Drug metabolism (2)

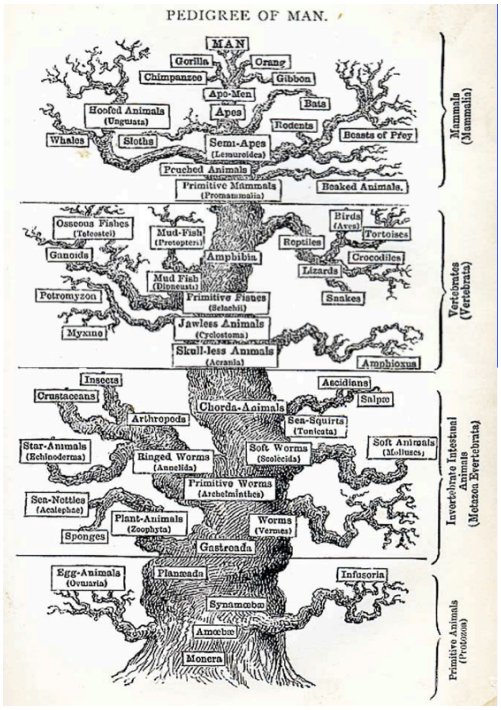
Sheila Sadeghi
Metabolic Biochemistry

Human cytochromes P450 in phase-1 DM

Number of named P450 Sequences

(Feb. 7, 2014)

Animals	6,502
Insects	3,571
Mammals	1,056
Other vertebrates	922
Non-insect invertebrates	953
Plants	7,533
Fungi	6,418
Protozoa	247
Bacteria	1,306
Archaea	48
Viruses	2
Total	22,056



Courtesy: David Nelson

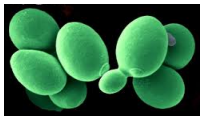
Number of P450s per genome

- *Arabidopsis thaliana*: 275
- *Drosophila melanogaster*: 90
- *Caenorhabditis elegans*: 80
- *Homo sapiens*: 57
- *Mycobacterium tuberculosis:* 20
- *Bacillus subtilis:* 7
- *Saccharomyces cerevisiae*: 3
- *Escherichia coli*: 0

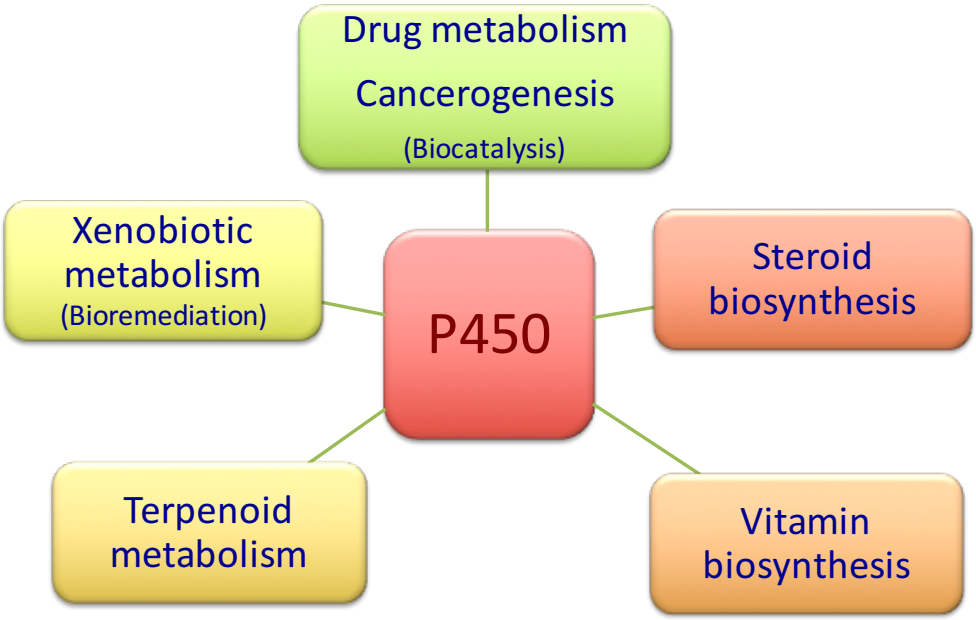








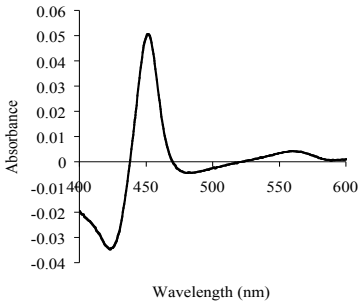

Relevance



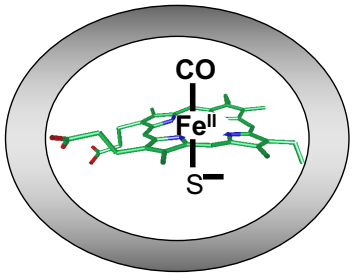
Introduction to cyt P450

- Superfamily of haem-thiolate monooxygenases
- 45-55 kDa
- Present in humans, mammals, insect, plants, bacteria, most species with the *exception of Escherichia coli*
- Generally found in most organs in mammals
  - Especially at high levels in liver: detoxification of xenobiotics (includes drugs)
- Extremely important for:
  - Drug metabolism
  - Synthesis and metabolism of:
    - steroids
    - Vitamin D
    - Prostaglandins

History



- Discovered in 1958 by Klingenberg and Garfinkel in liver microsomes (ER) of rat (Klingenberg, 1958) and pigs (Garfinkel, 1958)
- Described as *"carbon monoxide binding pigment"*
- Detection by difference absorption spectra between reduced and, reduced and CO-bound samples
- In 1964 Omura and Sato identified the 'pigment' as a haem and gave the name "cytochrome P450"
- Name still used although inaccurate
- Mammalian P450s are bound to the ER: this has required much effort for their solubilisation

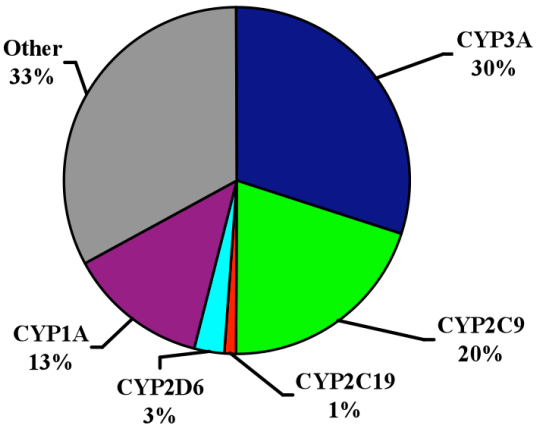


Nomenclature by AA sequence

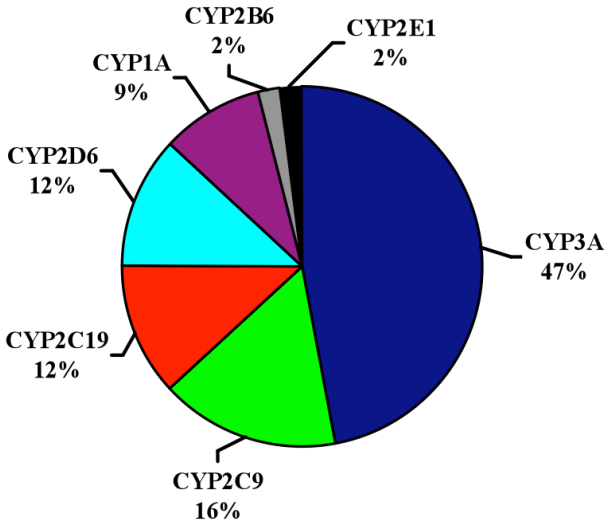
- From1989 a consistent nomenclature was introduced:
  - P450s (**CYP**) are now classified on the basis of AA identity, coded by letters and numbers that identifies what family and class they belong to:
    - Family: >40% sequence identity, **number: 3**
    - Subfamily: >55% sequence identity, **letter: A**
    - number of member in the subfamily, **number: 4**
  - There are > 50 P450s in human genome:
    - 17 families
    - 39 subfamilies

Conc. *versus* relevance to metabolism

- Out of 315 drugs studied:
  - 56% are cleared by CYP450
  - 85% of those by 3A4, 2D6, 2C9 and 2C19
- 2D6, 2C9, 2C19 and 2A6 are polymorphic and inducible:
  - inter-individual differences in drug metabolism
- CYP450 metabolism of a particular drug is studied with different inhibitors and activity is monitored with specific probes.



