



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



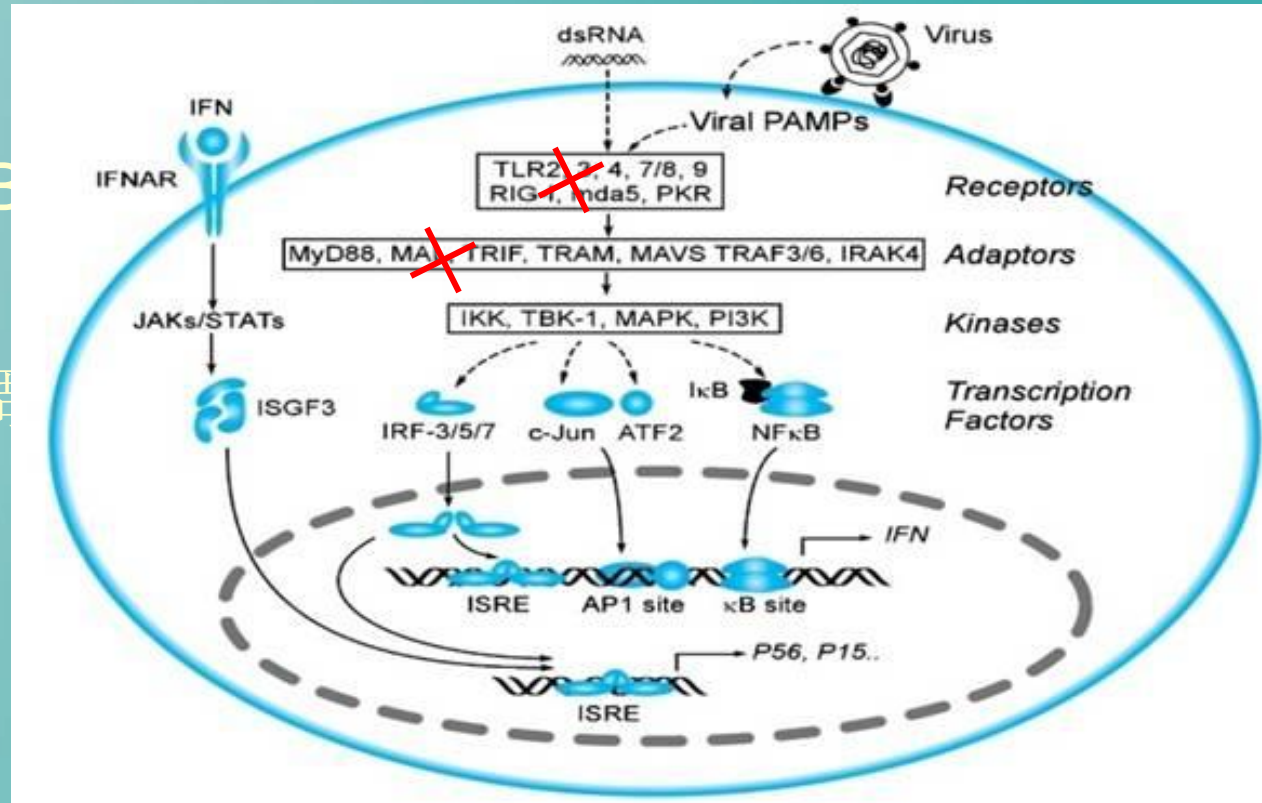
背景介绍

SARS-PLpro

功能: DUB

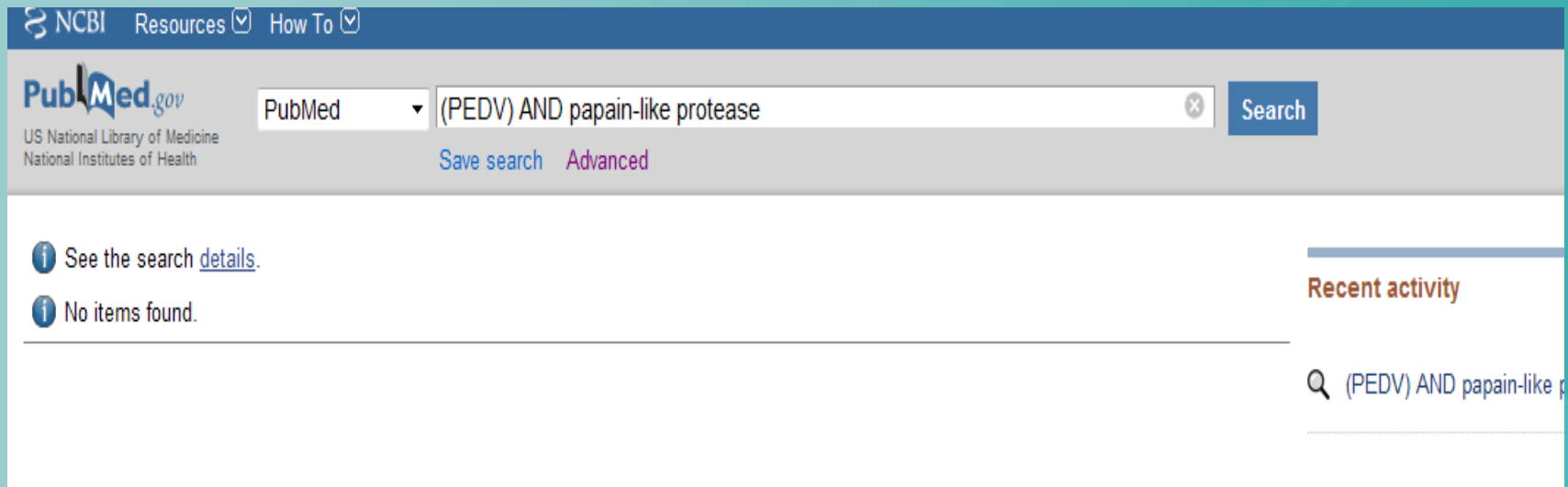
底物分子:

