Predicting Reactivity to Drug Metabolism: Beyond CYPs

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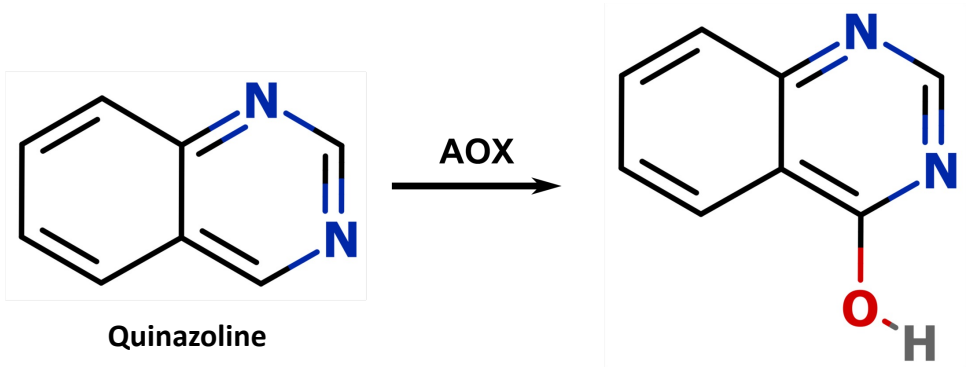
Enzymes

Aldehyde Oxidase (AOX)

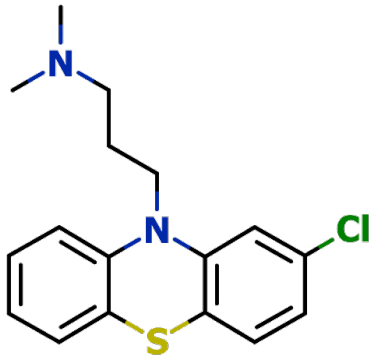
- Enzyme class in the modification phase
- Reaction types:
  - Oxidation of aldehydes and aromatic (hetero)cycles
  - Oxidation of iminium ions and amide hydrolysis
- Isoforms
  - **AOX1**
- Rationale
  - Azaheterocycles are common in drug-like molecules
  - Reduced CYP metabolism
  - Unexpected high metabolic clearance



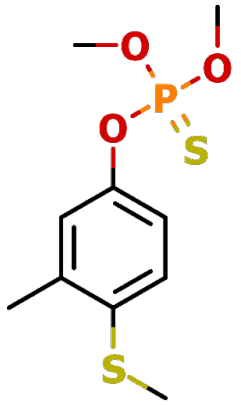
Quinazoline

Flavin-containing Monooxygenases (FMO)

- Works in conjunction with Cytochrome P450s in the modification phase
- Reaction types:
  - *N*- and *S*-oxidation
  - Demethylation, desulfuration and Bayer-Villiger oxidation
- Isoforms
  - **FMO1**, FMO2, **FMO3**, FMO4 and FMO5
- Rationale
  - Overlapping substrates with P450s (*e.g.* avoid DDI)
  - Preventing P450 metabolites
  - Toxic metabolites such as *S*-oxides and *S,S*-dioxides of thiocarbonyl (sulfines and sulfenes)



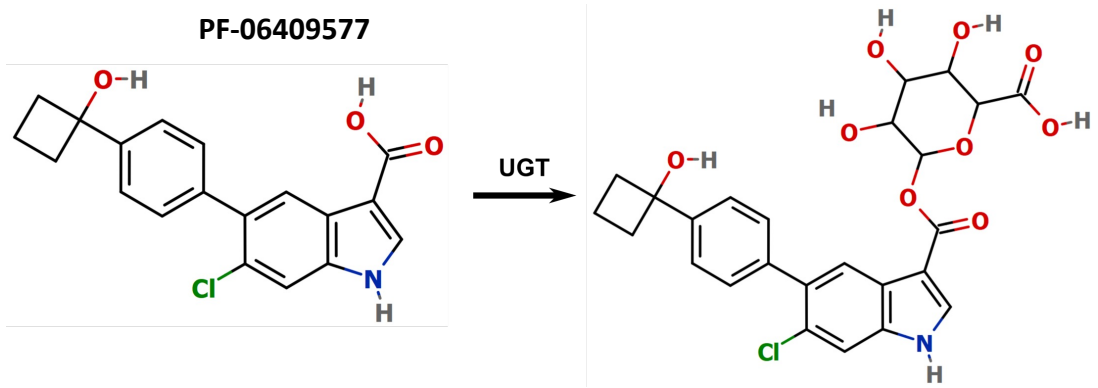
Chlorpromazine



Fenthion

Uridine Diphosphate Glucuronosyltransferase (UGT)

- Enzyme class in the conjugation phase
  - 40 per cent of known conjugation reactions
  - 15 per cent of drug metabolism reactions
- Reaction types:
  - *N*- and *O*-glucuronidation
  - *C*- and *S*-glucuronidation



- Isoforms
  - **1A1**, 1A3, **1A4**, 1A5, 1A6, 1A7, 1A8, **1A9**, 1A10, 2A1, 2A2, 2A3, 2B4, **2B7**, 2B10, 2B11, 2B15, 2B17, 2B28, 3A1, 3A2 and 8A1
- Rationale
  - The most important enzyme class in the conjugation phase

