Drug metabolism

Phase I metabolism

hydrolysis,

N-acetylation,

particularly

insertion of oxygen atom - cytochrome P450

Phase II metabolism

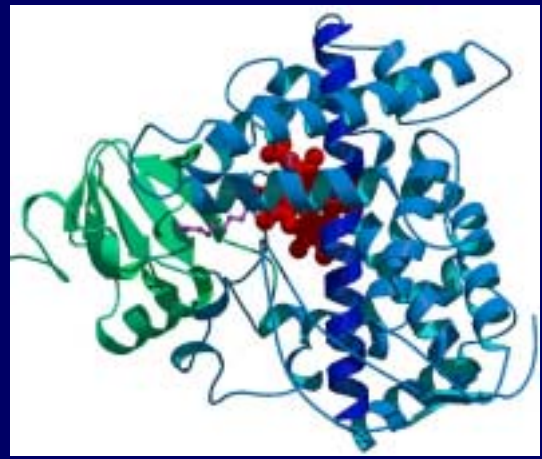
conjugation to:

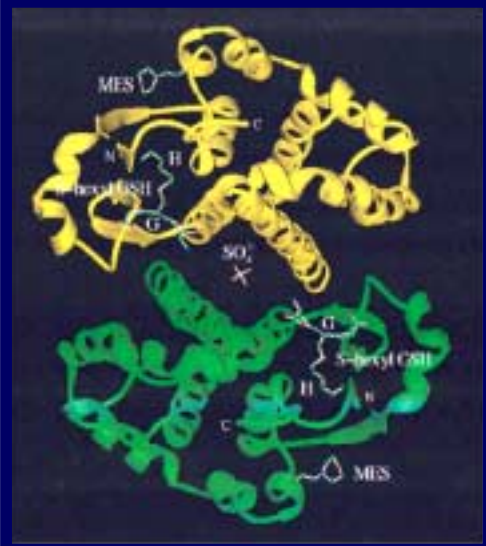
glutathione

glucuronic acid

sulphate

Increased polarity, ease of elimination





Cytochromes P_{450}

Superfamily' of ~1200 haem enzymes

Two classes according to redox partner

Catalyse mono-oxygenation of a wide variety of substrates - insertion of one atom of molecular oxygen, reduction of the other atom to water.

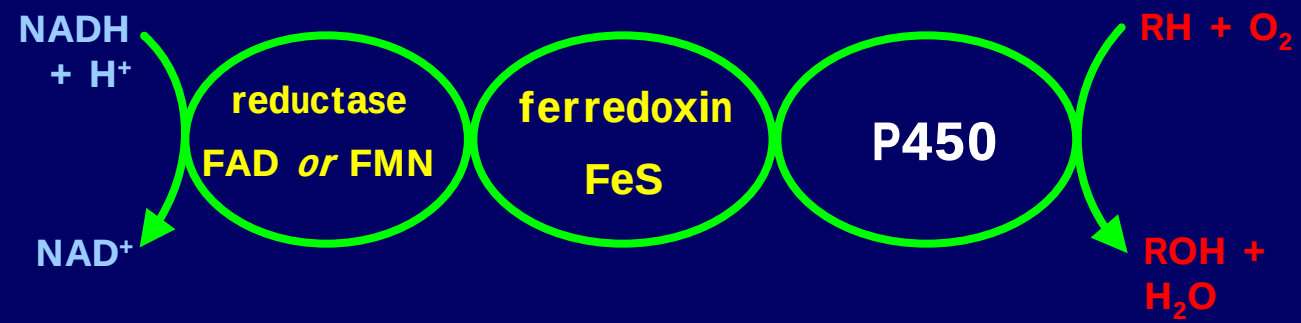
In mammals, the P450 / NADPH-P450 reductase system in the ER plays a **major role in determining the response of the organism to exogenous chemicals**.

Diversity of P450s allows them to deal with a wide variety of chemicals - many different P450s, each often has a broad substrate specificity.

http://drnelson.utmem.edu/CytochromeP450.html
http://www.icgeb.trieste.it/~p450srv/

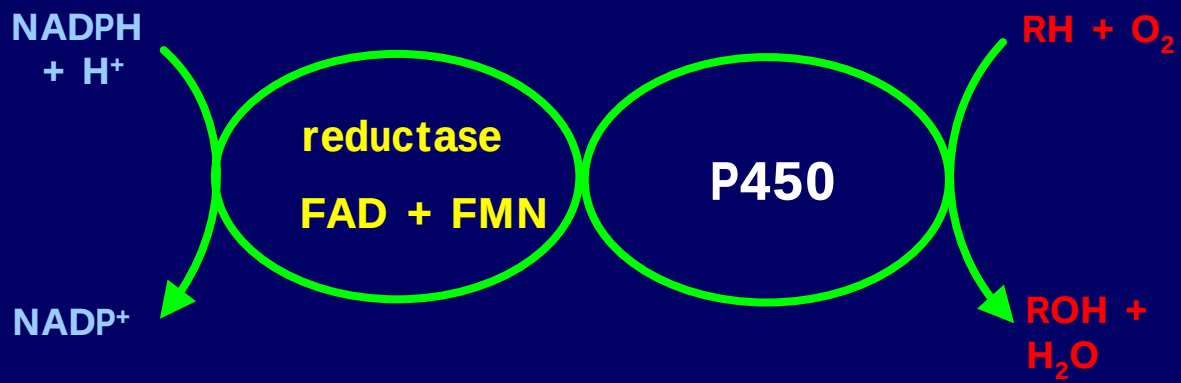
Two classes of cytochromes P_{450}

Class I Mitochondria and bacteria



Class II Endoplasmic reticulum

- and *Bacillus megaterium*



Cytochromes P450

Gene families

Currently > 1200 P450s in 215 families

S. cerevisiae has only 3 *CYP* genes

Arabidopsis thaliana has ~286 *CYP* genes

Drosophila has 94 *CYP* genes

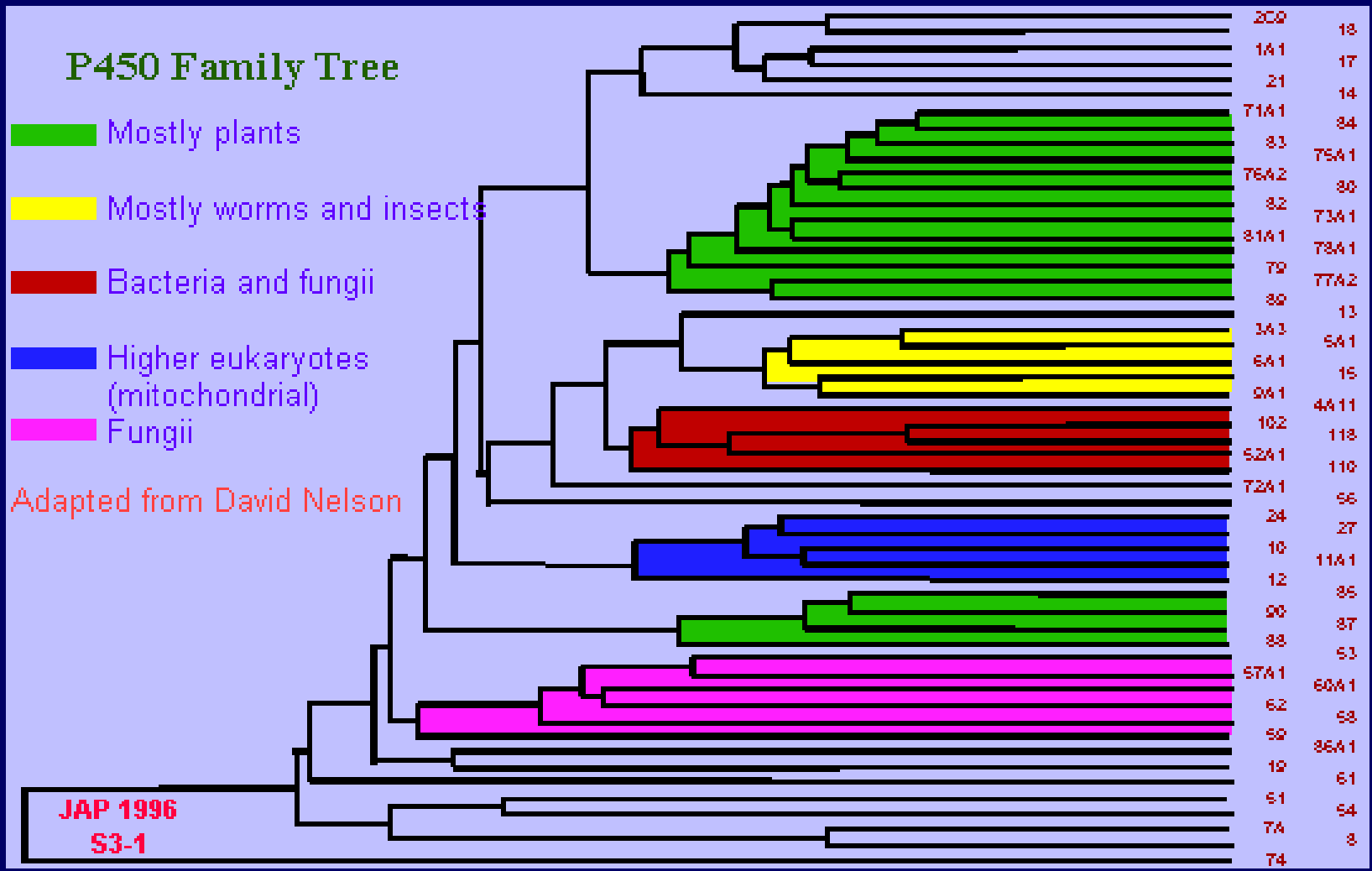
Caenorhabditis elegans has 73 *CYP* genes

