SKB 5814
UNDERGRADUATE PROJECT II

MOLECULAR MODELING OF CHITINASE FROM *Trichoderma virens*

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Objective of research

- *To predict* tertiary model of chitinase

SCOPE :

- ***Aimed*** to have sequence alignment and do comparison on several sequence.
- ***To predict*** the secondary structure
- ***To develop*** homology modeling of *Trichoderma virens*.

What is chitinase?

- An enzyme that will hydrolyze chitin & produce soluble chito-oligosaccharides (Boer *et al*., 2004)
- Cleave bond between C1 & C4 two consecutive N-acetylglucosamine of chitin.
- Found in various organisms. Eg:bacteria, plant& fungi.

Cont'd…

- Whereas plants produce chitinases as part of their defence against fungal pathogens.
- Many bacteria n fungi contain this enzyme convert chitin into compounds that can serve as energy source.
- The roles are diverse depends on the organisms.

Chitin….

- Major component of most fungal cell walls.
- An insoluble β-1,4 linked polymer of N-acetylglucosamine (GlcNac)
- 2nd nature's most abundant polymer on the earth.

Methodology

Do BLAST to know % of similarity n identical

↓

Multiple sequence alignment to search conserved region

↓

Predict secondary structure n tertiary model using computer-modeling

BLAST result

- *Coccidiodes immitis* have the highest score which is **52.3% identical and 71.0% similarity** to our amino acid sequences.
- *Bacillus circulans* WL-12 gave score **35.1% identical and 52.7% similarity**
- *Serratia marcescens* gave **30.4 % identical and 44.9 % similarity** in amino acid sequence.

- All the models that have been predicted were submitted to this website to do quality evaluation

http://nihserver.mbi.ucla.edu/SAVS

~all the result were received within 5-10 min

~using ERRAT n VERIFY 3D program

Quality validation result

Template	Minimization	VERIFY 3D	ERRAT
C. immitis	**1**	**97.49 %**	**87.62 %**
	2	96.73 %	93.24 %
	3	**96.98 %**	**87.88 %**
	4	97.74 %	82.73 %
	5	98.74 %	83.98 %
	6	**96.73 %**	**90.18 %**
B. circulans WL-12	1	87.19 %	67.37 %
	2	87.19 %	66.32 %
	3	**85.68 %**	**86.58 %**
	4	**84.67 %**	**91.03 %**
	5	85.68 %	86.58 %
	6	**84.67 %**	**90.77 %**
S. marcescens	**1**	**81.16 %**	**64.06 %**
	2	81.41 %	63.28 %
	3	91.21 %	63.09 %
	4	**82.16 %**	**68.06 %**
	5	**85.93 %**	**66.93 %**
	6	76.88 %	71.62 %

Cont'd

~From the quality validation result, the best 3 were selected from each template.

~ every models were analyzed using Ramachandran plot.

From *C. Immitis* template

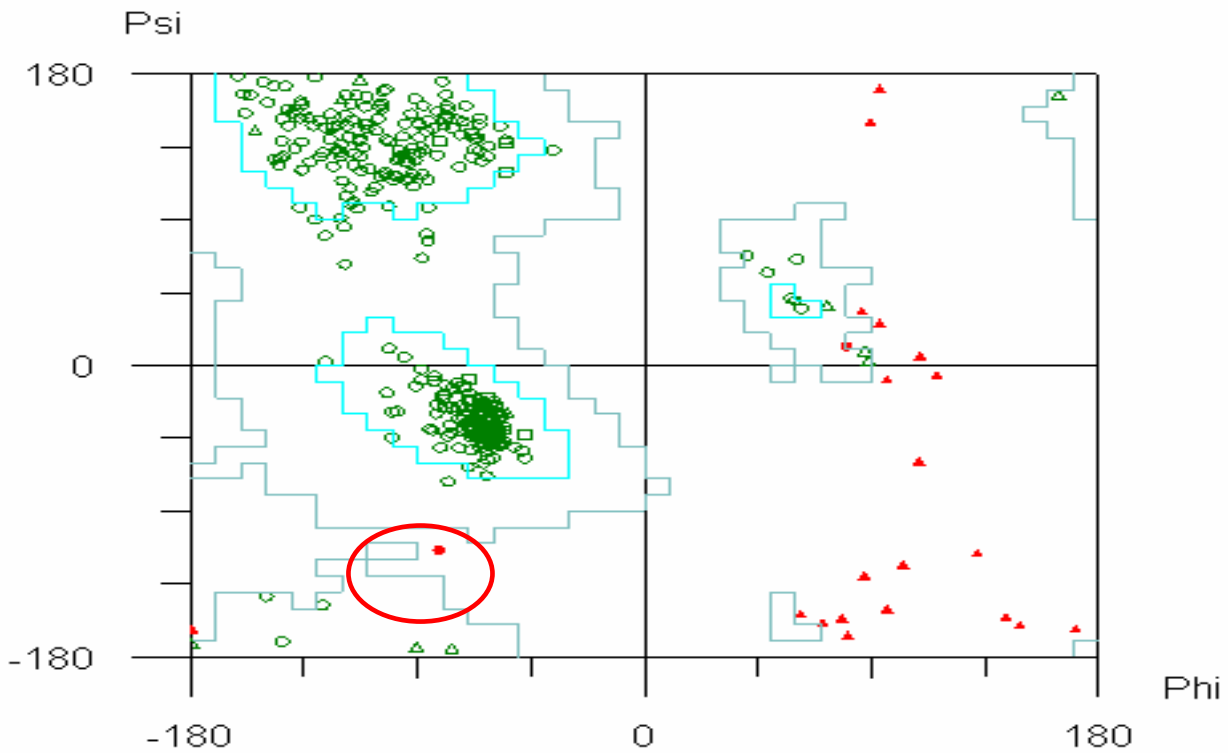


Figure 1: Ramachandran plot for Minimization 1 based on *C. immitis* template.

