Natural variation in the 'control loop' of BVMO$_{AFL210}$ and its influence on regioselectivity and sulfoxidation

Carmien Tolmie [1], Rodolpho do Aido-Machado [1], Felix Martin Ferroni [1,2], Martha Sophia Smit [1] and Diederik Johannes Opperman [1,*]

[1] University of the Free State, Department of Biotechnology, Bloemfontein, 9300, South Africa; tolmiec@ufs.ac.za (C.T.); machador@ufs.ac.za (R.d.A-M.); fferroni@fbcb.unl.edu.ar (F.M.F); smitms@ufs.ac.za (M.S.S.)

[2] Universidad Nacional del Litoral/CONICET, Departamento de Física, Santa Fe, S3000ZAA, Argentina

* Correspondence: opperdj@ufs.ac.za (D.J.O); Tel.: +27-51-401-2714

Supporting Information

Table S1. Data collection and refinement statistics for BVMO$_{AFL210}$.

Data Collection	
X-ray source	Beamline i04-1, Diamond Light Source
Wavelength (Å)	0.91587
Resolution (Å)	129.64 - 2.09 (2.36 – 2.09)[1]
Space group	$P2_1$
Unit cell parameters	
a/b/c (Å)	87.89 117.31 133.34
$\alpha/\beta/\gamma$ (°)	90.00 103.52 90.00
Unique reflections	72127 (3606)
Completeness (%)[2]	87.5 (73.7)
R_{merge}	0.120 (0.644)
Average $I/\sigma(I)$	6.6 (1.8)
Multiplicity	3.3 (3.1)
CC (1/2)	0.994 (0.620)
Refinement	
R_{work} / R_{free}	0.206/0.261
Molecules in ASU	4
Average B factor (Å):	
Protein	37.48
Ligand	46.96
Solvent	22.22
RMSD:	
Bond length (Å)	0.0127
Bond angle (°)	1.65
Ramachandran distribution (%) (favoured/allowed/outlier)	94.15/ 5.06/ 0.80
Molprobity score	2.01

[1] Values in parentheses are for the highest resolution shell. [2] Ellipsoidal

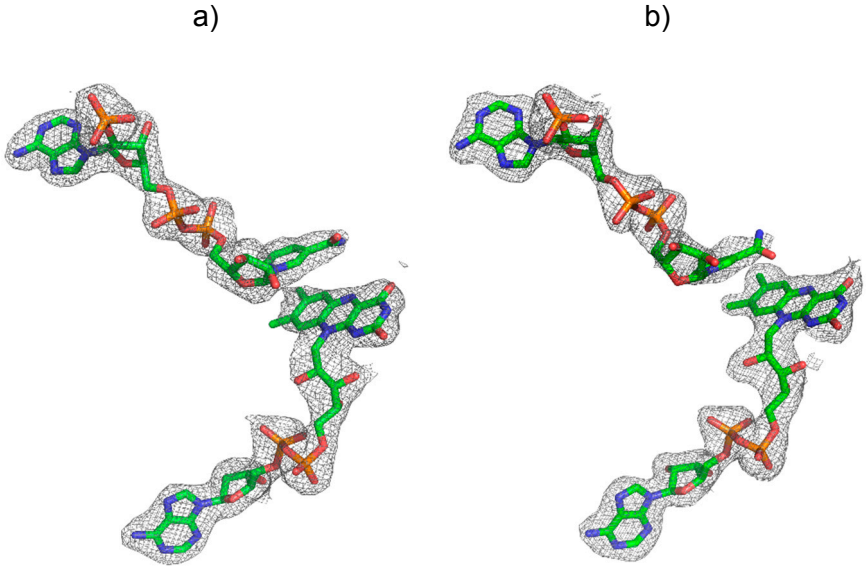


Figure S1. Electron density (2Fo-Fc) of the FAD and NADP moieties bound to BVMO$_{AFL210}$ in the 'open' (**a**, chain A) and 'closed' (**b**, chain B) conformation, contoured to 1 σ.