Mycobacterium tuberculosis has ~20 *CYP* genes

Humans have 55 *CYP* genes in 17 families

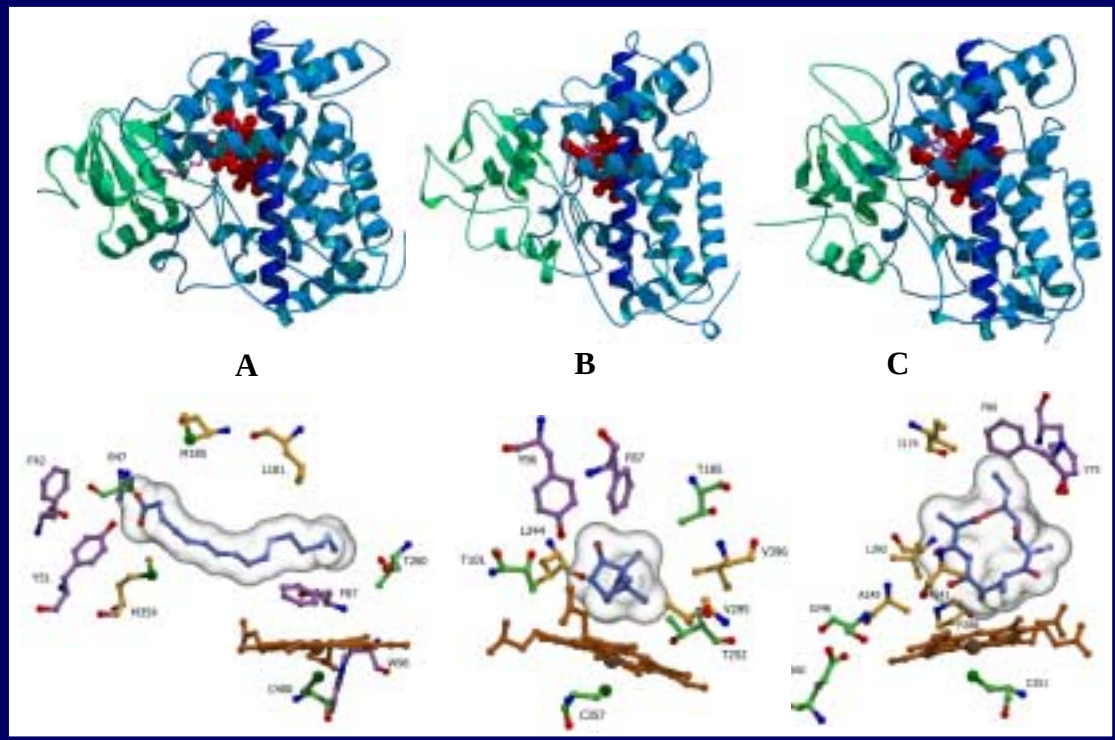
(rats ~60, mouse ~45)

The cytochrome P450 superfamily



Bacterial P450 structures

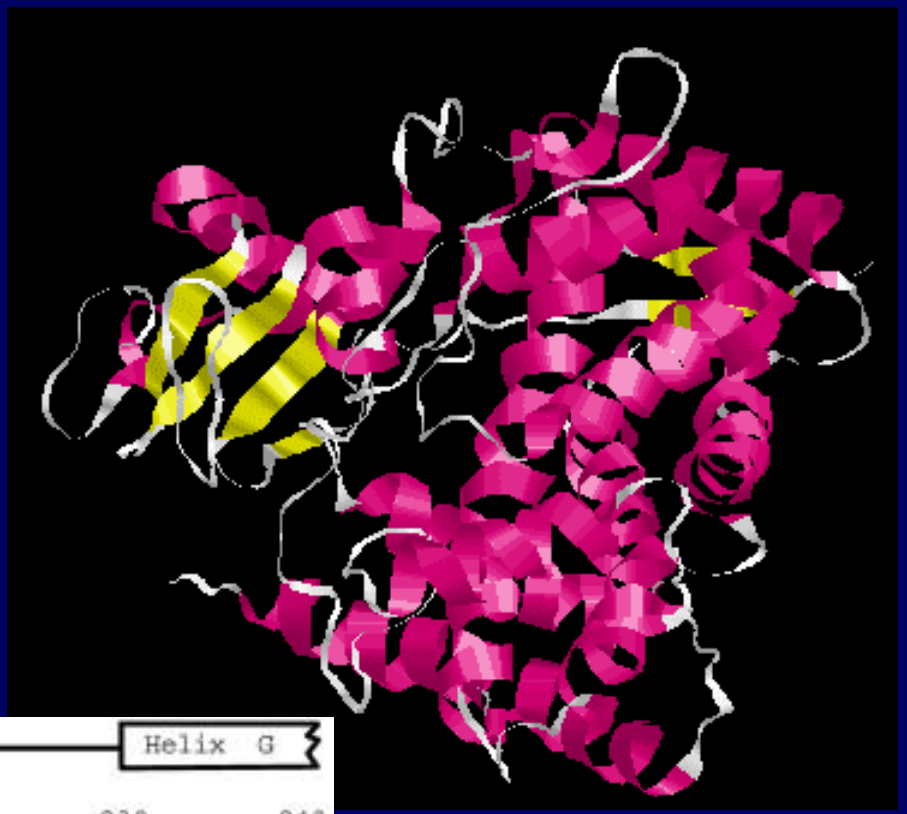
P450 BM3 P450cam P450eryF

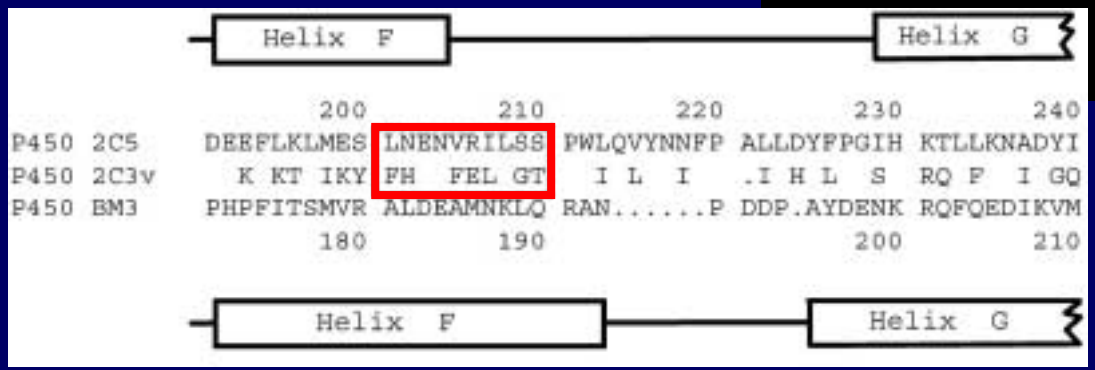


Common architecture; specificity determined by local amino-acid differences in the active site

Structure of CYP 2C5 / 2C3

Williams *et al.* (2000)
Mol. Cell, *5*, 121-131





Cytochromes P450 - catalytic activities

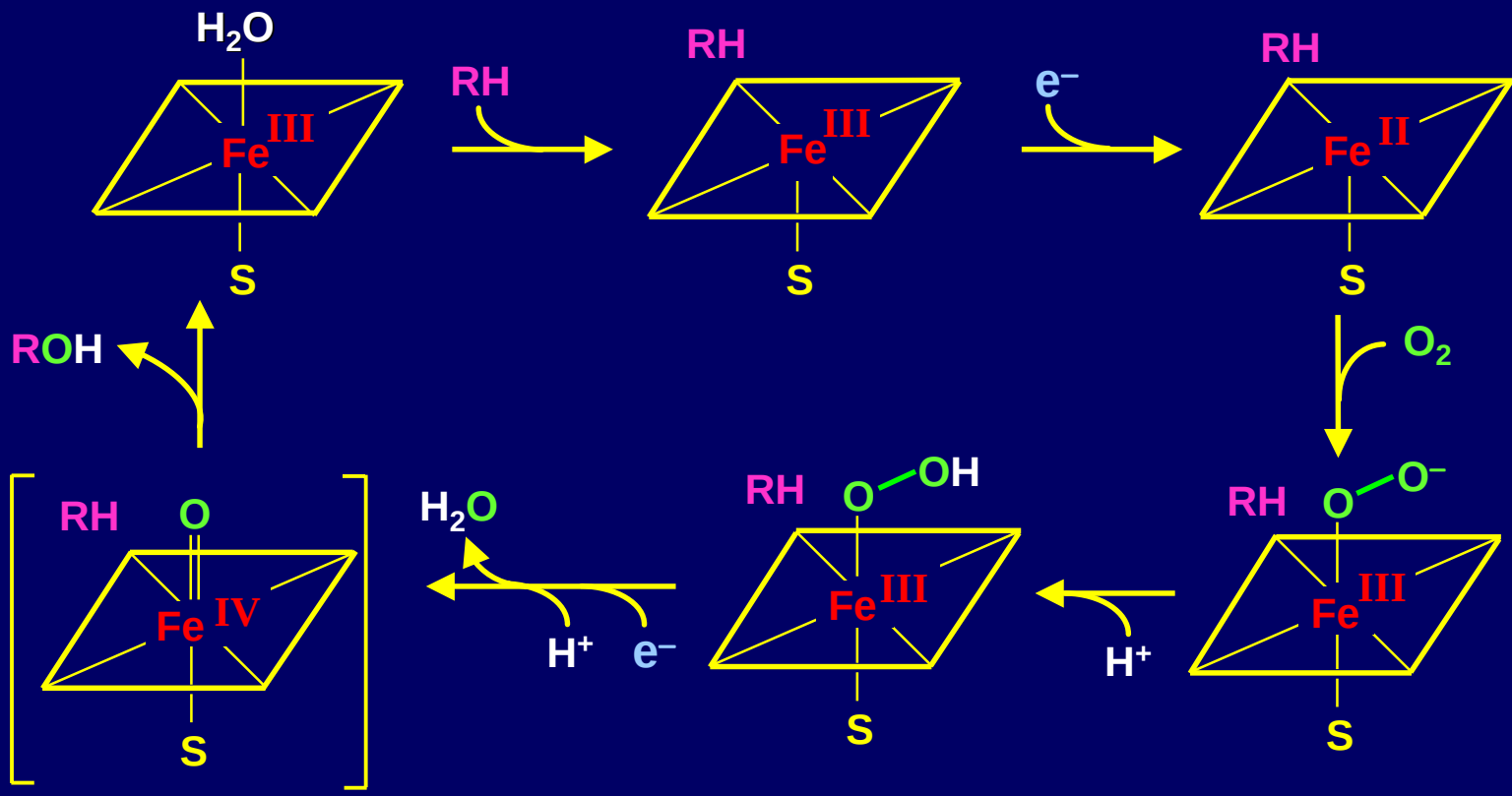
Oxygen-activation catalysts which incorporate one atom of molecular oxygen into a broad range of substrates with reduction of the other oxygen atom to water.

