What efficacy and safety profiles can we expect

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

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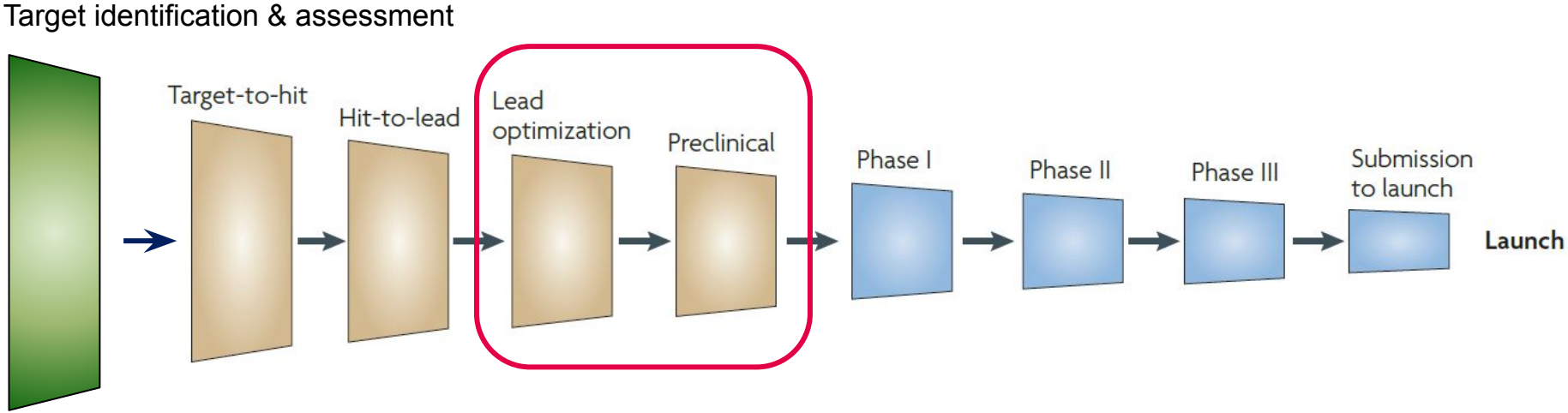
April-May 2023

Outline of Lecture 9

- Understanding pharmacology and toxicology with *in vitro*, *in vivo*, and *in silico* models
- Cell-type specific response to drugs
- Single-cell RNA sequencing for disease understanding and drug discovery

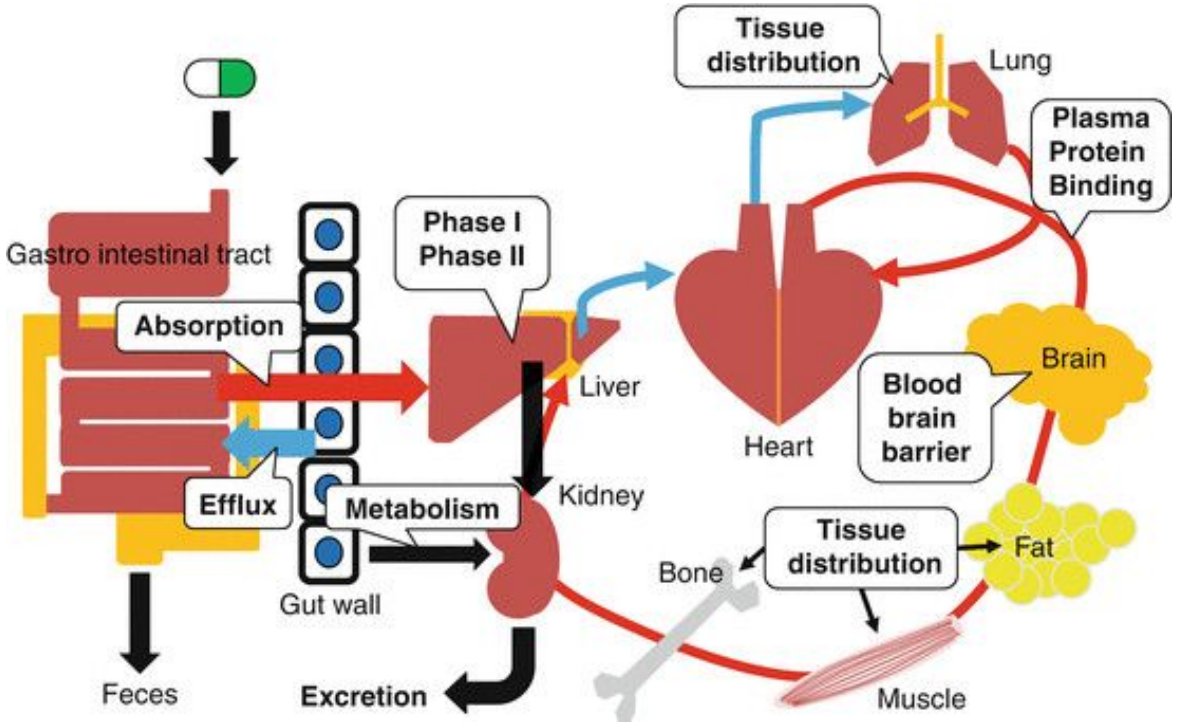
Where are we now



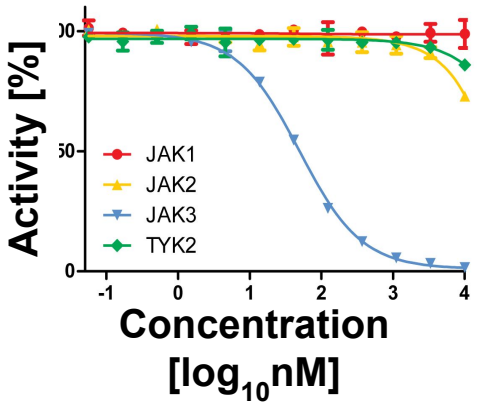
Goal: we want to select **one compound** from a few (~10^2-10^0) for entry in human.

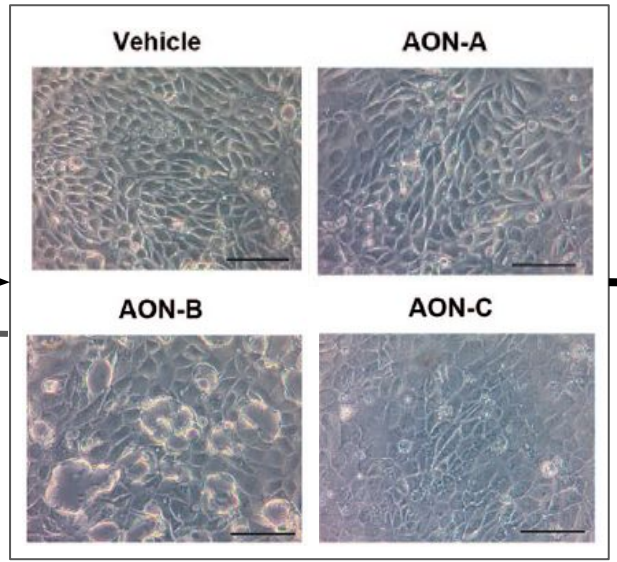
Factors that affect efficacy and safety profiles

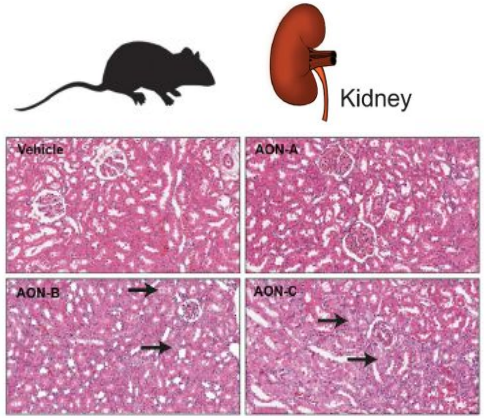
- Absorption
- Distribution
- **Pharmacology**
- **Toxicology**
- Metabolism
- Excretion



Classical workflow of efficacy and toxicity assessment







Biochemical & biophysical assays

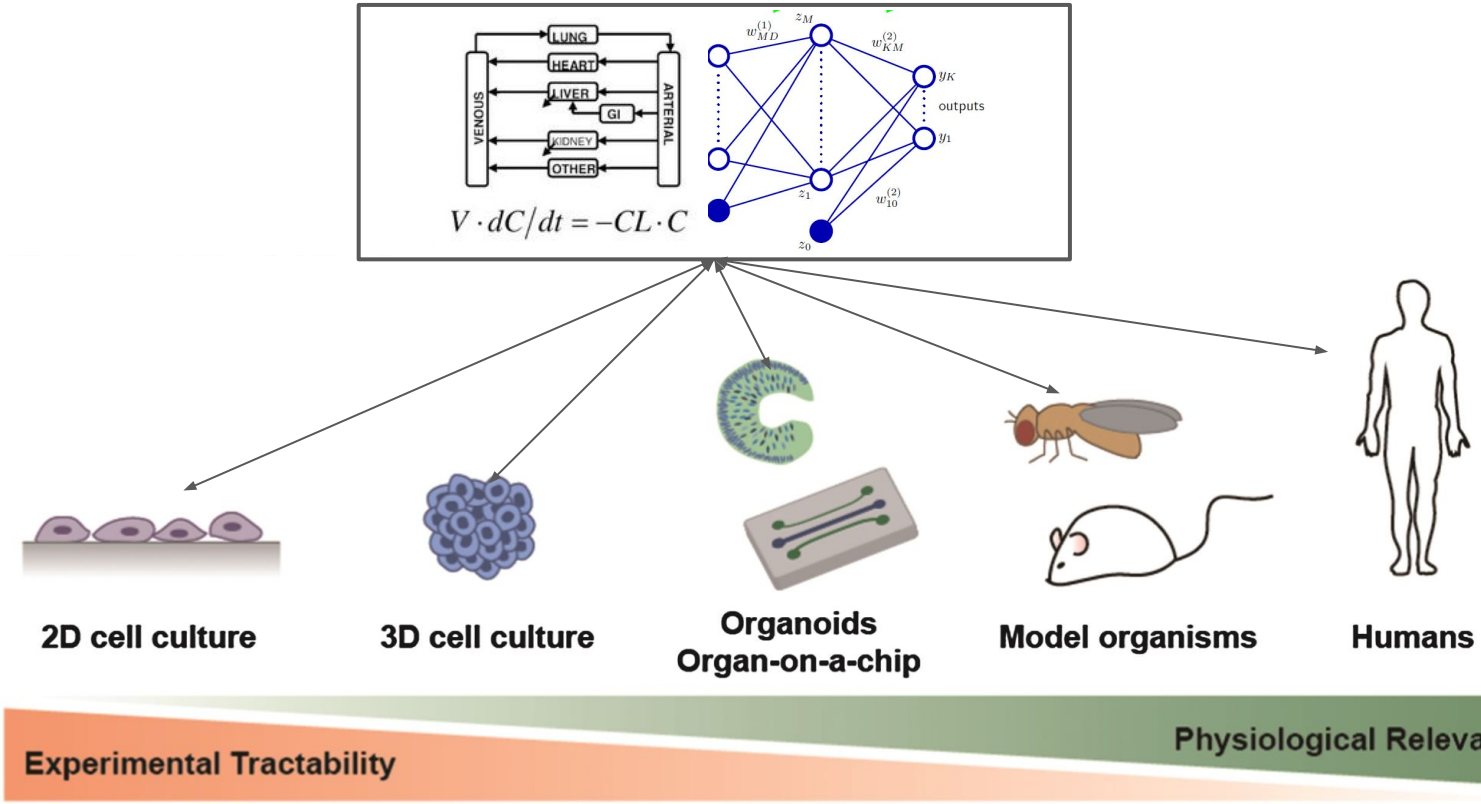
Cellular assays (*in vitro*)

Animal experiments (*in vivo*)

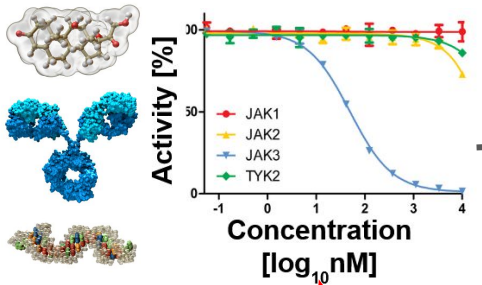
Usual workflow

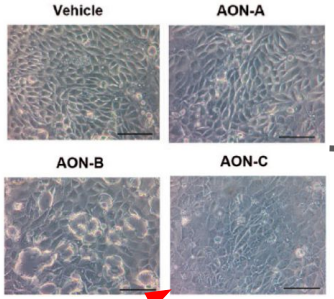
Assay development

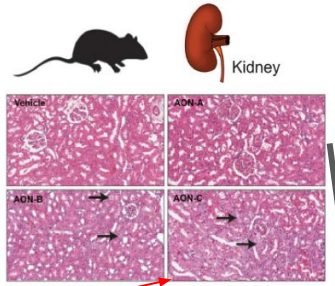
Biological and computational models of human diseases



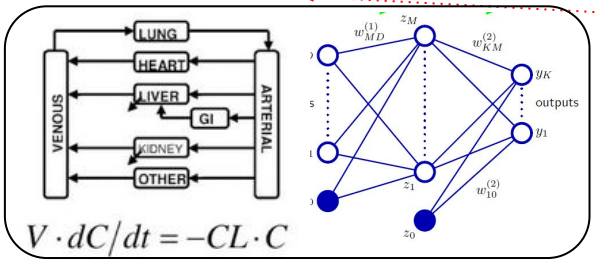
Computational methods empower efficacy and toxicity assessment





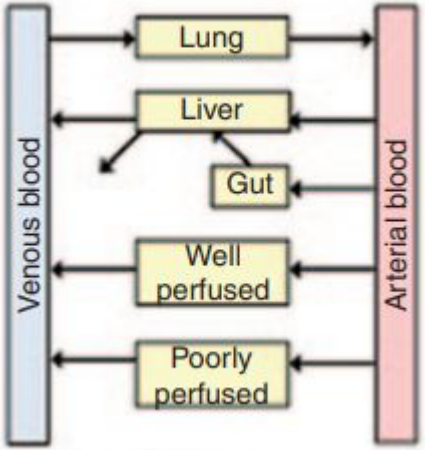


High-throughput technologies (omics, microscopy, etc.)

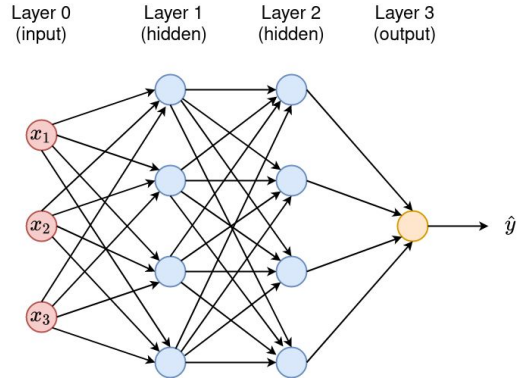


Mechanistic, causal, and statistical models

Three types of computational models

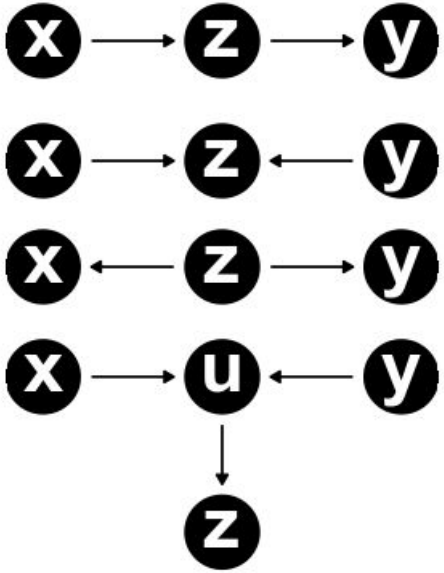


Mechanistic models



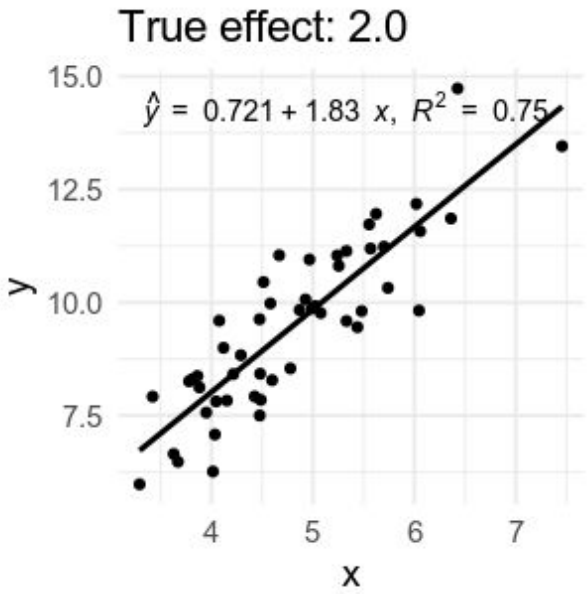
$$y = f(x) + \epsilon$$

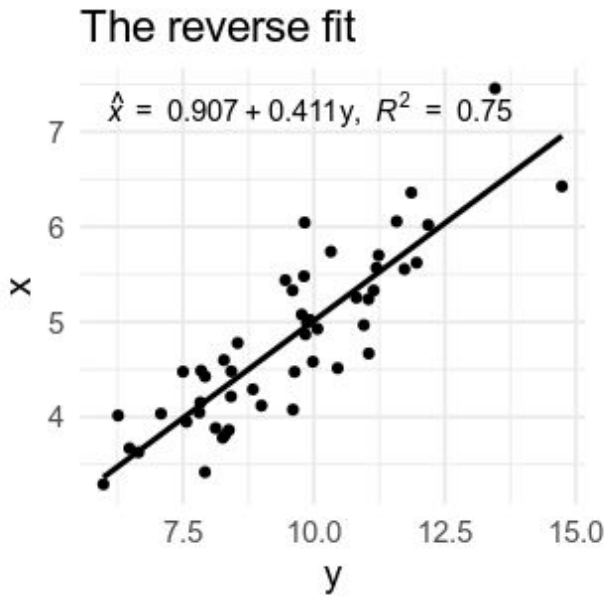
Statistical and machine-learning models

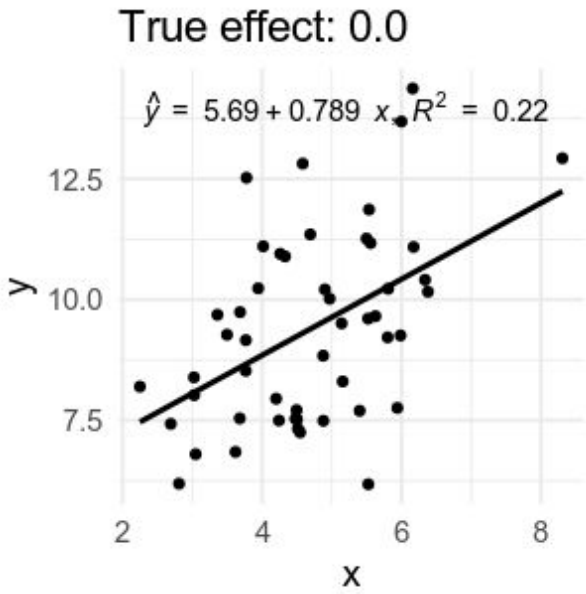


Causal models

Correlation is caused by causation, confounding, conincidence, or conspiracy

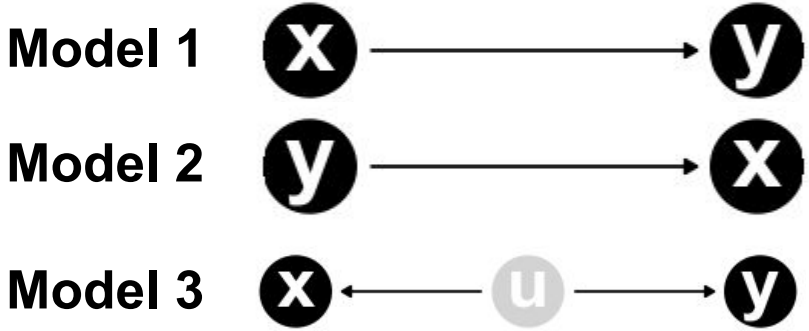






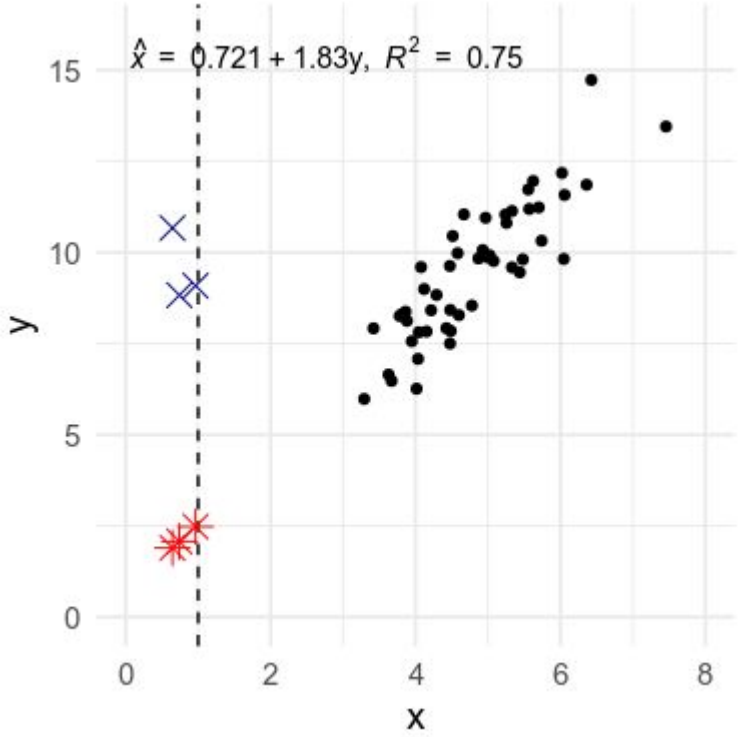
Statistical models alone cannot derive causality from correlation

We learn causality by (1) listing models explicitly and (2) manipulating a variable and observe the outcomes



Assume that the data is generated by either Model 1, or Model 2, or Model 3. And assume that we can manipulate the value of X by setting it to 1.0 (the dash line).

