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OPINION ARTICLE

Could Small Neurotoxins-Peptides be Expressed during SARS-CoV-2 Infection?

Concetta Cafiero[1,*], Alessandra Micera[2,*], Agnese Re[3], Loredana Postiglione[4], Andrea Cacciamani[2], Beniamino Schiavone[5], Giulio Benincasa[6] and Raffaele Palmirotta[7]

[1]*Medical Oncology, SG Moscati Hospital, Taranto, Italy;* [2]*Research Laboratories in Ophthalmology, IRCCS - Fondazione Bietti, Rome, Italy;* [3]*Department of Chemistry and Clinical Biochemistry, Catholic University of Sacred Heart, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy;* [4]*Department of Translational Medical Sciences, University of Federico II, Naples. Italy;* [5]*General Management Unit, Pineta Grande Hospital, Castel Volturno (CE), Italy;* [6]*Department of Clinical Pathology and Molecular Biology, Pineta Grande Hospital, Castel Volturno, (CE), Italy;* [7]*Interdisciplinary Department of Medicine, University of Bari 'Aldo Moro', School of Medicine, Bari, Italy*

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Abstract: SARS-CoV-2 pathogenesis has been recently extended to human central nervous system (CNS), in addition to nasopharyngeal truck, eye, lung and gut. The recent literature highlights that some SARS-CoV-2 spike glycoprotein regions homologous to neurotoxin-like peptides might bind to human nicotinic Acetyl-Choline Receptors (nAChRs). Spike-nAChR interaction can probably cause dysregulation of CNS and cholinergic anti-inflammatory pathways and uncontrolled immune-response, both associated to a severe COVID-19 pathophysiology. Herein, we hypothesize that inside the Open Reading Frame (ORF) region of spike glycoprotein, the RNA polymerase can translate small neurotoxic peptides by means of a "jumping mechanism" already demonstrated in other coronaviruses. These small peptides can bind the snAChRs instead of Spike glycoproteins. A striking homology occurred between these small peptides observed by sequence retrieval and proteins alignment. Acting as nAChRs antagonists, these small peptides (conotoxins) could be the explanation for the extrapulmonary clinical manifestations (neurological, hemorrhagic and thrombotic expressions, the prolonged apnea, the cardiocirculatory collapse, the heart arrhythmias, the ventricular tachycardia, the body temperature alteration, the electrolyte K+ imbalance and finally the significant reduction of butyryl cholinesterase (BuChE) plasma levels, as observed in COVID-19 patients. Several factors might induce the expression of these small peptides, including microbiota. The main hypothesis regarding the presence of these small peptides opens a new scenario on the etiology of COVID-19 clinical symptoms observed so far, including the neurological manifestations.

Keywords: SARS-CoV-2 genome, infection, neurotoxins, acetylcholine, sequence alignment, microbiome.

1. INTRODUCTION

The COVID-19 pandemic, a global emergency due to SARS-CoV-2 infection, has resulted in 187,827,660 confirmed cases of infection and 4,055,497 deaths from beginning to date (World Health Organization - WHO) [1]. The presence of previous chronic diseases represents a significant risk factor and influences the prognosis as making patients more vulnerable to COVID-19 disease, from progression towards worsening outcomes [2]. In addition, other lifestyle-related risk factors such as physical inactivity, obesity, excessive alcohol intake and smoking, have also been proposed [3, 4].

Particular attention has been devoted to smoking, as evidence indicate that smokers with COVID-19 are more likely to have serious illness and adverse outcomes once hospitalized [5]. However, the number of smokers requiring hospitalization is far lower than expected, according to population smoking rates [5, 6-9]. Since the beginning of the pandemic, several studies have shown a negative association between smoking prevalence and COVID-19 hospitalized patients, leading some researchers to hypothesize a possible therapeutic role of nicotine in the course of SARS-CoV-2 infection [7, 10, 11].

Based on these observations, some research groups have suggested an interaction between the human nicotinic acetylcholine receptor (nAChRs) and SARS-CoV-2virus, in line with the finding that viral Spike glycoprotein has specific motifs related to known nAChR antagonists [5, 7, 8, 11].

SARS-CoV-2 virus, initially isolated from ocular secretions of COVID-19 affected patients [12], is part of β-coronaviruses' group and contains a single-stranded positive RNA (~ 29.9kB) with 14 Open Reading Frames (ORFs) encoding for 27 different proteins [13, 14]. Spike glycoprotein is expressed on the surface of virus envelope, representing a fun-

*Address correspondence to this author at the Medical Oncology, SG Moscati Hospital, Taranto, Italy; E-mail: concettacafiero@gmail.com;
Research Laboratories in Ophthalmology, IRCCS - Fondazione Bietti, Rome, Italy; E-mail: alessandra.micera@fondazionebietti.it
[*]*These authors contributed equally to this work and share first authorship.*

damental key point to mediate the entry of SARS-CoV-2 into host cells by recognition of human ACE2 (hACE2) receptor through a region defined as Receptor-Binding Domain (RBD) [15, 16].

Using a molecular dynamics computer simulation based on data from a previous study by Changeux and coworkers, Oliveira and coworkers identified i. a region on Spike protein ranging from amino acids 674 to 685 with high homology to several neurotoxins as α-bungarotoxin, a nAChR antagonist directly competing with acetylcholine identified in the snake Bungarus multicinctus, and ii. a high affinity region for the human α4β2 and α7-nAChR subtypes [8, 11]. The same research team also hypothesized the interaction of spike protein with α7-nAChR [8, 11].

By sequence alignments, Farsalinos and coworkers identified homology between the Spike Glycoprotein area between the aa 375-390 and the neurotoxin homolog NL1. This *in-silico* study suggested a strong interaction of the amino acid motif with the nAChR α9 subunit [17]. The aa 375-390 peptide fragment is included in the RBD spanning aa 319-541, a domain that enables the spike protein to recognize ACE2 on the host cells [17]. Further advanced *in-silico* studies by the same group highlighted that the sequence of the aa 375-390 corresponded with the previously described cryptic epitope for human anti-SARS-CoV antibody CR3022, and through a particular and unusual folding of Spike glycoprotein, it might also bind to α7-nAChR [6].

The involvement of the nicotinic cholinergic system might explain some aspects of COVID-19 pathogenesis not well understood to date. Numerous evidence suggests a neurotropic action of SARS-CoV-2, a point also confirmed by a recent study in which it was demonstrated how the virus could penetrate through the olfactory mucosa and follow the neuro-anatomical structures reaching the primary respiratory and cardiovascular control centers of the medulla oblongata [18]. Retrospective studies have demonstrated neurological manifestations in more than 30% of COVID-19 patients with Central Nervous System (CNS) symptoms such as dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, and seizure, in addition to the loss of smell and taste that are specific to this disease [19].

Activation of α7-nAChRs, particularly expressed by B cells, T cells, and macrophages, reduces the production of proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) through a "cholinergic anti-inflammatory pathway" triggered by nicotine or nicotinic agonists [9, 20]. This nAChR antagonistic action of virus would therefore explain the "cytokine storm" and the hyperinflammatory syndrome observed in several COVID-19 patients [9, 11]. Numerous authors also hypothesized a prominent role of macrophages highlighting that the antagonistic action on α7-nAChRs by SARS-CoV-2 determines a dysregulation of the polarization mechanism and a permanence of M1 type macrophages, with a consequent increase in the concentration of inflammatory cytokines [21].

Finally, it is interesting to note that α7-nAChRs are also present on the surface of platelets and that, as previously demonstrated, their inhibition can lead to an increase in their activity and consequent clot formation, thus explaining coagulopathy and thrombus formation in COVID-19 patients [9, 11].

