Supplementary information for

Spatial requirement for phenylacetone monooxygenases for transformation of non-native linear substrates

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Table S1 BVMO species that show activity towards linear substrate 2-octanone

	Accession number	**BVMO group [1]**	**Activity**
mo4	ro03063	I	+ [2]
mo20	ro08137	I	+ [2]
CPMO	Comamonas sp. strain NCIMB 9872 (Q8GAW0)	I	++ [2]
BVMO $_{AFL838}$ (crystal structure PDB: 5J7X)	B8N653	I	k_{cat}/K_M = 5.3 x10^5 [M^{-1} s^{-1}] [3]
mo21	ro10187	I & II	+ [2]
PAMO quadruple variant (structure evolved from *T. fusca* BVMO, PDB: 2YLT)	Q47PU3	II	k_{cat}/K_M = 9.2 x10^3 [M^{-1} s^{-1}] [4]

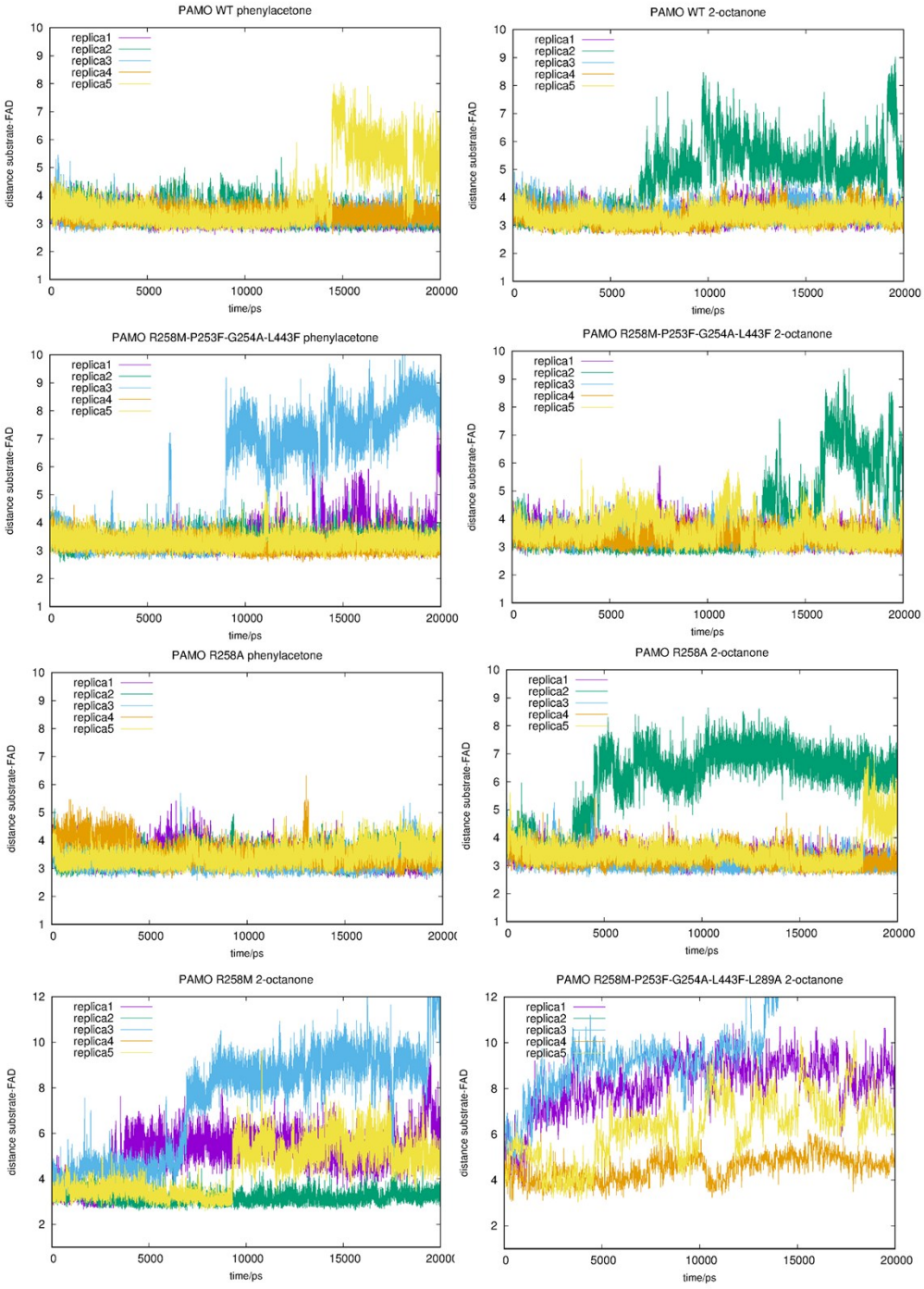

Figure S1 Distance between the peroxy-FAD oxygen and carbonyl carbon of phenylacetone and 2-octanone during the molecular dynamics simulations of the WT and variants of PAMO. 5 replicas of MD simulations were conducted for each of complex system.

