University of Basel

Small Molecules, Big Opportunities:

An *in silico* Platform for Off-target Profiling And Drug Repurposing

Martin Smieško
Computational Pharmacy Group, Department of Pharmaceutical Sciences, University of Basel
Swiss Center for Human And Applied Toxicology, Basel
Swiss Institute of Bioinformatics, Lausanne

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Drug Development Pipeline: costs 1-2 Billion USD per Drug

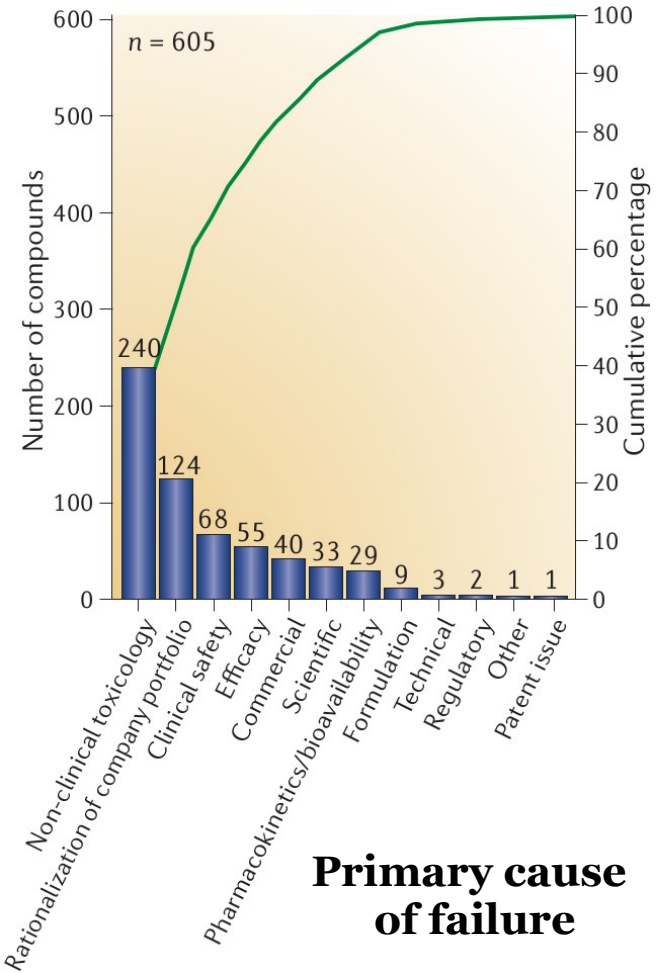
Target Discovery	Target Validation	Lead Discovery	Lead Optimization	Pre-clinical ADMET	Clinical Trials
Expression analysis *in vitro* function Bioinformatics Computational chemical biology	*in vivo* validation Druggability assessment	High-Throughput screening Combinatorial chemistry Fragments **Structure-based 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

Why New Approach Methodologies ?

- every single compound entering production must be thoroughly tested and characterized:
  - drugs (human & veterinary)
  - cosmetics (UV filters, fragrances…)
  - additives (flame retardants, plasticizers…)
  - food contact materials and polymers
  - agrochemicals (pesti-, fungi-, herbi-cides)
  - colorants, dyes, pigments…
- 3R: reduction, replacement and refinement of animal testing
- regulatory needs: EC, EPA, BLV… (REACH)
- drug attrition rates getting worse and development more costly

Knowledge gathered can be used to rationally
explain and **avoid toxic phenomena !**



Primary cause of failure

Waring M., Arrowsmith J., Leach A. et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat Rev Drug Discov* 14, 475–486 (2015)

Aim at the Right Target!

*"During preclinical testing, 75% of safety closures were compound-related – that is, they were due to '**off-target**' or other properties of the compound other than its action at the primary pharmacological target – as opposed to being due to the primary pharmacology of the target."*

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

On-target binding
(*primary pharmacology*)

- a **desired** interaction of a typically small molecule with biomacromolecular receptor(s) exhibiting preferably a **high** degree of specificity (e.g. astemizole at the histamine receptor)
- initiates a cascade of actions resulting in **curative** or at least illness **compensating** effect(s)

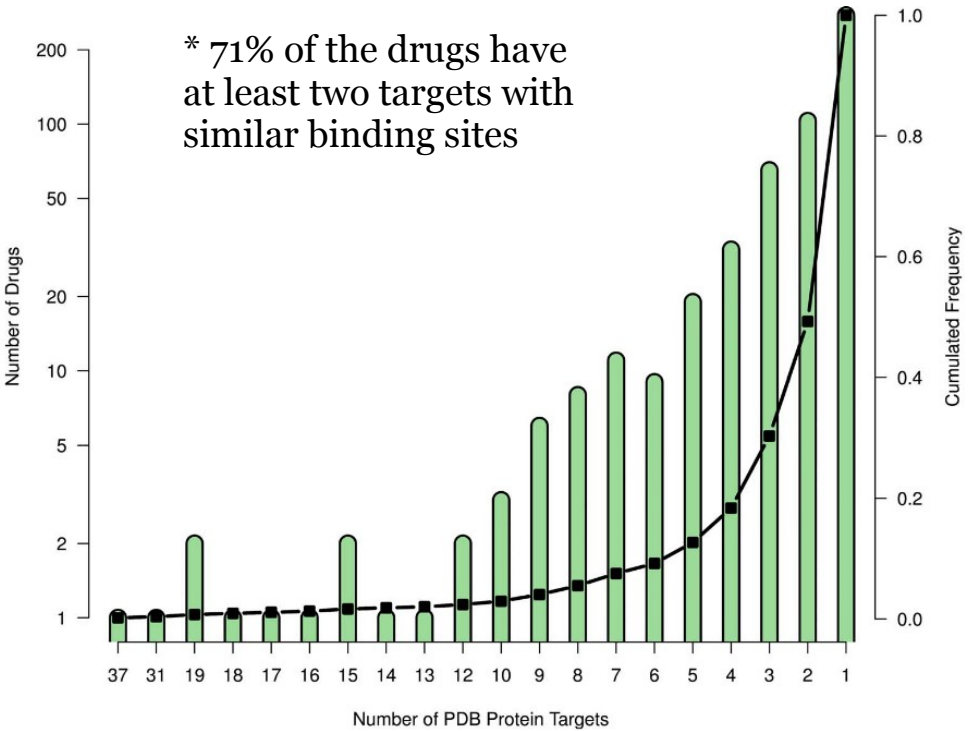
Off-target binding
(*anti-target, promiscuous binding, secondary pharmacology*)

- an **undesired** interaction of a typically small molecule with biomacromolecular receptor(s) exhibiting a **certain** degree of specificity (e.g. astemizole at the hERG ion channel)
- initiates a cascade of actions resulting in **adverse** effect(s) (astemizole → cardiotoxicity)

Even approved drugs bind to multiple on/off-targets

Diclofenac (cyclooxigenase inhibitor)	**PDB ID**
- human Transthyretin	3CFQ
- human PPARγ	4XTA
- human serum albumin	4Z69
- human MHC-class-I-related molecule MR1	5U1R
- sheep COX-1	3N8Y
- mouse COX-2	1PXX
- rabbit CYP450 2C5	1NR6
- viper Phosphilpase A2	1SV9
- bovine Lactoferin	3IB0

(R)-Ibuprofen (cyclooxigenase inhibitor)	
- human adipocyte binding protein FABP4	3P6G
- human aldo-keto reductase family 1 member C3	3R8G
- human carbonic anhydrase II	8DJ9
- human aldo-keto reductase family 1 member C2	4JTR
- bacterial CYP152B1	3VM4