Recently, the direct effect of inhibition of COVID-19 on critical survival genes has also been hypothesized, such as Sirt 1, that are linked to expression levels of α7-nAChRs [22, 23], resulting in effects that can range from inflammatory processes to programmed cell death [24].

2. SEQUENCE ALIGNMENT ANALYSIS

We mainly focused on the previously mentioned alignment studies that demonstrated a sequence homology between some regions of the virus Spike glycoprotein and nAChR antagonists such as α-bungarotoxin [8, 11] and Neurotoxin homolog NL1 [5, 6].

To verify the similarity between SARS-CoV-2 Spike glycoprotein sequence [6] and different neurotoxins with nAChR antagonistic action, we used EMBOSS Needle, a bioinformatic sequence analysis application provided by European Molecular Biology Laboratory - European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) [25, 26].

EMBOSS, acronym for European Molecular Biology Open Software Suite, is a free open-source software analysis package and contains a wide array of general-purpose bioinformatics programs for the needs of the molecular biology user community. Among these, EMBOSS Needle tool reads and compares two input sequences along with their entire length and writes their optimal global sequence alignment to file. The software uses the Needleman-Wunsch alignment algorithm, an automatic procedure for calculating the best possible alignment between two amino acid sequences [27]. This method allows to perform a fast and ingenious comparison between all the alignments among two sequences, considering every possible number of gaps in every possible position. Software's purpose is to choose the best alignment among all produced, simply the one that guarantees the highest 'score'. A short description of the steps used by this tool and an example of the output file are reported in Supplementary Fig. (**1**). Moreover, it is possible to view the entire procedure used by the software at https://www.youtube.com/watch?v=_lm1WOOuv5o&t=67s [28]. First, we performed a protein alignment between the 1273 aa SARS-CoV-2 spike glycoprotein (NCBI Gene ID: NC_045512.2) [14] and the mentioned above Neurotoxin homolog NL1 (UniProtKB - Q9DEQ3 - 3SO8_NAJAT), a polypeptide of 86 aa identified in Najaatra (Chinese cobra) [5, 6]. We noted that the presence of identical or functionally equivalent amino acids is not limited to the 375-390 region, as previously described, but it is also present in other areas of the Spike glycoprotein with a sequence homology of 47/86 aa (54.6%) (Fig. **1**, Supplementary Table **1**). Interestingly, we also found homology for the Muscarinic toxin-like protein, identified in Bungarus multicinctus (Many-banded krait; UniPro

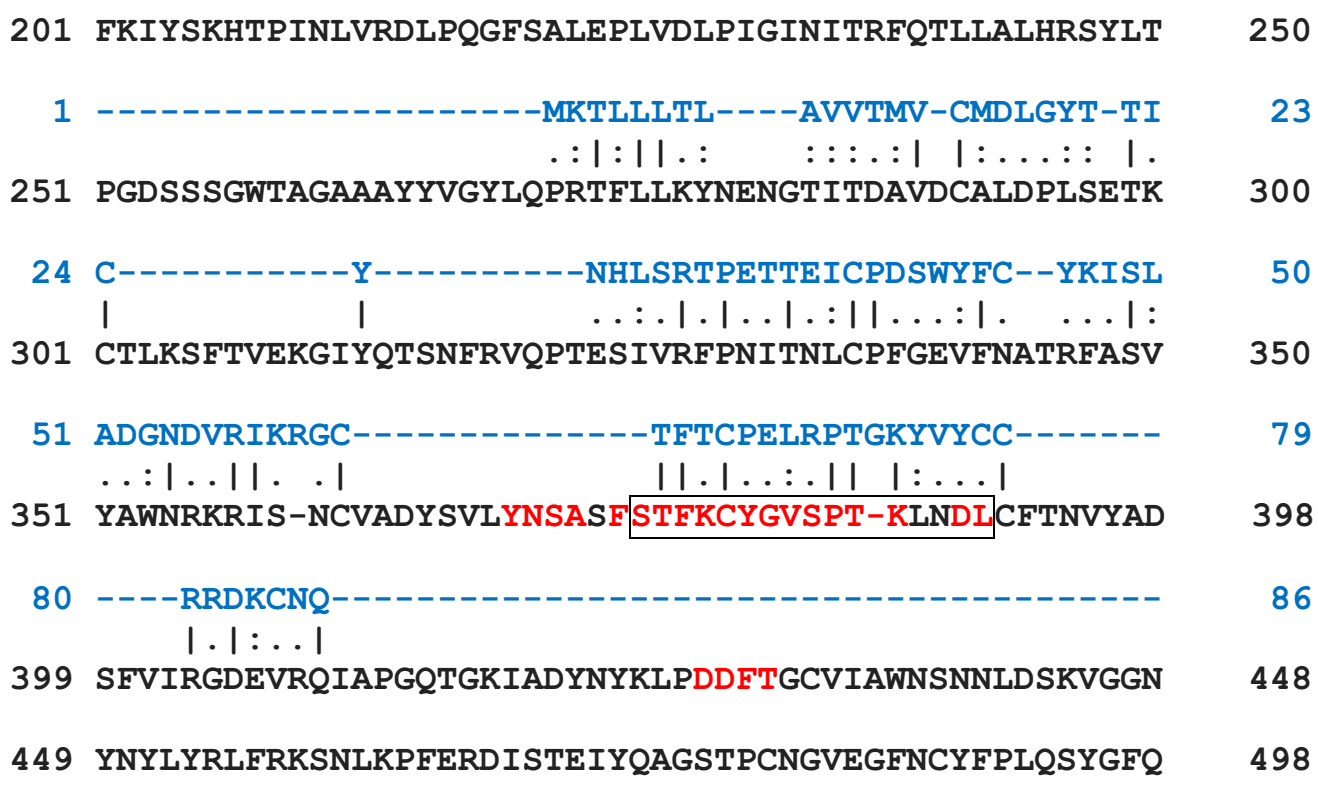

Fig. (1). Protein Sequence Alignment performed using EMBOSS Needle between SARS-CoV-2 Spike glycoprotein (GenBank: NC_045512.2) and Neurotoxin homolog NL1, Najaatra (Chinese cobra) (UniProtKB - Q9DEQ3 - 3SO8_NAJAT) protein. Black: Spike protein. Blue: Conotoxin proteins. Red: SARS-CoV-2 RBD epitope sequence for the CR3022 mAb. Framework: highly conserved region of SARS-CoVs' RBD (375–395 aa). (|): identities, (:) conservative replacements, (.): non-conservative replacements. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tKB: Q9W727 - 3SO8_BUNMU), which exhibits an entirely similar sequence to Neurotoxin homolog NL1. Furthermore, we found a homology between the cryptic epitope for the human antibody CR3022 and the 27 aa Kappa-conotoxin-like as14a, identified in Conus cancellatus (Cancellate cone; Conus austini; UniProtKB: P0C6S2 - CLEA_CONCF), with an overall sequence homology of 12/27 aa (44.4%) (Supplementary Table **1**). Later, we proceeded to find several conotoxins protein sequences from the UniProt Knowledgebase (UniProtKB) database (https://www.uniprot.org/uniprot/) [29] and always carrying out alignment with SARS-CoV-2 Spike glycoprotein. In all cases, we found a certain rate of homology with the Spike glycoprotein also in regions different from the cryptic epitope for the human antibody CR3022 and with percentages of homology ranging from 22.7% to 50.8%, with an average of 37% (Supplementary Table **1**).

Our *in-silico* analysis, through sequence retrieval and protein alignment, shows high positive correlation between small genome regions and different toxins, like conotoxin, that selectively could interact with nAChRs. Being nAChR antagonists, these toxins are used by cone snails to immobilize their prey [30]. The release of (oligo-) peptides almost identical to animal conotoxins could result in clinical manifestations such as neurological, hemorrhagic and thrombotic. This expression would explain the presence of symptoms of COVID-19 pathogenesis not yet fully clarified, such as prolonged apnea, cardiocirculatory collapse, heart arrhythmias, ventricular tachycardia, body temperature alteration, electrolyte imbalance (particularly K^+) and even the significant reduction of butyrylcholinesterase (BuChE) plasma levels.

