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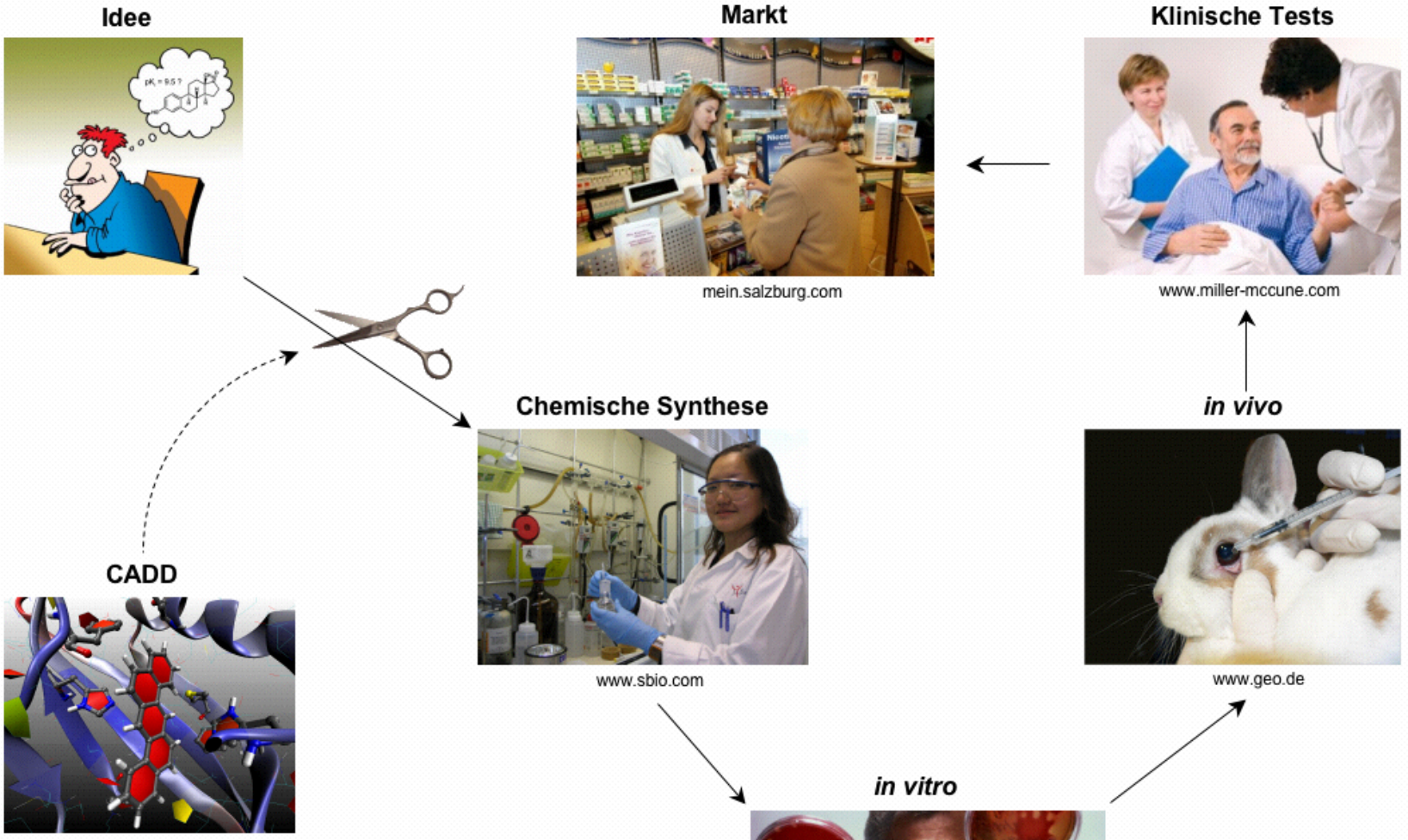
Computational Toxicology:

Computer-gestützte Voraussage des toxischen Potentials von Arzneistoffen und Umweltchemikalien

Ringvorlesung: “Computational Sciences” , Universität Basel, November 2017

Moderne (computer-gestützte) Entwicklung von Arzneistoffen

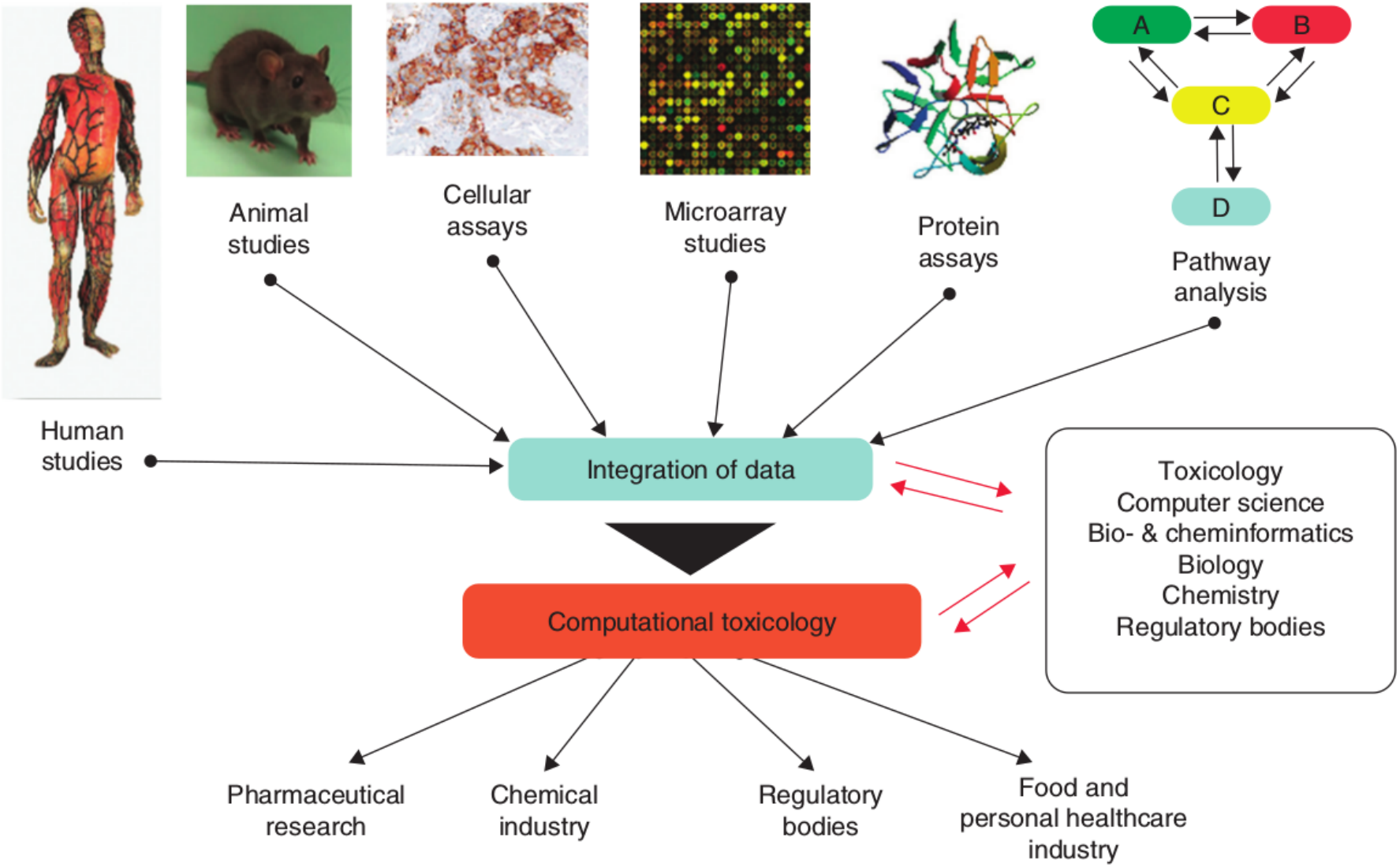


Aus tausenden von Ideen (*in cerebro*, Datenbanken, kombinatorische Chemie, etc.) werden einige hundert Substanzen chemisch synthetisiert und *in vitro* getestet. Eine kleinere Zahl von aussichtsreichen Kandidatsubstanzen werden anschliessend im Tierversuch getestet. Nur wenige Substanzen schaffen es in die klinische Prüfung. Bis eine neu(artig)e Wirksubstanz auf den Markt kommt, sind 10–15 Jahre Forschung & Entwicklung notwendig.

Arzneistoff: Entdeckung / Entwicklung Prozess

Target Discovery	Target Validation	Lead Discovery	Lead Optimization	Pre-clinical ADMET	Clinical Trials
Expression analysis *in vitro* function **Bioinformatics** **Computational chemical biology**	*in vivo* validation **Druggability assesment**	High-Throughput Screening CombChem Fragments **Structure-based design** **Virtual Screening** ***de novo* design**	Traditional Medicinal Chemistry **Rational drug design** **Virtual Screening** **Focused libraries** **Drug-likeness**	*in vitro* / in vivo Animal Studies **Physiologically-based modeling of bioavailability** ***in silico* ADMET** **Computational systems biology**	Phase I (I/II for cancer) Phase II Phase III

Computational Toxicology Methods



Nigsch F. et al. *Expert Opinion on Drug Metabolism & Toxicology (2009)* , 5, 1-14

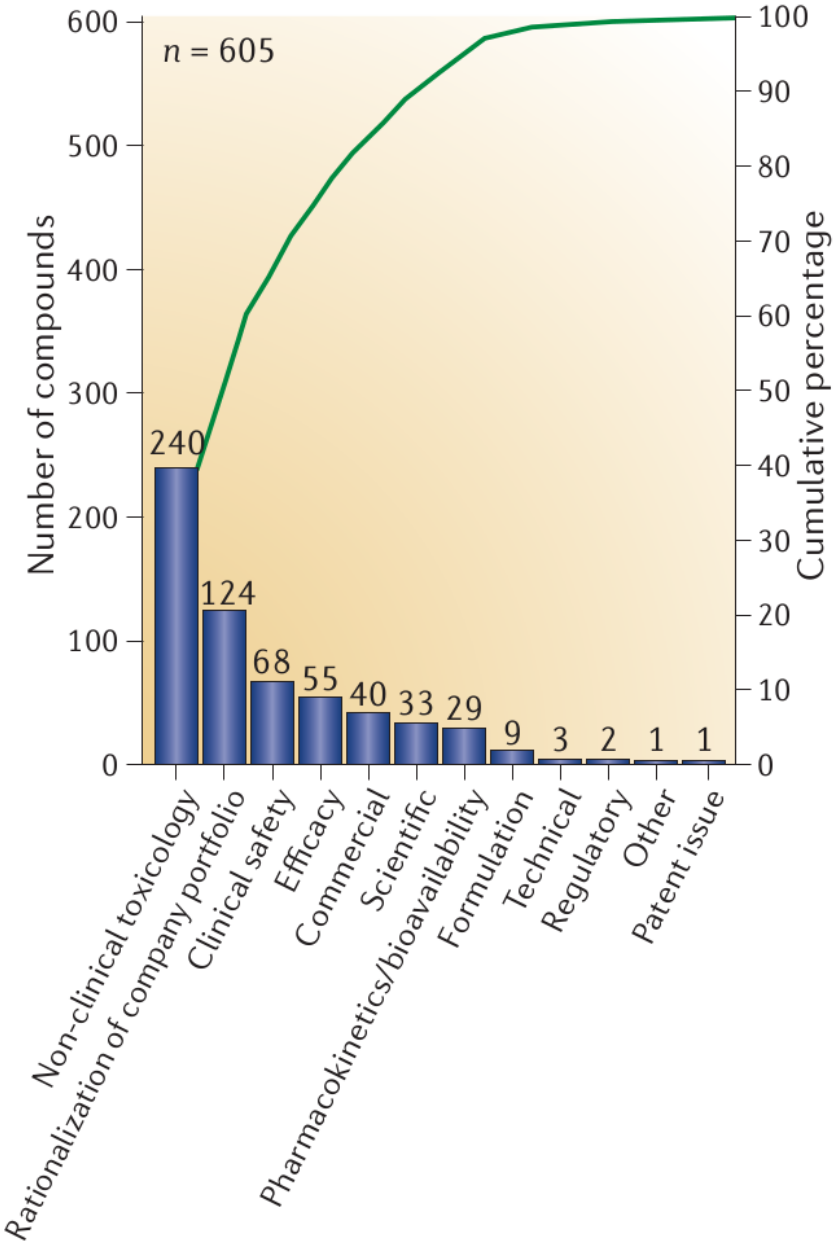
- Every single compound entering production must be throughly tested and characterized:
  - cosmetics (UV filters, fragrances…)
  - additives (polymer, flame retardants…)
  - agrochemicals
  - drugs
  - colorants & dyes

- 3R (reduction, replacement, refinement)

- Regulatory needs: EC, EPA, BLV… (REACH)

- knowledge gathered can be used to rationally **explain** and **avoid toxic phenomena**

- drug attrition rates



Waring M.J. et al. *Nature Reviews: Drug Discovery* (2015), 14, 475.

Side effect (or adverse effect) of drugs and chemicals

May occur as a result of the unwanted interaction between the compound and bio(macro)molecules involved in:

- Biosynthesis
- Signal transduction
- Transport
- Storage
- Metabolism

the nature of such an interaction can be **specific or unspecific**

biochemical pathway/intermediary metabolism → organelle → cell → organ → organism

Routes of exposure: can the compound get to the possible site of action?

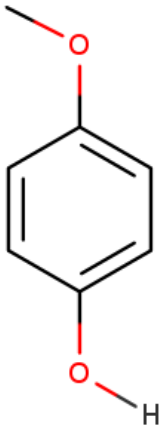
oral : most frequent and best studied because of pharma industry

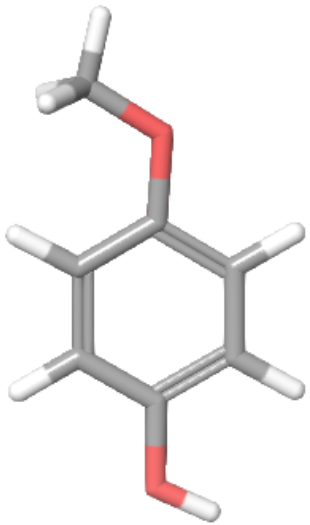
Lipinski's rule of 5

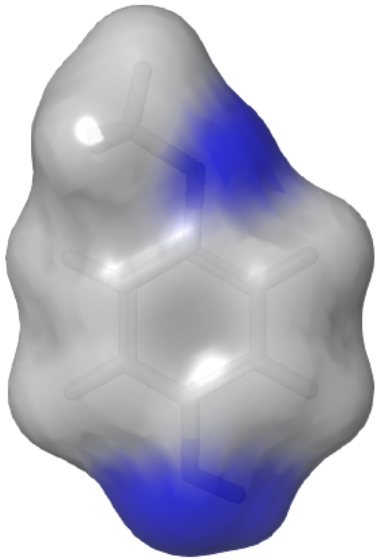
MW < 500
nHB-donor ≤ 5
nHB-acceptor ≤ 10
LogP < 5

Veber rules

- PSA < 140 Å^2
- nRotBond < 10







MW = 124, nHBdon = 1, nHBacc = 2, LogP = 1.2, PSA = 31 Å^2, nRotBond = 1

skin : transdermal patches (hormonal or opioid analgetic), cosmetics (shower gel, sunscreens), textile (dyes), plastics (e.g BPA from cash & bills)

inhalation : airborne particles (fumes, fentanyls), volatile chemicals, gases…

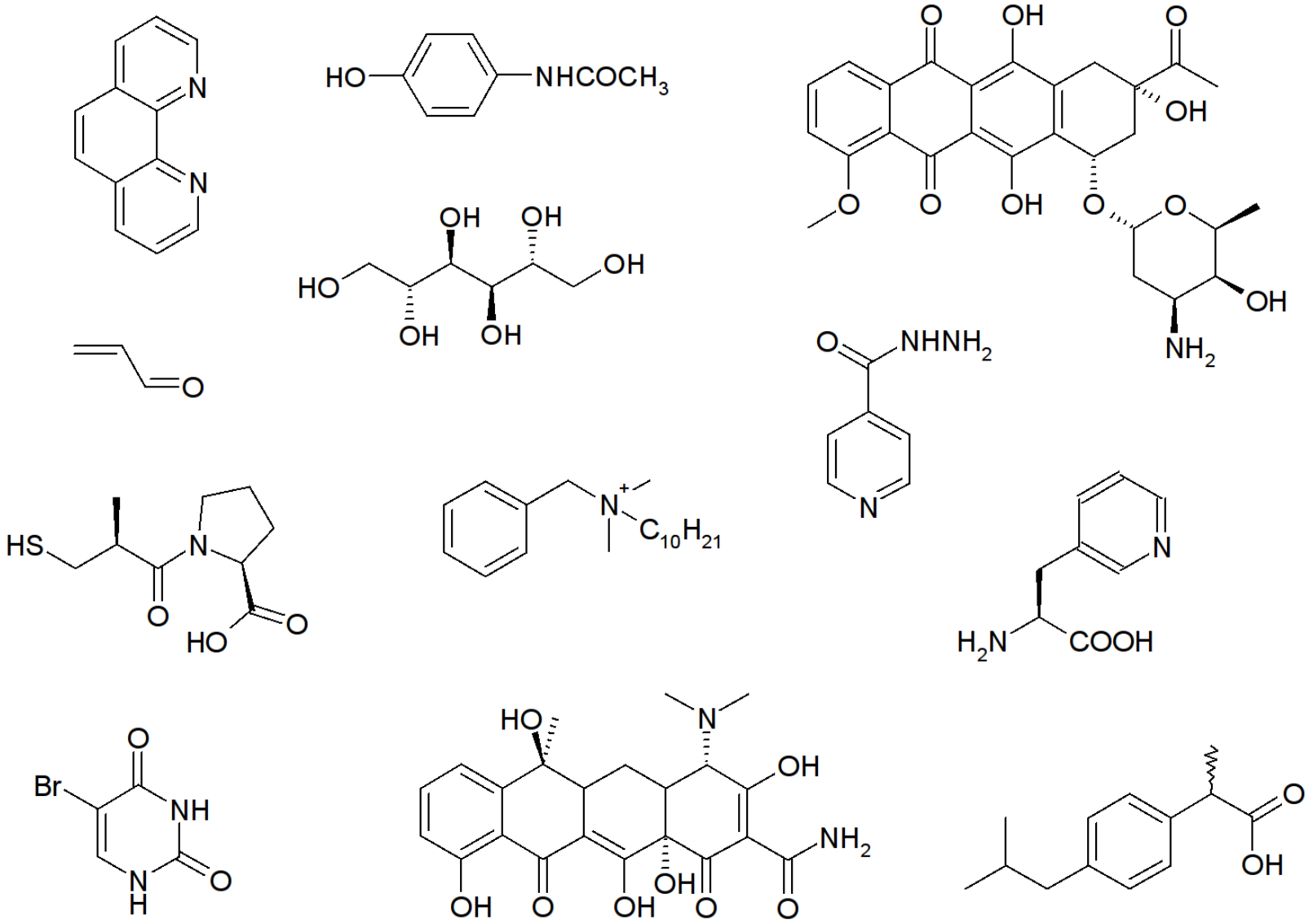
special : ocular, bucal…

The causal relationship has to be clear – a model has to be able to explain the the side effect and detect/predict similar behavior for new entities:

- ***reactive functional groups*** – interaction with biomacromolecules forming covalent bonds (electrophilicity, HOMO/LUMO)
- ***metabolically unstable groups, fragments, arrangements***
- ***chelating groups*** – interaction with trace elements (Ca, Fe)
- ***metabolites*** - interaction with biomacromolecules, off-target binding
- ***pharmacokinetics*** – compartments, accumulation, blood-brain barrier, placental barrier
- ***surface activity*** – cell lysis (saponins)
- ***isomery R/S, cist/trans*** – active/inactive ingredient
- ***off-target binding*** – anti-target Nr.1 hERG K+ (human ether-à-go-go related gene postassium) channel, cytochromes (inhibition/transformation), endocrine system

Many aspects can be detected by simple looking & thinking!