Question: which outcomes (red stars or blue crosses) would support which models? Why?



Causality is crucial for drug discovery

?

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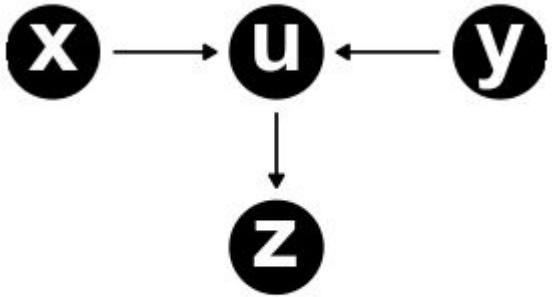
Biomarker, tox study, pathology, omics data, real-world data, …

	x	z	y
1	0.835386320	1	-0.73897252
2	-0.005354014	-1	-0.82972315
3	0.058788286	1	0.76213369
4	-1.015602246	-1	-0.05951719
5	-0.339569780	-1	-0.11745910
6	-0.041077979	-1	-1.28243716
7	0.363740407	1	-0.30570762
8	0.119496314	-1	-1.19932461
9	0.257108454	-1	-1.06044066
10	0.304537158	-1	-0.43396492

?

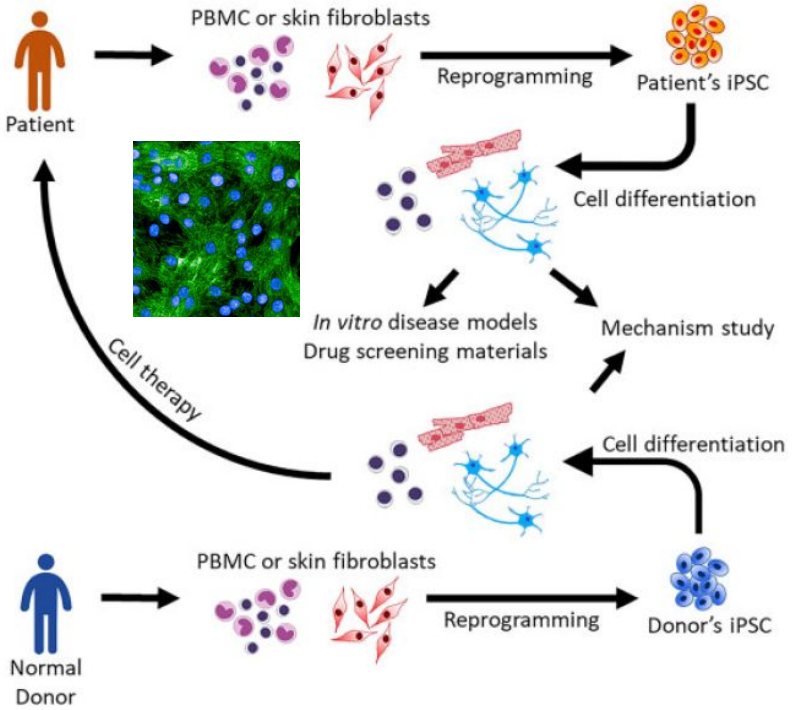
?

The descendant

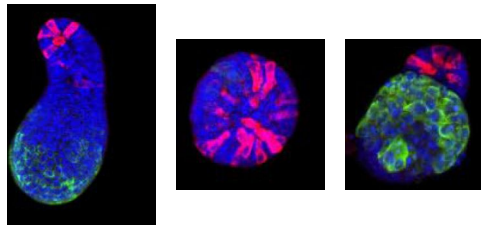


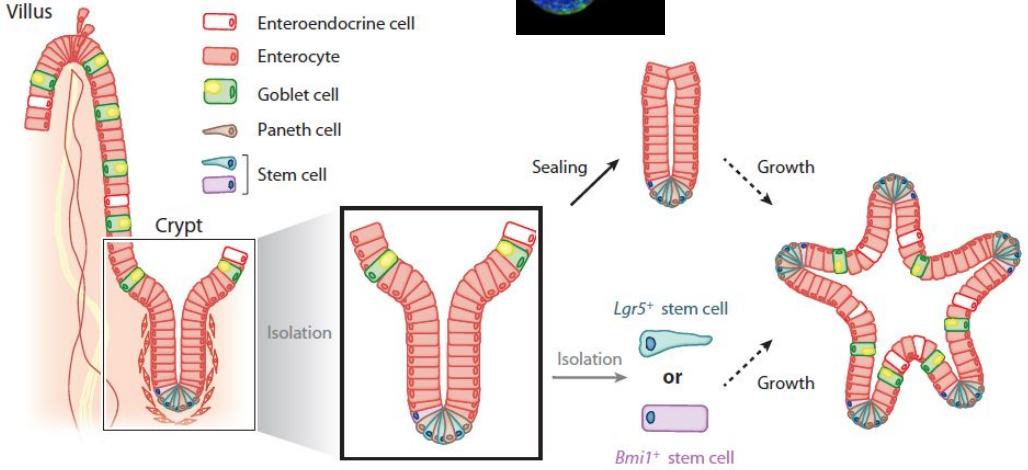
We need both models (knowledge + assumptions) and data to infer causality.

Stem cells and organoids empower efficacy and toxicity assessment



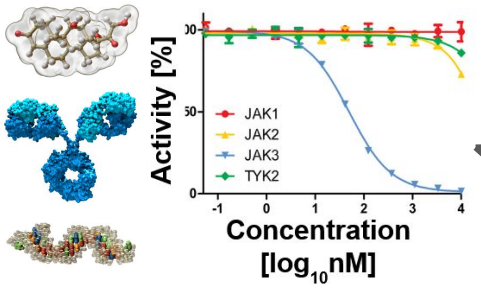
Induced pluripotent stem-cells

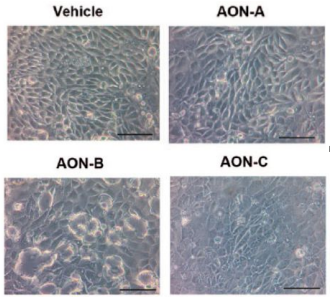


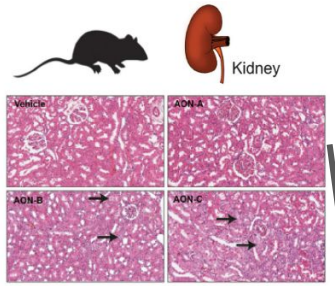


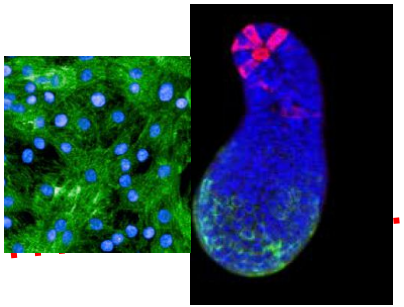
Small-intestinal organoids

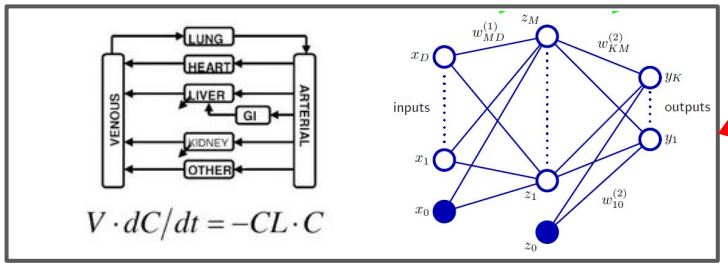
Computational methods and novel biological models empower efficacy and toxicity assessment



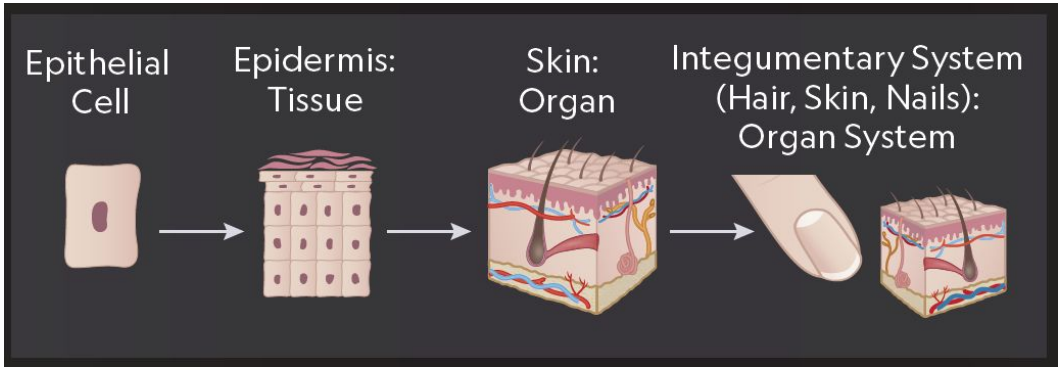








Complexity Increases Through a System

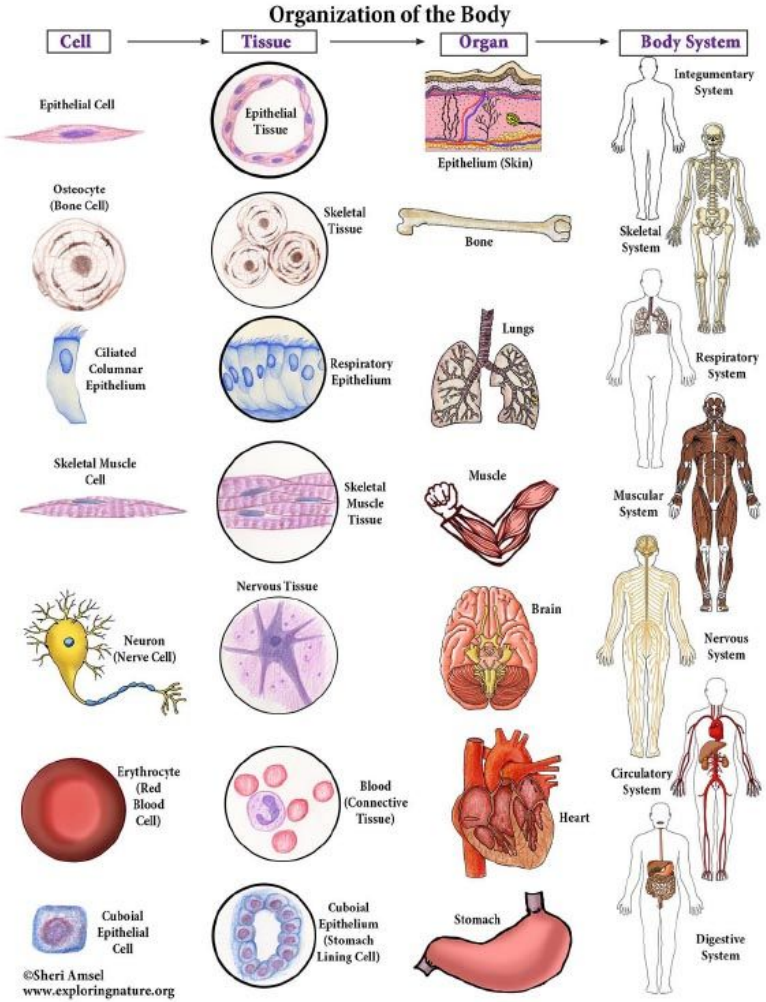


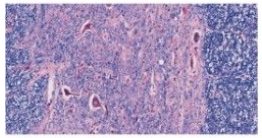
Cells: basic building blocks, variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

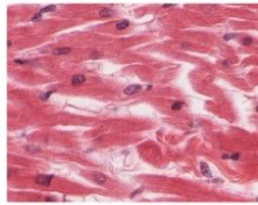
Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues



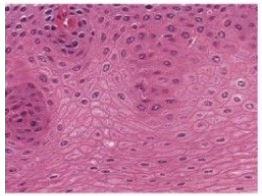


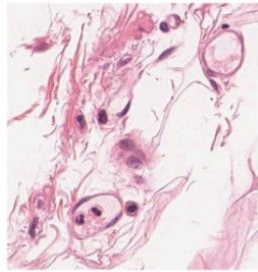
Nervous tissue
Brain
Spinal cord
Nerves



Muscle tissue
Cardiac muscle
Smooth muscle
Skeletal muscle

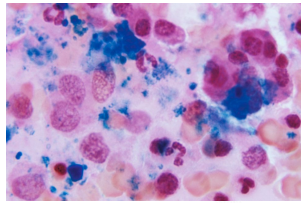
Epithelial tissue
Lining of GI tract organs and other hollow organs
Skin surface (epidermis)

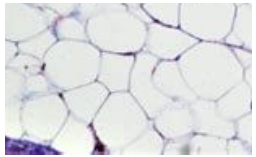


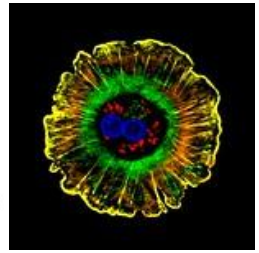


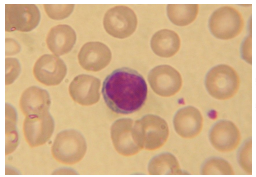
Connective tissue
Fat and other soft padding tissue
Bone
Tendon

Four major tissue types







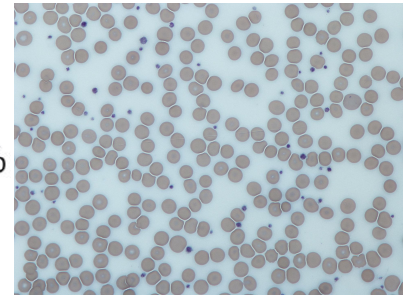


adipocytes 0.2%

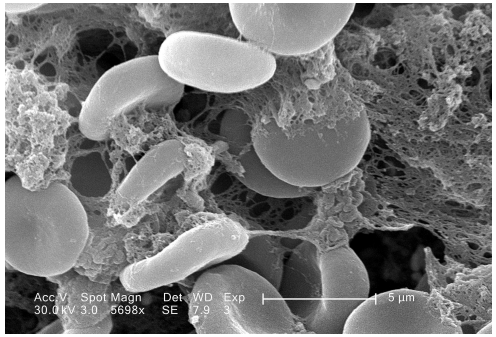
lymphocytes 1.5%

bone marrow cells 2.5%

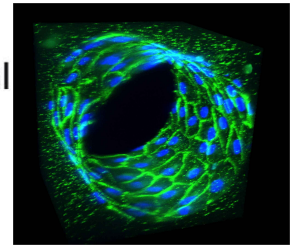
hepatocytes 0.8%



platelets 4.9%

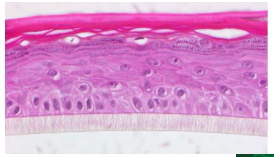


erythrocytes, 84%
25×10^{12} of 30×10^{12}
total human cell count



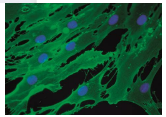
bronchial endothelial cells 0.5%

vascular endothelial cells 2.1%



epidermal cells 0.5%

respiratory interstitial cells 0.5%

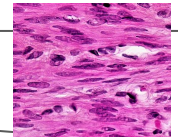


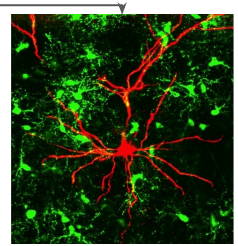
dermal fibroblasts 0.1%

muscle cells 0.001%

other 2.0%

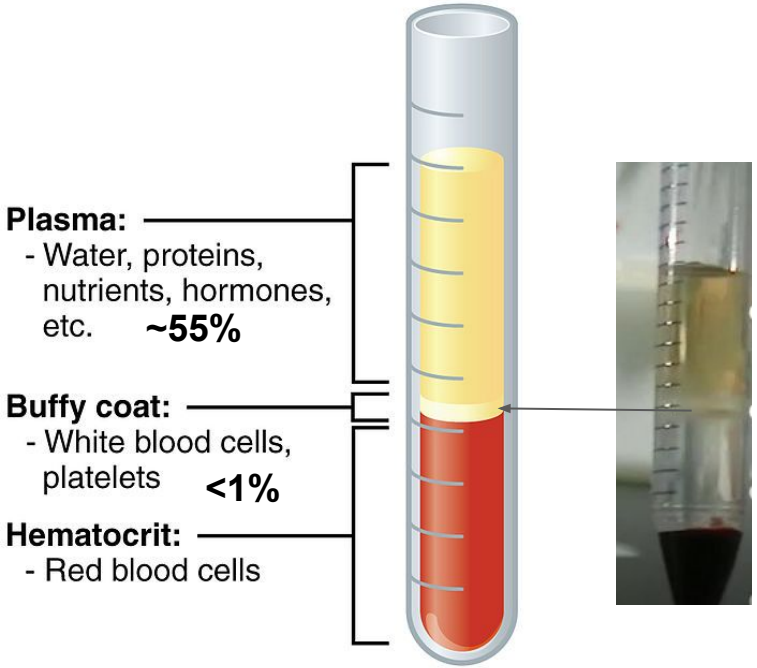
neurons and glia 0.6%

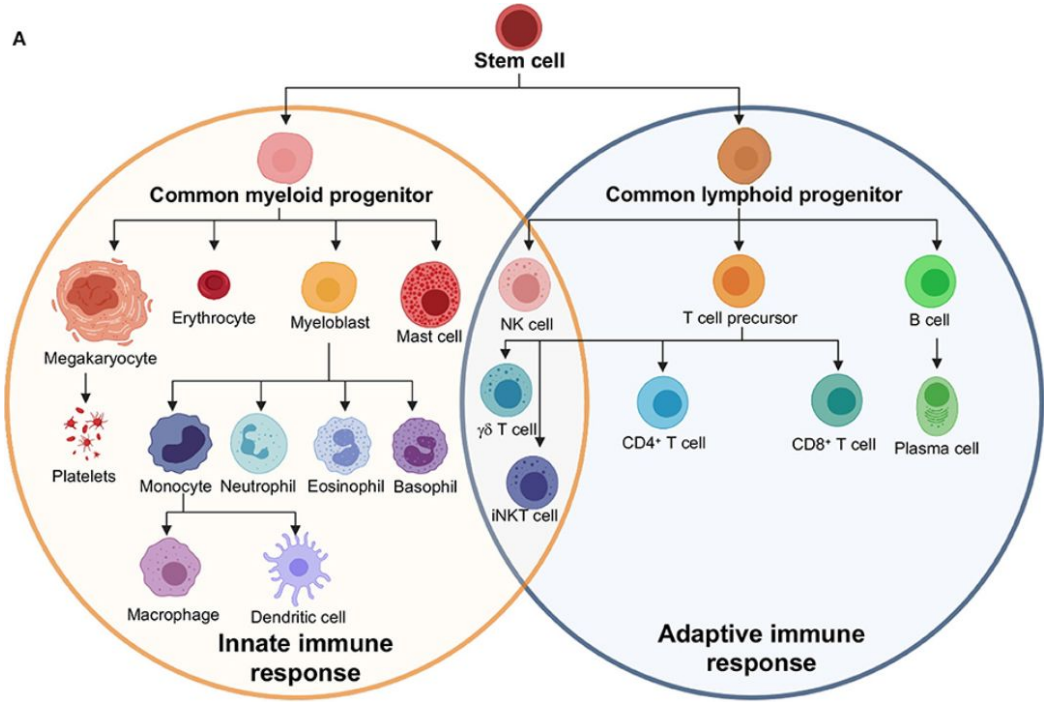




UNI BASEL

What's in a drop of blood? Ask a doctor or a biologist!