影响: 负调



文献检索

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



The screenshot displays the PubMed search interface. At the top, there are navigation links for 'NCBI', 'Resources', and 'How To'. The main search area includes the 'PubMed.gov' logo, the text 'US National Library of Medicine' and 'National Institutes of Health', a search box containing the query '(PEDV) AND papain-like protease', and a 'Search' button. Below the search box are links for 'Save search' and 'Advanced'. On the left side, there are two informational messages: 'See the search [details](#).' and 'No items found.'. On the right side, there is a 'Recent activity' section with a search icon and the text '(PEDV) AND papain-like p'.



文献检索

■ 关键词: (TGEV) AND papain-like protease

PubMed (TGEV) AND papain-like protease Search

RSS Save search Advanced Help

Display Settings: Summary, Sorted by Recently Added Send to:

Filters: [Manage Filters](#)

Results: 2

[Papain-like protease 1 from transmissible gastroenteritis virus: crystal structure and enzymatic activity toward viral and cellular substrates.](#)

1. [activity toward viral and cellular substrates.](#)
Wojdyla JA, Manolaridis I, van Kasteren PB, Kikkert M, Snijder EJ, Gorbalenya AE, Tucker PA.
J Virol. 2010 Oct;84(19):10063-73. Epub 2010 Jul 28.
PMID: 20668092 [PubMed - indexed for MEDLINE] [Free PMC Article](#)
[Related citations](#)

[Identification of protease and ADP-ribose 1"-monophosphatase activities associated with transmissible gastroenteritis virus non-structural protein 3.](#)

2. [transmissible gastroenteritis virus non-structural protein 3.](#)
Putics A, Gorbalenya AE, Ziebuhr J.
J Gen Virol. 2006 Mar;87(Pt 3):651-6.
PMID: 16476987 [PubMed - indexed for MEDLINE] [Free Article](#)
[Related citations](#)


1 free full-text article in PubMed Central

[Papain-like protease 1 from transmissible gastroenteritis virus: crystal structure \[J Virol. 2010\]](#)

Find related data

Database:

Search details



文献检索

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

NCBI Resources How To My NCBI

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed (hcov) AND papain-like protease Search

RSS Save search Advanced

[Choose additional filters](#) [Display Settings:](#) Summary, Sorted by Recently Added [Send to:](#)

Filters: [Manage Filters](#)

Text availability
Abstract available
Free full text available
Full text available

Publication dates
5 years
10 years
Custom range...

Species

Results: 5

[More and More Coronaviruses: Human Coronavirus HKU1.](#)

1. Woo PC, Lau SK, Yip CC, Huang Y, Yuen KY.
Viruses. 2009 Jun;1(1):57-71. Epub 2009 Jun 11.
PMID: 21994538 [PubMed - in process] [Free PMC Article](#)
[Related citations](#)

[Proteolytic processing and deubiquitinating activity of papain-like proteases of human coronavirus NL63.](#)


2. [NL63.](#)
Chen Z, Wang Y, Ratia K, Mesecar AD, Wilkinson KD, Baker SC.
J Virol. 2007 Jun;81(11):6007-18. Epub 2007 Mar 28.

3 free full-text articles in PubMed Central

More and More Coronaviruses: Human Coronavirus HKU1. [Viru...]

