

H5N1 NS1蛋白的功能验证

主讲人：王增

小组成员：高奇、王雪枝、慈彦鹏

目录

1. 研究背景
2. 研究目的
3. 对NS1的分析
 - 3.1 序列比对、进化树的构建
 - 3.2 信号肽分析
 - 3.3 跨膜分析
 - 3.4 运用weblab工具简单分析
 - 3.5 用SPdbViewer进行功能验证
4. 参考文献
5. 致谢

1.研究背景

NS1是AIV的一种非结构蛋白，在病毒侵染机体的过程中具有抑制先天性免疫应答的作用。

- N端作用：通过与dsRNA的结合，抑制IFN的产生、阻断siRNA的抗病毒作用。
- C端作用：抑制宿主细胞pre-mRNA的加工出核，最终抑制宿主细胞蛋白质的合成。

1.研究背景

- 已知N端发挥抗病毒作用的关键位点是35位、38位的Arg
- 已知C端的功能作用与182位的Trp具有很大的关系

2.研究目的

通过用生物信息学软件对具有代表性的H5N1的NS1序列、结构和功能的分析，来验证NS1在抑制先天性免疫应答中的重要性。

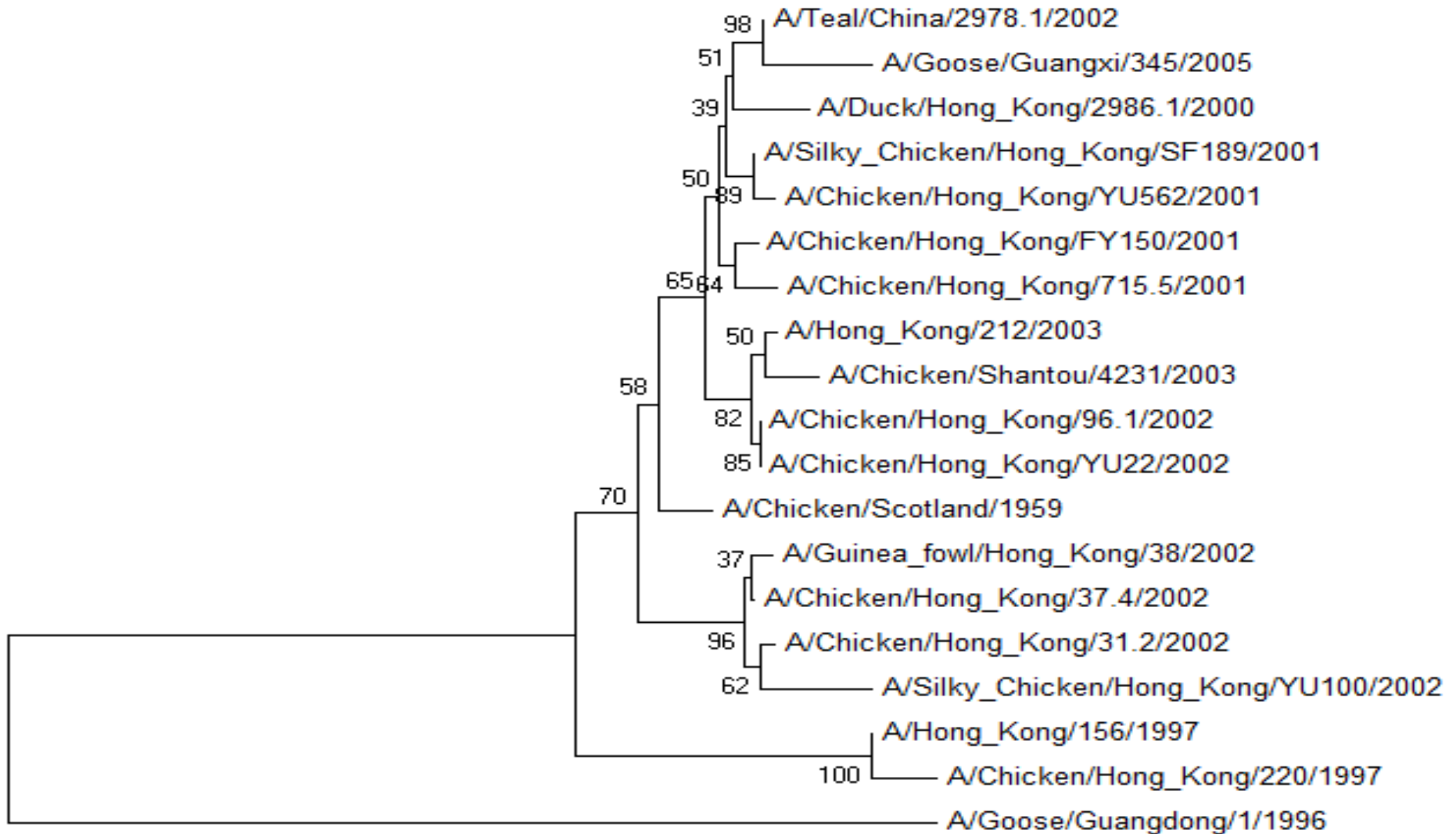
3.1 序列比对

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q56264	NS1_I97A1	★	Non-structural protein 1	NS	Influenza A virus (strain A/Hong Kong/156/1997 H5N1 genotype Gs/Gd)	230
<input type="checkbox"/> Q6DP37	NS1_I02A7	★	Non-structural protein 1	NS	Influenza A virus (strain A/Teal/China/2978.1/2002 H5N1 genotype W)	208
<input type="checkbox"/> Q6J880	NS1_I03A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Hong Kong/212/2003 H5N1 genotype Z+)	225
<input type="checkbox"/> Q6DP28	NS1_I03A1	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Shantou/4231/2003 H5N1 genotype V)	225
<input type="checkbox"/> Q2F4N6	NS1_I05A1	★	Non-structural protein 1	NS	Influenza A virus (strain A/Goose/Guangxi/345/2005 H5N1 genotype G)	219
<input type="checkbox"/> Q0A2H0	NS1_I59A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Scotland/1959 H5N1)	230
<input type="checkbox"/> Q9Q0L6	NS1_I96A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Goose/Guangdong/1/1996 H5N1 genotype Gs/Gd)	230
<input type="checkbox"/> Q8QPI8	NS1_I00A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Duck/Hong Kong/2986.1/2000 H5N1 genotype C)	225
<input type="checkbox"/> Q809X6	NS1_I01A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Silky Chicken/Hong Kong/SF189/2001 H5N1 genotype A)	225
<input type="checkbox"/> Q809Y0	NS1_I01A1	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/YU562/2001 H5N1 genotype B)	225
<input type="checkbox"/> Q809X8	NS1_I01A2	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/FY150/2001 H5N1 genotype D)	225
<input type="checkbox"/> Q809X2	NS1_I01A3	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/715.5/2001 H5N1 genotype E)	225
<input type="checkbox"/> Q6DP70	NS1_I02A1	★	Non-structural protein 1	NS	Influenza A virus (strain A/Guinea fowl/Hong Kong/38/2002 H5N1 genotype X0)	230
<input type="checkbox"/> Q6DP68	NS1_I02A2	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/31.2/2002 H5N1 genotype X1)	230
<input type="checkbox"/> Q6DP66	NS1_I02A3	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/37.4/2002 H5N1 genotype X2)	230
<input type="checkbox"/> Q6DP64	NS1_I02A4	★	Non-structural protein 1	NS	Influenza A virus (strain A/Silky Chicken/Hong Kong/YU100/2002 H5N1 genotype X3)	230
<input type="checkbox"/> Q6J871	NS1_I02A5	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/96.1/2002 H5N1 genotype Y)	225
<input type="checkbox"/> Q6DP62	NS1_I02A6	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/YU22/2002 H5N1 genotype Z)	225
<input type="checkbox"/> O89748	NS1_I97A0	★	Non-structural protein 1	NS	Influenza A virus (strain A/Chicken/Hong Kong/220/1997 H5N1 genotype Gs/Gd)	230