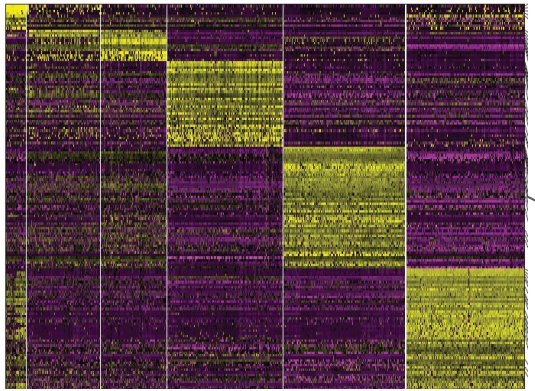




What's in a drop of blood? Count the genes!

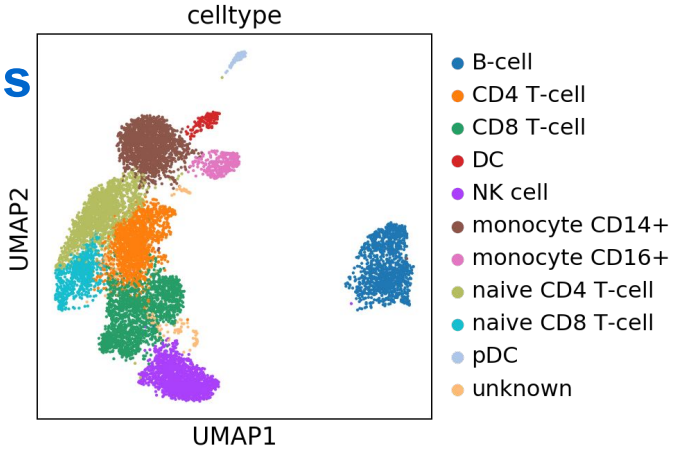
Sequencing

Genes



Cells

Data analysis



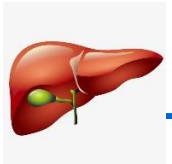
Low Expression

High Expression

Single-cell sequencing (scSeq) workflow

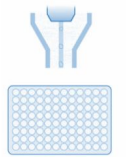
Tissue dissociation

Single cell capture and transcriptome sequencing



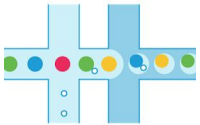
Solid tissue

Dissociated cells/nuclei



Flow cytometry 96-384 cells

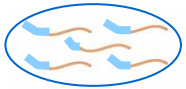
Cell/nuclei suspension



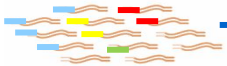
Droplet microfluidics 1000s of cells

Throughput

Single cells or nuclei



cDNA



Pooled cDNA libraries

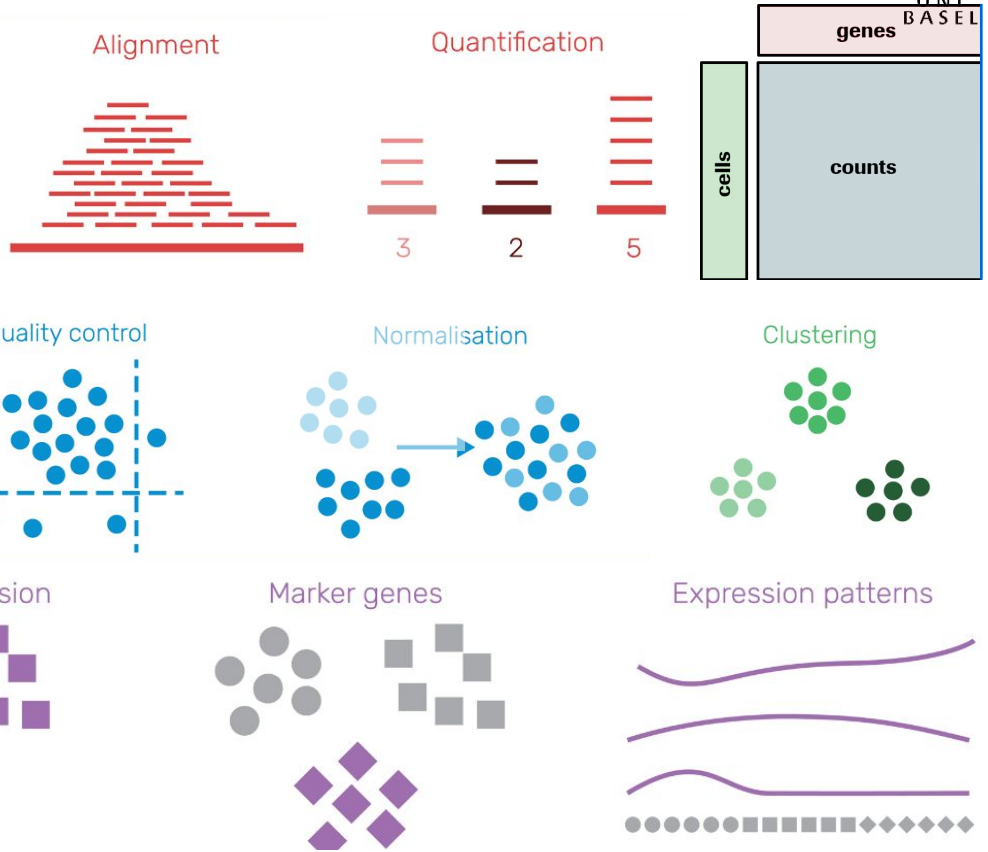
Next-Generation Sequencing

A linearized workflow of scSeq data analysis

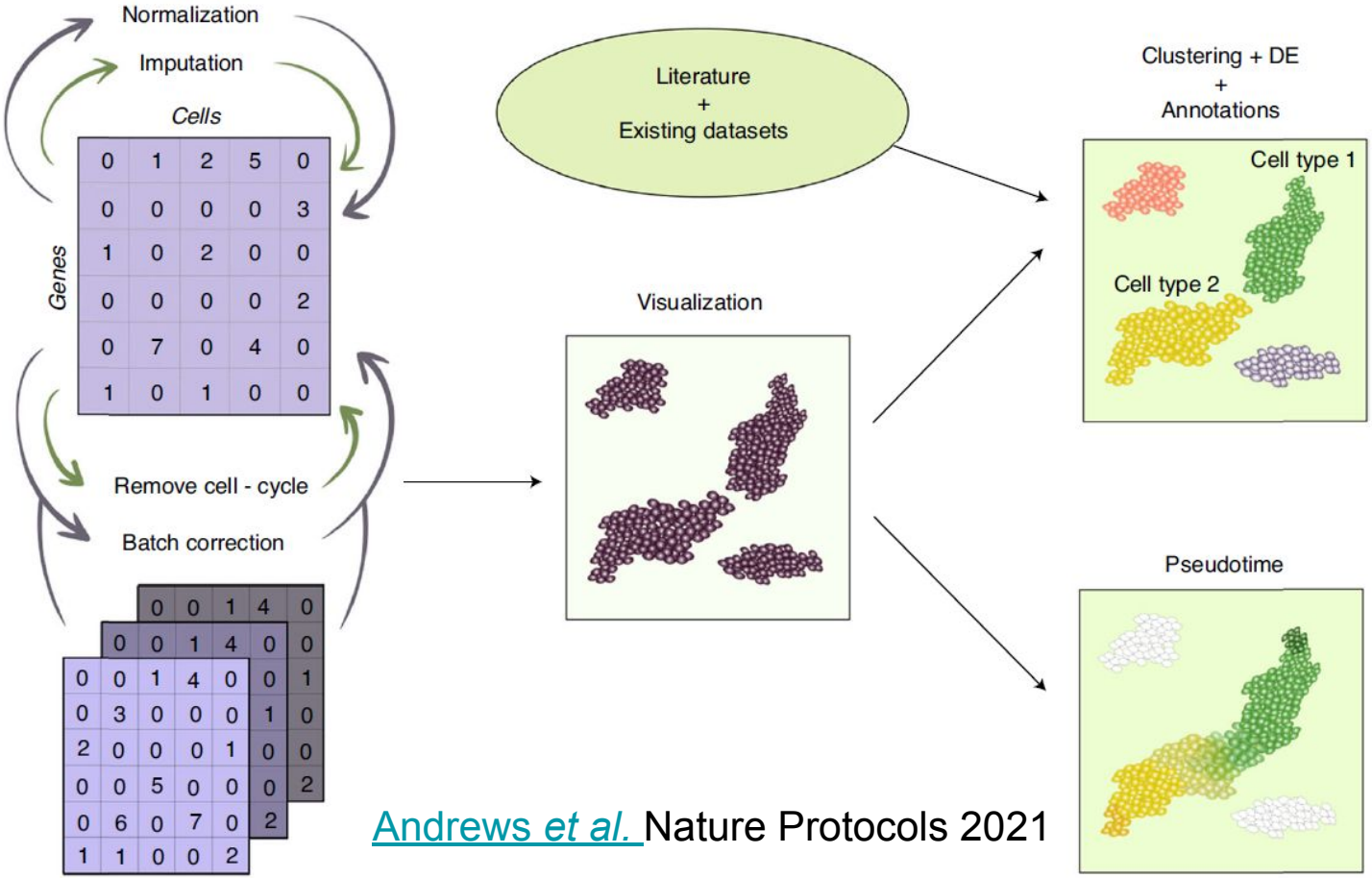
From short reads to gene-cell matrix

QC, filtering & normalization, dimensionality reduction, and clustering

Downstream analysis

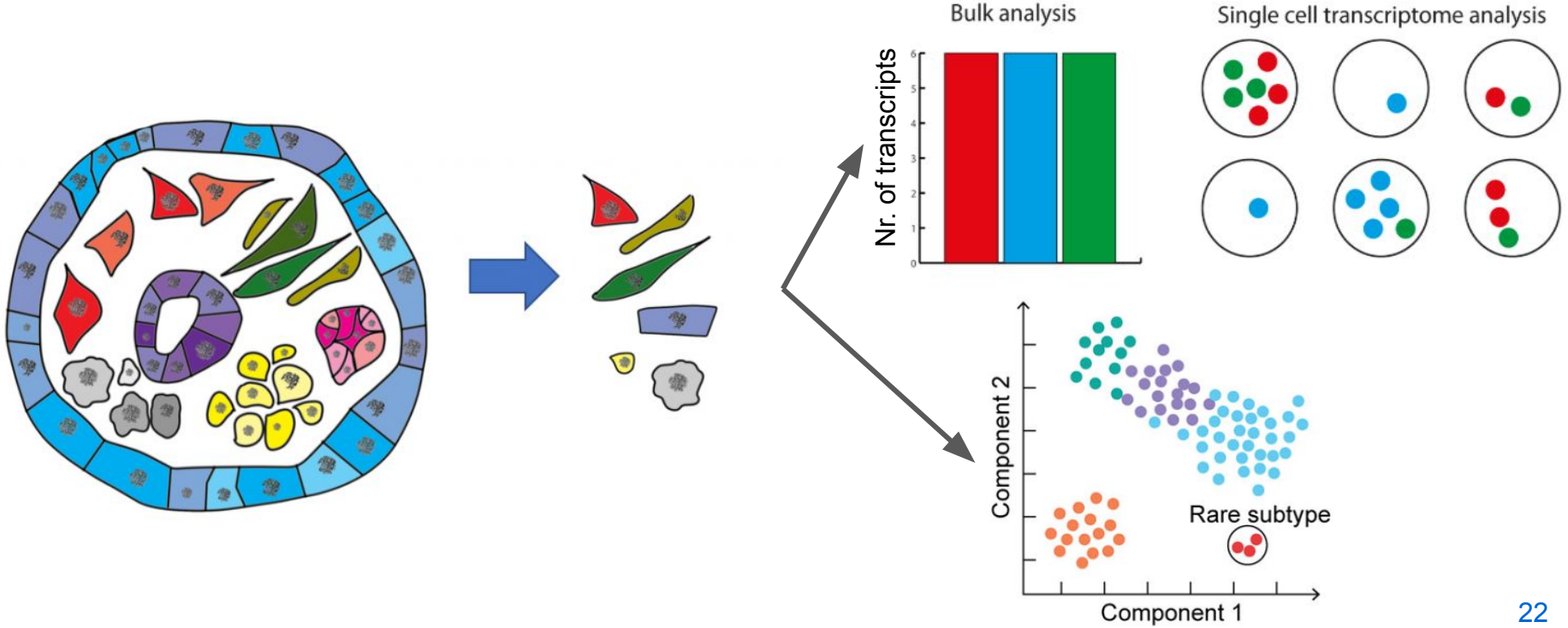


Overview of the computational workflow



Andrews *et al.* Nature Protocols 2021

Single-cell biology benefits both disease understanding and drug discovery

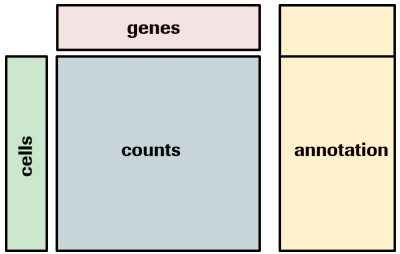


BESCA: An open-source Python package for single-cell gene expression analysis

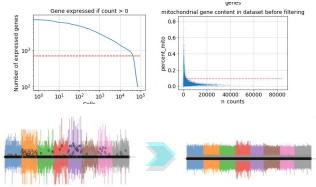
An automatized standard workflow

Data loading → Preprocessing → Clustering → Visualization → Analysis

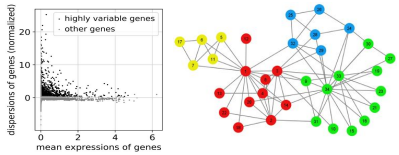
From FAIR format



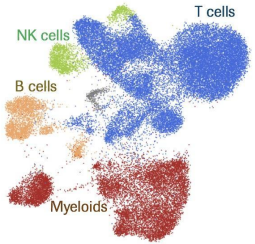
Quality Control
Filtering,
Normalization



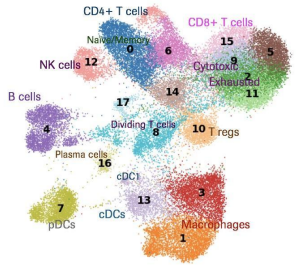
Dimensionality
reduction
Clustering



UMAP



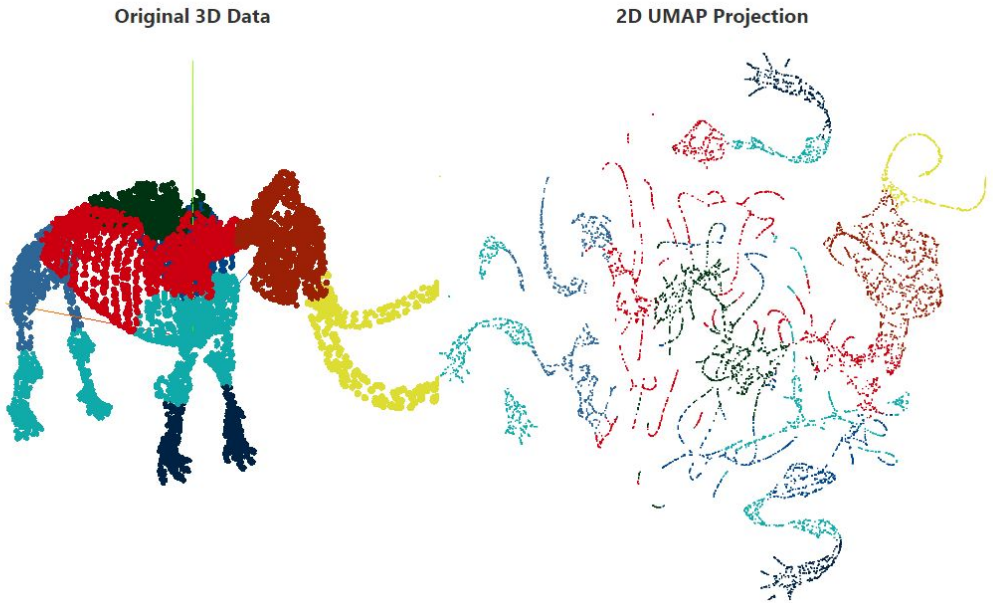
Cell type
characterisation

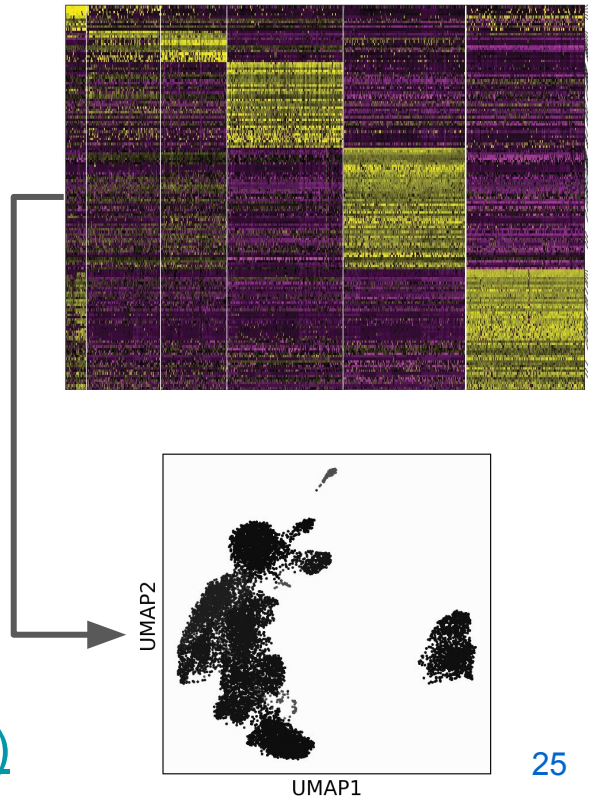


How to represent voxels with pixels?

