

南方根结线虫乙酰胆碱酯酶基因克隆 及其与噻唑磷抗药性分析

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一、研究背景

南方根结线虫(*Meloidogyne incognita*)是危害北方地区大棚蔬菜(黄瓜、番茄、芹菜等)的主要线虫,可以引起巨大的产量损失。其危害方式是线虫通过食道腺分泌水解酶类等,通过线虫口针分泌到宿主细胞中,破坏寄主细胞的正常代谢功能而产生病变,根系受害后形成瘤状根结。



★目前，噻唑磷(fosthiazate)等是使用较多的有机磷类杀线虫剂，其作用机制是抑制线虫神经系统传导中乙酰胆碱酯酶（Acetylcholinesterase，简称Ache)的活性，从而使神经元间神经递质乙酰胆碱无法分解成胆碱和乙酸，乙酰胆碱在突触的积累，使神经长时间的兴奋或者抑制，线虫正常的神经传导被破坏。

★但是，目前我们发现一些地区的南方根结线虫已对噻唑磷产生抗药性。



所以针对抗药性的产生，我们对南方根结线虫乙酰胆碱酯酶进行基因克隆，从基因序列、蛋白结构和酶活性的差异上进行研究，从分子水平上分析抗药性产生的原因，从而达到有效防治南方根结线虫的作用。



二、基因克隆

以Acetylcholinesterase *Meloidogyne incognita*为关键词搜索UniProt数据库

Search in Protein Knowledgebase (UniProtKB) Query Acetylcholinesterase Meloidogyne incognita Search Advanced Search » Clear

3 results for Acetylcholinesterase AND Meloidogyne AND incognita in UniProtKB sorted by score descending

[Browse by taxonomy, keyword, gene ontology, enzyme class or pathway](#) | [Reduce sequence redundancy to 100%, 90% or 50%](#)

Download

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Results [Customize](#)

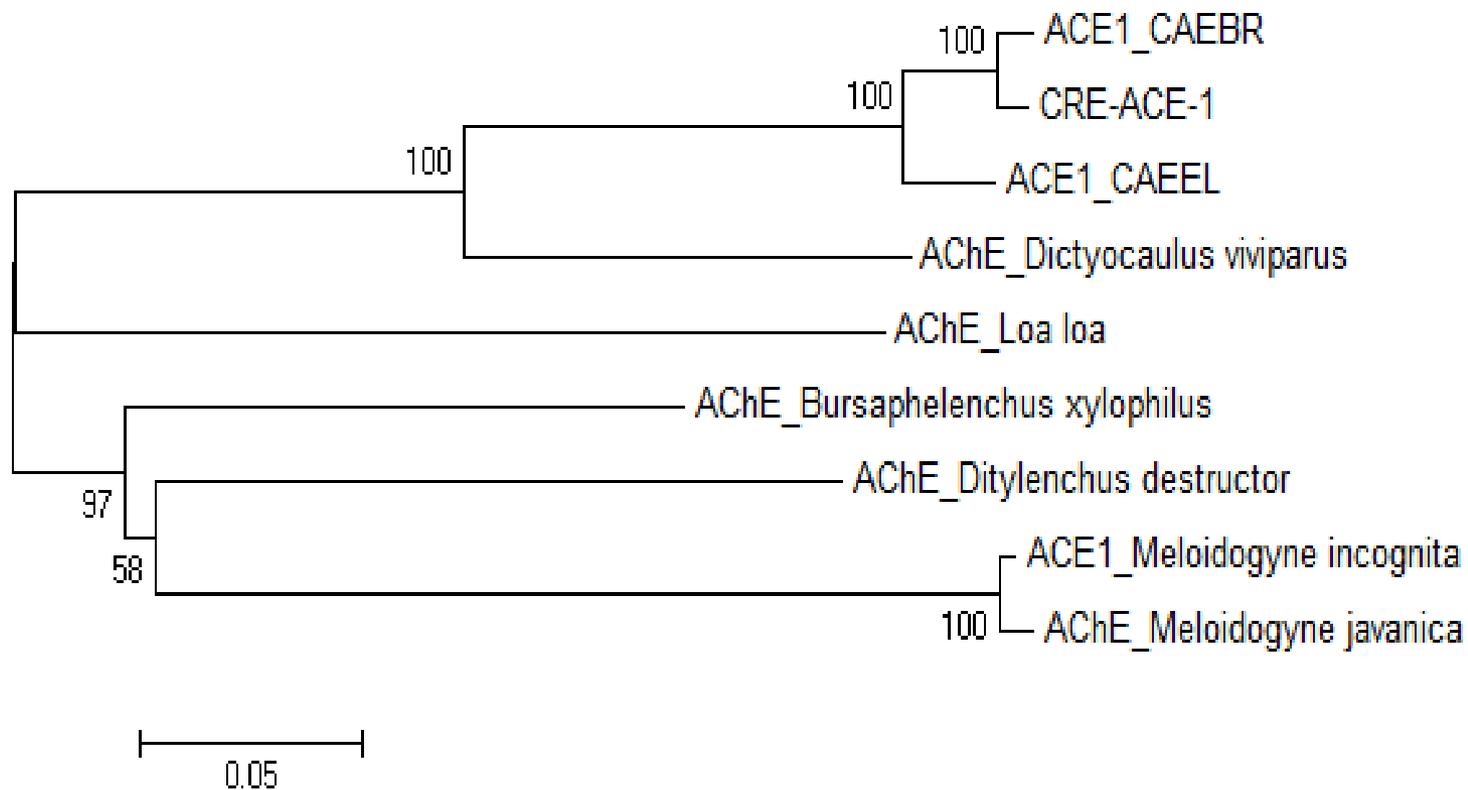
- › Quote terms: "meloidogyne incognita"
- › Restrict term "acetylcholinesterase" to [gene ontology](#) (2)
- › Restrict term "incognita" to [organism](#) (2)

