University of Basel

Martin Smieško
Molecular Modeling : Department of Pharmaceutical Sciences : University of Basel : Switzerland

Computer Modeling of Adverse Effects

Focus on Application of the Structure-Based Methods in Predicting Protein-Mediated Toxicity

- **Semester plan (21.9. 2017 - 2.11. 2017) : Thursdays**

September 21	*Introduction to Modeling of Drugs Side Effects (Part 1)*
	Introduction to Modeling of Drugs Side Effects (Part 2)
September 28	*VirtualToxLab – Predicting the Protein-Mediated Toxicity*
	Project Assignment & Introduction to the Software
October 5	*Standalone work, discussions*
October 12	*Standalone work, discussions*
October 19	*Standalone work, discussions*
October 26	*Standalone work, discussions*
November 2	*Presentations (~15 x 5-7 min, 5-7 slides)*

- **1 Credit Point (30 hours) – no exam – electronic report (PDF) instead**

- **Recommended literature**

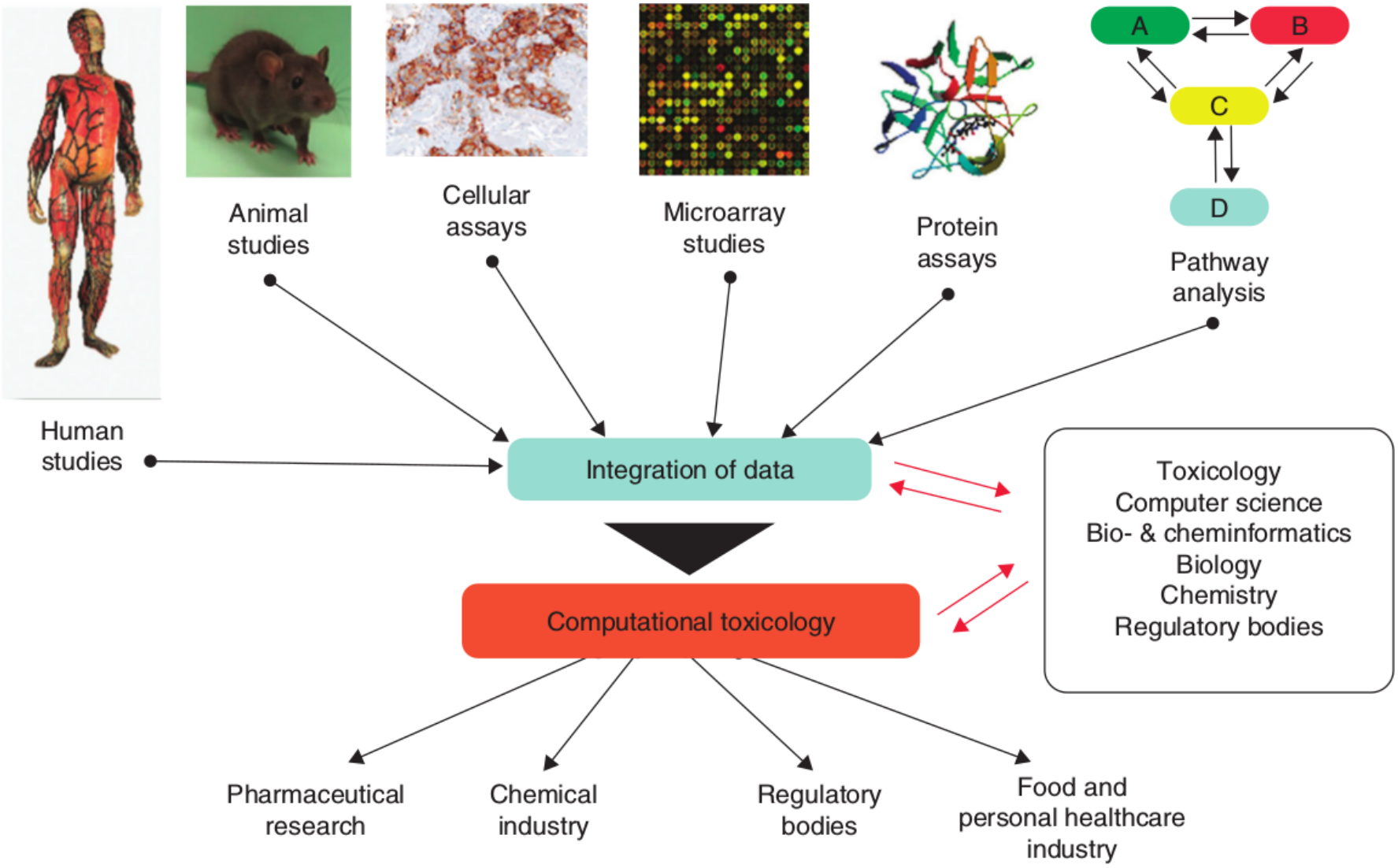
R.J. Vaz, T. Klabunde: Antitargets (ISBN: 978-3-527-31821-6)

N. Greene, W. Pennie: Computational toxicology, friend or foe? Toxicol. Res., 2015, 4, 1159–1172

The goal of the lecture series

Understanding the basic concepts of molecular simulations associated with toxicity endpoints. Use of the *VirtualToxLab* and other software to estimate the toxic potential of drugs and chemicals. Mechanistic interpretation of the results at the molecular level.

- Modeling toxic phenomena → simulation of underlying molecular processes (e.g. compound binding at the macromolecular receptor)
- Methods and technologies for predicting toxicity endpoints
- *VirtualToxLab* and other software
- Mechanistic interpretation
- Endocrine and metabolic disruption
- Interference with the hERG channel
- Comprehensive study of selected compound(s)



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

Why do we need computational (predictive) toxicology

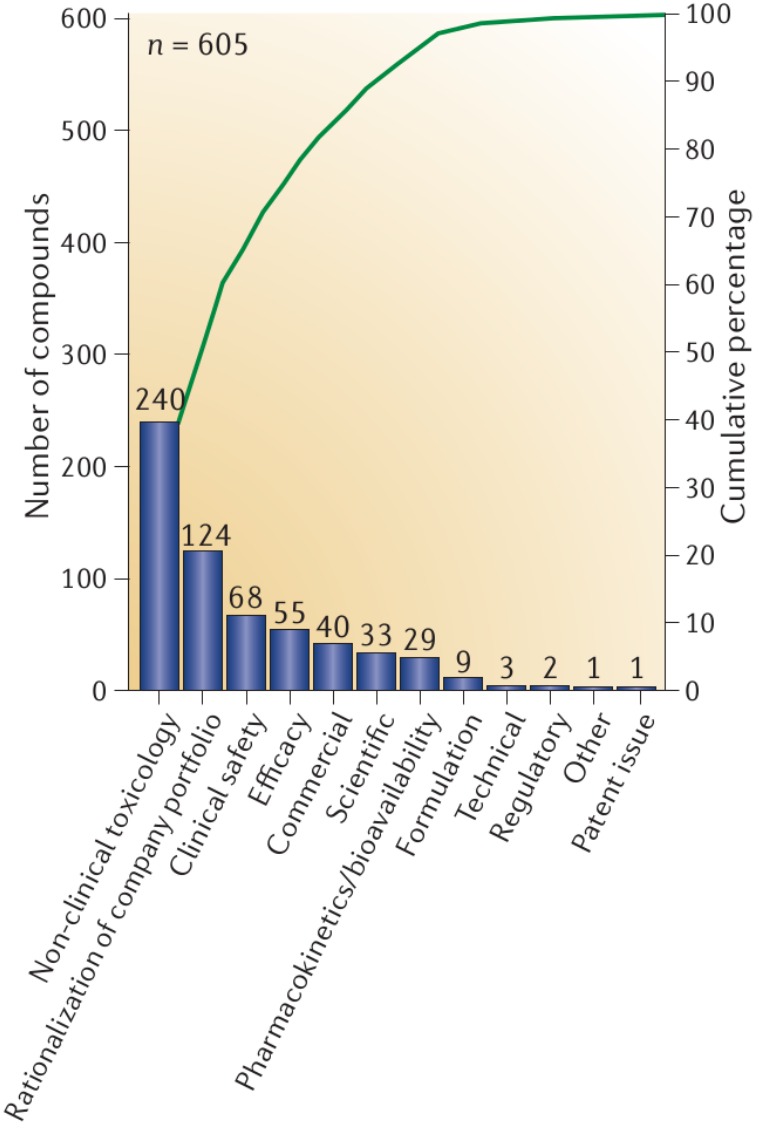
- Every single compound entering production must be throughly tested and characterized:
  - cosmetics (UV filters, fragrances...)
  - additives (polymer, flame retardants...)
  - agrochemicals
  - drugs
  - colorants & dyes

- 3R (reduction, replacement, refinement)

- Regulatory needs EC, EPA... (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)

- may occur as a **reaction to a medication** or as a result of **incorrect dosage** or **drug interactions**. Beginning treatment with a new medication, prolonged treatment, ceasing treatment or adjusting a patient's dosage may also cause a patient to experience unwanted reactions to a medication (e.g. antihypertensives, anticoagulants)

- result of the (unwanted) **interaction between the compound** and bio(macro)molecules involved in **biosynthesis, signal transduction, transport, storage, or metabolism**

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

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

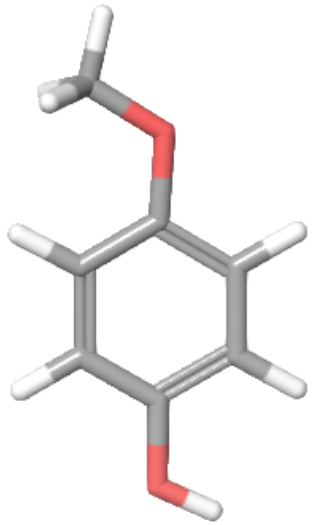
- **oral** : most frequent and best studied because of pharma industry

