Expanding the applicability of Baeyer-Villiger Monooxygenases

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Blending Baeyer–Villiger Mono–oxygenases: Using a Robust BVMO as a Scaffold for Creating Chimeric Enzymes with Novel Catalytic Properties

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The thermostable Baeyer–Villiger monooxygenase (BVMO) phenylacetone monooxygenase (PAMO) is used as a scaffold to introduce novel selectivities from other BVMOs or the metagenome by structure-inspired subdomain exchanges. This yields biocatalysts with new preferences in the oxidation of sulfides and the Baeyer–Villiger oxidation of ketones; all while maintaining most of the original thermostability.
Introduction

Baeyer–Villiger monooxygenases (BVMOs) represent a unique class of oxidative enzymes that are able to perform Baeyer–Villiger oxidations and sulfoxidations, often with high chemo-, regio-, and/or enantioselectivity. In the last few years a number of novel BVMOs have been identified and studied for their use as biocatalysts. However, only one of the BVMOs employed was shown to be robust: phenylacetone monooxygenase (PAMO). PAMO is relatively thermostable, displays activity at a broad pH range, and tolerates various organic solvents and ionic liquids. Unfortunately, PAMO shows a narrow substrate specificity, only accepting small aromatic ketones, sulfides, amines and boron compounds. This is in contrast with the most thoroughly studied BVMO, cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp., for which hundreds of substrates have been reported.

Both CHMO and PAMO have been the subject of enzyme engineering studies to alter their substrate specificity and enantioselectivity. Crystal structures of PAMO and CHMO are available to guide these enzyme engineering efforts. These have revealed that mutations in a loop at the substrate binding site (residues 440–446) have profound effects on substrate specificity and enantioselectivity. So far, engineering attempts on PAMO have focused on the conversion of novel substrates by introducing a limited number of mutations close to the active site.

To expand the substrate range of PAMO, we set out to blend the substrate specificities of sequence-related BVMOs into PAMO. Steroid monooxygenase (STMO) and CHMO display a high degree of sequence similarity (53% and 39% sequence identity) with PAMO, while they accept a much wider substrate range. STMO converts small aromatic compounds like phenylacetone and thioanisole but also the relatively bulky substrate such as progesterone. CHMO has been reported to act on a wide range of aliphatic compounds. As the sequence identities are too low for a classical gene shuffling approach, we decided to blend enzyme properties by performing structure-inspired subdomain exchanges. For this, the C-terminal part of PAMO was replaced by the respective subdomains of other BVMOs, including the 440–446 loop and part of the second sphere residues (Fig. 1).

**Figure 1**: Left: X-ray structure of PAMO with the exchanged C-terminal part in blue and the 440–446 loop residues as sticks around the pink MES molecule bound in the active site. FAD is shown in yellow and NADP⁺ in cyan. Right: a schematic view of the design of BVMO blends. PAMO is shown in green, the complementary BVMO subdomains in blue, yellow and magenta.
By blending BVMOs in this way, a large part of the thermostable PAMO is conserved, while a significant part of the substrate binding site is exchanged with that of another BVMO. It was anticipated that PAMO, due to its inherent stability, could act as a stable scaffold to accommodate a subdomain of another BVMO.

Subdomains were exchanged by introducing a suitable restriction site in the PAMO gene, allowing ligation-independent cloning. The single targeted crossover ensured an in-frame recombination while combining two genes with limited sequence identity. A limited set of chimeric BVMOs was created, allowing us to focus on the expansion of substrate specificity, instead of screening a large combinatorial library for conversions of a limited number of compounds. Specifically, the 106 C-terminal residues of PAMO were exchanged with the homologous regions of STMO or CHMO to create PASTMO and PACHMO, respectively. PASTPAMO was created by introducing 26 residues from STMO around the 440-446 loop in PAMO. Another created chimera, PAMEMO1, contained the C-terminal part of a putative BVMO (53% sequence identity with PAMO) for which the gene has been sequenced as part of a metagenome sequencing effort. The metagenome sequence database has so far hardly been exploited for novel biocatalysts and with this study we wanted to explore the potential of this source for BVMO activities. Several other chimeras were created using less similar metagenomic sequences, or using another BVMO as a scaffold and introducing the C-terminal part of PAMO. Unfortunately, these chimeric enzymes could not be expressed. All BVMO chimeras were fused to a thermostable and His-tagged phosphite dehydrogenase (PTDH) at the N-terminus. This facilitated effective expression and purification of the created BVMO blends while the fused PTDH can be exploited for cofactor regeneration.
Materials and methods

