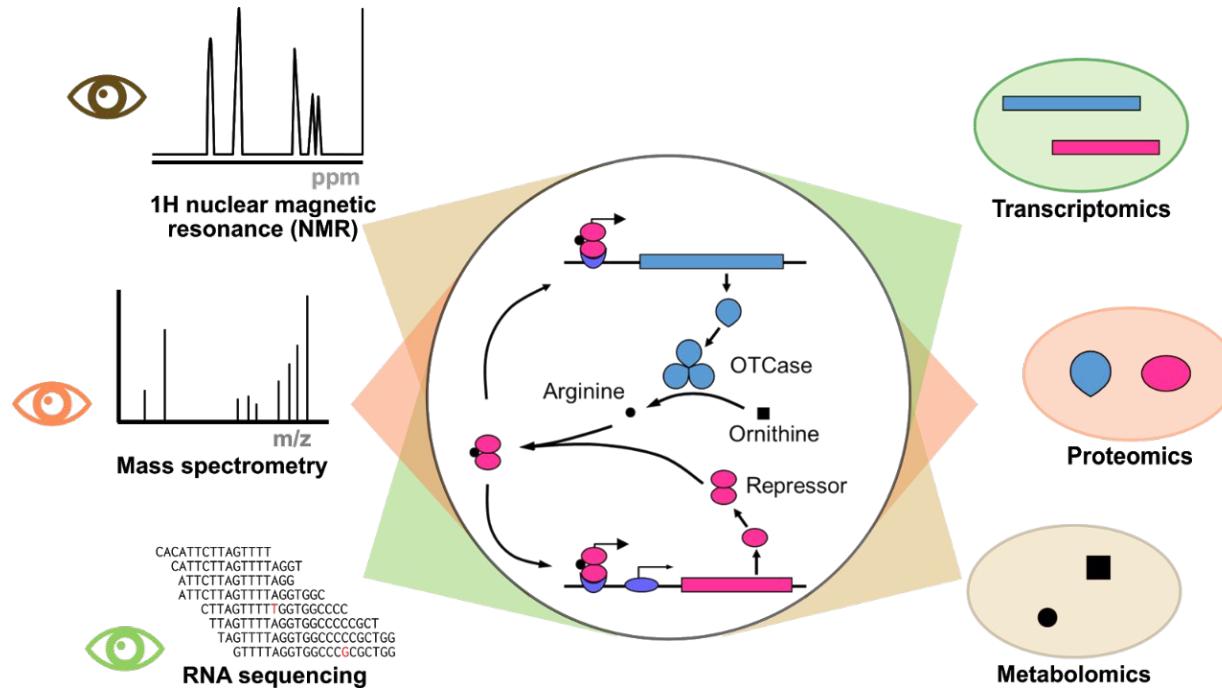


AMIDD 2025 Lecture 10: Biological networks and omics

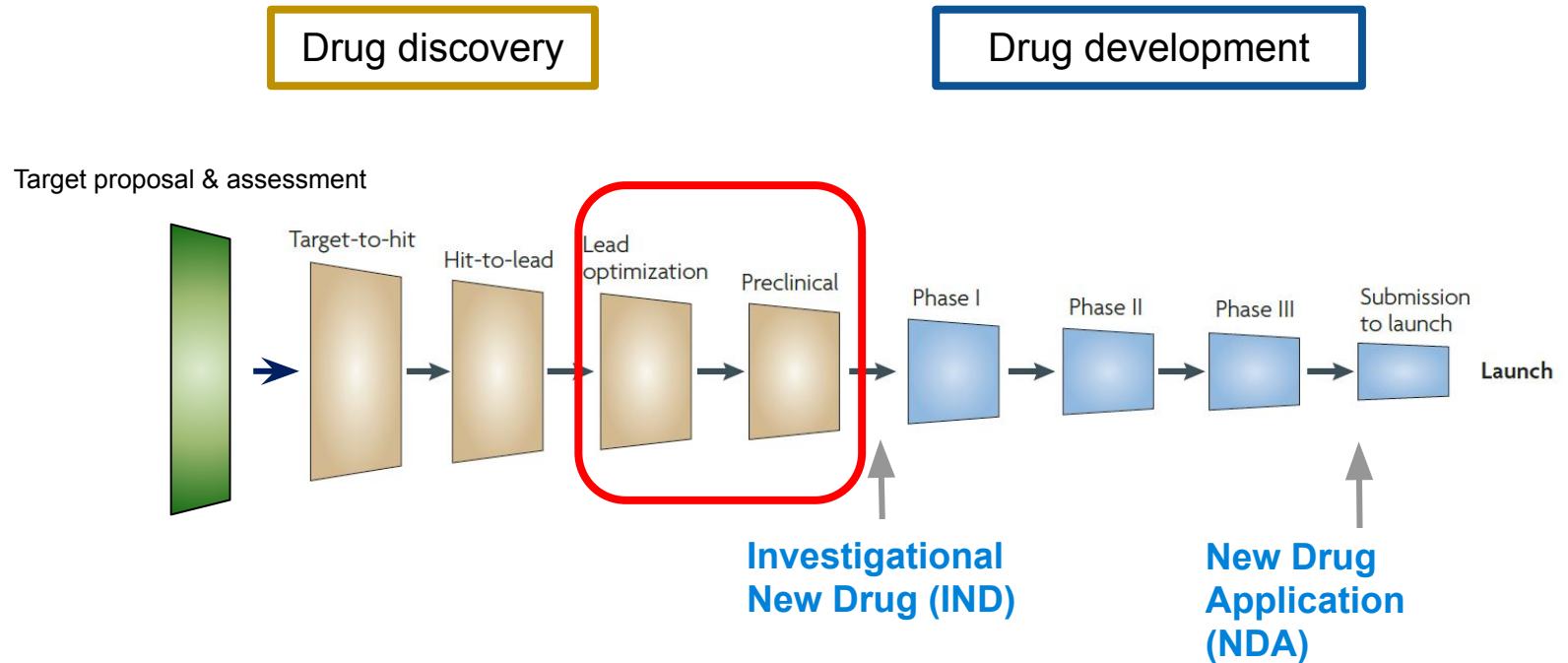


Dr. Jitao David Zhang, Computational Biologist

¹Pharmaceutical Sciences, Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche

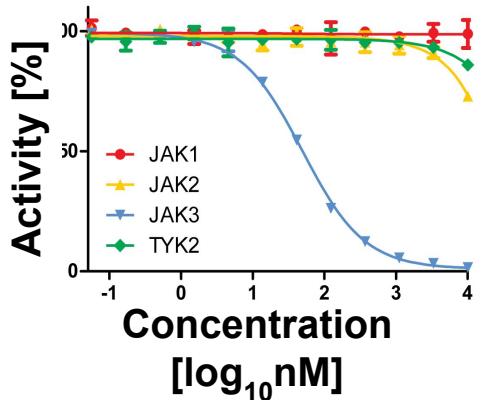
²Department of Mathematics and Informatics, University of Basel

Translational research makes molecules into medicines

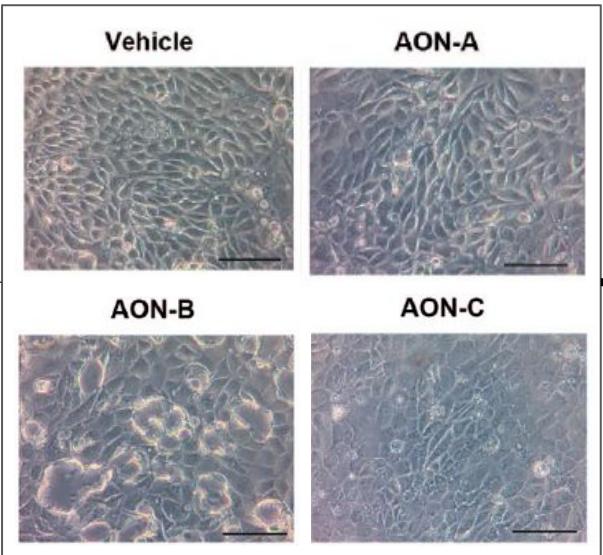


Adapted from Paul *et al.* "How to Improve R&D Productivity: The Pharmaceutical Industry's Grand Challenge." *Nature Reviews Drug Discovery*, 2010

