The Relationship Between Structure and Function

--take *Bacillus thuringiensis* Cry toxin as an example

CAAS10S2G

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Reporter： Deng Chao

June 13th ,2010

Outline

- 1. Introduction
- 2. Materials
- 3. Analysis and prediction of the general properties
- 4. Analysis of the structure and function

1. Introduction

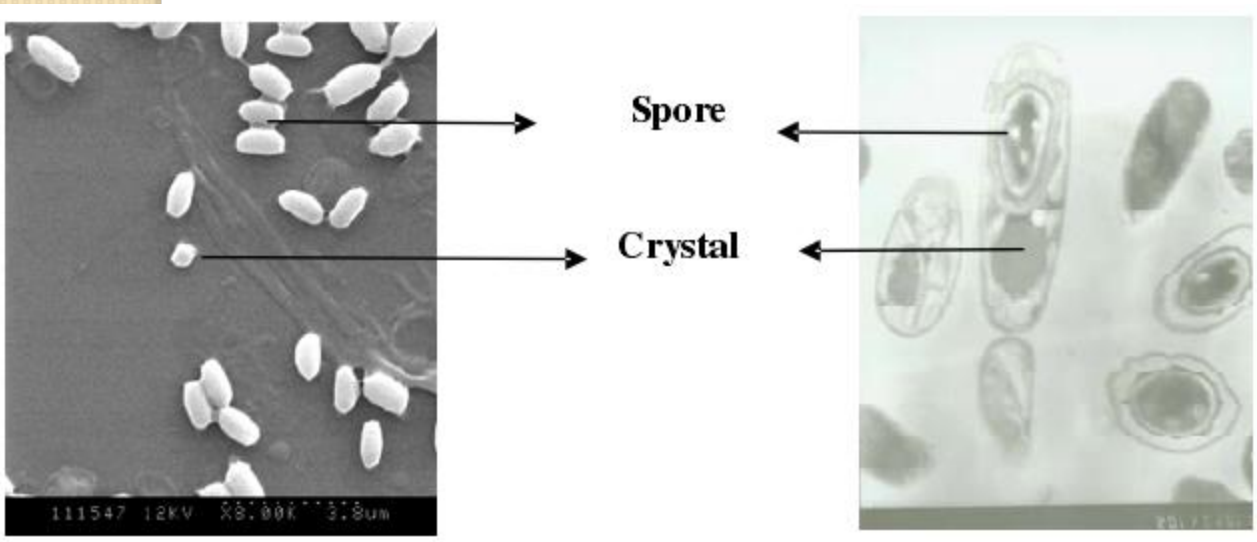
- *Bacillus thuringiensis* (BT)

  G^+

  Spore-forming

  Insecticidal crystal proteins

  (Cry toxin, delta-endotoxins)



Yan Guixin.2009 CAAS [D]

Kong-Ming Wu et al. *Science* 2008 (321): 1676-1678

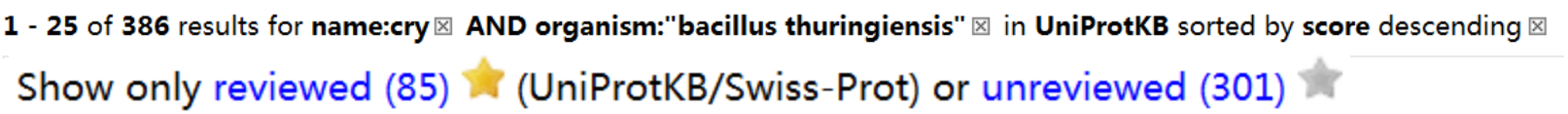
- BT toxins

  ~503 Bt toxins(Cry, Cyt and VIP)

  (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ Updated Feb 24th ,2010)

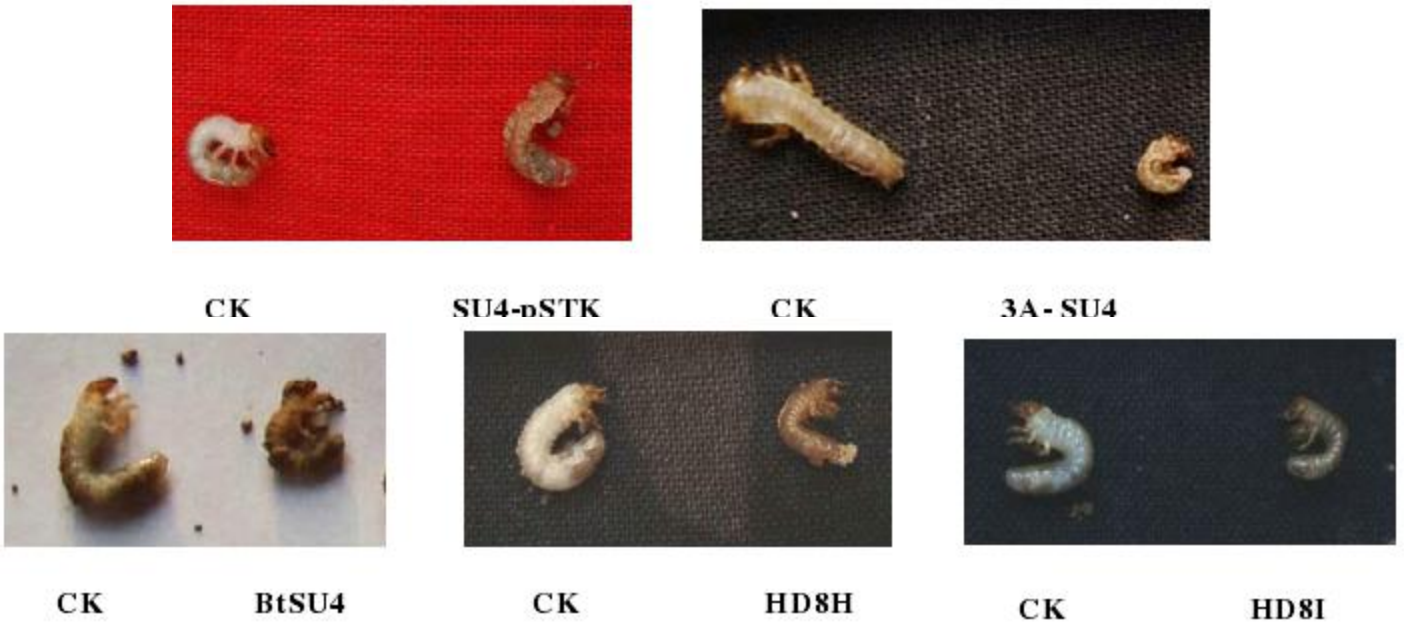
- Cry proteins

  ~ 386 (UniProt , with term "cry and *Bacillus thuringiensis* ")



- Cry proteins

  Toxic to many different species of insects larvae.



Yan Guixin.2009 CAAS [D]

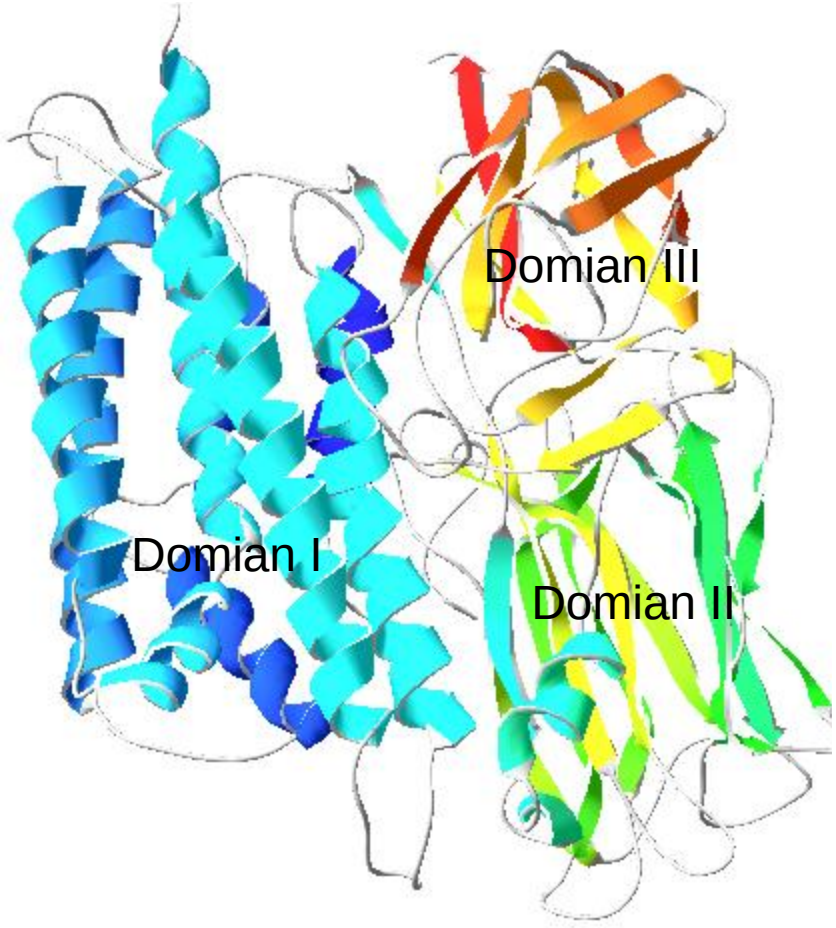
- The nomenclature of Cry proteins

Based on the similarity of the Cry protein sequences

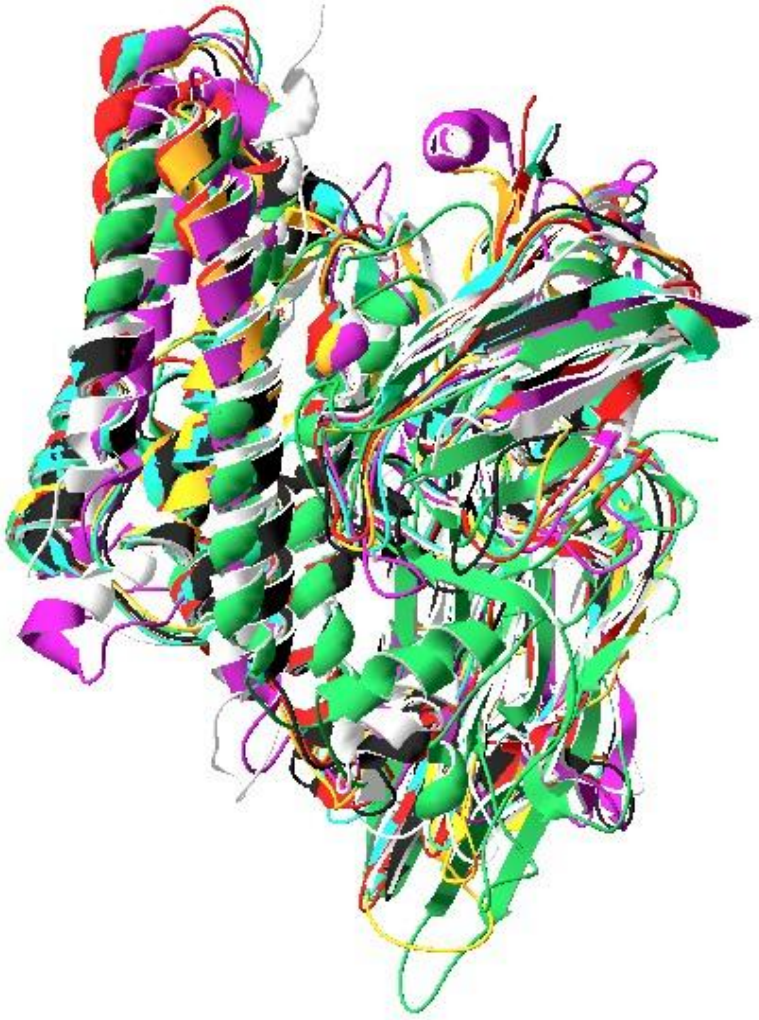
Similarity	Grade /symbol	Example(s)
<45%	I/ 1,2,3…	Cry1,Cry2…
45%-78%	II/ A,B,C…	Cry1A,Cry1B…
78%-95%	III/ a,b,c…	Cry1Aa,Cry1Ab…
>95%	IV/ 1,2,3…	Cry1Aa1, Cry1Aa2…

Crickmore N. et al. *Microbiol Mol Biol Rev.* 1998,62:807～813.

- Overview of Cry toxin

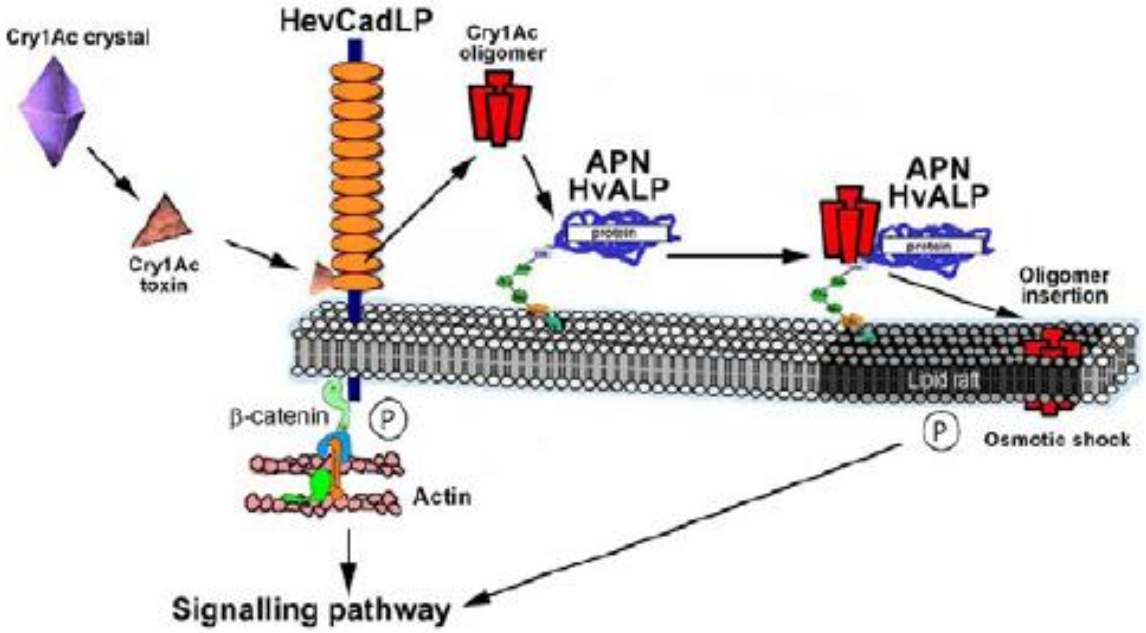


Cry4Aa(2C9K)



Superimposition of 7Cry toxins

- The mechanism of the toxic action to insects larvae



Jurat-Fuentes J.L. ,Adang M.J . *Journal of Invertebrate Pathology* 2006,92(3):166～171.

