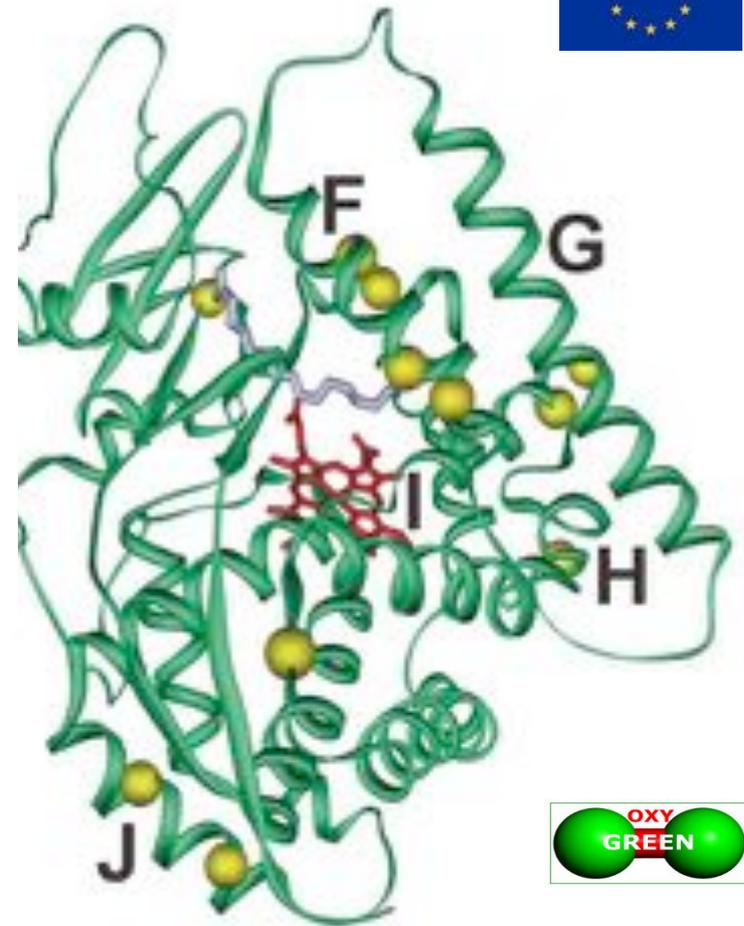


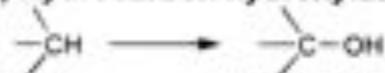
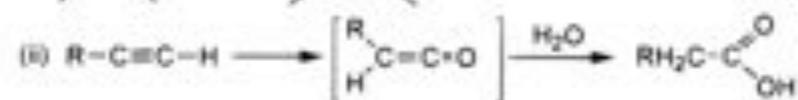
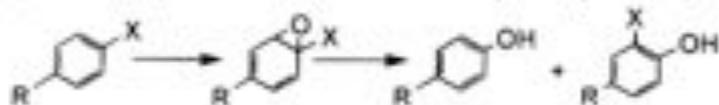
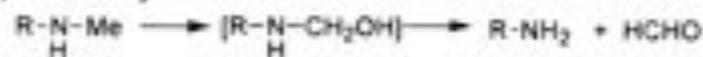
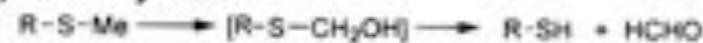
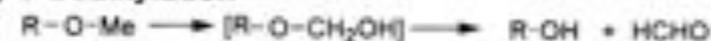
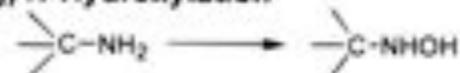
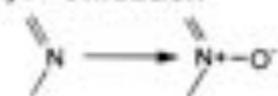
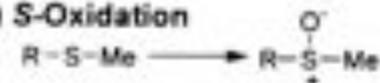
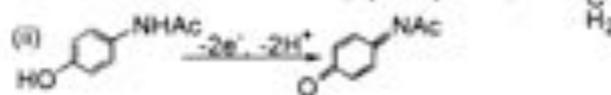
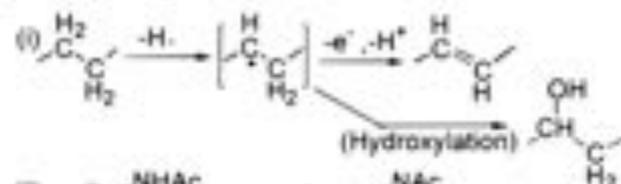
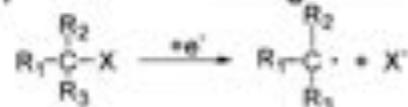
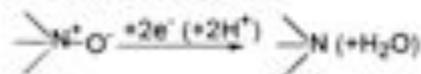
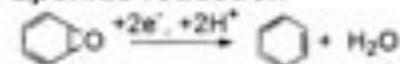
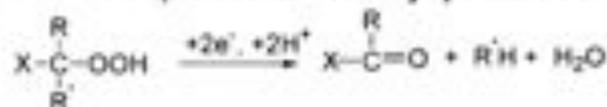


Heme dependent enzymes for biocatalysis and biosynthetic pathways

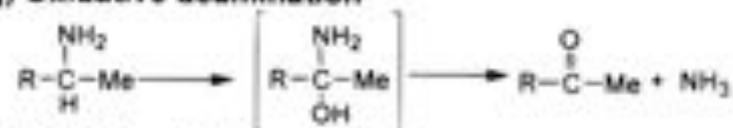


Heme enzymes

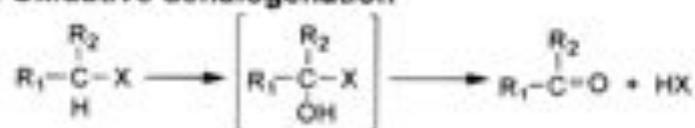
- Catalase
- P450 Cytochromes
- Chloroperoxidase/Peroxygenase
- Peroxidase

(a) Hydrocarbon hydroxylation**(b) Alkene epoxidation / Alkyne oxygenation****(c) Arene epoxidation, aromatic hydroxylation, NIH shift****(d) N-Dealkylation****(e) S-Dealkylation****(f) O-Dealkylation****(g) N-Hydroxylation****(h) N-Oxidation****(i) S-Oxidation****(m) Dehydrogenation****(n) Dehydrations****(o) Reductive dehalogenation****(p) N-Oxide reduction****(q) Epoxide reduction****(r) Reductive β -scission of alkyl peroxides**

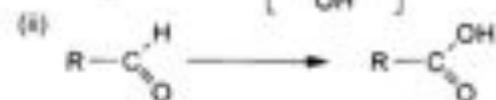
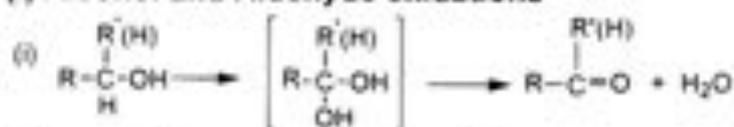
(j) Oxidative deamination



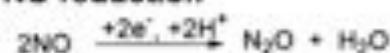
(k) Oxidative dehalogenation



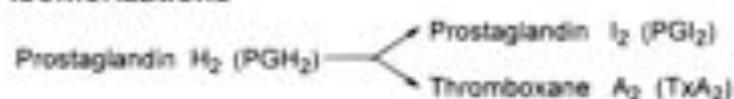
(l) Alcohol and Aldehyde oxidations



(s) NO reduction



(t) Isomerizations



(u) Oxidative C-C bond cleavage

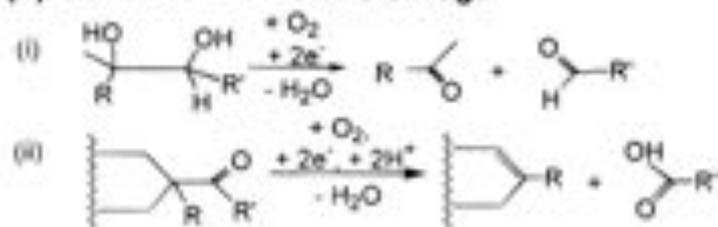
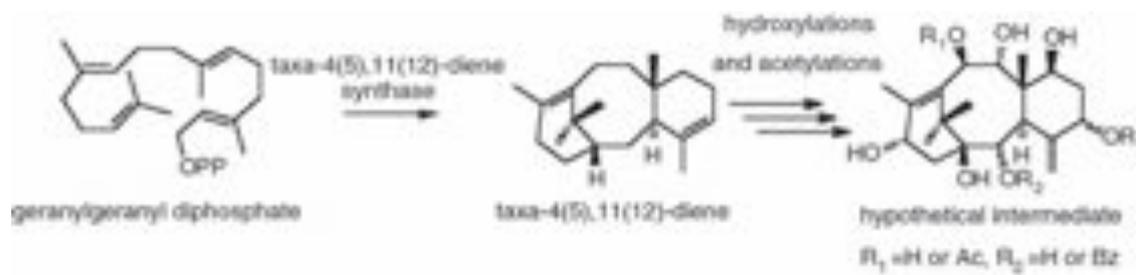
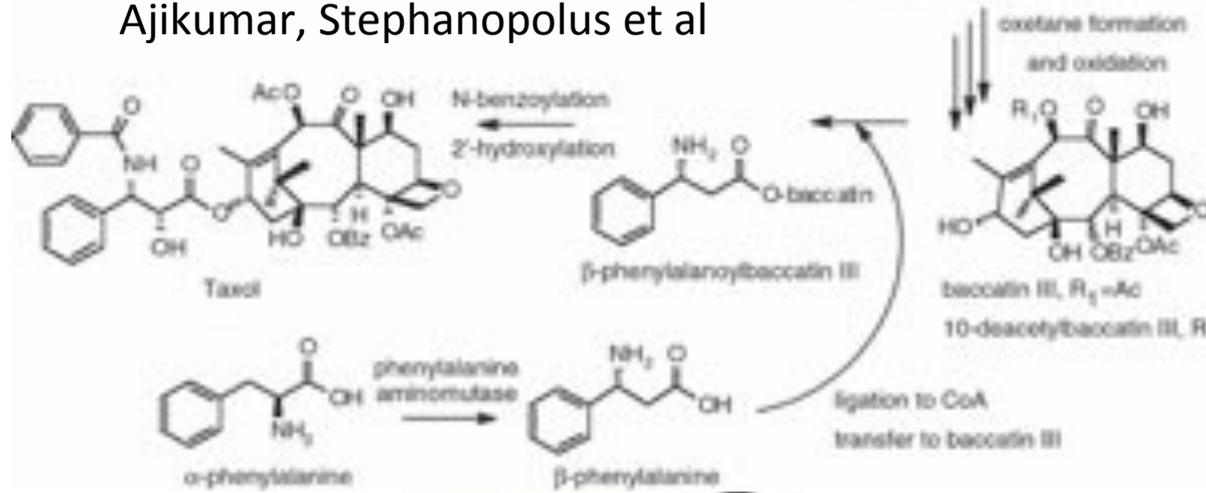


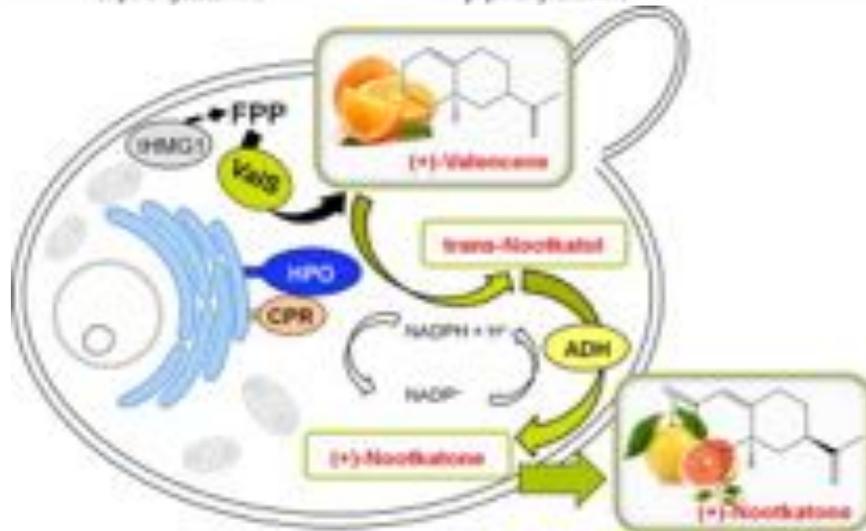
Figure 3. Schematic summary of the diverse P450-catalyzed reactions discussed in this review. See text (especially section IIIK) for references (for reactions *l* - *u*).