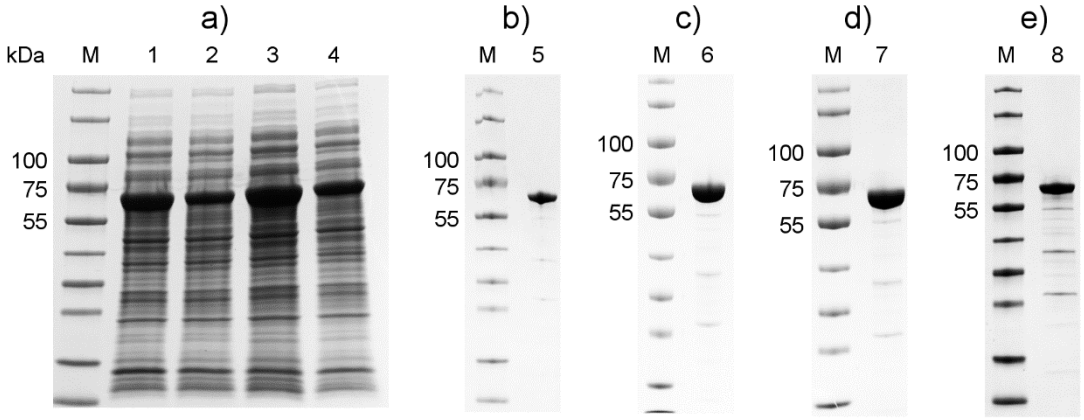
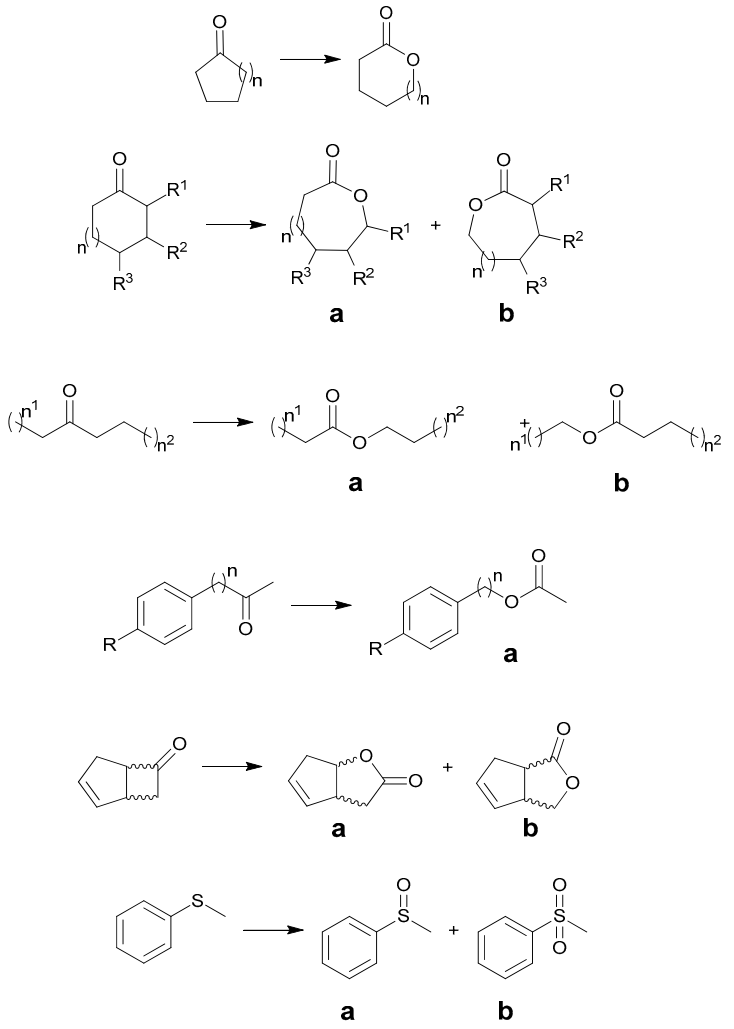


Figure S2. SDS-PAGE analysis of soluble expression of BVMO$_{AFL210}$ and mutants in *E. coli* cell free extracts (**a**). Lane M: molecular weight marker, 1: WT, 2: T513G, 3: T513Y, 4: T513W. Purified BVMO$_{AFL210}$ WT (**b**, lane 5), T513G (**c**, lane 6), T513Y (**d**, lane 7) and T513W (**e**, lane 8).