Materials

Restriction enzymes were obtained from New England Biolabs. Oligonucleotides were synthesized by Sigma-Aldrich and sequencing of the created constructs was done at GATC-Biotech. pCRE2-PAMO was mutated into pCRE2-PAMO-RsrII using site-directed mutagenesis, introducing a silent mutation in the codon for G436. The resulting plasmid was cut with PvuII/RsrII or RsrII/HindIII to create the backbone for the pCRE-xxx-PAMO and pCRE-PAMO-xxx chimeras respectively. Inserts were created using PCR, with 15 bp overlaps with the backbone to facilitate In-Fusion cloning (Clontech) with the cut vector, according to the manufacturer’s instructions. Plasmid and protein sequences are available upon request. The resulting constructs were transformed to CaCl₂ competent E. coli TOP10 cells. For the chimeras based on metagenomic sequences, three pieces were synthesized by GeneArt, cut with RsrII/HindIII and ligated into the backbone using Quickligase. Overexpression and purification of the enzymes was done as previously described.²

Substrates 1-5, as well as all other reagents were purchased from Sigma-Aldrich, Acros or Alfa Aesar. Gas chromatography was carried out on a Hewlett-Packard 6890 Series using a flame ionization detector. For all the analyses, the injector temperature was 225 ºC and the FID temperature was 250 ºC. HPLC was performed on a Shimadzu LC-10ADVP equipped with a Shimadzu SPD-M10AVP diode array detector. UV-Vis spectra were obtained using a Hewlet-Packard HP 8543 FT spectrophotometer in a 1.0 cm quartz cuvette.

Racemic methyl phenyl sulfoxide and benzyl phenyl sulfoxide were prepared by treatment of the starting sulfides with H₂O₂ in methanol at room temperature (yields higher than 80%). Lactones were synthesized from the starting ketones by treatment with mCPBA in CH₂Cl₂ at 0 ºC (yields range: 56-81%). The absolute configurations of the final sulfoxides and lactones are based on literature data.⁶ ¹⁵

General procedure for enzymatic oxidation

Unless otherwise state, substrates (5-10 mM) were dissolved in 50 mM Tris/HCl pH 7.5, containing 5% DMSO. Subsequently, 100 µM NADPH, 4 µM phosphite dehydrogenase, 10 mM sodium phosphite and 4 to 10 µM BVMO was added. The mixture was shaken o/n at 250 rpm at room temperature. Reactions were stopped by extraction with ethyl acetate (2 × 0.5 mL) with 0.1% mesitylene as internal standard, and the organic layer was dried over MgSO₄. Conversions and enantiomeric excesses of the oxidized products were determined by GC or HPLC.

