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Introduction to P450 Metabolism

Innovative Lead Optimization and Candidate Selection by *in Silico* Synthesis and ADMET Prediction. December 3rd 2015

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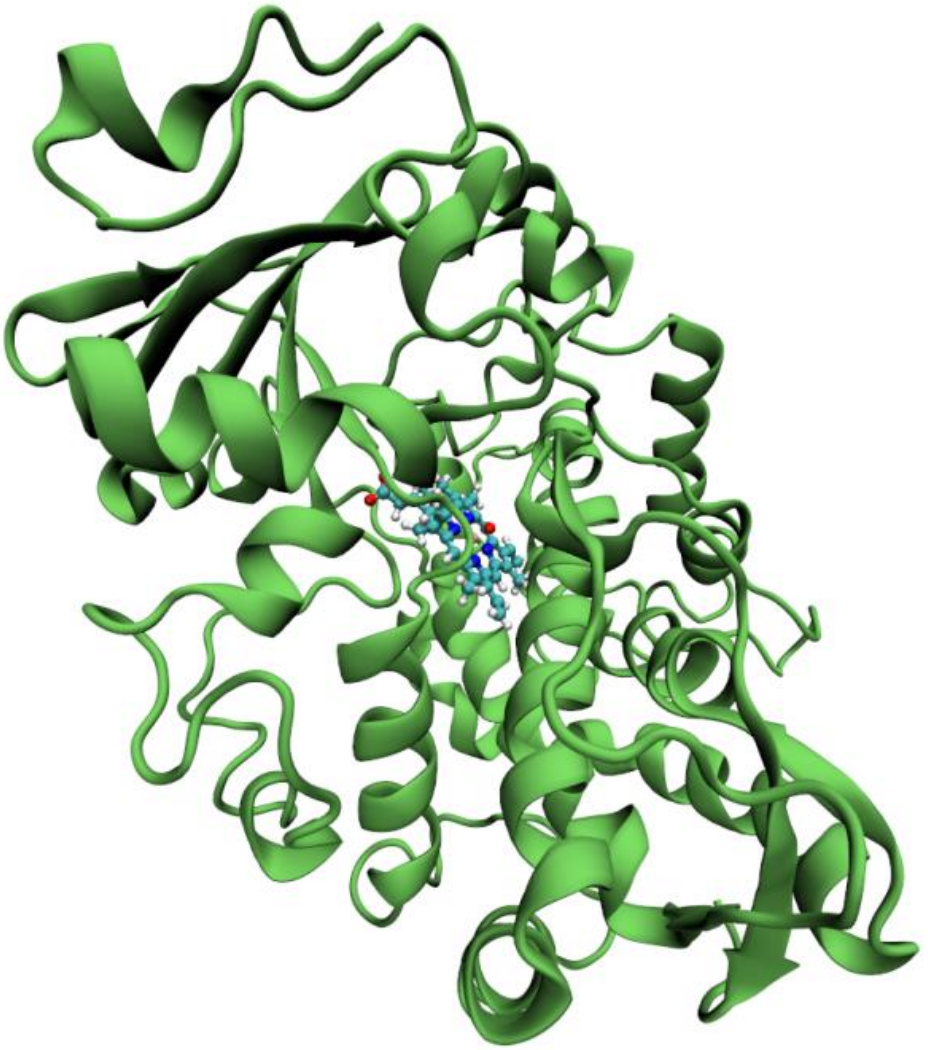
Overview

- Cytochrome P450
- Predicting P450 Metabolism
  - ‘Electronic’ contributions
  - Accessibility - steric and orientation
  - Generating metabolite structures
- Site Lability
- Conclusions

