Mechanism and Prediction of UGT Metabolism

27th August 2019

Mario Öeren¹ – mario@optibrium.com

Peter Hunt¹, David J. Ponting², Matthew D. Segall¹

¹ Optibrium Limited, Cambridge UK. ² Lhasa Limited, Leeds UK.
Overview

• UGT metabolism
  - A short overview

• Mechanistic studies
  - *Ab initio*
  - Semi-empirical

• QSAR models
  - Results from mechanistic studies
  - Steric and orientation descriptors

• Conclusions
UGT Metabolism
Uridine Diphosphate Glucuronosyltransferase (UGT)

• Metabolic enzyme
  – Conjugation (phase II)
  – 40% of conjugation reactions
  – Works with endo- and xenobiotics

• Human isoforms
  – Located in liver, kidneys, gut etc.
  – 19 known active isoforms
  – No full crystal structure available
  – 1A1, 1A4, 1A9 and 2B7
Reaction Types and Substrates

- **O-glucuronidation**
  - Phenols
  - Alcohols
  - Carboxylic Acids

- **N-glucuronidation**
  - Amines
  - Amides
  - N-heterocycles

- **S- and C-glucuronidation**
  - Thiols and thioketones
  - 3,5-pyrazolidinedione

[Diagram showing various types of glucuronidation reactions with substrates such as Simple Phenols, Complex Phenols, Aliphatic Alcohols, Anthraquinones and Flavones, Courmarins, Bilirubin, Bile Acids, Carboxylic Acids, Primary Amines, Secondary Amines, Tertiary Amines, Heterocyclic Amines, Opioids, C18 Steroids, C19 Steroids, C21 Steroids, Sapogenins.]

Modelling Approach

- Project goals
  - Isoform-specific site of metabolism models
  - Isoform-specific substrate classification models

- Model should be based on fundamental physical properties
  - The rate of product formation is correlated with the activation energy ($E_a$) of the rate limiting step of product formation
  - Models are based on quantum mechanics
  - Each site of metabolism is considered in the context of the whole molecule

- Pros
  - It should transfer well between chemical classes
  - It should be applicable beyond the training set

- Influence of the active site of each isoform
  - Steric and orientation descriptors
Reaction Mechanism of Glucuronidation
Reaction Mechanism of Glucuronidation

- Wide variety of experimental studies
  - Chemical modification
  - Photoaffinity labelling
  - Mutagenesis studies
  - Competitive inhibitors
  - Homology modelling
  - Docking studies
  - Different mechanisms

- No previous studies using quantum mechanical modelling methods
  - Density Functional Theory (DFT)
Mechanistic Studies – Simplification of the System

- Simplification of the system
  - Disregard the protein
  - Simplify the UDP-GA
Mechanistic Studies – *Ab Initio* Calculations

- Simplification of the system
  - Disregard the protein
  - Simplify the UDP-GA

- Identification of a transition state
  - *Ab initio* (B3LYP/SVP)
  - Generalizable for *N*- and *O*-glucuronidation
Mechanistic Studies – *Ab Initio* Calculations

- Simplification of the system
  - Disregard the protein
  - Simplify the UDP-GA

- Identification of a transition state
  - *Ab initio* (B3LYP/SVP)
  - Generalizable for *N*- and *O*-glucuronidation

Paracetamol
Mechanistic Studies – Validation

- Simplification of the system
  - Disregard the protein
  - Simplify the UDP-GA

- Identification of a transition state
  - Ab initio (B3LYP/SVP)
  - Generalizable for N- and O-glucuronidation

- Validation of the transition state
  - Experimental data ($V_{\text{max}}$)
  - $k = A e^{-\frac{E_a}{RT}}$
  - Data availability (O-glucuronidation)
  - Shape specific active sites
  - Noise in biological experiments

DFT vs $V_{\text{max}}$ (UGT1A1)

$E_a$ (kJ mol$^{-1}$)

