PEDV-PLpro木瓜样蛋白酶在先天性免疫中功能的预测

Prediction about the function of PEDV-Plpro in innate immunity

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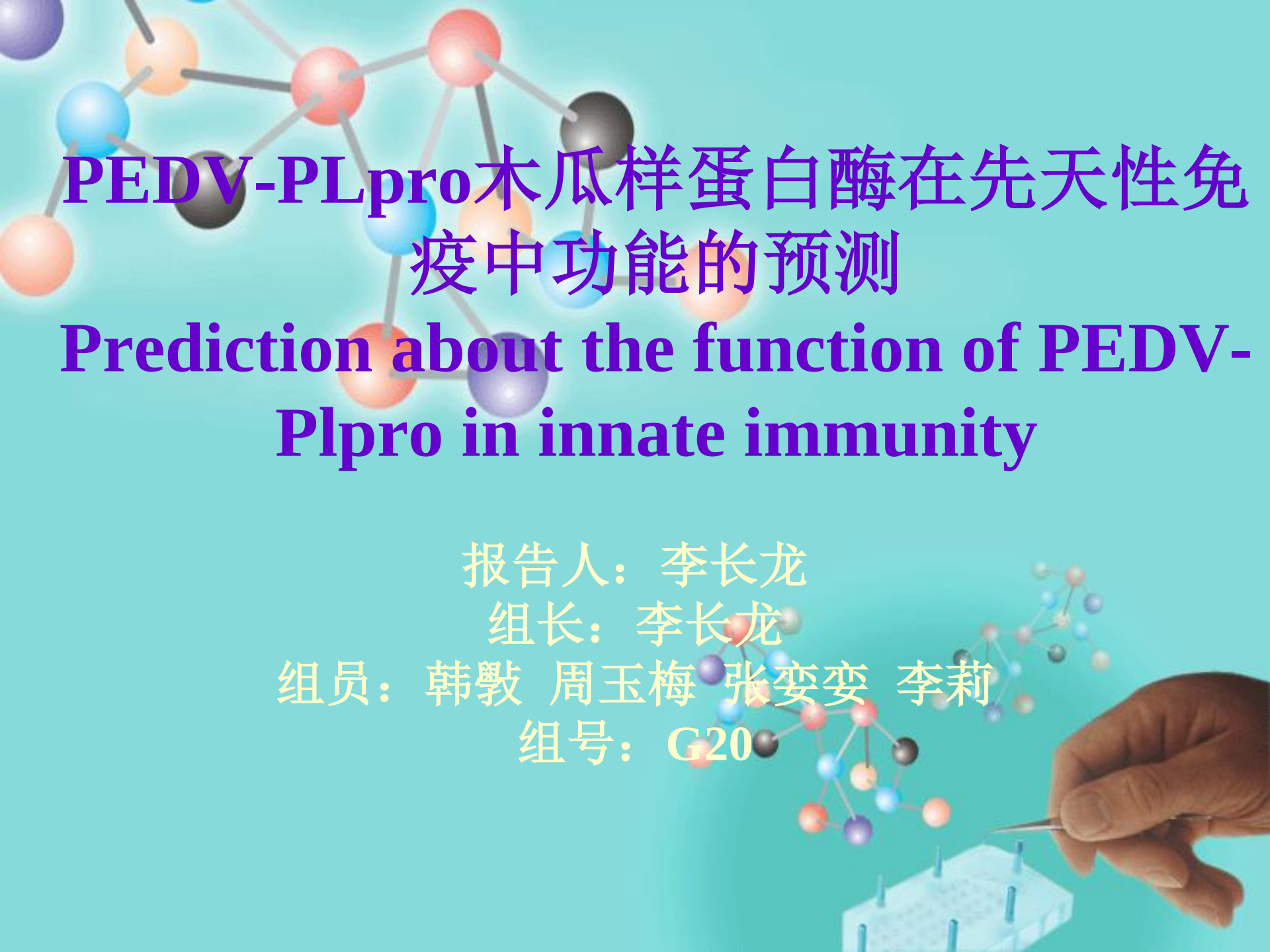
组号：G20



主要内容

- 背景介绍
- 文献检索
- 序列比对分析
- 同源建模及三维结构分析
- 结论
- 实验方案
- 致谢
- 参考文献

背景介绍

- 多种冠状病毒都有编码PLpro蛋白酶的基因
- **SARS病毒　nsp3-** PLpro
- **HCoV nsp3-** Plpro-1，Plpro-2
- **TGEV nsp3-** Plpro-1，Plpro-2
- **PEDV nsp3-** Plpro-1，Plpro-2
- **FCoV nsp3-** Plpro-1，Plpro-2
- PRRSV nsp1-Plpro