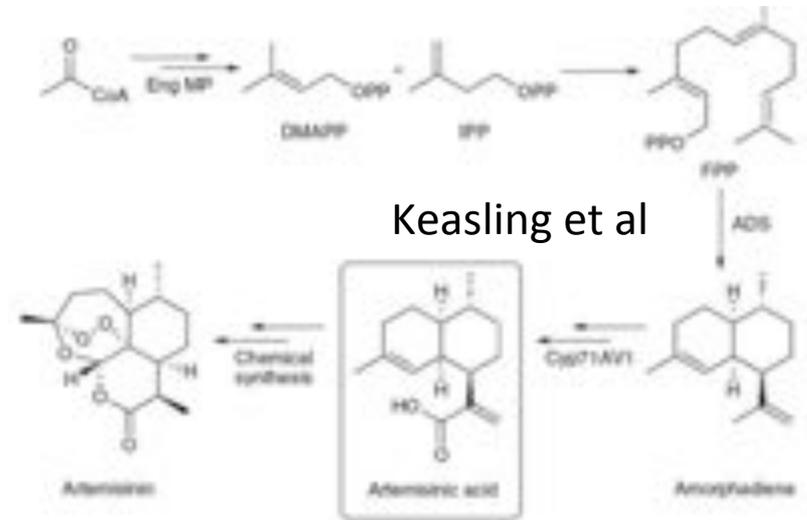
Ajikumar, Stephanopolus et al



Hydroxylation is key to natural compounds



Pichler et al., 2014



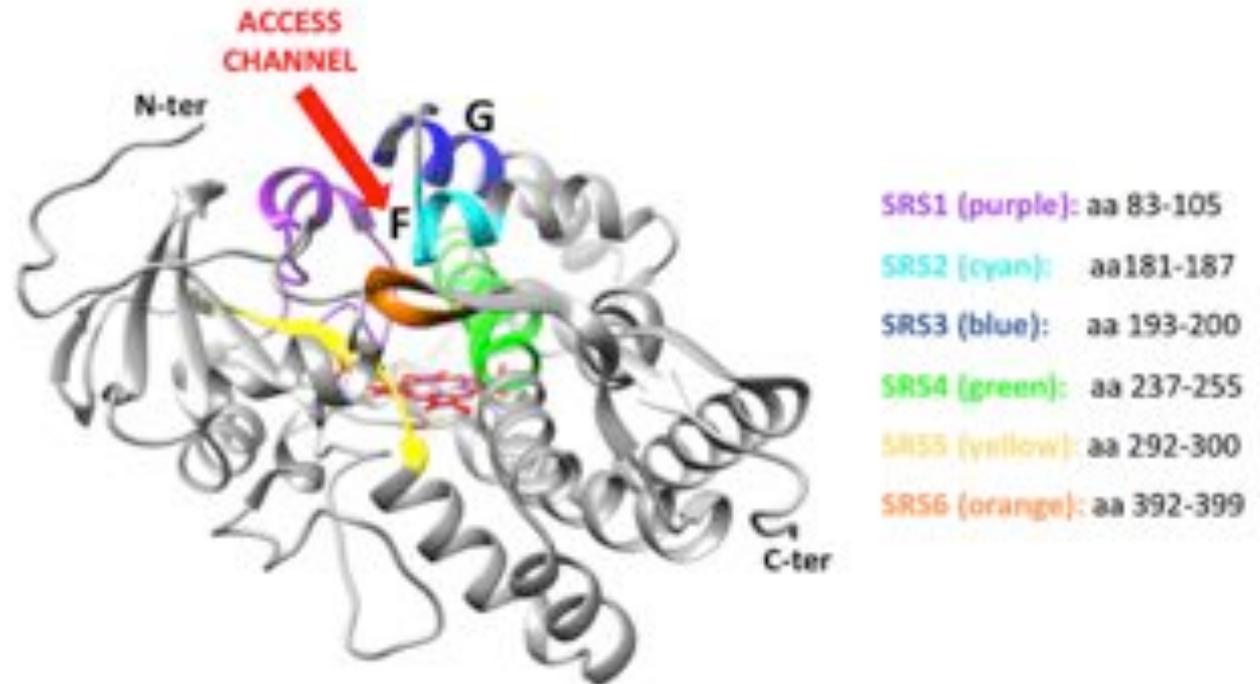
Keasling et al

Substrate recognition sites (SRS)

S172

Rend. Fis. Acc. Lincei (2017) 28 (Suppl 1):S169–S181

Fig. 2 SRSs regions in the P450cam (CYP101A) X-ray structure (PDB 2CPP) (Poulos et al. 1987)

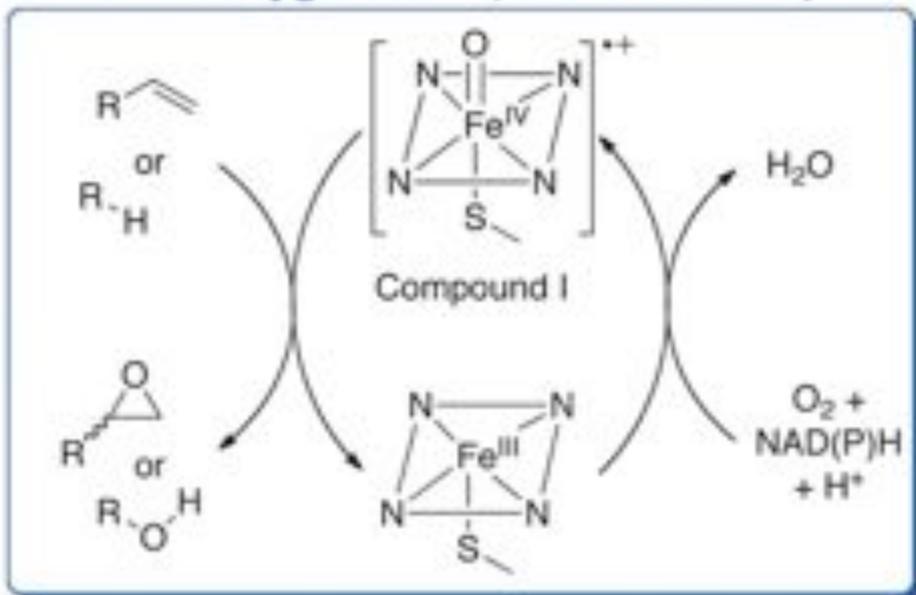


Pioneering work in structural biology on P450cam (CYP101) and BM3 (CYP102A1)

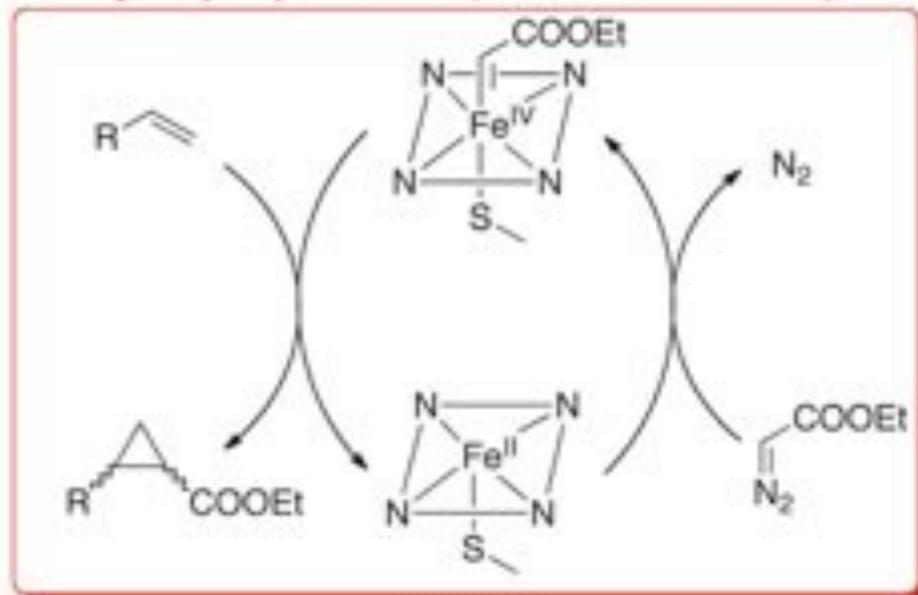
Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes

Pedro S. Coelho,^{1*} Eric M. Brustad,^{2*} Arvind Kannan,¹ Frances H. Arnold^{1†}

Monooxygenation (oxene transfer)



Cyclopropanation (carbene transfer)



SCIENCE VOL 339 18 JANUARY 2013

C400S mutation removes native monooxygenation (P411)

Nature Chemical Biology **9**, 485–487 (2013) doi:10.1038/nchembio.1278



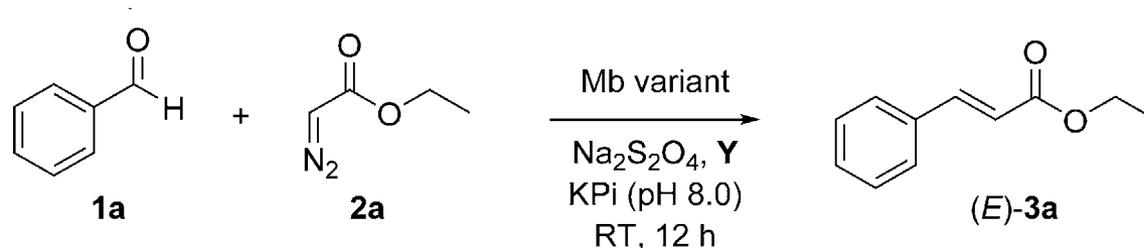
Nestl & Hauer, Nature Chemical Biology 9, 470–471 (2013)

Myoglobin/hemin catalyzed C-C and C-N bond formations

Olefination

DOI: 10.1002/anie.201508817

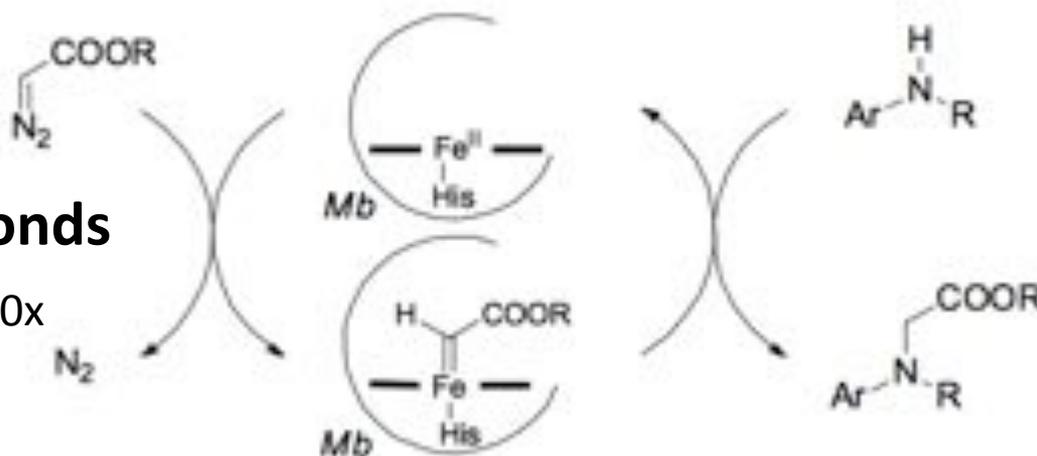
2016

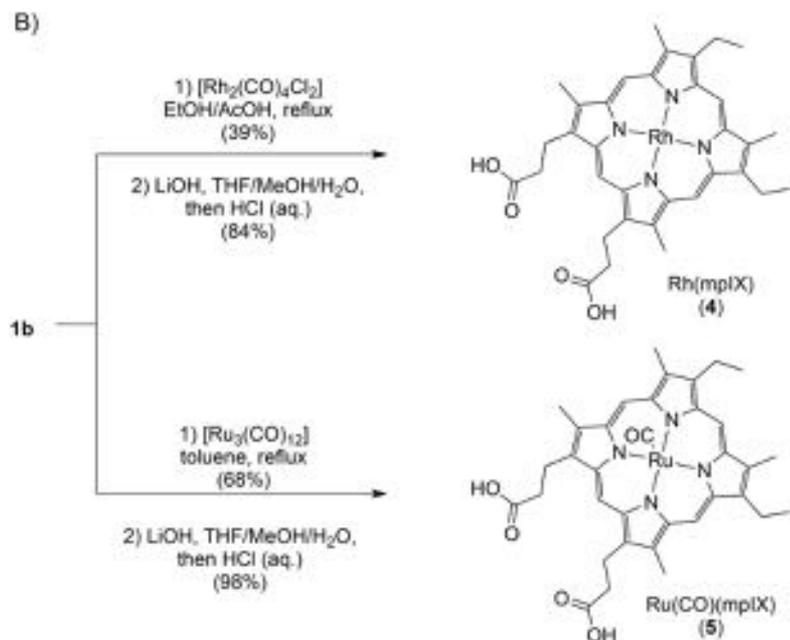
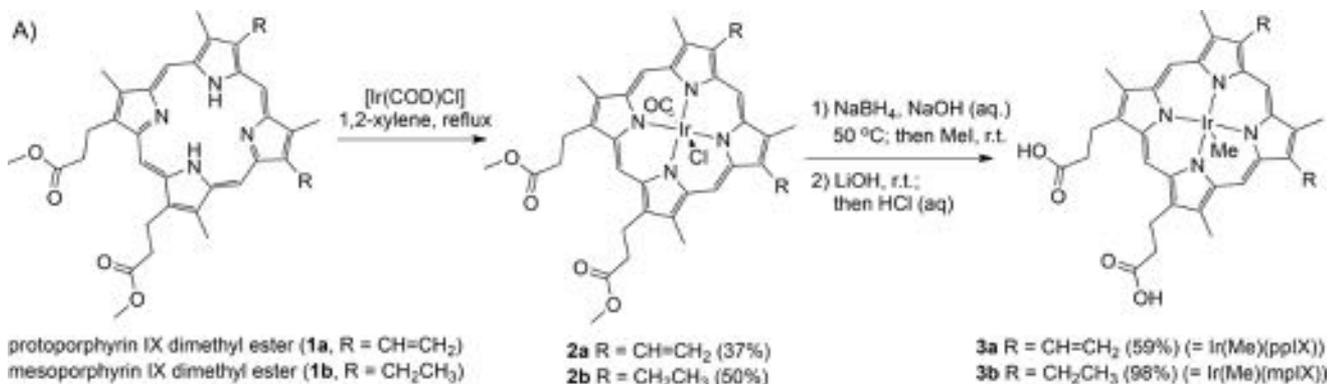


Formation of N-H bonds

DOI: 10.1039/x0xx00000x

2012



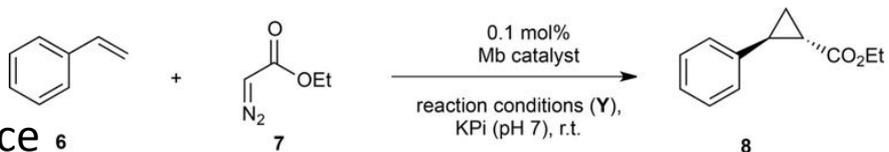


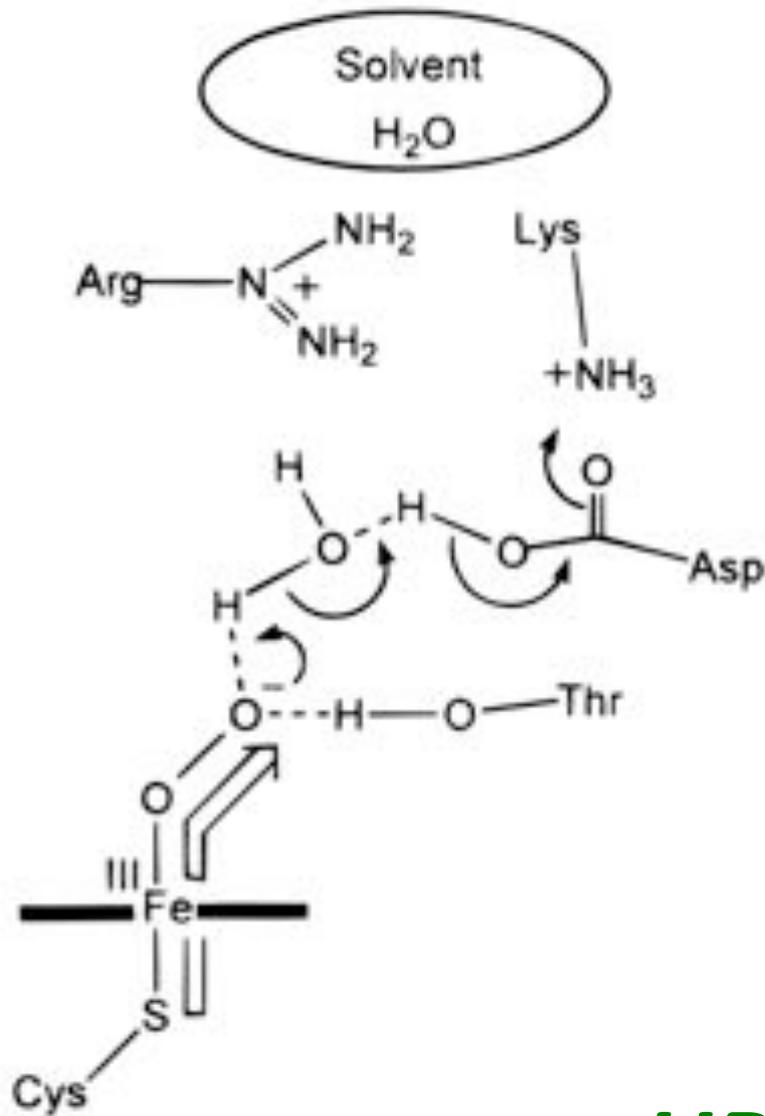
Iron replacement in heme + engineered myoglobin