$R^2 = 0.34$
Mechanistic Studies – Validation

• Simplification of the system
  – Disregard the protein
  – Simplify the UDP-GA

• Identification of a transition state
  – Ab initio (B3LYP/SVP)
  – Generalizable for N- and O-glucuronidation

• Validation of the transition state
  – Experimental data ($V_{\text{max}}$)
  – $k = Ae^{-\frac{E_a}{RT}}$
  – Data availability (O-glucuronidation)
  – Shape specific active sites
  – Noise in biological experiments

228 kJ mol$^{-1}$

243 kJ mol$^{-1}$

326 kJ mol$^{-1}$

Trifluoperazine

Observed
Non-Observed
From *Ab Initio* to Semi-empirical
B3LYP/SVP and AM1 Correlation

- Things to consider
  - AM1 is unable to detect weak interactions (H+ transfer)
  - AM1 systematic errors

- Fragment calculations
  - Aliphatic alcohols
  - Phenols
  - Carboxylic acids
  - Primary amines
  - Secondary amines
  - Tertiary amines

DFT vs Semi-Empirical

- AM1 (kJ mol⁻¹)
- B3LYP/SVP (kJ mol⁻¹)

- R² = 0.61
- R² = 0.90
B3LYP/SVP and AM1 Correlation

• Things to consider
  – AM1 is unable to detect weak interactions (H\textsuperscript{+} transfer)
  – AM1 systematic errors

• Fragment calculations
  – Aliphatic alcohols
  – Phenols
  – Carboxylic acids
  – Primary amines
  – Secondary amines
  – Tertiary amines

• Corrections for each class
  – \( R^2 = 0.95 \)
QSAR Models

- **Model order**
  - Isoform-specific site of metabolism models
  - Isoform-specific substrate classification models
  - General substrate classification models

- **Descriptors**
  - $E_a$
  - Site-specific descriptors
  - Whole-molecule descriptors

- **Methods**
  - Gaussian Processes

An example: atom-pair descriptor describing contribution of aromaticity.

Site of Metabolism Model of UGT1A1

- Compounds
  - Only compounds which are glucuronidated
  - Compounds with two or more sites

- Training and test sets
  - Split by molecule
  - 80:20 split
  - 0.7 Tanimoto Coefficient

- Training set
  - 79 molecules, 242 sites
  - 120 glucuronidated and 122 not

- Test set
  - 19 molecules, 52 sites
  - 26 glucuronidated and 26 not

<table>
<thead>
<tr>
<th>Model</th>
<th>Kappa</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPOPT</td>
<td>0.65</td>
<td>83%</td>
</tr>
</tbody>
</table>
Substrate Classification Model of UGT1A1

- **Compounds**
  - All compounds measured for UGT1A1
  - Compounds with no site-specific information

- **Training and test sets**
  - Split by molecule
  - 80:20 split
  - 0.7 Tanimoto Coefficient

- **Training Set**
  - 337 molecules
  - 171 glucuronidated and 166 not

- **Test set**
  - 67 molecules
  - 36 glucuronidated and 31 not

<table>
<thead>
<tr>
<th>Model</th>
<th>Kappa</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPOPT</td>
<td>0.64</td>
<td>82%</td>
</tr>
</tbody>
</table>

![Confusion Matrix](image)
Conclusions

- Mechanism of glucuronidation
  - Simplified transition state \((ab\ initio)\)
  - Validated against experimental data
  - Works with both \(N\)- and \(O\)-glucuronidation
  - Scalable using semi-empirical calculations

- QSAR models
  - Site of Metabolism Model
  - Substrate Classification Model
  - \(E_a\) and steric and orientation descriptors, whole molecule descriptors

- Future work
  - Tackle isoforms 1A4, 1A9 and 2B7
Thank You!

Matthew Segall
Peter Hunt

David Ponting

© 2019 Optibrium Ltd.