

# Predicting P450 Mediated Metabolism

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# Overview of Talk

Cytochrome P450s

Computational Set-up

Protein and Pharmacophore Models for CYP2D6

Combining Different Models for CYP2D6

Testing the Combined Model for CYP2D6

Conclusions & Acknowledgements



# Cytochrome P450s

- Superfamily of heme-containing enzymes
- Different P450s within gene family
  - > 40% amino acid identity
- Different P450s within sub-family
  - > 55% amino acid identity
- Involved in oxidising and reducing various substrates
- Involved in endogenous processes and (de)toxification processes



# CYP2D6 (P450 2D6)

CYP2D6 is polymorphically expressed

- e.g. absent in 5-9% of Caucasian population

**Common Characteristics in Substrates:**

- Basic Nitrogen atom
- Planar region near site of oxidation
- Negative Molecular Electrostatic Potential over the planar region
- Site of oxidation 5-7Å from basic nitrogen atom *or* adjacent to basic nitrogen atom



# P450 Metabolic Pathways

## Oxidative Pathways

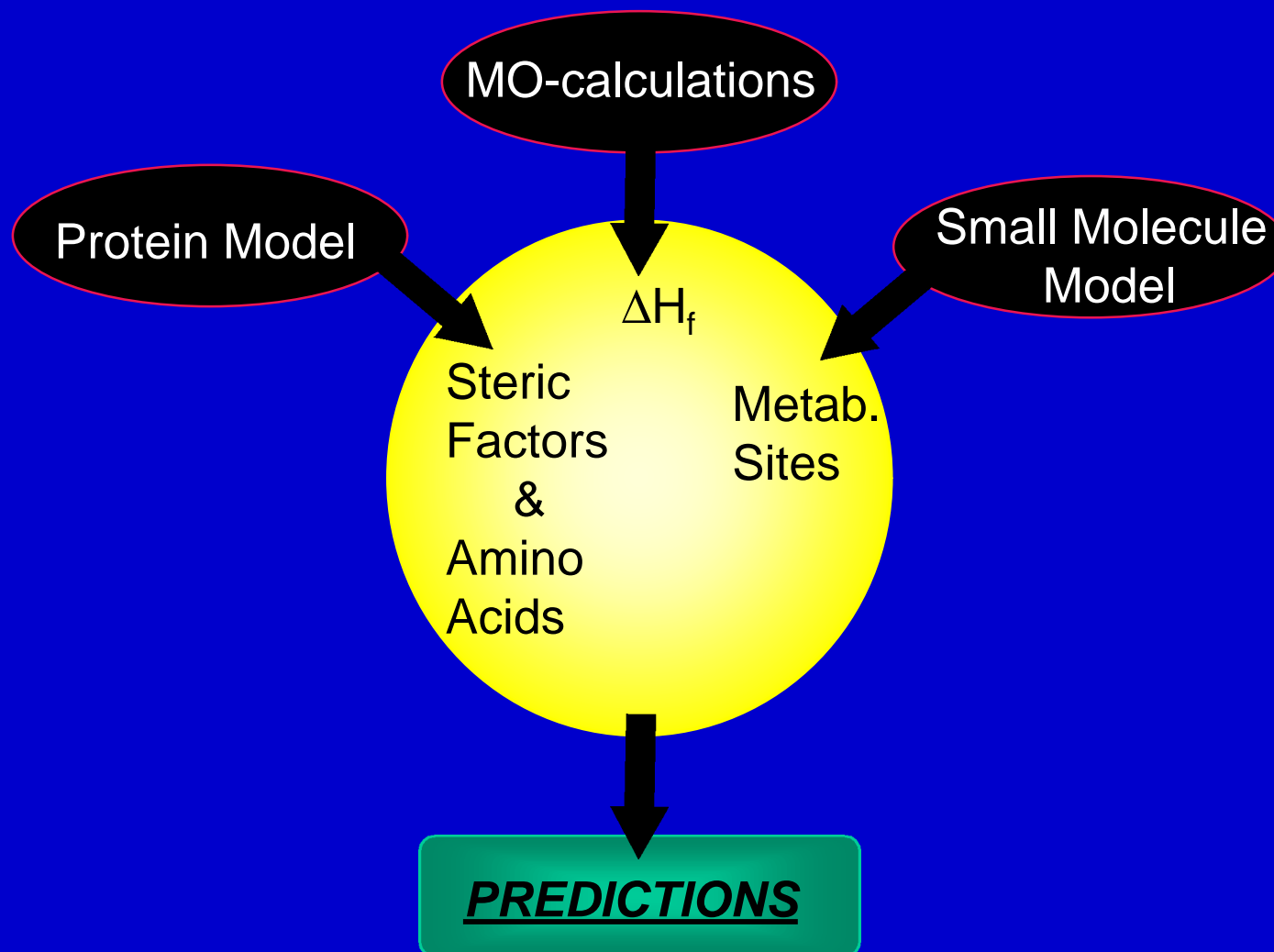
- Hydroxylation (e.g. aromatic or aliphatic rings)
- Oxidation (e.g. aromatisation or epoxidation)
- Hetero-atom dealkylation (e.g. O-dealkylation or N-dealkylation)

