20.109 Module 2

Lecture #4: **Introduction to screening: concepts & principles I**

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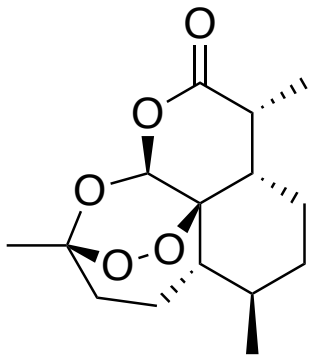


Modern framework for drug discovery and development

A. Basic science research and target identification

B. Target pharmacology and **biomarker development**

C. Lead identification

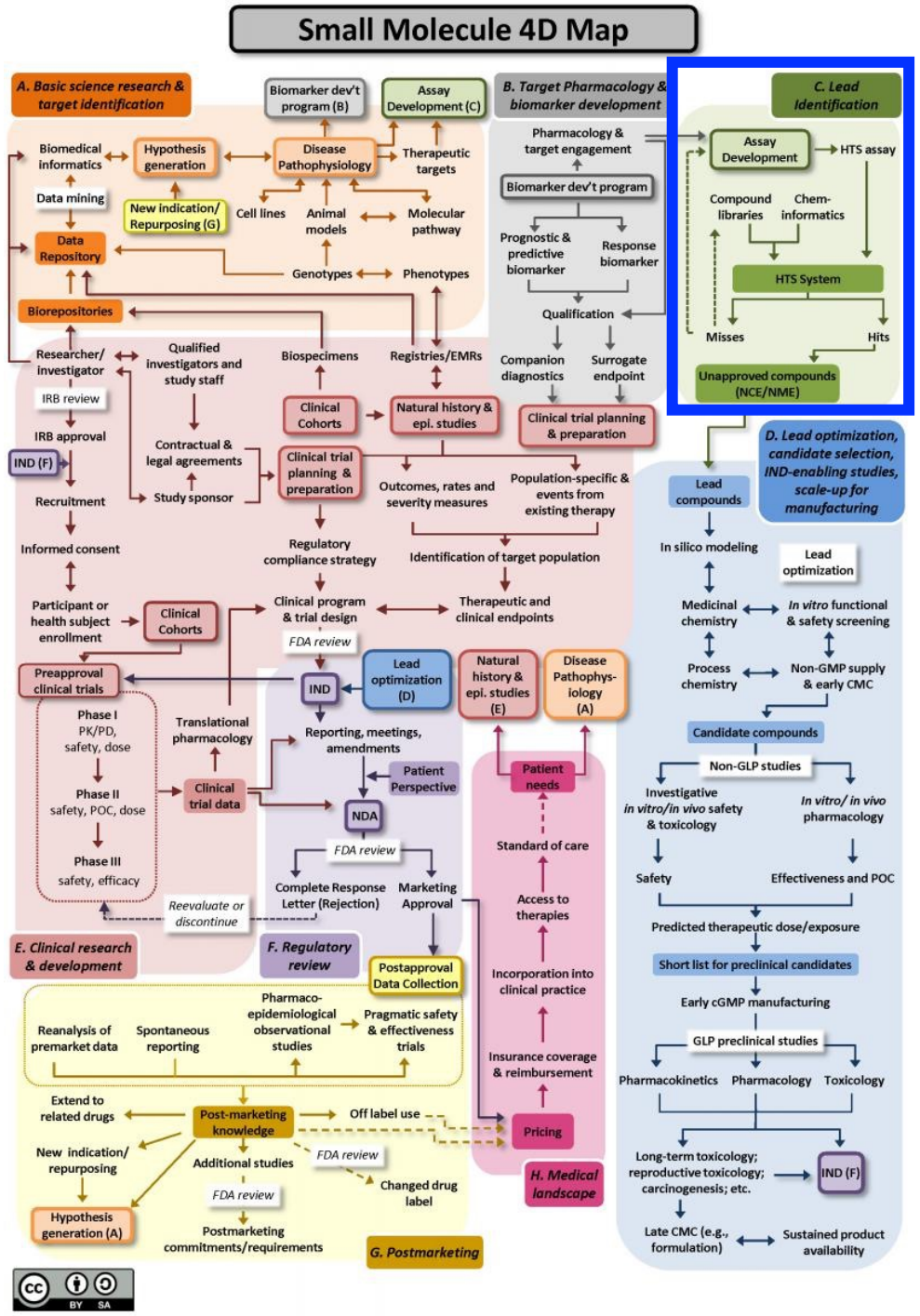
D. Lead optimization and candidate selection
- Improving pharmacologic, metabolic, safety profiles of lead toward use in humans

