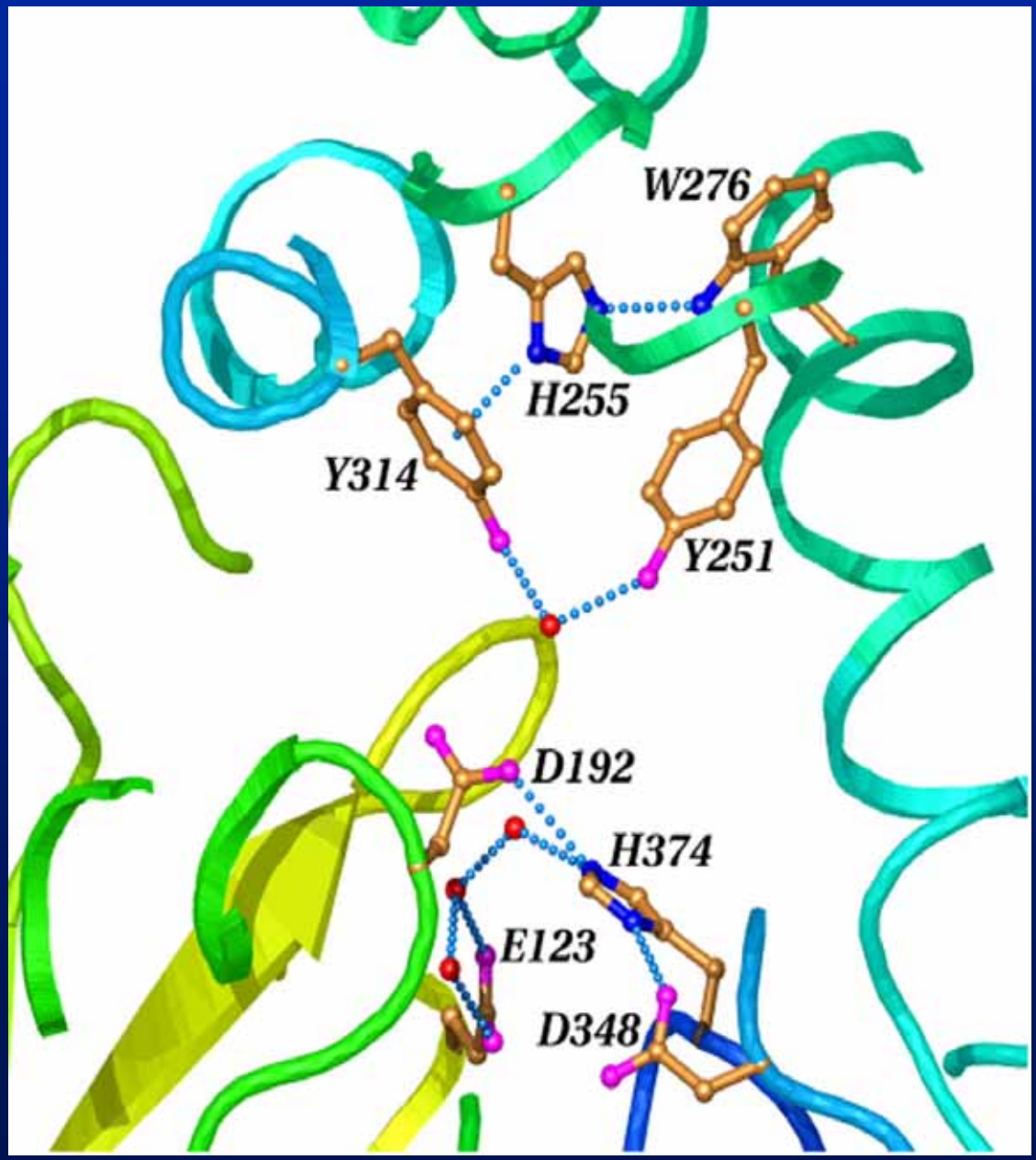
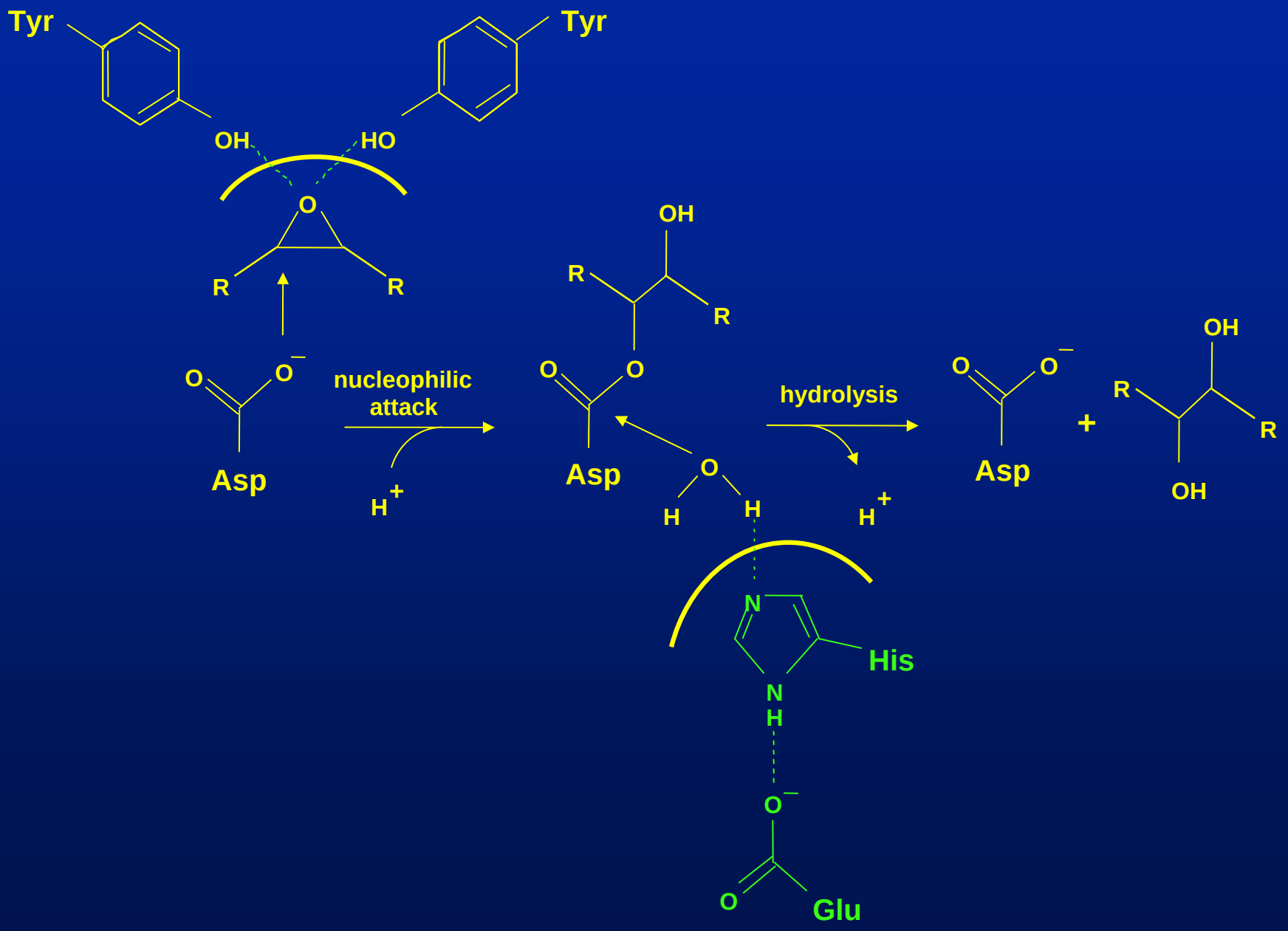
Types of Electrophilic Carcinogenic Intermediates

Procarcinogens	Reactive intermediates	Enzymes involved in control
Aromatic hydrocarbons	Epoxides	Cytochromes P450 Glutathione S-transferases Epoxide hydrolases
Aromatic amines	Reactive esters	Cytochromes P450 Sulfotransferases Acetyltransferases UDP-Glucuronosyltransferases Glutathione S-transferases
Dialkylnitrosamines	Carbonium ions/ electron-deficient alkyl groups	Cytochromes P450
Vicinal dihaloalkanes	Episulfonium ions	Glutathione S-transferases

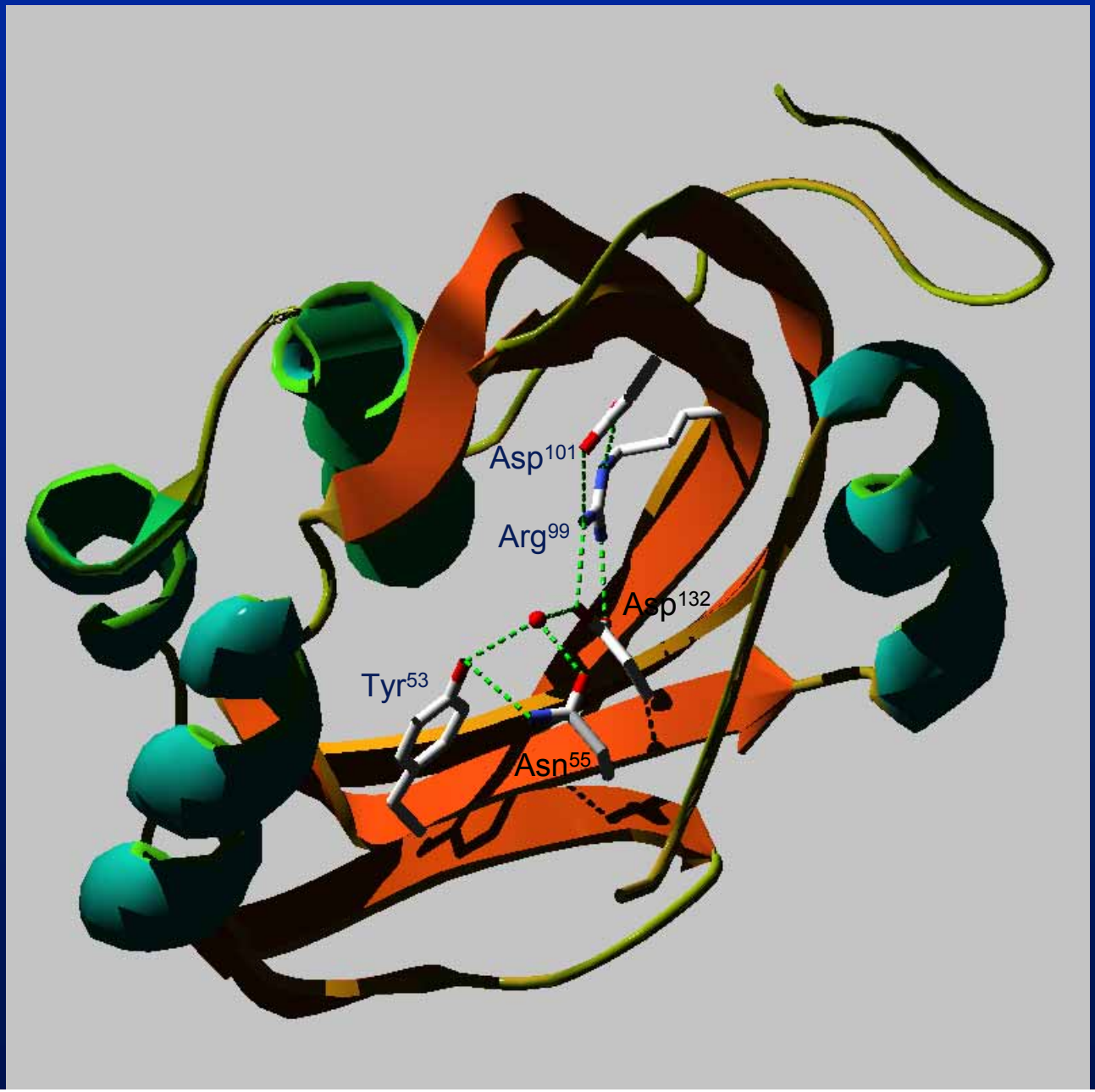
The Active Site of *Aspergillus niger* mEH