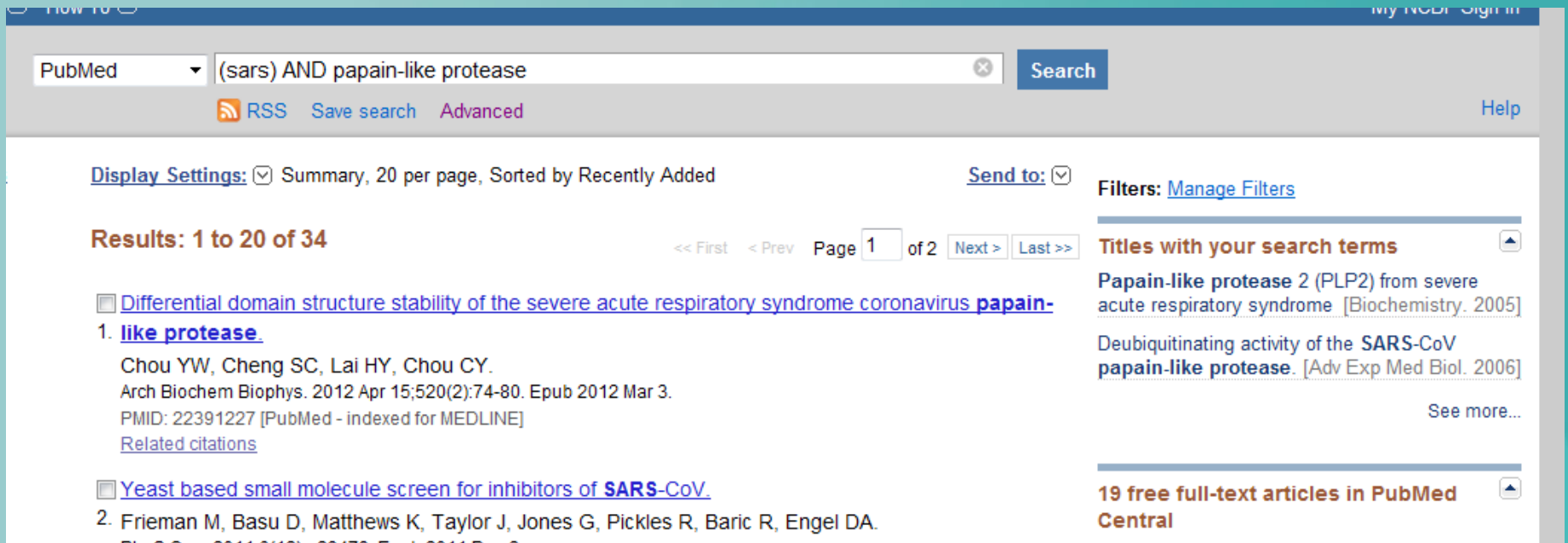
Proteolytic processing and deubiquitinating activity of papain-like proteases o [J V...]

Human coronavirus 229E papain-like proteases have overlapping specificities but di [J V...]



文献检索

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



The screenshot shows a PubMed search interface. At the top, there is a search bar with the query "(sars) AND papain-like protease" and a "Search" button. Below the search bar, there are links for "RSS", "Save search", and "Advanced". The search results are displayed in a list format. The first result is titled "Differential domain structure stability of the severe acute respiratory syndrome coronavirus papain-like protease" and is listed as "1. like protease." The authors are Chou YW, Cheng SC, Lai HY, and Chou CY. The publication is in Arch Biochem Biophys, 2012 Apr 15;520(2):74-80. The PMID is 22391227. The second result is titled "Yeast based small molecule screen for inhibitors of SARS-CoV" and is listed as "2. Frieman M, Basu D, Matthews K, Taylor J, Jones G, Pickles R, Baric R, Engel DA." The publication is in PLoS One, 2011;6(10):e25470. The PMID is 22111111. On the right side of the search results, there are sections for "Titles with your search terms" and "19 free full-text articles in PubMed Central".

PubMed (sars) AND papain-like protease Search

RSS Save search Advanced Help

Display Settings: Summary, 20 per page, Sorted by Recently Added Send to:

Filters: [Manage Filters](#)

Results: 1 to 20 of 34 << First < Prev Page 1 of 2 Next > Last >>

[Differential domain structure stability of the severe acute respiratory syndrome coronavirus papain-like protease.](#)

1. [like protease.](#)

Chou YW, Cheng SC, Lai HY, Chou CY.
Arch Biochem Biophys. 2012 Apr 15;520(2):74-80. Epub 2012 Mar 3.
PMID: 22391227 [PubMed - indexed for MEDLINE]
[Related citations](#)

[Yeast based small molecule screen for inhibitors of SARS-CoV.](#)

2. Frieman M, Basu D, Matthews K, Taylor J, Jones G, Pickles R, Baric R, Engel DA.
PLoS One. 2011;6(10):e25470. Epub 2011 Dec 9.

Titles with your search terms

[Papain-like protease 2 \(PLP2\) from severe acute respiratory syndrome \[Biochemistry. 2005\]](#)


[Deubiquitinating activity of the SARS-CoV papain-like protease. \[Adv Exp Med Biol. 2006\]](#)



[See more...](#)


19 free full-text articles in PubMed Central


文献检索

关键词：(SARS) AND papain-like protease
时间：2005至今

How To  My NCBI Sign In

PubMed  ((sars) AND papain-like protease) AND ("2005"[Date - Publication] : "3000"[Date - Publication])  Search Help

 RSS [Save search](#) [Advanced](#)

[Display Settings:](#) Summary, 20 per page, Sorted by Recently Added [Send to:](#) 


Results: 1 to 20 of 31 << First < Prev Page of 2 Next > Last >>

[Differential domain structure stability of the severe acute respiratory syndrome coronavirus papain-like protease.](#)
1. [like protease.](#)
Chou YW, Cheng SC, Lai HY, Chou CY.
Arch Biochem Biophys. 2012 Apr 15;520(2):74-80. Epub 2012 Mar 3.
PMID: 22391227 [PubMed - indexed for MEDLINE]
[Related citations](#)


