University of Parma

3D molecular modelling meets toxicology
A useful tool to investigate the TD and TK of food related toxicants

Luca Dellafiora[1], Jean-Lou Dorne[2], Jochem Louisse[3], Chiara Dall'Asta[1]

[1] Department of Food and Drug, University of Parma, Parma, Italy
[2] Scientific Committee and Emerging Risks Unit, European Food Safety Authority, Parma, Italy
[3] Wageningen Food Safety Research, Wageningen, the Netherlands

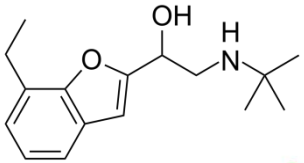
ESTIV - ASCCT Webinar, 30th August 2022

Terms of Reference

3D molecular modeling techniques refers to computational methods allowing to study the interaction between small molecules and biological (macro)molecules from a molecular standpoint

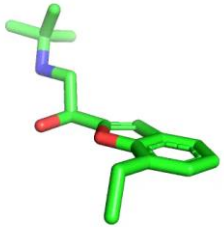
① PubChem

https://pubchem.ncbi.nlm.nih.gov



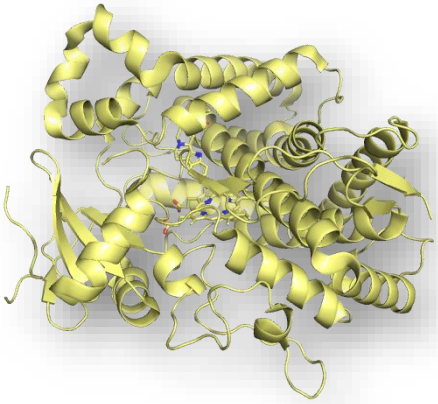
2D sketch

3D structure

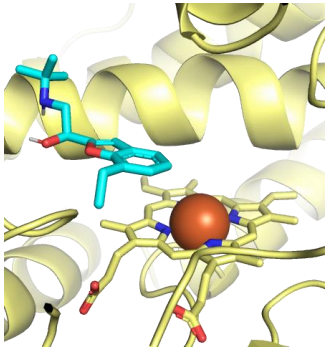


② RCSB PDB PROTEIN DATA BANK

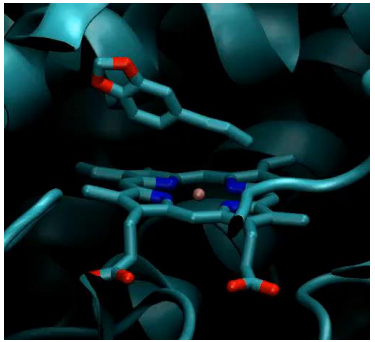
https://www.rcsb.org



③ Simulate the binding event



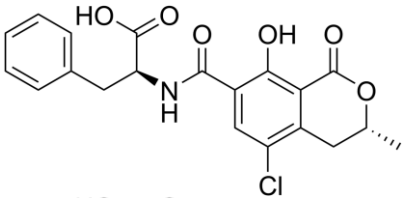
④ Check the stability over time



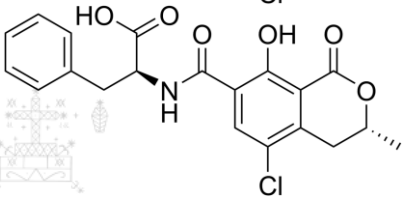
How to tackle TK and TD

3D modeling studies the interaction between small molecules and proteins

The type of protein under investigation switches the focus on TK or TD

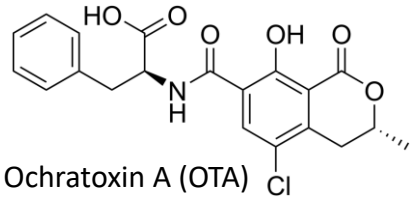


\+ CYPs, phase II enzymes and transporters → **TK**



\+ biological targets, proteins involved in MIE → **TD**

Addressing Ochratoxin A TK



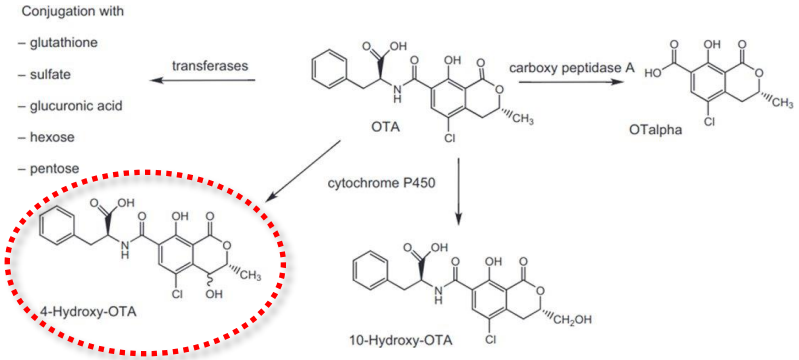
Ochratoxin A (OTA)

Rapidly absorbed after oral ingestion with a bioavailability ranging from 40% to 66% depending on the species and the dose