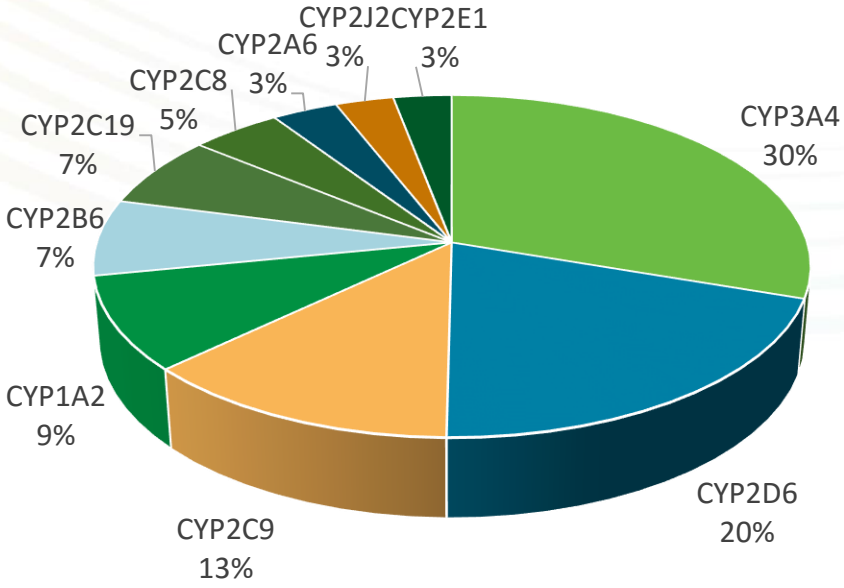
Cytochrome P450s

- Ubiquitous superfamily of haem-containing monoxygenase enzymes



Cytochrome P450s

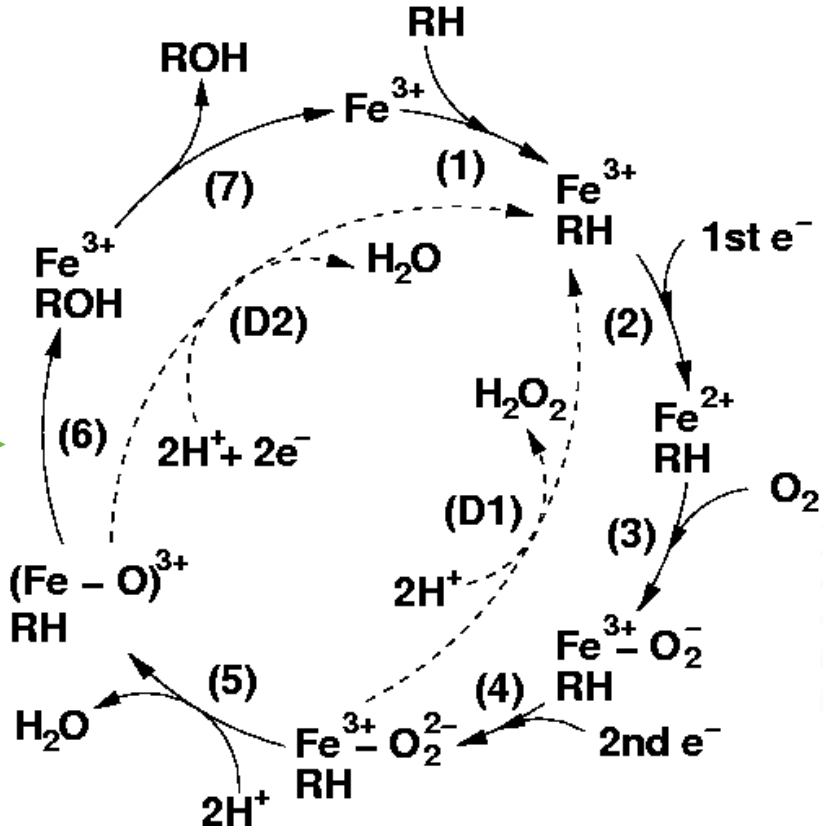
- Responsible for ~70-80% of drug metabolism, leading to:
  - Rapid clearance or low bioavailability
  - Potential for drug-drug interaction
  - Impact of P450 polymorphism
  - Bioactivation to form reactive/toxic metabolites
- Primary isoforms responsible for drug metabolism in human



P450 Catalytic Cycle

- Insertion of oxygen into ligand
  - Increase hydrophilicity
  - Facilitate secondary metabolism
  - Facilitate excretion

Product formation step



Shaik *et al.* Chemical Reviews, 110, 949-1017 (2010)