[Yeast based small molecule screen for inhibitors of SARS-CoV.](#)
2. Frieman M, Basu D, Matthews K, Taylor J, Jones G, Pickles R, Baric R, Engel DA.
PLoS One. 2011;6(12):e28479. Epub 2011 Dec 2.
PMID: 22164298 [PubMed - in process] **Free PMC Article**
[Related citations](#)

[Comparison of SARS and NL63 papain-like protease binding sites and binding site dynamics: inhibitor design implications.](#)
3. [inhibitor design implications.](#)

Filters: [Manage Filters](#)

Titles with your search terms 

Papain-like protease 2 (PLP2) from severe acute respiratory syndrome [Biochemistry. 2005]
Deubiquitinating activity of the SARS-CoV papain-like protease. [Adv Exp Med Biol. 2006]
[See more...](#)

18 free full-text articles in PubMed Central 

Yeast based small molecule screen for inhibitors of SARS-CoV. [PLoS One. 2011]
PLP2 of mouse hepatitis virus A59 (MHV-A59) targets TBK1 to negatively regulate [PLoS One. 2011]
The leader proteinase of foot-and-mouth disease

序列比对分析

BLAST结果

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

多序列比对

N110、C112

	F	A	F	D	F	V	S	Y	G	G	L	K	V	L	R	Q	S	H	N	N	D	W	V	T	S	T	L	V	Q	L	Q	L	
✓ 1. pedv
✓ 2. tgev	P	P	.	K	T	T	N	L	N	.	K	I	I	.	K	.	G	D	I	N	A	C	C	Y	.	.	A
✓ 3. hcov 1039-1250	.	S	.	S	.	R	D	E	L	.	V	R	.	.	D	.	.	D	I	S	T	
✓ 4. sars 315	K	K	W	K	.	P	Q	V	.	.	.	T	S	I	K	W	A	D	Y	L	S	.	V	.	L	A	.	G
✓ 5. HCoV-229E	.	E	M	P	.	E	E	L	N	.	.	.	I	.	K	.	L	D	N	.	V	M	L	.	I	.	.
✓ 6. FCoV	T	P	.	K	T	T	N	L	N	.	K	I	I	.	K	.	Q	D	I	N	A	C	C	Y	.	.	A

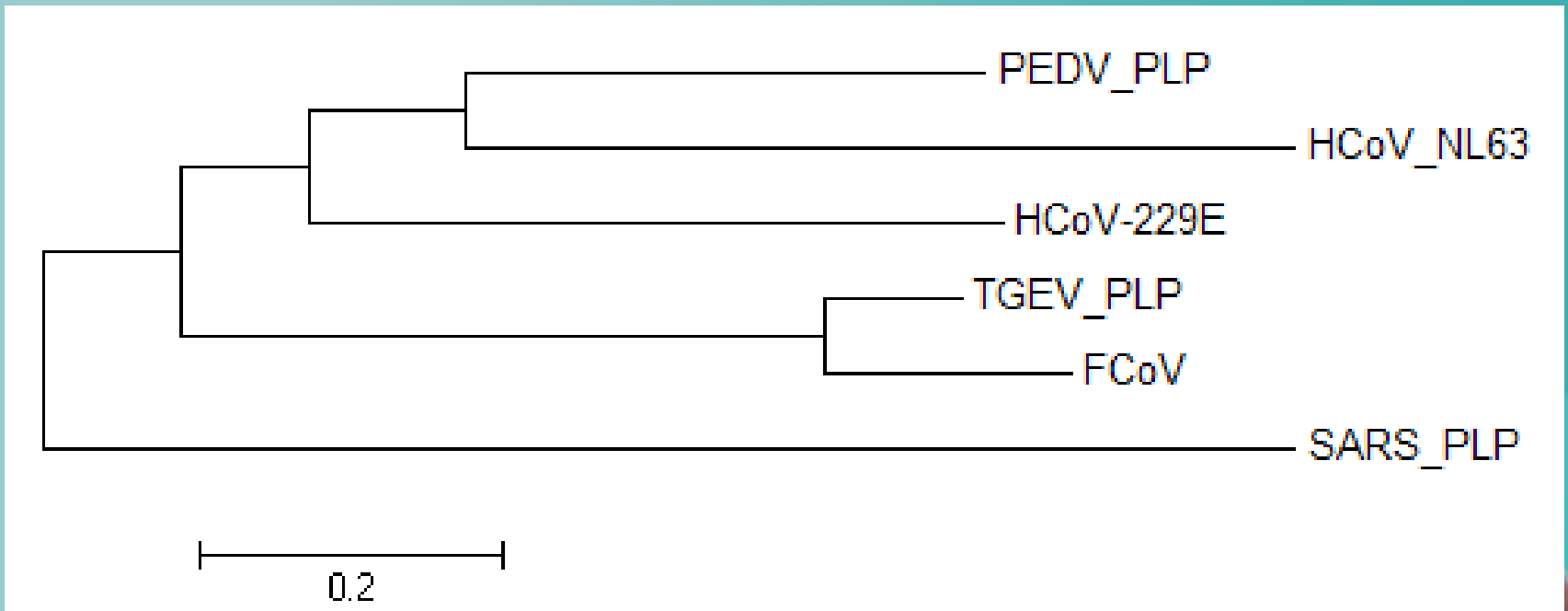
	A	F	I	G	.	K	D	S	G	H	Y	.	V	T	N	F	Y	D	A	A	M	A	I	D	G	Y	G	R	H	Q	I	K	.	
.
V	Y	T	.	T	T	Q	N	M	V	D	D	I	E	H	G	Y	C	V	.	.	M	.	I	K	P	L	.	K	
V	Y	L	.	V	.	G	Q	.	L	.	S	F	N	K	F	.	V	F	D	.	.	.		
E	Y	T	.	N	Y	Q	C	T	H	I	T	A	K	E	T	L	Y	R	.	.	.	A	H	L	T	K	M	S	E	
I	.	R	.	A	V	S	C	Q	.	I	.	S	Q	N	L	C	V	.	.	F	.	V	N	K	.	Q	P		
.	