E. Clinical research & development
- Clinical trials to establish efficacy and safety

F. Regulatory review (FDA approval)

G. Post-marketing
- Surveillance (adverse effects)
- Repurposing
- Off-label use

H. Medical landscape

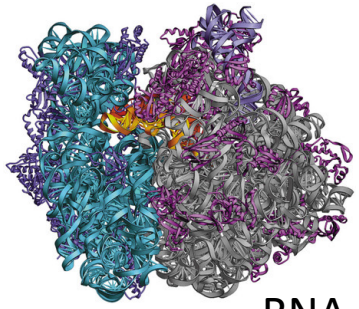


References:

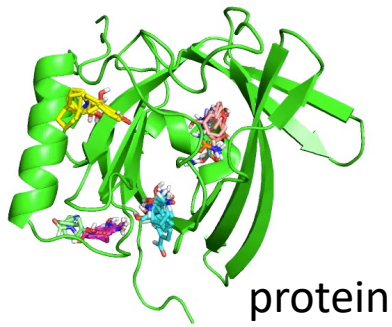
1) Wagner et al; Nature Reviews Drug Discovery; 2018;
2) https://ncats.nih.gov/translation/maps
3) 4D Map (interactive): https://4dmap.ncats.nih.gov/#/

Protein target selected for drug discovery effort …

DNA



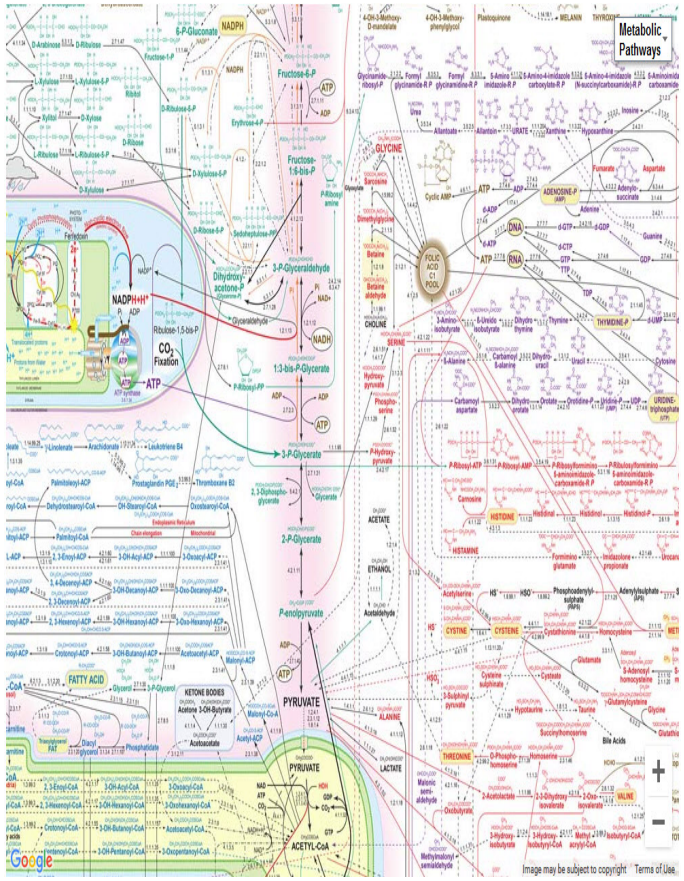
RNA



protein

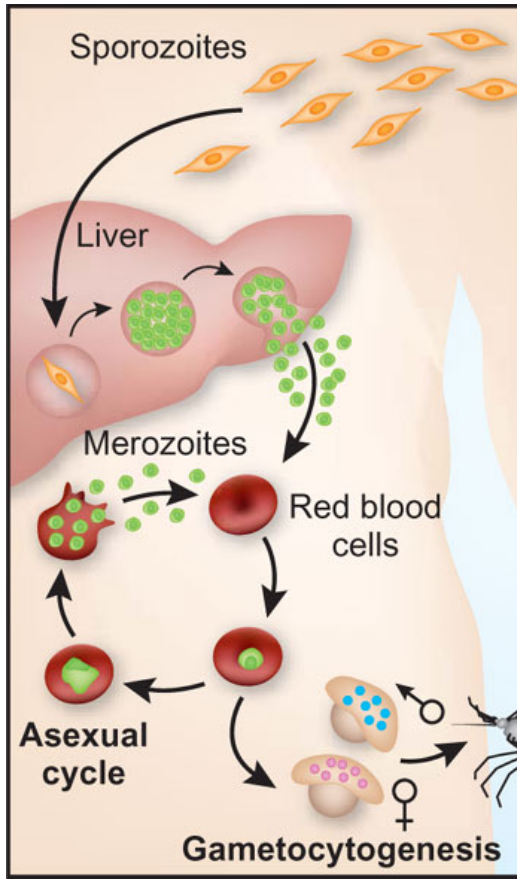
Molecular

- DNA, RNA, protein
- Carbohydrates



Biochemical/ Metabolic pathways

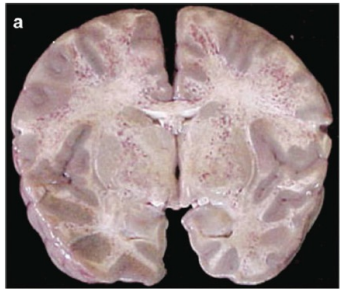
- Enzymes
- Structural proteins



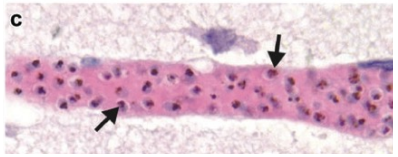
Cellular behavior

- Replication
- RBC invasion/ egress
- Differentiation

Cerebral malaria



Microhemmorhages



Vascular occlusion

Microbe-host Interactions

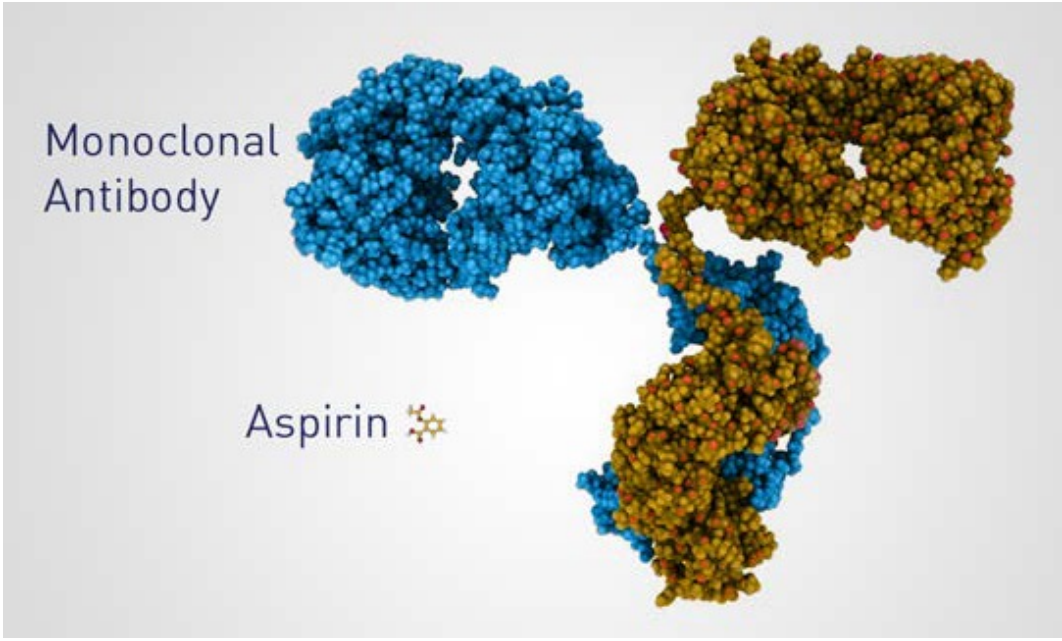
- Disease outcomes

… *choosing a therapeutic modality*

Strategies available for disrupting target function

- Small molecules ($M_w \leq 500$ Da)

- Peptides (500 Da < Mw < 5,000 Da)
- Nucleic acids (Mw ~ kDa)
  - Aptamers;
  - Antisense oligonucleotides
  - siRNAs

