猪繁殖与呼吸综合征病毒非结构蛋白NSP1β的PCPβ活性分析

CAAS11S G16

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LOGO

提纲

- 研究背景
- 序列比对与分析
- 系统进化分析
- 结构分析
- 参考文献

研究背景

PRRSV

>尼多病毒目

>>动脉炎病毒科

>>>动脉炎病毒属

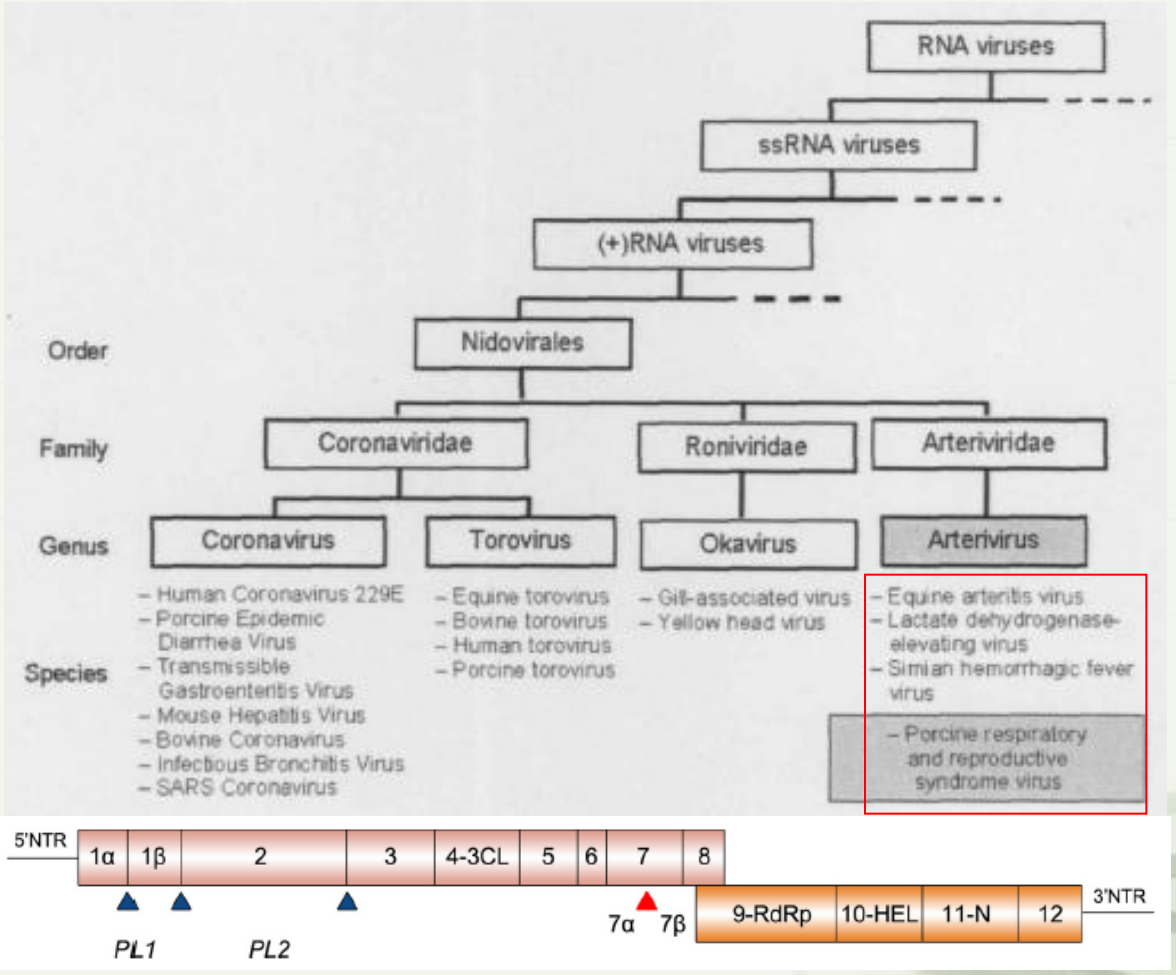
LV----欧洲株原型

VR2332----美洲株原型

EAV----马动脉炎病毒

SHFV----猴出血热病毒

LDV----乳酸脱氢酶增高症病毒



NSP1β是在病毒侵入宿主细胞后复制起始时产生的，通过自身的C 末端PCP 结构域从NSP2下游自动切割下来产生的。已有研究表明，该蛋白在病毒的生命循环及其毒力维持方面具有重要作用。

ELSEVIER

Virology

Volume 380, Issue 2, 25 October 2008, Pages 371–378

Identification of virulence determinants of porcine reproductive and respiratory syndrome virus through construction of chimeric clones

Byungjoon Kwon, Israrul H. Ansari, Asit K. Pattnaik, Fernando A. Osorio

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除此之外，已有报道NSP1β还具有抑制感染细胞内先天性免疫反应信号传导的作用，而NSP1β的结构在PDB中已经存在，在此我们试图对其结构及其蛋白酶活性进行分析。

The Crystal Structure of Porcine Reproductive and Respiratory Syndrome Virus Nonstructural Protein Nsp1β Reveals a Novel Metal-Dependent Nuclease[▽]

Fei Xue,[1]† Yuna Sun,[2]† Liming Yan,[1] Cong Zhao,[1] Ji Chen,[1] Mark Bartlam,[3] Xuemei Li,[2] Zhiyong Lou,[1]* and Zihe Rao[1,2,3]

Porcine Reproductive and Respiratory Syndrome Virus Inhibits Type I Interferon Signaling by Blocking STAT1/STAT2 Nuclear Translocation[▽]

Deendayal Patel,[1]†‡ Yuchen Nan,[1]‡ Meiyan Shen,[1]§ Krit Ritthipichai,[1] Xiaoping Zhu,[2] and Yan-Jin Zhang[1]*

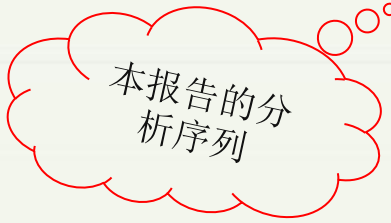
序列比对与分析

1. 序列检索

UniProtKB:

Arterivirus→ reviewed → Replicase polyprotein 1ab

登录号依次为： LV （Q04561）



HB-1 （Q8B912 ）

16244B （Q9YN02 ）

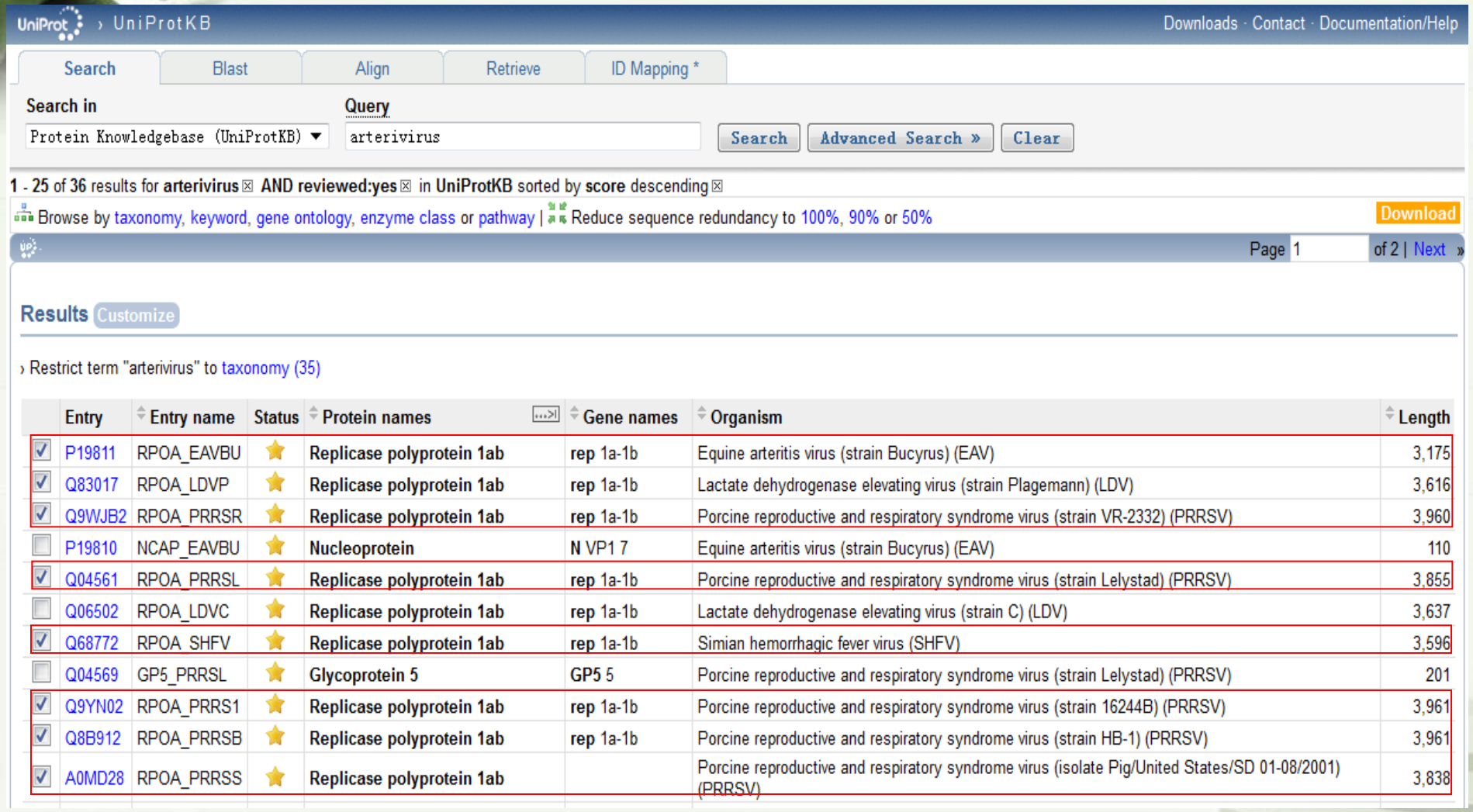
VR2332 （Q9WJB2 ）

USA/SD（A0MD28 ）

EAV （P19811 ）

SHFV （Q68772）

LDV （Q83017）



Entry	Entry name	Status	Protein names	Gene names	Organism	Length
P19811	RPOA_EAVBU	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Equine arteritis virus (strain Bucyrus) (EAV)	3,175
Q83017	RPOA_LDVP	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Lactate dehydrogenase elevating virus (strain Plagemann) (LDV)	3,616
Q9WJB2	RPOA_PRRSR	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Porcine reproductive and respiratory syndrome virus (strain VR-2332) (PRRSV)	3,960
P19810	NCAP_EAVBU	★	**Nucleoprotein**	**N** VP1 7	Equine arteritis virus (strain Bucyrus) (EAV)	110
Q04561	RPOA_PRRSL	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Porcine reproductive and respiratory syndrome virus (strain Lelystad) (PRRSV)	3,855
Q06502	RPOA_LDVC	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Lactate dehydrogenase elevating virus (strain C) (LDV)	3,637
Q68772	RPOA_SHFV	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Simian hemorrhagic fever virus (SHFV)	3,596
Q04569	GP5_PRRSL	★	**Glycoprotein 5**	**GP5** 5	Porcine reproductive and respiratory syndrome virus (strain Lelystad) (PRRSV)	201
Q9YN02	RPOA_PRRS1	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Porcine reproductive and respiratory syndrome virus (strain 16244B) (PRRSV)	3,961
Q8B912	RPOA_PRRSB	★	**Replicase polyprotein 1ab**	**rep** 1a-1b	Porcine reproductive and respiratory syndrome virus (strain HB-1) (PRRSV)	3,961
A0MD28	RPOA_PRRSS	★	**Replicase polyprotein 1ab**		Porcine reproductive and respiratory syndrome virus (isolate Pig/United States/SD 01-08/2001) (PRRSV)	3,838

*注：UniProtKB 中所得EAV、LDV、SHFV并非NSP1β，而是其前体包括NSP1α在内，于是我们便按照**Fei Xue et al, 2010, JVI** 文献中的序列进行了截短。*

以下截图为UniProt 中HB-1 的结构分析，存在一结构域肽酶_C32，起止位点为NSP1β的83和203位氨基酸，这与后面SMART的预测结果有差别。

Names · Attributes · General annotation · Ontologies · Alt products · Sequence annotation · Sequences · References · Cross-refs · Entry info · Documents · Customize order

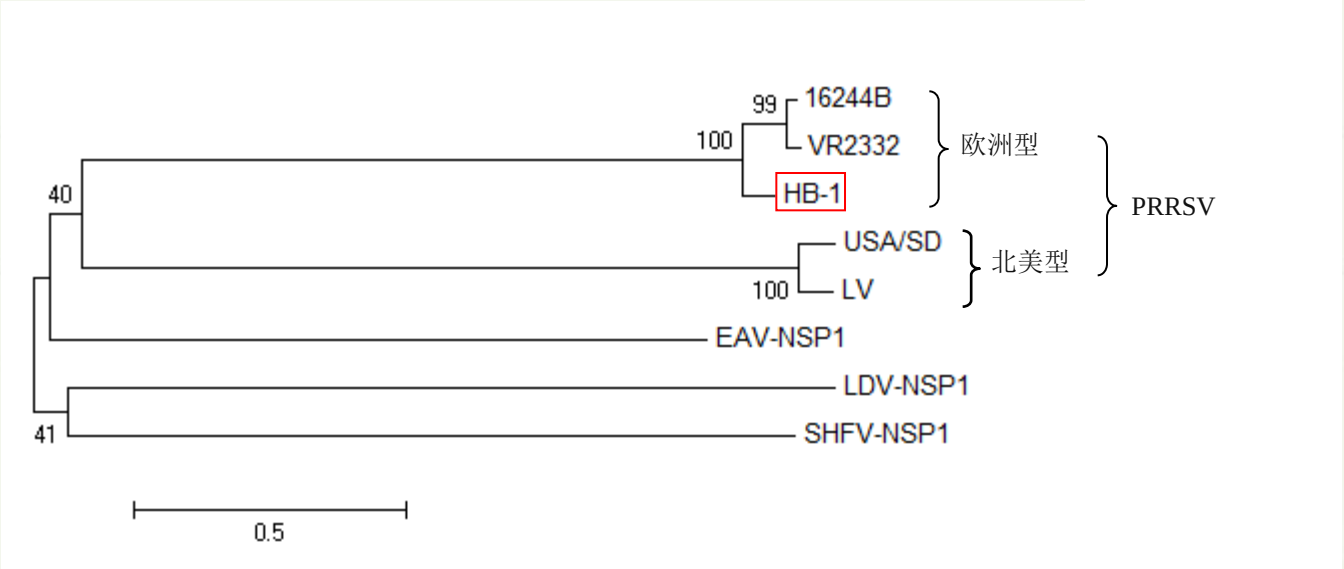
Regions

☐	Transmembrane	1266 – 1286	21	Helical; Potential	
☐	Transmembrane	1296 – 1316	21	Helical; Potential	
☐	Transmembrane	1368 – 1388	21	Helical; Potential	
☐	Transmembrane	1583 – 1603	21	Helical; Potential	
☐	Transmembrane	1648 – 1668	21	Helical; Potential	
☐	Transmembrane	1685 – 1705	21	Helical; Potential	
☐	Transmembrane	1719 – 1739	21	Helical; Potential	
☐	Transmembrane	2036 – 2056	21	Helical; Potential	
☐	Transmembrane	2060 – 2080	21	Helical; Potential	
☐	Transmembrane	2092 – 2112	21	Helical; Potential	
☐	Transmembrane	2137 – 2157	21	Helical; Potential	
☐	Transmembrane	2162 – 2182	21	Helical; Potential	
☐	Domain	69 – 180	112	Peptidase C31	
☑	Domain	263 – 383	121	Peptidase C32	
☐	Domain	428 – 535	108	Peptidase C33	
☐	Domain	1810 – 2013	204	Peptidase S32	
☐	Domain	2890 – 3024	135	RdRp catalytic	
☐	Domain	3290 – 3517	228	Helicase ATP-binding	
☐	Zinc finger	8 – 28	21	C4-type; atypical	
☐	Zinc finger	3151 – 3197	47	Viral helicase-type-like Potential	
☐	Nucleotide binding	3293 – 3300	8	ATP By similarity	
☐	Region	69 – 182	114	PCP1-alpha	
☐	Region	263 – 382	120	PCP1-beta	
☐	Region	426 – 513	88	OTU-like	
☐	Region	1266 – 1388	123	HD1	
☐	Region	1583 – 1745	163	HD2	
☐	Region	2036 – 2157	122	HD3	
☐	Compositional bias	808 – 924	117	Pro-rich	
☐	Compositional bias	2329 – 2344	16	Pro-rich	

