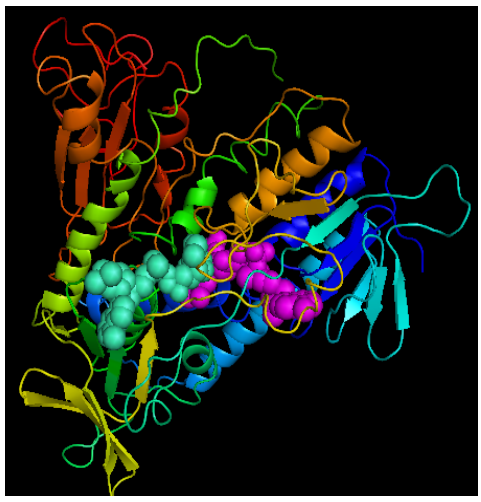
Drug metabolism (4)

Flavin-containing monooxygenases (FMO)

Sheila Sadeghi
Metabolic Biochemistry

Introduction hFMO

– Second most important family of monooxygenases in terms of drug metabolism
  - family of flavin (FAD) monooxygenases
  - Involved in metabolism of xenobiotics (drugs)
  - Catalyse the NADPH-dependent oxygenation of soft nucleophiles
  - No crystal structures available
  - 5 different isoforms, most important one is FMO3
    – Present in adult liver
    – Membrane-bound

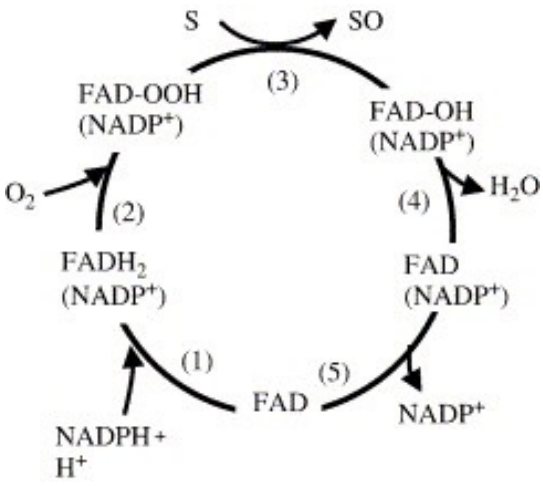


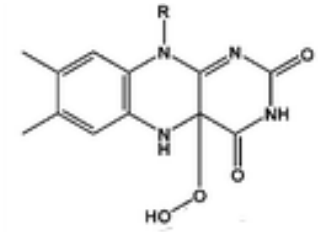
Properties

- Phase I drug metabolising enzyme
- Microsomal like CYPs
- NADPH dependent enzyme
  - FAD co-factor
- Oxidation of nucleophillic heteroatom contaning small molecules
  - soft centres such as nitrogen and sulfur i.e. N-oxidation and S-oxidation
- Cannot oxidise carbon – not as powerful as CYPs
- 5 genes (FMO 1-5) and 6 pseudogenes in humans

Model of human FMO1 showing FAD (pink) and NADPH (green) bound

Reaction mechanism





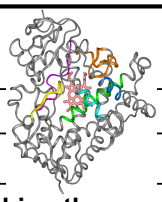
C4a-hydroperoxyflavin

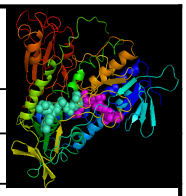
"Loaded gun"

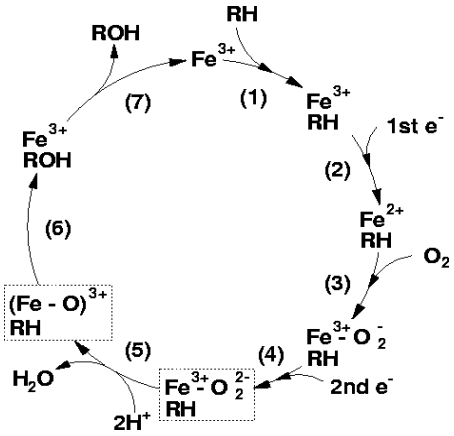
- Enzyme is reduced by NADPH and binds oxygen to form a stable C4a-hydroperoxyflavin prior to substrate binding
- Substrate spends very little time in active site
  - Higher turn-over number than human CYPs
- C4a-hydroperoxyflavin stable unlike compound I of P450s
  - Protein environment prevents decomposition of hydroperoxyflavin ?
  - Minimises uncoupling and formation of reactive oxygen species
  - Conservation of NAPDH but unproductive cycles can occur

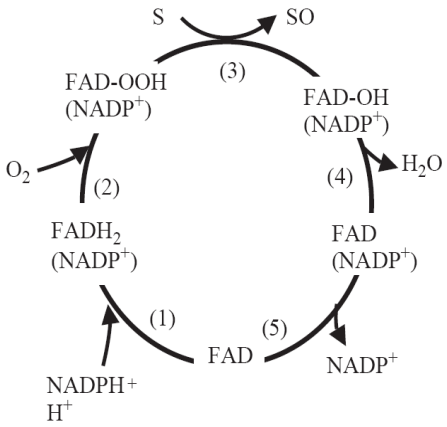
P450 versus FMO

P450	FMO
Huge family	Small family
Active site haem	Active site FAD
Binds the substrate before reaching the active form	Ready to oxidise before substrate binds
Induced by substrate	Not induced by substrate









Reaction mechanism

- Very few true competitive inhibitors of FMOs
  - Dietary indoles
  - (dimethylamino)stilbene carboxylic acids
  - Less potential for drug-drug interactions

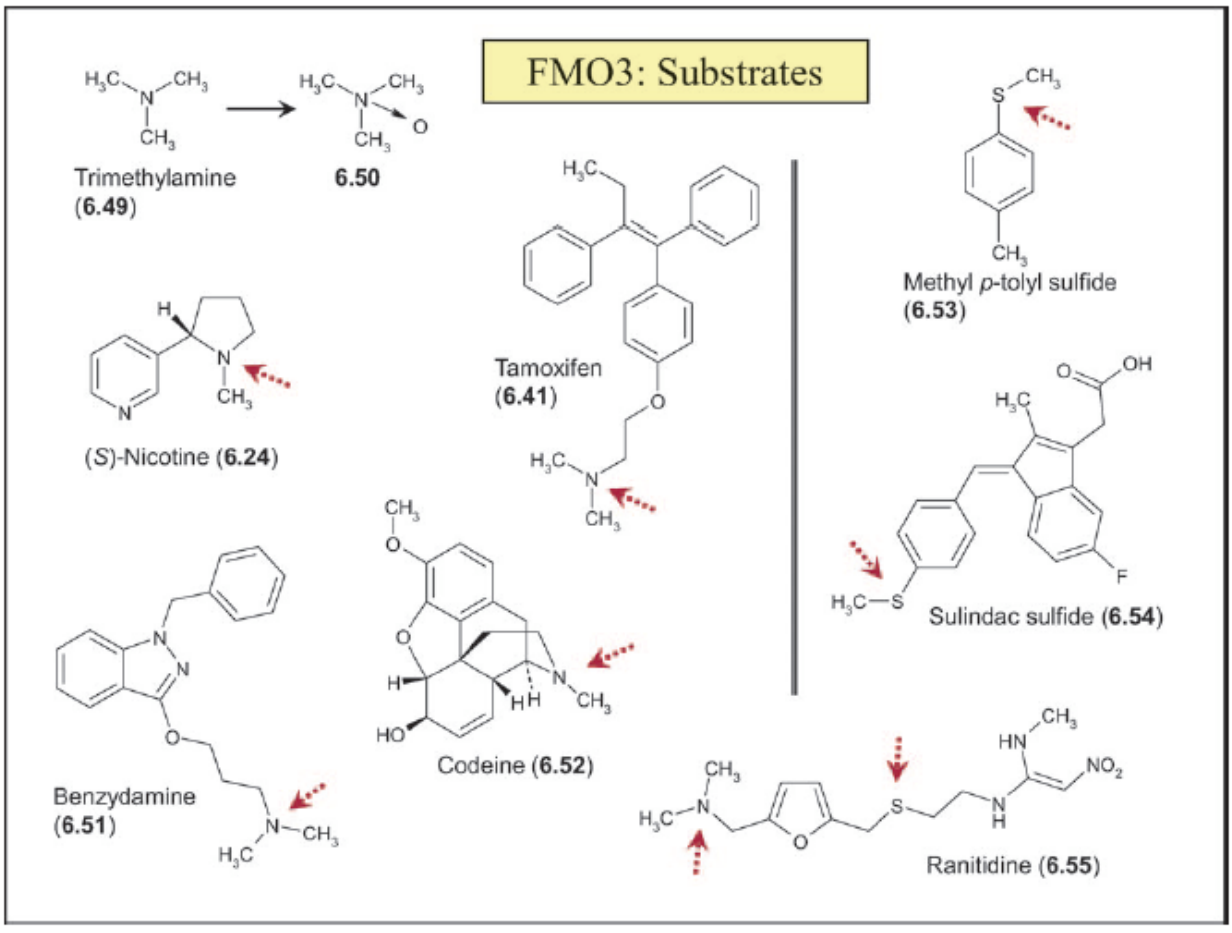
- Enzyme not inactivated by reactive metabolites
- Enzyme not inducible
- FMOs could be used as detoxification route instead of P450s but very limited substrate specificity and reactions carried out.