Comparative (Recognition) approach: shape, polarity, chelating capabilities, solubility, similarity to primary metabolites, Lipinski and Veber rules...



Database searching (1):

The Binding Database: compounds with activities, http://www.bindingdb.org/bind/index.jsp

ChEMBL Database: > 620k compounds, > 2.4M activities, http://www.ebi.ac.uk/chembldb/

TOXNET: general toxicity database, many sub databases, http://toxnet.nlm.nih.gov/index.html
sub-databanks:

ChemIDplus	Chemical Identification/Dictionary
HSDB	Hazardous Substances Data Bank
CCRIS	Chemical Carcinogenesis Information
CPDB	Carcinogenic Potency Database
GENETOX	Genetic Toxicology Data
IRIS	Integrated Risk Information, quantitative human carcinogenic/hazard data
ITER	International Toxicity Estimates for Risk
LactMed	Drugs and Lactation Database
TRI	Toxics Release Inventory
TOXMAP	Environmental Health e-Maps
Haz-Map	Occupational Exposure/Toxicology
Household Products	Health & Safety Information on Household Products

Database searching (2):

ToxCast Program - http://epa.gov/ncct/toxcast/

DSSTox - http://www.epa.gov/dsstox_structurebrowser/

Acute Toxicity Database - for Aquatic Species http://www.cerc.usgs.gov/data/acute/acute.html

ECOTOX - toxicity data derived predominantly from peer-reviewed literature for aquatic organisms, terrestrial plants and wildlife species, http://cfpub.epa.gov/ecotox/

SKIN DEEP - http://www.cosmeticsdatabase.com/index.php

Drug-Induced Toxicity Related Proteins Database
http://bioinf.xmu.edu.cn/databases/DITOP/index.html

PAN Pesticide Database - http://www.pesticideinfo.org/

ACuteTox - Predicting Human Acute Toxicity, http://www.acutetox.eu/

ZINC - free database of commercially-available compounds for virtual screening
http://zinc.docking.org/choose.shtml

Chemical Structure Lookup Service - 46 million unique structures
http://cactus.nci.nih.gov/cgi-bin/lookup/search

EC inventory – a database of the existing chemical substances
http://ecb.jrc.ec.europa.eu/qsar/information-sources/ec_inventory/

Das Modell!

„It can scarcely be denied that the supreme goal of all theory is to make the irreducible basic elements as simple and as few as possible without having to surrender the adequate representation of a single datum of experience."

Albert Einstein 1933

z.B.:

Separates Kleinmolekül:

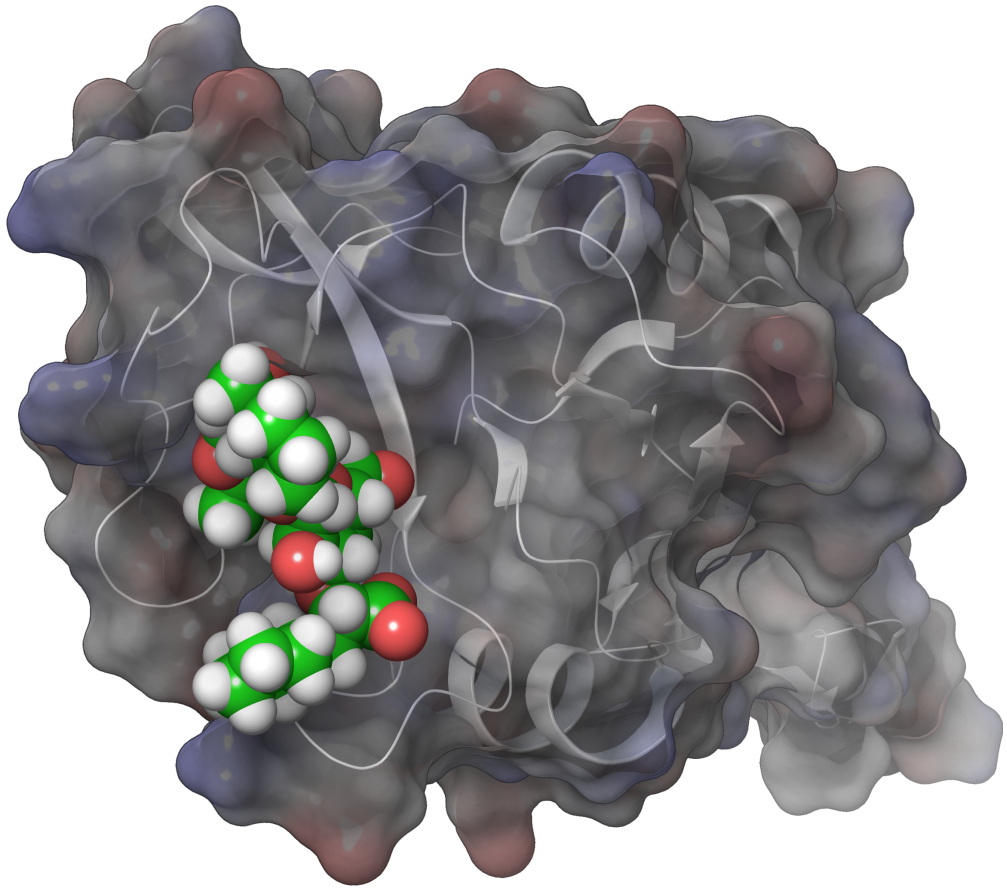
- Physikochemische Eigenschaften
- Deskriptoren
- Bioverfügbarkeit

Protein-Ligand Komplex:

- Wechselwirkungsenergie
- Interne Spannung

Protein-Ligand Komplex im Wasser:

- Bindungsaffinität
- Halbwertszeit (Residenzzeit)



Nur ein Modell!

"This is not a pipe" By René Magritte, 1898-1967

Nie vergessen: dass was wir sehen ist nur ein Modell, nicht die Realität!

Modeling = Creating a (computer) model for observed phenomena at various levels

- qualitative models: simple rule based, decision trees (e.g. *if soluble and contains a C=N-OH functional group…)*, expert systems, artificial intelligence
- quantitative models (QSARs):

$$f(\mathbf{x}) = \text{(side) effect} \overset{?}{\rightarrow} \text{toxicity}$$

- Where **x** can be:

  1-dimensional information, e.g. LogP, molecular weight

  2-dimensional information, e.g. connectivity, branched vs. linear

  3-dimensional information, e.g. conformation of a ligand

  multi-dimensional information (multiple conformers, protonation states)

- Setubal principles:

  defined endpoint, unambiguous algorithm, defined domain of applicability, appropriate measures of goodness-of-fit, robustness and predictivity, mechanistic interpretation

Free modeling resources (1):

OpenTox (interoperable predictive toxicology framework) - http://www.opentox.org/

LAZAR - http://lazar.in-silico.de/

Molinspiration - gives Nuclear Receptor Ligand likeness (also Kinase, GPCR and Ion Channel Ligand likeness), http://www.molinspiration.com/cgi-bin/properties

QSPR/OCHEM - build online QSARs, http://qspr.eu/

European Joint Research Center (Ispra, Italy) :
DART - designed for the ranking of chemicals according to their environmental and toxicological concern
Toxtree - places chemicals into categories and predicts various kinds of toxic effect by applying decision tree approaches
Toxmatch - encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and read-across,

Virtual Computational Chemistry Laboratory - property calculations
http://www.vcclab.org/

Free modeling resources (2):

EPI Suite - suite of physical/chemical property and environmental fate estimation, US EPA, http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

OncoLogic® - A Computer System to Evaluate the Carcinogenic Potential of Chemicals, http://www.epa.gov/oppt/sf/pubs/oncologic.htm

T.E.S.T. - estimate acute toxicity using the QSAR methodologies

http://www.epa.gov/nrmrl/std/cppb/qsar/#TEST

OECD QSAR Toolbox - tool for profiling mechanisms, chemical grouping and readacross, http://www.oecd.org/env/ehs/risk-assessment/theoecdqsartoolbox.htm

CAESAR – Computer Assisted Evaluation of Industrial chemical substances http://www.caesar-project.eu/

Commercial modeling resources:

ADMET predictor - http://www.simulations-plus.com

TOPKAT from Accelrys - http://www.accelrys.com

Pallas - http://www.compudrug.com

Derek - http://www.lhasalimited.org

MultiCASE - http://www.multicase.com

MDL QSAR - http://www.symyx.com

BioEpisteme - http://www.prousresearch.com

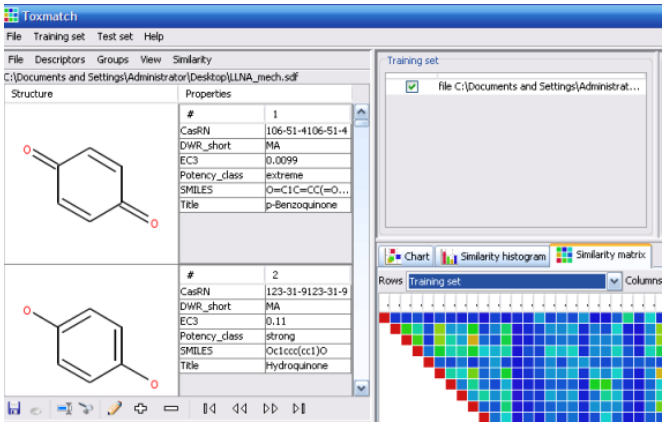
ACD ToxSuite - http://www.acdlabs.com

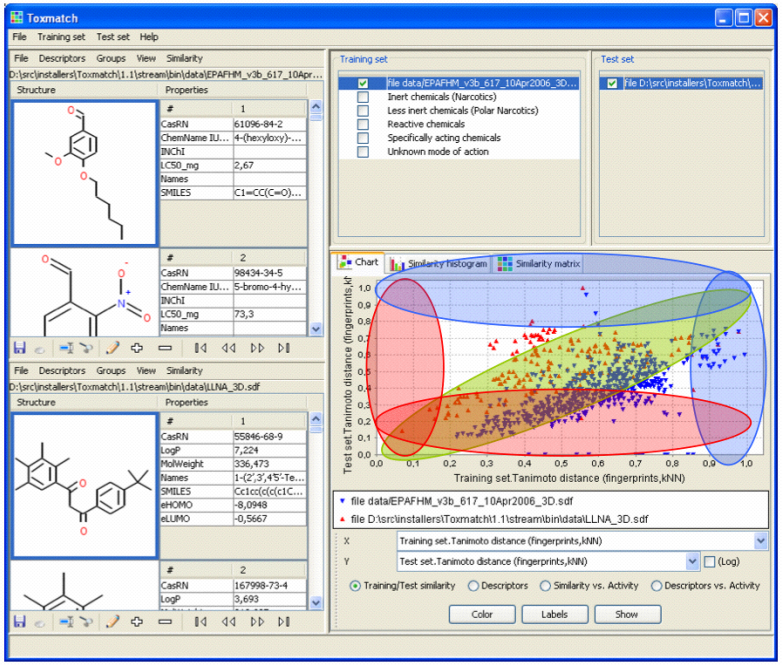
OASIS TIMES - http://www.oasis-lmc.org

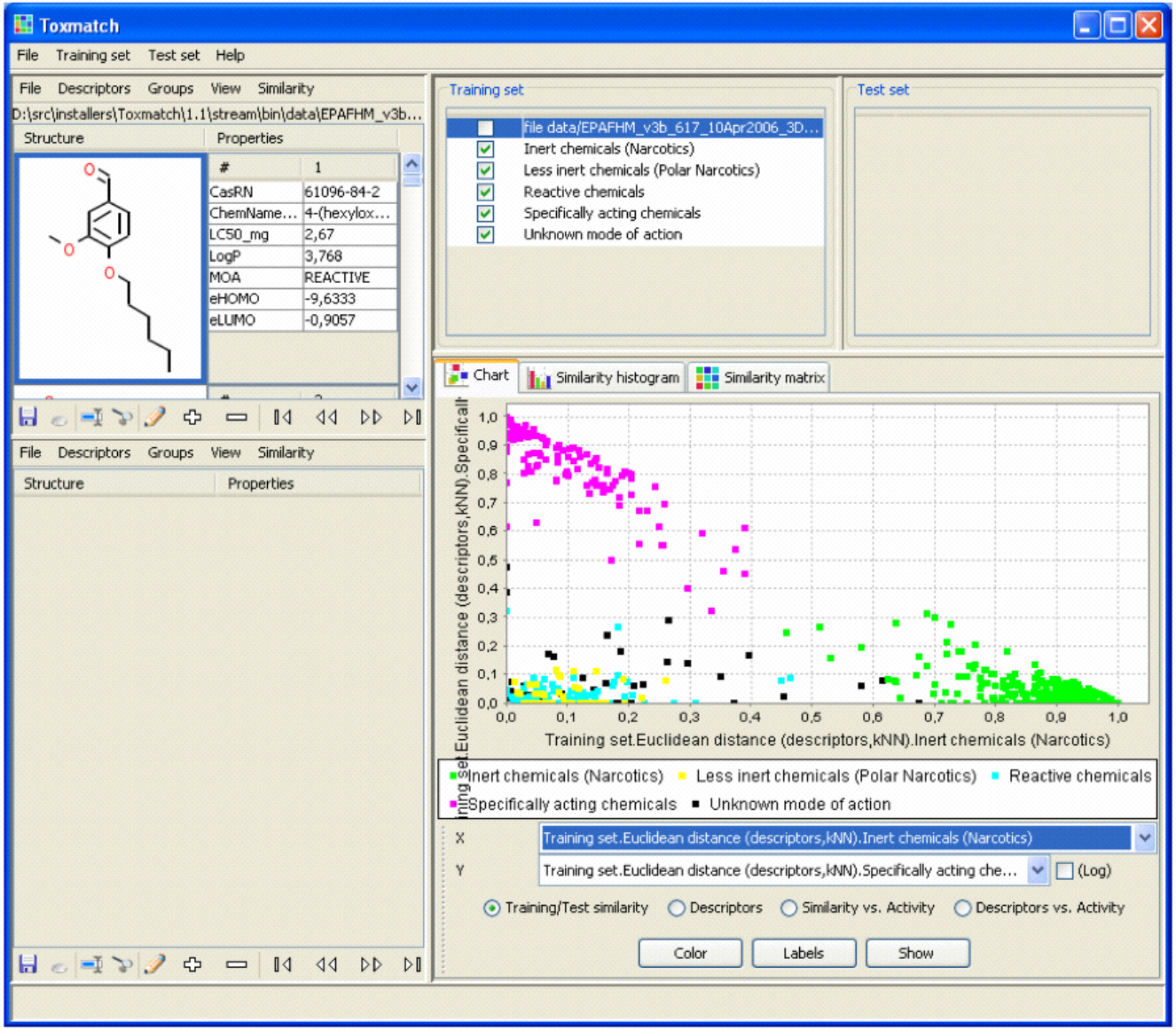
Molcode Toolbox - http://molcode.com

...

Statische Modellierung — ToxMatch

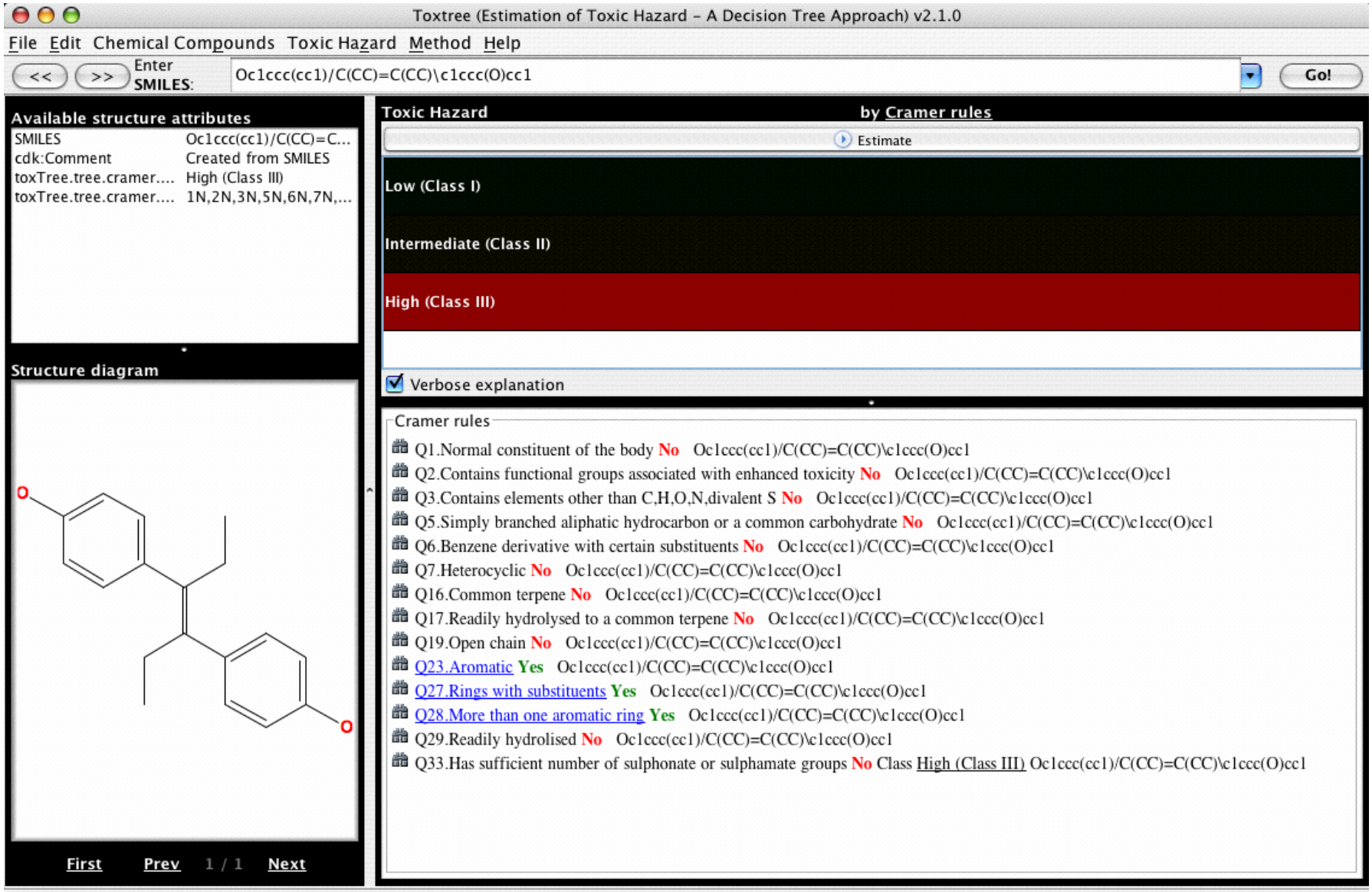






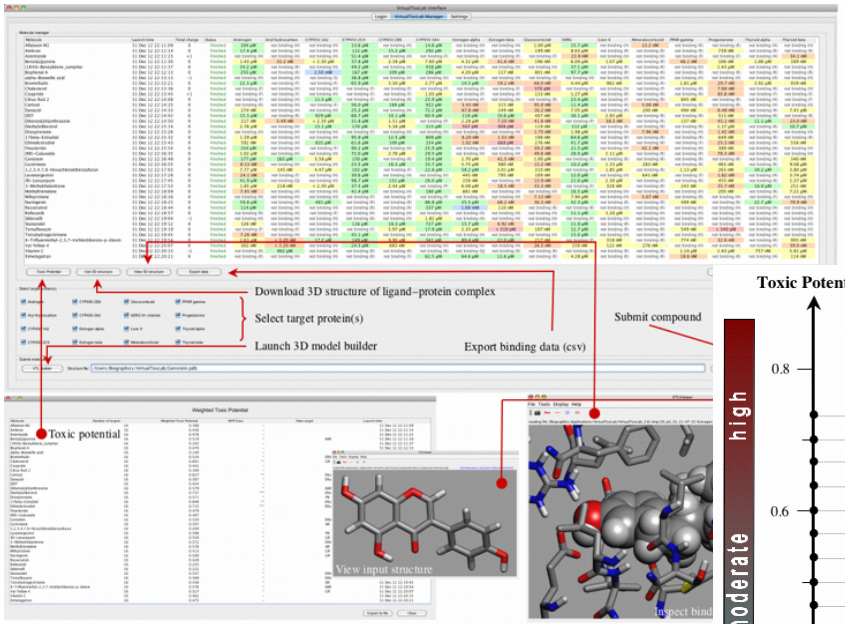
☞ http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxmatch

