

Supporting information

The substrate specificity in the O-demethylation of 4-alkylguaiacols by Cytochrome P450 AgcA_{P450}

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Amino acid sequence of P450 AgcA_{P450}
>tr|A0A385L6C3|A0A385L6C3_RHORH Cytochrome P450 OS=Rhodococcus
rhodochrous OX=1829 GN=C6369_001540 PE=3 SV=1

MTSTHSFIDEITIEELEADPYPFYERLRKEAPIAYVPALGMYIVSTKELCAEISKDDANW
 PAVISAAAGGRTFGPQALLNTNGDEHRNLRDMVEPHLQPSAVDKYIDDLVRPFARQRIAEF
 ENDGHADIVAAYCEPVSVRALGDDLGLGDVSTEKLEWFFHNLVSFTNAAVDENGEFANP
 EGFAPGDRAKAEIIAHVDPKIDKWIVEPDHSAISHWLHDGMPEGQTRSRDVIYPNLYVFL
 LGAMQEPGHAMATTLAFLSRPDQLERVIDDPTLIPRAASEGMRWVAPIWSAAVKRAARE
 VTVGGVTLPEGSIVMLSYGSANQDENAYNAPTEYDLDRALVPNMFTFGGKHCACAGTYFAN
 AVVRIGLEELLEAIIPNIERDETDFWGWGFRGPKQLFVKWEV

Sequence Alignment

Sequence alignment of the AgcA_{P450} and GcoA_{P450} enzymes. GcoA_{P450} was used as a template for the construction of the model by homology.

sp P0DPQ7 GCOA_AMYS7	MTTTERPDLAWLDEVMTQLERNPYEVYERLRAEAPLAFVPLGGSYVASTAEVCREVAT-	59
tr A0A385L6C3 A0A385L6C3_RHORH	----MTSTHSFIDEITIEELEADPYPFYERLRKEAPIAYVPALGMYIVSTKELCAEISKD	56
	::*:**:* :** :** .***** ***:**:* *:.** :* :* :*	
sp P0DPQ7 GCOA_AMYS7	SPDFEAVITPAGGRTFGHPAIIIGVNGDIHADLRSMVEPALQPAEVDRIIDDLVRPIARRY	119
tr A0A385L6C3 A0A385L6C3_RHORH	DANWPAVISAAGGRTFGPOALLNTNGDEHRNLRDMVEPHLQPSAVDKYIDDLVRPFARQR	116
	. : : ***:***** :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :* :*	
sp P0DPQ7 GCOA_AMYS7	LERFENDGHAELVAQYCEPVSVRSLGDDLGLQEVDSDKLEWFAKLNRSFTNAAVDENGE	179
tr A0A385L6C3 A0A385L6C3_RHORH	IAEFENDGHADIVAAYCEPVSVRALGDDLGLGDVSTEKLEWFFHNLVSFTNAAVDENGE	176
	: .*****:*** :*****:***** :*:***** :* :*****:*****	
sp P0DPQ7 GCOA_AMYS7	FANPEGFAEGDQAKAEIRAVVDPKIDKWIHPDASAIHNLHDMPPGQTRDREYIYPTI	239
tr A0A385L6C3 A0A385L6C3_RHORH	FANPEGFAPGDRAKAEIIAHVDPKIDKWIVEPDHSAISHWLHDGMPEGQTRSRDVIYPNL	236
	***** **:* ** :* :* :*	
sp P0DPQ7 GCOA_AMYS7	YVLLGAMQEPGHMASTLVGLFSRPEQLEEVDDPTLIPRAIAEGLRWTSPINSAFARI	299
tr A0A385L6C3 A0A385L6C3_RHORH	YVLLGAMQEPGHAMATTLAFLSRPDQLERVIDDPTLIPRAASEGMRWVAPIWSAAVKR	296
	** :***** **:* ** :*****:***** :*:***** :* :* :* :* :*	
sp P0DPQ7 GCOA_AMYS7	STKPVTIAGVDLPAGTPVMLSYGSANHDTGKYEAPSQYDLHRPPLPHLAFGAGNHACAGI	359
tr A0A385L6C3 A0A385L6C3_RHORH	AAREVTVGGVTLPEGSIVMLSYGSANQDENAYNAPTEYDLDRALVPNMFTFGGKHCACAGT	356
	:: * : * * * * * :*****:*** :* :* :* :* :* :* :* :* :* :* :* :* :*	
sp P0DPQ7 GCOA_AMYS7	YFANHVMRIALEELFEAIPLNERDTRREGVEFWGWFVFRGPTSLHVTWEV	407
tr A0A385L6C3 A0A385L6C3_RHORH	YFANAVVRIGLEELLEAIIPNIERDETDFWGWGFRGPKQLFVKWEV	404
	*** :*	