Thus, the data obtained from our analyses indicate that regions of homology with neurotoxin-like peptides are more numerous and more widely distributed on the Spike glycoprotein than previously observed by others [31].

3. DISCUSSION

Based on these high levels of homology, we hypothesize that inside the last ORF of viral genome encoding for Spike glycoprotein, the RNA polymerase can encode these small proteins. This hypothesis is supported by several pieces of evidence reporting that SARS-CoV-2 viral RNA polymerase, through a "jumping mechanism" already demonstrated in the other coronavirus, results in numerous discontinuous transcription events, which are likely to include small neurotoxin-like proteins such as conotoxins [32, 33]. Indeed,

while the role of many structural SARS-CoV-2 proteins is well described in the literature, several sgRNAs encoded by RNA polymerase jumping unusual display structure and a still unknown function [34]. The inhibition of AChRs can play a central role in cholinergic CNS and PNS synapses [11] and particularly the AChRs:nicotine binding might block nAChRs, supporting the hypothesis of a protective role for nicotine and other cholinergic agonists, in line with the observation that smokers are almost "protected" against SARS-CoV-2 hospitalization [17, 19, 35]. The absence of nAChRs:nicotine binding could provide logical explanations for acute inflammatory disorder in these patients, as COVID-19 pathology may be linked to severe dysregulation of CNS [10]. Finally, this hypothesis could explain the detection of some toxic products into bloodstream and tissues [36] and the correlation between different taxa compositions of nasopharyngeal or gastrointestinal microbiota and the severity of COVID-19 disease [37].

Certainly, the hypothesis of synthesis of small neurotoxic peptide herein described opens a new scenario on the etiology of COVID-19 clinical symptoms observed so far, including the neurological manifestations. Indeed, the presence of conotoxins-like peptides could explain the appearance of many symptoms including hyposmia, hypogeusia and the typical signs of Guillain Barre syndrome observed in some patients with COVID-19 [38]. As stated, the presence of toxic peptides can alter the normal functioning of ion channels, nicotinic AChR as well as ACh levels and induce a significant reduction of plasmatic BuChE levels [39].

However, the reason for production of these oligopeptides binding to nAChR still remains poorly understood and many doubts remain about the mechanisms related to transcriptional processes of the virus [33, 40]. The above-mentioned literature on computational modeling suggested that SARS-CoV-2 spike glycoprotein might bind to nAChRs by a particular and unusual folding, also through a cryptic epitope that coincides with the well-described cryptic epitope for the human SARS-CoV antibody CR3022 [5, 6, 8, 11]. By the way, it is well known that these peptides are functional macromolecules characterized by a specific 3D "native" structure that allows their functions correctly, once at final conformation [41]. The reaching of the final 3D-folding stability is assisted by specific protein complexes (chaperones) that guarantee the conservation of protein functions [41, 42]. Therefore, it is strongly unlike that Spike protein, specifically a 1273 aa large protein, can totally change its folding to express a small peptide portion of 30 aa able to bind the nicotinic receptor [6]. We strongly support the hypothesis that, through a "jumping mechanism" [33, 40], the RNA polymerase can prime the transcription of small peptides, like conotoxins, whose sequence is contained in the ORF region encoding for Spike glycoprotein. This possibility could be primed by external biological factors such as the host's microbiota [43]. Indeed, recent studies identified a different microbiome depending on ageing and their tissue distribution (gut, lung and eye), allowing us to hypothesize that microbiota can play a crucial role in directing the expression of this small protein by SARS-CoV-2 [32, 43-45]. As observed in COVID-19, elderly subjects are affected by more severe forms of disease, and most probably COVID-19 disease worsens in youngers in the presence of dysbiosis and/or when certain bacterial taxonomies prevail [46-50]. In this context, it is interesting the hypothesis recently proposed that bacterial lipopolysaccharides (LPS) may repress sirt 1 with a consequent effect on nACHRs resulting in a greater severity of covid-19 infection in elderly individuals [24].

Therefore, the microbiome, belonging to specific body districts, represents a valuable "new entry" in the biomedical and therapeutical fields for COVID-19 patients [40, 46, 50].

CONCLUSION

In view of the above reported considerations, some therapeutic solutions can be prospected. First, we must consider that conus venom is highly toxic and lethal as it is composed of many different types of conotoxins (α, δ, κ, μ and ω) which have neurological effects, due to different receptor targets [51, 52]. Indeed, the conotoxins are generally weakly immunogenic and therefore, not effectively targeted by current polyclonal anti-venom therapies [53]. Although the use of monoclonal anti-toxin directed either towards the virus surface antigens and/or the released virus products could be a quick solution to reduce mortality rates, it remains difficult to apply. On the contrary, nAChR can bind these toxins and therefore, it can be used against neurotoxic envenoming [53-56]. The administration of cholinesterase-derived human BuChE could be hypothesized as a potential therapeutic approach [51]. On the other hand, a therapeutic approach using nAChRs agonists, as hypothesized by a previous *in silico* study, cannot be ruled out [10].

Undoubtedly, our hypothesis remains to be proven and numerous efforts still need to be made by researchers to eradicate this serious pandemic that is affecting health and the economy globally.

LIST OF ABBREVIATIONS

hACE2	=	human Angiotensin-Converting Enzyme 2
CNS	=	central Nervous System
nAChRs	=	nicotinic Acetyl-Choline Receptors
ORF	=	Open Reading Frame
BuChE	=	butyryl Cholinesterase
WHO	=	World Health Organization
RBD	=	Receptor-Binding Domain
IL-1	=	interleukin-1
IL-6	=	interleukin-6
IL-8	=	interleukin-8
SIRT1	=	Sirtuin 1
TNF-α	=	Tumor Necrosis Factor-α
EMBL-EBI	=	European Molecular Biology Laboratory - European Bioinformatics Institute

EMBOSS = European Molecular Biology Open Software Suite

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. Geneva: WHO. Available from: https://-covid19.who.int Accessed, July 15, 2021

[2] Wang, D.; Hu, B.; Hu, C.; Zhu, F.; Liu, X.; Zhang, J.; Wang, B.; Xiang, H.; Cheng, Z.; Xiong, Y.; Zhao, Y.; Li, Y.; Wang, X.; Peng, Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA,* **2020**, *323*(11), 1061-1069. http://dx.doi.org/10.1001/jama.2020.1585 PMID: 32031570

[3] Lassen, M.C.H.; Skaarup, K.G.; Sengeløv, M.; Iversen, K.; Ulrik, C.S.; Jensen, J.U.S.; Biering-Sørensen, T. Alcohol consumption and the risk of acute respiratory distress syndrome in COVID-19. *Ann. Am. Thorac. Soc.,* **2021**, *18*(6), 1074-1076. http://dx.doi.org/10.1513/AnnalsATS.202008-988RL PMID: 33315543

[4] Hamer, M.; Kivimäki, M.; Gale, C.R.; Batty, G.D. Lifestyle risk factors, inflammatory mechanisms, and COVID-19 hospitalization: A community-based cohort study of 387,109 adults in UK. *Brain Behav. Immun.,* **2020**, *87*, 184-187. http://dx.doi.org/10.1016/j.bbi.2020.05.059 PMID: 32454138

[5] Farsalinos, K.; Angelopoulou, A.; Alexandris, N.; Poulas, K. COVID-19 and the nicotinic cholinergic system. *Eur. Respir. J.,* **2020**, *56*(1), 2001589. http://dx.doi.org/10.1183/13993003.01589-2020 PMID: 32444400