H273

D287

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 Å)

Sequence Identity [%]: 31.22

Evalue: 0

Quality information:

QMEAN Z-Score: -3.358

Quaternary structure information:

Template (3mp2): MONOMER

Model built: SINGLE CHAIN

Ligand information:

Ligands in the template: ZN: 1.

Ligands in the model: ZN: 1



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	FDFVSYGGLKVL RQ SHNNCWV ST LV QL QLLGIVDDPAME LF SAG RV GPM	121
	 : .. :..... :.....:	
tgev	5	F KT TNLNGKIIL KQ GDNNCW IN ACC YQ L Q AFDFFN EA WE KF KKGD VM DF	54
pedv	122	VR KCY ES Q KAILG SL GDVSAC LE SLTKDLHTL KIT CSVVC GC GTGERI YE	171
		.. :..... :.. :..	
tgev	55	V N LCYA AT TLAR GH SGDA EY LL EL MLND YS TAKIVLAA KC GC GE KEIV LE	104
pedv	172	G CA FR MT PT LE PF PY GACA Q CA Q VL MHT FK SIV GT GIF CRD IT AL SL DS-	220
		... : : : : ::	
tgev	105	RA V FK L T PL KE SF NYG VC GDC MQ V NT CR FL S VE GS GV F VH D--IL SK Q TP	152
pedv	221	---LV VK PL CA AA FI G-- KD SG HY V TN F Y DA AM AID GY GR HQ IK	259
		: ... :.. ..: ...:.....::	
tgev	153	E AM F VV K PV M H AV Y T G TT Q NG H Y M V DD IE H GY C V D G M G I K PL K	195