GC and HPLC analyses. The following columns were used for the determination of conversions and enantiomeric excesses of the sulfoxides by GC: A: GT-A (Alltech, 30 m x 0.25 mm x 0.25 µm), B: Hewlett Packard HP-1 (Agilent, 30 m x 0.32 mm x 0.25 µm) or C: Chiralsil Dex CB (Varian, 25 m × 0.32 mm × 0.25 µm).
Table 1: Determination of conversion and enantiomeric excess values by GC.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Program(^a)</th>
<th>Column</th>
<th>(t_R) (min) substrates</th>
<th>(t_R) (min) products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40/5/10/200</td>
<td>A</td>
<td>7.3</td>
<td>13.6 (R); 15.6 (S)</td>
</tr>
<tr>
<td>2</td>
<td>100/5/200/5</td>
<td>B</td>
<td>23.1</td>
<td>28.9</td>
</tr>
<tr>
<td>3</td>
<td>35/0/130/15/200</td>
<td>A</td>
<td>9.7</td>
<td>16.9 (normal)</td>
</tr>
<tr>
<td>3</td>
<td>130 isothermal</td>
<td>C</td>
<td>—</td>
<td>17.1 (abnormal)</td>
</tr>
<tr>
<td>4</td>
<td>40/10/3/180/5</td>
<td>A</td>
<td>23.5 (R)</td>
<td>30.6 (S)</td>
</tr>
<tr>
<td>4</td>
<td>40/10/3/180/5</td>
<td>A</td>
<td>23.6 (S)</td>
<td>30.9 (R)</td>
</tr>
<tr>
<td>5</td>
<td>190/1/4/235/5/30/300/3</td>
<td>B</td>
<td>19.4</td>
<td>18.9</td>
</tr>
</tbody>
</table>

\(^a\) Program: initial T (ºC)/ time (min)/ slope (ºC/min)/T (ºC)/ time (min).

For the determination of the enantiomeric excesses of the benzyl phenyl sulfoxide by HPLC, a Chiralcel OD (0.46 cm x 25 cm) column from Daicel was employed.

Table 2: Determination of enantiomeric excess values by HPLC.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Flow rate (mL min(^{-1}))</th>
<th>T (ºC)</th>
<th>Eluent(^a)</th>
<th>(t_R) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.5</td>
<td>25</td>
<td>n-hexane-IPA 9:1</td>
<td>19.2 (R); 22.4 (S)</td>
</tr>
</tbody>
</table>

\(^a\) The experiments were performed with isocratic eluent.

Kinetic measurements. Oxidation rates for the three wild type enzymes as well as for the three chimeric enzymes were determined by observing the depletion of NADPH, monitoring its absorbance at 340 nm in a solution containing 0.05 to 0.5 µM BVMO, 100 µM NADPH and 0 to 1 mM phenylacetone (employed as model substrate) dissolved in 50 mM Tris-HCl pH 7.5. Observed rates were fitted to the Michaelis-Menten equation, using the SigmaPlot software package.

**ThermoFAD method.** Experiments were performed using a BioRad RT-PCR machine and BioRad PCR plates using 5 µM enzyme. The excitation wavelength was set between 470 and 500 nm and a SYBR-green filter (523–543 nm) was used for fluorescence emission. Unfolding curves were measured between 20 ºC and 95 ºC (1 ºC/min) with fluorescence intensity measured every 0.5 ºC after a 10 s delay for temperature stabilization. The fluorescence intensity was plotted against the temperature to obtain a sigmoidal curve. The reported \(T_m\) values were determined as the temperature with the steepest slope (the maximum of the derivative of this curve).

**Results and discussion**

Four chimeric BVMOs were successfully overexpressed as soluble proteins. Purification revealed that all proteins contained the flavin cofactor, indicative of proper protein folding. To probe the stability of all created BVMO-blends, the ThermoFAD method was employed. Melting temperatures of the chimeric BVMOs were lower (49–58 ºC) when compared to PAMO (61 ºC), but significantly higher than those of...
STMO and CHMO (both 39 °C) (Table 3). This shows that the subdomain exchange yields stable BVMO variants, with the most stable chimera being the PASTPAMO mutant where of the exchanged part of 26 residues only 15 residues are different compared to PAMO. Two other metagenome-based chimeras also suggest that homology is important, two fusions with 44% and 35% identity were not expressed, while the metagenomic chimera with 53% identity was expressed in significant quantities. The inverse chimeras, CHPAMO and STPAMO, were also created. However, these chimeric proteins could not be expressed, probably due to the relatively labile N-terminal scaffold. Finally, we created chimeric BVMOs based on PAMO and parts of the second BVMO from *Thermobifida fusca* and two BVMOs from *Thermomonospora curvata*. Unfortunately these could not be expressed.