Predicting P450 Metabolism

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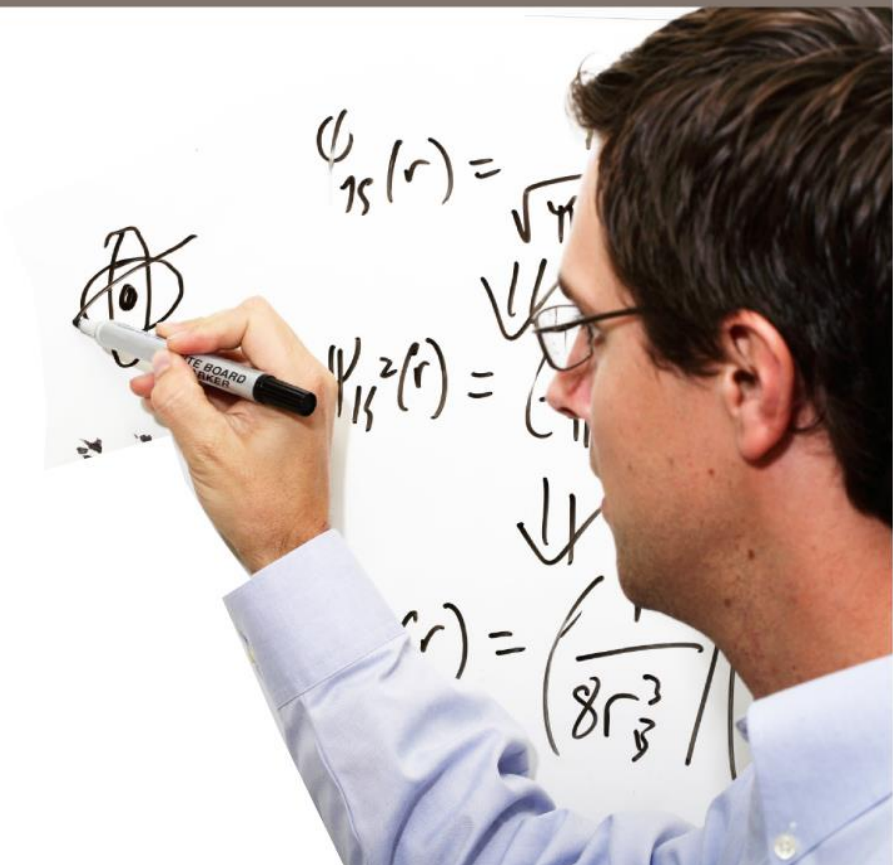


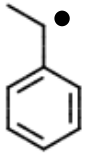
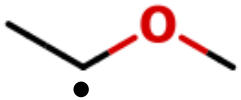
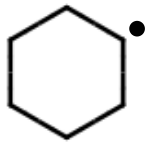
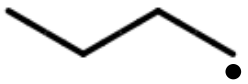
Methods

Two primary factors determine the site of metabolism:

- Electronic properties of substrate
  - H abstraction – aliphatic oxidation, N-dealkylation, O-dealkylation
  - Direct oxidation – aromatic oxidation, epoxidation, N-oxidation, S-oxidation
  - Activation barrier to abstraction of H and direct oxidation
  - Independent of isoform
- Orientation of substrate in active site
  - Electrostatic interactions with between protein and substrate
  - Freedom to move
  - Steric accessibility
  - Dependent on isoform and substrate

Electronic Effects

Trends in Metabolism Correlate with Radical Stability

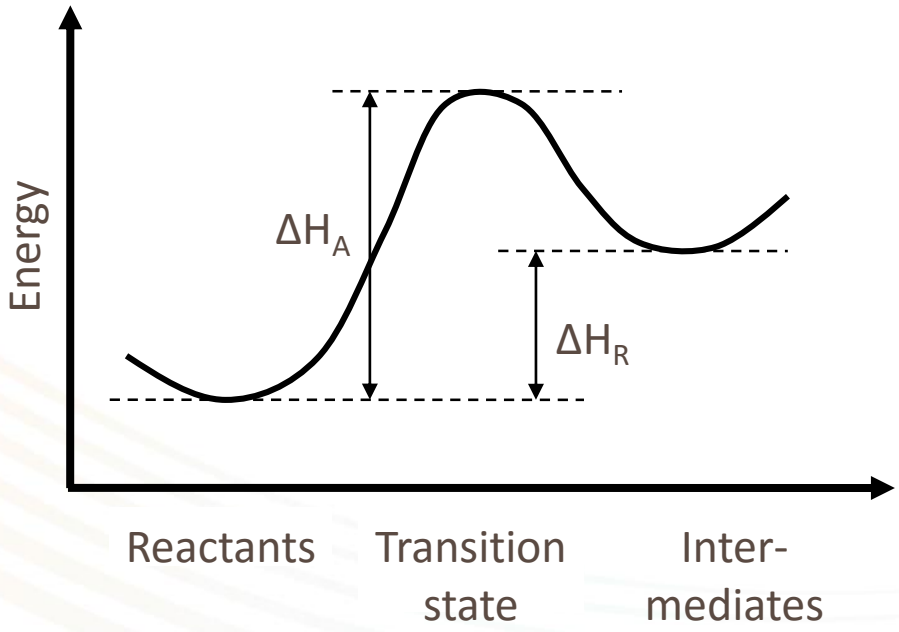
Radical	$\delta\Delta H_f$ (kcal/mol)	Reaction Type
	17.3	N-dealkylation
	19.6	benzylic hydroxylation
	26.6	O-dealkylation
	27.7	aliphatic hydroxylation
	28.6	aliphatic hydroxylation
	33	ω-hydroxylation