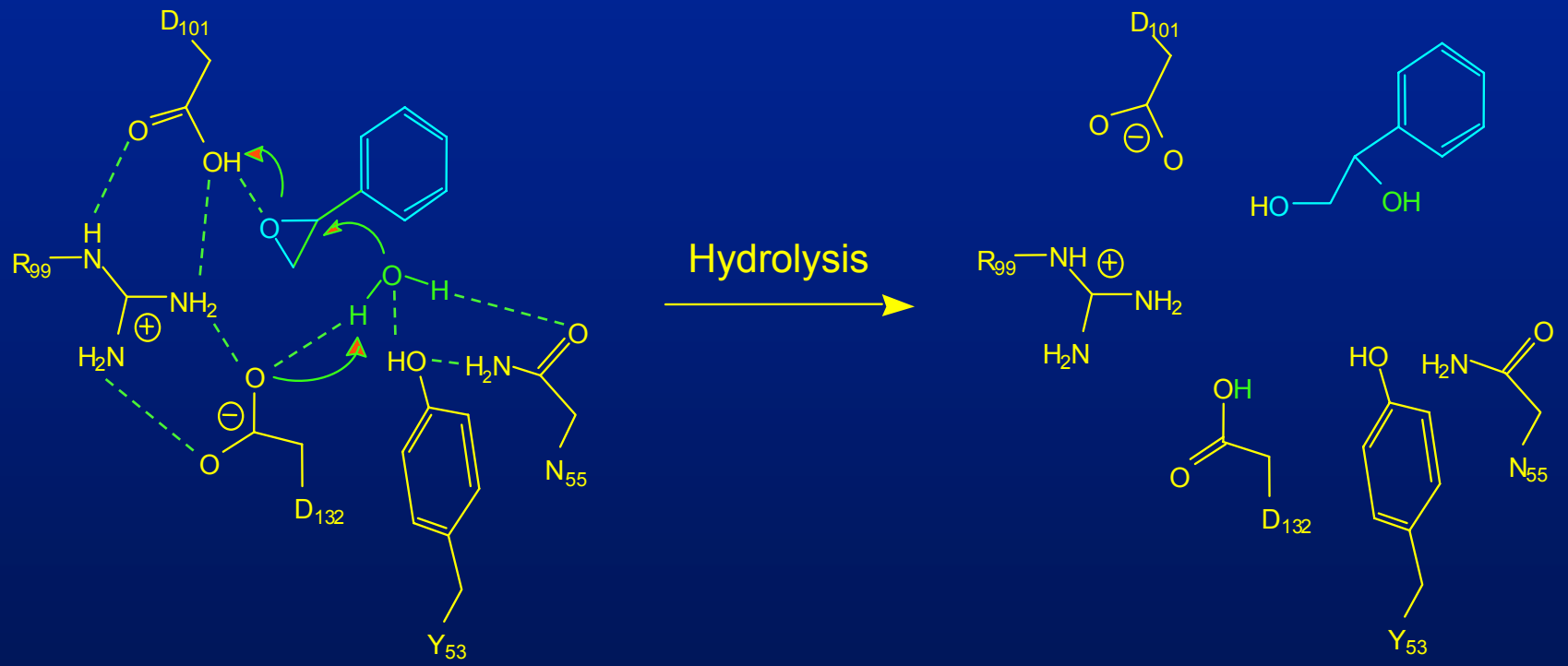
Extended Enzymatic Mechanism of Microsomal Epoxide Hydrolase



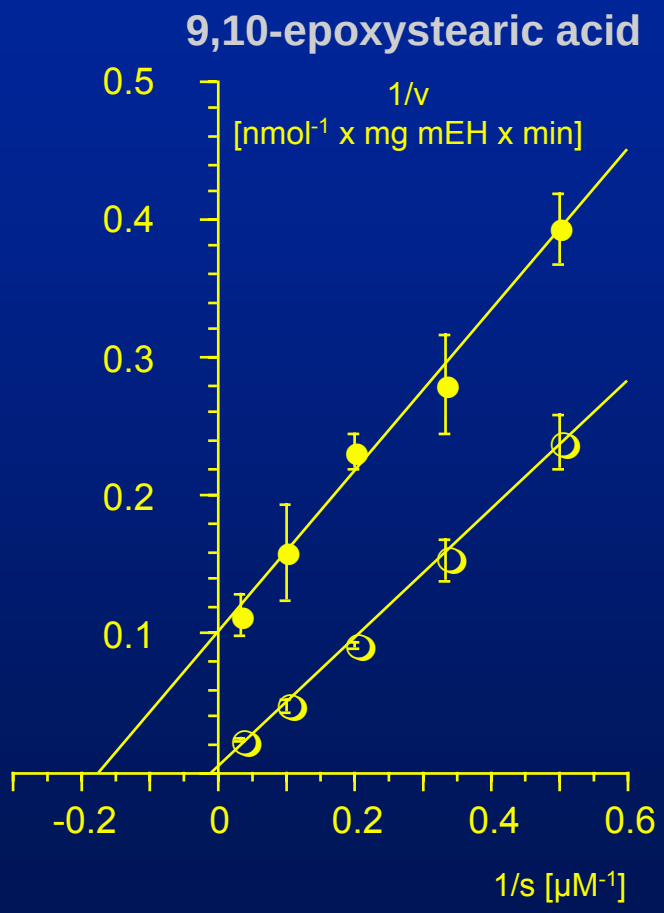
3D Structure of Bacterial Limonene Epoxide Hydrolase



Enzymatic Mechanism of Limonene Epoxide Hydrolase



Kinetic Analysis of mEH Mutant $Glu_{404}Asp$



● = wild type mEH

❍ = mEH $Glu_{404}Asp$

	$K_{M\ [\mu M]}$	$V_{max\ [nmol/mg/min]}$
Wild type mEH	6	9
mEH $Glu_{404}Asp$	180	330

Relationship between K_M, K_D and Rate Constants of the mEH Enzymatic Mechanism

$$E + S \underset{}{\overset{K_D}{\rightleftharpoons}} E\,S \xrightarrow{k_1} E \bullet S \xrightarrow{k_2} E + P$$

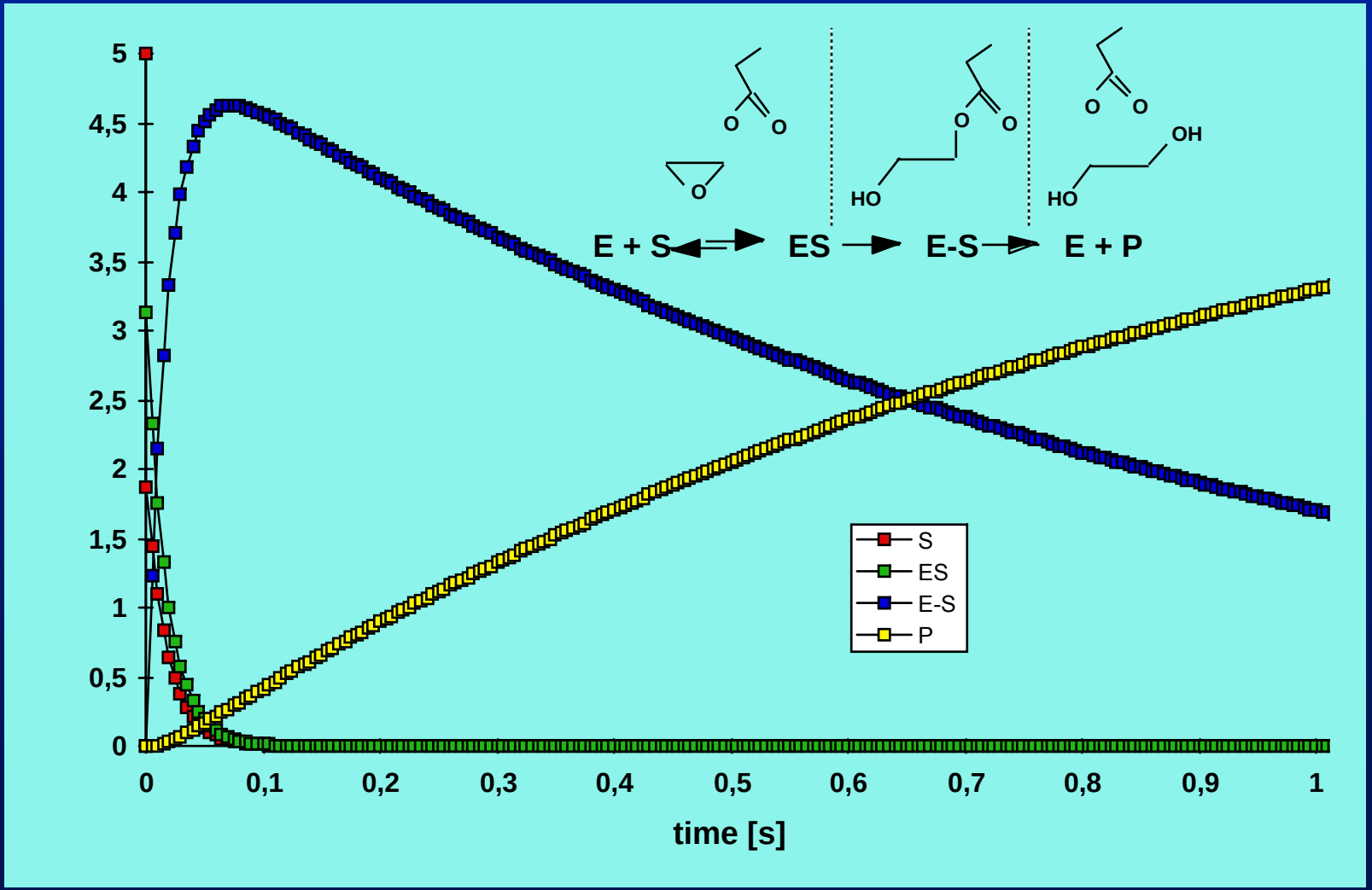
$$K_M = K_D \bullet \frac{k_2}{k_1 + k_2}$$

$$k_1 << k_2 \quad => \quad \frac{k_2}{k_1 + k_2} \approx 1 \quad => \quad K_M = K_D ; V_{max} \sim k_1$$

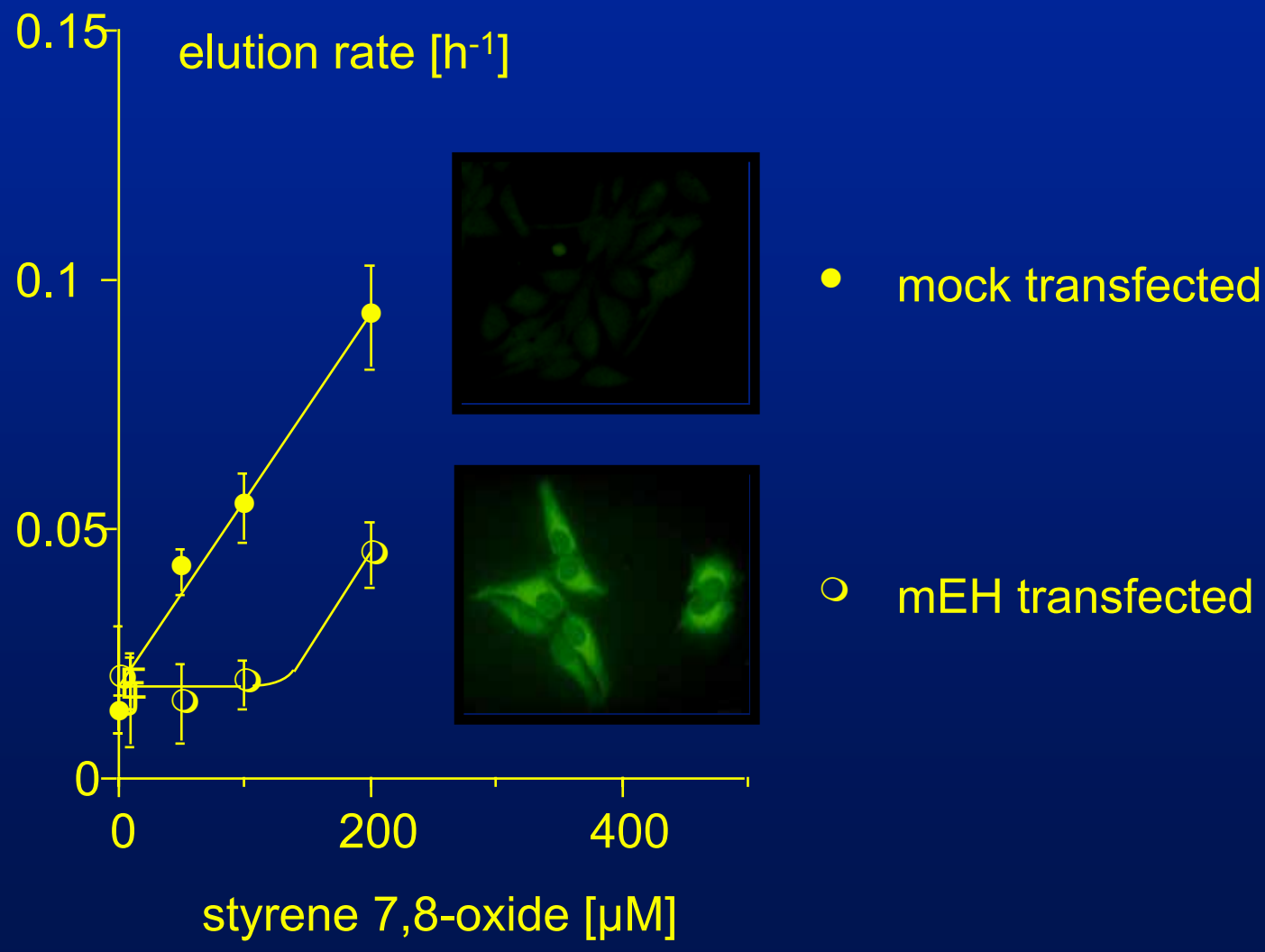
$$k_1 >> k_2 \quad => \quad \frac{k_2}{k_1 + k_2} \approx \frac{k_2}{k_1} \quad => \quad \frac{K_M}{K_D} = \frac{k_2}{k_1} ; \quad V_{max} \sim k_2$$

$$k_1 = k_2 \quad => \quad \frac{k_2}{k_1 + k_2} = \frac{1}{2} \quad => \quad K_M = \frac{1}{2} \bullet K_D ; \; V_{max} \sim k_1, k_2$$

