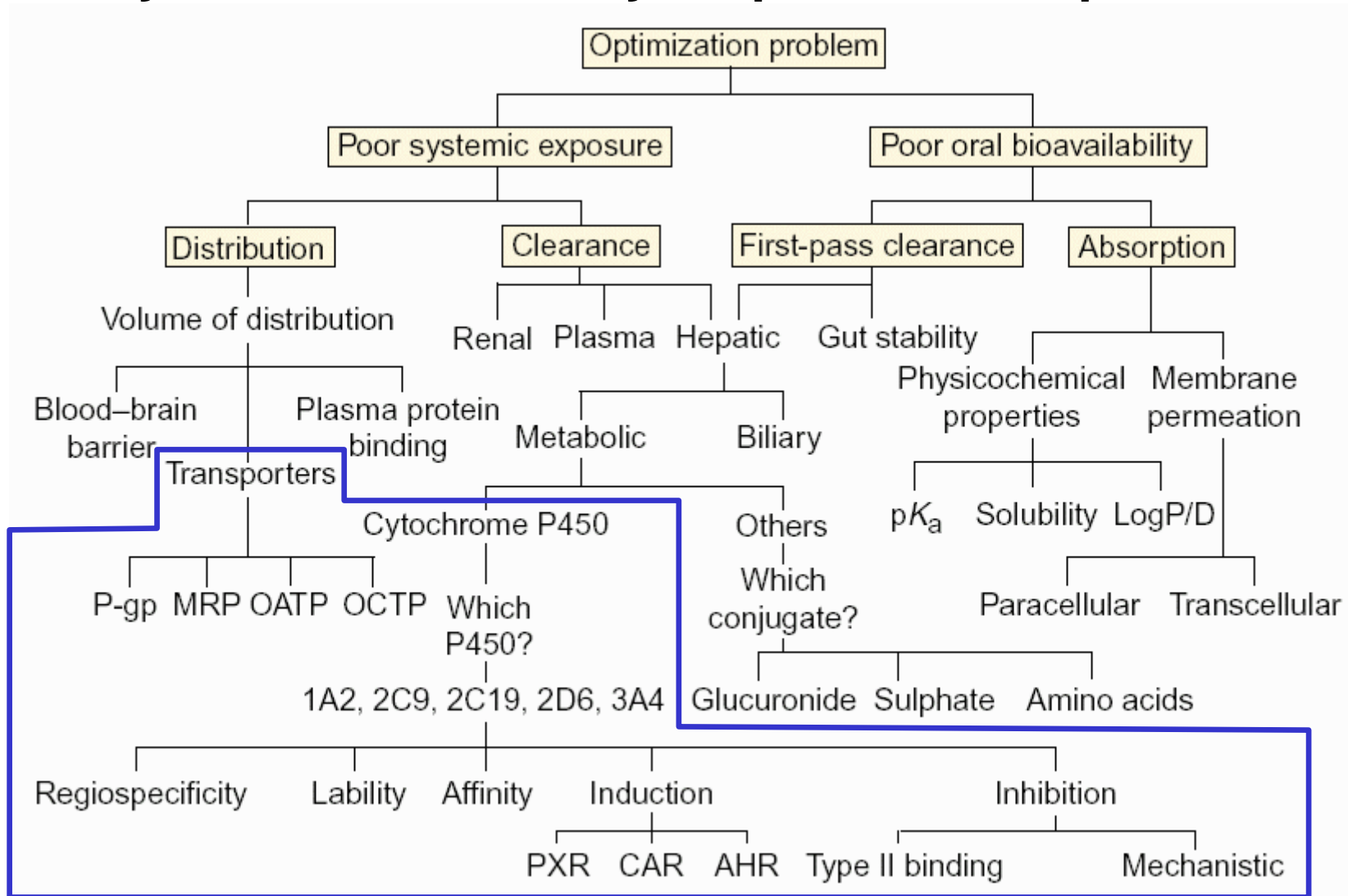


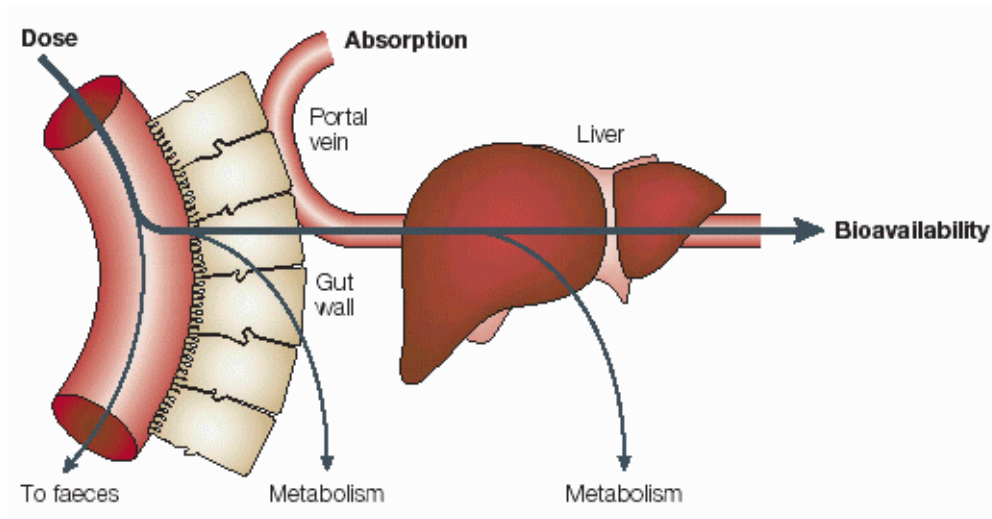
# Cytochrom P450, Polymorphism, Transporters



*Drug Discovery Today*

# Absorption and Metabolism

Nutrients as well as xenobiotics enter the blood circulation via the portal vein from the small intestine and reach the liver. Here, a variety of biochemical conversions of all substances is carried out.



# Enzyme Systems That Metabolize Xenobiotics

## Enzymatic System

## Main Site of Location

Cytochrome P450

Endoplasmatic Reticulum (5, 8)

FAD-Monooxygenase

Endoplasmatic Reticulum

Monoamine Oxidase

Mitochondria (9)

Alcohol/Aldehyde Dehydrogenase

Cytosol

Epoxide Hydrolase

Endoplasmatic Reticulum

Gluthathione S-Transferase

Cytosol

Sulfotransferase

Cytosol

Acetyltransferase

Cytosol

Methyltransferase

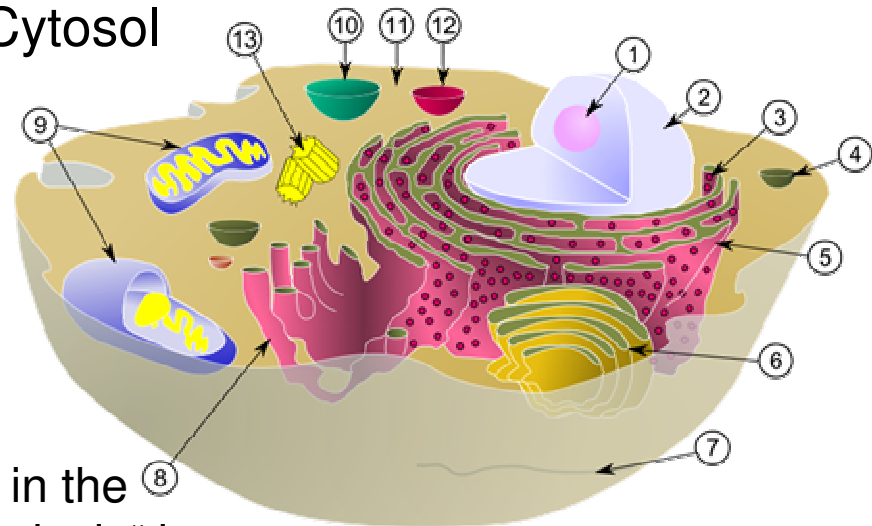
Cytosol

Oxidoreductase

Cytosol

Xanthine Oxidase

Cytosol



Lit: C. Ioannides „Cytochromes P450 in the Metabolism and Bioactivation of Chemicals“ in

Picture: Wikipedia

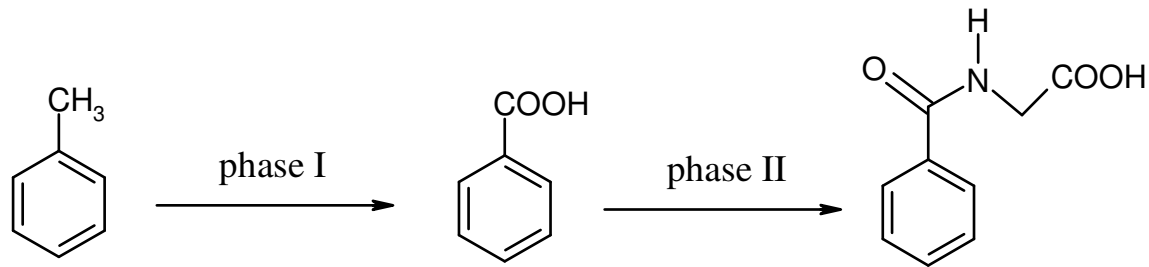
*Chemistry and Molecular Aspects of Drug Design and Action,*

Eds. E.A. Rekká, P.N. Kourounakis, CRC Press, Boca Raton, FL, 2008.

