# AMIDD 2025 Lecture 10: Biological networks and omics
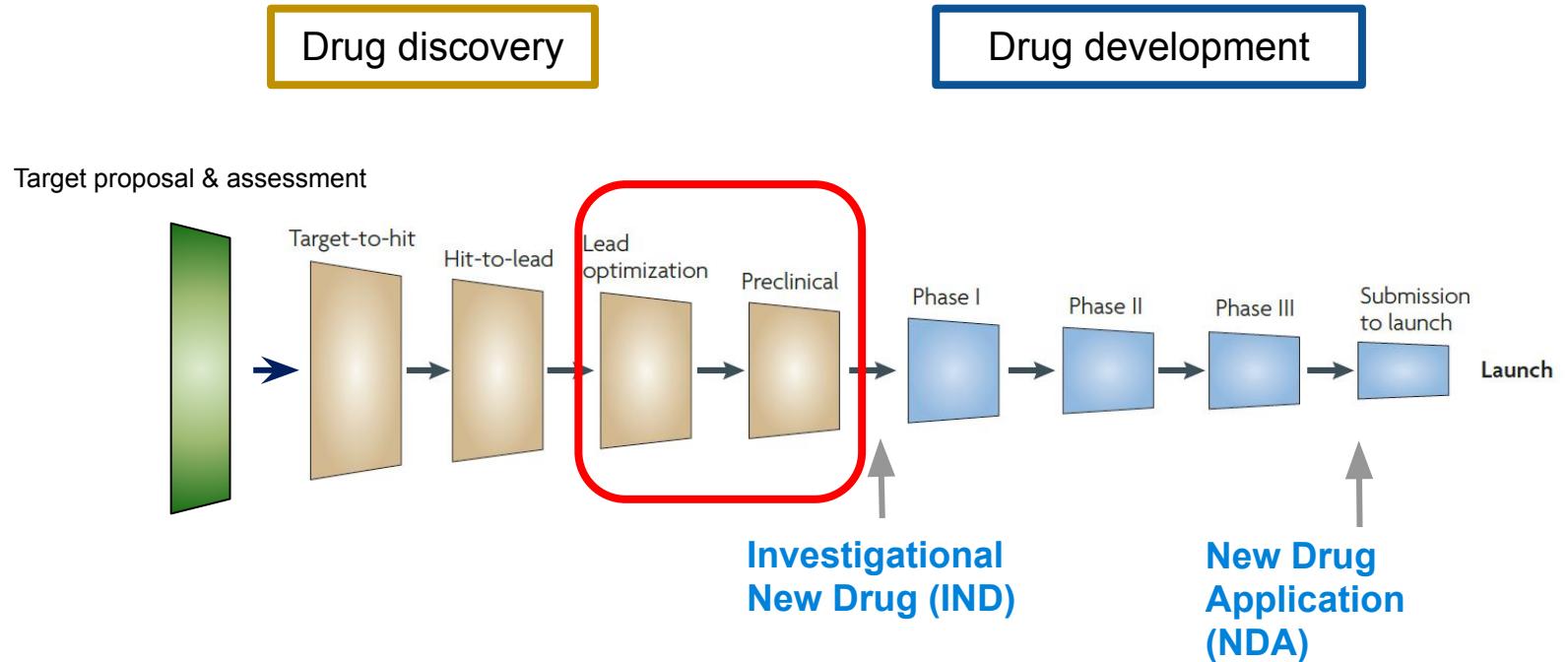
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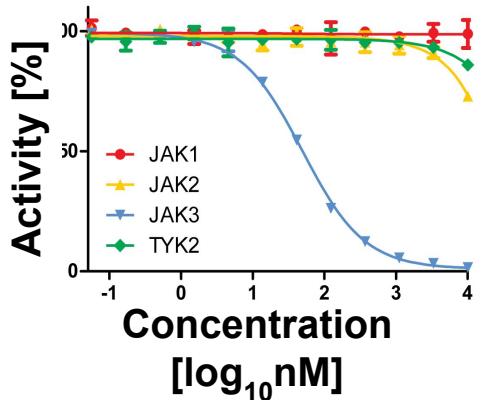
Translational research makes molecules into medicines
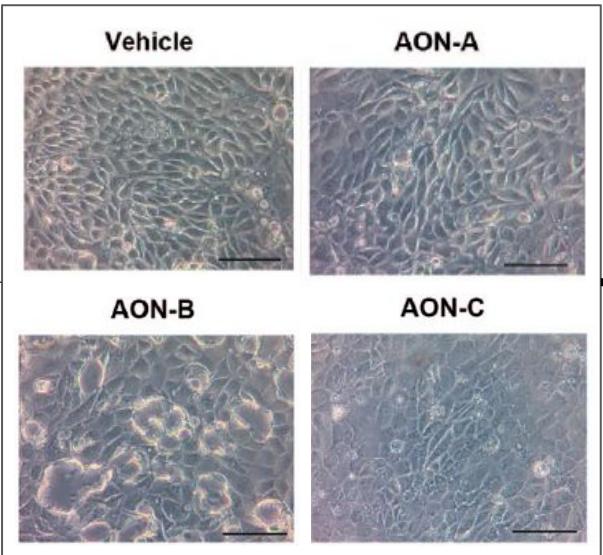
Adapted from Paul *et al.* "How to Improve R&D Productivity: The Pharmaceutical Industry's Grand Challenge." Nature Reviews Drug Discovery, 2010
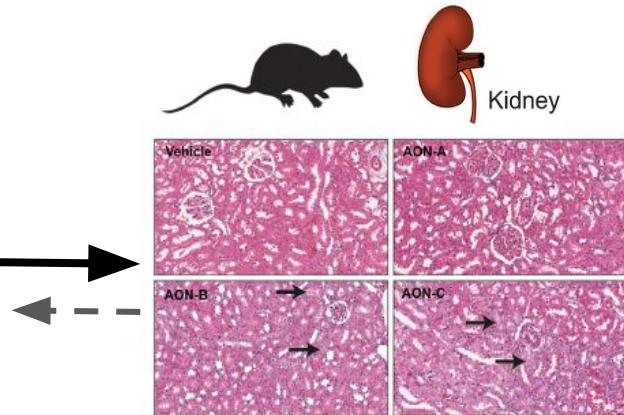
Classical workflow of efficacy and toxicity assessment

Biochemical & biophysical assays

Cellular assays (*in vitro*)
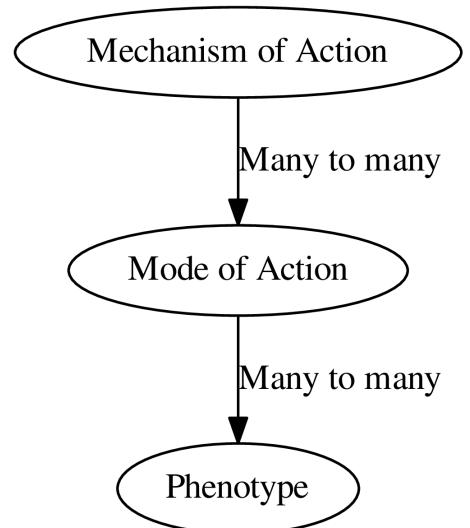
Animal experiments (*in vivo*)