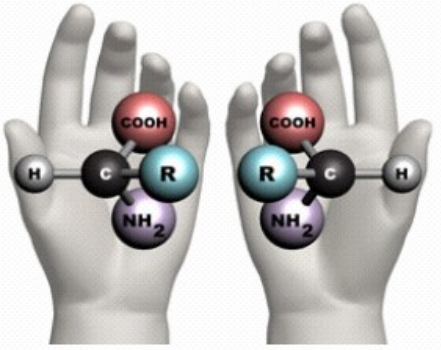


*Haupt J., Daminelli S., Schroeder M.: Drug Promiscuity in PDB: Protein Binding Site Similarity Is Key. *PloS ONE* 8(6):e65894 (2013)

On-target Design: Ligand-Protein Complementarity

nobelprize.org

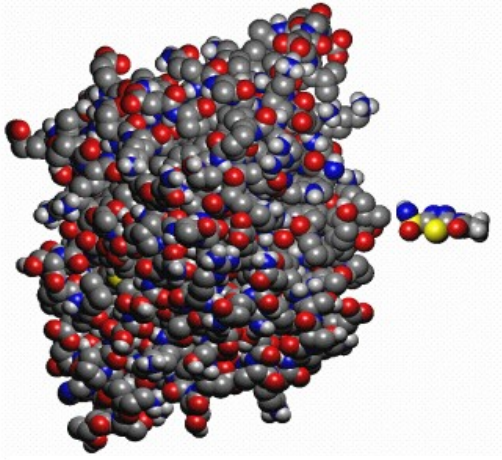
Emil Fischer
key-lock concept (1894)



enantiomorphic.blogspot.com

oldlockandkeyco.co.uk

muarchives.missouri.edu



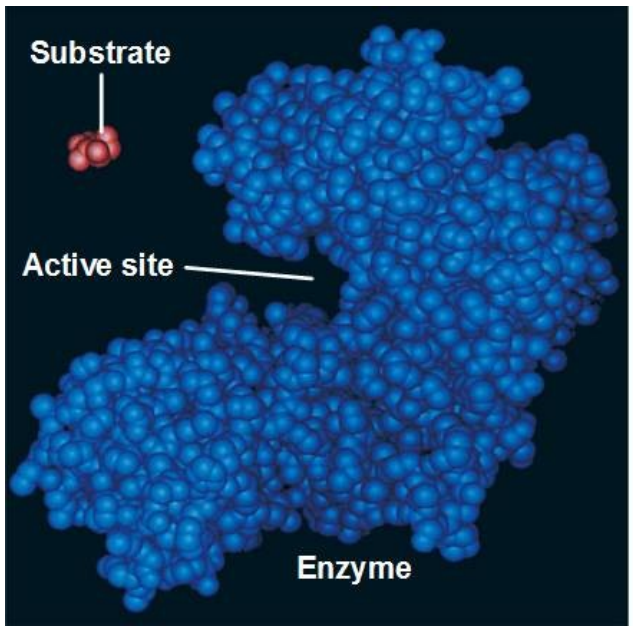
Lock: Carbonic anhydrase + Key: Acetazolamide

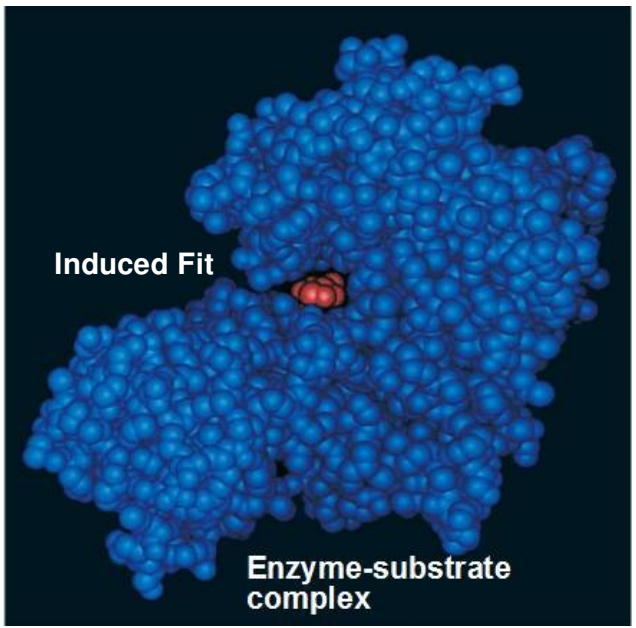
"Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glykosid zueinander passen müssen, wie Schloss und Schlüssel, um eine chemische Wirkung aufeinander ausüben zu können."

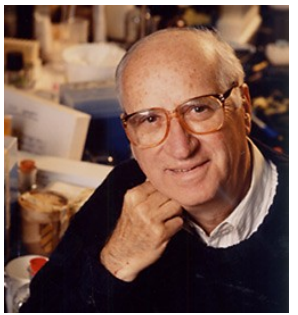
Emil Fischer (1894)

Off-target Effects: Induced-Fit

Both, the biological lock and the key are flexible !







Daniel Koshland
Induced-Fit (1958)

This mutual adaptation of protein and ligand is the origin of side effects.

Current safety profiling strategy = start early !

Target identification → Hit identification → Lead identification → Lead optimization → Pre-clinical development → Clinical development

Hazard identification

Hazard elimination → Mechanistic understanding

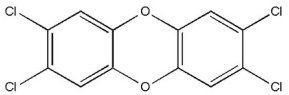
Core panel(s) of **off-targets** (30-60)	Broader panel(s) of **off-targets** (60-100's)

Early identification of promiscuity and liabilities; based on chemical series	Design out liabilities by SAR for individual targets	Mechanistic understanding of remaining liabilities

Valentin J.P., et al.: In vitro off-target profiling: impacting drug design and guiding quantitative translation risk assessment. *EuroTox 2018 Conference*, oral presentation on behalf of UCB, Novartis, Pfizer, Allergan & AstraZeneca (2018); *Tox Lett*. S1, 295, S21 (2018).

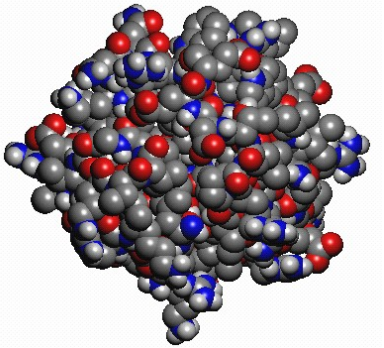
Simulation of off-target–mediated side effects

TCDD



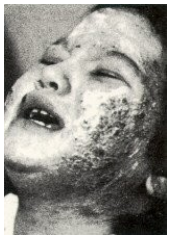
Exposure

Binding



Signaling

Manifestation of toxicity

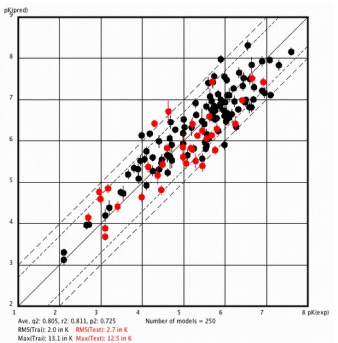


Seveso-casualty 1976

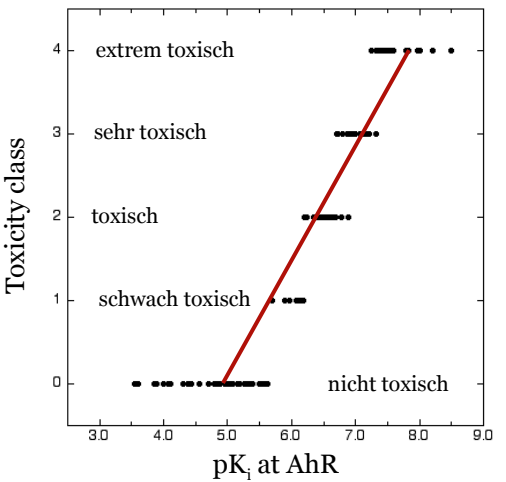
V. Juschtschenko 2004

Simulation of binding at target protein (AhR)

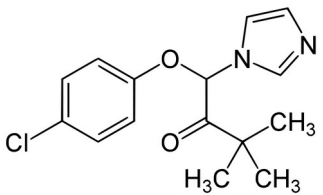
Consequence: from quantification of binding affinity to a protein, that triggers the undesired affect, we can evaluate the "toxic potential" of a compound, but not its toxicity due to unknown parameters like exposure, compensatory, mechanisms, clearance, metabolism etc.



