SUPPLEMENTAL MATERIAL to AEM 07694_11:

Camphor Pathway 2-Oxo-Δ^3-4,5,5-trimethylcyclopentenylacetyl-CoA Monooxygenase of *Pseudomonas putida* ATCC 17453: Cloning, Baeyer-Villiger Biooxidations, and Structures.

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SM1. Protein purification. *E. coli* BL21[OTEMO] cells were cultivated at 30 °C and enzyme production was induced by addition of 1 mM IPTG at an OD_{600} of about 0.4. After 4 h of induction, cells were harvested by centrifugation, washed with 20 mM sodium phosphate buffer (pH 7.5) and broken using a French press cell operating at 20,000 psi. The crude extract was obtained by centrifugation (20,000 g) followed by sterile filtration using a 0.22 µm membrane. All purification procedures were performed at 4 °C on an ÄKTA Explorer™ 100 Air chromatography system (GE Healthcare).

Crude cell extract was loaded onto a DEAE-Sepharose FF column (XK 26/13) that had been equilibrated with 20 mM sodium phosphate buffer, pH 7.5. OTEMO was eluted using a linear gradient of 0 to 300 mM NaCl in the same buffer. Active fractions were collected and solid ammonium sulfate was slowly added to a final concentration of 40% (w/v). Precipitated protein was removed by filtration (0.22 µm) and the clear filtrate subsequently loaded onto a Butyl-S Sepharose column (XK26/20) that had been equilibrated with 40% (w/v) ammonium sulfate in 20 mM sodium phosphate buffer (pH 7.0). Bound enzyme was eluted using a linear gradient, 40 to 0 % (w/v) ammonium sulfate, in phosphate buffer. Active fractions were collected, concentrated by ultrafiltration using a stirring cell (250 ml cell , Amicon, YM 10 membrane) and applied to a HiLoad Superdex 75 pg (16/60) column equilibrated with 150 mM NaCl in Na-HEPES (pH 7.5) buffer. Eluted active fractions were concentrated by ultrafiltration and used for crystallization experiments and enzymatic analysis. OTEMO mutants as

specified below were purified using the same protocol. Inactive mutants were identified by their intense yellow color monitored at 435 nm during purification.

SDS-PAGE) containing 0.1% (w/v) SDS was carried out using the discontinuous buffer system and silver-stained as previously described (Iwaki et al. 2006). Molecular weight marker proteins were from Bio-Rad, USA. The molecular mass of the native enzyme was determined by gel filtration on a HiLoad Superdex 200 prep grade column with reference to standard proteins (GE Healthcare).

SM2. Chiral GC analysis. Chiral GC analysis of lactones and recovered ketones was carried out on a Hewlett Packard 6890 Series gas chromatograph, Hewlett Packard 6890 Series autosampler equipped with a flame ionization detector. 1 µL was injected under pulse split (1:5) condition with 8 mL/min helium flow. The following GC-conditions were used: chiral GC program A: Varian Chirasil-Dex CB column (25 m x 0.25 mm x 0.25 µm); 75 ºC for 5 min; 2.5 ºC/min to 175 ºC, 10 min at 175 ºC, 10 ºC/min to 200 ºC, 2.5 min at 200 ºC; chiral GC program B: Varian Chirasil-Dex CB column (25 m x 0.25 mm x 0.25 µm); 75 ºC for 5 min; 1.0 ºC/min to 175 ºC, 10 min at 175 ºC, 10 ºC/min to 200 ºC, 2.5 min at 200 ºC; chiral GC program C: Supelco Inc. ß-Dex 225 column (30 m x 0.25 mm x 0.25 µm). 75 ºC for 5 min; 2.5 ºC/min to 175 ºC, 10 ºC/min to 225 ºC, 10.0 min at 200 ºC; chiral GC program D: Varian Chirasil-Dex CB column (25 m x 0.25 mm x 0.25 µm); 75 ºC for 5 min; 2.5 ºC/min to 100 ºC, 1.0 ºC/min to 150 ºC, 5 ºC/min to 200 ºC, 5.0 min at 200 ºC; chiral GC program E: Varian Chirasil-Dex CB column (25 m x 0.25 mm x 0.25 µm); 75 ºC for 5 min; 2.5 ºC/min to 100 ºC, 0.3 ºC/min to 190 ºC, 5 ºC/min to 200 ºC, 5.0 min at 200 ºC.

SM3. Spectroscopic data:

5-Methyloxepan-2-one (12a)

5-Methyloxepan-2-one (**12a**) was obtained as colorless oil in 94% yield (120 mg) following the general procedure for the preparative scale biotransformation using 4-methylcyclohexanone (**12**) (114 mg, 0.1 mmol) as starting material. *ee* = 62% *R* (chiral GC program B; *R*-isomer = 50.38 min, *S*-isomer = 49.84 min, SM = 19.42 min); α_D^{20} = +

25.42 (*c* 1.20, $CHCl_3$); α_D (> 98% *ee S*) = - 44.23 (*c* 1.20, $CHCl_3$); GC-MS (EI) = 9.34 min, 128 *m/z*.