# Cytochrome P450 Metabolism (I)

First reactions: *First pass effect*

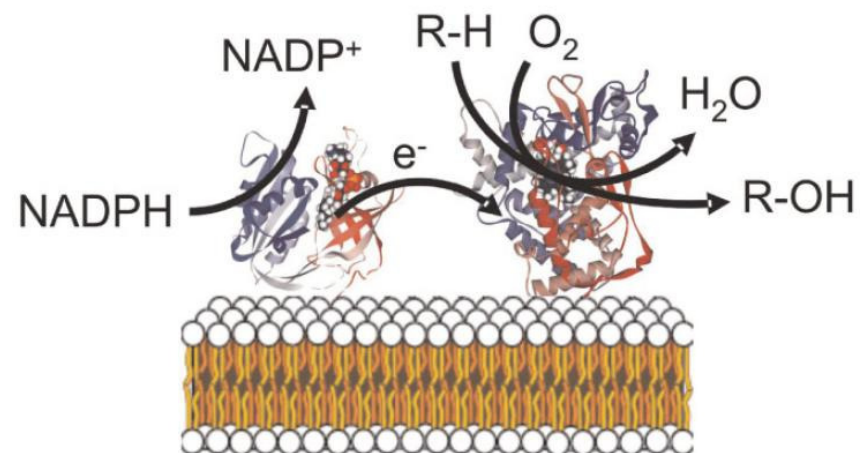
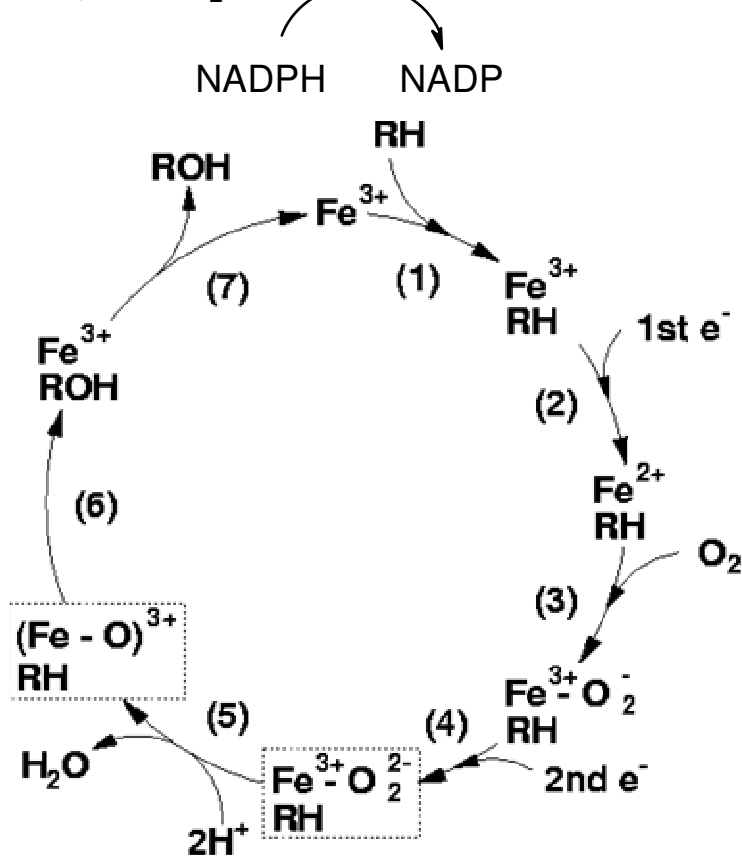
predominately lipophilic or heavy (MW >500) compounds are metabolized excessively, whereby they become more hydrophilic and thus easier to excret.



For the reactions comprising Phase I mainly the group of cytochrome P450 enzymes (CYP) is responsible. Usually substances are oxidized (formal addition of oxygen; redox reaction)

# Cytochrome P450 Metabolisms (II)

This mono-oxygenation of the substrates occurs in a catalytic cycle mediated by a hemoglobin-iron (Fe)

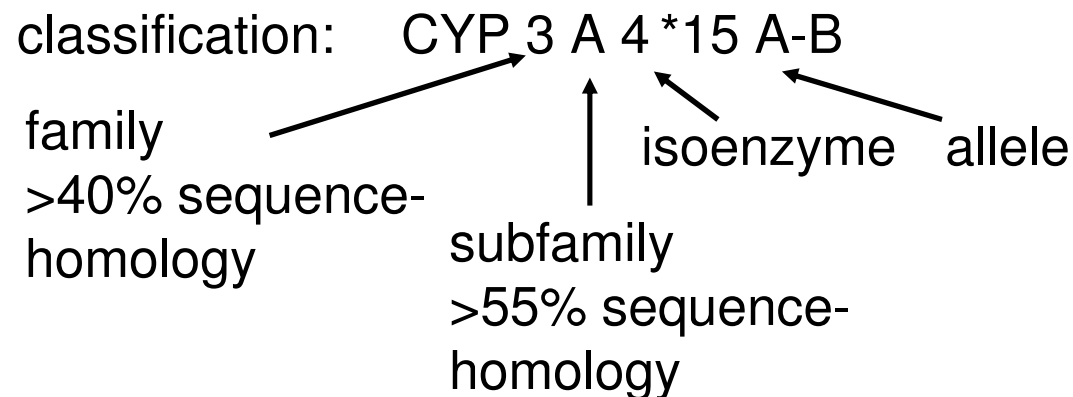


The electrons are provided by the cytochrome reductase

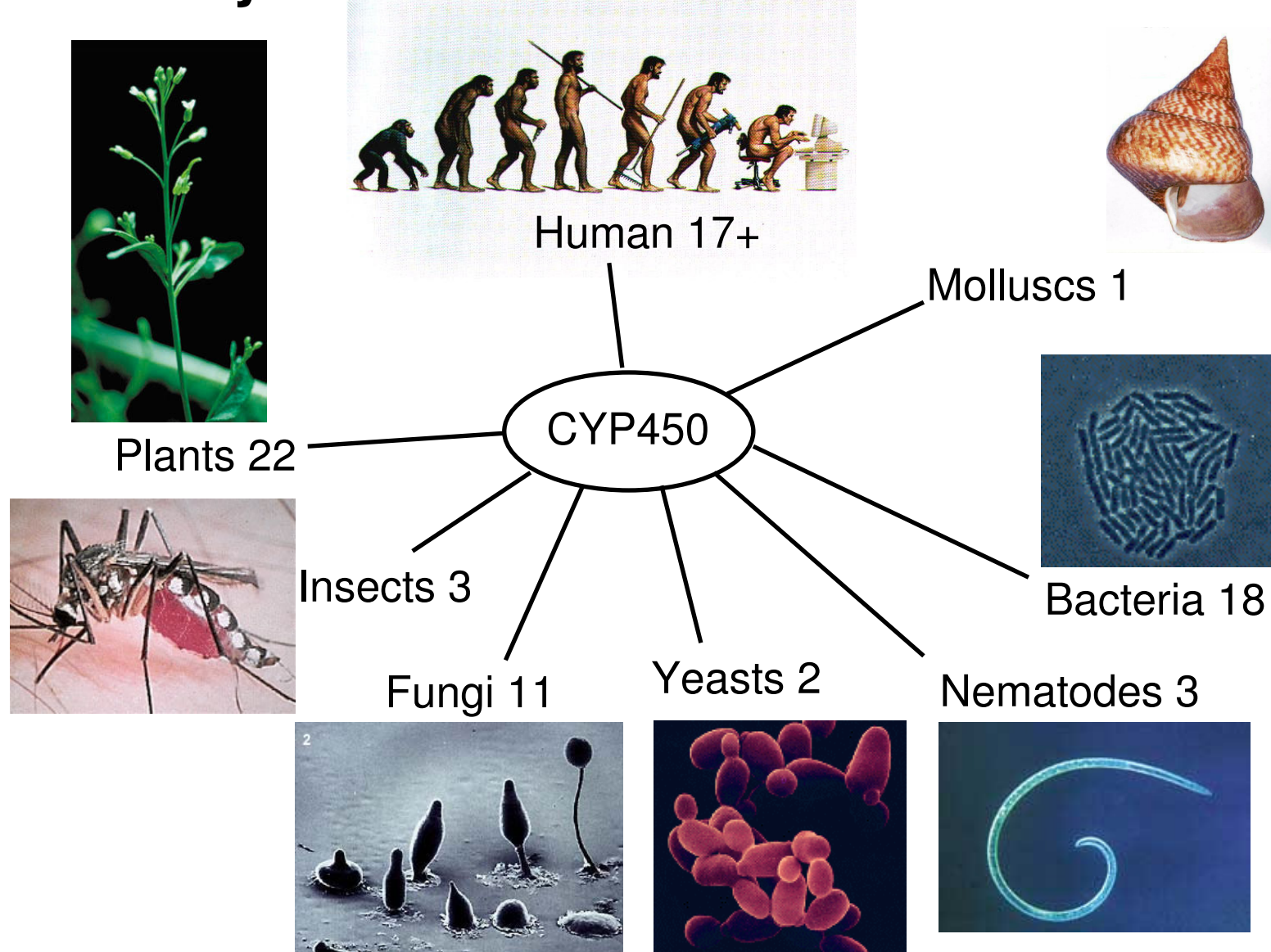
# Cytochrome P450 Metabolismus (III)

The cytochrome enzymes that account for the metabolism are predominately mono-oxygenases that evolved from enzymes for steroid and fatty acid synthesis.

In human 17 CYP-families containing about 50 isoforms have been characterized so far



# Cytochrome P450 Gene families



# Human cytochrome P450 family

From the super-family of the cytochromes, the following families have been found in human:

CYP 1-5, 7, 8, 11, 17, 19, 21, 24, 26, 27, 39, 46, 51

CYP 1, 2A, 2B, 2C, 2D, 2E, 3      metabolisms of  
xenobiotics

CYP 2G1, 7, 8B1, 11, 17, 19, 21, 27A1, 46, 51      steroid  
metabolisms

CYP 2J2, 4, 5, 8A1      fatty acids metabolisms

CYP 24 (vitamine D), 26 (retinoic acid), 27B1 (vitamine D), ...  
synthesis



# Cytochrome P450 Enzymes (I)

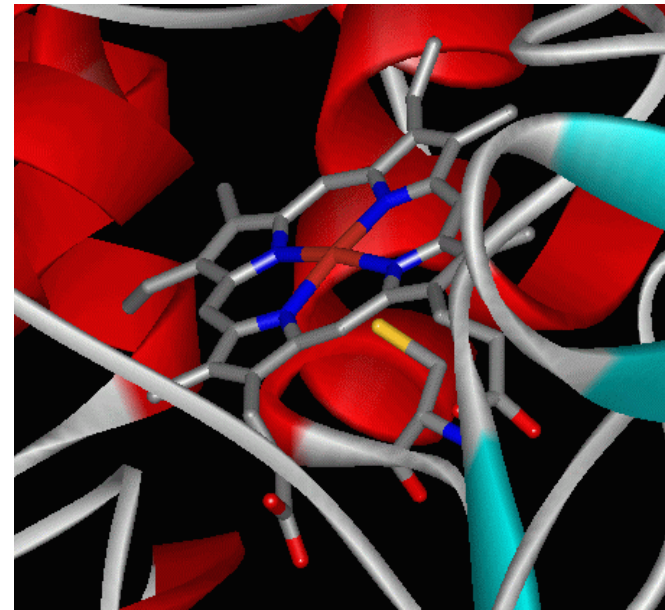
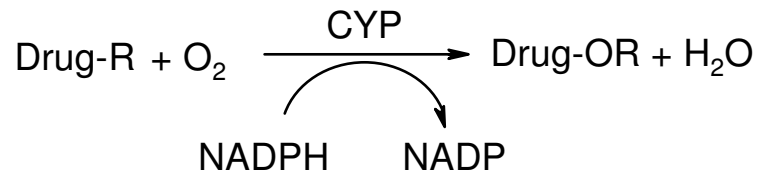
flavin monooxygenase isoenzyme

alcohol dehydrogenase

aldehyde oxidase

monoamine dehydrogenase (MAO)

} Further phase I enzymes,  
where the redox activity  
is mediated by an iron  
porphyrin in the active  
site