Increasing occurrence of metabolism

Korzekwa, Jones and Gillette, J. Am. Chem. Soc., **112**, p. 7042 (1990)

Electronic Models for CYP Reactivity

- Semi-empirical QM methods used to calculate energies of substrate and reaction intermediates
- Brönsted relationship to generate activation energy
- Fragment considered in context of molecular environment
  - Not considered as a discrete uniform entity
  - Subtle longer range effects can be captured
  - Important when developing a lead series



$$\Delta H_A \propto \Delta H_R$$

Electronic Models for CYP Reactivity

- Free energy relationships have been developed to predict activation energies for oxidation reactions
  - Hydrogen atom abstraction
  - Aromatic oxidation
  - S-oxidation
  - N-oxidation
  - Epoxidation
  - ….
- Models have been parameterized with:
  - Experimental data*
  - *Ab initio* calculations†

*Jones, Mysinger & Korzekwa, Drug Metab. Dispos., **30**(1) p. 7 (2002)

†Rydberg *et al.* ACS Med. Chem. Lett. **1** p. 96 (2010)

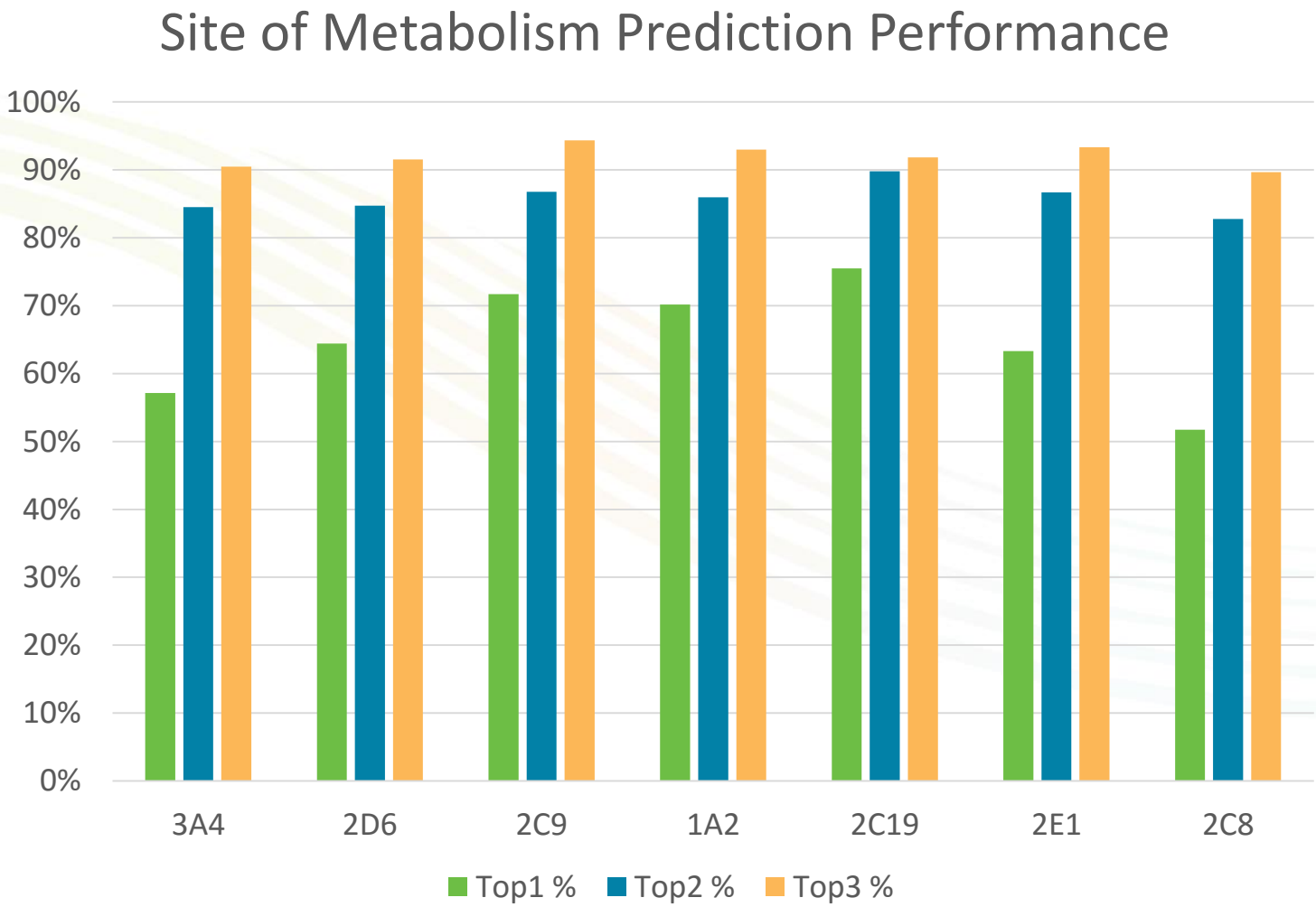
Steric and Orientation Effects

- Binding within active site restricts the accessibility of sites to the active oxy-haem species
