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SUPPORTING INFORMATION

Domain-swap dimerization of *Acanthamoeba castellanii* CYP51 and a unique mechanism of inactivation by isavuconazole

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Data S1 - Recombinant AcCYP51 amino acid sequence

MAKKTSSKGKLPPVVSSLIPFVGSGLSFAGGPLQYTTDAYKKYGDIFTMKVFGQRLTFLVGPDAHVPFFSQGDAELSQDEPYQ
FSVPIFGPNVVYGADLAHRNQQLKFIAASLSTKALQSYVPLIVKEAEDFFAKWDKSGTVDIRDALAELIILTASRCLMGKEIR
ENLFTEVAKLYQTLDEGLLPISVFFPYLPIPAHKRRDEARLAMVRMFKKIIDERRANPEVKHNDCLQVFMDARYRGEEQALND
EEITGLMIALLFAGQHTSSVTGSWTGLLLFEANNKKKFLPGVLEEQEEIRKEFGDELTMEALNKMDKLHRCVKEALRMYPPLL
FVMRKVIKPFSYKDYYVPEGDTVFVSPALSMRVEEVFPNADQYNPERFVEEDKQAQKYRFVGFGAGRHGCMGENFAYLQIKTI
WSVLLRNFDIELVGELPKPDYTAMVVGPAHPCLLRYTRKHHHHHH

Codon-optimized DNA sequence:

ATGGCCAAAAAGACGTCTTCAAAGGGTAAATTACCACCGGTTGTGAGCAGTTTGATTCCATTTGTGGGTAGCGGCCTGAGTTT
CGCAGGTGGTCCGTTGCAATACACCACTGATGCATACAAAAAGTACGGCGACATCTTTACGATGAAAGTTTTCGGCCAAAGAT
TAACCTTTTTGGTCGGTCCAGATGCCCATGTACCGTTTTTCTCTCAGGGCGATGCGGAACTGTCACAAGACGAACCTTATCAG
TTTTCCGTTCCGATTTTCGGCCCTAATGTCGTATACGGTGCTGATTTGGCACACCGCAACCAACAGCTGAAGTTTATTGCTGC
ATCTCTGTCAACTAAAGCGTTACAATCGTACGTCCCACTGATCGTAAAAGAAGCCGAAGATTTCTTTGCGAAGTGGGACAAAA
GCGGCACTGTGGATATTCGTGACGCCCTGGCGGAATTAATTATCTTGACAGCCAGTCGTTGTTTGATGGGCAAGGAAATCAGA
GAAAACCTGTTTACCGAAGTTGCGAAACTGTATCAGACATTAGATGAAGGTCTGTTGCCTATTTCTGTATTTTTCCCATACTT
ACCGATCCCTGCTCATAAACGTAGAGATGAAGCCCGCCTGGCGATGGTTCGTATGTTCAAAAAGATTATCGATGAACGCCGTG
CAAATCCGGAAGTTAAACACAACGATTGCCTGCAAGTGTTCATGGACGCCCGTTATCGTGGTGAAGAACAGGCGTTAAATGAT
GAAGAAATTACAGGCTTGATGATCGCTTTGCTGTTTGCAGGTCAACATACATCCTCGGTTACGGGCAGTTGGACCGGTTTGTT
GCTGTTCGAAGCTAACAACAAAAAGAAATTCTTGCCAGGCGTGCTGGAAGAACAGGAAGAAATTCGTAAAGAATTTGGCGATG
AATTGACTATGGAAGCTCTGAACAAGATGGACAAACTGCATAGATGTGTCAAAGAAGCATTACGCATGTATCCTCCATTATTG
TTCGTTATGAGAAAAGTTATTAAACCATTCTCTTACAAAGATTATTACGTTCCGGAAGGTGACACAGTCTTTGTATCCCCTGC
CCTGTCGATGAGAGTTGAAGAAGTGTTTCCGAATGCGGATCAATATAACCCTGAACGCTTCGTTGAAGAAGACAAGCAAGCTC
AGAAATACCGCTTTGTGGGTTTCGGCGCAGGTCGTCATGGCTGTATGGGTGAAAATTTTGCTTATCTGCAGATTAAGACCATC
TGGTCTGTCCTGTTACGCAACTTCGATATTGAATTAGTAGGCGAATTGCCAAAACCGGACTATACGGCTATGGTTGTGGGTCC
TGCACACCCATGCTTGCTGCGTTACACCAGAAAACATCACCATCACCATCACTAA

Data S1 Legend. Recombinant AcCYP51. The codon-optimized cDNA sequence of AcCYP51 (NCBI Reference: XP_004334294.1) was generated synthetically (GenScript, Piscataway, New Jersey). The 42 N-terminal residues were replaced with the MAKKTSSKGK lead sequence and the 6xHis tag is added at the C-terminus. The synthetic DNA construct was cloned between the NdeI and HindIII restriction sites into the pCW-LIC expression vector obtained from the non-profit plasmid repository (Addgene, Cambridge, MA). Engineered sequences are shaded in cyan and region involved in the domain swap interactions is underlined.