Figure S1 Sequence alignment of the AgcA_{P450} and GcoA_{P450} enzymes using Clustal-Omega webserver developed by the European Bioinformatics Institute. Marked in green we have the active centre conserved residues between these two proteins. Marked in red we have the non-conserved residues, an Isoleucine (GcoA_{P450}) replaced by a Leucine (AgcA_{P450}) and a Threonine (GcoA) replaced by an Alanine (AgcA_{P450}).

RMSD of AgcA_{P450} in complex with each substrate

AgcA_{P450} with guaiacol

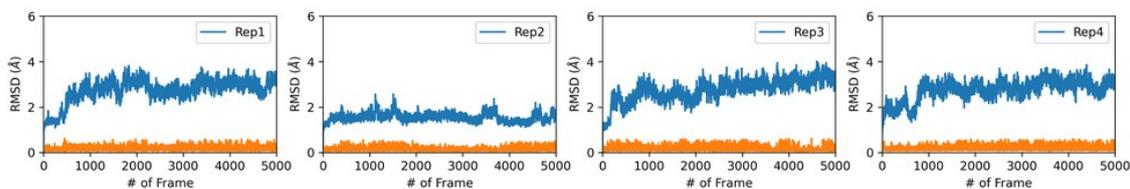


Figure S2 RMSD for the backbone C α atoms of AgcA_{P450} protein (in blue) and the RMSD of the heavy atoms of substrate Guaiacol (in orange).

AgcA_{P450} with 4-methylguaiacol

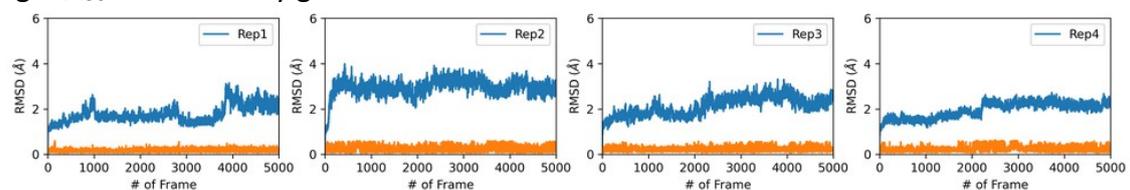


Figure S3 RMSD for the backbone C α atoms of AgcA_{P450} protein (in blue) and the RMSD of the heavy atoms of substrate 4-methylguaiacol (in orange).

AgcA_{P450} with 4-ethylguaiacol

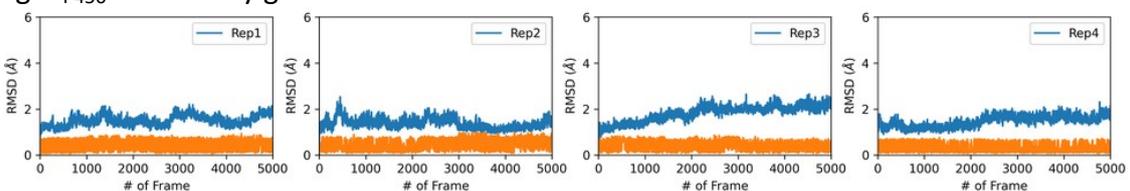


Figure S4 RMSD for the backbone C α atoms of AgcA_{P450} protein (in blue) and the RMSD of the heavy atoms of substrate 4-ethylguaiacol (in orange).

AgcA_{P450} with 4-propylguaiacol

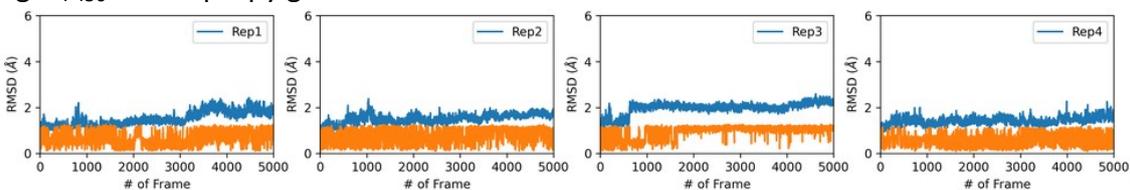


Figure S5 RMSD for the backbone C α atoms of AgcA_{P450} protein (in blue) and the RMSD of the heavy atoms of substrate 4-propylguaiacol (in orange).