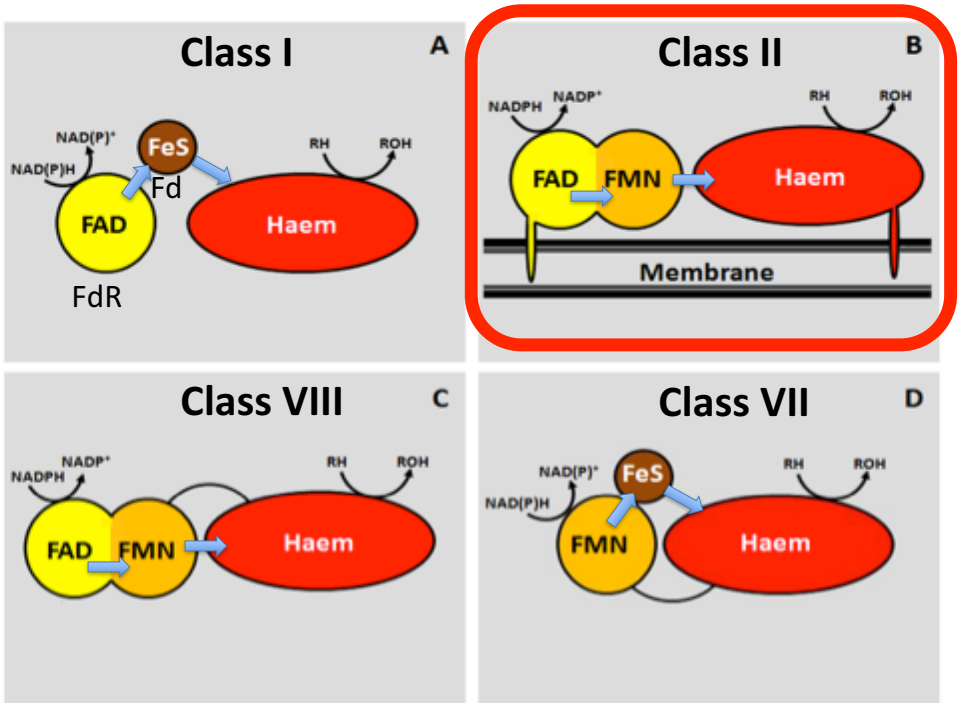
Conc. in liver



Relevance to metab.

Example: 2D6 is only 3% of hepatic P450 but it metabolises 12% of drugs!

Human P450s in DM are class II:



Sadeghi and Gilardi, Biotechnol. Appl. Biochem. 2013, **60**, 102-110.

the diameter of the cell body.

Pull-down assay and immunoprecipitation

Purification of recombinant GST fusion proteins from *Escherichia coli*, *in vitro* binding assays, and immunoprecipitation were done as described[11]. We loaded recombinant GST–RhoG with guanine nucleotides by incubating the protein with 100 μM GTP-γS or GDP in loading buffer (20 mM Tris-HCl, pH 7.5, 0.1 mM dithiothreitol and 5 mM EDTA) at 30 °C for 10 min. The reaction was stopped by adding $MgCl_2$ to final concentration of 10 mM. Activation of Rac1 in cells was determined by using the GST-fused CRIB domain of Pak1 as described[9].

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Crystal structure of human cytochrome P450 2C9 with bound warfarin

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Cytochrome P450 proteins (CYP450s) are membrane-associated haem proteins that metabolize physiologically important compounds in many species of microorganisms, plants and animals. Mammalian CYP450s recognize and metabolize diverse xenobiotics such as drug molecules, environmental compounds and pollutants[1]. Human CYP450 proteins CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are the major drug-metabolizing isoforms, and contribute to the oxidative metabolism of more than 90% of the drugs in current clinical use[2]. Polymorphic variants have also been reported for some CYP450 isoforms, which has implications for the efficacy of drugs in individuals, and for the co-administration of drugs. The molecular basis of drug recognition by human CYP450s, however, has remained elusive. Here we describe the crystal structure of a human CYP450, CYP2C9, both unliganded and in complex with the anti-coagulant drug warfarin. The structure defines unanticipated interactions between CYP2C9 and warfarin, and reveals a new binding pocket. The binding mode of warfarin suggests that CYP2C9 may undergo an allosteric mechanism during its function. The newly discovered binding pocket also suggests that CYP2C9 may simultaneously accommodate multiple ligands

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Human 2C9 structure

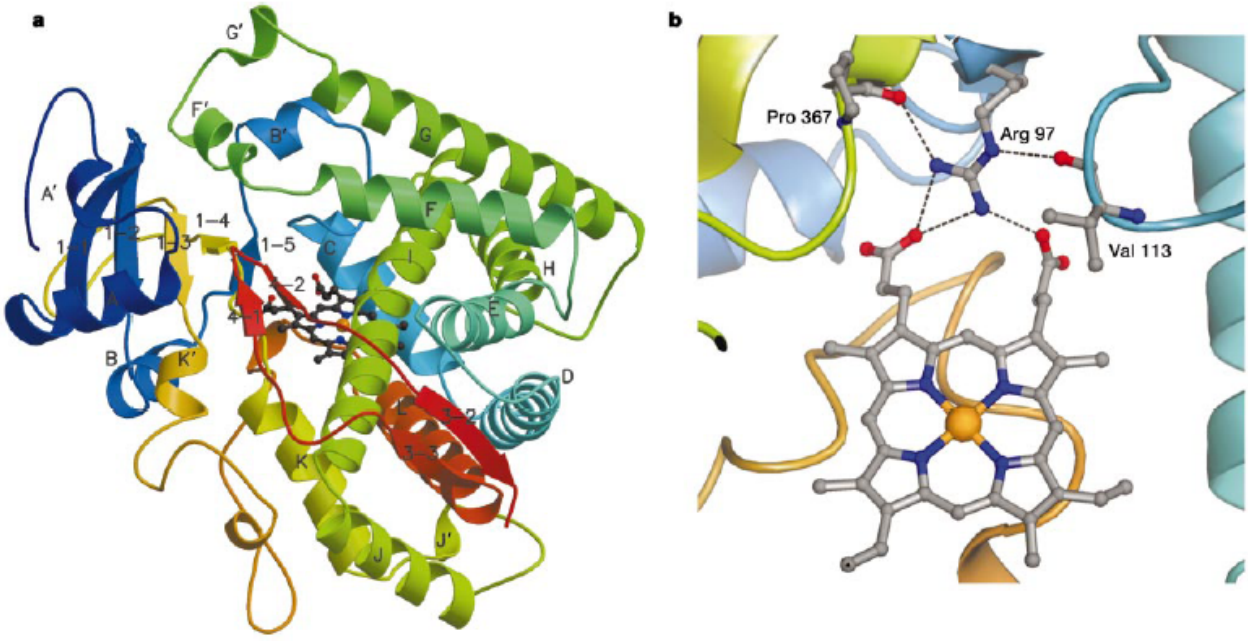


Figure 1 Structure of P450 CYP2C9. **a**, Overall fold of CYP2C9, coloured from blue at the N terminus to red at the C terminus. The haem group is depicted as a ball-and-stick model in the centre of the molecule, flanked by helices I and L. There is a slight distortion in helix I, close to the haem. The substrate access channel is widely acknowledged to involve the loops between helices B and C, and helices F and G. The figure was produced using Molscript (http://www.avatar.se/molscript). **b**, View of Arg 97 and the haem group (shown at the bottom). Arg 97 is held in position by hydrogen bonds (indicated by dashed lines) to the haem propionates and to the carbonyl oxygen atoms of Val 113 and Pro 367. Figures 1b–4b were produced using Aesop 2.5 (M. Noble, unpublished work).

