Combinatorial Chemistry:

Drug design approach:

1. Classical approach: make a change on an existing compound or synthesize a new structure and see what happens.

Most drug compounds were synthesized in milligram quantities in a serial one-at-a-time fashion. After synthesis, the compound was sent to a biologist, who tested it in several in vitro assays and returned the results to the chemist. Based on the assay results, the chemist would apply some structure activity relationship (SAR) or use chemical intuition to decide what changes to make in future versions of the molecule to improve activity.

Using this iterative process, a chemist would be able to synthesize only a handful of structures per week. Since the yield of marketable drugs from compounds synthesized and tested is only about 1 in 10,000, the road to success has been a long and expensive one, taking 6 to 12 years and costing $500 to $800 million per drug.

2. Combinatorial chemistry approach: In the mid- 1980s, classical approach to drug synthesis changed dramatically with the introduction of combinatorial chemistry.

The drug discovery process became a highly parallel one, in which hundreds or even thousands of structures could be synthesized at one time. Interestingly, biologists had for some time been using high-throughput screening (HTS) to perform their in vitro assays, running assays in 96-well microtiter plates and even using laboratory robotics for pipetting and analysis.

The term combinatorial chemistry was coined to refer to the parallel generation of all possible combinations of substituents or components in a synthetic experiment. Whereas the yield from a serial synthesis is a single compound, the yield from a combinatorial synthesis is a chemical library.

Figure 1 shows two common types of chemical libraries:

1- Generic library, based on a single parent or scaffold structure and multiple substituents or residues.
2- Mixture library, containing a variety of structure types.

The total number of structures in a library is either the product of the various numbers of substituents (for a generic library) or the total number of structures in a mixture.

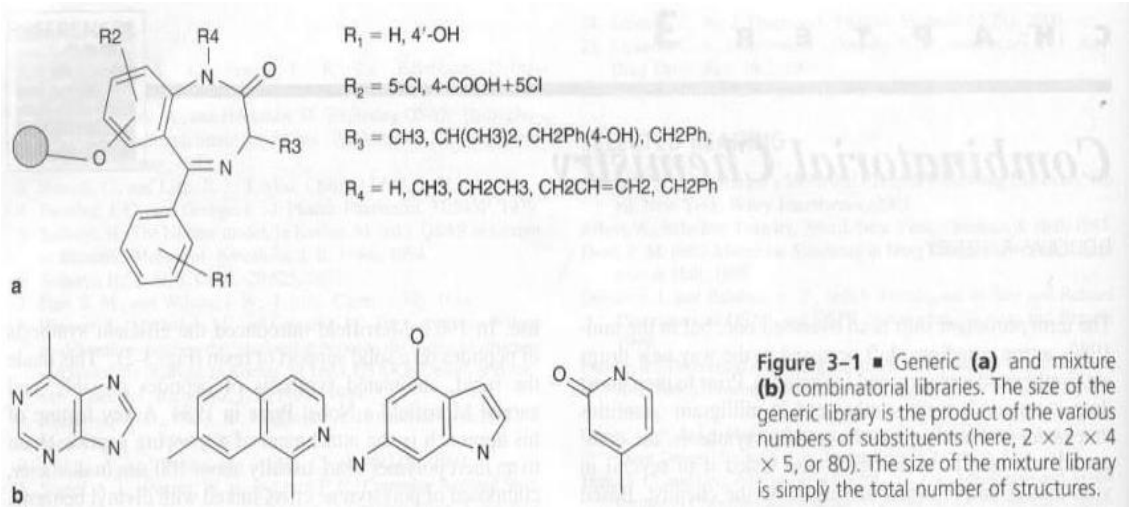


Figure 3–1 ■ Generic **(a)** and mixture **(b)** combinatorial libraries. The size of the generic library is the product of the various numbers of substituents (here, 2 × 2 × 4 × 5, or 80). The size of the mixture library is simply the total number of structures.

The medicinal chemist can select subsets of substituents that vary in lipophilicity, steric bulk, induction, and resonance effects.
This "rational" approach to drug design assumes that there is some understanding of the target receptor and that there is a lead molecule, commonly called the prototype molecule.
A classic example is the dihydrofolate reductase inhibitor methotrexate, which has been one of the prototypes that laboratories have used to synthesize and test new inhibitors

The goal of combinatorial chemistry is to be able to synthesize, purify, chemically analyze, and biologically test all the structures in the library, using as few synthetic experiments as possible.

3. Computer aided drug design: drug design increasingly is based on modern computational chemical techniques; it also uses sophisticated knowledge of disease mechanisms and receptor properties.

HOW IT BEGAN:
PEPTIDES AND OTHER LINEAR STRUCTURES

Combinatorial chemistry was first applied to the synthesis of peptides, since a convenient method for the automated synthesis of these compounds was already in wide spread use. In 1963, Merrifield introduced the efficient synthesis of peptides on a solid support or resin as shown in figure 2:

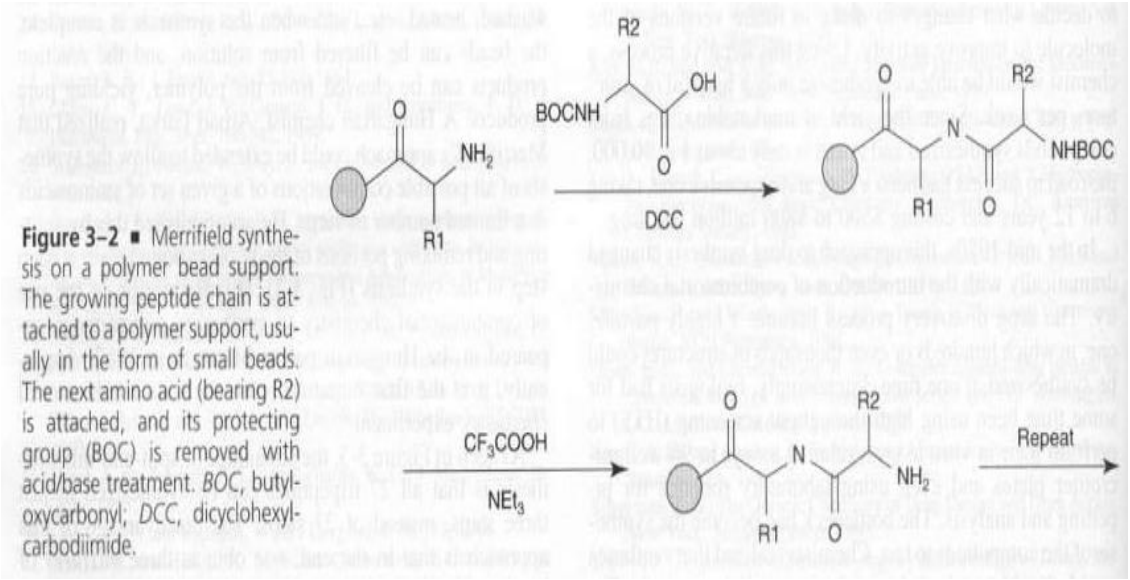


Figure 3–2 ■ Merrifield synthesis on a polymer bead support. The growing peptide chain is attached to a polymer support, usually in the form of small beads. The next amino acid (bearing R2) is attached, and its protecting group (BOC) is removed with acid/base treatment. *BOC*, butyloxycarbonyl; *DCC*, dicyclohexylcarbodiimide.