3.1 序列比对

保守位点	131	57%
变异位点	99	43%
只有一个突变的位点	66	29%

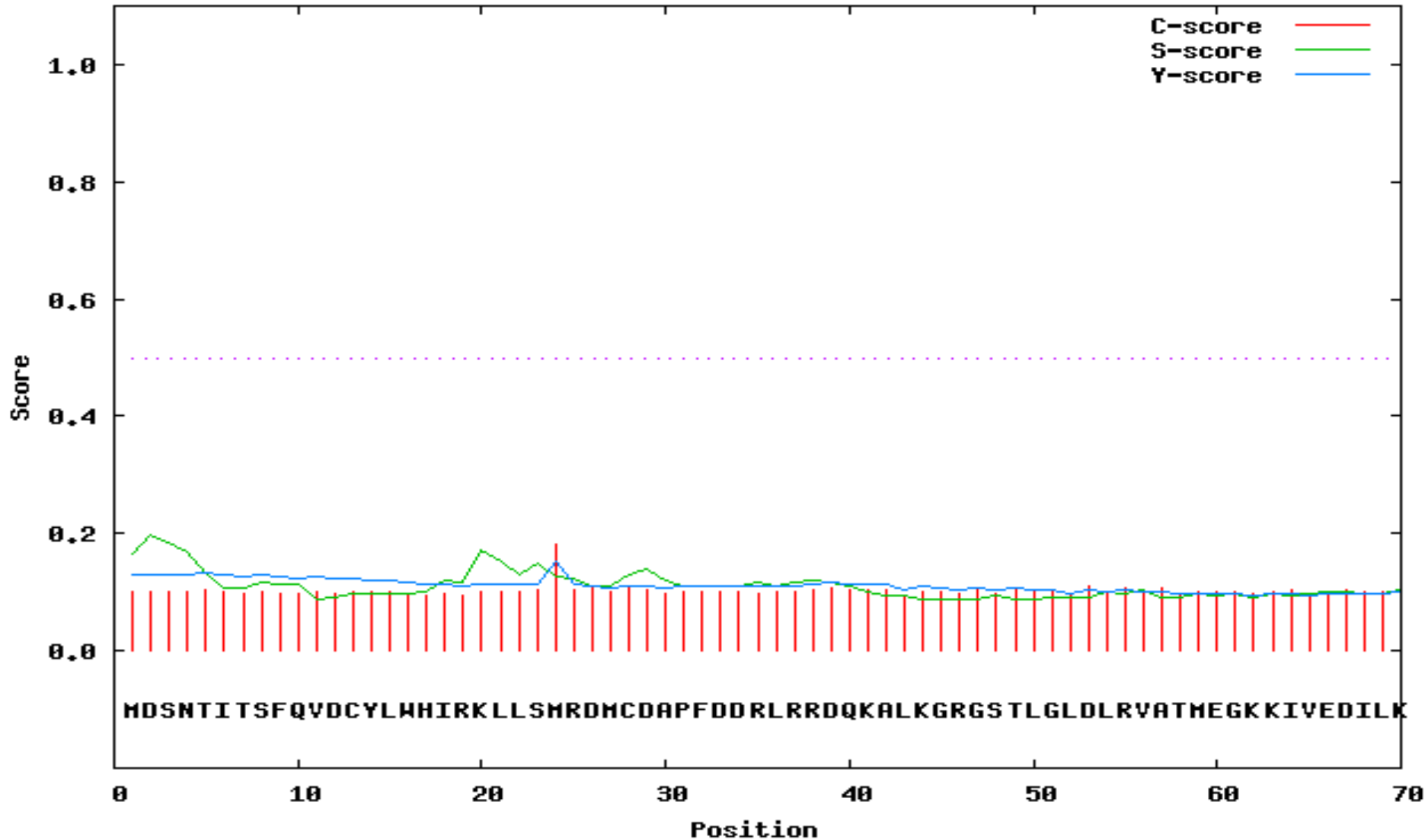
3.1 构建进化树(H5N1)