→	Usual workflow
← – – –	Assay development

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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.

```
   ( Mechanism of Action )
            |
        Many to many
            ↓
     ( Mode of Action )
            |
        Many to many
            ↓
       ( Phenotype )
```

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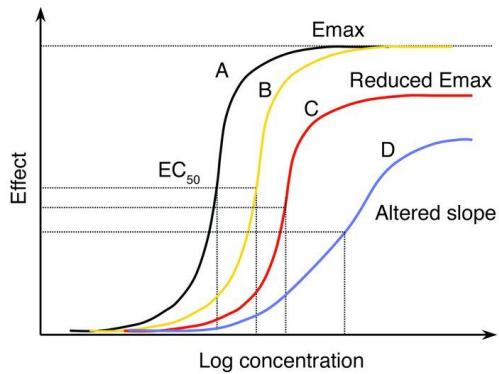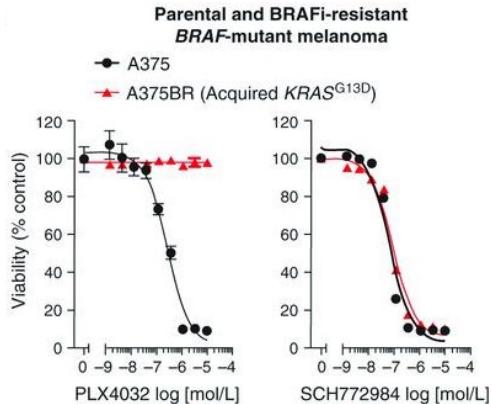
$$H = H_{max} \frac{x^n}{k^n + x^n}$$

The general form of the Hill function

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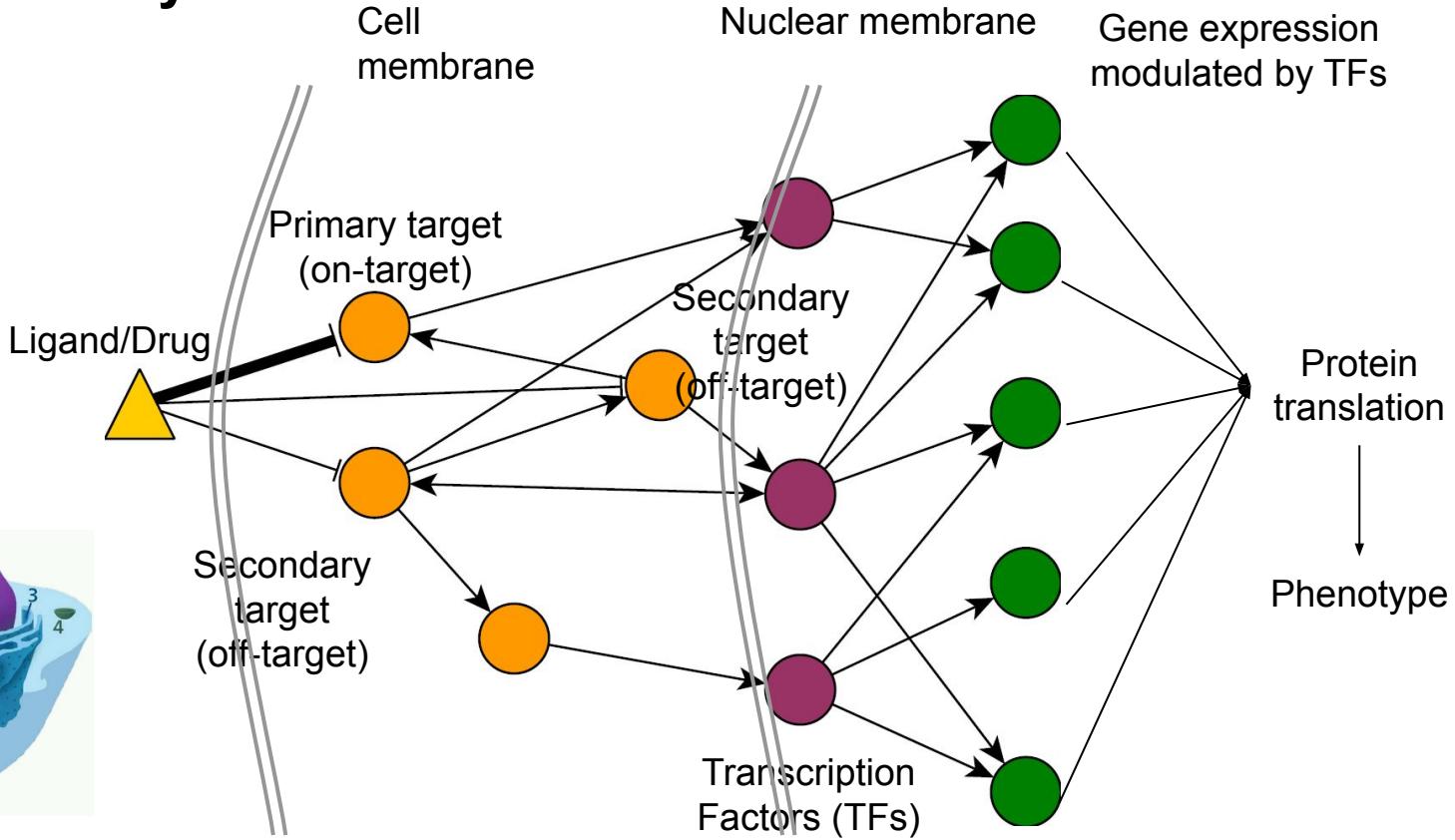
$$= E_{max} \frac{1}{1 + \left(\frac{EC_{50}}{[L]}\right)^n}$$

Modelling the dose-dependent effect

Parental and BRAFi-resistant *BRAF*-mutant melanoma
- A375
- A375BR (Acquired *KRAS*^{G13D})

Viability (% control) vs PLX4032 log [mol/L]
SCH772984 log [mol/L]

Emax, A, B, Reduced Emax, C, D, Altered slope, EC_{50}, Effect, Log concentration
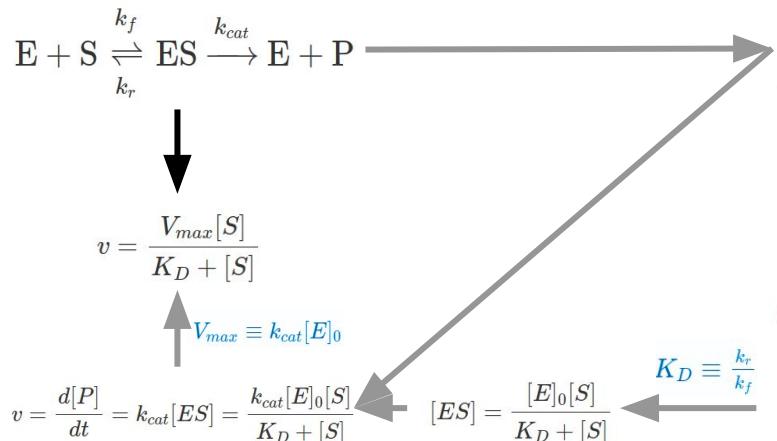
Suppose it is an antiviral drug, compared with curve B, what does curve A, C, and D suggest?

