Drug Discovery & Development

Discovery Phase

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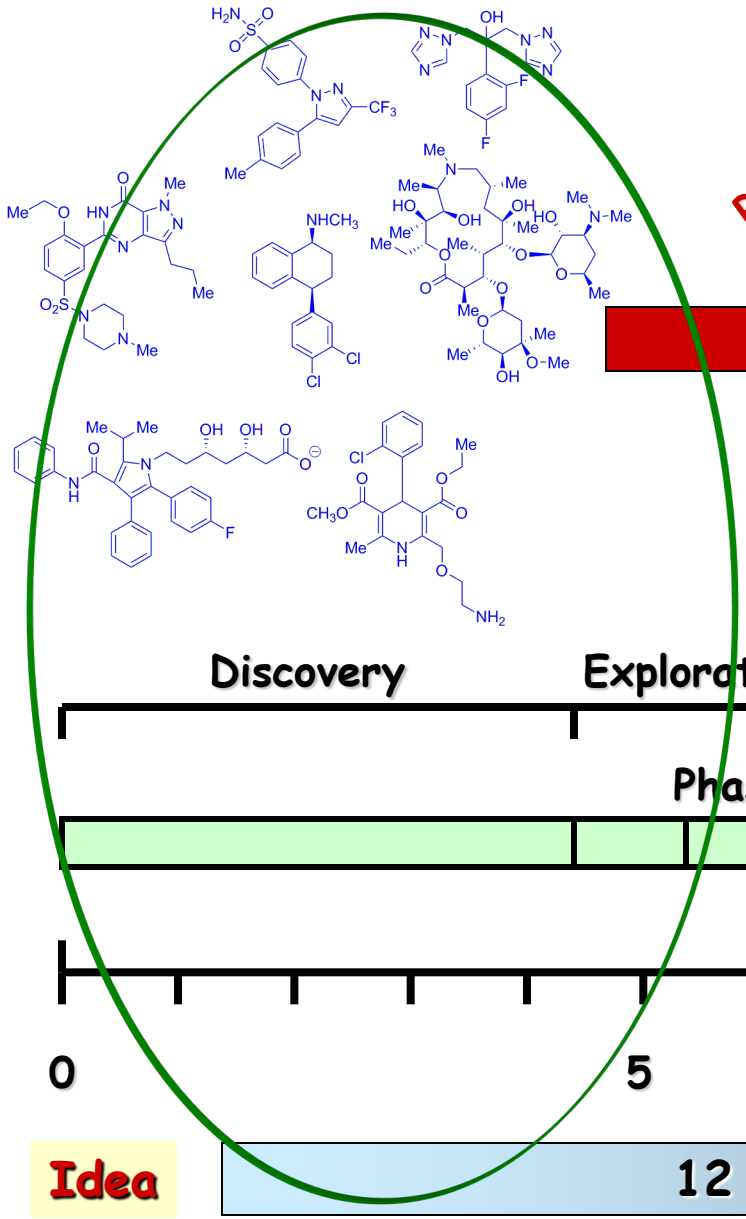
Drug Discovery & Development: Timeline



Pre-Clinical Pharmacology

Pre-Clinical Safety

Clinical Pharmacology & Safety

Discovery

Exploratory Development

Full Development

Phase I

Phase II

Phase III

0

5

years

10

15

Idea

12 – 15 years

Drug

Lecture Outline

- Target Identification & Verification
  - Basic Principles
  - Case I: off-target, cGMP PDE
  - Case II: HIV targets
- Lead Generation
  - Rational Design: HIV-protease Inhibitor
  - Concepts of Library Screen
  - Expansion of Small Molecule Inventory
  - High-Throughput Screen (HTS)
- From Lead to Drug:
  - Lead Optimization
  - Lipinski's Rule of Five & DEMET
- Macromolecule Drugs: Proteins & Nucleic Acids

Initial Stages: Drug Discovery

Discovery: 3-5 years

- Target selection
- Target verification

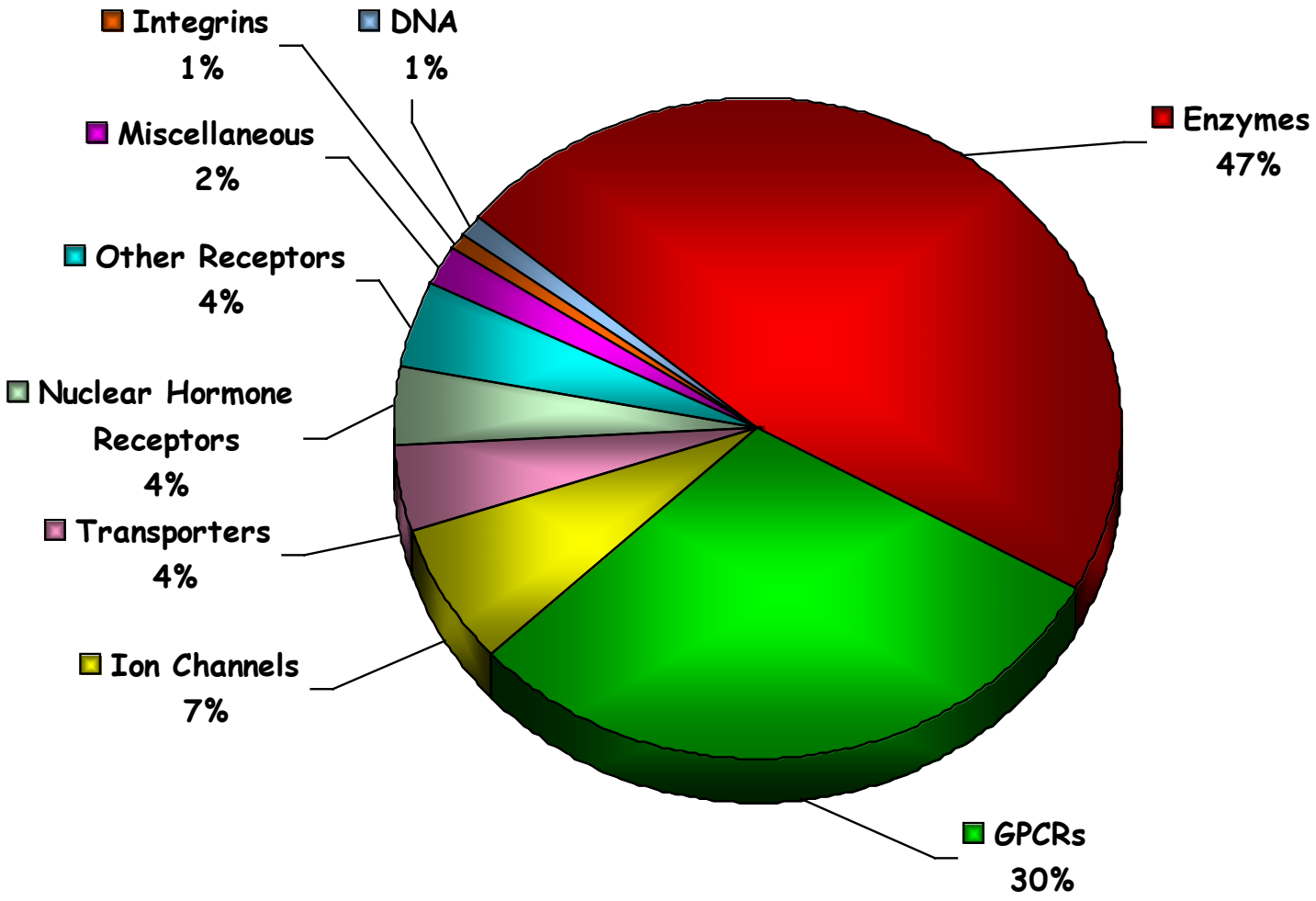
- High-throughput synthesis
- High-throughput screening
- Crystallography
- Computational chemistry

- Medicinal chemistry
- Process chemistry
- Pharmacokinetics
- Early toxicology

"A target is a molecular structure (chemically definable by at least molecular mass) that will undergo a specific interaction with ~~chemicals~~(chemical or biological reagents) that we call drugs because they are administrated to treat or diagnose a disease. This interaction has a connection with the clinic effects" **(*Nat. Rev. Drug Discov.* 2007, 6, 821-834).**

Target Identification & Validation

Marketed Small-Molecule Drug Targets



Nat. Rev. Drug Discov. **2002**, *1*, 727-730.