2. Materials

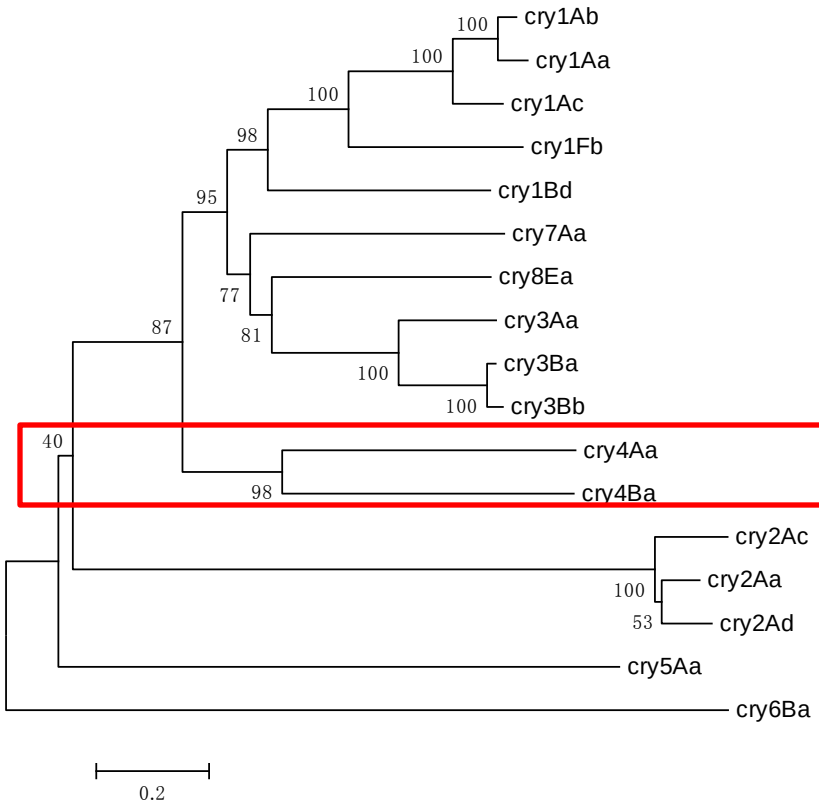
- Get the total 7 Cry proteins structures in PDB.

  [Cry1Aa(1CIY), Cry2Aa(1I5P), Cry3Aa(1DLC), Cry3Bb1(1JI6), Cry4Aa(2C9K), Cry4Ba (1W99), Cry8Ea1(3EB7)]

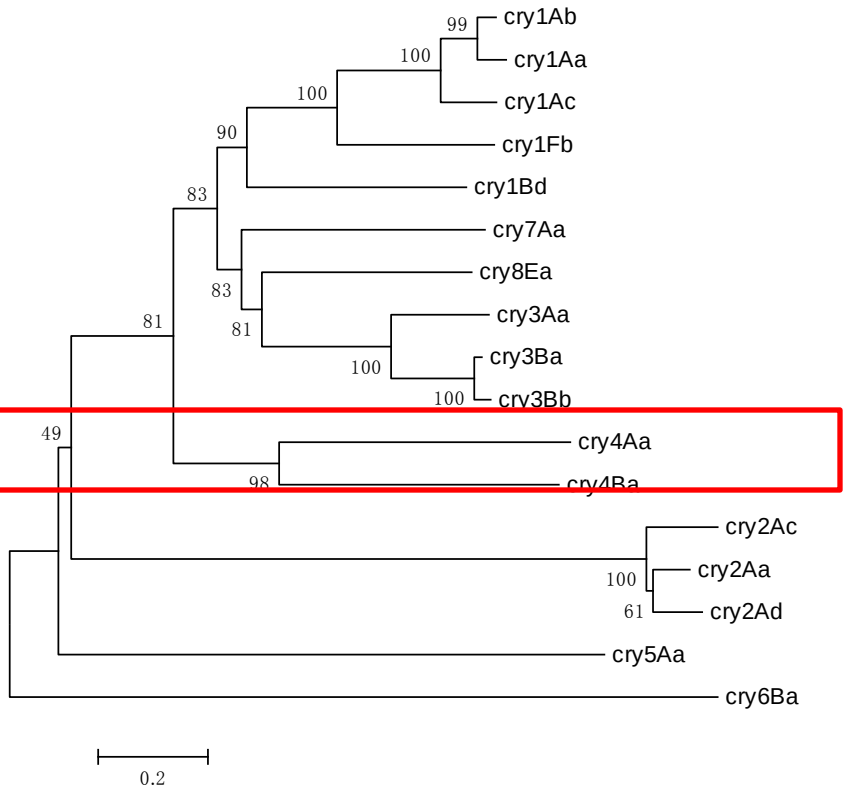
- Get full sequences of 17 Cry proteins in UniProt (including the full sequences of the 7 PDB Cry proteins and their toxic sequences) .

  (Cry1Aa, Cry1Ab, Cry1Ac, Cry1Bd, Cry1Fb, Cry2Aa, Cry2Ac, Cry2Ad, Cry3Aa, Cry3Ba, Cry3Bb, Cry4Aa, Cry4Ba, Cry5Aa, Cry6Ba,Cry7Aa, Cry8Ea1)

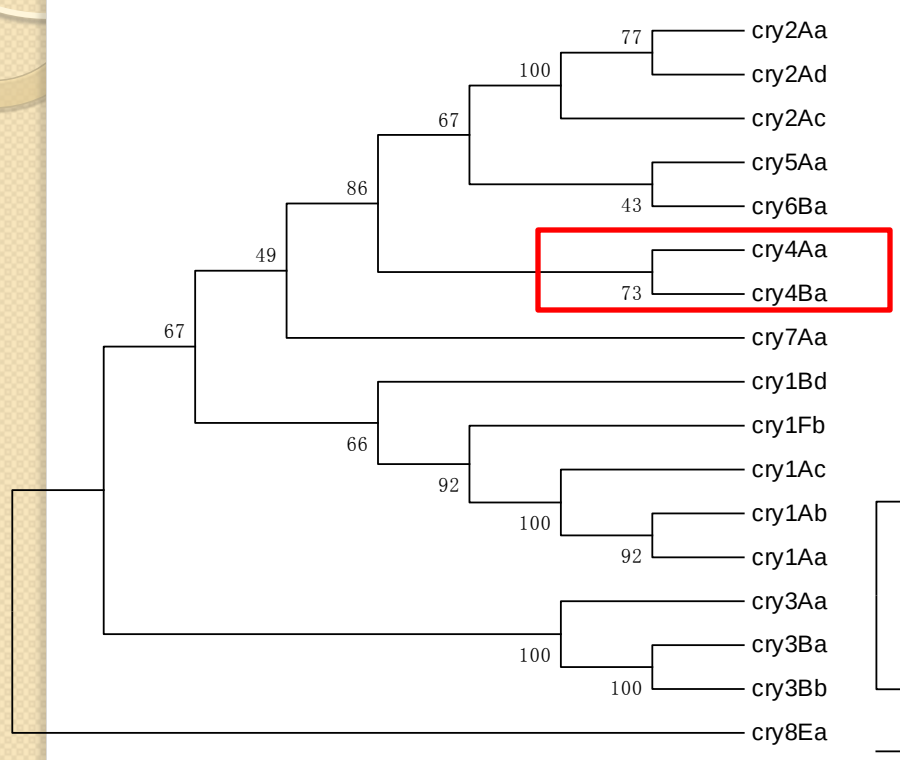
- Phylogenetic tree of 17 Cry proteins. (by MEGA 4.0)



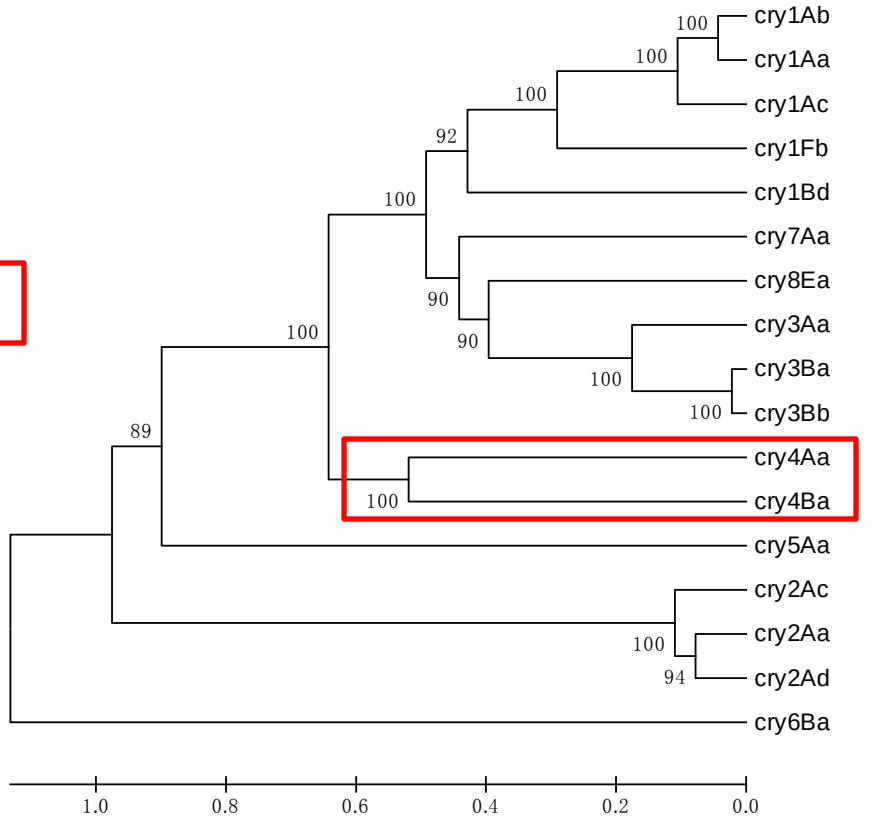
Neighbor-Joining method



Minimum Evolution method



Maximum Parsimony method



UPGMA method

- Choose Cry4Aa(P16480) and Cry4Ba(P05519) for further analysis

-both with PDB structures [Cry4Aa(2C9K), Cry4Ba (1W99)]

-both have toxic to Aedes（伊蚊），Culex（库 蚊） and Anopheles（按蚊） larvae but significantly differ in toxicity level

Table 1. Larvicidal activity of *Bacillus thuringiensis* svar. *israelensis* powders containing individual spore/crystals mixtures, against *Aedes aegypti* early 4th instar larvae

Powder	No. assays	No. larvae	LC_{50}[a] (95% fiducial limits)
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Bti[b]	3	1080	0.013 (0.011–0.016)

Henrique de Barros Moreira Beltrao , Maria Helena Neves Lobo Silva-Filha . *FEMS Microbiol Lett* 2007 ,(266) 163–169

3. General property(full length)

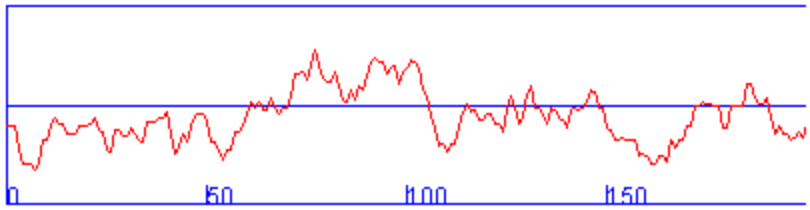
	Cry4Aa	Cry4Ba	Program/method
Length (aa)	1180	1136	ProtParam
M(Da)	134538.7	127764.2	ProtParam
PI(theoretical)	5.04	4.87	ProtParam
Hydrophobicity	-0.424152	-0.346919	SOSUI
SecP score	0.901246	0.822789	SecretomeP
Identity	56.5%		Jemboss
Similarity	68.1%		Jemboss

Conclusion 1: The acidity of the Cry ptoteins make it easier to dissolve in the midgut where is alkaline.

- Transmembrane prediction （SOSUI）

Cry4Aa

[Hydropathy profile]



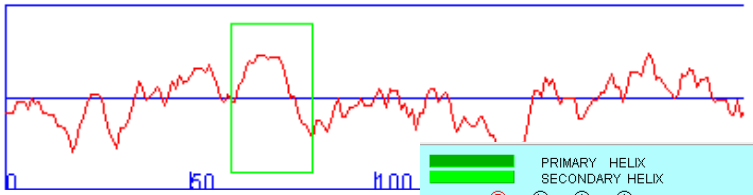
This amino acid sequence is of a SOLUBLE PROTEIN.

Cry4Ba

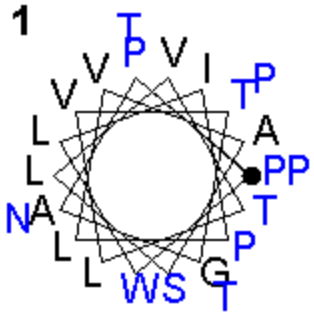
This amino acid sequence is of a MEMBRANE PROTEIN which have 1 transmembrane helix.