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
<input type="checkbox"/> O96529	O96529_MELIC	★	Acetylcholinesterase	ACHE ace-1	Meloidogyne incognita (Southern root-knot nematode)	656
<input type="checkbox"/> Q9XYA9	Q9XYA9_MELJA	★	Acetylcholinesterase	ACHE	Meloidogyne javanica (Root-knot nematode worm)	656
<input type="checkbox"/> Q71JB7	Q71JB7_MELIC	★	Acetylcholinesterase	ace-2	Meloidogyne incognita (Southern root-knot nematode)	686

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系统发育树

Neighbor-Joining tree法构建



Primer premier5.0引物设计

The screenshot displays the Primer Premier 5.0 software interface. The main window shows a DNA sequence editor with a sequence: 3'GGCGTACACCCACTTAAGGTCA 5' and 5'CGCCAGTGTGGTAATCCAGTTTAAAGTTTGTGCAATTTATTATTGTTTTTTCACCAATTTAAATATGCG 3'. A search criteria dialog box is open, showing the following settings:

- Search For: PCR Primers, Sequencing Primers, Hybridization Probes
- Search Type: Sense Primer, Compatible with Sense Primer, Anti-sense Primer, Compatible with Anti-sense Primer, Both, Pairs
- Search Ranges: Sense Primer: 1 to 2133; Anti-sense Primer: 1 to 2133; PCR Product Size: 100 bp to 500 bp
- Primer Length: 21 bp to 2 bp
- Search Mode: Automatic, Manual

The background window shows a table of search results:

	Rating	Seq No	Length	Tm [°C]	GC%	ΔG [kcal/mol]	Actn [μg/°C]
Sense	36	1	25	71.9	56.0	-50.9	32.6
Anti-sense	38	25	25	71.9	56.0	-50.9	32.9
Product	0	--	25	72.1	56.0	--	--

	Hairpin	Dimer	False Priming	Cross Dimer	Most Stable Hairpin ΔG = -1.9 [kcal/mol]
Sense	Found	Found	Found	Found	GGTGGTGGACCG
Anti-sense	Found	Found	Found		AATTCCAAGT 3'

Primer Premier

Primer: [SA] [Search] [Results] [Edit Primers]

Direct Select:

```

5' CCGCCAGTGGTGGAAATTC AAGT 3'
|||||
3' GCGGTCACACCACCTT AAGGTTCAAATCA

```

10 20 30

P P V W W N S K F K F

	Rating	Seq No	Length
Sense	36	1	25
Anti-sense	38	25	25
Product	0	--	25

	Hairpin	Dimer	False Priming
Sense	Found	Found	Found
Anti-sense	Found	Found	Found

Search Results

Sense Anti-sense Pairs

1058 sense primers found.

