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# Triggering type 1 diabetes post-covid: molecular mimicry?

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#### Short Report

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# Abstract Objective

To evaluate the possible similarity between the AA sequences of human insulin and human glutamic acid decarboxylase-65 (GAD65) with the SARS-CoV-2/COVID proteins to explain the possible trigger of DM1.

# Methods

AA sequences of human insulin, GAD65 and SARS-CoV-2 were obtained from the Protein Data Bank archive information database (RCSB PDB). NetMHCpan v4.1 was used for epitope prediction. Sequences were compared using BLAST for epitope comparison and Pairwise Structure Alignment to assess protein similarity. The AA sequences of human insulin (4F0N) and GAD65 (20KK) were compared with the sequences of the following SARS-CoV-2 proteins: SARS-Cov2 S protein at open state (7DDN), SARS-Cov2 S protein at close state (7DDD), SARS CoV-2 Spike protein (6ZB5), Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain (6M3M), Crystal structure of SARS-CoV-2 nucleocapsid protein C-terminal RNA binding domain (7DE1), Crystal structure of NSP1 from SARS-CoV-2 (7K3N), and SARS-CoV-2 S trimer (7DK3)).

# Results

The percent similarity between epitopes ranged from 45 to 60% (P 0.048) between both human insulin and SARS-CoV2 and for GAD 65 and SARS-CoV2, while the AA similarity of the evaluated samples ranged from 5.00–45.45% between human insulin and SARS-CoV2 and from 10.45–22.22% between GAD65 and SARS-CoV2.

# Conclusion

Immunoinformatics data suggest a potential pathogenic link between SARS-CoV-2/COVID and DM1. Thus, by molecular mimicry, we found that sequence similarity between epitopes and AA sequence between SARS-CoV-2 / COVID and human insulin and GAD65 could lead to the production of an immune cross-response to self-antigens, with self-tolerance breakdown, which could thus trigger DM1.

# Introduction

Coronavirus disease (COVID-19) and diabetes mellitus (DM) are two pandemics with high impact on public health worldwide.

Coronaviruses (CoV) are enveloped, positive single-stranded RNA viruses, widely dispersed in animals and humans worldwide, and responsible for causing respiratory infections in humans, notably severe

acute respiratory syndrome (SARS).<sup>1</sup> CoV is formed from the following structural proteins: spicule (S), membrane (M), nucleocapsid (N), and envelope (E). CoV after its entry into the host cell through the intrinsic action of the cell receptor and the viral envelope glycoprotein spicule (S), replicates in the cytoplasm in the same way as other positive single-stranded RNA viruses.<sup>2</sup> Human CoV can lead to potential complications such as SARS-CoV-2, also called COVID-19.<sup>3</sup>

Type 1 diabetes (T1DM) occurs due to an imbalance between Th1 and Th2 immunity resulting in insulitis and destruction of pancreatic beta cells ( $\beta$ -cells) and consequent reduced or absent insulin production.<sup>4</sup> Several factors encompassing genetic factors, environmental factors, and multiple viral infections may contribute to the development of T1DM.

A recent observational cohort study of DM1 pathogenesis monitored clinical infections and pancreatic islet autoantibodies, assessing the association between respiratory infections with islet autoimmunity, and CoV was identified as one of the viruses present in the study.<sup>5</sup>

 $\beta$ -cells can be destroyed by viral antigens, and possibly SARS-CoV-2 can bind to angiotensin-converting enzyme 2 in the pancreas, particularly in COVID-19 with clinical complications, leading to the triggering of DM1 in susceptible individuals.<sup>6,7</sup> Thus, it is likely that an increase in the incidence of DM1 is triggered by COVID-19 through the production of antibodies against  $\beta$ -cells by cross-reaction with SARS-CoV-2.

Most patients with DM1 develop cellular immune responses to autoantigens to glutamic acid decarboxylase 65 (GAD<sub>65</sub>), proinsulin, insulin, and a to tyrosine phosphatase.<sup>8</sup>

Theoretically, molecular mimicry would present the possibility in which similarities between foreign peptide sequence and autopeptides trigger a cross-reaction activating T or B lymphocytes developing autoimmune diseases. Thus, the aim of the present study was to evaluate the possible similarity between the epitopes of human insulin and  $GAD_{65}$  with the epitopes of SARS-CoV-2 / COVID, in addition to evaluating the possible similarities of the amino acid (AA) sequences of human insulin and  $GAD_{65}$  with the AA sequences of SARS-CoV-2 / COVID proteins, to explain the possible triggering of DM1 through molecular mimicry.