- Structure of ligand introduces steric hindrance
- Corrections to activation energy estimated with statistically trained model using 2D descriptors, including
  - Distances to charged functionalities, H-bond acceptors/donors, lipophilic groups
  - Distances to rings, flexible linkers, 'bulky' groups
- Trained and tested using high-quality regioselectivity data sets carefully curated from the literature

Isoform	Number of Molecules
3A4	305
2D6	202
2C9	193
1A2	201
2C19	184
2E1	105
2C8	106

Validation

Independent test sets of 30% of data



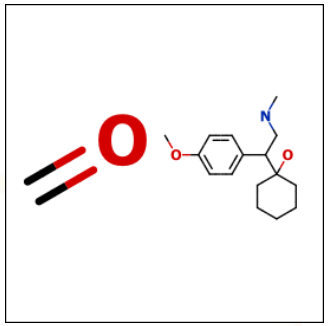
Metabolite Structure Generation

- SMIRKS patterns used to generate metabolite structures, e.g.

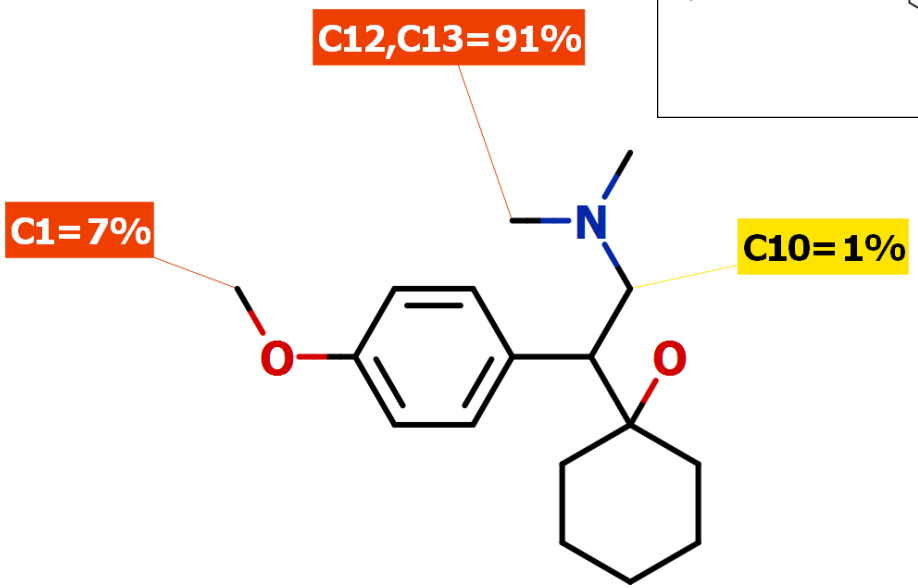
Reaction	SMIRKS
Aliphatic hydroxylation	[C;X4:1][H:2]>>[C:1][O][H:2]
Aromatic hydroxylation	[c:1][H:2]>>[c:1][O][H:2]
N dealkylation	[#7:1][C:2]([H])>>[#7:1][H].[C:2]=[O]
S oxidation	[#16:1] >> [#16:1](=[O])

- Metabolites can be exported into a new data set for further analysis, e.g. activity, physicochemical properties, etc.
  - Output exact mass to aid metabolite ID experiments