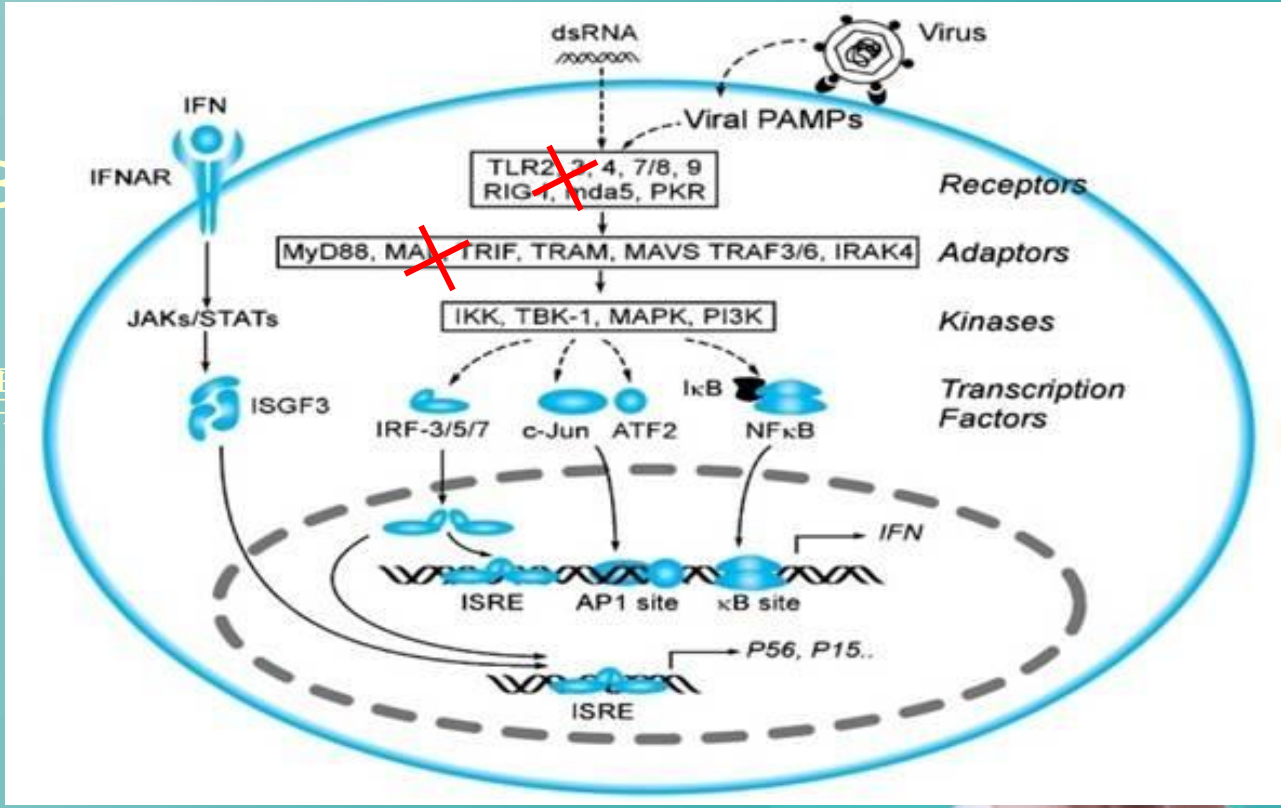
背景介绍

SARS-PLpro

功能：DUB

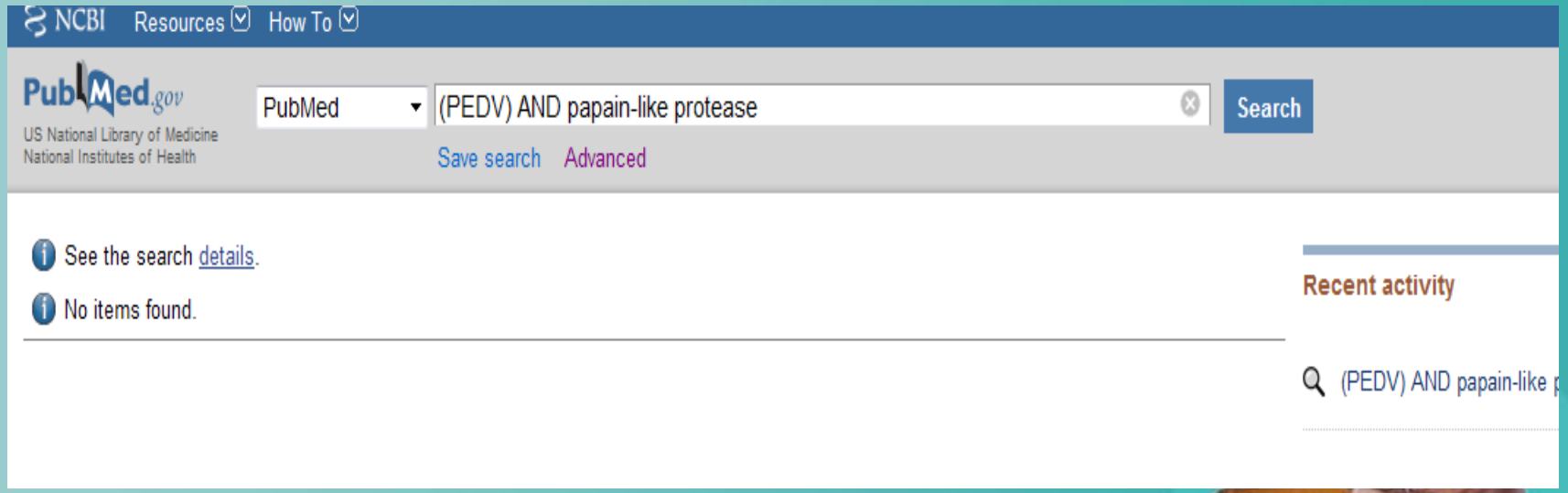
底物分子：

影响：负调



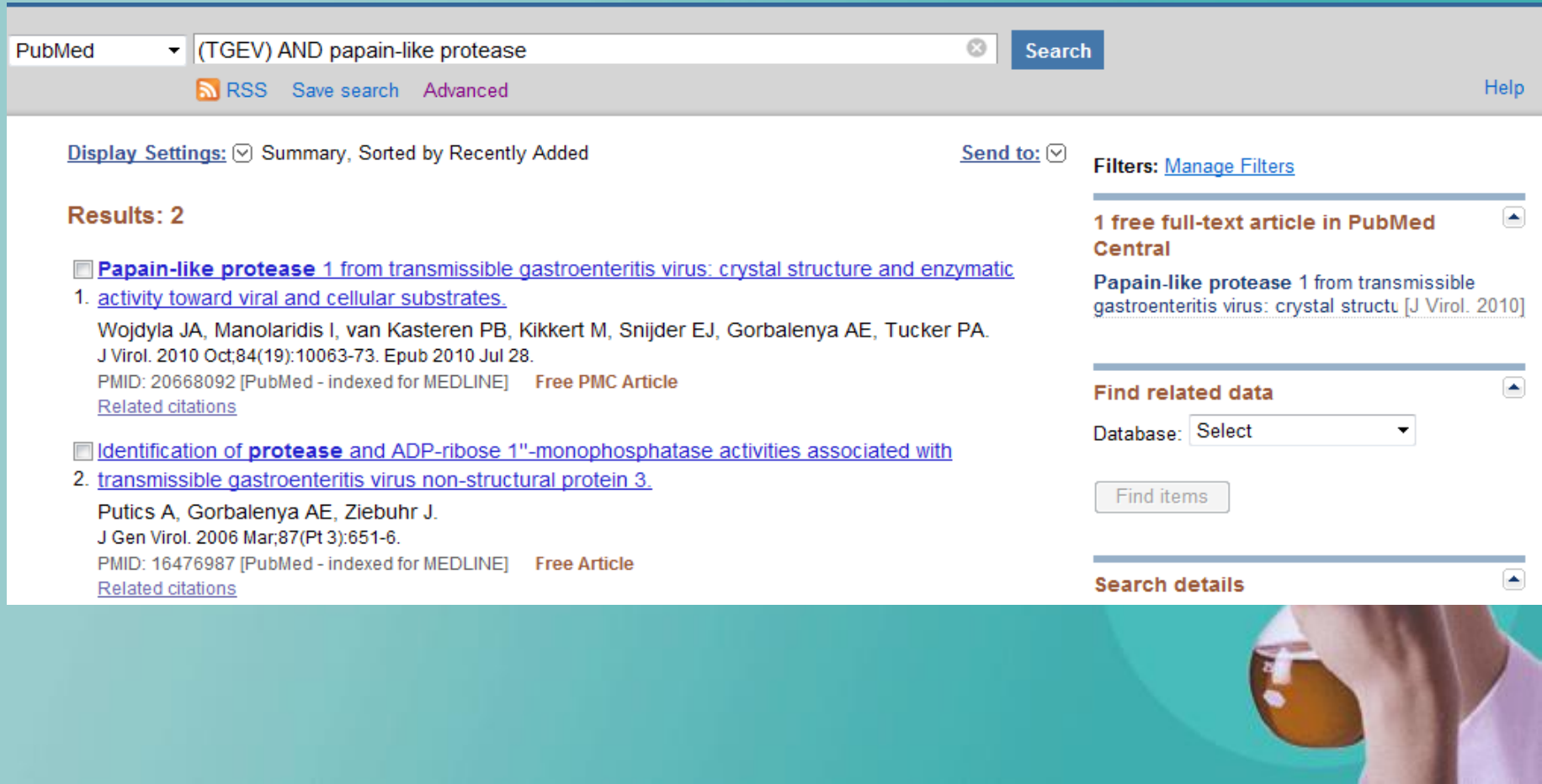
文献检索

- 关键词： (PEDV) AND papain-like protease



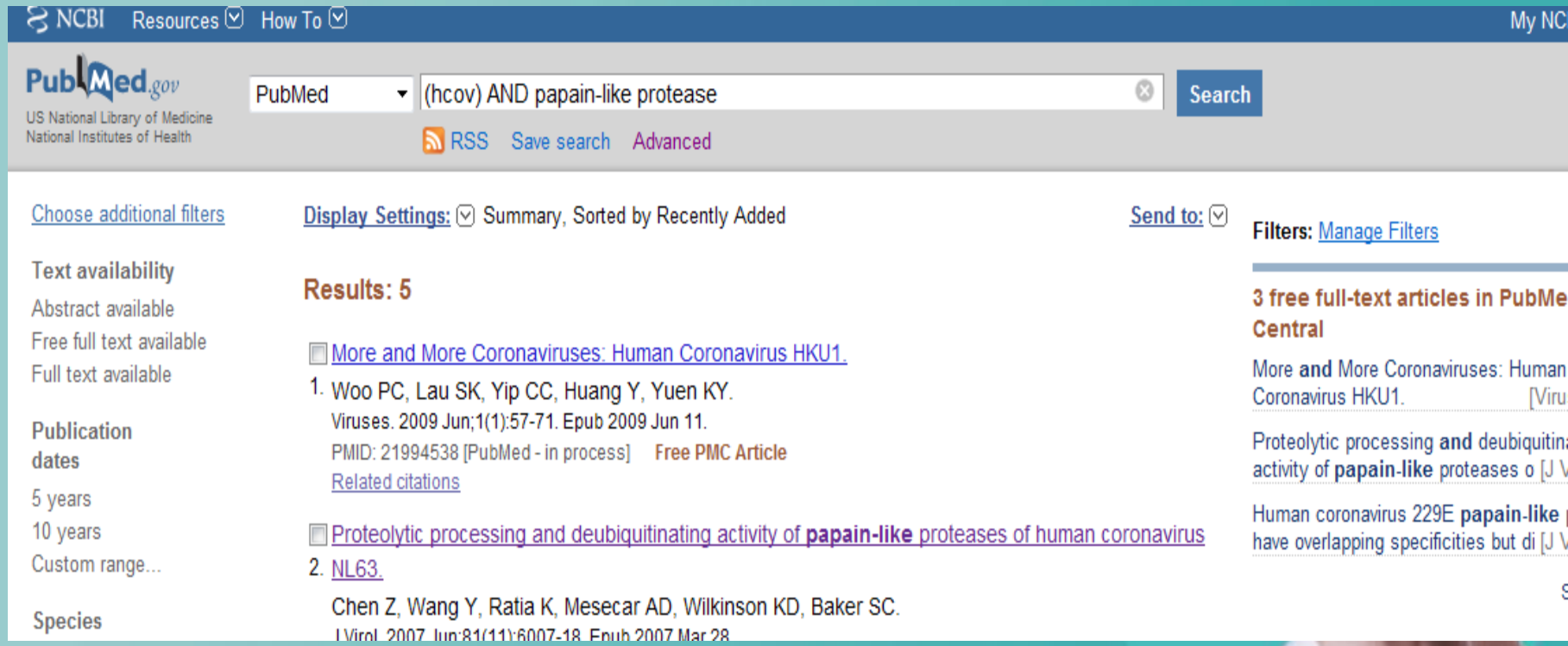
文献检索

- 关键词：(TGEV) AND papain-like protease



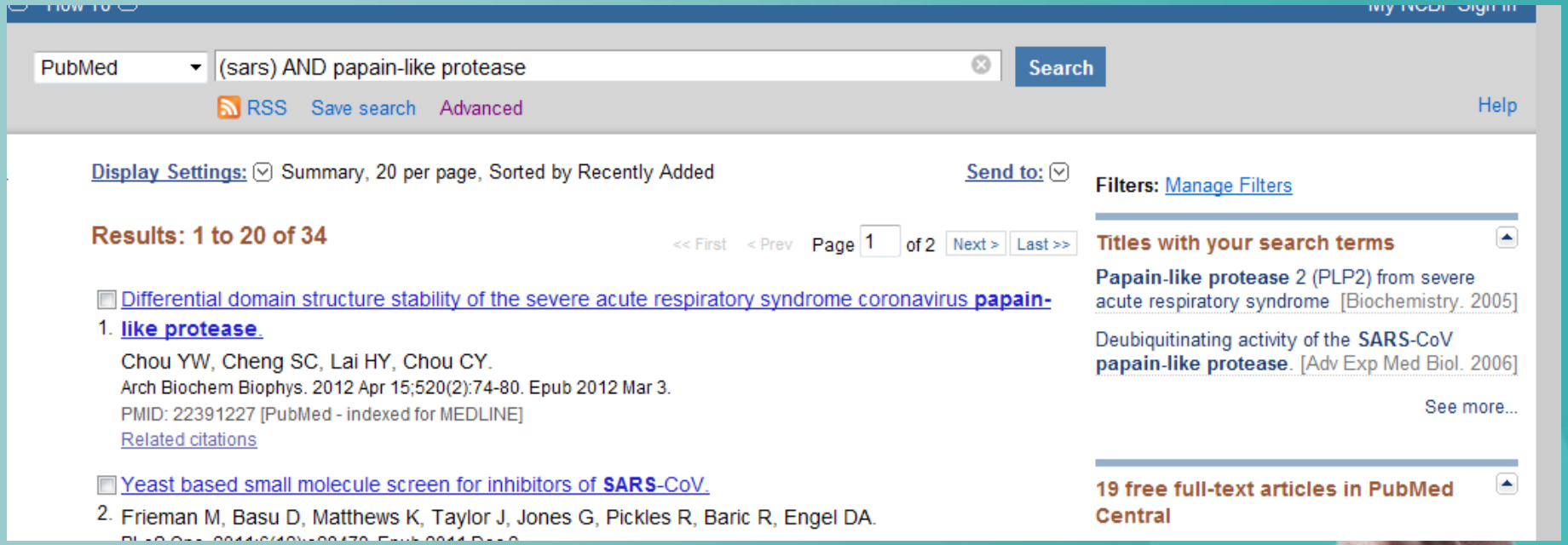
文献检索

- 关键词：(HCoV) AND papain-like protease



文献检索

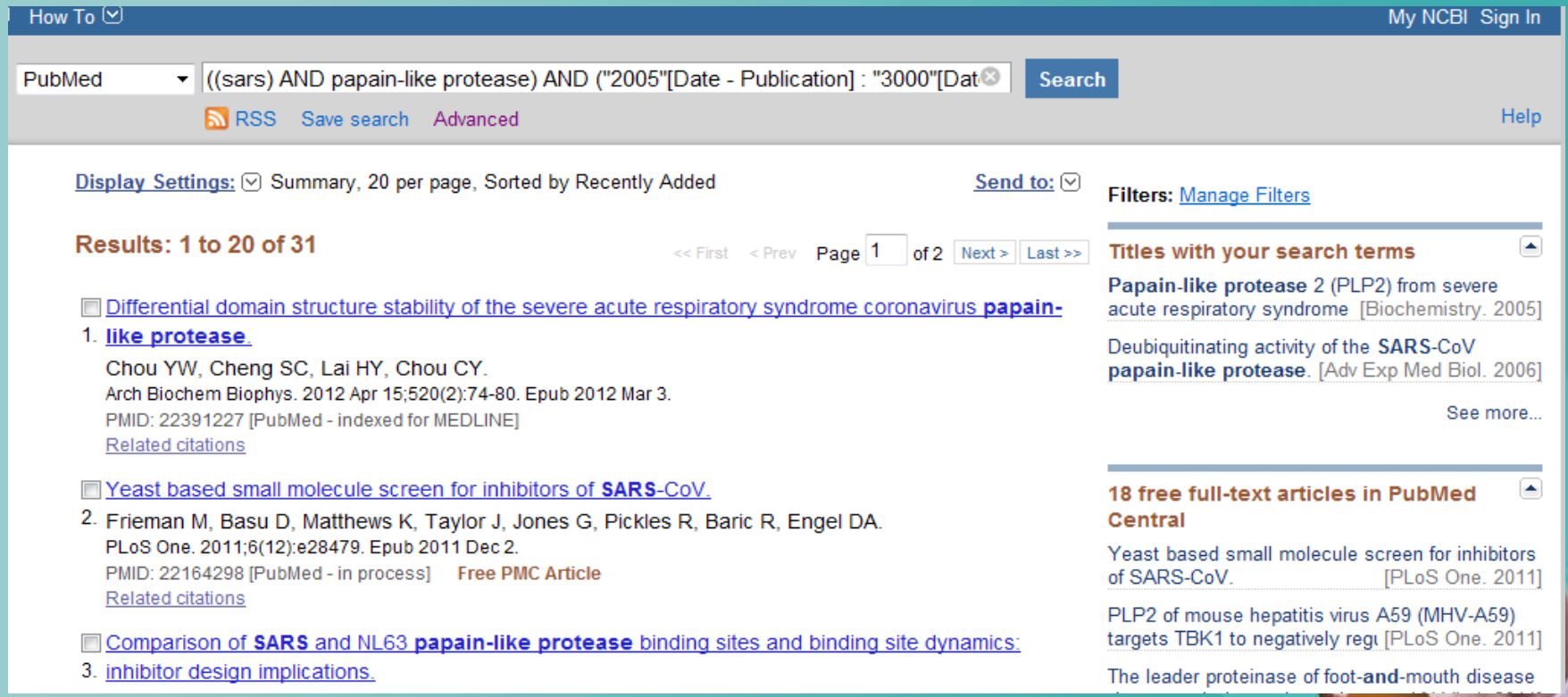
- 关键词：(SARS) AND papain-like protease



文献检索

关键词：(SARS) AND papain-like protease

时间：2005至今



序列比对分析

- BLAST结果

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Link
NEW NP_839958.1	putative coronavirus nsp1 [Porcine epidemic diarrhea virus]	583	583	100%	0.0	100%	G
NEW P0C6V6.1	RecName: Full=Replicase polyprotein 1a; Short=pp1a; AltName: Full=	584	584	100%	0.0	100%	
