Mechanism and Prediction of UGT Metabolism

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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

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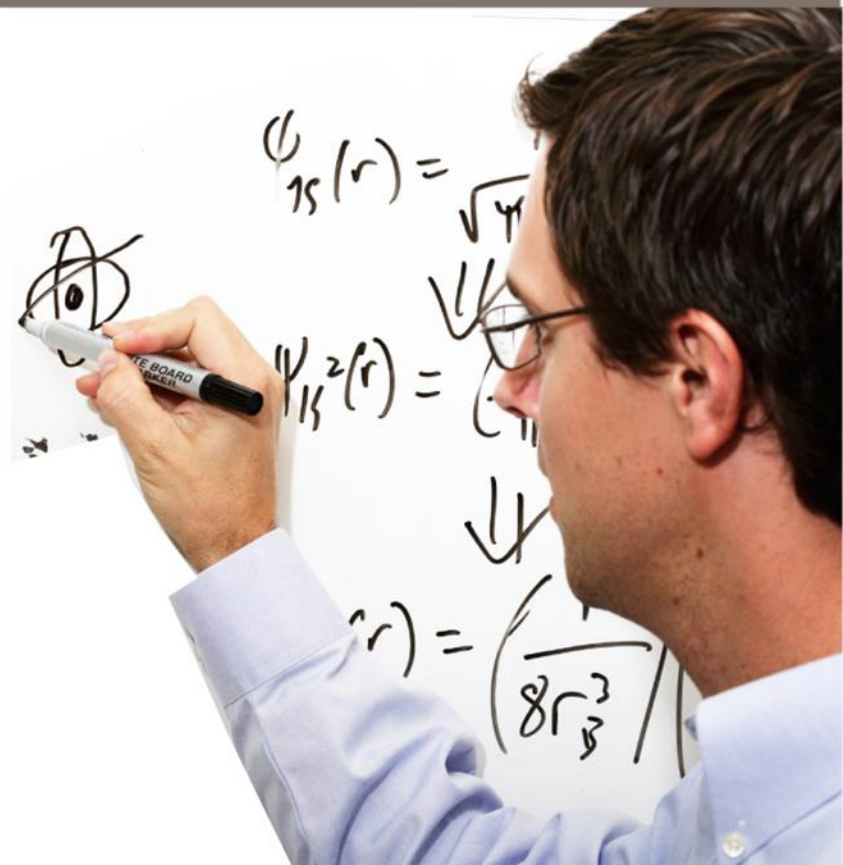
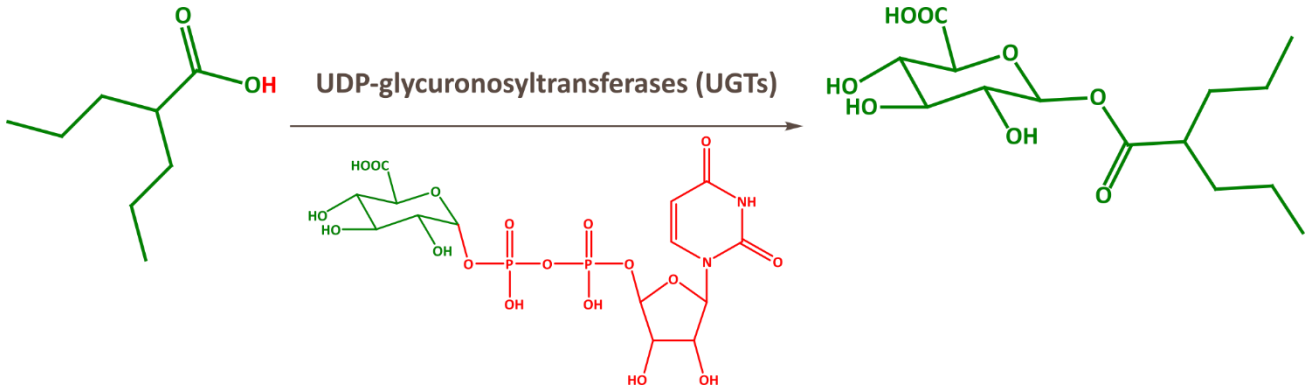
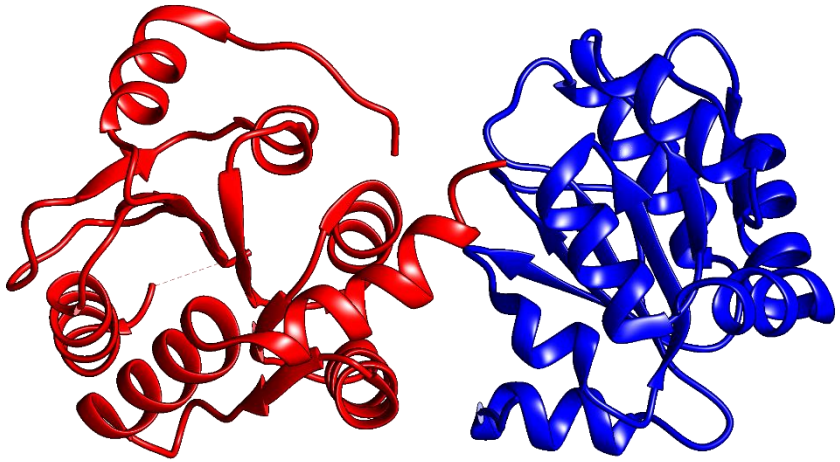


Uridine Diphosphate Glucuronosyltransferase (UGT)