Reactivity-accessibility Approach

Approaches to Predicting Drug Metabolism

Empirical

Pros

Fast

Easy to set up

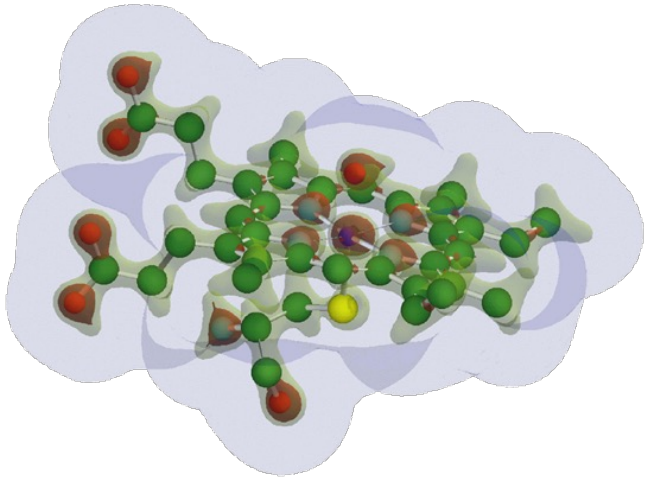
Cons

Needs lots of data

Non-transferable

Qualitative

Mechanistic



Pros

Can be built on smaller high-quality data sets

Transferable – based on physical principles

(Semi) quantitative

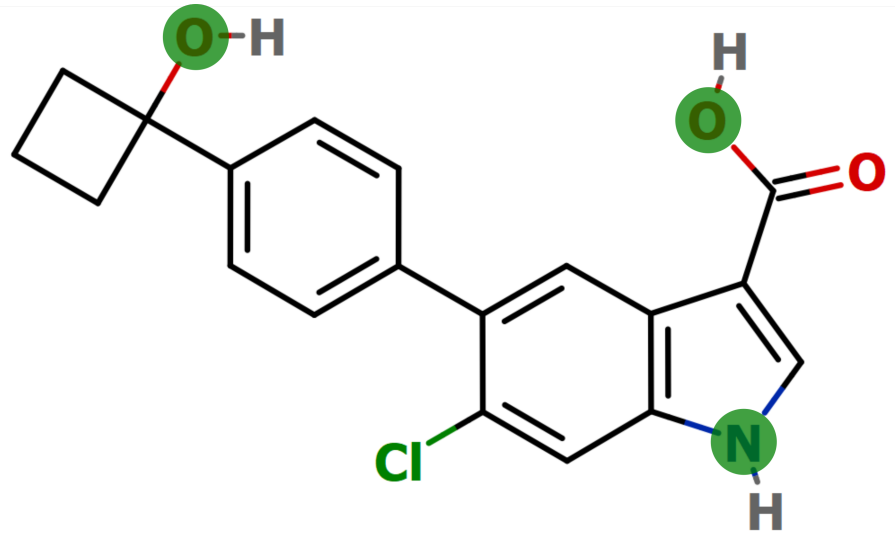
Cons

(Very) slow

Requires detailed understanding

Reactivity-accessibility Approach

- Aim
  - Which atom interacts with the enzyme
  - The potential site of metabolism and the observed site of metabolism
- Reactivity
  - Calculate the Ea of the rate limiting step
  - Simplified protein, but full substrate
  - Independent of isoform
- Accessibility
  - Steric accessibility
  - Electrostatic interactions
  - Dependent on isoform and substrate



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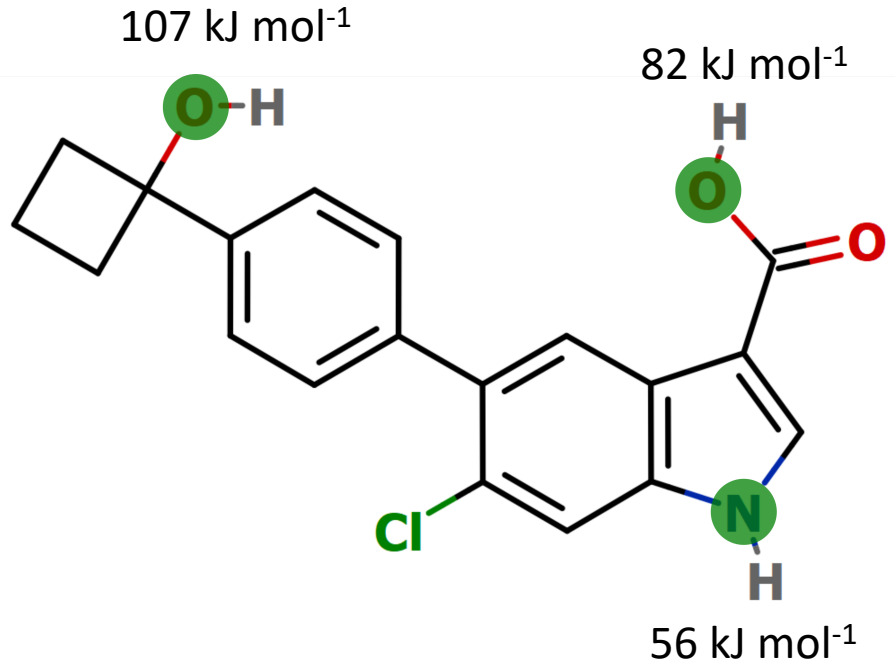