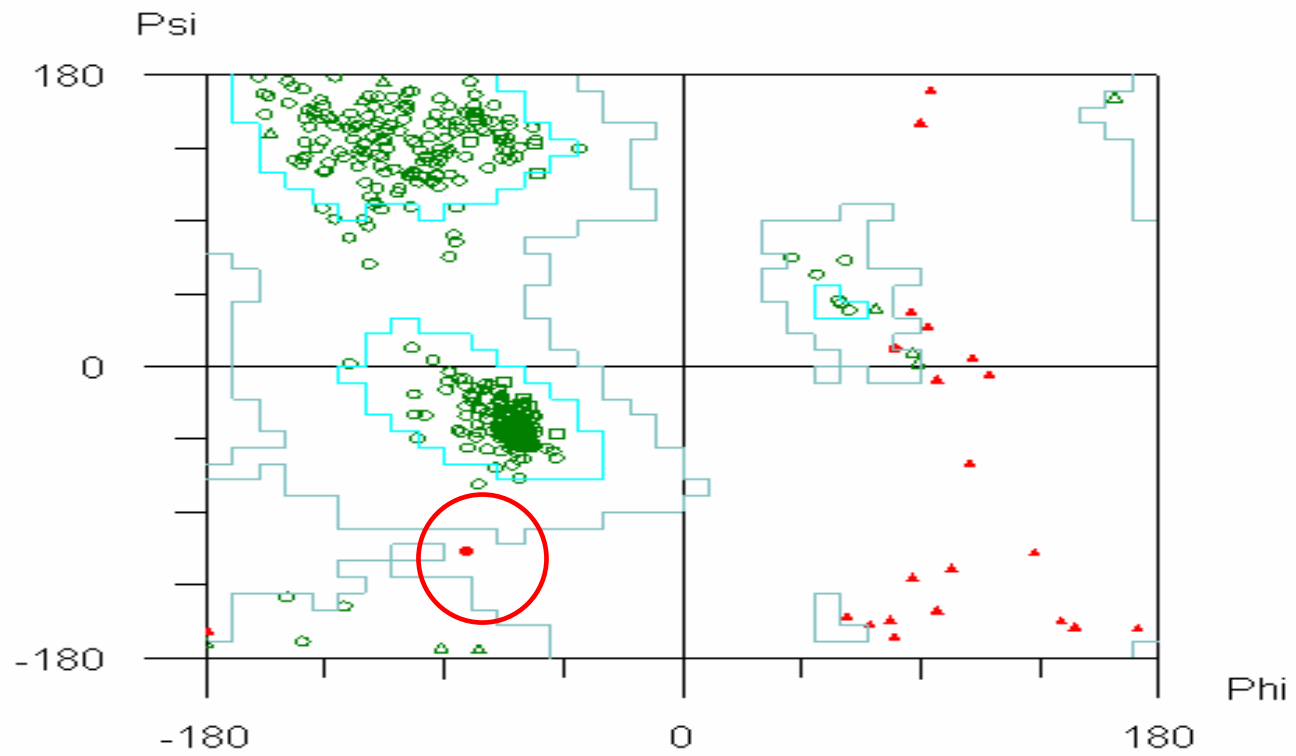


Figure 2: Ramachandran plot for Minimization 3 based on *C. immitis* template.

