

Supplementary data

Promising new antifungal treatment targeting chorismate synthase from *Paracoccidioides brasiliensis*

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	10	20	30	40	50	60	
1R53A	MSTFGKFLFRVITYGESEHCKSVGCIVDGVPPGMSLTEADIQPOLTR						PDRVEIQSG
1UM0A	MNTLGRFLRLITTFGESMGDVGVLGMPGSKIDYALLENEENKR						KEDDKVEITSG
1QX0B	-----MRYLTAGEHSMSPRLTAIIIEGIPAGLPLTAEDIINEDLRR						QGGYGRGGRMKIEENDQVVFISG
PbCS	-STFGEYFRVITYGESEHCKSVGCIVDGCPPGMALTEADIQPQNTRE						BPGQSALETPRNKEKDRVEIQSG
	***	***	***	***	***	***	***
	70	80	90	100	110	120	130
1R53A	TEFGKTILGTPIAMMIK-						
1UM0A	VFEDFSTGTGPFLHHNQRARS-						KDYDNHKNLPRPSHADFTYFHKGIRD
1QX0B	VRHSGTTGAPITMDVINKDQHK--WLDIMSAEDIEDIRLSR-----RKITHPRPGRHADLVGGIKYRFDD						
PbCS	TEFGITLGLPIGMIVRNEDQRPKD-						YGGSTMIDLPRPSHADFTYLEKYGI-K
	***	***	***	***	***	***	***
	140	150	160	170	180	190	200
1R53A	-----RETIGRVASGAIAKFLAQMSNVIEAVFTQIG-						EIKMMNRDSFDPEFQHLLN
1UM0A	FR-G GG ARESAAIRVAAGSAFAEMLLRLI--GIVCESGGIEIG-						GIRAHN-----
1QX0B	LRNSL KS ARETETMRVAVGAVAKRLLAEL-DMEIANHHVVFG-						GREIDV-----PENLTV
PbCS	ASS GG ARETIGRAAAGIAEKGYLRLSHDIDIVAFVSSVG MEHLFPPTA AHPNPITNPEFLKLIE						
	***	***	***	***	***	***	***
	210	220	230	240	250	260	270
1R53A	TITRERVDSMGPICRCPDASVAGLMVKEIEKYRGNKDSIGGVVTCVVRN-----LPTGLGEP--CFDK						
1UM0A	-YDFNHALK-SEIFALDEEGEEAQKTAIQNAIKNNHSIGGVALIRARSIKTNQKLPIGLGQQ--LYAK						
1QX0B	AEIKQRAAQ-SEVSIIVHQEREQEIKDVIDQIKRDGDTIGSVETVWGG-----VVFGLGSYVQWDRK						
PbCS	TIDRPTVDSFAPVRCPEASAAAERHTRVIEFNFRDQNDSIGGTVSCVIRN-----VVFGLGEP--CFDK						
	***	***	***	***	***	***	***
	280	290	300	310	320	330	340
1R53A	LEAMLAHAMLSIPASKGFEIGSGOFQGVSVPGSKHNDPFYRT						KINNSGGVQGG
1UM0A	LDAKIAZAMMGLNGVKAVEIGKGVESESSLKGSEYNDL-----MDQ-----KGFLSNRSQGVLG						
1QX0B	LDARLAGAVVSINAFKGVEIGLGFEGAGSYRKGSQVMDE--ILWSR-----EDGYTRATNNLGGFEGG						
PbCS	LEAKLAHAMLSIPATIGKFGFLGCGEIPGSVHNDFPVVTPDTRNG QNP D SKRLITIKINNSGGI QGG						
	***	***	***	***	***	***	***
	350	360	370	380	390	400	
1R53A	ISNGENIYFSVFFK5VRH-----DPAVIFPAIPIVEAMIALVIA DALLIQ						
1UM0A	HSNSEEZIIVRVHFKPTPSIIFQPGORIIDIN-GNECE-----DPCIAI TS SVVCESSLALVLA DAVVLLN						
1QX0B	HTNQQPIVVVRQVNGKPIPTLYKPKLMSVDIEZTHEPYKATVE-----SDPTALPAGMVHEAVVVAIVLAQEILEK						
PbCS	ISNGASIYFRVAFKFPATIQQAQTTIKYDFFEGGVLEAKC GD OPCVVVP AV APIVEMALVITDALMAQ						
	***	***	***	***	***	***	***
	410	420	430				
1R53A	KARDFS-----						
1UM0A	FSSDWLLEELKEAVAKMRDYTKNY-----						
1QX0B	FSSDWLLEELKEAVAKMRDYTKNY-----						
PbCS	NARETAKNLLPFLPG-----						

Figure S1. Alignment of the amino acid sequences from templates with *PbCS*. The green square highlight residues that compose the 5-EPSP site in *P. brasiliensis*. Residues that do not have correspondence in the templates are marked in yellow. Conserved residues are represented by an asterisk and the ligands are represented by the points at the end of alignment.