# Methodology

In the present study, we evaluated the similarities between the epitopes of human insulin and GAD65 with the epitopes of SARS-CoV-2 / COVID, and the similarities of the amino acid (AA) sequences of human insulin and GAD65 with the AA sequences of SARS-CoV-2 / COVID proteins.

The biological structures of GAD<sub>65</sub>, human insulin and nucleotides of SARS-CoV-2 were obtained from the Protein Data Bank archive information database (RCSB PDB) (https://www.rcsb.org/).

We performed the immunoinformatic prediction of epitopes of insulin, GAD65 and SARS-CoV-2 using NetMHCpan v4.1 software (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1), based on

artificial neural networks, following the recommended methodology and filtering the high affinity epitopes based on an IC50  $\leq$  50 nM and percentage rank  $\leq$  0.20 for the respective alleles analyzed, and subsequently performed the comparison of the epitopes to verify the hypothesis of cross-recognition. The similarity analyses were complemented with Parwise an extension of the Protein Data Bank program.

NetMHCpan 4.1 is a server that uses artificial neural networks to filter high affinity epitopes.<sup>9</sup>

We selected the predicted epitopes for the following sequences available in the RCSB PDB: GAD<sub>65</sub> (20KK), human insulin (4F0N), and SARS-CoV-2 (SARS-Cov2 S protein at open state (7DDN), SARS-Cov2 S protein at close state (7DDD), SARS CoV-2 Spike protein (6ZB5), Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain (6M3M), Crystal structure of SARS-CoV-2 nucleocapsid protein C-terminal RNA binding domain (7DE1), Crystal structure of NSP1 from SARS-CoV-2 (7K3N), and SARS-CoV-2 S trimer (7dk3)). For epitope extraction through NetMHCpan 4.1 we used the FASTA format obtained from the National Center for Biotechnology Information (NCBI) protein database, selected 11mer peptides and the human HLA-A\*01:01 allele.

For comparison of epitope candidates we used those with the strongest binding, programmed in NetMHCpan 4.1 between 0.5 and 2.0. The similarity between the sequences of the epitopes of GAD<sub>65</sub>, human insulin and the epitopes of SARS-Cov2 was the evaluated with the BLAST tool from NCBI.

Pairwise structure alignment is an application to evaluate the similarity between two proteins in homology search,<sup>10</sup> was used as a complementary tool in the evaluation of the homology and similarity between the study proteins on the RCSB PDB website.

The following sequences available in the RCSB PDB were used to assess the similarity between the proteins: GAD<sub>65</sub> (20KK), human insulin (4F0N), and the following SARS-CoV-2: SARS-Cov2 S protein in open state (7DDN), SARS-Cov2 S protein in closed state (7DDD), SARS CoV-2 Spike protein (6ZB5), crystal structure of SARS-CoV-2 protein nucleocapsid N-terminal RNA binding domain (6M3M), Crystal structure of the C-terminal nucleocapsid protein of the SARS-CoV-2 RNA binding domain (7DE1), Crystal structure of the NSP1 of SARS-CoV-2 (7K3N), and SARS-CoV-2 S trimer (7dk3).

# Results

The prediction of epitopes for GAD65 (20KK) was found to be 11 candidate epitopes, with three of them having threshold for stronger binding peptides (WB: 0.693-1.302) (Table 1).

For epitope prediction for human insulin (4F0N), 20 candidate epitopes were found, with two epitopes with threshold for stronger binding peptides (WB: 1.153-1.892) (Table 2).

Prediction of epitopes for the SARS-Cov2 S protein at open state (7DDN), 28 candidate epitopes were found, with two of them (SB) having threshold for peptide binding with highly strong (SB: 0.116 and 0.142) (Table 3).

For the prediction of epitopes for the SARS-Cov2 S protein at close state (7DDD), 33 candidate epitopes were found, with four having threshold for high binding power peptides (SB: 0.044 - 0.178) (Table 4).

Prediction of epitopes for the SARS CoV-2 Spike protein (6ZB5), 32 candidate epitopes were found, with three having a threshold for highly strongly binding peptides (SB: 0.116 - 0.178) (Table 5).

No epitopes were found, within the binding pattern established in the software used, for the Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain (6M3M) and for the Crystal structure of SARS-CoV-2 nucleocapsid protein C-terminal RNA binding domain (7DE1).

For epitope prediction for the Crystal Structure of NSP1 from SARS-CoV-2 (7K3N), 29 candidate epitopes were found, with five having thresholds for highly strong binding peptides (SB: 0.015 and 0.096) (Table 6).