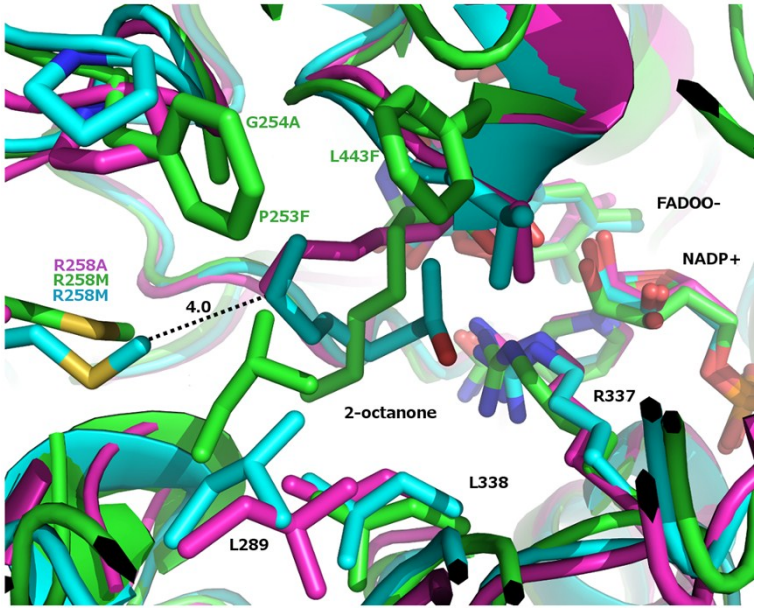

Figure S2 Superposition of the reference structures of the mutants R258A (purple, replica 3), R258M (cyan, replica 1) and P253F/G254A/R258M/L443F (green, replica 1) in complex with 2-octanone. The structures correspond to the ones with lowest RMSD compared to the respective average MD structures. In these replicas the substrate still maintains a catalytically favorable pose (carbonyl close to the C4a-peroflyflavin). Note that in the quadruple mutant L289 moves substantially towards P253F and G254A, preventing the approach of 2-octanone to R258M at the pocket entrance. As a result, 2-octanone adopts a pose similar to that in the WT enzyme with the alkyl tail nested in a hydrophobic pocket formed by L289, L338 and L340. In contrast, in the R258A and R258M mutants 2-octanone adopts such a pose that the alkyl tail moves away from the hydrophobic pocket and towards the entrance of the active site.