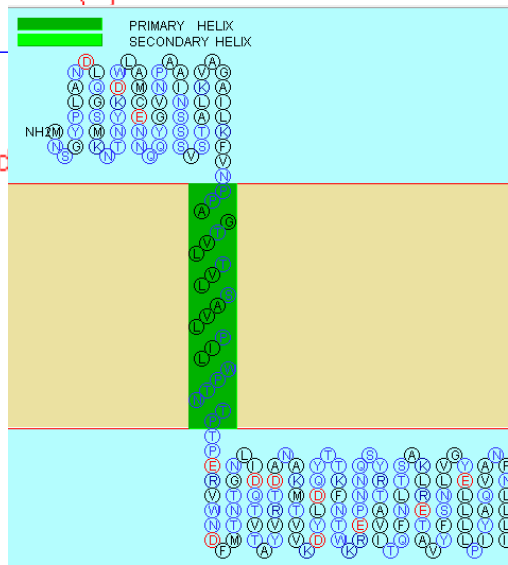
No.	N terminal	transmembrane region	C terminal	type	length
1	61	PPAGTVLTVLSAVLPILWPTNTP	83	PRIMARY	23

[Hydropathy profile]

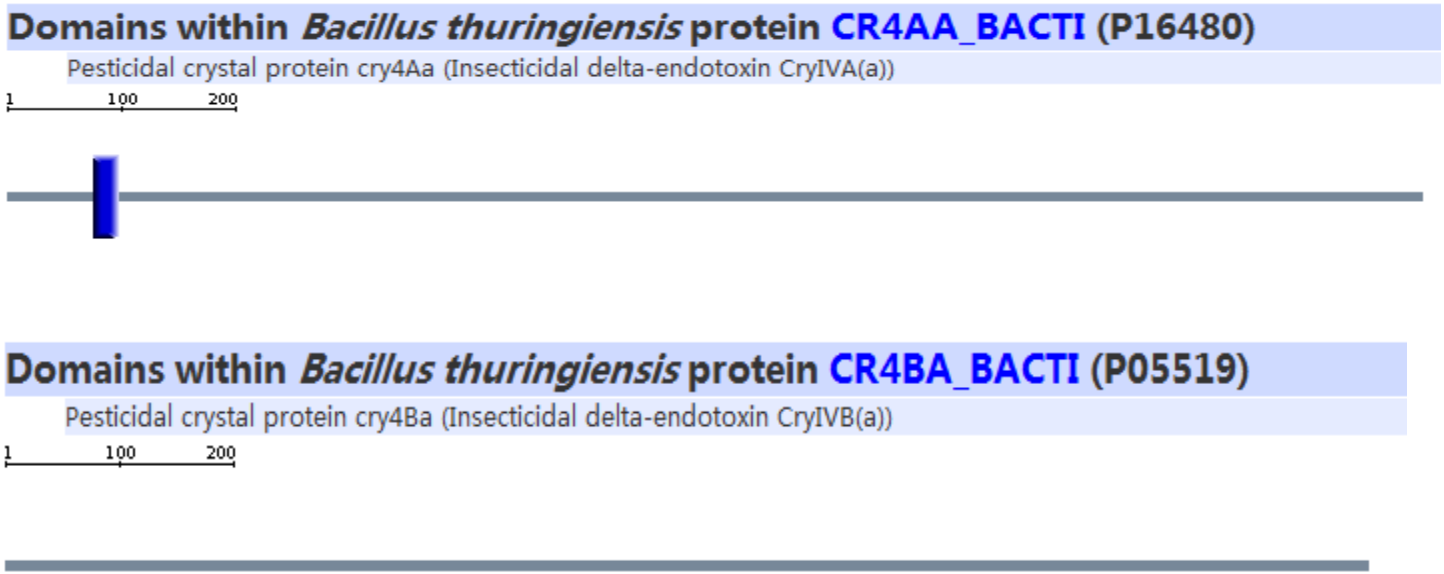


Hydrophobic residue: Black
Polar residue: Blue
Charged residue: Bold blue(+) Bold





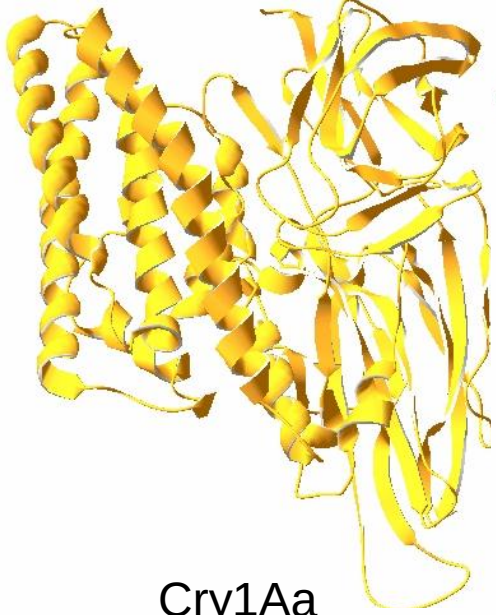
- Smart: different results!



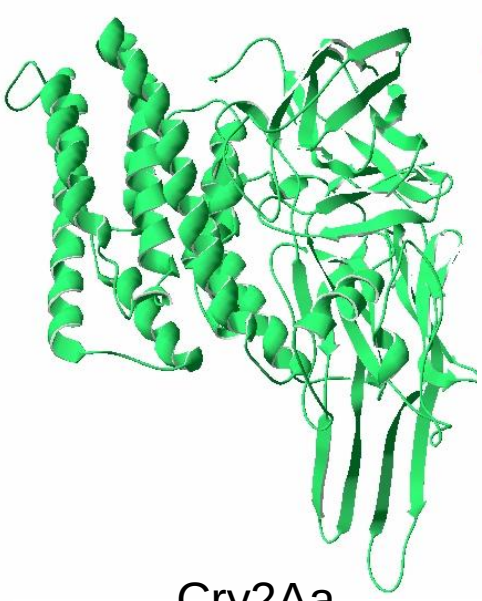
4. Structure and function (toxin sections)

- The full length protein (protoxin) dissolved and cleaved in the midgut of larvae, produce the active toxin.

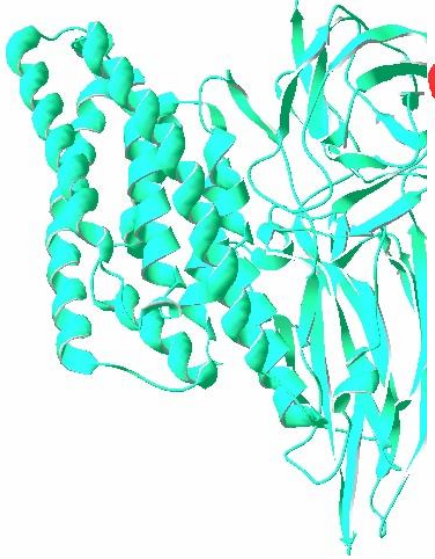
- Cry4Aa toxin: 68-679
- Cry4Ba toxin: 84-641



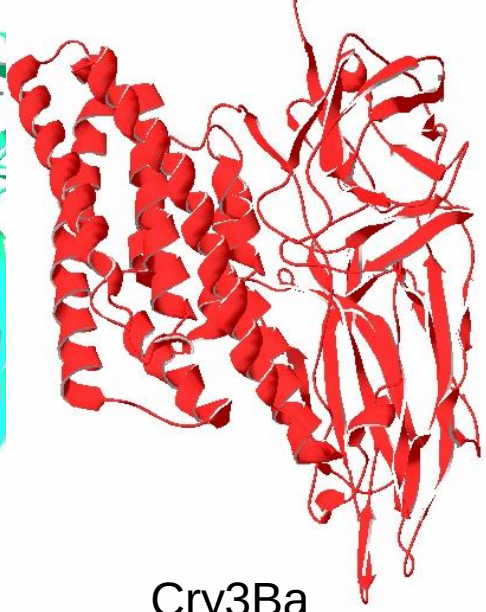
Cry1Aa



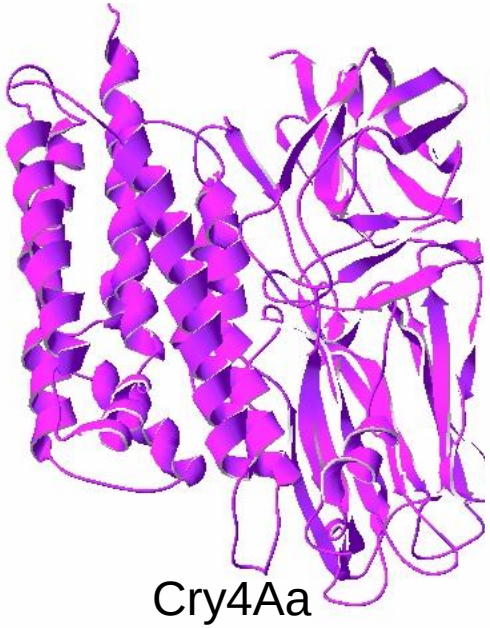
Cry2Aa



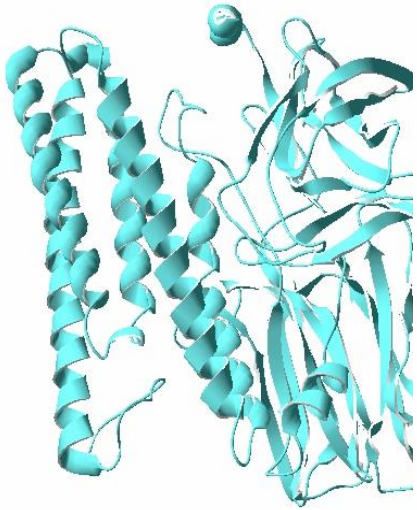
Cry3Aa



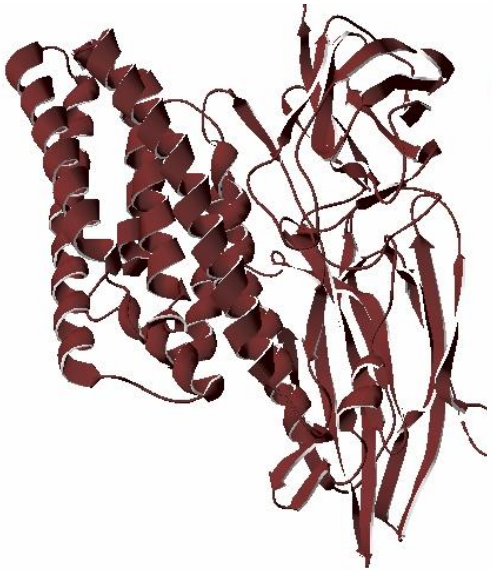
Cry3Ba



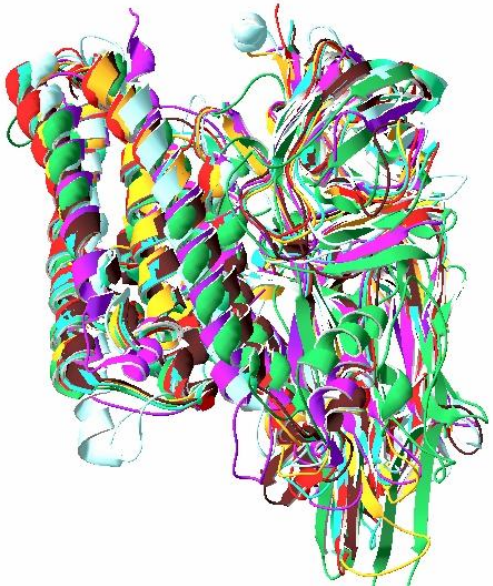
Cry4Aa



Cry4Ba

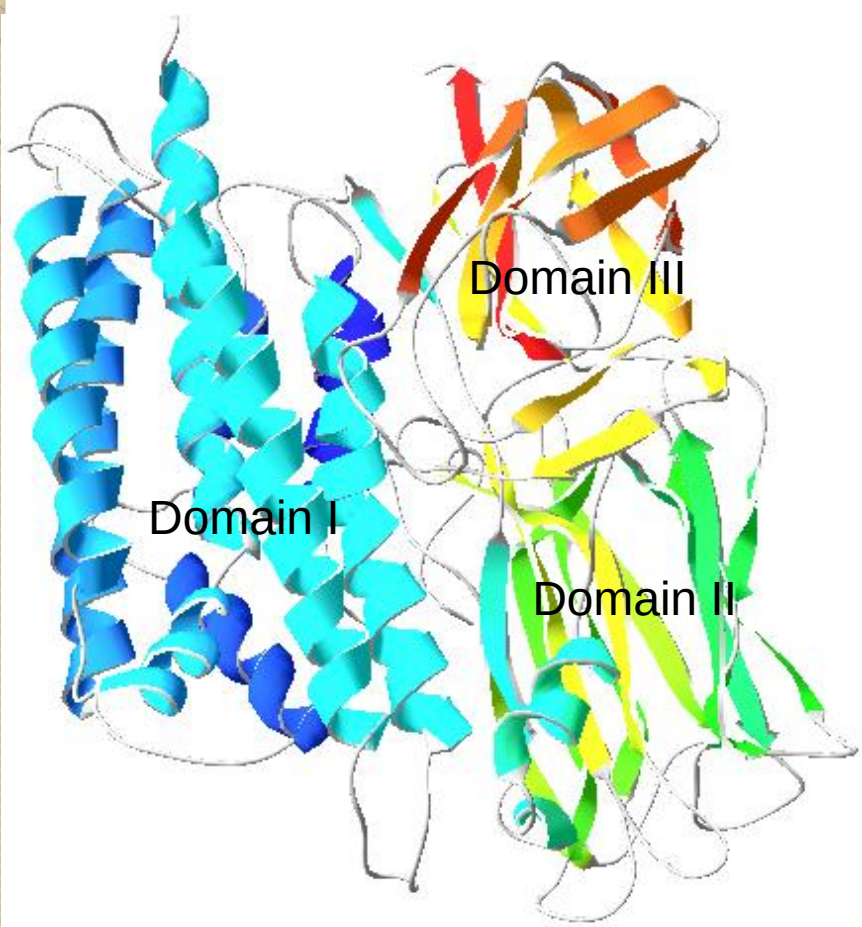


Cry8Ea1



Superimposition

Conclusion 2: the 3D structures of the Cry toxins seem to be highly similarity.