Tissue specific expression of hFMO

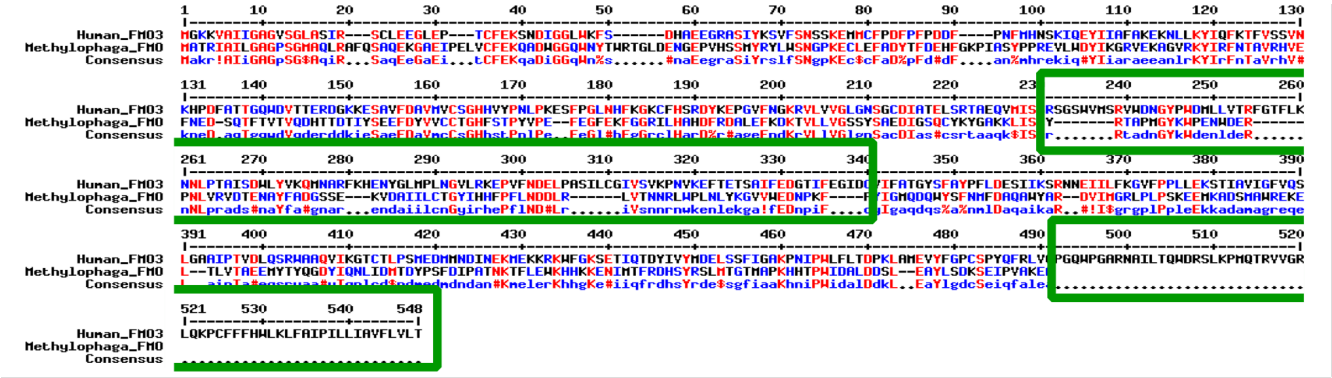
	FMO1	FMO2	FMO3	FMO4	FMO5
Fetal brain	56.4	17.6	5.6	14.6	21.0
Adult brain	3.1	140.9	10.7	19.6	56.5
Fetal liver	945.7	93.1	445.6	488.3	4406.8
Adult liver	96.0	988.7	**23088.6**	4881.7	**26539.5**
Adult kidney	**6198.2**	4682.7	530.9	**2509.9**	1628.3
Adult lung	595.7	**115895.5**	2223.9	738.1	2274.9
Adult small intestine	522.9	928.7	74.2	403.3	2586.3

	Tissue
FMO1	kidney
FMO2	lung
FMO3	liver
FMO4	kidney
FMO5	liver

Tissue specific expression of the FMO isoforms in humans expressed as copies per ng RNA

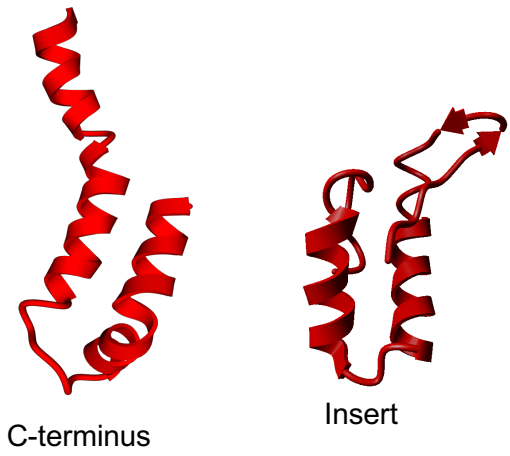


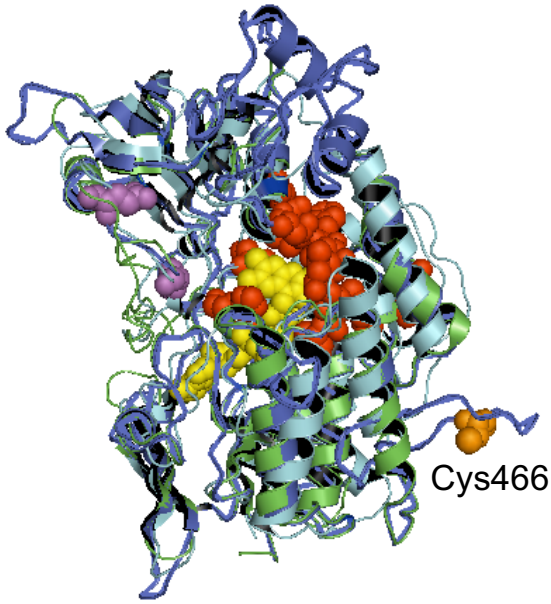
Molecular Modeling



Known crystal structures (≈28% homology)
- Yeast FMO (Eswaramoorthy et al., PNAS, 2006)
- Bacterial FMO (Alfieri et al., PNAS, **2008**)

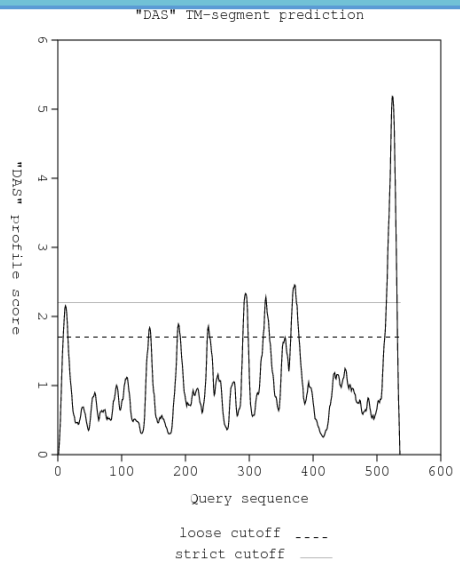
Molecular Modeling
Ab initio and homology modeling

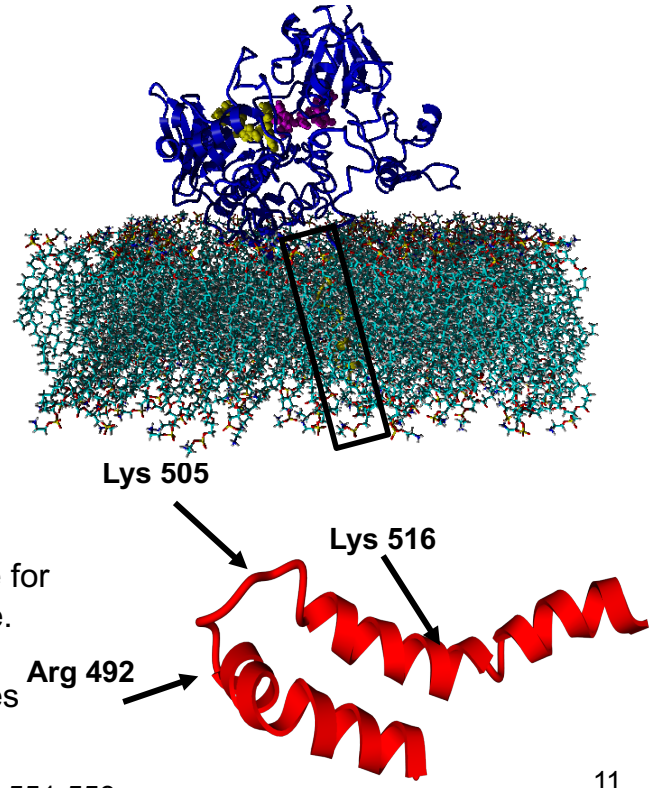




Superimposition of yeast (green, PDB:2GV8), bacterial (cyan, PDB:2VQ7) and human FMO3 (model; blue); RED = active site, YELLOW = FAD, PURPLE = access channel

Deletion of the membrane anchor





C-terminal region of hFMO3 responsible for the insertion of the enzyme in membrane. Three different clones were generated carrying a stop codon at different residues

Ref: Catucci et al., Biochem Pharm (2012) 83:551-558.

