# What can we do if there are no good targets

Mathematical and Computational Biology in Drug Discovery (MCBDD) Module II

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March-April 2021

The linear view of drug discovery builds on target-based approaches

Target identification & assessment
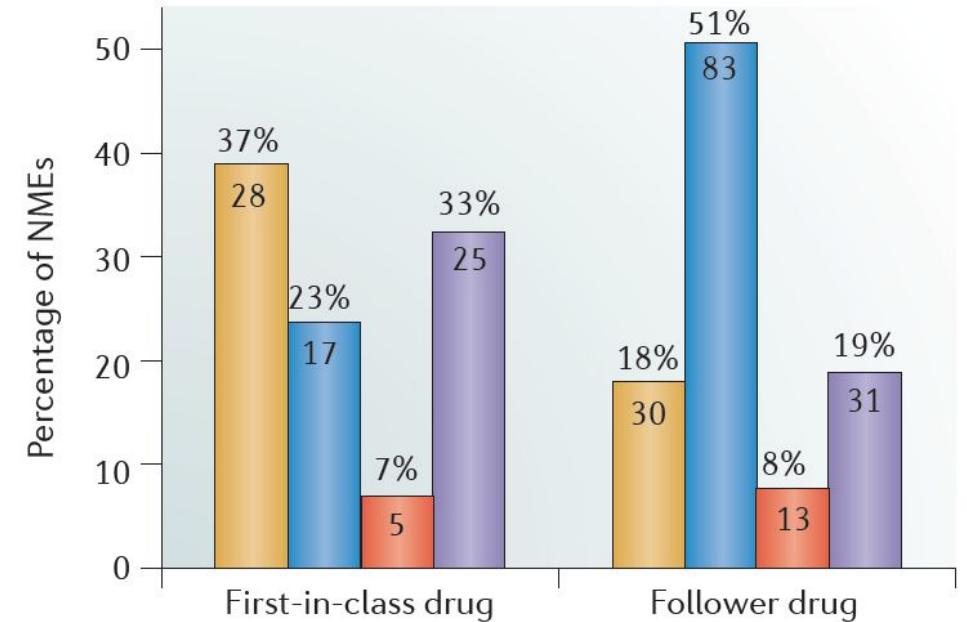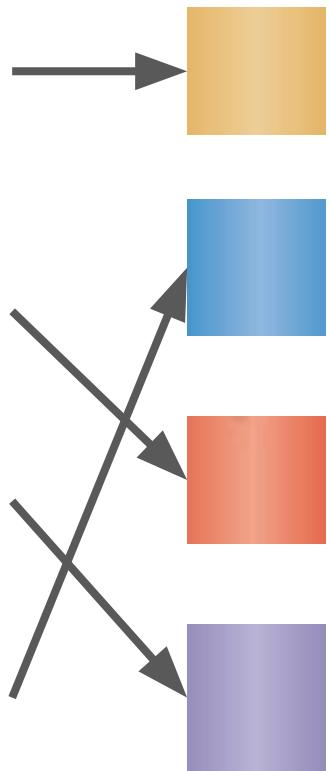
Five strategies when no good target is found
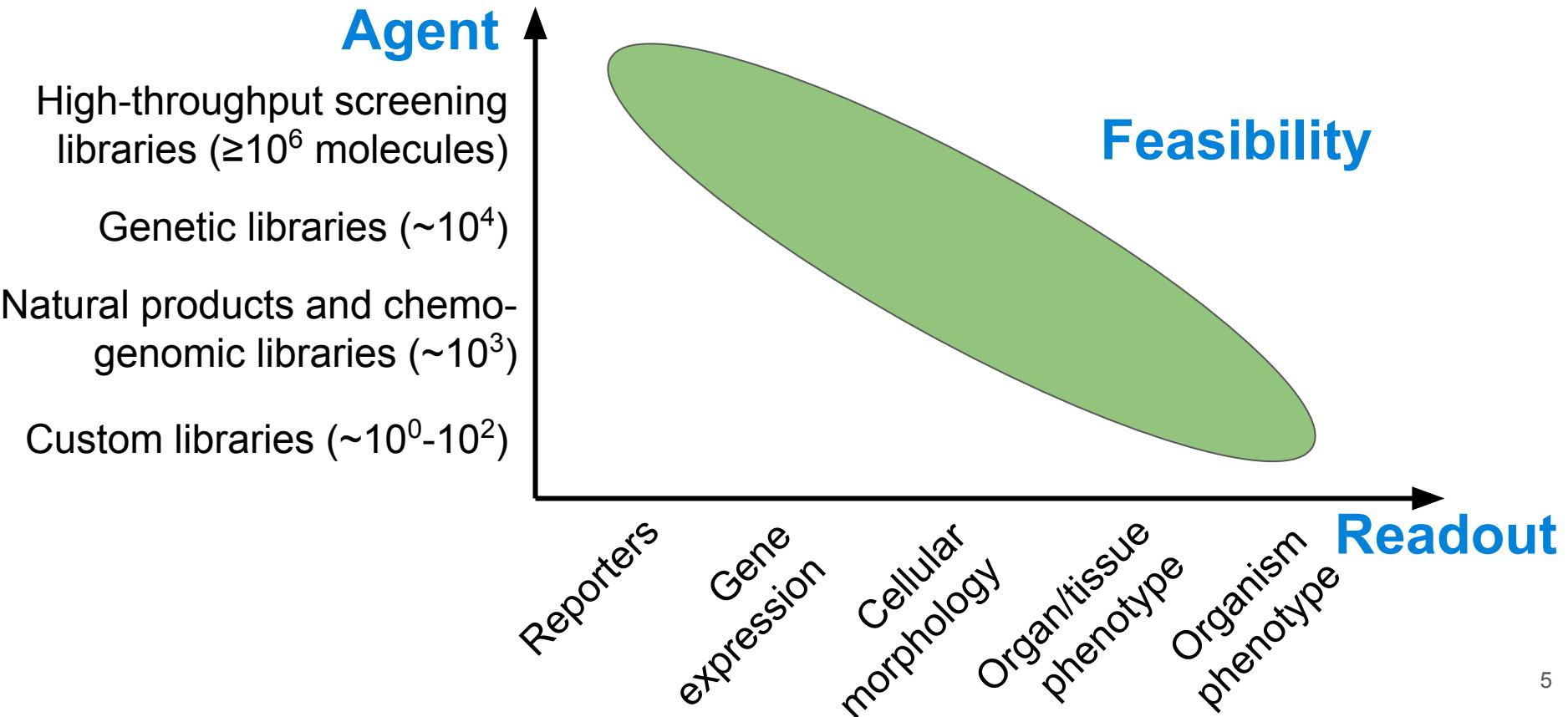
1. Phenotypic drug discovery

2. Natural products

3. Biologics

4. Interaction-based (multispecific) drug discovery

5. Drug repurposing or combination studies

Connect the lines!
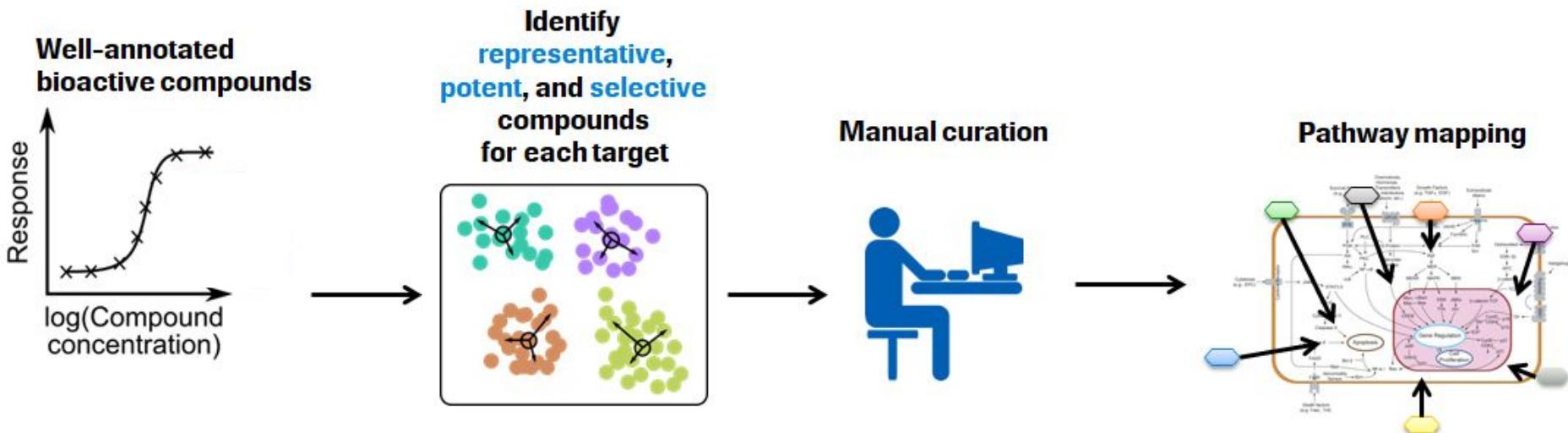
- Phenotypic screening

- Modified natural products

- Biologics

- Target-based screening

Phenotypic screenings by agent and readout
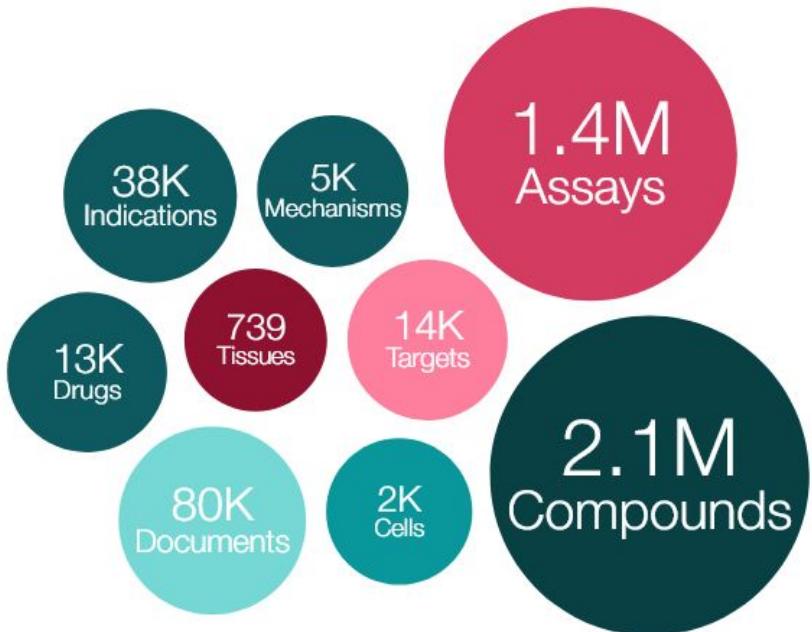
The <u>S</u>mall-molecule <u>PA</u>thway <u>R</u>esearch <u>K</u>it (SPARK)

The ChEMBL database

- An example of query: aspirin.

- Systematic and programmatic accession via ChEMBL API (source code).

- We can use **dose-response data** to annotate the *triplets* of compound, assay activity, and targets.
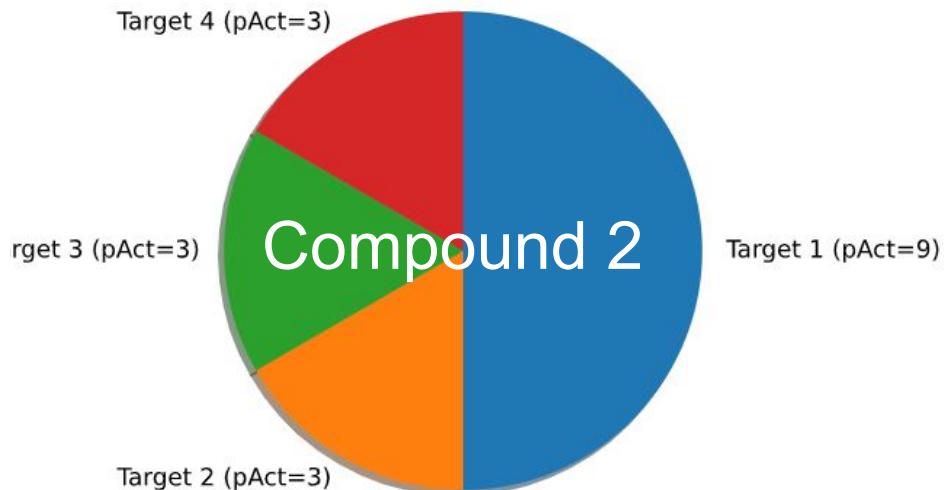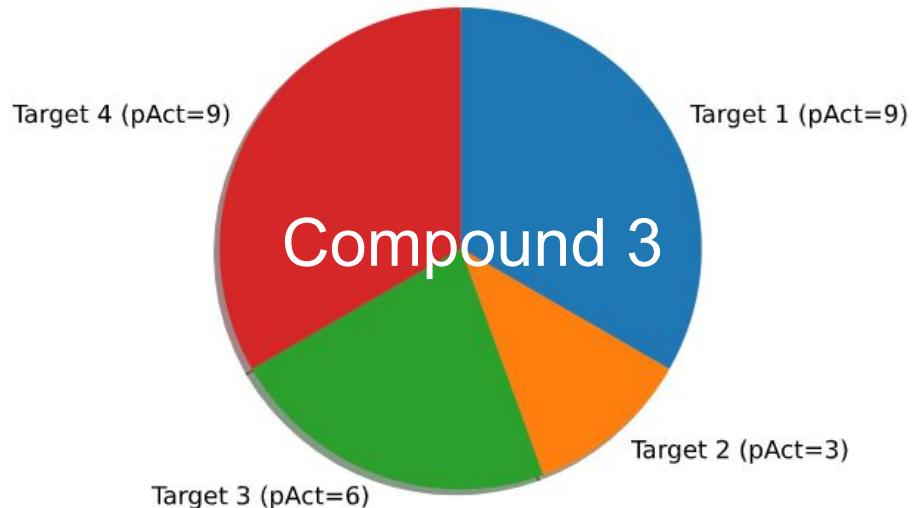
March 2021

Discussion
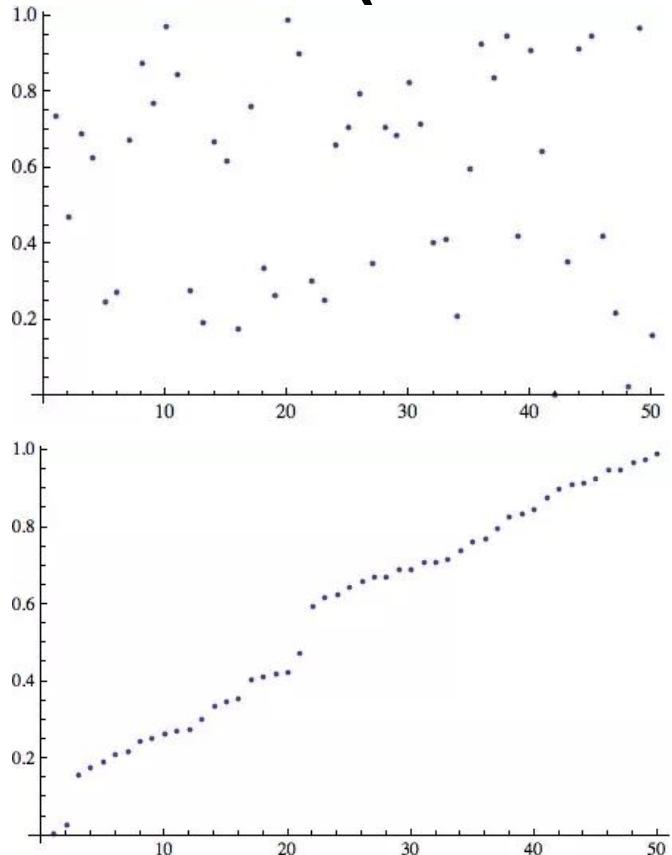
1. Why do we care selecting *representative*, *potent*, and *selective* compounds for each target?