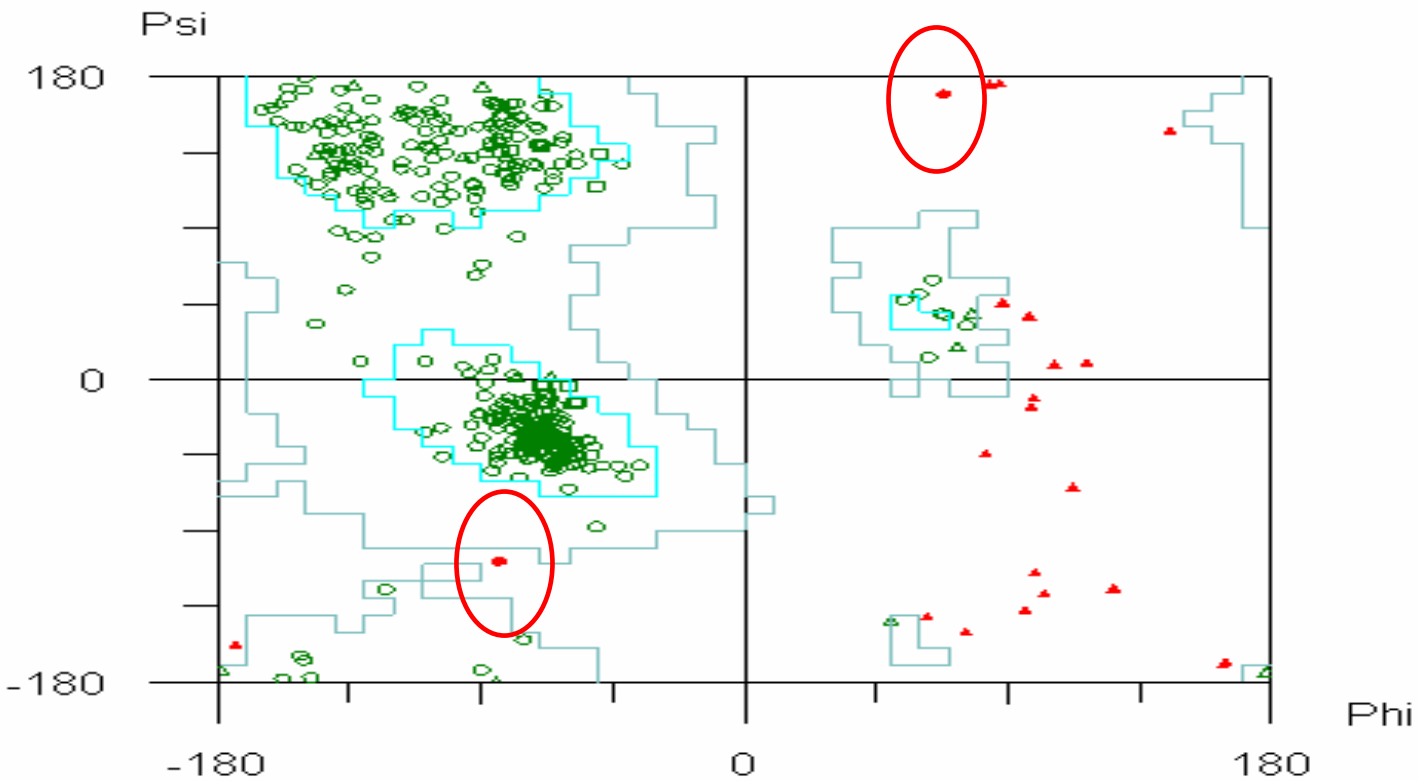


Figure 3: Ramachandran plot for Minimization 6 based on *C. immitis* template.

B. Circulans template

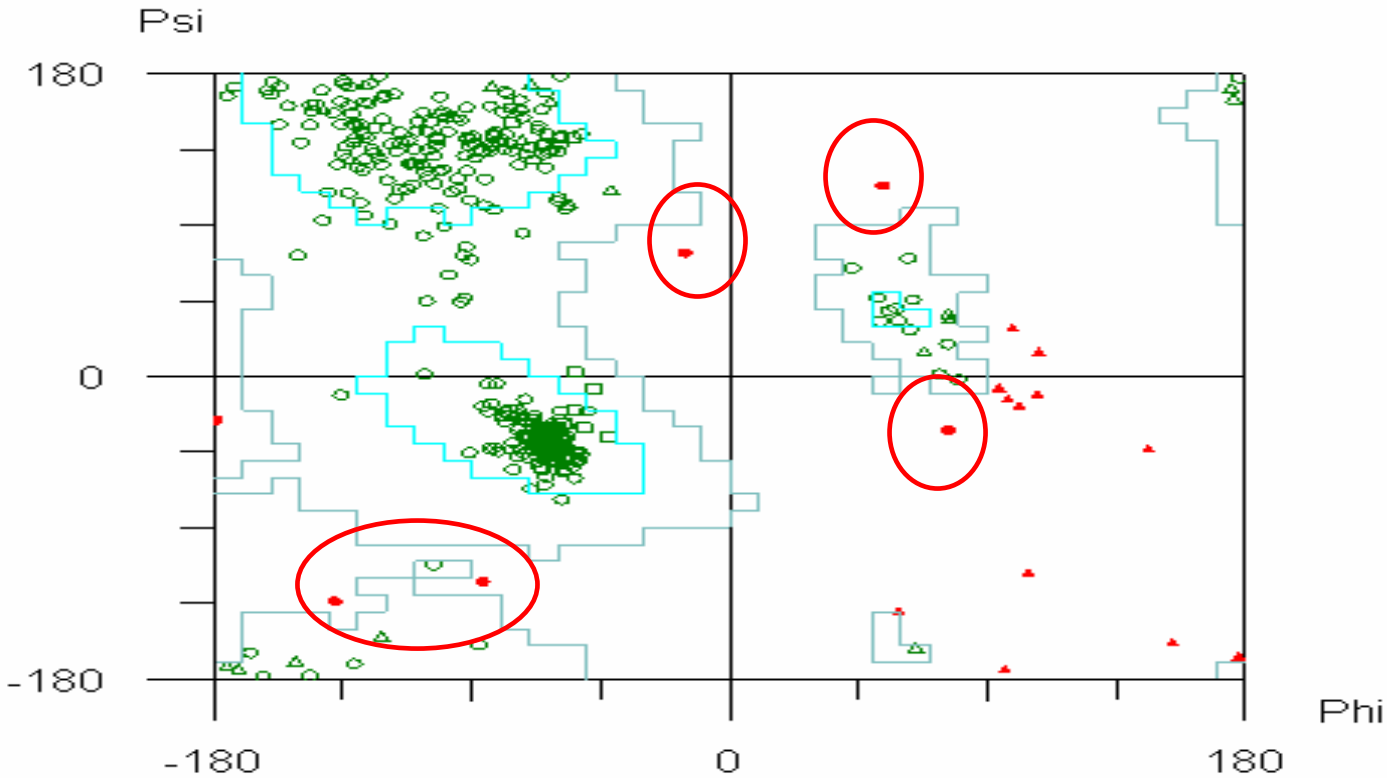


Figure 4: Ramachandran plot for Minimization 3 based on *B. circulans* template.