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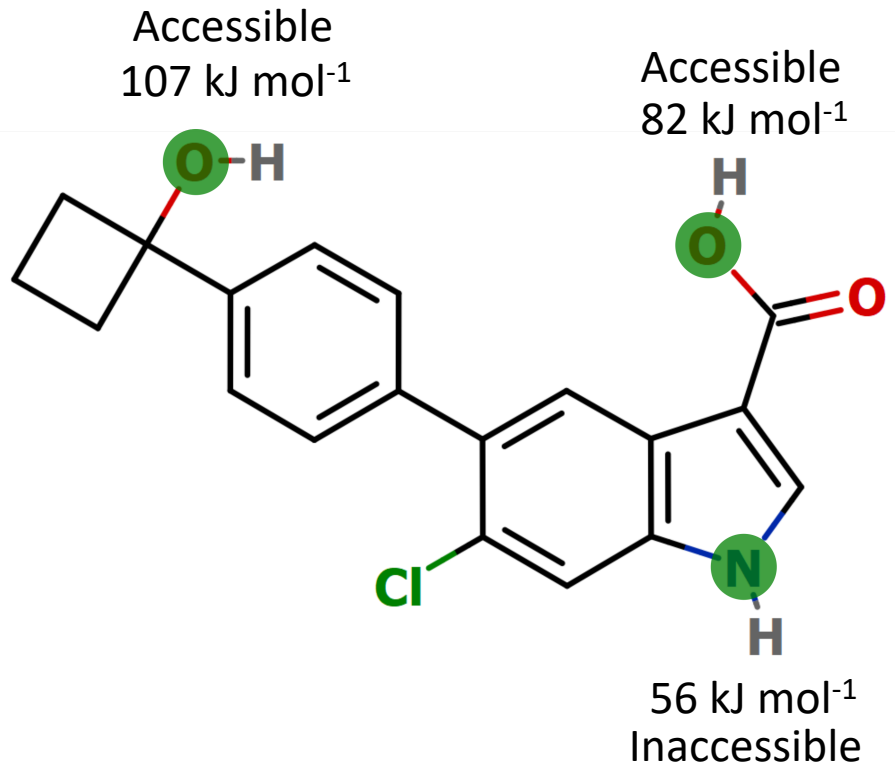
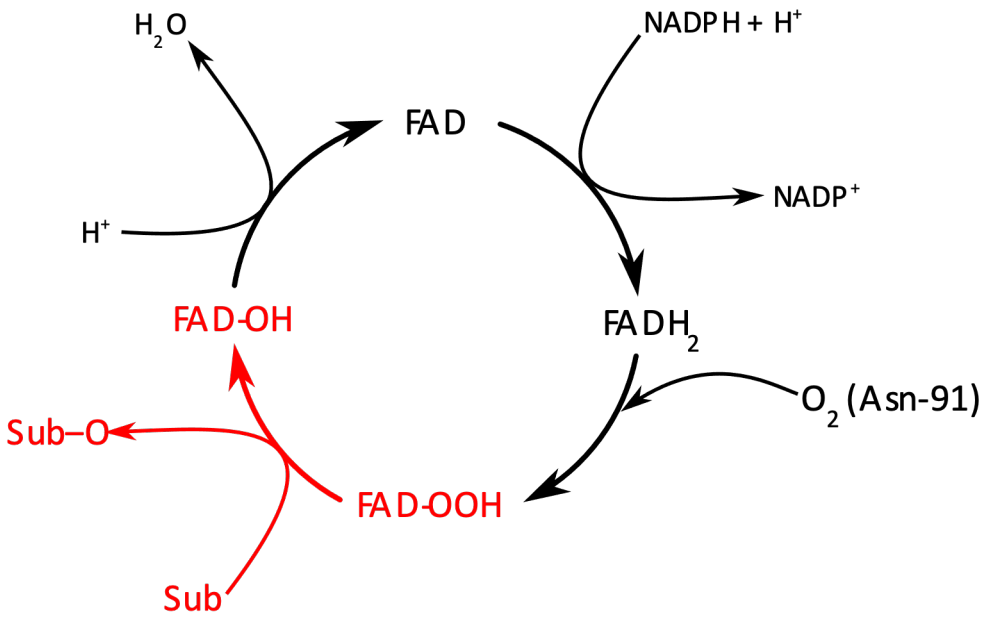


Simplifications for Reactivity

The Simplified Mechanism for FMO

- Rate-limiting step
  - Oxidation
  - S_N2 reaction mechanism



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The Simplified Mechanism for FMO

- Rate-limiting Step
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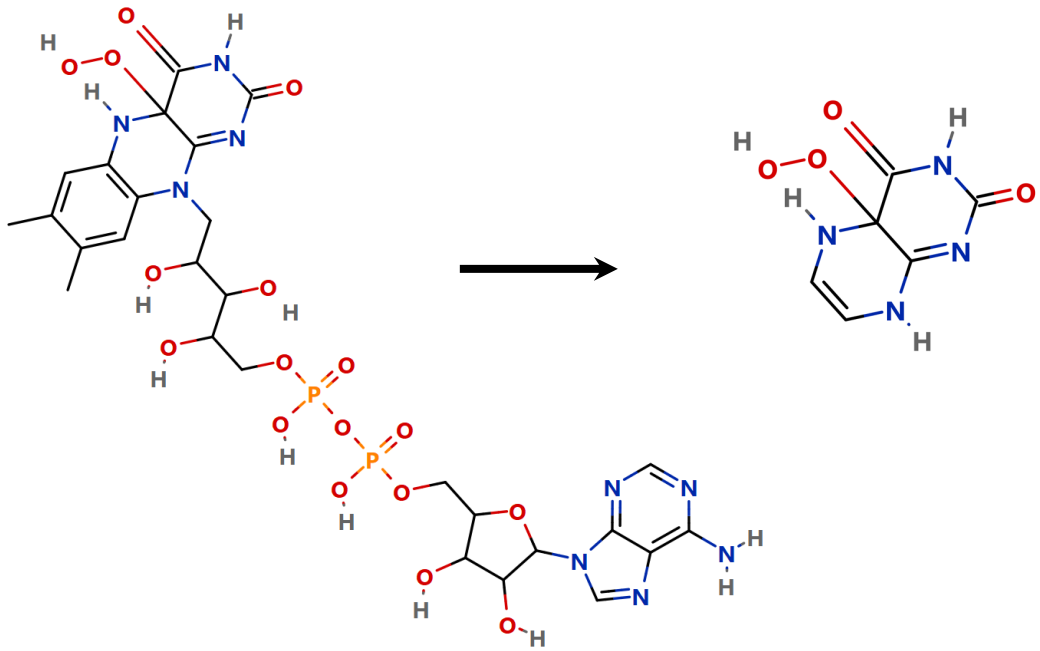
  - Discarded the protein
  - Full co-factor too large for practical calculations
  - “Tail” buried in groove and does not interact with substrate
  - Behaviour of simplified system at peroxide site analogous to full reaction centre