2. How to define following terms mathematically ...

   a. Representativity?
   b. Potency?
   c. Selectivity?

A toy example about how to quantify a compound's potency and selectivity
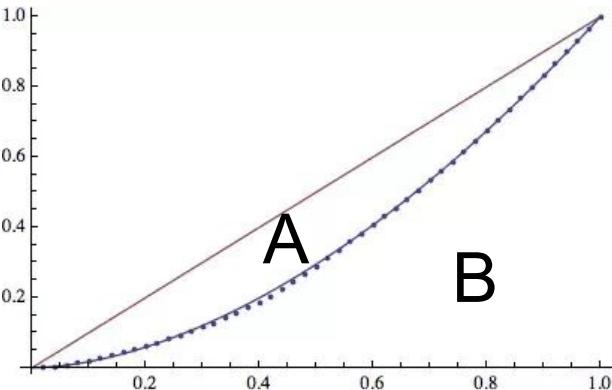
The Gini Index (a.k.a. Gini Coefficient)

A random vector of 50 values

Sorted from low to high
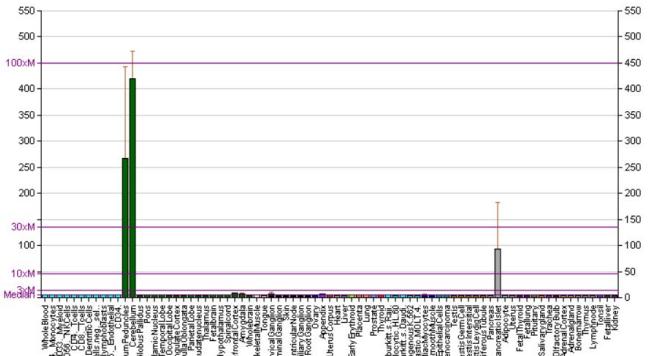
The Gini Index is calculated based on the cumulative distribution

$G=A/(A+B)$

The Gini Index quantifies inequality/ selectivity
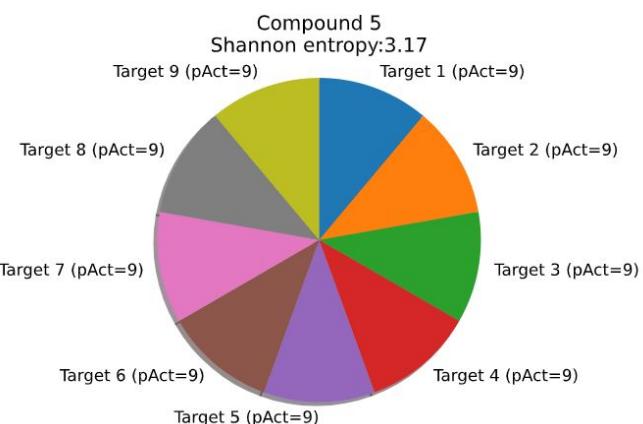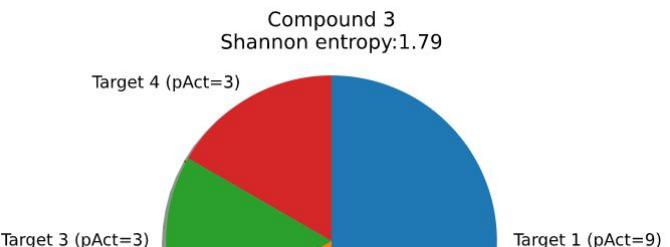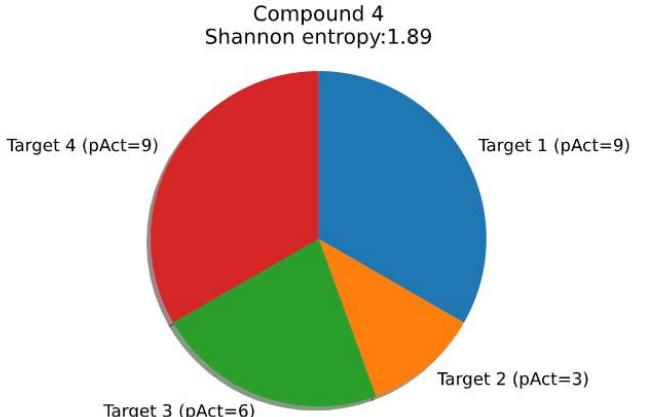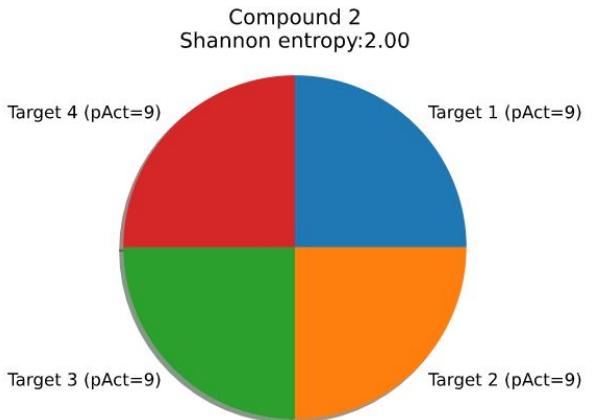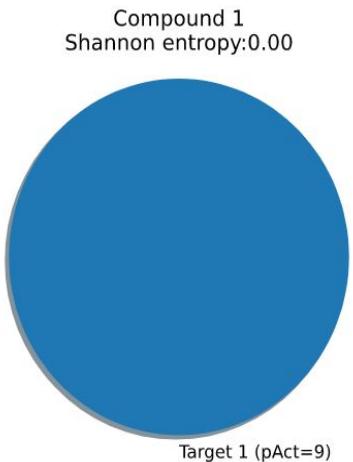
NEUROD1
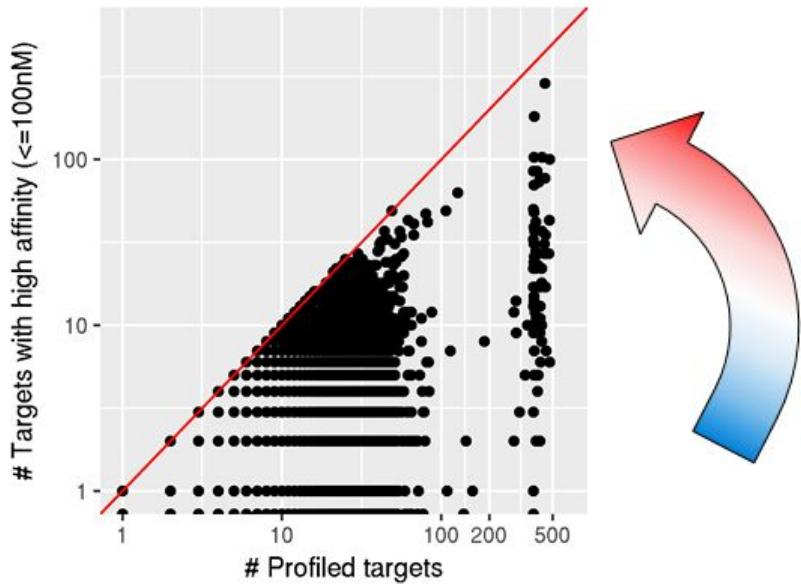
RBL1

The Gini Index of expression of *NEUROD1* across tissues is near 1, whereas that of *RBL1* is near 0.

11
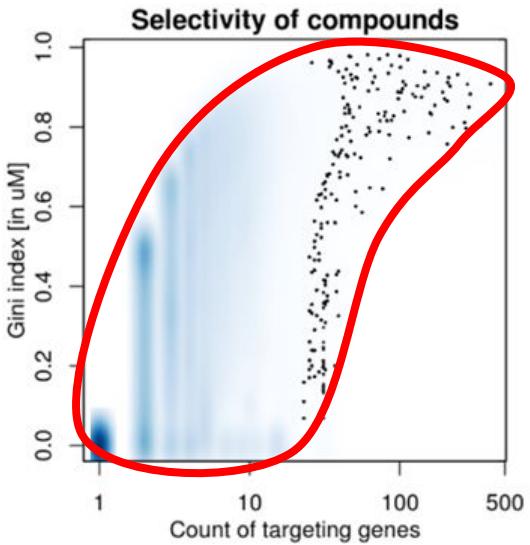
An alternative metric: Shannon's Entropy

Compound 1
Shannon entropy:0.00

Compound 2
Shannon entropy:2.00

Compound 3
Shannon entropy:1.79

Compound 4
Shannon entropy:1.89

Compound 5
Shannon entropy:3.17

Count of targets and selectivity of ChEMBL molecules
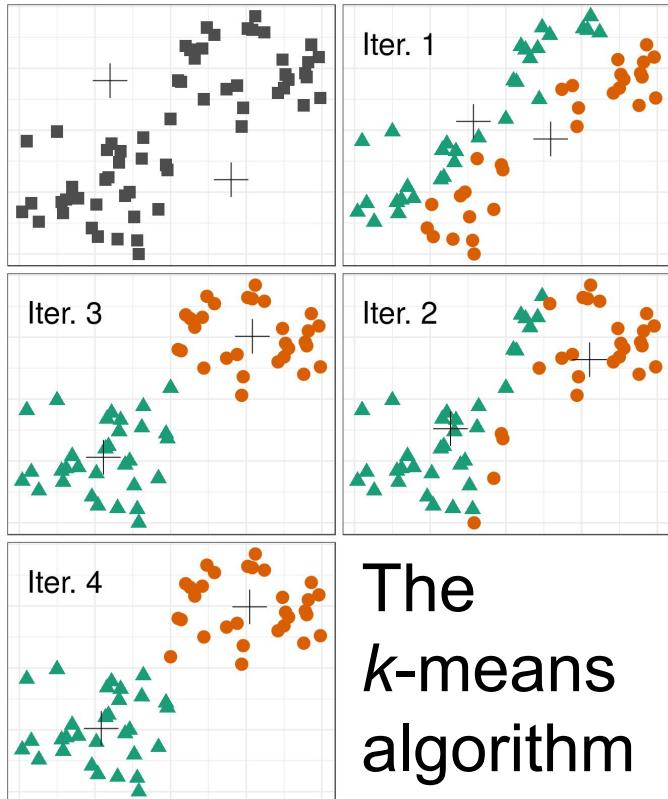
With some exceptions, most compounds are profiled against <100 targets. We distinguish between specific and pleiotropic compounds.