R. Fasan group

Adv. Synth. Catal. **2017**, 359, 2076–2089

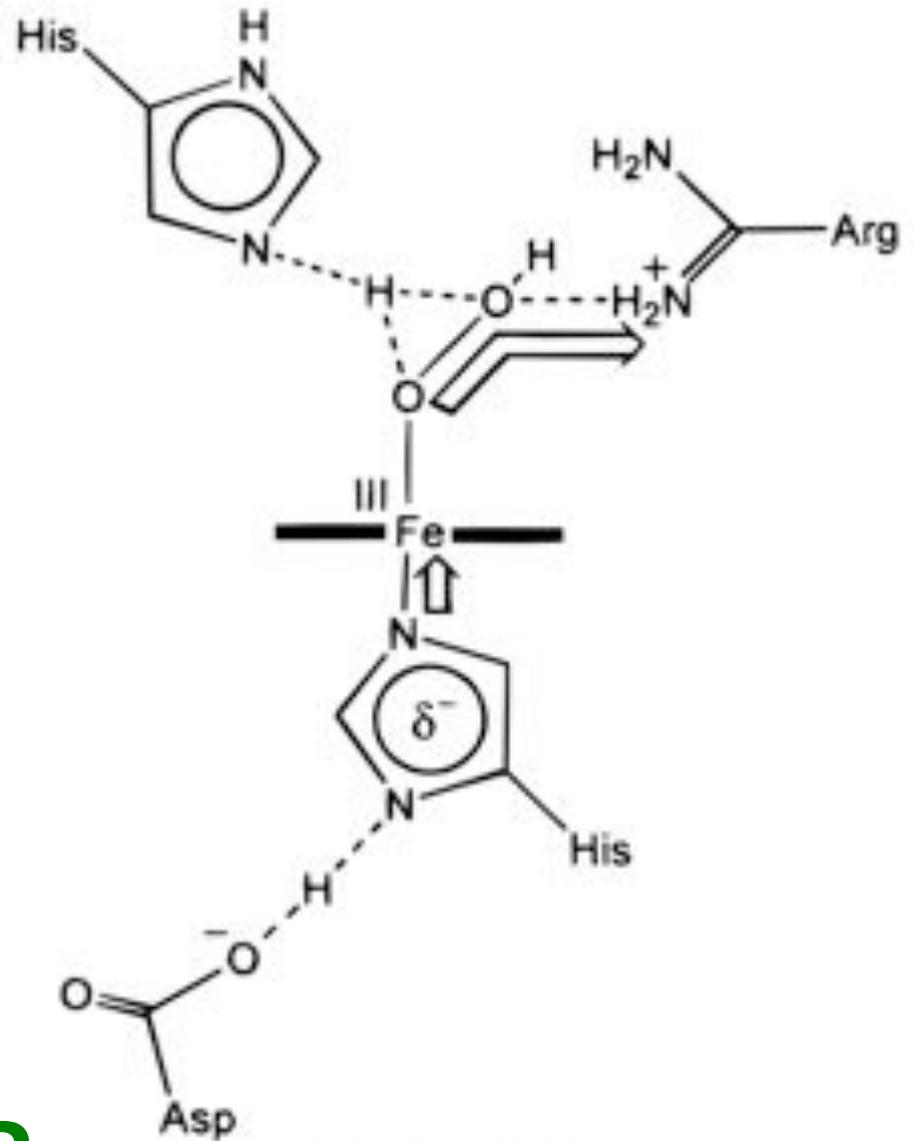
Cyclopropanation – increased O₂ tolerance **6**





