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Heme-iron oxygenases: powerful industrial biocatalysts?

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Are cytochrome P450 enzymes powerful industrial biocatalysts? Next to market demands, well-defined enzyme functionalities and process parameters allow generalizations on the basis of process windows. These can provide useful guidelines for the design of improved biocatalysts. Oxygenase-catalyzed reactions are of special interest for selective C–H bond oxidation. The versatile class of cytochrome P450 monooxygenases attracts particular attention, and impressive advances have been achieved with respect to mechanistic insight, enzyme activity, stability, and specificity. Recent major achievements include significant increases in productivities, yields, and rates of catalytic turnover as well as modification of substrate specificity and efficient multistep reactions in whole-cell biocatalysts. For some biocatalysts, these parameters are already of an industrially useful magnitude.

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Introduction

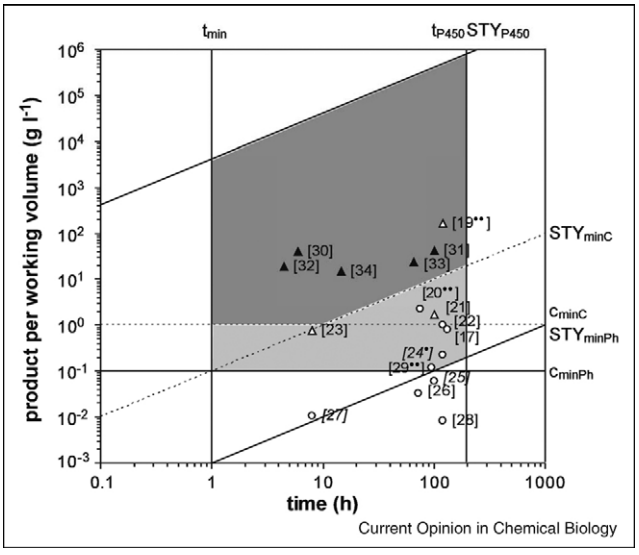
Cytochrome P450 (CYP450) oxygenases are generally acknowledged to have a high potential as biocatalysts in chemical and pharmaceutical industries [1•,2,3••]. CYP450 enzymes catalyse a diverse set of often highly specific oxidation reactions. They are involved in the biosynthesis of several pharmaceuticals, such as polyketide antibiotics [4], artemisinin [5], and paclitaxel [6,7]. Mammalian CYP450 enzymes are key enzymes in hepatic drug metabolism and detoxification and thus accept a broad range of complex substrates. The ability to catalyse the oxidation of unactivated carbon atoms under mild reaction conditions, a feature unparalleled in chemical synthesis, makes CYP450 enzymes interesting candidates for productive biocatalysis.

Although the interest of scientists in CYP450 enzymes has increased tremendously during the past decades, the application of these enzymes as biocatalysts in industrial processes is still limited to few examples. Research focuses mainly on drug metabolism and metabolite identification. Often the low activity and the multicomponent nature of these enzymes make their use challenging and often result in poor productivities. Different aspects including membrane integration, folding, cofactor regeneration, and substrate availability have to be dealt with. The use of whole-cell systems seems to be the best solution for the synthetic application of such complex enzymes [8]. Besides the application of CYP450 enzymes for synthetic purposes, their use in biosensors and biochips has great potential in medical diagnostics, environmental monitoring, and food quality control [9,10]. Based on recent literature, this review discusses the current status and the perspectives of CYP450 enzymes in synthetic applications focusing on productive biocatalysis targeted to fine chemical and pharmaceutical industries.