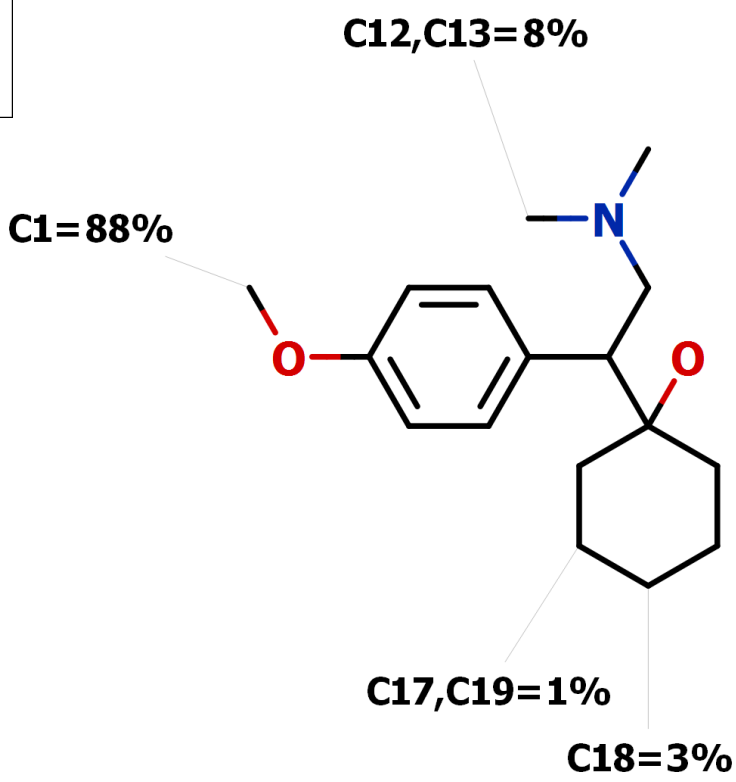
Example Regioselectivity Prediction
Venlafaxine



CYP3A4



CYP2D6



Site Lability

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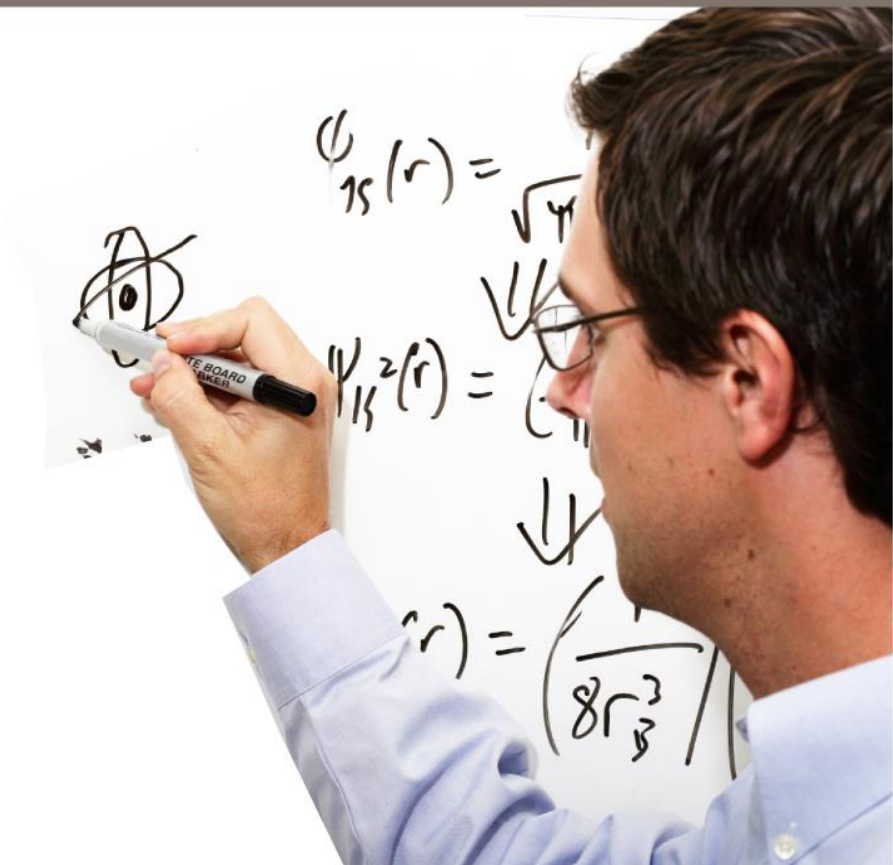
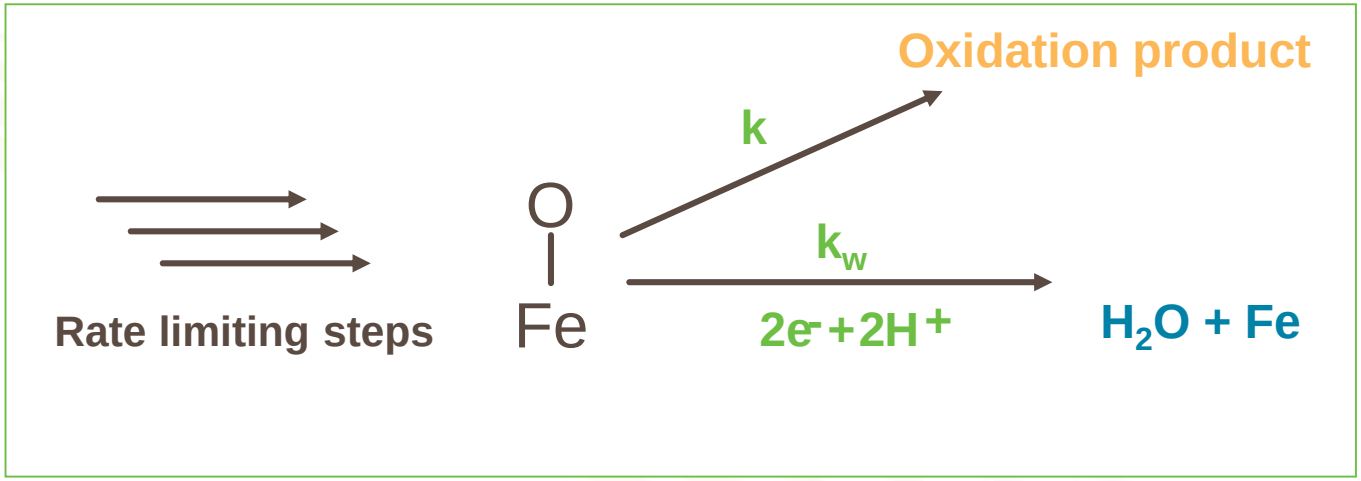