This leads to catalysis of a wide variety of reactions:

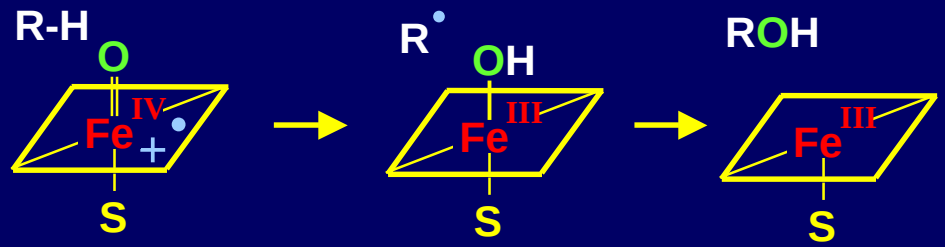
Hydroxylation of aliphatic & aromatic carbons
Epoxidation **N, O- and S-dealkylation**
Dehalogenation **Oxidative deamination**
N-oxidation and N-hydroxylation
Sulphoxide formation

>40 reactions, >10,000 substrates

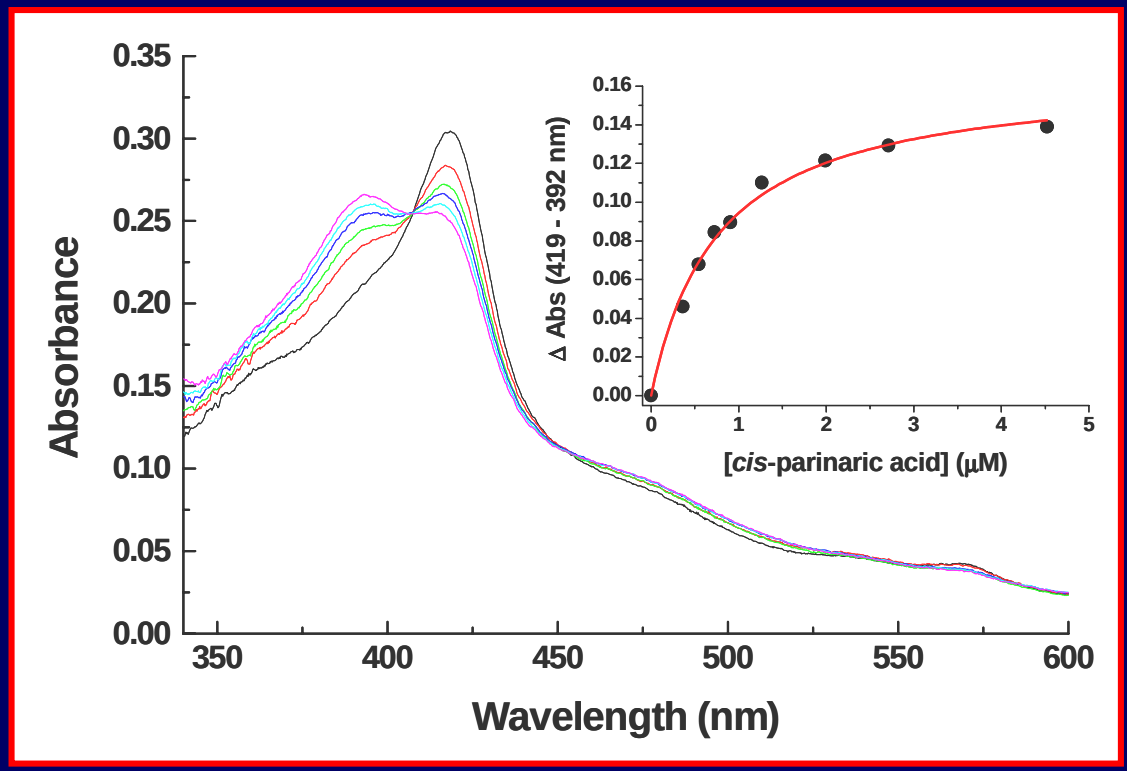
Cytochrome P450 - catalytic cycle



Radical rebound mechanism



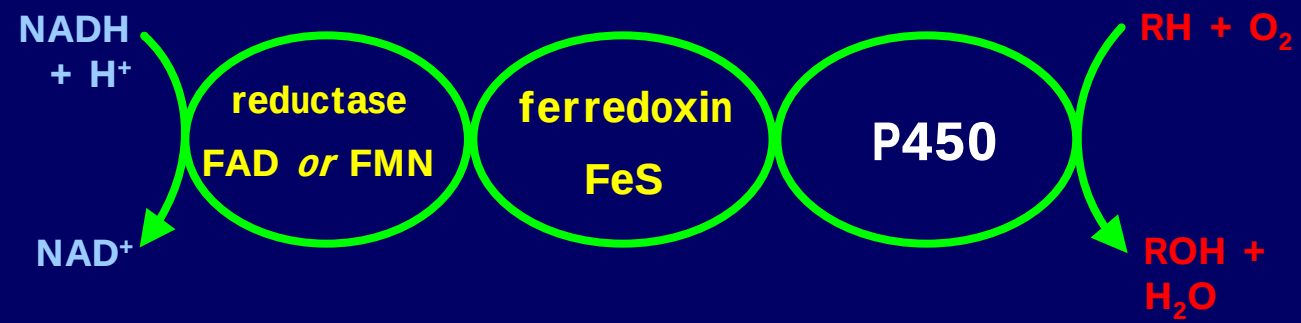
Substrate binding measured optically - low-spin to high-spin shift



