

# 12. Scientific Activities under HRD

1. Molecular modeling of Glutathione S-transferase of the filarial parasite *Wuchereria bancrofti* - a target for drug development against adult worm Trainee: S. T. Nathan; Supervisor: Nisha Mathew; Project Duration: 6 months, Dec 2003 – Jun 2004)

The current approach in anti-parasitic drug discovery process involves the identification of novel targets from databases on the parasite genome and metabolic pathways. Glutathione S-transferase enzymes (GST enzymes) are a family of detoxification enzymes that catalyze the conjugation of glutathione (GSH) with various endogenous and xenobiotic electrophiles. GSTs have been considered as good targets for anti-parasitic drug development. At the VCRC, Pondicherry, a model 3D-structure of the enzyme of the *W. bancrofti*, viz., *wbGST*, has been generated by utilizing information on the amino acid sequences and applying automated comparative protein modelling and bioinformatics tools.

The results of the protein-protein Basic Local Alignment Search Tool (BLAST) search for suitable template structure related to the target sequence (*wbGST*) showed porcine  $\pi$ -class GST 2gsr-A chain with 42% sequence similarity as the most suitable template for modelling. ClustalW pairwise alignment results of *wbGST* and 2gsr-A sequences showed 42% sequence similarity and 0.005% gap frequency with the porcine  $\pi$ -class GST (2gsr-A) sequence as shown in fig.1.

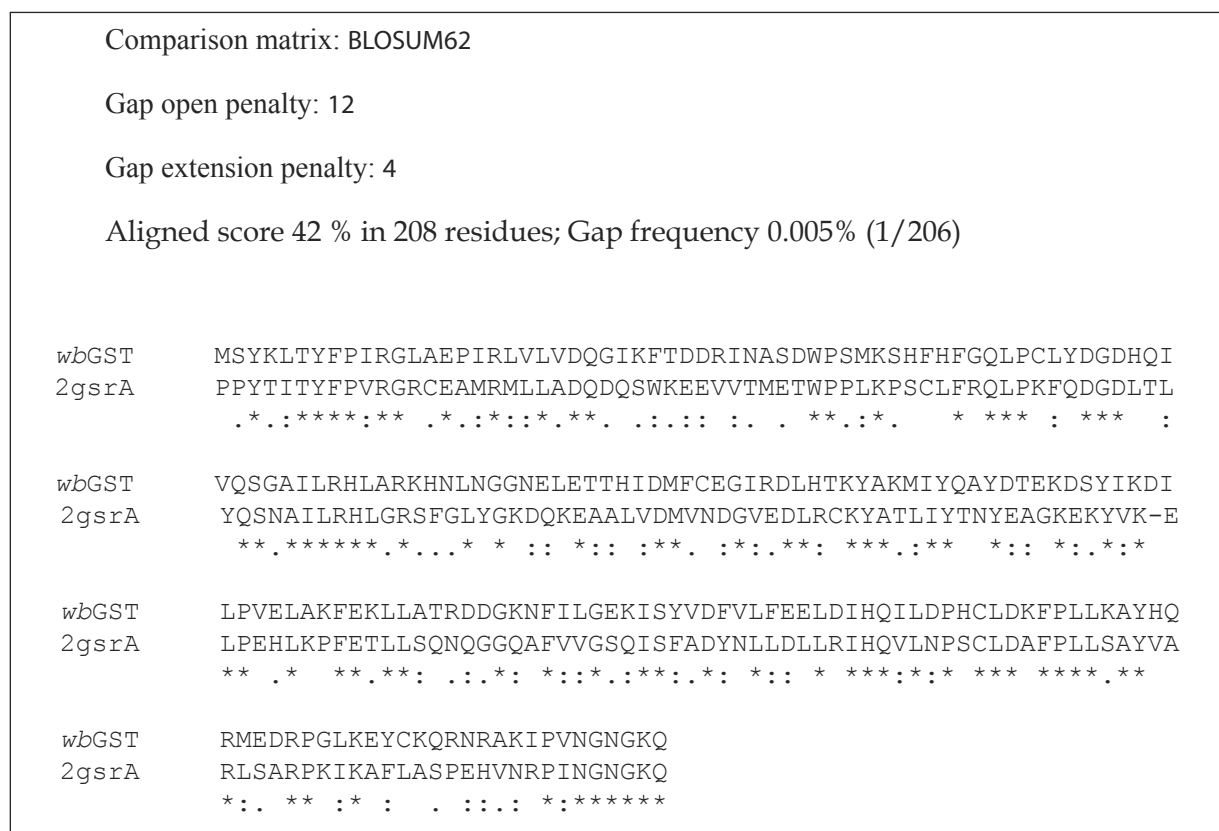
The model of *wbGST* built by the MODELLER6v2 was analyzed by the PROCHECK programmes. Ramachandran plot analysis (fig.2) showed that 93.5% of the residues are in core region, followed by 5.4% and 1.1% residues in the allowed and generously allowed regions respectively. None of the non-glycine residues is in disallowed regions. The PROSA II z-score and the energy graph (fig.2) for the final model further confirmed the quality of the modelled structure. It can be seen that in the modelled structure 1SFM, except for a few residues showing slightly positive