[6] Lagoumintzis, G.; Chasapis, C.T.; Alexandris, N.; Kouretas, D.; Tzartos, S.; Eliopoulos, E.; Farsalinos, K.; Poulas, K. Nicotinic cholinergic system and COVID-19: *In silico* identification of interactions between α7 nicotinic acetylcholine receptor and the cryptic epitopes of SARS-Co-V and SARS-CoV-2 spike glycoproteins. *Food Chem. Toxicol.,* **2021**, *149*, 112009. http://dx.doi.org/10.1016/j.fct.2021.112009 PMID: 33503469

[7] Farsalinos, K.; Eliopoulos, E.; Leonidas, D.D.; Papadopoulos, G.E.; Tzartos, S.; Poulas, K. Nicotinic cholinergic system and COVID-19: *In silico* identification of an interaction between SARS-COV-2 and nicotinic receptors with potential therapeutic targeting implications. *Int. J. Mol. Sci.,* **2020**, *21*(16), 5807. http://dx.doi.org/10.3390/ijms21165807 PMID: 32823591

[8] Oliveira, A.S.F.; Ibarra, A.A.; Bermudez, I.; Casalino, L.; Gaieb, Z.; Shoemark, D.K.; Gallagher, T.; Sessions, R.B.; Amaro, R.E.; Mulholland, A.J. A potential interaction between the SARS-CoV--2 spike protein and nicotinic acetylcholine receptors. *Biophys. J.,* **2021**, *120*(6), 983-993. http://dx.doi.org/10.1016/j.bpj.2021.01.037 PMID: 33609494

[9] Tizabi, Y.; Getachew, B.; Copeland, R.L.; Aschner, M. Nicotine and the nicotinic cholinergic system in COVID-19. *FEBS J.,* **2020**, *287*(17), 3656-3663. http://dx.doi.org/10.1111/febs.15521 PMID: 32790936

[10] Alexandris, N.; Lagoumintzis, G.; Chasapis, C.T.; Leonidas, D.D.; Papadopoulos, G.E.; Tzartos, S.J.; Tsatsakis, A.; Eliopoulos, E.; Poulas, K.; Farsalinos, K. Nicotinic cholinergic system and COVID-19: *In silico* evaluation of nicotinic acetylcholine receptor agonists as potential therapeutic interventions. *Toxicol. Rep.,* **2020**, *8*, 73-83. http://dx.doi.org/10.1016/j.toxrep.2020.12.013 PMID: 33425684

[11] Changeux, J.P. Discovery of the first neurotransmitter receptor: The acetylcholine nicotinic receptor. *Biomolecules,* **2020**, *10*(4), 547. http://dx.doi.org/10.3390/biom10040547 PMID: 32260196

[12] Colavita, F.; Lapa, D.; Carletti, F.; Lalle, E.; Bordi, L.; Marsella, P.; Nicastri, E.; Bevilacqua, N.; Giancola, M.L.; Corpolongo, A.; Ippolito, G.; Capobianchi, M.R.; Castilletti, C. SARS-CoV-2 isolation from ocular secretions of a patient with COVID-19 in Italy with prolonged viral RNA detection. *Ann. Intern. Med.,* **2020**, *173*(3), 242-243. http://dx.doi.org/10.7326/M20-1176 PMID: 32302380

[13] V'kovski, P.; Kratzel, A.; Steiner, S.; Stalder, H.; Thiel, V. Coronavirus biology and replication: Implications for SARS-CoV-2. *Nat. Rev. Microbiol.,* **2021**, *19*(3), 155-170. http://dx.doi.org/10.1038/s41579-020-00468-6 PMID: 33116300

[14] Wu, A.; Peng, Y.; Huang, B.; Ding, X.; Wang, X.; Niu, P.; Meng, J.; Zhu, Z.; Zhang, Z.; Wang, J.; Sheng, J.; Quan, L.; Xia, Z.; Tan, W.; Cheng, G.; Jiang, T. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe,* **2020**, *27*(3), 325-328. http://dx.doi.org/10.1016/j.chom.2020.02.001 PMID: 32035028

[15] Shang, J.; Wan, Y.; Luo, C.; Ye, G.; Geng, Q.; Auerbach, A.; Li, F. Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(21), 11727-11734. http://dx.doi.org/10.1073/pnas.2003138117 PMID: 32376634

[16] Cafiero, C.; Rosapepe, F.; Palmirotta, R.; Re, A.; Ottaiano, M.P.; Benincasa, G.; Perone, R.; Varriale, E.; D'Amato, G.; Cacciamani, A.; Micera, A.; Pisconti, S. Angiotensin system polymorphisms' in SARS-CoV-2 positive patients: Assessment between symptomatic and asymptomatic patients: A pilot study. *Pharm. Genomics Pers. Med.,* **2021**, *14*, 621-629. http://dx.doi.org/10.2147/PGPM.S303666 PMID: 34079337

[17] Farsalinos, K.; Niaura, R.; Le Houezec, J.; Barbouni, A.; Tsatsakis, A.; Kouretas, D.; Vantarakis, A.; Poulas, K. Editorial: Nicotine and SARS-CoV-2: COVID-19 may be a disease of the nicotinic cholinergic system. *Toxicol. Rep.,* **2020**, *7*, 658-663. http://dx.doi.org/10.1016/j.toxrep.2020.04.012 PMID: 32355638

[18] Meinhardt, J.; Radke, J.; Dittmayer, C.; Franz, J.; Thomas, C.; Mothes, R.; Laue, M.; Schneider, J.; Brünink, S.; Greuel, S.; Lehmann, M.; Hassan, O.; Aschman, T.; Schumann, E.; Chua, R.L.; Conrad, C.; Eils, R.; Stenzel, W.; Windgassen, M.; Rößler, L.; Goebel, H.H.; Gelderblom, H.R.; Martin, H.; Nitsche, A.; Schulz-Schaeffer, W.J.; Hakroush, S.; Winkler, M.S.; Tampe, B.; Scheibe, F.; Körtvélyessy, P.; Reinhold, D.; Siegmund, B.; Kühl, A.A.; Elezkurtaj, S.; Horst, D.; Oesterhelweg, L.; Tsokos, M.; Ingold-Heppner, B.; Stadelmann, C.; Drosten, C.; Corman, V.M.; Radbruch, H.; Heppner, F.L. Olfactory transmucosal SARS-CoV--2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat. Neurosci.,* **2021**, *24*(2), 168-175. http://dx.doi.org/10.1038/s41593-020-00758-5 PMID: 33257876

[19] Mao, L.; Jin, H.; Wang, M.; Hu, Y.; Chen, S.; He, Q.; Chang, J.; Hong, C.; Zhou, Y.; Wang, D.; Miao, X.; Li, Y.; Hu, B. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.,* **2020**, *77*(6), 683-690. http://dx.doi.org/10.1001/jamaneurol.2020.1127 PMID: 32275288

[20] Changeux, J.P.; Amoura, Z.; Rey, F.A.; Miyara, M. A nicotinic hypothesis for Covid-19 with preventive and therapeutic implications. *C. R. Biol.,* **2020**, *343*(1), 33-39. PMID: 32720486

[21] Tanmay, S.; Labrou, D.; Farsalinos, K.; Poulas, K. Is SARS-CoV--2 Spike glycoprotein impairing macrophage function *via* α7-nico-

tinic acetylcholine receptors? *Food Chem. Toxicol.,* **2021**, *152*, 112184.
http://dx.doi.org/10.1016/j.fct.2021.112184 PMID: 33838172

[22] Huarachi Olivera, R.E.; Lazarte Rivera, A. Coronavirus disease (COVID-19) and sirtuins. *Rev. Fac. Cien. Med. Univ. Nac. Cordoba,* **2020**, *77*(2), 117-125.
http://dx.doi.org/10.31053/1853.0605.v77.n2.28196 PMID: 32558516

[23] Cao, K.; Dong, Y.T.; Xiang, J.; Xu, Y.; Li, Y.; Song, H.; Yu, W.F.; Qi, X.L.; Guan, Z.Z. The neuroprotective effects of SIRT1 in mice carrying the APP/PS1 double-transgenic mutation and in SH-SY5Y cells over-expressing human APP670/671 may involve elevated levels of α7 nicotinic acetylcholine receptors. *Aging (Albany NY),* **2020**, *12*(2), 1792-1807.
http://dx.doi.org/10.18632/aging.102713 PMID: 32003755

[24] Martins, I.J. Infection and anti-aging gene inactivation. *Acta Sci. Nutr Health,* **2020**, *4*, 01-02.