## Reductive Pathways

- Reduction



# Computational Approach



# Substrates Used

## Hydroxylation Pharmacophore

Amitriptyline	MDMA
Aprindine	Methamphetamine
Brofaremine	4-Methoxyamphetamine
Bufuralol	Methoxyphenamine
Carvedilol	Metoprolol
Codeine	Mexiletine
Cinnarizine	Mianserine
Clomipramine	Mirtizapine
Debrisoquine	Nortriptyline
Desipramine	Ondansetron
Dextromethorphan	Paroxetine
Dihydrocodeine	Perhexiline
Ethylmorphine	Phenformine
Flunarizine	Propafenone
Fluperlapine	Propranolol
GBR-12,909	Tamoxifen
Guanoxan	Terfenadine
Hydrocodone	Tiricazine
Imipramine	Tropisetron
Indoramine	Venlafaxine

## N-dealkylation Pharmacophore

Amitriptyline	Methamphetamine
Citalopram	Methoxyphenamine
Clozapine	Mianserine
Deprenyl	Mirtizapine
Fluoxetine	MPTP
10-Hydroxyamitriptyline	Nortriptyline
Imipramine	Propranolol

## Test Set

Betaxolol  
Fluoxetine  
Loratidine  
MPTP  
Procainamide  
Ritonavir  
Sumatriptan



# Molecular Orbital Calculations

## Before Docking

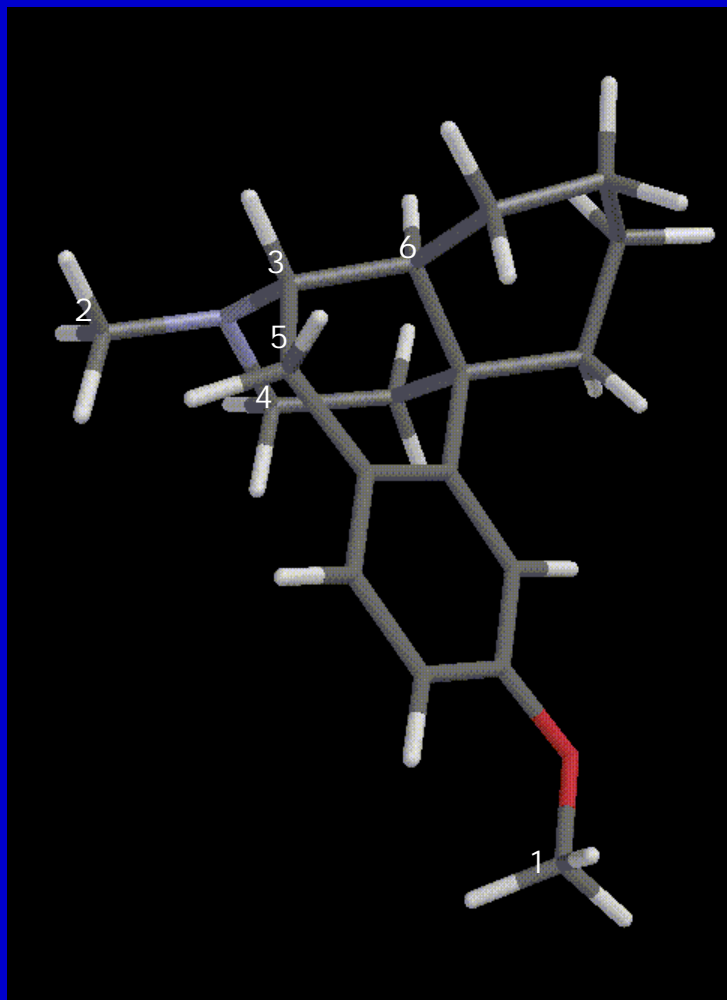
- Conformational search & AM1 geometry optimisation
- Geometry optimisation of all possible hydroxylated products, radicals and the radical cation
- Calculation of HOMO localisation