interaction energies, all other residues show negative interaction energies. The z-scores of pair, surface and combined energy were  $-7.47$ ,  $-5.00$  and  $-5.33$  for *wbGST* while for the template 2gsrA the values were  $-10.03$ ,  $-7.68$  and  $-6.28$  respectively. The comparable z-score values and interaction energies further confirm the quality of the modelled structure. The modelled 3D structure 1SFM of *wbGST* is given in fig. 4 as Hex view. The overall topology of the *wbGST* model consists of four-stranded  $\beta$ -sheet and eight- $\alpha$ -helices arranged in a smaller N-terminal domain I ( $\alpha_1$ - $\alpha_3$ ,  $\beta_1$ - $\beta_4$ ) and a larger C-terminal domain II ( $\alpha_4$ - $\alpha_8$ ). The N-terminal domain I is an  $\alpha/\beta$  structure built up of the four-stranded  $\beta$ -sheet and three  $\alpha$ -helices showing a  $\beta\alpha\beta\alpha\beta\alpha$  folding pattern. Domain II is composed of five  $\alpha$ -helices with all  $\alpha$  helical fold.

The 1SFM has been used for docking with GST inhibitors by Hex4.2 macromolecular docking using spherical polar Fourier correlations. Total number of solutions observed in docking 1SFM as receptor and known GST inhibitors ethacrynic acid, curcumine, plumbagin, GSH analogue, Terrapin-199 and the substrate CDNB as ligands were 53, 108, 25, 113, 134 and 53 respectively. From the results, the primary ligand-binding site was found to be tyrosine 116 for all the five inhibitors whereas the substrate CDNB was binding at tyrosine 106. The binding energies varied from  $-116.1$  to  $-196.5$  for the compounds studied, corresponding to their first docking solutions. Among the compounds studied the GSH analogue showed maximum affinity towards *wbGST* as it exhibited the least binding energy. Figure 5 illustrates the key-binding site (tyrosine 116) for the GSH analogue in *wbGST*.

The docking results of the present study showed that in 1SFM, the site near tyrosine residue at 116 of *wbGST* is the primary site of ligand binding for ethacrynic acid, curcumine, plumbagin and Terrapin-199 while the substrate CDNB was binding near the tyrosine residue at 106. Both these residues are located at the top of the  $\alpha_4$  helix in domain II of the

Fig.1. ClustalW pairwise alignment of *wbGST* and *2gsrA* sequences using user-defined parameters of gap open and extension penalties with BLOSUM62 matrix. The highly aligned regions (\*) are showed in blue color.



*wbGST*. In the case of CDNB the tyrosine (residue at 106) assisted binding by a nucleophilic substitution reaction and for other molecules a Michael type of addition assisted by another tyrosine (residue at 116) might have taken place.

The three-dimensional (3D) structure of *wbGST* has been submitted to the Protein Data Bank (PDB) (PDB ID: 1SFM and RCSB ID: RCSB021668). The modelled 3D structure of *wbGST*, 1SFM may be used for the design and development of specific inhibitors leading to macrofilaricidal molecules.