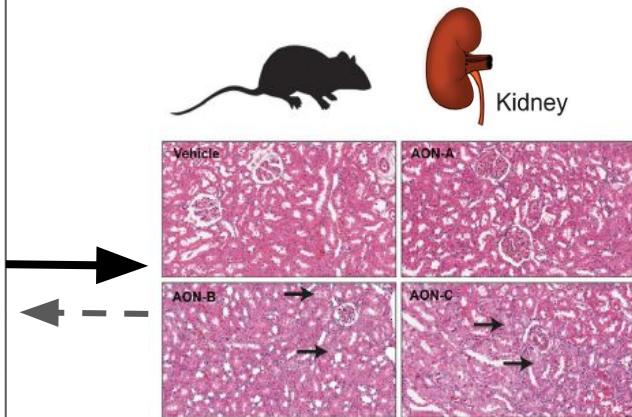
Classical workflow of efficacy and toxicity assessment



Biochemical &
biophysical assays



Cellular assays
(*in vitro*)

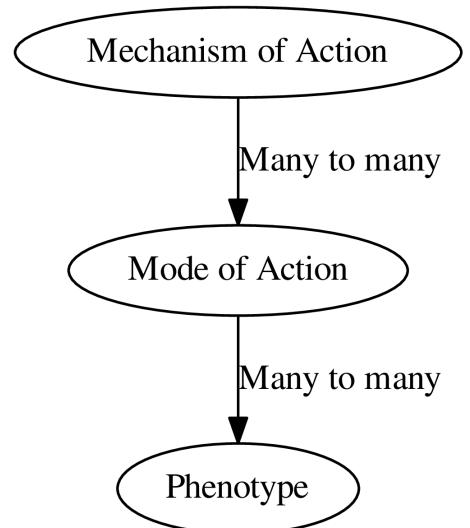


Animal
experiments
(*in vivo*)

→ Usual workflow
← - - Assay development

Mechanism of Action and Mode of Action

- **Mechanism of Action:** The specific biochemical interaction through which a drug substance produces its pharmacological effect, *at the molecular level*.
- **Mode of Action:** Functional or anatomical changes, *at the cellular level*, resulting from the exposure of a living organism to a substance.
- For instance, a mechanism of action of a drug can be “*binding to Monoacylglycerol lipase (MAGL)*” while its mode of action would be “*regulating endocannabinoid signaling*” and “*reducing inflammation*”.
- In lead optimization (LO) and early development, our goal is to understand both the mechanism of action and the mode of action *in vitro*, *in vivo*, and in human. The term *MoA* is used to refer both.



The Hill function is a common model of *in vitro* pharmacology

- The Hill function is one of the mostly useful non-linear functions to model biological systems.
- In its general form, H_{max} indicates the maximal value to which the function is asymptotic, n is the shape parameter (known as the Hill's coefficient), and k is the reflection point, often abbreviated as XC_{50} (X=I, E, C, ...), the half-saturation constant.
- The Michaelis-Menten model is a special case of the Hill function ($n=1$).

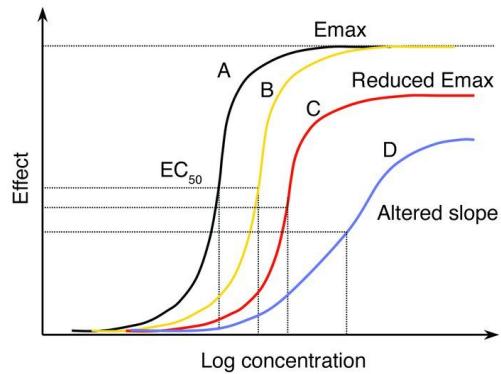
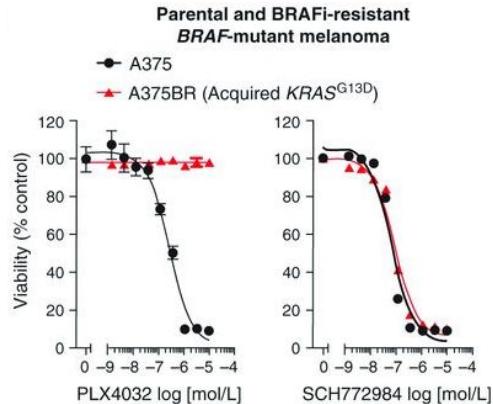
$$H = H_{max} \frac{x^n}{k^n + x^n}$$

The general form of the Hill function

$$E = E_{max} \frac{[L]^n}{EC_{50}^n + [L]^n}$$

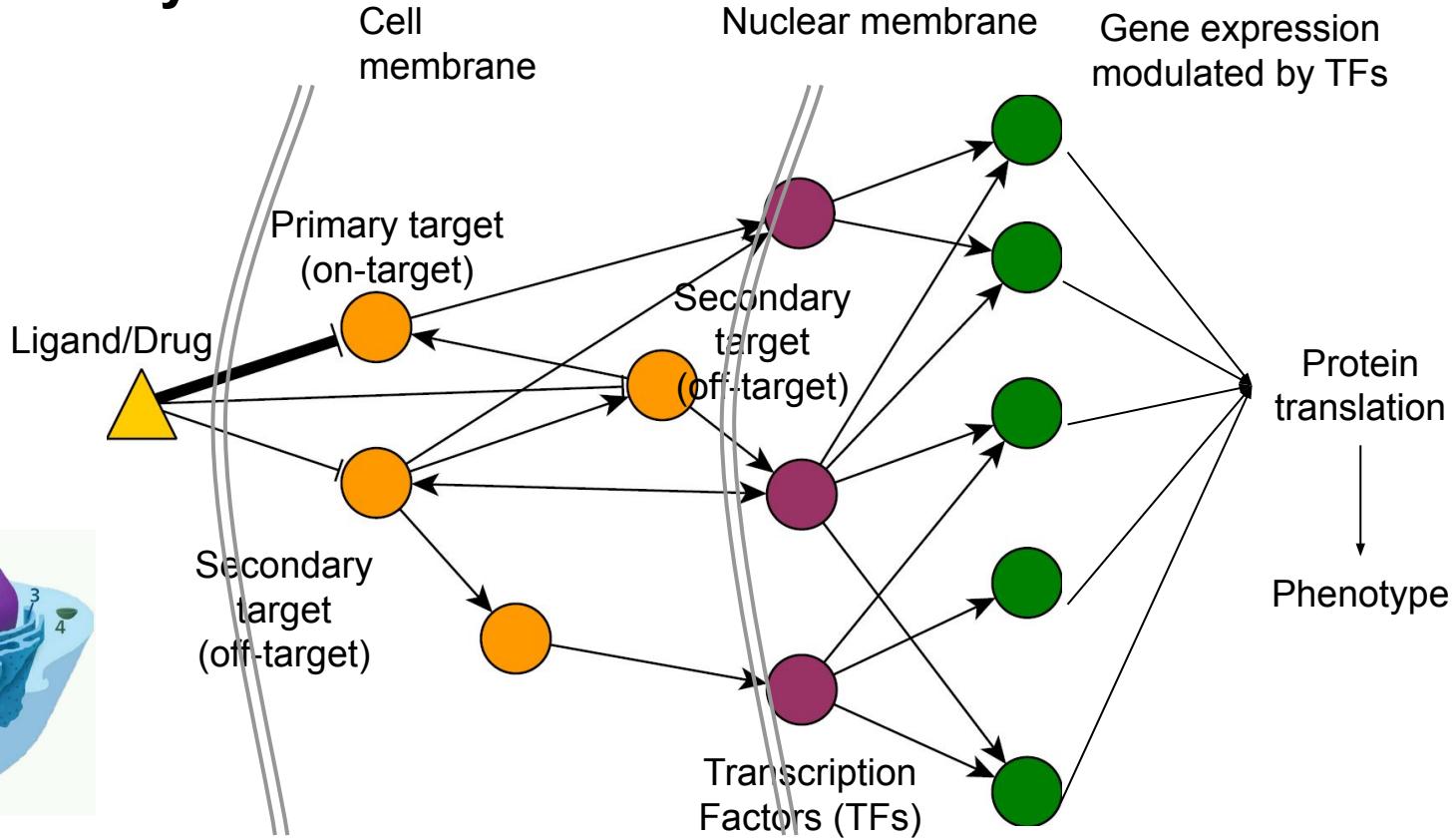
$$= E_{max} \frac{1}{1 + \left(\frac{EC_{50}}{[L]}\right)^n}$$

Modelling the dose-dependent effect

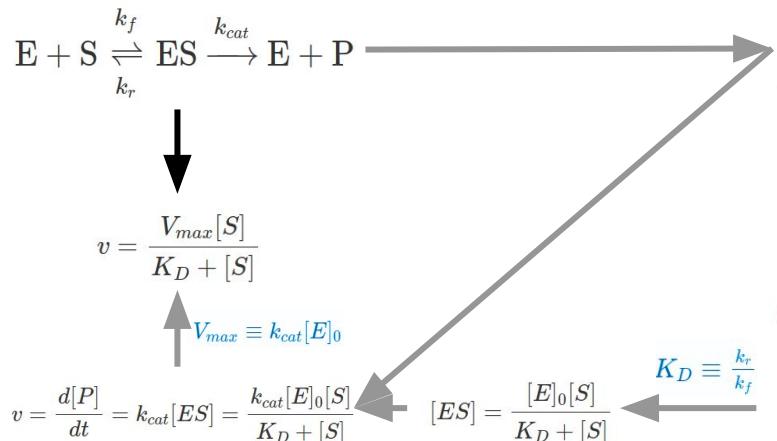


Suppose it is an antiviral drug, compared with curve B, what does curve A, C, and D suggest?