n = 1 **cyclopentanone**
n = 2 **cyclohexanone**
n = 4 **cyclooctanone**
n = 8 **cyclododecanone**

n = 0; R^1 R^2 R^3 = Me H H **2-methylcyclopentanone**
n = 1; R^1 R^2 R^3 = Me H H **2-methylcyclohexanone**
n = 1; R^1 R^2 R^3 = H Me H **3-methylcyclohexanone**
n = 1; R^1 R^2 R^3 = H H Me **4-methylcyclohexanone**
n = 1; R^1 R^2 R^3 = H H Et **4-ethylcyclohexanone**
n = 1; R^1 R^2 R^3 = H H *n*-Pr 4-***n*-propylcyclohexanone**

n^1 = 0; n^2 = 3 **2-heptanone**
n^1 = 0; n^2 = 4 **2-octanone**
n^1 = 1; n^2 = 3 **3-octanone**
n^1 = 0; n^2 = 6 **2-decanone**
n^1 = 0; n^2 = 7 **2-undecanone**
n^1 = 0; n^2 = 8 **2-dodecanone**

n = 0; R = H **acetophenone**
n = 1; R = H **phenylacetone**
n = 2; R = H **4-phenyl-2-butanone**
n = 2; R = OH **4-(4-hydroxyphenyl)-2-butanone**
n = 2; R = OMe **4-(4-methoxyphenyl)-2-butanone**

***rac*-cis-bicyclo[3.2.0]hept-2-en-6-one**

thioanisole

Scheme S1. Substrates screened.

Table S2. Whole-cell biotransformations.

Substrate	Conversion (GC %)			
	WT	**T513G**	**T513Y**	**T513W**
Cyclopentanone	45.3	80.9	26.8	29.7
Cyclohexanone	23.4	64.4	11.5	18.0
Cyclooctanone	-	-	-	-
Cyclododecanone	-	-	-	-
2-Methylcyclopentanone	34.5**a** / 9.9**b**	80.6**a** / 7.9**b**	22.3**a** / 14.5**b**	25.1**a** / 15.7**b**
2-Methylcyclohexanone	11.1**a** / 4.2**b**	44.9**a** / 9.0**b**	20.8**a** / <1**b**	25.7**a** / <1**b**
3-Methylcyclohexanone	22.8**a**	44.2**a**	16.7**a**	15.8**a**
4-Methylcyclohexanone	16.0	49.1	11.6	15.3
4-Ethylcyclohexanone	32.1	52.1	5.1	7.0
4-*n*-propylcyclohexanone	94.3	91.0	46.3	28.3
2-heptanone	47.7**a**	45.5**a**	38.5**a**	35.5**a**
2-octanone	97.8**a**	96.4**a**	93.4**a**	81.7**a**
3-octanone	>99**a**	>99**a**	96.9**a**	87.2**a**
2-decanone	87.4**a**	97.8**a**	67.7.4**a**	74.4**a**
2-undecanone	81.3**a**	94.2**a**	60.4**a**	62.2**a**
2-dodecanone	59.1**a**	93.1**a**	46.9**a**	53.7**a**
Acetophenone	13.6	14.8	<1	<1
Phenylacetone	30.7	42.4	27.2	16.2
4-phenyl-2-butanone	45.0	39.1	30.2	8.4
4-(4-hydroxyphenyl)-2-butanone	8.8	1.4	1.1	1.6
4-(4-methoxyphenyl)-2-butanone	42.5	52.6	53.7	24.3
rac-cis-bicyclo[3.2.0]hept-2-en-6-one	24.7**a** / 5.4**b**	53.0**a** / 17.8**b**	23.8**a** / 12.6**b**	27.0**a** / 18.5**b**
Thioanisole	21.8**a** / 29.1**b**	37.4**a** / 5.3**b**	31.6**a** / 1.5**b**	31.9**a** / 2.3**b**

