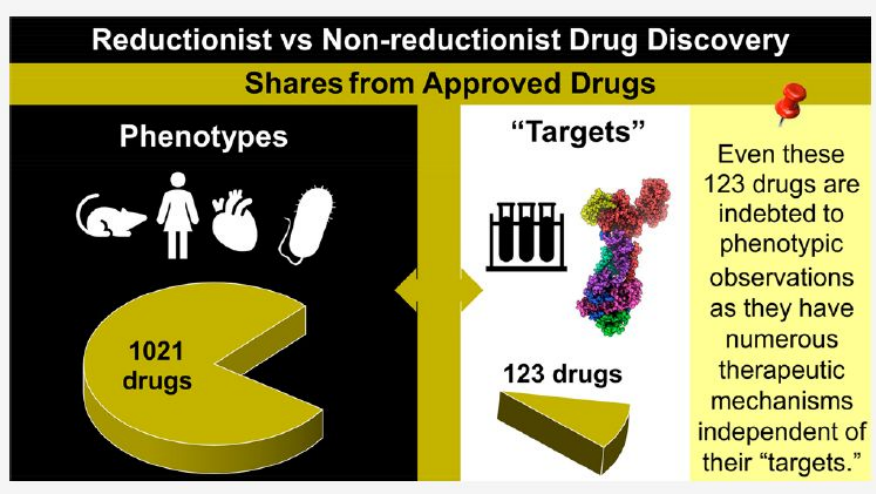
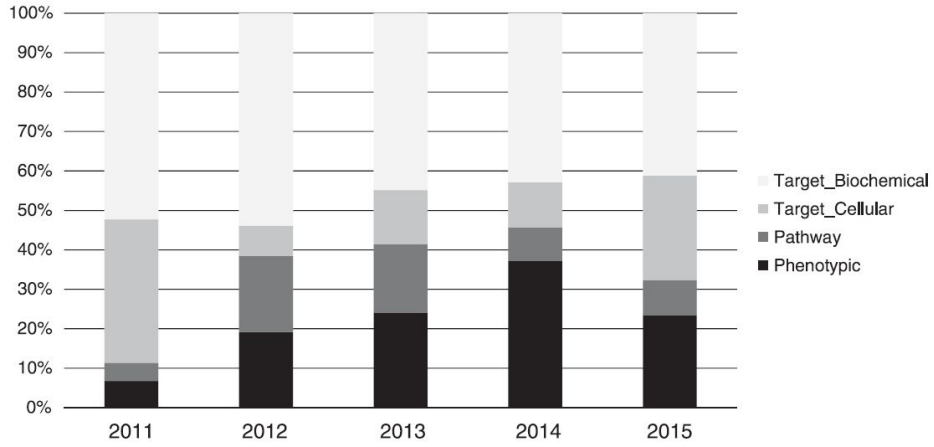
What can we do if there are no good targets

Mathematical and Computational Biology in Drug Discovery Module II

Dr. Jitao David Zhang
March-April 2025

Is target-based drug discovery the only way?



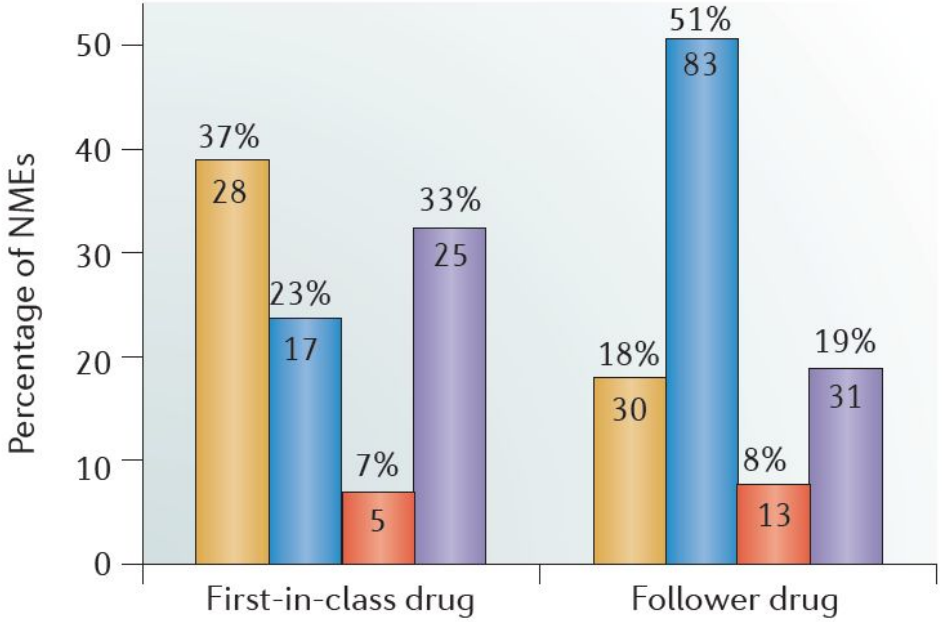


Sadri, Arash. 2023. "Is Target-Based Drug Discovery Efficient? Discovery and 'Off-Target' Mechanisms of All Drugs." Journal of Medicinal Chemistry 66 (18): 12651–77.

Haasen, Dorothea, Ulrich Schopfer, Christophe Antczak, Chantale Guy, Florian Fuchs, and Paul Selzer. 2017. "How Phenotypic Screening Influenced Drug Discovery: Lessons from Five Years of Practice." ASSAY and Drug Development Technologies 15 (6): 239–46.

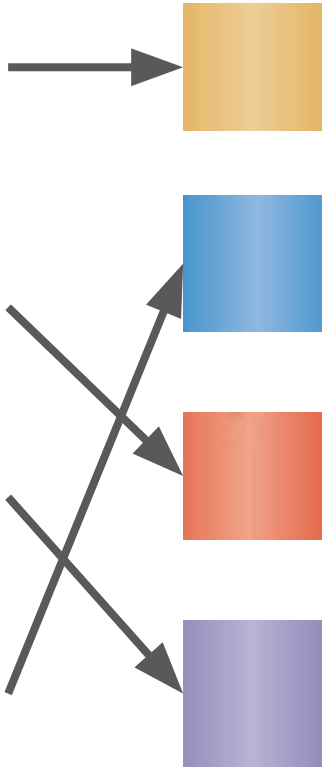
Five strategies when no good target is found

1. Phenotypic drug discovery
2. Natural products
3. Biologics
4. Interaction-based (multispecific) drug discovery
5. Drug repurposing or combination studies

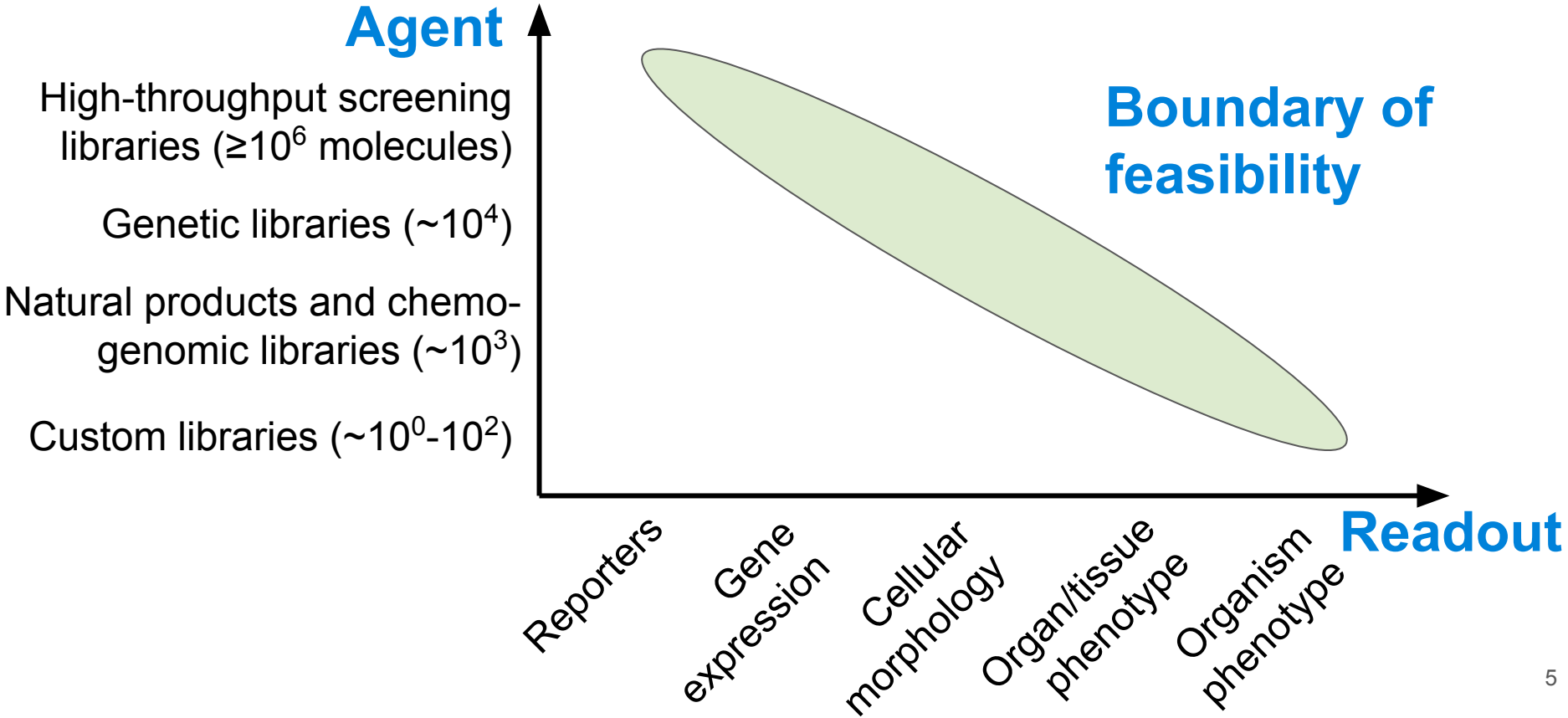


Connect the lines!

- Phenotypic screening
- Modified natural products
- Biologics
- Target-based screening

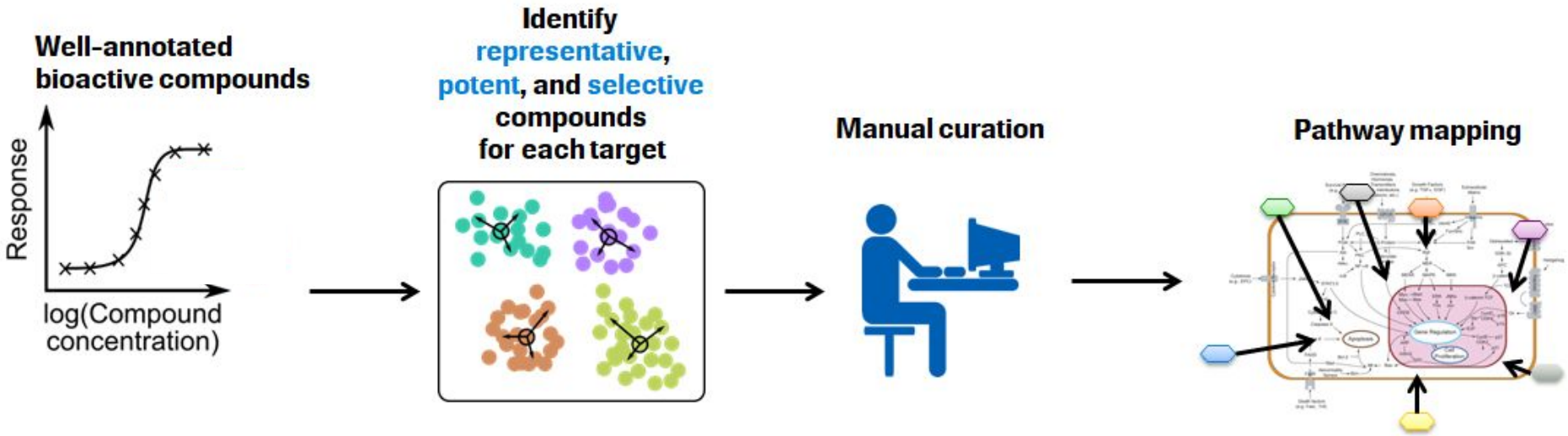


Phenotypic screenings by agent and readout



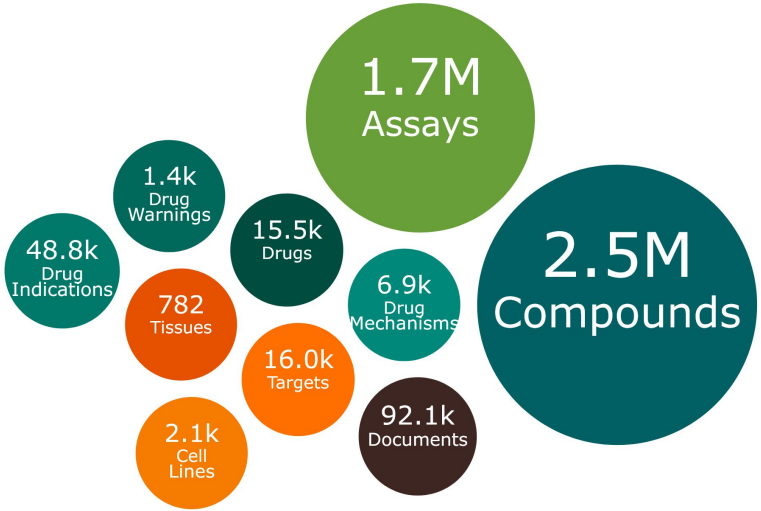
The Small-molecule PAthway Research Kit (SPARK)

Now known as the Pathway Annotated Chemical Ensemble (PACE) *library*



The ChEMBL database

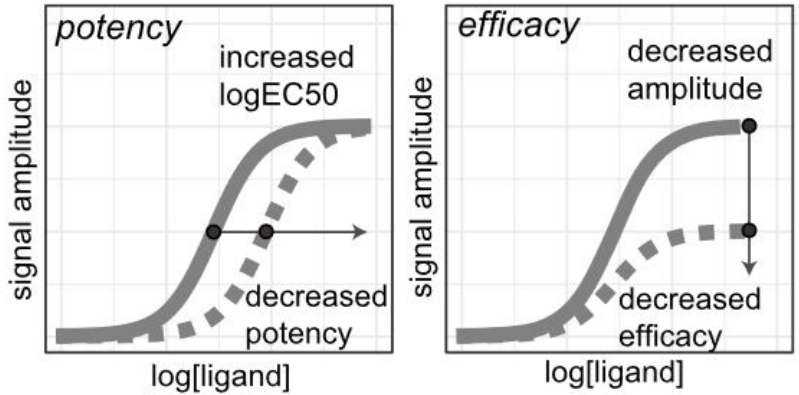
- An example of query: aspirin.
- Systematic and programmatic accession via ChEMBL API (source code).
- We can use **dose-response data** to annotate the *triplets* of compound, assay activity, and targets.