Productive biocatalysis

The economic feasibility of a biotransformation depends on its productivity and yield. For industrial fine chemical production, biotransformations have been considered to require a minimum space–time yield (STY_{minC} in Figure 1) of 0.1 g l^{-1} h^{-1} and a minimum final product concentration (c_{minC}) of 1 g l^{-1} [11,12•]. For pharmaceuticals, an efficient time-to-market strategy is more crucial than the production costs [8,13], with estimated minimum process requirements of 0.001 g l^{-1} h^{-1} and 0.1 g l^{-1} for volumetric productivity and product concentration, respectively [12•]. Using these minima, we defined an operational window for CYP450 processes (Figure 1) based on a previously described general concept [11,14]. Processes within the operational window are considered to have industrial potential. For both pharmaceutical as well as fine chemical production, the minimum reasonable process time (t_{min}) was set at 1 h [12•]. The upper boundaries of the operational window are defined by the maximally achievable process performance, which is calculated from the highest activity, expression level, and process time reported for CYP450 enzymes. To the best of our knowledge, the highest k_{cat} (285 s^{-1}, determined based on NADPH oxidation) has been reported for the oxidation of arachidonic acid catalysed by bacterial CYP102A1 [15]. It should be noted that CYP102A1 contains the oxygenase and reductase functionality fused in one polypeptide chain and that k_{cat} values for multicomponent enzymes are lower (0.3–20 s^{-1}) [16]. The highest expression level (12 500 nmol l^{-1}) was reported for the same enzyme in *Escherichia coli* [17•]. Combining

Figure 1



Operational window for CYP450-catalyzed bioprocesses. The dark grey area represents the operational window for bioprocesses in the fine-chemical industry. The light and dark grey areas together represent the operational window for bioprocesses in the pharmaceutical industry. (○) CYP450-based production of pharmaceutical products. (△) CYP450-based production of a fine chemicals. (▲) Production of fine chemicals based on oxygenases containing nonheme-iron or flavin centers. t_{min}: minimum time a bioprocess should run. t_{P450}: longest reported time a CYP450 enzyme was active in a (fed-) batch process. STY_{P450}: theoretical maximum space–time yield for CYP450-based processes. STY_{minC}: minimum required space–time yield for bioprocesses in the chemical industry. STY_{minPh}: minimum required space–time yield for bioprocesses in the pharmaceutical industry. c_{minC}: minimum required product concentration for bioprocesses in the fine-chemical industry. c_{minPh}: minimum required product concentration for bioprocesses in the pharmaceutical industry. Numbers in brackets correspond to literature in the reference list. Numbers in italics belong to a process based on a mammalian CYP450.

these two maxima, a volumetric productivity of 4.1×10^3 g l^{-1} h^{-1} (STY_{P450}) could theoretically be reached. The upper limit for the process time (t_{P450} = 195 h) was derived from the hydroxylation of simvastatin by a *Nocardia* species [18].

This operational window was used to evaluate CYP450 catalysed biotransformations carried out at a scale ≥1 l. We found 12 examples in literature [18,19••,20••,21–23,24•,25–28,29••] and compared them with 5 efficient bioconversions catalysed by nonheme oxygenases (Figure 1) [30–34]. Interestingly, the operation time of CYP450-based processes is high as compared to processes based on nonheme oxygenases. However, the performance of the latter is often limited by toxic or inhibiting product concentrations. For the same reason, an increase of the volumetric productivities of CYP450-based processes might result in lower process stabilities. The production of dicarboxylic acids from alkanes or fatty acids [19••,35,36] is the only CYP450-based biotransformation that fulfills the minimal requirements defined for fine chemical synthesis. This indicates that CYP450 mono-oxygenases in principle allow the same process efficiency as it is reached with oxygenases containing flavins or nonheme-iron centers in the active site. However, such high process efficiencies often are not achieved with CYP450 enzymes. Four CYP450-based biotransformations do not even fulfill the minimum requirements set for the pharmaceutical industry (Figure 1). Clearly, CYP450-based biocatalysis has to become more efficient in terms of productivity to be of industrial significance.

The three processes based on mammalian CYP450 enzymes showed particularly low productivities [24•,25,27] (Figure 1). This reflects the lower activity of mammalian as compared to bacterial CYP450 enzymes [1•], which might be explained by differences in structure and molecular dynamics [37]. Computational modeling indicated that the buried active site of CYP450 enzymes is accessible from the protein surface by multiple channels, which can merge to form large clefts [37]. For mammalian CYP2C5, two distinct channels have been proposed for substrate uptake from the membrane and the cytoplasm, the latter also allowing product egress, whereas bacterial enzymes are thought to contain one dominant channel for substrate access and product egress [38]. The high structural flexibility of mammalian CYP450s including the variable merger of substrate channels makes uptake of diverse and large substrates possible [37]. Their broad substrate spectrum is important for the natural function of these enzymes either in degradative or biosynthetic reactions, but might be responsible for the low activity.

