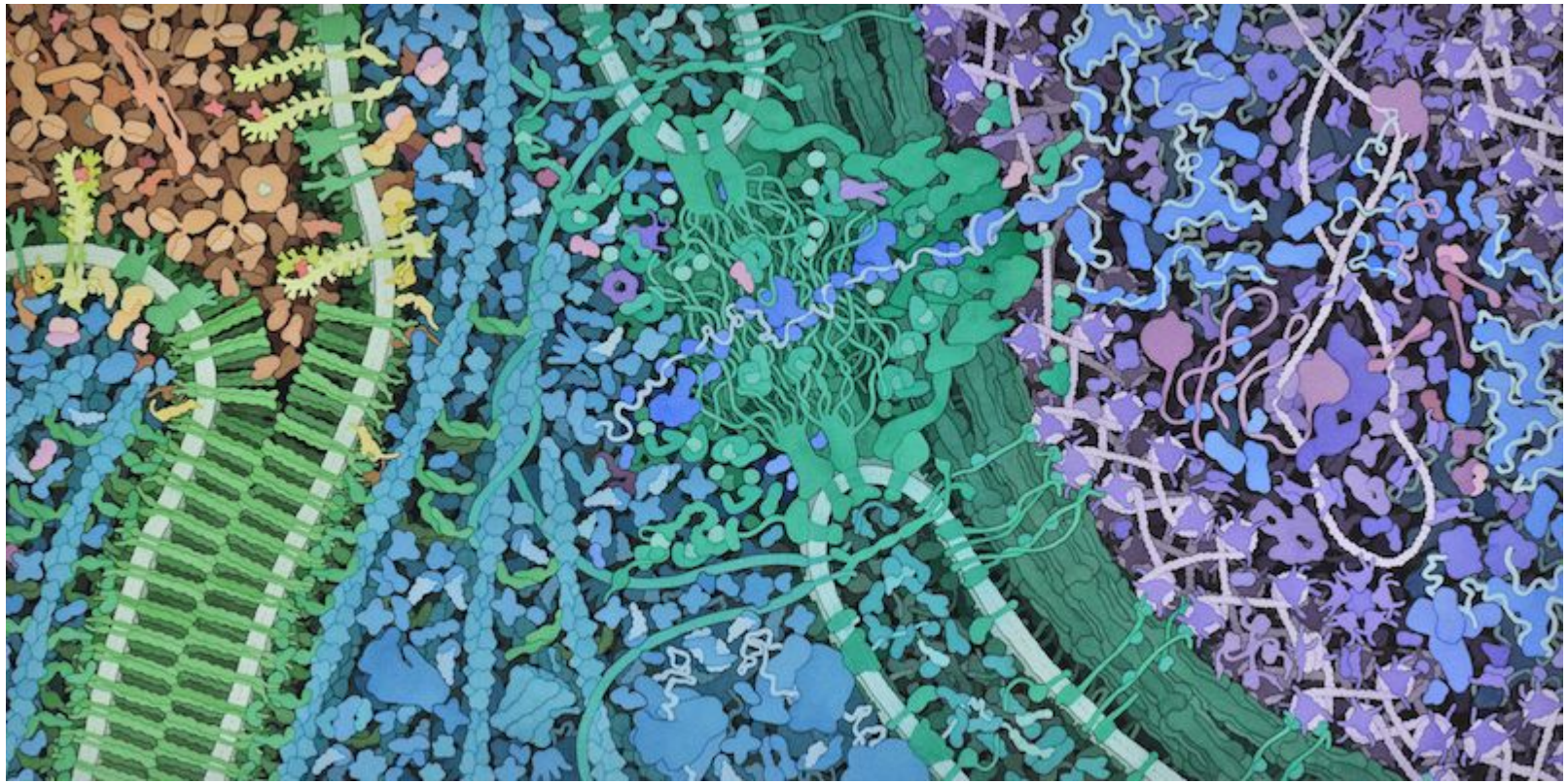
AMIDD Lecture 7: From individual interactions to networks



Vascular Endothelial Growth Factor (VegF) Signaling, David S. Goodsell, 2011

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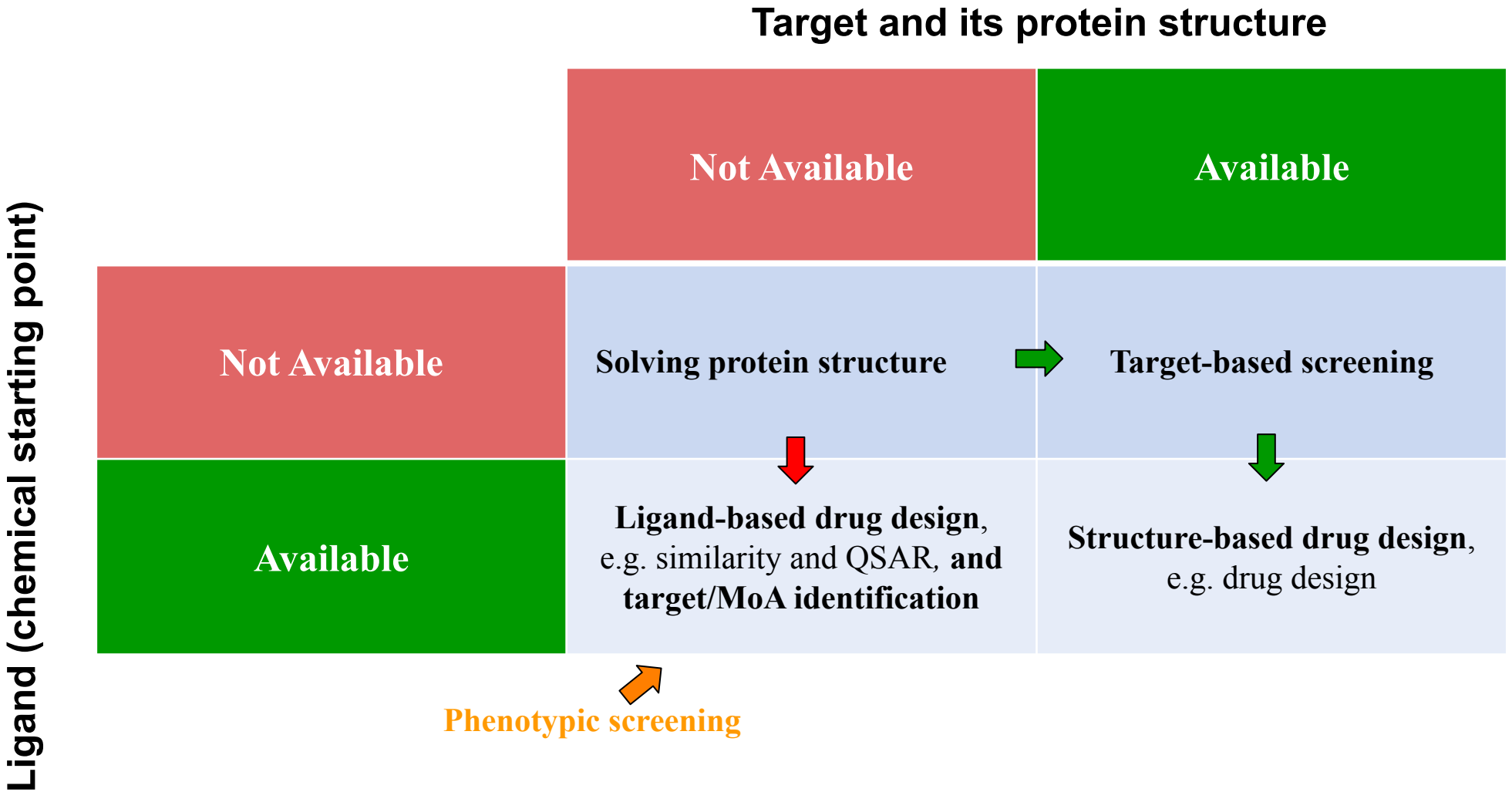
Topics

- **QSAR, machine learning, and causal inference**
- **Drug-target interaction: biophysical and biochemical views**
- **Biological networks interact with drugs and manifest its efficacy and safety**

Structure-based and ligand-based drug design



QSAR= quantitative structure activity relationship; MoA= mechanism of action, or mode of action

Quantitative Structure-Activity Relationships (QSARs)

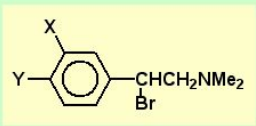
QSAR is a statistical modelling of correlation between biological activity and physicochemical properties, or $\Delta\phi=f(\Delta S)$, where ϕ indicates a biological activity and S indicates a chemical structure (1868-1869).

Molecular Descriptors (MD)

Compounds (C)	Target property	MD_1	MD_2	...	MD_M
C_1	y_1	$x_{1,1}$	$x_{1,2}$	...	$x_{1,M}$
C_2	y_2	$x_{2,1}$	...	...	...
C_3	y_3	...	...	...	...
C_4	y_4	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
C_N	y_N	$x_{N,1}$	$x_{N,2}$	...	$x_{N,M}$

The basic form of a QSAR model: find a function *f* that predicts *y* from *x*, y~f(x)

An example: **The Free-Wilson analysis.** The assumption: the biological activity for a set of analogues could be described by the contributions that substituents or structural elements make to the activity of a parent structure.



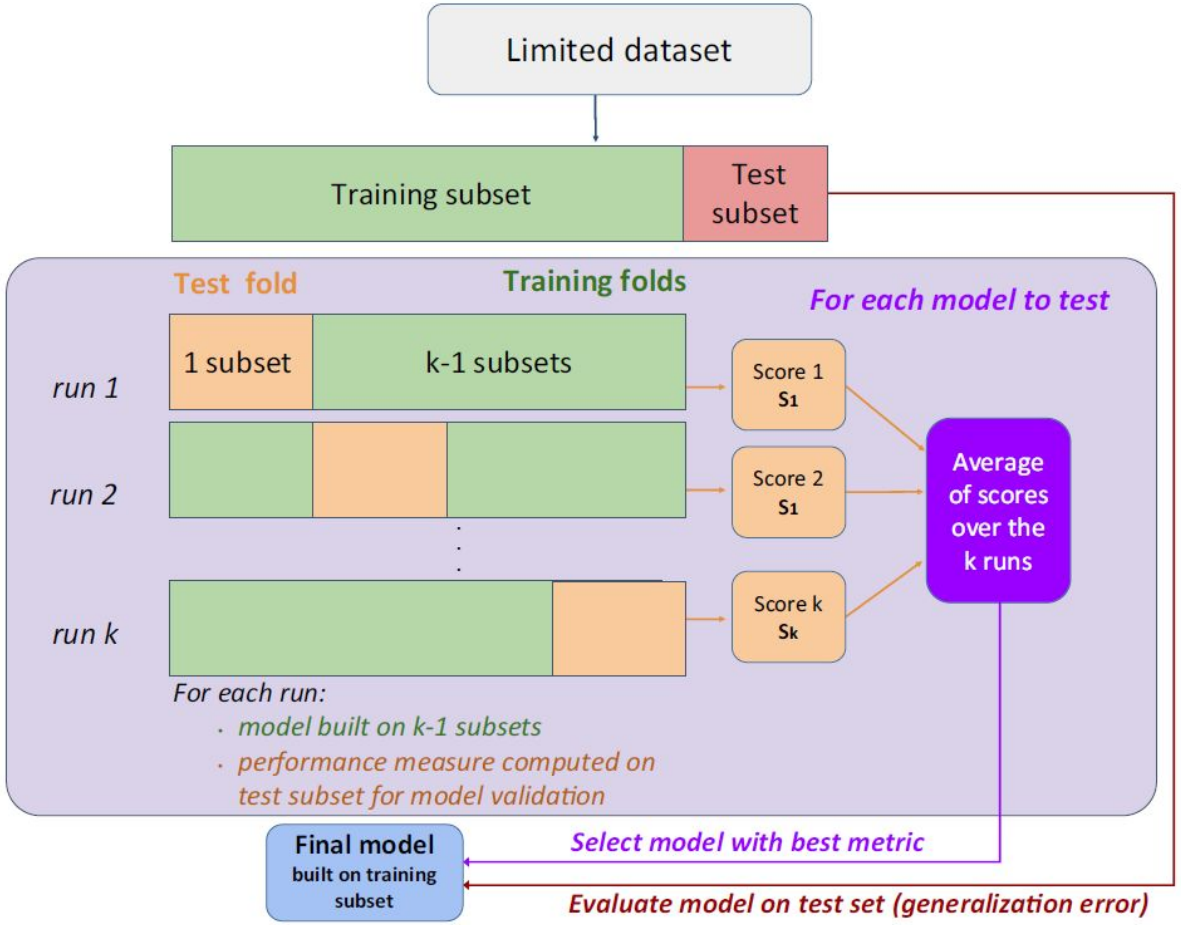
meta	para	meta-					para-					log 1/C	log 1/C
(X)	(Y)	F	Cl	Br	I	Me	F	Cl	Br	I	Me	obsd.	calc.a)
H	H											7.46	7.82
H	F						1					8.16	8.16
H	Cl							1				8.68	8.59
H	Br								1			8.89	8.84
H	I									1		9.25	9.25
H	Me										1	9.30	9.08
F	H	1										7.52	7.52
Cl	H		1									8.16	8.03
Br	H			1								8.30	8.26
I	H				1							8.40	8.40
Me	H					1						8.46	8.28
Cl	F		1				1					8.19	8.37
Br	F			1			1					8.57	8.60
Me	F					1	1						
Cl	Cl		1					1					
Br	Cl			1				1					
Me	Cl					1		1					
Cl	Br		1										
Br	Br			1									
Me	Br					1							
Me	Me					1							
Br	Me			1									

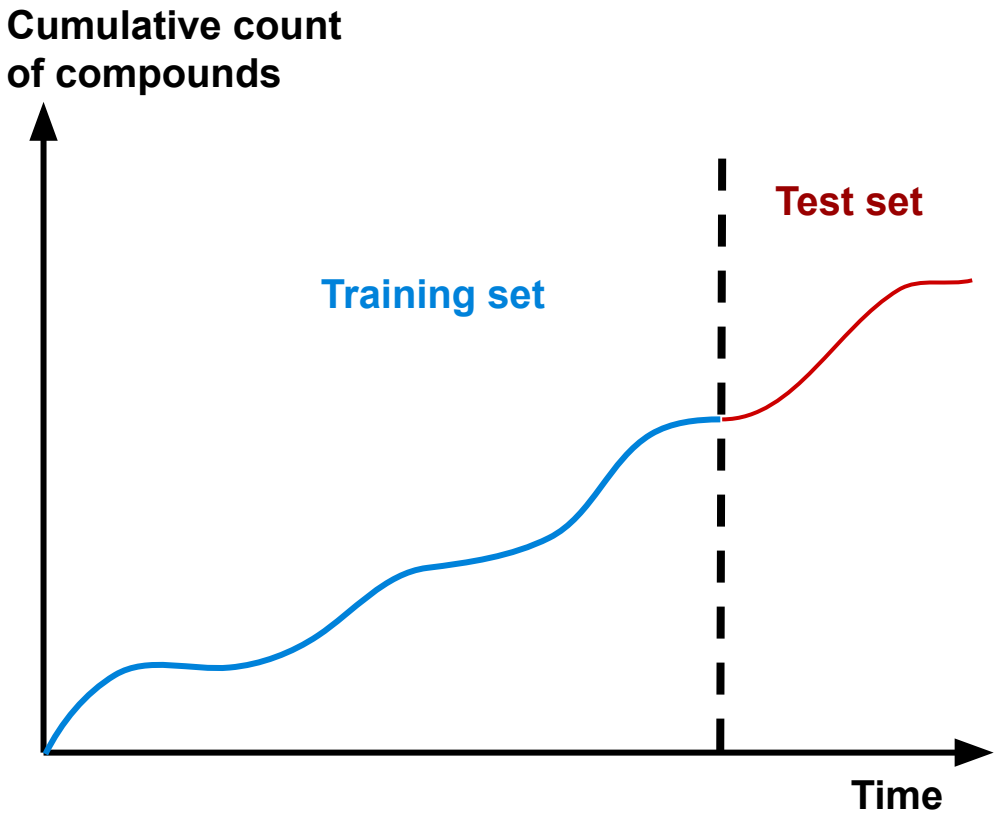
Multivariate regression analysis

$$\log(1/ED_{50}) = -0.301[m\text{–F}] + 0.27[m\text{–Cl}] + 0.434[m\text{–Br}] + 0.579[m\text{–I}] + 0.454[m\text{–Me}] + 0.340[p\text{–F}] + 0.768[p\text{–Cl}] + 1.020[p\text{–Br}] + 1.429[p\text{–I}] + 1.256[p\text{–Me}] + 7.821$$

$$n = 22,\ r^2 = 0.94,\ s = 0.194,\ F = 17.0$$

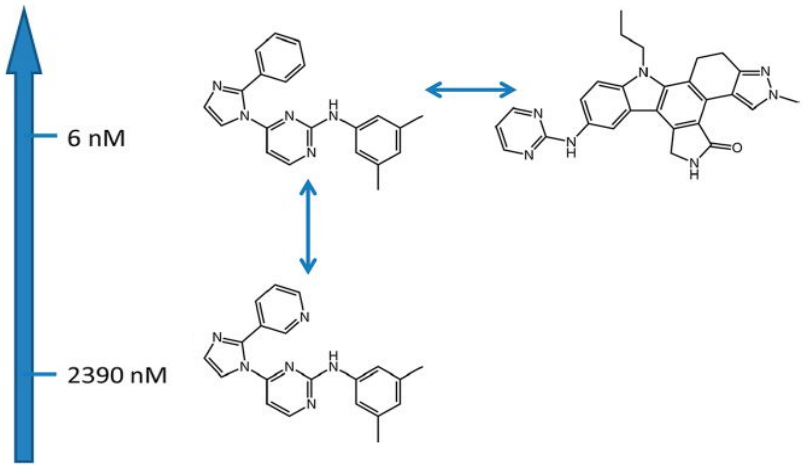
Watchout 1: Temporal validation is essential for drug discovery

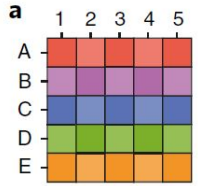




(Left) To assess the generalization ability of a supervised learning algorithm, data are separated into a training subset used for building the model and a test subset used to assess the generalization error. (Right) Temporal validation is especially important for drug discovery, because chemical structures used in the training set may differ substantially from those that will be tested.