B3LYP/SVP

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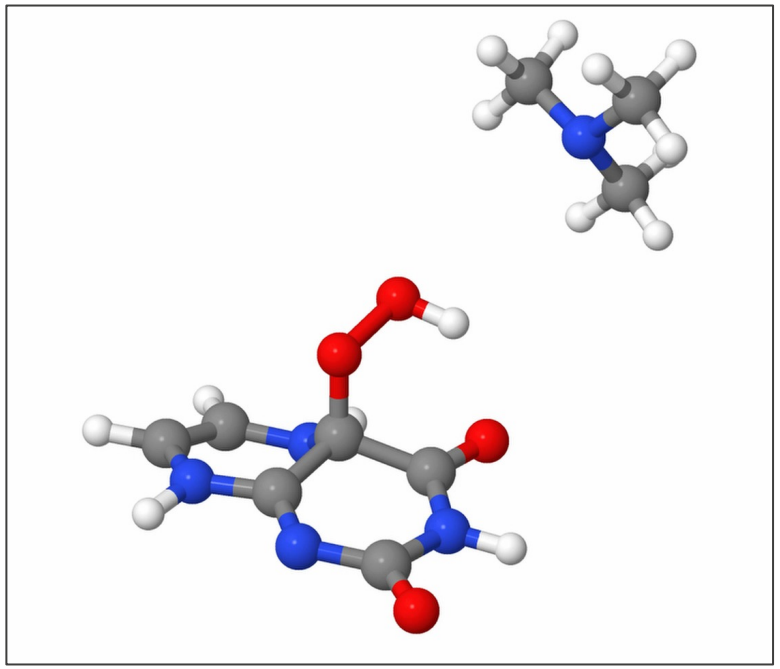
  - Interested in the activation energy of the reaction



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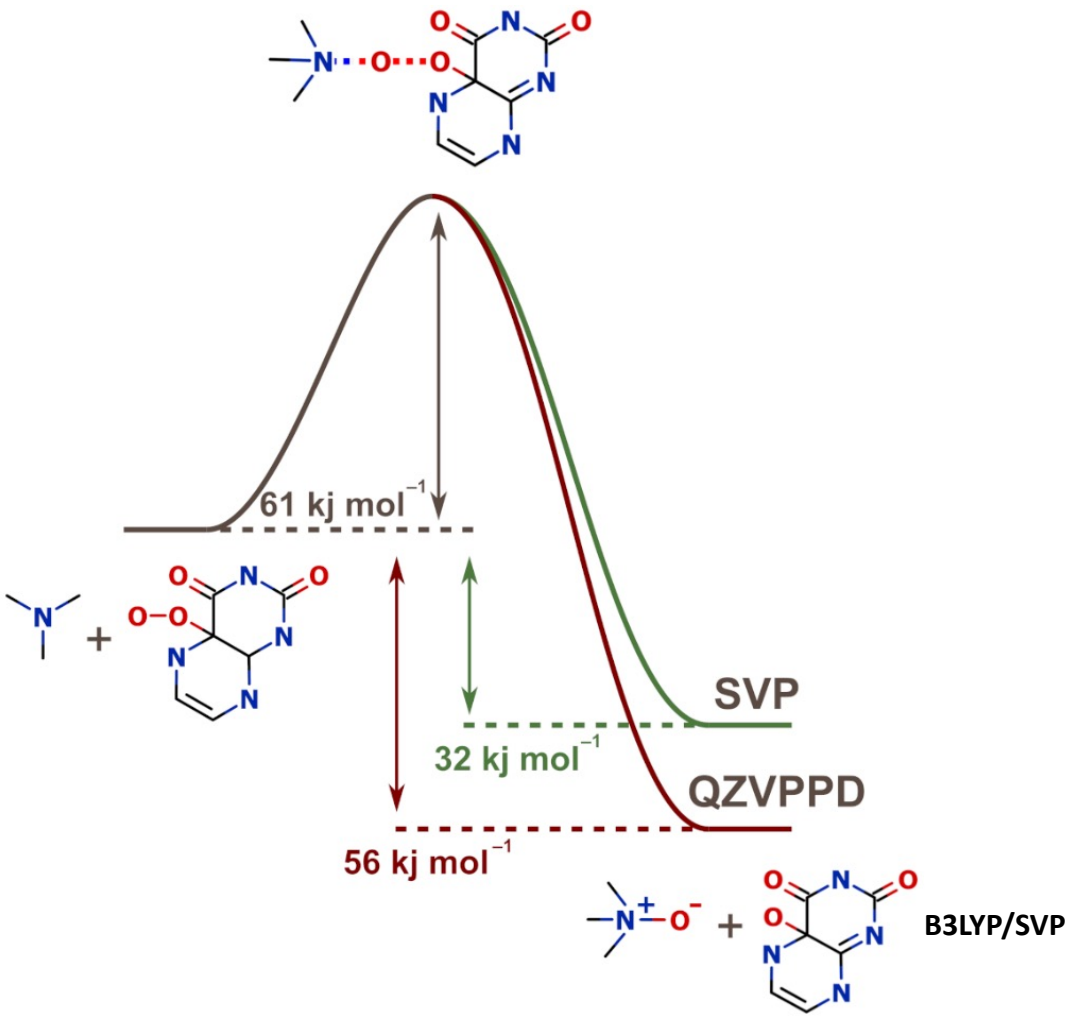