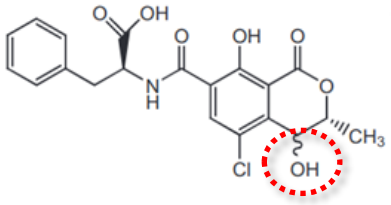
Extensively bound to albumin and other serum proteins (up to 99.98% in humans)

In humans and monkeys prevails renal excretion involving Organic Anion Transporters (OATs)

Biotransformation of OTA appears to be low and mostly limited to hydrolysis of the amide bond to form OTalpha *in vivo*



Addressing Ochratoxin A TK

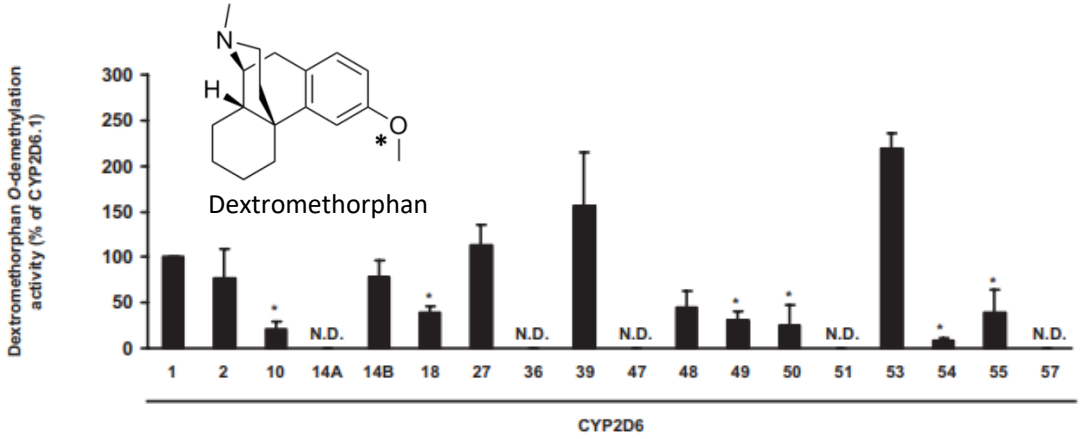


Described *in vitro* and *in vivo* as a minor metabolic route

Less toxic than OTA, unclear the effects of 4-OH on OTA's TK and TD

CYP2D6 together with CYP2B6 have been shown to be involved in the formation of 4-OH-OTA

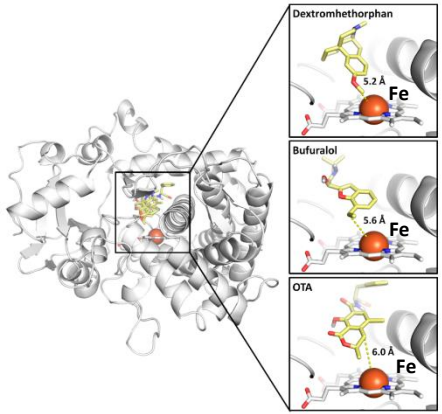
CYP2D6 is highly polymorphic, and polymorphism may change drastically change the transformation of given substrates

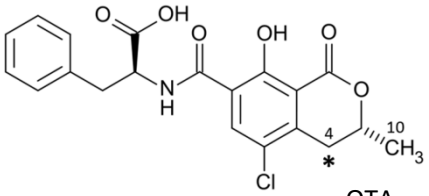


IARC. 2012. Chapter 6. In *IARC Scientific Publication N. 158*
Sakuyama et al., 2008. Drug Metab Dispos. 36 (12), 2460-7

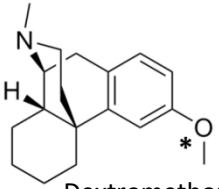
Is there any CYP2D6 polymorphism with an altered capability to form 4-OH-OTA?

A computational workflow was setup to estimate the capability of CYP2D6 polymorphisms to transform probe substrates

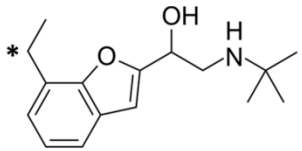




OTA



Dextromethorphan



Bufuralol

* Atom undergoing the reaction

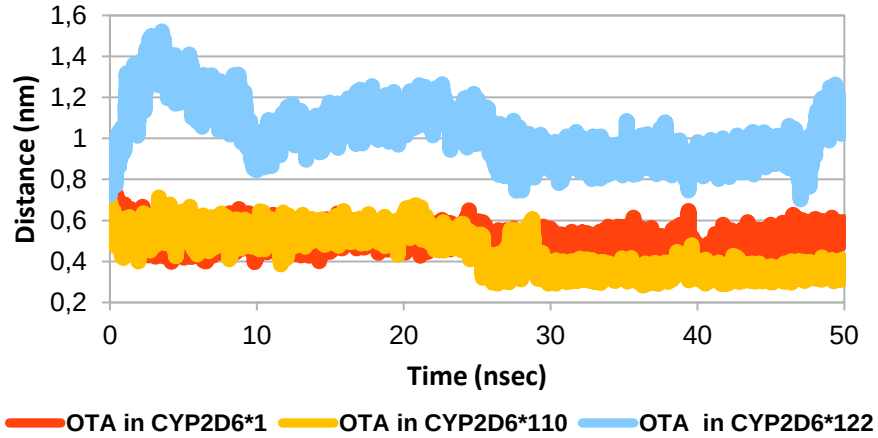
The atom undergoing the reaction must be closely and stably oriented toward the Fe-Heme [1]