系统进化分析

1. 进化树的构建

使用软件：MEGA 5.05



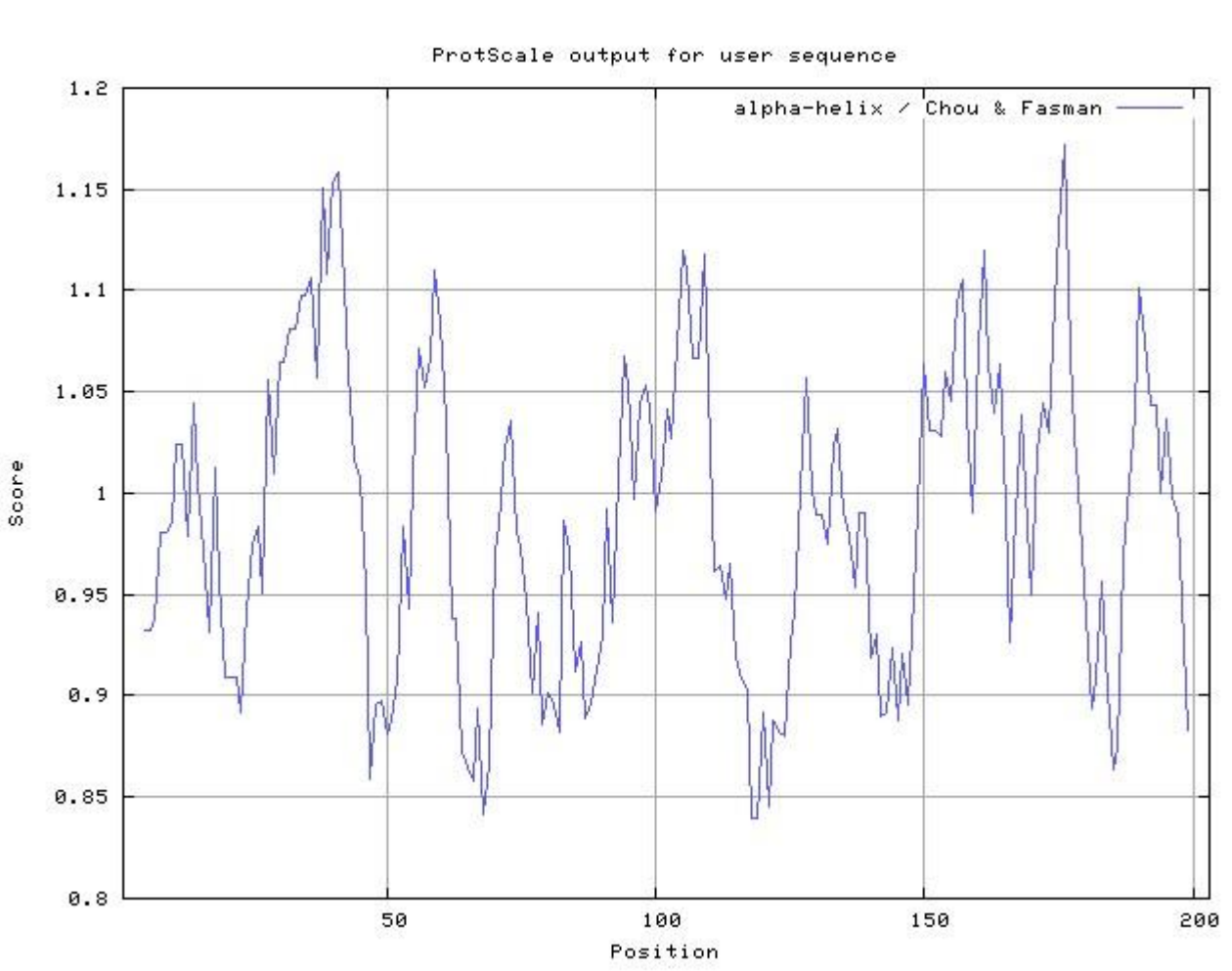
2. 结果分析：

虽然已有报道说明PRRSV 中的NSP1β和NSP2 的变异率最高，但是此进化树结果表明本报告所选毒株的变异率不是很高，并与全基因组序列的进化分析结果相一致。

16244B株、VR2332株、HB-1株位于同一分支，均为欧洲型PRRSV毒株，LV株与UAS/SD株位于同一分支，均为北美型PRRSV毒株，其bootstrap 值均达99％以上。