#	Rating	Seq No	Length	Tm	GC%	Mark
39	91	185	19	48.4	52.6	<input type="checkbox"/>
40	91	387	19	51.5	47.4	<input type="checkbox"/>
41	91	389	19	43.5	42.1	<input type="checkbox"/>
42	91	643	19	41.5	31.6	<input type="checkbox"/>
43	91	1227	19	48.3	36.8	<input type="checkbox"/>
44	91	1299	19	37.9	26.3	<input type="checkbox"/>
45	91	1469	19	51.3	36.8	<input type="checkbox"/>
46	91	1641	19	48.5	42.1	<input type="checkbox"/>
47	91	1707	19	44.1	31.6	<input type="checkbox"/>
48	90	75	19	42.1	21.1	<input type="checkbox"/>
49	90	139	19	54.4	47.4	<input type="checkbox"/>
50	90	179	19	44.5	36.8	<input type="checkbox"/>
51	90	295	19	56.9	52.6	<input type="checkbox"/>

TTGGGCAAAAT
TGAITGGCCCT
TTATATACAA
TTGGAAATTT
ATGGAARCAA
TGAGCAATAT
TTGIGGAATA
GTTCTACACA

三、蛋白质序列分析

Number of amino acids: 656

Molecular weight: 75962.3

Theoretical pI: 5.93

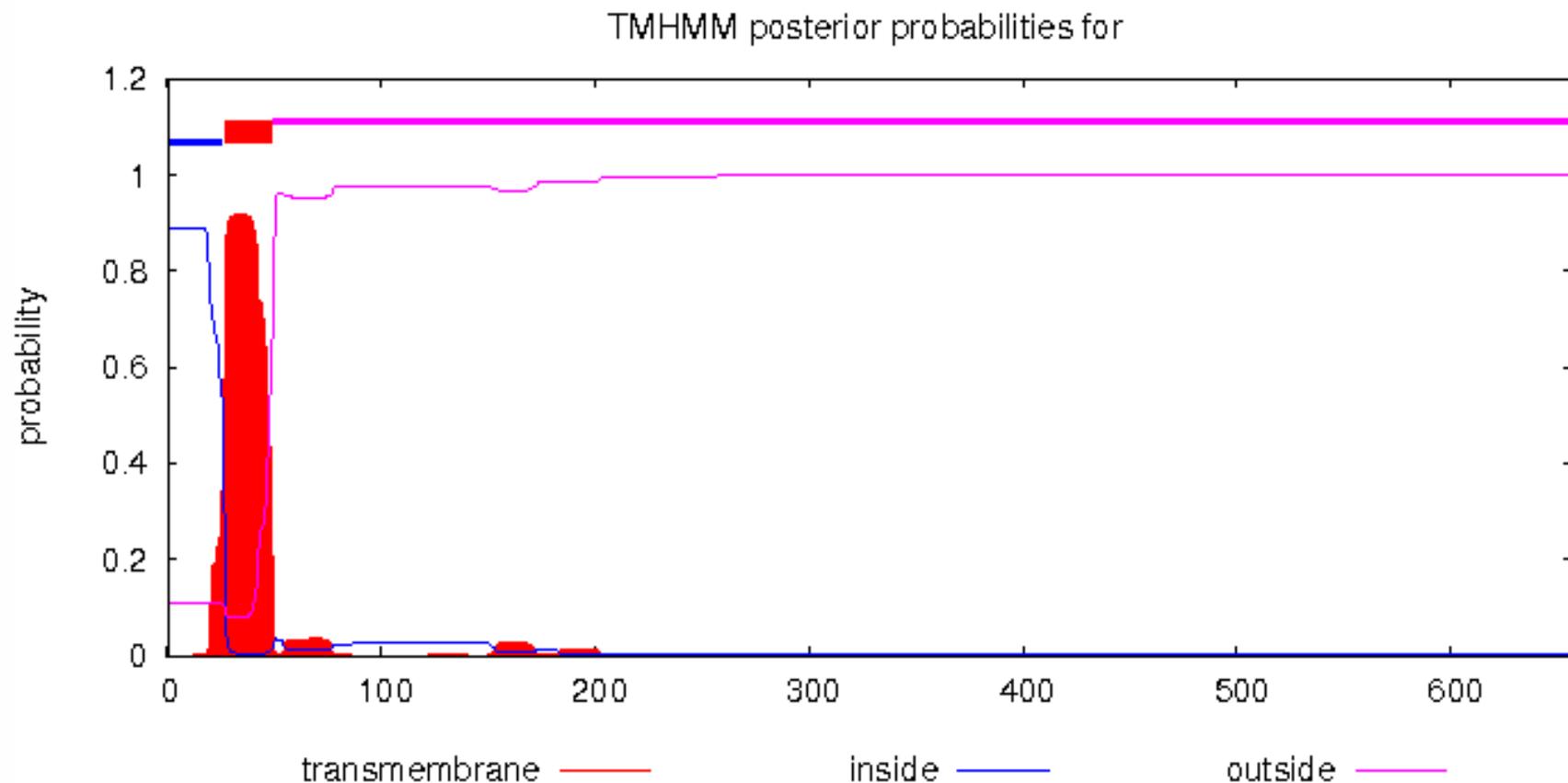
Amino acid composition: CSV format

Ala (A)	37	5.6%
Arg (R)	33	5.0%
Asn (N)	34	5.2%
Asp (D)	36	5.5%
Cys (C)	9	1.4%
Gln (Q)	24	3.7%
Glu (E)	41	6.2%
Gly (G)	43	6.6%
His (H)	12	1.8%
Ile (I)	38	5.8%
Leu (L)	52	7.9%
Lys (K)	36	5.5%
Met (M)	18	2.7%
Phe (F)	44	6.7%
Pro (P)	35	5.3%
Ser (S)	50	7.6%
Thr (T)	23	3.5%
Trp (W)	21	3.2%
Tyr (Y)	34	5.2%
Val (V)	36	5.5%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

protparam软件预测Ache蛋白质的理化性质

- ◇带负电的氨基酸(Asp + Glu) 总数为77个
- ◇带正电的氨基酸(Arg + Lys) 总数为69个
- ◇亲水性平均系数为-0.344, 说明此蛋白质为亲水性蛋白