Target Identification & Validation

A Target May Be Identified From:

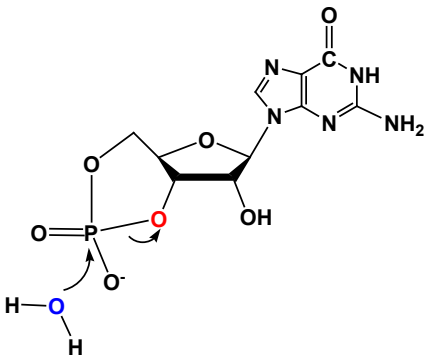
- Knowing the mechanism of existing drugs.
- An understanding of disease mechanism.
- Mutations in specific disease-related genes.
- Sequence or structural homology to known targets.
- Serendipity.

Target validation requires a demonstration that a molecular target is critically involved in a disease process, and that modulation of the target is likely to have a therapeutic effect.

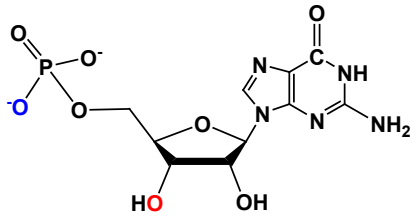
- Genetic manipulation:
  - Knockout/in, transgenic models
  - Antisense RNA
- Chemical manipulation:
  - Chemical knockouts and chemical genetics
  - Small molecule modulars

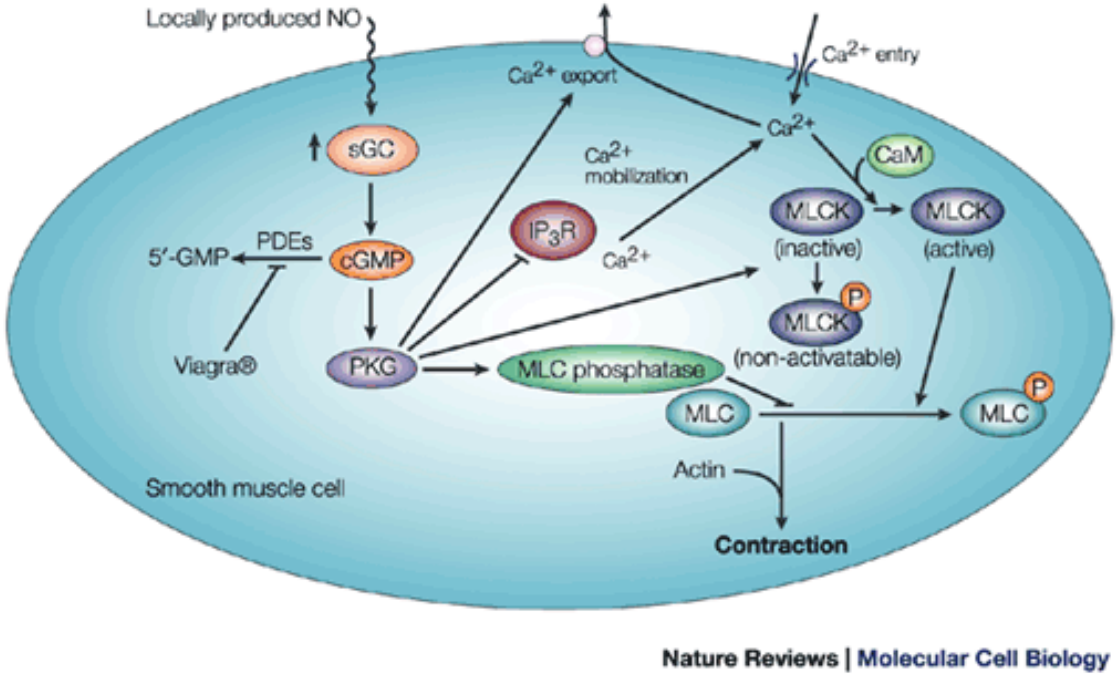
Mechanism of Pfizer's Drug --- Viagra

A drug was originally tested for cardiovascular disease



Cyclic-GMP Phosphodiesterase (cGMP PDE)





Penile erection occurs when blood swells the corpus cavernosum, an effect facilitated by relaxation of regional smooth muscle. The nitric oxide (NO) pathway leads to relaxation of smooth muscle by stimulating the soluble guanylyl cyclase (sGC), which results in the production of cyclic GMP (cGMP) and the activation of cGMP-dependent protein kinase (PKG). PKG causes smooth-muscle relaxation by mechanisms that are still being defined. Viagra® specifically inhibits the breakdown of cellular cGMP by PDE5 (an isoform of phosphodiesterase that is localized to erectile tissue), and thereby prolongs and enhances the effects of NO/cGMP (*Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 710-718)

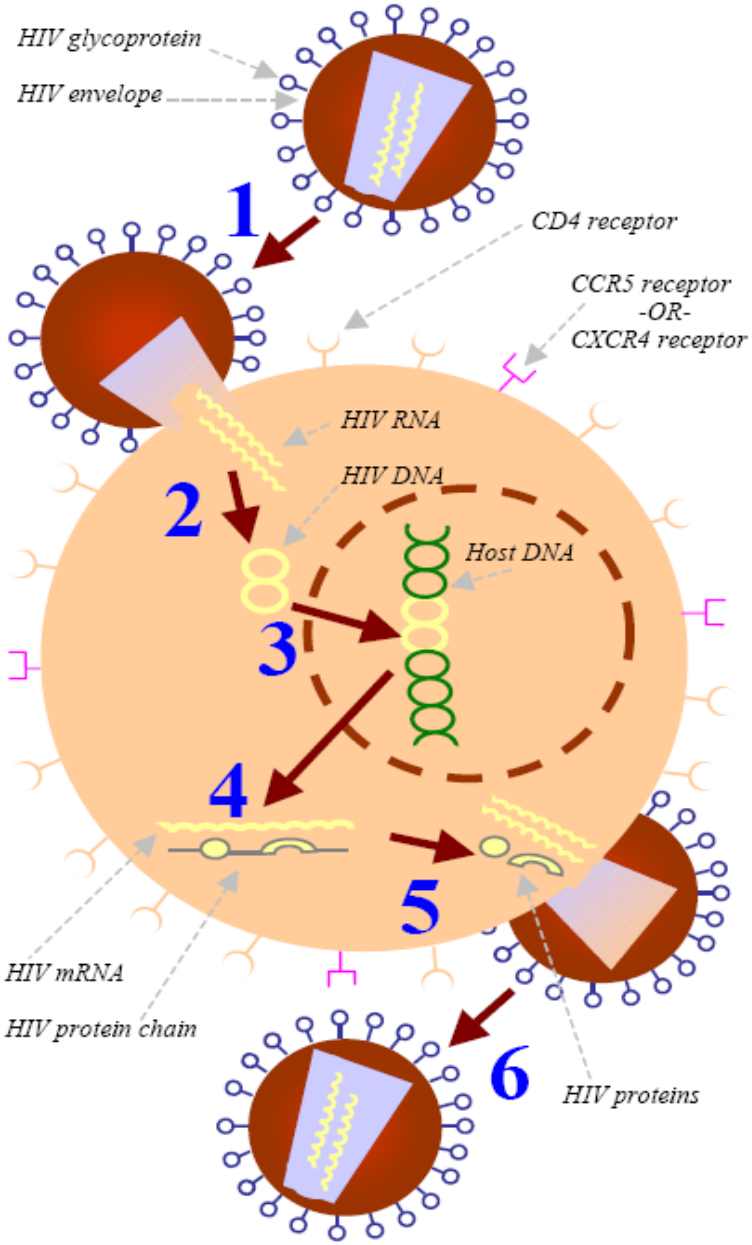
Target Identification & Validation

Consider HIV:

