Combinatorial chemistry

* Collection of techniques for synthesis of multiple compounds at the same time
* Systematic and repetitive, covalent connection of a set of building blocks of varying structures, to yield a large array of diverse molecules
* Mimics natural sources 🡪 produces pool of chemicals 🡪 one of them may be proved as lead compound
* Basic principle: Prepare large number of different compounds at once 🡪 identify the most promising compound 🡪 high throughput screening
* Characteristic: Different compounds generated under same reaction conditions in systematic manner 🡪 products of all possible combinations are obtained at same time
* Collection of synthesized compounds is referred to as combinatorial library
* Library is then screened 🡪 active compounds identified
* Approach has 2 phases
  + Making a library
  + Finding active compound
* All combinatorial libraries are structurally related: Have the same scaffold (core structure) / have the same backbone. Dissimilarities depend on building blocks.
* Combinatorial chemistry was developed to reduce time and costs of producing new drugs
* Example of effect on drug discovery due to acceleration of chemical synthesis:
  + Conventional synthesis: Material A + Material B -> Product AB
  + Combinatorial synthesis: Building blocks A (A1-An) + building blocks B (B1-Bn) --each starting A reacts with B🡪 A1-n+B1-n
  + Combinatorial covers many reactions compared to conventional
* Need for combinatorial chemistry:
  + Why?
    - Only one molecule can be synthesized at once: time consuming, cost ineffective
    - Low yields
    - Slower lead generation
    - High risk of failure: only hundreds of molecules generated each month
  + How?
    - Multiple molecules can be synthesized at once: time efficient, cost effective
    - High yields
    - Faster lead generation
    - Low risk of failure: Thousands of molecules generated each month
* Advantages of combinatorial chemistry
  + Creation of large libraries can be done in a short time (main advantage)
  + Compounds that can not be synthesized by traditional methods 🡪 can be done using combinatorial chemistry
  + Cost of combinatorial is high but compared to per compound analysis it is very low
  + High yields 🡪 many molecules for testing 🡪 faster lead generation
  + Low risk of failure
  + Speeds up drug discovery process
* Disadvantages of combinatorial chemistry
  + Even though synthesis is faster, one needs to synthesize the right compound
  + Large number of compounds 🡪 libraries not focused 🡪 inability to generate sufficient number of hits during assay
  + Chemistry is limited while using solid phase synthesis 🡪 Resin used is affected by reaction types 🡪 Each reaction step has to be carefully planned out.
* History
  + Very young science, 20 years old
  + Origins can be traced back to as far as 1963 🡪 professor R. Bruce Merrifield developed a way to make peptides by solid-phase synthesis
  + Modern definition of the field started taking shape in 1980s 🡪 H. Mario Geysen Wellcome developed 🡪 synthesize arrays of peptides on pin-shaped solid supports. 1985 🡪 Richard Houghten 🡪 technique for creating peptide libraries in tiny mesh "tea bags" by solid parallel synthesis
  + Dr Arpad Furka 🡪 1988 🡪 split and pool method
  + 80s-90s 🡪 peptide synthesis and later oligonucleotide synthesis
  + 90s 🡪 synthesis of small drug like organic compounds
  + Current 🡪 Drug discovery by pharmaceutical and biotechnology companies
* Types of combinatorial libraries
  + Range of techniques is very diverse, products can be made:
    - Individually
    - Parallelly
    - In mixtures
  + Combinatorial is of two types
    - Solid Phase Combinatorial Chemistry
      * Compound library synthesized on solid phase (resin bead)
    - Solution Phase Combinatorial Chemistry
      * Compound library synthesized on solvent (in reaction flask)
  + Solid phase combinatorial chemistry
    - Steps
      * Starting compound attached to inert solid / resin bead
      * Excess reagents added to solution
      * Products attached to resin beads separated by simple filtration
      * Cleavage and isolation of products form beads
    - Requirements
      * Cross-linked insoluble polymeric support 🡪 should be inert in synthetic conditions (resin bead)
      * Anchor / Linker, linked to resin 🡪 should have reactive functional group 🡪 can be used to attached substrate
      * Bond that links substrate to linker 🡪 should be stable to reaction conditions
      * Means to cleave product from linkers
      * Protective groups that are not involved in reaction to protect functional groups
    - Example of solid supports
      * Partially cross-linked polystyrene beads (polystyrene X divinyl benzene) 🡪 causes problems in peptide synthesis
      * Sheppard’s polyamide resin 🡪 more polar
      * Tentagel resin 🡪 similar environment to ether