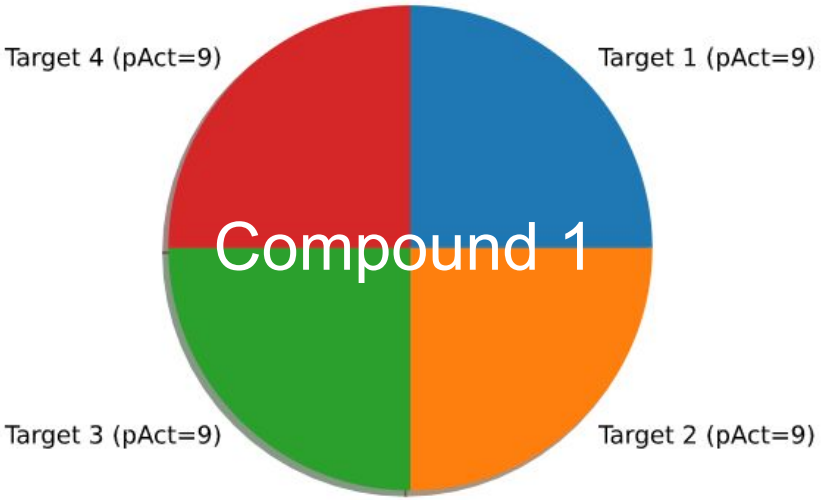
Visualization of ChEMBL
(version 35; Dec 2024)

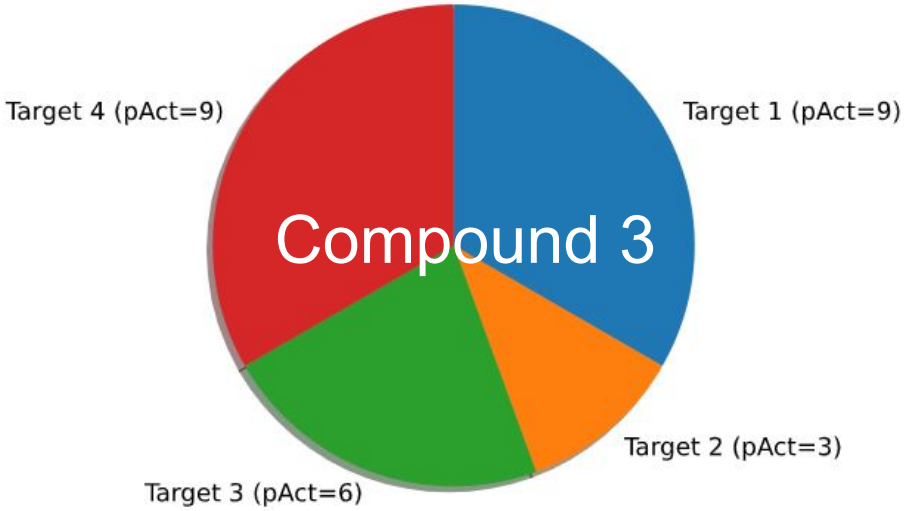
Discussion

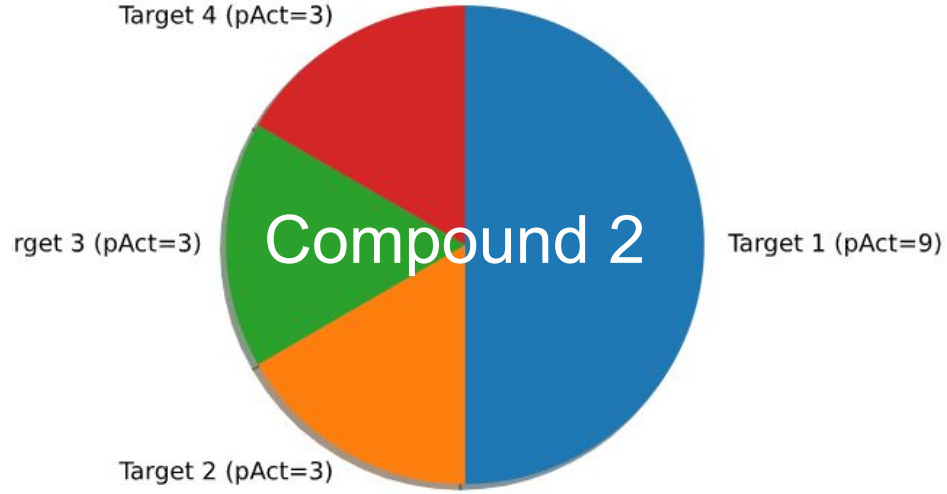
1. Why do we care selecting *representative*, *potent*, and *selective* compounds?
2. How to define following terms mathematically ...
   a. Representativity?
   b. Potency?
   c. Selectivity?



A toy example about how to quantify a compound's potency and selectivity

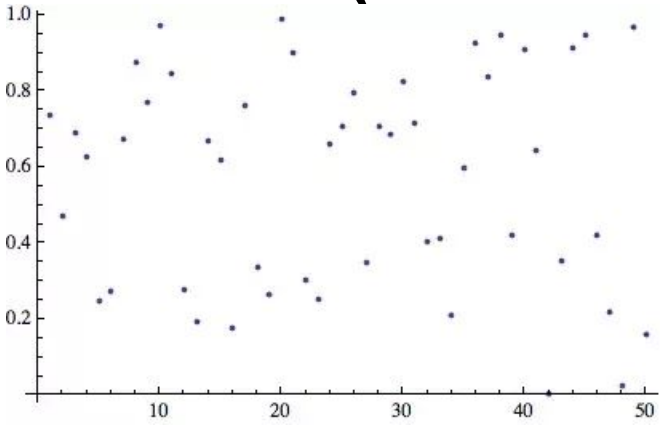




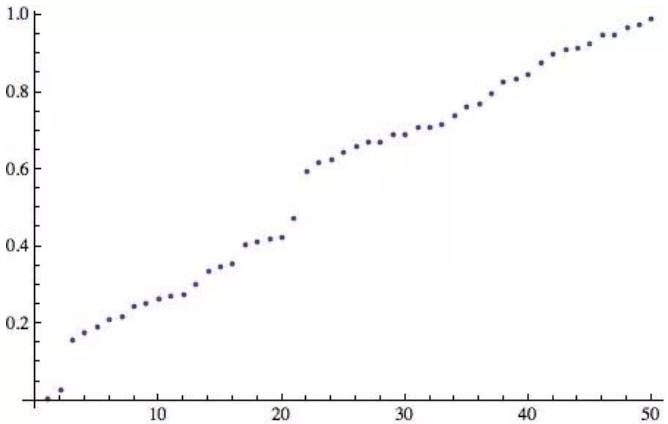


The Gini Index (a.k.a. Gini Coefficient)

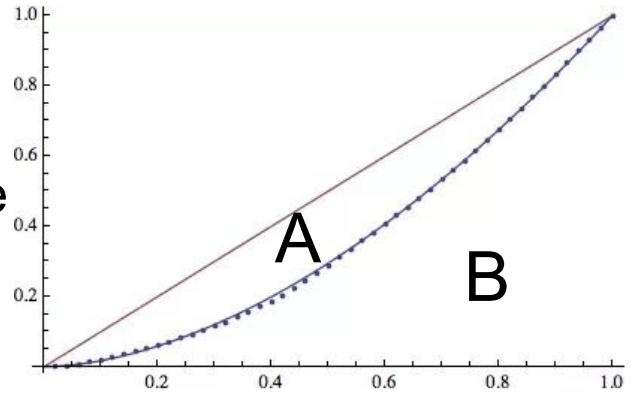
A random vector of 50 values



Sorted from low to high



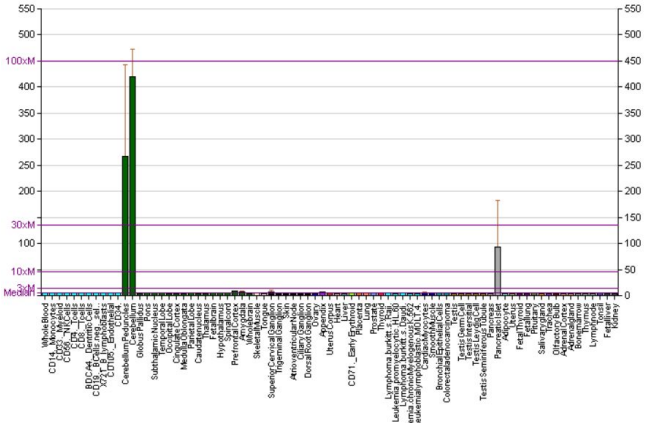
The Gini Index is calculated based on the cumulative distribution: larger value→large inequality



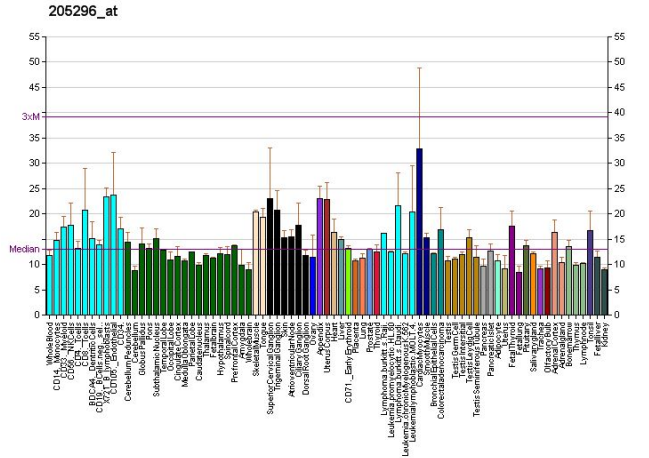
$G=A/(A+B)$

The Gini Index quantifies inequality/ selectivity

NEUROD1

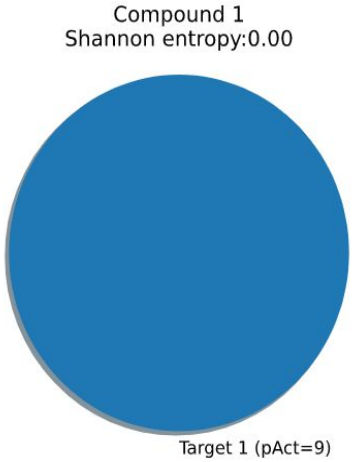


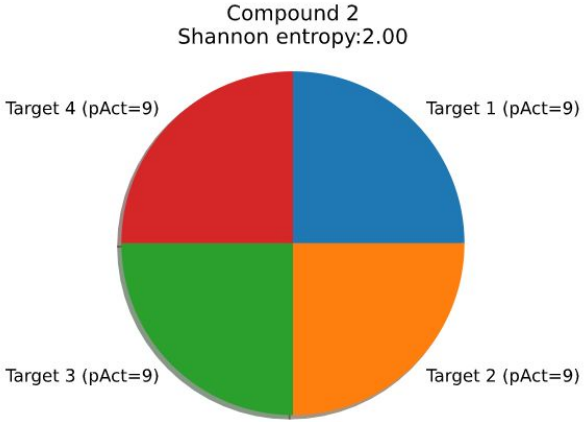
RBL1

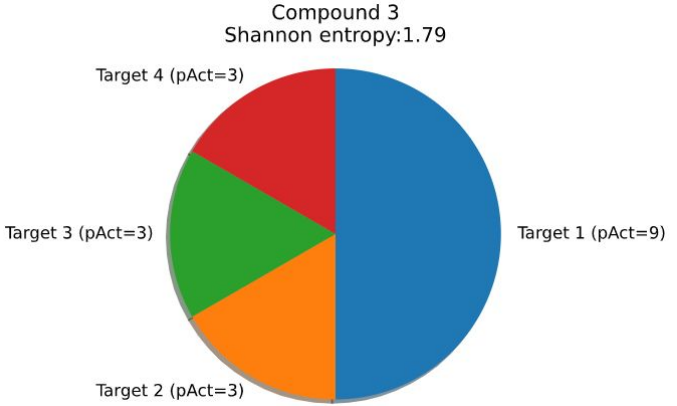


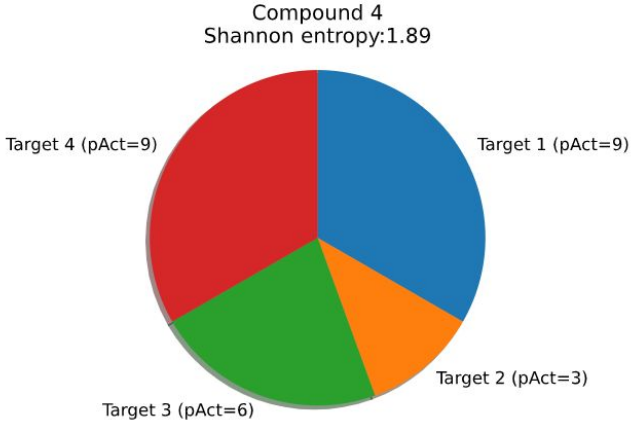
The Gini Index of expression of *NEUROD1* across tissues is near 1, whereas that of *RBL1* is near 0.

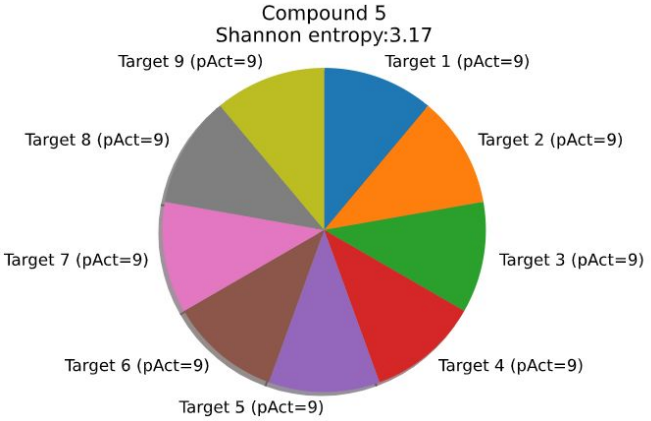
An alternative metric: Shannon's Entropy