结构分析

1. 二级结构预测　　使用软件：ExPASy



六个螺旋大约分

在

α1：36-49

η1：55-57

η2：78-81

α2: 86-92

α3：100-110

α4：120-128

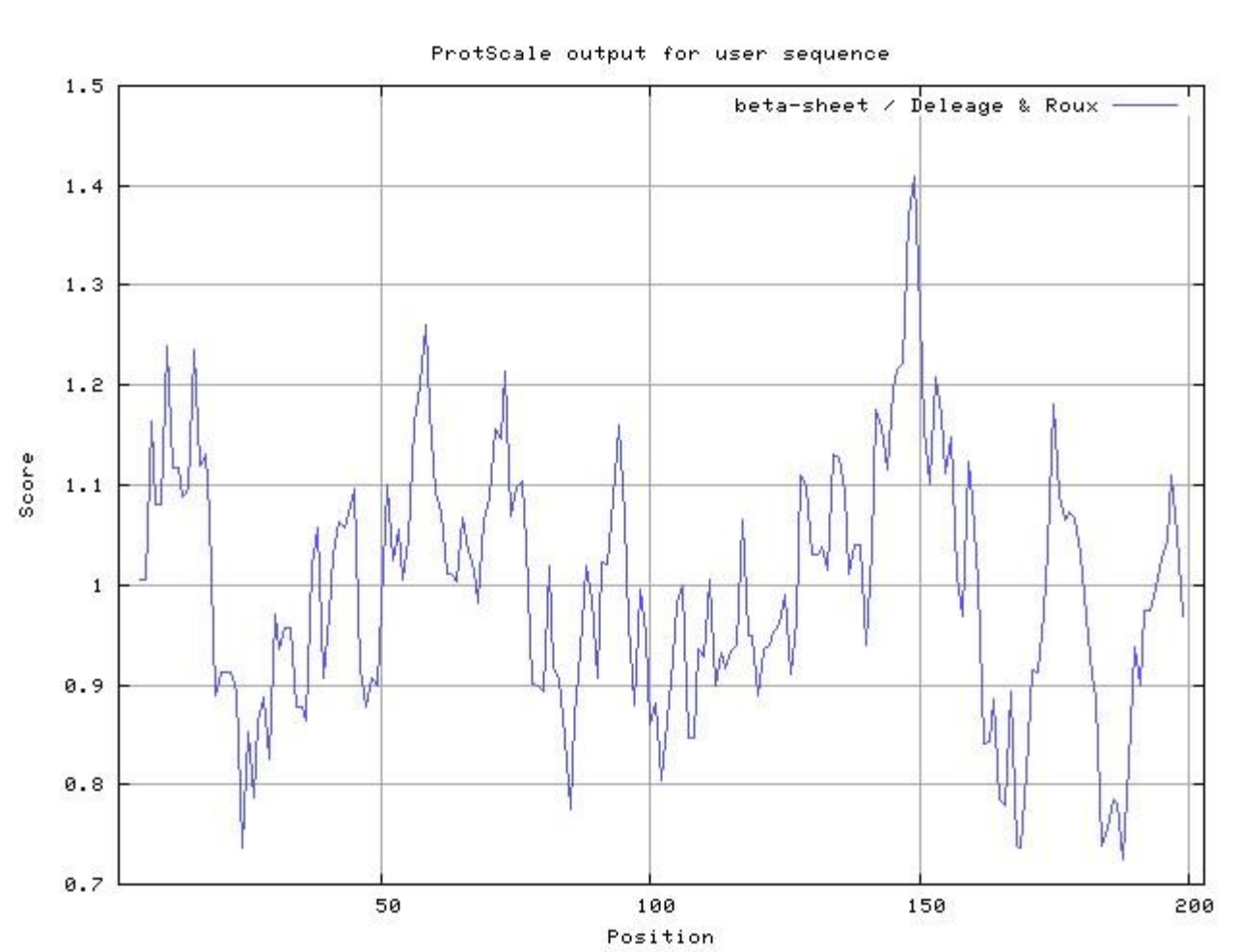
不同于文献中已研究的毒株，**HB**毒株还有另外的**α**螺旋

157-160

163-166

170-175

183-186



七个折叠大约分别在

β1:2-7

β2:10-15

β3:17-23

β4：135-140

β5:145-150

β6:153-158

β7:165-176

不同于文献中已研究的毒株，**HB**毒株还有另外的**β**折叠

55-60

70-75

170-175

SOPMA预测结果

```
        10         20         30         40         50         60         70
         |          |          |          |          |          |          |
ADVYDIGRGAVMYVAGGKVSWAPRGGDEVKFEPVPKELKLVANRLHTSFPPHHVVDMSKFTFMTPGSGVS
eeeeettttteeeeeetceeeecccccccceeecccchhhhhhhhhhhtccccccceeeccceeeeccccee
MRVEYQYGCLPADTVPEGNCWWRLFDLLPPEVQNKEIRHANQFGYQTKHGVPGKYLQRRLQVNGLRAVTD
eeecccttccccccccttchhhhhhhtcctttcchhhhhhhhhhcccccccccthhhhhhhhhttceeeec
THGPIVIQYFSVKESWIRHLKPVEEPSLPGFEDLLRIRVEPNTSPLAGKNEKIFRFGSHKWYG
ttcceeeeeeecccchhhhhhecccccccccccchhheeeeecttcccccccccceeeeeccttеec

Sequence length :   203

SOPMA :
   Alpha helix      (Hh) :    46 is  22.66%
   3₁₀ helix        (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :    53 is  26.11%
   Beta turn        (Tt) :    22 is  10.84%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :    82 is  40.39%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

α螺旋：6 个　　β折叠：12个

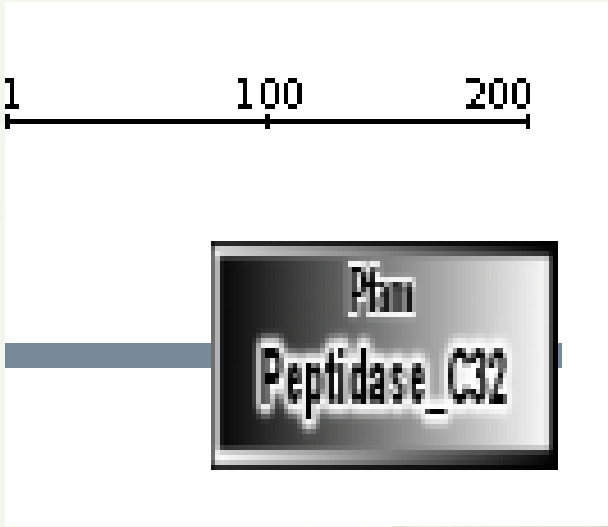
两种软件预测结果均与文献报道中的数据有差别，这恰恰说明“即使最著名的算法也大约只有60％的预测准确率”，因此大家不要过于迷信软件，只能将其作为一种辅助工具，万不能直接作为最终的实际结果，正确的结果是需要通过实验手段验证的。

2. 结构域分析

使用软件：SMART

Confidently predicted domains, repeats, motifs and features:

Name	Begin	End	E-value
Pfam:Peptidase_C32	75	202	1.90e-56



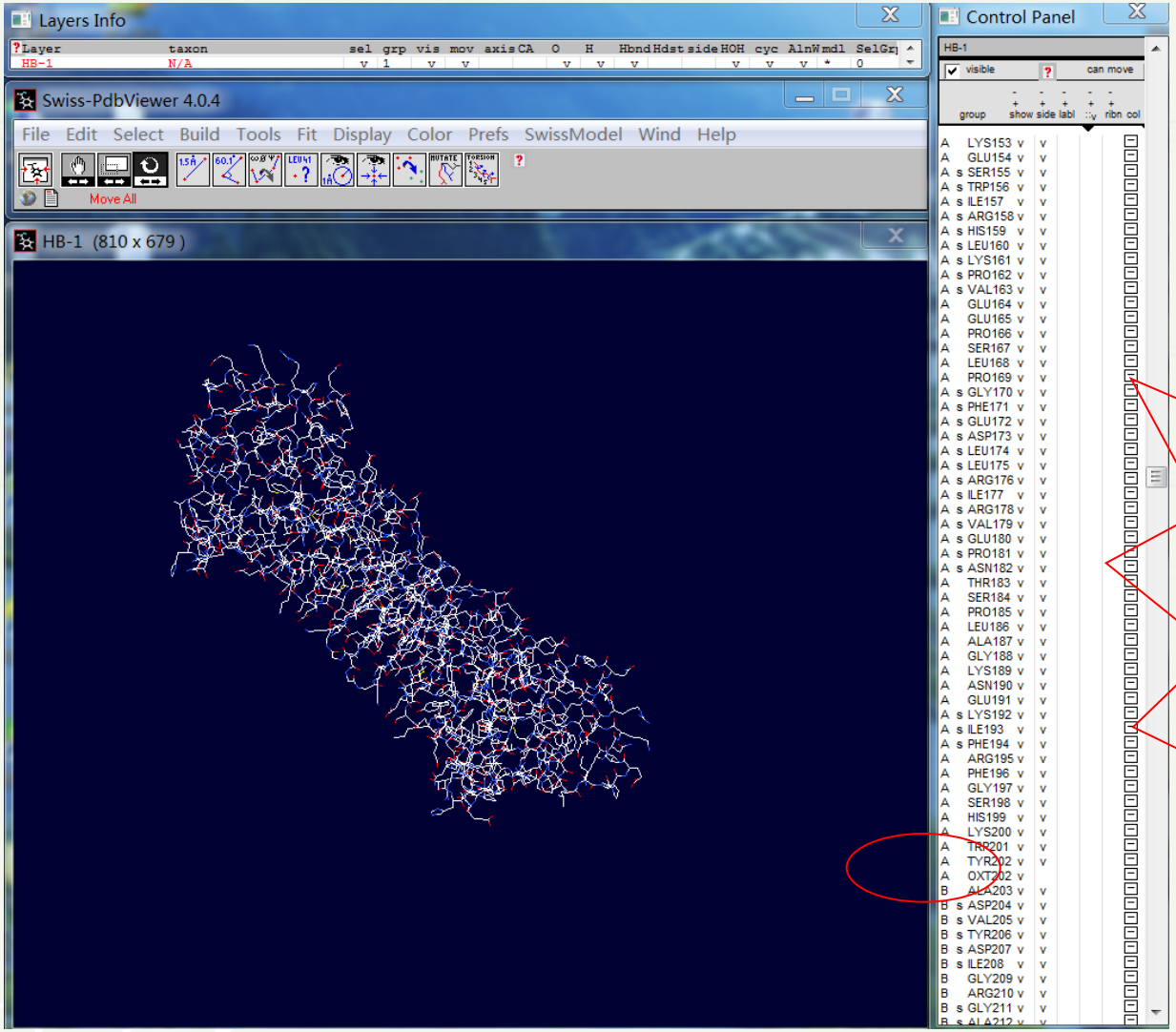
预测结果：

存在一个结构域 肽酶 _C32，从75位氨基酸开始，到202位氨基酸终止，E值<0.001，可信度较高。

该结果与UniProtKB中的公布结果（非预测，见第八页，为83和203）在起止氨基酸位点上有差异！

3. 同源建模

使用软件： Swiss-Model



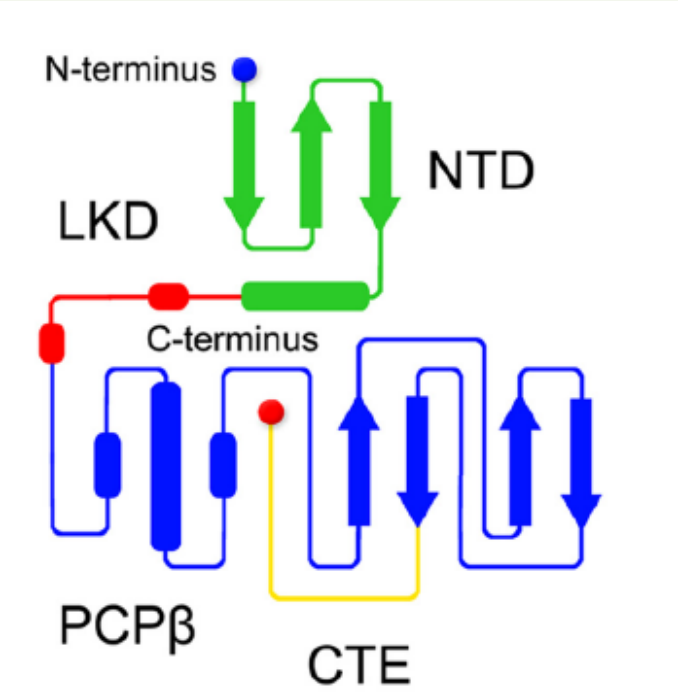
建模建模，均发现G203丢失，不只是什么原因！其所用模版为3MTV（PRRSV NSP1β，即前面提到的已解析出来的结构，只是毒株不同），两者序列一致性达96.6%，所以我们索性就直接用模板在此进行分析。

Pairwise Alignment Result

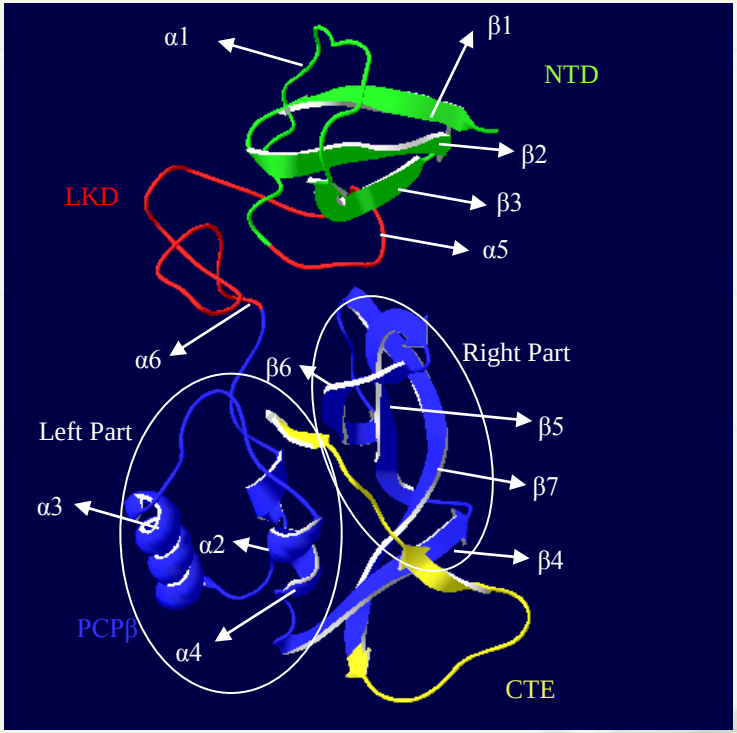
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
203	1066.0	196/203 (96.6%)	199/203 (98.0%)	0/203 (0.0%)