This made the rapid and automated synthesis of peptides possible. A key feature of his approach is the attachment of a growing peptide chain to an inert polymer bead, usually about 100 μm in diameter, composed of polystyrene cross-linked with divinyl benzene. Such beads were originally designed for size exclusion chromatography.
The beads can be immersed in solvents, washed, heated, etc...and when the synthesis is complete, the beads can be filtered from solution, and the reaction products can be cleaved from the polymer, yielding pure products.

A Hungarian chemist, Arpad Furka, realized that Merrifield's approach could be extended to allow the synthesis of all possible combinations of a given set of amino acids in a limited number of steps.

He accomplished this by splitting and remixing portions of the peptide-bound resin at each step in the synthesis (Fig: 3).

His description of the use of combinatorial chemistry to synthesize polypeptides appeared in the Hungarian patent literature in 1982. Apparently, it is the first literature reference to a combinatorial chemistry experiment.

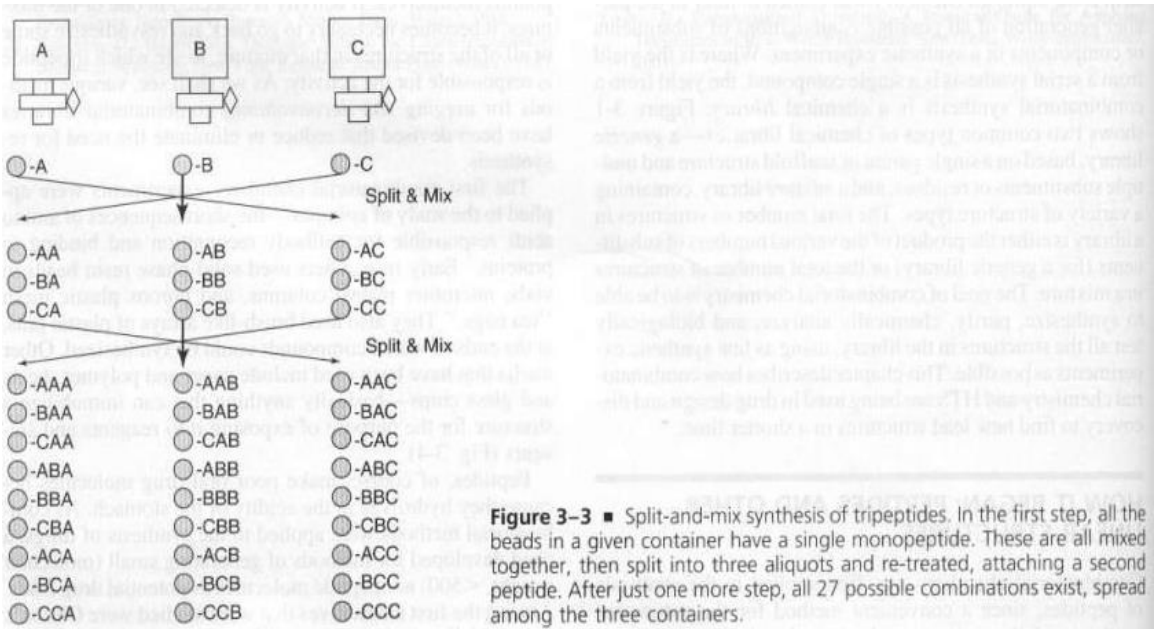


Figure 3–3 ■ Split-and-mix synthesis of tripeptides. In the first step, all the beads in a given container have a single monopeptide. These are all mixed together, then split into three aliquots and re-treated, attaching a second peptide. After just one more step, all 27 possible combinations exist, spread among the three containers.

As seen in Figure 3, the advantage of split-and-mix synthesis is that all 27 tripeptides can be synthesized in just three steps, instead of 27 steps.

The disadvantage of this approach is that in the end, one obtains three mixtures of beads with tripeptides attached, rather than the pure compounds themselves.

If activity is detected in one of the mixtures, it becomes necessary to go back and resynthesize some or all of the structures in that mixture, to see which tripeptide is responsible for the activity.

In a process called deconvolution, the synthesis is repeated in an iterative manner, producing smaller and sometimes overlapping mixtures.

The screening is repeated until the active compounds are identified.

(Deconvolute: To make the results of a combinatorial experiment less complex, usually by backtracking and reanalyzing or resynthesizing a subset of the structures in the library. The goal of deconvolution is to determine which of a mixture of compounds in actually responsible for activity.)

Examine the following Table, this simplified outline shows how four steps will identify the three active components in a 20-compound investigation. (Keep in mind that the actual combinatorial process will produce hundreds or thousands of compounds for testing.)

TABLE 2–12 Simplified Deconvolution Scheme for a 20-Compound Combinatorial Chemistry Screen

A	B[a]	C	D	E	F	G	H[a]	I	J	K	L	M	N[a]	O	P	Q	R	S	T
Carry out the synthesis producing four five-component mixtures. Screen the mixtures.																			
AB[a]CDE					FGH[a]IJ					KLM[a]NO					PQRST				
Retain only the three mixtures containing active components. Repeat the synthesis producing three-component mixtures and repeat the screening.																			
AB[a]C				DEF				GH[a]I					JKL					MN[a]O	
Discard the inactive mixtures. Repeat the synthesis producing overlapping two-component products and repeat the screening.																			
AB[a]			B[a]C				GH[a]				H[a]I				MN[a]			N[a]O	
Only compounds B, H, and N need to be chemically characterized.																			

Assume that the project calls for synthesizing 20 compounds, A to T. Rather than carry out 20 distinct syntheses followed by 20 separate screening experiments, all of which can take weeks, four combinatorial syntheses are carried out such that four mixtures containing live compounds each are obtained. Only the three mixtures that test positive in the screening assay are retained. The synthesis is repeated producing five mixtures of three components each, and the testing is repeated. Six more syntheses are carried out this time, producing overlapping two-component mixtures, and the assays are repeated. It is now possible to determine that compounds B. H. and N are active.

Instead of 20 syntheses and 20 assays, only 15 were required. Further, time-consuming purification of each mixture was not required. This process is very similar to that carried out by natural-product chemists. The microbial, plant, or animal tissue is extracted with a variety of solvents, beginning with nonpolar hydrocarbons and ending with an alcohol or water, and the fractions are screened for activity. Only the active fractions are retained.

The latter are more carefully fractionated, using biological assays to follow the purification. In either combinatorial synthesis or natural product isolation, once active compounds are identified, larger-scale, more focused syntheses can be done, using QSAR-derived experimental design and/or molecular modeling to yield compounds different from those produced from the combinatorial library of chemical fragments.

Early researchers used solid-phase resin beads in vials, microtiter plates, columns, and porous plastic mesh "tea bags" They also used brush-like arrays of plastic pins, at the ends of which compounds could be synthesized.

Other media that have been used include paper and polymer sheets and glass chips—basically anything that can immobilize a structure for the purpose of exposing it to reagents and solvents (Fig. 4).

