Combinatorial Chemistry

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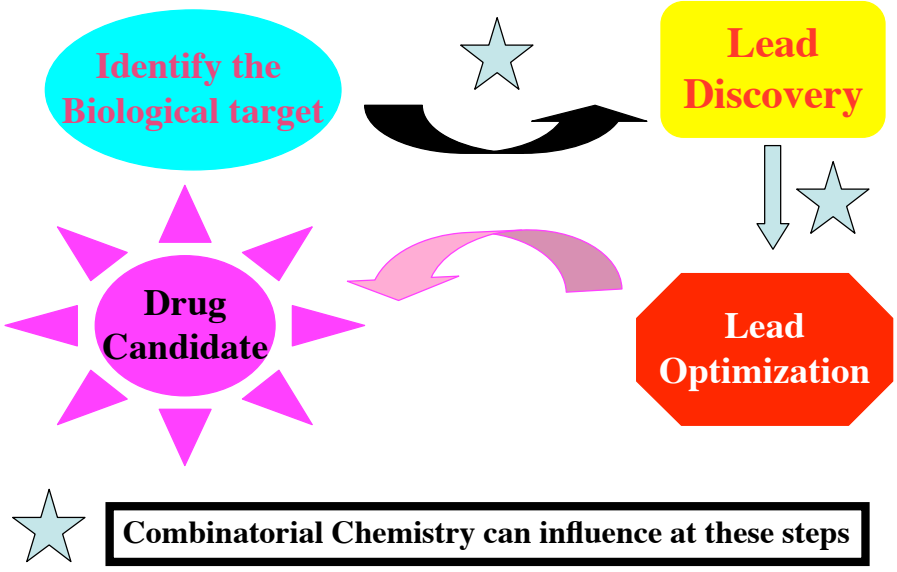
Drug Discovery Process



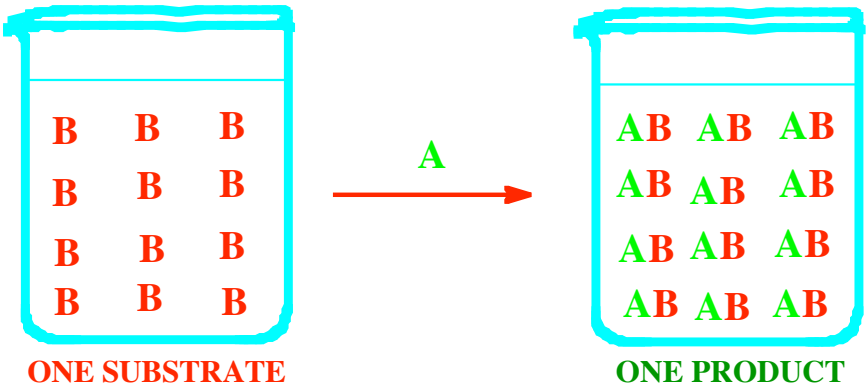
Principle

What is combinatorial synthesis?

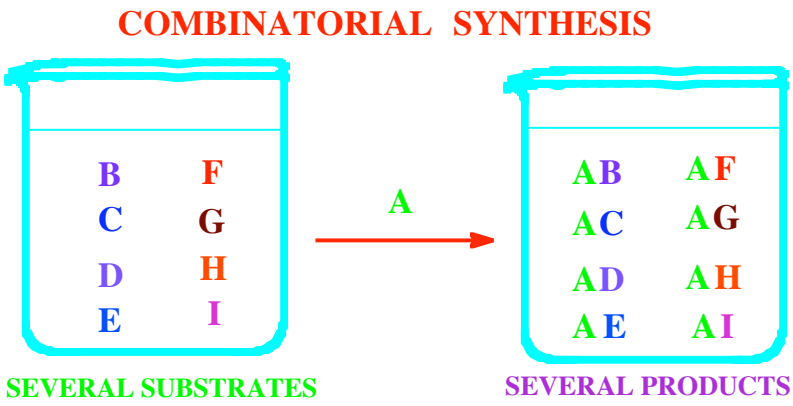
In conventional synthesis one compound is made at a time. In combinatorial chemistry several compounds are made simultaneously.