Expertensysteme — ToxTree



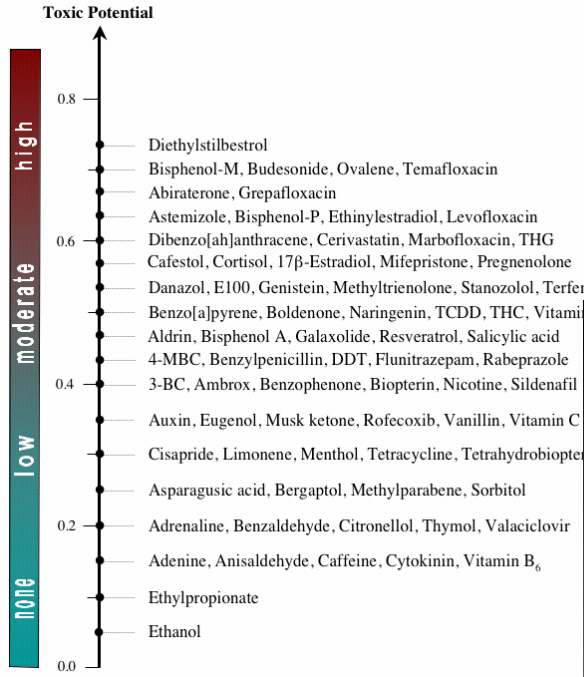
☞ http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree

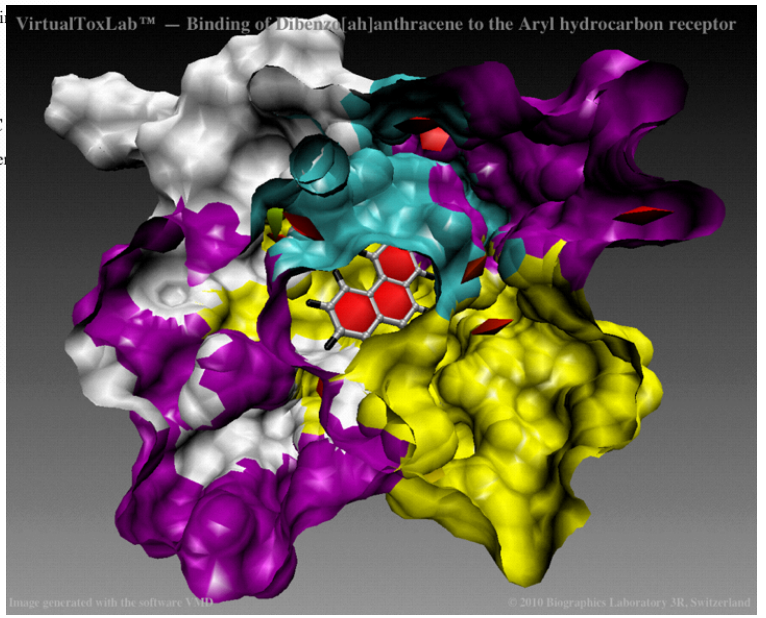
"Ab initio" 3D-Modellierung — VirtualToxLab



☞ http://www.virtualtoxlab.org

Das toxische Potential einer Substanz wird nicht durch Vergleich mit anderen Verbindungen (deren Toxizität bekannt ist) abgeleitet, sondern durch die **Simulation** und **Quantifizierung** von **Protein–Ligand-Wechselwirkungen** auf atomarer Ebenen abgeschätzt. Proteine, welche unerwünschte Effekte vermitteln, werden als "off-targets" bezeichnet.



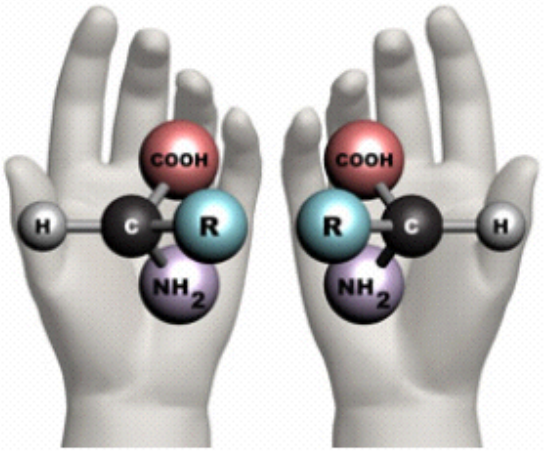


Emil Fischer — „Vater" des Molecular Modeling

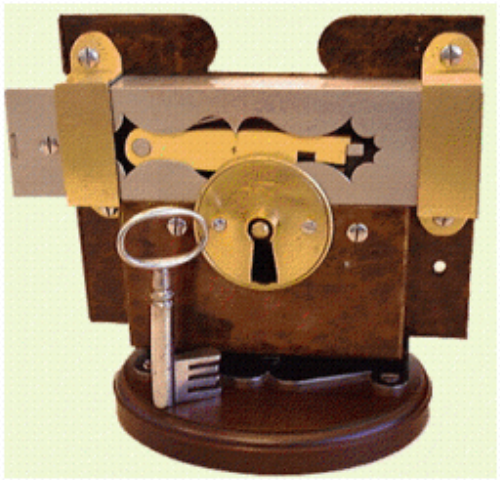
nobelprize.org

Schloss-Schlüssel-Prinzip (1894)

Emil Fischer (1852–1919)
1902: Nobelpreis für Chemie

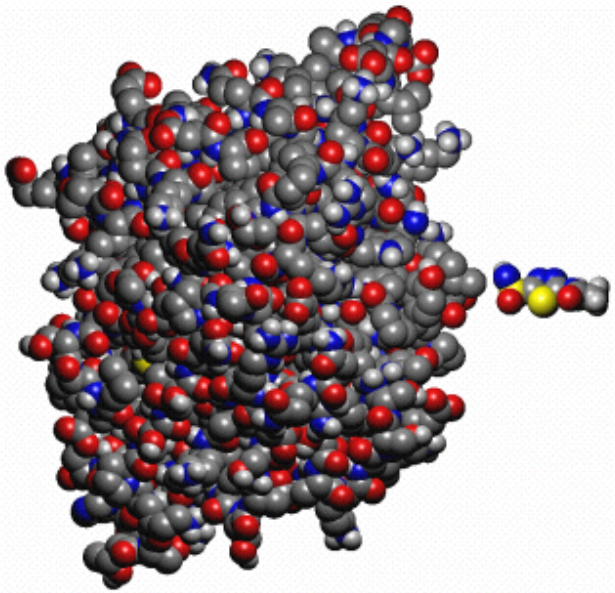


enantiomorphic.blogspot.com



oldlockandkeyco.co.uk

muarchives.missouri.edu

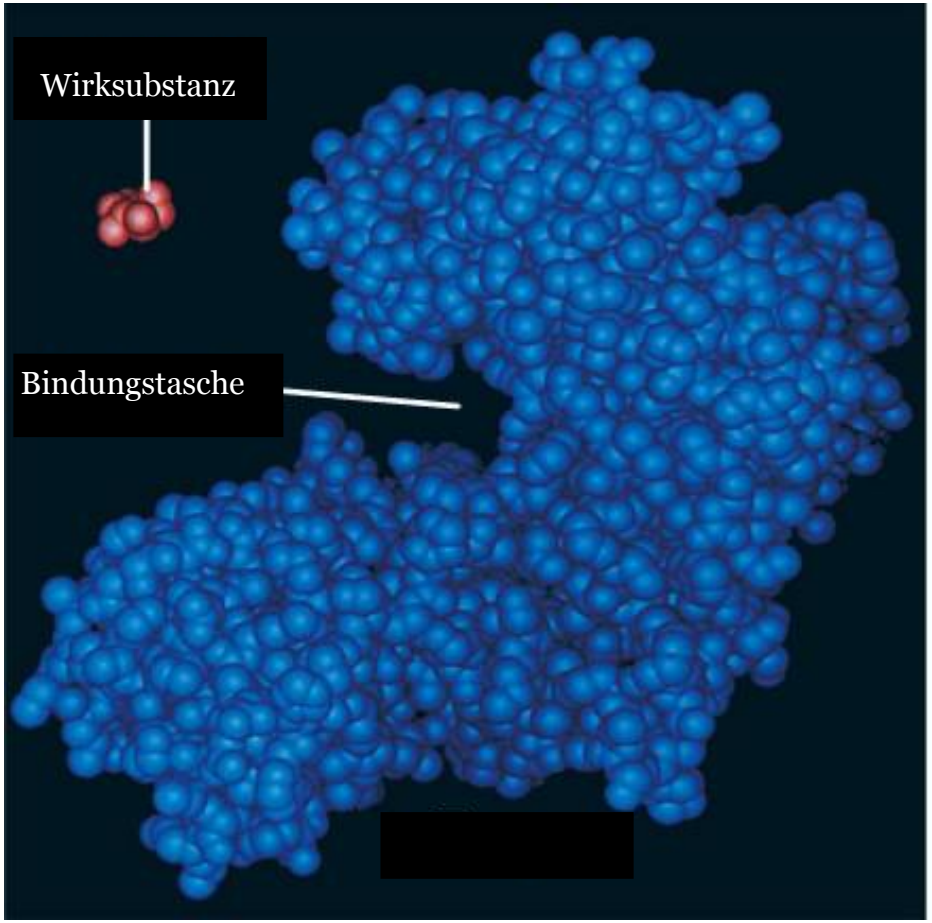


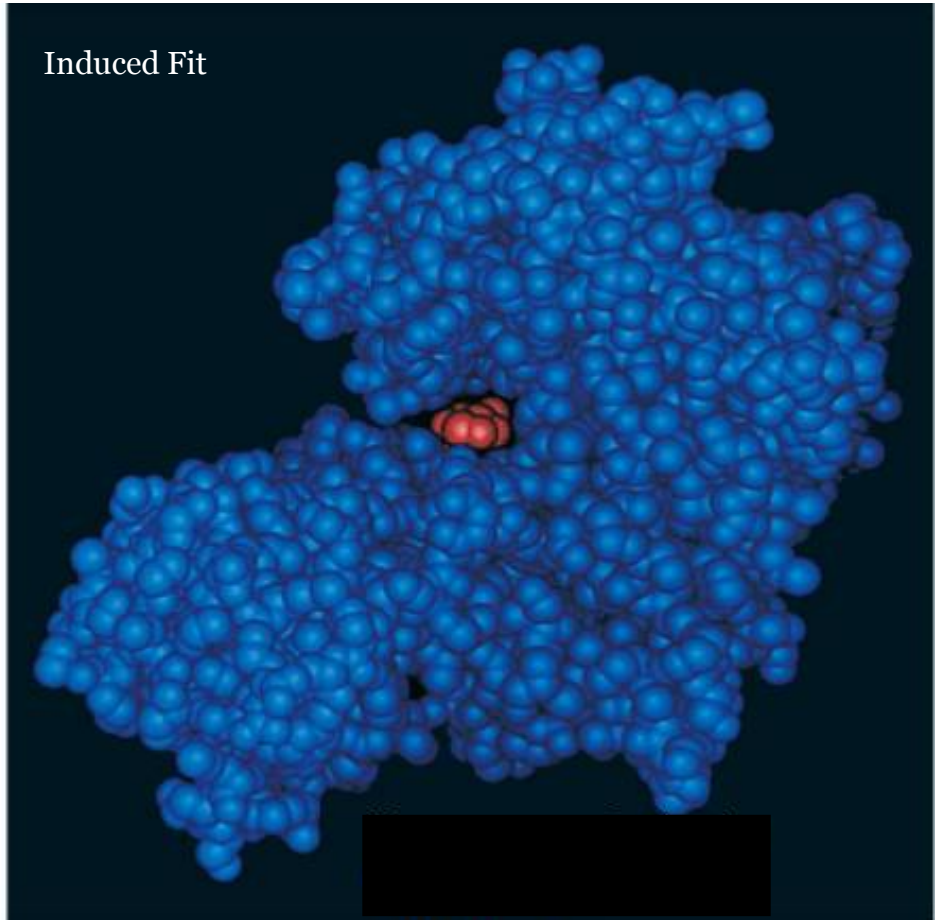
Carboanhydrase (Schloss) + Acetazolamid (Schlüssel)

"Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glykosid zueinander passen müssen, wie Schloss und Schlüssel, um eine chemische Wirkung aufeinander ausüben zu können."

Emil Fischer (1894)

Induced Fit: Biologisches Schloss+Schlüssel sind flexibel

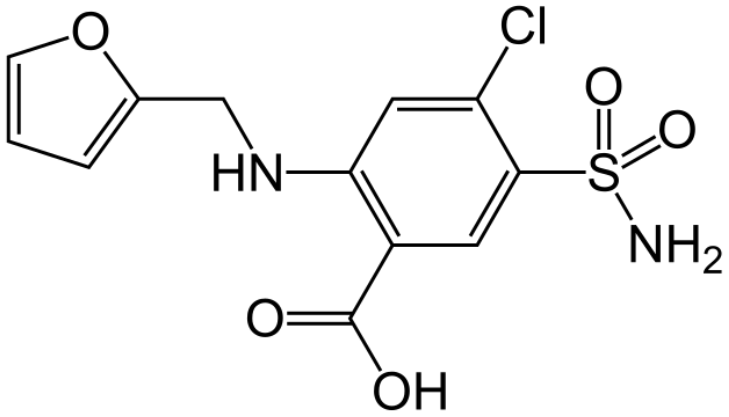


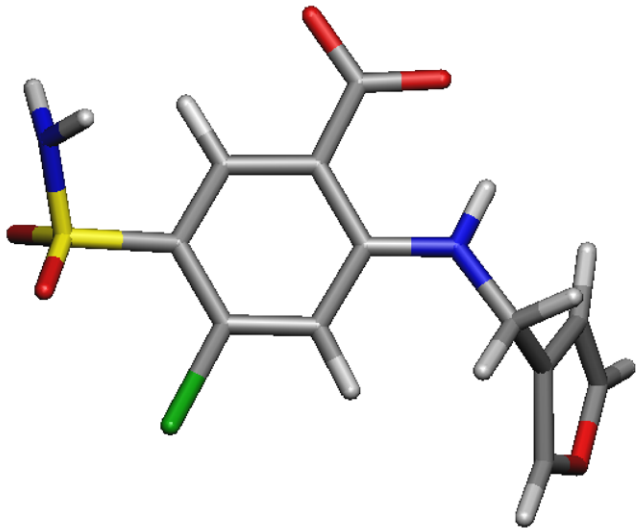


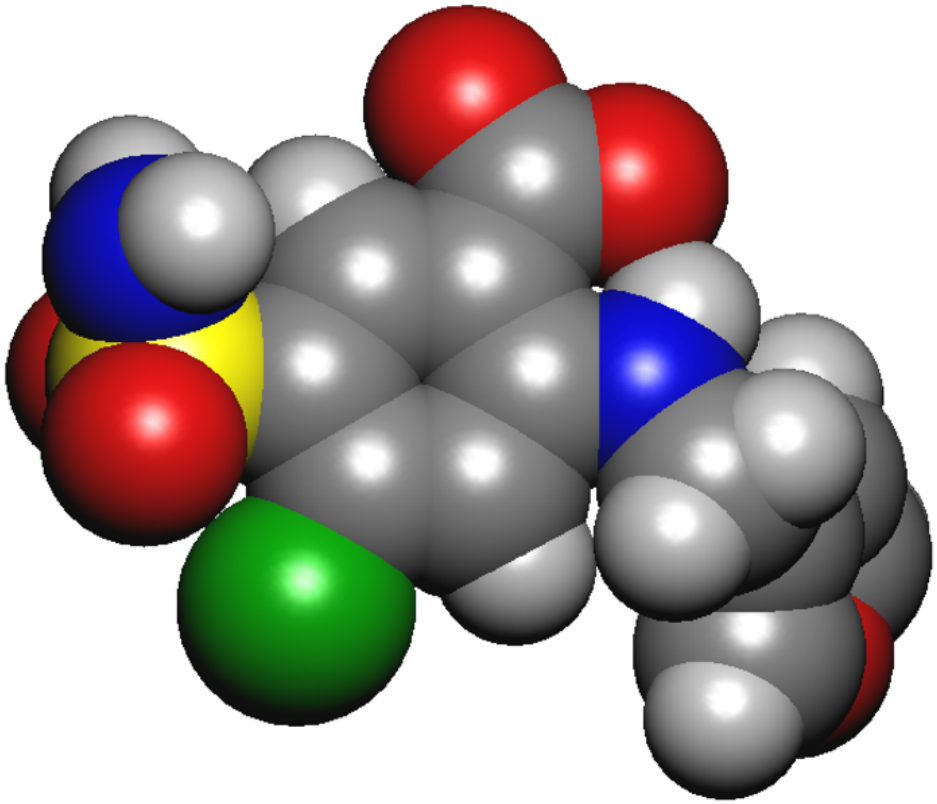
bio1151.nicerweb.com

Diese gegenseitige Anpassung ist eine Ursache von Arzneistoffnebenwirkungen

Biologische Schlüssel — interaktives 3D-Modelbuilding

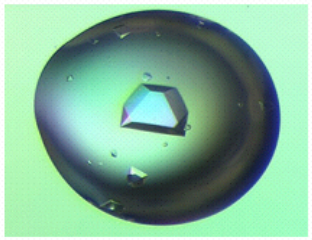






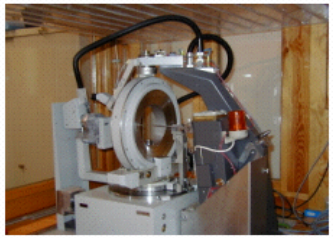
Furosemid — ein Diuretikum

> 135 000 Biologische Schlösser — Protein Data Bank (PDB)



