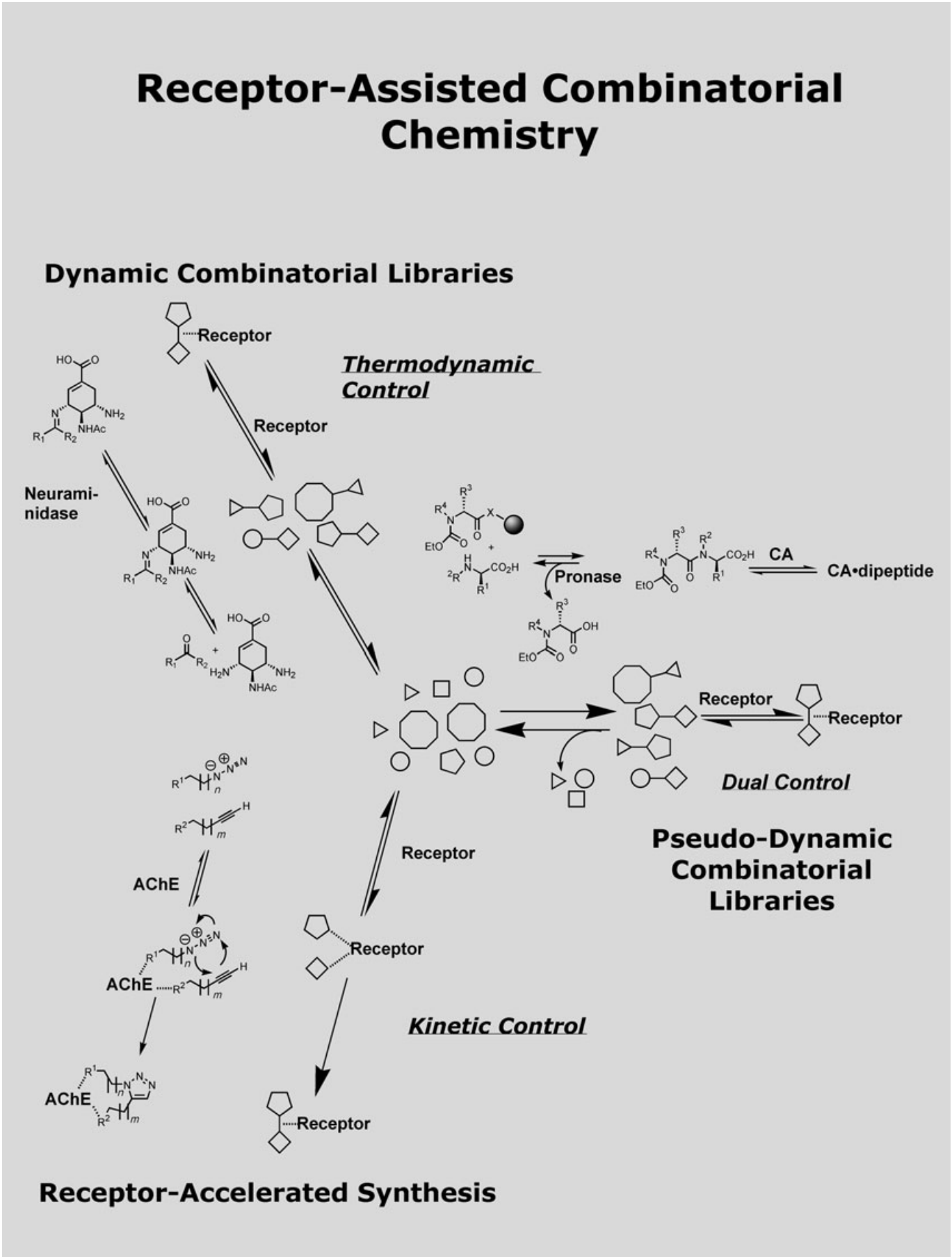

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Receptor-Assisted Combinatorial Chemistry: Thermodynamics and Kinetics in Drug Discovery

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Abstract: Current drug discovery using combinatorial chemistry involves synthesis followed by screening, but emerging methods involve receptor-assistance to combine these steps. Adding stoichiometric amounts of receptor during library synthesis alters the kinetics or thermodynamics of the synthesis in a way that identifies the best-binding library members. Three main methods have emerged thus far in receptor-assisted combinatorial chemistry: dynamic combinatorial libraries, receptor-accelerated synthesis, and a new method, pseudo-dynamic libraries. Pseudo-dynamic libraries apply both thermodynamics and kinetics to amplify library members to easily observable levels, and attain selectivity heretofore unseen in receptor-assisted systems.

Keywords: combinatorial chemistry · drug design · inhibitors · receptors · template synthesis

Introduction

Combinatorial chemistry methods in drug discovery currently favour focused libraries, which use templates or functional groups to provide affinity for the desired receptor.[1,2] An emerging method in combinatorial chemistry, receptor-assisted combinatorial chemistry (RACC), not only uses focused libraries, but also adds stoichiometric amounts of the receptor during the library synthesis. Addition of the receptor biases the synthesis toward the best binding compounds, thereby combining the synthesis and screening into one step. In addition, analysis avoids specific receptor assays, but detects increased amounts of the best-binding compounds with established analytical methods such as HPLC, mass spectrometry, NMR spectroscopy or even X-ray crystallography.

Three RACC methods have emerged: dynamic combinatorial libraries, receptor-accelerated synthesis, and a new method, pseudo-dynamic combinatorial libraries (Table 1, Figure 1). In this review, we discuss how these methods use thermodynamic control, kinetic control or both to increase the relative amounts of the best binding compounds during synthesis. We will emphasize the new pseudo-dynamic combinatorial library method since recent reviews of the other methods, especially dynamic combinatorial libraries, are available.[3]

Table 1. Drug discovery approaches using receptor-assisted combinatorial chemistry.