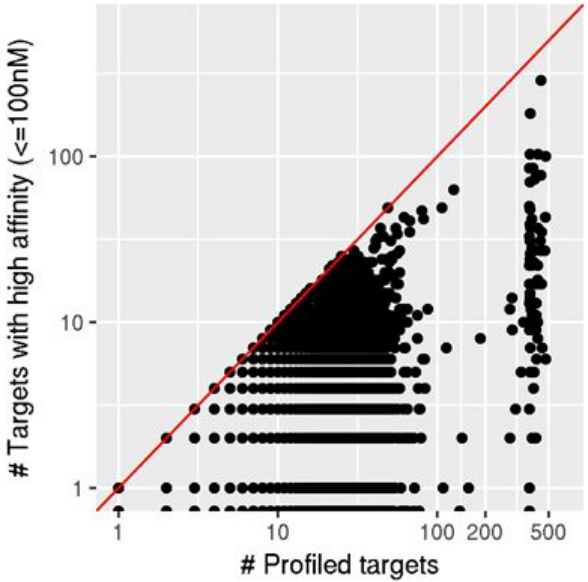


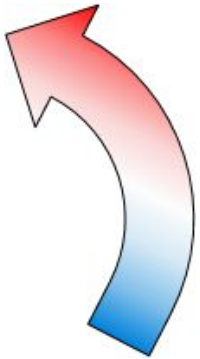


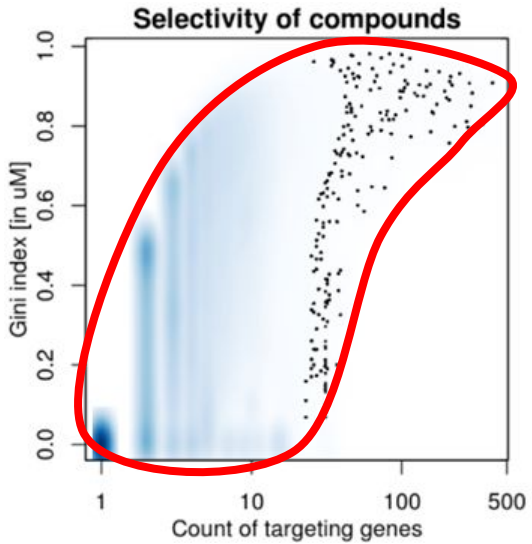


$$\mathrm{H}(X) := -\sum_{x \in \mathcal{X}} p(x) \log p(x)$$

Count of targets and selectivity of ChEMBL molecules



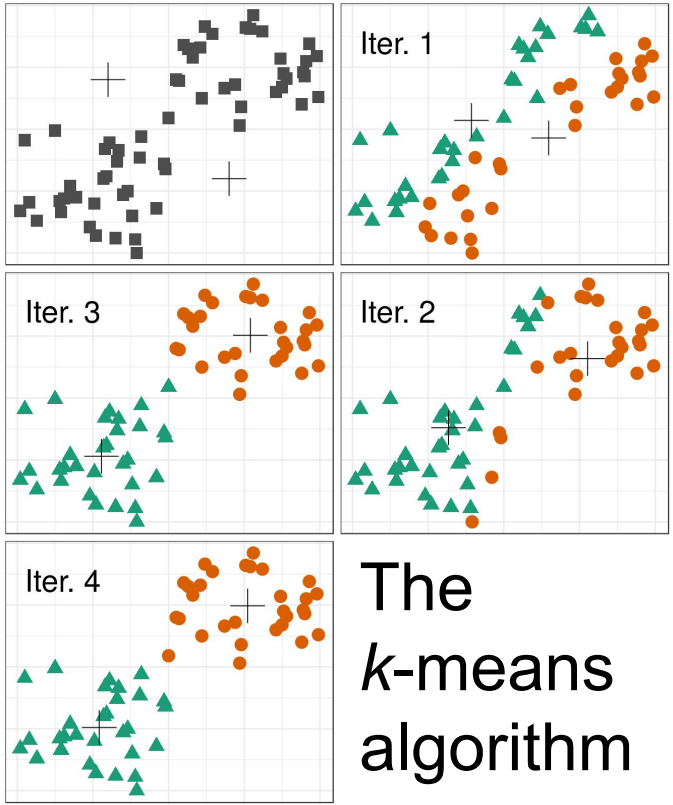




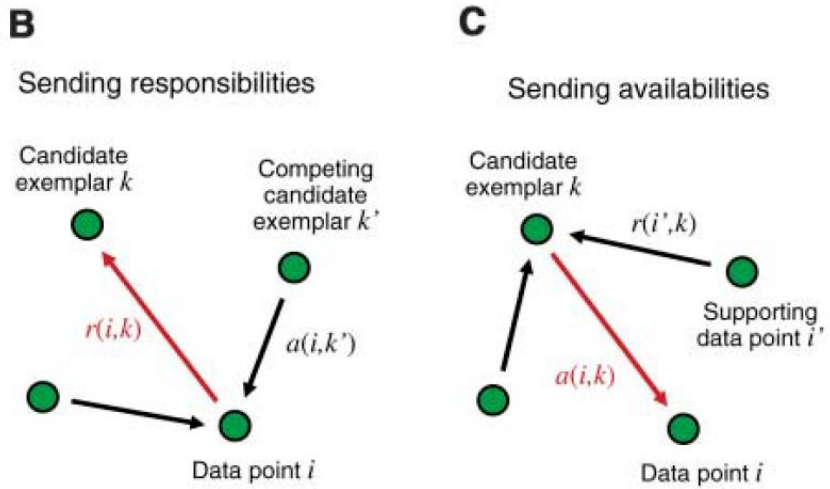
With some exceptions, most compounds are profiled against <100 targets. We distinguish between specific and pleiotropic compounds.

The **shark-fin shape** curve suggests that frequently profiled compounds tend to be more selective (and *vice versa*).

Unsupervised clustering

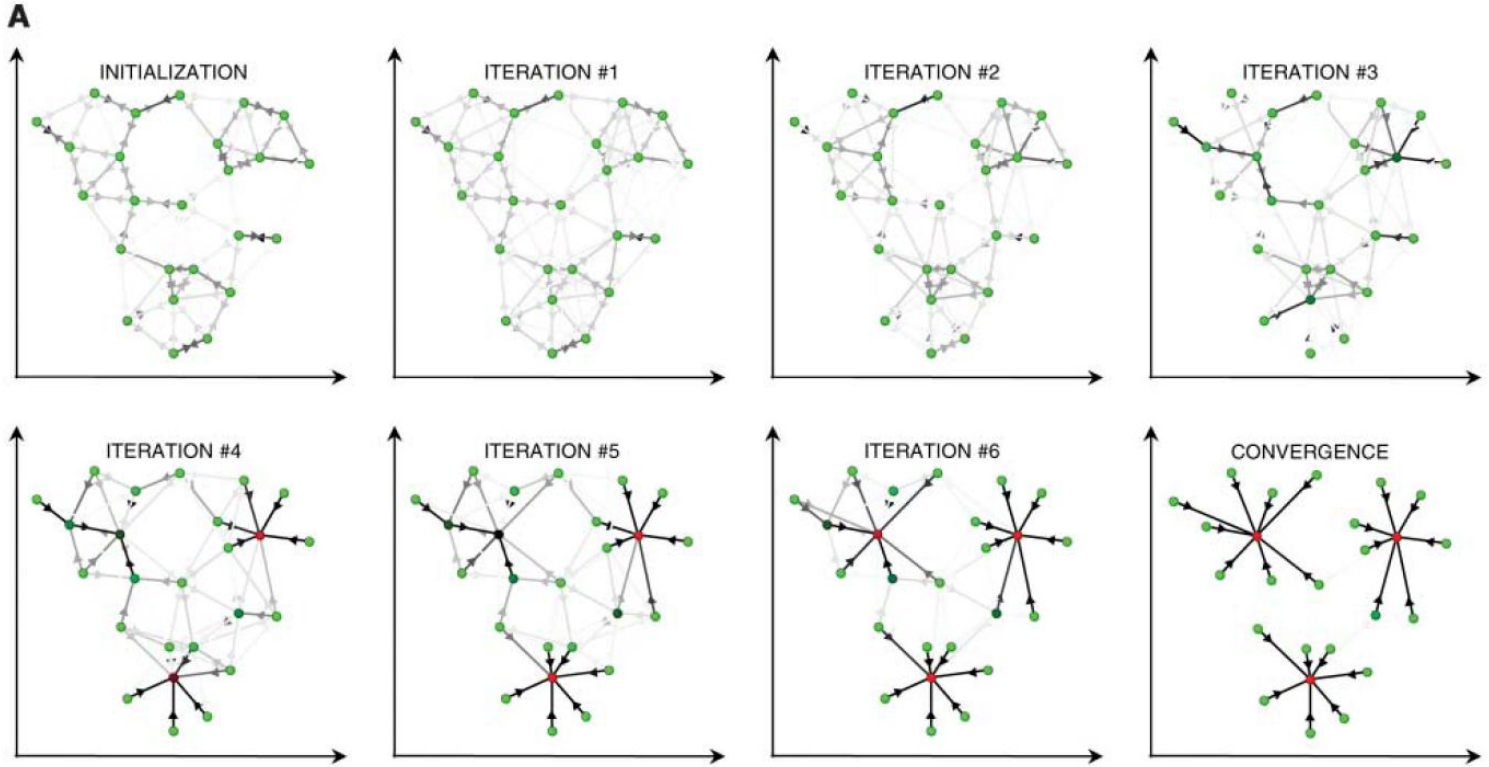


The *k*-means algorithm



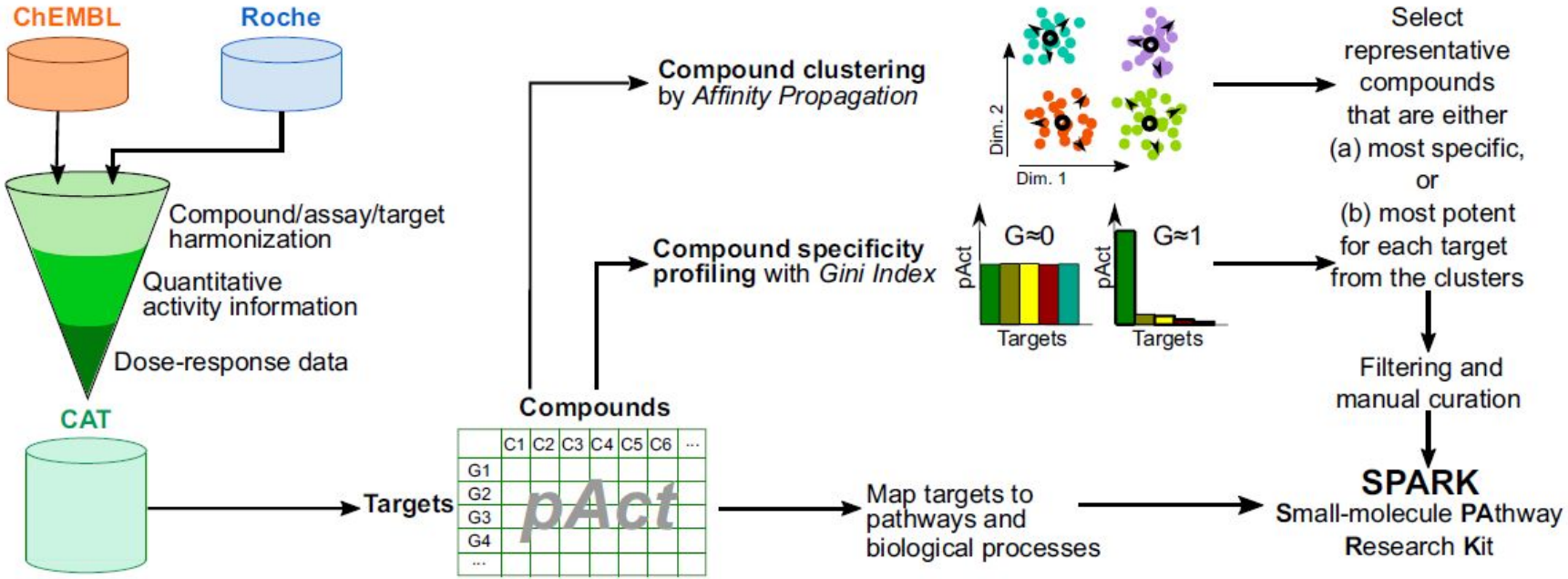
Affinity Propagation updates **responsibilities** and **availabilities** iteratively

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

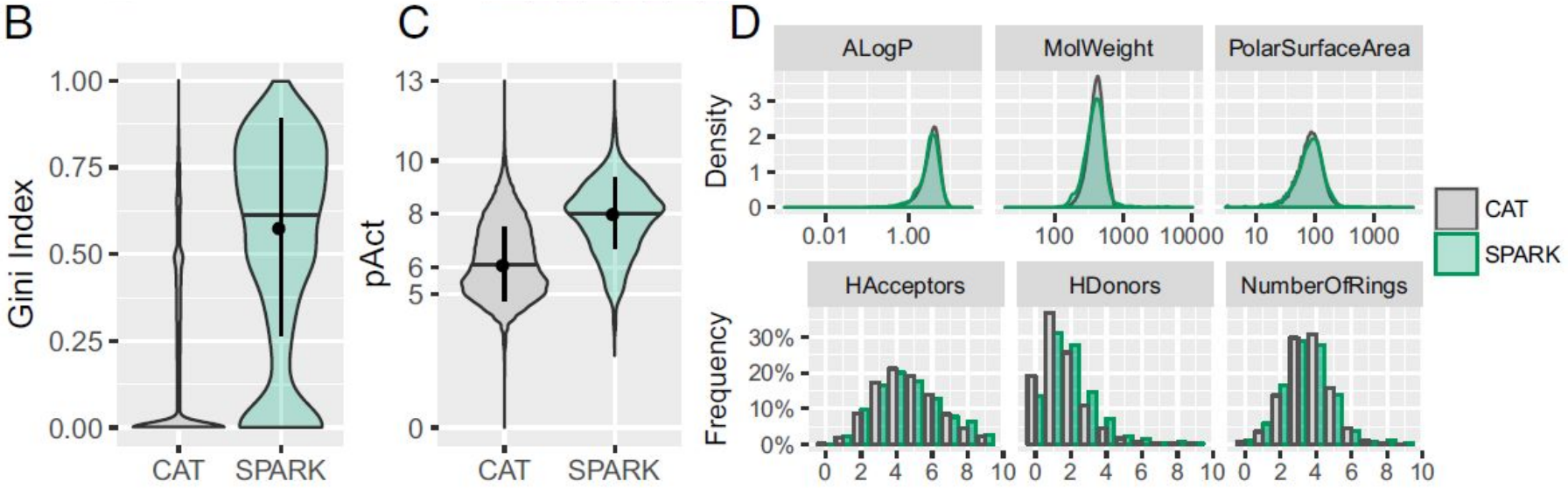
Pathways

… mapped to compounds

Curation

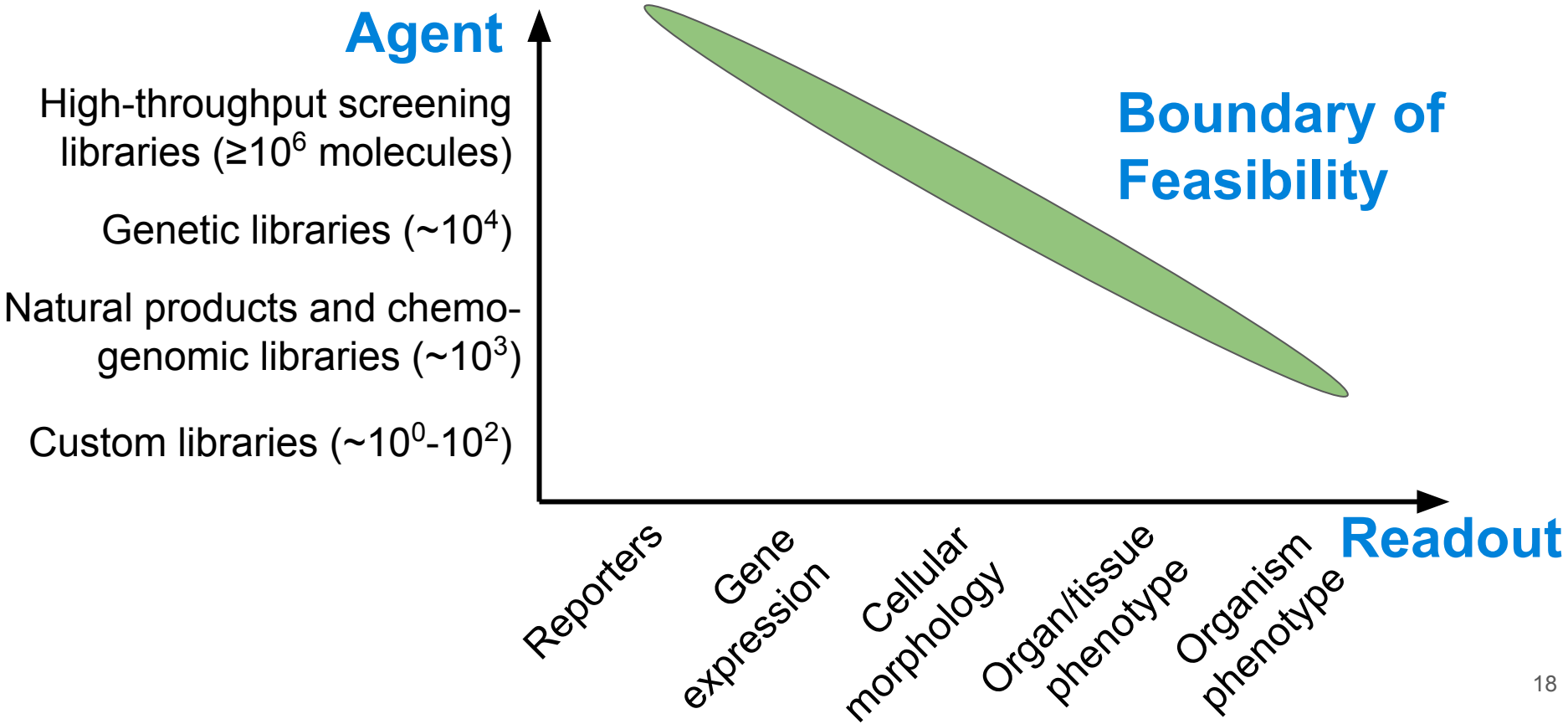
… to enrich quality compounds

SPARK covers the chemical space evenly with representative, potent, and specific compounds



