Offline activities of Lecture 6

Q1. What is the method commonly used to benchmark performance of different techniques of computer-aided drug design (CADD)? (Receiver Operating Characteristic curves)

Q2: What do we mean by molecular dynamics? (A computer simulation method to analyze the movements of atoms and molecules using Newtonian mechanistics)

Q3: What are the three basic methods to represent target and ligand structures *in silico*? (atomic, surface, and grid representations)

Q4: What sampling algorithms are there for protein-ligand docking? Can you explain one of them using your words? (systematic algorithms, molecular dynamics simulations, Monte Carlo search with Metropolis Criterion and genetic algorithms)

Q5: What are the key steps in structure-based virtual high-throughput screenings (SB-vHTS)? (preparing structures, posing, scoring)

Q6: What is the usual starting point of structure-based CADD campaign? (Experimentally determined protein structures, preferably in complex with ligands)

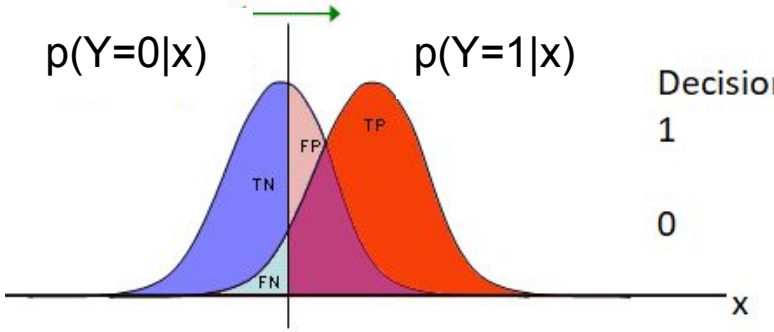
Q7: What do we mean by 'pharmacophore'? (model of the target binding site which summarizes steric and electronic features needed for optimal interaction of a ligand with a target, a "subgraph" of a molecule with interesting properties for drug design/protein binding)

Q8: In QSAR analysis, why it is important to select optimal descriptors/features? (to reduce noise, to increase generalized performance, and for hypothesis generation)

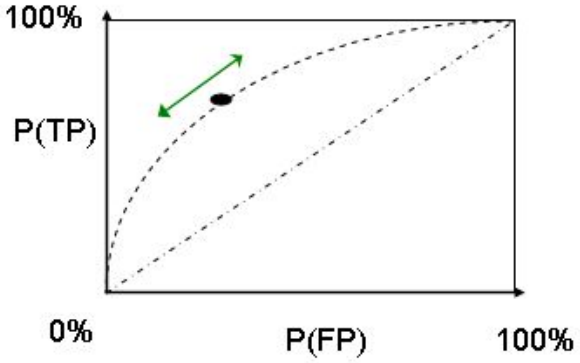
Q9: What do we mean by the acronyms *DMPK* and *ADMET*? (DMPK=drug metabolism and pharmacokinetics; ADMET= absorption, distribution, metabolism, excretion, and the potential for toxicity)

Q10: Why common CADD methods have difficulties handling protein-protein interaction and protein-DNA interactions? (large interaction size, lack of user-friendly tools, and comparably little training data)

Question about the ROC curve

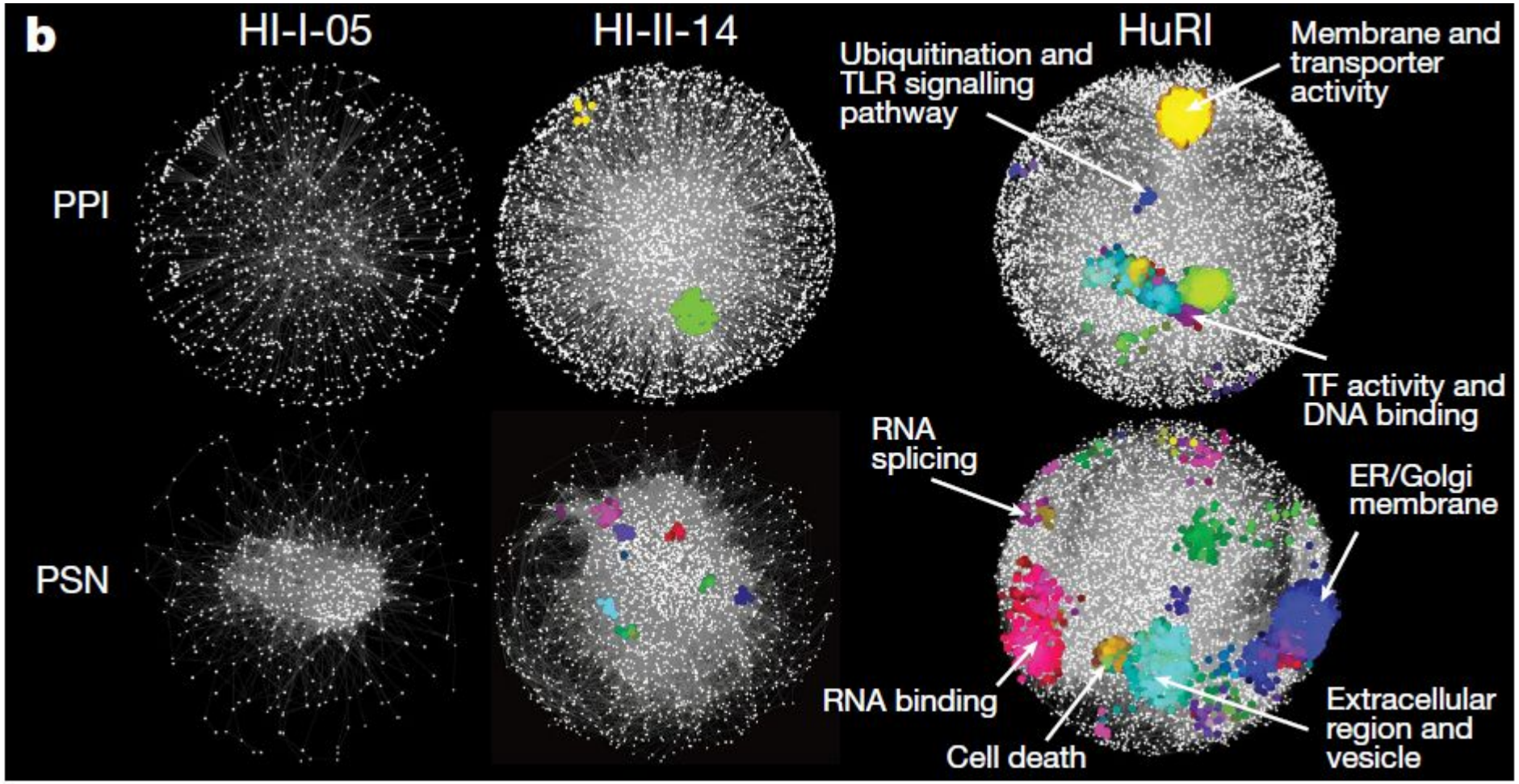


Decision	Y = 1	0
1	TP	FP
0	FN	TN
	1	1



		Predicted labels: 1	Predicted labels: 0	
Actual labels (observations)	1	True Positive (TP)	False Negative (FN)	Recall=TPR (True Positive Rate) TPR = $\frac{TP}{TP+FN}$
	0	False Positive (FP)	True Negative (TN)	Specificity = $\frac{TN}{TN+FP}$ False Positive Rate: FPR = $\frac{FP}{FP+TN}$
		Precision $\frac{TP}{TP+FP}$	False Negative Rate $\frac{FN}{TN+FN}$	Accuracy $\frac{TP+TN}{TP+TN+FP+FN}$

AMIDD Lecture 7: From individual interactions to networks



Luck *et al.* "A Reference Map of the Human Binary Protein Interactome." Nature, 2020

Dr. Jitao David Zhang, Computational Biologist

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Topics

- **From molecular models to cellular models**
- **Gene expression profiling: a case study of omics and cellular modelling**
- **Applications for drug mechanism of action: molecular phenotyping**

Four classical classes of mathematical models

Compartment models

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

Kinetics of ligand-target interaction

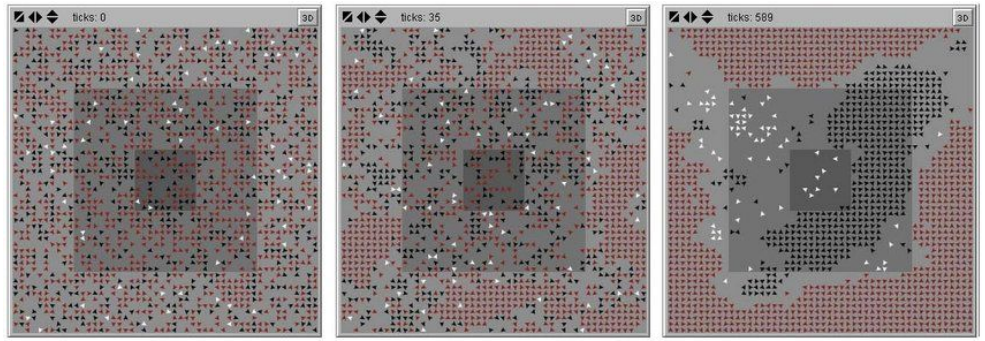
$$\frac{dx}{dt} = \alpha x - \beta xy,$$
$$\frac{dy}{dt} = -\gamma y + \delta xy,$$

The Lotka-Volterra equations modelling predator-prey relationships.

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

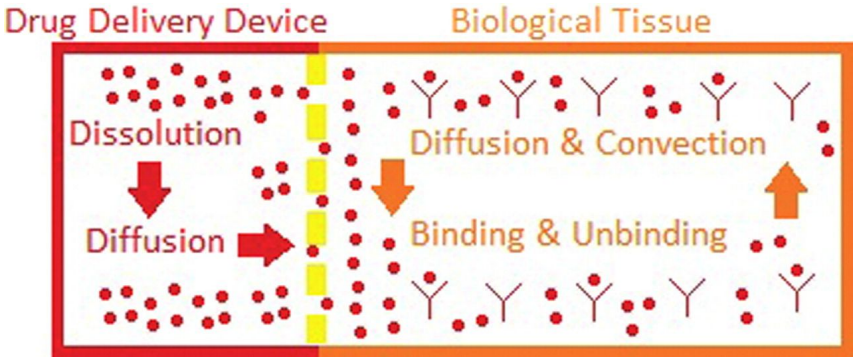
The SIR (S=susceptible, I=infectious, R=removed) model of epidemiology

Particle models



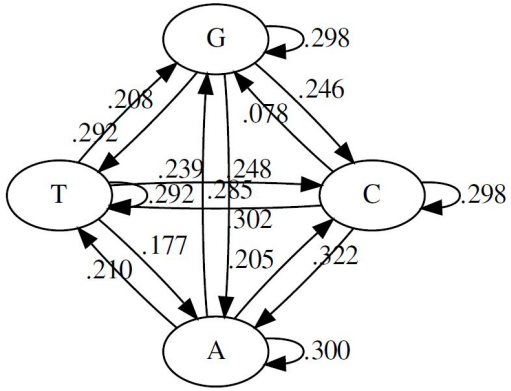
A Study on Socio-spatial Segregation Models Based on Multi-agent Systems by Quadros *et al*. (2012). 10.1109/BWSS.2012.14.