- 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





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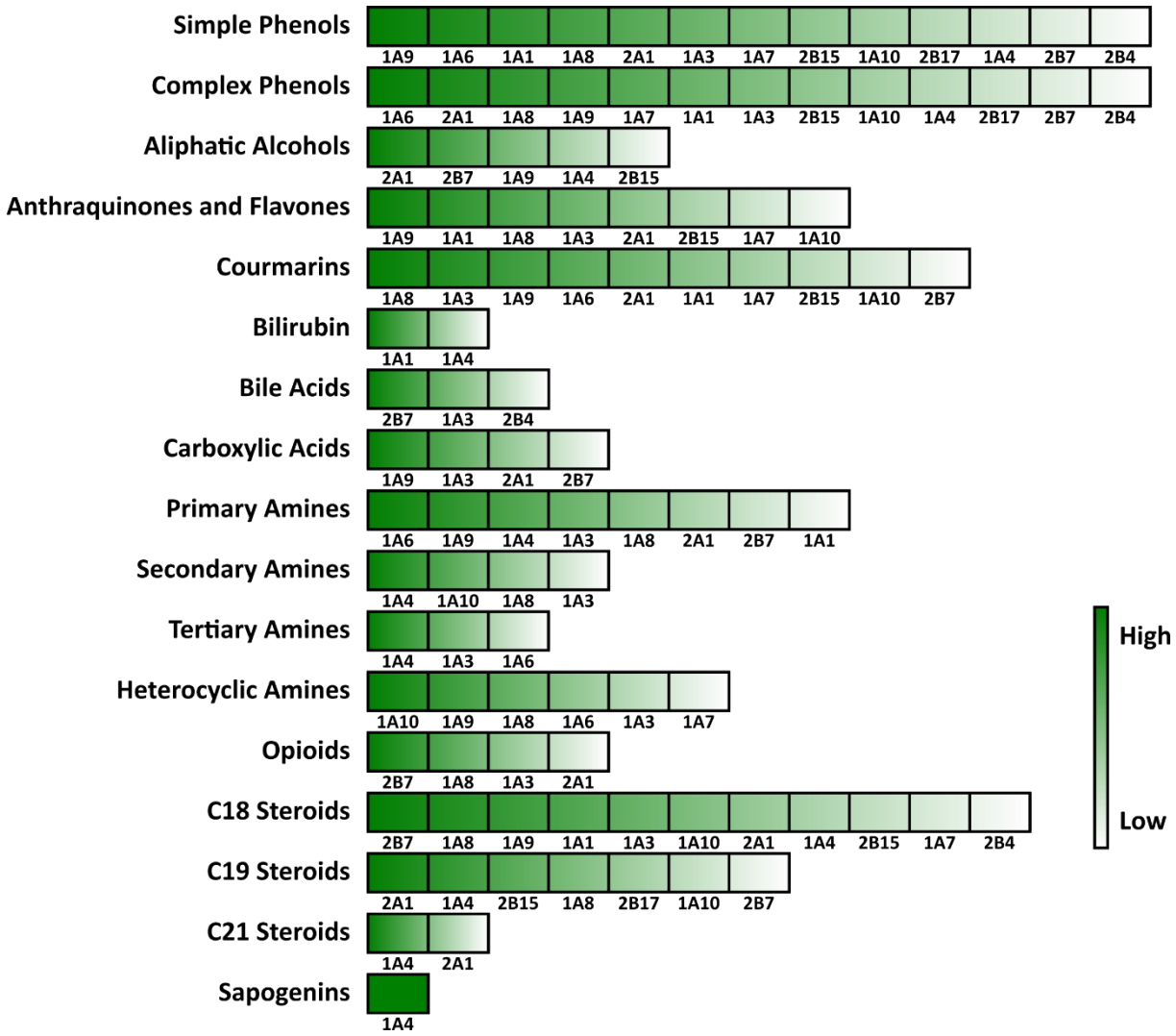
Reaction Types and Substrates

- *O*-glucuronidation
  - Phenols
  - Alcohols
  - Carboxylic Acids
- *N*-glucuronidation
  - Amines
  - Amides
  - *N*-heterocycles
- *S*- and *C*-glucuronidation
  - Thiols and thioketones
  - 3,5-pyrazolidinedione



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

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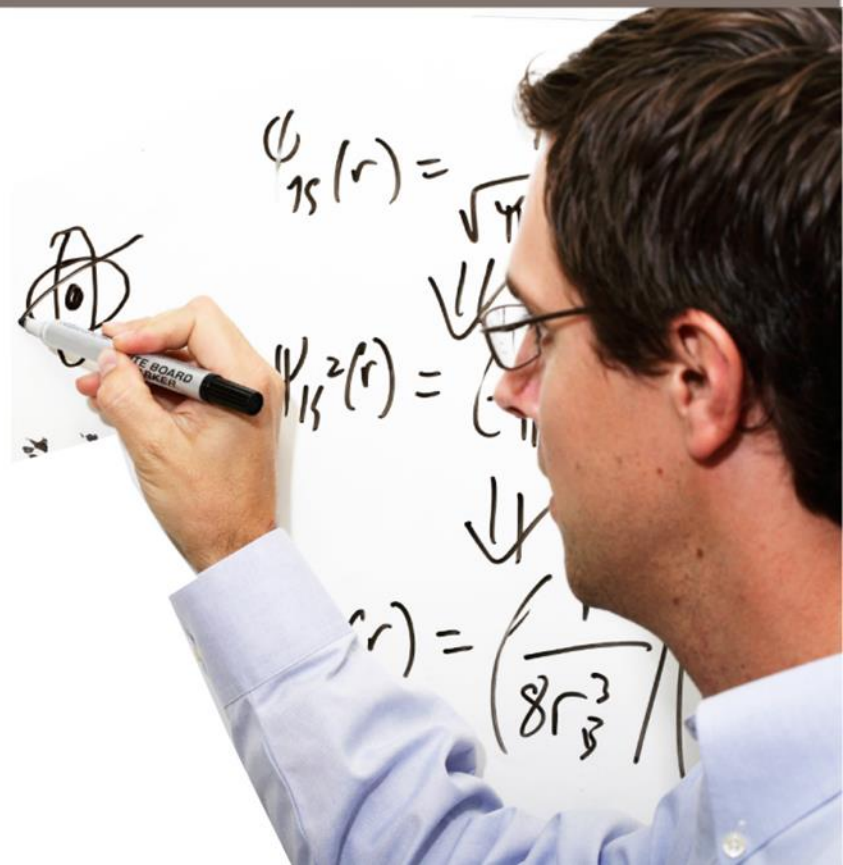
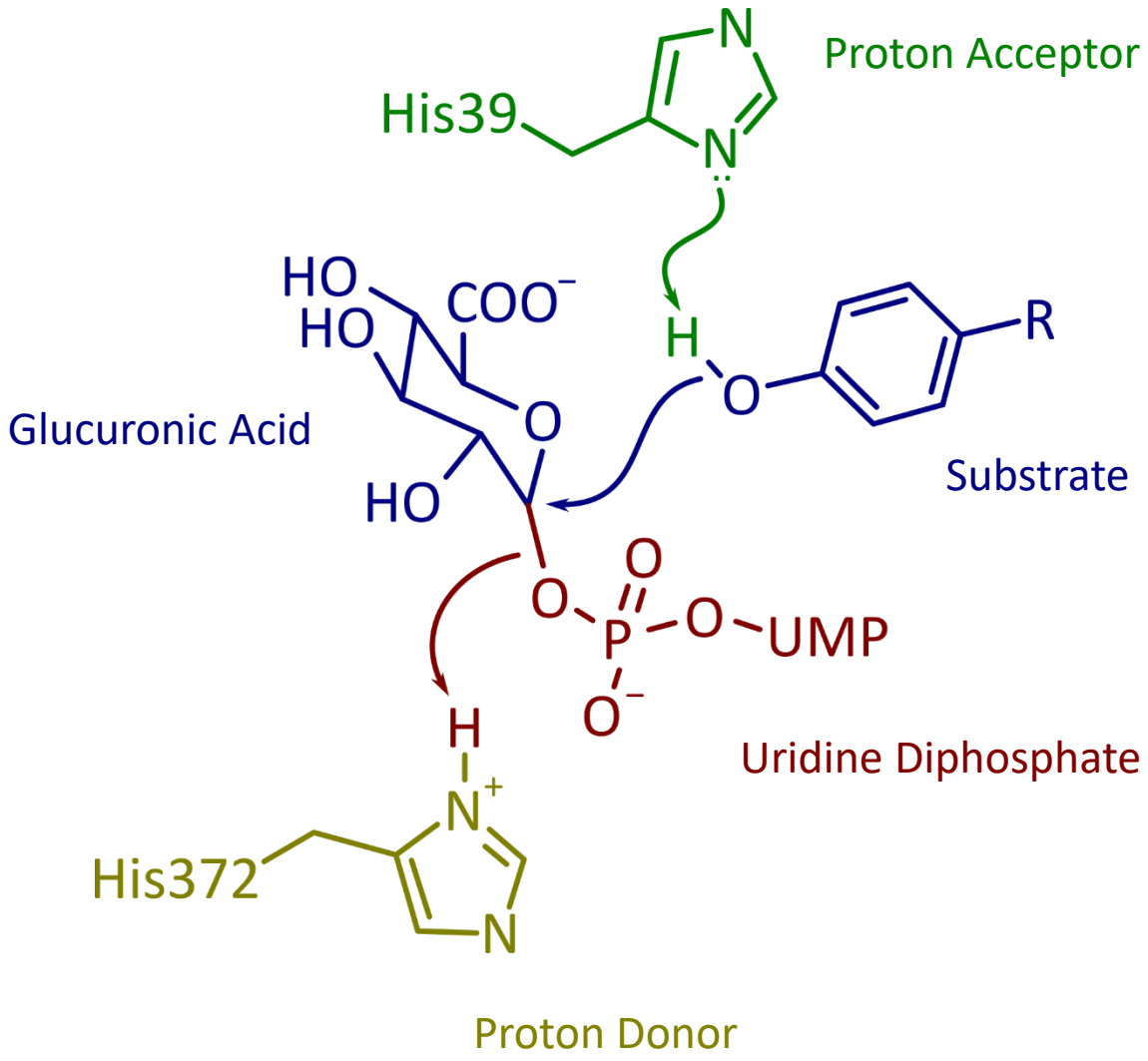


