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Research Article

Conformational Epitope Prediction of Envelope Protein for Novel Wuhan Pneumonia Coronavirus (2019-nCoV)

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Abstract

Previously, there were a number of Coronavirus Outbreaks that took place in 2003 and 2012 but were not as lethal as current 2019-nCoV. As the Envelope Protein Plays an Important role in forming the outer protective cover, So we took a closer look into the mutation that took place in 2019-nCoV that makes it different from SARS And MERS-CoV.

Keywords: *2019-nCoV; SARS-CoV; MERS-CoV; BLAST; CLUSTAL W; ELLIPRO*

Abbreviations

2019-nCoV: 2019 Novel Coronavirus; SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; MERS-CoV: Middle East Respiratory Syndrome Coronavirus; BLAST: Basic Local Alignment Search Tool

Introduction

In December 2019, suddenly there was an increase in the number of pneumonia cases in china [1,2]. On investigation by D.R Lee, who was a doctor in a hospital of Wuhan, Hubei province of china found that the disease was caused by previously existing virus with a modified and mutated version called as 2019 novel coronavirus (2019-nCoV) [3,4].

Corona viruses are a large group of RNA Viruses in the nidovirales order and family coronaviridae and have one of the largest RNA Genome. This virus has +ve single stranded RNA. Corona prefix came for the Latin origin and means "crown like appearance". Club like or the Crown like appearance is caused due to spike protein (S) present in the lipid bilayer protruding outwards [5]. There are four classification of corona viruses:

1. Alpha coronaviruses
2. Beta coronaviruses
3. Gamma coronaviruses
4. Delta coronaviruses.

Alpha and beta coronaviruses are the coronaviruses that infect humans and get transmitted from bats and live animals.

Early cases of coronaviruses occurred in 2002 - 2003 and was called severe acute respiratory syndrome (SARS-CoV) and was in southern china, Guangdong province of china. SARS coronaviruses were transmitted from bats to civets and then to humans, with a mortality rate of 9.6%. SARS-CoV has an incubation period of 8 to 14 days [6].

Another outbreak was in 2012 which occurred in Saudi Arabia which was called Middle East respiratory syndrome (MERS-CoV). It was transmitted from camels to humans and has an incubation period of 3 days [7].

Another outbreak took place in 2019, Wuhan, Hubei Province of china, and was identified as novel beta corona virus (2019-nCoV) which originated from live and Dead Sea animals market of Wuhan city [8,9].

Corona viruses has 4 structural proteins:

1. Spike protein(S).
2. Envelope protein (E).
3. Membrane protein (M)
4. Nucleocapsid protein (N).

Spike protein(S) is a trimeric protein which is a receptor binding protein and helps in fusion of the envelope of virus to the host cell. This spike protein is responsible for the crown like appearance like a king's crown. Envelope protein (E) is a pentameric protein that functions as an ion channel protein and allows flow of ions from host to virus and vice-versa. Membrane glycoprotein (M) is the protein that forms a lipid bilayer membrane for the corona virus. Nucleocapsid protein (N) is the RNA binding protein and helps in synthesis of more number of RNA and in the process of translation [10,11].

Immune system

A substance that is recognized by the immune system as foreign and invokes or introduces an immune response is called as antigen [12]. Antigens are usually proteins that are too large to bind as a whole to any receptor, so only specific site on the antigen bind with a specific antibody. These segments are called epitopes. Likewise, it is only paratope of the antibody that comes in contact with the epitope.

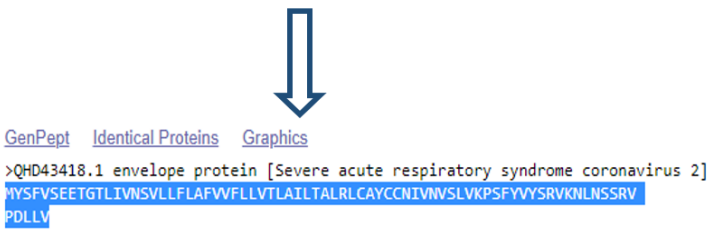
A linear or a sequential epitope is an epitope that is recognized by antibodies by its linear or straight sequence of amino acids. These have primary structure present in them. In contrast, most antibodies recognize a conformational epitope that has a specific three-dimensional shape as its protein structure.