Biological networks interact with drugs and manifest its efficacy and safety

Cell membrane

Nuclear membrane

Gene expression modulated by TFs

Primary target (on-target)

Ligand/Drug

Secondary target (off-target)

Secondary target (off-target)

Protein translation

Phenotype

Transcription Factors (TFs)
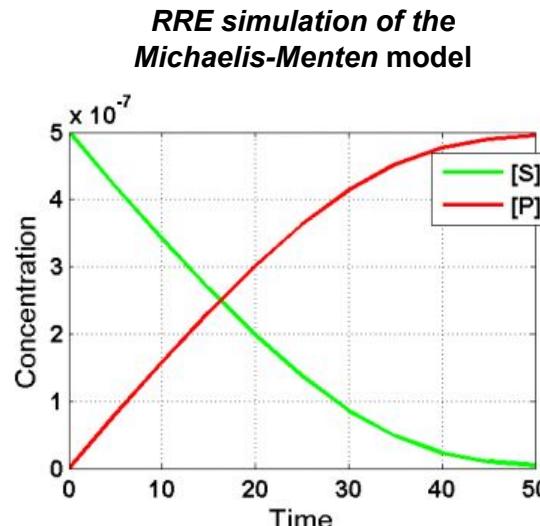
Reaction Rate Equations: a compartment/ODE model of biological chemical reaction

$$\mathrm{E + S} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathrm{ES} \overset{k_{cat}}{\longrightarrow} \mathrm{E + P}$$

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

$$\frac{d[S]}{dt} = -k_f[E][S] + k_r[ES],$$

$$\frac{d[ES]}{dt} = k_f[E][S] - k_r[ES] - k_{cat}[ES],$$

$$\frac{d[P]}{dt} = k_{cat}[ES],$$

$$k_f[E][S] = k_r[ES]$$

$$k_f([E]_0 - [ES])[S] = k_r[ES]$$
$$k_f[E]_0[S] - k_f[ES][S] = k_r[ES]$$
$$k_f[E]_0[S] = k_r[ES] + k_f[ES][S]$$
$$k_f[E]_0[S] = [ES](k_r + k_f[S])$$

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

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

$$v = \frac{V_{max}[S]}{K_D + [S]}$$

$$V_{max} \equiv k_{cat}[E]_0$$

$$v = \frac{d[P]}{dt} = k_{cat}[ES] = \frac{k_{cat}[E]_0[S]}{K_D + [S]}$$

$$[ES] = \frac{[E]_0[S]}{K_D + [S]}$$

$$K_D \equiv \frac{k_r}{k_f}$$

***RRE simulation of the Michaelis-Menten* model**
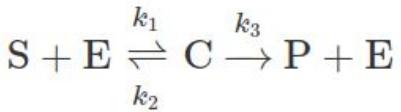
Source: Systems Engineering Wiki (tue.nl)

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

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \overset{k_3}{\rightarrow} P + E$$
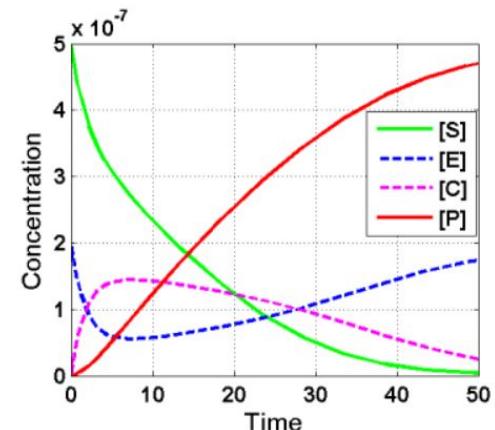
Given the initial values and rate constants

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

According to the law of mass action

$$\frac{d[S]}{dt} = -k_1[E][S] + k_2[C],$$

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

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

$$\frac{d[P]}{dt} = k_3[C],$$

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.


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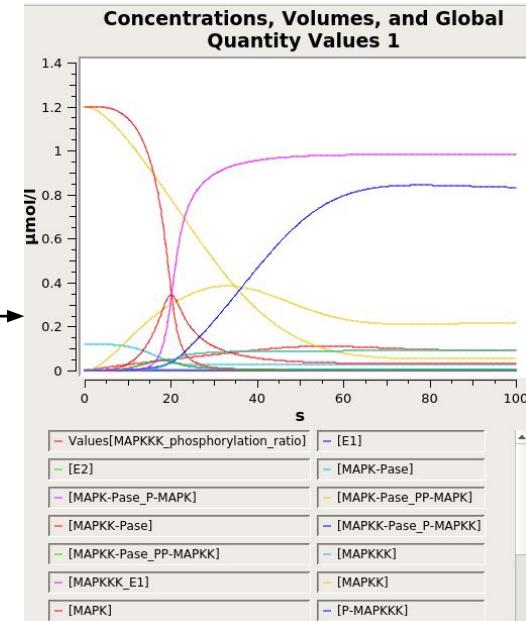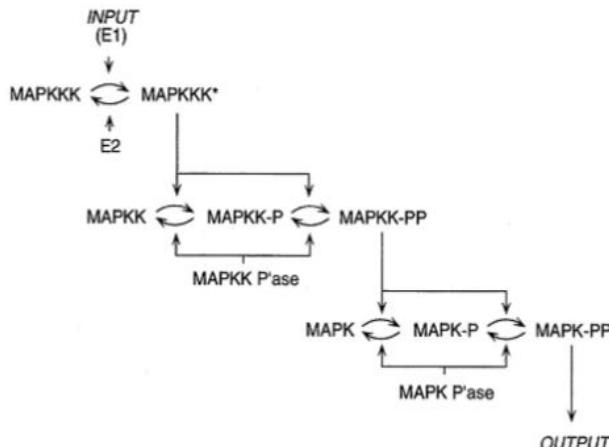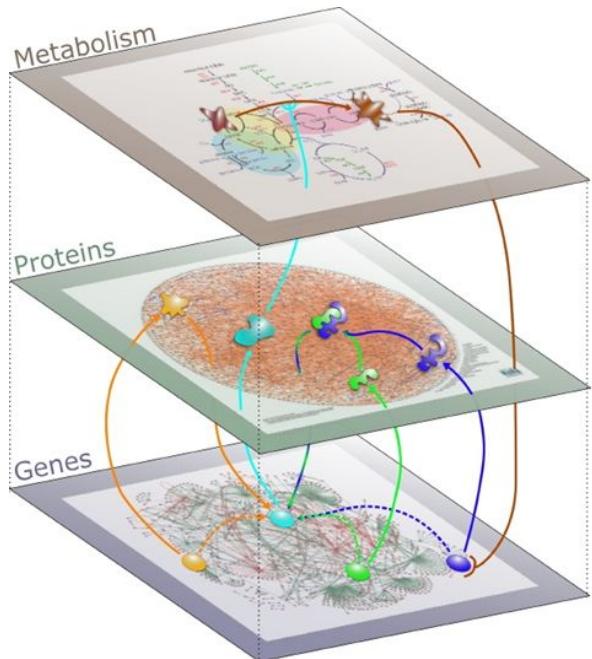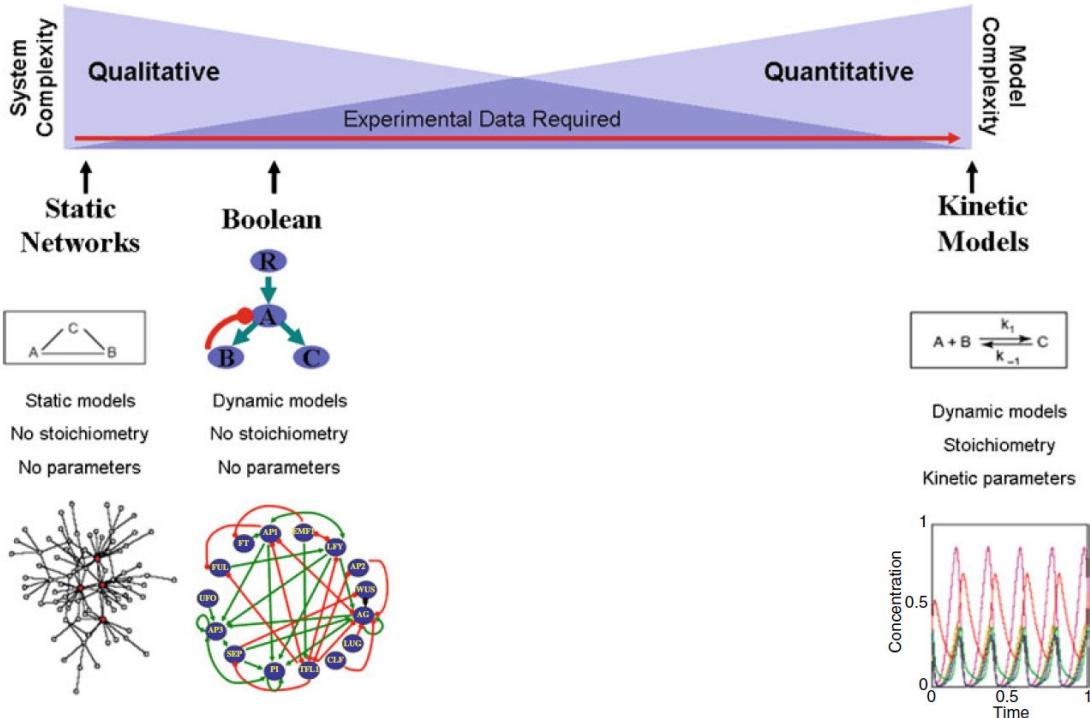
Figure: Huang and Ferrell, PNAS, 2006. Resources to learn more about stochastic modelling: MIT OpenCourseWare by Jeff Gore, and Stochastic Processes: An Introduction, Third Edition by Jones and Smith. Tutorials also available on the website of European Bioinformatics Institute (EBI)