Roudnicky *et al.*, PNAS, 2020, https://www.pnas.org/content/early/2020/08/04/1911532117

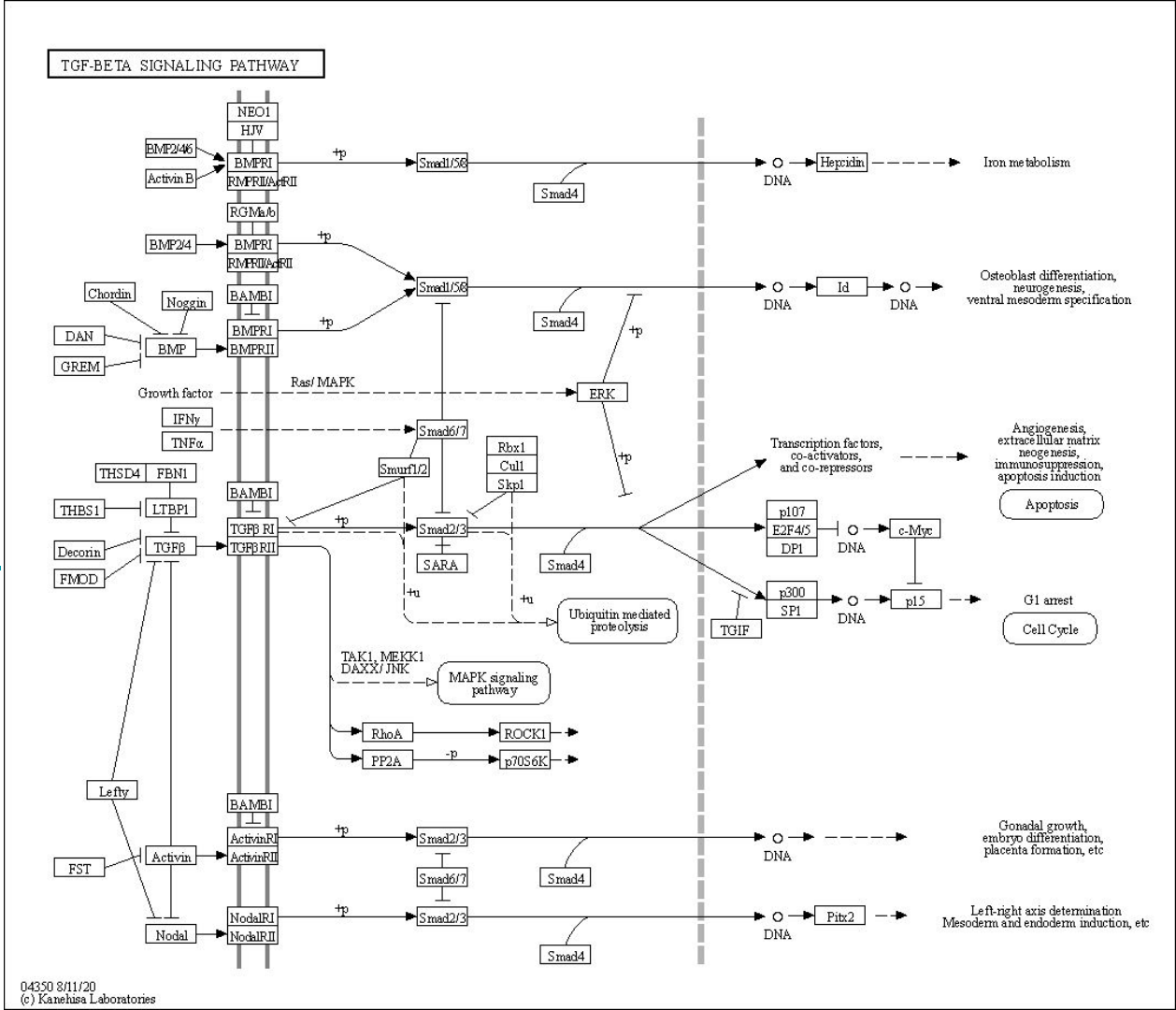
Phenotypic screenings by agent and readout



Mapping genes to biological pathways

Option 1: KEGG pathways, with the example of TGF-β signaling pathway.

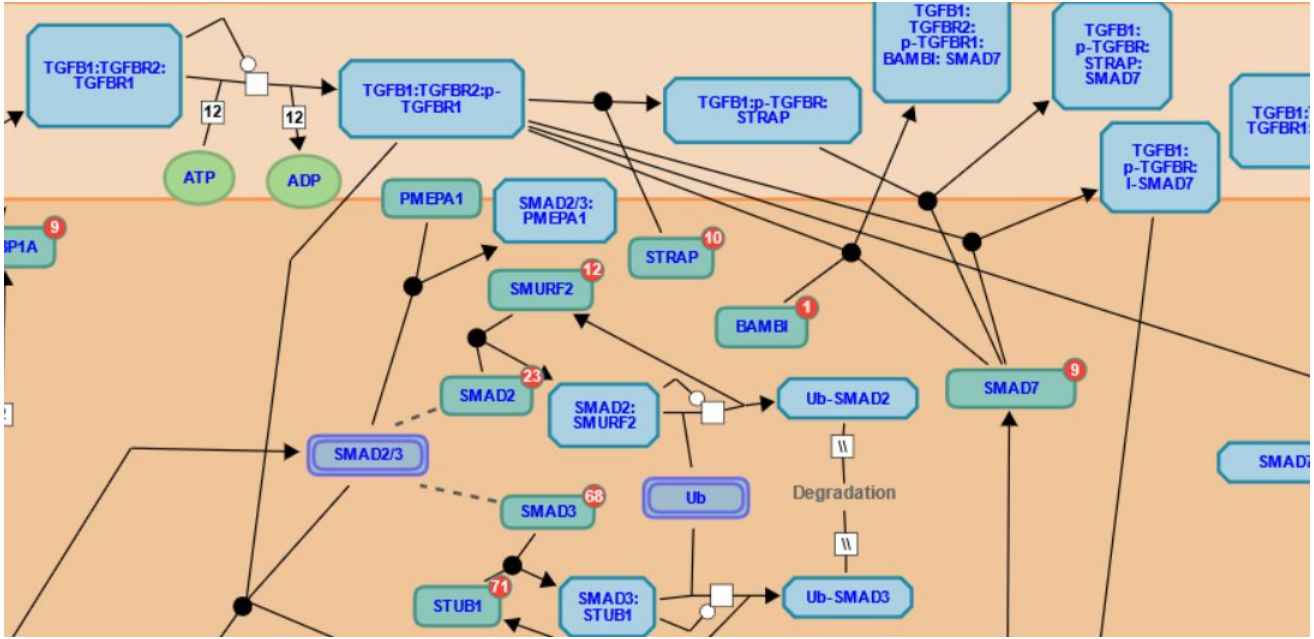
A RESTful API is available for academic use, with clients in Python and R.



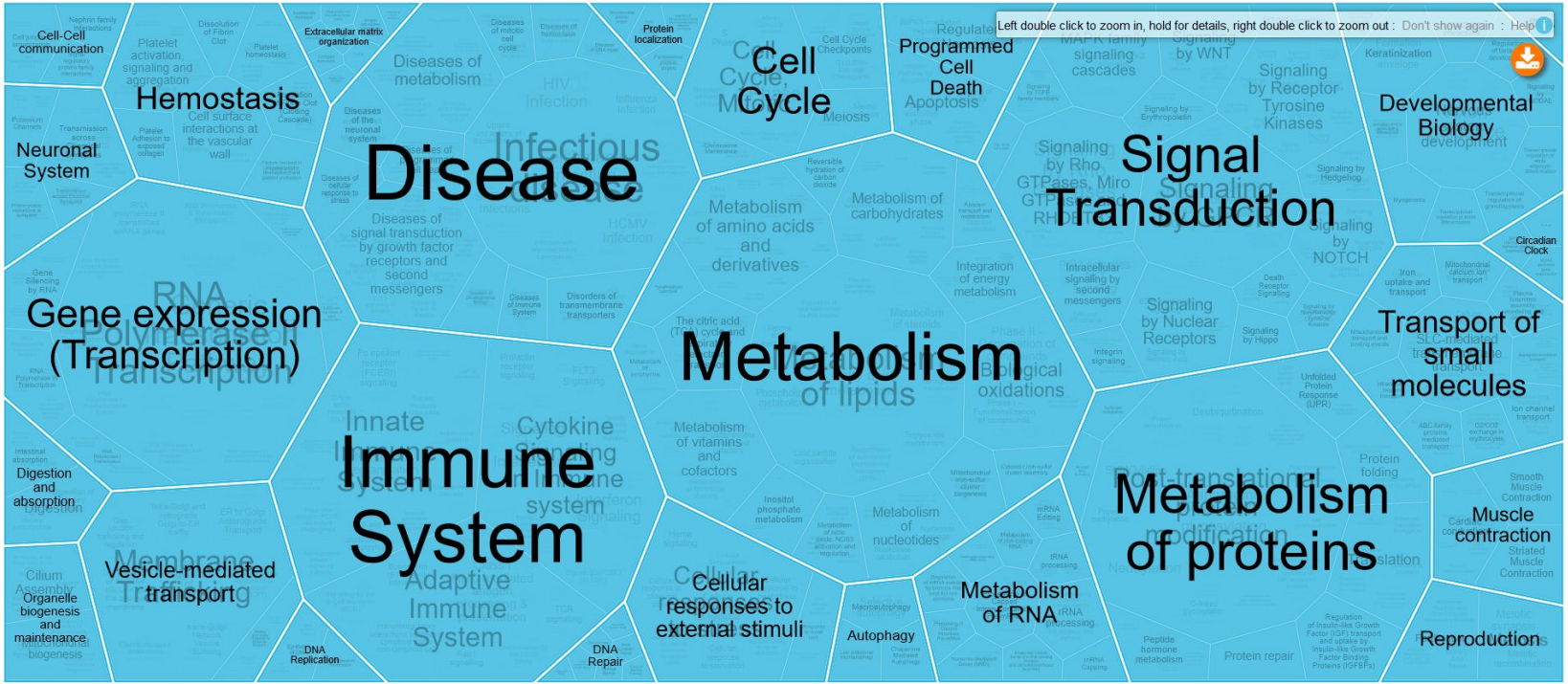
Mapping genes to biological pathways

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

Developer's Zone provides API and graph database interfaces.



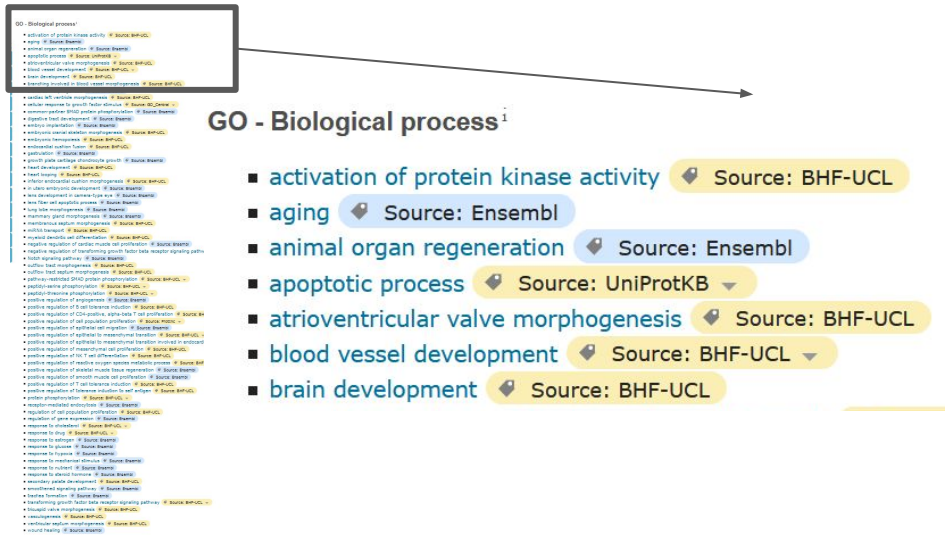
Overview of pathways captured by Reactome



The Voronoi (Reacfoam) view of all pathways in Reactome

Mapping genes to biological processes

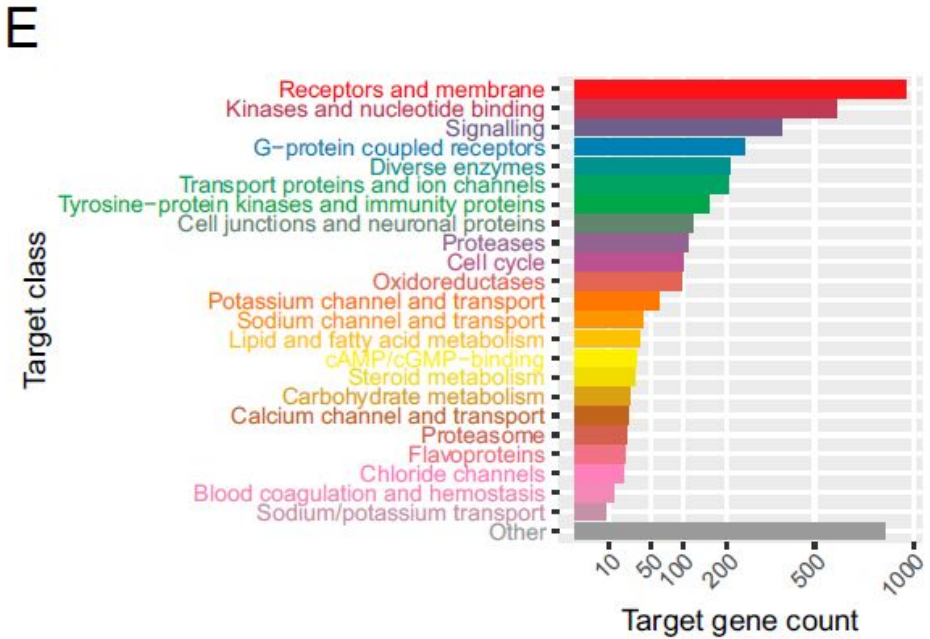
- Gene Ontology
- UniProtKB keywords
- Example: TGFBR2_HUMAN (TGF-beta receptor type -2, P37173)