NEW NP_598309.1	Pol1 [Porcine epidemic diarrhea virus] >gb|AAK38661.1| Pol1 [porcin	583	583	100%	0.0	100%	
NEW P0C6Y4.1	RecName: Full=Replicase polyprotein 1ab; Short=pp1ab; AltName: Fu	583	583	100%	0.0	100%	
NEW ABM64775.1	Pol1 [Porcine epidemic diarrhea virus]	583	583	100%	0.0	100%	
NEW AFC98503.1	polyprotein [Porcine epidemic diarrhea virus]	568	604	100%	0.0	97%	
NEW AFB77239.1	polyprotein [Porcine epidemic diarrhea virus]	565	601	100%	0.0	97%	
NEW AEQ55003.1	polyprotein [Porcine epidemic diarrhea virus]	560	597	100%	3e-180	97%	
NEW AFE85967.1	polyprotein [Porcine epidemic diarrhea virus]	559	596	100%	8e-180	96%	
NEW AFE85968.1	polyprotein [Porcine epidemic diarrhea virus]	540	576	100%	5e-173	94%	
NEW P0C6F6.1	RecName: Full=Replicase polyprotein 1a; Short=pp1a; AltName: Full=	315	355	86%	8e-95	60%	
NEW YP_001351683.1	ORF1 [Scotophilus bat coronavirus 512] >sp|P0C6W0.1|R1AB_BC512	314	354	86%	2e-94	60%	G