Approach	Description	How $\Delta G_{binding}$ is used
Dynamic combinatorial chemistry	Reversible reaction creates a library; binding to the receptor shifts the equilibrium	Thermodynamic binding shifts the equilibrium
Receptor-accelerated synthesis	Binding of components to receptor accelerates synthesis of best inhibitors	Binding and proximity increases rate of coupling
Pseudo-dynamic combinatorial chemistry	Formation and destruction of library are separate irreversible reactions; binding slows destruction reactions	Kinetic destruction enhances the thermodynamic selectivity

An ideal RACC library will both amplify the tightest-binding library member and show high selectivity versus other components in the library. For this review, we define amplification as the relative increase of the amount of a li-

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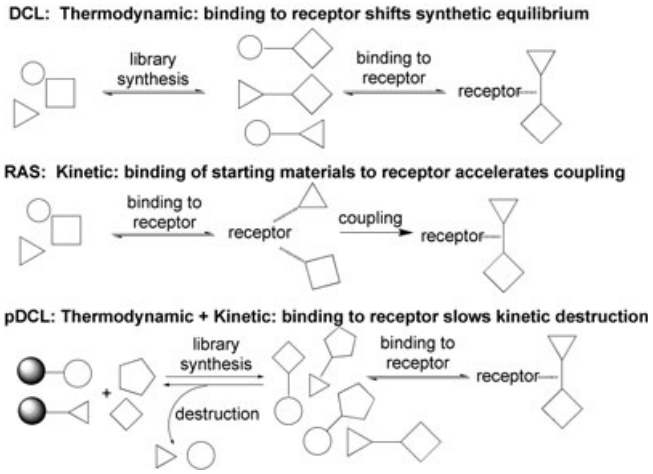

Figure 1. Three receptor-assisted combinatorial methods. In dynamic combinatorial libraries (DCLs), because library synthesis and binding to the receptor are reversible, thermodynamics control both amplification and selectivity. In receptor-accelerated synthesis (RAS), starting materials with strong receptor affinity come together in the active site and couple due to their proximity, forming a stronger inhibitor due to kinetic control. In pseudo-dynamic combinatorial libraries (pDCLs), kinetic destruction enhances the thermodynamic selectivity.

brary member upon addition of a receptor. We define the selectivity for a pair of library members as their relative amplification divided by their relative binding constants (see Table 2). Selectivities beyond one make it easier to distinguish between library members with similar binding constants.

Dynamic combinatorial libraries (DCLs) were the first RACC method to be developed. These libraries use synthetic equilibria to form mixtures of library members. The receptor binds the tightest-binding library members, removing them from solution. The synthetic equilibrium shifts to increase the amounts of these tightest binding library members according to LeChatelier's principle.

Balancing both the synthetic and receptor-binding equilibria in DCLs causes amplification. For a simple case—a compound isomerizes to form a library of compounds (e.g. through structural isomerization) that bind the receptor tightly, but with varying strengths—the product of synthesis and binding equilibrium constants for each isomer give the relative amounts of each isomer.[4] Thus, the selectivity between two isomers in such a system cannot be greater than one.

However, most DCLs contain not only a single isomerizing starting material, but several components combining to form hetero- and/or homodimers (or hetero- and homo-oligomers). In these cases, selectivity may be either greater or less than one. In certain cases, the selectivity may even invert, where a less tightly bound library member is amplified more than more tightly bound species. Computational simulations indicate that these undesirable cases are possible when homo- and heterodimers compete and the homodimer binds more tightly than the heterodimer, and where a series of oligomers compete and an oligomer containing many building blocks binds more tightly than a library member formed from only a few building blocks.[5]

Table 2. Terms in receptor-assisted combinatorial chemistry.

Amplification	Ratio of the amount of a library member synthesized in the presence of a receptor as compared to its amount in the absence of the receptor, $[I_A]_{receptor}/[I_A]_{no\ receptor}$. In *pseudo*-dynamic libraries, all compounds are eventually destroyed in the absence of the receptor, so the amplification is infinite. In these cases, it is more useful to compare yield, which is the concentration of a compound in the binding chamber compared to the concentration of the receptor, $[I_A]/[R]$. The maximum yield is 100%.
Casting versus molding	Casting forms a small molecule using a receptor-binding site as a template. Drug discovery seeks to cast a drug lead using the receptor. Molding forms a receptor by surrounding a small molecule target. For example, molding forms a crown ether around an ion.
Receptor	Entity to which the library members should bind. In drug discovery applications, receptors can be cell membrane receptors, enzymes, interfaces for protein–protein interaction, sites on RNA or DNA, etc. In supramolecular receptor-building applications, receptors are the supramolecules that are evolved to bind the guest molecule.
Selectivity	In DCL and RAS selectivity is the amplification of two library members I_A and I_B, where I_A is the stronger binder, compared with their relative binding constants, $[([I_A]_{receptor}/[I_A]_{no\ receptor})/([I_B]_{receptor}/[I_B]_{no\ receptor})]$ $[(K_{a(I_A)})/(K_{a(I_B)})]^{-1}$. In pDCL, since all compounds are eventually destroyed in the absence of the receptor, any detectable amount of a library member would result in an amplification of infinity. For pDCL, the selectivity is $[[I_A]_{receptor}/[I_B]_{receptor}][(K_{a(I_A)})/(K_{a(I_B)})]^{-1}$, which compares the relative concentrations of the inhibitors to their relative binding constants.
Tethering	A dynamic combinatorial method often involving formation of disulfides where one component is a cysteine residue on the receptor. Tethering focuses the binding to the region near the cysteine.