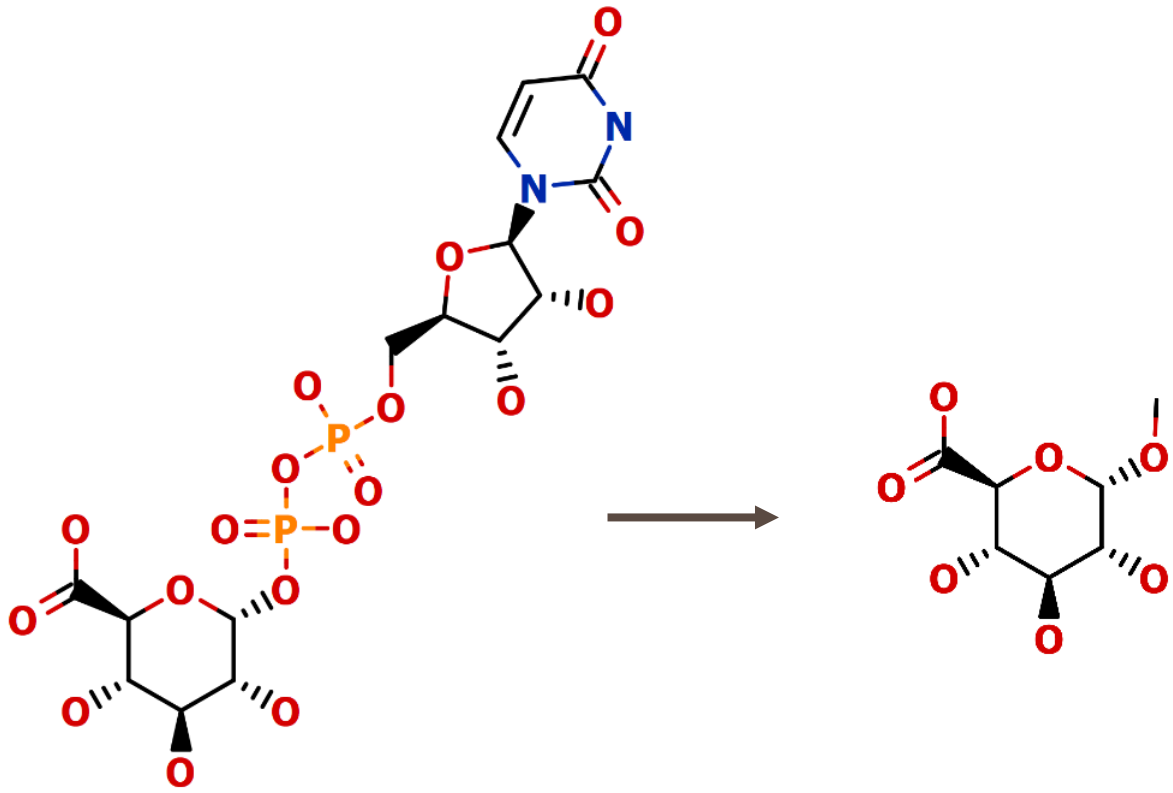
Reaction Mechanism of Glucuronidation

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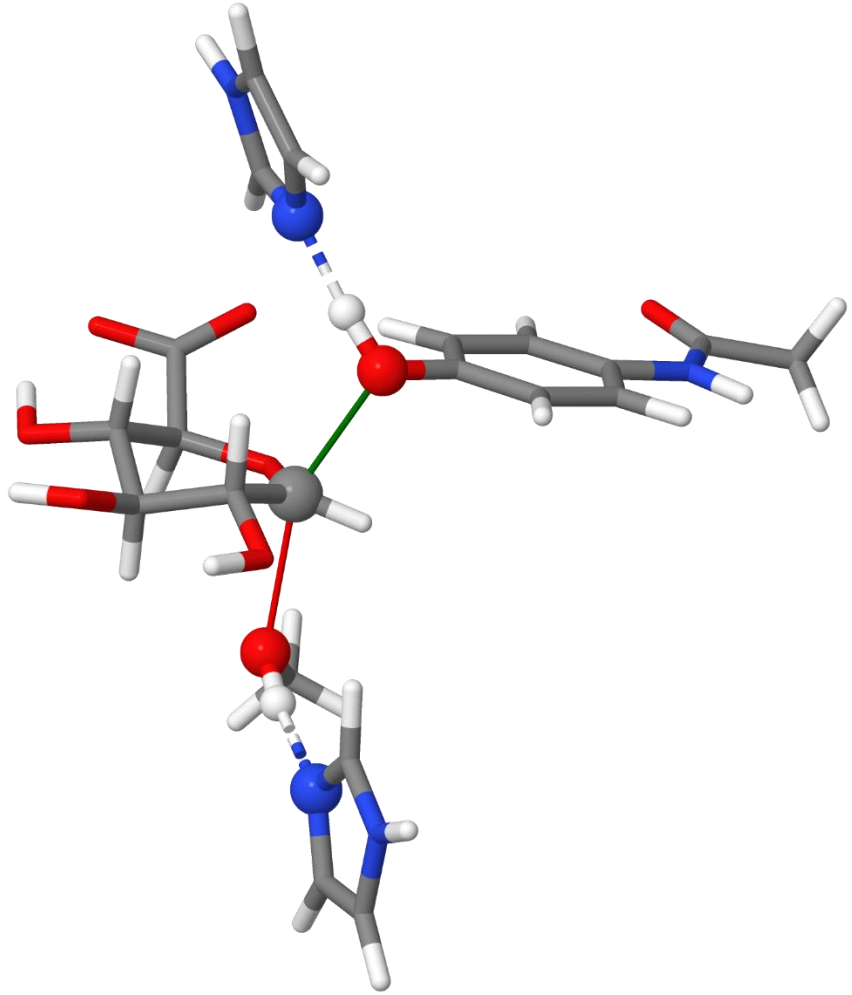
- Identification of a transition state
  - *Ab initio* (B3LYP/SVP)
  - Generalizable for *N*- and *O*-glucuronidation



Paracetamol

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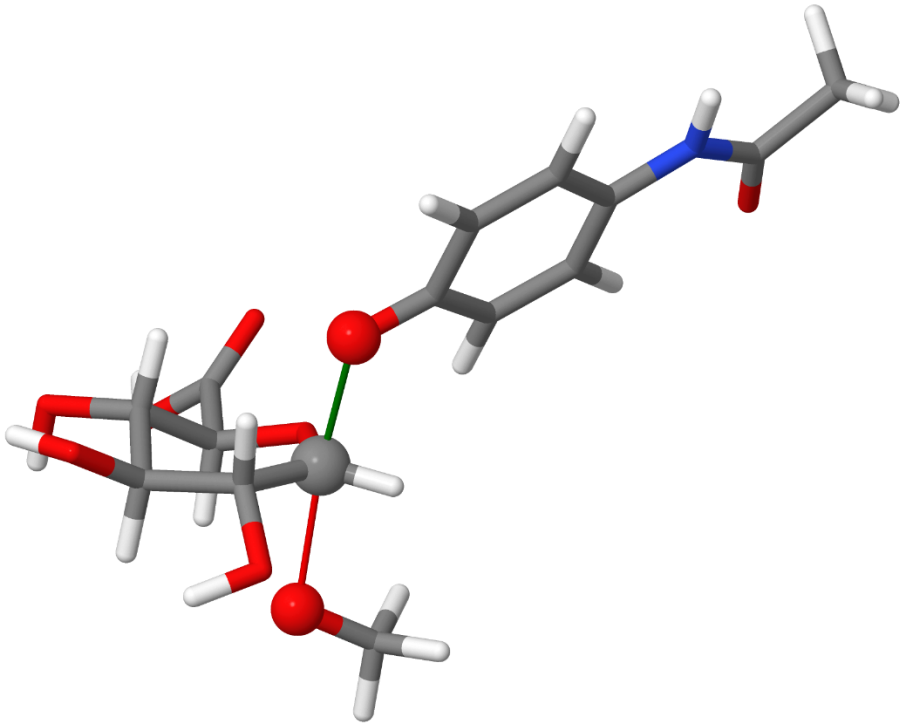

