REPORT

WORKSHOP ON NEW DEVELOPMENTS IN CHEMICAL SEPARATIONS FROM COMBINATORIAL CHEMISTRY AND RELATED SYNTHETIC STRATEGIES

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INTRODUCTION

The power of combinatorial chemistry and related high throughput synthetic strategies is currently being pursued as a fruitful way to develop molecules and materials with new properties. The strategy is motivated, for example in the pharmaceutical industry, by the difficulty of designing molecules to bind to specific sites on target biomolecules. By synthesizing a variety of similar structures, and then finding the one that has the most potent activity, new so-called lead structures will be found rapidly. Existing lead structures can be optimized.

This relatively new approach has many implications for separation science. The most obvious is the call for more separations power: higher resolution, lower concentrations, higher speed. This pressure butresses the traditional directions of research into the development of more useful separations. The advent of chip-based, electroosmotically pumped systems[1] will certainly accelerate progress in this traditional direction.

The progress in combinatorial chemistry and related synthetic strategies gives rise to two other, broadly significant possibilities for large changes in separation science. One possibility results from the unique requirements of the synthesis of a huge number of products simultaneously. Can syntheses and separations be designed to work together to create *strategies* that lead to mixtures containing only desired products but without side products? The other possibility results from the need for molecular selectivity in separations. Can combinatorial syntheses and related strategies be used in the development of *better separations media*?

A workshop in two parts was held. In one half-day session, pedagogical presentations educated across the barriers of discipline and scale. The speakers were Dr. Daniel Flynn, Monsanto; Prof. Dennis Curran, Department of Chemistry (DOC) University of Pittsburgh; Prof. Richard Willson, Department of Chemical Engineering (DOCE) University of Houston; Prof. Peter Carr, DOC, University of Minnesota. In the second half-day session, the participants broke into small groups to flesh out new ideas. A panel summarized the breakout discussions. Panelists included, Prof Steve Cramer,

DOCE, RPI; Prof. Ruben Carbonell, DOCE, North Carolina State University, Yen-Ho Chu, DOC, Ohio State University, DOC, Abraham Lenhoff, DOCE, University of Delaware, DOCE, Ralph Nielsen, Symyx Technologies, Frantisec Svec, DOC, University of California, Berkeley.

BACKGROUND

Chemical selectivity in separations.

Historically, many media have been used in chemical separations[2]. Despite this sustained effort of hundreds of scientists over decades, it has recently been suggested that, for analytical separations, the most sensible way to achieve a "clean" separation of a desired component from undesired components is not to play with the chemistry of the system of choice, but rather to make the separation more efficient[3]. The simple reasoning is that, in a difficult separation (taking chromatography as the technique, a "crowded chromatogram"), a change in the chemistry will only serve to rearrange the peaks. The advantage gained by resolving formerly unresolved components is lost by formerly resolved components becoming mixed.

This is not a rewarding message for a chemist. It is an even less appealing message for the engineer doing preparative chromatography where theoretical plates are sacrificed for throughput. Can we not do better by preparing phases with a certain selectivity to carry out a particular operation? Without question, the answer is yes. This has been demonstrated most convincingly in the developments in chiral separations, e.g., the development of Pirkle[4] phases. In these phases, detailed knowledge of solute properties allows for the estimation of the separability of R and S forms. Thus separation scientists, given sufficient time, can develop phases that are tailored to a particular separation problem.

Perhaps a little more subtle is the question of what a phase will not do. Can one prepare a membrane that will be a good environment for one solute and a poor environment for another, or can one design a surface with similar adsorptive characteristics? It is much easier to design for strong interactions with a particular solute than for weak interactions with the *n* solutes that are not desired.

Combinatorial synthesis.

The burgeoning interest in combinatorial[5] methods for the synthesis of new compounds and materials has resulted in the articulation of a number of remarkable strategies for obtaining the desired compounds from a mixture, or for understanding the structure of lead compounds. A brief overview of the strategies involved is provided below. The excellent review by Balkenhohl *et al*. is highly recommended[6]

The objective of high throughput synthesis is to find efficiently new compounds or materials that have a defined purpose. Initially conceived as a method whereby biologically active molecules could be found rapidly, the idea has been shown to be applicable in other spheres, notably in materials science;[7] superconducting materials;[8] magnetoresistance materials;[9] phospors;[10] metal complexing ligands;[11] catalysts;[12]

polymers;[13] sensors.[14] In both sorts of endeavors, synthesis geared towards biologically active compounds or new materials, there is a similar strategy involved.

- The synthesis must create a large number of candidate molecules/materials to maximize the probability of finding something useful. However, if the synthesis results in a mixture of all of the products in one solution, the larger the number of products, the more dilute each one is in the final solution.
- Separations that remove reagents and/or byproducts from products must be integrated into the overall process. While it is not at all unusual to consider the separation of the products in designing a scheme for a synthetic procedure, the level of complexity provided by combinatorial mixtures has focused new attention on the simultaneous design of synthesis and separations[15 16] Perhaps the Merrifield synthesis of peptides[17] is the most apt model for one aspect of this problem. At each stage in the synthesis of a support-bound species, the reagents can be removed from the product. Of course, the solid phase creates certain problems, namely the removal of the product from the phase, and mass transfer limitations
- A process that reveals molecules/materials with the desired properties must be designed and carried out. This is generally called "screening";
- Analysis of the useful molecules/materials must be done in order to be able to design the synthesis of macroscopic quantities of the lead compound/material;

The synthesis and screening procedures can take a variety of forms. Briefly, the synthesis can be on a solid support, in free solution, or attached to a polymer or other molecule that makes selective separation possible. There are several approaches to adding building blocks[18] that apply to each of the "support options" above. *Mixture* synthesis refers to the case in which a polyfunctional molecule is exposed to a mixture containing many varieties of orthogonally reactive species (Figure 1A). If there are n_a varieties of species A, and n_b of B, and so on, then in principle there will be $n_a \bullet n_b \bullet n_c$ different products. The attraction of the approach is its simplicity. The major drawback is that the reaction rates of all molecules of type A will not be the same, so that a statistical mixture will not result. This problem was solved by Furka, who developed the *split* or *split bead* approach. Here, let us take an example of a synthesis, like a peptide synthesis, in which the elongation of a chain results at each step. A mixture of n_a varieties of A is exposed, in n_b separate containers, to an excess of each variety of B. Following completion of the reaction and separation of excess B, the products are combined. The result is a mixture of all combinations of A and B. This mixture is then reseparated into n_c containers, etc. (Figure 1B). Finally, the completely parallel synthesis is that in which $n_a \bullet n_b \bullet n_c$ different "containers" are used. Each molecule of a mixture ends up isolated in space.