**Table 3:** Apparent melting points of the different BVMOs as determined by ThermoFAD, and catalytic constants employing phenylacetone as substrate.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>( T_m ) (°C)</th>
<th>( k_{cat} ) (s(^{-1}))</th>
<th>( K_M ) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMO</td>
<td>61</td>
<td>2.3</td>
<td>80</td>
</tr>
<tr>
<td>STMO</td>
<td>39</td>
<td>0.59</td>
<td>110</td>
</tr>
<tr>
<td>CHMO</td>
<td>39</td>
<td>1.5</td>
<td>630</td>
</tr>
<tr>
<td>PASTMO</td>
<td>49</td>
<td>0.58</td>
<td>160</td>
</tr>
<tr>
<td>PASTPAMO</td>
<td>58</td>
<td>1.2</td>
<td>250</td>
</tr>
<tr>
<td>PACHMO</td>
<td>55</td>
<td>n/a(^a)</td>
<td>n/a(^a)</td>
</tr>
<tr>
<td>PAMEMO1</td>
<td>51</td>
<td>0.51</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\) Observed rate similar to the uncoupling rate (0.1 s\(^{-1}\)).

Different compounds were used to compare the activity and specificity of the PAMO-based chimeric BVMOs with the parent enzymes (Scheme 1). The substrates were chosen in such a way that effects on substrate specificity, regio- and/or enantioselectivity could be assessed. All chimeric BVMOs prepared were shown to be active on most of the tested compounds (see Scheme 1). We used phenylacetone as a generally accepted substrate to determine enzyme activity (Table 3), and most purified enzymes were at least as active as the PTDH-STMO fusion enzyme, showing the chimeric enzymes were not only folded correctly as already shown by the ThermoFAD experiment, but also functional. Additionally, affinities for phenylacetone were not significantly decreased in the blended enzymes, only PACHMO did not accept this substrate at all.
Table 4: Enantioselective sulfoxidation of thioanisole 1 catalyzed by wild type and chimeric BVMOs

<table>
<thead>
<tr>
<th>Entry</th>
<th>Biocatalyst</th>
<th>Conv.(^b,c) (%)</th>
<th>ee(^b) (%)</th>
<th>Config.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAMO</td>
<td>93</td>
<td>16</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>STMO</td>
<td>87</td>
<td>27</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>CHMO</td>
<td>70</td>
<td>$\geq 99$</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>PASTMO</td>
<td>79</td>
<td>62</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>PASTPAMO</td>
<td>28</td>
<td>6</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>PACHMO</td>
<td>45</td>
<td>60</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>PAMEMO(^1)</td>
<td>82</td>
<td>60</td>
<td>S</td>
</tr>
</tbody>
</table>

\(^a\) 5.0 mM substrate incubated with 10 μM BVMO at pH 7.5 and 24 °C. Reaction time 24 h. \(^b\) Determined by GC. \(^c\) Only small amounts of the sulfone overoxidation product (≤ 5%) were observed.

Thioanisole 1 was used as a prochiral substrate to probe the enantioselectivity of native and chimeric BVMOs (Table 4). PAMO and CHMO preferentially form the (R)-sulfoxides with poor and excellent enantioselectivity, respectively (entries 1 and 3). The enantioselectivity of the PAMO–CHMO hybrid behaves as a blend of the parent enzymes as it forms the (R)-sulfoxide with moderate enantioselectivity, as shown in entry 6. STMO is the only native BVMO which is able to form the (S)-enantiomer, albeit with moderate enantioselectivity. Interestingly, PASTMO and PAMEMO\(^1\) exhibited improved S enantioselectivities respecting wild type STMO, showing that the exchanged subdomain plays a dominant role in the enzymatic selectivity.

The bulkier compound benzyl phenyl sulfide 2 was also tested to probe the effect of BVMO blending. While this sulfide was not accepted by both STMO and CHMO, incubation with PAMO led to poor conversion and selectivity (7% (R)-sulfoxide with 36% enantiomeric excess (ee) after 48 h). Chimera PASTMO did not convert this substrate. Conversion by PACHMO resulted in 14% of predominantly the (R)-enantiomer with a good enantioselectivity (70% ee). Intriguingly, oxidation of 2 by PAMEMO\(^1\) resulted in the most effective conversion (39% sulfoxide formed), generating the opposite S enantiomer with a moderate optical purity (56% ee).