5-Ethyloxepan-2-one (13a)

5-Ethyloxepan-2-one (**13a**) was obtained as colorless oil in 95% yield (135 mg) following the general procedure for the preparative scale biotransformation using 4-ethylcyclohexanone (**13**) (126 mg, 0.1 mmol) as starting material. *ee* = 99% *R* (chiral GC program B; *R*-isomer = 61.59 min, *S*-isomer = 60.65 min, SM = 29.71 min); α_D^{20} = + 37.18 (*c* 1.40, $CHCl_3$); α_D (> 98% *ee S*) = - 38 (*c* 5.55, $CHCl_3$); GC-MS (EI) = 12.14 min, 142 *m/z*.

5-Propyloxepan-2-one (14a)

5-Propyloxepan-2-one (**14a**) was obtained as colorless oil in 92% yield (144 mg) following the general procedure for the preparative scale biotransformation using 4-propylcyclohexanone (**14**) (140 mg, 0.1 mmol) as starting material. *ee* = 99% *R* (chiral GC program B; *R*-isomer = 70.34 min, *S*-isomer = 69.53 min, SM = 38.55 min); α_D^{20} = + 38.78 (*c* 1.75, $CHCl_3$); α_D (> 98% *ee S*) = - 38 (*c* 6.41, $CHCl_3$); GC-MS (EI) = 15.20 min, 156 *m/z*.

5-Pentyloxepan-2-one (15a)

5-Propyloxepan-2-one (**15a**) was detected by chiral GC in the organic layer after extraction. The conversion (9%) was calculated based on relative areas of the GC chromatogram and the determination of the absolute configuration was based on the elution pattern of compounds **12**, **13**, and **14**. *ee* = 96% *R* (chiral GC program B; *R*-isomer = 87.02 min, *S*-isomer = 86.47 min, SM = 57.94 min). GC-MS (EI) = 22.45 min, 184 *m/z*.

5-*tert*-butyloxepan-2-one (16a)

5-*tert*-butyloxepan-2-one (**16a**) was obtained as colorless solid in 14% yield (12 mg) following the general procedure for the preparative scale biotransformation using 4-*tert*-butylcyclohexanone (**16**) (77 mg, 0.05 mmol) as starting material. *ee* = 99% *R* (chiral GC program B; *R*-isomer = 72.58 min, *S*-isomer = 72.92 min, SM = 45.58 min); α_D^{20} = + 33.82 (*c* 1.75, $CHCl_3$); α_D (> 98% ee *S*) = - 34.9 (*c* 0.78, $CHCl_3$); GC-MS (EI) = 17.20 min, 155 (- CH_3) *m/z*.

7-Oxo-oxepane-4-carboxy ethyl ester (17a)

7-Oxo-oxepane-4-carboxy ethyl ester (**17a**) was obtained as colorless oil in 78% yield (73 mg) following the general procedure for the preparative scale biotransformation using 4-oxo-cyclohexyl carboxy ethyl ester (**17**) (85 mg, 0.05 mmol) as starting material. *ee* = 96% *R* (chiral GC program B; *R*-isomer = 83.46 min, *S*-isomer = 82.81 min, SM = 55.64 min); α_D^{20} = + 60.10 (*c* 0.80, $CHCl_3$); α_D (64% ee *R*) = + 37.5 (*c* 2.0); GC-MS (EI) = 18.30 min, 186 *m/z*.

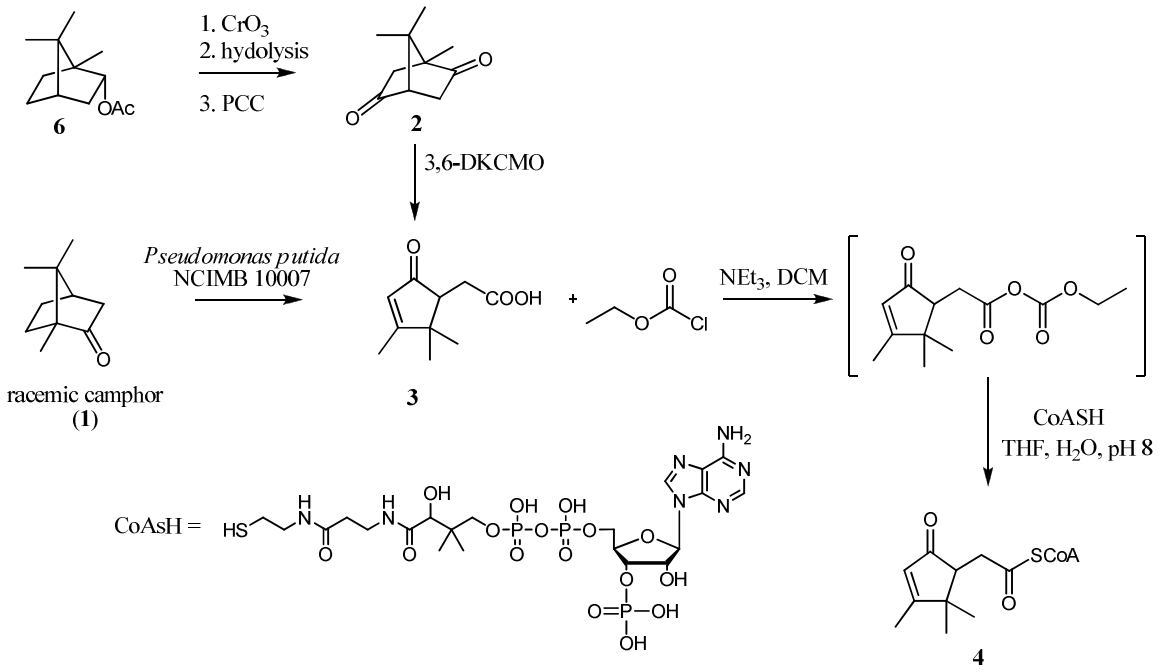
1,4,8-Trioxaspiro[4.6]undecan-9-one (18a)

1,4,8-Trioxaspiro[4.6]undecan-9-one (**18a**) was obtained as colorless oil in 52% yield (44 mg) following the general procedure for the preparative scale biotransformation using 1,4-dioxaspiro[4.5]decan-8-one (**18**) (78 mg, 0.05 mmol) as starting material. GC-MS (EI) = 17.87 min, 172 *m/z*.