Kinetics of Enzymatic Epoxide Hydrolysis



Threshold of the Genotoxic Effect of Styrene 7,8-oxide in hmEH-transgenic V79 Chinese Hamster Lung Fibroblasts



Conclusions

- **Xenobiotic-metabolizing EHs are α/β hydrolase fold enzymes working with a catalytic triad**
- **The first step of enzymatic epoxide hydrolysis is optimized for speed, allowing efficient detoxification with broad substrate specificity**
- **Experimental verification showed that this high speed detoxication introduces a practical threshold of genotoxicity, at least for the epoxides investigated**

Alwyn Jones
Sherry Mowbray
Terese Bergfors
Martin Hallberg
Jinyu Zou

Roland Furstoss
Jacques Baratti
Christophe Morisseau

Dick Janssen
Manfred Reetz

Ari Hirvonen
Marek Jakubowski
Dominique Lison
Vincent Haufroid
Pavel Vodicka
& Colleagues

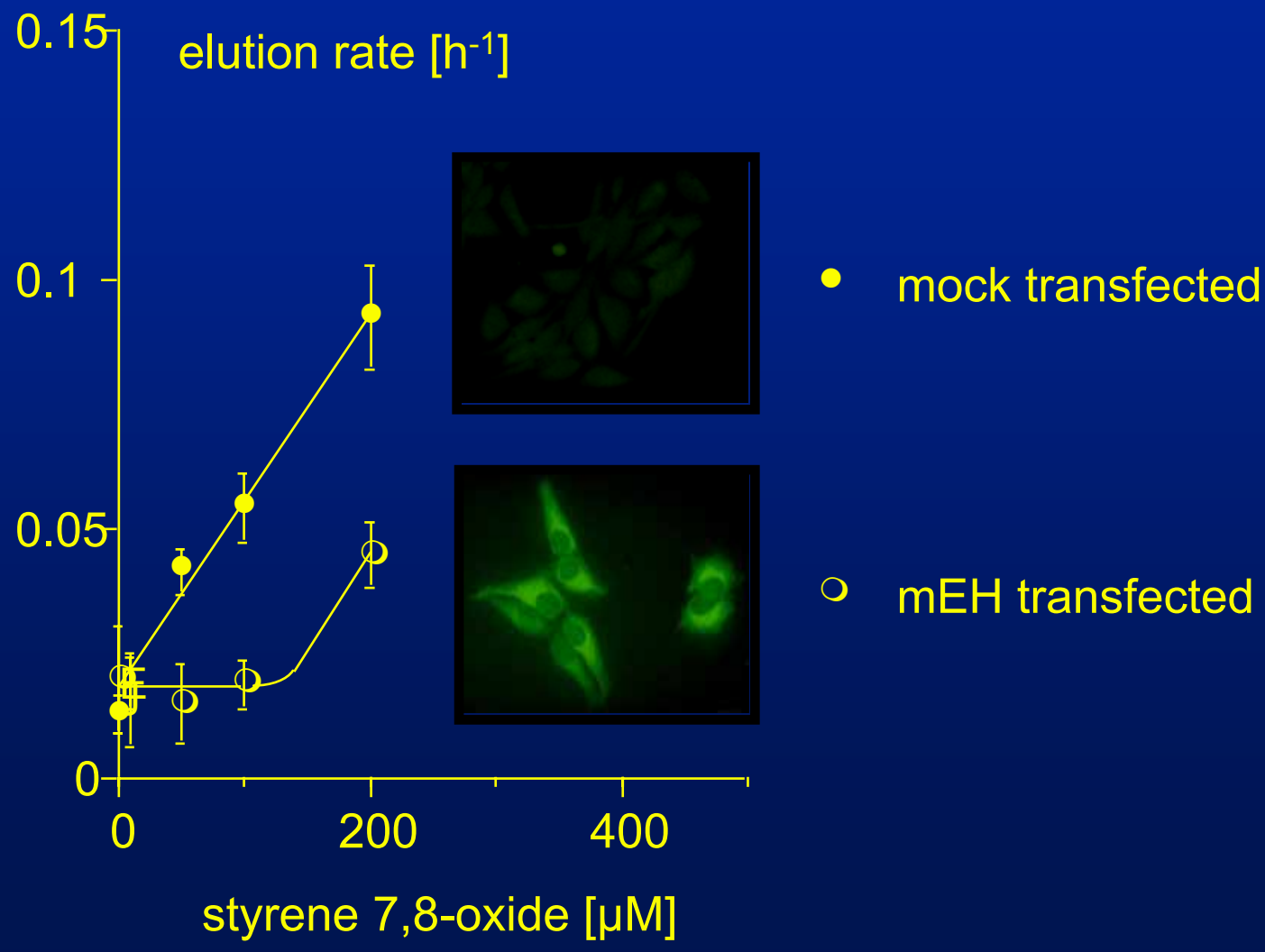
Michael Arand
Annette Cronin
Heike Dürk
Karen Hänel
Jan Georg Hengstler
Maria Elena Herrero
Shirli Homburg
Michael Knehr
Matthias Lohmann
Astrid Mecky
Frank Müller
Heike Nagel

EU Network of Excellence
ECNIS

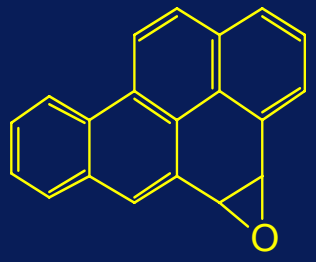
Bruce Hammock
Jeff Beetham
David Grant
Christophe Morisseau

Richard Armstrong
Conny Cassidy

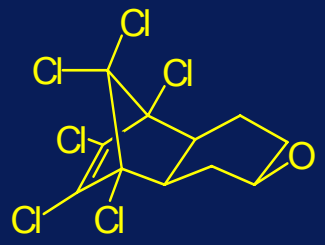
Threshold of the Genotoxic Effect of Styrene 7,8-oxide in hmEH-transgenic V79 Chinese Hamster Lung Fibroblasts