      * Beads, pins and functionalized glass surfaces
    - Characteristics of Solid supports:
      * Beads must be able to swell, and remain stable
      * Most reactions occur in bead interiors
    - Advantages of solid phase combinatorial chemistry
      * Reaction happens on solid supports 🡪 range of starting materials can be bound to separate resin beads and mixed 🡪 all starting material (all beads) can be treated at the same time in the same reactions 🡪 multistep synthesis is possible
      * Product bound to solid support 🡪 excess reagent can be washed off 🡪 excess reagent can be added to speed up reaction
      * Individual beads 🡪 individual products
      * Support can be regenerated / reutilized
      * Automation possible
    - Disadvantages of solid phase combinatorial chemistry
      * Not all synthesis can be done in solid phase
      * Some molecules don’t attach to beads
      * Some chemistry doesn’t work
      * Removing product from bead can sometimes damage product
      * Monitoring progress of reaction is difficult
      * Purity assessment and purifying of product is difficult
  + Solution Phase Combinatorial Chemistry
    - All reactions are conducted simultaneously, in well-ordered arrays of reaction vessels
    - Soluble polymer used as support
    - Chemistry takes place in solution phase
    - Solution phase is explored as alternative to solid phase
    - Advantages
      * Handling of material is each and can be automated
    - Disadvantages
      * Purification is a big disadvantage
      * Number of reagents in a solution result in several side reactions (main disadvantage)
      * PEG & liquid Teflon is used as common vehicle for solution phase synthesis. (IDK how tf this is a disadvantage)
    - Limitations
      * Number of reagents in a solution
        + Several side reactions
        + Leads to polymerization (tarry mass)
* Resin (solid support)
  + Bead size ranges from 10 micro meter to 750 micro meter
  + Solid support must have these characteristics
    - Physical stability / allow liquid handling and filtration
    - Chemical inertness
    - Ability to swell under reactive condition / allow solvents and reagents to permeate
    - Derivatization to allow for attachment of linker (covalent)
  + Solid support has two parts
    - Core
    - Linker
  + Starting compound attached to support via linker
  + Compounds not attached directly to beads / attached by “linker moiety” / enables attachment in reversible way without destroying molecules
  + Core 🡪 insolubility and swelling
  + Linker 🡪 functional group attachment and reaction conditions (cleavage)
  + Linker and bond with compound must be stable
  + Bead should swell but remain stable
  + Swelling is important (reactions take place inside bead)
  + Beads have bead shaped / developments in pins shapes (maximize surface area)
  + Types of solid support used
    - Polystyrene resin
    - Tenta Gel Resin
    - Glass and ceramic beads
* Linkers / anchors
  + Initial building block
  + Covalently attached to solid support with reactive function group
  + Allows attachment of first reactant
  + Bond between linker and substrate must be stable and should be easily cleavable
  + Bi-functional molecule 🡪 one group: irreversible attachment to resin 🡪 other group: reversible covalent bond to initial building lock
  + Different linkers available depending on product
  + Resins are named to define linker
    - Merrifield resin 🡪 Binds carboxylic acids 🡪 Cleaved using HF
    - Wang resin 🡪 Binds carboxylic acids 🡪 Cleaved using 95% TFA
    - Rink resin 🡪 Binds carboxylic acids 🡪 cleaves in carboxamide form
    - Hydroxymethyl resin 🡪 Binds activated carboxylic acids 🡪 Cleaves like Merrifield resin
    - Photolabile anchors 🡪 Allows cleavage by irradiation 🡪 2-nitronemzhydrylamine resin 🡪 absorbs UV light
    - Traceless anchors 🡪 who the fuck knows I’m not reading that confusing ass shit
* Protecting groups used in Solid phase synthesis
  + Primary function 🡪 protect portion of molecule that is not bound to resin (avoids subsequent polymerization)
  + Reversibly attached to convert to less reactive form
  + Protecting group cleaved when it is no longer needed
  + For peptide synthesis 🡪 large number of protecting groups developed
  + Needs to be stable under expected reaction conditions
  + After coupling 🡪 protecting group removed 🡪 synthesis continues in repetitive fashion
  + Cleavage condition => linker used
  + Two protecting groups can be removed without affecting stability to each other = orthogonal groups
  + Widely used protecting groups
    - Benzyl carbonyl (Z) group
    - 1 butoxy carbonyl (Boc) group
    - 9-fluorenyl methoxy carbonyl (9-fmoc) group
* Difference between solid phase and solution phase combinatorial chemistry (characteristics)