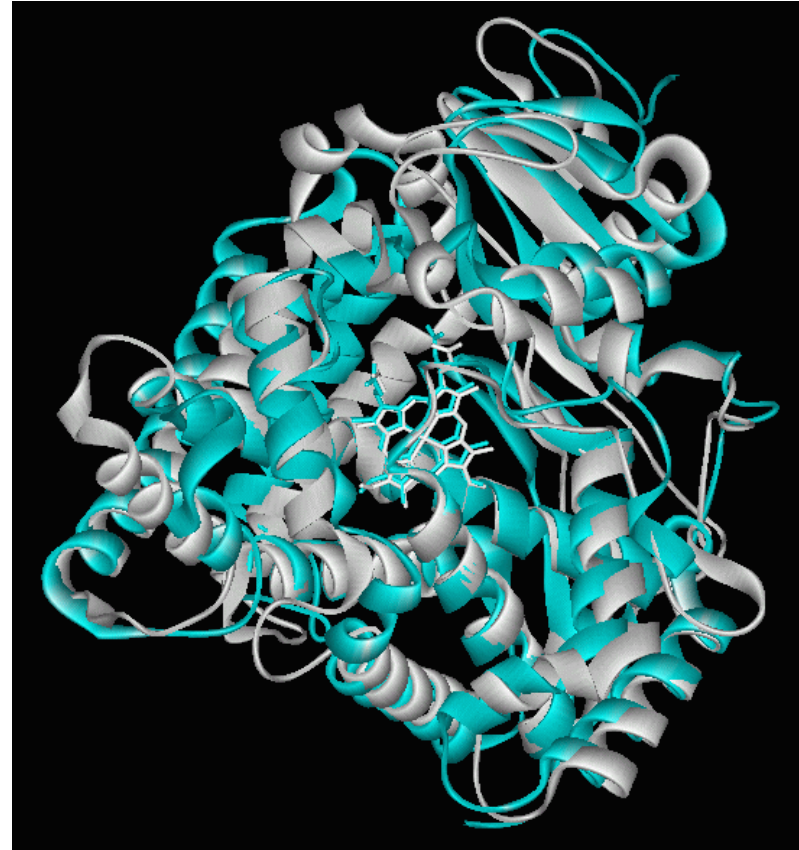


# Cytochrome P450 Enzymes (II)

Despite the low sequence identity of CYPs from different species, the overall tertiary structure is conserved, esp. in the active center. In the outer regions, however, strong deviations occur.

Nevertheless, substrate and product specificity is governed by mutations.

Superposition of human *h*CYP 2C9 (1OG5.pdb) and CYP 450 BM3 (2BMH.pdb) *Bacillus megaterium*

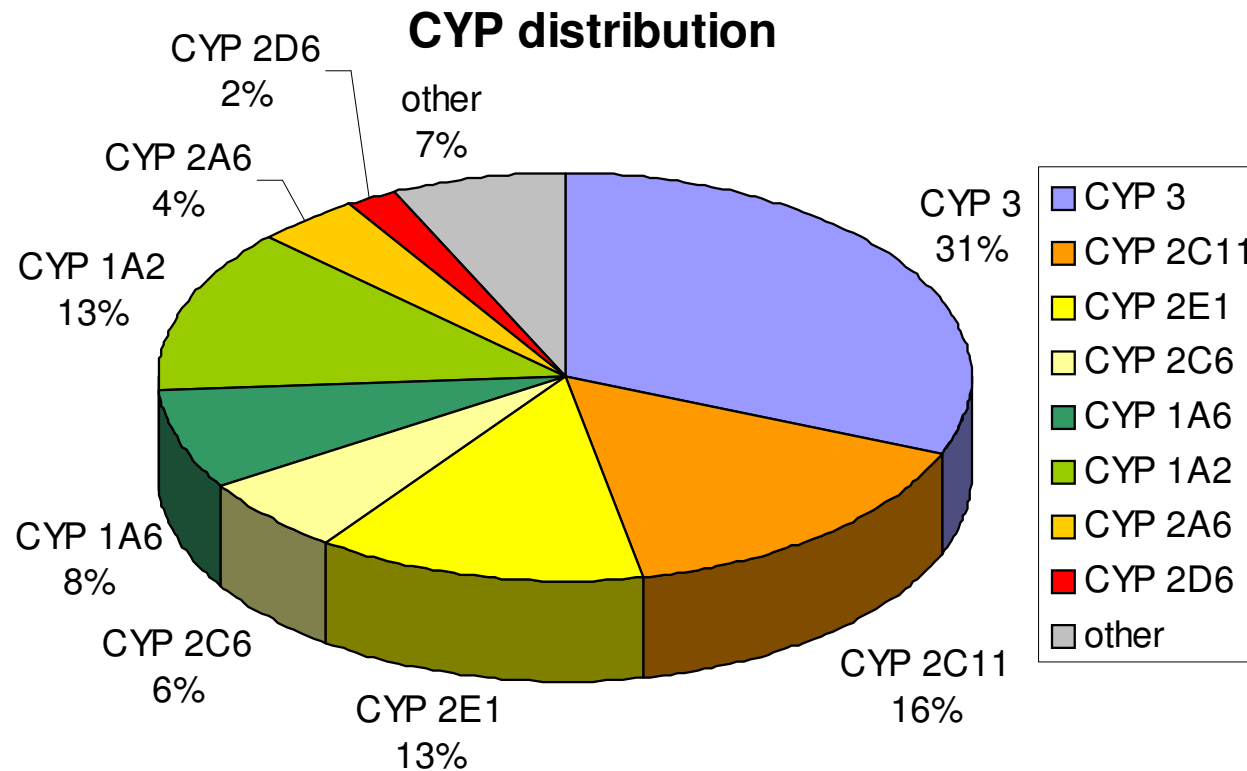


In contrast to bacterial CYPs, the microsomal *mammalian* CYPs possess an additional transmembrane helix that serves as an anchor in the membrane.

# Cytochrome P450 Enzymes (III)

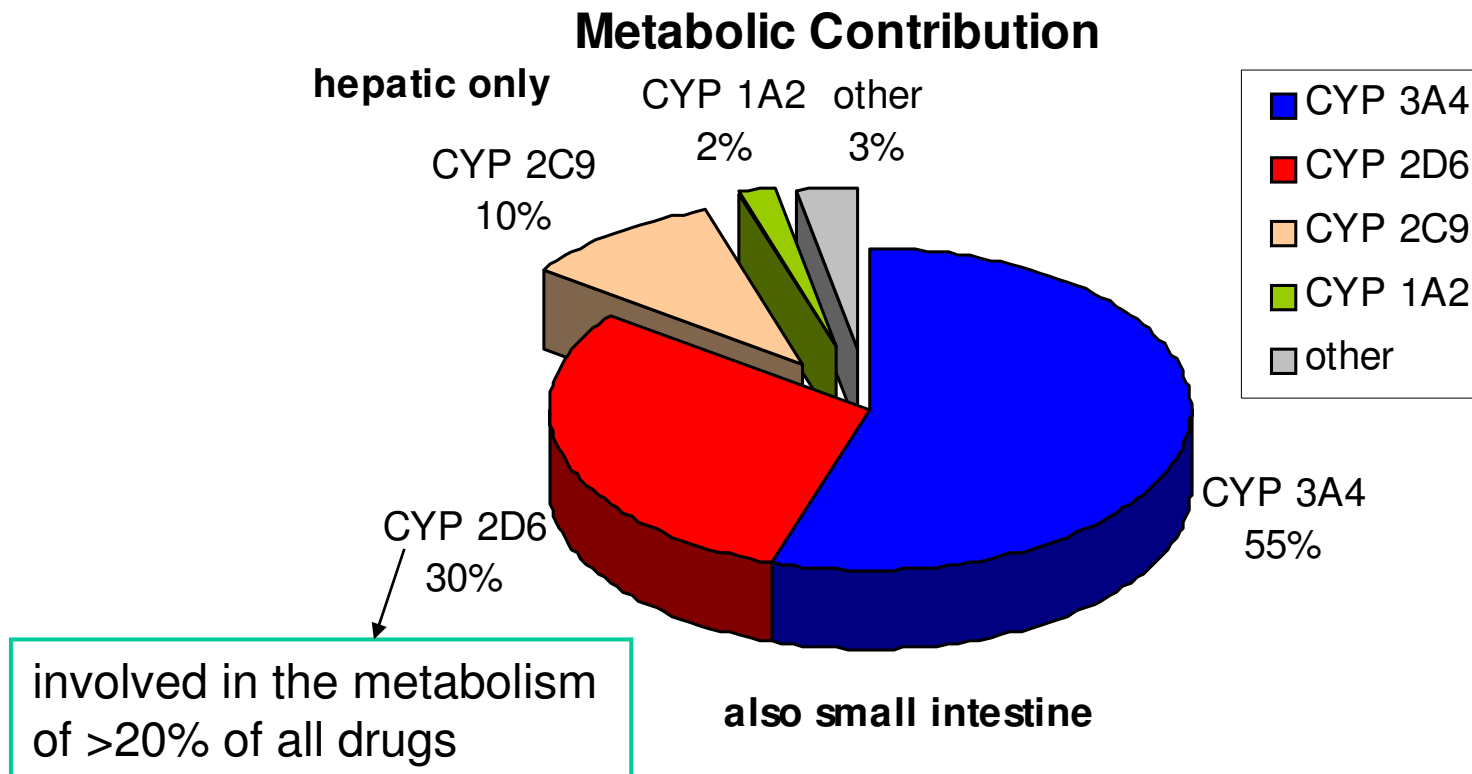
The prevailing amount of CYPs is present in the liver, however, certain CYPs are also found in cells of the intestine wall, the lung, and the brain.

the *mammalian* CYPs are bound to the endoplasmatic reticulum and thus membrane bound.



# Cytochrome P450 Enzymes (IV)

The metabolism of endogenous substances (xenobiotics) is carried out predominately by CYP 3A4, CYP 2D6, and CYP 2C9.



