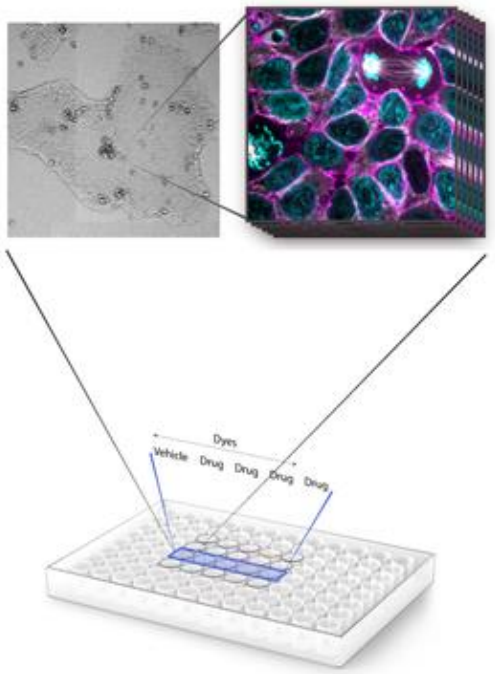
Phenotypic screens and cell perturbations

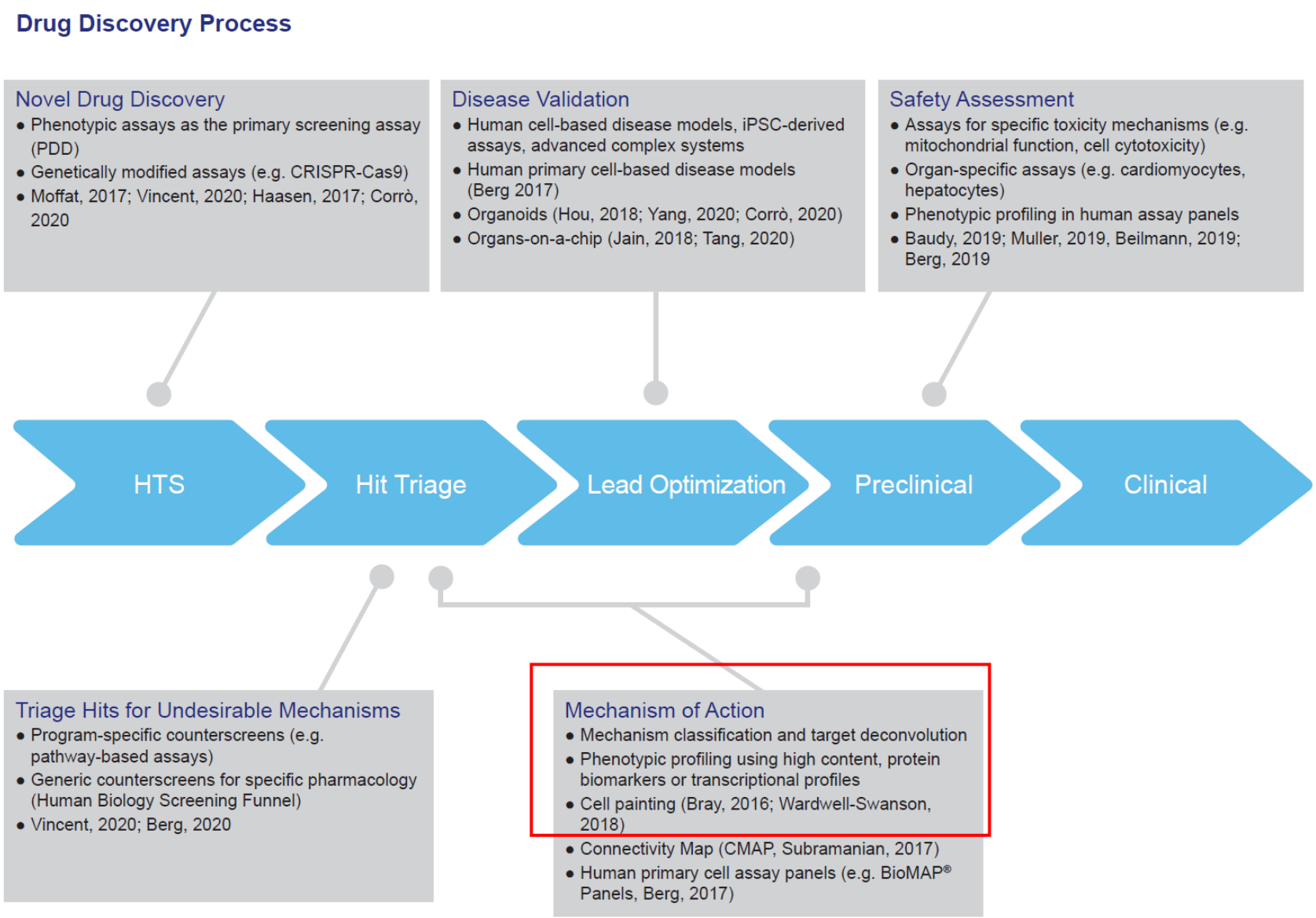
Rebecca Laposa



Outline

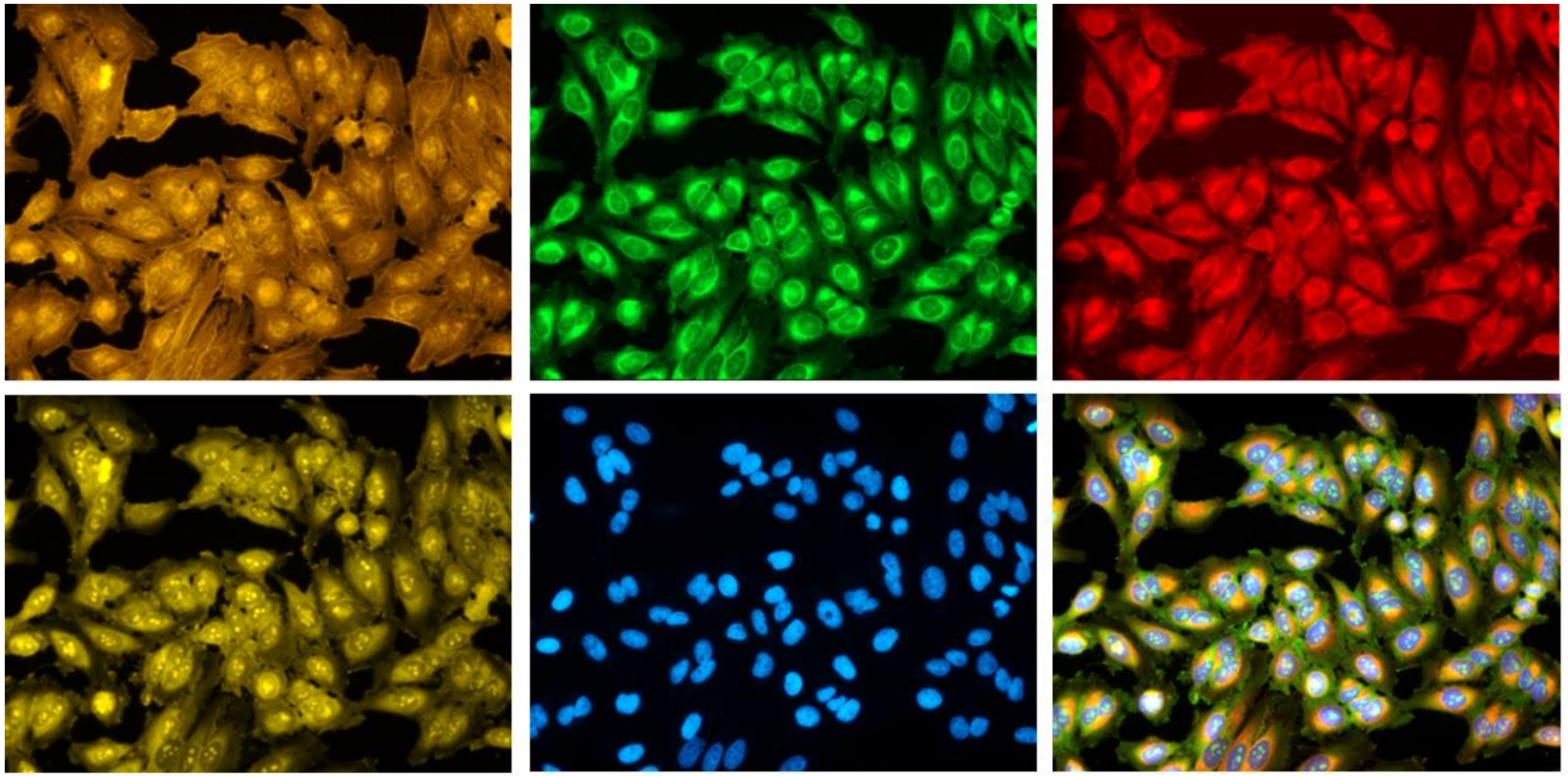
- High Content Screening
- RxRx3 benchmark dataset
- Contiuum of phenotypes – drugs and poisons
- Mechanisms of toxicity
- Tools for perturbing phenotypes

Phenotypic assays in drug discovery



Berg, Cell Chem Bio 2021

Cell Painting:
Various organelle/cell structure dyes



5 individual dyes that stain cell structures found in most cells

Eg: nuclei (blue)
Mitochondria (red)