Biological networks interact with drugs and manifest its efficacy and safety

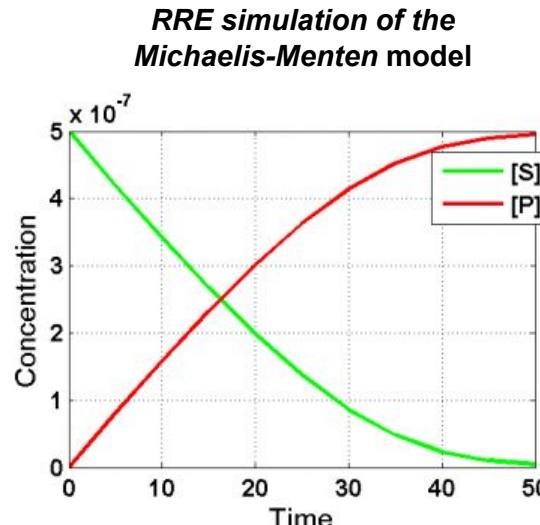


Reaction Rate Equations: a compartment/ODE model of biological chemical reaction



$$\begin{aligned}\frac{d[E]}{dt} &= -k_f[E][S] + k_r[ES] + k_{cat}[ES], \\ \frac{d[S]}{dt} &= -k_f[E][S] + k_r[ES], \\ \frac{d[ES]}{dt} &= k_f[E][S] - k_r[ES] - k_{cat}[ES], \\ \frac{d[P]}{dt} &= k_{cat}[ES],\end{aligned}$$

$k_f[E][S] = k_r[ES]$
 $k_f([E]_0 - [ES])[S] = k_r[ES]$
 $k_f[E]_0[S] - k_f[ES][S] = k_r[ES]$
 $k_f[E]_0[S] = k_r[ES] + k_f[ES][S]$
 $k_f[E]_0[S] = [ES](k_r + k_f[S])$
 $[ES] = \frac{k_f[E]_0[S]}{k_r + k_f[S]}$
 $[ES] = \frac{k_f[E]_0[S]}{k_f(\frac{k_r}{k_f} + [S])}$

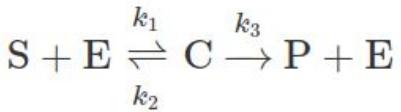


Source: [Systems Engineering Wiki \(tue.nl\)](#)

RRE is a set of ODEs, with each ODE representing one chemical species. Solution of the j th equation at time t is a real number representing the concentration of species j at time t .

Simulation of biological networks with ordinary differential expression

Given the reaction



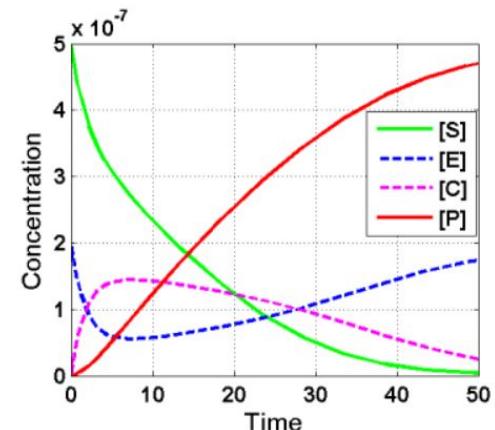
Given the initial values
and rate constants

- $S(0) = 5e^{-7}$
- $E(0) = 2e^{-7}$
- $C(0) = P(0) = 0$
- $k_1 = 1e^6$
- $k_2 = 1e^{-4}$
- $k_3 = 0.1$

According to the law
of mass action

$$\begin{aligned}\frac{d[S]}{dt} &= -k_1[E][S] + k_2[C], \\ \frac{d[E]}{dt} &= -k_1[E][S] + (k_2 + k_3)[C], \\ \frac{d[C]}{dt} &= k_1[E][S] - (k_2 + k_3)[C], \\ \frac{d[P]}{dt} &= k_3[C],\end{aligned}$$

It is possible to
simulate the
concentration
changes by time
deterministically.



See [Systems Engineering Wiki \(tue.nl\)](#) for MATLAB/COPASI codes and
Stochastic Modelling for Systems Biology by Darren J. Wilkinson

Simulating behavior of complex ODE systems with COPASI

- COPASI, freely available at <http://COPASI.org/>, supports both **ordinary differential equation (ODE)** based simulation as well as stochastic kinetic simulation.
- Such tools are important for detailed analysis of enzymatic reactions, for instance in the presence of drugs and/or disease-relevant mutation.

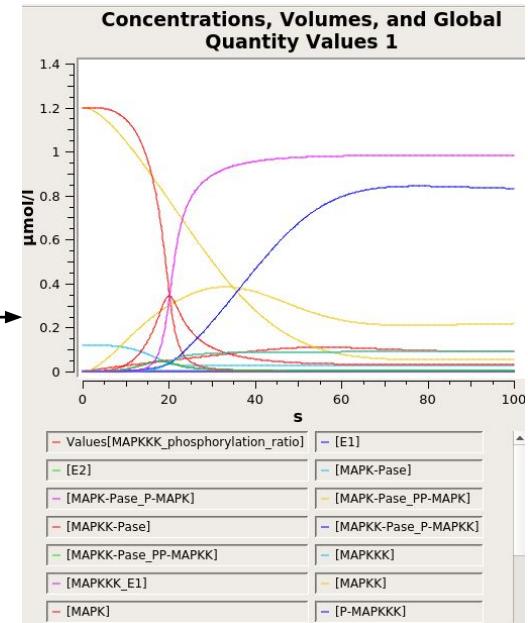
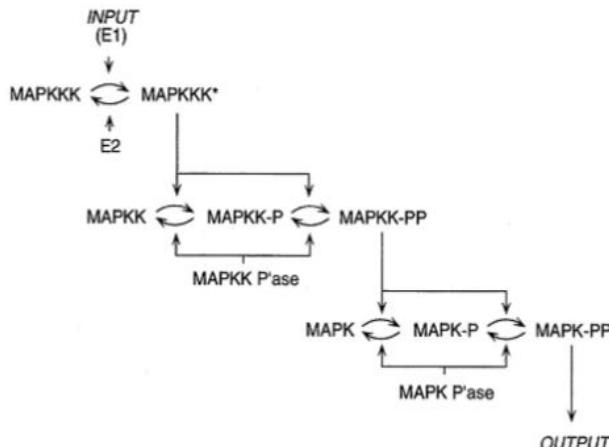
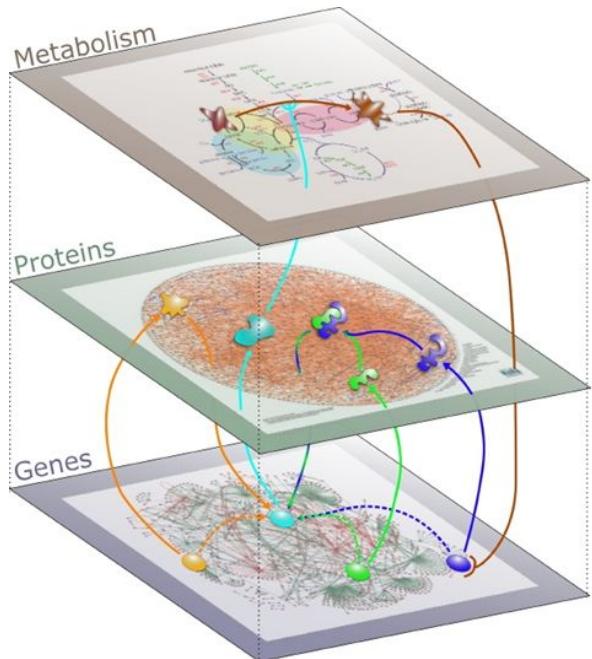