NEW YP_001718597.1	ORF1a polyprotein [Bat coronavirus 1B] >gb|ACA52155.1| ORF1a pol	231	270	93%	9e-66	45%	G
NEW YP_001718596.1	ORF1ab polyprotein [Bat coronavirus 1B] >gb|ACA52156.1| ORF1ab	231	270	93%	2e-65	45%	G
NEW YP_001718604.1	ORF1a polyprotein [Bat coronavirus 1A] >gb|ACA52162.1| ORF1a po	224	267	99%	3e-63	44%	G
NEW YP_001718603.1	ORF1ab polyprotein [Bat coronavirus 1A] >gb|ACA52163.1| ORF1ab	224	267	99%	3e-63	44%	G
NEW YP_001718611.1	ORF1a polyprotein [Bat coronavirus HKU8] >gb|ACA52169.1| ORF1a	218	261	81%	3e-61	49%	G
NEW YP_001718610.1	ORF1ab polyprotein [Bat coronavirus HKU8] >gb|ACA52170.1| ORF1a	218	261	81%	4e-61	49%	G
NEW YP_001552235.1	orf1a polyprotein [Bat coronavirus HKU2] >gb|ABQ57214.1| orf1a po	179	218	97%	9e-48	38%	G
NEW YP_001552234.1	orf1ab polyprotein [Bat coronavirus HKU2] >gb|ABQ57207.1| orf1ab	179	218	97%	1e-47	38%	G