Watchout 2: Molecular similarity does not equal biological similarity

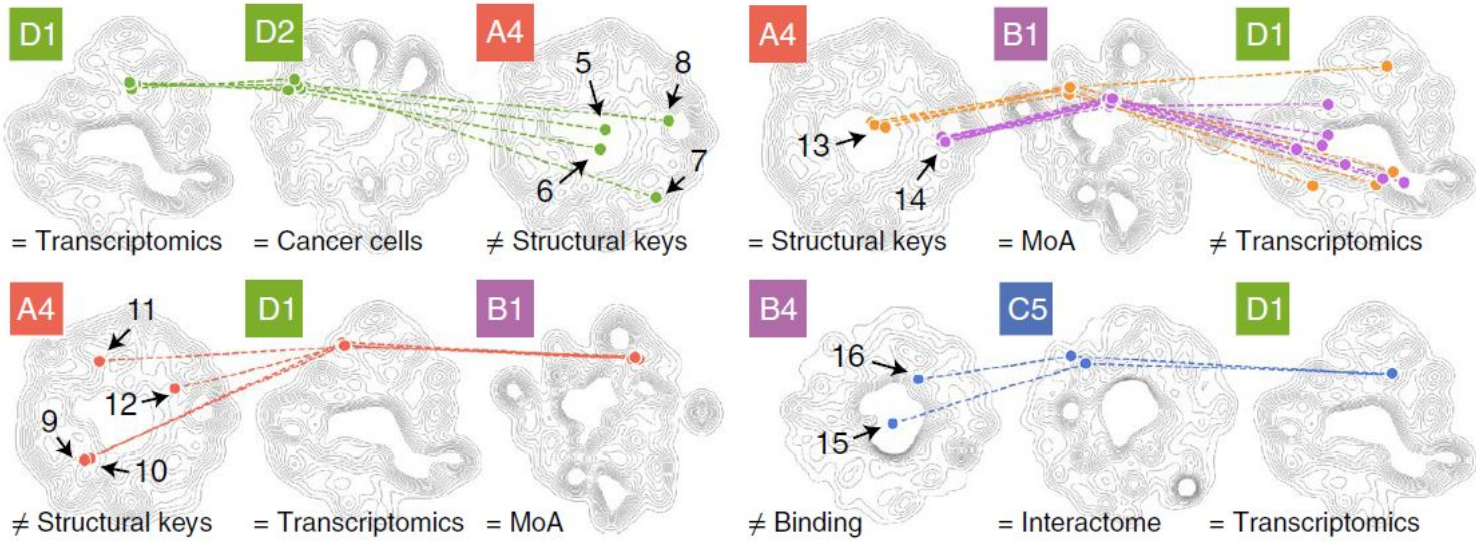




A1: 2D fingerprints	A2: 3D fingerprints	A3: Scaffolds	A4: Structural keys	A5: Physicochemistry
B1: Mechanisms of action	B2: Metabolic genes	B3: Crystals	B4: Binding	B5: HTS bioassays
C1: Small molecule roles	C2: Small molecule pathways	C3: Signaling pathways	C4: Biological proceses	C5: Interactome
D1: Transcription	D2: Cancer cell lines	D3: Chemical genetics	D4: Morphology	D5: Cell bioassays
E1: Therapeutic areas	E2: Indications	E3: Side effects	E4: Diseases & toxicology	E5: Drug–drug interactions

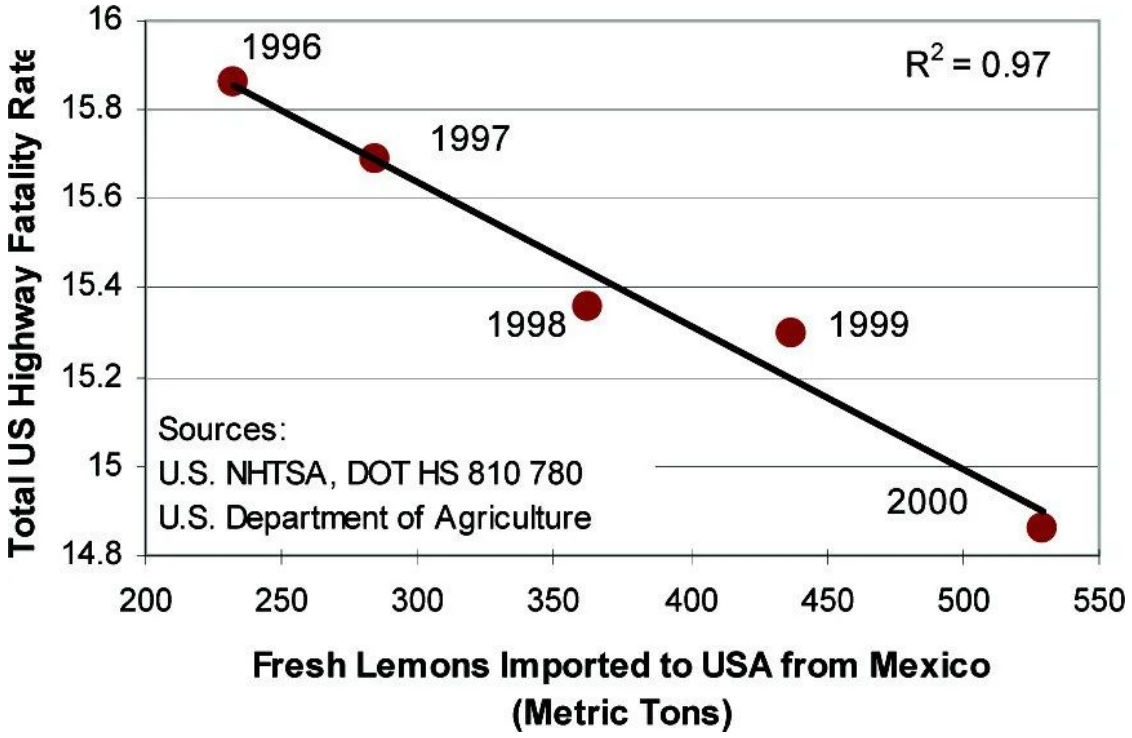
A: Chemistry
B: Targets
C: Biological network
D: Cells
E: Clinical readout

Watch out biological activity cliffs!
Similarity does not imply activity. Three vascular endothelial growth factor receptor 2 (VEGFR2) ligands are shown that represent different similarity−activity relationships.



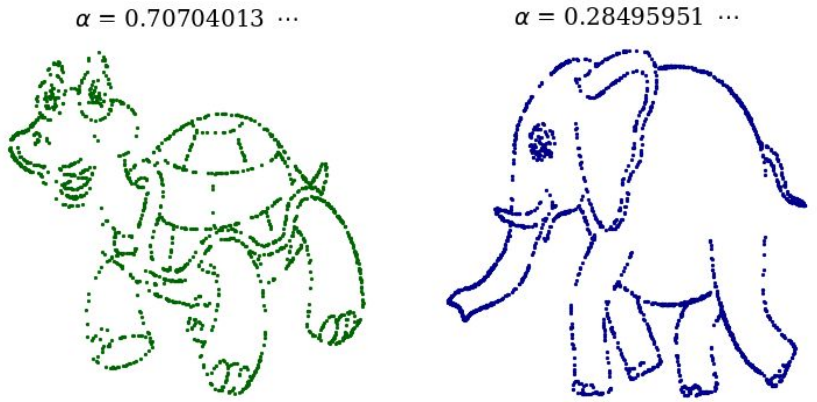
Duran-Frigola, Miquel, Eduardo Pauls, Oriol Guitart-Pla, Martino Bertoni, Víctor Alcalde, David Amat, Teresa Juan-Blanco, and Patrick Aloy. 2020. "Extending the Small-Molecule Similarity Principle to All Levels of Biology with the Chemical Checker." Nature Biotechnology, May, 1–10.

Watchout 3: Correlation can be caused by causation, confounding, coincidence, and conspiracy. Do we need correlation or causation?



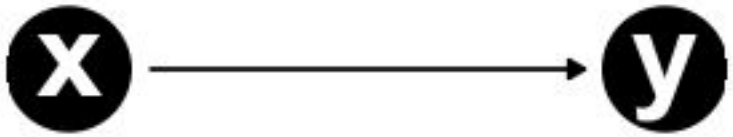
Johnson, Stephen R. “The Trouble with QSAR (or How I Learned To Stop Worrying and Embrace Fallacy).” Journal of Chemical Information and Modeling 48, no. 1 (January 1, 2008): 25–26. https://doi.org/10.1021/ci700332k

$$f_\alpha(x) = \sin^2\left(2^{x\tau} \arcsin\sqrt{\alpha}\right)$$

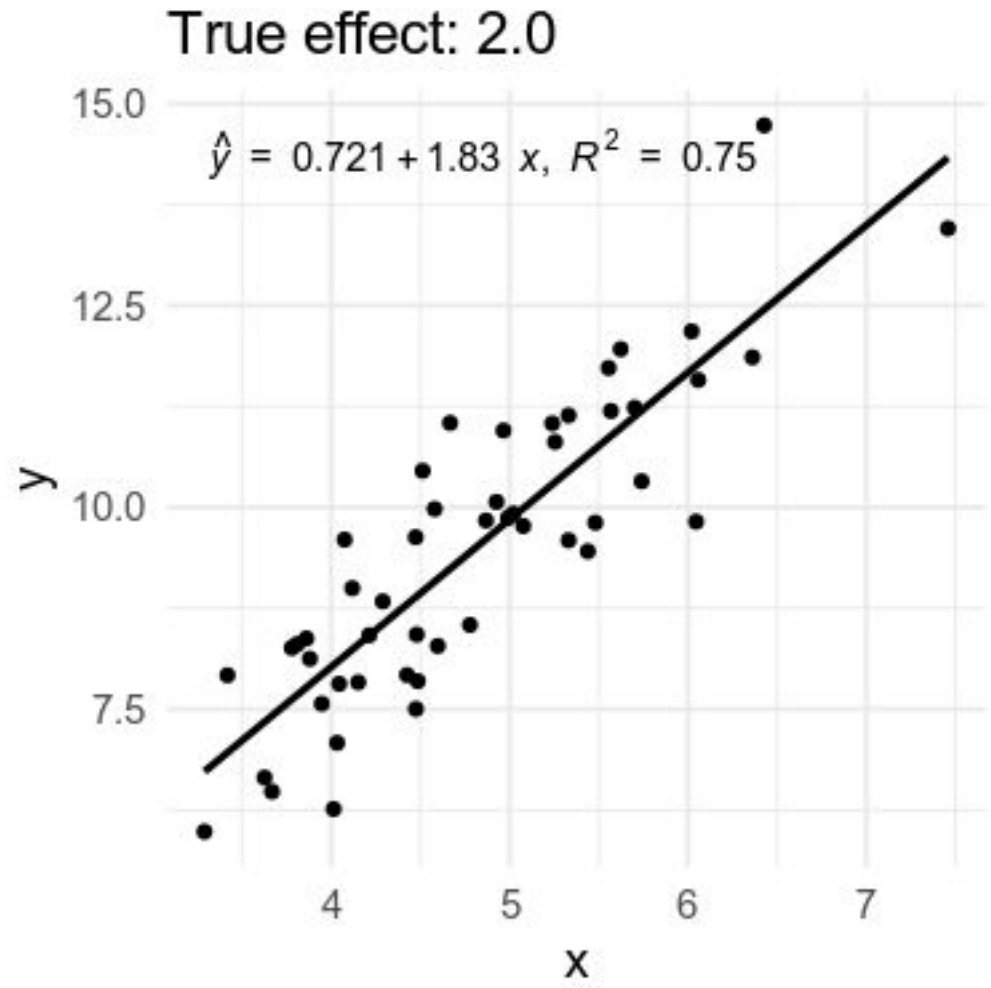


Boué, Laurent. “Real Numbers, Data Science and Chaos: How to Fit Any Dataset with a Single Parameter.” ArXiv:1904.12320 [Cs, Stat], April 28, 2019. http://arxiv.org/abs/1904.12320.GitHub Repo. Also see: Drawing an elephant with four complex parameters

Generative models shed light on correlation and causality



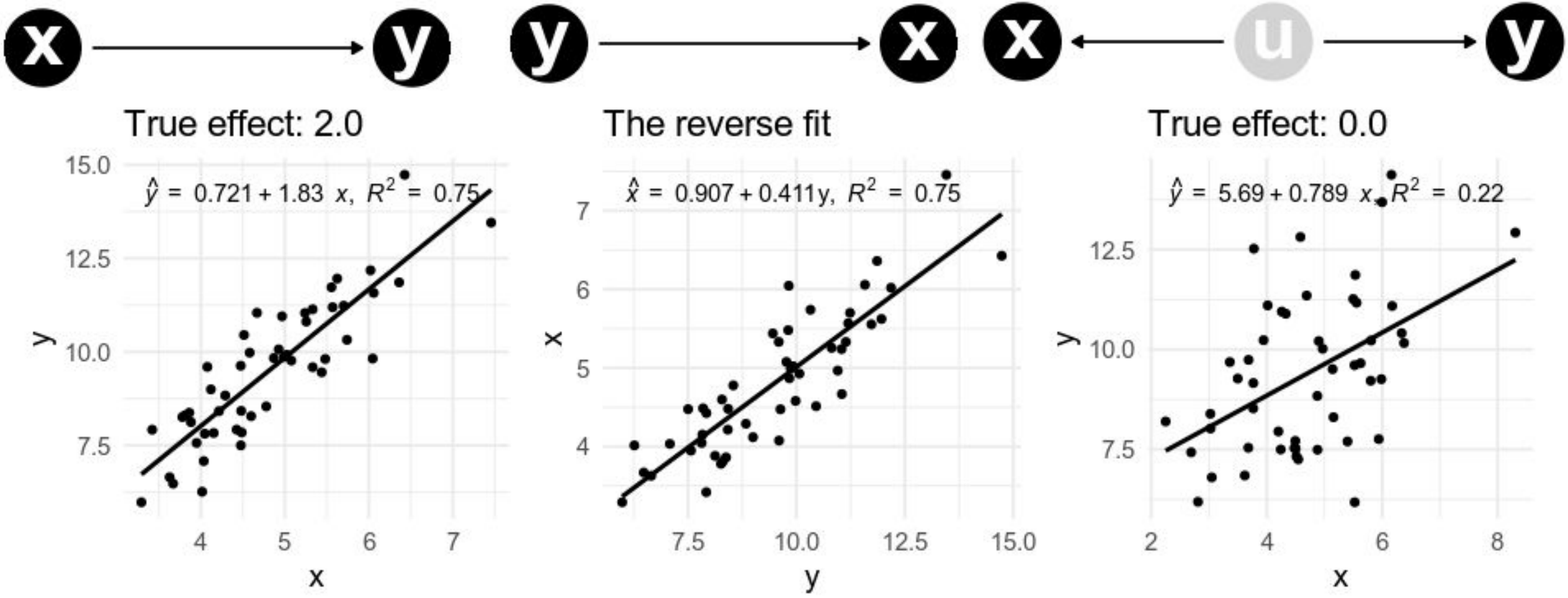
	x	y
1	4.926791	10.067779
2	4.479734	8.424283
3	4.289686	8.835629
4	4.474023	9.630499
5	4.214551	8.416680
6	6.057431	11.578080
7	4.597903	8.283025
8	5.021571	9.922731
9	3.627323	6.651222
10	5.622794	11.959972
11	5.555025	11.727815
12	4.966007	10.951562
13	5.076791	9.768299



Assumptions of the **generative model**:

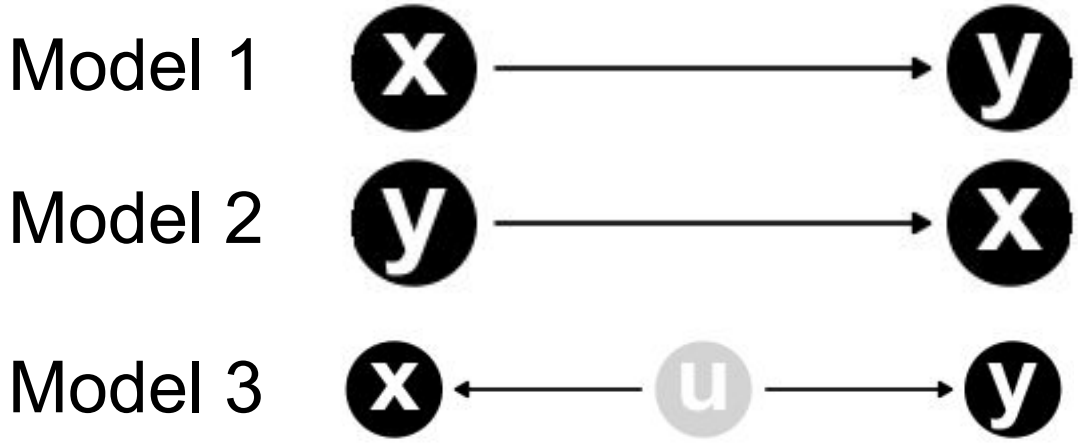
1. X is a random variable;
2. Every unit change of X induces a change of 2 units in Y.