QSAR

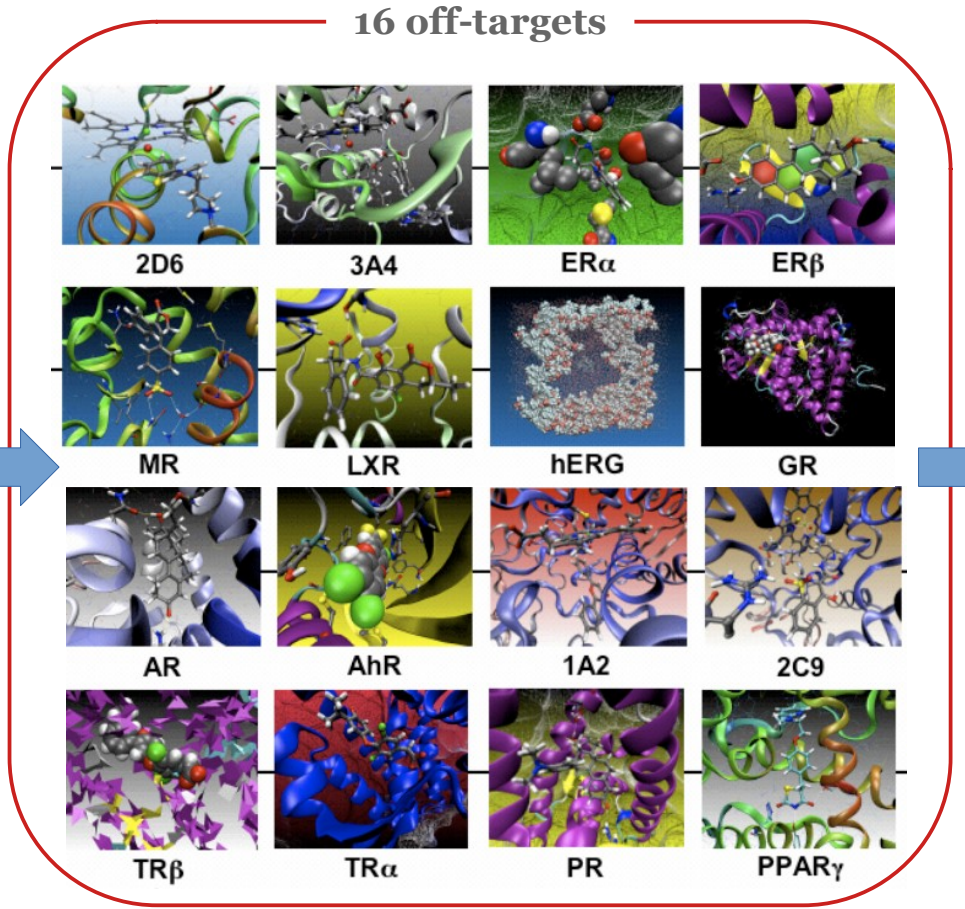


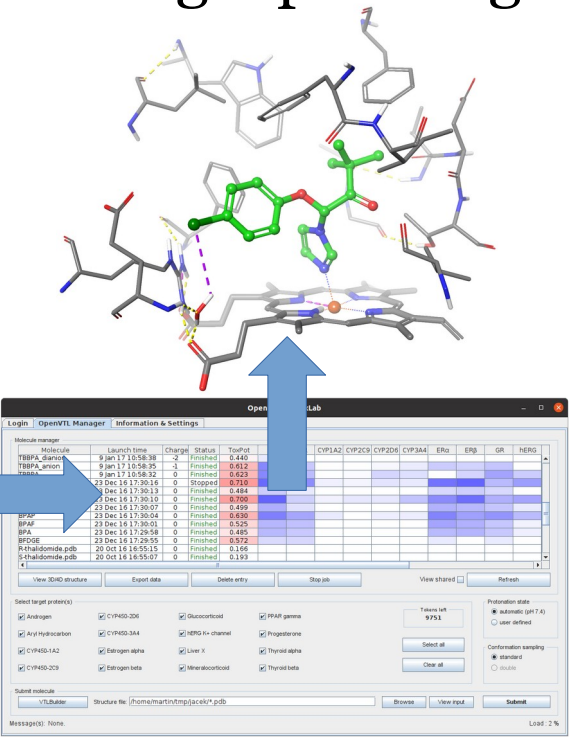
VirtualToxLab: an automated SBD platform for off-target profiling