Fatty acid binding to P450 BM3

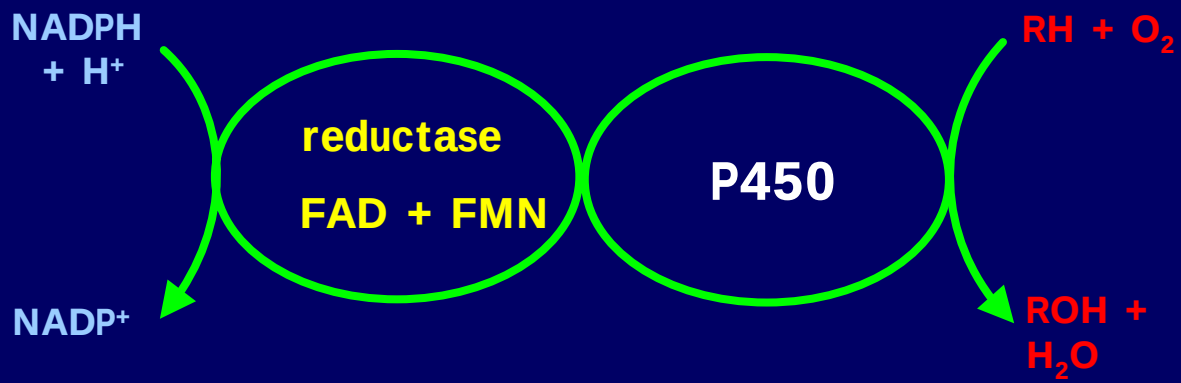
Two classes of cytochromes P_{450}

Class I Mitochondria and bacteria

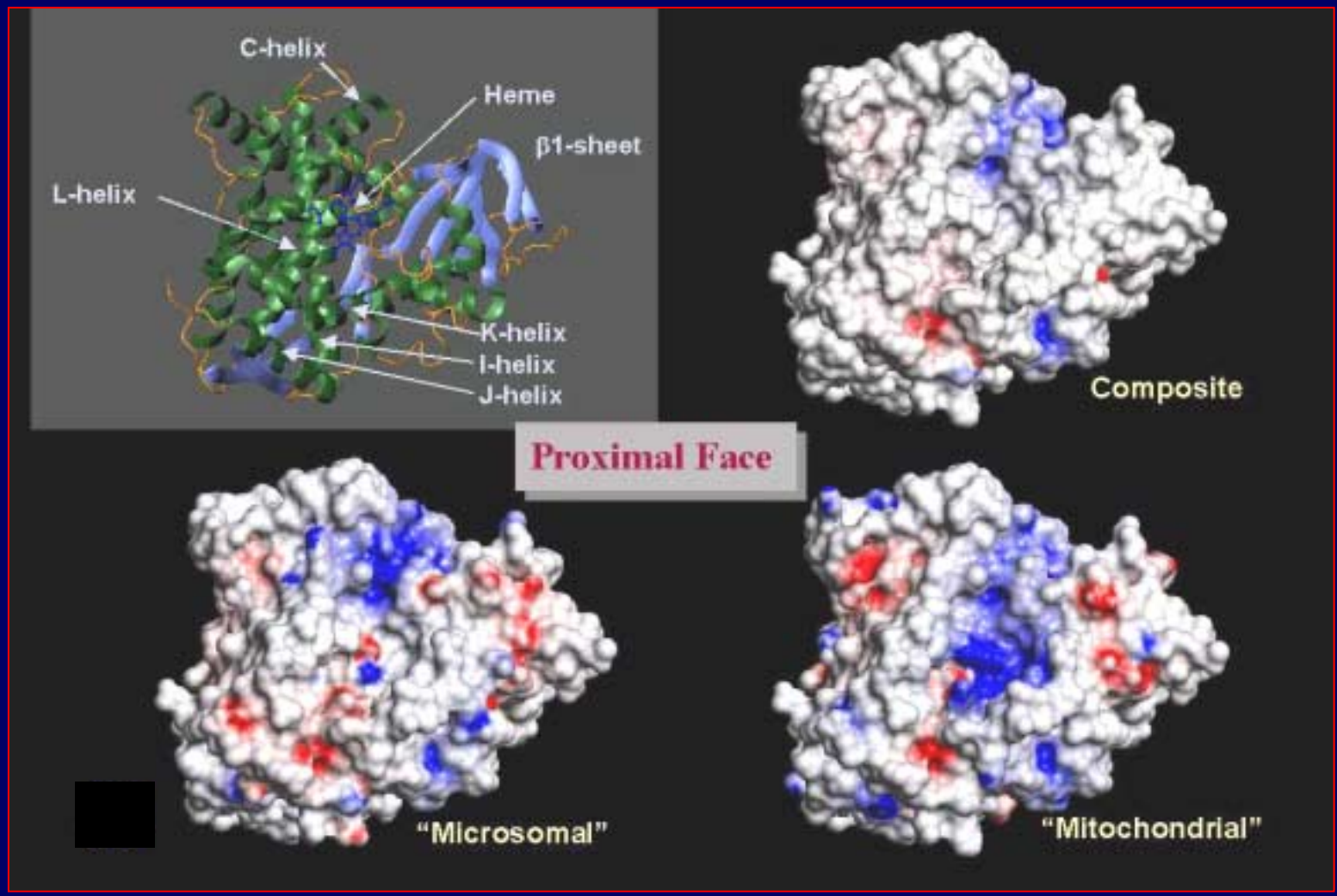


Class II Endoplasmic reticulum

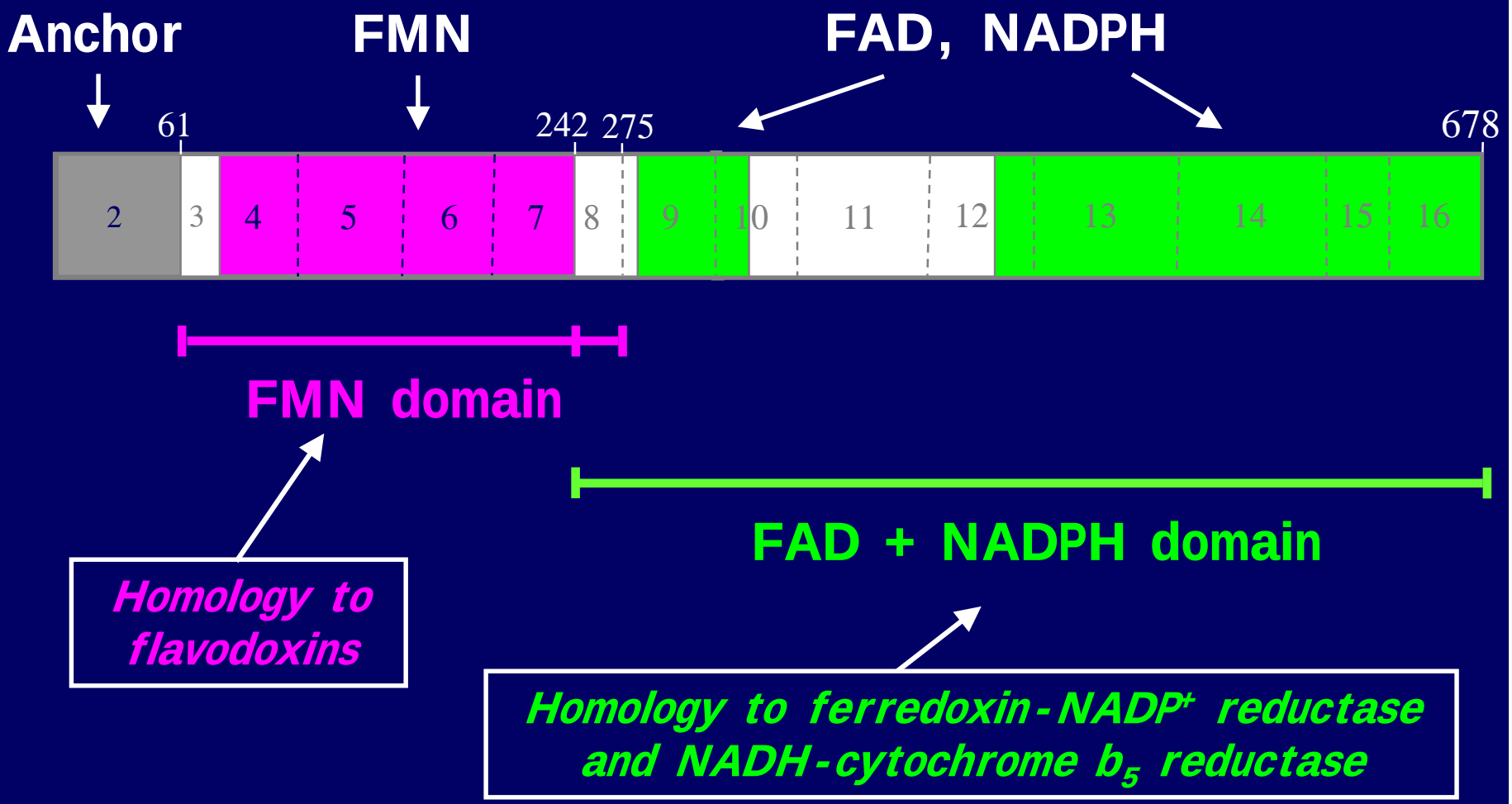
- and *Bacillus megaterium*



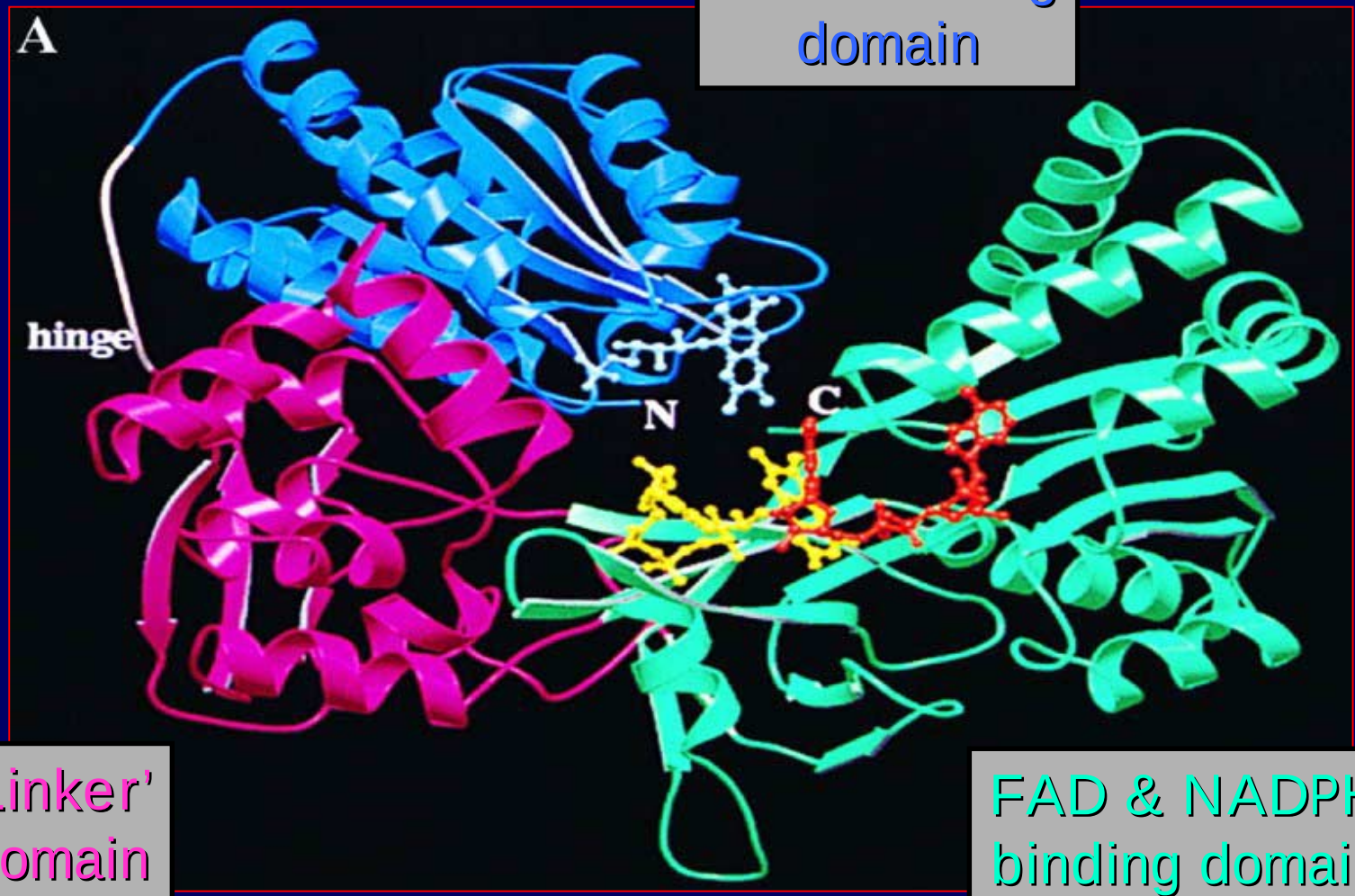
Redox partner binding sites in the two classes of P450s



Human NADPH-cytochrome P450 reductase



NADPH-cytochrome P450 reductase (CPR)



FMN binding domain

'Linker' domain

FAD & NADPH binding domain

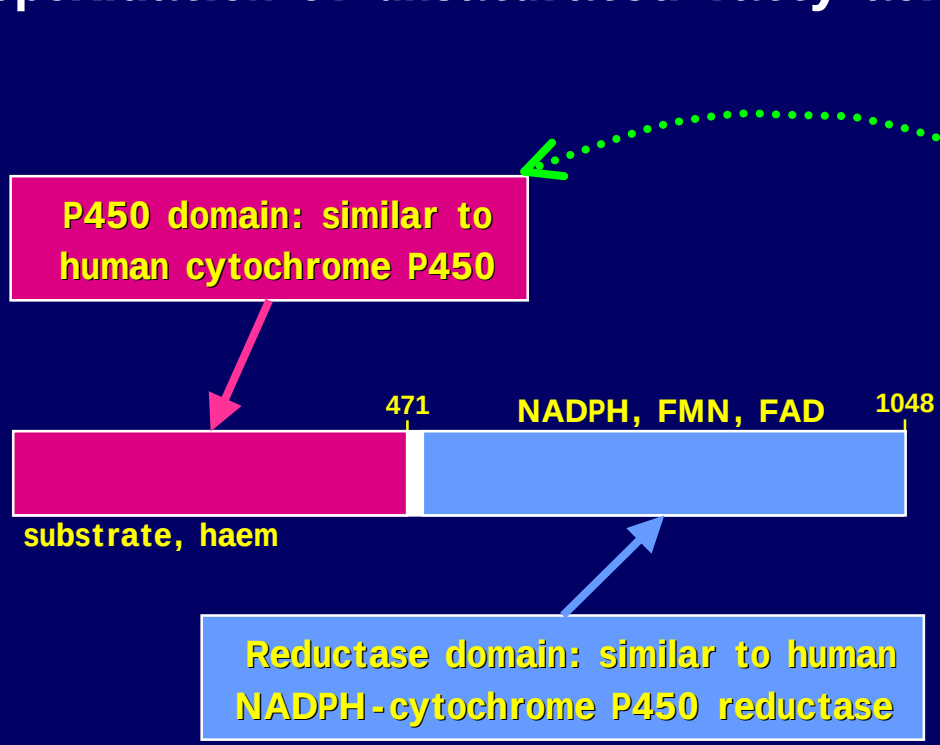
Wang et al., PNAS, *94*, 8411-8416, 1997