P450

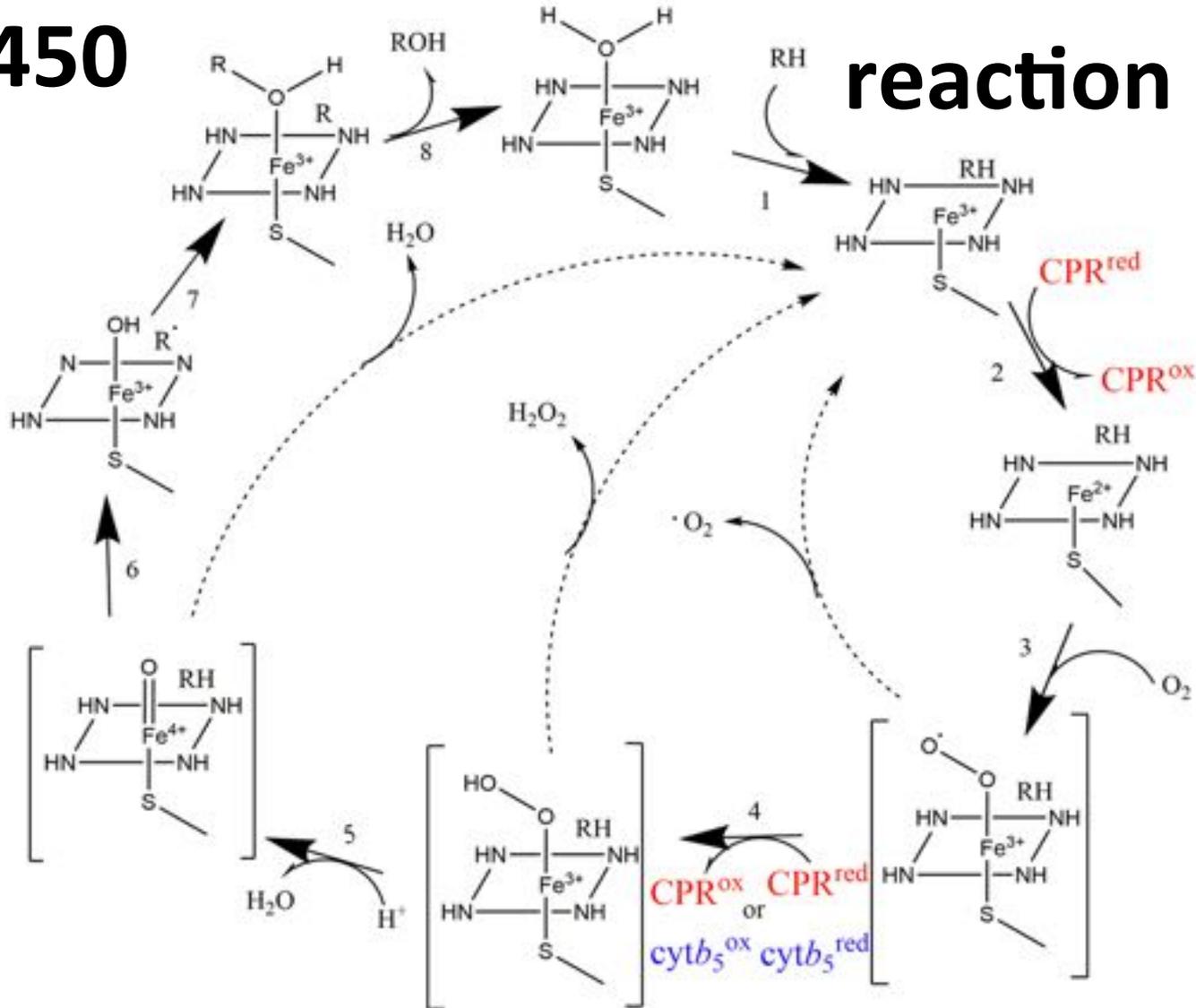
UPO

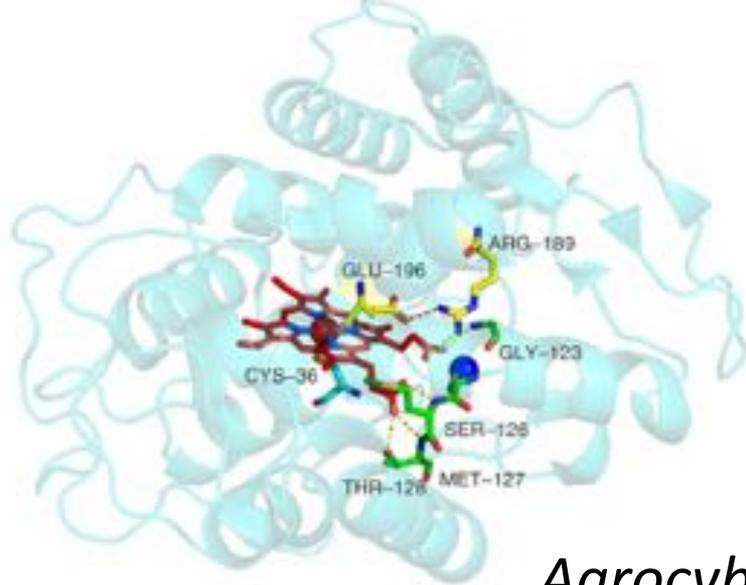


Peroxidase

P450

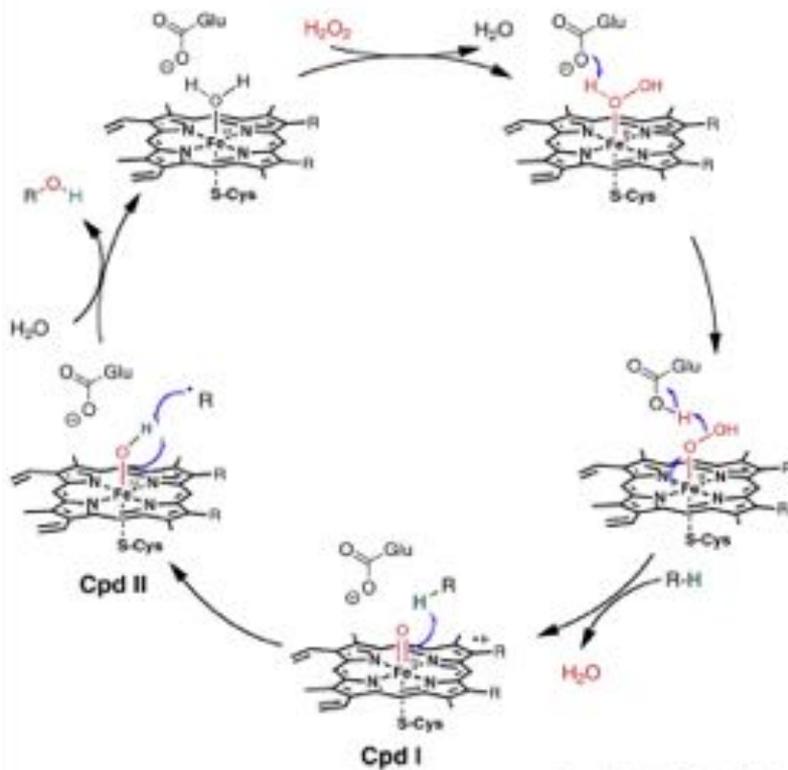
reaction cycle





Unspecific peroxygenases UPOs

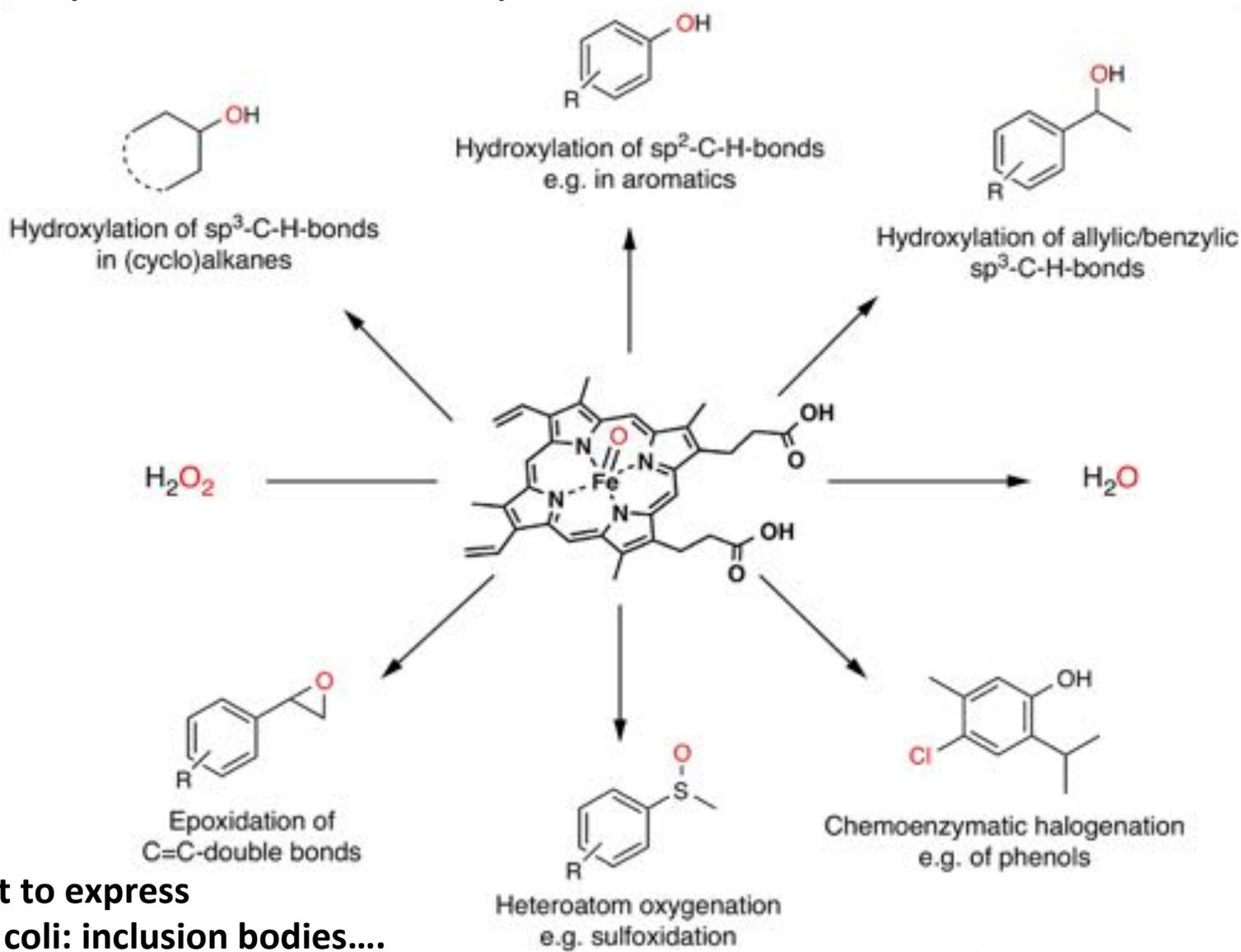
Agrocybe aegerita UPO



His instead of Glu 196
in *Cfu* CPO

Different activity,
selectivity, stability,
secreted enzymes

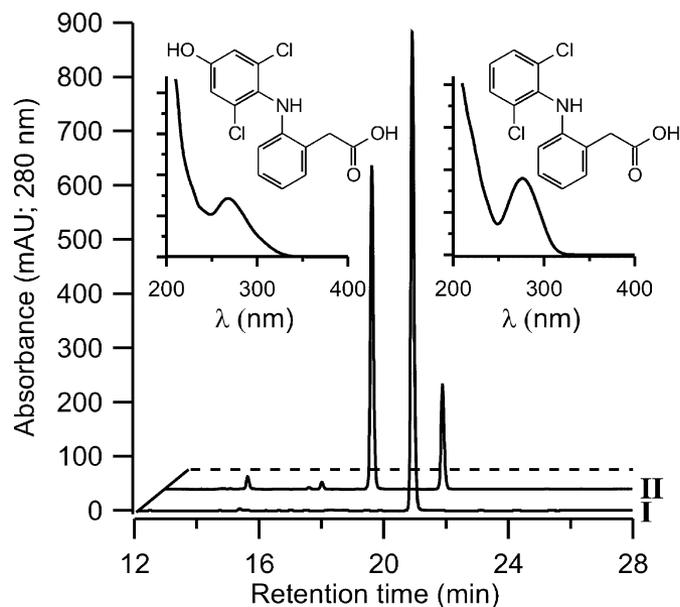
UPOs catalyze similar chemistry as CYPs



difficult to express

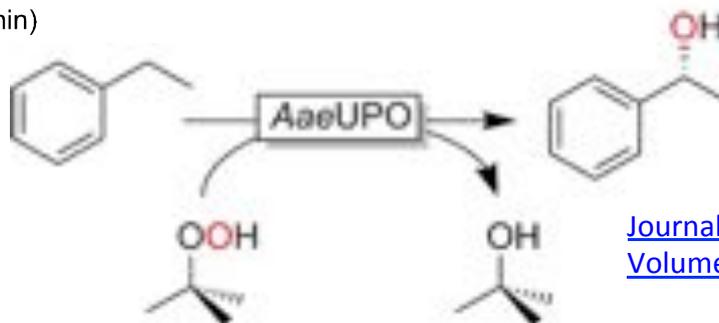
E. coli: inclusion bodies....

1 active enzyme made by *Pichia* after mutagenesis

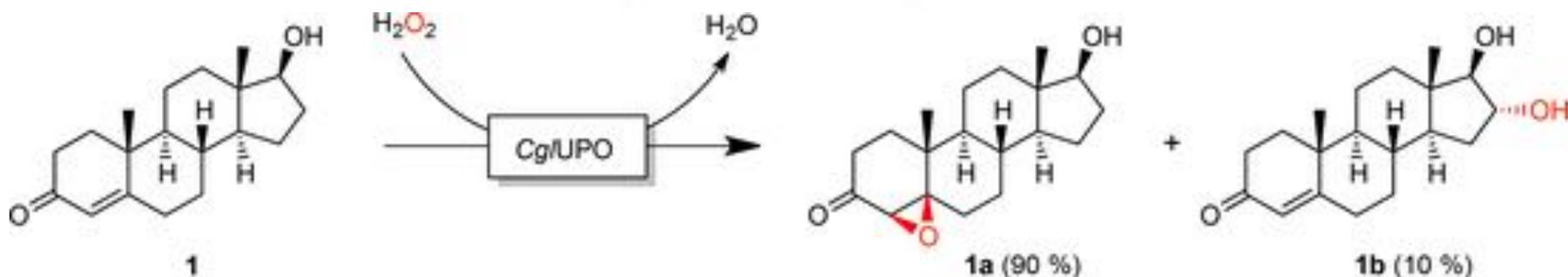


UPO catalyzed human drug metabolite synthesis

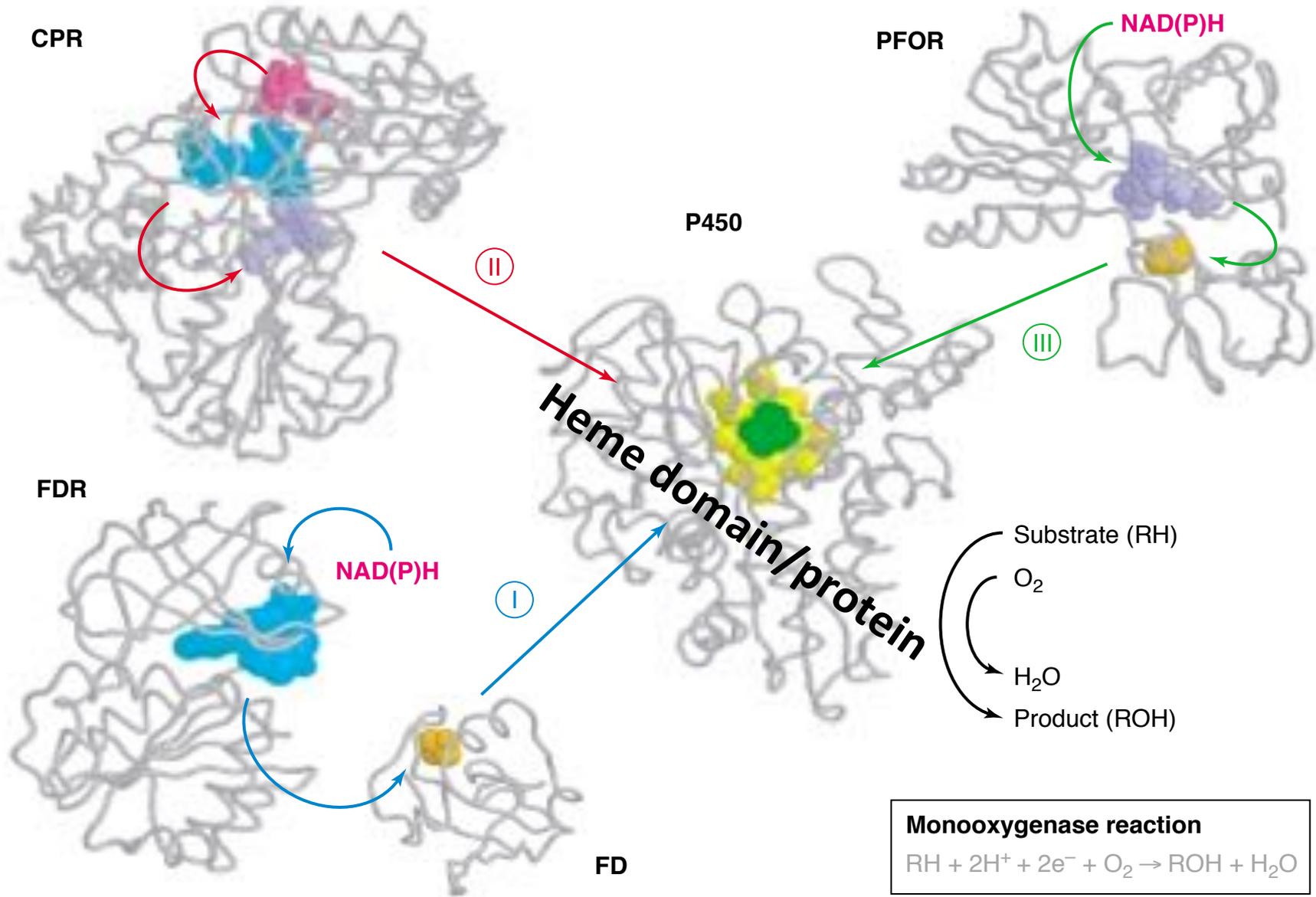
Bioorganic & Medicinal Chemistry Letters 19 (2009) 3085–3087



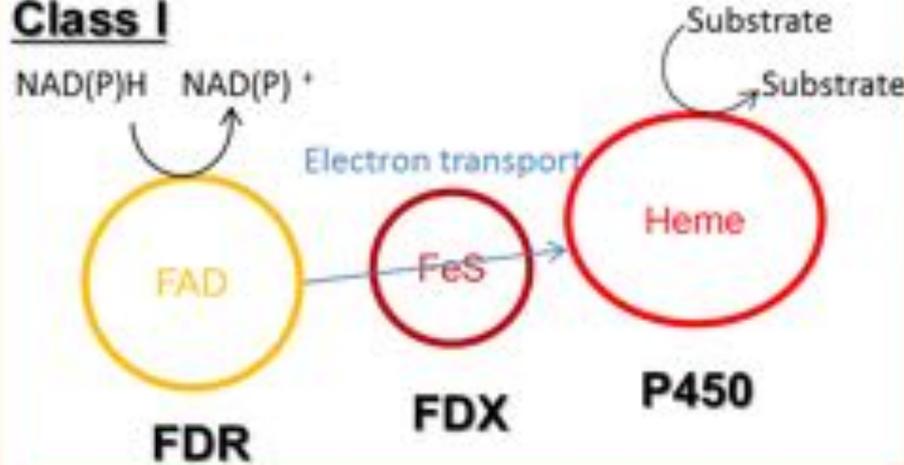
[Journal of Molecular Catalysis B: Enzymatic](#)
 Volume 134, Part B, 68-69, 2016, 70, 347-352



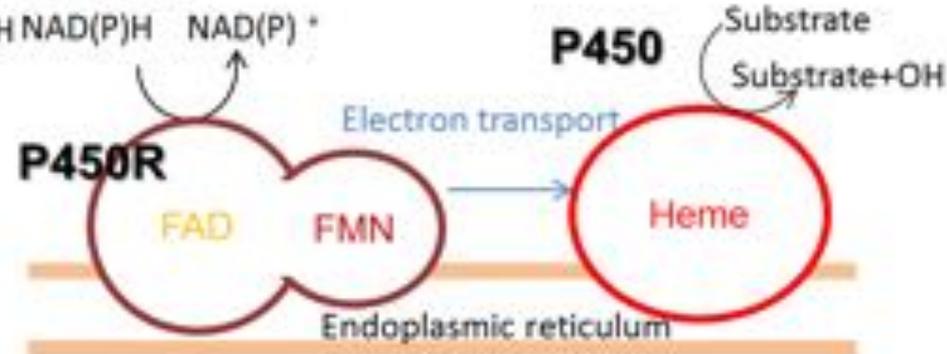
J. Kiebitz, K.-U. Schmidtke, J. Zimmermann, H. Kellner, N. Jehmlich, R. Ullrich, D. Zänder, M. Hofrichter, K. Scheibner, *ChemBioChem* 2017, 18, 563.



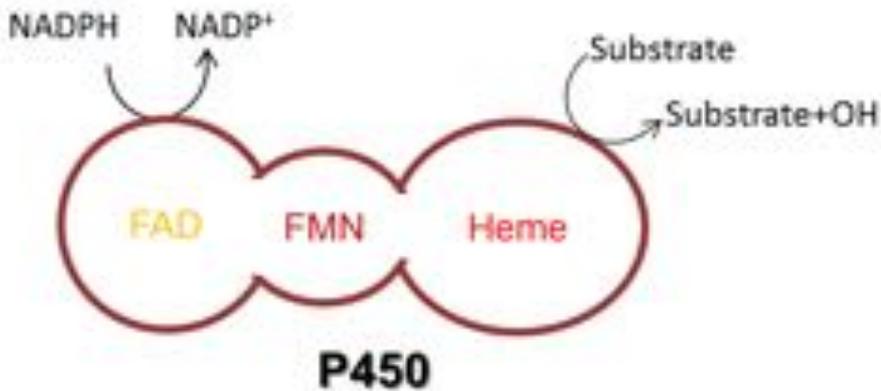
Class I



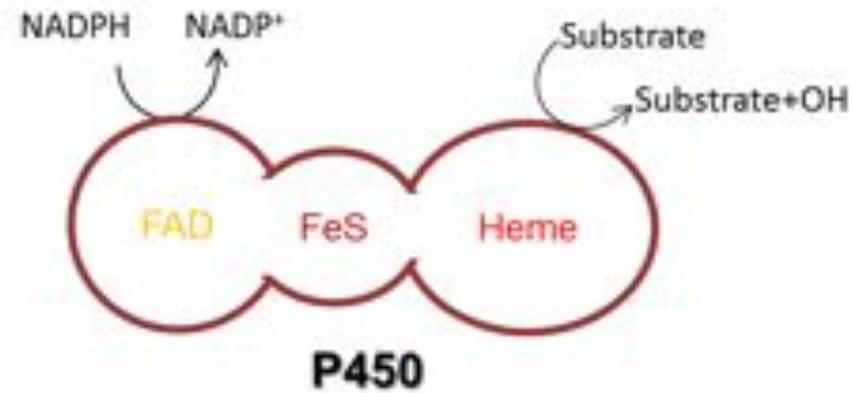
Class II



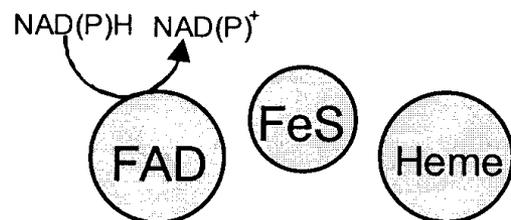
Class III



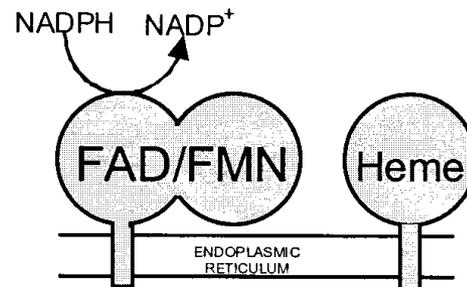
Class IV



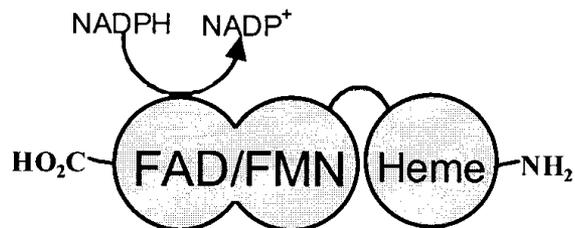
Class I



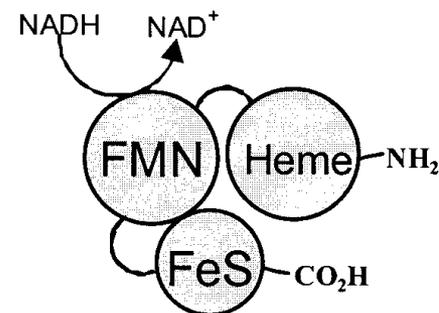
Class II



Class III



Class IV



Narhi, L. O., and A. J. Fulco. 1986. Characterization of a catalytically self-sufficient 119,000 dalton cytochrome P450 monooxygenase induced by barbiturates in *Bacillus megaterium*. *J. Biol. Chem.* **261**:7160–7169.