Compound of interest

drug / candidate
natural compound
agrochemical
food additive
fragrance

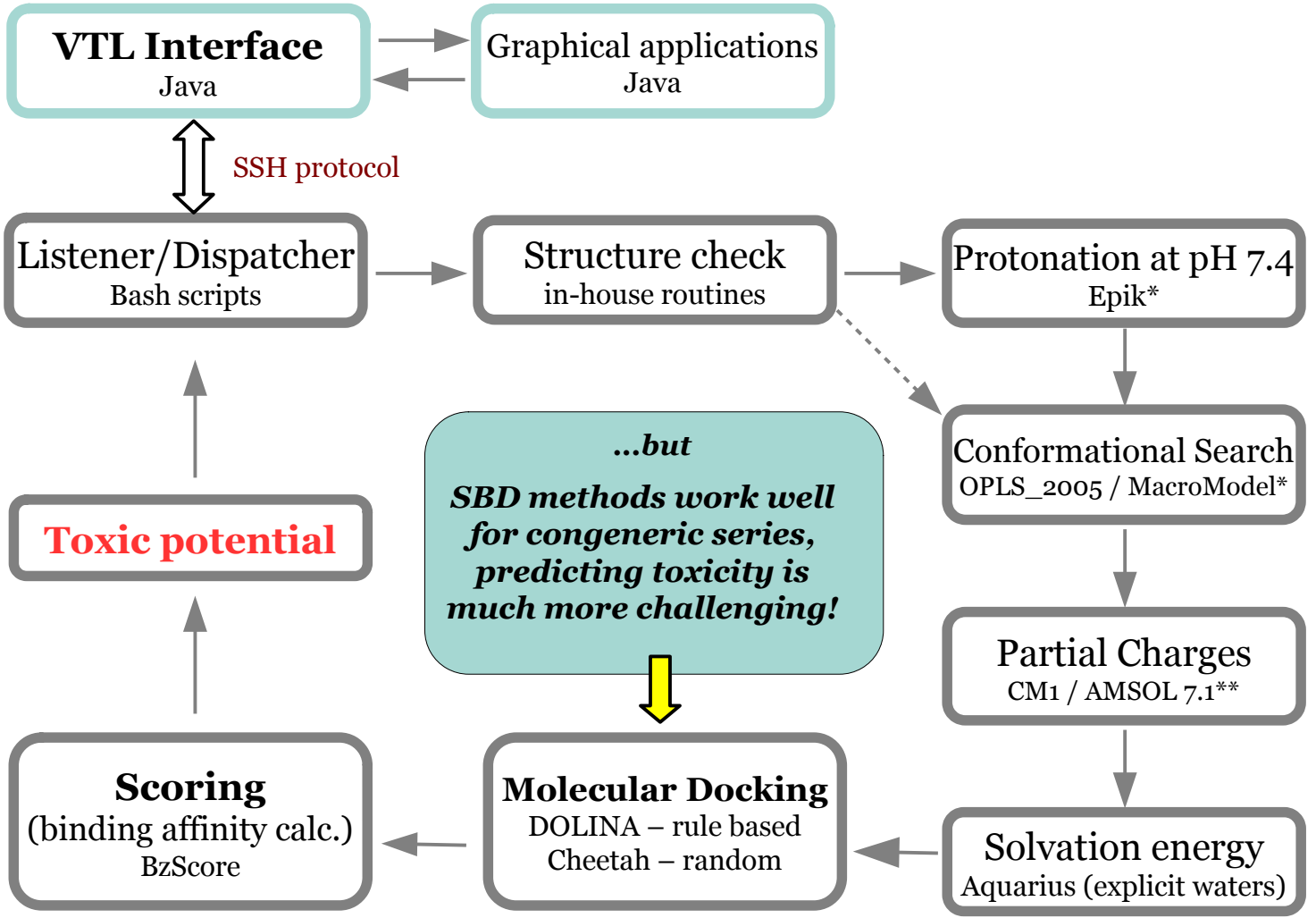




Off-target binding profile

Toxic Potential

VirtualToxLab
technical flow diagram



* Schrodinger Inc.
** University of Minnesota

DOLINA: Docking with Local INduced-fit Algorithm

Template–Protein Complex

Pharma-cophore ⟷

- Unmovable atoms
- Binding-site residues
- Side-chain conformers
- Forbidden zones

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

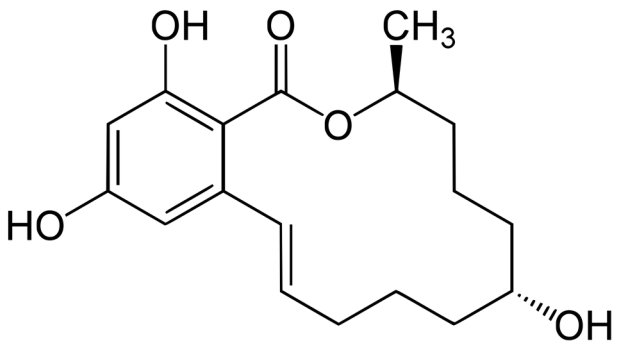
Scoring: H-bonding & lipophilic interaction energy

⇩

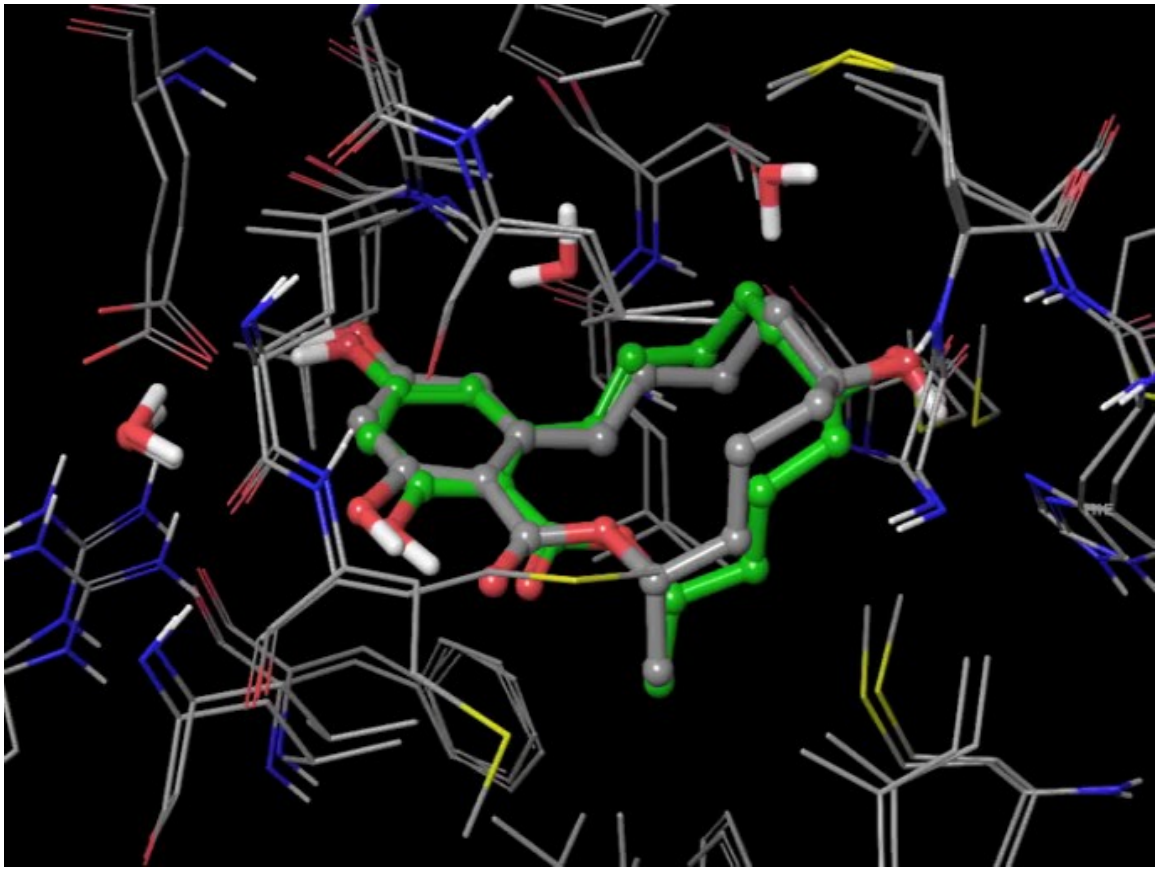
Top Ligand–Protein Complexes

Smieško M.: DOLINA – Docking Based on a Local Induced-Fit Algorithm: Application toward Small-Molecule Binding to Nuclear Receptors. *J Chem Inf Model*, 53, 6, 1415 – 1423 (2013).