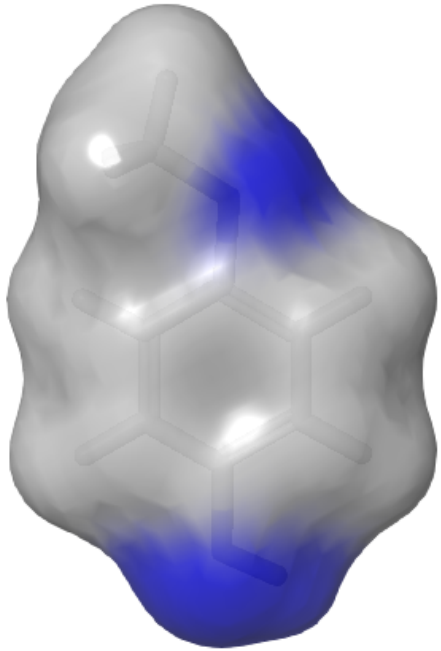
Lipinski's rule of 5

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

Veber rules

- PSA < 140 Å²
- nRotBond < 10





MW = 124, nHBdon = 1, nHBacc = 2, LogP = 1.2, PSA = 31 Å², nRotBond = 1

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

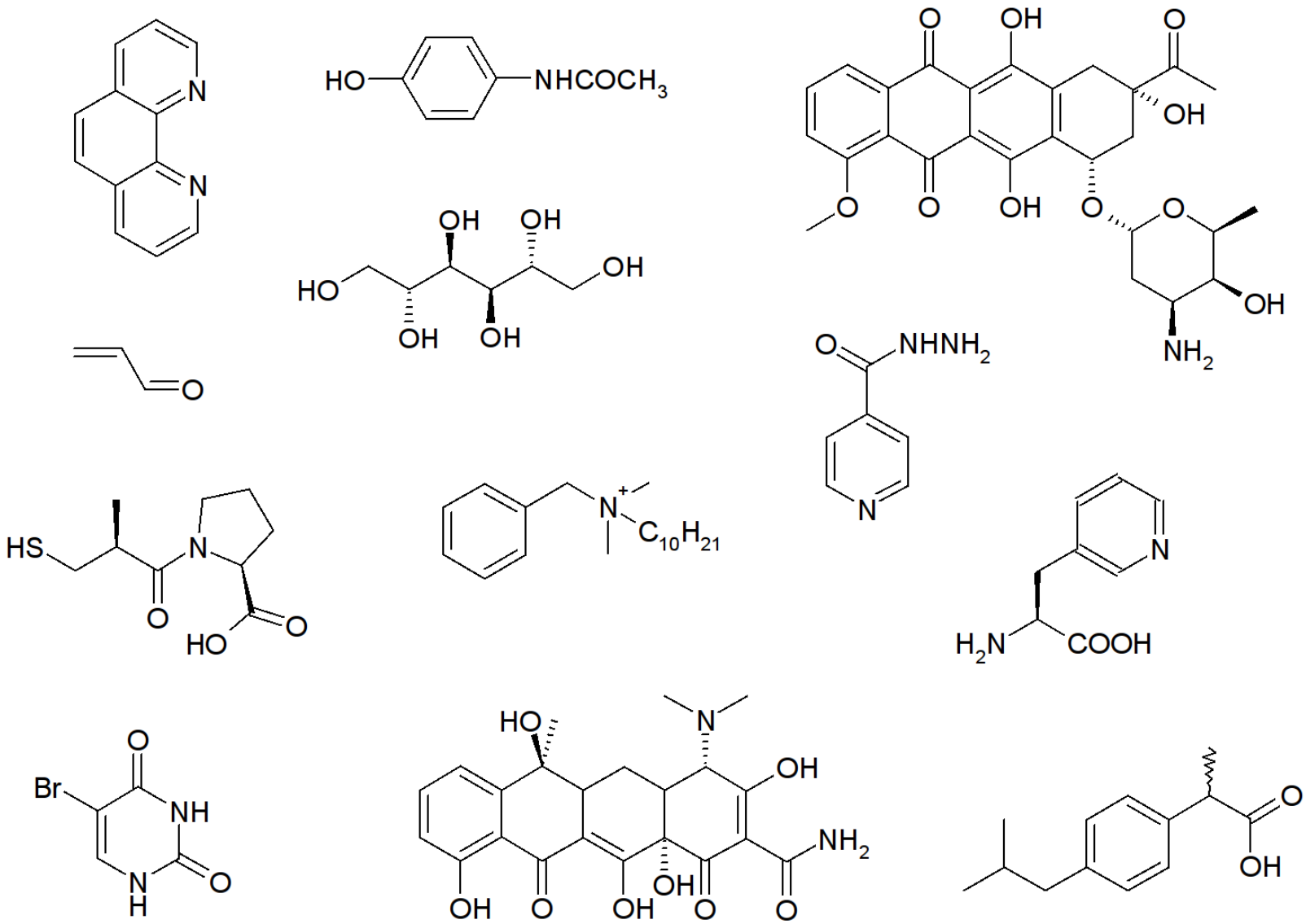
- **inhalation** : airborne particles (fumes, fentanyls), volatile chemicals, gases…

- **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

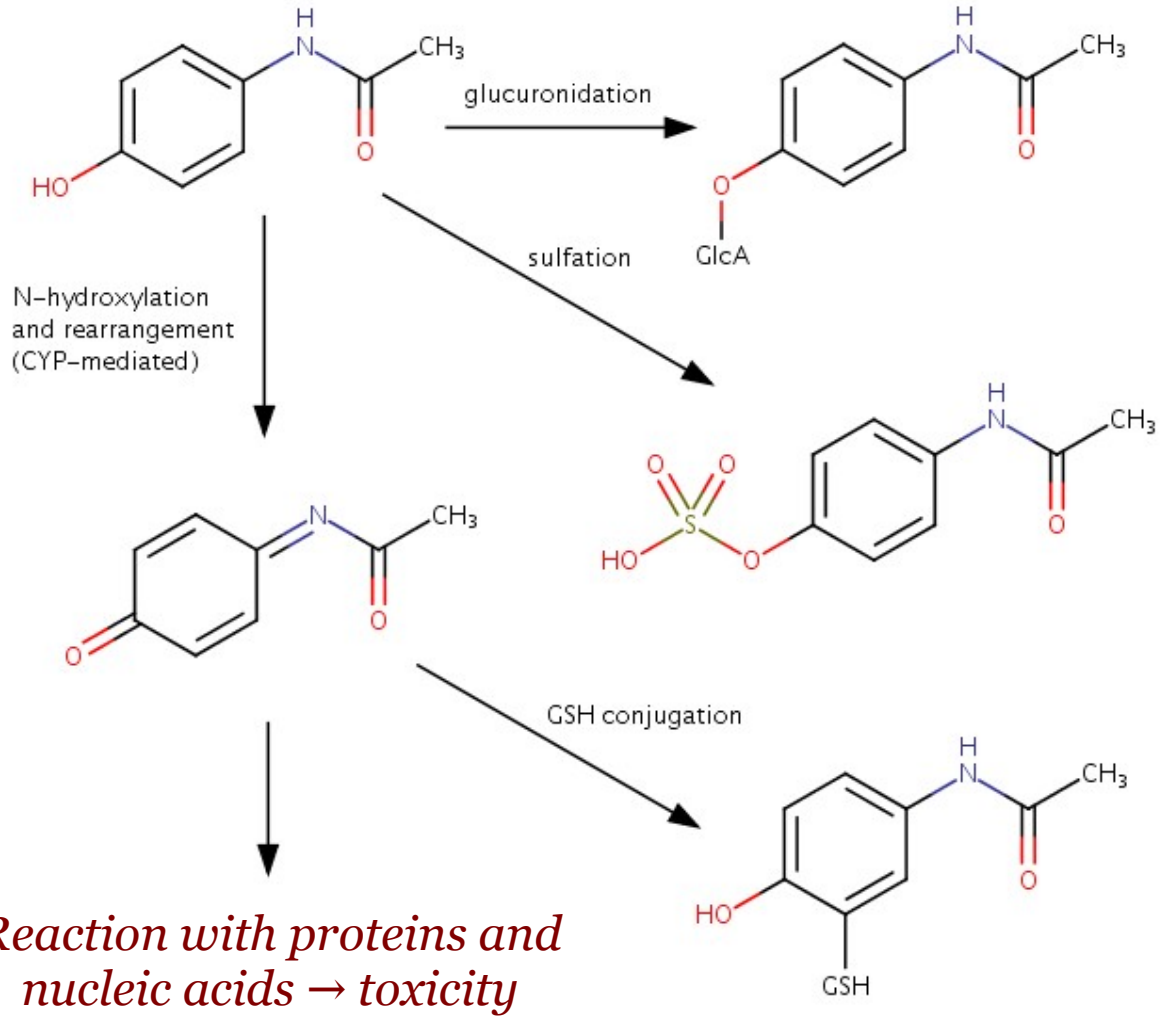
Many aspects can be detected by simple looking & thinking!



Properties to note: shape, polarity, lipophilicity, molecular weight, chelating capabilities, solubility, similarity to primary metabolites, Lipinski and Veber rules...

... however, side effects may vary, as individuals often will respond differently to medications depending on a variety of factors such as: age, overall health, ethnicity, gender, severity of the condition or disease being treated...