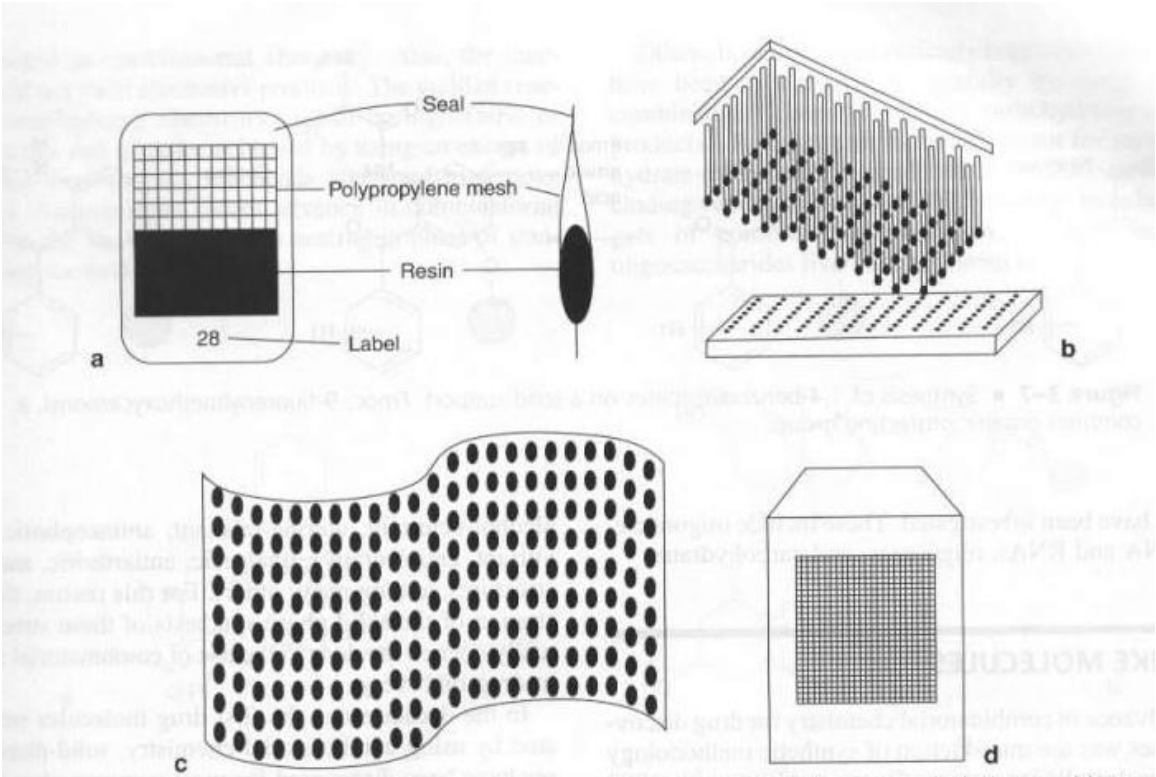


Figure 3–4 ■ Various approaches to immobilizing and separating chemical compounds during combinatorial synthesis have been devised. **a.** Tea bag synthesis. **b.** Pins and "lollypops." **c.** Dots on cellulose. **d.** Spatial arrays on microchips.

Peptides, of course, make poor oral drug molecules because they hydrolyze in the acidity of the stomach. As combinatorial methods were applied to the synthesis of drugs, a need developed for methods of generating small (molecular weight. <500) nonpeptide molecules as potential drug leads.

Among the first alternatives that were studied were Chiron's "peptoids"—molecules in which the variation occurs in (molecular weight <500) the attachment to the amide nitrogen (Fig.5)

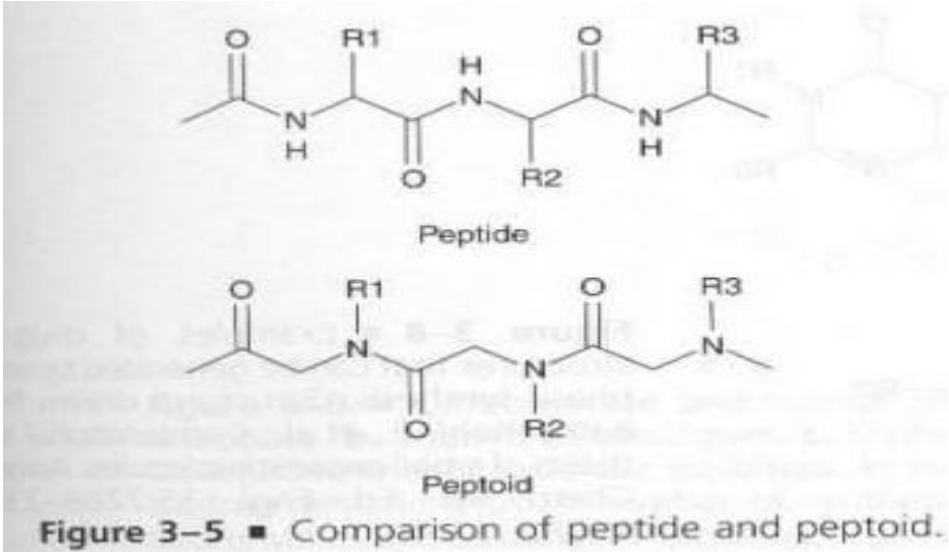


Figure 3–5 ■ Comparison of peptide and peptoid.

Although these structures could potentially place side chain functional groups in positions similar to those on the corresponding peptides, they differ significantly in that:

1- They lack peptide hydrogen bonds.
2- They lack chiral centers.
3- They also show more rotational flexibility than the corresponding peptides, since the peptoid amide bonds show less double-bond character than those in peptides.

Several potent peptoid ligands were found, including:
A nanomolar α-adrenergic inhibitor and a similarly active μ-opiate receptor ligand. Because of the ease of synthesis, other classes of linear chain molecules have been investigated. These include oligonucleotides (DNA and RNA), oligoureas, and carbohydrates.

DRUG-LIKE MOLECULES:

The real advance in combinatorial chemistry for drug discovery purposes was the introduction of synthetic methodology to yield true drug-like structures. Bunin and Ellman in 1992 demonstrated the synthesis of I .4-benzodiazepine compounds, using three components: a 2-aminobenzophenone, a protected amino acid, and an alkyl halide as in the following figure:

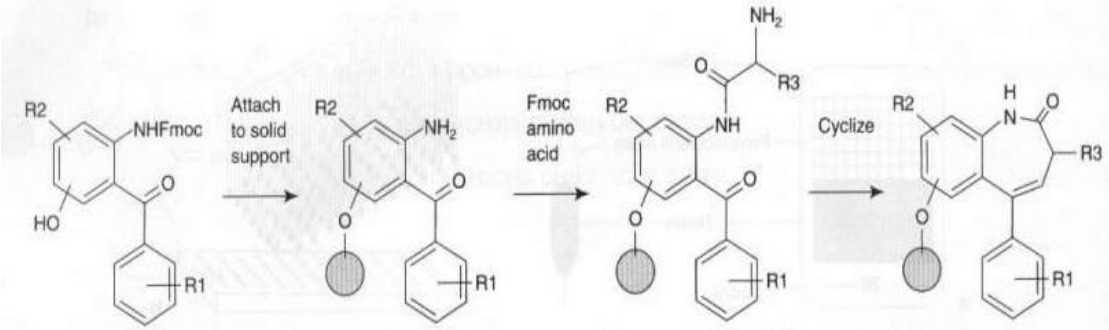


Figure 3–7 ■ Synthesis of 1,4-benzodiazepines on a solid support. *Fmoc*, 9-fluorenylmethoxycarbonyl, a common organic protecting group.