Human FMO1

- Primarily expressed in adult kidneys and fetal liver
  - Expression in liver drops immediately after birth
- Polymorphic with 20 allelic variants
  - Most result in increased K_m and/or altered V_{max}
  - FMO1*6 variant – low expression of enzyme
- Does not oxygenate primary amines
- Broadest specificity of all human FMOs

- Substrates include
  - Imipramine and chlorpromazine (anti-depressants)
  - Disulfiram (used to treat alcohol dependance)

- Purified human enzyme thermolabile and inhibited by low concentration of anionic detergents

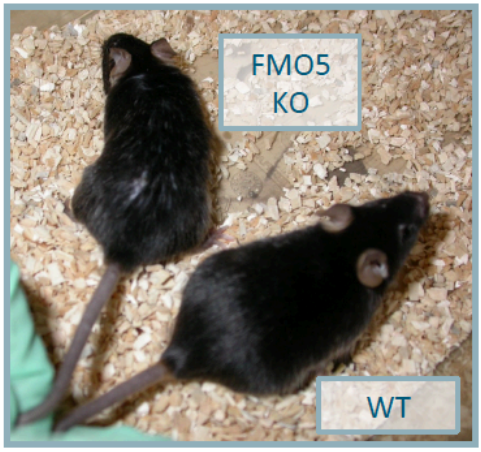
Human FMO2

- Primarily expressed in the lung
- Polymorphic with 5 allelic variants
  - Most result in no activity at all
- Very active towards bioactivation of small MW thioureas and detoxification of thioethers
  - Increased risk of toxicity following thiourea exposure in individuals with wild-type alelle
  - Decreased risk of toxicity following thioether containing organophosphate exposure in individuals with wild-type alelle
- Restricted active site and therefore very substrate specific enzyme
  - Substrate access channel estimated to be 8 Å long by 8 Å wide cylinder.
- Tertiary amines are excellent substrates
- Purified enzyme is thermostable compared to FMO1 and FMO3 and not inhibited by anionic detergents like FMO1 and FMO3

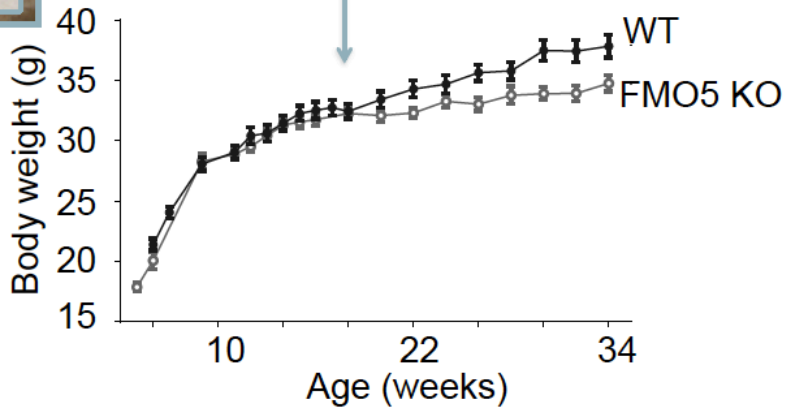
Human FMO4 and FMO5

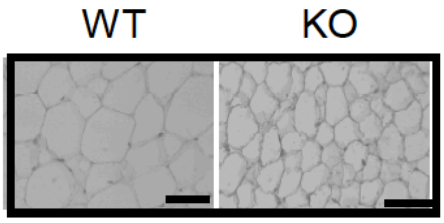
- Primarily found in adult liver and kidney
- Polymorphic but few variants reported to date
- Very limited substrate specificity and little contribution to drug metabolism identified to date
  - Difficult to express
  - Might not be involved in drug metabolism???

FMO5 – a regulator of metabolic ageing



Reduced weight gain in FMO5 KO mice





White adipose tissue,
30 weeks

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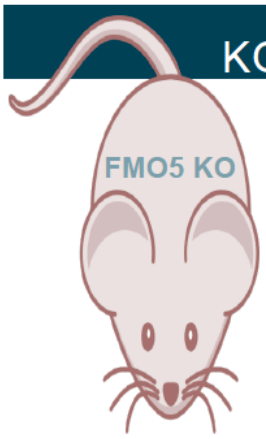
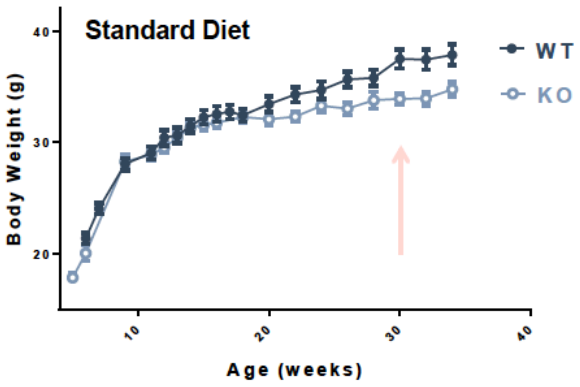
KO mice gain less weight with age

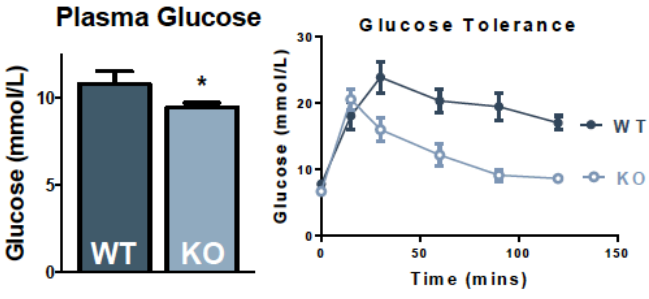




30 weeks of age

- WT ↑ plasma glucose
- KO ↑ glucose tolerance
- KO ↑ insulin sensitivity

30 weeks



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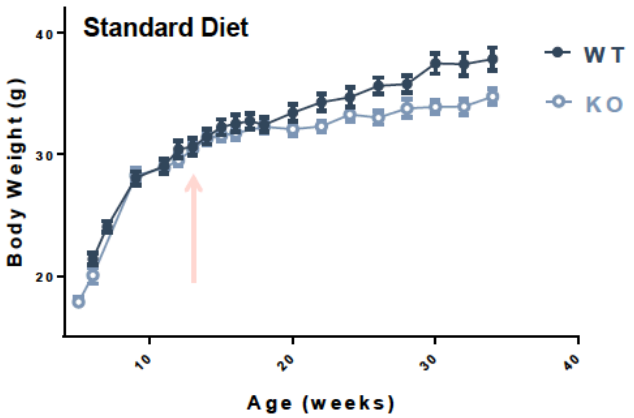
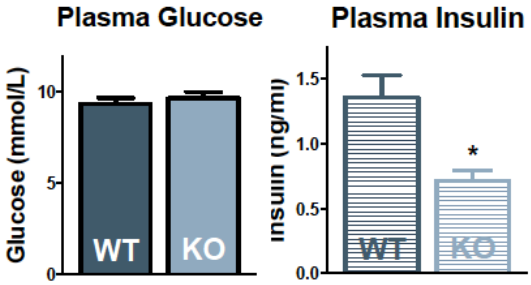
KO Mice have reduced plasma insulin



15 weeks of age



15 weeks of age

Plasma glucose WT=KO

Plasma insulin reduced In KO

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Most relevant to drug metabolism:

Human FMO3

- Primarily expressed in the liver
  - Expression levels 60% of human CYP3A sub-family
- Polymorphic with 26 allelic variants
  - Most result in reduced activity
- Most relevant to both drug metabolism and metabolism of endogenous compounds
- Intermediate substrate specificity compared to FMO1
- Substrates include
  - Tamoxifen (breast cancer treatment)
  - Clozapine (antipsychotic)
  - Nicotine
  - Trimethylamine (dietary compound)
  - Ranitidine (anti-ulcer)

Human FMO3 and Trimethylaminuria

- Trimethylamine –smelly compound found in diet (eggs, legumes, certain meats, fish)
- Excreted from body *via* urine after oxidation to trimethylamine *N*-oxide by FMO3
- Genetic polymorphisms leading to low FMO3 activity result in an inability to secrete trimethylamine *via* urine (trimethylaminuria)
  - Secreted in sweat and urine as parent compound (trimethylaminuria)
  - Leads to odour – "Fish-odour" syndrome
  - First reported as early as 1400 BC.

FMO and Disease

- Polymorphisms in FMO3 have been shown to cause disease
- Fish Odour Syndrome or Trimethylaminuria (TMAU)
  - caused by a rare genetic defect :
    - TMAU is a metabolic disorder whereby abnormal amounts of TMA are present in the urine, sweat, expired air, and other bodily secretions
    - TMA has a powerful smell of rotting fish which causes patients suffering from TMAU to have highly objectionable body odour
  - 2 relatively common polymorphisms, P153L and E305X, result in a large decrease in turnover of Trimethylamine (TMA) to Trimethylamine N-oxide (TMA-NO)
  - TMAU patients excrete up to 80% of their TMA (from diet) as free amine
    - healthy individuals convert 96% of the TMA into TMANO before excreting them
- single M82T mutation in FMO3
  - completely abolished enzyme function leading to TMAU.