Correlation is caused by causation or confounding



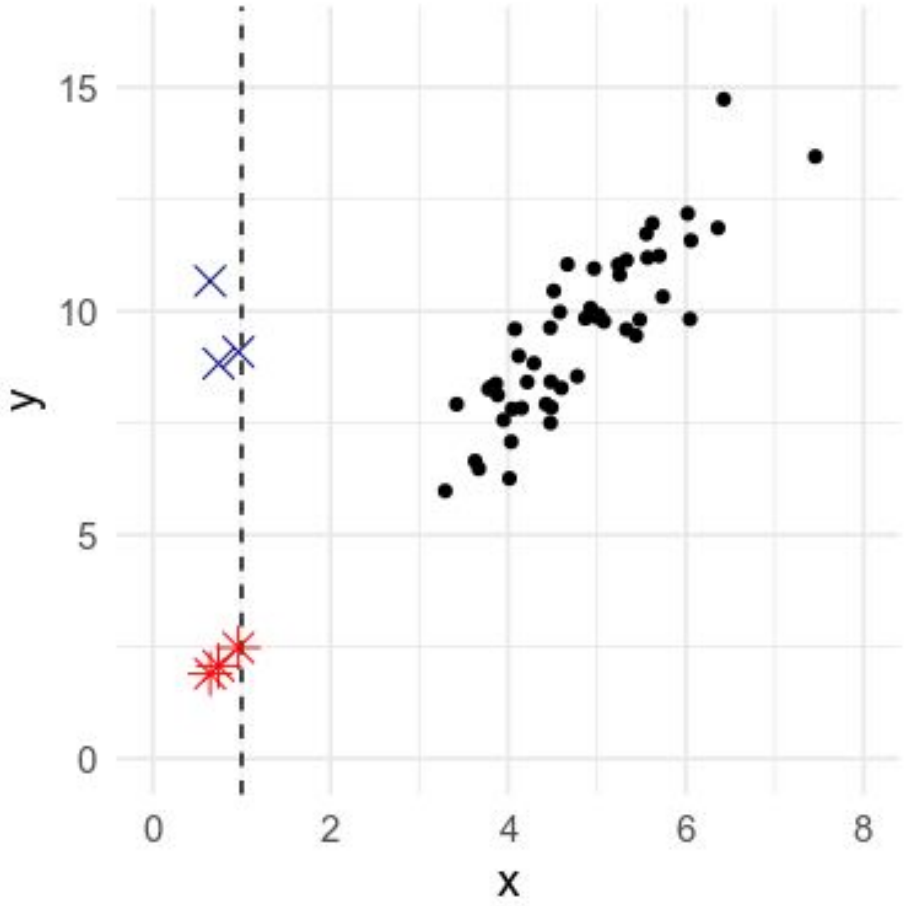
Statistical models alone cannot derive causality from correlation

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?



Answers

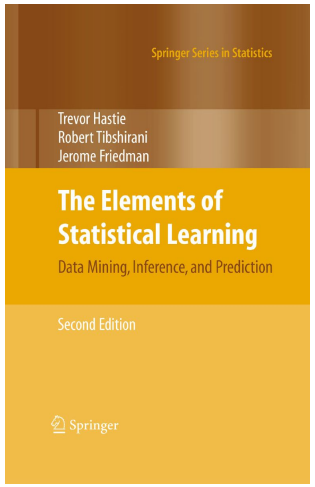
Red stars are supported by Model 1.

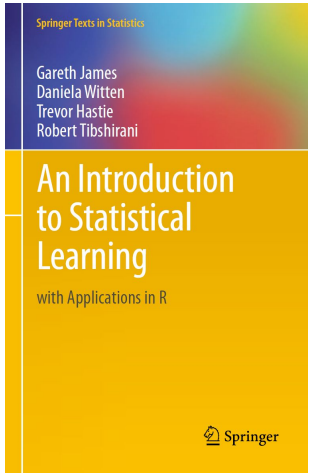
Blue crosses are supported by both Model 2 and Model 3.

Reason: causality (C→E, from cause to effect) is directional. Manipulating C has an effect on E, while manipulating E has no effect on C. Blue crosses are around mean values of Y. If Y causes X, manipulating X has no effect on Y. Then the most likely values of Y will be around the mean of existing samples.

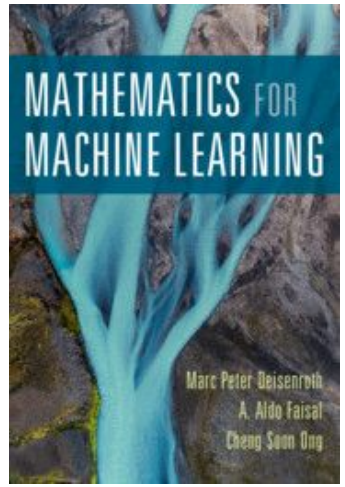
Resources for learning about machine learning

ESL and *ISL*: *From a frequentist view (almost)*

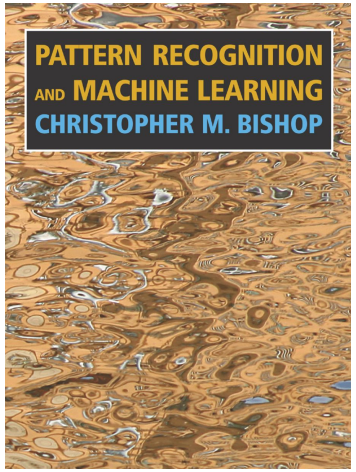


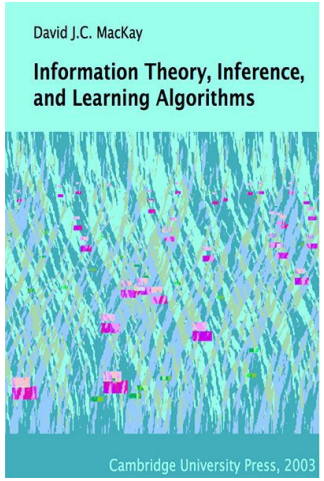


Mathematical foundations

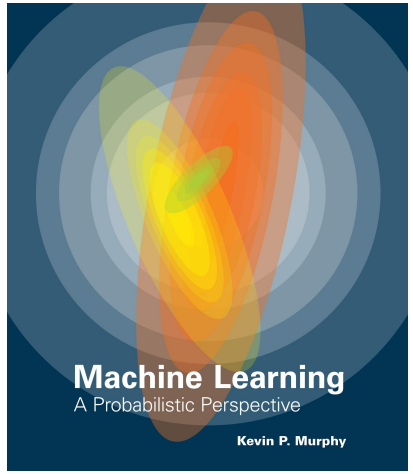


PRML and *ITILA*: *From a Bayesian view*



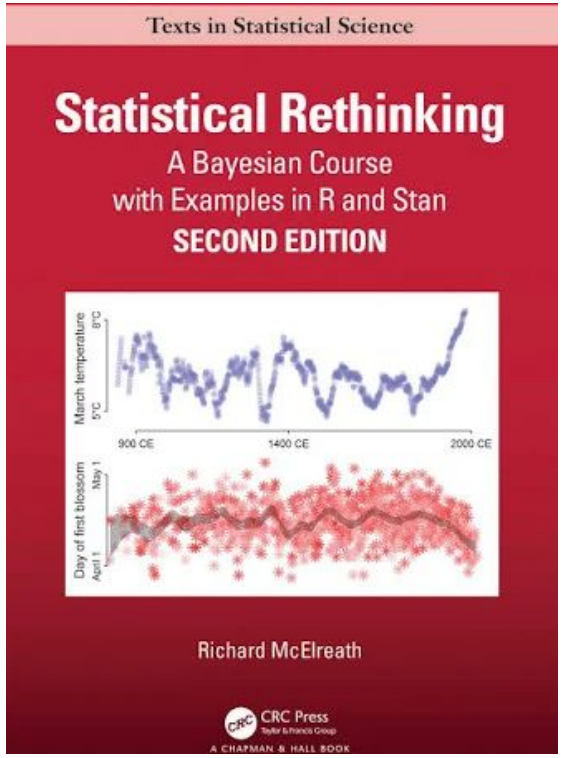


MLaPP: *Application oriented, more accessible, and balanced views*

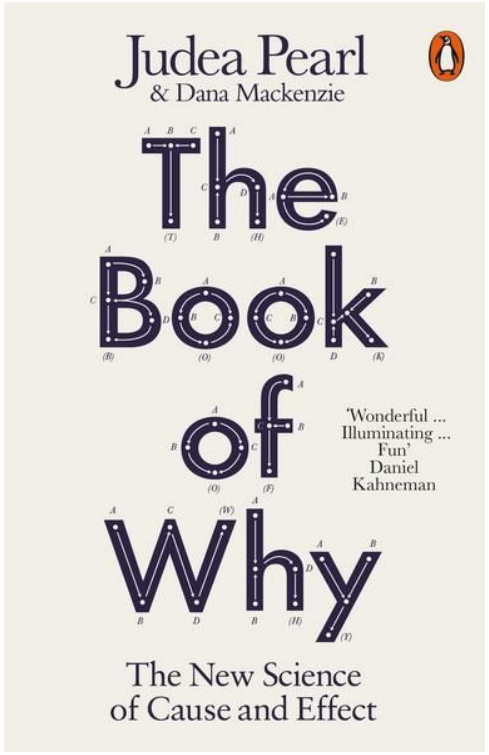


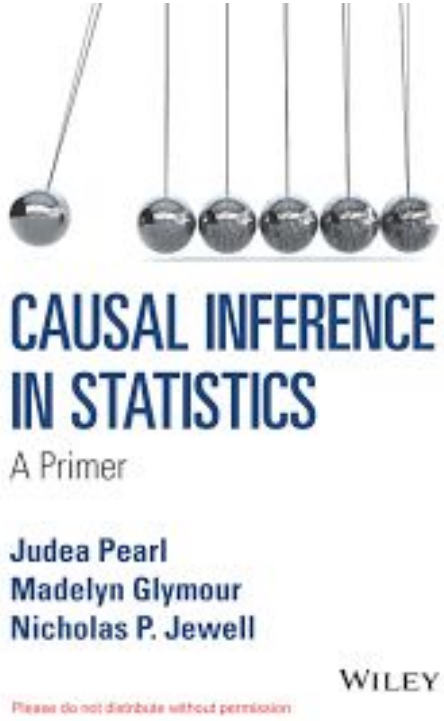
Resources for learning about causal inference

Causal inference in drug discovery and development, Michoel and Zhang, 2022

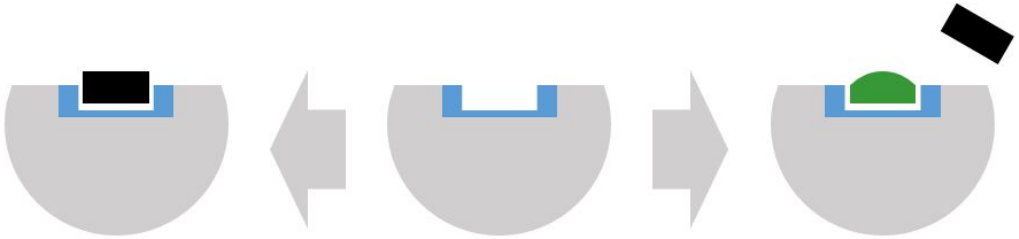


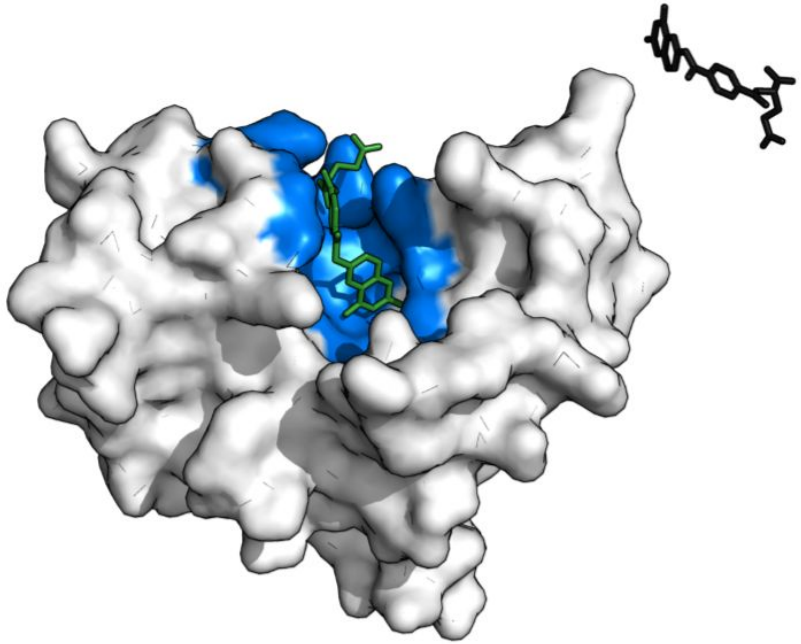
Lectures available on YouTube

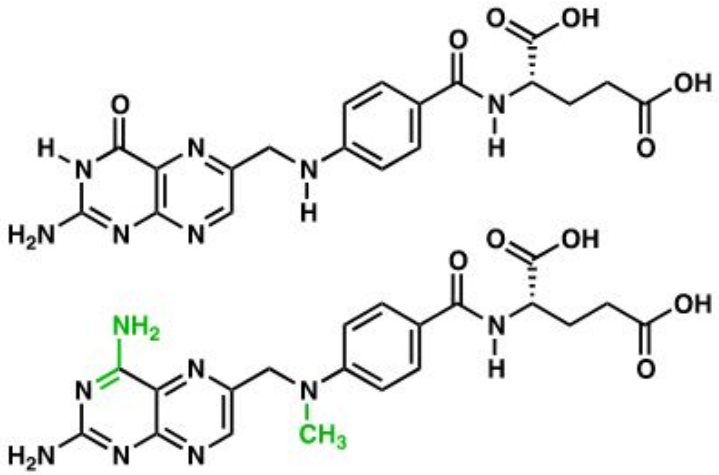




Seeing how a drug works







Dihydrofolic acid

MTX

The protein: Dihydrofolate reductase (DHFR), which converts dihydrofolic acid into tetrahydrofolate. The process is important for cell proliferation and cell growth. DHFR is a drug target for cancer and autoimmune diseases.

The natural substrate: dihydrofolic acid (vitamin B9), in black. Dihydrofolic acid is the *natural ligand* of DHFR.

The drug: methotrexate (MTX), in green. MTX is a *synthesized ligand* of DHFR, and it is a competitive inhibitor of DHFR with regard to its natural substrate.

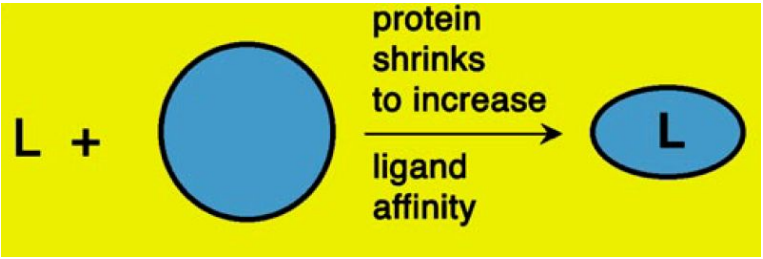
The binding site: where the enzyme binds its substrate and catalyses the chemical reaction, in blue.