3,7-Dimethyloxepan-2-one (19a)

3,7-Dimethyloxepan-2-one (**19a**) was obtained as colourless oil in 41% yield (58 mg; 53% conversion) following the general procedure for the preparative scale biotransformation using *cis*-2,6-dimethylcyclohexanone (**19**) (126 mg, 0.1 mmol) as starting material. *ee* = 97% *S* (chiral GC program A; *R*-isomer = 27.02 min, *S*-isomer = 26.23 min, SM = 15.78 min); α_D^{20} = - 10.56 (*c* 0.80, $CHCl_3$); lit. α_D (>98% ee *S*) = - 10.90 (*c* 1.56); GC-MS (EI) = 11.47 min, 142 *m/z*.

Fig. S1. Synthesis of 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid **(3)** and Synthesis of 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl CoA-ester **(4).**



Briefly, two chemoenzymatic syntheses of 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid (**3**) were explored. The first route started from the commercially available bornyl acetate (**6**) that was converted to 3,6-diketocamphane (**2**) in three steps according to the literature (Allen et al. 1979; Corey and Suggs, 1975). Biocatalytic transformation of 3,6-diketocamphane was performed with whole cells overexpressing recombinant 3,6-diketocamphane monooxygenase and the desired compound was isolated in 64 % yield. Alternatively, 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid (**3**) was isolated after biotransformation of racemic camphor with strain PpCam following recrystallization of the obtained residue. 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl CoA-ester (**4**) was prepared according to a modified procedure of the group of Hoppel (Sobhi et al. 2011). First the mixed anhydride was prepared from 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid (**3**) with ethylchoroformate in the presence of triethylamine. Subsequent treatment of the activated acid with coenzyme A in an aqueous THF solution led to formation of the desired compound **4** in good purity after purification by gel permeation chromatography, although resulting in low yield.

3,6-Diketocamphane was prepared in three steps from bornyl acetate (**6**) following literature procedures. First, bornyl acetate (10.0 g, 51 mmol) was treated with chromium trioxide (25.0 g, 250 mmol) in glacial acetic acid (85 mL) according to the procedure of Allen et al. (1979). Purification of the crude residue by flash column chromatography (100 g SiO_2, ethyl acetate - hexanes gradient) gave oxobornyl acetate (1.4 g, 13% yield) as colorless solid. mp 76 - 78 ºC (ethyl acetate/hexanes), lit. 78 ºC (hexanes) Subsequently, the acetate derivative (1.0 g, 4.76 mmol) was treated with sodium carbonate in a mixture of water and methanol according to the literature. The desired hydroxy ketone was isolated as white crystals after recrystallization from ethyl acetate hexane (0.55 g, 69% yield). mp > 200 ºC (ethyl acetate/hexanes), lit. 239 - 241 ºC; α_D^{20} = - 75.43 (*c* 1.25, $CHCl_3$), lit. α_D = - 87.0; Pyridinium chlorochromate (PCC) mediated oxidation of this intermediate (0.37 g, 2 mmol) according to a procedure of Corey and Suggs, gave 3,6-diketocamphane (**2**) (0.21 g, 57% yield) as white crystals after flash column chromatography (15 g SiO_2, ethyl acetate - hexanes gradient). m.p. >200 ºC (ethyl acetate/hexanes), lit. 190 - 192 ºC; α_D^{20} = - 115.61 (*c* 1.05, $CHCl_3$); lit. α_D = - 113.0; 3,6-Diketocamphane (100 mg) was subjected to an enzymatic Baeyer-Villiger oxidation using recombinant *E. coli* cells overexpressing 3,6-diketocamphane monooxygenase (this laboratory, manuscript in preparation). The obtained cell suspension was centrifuged and the pH of the supernatant was adjusted to pH 2 with 1N hydrochloride acid. The acidic aqueous layer was extracted with ethyl acetate and the layers were combined, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated to yield the title compound as white solid (71 mg, 64% yield). Alternatively, 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid (**3**) could be isolated from *P. putida* NCIMB 10007 whole cell mediated fermentations of camphor. Extraction of the acidified supernatant obtained after centrifugation of the cell broth gave a yellow solid. Recrystallizaton of the obtained residue in ethyl acetate yielded the title compound. mp 108 - 110 ºC (ethyl acetate), 102 - 104 ºC (diethylether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00-10.00 (bs, 1H), 5.84 (s, 1H), 2.79-2.91 (m, 1H), 2.64-2.77 (m, 1H), 2.31-2.48 (m, 1H), 2.08 (s, 3H), 1.22 (s, 3H), 1.04 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.1, 185.1, 176.9, 127.0, 53.3, 45.7, 30.8, 25.1, 22.7, 14.0 ppm.