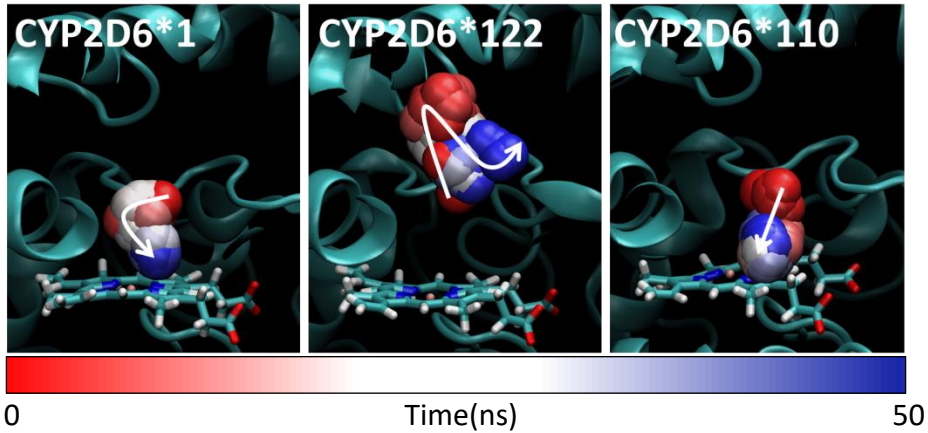
Molecular modeling could estimate the likeliness of molecules to be transformed monitoring their geometry of binding to Fe-Heme

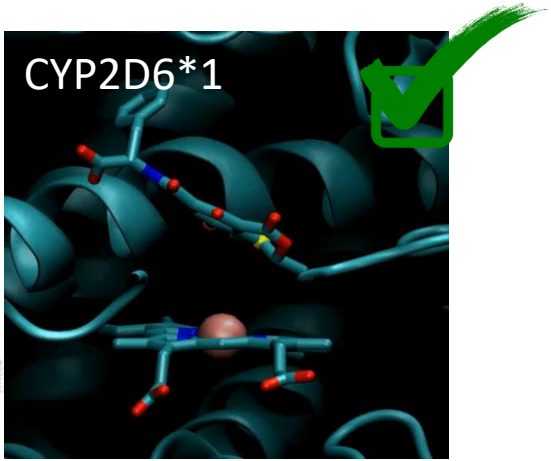
Molecular modeling may estimate whether the residue substitution of CYP polymorphisms may influence the proper orientation of molecules to undergo the reaction

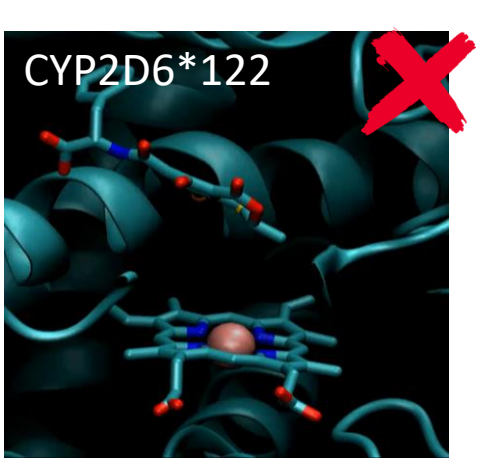
[1] Xu and Chen, 2020. Sci. Rep. 10, 1–14

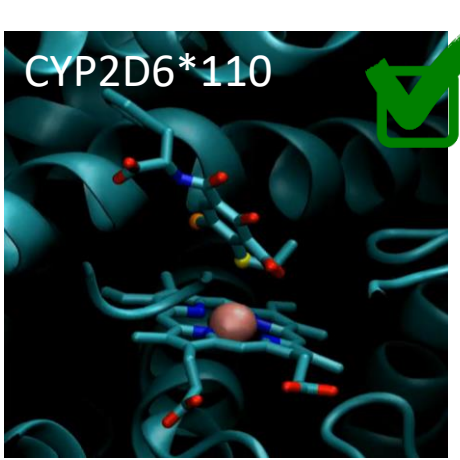
Polymorphisms from Pharmacogene Variation Consortium (PharmVar; www.pharmvar.org)











Is there any mutated transporters with an altered capability to transport OTA?