Transport models



McGinty, Sean, and Giuseppe Pontrelli. 2015. "*A General Model of Coupled Drug Release and Tissue Absorption for Drug Delivery Devices*." Journal of Controlled Release 217 (November): 327–36.

Finite state models



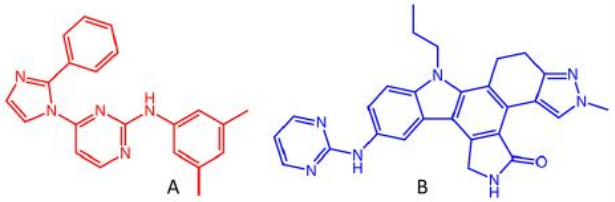
A finite-state Markov chain modelling DNA sequences

Molecular similarity and similarity measures

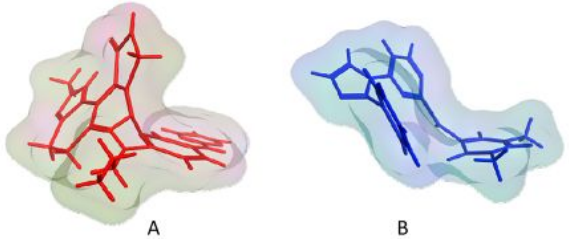
Chemical similarity

	Mol. weight	LogP	Rotatable bonds	Aromatic rings	Heavy atoms
A	341.4	5.23	4	4	26
B	463.5	4.43	4	5	35

Molecular similarity / **2D similarity**



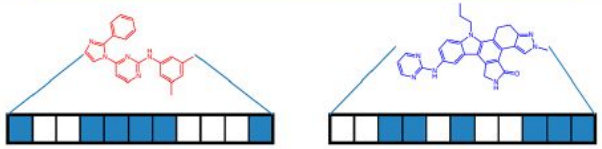
3D similarity



Biological similarity

	Vascular endothelial growth factor receptor 2	Tyrosine-protein kinase TIE-2
A	active	inactive
B	active	active

Global similarity



Local similarity

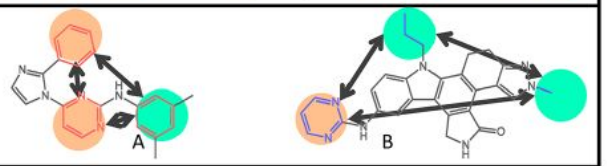


Table 2 Formulas for the various similarity and distance metrics

Distance metric	Formula for continuous variables[a]	Formula for dichotomous variables[a]
Manhattan distance	$D_{A,B} = \sum_{j=1}^{n} \lvert x_{jA} - x_{jB} \rvert$	$D_{A,B} = a + b - 2c$
Euclidean distance	$D_{A,B} = \left[\sum_{j=1}^{n} (x_{jA} - x_{jB})^2\right]^{1/2}$	$D_{A,B} = [a + b - 2c]^{1/2}$
Cosine coefficient	$S_{A,B} = \left[\sum_{j=1}^{n} x_{jA} x_{jB}\right] / \left[\sum_{j=1}^{n} (x_{jA})^2 \sum_{j=1}^{n} (x_{jB})^2\right]^{1/2}$	$S_{A,B} = \frac{c}{[ab]^{1/2}}$
Dice coefficient	$S_{A,B} = \left[2\sum_{j=1}^{n} x_{jA} x_{jB}\right] / \left[\sum_{j=1}^{n} (x_{jA})^2 + \sum_{j=1}^{n} (x_{jB})^2\right]$	$S_{A,B} = 2c/[a + b]$
Tanimoto coefficient	$S_{A,B} = \frac{\left[\sum_{j=1}^{n} x_{jA} x_{jB}\right]}{\left[\sum_{j=1}^{n} (x_{jA})^2 + \sum_{j=1}^{n} (x_{jB})^2 - \sum_{j=1}^{n} x_{jA} x_{jB}\right]}$	$S_{A,B} = c/[a + b - c]$
Soergel distance[b]	$D_{A,B} = \left[\sum_{j=1}^{n} \lvert x_{jA} - x_{jB} \rvert\right] / \left[\sum_{j=1}^{n} max(x_{jA}, x_{jB})\right]$	$D_{A,B} = 1 - \frac{c}{[a+b-c]}$

S denotes similarities, while *D* denotes distances. The two can be converted to each other by *similarity=1/(1+distance)*. x_{jA} means the j-th feature of molecule A. a is the number of *on* bits in molecule A, b is number of *on* bits in molecule B, while c is the number of bits that are *on* in both molecules.

(Left) Maggiora, Gerald, Martin Vogt, Dagmar Stumpfe, und Jürgen Bajorath. „Molecular Similarity in Medicinal Chemistry“. *Journal of Medicinal Chemistry* 57, Nr. 8 (24. April 2014): 3186–3204. (Right) Bajusz, Dávid, Anita Rácz, and Károly Héberger. 2015. “Why Is Tanimoto Index an Appropriate Choice for Fingerprint-Based Similarity Calculations?” Journal of Cheminformatics 7 (1): 20.

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).

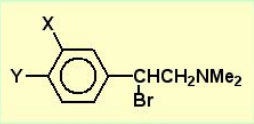
Molecular Descriptors (MD)