Although we normally think of benzodiazepines as muscle relaxants and tranquilizers, a search for drug structures containing the benzodiazepine scaffold returns antiviral, alcohol-deterrent, uterine-relaxant, antineoplastic, anticonvulsant, antiulcerative, analgesic, antiarthritic, and sedative structures, among many others. For this reason, the demonstration of the solid-phase synthesis of these structures virtually opened the door to the use of combinatorial chemistry in drug discovery for most common classes of drug structure including carbohydrates and natural

products. Carbohydrate antibiotics, including vancomycin and aminoglycosides, have been the targets of combinatorial chemistry. Natural products that have been studied, including vitamin D analogues, erythromycin-like antibiotics, anticancer, and galanthamine, a cholinesterase inhibitor.

The reactions that can be performed in combinatorial chemistry are:

1. Simpler than many reactions in standard synthetic procedures that a chemist can perform. Generally the following conditions avoided in combinatorial chemistry reaction:
   A/ Extremes of temperature and pressure.
   B/ the use of highly caustic reagents.
   C/ inert atmospheres.
   D/ multistep reactions.

2. Not yield alternative products. The yield of reactions in combinatorial chemistry should be high (80% or more) but this can often be achieved by using an excess of reagent and then washing the beads afterward to remove the excess.
3. An important recent advance in combinatorial chemistry is the use of microwave heating in place of standard heating methods.

SUPPORTS AND LINKERS:

Most solid-state combinatorial chemistry is conducted by using polymer beads 10 to 750μm in diameter. These heads swell in organic solvents, allowing the free diffusion of solvent and reagent into the interior of the bead and greatly expanding the available area for the attachment of product.

The polymers are inert, except for the functional groups to which the molecules arc attached.

In general, the compounds to he synthesized are not attached directly to the polymer molecules. They are usually attached using a "linker" moiety that:

(a) enables attachment in a way that can be easily reversed without destroying the molecule that is being synthesized
(b) allows some room for rotational freedom of the molecules attached to the polymer.

Sometimes, the molecules attached to the polymer are used directly as substrates in in vitro assays without removing them from the polymer.

In such cases, if the molecules are too tightly packed on the polymer, the enzyme molecules cannot gain access to the substrate.

In general, about 1 mmnol of linker is attached per gram of solid support.
The types of solid supports that are used include:

1- Polystyrene resins. Polystyrene cross-linked with divinyl benzene (about 1% cross-linking). These are common resins used in site exclusion chromatography.
2- TentaGel resins. Polystyrene in which some of the phenyl groups have polyethylene glycol (PEG) groups attached in the para position. The free OH groups of the PEG allow the attachment of compounds to be synthesized.
3- Polyacrylamide resins. Like "super glue.' these resins swell better in polar solvents and, since they contain amide bonds, more closely resemble biological materials.
4- Glass and ceramic beads. Not a type of organic resin but sometimes used when high-temperature or high-pressure reactions are needed.

To support the attachment of a synthetic target, the polymer is usually modified by equipping it with a linker or anchor group. Such groups must be stable under the reaction conditions, but they must be susceptible to a "cleavage" reaction that allows removal of the product.
Some common linkers are shown in the following figure, along with the reagents that cleave the product from the resin.

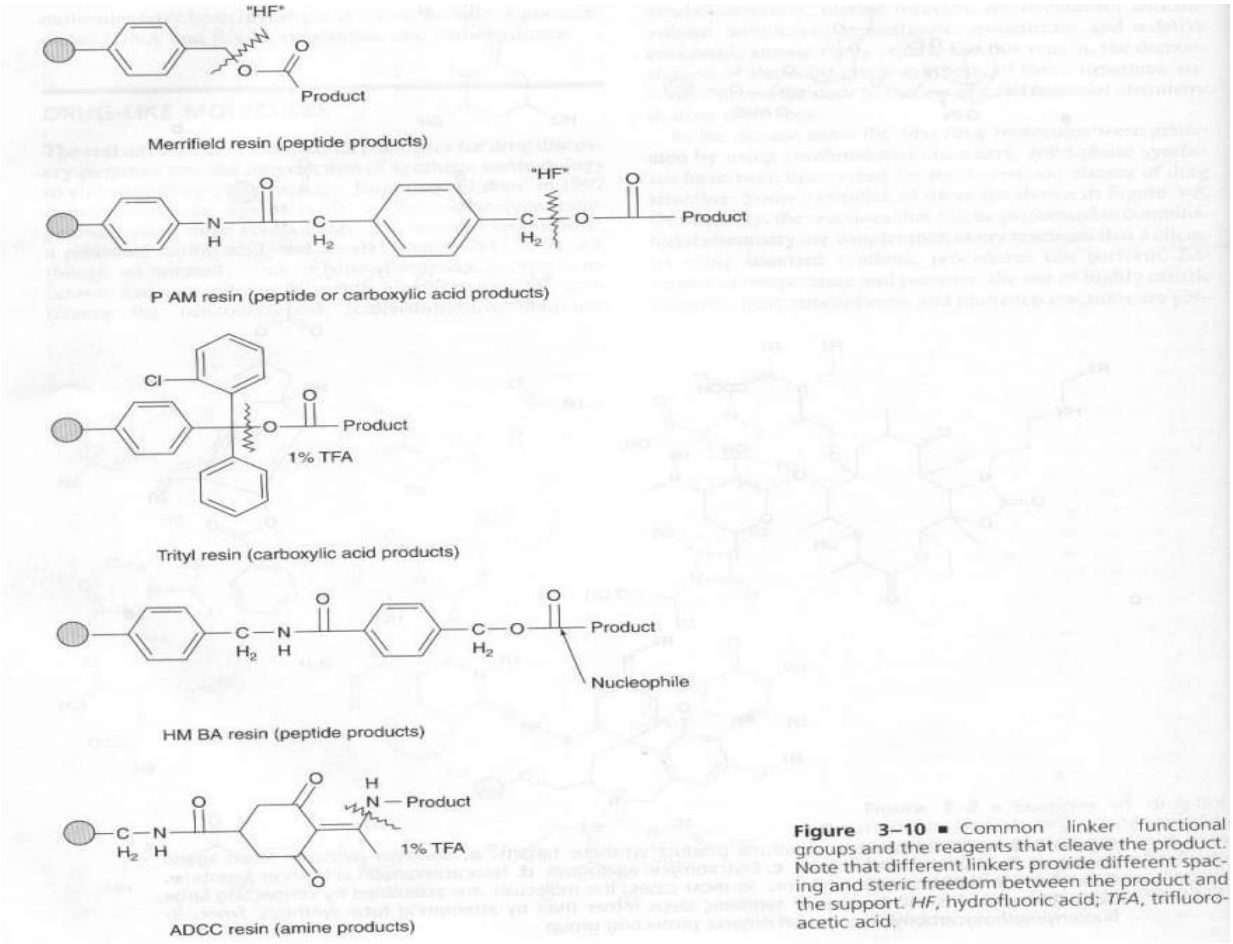


Figure 3–10 ■ Common linker functional groups and the reagents that cleave the product. Note that different linkers provide different spacing and steric freedom between the product and the support. *HF*, hydrofluoric acid; *TFA*, trifluoroacetic acid.

Some specialized linkers have been developed to meet particular reaction or product conditions:

1- Traceless linkers can be cleaved from the resin with no residual functionality left. This allows the attachment of aryl and alkyl products that do not have OH or NH functionality. These linkers usually include a silyl group (-Si(CH_3)$_2$-) that is sensitive to acids and can be cleaved to give unsubstituted phenyl or alkyl products.

2- "safety-catch" linkers are inert to the synthesis conditions but have to be chemically transformed to allow final liberation of the product from the resin.

Typically, two reactions are required to break the linker (hence the name).