Homodimers are not common in drug discovery where the goal is to fill a complicated, nonsymmetrical pocket in a biomolecule. In these cases, selectivity close to one is expected, although they may be greater or less than one. In practice, no one has reported selectivities greater than one in a drug-discovery context for thermodynamically controlled systems. However, adding a kinetic component (see below) can raise the selectivity beyond one.

In receptor-accelerated synthesis (RAS) the receptor binds several starting components and promotes their coupling due to proximity, forming a new, presumably tighter-binding species. The rate-acceleration of this coupling reaction identifies the best binding compounds and determines the amplification. Selectivity arises from two factors: binding of the starting components to the receptor and the ability of the receptor to catalyze their coupling, but both of these are difficult to predict. Upon coupling to form product, the binding interactions of the starting materials to the receptor may strengthen or weaken. Similarly, the rate acceleration due to proximity is difficult to predict because the receptor is not normally a coupling catalyst. On the

other hand, the reaction is kinetically controlled, thus, the selectivity may be very high.

A new receptor-assisted method, pseudo-dynamic combinatorial chemistry, uses an irreversible library synthesis, combined with an irreversible destruction reaction that regenerates some of the starting materials. These starting materials are then re-used in a new round of synthesis. Thermodynamically controlled binding to a receptor protects strong binding library members from the kinetically controlled destruction process. Amplification results from the receptor protecting bound library members from the destruction because in the absence of receptor, the destruction reaction removes all library members. Iterative synthetic cycles allow the library members to build up in the system, thus increasing their absolute amounts. The selectivity comes partially from the initial reversible binding of the library members to the receptor, but mainly from the kinetically controlled destruction of the weaker binders. The selectivity increases as the destruction reaction proceeds and can significantly exceed one.

Besides their potential for drug discovery, receptor assisted combinatorial chemistry may also be an important step in extending molecular evolution to small organic molecules. Humans have controlled biological evolution for centuries by breeding crops and domestic animals, and only recently extended their control of evolution to the molecular level. So far, this molecular evolution evolved only biomolecules and still used nature's biochemical machinery. For example, researchers used directed evolution (recursive mutagenesis and screening) to evolve proteins with increased stereoselectivity, stability or substrate specificity.[6] One current frontier of molecular evolution is to evolve non-biochemical molecules. No one has yet reached this goal, but a number of groups have reported steps in that direction.[7] The RACC methods described here also contribute to these efforts.

Dynamic Combinatorial Chemistry: A Thermodynamic Method

In the early 20th century LeChatelier showed that secondary reactions could shift an equilibrium. Using this principle to discover tight binding molecules is a more recent development. For example, binding of short oligonucleotide sequences to a longer DNA strand[8] or binding of a tripeptide by a cyclic diisophthalamide[9] shifted an imine and a disulfide synthetic equilibrium, respectively. Huc and Lehn identified the key requirements of DCL in 1997.[10] The creation of a library should be reversible and occur in aqueous media in the presence of a receptor which induces a detectable shift in equilibrium. Huc and Lehn demonstrated the concept by identifying inhibitors of carbonic anhydrase using a DCL of imines formed from amines and aldehydes. To detect these imines by HPLC, they "locked-in" the equilibrium by irreversible reduction of the imines with $NaBH_3CN$ to the corresponding amines. Analysis methods that involve separation of the library components (e.g. HPLC) require a lock-in reaction to fix the library composition, but analysis methods that detect library composition in situ (e.g. NMR,[11] X-ray crystallography[12]) avoid a lock-in reaction.

Most dynamic combinatorial libraries reported so far use formation of hydrazones, imines or disulfides as the linking reaction, with disulfide exchange being the most common. Disulfides rapidly equilibrate at pH values greater than 8, but are "locked in" below pH 5. For example, a library of disulfide-linked sugar dimers equilibrated at pH 7.4 in the presence of the plant lectin, concanavalin A, which binds mannose-rich oligosaccharides.[13] Lowering the pH locked in the equilibrium. Subsequent separation on an affinity column revealed an increase in the mannose-containing homodimer.[1]

Researchers from Therascope Pharmaceuticals (now Alantos Pharmaceuticals) created an imine library by condensing a diamine with more than fifty different ketones (Figure 2)[14] in the presence of neuraminidase, a key influenza virus enzyme. After reduction of the imines, LC/MS analysis identified several hits. Control experiments—library synthesis in the presence of bovine serum albumin (BSA) and in the presence of neuraminidase and a known potent inhibitor, Zanamavir—eliminated one of the initial hits (bottom right of Figure 2). The relative amplifications of the remaining true hits did not correspond directly to their binding affinities. One highly amplified compound was not a potent inhibitor and the strongest inhibitor was amplified approximately three-fold less. The authors suggest that this puzzling result can be explained by the lock-in reaction. The actual species undergoing equilibration are imines and hemiaminals. The receptor amplifies the amounts of these transient species and they are then trapped out, irreversibly, by reduction. The reduced products have different structural and electronic properties and thus their interaction with the receptor may be worse, or better, than the intermediates from which they are derived.

A recent DCL variation called "tethering" also used disulfide exchange as the linking reaction.[15] The key advantage of tethering is the ability to focus on particular region on a receptor. In this method the sulfur of a cysteine residue (either existing near the site of interest or added by protein engineering) underwent disulfide exchange with a library of disulfides. Those fragments that bind tightly to the receptor will form a more stable disulfide with the cysteine residue. By screening 7000 disulfides in batches of 5–20 compounds, MS analysis of the receptor-disulfides identified fragments that bind tightly to a potent inhibitor of interleukin 2, a target in immune-disorder therapy (Figure 3).[16] Adding these fragments to a known inhibitor improved binding up to 15-fold and with additional modifications up to 50-fold. The screening required small batches to distinguish between thiol fragments with identical molecular weight and between thiol fragments with similar binding constants.

