, G. Durner, M. W. Gobel, *Liebigs Ann. Chem.* **1994**, 49 – 58.

[30] R. P. Dixon, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1992**, *114*, 366 – 367.

[31] J.-M. Lehn, E. Sonveax, A. K. Willard, *J. Am. Chem. Soc.* **1978**, *100*, 4914 – 4916.

[32] A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682 – 684; *Angew. Chem. Int. Ed.* **1998**, *37*, 649 – 652.

[33] a) L. Fabbrizzi, M. Licchelli, P. Pallavicini, L. Parodi, *Angew. Chem.* **1998**, *110*, 838 – 841; *Angew. Chem. Int. Ed*. **1998**, *37*, 800 – 802; b) L. Fabbrizzi, M. Licchelli, L. Parodi, A. Poggi, A. Taglietti, *Eur. J. Inorg. Chem*. **1999**, 35 – 39; c) L. Fabbrizzi, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, *Chem. Commun*. **1997**, 581 – 582; d) J. Buegler, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem*. **1998**, *63*, 5339 – 5344; e) B. M. M. G. Antonisse, B. H. M. Snellink-Ruel, J. F. J. Engbersen, D. N. Reinhoudt, *J. Chem. Soc. Perkin Trans.* **1998**, 773 – 777; f) M. M. G. Antonisse, B. H. M. Snellink-Rueel, I. Yigit, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem*. **1997**, *62*, 9034 – 9038.

[34] a) G. Schwarzenbach, *Analyst* **1955**, *80*, 713 – 714; b) J. Korbl, R. Pribil, *Chem. Ind. (London)* **1957**, 233 – 234.

[35] a) L. R. Sousa, B. Son, T. E. Trehearne, R. W. Stevenson, S. J. Ganion, B. E. Beeson, S. Barnell, T. E. Mabry, M. Yao, C. Chakrabarty, P. L. Bock, C. C. Yoder, S. Pope, *ACS Symp. Ser.* **1993**, *538*, 10 – 24; b) M. Takagi, K. Nakano, N. Nakashima, *Pure Appl. Chem*. **1989**, *61*, 1605 – 1612.

[36] G. Kessler, M. Wolfman, *Clin. Chem.* **1964**, *10*, 686 – 703.

[37] R. P. Haugland in *Handbook of Fluorescent Probes and Research Chemicals* (Ed.: M. T. Z. Spence), 6th ed., Molecular Probes, Eugene, **1996**, chap. 24.

[38] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515 – 1566.

[39] D. H. Vance, A. W. Czarnik, *J. Am. Chem. Soc.* **1994**, *116*, 9397 – 9398.

[40] T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982 – 8987.

[41] Z. Chen, A. J. Pilgrim, P. D. Beer, *J. Chem. Soc. Faraday Trans.* **1995**, *91*, 4331 – 4333.

[42] S. Wisker, E. V. Anslyn, *Acc. Chem. Res.*, in press.

[43] M. H. Wade, A. W. de Feijter, M. K. Frame, M. Schindler, *Methods Biochem. Anal*. **1994**, *37*, 117 – 141.

[44] a) H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda, A. Ueno, *J. Am. Chem. Soc.* **1996**, *118*, 10980 – 10988; b) R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia, G. Sartor, *J. Org. Chem*. **1997**, *62*, 6283 – 6289.

[45] J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903 – 3906; *Angew. Chem. Int. Ed.* **1999**, *37*, 3666 – 3669.

[46] a) M. Inouye, K. Hashimoto, K Isagawa, *J. Am. Chem. Soc.* **1994**, *116*, 5517 – 5518; b) K. N. Koh, K. Araki, A. Ikeda, H. Otsuka, S. Shinkai, *J. Am. Chem. Soc.* **1996**, *118*, 755 – 758.

[47] A. P. Bisson, V. M. Lynch, M.-K. C. Monahan, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 2435 – 2437; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2340 – 2342.

[48] A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682 – 684; *Angew. Chem. Int. Ed.* **1998**, *37*, 649 – 652.

[49] K. Niikura, A. Metzger, E. V. Anslyn, *J. Am. Chem. Soc.* **1998**, *120*, 8533 – 8534.

[50] R. M. Pangborn in *The Chemical Senses and Nutrition* (Eds.: M. R. Kare, O. Maller), John Hopkins, Baltimore, **1967**, p. 45.

[51] A. A. Schleppnik, *ACS Symp. Ser.* **1981**, *148*, 161 – 175.