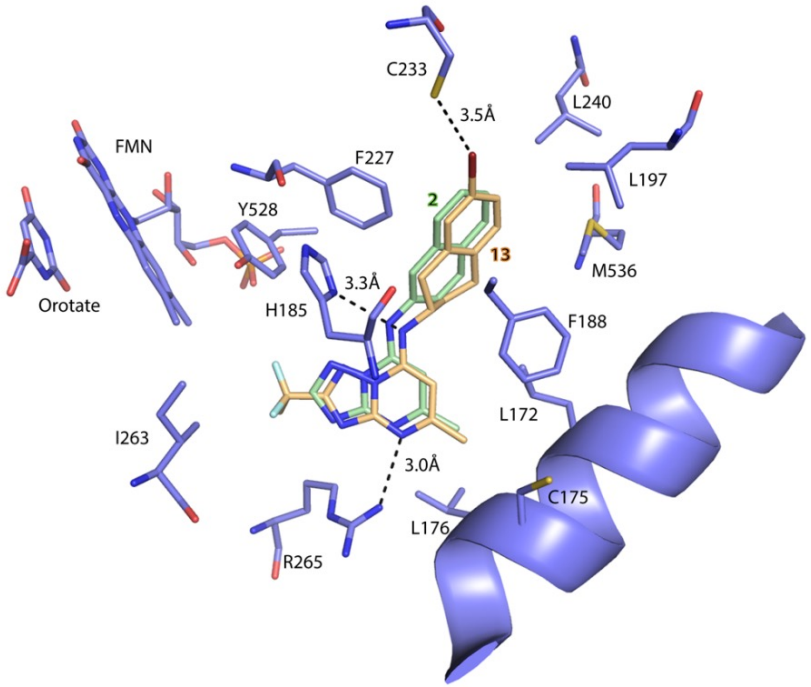


- Biologics* (M_w ~ kDa)
  - Proteins (antibodies, enzymes …)

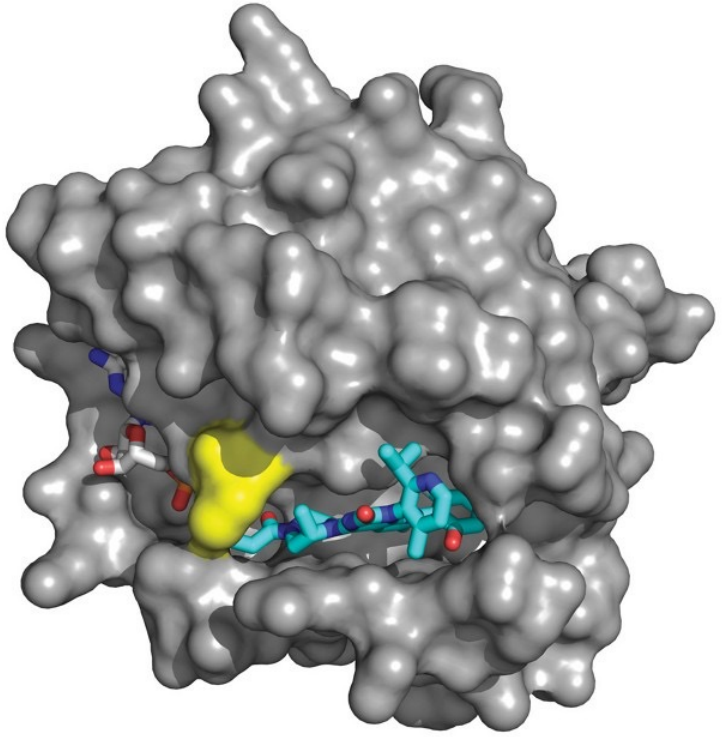
– Biological products are a diverse category of products and are generally large, complex molecules.
– Usually produced through biotechnology in a living system or cells (microorganisms, plants or animals)

Properties favoring small molecule therapeutics

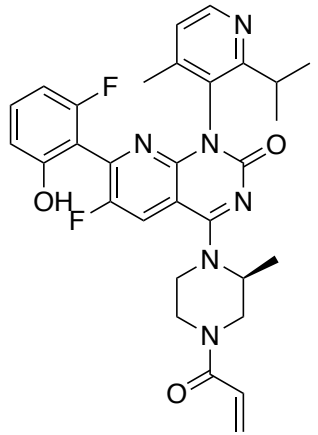
- Can make intimate molecular contact with relevant target protein surface features



Flavin and substrate binding sites in the *Plasmodium* DHODH protein



AMG510 bound to KRAS



AMG510 (2D)

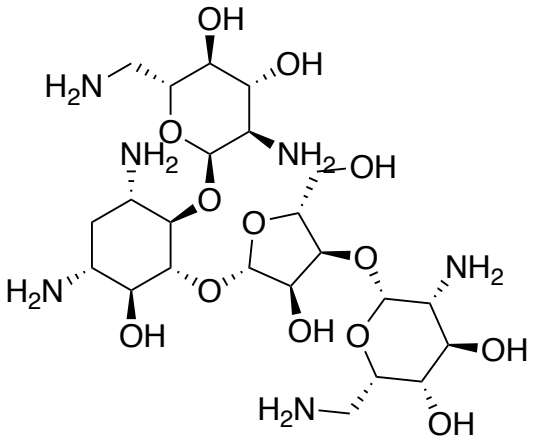
References:
DOI: 10.1038/s41586-019-1694-1
DOI: 10.1021/acs.jmedchem.6b00275

Properties favoring small molecule therapeutics

- Can make intimate molecular contact with relevant target protein surface features

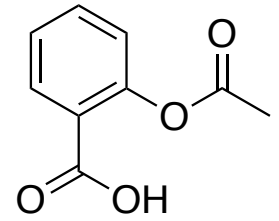
- Cell membrane permeable
  - Intracellular
  - Extracellular targets