Paracetamol
cyclooxygenase inhibitor



Software Tools for Toxicity Evaluation - Databases

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

Software Tools for Toxicity Evaluation - Databases

ToxCas 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/

Software Tools for Toxicity Evaluation - Databases

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/

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):

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

- 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

Software Tools for Toxicity Evaluation – Online Tools

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/

Software Tools for Toxicity Evaluation (Free)

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/

Software Tools for Toxicity Evaluation (Free)

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

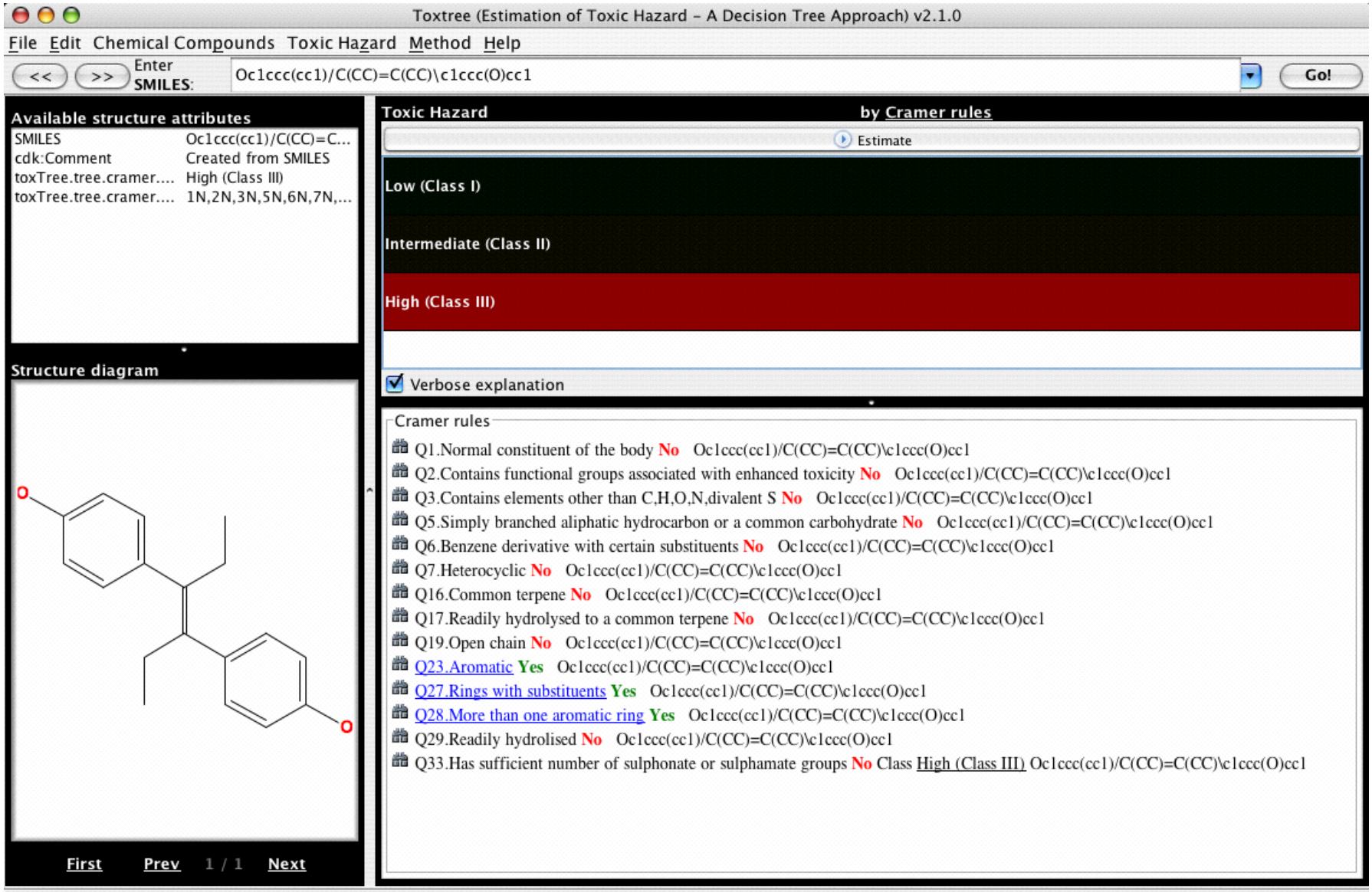
BioEpisteme - http://www.prousresearch.com