1. Binding and Fusion
2. Reverse Transcription
3. Integration
4. Transcription-Translation
5. Assembly
6. Budding and Release

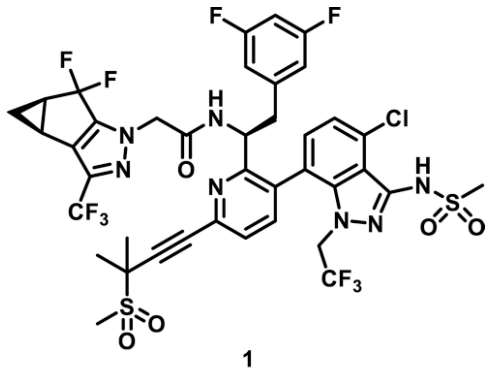
Criteria:

- Function critical for replication
- Druggable target (subjective)
  Known small molecule inhibitors?
- Conservation across virus variants
- Selectivity versus human proteins
- Difficult to incorporate all criteria in a single target!



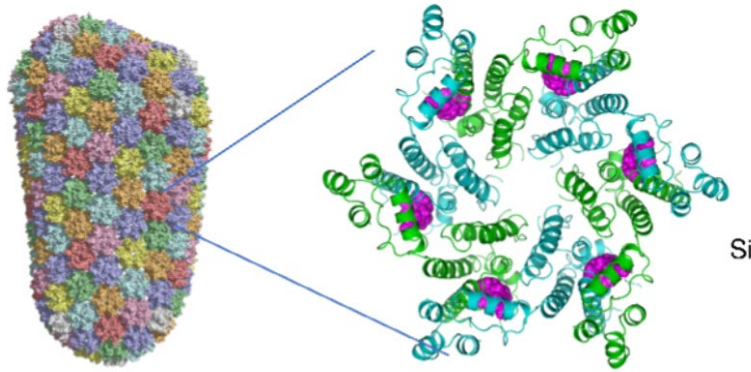
Discovery of Lenacapavir (2025)

First-in-Class Twice-Yearly Capsid Inhibitor for HIV-1 Treatment

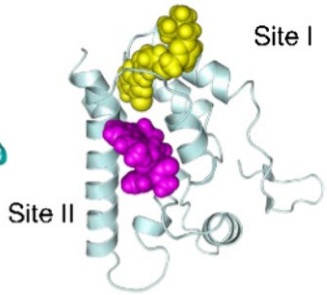


1
Lenacapavir

HIV-1 MT4 EC_{50} = 105 pM
Human hepatocyte predCL= 0.01 L/h/kg



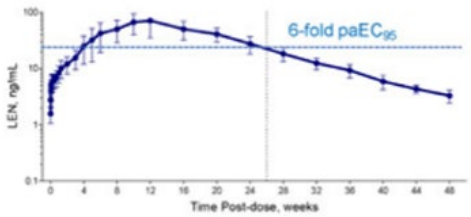
CA hexamer
Site II binding site



CA NTD
Site I and Site II binding sites

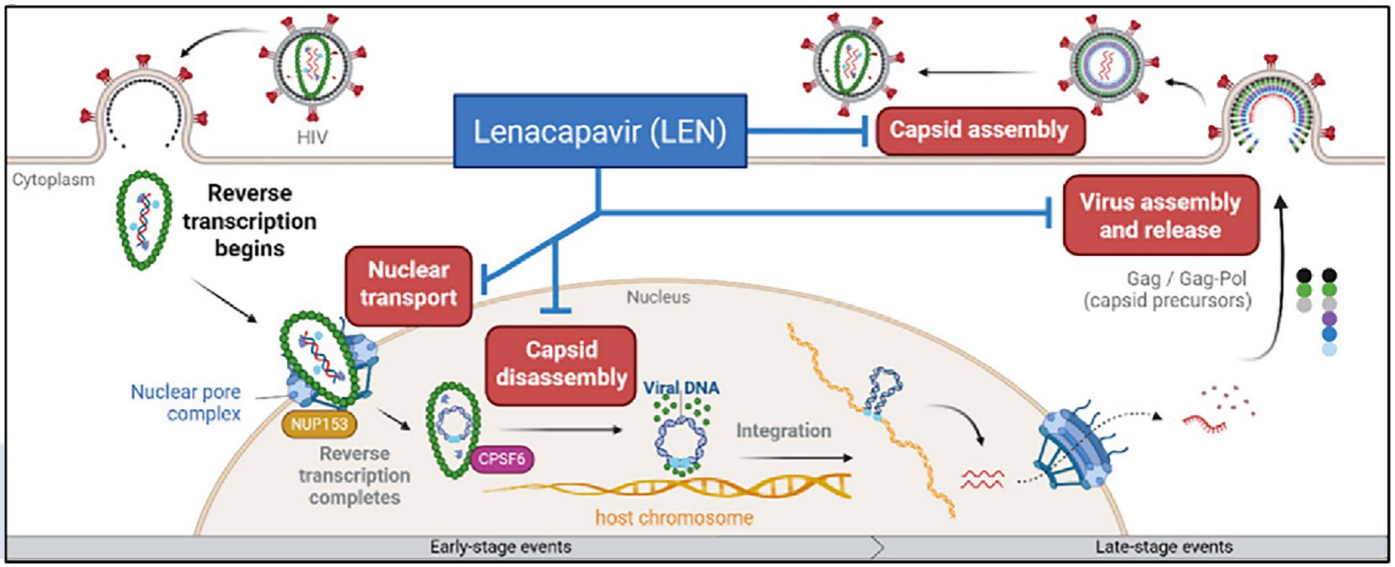
Twice-Yearly Dosing

Phase 1 single subcutaneous 900 mg dose



Begley, AIDS 2020

Maintains plasma concentrations >paEC$_{95}$ over 6 months



https://doi.org/10.1021/acs.jmedchem.5c01625?urlappend=%3Fref%3DPDF&jav=VoR&rel=cite-as