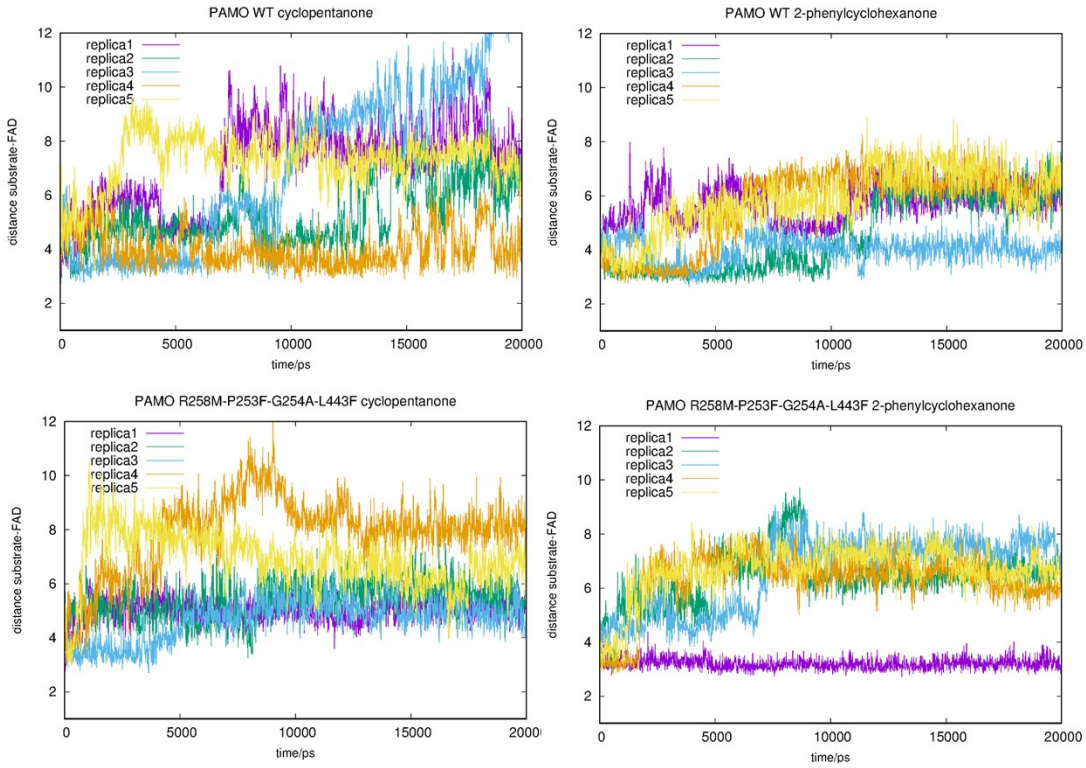

Figure S3 Distance between the peroxy-FAD oxygen and carbonyl carbon of cyclopentanone and 2-phenylcyclohexanone during the molecular dynamics simulations of the WT and variants of PAMO. 5 replicas of MD simulations were conducted for each of complex system.