Besides enzyme activity, aspects influencing the efficiency of CYP450-based bioconversions include specificity, stability, coupling efficiency, interaction with electron transport proteins, membrane integration, and cofactor regeneration. These features typically are optimized on three different levels: enzyme engineering, cell engineering, and reaction engineering. In order to implement a biotransformation into an industrial process, these three levels are completed by process engineering, which considers scaling down-stream processing issues. Recent achievements in whole-cell P450-catalysis are summarized in Table 1 and discussed below.

Enzyme engineering

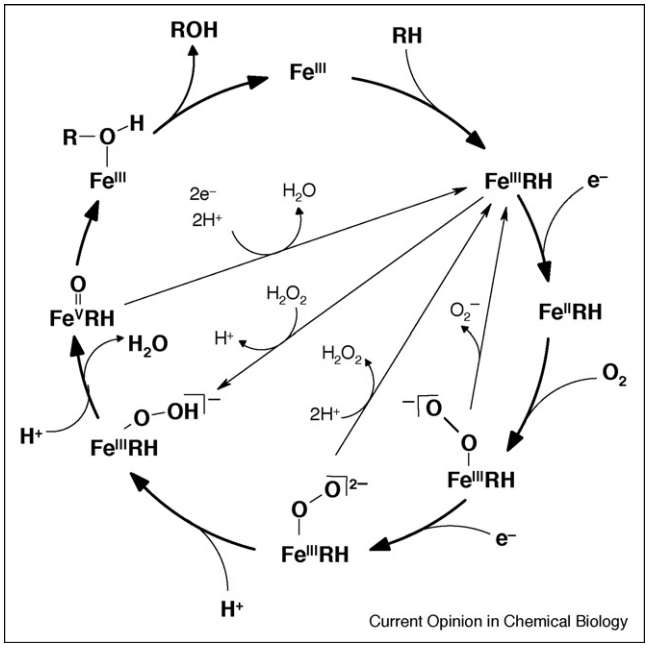
CYP450 enzymes have been engineered to increase expression levels, activities, and stabilities and to change their substrate specificities. Most of this work has been performed with the bacterial enzymes CYP102A1 (P450 BM3) and CYP101A1 (P450cam) as reviewed recently [1•,2,39], but also the engineering of mammalian enzymes has been reported [3••]. Here, we will focus on recent examples, which are of interest for the development of industrial biocatalysts.

In order to increase expression levels of mammalian CYP450s in *E. coli*, the replacement of the 5′-sequence of wild-type CYP450 genes by a specific amino acid sequence (MALLLAVF-) or truncation of the hydrophobic N-terminus have developed into standard procedures [40•]. However, these changes might negatively influence the interaction of the CYP450 oxygenase with the corresponding reductase [41•]. Kim *et al.* reported that there was no necessity to modify the N-terminal sequence of human CYP1A2 for high-level expression in *E. coli* [42]. Alternatively, the 5′-sequence was fused with the leader sequence of the bacterial *ompA* gene, thus preventing any modification of the CYP450 cDNA sequence [43]. This strategy improved the expression levels of recombinant CYP3A4 from 90 to 500 nmol l^{-1} without any loss of activity for testosterone hydroxylation determined in membrane fractions. Furthermore, codon usage optimization enabled expression of human CYP2W1 to significant levels in *E. coli* (350 nmol l^{-1}) [44]. Keasling and coworkers successfully used the same strategy for plant enzymes [45••]. Although no single strategy exists, optimization of codon usage and introduction of a signal sequence are promising strategies to improve CYP450 expression levels.