In view of the promising results in the sulfoxidation processes, we analyzed different racemic ketones. Bicyclic ketone (±)-bicyclo[3.2.0]hept-2-en-6-one 3 has often been used to investigate the stereoselectivity of BVMOs because four distinct chiral products can be formed from this compound. The so-called normal lactones are formed when the oxygen is inserted next to the tertiary carbon, as opposed to the secondary carbon for the abnormal lactones. This gives two chemically distinct products for both the (1\(S\),5\(R\))- and (1\(R\),5\(S\))-enantiomers. This conversion provides information about differences in regio- and enantioselectivities of the different biocatalysts. The three chimeric BVMOs were found to be just as effective as the native BVMOs in converting 3 (Table 5, entries 1–3). PASTMO shows an intermediate enantioselectivity when compared with the parent enzymes, while retaining the regioselectivity of STMO. Interestingly, PASTPAMO showed even higher enantioselectivity, not found in both of the parents or the PASTMO chimera and also the highest normal to abnormal ratio. PACHMO displays the opposite behavior: it has a small preference for the abnormal lactone, something that is not observed with any of the parent BVMOs. Both major enantiomers are obtained with complete enantioselectivity. The introduction of the metagenomic gene fragment renders a PAMO variant, PAMEMO\(^1\), which shows novel properties by forming predominantly the normal lactone with excellent selectivity (entry 7). These
data show that by the design of chimeric BVMOs, thermostable PAMO variants can be made that display novel catalytic properties when compared with native BVMOs.

### Table 5: Baeyer-Villiger oxidation of 3 employing wild type and chimeric enzymes

<table>
<thead>
<tr>
<th>Entry</th>
<th>Biocatalyst</th>
<th>Conv.(^{b}) (%)</th>
<th>Ratio norm./abn.</th>
<th>ee ((1S,5R)) normal(^b) (%)</th>
<th>ee ((1R,5S)) abnormal(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAMO</td>
<td>60</td>
<td>50:50</td>
<td>29</td>
<td>(\geq 99)</td>
</tr>
<tr>
<td>2</td>
<td>STMO</td>
<td>58</td>
<td>65:35</td>
<td>79</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>CHMO</td>
<td>69</td>
<td>50:50</td>
<td>97</td>
<td>(\geq 99)</td>
</tr>
<tr>
<td>4</td>
<td>PASTMO</td>
<td>75</td>
<td>66:34</td>
<td>76</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>PASTPAMO</td>
<td>51</td>
<td>88:12</td>
<td>(\geq 99)</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>PACHMO</td>
<td>38</td>
<td>39:61</td>
<td>(\geq 99)</td>
<td>(\geq 99)</td>
</tr>
<tr>
<td>7</td>
<td>PAMEMO1</td>
<td>58</td>
<td>76:24</td>
<td>(\geq 99)</td>
<td>49</td>
</tr>
</tbody>
</table>

\(^a\) 5.0 mM substrate incubated with 10 \(\mu\)M BVMO at pH 7.5 and 24 °C. Reaction time 24 h. \(^b\) Determined by GC.

For a further exploration of the created BVMO blends, also the racemic ketone \((\pm)-2\)-phenylcyclohexanone 4 was tested (Table 6). The corresponding enantiomerically pure lactones formed represent attractive building blocks in organic chemistry. It has been shown before that CHMO is capable to form the \((R)\)-lactone with excellent optical purity (entry 3). In contrast, we found that STMO does not act on this ketone while PAMO forms the \((S)\)-enantiomer with poor selectivity (entry 1). The use of chimeric enzymes PASTMO and PASTPAMO led to the formation of the \((S)\) lactones with good enantioselectivities, as observed in entries 4 and 5, while PACHMO and PAMEMO1 created the \((R)\)-lactones with excellent enantioselectivity (entries 6 and 7). Conversions achieved with the chimeric enzymes are equal to or higher than that obtained with wild type PAMO.