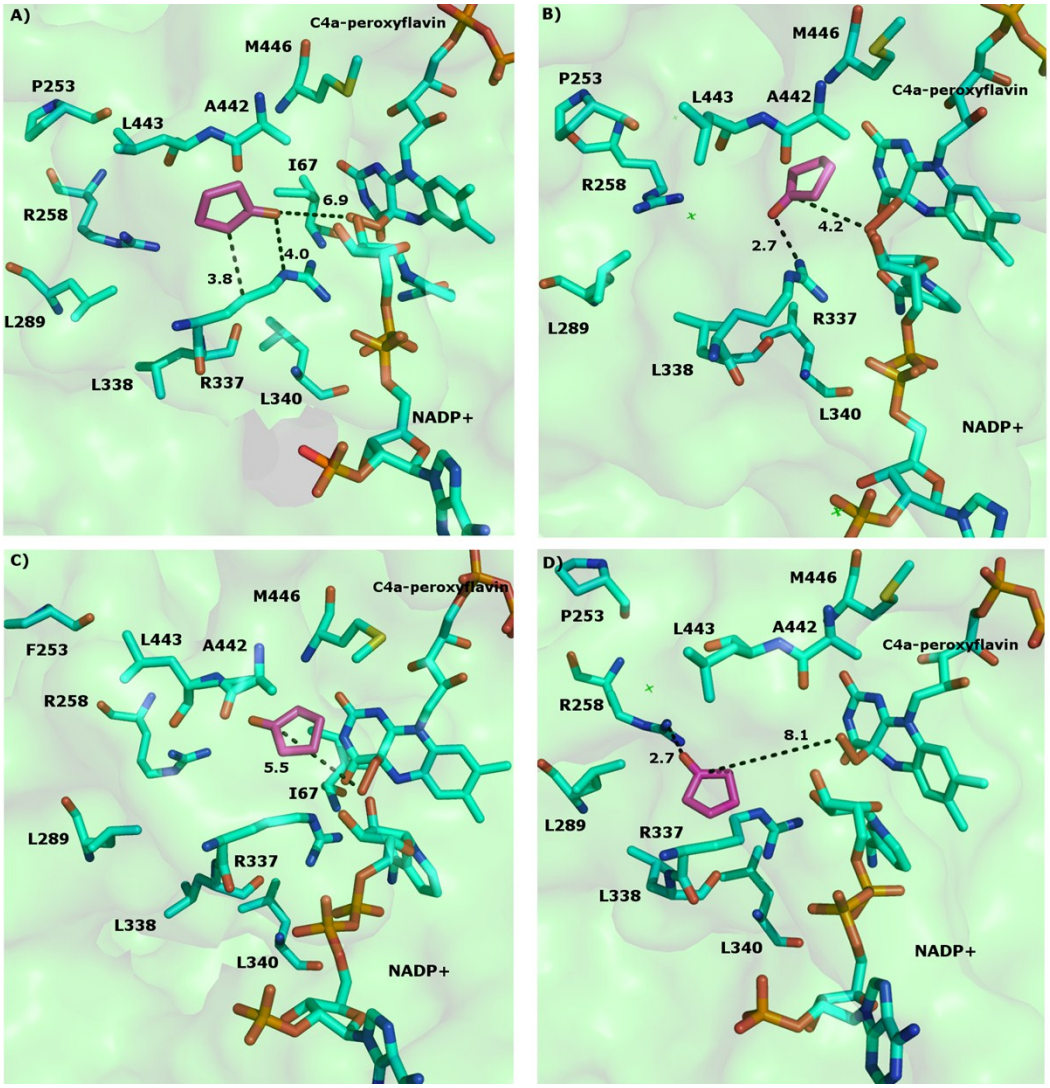

Figure S4 MD reference structures of the PAMO WT in complexe with cyclopentanone: A) replica 1; B) replica 2; C) replica 4 (replica 3 is similar to replica 5); D) replica 4.

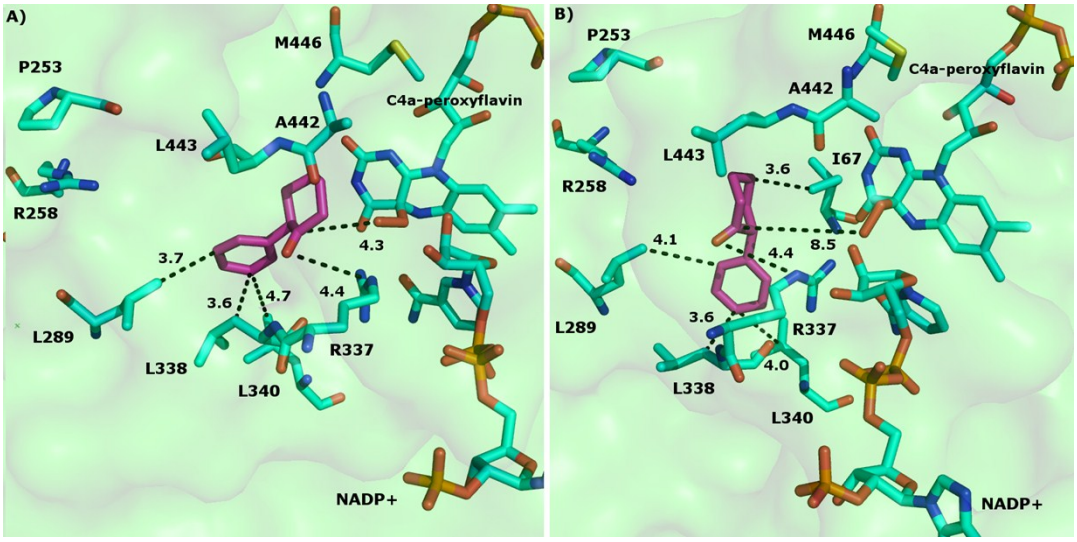

Figure S5 MD reference structures of the WT PAMO complexed with 2-phenylhexanone: A) replica 2 (replica 1 and replica 3 are similar to replica 2); B) replica 4 (5 is similar to replica 4);