[1] The computational simulations mentioned above suggest that this type of DCL where homodimers compete with heterodimers can give unusual results, however, this experiment did indeed identify the strongest binder. Please refer to ref. [5] for more details.

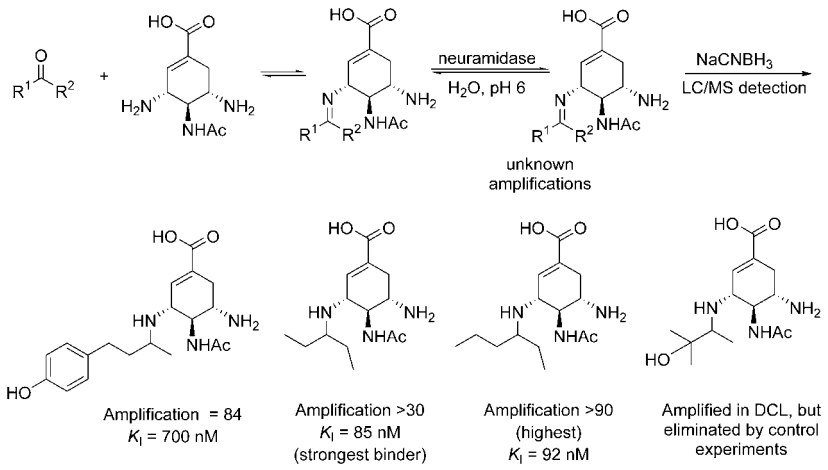

Figure 2. Condensation of a diamine with several ketones formed a library of potential neuraminidase inhibitors.[14] Reduction followed by HPLC analysis identified several inhibitors, but the most amplified species were not the strongest binders.

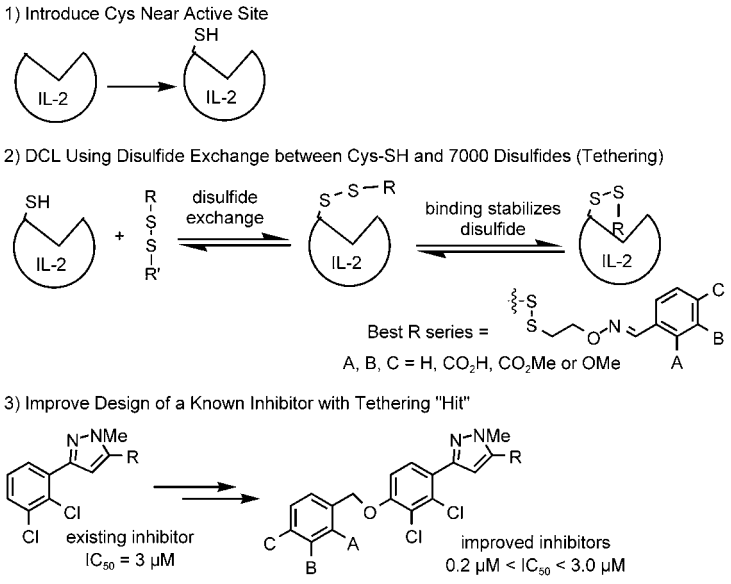

Figure 3. An example of "tethering" using DCL principles from Sunesis pharmaceuticals.[16] 1) Crystal structure-guided mutations introduced a cysteine near the binding site of IL-2. 2) Each mutant is individually screened against 7000 disulfide-containing fragments in batches of 5–20 using dynamic combinatorial interactions. MS identified the most stable disulfides. 3) Traditional medicinal chemistry approach added the tethering hit to an existing inhibitor of IL-2 thereby improving binding 15-fold.

Researchers have already made DCLs containing hundreds of compounds,[2] but current research seeks to further increase the size and complexity of these libraries and well as improve the screening strategies. Using a wider range of equilibration reactions, researchers hope to generate libraries rivaling diverse, natural product-based combinatorial libraries. In this regard, the use of organic solvents could have significant benefit as there are more reactions that have been developed in organic solvent than under aqueous conditions. While organic phases have been used in DCLs focused on molding receptors around small targets, drug discovery applications are hindered by the tendency of organic solvents to denature the target (enzyme, receptor, etc.). Thus methods of phase separation will need to be developed in order to benefit from the full force of organic synthesis.[17]

Although large libraries with higher diversity are desireable, screening larger libraries is harder because it is increasingly likely to find library members with similar binding constants and thus similar changes in concentration. In addition, the changes in concentration are smaller in larger libraries because of this competition.[4] One solution, mentioned in the tethering example above, is to screen compounds in small batches.[15,16] Another solution is to make sub-libraries in which each sub-library lacks one of the starting compounds. Starting materials whose absence eliminated amplification were those essential for binding, thus "deconvoluting" the results.[18]

Receptor-Accelerated Synthesis: A Kinetic Method

In receptor-accelerated synthesis (RAS), a library of starting materials competitively binds to a receptor, and cross-couples irreversibly to form a tight-binding ligand. The binding of the starting materials to the receptor brings them close to one another, speeding up a reaction that would not have occurred in solution. Amplification and selectivity of product formation come from both receptor affinity and the ability of the receptor to accelerate the coupling.