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Different ways of modelling biological networks
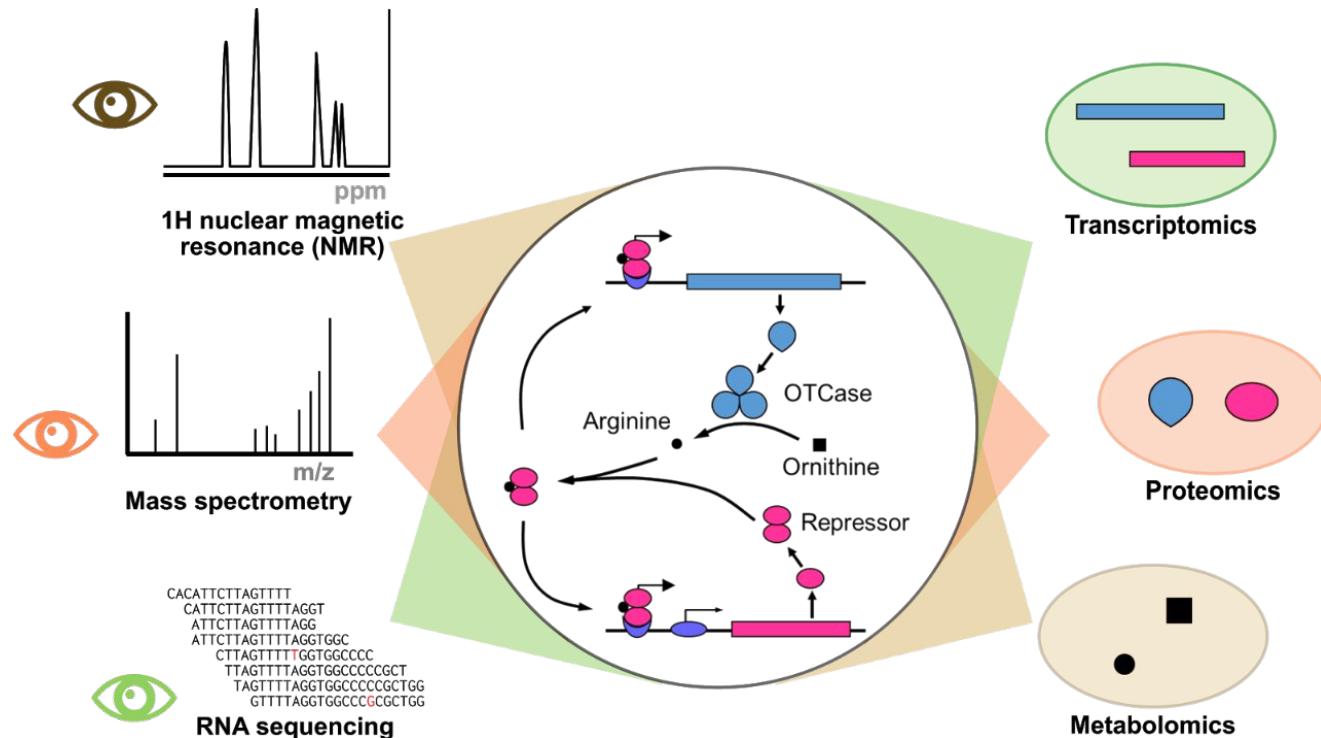