As implied above by the manifold applications of high throughput synthesis, the screening procedures depend upon the application. Screening for catalytic activity may require product analysis, or detection of heat generation; screening for enzyme inhibition may require the same. Screening for artificial receptors requires that binding to a substrate be detected. The identification of the most promising compounds in a library is fairly straightforward in the parallel synthesis case. There must be a way to detect binding, e.g., by a fluorescent label, but once binding is discovered, the structural analysis of the artificial receptor may proceed without further effort. In the mixture and

split approaches, it is typical to synthesize sublibraries, and analyze binding of the sublibraries to determine the most active compounds in the library. For example, in the split approach, assume that this is a polymer bead supported synthesis with the first step being to prepare bead-bound A_1, A_2, … A_{n_a}. It will help to use the designation "R_A" to denote all varieties of reactant A. Instead of mixing all of these beads together, leave a portion separated. Thus, one has a container of supported A_1, a container of supported A_2 …. A_{na}, and a mixture of all of them. Divide *each* of these into n_b containers, do the reaction with each of the n_b varieties of B. Remix the n_b containers that have a known A residue, but set aside n_b containers that have all varieties of A and a known B. At this point, one has A_1R_B, A_2R_B,… $A_{n_a}R_B$; R_AB_1, R_AB_2, …$R_AB_{n_b}$, and R_AR_B. A screen carried out at this stage might identify mixtures A_1R_B and R_AB_2 as being active. The conclusion, which can be confirmed by synthesis, is that A_1B_2 is an active species. This is one example of a process known as *deconvolution* whereby key residues are discovered through the activity of sublibraries.

QUESTIONS

The conjunction of fields, separation science and synthesis of compounds and materials, has brought to light new questions, namely

Can combinatorial chemistry be used to develop new methods of separation?

It should be possible to create new separations from libraries. Bioseparations could be based on new protein or DNA binding ligands. New membranes could be envisioned with molecular transporters that result from libraries. Properties beyond simple molecular recognition could be developed, for example, antifouling surfaces for membrane separations.

Can separations be easily designed and implemented for the generation of large libraries free of side products?

It should be possible to design phases that will remove completely all spent reagents from a reaction mixture, or to remove other undesired products. It would be very important to have synthesis/separation strategies that yield good results at industrial or chip scale.

DISCUSSION

New Separations

The question of whether high throughput synthesis can be used to developed new methods of separation can be answered in the affirmative.

For example, the UC Berkeley group[19] synthesized a solution library of 36 L-amino acid anilides, which are potential selectors for chiral HPLC, and attached them to functionalized macroporous polymer beads. The best selector from the library was identified by a deconvolution process using the HPLC separation of racemic α-amino acid alkyl amides as a probe. The best chiral stationary phase (CSP) with a remarkable separation factor of 26 for the desired separation was identified in only a few

deconvolution steps. As a result of the "parallelism advantage", the number of columns that have to be screened in this combinatorial approach on the road down to the most selective, single selector CSP is much smaller than the number of actual selectors in the initial library (Figure 2). In a different approach, the Berkeley group used the principle of *reciprocity* (Figure 2) which has been defined by Pirkle. Thus, they used the single step Biginelli multicomponent condensation to prepare a parallel library of 108 4-aryl-1,4-dihydropyrimidine (DHPM) enantiomers which are potential selectors for chiral HPLC separations. The individual compounds were screened by observing the enantioselectivity on a "brush-type" chiral stationary phase with immobilized *target* enantiomer. Separation factors of up to 12 were achieved. The best candidates from the library were prepared as a single enantiomers and attached to monodisperse macroporous beads affording novel polymer based SCP with excellent enantioselectivities for the target.

Chemical libraries offer great potential for developing novel methods of separating pure components from complex mixtures by molecular recognition. For example, the large-scale affinity purification of human proteins destined for pharmaceutical applications can be done using immobilized antibodies. However, antibodies are immunogenic, costly, and chemically fragile. Carbonell, Hammond and co-workers have shown that combinatorial peptide libraries can be used to identify peptide ligands that can be used for the affinity purification of pharmaceutical proteins from human plasma.[20,21,22,23,24,25] Phage display libraries[23] as well solid phase chemical libraries of small peptides have been screened using antibody detection[22] and amplication of radiolabels[25]. Soluble libraries have also been screened using MS/MS sequencing of peptides eluted from a chromatographic column with immobilized target protein[24]. Peptides have several potential advantages over the use of antibodies for large scale affinity purification[20,21]. They are non-immunogenic, they can be produced under GMP conditions at relatively low cost compared to an antibody, and they can withstand the harsh conditions required for elution and washing of columns. Several peptides have been identified so far that are able to bind to Fibrinogen[25], Faxtor IX[22], van Willebrand Factor[24], α1 Proteinase Inhibitor and recombinant Factor VIII. Some impressive purification factors from real mixtures have resulted from this type of separation[20,21,22,23], and the technique seems applicable to a large number of different systems for various other applications.

Strategic Separations

Syntheses that yield high purity products are required for the generation of clean libraries. It makes sense to develop synthetic approaches that allow for rapid, "binary" separations in which the desired product is completely separated from other compounds.[26] One series of approaches is termed polymer-assisted solution-phase (PASP) synthesis. [27,28,29,30] In this strategy, advantage is taken of the inherent reactivity of reagents and molecular recognition of byproducts by specific receptors to remove reagents and byproducts from a solution containing product. If catalysts or other less reactive reagents are used, then they may be labeled to give them properties that allow their effective removal from solution[31]. For example, in a reaction of a nucleophilic substrate such as an amine, with *excess* electrophilic reagents (isocyanate, acid chloride, etc.), mass action forces a high yield of desired product, but it ends up in a sea of

unreacted electrophiles. These may be removed through the addition of a polymer-bound amine. The excess reagents, reacting with the polymer, become polymer-bound, thus easily removed (Figure 3) from the solution.