Several groups have used directed evolution and site-directed mutagenesis to change the substrate specificity of CYP450 enzymes. CYP101A1 and CYP102A1 were engineered to accept short chain alkanes as substrates. Xu *et al.* even reported the oxidation of ethane to ethanol by a mutant of CYP101A1 [46•]. However, ethane was oxidized at a significantly lower rate (78.2 nmol min^{-1} $nmol^{-1}$) than propane (505 nmol min^{-1} $nmol^{-1}$), which correlated with the coupling efficiencies of 10.5 and 85.6% for ethane and propane, respectively. For propane oxidation by different CYP102A1 mutants, the same relation between enzyme activity and coupling efficiency was found [47••]. Uncoupling, a common phenomenon in CYP450 catalysis, results in the unproductive oxidation of NAD(P)H and the formation of water, superoxide, or hydrogen peroxide (Figure 2), which can be harmful for the enzyme and the cell. Directed evolution of a CYP102A1 mutant capable of oxidizing alkanes increased the propane oxidation rate from 201 to 370 nmol min^{-1} $nmol^{-1}$, the total turnover number from 5650 to 45 800, and the coupling efficiency from 17 to 98.2% [47••]. The presented data clearly indicate that a high coupling efficiency is crucial for productive CYP450 catalysis. The improved coupling efficiency was not only accompanied by an activity increase but also by a higher stability. In an independent study, the thermostability (in terms of half-life) of CYP102A1 at 57 °C could be increased 108-fold by the recombination of stabilizing fragments [48•].

CYP450 enzymes can also oxidize their substrates via the so-called shunt pathway (Figure 2) with peroxides instead of molecular oxygen as the oxygen donor. In general, this pathway is less efficient, but has the advantage of being independent of NAD(P)H. Arnold and coworkers successfully engineered CYP101A1 by directed evolution to efficiently catalyse naphthalene hydroxylation via the peroxide shunt pathway, for which the activity was increased more than nine times with respect to wild-type CYP101A1 [49]. Unfortunately, the peroxide shunt and the O_2-dependent and NADH-dependent reaction have not been compared in terms of reaction rate.

Figure 2



Generalized CYP450 reaction cycle showing the three possible uncoupling reactions and the peroxide shunt pathway.

In more recent studies, the peroxygenase activity of CYP102A1 also has been improved by directed evolution [50]. Thereby, a maximum H_2O_2-driven 12-*p*-nitrophenoxycarboxylic acid oxidation rate of 800 min^{-1} has been reached, which is in the same order of magnitude as the activity of the parental F87A mutant with oxygen and NADPH (k_{cat} = 508 min^{-1}) [51]. The use of the hydrogen peroxide shunt has also been described for the human enzymes CYP2D6 and CYP3A4, which were specifically selected because of their high substrate promiscuity and potential for biocatalysis [52]. Kumar *et al.* engineered human CYP3A4 by directed evolution and site-directed mutagenesis to increase the efficiency of the peroxide shunt [53]. A mutant with an 11-fold increase in k_{cat}/K_m for 7-benzoylquinoline debenzylation using cumene hydroperoxide as oxygen donor was found. However, the mutants showed 10–15 times lower activities using cumene or hydrogen peroxide in comparison to the

NADPH-dependent reaction (wild-type enzyme showed a 25-fold decrease in activity). By contrast, Chefson *et al.* [52] described a 2.1-fold higher activity towards dextromethorphan for CYP2D6 (initial rate: 2.4 nmol min^{-1} $nmol^{-1}$; total turnover number: 107) and a 1.3-fold higher hydroxytestosterone formation rate for CYP3A4 (9.1 nmol min^{-1} $nmol^{-1}$; 42) by adding cumene hydroperoxide instead of NADPH and the human NADPH-reductase. Remarkably, in this study, no protein engineering to improve the efficiency of the peroxide shunt pathway was performed. Moreover, the addition of exogenous hydrogen peroxide did not result in detectable product formation [52]. The engineering of CYP450 enzymes for a more efficient peroxide shunt pathway is a promising strategy for the development of biocatalysts based on isolated enzymes. The addition of peroxides solves the cofactor regeneration problem but might destabilize the enzyme. The addition of peroxides to whole-cell systems is expected to be complicated by factors such as cytotoxicity to the cells, peroxide breakdown by catalases, and limited uptake of organic peroxides.

Cell engineering

CYP450 enzyme systems often consist of multiple and membrane-bound components, which complicates the handling of isolated enzymes and often results in unstable activities. Thus, whole-cell systems are typically used to guarantee continuous cofactor regeneration and enzyme synthesis. Nevertheless, all necessary components have to be available at the right time, concentration, and location in the cell for optimal reaction conditions.

