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 (A_1-A_n) + building blocks B (B_1-B_n) --each starting A reacts with B→ A_{1-n}+B_{1-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-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