Byproducts are often unremarkable and unreactive, so that covalent sequestration is not possible. However, they may be ionic, in which case simple ion exchange resins can be used for their removal. They may also be reactive, but weakly. There are clever strategies for making weakly reactive species sequesterable through the use of bifunctional reagents. For example, tetrafluorophthalic anhydride (TFPA) reacts with weak nucleophiles such as anilines and alcohols to form a product that is a weak acid. The weak acid can be deprotonated leading to an anionic product, separable using ion exchange. Furthermore, the excess TFPA can be removed through the use of an amino resin!

In ideal circumstances, it would be a simple matter to sequester, reversibly, the product, leaving behind all other compounds. This may be possible if there is a reversibly reactive functional group on the product, or if one can take advantage of acid/base/ion exchange sorts of separations.

Labeling strategies have been mentioned. One particularly interesting strategy[32] takes advantage of the low polarizability of fluoroalkanes. Fluoroalkanes have the added advantage that they are unreactive. The strategy is to label components of the reaction with a fluorocarbon, or fluorous, tail. Then, by virtue of the tail, fluorously labeled molecules will partition into fluorous liquids, such as perfluorohexane. This leads to a separation strategy, as fluorous liquids are generally immiscible in organic solvents and water. Thus, three phase extractions can be carried out. A problem, of course, is that the fluorously labeled reagent is not soluble in many useful solvents, and so doing chemical reactions may seem next to impossible. Fortunately, there are solvents and solvent mixtures that can be used to dissolve compounds with fluorous tails and more common organic reagents. Benzotrifluoride (α,α,α-trifluorotoluene) is one, tetrahydrofuran may be useful. An example of how this separation/synthesis works is shown in Figure 4.

An interesting problem arises. How many fluorines on a molecule will render it fluorophilic, hydrophobic, and organophobic? This must depend on the size of the organic portion of the molecule. The good news is that the partition coefficient (fluorocarbon/organic solvent) increases as the number of fluorines on a molecule increases. The bad news is that this results from a decreasing solubility of the molecule in the fluorous phase, and a more steeply decreasing solubility in the organic phase, as the number of fluorines increases.

It is evident from the foregoing paragraphs that concepts, materials, unit operations, and molecular behavior common to separation science will be integrated into synthetic protocols. New approaches to these sorts of separations are urgently required. The chemistry must be reliable, must not place undo restrictions on the syntheses used, should ideally involve recycling of resins/polymers/solvents, and should be amenable to automation.

Screening

A challenge to separation science also exists when the library is complete. How do you know what you have? How do you find what is interesting and analyze it? These questions revolve around screening: the operation during which useful species are

recovered from the library. The simplest screen is that in which specific binding is sought. Then, by merely exposing the library components to the desired complementary chemical species, the components that interact can be picked out and analysed. This sounds simple, but in practice there are subtle issues. For example, in using phage display libraries, in which viruses express the protein products on their external surface, multidentate binding should be avoided. The virus particles that are wearing molecules that bind to the chosen complement are fetched out of solution by virtue of this binding ability. But they must be dissociated from the medium containing the chosen complement, e.g., magnetic particles. If the binding is too tight, as it may be if several molecules are expressed on the virus, then the virus will not be recoverable. Another example is in affinity capillary electrophoresis[33], where relative migration velocities, concentrations, and detectability all must be working in one's favor to carry out the experiment successfully (Figure 5).

Instruments that process samples rapidly are attractive for screening. Flow cytometry and electroosmotically pumped chip-like instruments have the capability to pass many samples per unit time. These can be expected to be adapted to the screening problem more in the future.

The real challenges, however, are in screening materials for things other than binding.[34] For example, infrared detectors that can be used to determine local heating, therefore the presence of a reaction; or the IR could be used to detect a key reaction product, like CO_2 to screen for catalysts or membrane permeation (Figure 6).

The issue of the power of separations arises. Whether it is the power to analyze libraries, or the anticipation of new power arising from new separations media developed with high throughput processes, it is important to understand what the limits are. Giddings and others have pointed out the dynamics aspects of separations power, but where do the chemical limitations lie? This issue can be discussed in the context of examples from liquid chromatography, on the foundation of solvatochromism.[35]

There is tremendous power to be gained by doing separations in multiple dimensions. This has been demonstrated most convincingly by the 2-D electrophoresis experiment in which proteins are separated by isoelectric focusing in one dimension, and by SDS-PAGE in the perpendicular direction. Staining and imaging are used for detection and data storage. More recently two-column techniques have been developed for comprehensive 2-D separations.[36] To be successful, the separations that are combined must be, in an abstract sense, orthogonal. This is widely recognized. But what does this mean in chemical terms? There is a related question. Most separations involve several retention controlling variables. If an initial attempt at a separation is broadly successful – all injected components elute, there is enough peak capacity for the sample – but locally unsuccessful because a couple of peaks overlap, which variables should be altered? Again in the abstract, the variable altered should be the one that is orthogonal to the major retention chemistry – only in this way will differential changes in retention occur. But, again, what does this mean in the laboratory?

Values of log(k') (from 22 aromatic compounds) on several hplc systems can be correlated to reveal how similar the systems are. If a single solute-dependent factor controls retention, then changes in the strength of that interaction cannot give rise to changes in the order of elution of the components. Thus, in simple reversed-phase systems without secondary equilibria, changing the mobile phase strength by adding or

removing the organic phase, cannot rearrange the peaks. Changing the solvent may have a modest influence – from among the most commonly used three solvents of methanol, acetonitrile and tetrahydrofuran, the first and third are the most different. On the other hand, carbon-based reversed phases are much different than alklysilane-based phases. For these 22 solutes, the correlation coefficient among values of log(k') on a carbon (on zirconia) phase and a polybutadiene (on zirconia) phase was only 0.38. Compare this to the correlation coefficients for different aqueous/organic mobile phases on the same C8 silica-based reversed phase, which were all over 0.9 (Figure 7).

Values of log(k'), being proportional to the free energy of the retention process, can be correlated to a small series of numerical estimates of certain solute properties X_i, Y_i, …, such as molar volume, dipolarity, acidity and basicity. The correlation equation that results from correlating log(k') and the solute properties yields regression coefficients (a,b,…) for each of the solute properties ($\log(k_i') = aX_i + bY_i + \ldots$). The magnitude and statistical significance of the coefficients (a,b,…) reflect the degree to which a particular separations system uses (responds to, takes advantage of each molecular property (X_i, Y_i). These coefficients, then, are quantitative measures of the orthogonality of separations techniques, or separations conditions. The most different techniques would have the most "different" sets of parameters. Two techniques that were correlated to 4 solute properties that had coefficients 0,1,0,0 and 0,0,1,0 would be as orthogonal as the chemical property represented by the 2nd and 3rd parameters was. On the other hand, separations techniques that had coefficient values of 0.4, 0.5, 0.5, 0.4 and 0.2, 0.25, 0.25, 0.2 could not be used together to improve a separation based on one of the techniques alone.