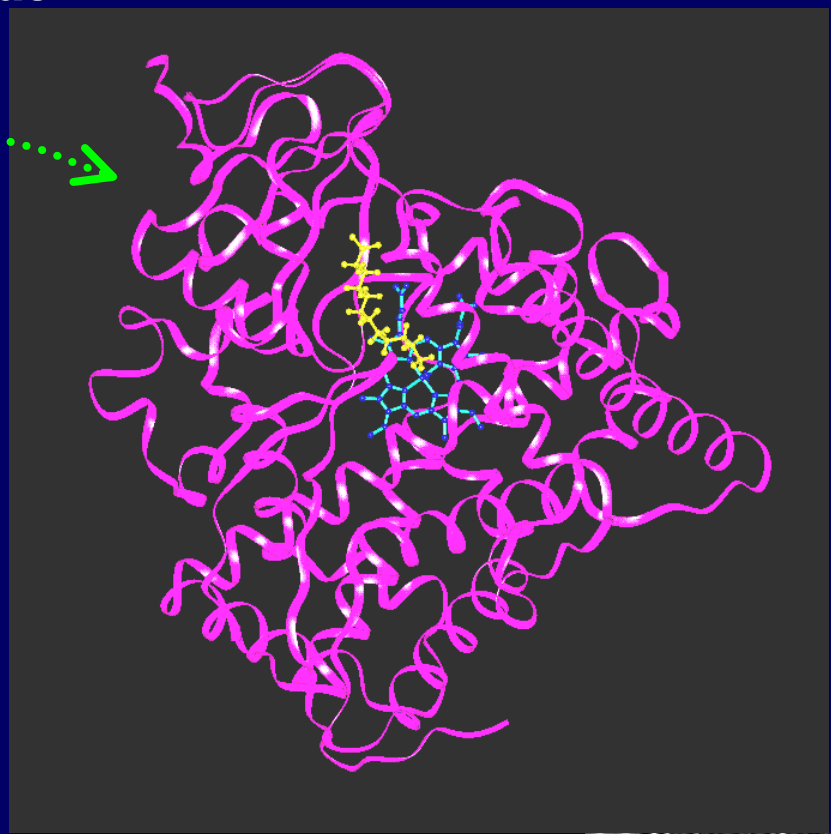
Cytochrome P450 BM3 (CYP102)

From *Bacillus megaterium* (expressed in *E. coli* - up to 250mg/l)

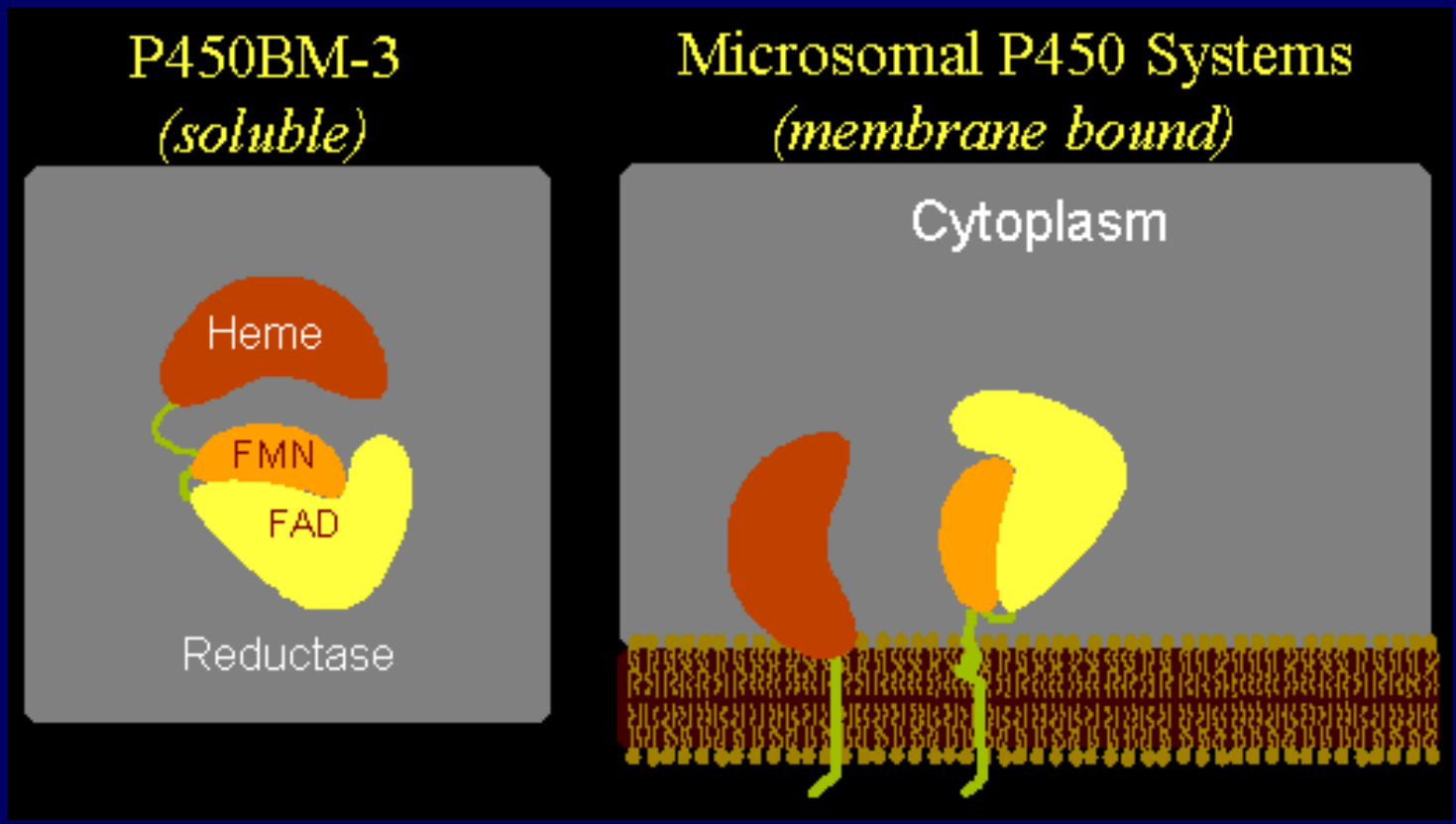
"Self-contained" enzyme

Catalyses ω-1, ω-2 and ω-3 hydroxylation of fatty acids, and epoxidation of unsaturated fatty acids