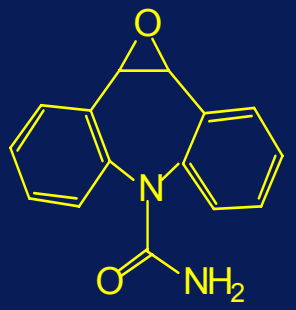
“Typical” mEH Substrates



benzo[a]pyrene 4,5-oxide

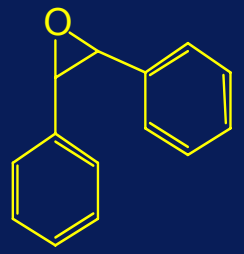


HEOM



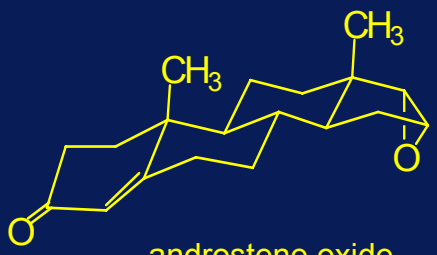
carbamazepine 10,11-ox

cis-9,10-epoxystearic acid



cis-Stilbene oxide

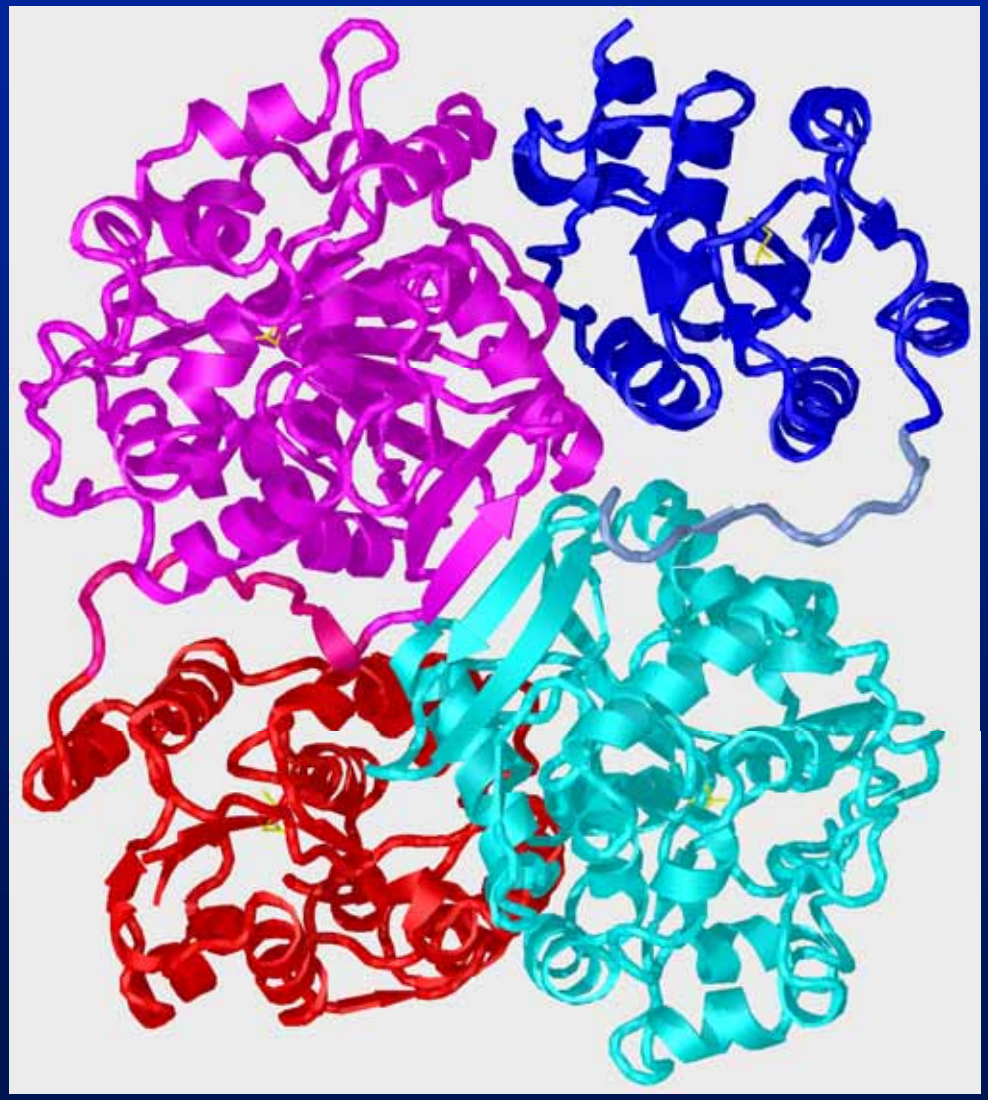
styrene 7,8-oxide



androstene oxide

octane-1,2-epoxid

Mouse sEH Structure



EH from Agrobacterium at 2.1 Å

Nardini et al., J.Biol.Chem. 1999

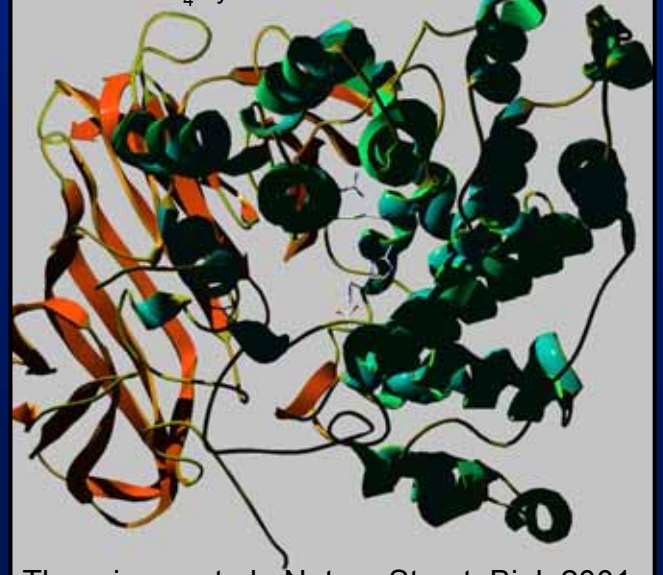
sEH from mouse at 2.8 Å

Argiriadi et al., PNAS 1999

EH from Aspergillus at 1.7 Å

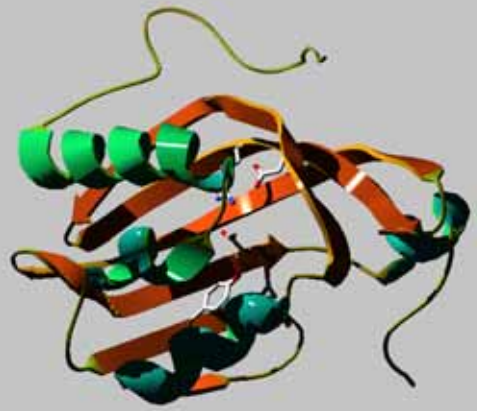
Zou et al., Structure 2000

Leukotriene A_4 hydrolase from human at 1.95 Å



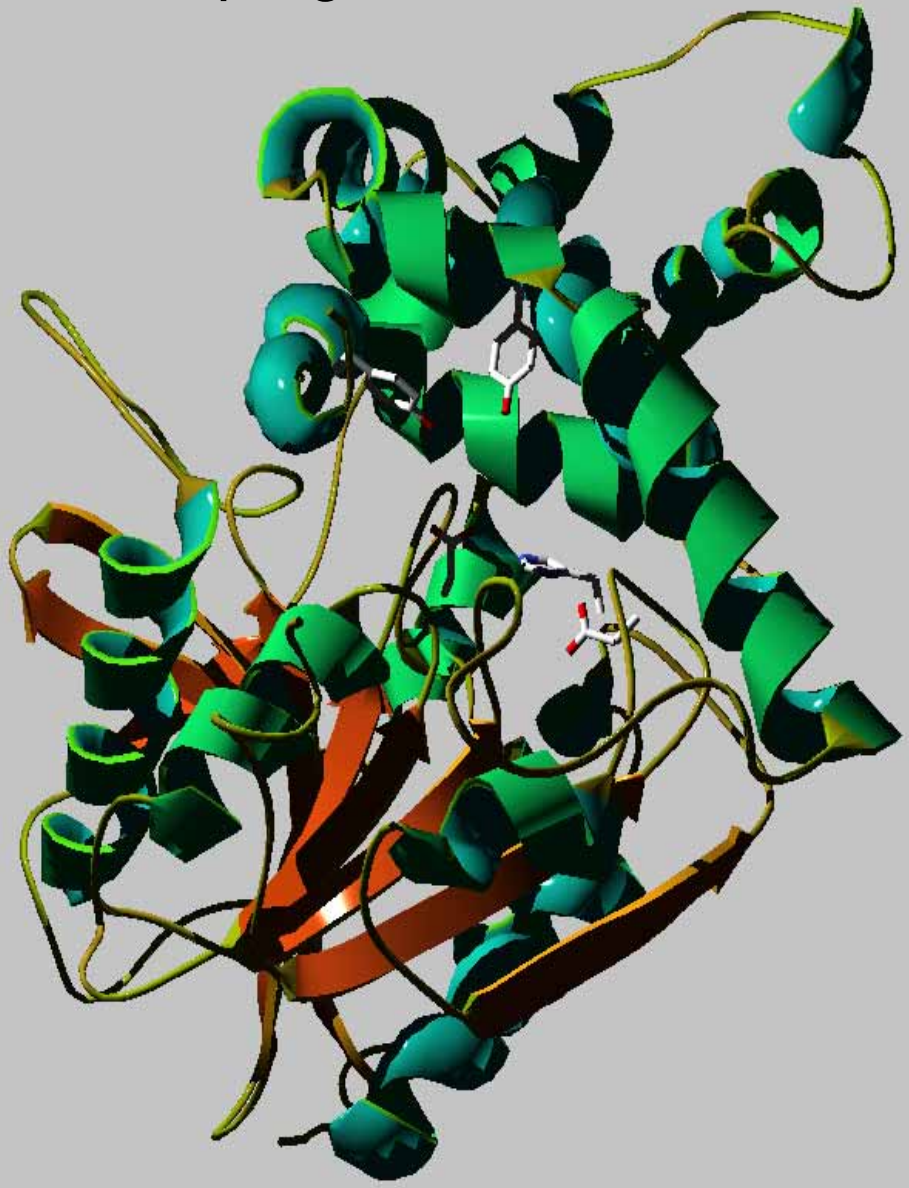
Thunnissen et al., Nature Struct. Biol. 2001