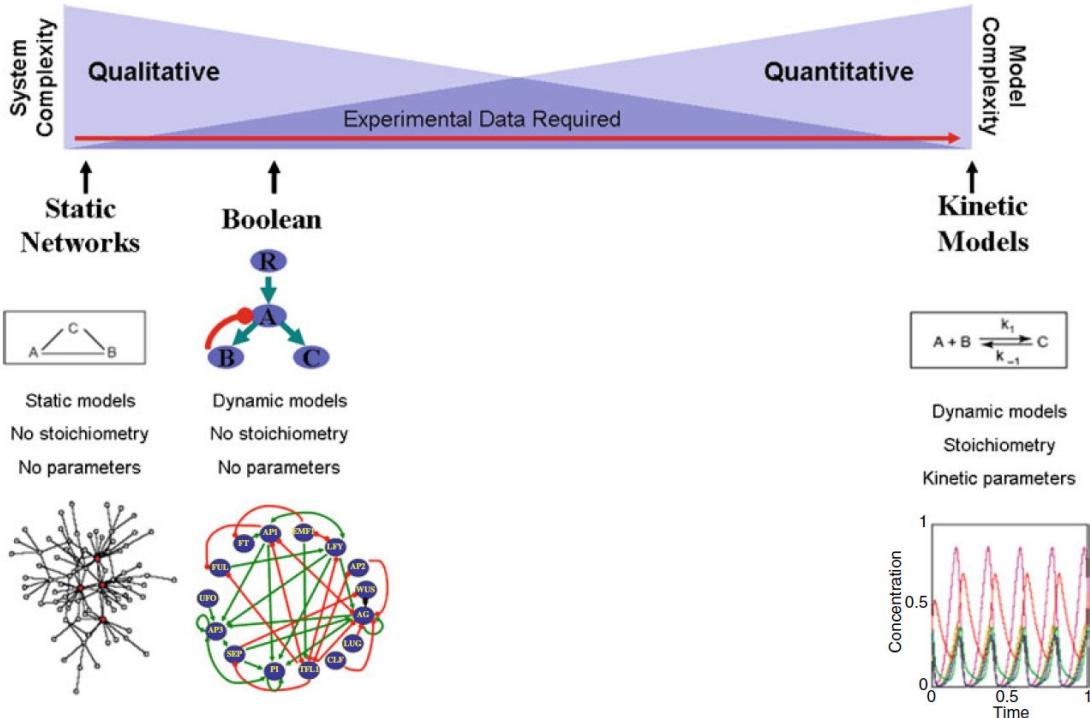
Figure: Huang and Ferrell, PNAS, 2006. Resources to learn more about stochastic modelling: [MIT OpenCourseWare](#) by Jeff Gore, and [Stochastic Processes: An Introduction, Third Edition](#) by Jones and Smith. Tutorials also available on the [website of European Bioinformatics Institute \(EBI\)](#).