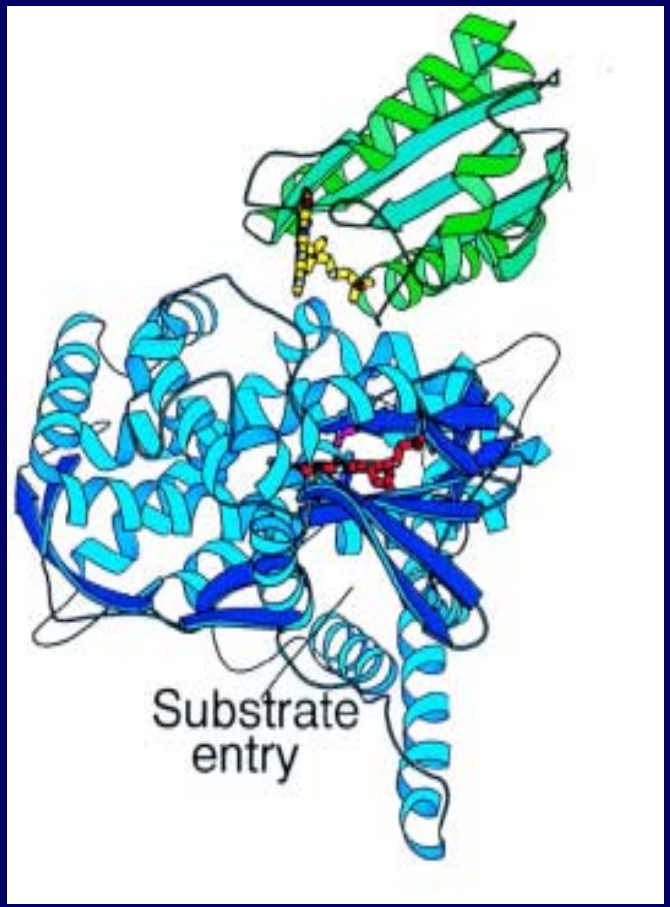


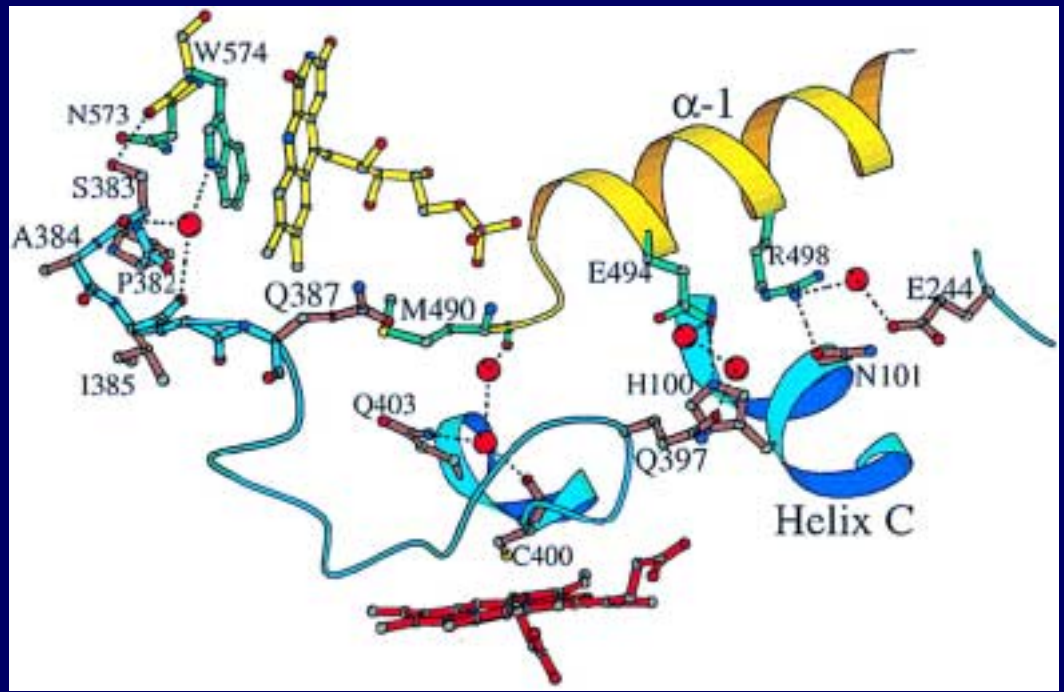


Class II cytochrome P450s



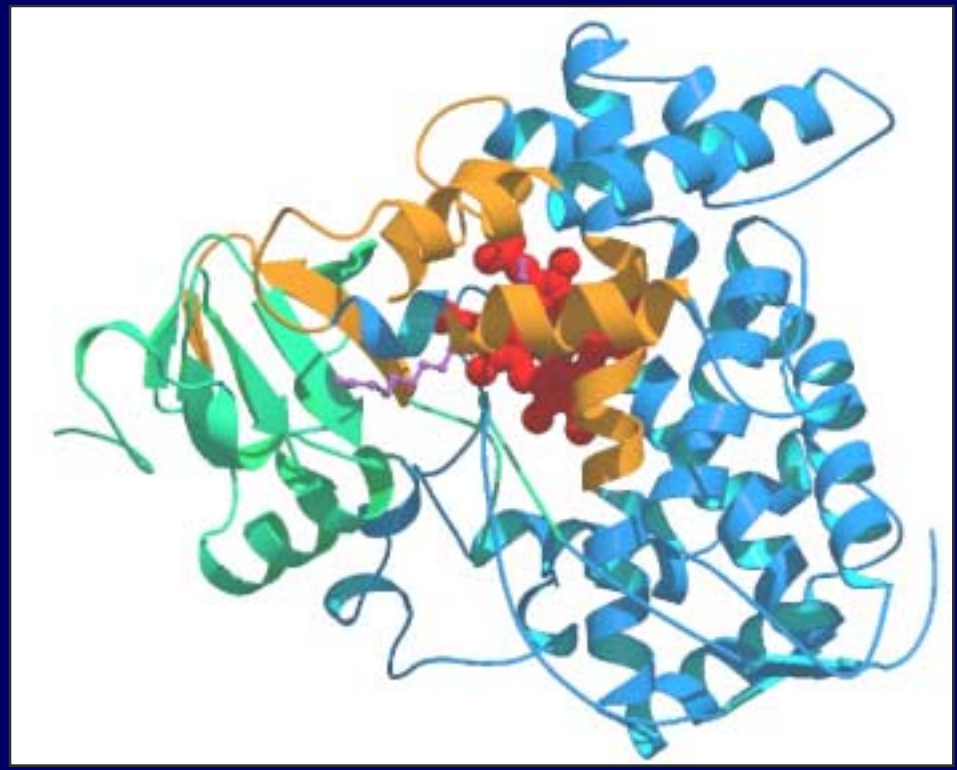
P450 BM3 haem domain and FMN domain





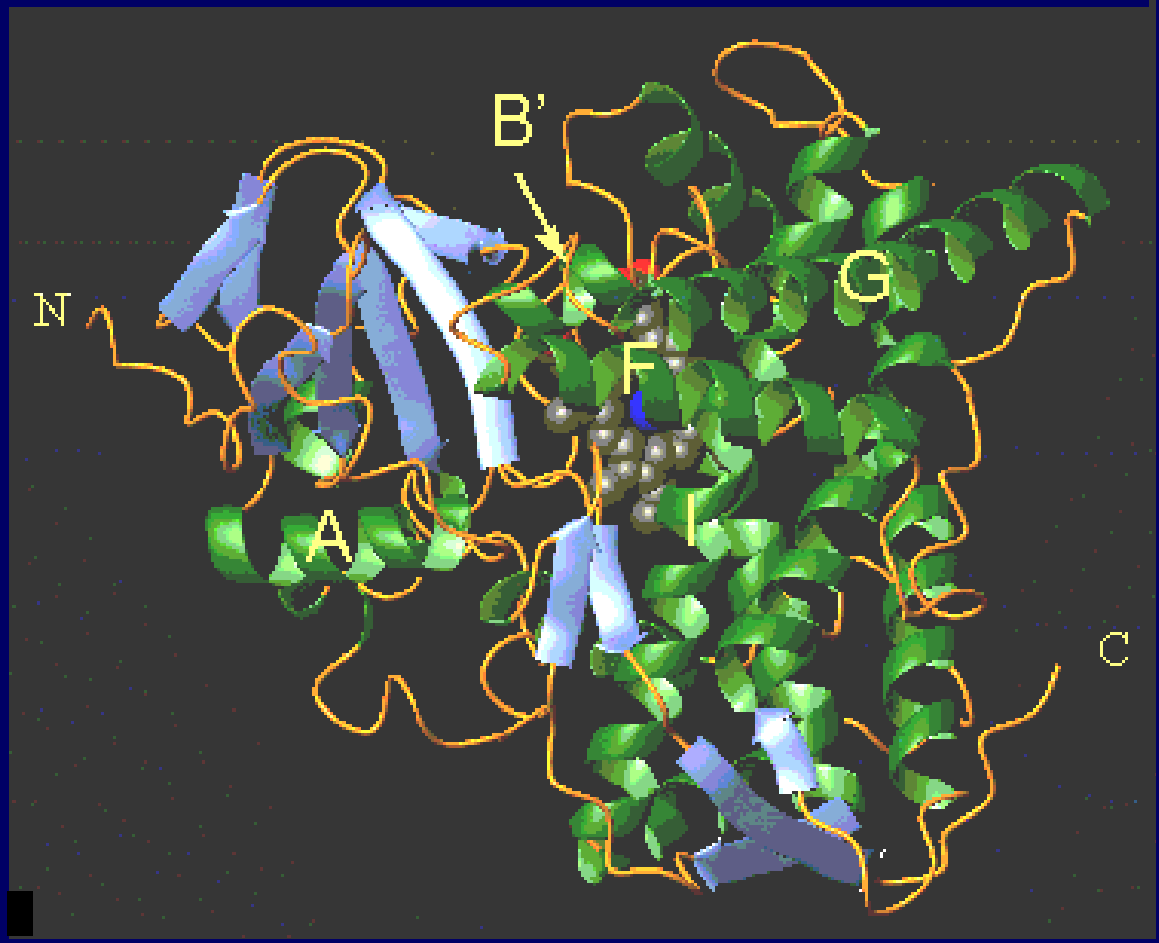
Sevrioukova *et al.* (1999) PNAS, *96*, 1863-1868

Substrate recognition sequences in P450s



SRS1: Residues L75 to N95 across the B' helix and B'-C loop.
SRS2: Residues P172 to E183 at the C-terminus of the F helix.
SRS3: Residues L188 to E207 at the N-terminus of the G helix.
SRS4: Residues in the I helix, particularly between T260 and L272.
SRS5: Residues in the β6-1/β1-4 region between A330 and L341.
SRS6: Residues in the β4 hairpin region between T436 and E442.

Cytochrome P450 BM3 haem domain



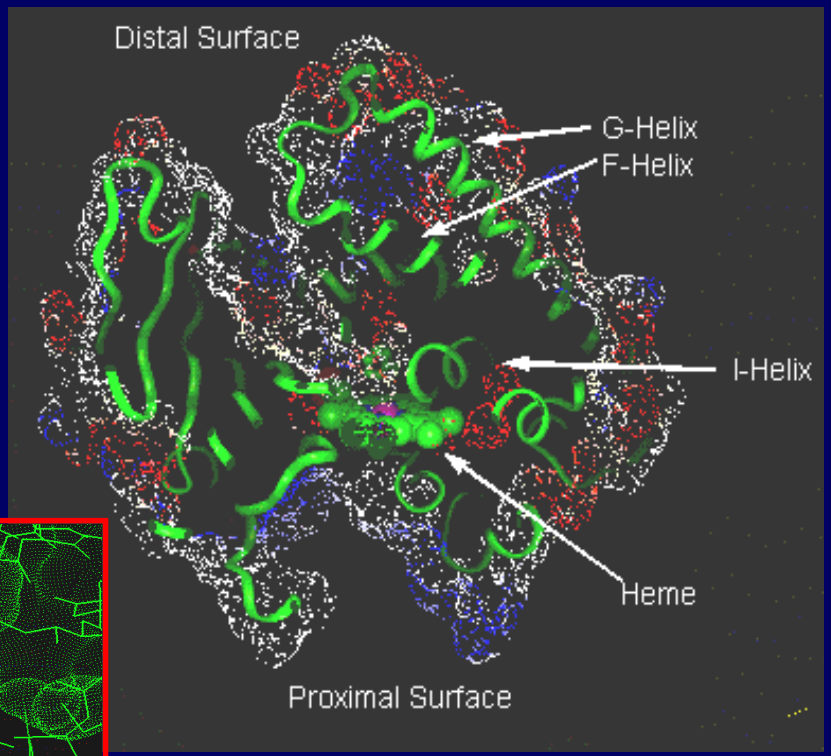
Specificity of P450 BM3 - fatty acids

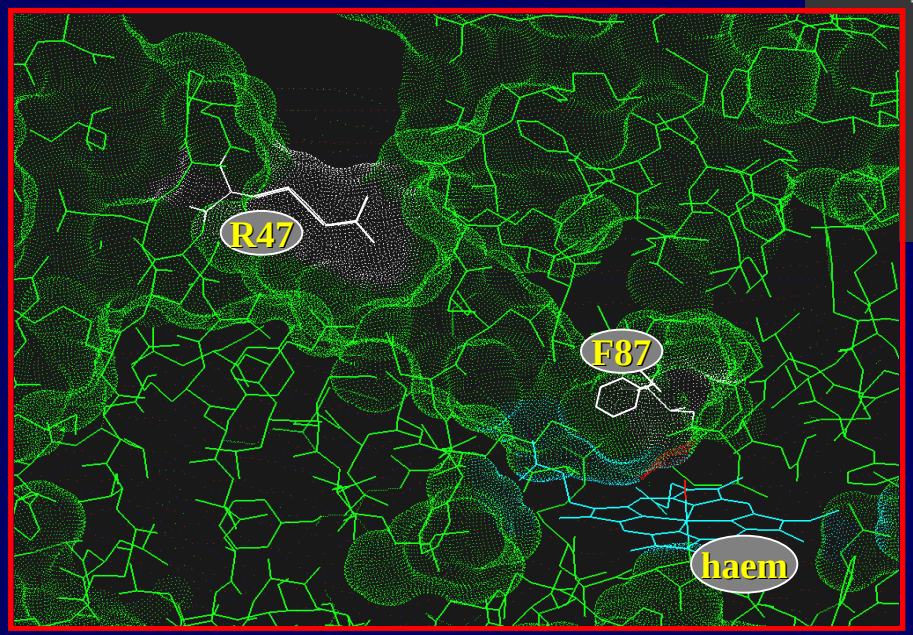
Substrate	k_{cat}, s^{-1}	K_M, μM
Laurate C12	25	136
Myristate C14	53	7
Palmitate C16	84	1.4

	ω	ω–1	ω–2	ω–3
laurate	**0**	**30%**	**35%**	**35%**
palmitate		**98%R**	**98%R**	**72%R**

Oliver et al. (1997) Biochemistry, *36*, 1567;
Truan etal. (1999) Arch. Biochem. Biophys., 366, 192

The cytochrome P450 BM3 active site channel





Relaxation measurements of iron-substrate distances

$$\frac{1}{T_{1,\,obs}} = \frac{f_B}{T_{1B}} + \frac{f_F}{T_{1F}}$$

The measured relaxation rate is the average of the rates in the bound and in the free ligand.