[52] a) R. J. Elliott-Martin, P. N. Bartlett, J. W. Gardner, T. T. Mottram in *Sensors and Their Applications*, *Vol. VII* (Ed.: A. T. Augousti), Institute of Physics, Bristol, **1995**, pp. 12 – 20; b) *Handbook of Biosensors and Electronic Noses: Medicine*, *Food and the Environment* (Ed.: E. Kress-Rogers), CRC, New York, **1997**.

[53] K. J. Albert, N. S. Lewis, C. L. Schauer, G. A. Sotzing, S. E. Stitzel, T. P. Vaid, D. R. Walt, *Chem. Rev.* **2000**, *100*, 2595 – 2626.

[54] a) T. Nakamoto, K. Fukunishi, T. Moriizumi, *Sens. Actuators B* **1990**, *1*, 473 – 476; b) T. Nakamoto, A. Fukuda, T. Moriizumi, *Sens. Actuators B* **1993**, *10*, 85 – 90; c) S. Hanaki, T. Nakamoto, T. Moriizumi, *Sens. Actuators A* **1996**, *57*, 65 – 71.

[55] a) T. Nakamoto, K. Nakamura, T. Moriizumi, *IEEE* **1996**, *1*, 351 – 354; b) J. A. J. Brunick, C. Di Natale, F. Bungaro, F. A. M. Davide, A. D'Amico, R. Paolesse, T. Boschi, M. Faccio, G. Ferri, *Anal. Chim. Acta* **1996**, *325*, 53 – 64.

[56] a) "Sensors and Sensory Systems for an Electronic Nose": D. Kohl in *NATO ASI Ser. Ser. E*, *Vol. 12* (Eds.: J. W. Gardner, P. N. Bartlett), Kluwer, Dordrecht, **1992**, chap. 5; b) P. Corcoran, H. V. Shurmer, J. W. Gardner, *Sens. Actuators B* **1993**, *15*, 32 – 37; c) J. W. Gardner, A. Pike, N. F. de Rooij, M. Koudelka-Hep, P. A. Clerc, A. Hierlemann, W. Gopel, *Sens. Actuators B* **1995**, *26*, 135 – 139.

[57] a) M. C. Logergan, E. J. Severin, B. J. Doleman, S. A. Beaber, R. H. Grubbs, N. S. Lewis, *Chem. Mater.* **1996**, *8*, 2298 – 2312; b) N. S. Lewis, M. C. Logergan, E. J. Severin, B. J. Doleman, R. H. Grubbs, *Proc. SPIE Int. Soc. Opt. Eng*. **1997**, *3079*, 660 – 670.

[58] a) K. C. Persaud, P. Travers, *Intell. Instrum. Comput.* **1991**, *4*, 147 – 154; b) K. C. Persaud, *Trends Anal. Chem.* **1992**, *11*, 61 – 67; c) J. V. Hatfield, P. Neaves, P. J. Hicks, K. C. Persaud, P. Travers, *Sens. Actuators B* **1994**, *18*, 221 – 228; d) M. E. H. Amrani, K. C. Persaud, P. A. Payne, *Meas. Sci. Tech.* **1995**, *10*, 1500 – 1507.

[59] A. I. Spielman, J. G. Brand, M. R. Kare in *Encyclopedia of Human Biology*, *Vol. 7* (Ed.: R. Dulbecco), Academic Press, San Diego, **1991**, pp. 527 – 535.

[60] a) K. Chen, D. Liu, L. Nie, S. Yao, *Biosens. Bioelectron.* **1996**, *11*, 515 – 519; b) K. Chen, D. Wang, *IEEE* **1998**, *1*, 619 – 624.

[61] H. Sangodkar, S. Sukeerthi, R. S. Srinivasa, R. Lal, A. Q. Contractor, *Anal. Chem.* **1996**, *68*, 779 – 783.

[62] J. H. Holtz, J. S. W. Holtz, C. H. Munro, S. A. Asher, *Anal. Chem.* **1998**, *70*, 780 – 791.

[63] a) C. A. Amatore in *Physical Electrochemistry: Principles*, *Methods and Applications* (Ed.: I. Rubenstein), Marcel Dekker, New York, **1995**, pp. 131 – 208; b) I. R. Lauks, *Acc. Chem. Res.* **1998**, *31*, 317 – 324; c) R. M. Wightman, D. O. Wipf in *Electroanalytical Chemistry* (Ed.: A. J. Bard), Marcel Dekker, New York, **1989**, pp. 267 – 353.