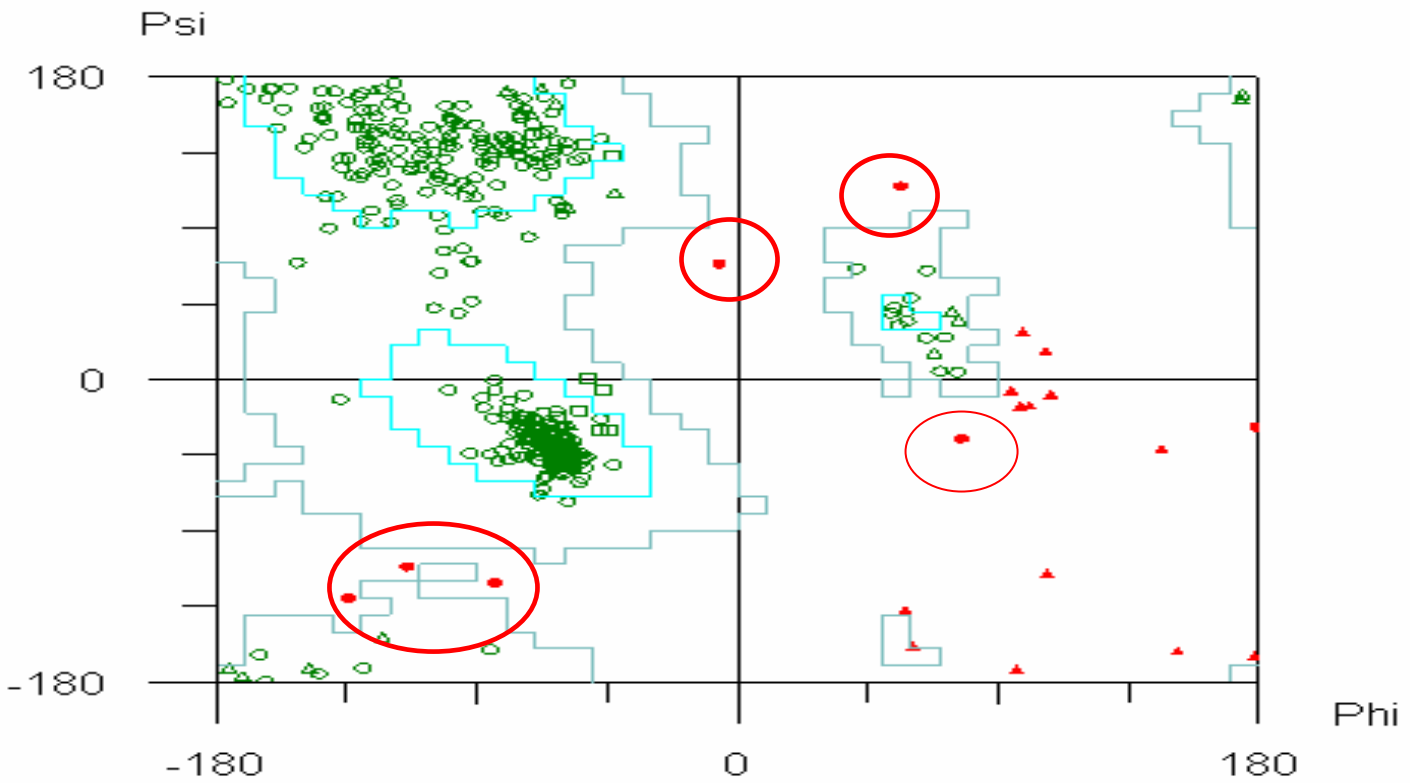


Figure 5: Ramachandran plot for Minimization 4 based on *B. circulans* template.