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

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

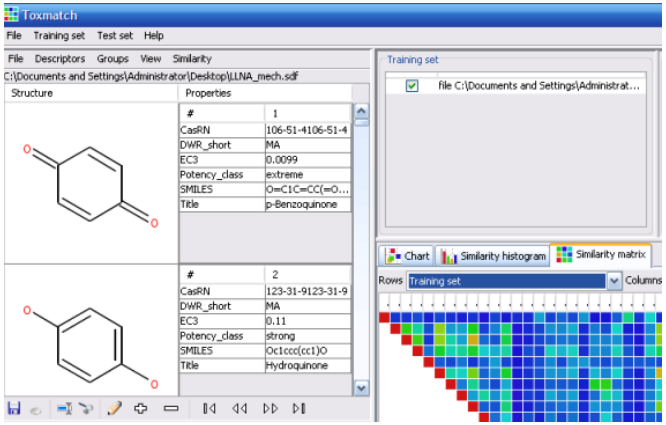
Molcode Toolbox - http://molcode.com

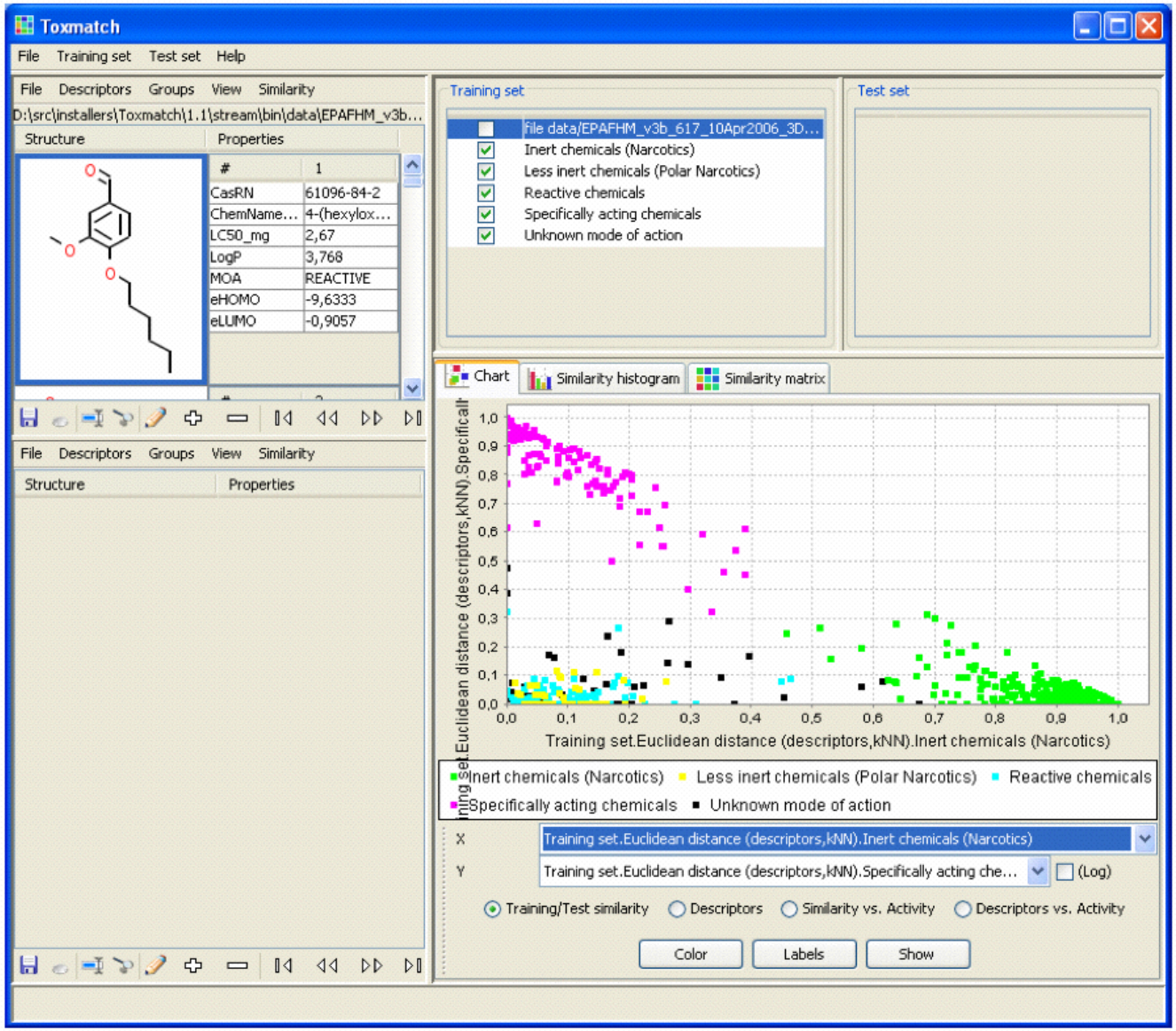
Expert System — ToxTree



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

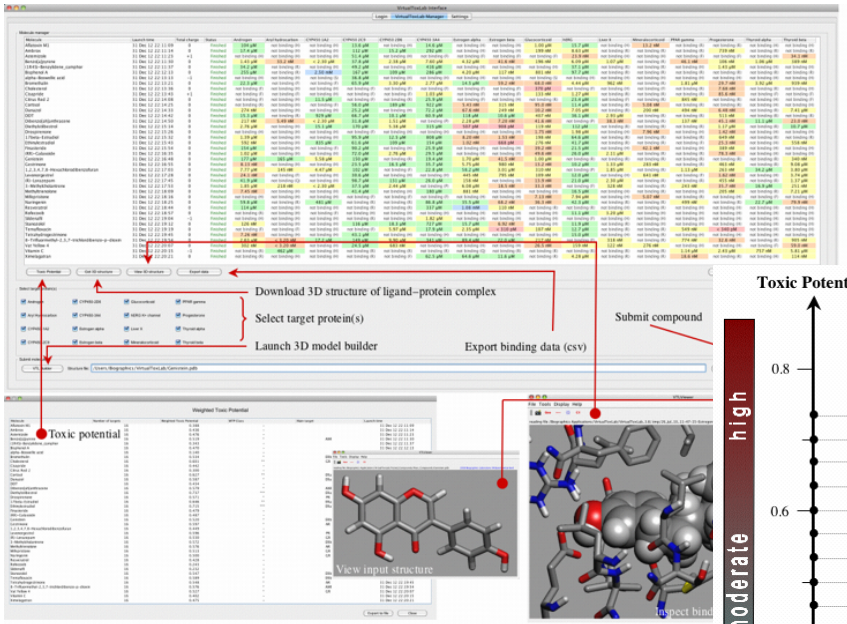
Static Modeling – ToxMatch





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

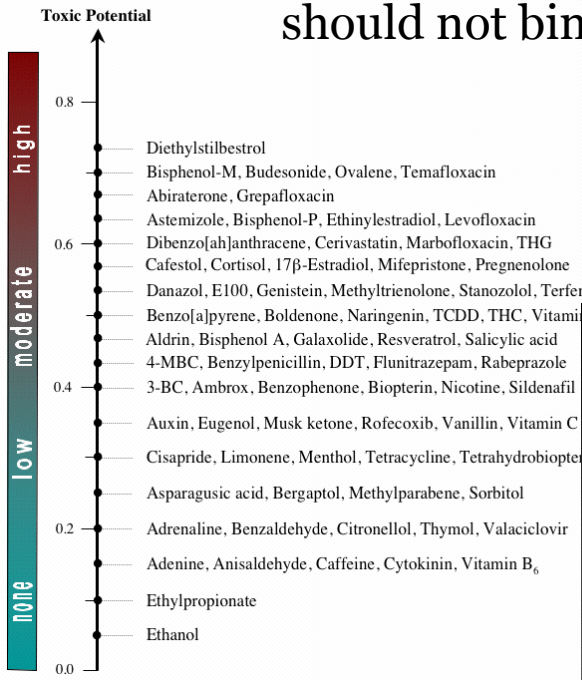
“Ab initio” 3D-Modeling — VirtualToxLab

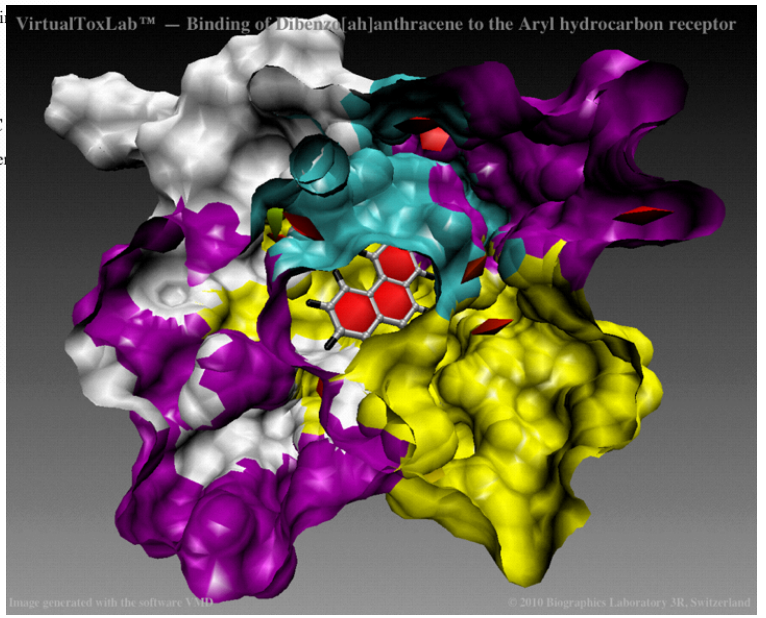


☞ http://www.virtualtoxlab.org

Simulation and Quantification of the protein-ligand interaction at the atomic level

In toxicology, proteins to which ligands should not bind are termed anti-targets



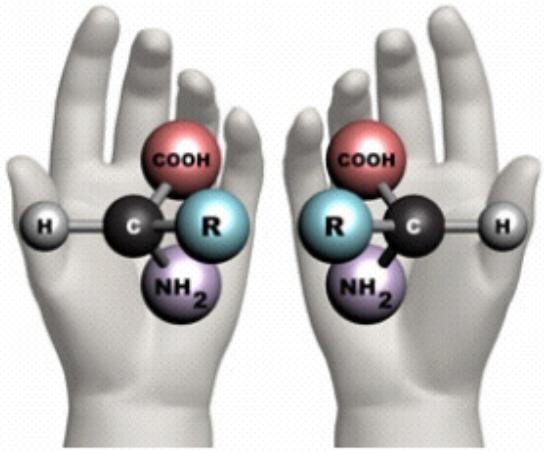


Key-lock Concept & Protein-mediated Toxicity

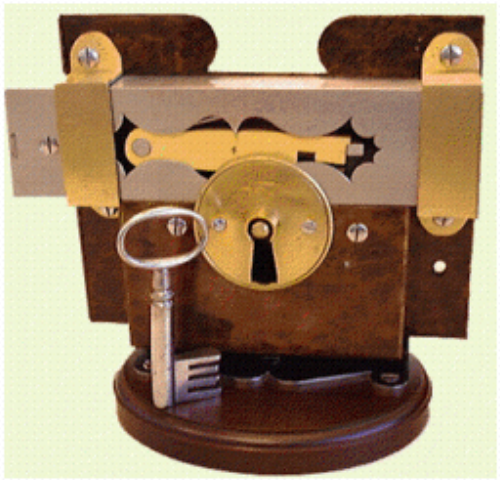
nobelprize.org

Schloss-Schlüssel-Prinzip (1894)

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

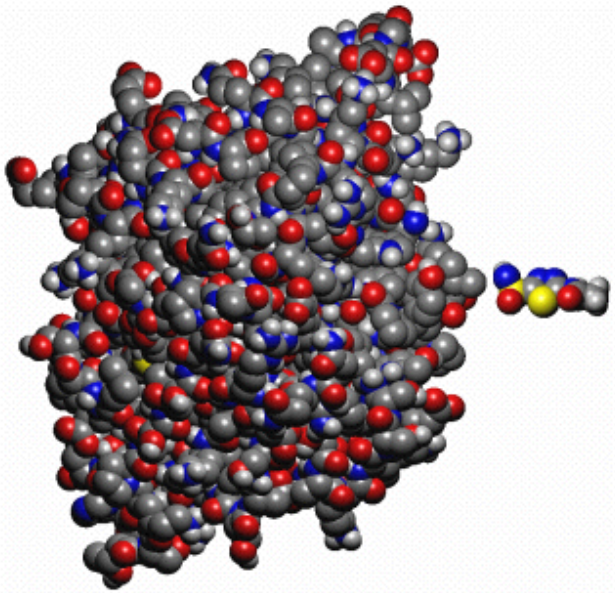


enantiomorphic.blogspot.com



oldlockandkeyco.co.uk

muarchives.missouri.edu



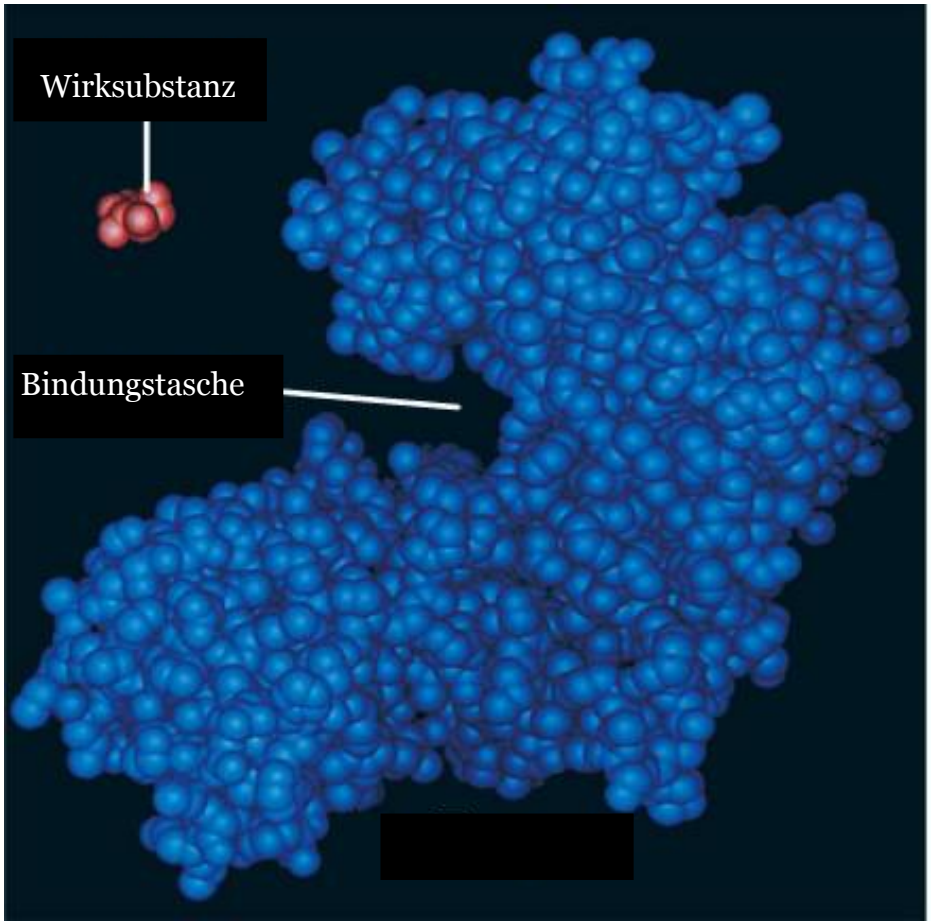
Carboanhydrase (Schloss) + Acetazolamid (Schlüssel)