Molecular Docking - Accuracy



α-Zearalenol
(mycotoxin)



Binding at the Estrogen Receptor-α

- grey carbons – docked pose → VirtualToxLab
- green carbons – reference X-ray structure (4TUZ)

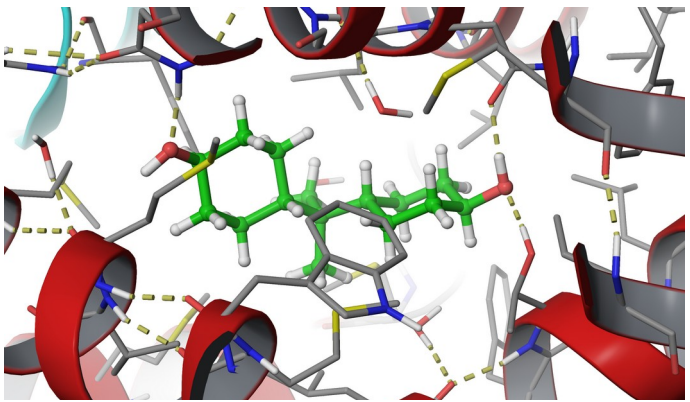
VirtualToxLab – a few highlights

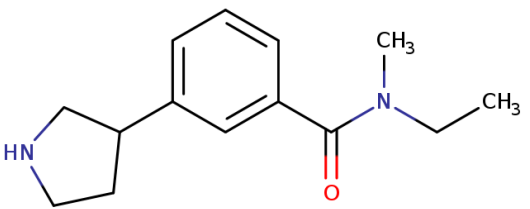
- used by more than 200 academic, non-profit and regulatory organizations worldwide
- recommended tool for identification of endocrine disruptors by EU/EC institutions

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

- used by five global food and cosmetic companies
- in cooperation with the Swiss Federal Food Safety and Veterinary Office: screening study of 3.5k substances in inks for food packaging materials (3 months / 128 cores); Projects ToxOligo 1 & 2 ongoing (~500 plastic oligomers)

hydrogenated Bisphenol-A
(bound at the AR)
CAS Nr. 80-04-6



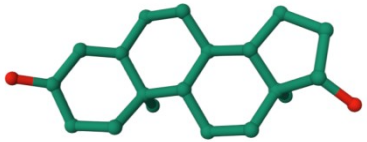


Solvent Red 119
CAS Nr. 1223748-27-3

Smieško M, Don CG, Meuwly R, Kucsera S, Brüschweiler BJ: Large-scale in silico screening of compounds contained in printing inks for food packaging materials. *EuroTox 2018*, poster presentation; manuscript in preparation.

Our Newest Solution to the Problem: *PanScreen*

User-submitted compound



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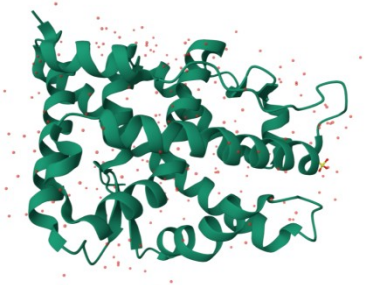
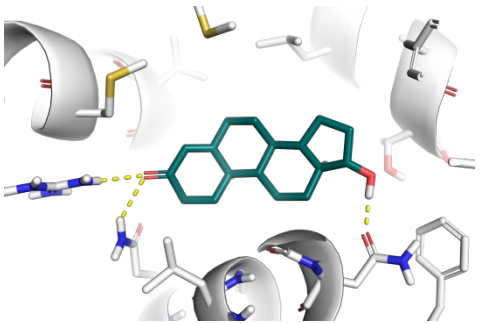
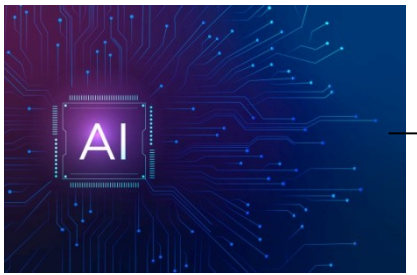


Implemented off-target(s)

Molecular docking
- Smina
- Glide
- LeDock



Complex analysis and re-scoring
- Gnina
- **Po-sco** interaction analyzer



AI-enabled interpretation

ΔG_{bind}

Predicted binding affinity

- Java **independent**
- **modular** implementation of off-targets & computational tools for easy upgradeability
- developed as a **portable** python package, C++ utilities
- Back-end: easily **distributable** as Docker containers
- Front-end: **user-friendly** web interface for non-commercial users at https://www.panscreen.ch/

PanScreen: Off-target Dataset Details

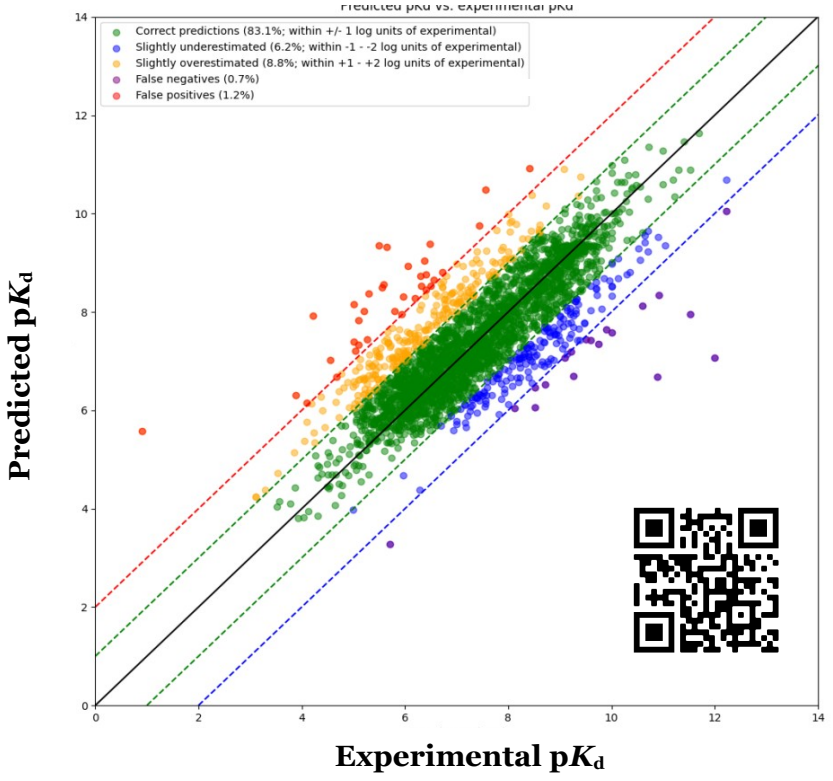
Affinity data: ChEMBL repository (14 000 molecule/affinity pairs); Structural data: Protein Data Bank (up to 3 per off-target)

UniProt ID	Off-target Name	Affinity range [kcal/mol]	Ratio active/inactive	# size validation set (20% of total)
P10275	AR	[-4.8, -13.0]	2.8	169
P23458	JAK1	[-7.0, -13.6]	20.1	148
O60674	JAK2	[-5.7, -16.1]	9.0	180
P25103	Substance-P	[-6.2, -14.4]	12.7	137
P28222	5HT1B	[-6.7, -13.4]	6.6	145
P49286	Melatonin	[-6.6, -16.4]	15.2	178
Q9Y233	PDE10A	[-4.4, -16.4]	20.0	189
P04150	GR	[-6.3, -13.4]	8.4	150
P07550	b2-ADR	[-5.2, -14.6]	2.4	131
Q08499	PDE4D	[-5.2, -13.7]	1.6	55
P03372	ERa	[-4.2, -14.2]	3.3	78
Q92731	ERb	[-4.2, -14.2]	4.4	65
P37231	PPARg	[-1.2, -13.4]	2.1	87
P14416	Dopamine-2 R	[-6.7, -15.5]	4.1	1120