Radial distribution functions in relation to the Cpd I complex in the active site

AgcA_{P450} with guaiacol

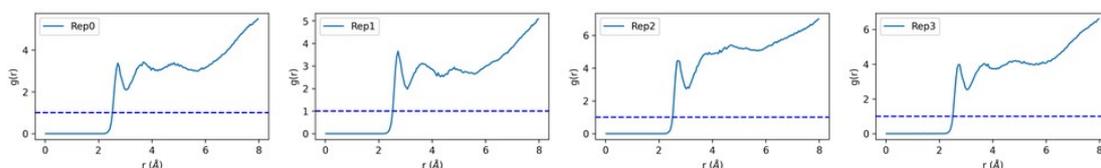


Figure S6 The radial distribution function of AgcA_{P450} protein in complex with guaiacol. The dashed blue line represents at least one water molecule near the Iron-oxo complex of Cpd I during the MD simulations.

AgcA_{P450} with 4-methylguaiacol

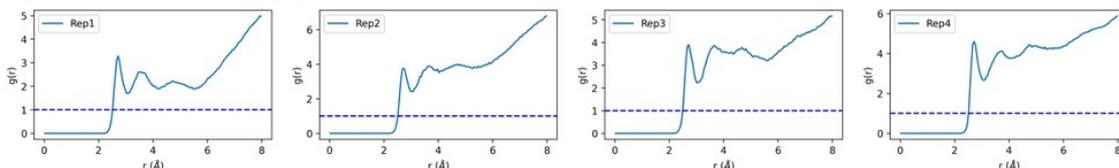


Figure S7 The radial distribution function of AgcA_{P450} protein in complex with 4-methylguaiacol. The dashed blue line represents at least one water molecule near the Iron-oxo complex of Cpd I during the MD simulations.

AgcA_{P450} with 4-ethylguaiacol

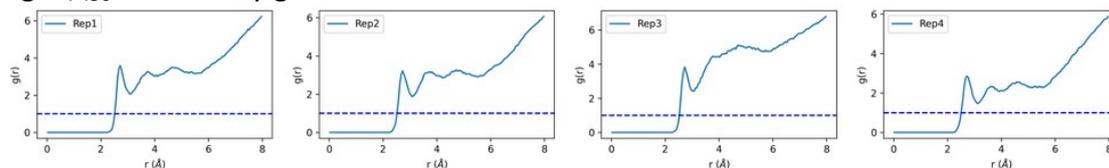


Figure S8 The radial distribution function of AgcA_{P450} protein in complex with 4-ethylguaiacol. The dashed blue line represents at least one water molecule near the Iron-oxo complex of Cpd I during the MD simulations.

AgcA_{P450} with 4-propylguaiacol

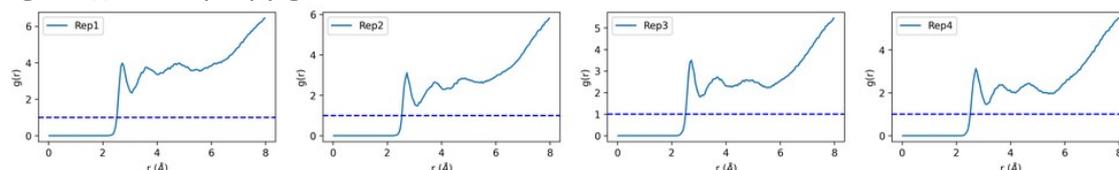


Figure S9 The radial distribution function of AgcA_{P450} protein in complex with 4-propylguaiacol. The dashed blue line represents at least one water molecule near the Iron-oxo complex of Cpd I during the MD simulations.

Distance of the residues A169 and I13 acting as gate of the substrate access channel

AgcA_{P450} with guaiacol

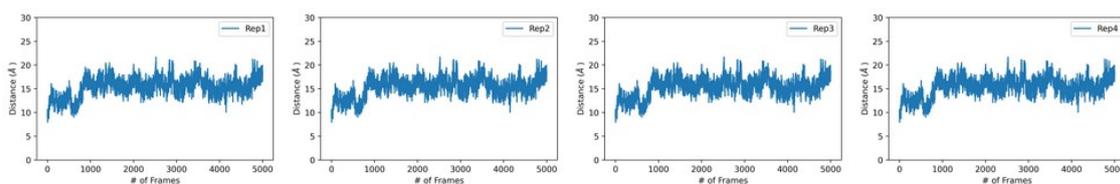


Figure S10 Distance between I13 and A169 during the MD simulation of AgcA_{P450} protein in complex with Guaiacol.