Early examples of RAS successfully identified products that bind to the target, but the selectivities were similar to those for DCLs. Carbonic anhydrase accelerated the coupling of alkyl chlorides to α-mercaptotosylamide, which contains a sulfonamide group that binds to the active site zinc.[19] This acceleration gave a two-fold increase for products that had nine-fold differences in binding constants, or a selectivity of ~0.2. In a second example, vancomycin receptor peptides accelerated dimerization of vancomycin analogues by either disulfide formation or olefin metathesis.[20] The dimerization rate accelerations correlated with the receptor affinity, suggesting selectivity near one. In a third example, the protease kallikrein accelerated the rate of a nucleophilic aromatic substitution with a five-amine library.[21] The selectivities approached 1.5, indicating that the relative amount of a binder to its closest competing compound was 1.5 times higher than their relative binding constants.

The most successful example of RAS yielded a femtomolar inhibitor of acetylcholine esterase (AChE) by optimizing the linker length and orientation between a micromolar and a nanomolar inhibitor of AChE.[22] In 49 parallel experiments, binary mixtures of two polyaromatic AChE inhibitors, one with a linker containing a terminal azide and the other with a linker containing a terminal acetylene, were incubated with AChE. Only the TZ2 + PA6 pair formed a product, presumably because binding this pair of inhibitors brought the azide and acetylene moieties close enough to promote a [3+2] dipolar cycloaddition (Figure 4). The selectivity in this reaction may be high since only one compound formed, but the binding constants for all other potential products are unknown. These researchers likely used parallel experiments instead of a one-pot experiment because the starting materials are such good inhibitors that exchange on the receptor is slow.

The two challenges of RAS are binding the starting materials to the receptor and significantly accelerating their coupling. For amplification to occur, the receptor must tightly bind the two starting materials simultaneously. This requirement eliminates cases where two weakly binding species link to form a strong binding one. Similarly, optimization of substituents around a tightly binding core may be difficult with RAS if the substituent fragments do not bind well. RAS is probably best suited to optimize linkers between two molecules that bind well at adjacent sites. The need to significantly speed up coupling is also challenging because the receptor is not a catalyst for the desired reaction, but only holds the two reactants close to one another.

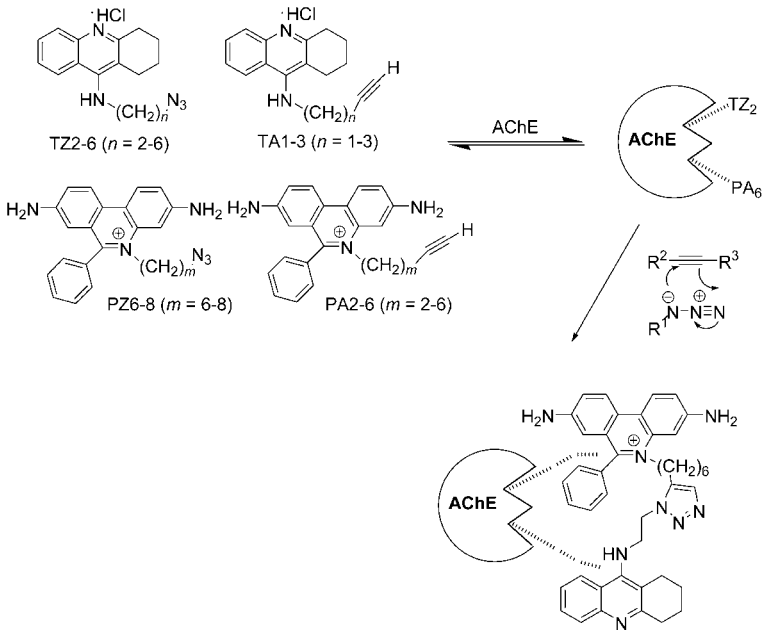

Figure 4. An example of receptor-accelerated synthesis from Lewis et al.[22] Eight azides and eight acetylenes were combined in the presence of acetylcholine esterase (AChE). Optimizing the linker length and geometry between a micromolar and a nanomolar inhibitor allowed a [3+2] dipolar cycloaddition to form a femtomolar inhibitor. This was possible because both starting materials could bind to adjacent sites on the receptor.

Adding Dynamics to Affinity Chromatography Methods

For decades researchers have used affinity chromatography to isolate tight binding compounds from a static mixture. However, several groups have recently used affinity chromatography in conjunction with DCLs. For example, Miller and co-workers combined eight salicylaldimines with zinc dichloride to form a library of 36 bis(salicylaldiminato)zinc complexes on an affinity column of immobilized poly (dA-T) DNA.[23] After equilibration, elution and chemical analysis correctly revealed which salicylaldimines did not bind to the DNA. The missing salicylaldimine was the one that formed a tight binding complex.

In another example, Eliseev and Nelen used iterative affinity chromatography in conjunction with UV-induced isomerization of dienoic acids to enrich the arginine-binding compounds in a mixture. The *cis* and *trans* alkene isomers of dienoic acids were passed through a column where they could bind to immobilized arginine (Figure 5).[24] The eluted dienoic acids, enriched in the less-tightly binding *trans* isomers, were photoisomerized and passed through the column again. After thirty cycles of Arg-binding and UV-induced isomerization of eluted library members, the amount of the tightest binding, *cis*,*cis* compound on the column was 50% greater than it was after one cycle. The selectivity was >2 for the tightest binding *cis*,*cis* compound. As this is a single component DCL, one would normally expect the selectivity to be close to one. The selectivity is higher because this experiment included a kinetic component—the rate of elution of more weakly bound compounds, and used iterative cycles to increase the selectivity achieved through one binding/elution/isomerization cycle. Iterative chromatography systems require immobilization of a receptor onto a column, and a process that can equilibrate the compounds in the column eluate. This method has not yet been used in a drug discovery application, but needs only the above two conditions to be fulfilled before it can be.