0.02

3.2 信号肽分析

SignalP-4.0 prediction (euk networks): seq1

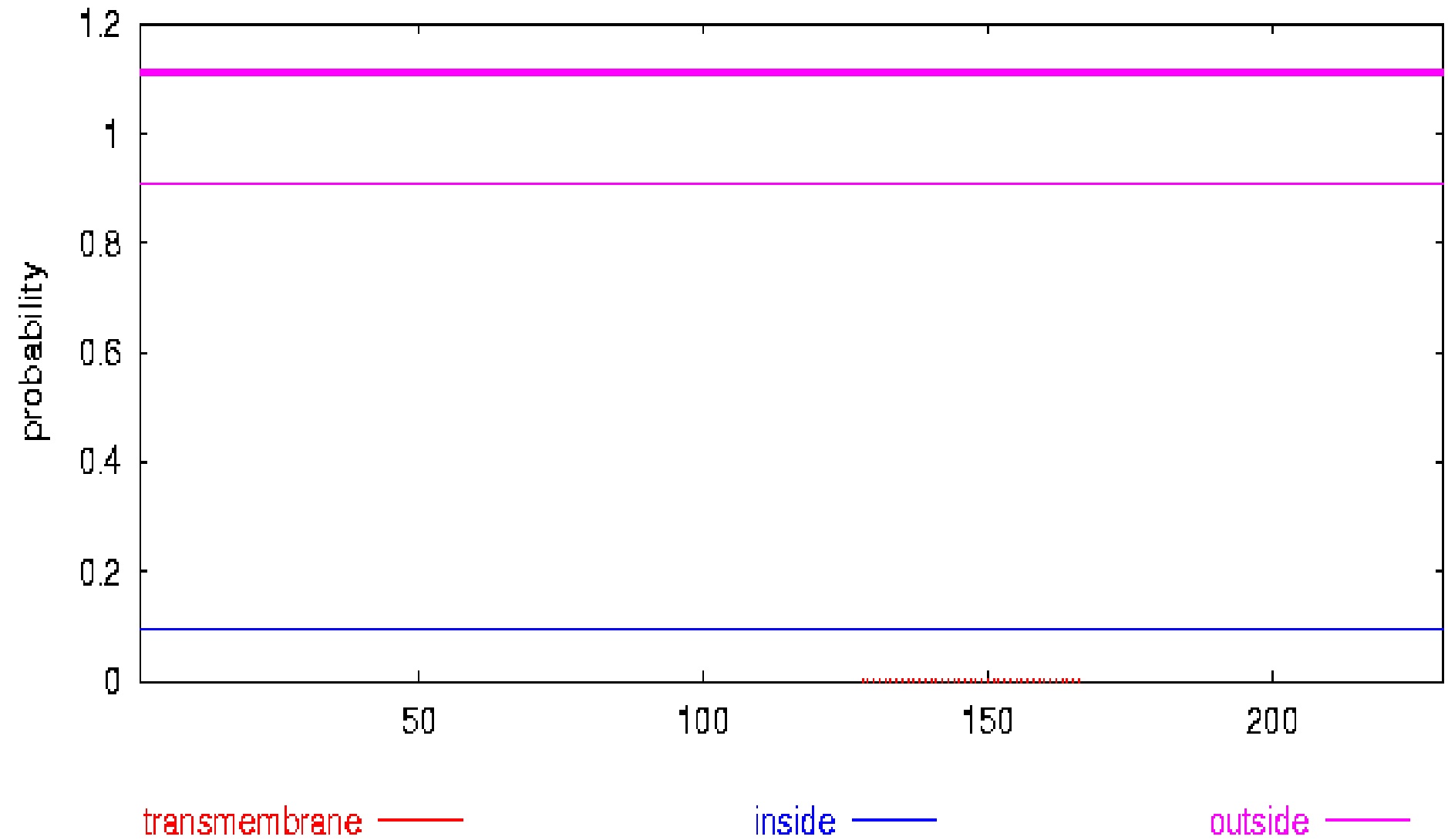


3.2信号肽分析

- 从以上结果可以分析得出，NS1没有信号肽的存在，但是在Uniprot中有核定位信号，这样看来两者结果不符。
- 解析：NS1不是分泌蛋白，核定位信号依旧存在于成熟的NS1蛋白中，是不需要切割的，因此检测不到切割位点。