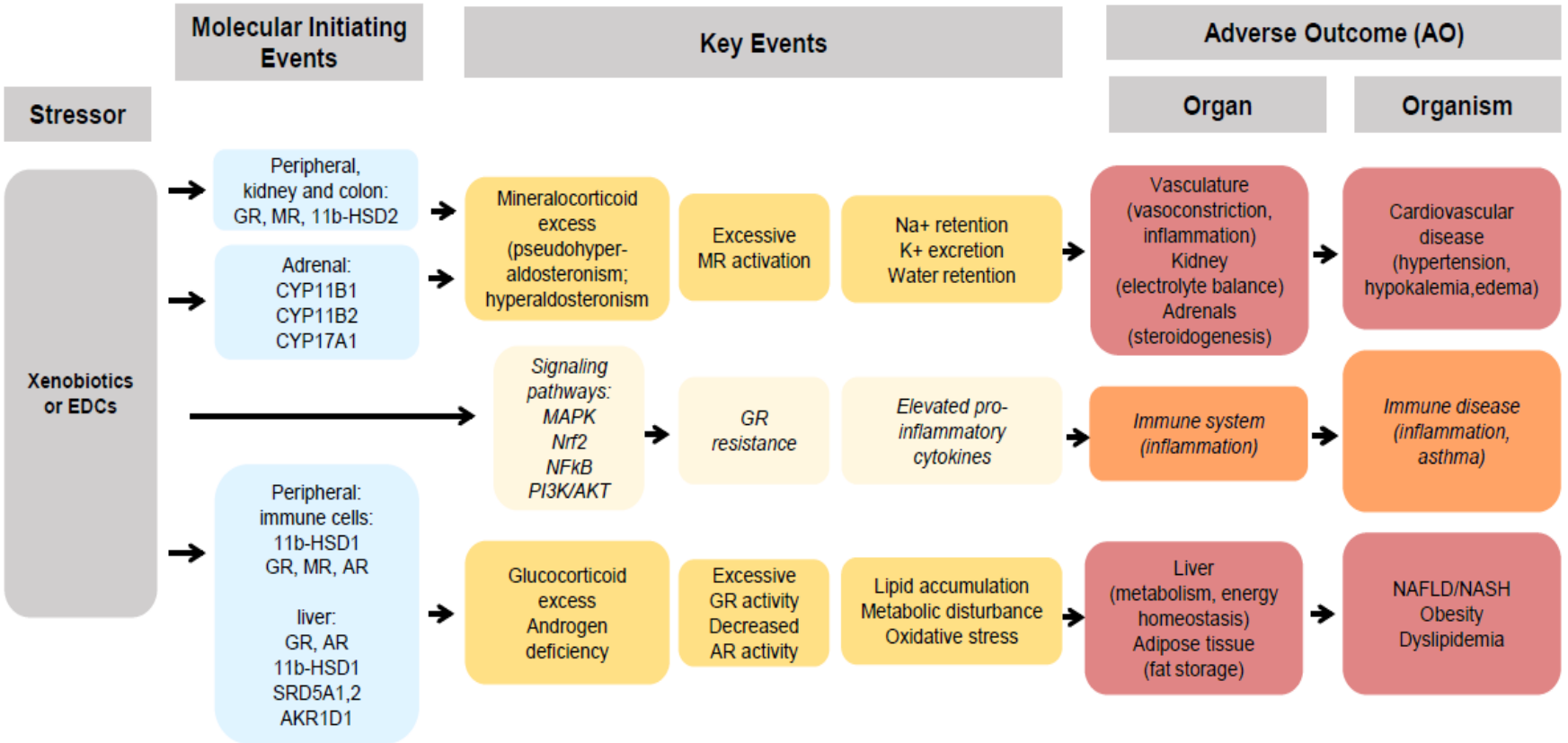
PanScreen: Performance Metrics

Predictive performance of the implemented off-targets for the validation set

Name	PCC	MUE [kcal/mol]	RMSE [kcal/mol]	AUROC
Tyrosine-protein kinase JAK2	0.81	0.68	1.10	0.94
Estrogen receptor alpha	0.84	0.94	1.18	0.89
Glucocorticoid receptor	0.79	0.70	0.95	0.89
Beta-2 adrenergic receptor	0.79	0.92	1.17	0.91
Androgen receptor	0.81	0.79	1.03	0.88
Dopamine receptor D2	0.75	0.66	0.87	0.88
Tyrosine-protein kinase JAK1	0.81	0.55	0.85	0.94
Substance-P receptor	0.80	0.77	1.02	0.91
5HT receptor 1B	0.77	0.85	1.06	0.87
PPARγ	0.68	0.89	1.33	0.84
Melatonin receptor 1B	0.72	1.06	1.35	0.84
Phosphodiesterase 4D	0.80	0.95	1.36	0.82
Estrogen receptor beta	0.75	1.00	1.29	0.85
Phosphodiesterase 10A	0.80	0.88	1.18	0.93



Search for xenobiotics interfering with the corticosteroid–androgen balance



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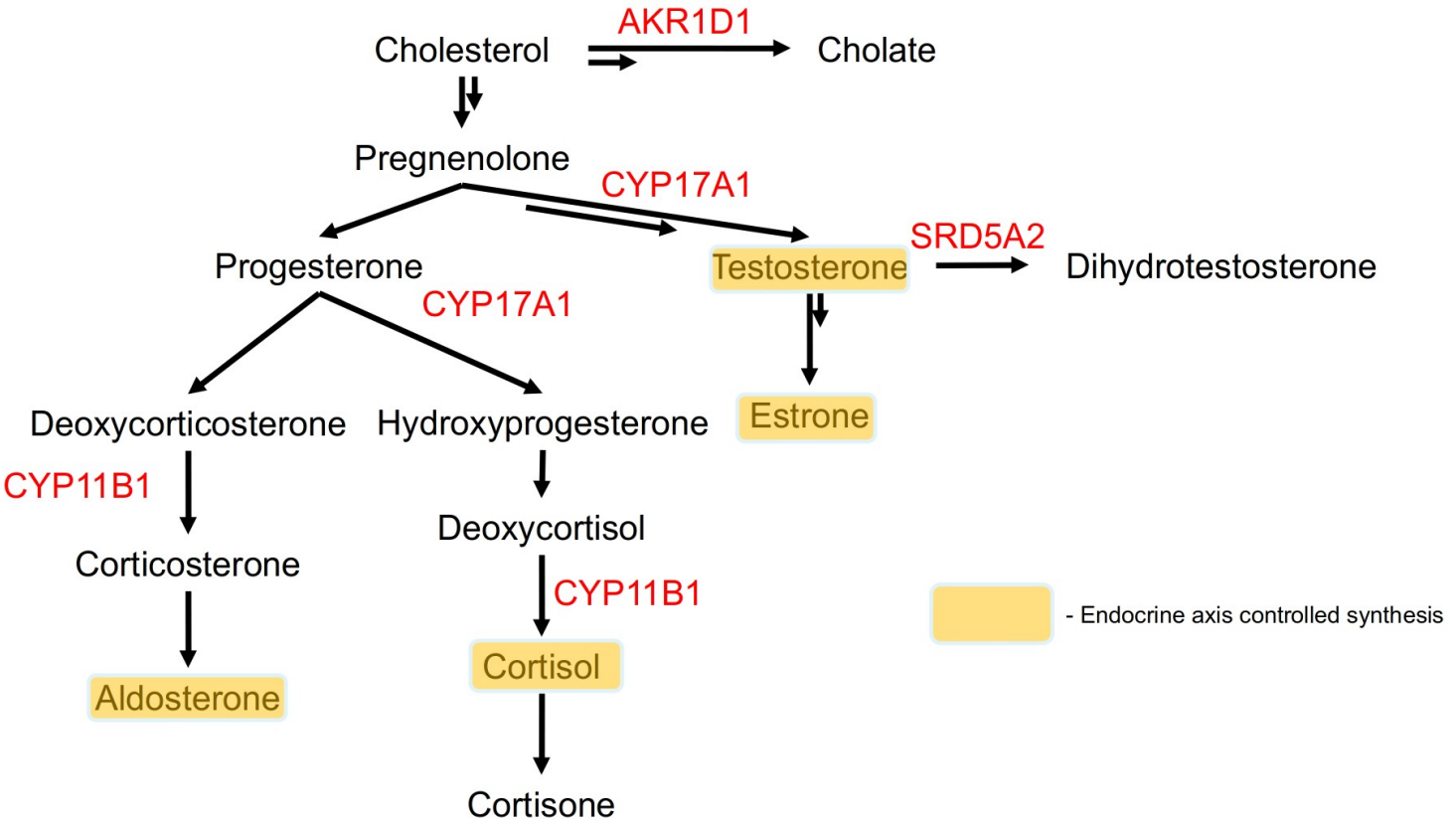


Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, Second Edition

Search for xenobiotics interfering with the corticosteroid–androgen balance

Preparation

- four anti-targets selected: CYP11B1, CYP17A1, 5-α reductase (SRD5A2), 5-β reductase (AKR1D1)
- ensembles of multiple protein structures created for each off-target
- several docking engines employed: Glide, smina, GOLD, Dolina
- docking protocols validated using known actives and inactives, decoys, re-docking & cross-docking

Virtual Screening

- screened ligands compiled from the DrugBank (FDA approved, experimental, investigational + metabolites)
- post-docking evaluation: MM-GB/SA, visual analysis, similarity searches within the compound group

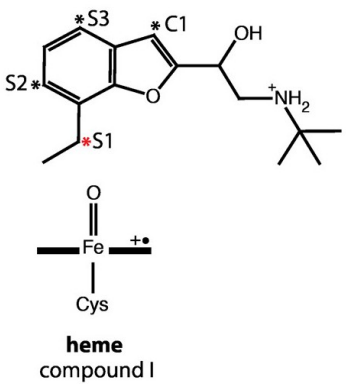
Results

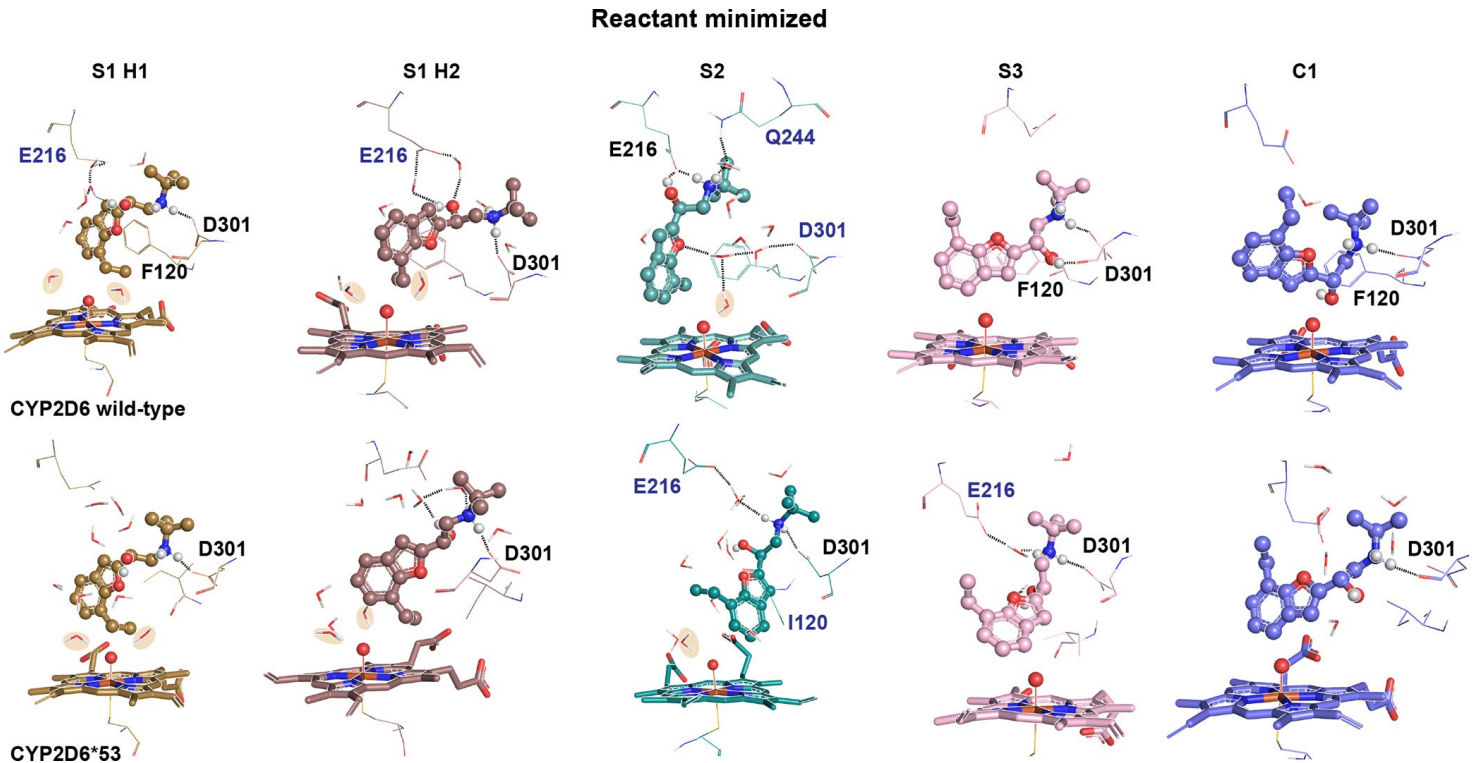
- CYP11B1 (39): 19 novel inhibitors, several < 10 nM, 3 < 1 nM!!! → 10.1016/j.taap.2023.116638
- CYP17A1 (11): 2 novel hits found (IC50=1.47 μM & IC50=2.2 μM) → in preparation
- SRD5A2 (11): found 1 inhibitor (IC50 to be determined)
- AKR1D1 (46): found 1 inhibitor (IC50=2.3 μM), 2 weak inhibitors (60% activity at 10μM); 6 anabolic steroids → 10.1016/j.toxlet.2023.07.006

(Phase 1) Metabolism – Enzyme (CYP450) based

1) Identification of poses by molecular docking and molecular dynamics that expose “vulnerable” ligand regions for the oxidation from the oxyferryl group (Fe=O) at the active site of CYP2D6 wild type / variant *53 → UM phenotype

Bufuralol

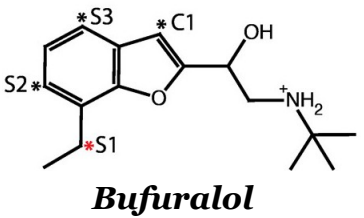




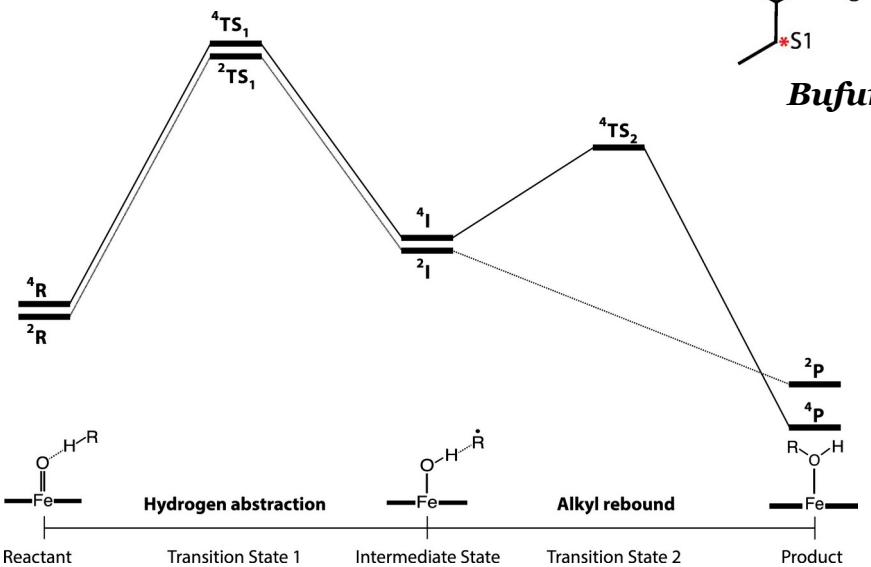
Don C.G., Smieško M.: Deciphering Reaction Determinants of Altered-Activity CYP2D6 Variants by Well-Tempered Metadynamics Simulation and QM/MM Calculations. *J Chem Inf Model* 60 (12), 6642-6653, (2020)