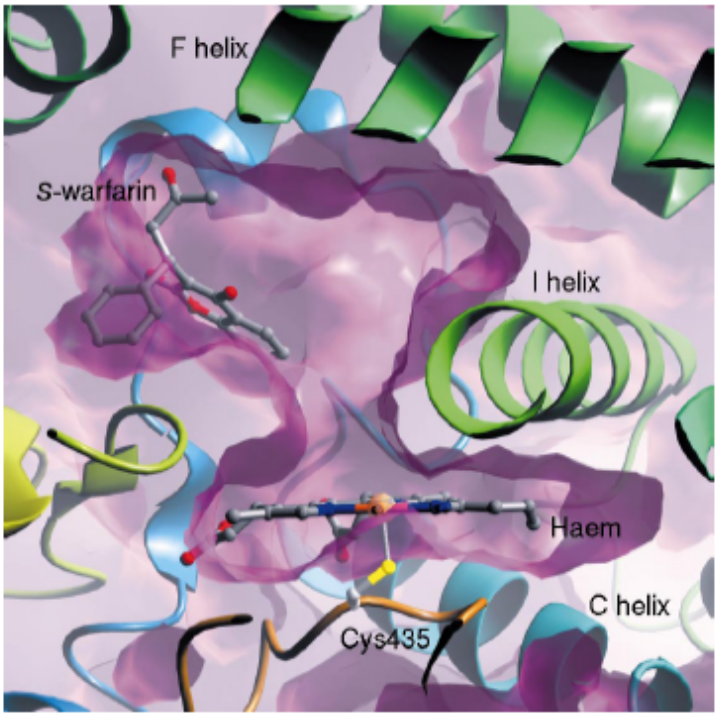


Figure 3 The surface of the active site of CYP2C9. The haem is shown at the bottom, and the *S*-warfarin molecule above and to the left. The active site of CYP2C9 is large and could potentially accommodate either compounds larger than *S*-warfarin or multiple ligands of comparable size to *S*-warfarin without substantial conformational movement. With *S*-warfarin bound in this location the haem group remains available to metabolize additional substrate molecules.

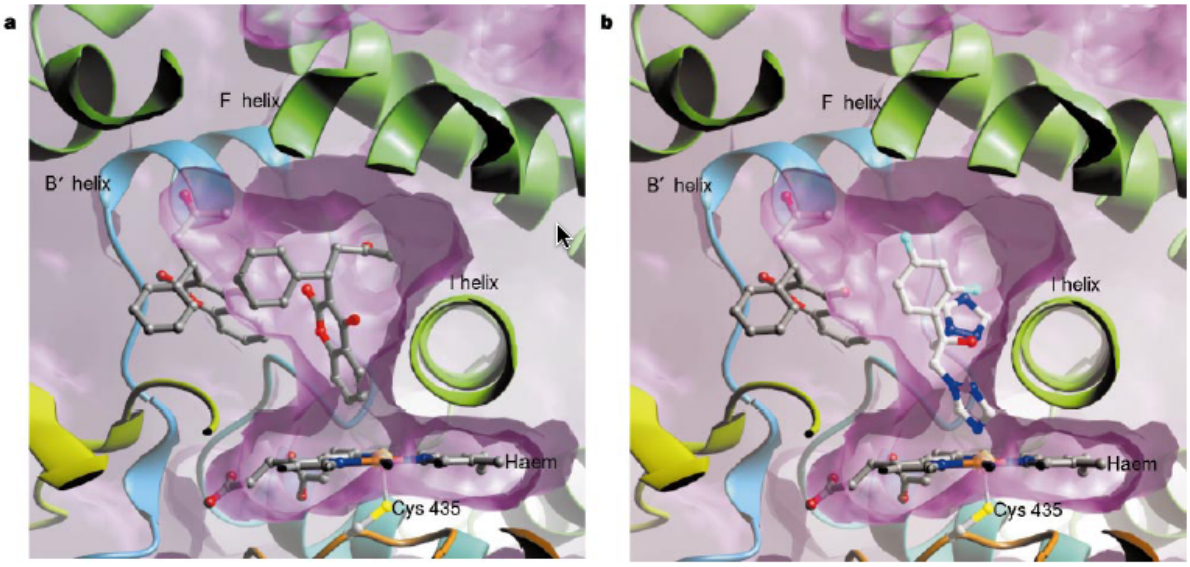
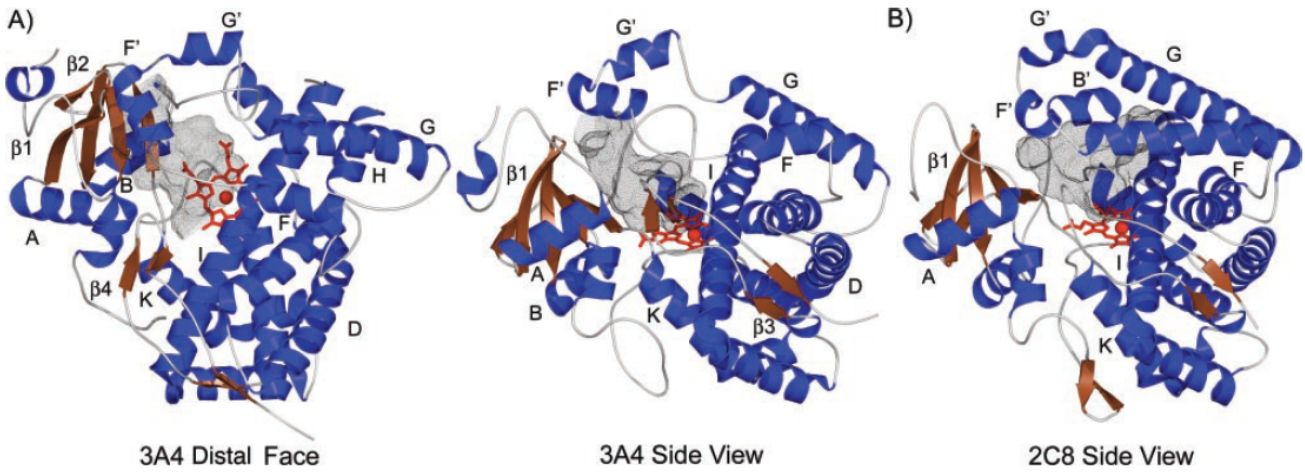
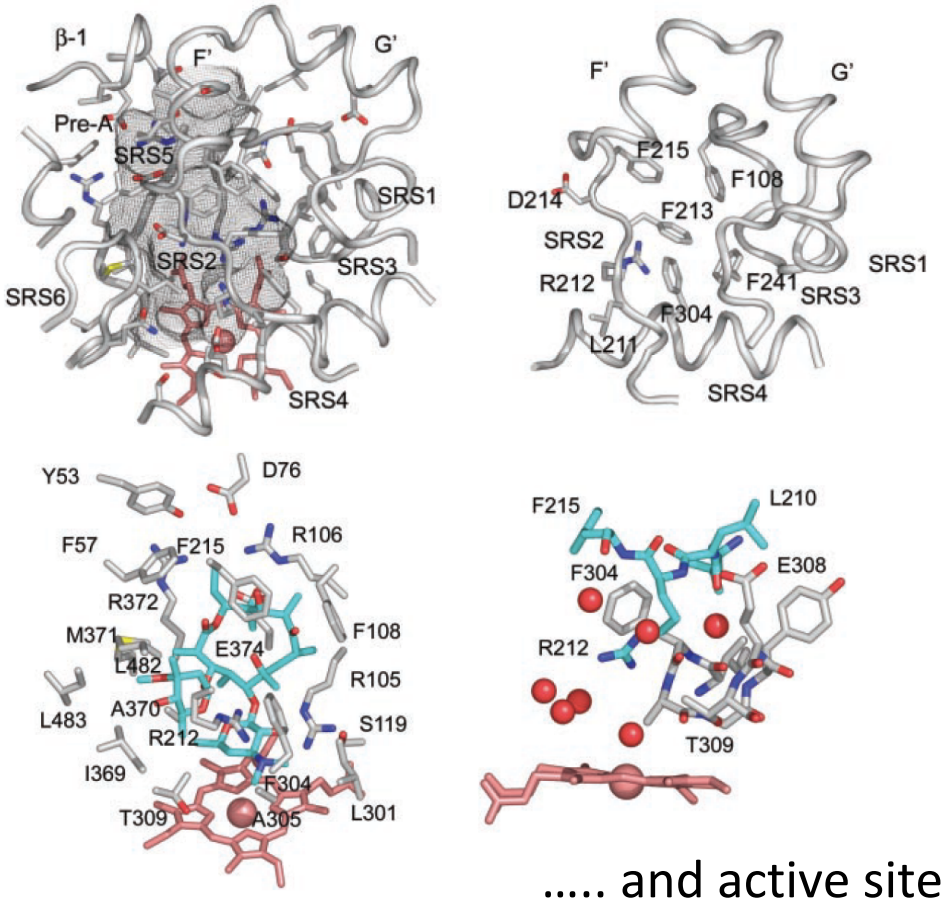


Figure 4 View of the region of the active site of CYP2C9 that remains available to accommodate additional ligand(s) after *S*-warfarin. The bound *S*-warfarin molecule is shown as in Fig. 3. **a**, A second molecule of *S*-warfarin has been modelled into the active with the site of hydroxylation closest to the haem iron. **b**, A known haem binder fluconazole has been modelled into the cavity in a similar conformation to that observed in the complex of CYP51 with fluconazole (Protein Data Bank code 1EA1).