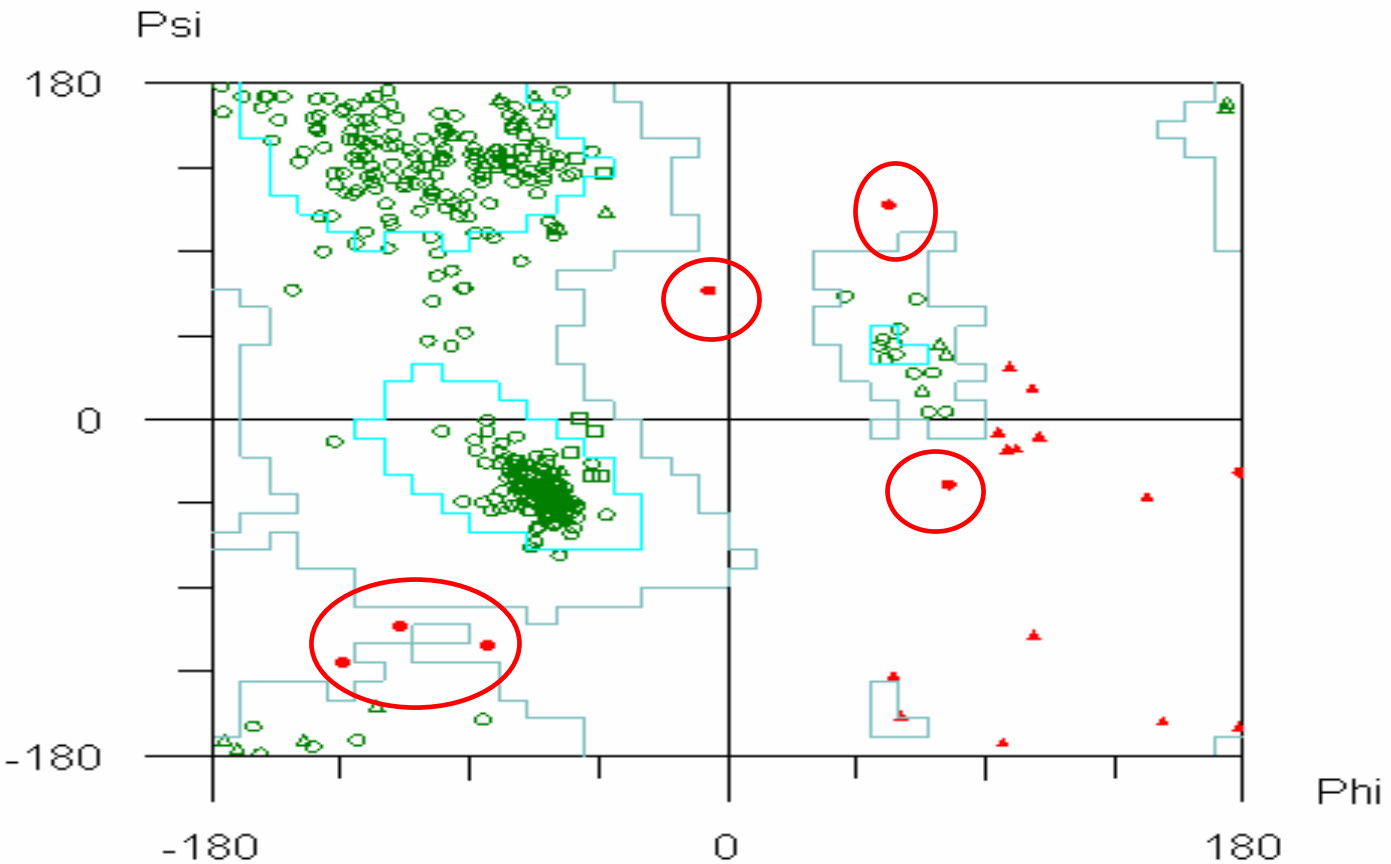


Figure 6: Ramachandran plot for Minimization 6 based on *S. marcescens* template.

From *S. marcescens* template

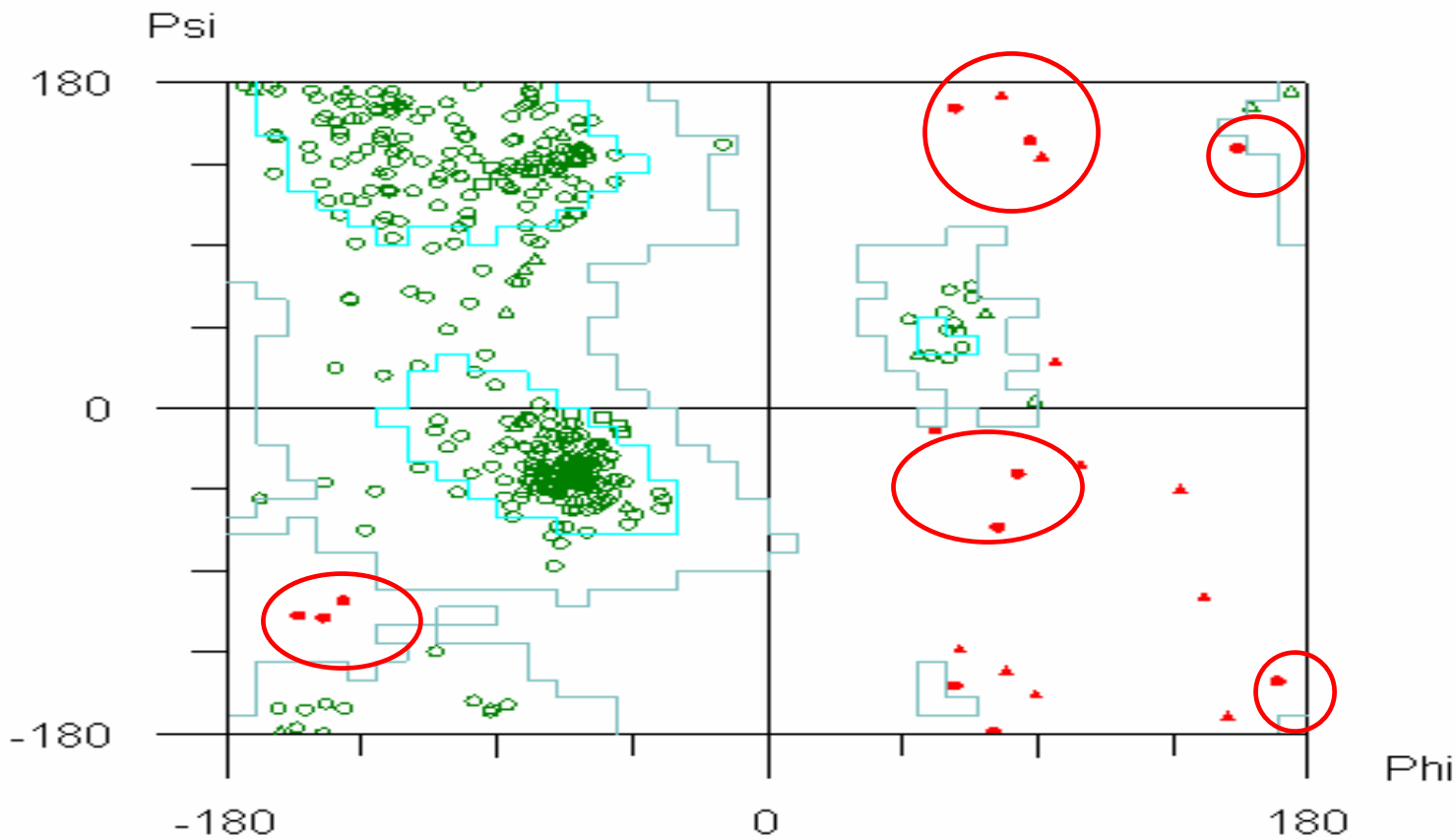


Figure 7: Ramachandran plot for Minimization 1 based on *B. circulans* template.