Prediction of epitopes for the SARS-CoV-2 S trimer (7dk3), 18 candidate epitopes were found, with three having thresholds for highly strongly binding peptides (SB: 0.044 and 0.142) (Table 7).

#### Similarity analyses between the GAD<sub>65</sub> epitope and the SARS-CoV-2 epitopes

The percent similarity between the strongest binding GAD<sub>65</sub> epitope and the SARS-Cov2 epitopes are presented in Table 8.

GAD <sub>65</sub> EPITOPE (20KK)	% SIMILARITY	E-VALUE
7DDN EPITOPE	45-60	0.048
<b>7DDD EPITOPE</b>	45-60	0.048
6ZB5 EPITOPE	45-60	0.048
6M3M EPITOPE	-	-
7DE1 EPITOPE	-	-
7K3N EPITOPE		NON-SIGNIFICANT
7dk3 EPITOPE	45-60	0.048

**Table 8**. Similarity between the  $GAD_{65}$  epitope and the SARS-Cov2 epitopes.

Similarity analyses between the human insulin epitope and the SARS-CoV-2 epitopes

The percent similarity between the strongest binding human insulin epitope and the SARS-Cov2 epitopes are presented in Table 9.

 Table 9. Similarity between the Insulin epitope and the SARS-Cov2 epitopes.

INSULIN EPITOPE (KF0N)	% SIMILARITY	E-VALUE
7DDN EPITOPE	45-60	0.048
7DDD EPITOPE	45-60	0.048
6ZB5 EPITOPE		NON-SIGNIFICANT
6M3M EPITOPE	-	-
7DE1 EPITOPE	-	-
7K3N EPITOPE		NON-SIGNIFICANT
7dk3 EPITOPE		NON-SIGNIFICANT

Sequence identity percentages (SI%) and sequence similarity percentages (SS%) were found between 4F0N, 20KK and SARS-CoV-2.

#### Amino acid sequence similarity analyses between human insulin and SARS-CoV-2

The similarity between 4F0N and SARS-CoV-2 ranged from 18.75% to 21.74%, distributed thus:

- 4F0N and 7DDN = SI% 4.76 and SS% 28.57 (Figure 1)
- 4F0N and 7DDD = SI% 14.39 and SS% 23.81 (Figure 2).
- 4F0N and 6ZB5 = SI% 4.76 and SS% 28.57 (Figure 3).
- 4F0N and 6M3M = SI% 5.00 and SS% 5.00 (Figure 4).
- 4F0N and 7DE1 = SI% 4.76 and SS% 9.21 (Figure 5).
- 4F0N and 7K3N = SI% 9.09 and SS% 45.45 (Figure 6).
- 4F0N and 7DK3 = SI% 4.76 and SS% 28.57 (Figure 7).

#### Amino acid sequence similarity analysis between GAD<sub>65</sub> and SARS-CoV-2

The similarity between 20KK and SARS-CoV-2 ranged from 10.45% to 22.22%, distributed thus:

- 20KK and 7DDN = SI% 6.70 and SS% 15.64 (Figure 8).
- 20KK and 7DDD = SI% 7.53 and SS% 18.84 (Figure 9).
- 20KK and 6ZB5 = SI% 6.68 and SS% 17.38 (Figure 10).

- 20KK and 6M3M = SI% 4.48 and SS% 10.45 (Figure 11).
- 20KK and 7DE1 = SI% 6.67 and SS% 22.22 (Figure 12).
- 20KK and 7K3N = SI% 3.19 and SS% 15.97 (Figure 13).
- 20KK and 7DK3 = SI% 3.95 and 17.98 (Figure 14).

# Discussion

Although SARS-CoV-2 is not yet listed among the viruses involved in the etiology of DM1, our study proposed to evaluate the triggering of DM1 by molecular mimicry through protein similarity. Our results, using immuno-informatics tools, in assessing the similarity between the epitopes and the AA sequence of GAD<sub>65</sub>, human insulin and 7 select proteins of SARS CoV-2, can substantiate that DM1 induction can be triggered by the 7DDN, 7DDD and 7dk3 proteins of SARS-CoV-2.

The basis of the pathogenesis of DM1 is founded on two pillars: genetic predisposition and the existence of anti- $\beta$ -cell, anti-GAD<sub>65</sub>, anti-insulin, and anti-tyrosine phosphatase autoantibodies.<sup>11</sup>

It is possible that SARS-CoV-2 has the ability to lead to pleiotropic modifications in glucose metabolism, complicating existing diabetes or triggering it.<sup>12</sup> There are several reports of a viral etiology of DM1, including coronaviruses that relate to angiotensin-converting enzyme 2 (ACE2) receptors, and it has been observed that a higher incidence of hyperglycemia and DM1 have been reported among patients with SARS-CoV pneumonia than among those with pneumonia of another etiology.<sup>13</sup> Our analyses may support the hypothesis of molecular mimicry as a potential diabetogenic effect of SARS-CoV-2 for triggering DM1.