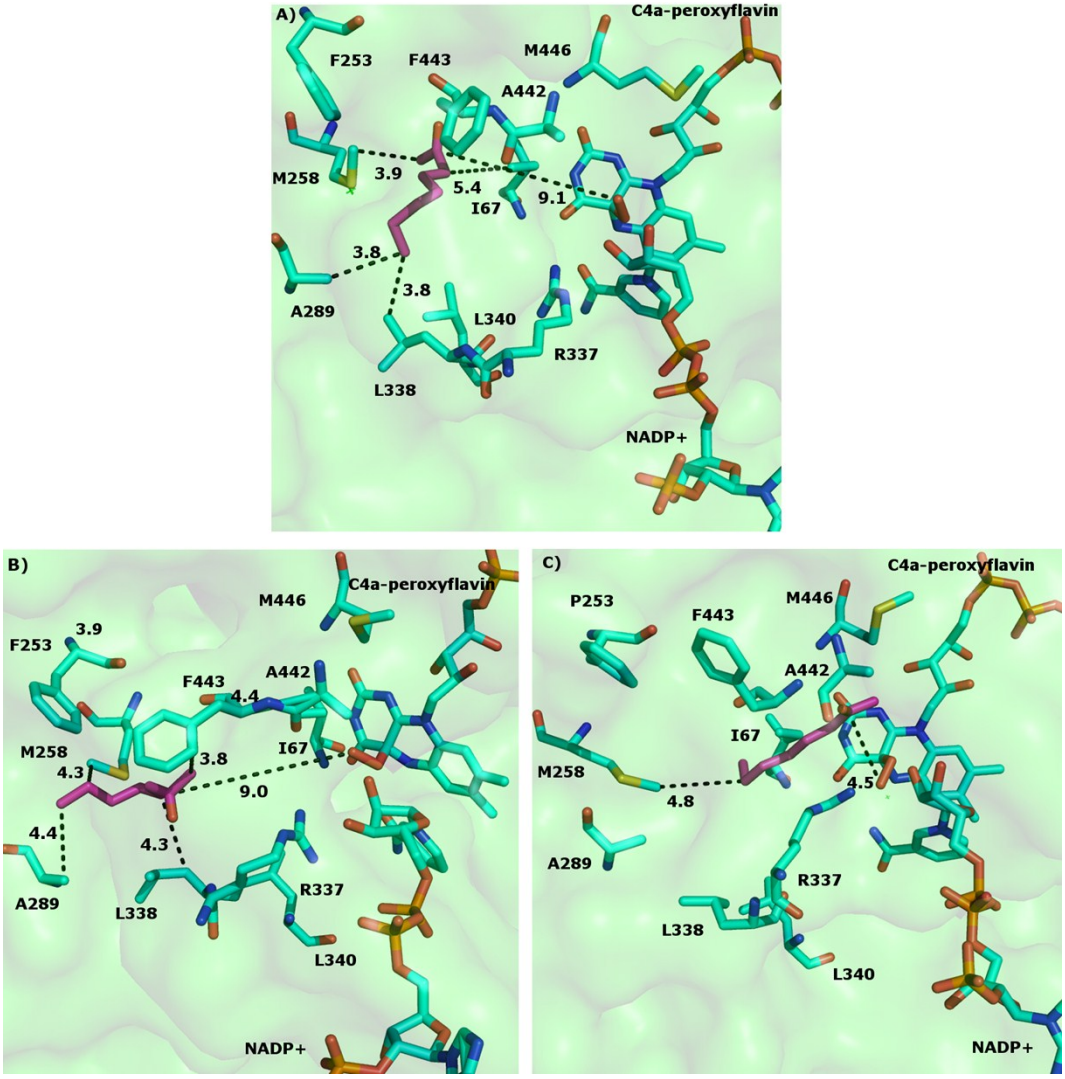

Figure S6 MD reference structures of the P253F/G254A/L289A/R258M/L443F variant in complex with 2-octanone: A) replica 1; B) replica 3 (replica 2 is similar to replica 3); C) replica 4 (replica 5 is similar to replica 4).

References:

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2. Riebel A, Dudek HM, de Gonzalo G, Stepniak P, Rychlewski L, Fraaije MW. Expanding the set of rhodococcal Baeyer-Villiger monooxygenases by high-throughput cloning, expression and substrate screening. *Appl Microbiol Biotechnol*. 2012,**95**,1479-89.
3. Ferroni FM, Tolmie C, Smit MS, Opperman DJ. Structural and Catalytic Characterization of a Fungal Baeyer-Villiger Monooxygenase. *PLoS One*. 2016,**11**,e0160186.
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