Cry4Aa(2C9K)

- Domain I

Helixes rich （usually 7 ） , responsibility for the formation of ion channel in the midgut cell membrane (the toxic domain).

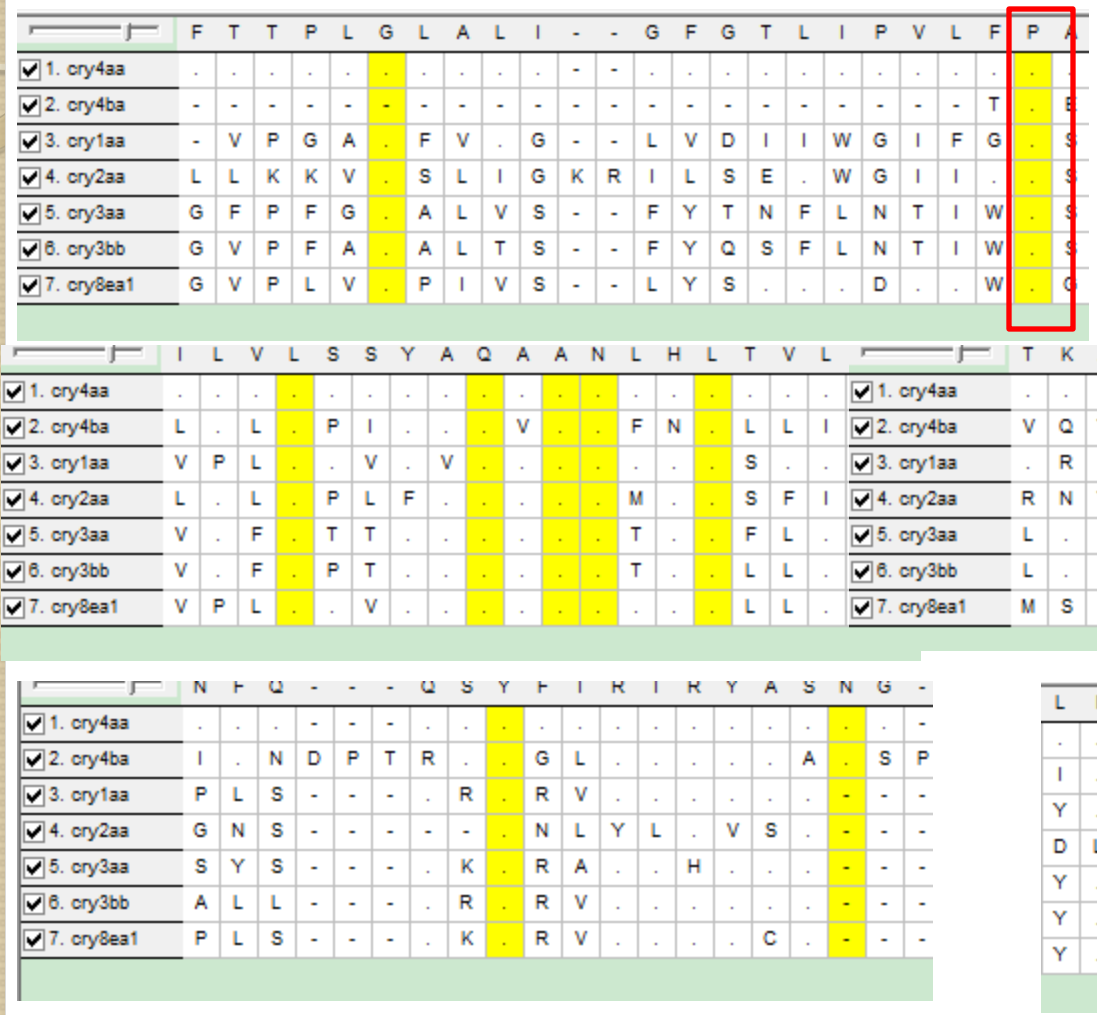
- Domain II

Beta-sheet rich, involved in the specific binding to the receptor.

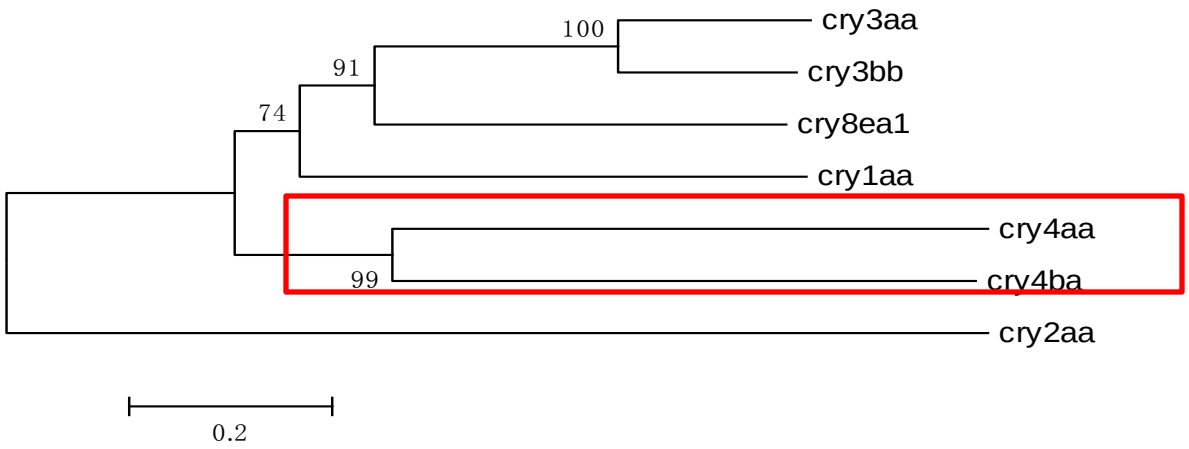
- Domain III

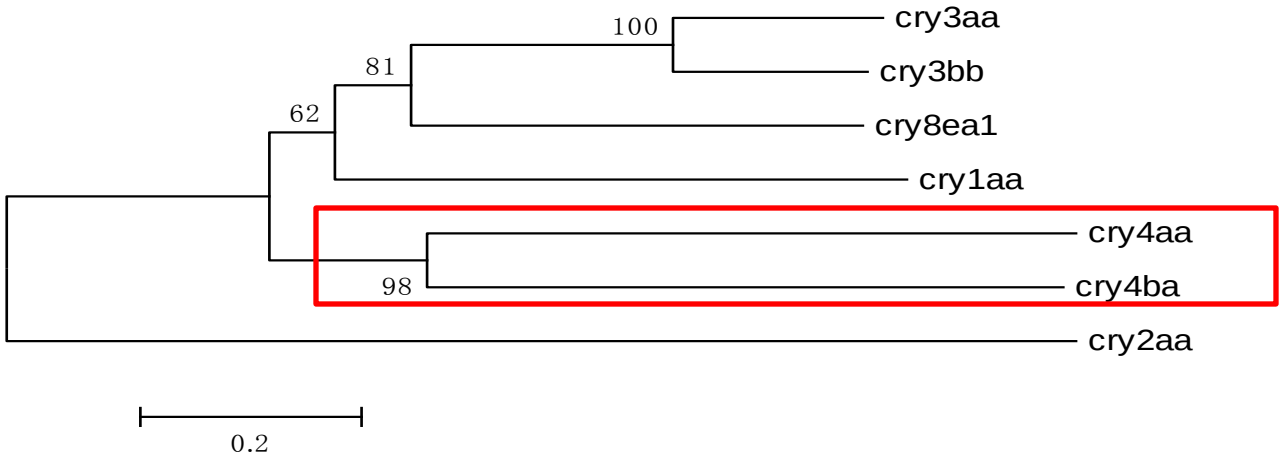
Not very clear, maybe with both function.

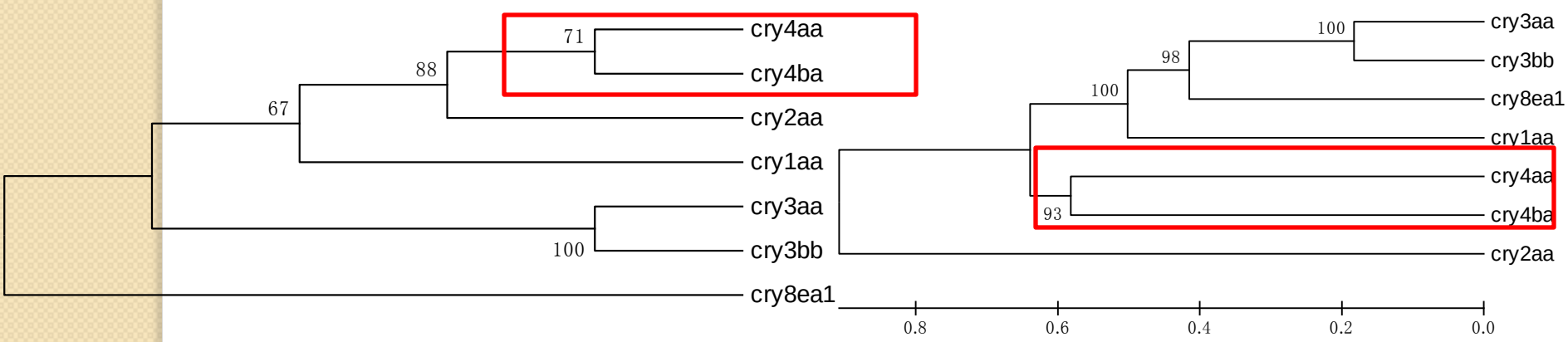
- Multiple alignment of 7 Cry toxins (by MEGA 4.0)