3-A rather elegant approach to linker chemistry is to use linkers that are sensitive to ultraviolet (UV) light. The Affymax group has used these in the synthesis of carboxylic acid and carboxamide products.

4- Finally, some groups have used linkers that can only he cleaved by enzymes.

SOLUTION-PHASE COMBINATORIAL CHEMISTRY:

Most ordinary synthetic chemistry takes place in solution. When a reaction must be modified to accommodate a solid support, it takes time and resources to develop and optimize the reaction conditions. Indeed, a combinatorial chemist may spend months designing a solid-phase reaction and gathering the necessary materials but then conduct the entire synthesis in a matter of hours or days!

Many reactions cannot ever be run on solid supports because of poor yields or failed reactions.
For these reasons, there has been much interest in using solution-phase chemistry for the preparation of combinatorial libraries. Unlike one-bead one-compound synthesis described above, solution-phase combinatorial chemistry often leads to a mixture of products.

Imagine reacting a set of 10 amines with 10 acid chlorides, all in one flask, and with the reactants and conditions chosen so that no reaction of amines with amines or chlorides with chlorides occurs, only reactions between amines and chlorides. The result would be a mixture of 100 amides, one for each possible combination of amine and acid chloride. The resulting mixture could then be tested for activity, under the assumption that the inactive amides did not interfere with binding of active molecules (not always a valid assumption).
If activity is found, smaller subsets of amines and chlorides can be tested to eventually find the structure(s) responsible for activity.

Researchers have gone one step further by reacting multiple kinds of reactants together to produce some rather amazing mixtures. Figure below shows an example of a four-component Ugi reaction that yields, alter appropriate further transformation of the intermediate product, a mixture of carboxylic acids, esters and thioesters, pyrroles, I .4-benzodiazepine- 2,5-diones, and even a monosaccharide.

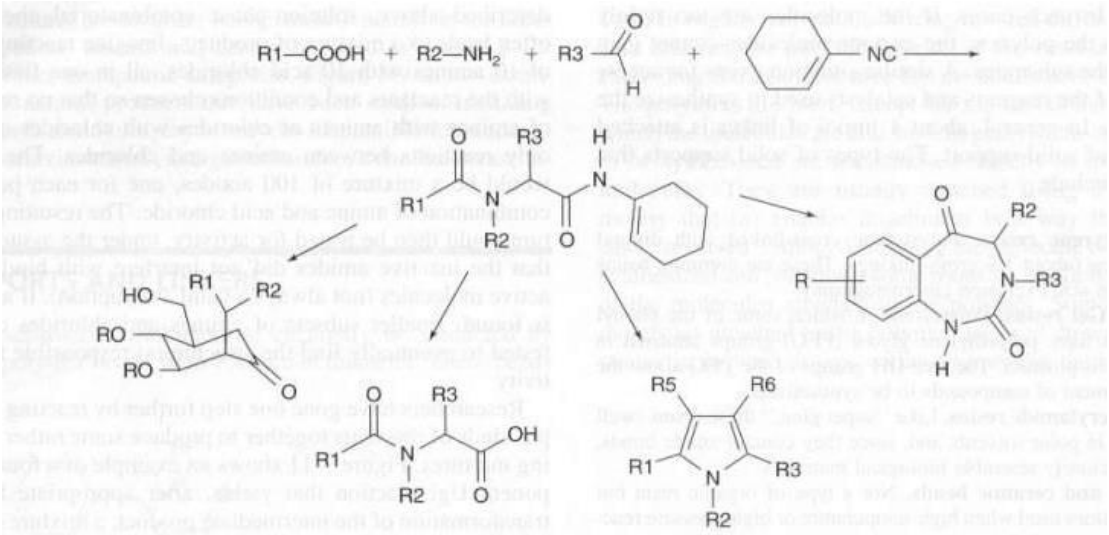


Figure 3–11 ■ Four-component Ugi reaction reported by Keating and Armstrong.[14] A combinatorial mixture of the intermediate cyclohexenyl amide can be split into several portions, and each can be further reacted to give a variety of products, all of which will be combinatorial. (Reaction redrawn from description from Keating, T. A., and Armstrong, R. W.: Molecular diversity via a convertible isocyanide in the Ugi four-component condensation. J. Am. Chem. Soc. 117:7842–7843, 1995.)

Despite the diversity of the chemistry, the yields of products in such mixture-based experiments are often found to be about 90% or better.

Although this is an extreme example of a multicomponent reaction, it illustrates the utility of solution-phase chemistry for generating great diversity in chemical libraries.

An approach that is intermediate between solid-phase chemistry and solution-phase chemistry is to use soluble polymers as a support for the product.

Types of soluble polymers:

1. PEG is a common vehicle in many pharmaceutical preparations. Depending on the degree of polymerization. PEG can be liquid or solid at room temperature and show varying degrees of solubility in aqueous and organic solvents.

Each molecule of PEG has an OH group at either end:

$$HOCH_2CH_2O\text{-}[CH_2CH_2O]_n\text{-}CH_2CH_2OH$$

By converting one OH group to a methyl ether (MeO—PEG—OH), it is possible to attach a carboxylic acid functionality to the free OH and use solution-phase combinatorial chemistry to synthesize, for example, N-aryl-sulfonamide structures.

The resulting mixture of PEG-bound sulfonamides can be separated by use of chromatography.

2. Dendrimers: These are large, highly branched molecules with terminal amino groups that can be used like the OH groups of PEG for the attachment of products.

3. A class of molecules known as fluorous phases are a form of "liquid Teflon," consisting mainly of long chains of (—CF2-) groups attached to a silicon atom. When these phases are used as a soluble support for synthesis, the resulting product can be readily separated from any organic solvents and reaction by-products by extracting the reaction mixture with fluorocarbon solvents."

4. A unique application using complementary DNA as a "support" has been reported by Harvard researchers. To "encourage" pairs of molecules in solution to react under mild conditions, they attach short strands of complementary DNA or RNA to the structures to "zip" the structures together and promote reaction. The DNA is then removed, yielding product that would not otherwise be synthesized. Using this method makes it possible to prevent reaction of certain pairs of structures as well.

DETECTION, PURIFICATION, AND ANALYSIS:

Detection, analysis, and purification of combinatorial libraries places high demands on existing analytical techniques because:
(a) the quantities to be analyzed are very small, sometimes pico moles of material.
(b) The analysis should be nondestructive, to allow recovery of the compound if possible.
(c) The methods must be suitable for rapid, parallel analysis—analysis cannot be the rate-limiting step in the procedure.

No single analytical technique can fit all the requirements, so usually some "hyphenated" analytical techniques are used, for example, high-performance liquid chromatography with a mass spectrometer detection system (HPLC-MS).

Chromatography is usually the first step in the analysis of a combinatorial mixture. If we start with solid-phase chemistry, we chemically cleave the compounds from the support and filter off the beads, giving a solution containing the compounds we synthesized.
If the solution contains just a single compound, we might use a spectrophotometer, to measure infrared (IR) and ultraviolet (UV) absorbance or fluorescence directly, or even nuclear magnetic resonance (NMR) spectroscopy, to determine the structure of the compound in solution.
If the solution contains a mixture of compounds, one must separate them before determining their structures. HPLC is a standard approach. The components in the mixture are detected by some optical method (UV absorption. fluorescence, refractive index. etc.) that gives rise to peaks on a graphical readout.
Sometimes, the output from the column is passed into a spectrophotometer or mass spectrometer to generate a spectrum for each fraction of the output. These spectra can be interpreted to determine the structure of the compound that caused a given peak.