Orthodox synthesis - one product at a time

ORTHODOX SYNTHESIS



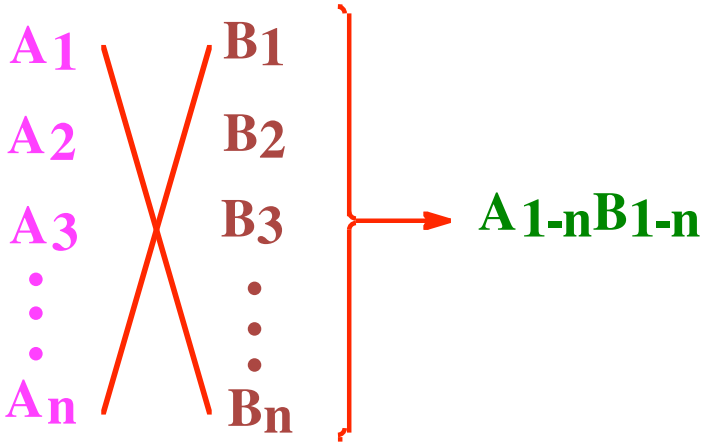
Combinatorial synthesis -
Several products at the same time



CONVENTIONAL SYNTHESIS

A + B ⟶ A—B

COMBINATORIAL SYNTHESIS



Conventional Synthesis:

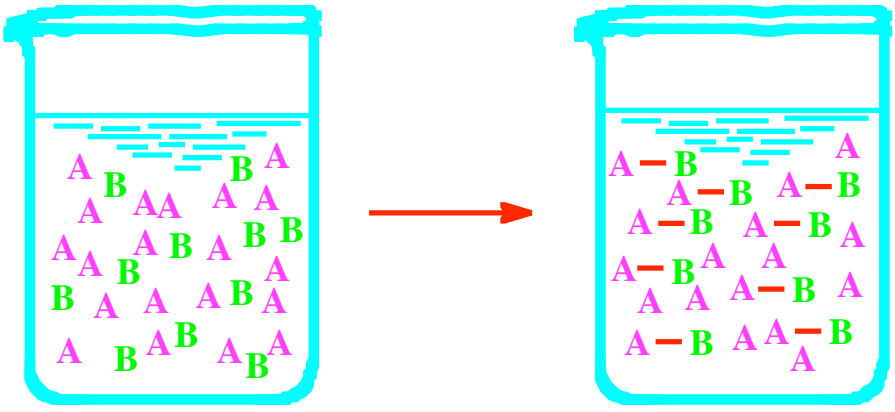
A + B ----> A-B Few substrates ---> single product

Combinatorial Synthesis:

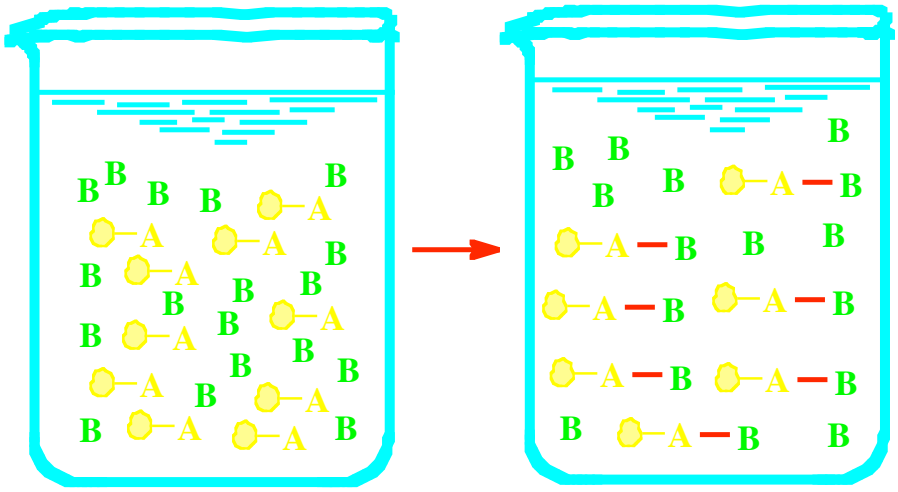
A1	+	B1	A1B1	+	A2B1	+	A3B1 ....AnB1
A2	+	B2	A1B2	+	A2B2	+	A3B2 ....AnB2
A3	+	B3	A1B3	+	A2B3	+	A3B3 ....AnB3
A4	+	B4	A1B4	+	A2B4	+	A3B4 ....AnB4
...		...	....		....		....
...		...	....		....		....
...		...	....		....		....
An	+	Bn	A1Bn	+	A2Bn	+	A3Bn ....AnBn