PROBLEMS, CHALLENGES, AND OPPORTUNITIES.

Breakout sessions. Sessions were chaired by Abraham Lenhoff (U. of Delaware) and Peter Carr (U. of Minnesota) (*The limits of chemical selectivity in separations*); Ralph Neilsen (Symyx) and Richard Willson (U. of Houston) (*Merging screening and separations in materials synthesis);* Frantisek Svec (Berkeley) and Ruben Carbonell (NCSU) (*Managing libraries for specific ligands*); Steve Cramer (RPI) and Dennis Curran (U. of Pittsburgh) (*Engineering aspects of sequestering*),; Yen-Ho Chu (Ohio State University) (*Electroosmotically driven separations*) and Daniel Flynn (Monsanto) (*How pure does a lead compound need to be?*).

The limits of chemical selectivity in separations.

Separation science can provide support for combinatorial chemistry in several ways. Traditional separations ideas can be successfully applied to many easy and even some difficult separations, but specific new challenges that are posed by the new applications must be addressed. Adequate specificity must be provided for, particularly in making possible separations of very similar materials. Both thermodynamic versatility, e.g., in providing tunable selectivity (most conveniently via the mobile phase), as well as dynamic behavior in providing for adequate peak capacity, can be exploited in seeking such capabilities. Longer term goals should include better capabilities for predicting the outcome of separations in order to rationalize separation strategies and tactics, ways to

model and design novel media, and exploration of the feasibility of novel principles of interaction that can be harnessed in separations.

New fundamental information should be coming from libraries. Separation scientists often study the influence of molecular structure on the behavior of solutes in separations systems. Generally, the variations in molecular structure are limited by the availability of pure compounds. Let us turn this around. Do we study homologous series so frequently because we have available at a reasonable cost and at high purity a significant number of members of the series R-$(CH_2)_n$-R' ? What could we learn if we had dozens of series of compounds equally rich? High throughput methods are an ideal way to obtain these hypothetical series.

Merging screening and separations in materials synthesis.

Screening for processes rather than molecular interactions is a particularly significant challenge. Many materials that give rise to devices or elements in a process are mixtures. The synthesis of libraries of mixtures could be screened by separations techniques. Clearly this will be time consuming, however it probably saves time over constructing process elements and screening them for behavior. For example, screening polymer mixtures for molecular weight distribution or some other property probably will take less time than preparing semipermeable membranes from them and testing their efficacy in a particular process. One must, of course, understand the relationship between the polymer properties and the membrane function. Perhaps libraries of materials could aid in composing this sort of composition/function relationship. In any case, with in this arena there is a very clear time/information tradeoff.

Potential targets for this kind of multicomponent library are catalysts, inorganic materials, and polymers. Success has been achieved in inorganic materials synthesis. Polymer libraries have been screened for molecular weight distribution. Obstacles to progress include, besides the incredible challenges of screening, the interdisciplinary nature of the problem. At the same time, there are opportunities, especially in inorganic materials chemistry, where high throughput approaches will replace "shake and bake".

Engineering aspects of sequestering.

In the preparation of small molecule libraries, there are many engineering aspect that accompany the chemical challenges. Sequestering (of reagents, product, byproducts) can be carried out in three ways: extraction (liquid/liquid), packed bed (solid/liquid) and by membranes. Issues that arise in deciding which route to take are: the affinity of the sequestration chemistry, the need (or lack of) for recycling the sequestering reagent, the type of operation (flow through or batch), and the kinetics of mass transport. Curran has shown that fluorous phases may be used with some selectivity. Are there other chemical label/phase pairs that will allow selective extraction of only those molecules labeled with a particular moiety? The His-tag used in immobilized metal ion affinity chromatography might be applied outside the sphere of protein recovery. Ag^+ and Hg^{2+} are well known to associate with unsaturated functional groups. Can this provide the bases for a "binary" separation?

Managing libraries for specific ligands.

Perhaps it would be simpler to combine partitioning and reaction to improve selectivity. Of course if the reaction is covalent, then the issue of recovery and recycling enters. The reaction used to augment selectivity in partitioning should be reversible. Perhaps there are ways to cause the ligand/product of choice in a library to precipitate under particular conditions, such as temperature, that are easily controlled.

Polymeric sequestering auxiliaries are typically based on swellable polystyrene beads (1-2% crosslinked) although macroporous scavenger resins have also been introduced. However, manipulation with beads is cumbersome. Researchers at UC Berkeley have developed macroporous polymers in entirely new monolithic format[37]. The flow-through nature of these materials is intrinsically amenable to automated processes required for creation of large combinatorial libraries. For example, monoliths can be prepared by in-situ polymerization within the well of any shape (square, round,…) of a suitable microplate. The purification can be then easily reduced to a simple, single-step reactive filtration.

Electroosmotically driven separations

Separations are also important to small molecule libraries. For library characterization, important issues such as reaction stereoselectivity and racemization during library synthesis have hardly been addressed. In comparison to chiral HPLC, affinity capillary electrophoresis should be able to contribute significantly in addressing these issues, since it offers the advantages of low cost, hight efficiency, and , particulary, rapid method development and optimization. Affinity capillary electrophoresis can now be used to marshal 96 samples from injector to detector with good resolution. Thus, in one continuous run, samples from the 96 wells of a microtiter plate can be injected and exposed to a potential receptor molecule. Missing peaks in the 96 peak parade identify leads. Molecules and materials of use in sensors could be useful in separations, and *vice versa*. Chip-based manipulation of reagents and separations will be welome. The scaling down of volumes should allow for easier management of libraries. Processes that employ this "fishing out" concept are around for more than 2 decades[38] and only recently have been used in the combinatorial synthesis[39,40]

How pure does a lead compound need to be?

The purity of a compound that has come from a library depends on purpose of the project. For lead generation, purity does not need to be very high. However for something like quantitative structure activity relationships, purity should be very high. Deconvolution strategies, as in the early Geysen work, are still useful. Mass spectrometry is useful for understanding impure leads. Leveling of selectivity can be achieved by determining binding in a medium containing a large quantity of a somewhat weakly bound ligand.