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									

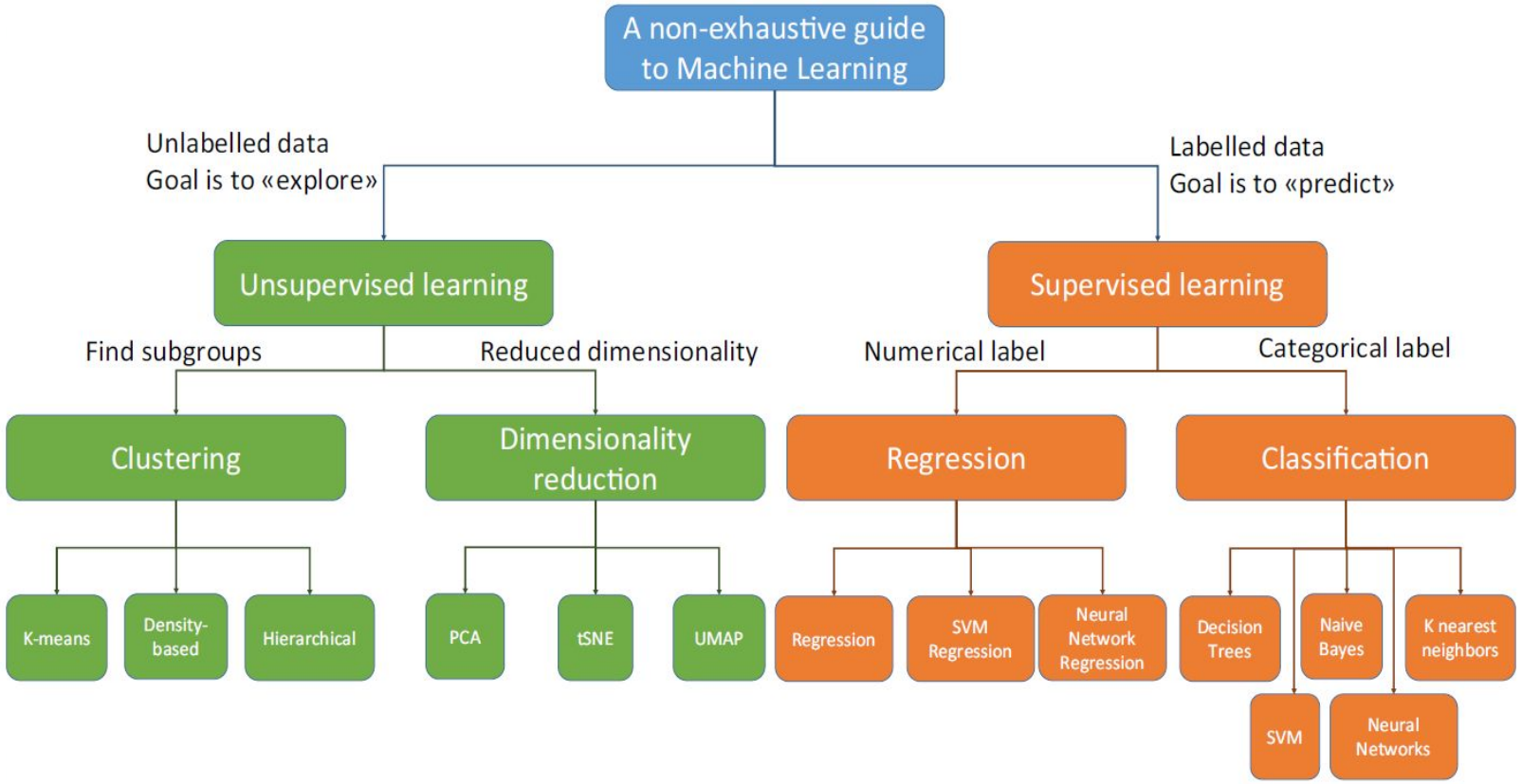
Multivariate regression analysis

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

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

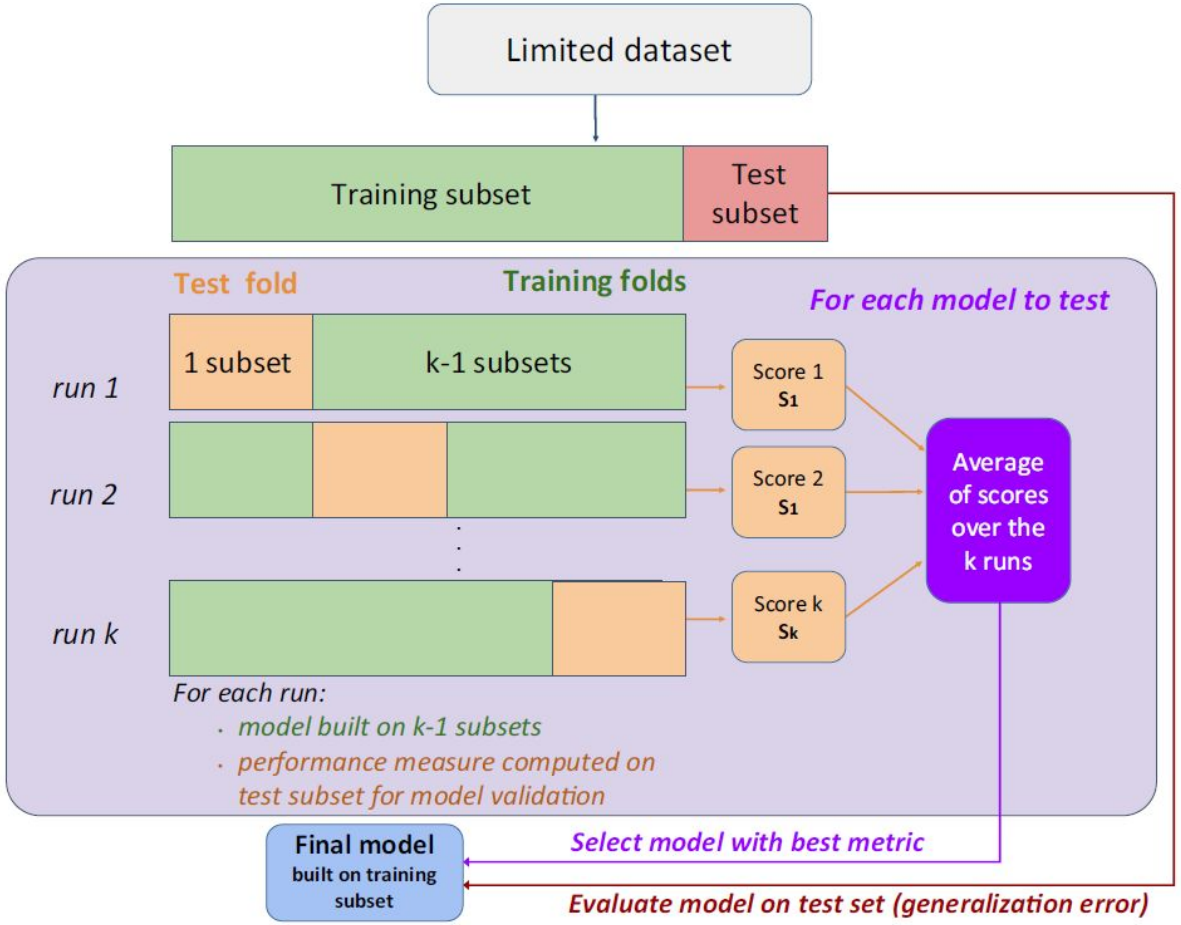
QSAR models mark the early adoption of statistical modelling and machine learning in drug discovery, the fifth type of mathematical modelling

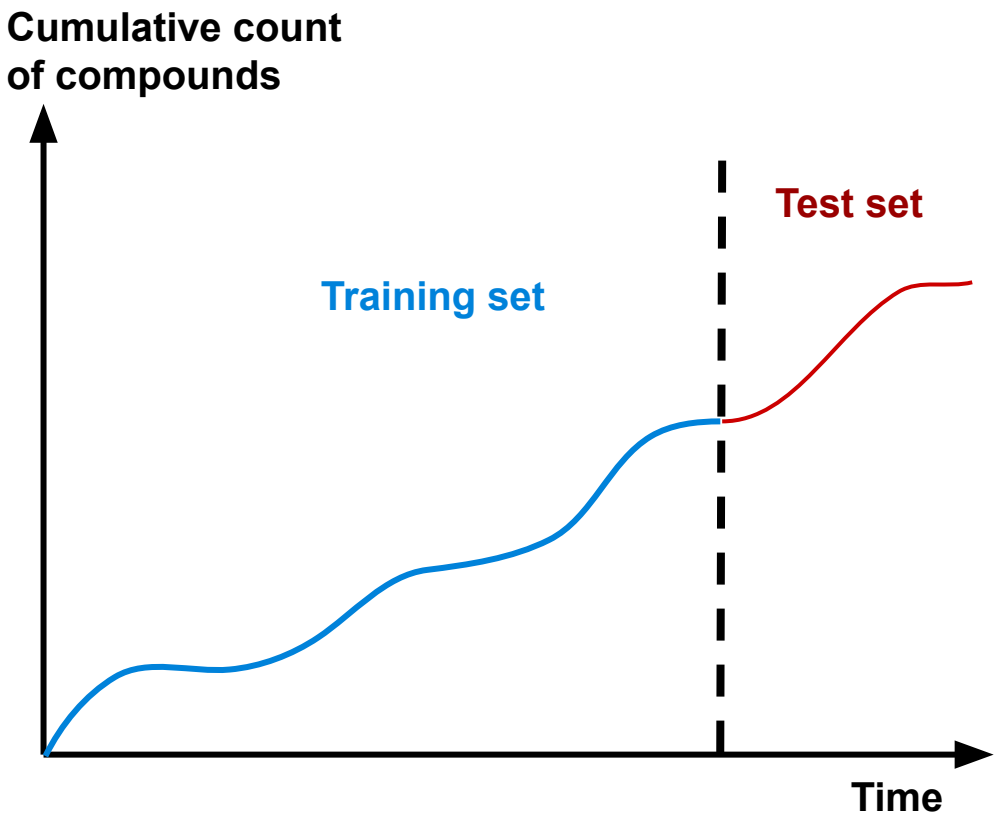
- QSAR is among the earliest subjects that used machine learning and pattern recognition in drug discovery.
- **Advantages**: technically easy, fast, and many models are useful as filters.
- **Disadvantages**: statistical models cannot capture mechanistic aspects of biochemical interactions, limited ability to debug when a model fails to work, and findings may not be generalizable.



Badillo, Solveig, *et al.* 2020. “An Introduction to Machine Learning.” *Clinical Pharmacology & Therapeutics*.

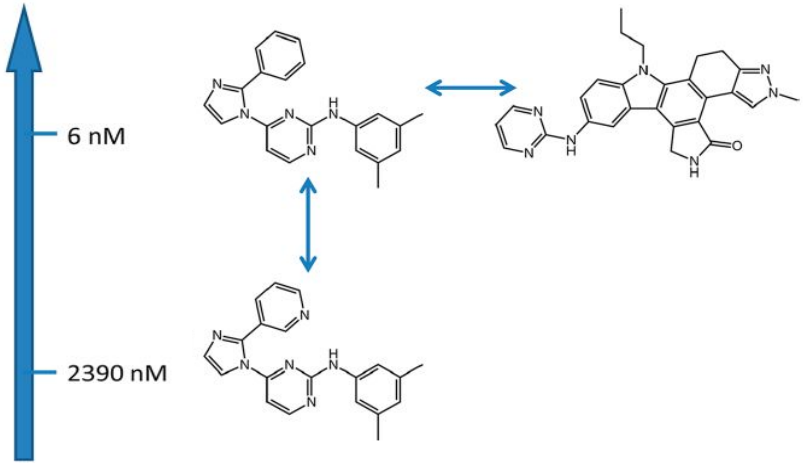
The general practice of training a supervised learning model

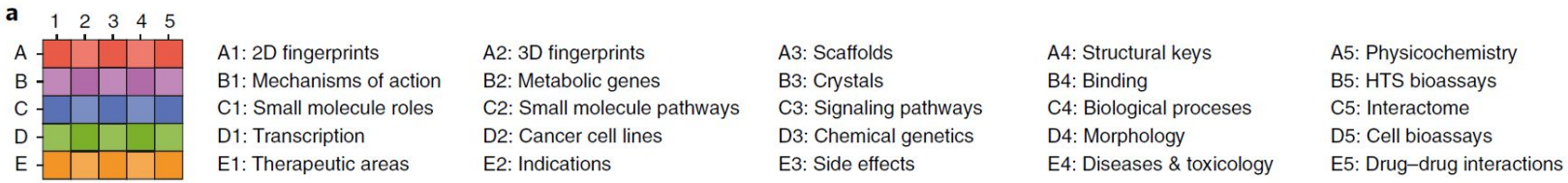




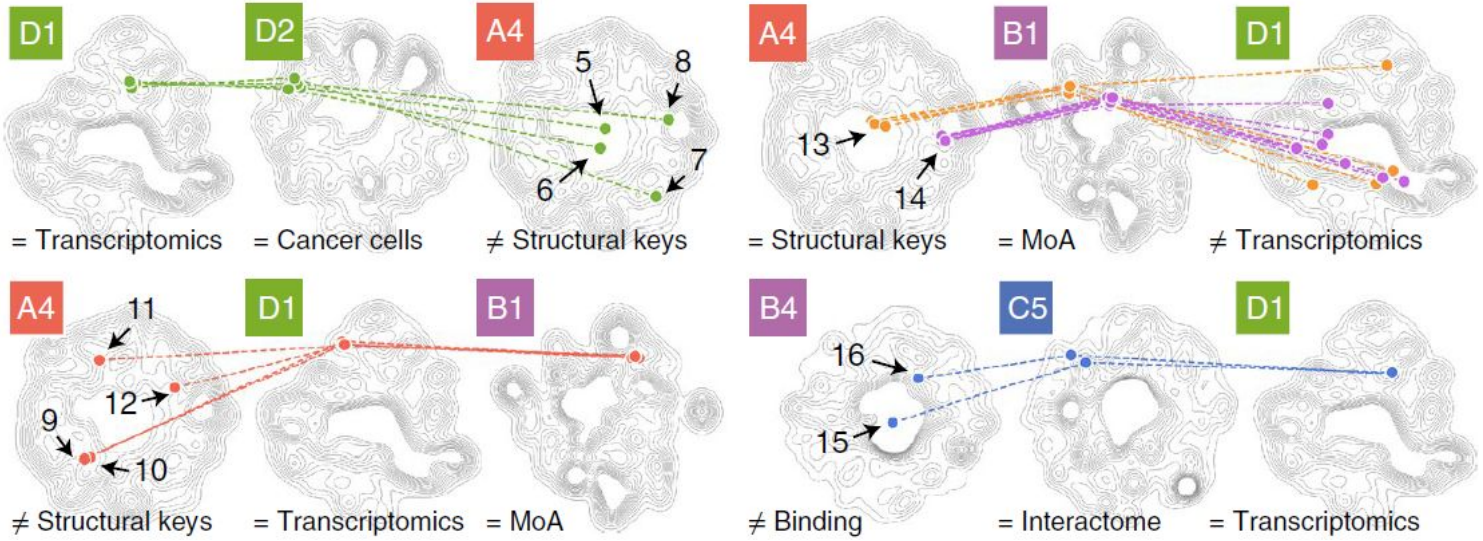
(Left) To assess the generalization ability of a supervised learning algorithm, data are separated into a training subset used for building the model and a test subset used to assess the generalization error (from Badillo *et al.*, 2020) (Right) Temporal validation is especially important for drug discovery, because chemical structures used in the training set may differ substantially from those that will be tested.