It is also possible to use much larger chromatographic columns and run preparative HPLC to separate up to several milligrams of material for further analysis or biological assay.

Chromatographic separations and analyses can be fully automated. Thus, a chemist can place all the reaction vessels, microtiter plates, etc. from a combinatorial experiment into racks and use a robotic system to draw samples, inject them into the HPLC, and collect the data output into computer files or databases—all without further intervention from the chemist (except to wash the dishes!).
For this reason, speed and solvent handling are special concerns with combinatorial experiments. One approach that has been adopted to speed up analyses and reduce the amount of solvent that must be consumed is, supercritical fluid chromatography (SFC), their characteristic:

1. The solvent is not a common organic solvent such as acetone or ethanol. Instead, it is a pressurized gas like CO_2 that evaporates from the output, leaving pure compound behind.
2. Another advantage of SFC is speed: since the solvent molecules are small, diffusion is rapid, and separations take place in about half the time of ordinary HPLC separations or less.
3. Finally, the amount of "solvent" that is consumed is significantly lower with SFC.

A disadvantage is that certain compounds may not separate as well under SFC as under HPLC.

IR spectroscopy is often applied in combinatorial chemistry with special consideration, these are:

1. Since IR light can be reflected from materials, one can analyze resin beads directly, without cleaving the products from them.
2. Since the loading of product on any given bead is very small, usually computer-enhanced methods like Fourier transform IR (FTIR) are needed to enhance the very small spectral signal from one or a few beads.
3. Interestingly, the shape of the beads has been found to affect the IR spectra results, and flattened rather than spherical beads give stronger IR signals.

NMR spectroscopy; their characteristic are:

1. Gives more structural information than IR or UV spectroscopy, but it has traditionally not been nearly as sensitive.

2. Compounds are normally cleaved from solid support before analysis by NMR, since NMR on solid resin or on resin swollen by solvent give broadened peaks and low resolution.
3. A type of NMR called magic angle spinning NMR, in which the sample is inserted into the magnetic field at an angle of about 55^0, reduces the peak broadening and has been used to analyze swollen polymer beads directly.

NMR techniques that have been used to analyze combinatorial mixtures include:

1. Magic angle spinning NMR.
2. Various "two-dimensional" (2D) NMR techniques that use multiple magnetic fields.
3. HPLC-NMR.
4. Capillary electrophoresis coupled to NMR (CE-NMR).
5. NMR to detect the binding of drugs to receptors to identify active agents. This latter technique has been termed SAR with NMR.

Mass spectrometry (MS):

1. The technique most widely used for combinatorial library analysis.
2. The measurements can he made on resin beads directly.
3. A wide range of compounds can be analyzed.
4. MS analyses can be highly automated.
5. A very important use of MS in combinatorial chemistry is in quality control of combinatorial libraries. (Only MS provides the sensitivity and versatility to perform this checking with both solid-phase and solution-phase libraries.)

ENCODING COMBINATORIAL LIBRARIES:

Once we have found a mixture or sub library that shows biological activity, how do we determine exactly which structure or structures are responsible for the activity?
We can purify and analyze as described in the previous section. But if no direct analysis is available, we need to encode(شفرة) or tag(رقعة) the support or the molecules themselves, using physical or molecular "barcodes."(شفرة كومبيوترية توضع على السلع) An obvious approach that can be used only with small libraries is to physically label each vial of one-bead one-compound resin. This may be practical for a few tens of compounds, but what if we have a library of 32000 compounds, or even I000000 compounds, in a mixture?

Clearly, there is a need for a more automated means of identifying the structures that are in the library.
The most common approach to encoding solid-phase libraries is to attach a chemical tag to the resin beads as the target molecule gets synthesized. Typically, at each step in the reaction, a tag is attached that is unique for the given step.

For example, if we are creating a tripeptide and we have 10 possible amino acids at each position, we need to attach either a single tag that says the tripeptide on this bead has amino acid Ala at position 1, Phe at position 2, and Gly at position 3." or we need to attach three different tags, one for each position.

Types of chemical encoding:

1. The earliest types of chemical encoding were the attachment of oligonucleotides (usually single-strand DNA) to beads on which peptides or peptoids were being built.
Since there are 20 possible amino acids and only four nucleotide bases, enough bases must be attached at each amino acid addition step to identify properly the amino acid being attached. Although three bases are used in the DNA genetic code, it is customary(مالوف) to use up to six bases for library tagging.
For decoding, the DNA tag is amplified by use of the polymerase chain reaction (PCR). The same reaction that is used in forensic (شرعي)DNA analysis. For this reason, the chemical tag must also bear PCR primer sequences.

Two types of anchors have been used to connect the DNA tags to the solid support as shown in this figure:

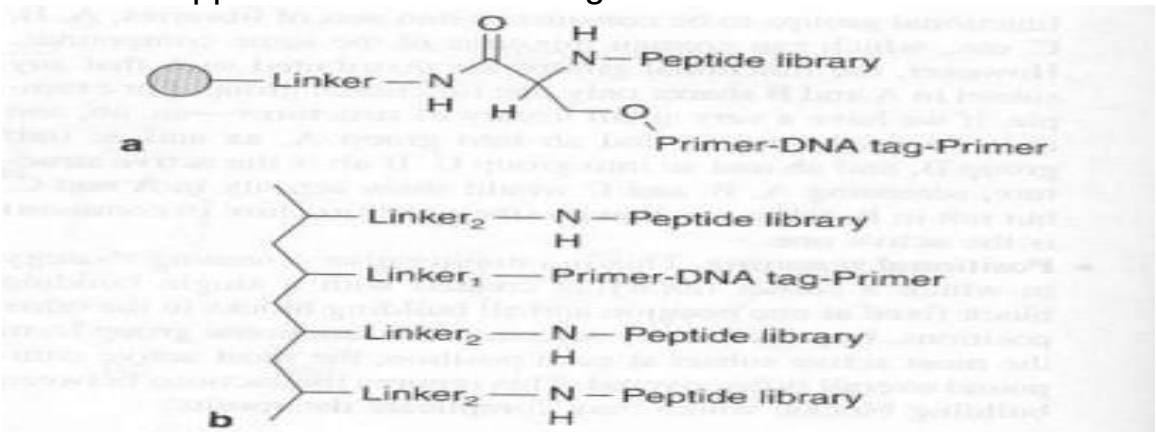


Figure 3–12 ■ Two ways of attaching DNA tags to solid supports. **a.** A DNA tag is attached with each peptide molecule via a bifunctional serine residue. **b.** The DNA and peptide groups have separate linkers.

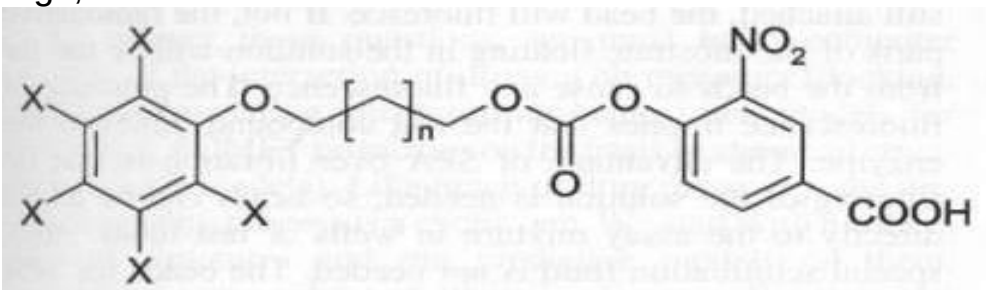
1. The growing DNA chain is attached to the α carbon of a serine group that is anchored to the solid support by a linker molecule. The growing peptide chain is attached to the serine amino group, possibly through a spacer.
2. The DNA chain has its own anchor to the solid support. In this case, fewer DNA tags are attached than the number of polypeptide molecules.