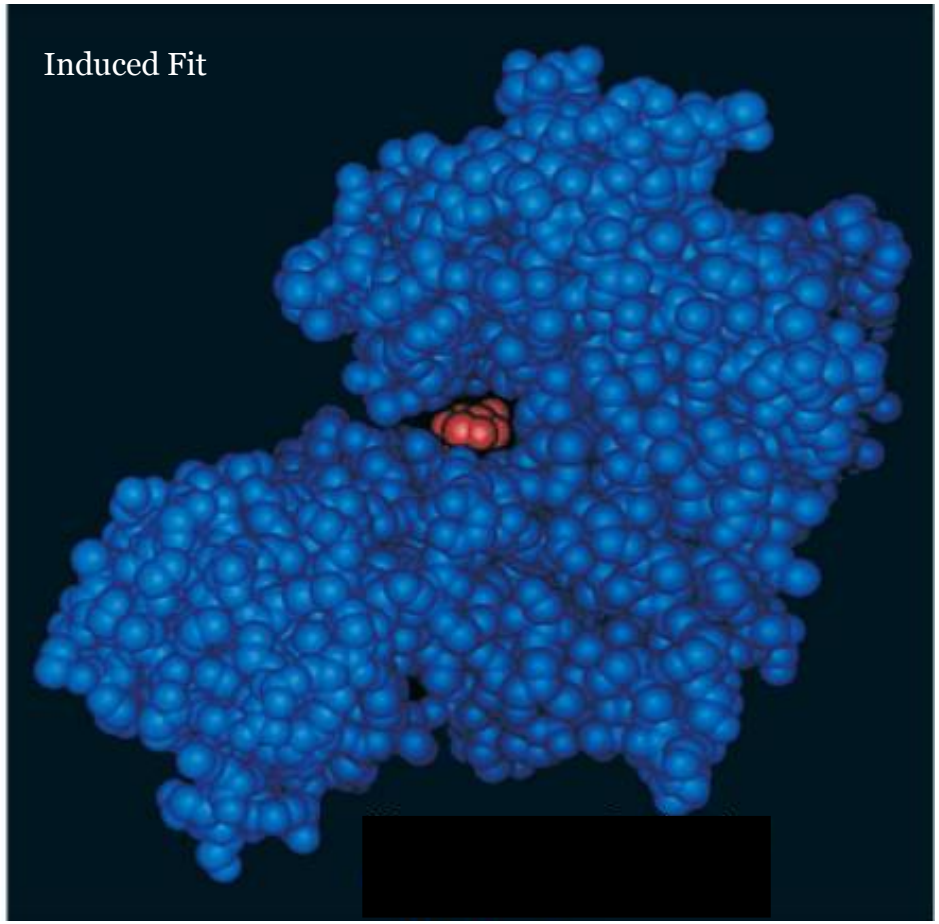
"Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glykosid zueinander passen müssen, wie Schloss und Schlüssel, um eine chemische Wirkung aufeinander ausüben zu können."

Emil Fischer (1894)

Key-lock Concept & Protein-mediated Toxicity

Induced-fit



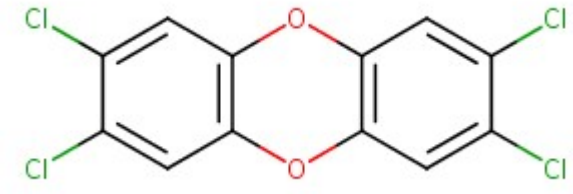


bio1151.nicerweb.com

Protein-mediated Toxicity

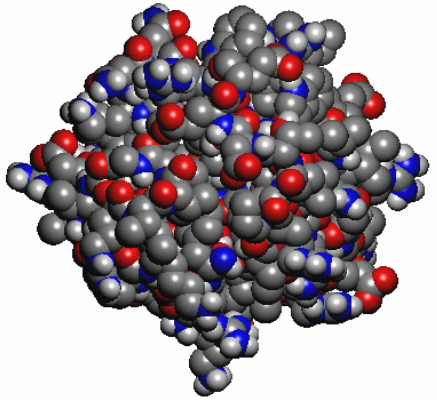
- Most studied, most relevant, most specific
- Central concept : effects are dose dependent

2,3,7,8-tetrachlorodibenzo-p-dioxin (AhR agonist)

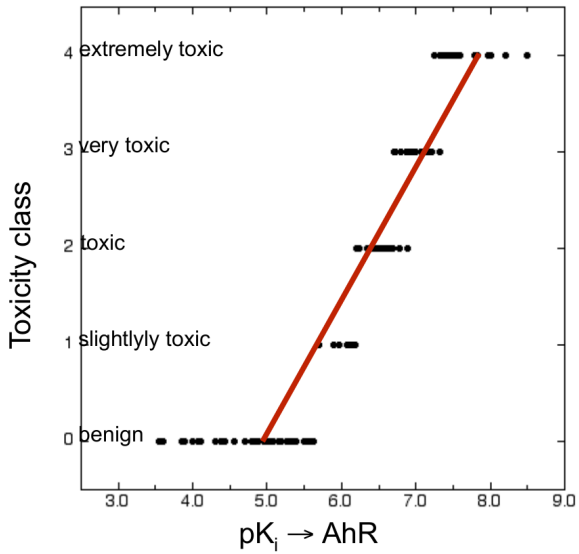


binding

Aryl hydrocarbon receptor



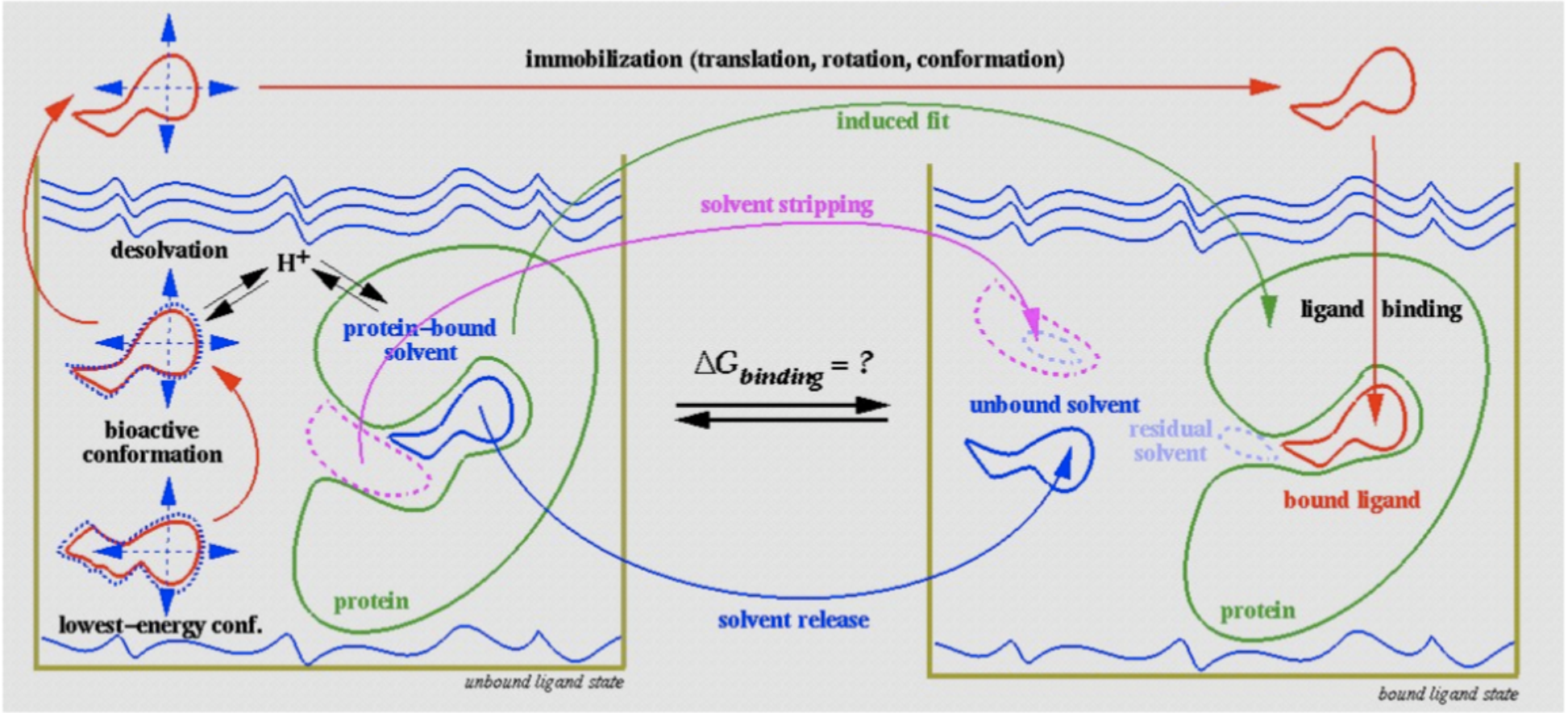
signal transduction & response



before *acute* *after*

Sorg, O. et al. *The Lancet* (2009), 374, 1179–85.

Biophysical model for 3-dimensional concepts



$$\Delta G_{binding} = E_{ligand\text{-}protein} - E_{ligand\text{-}solvent} - E_{ligand\text{-}strain} - E_{linduced\text{-}fit} - T\Delta S - E_{protein\text{-}solvent}$$

Force field (Molecular Mechanics)

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

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

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

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

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

Vedani et al. *J Am Chem Soc* (1990), 112, 4759

Compound of interest

drug candidate, natural compound, agrochemical...