The **shark-fin shape** curve suggests that frequently profiled compounds tend to be more selective (and *vice versa*).
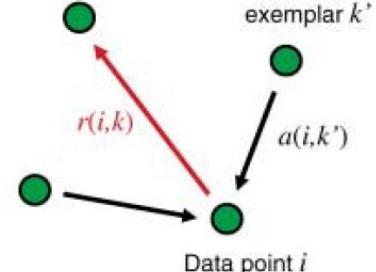
Unsupervised clustering
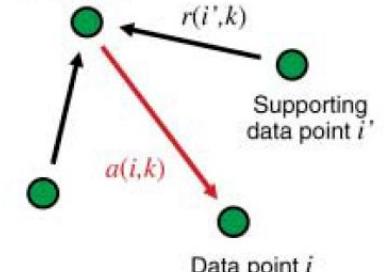
The
k-means
algorithm
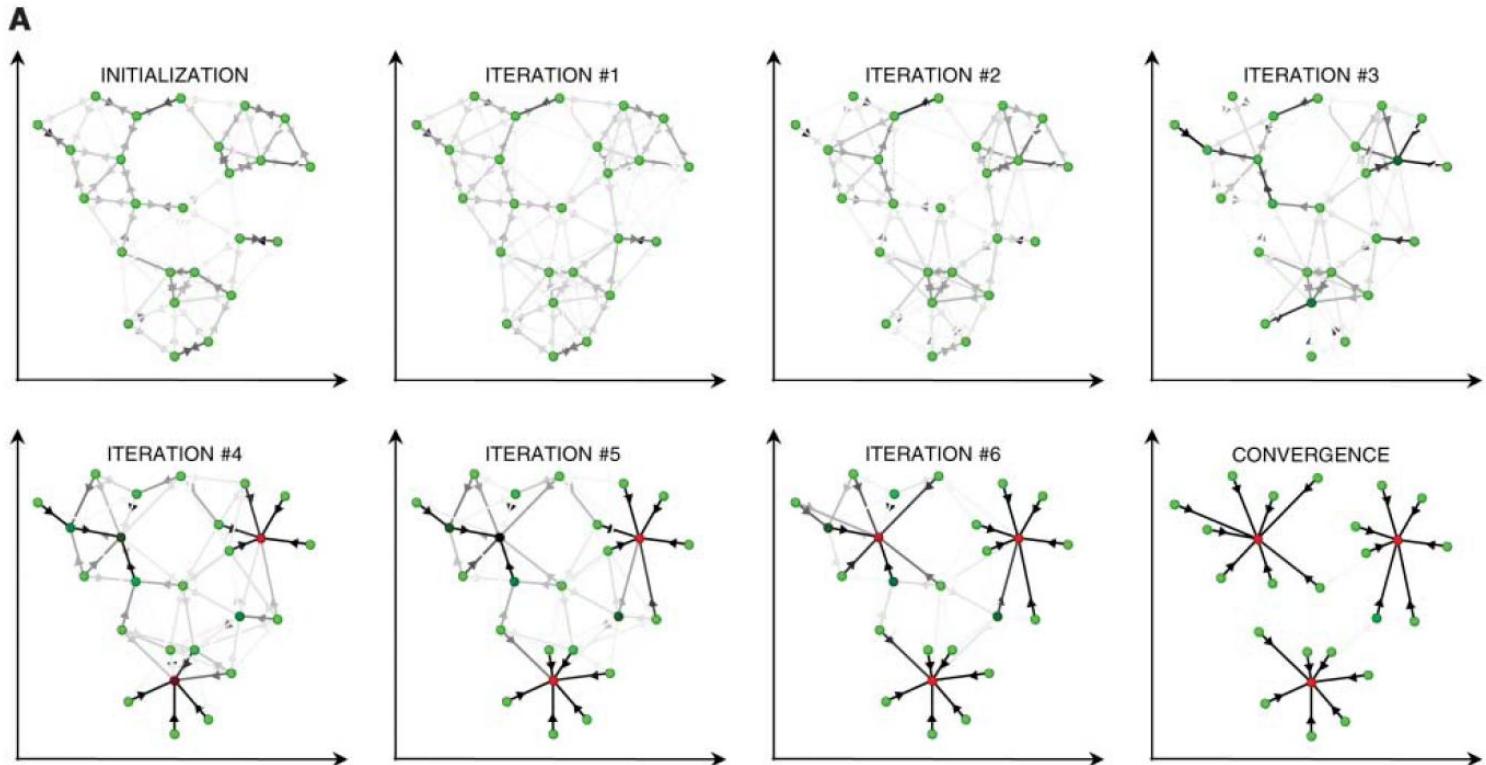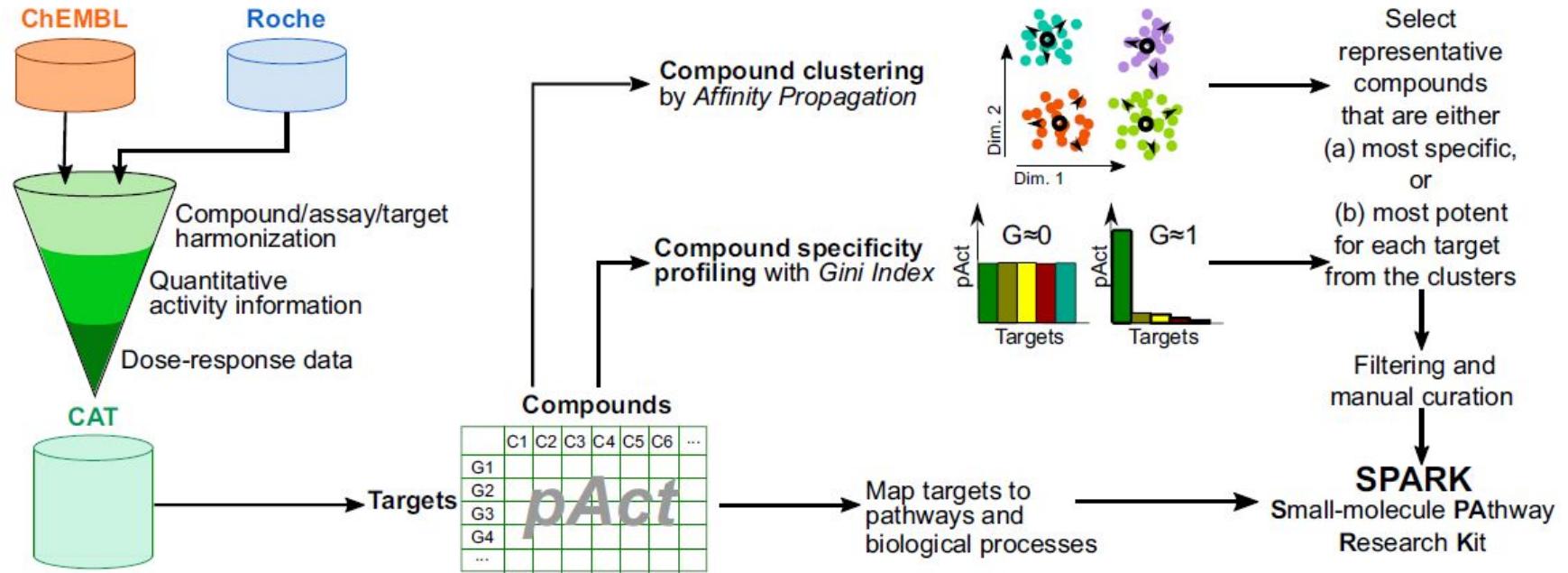
Affinity Propagation updates
responsibilities and
availabilities iteratively

Affinity Propagation in action

[A movie of iterations](#)

Construction of SPARK in detail

Harmonization

… of public and Roche internal data

Machine learning

… to select compounds

Pathways
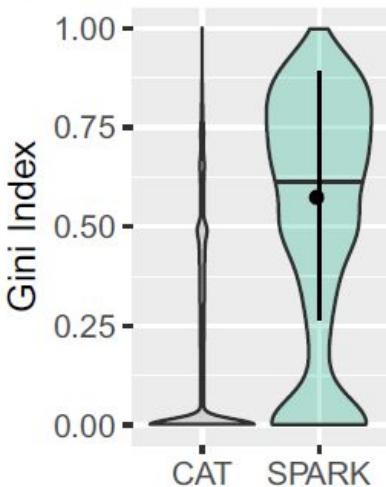
… mapped to compounds

Curation
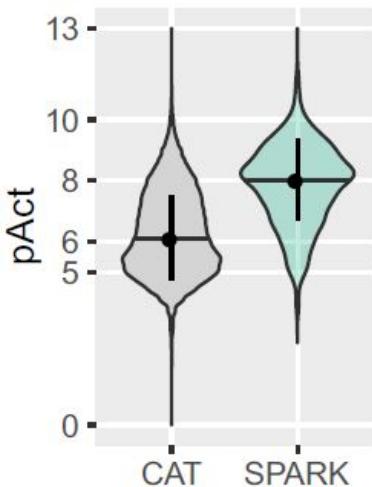
… to enrich quality compounds

SPARK covers the chemical space evenly with representative, potent, and specific compounds
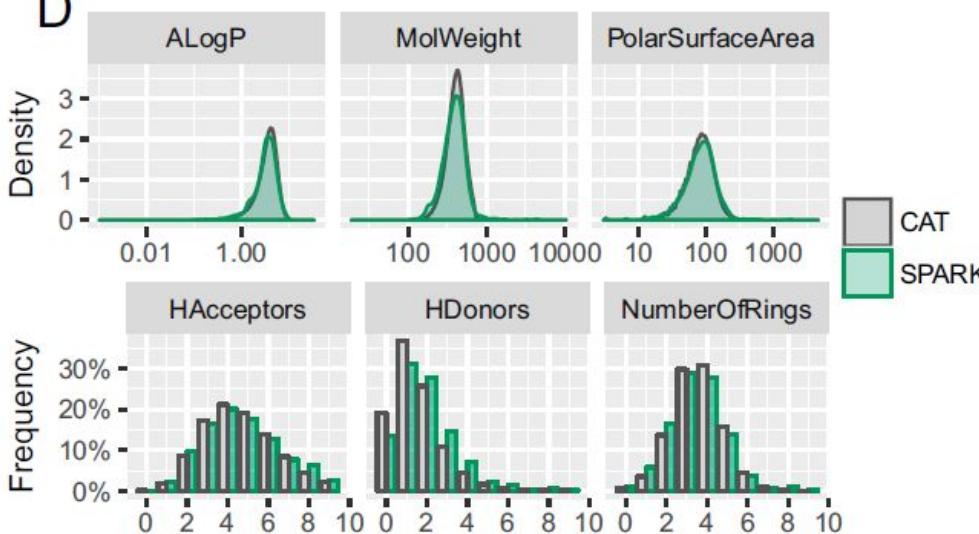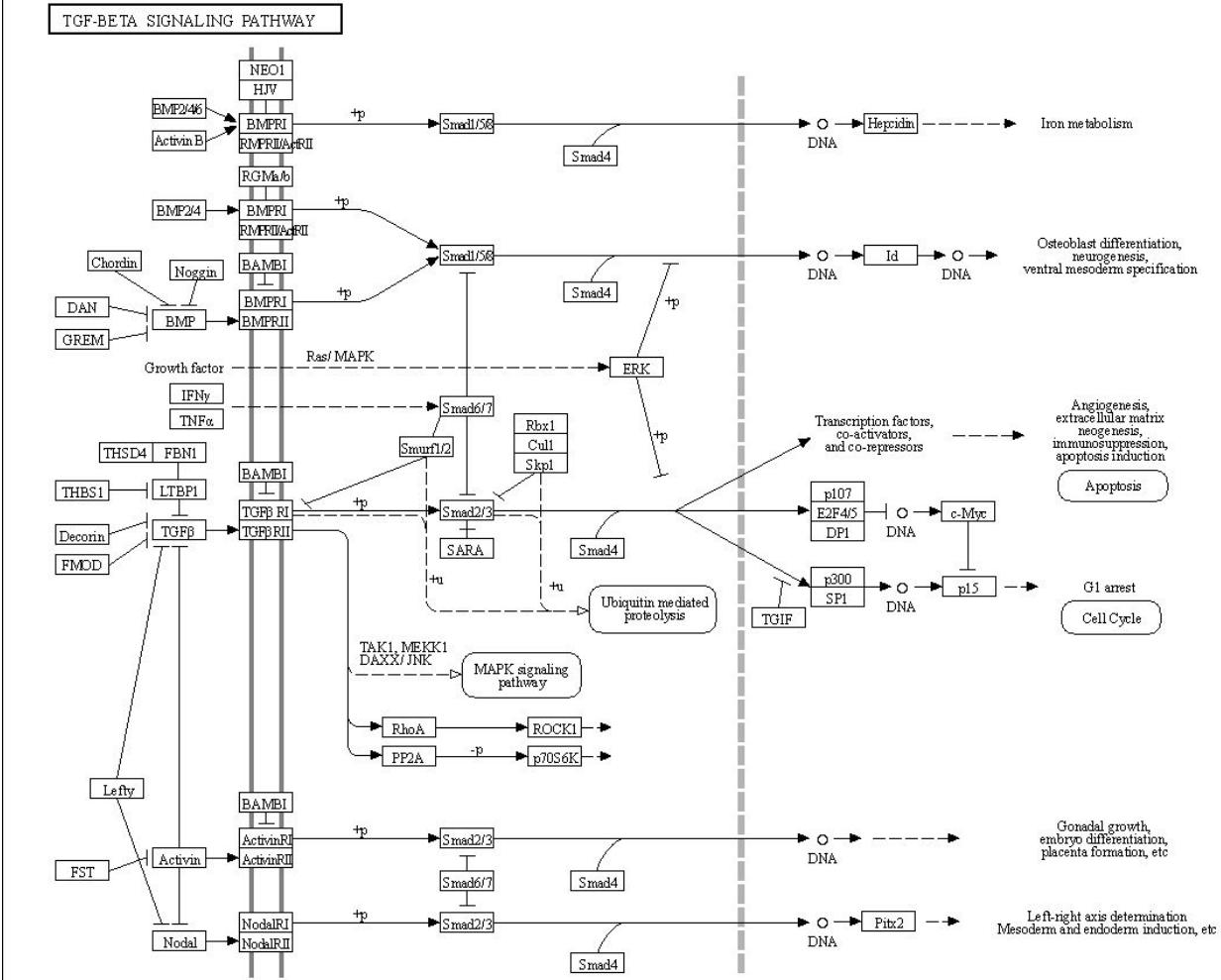
Mapping genes to biological pathways