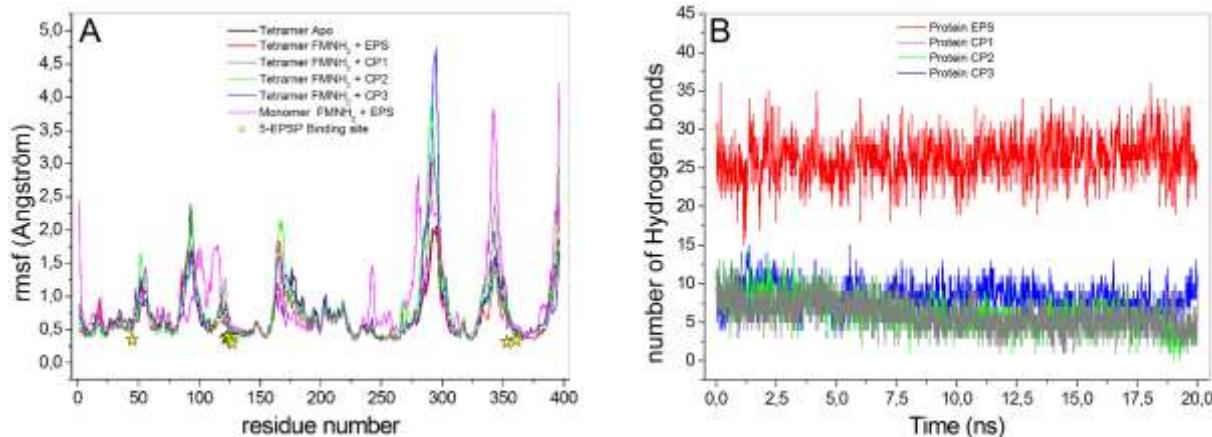


Figure S2. A) Root-mean-square fluctuation (rmsf) of C_α atoms from *PbCS* residues. The yellow stars mark the position of the active site residues described in **Table S1**. B) Average number of hydrogen bonds formed between the protein and ligand along the simulation. *PbrasiliensisCS* structures: The A chain bonded to FMNH₂ + 5-EPSP (**pink**), tetramer apo (**black**), tetramer bonded to FMNH₂ + 5-EPSP (**red**), tetramer bonded to FMNH₂ + CP 1 (**gray**), tetramer bonded to FMNH₂ + CP2 (**green**), and tetramer bonded to FMNH₂ + CP3 (**blue**). The root-mean-square deviation (A) and radius of gyration (B) were obtained from the oscillation of the protein main chain atoms relative to the full minimized structure.

Table S1. Contact frequency up to 4 Å between *PbCS* and the evaluated ligands.

Residue	EPSP	CP1	CP2	CP3	Residue	EPSP	CP1	CP2	CP3
Ser-15	–	0.24	–	0.12	Tyr-110	–	0.23	–	–
Arg-45	0.99	0.67	0.73	0.80	Ala-118	–	0.08	–	–
Arg-46	0.21	–	0.29	0.01	Ser-119	–	0.56	–	0.35
Ser-50	–	0.19	–	–	Ser-120	–	0.59	0.17	0.22
Leu-52	–	0.20	0.10	–	Gly-121	0.55	0.23	0.32	0.20
Thr-53	0.32	0.58	0.46	0.26	Gly-122	0.78	0.34	0.74	0.83
Thr-54	0.42	0.43	0.46	0.45	Gly-123	0.97	0.67	0.99	0.90
Pro-55	0.45	0.31	0.26	0.23	Arg-124	0.99	1.00	0.60	0.53
Asn-57	–	–	0.22	0.24	Ser-125	0.52	0.76	–	–
Glu-58	–	0.09	–	0.10	Ser-126	0.97	0.99	0.65	0.49
Asn-84	0.01	0.04	0.11	0.27	Ala-127	0.73	0.61	0.43	0.41
Asp-86	0.22	0.03	0.09	0.38	Thr-128	0.12	–	–	0.24
Gln-87	–	–	0.19	0.28	Thr-130	–	0.36	0.50	0.32
Asp-91	–	–	–	0.10	Ile-131	0.09	0.06	0.02	0.24
Tyr-92	0.10	–	0.05	–	Pro-258	–	0.15	–	–
Gly-93	–	–	–	0.12	Ala-330	–	–	0.06	0.39
Gly-94	–	–	–	0.40	Thr-331	0.08	0.19	0.44	0.48
Ser-95	–	0.20	0.11	0.05	Lys-351	–	–	0.02	0.09
Thr-96	–	–	–	0.06	Gly-352	0.31	0.26	0.28	0.48
Asp-98	–	0.24	–	–	Arg-353	1.00	0.97	0.95	0.99
Arg-102	0.25	0.37	–	0.88	His-354	–	0.27	0.59	0.07
Ser-104	–	–	0.24	–	Asp-355	0.04	0.55	0.52	0.21
His-105	0.25	0.7	0.69	0.64	Val-358	–	–	–	0.05
Ala-106	–	0.27	0.28	–	Arg-361	0.93	0.72	0.95	1.00
Asp-107	–	0.24	–	0.36	FMNH₂	1.00	1.00	1.00	1.00

Obs: only contacts with significant frequently (p <0.05) are presented plementary Table Y.