Paracetamol

Mechanistic Studies – Validation

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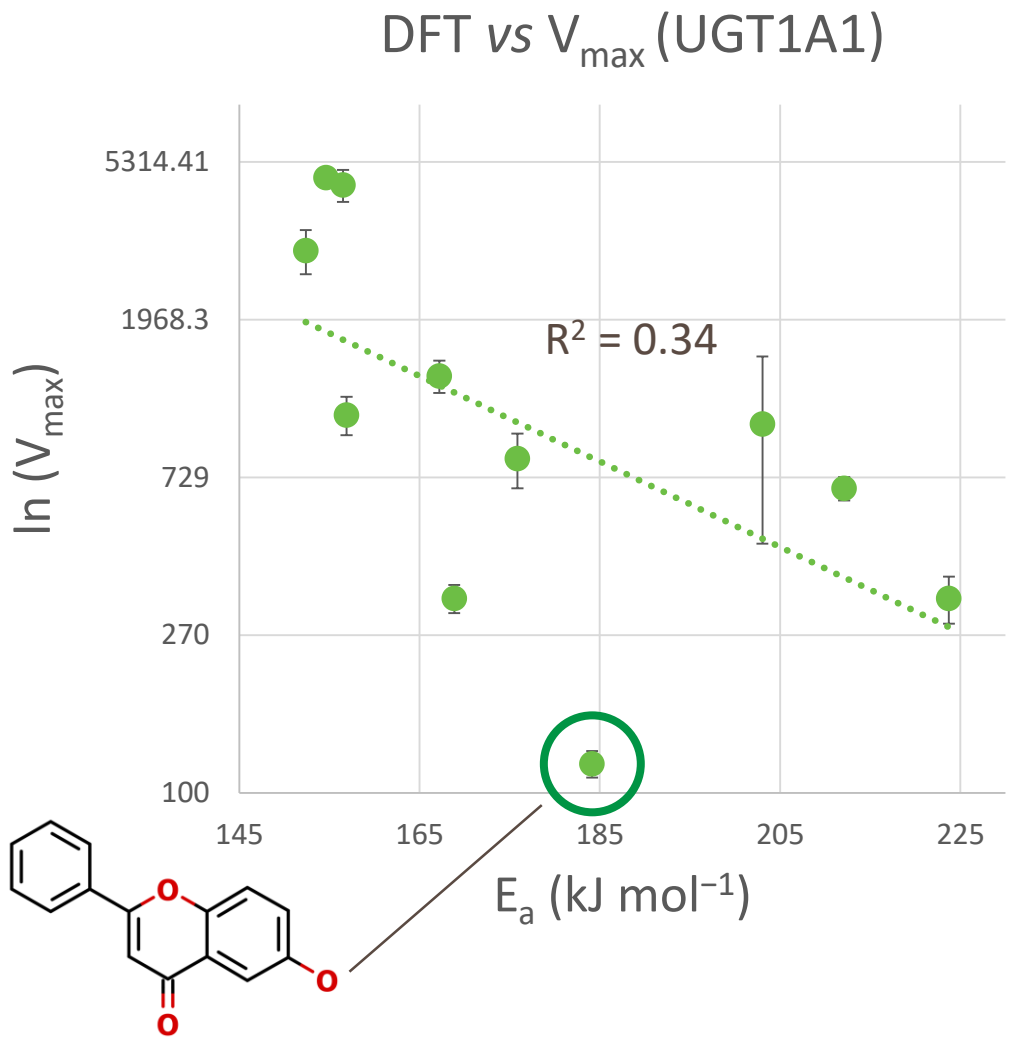
- Validation of the transition state
  - Experimental data (V_{max})
  - $k = Ae^{\frac{-E_a}{RT}}$
  - Data availability (*O*-glucuronidation)
  - Shape specific active sites
  - Noise in biological experiments