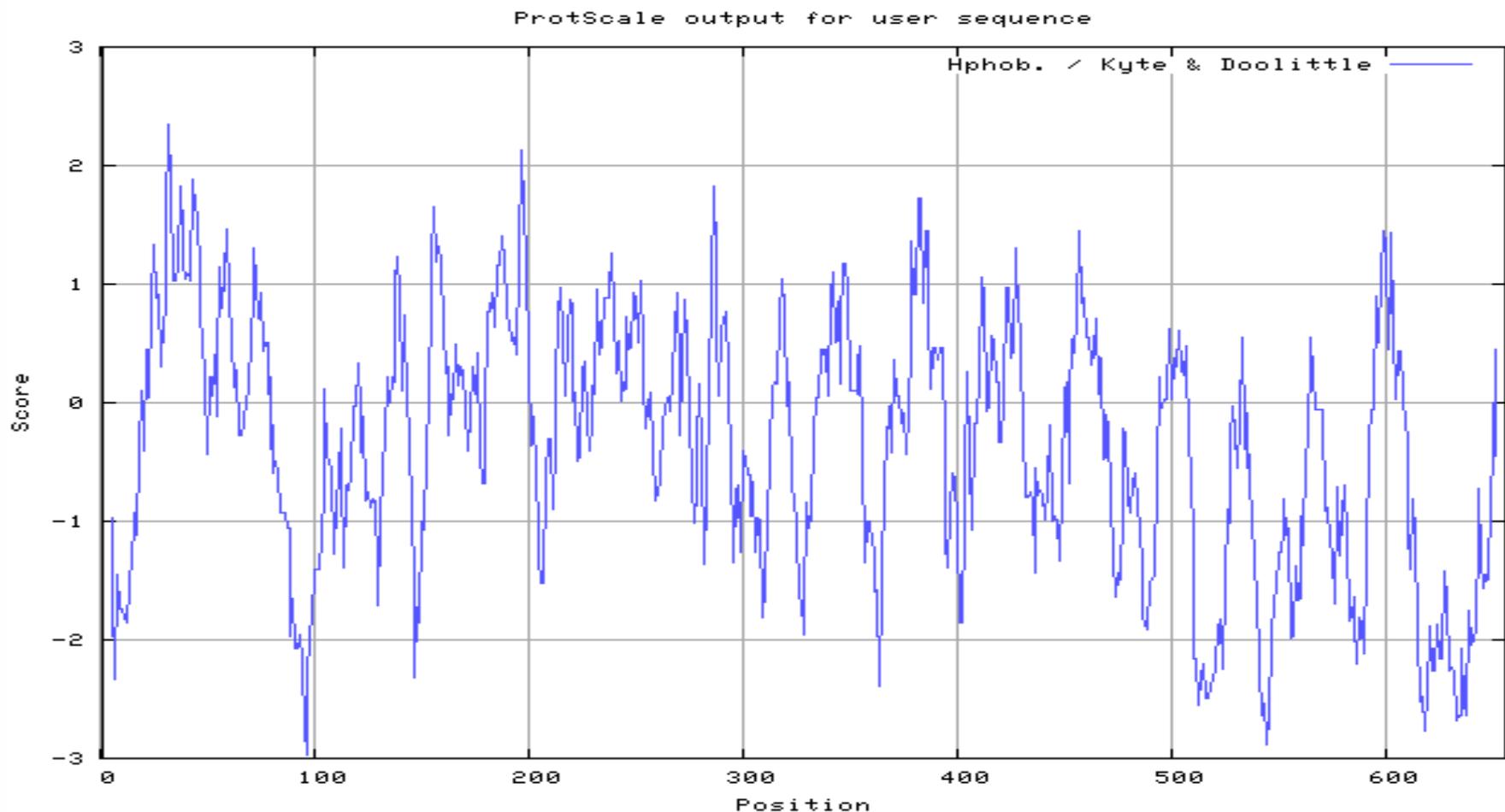
TMHMM Server v. 2.0程序预测跨膜结构



红色表示跨膜区，共23个氨基酸序列，从第27个氨基酸开始到第49个氨基酸结束



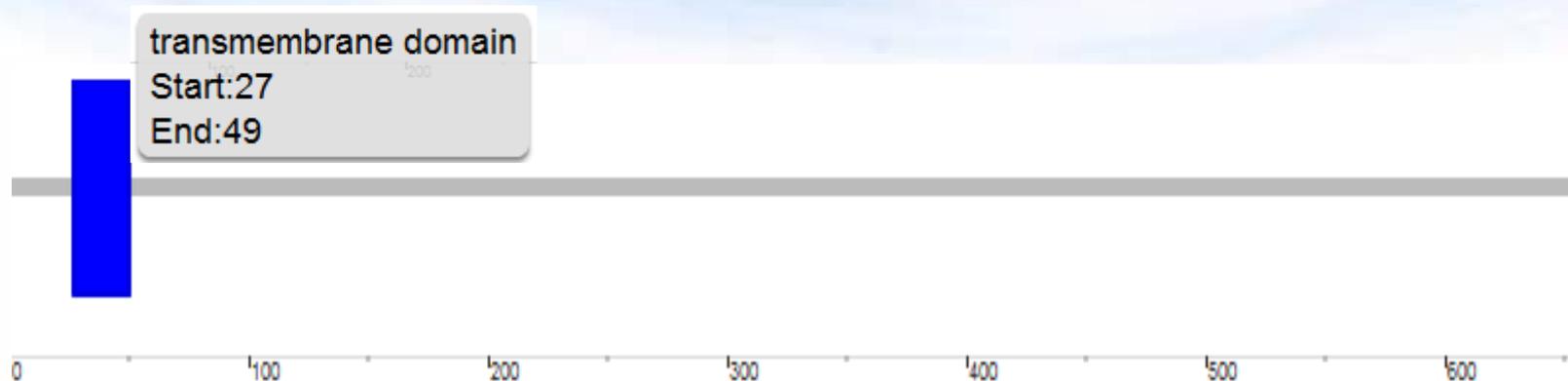
protscale软件进行疏水性分析



氨基酸分值越低亲水性越强的规律推测蛋白疏水性



SMART结构功能域的预测



Information	
Length	656 aa
Source database	UniProt
Identifiers	O96529_MELIC, O96529

只有一个跨膜结构域，长度为23个氨基酸



四、蛋白三维结构模型构建和分析

swiss-model进行同源模建

进入swiss model workspace, 提交蛋白质的序列, 根据Automated Mode同源模建找到

[myWorkspace]

[login]

Workunit: P000003 Ache protein model - Overview



Print/Save this page as 

Model Summary



Model information:

Modelled residue range: 43 to 603
Based on template: [3i6mA] (2.26 Å)
Sequence Identity [%]: 41.95
Evaluate: 0.00e-1

Quality information: [details]▼

QMEAN Z-Score: -3.78



Quaternary structure information: [details]▼

Template (3i6m): DIMER
Model built: SINGLE CHAIN

Ligand information: [details]▼

Ligands in the template: G3X: 1, NAG: 1.
Ligands in the model: none.