```
GD-XH      1 ADVYDIGRGAVMYVAGGKVSWAPRGGNEVKFEPVPKELKLVANRLHTSFP     50
             ||||||||||||||||||||||||||:||||||||||||||||||||||||
HB-1       1 ADVYDIGRGAVMYVAGGKVSWAPRGGDEVKFEPVPKELKLVANRLHTSFP     50

GD-XH     51 PHHVVDMSKFTFITPGSGVSMRVEYQYGCLPADTVPEGNCWWRLLDSLPP    100
             ||||||||||||:||||||||||||||||||||||||||||||.|.|||
HB-1      51 PHHVVDMSKFTFMTPGSGVSMRVEYQYGCLPADTVPEGNCWWRLFDLLPP    100

GD-XH    101 EVQYKEIRHANQFGYQTKHGVPGKYLQRRLQVNGLRAVTDTHGPIVIQYF    150
             |||.||||||||||||||||||||||||||||||||||||||||||||||
HB-1     101 EVQNKEIRHANQFGYQTKHGVPGKYLQRRLQVNGLRAVTDTHGPIVIQYF    150

GD-XH    151 SVKESWIRHLKLVEEPSLPGFEDLLRIRVEPNTSPLAGKDEKIFRFGSHK    200
             |||||||||||.|||||||||||||||||||||||||||:||||||||||
HB-1     151 SVKESWIRHLKPVEEPSLPGFEDLLRIRVEPNTSPLAGKNEKIFRFGSHK    200