Intestinal microbiota metabolism of *L*-carnitine, a nutrient in red meat, promotes atherosclerosis

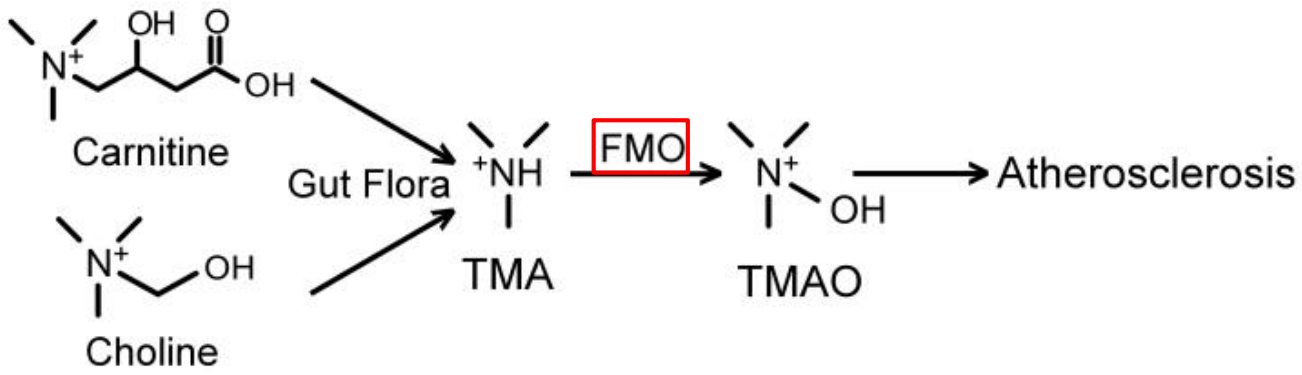
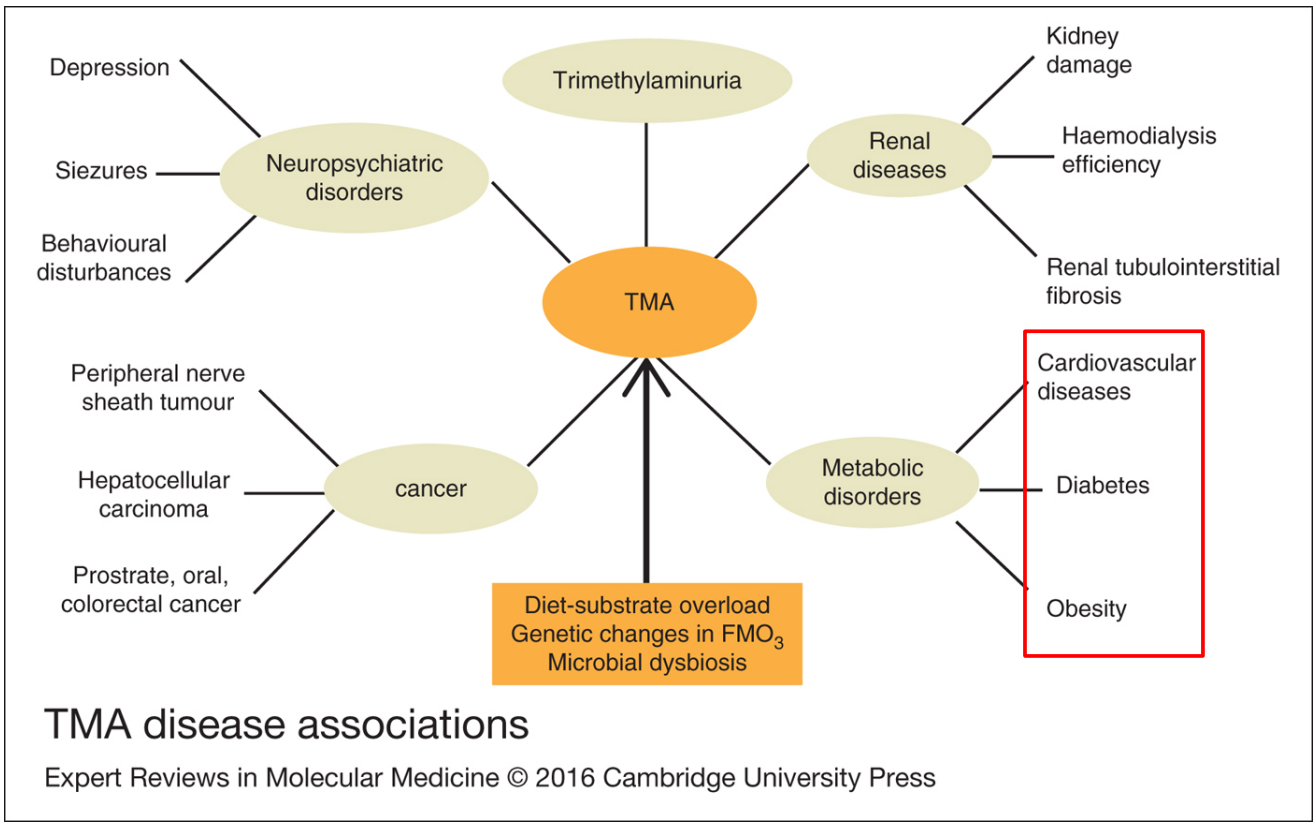


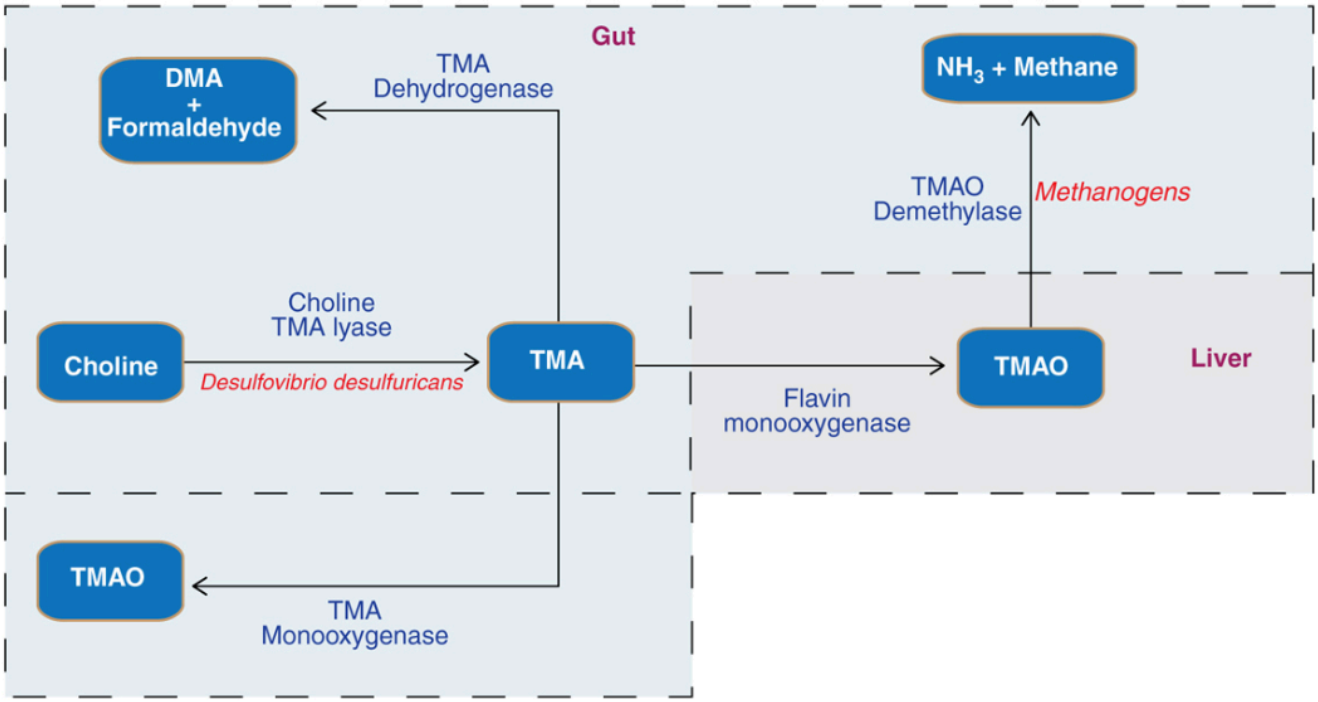
Figure 1. TMAO production from carnitine is a microbiota dependent process in humans
(**a**) Structure of carnitine and scheme of carnitine and choline metabolism to TMAO. L-Carnitine and choline (are both dietary trimethylamines that can be metabolized by microbiota to TMA. TMA is then further oxidized to TMAO by flavin monooxygenases

Koeth et al., *Nat Med*. 2013 May ; 19(5): 576–585. doi:10.1038/nm.3145.