3.3跨膜分析

TMHMM posterior probabilities for sp_Q9QQL6_NS1_I96A0

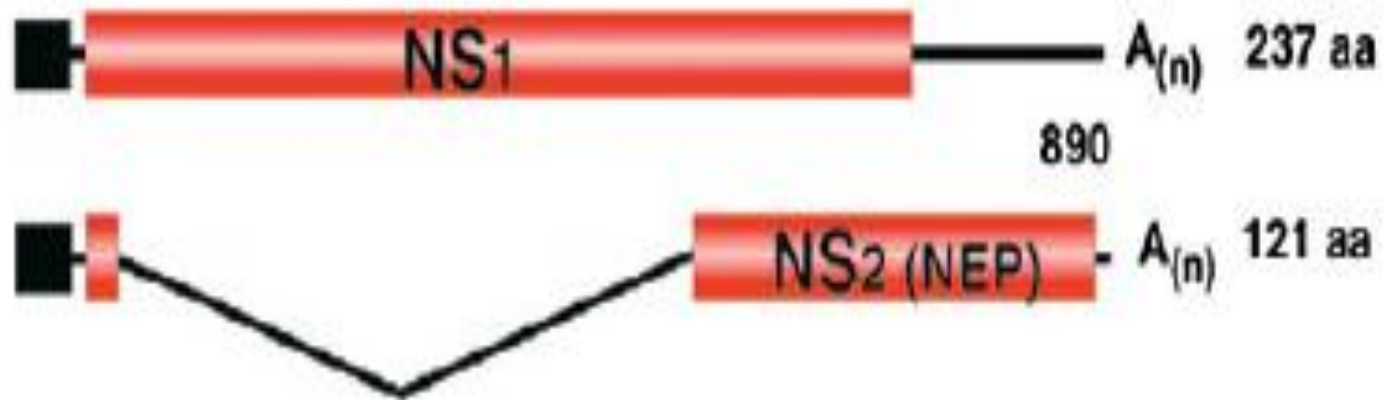


3.3跨膜分析

- NS1是一个内部非结构蛋白，通过与宿主蛋白或dsRNA的结合发挥作用，没有跨膜为正常现象。

3.4用weblab进行分析

Influenza A virus segment 8

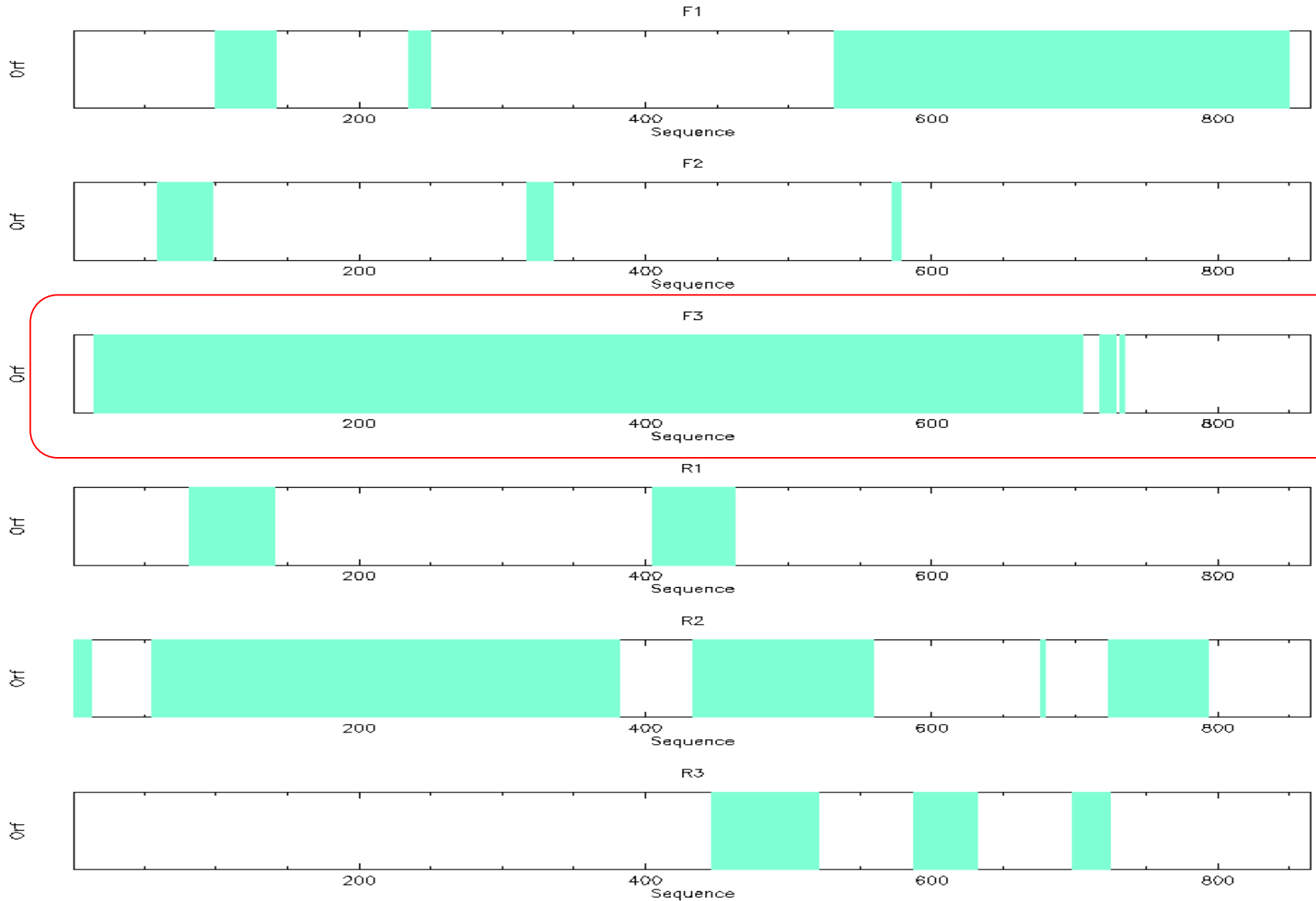


Segment 8 full length: 865 bp (RefSeq: A/Goose/GD/1/96)

NS1: 15-707

NS2 (NEP): 15-44,517-852

3.4.1 Plotorf



3.4.2 Sixpack

```
>A/Goose/Guangdong/1/96(H5N1),_1_ORF1 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 1, ORF 1, threshold 1,
4aa
VTKT
>A/Goose/Guangdong/1/96(H5N1),_1_ORF2 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 1, ORF 2, threshold 1,
14aa
MPPLMTGSEETKRH
>A/Goose/Guangdong/1/96(H5N1),_1_ORF3 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 1, ORF 3, threshold 1,
5aa
MKTSK
>A/Goose/Guangdong/1/96(H5N1),_1_ORF4 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 1, ORF 4, threshold 1,
106aa
MSKMQLESSVDLNGMITQFERLKIYRDSLGESMMRMGDLHSLQNRNATWRNELSQKFEE
IRWLIAECRNILTKTENSFEQITFLQALQLLLEVESEIRTFSPQLI
>A/Goose/Guangdong/1/96(H5N1),_2_ORF1 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 2, ORF 1, threshold 1,
13aa
MAHKKATQYERHV
>A/Goose/Guangdong/1/96(H5N1),_2_ORF2 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 2, ORF 2, threshold 1,
6aa
MVHADA
>A/Goose/Guangdong/1/96(H5N1),_2_ORF3 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 2, ORF 3, threshold 1,
2aa
ME
>A/Goose/Guangdong/1/96(H5N1),_3_ORF1 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 3, ORF 1, threshold 1,
230aa
MDSNTITSFQVDCYLWHIRKLLSMRDMCDAPFDDRLRRDQKALKGRGSTLGLDLRVATME
GKKIVEDILKSETNENLKIAIASSPAPRYITDMSIEEMSREWYMLMPRQKITGGLMVKMD
QAIMDKRIILKANFSVLFQLETLVSLRAFTESGAIVAEIFPIPSVPGHFTEDEVKNAIGI
LIGGLEWWDNSIRASENIQRFQAWGIHDENGGPSLPPKQKRYMAKRVESEV
>A/Goose/Guangdong/1/96(H5N1),_3_ORF2 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 3, ORF 2, threshold 1,
4aa
MAHC
>A/Goose/Guangdong/1/96(H5N1),_3_ORF3 Translation of A/Goose/Guangdong/1/96(H5N1), in frame 3, ORF 3, threshold 1,
1aa
M
```


3.4.3 Getorf







```
>A/Goose/Guangdong/1/96(H5N1)),_1 [15 - 704] NS full length
ATGGATTCCAACACGATAACCTCGTTTCAGGTAGATTGTTATCTATGGCACATAAGAAAG
CTACTCAGTATGAGAGACATGTGTGATGCCCCCTTTGATGACAGGCTCCGAAGAGACCAA
AAGGCATTAAGGGGAAGAGGCAGCACACTTGGACTCGATTTAAGAGTGGCTACAATGGAG
GGGAAAAAGATCGTTGAGGACATCCTGAAGAGTGAGACAAATGAAAACCTCAAAATAGCC
ATTGCTTCCAGTCCCTGCTCCTCGGTATATCACCGATATGAGCATAGAGGAGATGAGCCGA
GAATGGTACATGCTGATGCCTAGGCAGAAAATAACTGGAGGCCTTATGGTGAAAATGGAC
CAAGCCATAATGGATAAAAGAATTATCCTTAAAGCAAATTTCTCAGTTCTATTTGATCAA
CTAGAGACATTAGTCTCTCTGAGGGCATTACAGAAAGTGGTGCTATTGTGGCTGAAATA
TTTCCCATTCCCTCCGTACCAGGACATTTTACAGAGGATGTCAAAAATGCAATTGGAATC
CTCATCGGTGGACTTGAAATGGAATGATAACTCAATTCGAGCGTCTGAAAATATACAGAGA
TTCGCTTGGGGAAATCCATGATGAGAAATGGGGGACCTTCACTCCCTCCAAAACAGAAACGC
TACATGGCGAAACGAGTTGAGTCAGAAGTT
>A/Goose/Guangdong/1/96(H5N1)),_2 [532 - 849] NS full length
ATGTCAAAAATGCAATTGGAATCCTCATCGGTGGACTTGAATGGAATGATAACTCAATTC
GAGCGTCTGAAAATATACAGAGATTCGCTTGGGGAAATCCATGATGAGAAATGGGGGACCTT
CACTCCCTCCAAAACAGAAACGCTACATGGCGAAACGAGTTGAGTCAGAAGTTTGAAGAG
ATCAGATGGCTCATTGCTGAATGTAGAAATATACTGACAAAGACTGAAAATAGCTTTGAA
CAGATAACATTTTTGCAAGCATTGCAACTCTTACTTGAAGTTGAGAGTGAGATAAGGACC
TTCTCTTTTTCAGCTTATT
```