The term molecular mimicry was first used in 1964 by Damian to describe the expression of similarity between antigens expressed by microorganisms and their human hosts and the immune response.<sup>14</sup> Molecular mimicry is an evolving concept, involving not only similarity between the molecular structure of the microorganism's protein and the human proteome, as well as environmental and genetic factors, and mechanisms concomitant to positive / negative selection of T cells.<sup>15</sup>

The following criteria may be related to molecular mimicry: "similarity between a host epitope and an epitope of a microorganism or environmental agent," "epidemiological link between exposure to the environmental agent or microorganism and the development of autoimmune disease," "reproducibility of autoimmunity in an animal model after sensitization with the appropriate epitopes after infection with the microorganism or exposure to the environmental agent," and "detection of antibodies or T cells that cross-react with both epitopes in patients with autoimmune disease."<sup>16</sup> In our study, we attributed the triggering of SARS-CoV-2-related DM1 to the similarity between the epitopes and the molecular structure of human insulin and GAD65 with the molecular structure of the SARS-CoV-2 protein.

Studies have suggested that molecular mimicry plays an important role in generating autoimmunity in patients with COVID-19.<sup>17,18</sup> To trigger autoimmunity secondary to infection, only 5 to 6 identical AA

between a viral protein and a host protein are required to induce an immune response.<sup>19</sup> In this work, we analyzed the similarity between the epitopes of 7 SARS-CoV-2 polyprotein sequences and 2 specific human beta cell protein antigens, and observed that SARS-CoV-2, human insulin, and GAD65 share AA sequence similarity, and that some regions contain epitopes of SARS-CoV-2 and $\beta$ -cell autoantigens with high similarities. We did not find any study in the literature that evaluated the similarity we propose in our study.

 $GAD_{65}$  is a neuroendocrine enzyme and is a key autoantigen for triggering DM1, and anti-GAD<sub>65</sub> autoantibodies are positive in the serum of 70–80% of DM1 patients, and is therefore an important marker in the prediction and diagnosis of DM1.<sup>20</sup> The high-resolution crystal structure of  $GAD_{65}$  was created in 2007, thus making available various insights into the molecular determinants of antigenicity, as well as an atomic positioning of epitope mapping data.<sup>21</sup> GAD65 was employed as a function of the various information on molecular determinants of antigenicity triggering DM1. In our study,  $GAD_{65}$  showed a higher similarity to the SARS-CoV-2 proteins 7DDN, 7DDD and 7dk3, while the similarity to the other proteins evaluated was not significant, thus supporting the hypothesis that a molecular mimicry could occur between  $GAD_{65}$  and SARS-CoV-2.

Insulin is secreted by  $\beta$ -cells, which are indispensable for the body's glycemic homeostasis. In the individual with DM1, an autoimmune insulinitis occurs resulting in the destruction of the  $\beta$ -cells and consequent lack of insulin secretion. Proposed pathogenic mechanisms for DM1 include molecular mimicry, non-autoimmune $\beta$ -cell infection, alterations in thymic T cells, and immune activation.<sup>22</sup> Thus, there is evidence that viral infection in genetically predisposed individuals can activate the immune system through molecular mimicry, triggering DM1. In our study, human insulin showed high similarity to SARS-CoV-2 proteins 7DDN and 7DDD, coinciding with GAD<sub>65</sub> already described above and also supporting the possible molecular mimicry between human insulin and SARS-CoV-2.

# Conclusion

Immunoinformatics data suggest a potential pathogenic link between SARS-CoV-2/COVID and DM1. Thus, by molecular mimicry, we found that sequence similarity between the epitopes and the AA sequence between SARS-CoV-2 / COVID and human insulin and GAD<sub>65</sub> could lead to the production of an immune cross-response to self-antigens, with self-tolerance breakdown, which could thus trigger DM1.

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### Declarations

Competing interests: The authors declare no competing interests.

# Tables

Tables 1-7 are available in the supplementary files section.

# Figures



### Source: Research result.

#### Figure 1

Similarity between 4F0N and 7DDN.