# Substrate specificity of CYPs (I)

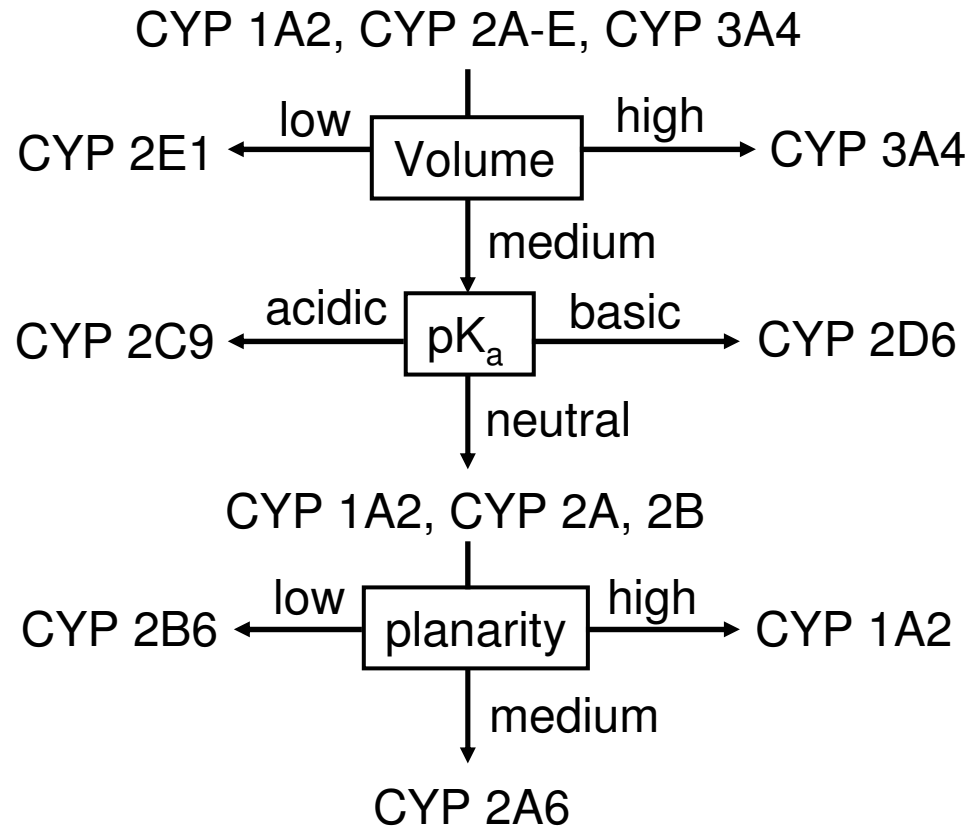
Specific substrates of certain human CYPs

CYP 1A2	verapamil, imipramine, amitryptiline, caffeine (arylamine <i>N</i> -oxidation)
CYP 2A6	nicotine
CYP 2B6	cyclophosphamid
CYP 2C9	diclofenac, naproxen, piroxicam, warfarin
CYP 2C19	diazepam, omeprazole, propranolol
CYP 2D6	amitryptiline, captopril, codeine, mianserin, chlorpromazine
CYP 2E1	dapsone, ethanol, halothane, paracetamol
CYP 3A4	alprazolam, cisapride, terfenadine, ...

see <http://medicine.iupui.edu/flockhart/>

# Substrate specificity of CYPs (II)

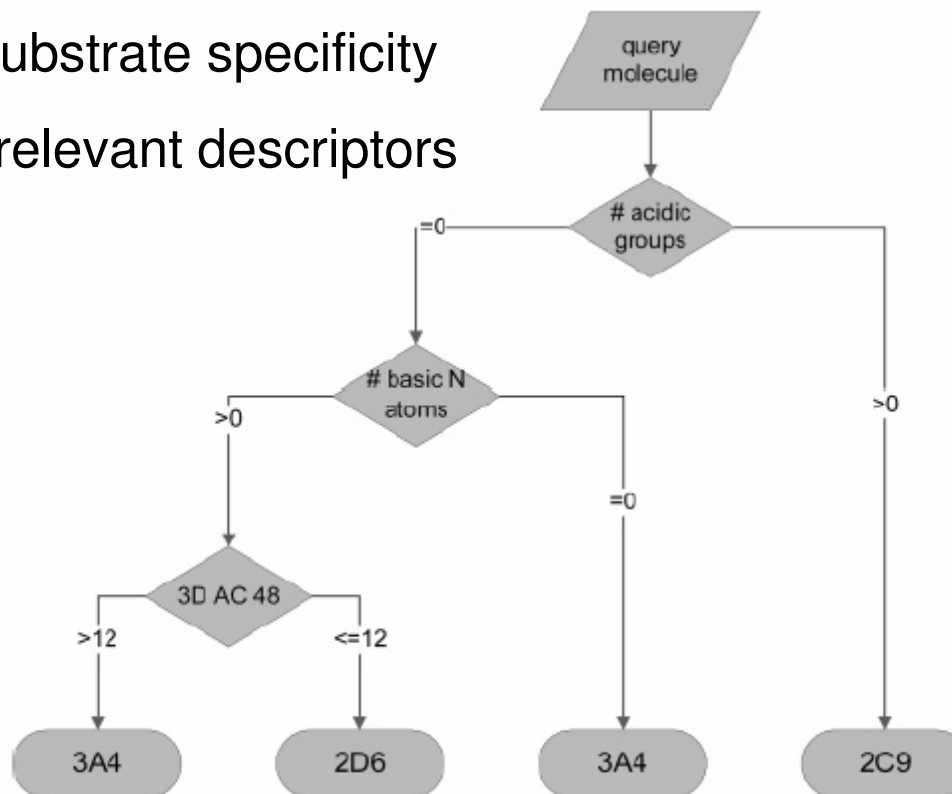
Decision tree for human P450 substrates



Lit: D.F.V. Lewis *Biochem. Pharmacol.* **60** (2000) 293

# Prediction Models for Cytochrome P450 Metabolism (I)

Decision Tree for substrate specificity  
→ Identification of relevant descriptors



Lit. L.Terfloth et al. *J.Chem.Inf.Model.* **47** (2007) 1688-1701.

Major source of experimental data:  
S.Rendic *Drug Metabol.Rev.* **34** (2002) 83-448.

# Prediction Models for Cytochrome P450 Metabolism (II)

Qualitative prediction of metabolism for specific CYPs:

Binary classification into substrates / non-substrates

inhibitors / non-inhibitors

Problems: partial overlap of inhibitors and non-substrates

variability of data sets (how much of a non-substrate is metabolized?), unbalanced data sets (one class dominating)

Used machine learning algorithms: decision trees, neural networks, support vector machines, *k*-nearest neighbor, naïve Bayes

Lit. C.W.Yap & Y.Z.Chen *J.Chem.Inf.Model.* **45** (2005) 982-992.

J.M.Kriegl et al. *QSAR Comb.Sci.* **24** (2005) 491-502.

P.S.Bazeley et al. *J.Chem.Inf.Model.* **46** (2006) 2698-2708.

B.F.Jensen et al. *J.Med.Chem.* **50** (2007) 501-511.

M.Carbon-Mangels & M.C.Hutter. *J.Mol.Inf.* **30** (2011) 885-895.



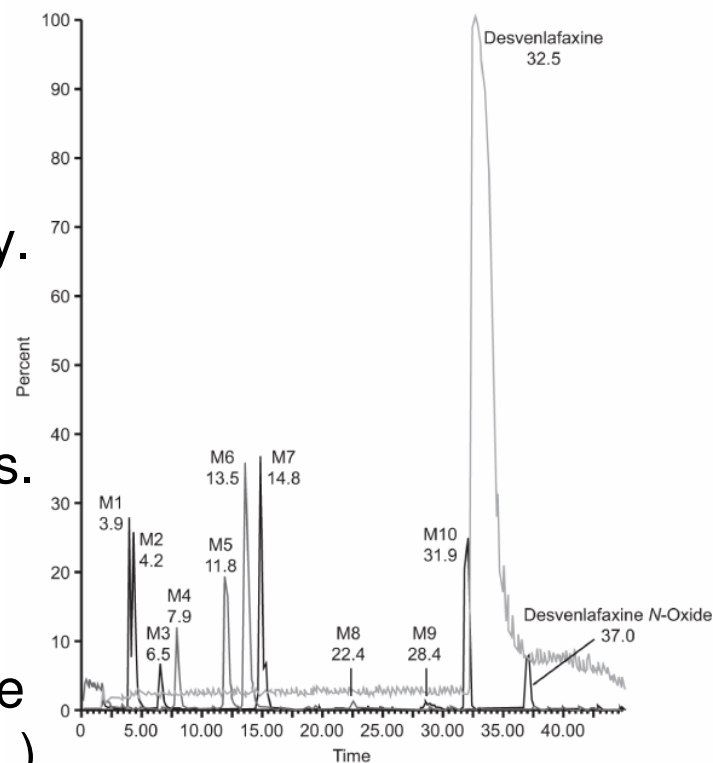
# Cytochrome P450 Metabolism (IV)

During pre-clinical development it is of importance to characterize also the metabolic products of drugs since these might be toxic themselves.

Experimentally, the according (human) CYP-enzymes are expressed in *E. coli*, and the conversion is monitored by gas chromatography and mass spectroscopy.

This allows the selective determination of metabolites by single cytochrome P450 enzymes and their genetic variants.

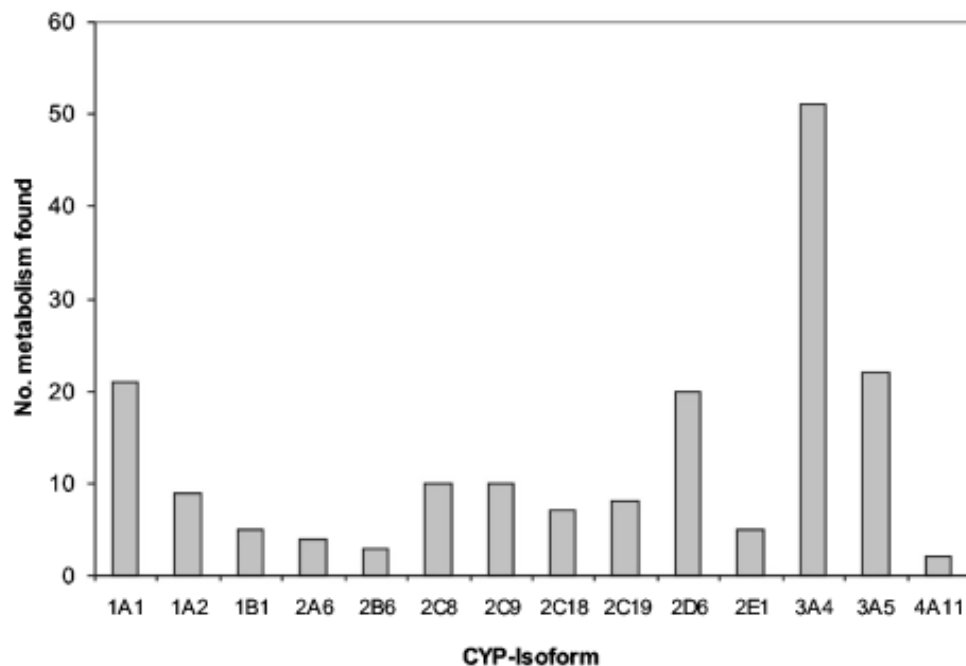
The results are used to compared with corresponding *in vivo* results from animals in order to chose the appropriate animal model (mouse, dog, guinea pig,...).



Lit. K.Schroer, M.Kittelmann, S.Lütz *Biotechnol. & Bioengin.* **106** (2010) 699.