ODE-based simulation of dynamics

Different ways of modelling biological networks

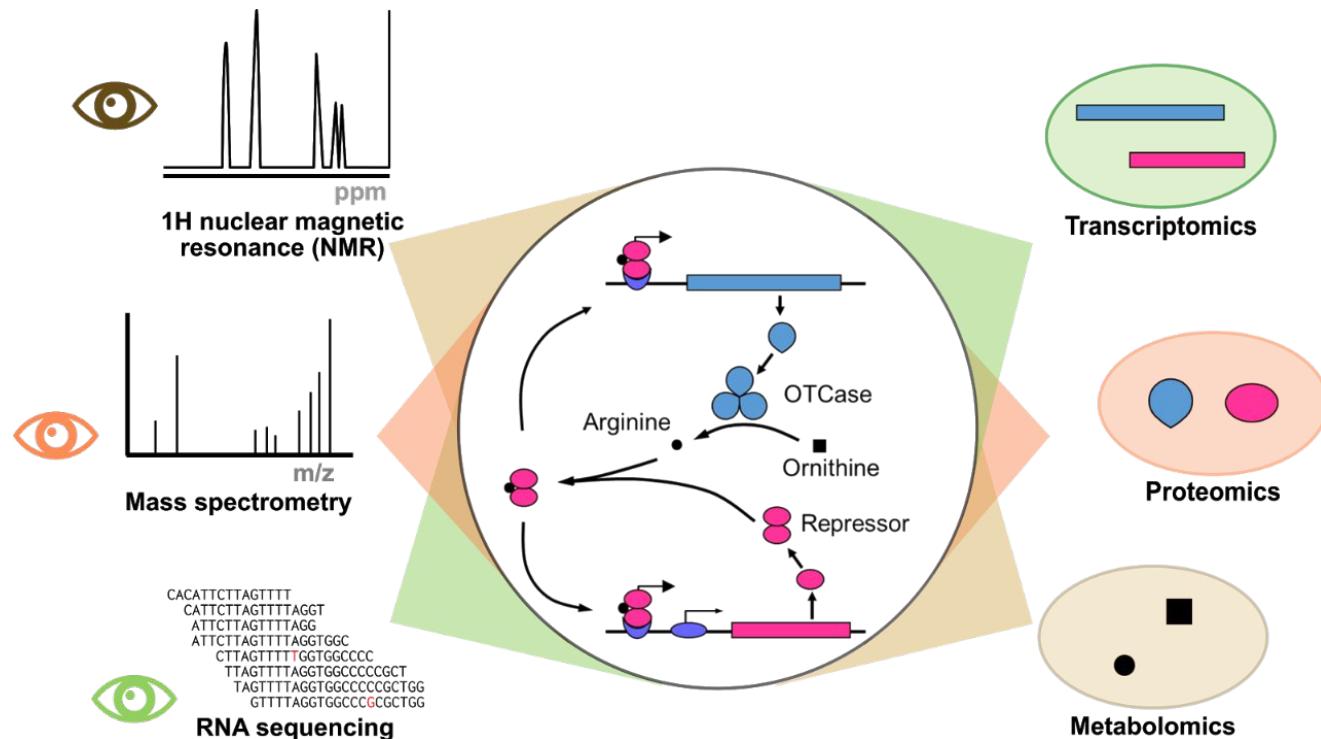


Stéphane CHÉDIN & Jean LABARRE, www-dsv.cea.fr

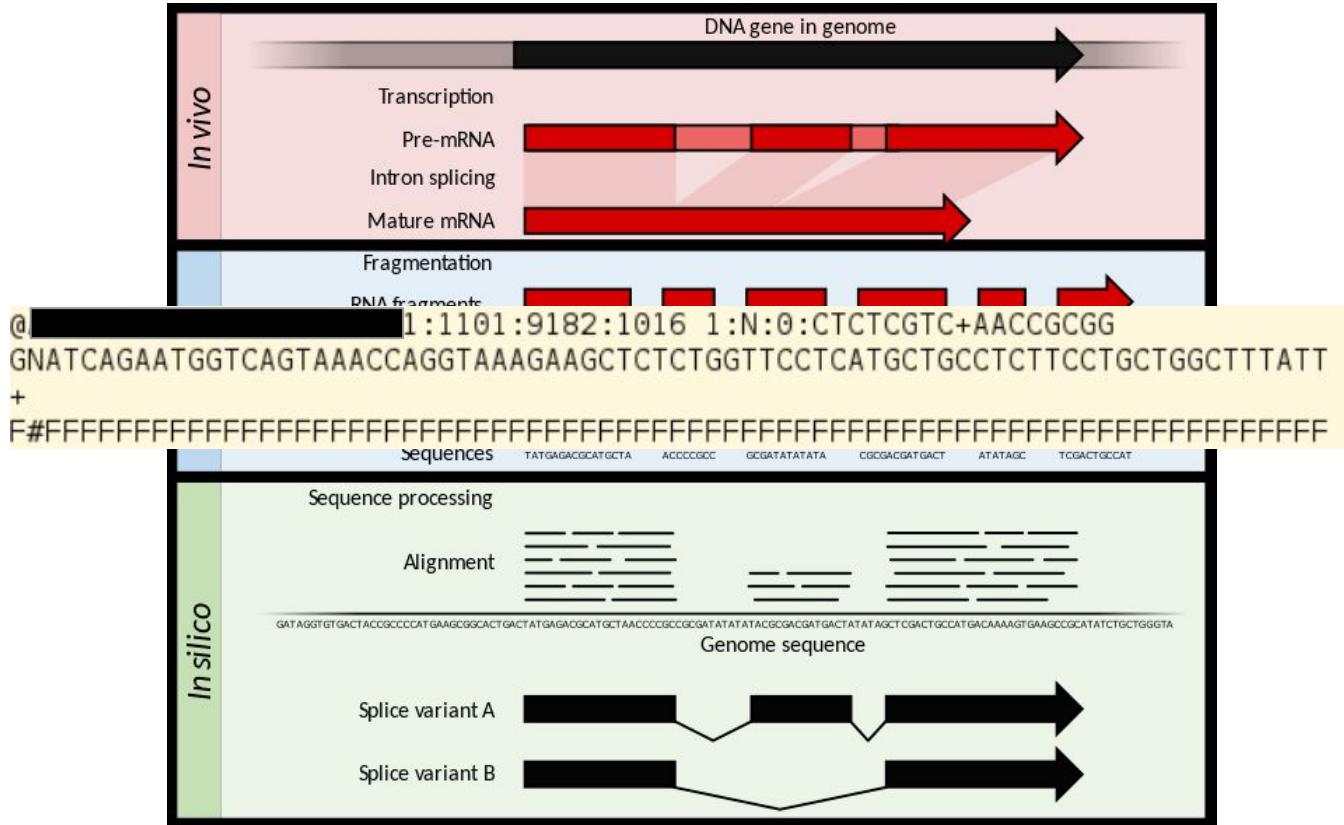


Garg, Abhishek, Kartik Mohanram, Giovanni De Micheli, and Ioannis Xenarios. 2012. "[Implicit Methods for Qualitative Modeling of Gene Regulatory Networks](#)." In *Gene Regulatory Networks: Methods and Protocols*, edited by Bart Deplancke and Nele Gheldof, 397–443. Methods in Molecular Biology. Totowa, NJ: Humana Press.

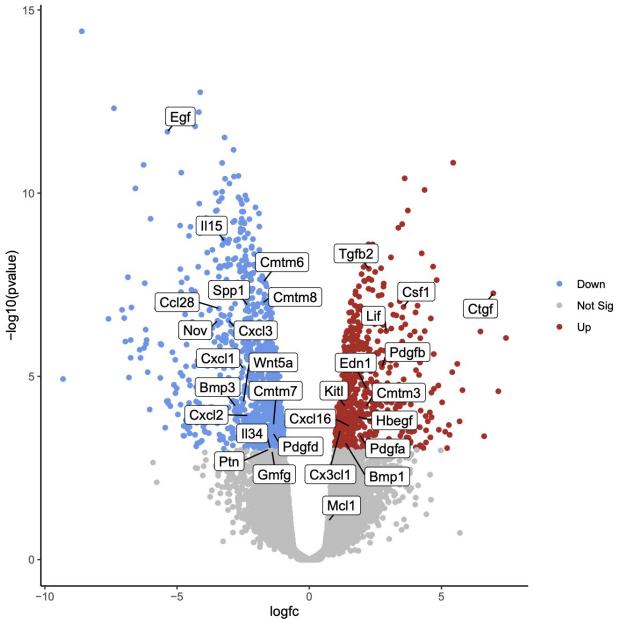
Biological networks can be studied with omics technologies



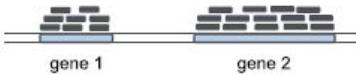
Principle of next-generation RNA sequencing (NGS)



We can reveal compound's effect on gene expression by performing differential gene expression analysis



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

	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

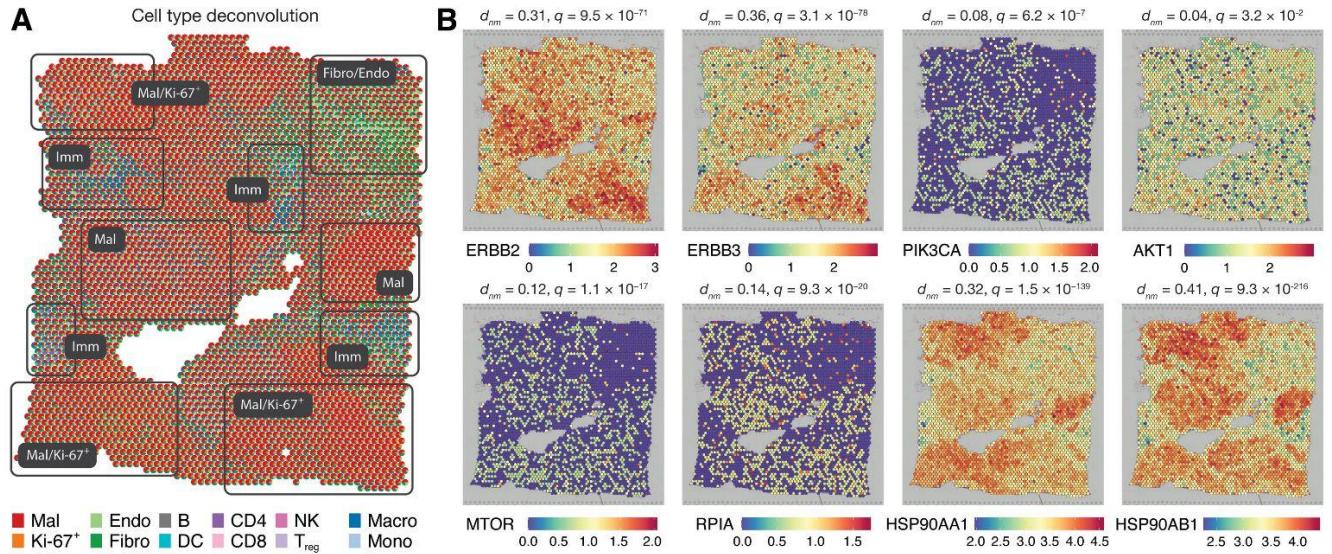
Tot. reads:
5 millions

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

The principle can be applied to individual cells in spatiotemporal contexts



Mal: malignant cells

Ki-67⁺: proliferating cells
(Ki-67 positive)

Endo: endothelial cells

Fibro: fibroblasts

B: B cells

DC: dendritic cells

CD4: CD4⁺ T cells

CD8: CD8⁺ T cells

T_{reg}: regulatory T cells

NK: natural killer cells

Macro: macrophages

Mono: monocytes

[SOAR elucidates biological insights and empowers drug discovery through spatial transcriptomics](#), Li et al., Science Advances, 2024. Breast cancer tissue H&C (Hematoxylin and eosin) staining figure was generated with Gemini 2.5.

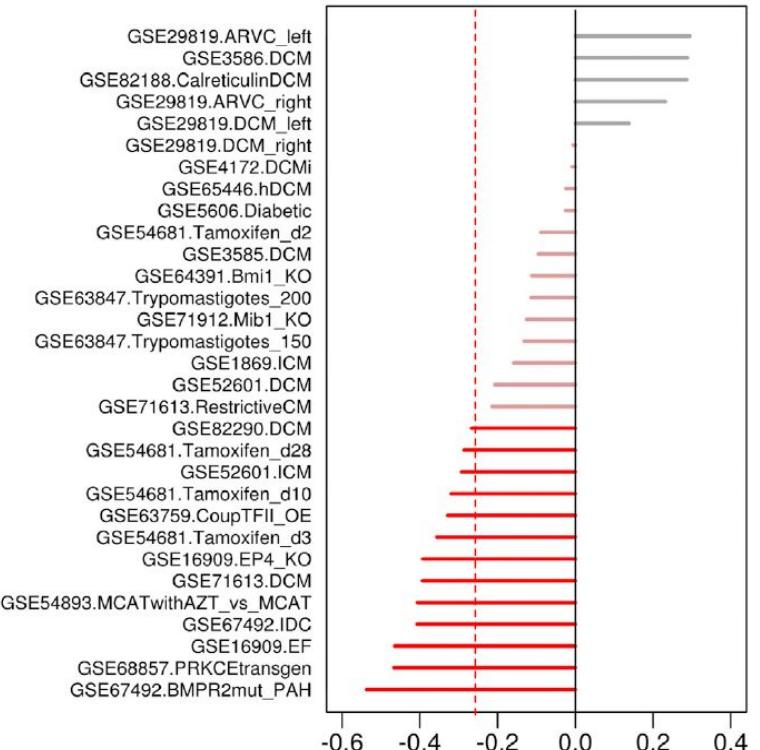
Opportunities and challenges of using omics to study MoA

Opportunities:

1. Patient samples can be profiled with omics. It is possible to compare a compound's effect with the changes induced by disease progression (right).
2. Well-designed omics study can reveal both strong and subtle effects of the compound (the example with splicing modifier).