_									
Struct	ture ID		Descrip	noite		Sequence Leng	gth Modeled	Residues	Alignment Coverage
4	FON.A		Insulin	A chain		21	21		100%
<b>7</b>	A.DOD		Spike g	lycoprot	ein	1261	1088		2%
ALIGNM	IENT								
RMSD	TM-score	Score	SI%	\$\$%	Length				
2.98	0.02	33.34	14.29	23.81	21				
1:A	GIVEQCCT	SIC	SLYQU	ENYCN	21:A				
835:A	KQYGDCLG	DIAAR	DLICA	QKFNG	857:A			•	
								X	-
								S.	

### Figure 2

Similarity between 4F0N and 7DDD.

Structure ID	Description	Sequence Length	Modeled Residues	Alignment Coverage
4F0N.A	Insulin A chain	21	21	100%
6Z85.A	Spike glycoprotein	1259	1032	2%
ALIGNMENT RMSD TM-score Sco 2.23 0.02 343 1:A GIVEQCCTSI - 976:A VLNDILSRLDKY	re SI% SS% Length 35 4.76 28.57 21 •CSLYQEENYCN 21:A VEAEVQIDRLIT 998A		Š	

Source: Research result.

### Figure 3

Similarity between 4F0N and 6ZB5.

Struct	ure ID	Desc	ription		Sequence Le	ngth	Modeled Residue	5	Alignment Coverage	
4	ON.A	Insuli	n A cha	in	21		21		86%	
<b>6</b>	/3M.A	Nuck	oprotei	in	136		121		15%	
ALIGNM	ENT									
RMSD	TM-score	Score	\$1%	\$\$%	Length					
3.69	0.09	22.91	5	5	18					
2A	IVE QC	CTS	ICSLY	QLENY	CN 21:A					
50:A	RRA <mark>trrirmko</mark>	I SP <mark>RWY</mark>	FYYLG	TGPEA	GL 83:A			•		
										k v

Source: Research result.

### Figure 4

Similarity between 4F0N and 6M3M.

Struc	ture ID	Description		Sequence Length	Modeled Residues	Alignment Coverage
<b>=</b> 4	FONA	Insulin A chain		21	21	100%
<b>7</b>	DELA	Oxysterol-binding pr	otein-related protein 3	388	376	6%
RMSD	MENT	Score SI% SS%	Length			
2.32	0.05	27.89 4.76 9.52	21			
12A 314:A	PDORFLEEC	SICS - EVQLENVC	210A 336A			

### Figure 5

Similarity between 4F0N and 7DE1.

Structure ID	Description	Sequence Length	Modeled Residues	Alignment Coverage
4F0N.A	Insulin A chain	21	21	52%
7K3N.A	Host translation inhibitor nsp1	180	106	10%
IGNMENT	Score SI% SS% Length			
1,49 0.09	23.28 9.09 45.45 11			
ACSLYQLE	NYCN 21:A			
DSVEEVI	SEAR 43A			
				4

Source: Research result.

### Figure 6

#### Similarity between4F0N e o 7K3N.

Structure ID	Description	Sequence Length	Modeled Residues	Alignment Coverage
4F0NA	Insulin A chain	21	21	100%
70K3.A	Spike glycoprotein	1261	1062	2%
ALIGNMENT RMSD TM-score S 2.26 0.02 3 1:A GIVEQCCTSI 976:A VENDILSRED	core SI% SS% Length 12.52 4.76 28.57 21 CSLYQLENYCH 21:A PPEAEVQIDRLIT 998:A			

Source: Research result.

Figure 7

Similarity between 4F0N and 7DK3.