Stéphane CHÉDIN & Jean LABARRE, www-dsv.cea.fr
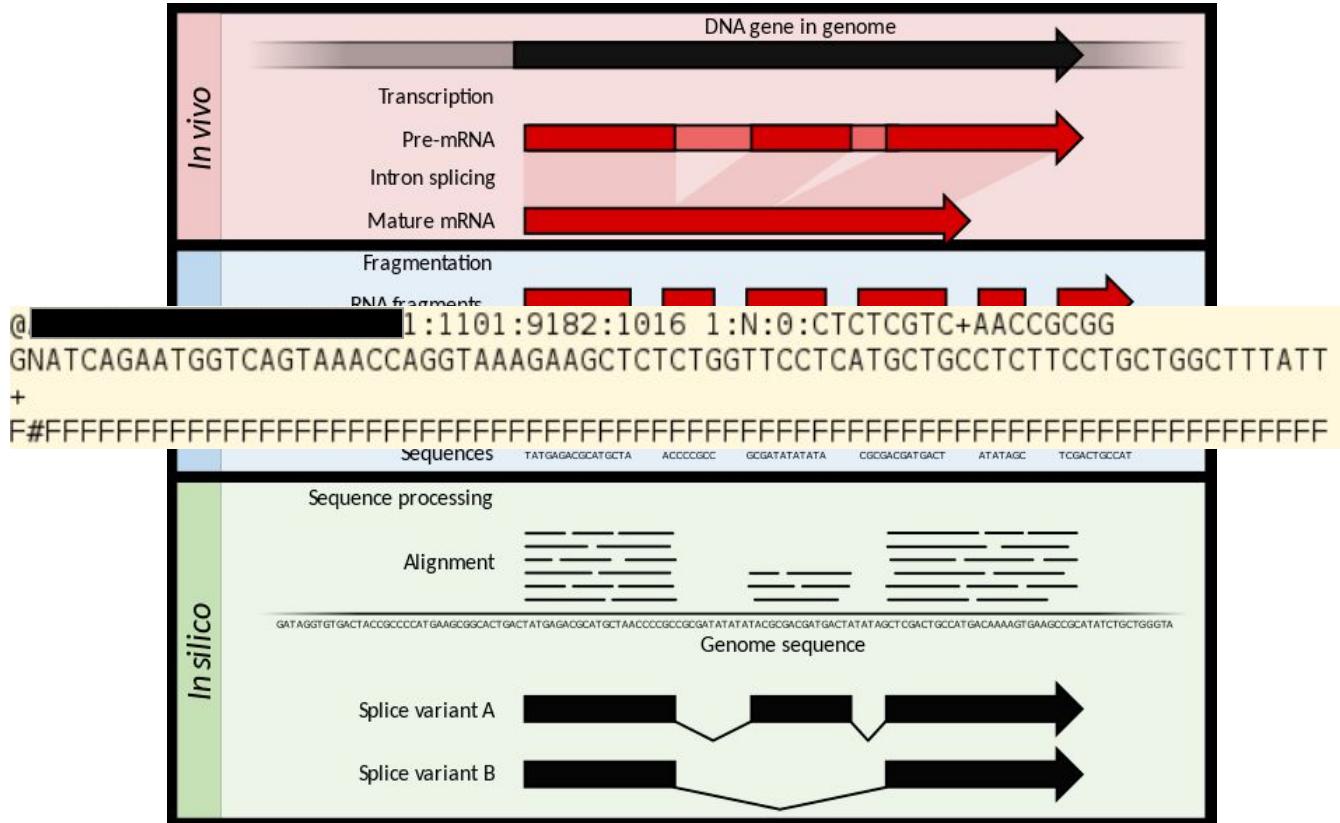
Garg, Abhishek, Kartik Mohanram, Giovanni De Micheli, and Ioannis Xenarios. 2012. "Implicit Methods for Qualitative Modeling of Gene Regulatory Networks." In *Gene Regulatory Networks: Methods and Protocols*, edited by Bart Deplancke and Nele Gheldof, 397–443. Methods in Molecular Biology. Totowa, NJ: Humana Press.
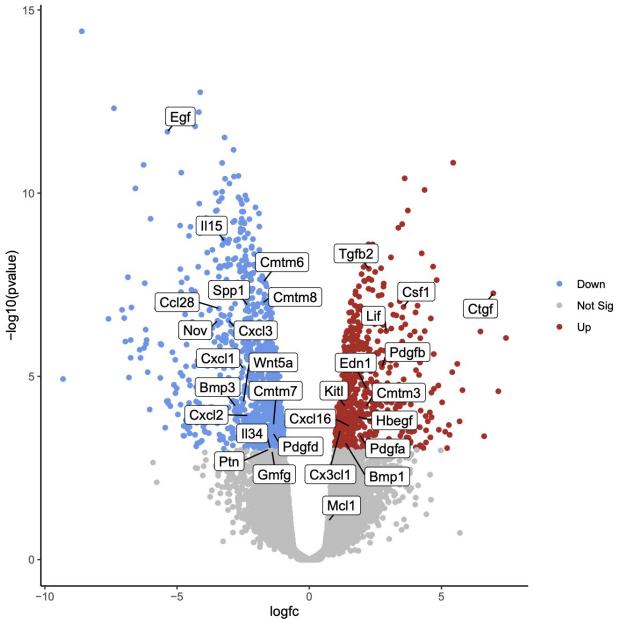
Biological networks can be studied with omics technologies
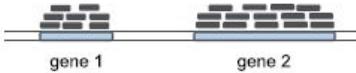
Principle of next-generation RNA sequencing (NGS)

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

Read Mapping

Count collection

Normalization

Differential Gene Expression Analysis

Visualization (e.g. volcano plot)

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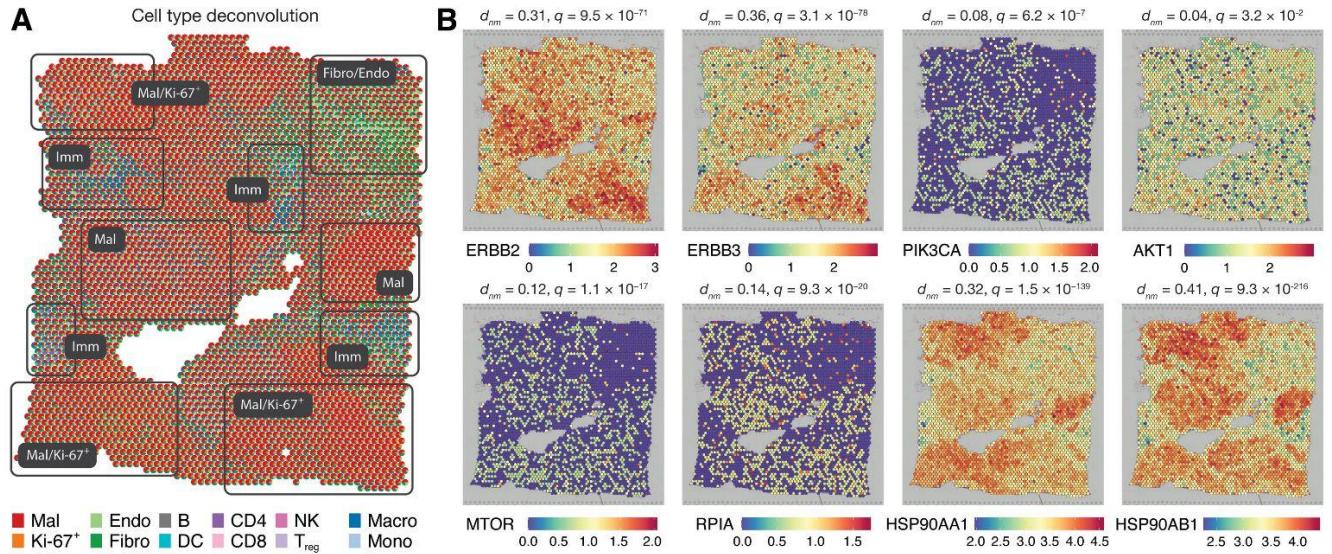
The principle can be applied to individual cells in sptiotemporal contexts