Molecular similarity does not equal biological similarity





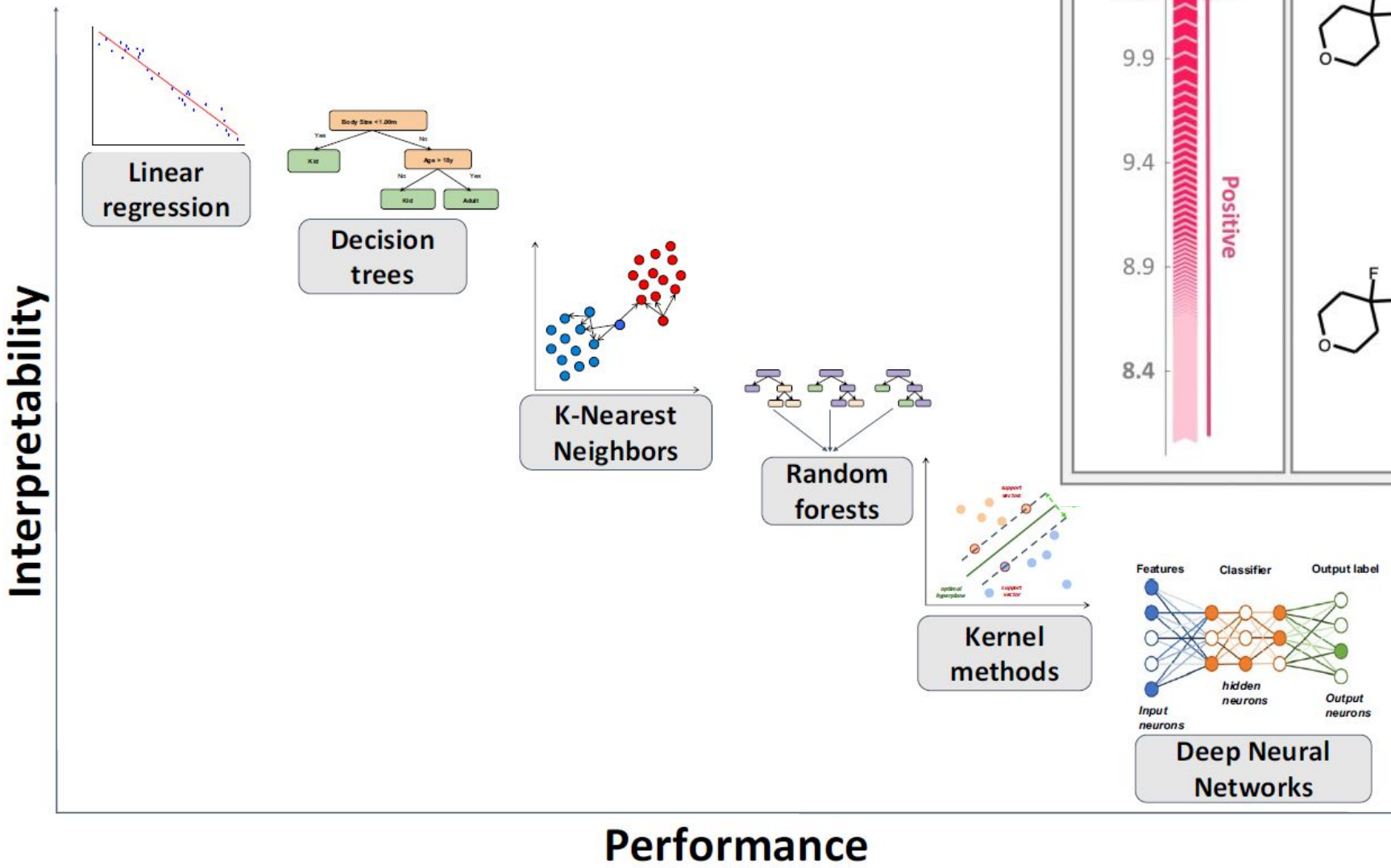
A: Chemistry
B: Targets
C: Biological network
D: Cells
E: Clinical readout

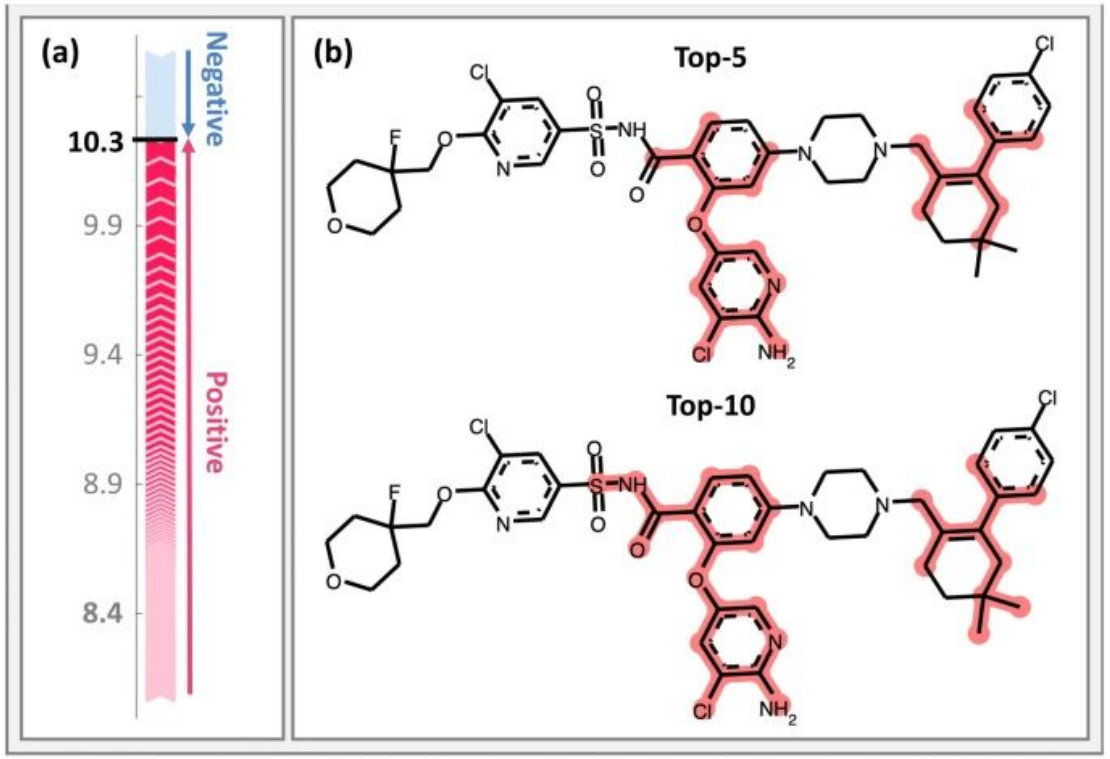


Watch out biological activity cliffs!
Similarity does not imply activity. Three vascular endothelial growth factor receptor 2 (VEGFR2) ligands are shown that represent different similarity−activity relationships.

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.

Interpretable and Causal Models will become more important

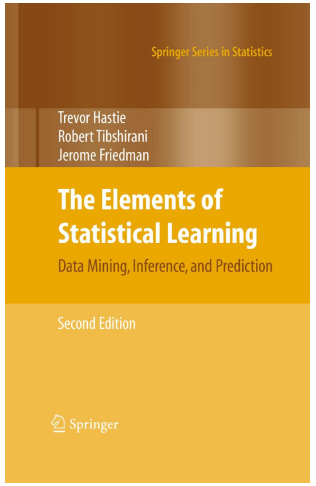


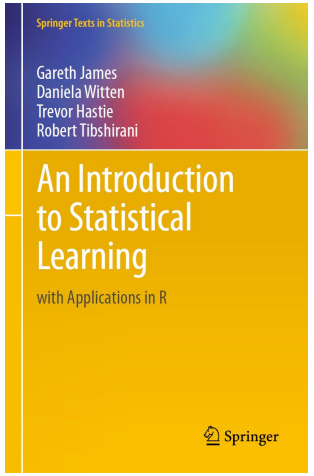


Rodríguez-Pérez, Raquel, and Jürgen Bajorath. "Interpretation of Machine Learning Models Using Shapley Values: Application to Compound Potency and Multi-Target Activity Predictions." Journal of Computer-Aided Molecular Design 34, no. 10 (October 1, 2020): 1013–26..

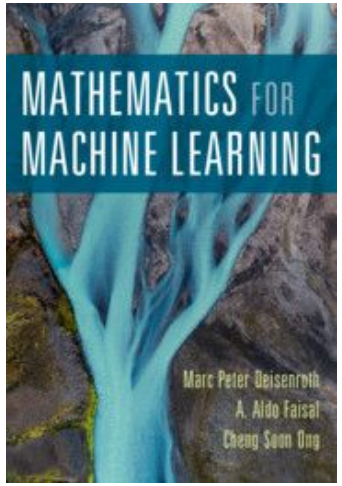
Resources for learning about machine learning

ESL and ISL: From a frequentist view (almost)