结论：NS1完整，NS2不完整

3.4.4 分析

- 因为编码NS2的核苷酸序列分为两部分（15-44,517-852），第二段开头有没有起始密码子，所以软件不能把这两部分识别成一段完整的序列，导致无法分析出结果。但对于编码连续的氨基酸可以分析出结果。

3.5.1 背景

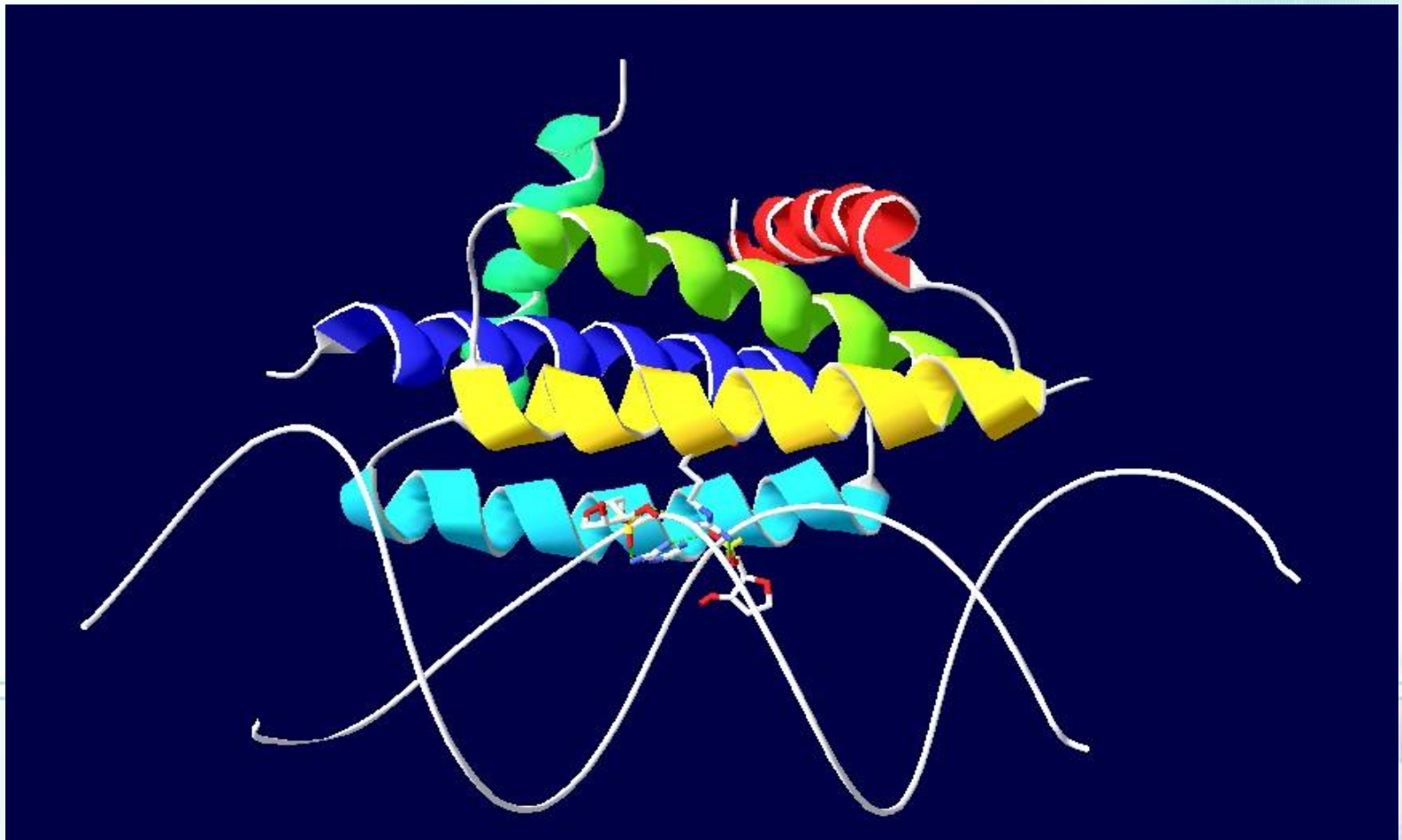
<input type="checkbox"/>	Region	1 – 73	73	RNA-binding By similarity	
<input type="checkbox"/>	Region	180 – 215	36	CPSF4-binding By similarity	
<input type="checkbox"/>	Region	223 – 230	8	PABPN1-binding By similarity	
<input type="checkbox"/>	Motif	34 – 38	5	Nuclear localization signal 1 By similarity	
<input type="checkbox"/>	Motif	137 – 146	10	Nuclear export signal By similarity	
<input type="checkbox"/>	Motif	216 – 221	6	Nuclear localization signal 2 By similarity	