Fig.2. Ramachandran plot of phi/psi distribution of wbGST model 1SFM produced by PROCHECK

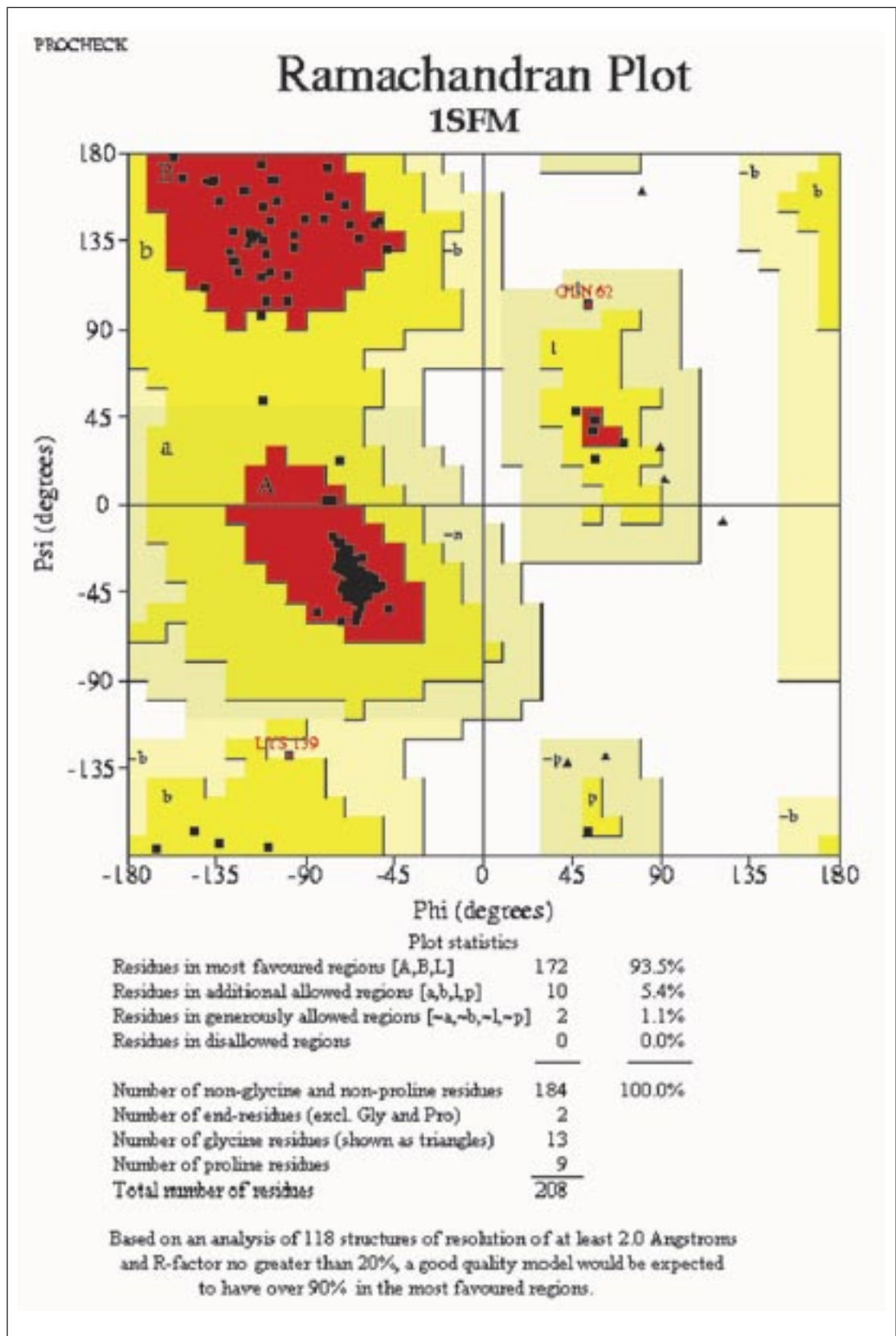
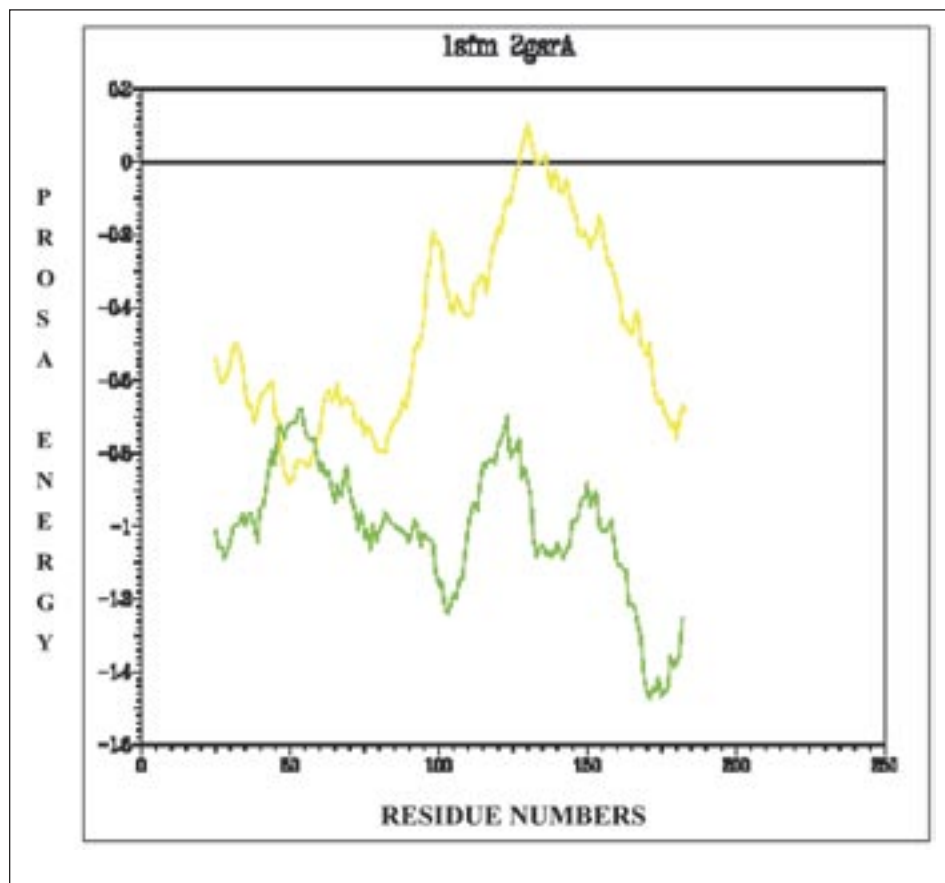