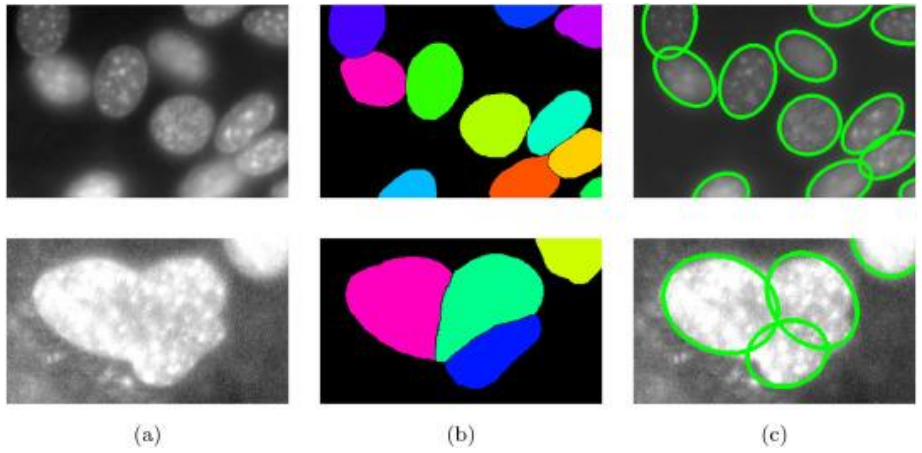
Plus overlay (panel 6)

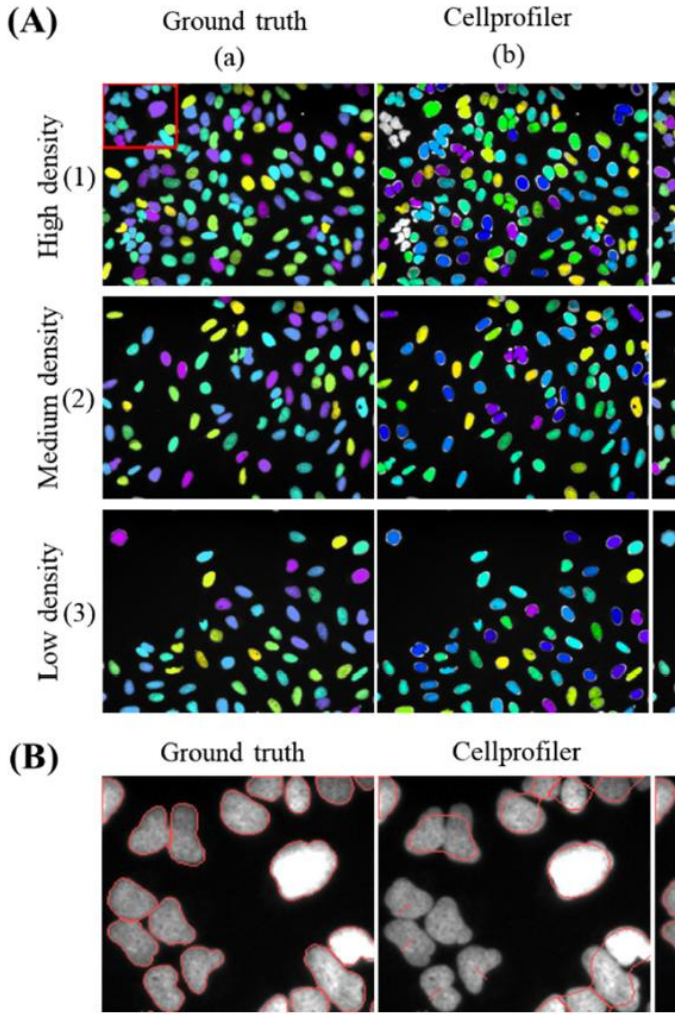
Images: Wikipedia

Traditional HCS pipelines:
Human subject matter expertise guides assumptions about features

- Example: nuclear segmentation
- nuclei are assumed to have certain attributes
  - size range
  - minimum brightness intensity
  - signal over baseline

- Algorithms:
  - Thresholding
  - Watershed algorithm
  - etc

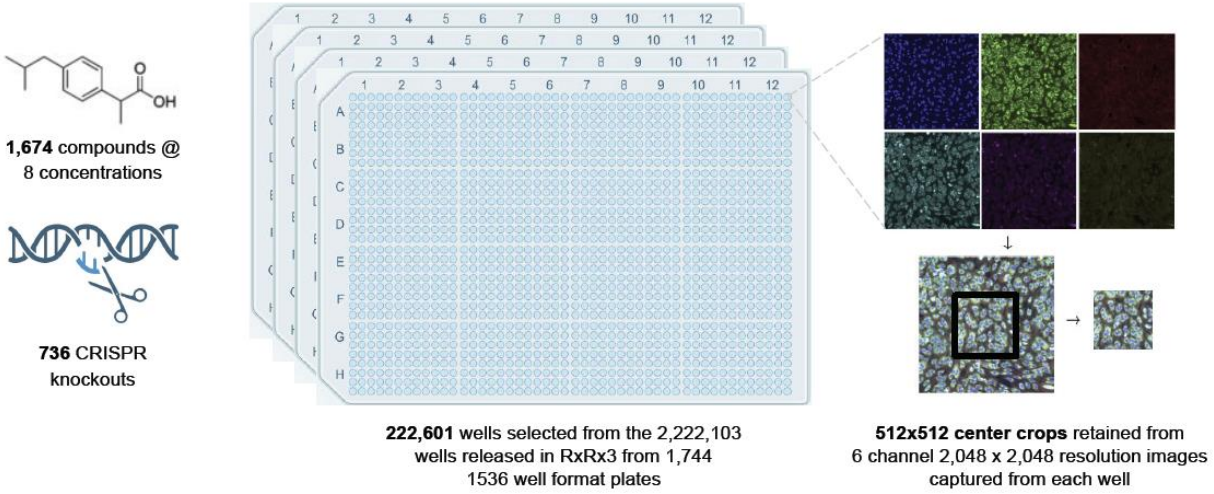




Pan et al. Genes 2022

Contrast: AI/ML for phenomic models

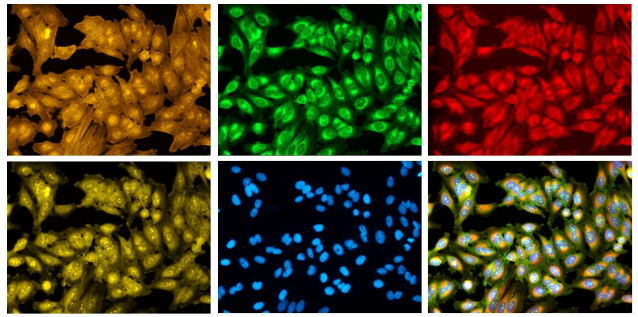
- Uses deep learning to learn feature representation directly from pixel values
- Example benchmarking: RxRx3 Core (Module)



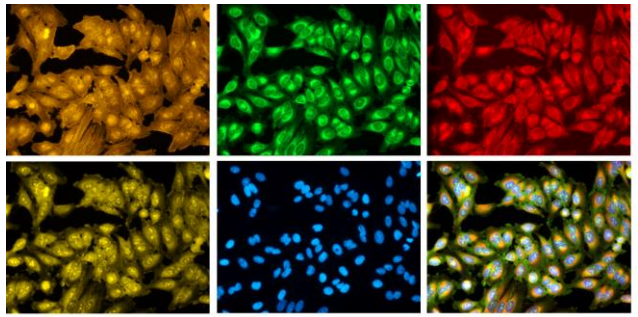
- 1674 compounds
- 736 CRISPR gene knockouts
- replicates

Perturbations: Combining phenotypic screening of compounds and genome-wide CRISPR screening

Perturb cells with small molecules



Perturb cells with gene editing

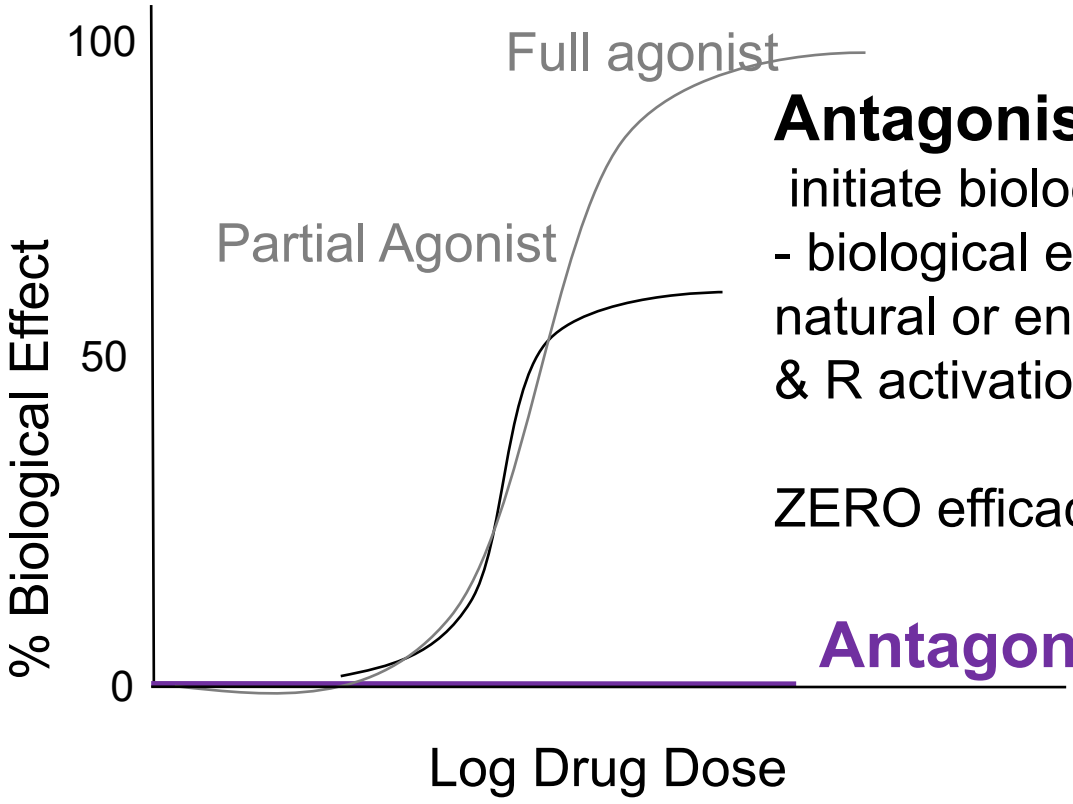


- Compare learned features to uncover similarities.
- If the representation of two perturbations in the neural network is similar, the network indicates that these perturbations lead to similar infilling (autoencoder)
- Infer mechanisms to drugs (what is the drug's target) → inferred causality

Issues with RxRx3 dataset – Pharmacologist perspective

- Is the target present in the cell type?
  - Human umbilical epithelial cell line, HUVEC
  - All cell types same DNA → very different transcripts, proteins
- Only very few genes studied (~750 out of 25,000)
- Unlike a large phenotypic screen, this project involves FDA-approved drugs only (1674 compounds vs HTS library of >100K compounds)
- If our drug development program seeks antagonists, is the endogenous ligand present?
- What about dose-response?