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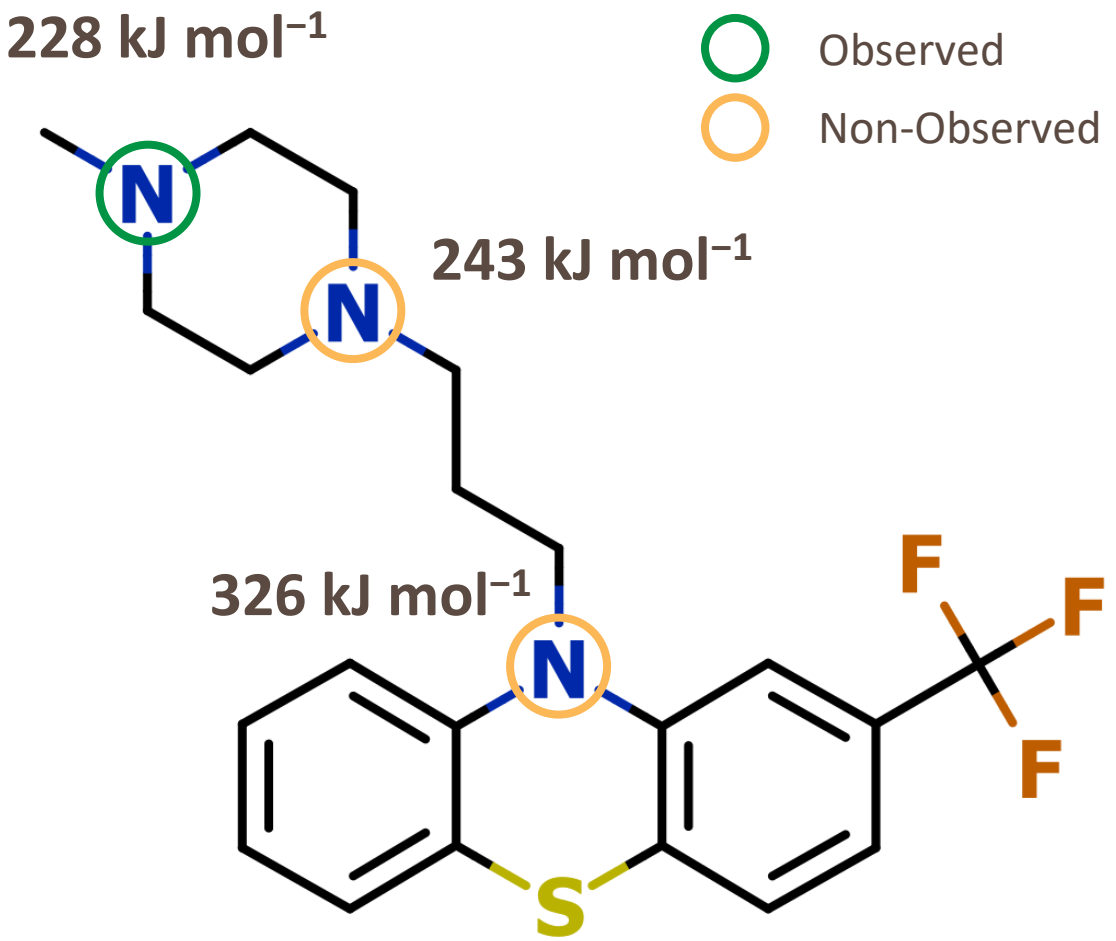