## After Docking & Minimisation in Protein

- Geometry optimisation of docked molecule



# Dextromethorphan



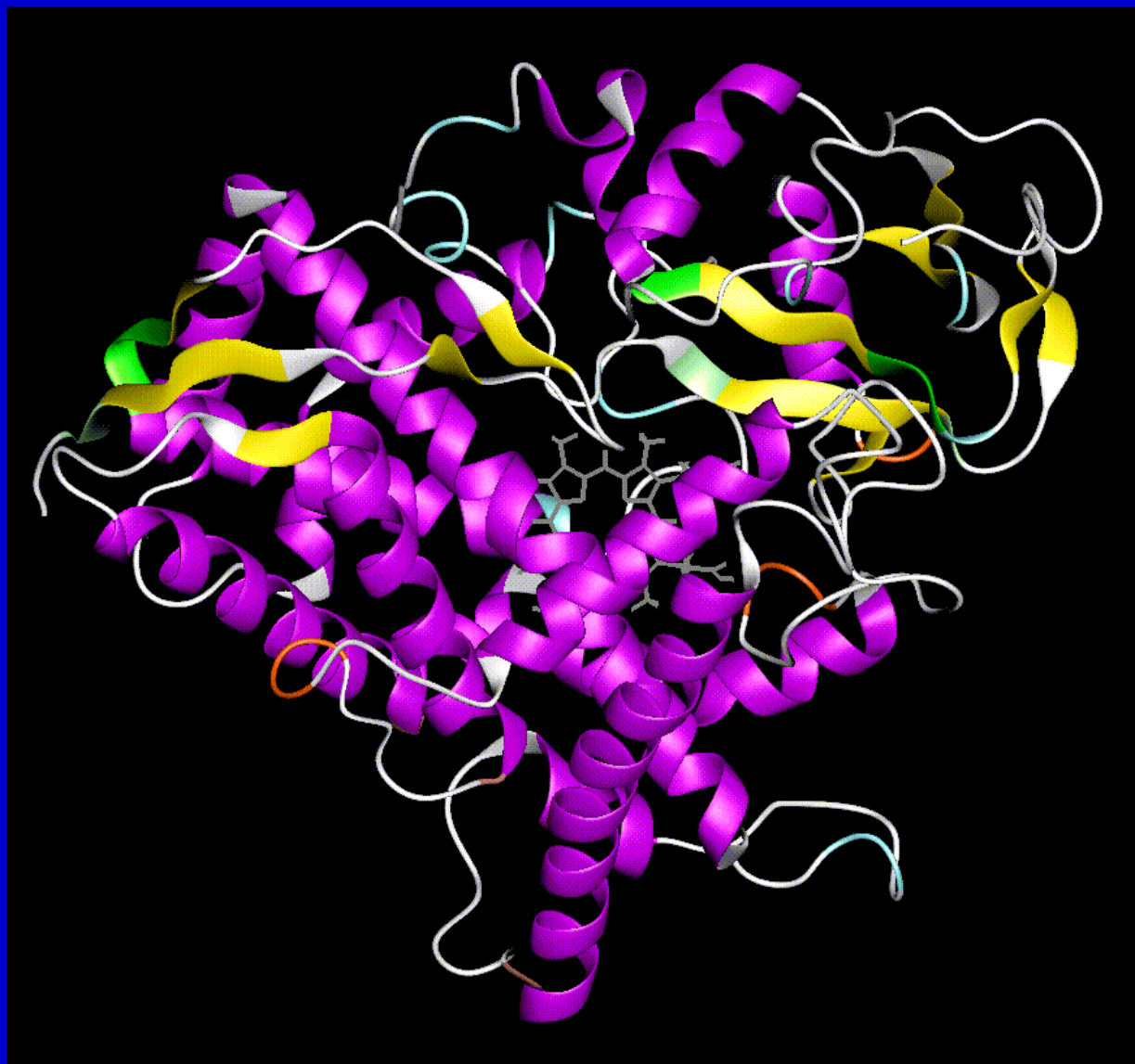
Site	DE (kcal/mol)	distance (Å)
1	0.0	7.8
2	8.6	1.4
3	16.6	1.5
4	10.2	1.5
5	12.4	2.6
6	17.4	2.5

# Protein Model

- Constructed 20 homology models using CYP101 (CAM), CYP102 (BM<sub>3</sub>) and CYP108 (TERP) and selected best one
- Arg<sup>132</sup>, His<sup>376</sup>, Ser<sup>413</sup> & Arg<sup>441</sup> interact with propionate groups of heme
- Asp<sup>301</sup>, Glu<sup>216</sup> and Phe<sup>481</sup> are key residues in the active site

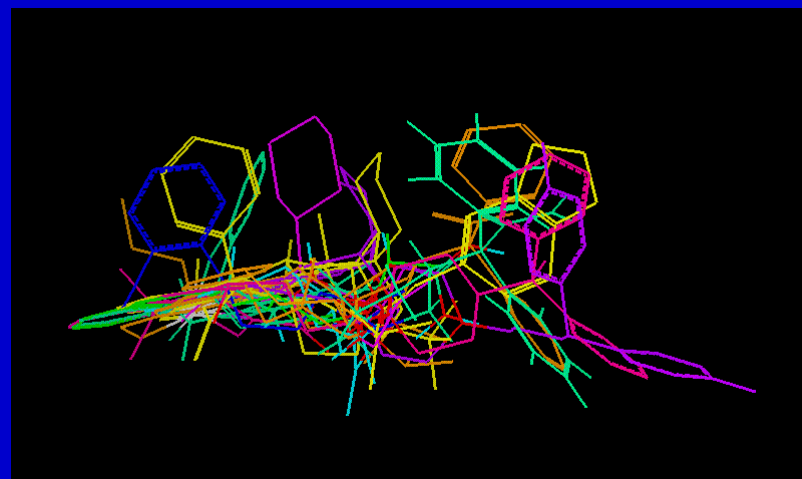
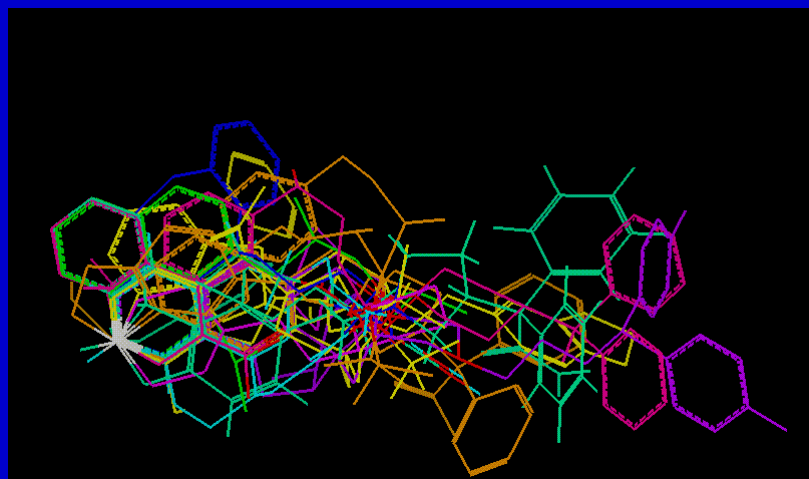