RESEARCH NEEDS:

1. Basic chemistry to determine the nature of specific interactions between atoms and molecules and the factors that contribute to molecular recognition, specificity and avidity, including the role of solvents.
2. Studies of novel engineering principles for the development of high throughput methods to screen libraries.
3. Molecular modelling efforts to try to understand the factors in item 1.
4. Interfacial science studies to understand the role of the support of a solid phase library on specificity and avidity to the ligand.
5. Applications of green chemistry and atom economy to make the synthesis of chemical libraries more efficient and environmentally friendly.
6. Novel applications of chemical libraries to the development of new separation methods, sensors, catalysts, materials, etc.
7. Mathematical, statistical, modelling and/or chemical approaches to lead optimization. How does one minimize the number of variables in the library to arrive at a good hit?
8. The development of robust, reversible selective extraction media.

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FIGURE LEGENDS

1. A. In the mixture synthesis, a polyfunctional core is exposed to a mixture containing n_a different compounds that react at one of the sites on the core, n_b of another, and so on. B. The split synthesis strategy has separate containers for the preparation of supported A. The As are mixed, and reseparated into n_b containers where the A-mixture reacts with B_1, the A-mixture reacts with B_2 and so on.

2. A schematic picture of the development of a chiral column using a high throughput synthesis. The library of potential selectors is generated. It is screened by using an affinity column with the target, enantiomerically pure analyte. Racemic members of the library are separated on the target column. Those racemates that are well separated are candidates for the preparation of a column that is capable of separating racemic target analyte.

3. Polymer or bead-bound reagents sharing reactivity properties with the substrate can be used to remove unreacted reagents.

4. The three-phase partitioning puts the reaction products in the fluorous phase and byproducts in the organic and aqueous phases until the last step when the fluorous silane ends up in the fluorous phase with the product alone in the organic phase.

5. ACE of an all-D library of Fmoc-DDXXX (10^2 = 100) tetrapeptides to search for ligands that bind tightly to vancomycin. In the procedure, vancomycin (70 μM) used as the receptor was first introduced into the electrophoresis buffer as a plug (10-50 s pressure injection), followed by a short plug of the library (3 s pressure injection), and the ACE experiment was carried out using a PVA coated capillary (27 cm total length, 20 cm effective length, 50 μM inner diameter) in 50 mM Tris-acetate buffer (pH 8.1) at 13 kV. Since vancomycin was slightly positively charged under experimental conditions, it migrated away from the detector. The whole library traveled towards the MS detector, and any ligands recognized by the receptor were retained and separated from noninteracting species in the library. This separation of ligands from the library can be readily manipulated by changing both the length of the receptor plug and the receptor concentration. Using 40-s injection of vancomycin (70 μM), three ligands in two peaks were detected and structurally identified by MS: Fmoc-DDFA, Fmoc-DDYA and Fmoc-DDHA. The electrophoresis buffer was used as the control to introduce into the capillary at corresponding plugs to ensure the specific binding of lead compounds to the receptor. The total electrophoresis time for each library screening was less than 4 min. Copied, with permission, from Chu, Y.-H.; Cheng, C. C. *Cellular and Molecular Life Sciences*, **1998**, *54*, 663.

6. IR thermographic image of candidate catalyst formulations at (a) 200°C under nonreactive conditions (O_2-free H_2 feed), (b) before ignition of the Rh-loaded pellet (reactor

temperature, 80°C), and (c) after the ignition of the Rh-loaded pellet (reactor temperature, 85°C).

7. A matrix of correlation plots. Seven separation systems (C8 bonded phase, Acetonitrile/Water 50/50; C8 bonded phase, Acetonitrile/Water 70/30; C8 bonded phase, Tetrahydrofuran/Water 50/50; C8 bonded phase, Methanol/Water 50/50; Polystyrene, Acetonitrile/Water 50/50; Polybutadiene-zirconia, Acetonitrile/Water 50/50, Carbon on zirconia, Acetonitrile/Water, 50/50) and 22 solutes. (P. W. Carr, unpublished)