Fig.3. PROSA energy profiles calculated for the refined model 1sfm and its template 2gsr A



In X-axis: Residue numbers (50/cm), Y-axis: Prosa energy (0.2/cm).  
Yellow lines-Target 1sfm (wbGST); Green lines- Template 2gsrA.

Fig.4. Computationally modelled 3D Structure of wbGST submitted to Brookhaven Protein data bank (PDB ID: 1sfm) under theoretical models category. (Helices are Red, strands are Yellow, coils are Blue, Turns are Green and 310 Helices are Brown. The ribbon structure shown in the representation is HEX4.2 view.)

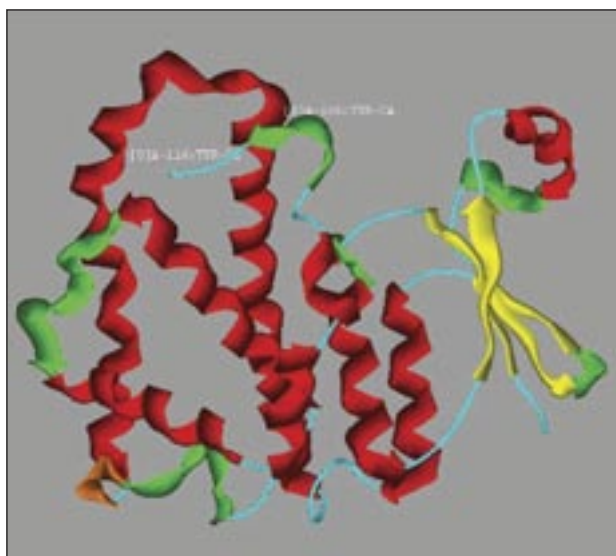


Fig. 5. Primary binding site of GSH analogue in 1sfm obtained by Hex 4.2 docking.