Conformational epitope

A conformational epitope consists of amino acid residues that are discontinuous and not continuous which will be recognized by the antibody and which are present in an antigen that come in direct contact with a receptor of the immune system and binds to an antibody. These generally exist as tertiary or quaternary structure. There were some studies that led to the conclusion that envelope protein (E) of Coronavirus serves as an Epitope [13].

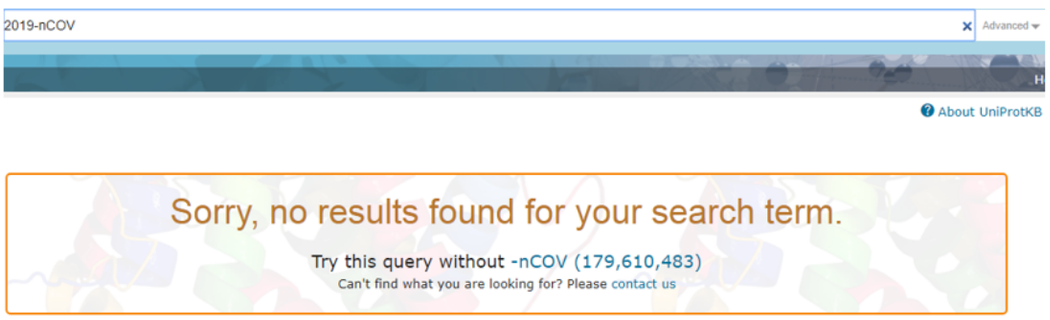
Materials and Methods

a. Initially 2019-nCoV corona virus was thoroughly studied and it's properties of the epitope of the envelope protein was studied.

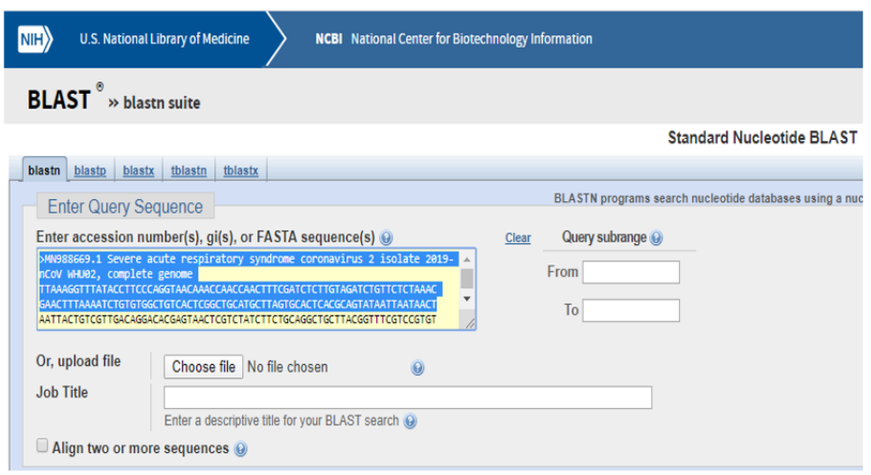


b. Fasta sequence of the envelope (E) protein [Wuhan Seafood Market Pneumonia Virus] was studied and extracted.

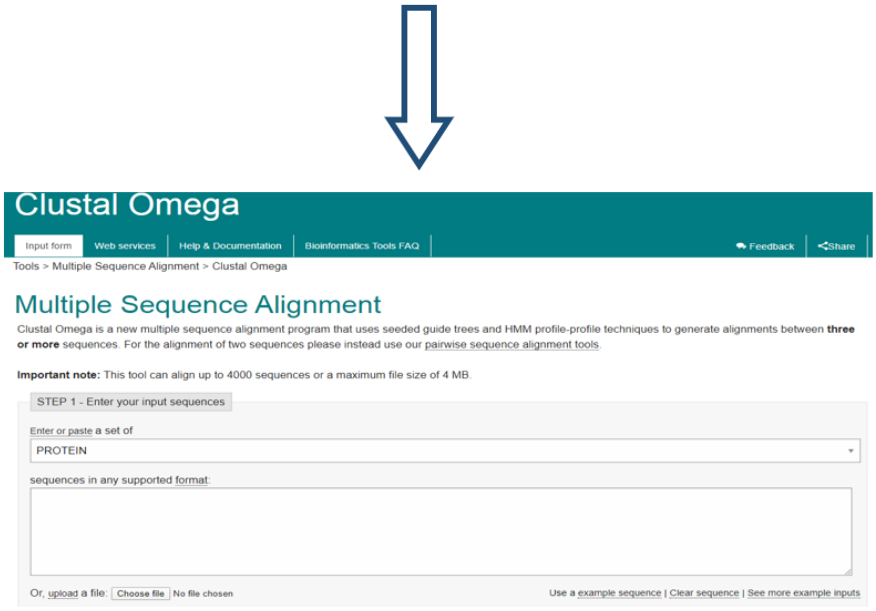
c. Structure of the above protein was searched in Protein Data Bank.