2. "Binary" approach (if DNA tags cannot be used, one can label beads by using a "binary" approach(مزدوج رقم ثنائي).
Suppose we are building a tripeptide with four possible amino acids at each position. We can use binary digits to encode which amino acid is at a given position us follows (each binary "number" is read from the right) (Table -I):

TABLE 3–1 Binary Encoding of a Tripeptide, Using 18 Possible Tags

Amino Acid	Position 1	Position 2	Position 3
Ala	00 00 00	00 00 00	00 00 00
Phe	00 00 01	00 01 00	01 00 00
Gly	00 00 10	00 10 00	10 00 00
Lys	00 00 11	00 11 00	11 00 00

Thus, using 18 different tags (3 x 6) we can encode for any of the (4^3 = 64) members of the library.
For example, if the product is Ala-Gly-Lys, the encoding would be 00000000100011 0000.
3. It is common to use poly halogenated aromatic compounds as tags, such as



Where X represents some combination of halogen atoms. The halogens make the tags show up clearly in MS analysis of the mixture, and by varying the chain length a, the tag can be made flexible enough not to interfere with attachment of the product.

4. Isotopically labeled peptides and dyes.

When it is not possible to use chemical tags, one must physically label the solid or liquid support itself.

Types of physically label:

1. Radiofrequency encoding, in which tiny microchips are added to the resin or to the solution phase. As various reactions are conducted to generate the products, at each step a radiofrequency signal is stored in the microchip.

This signal can be recalled to identify the sequence of reactions that generated the product.

2. Laser optical encoding is yet another approach, in which the solid support consists of a ceramic chip covered with a polypropylene—polystyrene polymer solid phase. The barcode pattern is actually burned into the ceramic at each step in the reaction and is decoded visually with use of a microscope.

3. Finally, one can embed semiconductor particles into the solid phase that fluoresce at different wavelengths. These are called "quantum dots" by their manufacturer.

HIGH-THROUGHPUT SCREENING (HTS):(حاجز،غربال عالي)

Without the ability to screen libraries rapidly for activity, there would be no combinatorial chemistry. Fortunately, the biologists are just as adept at developing rapid high-throughput assays as the chemists are at generating structures.

(HTS): The process for rapidly assessing the activity of samples from a combinatorial library or other compound collection, usually done by running parallel assays in plates of 96 or more wells. A screening rate of 100.000 assays per day is termed ultrahigh-throughput screening.

HTS is an extremely broad topic, encompassing enzymes, organelles, cells, various tissues, whole organs, and even whole animal testing, via cassette dosing. This section briefly discusses only a part of the role of HTS in drug discovery, with emphasis on a few recent developments.

Successful HTS programs integrate(يدمج، يوحد) several activities, including:

1. Target identification (genomics and molecular boilogy groups).
2. Reagent preparation (protein expression and Purification groups).
3. Compound management (تعامل) (information management group).
4. Assay development (biologist and pharmacologist).
5. High-throughput library screening (biologists and chemists). Formerly, these activities were handled separately, and multiple handoffs of samples were involved.

It is becoming more common to integrate the activities and share expertise. This increases efficiency of the screening process.

Another route to increasing efficiency is a move to higher-density screening platforms (خطط ، برامج).

The standard layout for HTS has been a 96-well micro titer plate (12 x 8). Denser formats, up to 1,536 wells per plate, are increasingly being used. This requires advances in liquid handling, precision of detection, and laboratory automation.

One of the first activities in developing a HTS assay is selecting the target. About 500 targets are currently being used by drug companies. Of these:

1. Cell membrane receptors, mostly G-protein—coupled receptors make up the largest group (about 45% of the total).
2. Enzymes make up the next largest group (28%).
3. Hormones (11%).
4. Unknowns (7%).
5. Ion channels (5%).
6. Nuclear receptors (2%).
7. DNA (2%).

It is expected that the annotation (حاشية) of the human genome will add additional targets. Although the rate of this addition is not known.

In fact, much HTS involves screening compounds that are part of the corporate storehouse of compounds synthesized in the past, or they may be a library purchased from a vendor(بائع). Such libraries usually consist of microtiter plates containing frozen or dried samples of compound—perhaps only micrograms per well.

The size of such libraries may range from a few thousand compounds to nearly a million.
The cost of completely screening such a library against just a single assay may amount to over $300,000, so such large scale screens are conducted rather infrequently, compared with routine day-to-day screens. It has been estimated that one must screen at least 120,000 "quality" compounds (i.e.. diverse drug-like structures) to discover a single-lead series for a therapeutically sound target.

As discussed above in the section on pooling strategies, one can reduce the screening effort by pooling groups of structures and running assays on mixtures of compounds.
This also led to:

1. Conserves reagents and biological material.
2. Have smaller storage requirements.
3. Requires fewer personnel.

There are potential problems with pooling:

1. A number of factors limit the number of different compounds we can test in a given well, including ionization, reactivity, and solubility.
2. Compounds can enter a screening program in a nonrandom order, such that a given assay plate may have compounds that are highly similar structurally. This may give rise to false-positive hits. False-negative hits are less likely to arise from pooling.
3. The use of replicates— compounds from the same series— in a given assay.

If only one representative of a given series is present. The chance of missing that series as a possible lead series is greater than if multiple members are present. Therefore, it is common to include several members of each series in a given assay when possible.

To be effective, a given compound must dissolve completely in the assay medium. It is common to add a small amount (1%) of dimethyl sulfoxide (DMSO) to the assay to assist solvation. The best concentration of compound to use is somewhat debatable.

High concentrations (10 µM and above) often lead to more false positives than screening at a low concentration (3 µM). The reason for this may be nonspecific binding at the higher concentration.

Just as there are several ways to detect and identify members of a combinatorial library, there are many ways so measure activity in HTS assays. Any such method must be:

1. Accurate.
2. Reproducible.
3. Have a high signal, to-noise ratio (S/N).

Typically, the result of HTS is a qualitative (yes/no) or semi quantitative one (high-medium-low), rather than a precise value (e.g.. KI_{50} or LD_{50}).
The methods for detection in HTS fall into the categories of nonradiometric and radiometric.

Nonradiometric methods include absorbance, fluorescence, and luminescence(صفة ذو علاقة بالتالق) spectroscopy. Enzyme assays are a common example.
Radiometric methods include filtration and scintillation (يومض، يطلق شررا) proximity assay (SPA). These assays use radioisotopes. So safe storage and handling are of concern.

In filtration assay, a radioactive substrate bound to a capture group is cleaved by its enzyme, removing the radioactivity from the capture group. The mixture is filtered through special filter paper that the capture group sticks to, but everything else passes through. A scintillation fluid is added, and the radioactivity of the filter is measured. The degree to which the radioactivity is retained measures the strength of the inhibition (Fig. 3-I3a).