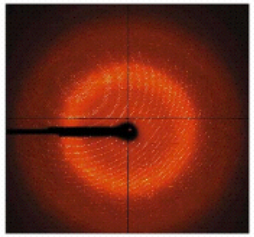
Proteinkristall
ttplabtech.com

$$\rho(x,y,z) = 1/V \cdot \sum_{hkl} F_{hkl} \cdot e^{-2\pi i(hx+ky+lz)}$$

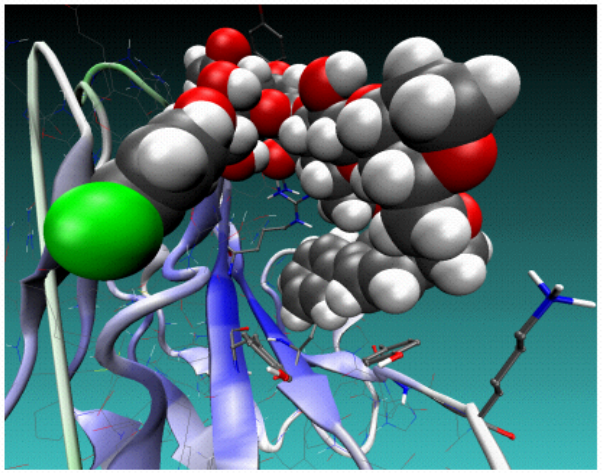


Diffraktometer
lks.physik.uni-erlangen.de

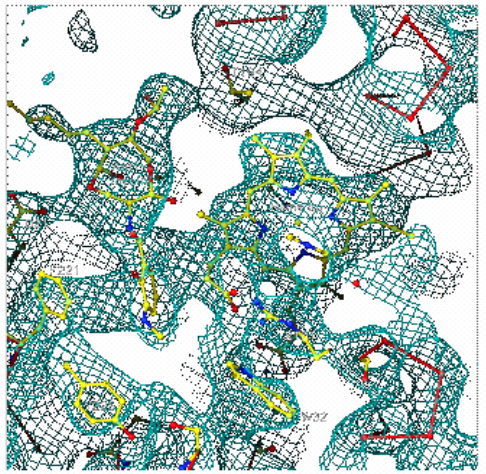
Max Perutz und John Kendrew: Nobelpreis 1964
nobelprize.org



Beugungsmuster
lbl.gov



Atomistisches Modell

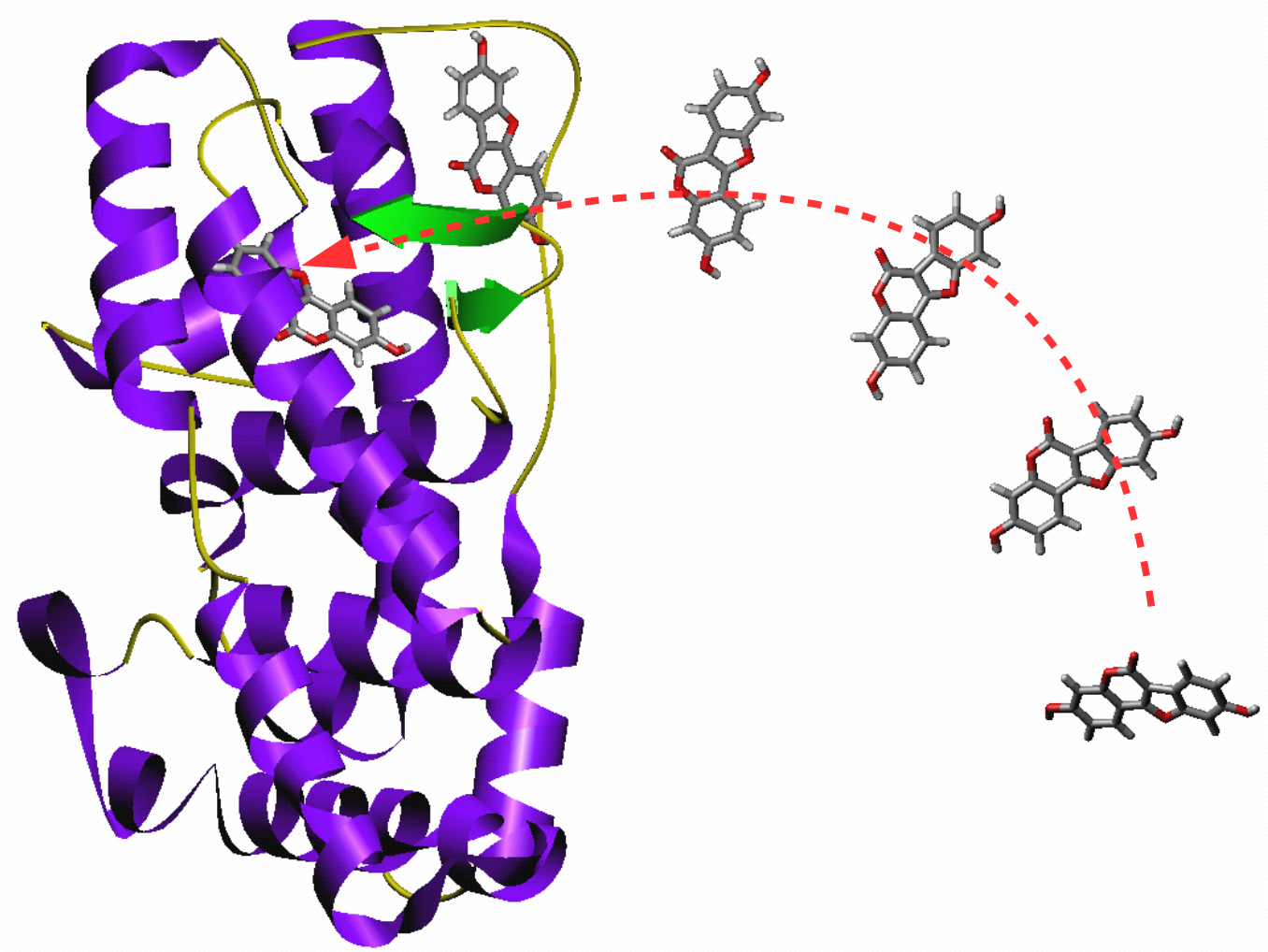


Elektronendichtekarte
lbl.gov

X-ray + NMR + Cryo-EM + …

Molecular Docking in Computational Toxicology

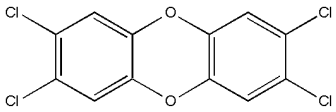
Methoden des struktur-basierten Designs im Einsatz



Andocken einer Substanz in die Bindungstasche (3D-Struktur) eines Proteins

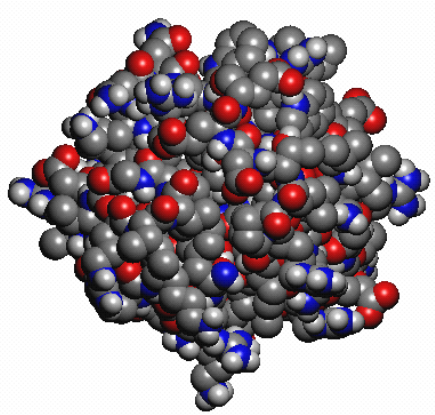
Simulation von Rezeptor-vermittelten Nebenwirkungen

TCDD



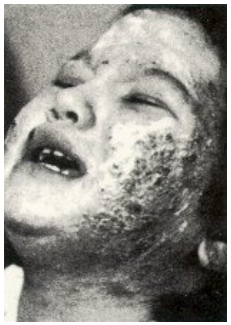
Einnahme/Hautkontakt

Bindung

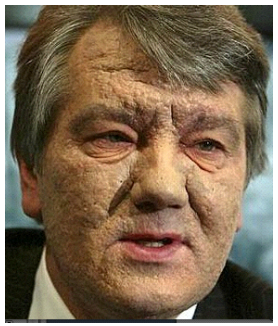


Signalweiterleitung

Manifestation der Toxizität



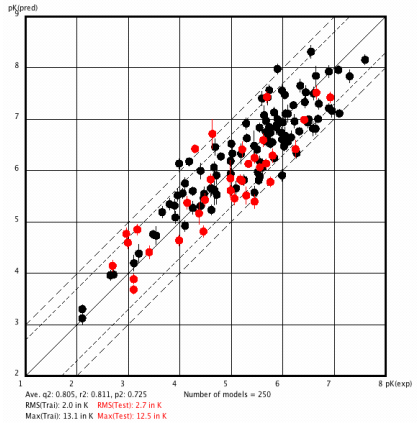
Seveso-Opfer 1976
www.brooklyn.cuny.edu



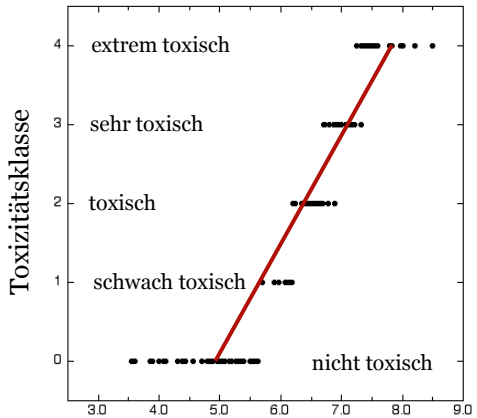
V. Juschtschenko 2004
www.blick.ch

Simulation der Bindung ans Zielprotein (AhR)

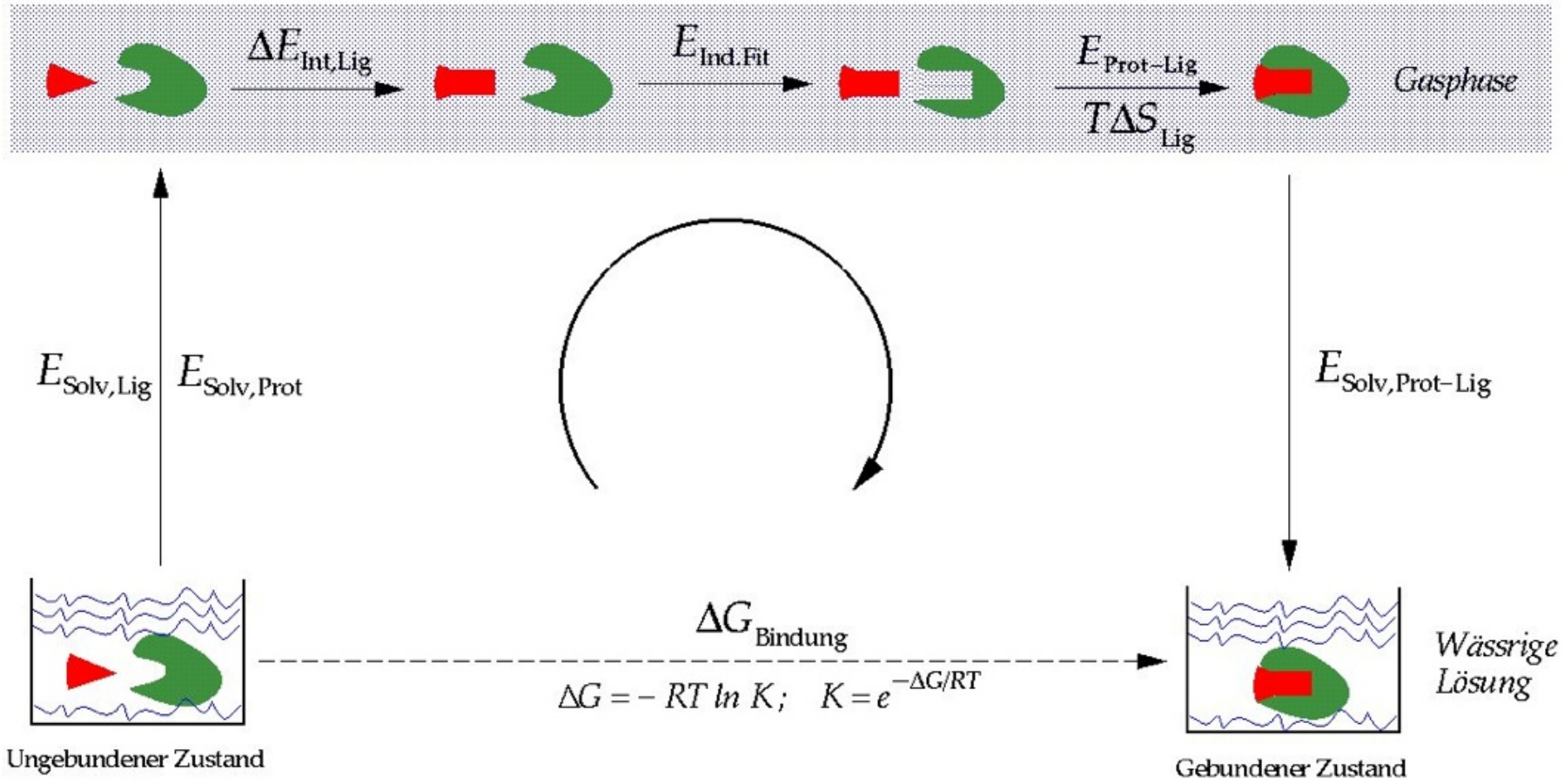
Konsequenz: Aus der Quantifi-zierung der Bindungsaffintiät zu einem Protein, das unerwünschte Wirkungen vermittelt lässt sich das "toxische Potential" der Ver-bindung, nicht aber deren Toxizität abschätzen.



QSAR



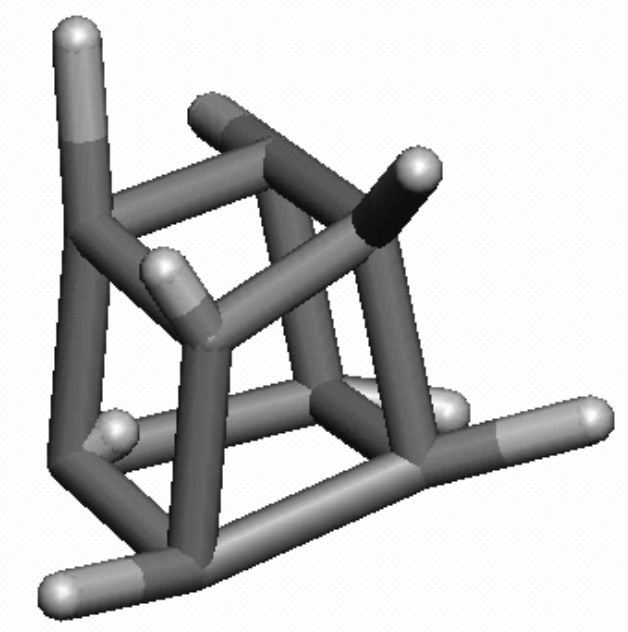
Berechnung der Bindungsaffinität (ΔG) der Wirksubstanz



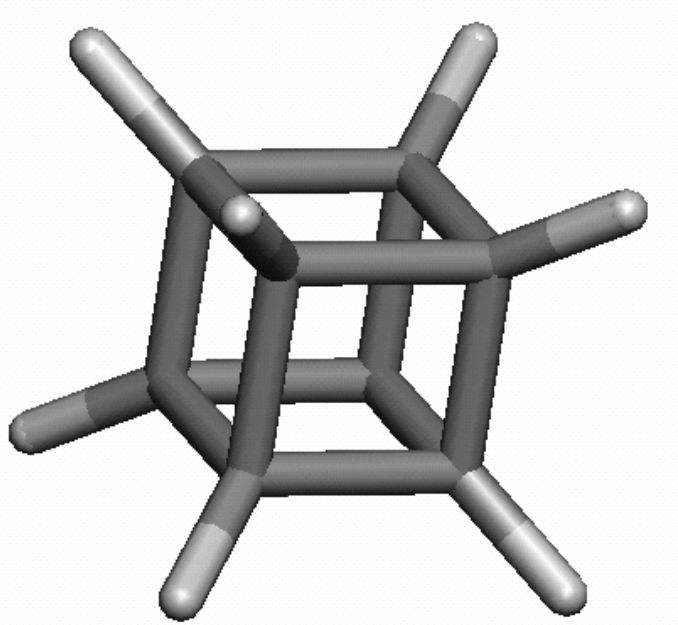
ΔG ist eine Zustandsgrösse, d.h. sie hängt nicht vom eingeschlagenen Weg ab.

Zentrale Philosophie:

Struktur und Energie eines Moleküls sind in engem Zusammenhang



Energieminimierung
Strukturoptimierung



Definition: Hohe Energie = Instabiles System

Die Energie von Molekülen lässt sich mit Molekülmechanikrechnungen (Kraftfeldrechnungen) minimieren. Dabei wird deren Struktur optimiert.

Tiefe Energie = Stabiles System

Das Kraftfeld — das Gehirn von Molekülоptimierungen

$$E_{total} = \sum_{bonds} K_r (r - r_{eq})^2 + \sum_{angles} K_\theta (\theta - \theta_{eq})^2 + \sum_{torsions} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] +$$

$$\sum_{nb\ pairs} \frac{q_i \cdot q_j}{4\pi\varepsilon_0 D(r) r_{ij}} + \sum_{nb\ pairs} \left(\frac{A}{r_{ij}^{12}} - \frac{B}{r_{ij}^{6}}\right) +$$

$$\sum_{H\ bonds} \left(\frac{C}{r_{ij}^{12}} - \frac{D}{r_{ij}^{10}}\right) \cdot \cos^2(\theta_{Don-H\cdots Acc}) \cdot \cos^n(\omega_{H\cdots Acc-LP}) +$$