		~					
Structure ID	Description	•		Seque	nce Length	Modeled Residues	Alignment Coverage
20KKA	Glutamate	decarboxylase 2		497		483	37%
7DDN.A	Spike glyco	protein		1261		1066	17%
LIGNMENT							
RMSD	TM-score	Score	S1%	\$\$%	Length		
10.97	0.1	187.01	6.7	15.64	178		5
4:A COGER	PTLAFLQDVMNI				30:A		550
13:A AIPTN	FTISVTTEILPVSM	TKTSVDCTM	ICGDSTEC	SNLLLQYGS	SFCTQ 762A		
1:A	LLQYV	VKSFDRSTKV	IDFHYPNE	LLQEYNHE	ADQP 66:A		
63:A LNRAL	TGIAVEQDENTQEN	FAQVKQIYKT	PP	<b>IK</b>	795:A		<b>S</b>
7:A QNLEE	ILMHCQTTLKYAIK	TGH		PRI	FINQL 95:A		<b>3</b>
96:A DFGGF	NFSQILPDPSKPSK	RSFIEDLLFA	KVTICAQ	FNGLTVLP	PLLTD 867:A		
6:A STGLO	HVGLAAD				107:A		a = (k + 1)
68:A EMIAC	YTSALLAGTITSGN	TEGAGAALQI	PFANQHAT	RENGIGVE	NVLY 917:A		
08:A	WLTS	TANTNHETYE	IAPVFVLU	LEYVTLKKM	REIIG 142:A		
18:A ENQKL	IANQFNSAIG	SLSSTAS	ALGKLQ-		949:A		
43:A WPGGS	GDGIFSPGGAISNM	YAMMIAREKE	FPEVKEK	MAALPRLI	AFTSE 192:A		
50:A	DVVNQNAQALN	TLVKQLSSNP	G	AISSV	976:A	_	
93:A HSHES	LKKGAAALGIGTDS	VILIKCDERG	KHIPSOL	RRILEAKO	GEVP 242A		
77:A				NDILSRLDP	PPEAE 990:A		
43:A FLVSA	TAGTTVYGAFDPLL	AVADICKKY	INMHVDAA	MGGGLLMSP	ККНКЫ 292 <del>.</del> А		
91:A VQ		IDRLIT	GRLQSL		1004:A		
93:A KLSGV	ERANSVTWNPHXMM	GVPLQC	VREEGLMO	NCNQMHDLS	5YDTG 353:A		
005:A	QTYVTQQL	IRAA			1016A		
	CGRHVDVFKLWLMM	RAKGTTGF			380:A		
And a state of the							

Figure 8

Similarity between 20KK e 7DDN.

Stre	ecture ID	Descriptio	<b></b>		Seq	sence Leng	petho	Modeled Residues	Alignm	ent Coverage
-	20KKA	Glutamate	e decarboxytase 2		497			483	49%	
	7000.A	Spike glys	oprotein		1261			1088	22%	
IGN	MENT									
							_			
	MSD	TM-score	Score	SING	\$\$\$6	Lengt	•			
1	0.85	0.12	204.48	7.53	18.84	235	12.1			
-	LAFLODV	MNTLLQVVVKS	FDRST	THEFT	PULPEND.	SVYFAC.	43:4		11000	-
					CPOLPT NO					
	TEKSNII	RGWIFGTTLDS	KTQSLLIVNNAT	INVVIEN	EFQFCND	FLGVY	144A			5 >
									364	5 5
5:A	YHKNNKS	RMESEFRVYSS	ANNETFEYVSQU	PFLMDLEG	SKQGNEKN	LREFVF	194:A			
<b>A</b> .			KVIDF HY	PNELLQEY	ELADQ	PONLEE	71;A			
S:A	KNIDGYF	KIYSKHTPINL	VRDL POGES	A1		PLVD	228 A			
÷.					TLM	REQTTL	A.08	5		Jan San
-	EVAT	THE OTLLALHE	PRVENOLSTCH	NW	L.C.		102.4	-		
6 A	TOAVOCA	LOPUSETKETL	KSETVEKGIYOT	ISNERVOR	TESTVEF	INTTNE	335 A			
								-		5
6:A	CPFGEVF	NATREASVYAN	NRKRISNOVADY	SVLYNSA	SESTERC	GVSPT	385:A			6
đ:A	KUNDLEF	TNVVADSEVIE	GDEVRQIAPGQ	TGKIADYN	WYKLPDDF	IGCVIA	435:A			
6.A.	WNSNNLD	SKVGGNVNVLV	RUFRKSNUKPFI	CROISTEI	VQAGSTPO	NGVEG	485 A			
	PACTEPL	621.01 Ghives	GTOPTRVVVLSI	LLHAPA	AT VCGPKK	- NLVK	222.0			
0:A	NKCVNEN	NGLIGTOVLI	ESNKKELPEOOL	GROIADI	TDAVEDR	TLEIL	585:A			
d:A	DITPOSE	GOVSVITEGTN	TSNQVAVLYQDY	VNCTEVNY	FOTRAGE	LIGACH	655:A			
6:A	VNNSYEC	DIPIGAGICAS	VQTSQSIIAVT	ISLGAENS	SVAYSNNS:	TATPTN	717;A			
3:A		GLA	ADWLTSTANTN	HETYS	TAPVEVL	LEVVIL	134 A			
A.S	FTISVTT	EILPVSHTKTS	VDCTMVICGDS	TECSNELL	QYGSFCT	LINRAL	767:A			
3.4	KKMREII	GWPGGS GOGIF	SPGGAISNAVAN	ANTARFER	<b>TPPEV</b> KEK		178:A			
		CHANNEL PROTAV	EQUENT	CARA CO	ROIV KTPI	IKOFG	204.4			
-	GENESOT	PDPSKPSKPS	FIEDLLENEWT	ADAG	OVERCIE	TAARD	848.4			
5:A		LGI	GTOSVILIKCO	REKMIPS	DLEBBIL	AKOKG	239.A			
9:A	LICAQUE	NGLTVLPPELT	DEMIAQVT		SALLAGT	TSGWT	887:A			
0:A	FUPFLY					SATA	249 A			
a:A	FGAGAAL	QIPFAMQMAYR	FNGIGVTQNVL	VENQKLIA	NOFNSAI	SKIQDS	937:A			
0:A	GTTVYGA	<b>FD</b> PLLAVADIC	KKYKIMMHV <b>DA</b>	ANGGGLLM	4SRKHKWK	SOVER	299:A			
a:A	LSSTASA	LG	KLC	204			951:A			
0.A.	ANSVIEN	PHAMMGVPLQC	SALLVREEGLMO	ONCNOMHE	LSYDTGD	CALQCG	360:A			
-	B M M D M F M	QNAQALNTLVK	CERTIFICATION CONTRACTOR	LNDILS	LOFFEAE	OIDEL.	410.4			
2.4	TTOBLOS	LOTYYTOOLIE	AATTOATANIA	TEREFOR	LCOSKBY	CONC.	1046.4			
	The second secon		and a second tax			1.				