# Cytochrome P450 Metabolism (V)

The most prominent CYP-Enzymes during pre-clinical development used for generating metabolites



Number of metabolism events found (60 compounds tested)

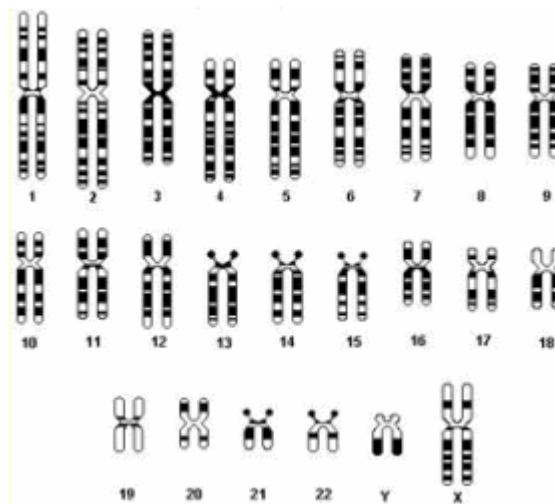
Lit. K.Schroer, M.Kittelmann, S.Lütz *Biotechnol. & Bioengin.* **106** (2010) 699.

# Cytochrome P450 polymorphism

„Every human differs (more or less)“

The phenotype can be distinguished by the actual activity or the amount of the expressed CYP enzyme.

The genotype, however, is determined by the individual DNA sequence. Human: two sets of chromosomes (diploid)



That mean: The same genotype enables different phenotypes

Depending on the metabolic activity, three major categories of metabolizers are separated: *extensive metabolizer* (normal), *poor metabolizer*, and *ultra-rapid metabolizer* (increased metabolism of xenobiotics)

Lit: K. Nagata et al. *Drug Metabol. Pharmacokin* **3** (2002) 167

# Single Nucleotide Polymorphism (SNP)

SNPs are differences of single bases in the DNA that can be observed between individuals in a population.

Alleles occurring in at least 1% of the population are defined as polymorphism. E.g. these genotypes are ordinary.

Conversely, differences in the genome that occur in less than 1% are referred to as mutations.

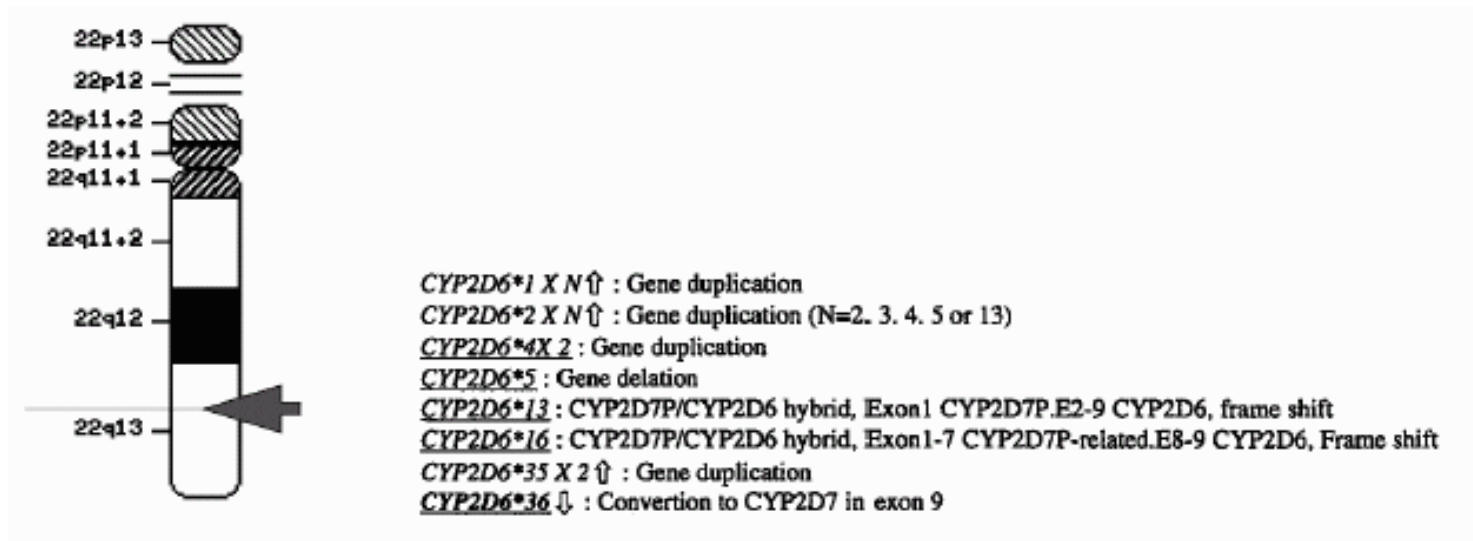
In the case of rare inherited diseases, typically mutations in the coding region of DNA sequences are observed.

Lit: A.D. Rose *Nature* **405** (2000) 857.

# CYP 2D6 Polymorphism (I)

The polymorphism of CYP 2D6 (debrisoquine 4-hydroxylase) has been studied in great detail, as metabolic differences have first been described for debrisoquine and sparteine (antipsychotics)

See: D.B.Goldstein et al. *Nature Rev. Genetics* 4 (2003) 937.



localized on chromosome 22

Of the 75 alleles, 26 exprime CYP2D6 proteines

see <http://www.imm.ki.se/CYPalleles/cyp2d6.htm>

## CYP 2D6 Polymorphisms (II)

Designation	Characteristic mutation(s)	Enzyme activity	Allelic frequency (%)
<i>CYP2D6*1</i>	Wild type	Normal	
<i>CYP2D6*2</i>	G <sub>1749</sub> C, C <sub>2938</sub> T, G <sub>4268</sub> C substitutions	Normal	30
<i>CYP2D6*3</i>	A <sub>2637</sub> deletion	Deficient	2
<i>CYP2D6*4</i>	G <sub>1934</sub> A substitution	Deficient	22
<i>CYP2D6*5</i>	Gene deletion	Deficient	2
<i>CYP2D6*6</i>	T <sub>1795</sub> deletion	Deficient	2
<i>CYP2D6*7</i>	A <sub>3023</sub> C substitution	Deficient	0·1
<i>CYP2D6*8</i>	G <sub>1846</sub> T substitution	Deficient	0·1
<i>CYP2D6*9</i>	(A <sub>2701</sub> -A <sub>2703</sub> ) or (G <sub>2702</sub> -A <sub>2704</sub> ) deletion	Decreased	1·5
<i>CYP2D6*10</i>	C <sub>188</sub> T, G <sub>1749</sub> C, G <sub>4268</sub> C substitutions	Decreased	1·5
<i>CYP2D6*11</i>	G <sub>971</sub> C substitution	Deficient	0·1
<i>CYP2D6*12</i>	G <sub>212</sub> A substitution	Deficient	0·1
<i>CYP2D6*13</i>	Hybrid: 2D7 exon 1, 2D6 exons 2-9	Deficient	0·1
<i>CYP2D6*14</i>	G <sub>1846</sub> A substitution	Deficient	0·1
<i>CYP2D6*15</i>	T <sub>226</sub> insertion	Deficient	0·1
<i>CYP2D6*16</i>	Hybrid: 2D7 exons 1-7, 2D6 exons 8-9	Deficient	0·1
<i>CYP2D6*1</i> × 2	Gene duplication	Increased	1
<i>CYP2D6*2</i> × 2	Gene duplication	Increased	1·5
<i>CYP2D6*4</i> × 2	Gene duplication	Deficient	0·5

Lit: J. van der Weide et al. *Ann. Clin. Biochem* **36** (1999) 722

# CYP 2D6 Polymorphism (III)

MGLEALVPLAVIVVAIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQ

poor debrisoquine metabolism **S** **R** impaired mechanism of sparteine

LRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVF

poor debrisoquine metabolism **I**

LARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDK

poor debrisoquine metabolism **R**

AVSNVIASLTCGRRFEYDDPRFLRLDLAQEGLKEESGFLREVLNAVVPVLLHIPALAGKV

LRFQKAFLLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEME~~K~~AKGNPESFNDENLRIVVA

missing in CYP2D6\*9 allele

DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVI

**P** loss of activity in CYP2D6\*7

HEVQRFGDIVPLGMTHMTRSRIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRRFHPEHF

LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELELFFTSLLQHFSFSVPTGQPRPSHHGV

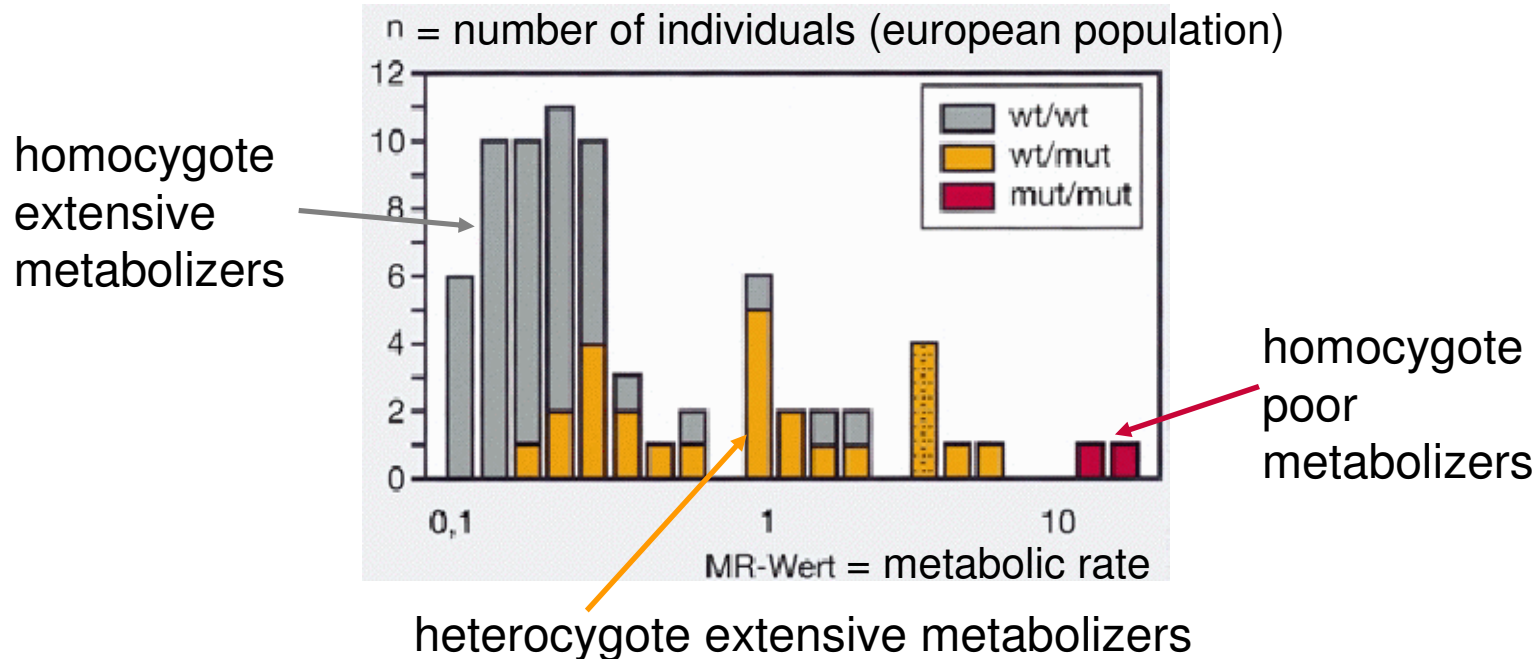
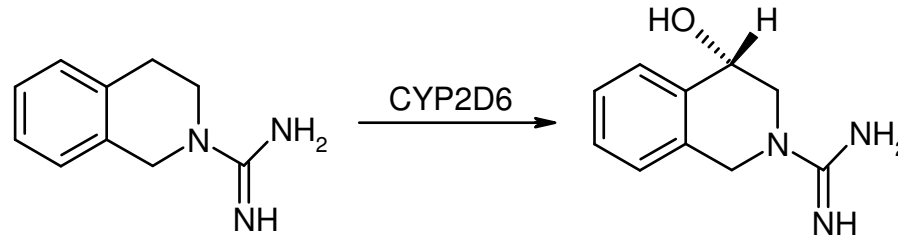
FAFLVSPSPYELCAVPR