序列比对分析

- 从文章中找到相关序列

PEDV、TGEV、HCoV、FCoV、SARS

- 两两比对
- 多序列比对

序列比对分析

- **SARS-PEDV**

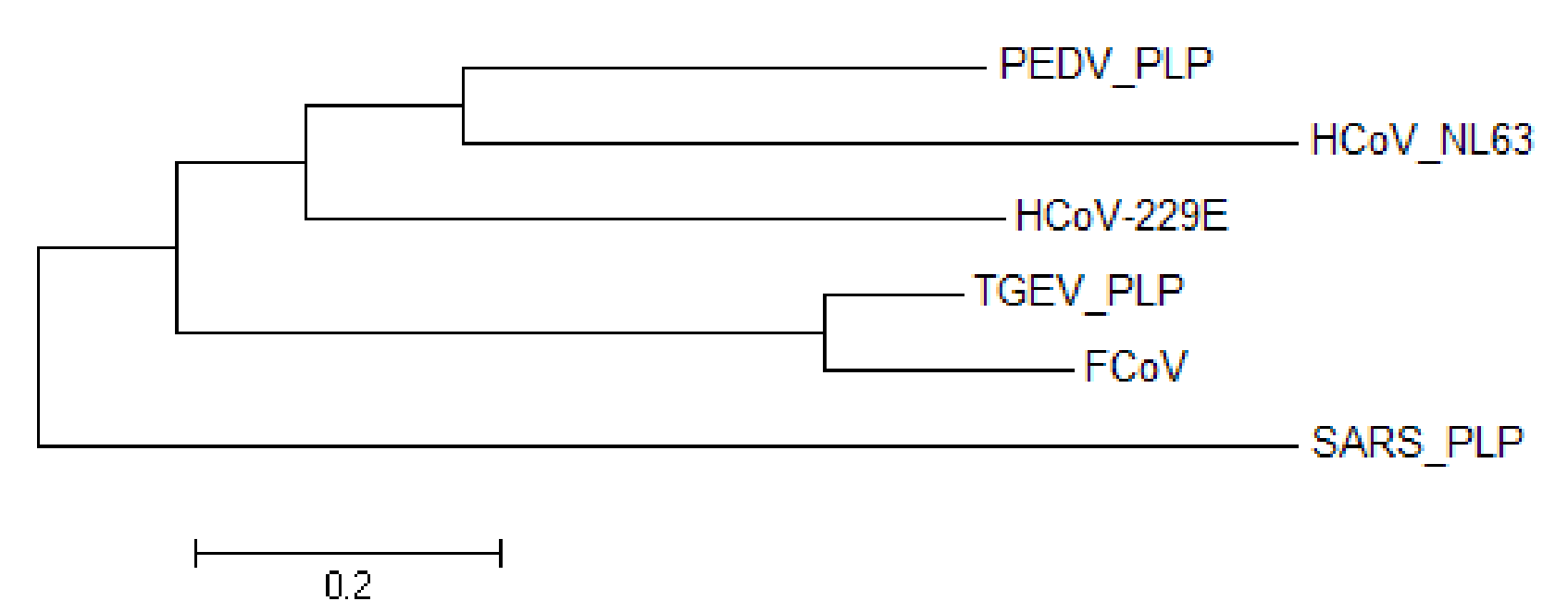
LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
292	220.0	76/292 (26.0%)	106/292 (36.3%)	83/292 (28.4%)

序列比对

- 多序列比对

序列比对

- 进化树



同源建模

Model Summary:

Model information:

Modelled residue range:	68 to 269
Based on template:	3mp2A (2.50 A)
Sequence Identity [%]:	31.22
Evalue:	0

Quaternary structure information:

Template (3mp2): MONOMER
Model built: SINGLE CHAIN

Ligand information:

Ligands in the template: ZN: 1.
Ligands in the model: ZN: 1

Quality information:

QMEAN Z-Score: -3.358

同源建模

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
193	381.0	66/193 (34.2%)	93/193 (48.2%)	7/193 (3.6%)