(Phase 1) Metabolism – Enzyme (CYP450) based

2) calculation of the activation barrier for oxidation reactions at these sites using **quantum mechanics** (DFT)



Bufuralol



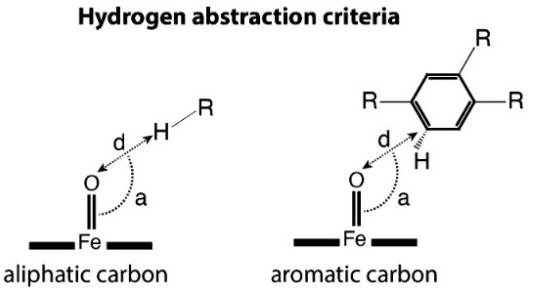


Table 1. QM/MM Activation Barriers (kcal/mol, M06-2X/LACV3P*) of the Hydrogen Abstraction of Bufuralol's SoMS Studied in CYP2D6 WT and *CYP2D6*53*[a]

activation energy ΔE [kcal/mol]			Δrmsd (Å)
SoM	CYP2D6 WT	*CYP2D6*53*	bufuralol
S1_H1	13.3	12.4	1.8
S1_H2	22.8	31.9	2.7
S2	22.9	21.2	6.4
S3	28.8	61.8	4.0
C1	57.2	42.4	4.7

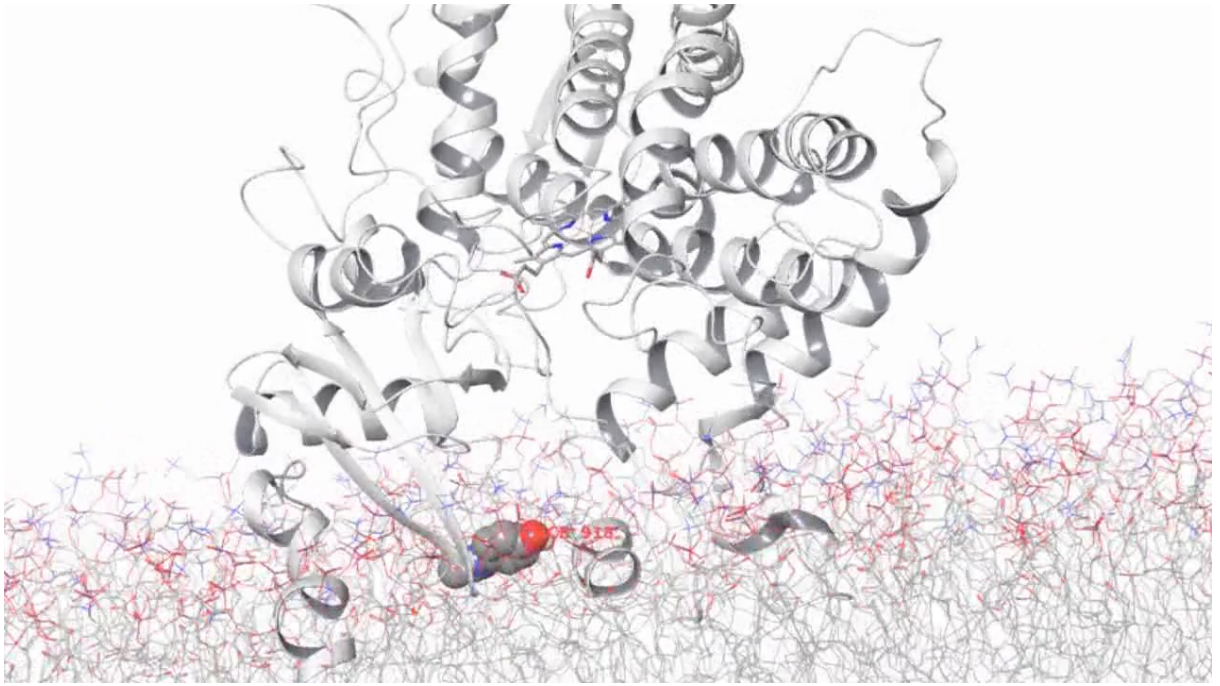
Calculated trends in agreement with experiment → personalized metabolism prediction is possible!

Don C.G., Smieško M.: Deciphering Reaction Determinants of Altered-Activity CYP2D6 Variants by Well-Tempered Metadynamics Simulation and QM/MM Calculations. *J Chem Inf Model* 60 (12), 6642-6653, (2020)

Rare event MD simulation: *Paracetamol* binding to CYP2D6*53

A rare event in atomic detail:

- ligand interaction with the membrane
- recognition at the protein surface
- ligand entry to the tunnel
- tunnel bottleneck/gating residues
- passage toward the active site
- intermolecular interactions and dynamics of the binding mode within the active site
- residence time
- solvent effects/displacement
- protein fluctuations/ rearrangement/ local & long range induced-fit effects
- membrane influence
- allosteric binding sites
- …



periodic boundary system of CYP2D6*53 within the membrane (80 x 100 x 130 Å), simulation software: Desmond, hardware accelerator: nVidia Titan X

Fischer, A., Smieško, M. Spontaneous Ligand Access Events to Membrane-Bound Cytochrome P450 2D6 Sampled at Atomic Resolution. *Sci Rep* 9, 16411 (2019)

Conclusions

- compound safety is still an open issue in pharma, food, agro/chemical, cosmetic industries
- even some approved (!) drugs show off-target binding

- structure-based design methods are effective in screening for the off-target binding
- if properly applied, docking & scoring-based methods can identify off-target binding
  - molecular dynamics offer a comprehensive view of the on/off-target binding
  - QM/MM approaches can be used to accurately model reactivity of ligands in CYPs (→ metabolism), even for SNP variants → precision toxicology
  - offer mechanistic interpretation with direct hints to avoid (design out) the off-target binding

The Future – Near and Far

- **more accurate** force fields and parameters (polarized, AI-based)
- growing toxicological evidence, new *in vitro* data
- faster performance CPUs / GPUs
- **smarter algorithms:** docking and scoring + machine learning approaches
- **complex simulations** → larger and more realistic molecular systems
- **new targets** – cover all relevant human macromolecules and their complexes available
- complete **adverse outcome pathways** (AOPs)
- physiology-based **pharmacokinetic modeling** (PBPK)
- **personalised medicine** – genetic information explicitly included in the computer simulation
- **linking** to "omics", clinical (→ drug repurposing) and toxicological data
- tox-enabled medicinal chemistry optimization → **drugs safe by design**

More accurate simulations → Reliable predictions → Safer compounds !

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