AgcA_{P450} with 4-methylguaiacol

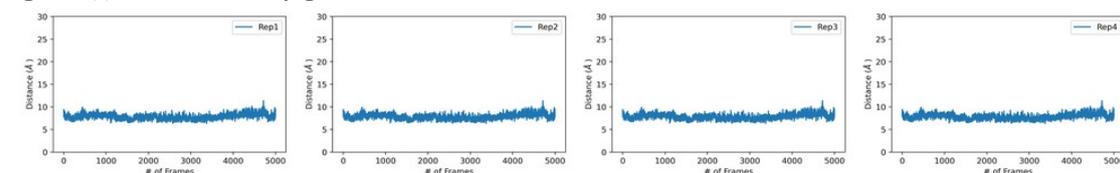


Figure S11 Distance between I13 and A169 during the MD simulation of AgcA_{P450} protein in complex with 4-methylguaiacol.

AgcA_{P450} with 4-ethylguaiacol

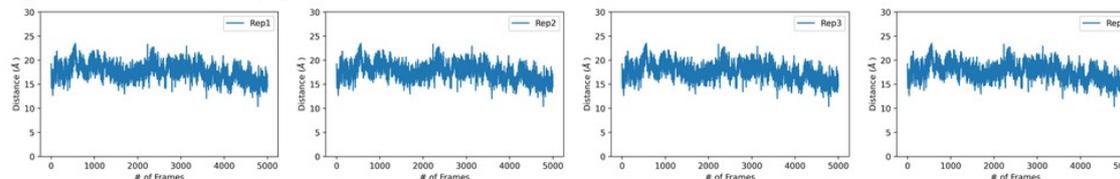


Figure S12 Distance between I13 and A169 during the MD simulation of AgcA_{P450} protein in complex with 4-ethylguaiacol.

AgcA_{EP4} with 4-propylguaiacol

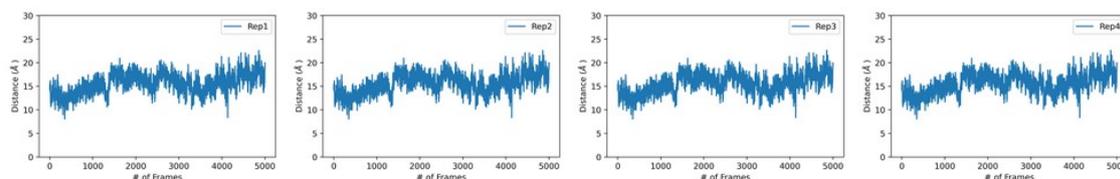


Figure S13 Distance between I13 and A169 during the MD simulation of AgcA_{P450} protein in complex with 4-propylguaiacol.

Distance of the methyl group and the Iron-oxo complex of each complex

AgcA_{P450} with guaiacol

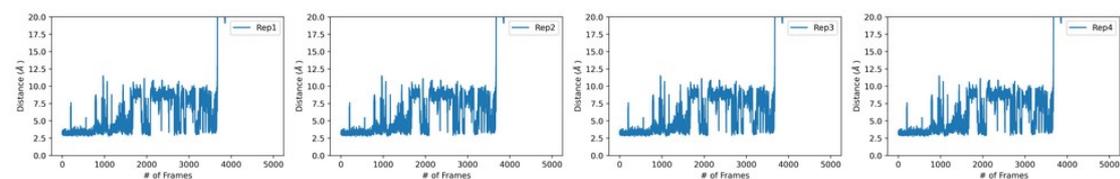


Figure S14 Distance between the methoxy group of guaiacol and the oxygen atom of Fe(IV)=O.

AgcA_{P450} with 4-methylguaiacol

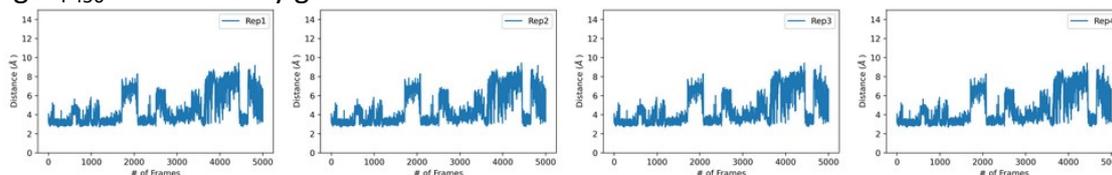


Figure S15 Distance between the methoxy group of 4-methylguaiacol and the oxygen atom of Fe(IV)=O.