The relaxation rate depends on the distance from the iron and on the correlation time of the interaction, here the electron spin relaxation time.

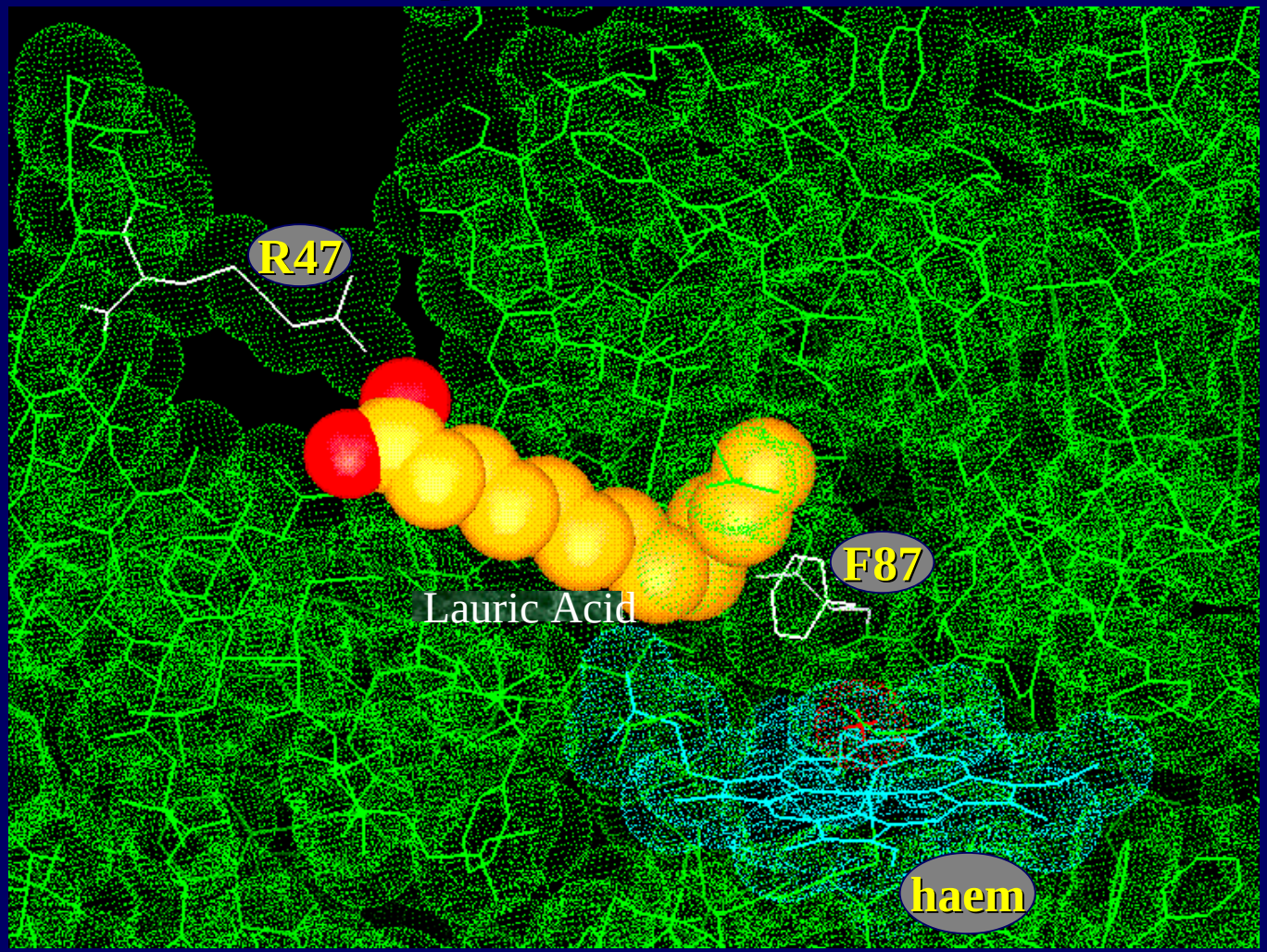
$$\frac{1}{T_1} = \frac{K}{\mathbf{r^6}}\left[\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right]$$

Ferric complex of P450 BM3 with 12-bromolaurate

	C2	C3	C10	C11	C12
$T_{1,M}$ (ms)	140 ± 10	90 ± 11	5.1 ± 0.5	3.8 ± 0.4	1.7 ± 0.3
r (Å)	16.3 ± 0.2	15.1 ± 0.2	9.4 ± 0.2	8.9 ± 0.2	7.8 ± 0.2

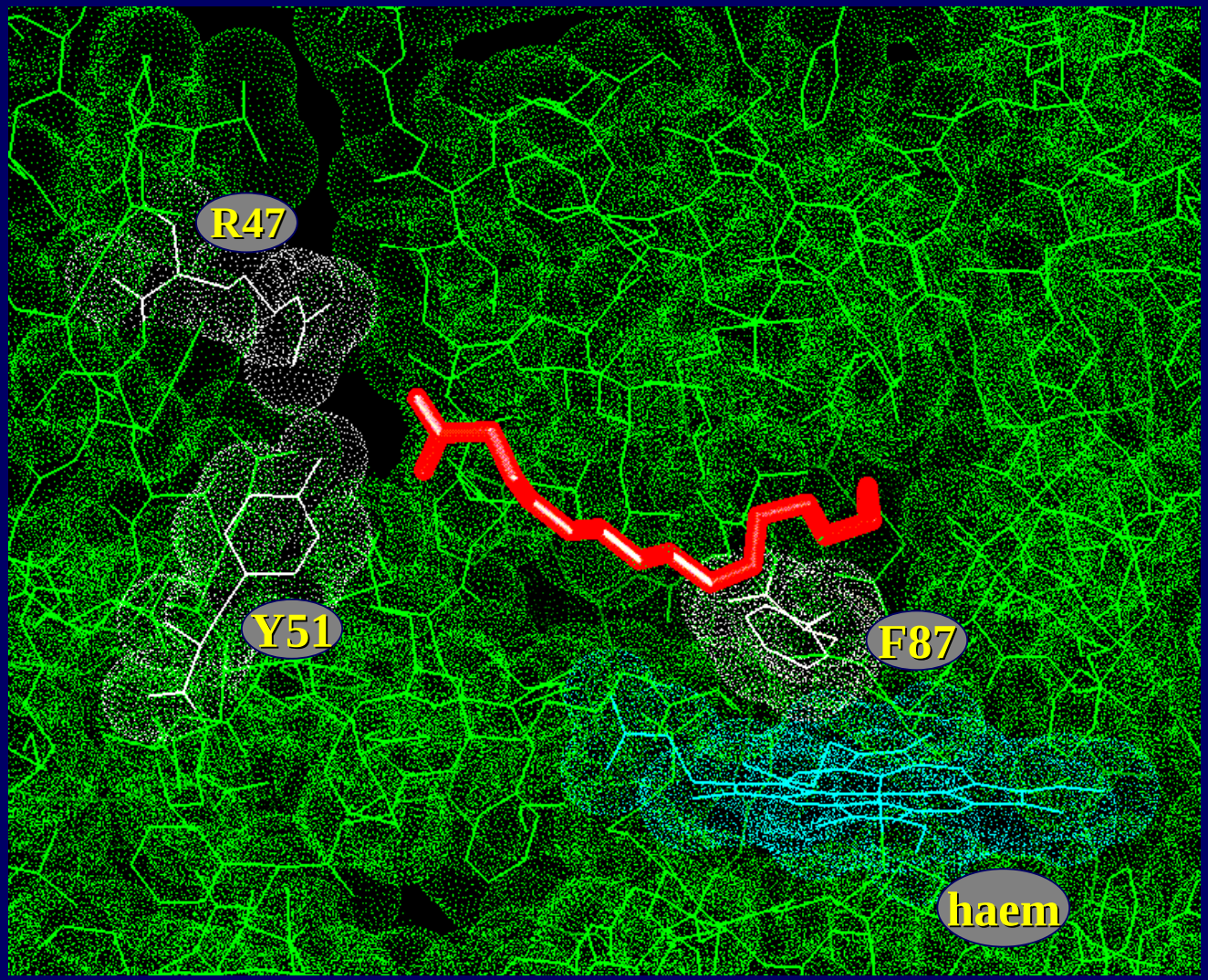
$T_{1,M} \propto r^6$

Cytochrome P450 BM3 active site channel with bound substrate positioned from NMR data