Pseudo-Dynamic Combinatorial Chemistry

Pseudo-dynamic combinatorial libraries (pDCLs) involve irreversible synthesis and destruction of library members in the presence of a receptor. The term "pseudo-dynamic" refers to the irreversible synthesis and destruction reactions, in place

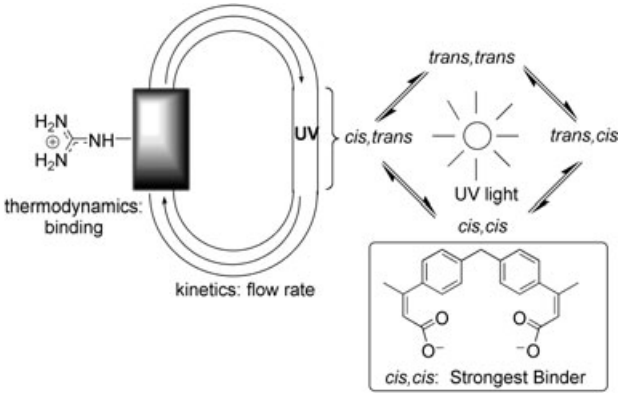

Figure 5. The affinity column-UV generator loop by Nelen and Eliseev.[24] A mixture of isomers is passed through the arginine column. UV-induced isomerization to their most thermodynamically stable state occurs only to compounds that do not bind. Re-introduction of the mixture to the column adds more tight binders. Combining the thermodynamic binding with the rate of introduction of the new library compounds (kinetic) results in amplification and high selectivity for the strongest arginine binder.

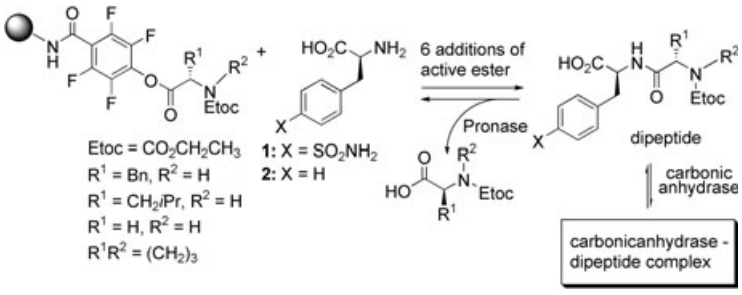

Figure 6. An example of a pseudo-dynamic combinatorial library.[26] A non-selective hydrolysis weans away weakly bound inhibitors. An iterative addition of fresh activated esters recycles the sulfonamide-containing amino acid. Each round of synthesis and destruction amplifies the amount of best inhibitor at the expense of poorer ones. Optimization of the relative synthesis and destruction rates yielded only the best-binding library member.

of an equilibrium in a normal dynamic combinatorial library. After library synthesis, the members associate with the receptor in a thermodynamically governed process. Next, the destruction reaction removes unbound compounds rapidly, weak binding members more slowly, and tight binding members slowest of all. Thus, pDCLs exploit the relative affinities of library members to a receptor (thermodynamics) to protect strong inhibitors from a kinetic destruction reaction.

A new round of synthesis recycles some of the components released in the destruction reaction. This recycling reintroduces all library members to the receptor, allowing strong binders to take up binding sites vacated by weaker binders. This increases the relative amounts of strong binders in the system at the expense of weaker ones. Extending the time between successive synthetic cycles decreases the amount of synthesis relative to destruction. The ability to adjust these relative rates allows tuning to yield only the best inhibitor, giving pDCLs a potential advantage over other RACC methods.

Creation of a pseudo-dynamic library required complementary and compatible combinatorial synthesis and destruction process. In the first model pseudo-dynamic library, the combinatorial synthesis of dipeptide inhibitors of the receptor, carbonic anhydrase (CA), used an aqueous, solid-phase approach.[25] Nucleophiles **1** (Phe_{sa}) and **2** (Phe) reacted with solid-supported active esters of N-Etoc protected amino acids to release dipeptides into solution (Figure 6). Nucleophile **1** contained a sulfonamide that was expected to bind to the active site zinc of CA. A non-selective protease (Pronase from *S. griseus*) catalyzed hydrolysis of the unbound dipeptides. Preliminary theoretical and experimental studies showed that for a static library in the presence of a receptor, a destruction reaction could increase the ratio of the best binder relative to a weaker binder to levels beyond the ratios of their inhibition constants.[4]

Dialysis membranes separated the active ester resin and the protease from the CA in a three-compartment vessel to prevent modification of the CA (Figure 7). Coupling of nucleophiles **1** and **2** to active esters of Gly, Pro, Leu and Phe made an eight-membered library. After optimizing the time allowed for destruction between additions of active ester, the ratio of the strongest binding dipeptide, $EtocProPhe_{sa}$, over the second strongest was greater than 100:1, compared with the ~2:1 ratio of their inhibition constants, giving a selectivity greater than 50. HPLC did not detect any other dipeptides. Furthermore, $EtocProPhe_{sa}$'s concentration rose over the course of six additions of active ester to eventually occupy 30% of all available CA binding sites.[26] Although potential selectivity in the destruction step could have complicated the interpretation, these experiments, used large amounts of Pronase so that the rate-limiting step in the destruction was nonselective diffusion across the membrane.