When screening for antagonists, need natural (endogenous) agonist present



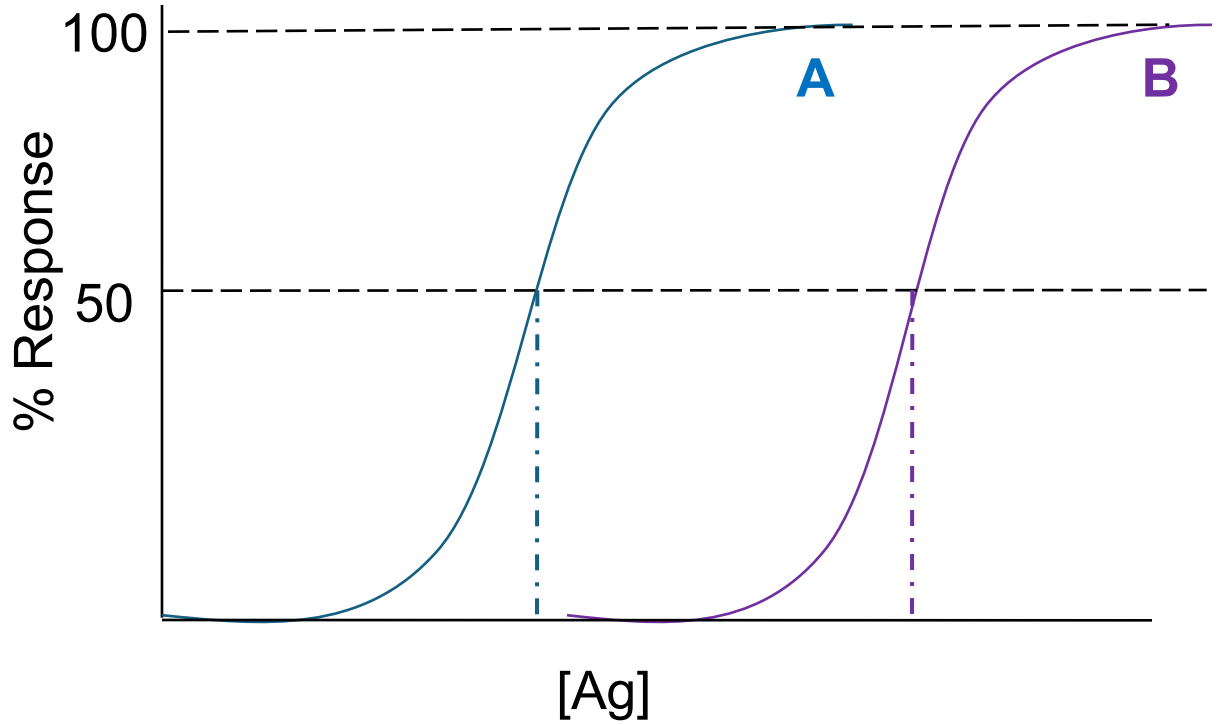
Antagonists- binds R but does not initiate biological activity/signaling cascade
- biological effects from "***preventing***" natural or endogenous agonist from binding & R activation

ZERO efficacy on its own

Antagonist alone

Competitive Antagonist

- Bind same site on R as Agonist
- Inhibition can be overcome by ↑ [Ag]
- Affects Ag potency (ie shifts D-R curve RIGHT)
  - Clinically useful

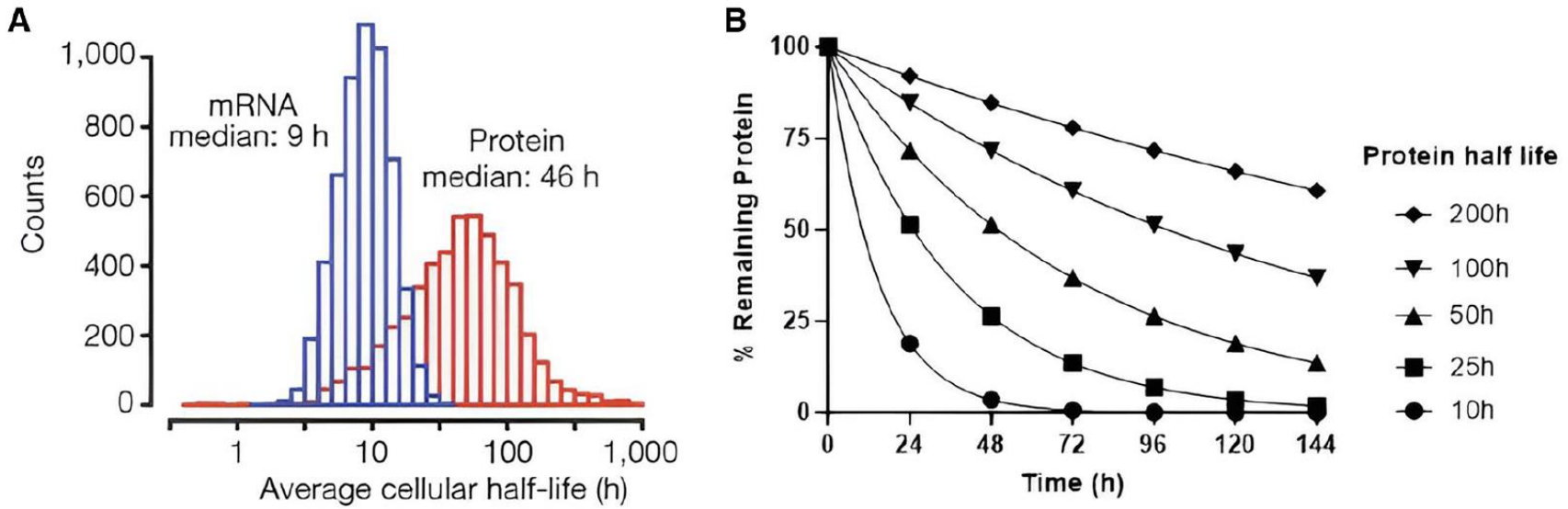


A: Ag alone
maximal potency & efficacy

B: Ag + comp ANT
reduced potency but maximal efficacy still achievable

What about timing?

- Timing of assay (microscope image capture post-intervention) reveals effects of some proteins but not others…



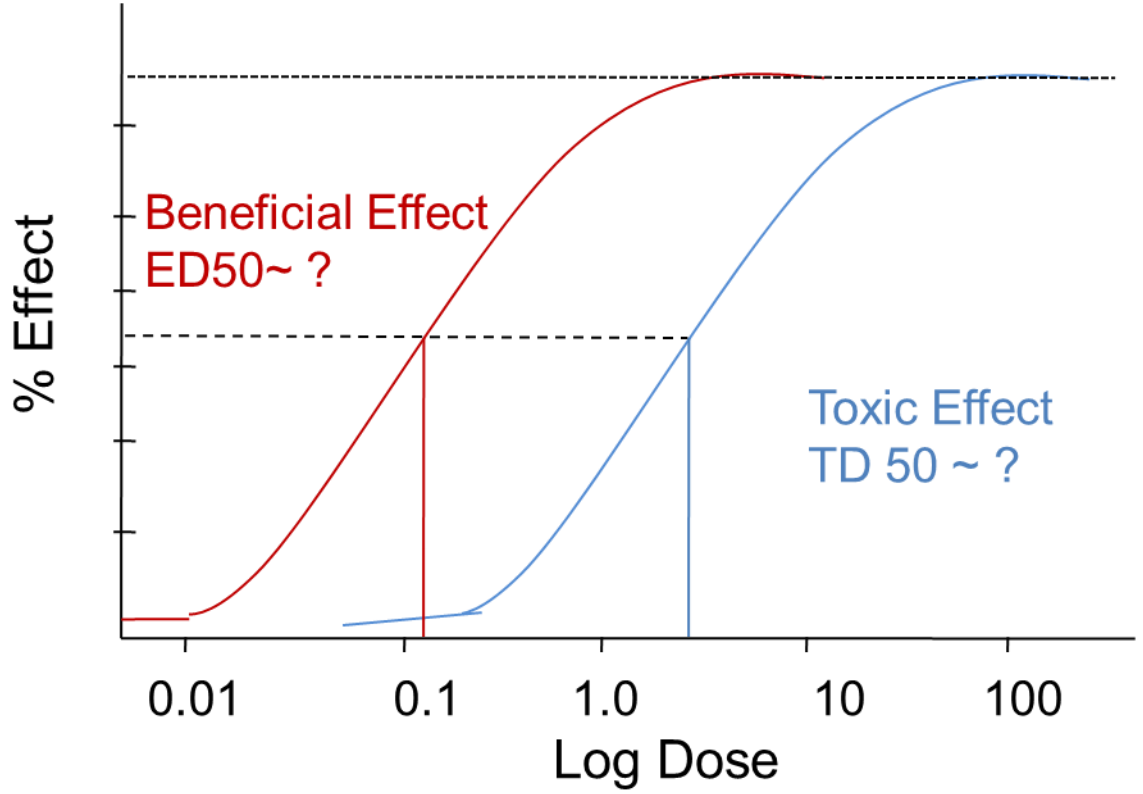
Vincent and Gianni, Cell Chemical Biology 2025

Is my molecule toxic?

- This question has a dose-dependent answer....