Option 1: KEGG pathways, with the example of TGF-β signaling pathway.
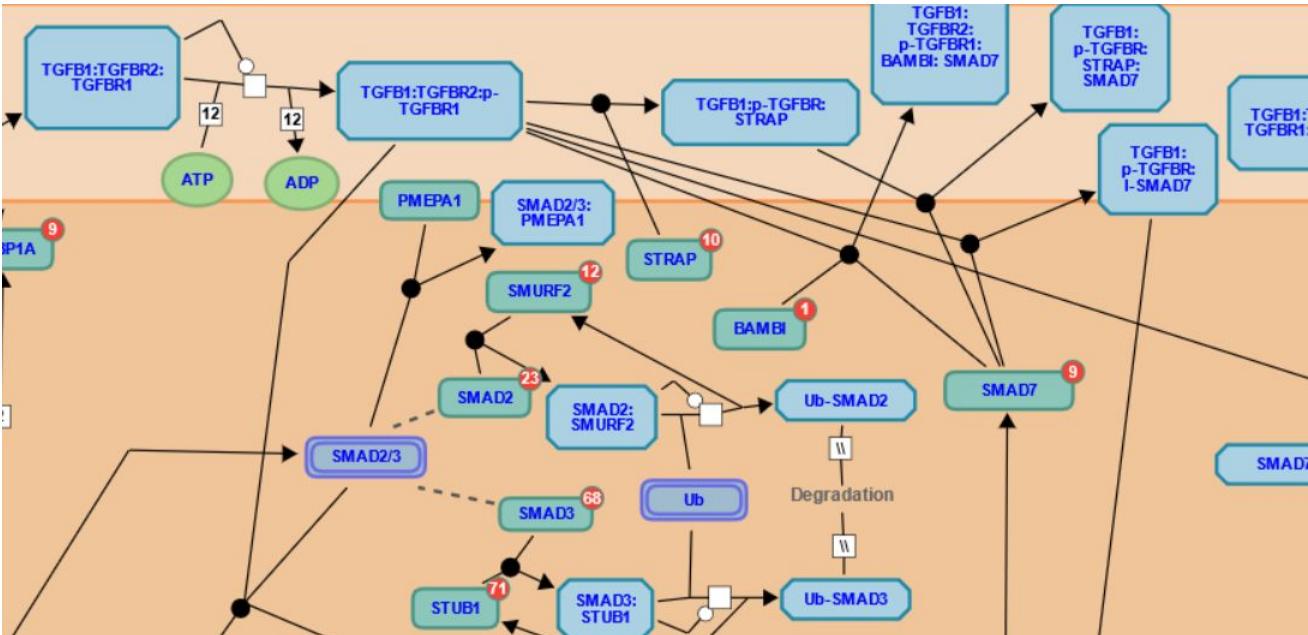
A RESTful API is available for academic use, with clients in Python and R.
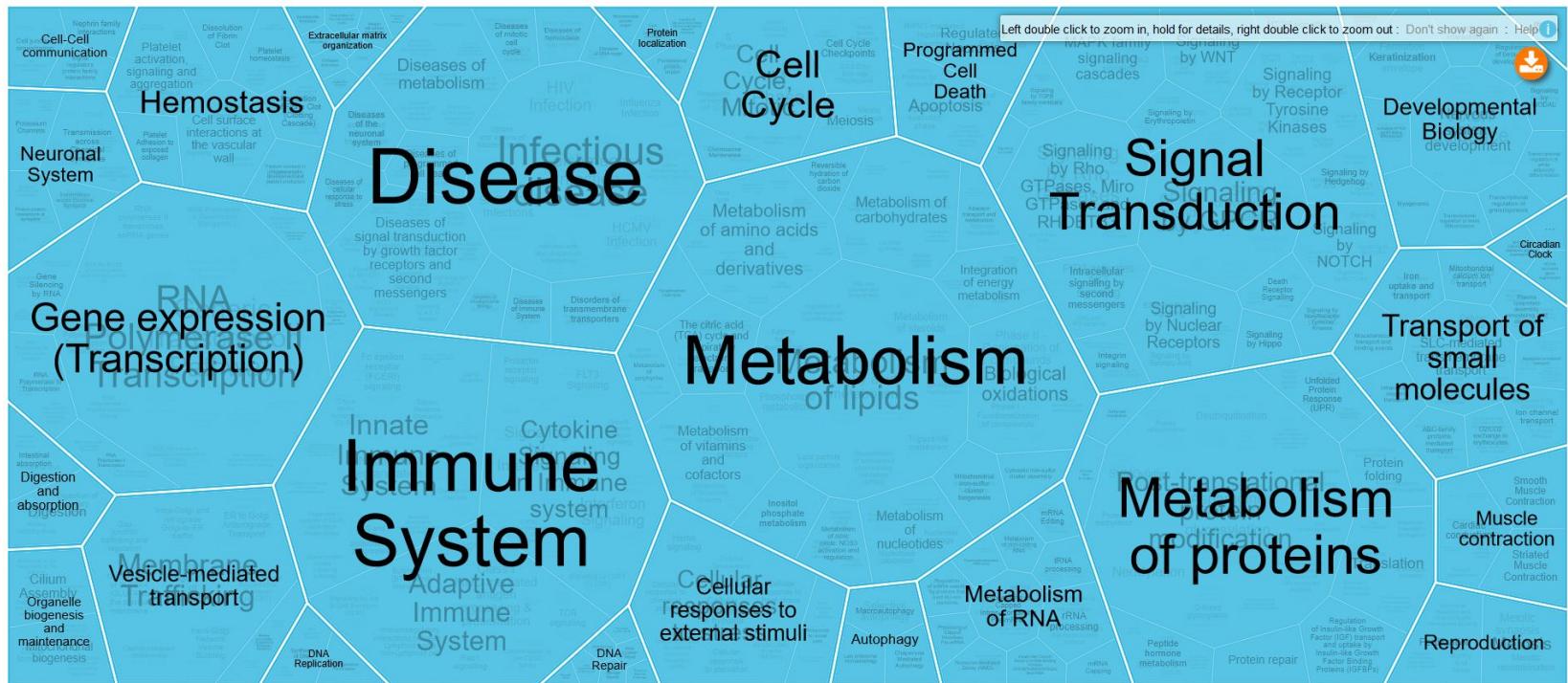
Mapping genes to biological pathways

Option 2: Reactome pathways, with the example of the TGF-β signaling pathway.

Developer's Zone provides API and graph database interfaces.

Overview of pathways captured by Reactome
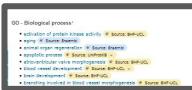
The Voronoi (Reacfoam) view of all pathways in Reactome

Mapping genes to biological processes

- [Gene Ontology](#)
- [UniProtKB keywords](#)
- Example:
  [TGFBR2_HUMAN](#)
  (TGF-beta receptor type
  -2, P37173)

GO - Biological process [i]

- activation of protein kinase activity — Source: BHF-UCL
- aging — Source: Ensembl
- animal organ regeneration — Source: Ensembl
- apoptotic process — Source: UniProtKB
- atrioventricular valve morphogenesis — Source: BHF-UCL
- blood vessel development — Source: BHF-UCL
- brain development — Source: BHF-UCL
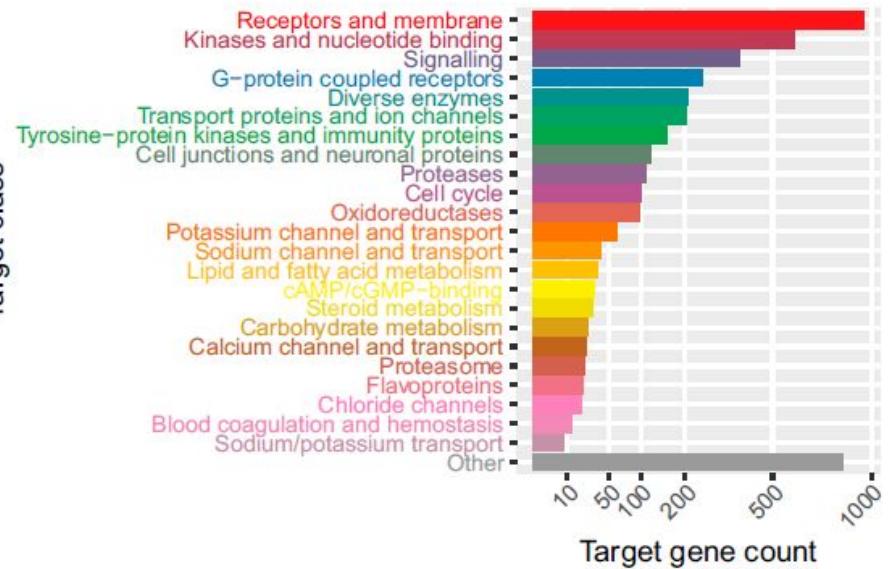
Keywords [i]
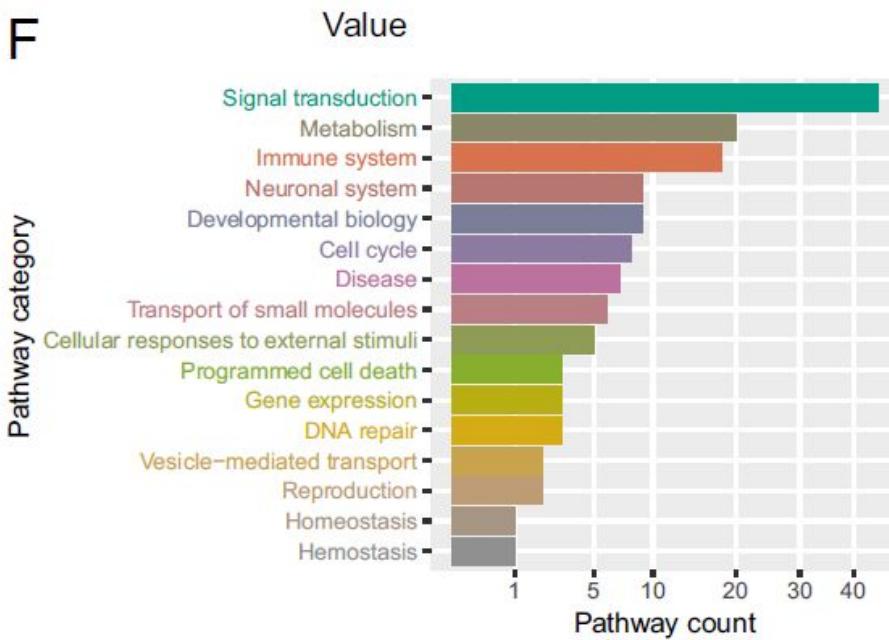
Molecular function	Kinase, Receptor, Serine/threonine-protein kinase, Transferase
Biological process	Apoptosis, Differentiation, Growth regulation
Ligand	ATP-binding, Magnesium, Manganese, Metal-binding, Nucleotide-binding

SPARK covers the target space evenly with representative, potent, and specific compounds
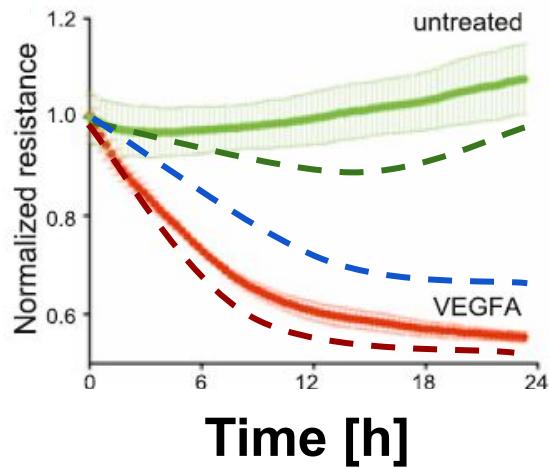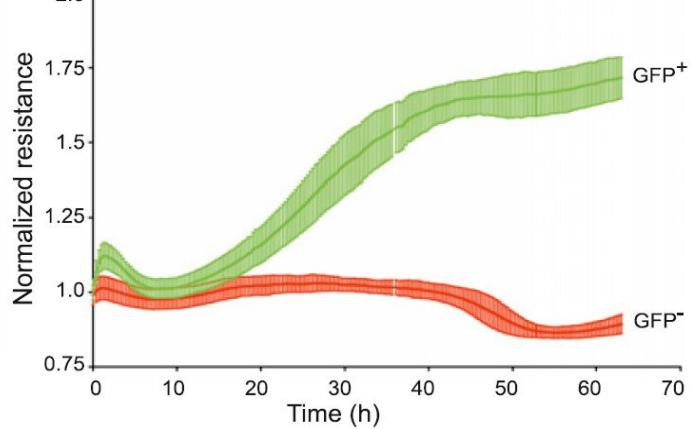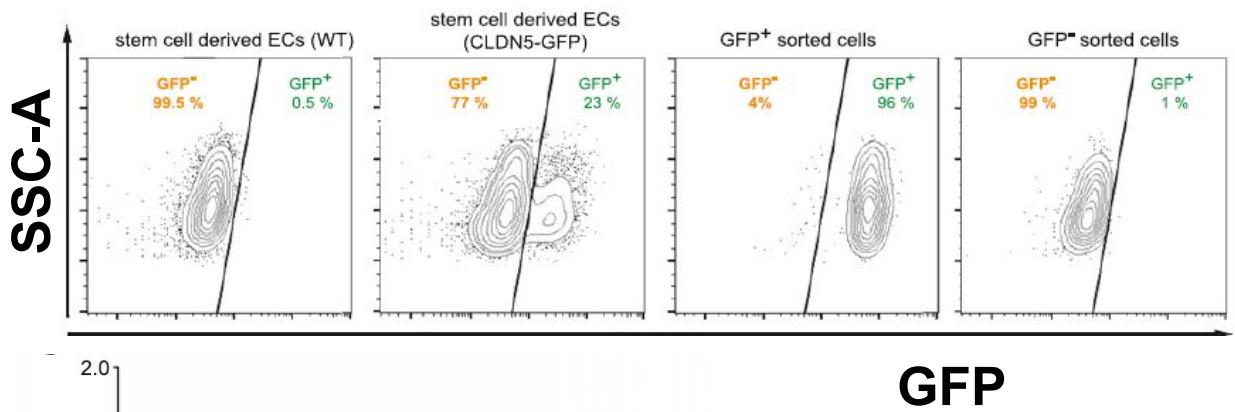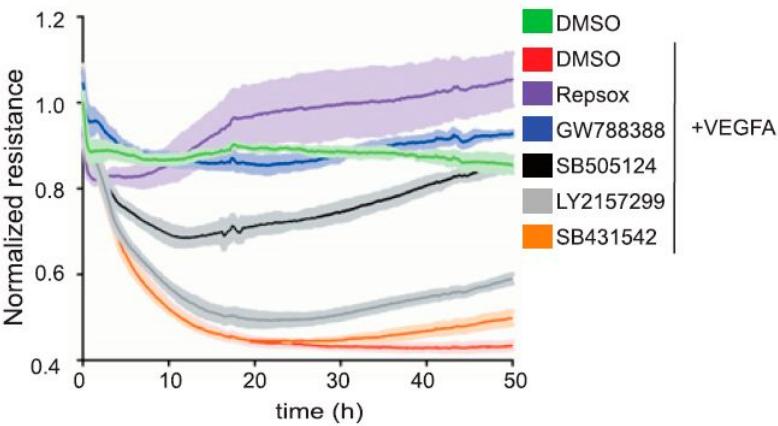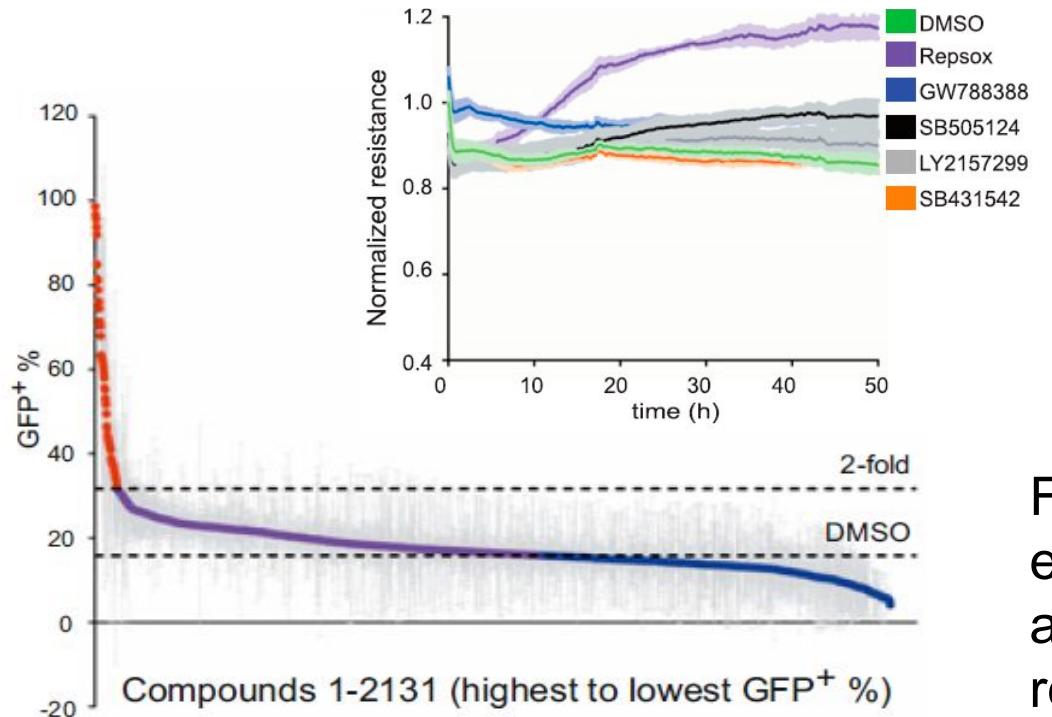
Screening with stem-cell-derived endothelial cells with a reporter added by genome editing