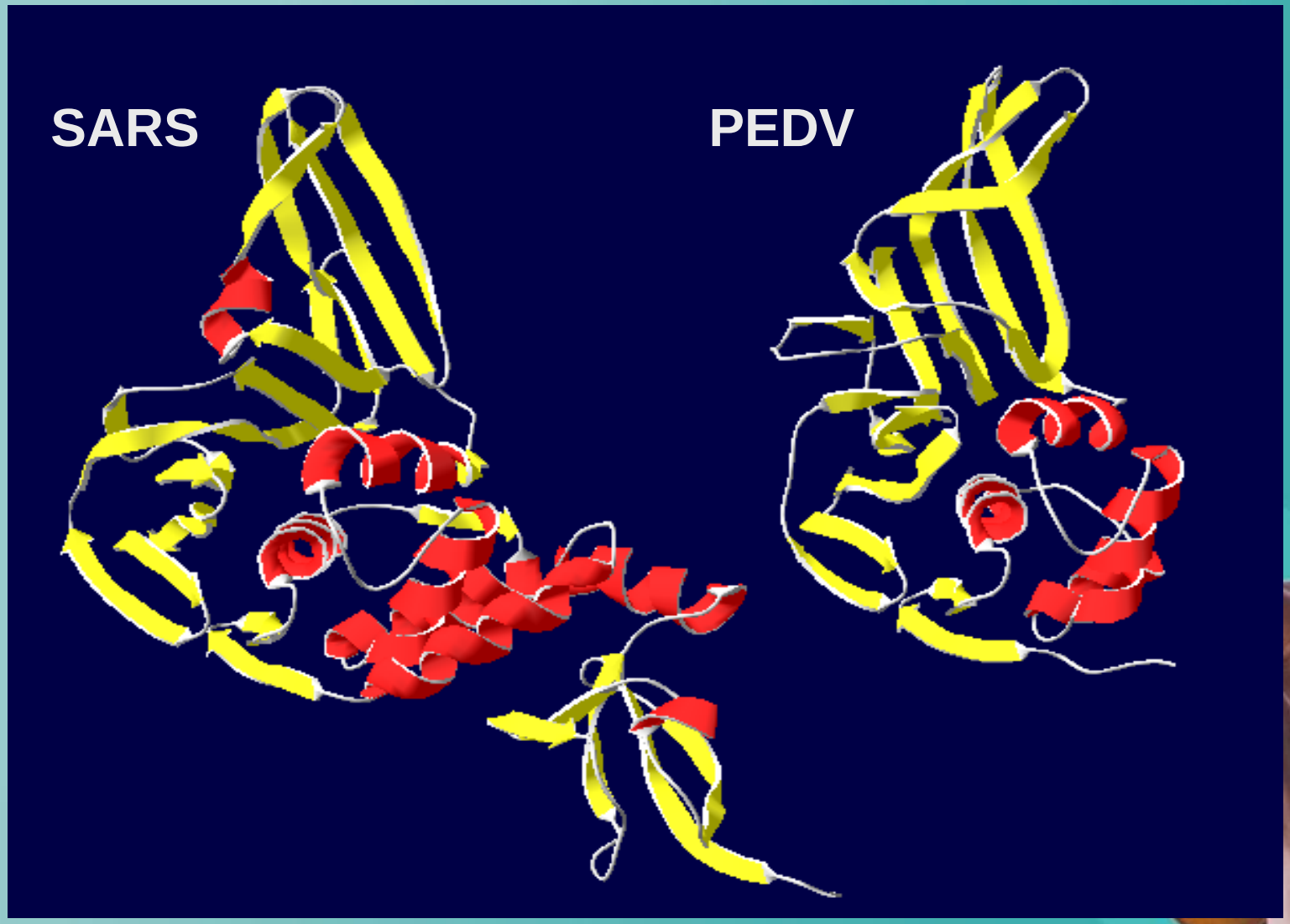
```
pedv        72 FDFVSYGGLKVLRQSHNNCWVTSTLVQLQLLGIVDDPAMELFSAGRVGPM    121
               |...:..|..:|:|..||||:.:...|||.:...::.|.|.|..|.|...
tgev         5 FKTTNLNGKIILKQGDNNCWINACCYQLQAFDFFNNEAWEKFKKGDVMDF     54

pedv       122 VRKCYESQKAILGSLGDVSACLESLTKDLHTLKITCSVVCGCGTGERIYE    171
               |..||.:.....|..||....||.:..|..|.||..:..||||..|.:.|
tgev        55 VNLCYAATTLARGHSGDAEYLLELMLNDYSTAKIVLAAKCGCGEKEIVLE    104

pedv       172 GCAFRMTPTLEPFPYGACAQCAQVLMHTFKSIVGTGIFCRDTTALSLDS-    220
               ...|::||..|.|.||.|..|.||....|.|:.|:|:|..|  .||..:
tgev       105 RAVFKLTPLKESFNYGVCGDCMQVNTCRFLSVEGSGVFVHD--ILSKQTP    152

pedv       221 ---LVVKPLCAAAFIG-KDSGHYVTNFYDAAMAIDGYGRHQIK    259
                  :||||:..|.:.| ..:|||:.:..:....:||.|...:|
tgev       153 EAMFVVKPVMHAVYTGTTQNGHYMVDDIEHGYCVDGMGIKPLK    195
```