JOURNAL OF BACTERIOLOGY, July 2002, p. 3898–3908
0021-9193/02/304.00+0 DOI: 10.1128/JB.184.14.3898–3908.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

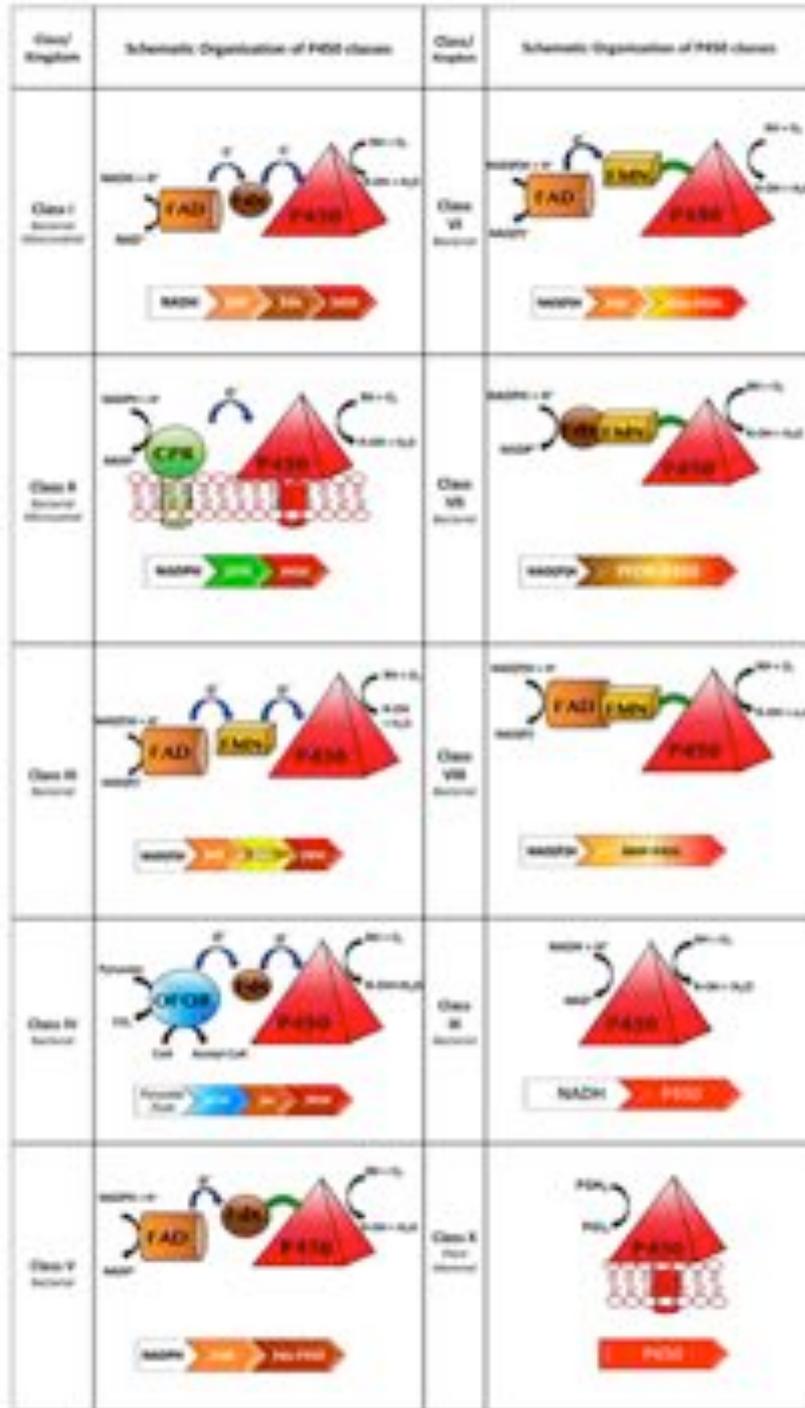
Vol. 184, No. 14

Identification of a New Class of Cytochrome P450 from a *Rhodococcus* sp.

Gareth A. Roberts, Gideon Grogan,† Andy Greter, Sabine L. Flitsch, and Nicholas J. Turner*

*The Edinburgh Centre for Protein Technology, Department of Chemistry, University of Edinburgh,
Edinburgh EH9 3JF, United Kingdom*

Received 22 January 2002/Accepted 26 April 2002



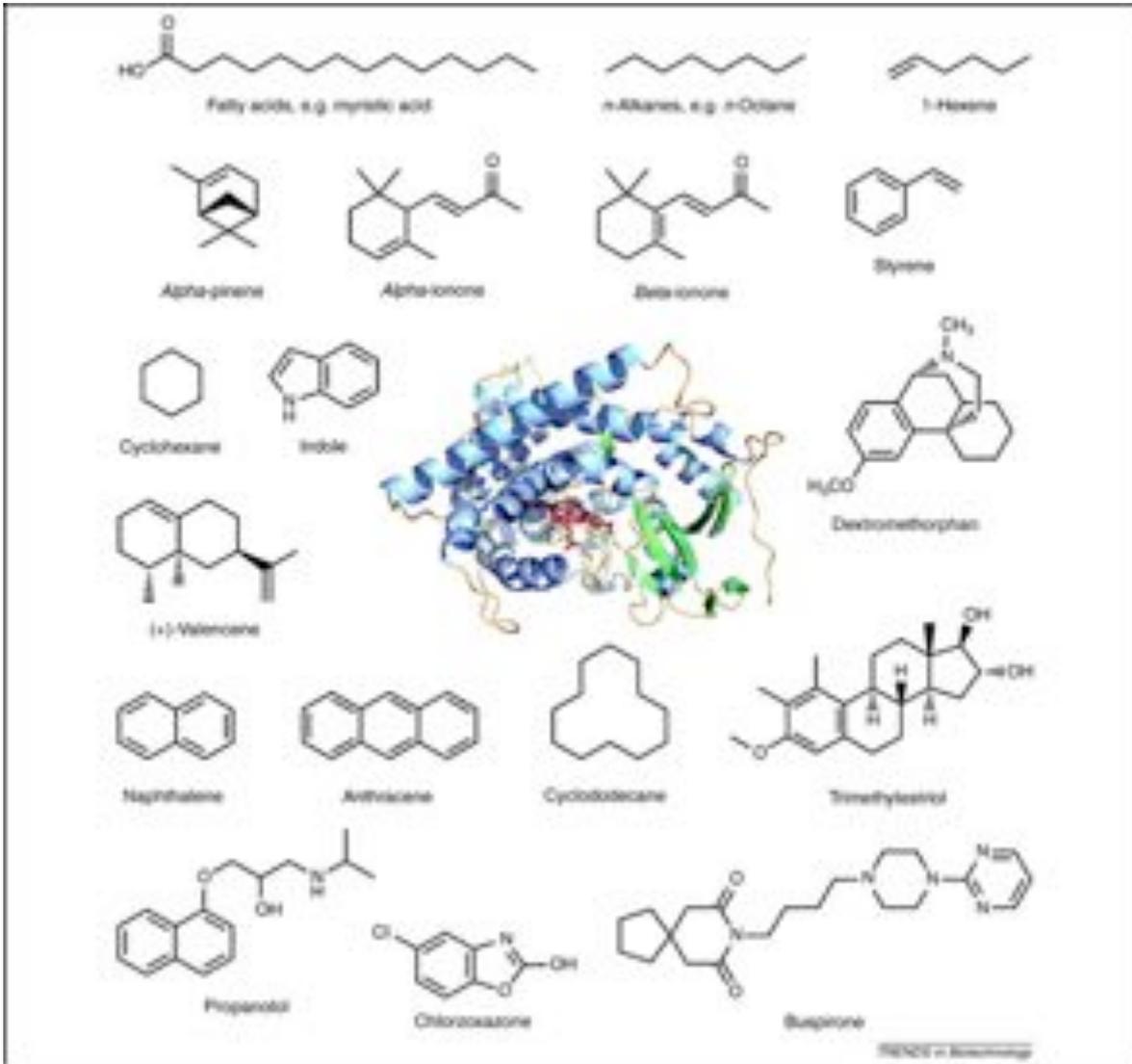
Natural Self Sufficient P450s

Rend. Fis. Acc. Lincei (2017) 28 (Suppl 1):S169–S181 DOI 10.1007/s12210-016-0581-z

CONCEPTS IN CATALYSIS

Catalytically self-sufficient cytochromes P450 for green production of fine chemicals

Alberto Ciaramella¹ • Daniela Minerdi¹ • Gianfranco Gilardi¹



P450 BM3

Can do anything?
or nothing?

*(Alkanes/terminal
steroids
natural compounds
aromatics, heterocycles,....)
not satisfying.....*

Alternatives

CYP153: 1st from HXN Stämmen (Engesser, Schmid, Withold, van Beilen): e.g. M. Smit, Zhi Li, B. Hauer,.... *Uni Pavia*

P450RhF: N. Turner



Human liver P450s: D. Rozzell, Guengerich,



Plant P450s, fungal P450s



(?Unspecific?) Peroxygenases

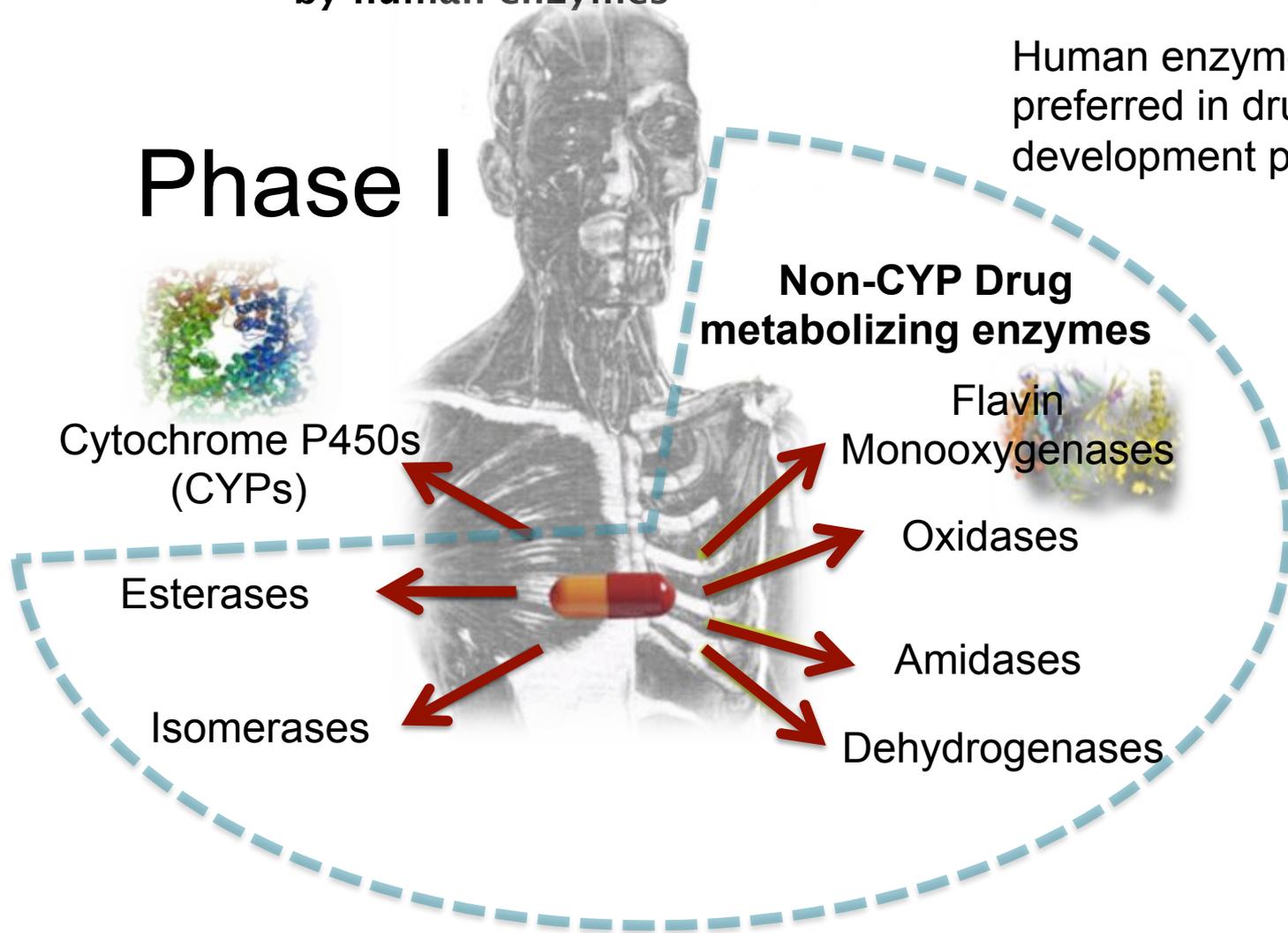


Di-iron dioxygenases: NON HEME

Human drug metabolism by human enzymes

Human enzymes often preferred in drug development process

Phase I



Cytochrome P450s (CYPs)