[25] Madeira, F.; Park, Y.M.; Lee, J.; Buso, N.; Gur, T.; Madhusoodanan, N.; Basutkar, P.; Tivey, A.R.N.; Potter, S.C.; Finn, R.D.; Lopez, R. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.,* **2019**, *47*(W1), W636-W641.
http://dx.doi.org/10.1093/nar/gkz268 PMID: 30976793

[26] EMBOSS Needle. Available from: https://www.ebi.ac.uk/Tools/psa/emboss_needle/ (Accessed, July 15, 2021).

[27] Needleman, S.B.; Wunsch, C.D. A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.,* **1970**, *48*(3), 443-453.
http://dx.doi.org/10.1016/0022-2836(70)90057-4 PMID: 5420325

[28] Global Alignment Using EMBOSS Needle. Youtube. Available from: https://www.youtube.com/watch?v=_lm1WOOuv5o&t=67s (Accessed, December 5, 2021).

[29] UniProt Knowledgebase (UniProtKB) database. Available from: https://www.uniprot.org/uniprot/ Accessed, July 15, 2021

[30] Becker, S.; Terlau, H. Toxins from cone snails: Properties, applications and biotechnological production. *Appl. Microbiol. Biotechnol.,* **2008**, *79*(1), 1-9.
http://dx.doi.org/10.1007/s00253-008-1385-6 PMID: 18340446

[31] Mir, R.; Karim, S.; Kamal, M.A.; Wilson, C.M.; Mirza, Z. Conotoxins: Structure, therapeutic potential and pharmacological applications. *Curr. Pharm. Des.,* **2016**, *22*(5), 582-589.
http://dx.doi.org/10.2174/1381612822666151124234715 PMID: 26601961

[32] Kim, S.; Jazwinski, S.M. The gut microbiota and healthy aging: A mini-review. *Gerontology,* **2018**, *64*(6), 513-520.
http://dx.doi.org/10.1159/000490615 PMID: 30025401

[33] Sola, I.; Almazán, F.; Zúñiga, S.; Enjuanes, L. Continuous and discontinuous RNA synthesis in coronaviruses. *Annu. Rev. Virol.,* **2015**, *2*(1), 265-288.
http://dx.doi.org/10.1146/annurev-virology-100114-055218 PMID: 26958916

[34] Romano, M.; Ruggiero, A.; Squeglia, F.; Maga, G.; Berisio, R. A structural view of SARS-CoV-2 RNA replication machinery: RNA synthesis, proofreading and final capping. *Cells,* **2020**, *9*(5), 1267.
http://dx.doi.org/10.3390/cells9051267 PMID: 32443810

[35] van Westen-Lagerweij, N.A.; Meijer, E.; Meeuwsen, E.G.; Chavannes, N.H.; Willemsen, M.C.; Croes, E.A. Are smokers protected against SARS-CoV-2 infection (COVID-19)? The origins of the myth. *NPJ Prim. Care Respir. Med.,* **2021**, *31*(1), 10.
http://dx.doi.org/10.1038/s41533-021-00223-1 PMID: 33637750

[36] Li, C.X.; Chen, J.; Lv, S.K.; Li, J.H.; Li, L.L.; Hu, X. Whole-transcriptome RNA sequencing reveals significant differentially expressed mRNAs, miRNAs, and lncRNAs and related regulating biological pathways in the peripheral blood of COVID-19 patients. *Mediators Inflamm.,* **2021**, *2021*, 6635925.
http://dx.doi.org/10.1155/2021/6635925 PMID: 33833618

[37] Brogna, B.; Brogna, C.; Petrillo, M.; Conte, A.M.; Benincasa, G.; Montano, L.; Piscopo, M. SARS-CoV-2 detection in fecal sample from a patient with typical findings of COVID-19 pneumonia on CT but negative to multiple SARS-CoV-2 RT-PCR tests on oropharyngeal and nasopharyngeal swab samples. *Medicina (Kaunas),* **2021**, *57*(3), 290.
http://dx.doi.org/10.3390/medicina57030290 PMID: 33804646

[38] Abdullahi, A.; Candan, S.A.; Soysal Tomruk, M.; Elibol, N.; Dada, O.; Truijen, S.; Saeys, W. Is Guillain-Barré Syndrome associated with COVID-19 infection? A systemic review of the evidence. *Front. Neurol.,* **2021**, *11*, 566308.
http://dx.doi.org/10.3389/fneur.2020.566308 PMID: 33519663

[39] Lebbe, E.K.; Peigneur, S.; Wijesekara, I.; Tytgat, J. Conotoxins targeting nicotinic acetylcholine receptors: An overview. *Mar. Drugs,* **2014**, *12*(5), 2970-3004.
http://dx.doi.org/10.3390/md12052970 PMID: 24857959

[40] Kim, D.; Lee, J.Y.; Yang, J.S.; Kim, J.W.; Kim, V.N.; Chang, H. The architecture of SARS-CoV-2 transcriptome. *Cell,* **2020**, *181*(4), 914-921.e10.
http://dx.doi.org/10.1016/j.cell.2020.04.011 PMID: 32330414

[41] Best, R.B. Race to the native state. *Proc. Natl. Acad. Sci. USA,* **2018**, *115*(10), 2267-2269.
http://dx.doi.org/10.1073/pnas.1722622115 PMID: 29472450

[42] Englander, S.W.; Mayne, L. The nature of protein folding pathways. *Proc. Natl. Acad. Sci. USA,* **2014**, *111*(45), 15873-15880.
http://dx.doi.org/10.1073/pnas.1411798111 PMID: 25326421

[43] Zhang, D.; Li, S.; Wang, N.; Tan, H.Y.; Zhang, Z.; Feng, Y. The cross-talk between gut microbiota and lungs in common lung diseases. *Front. Microbiol.,* **2020**, *11*, 301.
http://dx.doi.org/10.3389/fmicb.2020.00301 PMID: 32158441

[44] Bischoff, S.C. Microbiota and aging. *Curr. Opin. Clin. Nutr. Metab. Care,* **2016**, *19*(1), 26-30.
http://dx.doi.org/10.1097/MCO.0000000000000242 PMID: 26560527

[45] Li, J.J.; Yi, S.; Wei, L. Ocular microbiota and intraocular inflammation. *Front. Immunol.,* **2020**, *11*, 609765.
http://dx.doi.org/10.3389/fimmu.2020.609765 PMID: 33424865

[46] Ventero, M.P.; Cuadrat, R.R.C.; Vidal, I.; Andrade, B.G.N.; Molina-Pardines, C.; Haro-Moreno, J.M.; Coutinho, F.H.; Merino, E.; Regitano, L.C.A.; Silveira, C.B.; Afli, H.; López-Pérez, M.; Rodríguez, J.C. Nasopharyngeal microbial communities of patients infected with SARS-CoV-2 that developed COVID-19. *Front. Microbiol.,* **2021**, *12*, 637430.
http://dx.doi.org/10.3389/fmicb.2021.637430 PMID: 33815323