The elephant bull *Tusker* at Zolli Basel plays with a tree trunk on a post (2022)

Uniform Manifold Approximation and Projection (UMAP) for dimension reduction

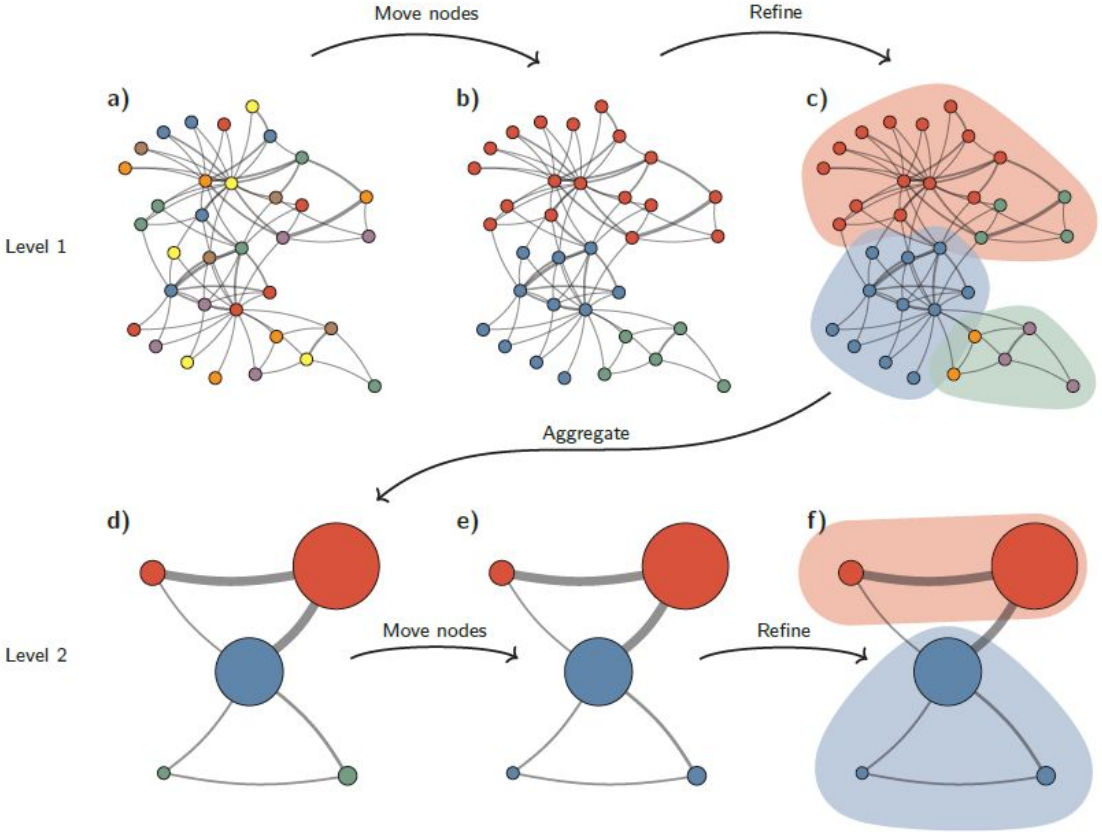


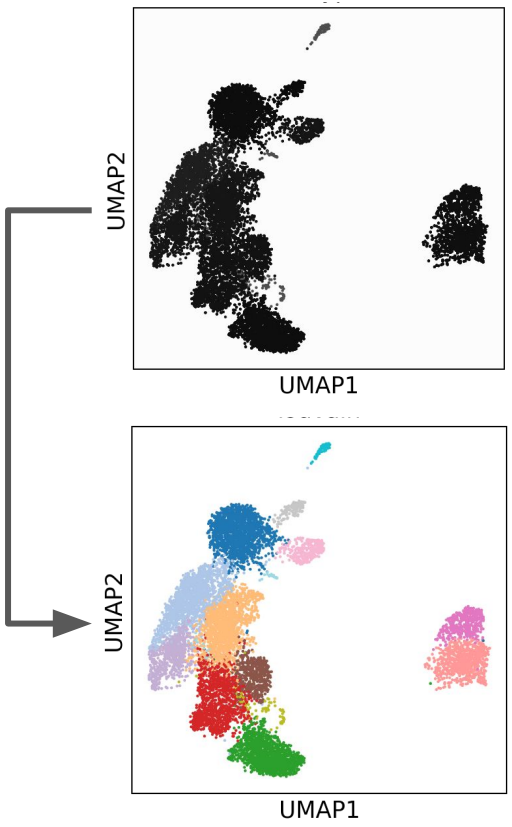


Understanding UMAP by A. Coenen and A. Pearce

UMAP by Leland McInnes on SciPy 2018 (YouTube)

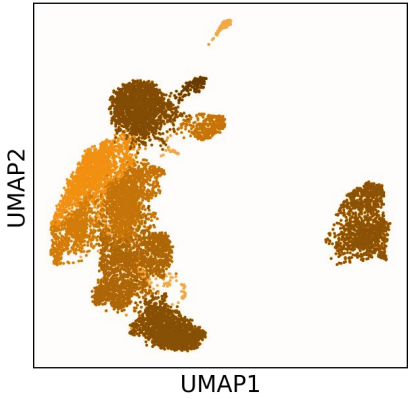
The Leiden Algorithm for Community Detection



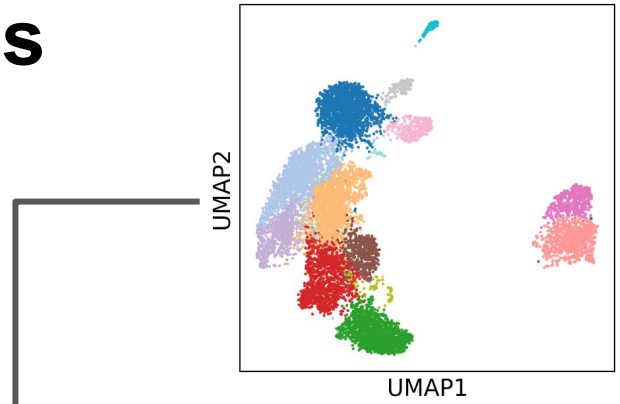


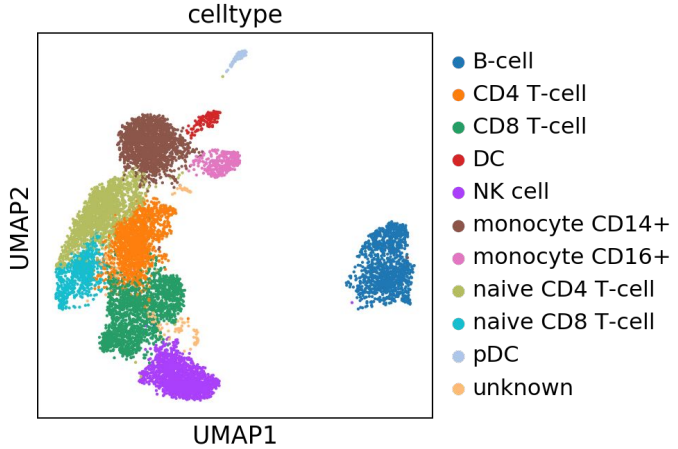
Biological knowledge and visual inspection is used to annotate cell types

Heatmap of gene X

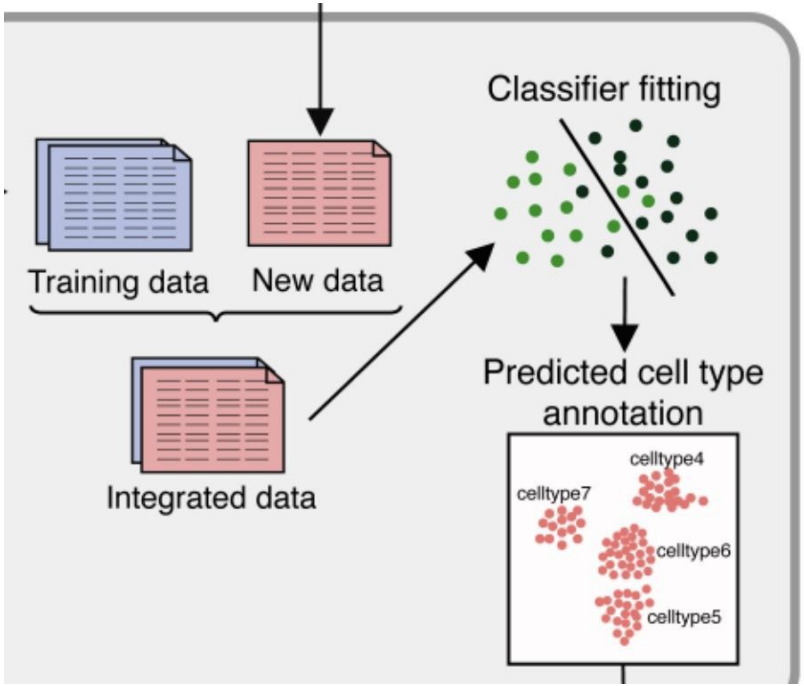


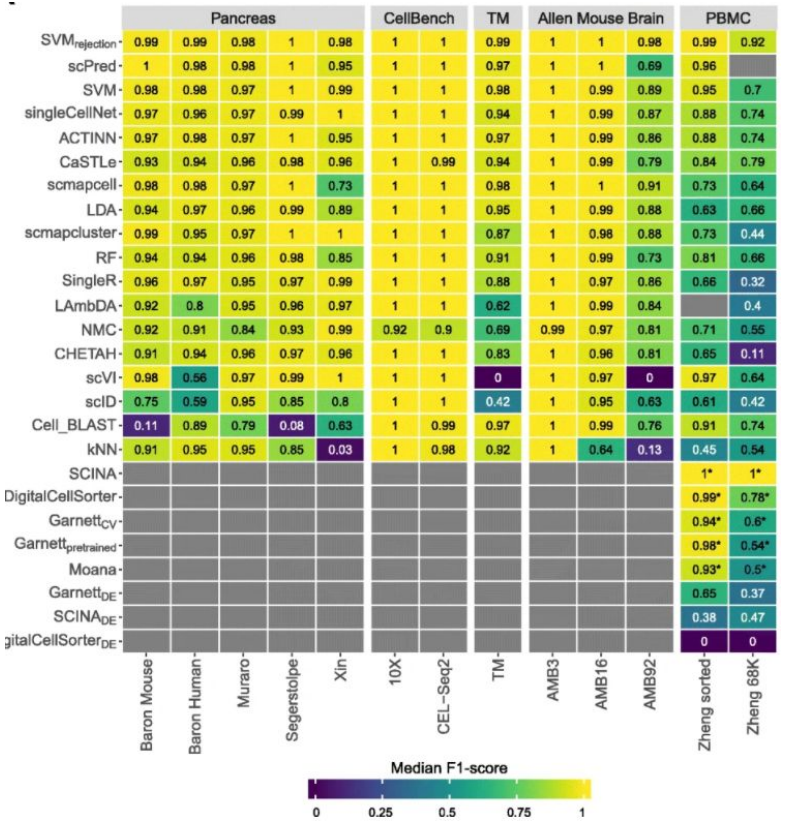
lymphocyte	PTPRC							
myeloid	S100A8	S100A9	CST3					
Bcell	CD19	CD79A	MS4A1					
Tcells	CD3E	CD3G	CD3D					
CD4	CD4							
CD8	CD8A	CD8B						
NKcell	NKG7	GNLY	NCAM1					
monocyte	CST3	CSF1R	ITGAM	CD14	FCGR3A	FCGR3B		
macrophage	CD14	IL1B	LYZ	CD163	ITGAX	CD68	CSF1R	FCGR3A



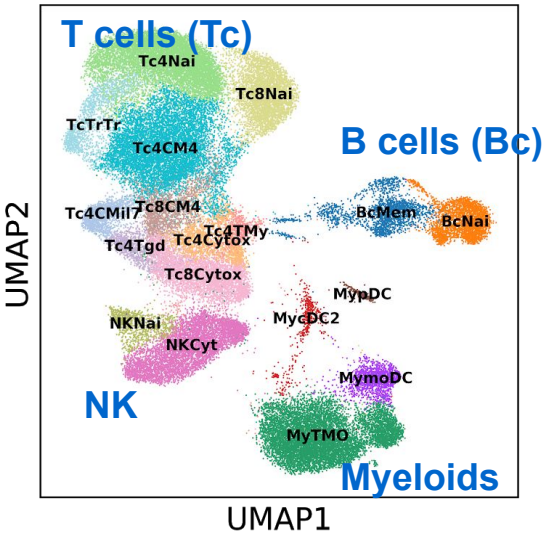


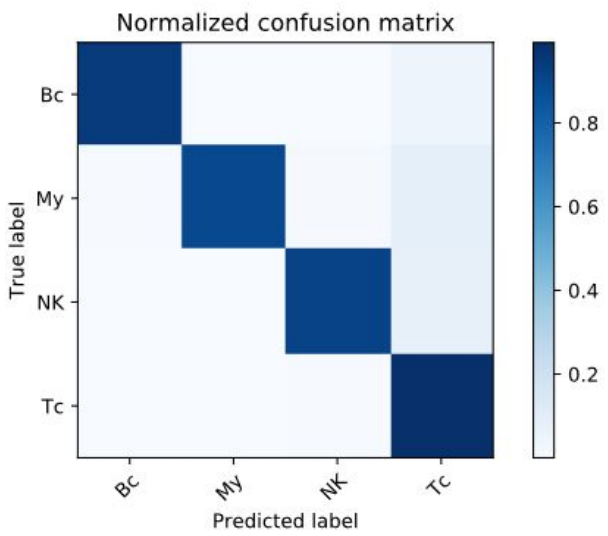
Cell type annotation with machine learning

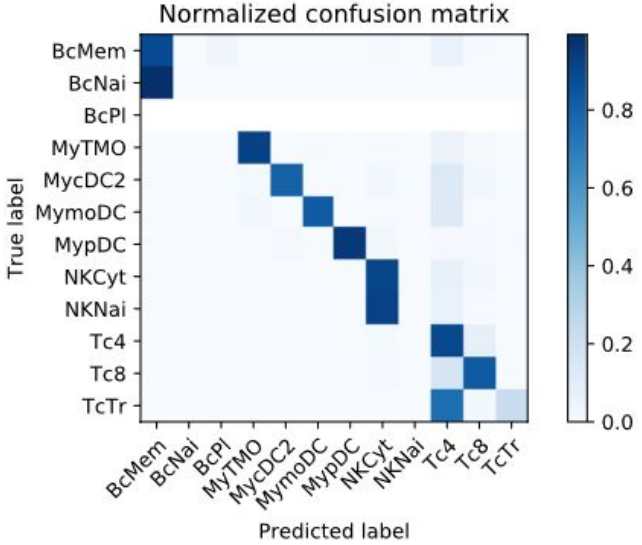




A PBMC example of cell type annotation







- Broad level cell types, including B cells (Bc), Myeloid (My), NK cells (NK) and T cells (Tc), are successfully predicted.
- Missing and highly similar cell types cause challenges with increased granularity. Essential: reference data quality and knowledge of cell types.

Single-cell biology is important in drug discovery

Disease understanding: disease-specific cell types and states

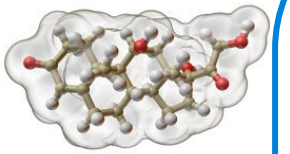
Biomarker and patient stratification: which genes should we measure in which cell type(s)?

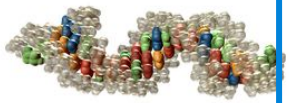
Target identification: expression pattern in health and disease across cell types

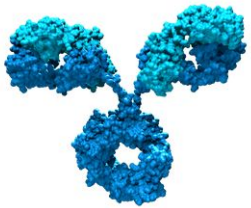
MoA and safety modelling: perturbation effect at single-cell level

End of Lecture 8

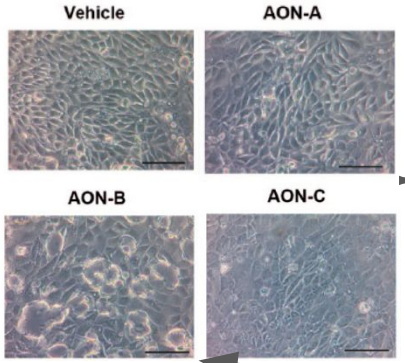
Proteomics plays an important role in *in vitro*/*in vivo* translation



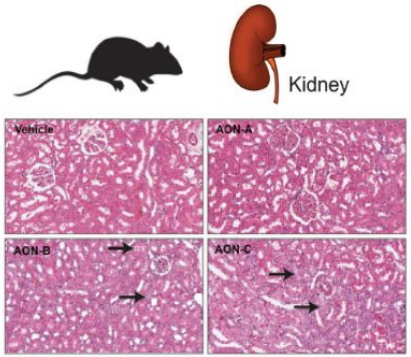




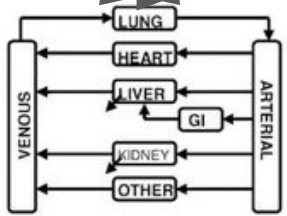
in vitro



in vivo



Clinical trial

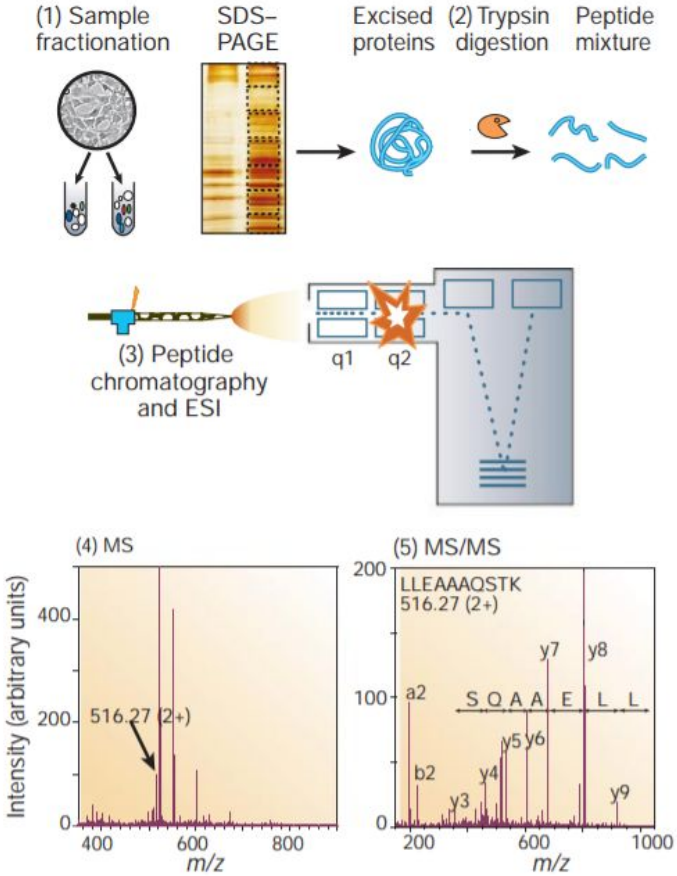


in silico

$$V \cdot dC/dt = -CL \cdot C$$

Mass-spectrometry based proteomics

- **SDS-PAGE**: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
- **ESI**: Electrospray ionization
- **q1/q2**: selection/collision/separation cells
- **MS**: Mass spectrometry
- **MS/MS**: tandem mass spectrometry