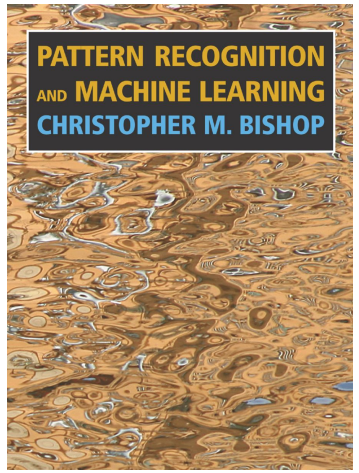


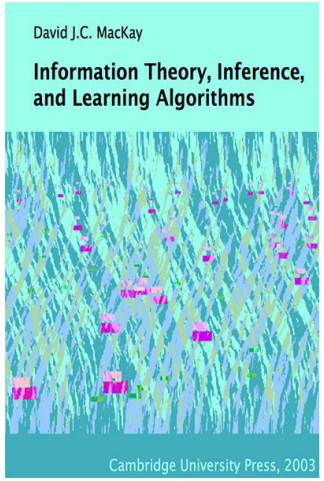


Mathematical foundations

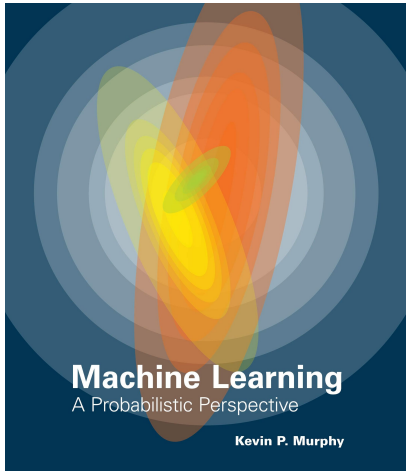


PRML and ITILA: From a Bayesian view



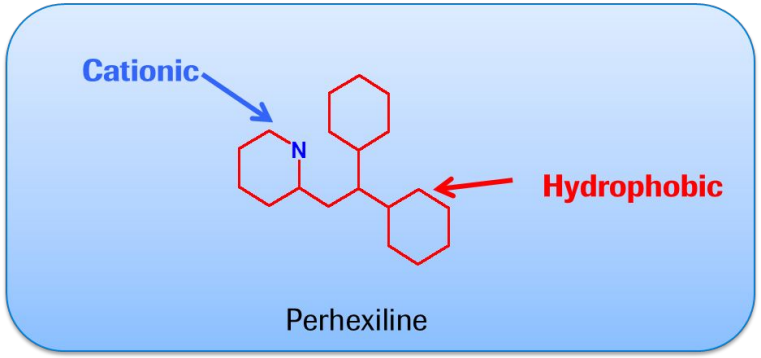


MLaPP: Application oriented, more accessible, and balanced views

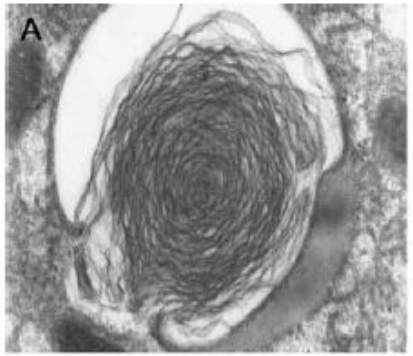


Drug-induced phospholipidosis is correlated with amphiphilicity

- 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.

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

$\vec{A}$: 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

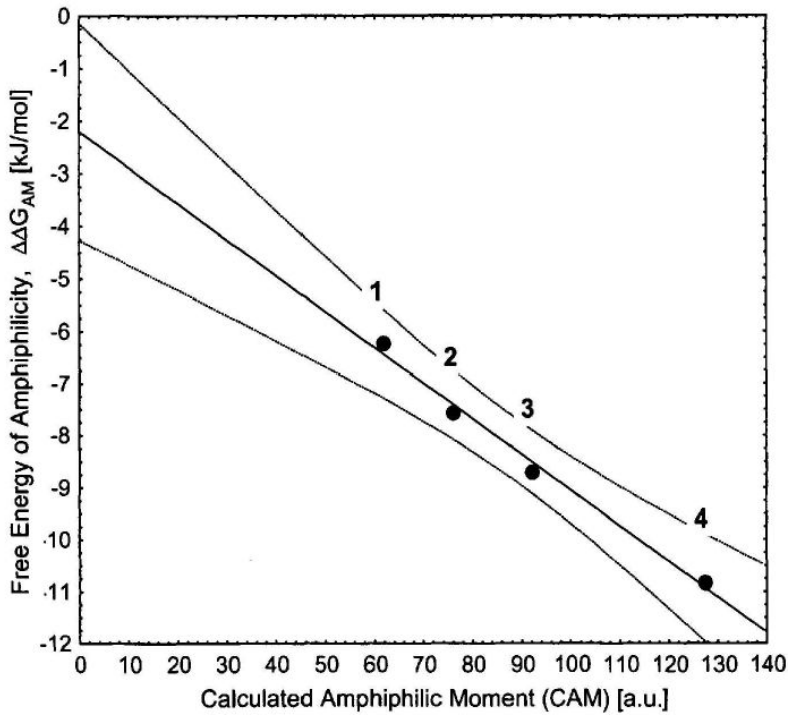
$\vec{\alpha}_i$: the hydrophobic/hydrophilic contribution of atom/fragment *i*

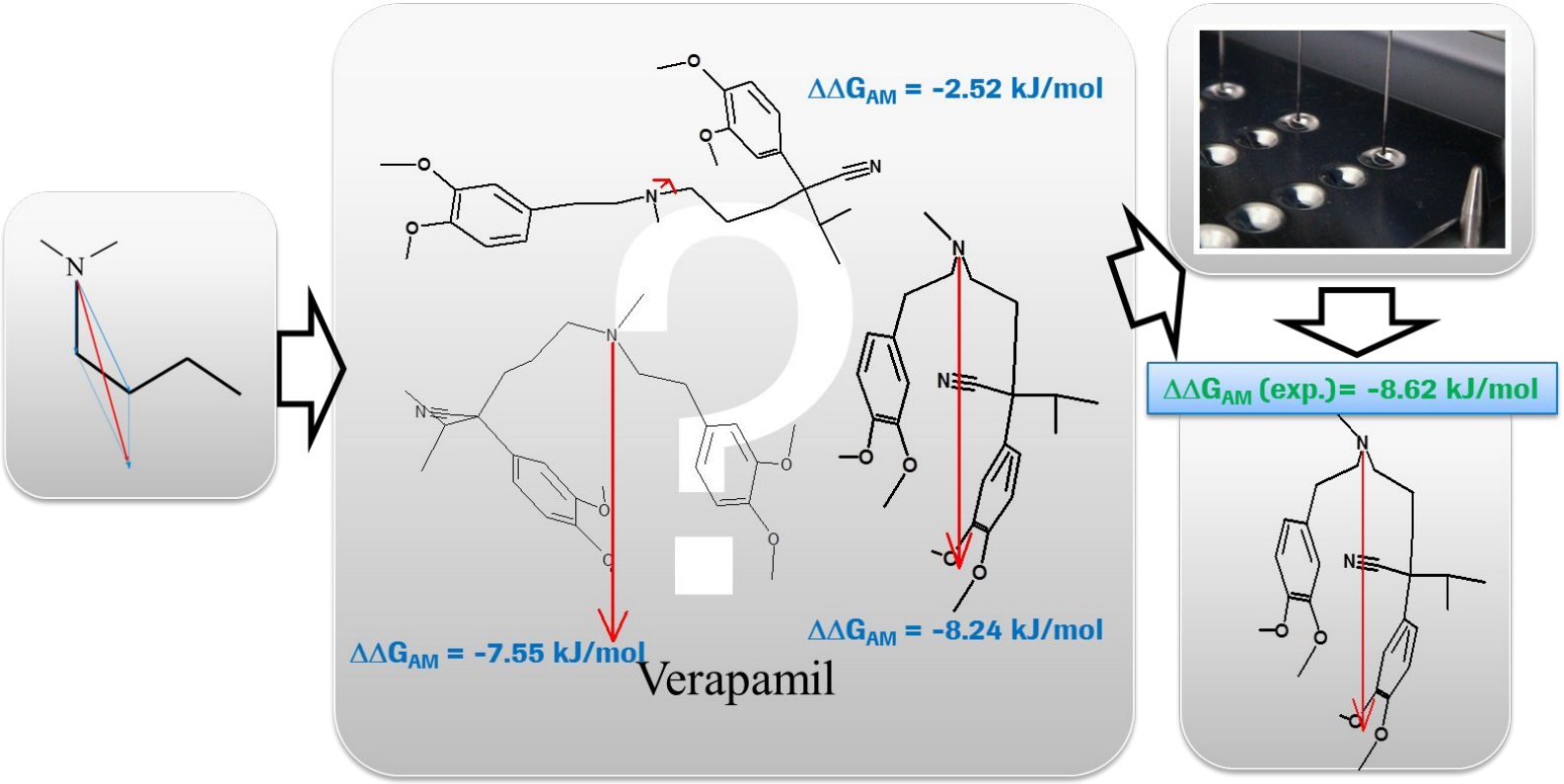
Fischer *et al.* (Chimia 2000) discovered that it is possible to predict the amphiphilicity property of druglike molecules by calculating the amphiphilic moment using a simple equation.

***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)

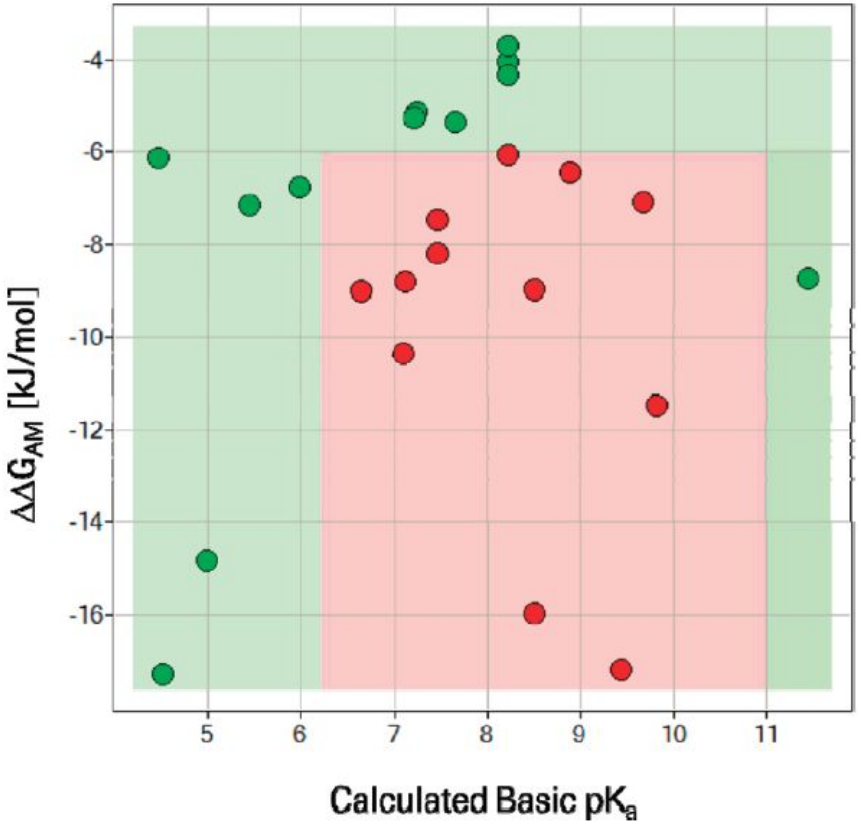




Iterative model building, experimentation, and model refining led to the predictive tool CAFCA

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 a positive PLD response 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).
- **Generally, some safety liabilities, despite complex underlying biological and chemical mechanisms, can be predicted by molecular modelling well, sometimes with surprisingly elegant models!**