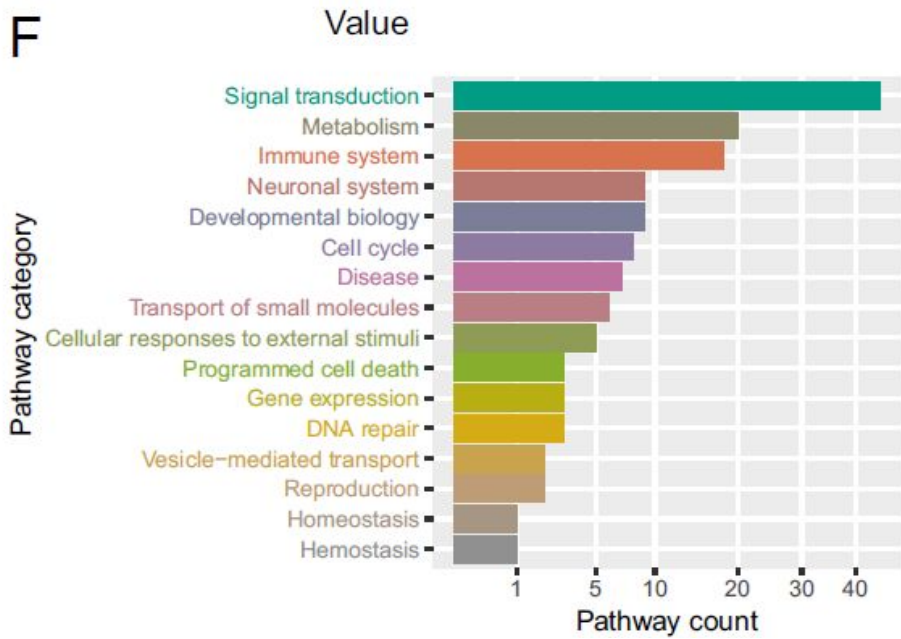


Keywords

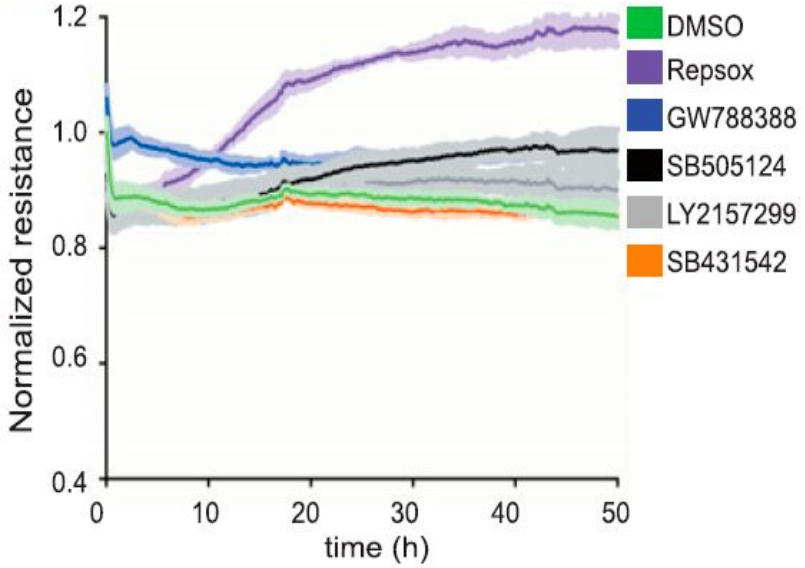
Molecular function	Kinase, Receptor, Serine/threonine-protein kinase, Transferase
Biological process	Apoptosis, Differentiation, Growth regulation
Ligand	ATP-binding, Magnesium, Manganese, Metal-binding, Nucleotide-binding

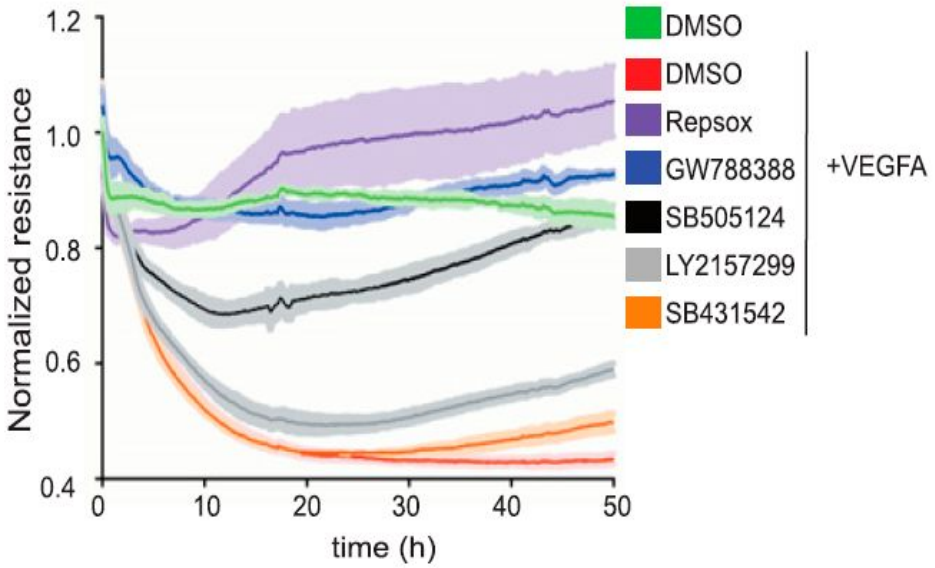
SPARK covers the target space evenly with representative, potent, and specific compounds





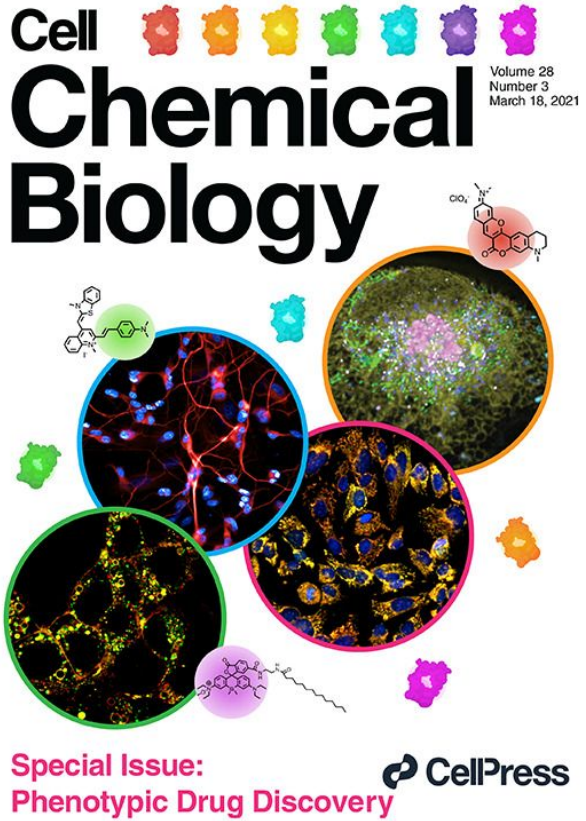
Screening with SPARK in endothelial cells identified TGF-β pathway genes as potential targets for diabetic retinopathy





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.

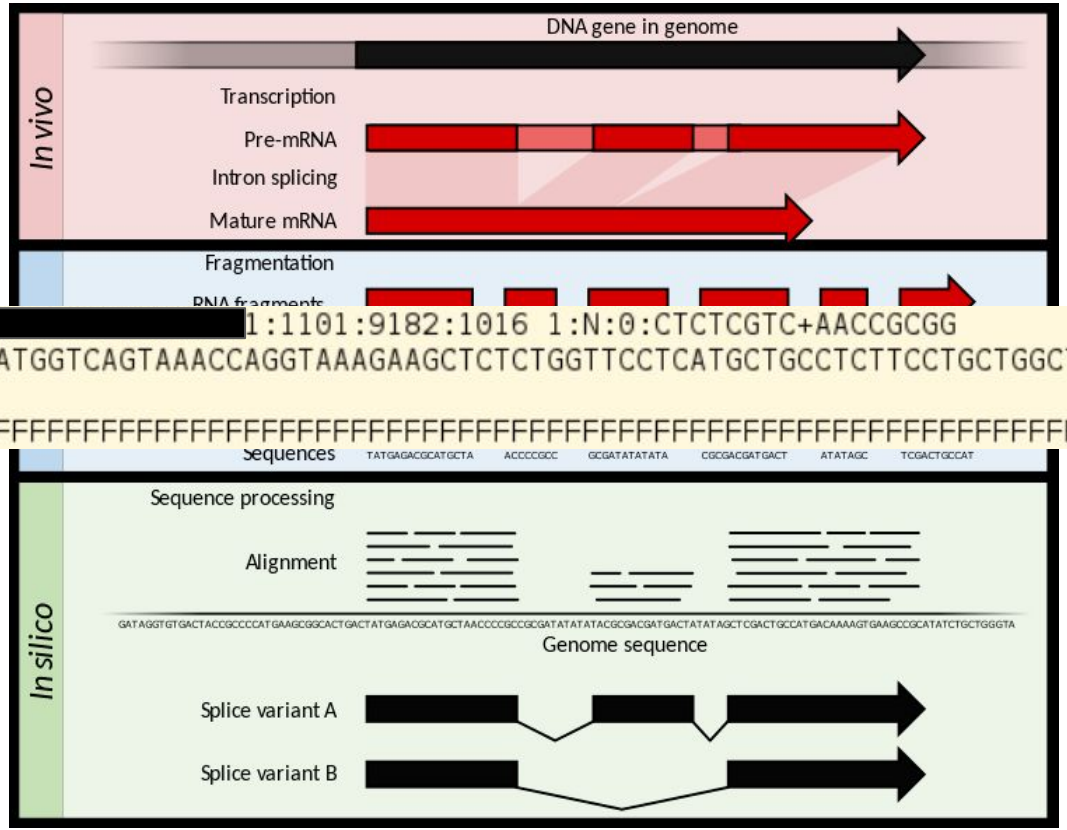


Offline activities of Module II

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.

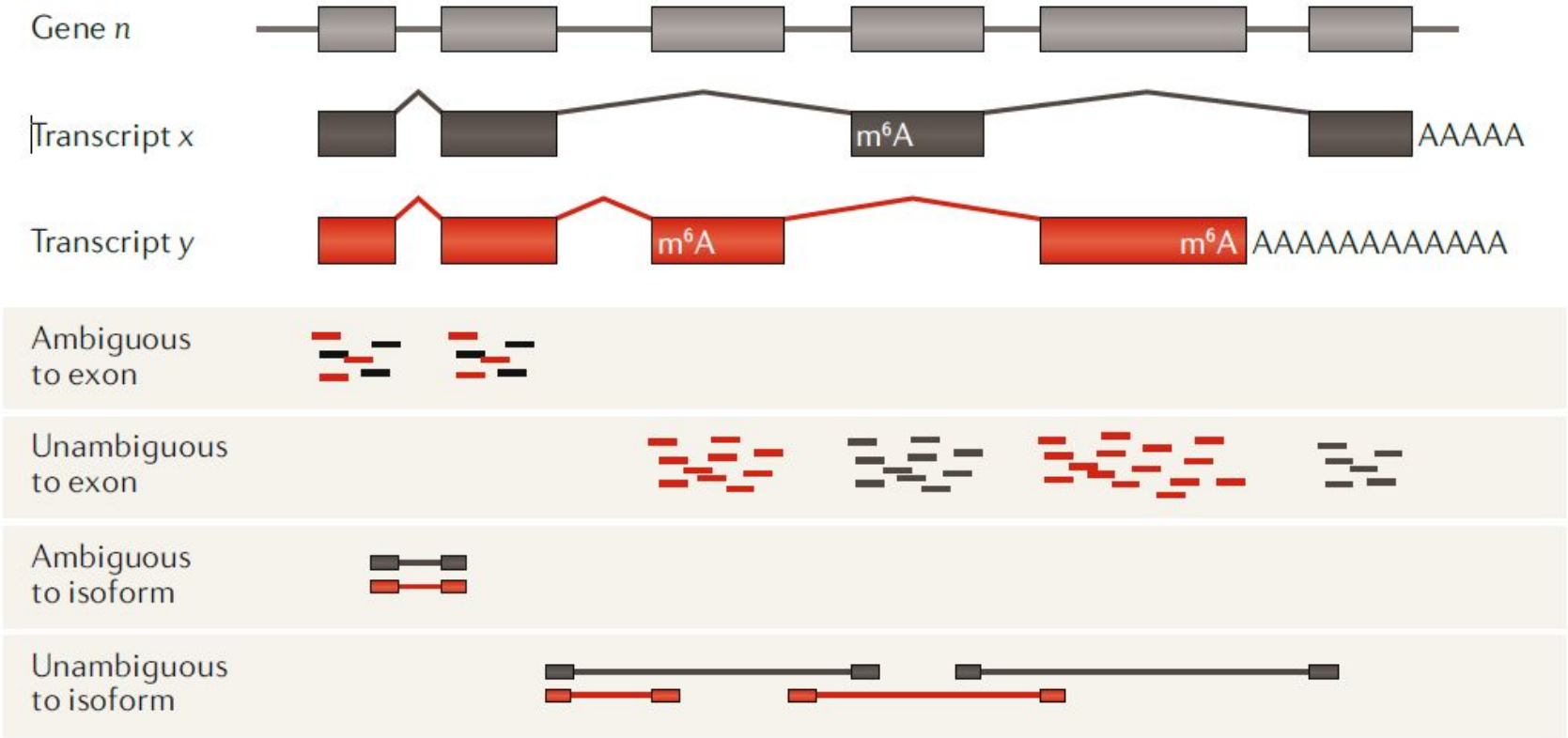
1. Retrieve all approved drugs from the ChEMBL database, sort them by approval year and name (a Python example is here; documentations of the ChEMBL API can be found here);
2. For each approved drug **since 2019** that you identified in step (1), retrieve a list of UniProt accession numbers, namely protein 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



@[redacted]1:1101:9182:1016 1:N:0:CTCTCGTC+AACCGCGG
GNATCAGAATGGTCAGTAAACCAGGTAAAGAAGCTCTCTGGTTCCTCATGCTGCCTCTTCCTGCTGGCTTTATT
+
F#FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF

Transcriptome profiling by RNA sequencing



Differential gene expression

Read Mapping

Count collection

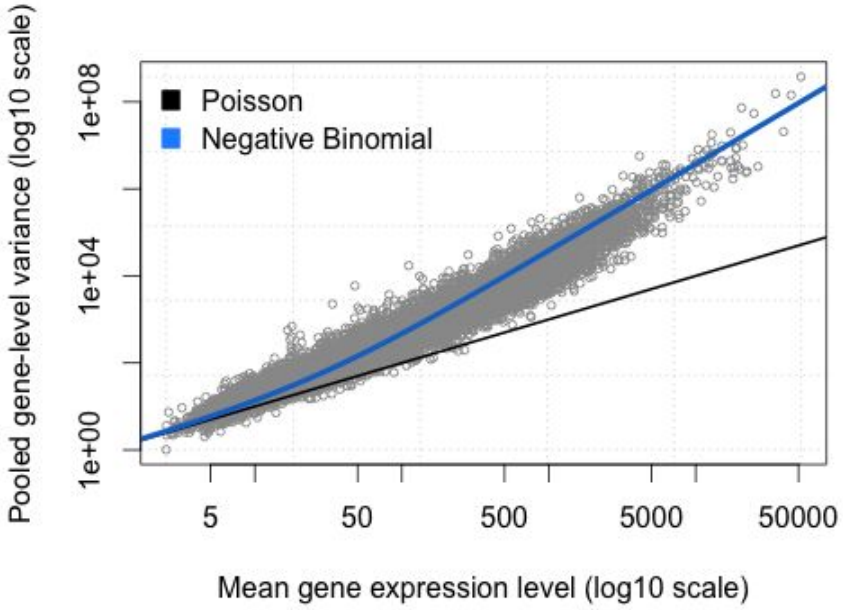
	sample A1	sample A2	sample B1	sample B2
gene 1	8	10	100	200
gene 2	14	15	15	40
gene 3	33	40	35	70
...	...	...	...	...
gene N	100	120	105	220