d. Peptide Sequence was searched in Uniprot Yielded no results.

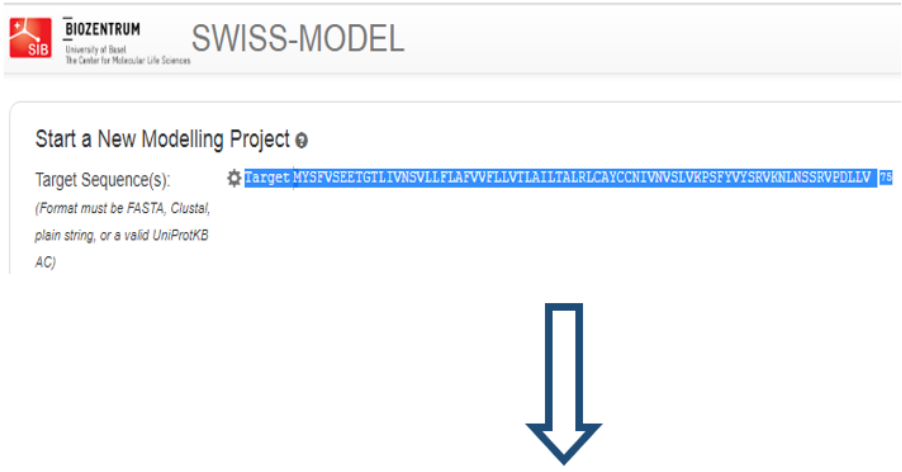


e. Blast was done for the 2019-nCoV.

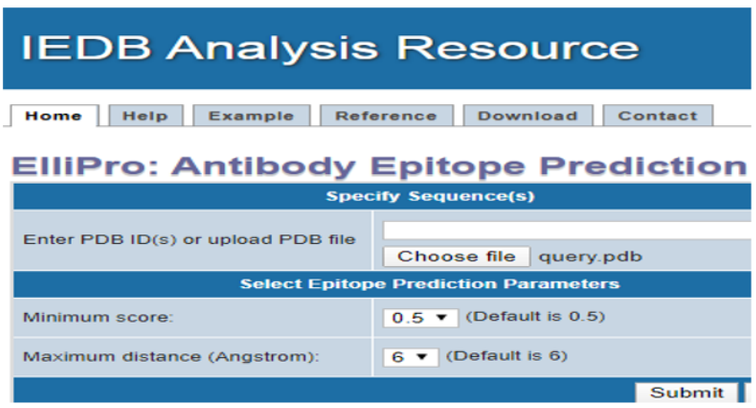


f. Multiple sequence alignment of the protein with existing SARS corona virus and Bat Coronavirus was done for identifying the degree of similarity.

g. 3d model of the envelope protein model was built using swiss model server.



h. Model generated through Swiss Model was uploaded in Ellipro.



i. Epitope Sequences for the Envelope protein were determined through Ellipro.

j. Epitope sequence was searched in Scanprosite which yielded no hits.

k. Amino acids for the epitope were analyzed by Discovery Studio.

l. Secondary structure was analyzed through PROTEIN MODEL CHECK Database that yielded composition of helix and turns.