Non-CYP Drug metabolizing enzymes

Flavin Monooxygenases

Oxidases

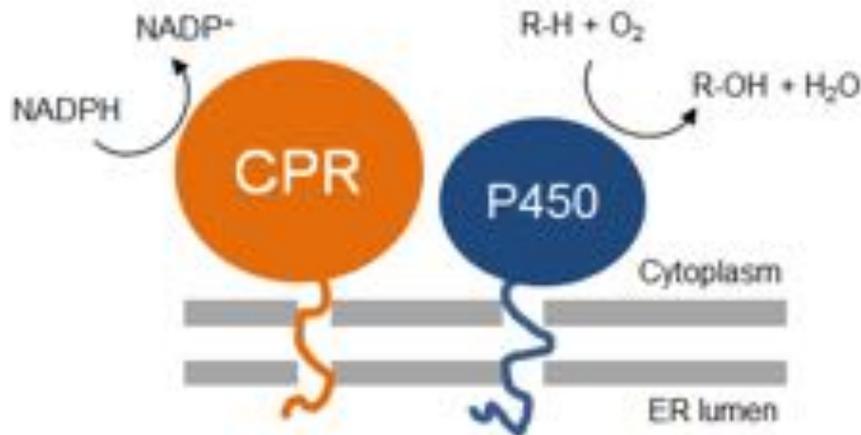
Esterases

Amidases

Isomerases

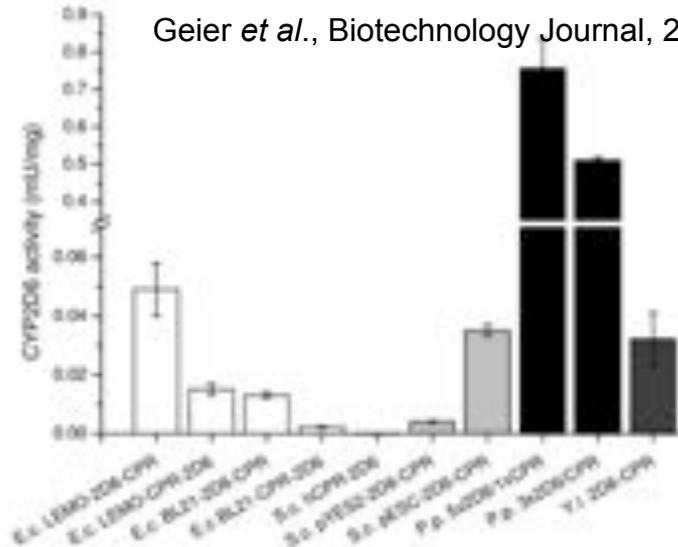
Dehydrogenases

Case study: Human P450 expression in *Pichia pastoris*

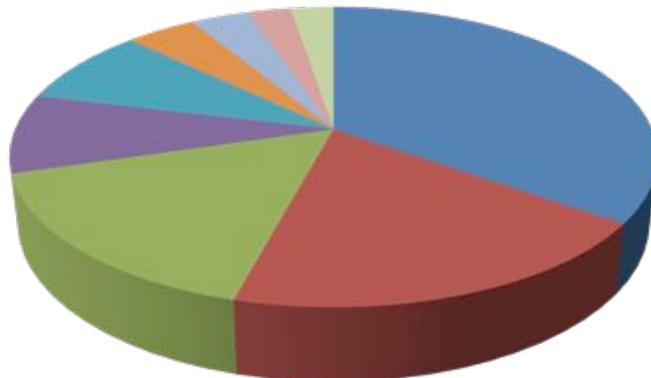


A comparative study of hP450 production in recombinant microbes

Geier *et al.*, Biotechnology Journal, 2012;

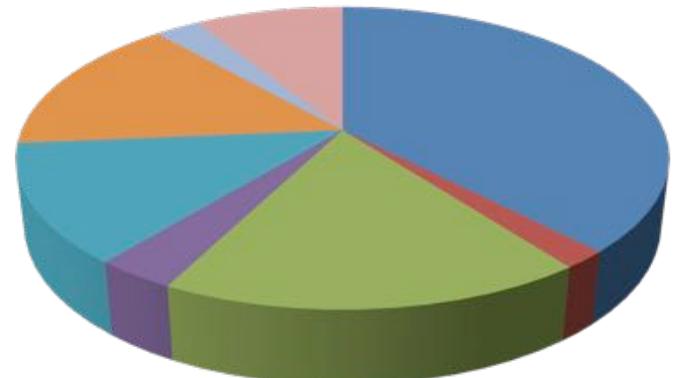


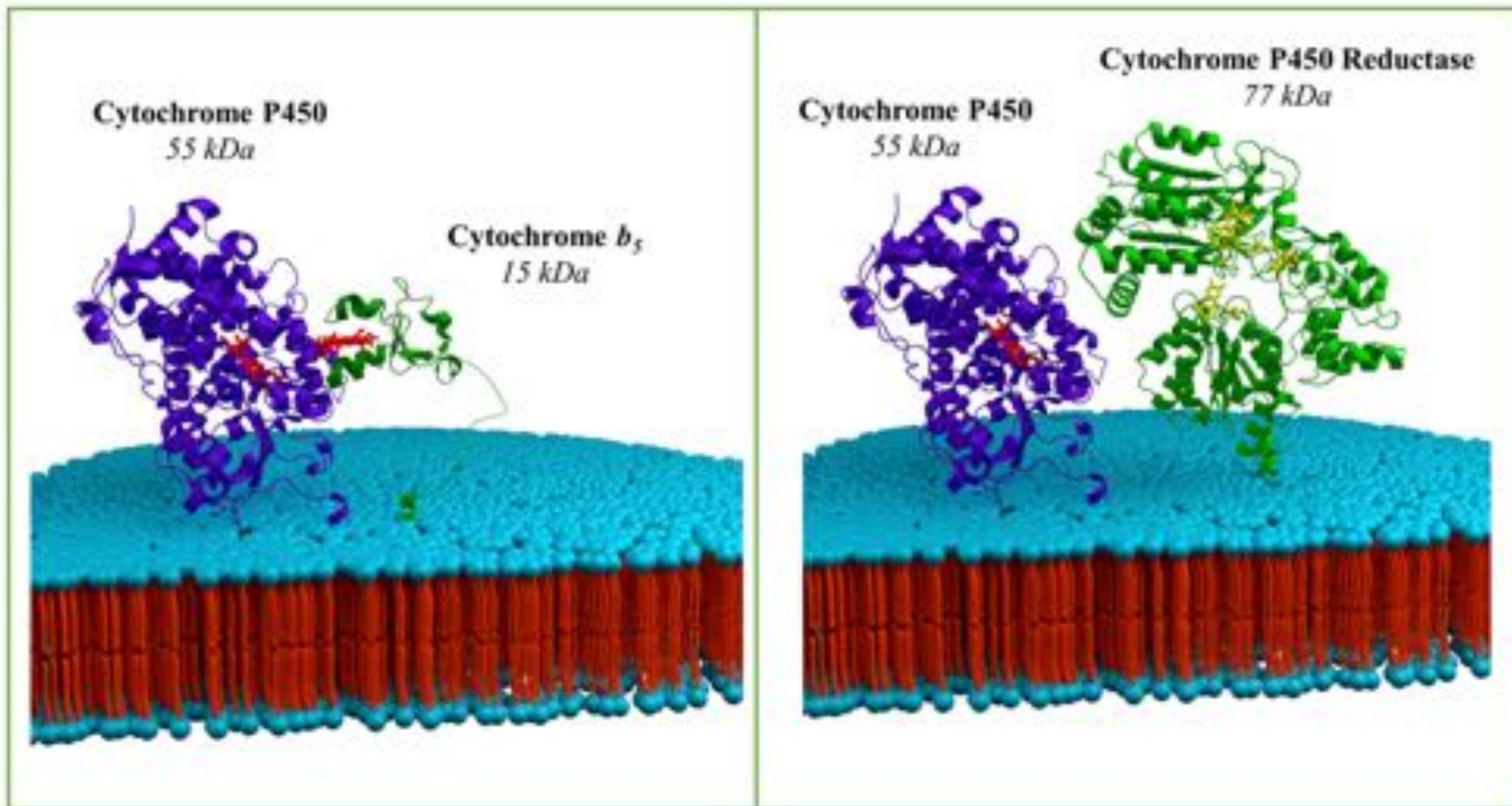
Drug metabolism



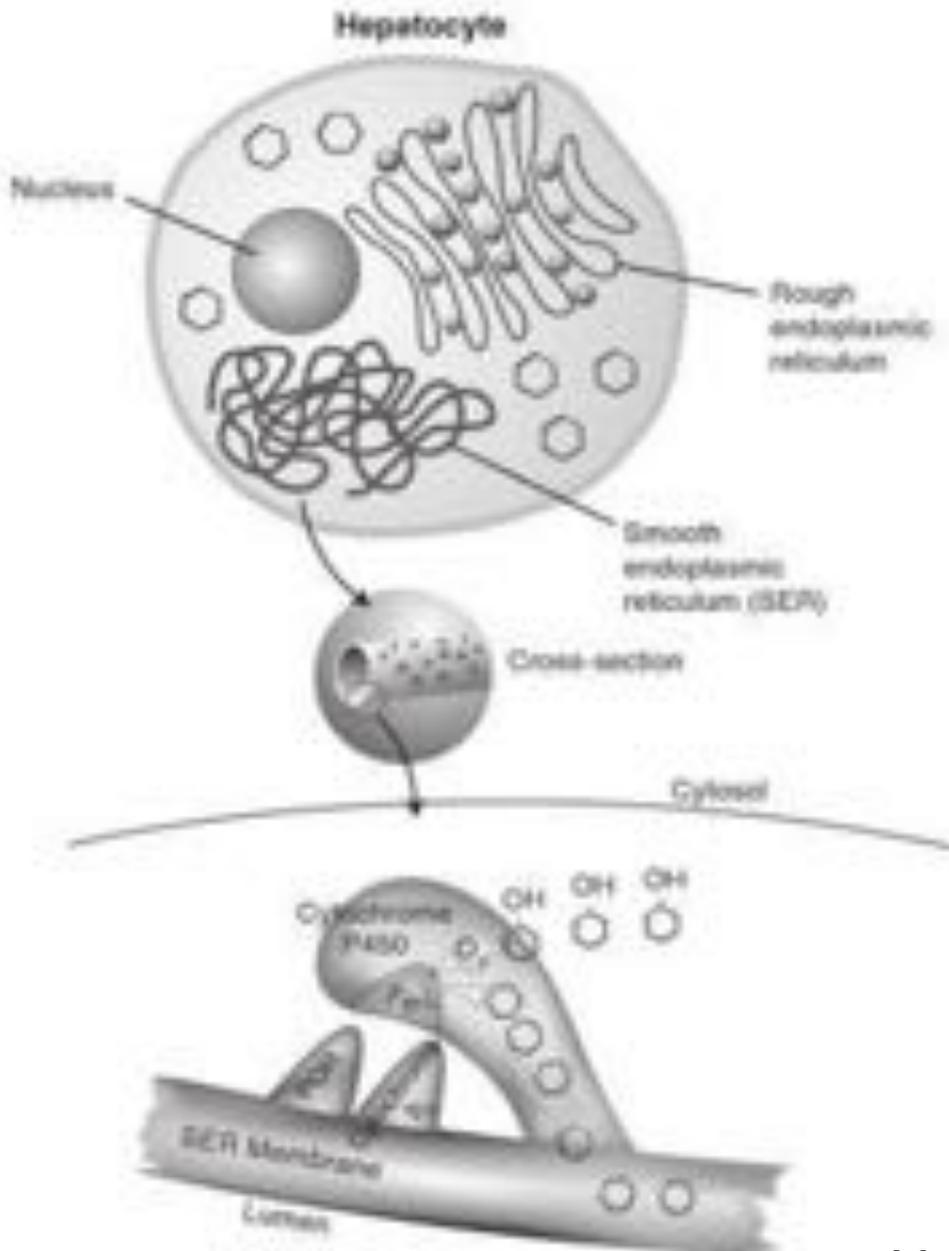
- CYP3A4,5
- CYP2D6
- CYP2C8,9
- CYP2C18,19
- CYP1A2
- CYP2E1
- CYP2B6
- CYP2A6
- CYP1A1

Abundance in human liver





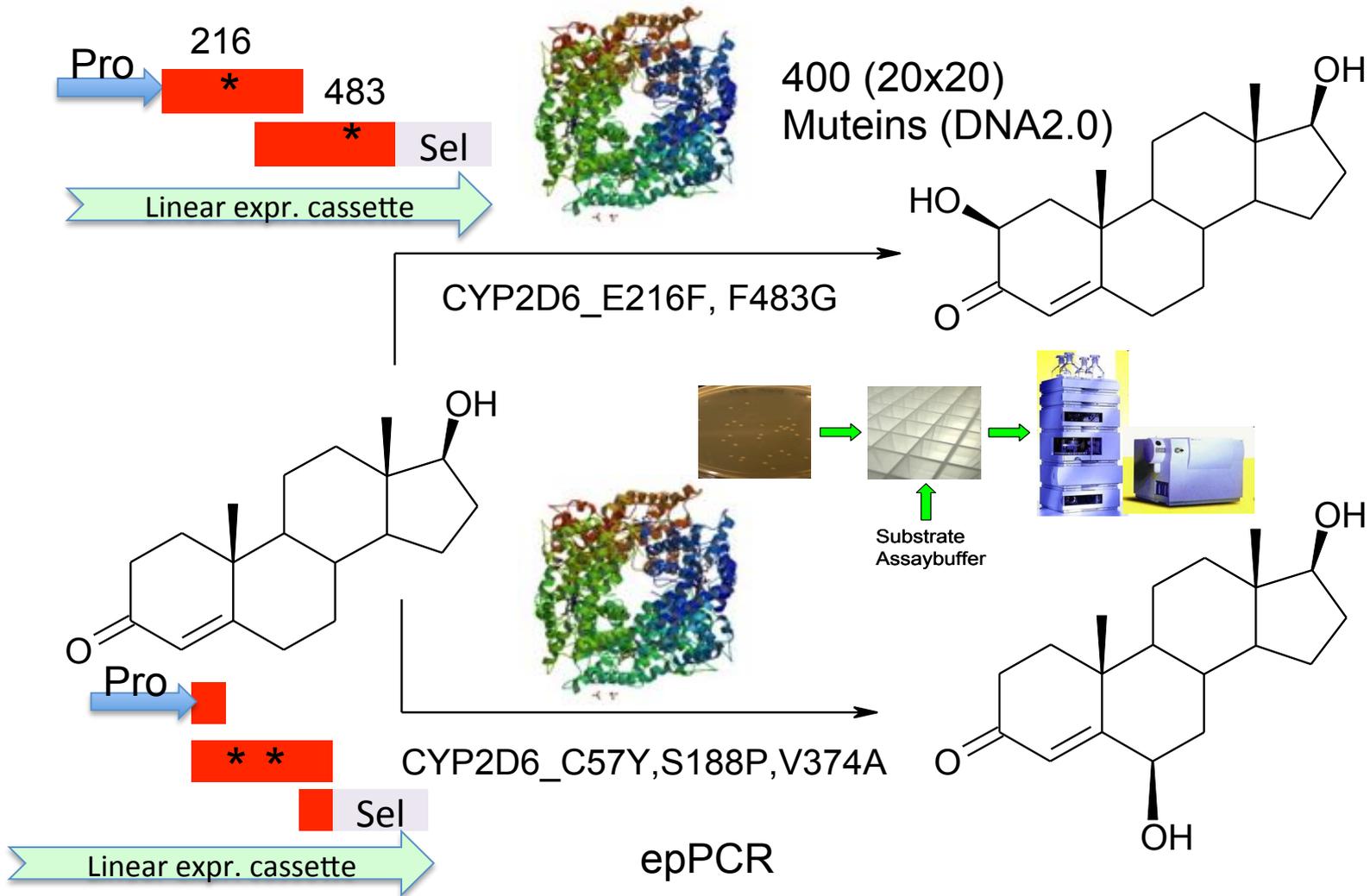
Barnaba C, Gentry K, Sumangala N and Ramamoorthy A. The catalytic function of cytochrome P450 is entwined with its membrane-bound nature [version 1]. F1000Research 2017, 6:662 (doi: 10.12688/f1000research.11015.1)