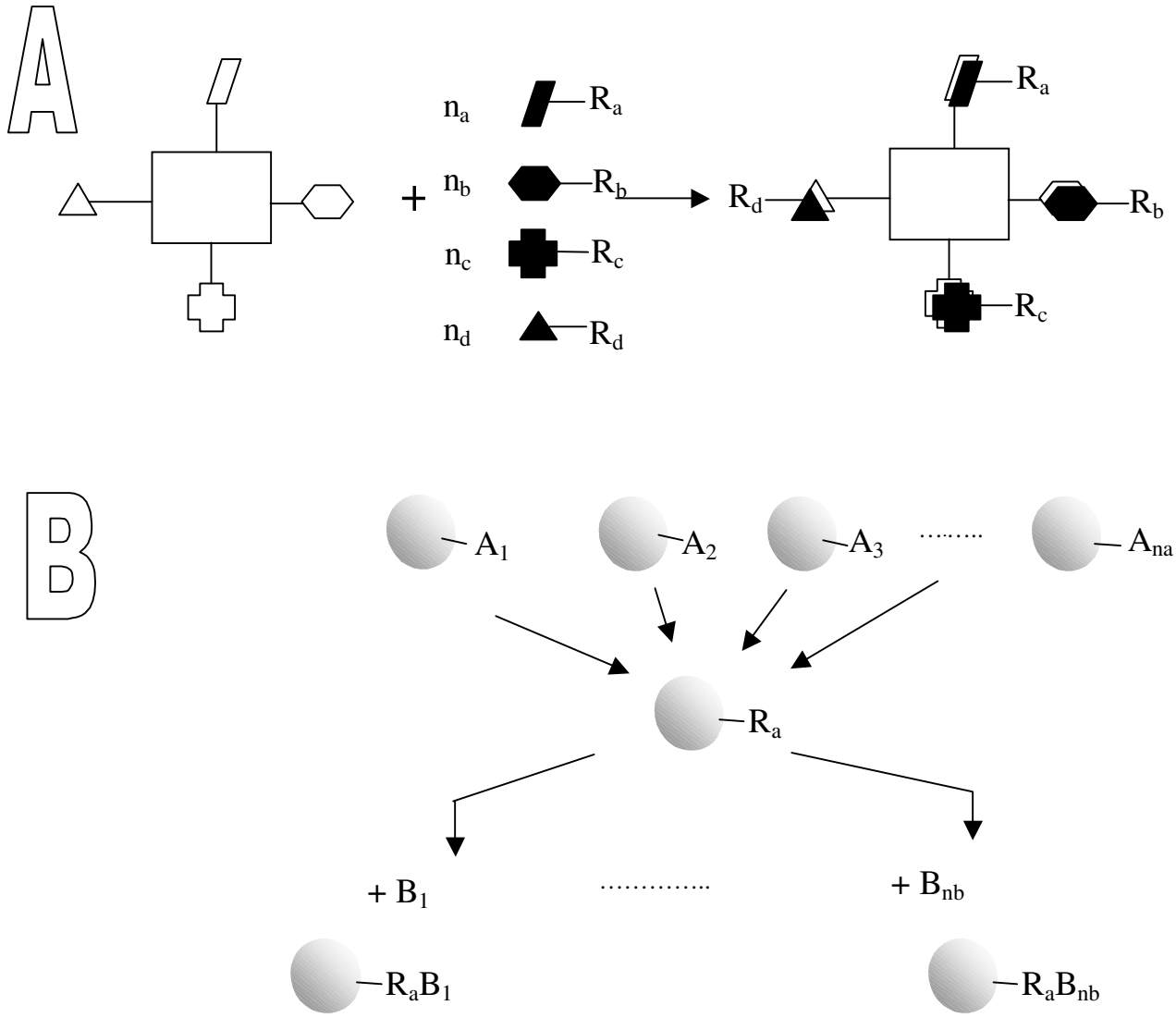

A
n_a
R_a
n_b
R_b
n_c
R_c
n_d
R_d
R_d
R_a
R_b
R_c
B
A_1
A_2
A_3
……..
A_{na}
R_a
+ B_1
……..
+ B_{nb}
R_aB_1
R_aB_{nb}

Figure 1

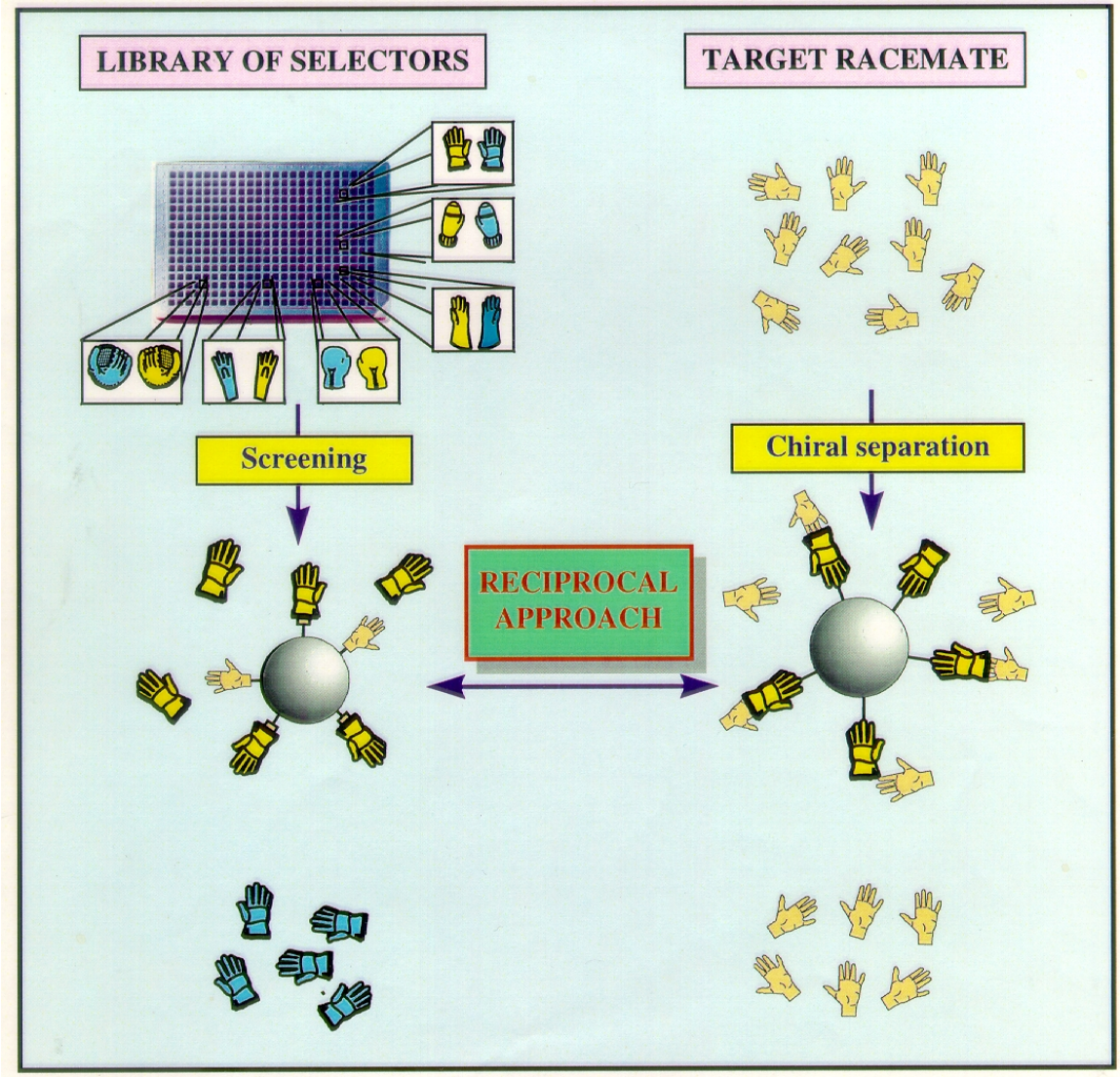

LIBRARY OF SELECTORS
TARGET RACEMATE
Screening
Chiral separation
RECIPROCAL APPROACH