# CYP2D6 Protein Model



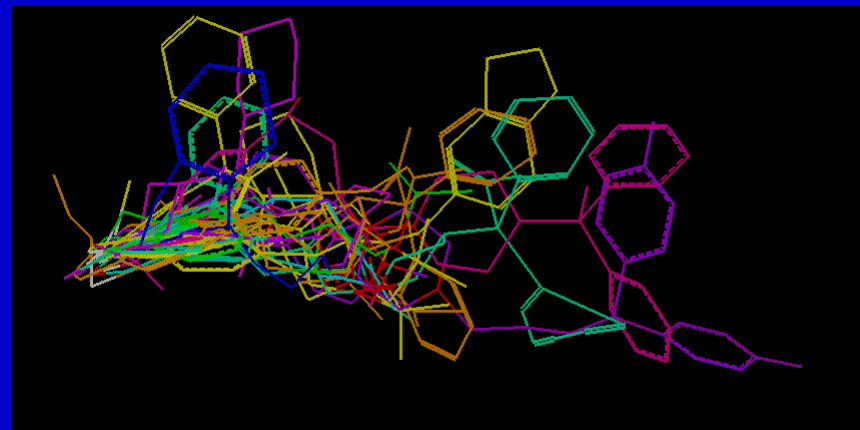
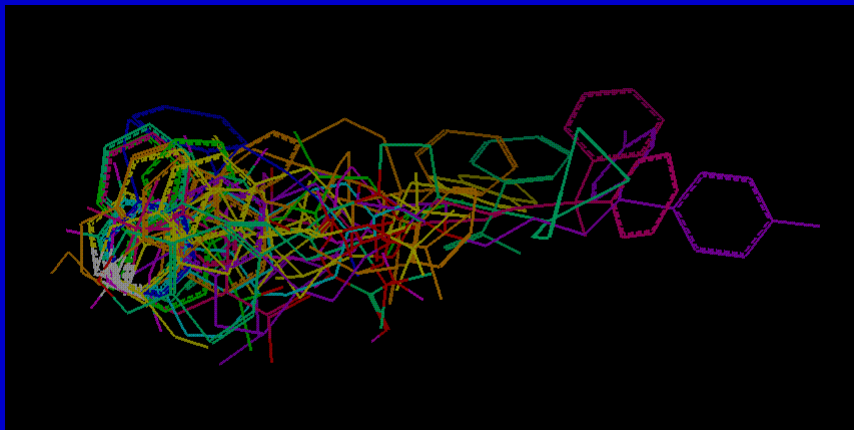
# Hydroxylation/O-demethylation Pharmacophore Model

- Used 57 metabolic pathways in 40 CYP2D6 substrates
- Used dextromethorphan, debrisoquine and GBR-12,909 as templates
- Overlaid basic nitrogen, site of oxidation & planar region



# Combined Model

- Combined Pharmacophore and Protein Model
- Minimised all substrates in presence of protein