2-Oxo-Δ3-4,5,5-trimethylcyclopentynyl CoA-ester **4** was prepared according to a modified procedure of the group of Hoppel (Sobhi et al. 2010). Triethylamine (81 mg, 0.80 mmol) was added to a solution of 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl acidic acid (**3**) (73 mg, 0.40 mmol) in dry dichloromethane (10 mL) under nitrogen atmosphere. The reaction mixture was cooled to 0 ºC and ethyl choroformate (87 mg, 0.80 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred at the same temperature for 2 hours. The solvent was evaporated and the solid residue was extracted with dry THF (7 mL). In a separate flask coenzyme ASH (30 mg, 0.04 mmol) was dissolved in a mixture of THF and water (4:1, 7 mL) and the pH was adjusted to 7.5 with a 0.1M aqueous solution of potassium bicarbonate. The THF extract, containing the mixed anhydride, was added to the CoA-SH solution and the pH was adjusted to 8.0 using a 0.1M aqueous solution of potassium bicarbonate. The reaction mixture was allowed to stir at room temperature for 1 hour, before the pH of the reaction mixture was adjusted to 4.0. THF was evaporated under reduced pressure and the remaining aqueous solution was diluted with water (3 mL). The pH of the aqueous solution was adjusted to pH 2.0 with 0.2 M HCl. The acidified aqueous layer was extracted three times with diethyl ether (10 mL). The aqueous solution was neutralized (pH 6.8), frozen, and lyophilized. The residue (100 mg) was dissolved in water and separated on a Sephadex G15 column (15x20) with water as eluent. The relevant fractions (detected at 280 nm) were pooled and lypholyzed, to give 4 mg of the desired compound as white solide. LC/HRMS analysis confirmed the structure of 2-oxo-Δ3-4,5,5-trimethylcyclopentynyl CoA-ester **4** in 75% purity (based on relative areas detected at 265nm). No free CoA-ester was detected in the sample. LC/HRMS analysis was performed on a mass spectrometer (MS, Bruker MicroTOFQ mass analyzer) attached to an HPLC system (Hewlett Packard 1200 Series) equipped with a DAD detector. Aliquots (10 μL) were injected into a 3 micron-pore size Gemini-NX-C18 column (2.6 mm ID × 150 mm; Phenomenex) at 30 °C. The solvent system was composed of a CH_3OH/CH_3COONH_4 100 mM gradient (MeOH % v/v: 10 to 25 for 15 min; 25 to 80 for 20 min; 80 for 5 min; 80 to 10 for 2 min; 10 for 18 min) at a flow rate of 0.2 mL min^{-1}. For mass analysis, both negative and positive electrospray ionization (ES–, ES+) were used to produce. Mass range was scanned from 40 to 1000 *m/z*. The retention time of

compound **4** was 37.45 min. HRMS (ES+) calculated for $C_{31}H_{49}N_7O_{18}P_3S$ 932.2068, found 932.2064.

Fig. S2. Structure-based sequence alignment of OTEMO, CHMO and PAMO based on their structure superposition. The PAMO, and the open and closed forms of CHMO, respectively are identified by their PDB accession numbers at http://www.rcsb.org. The structures are superposed using Deep View (Swiss PDB viewer). High-lighted in yellow are the structurally conserved key active site residues.

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OTEMO_A          6        ASPALDA VVIGAGVTGI YQAFLINQA- GMKVLGIEAG EDVGGTWYWN
OTEMO_B          6        ASPALDA VVIGAGVTGI YQAFLINQA- GMKVLGIEAG EDVGGTWYWN
1W4X_aligned     10    RRQPPEEVDV LVVGAGFSGL YALYRLREL- GRSVHVIETA GDVGGVWYWN
3GWD_aligned     5        TTHTVDA VVIGAGFGGI YAVHKLHHEL GLTTVGFDKA DGPGGTWYWN
3GWF_aligned     6         THTVDA VVIGAGFGGI YAVHKLHHEL GLTTVGFDKA DGPGGTWYWN
                            .  .*  .*.***  *. *    . .   *     .. .    ** ****

OTEMO_A          52    RYPGCRLDTE SYAYGYFALK GI-IP-EW-E WSENFASQPE MLRYVNRAAD
OTEMO_B          52    RYPGCRLDTE SYAYGYFALK GI-IP-EW-E WSENFASQPE MLRYVNRAAD
1W4X_aligned     59    RYPGARCDIE SIEYCYSF-- SEEVL-QEWN WTERYASQPE ILRYINFVAD
3GWD_aligned     52    RYPGALSDTE SHLYRF--SF DRDLLQES-T WKTTYITQPE ILEYLEDVVD
3GWF_aligned     52    RYPGALSDTE SHLYRF--SF DRDLLQES-T WKTTYITQPE ILEYLEDVVD
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OTEMO_A          99    AMDVRKHYRF NTRVTAARYV ENDRLWEVTL DNEEVVTCRF LISATGPLSA
OTEMO_B          99    AMDVRKHYRF NTRVTAARYV ENDRLWEVTL DNEEVVTCRF LISATGPLSA
1W4X_aligned     106   KFDLRSGITF HTTVTAAAFD EATNTWTVDT NHGDRIRARY LIMASGQLSV
3GWD_aligned     99    RFDLRRHFKF GTEVTSALYL DDENLWEVTT DHGEVYRAKY VVNAVGLLSA
3GWF_aligned     99    RFDLRRHFKF GTEVTSALYL DDENLWEVTT DHGEVYRAKY VVNAVG-LLS
                         *.*    *  * **.* .  .    * *   .. .    .. .. * * *