Trifluoperazine

From *Ab Initio* to Semi-empirical

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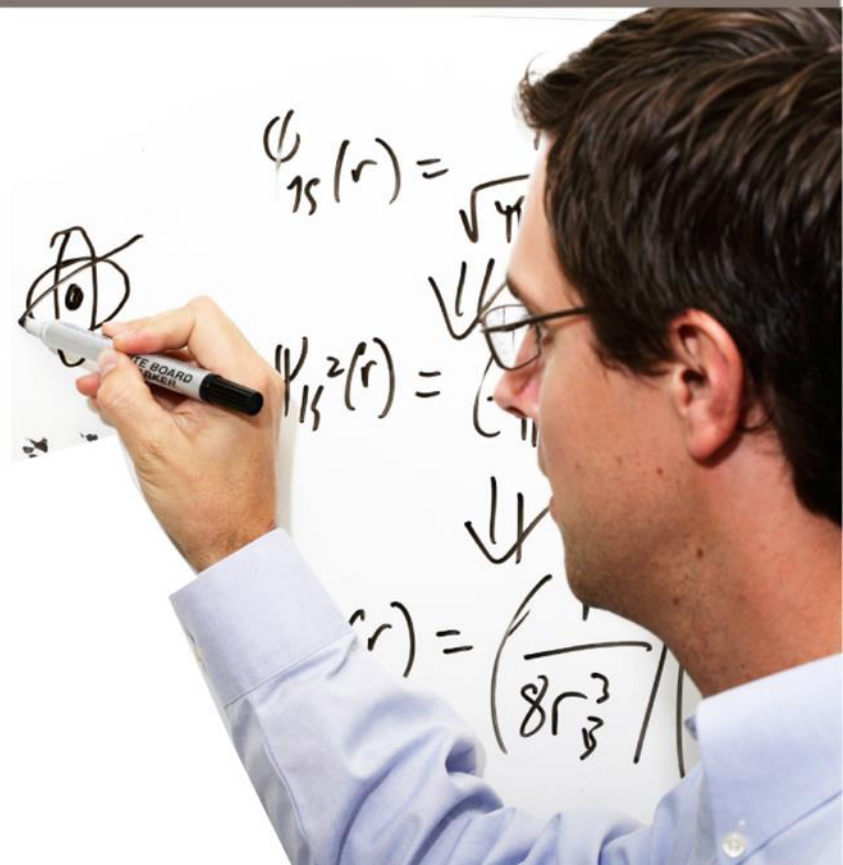
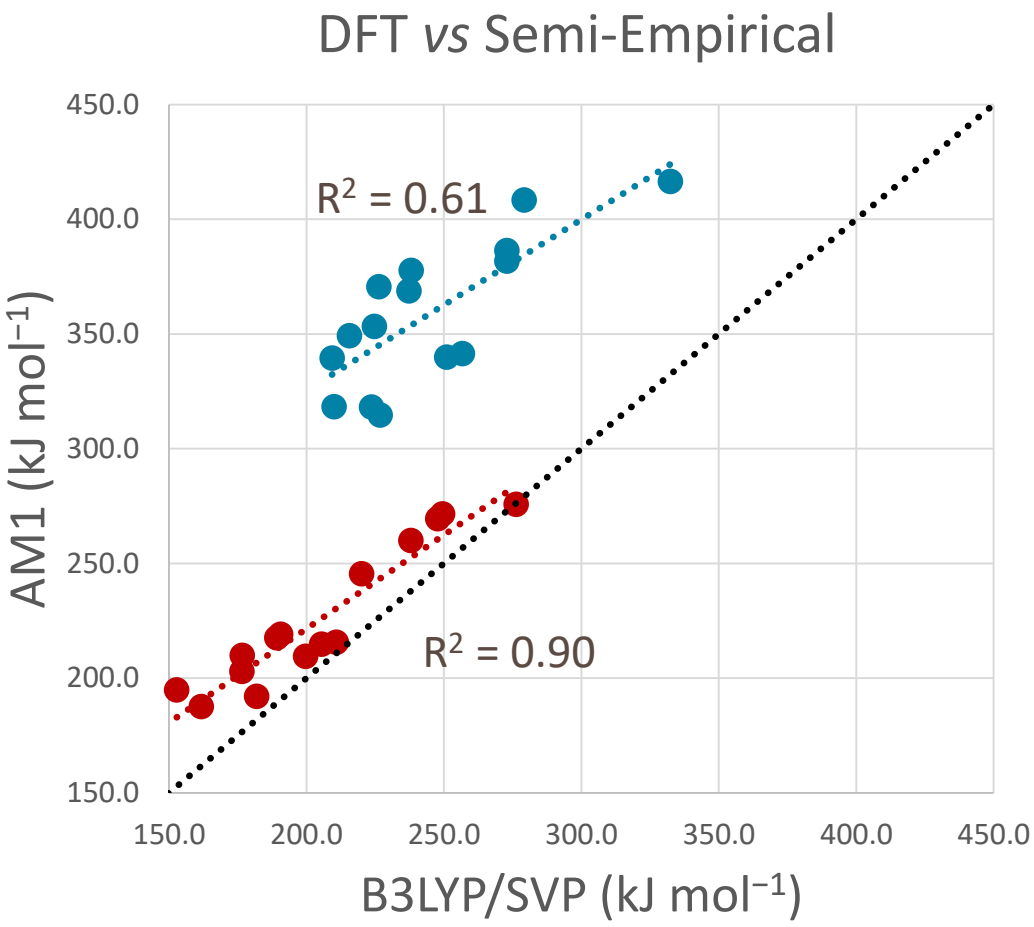


B3LYP/SVP and AM1 Correlation

- 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



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- Fragment calculations
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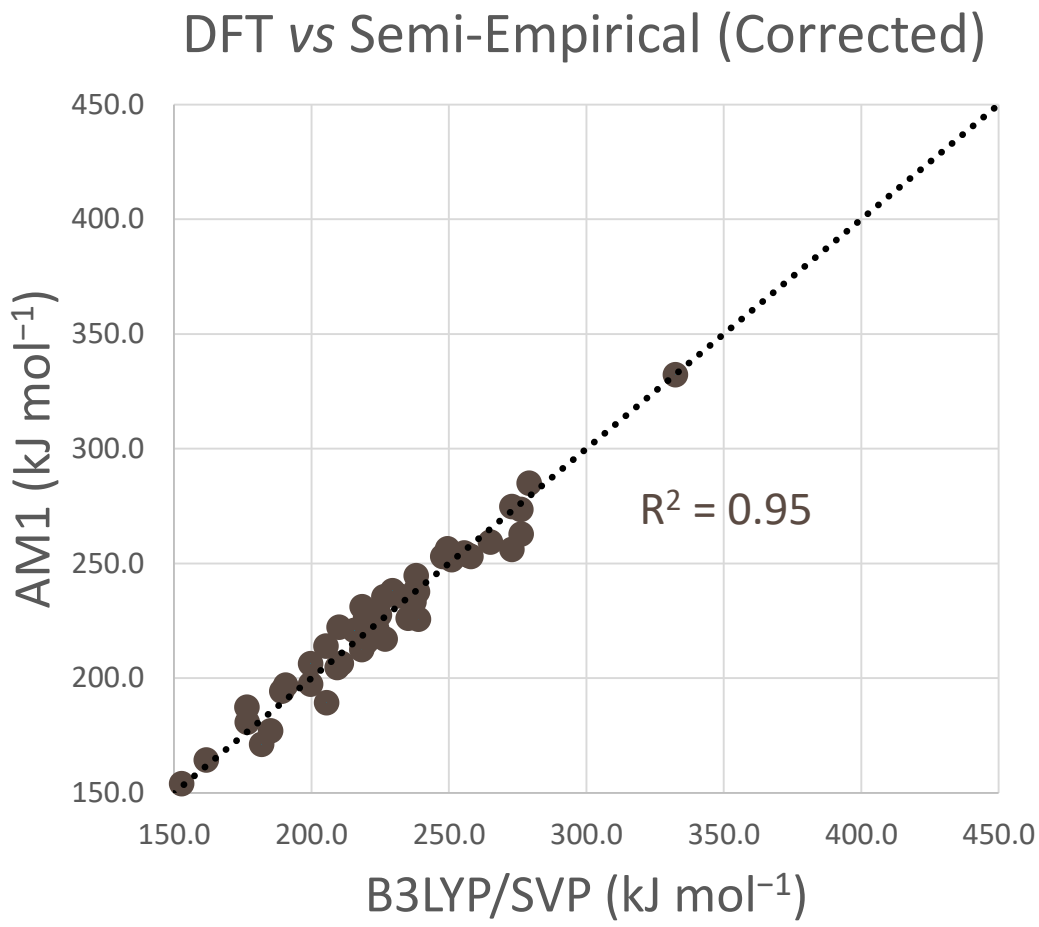
  - Carboxylic acids
  - Primary amines
  - Secondary amines
  - Tertiary amines
- Corrections for each class
  - $R^2 = 0.95$