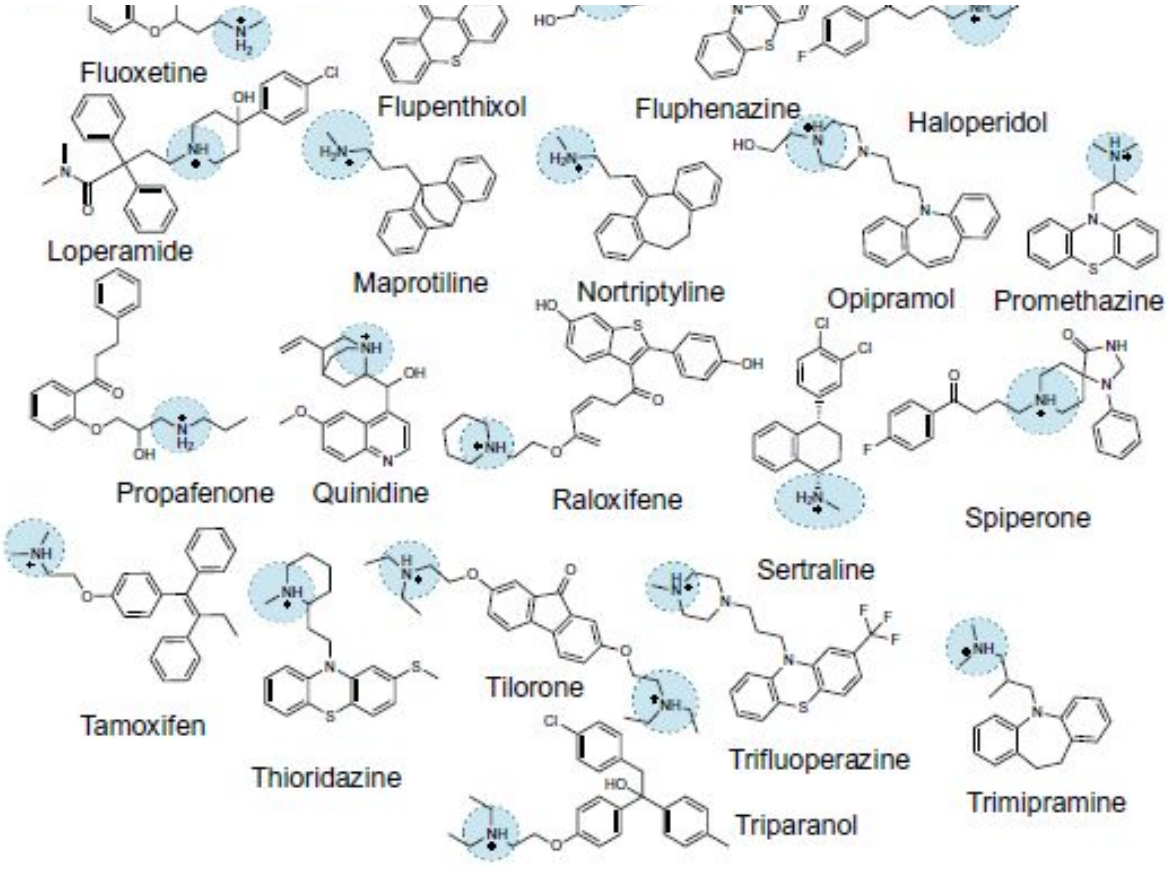
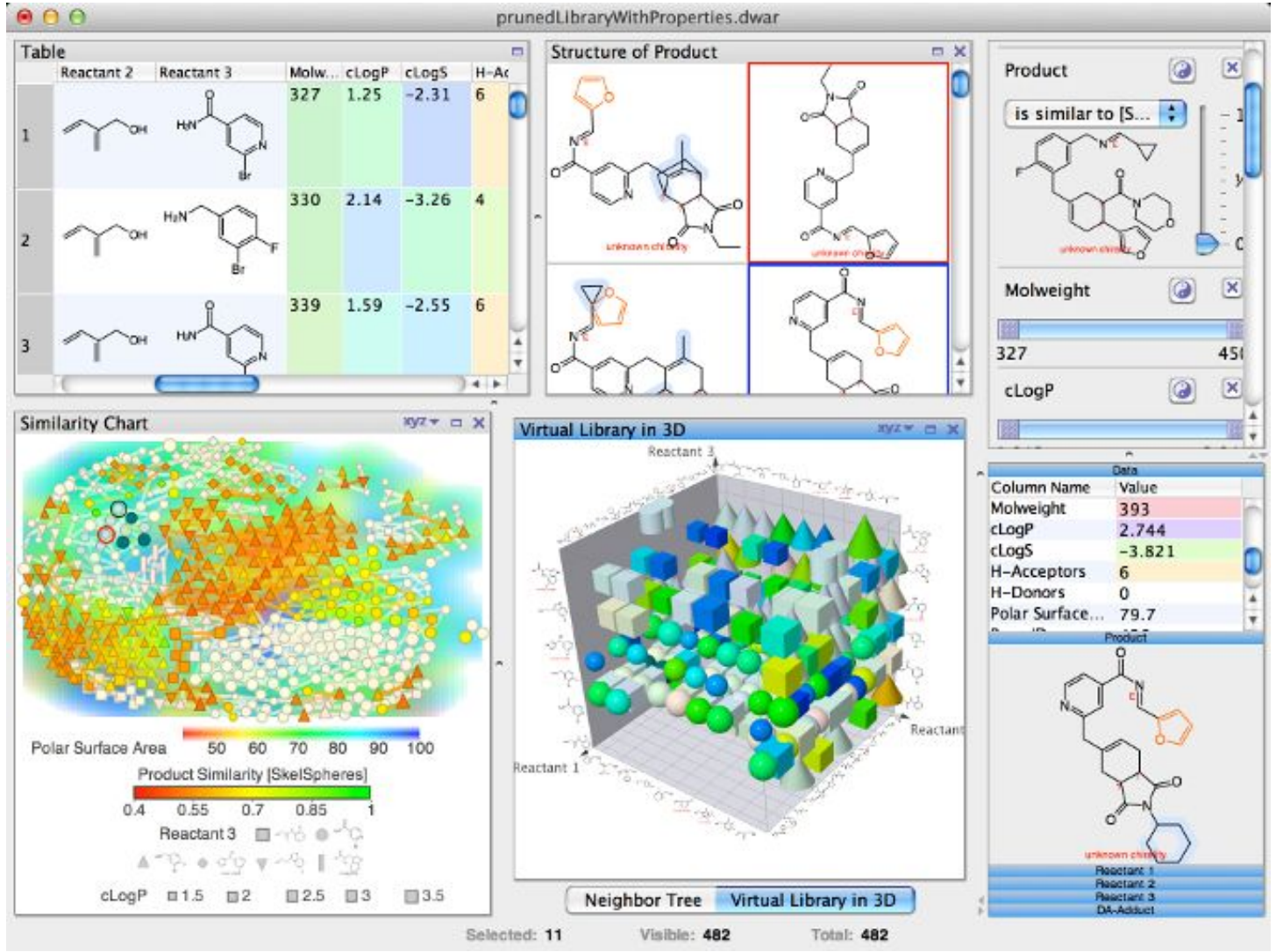


Fig. 1. Representative examples of CADs that are identified in SARS-CoV-2 drug repurposing screens.

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.

DataWarrior: an open-source program for data visualization and analysis with chemical intelligence



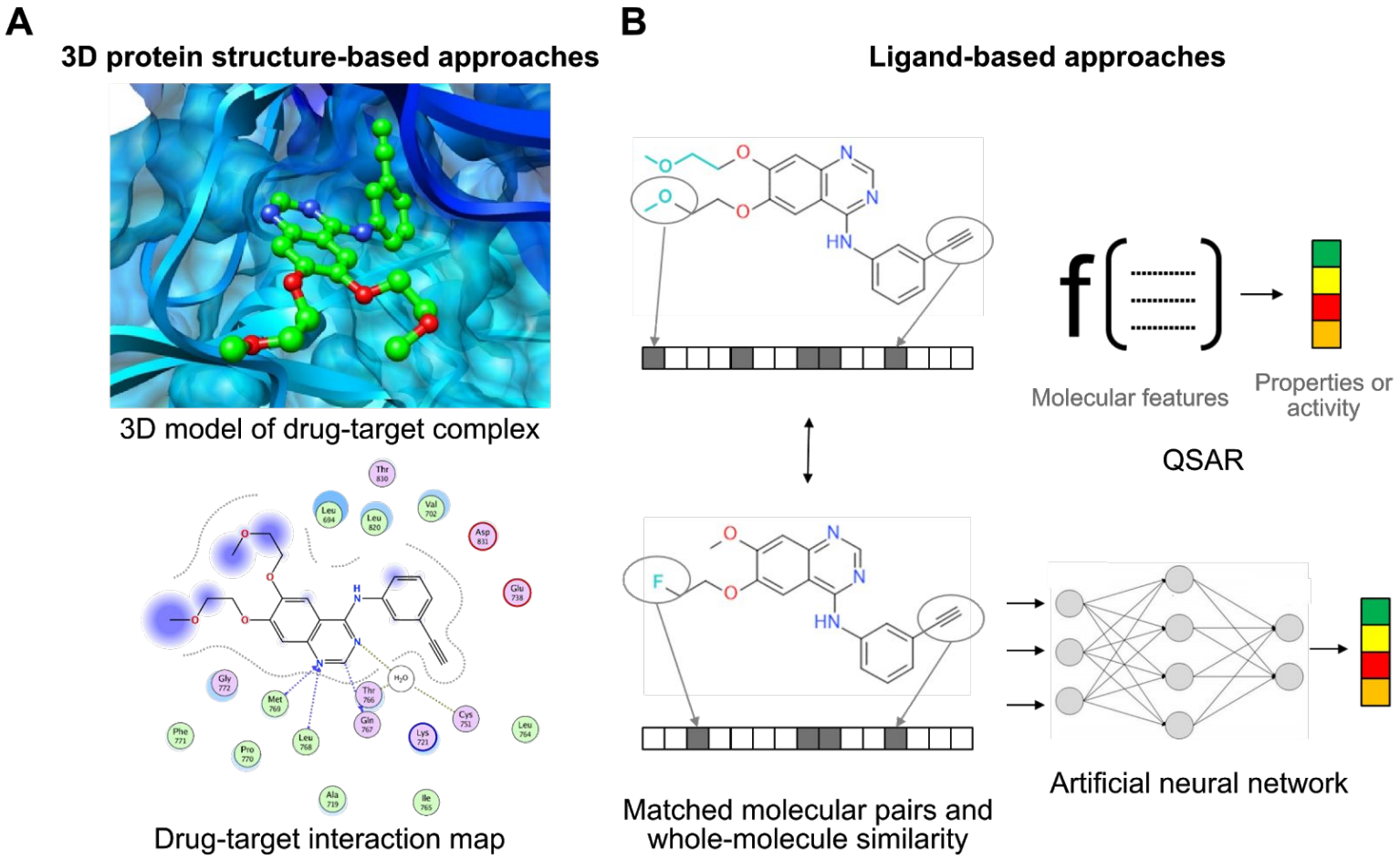
DataWarrior was and still is developed at Actelion/Idorsia Pharmaceuticals Ltd.