3.5.1 结论

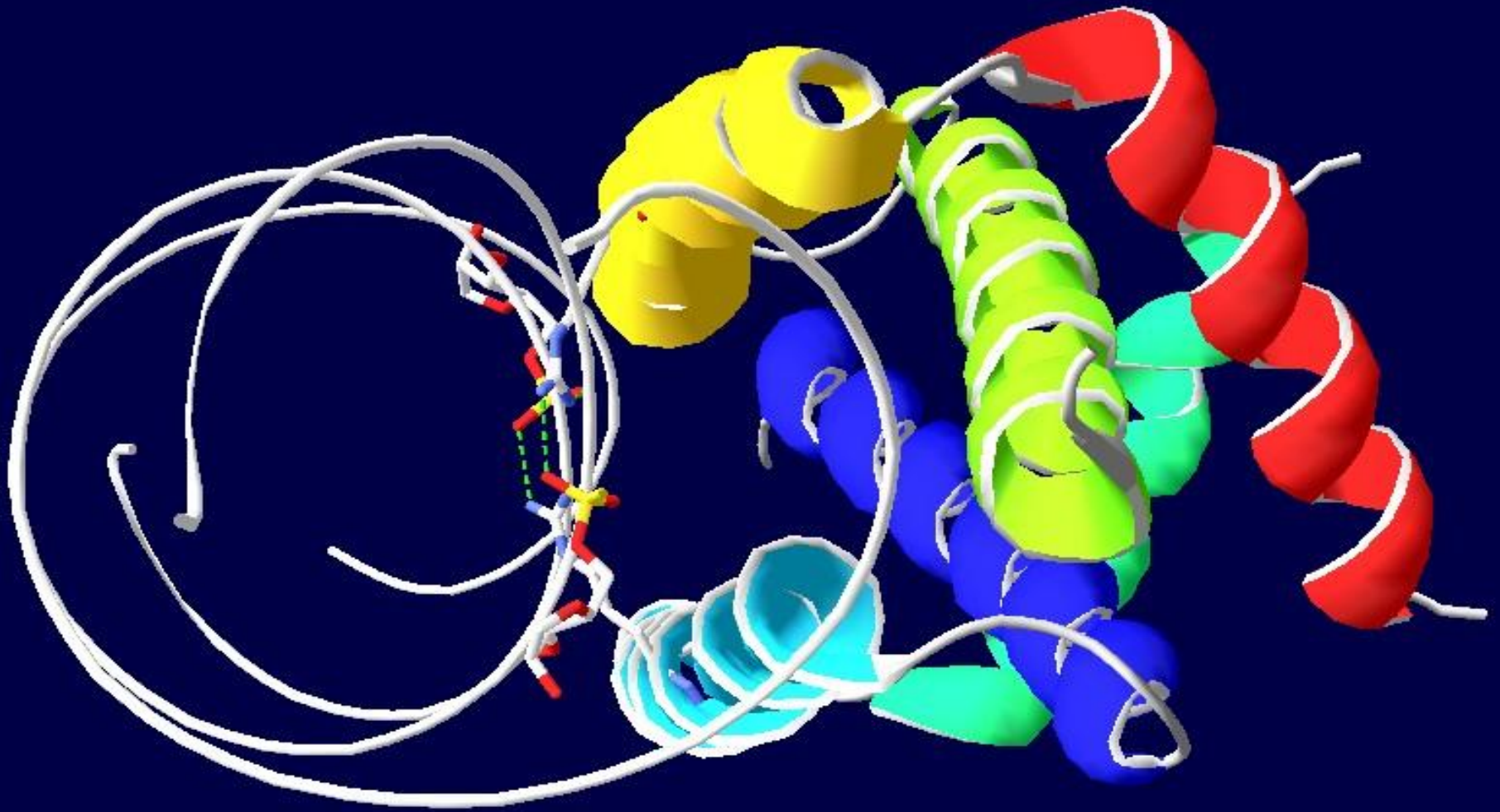
- 可以看出NS1的RNA-binding domain 中的35和38的Arg在所有的序列中都比较保守，对功能具有很重要的影响。

3.5.2 N端

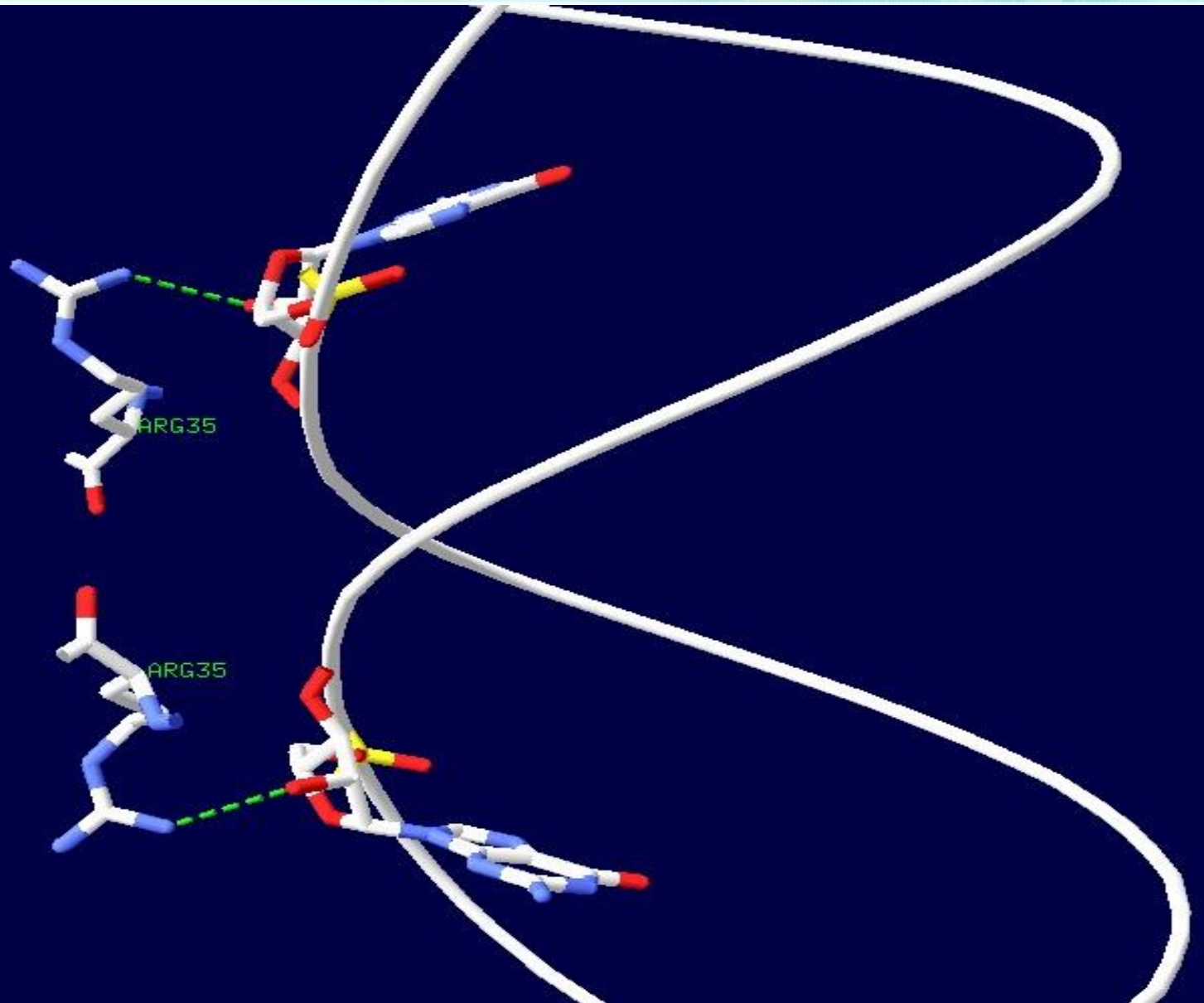
- 从PDB中找到相应的结构（PDB ID: 2ZKO），分析其与dsRNA结合的关键位点。