Proteomics approaches for drug discovery

Affinity purification

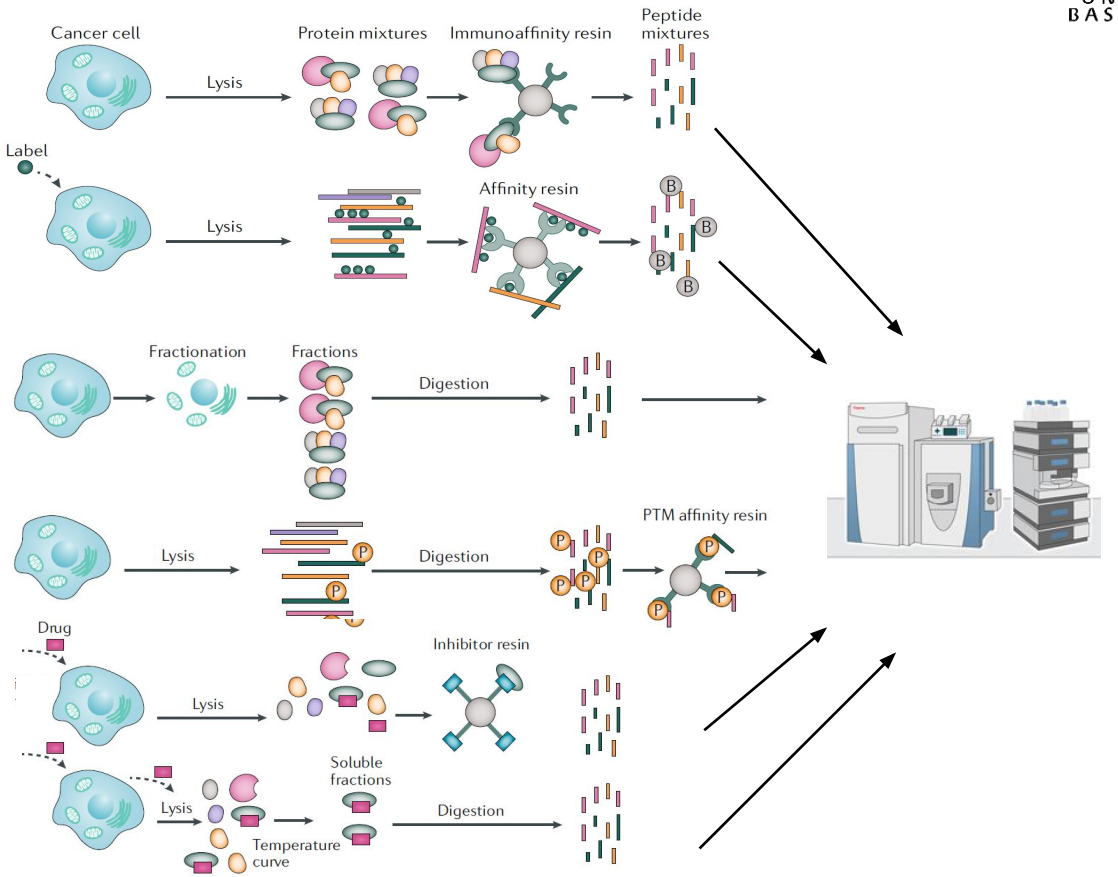
Proximity labelling

Organelle proteome profiling

Post-translational modification (PTM) profiling

Chemoaffinity enrichment

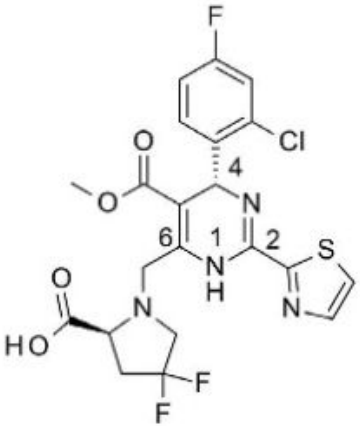
Thermal proteome profiling



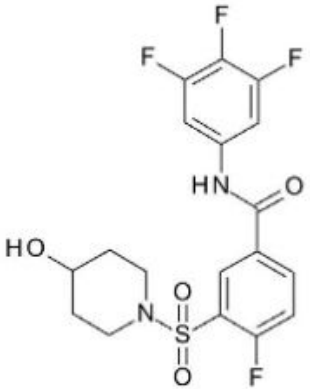
Case 1: Differentiate two compounds that inhibit Hepatitis B Virus with similar mode of action

a

HAP_R01

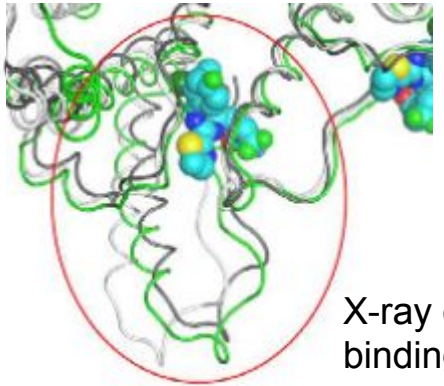


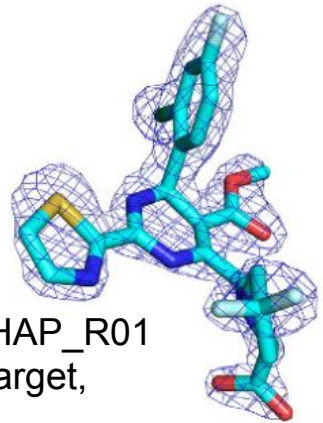
SBA_R01



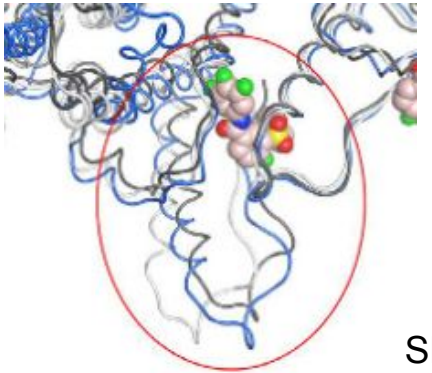
b

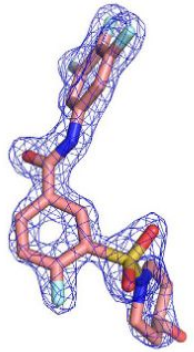
Compound	IC_{50} (μM)	HepG2.2.15 EC_{50} (μM)	CC_{50} (μM)
HAP_R01	0.39±0.13	0.0064±0.0006	34.8±1.8
SBA_R01	1.90±0.22	0.26±0.02	8.05±0.92





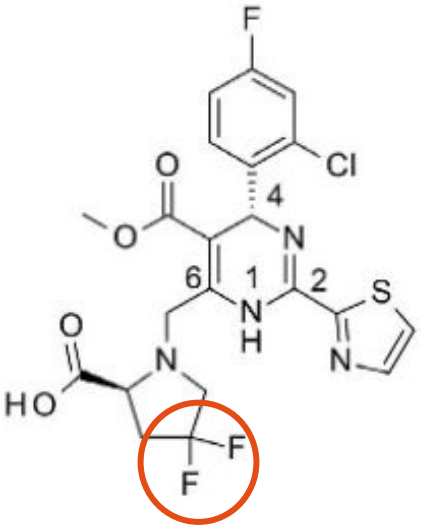
X-ray data of HAP_R01 binding to its target, HBV capsid



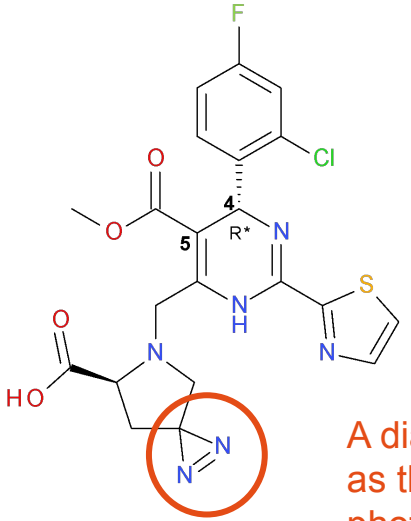


SBA_R01

Chemical probes: drug-like molecules to probe its mode of action

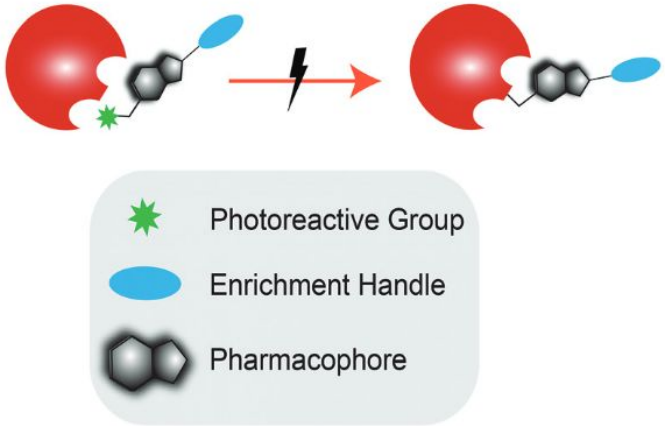


Compound	IC_{50} (μM)
HAP_R01	0.39±0.13

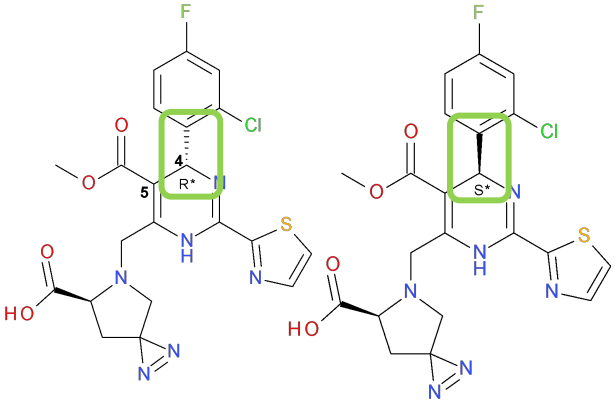


RO-A
EC_{50}: **0.040 μM**
IC_{50}: **0.47 μM**

A diazirine group as the photoreactive group



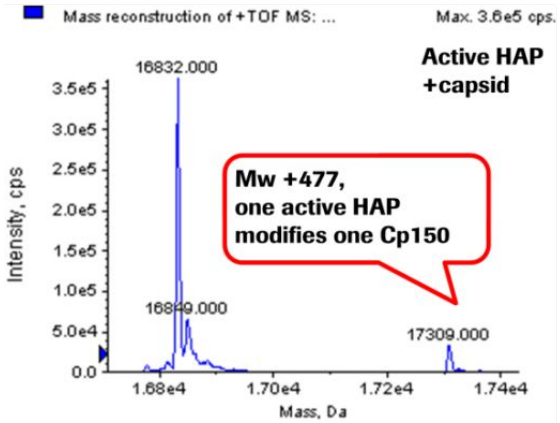
Case 1 solved: Proteomics confirmed target binding and mapped the small molecule binding pocket

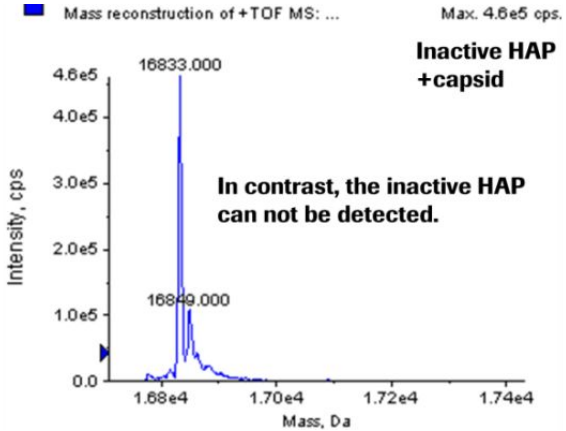


RO-A
EC_{50}: **0.040 µM**
IC_{50}: **0.47 µM**

RO-B
EC_{50}: **>1 µM**
IC_{50}: **>100 µM**

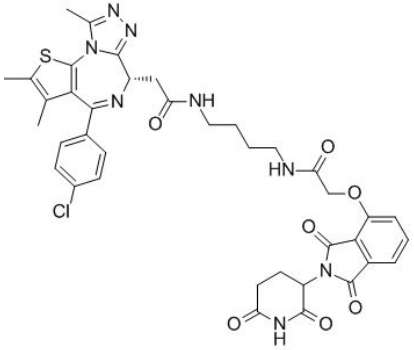
+Cp150, UV, MS



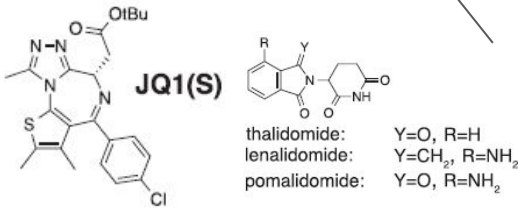


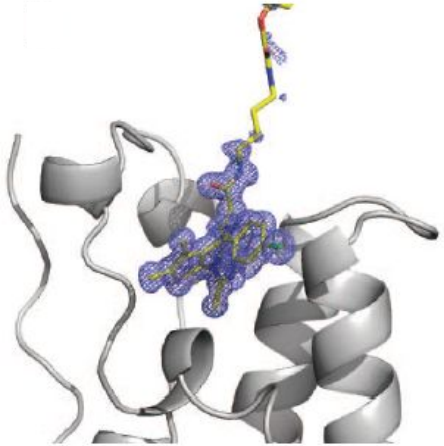
Proteolytic digestion/LC-MS/MS identified labelling site Y118 (Y=Tyrosine) of HBV capsid protein. More photoaffinity probes identified labelling sites at R127 (R=Arginine) and Y38.

Case 2: Confirmation of selective degradation of protein target *in vivo*

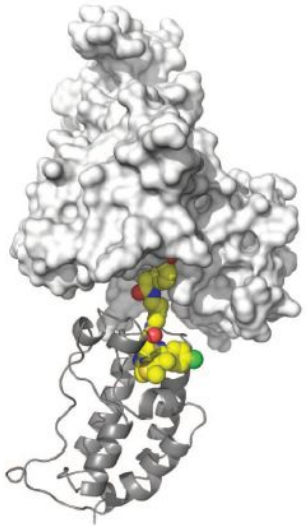


dBET1

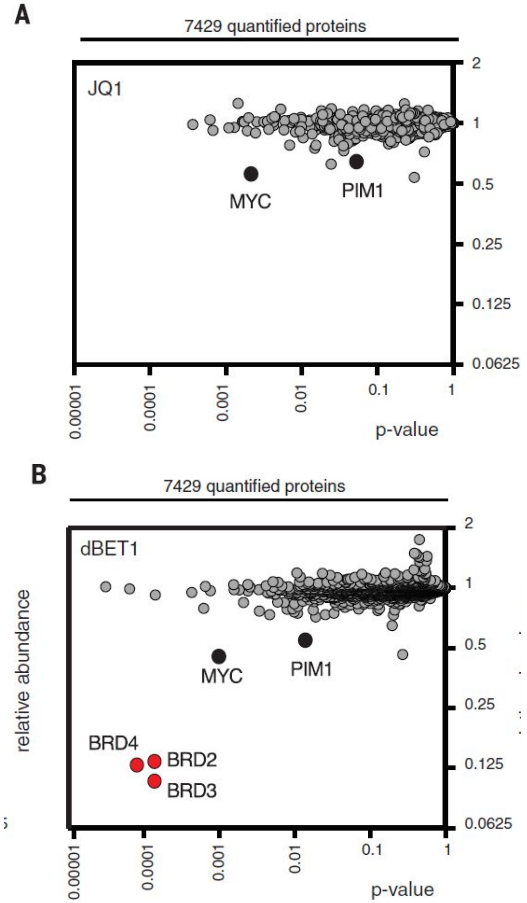




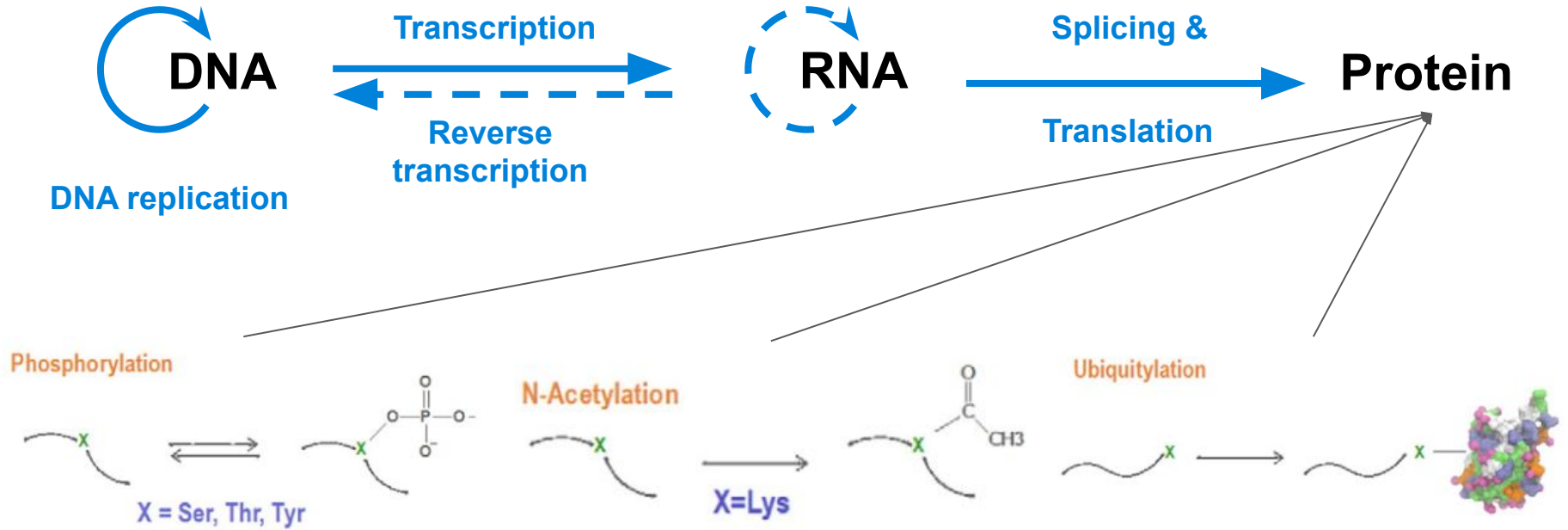
Crystal structure of dBET1 binding to its target BRD4



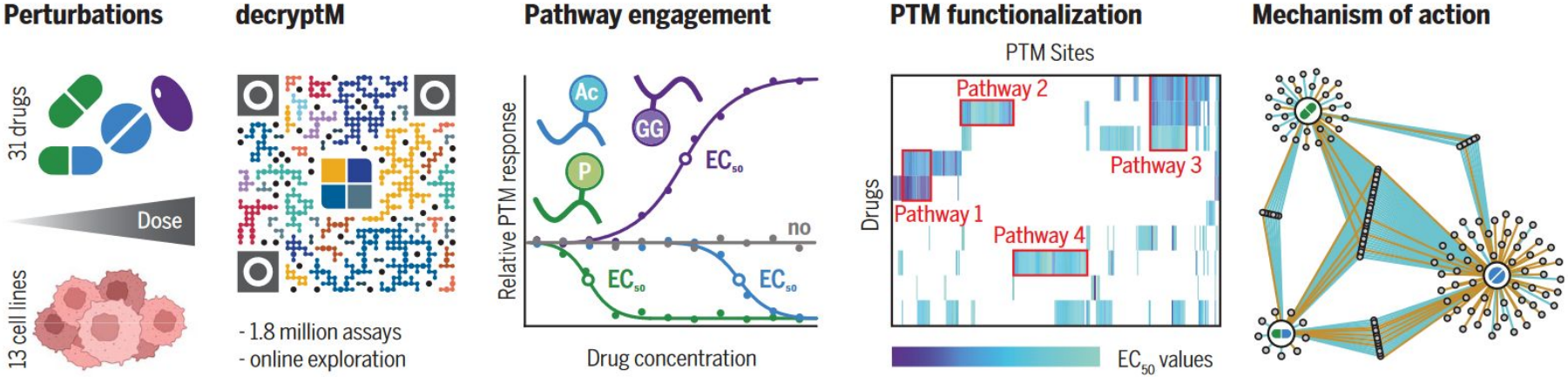
Docking of dBET1-BRD4 to DDB1-CRBN structure



Protein post-translational modifications (PTMs) offer an additional layer of regulation