OTEMO_A          149   SAAPDI-KGI DSFKGESFHS SRWPTDAEGA PKGVDFTGKR VGVIGTGATG
OTEMO_B          149   SRMPDI-KGI DSFKGESFHS SRWPTDA-GAP KGVDFTGKRV GVIGTGATG
1W4X_aligned     156   PQL-PNFPGL KDFAGNLYHT GNWPHE---- --PVDFSGQR VGVIGTGSSG
3GWD_aligned     149   INFPNL-PGL DTFEGETIHT AAWPE----- GKSLAG--RR VGVIGTGSTG
3GWF_aligned     148   AINFPNLPGL DTFEGETIHT AAWPE----- -GKSLAG-RR VGVIGTGSTG
                             *.   * *. .*. . **                  .   ..

OTEMO_A          198   VQIIPIAAET AKELYVFQRT PNWCTPLGNS PMSKEKMDSL RNRYPTILEY
OTEMO_B          199   VQIIPIAAETA KELYVFQRTP NWCTPLGNSP MSKEKMDSLR NRYPTILEY
1W4X_aligned     199   IQVSPQIAKQ AAELFVFQRT PHFAVPARNA PLDPEFLADL KKRYAEFREE
3GWD_aligned     191   QQVITSLAPE VEHLTVFVRT PQYSVPVGNR PVNPEQIAEI KADYDRIWER
3GWF_aligned     191   QQVITSLAPE VEHLTVFVRT PQYSVPVGNR PVNPEQIAEI KADYDRIWER
                 .                          .                 .

OTEMO_A          248   VKSTDTAFPY HRDPRKGTDV SESERDAFFE ELYRQPGY-G IWLSGFRDLL
OTEMO_B          249   VKSTDTAFPYH RDPRKGTDVS ESERDAFFEE LYRQPGYG-I WLSGFRDLL
1W4X_aligned     249   SRNTPGGTHR YQGPKSALEV SDEELVETLE RYWQEGGP-D ILA-AYRDIL
3GWD_aligned     241   AKNSAVAFGF EESTLPAMSV SEEERNRIFQ EAWDHGGGFR FMFGTFGDIA
3GWF_aligned     241   AKNSAVAFGF EESTLPAMSV SEEERNRIFQ EAWDHGGGFR FMFGTFGDIA
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OTEMO_A          297   LNKESNKFLA DFVAKKIRQR VKDPTVAEKL IPKDHPFGAK RVPMETNYYE
OTEMO_B          298   LNKESNKFLA DFVAKKIRQR VKDPTVAEKL IPKDHPFGAK RVPMETNYYE
1W4X_aligned     297   RDRDANERVA EFIRNKIRNT VRDPEVAERL VPKGYPFGTK RLILEIDYYE
3GWD_aligned     291   TDEAANEAAA SFIRAKVAEI IEDPETARKL MPK-GLF-AK RPLCDSGYYE
3GWF_aligned     291   TDEAANEAAA SFIRAKVAEI IEDPETARKL MPK--GLFAK RPLCDSGYYE
                        .  .*  .*  *.  *     . **  * **  **     ** *      ***

OTEMO_A          347   TYNRDNVHLV DIREAPIQEV TPEGIKTAD- AAYDLDVIIY ATGFD---G-
OTEMO_B          348   TYNRDNVHLVD IREAPIQEVT PEGIKTADA- AYDLDVIIYA TGFDA--G-
1W4X_aligned     347   MFNRDNVHLV DTLSAPIETI TPRGVRTSE- REYELDSLVL ATGFDAL---
3GWD_aligned     339   VYNRPNVEAV AIKENPIREV TAKGVVTEDG VLHELDVLVF ATGFDAVDG-
3GWF_aligned     339   VYNRPNVEAV AIKENPIREV TAKGVVTEDG VLHELDVLVF ATGFDAVDG-
                                           .         .          .   .       *

OTEMO_A          395   --SLDRIDIR GKDNVRLIDA WAEGPSTYLG LQARGFPNFF TLVGPHNGST
OTEMO_B          395   --SLDRIDIR GKDNVRLIDA WAEGPSTYLG LQARGFPNFF TLVGPHNGST
1W4X_aligned     393   TGALFKIDIR GVGNVALKEK WAAGPRTYLG LSTAGFPNLF FIAGPGSPSA
3GWD_aligned     388   --NYRRIEIR GRDGLHINDH WDGQPTSYLG VSTANFPNWF MVLGPNGPF-
3GWF_aligned     388   --NYRRIEIR GRDGLHINDH WDGQPTSYLG VSTANFPNWF MVLGPNGPF-
                         .  .*.** *   . . .  *   * .*** . .  *** *  . **