|  |  |
| --- | --- |
| Solid Phase | Solution Phase |
| Makes a mixture of products | Makes only one product |
| Small amounts of product forms | Large amounts of product formed |
| Simple isolation (filtration) works | Purification is more difficult |
| Requires two extra reaction steps: linkage and cleavage | No extra steps for linkage and cleavage |
| Limits to chemistry which can be performed | Wide range of reactions can be utilized |
| Automation possible | Automation difficult |
| Large excess of reagent can be used to drive reaction | Large excess of reagent can’t be used as it causes subsequent separation problem |
| Longer reaction time | Shorter reaction time |
| Monitoring of reaction is difficult | Monitoring of reaction is easy |
| Split and mix / parallel technique can be applied | Split and mix can’t be applied. parallel technique is possible |

* Types of combinatorial libraries
  + Scaffold based libraries
    - Core structure is common for all compounds
    - Scaffold consists of several building blocks
    - Eg: Animo acid and amino benzophenone
  + Backbone-based libraries
    - Example – nucleic acid and carbohydrate
* Approaches to build libraries
  + Random / diverse approach
    - Synthesis of diverse compounds 🡪 large number of molecules 🡪 more hits
    - Little is known about target 🡪 more diverse library
  + Focused libraries
    - Synthesis of focused compounds 🡪 small number of molecules
    - Incorporate as much information about the target as possible
* Library preparation
* Split and mix
  + Dr. Arpad Furka 1988
  + Steps
    - Ingredients assembled on surface of beads
    - Each step 🡪 beads from last step partitioned into new building block 🡪 formation of new groups 🡪 beads from this group are split again 🡪 step is repetitive until active compound is found
    - One compound is bound to each resin
    - Requires solid support
    - Only employed for solid phase synthesis
  + Advantages
    - Only few reaction vessels needed
    - Large libraries can be quickly generated
  + Disadvantages
    - Threefold the number of beads necessary
    - Amount of synthesized product is low
    - Complex mixtures formed
* Parallel synthesis
  + Steps
    - Each compound in specific reaction vessel
    - Each material reacts with each building block
    - Product is split into portions 🡪 reacted with different building block
    - Methods include
      * Houghton’s tea bag
      * Automated parallel synthesis
  + Advantages
    - Individual compounds in own vessel, identification of product easy
    - Each compound is substantially pure
    - Biological evaluation is easy
  + Disadvantage
    - Only for medium libraries
    - Large number of vessels
    - Large number of reactions

Houghton Teabag method (parallel synthesis)