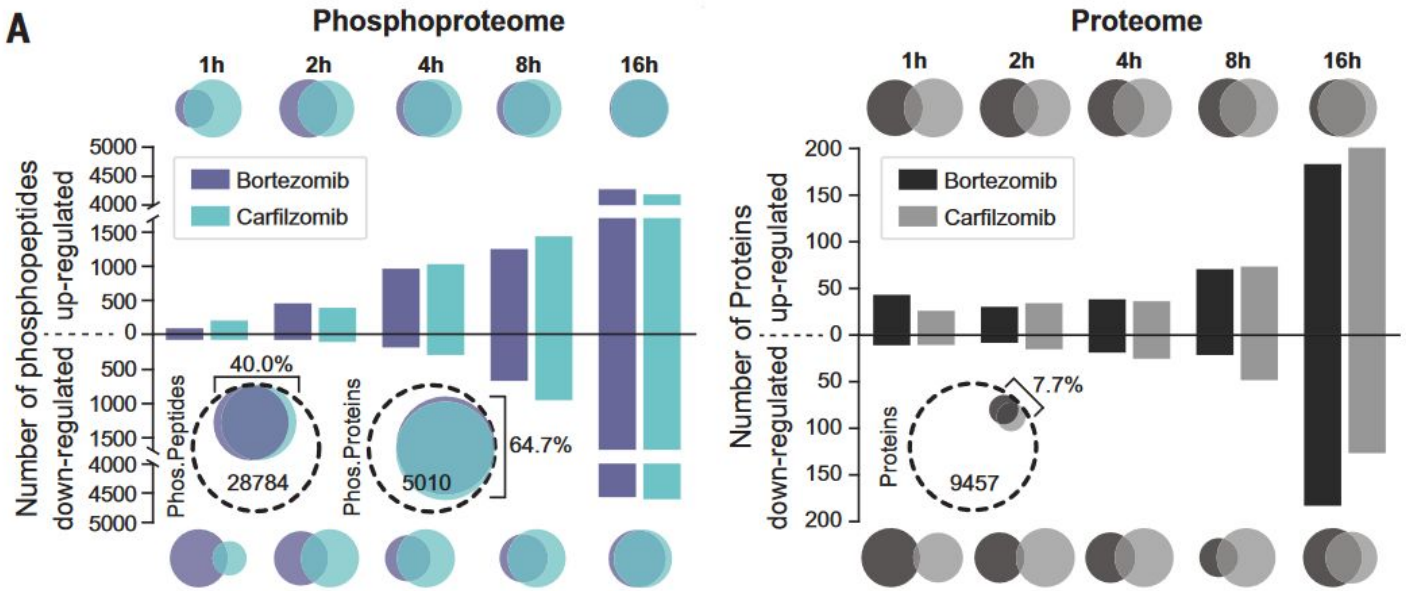


Case 3: Millions of PTM profiles induced by drugs in cancer cell lines



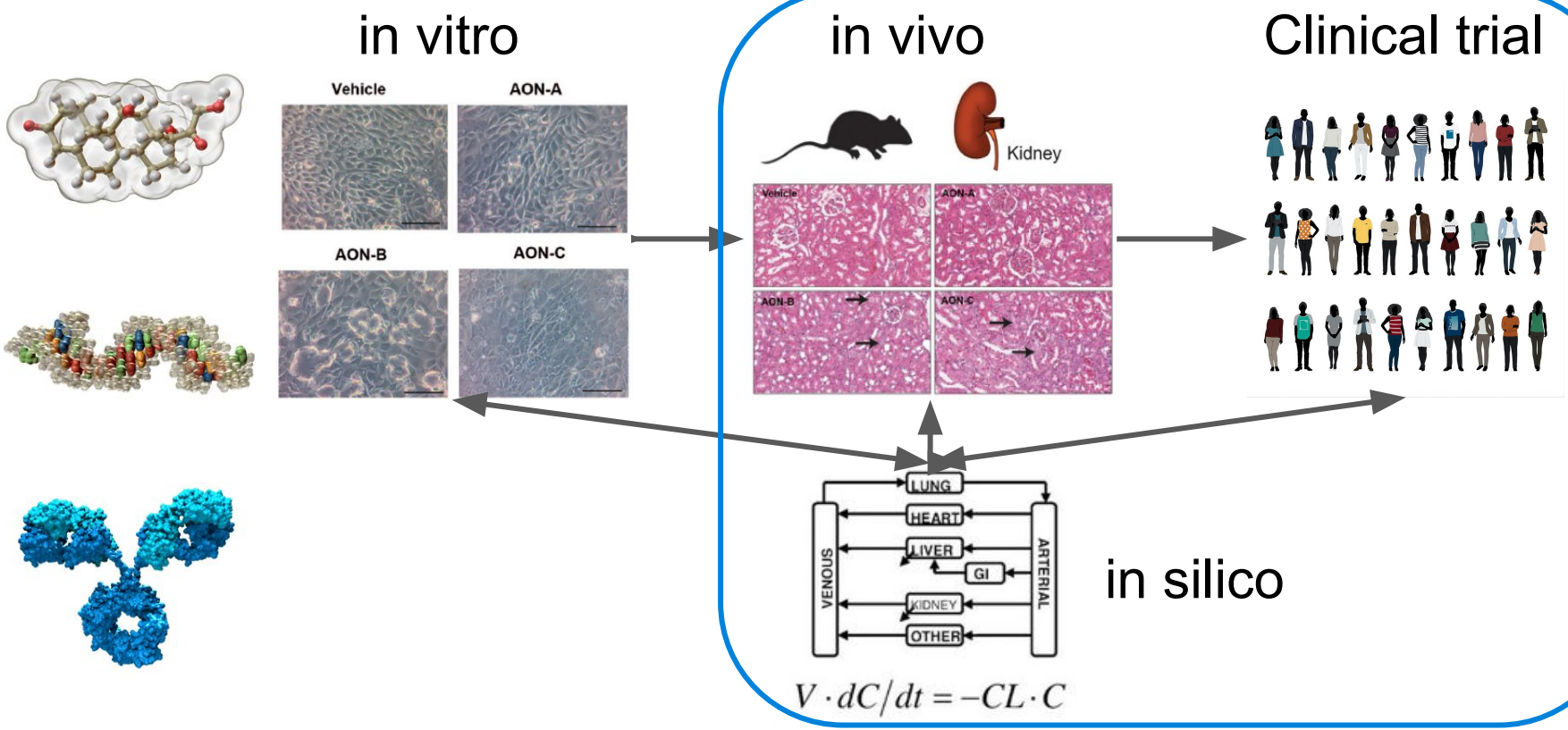
decryptM (Nature 2023): Following the dose-dependent treatment of cancer cells with drugs, quantitative mass spectrometry records dose-response of thousands of posttranslationally modified peptides. EC50: half-maximal effective concentration; Ac, acetylation; GG, ubiquitinylation; P, phosphorylation.

PTM and proteomics characterize MoA of drugs

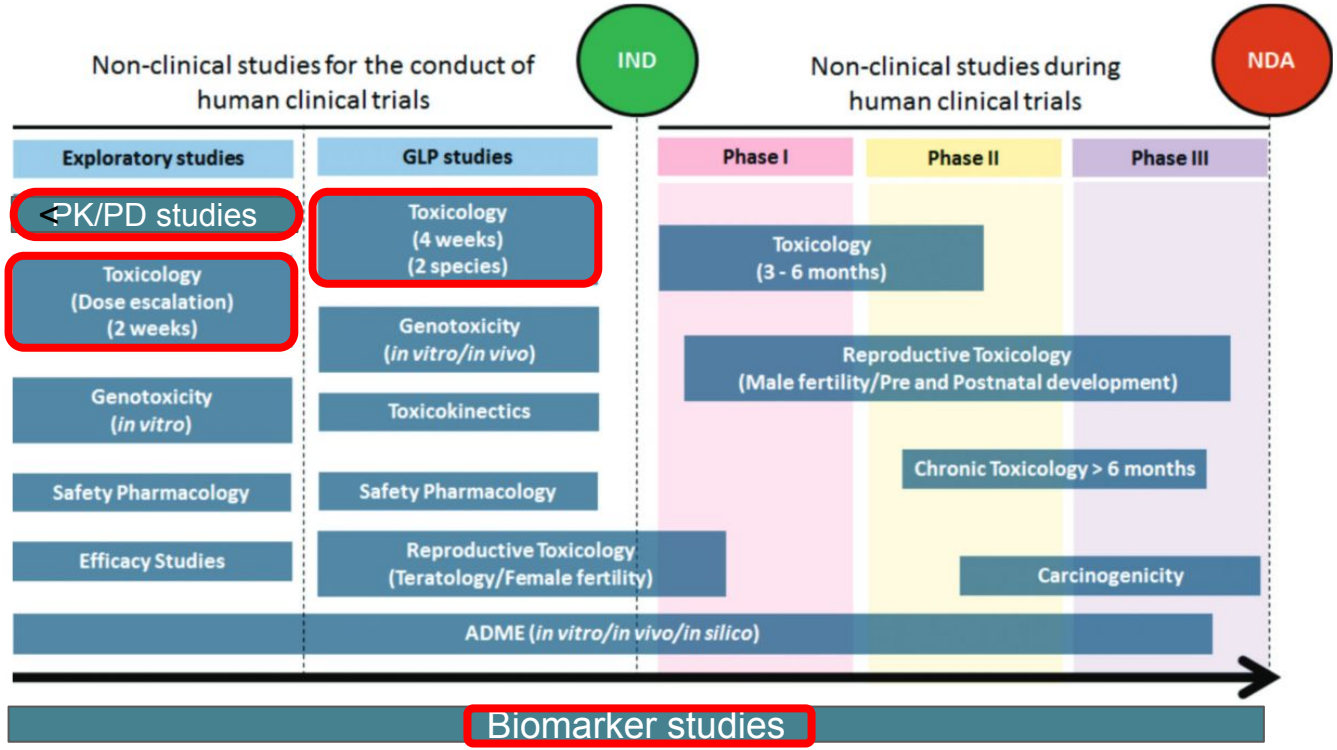


Bortezomib (BTZ) and carfilzomib (CFZ) both treat multiple myeloma by inhibiting the proteasome by reversible covalent (BTZ) or irreversible (CFZ) binding to the protease PSMB5. Time-series data show both the dynamics and the converging signaling.

Dose prediction based on pharmacology and toxicology before entry into human

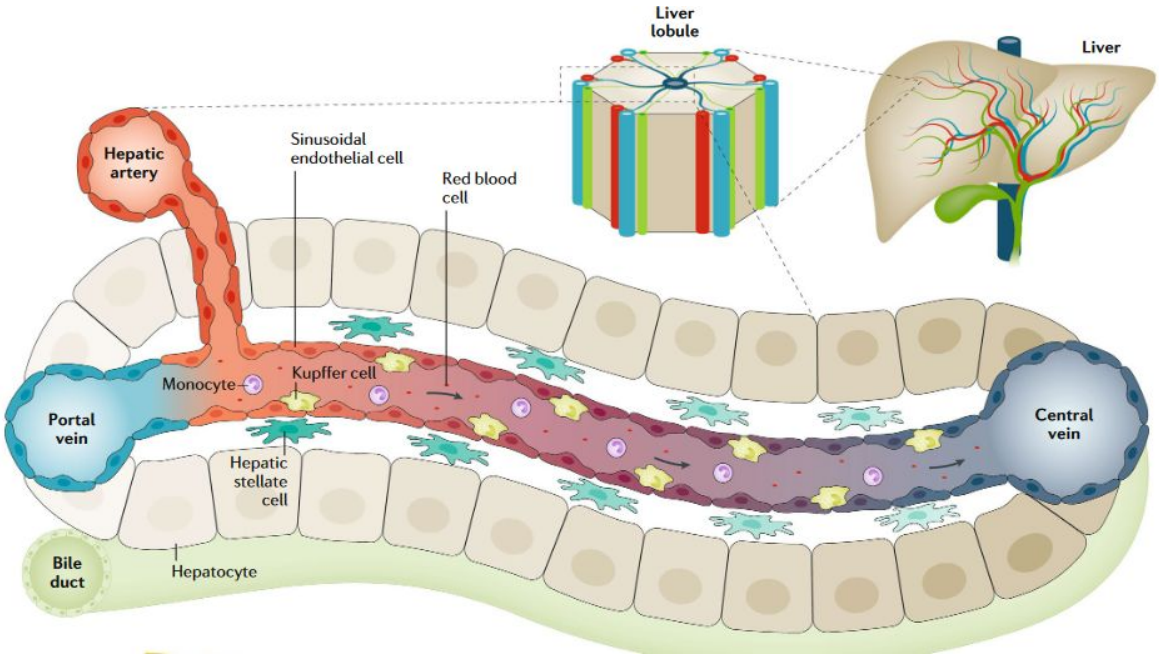


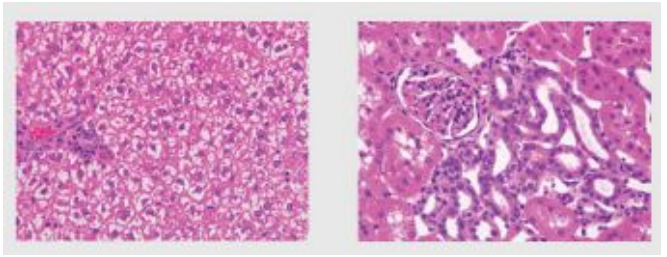
Current practices of non-clinical studies in drug development



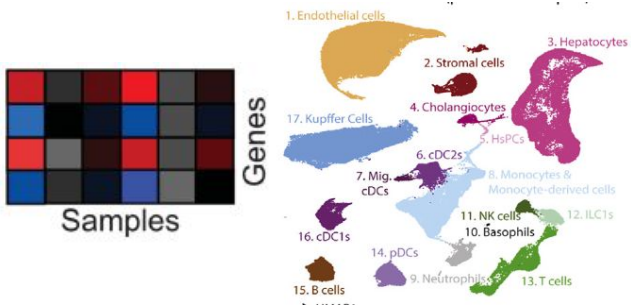
- IND: Investigational New Drug application
- NDA: New Drug Application
- GLP: Good Lab Practice
- Red boxes: Focus areas of this and coming lectures

Current practices of profiling and understanding toxicology: an example with liver





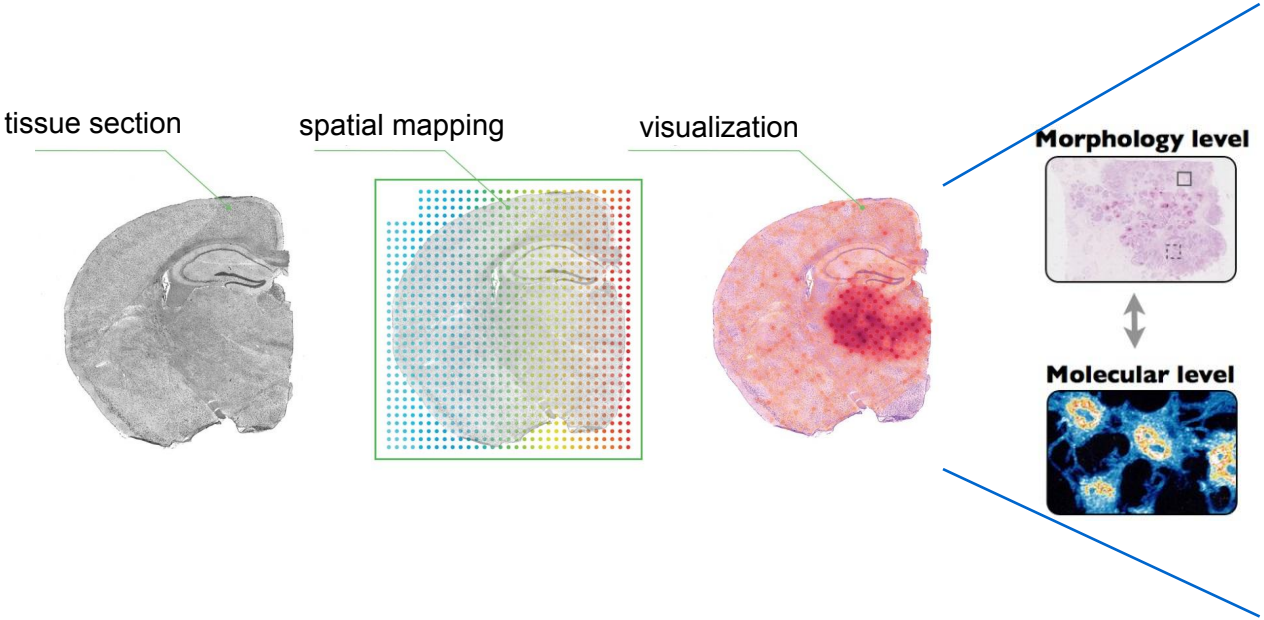
Histopathology

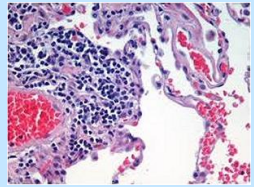


Omics

[Liver structure and anatomy (YouTube Video)]