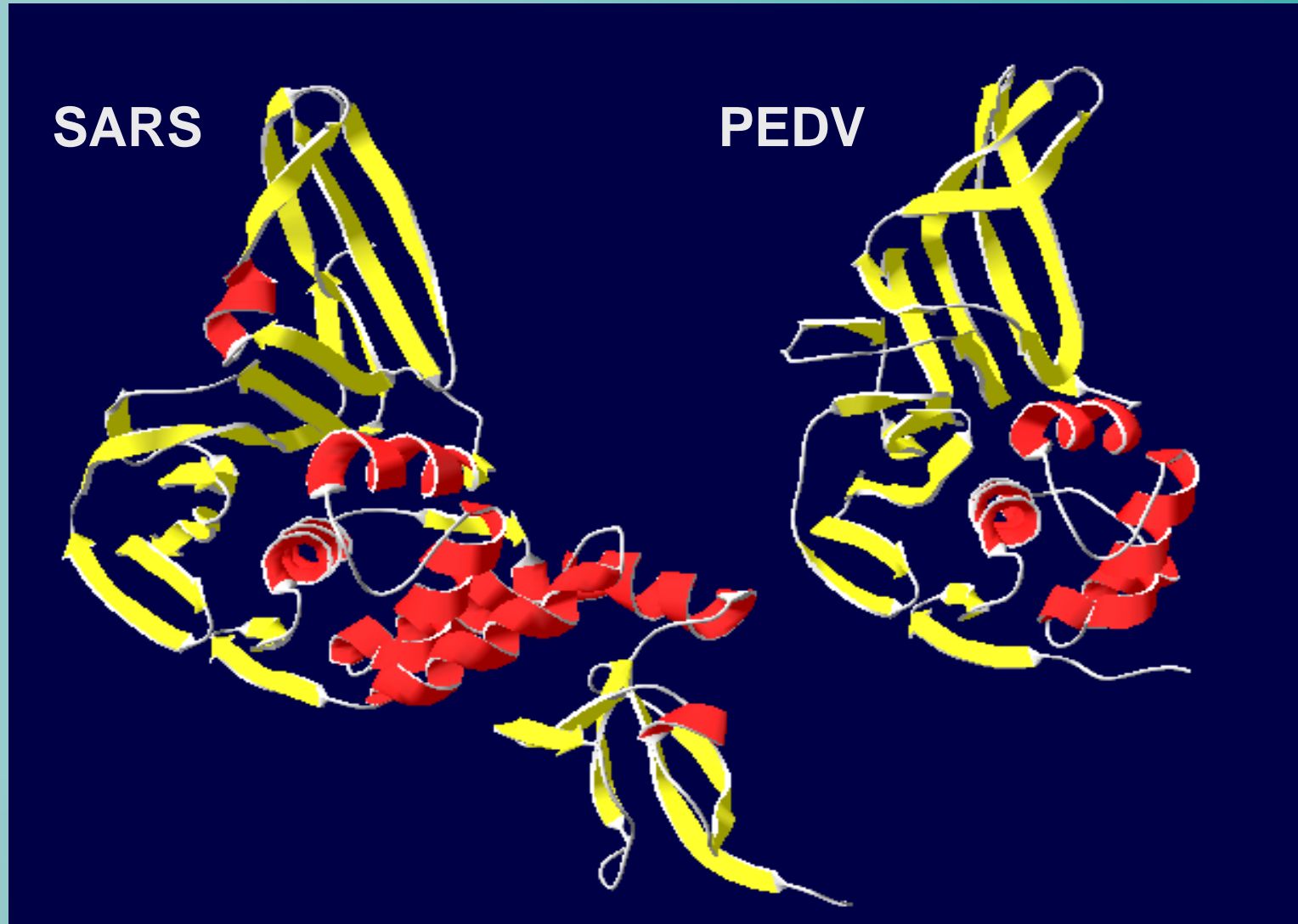
三维结构分析

SARS-plpro、PEDV-Plpro

Magic Fit 均方根误差 5.32埃



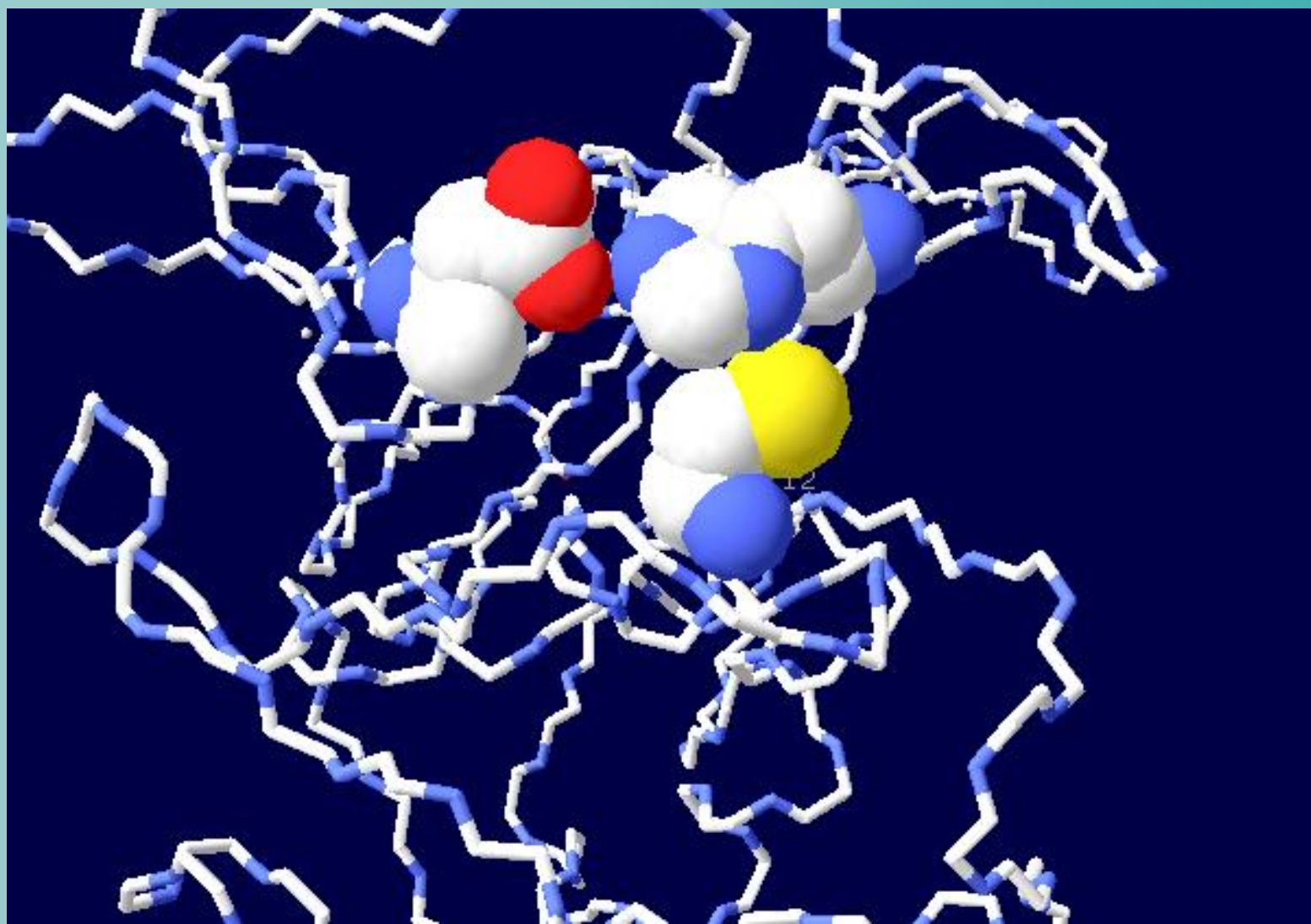
三维结构分析

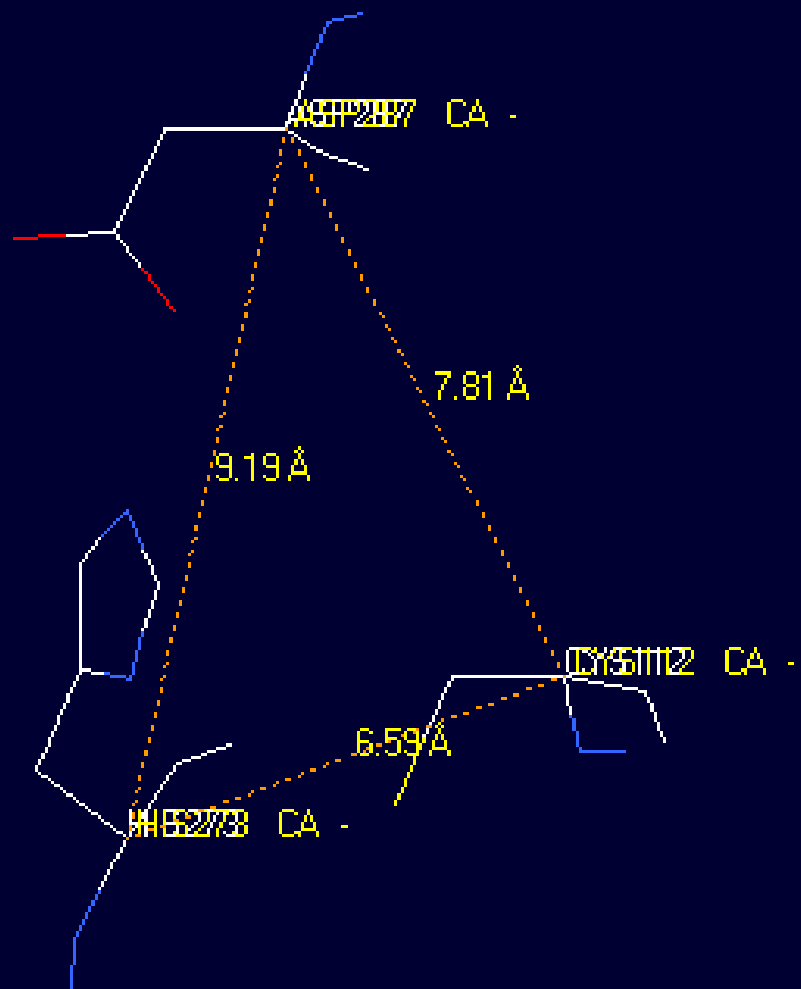


三维结构分析

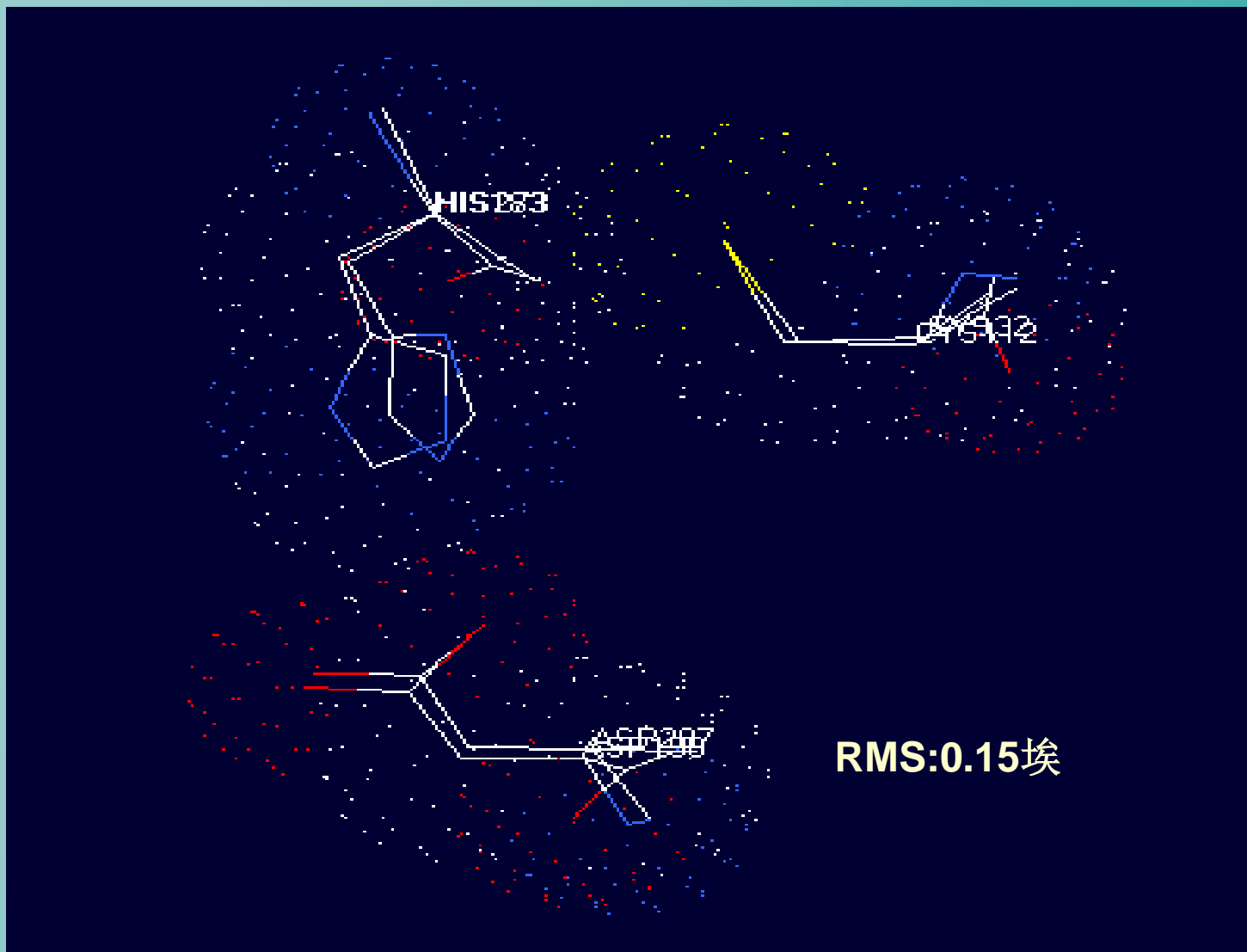
- 特异性位点FIT
- SARS:C112, H273, D287
- PEDV: C34, H183, D196







三维结构分析



结论

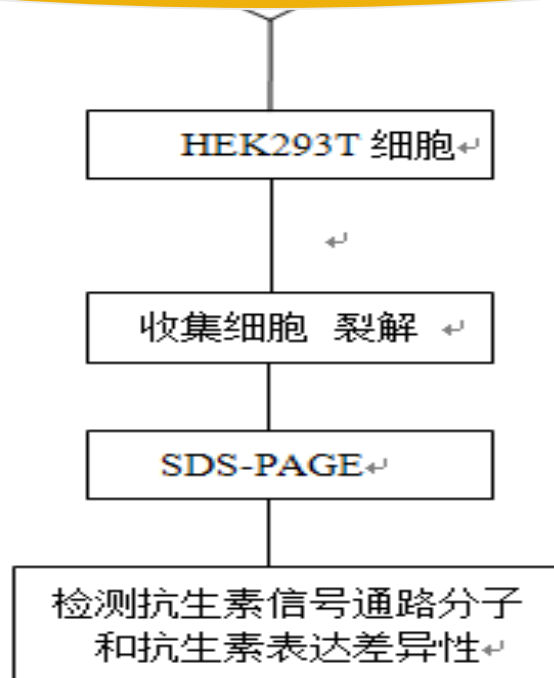
- SARS-plpro和PEDV-plpro在结构上很相似，而且从酶的角度来讲，他们具有相同的活性位点，那么，我们就有理由认为，他们具有相似的活性，即：

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