logs: [Templates]▼ [Alignment]▼ [Modeling]▼

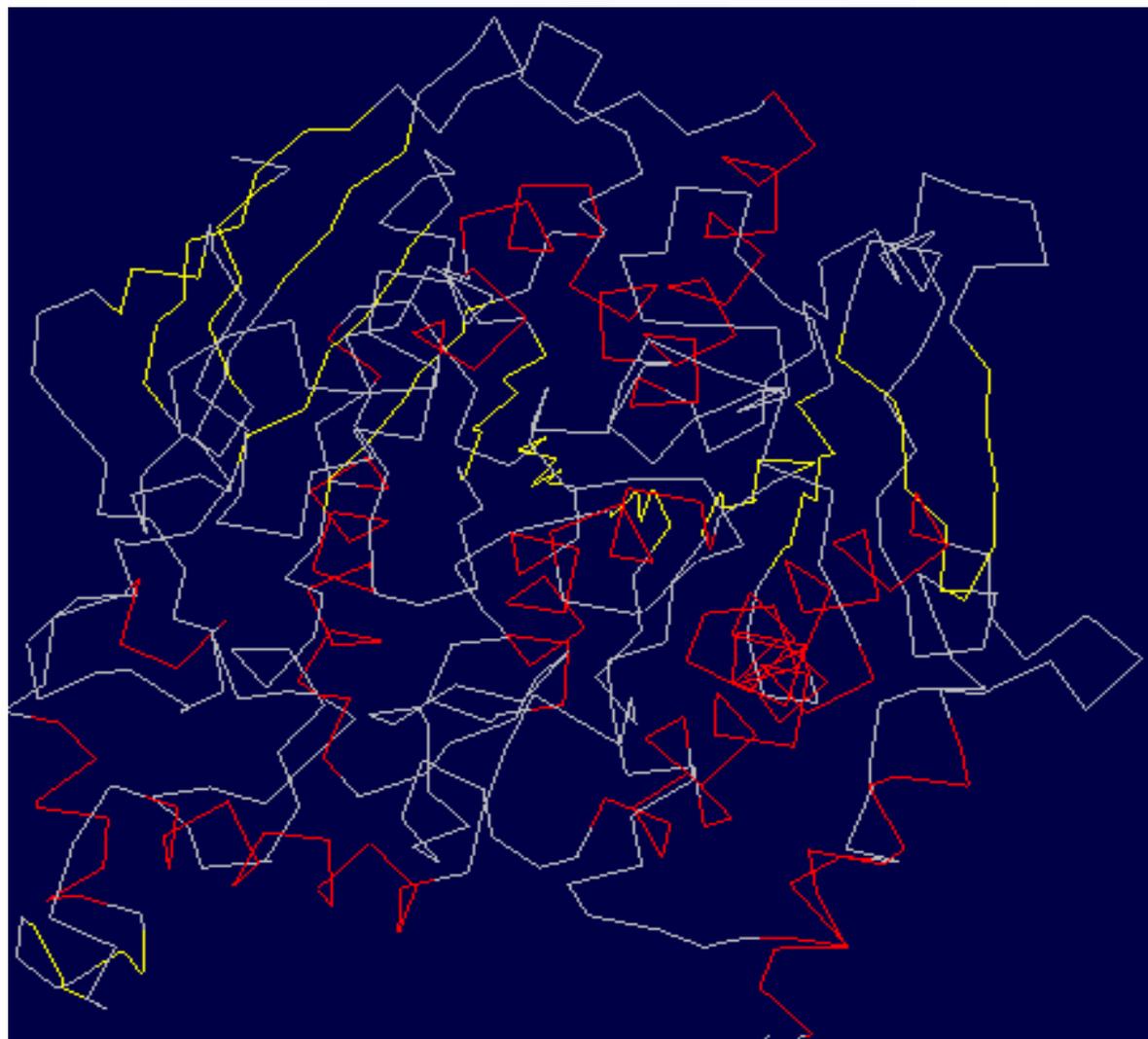
display model: as [pdb]▼ - as [DeepView project]▼ - in [AstexViewer]▼