Structure of 3A4

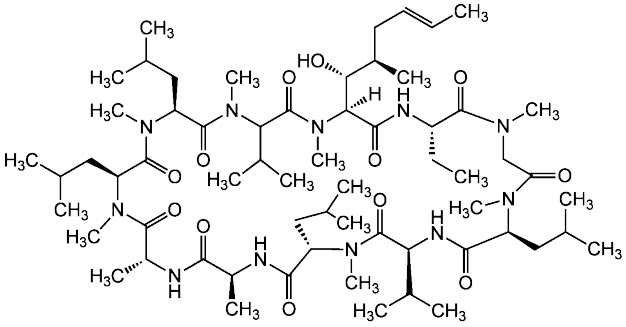


3A4 substrate recognition sites (SRS) …..

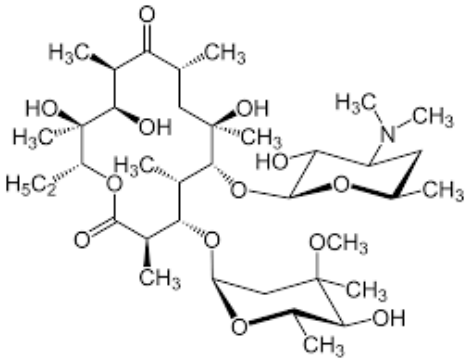


….. and active site

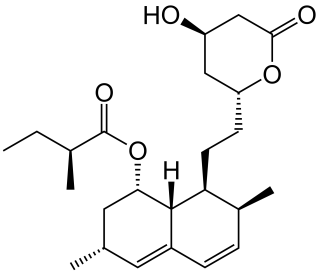
Substrates recognised by 3A4



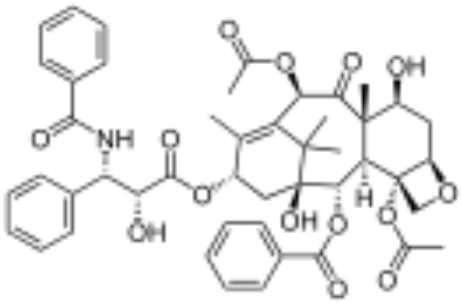
cyclosporine



erythromycin

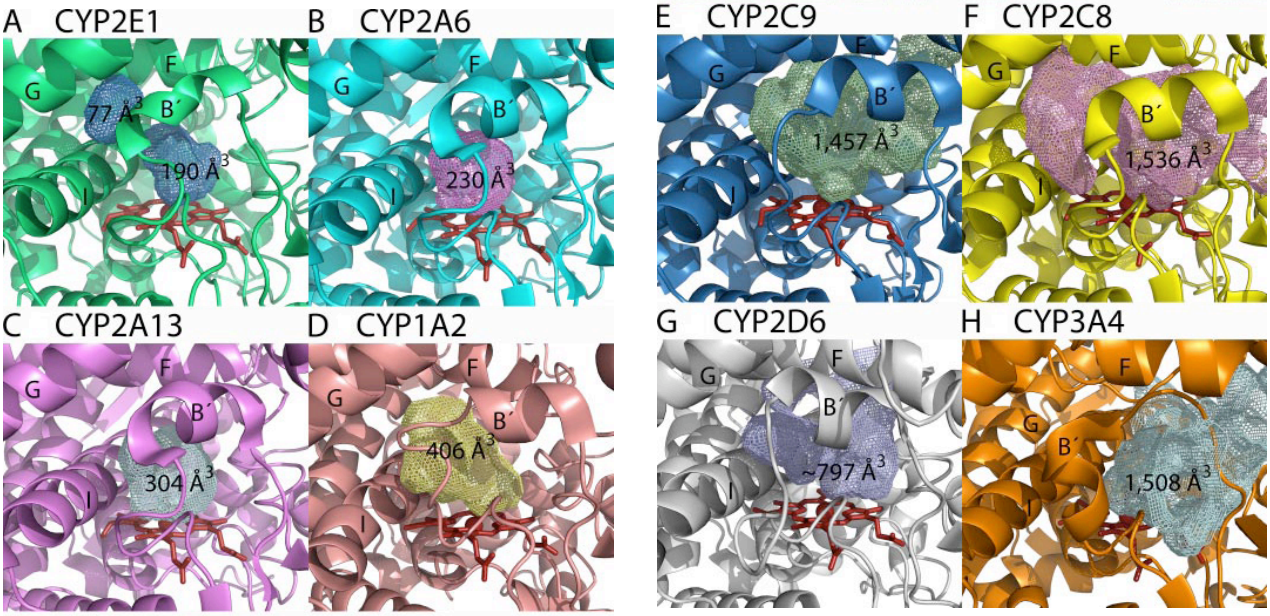


statins (lovastatin)



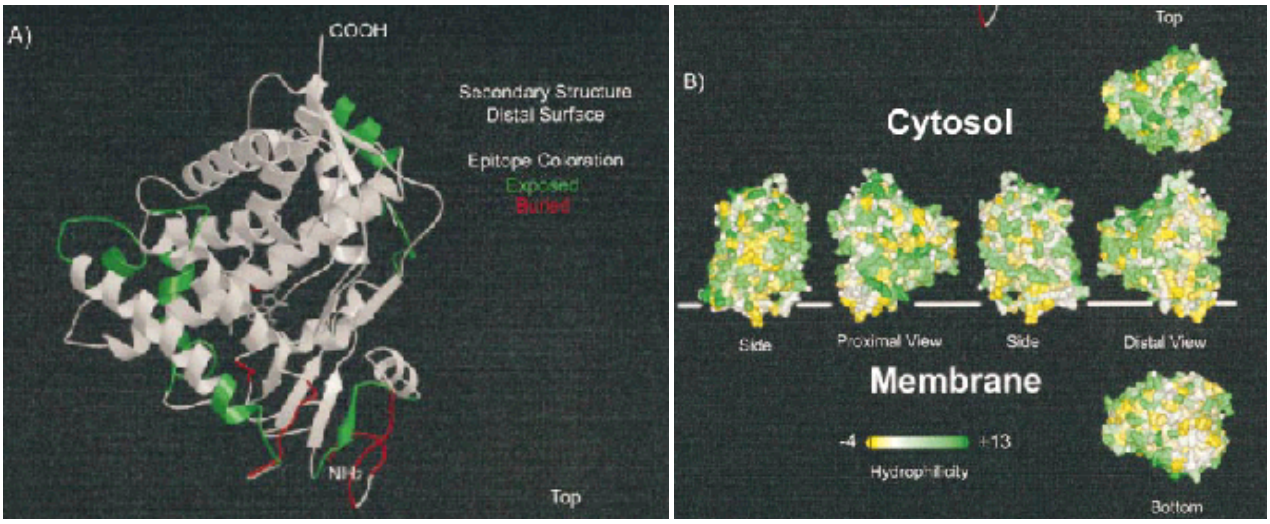
taxanes (paclitaxel)