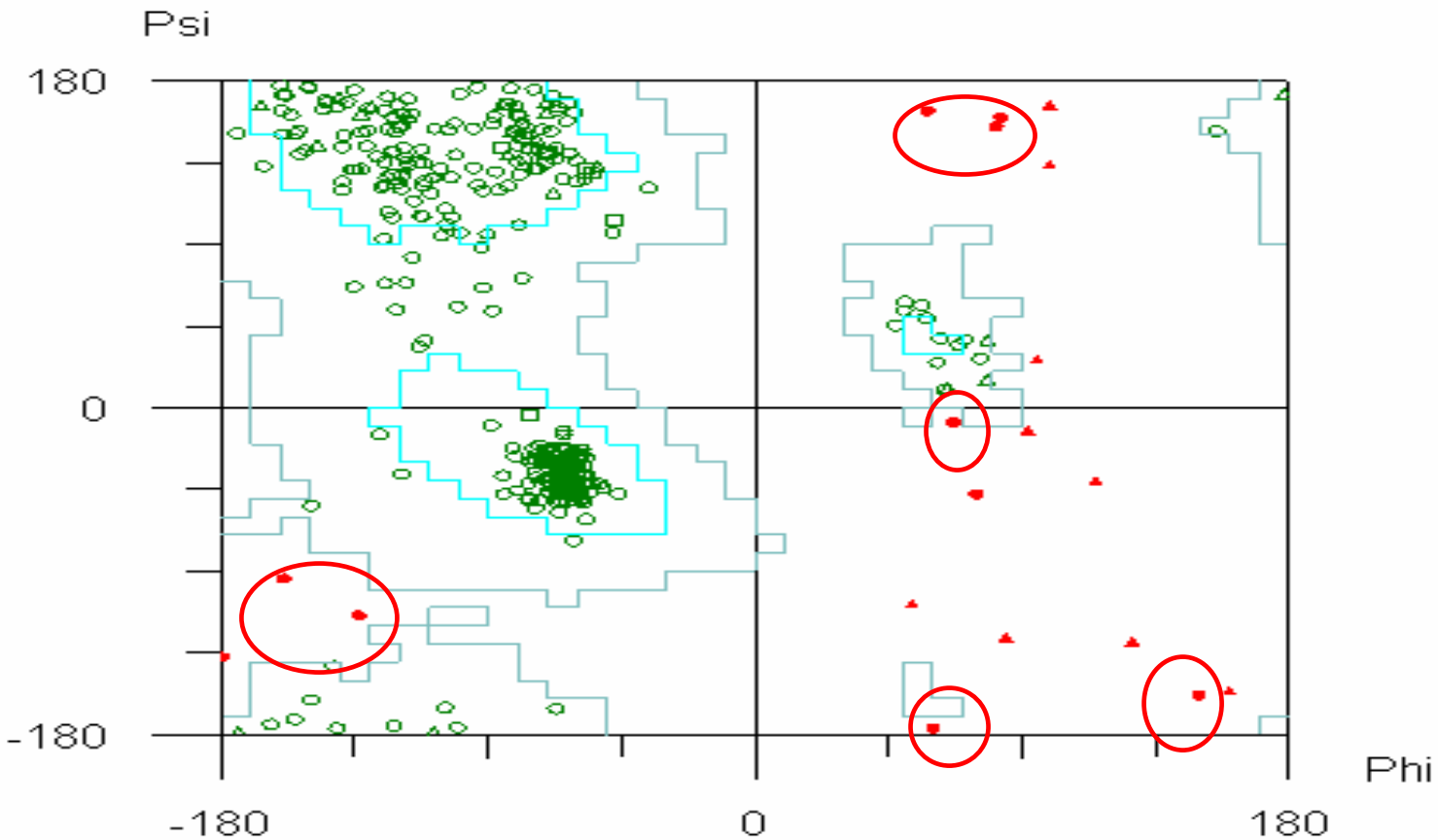


Figure 8: Ramachandran plot for Minimization 4 based on *B. circulans* template.