[47] Ferreira, C.; Viana, S.D.; Reis, F. Gut Microbiota dysbiosis-immune hyperresponse-inflammation triad in coronavirus disease 2019 (COVID-19): Impact of pharmacological and nutraceutical approaches. *Microorganisms,* **2020**, *8*(10), 1514.
http://dx.doi.org/10.3390/microorganisms8101514 PMID: 33019592

[48] de Oliveira, G.L.V.; Oliveira, C.N.S.; Pinzan, C.F.; de Salis, L.V.V.; Cardoso, C.R.B. Microbiota modulation of the gut-lung axis in COVID-19. *Front. Immunol.,* **2021**, *12*, 635471.
http://dx.doi.org/10.3389/fimmu.2021.635471 PMID: 33717181

[49] Di Stadio, A.; Costantini, C.; Renga, G.; Pariano, M.; Ricci, G.; Romani, L. The microbiota/host immune system interaction in the nose to protect from COVID-19. *Life (Basel),* **2020**, *10*(12), 345.
http://dx.doi.org/10.3390/life10120345 PMID: 33322584

[50] Sundararaman, A.; Ray, M.; Ravindra, P.V.; Halami, P.M. Role of probiotics to combat viral infections with emphasis on COVID-19. *Appl. Microbiol. Biotechnol.,* **2020**, *104*(19), 8089-8104.
http://dx.doi.org/10.1007/s00253-020-10832-4 PMID: 32813065

[51] Mumford, H.; Docx, C.J.; Price, M.E.; Green, A.C.; Tattersall, J.E.; Armstrong, S.J. Human plasma-derived BuChE as a stoichiometric bioscavenger for treatment of nerve agent poisoning. *Chem. Biol. Interact.,* **2013**, *203*(1), 160-166.
http://dx.doi.org/10.1016/j.cbi.2012.08.018 PMID: 22981459

[52] Wu, Y.; Zhangsun, D.; Zhu, X.; Kaas, Q.; Zhangsun, M.; Harvey, P.J.; Craik, D.J.; McIntosh, J.M.; Luo, S. α-Conotoxin [S9A]TxID potently discriminates between α3β4 and α6/α3β4 nicotinic acetylcholine receptors. *J. Med. Chem.,* **2017**, *60*(13), 5826-5833.
http://dx.doi.org/10.1021/acs.jmedchem.7b00546 PMID: 28603989

[53] Albulescu, L.O.; Kazandjian, T.; Slagboom, J.; Bruyneel, B.; Ainsworth, S.; Alsolaiss, J.; Wagstaff, S.C.; Whiteley, G.; Harrison, R.A.; Ulens, C.; Kool, J.; Casewell, N.R. A Decoy-receptor approach using nicotinic acetylcholine receptor mimics reveals their potential as novel therapeutics against neurotoxic snakebite. *Front. Pharmacol.,* **2019**, *10*, 848.

http://dx.doi.org/10.3389/fphar.2019.00848 PMID: 31417406

[54] Chen, H.Y.; Wang, W.W.; Chaou, C.H.; Lin, C.C. Prognostic value of serial serum cholinesterase activities in organophosphate poisoned patients. *J. Emerg. Med.,* **2009**, *27*(9), 1034-1039. http://dx.doi.org/10.1016/j.ajem.2008.07.006 PMID: 19931747

[55] Nakajima, K.; Abe, T.; Saji, R.; Ogawa, F.; Taniguchi, H.; Yamaguchi, K.; Sakai, K.; Nakagawa, T.; Matsumura, R.; Oi, Y.; Nishii, M.; Takeuchi, I. Serum cholinesterase associated with COVID-19 pneumonia severity and mortality. *J. Infect.,* **2021**, *82*(2), 282-327. http://dx.doi.org/10.1016/j.jinf.2020.08.021 PMID: 32822684

[56] Courties, A.; Boussier, J.; Hadjadj, J.; Yatim, N.; Barnabei, L.; Péré, H.; Veyer, D.; Kernéis, S.; Carlier, N.; Pène, F.; Rieux-Laucat, F.; Charbit, B.; Bondet, V.; Duffy, D.; Berenbaum, F.; Terrier, B.; Sellam, J. Regulation of the acetylcholine/α7nAChR anti-inflammatory pathway in COVID-19 patients. *Sci. Rep.,* **2021**, *11*(1), 11886. http://dx.doi.org/10.1038/s41598-021-91417-7 PMID: 34088975

Supplementary Material

Could Small Neurotoxins-Peptides be Expressed during SARS-CoV-2 Infection?

Concetta Cafiero[1,*], Alessandra Micera[2,*], Agnese Re[3], Loredana Postiglione[4], Andrea Cacciamani[2] , Beniamino Schiavone[5], Giulio Benincasa[6] and Raffaele Palmirotta[7]

[1]Medical Oncology, SG Moscati Hospital, Taranto, Italy; [2]Research Laboratories in Ophthalmology, IRCCS - Fondazione Bietti, Rome, Italy; [3]Department of Chemistry and Clinical Biochemistry, Catholic University of Sacred Heart, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [4]Department of Translational Medical Sciences, University of Federico II, Naples. Italy; [5]General Management Unit, Pineta Grande Hospital, Castel Volturno (CE), Italy; [6]Department of Clinical Pathology and Molecular Biology, Pineta Grande Hospital, Castel Volturno, (CE), Italy; [7]Interdisciplinary Department of Medicine, University of Bari 'Aldo Moro', School of Medicine, Italy

Supplementary Table 1. Protein Sequence Alignment performed using EMBOSS Needle between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and several conotoxines protein. Black: spike structural protein. Blue: Conotoxin proteins. Red: SARS-COV-2 RBD epitope sequence for the CR3022 mAb. Framework: highly conserved region of SARS-CoVs RBD (aa 375–395). (|): identities, (:) conservative replacements, (.): non-conservative replacements.

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Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Neurotoxin homolog NL1, *Naja atra (Chinese cobra)* (UniProtKB - Q9DEQ3 - 3SO8_NAJAT)* protein *[ref. Farsalinos et al. 2020c*].

*This sequence is similar to Muscarinic toxin-like protein, *Bungarus multicinctus (Many-banded krait)* (UniProtKB: Q9W727 - 3SO8_BUNMU)

MKTLLLTLVVVTMVCMDLGYTTICYNHLSRTPETTEICPDSWYFCYKIS-LADGNDVRIKRGCTFTCPELRPTGKYVYCCRRDKCNQ

```
EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001         1 ----------------------MKTLLLTL----AVVTMV-CMDLGYT-TI     23
                                           .:|:||.:    ::::.:| |:...:: |.
EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001        24 C-----------Y----------NHLSRTPETTEICPDSWYFC--YKISL     50
                     |           |          ..:.|.|..|.:||...:|.  ...|:
EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001        51 ADGNDVRIKRGC--------------TFTCPELRPTGKYVYCC-------     79
                     ..:|..||. .|               ||.|..:.|| |:...|
EMBOSS_001       351 YAWNRKRIS-NCVADYSVLYNSASFSTFKCYGVSPT-KLNDLCFTNVYAD    398
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1875-5488/21

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EMBOSS_001         80 ----RRDKCNQ----------------------------------------      86
                        |.|:..|
EMBOSS_001        399 SFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGN     448

EMBOSS_001        449 YNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQ     498

EMBOSS_001        499 PTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTG     548

EMBOSS_001        549 TGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVI     598

EMBOSS_001        599 TPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAG     648

EMBOSS_001        649 CLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMS     698

EMBOSS_001        699 LGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTE     748

EMBOSS_001        749 CSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG     798

EMBOSS_001        799 GFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARD     848

EMBOSS_001        849 LICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF     898

EMBOSS_001        899 AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKL     948

EMBOSS_001        949 QDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLIT     998

EMBOSS_001        999 GRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYH    1048

EMBOSS_001       1049 LMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSN    1098

EMBOSS_001       1099 GTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSF    1148

EMBOSS_001       1149 KEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLI    1198

EMBOSS_001       1199 DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCC    1248

EMBOSS_001       1249 SCGSCCKFDEDDSEPVLKGVKLHYT    1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and **Kappa-conotoxin-like as14a, *Conus cancellatus (Cancellate cone) (Conus austini)*** (UniProtKB: *P0C6S2 - CLEA_CONCF*) protein.

GGVGRCIYNCMNSGGGLNFIQCKTMCY