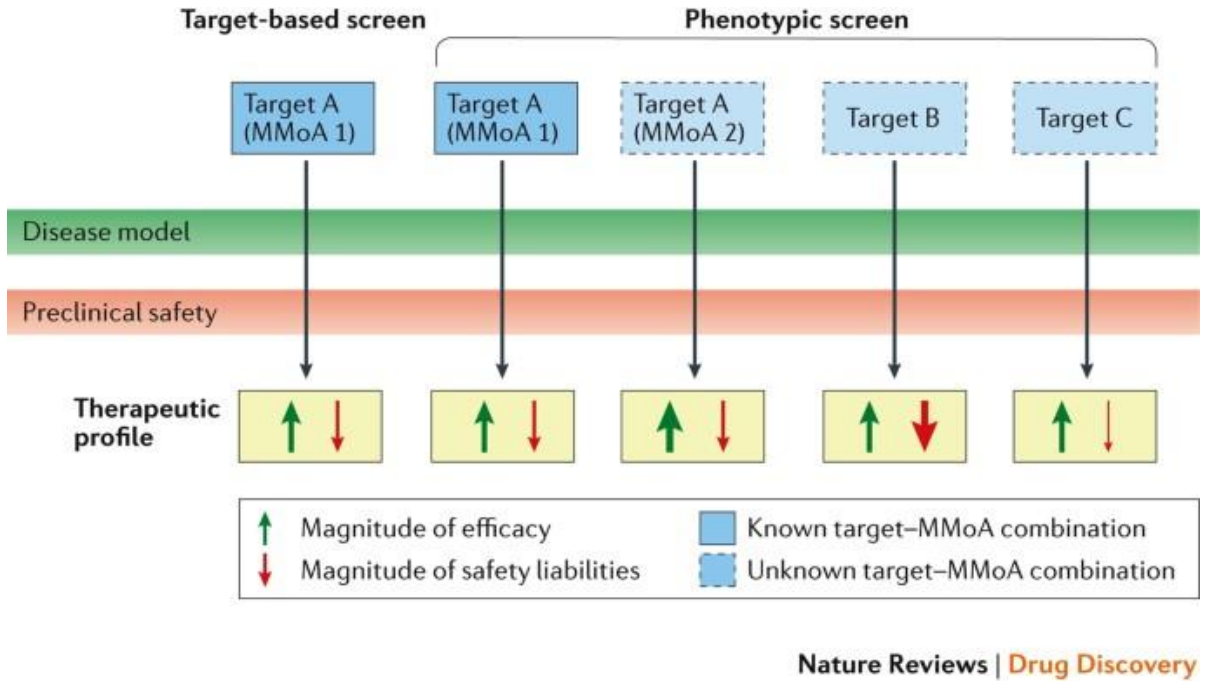
- Need to move beyond simple classification problem (hit / not-hit)
- Reframe drugs/toxins as “bioactive molecules” with effect x at concentration y

Therapeutic index
Ratio of concentration for toxic effect vs concentration for therapeutic effect (TD_{50}/ED_{50})



High therapeutic index:
Clinical utility

Phenotypic screens:
Implications for drug safety and unknowns



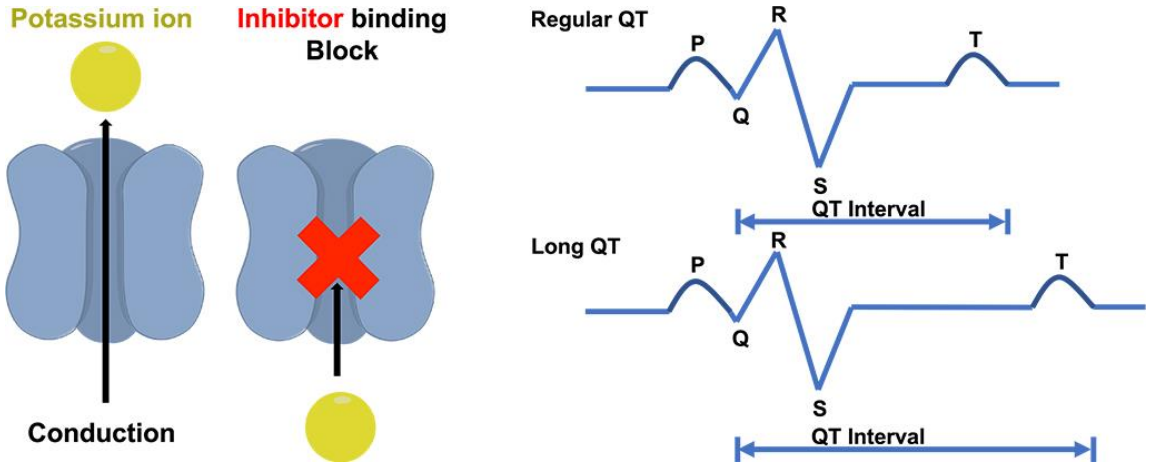
Toxicity depends on dose

3 major mechanisms

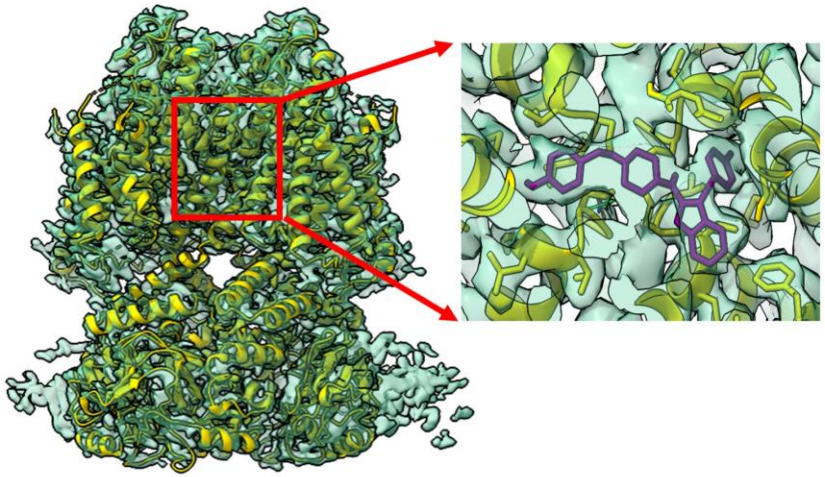
1. Off-target toxicity
2. On-target toxicity
3. Reactive intermediates

1. Off-target toxicity

- Some off-targets are so concerning that all new drugs are counter-screened against these targets
- Drug Development goal: “kill bad drugs early”
- Largest reason for attrition in preclinical drug dev: potential for cardiotoxicity
- Common example: cardiac ion channel → cardiotoxicity



hERG potassium channel in the heart

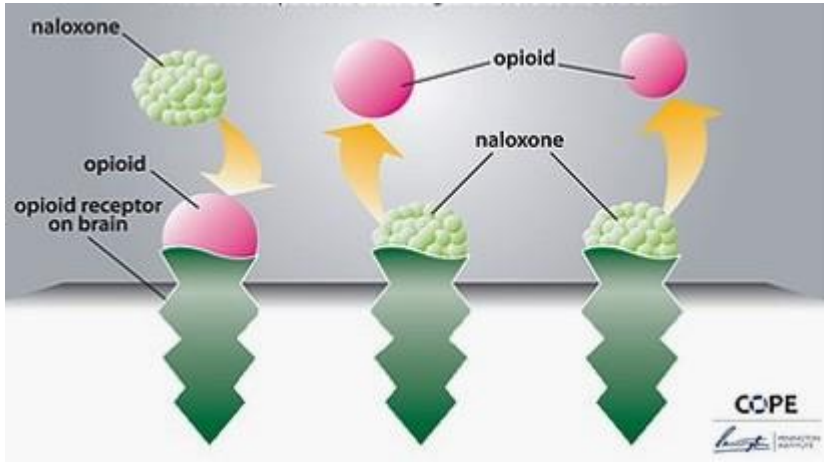


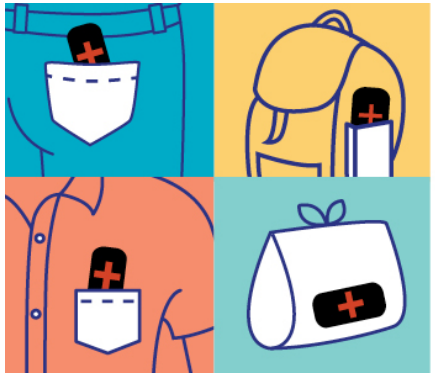
CryoEM of hERG potassium channel

Images: Nanoimaging

2. **On-target** toxicity

- Exacerbation of therapeutic effects
- Example: opioid drugs and opioid mu receptor
- On-target toxicity:
  - Respiratory depression
  - Sedation
- Eg. Fentanyl, opioid overdoses
- Counteract with naloxone
  - Fast-acting antagonist





3. Drug metabolism

- Protective
- Evolutionarily conserved enzymes
- Remove “foreign” small molecules (chemicals) from the body
- Broad substrate specificity
  - Protection against chemicals the body has never encountered previously
  - Plant poisons
  - Including new and investigational drugs
- Abundant in liver but other sites in body also