Results and Discussion

Figure 1: *Figure showing no yet available structures for the Accession number QHD43418 which is the envelope protein of 2019-nCoV Wuhan pneumonia SARS corona virus.*

```
CLUSTAL O(1.2.4) multiple sequence alignment


QHD43418.1      MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS    60
ACZ72287.1      MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPTVYVYS    60
ABD75324.1      MYSFVSEETGTLIVNSVLLFFAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPTVYVYS    60
                **********************:**********************************:.****

QHD43418.1      RVKNLNSSR-VPDLLV        75
ACZ72287.1      RVKNLNSSEGVPDLLV        76
ABD75324.1      RVKNLNSSEGVPDLLV        76
                ********. ******
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Figure 2: *Figure showing the multiple sequence alignment of envelope protein of Wuhan pneumonia corona virus (2019-nCoV), existing SARS corona virus and bat SARS corona virus.*

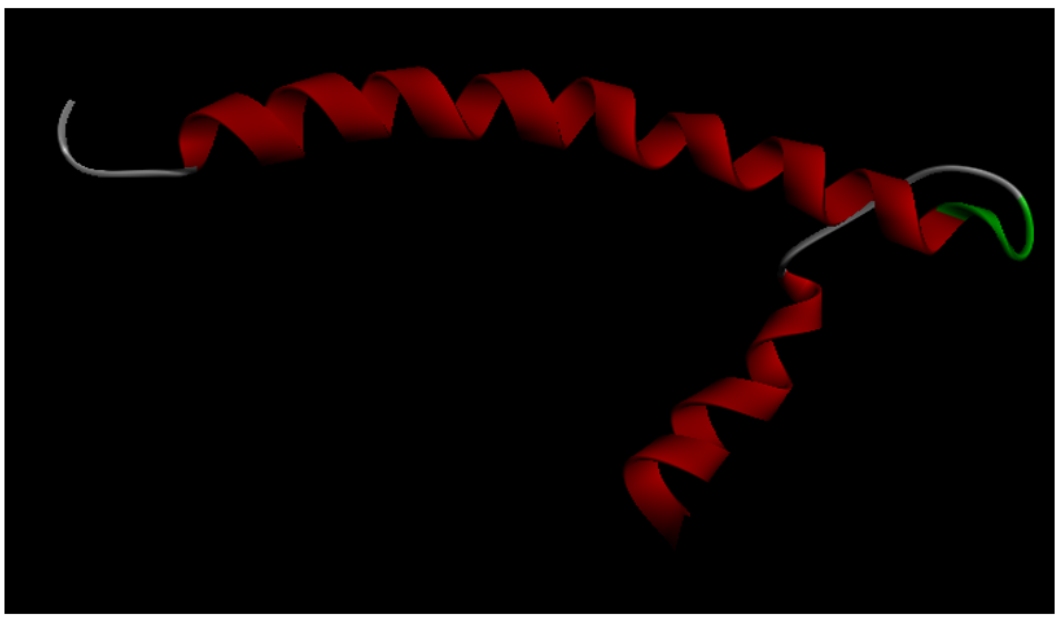

Figure 3: *Figure showing the 3d structure of envelope protein generated using Swiss model database by choosing appropriate templates which was viewed in Discovery Studio.*

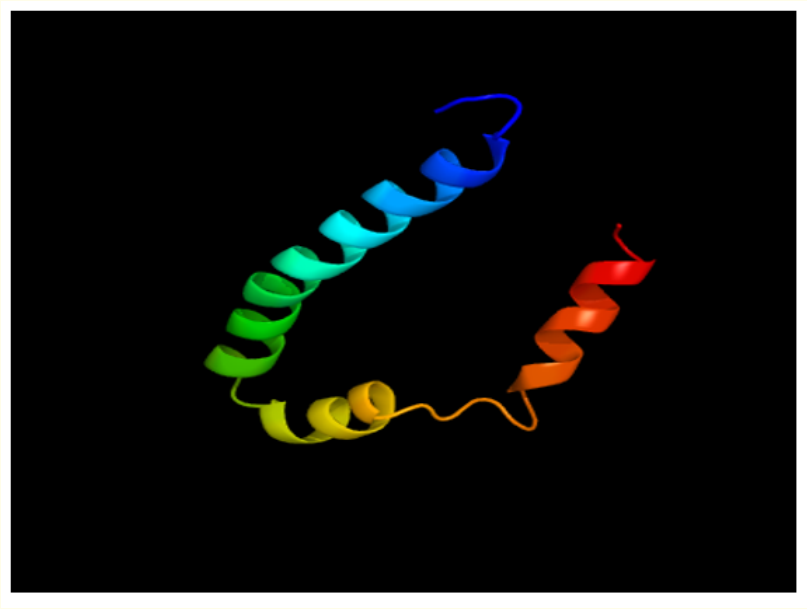

Figure 4: *Figure showing the predicted secondary structure of the envelope protein using phyre2. In a colour picture, the residues that are part of a helix are shown in blue, strand residues in red. Preferred regions for helical residues are drawn in blue, for strand residues in red, and for all other residues in green.*