GD-XH    201 WYG     203
             |||
HB-1     201 WYG     203
```

PDB ID：3MTV

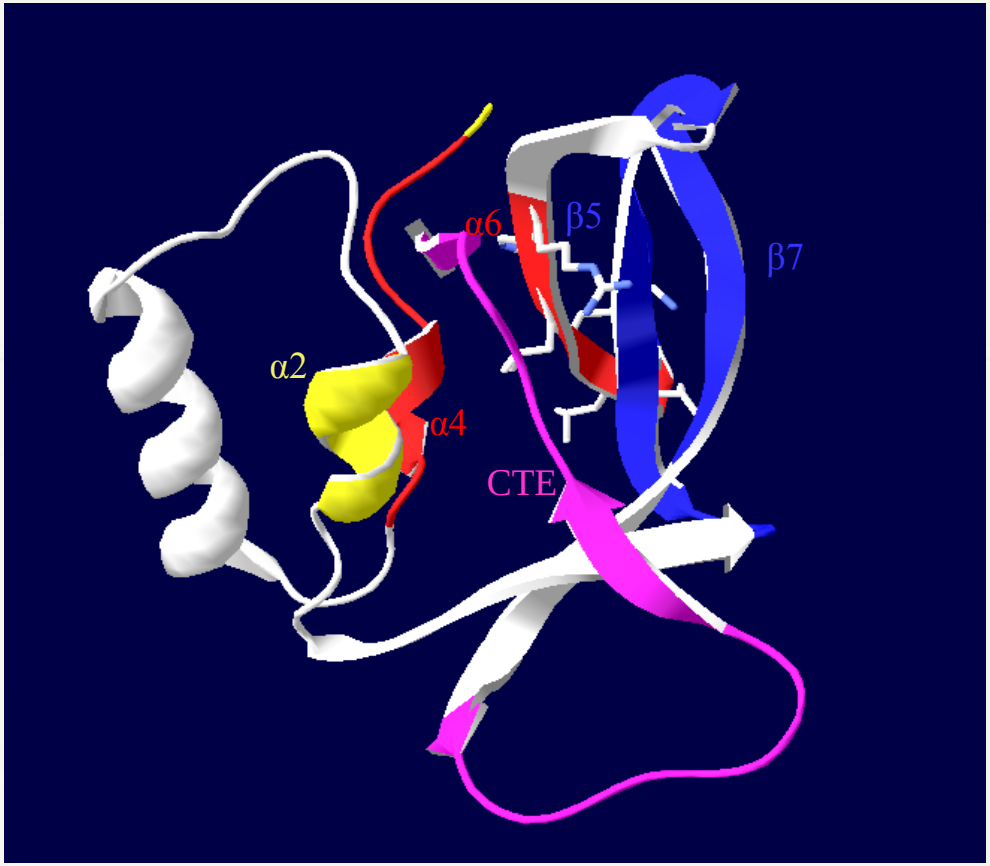


Fei Xue et al, 2010, JVI



4. PCPβ 结构域和 CTE 结构域

由于我们想分析的是NSP1β的蛋白酶活性，所以在此重点介绍与其相关的的PCPβ 结构域和 CTE 结构域。



活性口袋呈U型

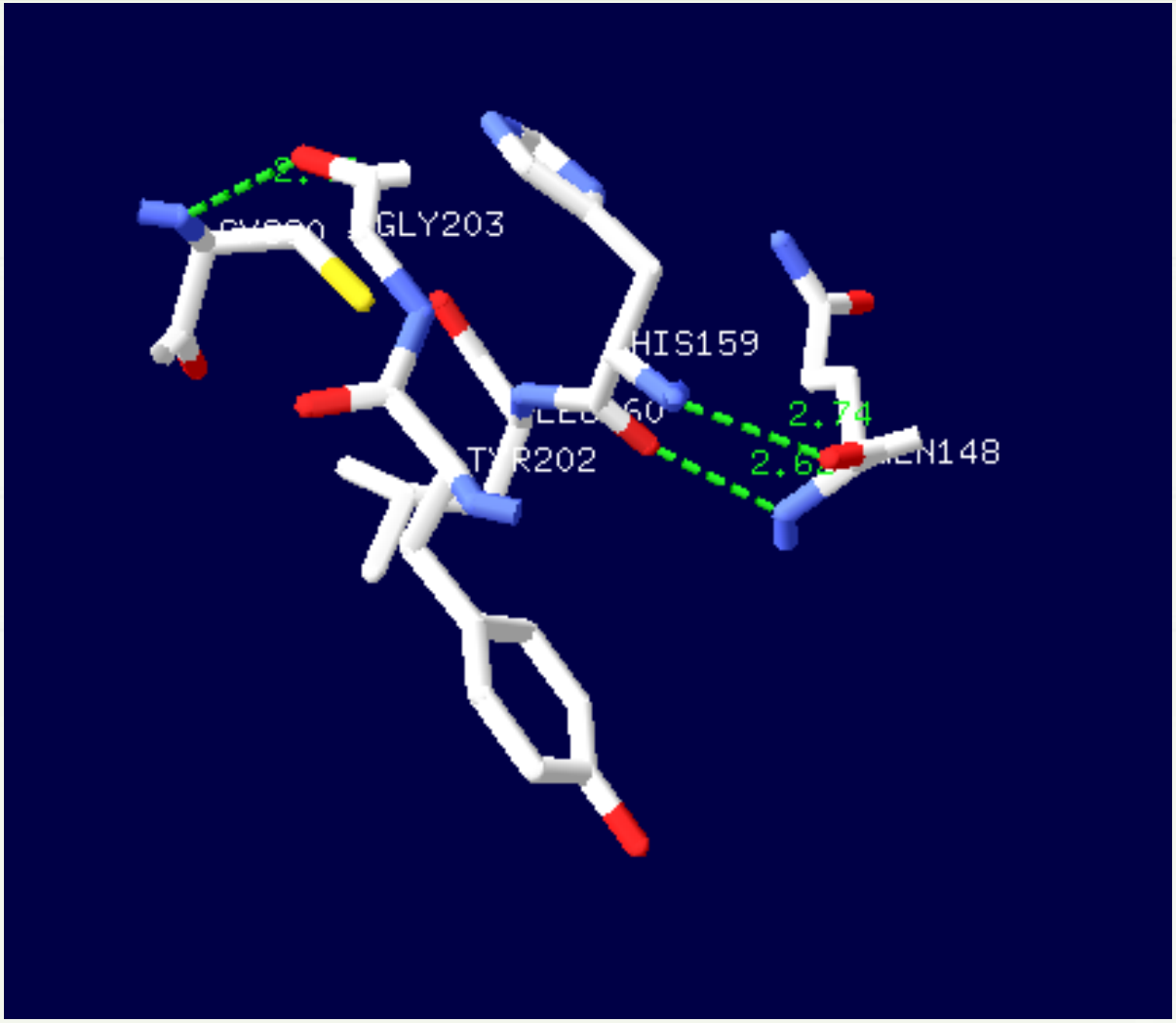
左侧为α2螺旋（黄色）；

底部为α4螺旋和α6螺旋部分残基的侧链共同组成（红色）；

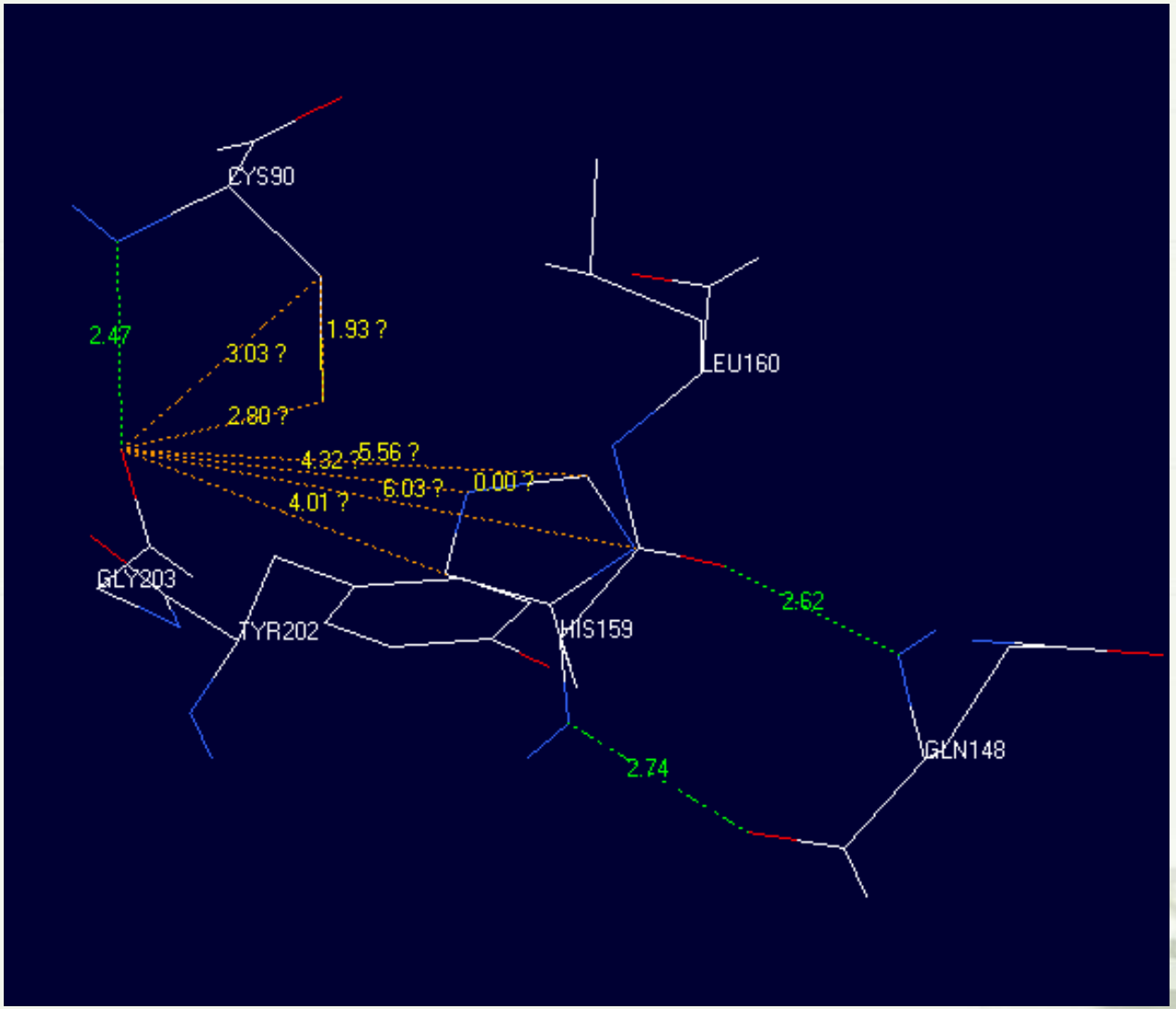
右侧为β5和β6共同组成（蓝色）；

活性中心为位于结合该口袋的底物得得顶端，为C90和H159；

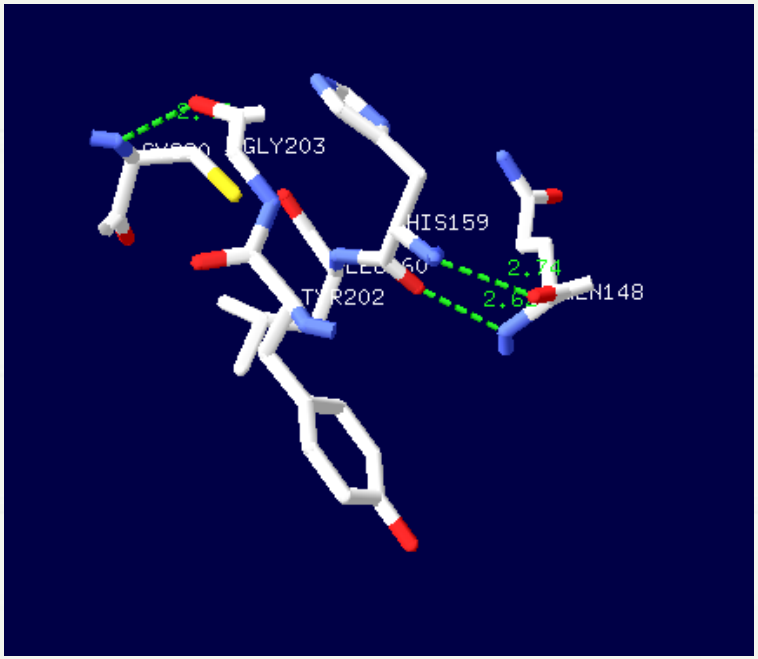
而该蛋白的CTE就像底物一样紧紧结合于该口袋中！



验证是否靠氢键将CTE固定于该口袋中！发现活性中心存在三条氢键！

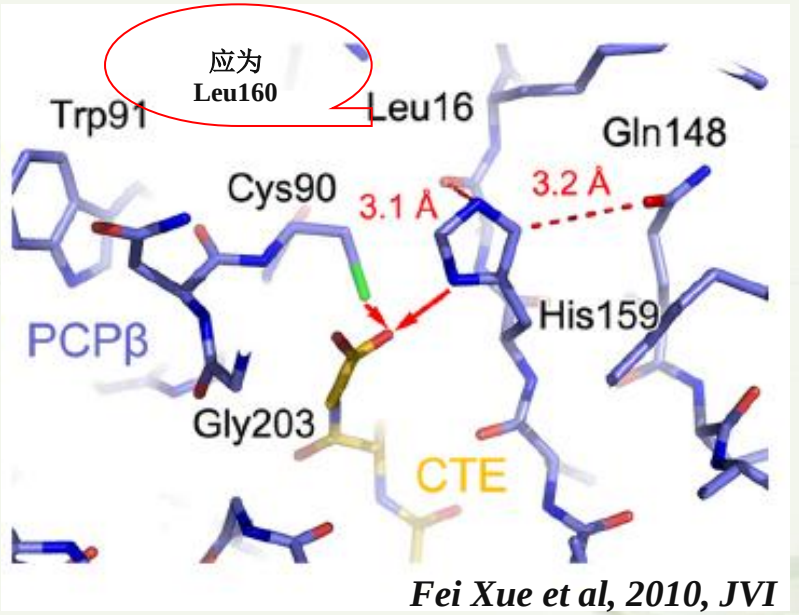


测量G203原子与其周围各原子的距离！最小距离为2.80埃！



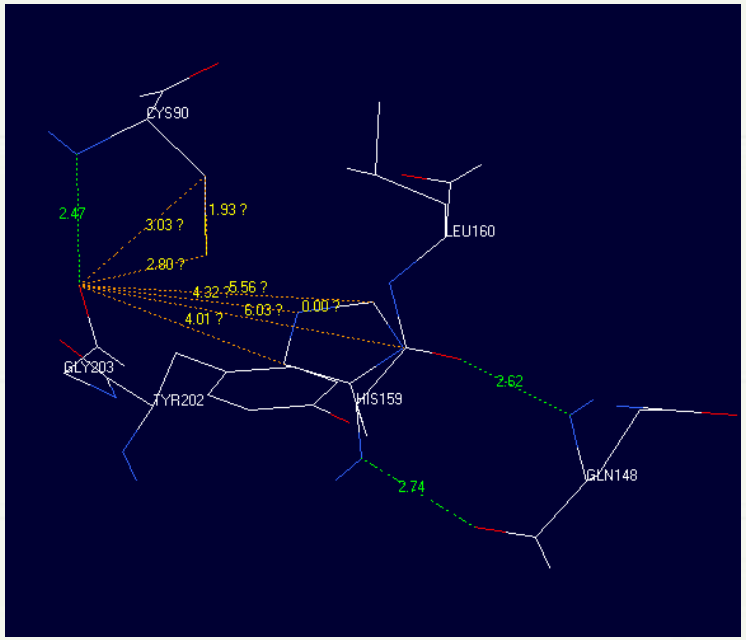
我们所做的结果与文献报道有一定出入，发现H159 与Q148 之间存在2个氢键，而C90 与 G203之间存在1个氢键