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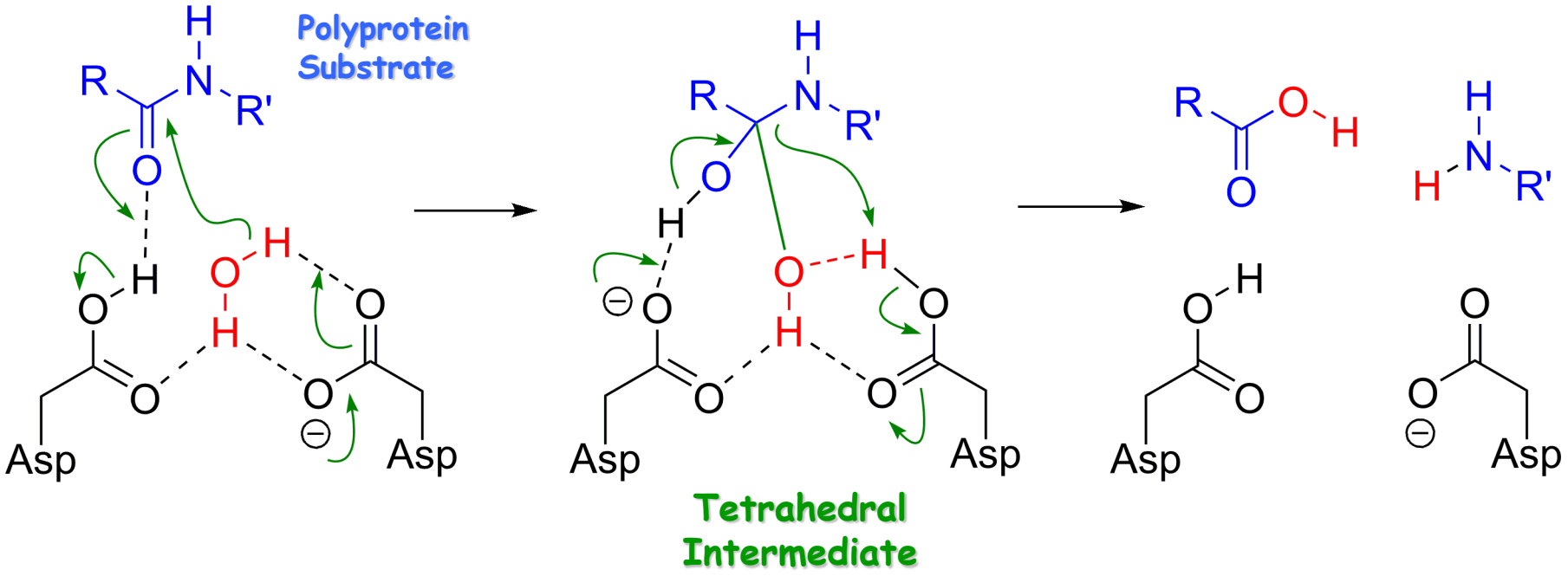
"Rational (Mechanism-Based)" Design of Target Inhibitors

Consider HIV Protease:

The replication of HIV requires proteolytic cleavage of a polyprotein that contains several key viral proteins. Proteolysis (amide hydrolysis) is performed by a virus-encoded protein, HIV-protease, that is itself contained within the polyprotein.

HIV Protease is an Aspartic Protease



"Rational" Design of Target Inhibitors

Consider HIV-Protease

HIV-protease inhibitor design began with the study of the natural cleavage sites on the polyprotein.

I	---Ser-Gln-Asn-**Tyr–Pro**-Ile-Val-Gln---
II	---Ala-Arg-Val-**Leu–Ala**-Glu-Ala-Met---
III	---Ala-Thr-Ile-**Met–Met**-Gln-Arg-Gly---
IV	---Pro-Gly-Asn-**Phe–Leu**-Gln-Ser-Arg---
V	---Ser-Phe-Asn-**Phe–Pro**-Gln-Ile-Thr---
VI	---Thr-Leu-Asn-**Tyr–Pro**-Ile-Ser-Pro---
VII	---Ala-Glu-Thr-**Phe–Tyr**-Val-Asp-Gly---
VIII	---Arg-Lys-Ile-**Leu–Phe**-Leu-Asp-Gly---

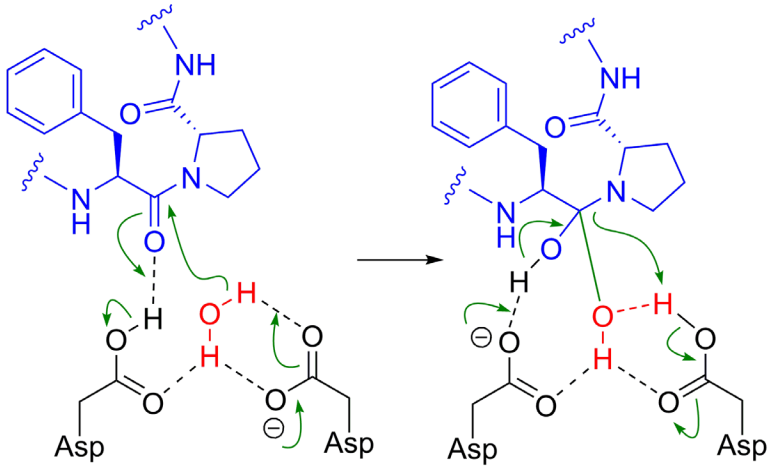
Three peptide sequences cleaved by HIV-protease occur at Phe-Pro and Tyr-Pro linkages. Since no known human aspartic protease cleaves at this linkage, these substructures were chosen as scaffolds from which to derive peptide-like inhibitors.

"Rational" Design of Target Inhibitors

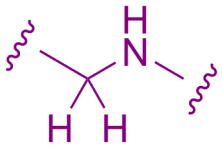
Consider HIV-Protease

Polyprotein Substrate

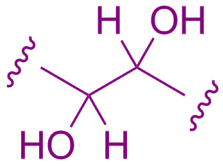
Tetrahedral Intermediate



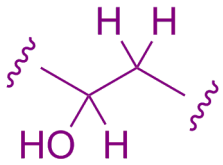
Structural Mimics of the Tetrahedral Intermediate



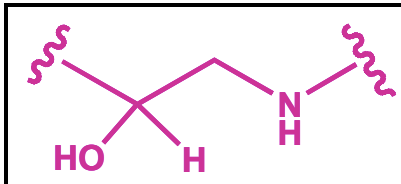
amine



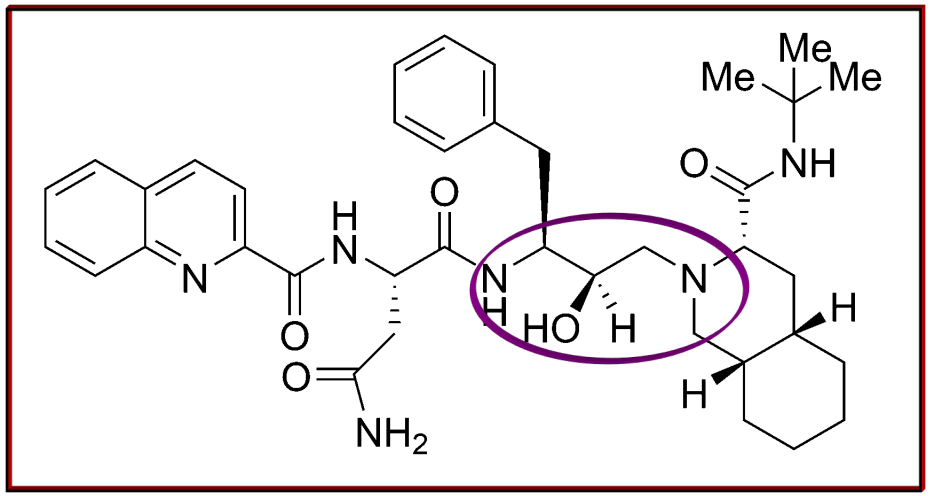
dihydroxyethylene



hydroxyethylene



hydroxyethylamine



Saquinavir

The first HIV-protease drug.
(Hoffman-La Roche)

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Lead Generation – Library Screening

Building on a Successful Tradition at MSKCC

The day after a happy Thanksgiving, Mike's nosebleeds began. The first came while Mrs. Parker was rushing to work. Desperately, she wet Mike's neck, tucked a bit of napkin under his lip and gave him gum to chew. Nothing worked, and finally she took him to her mother's while she rushed off to her job. It was an hour before the bleeding stopped.

the bathroom door to keep from falling on the floor. His nose was bleeding again. She woke Reuben and he ran out to call their older daughter in Stamford, telling her they were rushing Mike to Stamford Hospital.