OTEMO_A          443   FCNVGVCGGL QAEWVLRMIS YMKDNGFTYS EPTQAAENRW TEEVYADFSR
OTEMO_B          443   FCNVGVCGGL QAEWVLRMIS YMKDNGFTYS EPTQAAENRW TEEVYADFSR
1W4X_aligned     443   LSNMLVSIEQ HVEWVTDHIA YMFKNGLTRS EAVLEKEDEW VEHVNEIADE
3GWD_aligned     435   -TNLPPSIET QVEWISDTIG YAERNGVRAI EPTPEAEAEW TETCTEIANA
3GWF_aligned     435   -TNLPPSIET QVEWISDTIG YAERNGVRAI EPTPEAEAEW TETCTEIANA
                         *.       . **.   *. *   **     *.    *  *  *

OTEMO_A          493   TLLAEANAWW VKTTTKPDGS VVRRTLVH-V SGGPEYRKRC EQVAYNNYNG
OTEMO_B          493   TLLAEANAWW VKTTTKPDGS VVRRTLVH-V SGGPEYRKRC EQVAYNNYNG
1W4X_aligned     493   TLYPMTASWY T---GANVPG KPRVFML-YV GGFHRYRQIC DEVAAKGYEG
3GWD_aligned     484   TLFTKGDSWI FGAN---IPG KKPSVLF-YL GGLRNYRAVM AEVAADGYRG
3GWF_aligned     484   TLF------- ---------- -----VLFYL GGLRNYRAVM AEVAADGYRG
                       **                          .   . .*   **     .** . * *

OTEMO_A          542   FELA
OTEMO_B          542   FELA-
1W4X_aligned     539   FVLT-
3GWD_aligned     530   FEVKSA
3GWF_aligned     530   FEVKS
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Fig. S3. SDS-PAGE of purified OTEMO and mutants. Lanes 1-4: wild-type protein, Y53F, D59A, and D59N.

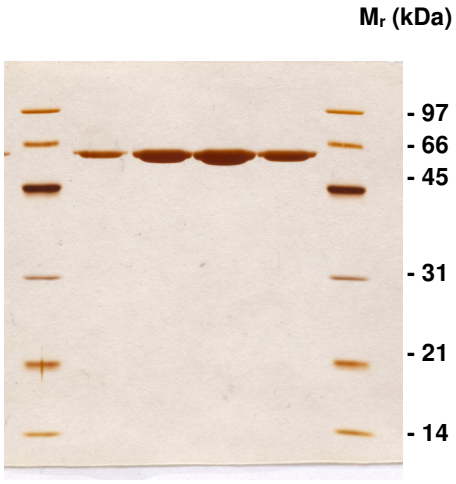


Fig S4. Structural superposition of OTEMO [green, PDB 3UP4, chain A], CHMO [pink, PDB 3GWD] and PAMO [grey, PDB 2YLR]. The FAD and NADP molecules are shown in stick representation.

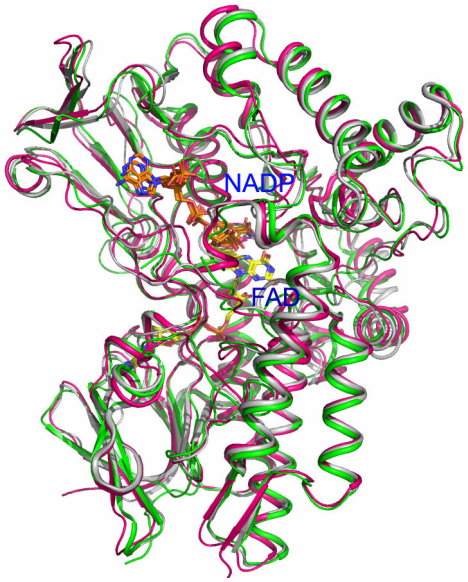
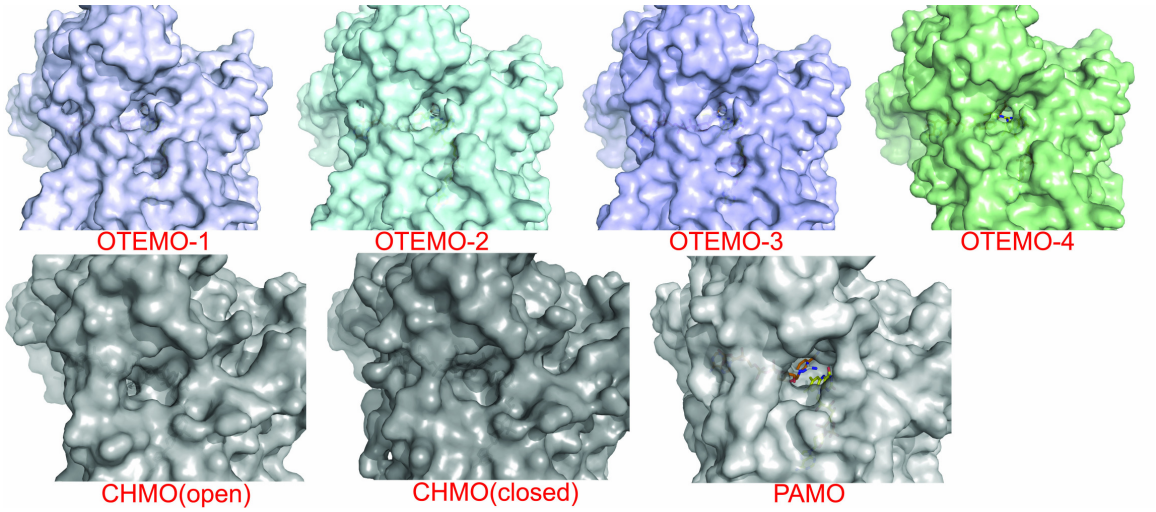