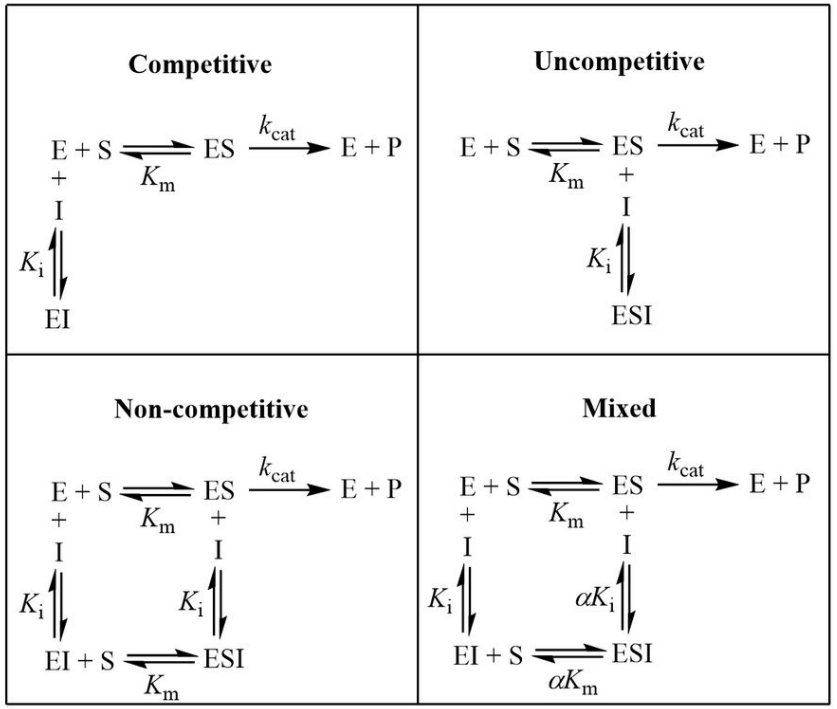
Biophysical and biochemical views of ligand-target binding

The **biophysical view** of binding: Both enthalpy (heat transfer) and entropy (disorder) contribute to the binding energy ($\Delta G=\Delta H-T\Delta S$).

- Binding occurs in favourable steric*, i.e.* spatial, configurations. A simplification is **the 'lock-and-the-key' model**, however, in reality enzyme **undergoes changes in its shape**.
- Binding is mediated by intermolecular forces, such as electrostatic interactions (*e.g.* hydrogen bonds), Van der Waals forces (dipole interactions), π-effects (interactions of π-orbitals of a molecular system), and hydrophobic effect.
- Binding opposes motion, and motion opposes binding: there is enthalpy/entropy compensation in ligand-substrate binding.

The **biochemical view** of binding: The *rate* of binding is called affinity, often expressed in K_d or, for inhibitors, K_i. A closely related, and often confusing, concept is IC_{50}.





Four basic types of kinetic mechanism of inhibition, source: sciencesnail.com

From the law of mass action to ligand-target interaction

$$\mathrm{L} + \mathrm{R} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{LR}$$

The law of mass action

$$\frac{d[LR]}{dt} = k_1[L][R] - k_2[LR]$$

At equilibrium, no net change of [LR]

$$k_1[L][R] = k_2[LR]$$

$$R_{total} = [R] + [LR]$$

$$k_1[L]([R_{total}] - [LR]) = k_2[LR],$$

$$[LR] = \frac{k_1[L][R_{total}]}{k_1[L] + k_2}$$

$K_D \equiv k_2/k_1$

$$[LR] = \frac{[R_{total}][L]}{[L] + K_D}$$

Hidden or skipped in the lecture

The biophysical (thermodynamic) view of binding affinity: enthalpy and entropy

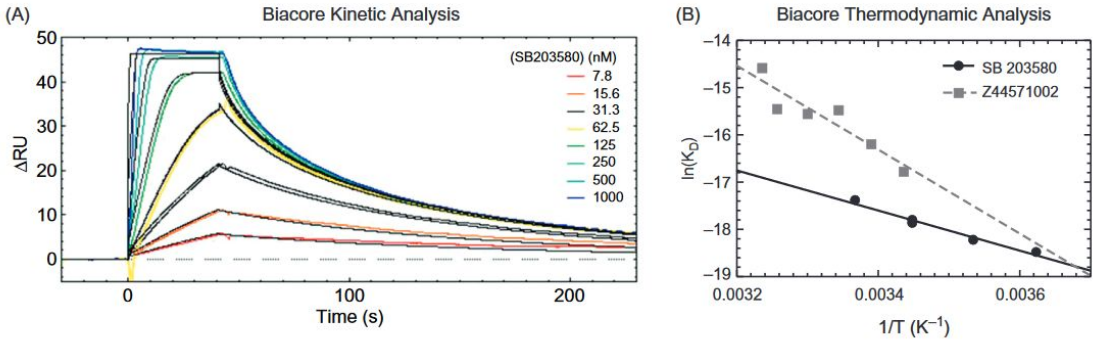
Gibbs free energy

$$\Delta G = \Delta H - T\Delta S$$

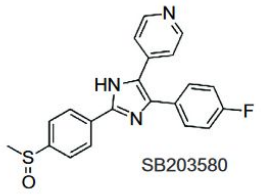
Van't Hoff Equation

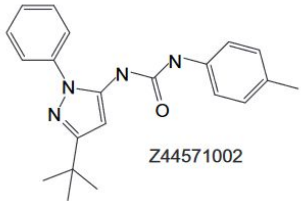
$$\Delta G = -RT \ln K$$

$$-ln(K_D) = \frac{\Delta H}{R}\frac{1}{T} - \frac{\Delta S}{R}$$



Compound Name	k1	k2	KD	ΔG	ΔH	TΔS
Z44571002	2.2e4±3e2	0.001±8.0e6	5.2e−8	−40	−75	−35
SB203580	1.7e6±1.7e5	0.130±0.014	7.8e−8	−43	−36	7.5



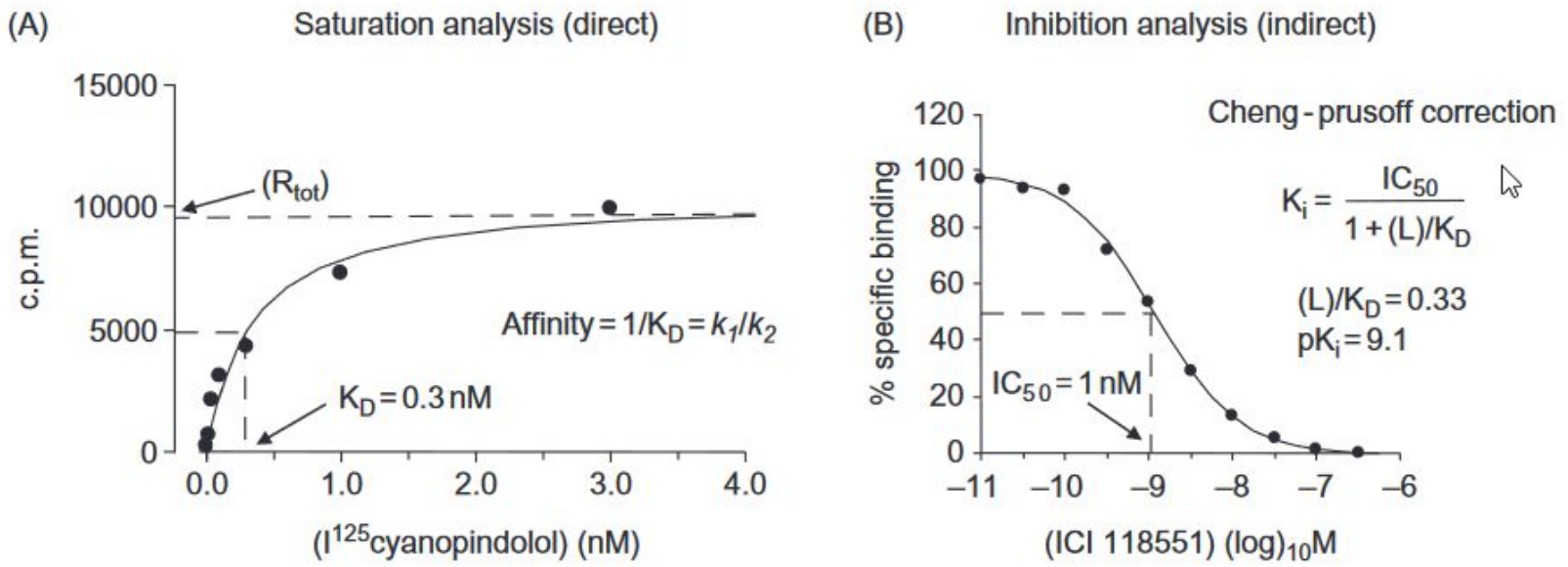


Kinetic and thermodynamic measurements of two p38α inhibitors. (A) The time course of SB203580 binding to immobilized mitogen activated kinase p38α. The y-axis shows the mass change resulting from compound binding to p38α. At t=0, a range of SB203580 concentrations were passed across the immobilized p38α to measure net association, and then at t=50s, the compound is replaced with buffer to initiate dissociation. The table shows the association and dissociation rate constants as well as the equilibrium dissociation constants (KD(M)) for two compounds. (B) Thermodynamic analysis. Enthalpy and entropy components of binding derived from the Van't Hoff analysis are detailed in the attached table. ΔG, ΔH and TΔS values are in kJ/mol.

For a thorough discussion about enthalpic and entropic contributions to molecular interactions, see *A Medicinal Chemist's Guide to Molecular Interactions* (Journal of Medicinal Chemistry 53 (14): 5061–84) by Bissantz *et al.*

Hidden or skipped in the lecture

The biochemical (kinetic) view of binding affinity: the hyperbola curve and the dissociation constant K_D



Binding assays with direct and indirect measurements. (A) A direct binding assay using I^{125} labelled cyanopindolol as a β2-adrenoceptor ligand. The curve describes a rectangular hyperbola which saturates at high ligand concentration. The ligand dissociation constant (KD) was estimated as 0.3 nM and is a measure of the ligand affinity. (B) A typical inhibition analysis using membranes expressing the human β2-adrenoceptor and employing 0.1 nM I^{125} cyanopindolol as the labeled ligand. The displacing ligand, the selective β2-adrenoceptor antagonist ICI 118551, produces complete inhibition of the specific binding yielding an IC50 of 1 nM. From *Evaluation of the Biological Activity of Compounds: Techniques and Mechanism of Action Studies*, by Iain G. Dougall and John Unitt.

Questions: (1) how can we interpret the hyperbola curve? (2) if f(x) is a function with the form of Ax/(k+x), what will be the form of function g(f(x)) where g(x)=Bx/(k'+x)? What implications does this have?

Modelling enzyme kinetics with the Michaelis-Menten model

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

The law of mass action

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

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

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

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

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

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

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

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

The dose-response curve and IC50: The Hill function and *in vitro* pharmacology

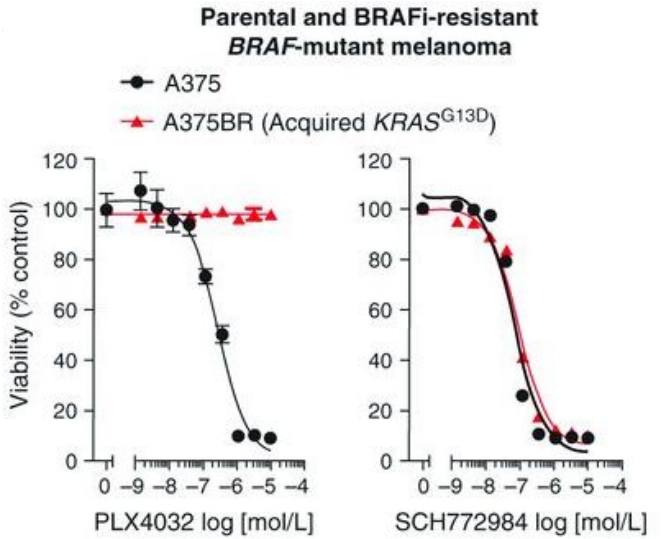
- The Hill function is one of the mostly useful non-linear functions to model biological systems.
- In its general form, H_{max} indicates the maximal value to which the function is asymptotic, *n* is the shape parameter (known as the Hill's coefficient), and *k* is the reflection point, often abbreviated 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)*.

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

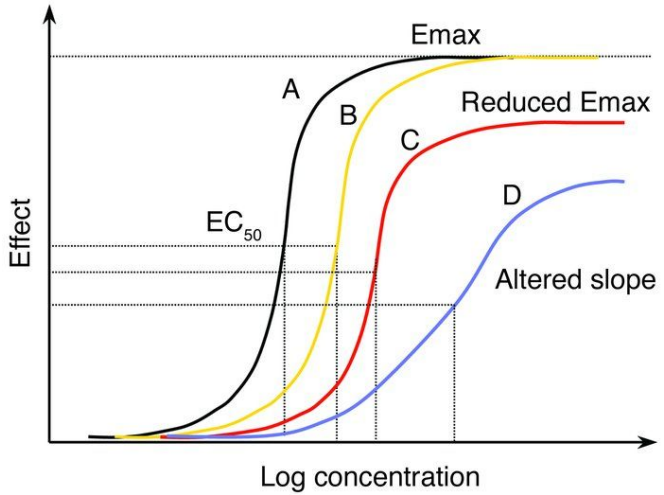
The general form of the Hill function

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

Modelling the dose-dependent effect



Morris *et al.* Cancer Discov; 3(7); 742–50. ©2013 AACR.

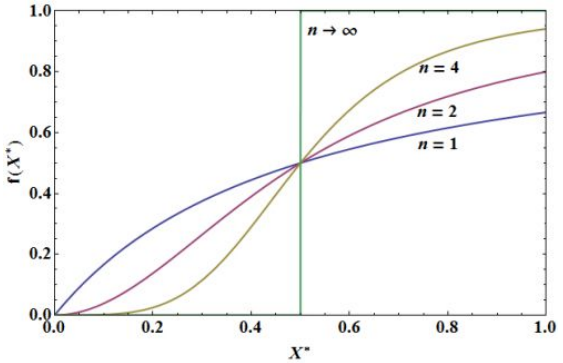


White. *J Clin Invest.* 2004;113(8):1084-1092. https://doi.org/10.1172/JCI21682.

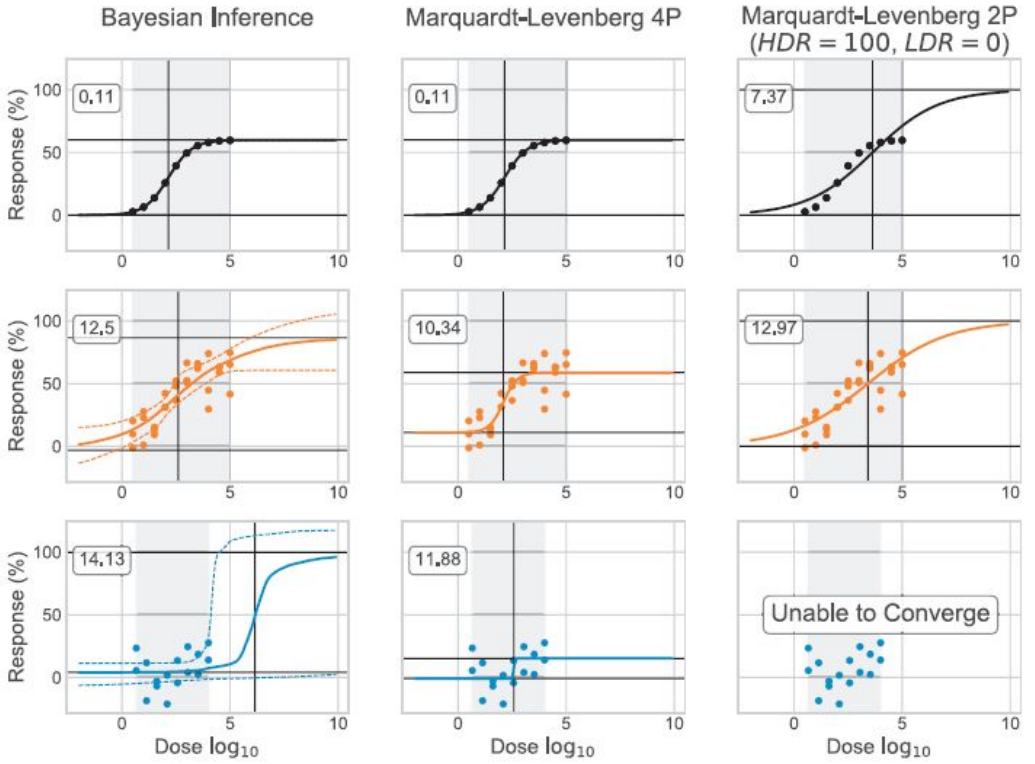
Suppose it is an antiviral drug, compared with curve B, what does curve A, C, and D suggest?

Theoretical and practical considerations about the Hill function

- The Hill function can be deduced from statistical mechanics of binding, a particle modelling approach. See for instance an article on Biophysics Wiki by Andreas Piehler for details.
- The Hill function is often used to model either *target occupancy* or *tissue response* (pharmacology).
- The Hill function can be approximated by a step function when *n* goes towards infinity (top panel). This can be seen as one of the theoretical foundations of Boolean network modelling.
- Dose-response data may look quite different from the ideal curve (bottom panel). By using a Bayesian inference approach, it is possible to perform inference even with ill-looking data.