Toxicology Letters 355 (2022) 19–30

Contents lists available at ScienceDirect

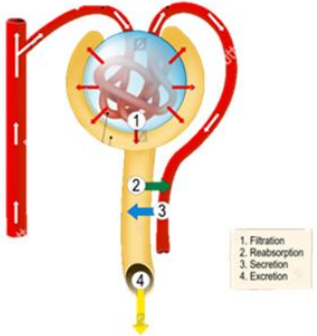
Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

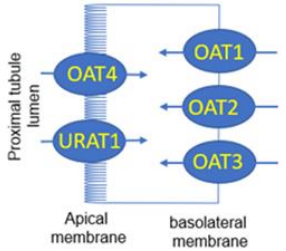
Investigating the interaction between organic anion transporter 1 and ochratoxin A: An *in silico* structural study to depict early molecular events of substrate recruitment and the impact of single point mutations

Jochem Louisse[a], Jean Lou C.M Dorne[b], Luca Dellafiora[c,*]

Nephrone



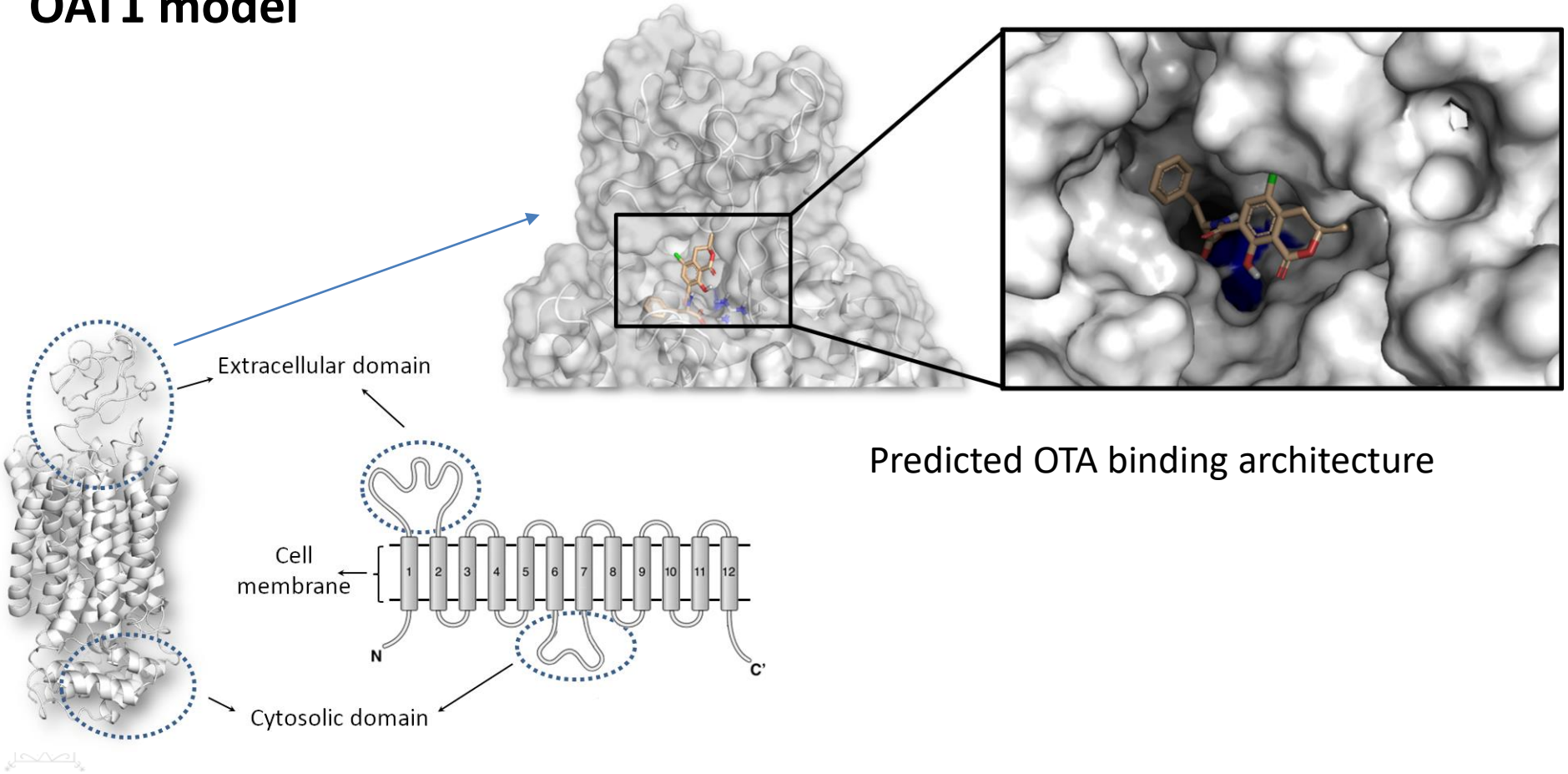
Proximal tubular cells



The excretion pathway of OTA via urine involves OAT1, OAT2 and OAT4

OAT1 has the highest affinity, and a prominent role has been inferred accordingly