TMA disease associations

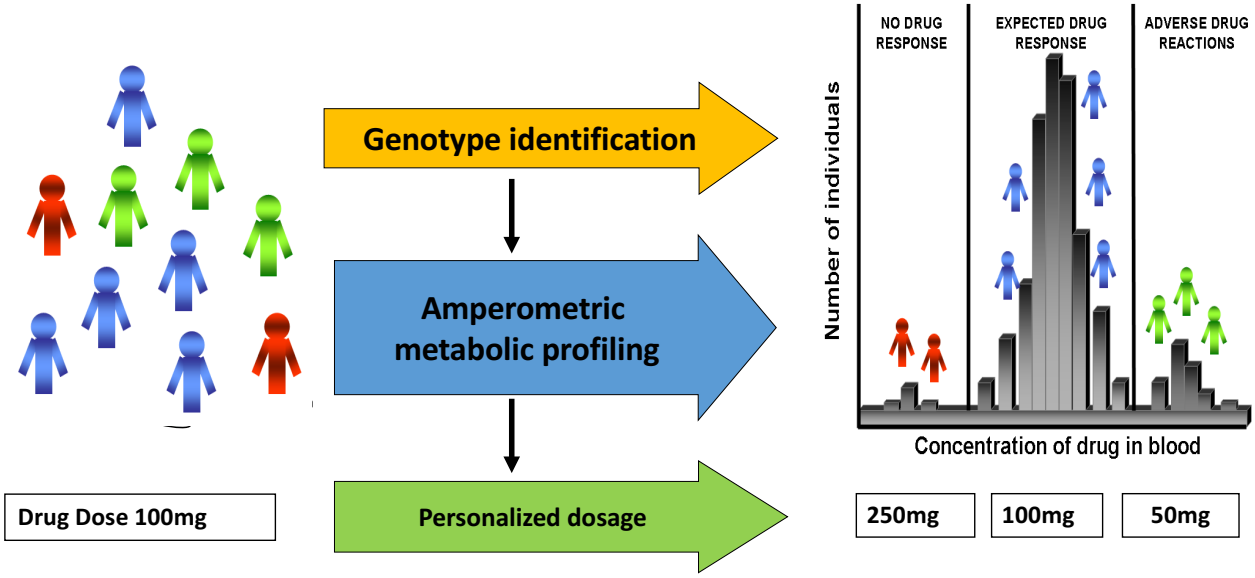
Schematic representation of the origin and fate of human gut TMA, which is synthesised using dietary precursors such as choline, carnitine by gut microbial enzymes



Flavin-containing monooxygenases (FMO):

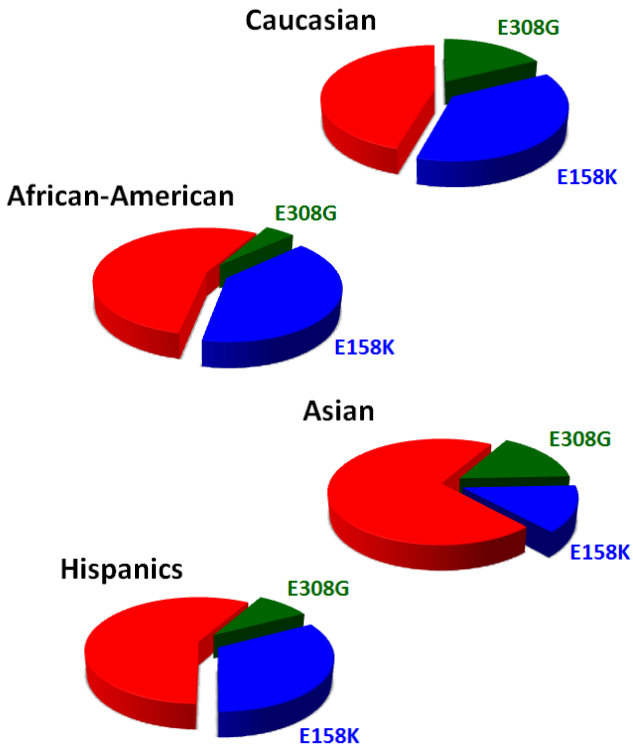
polymorphism

Polymorphic variants amongst us



Ref: Panico et al., Anal. Chem. 2011

hFMO3 common polymorphic variants



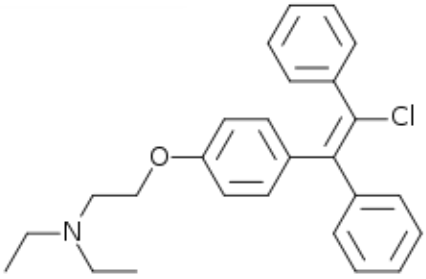
Ref: Gao et al., Gene 2016

hFMO3 common polymorphic variants

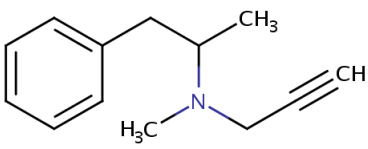
Polymorphic variant	Effect	Reference
V257M	Tyramine (diet component)	Cashman et al., 2000
	5-DPT (Phenothiazine)	Cashman et al.,2000
	Danusertib	Catucci et al., 2013
E158K	Tyramine	Cashman et al., 2000
	Benzydamine	Stormer et al., 2000
	Ranitidine (treatment of ulcers)	Park et al.,2002
	Methimazole (hyperthyroidism drug)	Lattard et al., 2003
	5-DPT	Treacy et al., 1998
	Sulindac sulfide	Hisamuddin et al., 2007
	Amphetamine and Metamphetamine	Cashman et al., 1998
E308G	Ranitidine	Park et al., 2002
	Methimazole	Lattard et al.,2003
	sulindac sulfide	Hisamuddin et al., 2007
	olanzapine (antipsychotic drug)	Söderberg et al., 2013

WORLD ANTI-DOPING AGENCY
play true

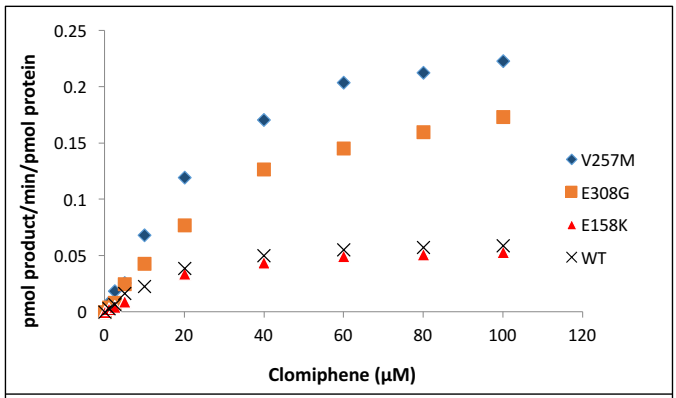
Effect of Polymorphic variants Performance-enhancing drugs

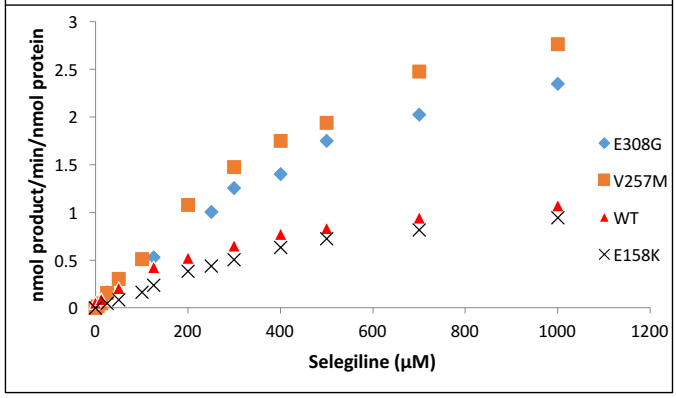


accelerates testosterone secretion

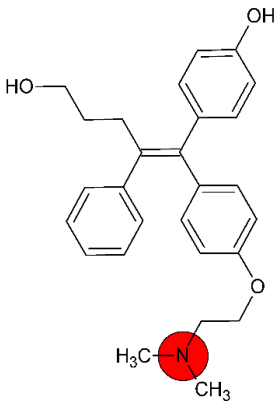


specific stimulants (amphetamine-type)