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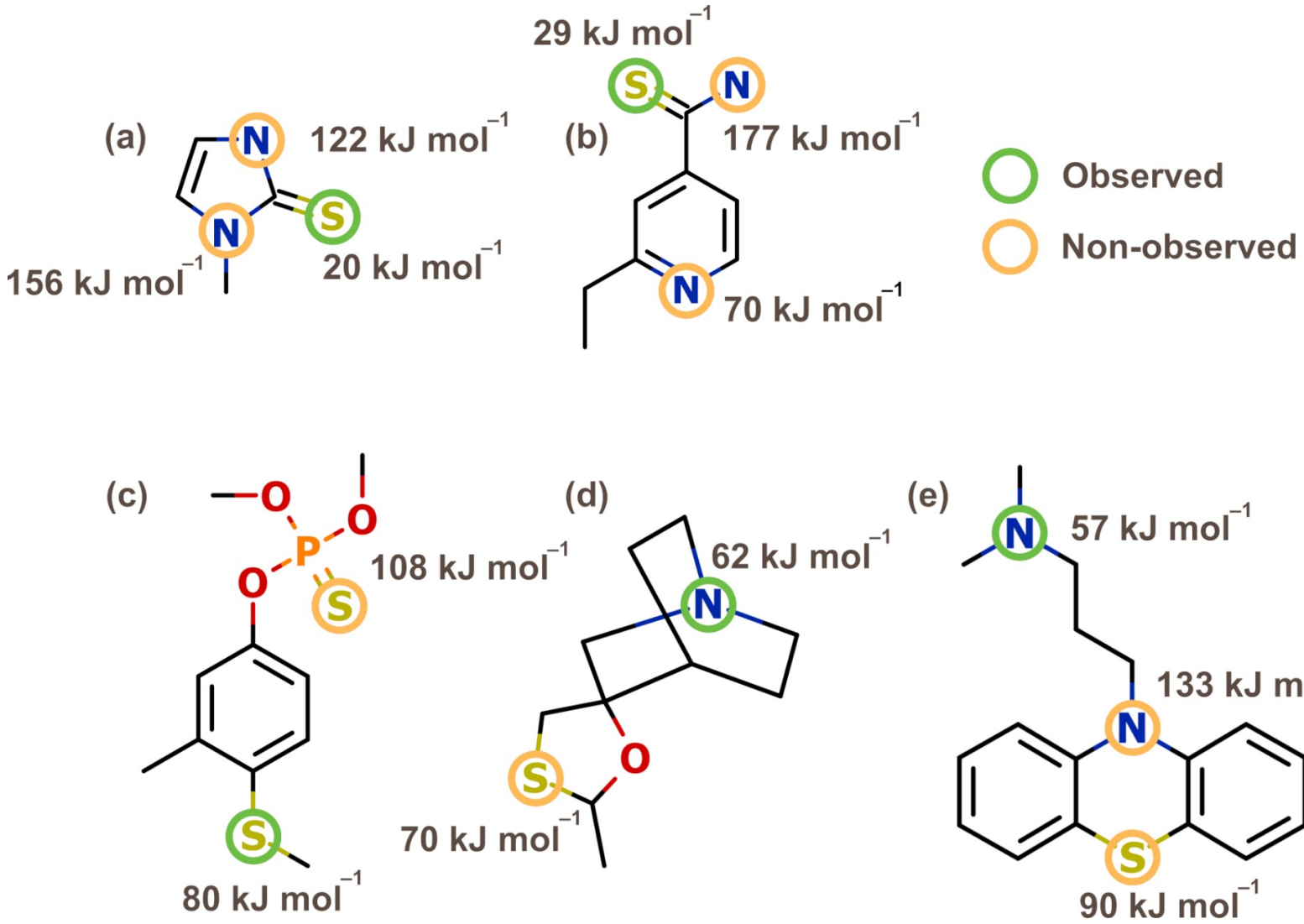
Application to Various Substrates