**Hydroxylation Pharmacophore after Optimisation in Protein Model**

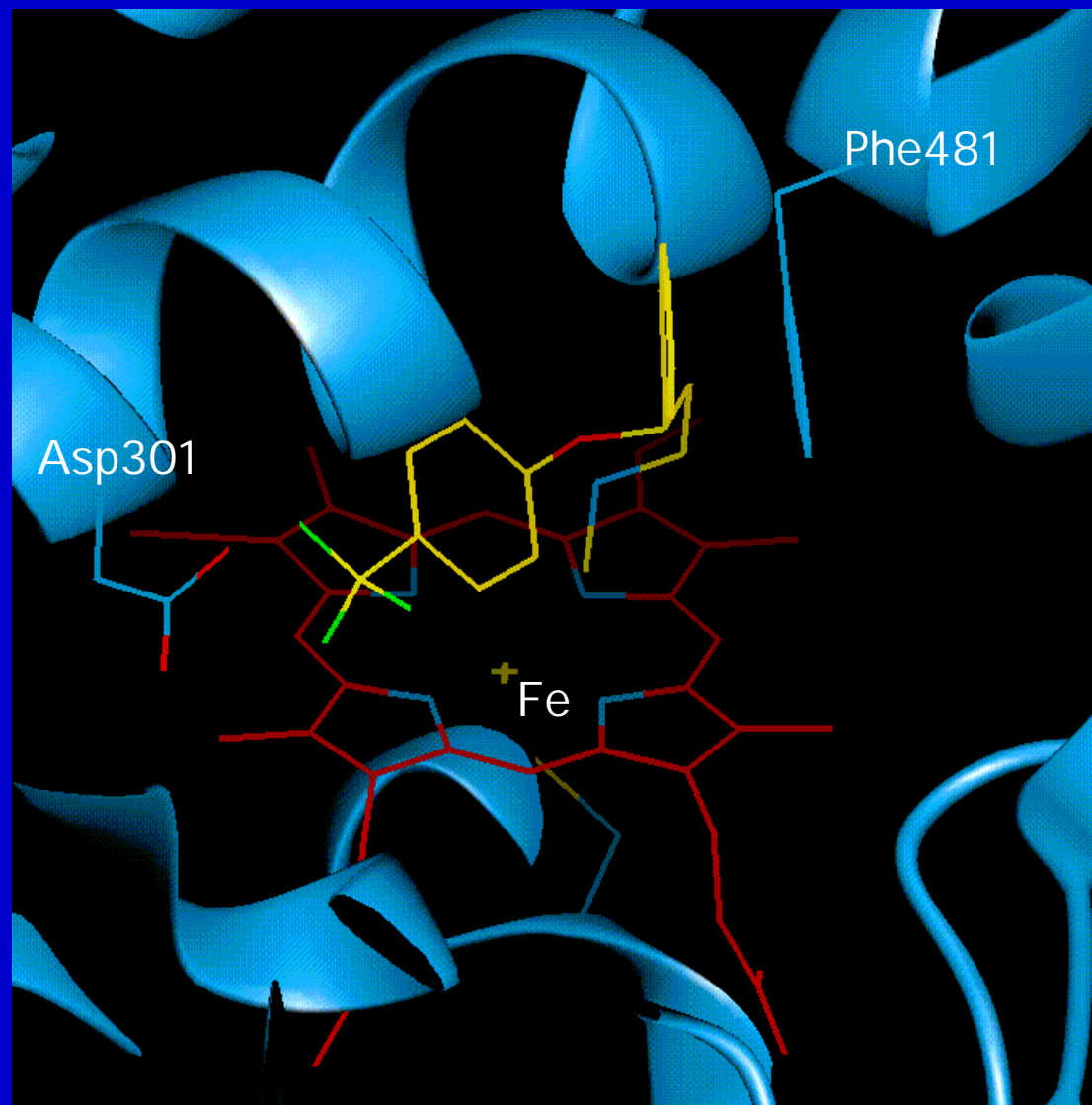


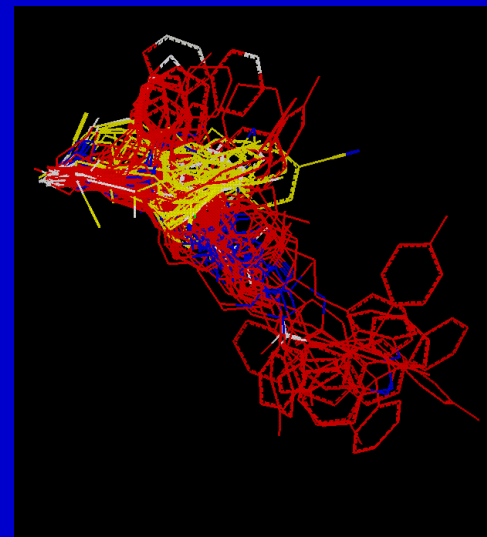
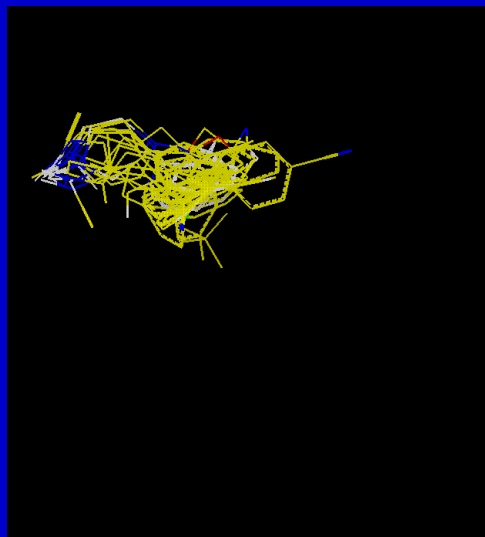
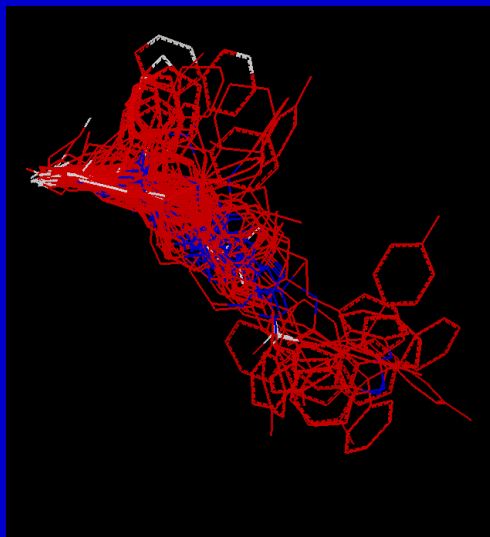
# N-dealkylation Pharmacophore & Combination with Model