Mike was carried outdoors wrapped in a blanket, and he remembers the dark, cold night air and seeing grease in the back of the Ford pickup before falling

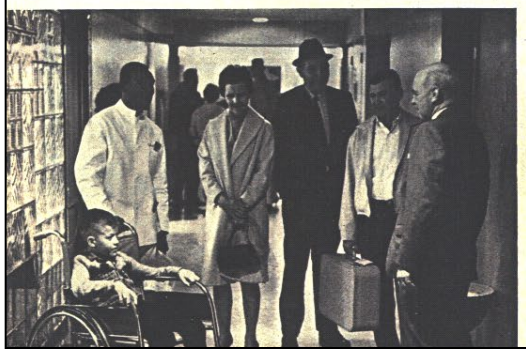
Picture taken at the SKI Cancer Research Center in Rye, NY.

28,000 Chemicals were screened for possible cure for cancer.

(Life Magazine, 1966)

right down." Mrs. Parker was sleeping badly for the first time in her life. Her husband, who had

N.Y., shows cabinets containing some 28,000 chemicals screened there for possible use in cancer. Labs elsewhere have tested more than 170,000. Of these, the eight in foreground are being used to treat leukemia.



Of 170,000 drugs tested, eight might help Mike Parker

Lead Generation – Library Screening

High-Throughput Screening of Small Molecule Libraries

High Throughput Screening → **Finding a Hit**

- "Chemical Space"
  - 10^{180} structures
- Likely Candidates
  - 10^{18}
- Known Chemicals
  - 10^{9}
- Commercial Chemicals
  - 10^{6}
- Commercial Drugs
  - 10^{3}
- Block-Buster Drugs
  - 10^{2}

Lead Generation

High-Throughput Screening of Small Molecule Libraries

How can one generate small molecule libraries?

Combinatorial Chemistry

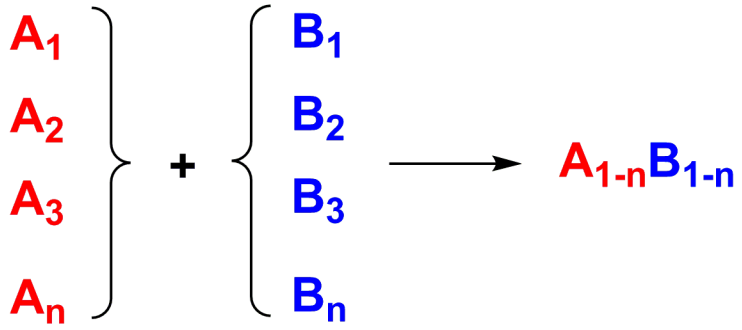
The synthesis of large numbers of compounds through preparing many single compounds in parallel or many compounds simultaneously in mixtures.

Orthodox Synthesis

$$A + B \longrightarrow C$$

- Stepwise directed synthesis of one specific product using solution chemistry.

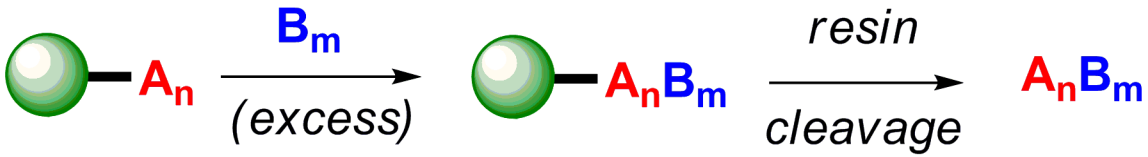
Combinatorial Synthesis



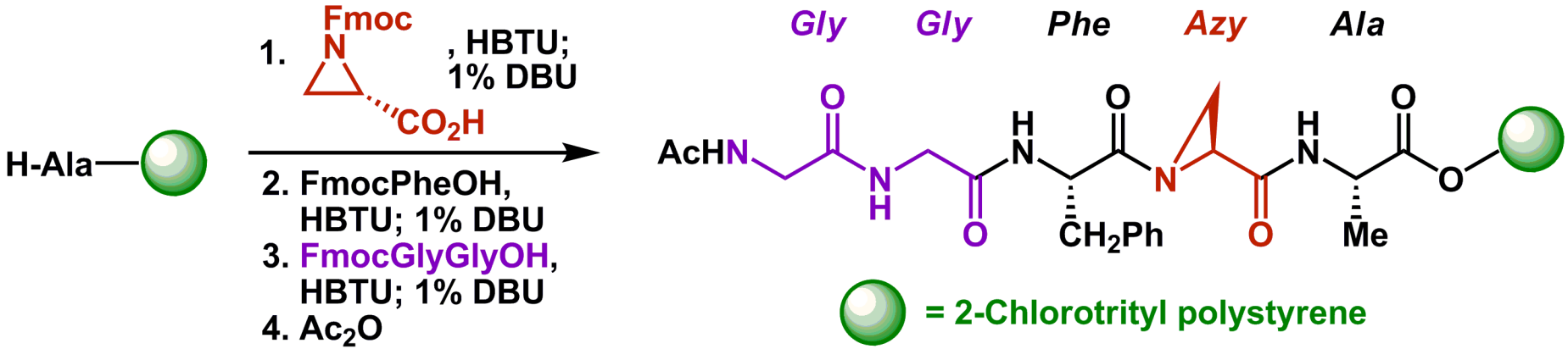
Lead Generation: Combinatorial Chemistry

Synthesis on solid supports:

- Reactions can be driven to completion by __use of excess substrate__.
- Products can be readily separated from reagents.
- Automation is simplified.