Several substrates ----> Several products

Conventional synthesis



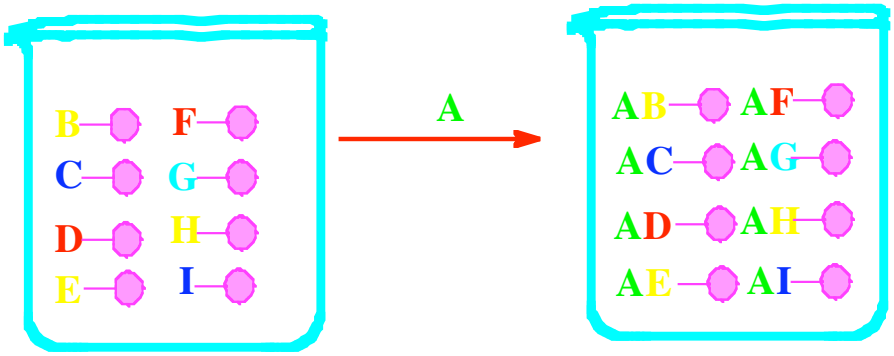
Solid Phase Synthesis



Combinatorial synthesis -

Synthesis can be easily achieved on solid phase.

COMBINATORIAL SYNTHESIS

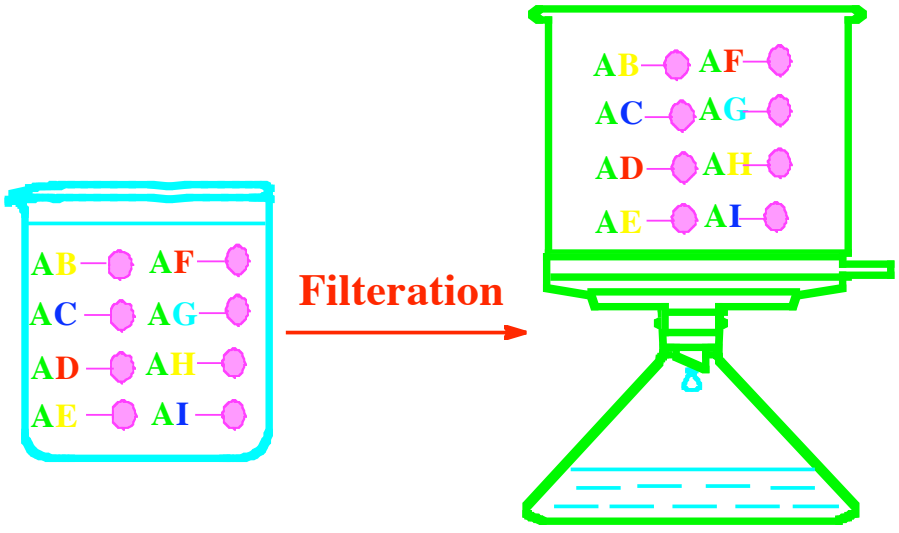


SEVERAL SUBSTRATES **SEVERAL PRODUCTS**

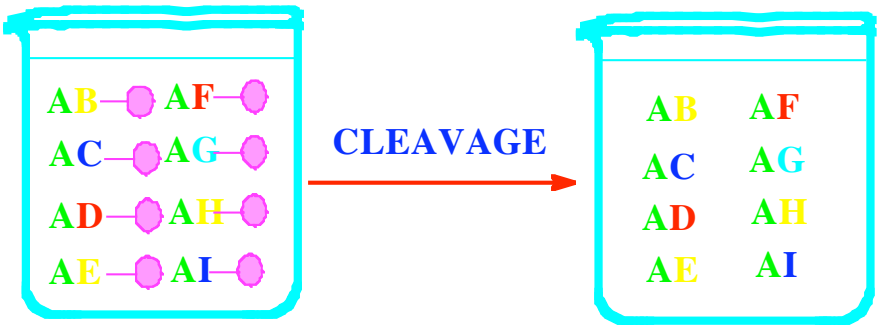
Solid Phase Synthesis - Advantages

- Reaction can be forced to go forward by excess reagents.
- Isolation of product by simple filtration.
- Purification of products is easy- simple washing.
- Several steps can be accomplished on the same compound.

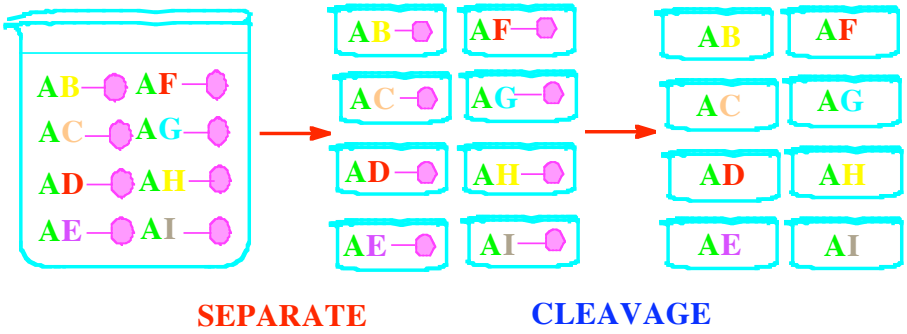
In solid phase synthesis, products are isolated easily by filtration