QSAR Models

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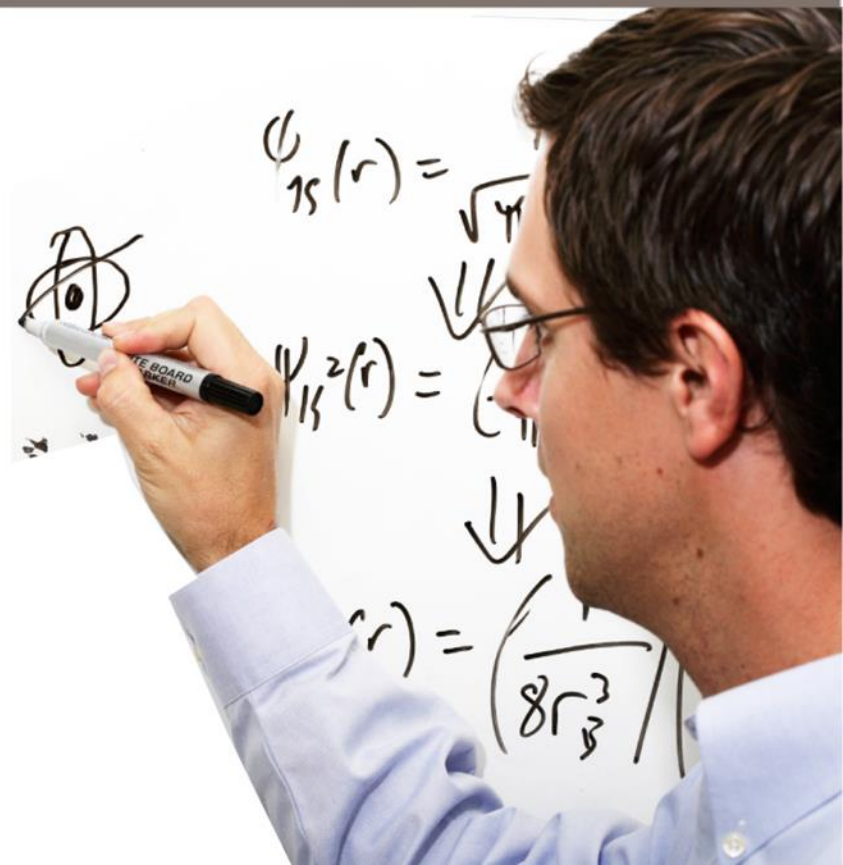
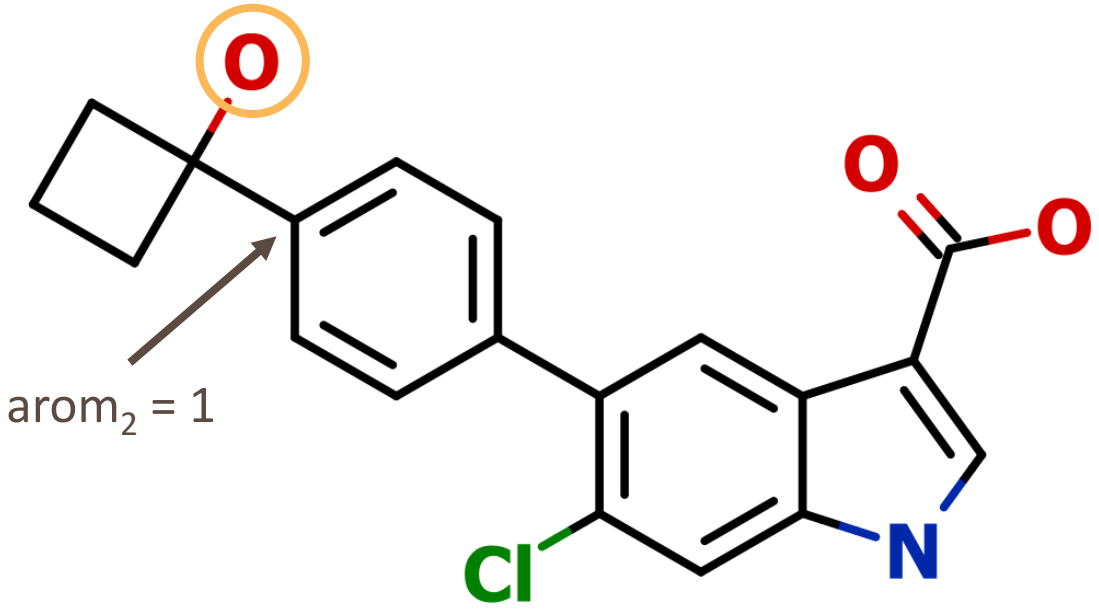


QSAR Models

- 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

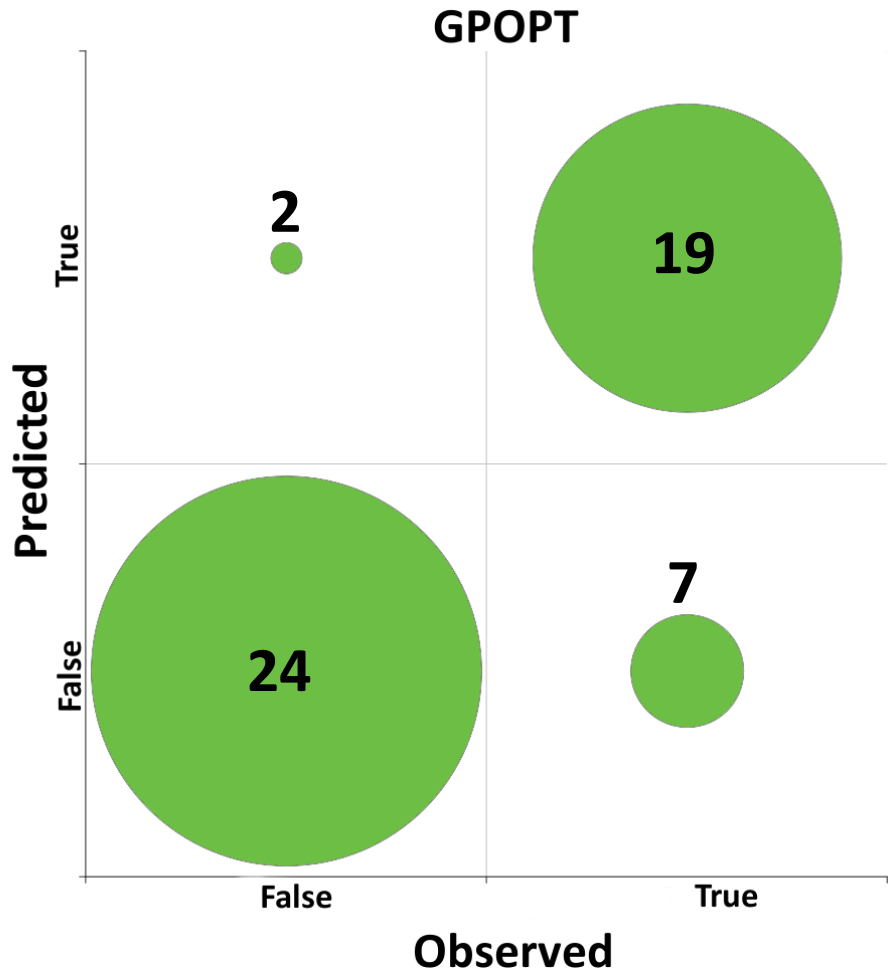
An example: atom-pair descriptor describing contribution of aromaticity.