Challenges:

1. Data from biological models that poorly reflect human disease can do more harm than benefits.
2. Curse of dimensionality.
3. It is intrinsically challenging to how drugs work.

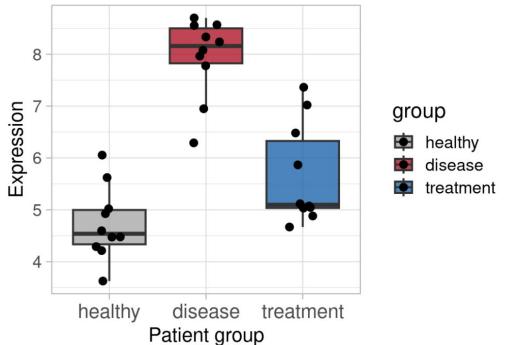


Pathway regulation by beneficial compounds
and in cardiomyopathy: the correlation

Why negative correlation is a good sign?

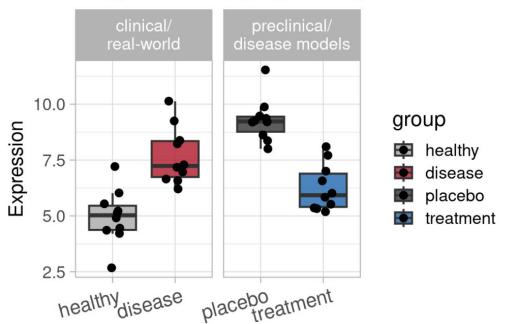
A A partially working treatment

The gene is causally regulated by the disease



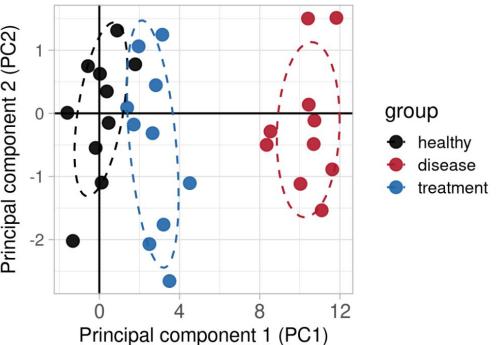
C Simulation: two comparisons

The gene is causally regulated by the disease



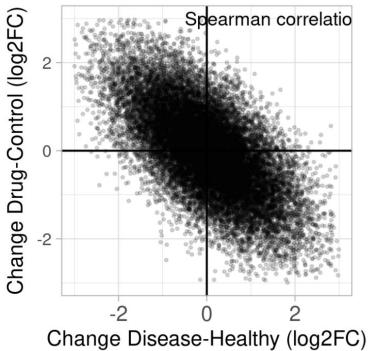
B Simulated clinical observations

10 samples/group, 5000 features (1% diff. exp.)



D Changes in two comparisons

Log₂ fold change (log2FC) of 20000 genes

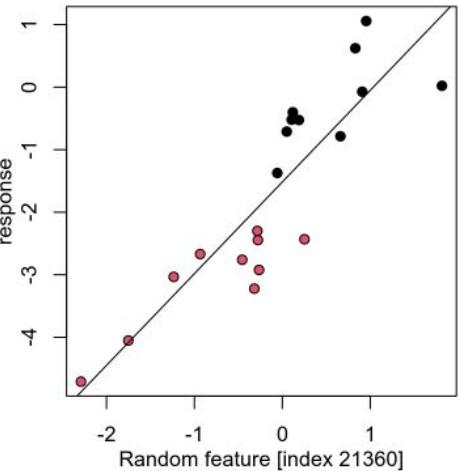
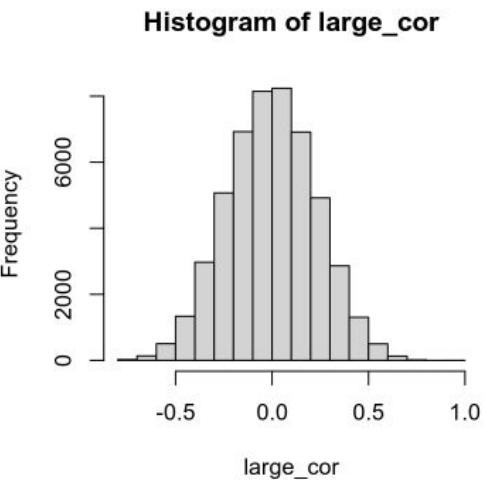


Panel A: A gene is causally up-regulated in patients by a disease. If its regulation is partially or completely reversed by the treatment, we increase our confidence in the drug. **Panel B:** the concept can be generalized to situations where many genes are regulated by the disease and reversed by the drug treatment, visualized with PCA (principal component analysis). **Panel C:** We can quantify biological features both in clinical settings (e.g. patients versus healthy donors) and in preclinical settings (e.g. drug's effect in disease models versus placebo). **Panel D:** a negative correlation between changes in two comparisons increases our confidence in drug's effectiveness, assuming that the causal structure is conserved between clinical and preclinical settings. [See more details about the simulation in AMIDD's repo..](#)

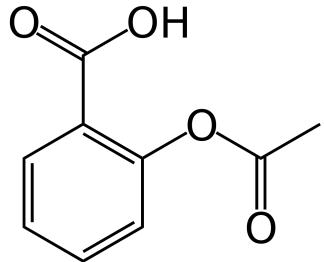
Given enough tests, there will be significant results

```
set.seed(1887)
patient_group <- gl(2, 10)
response <- c(rnorm(10, 0), rnorm(10, -3))
random_features_large <- matrix(rnorm(20*50000), nrow=20)
large_cor <- cor(response, random_features_large, method="spearman")
hist(large_cor)
```

```
largest_cor_ind <- which.max(large_cor)
{
  compactPar()
  plot(random_features_large[, largest_cor_ind],
       response,
       bg=patient_group, pch=21,
       xlab=sprintf("Random feature [index %d]", largest_cor_ind))
  abline(lm(response ~ random_features_large[, largest_cor_ind]))
}
```



The road of MoA understanding can be 120 year long



Dai *et al*, Cell, 2019

Acetylation blocks cGAS activity and inhibits self-DNA-induced autoimmunity



Aspirin
trademarked in
1899

- Acetylation suppresses cGAS activity
- Aspirin directly acetylates cGAS
- Aspirin inhibits cGAS-mediated interferon production
- Aspirin alleviates DNA-induced autoimmunity in AGS mouse models and patient cells

MoA understanding can be a long process full of surprises

Summary

1. In lead optimization and early development, we are interested in MoA of drug candidates *in vitro*, *in vivo*, and in human.
2. We can study MoA by modeling biological networks, for instance with ODE-based models and its variants.
3. We can also study MoA by performing omics experiments and analysing the data with statistical, machine-learning or AI tools. It is helpful to keep both advantages and challenges in mind.

WHEN YOU SEE A CLAIM THAT A COMMON DRUG OR VITAMIN "KILLS CANCER CELLS IN A PETRI DISH,"

KEEP IN MIND:



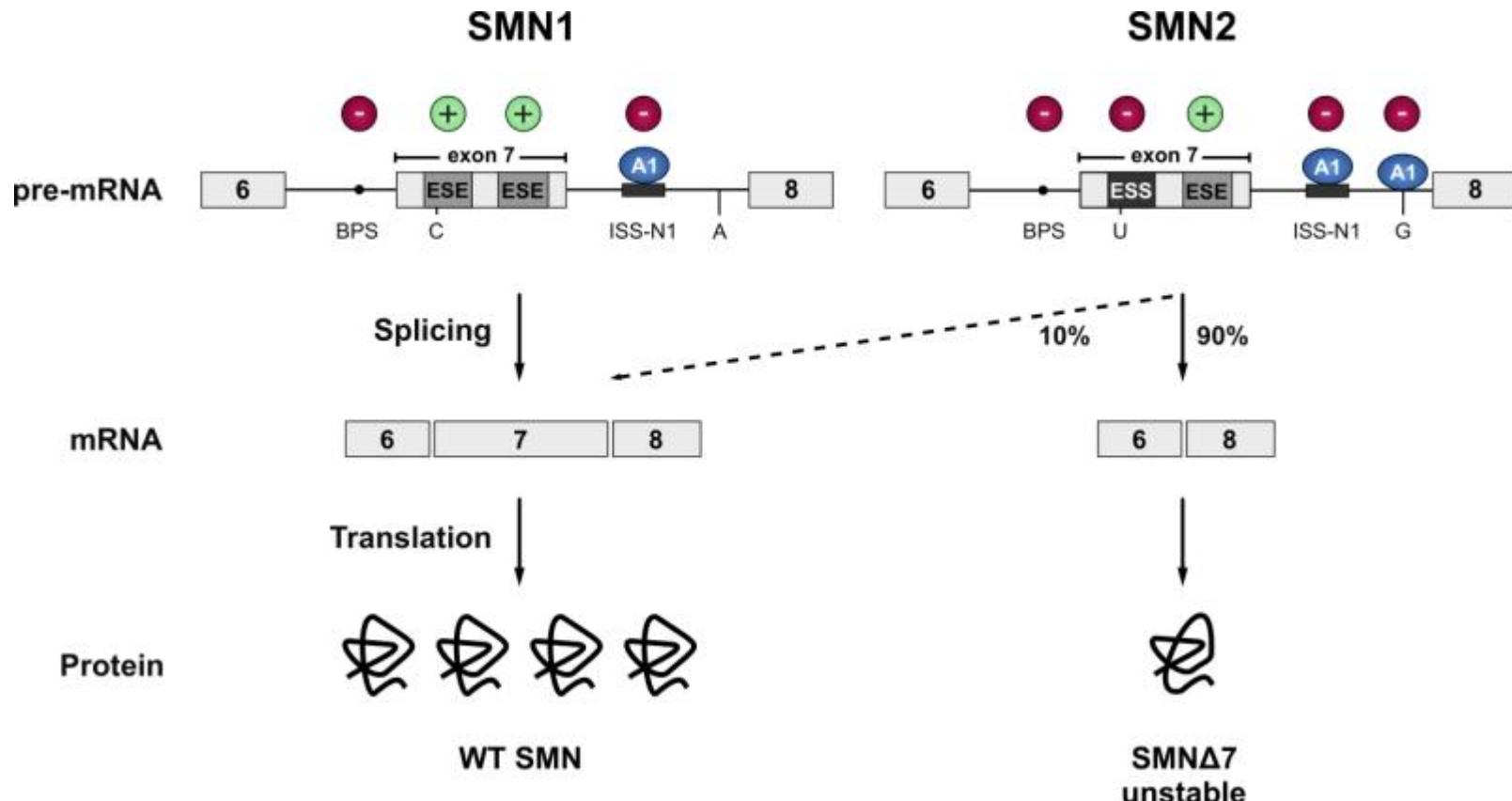
SO DOES A HANDGUN.

<https://xkcd.com/1217/>

Backup material

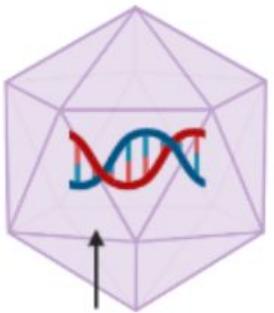
Splicing modifiers

Splicing of SMN1 and SMN2 genes: patients with mutations in SMN1 gene suffer from Spinal Muscle Atrophy (SMA)



Three drugs of different modalities are approved to treat SMA

AAV9 capsid

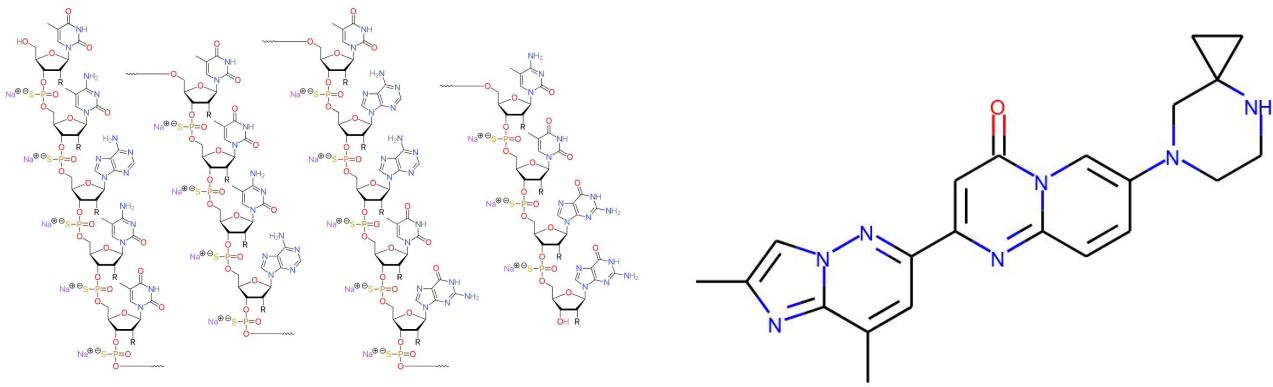


SMN1 gene

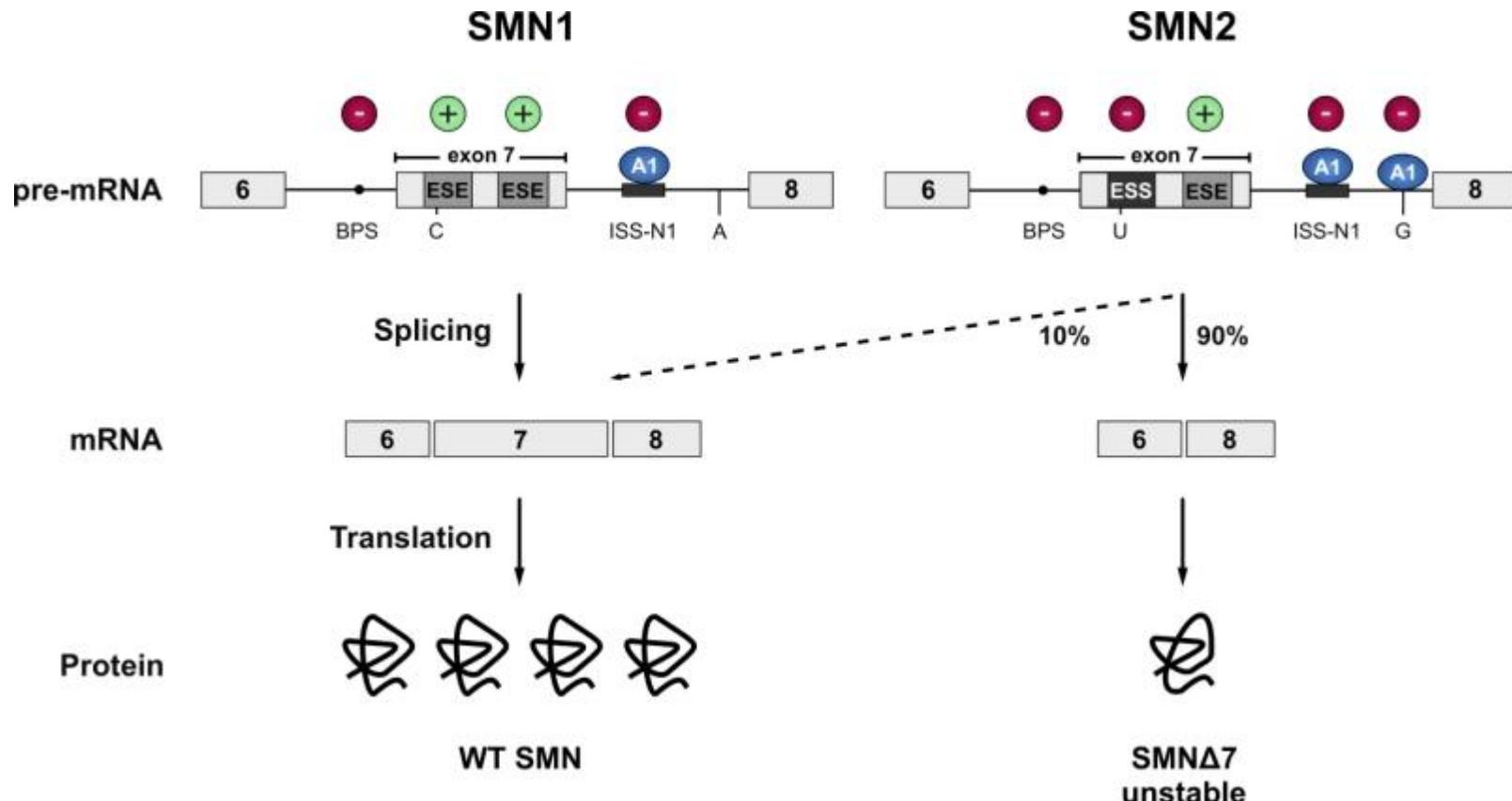
Onasemnogene
Abeparvovec/
Zolgensma

Nusinersen sodium/ Spinraza
[CHEMBL3833342](https://chembl.org/compound/CHEMBL3833342)