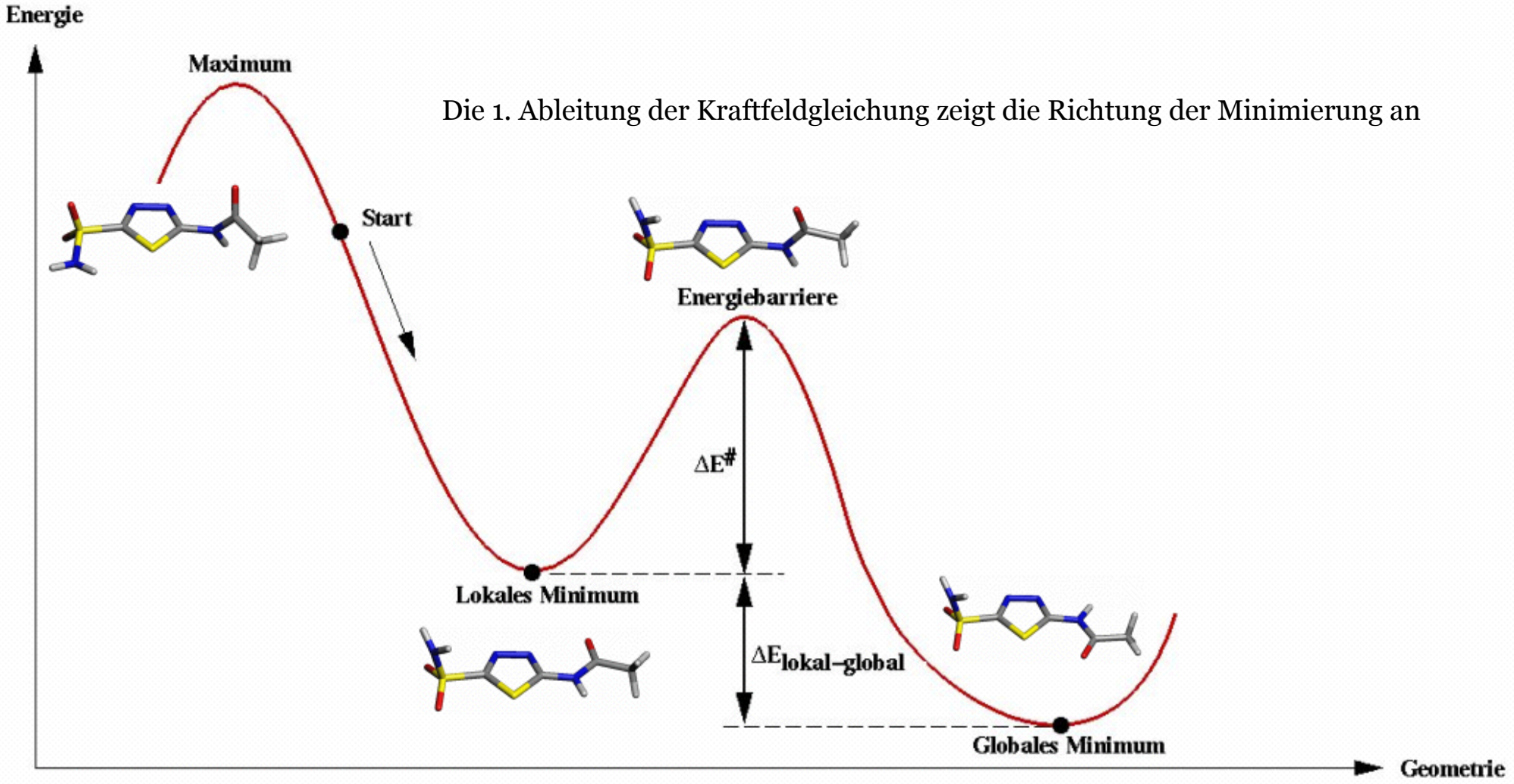
$$\sum_{metal\ pairs} \frac{q_i^{CT} \cdot q_j^{CT}}{4\pi\varepsilon_0 D(r) r_{ij}} + \sum_{metal\ pairs} \left(\frac{E}{r_{ij}^{12}} - \frac{F}{r_{ij}^{10}}\right) +$$

$$\sum_{atoms} -\frac{1}{2}\alpha_i \left[\vec{E}_i^{\,\circ} \cdot \vec{E}_i\right]$$

J. Am. Chem. Soc. 1990, 112, 4759–4767
ChemMedChem 2010, 5, 2088–2101

Die Kraftfeldgleichung erstellt die Beziehung zwischen Struktur und Energie

Der „Minimizer” — der Motor von Molekülоptimierungen

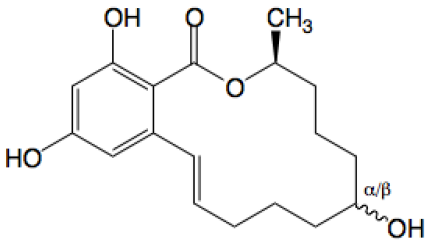


Die 1. Ableitung der Kraftfeldgleichung zeigt die Richtung der Minimierung an

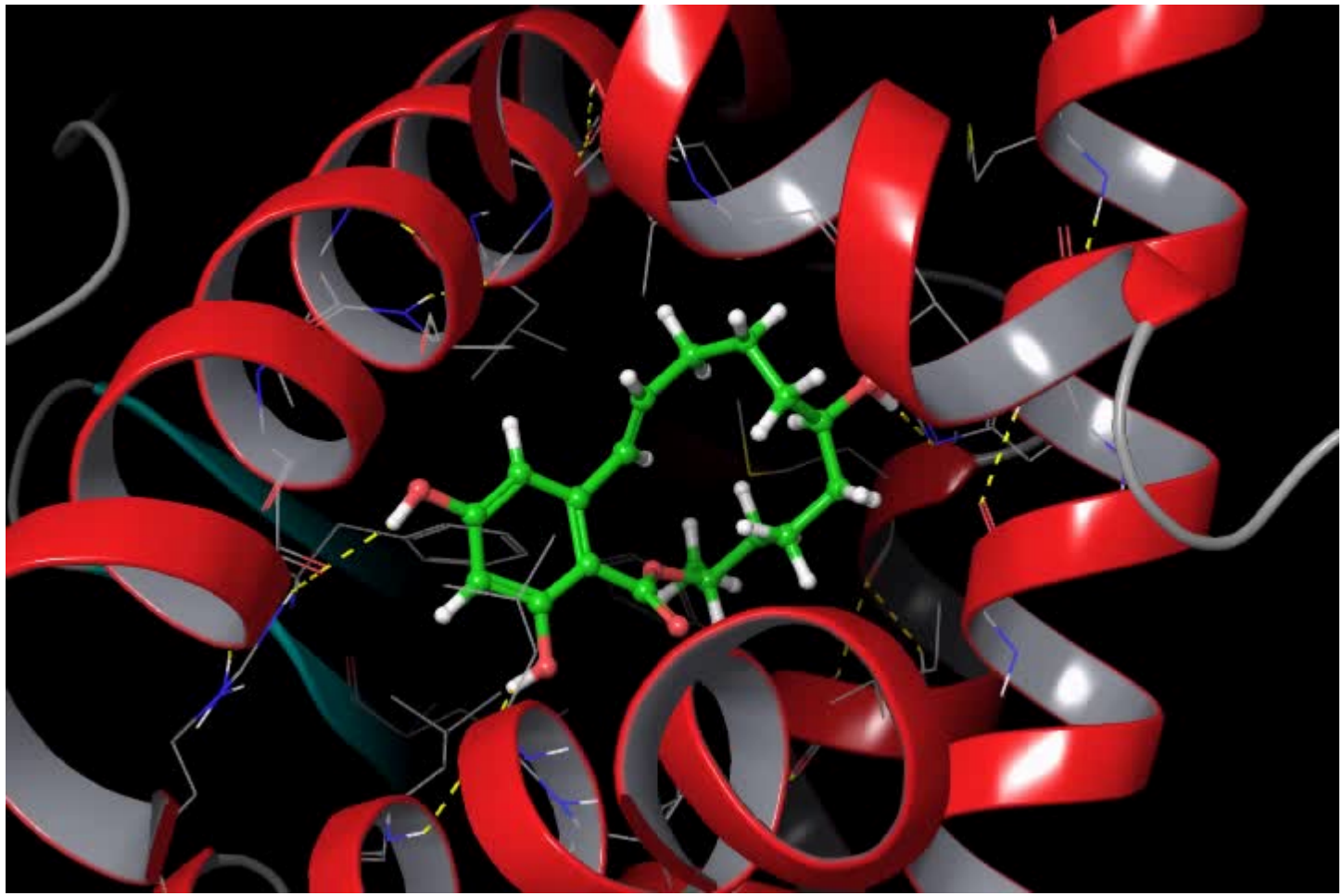
Molekülmechanik-Optimierungen enden immer im nächsten lokalen Minimum

Moleküldynamik-Simulationen

Newton'sche Bewegungsgleichung (1687): $r(t+\Delta t) = r(t) + \partial r/\partial t \cdot \Delta t + 0.5 \cdot \partial^2 r/\partial t^2 \cdot \Delta t^2$

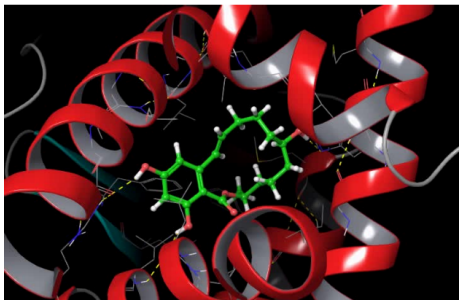


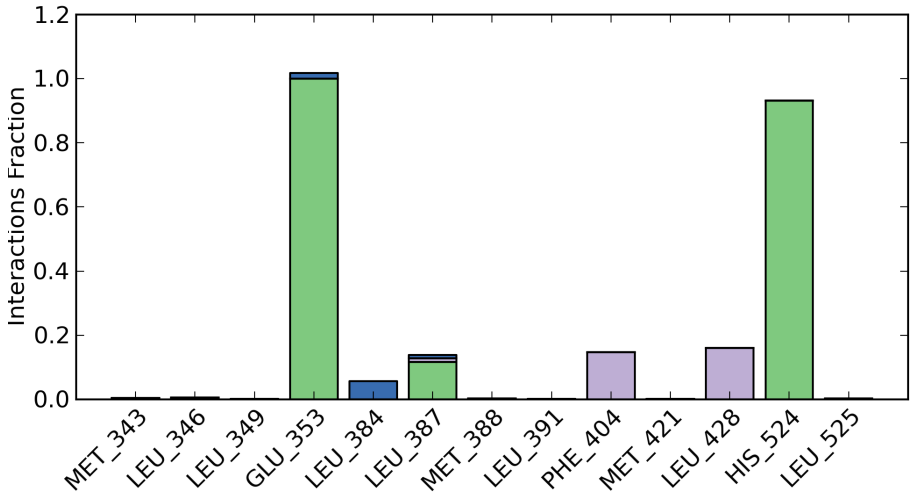
α-Zearalenol

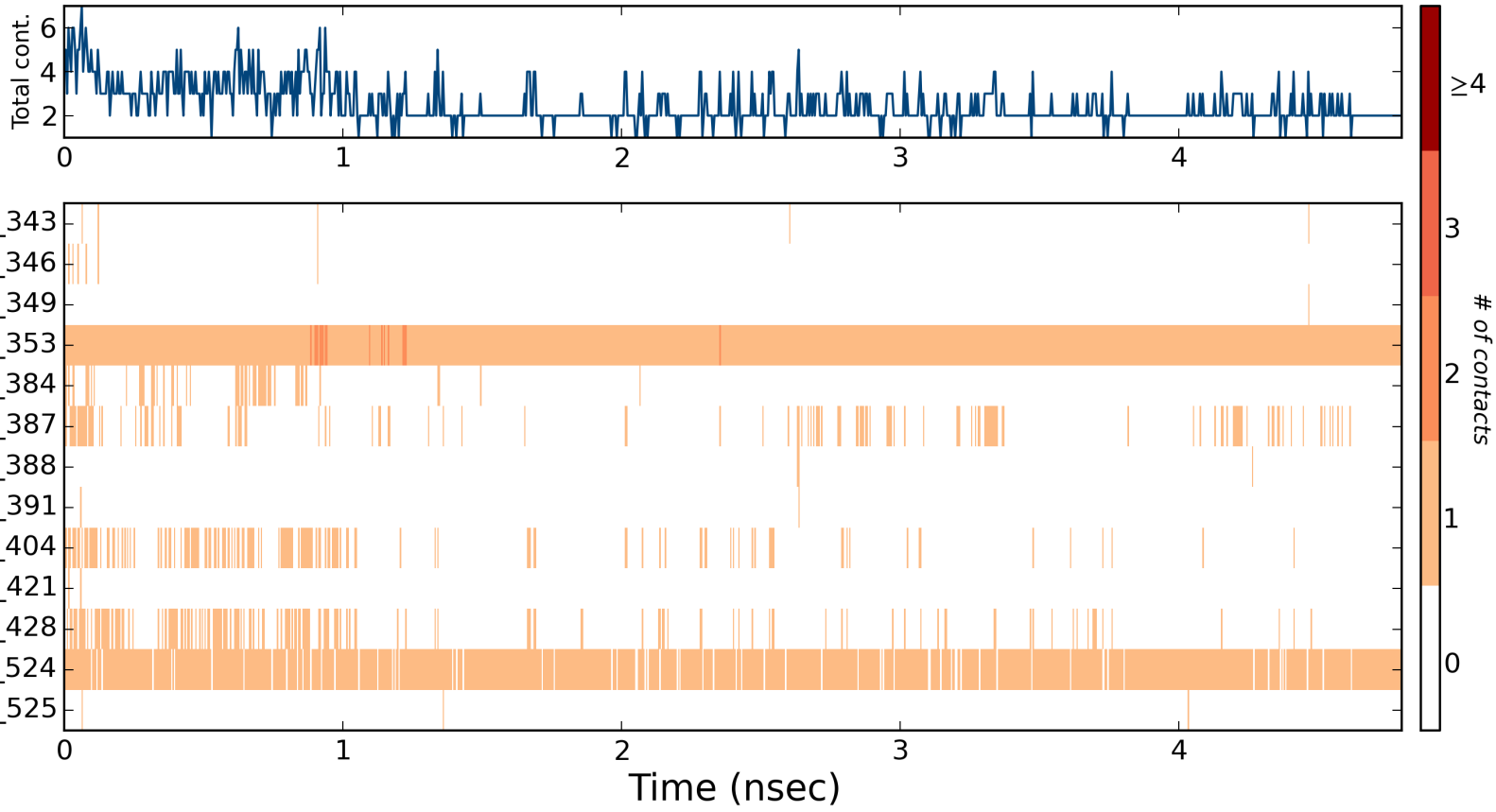


4.8 ns MD simulation of docked α-zearalenol at the Estrogen receptor α
(Software *Desmond, D.E.Shaw, New York)*

MD Simulation

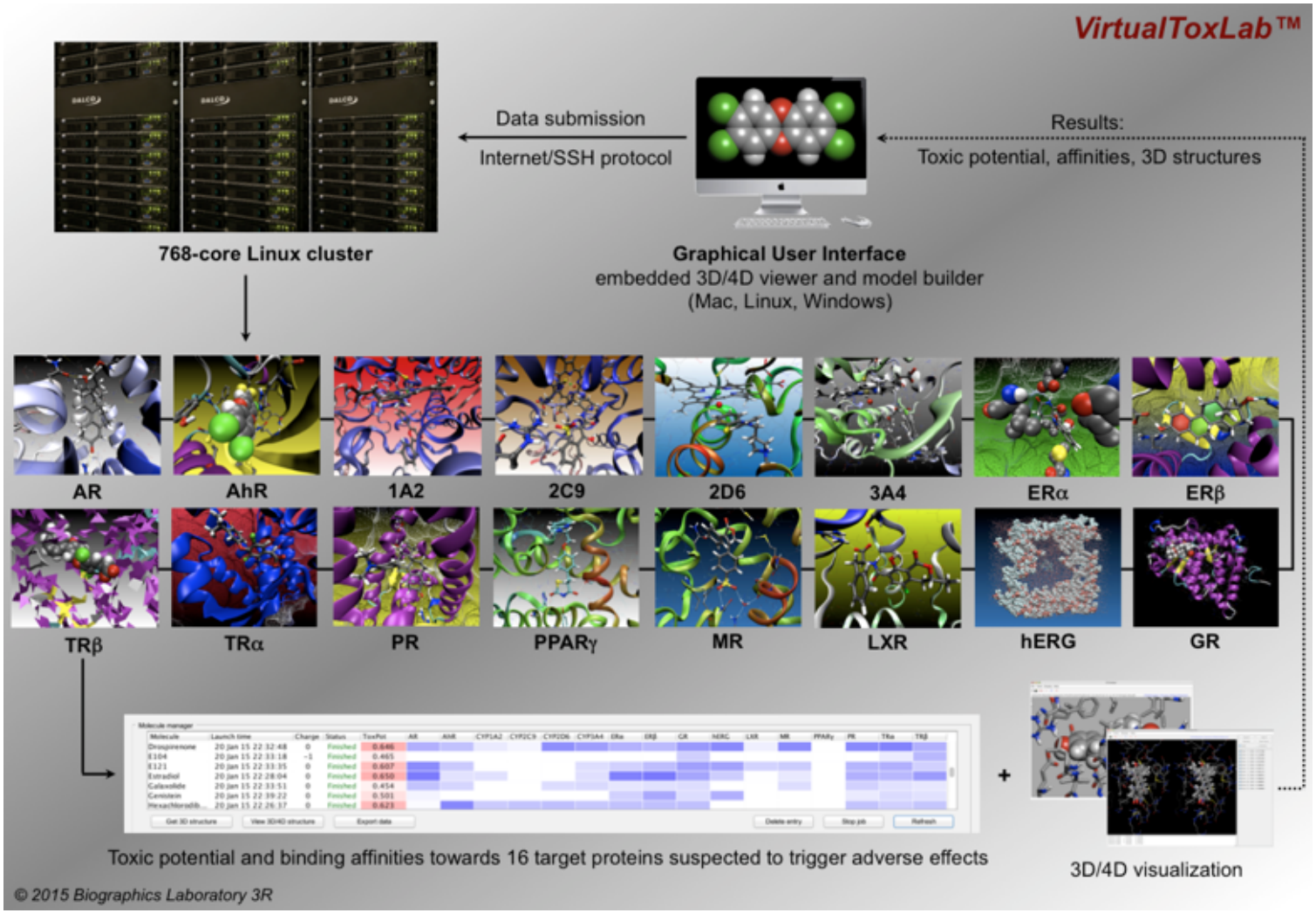






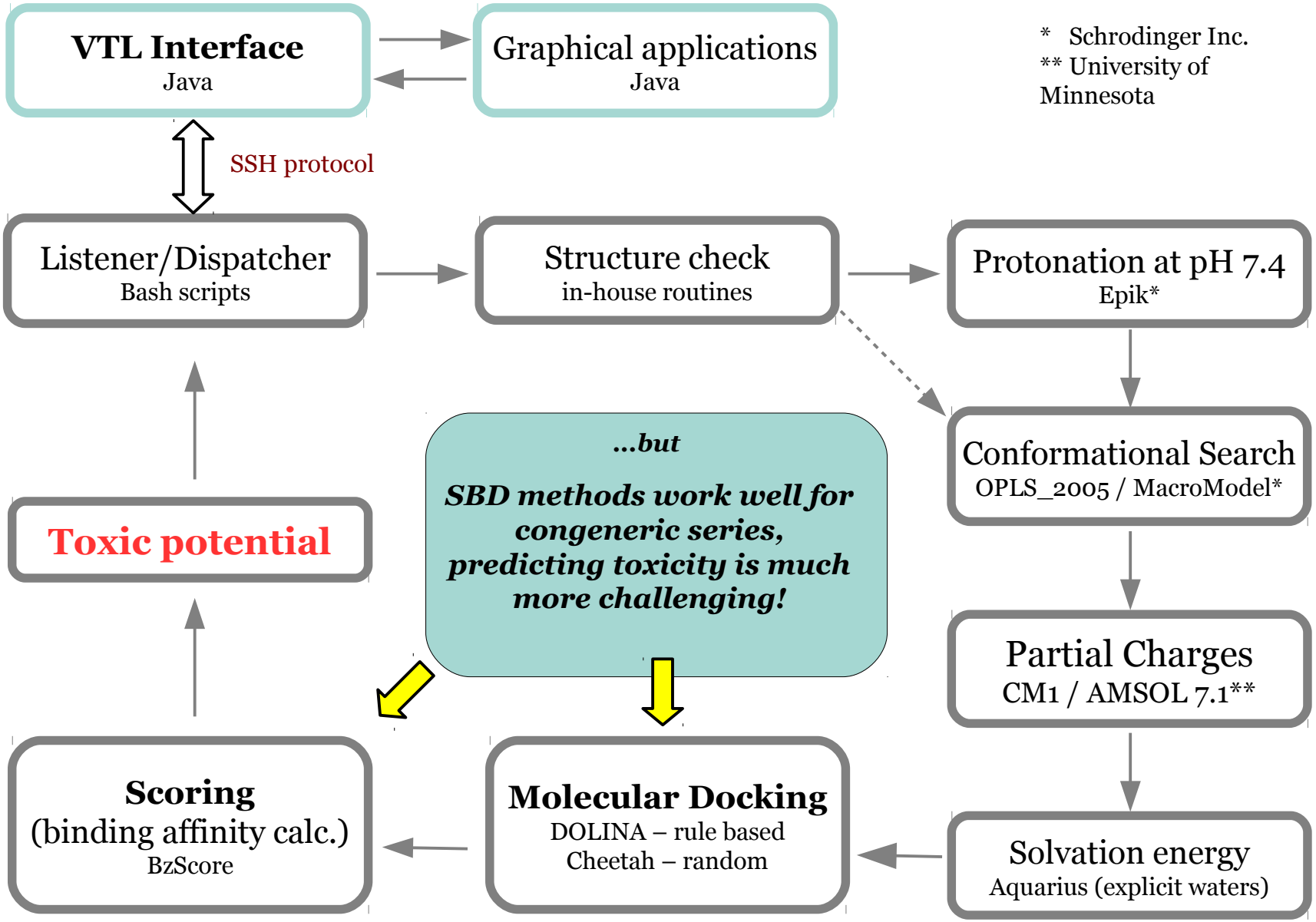
VirtualToxLab

Automatisierte Voraussage des toxischen Potentials
von Arzneistoffen, (Umwelt-) Chemikalien und Naturstoffen