- Orally bioavailable



Log P: -9.55
MR: 139.78 [cm3/mol]
tPSA: 353.11
CLogP: -6.46605

Log P: 1.18
MR: 43.29 [cm3/mol]
tPSA: 63.6
CLogP: 0.804



Lipinsky's 'Rule of 5': Predicting oral bioavailability likelihood

1. Molecular weight is less than ~500 Da

2. The calculated log P value is less than five
- Measure of lipophilicity (propensity to partition into cell membranes, fatty tissues)

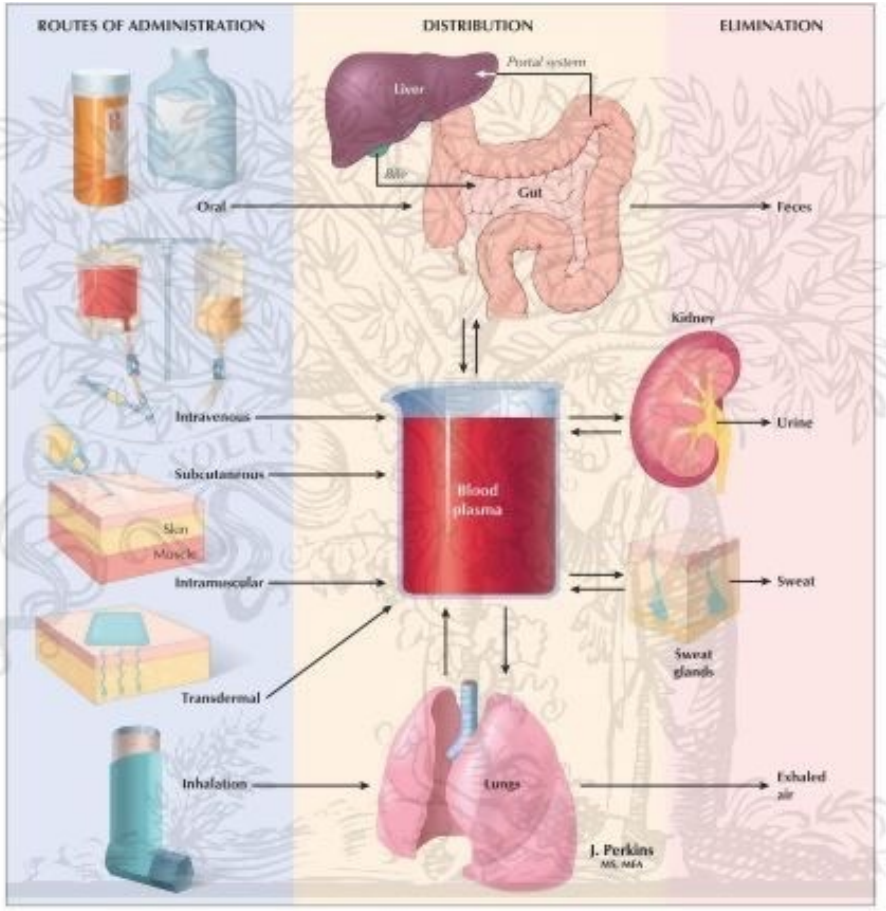
3. There are less than five hydrogen bond donors (–NH–, –OH)

4. The number of hydrogen bond acceptors (–N6-point double bond, –O–) is less than ten

Properties favoring small molecule therapeutics

- Can make intimate molecular contact with relevant target protein surface features
- Cell membrane permeable
  - Intracellular
  - Extracellular targets
- Oral bioavailability
- Stability
  - Gastrointestinal tract (e.g., pH, enzymes, …)
  - Metabolic transformation (liver, gut microbiome)
  - Excretion

Absorption, Distribution, Metabolism, Excretion (ADME) Concept



Properties favoring small molecule therapeutics

- Can make intimate molecular contact with relevant target protein surface features
- Cell membrane permeable
  - Intracellular targets
  - Extracellular targets
- Oral bioavailability
- Stability
  - Gastrointestinal tract (e.g., pH, enzymes, …)
  - Metabolic transformation (liver, gut microbiome)
  - Excretion
- Cost
  - Cheaper to manufacture on large scale
  - Cheaper to distribute (little need for refrigeration, etc.)

Learning Objectives

A. Discovering compounds (“hits”) that can interfere with the function of your defined target
   A. What, where, how to search?