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



Tools: *edgeR* and *DESeq2*

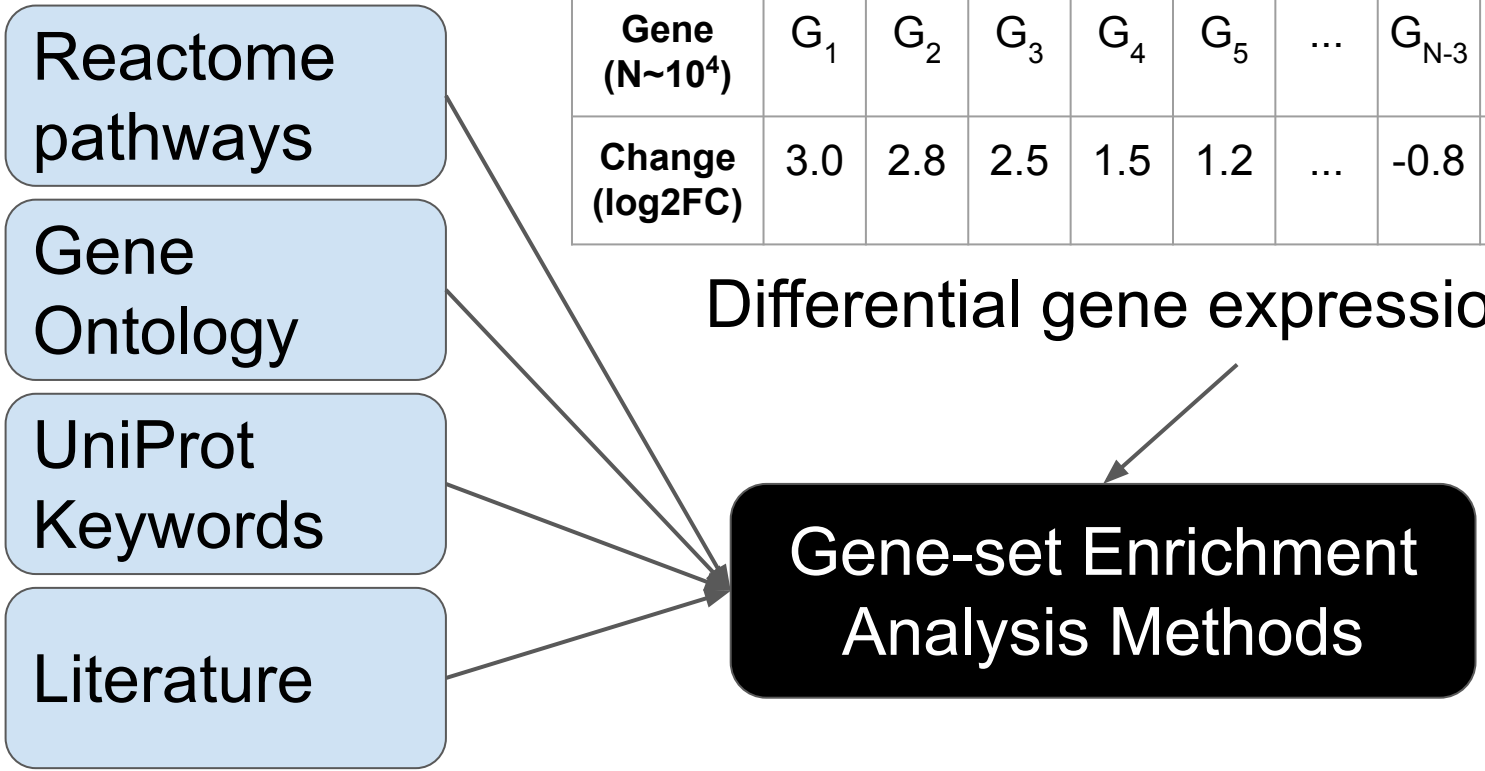
Probability theory and statistical tools discussed

- Distributions
  - Gaussian distribution (used in linear model)
  - Bernoulli distribution → Binomial distribution → Negative binomial distribution
  - Poisson distribution → Negative binomial distribution
  - Poisson distribution ←→ Exponential distribution
- Statistical methods
  - Bootstrapping method
  - Student’s t-test
  - Wilcoxon-Mann-Whitney test
  - Kolmogorov-Smirnov test

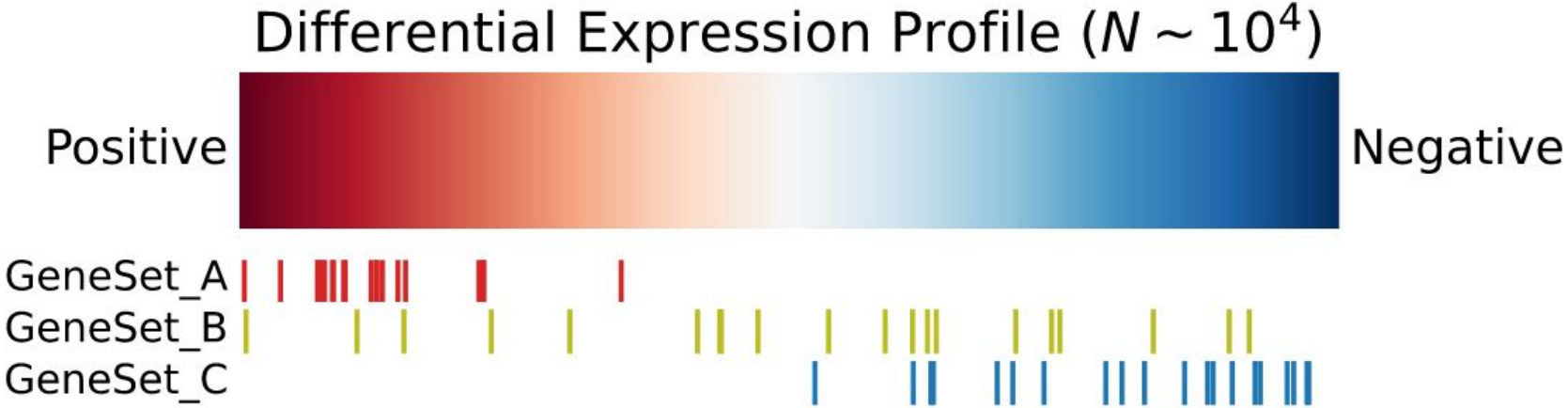
Interpret differential gene expression data with gene-set enrichment analysis

Gene ($N \sim 10^4$)	G_1	G_2	G_3	G_4	G_5	...	G_{N-3}	G_{N-2}	G_{N-1}	G_N
Change (log2FC)	3.0	2.8	2.5	1.5	1.2	...	-0.8	-1.2	-1.5	-2.2

Differential gene expression results



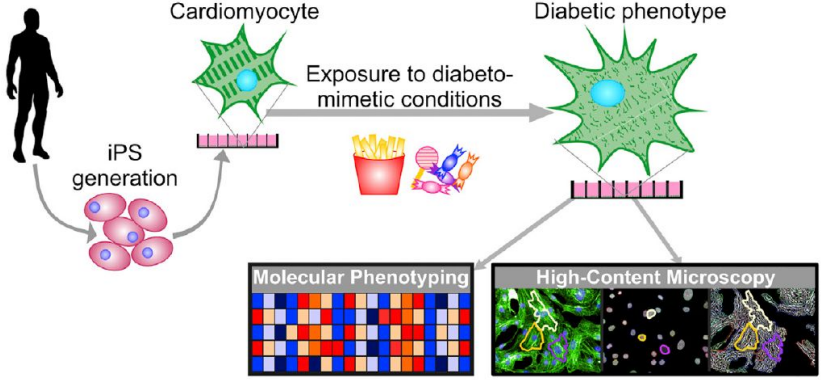
Gene-set enrichment analysis



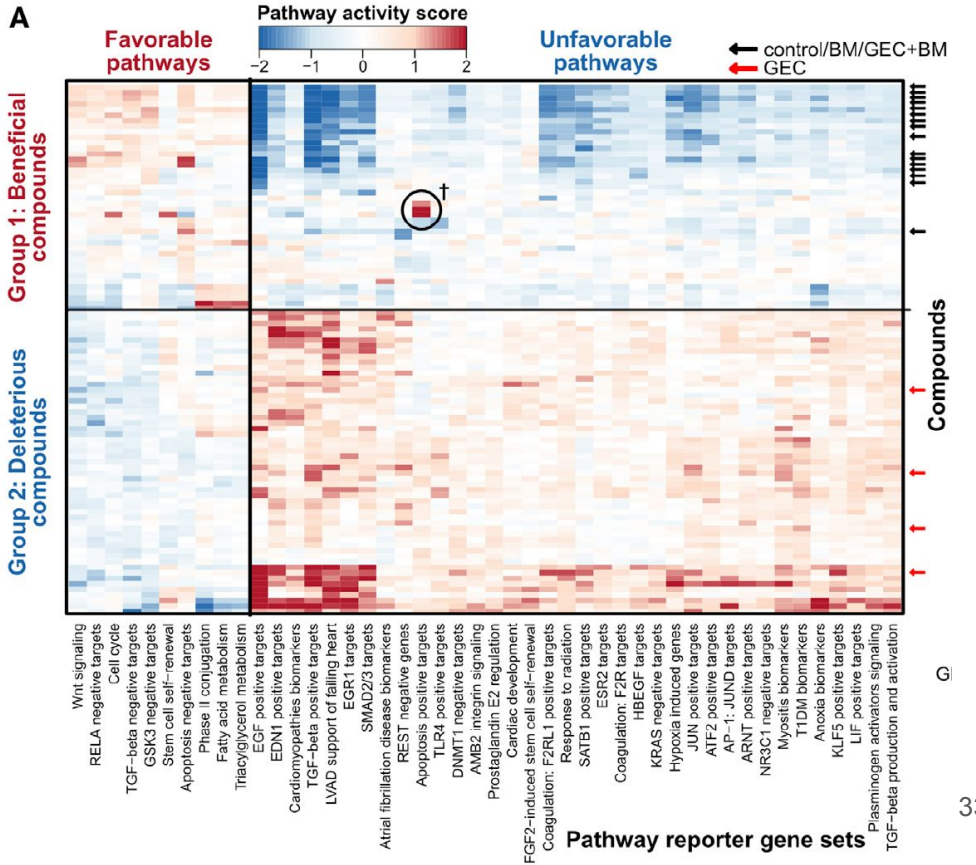
Input: (1) a differential gene expression profile; (2) a set of gene-sets {*G*}, each a set of genes.

Output: a ranked list of the input gene-sets by *enrichment*.

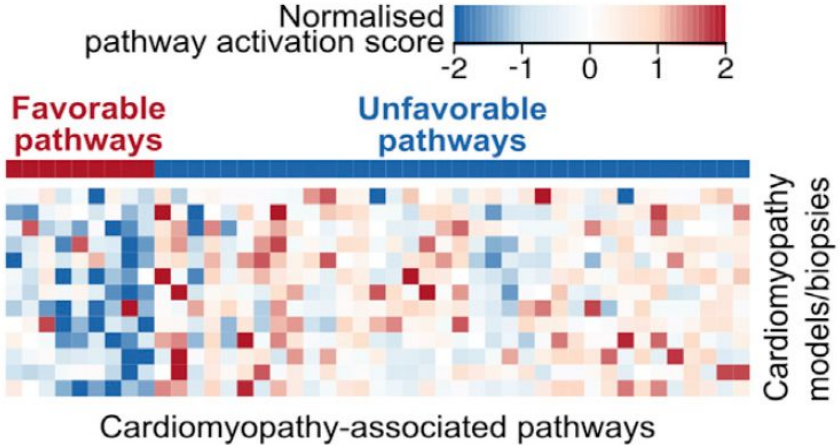
Gene expression as screening readout



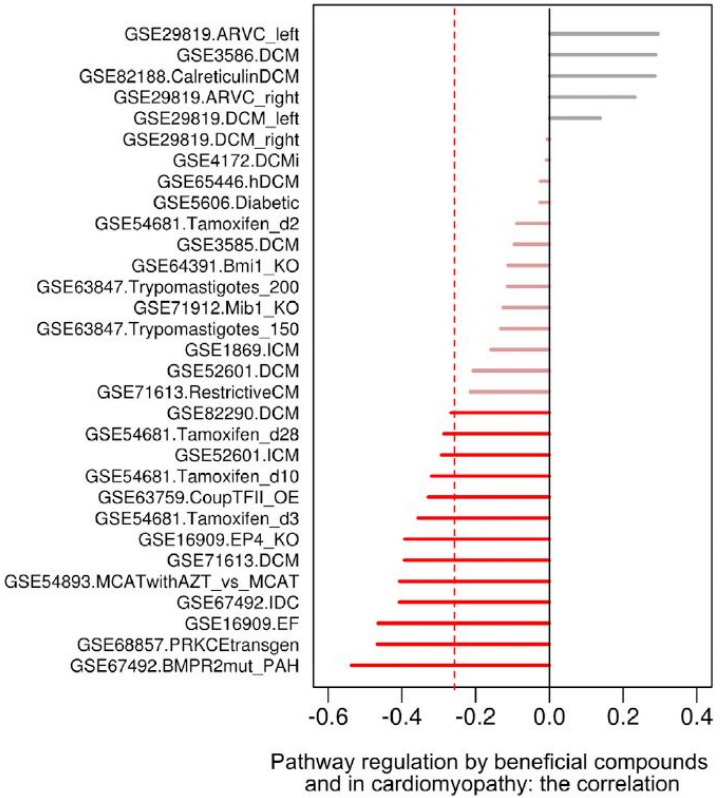
Differential gene expression profiles are molecular snapshots of drugs' action in the cell.



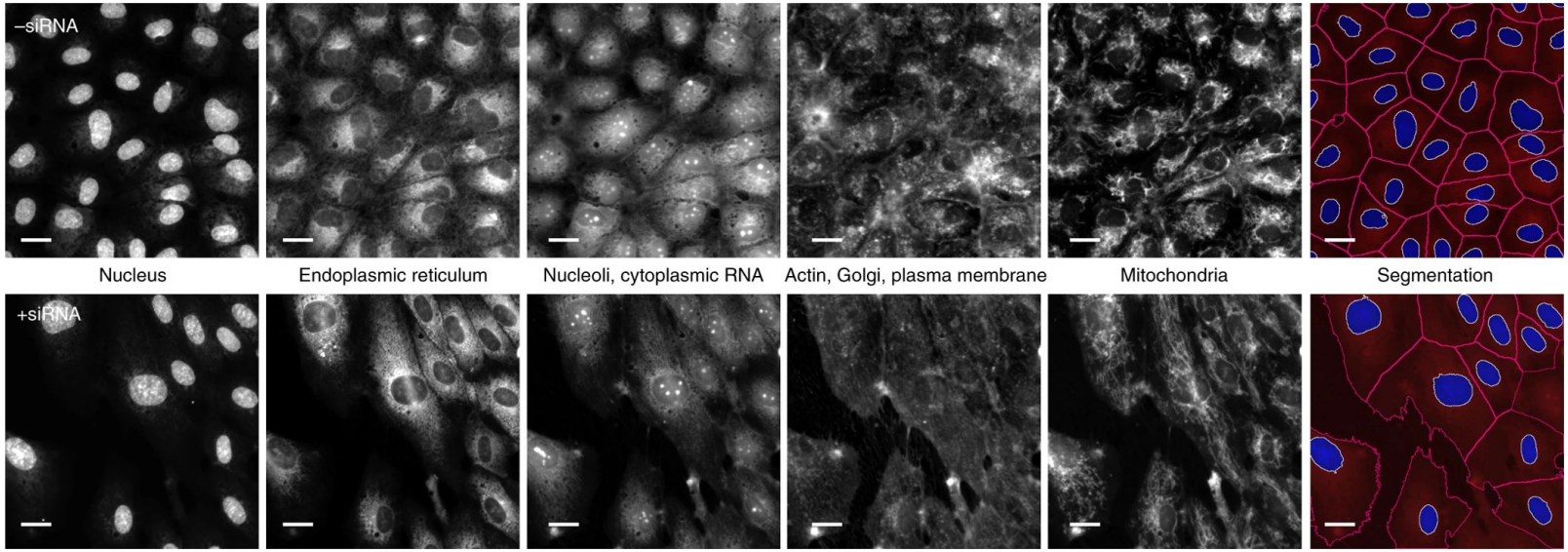
Gene expression from patient and animal models help compound selection

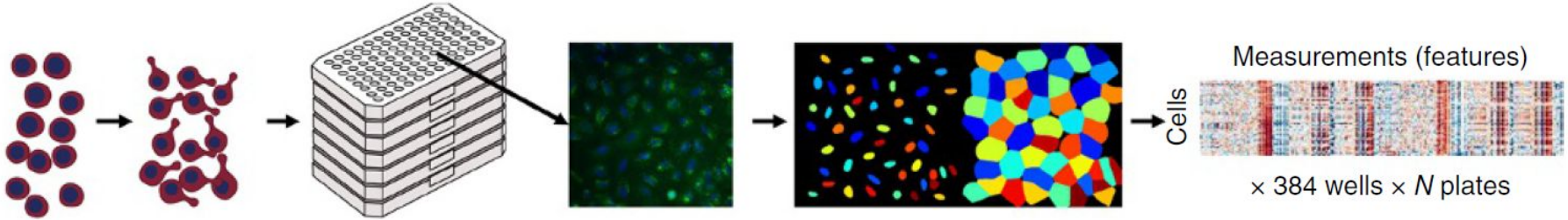


We can prioritise molecules that reverse disease-induced changes.