- Used 15 metabolic pathways in 14 substrates known to be N-dealkylated by CYP2D6
- Used MPTP as template
- Overlaid site of oxidation & planar region
- Combined N-dealkylation pharmacophore with the previously combined model (protein with hydroxylation/O-dealkylation pharmacophore)

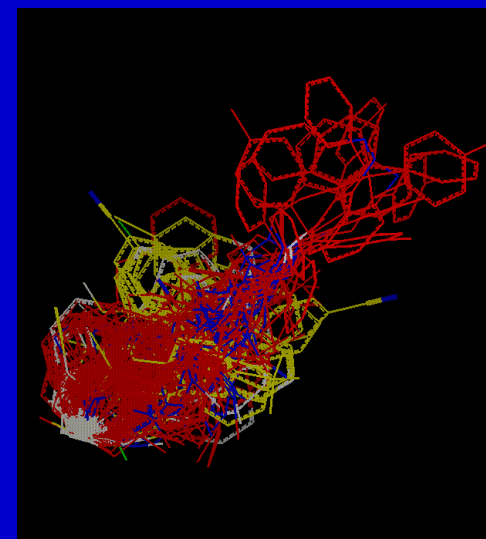
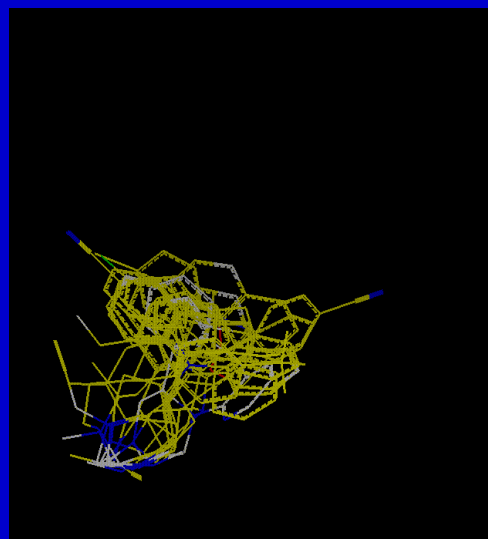


# Fluoxetine N-demethylation in Protein Model

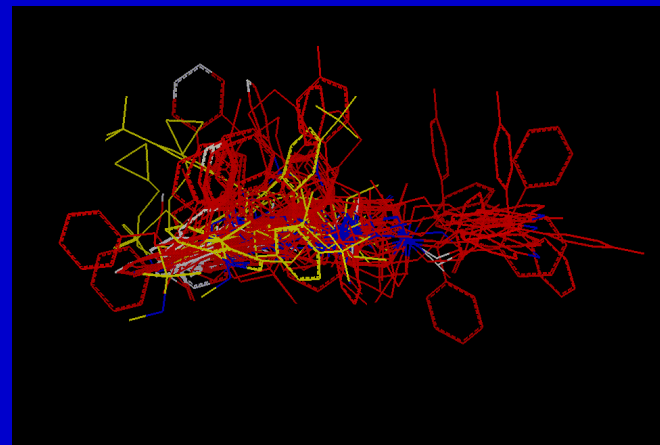
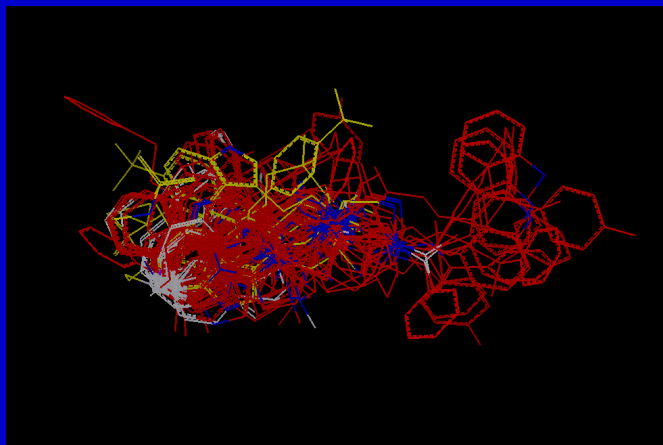