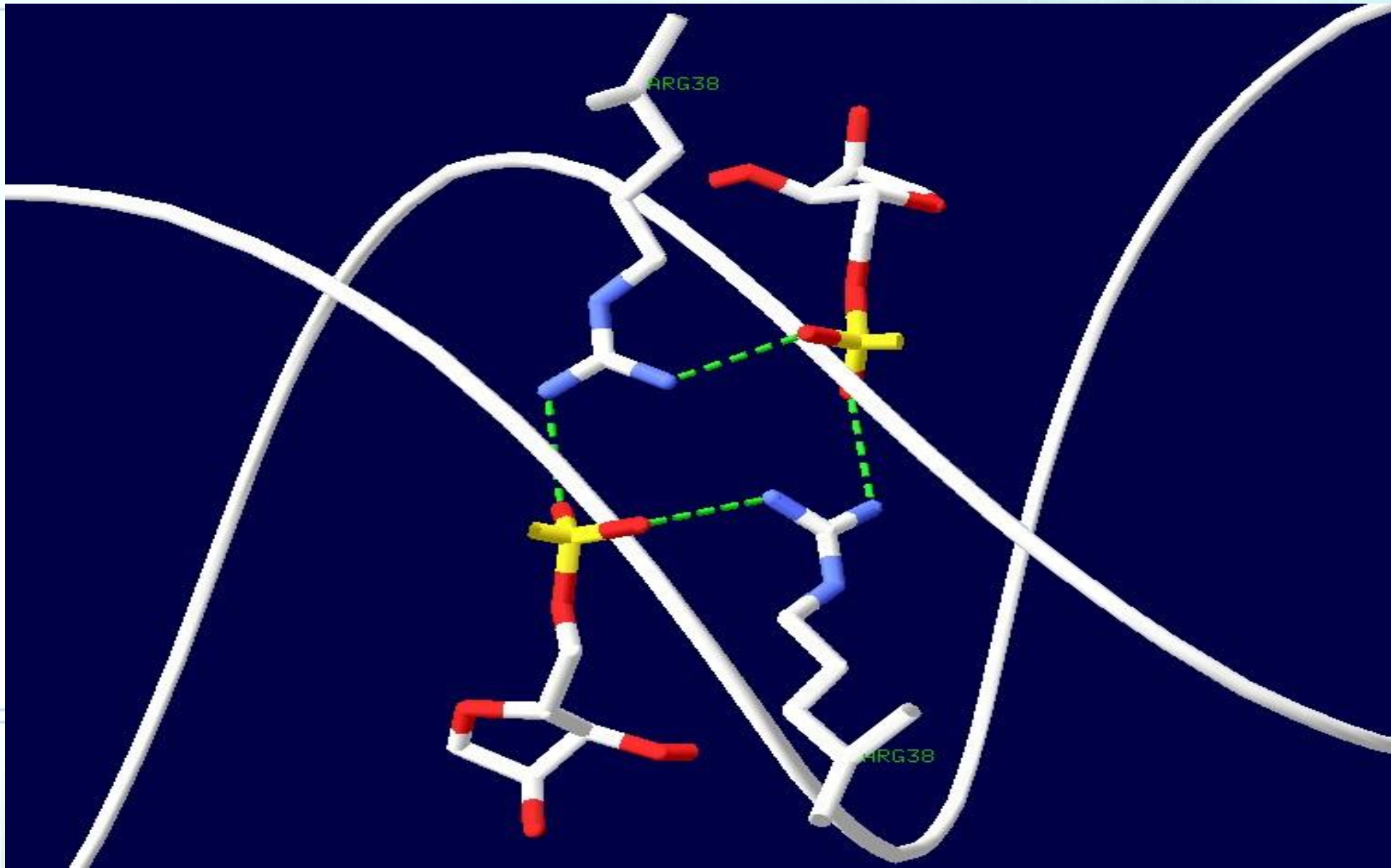
3.5.2 N端



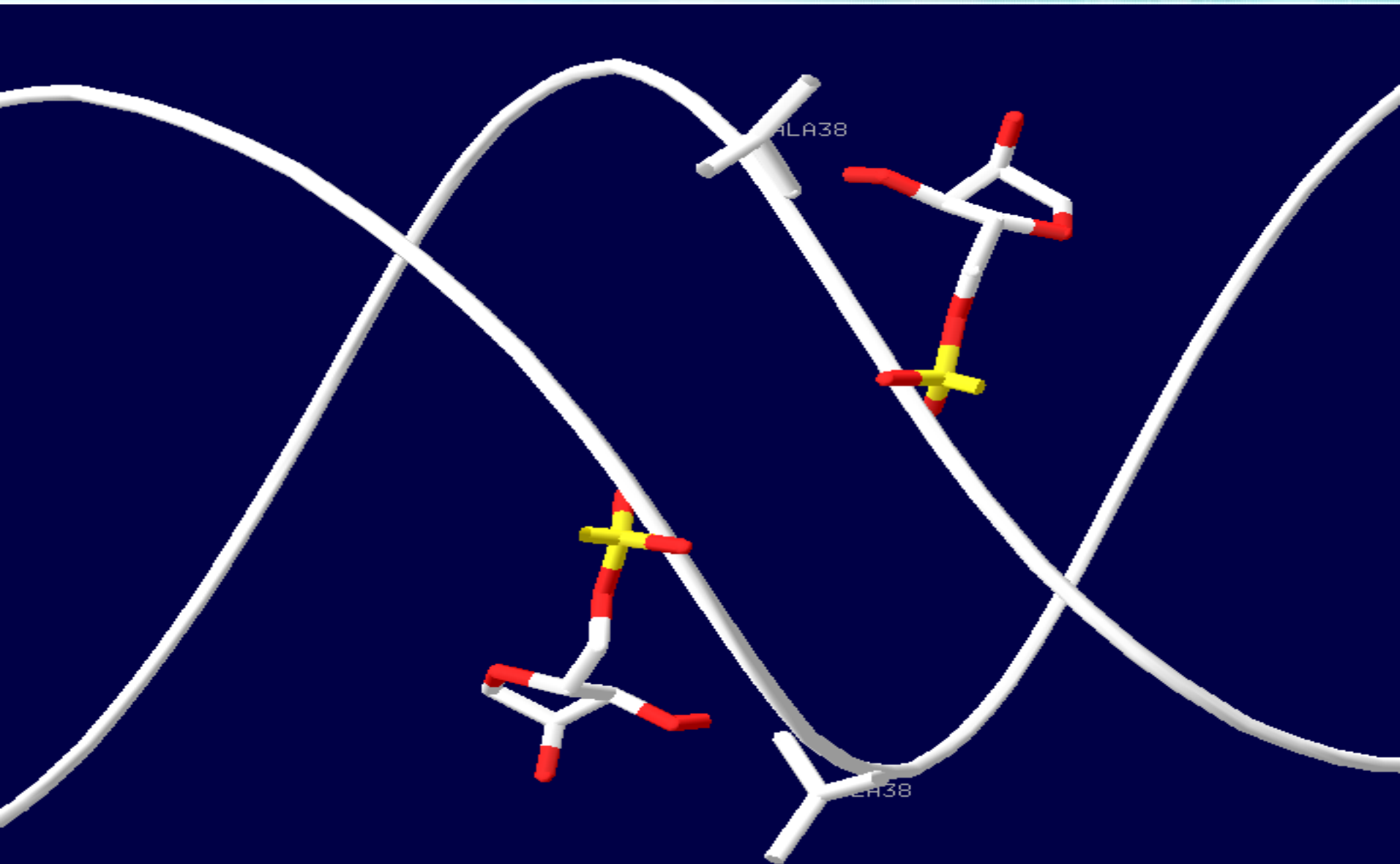
3.5.2 35Arg-G6



3.5.2 38Arg-C7



3.5.2 R38A



3.5.2 结论

- 由此证实了NS1的35和38位Arg是与dsRNA结合的关键位点

3.5.3 C端

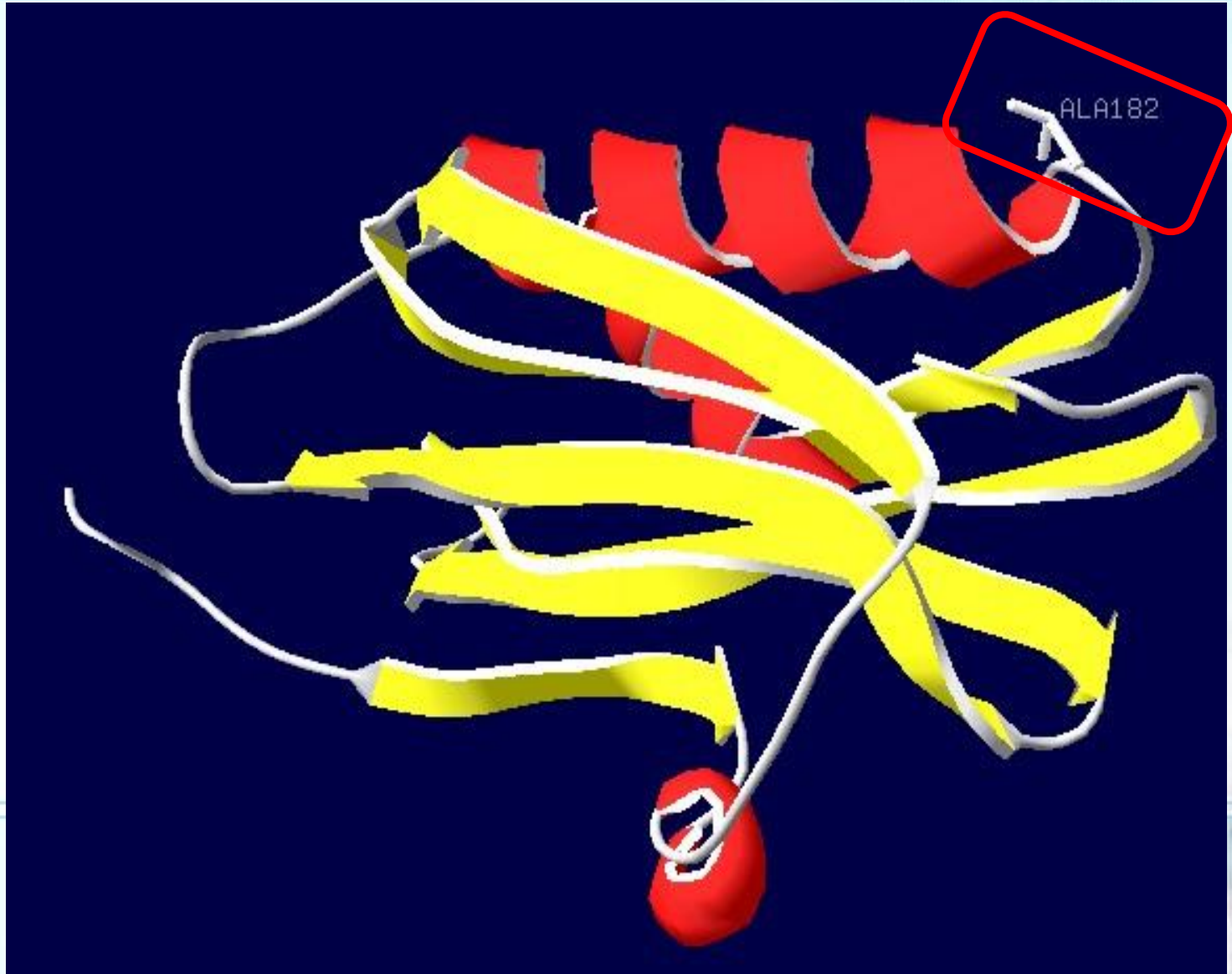
- 验证突变W182A对结构和功能的影响 (PDB ID : 3P38)

突变前



3.5.3 C端

突变后



3.5.3 分析

- Trp是极性、中性氨基酸，其侧链都伸出在外侧；Ala是非极性、疏水氨基酸，位于C端的内部。
- 如果为Trp，则因侧链太大则不易于蛋白质结合；如果为Ala，则可以与蛋白质结合。
- 突变为Ala之后，更容易与pre-mRNA的3'-末端加工蛋白结合，从而阻止宿主细胞的pre-mRNA的加工和出核。

4. 参考文献

- Ao Cheng et al. Structural basis for dsRNA recognition by NS1 protein of influenza A virus. *Cell Research*, 2009, 19:187-195
- Zachary A. Bornholdt et al. X-ray structure of NS1 from a highly pathogenic H5N1 influenza virus. *Nature*, 2008, 456:985-988
- Zachary A. Bornholdt et al. X-ray structure of influenza virus NS1 effector domain. *Nature Structural & Molecular Biology*, 2006, 13:599-560
- Benjamin G. Hale et al. Structural insights into phosphoinositide 3-kinase activation by the influenza A virus NS1 protein. *PNAS*, 2010, 107:1954-1959
- Yu J, Li X, Wang Y, Li B, Li H, et al. (2011) PDlim2 Selectively Interacts with the PDZ Binding Motif of Highly Pathogenic Avian H5N1 Influenza A Virus NS1. *PLoS ONE* 6(5): e19511. doi:10.1371/journal.pone.0019511

5.致谢

- 感谢罗老师在这一学期对我们的悉心指导
- 感谢我们小组成员的通力合作

谢谢！