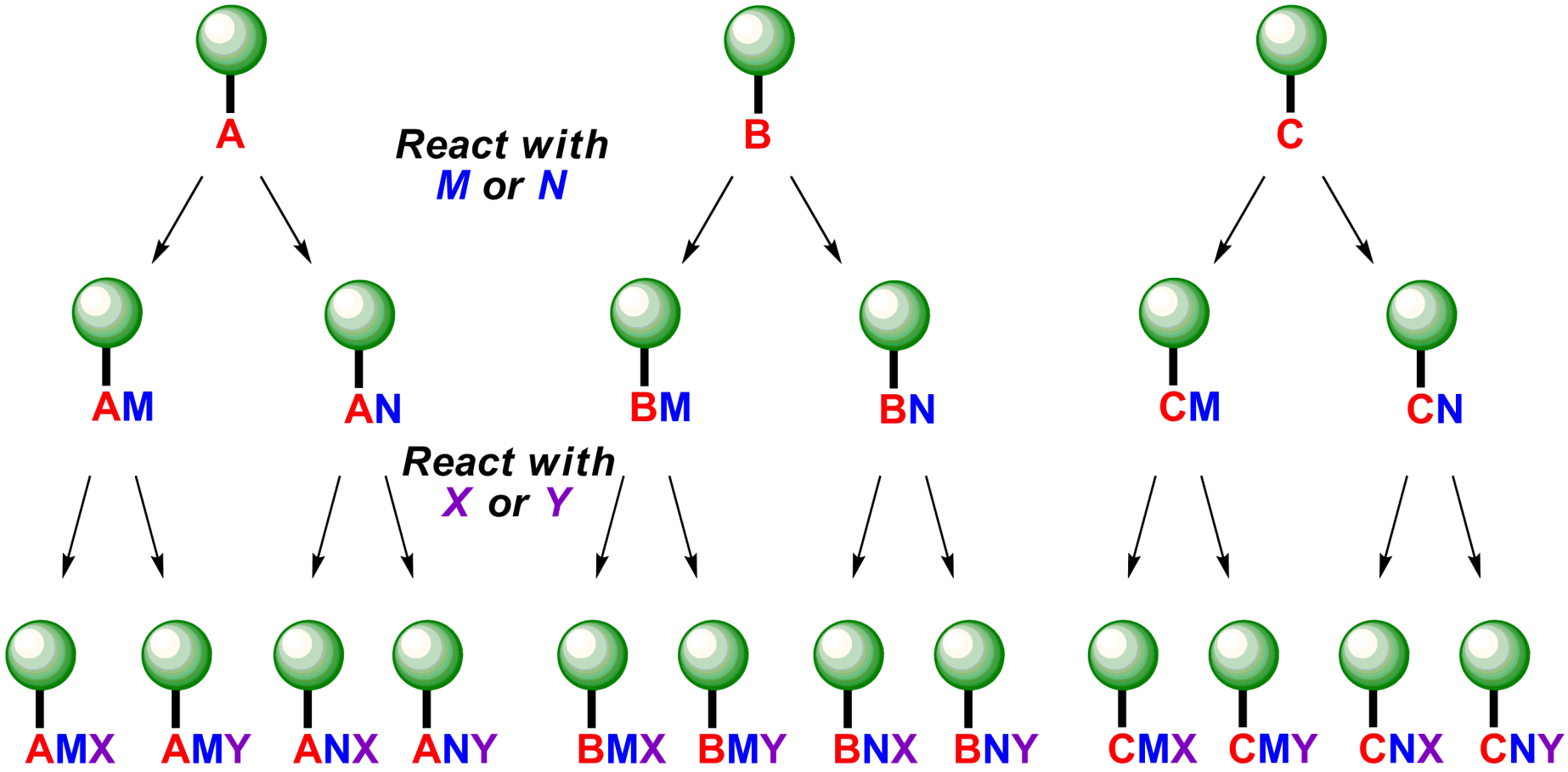


Solid-phase peptide synthesis is the classic example:



Lead Generation: Combinatorial Chemistry

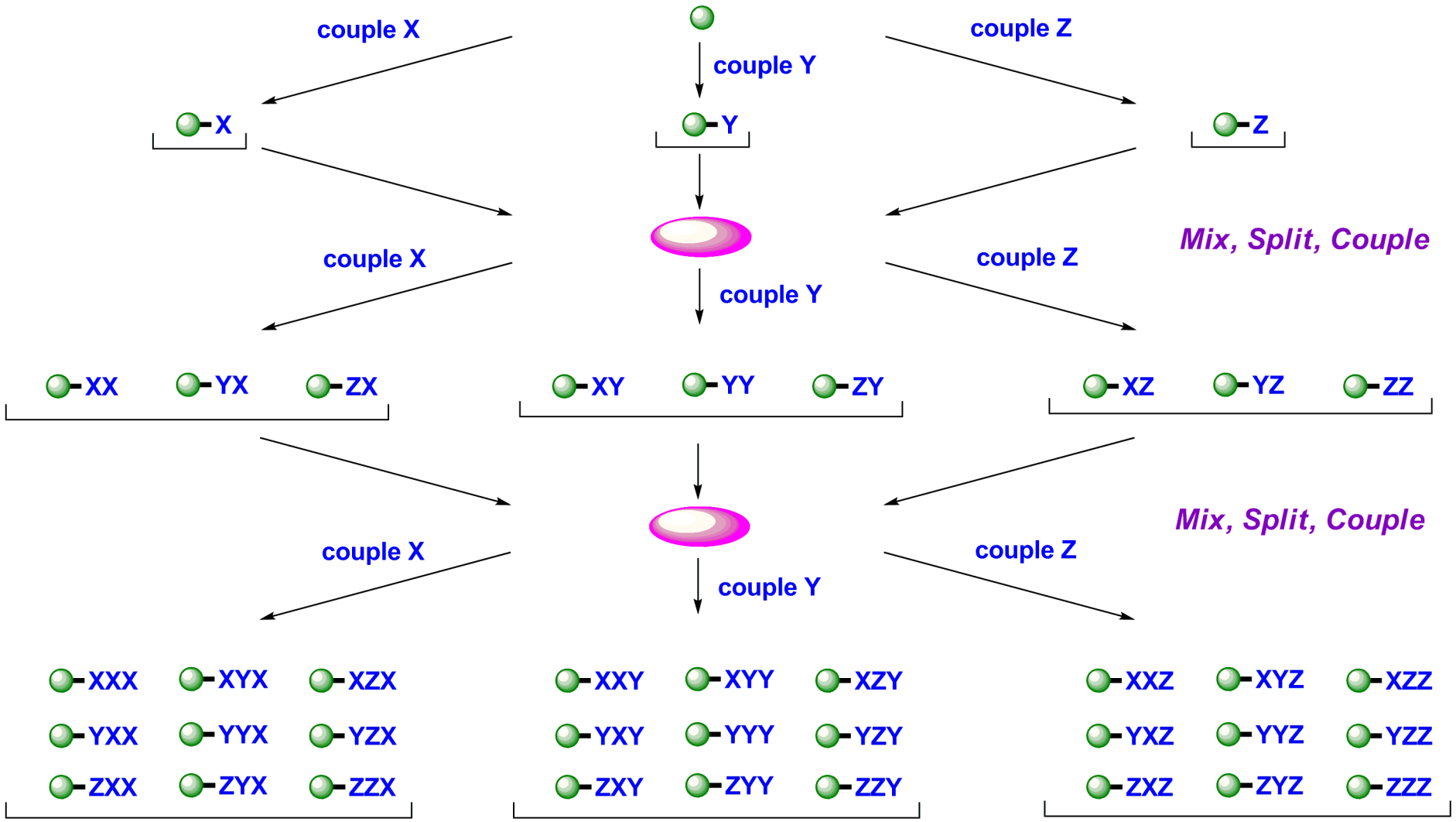
Dealing with Mixtures of Compounds: Spatially-Arrayed Libraries



Since each reaction is run is a separate vessel/well, products of each reaction should be homogeneous, provided that the chemistry is efficient.

Lead Generation: Combinatorial Chemistry

Dealing with Mixtures of Compounds: Split-Pool Synthesis



Split-pool synthesis arrives at higher diversity much more quickly.