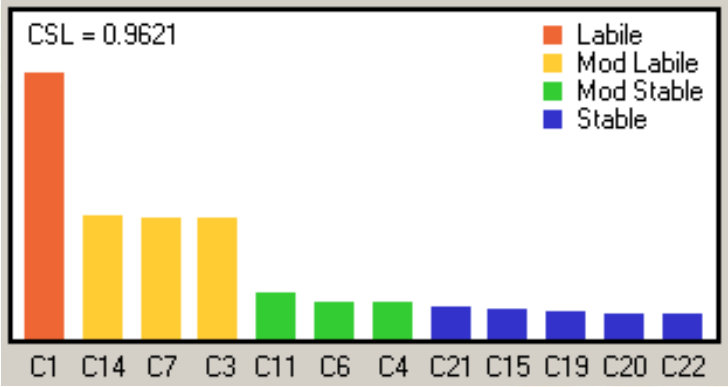
P450 Metabolic Lability



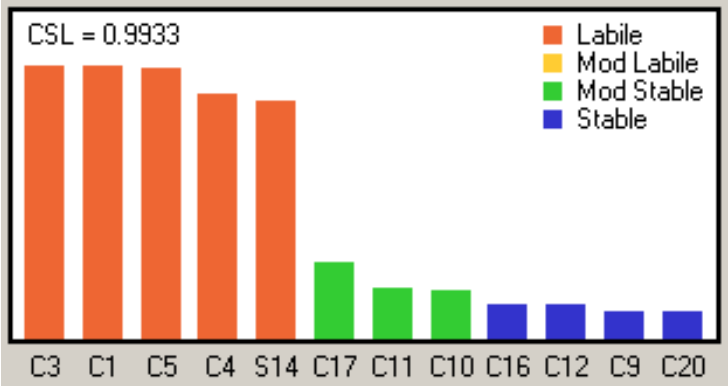
- Oxidation of a site on the molecule is in competition with water formation (and deactivation of the P450 active site).
- Site lability is a measure of how easily a site is oxidised compared to water formation, governing the efficiency of product formation.