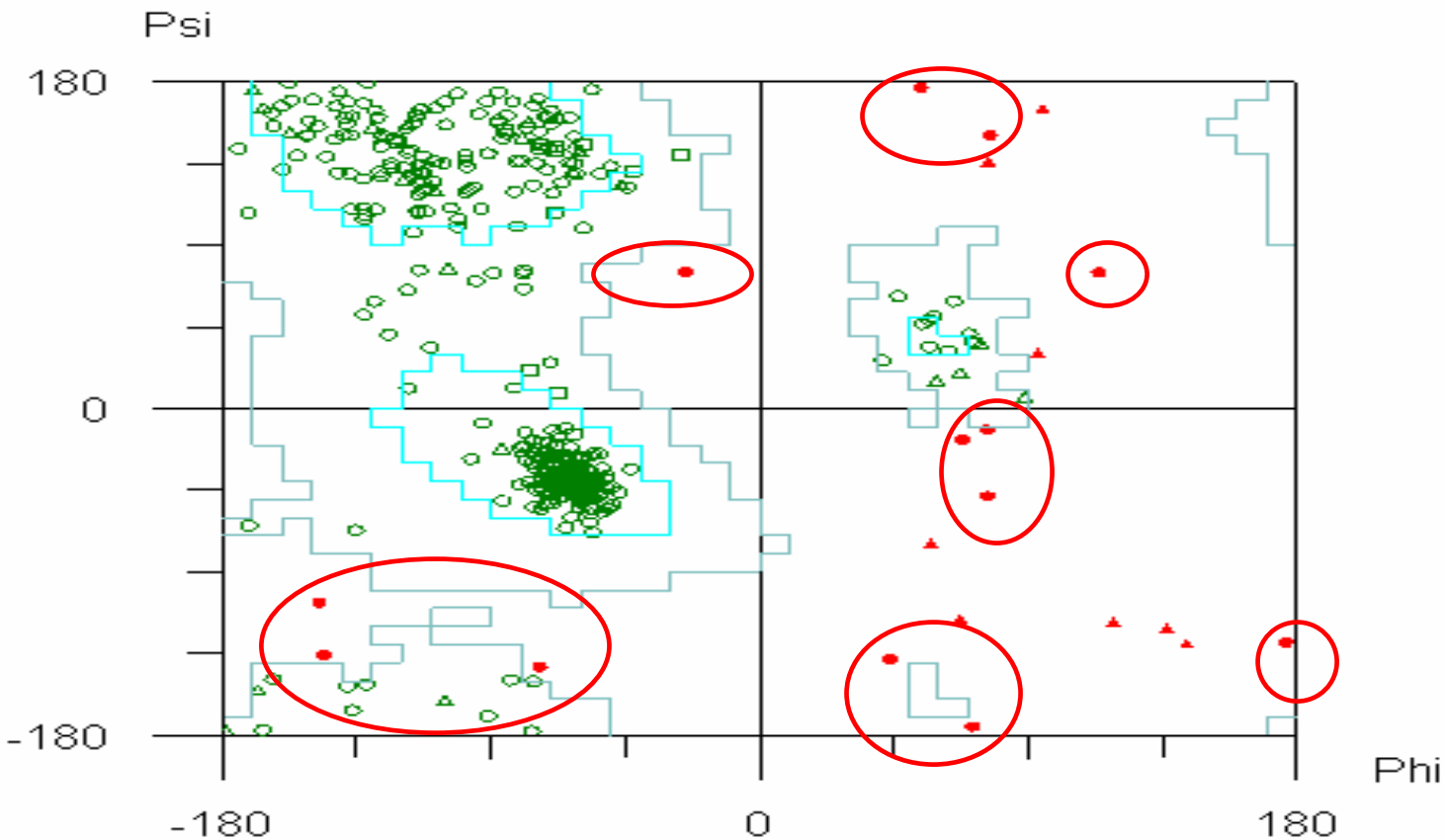


Figure 9: Ramachandran plot for Minimization 5 based on *S. marcescens* template.

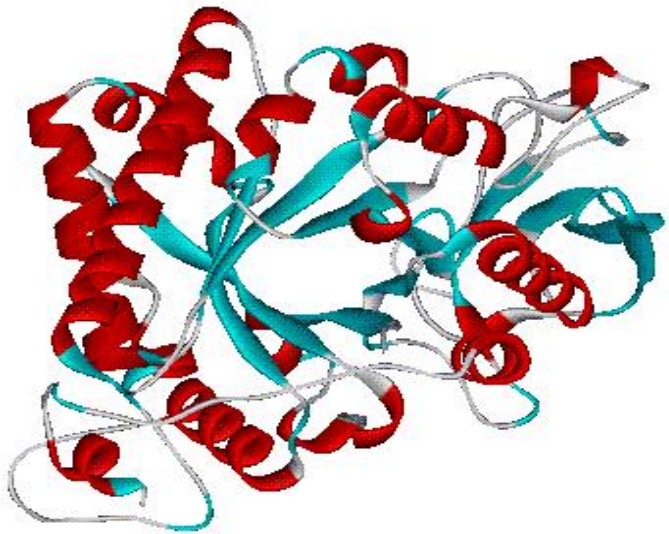
Multiple sequence alignment

SXGG

DXXDXDXE

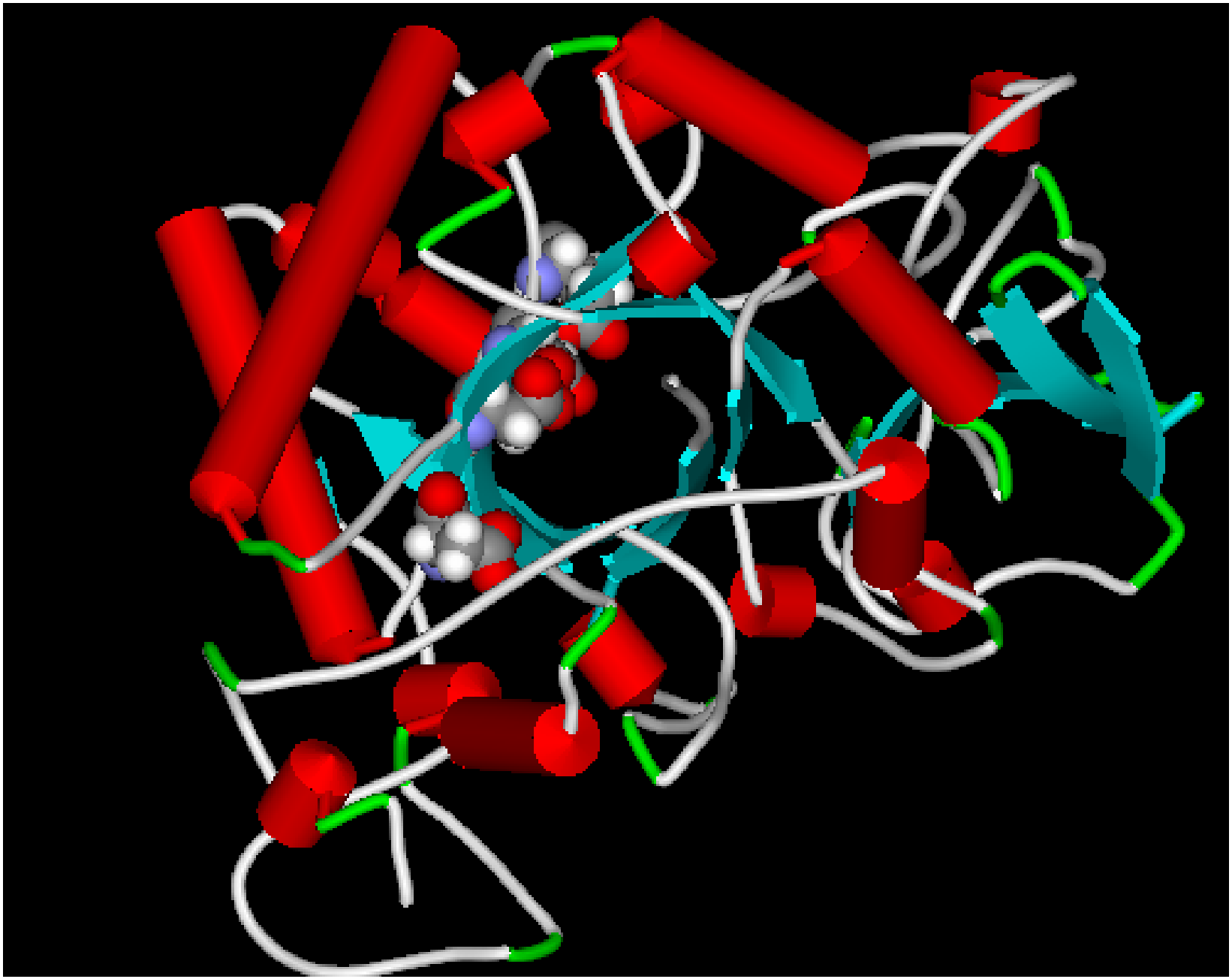
Figure 10: Sequence alignment shows the conserved sequence on family 18 glycosyl hydrolase.