Fig. S5. Surface comparison of all NADP-bound BVMO proteins (OTEMO in four different conformations as summarized in Table 5, CHMO in both open and closed forms, and PAMO). FAD, NADP and the conserved arginine residue are shown in stick mode. (a) The specific funnel observed in PAMO is not present in OTEMO and CHMO. (b) All BVMO proteins contain a small "channel" between the FAD and NADP domains, which is different from the "funnel" proposed for PAMO. This channel is more likely the substrate binding path for OTEMO/CHMO.

(a)



(b)

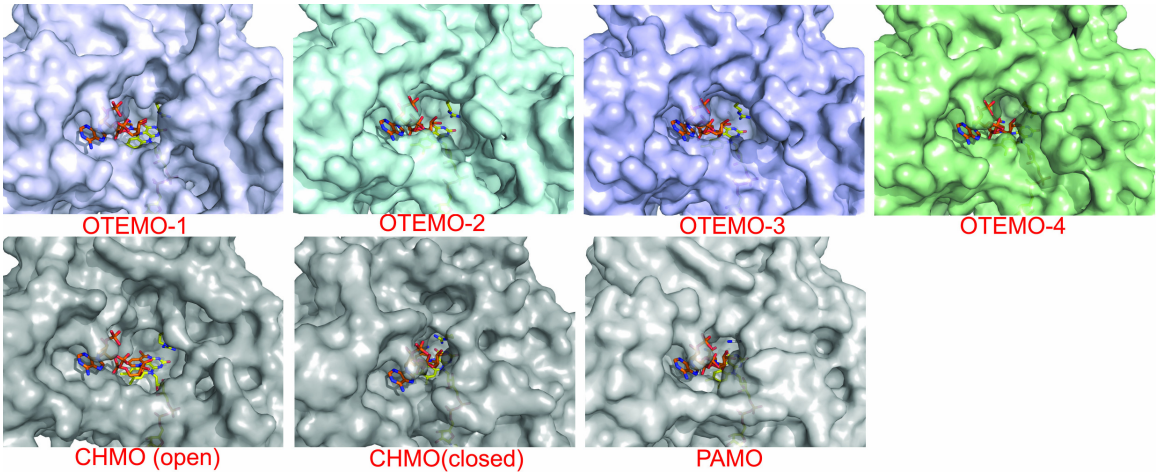


Fig. S6. Superposition of open (type 3, grey and magenta) and closed (type 4, grey and green) forms of the OTEMO-FAD-NADP complexes showing the putative cork-bottle mechanism upon substrate binding.

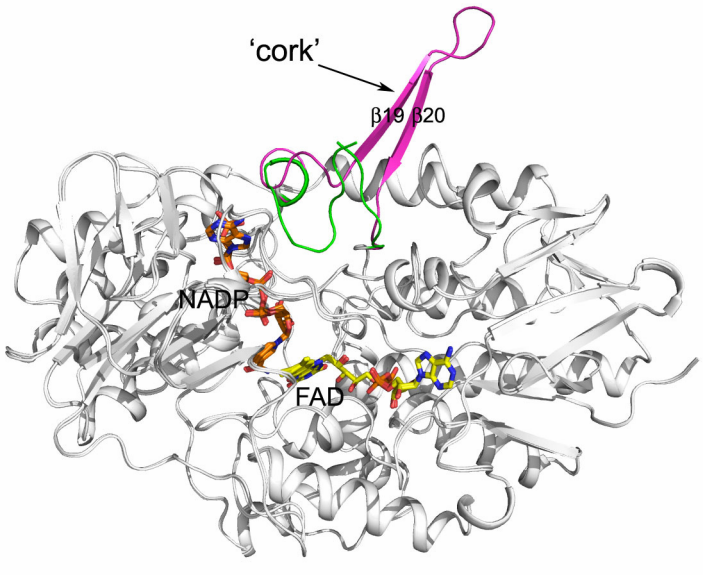


Fig S7. Electrostatic potential of the active site pocket viewed from the same orientation from Fig. S5(a). NADP (carbon in orange) and FAD (carbon in yellow) molecules are shown in stick mode.