From the biophysics wiki article by Andreas Piehler

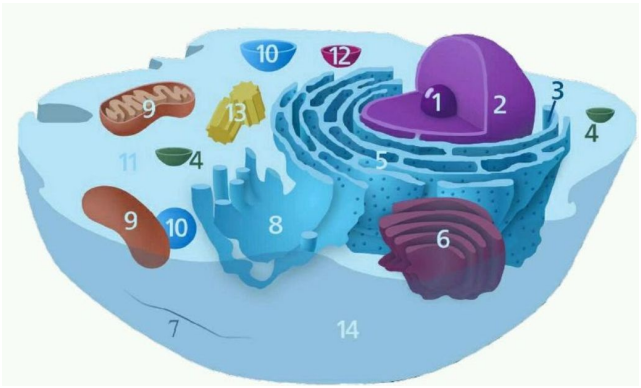


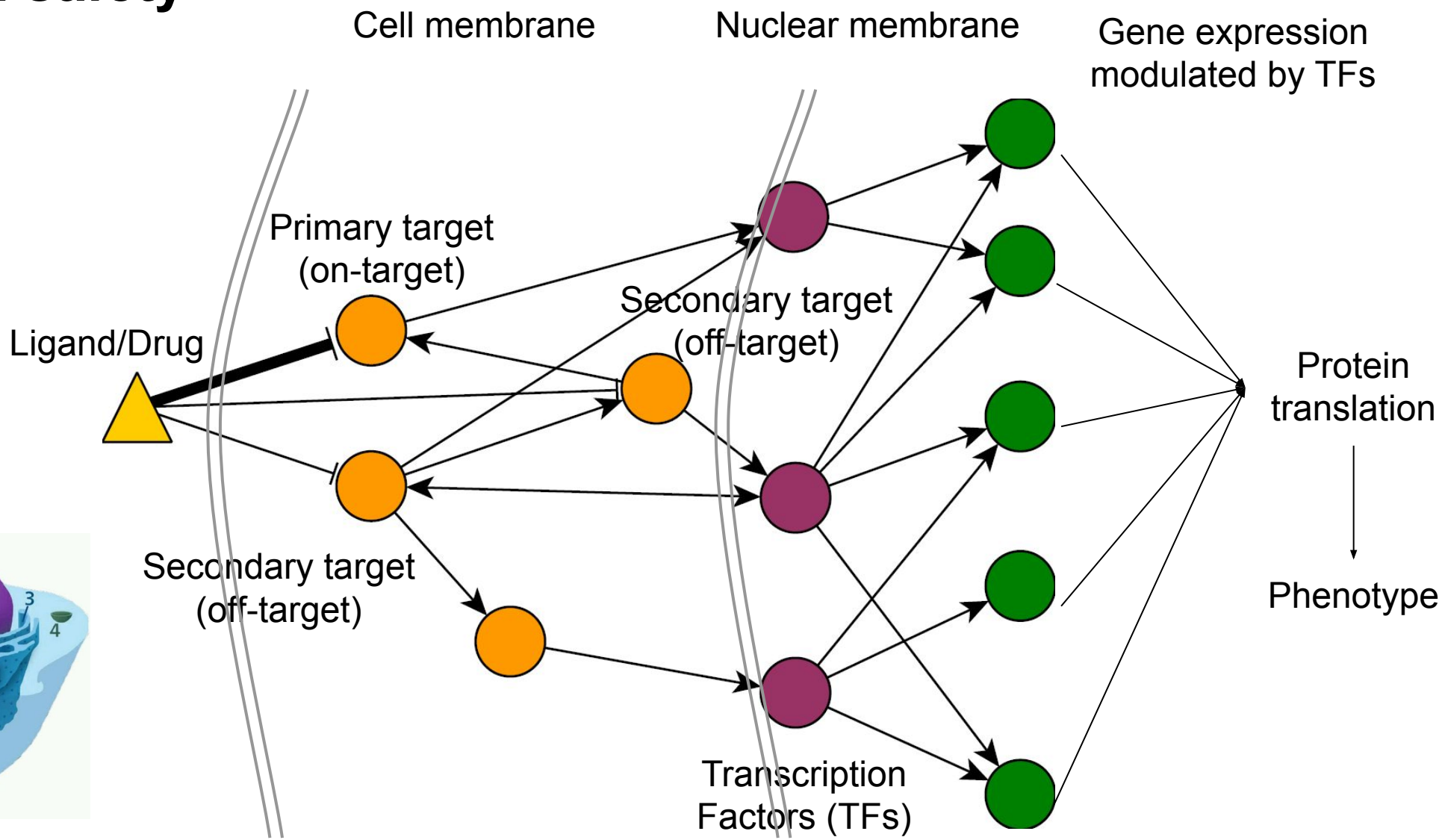
The Bayesian inference approach versus the non-Bayesian Marquardt-Levenberg algorithm for non-linear regression fitting. Labelle, Caroline, Anne Marinier, and Sébastien Lemieux. 2019. "Enhancing the Drug Discovery Process: Bayesian Inference for the Analysis and Comparison of Dose–Response Experiments." *Bioinformatics* 35 (14): i464–73.

Why modelling molecules is not enough for drug discovery?

The importance of networks

Biological networks interact with drugs and manifest its efficacy and safety





Summary

- **Machine learning is important in drug discovery. Machine learning should be guided by chemical and biological models to improve human understanding.**
- **ODE-based compartment models and stochastic models can be used to model small to moderate biochemical networks.**
- **The network nature of biology requires models beyond the molecular level.**

Five classes of mathematical models drug discovery

Compartment models

$$\frac{d[LR]}{dt} = k_1[L][R] - k_2[LR]$$

Kinetics of ligand-target interaction

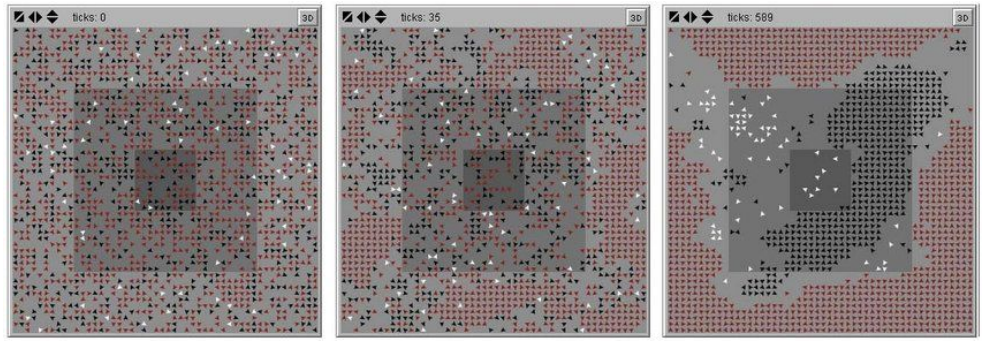
$$\frac{dx}{dt} = \alpha x - \beta xy,$$
$$\frac{dy}{dt} = -\gamma y + \delta xy,$$

The Lotka-Volterra equations modelling predator-prey relationships.

$$\frac{dS}{dt} = -\frac{\beta IS}{N},$$
$$\frac{dI}{dt} = \frac{\beta IS}{N} - \gamma I,$$
$$\frac{dR}{dt} = \gamma I$$

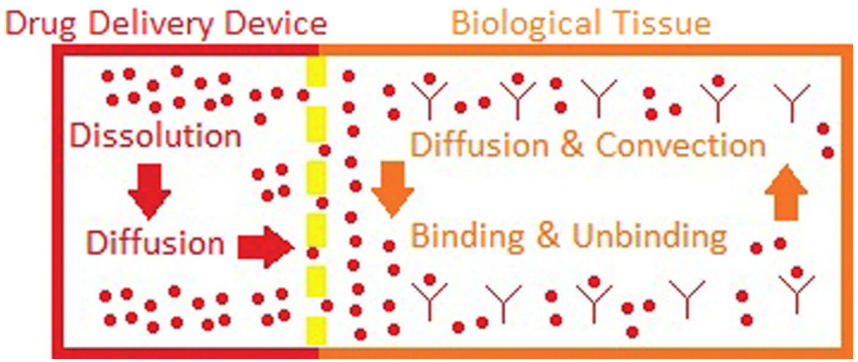
The SIR (S=susceptible, I=infectious, R=removed) model of epidemiology

Particle models

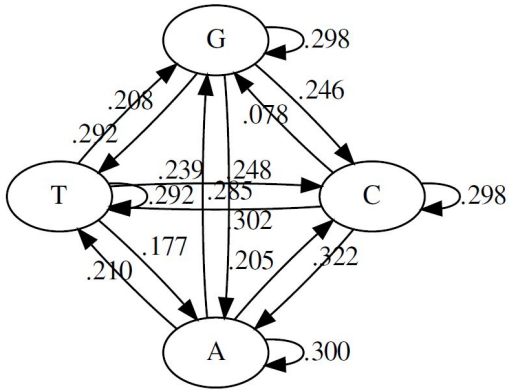


A Study on Socio-spatial Segregation Models Based on Multi-agent Systems by Quadros *et al*. (2012). 10.1109/BWSS.2012.14.

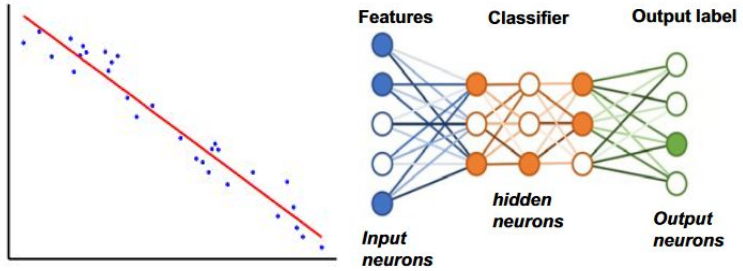
Transport models



Finite state models



Statistical/machine learning models



Backup slides

The principle of molecular docking, a case study of structure-based drug design

Docking is like a discotheque: it is all about posing and scoring – Roger Sayle (*NextMove* Software Limited)

Three basic methods to represent target and ligand structures *in silico*

- *Atomic*: used in conjunction with a potential energy function, computational complexity high
- *Surface*: often used in protein-protein docking
- *Grid representation*: the basic idea is that to store information about the receptor's energetic contributions on grid points so that it only needs to be read during ligand scoring.

In the most basic form, grid points store two types of potentials: **electrostatic** and **van der Waals forces**, for instance using Coulombic interactions and L-J 12-6 function.

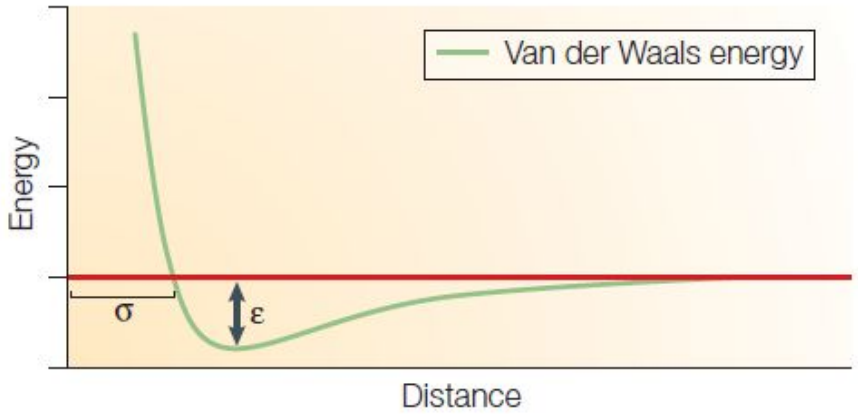
$$E_{coul}(r) = \sum_{i=1}^{N_A} \sum_{j=1}^{N_B} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}$$

Coulombic interactions (electrostatic interactions between electric charges)

$$E_{vdW}(r) = \sum_{j=1}^{N} \sum_{i=1}^{N} 4\varepsilon \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right]$$

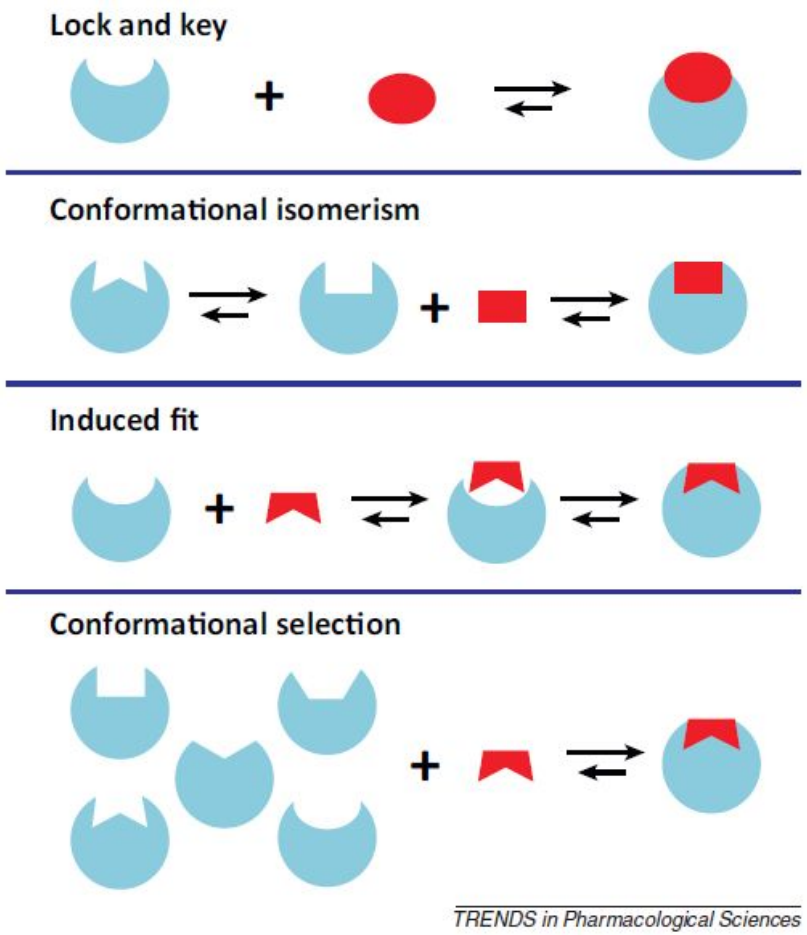
Lennard–Jones 12–6 function (intermolecular interactions without charge)

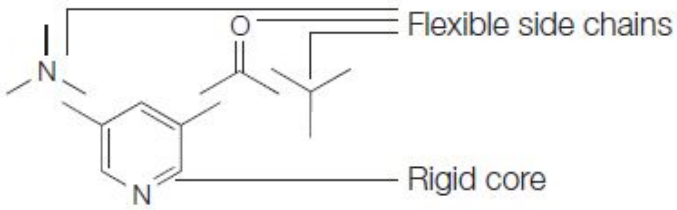
- ε is the **well depth** of the potential
- σ is the **collision diameter** of the respective atoms *i* and *j*.

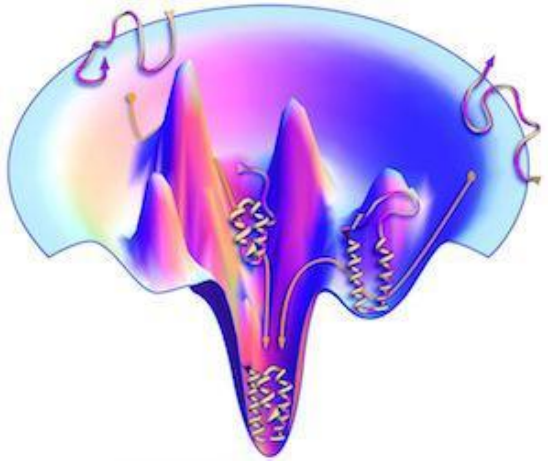


Kitchen, Douglas B., Hélène Decornez, John R. Furr, und Jürgen Bajorath. „Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications". *Nature Reviews Drug Discovery* 3, Nr. 11 (November 2004): 935–49. https://doi.org/10.1038/nrd1549.