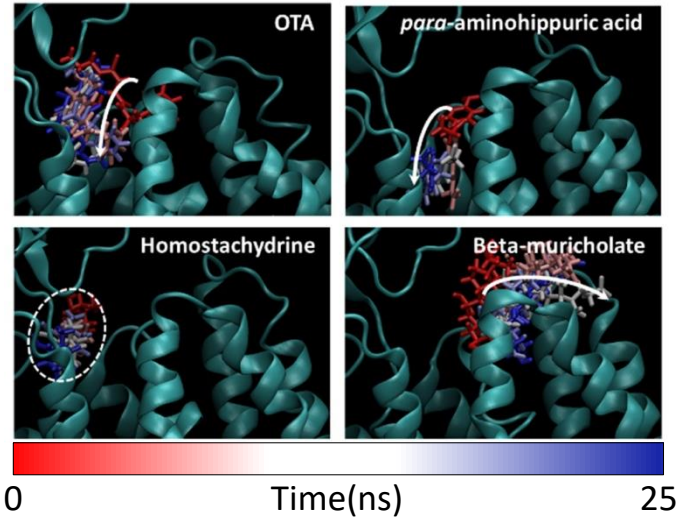
OAT1 model

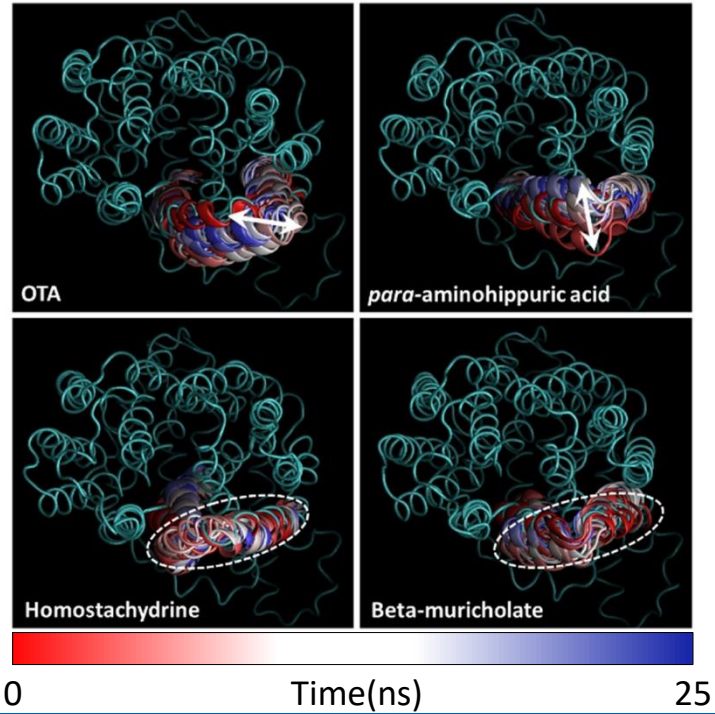


Predicted OTA binding architecture

Assessment of OAT1 model reliability

OAT1 was found to open when bound to probe substrates like OTA and p-amminohippuric drawing an inward trajectory, while it laid closed bound to non-substrates (like homostachydrine and beta-muricholate)



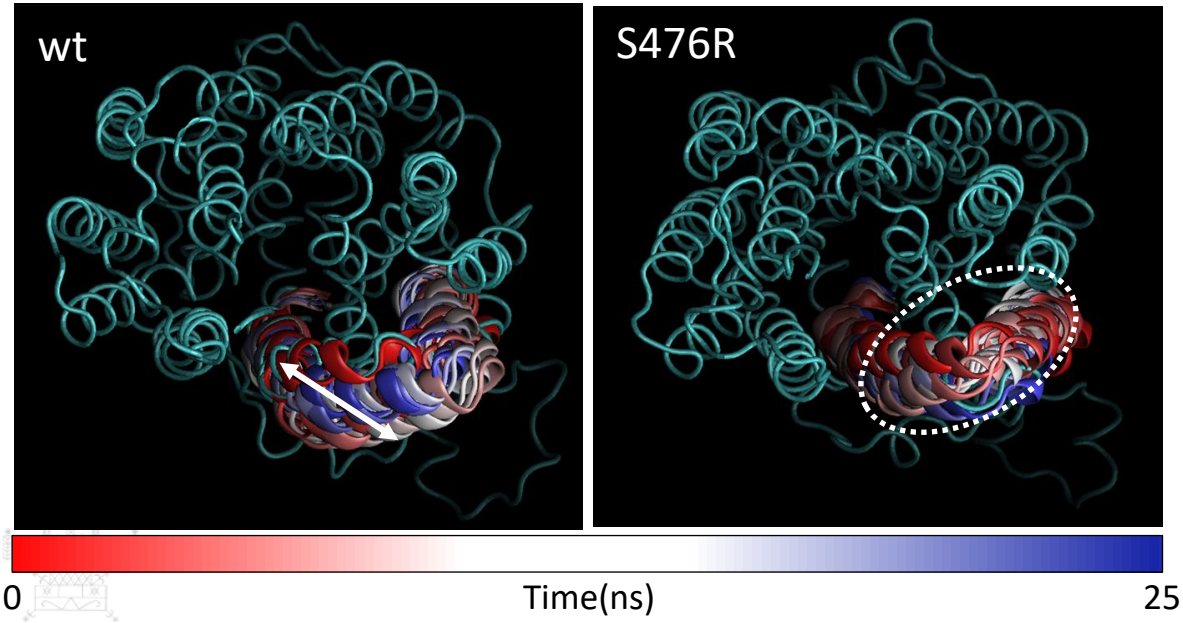


Geometrical features and ligand trajectories could be used to distinguish transported substrates from non-substrates