```
EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS      50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI     100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK     150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY     200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT     250

EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK     300

EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV     350

EMBOSS_001         1 -----GGVGRCI--YNCMNSGGGLNFIQC--------KTMCY--------      27
                         ..:..|:  |:.:.:....:..:|        ..:|:
EMBOSS_001       351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF     400
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```
EMBOSS_001       401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450
EMBOSS_001       451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500
EMBOSS_001       501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550
EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600
EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650
EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700
EMBOSS_001       701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750
EMBOSS_001       751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800
EMBOSS_001       801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850
EMBOSS_001       851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900
EMBOSS_001       901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950
EMBOSS_001       951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000
EMBOSS_001      1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050
EMBOSS_001      1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100
EMBOSS_001      1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE   1150
EMBOSS_001      1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL   1200
EMBOSS_001      1201 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSC   1250
EMBOSS_001      1251 GSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Rho-conotoxin TIA (CA1A_CONTU), Conus tulipa (Fish-hunting cone snail) (Tulip cone) (UniProtKB: P58811) protein.

MFTVFLLVVLATTGVSFTLDRASDGGNAVAKKSDVTARFNWRCCLIPACRRNHKKFCG

```
EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50
EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100
EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150
EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200
EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250
EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300
EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350
EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400
EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450
EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500
EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550
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```
EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650

EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001       701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750

EMBOSS_001       751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800

EMBOSS_001       801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850

EMBOSS_001         1 ----------------------MFTVFLLVVLATTGVSFTLDRASDGGNAV     29
                                           .:|..||....|:|.:|       |..|.
EMBOSS_001       851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF-------GAGAA    893

EMBOSS_001        30 AKKSDVTARFNWRCCLIPACRRNHKKFCG---------------------     58
                     .:               ||...:...:|.|
EMBOSS_001       894 LQ--------------IPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNS    929

EMBOSS_001       930 AIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLND    979

EMBOSS_001       980 ILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM   1029

EMBOSS_001      1030 SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAP   1079

EMBOSS_001      1080 AICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVV   1129

EMBOSS_001      1130 IGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNI   1179

EMBOSS_001      1180 QKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM   1229

EMBOSS_001      1230 VTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and **Kappa-conotoxin SIVA, *Conus striatus (Striated cone)*** (UniProtKB: P0C828 -CA4A_CONST) protein.

```
MGMRMMFTVFLLVVLATTVVSTPSDRASDGRNAAVHERQKSLVPSVITTCCGYDPGTMCPPCRCTNSCG

EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001       351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001       401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001       451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001       501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650
```

```
EMBOSS_001        651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001        701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750

EMBOSS_001        751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800

EMBOSS_001        801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850

EMBOSS_001        851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900

EMBOSS_001        901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950

EMBOSS_001        951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000

EMBOSS_001       1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050

EMBOSS_001       1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100

EMBOSS_001       1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE   1150

EMBOSS_001       1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL   1200

EMBOSS_001          1 ----------------MGMRMMFTVFLLVVLATTVVSTPSDRASDGRNA     33
                                       :|    |...|:.::..|::.
EMBOSS_001       1201 QELGKYEQYIKWPWYIWLG----FIAGLIAIVMVTIML------------   1234

EMBOSS_001         34 AVHERQKSLVPSVITTCCGYDPGTMCPPCRCTNSCG--------------     69
                                .:|:||....|  |  |.|.:.|.
EMBOSS_001       1235 -----------CCMTSCCSCLKG--C--CSCGSCCKFDEDDSEPVLKGVK   1269

EMBOSS_001       1270 LHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin Cl14.9, *Californiconus californicus (California cone) (Conus californicus)* (UniProtKB: D6C4J8 - CUE9_CONCL) protein.

MTAKATLLVLALVVMATSGVSSASVAGGPVVNSDTVSRSDPERLSTRGCVANCQANQTGIDCIKYCGIGIGRRDITQQ

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EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001        551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001        601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650
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EMBOSS_001         1 ----------------------------------MTAKATLLVLALVVM     15
                                                        ..|..:::...:.:.
EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001        16 ATSGV--SSASVAGGPVVNSDTVSRSDPERLSTRGCVANCQANQTGIDCI     63
                     |.:.|  |:.|:|   :..:.|:|      ::|.  :......:|.:||.
EMBOSS_001       701 AENSVAYSNNSIA---IPTNFTIS------VTTE--ILPVSMTKTSVDCT    739

EMBOSS_001        64 KY-CGIGIGRRDITQQ---------------------------------     78
                     .| ||      |.|:.
EMBOSS_001       740 MYICG------DSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFA    783

EMBOSS_001       784 QVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGF    833

EMBOSS_001       834 IKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTIT    883

EMBOSS_001       884 SGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGK    933

EMBOSS_001       934 IQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSR    983

EMBOSS_001       984 LDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECV   1033

EMBOSS_001      1034 LGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH   1083

EMBOSS_001      1084 DGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIV   1133

EMBOSS_001      1134 NNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEI   1183

EMBOSS_001      1184 DRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIM   1233

EMBOSS_001      1234 LCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Alpha-conotoxin SII, *Conus striatus (Striated cone)* (UniProtKB: P28879- CAS2_CONST) protein.

MGMRMMFTVFLLVVLATTVVSFPSDRASDGRDDEAKDERSDMHESDRNGRGCCCNPACGPNYGCGTSCSRTL

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EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001       351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001       401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001       451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001       501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650

EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001       701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750
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EMBOSS_001      751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF   800

EMBOSS_001      801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI   850

EMBOSS_001      851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM   900

EMBOSS_001      901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD   950

EMBOSS_001      951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR  1000

EMBOSS_001     1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM  1050

EMBOSS_001     1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT  1100

EMBOSS_001     1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE  1150

EMBOSS_001     1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1200

EMBOSS_001        1 ----------------MGMRMMFTVFLLVVLATTVVSFPSDRASDGRDD    33
                                   :|    |...|:.::..|::.
EMBOSS_001     1201 QELGKYEQYIKWPWYIWLG----FIAGLIAIVMVTIML----------  1234

EMBOSS_001       34 EAKDERSDMHESDRNGRGCCCNPACGPNYGCGTSCSRTL----------    72
                                   ||....|....|| .||....
EMBOSS_001     1235 ------------------CCMTSCCSCLKGC-CSCGSCCKFDEDDSEPVL  1265

EMBOSS_001     1266 KGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin 10, *Conus virgo (Virgin cone)* (UniProtKB: Q5K0C5 - O16A_CONVR) protein.