Data S2 - AcCYP51 expression and purification protocols.

Expression: Fresh transformants containing the pCW-AcCYP51 vector were obtained after transforming ~ 50 ng of vector DNA into HMS 174 *E.coli* cells pre-transformed with pGro7 plasmid (Takara) carrying GroEL/ES chaperones. After overnight incubation at 37 °C on an LB agar plate supplemented with 50 μg/ml ampicillin and 20 μg/ml chloramphenicol, a selected single colony was used to set up a primary culture in LB media supplemented with 50 μg/ml ampicillin and 20 μg/ml chloramphenicol. Primary culture was incubated overnight at 37 °C, 250 rpm.

10 ml of the primary culture were used to set up secondary culture in 1 liter of Terrific Broth media supplemented with 1 mM Thiamine, 50 μg/ml ampicillin, 20 μg/ml chloramphenicol, 1X rare salt solution (**Table S1**). After inoculation, the culture was incubated for 5 h at 37 °C with constant stirring at 200 rpm. When OD_{600} reached approximately 0.4-0.5 a.u., the incubation temperature was reduced to 25 °C and stirring was reduced to 145 rpm. Growth continued until OD_{600} reached 0.6-0.8 a.u., then AcCYP51 expression was induced with 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Simultaneously, arabinose was added to 0.5 mg/ml to induce chaperone expression. Finally, the media was also supplemented with a heme precursor - 1 mM delta-aminolevulinic acid - and incubated for 45-48 h at 25 °C, 145 rpm.

After incubation, the culture was harvested at 6000 g, 4 °C for 15 min and the cell pellet was suspended evenly in 10-15 ml of lysis buffer (**Table S2**) and 25 μl of lysozyme (from 50 mg/ml stock) was added per liter of culture. Cell suspension was stored at -80 °C.

Purification: Cells were disrupted using a fluid processor Microfluidics M-110P (Microfluidics Inc.) and cell debris were removed by centrifuging at 4 °C, for 30 min. at 15000 rpm.

The supernatant thus obtained was loaded onto a pre-equilibrated Ni-NTA column. Following loading, the column was washed with 10 column volumes (CV) of wash buffer I (refer **Table S3** for buffer compositions) followed by 10 CV of wash buffer II. Finally, column-bound His-tagged protein was eluted under a linear gradient of 0-100 mM L-histidine (Sigma).

Fractions containing AcCYP51 were pooled together and loaded onto a pre-equilibrated Q-Sepharose column. Protein was collected in the flow-through and re-loaded onto a pre-equilibrated hydroxyapatite (HAP) column. Protein bound to HAP column was eluted under a linear gradient of 0.05 – 1.0 M K-phosphate buffer, pH 8.0, supplemented with 10% glycerol and 0.5 mM EDTA. Protein fractions containing AcCYP51 were pooled and concentrated to 2-3 mg/ml. Concentrated AcCYP51 was then injected onto a pre-equilibrated Superdex 200 XK 26 size-exclusion chromatography (SEC) column and the AcCYP51 fractions corresponding to monomer and dimer populations were eluted and pooled separately. Each protein pool was concentrated to ~ 1 mM and stored at -80 °C.

Table S1: Composition of 4000X rare salt solution

$FeCl_3.6H_2O$	2.7 g
$ZnCl_2.4H_2O$	0.2 g
$CoCl_2.6H_2O$	0.2 g
$Na_2MoO_4.\ 2H_2O$	0.2 g
$CaCl_2.2H_2O$	0.1 g
$CuSO_4.5\ H_2O$	0.186 g
H_3BO_3	0.05 g
Add HCl until salts dissolve	
Deionized water	Make up to 100 ml

Table S2: Composition of lysis buffer

Reagents	Working concentration
Glycerol	5%
K-Phosphate, pH-8.0	50 mM
EDTA	0.5 mM
NaCl	300 mM
PMSF	0.5 mM
DTT	1 mM

Table S3: Composition of purification buffers

Component	Reagent	Working concentration
Ni-NTA		
Equilibration buffer	K-phosphate, pH 8.0 NaCl Glycerol	50 mM 300 mM 10 %
Wash buffer I	Same as equilibration buffer	
Wash buffer II	K-phosphate, pH 8.0 Glycerol	50 mM 10 %
Elution buffer	K-phosphate, pH 8.0 Glycerol L-Histidine	50 mM 10 % 0-100 mM
Q-Sepharose		
Equilibration and running buffer	K-phosphate, pH 8.0 Glycerol	50 mM 10 %
HAP		
Equilibration buffer	K-Phosphate, pH 8.0 Glycerol	50 mM 10%
Elution buffer	K-Phosphate, pH 8.0 Glycerol EDTA	0.05 – 1 M 10% 0.5 mM
Superdex 200 XK 26		
Equilibration and running buffer	K-Phosphate, pH 8.0 Glycerol	50 mM 5%