SSC-A: Side-scatter area of flow cytometry;

GFP: Green fluorescent protein;

VEGFA: vascular endothelial growth factor A

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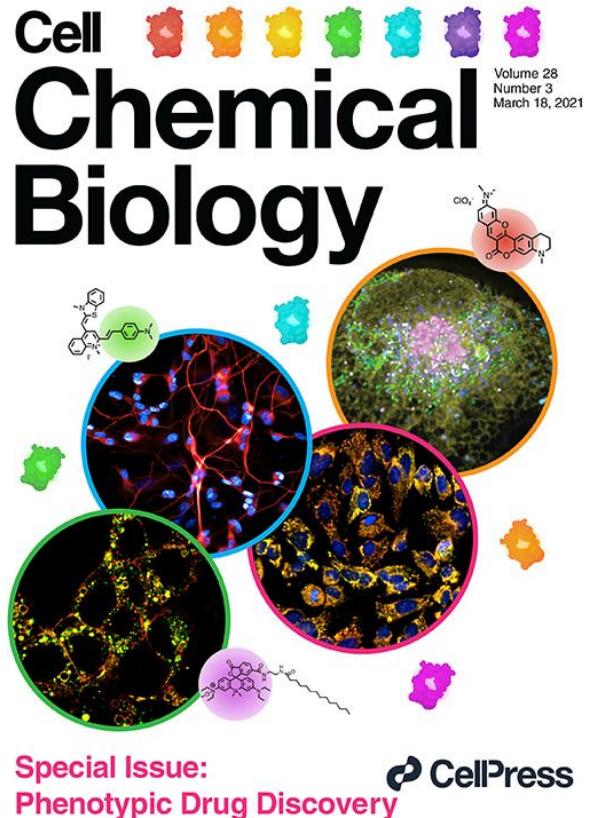
Compounds targeting the TGF-β pathway such as RepSox modulates endothelial cells

Further *in vitro* and *in vivo* experiments establish RepSox as a tool compound modulating retinopathy.

Conclusions about chemogenomic library

- Phenotypic drug discovery can lead to first-in-class drugs with novel mechanisms;
- Unsupervised machine learning and data modelling contribute to build chemogenomic libraries;
- We can link drug candidates via targets to biological pathways and processes.

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Special Issue:
Phenotypic Drug Discovery

CellPress

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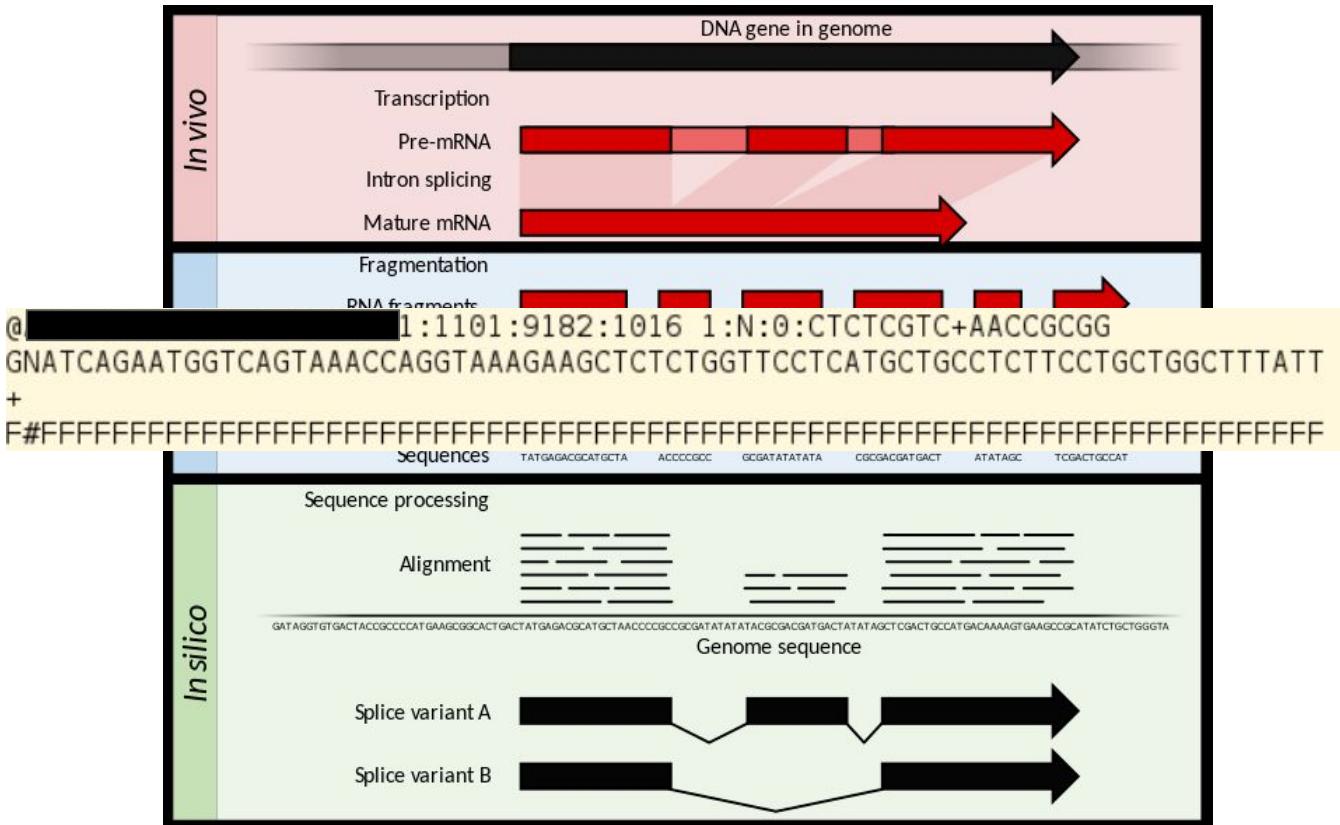
Offline activities of Module II
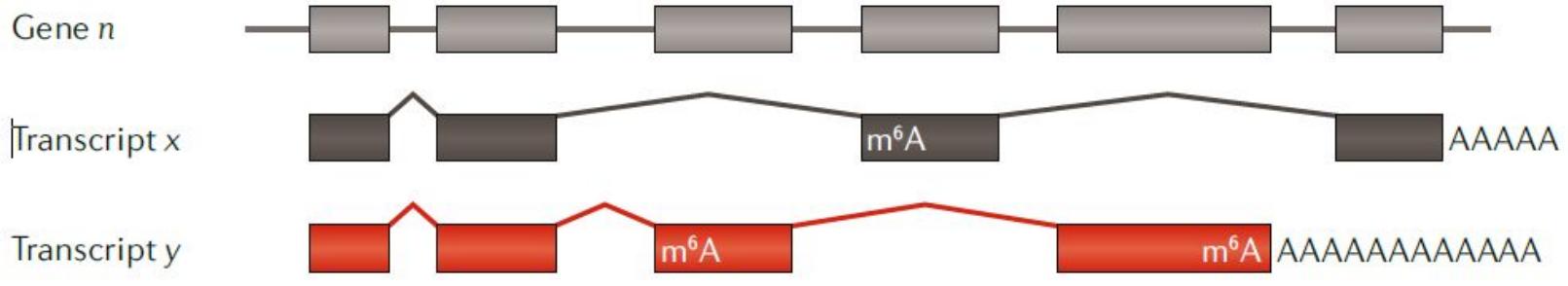
Please use your favourite programming language (shell scripts, python, R, for instance) and APIs (Application Programming Interfaces) of databases to perform following operations. Submit your code.

1. Retrieve all approved drugs from the ChEMBL database, sort them by approval year and name (a Python example is here; documentations of the ChEMBL API can be found here);
2. For each approved drug **since 2011** that you identified in step (1), retrieve a list of UniProt accession numbers, namely protein targets associated with the drug;
3. For each protein with a UniProt accession number that you identified in step (2), retrieve UniProt keywords associated with it. You can use the UniProt API, documented here. Python and R clients are also available.

Transcriptome profiling by RNA sequencing

Transcriptome profiling by RNA sequencing

Differential gene expression

UNI BASEL

Read Mapping

gene 1   gene 2
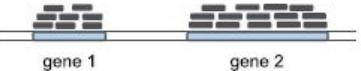
Count collection
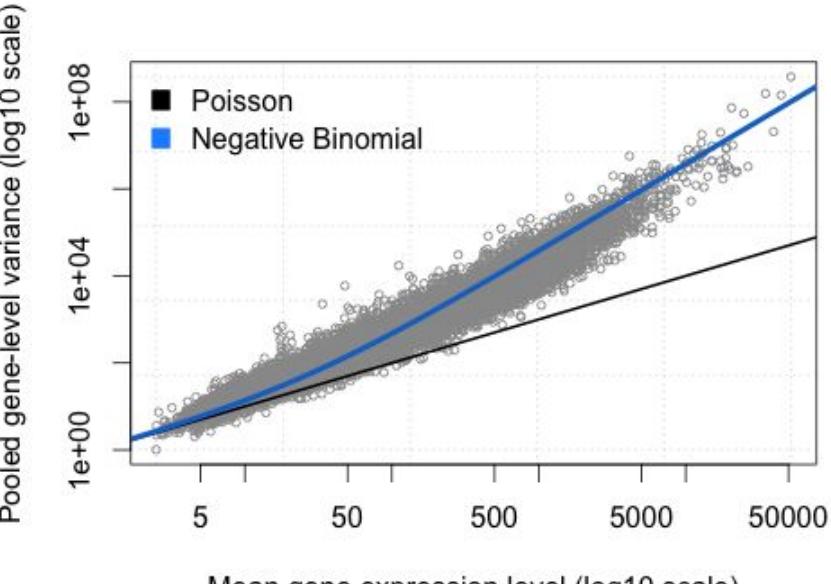
	sample A1	sample A2	sample B1	sample B2
gene 1	8	10	100	200
gene 2	14	15	15	40
gene 3	33	40	35	70
...	...	...	...	...
gene N	100	120	105	220

Normalization by library size

	sample A1	sample A2	sample B1	sample B2
gene 1	8	10	100	200
gene 2	14	15	115	40
gene 3	33	40	35	70
...	...	...	...	...
gene N	100	120	105	220