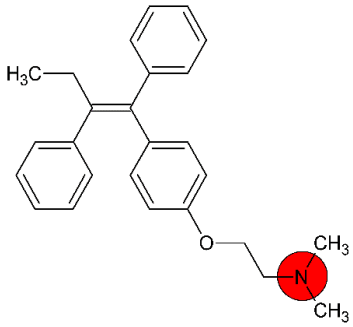




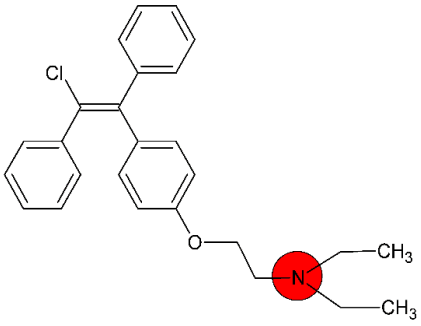
GSK5182

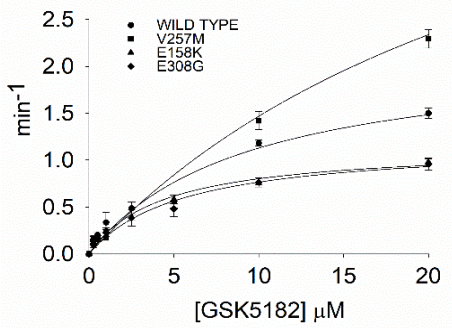


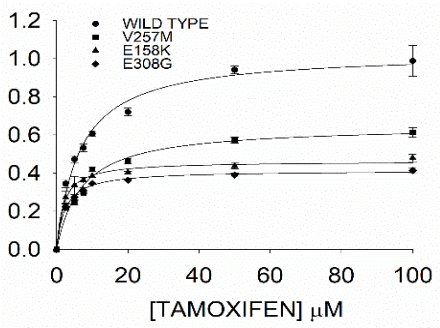
TAMOXIFEN

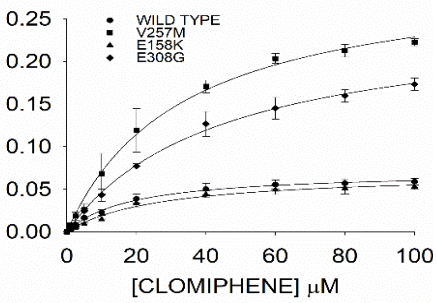


CLOMIPHENE









Diabetes

Breast cancer

Infertility

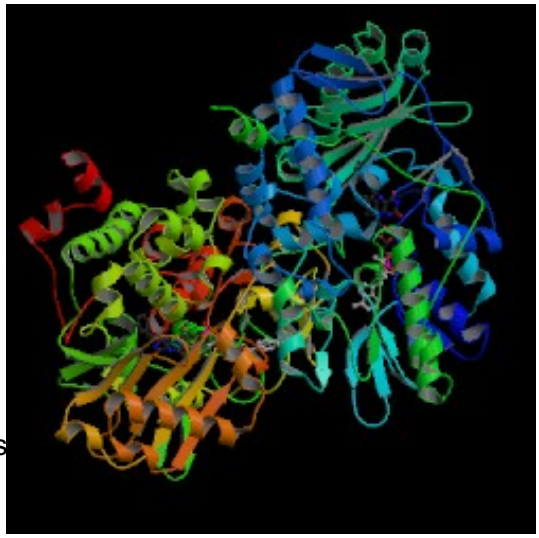
Substrate	Variant	K_m μM	k_{cat} min^{-1}	$CL_{int}(k_{cat}/K_m)$ $min^{-1}\mu M^{-1}$	Relative clearance (% of wild type)
GSK 5182	WT	9.82±1.85	2.22±0.22	0.22±0.048	100.00
	V257M	28.5±6.2*	5.69±0.47*	0.19±0.046	86.36
	E158K	4.57±0.42	1.16±0.05*	0.25±0.026	113.64
	E308G	5.87±0.92	1.20±0.08*	0.20±0.035	90.91
Tamoxifen	WT	6.4±0.7	1.13±0.7	0.18±0.02	100.00
	V257M	8.1±0.5*	0.6±0.02*	0.07±0.005* ↓	38.89
	E158K	1.56±0.03*	0.45±0.01*	0.29±0.01* ↑	161.11
	E308G	2.50±0.3*	0.38±0.02*	0.15±0.02	83.33
Clomiphene	WT	18.3±2.1	0.07±0.002	0.004±0.0005	100.00
	V257M	33.2±3.85*	0.30±0.01*	0.009±0.001* ↑	225.00
	E158K	20.46±3.29	0.06±0.003	0.003±0.0005	75.00
	E308G	44.4±1.67*	0.25±0.01*	0.006±0.0003* ↑	150.00

*$p < 0.05$ versus wild-type hFMO3

Other Phase-1 Drug metabolising enzymes

Monoamine Oxidase (MAO)

- Catalyses oxidation of monoamines.
- Covalently bound FAD co-factor
- Mitochondrial
- Two types in humans: MAO-A and MAO-B.
- **Vital to inactivation of neurotransmitters e.g. seretonin, adrenaline, noradrenaline**.
- Inhibitors used in treatment of depression
- Important in dietary tyramine metabolism
  - Drug - food interaction between MAO inhibitors and tyramine containing foods e.g. Chocolate, cheese, yeast extracts



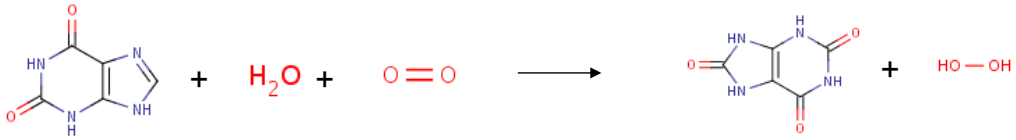
Human MAO-B (pdb: 1GOS)

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \rightarrow R\text{-}C(=O)\text{-}H + NH_3 + H_2O_2$$

Xanthine Oxidase (XO)

- Catalyses oxidation of hypoxanthines to xanthines and then to uric acid.
- Large (270 kDa) protein with 2 FAD, 4 2Fe-2S clusters and 2 molybdenum atoms.
- NADH dependent enzyme
- Uses water as source of oxygen atom
- Drugs metabolised include theophylline (asthma therapy) and 6-mercaptopurine (cancer and autoimmune disease therapy).

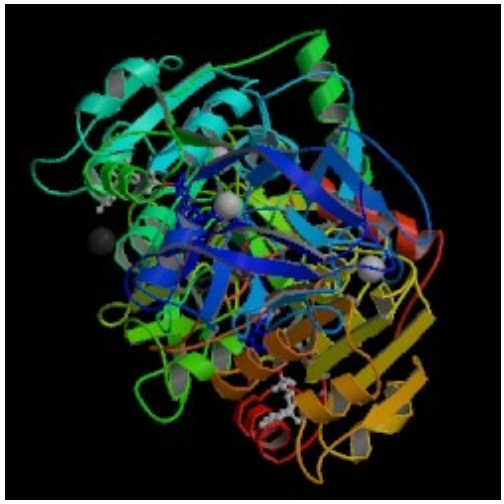
Bovine XO (pdb: 1FIQ)



XANTHINE URIC ACID

Alcohol and aldehyde dehydrogenases

- Multiple forms in humans
  - Smooth ER, Mitochondrial +Cytosolic
  - Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the major enzymes responsible for ethanol metabolism in humans.
  - Both enzymes exhibit genetic polymorphisms among racial populations.
  - About half of the Chinese population lack mitochondrial ALDH2 activity and such a deficiency has been believed to be a negative risk factor for the development of alcoholism.



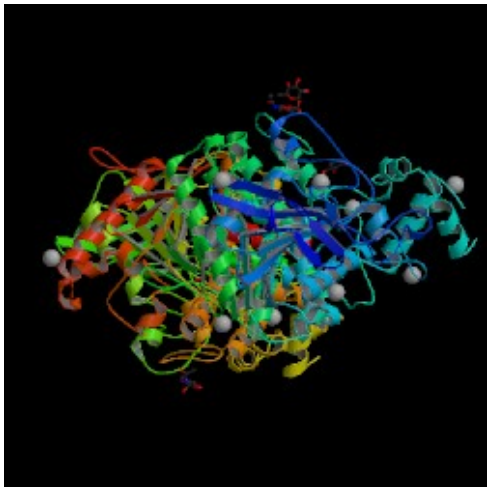
Human alcohol dehydrogenase (pdb: 1HDX)

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

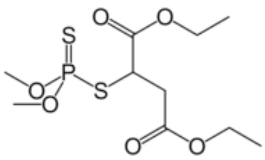
$$CH_3CHO + NAD^+ \longrightarrow CH_3COOH + NADH + H^+$$

Esterases

- Multiple forms in humans
  - Lipases
  - Acetylesterases
  - Thioesterases
  - Amidases
- Responsible for hydrolysis of ester and amide drugs e.g. Aspirin, procaine, lidocaine, peptide drugs
- ß-lactamase in bacteria responsible for penicillin resistance
- Inhibitors of acetylcholinesterase are potent neurotoxins (Chemical warfare) but also used clinically for anaesthesia and to treat glaucoma and Alzheimer's disease and also as pesticides
- Inhibitors e.g Malathion a pesticide
  - Phosphorus atom with two lipophillic groups, a leaving group (halide or thiocyanate) and terminal oxygen.