P450 Metabolic Landscape

A



B

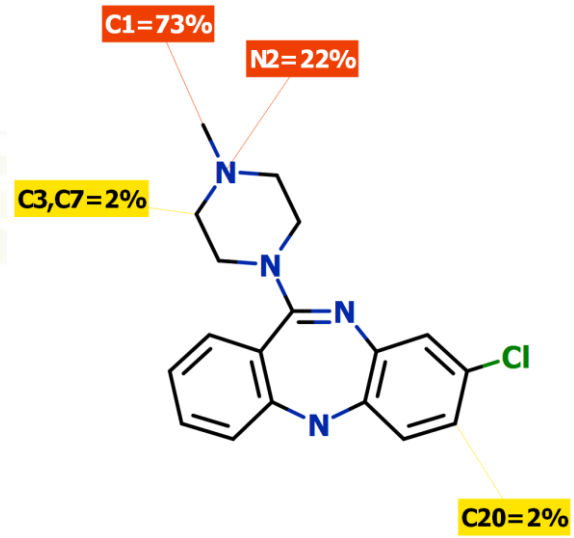


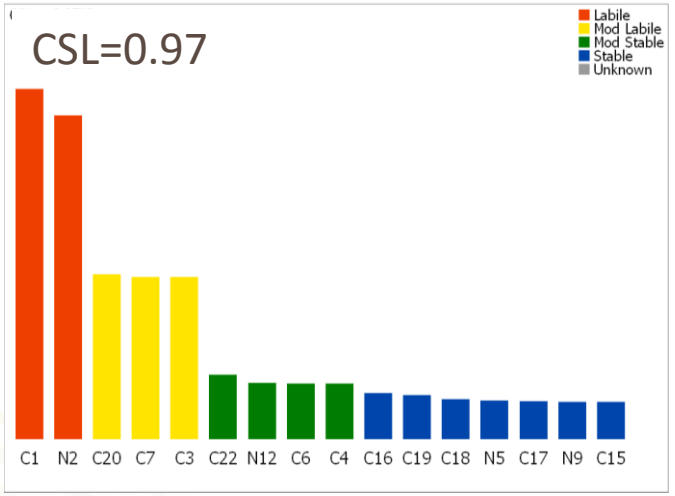
- This output indicates how **vulnerable** a molecule is to metabolism by CYP3A4, **if** it binds as a substrate
- Which compound is a better opportunity for optimisation?

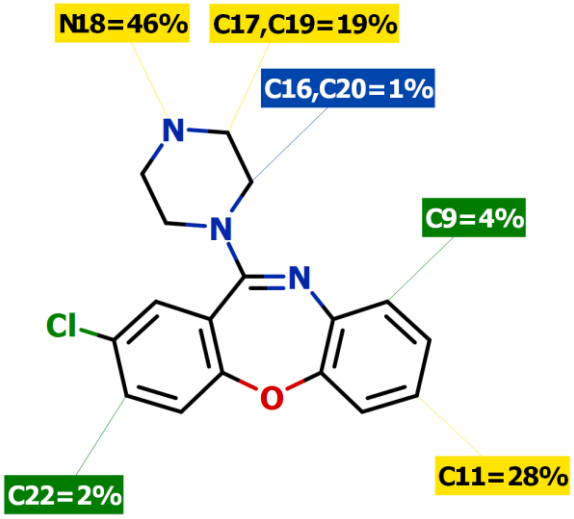
Composite Site Lability (CSL)

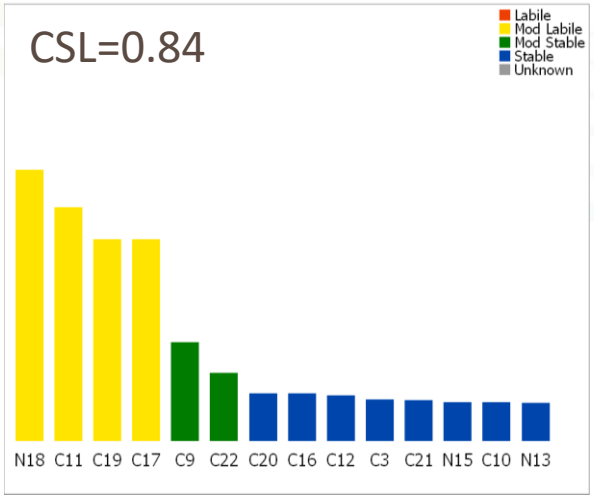
- The **Composite Site Lability** is a measure of the efficiency of metabolism of a molecule by CYP3A4
- CSL varies between 0.0 and 1.0
  - Lower values imply greater metabolic stability
- A **labile site** on a molecule may need modification to improve its stability
- Site lability is an important factor affecting rate of metabolism, but other factors are important
  - E.g. binding affinity, reduction rates (type I and type II binding)

Example
Clozapine vs Amoxapine









Conclusions

- Models of P450 metabolism can accurately predict site of metabolism and metabolites
- Predicting sites of metabolism is useful but not sufficient for compound design
- QM approaches can be used to estimate lability on an absolute scale
  - With corrections for steric accessibility and orientation
  - Fragments considered in their molecular environment
- Acknowledgement
  - This research has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under the grant agreement no602156