Tot. reads: 5 millions   Tot. reads: 10 millions
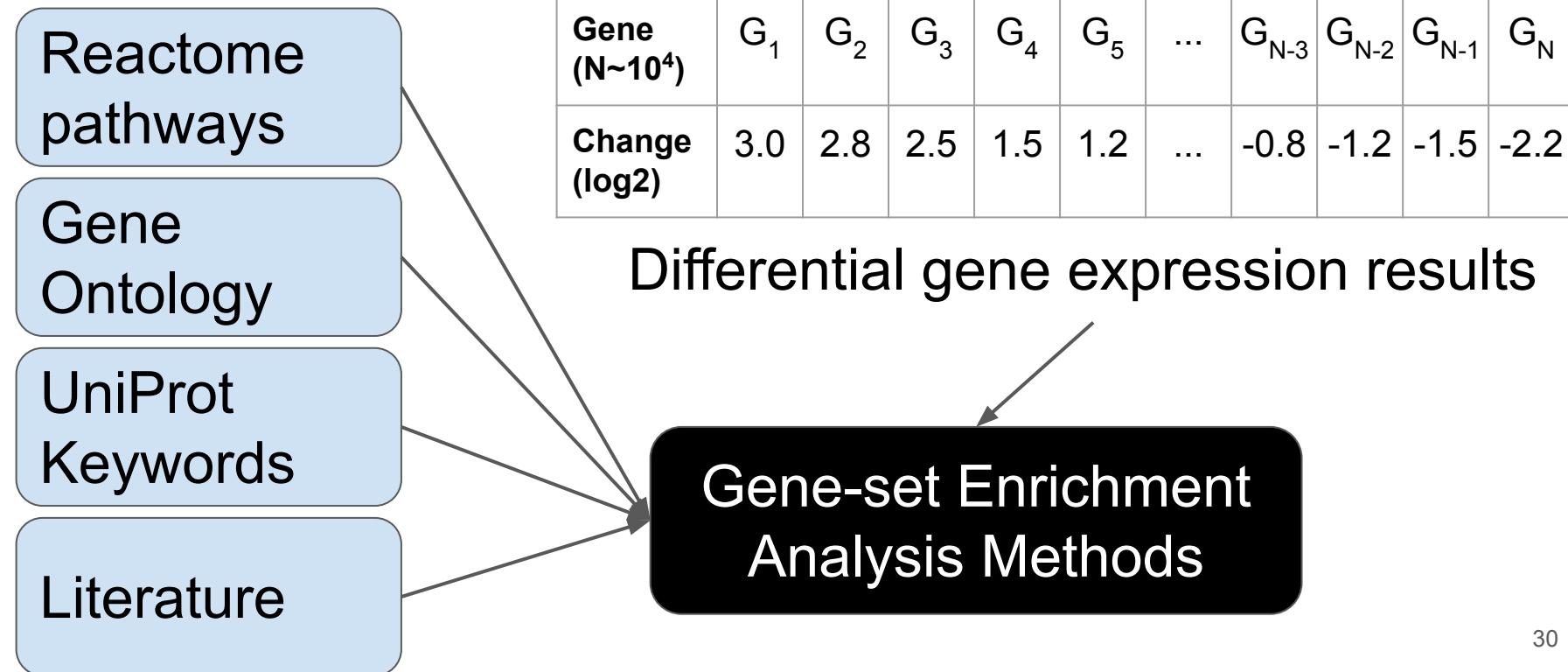
Differential Gene Expression Analysis
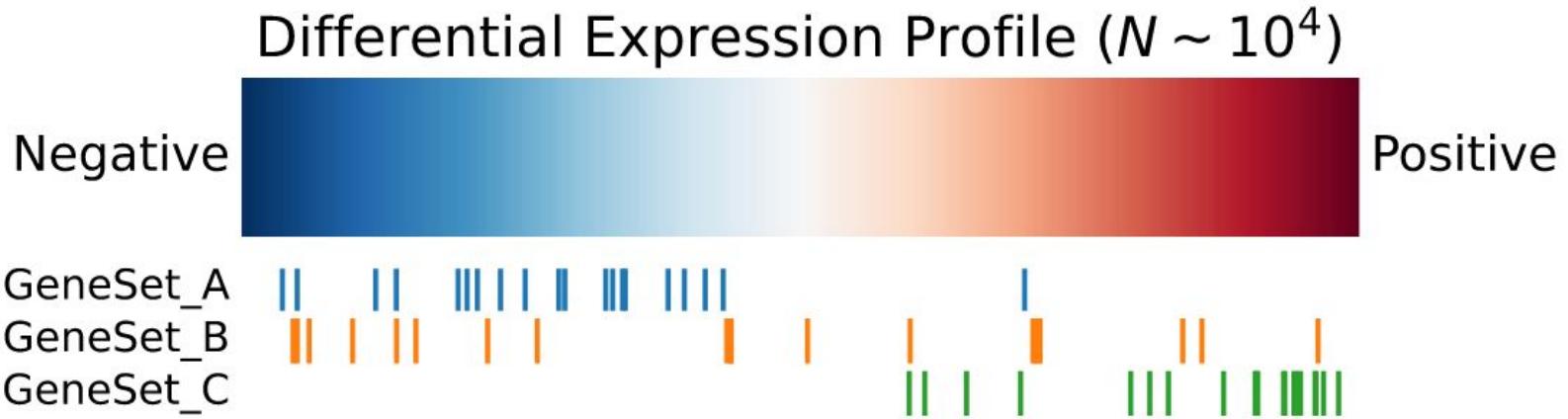
	sample A1	sample A2	sample B1	sample B2
gene 1	0.16	0.20	2.00	2.00
gene 2	0.28	0.30	0.30	0.40
gene 3	0.66	0.80	0.70	0.70
...	...	...	...	...
gene N	2.00	2.40	2.10	2.20

Mean gene expression level (log10 scale)

Tools: *edgeR* and *DESeq2*

Interpret differential gene expression data with gene-set enrichment analysis

Gene (N~10^4)	G_1	G_2	G_3	G_4	G_5	...	G_{N-3}	G_{N-2}	G_{N-1}	G_N
Change (log2)	3.0	2.8	2.5	1.5	1.2	...	-0.8	-1.2	-1.5	-2.2

Reactome pathways

Gene Ontology

UniProt Keywords

Literature

Differential gene expression results

Gene-set Enrichment Analysis Methods

Gene-set enrichment analysis

Differential Expression Profile ($N \sim 10^4$)

Negative ████████████████ Positive
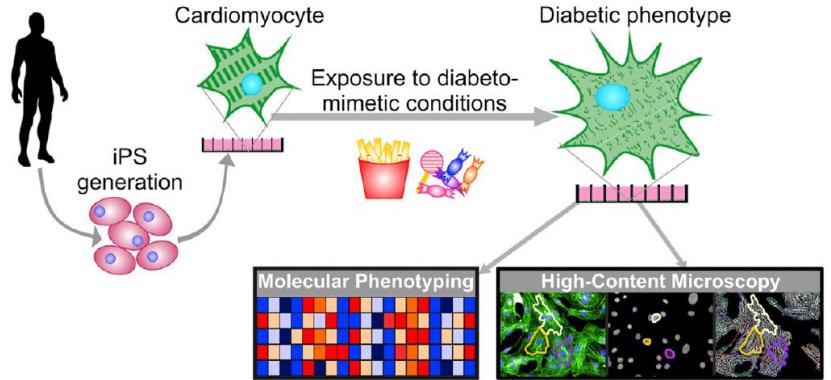
GeneSet_A
GeneSet_B
GeneSet_C
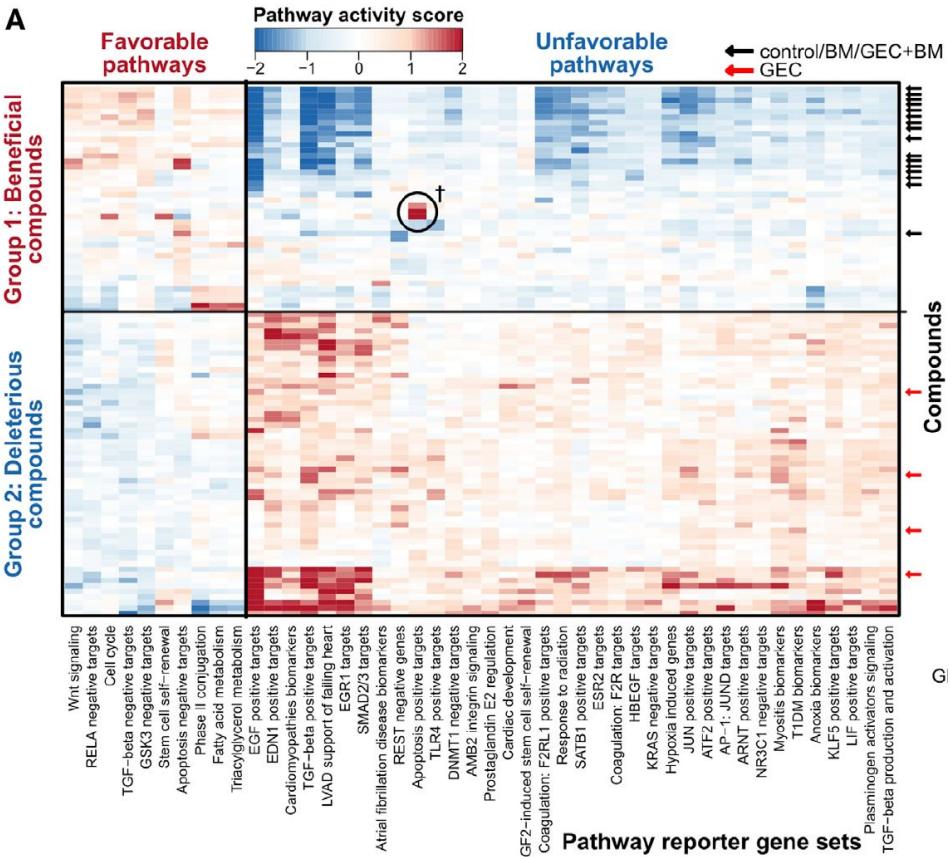
Input: (1) a differential gene expression profile; (2) a set of gene-sets {G}, each a set of genes.

Output: a ranked list of the input gene-sets by *enrichment*.
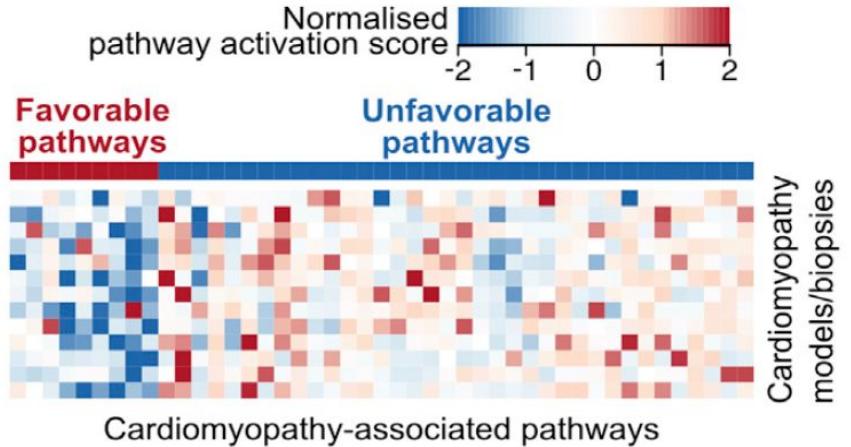
Gene expression as screening readout
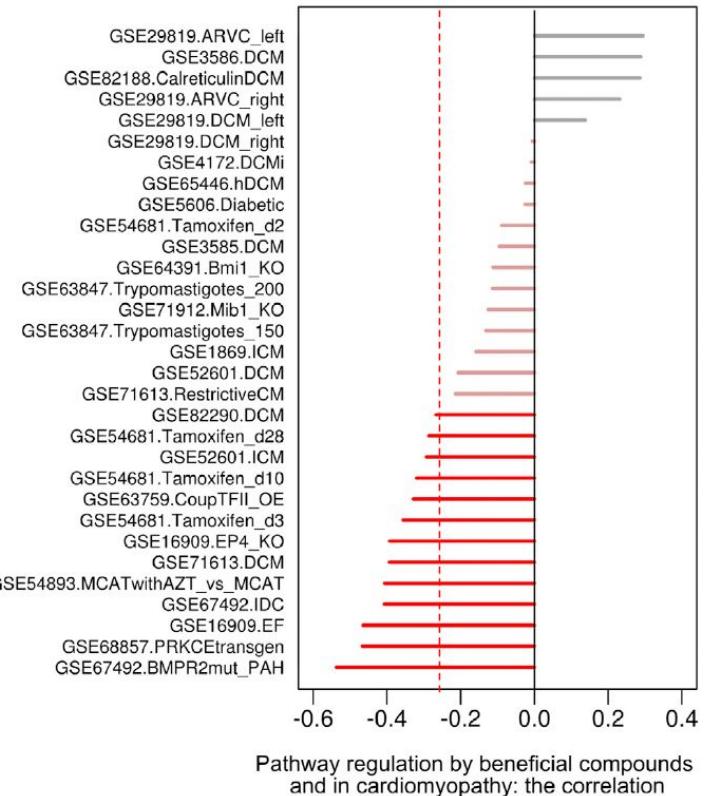
Differential gene expression profiles are molecular snapshots of drugs' action in the cell.
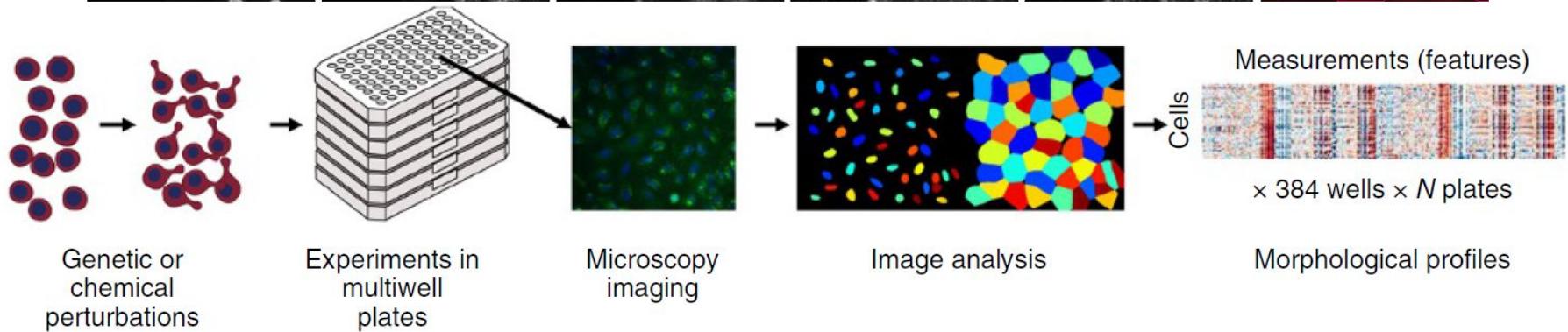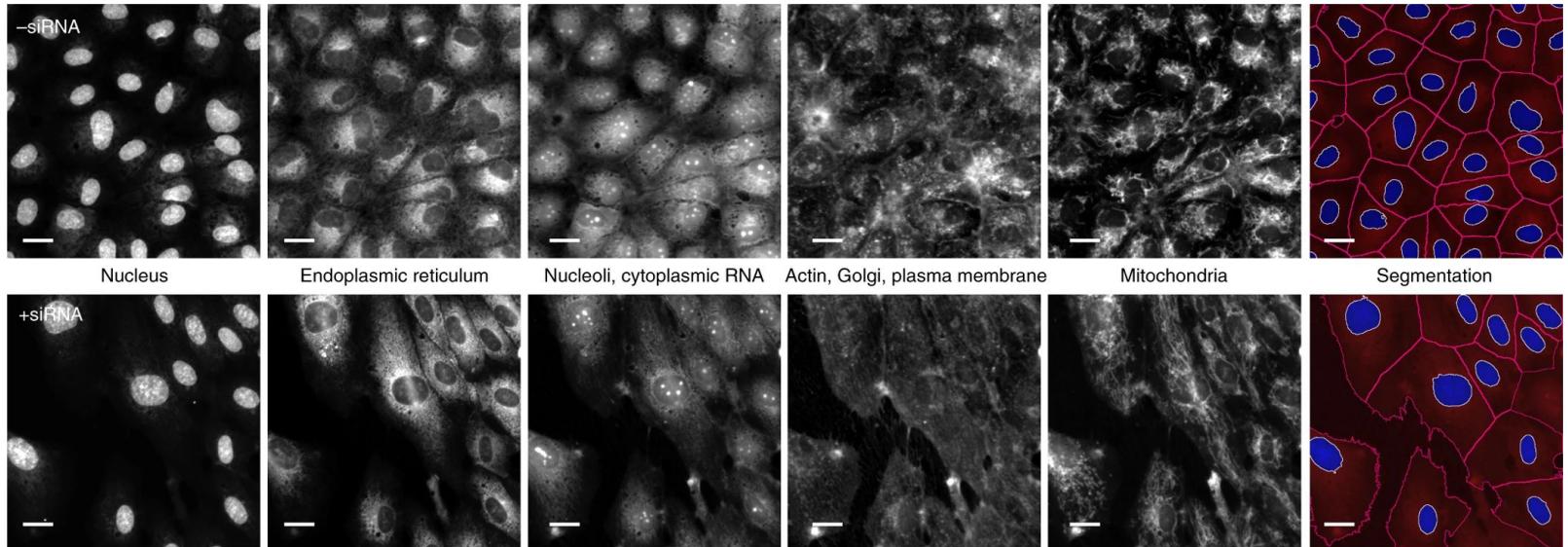
Gene expression from patient and animal models help compound selection

We can prioritise molecules that reverse disease-induced changes.