Mouse acetylcholinesterase (pdb: 1N5M)



35

Malathion

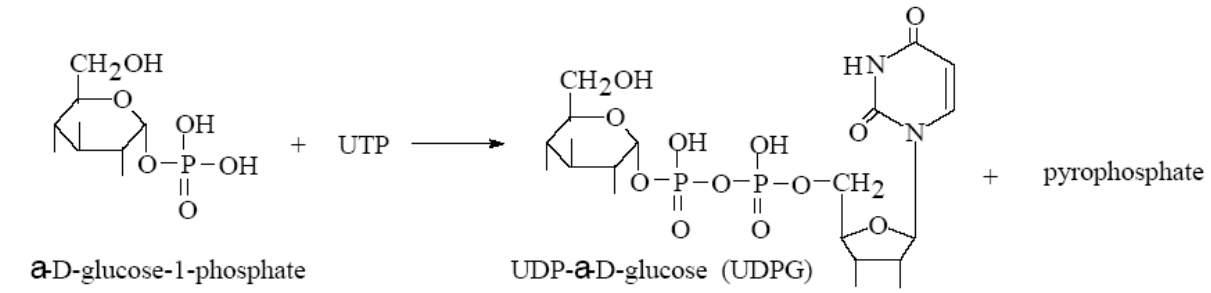
Phase 2
Drug Metabolising Enzymes

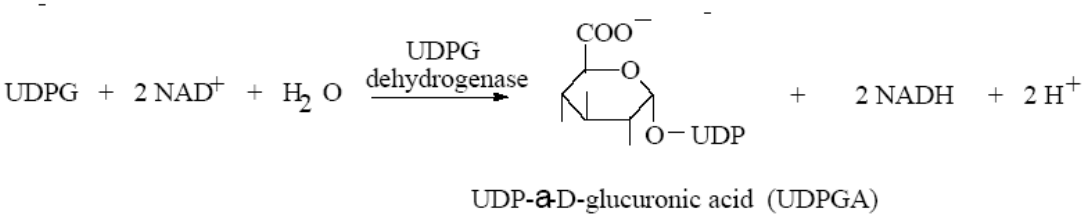
Enzymes involved

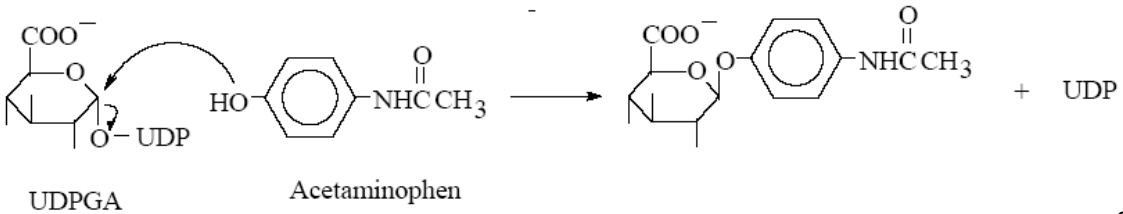
- UDP-glucuronosyltransferases (UGTs)
  - UDP-glucuronic acid conjugated to –OH, -COOH, -NH_2 and –SH groups
  - High capacity
- Sulphotransferases (SULT)
  - Phosphoadenosyl phosphosulphate (PAPS) conjugated to –OH, -NH_2 and –SO_2NH_2
  - Low capacity
- Glutathione S-transferases (GSTs)
  - Glutathione conjugated to electrophiles
  - Low capacity
- N-acetlytransferases (NAT)
  - Acetyl-CoA conjugated to –OH, -NH_2 and –SO_2NH_2
  - Variable capacity
- Methyltransferases
  - S-adenosyl methionine conjugated to catecholamines and phenols

UDP-glucuronosyltransferases (UGTs)

- Most important phase II enzyme
  - Results in very polar metabolite
  - enhances excretion
- Multiple isoforms in humans (21 identified to date)
  - Divided into 2 families UGT1 and UGT2
  - Wide substrate specificity
  - Involved in enterohepatic recirculation
    - Compound conjugated by liver and re-secreted into gut through gall bladder
- Several microsomal forms in human liver
- High capacity enzyme
  - Huge supply of glucuronic acid
- Inducible by phenobarbitone like CYPs
- Polymorphic and polymorphism associated to unconjugated hyperbilirubenemia
- Unusual gene structure as multiple products from one gene
  - Alternative splicing

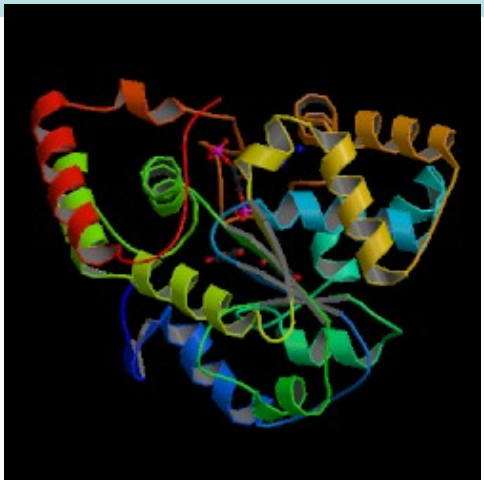




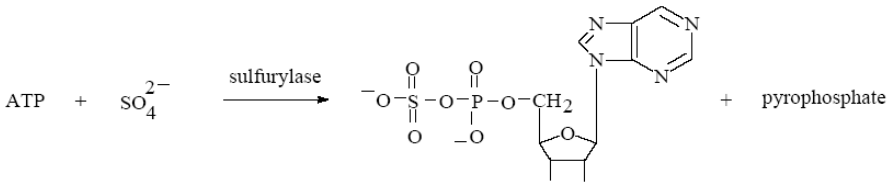


Sulphotransferases (SULTs)

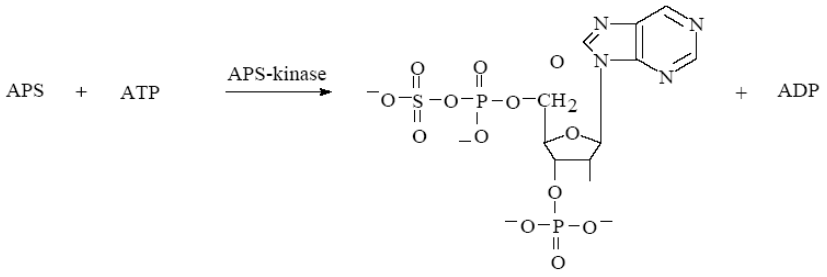
- Found in soluble fraction of liver
- Low capacity enzyme
  - Limited by amount of inorganic sulphate
- Multiple isoforms in humans (10 identified to date)
  - Wide tissue distribution
  - Multiple families
    - SULT1 - phenolic substrates
    - SULT2 - DHEA and steroid substrates
    - SULT4 - Minor family
  - Widest substrate specificity
  - Inhibition by drugs and dietary chemicals
- Conjugate is PAPS
- Energetically highly demanding
  - 2 Molecules of ATP required to make one molecule of PAPS
- Polymorphic enzyme
- Responsible for activation of promutagens such as 1-hydroxymethylpyrene,



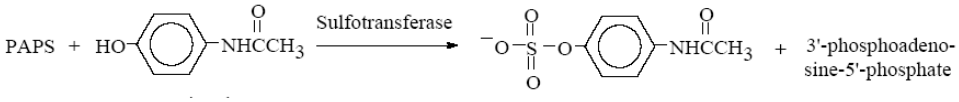
Human SULT1A1 (pdb: 1LS6)



Adenosine-5'-phosphosulfate (APS)



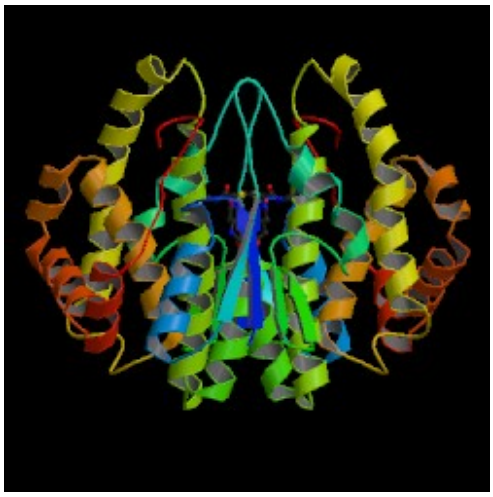
3'-phosphoadenosine-5'-phosphosulfate (PAPS)



Acetaminophen

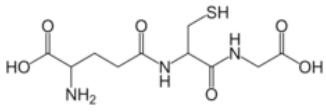
Glutathione S-transferases (GSTs)

- Two supergene families
  - Cytosolic GSTs – 16 genes
  - Membrane GSTs – 6 gene
  - Broad and overlapping substrate specificities
- Low capacity enzyme
  - Limited by amount of glutathione
- Conjugate is glutathione (Glu-Gly-Cys)
- Glutathione (GSH) Conjugated to activated epoxides and organic halides
- Very important detoxification mechanism against reactive epoxides
  - Biological hoover
  - Cellular protection vs. oxidative damage
- Conjugated compound further metabolised in kidney by γ-glutamyltransferase, cysteinyl glycinase and N-acetyl transferase