The biocatalyst can be a wild-type microorganism, but in most cases optimization of the biocatalyst is necessary to improve activity and/or stability. For the development of a recombinant biocatalyst, *E. coli* is in general the organism of choice because of the well-developed molecular biology techniques. Recently, Hanlon *et al.* compared expression levels and activities of human CYP450 enzymes in *E. coli* and *S. cerevisiae* and concluded that *E. coli* is the more suitable host, especially for CYP3A4 with a 115-fold higher catalytic activity for testosterone hydroxylation [12 nmol min^{-1} (mg protein)$^{-1}$] than *S. cerevisiae* [0.1 nmol min^{-1} (mg protein)$^{-1}$] [54]. However, other organisms could still offer a better alternative for a specific enzyme. Recombinant *Pseudomonas putida* have recently been used for the bioconversion of (−)-limonene to (−)-perillyl alcohol [20••]. CYP153A6 isolated from *Mycobacterium* showed higher activities in *P. putida* GPo12 as compared to *E. coli* (3.0 and 0.1 U g^{-1}, respectively, based on dry cell weight). The use of recombinant yeast strains offers the possibility and advantage to use the host NADPH-reductase. For example, human CYP2D6 producing *Schizosaccharomyces pombe* have been used for the synthesis of drug metabolites [24•]. This fission yeast strain was chosen, as it is a eukaryote, its genome is fully sequenced, and it has only two endogenous CYP450 genes. Industrial *Pichia pastoris* strains have also shown promising levels of mammalian CYP17A1 expression [55]. The high cell densities achieved by this organism in industrial fermentations represents another advantage. Although *E. coli* is still the standard host organism, other strains should be investigated when the desired activities for a specific biotransformation

Table 1

Major improvements achieved for whole-cell CYP450 processes

Engineering level	Methodology	Improved parameter	Fold increase achieved	Reference
Enzyme engineering	5′-Terminal sequence modifications of plant CYP450 genes	Enzyme expression level	5	[45••]
	Fusion of *ompA* signal sequence to the 5′-end of mammalian CYP450 genes	Enzyme expression level	5.6	[43]
	Codon usage optimization	Product yield	2.5	[45••]
	Site-directed mutagenesis	Activity towards an unnatural substrate	0–78.2 min^{-1}[a]	[46•]
	Directed evolution	Activity	1.8	[47••]
		Coupling efficiency	5.8	[47••]
		Total turnover number	8.1	[47••]
Cell engineering	Host selection	Specific activity	30[b]	[20••]
		Specific activity	115[c]	[54]
	Redox partner selection	Product yield	16	[56]
	Chaperone coexpression	Enzyme expression level	5.1	[44]
	Cofactor regeneration	Activity	10	[60]
Reaction engineering	Fed batch optimization	Enzyme expression level	25	[17•]
		Enzyme expression level	10	[66]
	pH optimization	Product yield	1.22	[19••]

[a] A mutant of CYP101A1 showed ethane hydroxylation activity whereas wild-type CYP101A1 is not able to perform this reaction.
[b] *P. putida* showed a higher specific activity than *E. coli*.
[c] *E. coli* showed a higher specific activity than *S. cerevisiae*.

are not reached. Further improvements of whole-cell activities may be achieved by coexpression and metabolic engineering strategies.

Coexpression strategies

The coexpression of genes encoding the redox partners of the CYP450 is necessary when the host cell lacks such electron transfer proteins (*E. coli*) or the activity using an endogenous system is low. Ruijssenaars *et al.* investigated the coexpression of different redox partners from *Bacillus* sp. in *P. putida* and found an up to 16-fold variation in the rate for testosterone hydroxylation catalysed by CYP106A2 [56]. For *E. coli*, coexpression of genes responsible for heme synthesis, protein folding, or cofactor regeneration has also been described.