A set of OAT1 mutations found occurring in the human population have been investigated

Mutated variants were retrieved from BioMuta DB[1] that collects mutations occurring in cancer

The analysis focused on mutations surrounding the calculated OTA initial binding site: S476R, R423P, D359N, Q361K, and E480K

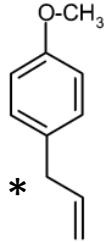


S476R may hinder OAT1 opening, reducing the transmembrane transport of OTA

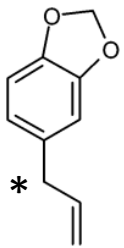
S476R bearing subjects may show differences in OTA TK

Addressing the bioactivation of alkenylbenzenes

Preliminary results



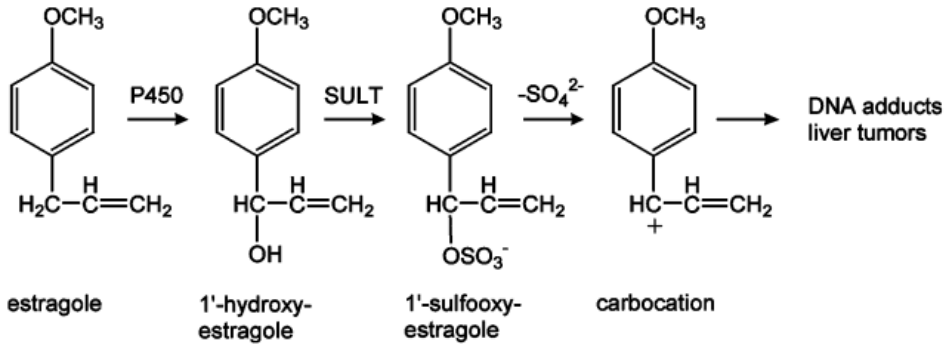
estragole



safrole

enzyme	enzyme efficiency k_{cat}/K_m (min^{-1} mM^{-1}) estragole	safrole
P450 1A2	59	ND[a]
P450 2A6	341	160

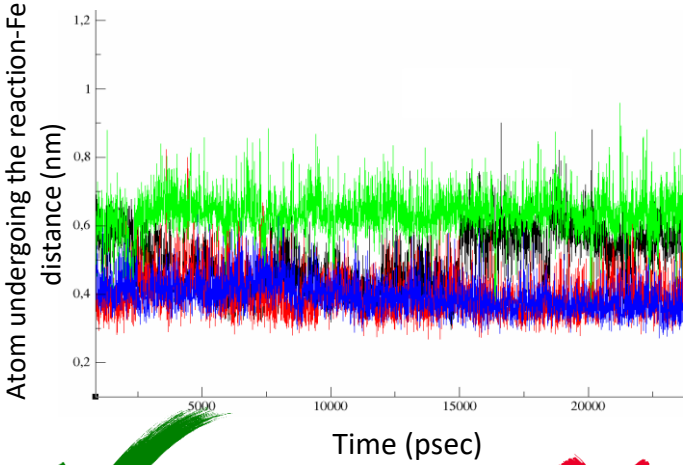
The CYP-dependent oxidation at the * position to form hydroxylated derivatives is important to activate compound toxicity

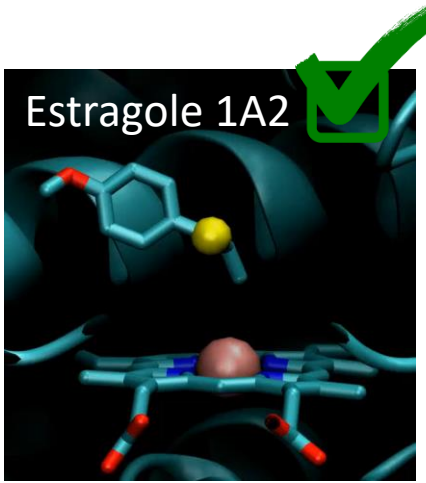


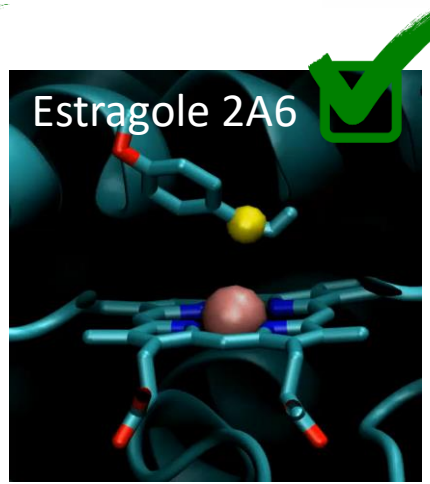
Addressing the bioactivation of alkenylbenzenes