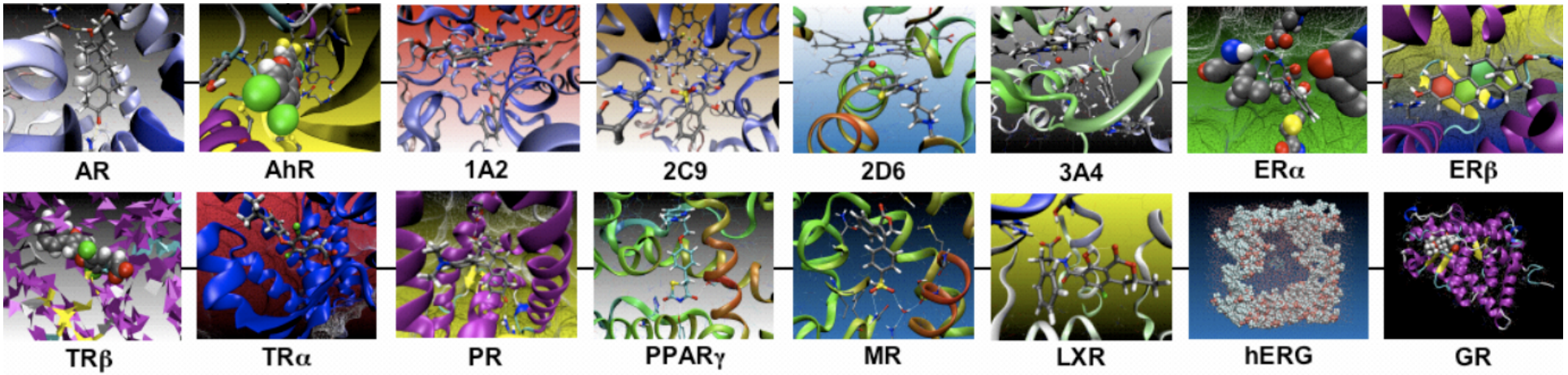
+

State-of-the-art structure based design methods

molecular docking + scoring

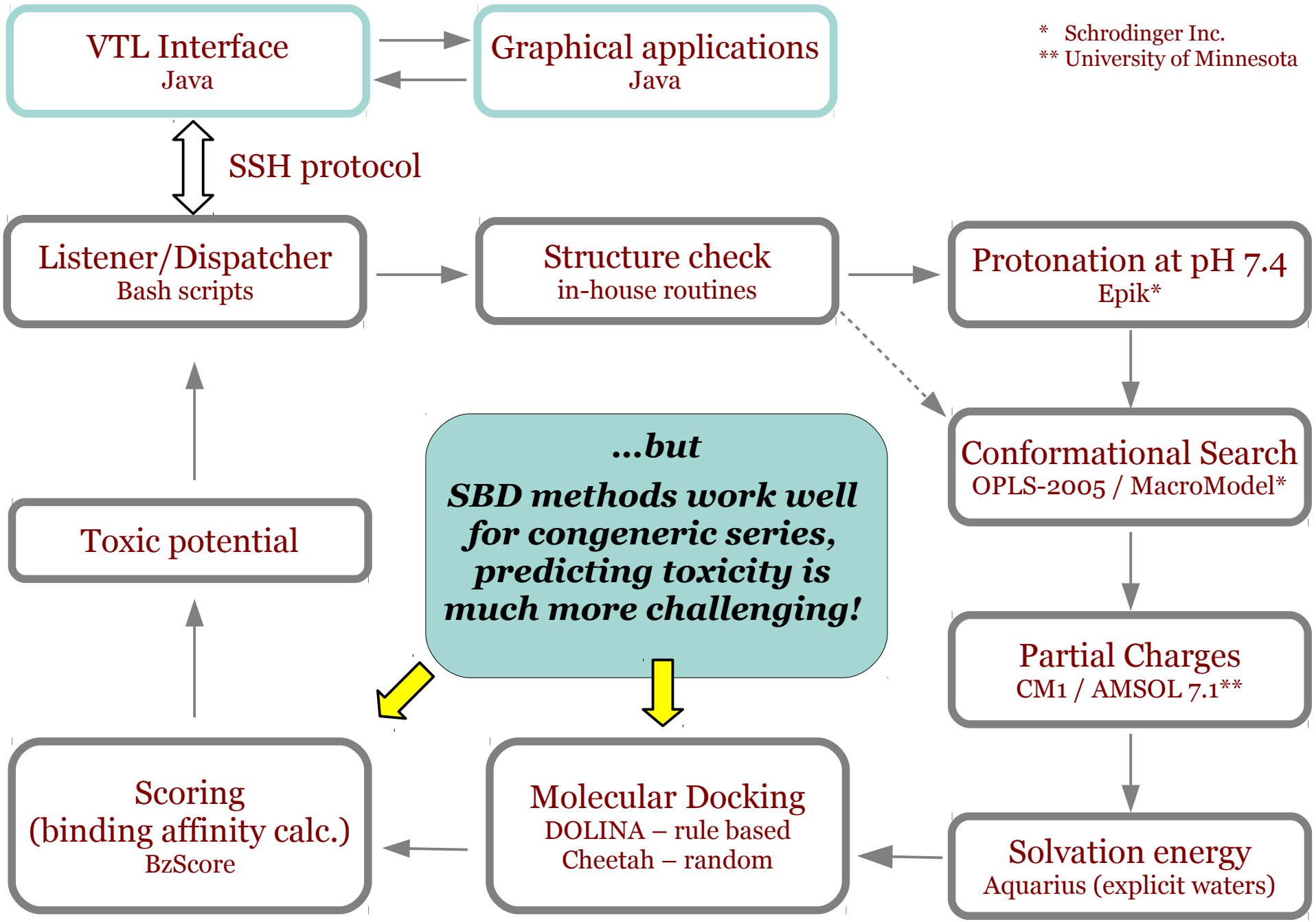
+

an array of relevant protein target structures



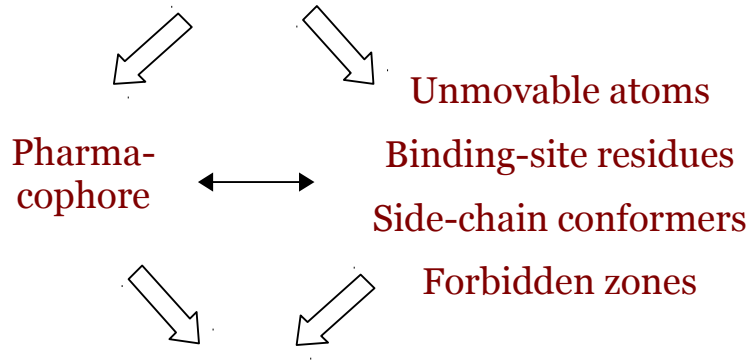
Off-target binding profile

Toxic Potential


VTL Interface
Java
Graphical applications
Java
* Schrodinger Inc.
** University of Minnesota
SSH protocol
Listener/Dispatcher
Bash scripts
Structure check
in-house routines
Protonation at pH 7.4
Epik*
Conformational Search
OPLS-2005 / MacroModel*
Partial Charges
CM1 / AMSOL 7.1**
Solvation energy
Aquarius (explicit waters)
Molecular Docking
DOLINA – rule based
Cheetah – random
Scoring
(binding affinity calc.)
BzScore
Toxic potential
...but
SBD methods work well for congeneric series, predicting toxicity is much more challenging!

How to dock (dissimilar) compounds?

Template–Protein Complex



Ligand conformers

⇩

Filtering duplicate &
self-folded structures
Accessibility check

⇩

Extended Pharmacophore ⇨ Matching to solutions ⇦ **Pharmacophores**

⇩

Pose Generation: rotations & translations + bump check

Induced-Fit: single & combinatorial side-chain rearrangement

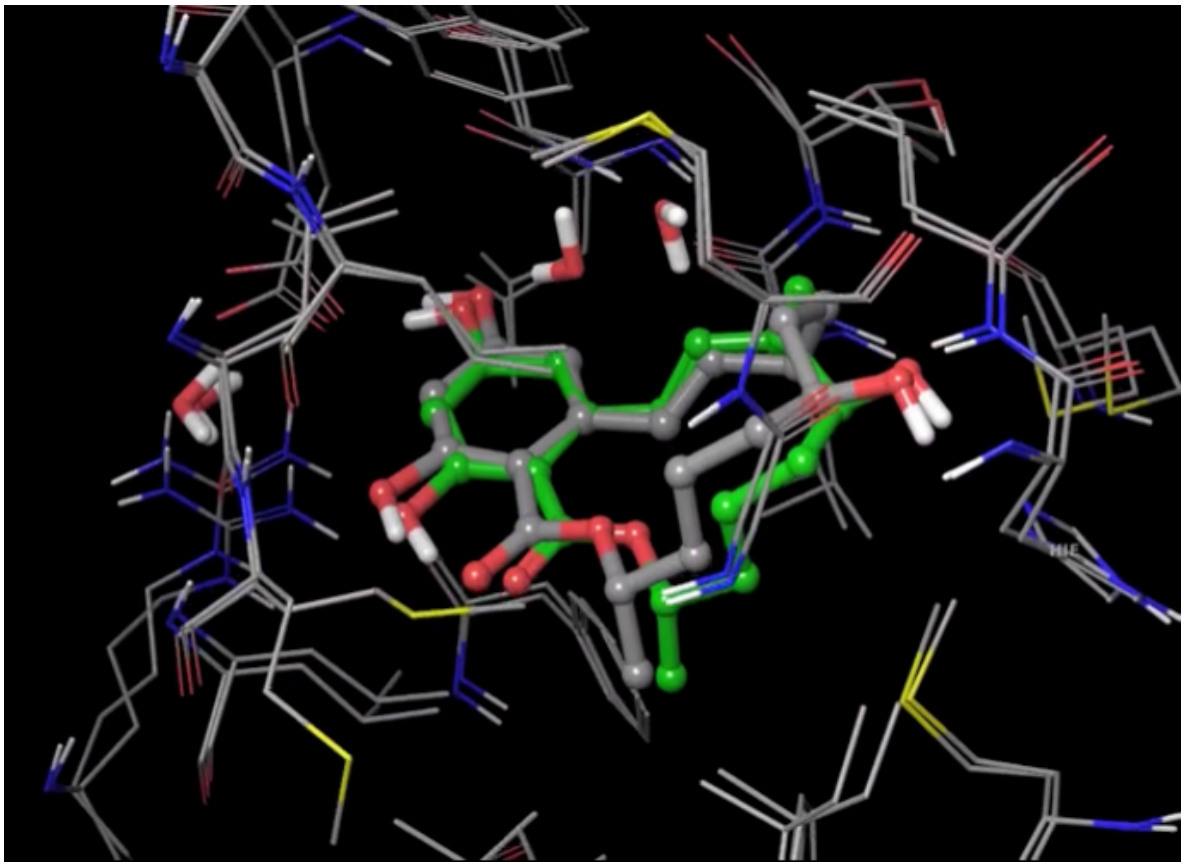
Scoring: H-bonding & lipophilic interaction energy