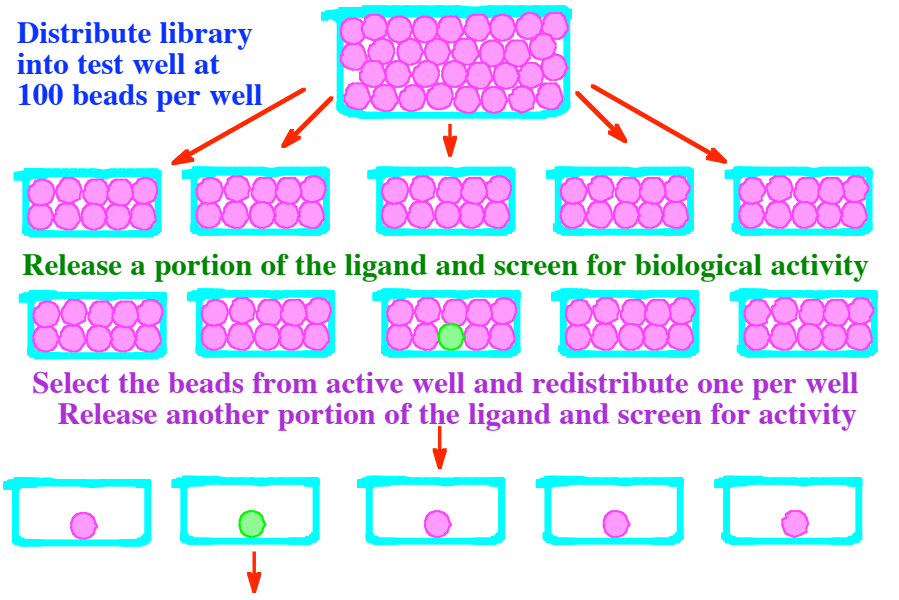
Products from solid phase is released by cleavage and used for further work



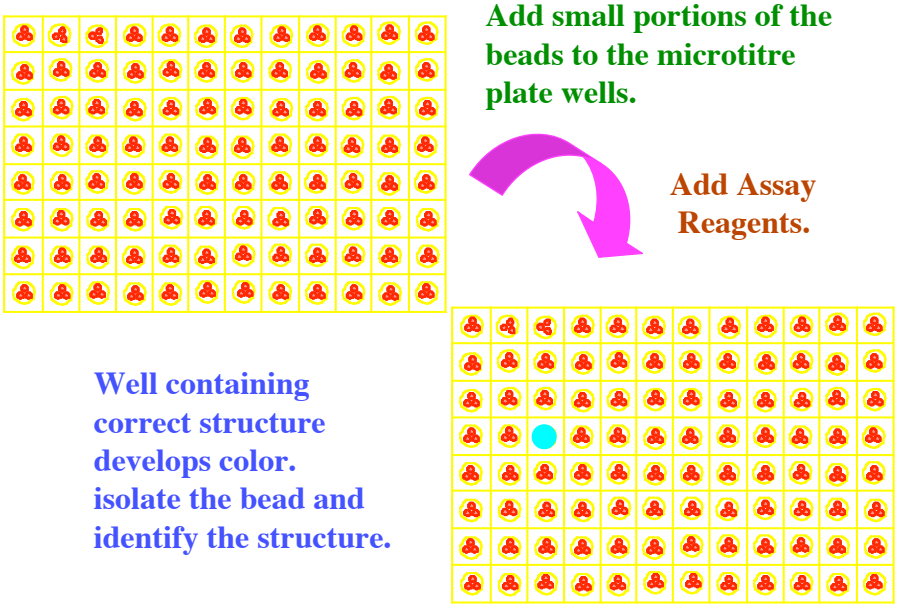
Products can be manually separated and then cleaved