Comparison of binding pockets

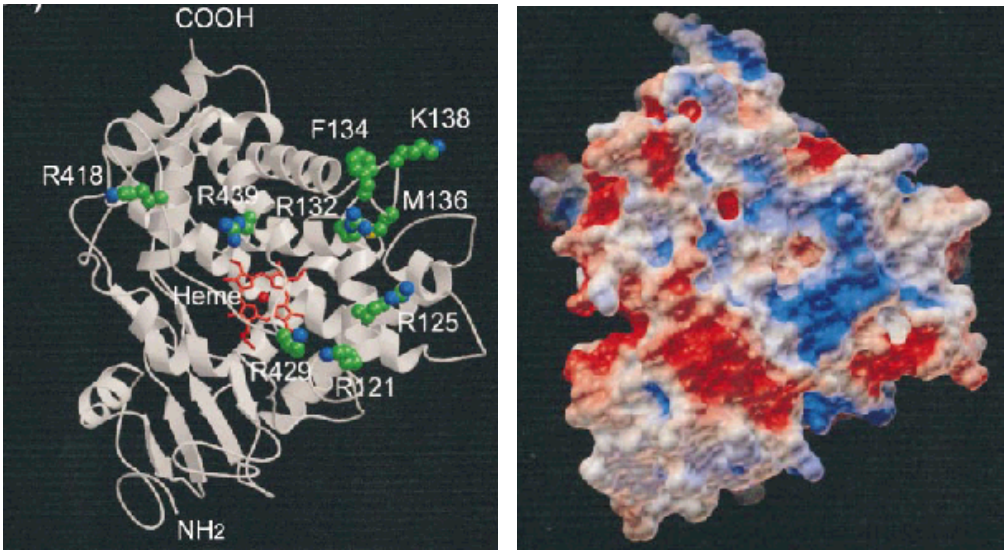


Ref: Porubsky et al. 2008, JBC 283:33698-707

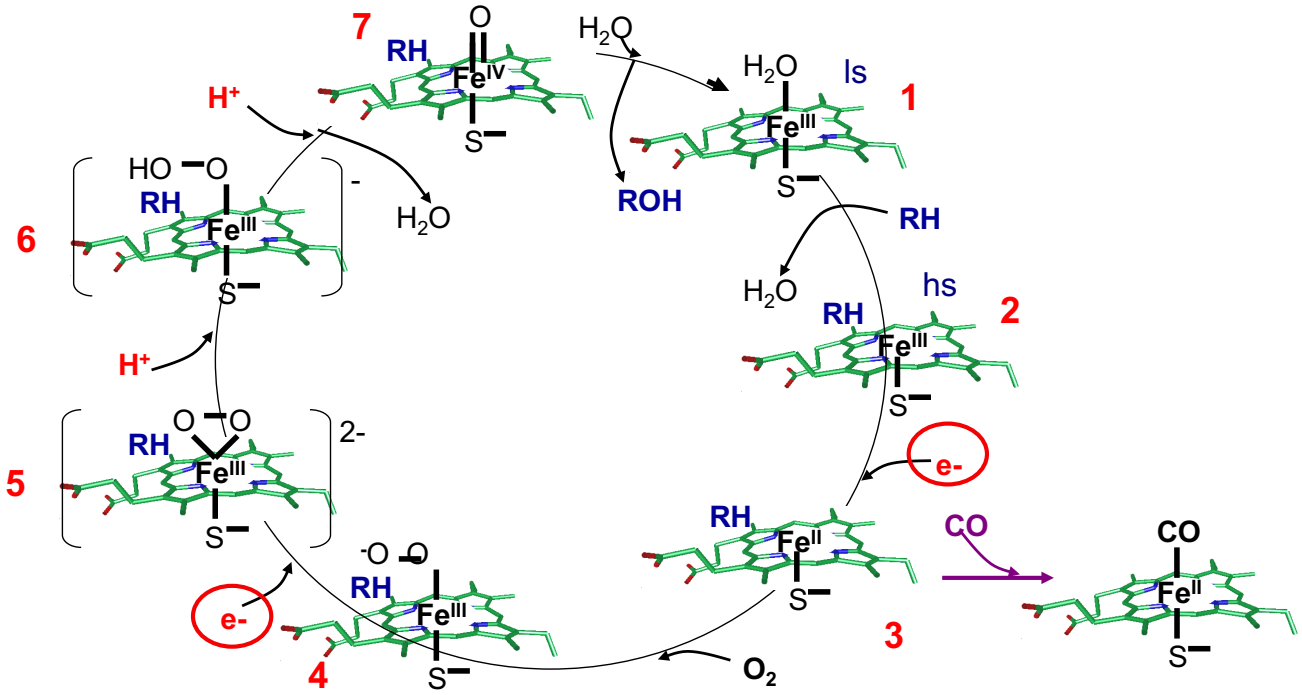
Membrane binding surface

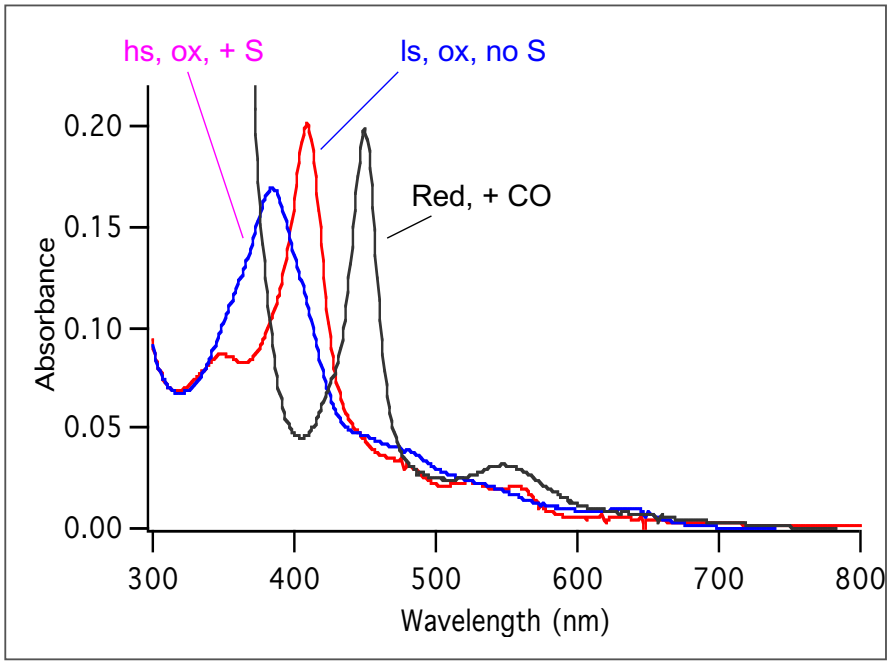


CPR interaction site

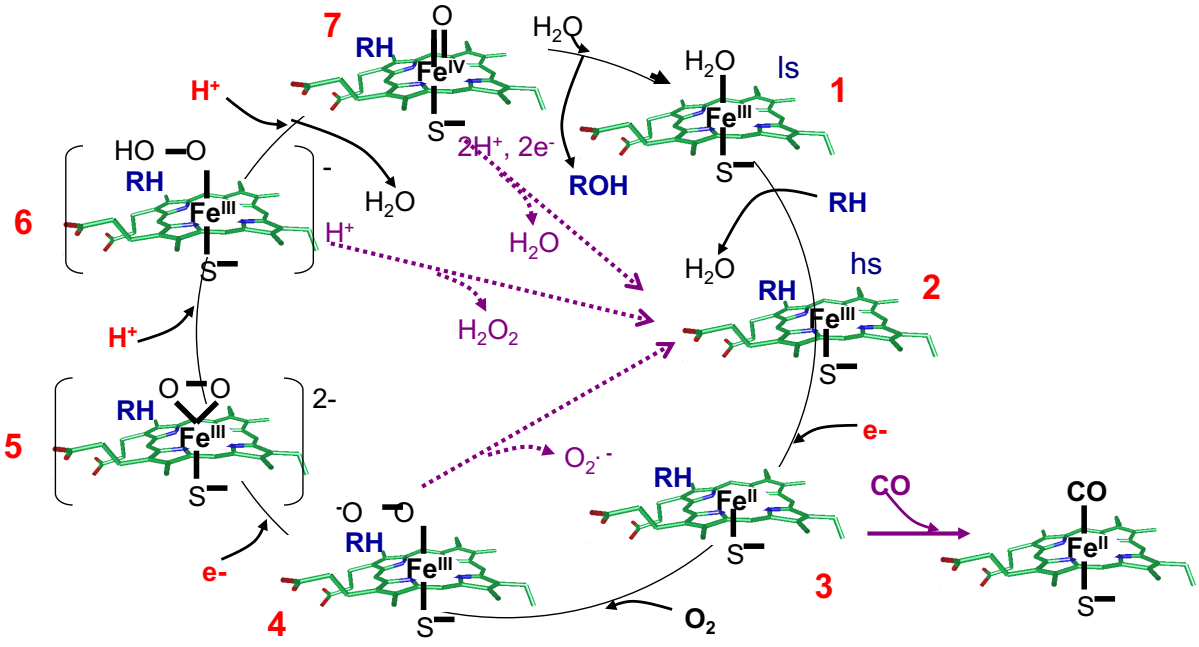


Cytochrome P450: Catalytic cycle





Uncoupling reactions



Uncoupling reactions

- Coupling between NADPH oxidation and substrate turnover in P450s varies widely: from greater than 95 % and less than 1 %
- High levels of coupling are usually reported for the bacterial P450s which are found to be close to 100 % coupled
- Values of less than 1 % are reported for some human P450s in a reconstituted system
- Uncoupling can happen at one of three points in the cycle, giving **reactive oxygen species or water**:
  - superoxide anion radical,
  - hydroperoxo iron complex disassociates to give hydrogen peroxide,
  - the single oxygen in the iron oxygen complex (compound I) gives a water molecule.
- Another form of uncoupling is also possible where for some reason electrons are not transferred from the P450s protein partner (i.e. CPR) but instead are transferred into solution, where they may form a variety of reactive species.