```
Secondary structure assignment
                      10         20         30         40         50         60
                       |          |          |          |          |          |
    1 -    60 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
(   1)-(   60)   TT     TTHHHHHHHHHHHHTHHHHT TTHHHHHHHHHHHHTT      TTTTT
                      70
                       |
   61 -    75 RVKNLNSSRVPDLLV
(  61)-(   75)  HHHHTT
```

Figure 5: *Figure showing the quality of the model generated by swiss-model server, Only helix (H), overwound or 3/10-helix (3), strand (S), turn (T) and coil (blank) are shown. This data is generated by PROTEIN MODEL CHECK DATABASE.*

Predicted linear epitope(s)

No.	Chain	Start	End	Peptide	Number of residues	Score
1	_	23	35	FVVFLLVTLAILT	13	0.664
2	_	5	10	VSEETG	6	0.66
3	_	42	49	YCCNIVNV	8	0.628
4	_	58	73	VYSRVKNLNSSRVPDL	16	0.608

Table: *Showing the predicted peptide sequences of the epitope of 2019-nCoV.*

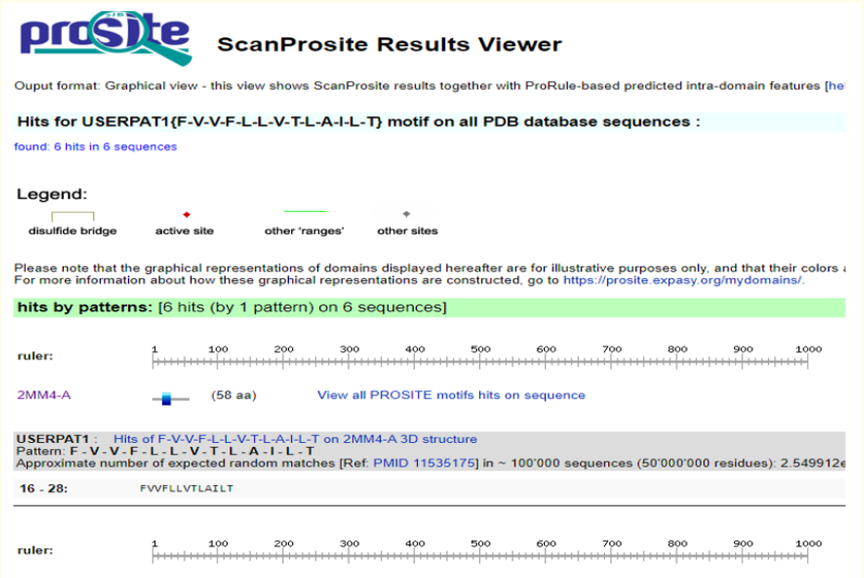