Pathway regulation by beneficial compounds and in cardiomyopathy: the correlation

Nucleus | Endoplasmic reticulum | Nucleoli, cytoplasmic RNA | Actin, Golgi, plasma membrane | Mitochondria | Segmentation

Measurements (features)

× 384 wells × N plates

Genetic or chemical perturbations | Experiments in multiwell plates | Microscopy imaging | Image analysis | Morphological profiles
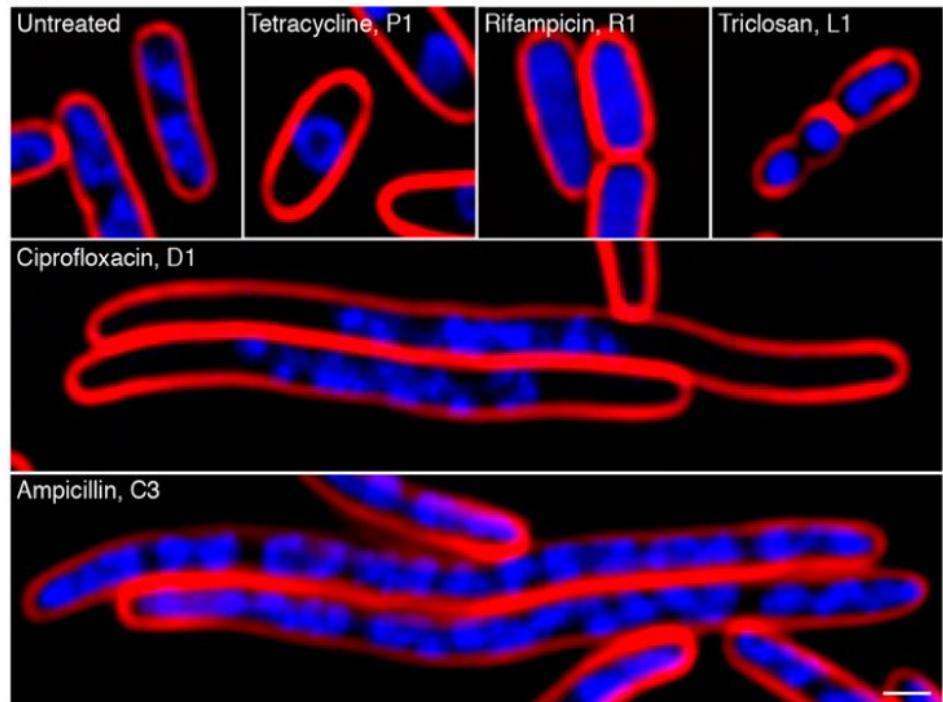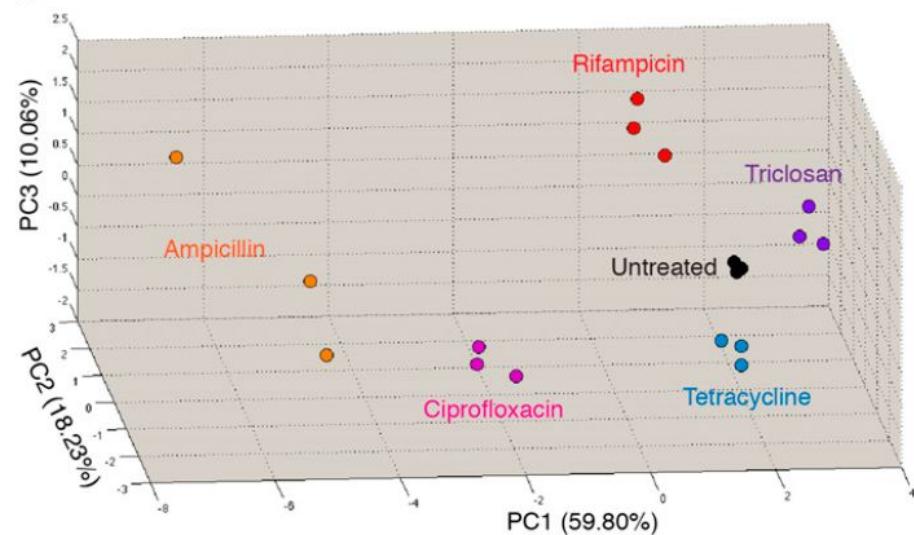
Cytological profiling for antibiotics discovery
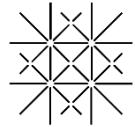
P: Protein translation inhibitors
R: RNA transcription inhibitors
D: DNA replication inhibitors
L: Lipid biosynthesis inhibitors
C: Cell-wall synthesis inhibitors (peptidoglycan)
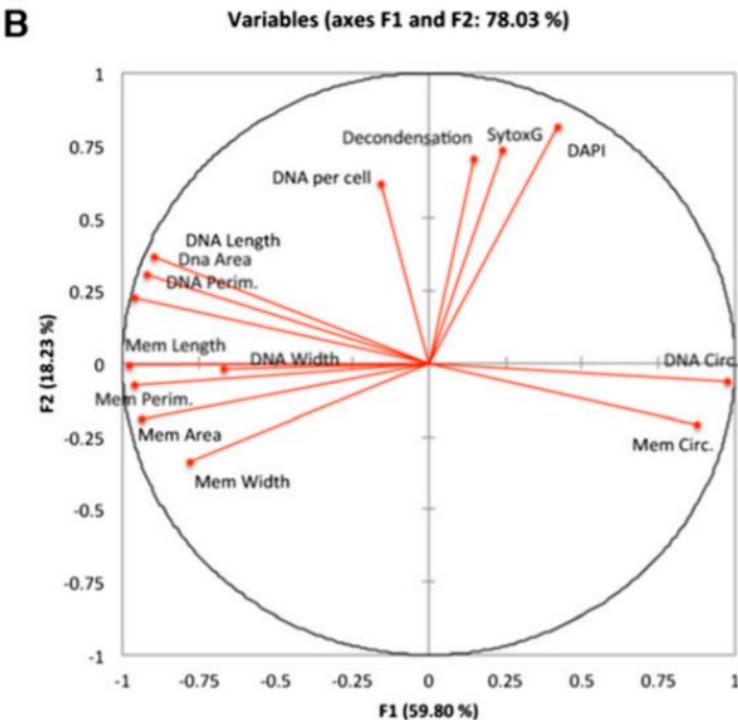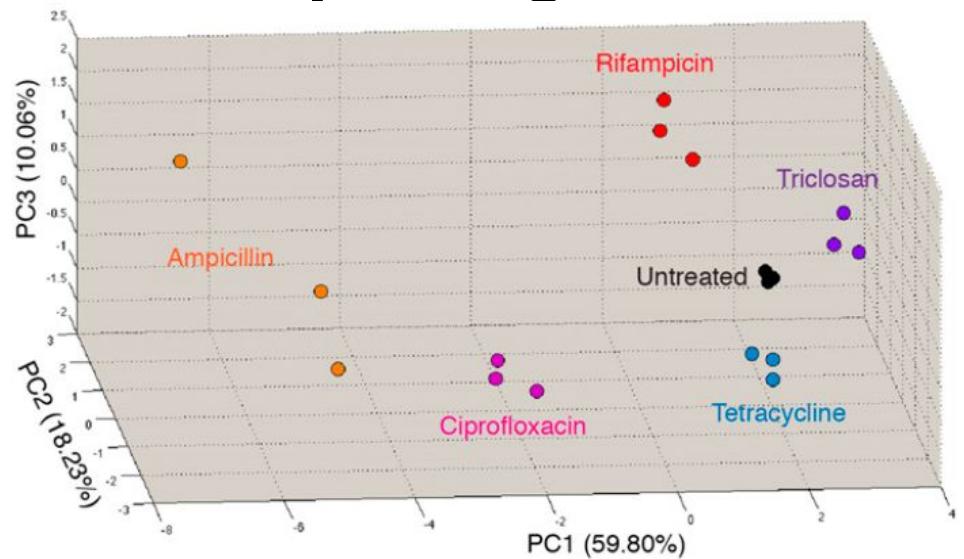
35
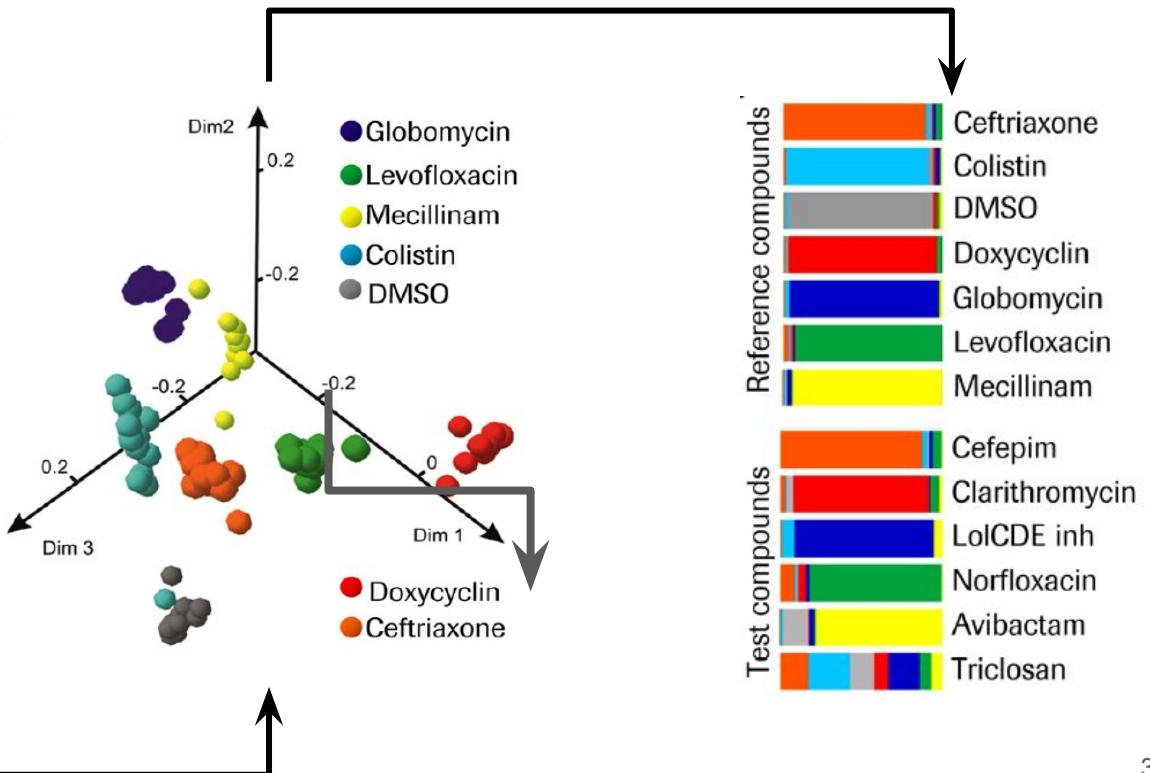
Principal components are linear combination of morphological features
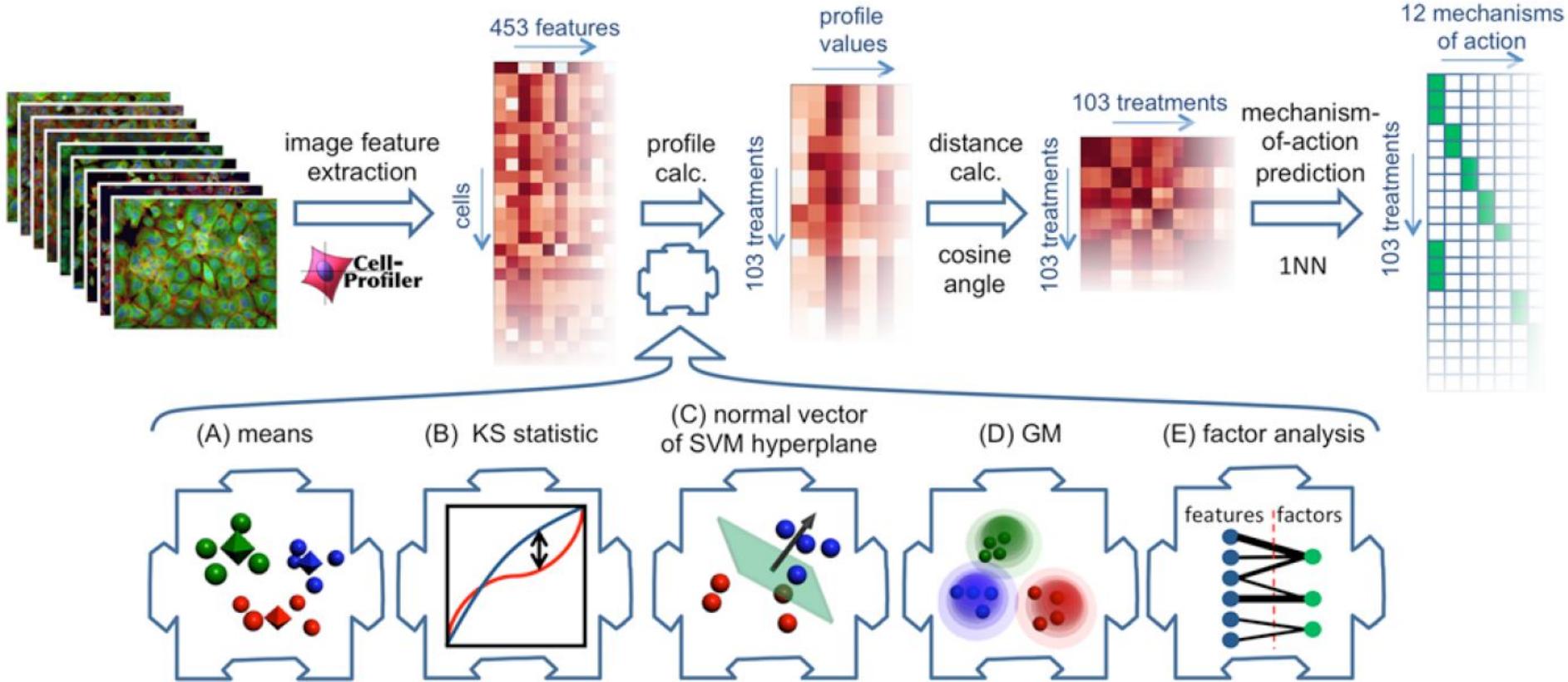
Membrane area, μm^2 DNA area, μm^2 Membrane perimeter, μm DNA perimeter, μm Membrane length, μm DNA length, μm No. of nucleoids per cell