⇩

Top Ligand–Protein Complexes

Molecular Docking

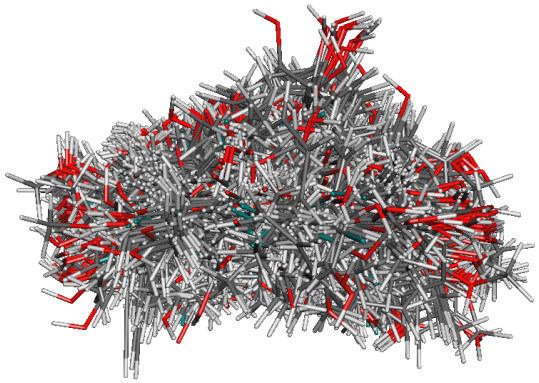
a-Zearalenol docked to the Estrogen receptor α

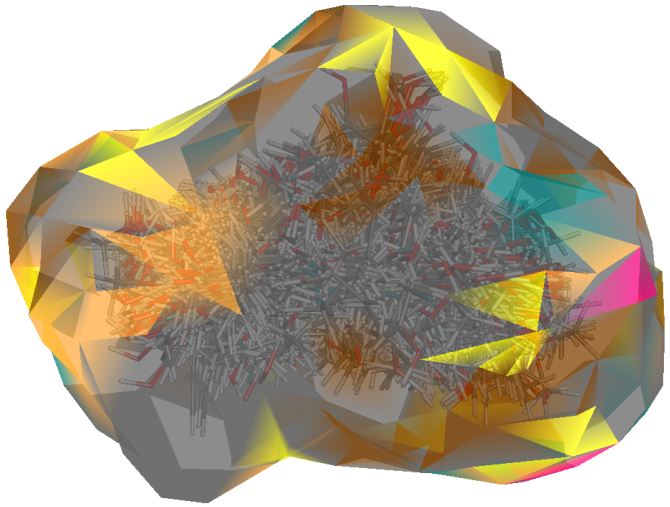


- Grey carbon atoms – docked pose
- Green carbon atoms – reference crystal structure (PDB ID: 4TUZ)