EH from Rhodococcus at 1.2 Å

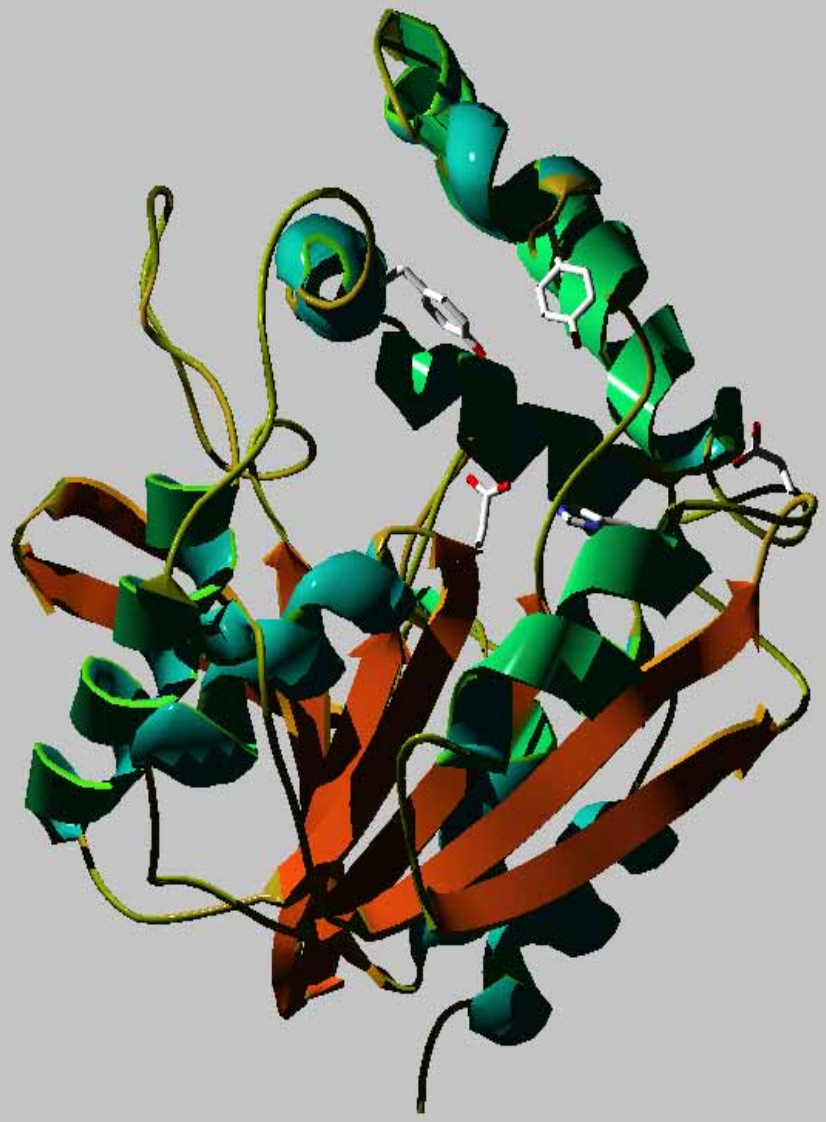


Arand et al., EMBO J., in press

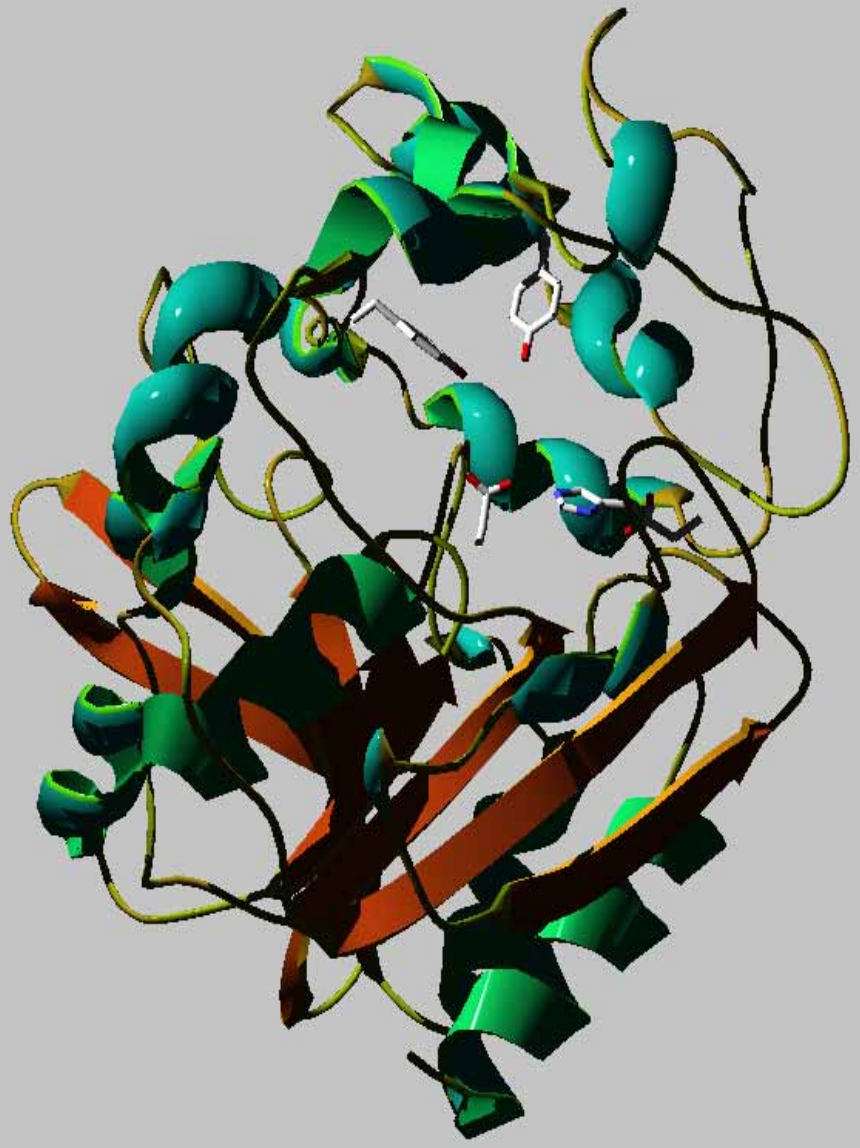
EH from Aspergillus

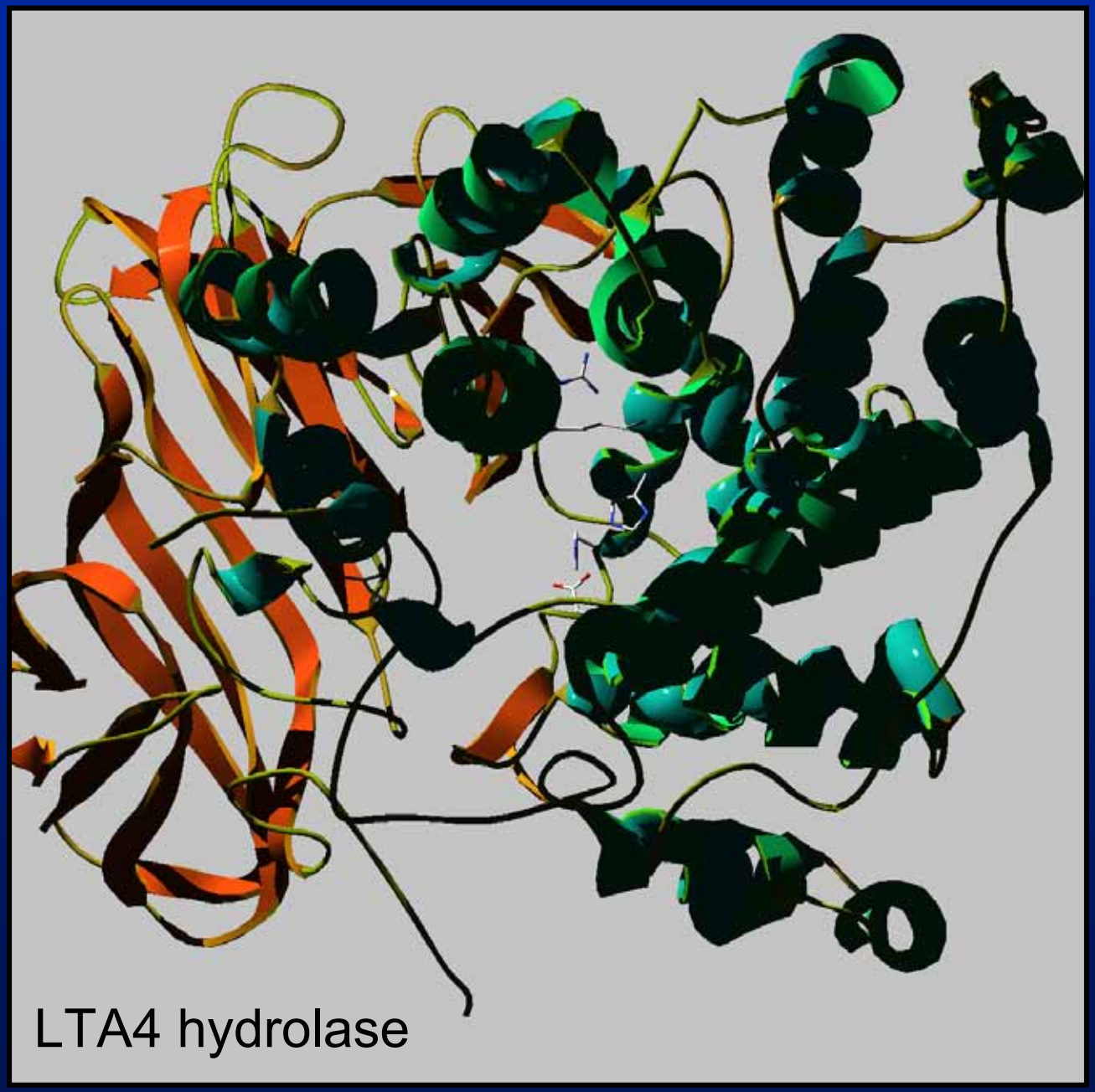


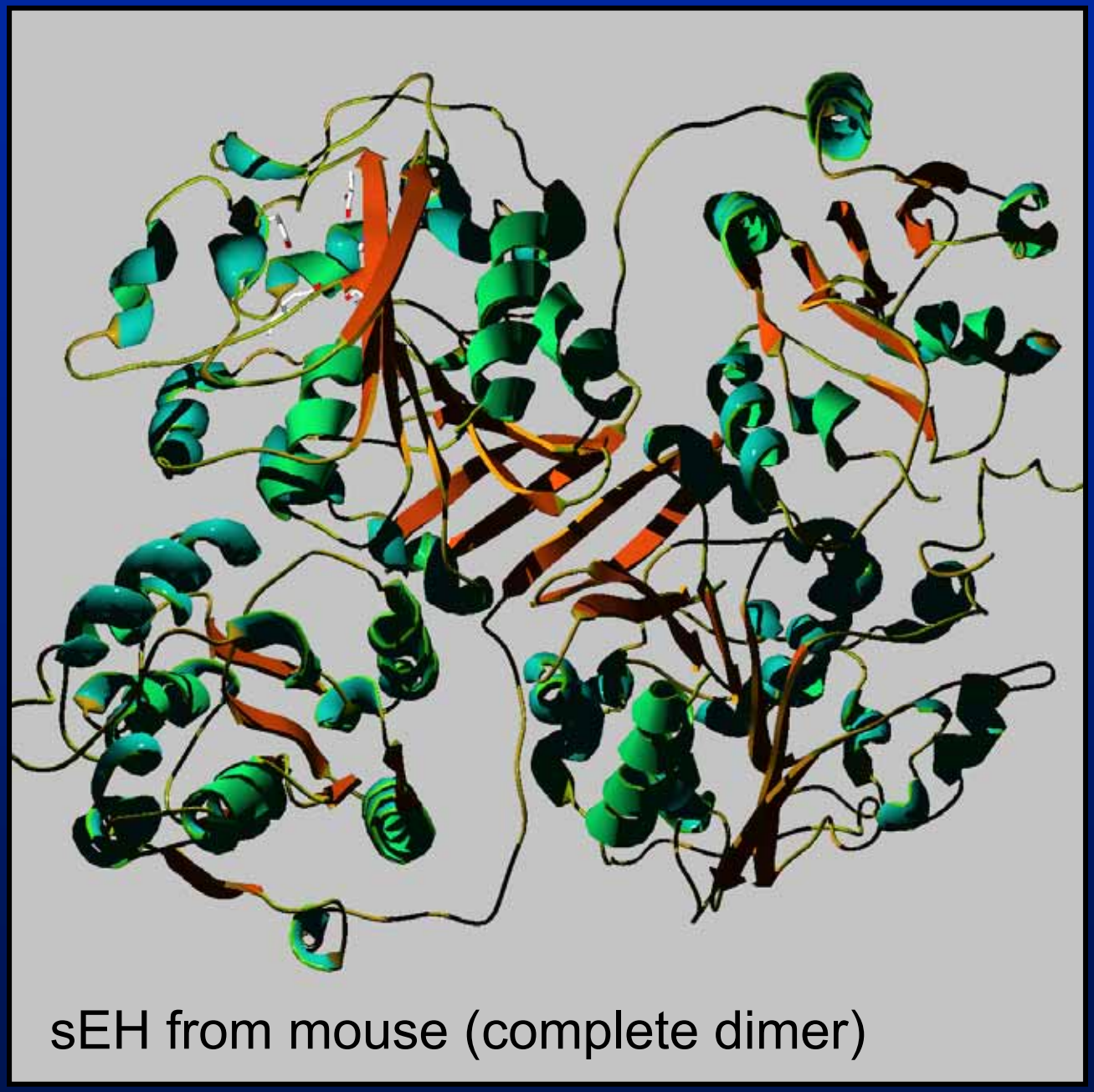
EH from Agrobacterium



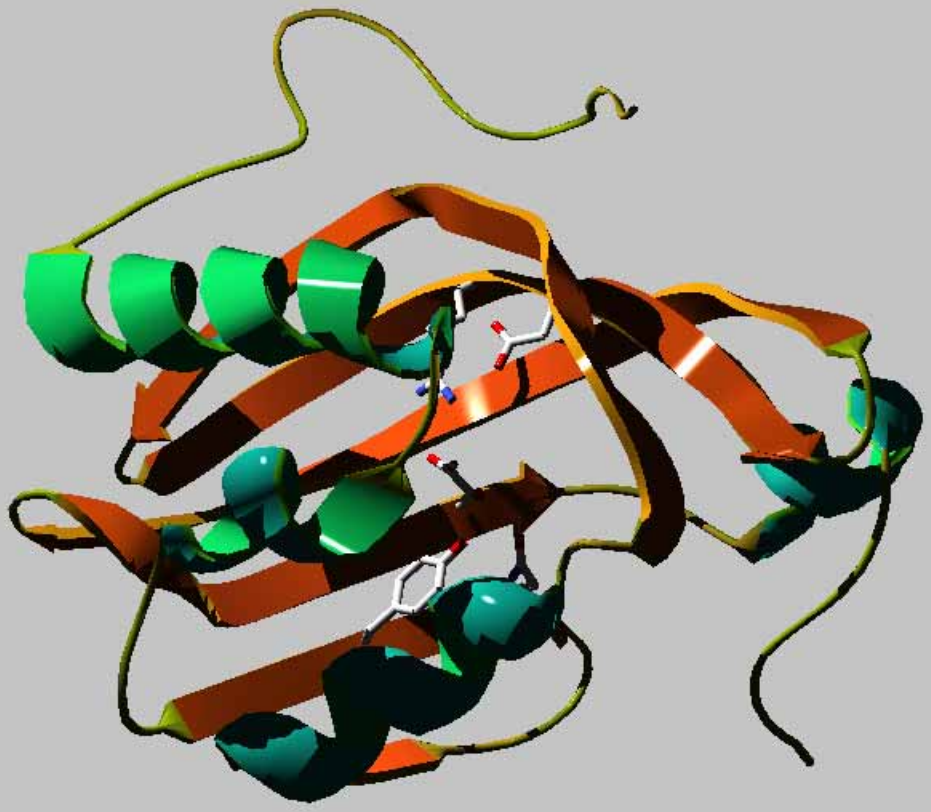
sEH from mouse (only the C-terminus displayed)



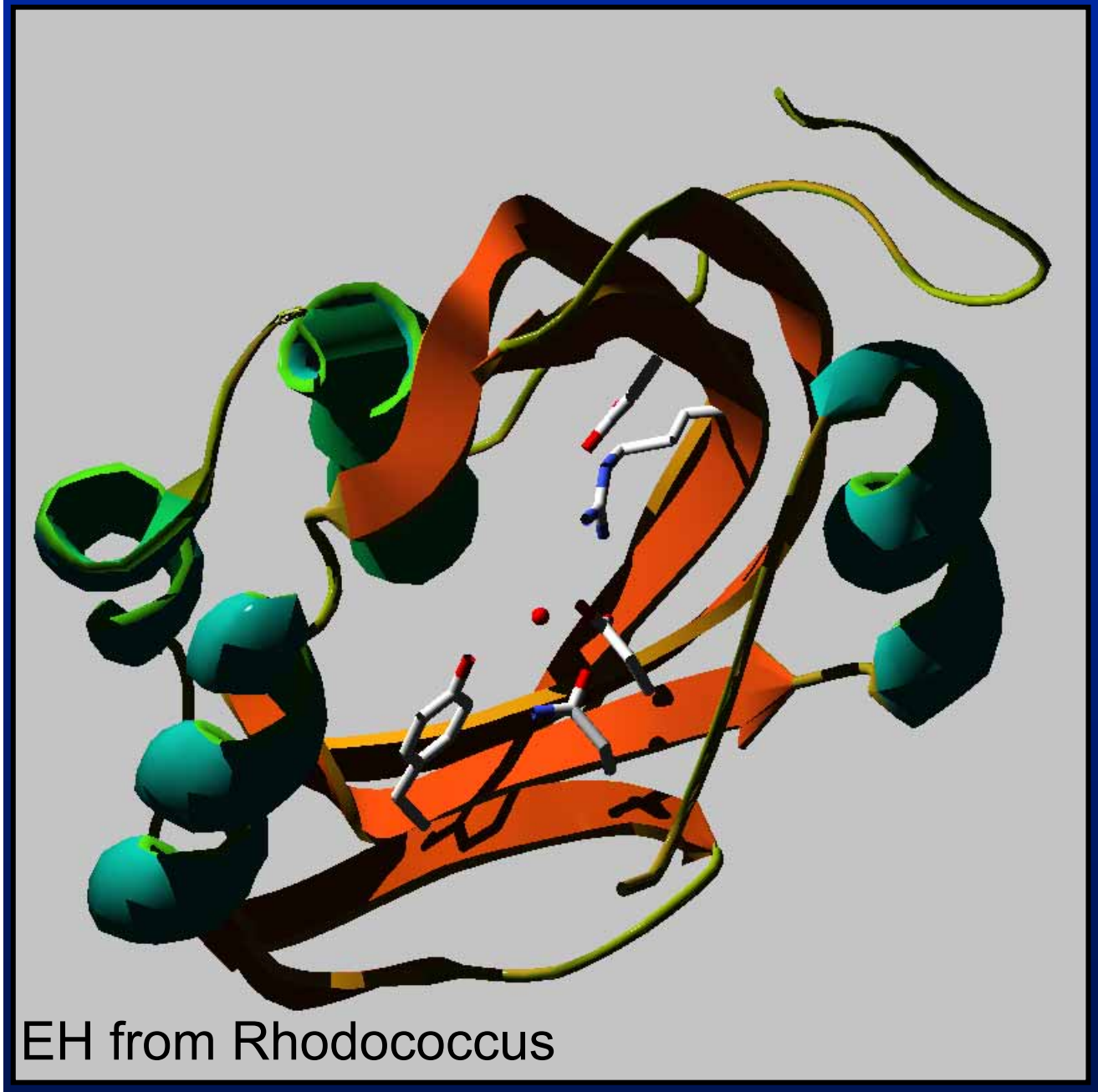

LTA4 hydrolase



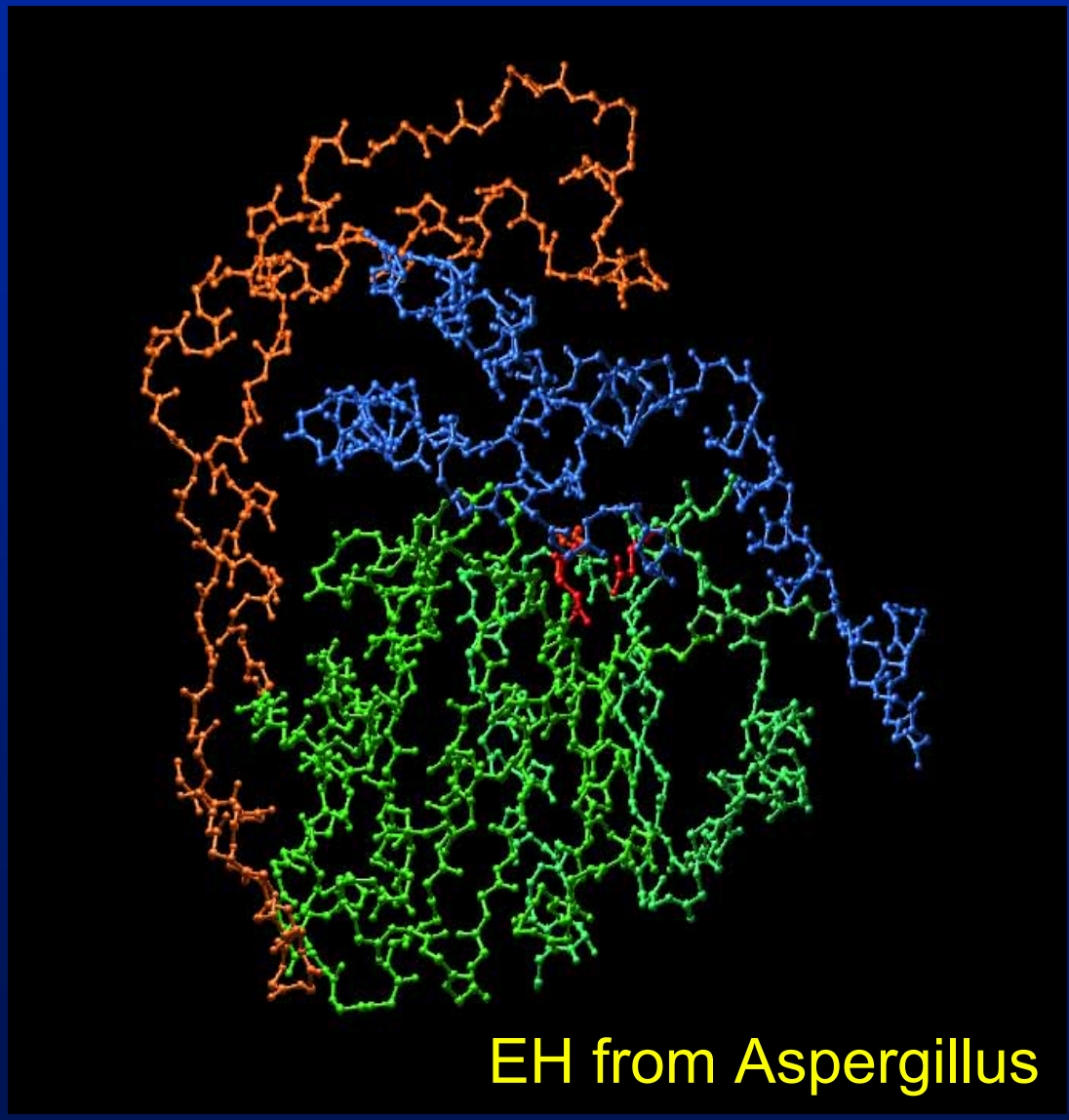
sEH from mouse (complete dimer)