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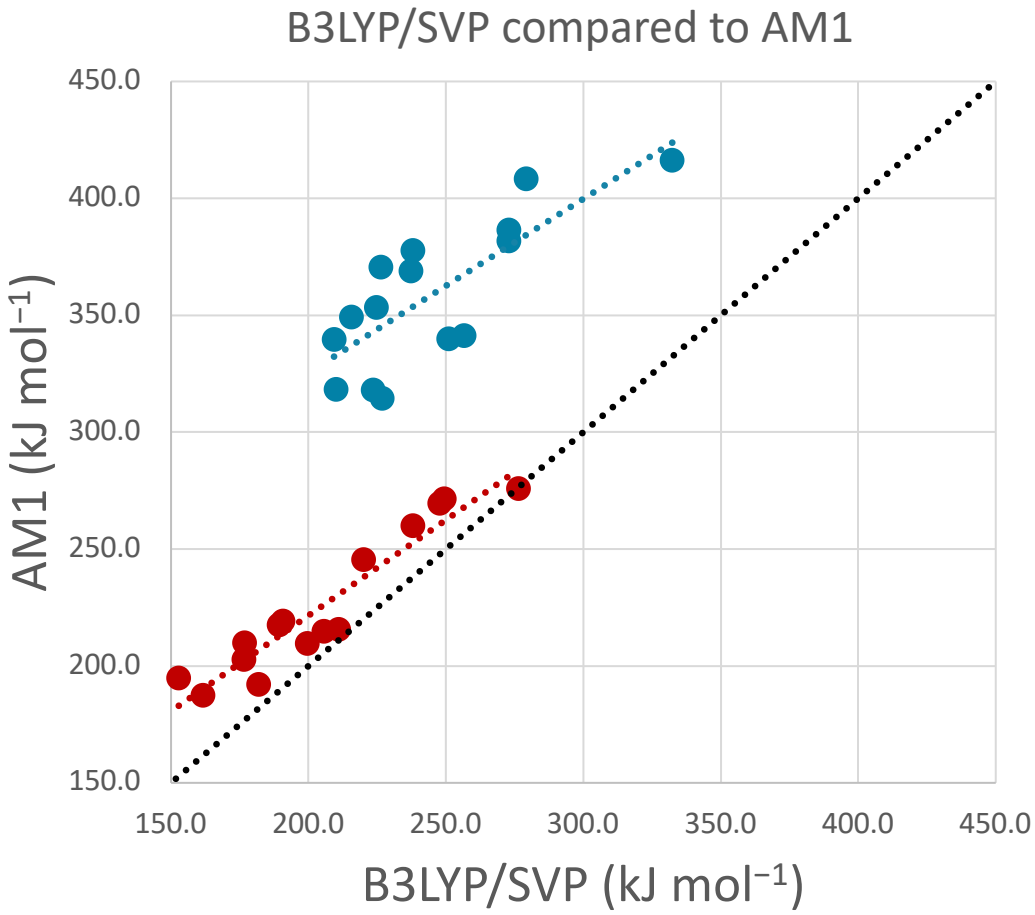
Transition from DFT to AM1 for UGT

- AM1 is Faster
- Things to Consider
  - AM1 is bad at detecting weak interactions
  - AM1 makes systematic errors
- Fragment calculations
  - Aliphatic alcohols
  - Phenols
  - Carboxylic acids
  - Primary amines
  - Secondary amines
  - Tertiary amines



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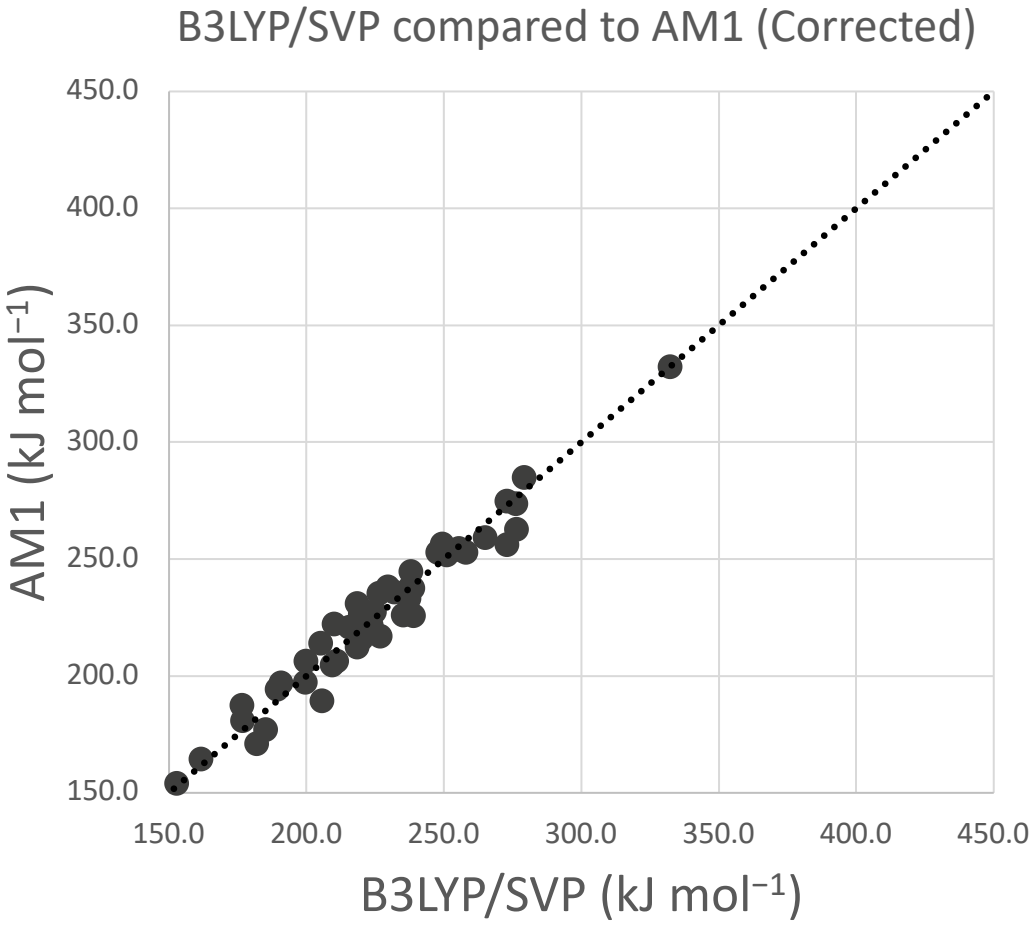
  - Primary amines
  - Secondary amines
  - Tertiary amines
- Corrections for each class
  - $R^2 = 0.95$



Training Models

Training and Testing Models

- Isoform-specific site of metabolism models
  - Experimental data for seven isoforms
  - Only compounds which are metabolised
  - Compounds with two or more sites