In the catalytic center, one of the carboxylate oxygen atoms of Gly203 (usually named the P_1 substrate residue) points toward the "oxyanion hole" and is stabilized by the sulfhydryl group of Cys90 and the imidazole group of His159, with distances of 2.1 Å and 2.5 Å, respectively. This is very similar to the substrate binding behavior in the crystal structure of PRRSV nsp1α. As with previously reported crystal structures of PCPs, His159 initiates a hydrogen bonding network involving Gln148 and Leu160 that might act as a charge relay system during catalysis (Fig. 5B).



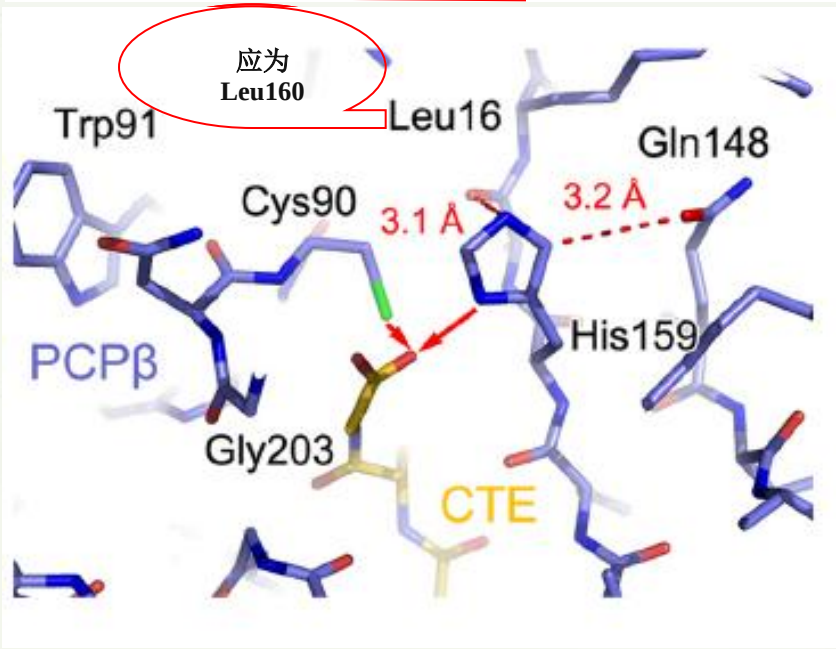
Fei Xue et al, 2010, JVI

文献：H159 与Q148 、L160分别形成1个氢键，构成氢键网络，作于类似于电荷中继系统。



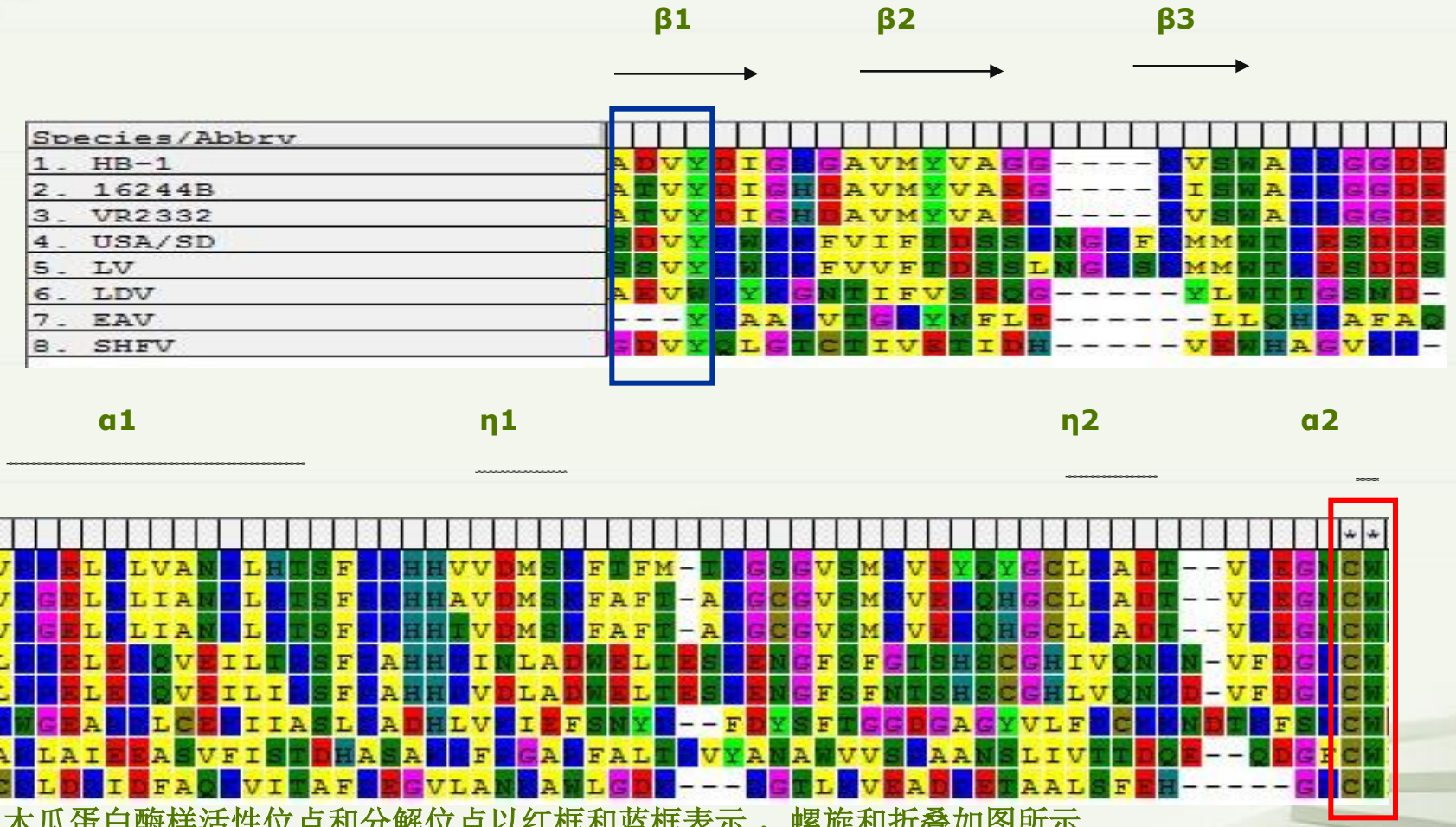
以甘氨酸残基羧基上的一个氧原子原子为基点，测量距离均在2.8埃以上

In the catalytic center, one of the carboxylate oxygen atoms of Gly203 (usually named the P_1 substrate residue) points toward the "oxyanion hole" and is stabilized