download model: as [pdb]⚡ - as [Deepview project]⚡ - as [text]⚡



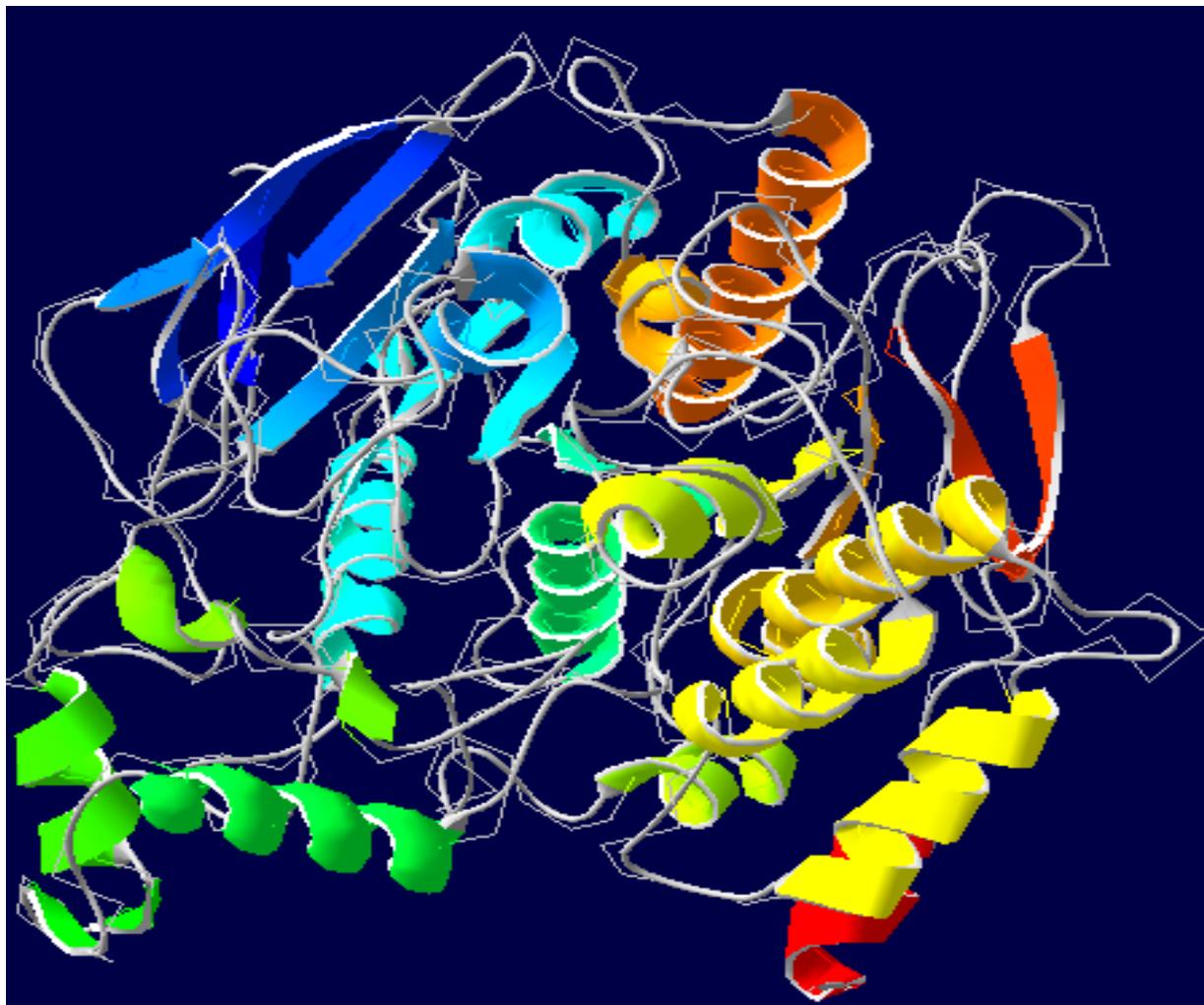
Swiss-PDB Viewer进行三维结构预测

color-----Secondary Structure



Color---SecondaryStructureSuccession

整个序列的每个二级结构用不同的颜色显示出来



以后将要进行的工作

- 利用材料克隆出敏感种群和抗性种群的基因
- 比较抗性种群和敏感种群在基因序列差异和蛋白序列差异
- 比较敏感种群和抗性种群乙酰胆碱酯酶的活力和对噻唑磷产生抗药性影响



致谢

- 谢谢罗老师为我们打开了生物信息技术的大门，面对海量的生物信息数据、软件和程序我们不再迷茫
- 谢谢G01小组的各位成员
- 谢谢大家



The end, thank you!