The insufficient endogenous heme synthesis is a main disadvantage of *E. coli* so that the expensive heme precursor 5-aminolevulinic acid has to be added as a substrate. Coexpression of the *hemA* gene, which encodes a glutamyl-tRNA reductase catalyzing the rate-limiting step in heme biosynthesis, circumvented the need to add 5-aminolevulinic acid enabling good expression levels for several mammalian CYP450 enzymes [57]. This strategy can reduce process costs, because 5-aminolevulinic acid addition is responsible for up to 60% of the enzyme production costs.

The coexpression of the chaperone genes *groEL/groES* in *E. coli* improved vitamin D3 hydroxylation by CYP27B1 [58]. This could be explained by higher expression levels and a more efficient folding. Coexpression of *groEL/groES* also led to a concentration increase of active human CYP2W1 (from 350 to 1800 nmol l^{-1}) and CYP2S1 (from below 120 to 600 nmol l^{-1}) [44]. However, it is unclear, whether this effect is caused by the chaperone proteins or by the osmotic stress induced by arabinose added to induce chaperone gene expression [59].

For CYP101A1-based whole-cell catalysis, coexpression of a glycerol dehydrogenase gene to increase intracellular NADH availability resulted in a 10-fold improvement of camphor bioconversion [60]. The production of indigo catalysed by CYP102A1 was improved by the coexpression of a glucose dehydrogenase gene to enhance NADPH regeneration [61]. Thereby, an indole oxidation activity of 8173 U (mg CYP450)$^{-1}$ and an indigo concentration of 760 mg l^{-1} were reached. Coexpression strategies have become powerful tools in the optimization of whole-cell CYP450 biocatalysis, especially with respect to NAD(P)H regeneration and heme synthesis, leading to higher productivities and lower costs.

New biosynthetic pathways

The use of whole cells provides the opportunity to construct artificial biosynthetic pathways, comprising several enzymatic steps, and to link these to primary metabolism. As such, metabolic engineering allows the replacement of complex and expensive substrates, for example, by simple sugars, which makes the processes economically more attractive. The work of Keasling and coworkers aimed at producing the antimalaria drug artemisinin in yeast and *E. coli* is an impressive example. An increased availability of endogenous precursors for terpenoid biosynthesis in *E. coli* and *S. cerevisiae* has been achieved by overexpressing genes from the mevalonate biosynthetic pathway from *S. cerevisiae* [29••,45••] resulting in an artemisinic acid concentration of 105 and 115 mg l^{-1}, respectively, levels suitable for an industrial production process (Figure 1). In a similar approach, a pathway for hydrocortisone biosynthesis in *S. cerevisiae* has been reconstituted [62••]. Eight mammalian proteins including four CYP450 enzymes were combined in the recombinant host producing 10 mg l^{-1} of hydrocortisone [62••,63]. Modified strains expressing terpenoid synthases and CYP450 genes may in future represent a very important platform for cost-efficient production of terpenoids from cheap and renewable carbon sources.

Mass transfer optimization

Mammalian CYP450s typically functionalize large and complex molecules in a specific way, which gives access to potentially interesting pharmaceuticals. For whole-cell biocatalysts, the transfer of such large molecules across the cell membrane can become a limiting factor. Several strategies have been followed to solve this problem.

Adrenodoxin displayed on the cell surface of *E. coli* was shown to be active together with separately added bovine CYP11A1 and adrenodoxin reductase [64]. Although these strains can be considered as whole-cell biocatalysts, the requirement for additional enzymes, their subunits, or cofactors gives these cells many properties of immobilized enzymes and may only be synthetically relevant, if all enzymes (and cofactors) are codisplayed.