B. Knowing you’ve found what you’re looking for …
   A. Assays
   B. Choosing the right assay for the question

Identifying a “hit” compound to a defined protein target …

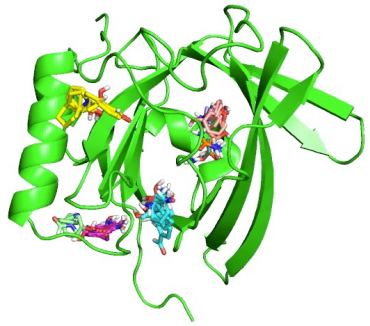
- Uses a “screening” process
- Involves querying diverse compound collections / libraries
  - Usually quite large (≥ 50,000)
- Must be able to identify ***rare, desired hits*** (signal)
- Reject uninteresting compounds (noise)

Question:
How would you go about doing this?
-Define:
1) your starting point;
2) process;
3) endpoint/ outcome

Types of screening processes we will consider in class …

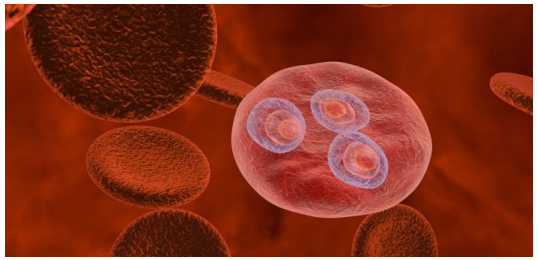
- Uses a “screening” process
- Involves querying diverse compound collections / libraries
  - Usually quite large (≥ 50,000)
- Must be able to identify rare, desired hits (signal)
- Reject uninteresting compounds (noise)

