

Engineering a pseudo-26-kDa *Schistosoma* glutathione transferase from *bovis/haematobium* for structure, kinetics and ligandin studies

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Supplementary Section

CLUSTAL O(1.2.4) multiple sequence alignment

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ENA|RTG84353|RTG84353.1      CTTGTACAACCGACTCGTCTTCTTTTGGAAATATGTTGGCGAAGTATATGAAGAGCGTTTG      60
ENA|KAF1314627|KAF1314627.1  CTTGTACAACCGACTCGTCTTCTTTTGGAAATATGTTGGCGAAGTATATGAAGAGCGTTTG      60
*****

ENA|RTG84353|RTG84353.1      TATGATCGCAATGATGGTGATGTCTGGCGAAACGAAAAGTTCAATTTAGGTCTGGAATTT      120
ENA|KAF1314627|KAF1314627.1  TATGATCGCAATGATGGTGATGTCTGGCGAAACGAAAAGTTCAATTTAGGTCTGGAATTT      120
*****

ENA|RTG84353|RTG84353.1      CCTAATCTACCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCTATTTTA      180
ENA|KAF1314627|KAF1314627.1  CCTAATCTACCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCTATTTTA      180
*****

ENA|RTG84353|RTG84353.1      CGTTATATAGCTGATAAACACAATATGTTGGGTGGTTGTCCCAAAGAACGTGCAGAAATT      240
ENA|KAF1314627|KAF1314627.1  CGTTATATAGCTGATAAACACAATATGTTGGGTGGTTGTCCCAAAGAACGTGCAGAAATT      240
*****

ENA|RTG84353|RTG84353.1      TCTATGCTTGAGGGAGCCATTTTGGATATCAGACTTGGTGTTTCAAGAATTGCATATAAT      300
ENA|KAF1314627|KAF1314627.1  TCTATGCTTGAGGGAGCCGTTTGGATATCAGACTTGGTGTTTCAAGAATTGCATATAAT      300
*****

ENA|RTG84353|RTG84353.1      AAAGAATTTGAAACTCTCAAAGTTGGTTTTCTCAATCAACTCCCTGGAATGCTGAAAATG      360
ENA|KAF1314627|KAF1314627.1  AAAGAATTTGAAACTCTCAAAGTTGGTTTTCTCAACCAACTCCCTGGAATGCTGAAAATG      360
*****

ENA|RTG84353|RTG84353.1      TTTGAAAATCGTTTATCTCACAAAATATATTTGAATGGTGATAATGTGACTCATGTTGAC      420
ENA|KAF1314627|KAF1314627.1  TTTGAAAATCGTTTATCTCAC-----                        381
*****

ENA|RTG84353|RTG84353.1      TTCATGCTGTACGACGCTCTTGATGTAGTTTTATACATGGACCCAAAGTGCTTGGATGCA      480
ENA|KAF1314627|KAF1314627.1  -----                        381

ENA|RTG84353|RTG84353.1      TTTCCAAAACATAATTAGTTTCAAACAGCGTATAGAAAATTTACCACCAATCAAGAACTAC      540
ENA|KAF1314627|KAF1314627.1  -----                        381

ENA|RTG84353|RTG84353.1      CTGAATTCTGACAGGCACATAAAATGGCCTCTGCAAGGCTGGTCCGCCATTTTGGTGGT      600
ENA|KAF1314627|KAF1314627.1  -----                        381

ENA|RTG84353|RTG84353.1      GGAGATGCTCCTCCAAAATAA      621
ENA|KAF1314627|KAF1314627.1  -----                        381

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Figure S1: The Clustal Omega multiple sequence alignment data showing 99.9% sequence conservation between the gene fragment encoding putative 26-kDa GST from *S. haematobium* (EBI ID KAF1314627) and *S. bovis* (EBI ID RTG84353).