by the sulfhydryl group of Cys90 and the imidazole group of His159, with distances of 2.1 Å and 2.5 Å, respectively. This is very similar to



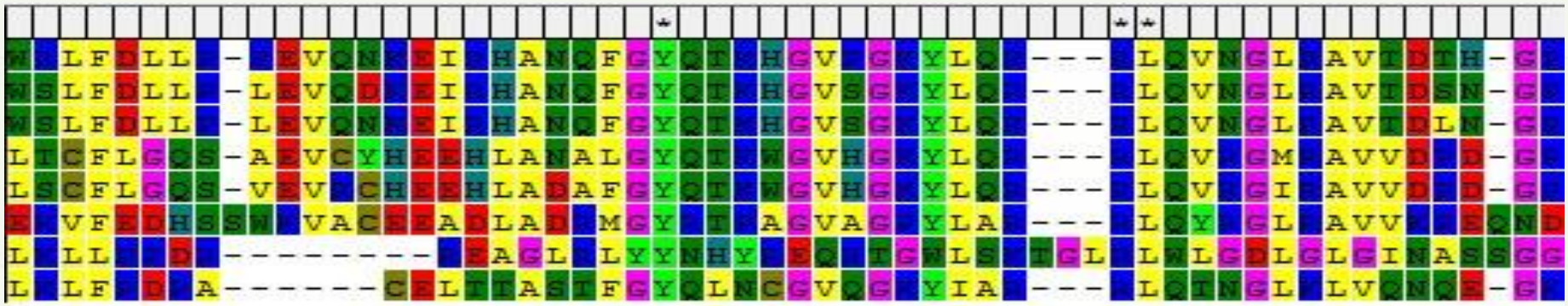
文献： G203 由C90 的巯基基团与H159的咪唑基团稳定，距离分别为2.1埃和2.5埃

5. 自切模型

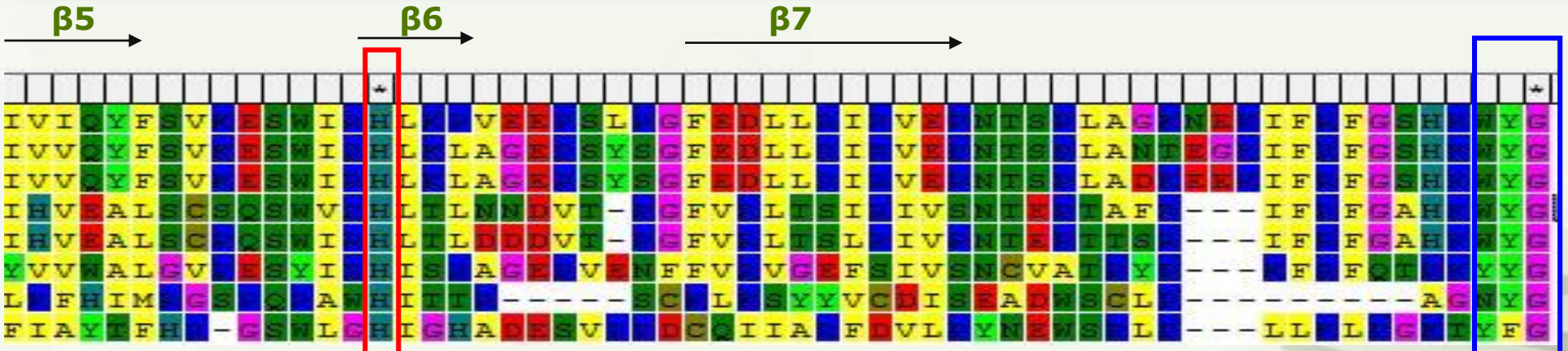


木瓜蛋白酶样活性位点和分解位点以红框和蓝框表示， 螺旋和折叠如图所示

α2 α3 α4 β4



β5 β6 β7



木瓜蛋白酶样活性位点和分解位点以红框和蓝框表示，螺旋和折叠如图所示

讨论

由于在利用我们的HB-1序列进行同源建模的时候发现，G203 残基总是自动丢失，而两者序列一致性达96.6％，并且活性位点未发生突变，所以我们便索性直接用解析出的结构（3MTV）进行分析，在我们试图本去还原饶子河老师的解析结果时，发现其中出现很多不同的地方，原因未知，这我们有待进一步学习研究！

主要参考文献

1. *Fei Xue et al. 2010. J. Virol. 84:6461-6471.*
2. *Deendayal et al. 2010. J. Virol. 84:11045-11055.*
3. *Lalit K. Beura et al. 2010. J. Virol. 84:1574-1584.*
4. *Byungjoon Kwon et al. 2008. Virology. 38:371-378.*

致谢

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