1. Target-based screening



Isolated protein target of interest
➢ Biologically validated

2. Phenotypic-based screening

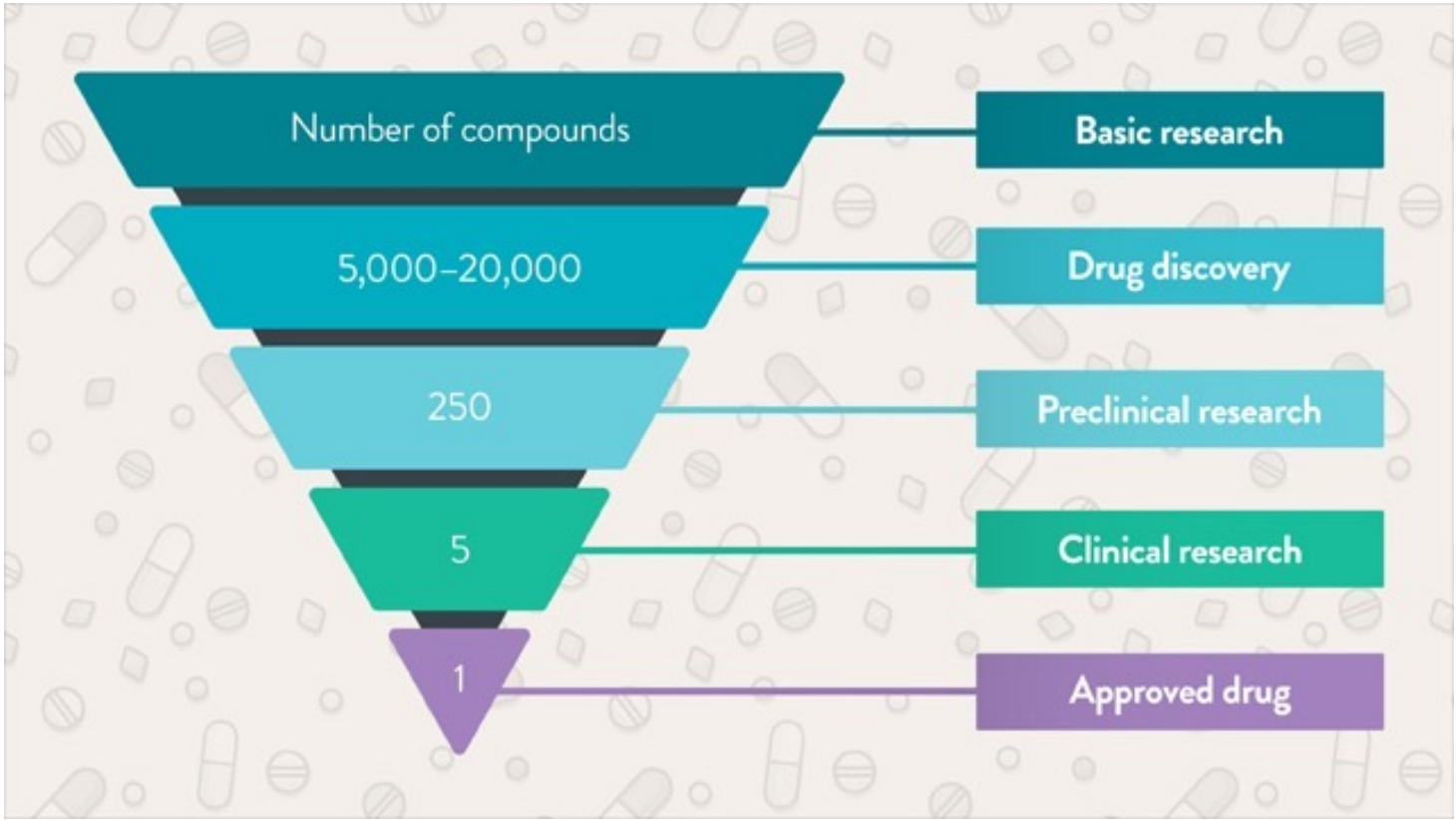


Cells or model organism
- Pathogen;
- cancer cell;
- Model organism

C. elegans

Hard truth: Must search broadly to find a *possible* solution

- Anti-plasmodium screen:
  - 100,000 molecules screened
  - 468 “hits” (0.5% hit rate)
- Substantial attrition at the first step in the screening process!



https://www.technologynetworks.com/drug-discovery/articles/exploring-the-drug-development-process-331894

Identifying a “hit” compound to a defined target …

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- **Public collections**
  - Universities
  - Commercial suppliers