Figure 6: *Figure showing the prosite search result of epitope peptide sequence "FVVFLLVTLAILT".*

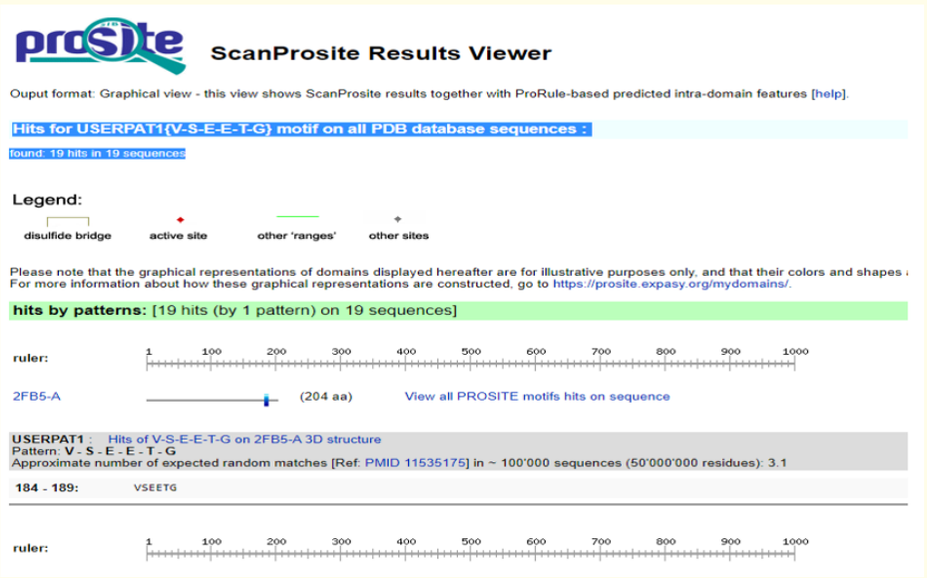

Figure 7: *Figure showing the prosite motif search result of epitope peptide sequence motif "VSEETG".*

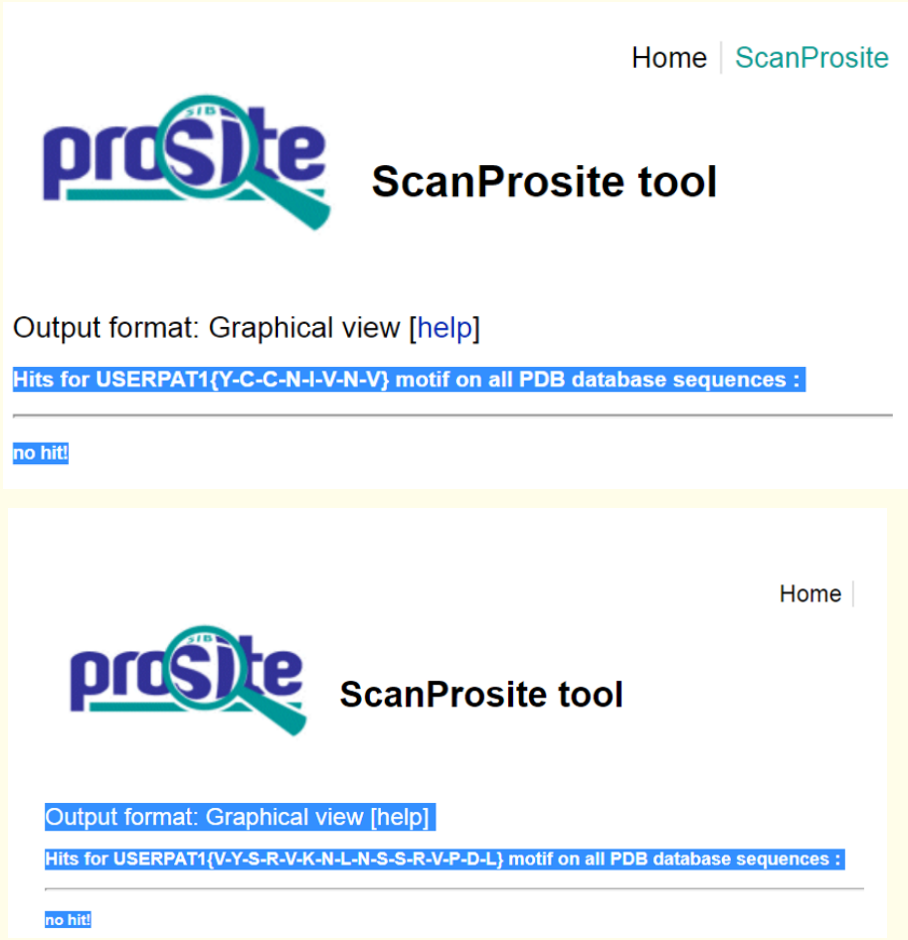

Figure 8: *Figure showing the prosite search result of epitope peptide sequence motif "YCCNIVNV" and "VYSRVKNLNSSRVPDL" which shows no hits indicating the presence of a unique sequence motif in the proteins compared in the database.*

Conclusion

Two unique conformational peptides were identified which were not there in the previous identified coronaviruses. The sequence is as follows:

1. YCCNIVNV
2. VYSRVKNLNSSRVPDL.

These two epitopes were predicted to be unique conformational epitope in Wuhan Pneumonia Coronavirus (2019-nCoV) also called SARS CoV-2.

Conflict of Interest

The authors have no conflict of interest.

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