AgcA_{P450} with 4-ethylguaiacol

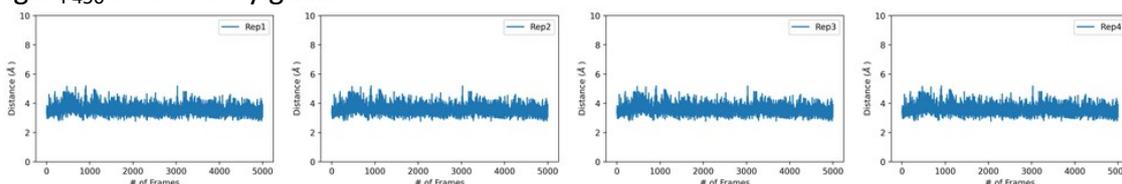


Figure S16 Distance between the methoxy group of 4-ethylguaiacol and the oxygen atom of Fe(IV)=O.

AgcA_{P450} with 4-propylguaiacol

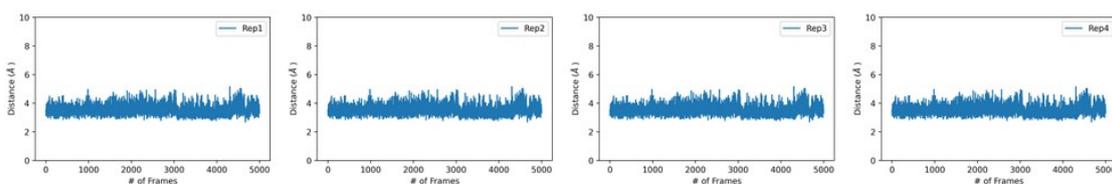


Figure S17 Distance between the methoxy group of 4-propylguaiacol and the oxygen atom of Fe(IV)=O.

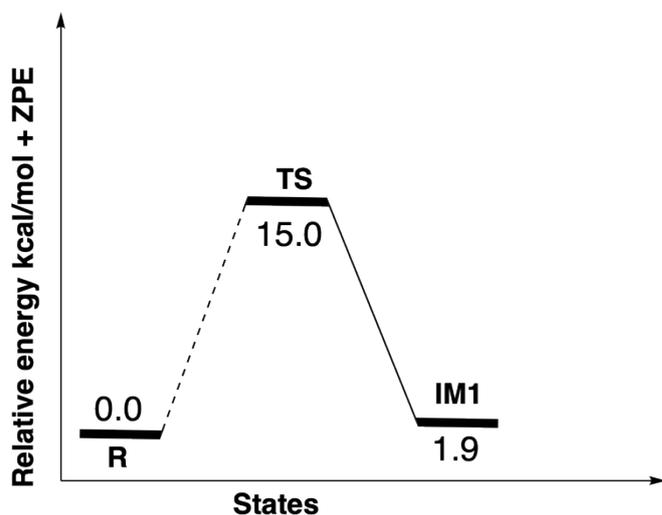


Figure S18 The oxidation 4-propylguaiacol in the active site of AgcA_{P450} enzyme calculated using QM/MM methods at M=4 spin of iron. The reaction profile of the hydrogen atom transfer (HAT) catalysed by Cpd I was computed using the UB3LYP functional with D3BJ dispersion correction and the def2-TZVP basis set for M=4.

Table S1 QM/MM calculated spin densities of the oxidation 4-propylguaiacol in the active site of AgcA_{P450} at M=2

	RC	TS	IM1	IM2
Fe(IV)=O	2.11	1.8	2.11	1.12
Porphyrin+Cysteine	-1.1	-0.36	-0.16	0.0
4-propylguaiacol	0.0	-0.44	-0.96	0.0

Table S2 QM/MM calculated spin densities of the oxidation 4-propylguaiacol in the active site of AgcA_{P450} at M=4

	RC	TS	IM1
Fe(IV)=O	2.04	2.11	2.14
Porphyrin+Cysteine	0.93	0.32	-0.12
4-propylguaiacol	0.0	0.55	0.97