Enzyme	Isoform	No. of Substrates	No. of Potential SoM	No. of SoM Metabolised
AOX	AOX1	157	865	160
FMO	FMO1	56	172	56
	FMO3	67	209	69
UGT	UGT1A1	98	297	146
	UGT1A4	54	146	66
	UGT1A9	137	390	187
	UGT2B7	90	223	115

Training and Testing Models

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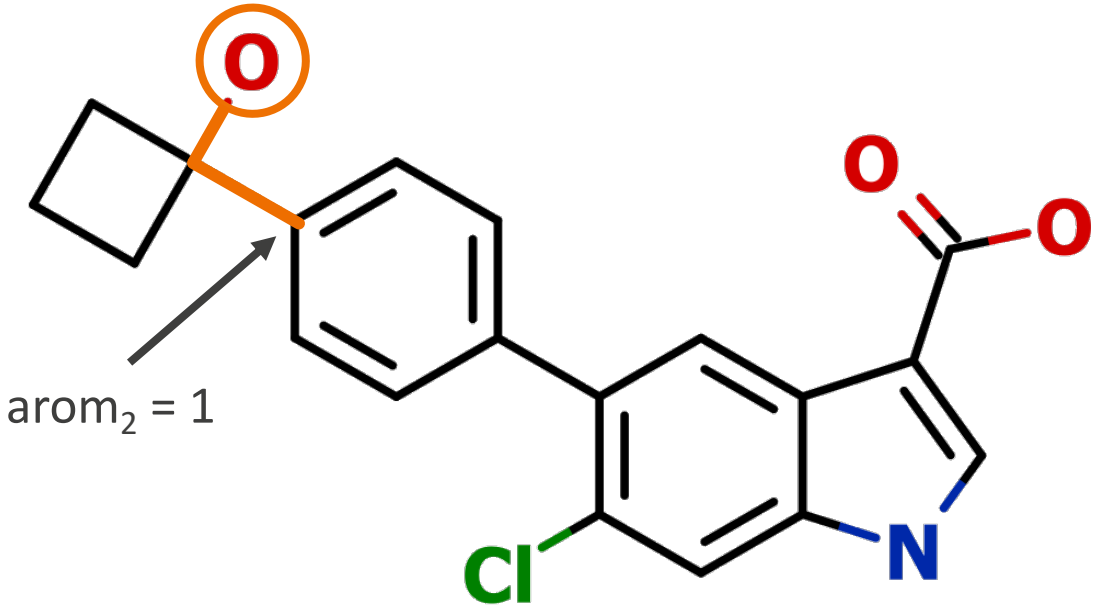
- Descriptors
  - E_a
  - Site-specific (rooted) descriptors

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



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  - Site-specific (rooted) descriptors
- Data Matrix
  - Training set, testing set
  - Split by molecule

Compound	Site	Experiment	E_a	Descriptor 1	...	Descriptor *N*
Comp1	0	True	78.9	3.00	...	5.16
Comp1	12	False	99.1	4.00	...	6.97
Comp1	17	False	103.7	9.00	...	3.25
Comp2	5	False	116.9	7.00	...	2.13
Comp2	6	True	94.5	1.00	...	1.87
Comp3	2	False	101.2	7.00	...	8.10
Comp3	14	True	108.0	4.00	...	2.33
...	...	...	...	...	...	...
Comp M	5	True	70.2	6.00	...	7.27

Training and Testing Models

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- Data Matrix
  - Training set, testing set
  - Split by molecule
- Methods and Statistics
  - Gaussian Processes
  - Balanced accuracy and kappa value

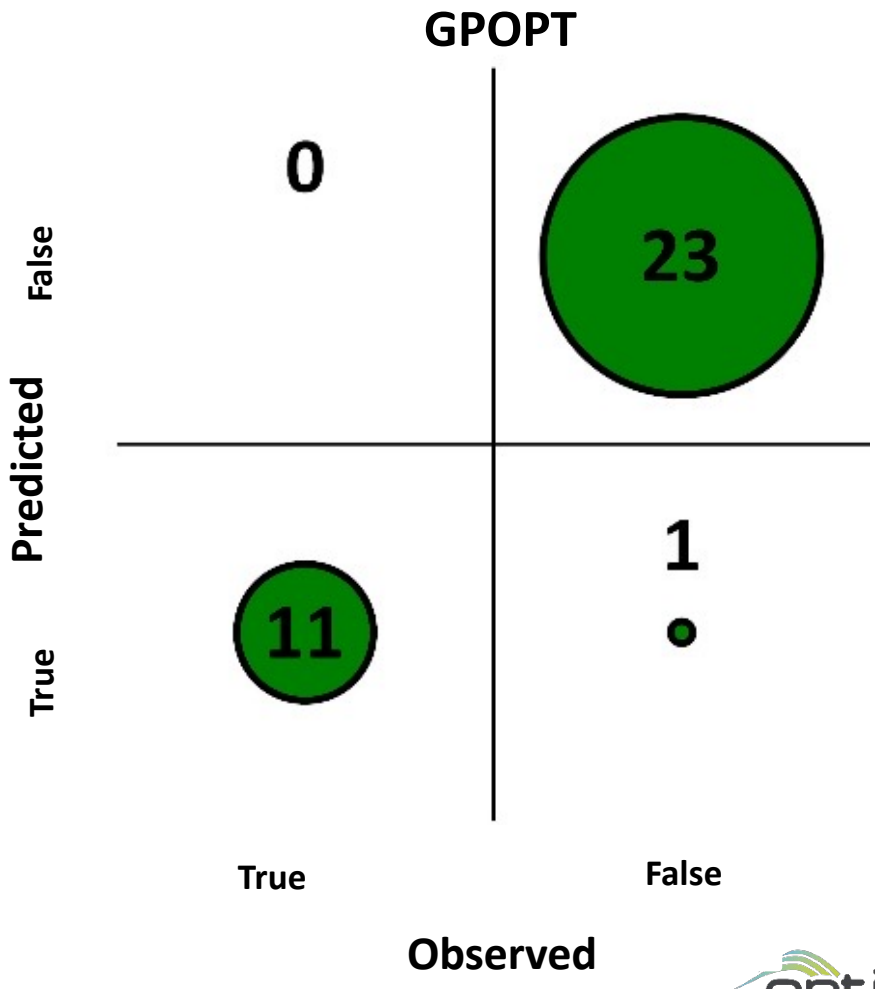
κ	Agreement
$\kappa < 0.5$	poor agreement
$0.5 \leq \kappa < 0.6$	moderate agreement
$0.6 \leq \kappa < 0.8$	good agreement
$0.8 \leq \kappa < 1.0$	very good agreement