Tertiary model of chitinase



~ a (β/α)8TIM-barrel (DXDXE motif)

~Structural studies have shown that the side chain of Asp137 (and its equivalents in other family 18 chitinase) may occur in two conformations, either pointing "down" onto TIM barrel (towards Asp135) or pointing "up" towards Glu139 (van Aalten *et al*., 2001).



Superimpose by sequence alignment

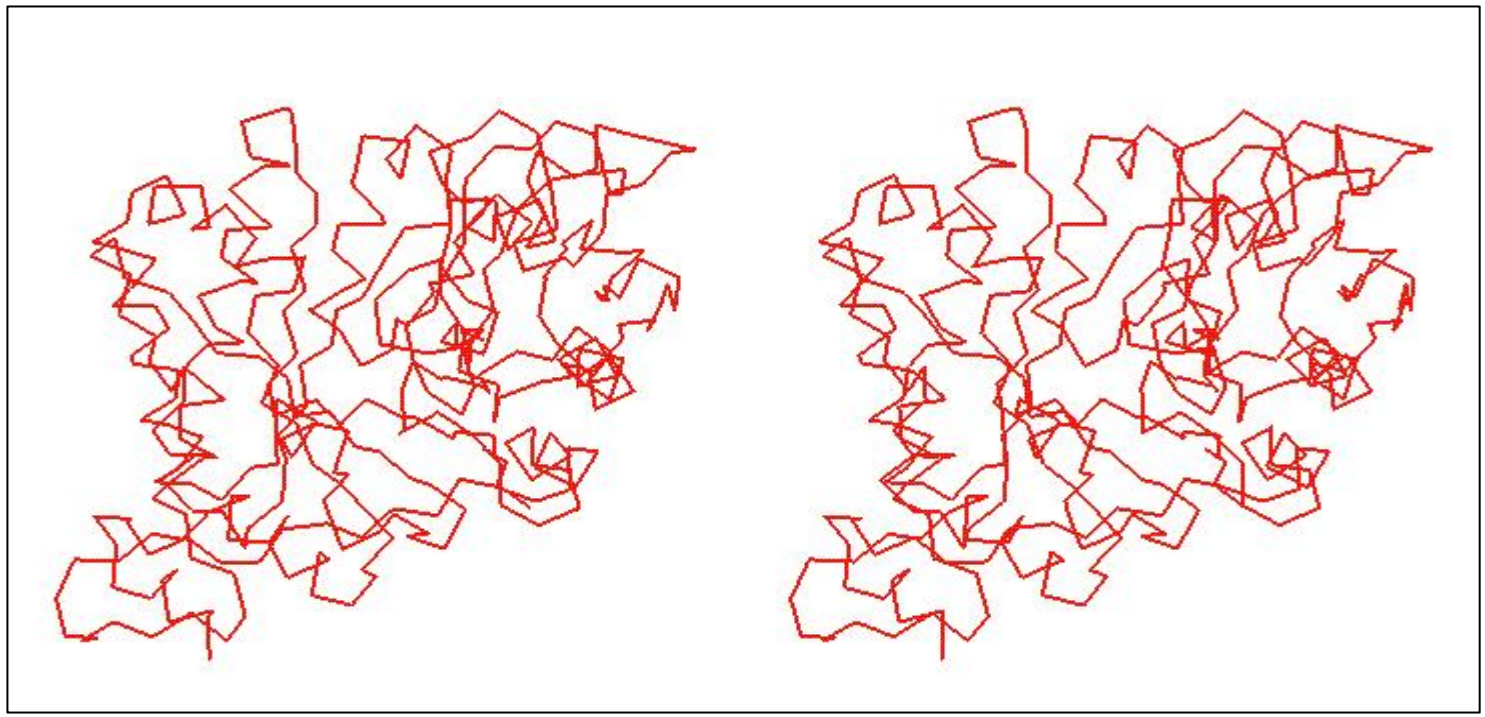


Figure 11 : Stereochemistry between crystal structure *C. immitis* (left hand side) & the model of chitUKM-1

~give value of 0.28 RMSD

Conclusion

- Multiple sequence alignment can detect the conserved motifs.
- A good quality model depends on %similarity & %identical. The higher the %, the good quality we made.
- Only 1 catalytic domain have been found.

Recommendation

- *Further research*

~do site-directed mutagenesis to know the contribution of conserved residues in enzyme activity

- *Upgrade software*

~ study can be done on another part of enzyme structure

~more user-friendly