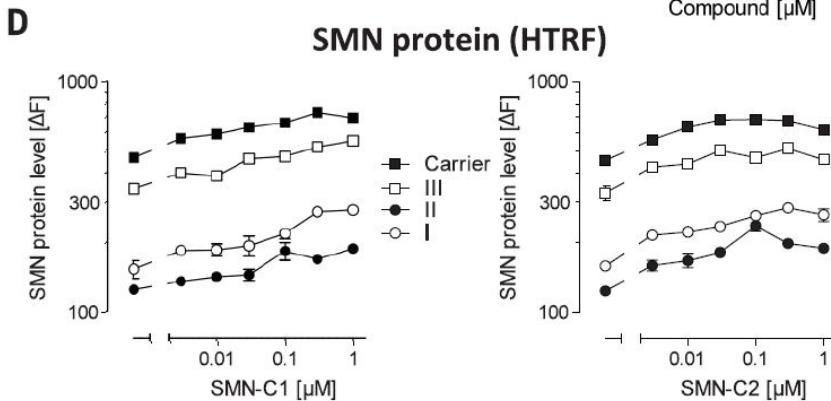
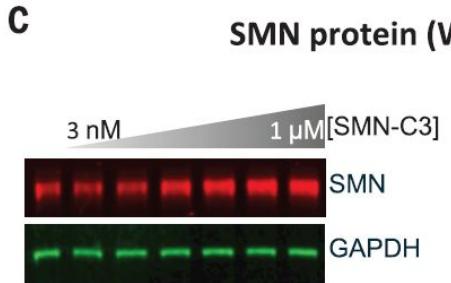
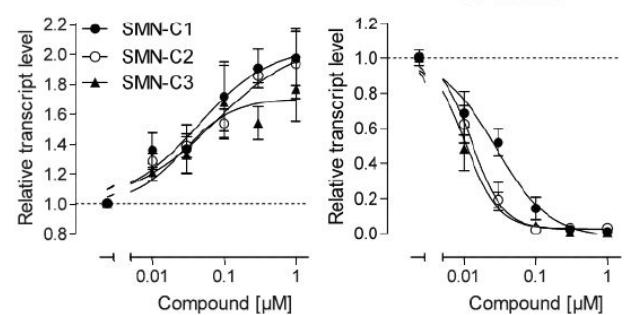
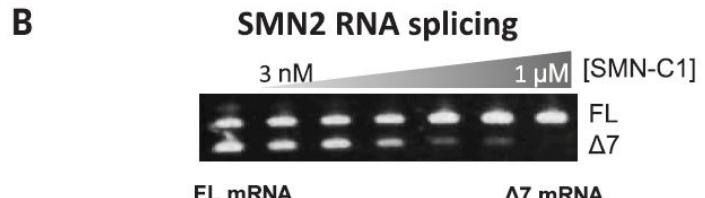
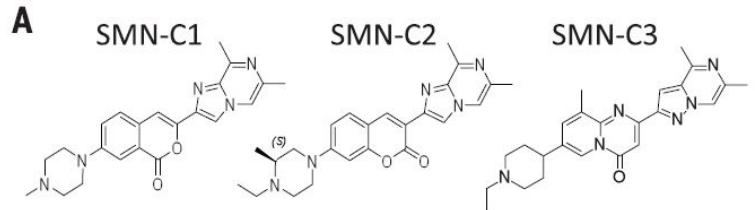
Risdiplam/ Evrysdi
[CHEMBL4297528](https://chembl.org/compound/CHEMBL4297528)



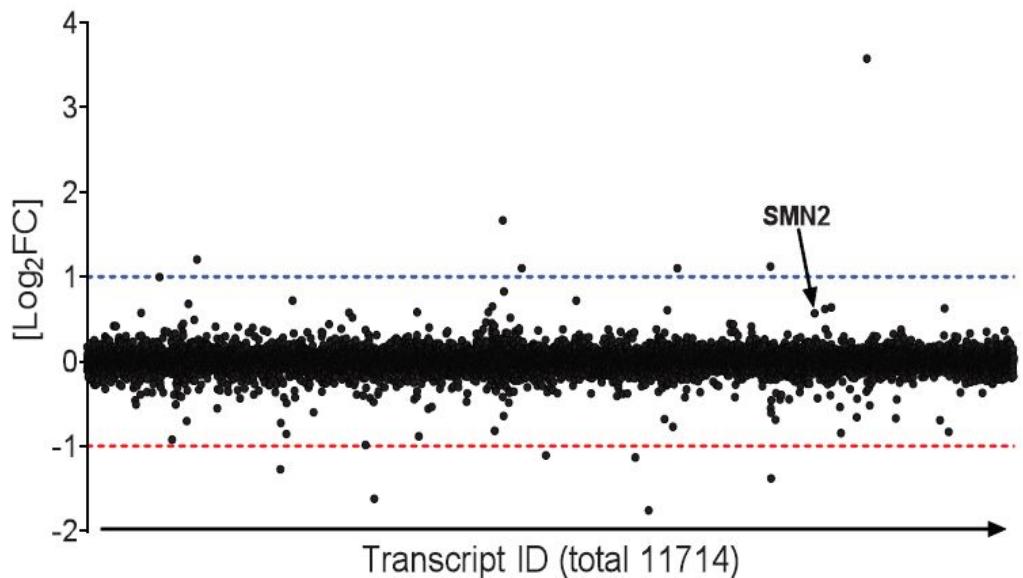
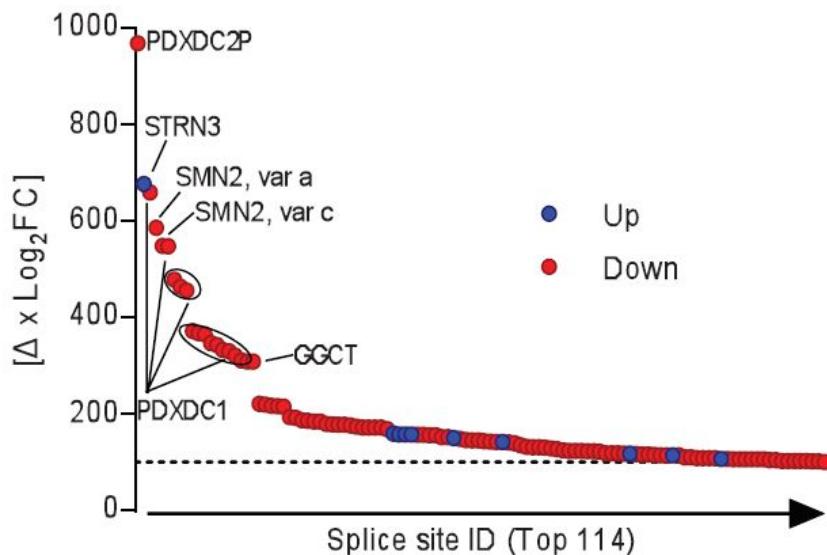
Splicing of SMN1 and SMN2 genes: patients with mutations in SMN1 gene suffer from Spinal Muscle Atrophy (SMA)



Small molecules were identified as RNA splicing modifiers

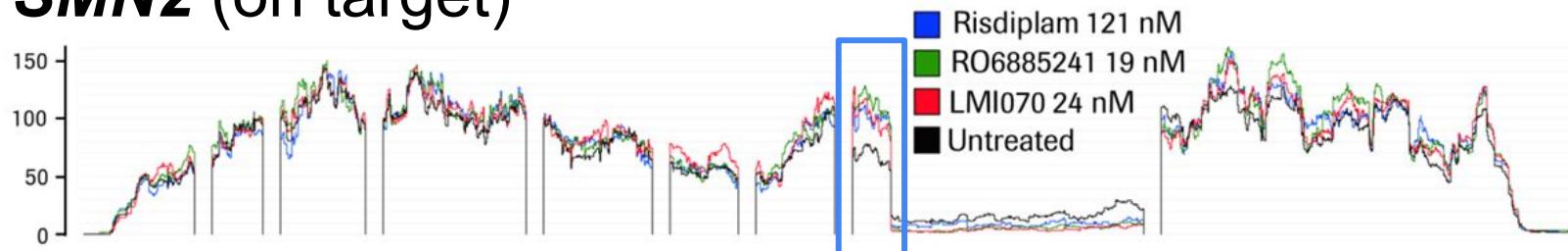


RNA sequencing confirms the specificity of SMN-C3

A**Transcriptional changes by SMN-C3****B****Splicing regulation by SMN-C3**

RNA sequencing confirms the superior safety profile of SMN-C3 over other compounds

SMN2 (on target)



FOXM2 (off target)