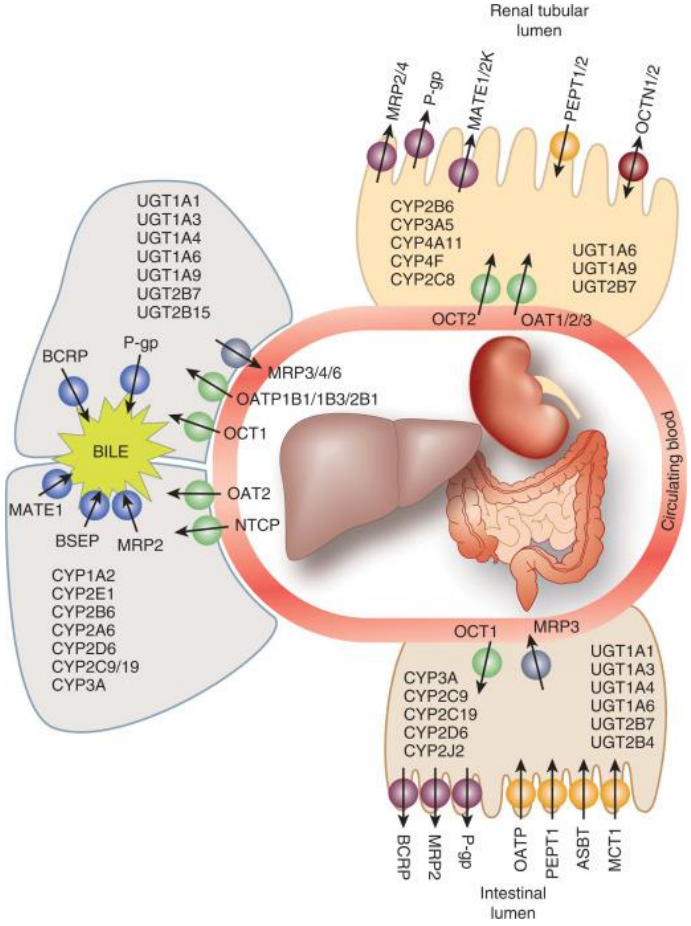
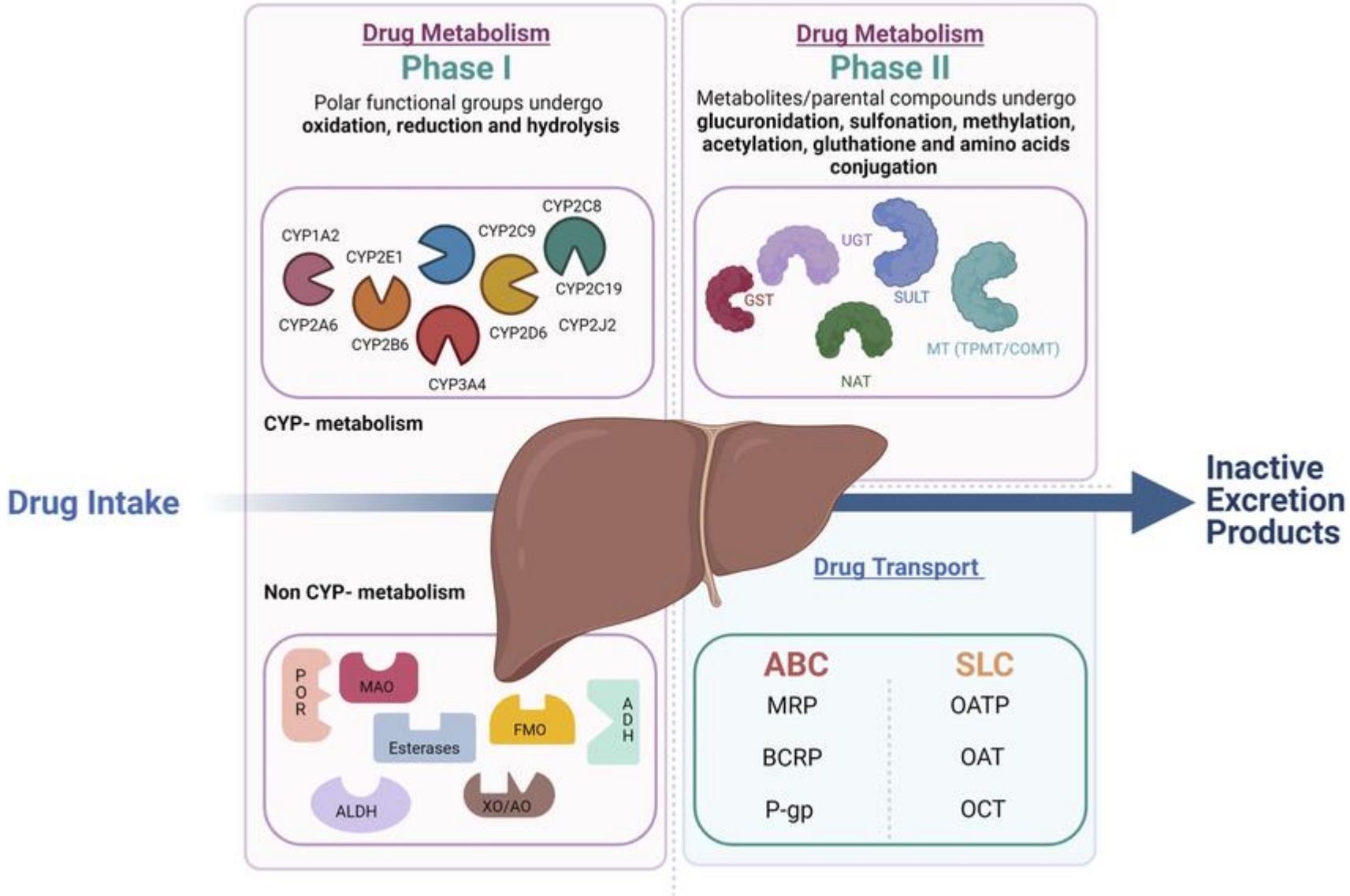


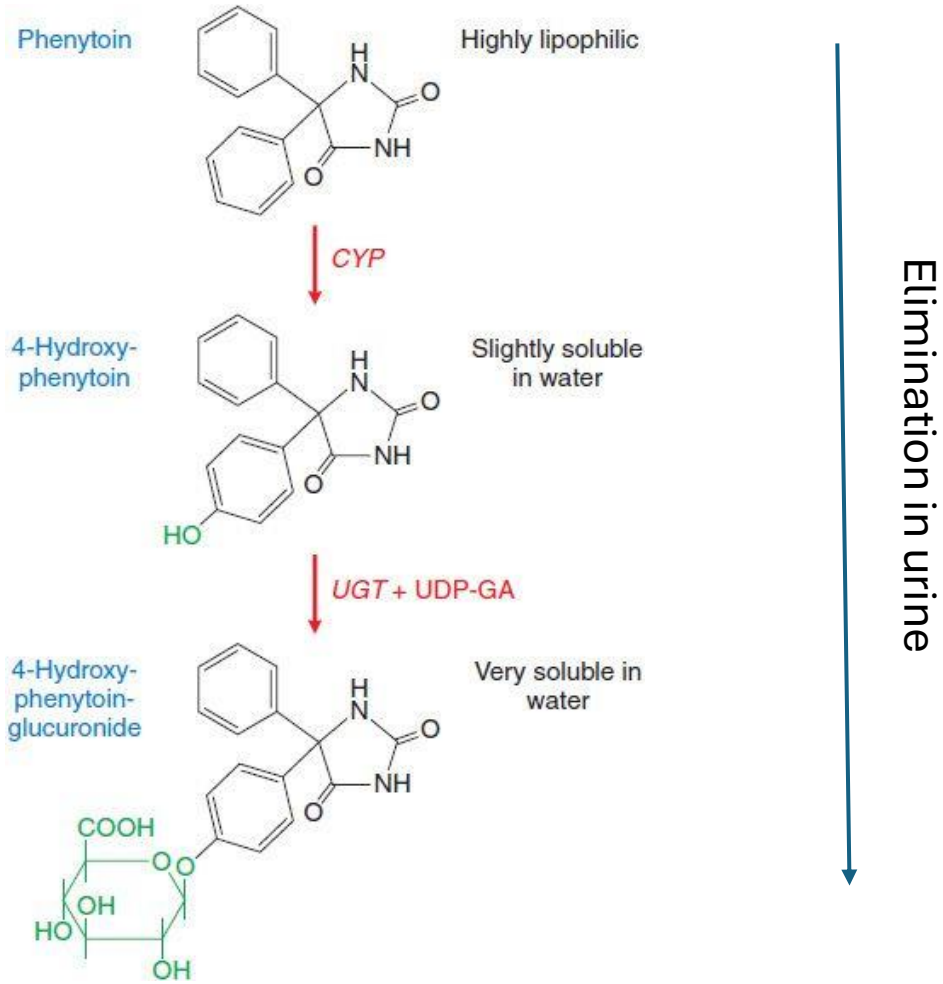
Image: Catherine Yeung

3. Drug metabolism and transport for drug elimination



Abouir et al., Frontiers Pharmacol 2021

Drug metabolism to enhance elimination

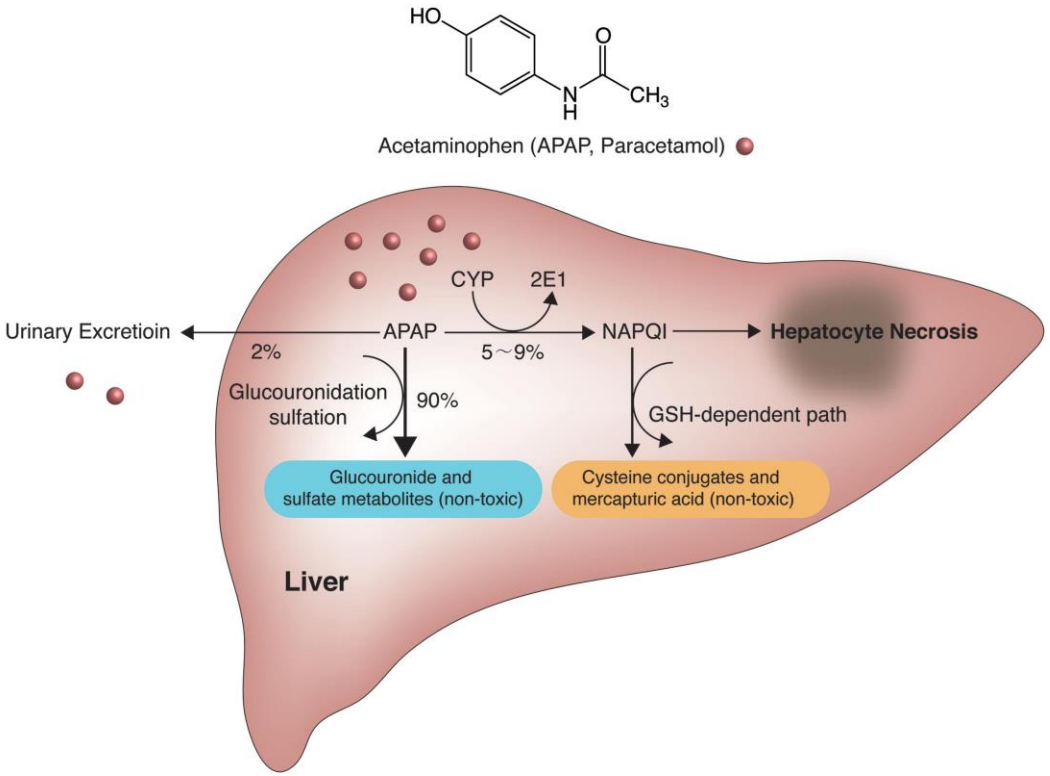


Anticonvulsant drug example

Source: BasicMedical

3. Toxicity from Reactive metabolites

- Chemically reactive metabolites sometimes produced during drug metabolism
- Some of the intermediates produced can be harmful
  - covalent reactions with protein, DNA
- Inter-individual differences