https://doi.org/10.1021/ci7000633

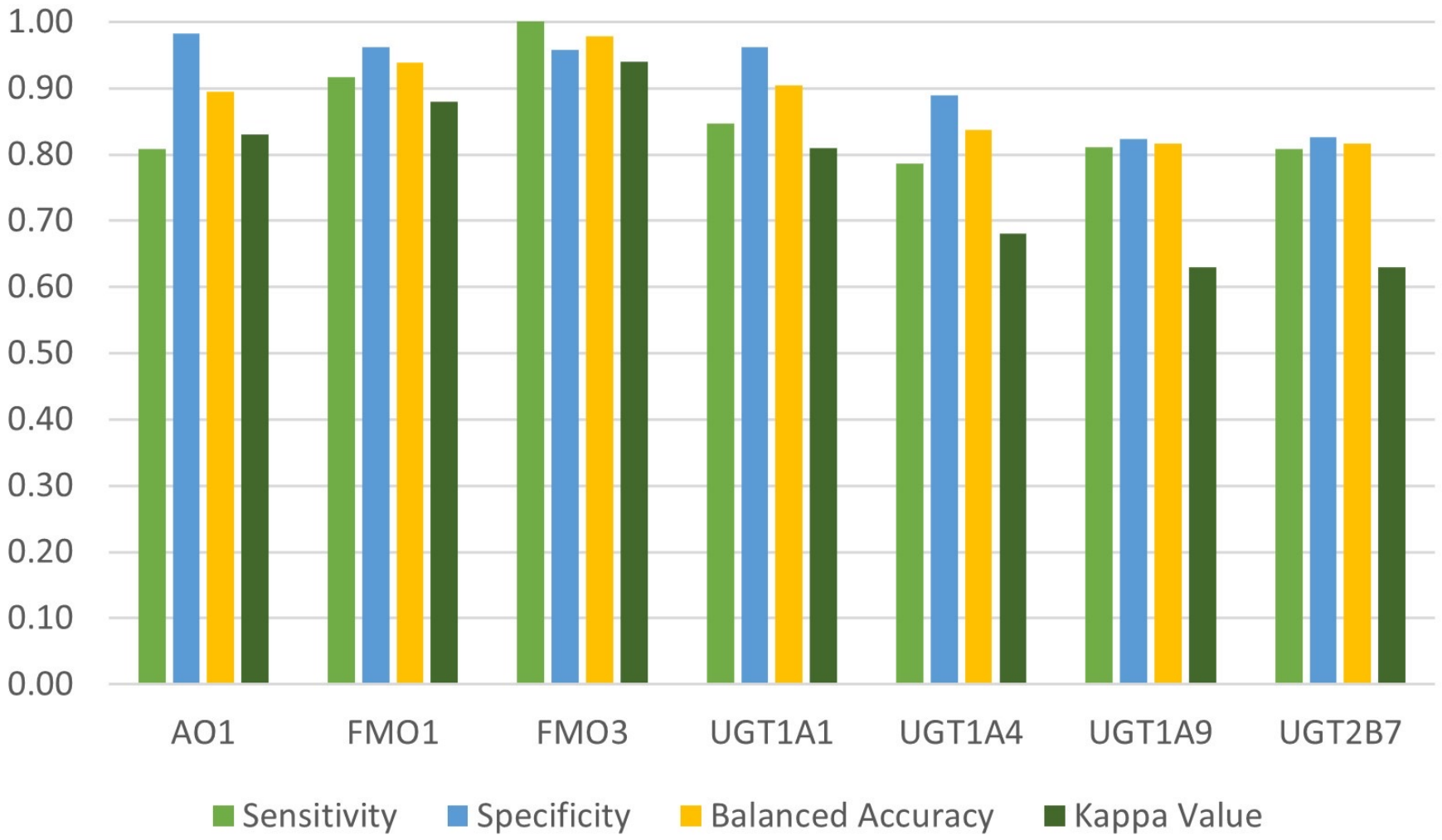
Site of Metabolism Model of FMO3

- Training set
  - 54 molecules, 173 sites
- Test set
  - 12 molecules, 35 sites
- E_a alone predicted 82% of the cases
  - AUC of 0.92

Model	Kappa	Balanced Accuracy
GPOPT	0.94	98%



Summary of Metabolism Models



Conclusions

- Gathered Isoform-specific Data
  - Seven isoforms of AOX, FMO, and UGT
- Simplified Mechanism for Reactivity
  - AOXs, FMOs and UGTs
  - Using DFT and semi-empirical methods
- Trained Reactivity-accessibility Models
  - AOX1
  - FMO1 and FMO3
  - UGT1A1, UGT1A4, UGT1A9, UGT2B7

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- Peter Walton
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- Matthew Segall
  - AOX, FMO, and UGT