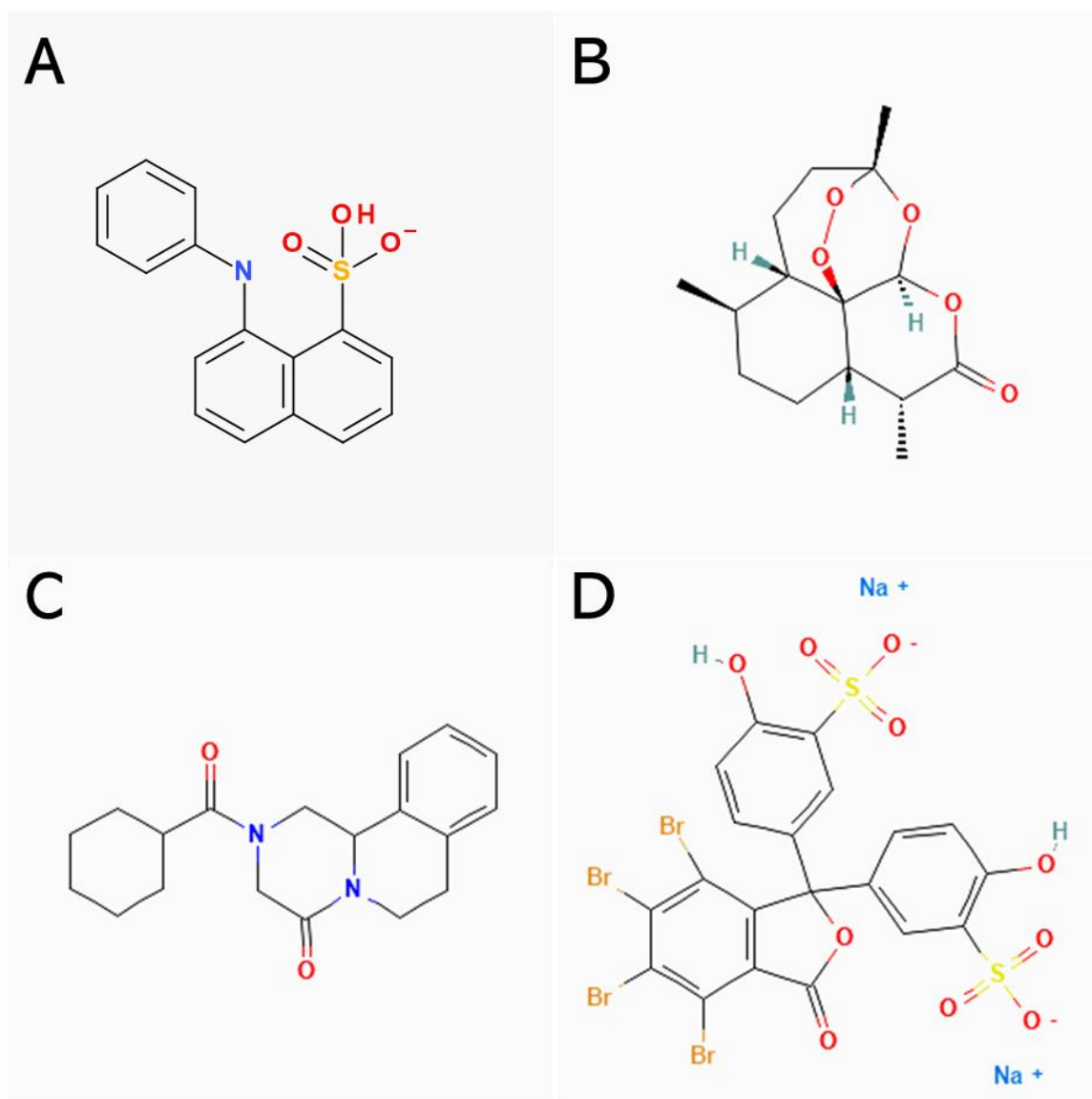


Figure S2: 2D illustration of the structures of the four ligands used in the study. *A.* ANS, *B.* Artemisinin, *C.* Praziquantel and *D.* Bromosulphthalein.

	1	2	3	4	5	6	7	8	9	10	11	12	
A													1mM GSH
B													
C													
D													
E													No GSH
F													
G													
H													
	No ligand				BSP				PZQ				

Figure S3: The 96-well plate plan used for the fluorescence-based thermal shift assay on the effect of PZQ and BSP on the stability of the pseudo Sbh26GST.