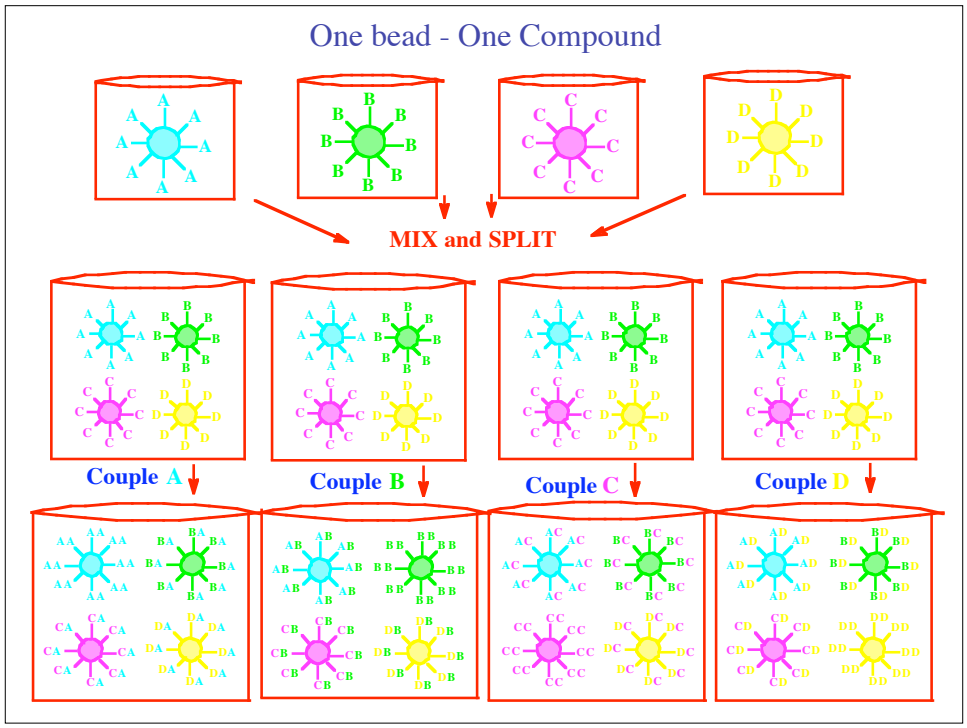


Multi-release approach to solution screening of the libraries



Assays can be made on the solid phase itself

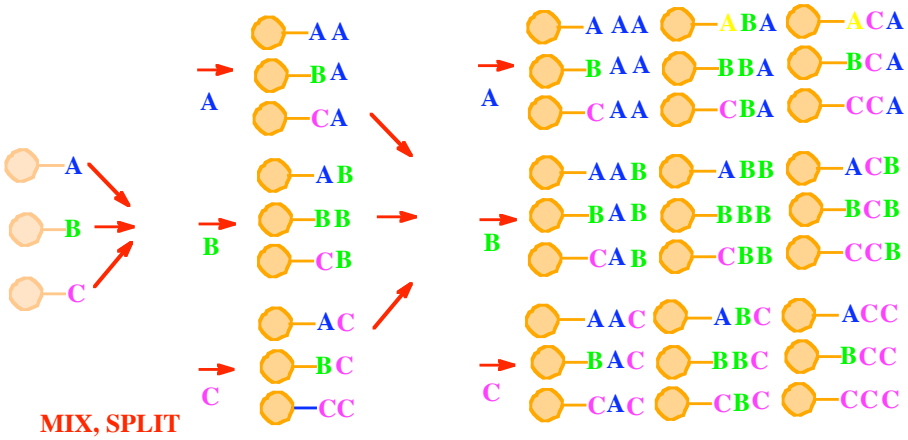




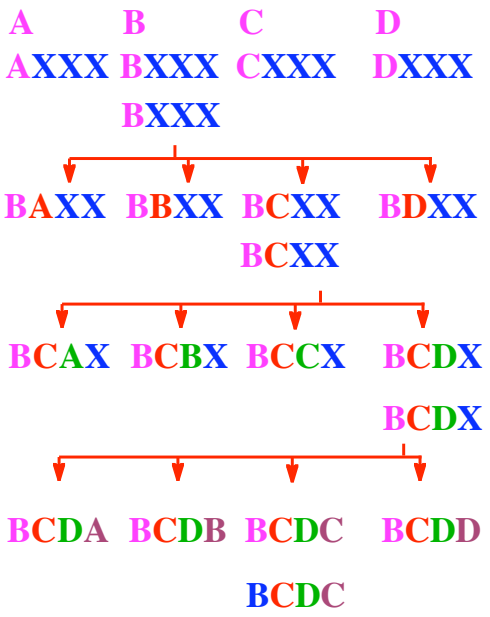
Tripeptide synthesis by combinatorial chemistry -

Split Mix synthesis

3 amino acids 9 dipeptides (3^2) 27 tripeptides (3^3)