Lipophilic substrates enter the active site via the membrane

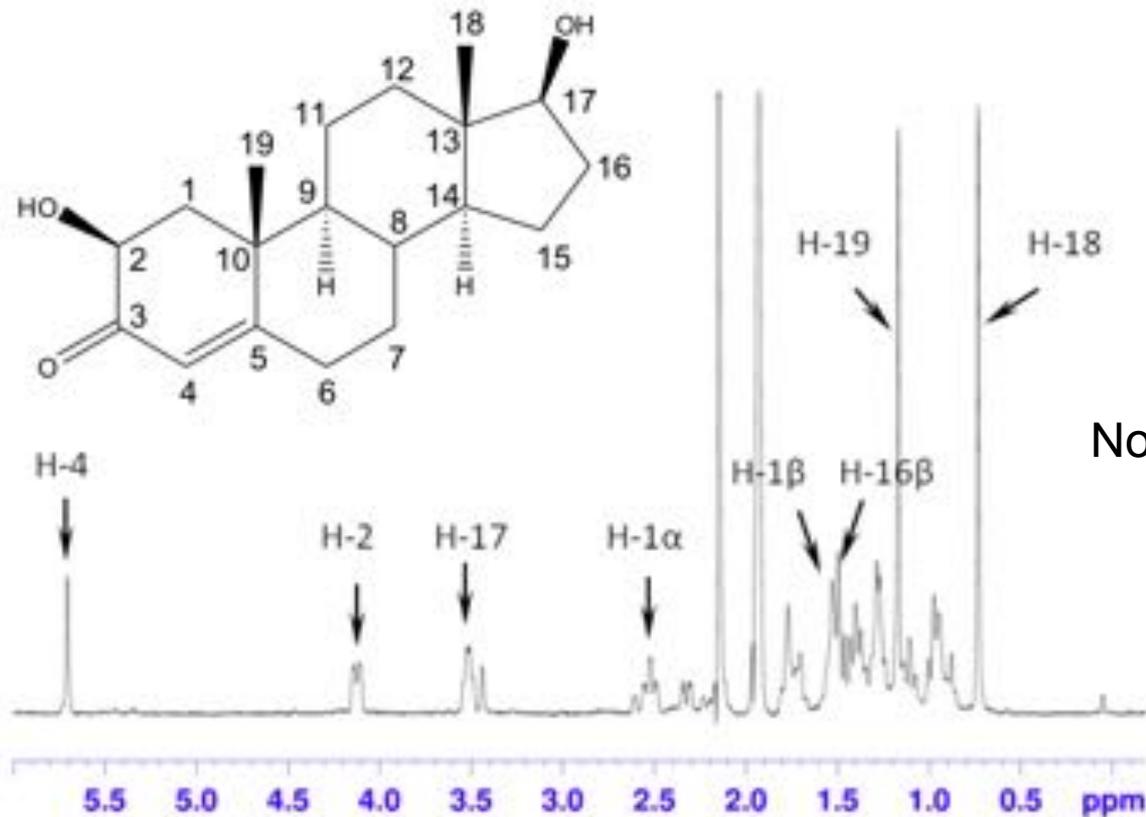
M. D. Coleman, 2010 Human drug metabolism- An Introduction (ed.2). Wiley-Blackwell

CYP2D6 engineering



Unknown metabolite is 2-hydroxy testosterone !

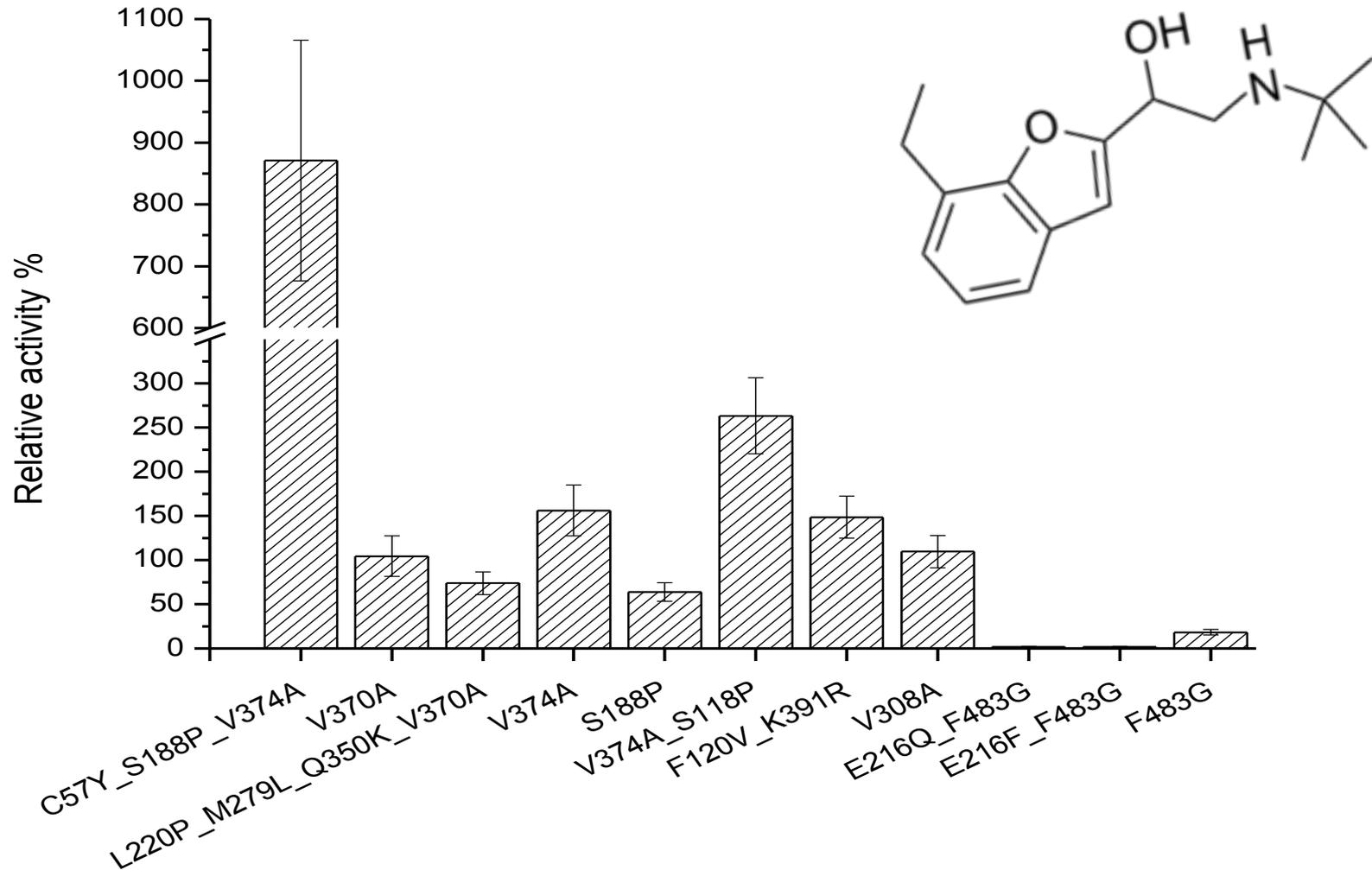
Identification of 2,17-dihydroxy-, (2 β ,17 β)-androst-4-en-3-one (2 β -hydroxytestosterone)



Not 15 hydroxytestosterone

Rudroff, Fink, Mihovilovic, TU Vienna

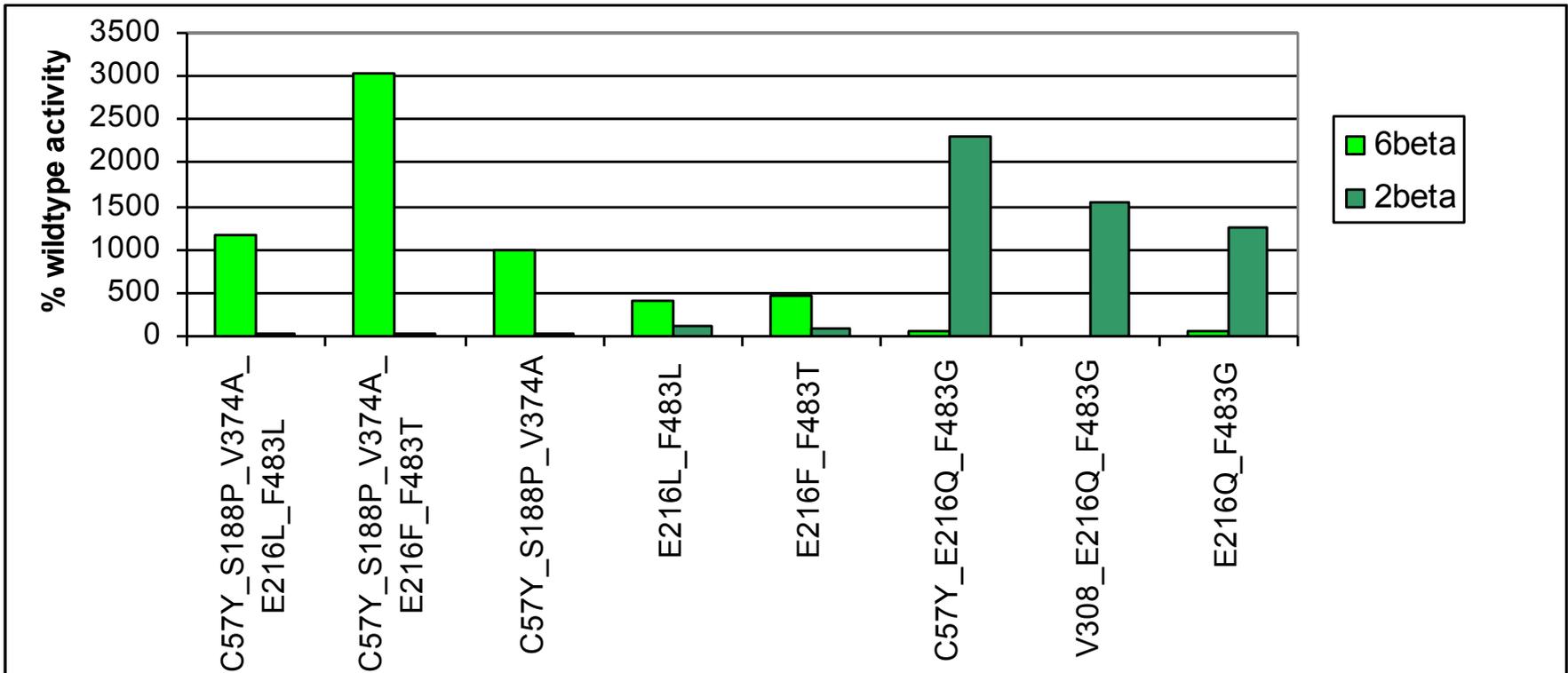
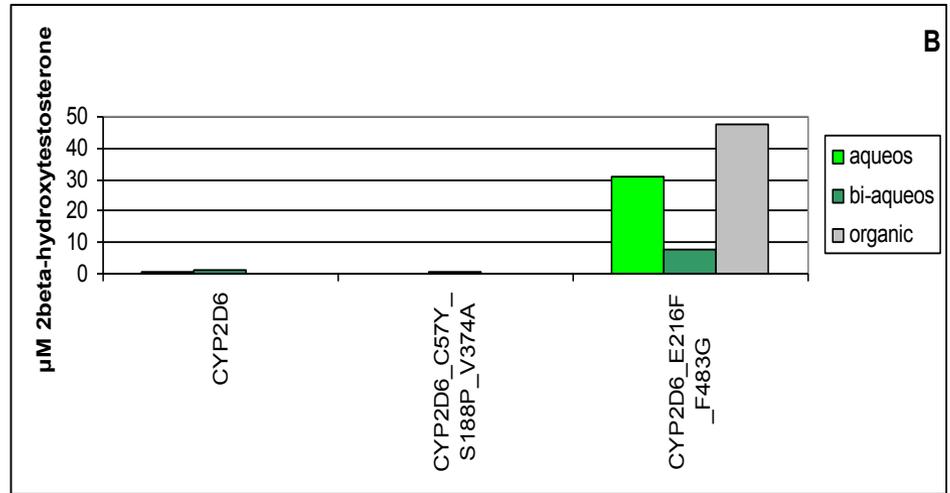
Bufuralol hydroxylation