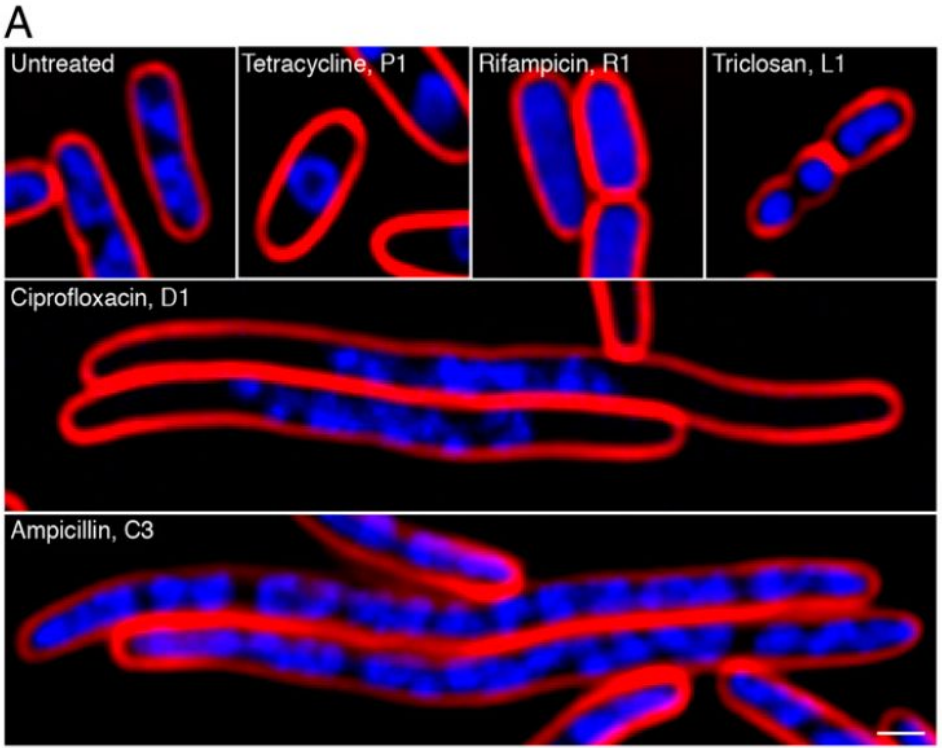


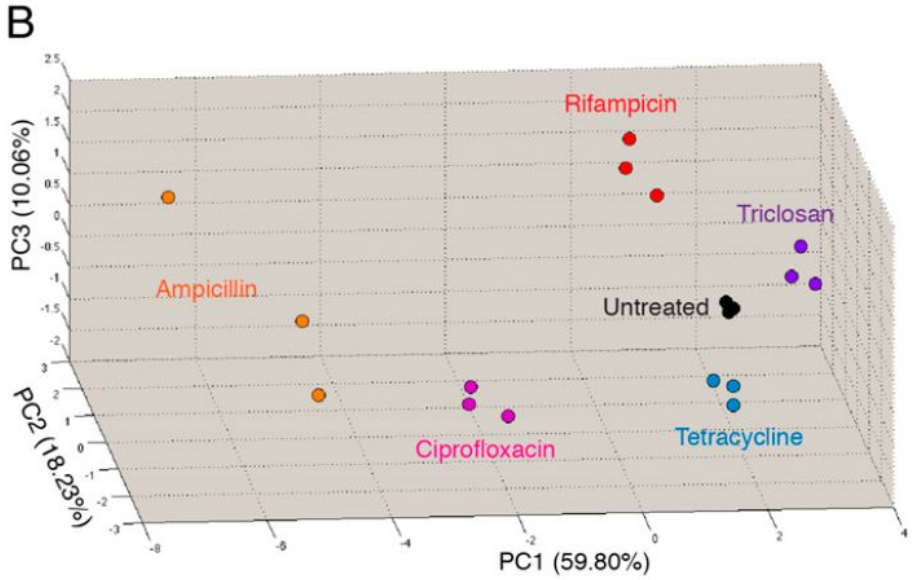
Morphology as screening readout





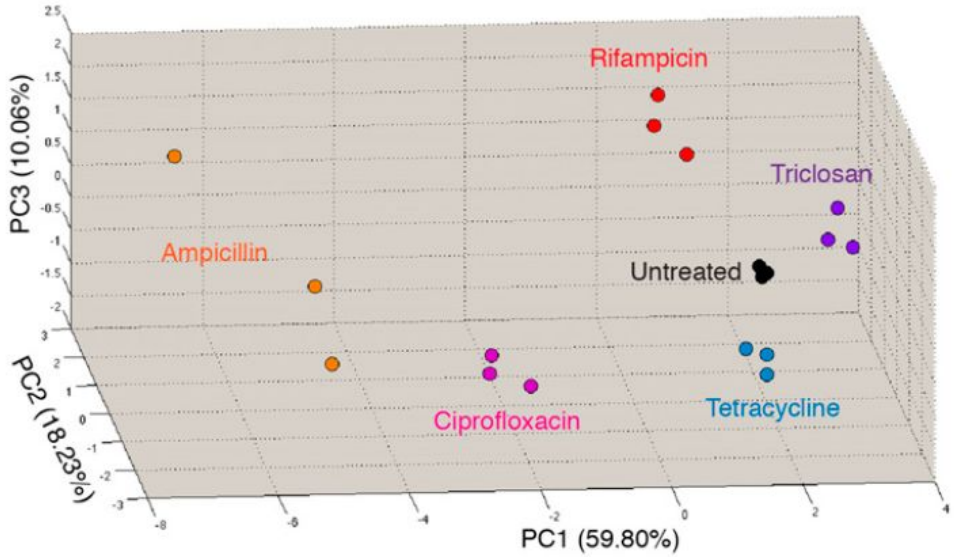
Cytological profiling for antibiotics discovery

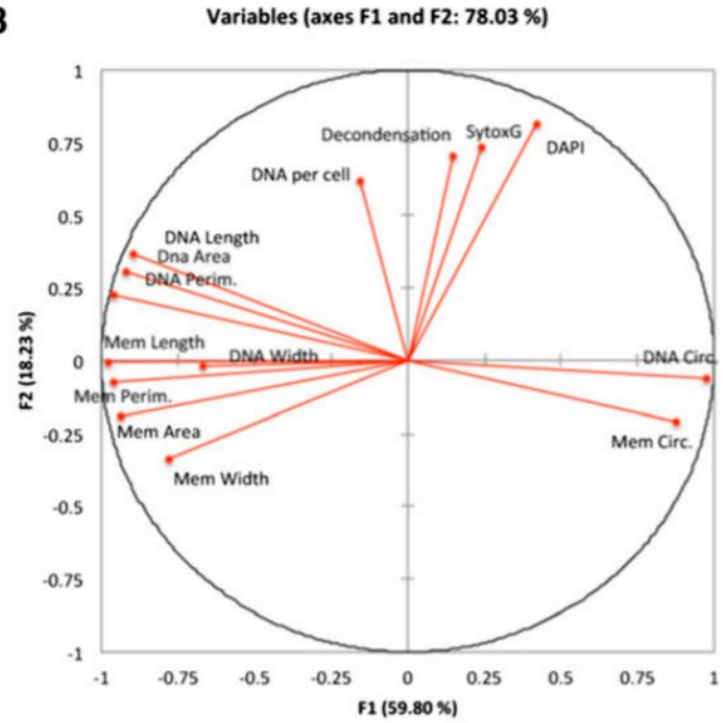




P: Protein translation inhibitors
R: RNA transcription inhibitors
D: DNA replication inhibitors
L: Lipid biosynthesis inhibitors
C: Cell-wall synthesis inhibitors (peptidoglycan)

Principal components are linear combination of morphological features

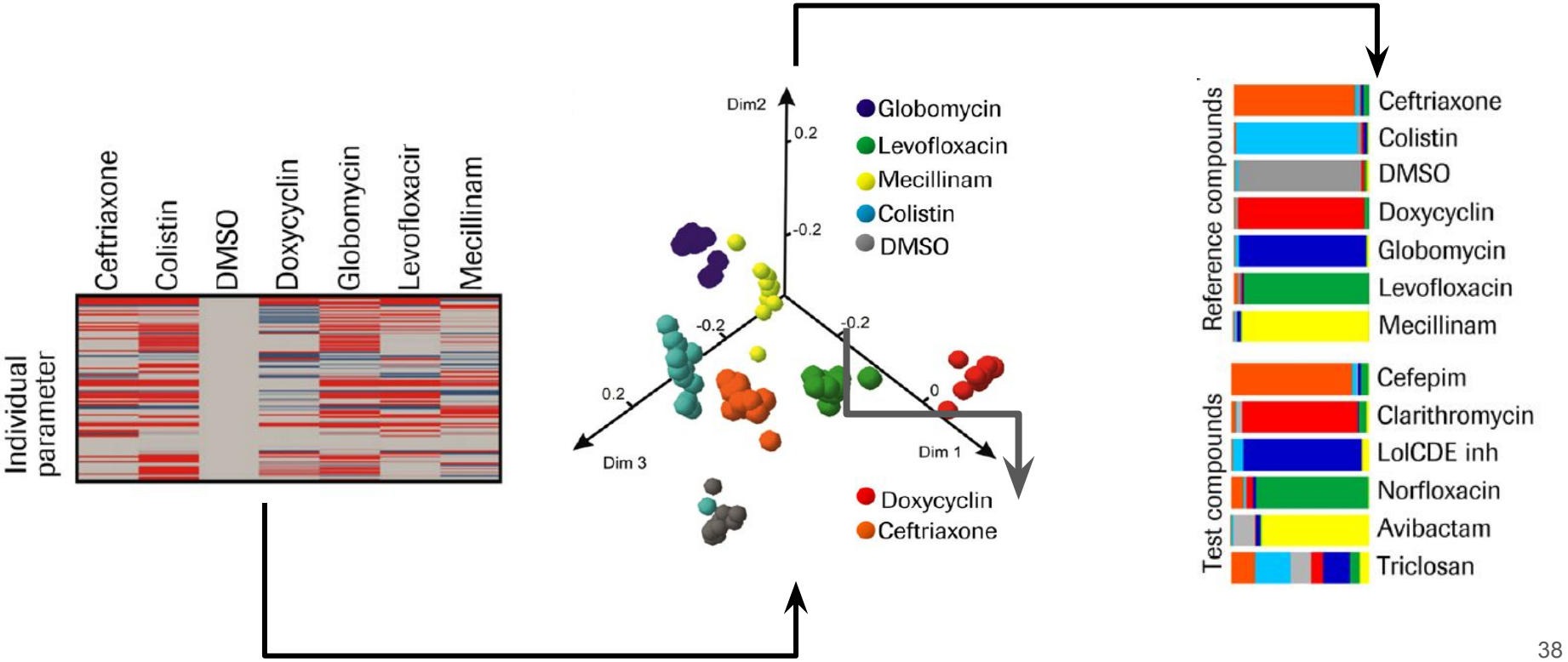




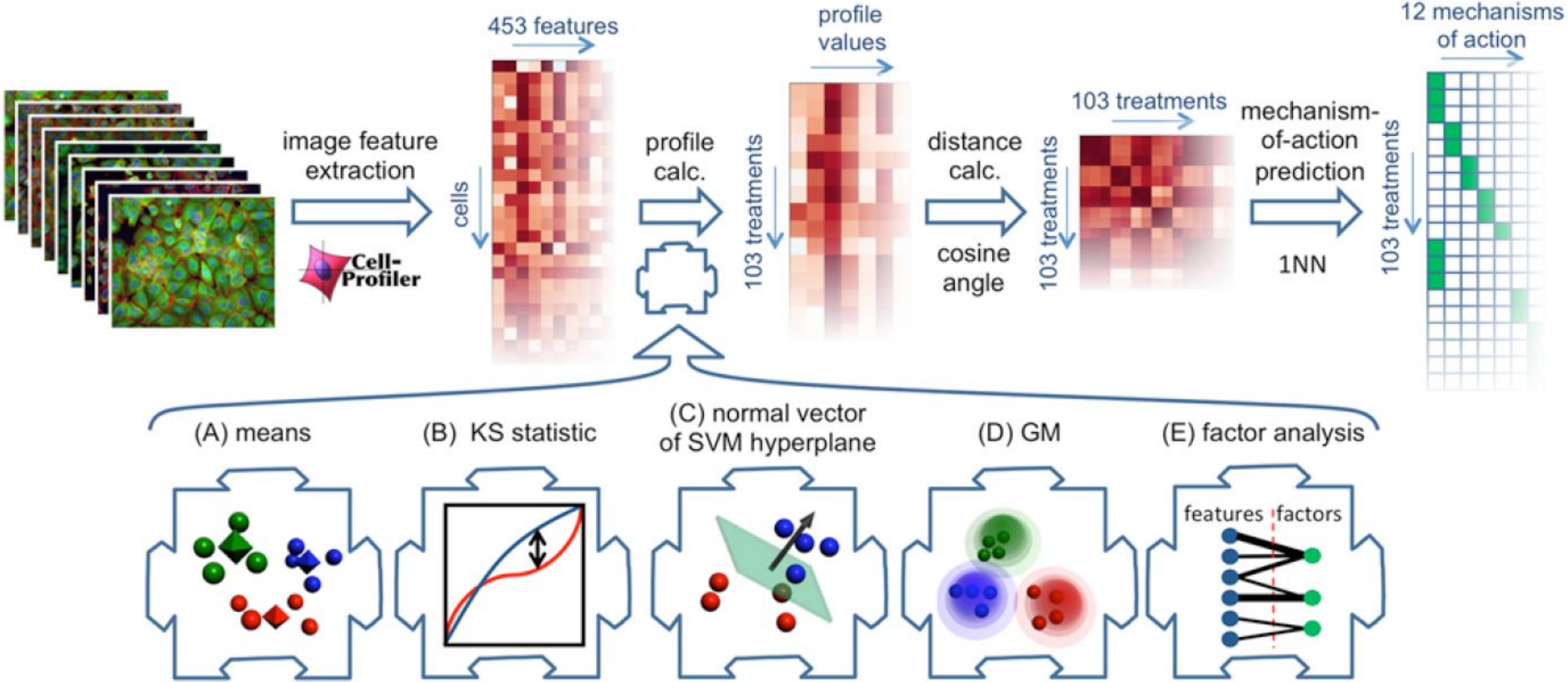
Membrane area, μm^2 | DNA area, μm^2 | Membrane perimeter, μm | DNA perimeter, μm | Membrane length, μm | DNA length, μm | No. of nucleoids per cell