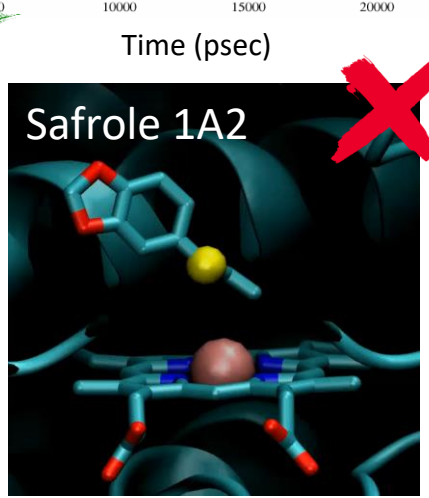
Applying the rule described for OTA-CYP2D6 we can distinguish substrates from non substrates

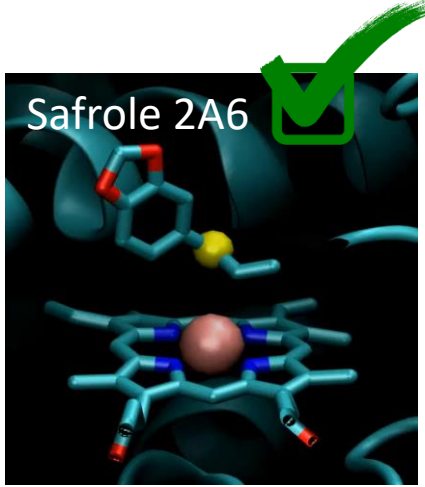
	enzyme efficiency k_{cat}/K_m (min^{-1} mM^{-1})	
enzyme	estragole	safrole
P450 1A2	59	ND[a]
P450 2A6	341	160











Alkenylbenzenes may be present in feed

Animal CYPs homologues to human CYP1A2 and CYP2D6 were screened for their theoretical efficiency to transform safrole

The study focused on: rat, mouse, dog, rabbit, pig, chicken, goat and sheep

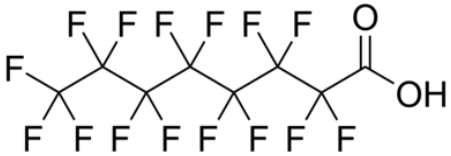
	enzyme efficiency k_{cat}/K_m (min^{-1} mM^{-1})	
enzyme	estragole	safrole
P450 1A2	59	ND[a]
P450 2A6	341	160

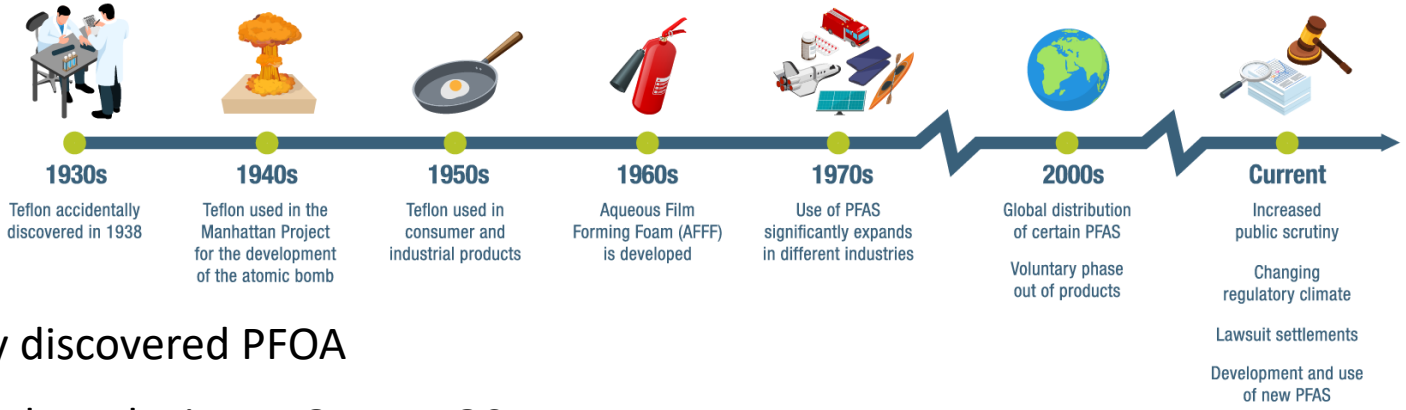
Safrole in animal homologues to human CYP2A6 showed a worse interaction compared to human suggesting a lower yield of transformation (lower toxicity?), with the exception of sheep and goat where the interaction was similar to that within human CYP2A6

Safrole in animal homologues to human 1A2 showed a better interaction compared to human suggesting a higher yield of transformation (higher toxicity?), with the exception of pig where the interaction was similar to that within human CYP2A6

Addressing the transport of PFAS

Polyfluoroalkyl substances (PFAS)