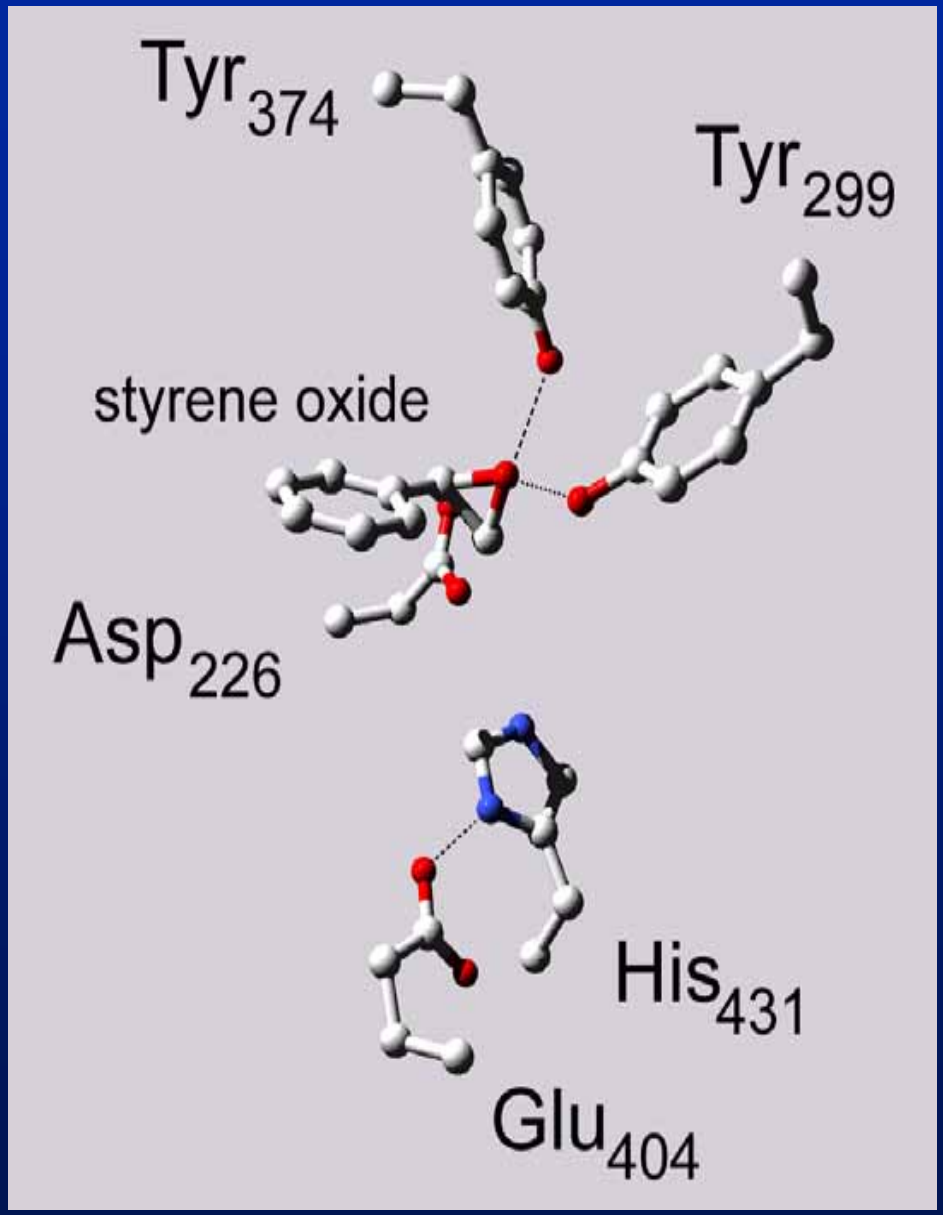


EH from Rhodococcus

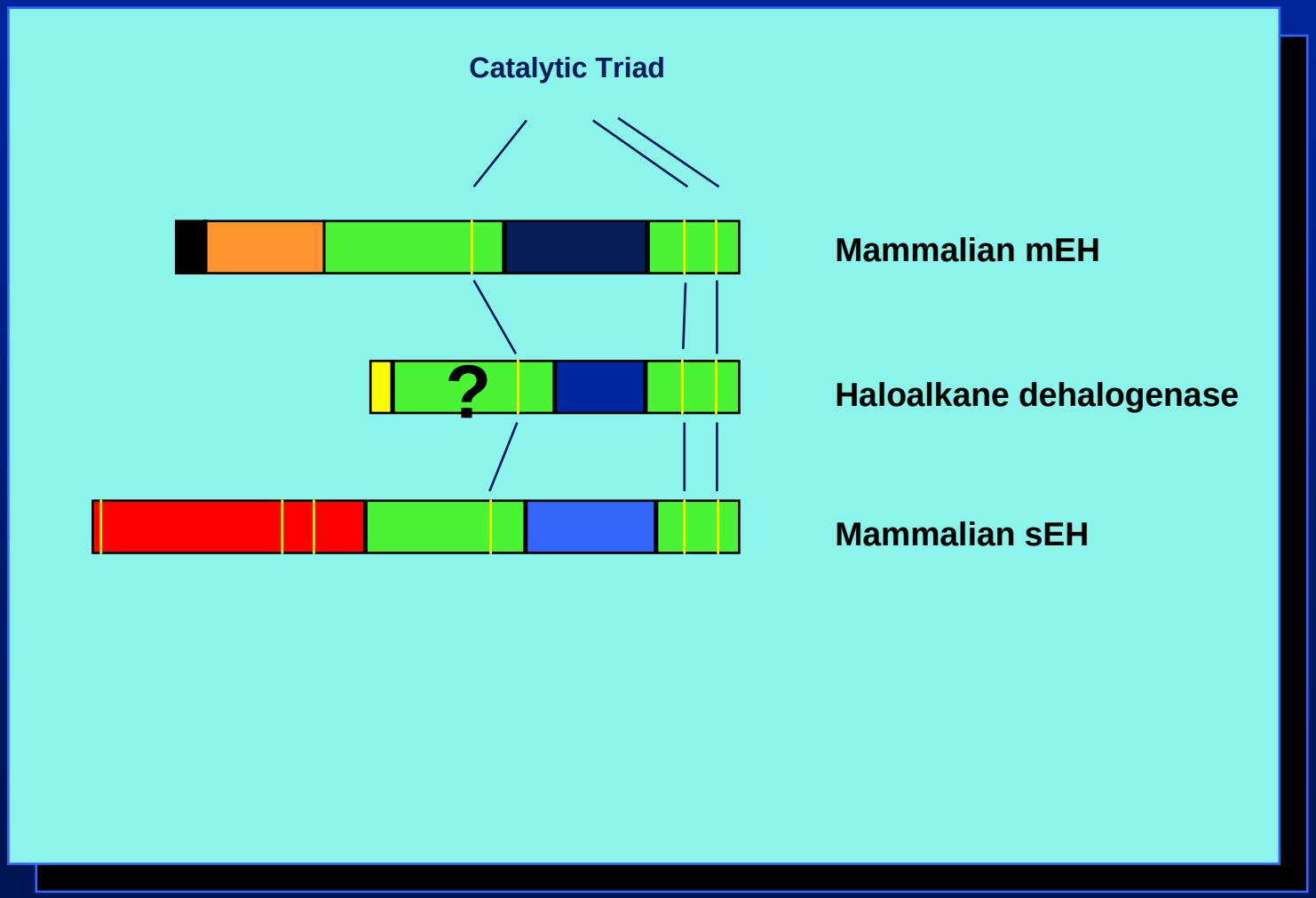


EH from Rhodococcus

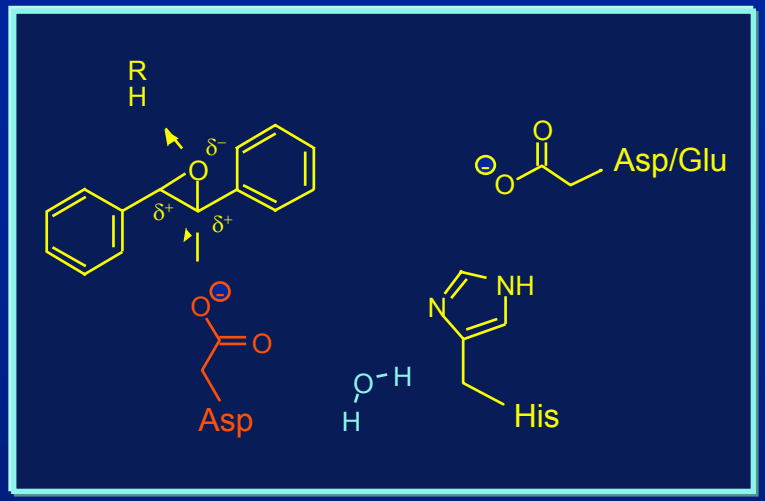

EH from Aspergillus

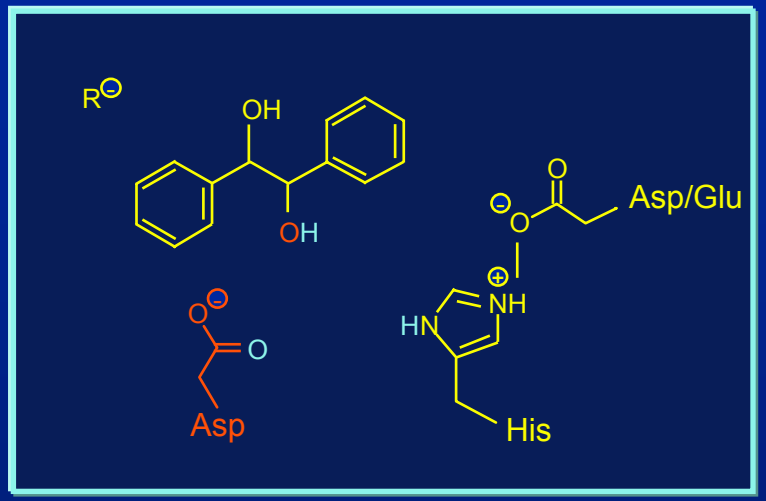

Tyr374
Tyr299
styrene oxide
Asp226
His431
Glu404

Structural Relationship between Mammalian Epoxide Hydrolases and other Enzymes Related by Sequence Similarity