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

Morphology classifies compounds by MoA



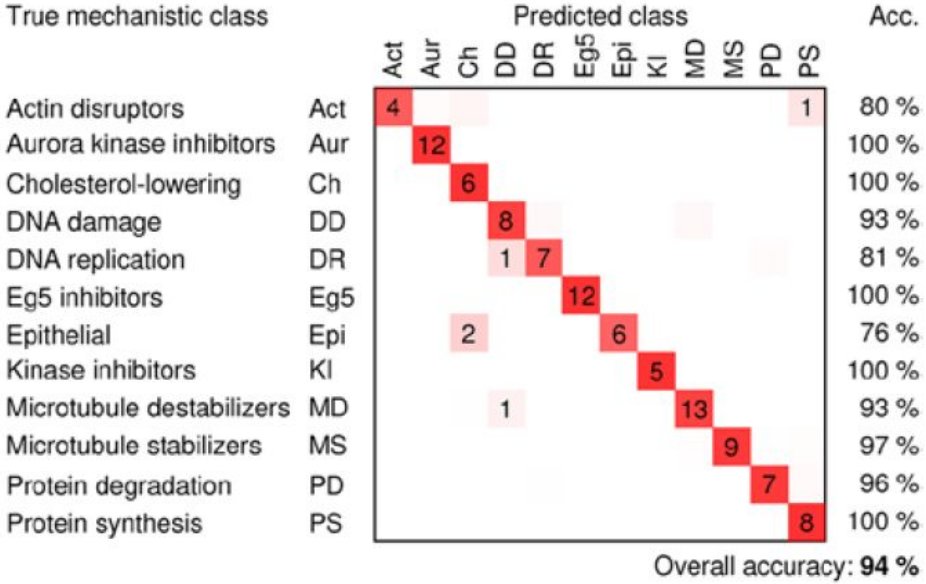
Comparison of computational methods



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



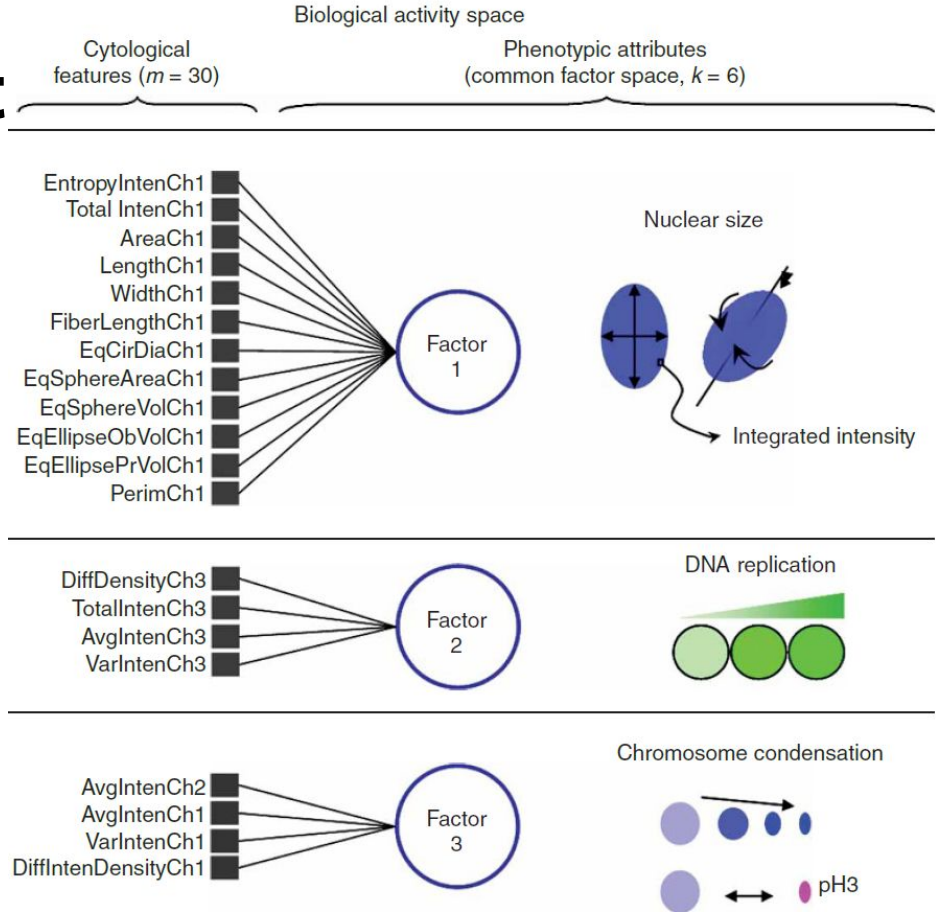
A possible explanation for the success of latent variable models

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

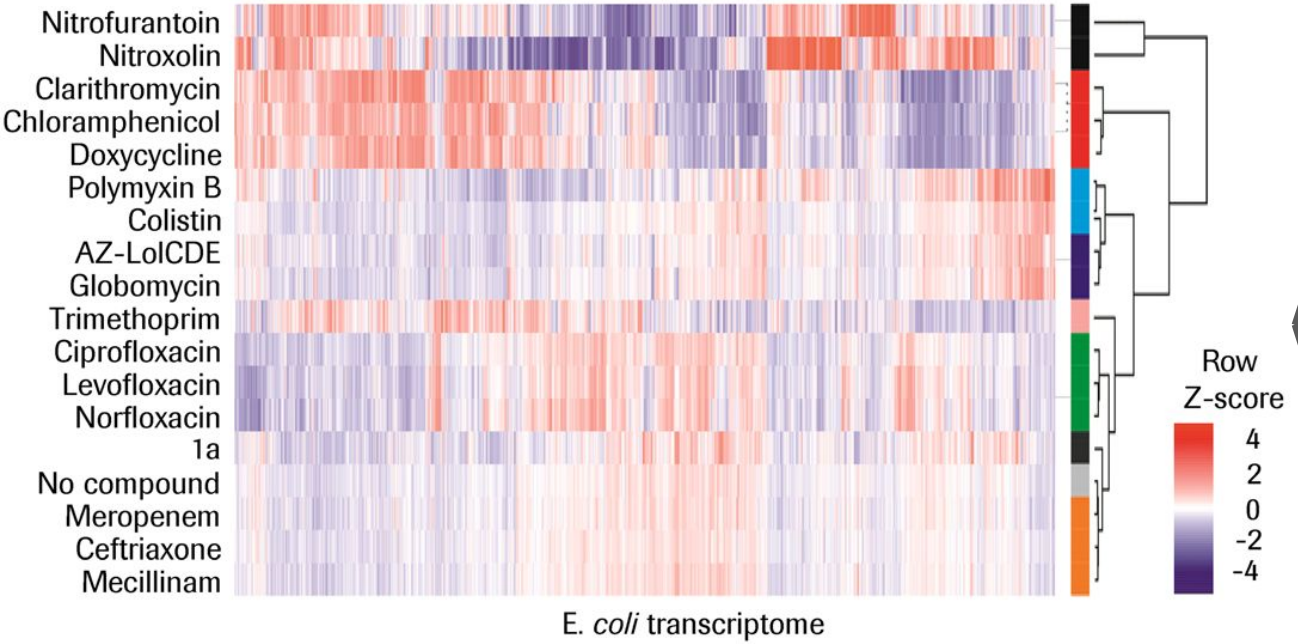
Cytological features

k-factor space

A common latent factor model



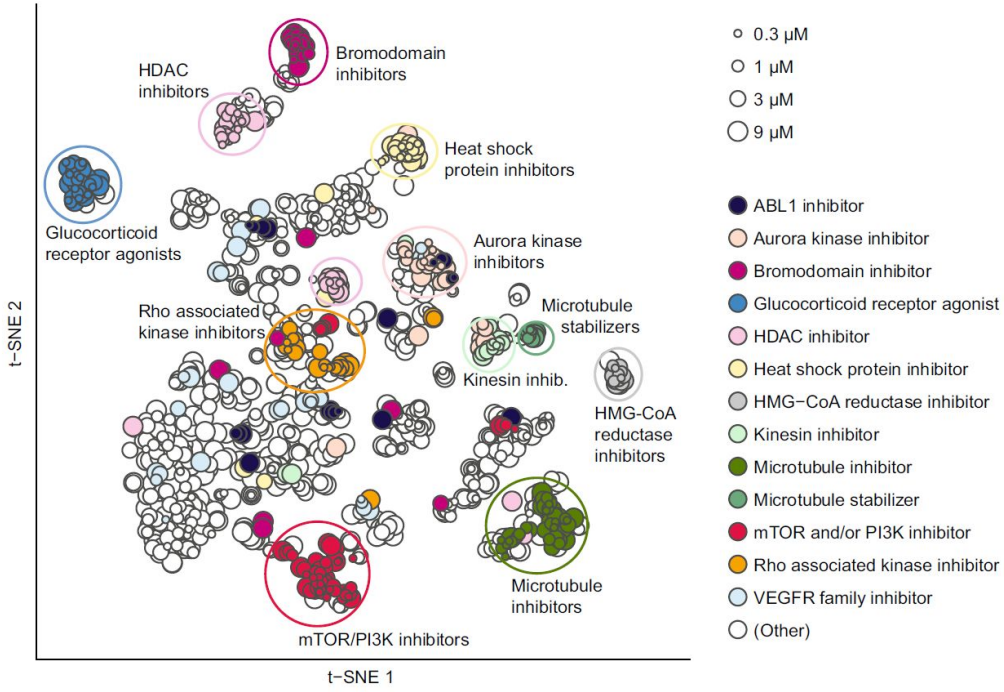
Morphology and gene expression used jointly



E. *coli* transcriptome

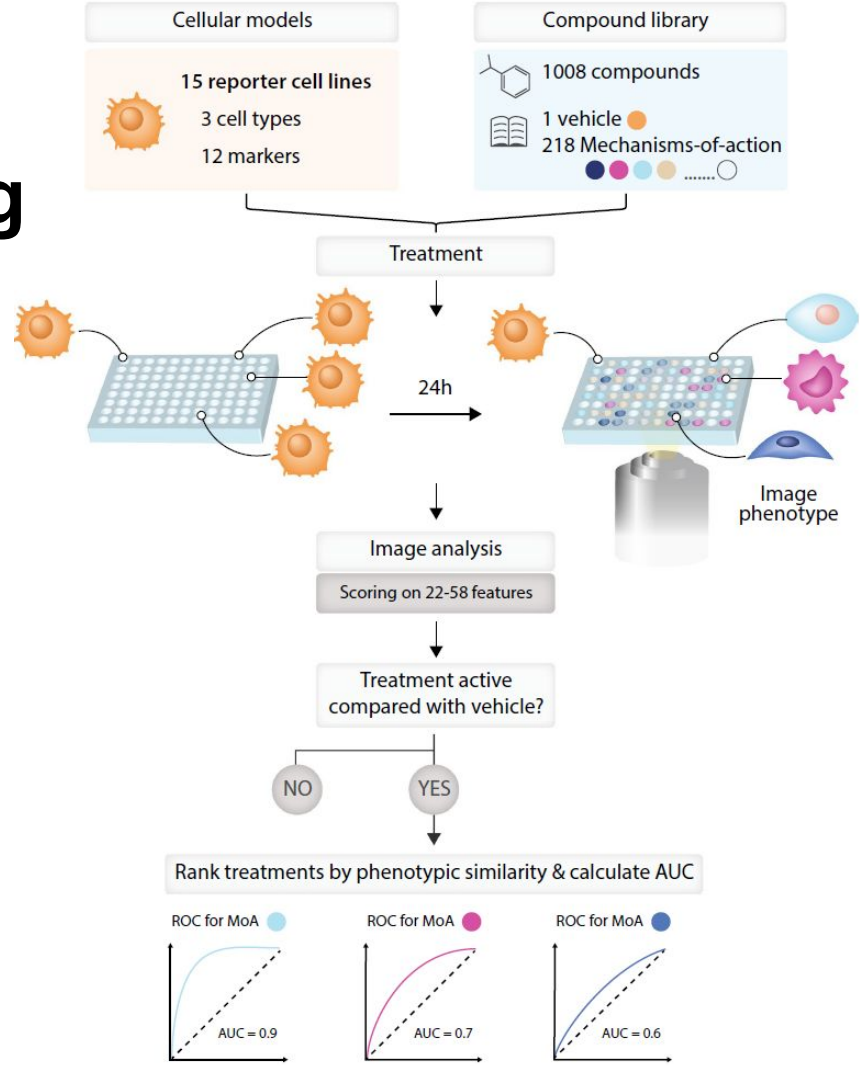
- Gene-set enrichment analysis
- Reporter assays
- Pathway-Phenotype associations

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



- 0.3 μM
- 1 μM
- 3 μM
- 9 μM

- ABL1 inhibitor
- Aurora kinase inhibitor
- Bromodomain inhibitor
- Glucocorticoid receptor agonist
- HDAC inhibitor
- Heat shock protein inhibitor
- HMG−CoA reductase inhibitor
- Kinesin inhibitor
- Microtubule inhibitor
- Microtubule stabilizer
- mTOR and/or PI3K inhibitor
- Rho associated kinase inhibitor
- VEGFR family inhibitor
- (Other)



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.

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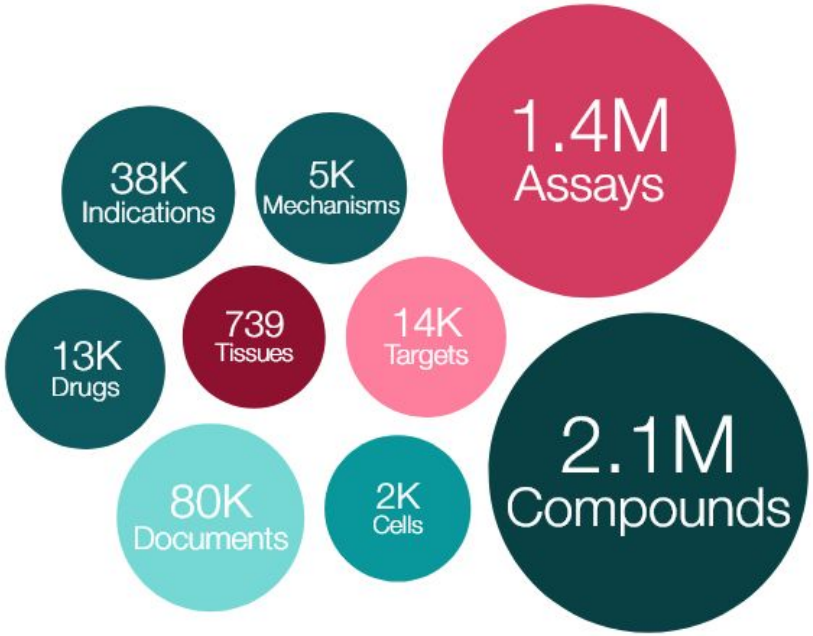
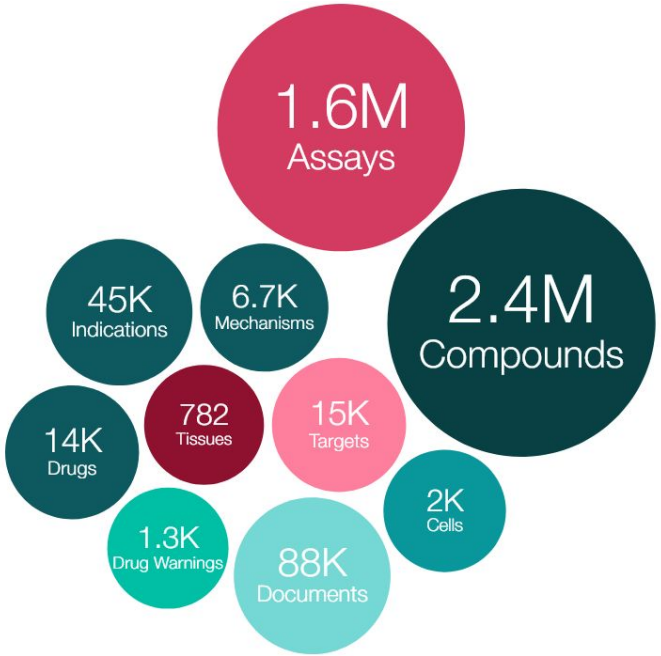
The evolution of ChEMBL database



Visualization of ChEMBL (2021)



Visualization of ChEMBL
(version 33; 2024)