Scoring – Trained QSAR Model

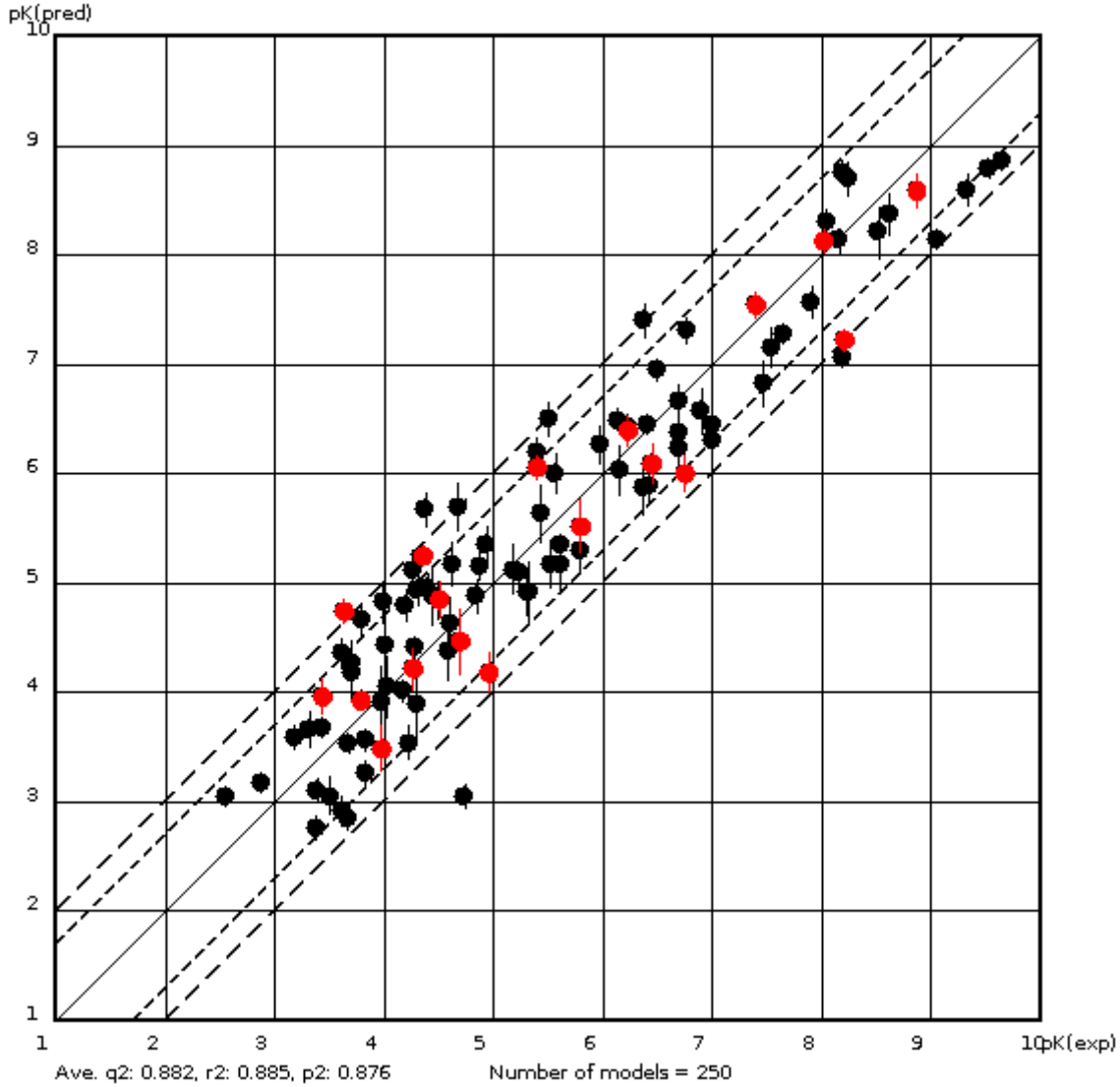
Protein-based alignment



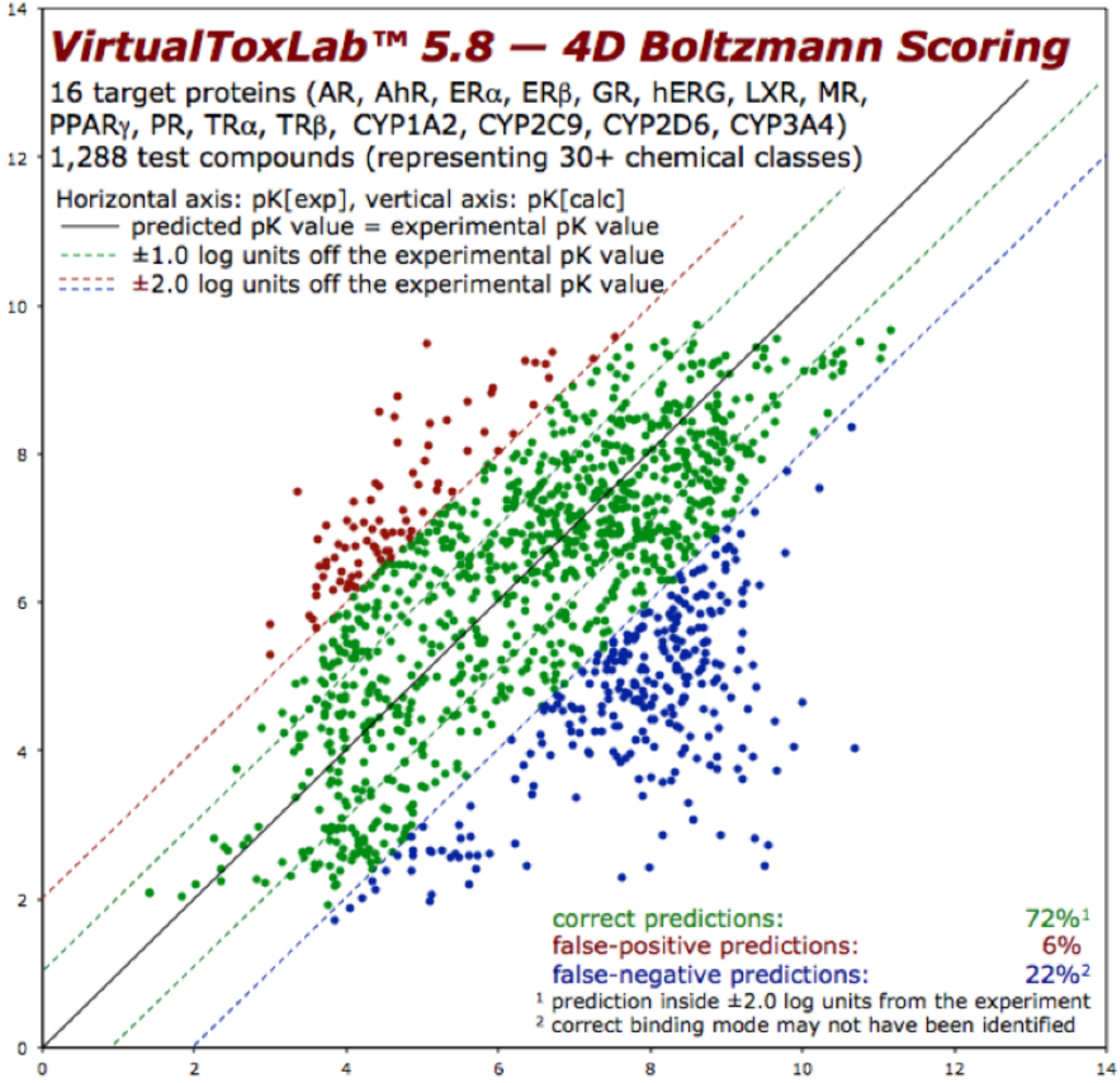


Receptor surrogate

Binding energy

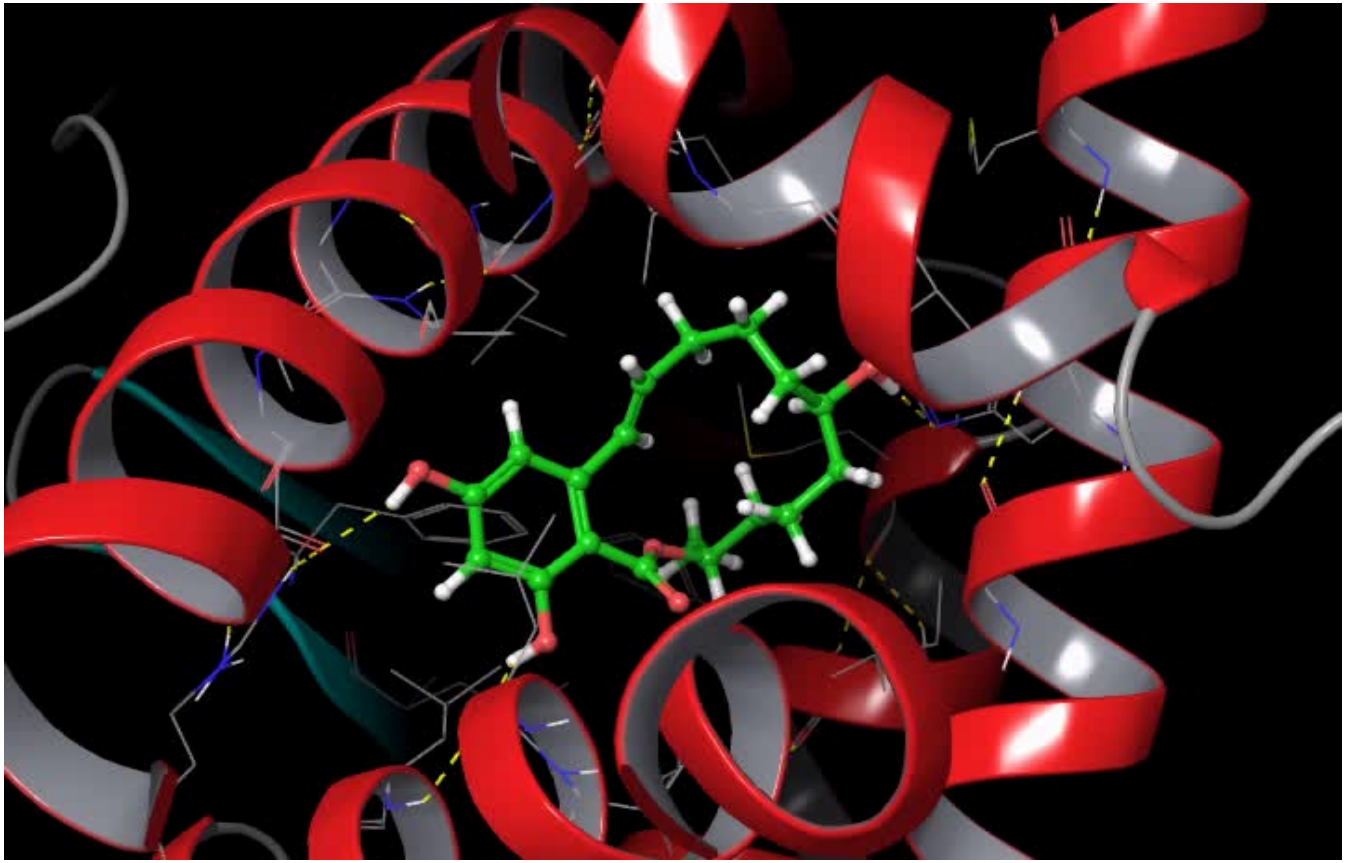


Direct Scoring



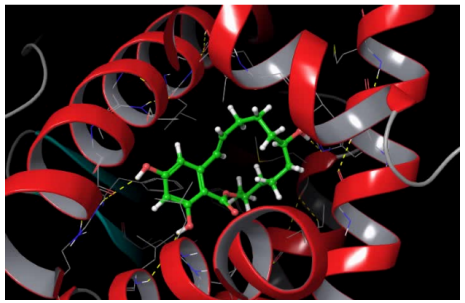
Molecular Dynamics

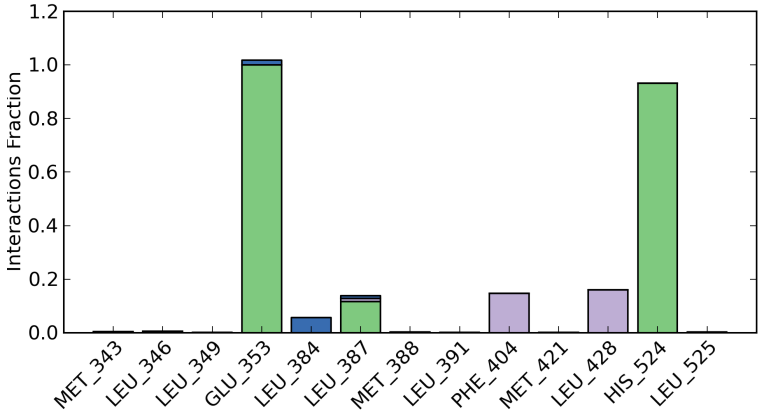
4.8 ns MD simulation of docked α-zearalenol at the Estrogen receptor α

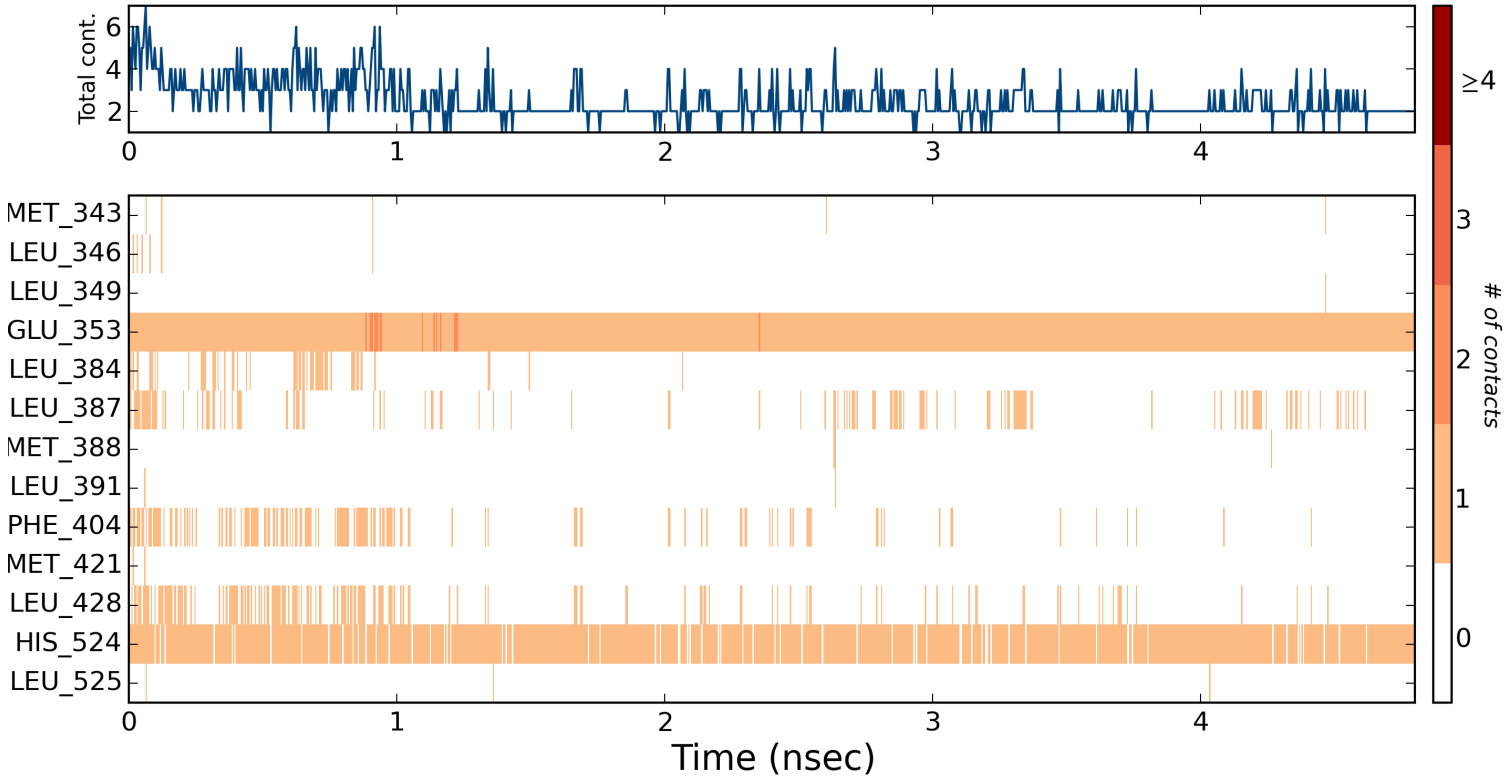


MD run using software Desmond, D.E.Shaw, New York

Molecular Dynamics







Predicting Protein-mediated Toxicity

Advantages

- cost-effective and ethical alternative to experimental testing
- mechanistic interpretation, hints for preventing the off-target binding
- human protein structures, no interspecies issues

Disadvantages

- needs an X-ray structure or a homology model
- needs large datasets if QSAR used
- usually not a high-throughput technology (yet)
- can lead to false positives / false negatives

Outlook

- continuously improve performance by smarter algorithms and methods
- improve accuracy of the scoring
- include new targets
- linking to “omics”

University of Basel – the oldest Swiss University (1460)

Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim) father of toxicology ("*Dosis facit venenum*"), studied medicine at University of Basel in 1509