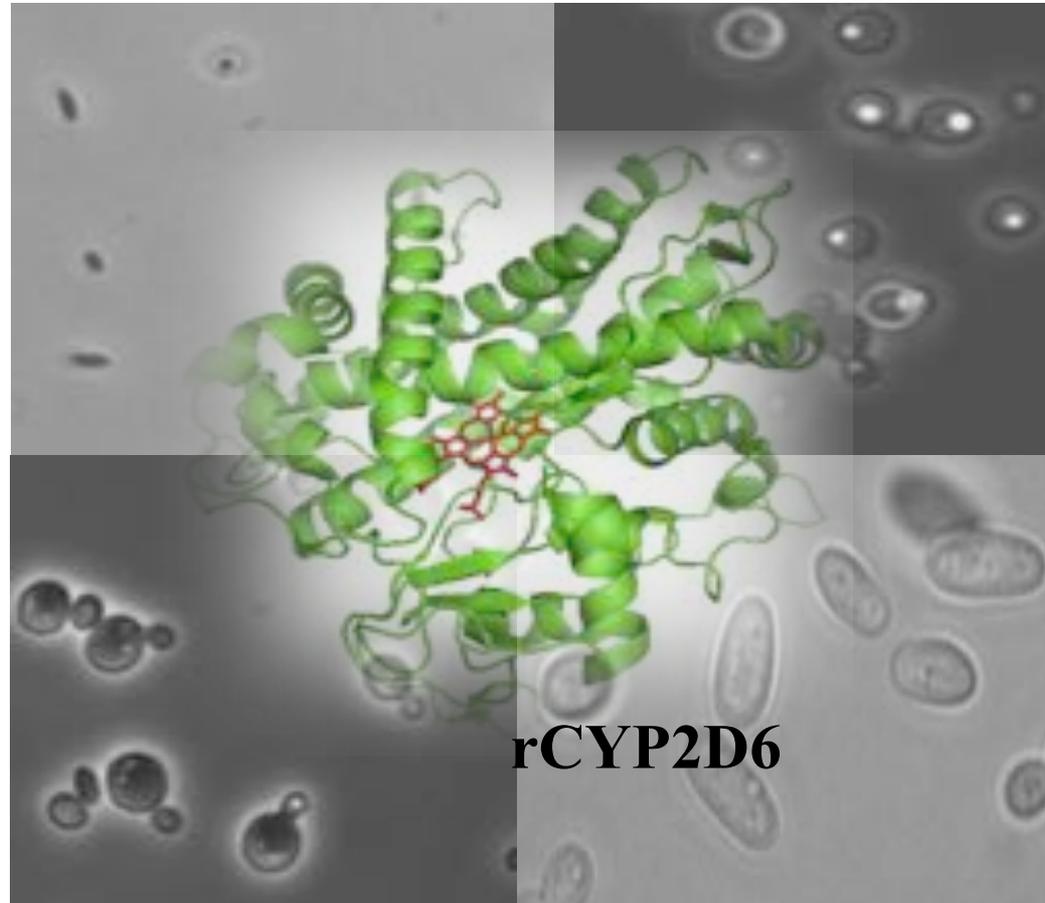
Conversion of CYP2D6 to steroid hydroxylase

Oxygreen (FP7)

Martina Geier, PhD thesis



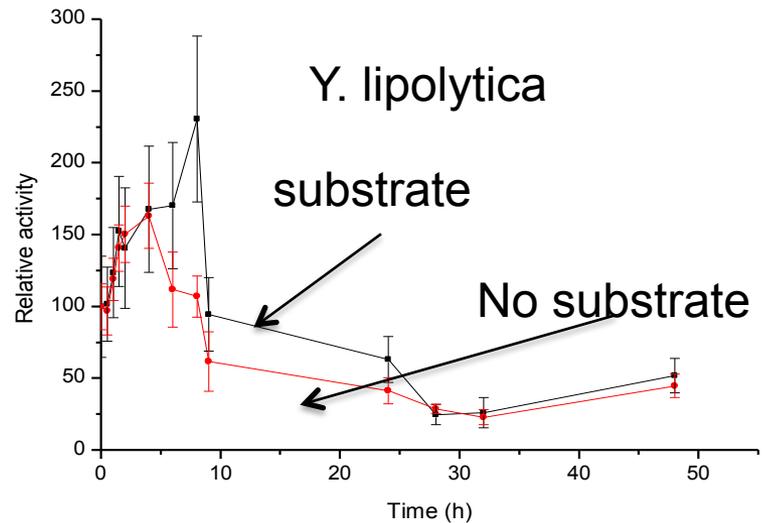
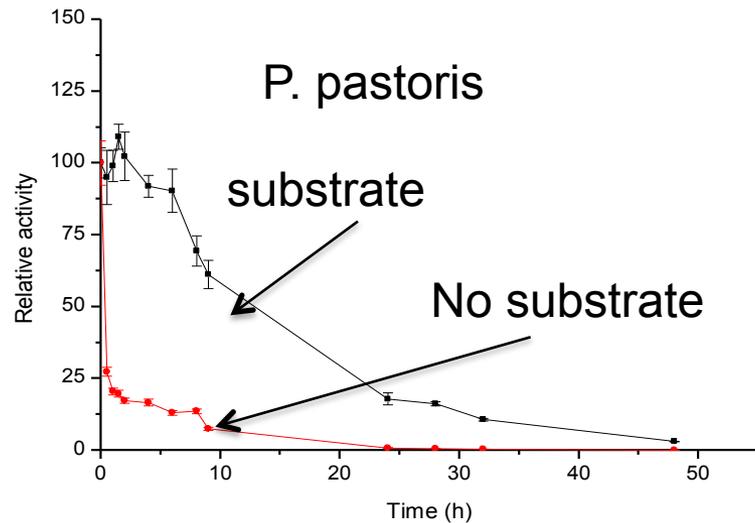
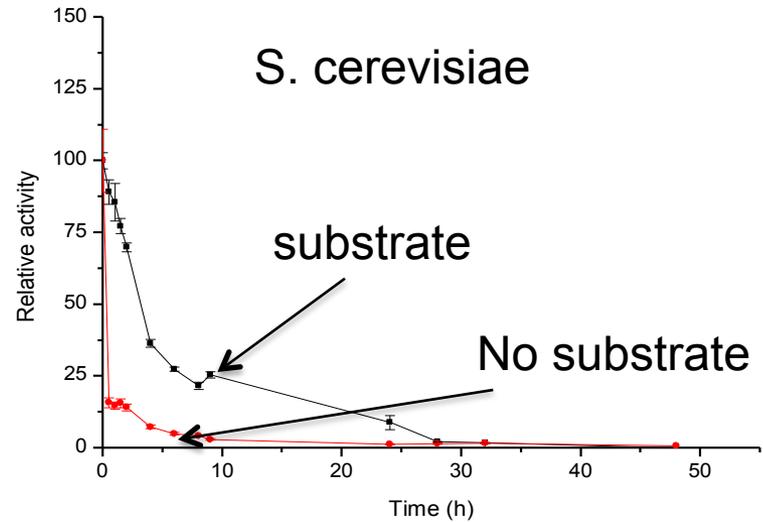
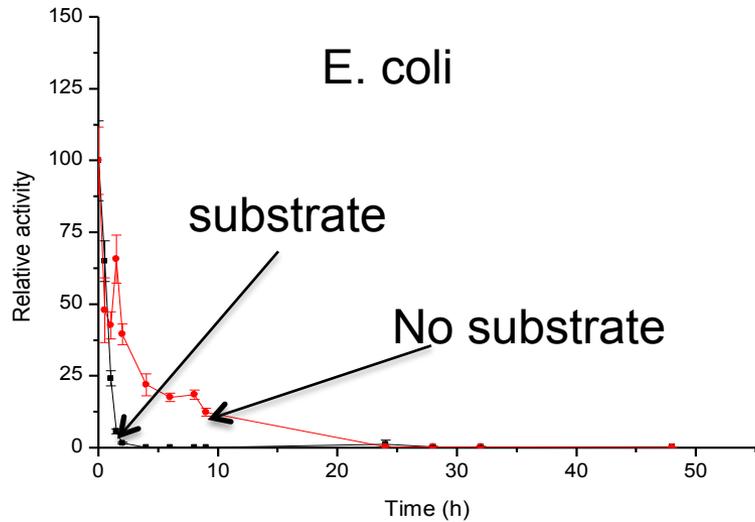
Whole cell processes



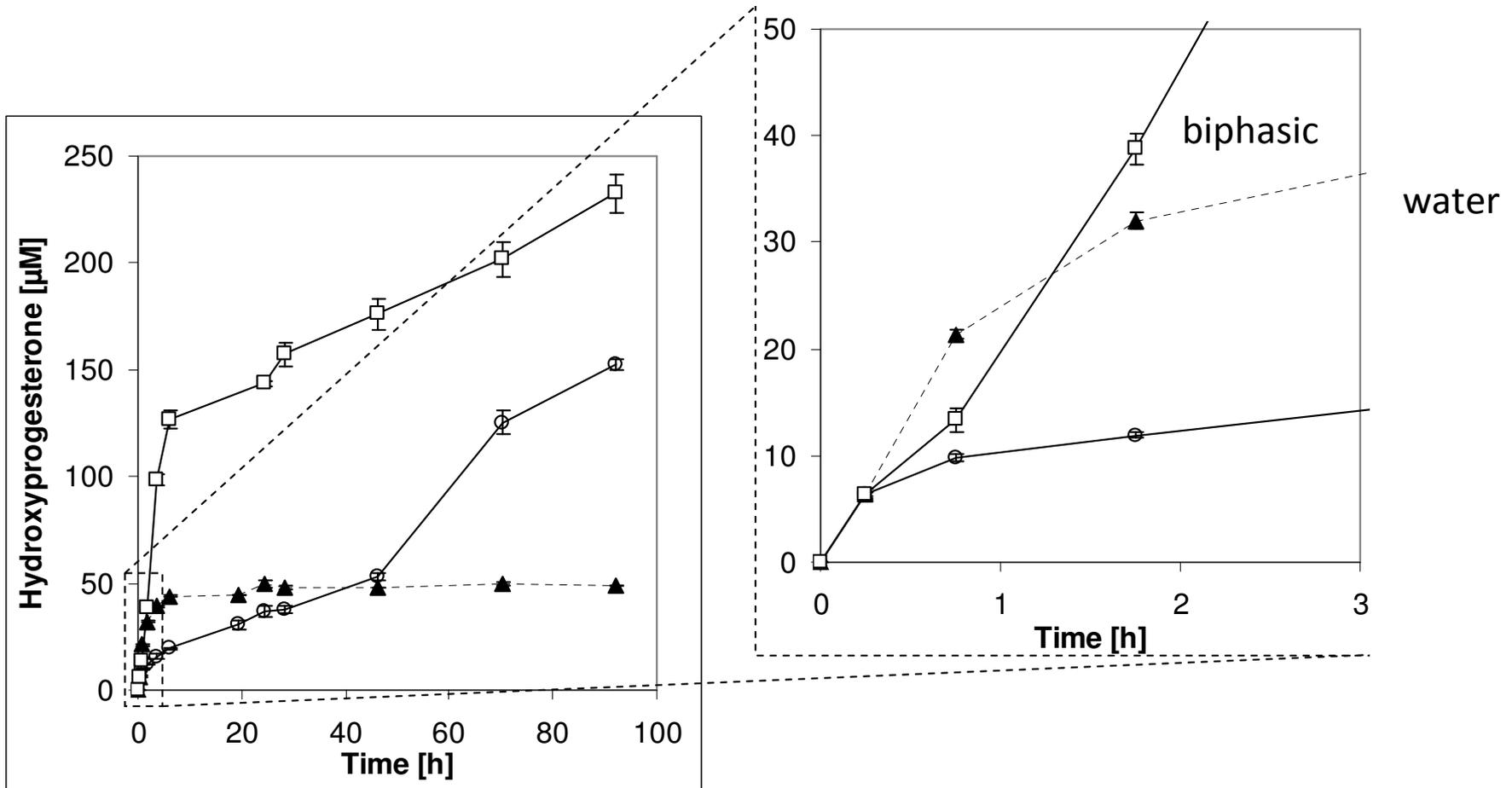
With Bruno Bühler & Andreas Schmid, TU Dortmund

Braun et al., Microbial Cell factories 2012

Whole cell catalyst stability



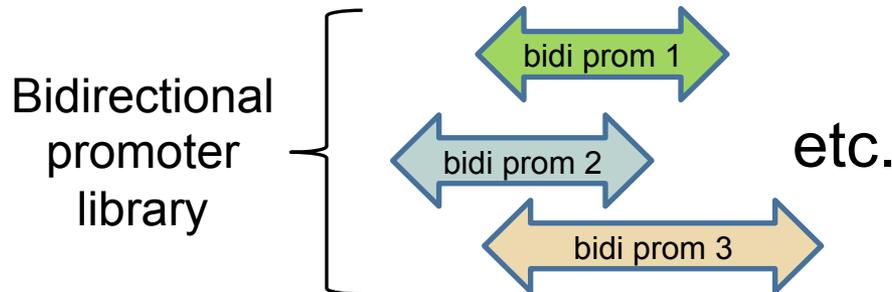
Progesterone hydroxylation by *Y. lipolytica* CYP3A4/hCYPR



aqueous / biphasic systems

Expression strategies: Bidirectional promoters

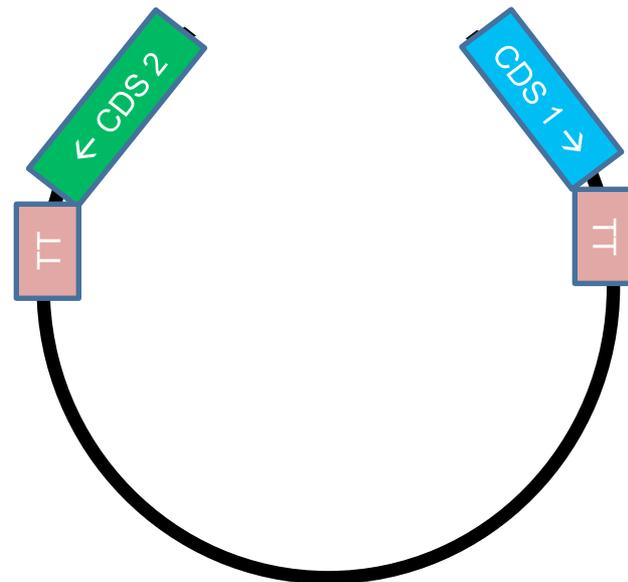
E. coli & *P. pastoris* promoter libraries



Vogl *et al.*,
EP 2862933 A2, 2015;

Gene coexpression

- Dimeric protein (Antibodies)
- Enzyme + redox partner (CYP+CPR)
- Folding helper (GOI+chaperone, PDI...)
- Metabolic pathways



ROBOX



Optimization of...
Transcription levels/strength
Ratio
„Time profile“
(= constitutive vs. inducible)

Main focus on CYP2C9

Thank you for your attention!



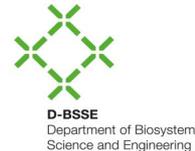
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