Posing: dealing with flexibility of ligand and of protein





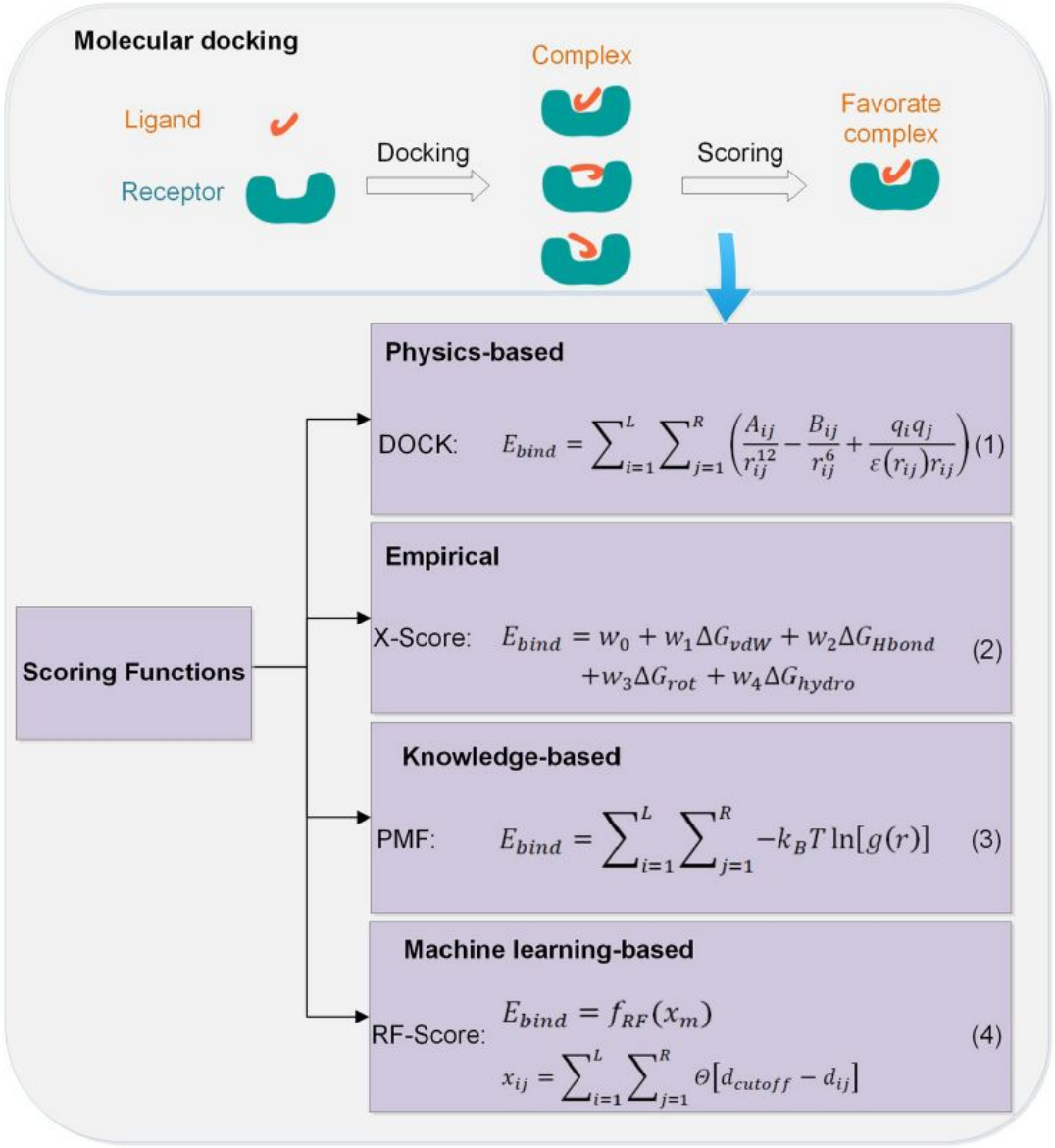


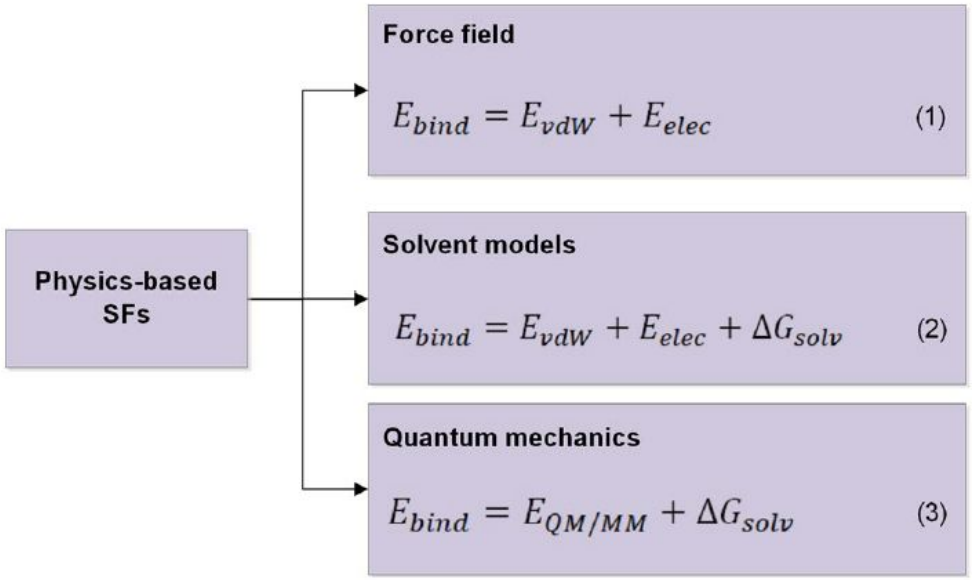
Methods to deal with ligand and protein flexibility

- Systematic search
- Random search, such as Monte-Carlo and genetic algorithms
- Simulation methods, such as molecular dynamics

Chen, Yu-Chian. „Beware of docking!“ *Trends in Pharmacological Sciences* 36, Nr. 2 (1. Februar 2015): 78–95. https://doi.org/10.1016/j.tips.2014.12.001.

Types of scoring functions

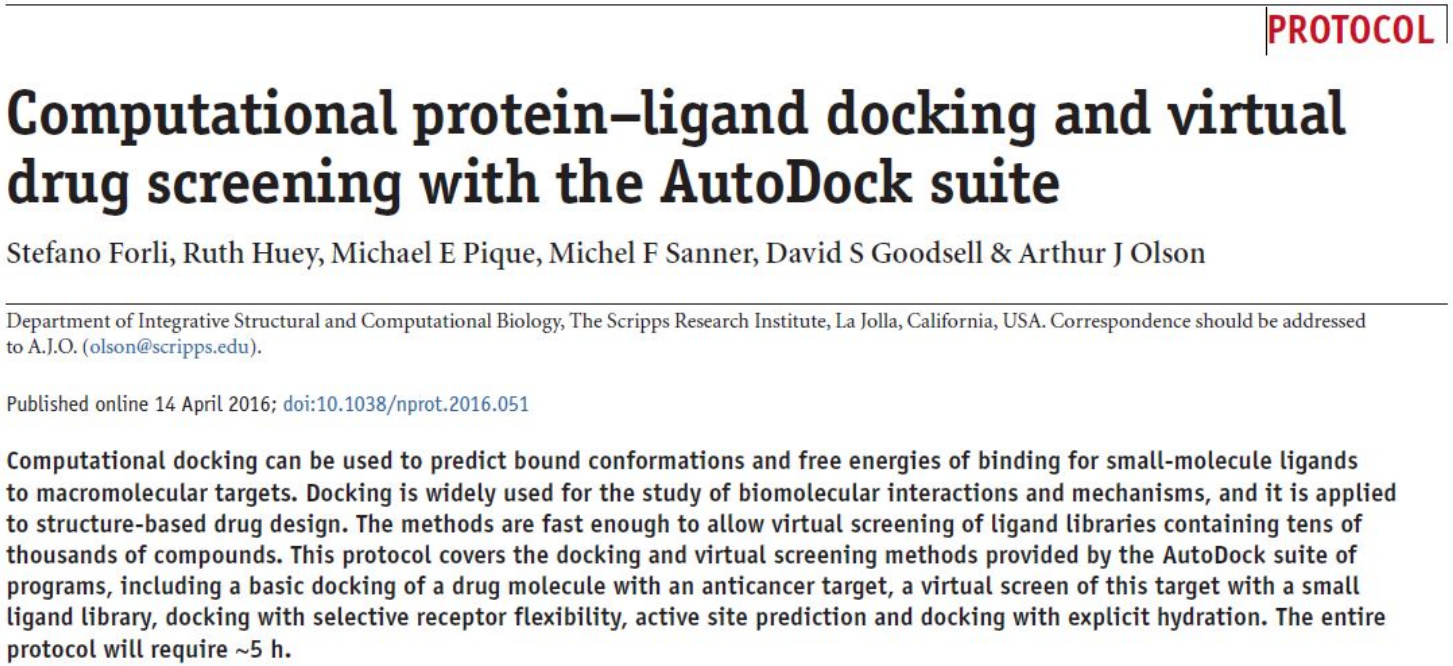




- Empirical scoring functions estimate the binding affinity of a complex by **summing up the important energetic factors for protein–ligand binding**, such as hydrogen bonds, hydrophobic effects, steric clashes, etc. It relies on training set and regression analysis.
- Knowledge-based scoring functions derive the desired pairwise potentials from three-dimensional structures of a large set of protein–ligand complexes based **on the inverse Boltzmann distribution**. It is assumed that the frequency of different atom pairs in different distances is related to the interaction of two atoms and converts the frequency into the distance-dependent potential of mean force.
- Machine learning-based scoring functions are usually used for rescoring to improve the initial docking.

Li, Jin, Ailing Fu, und Le Zhang. „An Overview of Scoring Functions Used for Protein–Ligand Interactions in Molecular Docking“. *Interdisciplinary Sciences: Computational Life Sciences* 11, Nr. 2 (1. Juni 2019): 320–28. https://doi.org/10.1007/s12539-019-00327-w.

Interested in learning more about molecular modelling?



PROTOCOL

Computational protein–ligand docking and virtual drug screening with the AutoDock suite

Stefano Forli, Ruth Huey, Michael E Pique, Michel F Sanner, David S Goodsell & Arthur J Olson

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, USA. Correspondence should be addressed to A.J.O. (olson@scripps.edu).

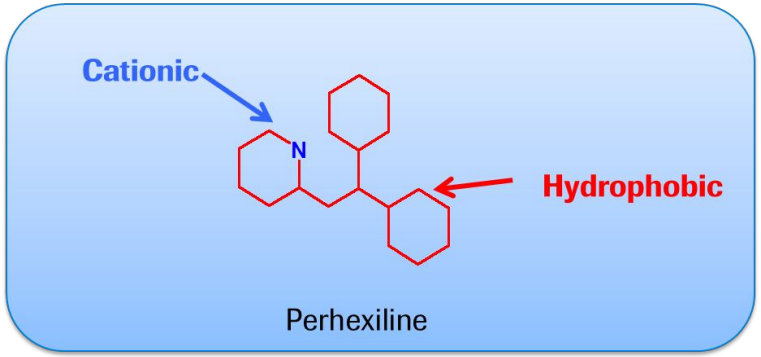
Published online 14 April 2016; doi:10.1038/nprot.2016.051

Computational docking can be used to predict bound conformations and free energies of binding for small-molecule ligands to macromolecular targets. Docking is widely used for the study of biomolecular interactions and mechanisms, and it is applied to structure-based drug design. The methods are fast enough to allow virtual screening of ligand libraries containing tens of thousands of compounds. This protocol covers the docking and virtual screening methods provided by the AutoDock suite of programs, including a basic docking of a drug molecule with an anticancer target, a virtual screen of this target with a small ligand library, docking with selective receptor flexibility, active site prediction and docking with explicit hydration. The entire protocol will require ~5 h.

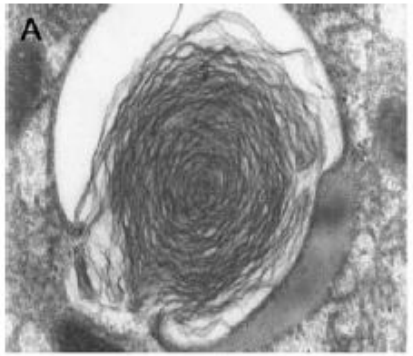
- Try docking yourself by following this protocol: Forli, Stefano, Ruth Huey, Michael E. Pique, Michel F. Sanner, David S. Goodsell, und Arthur J. Olson. „Computational Protein–Ligand Docking and Virtual Drug Screening with the AutoDock Suite“. *Nature Protocols* 11, Nr. 5 (Mai 2016): 905–19. https://doi.org/10.1038/nprot.2016.051.
- In-depth reading: Sliwoski, Gregory, Sandeepkumar Kothiwale, Jens Meiler, und Edward W. Lowe. „Computational Methods in Drug Discovery“. *Pharmacological Reviews* 66, Nr. 1 (1. Januar 2014): 334–95. https://doi.org/10.1124/pr.112.007336.
- A more advanced talk by Arthur Olson can be found here, Workshop on the Mathematics of Drug Design/Discovery, June 4 - 8, 2018, The Fields Institute. Courses available at the University of Basel and beyond.
- **Binding predicted by docking should always be challenged and verified by experimental testing! Docking scores seldomly correlate with binding affinity.**

Drug-induced phospholipidosis is correlated with amphiphilicity

- Phospholipidosis is a lysosomal storage disorder characterized by the excess accumulation of phospholipids in tissues.
- Drug-induced phospholipidosis is caused by cationic amphiphilic drugs and some cationic hydrophilic drugs.
- Clinical pharmacokinetic characteristics of drug-induced phospholipidosis include (1) very long terminal half lives, (2) high volume of distribution, (3) tissue accumulation upon frequent dosing, and (4) deficit in drug metabolism.



Lüllmann *et al.*, Drug Induced Phospholipidosis, *Crit. Rev. Toxicol. 4, 185, 1975*



Anderson and Borlak, Drug-Induced Phospholipidosis,. *FEBS Letters* 580, Nr. 23 (2006): 5533–40.

Fischer *et al.* (Chimia 2000) discovered that it is possible to predict the amphiphilicity property of druglike molecules by calculating the amphiphilic moment using a simple equation.

$$\vec{A} = \sum_i d \cdot \vec{\alpha}_i$$

$\vec{A}$: Caculated amphiphilic moment

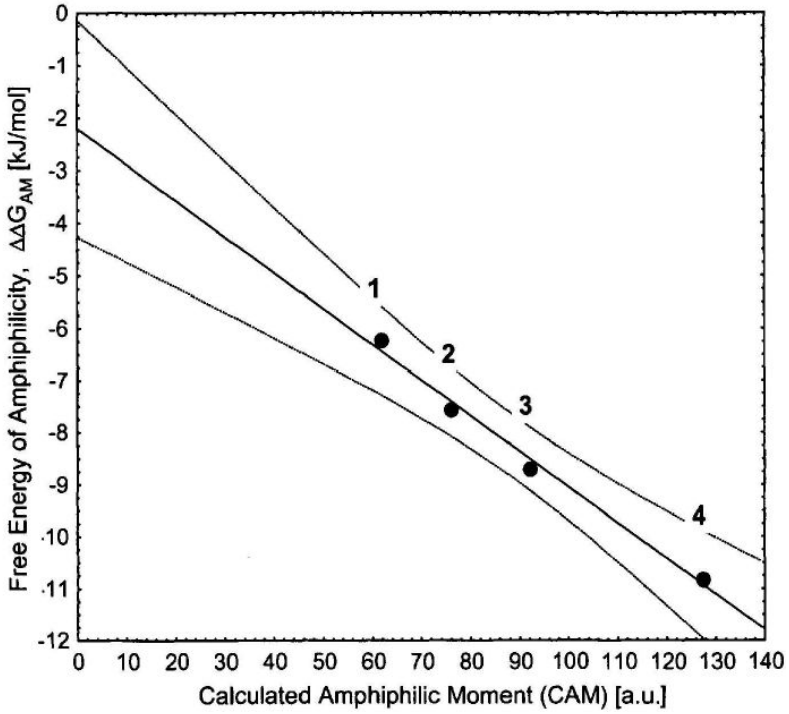
d: distance between the center of gravity of the charged part of a molecule and the hydrophobic/hydrophilic remnant of the molecule