SPA is a newer, simpler method (Fig. 3-I3b). We start with the same radioactive substrate, which may not necessarily need a capture group. The enzyme and potential drug are added, causing the cleavage of the substrate to some degree.
Now, instead of filtering, a special resin bead coated with a scintillant—a compound that fluoresces in the near presence of the radioactive substrate (near being about 20 μM)—is added to the mixture. The lysed and unlysed substrate binds to the beads, and if the radioactive part of the substrate is still attached, the bead will fluoresce. If not, the radioactive parts of the substrate floating in the solution will be too far from the beads to cause any fluorescence.
The presence of fluorescence implies that the test compound inhibited the enzyme.

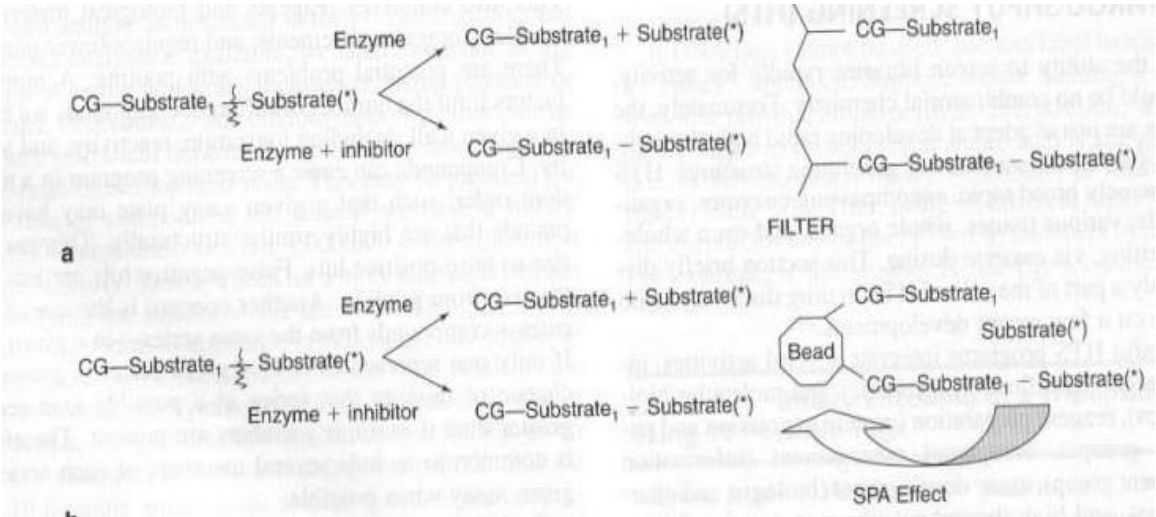


Figure 3–13 ■ Comparison of filtration and scintillation proximity assays. **a.** In filtration assay, the enzyme, substrate, and inhibitor are mixed; the uninhibited enzyme splits the radioactive portion (*) off the substrate, and filtering the mixture, followed by measuring the radioactivity of the filter, tells how much inhibition has occurred. **b.** In SPA, the same mixture is treated with resin beads containing a scintillant that fluoresces only in close proximity to the radioactive source. Any radioactivity that was split off by the enzyme does not need to be filtered in SPA.

The advantage of SPA over filtration is that:

1. No filtering of the solution is needed, so beads can be added directly to the assay mixture in wells or test tubes.
2. Special scintillation fluid is not needed.

The beads for SPA can be engineered to attach a variety of substrate types.

Other HTS assay advances include:

1. The use of microorganisms such as bacteria and yeast.
2. The cloning and expression of mammalian receptors in microorganisms.
3. Probing protein— protein interactions.
4. Very importantly, DNA and protein arrays.

VIRTUAL (IN SILICO) SCREENING: (افتراضي،عملي)

Virtual, or in silico, screening refers to the use of computers to predict whether a compound will show desired properties or activity on the basis of its two-dimensional (2D) or three dimensional (3D) chemical structure or its physicochemical properties.

The motivation (دافع، حافز) for using virtual screening arises from:

1. The flood (فيضـان، طوفـان) of new structures coming from combinatorial chemistry.
2. The expense and time required to run conventional HTS.

3. The ethical concerns about using animal tissue instead of predictive models.
4. An increasing failure rate for structures coming out of combinatorial programs.

In general, a virtual screening program attempts to answer one or both of these questions:

I. Will a particular compound show sufficient binding to a known receptor?
2. Will a particular compound possess any undesirable ADMET properties?

To answer these questions, we must build

- computer models of the interaction of drugs with receptors (docking, molecular modeling, quantum mechanics) and
- models for predicting ADMET properties on the basis of chemical structure (QSAR models).

Like much of drug discovery, the virtual screening process is a cyclic one composed from:

- A collection of structures and run predictive models on them.
- Generating some subset of "best" structures.
- Test these structures in real assays or screens to see if the predictions were accurate.
- Incorporate information learned from the real assays back into our predictive models to improve them for future use.

Models for the prediction of binding arise from the field of molecular modeling. This includes molecular mechanics (predicting the 3D structure of molecules from the standpoint of the atomic nuclei) and quantum mechanics (predicting the 3D structure of molecules from the standpoint of their electrons).
We can generate fairly accurate 3D structures of stand-alone molecules with either of these approaches.

If we know the 3D structure of a receptor, we can predict whether a given compound will "fit" into the receptor with sufficiently tight binding to present normal substrates from binding. i.e... an enzyme inhibitor (or drug). Typically, this does not consider any effects of transport, metabolism, interaction with solvent. etc. Consequently, the existing models for the binding of drugs to receptors are rather crude (فض، خشن), with errors of 50% or more.
Nevertheless, for the purpose of screening, this is often adequate.

If a structure is predicted to show tight binding, it will probably be synthesized and tested in a real HTS assay, to obtain a more accurate estimate of its activity.

We can combine the prediction of binding to a receptor with the design of molecules. If we start with a set of building blocks and a known receptor, we can align (يرصف) complementary building blocks with pockets in the receptor, in conformations that maximize hydrogen bonding and other interactions. When the fragments are aligned, we can then connect the fragments with appropriately sited spacer fragments to "build" a drug molecule within the confines (حدود، حاجز) of the receptor. This approach is sometimes called *de novo* or structure based drug design.

Pfizer researchers found that since the introduction of combinatorial chemistry, chemists had tended to design larger, more complex, and more lipophilic structures than in the past. Since the structures were supposed to be designed as lead structures rather than optimized drug molecules, it was becoming more difficult to optimize them into drugs. This led Lipinski to enumerate some rules for the rejection of structures. He proposed rejecting any structures that fail two or more of the following criteria:

• Molecular weight should be <500.
• Number of hydrogen bond donors (NH. OH should be fewer than 5.
• Number of hydrogen bond acceptors t—N—. —0—. —S-—) should be fewer than 10.
• Calculated log P value should be less than 5.

Since the number 5 shows up in many of these criteria, they have become known as the "rule of five." The "rule of five" is designed to be a yes/no filter for the rejection of structures.

More quantitative models designed to predict some value or levels of property have also been developed. Examples include
1. Predicting Caco-2 cell permeability (Caco-2 cells are human intestinal epithelial cells that can be layered and studied in vitro)
2. Predicting binding to cytochrome P-450.

Llplnski "rule of five": A set of criteria for predicting the oral bioavailability of a compound on the basis of simple molecular properties (molecular weight. <500: log P. <5; number of

hydrogen-bond donors. <5; and number of hydrogen-bond acceptors. <10). Typically, the criteria are applied to a library to filter structures from the library before any synthesis takes place. Any structure exceeding two or more criteria is rejected.