Selected subset of functionalities

- Molecular descriptor calculation
- Similarity calculation
- Compound clustering
- Docking
- …

Thomas Sander, Joel Freyss, Modest von Korff, Christian Rufener. DataWarrior: An Open-Source Program For Chemistry Aware Data Visualization And Analysis. J Chem Inf Model 2015, 55, 460-473, doi 10.1021/ci500588j

Summary of molecular modelling



Today we learned ligand-target interaction and **molecular modelling techniques:**

- (A) 3D protein structure-based approaches. An example with docking can be found in the backup slides.
- (B) Ligand-based approaches (similarity search). Another example of amphiphilicity can be found in the backup slides.

Zhang, Jitao David, Lisa Sach-Peltason, Christian Kramer, Ken Wang, and Martin Ebeling. 2020. "Multiscale Modelling of Drug Mechanism and Safety." *Drug Discovery Today* 25 (3): 519–34.

Why modelling molecules is not enough for drug discovery?

The importance of networks

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

Given the reaction

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

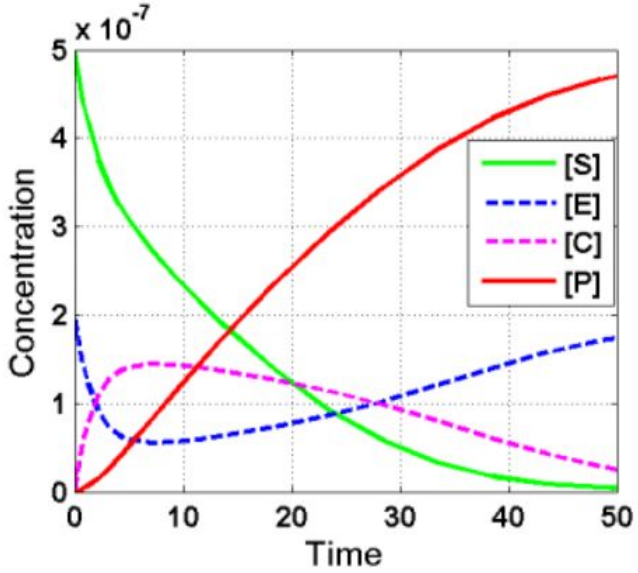
Given the initial values and rate constants

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

According to the law of mass action

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

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

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

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

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

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

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

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

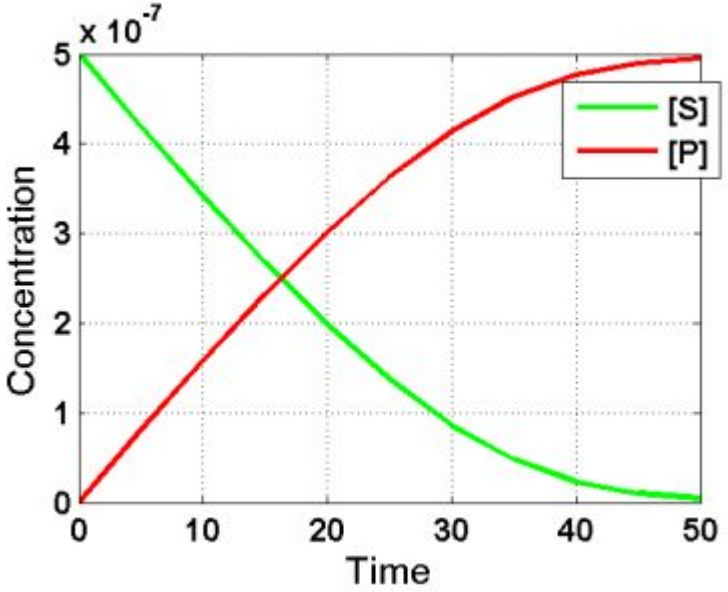
$$K_D \equiv \frac{k_r}{k_f}$$

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

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

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

***RRE simulation of the Michaelis-Menten* model**

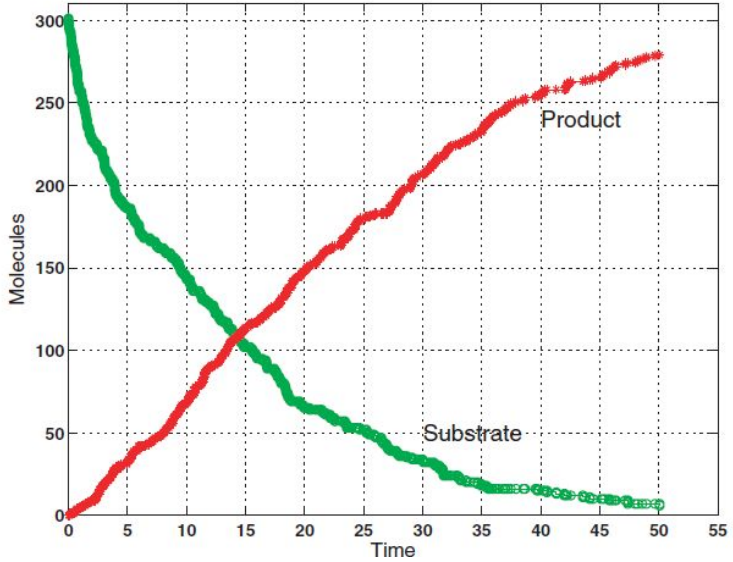


Source: Systems Engineering Wiki (tue.nl)

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 performed in four steps
  - **Initialize** the system with initial conditions
  - 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.
  - Let the randomly selected reaction happen and **update** the time.
  - **Iterate** until substrates are exhausted or simulation time is over.
- Further computation tricks such as 'tau-leaping' by lumping together reactions are possible. The chemical Langevin equation (CLE) replaces further accelerates stochastic simulation by approximating the Poisson distribution with the normal distribution.



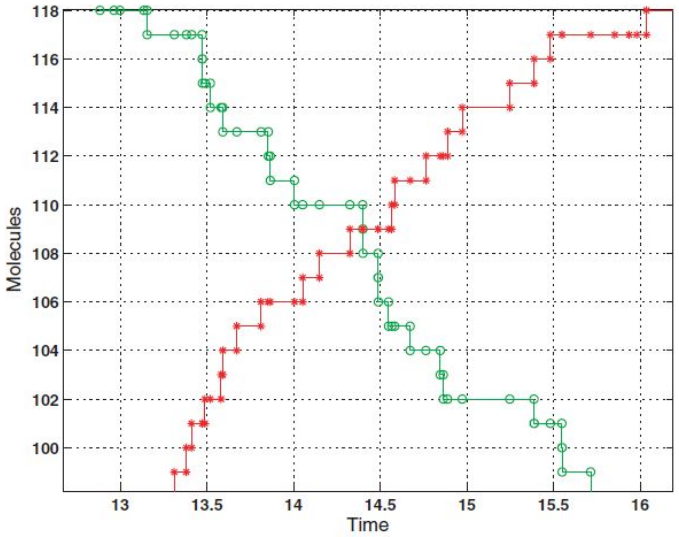
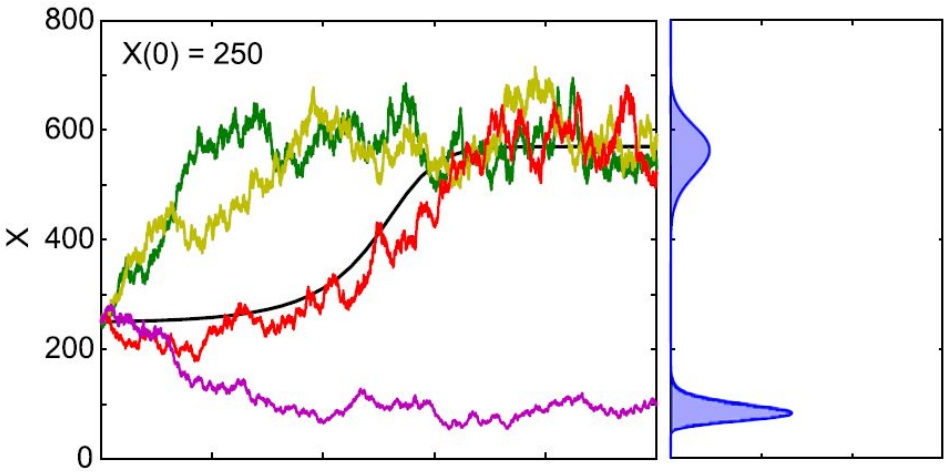
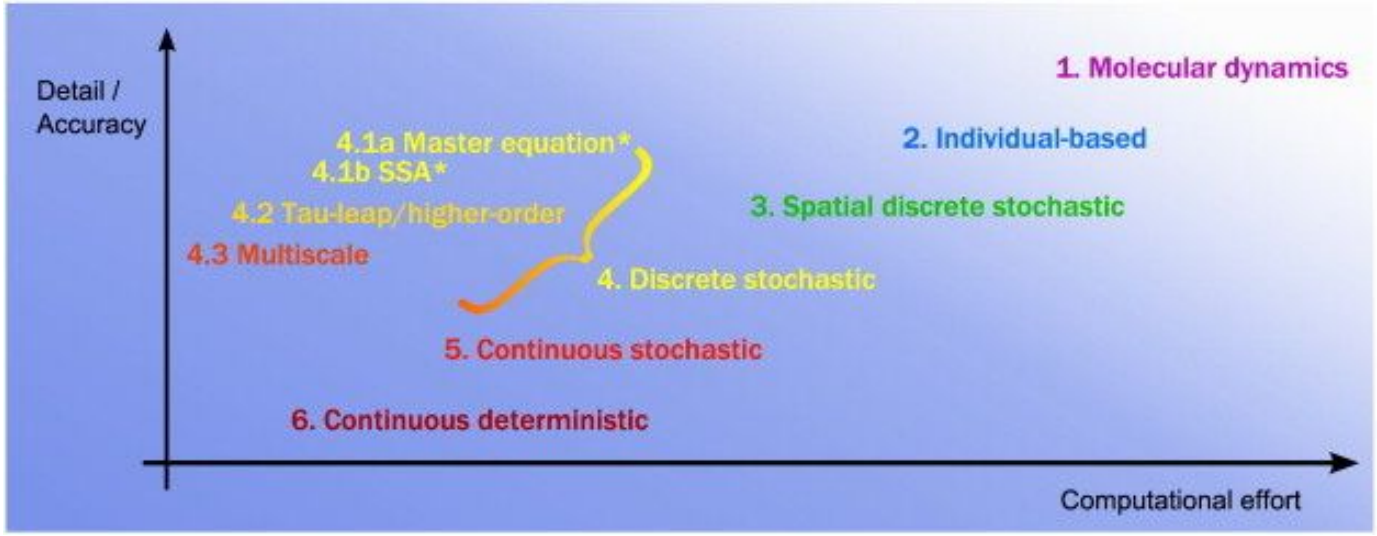


Figure source and further reading: Higham, Desmond J. 2008. "Modeling and Simulating Chemical Reactions." *SIAM Review* 50 (2): 347–68. https://doi.org/10.1137/060666457.