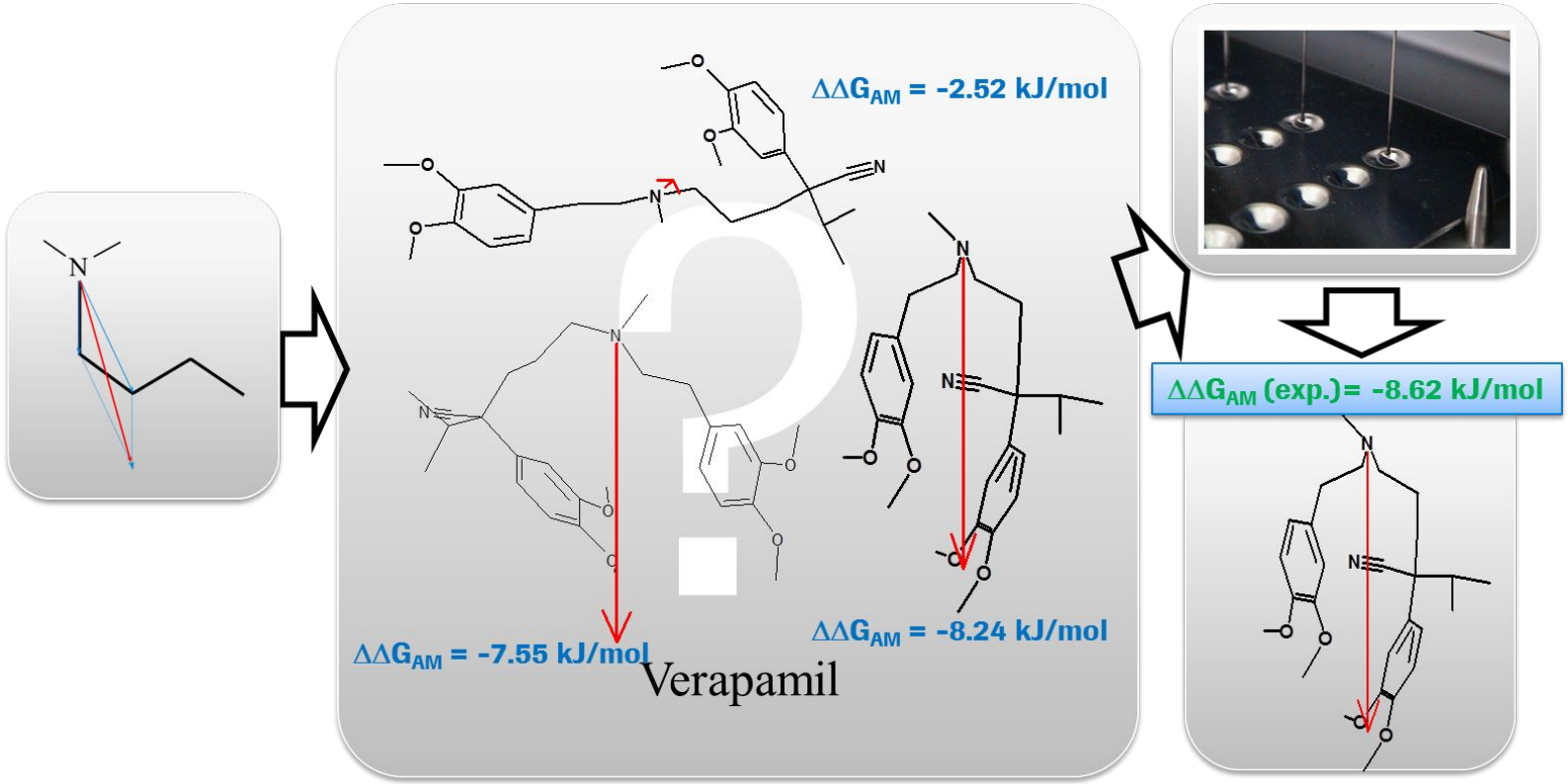
$\vec{\alpha}_i$: the hydrophobic/hydrophilic contribution of atom/fragment *i*

***In silico* calculation of amphiphilicity property may be used to predict phospholipidosis induction potential**

In silico prediction of amphiphilicity

Development of CAFCA (CAlculated Free energy of amphiphilicity of small Charged Amphiphiles)

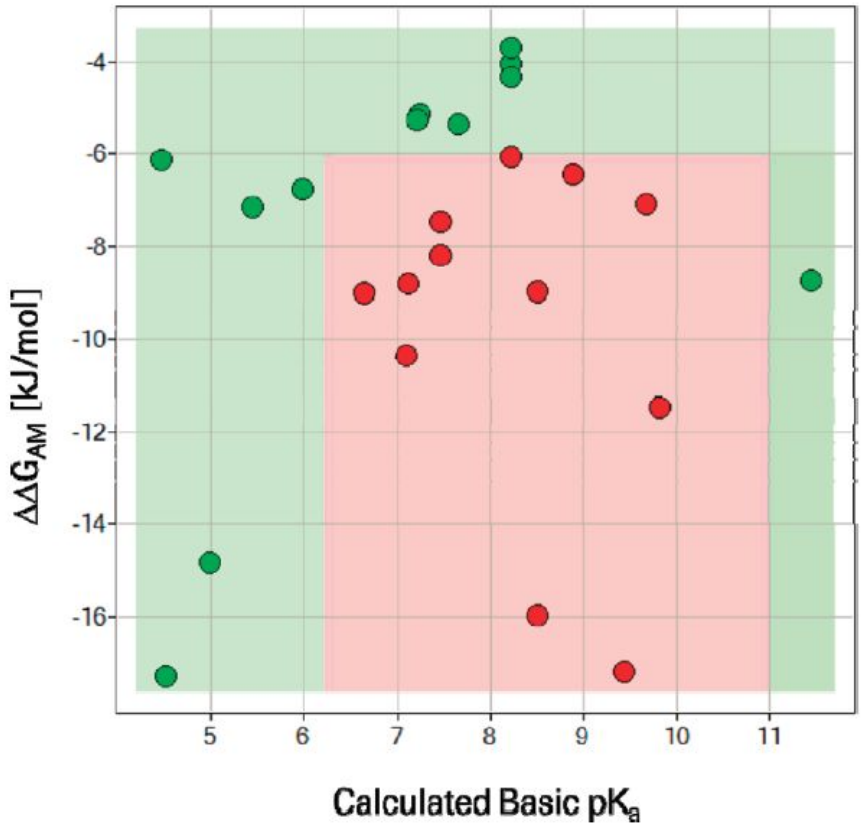




Iterative model building, experimentation, and model refining led to the predictive tool CAFCA

Validation of in silico phospholipidosis prediction

Model Validation from 1999-2004



Plot of amphiphilicity ($\Delta\Delta G_{AM}$) versus calculated basic pK_a for the training set of 24 compounds. The red area defines the region where phospholipidosis is expected, and the green area defines where a negative response is expected according to the tool.

in vitro/ in vivo	in silico/ in vivo	Exp. PC/ in vivo	In silico/ in vitro	n=36
94%	81%	89%	89%	

in vitro/in silico			n=422
Accuracy [(TP+TN)/ (P+N)]	Sensitivity [True Positive Rate]	Specificity [True Negative Rate]	Precision [TP/(TP+FP)]
86%	80%	90%	84%

Fischer et al., *J. Med. Chem, 55 (1), 2012*

We gained mechanistic insights of phospholipidosis induction by cationic amphiphilic drugs with the model

Phospholipidosis: lessons learned (and lessons not yet learned)

- Cationic amphiphilic properties of a molecule is an early marker for safety in drug discovery and early development.
  - Phospholipidosis in dose range finding studies
  - Cardiac ion channel interactions (hERG, natrium channel, …)
  - Receptor binding promiscuity
  - P-gp inhibition
  - Mitochondrial toxicity in case of safety relevant findings, *e.g.* in dose range finding studies
- Extreme basic amphiphilic properties should be avoided because of a higher risk of PLD, QT-prolongation, mitochondrial toxicity. However, basic compounds with moderate amphiphilic properties are still a preferred scaffold for many therapeutic areas (especially CNS).
- **Safety liabilities caused by physicochemical properties of the drugs may be well predicted by molecular modelling inspired by simple models.**

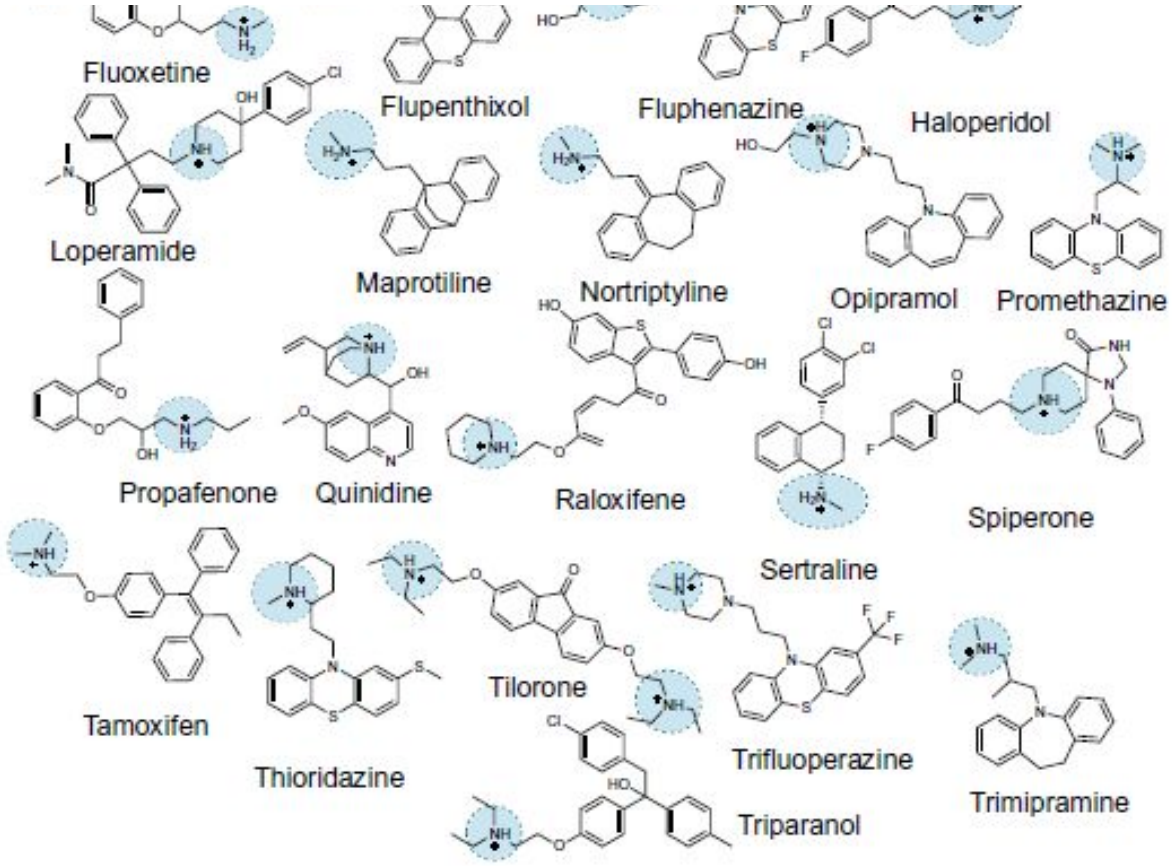


Fig. 1. Representative examples of CADs that are identified in SARS-CoV-2 drug repurposing screens.

Tummino, Tia A., Veronica V. Rezelj, Benoit Fischer, Audrey Fischer, Matthew J. O'Meara, Blandine Monel, Thomas Vallet, et al. "Drug-Induced Phospholipidosis Confounds Drug Repurposing for SARS-CoV-2." Science 373, no. 6554 (July 30, 2021): 541–47. https://doi.org/10.1126/science.abi4708.

More about the the Free-Wilson analysis

- *A Mathematical Contribution to Structure-Activity Studies* by Spencer M. Free and James W. Wilson, Journal of Medicinal Chemistry, 1964, and reviewed by Kubinyi, 1988.
- A Python implementation on GitHub, and a blog post going through examples, is shared by Pat Walters.
- Free-Wilson nonadditivity is a research topic, for instance see Cramer *et al.*, 2015
- Source of the example shown in the lecture: QSAR of the ACCVIP project (The Australian Computational Chemistry via the Internet Project)

Simulation of biological networks with ordinary differential expression: the simplest case

Given the reaction

$$\mathrm{S} + \mathrm{E} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_3} \mathrm{P} + \mathrm{E}$$

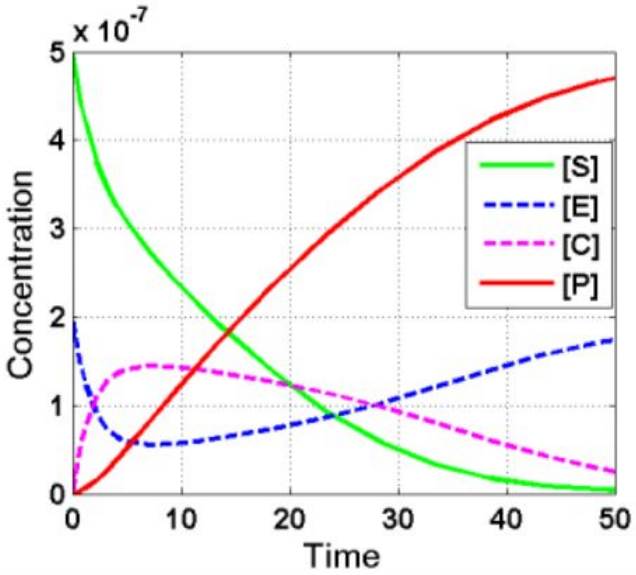
Given the initial values and rate constants

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

According to the law of mass action

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

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

Chemical Master Equations (CME): a particle model of chemical reaction

Given the reaction $\mathrm{A} + \mathrm{B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} + \mathrm{D}$ and the initial condition $X(0) = \begin{bmatrix} K \\ K \\ 0 \\ 0 \end{bmatrix}$ (*K* molecules of species A and of species B respectively)

The state vector $X(t)$ can take at any time point *one* of the values $\begin{bmatrix} K \\ K \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} K-1 \\ K-1 \\ 1 \\ 1 \end{bmatrix}, \begin{bmatrix} K-2 \\ K-2 \\ 2 \\ 2 \end{bmatrix}, \ldots, \begin{bmatrix} 0 \\ 0 \\ K \\ K \end{bmatrix},$

Theoretically we can build an ODE system with *K+1* equations to model *every state of the reaction*, down to every particle. In reality, the dimension is so high so that a simulation is not feasible.

CME is a set of ODEs, with each ODE representing one possible state of the system. Solution of the *k*th equation at time *t* is a real number giving the probability of system being in that particular state at that time.

Reaction Rate Equations (RRE): a compartment model

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

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

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

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

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

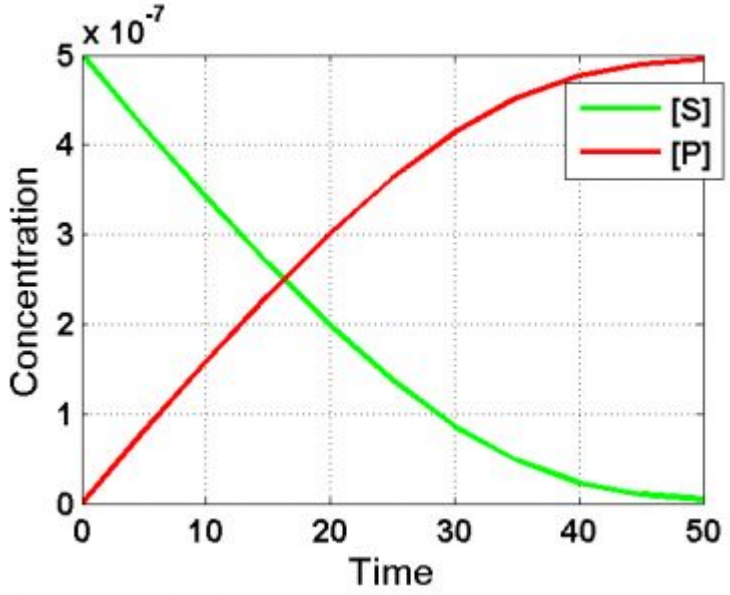
$K_D \equiv \frac{k_r}{k_f}$

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

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

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

***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*.

The Gillespie's algorithm and the chemical Langevin equation allow stochastic simulation of biological networks

- The *stochastic simulation algorithm* (exact SSA), also called *Gillespie's algorithm*, allows stochastic simulation of a reaction. It is done in four steps:
  1. **initialize** the system with initial conditions
  2. Given a state at time *t*, we can define a probability *p* that reaction *j* takes place in the time interval $[t+\tau, t+\tau+d\tau)$. It is the product of two density functions of two random variables: the probability of reaction *j* happens (proportional to the number of substrate molecules), multiplied by the time until next reaction, which is exponentially distributed. This is known as the ***Monte Carlo*** step.
  3. Let the randomly selected reaction happen and **update** the time.
  4. **Iterate** until substrates are exhausted or simulation time is over.
- Further computation tricks, .e. 'tau-leaping', are used to lump together reactions. The chemical Langevin equation (CLE) further accelerates stochastic simulation by approximating *Poisson* with normal distribution.