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T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=100
*
  BAD  AVG  GOOD
*
Sj26GST.      : 100
Sbh26GST.     : 100
cons          : 10

Sj26GST.      MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSM
Sbh26GST.     MAPILGYWKIKGLVQPTRLLLEYGVEVYEERLYDRNDGDVWRNEKFNLGLEFPNLPYYIDGDVKLTQSM
cons          *:*****: * ***,*:*,*: ***,*:*****

Sj26GST.      AIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLC
Sbh26GST.     AILRYIADKHNMLGGCPKERAISMLEGAILDIRLGVSRIAYNKEFETLKVGFLNQLPGMLKMFENRLS
cons          **:*****:*** ***,*:*****:*,*:** ***,*:

Sj26GST.      HKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQ
Sbh26GST.     HKIYLNGDNVTHVDFMLYDALDVVLYMDPKCLDAFPKLISFKQRIENLPPIKNYLNSDRHIKWPLQGWS
cons          ** ***,*** ***** *****:*,*:** *:*,*:*,*:*,*: ***,

Sj26GST.      ATFGGGDHPPK
Sbh26GST.     AIFGGGDAPPK
cons          * *****

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Figure S4. Computation of a 3-dimensional structural alignment between pseudo Sbh26GST and Sj26GST using the Espresso Alignment algorithm.

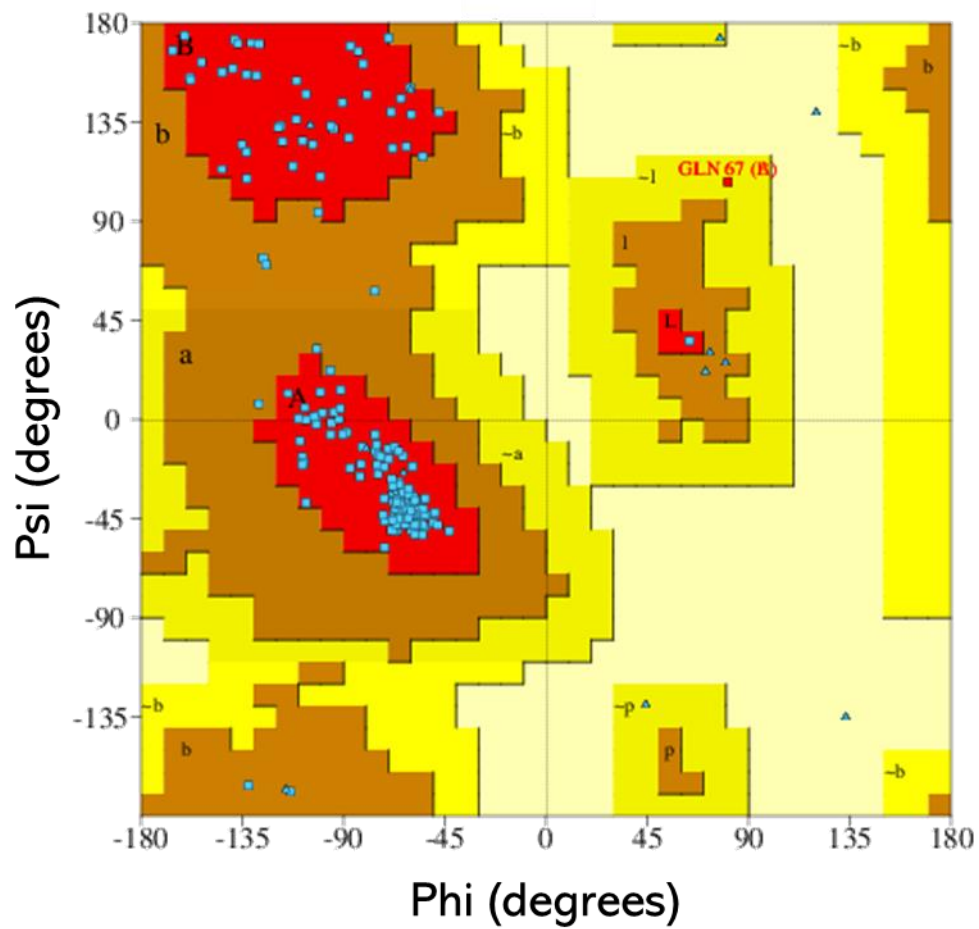


Figure S5. Ramachandran plot analysis of Sbh26GST. Here, the red region indicates favored region, yellow region for allowed and light-yellow shows generously allowed region and white for disallowed region. Phi and Psi angles define torsion angles in a figure. This analysis was done using the ProCheck algorithm [1, 2].