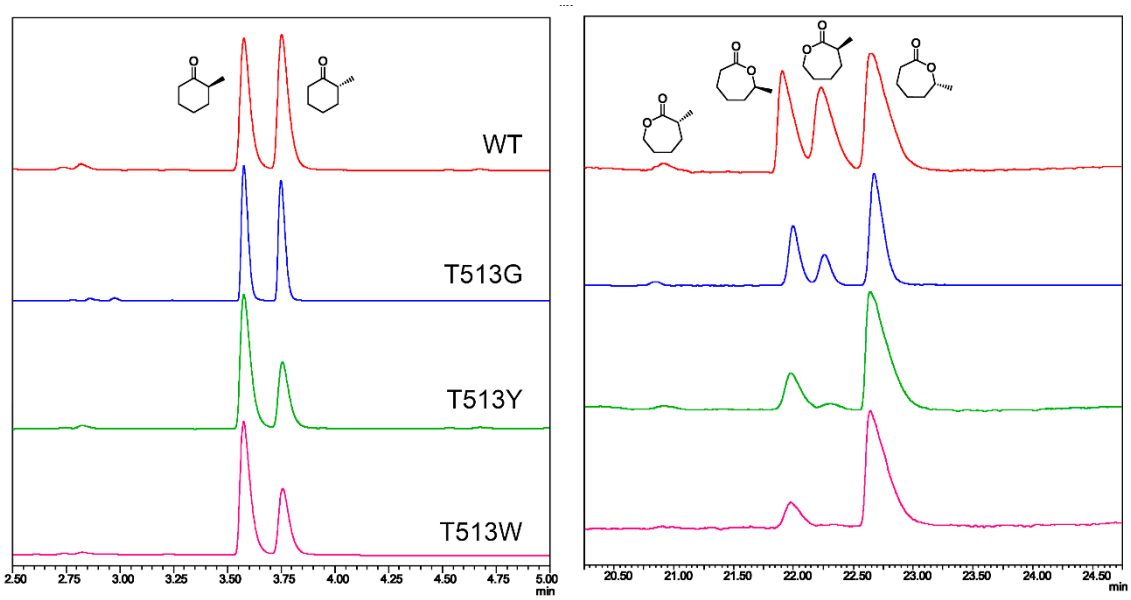


Figure S3. Chiral analysis of 2-methylcyclohexanone conversion by BVMO$_{AFL210}$ WT and mutants. *S*-2-methylcyclohexanone (3.59 min); *R*-2-methylcyclohexanone (3.76 min); *R*-"abnormal/distal" lactone (20.9 min), *S*-"normal/proximal" lactone (21.9 min); *S*-"abnormal/distal" lactone (22.2 min); *R*-"normal/proximal" lactone (22.6 min).

Table S3. GC Methods.

Substrates	Program[1]	Retention time (min)	
		Substrate	Products
Cyclopentanone	80/2/15/185	2.45	5.1
Cyclohexanone	60/1/10/220/5	4.28	8.64
Cyclooctanone	60/1/10/220/5	8.03	8.32
Cyclododecanone	60/1/10/220/5	9.79	9.87
2-Methylcyclopentanone	60/1/10/220/5	3.53	7.37 **b** 7.44 **a**
2-Methylcyclohexanone	60/1/10/220/5	5.03	9.65 **a** 9.81 **b**
3-Methylcyclohexanone	60/1/10/220/5	5.1	10.43 **a**
4-Methylcyclohexanone	60/1/10/220/5	5.19	10.56
4-Ethylcyclohexanone	60/1/10/220/5	7.04	12.35
4-*n*-propylcyclohexanone	60/1/10/220/5	9.39	13.34
2-heptanone	80/2/15/250	3.22	3.48 **a**
2-octanone	80/2/15/250	3.01	3.42 **a**
3-octanone	80/2/15/250	4.18	4.43 **a**
2-decanone	80/2/15/250	6.51	6.68 **a**
2-undecanone	80/2/15/250	7.54	7.68 **a**
2-dodecanone	80/2/15/250	8.49	8.60
Acetophenone	80/2/8/140/0/15/220/2	6.15	5.91
Phenylacetone	80/2/8/140/0/15/220/2	7.21	7.79
4-phenyl-2-butanone	80/2/8/140/0/15/220/2	9.28	9.45
4-(4-hydroxyphenyl)-2-butanone	60/5/5/160/0/25/250/2	24.63	24.73
4-(4-methoxyphenyl)-2-butanone	80/2/8/140/0/15/220/2	12.7	12.75
rac-cis-bicyclo[3.2.0]hept-2-en-6-one	60/1/10/110/4/25/200/2	3.85	6.6 **a** 6.56 **b**

[1] °C/min/min^{-1}/°C/min.