In pseudo-dynamic libraries, thermodynamics provide the essential initial selective binding to the receptor, but, as in dynamic libraries, this selectivity is often low. The kinetic destruction during the temporary absence of synthesis weans away non-, and poor inhibitors and greatly improves the selectivity for the best binders. Iteration of the synthesis–binding–destruction cycle allows better binders to build up in the system, giving amplification. Iteration also improves selectivity by re-introducing strong binders to the system, allowing them to replace weaker binders that were not completely removed in the previous destruction cycle.

In a pDCL all compounds are eventually destroyed in the absence of the receptor, so the definition of amplification used in Table 2 for DCLs and RAS implies that the amplification would be infinite in all pDCL experiments. This is misleading since some pDCL experiments generate more of the best binding compounds than others. It is more useful to discuss yield for pDCL, that is, the amount of a compound present in the screening chamber relative to the amount of receptor. This yield depends mainly on two factors: how strongly the compound binds, and the length of time the destruction reaction is allowed to proceed unabated by new

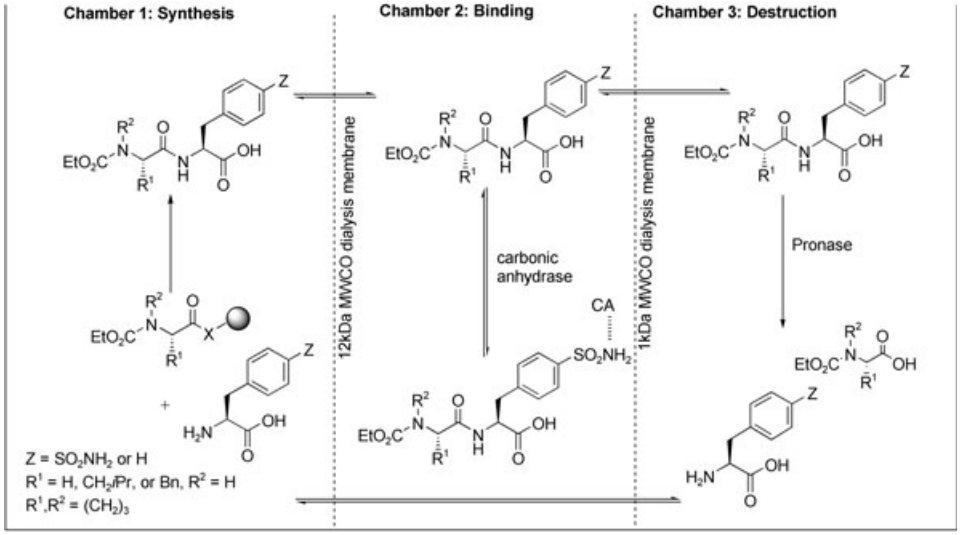

Figure 7. A three-chamber pseudo-dynamic combinatorial library.[26] Coupling of Phe_{sa} (**1**, Z = SO_2NH_2) and Phe (**2**, Z = H) to four active esters (N-Etoc-Phe, N-Etoc-Gly, N-Etoc-Leu and N-Etoc-Pro) makes an eight-member library. Dialysis membranes separate the chambers. One pseudo-dynamic cycle consists of addition of activated ester, stirring for X h (X defining the cycle time), then adding another portion of activated ester to start the next cycle. HPLC analysis of the binding chamber monitors progress. MWCO = molecular weight cut off.

synthesis. The optimum cycle time removes all but the best-binding compound, while keeping the yield as high as possible. Multiple cycles allow the yield to build up to a maximum of a stoichiometric amount compared to the receptor.

There are several potential advantages of pDCLs that may make them useful for drug discovery. First, by combining the inherent thermodynamic selectivity of a receptor towards a library of inhibitors of various strengths with a kinetic removal of weak binders, the selectivity of a receptor-assisted combinatorial system can be vastly increased relative to a normal DCL. In addition, the particular receptor type should not influence the success of these systems as it does in RAS. Further, no lock-in reaction is needed as the library members are synthesized irreversibly.

Further theoretical and experimental studies are essential to evaluate the potential advantages of pDCLs relative to other techniques. Importantly, the extension to large libraries containing high diversity will be critical to its potential success as a method for drug discovery. The key difference between pDCLs and other methods is the destruction reaction in pDCLs. When the library includes more tightly binding compounds, dissociation from the receptor may be slower, thus, requiring longer reaction times for the destruction reaction to proceed. Some receptors may not be stable long enough for this screening. Another design issue in pDCLs is the nature of the kinetic destruction component. It is not limited to peptide hydrolysis, but could include other chemical reactions that destroy unbound library members, or even physical separation steps that remove them. Different types of library synthesis will require designing new non-selective destruction methods to match.

Conclusion

Receptor-assisted combinatorial chemistry is an emerging field that can extend control of molecular evolution to small, organic molecules. It combines library synthesis and screening into one step by making focused libraries in the presence of the receptor. Thermodynamics and kinetics control the amplification and selectivity of the strongest-binding inhibitors in a library. Thermodynamic control often limits the amplification and selectivity to the binding constant differences. Kinetic control can give high selectivity, but it is harder to predict which systems will succeed. By alternating thermodynamic and kinetic control in pseudo-dynamic libraries one gains the predictability of a thermodynamically controlled system along with the high potential selectivity of a kinetically controlled system.