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.
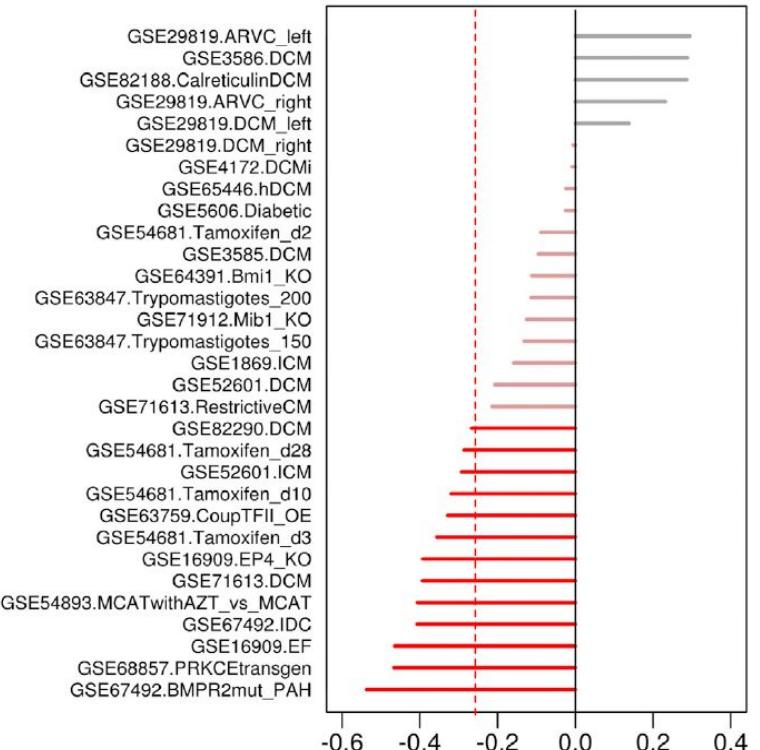
# Opportunities and challenges of using omics to study MoA

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.
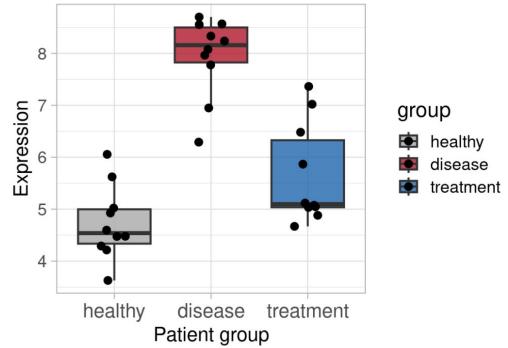
Pathway regulation by beneficial compounds and in cardiomyopathy: the correlation

Why is negative correlation a good sign?
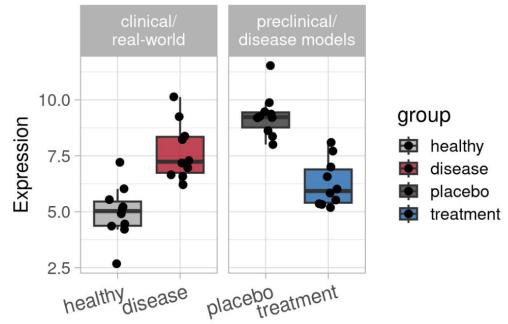
A A partially working treatment
The gene is causally regulated by the disease

**B** Simulated clinical observations
10 samples/group, 5000 features (1% diff. exp.)

C Simulation: two comparisons
The gene is causally regulated by the disease

D Changes in two comparisons
Log2 fold change (log2FC) of 20000 genes
Spearman cor.:-0.63
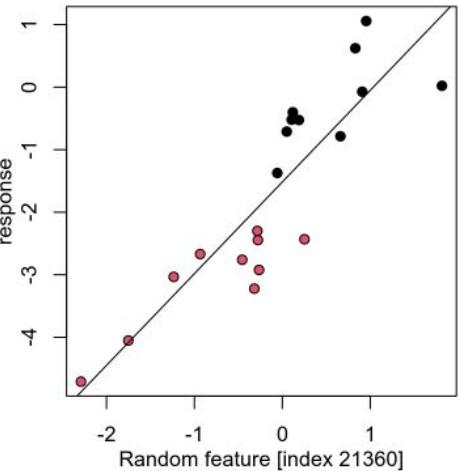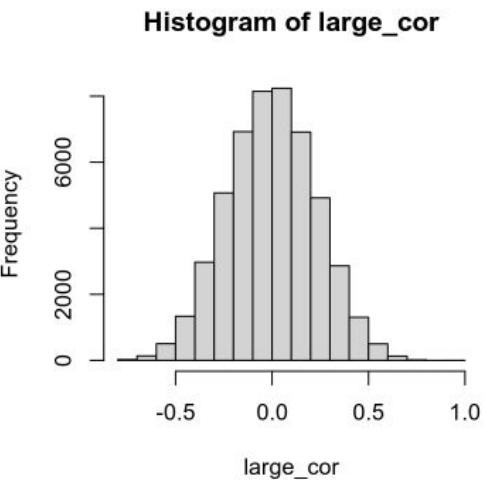
Panel A: A gene is causally up-regulated in patients by a disease. If its regulation is partially or completely reversed by the treatment, we increase our confidence in the drug. **Panel B**: the concept can be generalized to situations where many genes are regulated by the disease and reversed by the 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..

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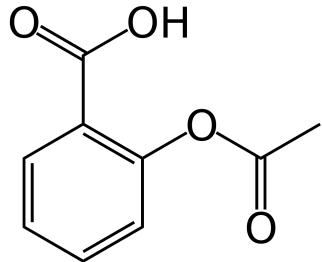
# Given enough tests, there will be significant results

```r
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)
```


Histogram of large_cor

```r
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]))
}
```


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The road of MoA understanding can be 120 year long

Aspirin trademarked in 1899

Dai *et al*, Cell, 2019

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

- 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/
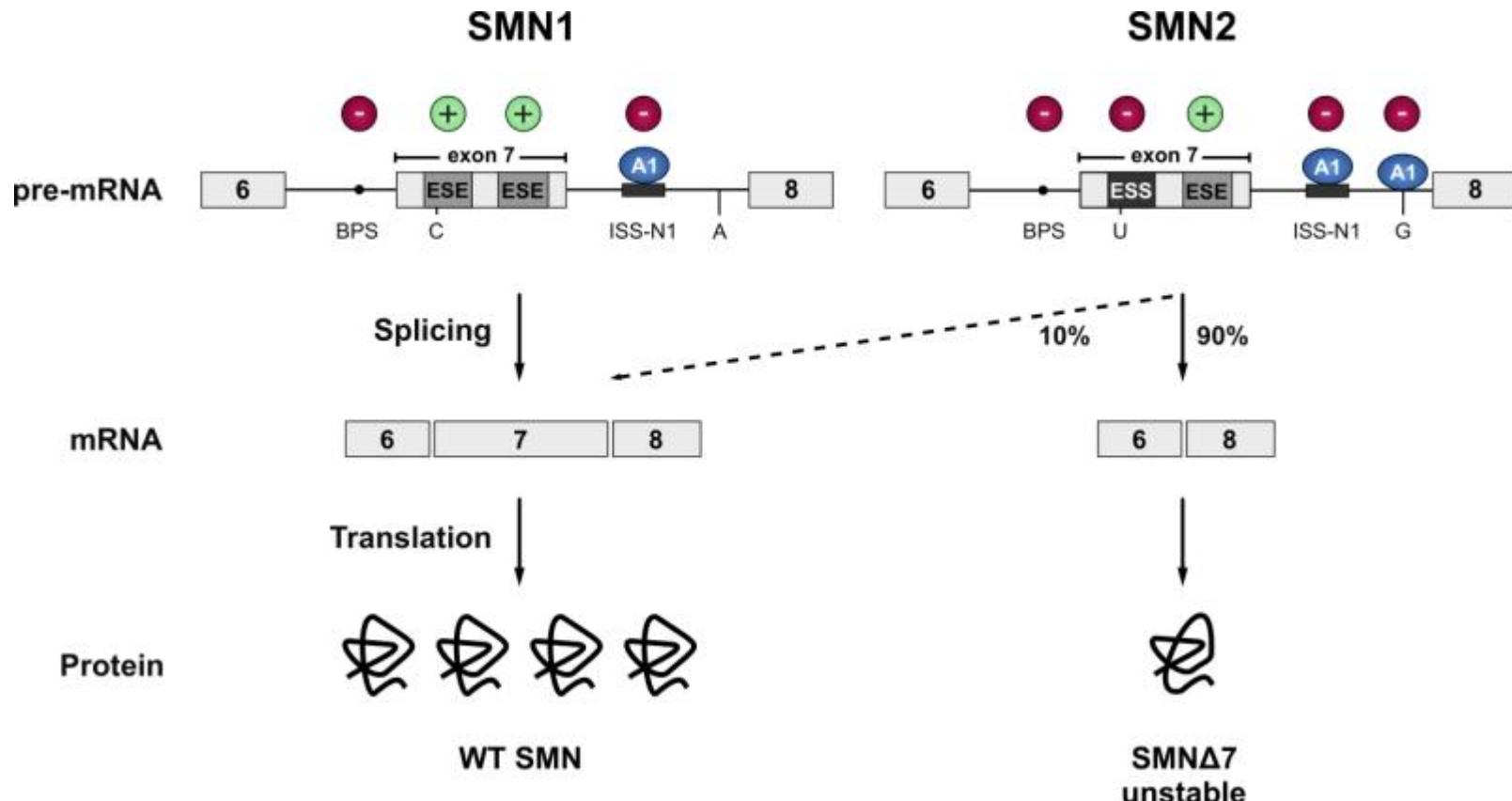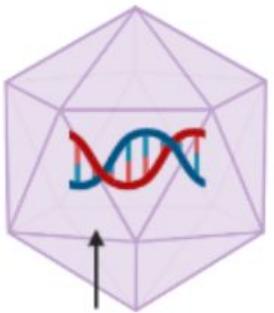
Backup material
Splicing modifiers