Spatially resolved omics complement histopathology





Spatial Omics

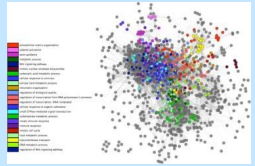
Histopathology

- Morphology

Molecular Biomarkers

- Genetics, Transcriptomics

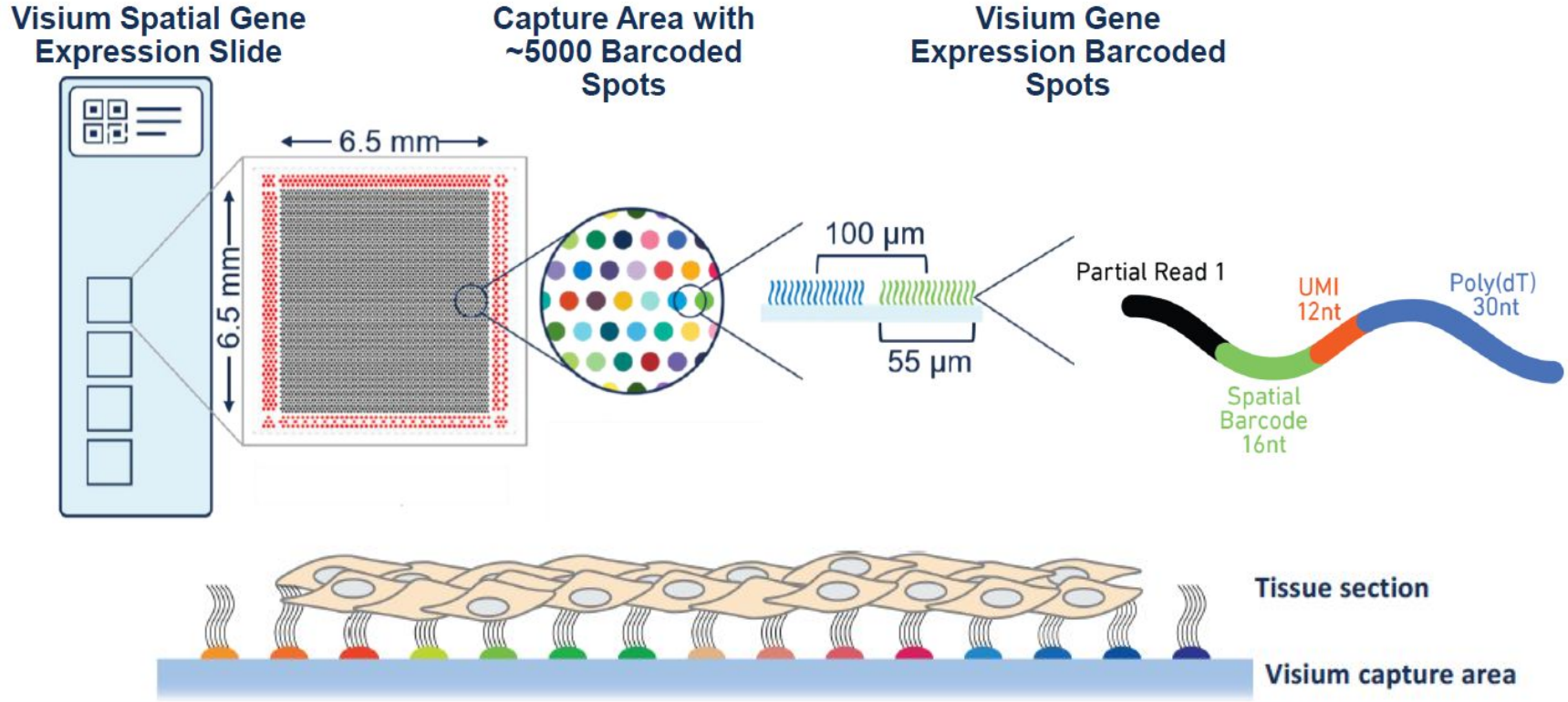
- Proteomics



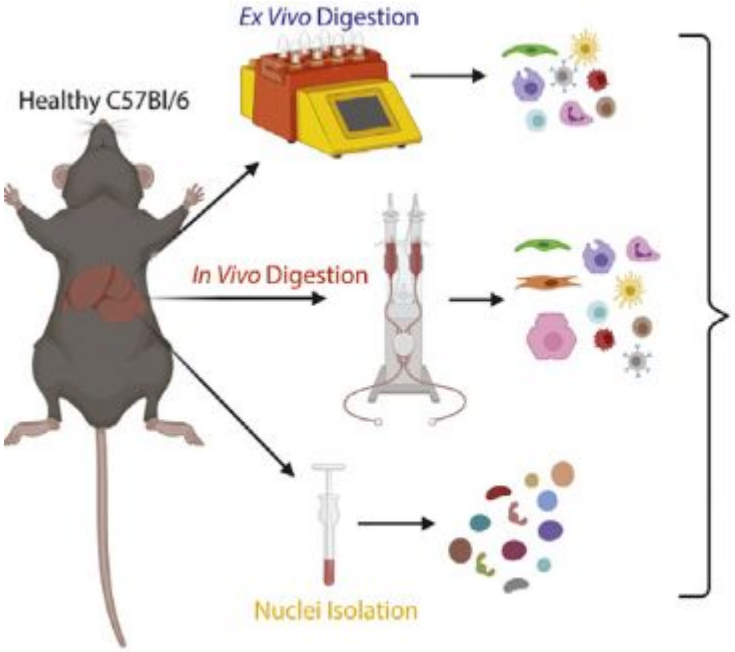
Data insights

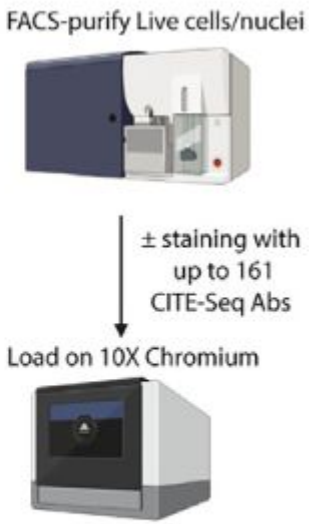
- Data analysis & integration

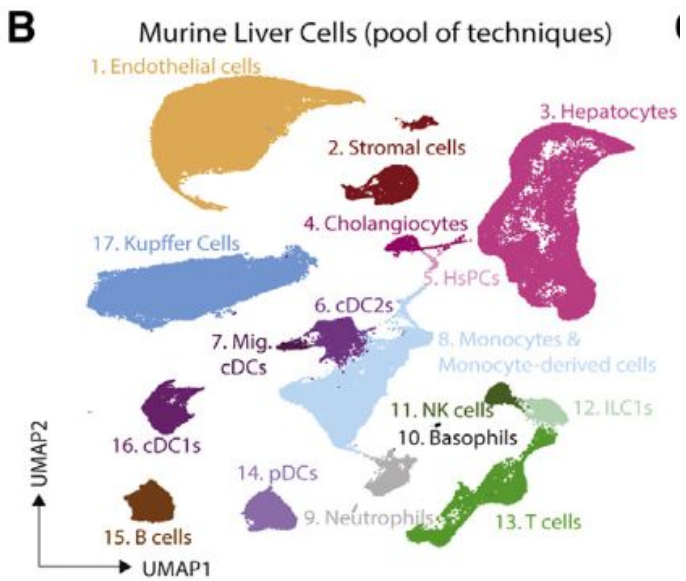
An example: 10x VISIUM Technology



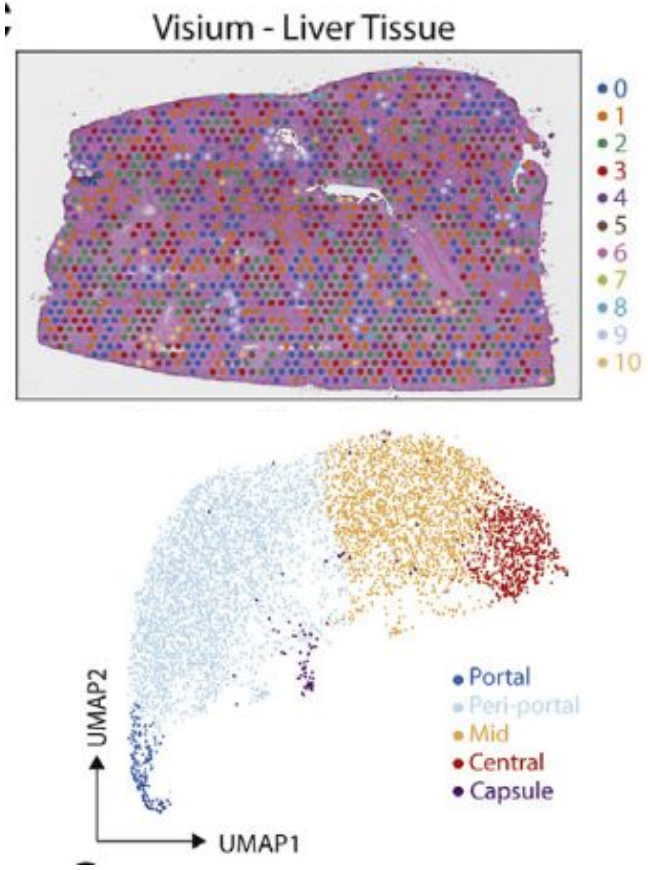
Spatial and single-cell expression of liver cells

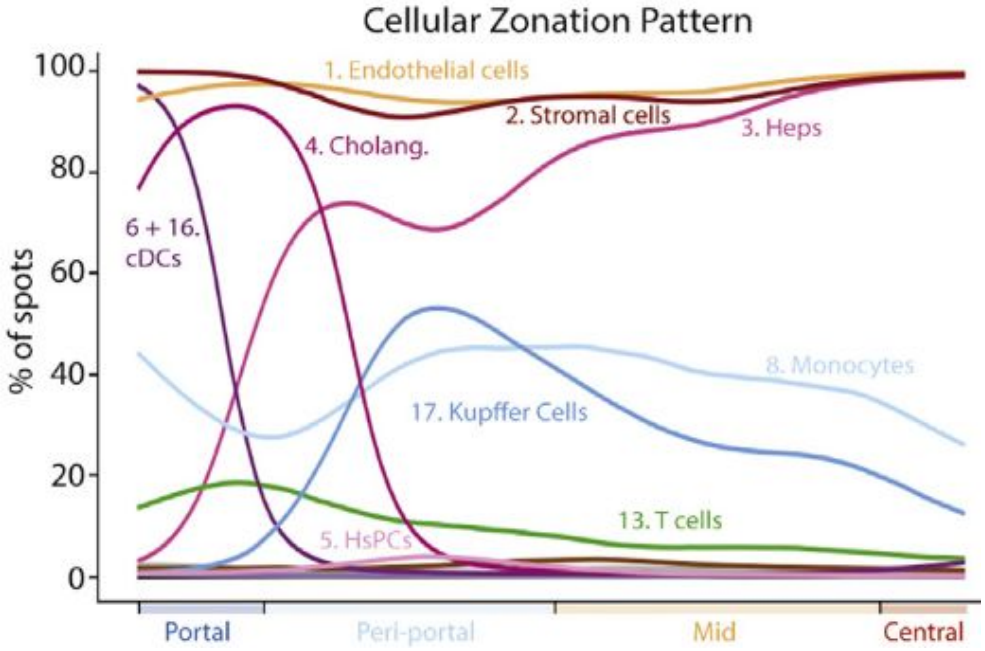




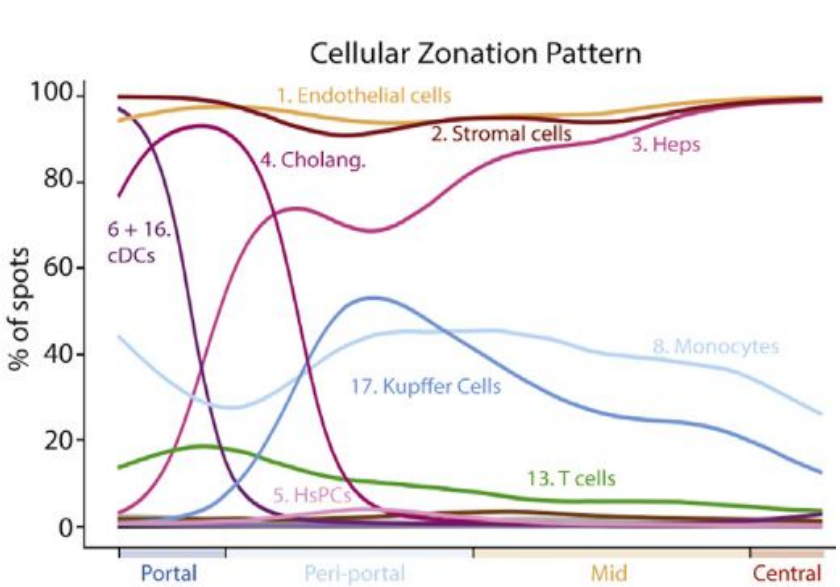


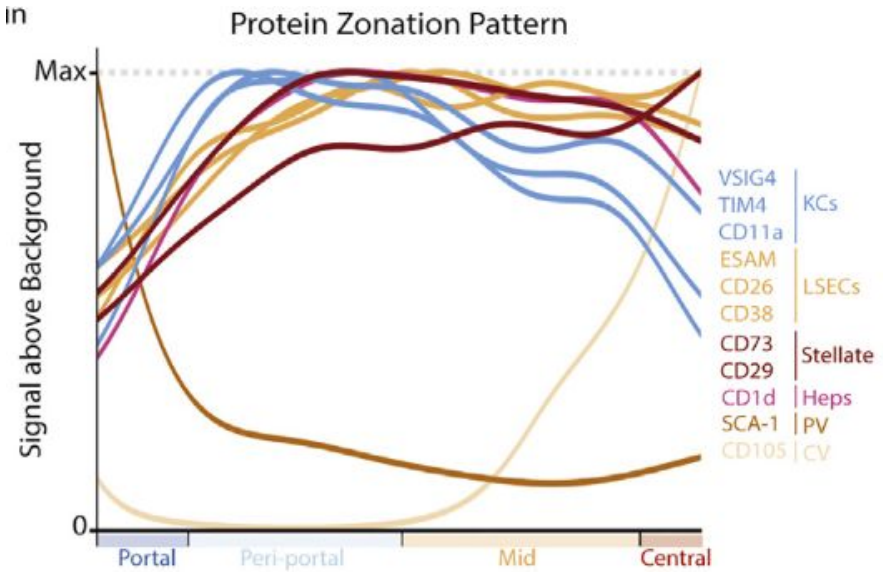
Spatial and single-cell expression of liver cells



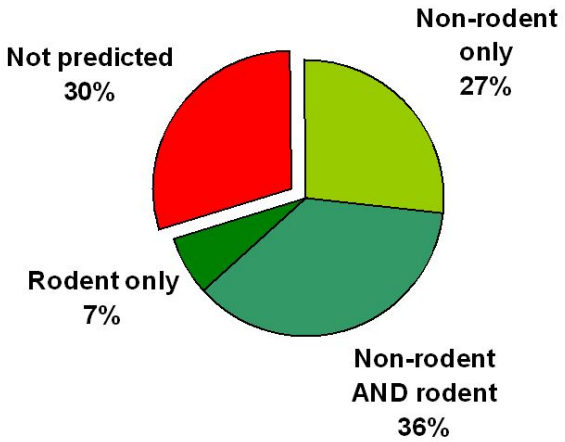


Spatial mRNA and protein expression data empowers digital pathology and biological understanding





How predictive is animal safety testing for humans? It depends on modality and therapeutic classes.



Regul Toxicol Pharmacol. 2000:32:56-67

Target organ of ADRs	Small molecule drugs		Large molecule drugs	
	% of ADRs	% of correlation	% of ADRs	% of correlation
Gastrointestinal	21	80	14	19
Neurological	20	34	11	4
Hepatobiliary	11	73	8	21
Hematological	8	75	8	80
Cutaneous	5	56	9	22
Systemic	5	45	8	20
Cardiovascular	4	61	6	0
Ocular	5	64	5	83
Musculoskeletal	3	16	5	0
Metabolic	4	50	3	43
Faucal/oral	4	41	3	38
Urinary	3	61	3	14
Respiratory	1	45	5	32
Infection	0.4	100	6	68
Nasal	1	27	2	33
Application site reaction	1	100	3	81
Others	3	45	1	80

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.38, No.4, 581-598, 2013

Conclusions

- We predict efficacy and safety profiles of drugs by studying the mechanism and mode of action (MoA).
- Bulk and single-cell RNA sequencing, and proteomics based on mass spectrometry (MS) are essential tools for understanding MoA of drug candidates.
- Spatial omics combines imaging and omics technologies to offer spatially resolved data of biological systems. Their use in animal models and human samples has the potential to improve translational studies.

Offline activities of Module IV (optional)

Perform your own single-cell data analysis to get first-hand experience working with high-dimensional biological data.

- If you are new to the topic, please use the PBMC tutorial of *Scanpy* (python) or the PBMC tutorial of Seurat (R).
- If you have experience with such data already, checkout the NBIS workshop on single-cell sequencing data analysis to cover advanced topics such as spatial transcriptomics and trajectory inference.

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1. Figures: Lumen Learning, Exploring Nature, National Geographics, Platelet cells (Graham Beards, CC-BY-SA 4.0), Lymphocytes (NicolasGrandjean, CC-BY-SA 3.0), Adipocytes (Public Domain), Hepatocytes (CC-BY-NC 2.0), Neurons and Glia (Public Domain), Blood (CC 3.0), Blood Cells (By A. Rad and M. Häggström. CC-BY-SA 3.0 license), A selective JAK3 inhibitor (London Lab/Weizmann institute)
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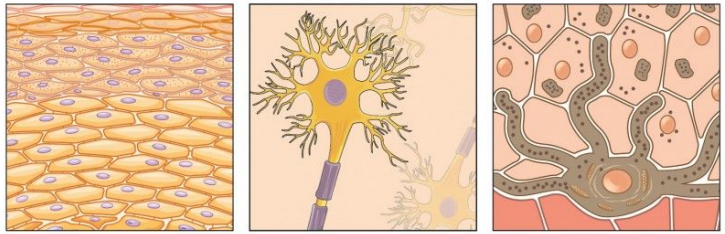
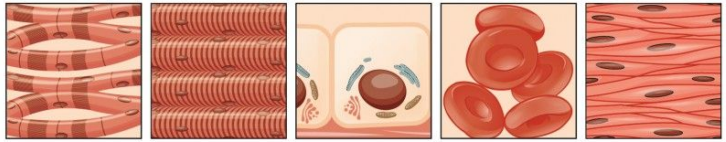
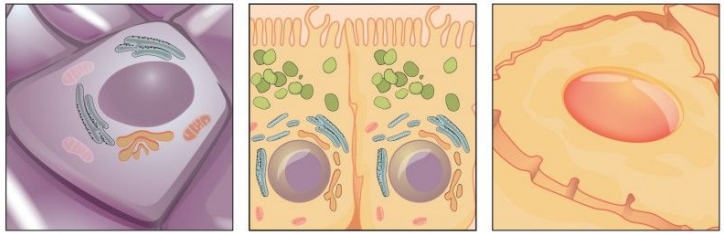
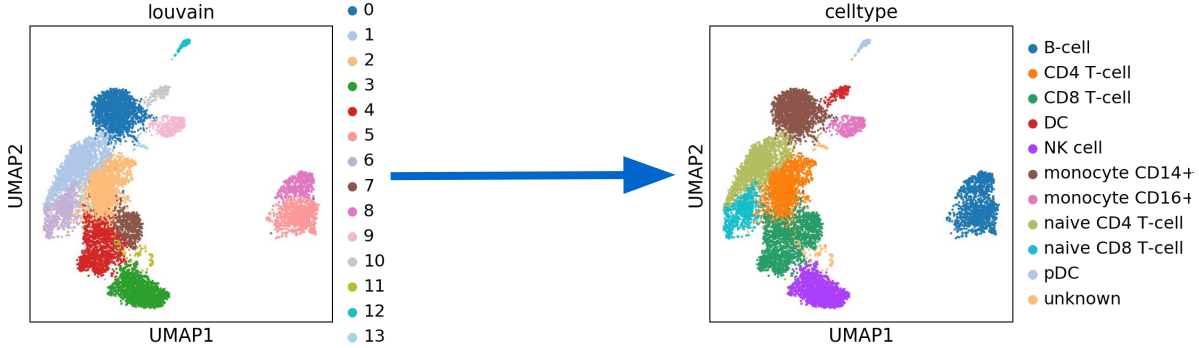
Supplementary Information

Embryonic origins of tissues

Germ Layer	Gives rise to:
Ectoderm	Epidermis, glands on skin, some cranial bones, pituitary and adrenal medulla, the nervous system, the mouth between cheek and gums, the anus  Skin cells Neurons Pigment cell
Mesoderm	Connective tissues proper, bone, cartilage, blood, endothelium of blood vessels, muscle, synovial membranes, serous membranes lining body cavities, kidneys, lining of gonads  Cardiac muscle Skeletal muscle Tubule cell of kidney Red blood cells Smooth muscle
Endoderm	Lining of airways and digestive system except the mouth and distal part of digestive system (rectum and anal canal); glands (digestive glands, endocrine glands, adrenal cortex)  Lung cell Thyroid cell Pancreatic cell

An intern project: Cell type annotation

From unsupervised clustering and cluster based annotation



Luis Wyss
RAAN intern 2019

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Label
Training Cell 1	10	50	0	12	4	Celltype A
Training Cell 2	8	45	78	3	23	Celltype B
Training Cell 3	14	55	78	65	55	Celltype B
Training Cell 4	78	12	13	9	58	Celltype A
Training Cell 5	45	23	65	98	11	Celltype C

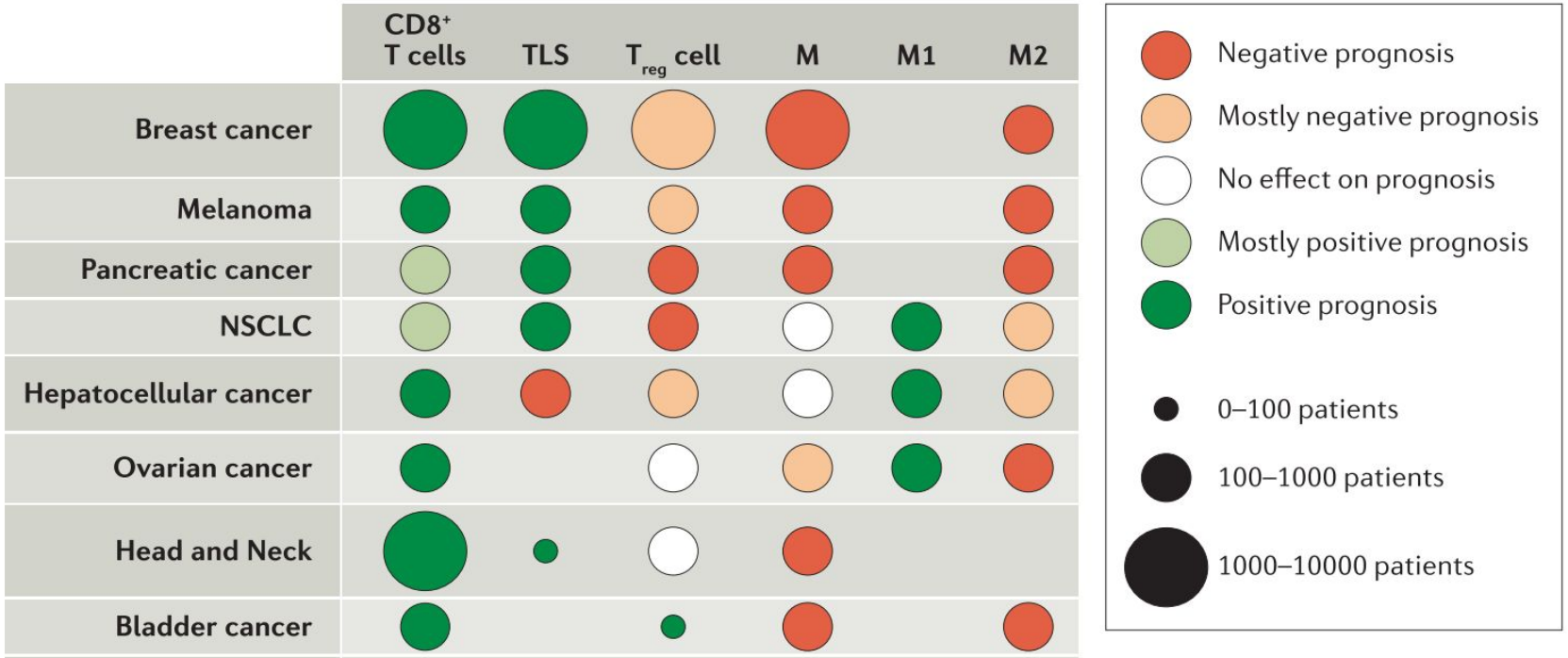
To supervised annotation at single-cell level:

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
Cell 1	45	45	8	56	3
Cell 2	65	120	78	45	12
Cell 3	79	12	34	65	88
Cell 4	7	59	32	47	62

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Prediction
Cell 1	45	45	8	56	3	Celltype A
Cell 2	65	120	78	45	12	Celltype B
Cell 3	79	12	34	65	88	Celltype C
Cell 4	7	59	32	47	62	Celltype B

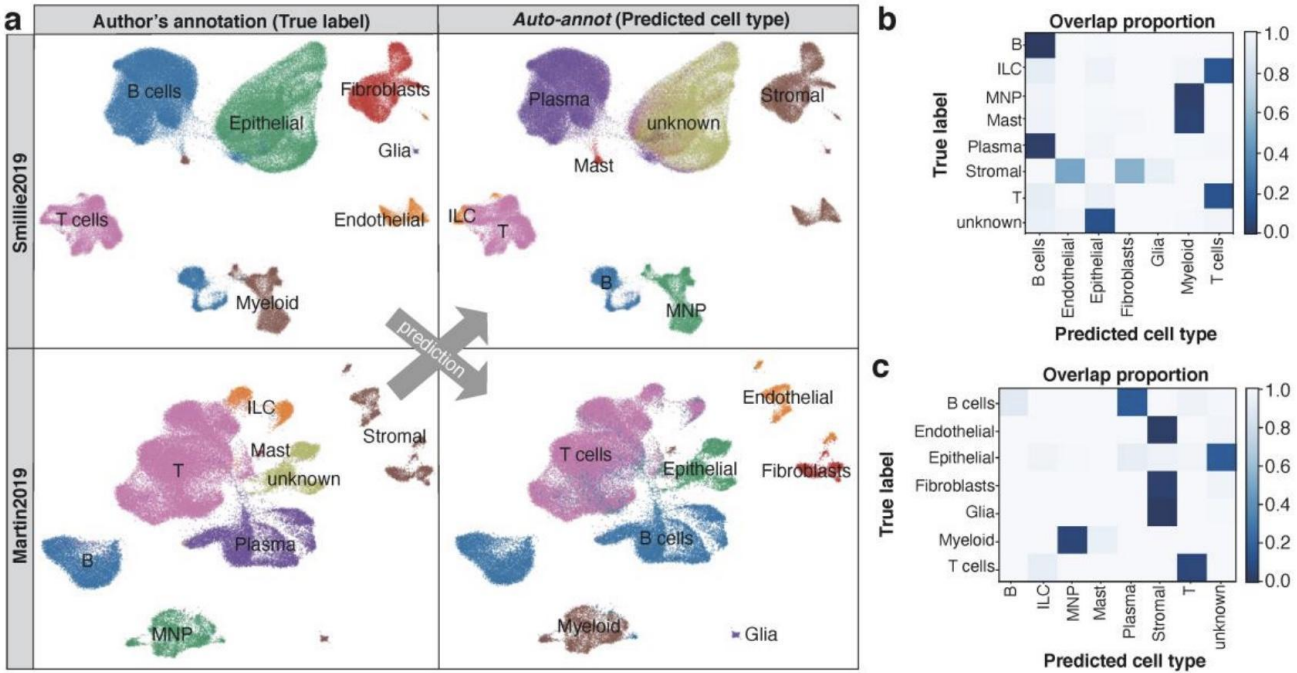
Advantages: (1) automation, (2) annotation independent from clustering, and (3) we can estimate the confidence of prediction

Abundance of immune cells in tumor microenvironments affect outcome



TLS: tertiary lymphoid structures; T_{reg}: regulatory T cells; M: macrophages; M1/M2: subtypes of macrophages

An example of Inflammatory Bowel Disease (IBD)



We observed Inconsistent cell type nomenclature across studies.
Machine learning allows us compare and integrate multiple studies.

We are living ecosystems

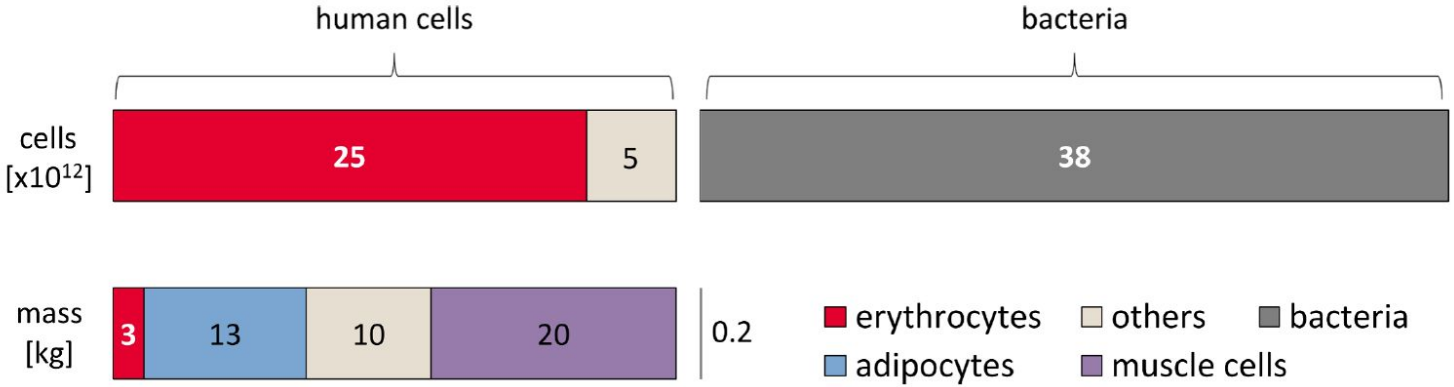
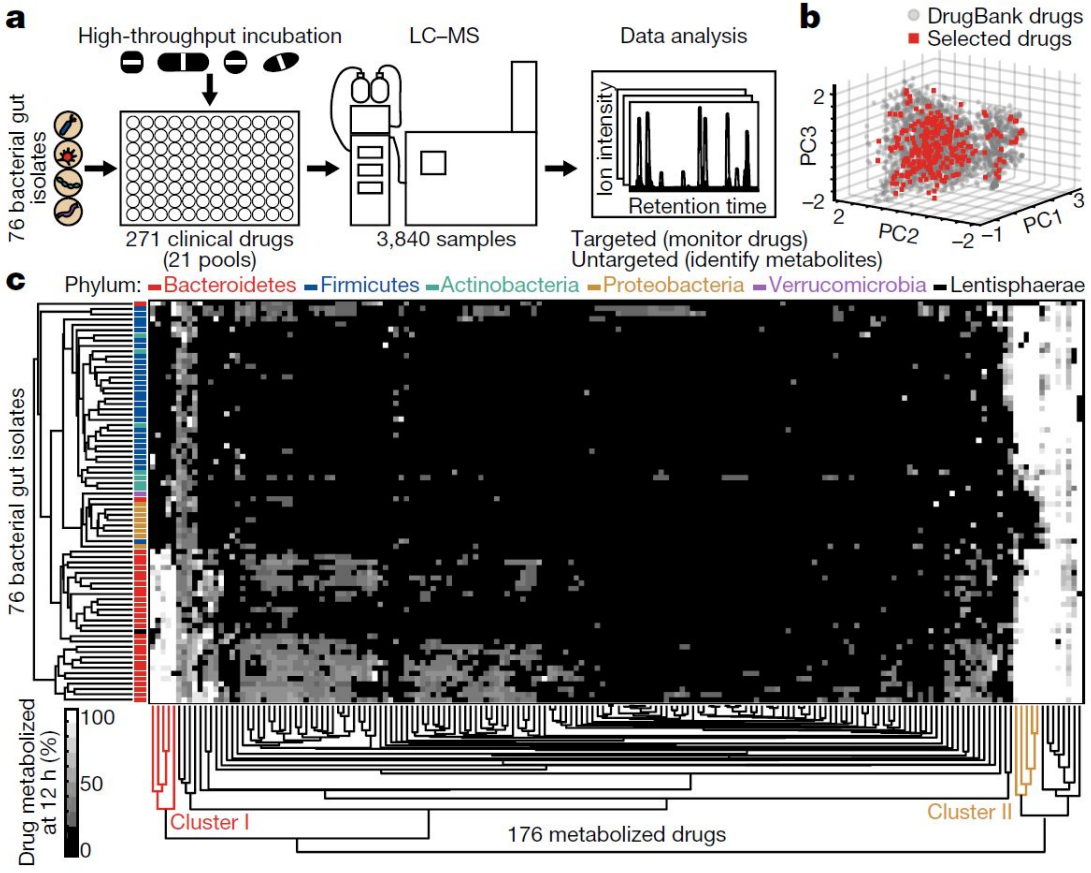
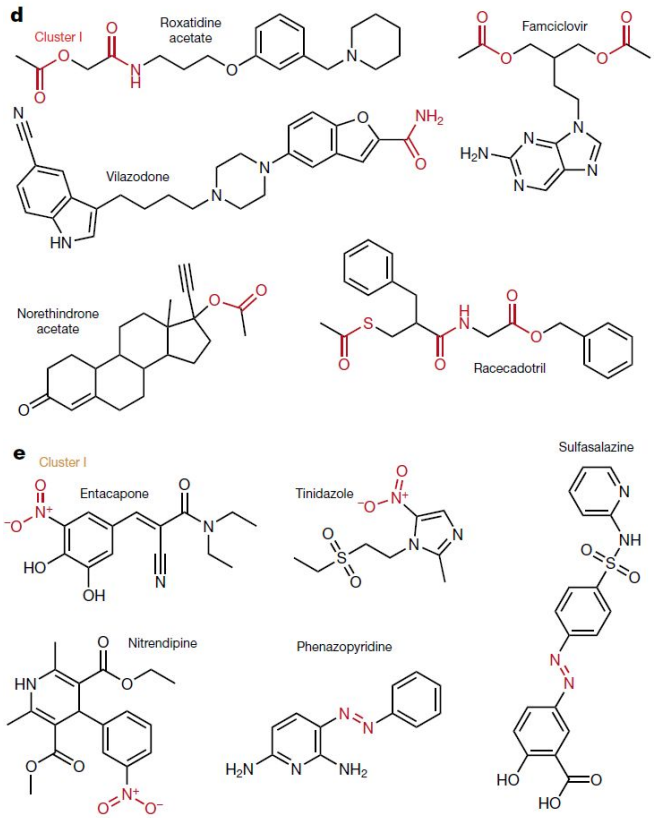


Table 3. B/H ratio for different population. See Table B in S1 Appendix for full references.

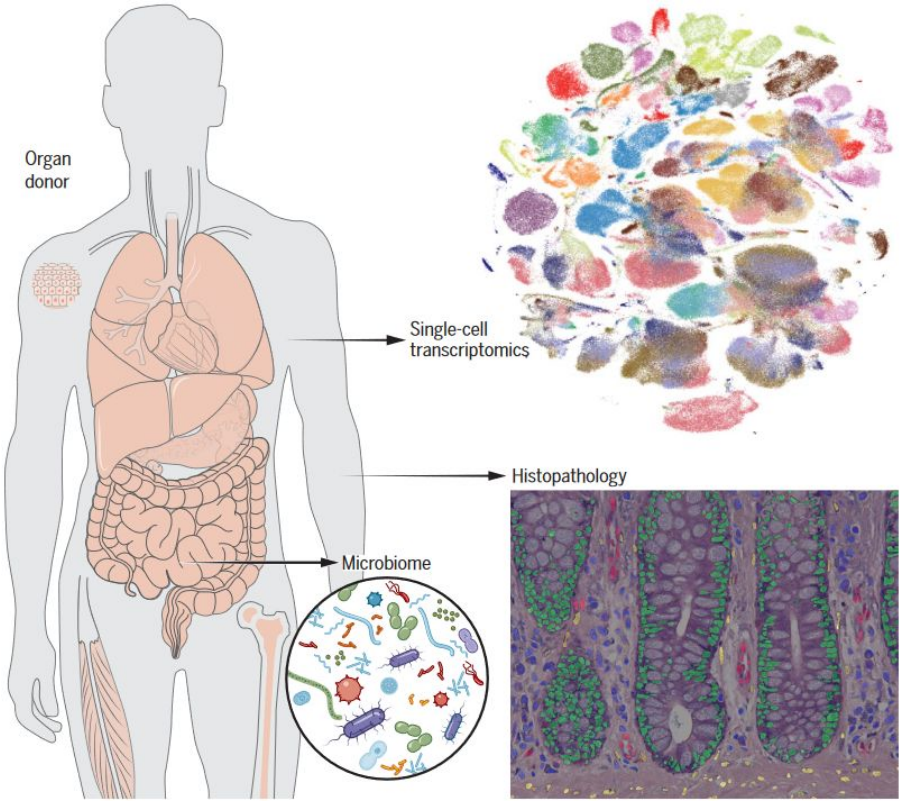
population segment	body weight [kg]	age [y]	blood volume [L]	RBC count [10^{12}/L]	colon content [g]	bac. conc. [10^{11}/ g wet] (1)	total human cells [10^{12}] (2)	total bacteria [10^{12}]	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	**1.3**
ref. woman	63		3.9	4.5	480	0.92	21	44	**2.2**
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	**2.3**
infant	9.6	1	0.8	4.5	80	0.92	4	7	**1.7**
elder	70	66	3.8 (3)	4.8	420	0.92	22	38	**1.8**
obese	140		6.7	5.0 (4)	610 (5)	0.92	40	56	**1.4**

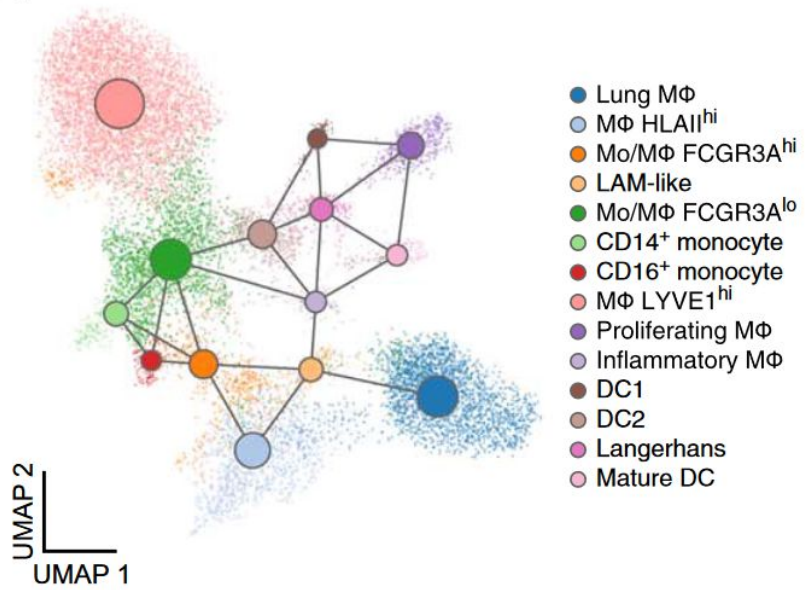
Gut microbiome can metabolize drugs differently





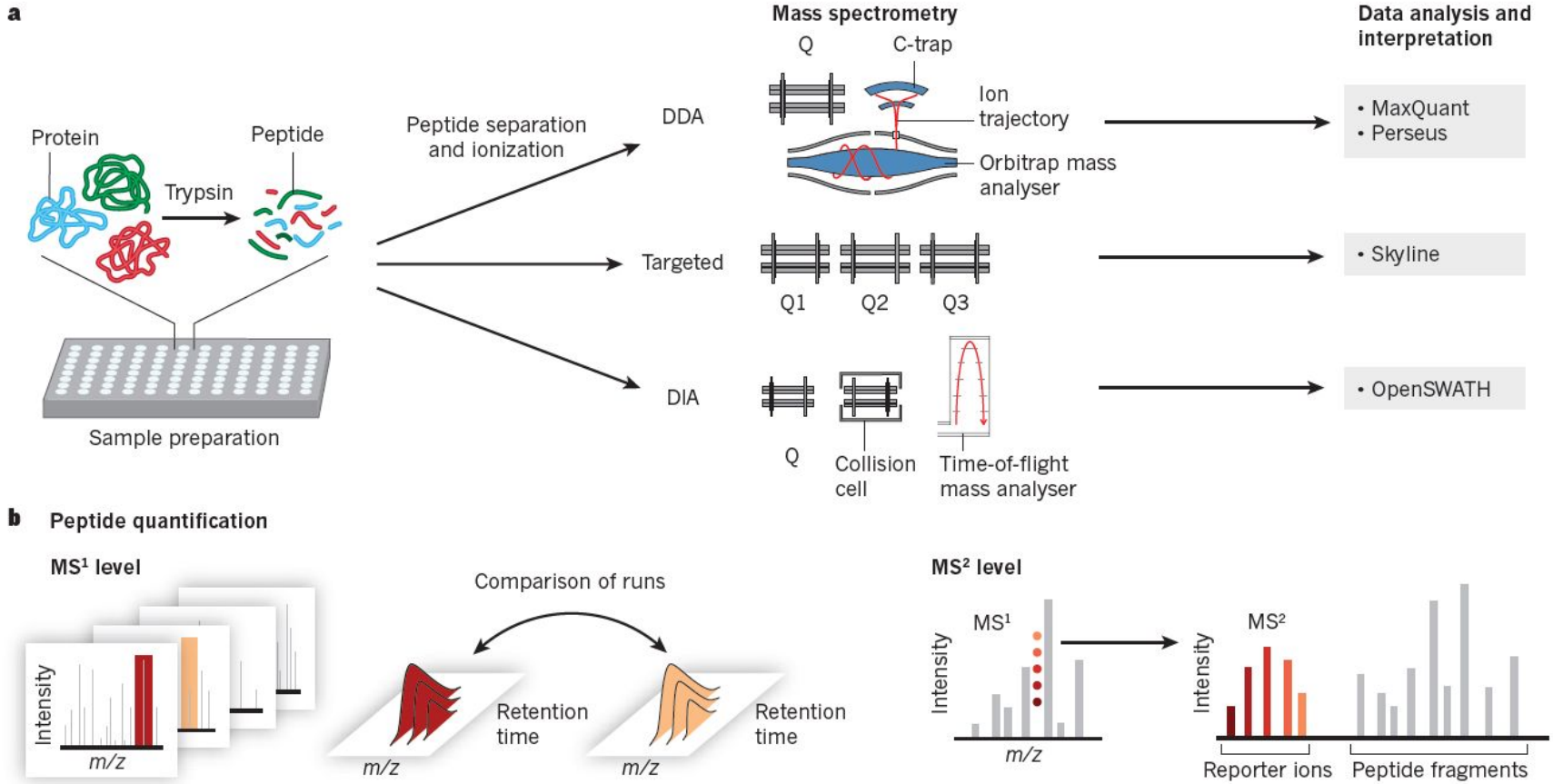
The *Tabula Sapiens* and other community projects offer reference expression data in healthy donors



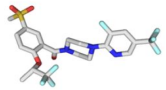


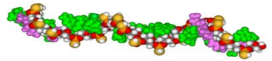
Left: the *Tabula Sapiens*. Right: Myeloid (Mϕ=macrophages, Mo/monocytes, LAM=lipid-associated macrophages, DC=dendritic cells) gene expression

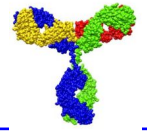
Mass-spectrometry based proteomics



Comparing modalities with regard to safety assessment







	Small molecules	Single Stranded Oligos	Biologics
Molecular weight	<1000 D	5000-7000 D	> 30000 D
Manufacture	Chemical synthesis	Chemical synthesis	Biologically-derived
Structure	Single entity, high purity	Single entity with 10-15% product-related impurities	Complex, heterogeneous
Chemical-driven toxicity	Yes	Yes	No
Metabolism	Species-specific	Species-independent catabolism by proteolytic degradation	Species-independent catabolism by proteolytic degradation
PK	Generally short $t_{1/2}$	Long (tissue) $t_{1/2}$	Long $t_{1/2}$
Some general aspects	High throughput screening/early safety testing of up to 500 small molecules	Biodistribution with consistent patterns	Fewer, yet complex due to biology/immunology

Adapted from Schubert et al, Nucl Acid Therap 2012, with input from Yann Tessier and Susanne Mohr

Proteomics enables the elucidation of protein relations in the protein communities