**T** impaired metabolism of sparteine in alleles 2, 10, 12, 14 and 17 of CYP2D6

see <http://www.expasy.org/cgi-bin/niceprot.pl?P10635>

# CYP 2D6 Polymorphism (IV)

variability of debrisoquine-4-hydroxylation



Lit: T. Winkler *Deutsche Apothekerzeitung* **140** (2000) 38



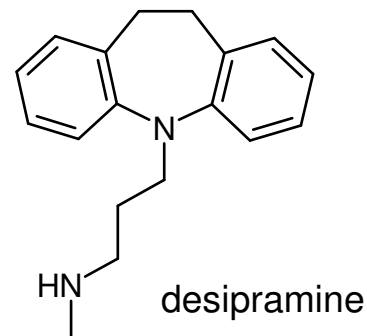
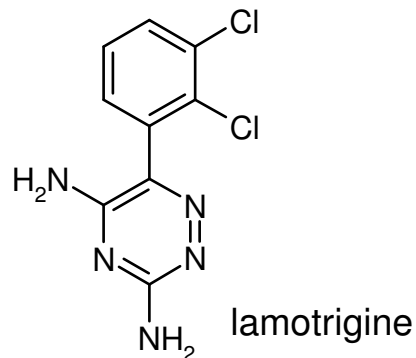
# CYP 2D6 Polymorphism (V)

the *poor metabolizer* phenotyp has consequences for the metabolism of more than 25% of all common drugs, since it causes an increased concentration of xenobiotics that are not metabolized.

Lit: H.K.Kroemer & M.Eichelbaum. *Life Sci.* **56** (1995) 2285.

Thus, CYP2D6 genotyping is already applied to select appropriate test candiates in phase II of clinical tests:

lamotrigine, desipramine (Antidepressants)



Lit: M.P.Murphy et al. *Pharmacogenetics* **10** (2000) 583.

# Polymorphism of further CYPs

CYP 1A2 individual; strong, medium, and slow conversion of caffeine

CYP 2B6 absent in 3-4 % of the caucasian population

CYP 2C9 deficit in 1-3 % of the caucasian population

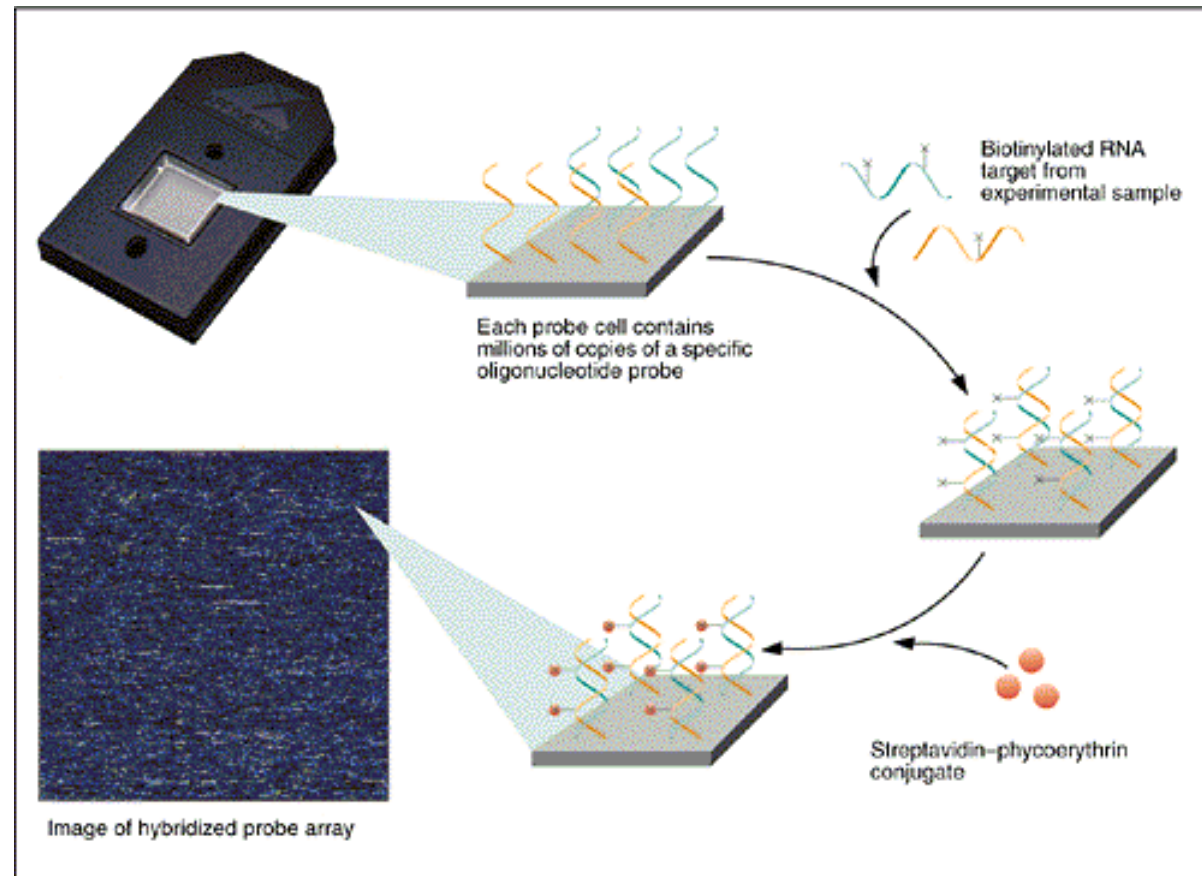
CYP 2C19 individuals with inactive enzyme (3-6 % of the caucasian and 15-20 % of the asian population)

CYP 2D6 poor metabolizers in 5-8 % of the european, 10 % of the caucasian and <1% in the japanese population. Overexpression (gene duplication) in parts of the african and oriental population

CYP 3A4 only few mutations known in connection with the polymorphism of the MDR1 transporter gene

# Genotyping for P450 alleles

Affymetrix (US) has developed microarrays (gene chips) using immobilized synthetic copies of P450 nucleotides, that allow the identification of all clinically relevant allelic variants.



# Induction and regulation of CYP3A (I)

A series of xenobiotics have been identified that lead to increased expression of enzymes of the CYP3A family.

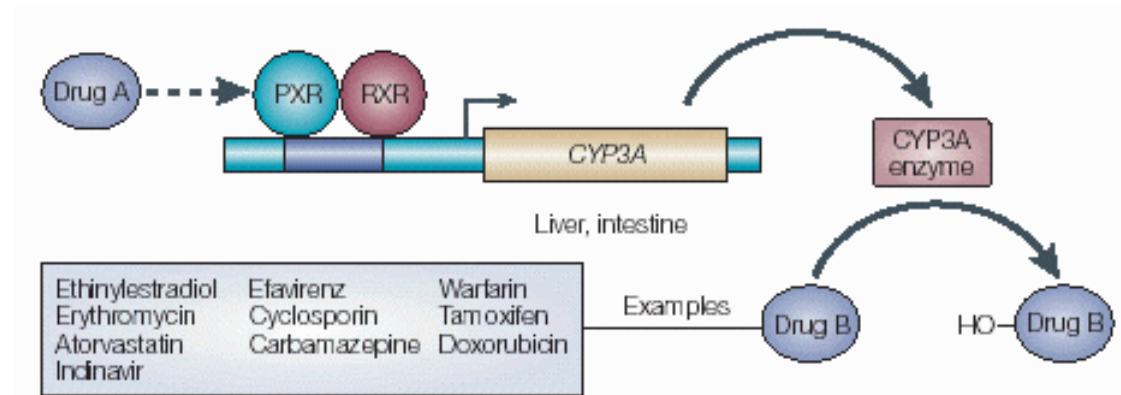
indinavir	antiviral
efavirenz	antiviral
cyclosporine	immuno-suppressant
carbamazepine	antipsychotic
atorvastatin	HMG CoA reductase inhibitor
tamoxifen	anti-hormone

These bind to the *pregnane X receptor* (PXR) which is the transcription factor for the regulation of the CYP3A gene expression.

Lit: T.M. Wilson et al. *Nature Rev. Drug Disc.* **1** (2002) 259

# Induction and regulation of CYP3A (II)

The PXR receptor operates together with the *retinoid X receptor* (RXR) as a heterodimer.



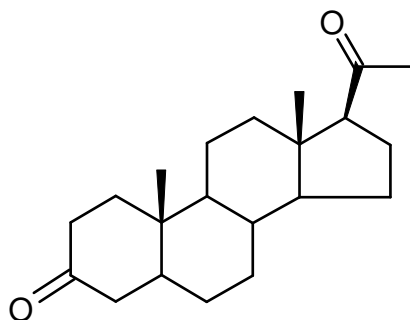
CYP3A induction leads to an increased metabolism of the administered substance due to upregulated enzymes. This can cause adverse reactions, like inflammation of the liver (hepatitis).

Lit: T.M. Wilson et al. *Nature Rev. Drug Disc.* **1** (2002) 259

# RXR and other nuclear receptors (I)

As a specific, endogenous activator of RXR, 5 $\beta$ -pregnane-3,20-dione has been identified.

In contrast, PXR is much less specific and is activated by glucocorticoids as well as by anti-glucocorticoids.