# 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**
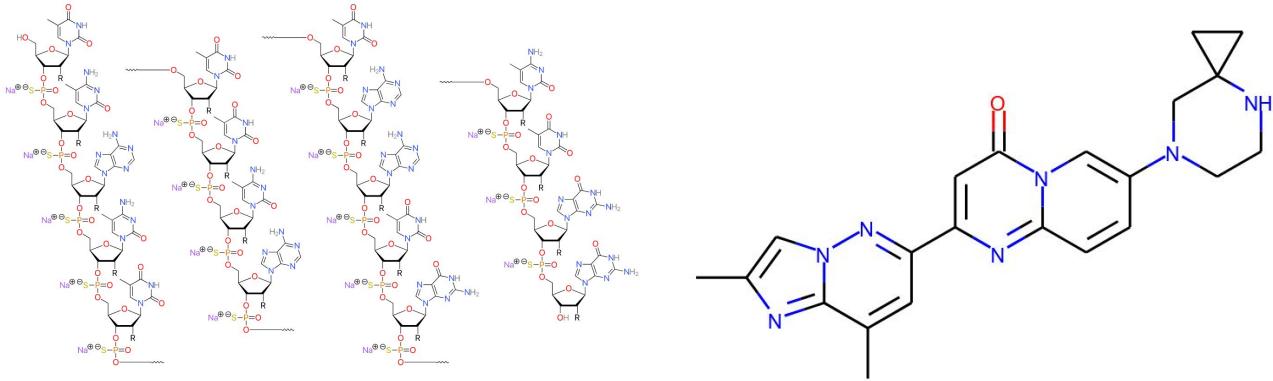
SMN1 gene
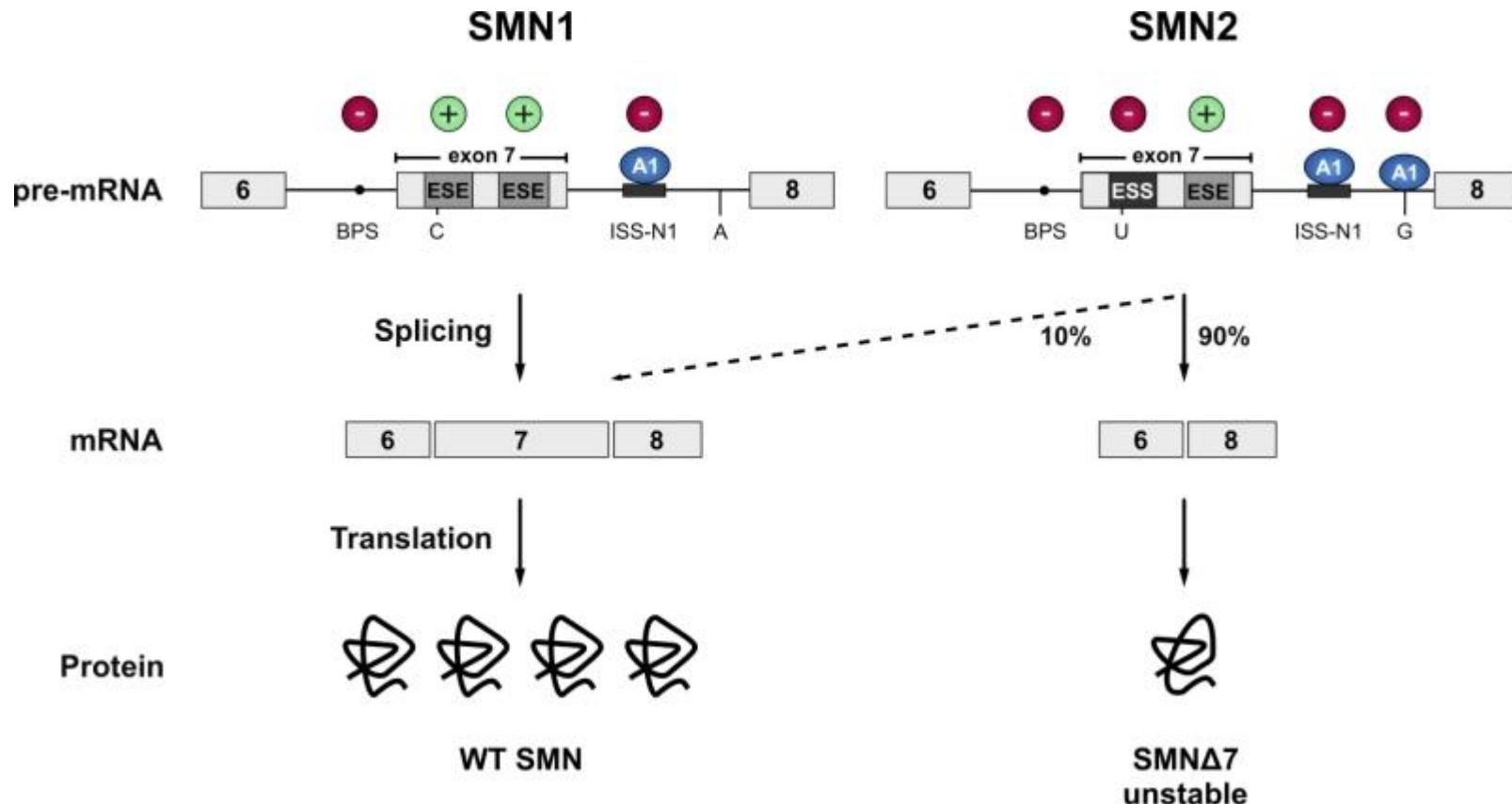
Onasemnogene Abeparvovec/ Zolgensma