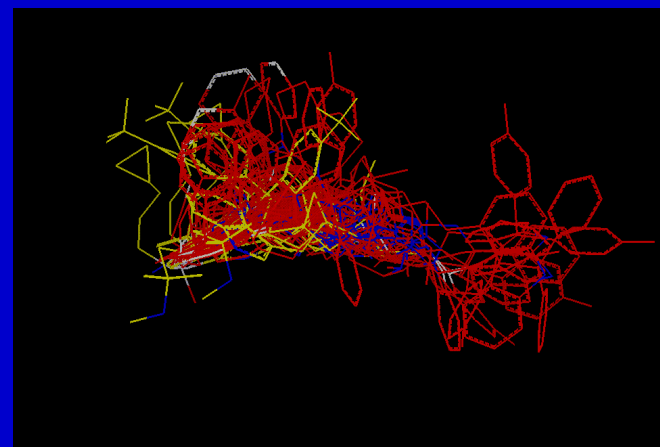
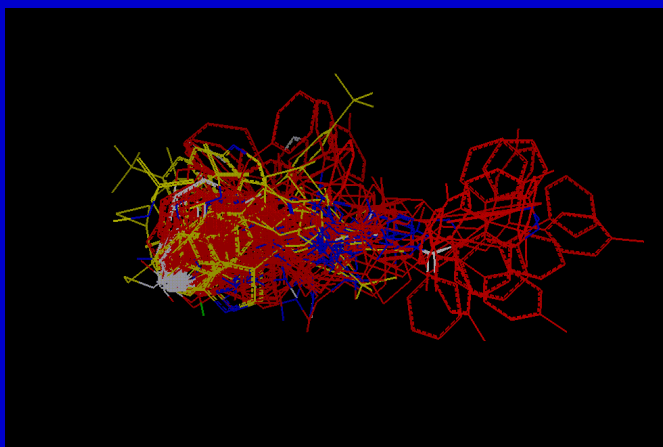
**Hydroxylation + N-dealkylation = Dual-Pharmacophore**



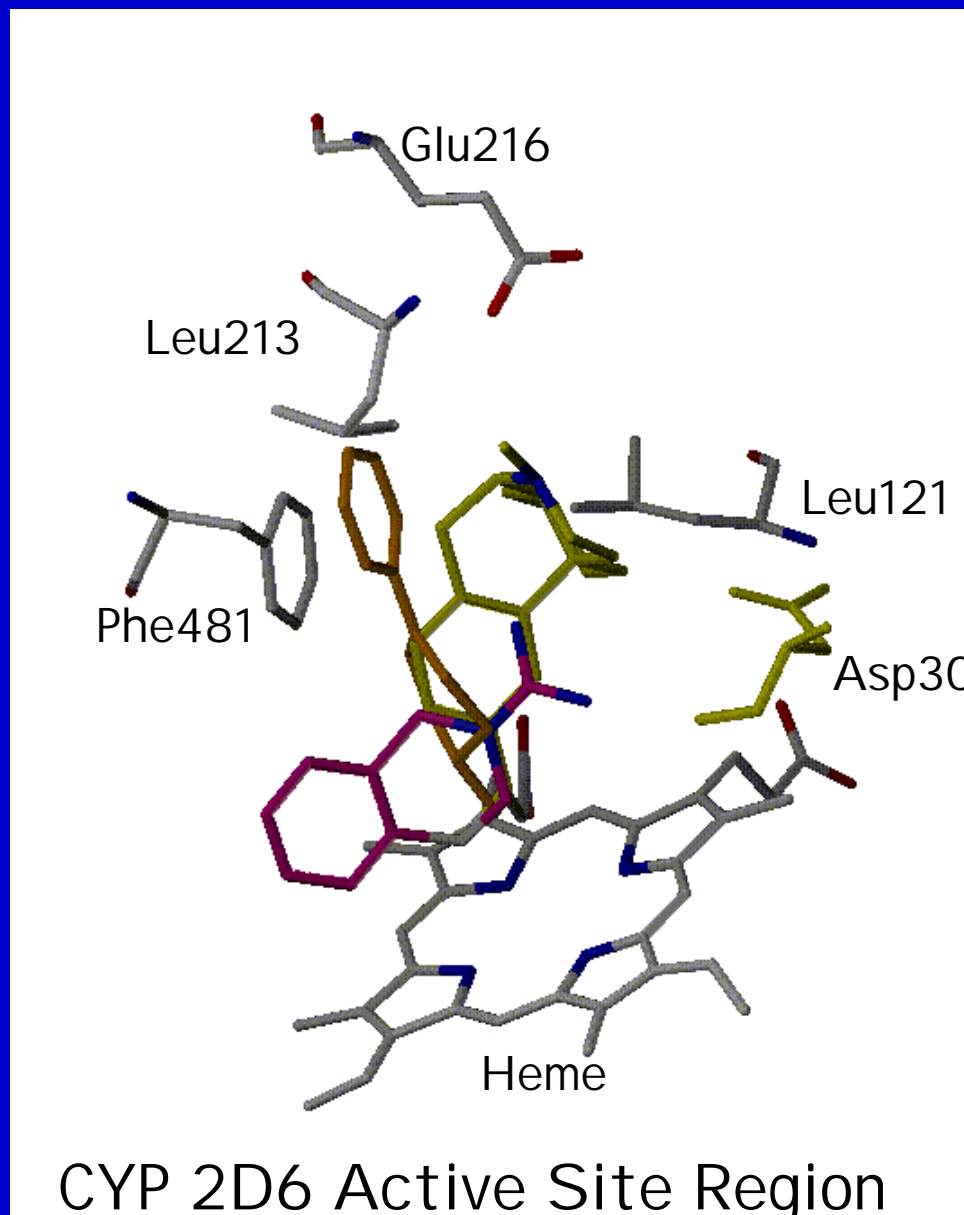
# N-dealkylation Pharmacophore (yellow) Added to Hydroxylation/O-dealkylation Pharmacophore (red)