☞ http://www.virtualtoxlab.org, Toxicol. Appl. Pharmacol. 2012, 261, 142–153 und Tox. Lett. 2015, 232, 519–532

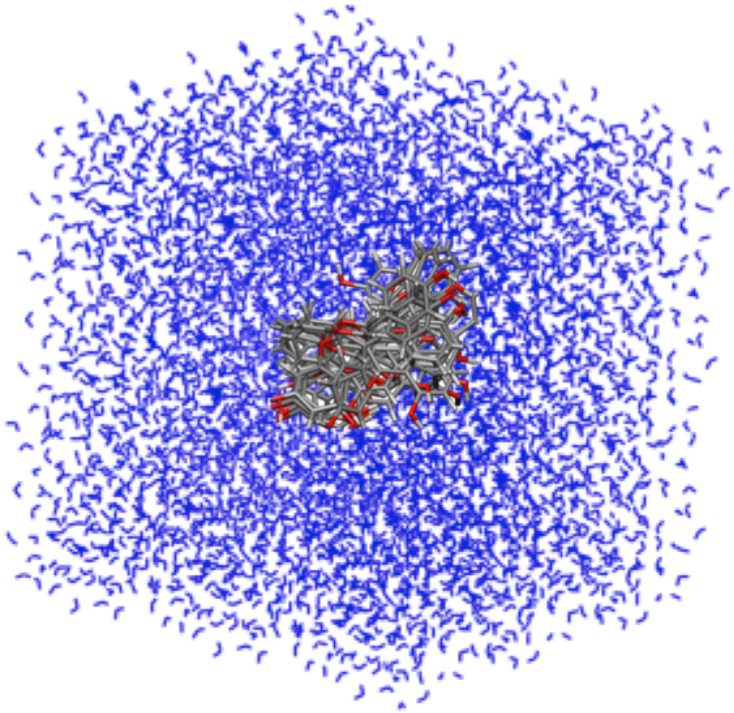
VirtualToxLab — Technisches Flussdiagramm

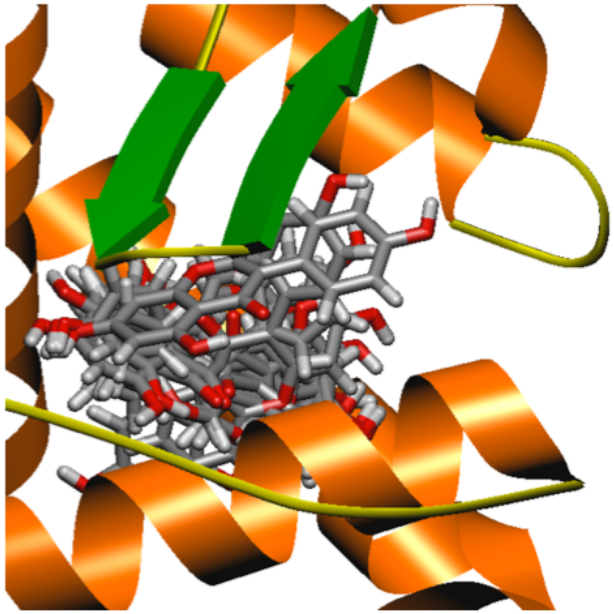


VirtualToxLab — Boltzmann Bewertung (4D)

Direkte Bewertung des Wasser-Protein-Übergangs einer Wirksubstanz (4D Bolzmann-Ensemble)

Beispiel: Genistein →Estrogen receptor β



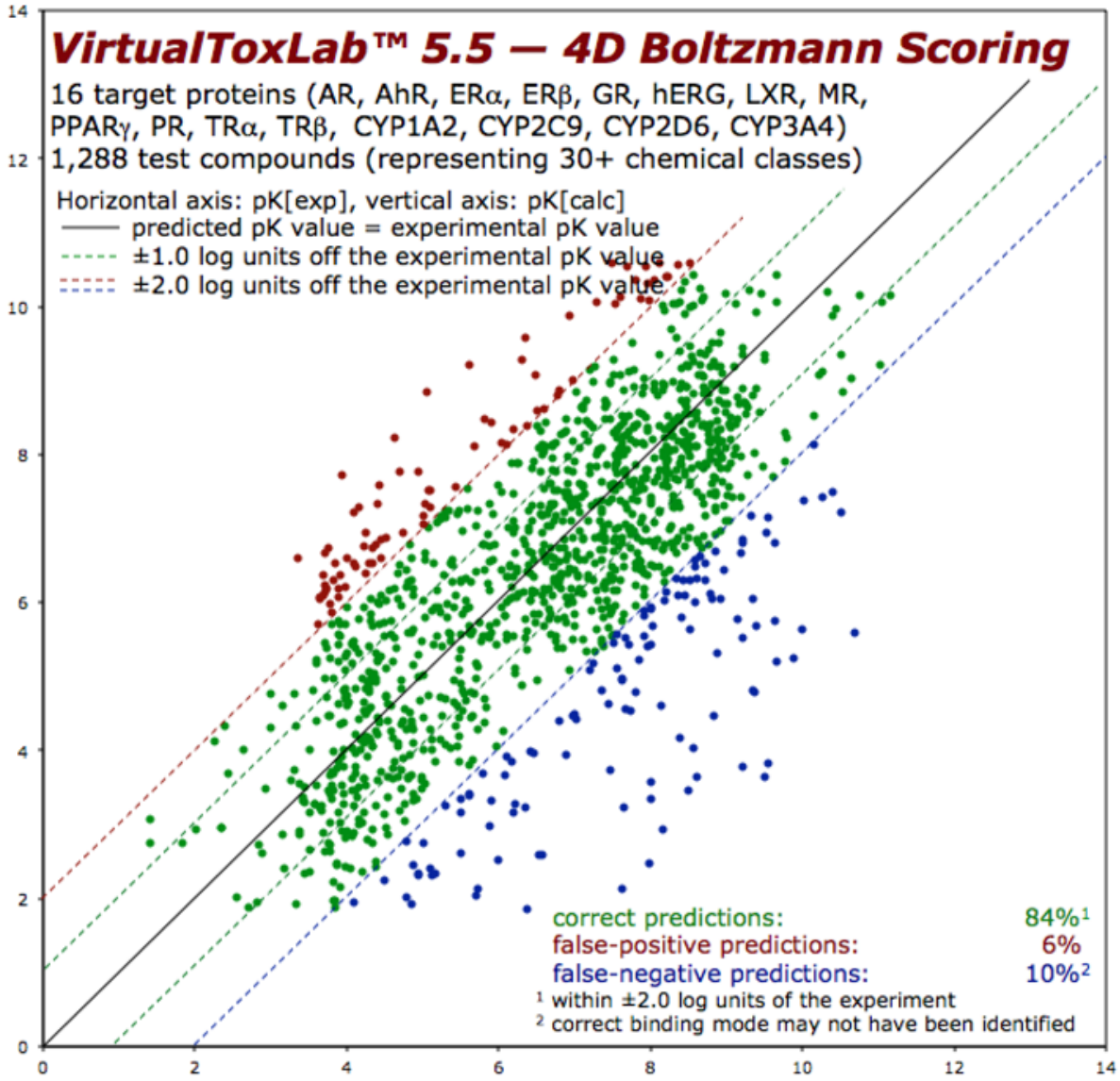


Water-bound ligand: E_{Bzw} = –36.262 kcal/mol

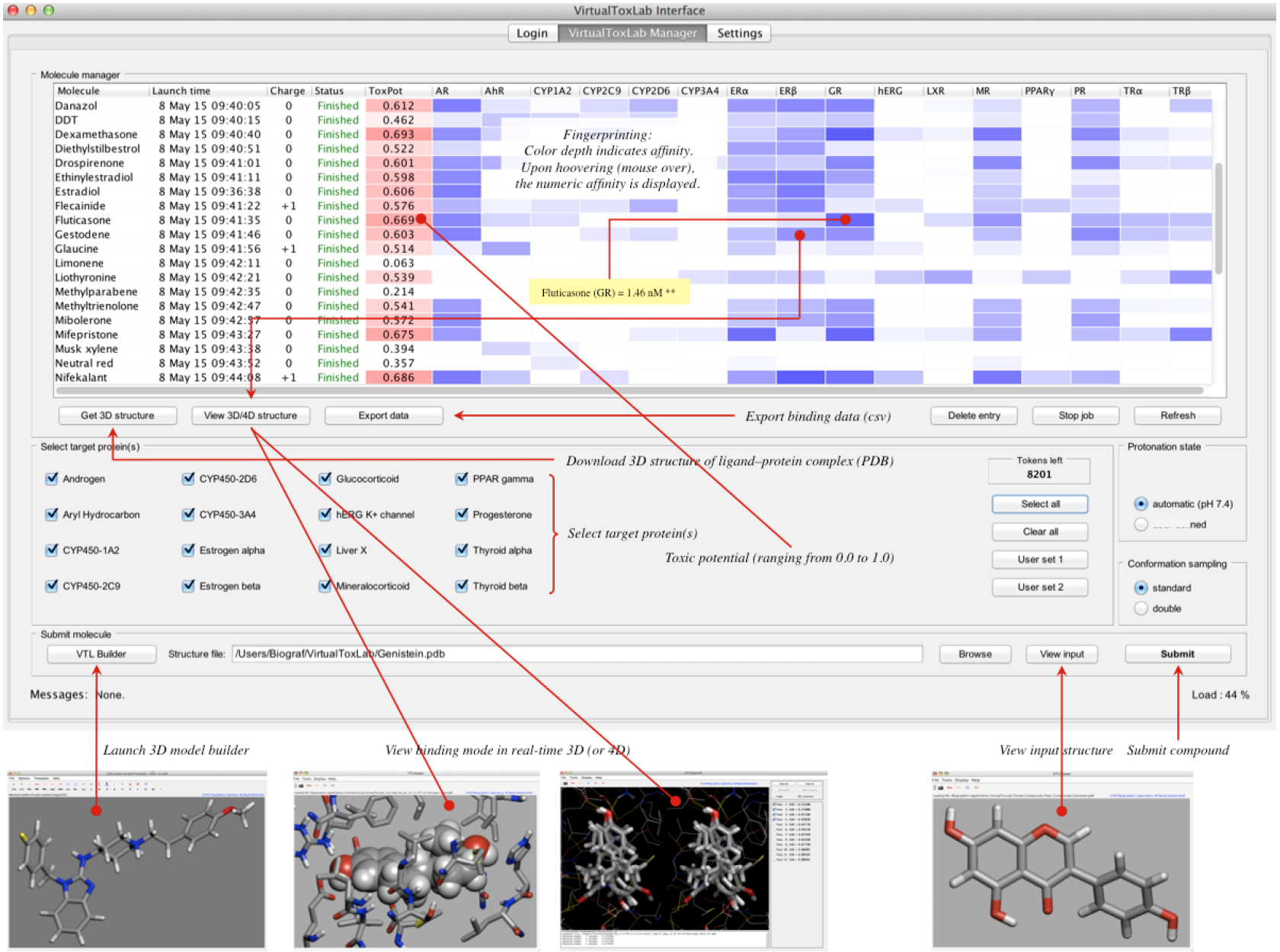
Protein-bound ligand: E_{Bzw} = –47.006 kcal/mol

ΔE_{Bzw} = –10.774 kcal/mol → converting ΔE into K using: $K = e^{-\Delta E / RT}$ → **K_{calc} = 9.66×10^{-9} M** (K_{exp} = 1.2×10^{-8} M)

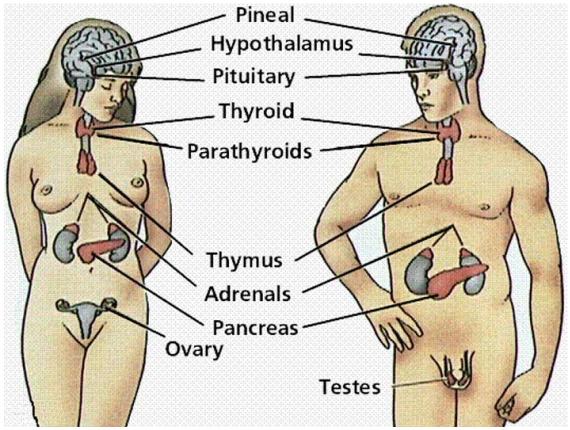
VirtualToxLab — Validierung anhand von 1,288 Substanzen



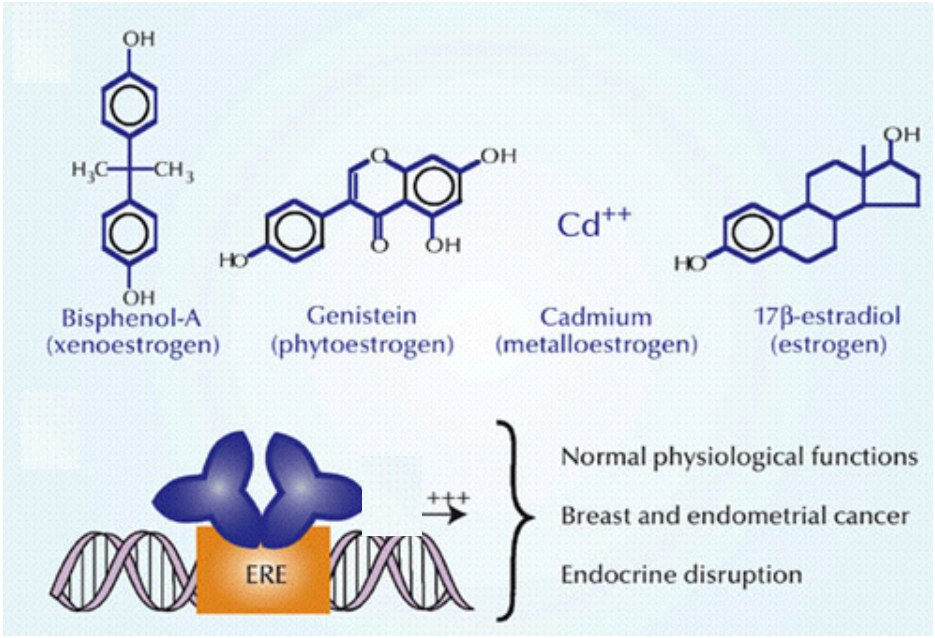
VirtualToxLab — Grafische Schnittstelle (Interface)



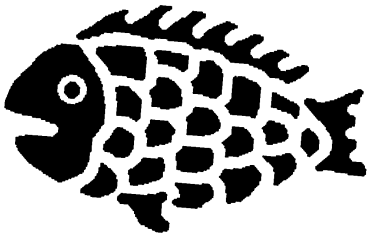
Nebenwirkungen/Toxizität: Endokrine Störungen



www.eco-thinker.com



www.medscape.com



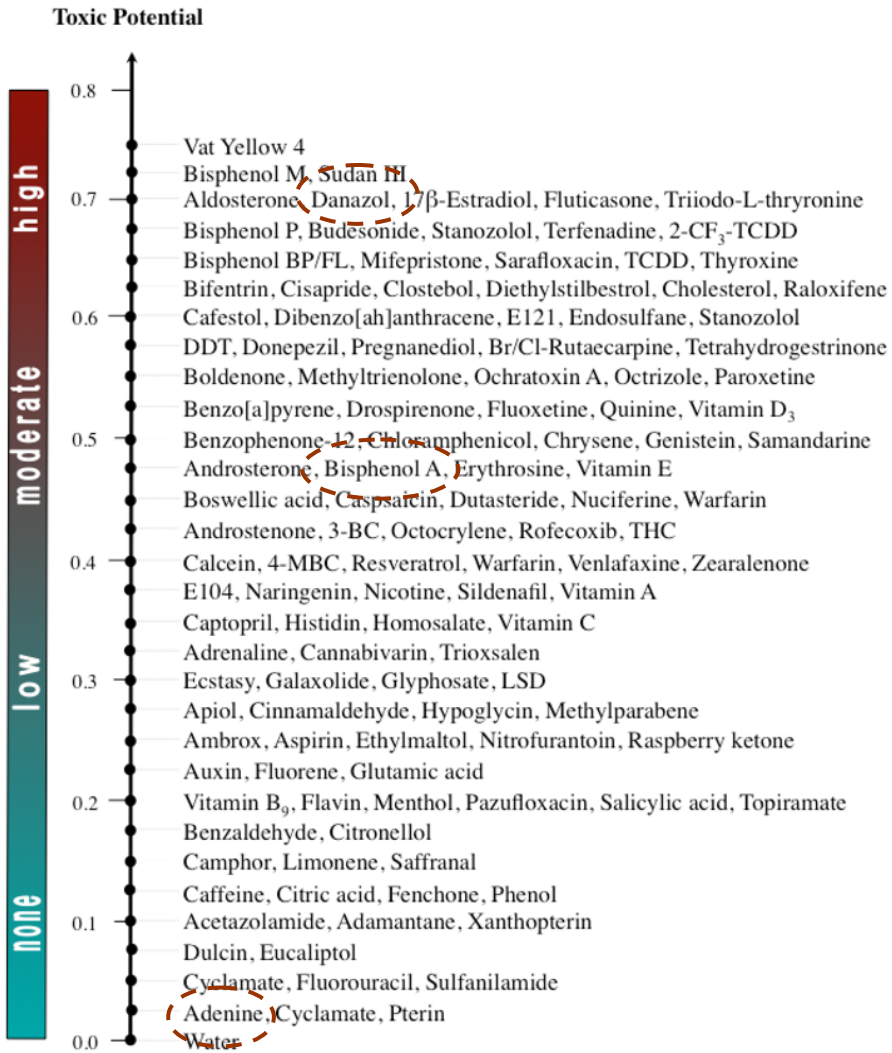
www.nutri-notes.com

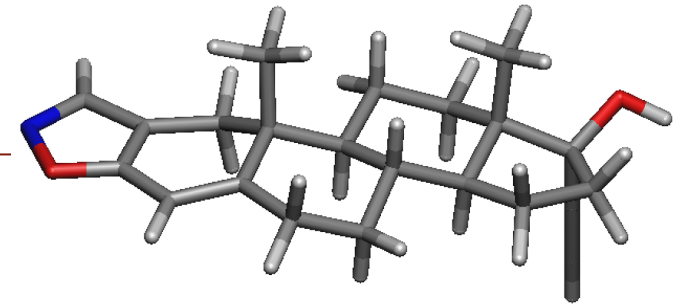
Endokrine Disruptoren (hormonell aktive Substanzen) — auch "Tarnkappenchemikalien" genannt — sind körperfremde Substanzen, die wie Hormone ins Endokrine System ein-greifen können und dort zerstörerische Wirkungen entfalten können. Solche Substanzen wurden mit toxischen Effekten in Tieren in Verbindung gebracht und lassen vermuten, dass kleinste Mengen beim Menschen vergleichbare Effekte hervorrufen können.