  - Public-private agreements
- **Proprietary collections**
  - Pharmaceutical companies
- **Composition**
  - Synthetic
  - Natural products
    - Microbial (bacterial, fungal…
    - Forests (e.g. plants, …)
    - Ocean (e.g. sponges, …)
    - Other environmental sources
- **Considerations**
  - Sampling of diverse chemical properties
  - Stability
  - Ease of synthesis/ production (cost)

Identifying a “hit” compound to a defined target: “Finding your needle in a haystack”

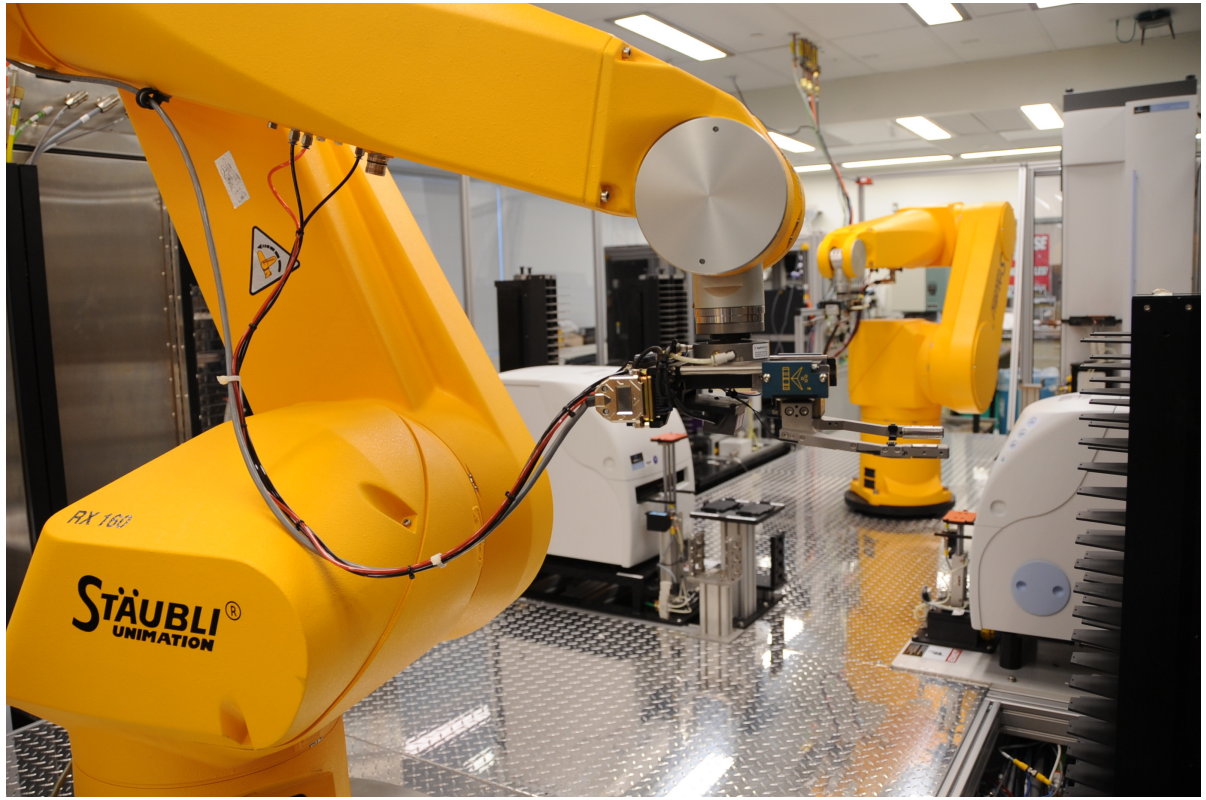
- Uses a “screening” process
- Involves querying diverse compound collections / libraries
  - Usually quite large (≥ 50,000)
- Must be able to identify ***rare, desired hits*** (signal)
- Reject uninteresting compounds (noise)