### Figure 9

Similarity between 20KK and 7DDD.

0			~							
Strue	ture ID	Description			Sequ	ence Leng	pth	Modeled Residues	Alignmer	nt Coverage
	OKKA	Glutamate d	lecarboxylase 2		497			483	70%	
	285.A	Spike glycog	joike glycoprotein		1259	•		1032	33%	
ALIGNE	MENT									
RJ	ISD TN	A-score	Score	5176	\$\$%	Longt	•			
1	1.67	0.14	179.3	6.68	17.38	339				
1:4	NYAFEHATDE -	- LPACOGE	PT····	LODVINT	LLOYVYKS	DB	41.4		3	
271:A	QPRTFLLKYN	NGTITDAVE	DCALOPLSET	KCTLKSF	TVERGINQT	ISNERV	320:A		Charles and the second	
										• <
321:A	QPTESIVEFPN	ITNLCPFG	EVENATREAS	VYAWNEK	RISNEVADY	SVLYN	370:A			
371:A	SASFSTFRCVG	VSPTKLND	LCFTNVYADS	FVIRGDE	VRQIAPGQ	TGKIAD	420:A	د.		
421-4	VNVKLEDDETG	CUTAUNSNI	NIDSKYGGNY	NYLYPLE	IKSNI KPER	ROIST	470.4	4		
471:A	EIVQAGSTPCN	GVEGENCY	FPLQSYGFQP	TNGVGYQ	PYRVVVLSP	FELLHA	\$20:A			22
										2
521:A	PATVCGPKKST	NUVKNKCVI	NENENGLIGT	GVLTESN	KKFLPFQQF	FGRDIA	570:A	_		
42:A			STEVIDE	HYPNELL	QEYNH CLAC	QP	66:A		1 -	
\$71:A	DTTDAVRDPQT	LEILDITPO	CSPEGVEVIT	PGTNTSN	QVAVE YODY	VNCTTW	633:A			
62.4.4	ONLEE	TRACELIC	CQT TER	VAIRTGH	ICASYODS1		607.4			
90:A	RYEN		OLSTGL	DHYGLAA	DHLTSTAN	INMET -	119.4			
698:A	SEGAENSVAYS	NNSIAIPT	NTTI SVTTEI	LPVSHTK	TSVDCTMVI	ICGDST	747:A			
120:A		YEIAPVE	VELEVVTERE	MREIIGH	PGGSGOGIF	SPGGA	157:A			
748:A	ECSNLLLQYGS	CTQLNRA	LTGIAVEQDK	NTQEVEA	QVKQ1		788:A			
158:A	ISNHYAMMIAR	FKMFPEVK	EKGMAAL PRL	TAFTSEN	SHFSLKKGA	AAALGI	207:A			
789:A	YKT		PPI	KDFGGFN	FSQILPDPS	SKPSKR	815:A			
208.4	GTDSVILIKC	FERGEMIPS	DLERRILEAK	QKG		VPFLVS	240.4			
247:6	ATAGTTVYGAP	DPLLAVAD	ICKEVEINMH	VDAANGG	GLLMSRKH	WELS-	295 A			
873:A	YTSALLAGTIT	SCHTFGAG	AALQIPFAMO	BAYREN		GVTON	914A			
296 A		- GVERANS	VTWNPHXMMG	VPLQCSA	LVREEGLP	QNCNQ	333:A			
915:A	VLYENQKLIAN	OFNSATGK	IQDSLSSALG	KLQDVVN	<b>Q -</b>		954:A			
334:A	MHDLSVDTGDX	AL QCGRHV					363:A			
955:A	NAQA-	LNTLVK	QLSSNFGAIS	SVENDIE	SRLDKVEAD	LADIDA	995:A			
304 A	L TTCPLOSIO	VEREWENNI	RAKGTTGFEA	NVDECLE	LAEVLYNII	RNREG	405A			
404-6	VENVEDGKED	HI COLLERS	INVEFATIPE	SLOTLE	CALOQSER'S		430 A			
1044 A	GKGYHLMSEP	SAPHGVV	LHVTYVPAQE	KNETTAP	AICHDGKAN	FPREG	1093;A			
434:A	ERMSRESKVAP	VIKARMHE	GTTMVSYO	LGDKVNF			469:A			
1094:A	VEVSNGTHWEV		🕱	QRNEVEP			1112:A			