Serial deconvolution synthetic combinatorial libraries



Fix the first position and prepare libraries

Screen & select the best subset to determine the first position

Fix the 1st & 2nd position and prepare libraries

Screen & select the best subset to determine the second position

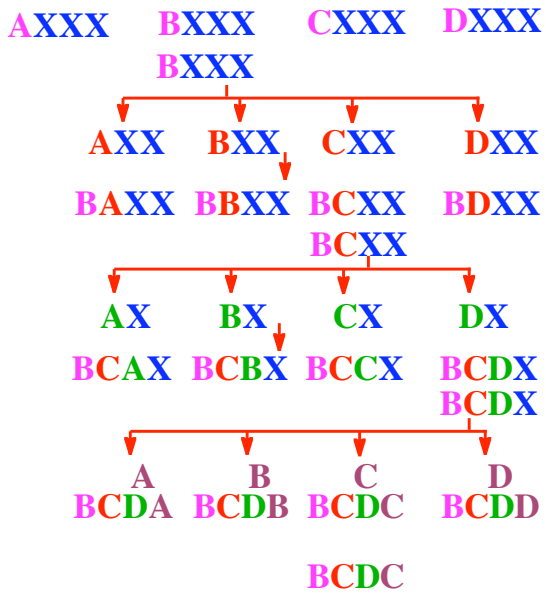
Fix the 1st three position and prepare libraries

Screen & select the best subset to determine the third position

Fix all positions; prepare libraries

Screen & select the best subset to determine the last position

Recursive deconvolution synthetic combinatorial library



Screen & select the best subset to determine the first position

Retrive intermediate tripeptide library and couple B to all.

Screen & select the best subset to determine the second position

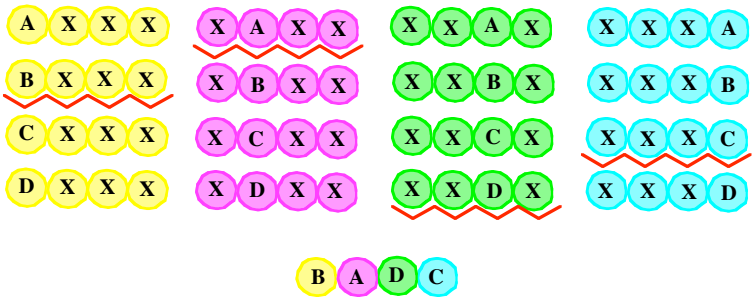
Retrive intermediate dipeptide library and couple BC to all.

Screen & select the best subset to determine the third position

Retrive the starting amino acid library and couple BCD to all.

Screen & select the best subset to determine the last position

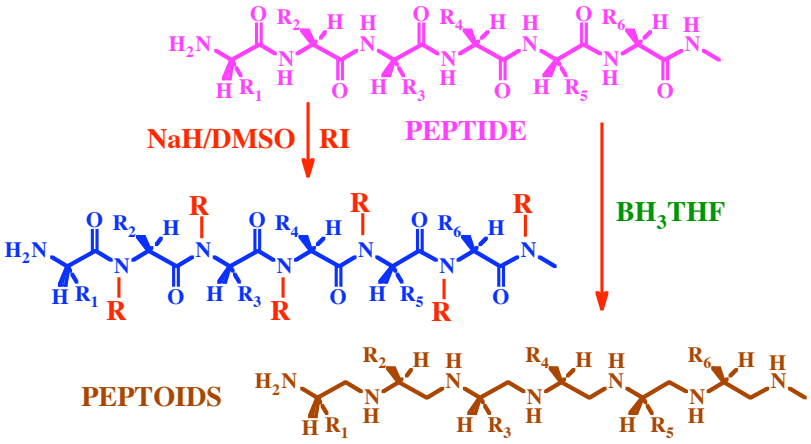
Positional Screening Library



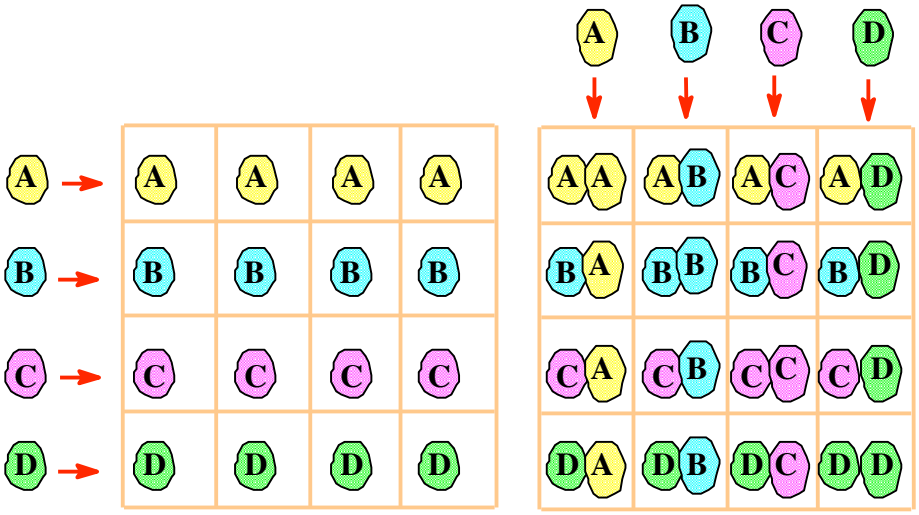
$O_1XXXXX\text{-}NH_2$
$XO_2XXXX\text{-}NH_2$
$XXO_3XXX\text{-}NH_2$
$XXXO_4XX\text{-}NH_2$
$XXXXO_5X\text{-}NH_2$
$XXXXXO_6\text{-}NH_2$