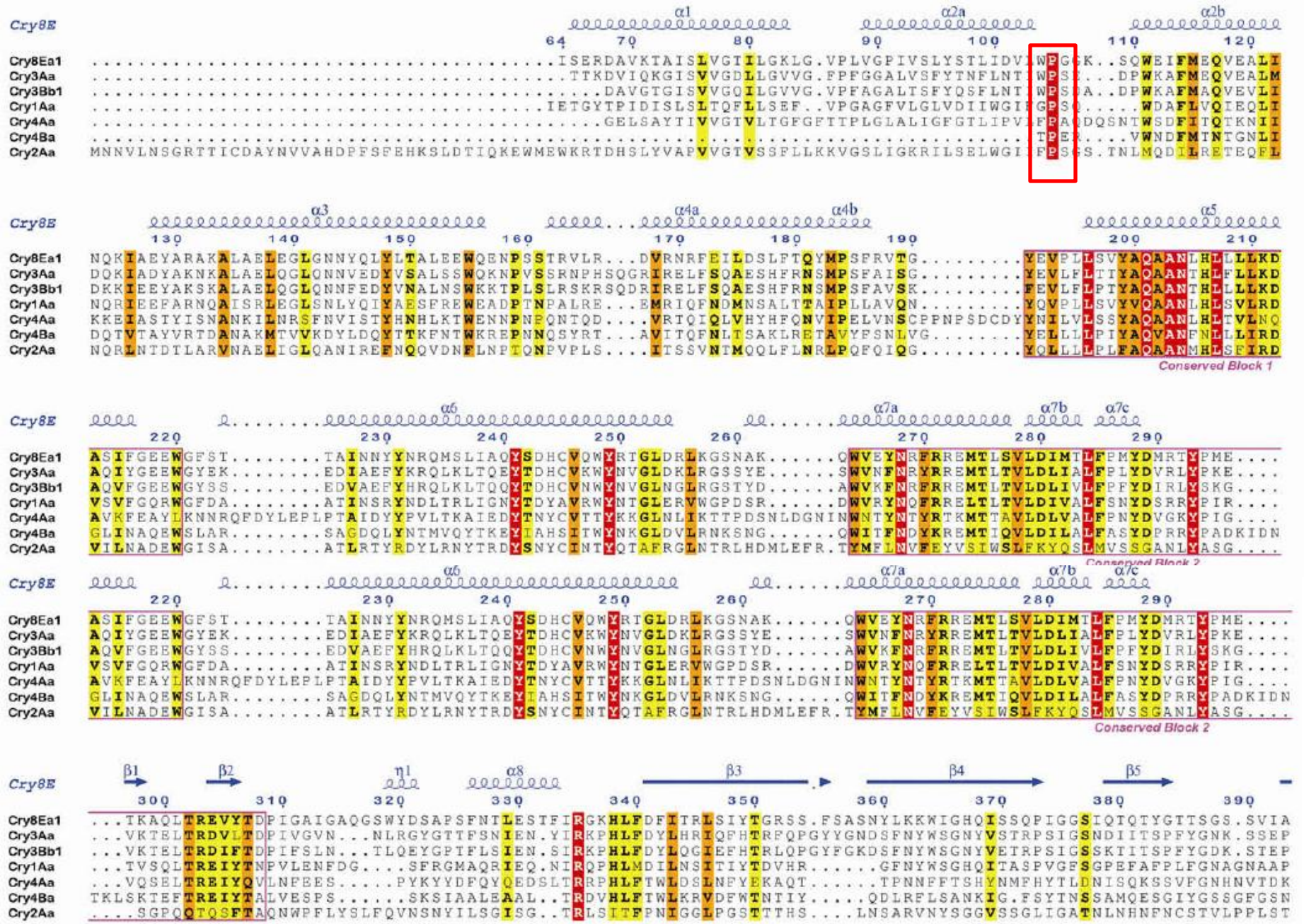


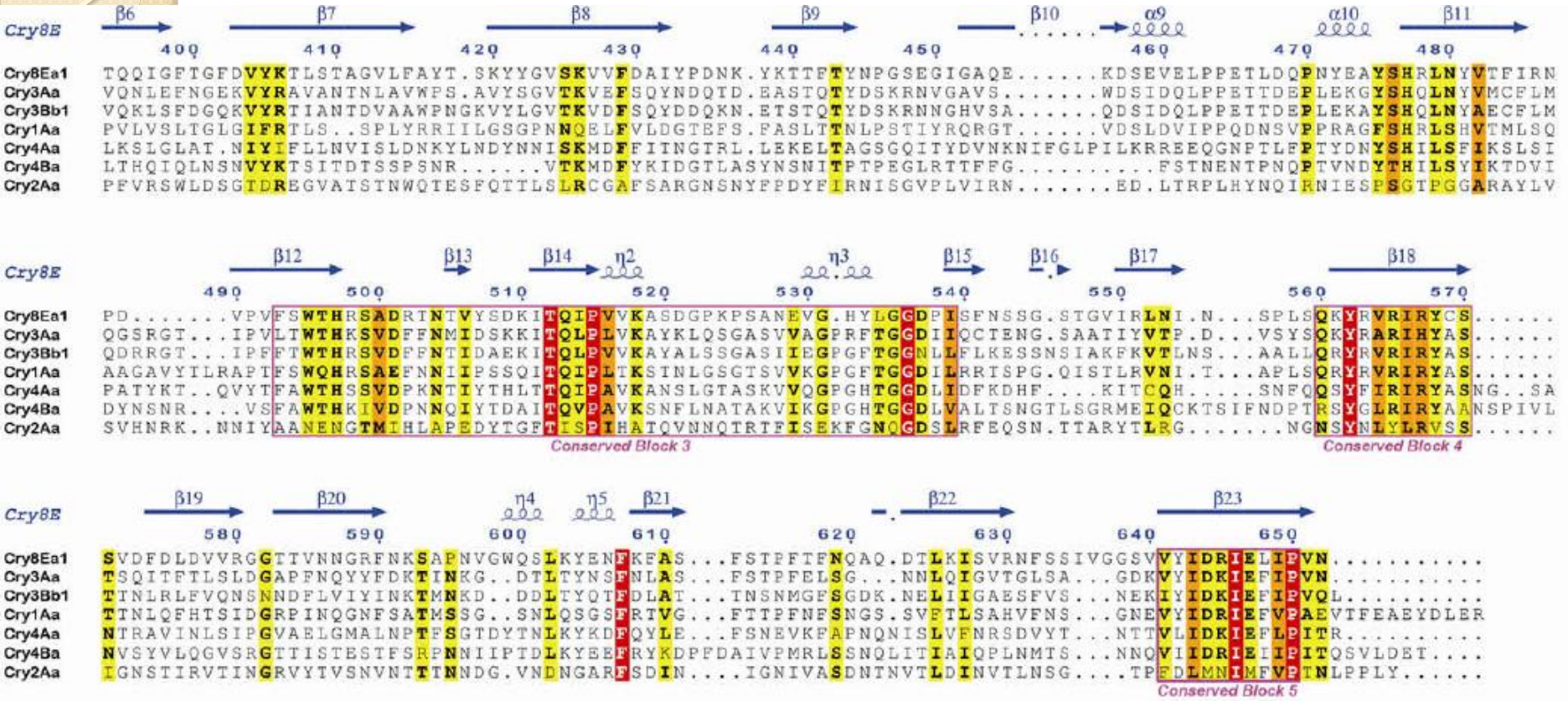
Some conserved blocks


100
91
74
99
cry3aa
cry3bb
cry8ea1
cry1aa
cry4aa
cry4ba
cry2aa
0.2


100
81
62
98
cry3aa
cry3bb
cry8ea1
cry1aa
cry4aa
cry4ba
cry2aa
0.2


71
88
67
100
cry4aa
cry4ba
cry2aa
cry1aa
cry3aa
cry3bb
cry8ea1
100
98
100
93
cry3aa
cry3bb
cry8ea1
cry1aa
cry4aa
cry4ba
cry2aa
0.8
0.6
0.4
0.2
0.0

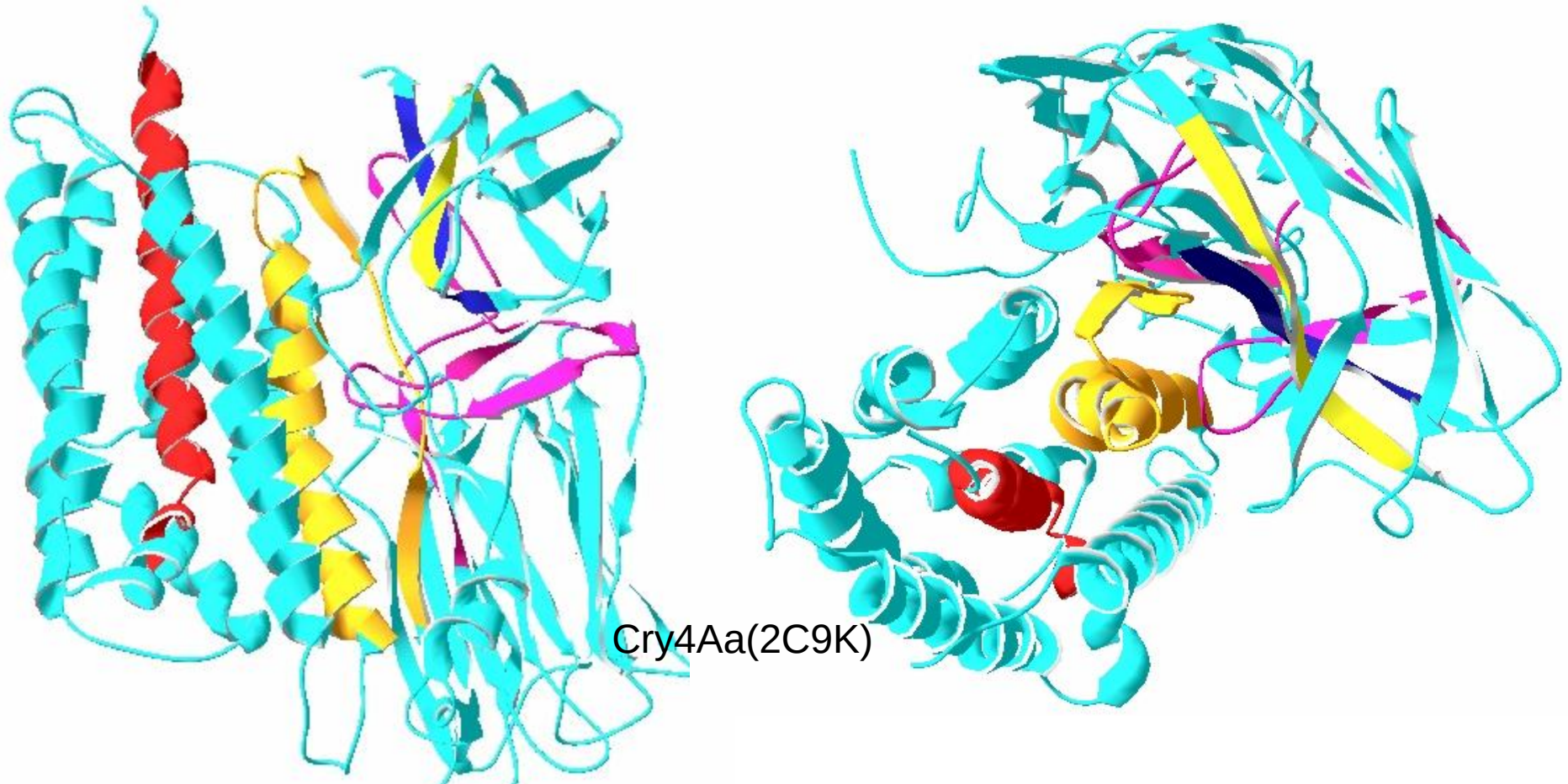

Cry8E
α1
α2a
α2b
Cry8Ea1
Cry3Aa
Cry3Bb1
Cry1Aa
Cry4Aa
Cry4Ba
Cry2Aa
α3
α4a
α4b
α5
Conserved Block 1
α6
α7a
α7b
α7c
Conserved Block 2
β1
β2
η1
α8
β3
β4
β5



Multiple alignment by DSSP: 5 conserved blocks

Shuyuan Guo, et al. *Journal of Structural Biology.* 168 (2009) 259–266

- The location of the conserved blocks



Cry4Aa(2C9K)

Block1 Tyr202-Leu231, Block2 Trp289-Val333
Block3 Phe520-Ile567, Block4 Gln584-Ser594， Block5 Val667-Pro676

Conclusion 3: All conserved blocks located in the *center* of molecular or the *interfaces* between 2 domains. Maybe important for the structure and stability.

- Comparison of domain I/ II between Cry4Aa and Cry4Ba

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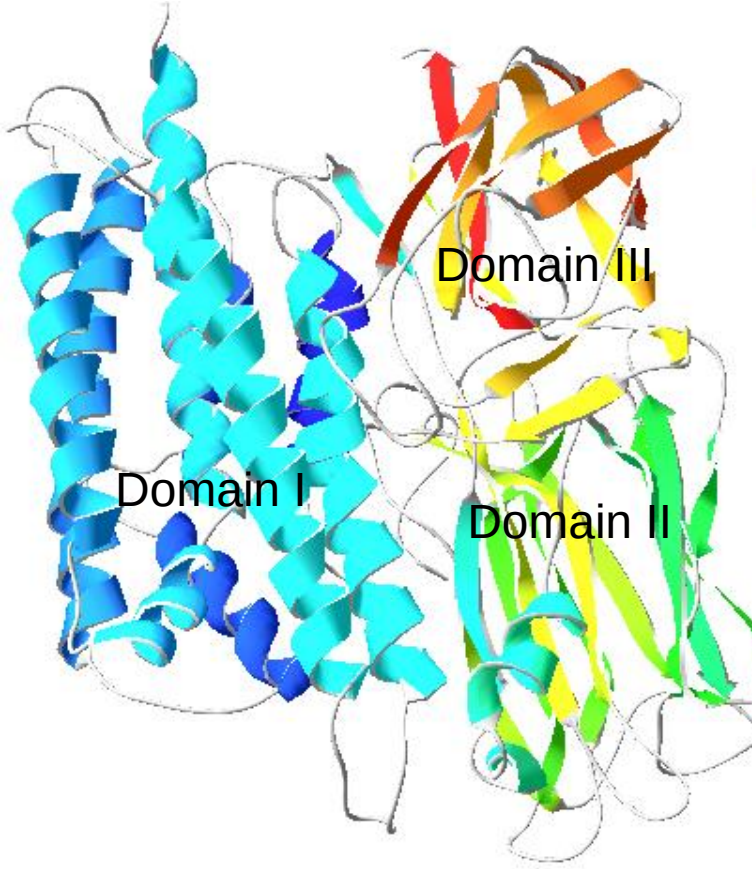
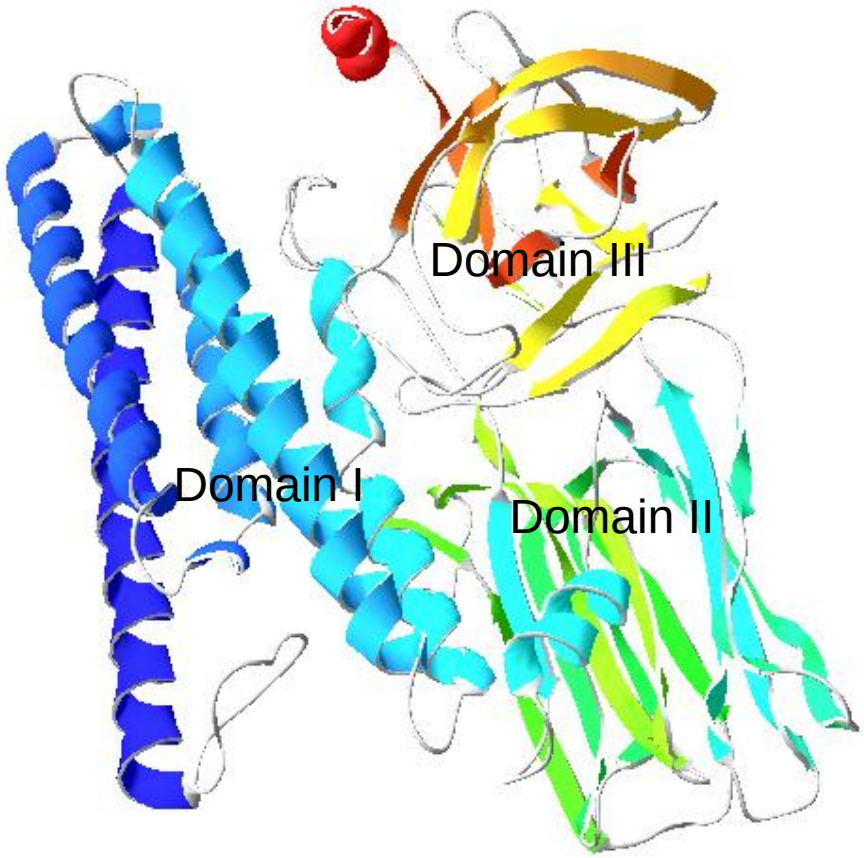
Henrique de Barros Moreira Beltrao , Maria Helena Neves Lobo Silva-Filha . *FEMS Microbiol Lett* 2007 (266) 163–169

	Domain I	Domain II	Domain III
Cry4Aa	68-321	322-524	525-679
Cry4Ba	84-282	283-466	467-641