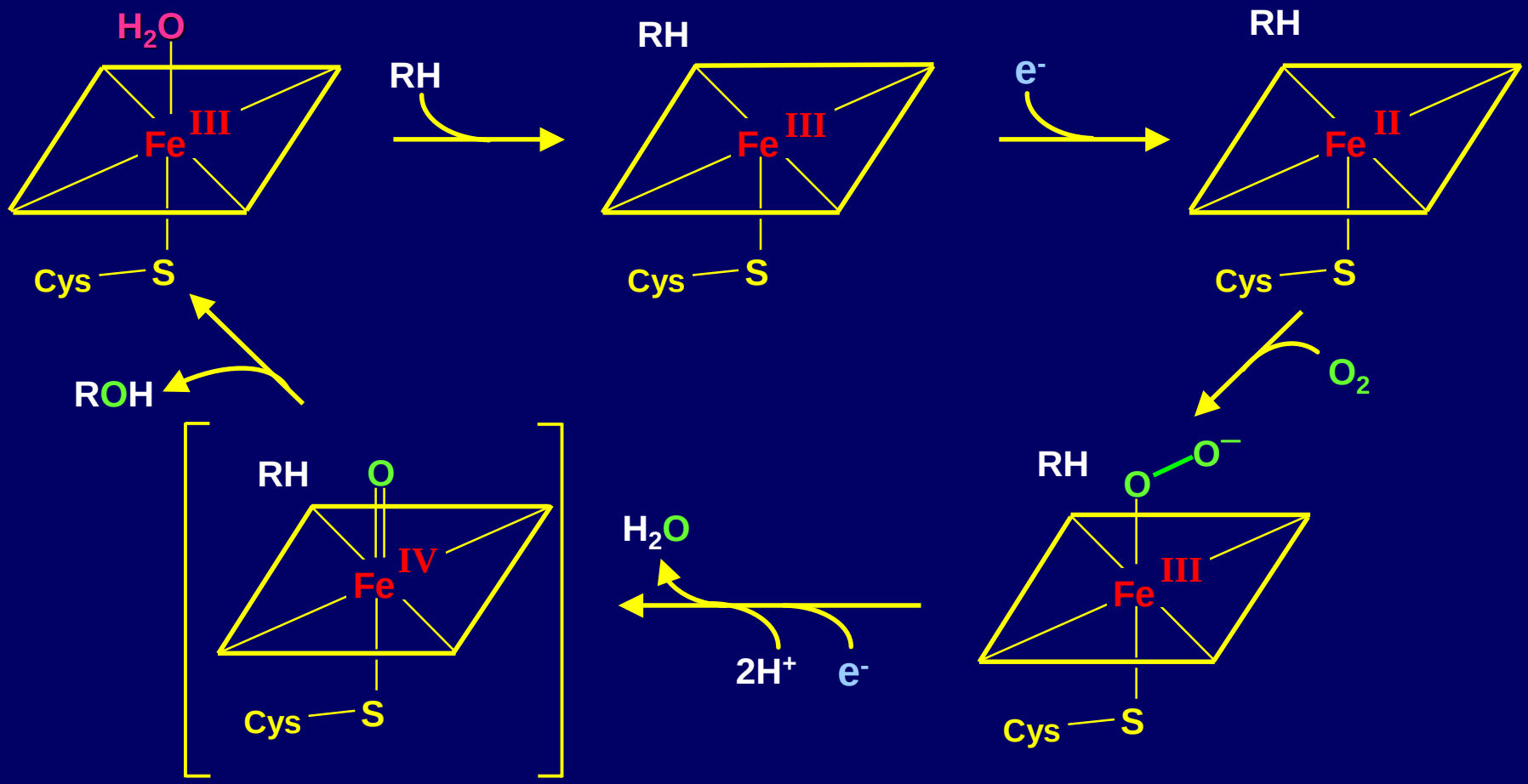
Modi *et al.* (1995) Biochemistry, *34*, 8982

Cytochrome P450 BM3 active site channel with bound substrate



Li & Poulos (1997) Nature Struct. Biol., *4*, 140-146

Cytochrome P450 - catalytic cycle



Substrate-iron distances in oxidised & reduced enzyme

Complex	r (Å)		
	C10	C11	C12
Ferric	9.4	8.9	7.8
Ferrous	3.0	3.1	5.1

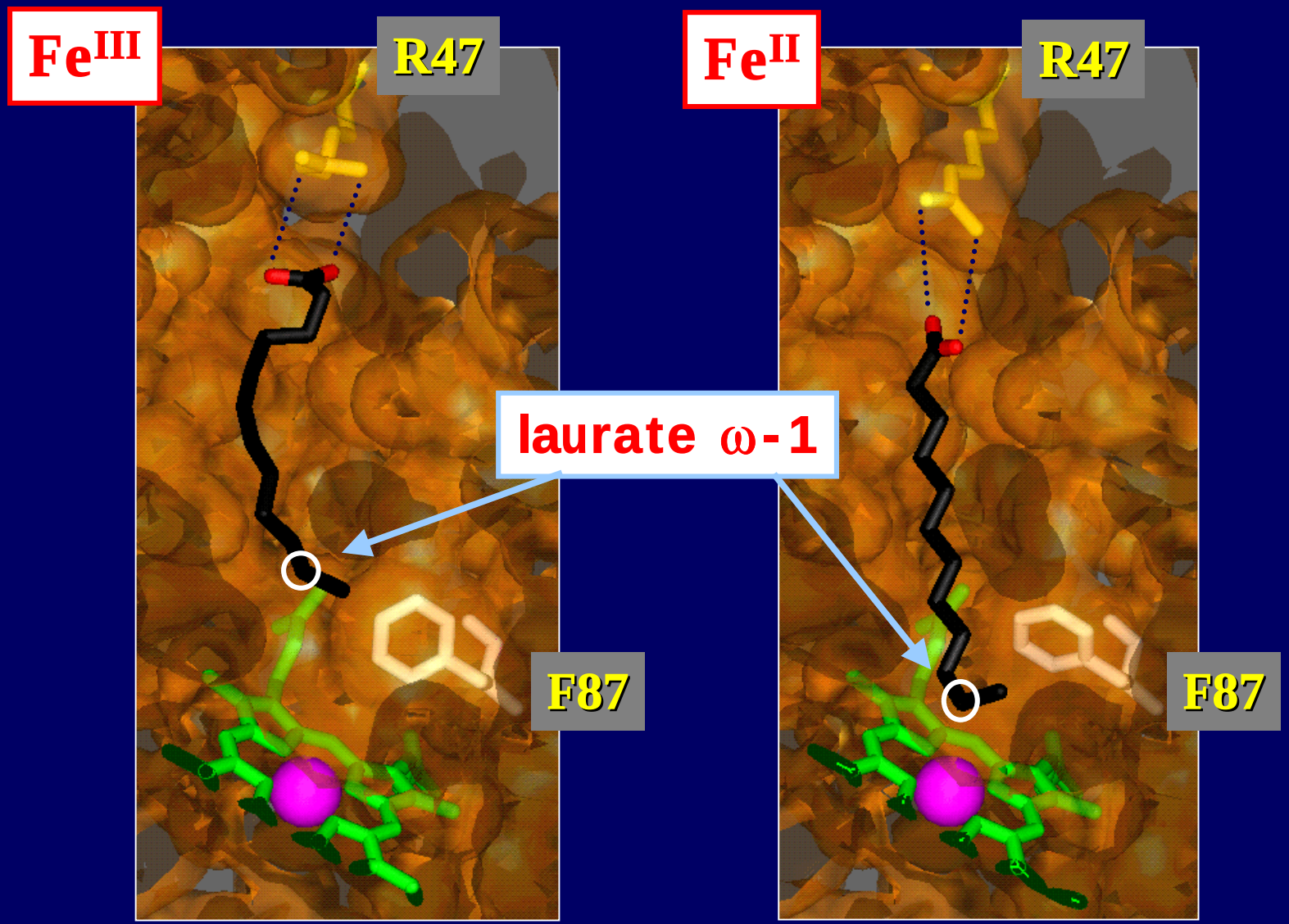
Substrate: 12-bromolaurate

Hydroxylated

Not hydroxylated

Modi *et al.* (1996) Nature Struct. Biol., *3*, 414

P450 BM3 - ferric & ferrous complexes



Altering the regiospecificity: Phe87Ala

Protein	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1}s^{-1}$)	Product ratio ω : ω-1 : ω-2 : ω-3
WT	26	136	$1.9\ 10^5$	0 : 30 : 35 : 35
F87A	25	167	$1.5\ 10^5$	>90 : <5 : <5 : 0

Data for laurate

Oliver *et al.* (1997) Biochemistry, *36*, 1567

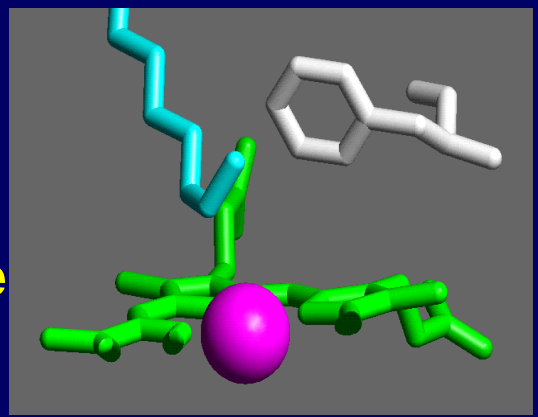
The mutation converts the enzyme from one which specifically suppresses hydroxylation at the ω position to one which specifically favours hydroxylation at this position.

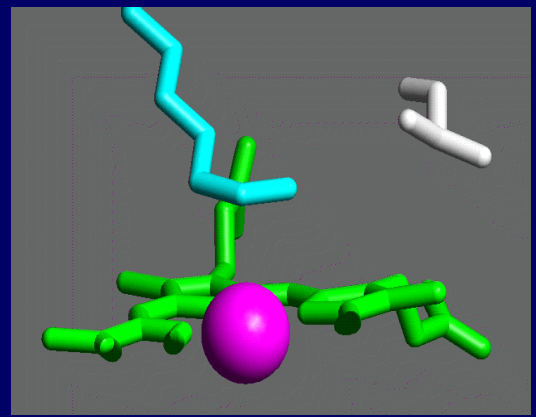
Phe87Ala mutant - altered substrate binding in a catalytic intermediate

	C2	C3	C10	C11	C12
Ferric complex					
Wild-type	16.3 ± 0.2	15.1 ± 0.3	9.4 ± 0.2	8.9 ± 0.2	7.8 ± 0.2
F87A	16.1 ± 0.1	15.5 ± 0.1	9.1 ± 0.2	8.6 ± 0.2	7.7 ±0.2
Ferrous complex					
Wild-type	-	-	3.0 ± 0.1	3.1 ± 0.1	5.1 ± 0.1
F87A	-	-	2.9 ± 0.06	3.1 ± 0.07	3.1 ± 0.05

Ferrous complex

Wild-type





Phe87Ala