- Appropriate phenotypic assays that "catch" toxicity require:
  - Source of drug metabolizing enzymes
  - Protein target expressed in cell type

Yoon et al., 2016

Reactive metabolite toxicity – acetaminophen (Tylenol)

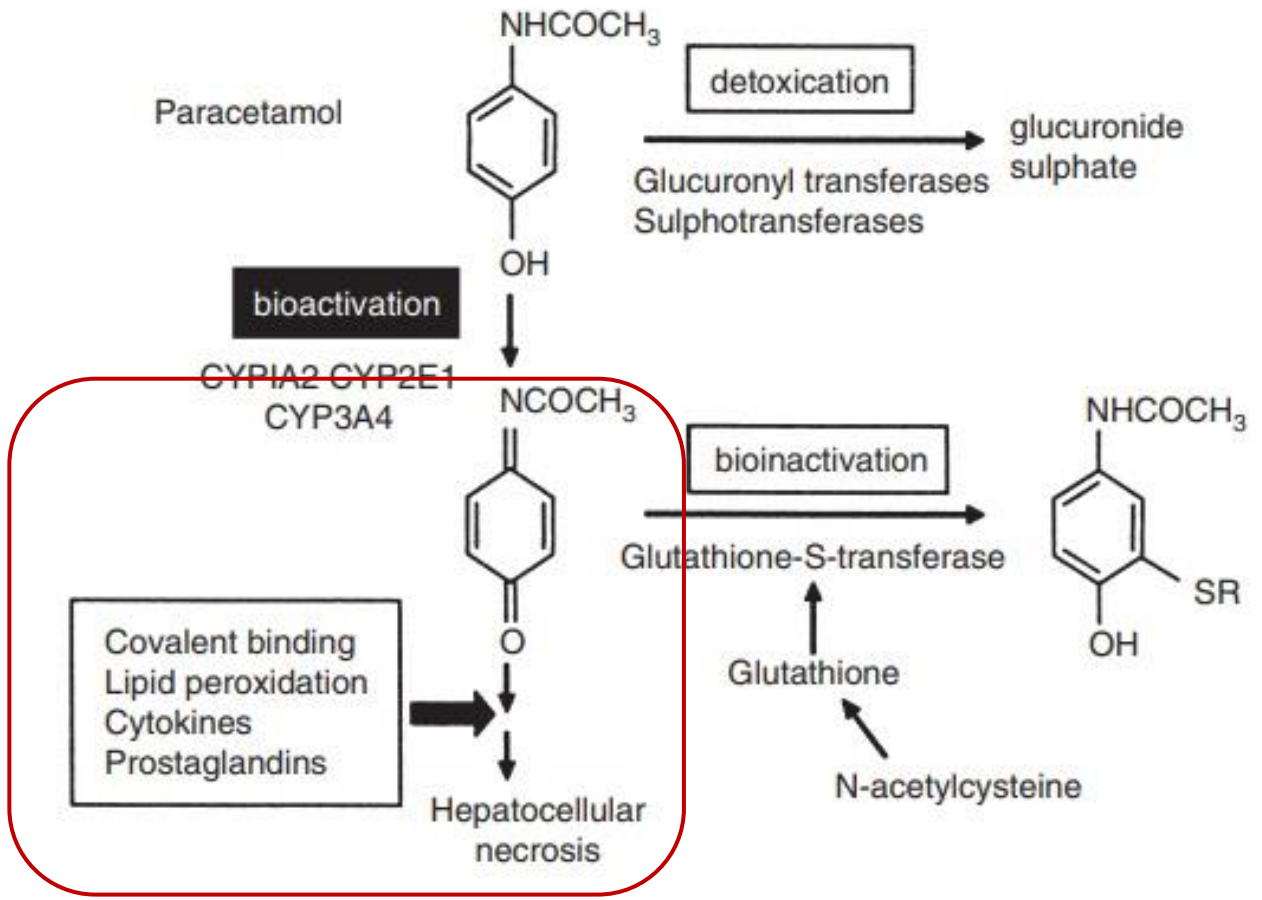
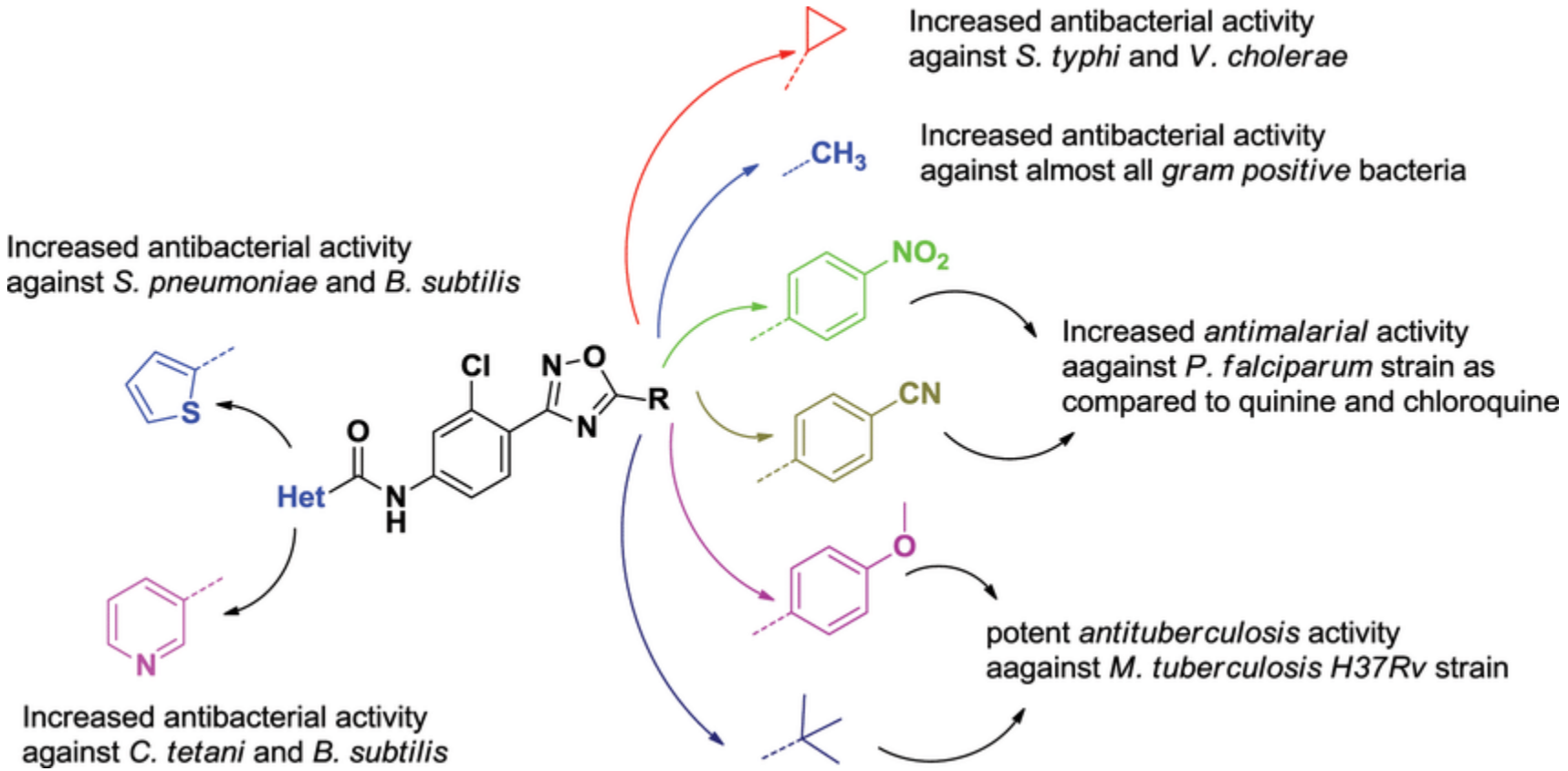


Figure 8.3. The role of metabolism in the hepatotoxicity associated with paracetamol.

Correlating chemical structure and phenotypic effect: Can your screen reveal structure-activity relationships?