Hexapeptide with 18 amino acid
6 sets of 18^5 (1,889,568) compounds
O is fixed at each position.
Total 18^6 (34 million) compounds

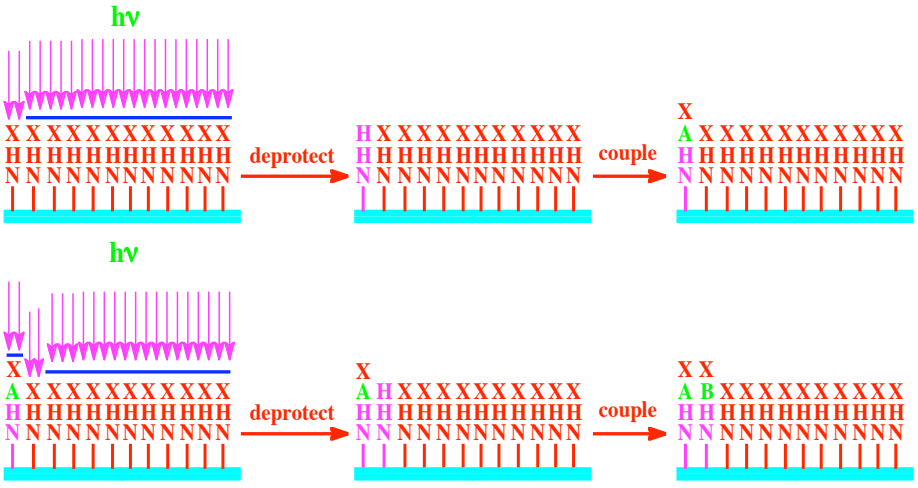
The peptide libraries are good in the test tube.
But they are ineffective as drug candidates
because they tent to undergo rapid enzymatic hydrolysis
in the body - " Fast clearence". Hence peptide libraries are
converted into peptoid compounds that resist enzymatic hydrolysis
These compounds are called library of libraries.



Spacially addressable synthesis

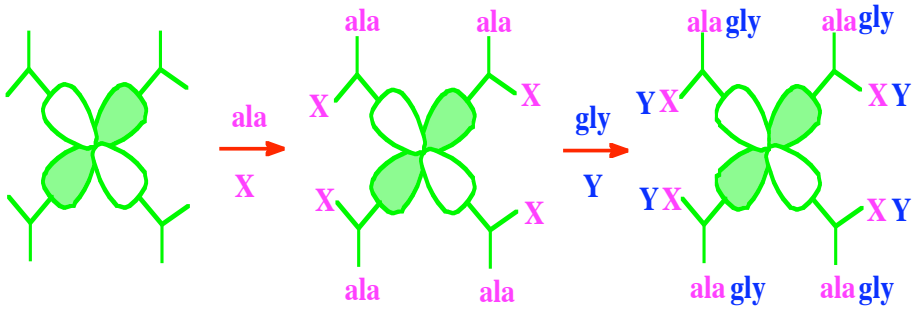


Use of photomask to spacially address the synthesis



Use of branched linkers for synthesis of a library using a peptide encoding system

Each time a compound is added, an encoding peptide is synthesized on the branch. The correct sequence of the unknown compound can then be determined by sequencing the encoding peptide on the bead.



Solution phase synthesis - Glaxo example

Smith et al., Biomed. Chem. Lett 4, 2821-2824 (1994).