[1] M. A. Rouhi, *Chem. Eng. News* **2003**, *81(41)*, 77–78, 82–83, 86, 88–91.
[2] S. Borman, *Chem. Eng. News* **2003**, *81(43)*, 45–56, and references therein.
[3] Selected reviews: a) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455–2463; b) G. R. L. Cousins, S.-A. Poulsen, J. K. M. Sanders, *Curr. Opin. Chem. Biol.* **2000**, *4*, 270–279; c) J.-M. Lehn, A. V. Eliseev, *Science* **2001**, *291*, 2331–2332; d) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Curr. Op. Chem. Biol.* **2002**, *6*, 321–327.
[4] J. D. Cheeseman A. D. Corbett, R. Shu, J. Croteau, J. L. Gleason, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2002**, *124*, 5692–5701.
[5] These counterintuitive results presumably arise from a combination of statistical effects, which favour the formation of products which reflect the composition of starting materials which make up the library, and entropic effects, which favour homo/heterooligomers which contain a small number of components. Empirical modeling of DCLs in which homooligomers compete with heterooligomers are addressed in the following articles. However, this remains a controversial subject, and an active area of research: a) J. S. Moore, N. W. Zimmerman, *Org. Lett.* **2000**, *2*, 915–918; b) Z. Grote, R. Scopelliti, K. Severin, *Angew. Chem.* **2003**, *115*, 3951–3955; *Angew. Chem. Int. Ed.* **2003**, *42*, 3821–3825; *Angew. Chem.* **2003**, *115*, 3951–3955; c) K. Severin, *Chem. Eur. J.* **2004**, *10*, 2565–2580; d) P. T. Corbett, S. Otto, J. K. M. Sanders, *Org. Lett.* **2004**, *6*, 1825–1827; e) P. T. Corbett, S. Otto, J. K. M. Sanders, *Chem. Eur. J.* **2004**, *10*, 3139–3143.
[6] Reviews: F. H. Arnold, *Acc. Chem. Res.* **1998**, *31*, 125–131; C. Schmidt-Dannert, *Biochemistry* **2001**, *40*, 13125–13136.
[7] See for example: Z. J. Gartner, D. R. Liu, *J. Am. Chem. Soc.* **2001**, *123*, 6961–6963.
[8] J. T. Goodwin, D. G. Lynn, *J. Am. Chem. Soc.* **1992**, *114*, 9197–9198.
[9] H. Hioki, W. C. Still, *J. Org. Chem.* **1998**, *63*, 904–905.
[10] I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110.

[11] Y. Kubota, S. Sakamoto, K. Yamaguchi, M. Fujita, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4854–4856.
[12] a) M. S. Congreve, D. J. Davis, L. Devine, C. Granata, M. O'Reilly, P. G. Wyatt, H. Jhoti, *Angew. Chem.* **2003**, *115*, 4617–4620; *Angew. Chem. Int. Ed.* **2003**, *42*, 4479–4482; b) R. E. Babine, M. J. Rynkiewicz, L. J. S. S. Abdel-Meguid, *Lett. Drug Design Discov.* **2004**, *1*, 173–177.
[13] O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41–48.
[14] M. Hochgürtel, R. Biesinger, H. Kroth, D. Piecha, M. W. Hofmann, S. Krause, O. Schaaf, C. Nicolau, A. V. Eliseev, *J. Med. Chem.* **2003**, *46*, 356–358.
[15] D. A. Erlanson, A. C. Braisted, D. R. Raphael, M. Randal, R. M. Stroud, E. M. Gordon, J. A. Wells, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9367–9372.
[16] A. C. Braisted, J. D. Oslob, W. L. Delano, J. Hyde, R. S. McDowell, N. Waal, C. Yu, M. R. Arkin, B. C. Raimundo, *J. Am. Chem. Soc.* **2003**, *125*, 3714–3715; see also: D. A. Erlanson, J. W. Lam, C. Wiesmann, T. N. Luong, R. L. Simmons, W. L. DeLano, I. C. Choong, M. T. Burdett, W. M. Flanagan, D. Lee, E. M. Gordon, T. O'Brien, *Nat. Biotechnol.* **2003**, *21*, 308–314.
[17] M. D. Burke, E. M. Berger, S. L. Schreiber, *Science* **2003**, *302*, 613–618.
[18] T. Bunyapaiboonsri, O. Ramström, S. Lohman, J.-M. Lehn, L. Peng, M. Goeldner, *ChemBioChem* **2001**, *2*, 438–444.
[19] R. Nguyen, I. Huc, *Angew. Chem.* **2001**, *113*, 1824–1826; *Angew. Chem. Int. Ed.* **2001**, *40*, 1774–1776; .
[20] K. C. Nicolaou, R. Hughes, S. Y. Cho, N. Winssinger, C. Smethurst, H. Labischinski, R. Endermann, *Angew. Chem.* **2000**, *112*, 3981–3986; *Angew. Chem. Int. Ed.* **2000**, *39*, 3823–3828.
[21] Y. Yu, L. Ye, K. Haupt, K. Mosbach, *Angew. Chem.* **2002**, *114*, 4639–4643; *Angew. Chem. Int. Ed.* **2002**, *41*, 4459–4463.
[22] W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radic, P. R. Carlier, P. Taylor, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 1095–1099; *Angew. Chem. Int. Ed.* **2002**, *41*, 1053–1057.
[23] B. Klekota, M. H. Hammond, B. L. Miller *Tetrahedron Lett.* **1997**, *38*, 8639–8642.
[24] a) A. V. Eliseev, M. I. Nelen, *J. Am. Chem. Soc.* **1997**, *119*, 1147–1148; b) A. V. Eliseev, M. I. Nelen, *Chem. Eur. J.* **1998**, *4*, 825–834.
[25] A. D. Corbett, J. L. Gleason, *Tetrahedron Lett.* **2002**, *43*, 1369–1372.
[26] A. D. Corbett, J. D. Cheeseman, R. J. Kazlauskas, J. L. Gleason, *Angew. Chem.* **2004**, *116*, 2486–2490; *Angew. Chem. Int. Ed.* **2004**, *43*, 2432–2436.

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