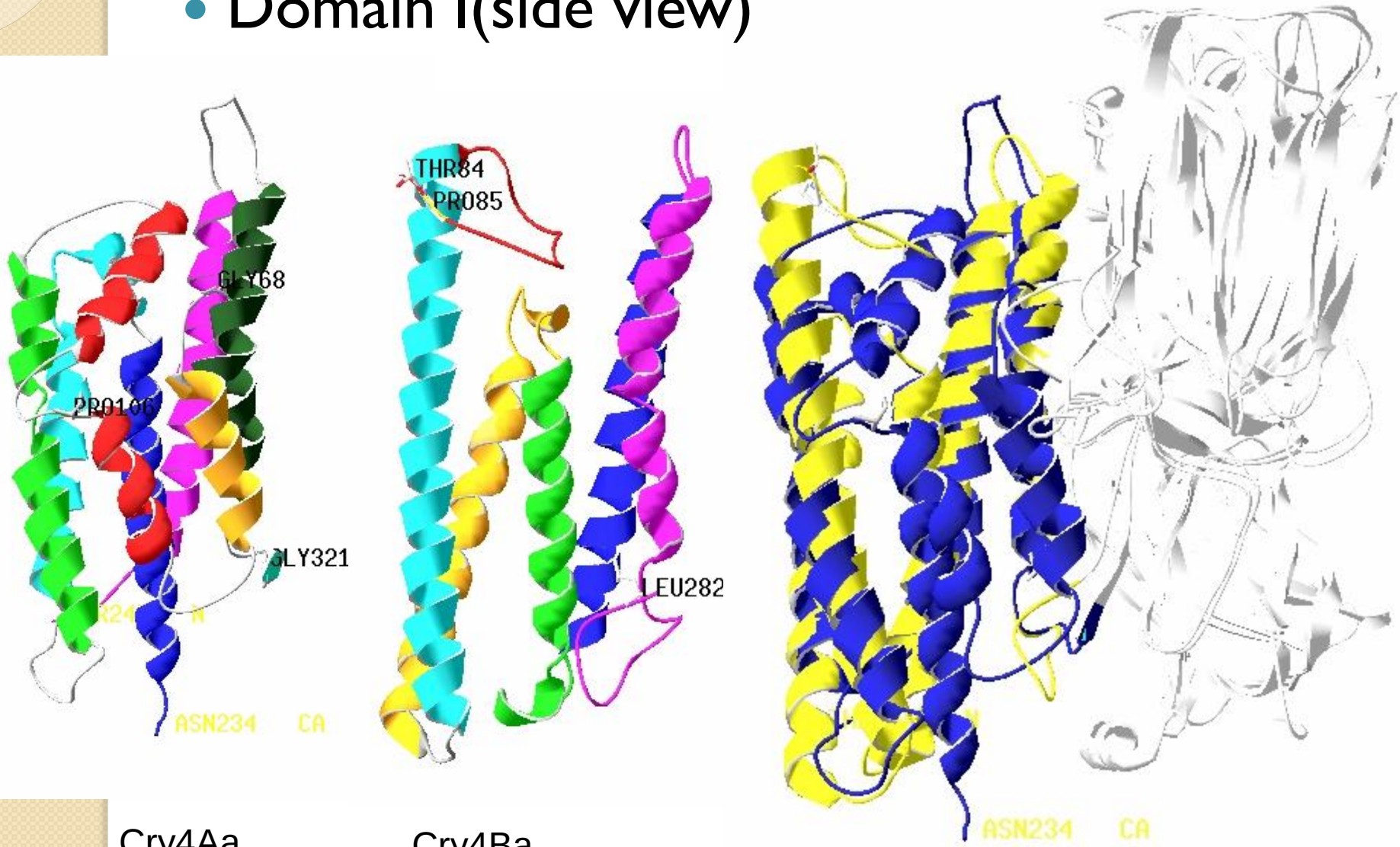


Cry4Aa(2C9K)



Cry4Ba (1W99)

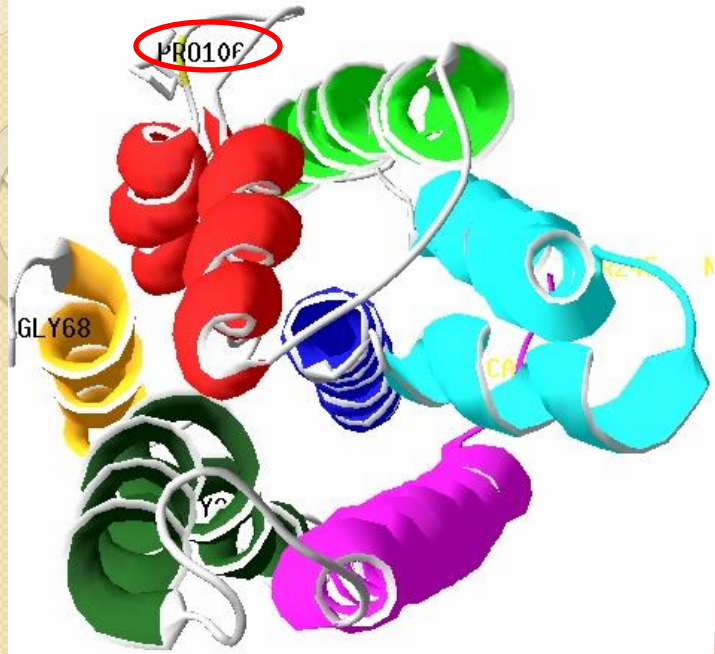
- Domain I(side view)



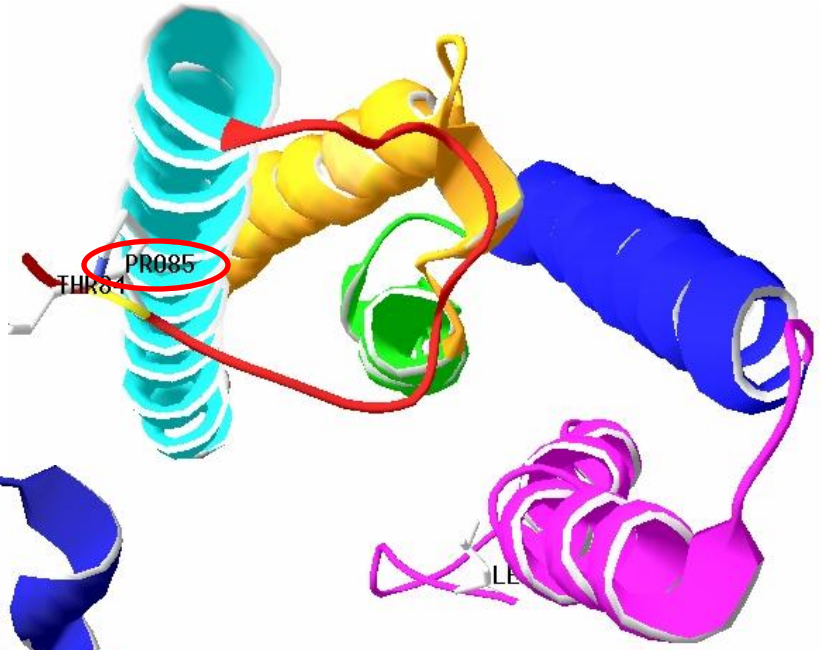
Cry4Aa
7 α-helixes

Cry4Ba
5 α-helixes
(lose of α1and α2)

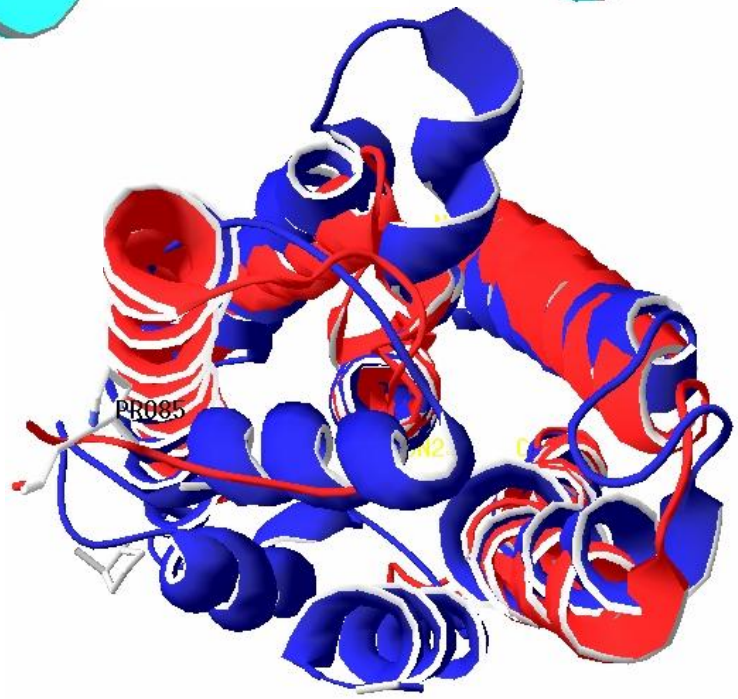
- Domain I(top view)



Cry4Aa
7 α-helixes

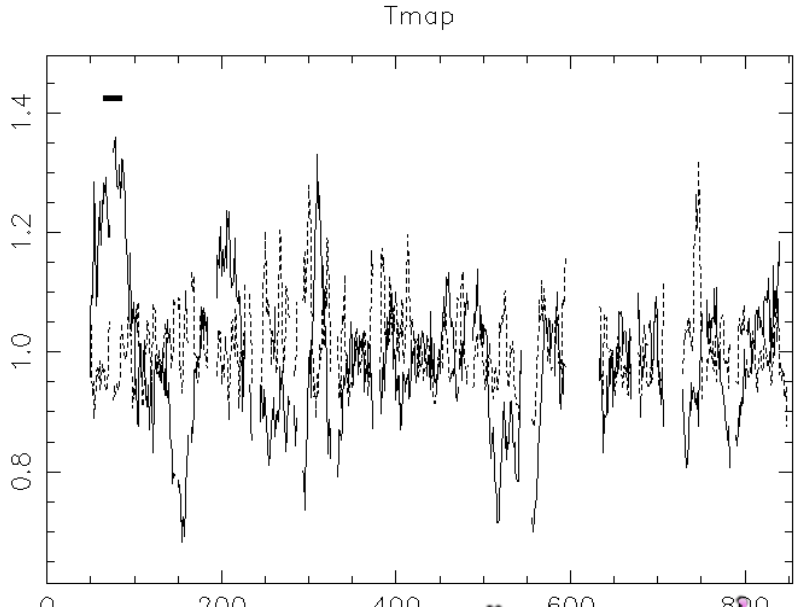


Cry4Ba
5 α-helixes



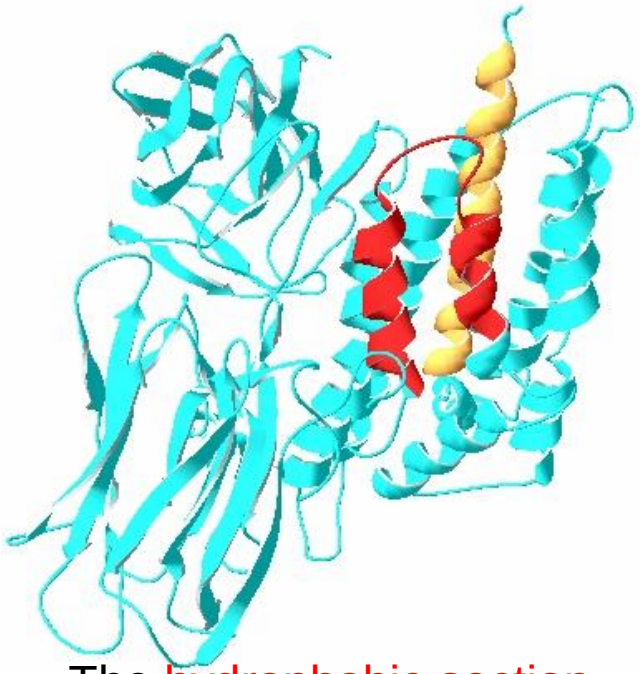
- α-4 and α-5 are the keys to pore-forming in the midgut membrane, maybe because of their high hydrophobicity

- T-map of Cry4Aa toxin(by Weblab)

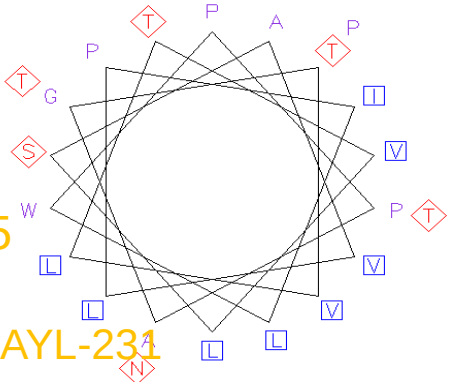


This amino acid sequence is of a **MEMBRANE PROTEIN** which have 1 transmembrane helix.