40 RCOCl + 40 amines ---> amides

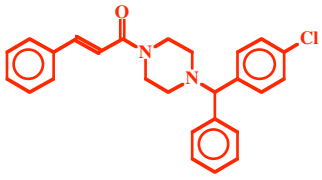
40 RCOCl + 40 alcohols ---> esters

Set 1: RCOCl + 40 R'NH_2 -----> 40 RCONHR' + HCl

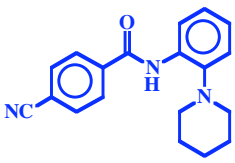
Set 2: 40 R"COCl + RNH_2 -----> 40 R"CONHR + HCl

Compounds identified:

Neurokinin-3 receptor inhibitor Ki = 60 μM



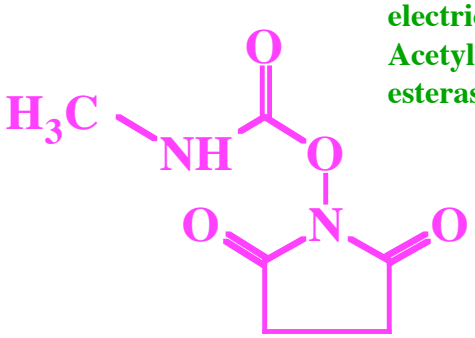
Matrix metalloprotease-1 inhibitor Ki = 55 μM



Identifying the inhibitor of acetylcholine esterase

9 alcohols + 6 isocyanates ---> 54 carbamates

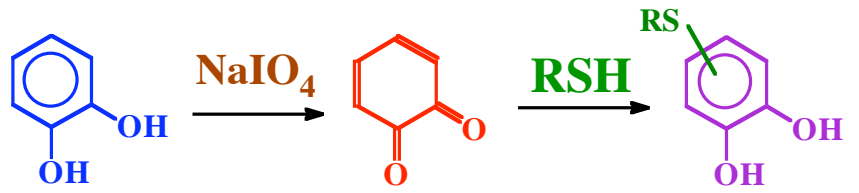
Test them on electric eel Acetylcholine esterase



A potent inhibitor was identified

In this lab you will make different substrates of tyrosinase by combinatorial type synthesis and test them as substrates for tyrosinase.

- **All *o*-dihydroxyphenols (called in general catechols) are easily and quantitatively oxidized by sodium periodate to their corresponding quinones.**
- **These quinones are unstable and polymerize rapidly. But they can be trapped by thiols instantaneously to form thiolated catechols.**



Catechol **Quinone** **Thiolated catechols**

In this lab, you will make different thiolated catechols using combinatorial synthesis and test them as substrates for tyrosinase

- **You will take eight differeent catechols - these are: catechol; 3-methylcatechol; 4-methylcatechol; 3,4-dihydroxy benzaldehyde; 3,4-dihydroxybenzoic acid; 3,4-dihydroxy phenylacetic acid; 3,4-dihydroxyhydrocinnamic acid and N-acetyldopamine.**
- **You will oxidize them with sodium periodate and allow the resultant quinones to react quickly with four different thiols -cysteine; N-acetylcysteine, glutathione and dithiothreitol.**
- **The resultant 32 compounds and the unmodified 8 originals (together 40 compounds) will be tested on tyrosinase as substrates.**

Tyrosinase is an important enzyme catalyzing the oxidation of the amino acid tyrosine to dopa and resultant dopa to dopaquinone

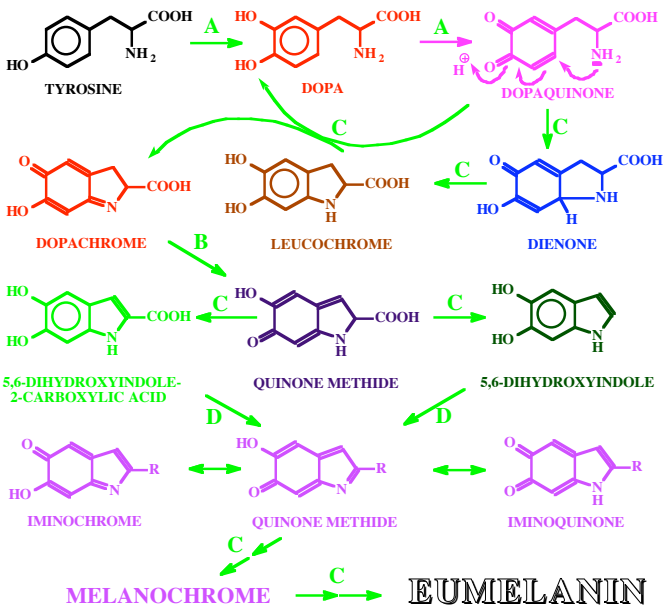
TYROSINE DOPA

TYROSINE HYDROXYLATION

DOPA DOPAQUINONE

DOPA OXIDATION

Tyrosinase is responsible for the biosynthesis of the melanin pigment in all living organism



References

- Terrett, N.K., Gardner, M., Gordon, D. W., Kobylecki, R. J., and Steele, J. Combinatorial Synthesis -The design of compound libraries and their application to drug discovery. Tetrahedron 51, 8135-8173 (1995).
- Sugumaran, M. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. Pigment cell Research. 15, 2-9 (2002).