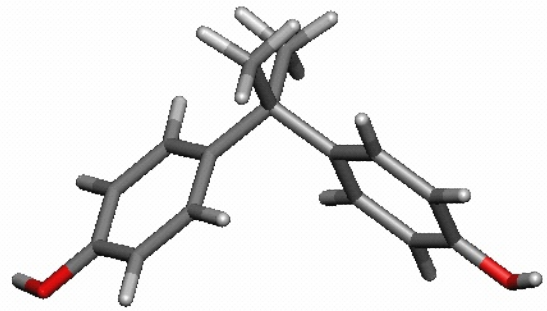
☞ Diethylstilbestrol, Thalidomid: vom Markt genommen

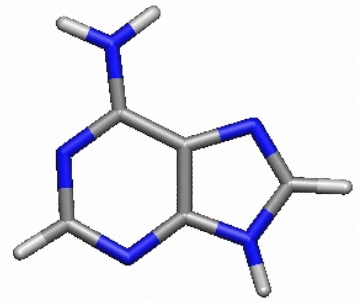
www.treehugger.com

VirtualToxLab — "Toxicity Alerts"

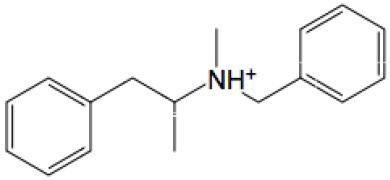




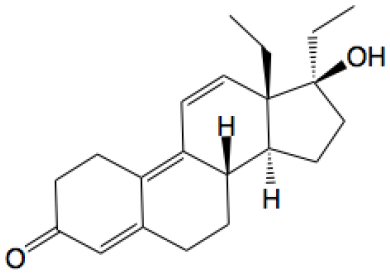




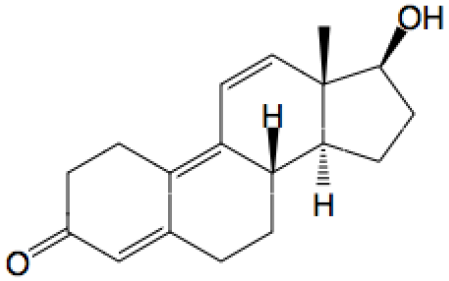
VirtualToxLab — Analyse von verbotenen Dopingsubstanzen



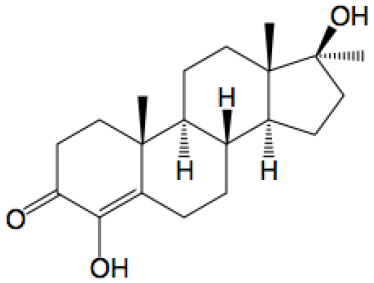
Benzphetamin



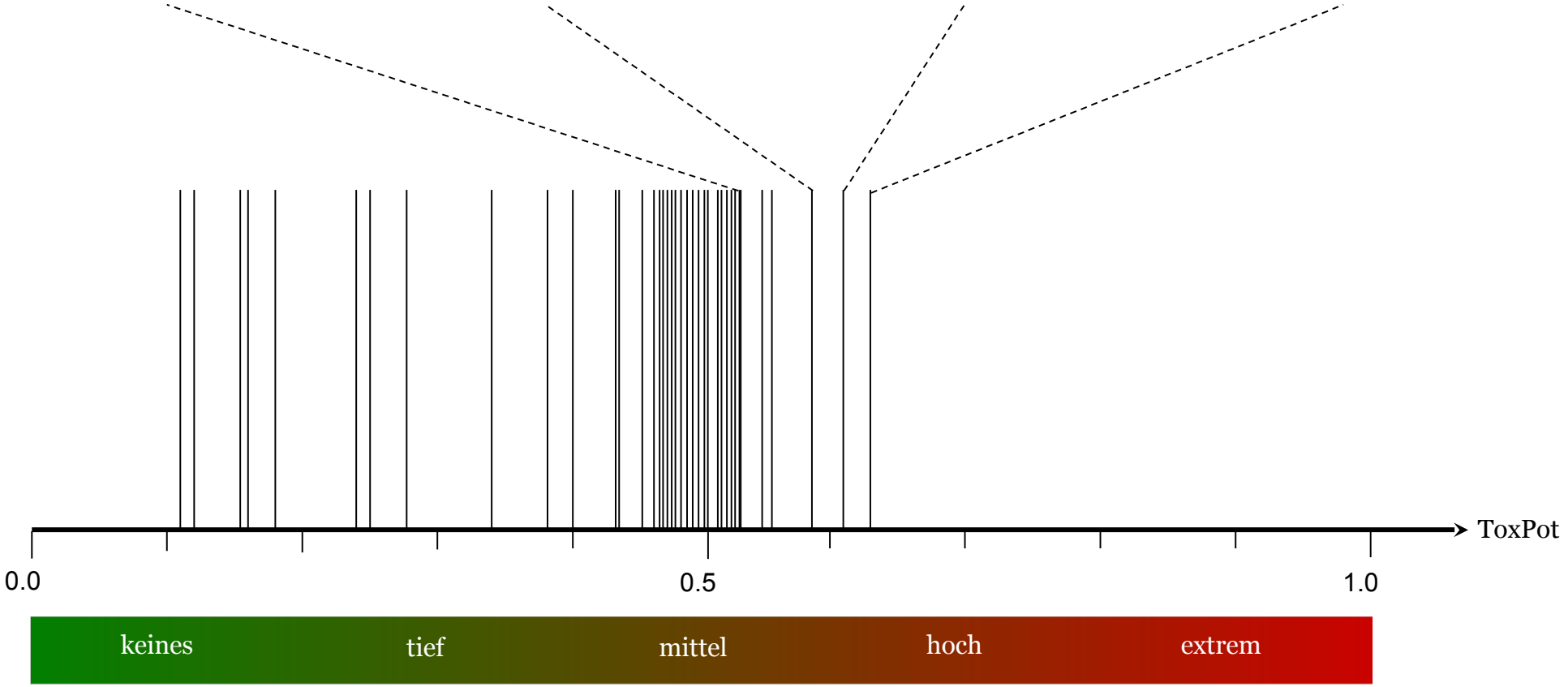
Tetrahydrogestrinon



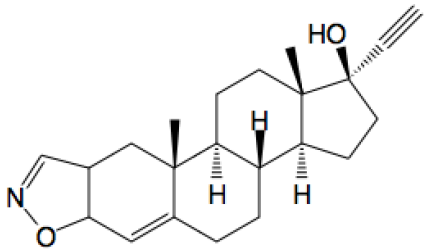
Trenbolon



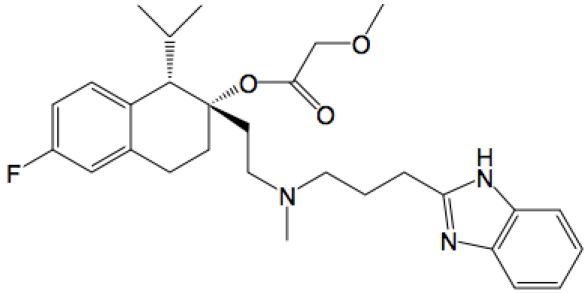
Oxymesteron



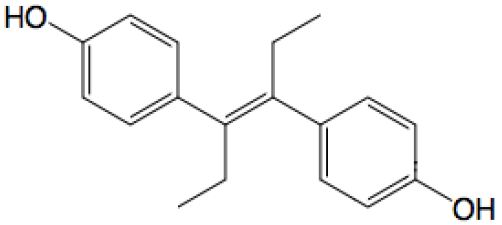
VirtualToxLab — Vom Markt zurückgezogene Substanzen



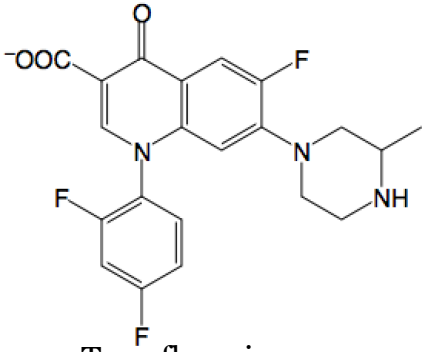
Danazol



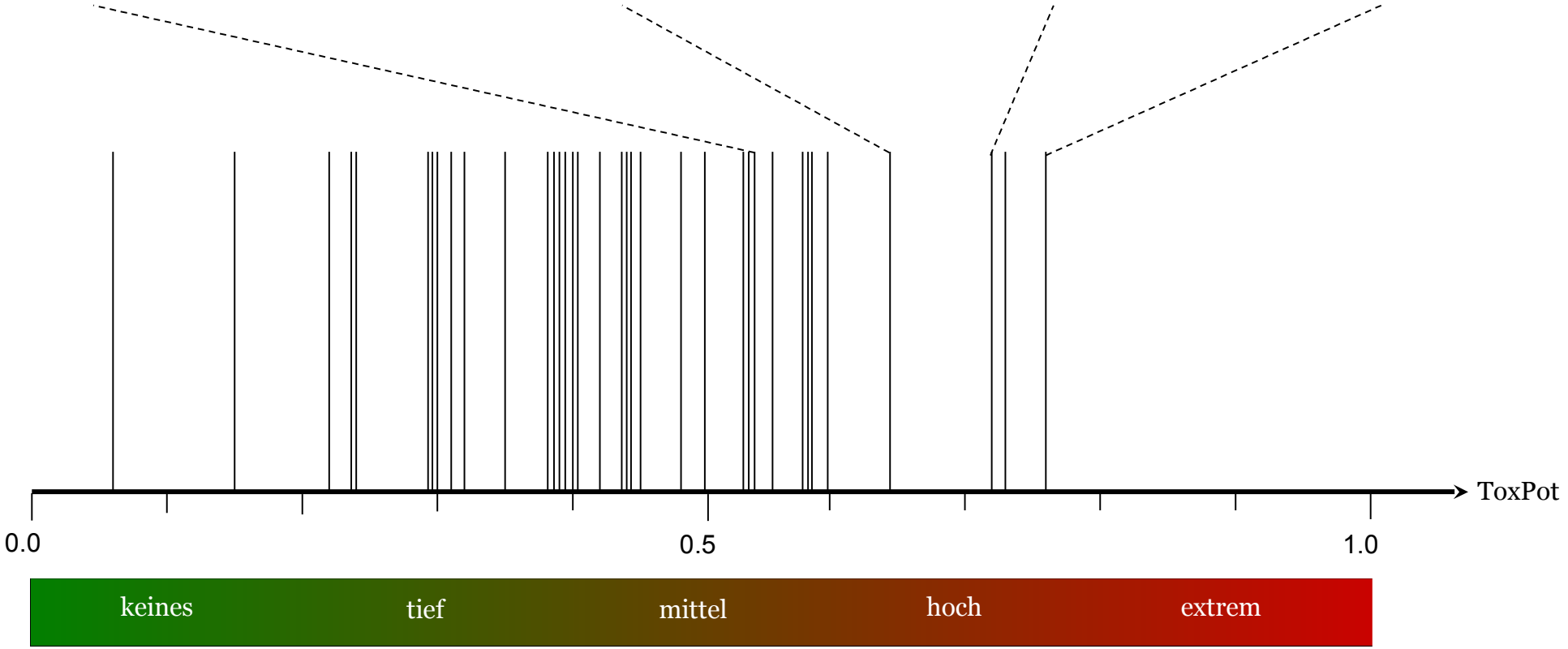
Mibefradil



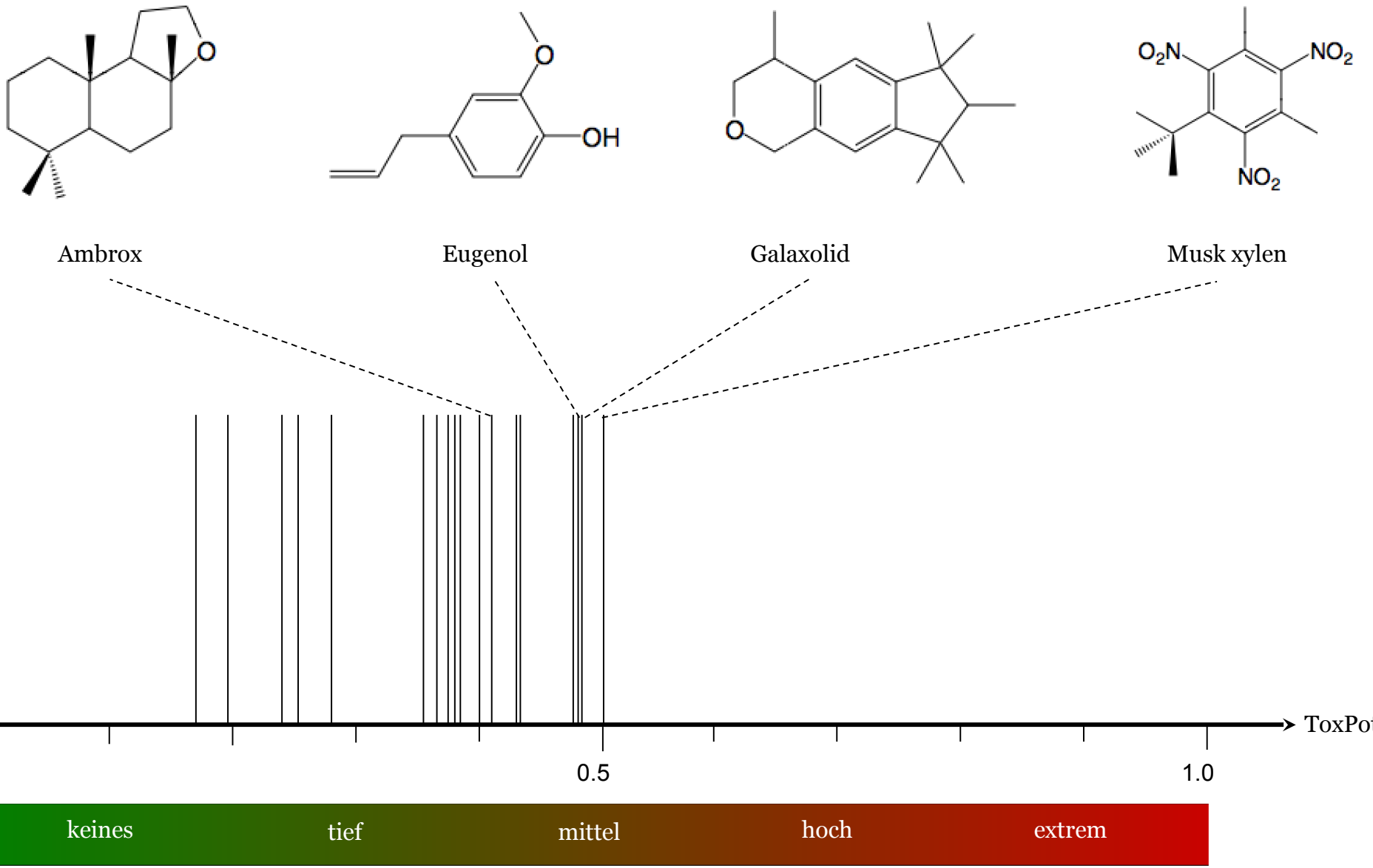
Diethylstilbestrol



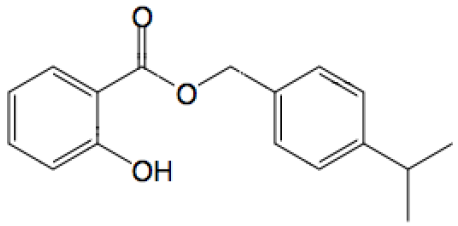
Temafloxacin



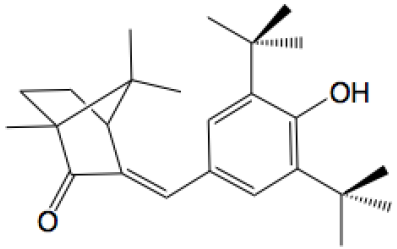
VirtualToxLab — Duftstoffe in Parfüms



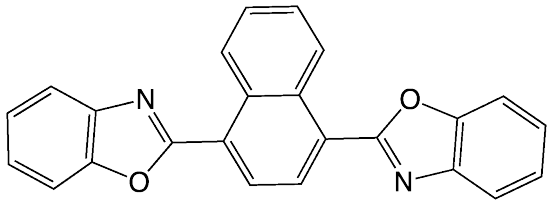
VirtualToxLab — UV-Filter und einige ihrer Metaboliten



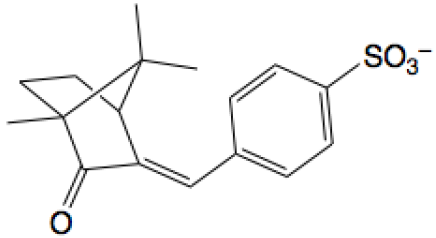
4-Isopropylbenzylsalicylate



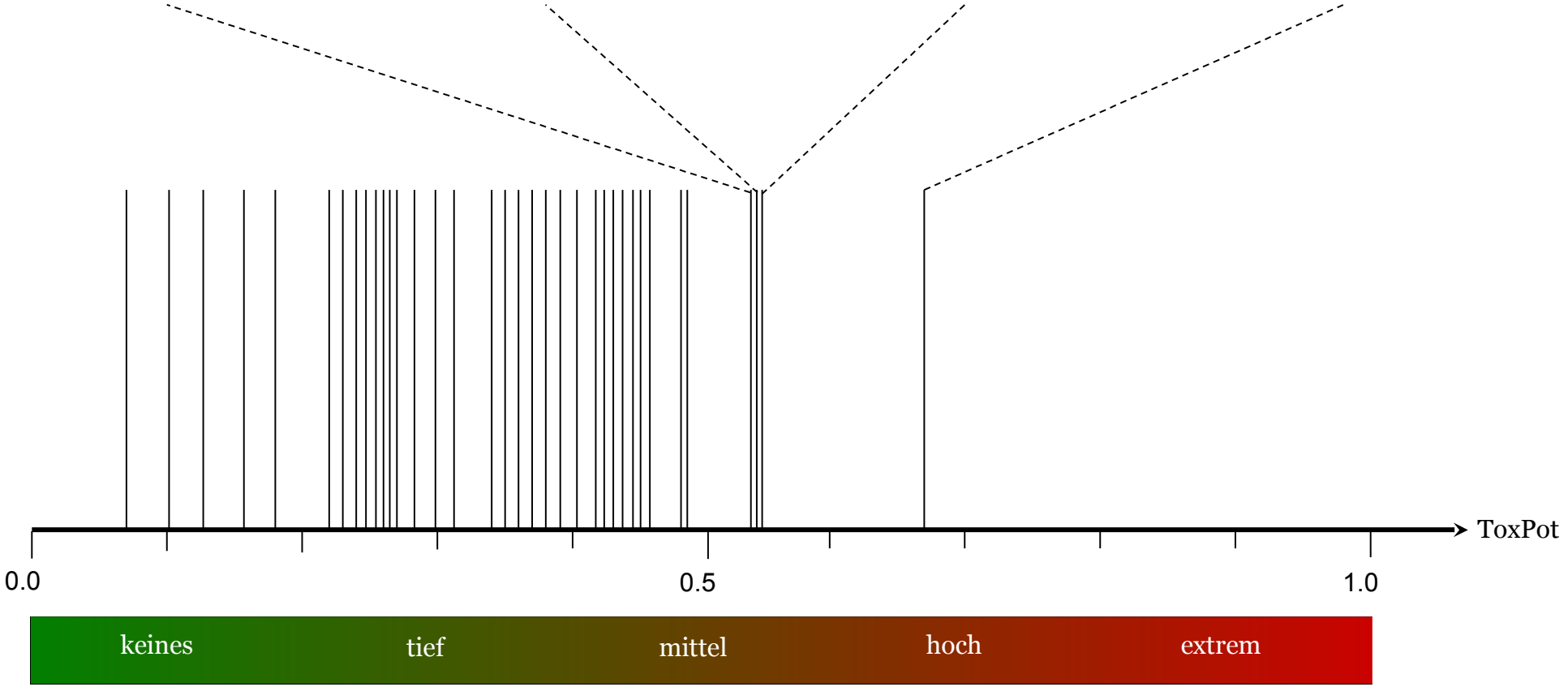
Di-tert.-butylhydroxy-benzylidenecamphor