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

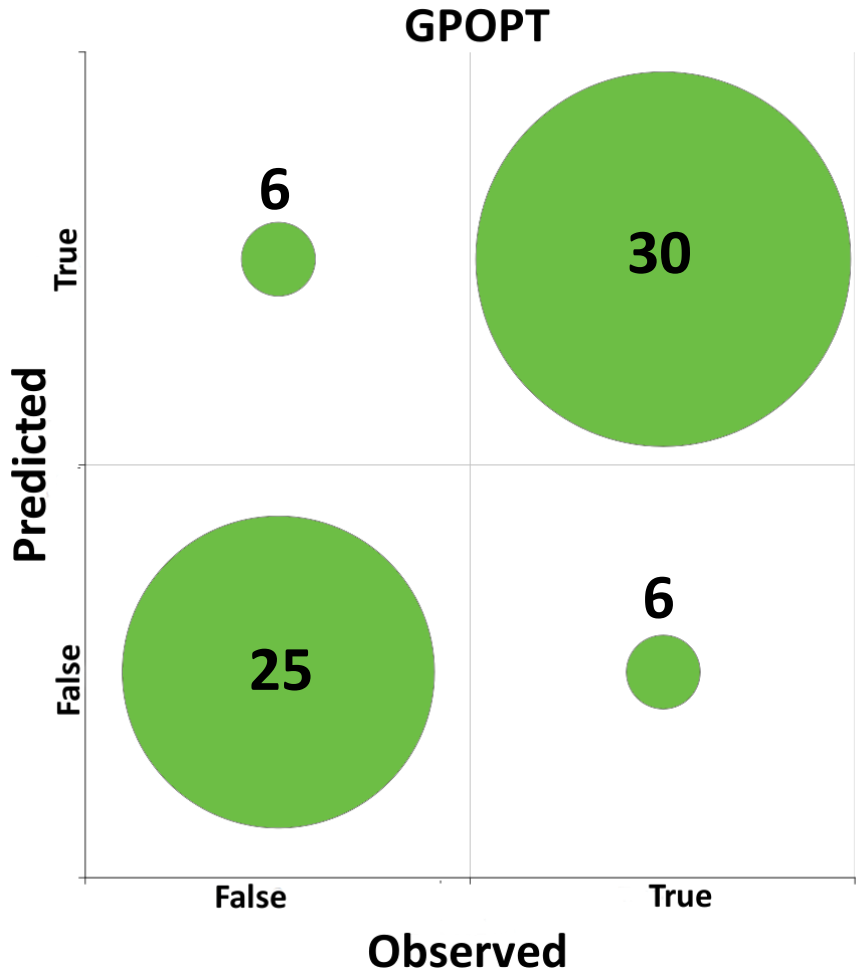
Model	Kappa	Accuracy
GPOPT	0.65	83%



Substrate Classification Model of UGT1A1

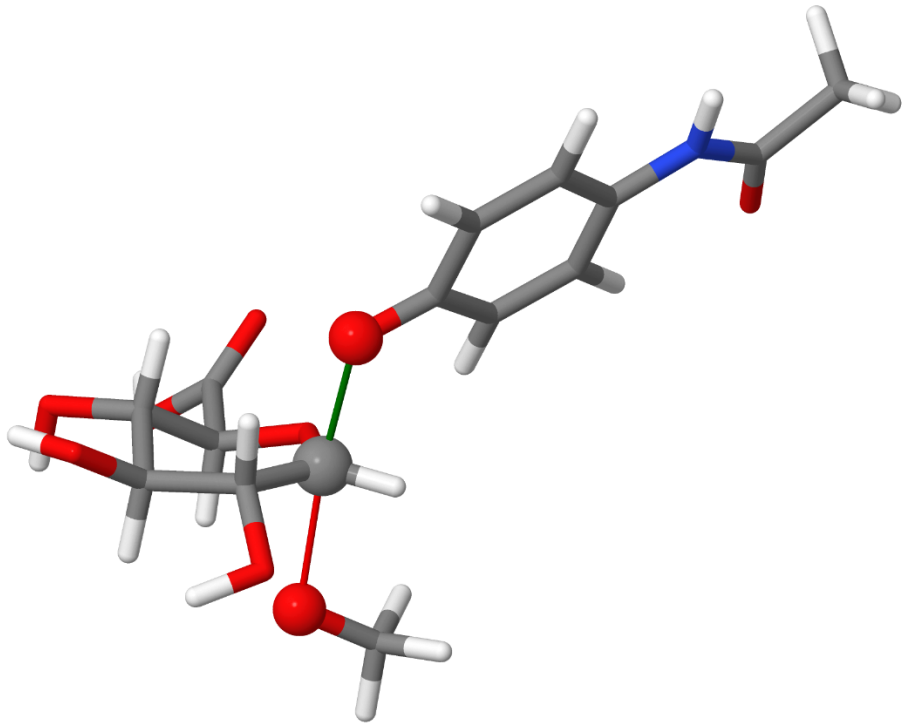
- Compounds
  - All compounds measured for UGT1A1
  - Compounds with no site-specific information
- Training and test sets
  - Split by molecule
  - 80:20 split
  - 0.7 Tanimoto Coefficient
- Training Set
  - 337 molecules
  - 171 glucuronidated and 166 not
- Test set
  - 67 molecules
  - 36 glucuronidated and 31 not

Model	Kappa	Accuracy
GPOPT	0.64	82%



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!

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