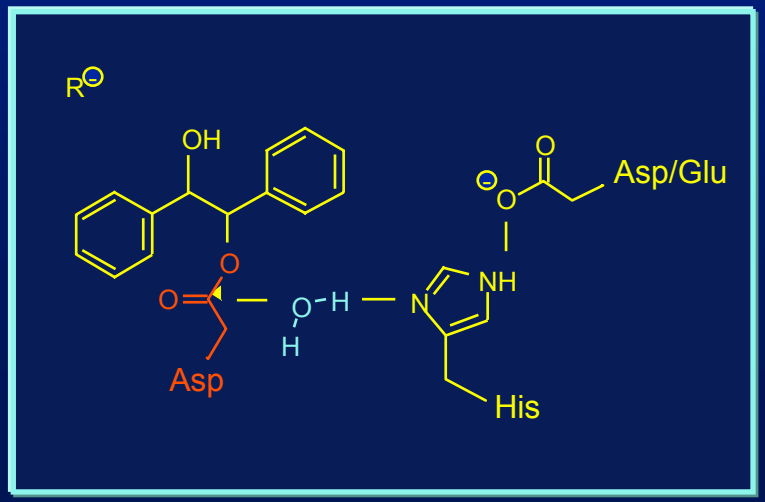


Enzymatic Mechanism of Epoxide Hydrolysis





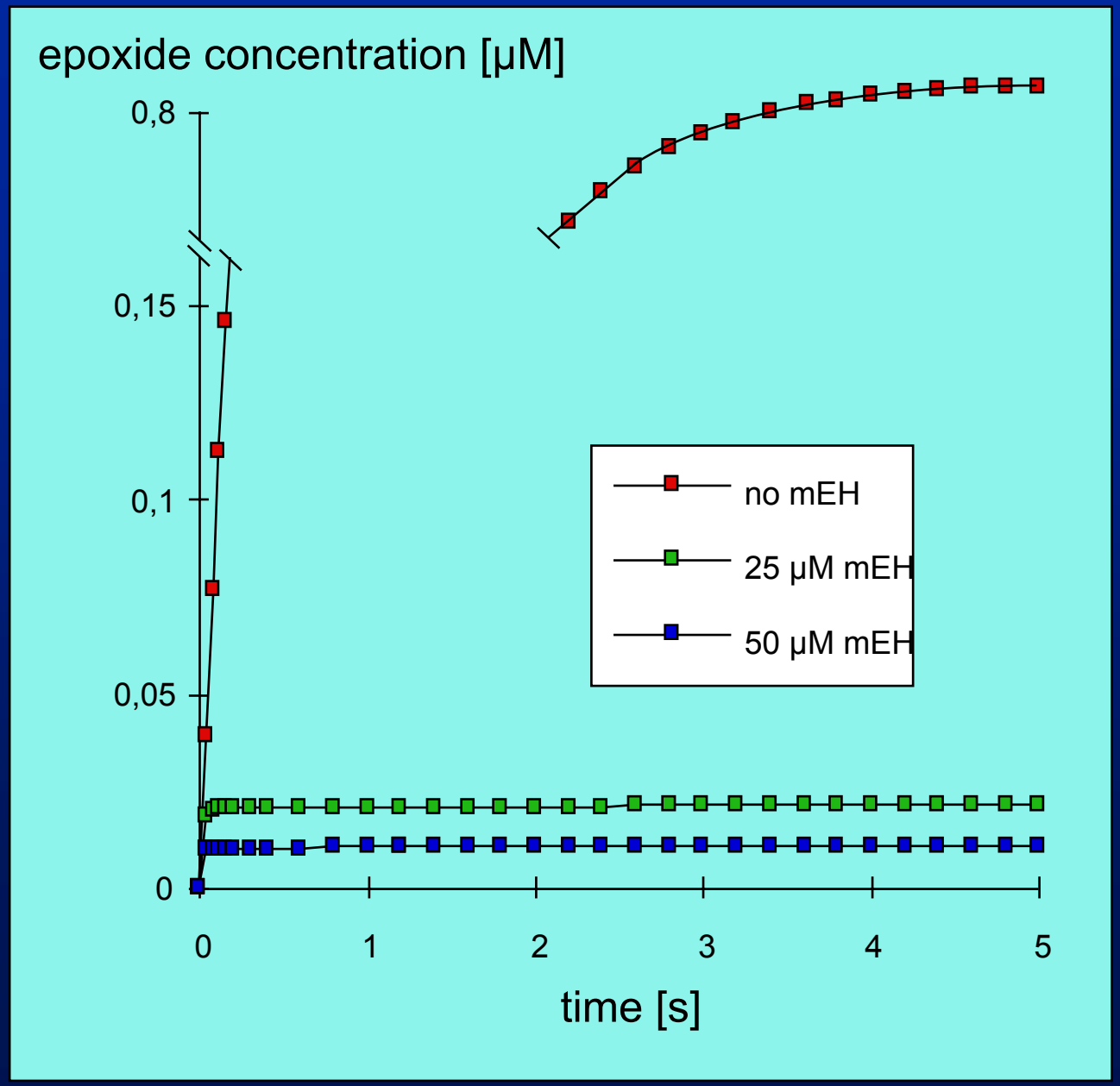
Step 1
Nucleophilic Attack



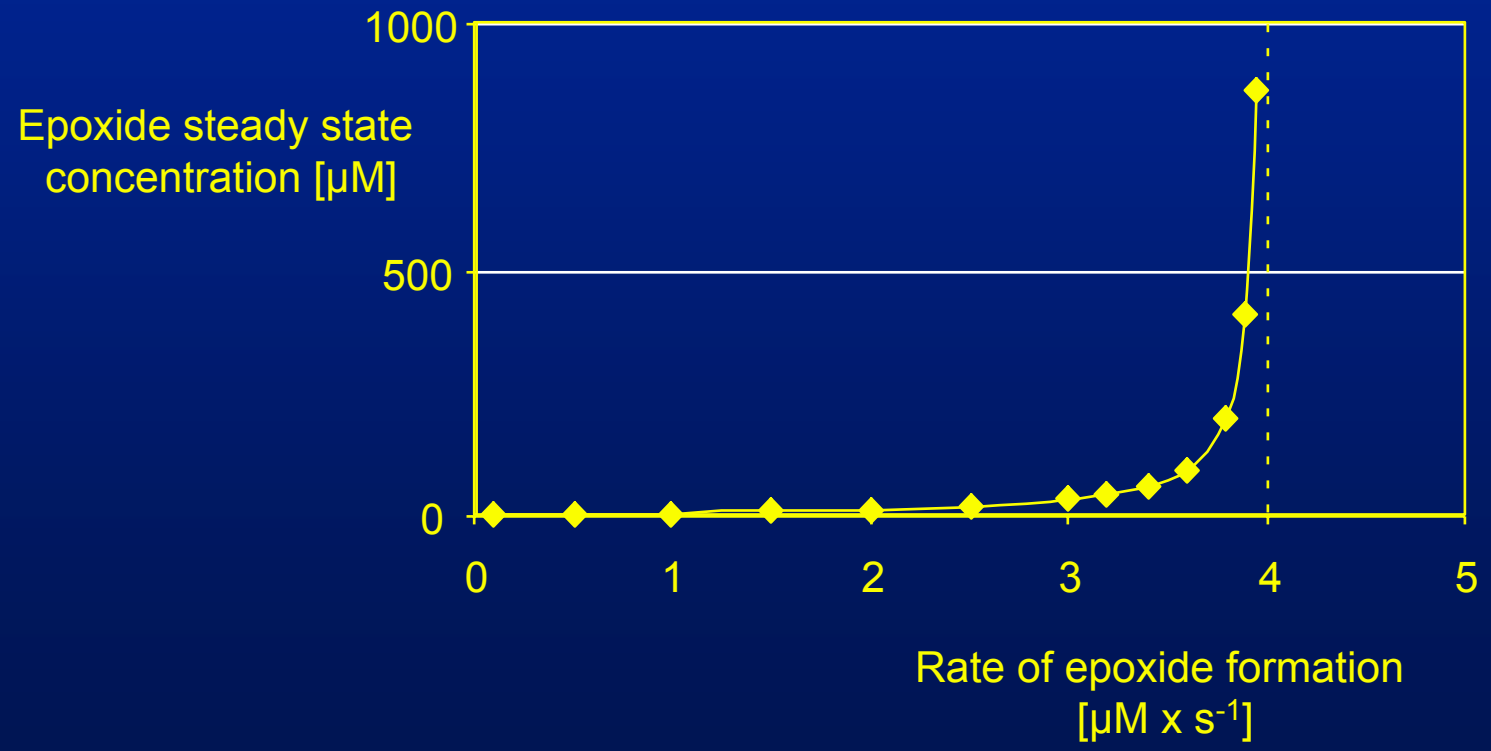
Step 2
Hydrolysis

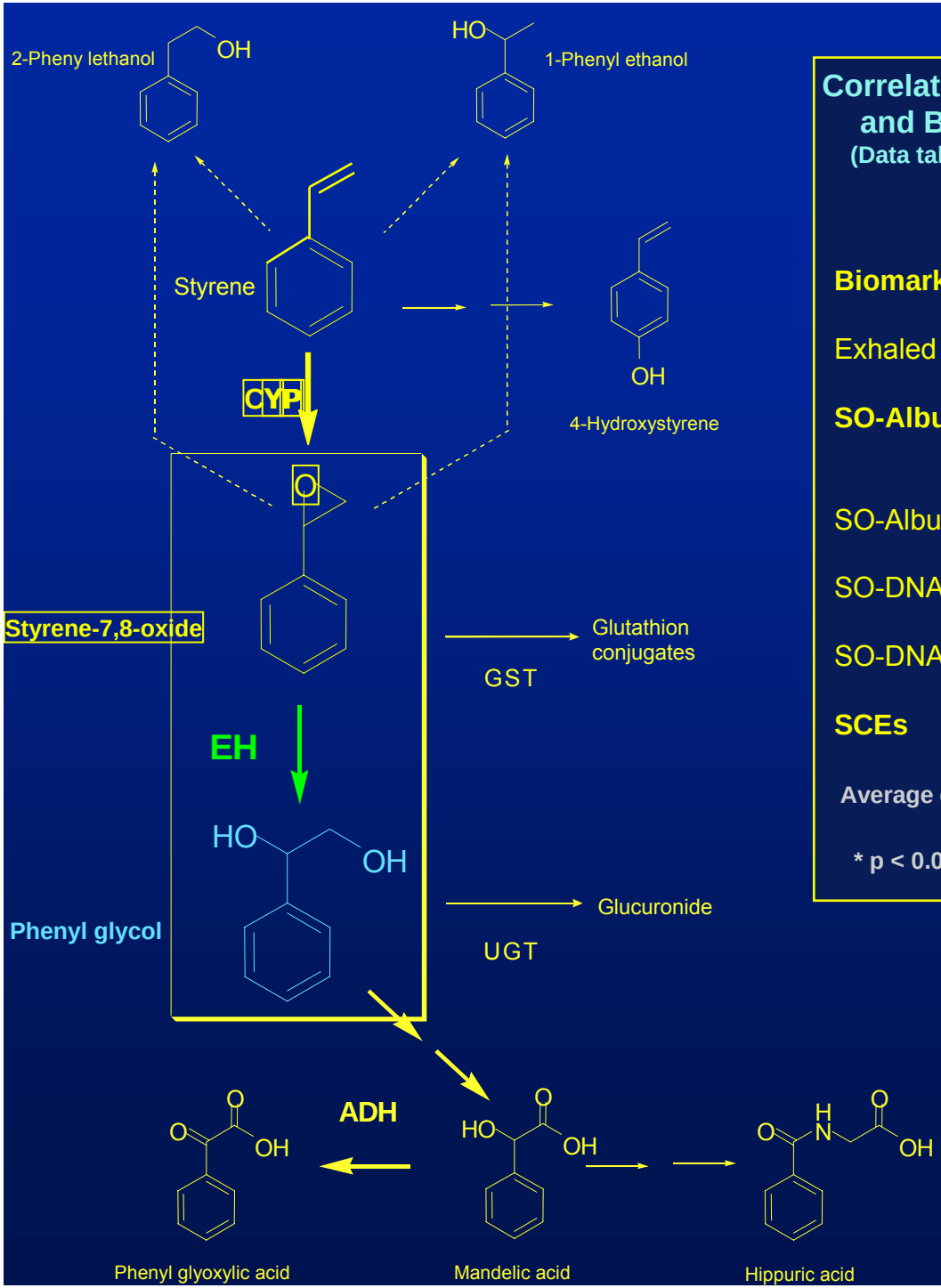
	sEH	mEH
Asp	333	226
His	524	431
Asp/Glu	495	404

Expected Effect of the mEH Concentration on the Epoxide Steady State Concentration



Steady State Kinetics of Epoxide Detoxification





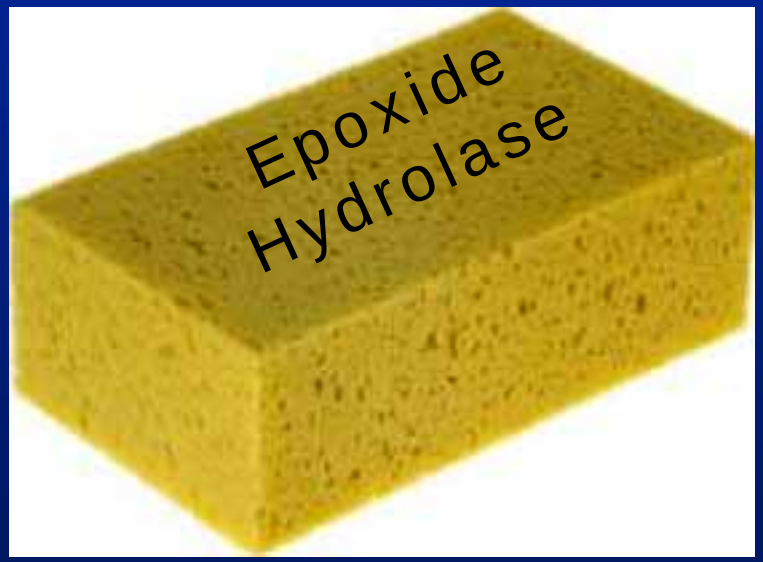
Correlation between Styrene- and Styrene Oxide-Exposure and Biomarker Blood Levels of Healthy Non-Smokers