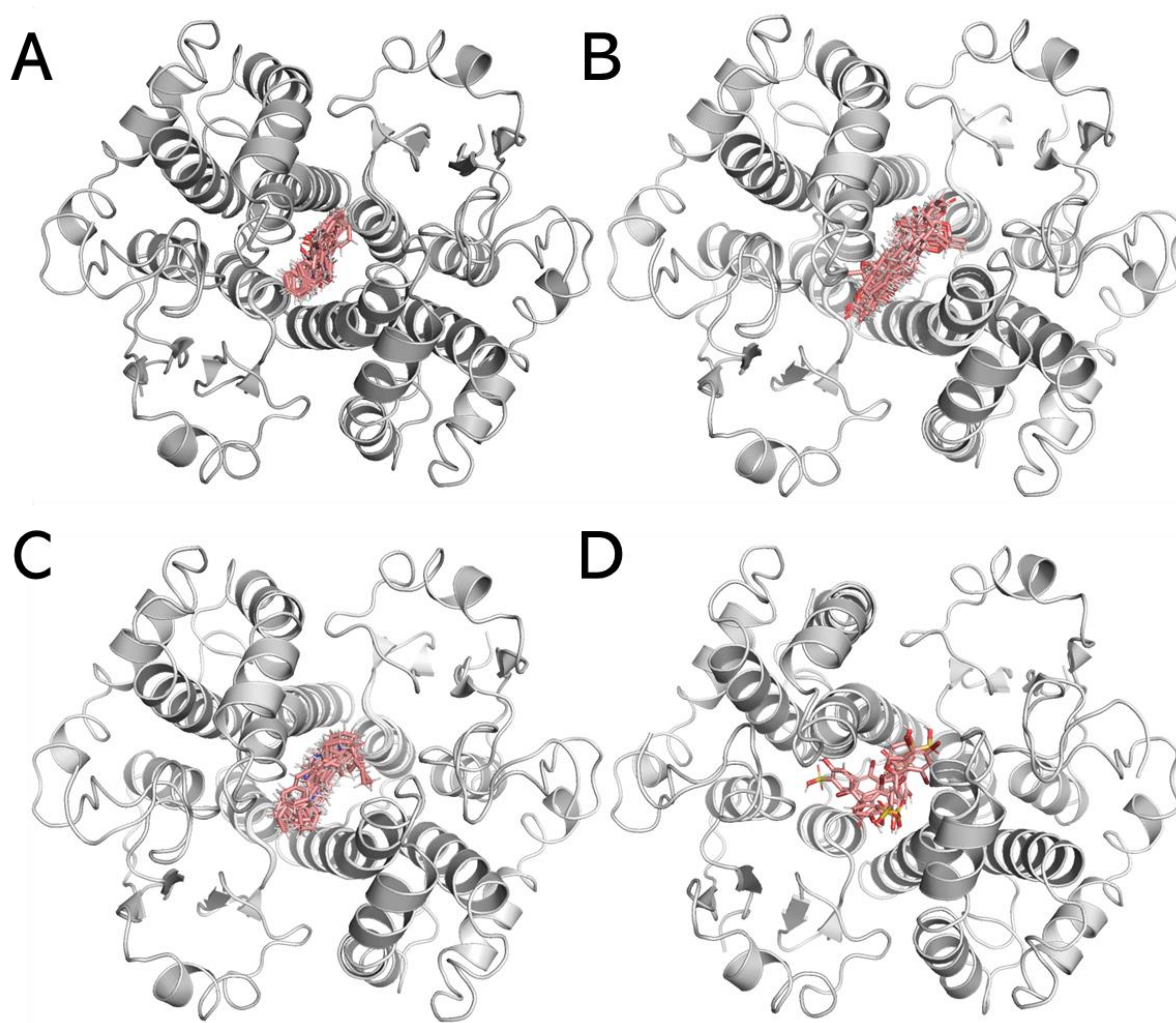


Figure S6. Ribbon representation of the 3D homology model of Sbh26GST indicating the preferred binding site for ANS (*A*), ART (*B*), PZQ (*C*) and BSP (*D*) based on the induced fit ligand docking implemented in Schrödinger Maestro v12.0. All the poses derived indicate that the ligands (in pink stick representation) preferred to bind at the dimer interface. The image was rendered using PyMol [3].