Uncoupling reactions

- There are 3 possibilities with the following stoichiometries:

$$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + 2\ O_2^{\cdot -} + H^+$$

$$NAD(P)H + O_2 + H^+ \rightarrow NAD(P)^+ + H_2O_2$$

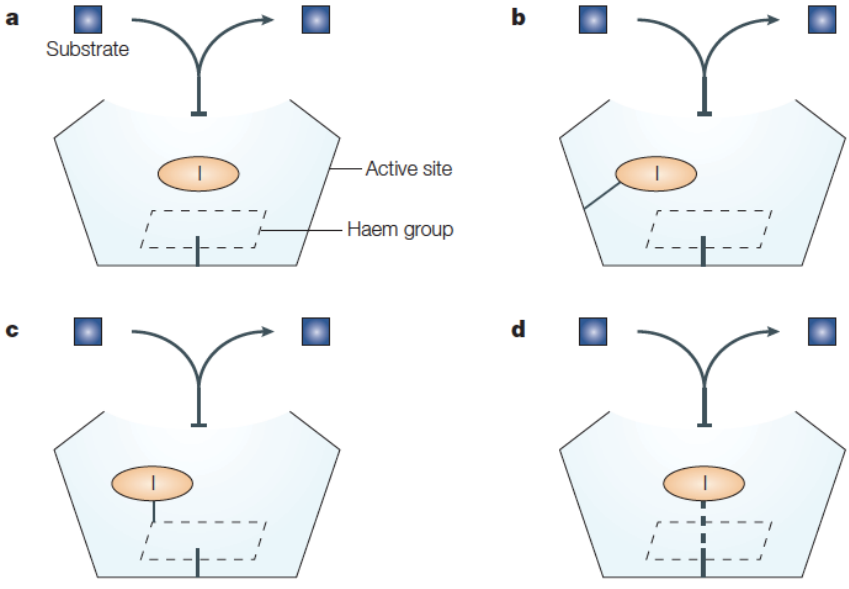
$$2NAD(P)H + O_2 + 2H^+ \rightarrow 2NAD(P)^+ + 2H_2O$$

Cytochrome P450: Inhibition

Modulation of activity : inhibition

A) Reversible inhibition

B) Irreversible inhibition - 1

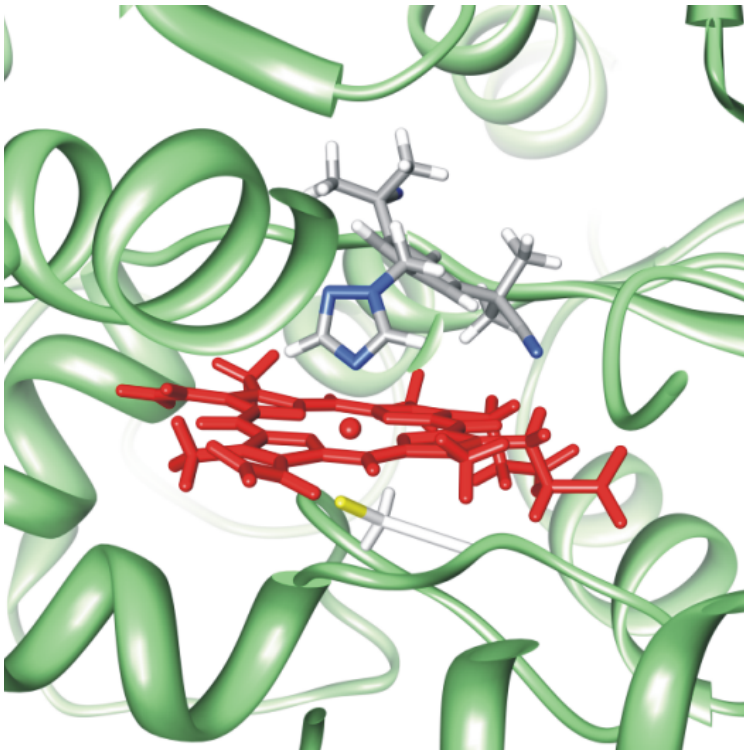


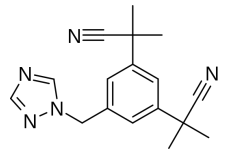
C) Irreversible inhibition - 2

C) Quasi-irreversible inhibition

Wienkers and Heath, Nature Reviews, 2005, 4, 825-833

Quasi-irreversible inhibition of aromatase

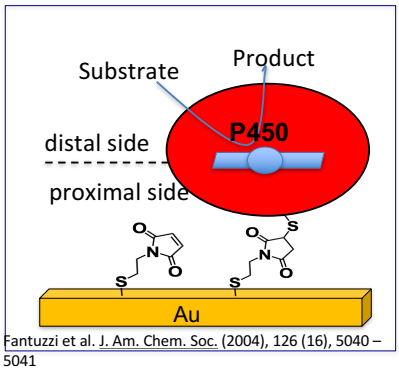




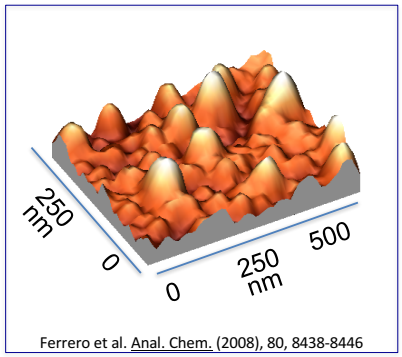
Anastrozole-bound Aro

Morelli *et al*, Chem. Commun.,2011, **47**, 10737–10739

Research in our lab: bioelectrochemistry of P450

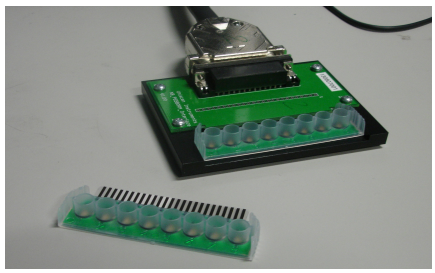


Fantuzzi et al. J. Am. Chem. Soc. (2004), 126 (16), 5040 – 5041

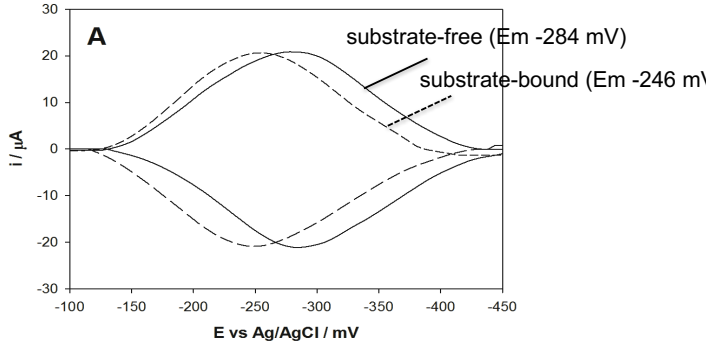


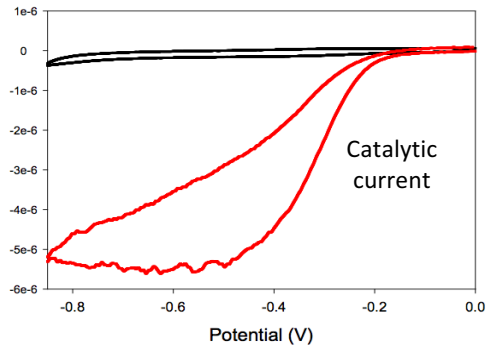
Ferrero et al. Anal. Chem. (2008), 80, 8438-8446

Electrode array



Fantuzzi et al. Anal. Chem. (2011), 83, 3831–3839





Cytochrome P450: drug-drug interactions

Xenobiotics (food components or drugs) that induce or inhibit P450 enzymes can change the rate or extent of drug metabolism

Bottom line: a greater concentration of drug remains in the plasma

- Seldane (terfenadine) and Hismanal (astemizole) were introduced to clinical practice approximately 25 years ago as the first non-sedating antihistamines.
- Because antihistamine dosing was no longer limited by unwanted sedation, dosages often were increased above approved limits to achieve therapeutic goals
- Anything that increases the serum concentration of terfenadine increases the **risk of arrhythmia**.
- CYP3A4 metabolizes 99% of terfenadine on its first pass through the liver and small bowel; terfenadine is almost completely eliminated before it reaches the systemic circulation.
- Drugs that block CYP3A4 will result in measurable and potentially toxic levels of terfenadine in the circulation.