Membrane width, μm DNA width, μm Membrane circularity   DNA circularity   SytoxG intensity   DAPI intensity   Decondensation

Morphology classifies compounds by MoA

Comparison of computational methods

Do the benchmark and use Occam's Razor

Table 1. Accuracies for classifying compound treatments into mechanisms of action.
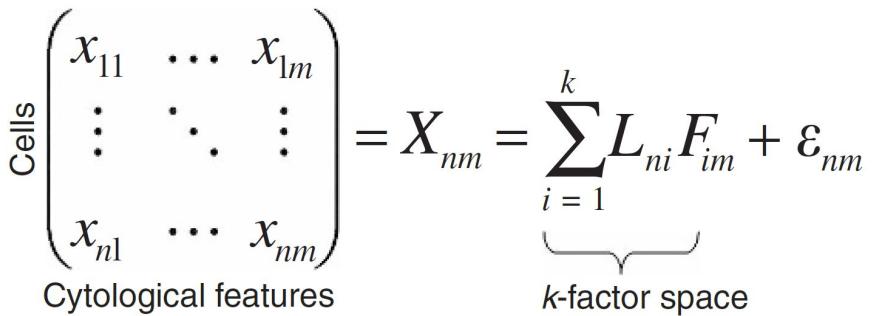
Method	Accuracy, %
Means	83
KS statistic	83
Normal vector to support-vector machine hyperplane	81
With recursive feature elimination	64
Distribution over Gaussian mixture components	83
Factor analysis + means	94

True mechanistic class		Act	Aur	Ch	DD	DR	Eg5	Epi	KI	MD	MS	PD	PS	Acc.
Actin disruptors	Act	4											1	80 %
Aurora kinase inhibitors	Aur		12											100 %
Cholesterol-lowering	Ch			6										100 %
DNA damage	DD				8									93 %
DNA replication	DR				1	7								81 %
Eg5 inhibitors	Eg5						12							100 %
Epithelial	Epi		2					6						76 %
Kinase inhibitors	KI								5					100 %
Microtubule destabilizers	MD					1				13				93 %
Microtubule stabilizers	MS										9			97 %
Protein degradation	PD											7		96 %
Protein synthesis	PS												8	100 %
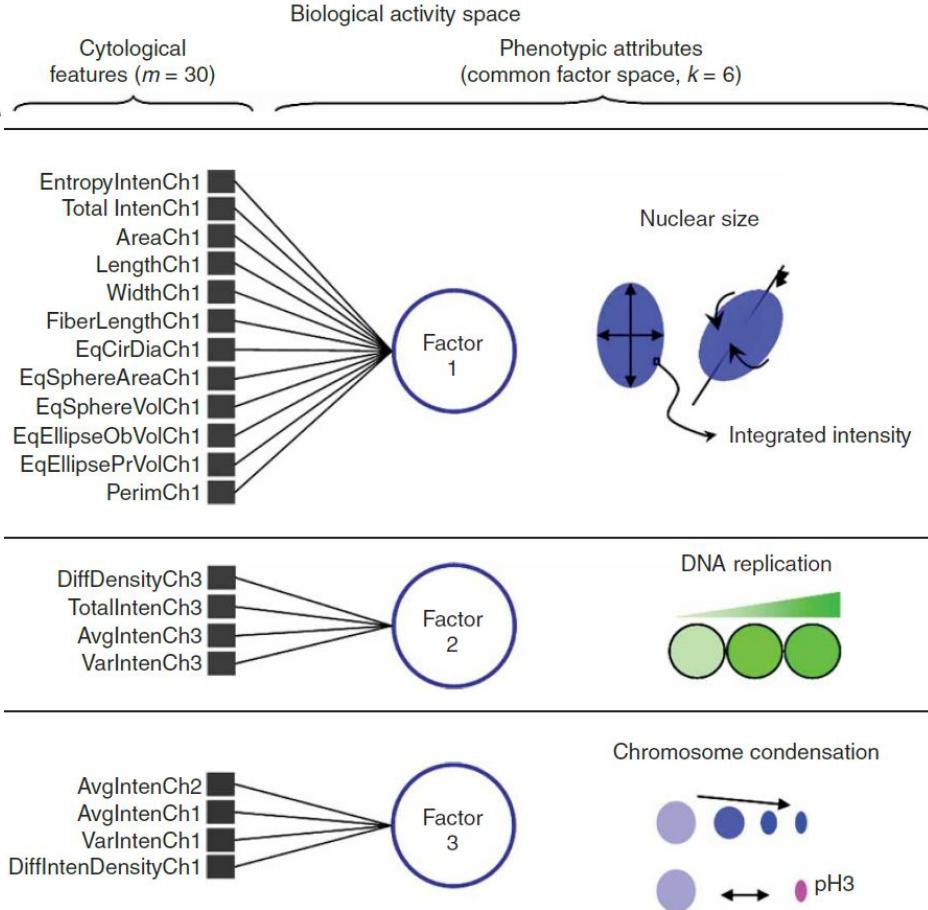
Overall accuracy: **94 %**
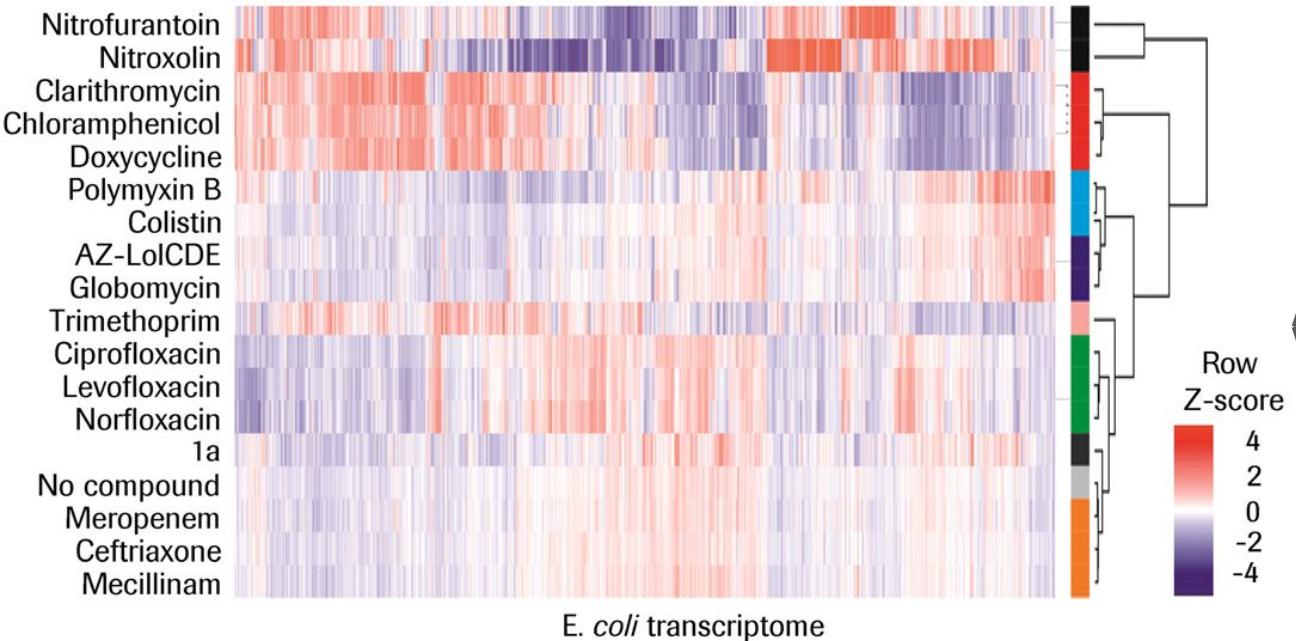
A possible explanation for the success of latent variable models

$$\text{Cells} \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix} = X_{nm} = \sum_{i=1}^{k} L_{ni} F_{im} + \varepsilon_{nm}$$

Cytological features

k-factor space

A common latent factor model

Morphology and gene expression used jointly

Nitrofurantoin
Nitroxolin
Clarithromycin
Chloramphenicol
Doxycycline
Polymyxin B
Colistin
AZ-LolCDE
Globomycin
Trimethoprim
Ciprofloxacin
Levofloxacin
Norfloxacin
1a
No compound
Meropenem
Ceftriaxone
Mecillinam

E. *coli* transcriptome

Row Z-score
4
2
0
-2
-4
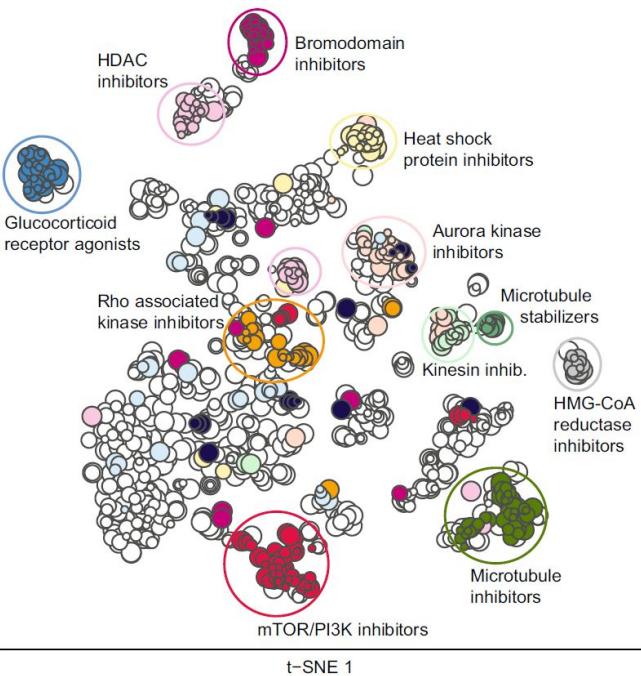
Gene-set enrichment analysis

Reporter assays
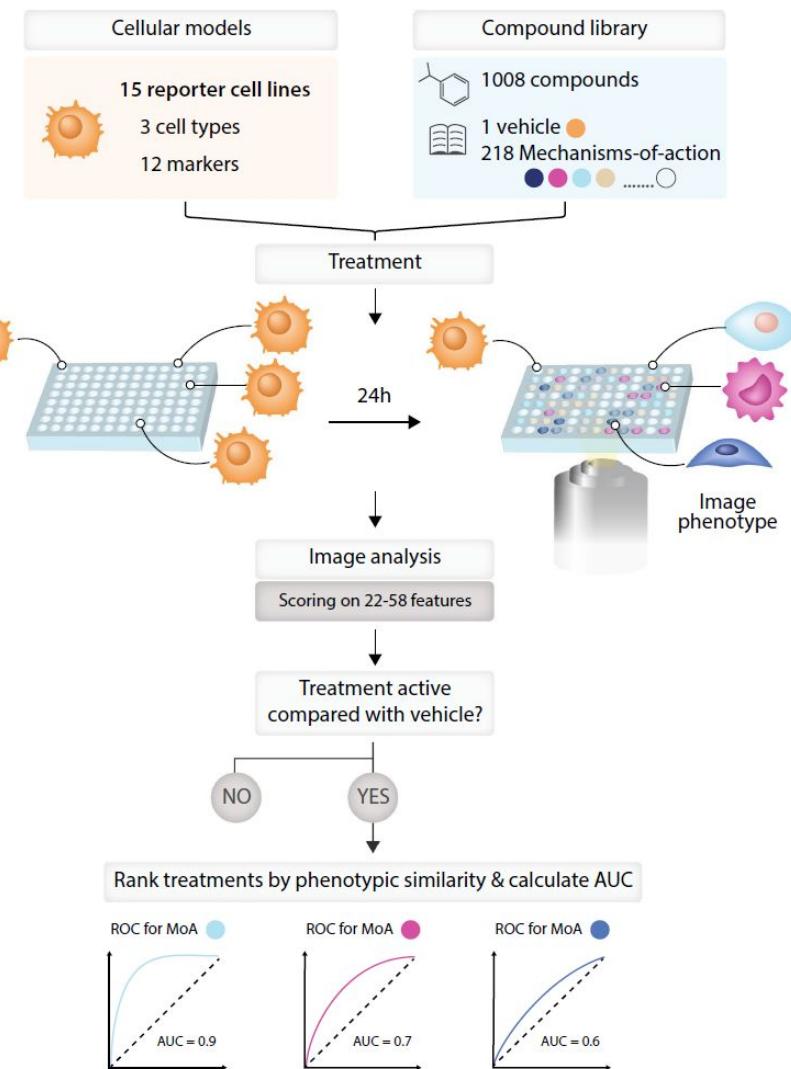
Pathway-Phenotype associations

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A multi-cell-type, 1008-compound screening by Cox *et al.* (2020)

Conclusions

- Gene expression and image-based profiling can be used individually or jointly for phenotypic screening;

- Integration of biological knowledge, high-throughput data, and statistical modelling empowers phenotypic drug discovery.

References

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