[64] K. Toko in *Characterization of Food: Emerging Methods* (Ed.: A. G. Gaonkar), Elsevier, Amsterdam, **1995**, pp. 377 – 401.

[65] a) S. M. Barnard, D. R. Walt, *Environ. Sci. Technol*. **1991**, *25*, 1301 – 1304; b) D. R. Walt, T. A. Dickinson, B. G. Healey, J. S. Kauer, J. White, *Proc. SPIE Int. Soc. Opt. Eng*. **1995**, *2508*, 111 – 116; c) J. White, J. S. Kauer, T. A. Dickinson, D. R. Walt, *Anal. Chem*. **1996**, *68*, 2191 – 2202.

[66] a) B. G. Healey, S. Chadha, D. R. Walt, F. P. Milanovich, J. Richards, S. Brown, *Proc. SPIE Int. Soc. Opt. Eng*. **1994**, *2360*, 10110 – 10112; b) S. R. Johnson, J. M. Sutter, H. L. Engelhardt, P. C. Jurs, J. White, J. S. Kauer, T. A. Dickinson, D. R. Walt, *Anal. Chem.* **1997**, *69*, 4641 – 4648; c) D. R. Walt, *Acc. Chem. Res.* **1998**, *31*, 267 – 278; d) K. L. Michael, L. C. Taylor, S. L. Schultz, F. Szurdoki, D. R. Walt, *Proc. SPIE Int. Soc. Opt. Eng*. **1998**, *3270*, 34 – 41.

[67] B. G. Healey, D. R. Walt, *Anal. Chem*. **1997**, *69*, 2213 – 2216.

[68] a) J. Mitrovics, H. Ulmer, U. Weimar, W. Gopel, *Acc. Chem. Res.* **1998**, *31*, 307 – 315; b) G. C. Osbourn, J. W. Bartholomew, A. J. Ricco, G. C. Frye, *Acc. Chem. Res.* **1998**, *31*, 297 – 305.

[69] X. Wu, J. Kim, J. S. Dordick, *Biotechnol. Prog.* **2000**, *16*, 513 – 516.
[70] a) T. V. Getchell, *Physiol. Rev.* **1986**, *66*, 772 – 777; b) *Chemosensory Information Processing* (Ed.: D. Schild), Springer, Berlin, **1990**.
[71] a) J. J. Lavigne, S. Savoy, M. B. Clevenger, J. E. Ritchie, M. Bridget, S.-J. Yoo, E. V. Anslyn, J. T. McDevitt, J. B. Shear, D. Neikirk, *J. Am. Chem. Soc.* **1998**, *120*, 6429 – 6430; b) A. Goodey, J. J. Lavigne, S. M. Savoy, M. D. Rodriguez, T. E. Curey, A. Tsao, G. Simmons, J. Wright, S.-J. Yao, Y. Sohn, E. V. Anslyn, J. B. Shear, D. P. Neikirk, J. T. McDevitt, *J. Am. Chem. Soc.* **2001**, *123*, 2559 – 2570; c) T. E. Curey, A. Goodey, A. Tsao, J. J. Lavigne, Y. Sohn, J. T. McDevitt, E. V. Anslyn, D. P. Neikirk, J. B. Shear, *Anal. Biochem.* **2001**, *293*, 178 – 184.
[72] A. Furka, F. Sebestyen, M. Asgedom, G. Dibo, *Int. J. Pept. Protein Res.* **1991**, *37*, 487 – 493.
[73] D. Leipert, D. Nopper, M. Bauser, G. Gauglitz, G. Jung, *Angew. Chem.* **1998**, *110*, 3503 – 3505; *Angew. Chem. Int. Ed.* **1998**, *37*, 3308 – 3311.
[74] P. Grandini, F. Mancin, P. Tecilla, P. Scrimin, U. Tonellato, *Angew. Chem.* **1999**, *111*, 3247 – 3250; *Angew. Chem. Int. Ed.* **1999**, *38*, 3061 – 3064.
[75] A. M. A. van Wageningen, R. M. J. Liskamp, *Tetrahedron Lett.* **1999**, 9347 – 9351.
[76] T. A. Dickinson, D. R. Walt, J. White, J. S. Kauer, *Anal. Chem.* **1997**, *69*, 3413 – 3418.
[77] S. E. Schneider, S. N. O'Neil, E. V. Anslyn, *J. Am. Chem. Soc.* **2000**, *122*, 542 – 543.