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

- 1 Justyna A. Wojdyla, et al. Papain-Like Protease 1 from Transmissible Gastroenteritis Virus:Crystal Structure and Enzymatic Activity toward Viral and Cellular Substrates. *J. Virol.* **67**:6056–6063
- 2 Zhongbin Chen,et al.Proteolytic Processing and Deubiquitinating Activity of Papain-Like Proteases of Human Coronavirus NL63. *JOURNAL OF VIROLOGY*, June 2007, p. 6007–6018
- 3 孙莉等, NL63 冠状病毒木瓜样蛋白酶去泛素化酶活性和对宿主抗病毒天然免疫反应调节作用, *生物化学与生物物理进展*, 2010, 37(8): 871~880
- 4 Sun L, Xing Y, Chen X, Zheng Y, Yang Y, et al. (2012) Coronavirus Papain-like Proteases Negatively Regulate Antiviral Innate Immune Response through Disruption of STING-Mediated Signaling. *PLoS ONE* 7(2): e30802.
- 5 **Kiira Ratia et al.Severe acute respiratory syndrome coronavirus papain-like protease: Structure of a viral deubiquitinating enzyme.** *PNAS*
- 6 张百灵等。猪流行性腹泻病毒(PEDV) 与抗病毒天然免疫, *中国生物化学与分子生物学报*, 2011 年6 月27(6) :516 ~ 523



Thanks for your attention!