Figure 2

1 eq RNH2 + 10 eq. R'COCl ⟶ 1 eq. RNCOR' +

9 eq. R'COCl

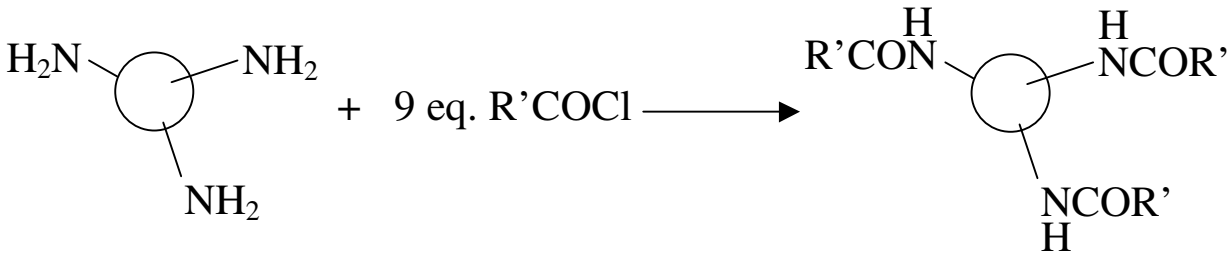


Figure 3

Fluorous Synthesis Illustrated in a Multi-layer Reaction Scheme

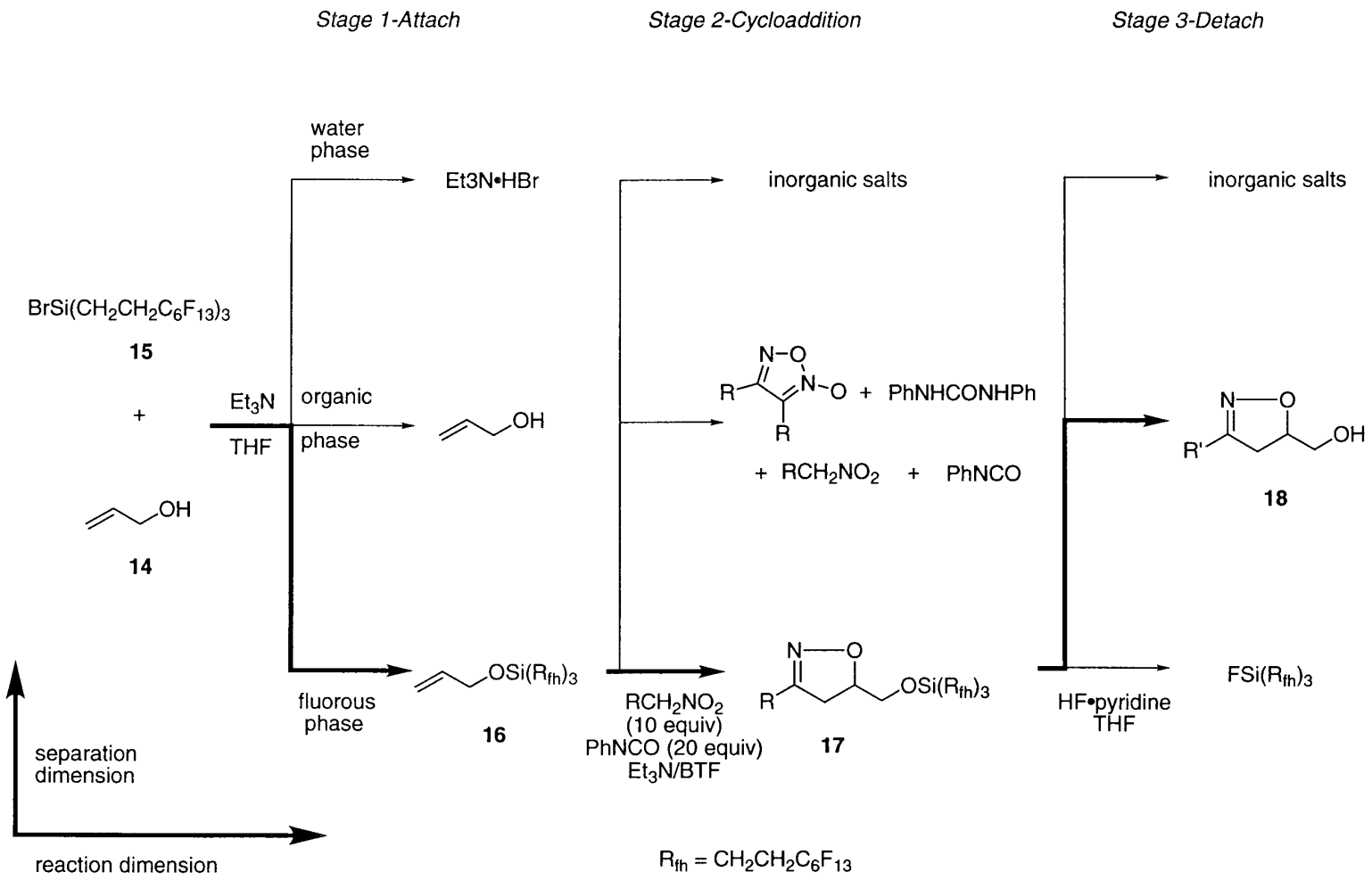


Figure 4

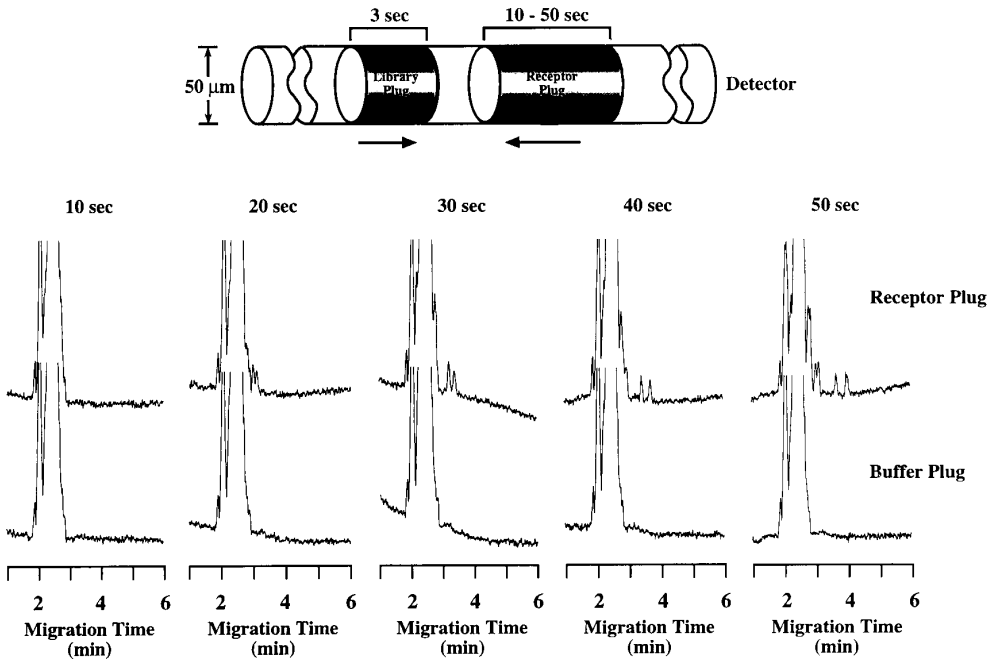
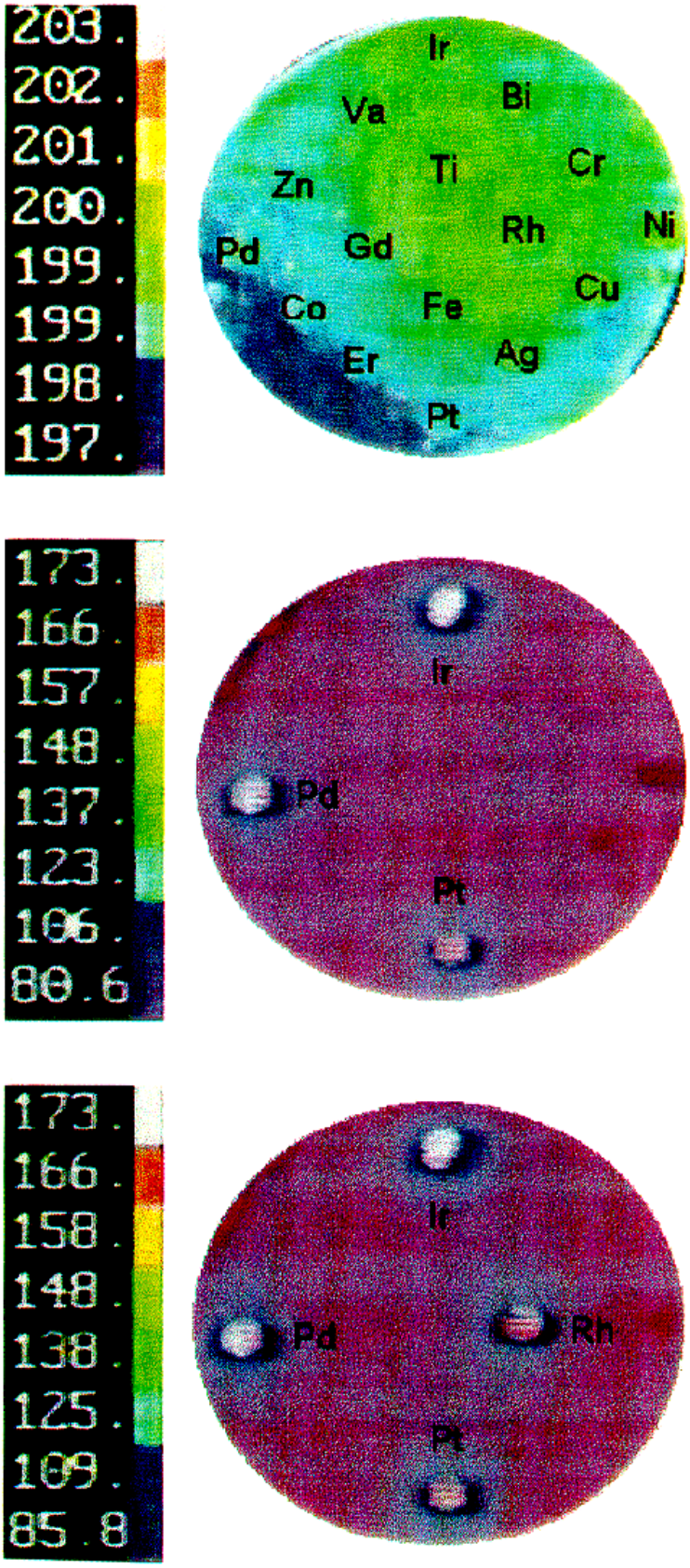

3 sec
10 - 50 sec
50 µm
Library Plug
Receptor Plug
Detector
10 sec
20 sec
30 sec
40 sec
50 sec