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]	

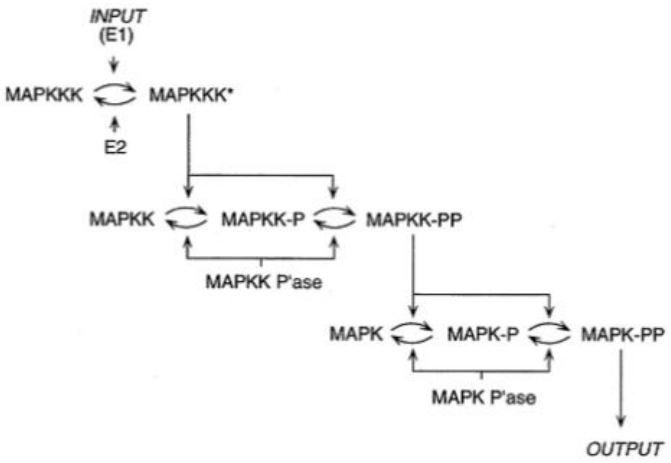
Cat. represents Category from Fig. 2. Abbreviations: SSA, stochastic simulation algorithm; SDE, stochastic differential equation; PDE (R-D), partial differential equation (classical reaction-diffusion equations); ODE, ordinary differential equation.

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

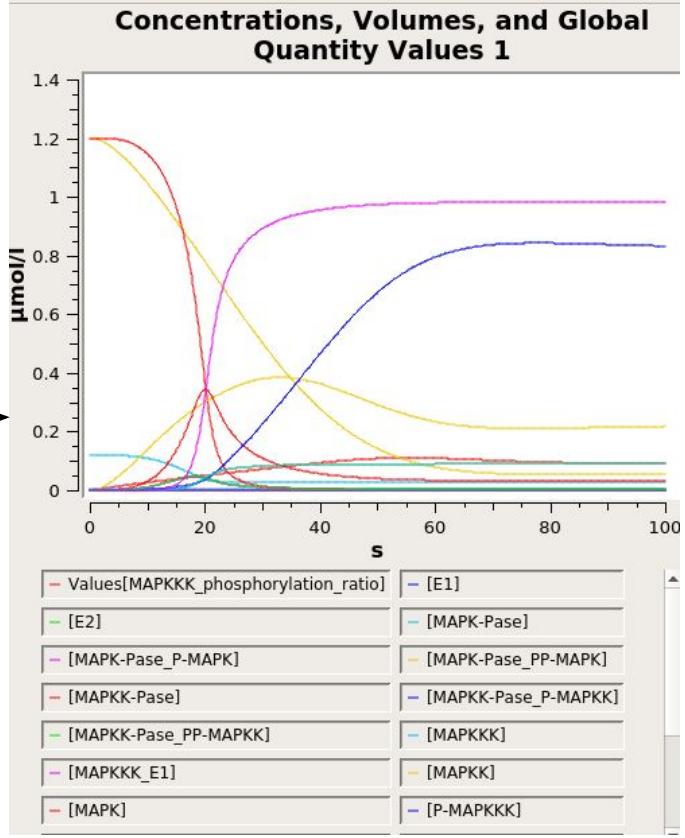
Also see *Stochastic Modelling for Systems Biology* by Darren J. Wilkinson.

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

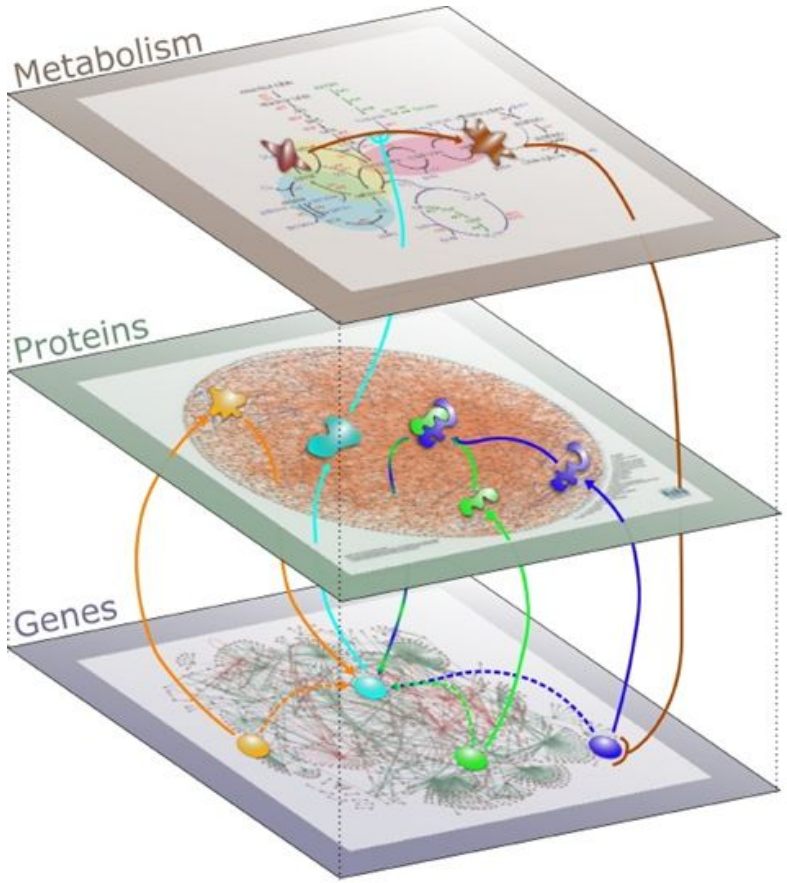


ODE-based simulation of dynamics

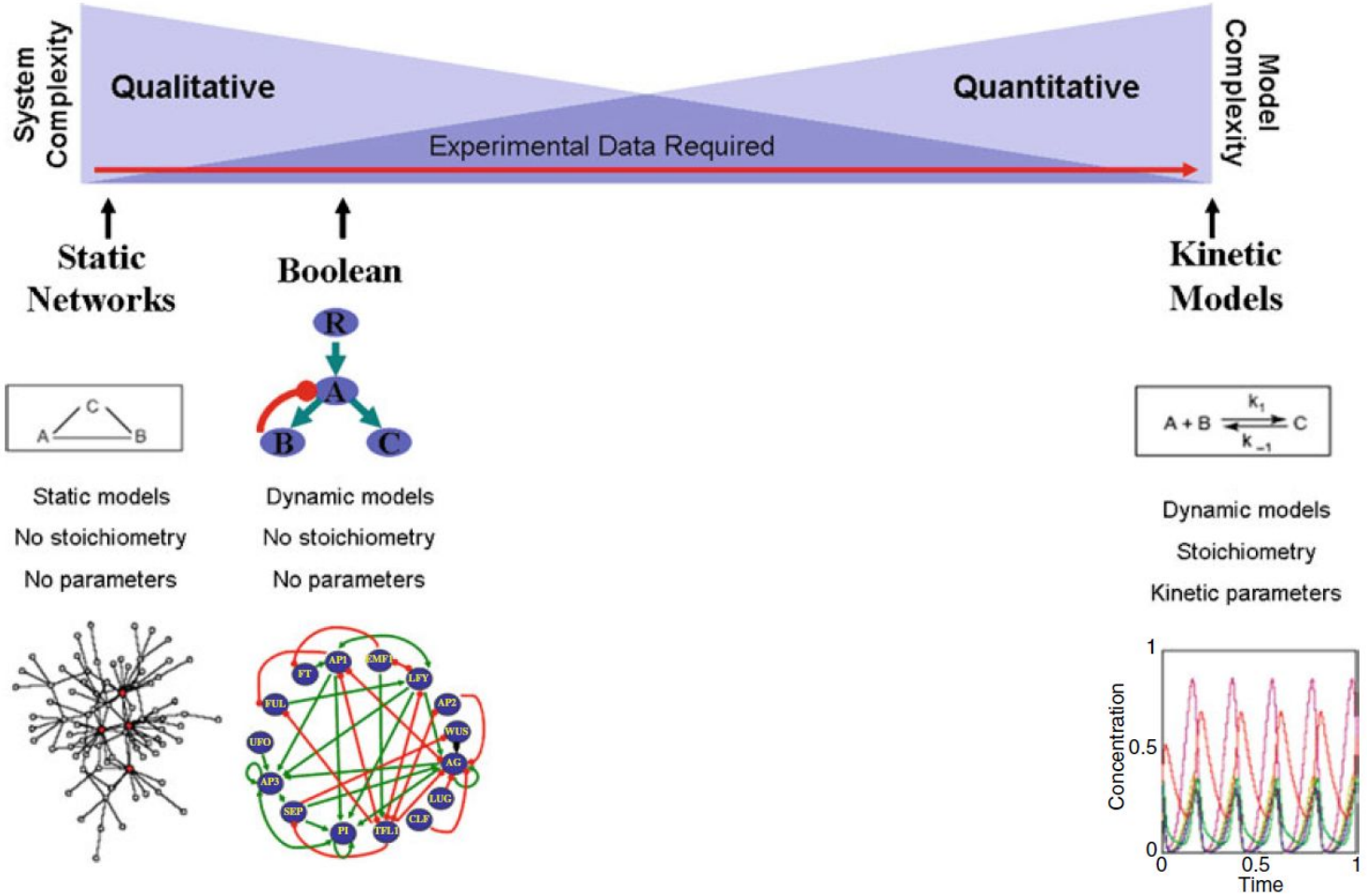
Summary

- **QSAR and machine learning models 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.**

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.