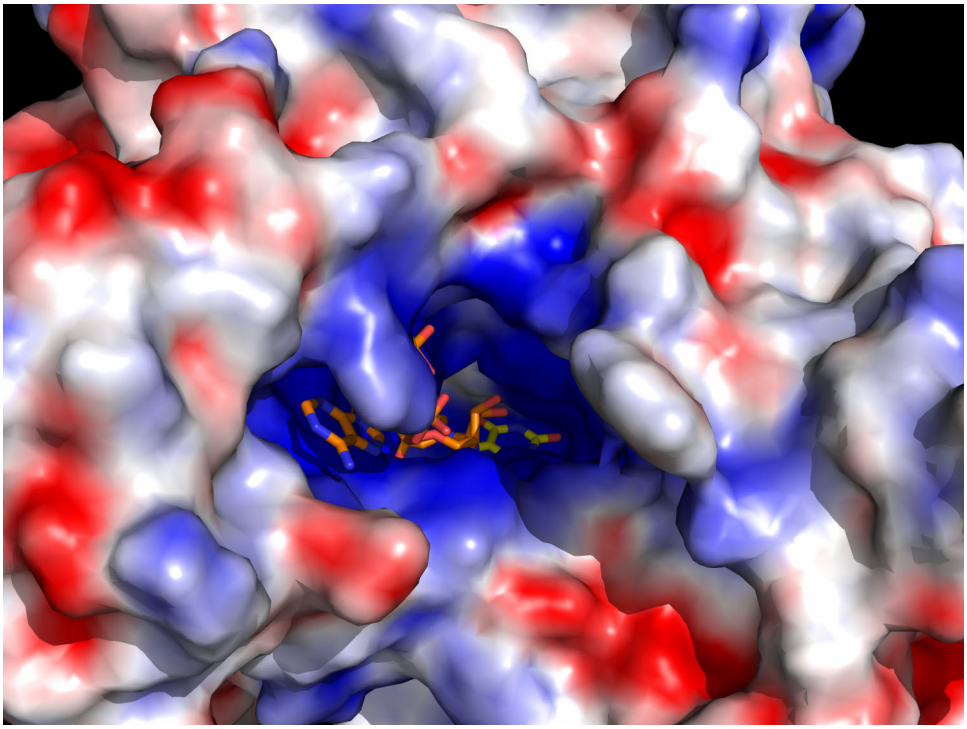


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Table S1. X-ray data collection and refinement statistics

Structure	FAD complex (form 1)	FAD complex (form 2)	FAD-NADP complex (form 1)	FAD-NADP complex (form 2)	FAD-NADP complex (form 3)	FAD-NADP complex (form 4)
Space group	P21	P21	P21	P21	P212121	P21
a, b, c (Å) α, β, γ (°)	59.0, 140.0,67.6 98.4	67.3,92.5,93.4 103.3	66.8, 95.0, 93.1 102.3	66.8,95.2,93.1 101.8	61.3,66.2,282.7	66.8, 94.4, 93.1, 102.4
wavelength(Å)	0.97929	0.97931	0.97929	0.97931	0.97949	0.97949
resolution (Å)	50-2.05(2.12-2.05)	50-1.95(2.02-1.95)	50-2.0(2.07-2.0)	50-2.42(2.51-2.42)	50-2.80(2.90-2.80)	50-2.45(2.54-2.45)
observed *hkl*	247778	243112	278353	109181	82445	106508
unique *hkl*	67586(6635)	79134(7809)	76439(7650)	42173 (3984)	25733(2543)	39964(4025)
Redundancy	3.7 (3.4)	3.1(2.8)	3.6 (3.3)	2.6 (2.5)	3.2(3.2)	2.7 (2.6)
completeness (%)	99.5 (98.6)	99.0(98.4)	99.7 (100)	95.8 (91.0)	88.2(88.6)	96.6 (98.2)
R_{sym} [a]	0.117(0.566)	0.098(0.541)	0.109(0.478)	0.105 (0.452)	0.128(0.520)	0.097(0.349)
I/(σI)	10.8 (2.1)	14.5 (2.0)	11.6 (2.5)	14.3 (2.9)	13.8(3.1)	9.5 (2.0)
Wilson B ($Å^2$)	20.6	20.6	18.9	36.9	43.5	35.6
R_{work} [b] (# *hkl*)	0.190(64069)	0.190 (75014)	0.196(72567)	0.196 (39979)	0.200(24255)	0.185(37916)
R_{free} (# *hkl*)	0.240(3416)	0.230 (3967)	0.245(3840)	0.247 (2122)	0.264(1295)	0.236(2017)
B-factors (# atoms)						
Protein	25.8(8538)	23.8 (8517)	24.5(8533)	26.5 (8596)	29.3(8462)	25.8(8504)
Solvent	32.3(593)	29.6 (405)	30.0(750)	15.4 (243)	26.2 (49)	27.5 (298)
ligands	18.1 (106)	17.7 (106)	20.0 (202)	20.8 (202)	23.9 (202)	26.3 (202)
Ramachandran						
allowed (%)	99.9	99.8	99.6	99.2	99.5	99.6
generous (%)	0.1	0.2	0.2	0.6	0.3	0.2
disallowed (%)	0	0	0.2	0.2	0.2	0.2
rmsd's						
bonds (Å)	0.010	0.015	0.010	0.014	0.013	0.019
angles (°)	1.22	1.51	1.26	1.58	1.55	1.84
PDB code	3UOV	3UOX	3UOY	3UOZ	3UP4	3UP5

[a] $R_{sym} = (\Sigma|I_{obs} - I_{avg}|)/\Sigma I_{avg}$. [b] $R_{work} = (\Sigma|F_{obs} - F_{calc}|)/\Sigma F_{obs}$

TABLE S2. Purification of OTEMO using a three-step chromatographic approach

Purification Step	Tot. Act[1] (U)	Spec. Act.[1] (U/mg)	Yield/Purif. (%/-fold)
Crude extract	1,035	3.14	100/1.0
DEAE-Sepharose	611	5.18	59/1.6
Butyl-Sepharose	176	5.64	17/1.7
Superdex 75	131	6.62	13/2.1

[1)] 2-n-hexyl cyclopentanone was used as substrate