# N-dealkylation Pharmacophore (yellow) and Hydroxylation/O-dealkylation Pharmacophore (red) after Optimisation



# Important Amino Acids

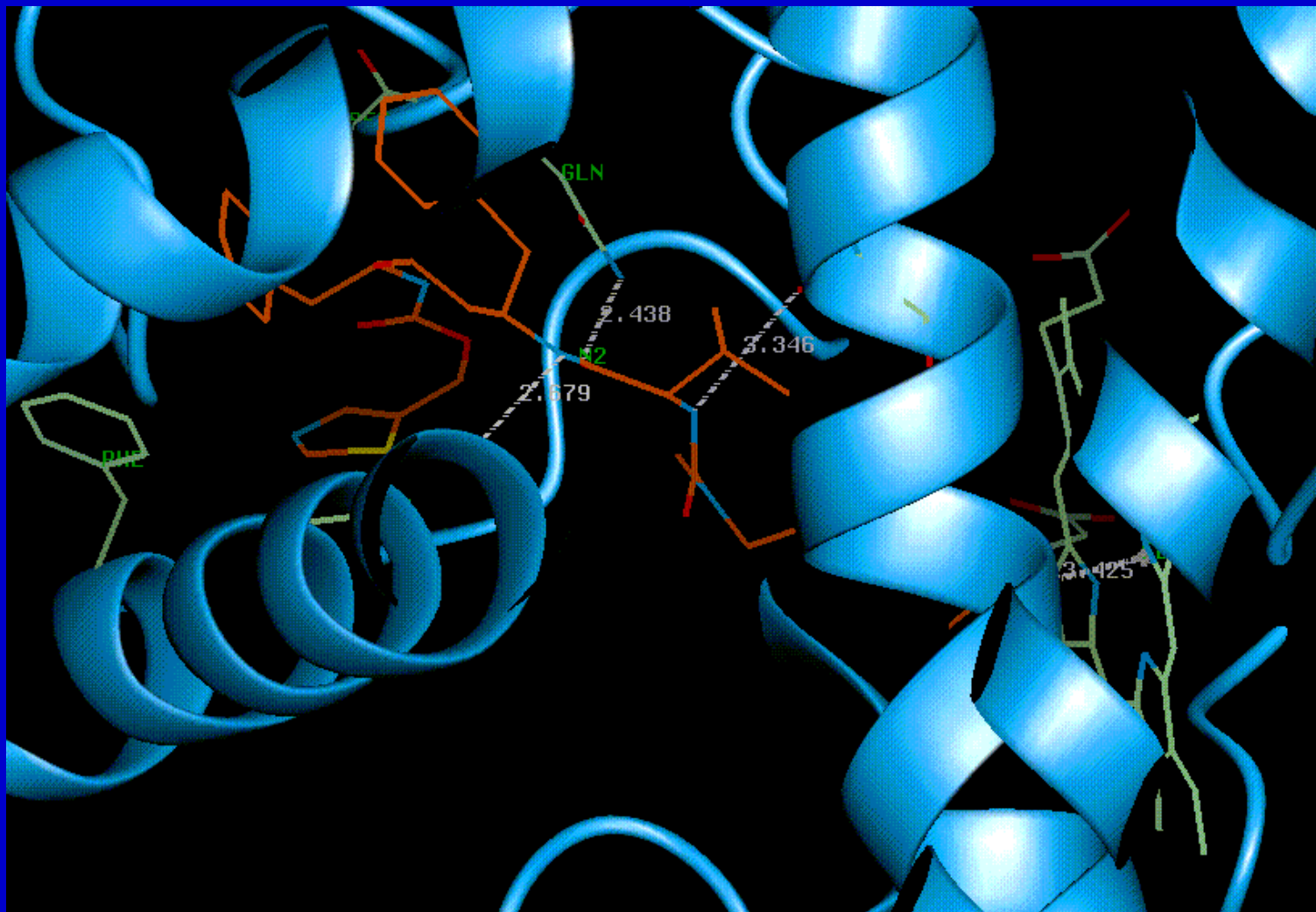


# Testing the Combined Model

- Used 8 substrates (CYP2D6 involvement in metabolism initially unknown)
- Attempted overlays with both pharmacophores
- Compared results with experimental data
- Well established CYP2D6 metabolic pathways correctly predicted
- Unusual pathways not predicted (e.g. ritonavir and procainamide)
- Additional metabolites predicted (not detected so far)



# Ritonavir in Combined CYP2D6 Model



# Future Prospects

- Extend model with 'new' metabolic pathways
- Application of Models in Rational Drug Design
- Using Methodology for Other P450s



# Conclusions

- Constructed a **Combined Model** consisting of a **Protein Model** and **two distinct Pharmacophore Models**
- **Hydroxylation reactions, O-dealkylation reactions and N-dealkylation reactions** involving CYP2D6 in new compounds can be predicted
- Metabolism through new metabolic pathways cannot be predicted



# Acknowledgements

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