No.	N terminal	transmembrane region	C terminal	type	length
1	14	VGTVLTGFGFTTPLGLALIGFGT	36	PRIMARY	23



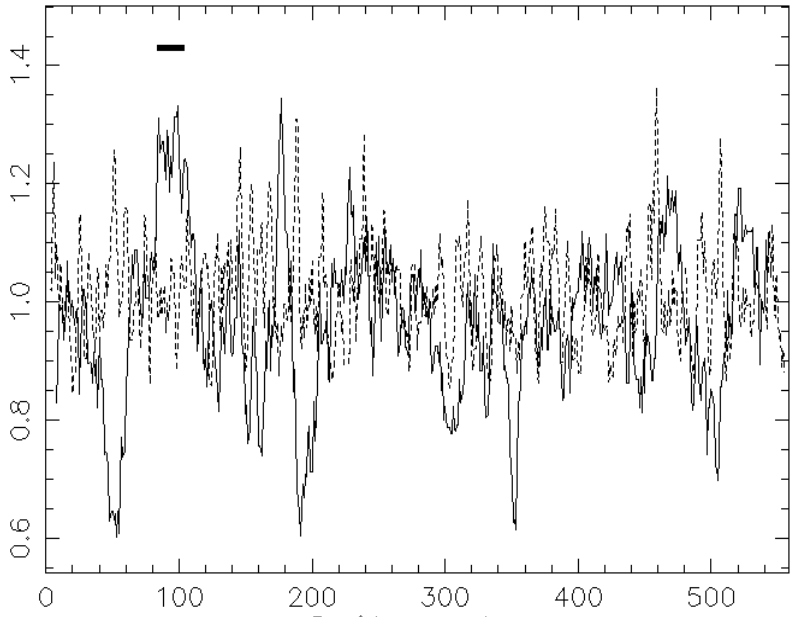
The hydrophobic section located in the α-1 and α-2



The helical wheel of Cry4Aa α-5 shows its hydrophobicity.

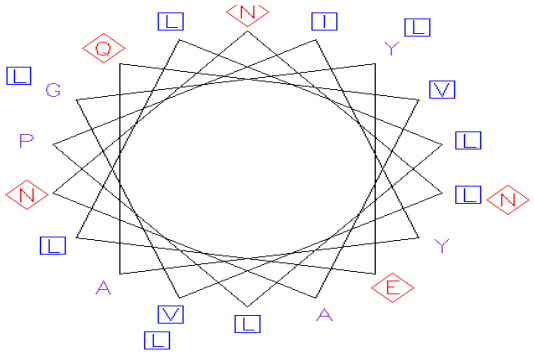
208-SSYAQAANLHLTVLNQAVKFEAYL-231

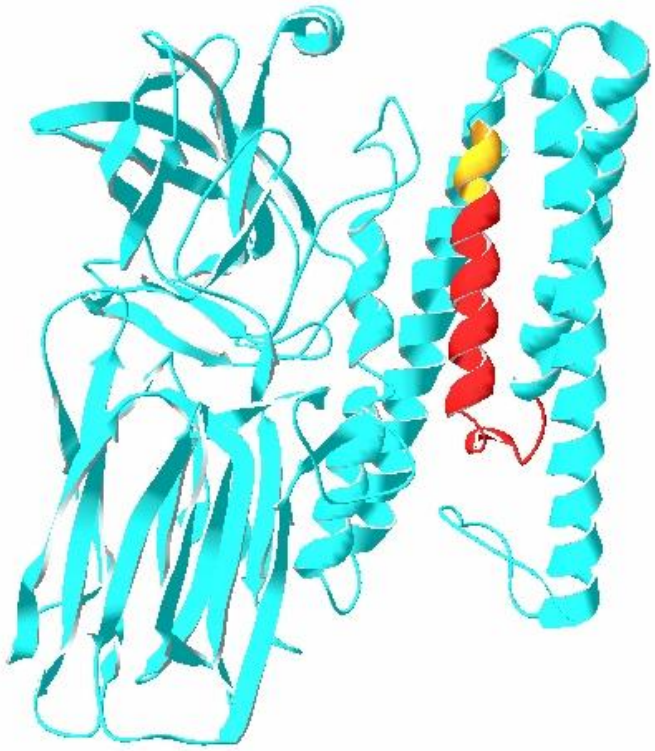
- T-map of Cry4Ba toxin(by Weblab)



This amino acid sequence is of a MEMBRANE PROTEIN which have 1 transmembrane helix.

No.	N terminal	transmembrane region	C terminal	type	length
1	4	NLVGYELLLLPIYAQVANFNLLL	26	PRIMARY	23



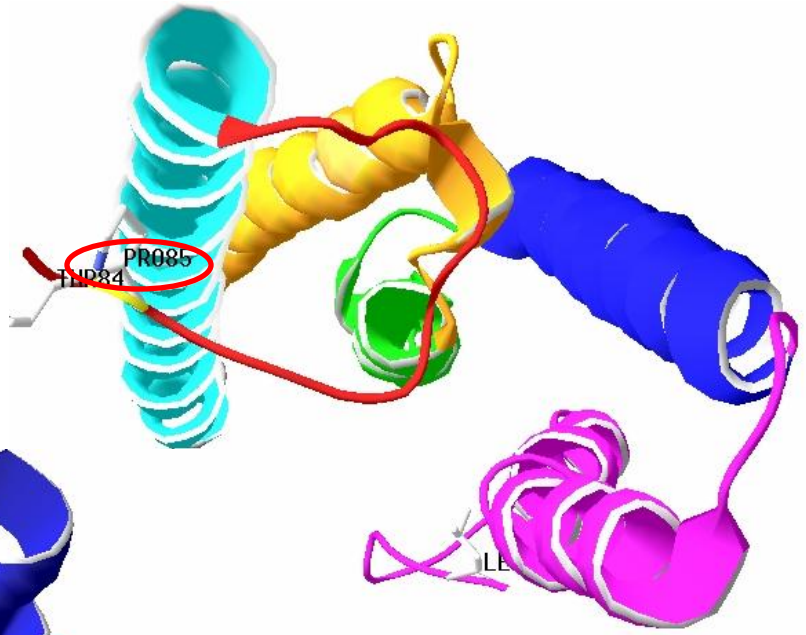


The hydrophobic section located in the α-5

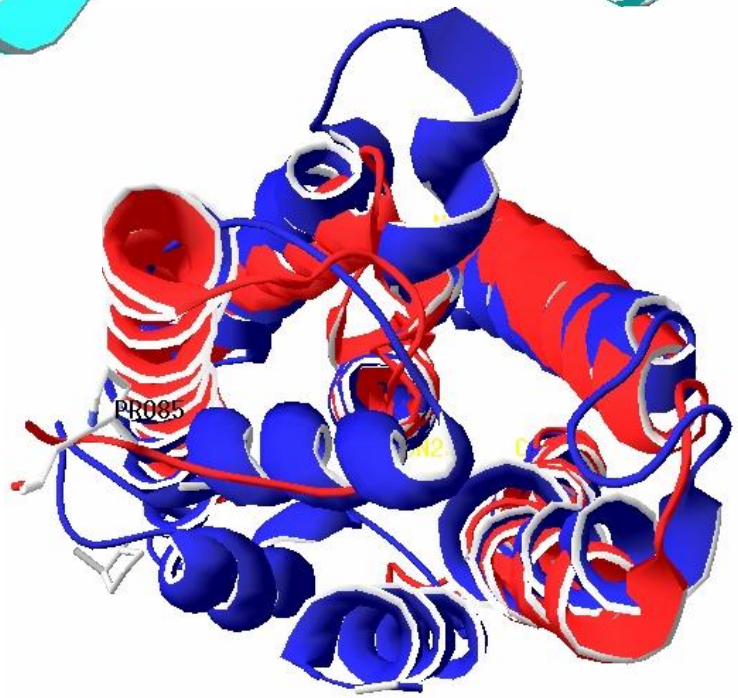
Conclusion4 :α-5 are highly hydrophobic

- Domain I(top view)

Cry4Aa
7 α-helixes



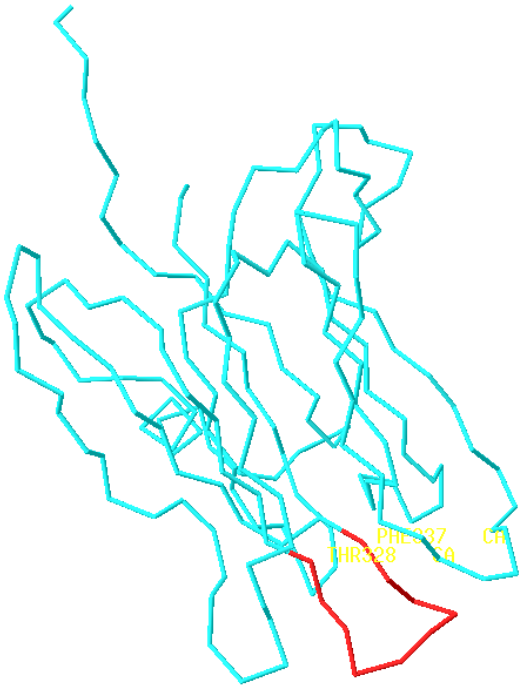
Cry4Ba
5 α-helixes

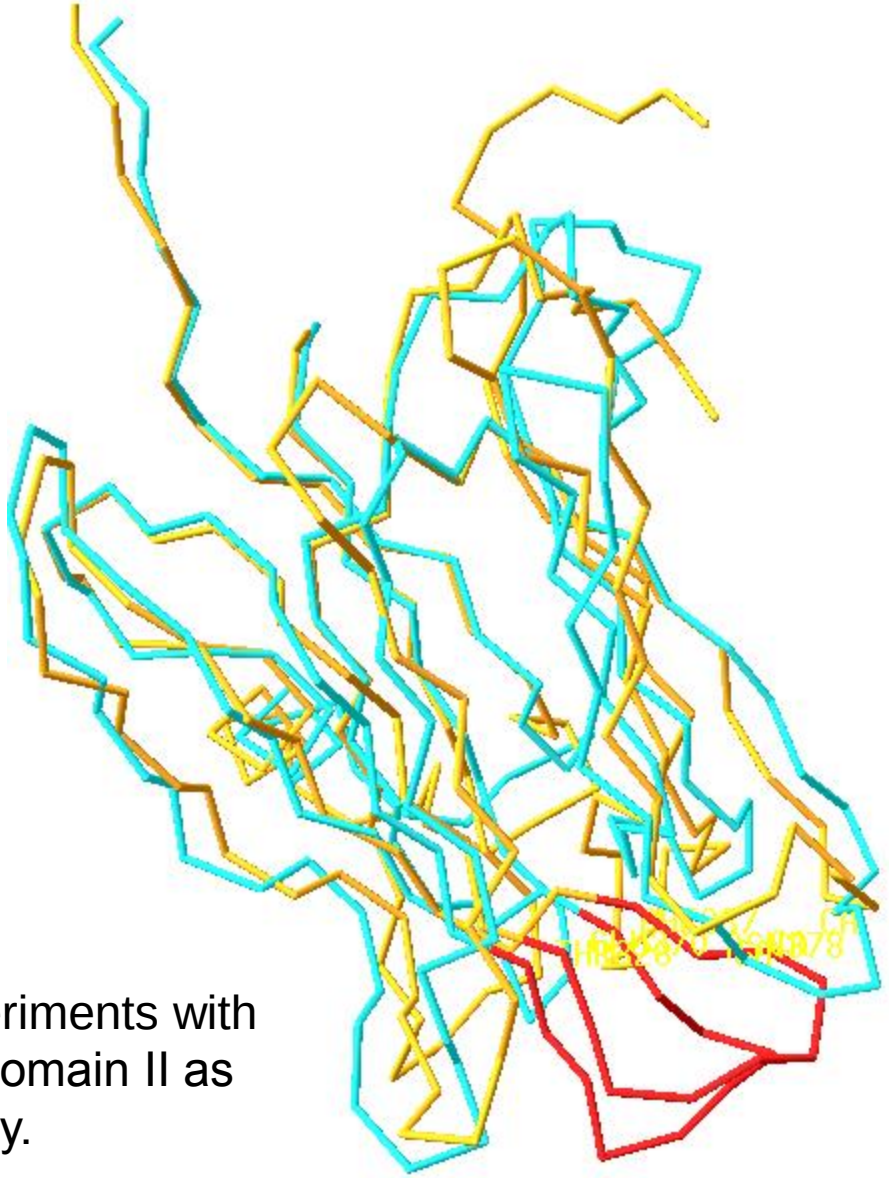


The α-1 and α-2 maybe not necessary for the toxicity of Cry toxin

Conclusion 5: the conserved Pro may play a important role as the link of the "lip", and maybe involved in the stability of Cry toxin. Shuyuan Guo, et al. *Journal of Structural Biology.* 168 (2009) 259–266

- Domain II(side view)





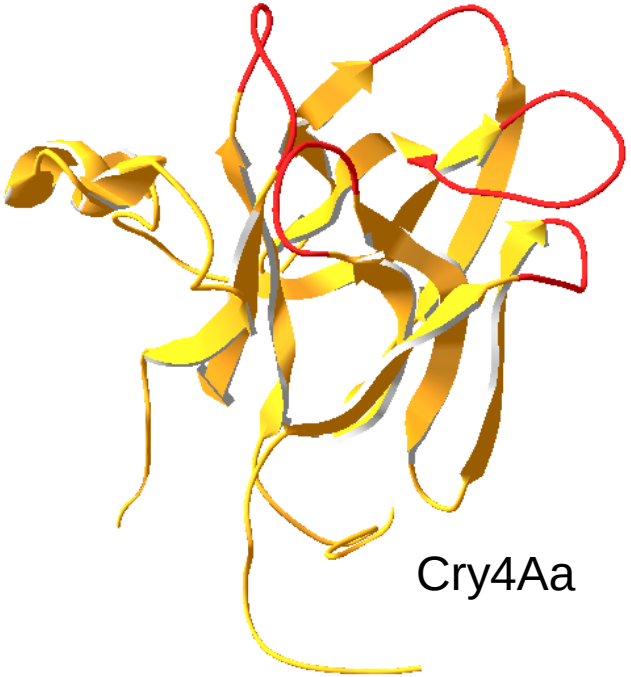
Cry4Aa

Cry4Ba

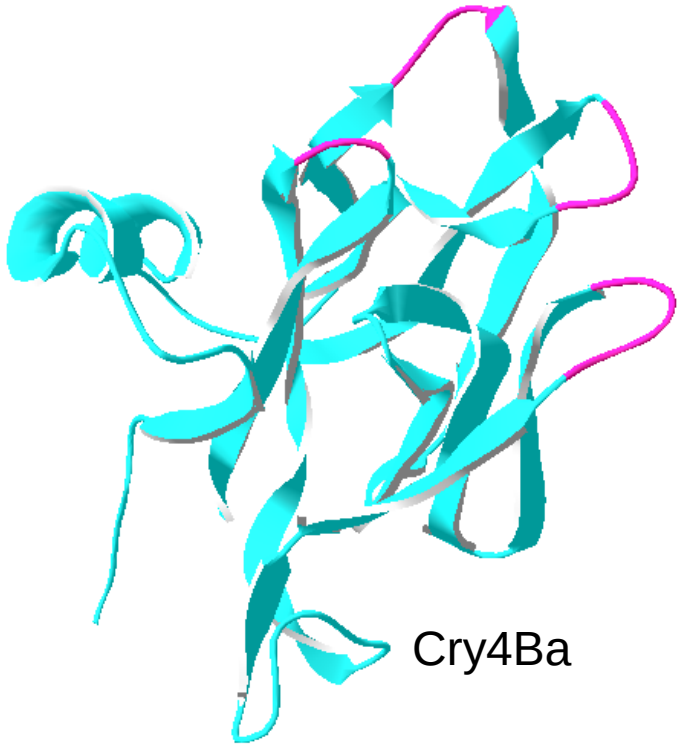
Mutagenesis and loop swapping experiments with Cry toxins have identified regions of domain II as major determinants of insect specificity.