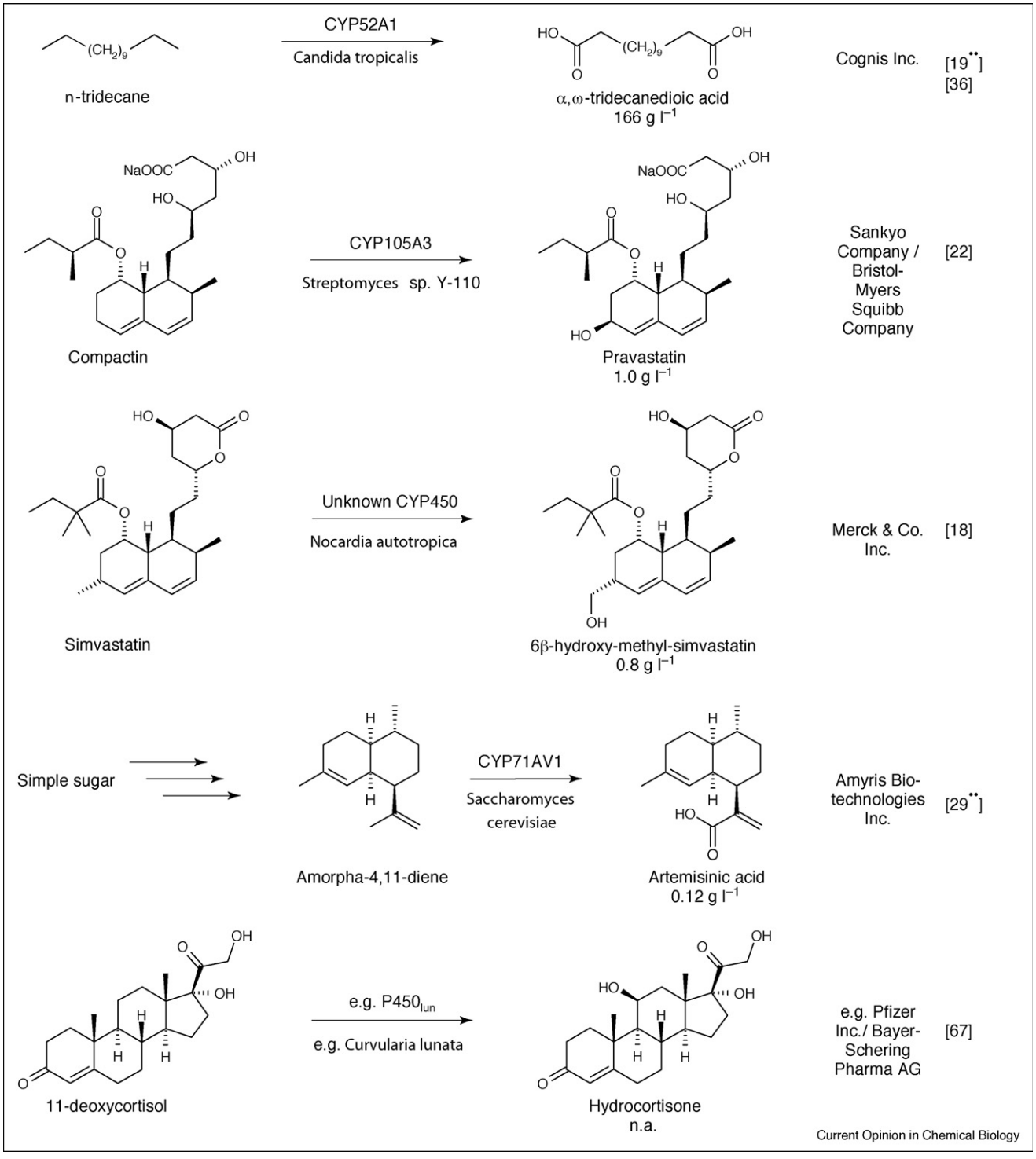
Mass transfer limitations have also been decreased by the supply of detergents such as Tween 80 to increase the permeability of cell membranes [54]. Detergents may however interfere with cellular energy metabolism (cofactor regeneration) and might also complicate product purification. From this perspective, the use of a membrane-permeable mutant strain as described for toluene dioxygenase, a nonheme-iron enzyme [65], could represent a better approach to solving mass transfer limitations than the use of detergents.