Devising a strategy to find your needle in a haystack …

- Assay
  - Investigative procedure for qualitatively or quantitatively assessing the presence, amount or functional activity of a target entity
- Can be used in:
  - Discovery
  - Validation
- Components needed for an assay
  - Input
  - ”operation” performed in a suitable “format”
  - Readout (to assess outcome)

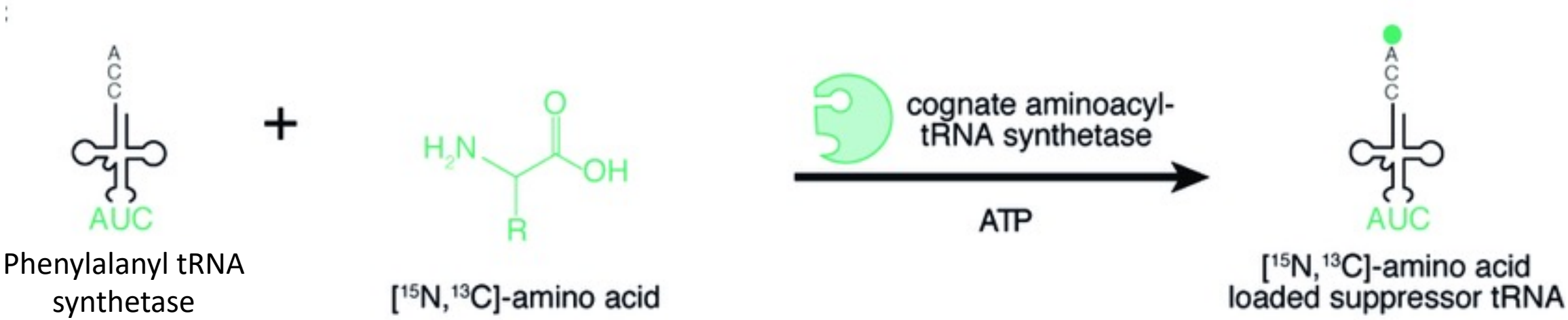
Some desirable assay features …

- Simple and inexpensive
- Fast
- Scalable
- Easily standardized
- Reproducible
  - Accurate
  - Precise
- Sensitive
- Specific



Automation can help with achieving speed, scale and reproducibility of screens

Case Study 1:
Discover inhibitors of the phenylalanyl tRNA synthetase enzyme



- Assay
  - Investigative procedure for qualitatively or quantitatively assessing the *presence*, *amount* or *functional activity* of a target entity

- Can be used in:
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  - Validation

- Components needed for an assay
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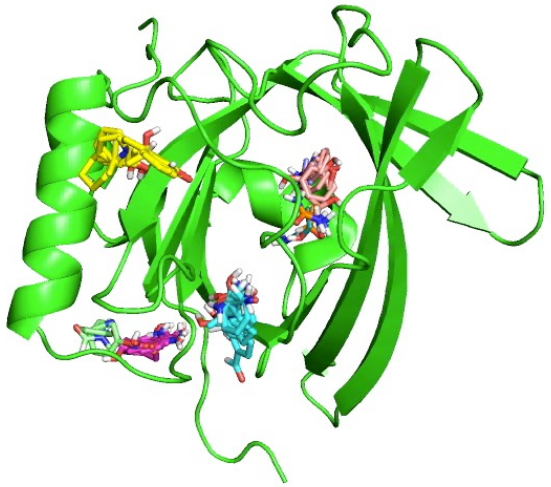
Case Study 2:
Discover inhibitors of an essential protein of unknown function



Cellular function – unknown, but **essential for survival**
Enzymatic activity -- unknown
Protein interactions -- unknown

- Assay
  - Investigative procedure for qualitatively or quantitatively assessing the *presence*, *amount* or *functional activity* of a target entity

- Can be used in:
  - Discovery
  - Validation

- Components needed for an assay
  - Input
  - ”operation” performed in a suitable “format”
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Summary

- Small molecule therapeutics make intimate molecular contact with relevant target protein surface features to interfere with their function(s)
- Libraries of small molecules from different sources and with diverse properties can be prospectively assembled to facilitate finding new small molecule drugs
- Screens can be effectively used to identify small molecules of therapeutic interest
- Important to select screening assays appropriate to the target of interest and, where possible, should incorporate what is known about its function