Nusinersen sodium/ Spinraza (CHEMBL3833342)
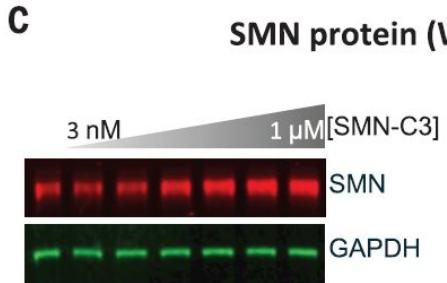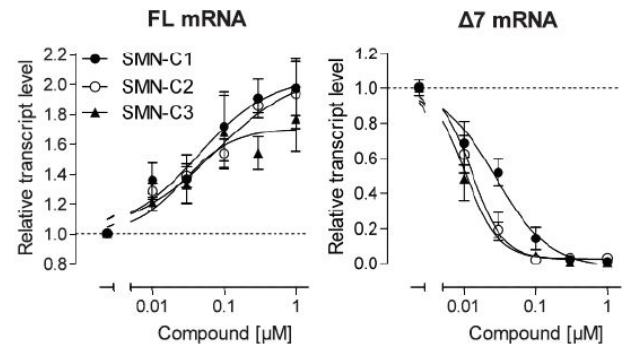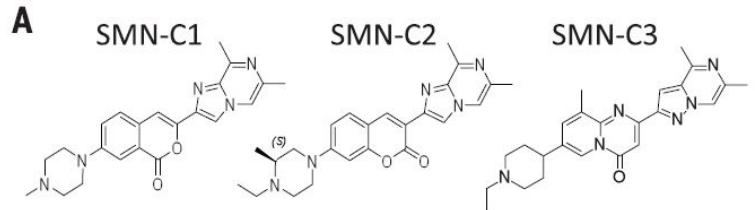
Risdiplam/ *Evrysdi* (CHEMBL4297528)
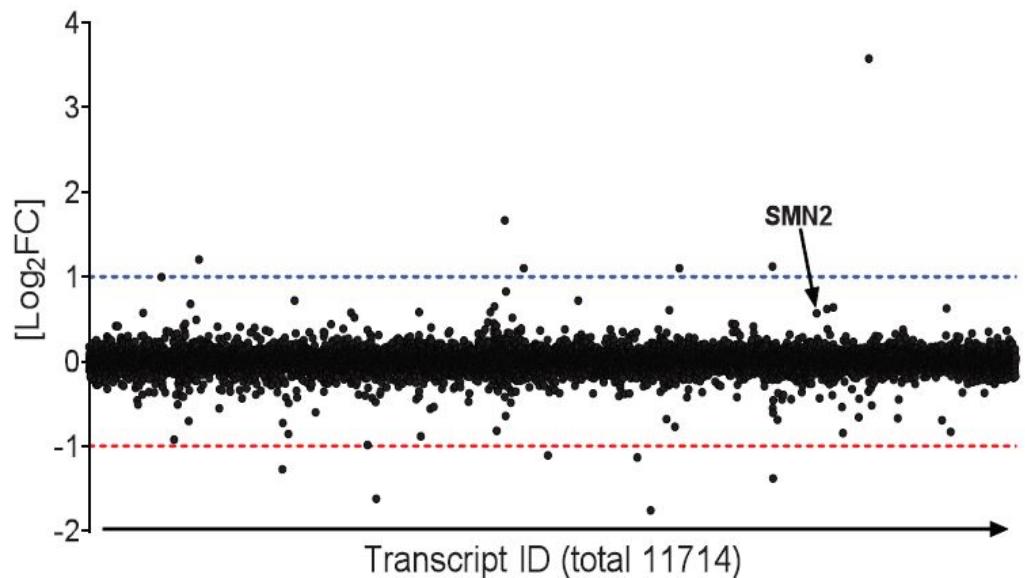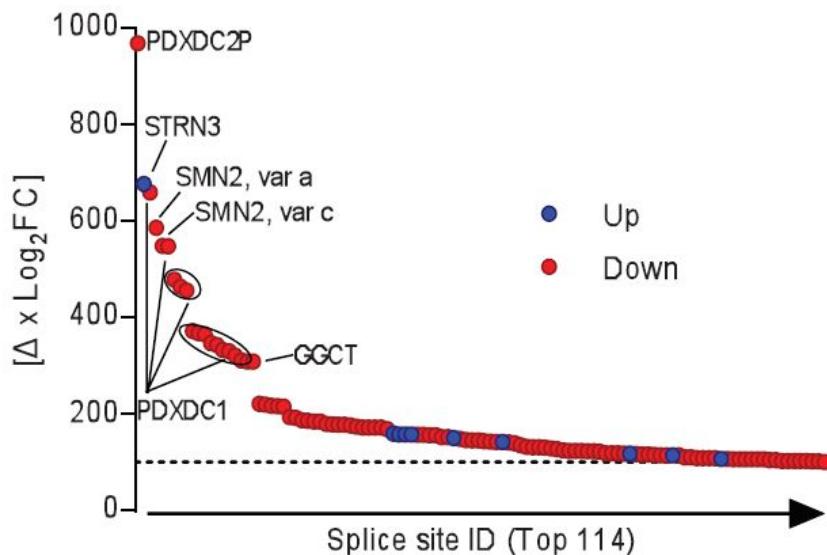
Splicing of SMN1 and SMN2 genes: patients with mutations in SMN1 gene suffer from Spinal Muscle Atrophy (SMA)

# Small molecules were identified as RNA splicing modifiers
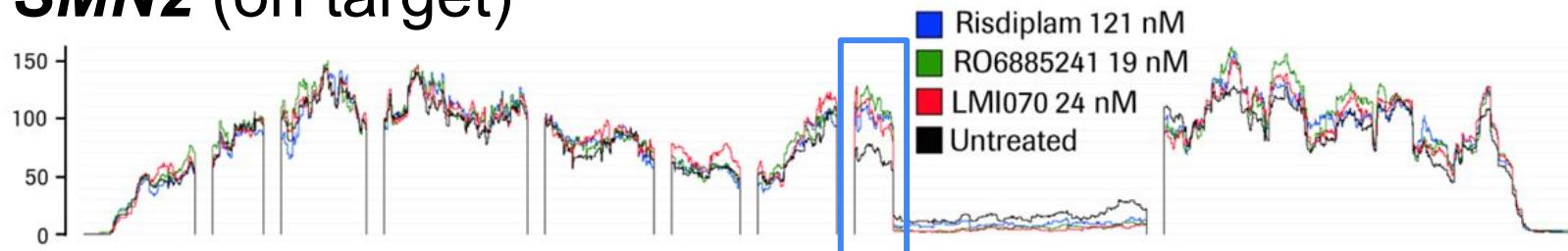
RNA sequencing confirms the specificity of SMN-C3

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)

Legend:
- Risdiplam 121 nM (blue)
- RO6885241 19 nM (green)
- LMI070 24 nM (red)
- Untreated (black)