三维结构分析

SARS-plpro、PEDV-Plpro

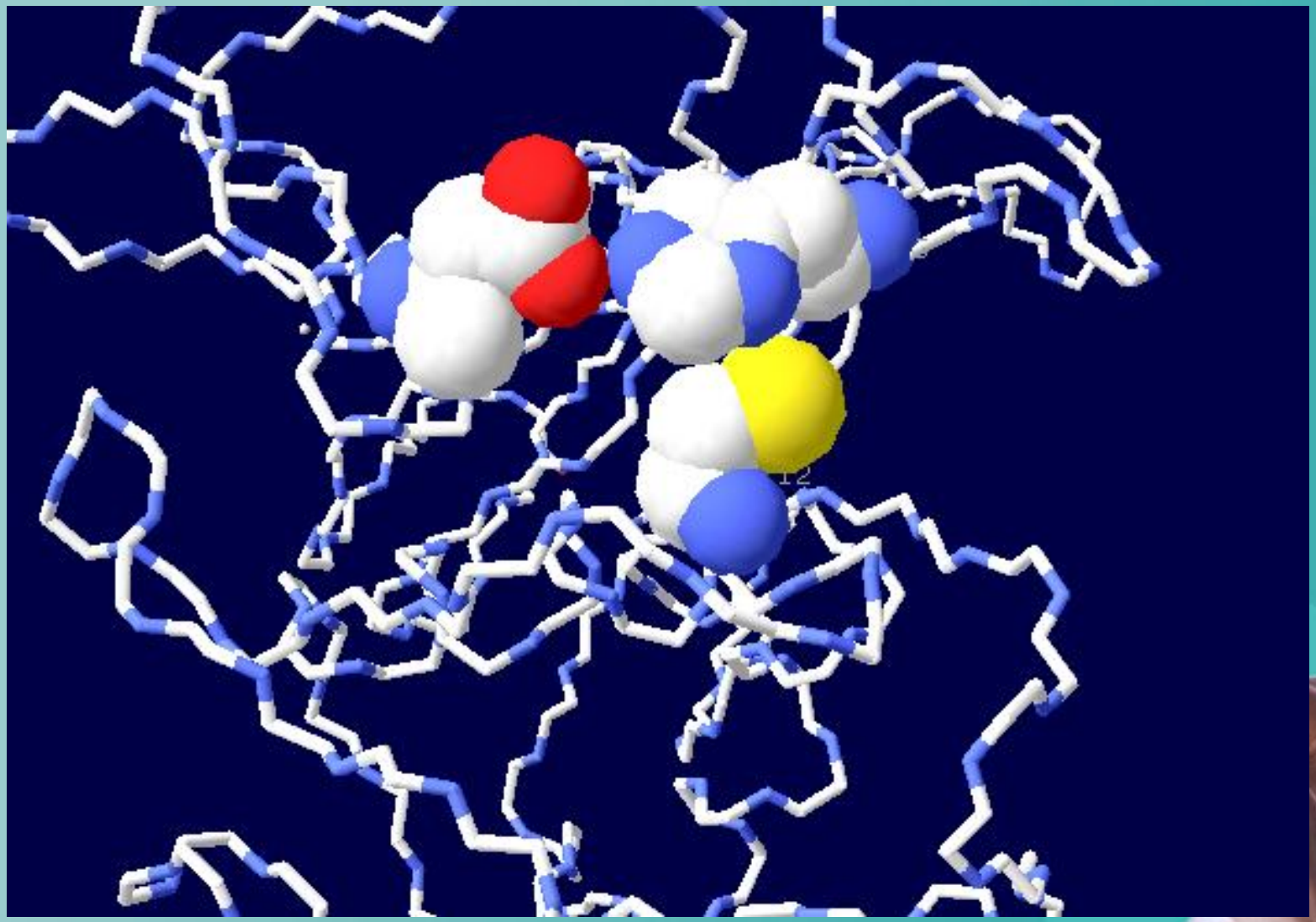
Magic Fit 均方根误差 5.32埃

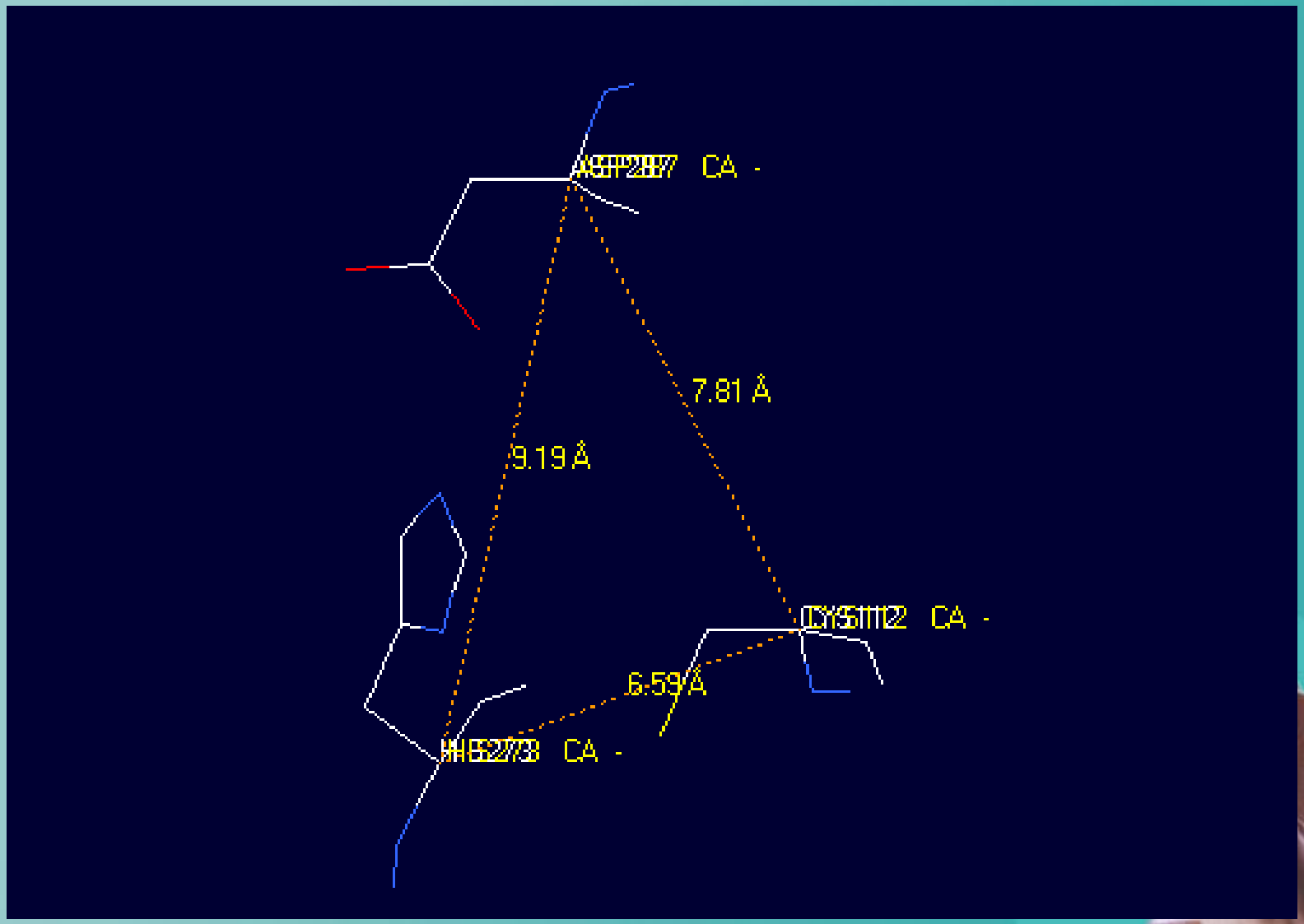
三维结构分析



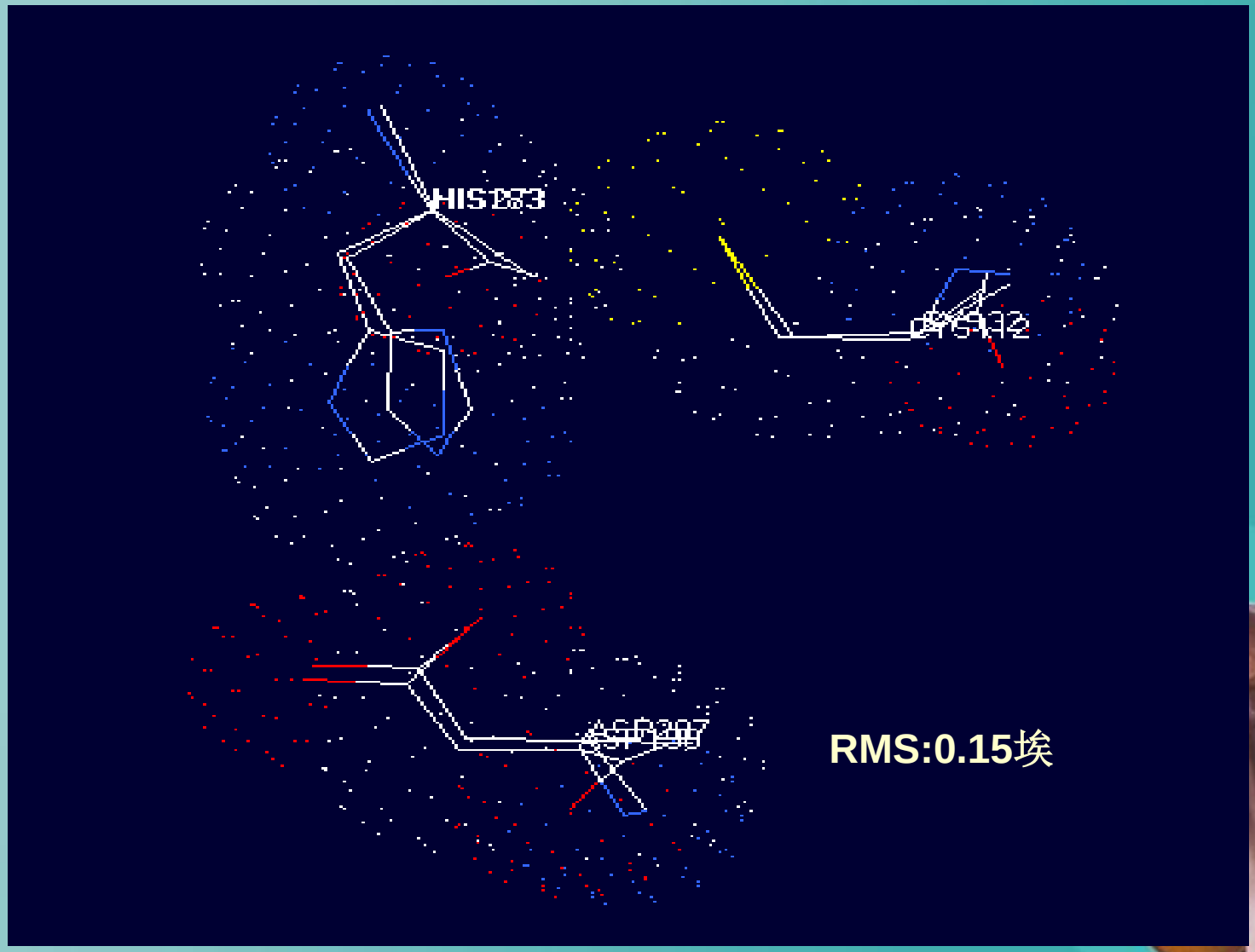
三维结构分析

- 特异性位点FIT
- SARS:C112，H273，D287
- PEDV: C34，H183，D196




CA -
7.81 Å
9.19 Å
CA -
6.59 Å
CA -

三维结构分析

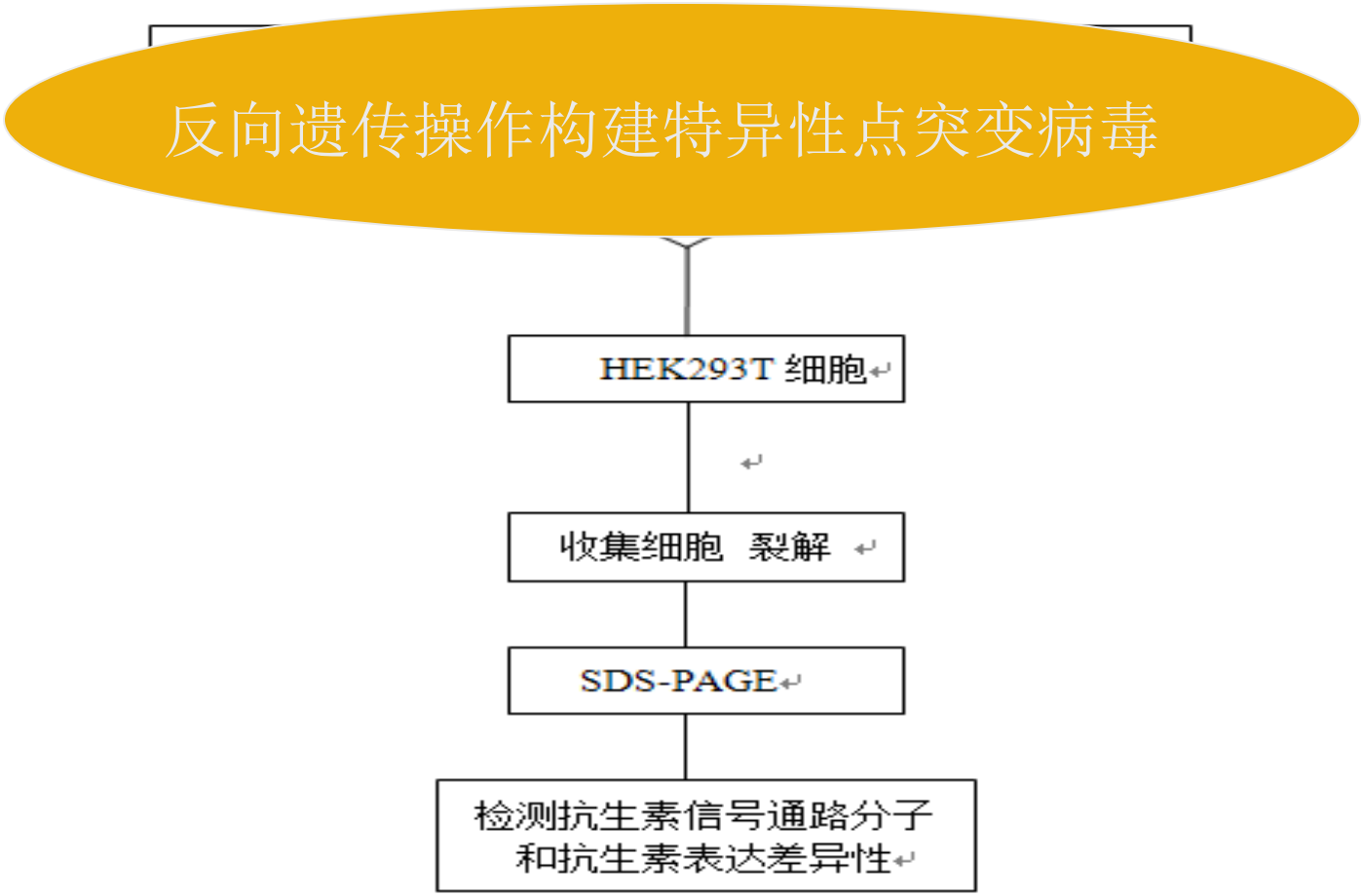


结论

- SARS-plpro和PEDV-plpro在结构上很相似，而且从酶的角度来讲，他们具有相同的活性位点，那么，我们就有理由认为，他们具有相似的活性，即：

PEDV-plpro在先天性免疫的功能是利用其DUB活性，破坏细胞干扰素调控信号通路，负调控宿主抗病毒反应

实验验证


反向遗传操作构建特异性点突变病毒
HEK293T 细胞
收集细胞 裂解
SDS-PAGE
检测抗生素信号通路分子
和抗生素表达差异性

致谢

- 感谢罗老师一学期来对我们学习的教导
- 感谢我们小组成员的合作与参与
- 感谢孙留克、张利杰等对本课题的指导与建议

参考文献

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- 2 Zhongbin Chen,et al.Proteolytic Processing and Deubiquitinating Activity of Papain-Like
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- 5 **Kiira Ratia et al.Severe acute respiratory syndrome coronavirus papain-like protease: Structure of a viral deubiquitinating enzyme.** PNAS
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Thanks for your attention!