Diversity in Chemical Space: Combinatorial Libraries, Natural Products, Drugs

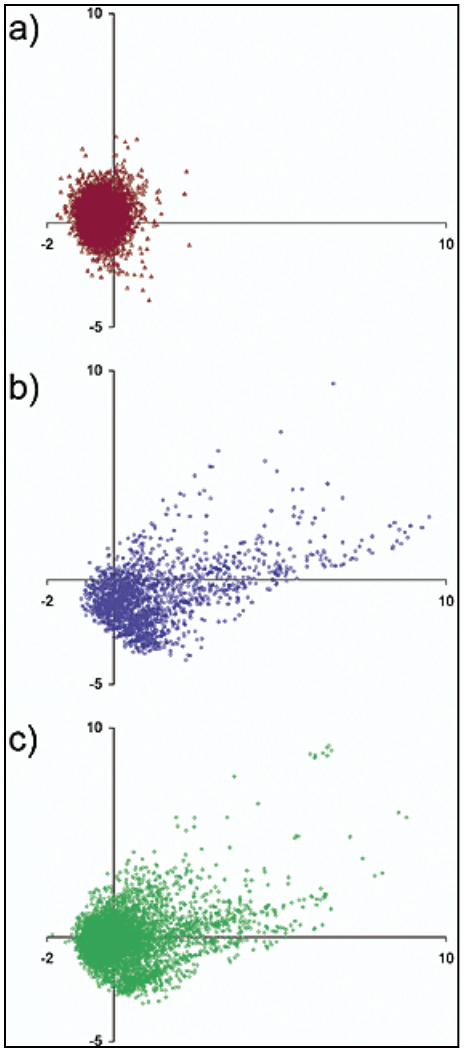
Principal Component Statistical Analysis of Molecular Structural Elements

- Number of chiral centers.
- Number of rotatable bonds.
- Content of aromatic moieties.
- Degree of ring fusion.
- # of solvated H-bond acceptors.
- # of solvated H-bond donors.
- # of C-N bonds.
- # of C-O bonds.
- # of C-halogen bonds.
- # of C-S bonds.
- Etc….

Combinatorial Compounds

Natural Products

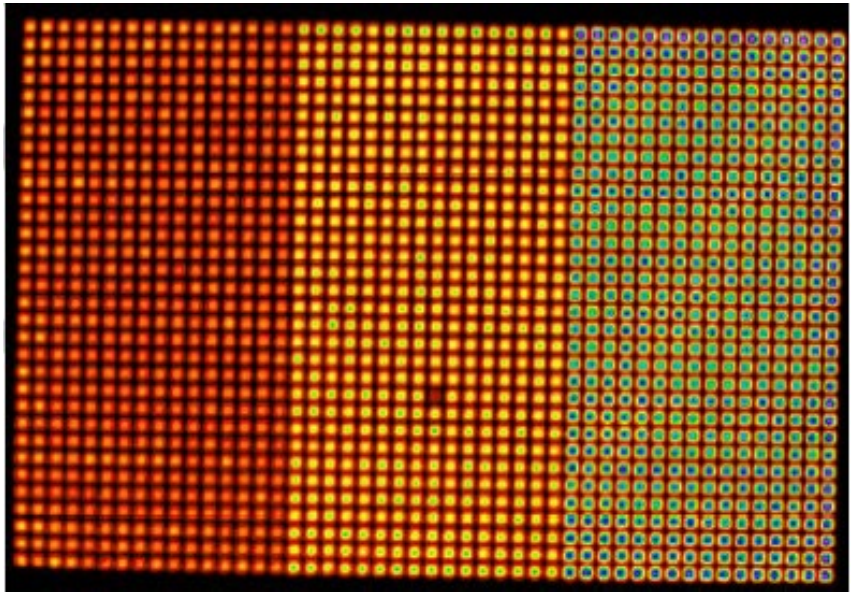
Drug Molecules



J. Chem. Inf. Comput. Sci. **2003**, *43*, 218-227.

Lead Generation: High-Throughput Screening (HTS)

- Automated biological testing with the capacity to investigate large chemical libraries.
  - Thousands to millions of compounds
- Assess binding interactions to target.
  - Potency of enzyme inhibition
- Cellular assays
  - Measure biological effect of compounds in cells of interest



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Lead Optimization and Drug Candidates

Privileged Classes of Small Molecule Drugs?

Lipinski's Rule of Five:
Features Commonly Found in Orally Active Drugs.

- Not more than 5 hydrogen bond donors (OH, NH)
- Not more than 10 hydrogen bond acceptors (N, O)
- Molecular weight under 500
- A LogP under 5 *

There are two common misconceptions with this rule of thumb:

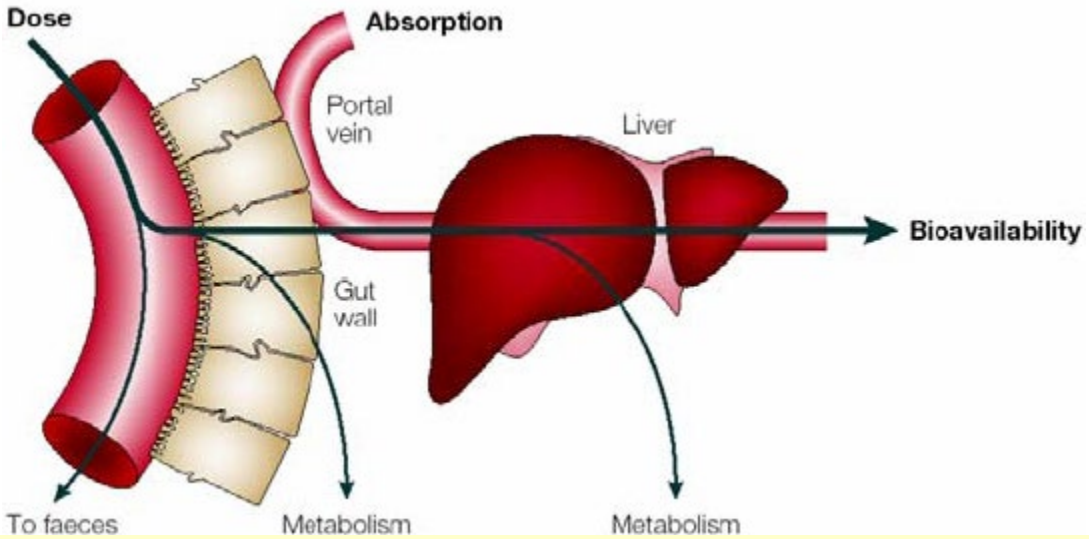
- Molecules that satisfy this criteria are not automatically drug-like.
- This rule was derived from surveying drugs, NOT LEAD STRUCTURES.

* A partition coefficient is a measure of differential solubility of a compound in two solvents. The log ratio of the concentrations of the solute in the solvent is called LogP. The best known of these partition coefficients is the one based on the solvents octanol and water. The octanol-water partition coefficient is a measure of the hydrophobicity and hydrophilicity of a substance. In the context of drug-like substances, hydrophobicity is related to absorption, bioavailability, hydrophobic receptor interactions, metabolism and toxicity.

Suitability of Drug Candidates

ADMET

- Absorption
- Distribution
- Metabolism
- Excretion
- Toxicity



Absorption: A compound has to be taken in to the bloodstream, usually via mucous surfaces like the digestive tract. Uptake into the target organs or cells needs to be ensured. This can be a serious problem at some natural barriers like the blood-brain barrier.

Distribution: The compound needs to be carried to its effector site, most often via the bloodstream. From there, the compound may distribute into tissues and organs, usually to differing extents.

Metabolism: Compounds begin to break down as soon as they enter the body. The majority of small-molecule drug metabolism is carried out in the liver by redox (cytochrome P450) enzymes.

Excretion: Compounds and their metabolites need to be removed from the body via excretion, usually through the kidneys (urine) or in the feces. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism.

Toxicity: The adverse effects of a drug on the body, ranging from nausea to death.

