University of Parma

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

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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
2. PDB
   - https://www.rcsb.org
3. Simulate the binding event
4. Check the stability over time

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

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

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Sakuyama et al., 2008. Drug Metab Dispos. 36 (12), 2460-7

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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

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

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

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

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

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

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Assessment of OAT1 model reliability

OAT1 was found to open when bound to probe substrates like OTA and p-aminohippuric 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

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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.

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The CYP-dependent oxidation at the * position to form hydroxylated derivatives is important to activate compound toxicity.
Applying the rule described for OTA-CYP2D6 we can distinguish substrates from non substrates.

<table>
<thead>
<tr>
<th>enzyme</th>
<th>estragole</th>
<th>safrole</th>
</tr>
</thead>
<tbody>
<tr>
<td>P450 1A2</td>
<td>59</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P450 2A6</td>
<td>341</td>
<td>160</td>
</tr>
</tbody>
</table>

Addressing the bioactivation of alkenylbenzenes

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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

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

<table>
<thead>
<tr>
<th>enzyme</th>
<th>$k_{cat}/K_m$ (min$^{-1}$ mM$^{-1}$)</th>
<th>Safrole</th>
</tr>
</thead>
<tbody>
<tr>
<td>P450 1A2</td>
<td>59</td>
<td>ND$^a$</td>
</tr>
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Addressing the transport of PFAS

Polyfluoroalkyl substances (PFAS)

1947: USA, 3M firstly discovered PFOA
1949: USA, 3M started producing PFOA and 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

<table>
<thead>
<tr>
<th>PFAS</th>
<th>Chain carbon number</th>
<th>Half-life based on literature</th>
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</thead>
<tbody>
<tr>
<td>PFHpA</td>
<td>7</td>
<td>1.2 years</td>
</tr>
<tr>
<td>PFOA</td>
<td>8</td>
<td>2.7 years</td>
</tr>
<tr>
<td>PFNA</td>
<td>9</td>
<td>4.3 years</td>
</tr>
<tr>
<td>PFDA</td>
<td>10</td>
<td>9.2 years</td>
</tr>
</tbody>
</table>

Some information on PFOA:

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

Most of PFAS need to be characterized in this sense!

3D modelling may help shedding light on TK/TD of PFAS
Addressing the transport of PFAS

3D molecular modeling integrated to experimtial 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>OAT4</th>
<th>URAT1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score (kcal/mol)</td>
<td>Substrate</td>
</tr>
<tr>
<td>PFHpA</td>
<td>-6.1</td>
<td>Yes</td>
</tr>
<tr>
<td>PFOA</td>
<td>-6.2</td>
<td>Yes</td>
</tr>
<tr>
<td>PFNA</td>
<td>-6.8</td>
<td>Yes</td>
</tr>
<tr>
<td>PFDA</td>
<td>-7.1</td>
<td>Yes</td>
</tr>
<tr>
<td>PFHxS</td>
<td>-6</td>
<td>Yes</td>
</tr>
<tr>
<td>PFOS</td>
<td>-7.3</td>
<td>Yes</td>
</tr>
<tr>
<td>PFBS</td>
<td>-5.5</td>
<td>No</td>
</tr>
<tr>
<td>Uric acid</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>-6.2</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Docking poses and MD results

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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 useful 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 informatively plan further experiments.
Thanks for your attention!

More details of related works @:

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