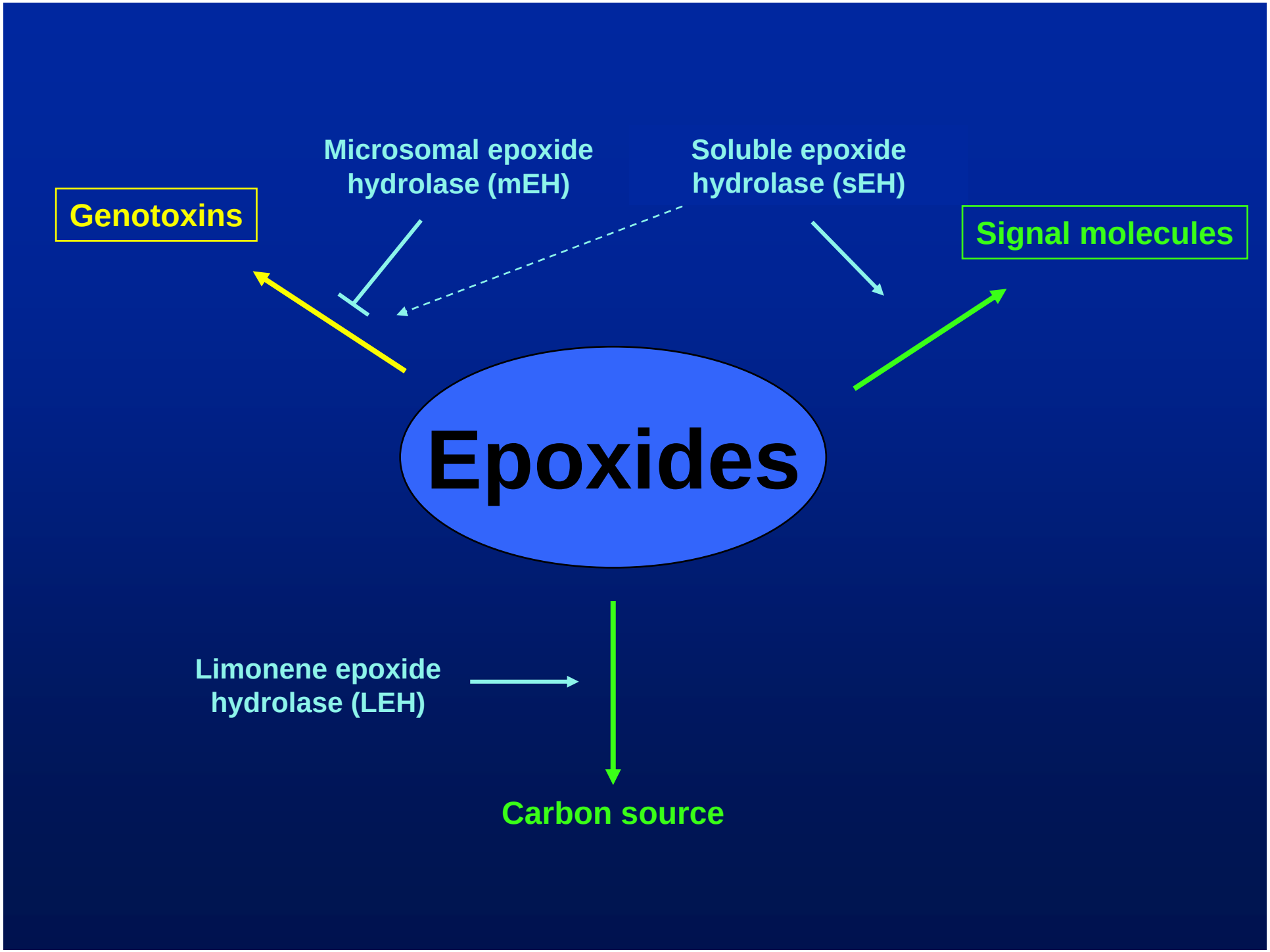
(Data taken from Rappaport et al., *Cancer Res*. (1996) 56, pp. 5410-5416)

Biomarker	Styrene (n = 21)	Styrene oxide (n = 8)
Exhaled Styrene	0.948***	0.442
SO-Albumin (α)	**0.016**	**0.811***
SO-Albumin (β)	0.045	0.667
SO-DNA (1)	0.356	0.528
SO-DNA (2)	0.434*	0.491
SCEs	**0.394**	**0.811***

Average exposure level: Styrene = 50.5 mg/m^3; Styrene oxide = 129 µg/m^3

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$**


Epoxide
Hydrolase


Microsomal epoxide hydrolase (mEH)
Soluble epoxide hydrolase (sEH)
Genotoxins
Signal molecules
Epoxides
Limonene epoxide hydrolase (LEH)
Carbon source

EH from Agrobacterium at 2.1 Å

Nardini et al., J.Biol.Chem. 1999

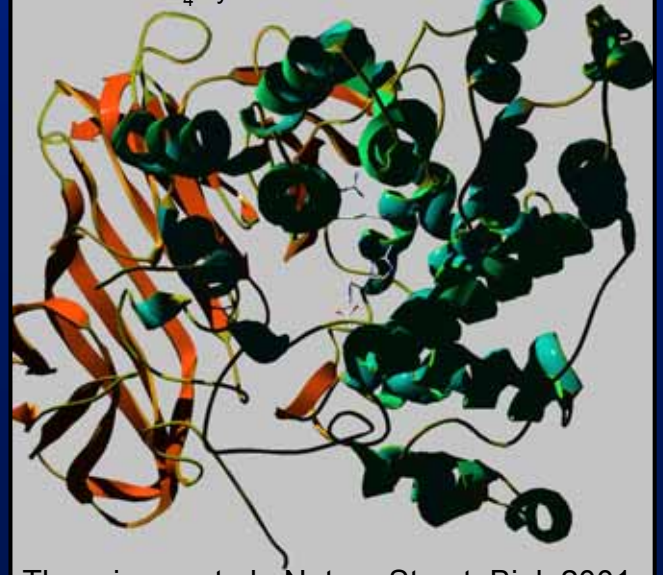
sEH from mouse at 2.8 Å

Argiriadi et al., PNAS 1999

EH from Aspergillus at 1.7 Å

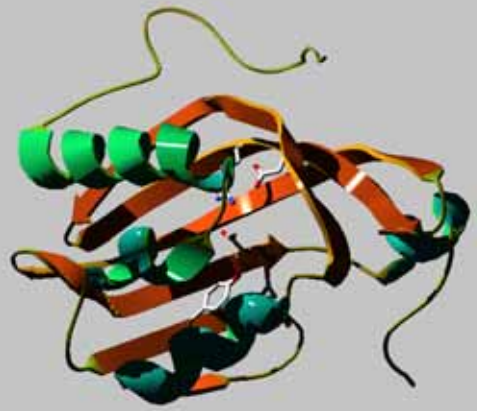
Zou et al., Structure 2000

Leukotriene A_4 hydrolase from human at 1.95 Å



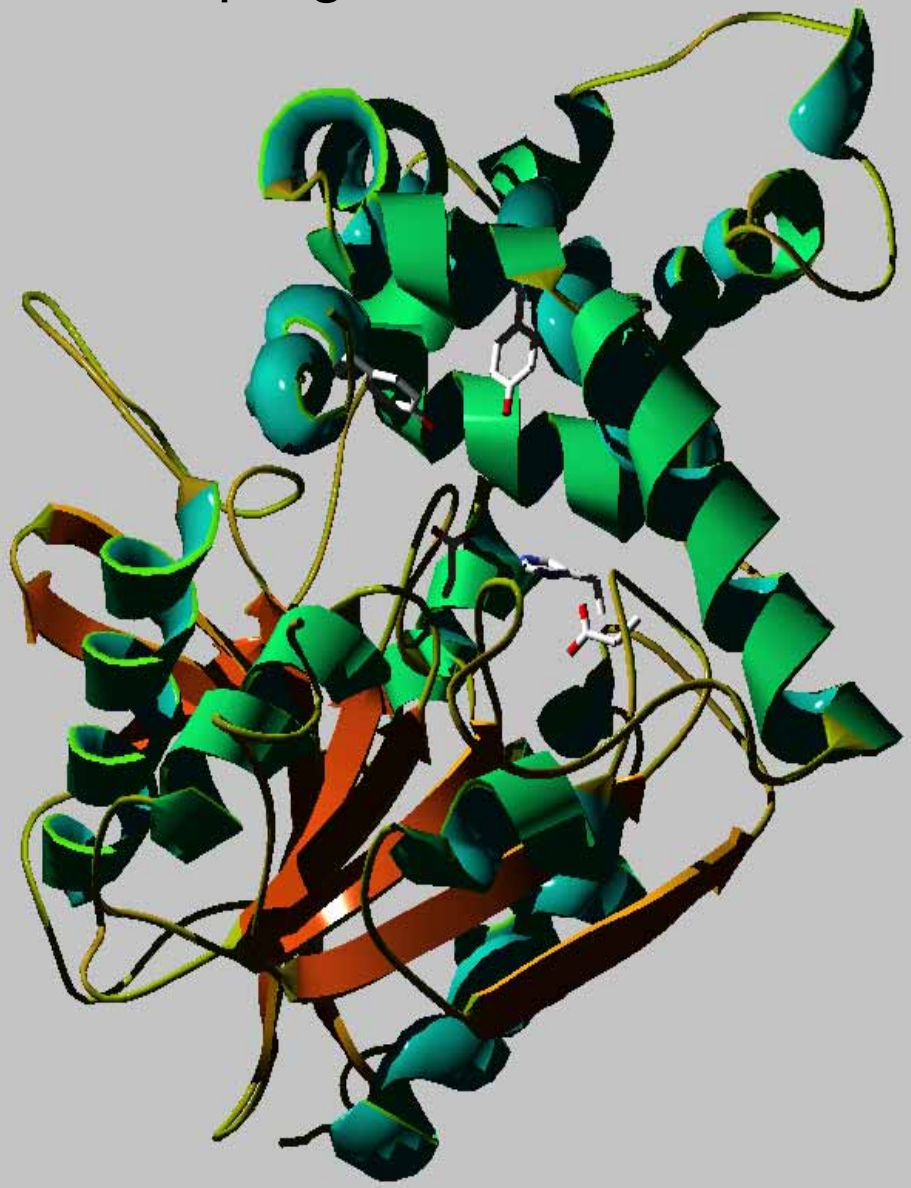
Thunnissen et al., Nature Struct. Biol. 2001

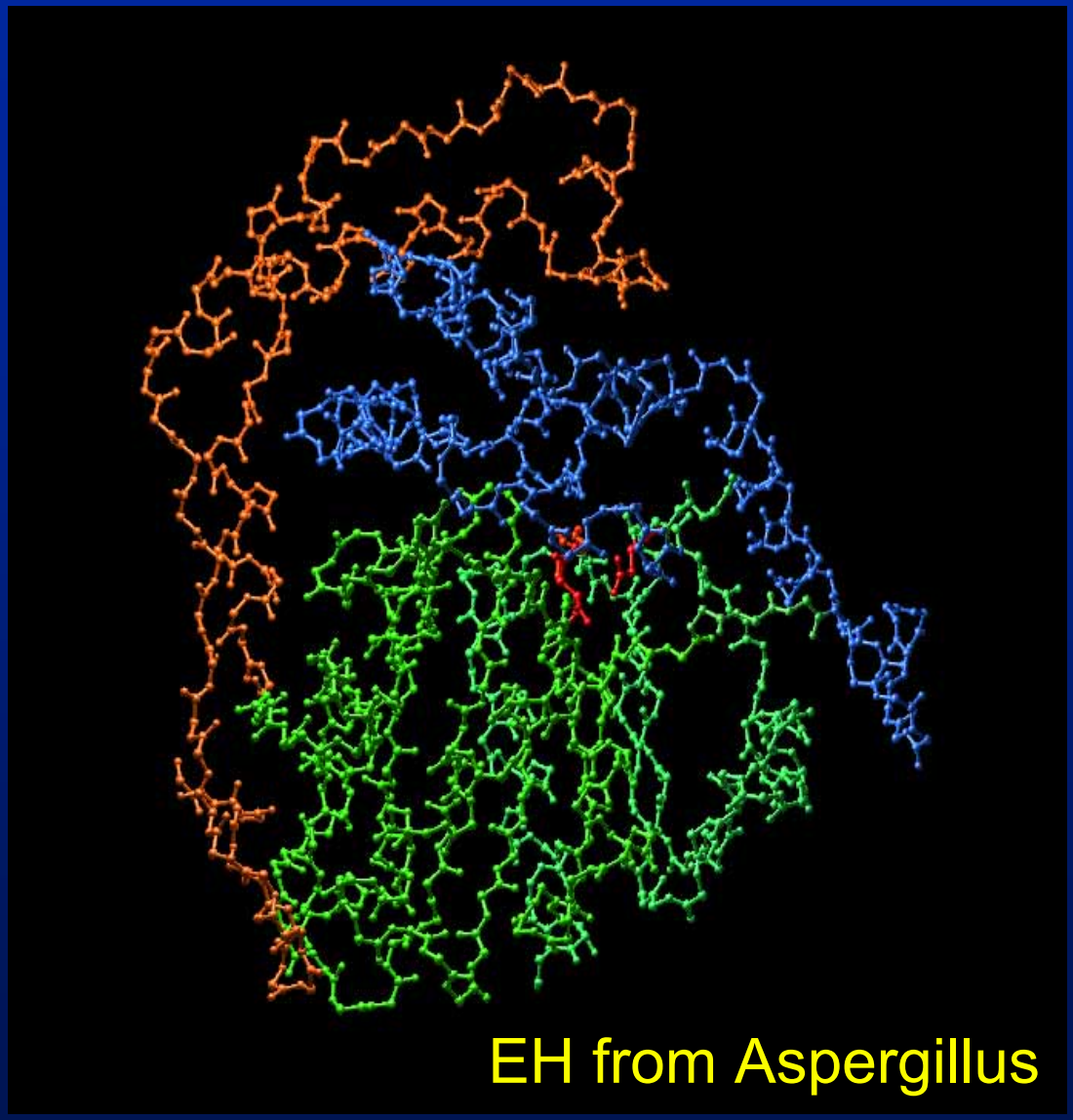
EH from Rhodococcus at 1.2 Å



Arand et al., EMBO J., in press

EH from Aspergillus




EH from Aspergillus

Evaluation of the Detoxification Efficacy of mEH