Reaction engineering

Reaction engineering focuses on the conditions under which the biocatalytic reaction takes place, aiming to optimize the productivity and stability of the process. The increase of pH during fermentation resulted in a 22% increase in the production of α,ω-dicarboxylic acids by *Candida tropicalis* [19••]. Temperature, pH, media, and

glycerol/glucose feed optimization allowed expression levels of 1010, 1800, and 890 nmol l^{-1} for human CYP1A2, CYP2C9, and CYP3A4, respectively, with *E. coli* in a 10 l bioreactor [66]. This already represents an up to 10-fold increase as compared to earlier literature data. Furthermore, dissolved oxygen concentrations below 1% were found to be optimal for the production of active CYP450. Higher dissolved oxygen levels allowed faster

Figure 3



Examples of industrial biotransformations using CYP450 enzymes. Achieved product titers are indicated below the product names when available. n.a.: not available.

cell growth, but led to higher absorptions at 420 nm in CO-spectra indicating incorrect folding of the CYP450 enzymes [66]. A final CYP102A1 concentration of 12 500 nmol l^{-1}, achieved in a 5 l fed-batch culture after 10 h of expression (~1.5 g CYP450 l^{-1}; 11% of cell dry weight), represents the highest expression level reported for a CYP450 enzyme and was 25-fold higher than reported before for the same enzyme [17•]. Also here, a low dissolved oxygen concentration in combination with glycerol as the sole carbon source was found to be optimal.

Whole-cell biotransformations are often hampered by low substrate solubilities in water or substrate and/or product toxicity. *In situ* product removal using an organic second phase can be a solution and was applied for the conversion of (−)-limonene by CYP153A6-containing *P. putida* GPo12 [20••]. Growth on octane in 1 l minimal medium and addition of 500 ml bis(2-ethylhexyl)phthalate as a second phase and 50 ml of the substrate (−)-limonene allowed the accumulation of 6.8 g l^{-1} (−)-perillyl alcohol in the organic phase.

Today, academic research on biocatalytic CYP450 application mainly focuses on enzyme and cell engineering. However, as shown by the examples described above, the optimization of reaction conditions is also important and should be combined with enzyme and cell engineering to understand and design processes with potential for industrial applications.

Process engineering — industrial processes

Although many developments towards new productive biocatalytic systems based on CYP450 enzymes have been described, only a limited number of CYP450-based processes are used in industry. Figure 3 shows some relevant examples. Although protein engineering has resulted in some impressive achievements, the relevance of the results for industrial biocatalyst development is often not addressed. Enzyme, cell, and reaction engineering are rarely combined and the development of suitable processes for the isolation and purification of products and the interaction of these down-stream processes with the chosen biotransformation conditions are often underestimated. However, efficient down-stream processing is essential to achieve an economically feasible CYP450-based process [54].

The production of dicarboxylic acids (important building blocks for polymer synthesis) from alkanes or fatty acids by *C. tropicalis* (Figure 3) is a good example for integrated process design combining both biocatalyst and process engineering [19••,35,36]. In *C. tropicalis* ATCC 20336, the terminal hydroxylations catalysed by CYP52A1 are followed by the oxidations catalyzed by fatty alcohol oxidase and fatty aldehyde dehydrogenase. Integrated process development enabled the accumulation of dicarboxylic acids to concentrations above 100 g l^{-1}, which makes it the CYP450 process with the highest productivity (up to 1.9 g l^{-1} h^{-1}, Figure 1).

Processes in the pharmaceutical industry include steroid derivatization [67] and the production of the cholesterol-lowering drug pravastatin [22] (Figure 3). Nowadays, pravastatin is produced via the CYP105A3-based hydroxylation of compactin by *Streptomyces* sp. The initially achieved production of 340 mg l^{-1} in 24 h was optimized by intermittent substrate feeding resulting in a product concentration of 1000 mg l^{-1} and a conversion rate of 10 mg l^{-1} h^{-1} [22]. Continuous feeding increased the productivity to 15 mg l^{-1} h^{-1}.

Productivities reached with mammalian CYP450s are considered not to be suitable for fine chemical production so far (Figure 1). However, based on their capability to specifically functionalize complex bioactive compounds, these enzymes may well find applications in the pharmaceutical industry, for example, for the production of pharmaceuticals and for drug derivatization. Specific applications include the production of human drug metabolites [24•,66] and radio-labeled compounds [68].

In conclusion, it can be stated that the productivity of CYP450-based biocatalysts is still often too low for industrial applications in organic syntheses. For successful implementations of whole-cell biocatalysts in industry, all four levels of catalyst and process design (protein, cell, reaction, and process engineering) and especially their interactions have to be considered.

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