Drug-drug interactions

- Early predictions are important:

  - (1) TERFENADINE: H_1-histamine antagonist
  - (2) KETOCONAZOLE: Antifungal agent

  (1) good substrate of CYP3A4, metabolised at normal rate;

  (2) potent inhibitor of CYP3A4;

  (1) + (2), terfenadine remains at high levels in plasma, leading to LETHAL VENTRICULAR ARRHYTHMIAS.

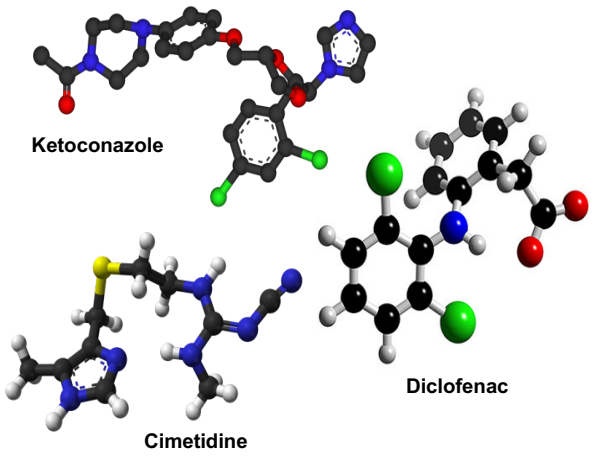
P450 related withdrawals

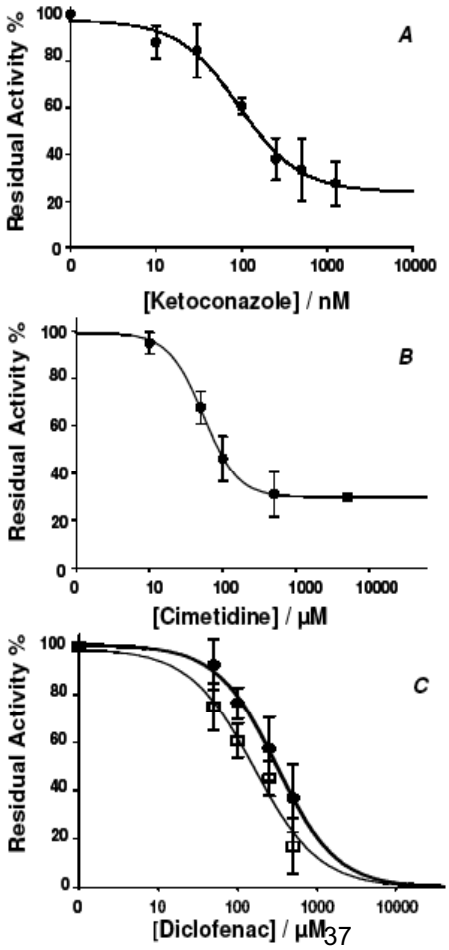
Drug (Company)	Use	P450 involved	Effect of interaction
Terfenadine (Hoechst Marion Roussel)	Antihistamine	CYP3A4	Fatal arrhythmias
Cisapride (Janssen)	Heartburn	CYP3A4	Fatal arrhythmias
Cerivastatin (Bayer)	Lipid lowering drug	CYP2C8	Rhabdomyolysis (muscle breakdown)
Astemizole (Janssen)	Antihistamine	CYP3A4	Fatal arrhythmias
Mibefradil (Roche)	Hypertension and angina	CYP3A4	Fatal arrhythmias
Perhexiline	Angina	CYP2D6	Nerve toxicity
Nefazodone (Bristol-Myers Squibb)	Antidepressant	CYP3A4	Liver toxicity
Grepafloxacin (Glaxo)	Antibiotic	CYP3A4	Fatal arrhythmias

Drugs Removed from or Restricted in the U.S. Market Because of Drug Interactions

- Terfenadine (Seldane®) February 1998
  - Anti-histamine
  - Interaction with drugs e.g. ketoconazole, erythromycin
  - Interaction with food e.g. grapefruit juice
- Mibefradil (Posicor®) June 1998
  - Calcium channel blocker
- Astemizole (Hismanal®) July 1999
  - Anti-histamine
- Grepafloxacin (Raxar®) October 1999
  - Anti-bacterial
- Cisapride (Propulsid®) January 2000
  - Treatment of constipation

3A4 Drug-drug interactions



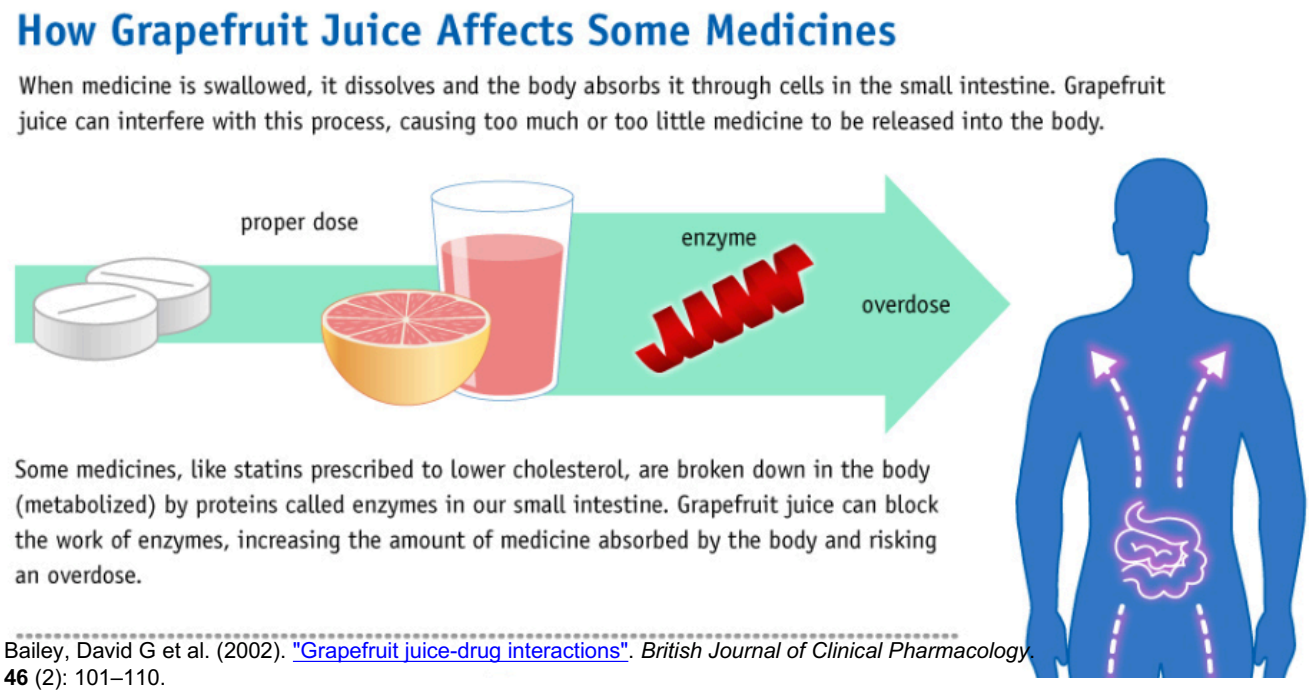


According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*.

Sadeghi et al.. Drug-drug interactions and cooperative effects detected in Electrochemically driven human cytochrome P450 3A4. Bioelectrochemistry (2012).