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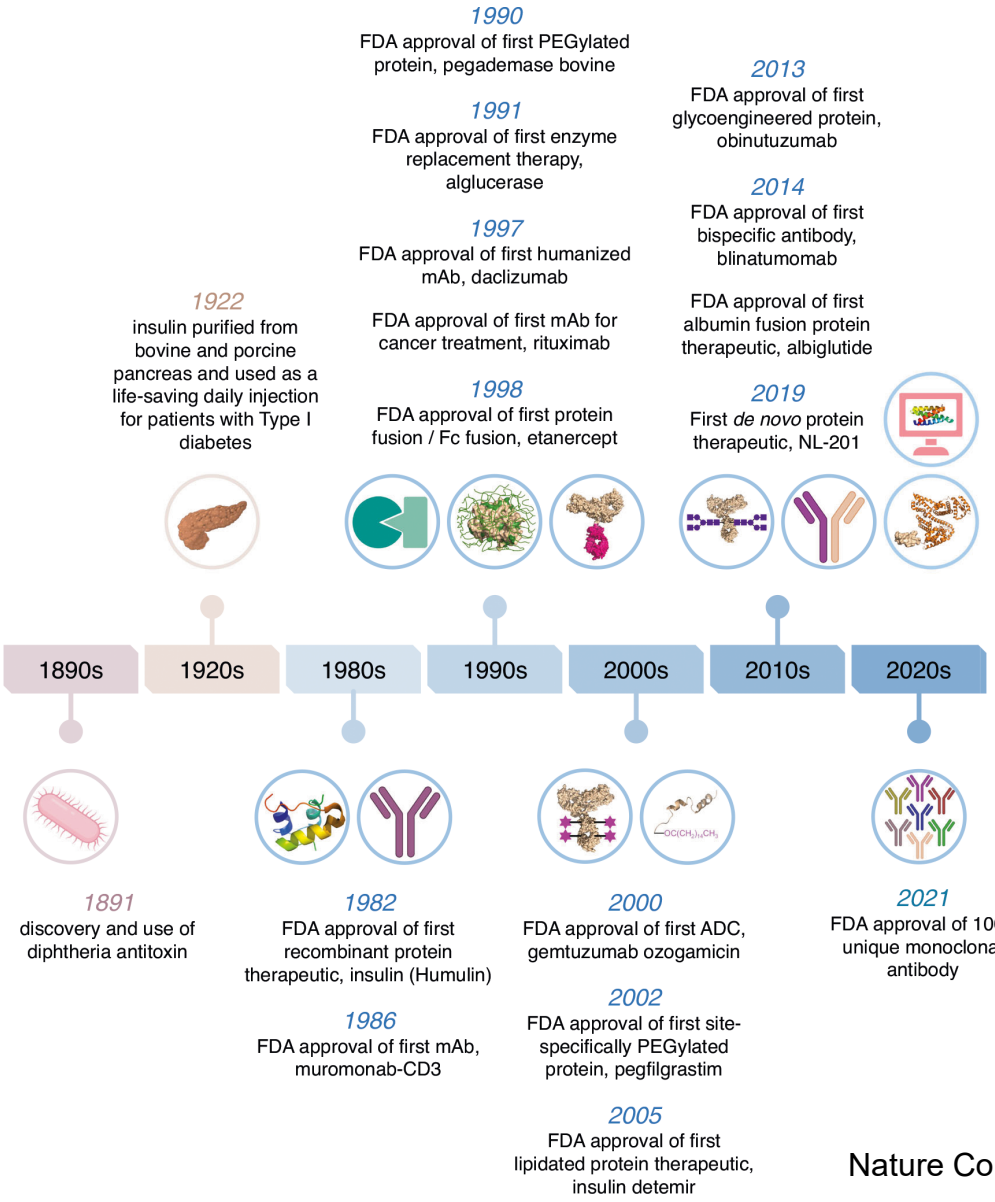
Protein-based Therapeutics

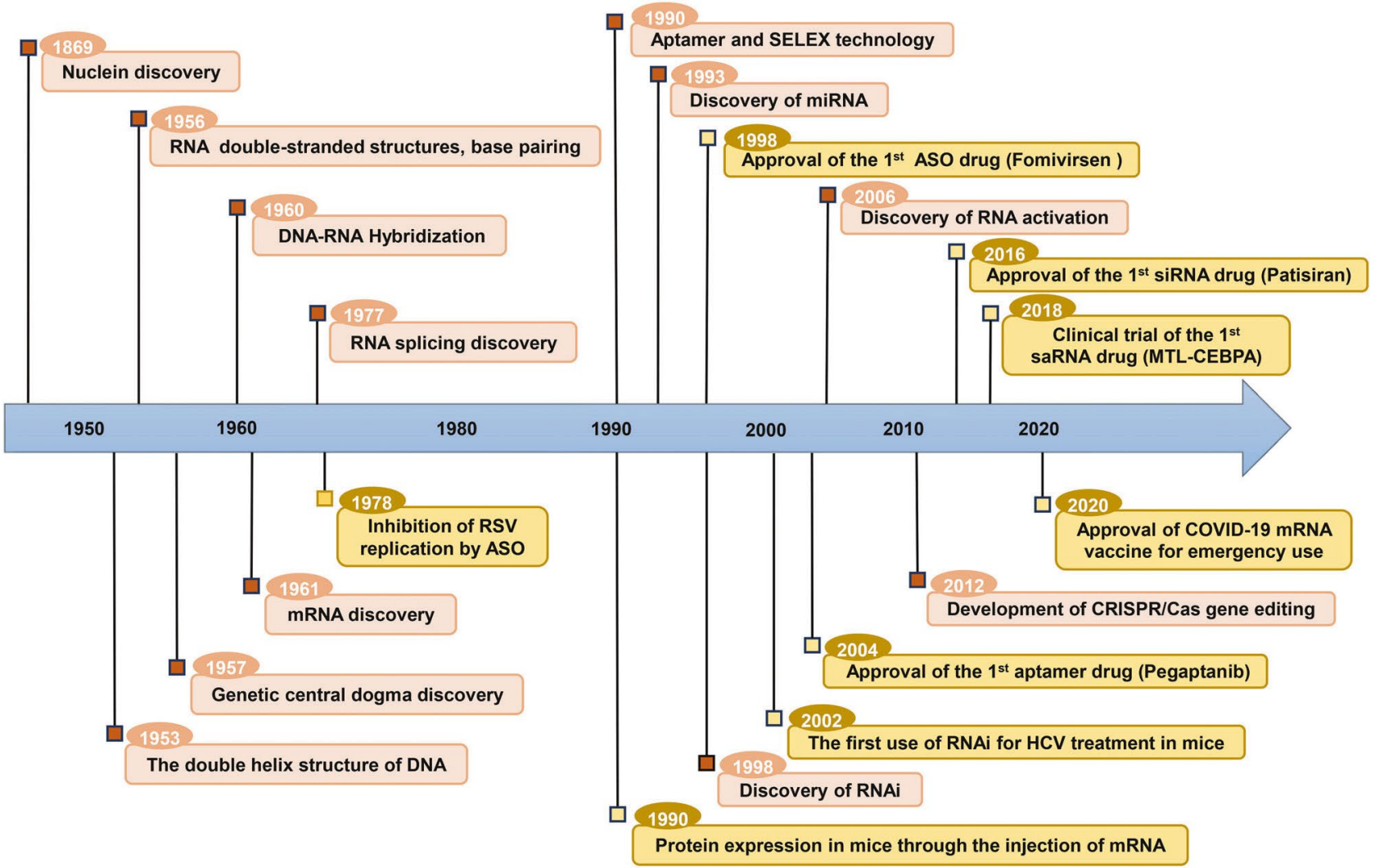
A timeline of significant advances of protein-based therapeutics



Nature Communications | (2023) 14:2411

Nucleic Acid Drugs (NADs)

Essential discoveries in fundamental theory and critical NADs therapy



Signal Transduction and Targeted Therapy (2024) 9:316