1947: USA, 3M firstly discovered PFOA

1949: USA, 3M strated producing PFOA e PFOS

1950: First use in Teflon

1950-2000: PFOA and PFOS have been used in many applications

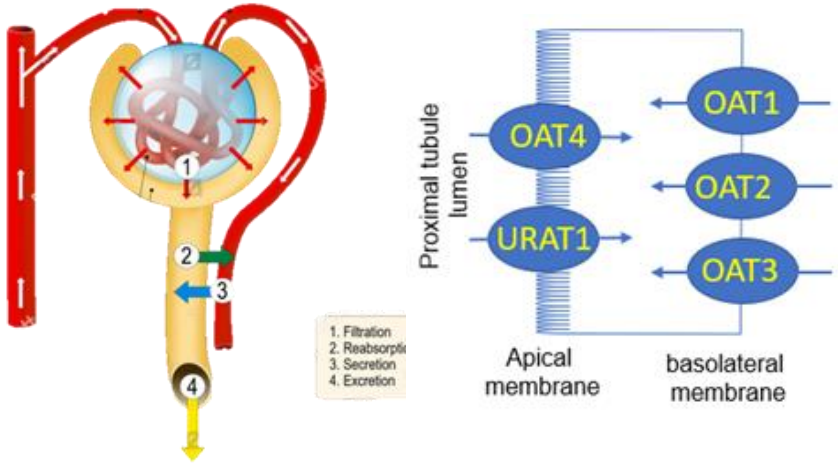
2000: USA, 3M ended producing long chain PFAS (like PFOA and PFOS)

Today: PFAS are super resistant food and environmental contaminants

Effects of PFAS on living organisms

They disrupt peroxisome activity, lipids metabolism, reproductive and nervous system, liver functionality

PFAS	Chain carbon number	Half-life based on literature
PFHpA	7	1.2 years
PFOA	8	2.7 years
PFNA	9	4.3 years
PFDA	10	9.2 years



Some information on PFOA:

- Active reabsorption possible via OAT4 and URAT1
- Active excretion may play a role as well via OAT1/OAT2/OAT3

3D modelling may help shading light on TK/TD of PFAS

Most of PFAS need to be characterized in this sense!

Addressing the transport of PFAS

Preliminary results

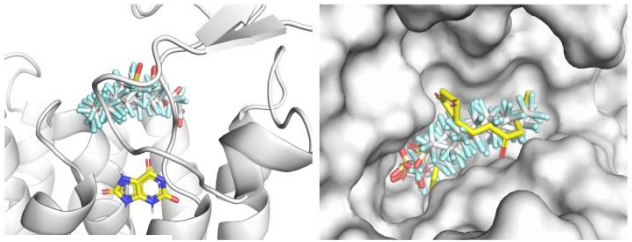
3D molecular modeling integrated to experimtal trials to provide more insights into the transport of PFASs by OAT4 and URAT1

Docking scores of PFAS and probe substrates of URAT1 and OAT4

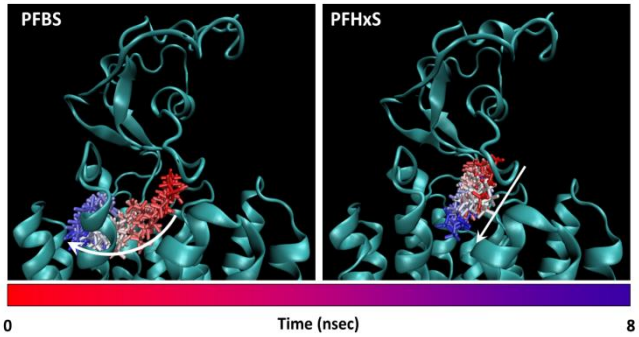
Compound	OAT4		URAT1	
	Score (kcal/mol)	Substrate	Score (kcal/mol)	Substrate
PFHpA	-6.1	Yes	-5.5	No
PFOA	-6.2	Yes	-6.0	No
PFNA	-6.8	Yes	-6.6	No
PFDA	-7.1	Yes	-6.7	No
PFHxS	-6	Yes	-7.3	No
PFOS	-7.3	Yes	-6.8	No
PFBS	-5.5	No	-4.9	No
Uric acid	---	---	-6.6	Yes
Prostaglandin E2	-6.2	Yes	---	---

Docking poses and MD results

URAT1 OAT4



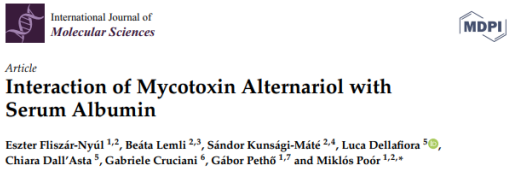
OAT4



Take home message

3D molecular modeling may investigate the interaction between toxicants and TK actors

Analysis has been already successfully applied to SULTs and serum transporters



The approach may provide usefull means to broadly study TK-related aspects of small molecules toxicology to:

- Achieve a more informed understanding of molecular basis underpinning toxicants action
- Predict toxicokinetics of poorly studied compounds and/or in scarcely characterized species
- May provide useful mechanistic insights either to refine other modeling techniques or to informedly plan further experiments

Thanks for your attention!

More details of related works @:

Further info: luca.dellafiora@unipr.it

UNIVERSITÀ DI PARMA

www.unipr.it