* Polypropylene mesh bag (15 x 20 mm) 🡪 filled with resin beads 🡪 sealed and labeled for identification (tea bag) 🡪 designed by Houghton in 1985
* Mesh too small for resin beads, enough for solvents and reagents
* Used to make multimiligram (500 micro moles) of single peptide sequence in each packet
* Manual approach
* Bags can be combined into same reactors (saves time and work)
* Example
  + Synthesis of 40 peptides 🡪 all bags are charged with beads (box-protected amino acid) 🡪 combined for resin deprotection 🡪 washing 🡪 neutralization
  + Bag sorted into groups 🡪 addition of next amino acid 🡪 combined for deprotection 🡪 washing 🡪 neutralization
  + Bags treated with HF / anisole to cleave peptides from beads
  + Classic example of combinatorial synthesis for speed and effectiveness
* Advantages
  + Easy to identify active hit (own bag + labelled)
  + New equipment (personal synthesizer / multi vial apparatus) allows parallel synthesis by one chemist
  + Can be automated
  + Large quantity of each compound obtained
* Disadvantages
  + Many impurities (unless very clean reactions)
  + Effective for 1-3 step reactions only
  + Makes smaller (focused) libraries

Automated parallel synthesis

* 42, 96, 144 vessel wells available
* Beads or pins used
* Automated reactions
* Same route different reagent for each vessel
* Different product per vessel
* Steps
  + Building block is separate vessel
  + Done in solid or solution
  + 96 well is commonly used
  + Reaction 🡪 product split into ‘n’ portion 🡪 reaction with new building blocks
  + Like split and pool produces multiple compounds at the same time
  + Unlike split and pool produces compounds individually and not in mixture

Mixed combinatorial synthesis

* Using standard synthetic route to produce large number of analogues, each vessel contains mixture of products
* Identities are not know
* Useful for finding lead compound
* Synthesizes large number of compounds quickly
* Inactive stored in combinatorial libraries 🡪 studied further for active component

Screening of combinatorial library

* 2 ways
  + Virtual screening
    - Uses computational methods
    - 3 screening methods
      * Molecular docking
      * Pharmacophore mapping
      * QSAR-QSPR
    - Disadvantages
      * Cannot replace real screening
      * Generated hits difficult to synthesize
  + Experimental real screening
    - High throughput screening, tests large number of compounds, real results
    - Disadvantage
      * Very expensive
      * Slower than virtual screening
* Most common assay 🡪 determines binding of library compounds to target protein
* Other common assays 🡪 Biochemical, enzymatic, cell based
* Cell based 🡪 direct cytotoxic, receptor binding, cell signaling
* Selection depends on
  + Nature of libraries
  + Parallel synthesized libraries can be screened with automation HTS
  + Solid support library can be screen with biological targes

Application of combinatorial chemistry

* Cancer research and drug discovery
* Build synthetic gene circuits: screening and selection strategies
* Approaches for improving soluble protein expression in E coli
* Combinatorial library based strategies to optimize proteins
* Rapid humanization of anticarcinoma br96 fab
* Synthetic peptide combinatorial libraries
* Anti-viral research

Strategies for library design

* Monomer-based selection
  + Small repeating unit are monomers
  + Monomer = a; polymer of monomer = a-a-a-a-a-a-a-a-a
  + Monomer-based 🡪 optimized subsets selected (disregard resulting product)
  + 3 component library 🡪 100 monomer at each position 🡪 aim 10x10x10 library 🡪 10 most diverse monomers from each set 🡪 subsets of size n contained without larger set N
  + More than 1013 subsets can not be examined
  + Fuck that
* Product based selection
  + More complex optimization (combinatorial optimization)
  + Properties of product monomer is taken into account
  + Enumeration of virtual lib 🡪 any subset selection applied
  + Called cherry picking 🡪 low synthetic efficiency
  + Efficiency improved by 🡪 taking combinatorial constraint into account 🡪 select subset 🡪 every reagent at each point 🡪 reacts with every other reagent at other points
  + More computationally demanding 🡪 more reliable
  + Has greater computational complexity but can also be more effective when optimizing properties of lib as whole

Not writing just reading the rest