Receptor Plug
Buffer Plug
2
4
6
Migration Time (min)

Figure 5


203.
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Ir
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Cu
Co
Fe
Er
Ag
Pt
173.
166.
157.
148.
137.
123.
106.
80.6
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125.
109.
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Ir
Pd
Rh
Pt

Figure 6

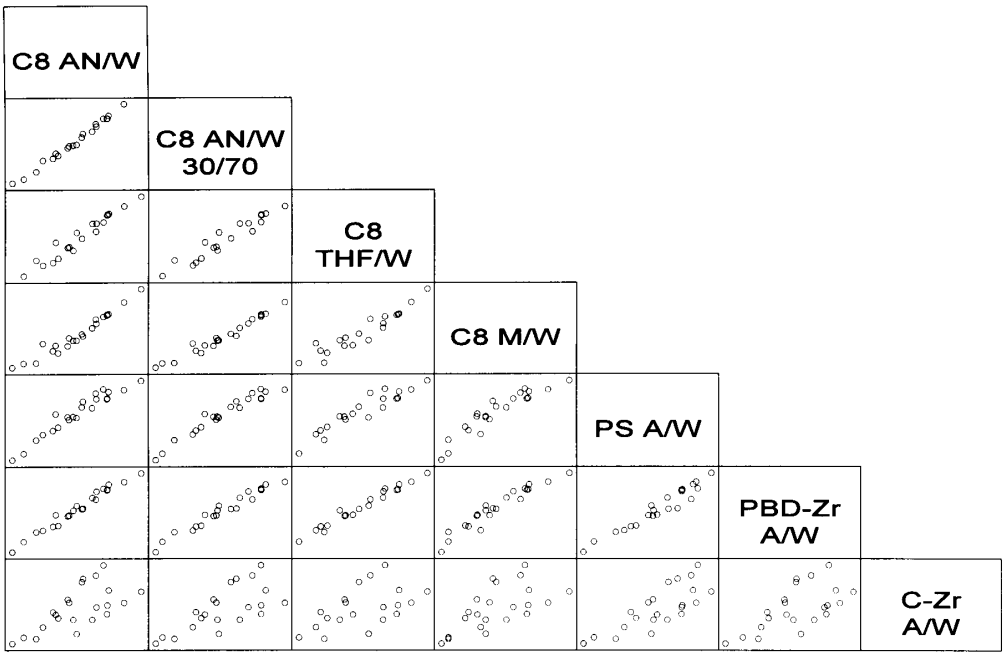

Figure 7