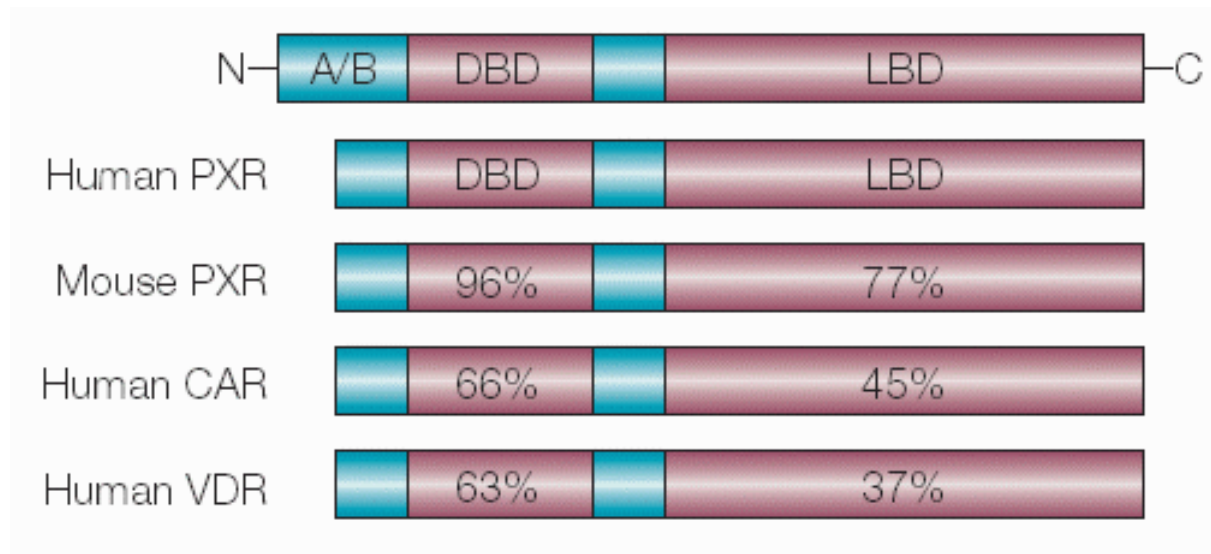
5-b-pregnane-3,20-dione

Conversely, the unspecific *constitutive androgen receptor* (CAR) is found in the cytoplasm and dimerizes with PXR in the nucleus. Analog to PXR, the CYP2B gene is regulated.

Likewise high sequence homology has been found for the *vitamine D receptor* (VDR) that regulates CYP27, and for the *arylhydrocarbon receptor* (AHR) (dioxin receptor).

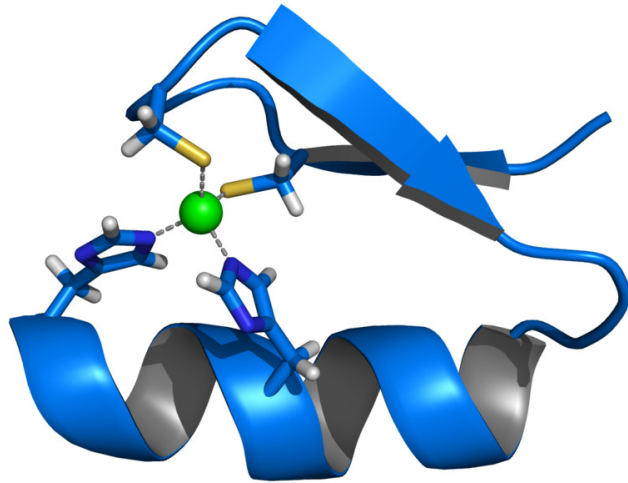
## RXR and other nuclear receptors (II)

These nuclear receptors all belong to a family of transcription factors. Each one possess a double zinc-finger DNA-binding domain (DBD), and a larger ligand binding domain (LBD) which is located at the carboxy terminal.



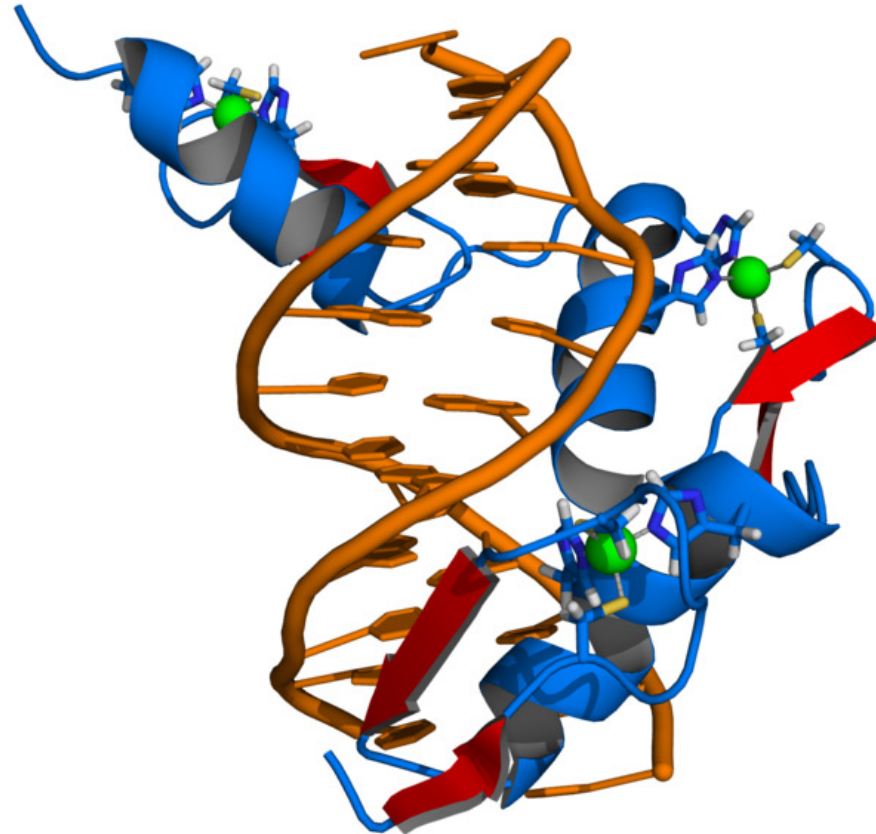
They have been called *orphan nuclear receptors* as their ligands have been found later.

# Zinc finger motif



The zinc ion is coordinated by two cysteines and two histidines.

Source: Wikipedia



The protein Zif268 contains three zinc fingers motives in complex with the DNA



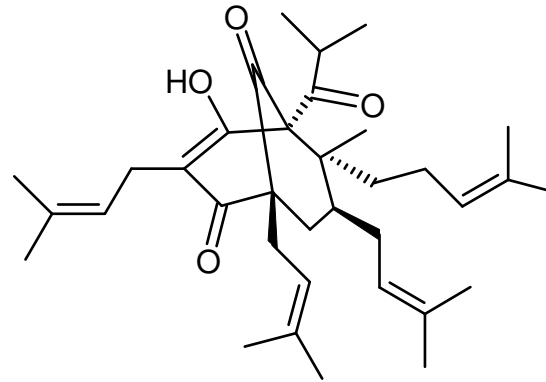
# Human Orphan Nuclear Receptors

receptor (gene ID)	natural ligand (synthetic ligand)
CAR (NR1I3)	3 $\alpha$ ,5 $\alpha$ -androstanol
COUP (NR2F1)	–
ERR (NR3B1)	(4-hydroxytamoxifen)
FXR (NR1H4)	chenodeoxycholic acid
HNF4 (NR2A1)	palmitic acid
LRH (NR5A2)	–
PPAR (NR1C1)	eicosapentaenoic acid
PXR (NR1I2)	5 $\beta$ -pregnane-3,20-dione, (rifampicin)
ROR (NR1F1)	stearic acid
RXR (NR2B1)	9- <i>cis</i> -RNA

(Selection only, for more see the cited reference)

Lit: T.M.Wilson & J.T. Moore *Mol. Endocrin.* **16** (2002) 1135.

## Induction and regulation of CYP3A (III)



hyperforin, a natural ingredient of St. John's wort (*Johanniskraut*, *Hypericum perforatum*) exhibits the highest measured affinity to PXR ( $K_d = 27$  nM) so far.

Application: remedy against cholestasis [Gallestauung], mild antidepressant (heavily debated if available concentration in preparations of St. John's wort is sufficiently high)

# Induction and regulation of CYP3A (IV)

X-ray structure of PXR complexed with hyperforin (1M13.pdb)



Lit: R.E. Watkins et al. *Biochemistry* **42** (2003) 1430

# Induction of further CYPs

CYP 1A2            omeprazole, insulin, aromatic hydrocarbons  
(cigarette smoking, charbroiled meat)

causes increased caffeine level in the plasma,  
if you quit smoking.

CYP 2C9            rifampicin, secobarbital

CYP 2C19          carbamazepine, prednisone

CYP 2D6            dexamethason

CYP 2E1            ethanol, isoniazid

CYP 3A4            glucocorticoides, phenobarbitone,  
rifampicin, nevirapine, sulfadimidine,  
nevirapine, sulfinpyrazone, troglitazone