Tools for perturbations

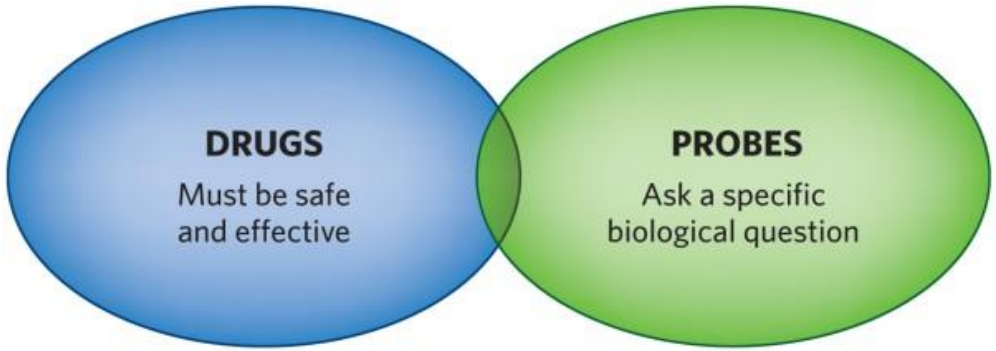
- Loss-of-function vs gain-of-function
  - CRISPR (loss)
  - Gene delivery (gain) → RNA → protein
- Binary vs continuous perturbation
  - CRISPR (DNA knockout, binary)
  - siRNA (acts on RNA transcripts, vary effect by varying dose of siRNA)
  - Recall: pharmacology focus on dose effects
- Chemical probes
  - **Cell-permeable** chemicals with clean mechanisms (single target)
  - Precise control of timing
  - Advantage when also structurally related inactive control (can do structure-activity relationships)

	RNAi	CRISPR
Benefits	· Pre-designed reagents readily available · Useful for studying the effect of essential genes on phenotypes · Studies where temporary loss-of-function is desired (e.g., to mimic the effect of a drug)	· Precise gene targeting with fewer off-target effects · Permanent gene disruption results in robust signal · Lower risk of immune response (some formats) · Flexible time frame for assay
Drawbacks	· Temporary gene disruption may require a narrow assay window · Incomplete silencing (knockdown) may not produce a strong signal · Associated with more off-target effects · Silencing of multiple transcripts possible (introducing noise) · Introduced RNA may stimulate immune response · Laborious analysis and verification of true hits	· Cannot be used to study essential genes

Chemical probes: tools for perturbation experiments

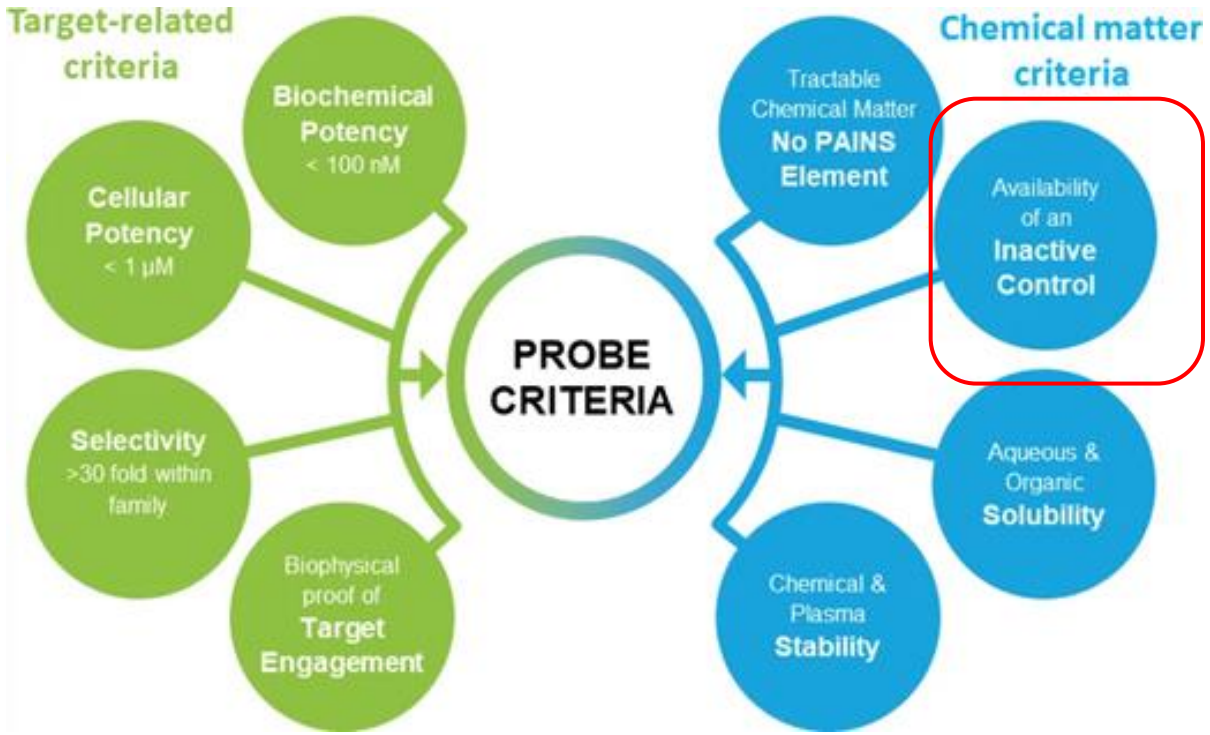
TARGET 2035

Goal: a chemical probe for every human protein



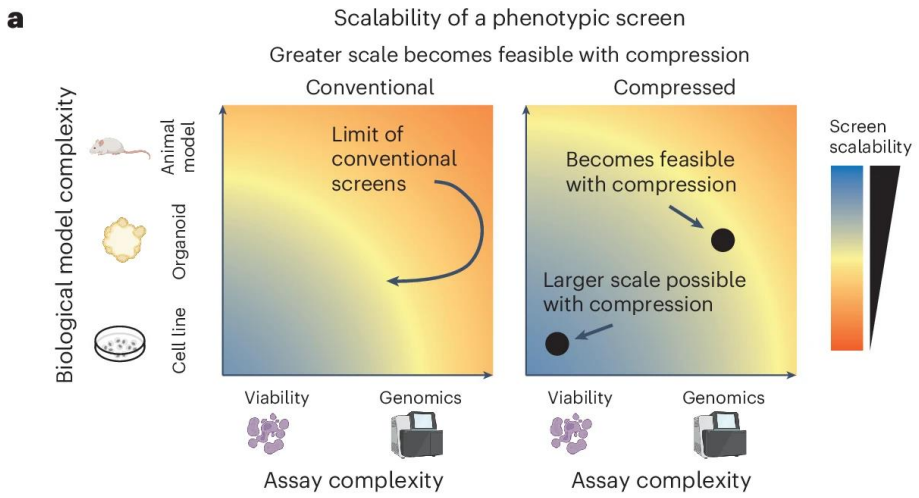
- May have undefined MoA
- IP restrictions; limited availability
- Must have human bioavailability
- High bar for physicochemical (guidelines for MW, lipophilicity, etc.) and pharmaceutic properties (stability, reasonable and economic synthesis, defined crystallization form, etc.)

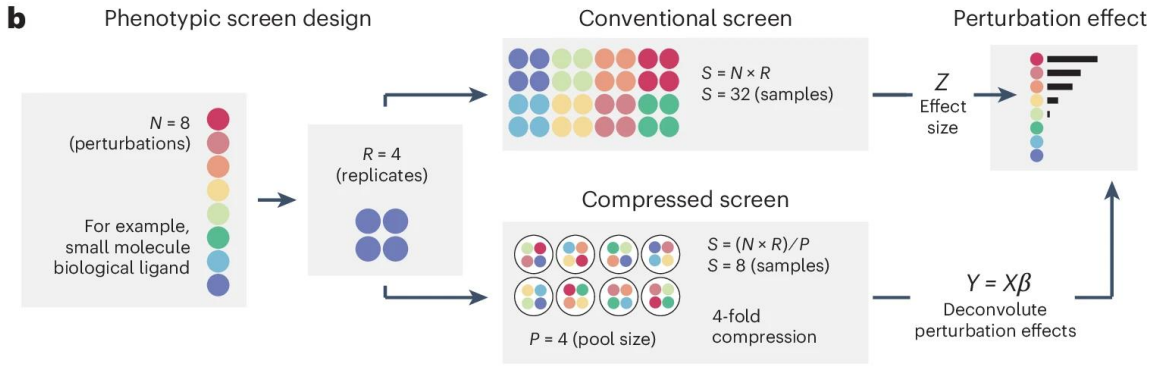
- Defined MoA is required
- Needs selectivity
- Freely available (both the physical compound itself and activity data)
- Drug-like properties, such as bioavailability, not necessarily required
- Value is markedly enhanced by use of structurally related inactive and structurally unrelated active compounds

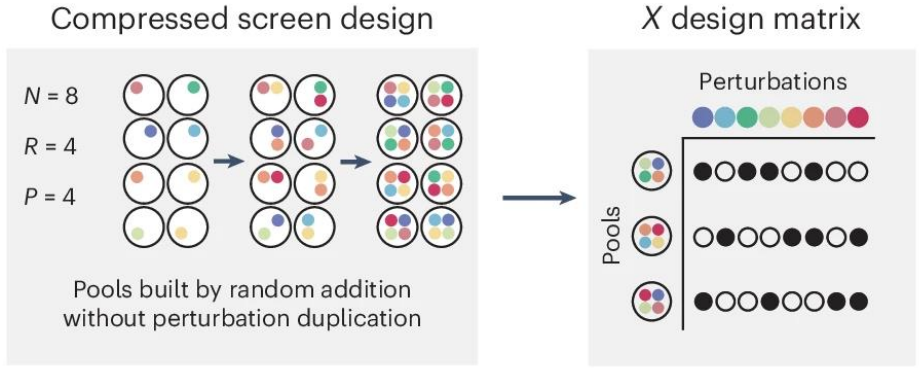


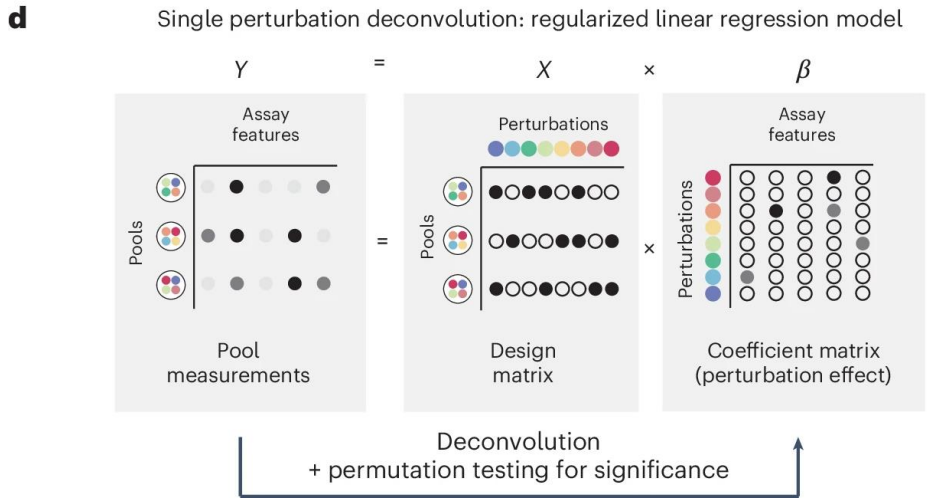
Images: Nature Chemical Biology, Frances Crick Institute