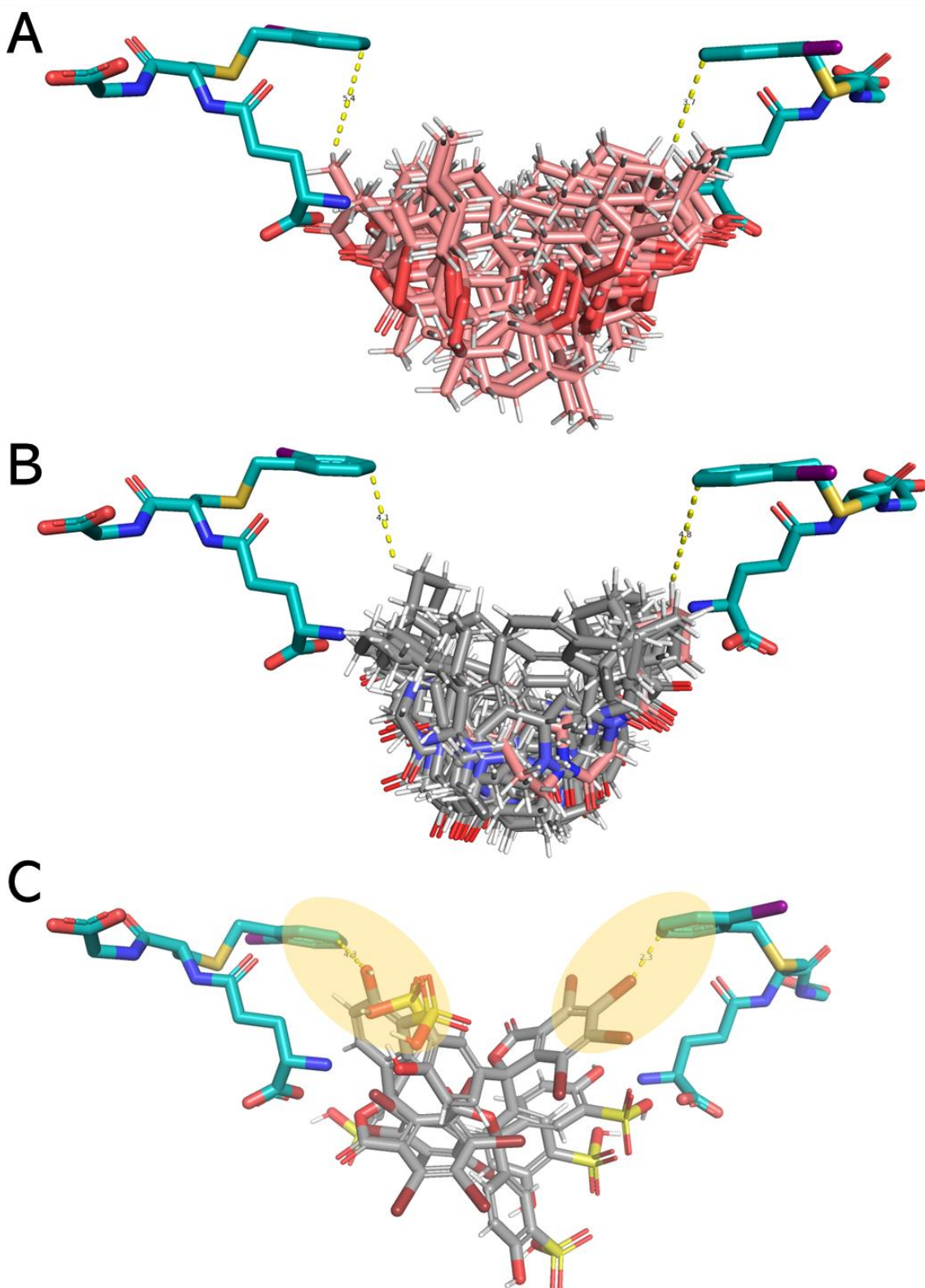


Figure S7. Stick representation of the proximity between the ligands bond within the dimer interface and the active sites occupied by the glutathione moiety in the G-site and the 2-iodobenzyl moiety occupying the H-site. *A.* ART, *B.* PZQ and *C.* BSP. The image was generated using PyMol.

References

1. Laskowski, R.A.; MacArthur, M.W.; Moss, D.S.; Thornton, J.M. PROCHECK: a program to check the stereochemical quality of protein structures. *J Appl Crystallogr* **1993**, *26*, 283-91.
2. Laskowski, R.A.; Rullmann, J.A.C.; MacArthur, M.W.; Kaptein, R.; Thornton, J.M. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J Biomol NMR* **1996**, *8*, 477-86.
3. DeLano, W.L. Pymol: An open-source molecular graphics tool. *CCP4 Newsletter on protein crystallography* **2002**, *40*, 82-92.