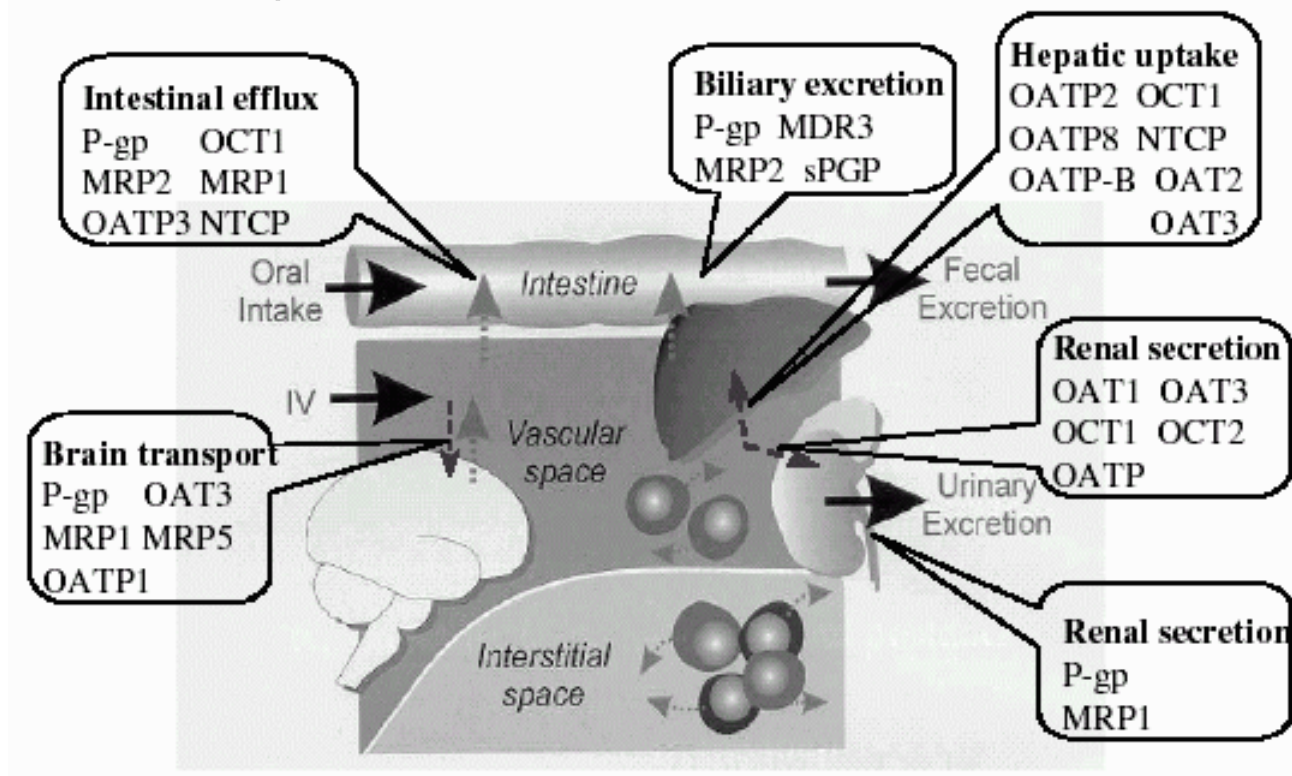
# Typical inhibitors of various CYPs

CYP 1A2	cimetidine, ciprofloxacin, enoxacin... grapefruit juice (naringin, 6',7'-dihydroxy-bergamottin)
CYP 2C9	chloramphenicol, amiodarone, omeprazole,...
CYP 2C19	fluoxetine, fluvastatin, sertraline,...
CYP 2D6	fluoxetine, paroxetine, quinidine, haloperidol, ritonavir,...
CYP 2E1	disulfiram, cimetidine,...
CYP 3A4	cannabinoids, erythromycin, ritonavir, ketoconazole, grapefruit juice

see <http://medicine.iupui.edu/flockhart/>

# Transporters (I)

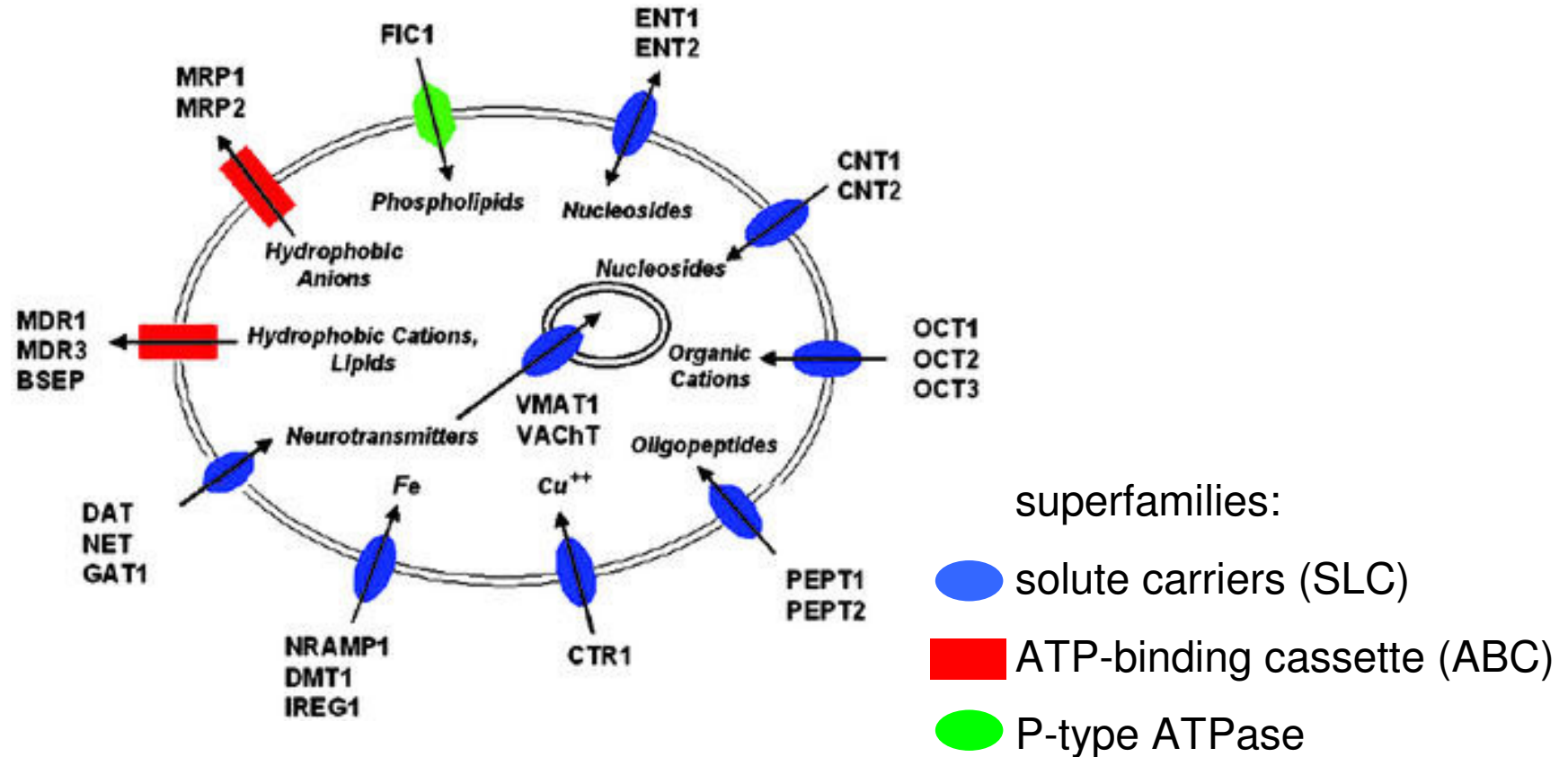
In contrast to the passive diffusion through membranes transporters cause increased *influx* into, or conversely *efflux* from compartments, whereby ATP is consumed. (active transport)



Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469

# Transporters (II)

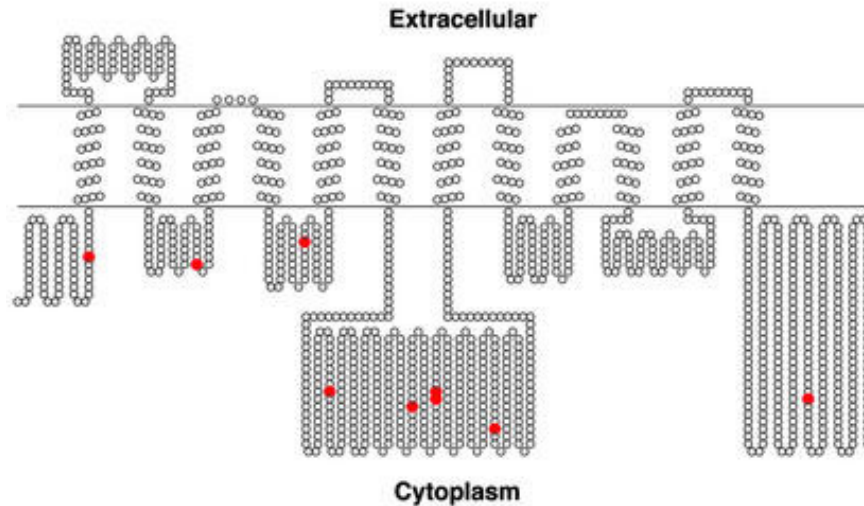
Membrane bound transporters involved in the pharmacokinetic of endogenous substances



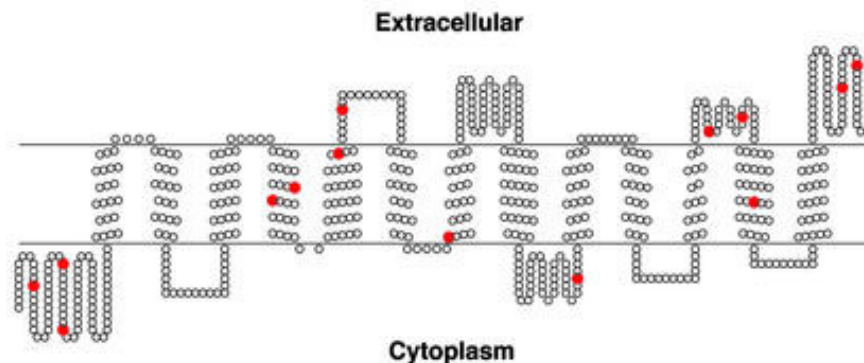
Lit: M.K.Leabman et al. *Proc.Nat.Acad.Sci.USA* **100** (2003) 5896

# Structure of membrane-bound transporters

**BILE SALT EXPORT PUMP (ABCB1)**



**CONCENTRATIVE NUCLEOSIDE TRANSPORTER 1 (SLC28A1)**



Membrane-bound transporters are proteins with up to 12 and more transmembrane helices that are connected by loops. So far no X-ray structure of a transporter has been achieved.

Lit: M.K.Leabman et al. *Proc.Nat.Acad.Sci.USA* **100** (2003) 5896



# P-glycoprotein

P-gp belongs to the group of *multidrug resistant proteins* (MDR) and is encoded by the MDR1 gene.

Especially the bioavailability of antipsychotics is limited by the mediated efflux from the brain and central nervous system back into the system blood circulation.

Likewise transport of substances from the liver into the gastrointestinal (*biliary excretion*) e.g. of indinavir

Overexpression of P-gp in cancer cells leads to resistance against antineoplastics.

Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469.  
A.Seelig *Eur.J.Biochem.* **251** (1998) 252.

# Transporter proteins for organic ions

Comprising the families of the

Organic Anion Transporters (OAT) and the

Organic Cation Transporters (OCT)

The contribute in particular to the excretion of hydrophilic metabolites and katabolites.

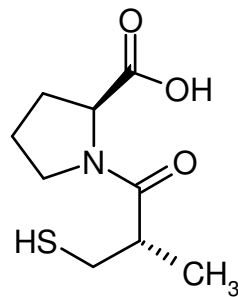
Lit: A.Ayrton et al. *Xenobiotica* **31** (2001) 469

# Transporter proteins for *influx*

There are also transporters that mediate the active uptake of substance from the intestine

Pept1 (intestinal peptide transporter)  
transmembrane protein possessing 12 TM-helices  
Responsible for the uptake of nitrogen !

substrates: small peptides (di- and tripeptide, as well as compounds exhibiting peptide-like features, e.g. captopril)



# Polymorphisms of transporters

Also transporters show considerable genetic variations:

gene    protein / function

ABCB1 (ATP-binding cassette subfamily B member 1) P-gp efflux

SLC6A3 (dopamine transporter) neurotransmitter

SLC6A4 (serotonin transporter) neurotransmitter

ADRB2 ( $\beta$ -adrenergic receptor) rezeptor for  $\beta$ -blocker

ALOX5 (arachidonate 5-lipoxygenase) biosynthesis of  
leukotrienes

See: D.B.Goldstein et al. *Nature Rev. Genetics* **4** (2003) 937.