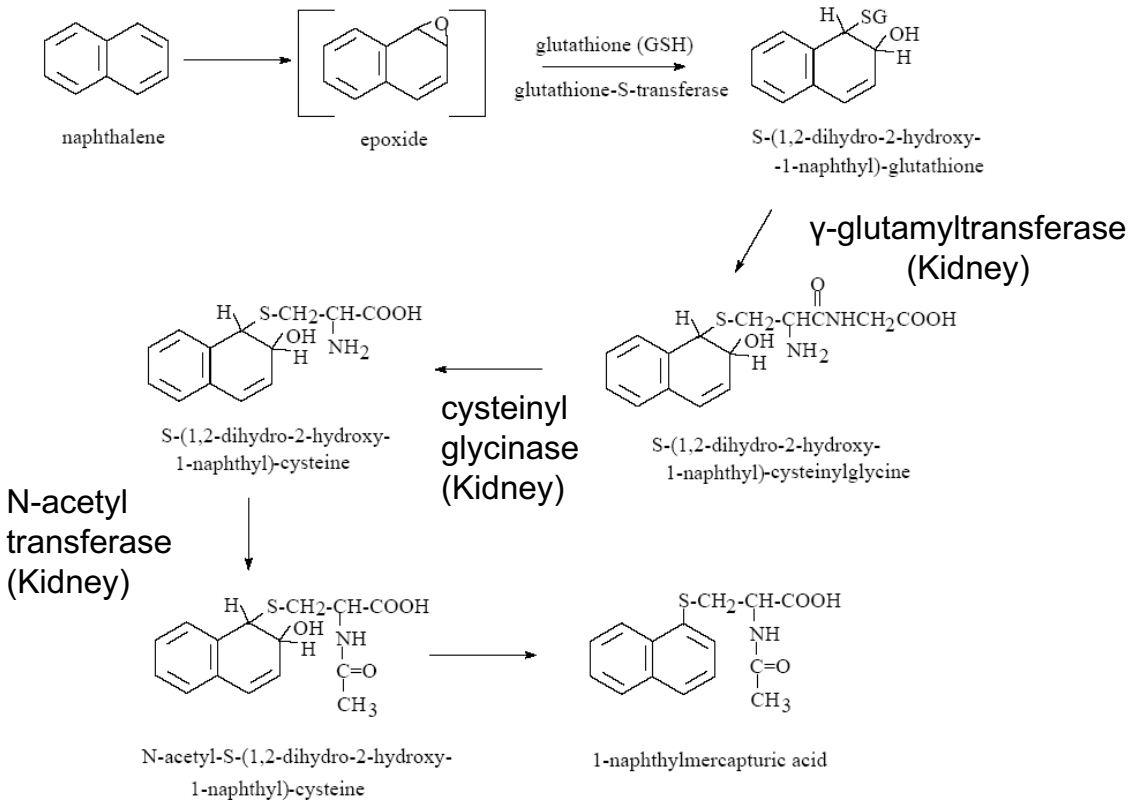


Human GSTM2-2

(pdb: 1HNA)



Glutathione

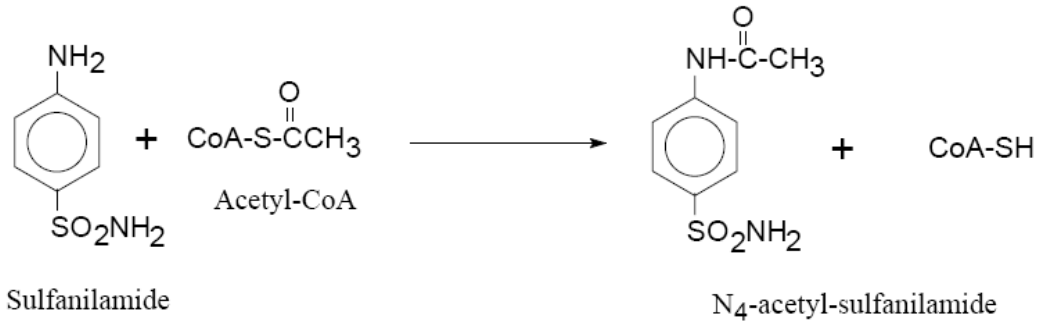


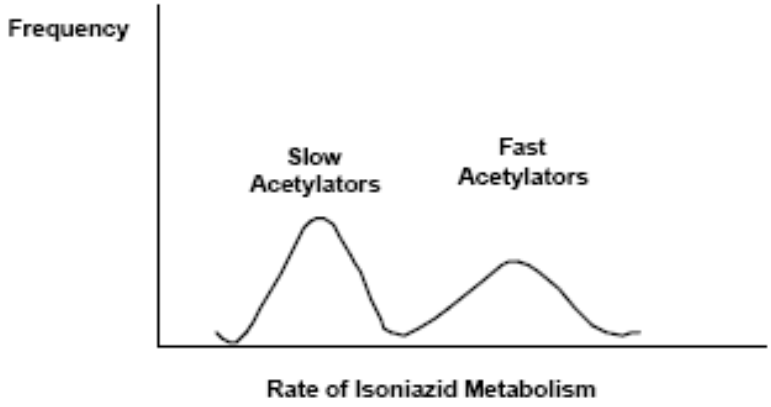
N-acetyltransferases (NATs)

- Acetly-CoA cojugate is used to transfer Acetyl group to –OH, -NH_2 and –SO_2NH_2
- Two enzymes located in soluble fraction of liver but also in other tissues
  - NAT1 and NAT2 both polymorphic
- Classic example of polymorphism (NAT2)
  - Determined 30 years ago
  - First noted because of marked bimodal distribution in antituberculosis drug isoniazid
  - Rapid acetylators
    - Isoniazid $T_{1/2}$ about 1 hr
    - 50 % Caucasians and 90 % Asians
    - More prone to liver injury with Isoniazid
  - Slow acetylators
    - Isoniazid $T_{1/2}$ about 3.5 hr – nerve ending damage with Isoniazid
    - Several mutations in NAT2 gene can give non-functional enzyme
    - Autosomal recessive
  - Other drugs affected e.g. Dapsone, procainamide
  - NAT2 polymorphism associated to bladder cancer risk

Human NAT1

(pdb: 2IJA)

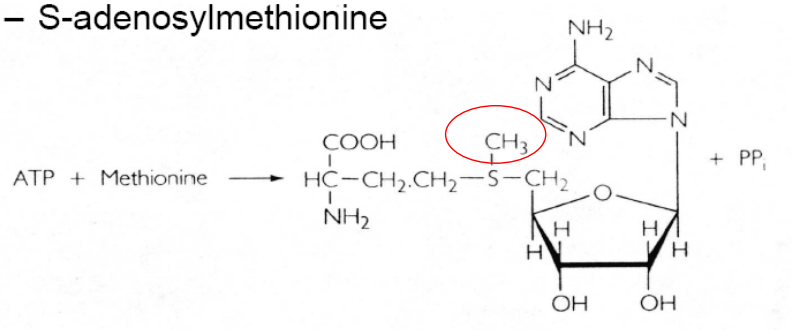




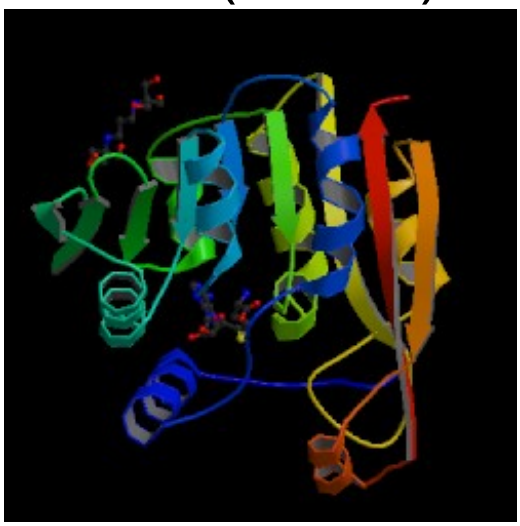
Methyltransferases (MTs)

- Methyltransferases conjugate methyl groups to –OH and NH_2
- Can potentially reverse demethylation by Phase I enzymes
- Numerous forms in human
  - DNA methylation
  - Catechol amine methylation
  - Thiopurine methylation (TPMTs)
- Conjugate is S-adenosylmethionine (SAM)
  - Generation of SAM is energetically unfavourable due to ATP requirement.

– S-adenosylmethionine



Thiopurine S-methyltransferase (TPMT)



Human thiopurine S-methyltransferase (pdb: 2BZG)

- Thiopurines used in cancer therapy
  - Azathioprine
  - 6-mercaptopurine
- Toxicity can be serious
  - Bone marrow suppression
  - Liver toxicity
- Detoxified by TPMT which is polymorphic
  - Inherited (autosomal co-dominant)
  - 4 mutant alleles identified
    - TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C
  - In most populations
    - 90% have normal activity (2 normal alleles)
    - 10% have intermediate activity (1 mutant allele)
    - 0.33 % are deficient (2 mutant alleles)
  - TPMT deficient individuals accumulate toxic thioguinine metabolites of azathioprine and 6-mercaptopurine
    - In these individuals dose should be lowered or drug avoided

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