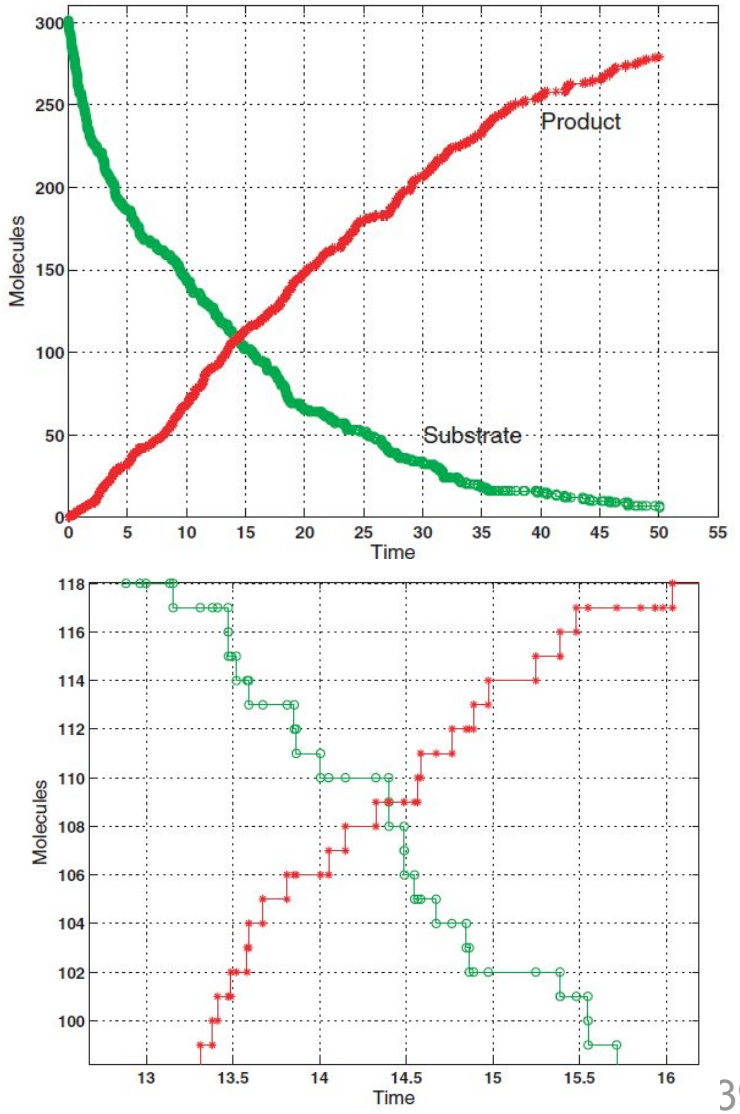
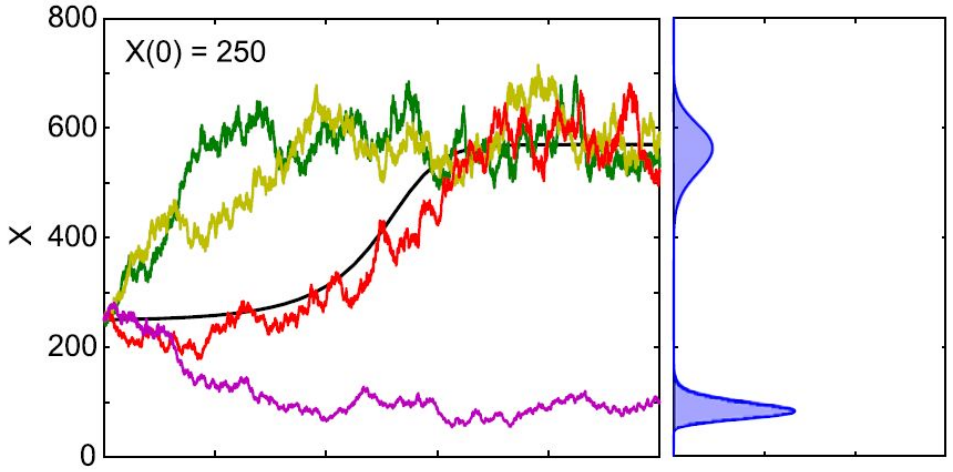
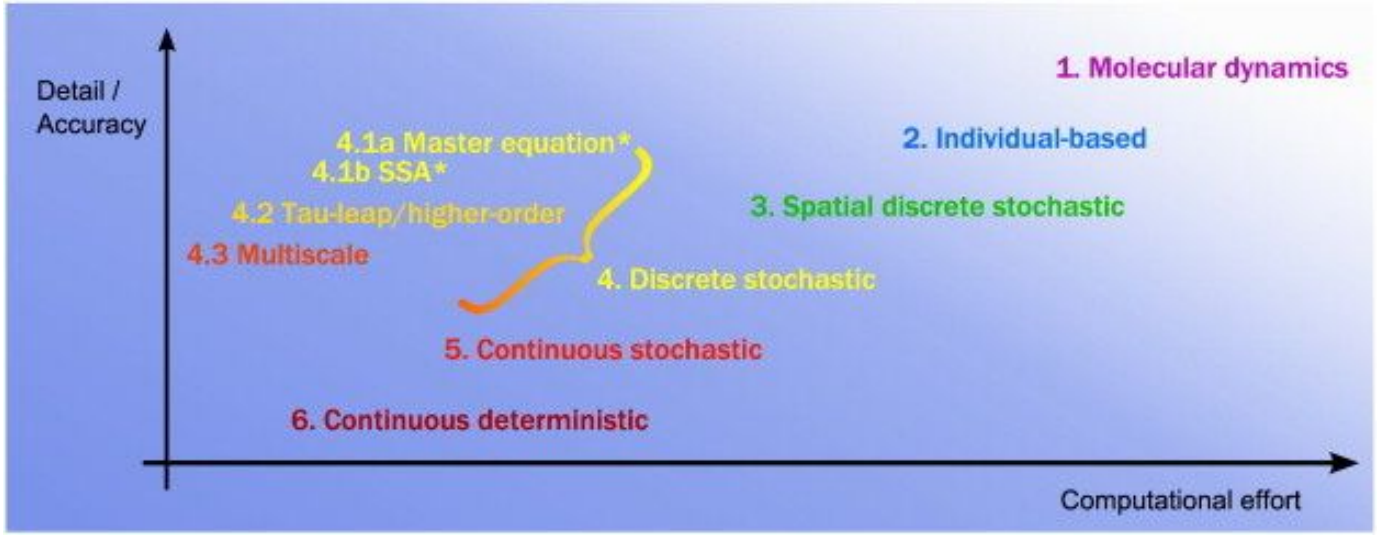


Figure source and further reading: Higham, Desmond J. 2008. "Modeling and Simulating Chemical Reactions." *SIAM Review* 50 (2): 347–68. https://doi.org/10.1137/060666457.

Why stochastic modelling?



- Stochastic modelling can reveal individual trajectories that are otherwise 'averaged' by ODE models.
- Small systems and single-molecule studies show stochastic behaviour.
- It is possible to consider both extrinsic and intrinsic factors and take them into the model.



Advantages and disadvantages of several modelling/simulation methods.

Simulation method	Cat.	Advantages	Disadvantages	References	Software
Master equation	4	Exact	Very computationally intensive	[85,143]	
SSA	4	Statistically exact	Very computationally intensive	[82,109]	COPASI [144] StochKit [145] STOCKS [146] BioNetS [147]
Tau-leap	4	Relatively fast	Approximate; too slow for large systems or frequent/multiscale reactions	[83,113,118]	StochKit [145]
Higher-order	4	Relatively fast; accurate	Approximate; too slow for large systems or frequent/multiscale reactions	[83,121,122,124,125]	
Multiscale/hybrid	4	Fast; good for systems with disparate reaction scales	Approximate; problems with coupling different scales	[131,132,137,139,148]	COPASI [144] BioNetS [147]
Brownian dynamics	2	Tracks individual molecules	Slow; molecule size must be artificially added	[149,150]	Smoldyn [149,151] MCell [152]
Compartment-based	3	Accounts for diffusion between homogeneous compartments	Slow; compartment size must be set manually; each compartment is homogeneous	[150,153,154]	MesoRD [153] URDME [155]
SDE	5	Fast	Continuous; Gaussian noise	[76]	BioNetS [147]
PDE (R-D)	6	Very fast; spatial	Continous; no noise	[156]	
ODE	6	Very fast	Continuous; no noise	[157]	

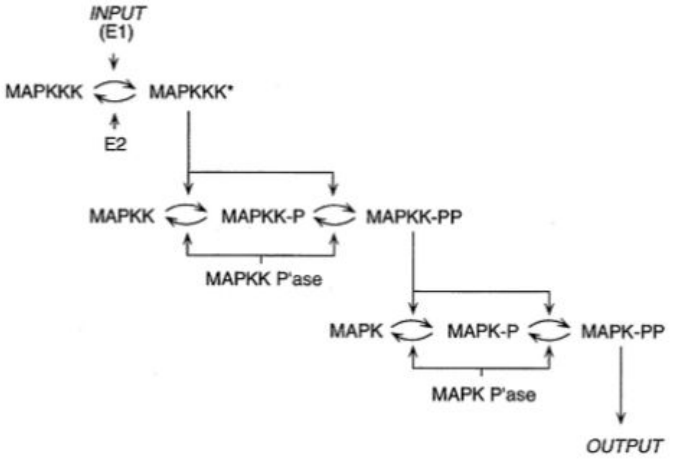
Cat. represents Category from Fig. 2. Abbreviations: SSA, stochastic simulation algorithm; SDE, stochastic differential equation; PDE (R-D), partial differential equation (classical reaction-diffusion equations); ODE, ordinary differential equation.

Székely and Burrage. 2014. "Stochastic Simulation in Systems Biology." *Computational and Structural Biotechnology Journal* 12 (20–21): 14–25.

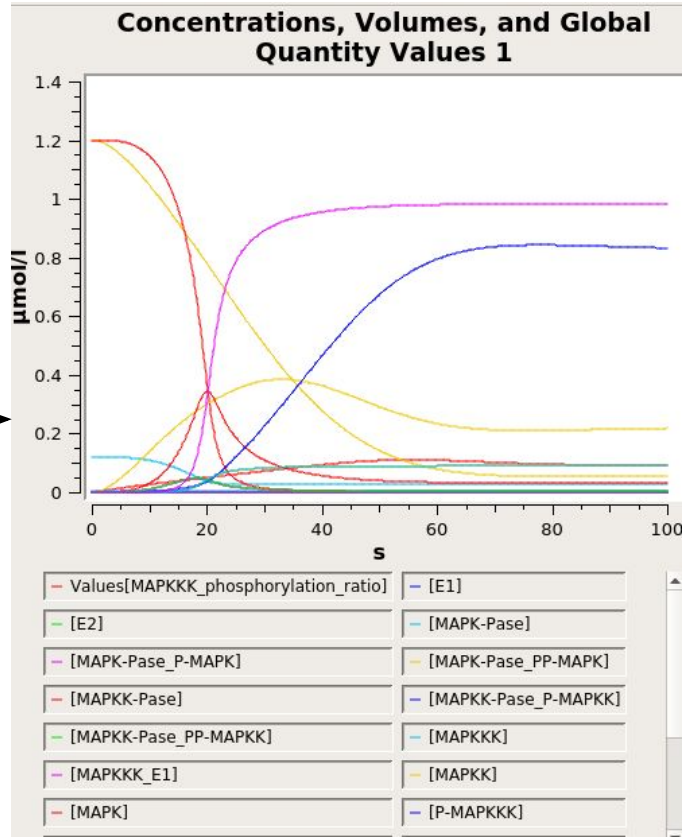
Also see *Stochastic Modelling for Systems Biology* by Darren J. Wilkinson.

Biochemical system simulator COPASI

- Freely available at http://COPASI.org/
- COPASI supports two types of simulations: (1) **ordinary differential equation (ODE)** based simulation**, (2) stochastic kinetic simulation**, among others using the stochastic Runge–Kutta method (RI5) and Gillespie's algorithm
    - 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)
- The mathematical concept and software tools are important for detailed analysis of enzymatic reactions, especially in the presence of drugs and/or disease-relevant mutation

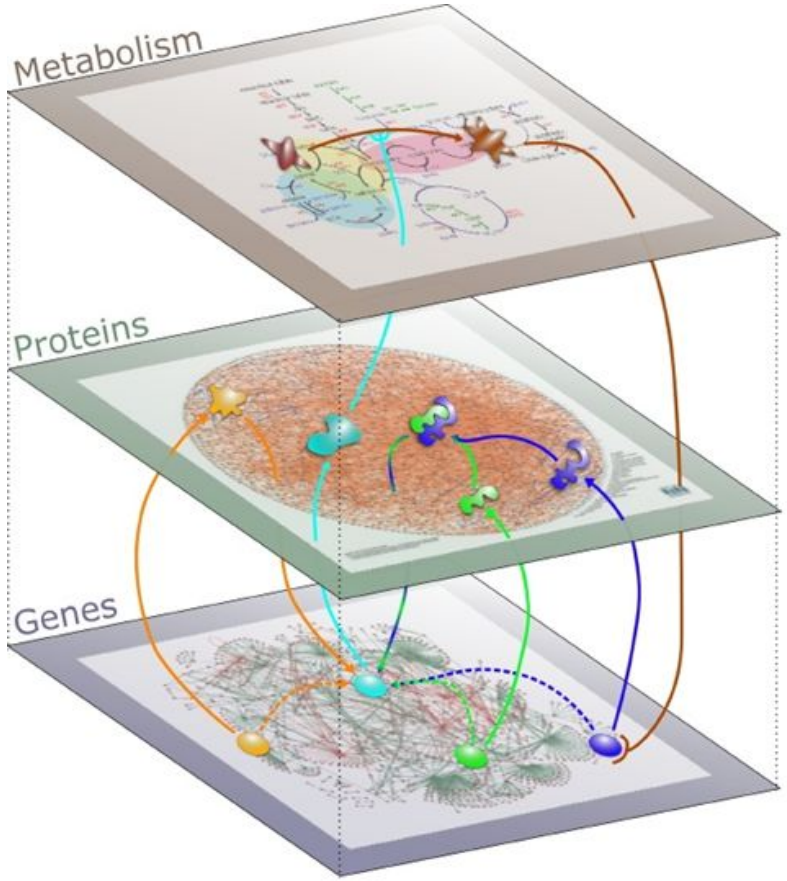


Huang and Ferrell, PNAS, 2006

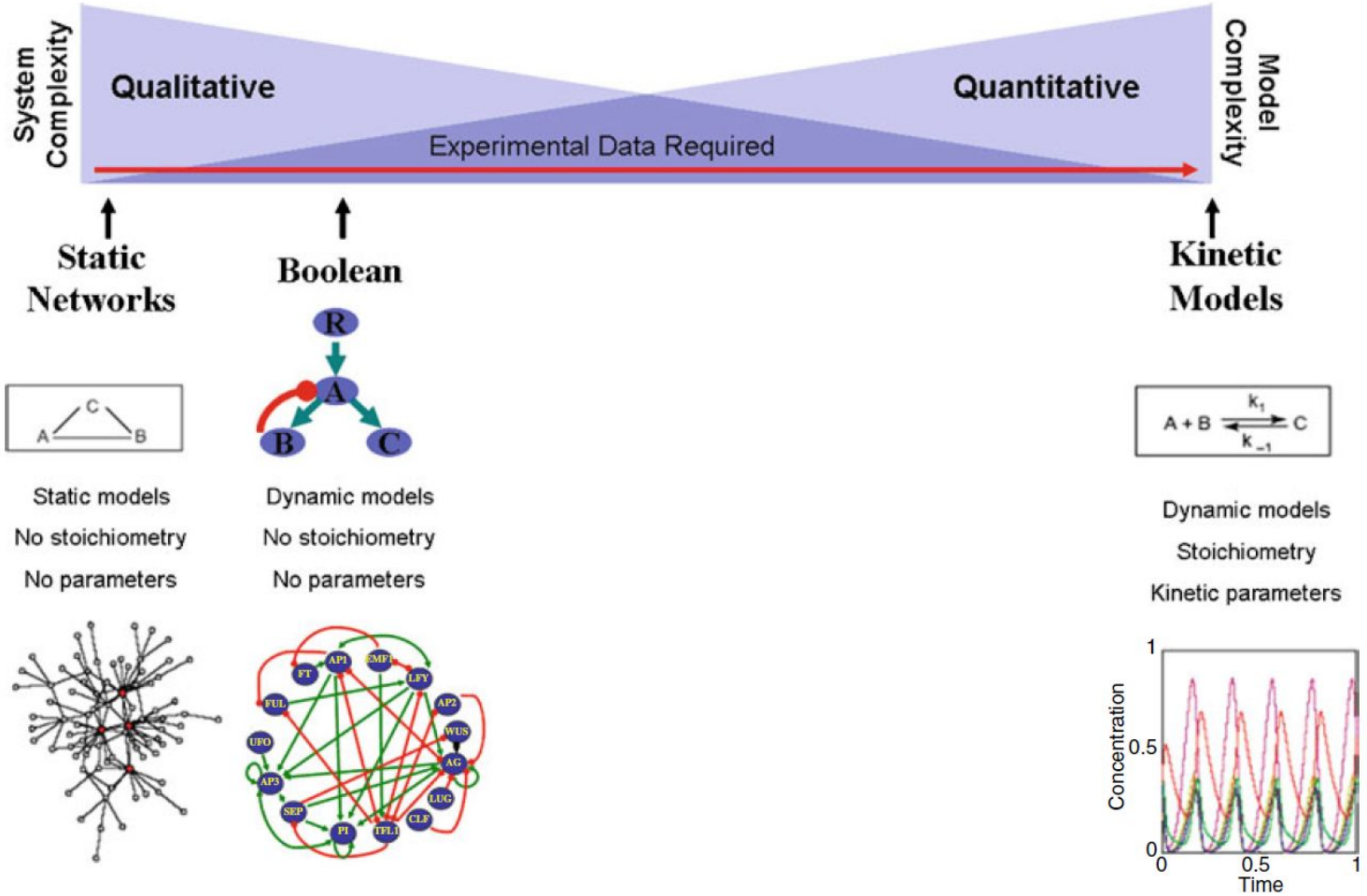


ODE-based simulation of dynamics

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.