### Table 6: Enzymatic oxidation of racemic ketone 4 by chimeric and wild type enzymes

<table>
<thead>
<tr>
<th>Entry</th>
<th>Biocatalyst</th>
<th>Conv.(^{b}) (%)</th>
<th>ee lactone(^b) (%)</th>
<th>Config.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAMO</td>
<td>11</td>
<td>22</td>
<td>(S)</td>
</tr>
<tr>
<td>2</td>
<td>STMO</td>
<td>(\leq 3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>CHMO</td>
<td>42</td>
<td>(\geq 99)</td>
<td>(R)</td>
</tr>
<tr>
<td>4</td>
<td>PASTMO</td>
<td>30</td>
<td>70</td>
<td>(S)</td>
</tr>
<tr>
<td>5</td>
<td>PASTPAMO</td>
<td>18</td>
<td>87</td>
<td>(S)</td>
</tr>
<tr>
<td>6</td>
<td>PACHMO</td>
<td>11</td>
<td>(\geq 99)</td>
<td>(R)</td>
</tr>
<tr>
<td>7</td>
<td>PAMEMO1</td>
<td>24</td>
<td>(\geq 99)</td>
<td>(R)</td>
</tr>
</tbody>
</table>

\(^a\) 10.0 mM substrate incubated with 4 \(\mu\)M BVMO at pH 7.5 and 24 °C. Reaction time 24 h. \(^b\) Determined by GC. \(^c\) Reaction performed with a substrate concentration of 5.0 mM and 10 \(\mu\)M BVMO.

Finally, progesterone 5 was tested as substrate of different BVMOs. This compound was oxidized by STMO with a high conversion (90% after 24 hours), while neither PAMO nor CHMO was capable of oxidizing this steroid. Incubation of 5 with PACHMO and PAMEMO1 led to the recovery of the starting material, while PASTMO afforded the final lactone with a moderate conversion (19% after 24 hours). PASTPAMO did even better with 36% conversion, showing again that the behavior of the enzymes is not a blend of both parent’s behavior, but new properties can emerge. The results with 4 and 5 indicate
that hybrid enzymes can show improved conversions or enantioselectivities compared to PAMO while retaining its thermostability.

The data above show that exchange of the C-terminal subdomain of PAMO is an effective approach to generate BVMOs that are relatively robust while exhibiting novel catalytic properties. This method of generating novel BVMOs by blending a robust BVMO with gene fragments of other BVMOs is different from the commonly employed methodologies of enzyme engineering or discovery. PAMO appears to be a very suitable scaffold for such a subdomain exchange approach as it allows the exchange of sequences with only 39% sequence identity while retaining thermostability, even after replacing more than 10% of its residues. All purified chimeras were more thermostable than the parent BVMO from which the C-terminal subdomain originated. The method also allows blending of gene fragments from genes that can be selected by an in silico search of (meta)genome sequence databases, exploring untapped sequence space. This permits a very rapid and relatively cheap access to new BVMO activities as such short gene fragments can be easily prepared by modern DNA synthesis protocols.

The substrate specificity of the BVMO blends was found to be at least partially based on the introduced C-terminal part. All chimeras show novel catalytic behaviour, especially concerning enantioselectivity. Not all activities from the parent BVMOs could be introduced in the chimeras. For example, conversion of cyclohexanone was not observed for PACHMO. On the other hand some of the chimeras show unanticipated behaviour by converting substrates with a higher yield or enantiomeric excess than either of both parent enzymes. Thus, PASTMO shows a better conversion and enantioselectivity with the relatively bulky ketones 3 and 4 when compared to the parent enzymes. The results obtained show that the catalytic properties of chimeras are not just the average of those of the parents, but that new properties can be introduced, not present in either of the parents. This represents a starting point to expand the possibilities for BVMO-catalyzed biocatalysis, by creating a portfolio of BVMO blends with suitable stability and expression, and with varying substrate specificities and regio- and/or enantioselectivities.

Acknowledgements

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References