Compressed phenotypic screens with pooled perturbations









Liu et al., Nat Biotech 2024

CellPress

Cell Chemical Biology

Perspective

The limitations of small molecule and genetic screening in phenotypic drug discovery

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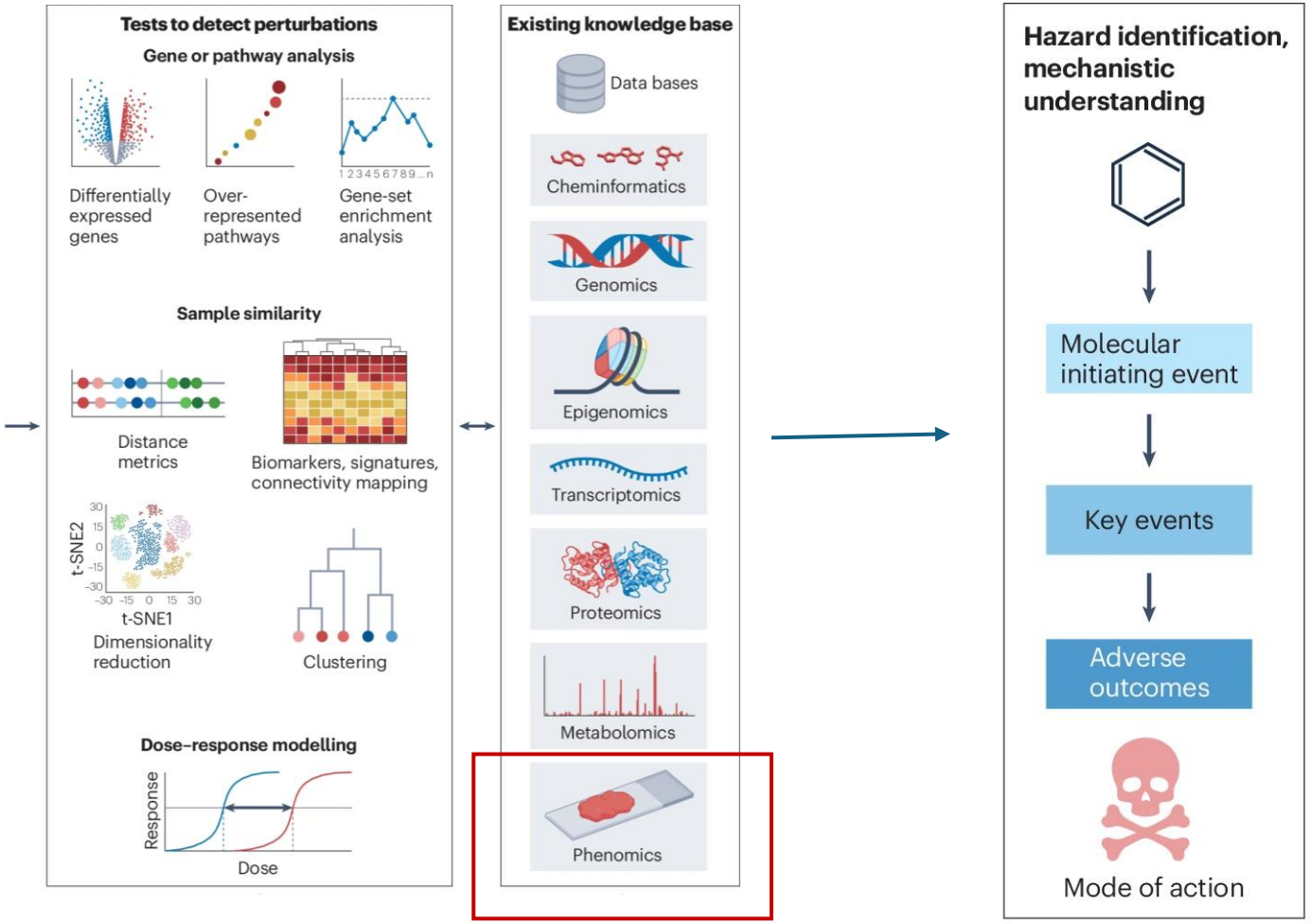
https://doi.org/10.1016/j.chembiol.2025.10.008

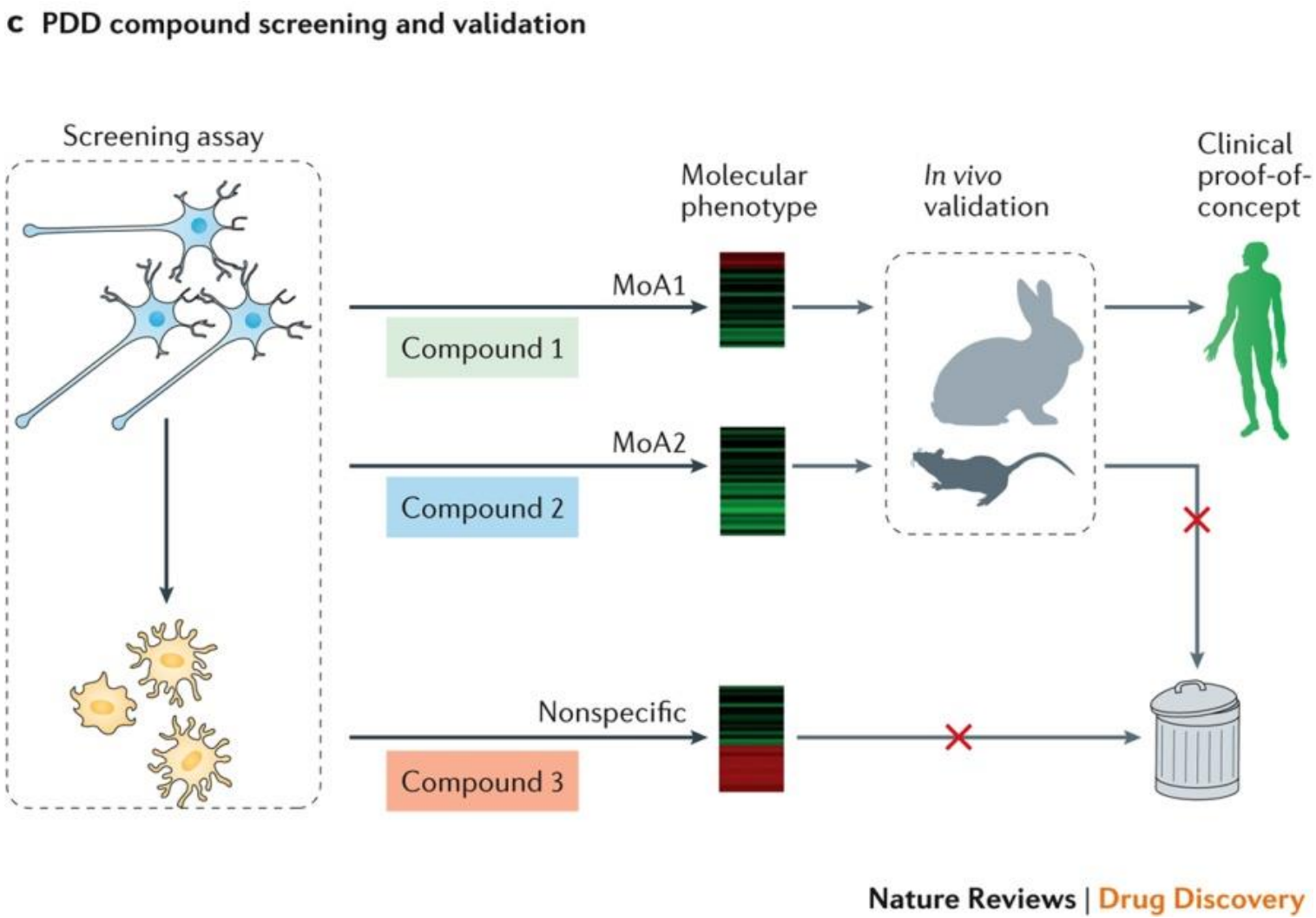
Table 2. Small molecule screening limitations and mitigation strategies

Small molecule screening limitations	Mitigation strategies
Chemogenomics libraries only cover ~5%–10% of the genome	Additional genome coverage via secreted proteins or receptor antibodies
Large legacy compound libraries only cover a fraction of the genome	New compound classes address a larger fraction of the genome (covalent, photoactivatable, and molecular glue compounds)
Limited throughput for complex assays, especially *in vivo* assays	Screening library compression, smaller compounds (e.g., fragments), biological diversity-driven libraries
Frequent lack of selectivity for related protein homologs	New compound classes may offer greater selectivity (covalent and molecular glue compounds)
Potential species translation hurdle	Additional species phenotypic assays, 10× *in vivo* dosing strategy, complex human models
Target and MoA deconvolution post-screen often challenging and may be required for successful outcome	New compound classes (covalent, photoactivatable, and glue) may facilitate target and MoA deconvolution through proteomics

Integrating phenomic approaches to understand toxicity



Where do we go from here in drug development?



Takeaways

- High content screening and phenotypic assays are useful in drug development
- Modeling approaches can leverage learned features that are distinct from subject matter expert-imposed features
- Phenotypic assays that combine genetic and chemical approaches are relevant for deconvoluting targets
- Drug toxicity is a phenotype of interest, amenable to AI/ML analysis