Dibenzoxazinylnaphtalene

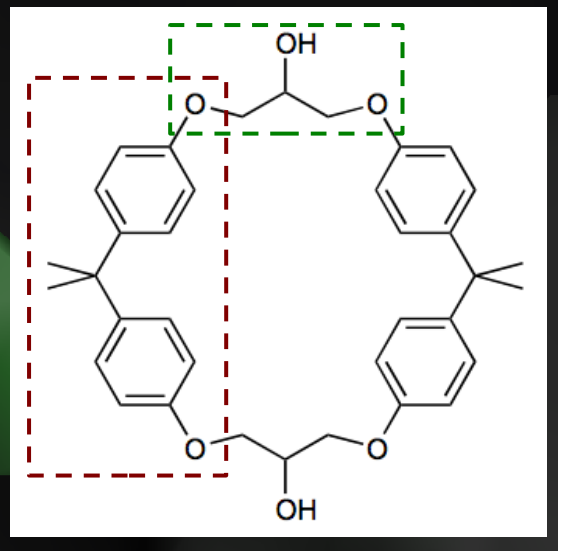


4-Methylbenzylidenecamphor sulfonic acid



Cyclo-diBA — Toxisches Nebenprodukt in Konservendosen

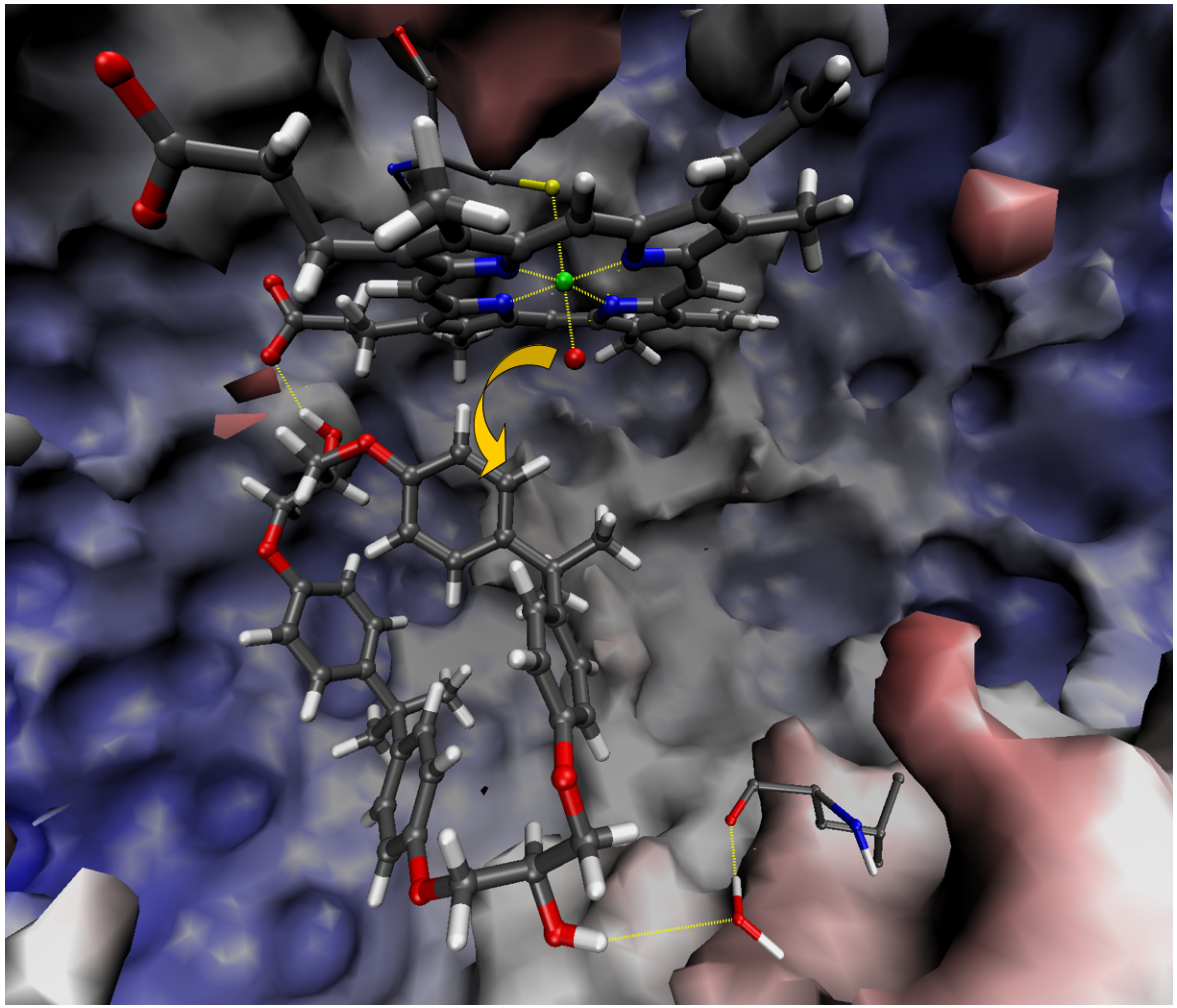
Cyclo-diBA, the cyclic product formed from bisphenol A and diglycidyl ether during the production of epoxy resins. Half of the samples of canned fish in oil collected in April 2010 contained cyclo-diBA with an average concentration of 1025 µg/kg and a maximum of 1980 µg/kg. The majority of the canned meat products contained cyclo-diBA at a mean concentration of 477 µg/kg and a maximum of 1050 µg/kg. All prepared meals, such as ravioli or soups, contained cyclo-diBA with a mean at 287 µg/kg. In canned tomatoes, peas and other vegetables in water or fruits in syrup, no cyclo-diBA was detected (<25 µg/kg). Since no experimental toxicity data are available except for its cytotoxicity, an in silico hazard profiling was performed. Cyclo-diBA seems to be stable and of low reactivity. There is indication for considerable oral bioavailability and for the potential to accumulate in the human body. Cyclo-diBA can be metabolized into cyclic and acyclic compounds.



Zusammenarbeit mit Dr. Beat Brüschweiler, BAG

☞ Food Chem. Toxicol. 2013, 58, 107–115

Cyclo-diBA: Mutanten können für Toxizität verantwortlich sein

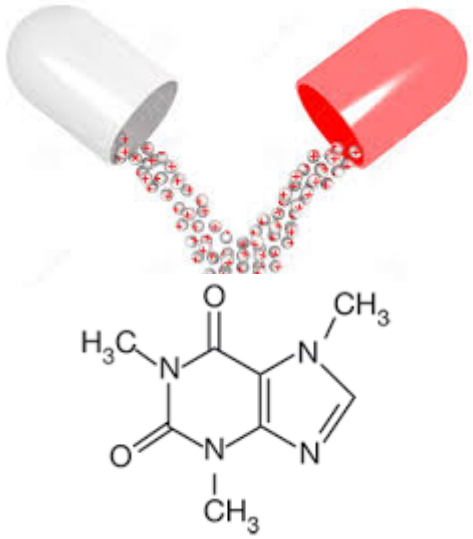


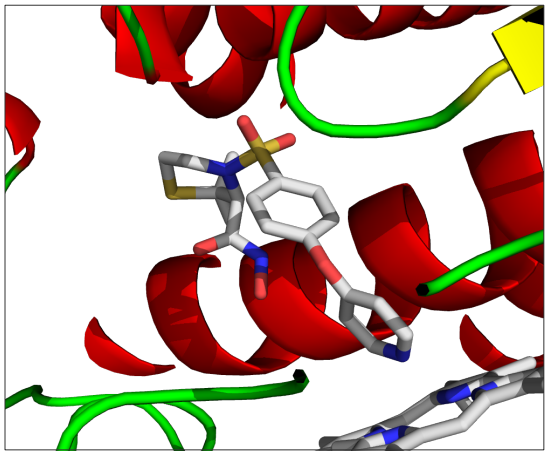
Bindung von Cyclo-diBA an CYP450 3A4: Erklärt Mutanten M5 und M6

Compound	Isomers	ToxPot	Target
Parent compound			
Cyclo-diBA	2	cis = 0.477 trans = 0.377	GR PR
Cyclic metabolites			
M1	1	0.380	PR
M4	4	0.339–0.621	ERβ
M5	4	0.371–0.625	GR
M6	4	0.267–0.295	GR
M7	1	0.369	3A4
Acyclic metabolites			
M2	4	0.359–0.587	PR
M3	4	0.420–0.641	GR
Reference compound			
Bisphenol A	1	0.474	ERβ [1]

[1] Calculated binding affinity = 67 nM (exp. = 93 nM)

☞ Food Chem. Toxicol. 2013, 58, 107–115





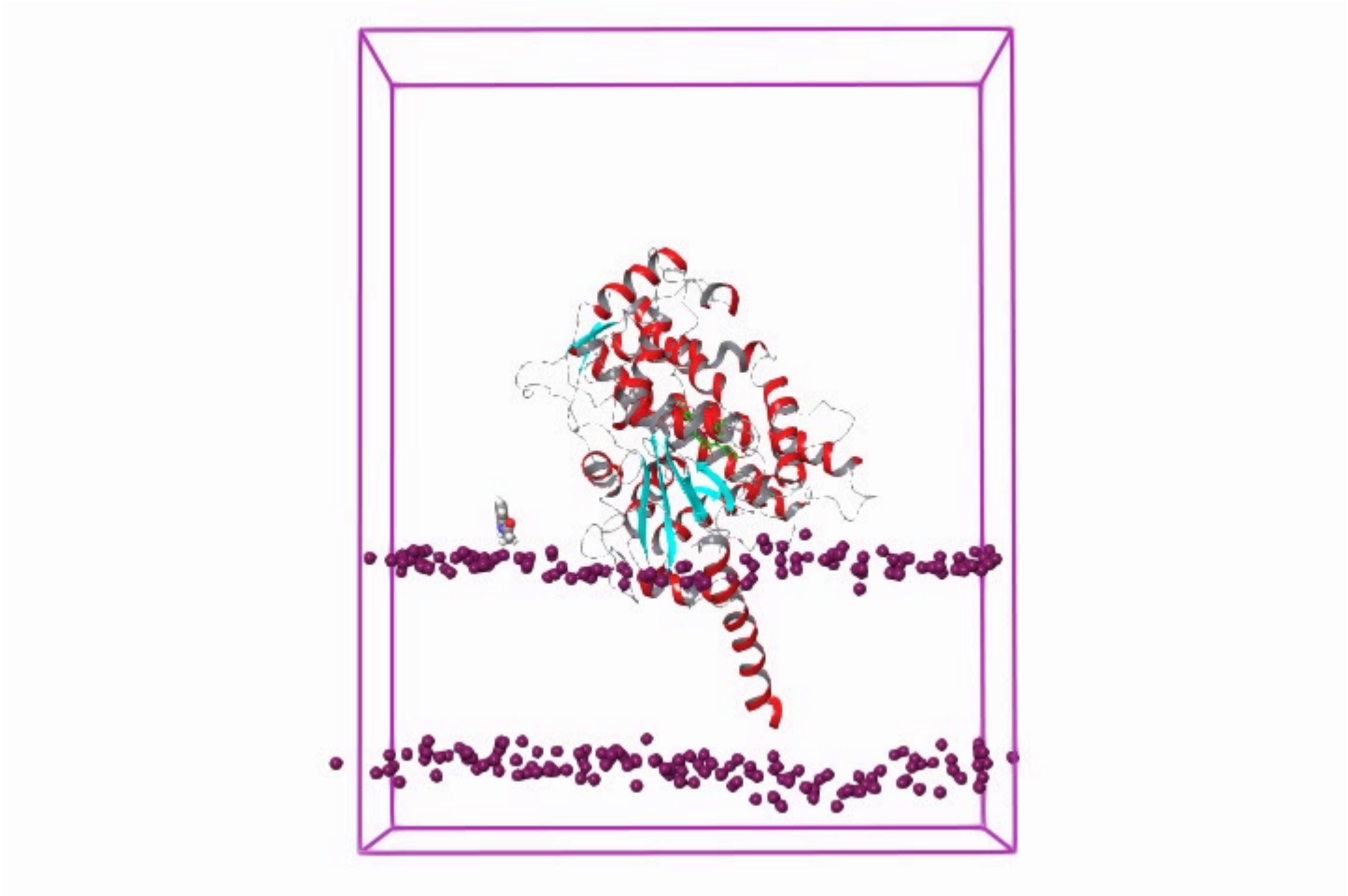
de-CYP-her

deciphering cytochrome issues in drug design

- molecular docking & scoring (VTL analogue)
- (post processing) microsecond scale MD simulations (GPU accelerated)
- structural and functional effects in (single nucleotide) polymorphs / variants
- substrate entrance / exit channels and their function
- residence time, mode of action
- substrate (incl. site of metabolism) / inhibitor prediction

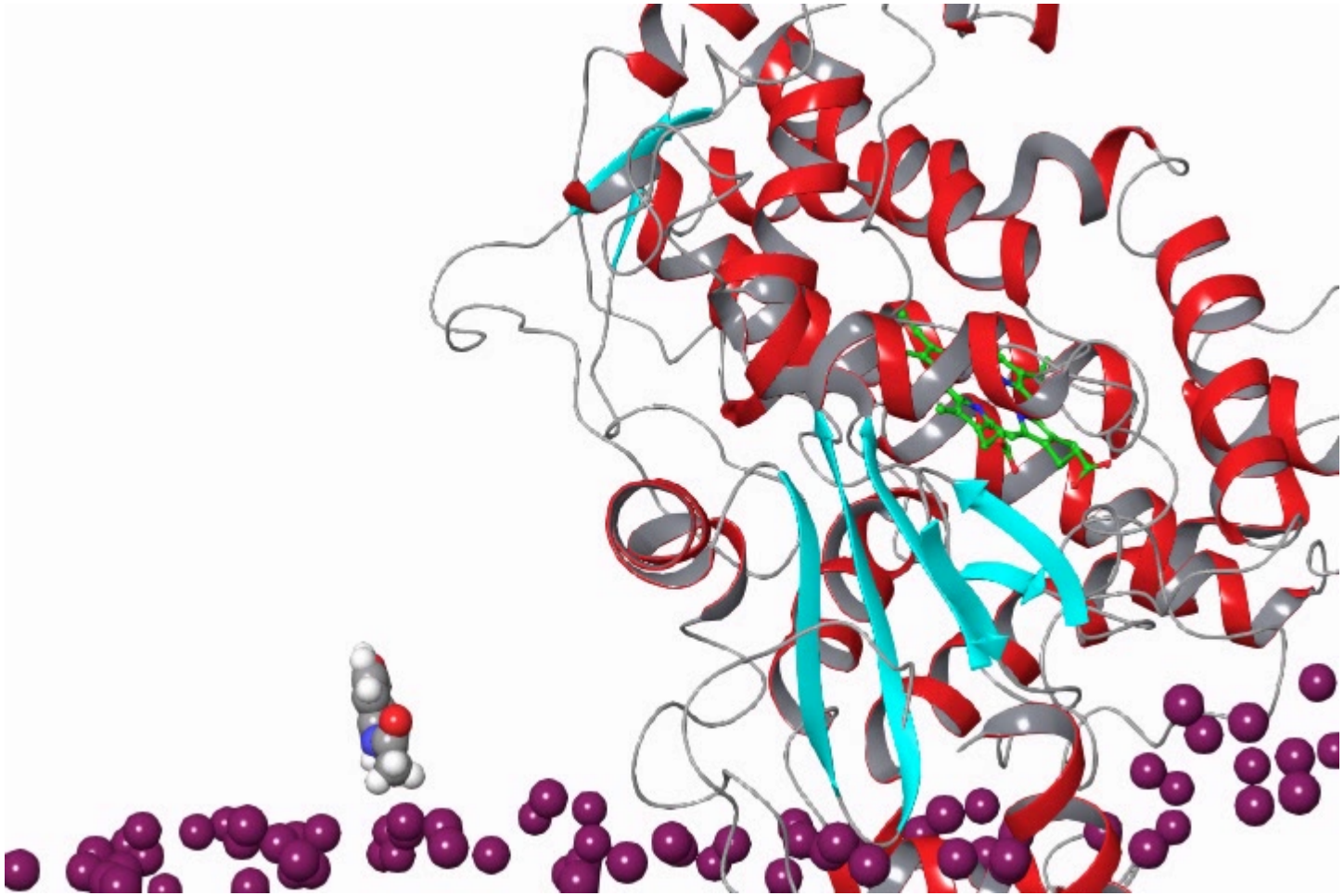
Microsecond-scale MD Simulations

- periodic boundary system with a membrane (80 x 100 x 130 Å)
- cytochrome 2D6 anchored in the membrane
- small-molecule ligand(s) interacting with the protein, membrane, channels…
- software: Desmond, hardware: nVidia Titan X (80 ns / day)



Microsecond-scale MD Simulations

- close-up on ***paracetamol*** entering the active site of the enzyme (heme colored green)
- huge amount of data generated (structure, mechanistic effects, function, theories…)

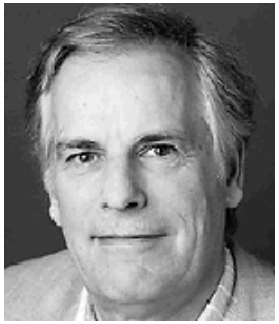


Was bring die Zukunft?

- bessere (akkurate) Kraftfelder, schnellere und schlaue Algorithmen
- noch mehr detaillierte und komplexere Simulationen (grössere Systeme)
- neue Targets (idealerweise: alle relevante Humane Makromoleküle und ihre Komplexe)
- längere Simulationszeiten bei der MDs → grössere Datenmengen
- Personalised Medicine – genetische Information wird in der Computersimulation berücksichtigt
- akkurate Simulationen → sichere Voraussagen → sichere Substanzen

Acknowledgements

Professor Angelo Vedani
Universität Basel &
BiografikLabor 3R



Professor Max Dobler
Emeritus ETH Zürich
Software-Entwicklung

Schweizerischer Nationalfonds
Universität Basel

Fleitmann Stiftung, Luzern
Gazan Stiftung, Zug
Doerenkamp-Zbinden Stiftung, Zürich
Biografiklabor 3R, Basel

nVidia Academic Grant Support