Cytochrome P450: drug-food interactions

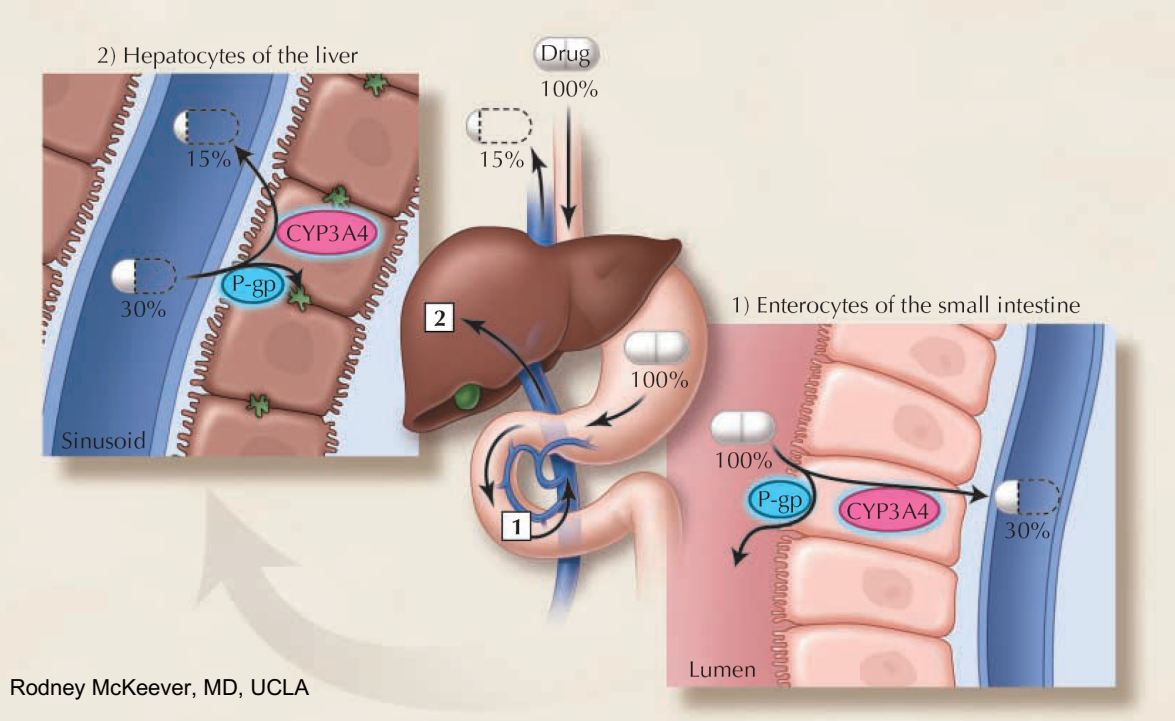
Food-drug interactions



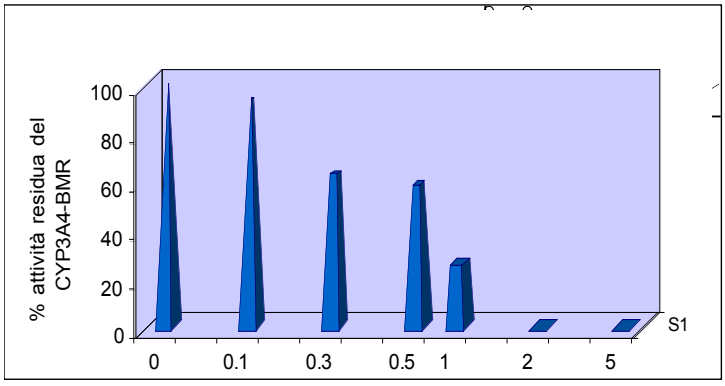
Bailey, David G et al. (2002). "Grapefruit juice-drug interactions". *British Journal of Clinical Pharmacology* **46** (2): 101–110.
https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm292276.htm

Drugs interacting with grapefruit juice

Drug class	Major Interactions	Minor interactions
Antiarrhythmic agents	amiodarone (Cordarone) dronedarone (Multaq)	
Antihistamines	terfenadine (Seldane) (off the market) diphenhydramine (Benadryl) (partially) astemizole (Hismanal) (off the market)	
Calcium channel antagonists		felodipine (Plendil) nicardipine (Cardene) nifedipine (Procardia) nimodipine (Nimotop) nisoldipine (Sular) isradipine (DynaCirc)
Statins (HMG-CoA reductase inhibitors)	simvastatin (Zocor) lovastatin (Mevacor)	atorvastatin (Lipitor) cerivastatin (Baycol) (off the market)
Cough Suppressant/NMDA Antagonist	dextromethorphan	
Erectile dysfunction drugs		sildenafil (Viagra) tadalafil (Cialis) vardenafil (Levitra)
HIV protease inhibitors		saquinavir (Invirase) ritonavir (Norvir) nelfinavir (Viracept) amprenavir (Agenerase)

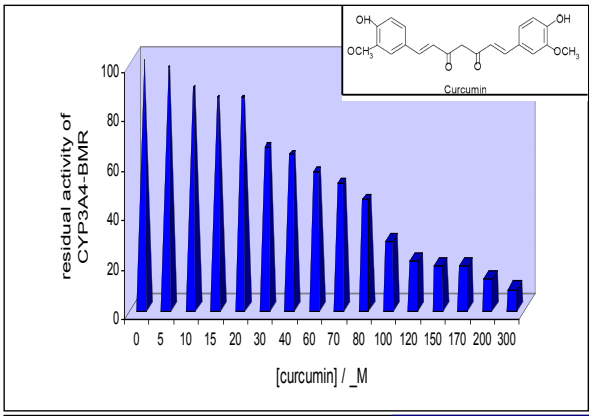


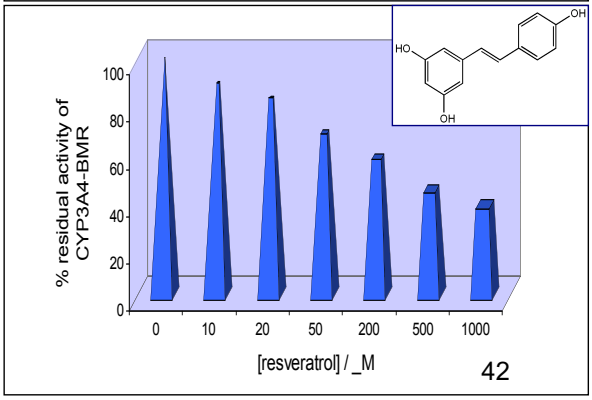
3A4: Drug-food interactions

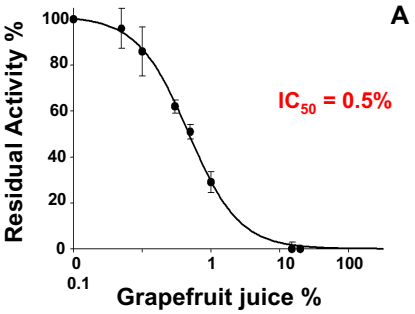


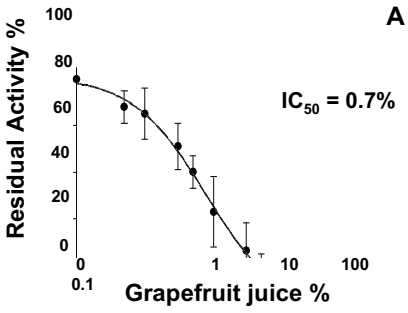
% Grapefruit juice

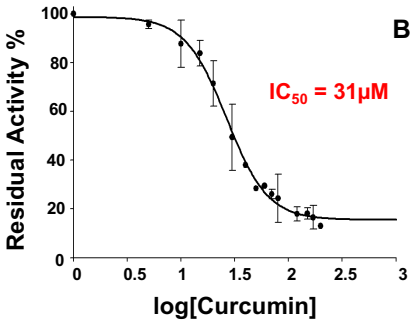
Drug = erythromycin
Food = grapefruit juice
red wine
curry powder

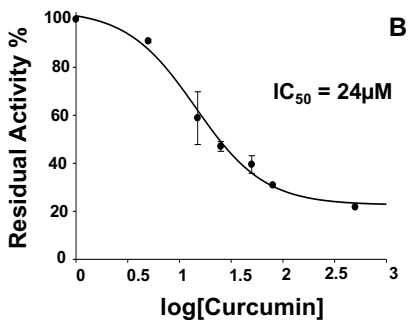


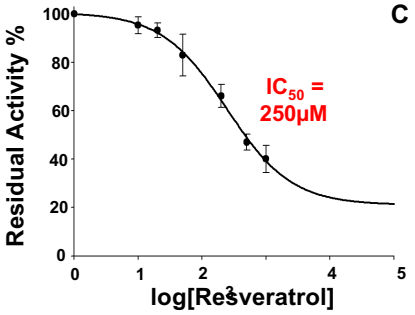


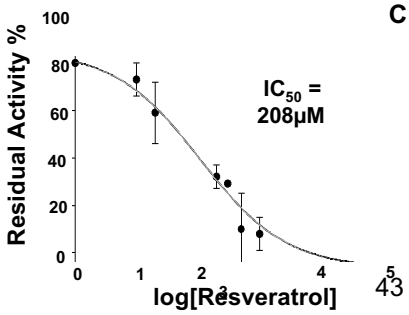












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