Poncet, S. *et al. J. Invertebr. Pathol.*1995,66:131–135.

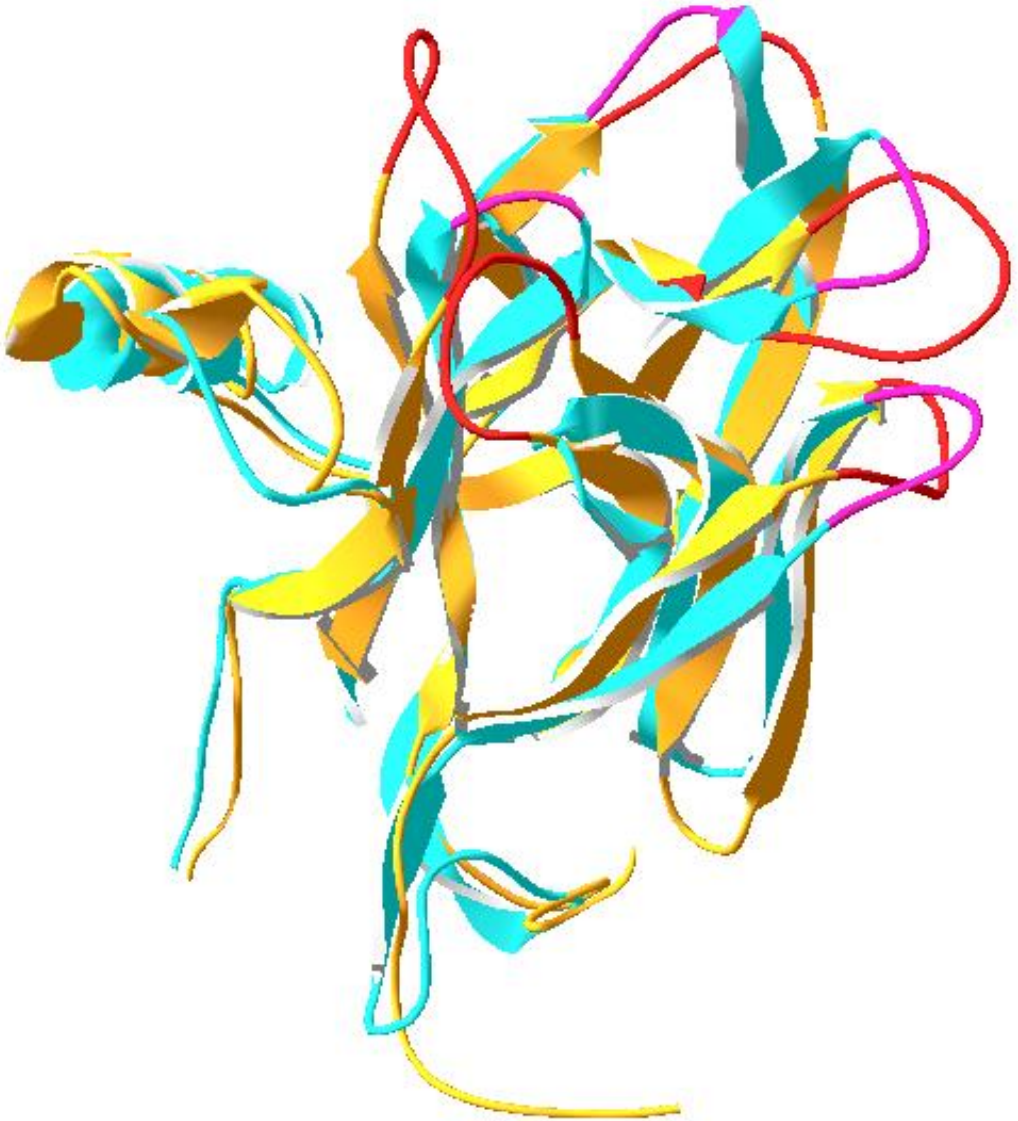
Abdullah, M. A, et al. *Appl. Environ. Microbiol.* 2003,69:5343–5353.



Cry4Aa



Cry4Ba

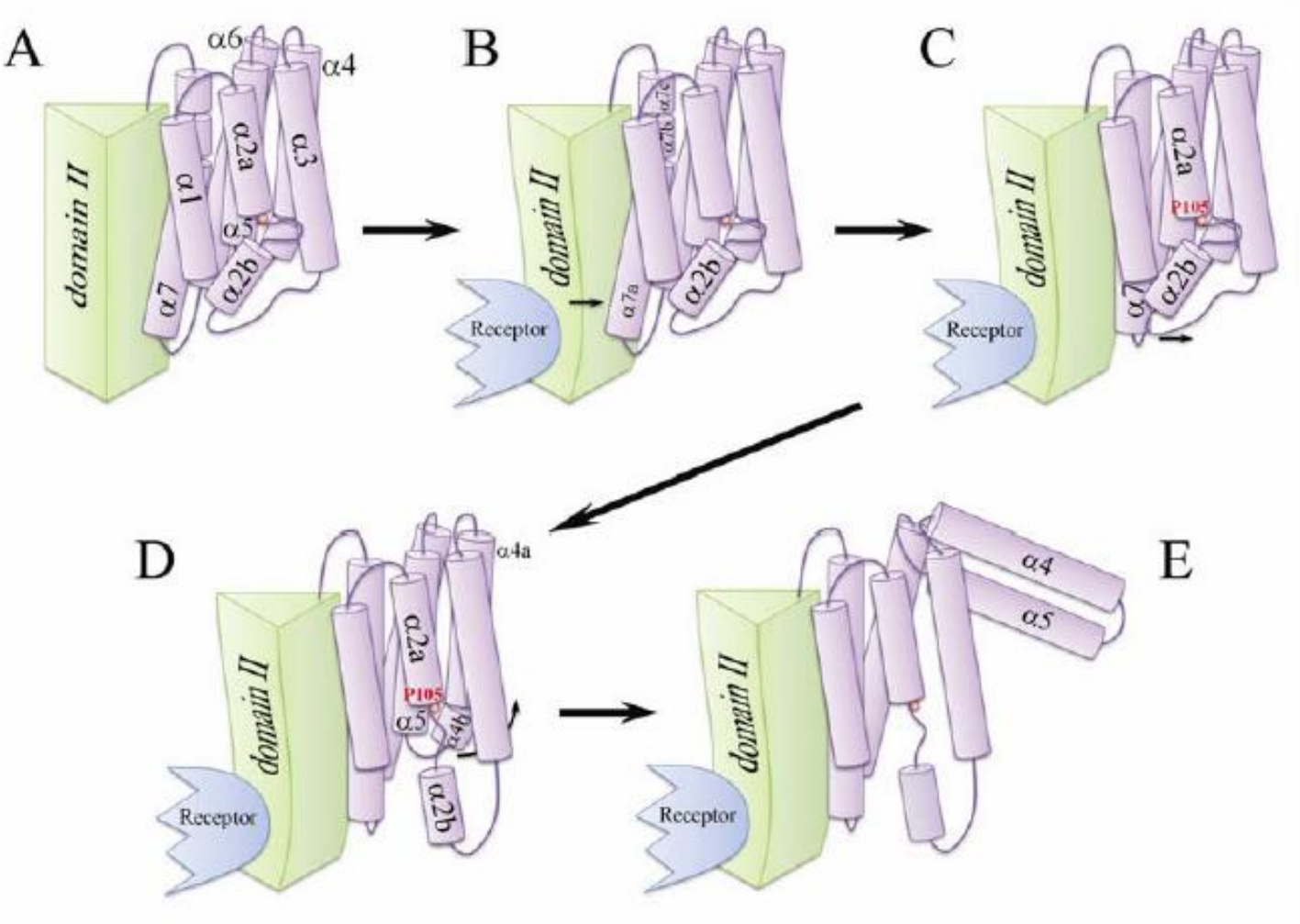


Conclusion 6: **The loops in domain II major determine the insect specificity.**

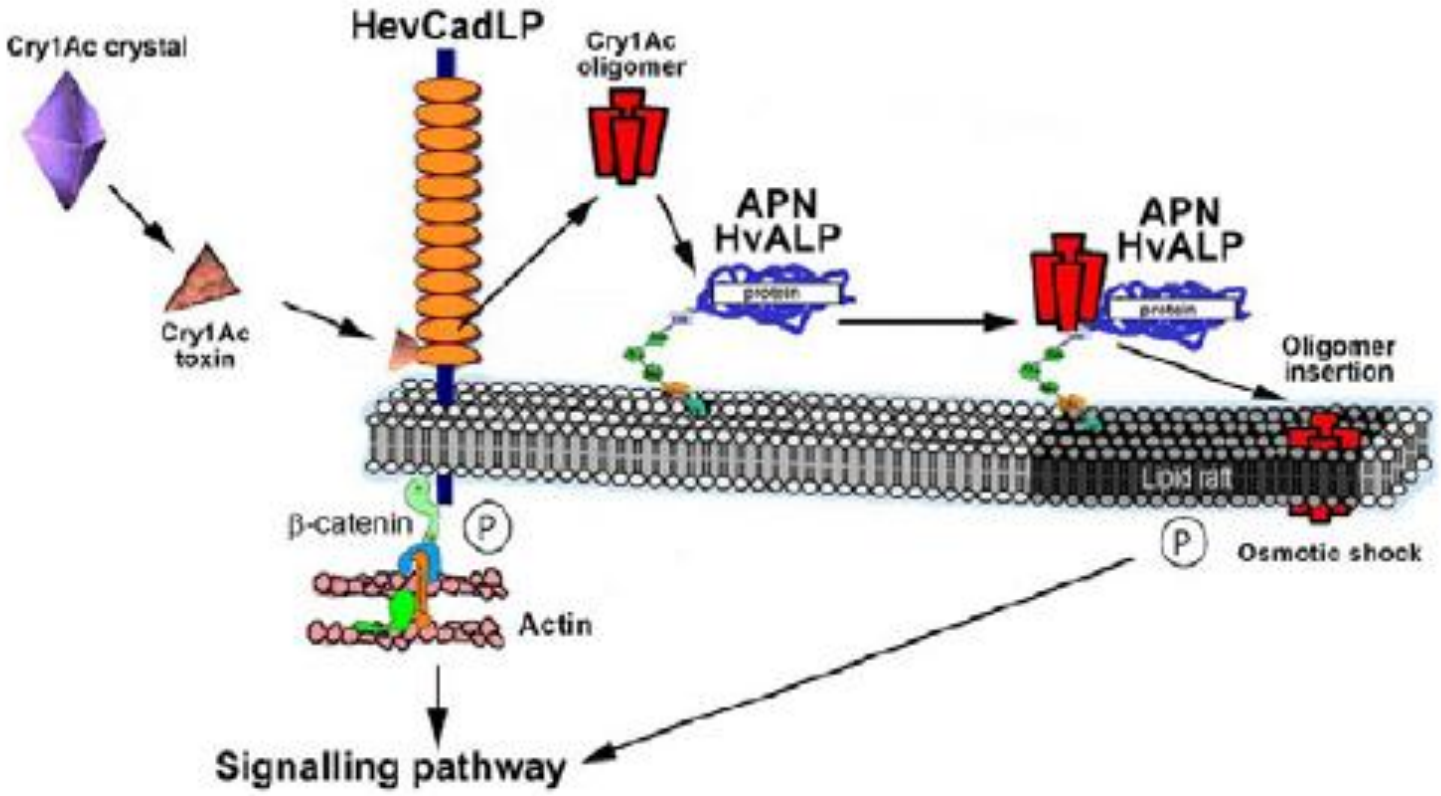
Summary:

- **The acidity of the Cry proteins make it easier to dissolve in the midgut where is alkaline.**
- **The 3D structures of the Cry toxins share high similarity.**
- **The conserved sections located in the center of molecular or the interfaces between 2 domains. Maybe important for the structure and stability.**
- **α-5 are highly hydrophobic thus it can easily insert into the midgut membrane**
- **The conserved Pro play a important role as the link of the "lip" which may protect the α-5 .**
- **The loops in domain II major determine the insect specificity and activity.**

- Details of the toxic mechanism



Shuyuan Guo, et,al. *Journal of Structural Biology* 168 (2009) 259–266



Jurat-Fuentes J.L. ,Adang M.J . *Journal of Invertebrate Pathology* 2006,92(3):166～171.

Acknowledgements

This work was done by the hands of my team members. Thank them for their hardworking and help.

Appreciate my classmates for their meaningful suggestions.

Pro. Jingchu Luo guided me to the gate of bioinformatics, thank him very much!

Thank you!