Source: Research result.

### Figure 10

Similarity between 20KK e 6ZB5.

Strue	cture ID	Description	n		Seque	nce Length	Modeled Residues	Alignment Coverage
•	ZOKKA	Glutamate	decarboxylase 2		497		483	6%
	(M3MA	Nucleoprot	ein		136		121	24%
ALIGN	MENT							
RJ	MSD	TM-score	Score	SI%	SS%	Length		
3	3.11	0.05	40.18	4.48	10.45	29		
280:A	<b>W</b> GGGLLM	SRKHKWKLSGVE	RANSVTWNP	HXMMG	VPLQCSALLV	REEGL 327:A		
25:A	DLKFPRG	QGVPINTNSSPO	DOQIGYYRRA	RRIRMK	DLSPRWYFYY	GTGP 79:A		<u> </u>
328:A	MQNCNQM	HDL			SYDTGDKAL	357:A		7
80:A	EAGLPYG	ANKDGIIWVATE	EGALNTPKOH	IGTRNPA	INAAIVLQL	123:A		
							<b>N</b>	
							1	<b>\</b>
								r s
								) ()
								~ ~
								<b>&gt;</b>

Figure 11

Similarity between 20KK e 6M3M.

Structure ID	Description			Sequ	ence Length	Modeled Residue	s Alignment Coverage
20KKA	Glutamate decar	boxylase 2		497		483	9%
7DE1.A	Nucleoprotein			115		108	39%
ALIGNMENT							
RMSD	TM-score	Score	SI%	\$\$%	Length		
7.05	0.05	71.08	6.67	22.22	42		
320A LLVREED	SEMQNCNQMHDESYDT	GOKALQ	GRHVDVI	FK1	368:A		
8:A KPRQKRT	TATKAYN	VTQAF	GRRGPEQ	T <mark>Q G</mark> N F G D Q E L	IRQGT 47:A		
369:A	<mark>w</mark> lmwr	AKGTTG	FEAHVD		385:A		
48:A DYKHWPQ	QIAQFAPSASA <mark>F</mark> FG <mark>MS</mark>	RIGMEV	TPSGTW		81:A		
							_

### Figure 12

Similarity between 20KK and 7DE1.

Strue	ture ID	Description			\$	equence Length	Modeled Residues	Alignment Coverage
<b>Z</b> 2	OKKA	Glutamate decarboxylase 2				97	483	13%
■ 7	KINA	Host translation inhibitor nsp1			1	80	106	60%
ALIGNM	MENT							
RA	NSD TI	M-score	Score	SI%	SS%	Length		
5	.94	0.09	74.17	3.19	15.96	64		
242:A	PFLVSATAGT	TVYGAFDPLLA	VA···DIC	KKYKIWM	HVDA	AWGGGL 284:A		
14:A	VQLSLPVLQV	ROVLVRGF	GDSVEEVL	SEARQHL	(DGT <mark>CGLV</mark>	<mark>- vekg</mark> 59:А		
285:A	LMSRKH <mark>KWKL</mark>	SGVERANSVTW	INPHXMMGV	PLQCSAL	VREEGLM	QNCNQM 334:A		
60:A	V L PQL	EQPYVFIKRSD	GHVMVELV	AELEG	QYGRSGE	LGVLV 108A		
335:A	HOLSYDTGOK	AL				357:A		
109:A	PHVGEIPVAY	RK				120:A	· ·	
		-						

### Figure 13

Similarity between 20KK and 7K3N.

#### Figure 14

Similarity between 20KK and 7DK3.

# **Supplementary Files**

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