MKLTCVLIITVLFLTASQLITADYSRDQRQYRAVRLGDEMRNFKGARDCGGQGEGCYTQPCCPGLRCRGGGTGGGACQL

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EMBOSS_001        1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS    50

EMBOSS_001       51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI   100

EMBOSS_001      101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK   150

EMBOSS_001      151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY   200

EMBOSS_001      201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT   250

EMBOSS_001      251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK   300

EMBOSS_001      301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV   350

EMBOSS_001      351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF   400

EMBOSS_001      401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN   450

EMBOSS_001      451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT   500

EMBOSS_001      501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG   550

EMBOSS_001      551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP   600

EMBOSS_001      601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL   650

EMBOSS_001      651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG   700

EMBOSS_001      701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS   750
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EMBOSS_001        751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800

EMBOSS_001        801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850

EMBOSS_001        851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900

EMBOSS_001        901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950

EMBOSS_001        951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000

EMBOSS_001       1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050

EMBOSS_001       1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100

EMBOSS_001       1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE   1150

EMBOSS_001       1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL   1200

EMBOSS_001          1 ----------------------MKLTCVLIITVLFLTASQLITADYSRDQR     29
                                             ..|..::::|::.
EMBOSS_001       1201 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIML----------------   1234

EMBOSS_001         30 QYRAVRLGDEMRNFKGARDCGGQGEGCYTQPCCPGLRCRGGGTGGGACQL     79
                                          |....||..|  :|..:.|..|:.
EMBOSS_001       1235 --------------------------CCMTSCCSCL--KGCCSCGSCCKF   1256

EMBOSS_001       1257 DEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Alpha-conotoxin-like Pu1.5, Alpha-conotoxin-like Pu1.5 (UniProtKB: P0C8U9 - CA15_CONPL) protein.

MFTVFLLVILATTVVPFPSDRDPASNHENSKGSNRNAWLTPEECCAAPACREMILEFCLAGEAFAAALDGFRRLPYRLSSE

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EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001          1 ------------MFTVFLLVILATTVV----PFPSDRDP---ASNHENSK     31
                                  .....|:|..||.||    .|....||   ...|:|:|
EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001         32 GSNRNAWLTPE--ECCAAPACREMILEFCLAGEAFAAALDG----FRRLP     75
                        :|:..|  ...:|..|     .|....:.|...|:|    |:.|.
EMBOSS_001        151 -----SWMESEFRVYSSANNC-----TFEYVSQPFLMDLEGKQGNFKNLR    190

EMBOSS_001         76 ----------YRLSSE-----------------------------------     81
                              :::.|:
EMBOSS_001        191 EFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT    240

EMBOSS_001        241 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVD    290

EMBOSS_001        291 CALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGE    340

EMBOSS_001        341 VFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL    390

EMBOSS_001        391 CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNN    440

EMBOSS_001        441 LDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF    490

EMBOSS_001        491 PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN    540

EMBOSS_001        541 FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPC    590

EMBOSS_001        591 SFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS    640

EMBOSS_001        641 NVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQ    690
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EMBOSS_001       691 SIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTM    740

EMBOSS_001       741 YICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYK    790

EMBOSS_001       791 TPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC    840

EMBOSS_001       841 LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGA    890

EMBOSS_001       891 GAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSS    940

EMBOSS_001       941 TASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAE    990

EMBOSS_001       991 VQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV   1040

EMBOSS_001      1041 DFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFP   1090

EMBOSS_001      1091 REGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP   1140

EMBOSS_001      1141 LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVA   1190

EMBOSS_001      1191 KNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSC   1240

EMBOSS_001      1241 CSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT   1273
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Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Chi-conotoxin CMrX, *Conus marmoreus (Marble cone)* (UniProtKB: P58809 - CTAX_CONMR) protein

MRCLPVLIILLLLTASAPGVDVLPKTEDDVPLSSVYGNGKSILRGILRKGICCGVSFCYPC

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EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001          1 ------------------------MRCLPVLIILLLLTASAPGVDVL-P     24
                                              :|....|.||.:...|..||.|: |
EMBOSS_001        551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001         25 KTE--------------DDVPLS----------SVYGNGKSILRGILRKG     50
                      .|.               .:||::          .||..|.::.:  .|.|
EMBOSS_001        601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQ--TRAG    648

EMBOSS_001         51 ICCG---VSFCYPC-----------------------------------      61
                      ...|   |:..|.|
EMBOSS_001        649 CLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMS    698
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EMBOSS_001        699 LGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTE    748

EMBOSS_001        749 CSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG    798

EMBOSS_001        799 GFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARD    848

EMBOSS_001        849 LICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF    898

EMBOSS_001        899 AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKL    948

EMBOSS_001        949 QDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLIT    998

EMBOSS_001        999 GRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYH   1048

EMBOSS_001       1049 LMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSN   1098

EMBOSS_001       1099 GTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSF   1148

EMBOSS_001       1149 KEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLI   1198

EMBOSS_001       1199 DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCC   1248

EMBOSS_001       1249 SCGSCCKFDEDDSEPVLKGVKLHYT    1273
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Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin Cl9.6, *Californiconus californicus (California cone) (Conus californicus)* (UniProtKB: D6C4M3 - CU96_CONCL) protein.

MSTLGMTLLILLLLLPLATPDDVGQPPKRDTLRNLLKIGTRGQGGCVPPGGGRCKANQACTKGGNPGTCGFQYDLCLCLRN

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EMBOSS_001          1 MSTLGMTLLILLLLLPLATPDDVGQPPKRDTLRNLLKIGTRGQGGCVPPG      50
                        :.:.|:||||.:...|              .:.||.|   :||
EMBOSS_001          1 -------MFVFLVLLPLVSSQCV-------------NLTTRTQ---LPP-      26

EMBOSS_001         51 GGRCKANQACTKGGNPGTCGFQY--------------DLCLCLRN-----      81
                        |.|   |..|.|..|               ||.|...:
EMBOSS_001         27 --------AYT---NSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWF      65

EMBOSS_001         66 HAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQ    115

EMBOSS_001        116 SLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANN    165

EMBOSS_001        166 CTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRD    215

EMBOSS_001        216 LPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAY    265

EMBOSS_001        266 YVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQT    315

EMBOSS_001        316 SNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADY    365

EMBOSS_001        366 SVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQT    415

EMBOSS_001        416 GKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFE    465

EMBOSS_001        466 RDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSF    515

EMBOSS_001        516 ELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQF    565

EMBOSS_001        566 GRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDV    615

EMBOSS_001        616 NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIP    665

EMBOSS_001        666 IGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIP    715

EMBOSS_001        716 TNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNR    765
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EMBOSS_001       766 ALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKR    815

EMBOSS_001       816 SFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLL    865

EMBOSS_001       866 TDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNV    915

EMBOSS_001       916 LYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQ    965

EMBOSS_001       966 LSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRA   1015

EMBOSS_001      1016 AEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHV   1065

EMBOSS_001      1066 TYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQII   1115

EMBOSS_001      1116 TTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVD   1165

EMBOSS_001      1166 LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWY   1215

EMBOSS_001      1216 IWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVL   1265

EMBOSS_001      1266 KGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin Vi15a, *Conus virgo (Virgin cone),* (UniProtKB: *B3FIA5 - CVFA_CONVR*) protein.

MMPVILLLLLSLAIRCADGKAVQGDSDPSASLLTGDKNHDLPVKRDCTTCAGEECCGRCTCPWGDNCSCIEWGK

```
EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001       351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001       401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001       451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001       501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650

EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001       701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750

EMBOSS_001       751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800

EMBOSS_001       801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850

EMBOSS_001       851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900

EMBOSS_001       901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950
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EMBOSS_001        951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000

EMBOSS_001       1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050

EMBOSS_001       1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100

EMBOSS_001       1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE   1150

EMBOSS_001       1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL   1200

EMBOSS_001          1 ----------------------------MMPVILLLLLS----LAIRCAD     18
                                                   |:.::|..:.|    |...|:.
EMBOSS_001       1201 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSC   1250

EMBOSS_001         19 GKAVQGDSDPSASLLTGDKNHDLPVKRDCTTCAGEECCGRCTCPWGDNCS     68
                      |...:.|.|.|...:|.|.|.|..
EMBOSS_001       1251 GSCCKFDEDDSEPVLKGVKLHYT----------------------------   1273

EMBOSS_001         69 CIEWGK     74

EMBOSS_001       1274 ------   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin Cal5a L3, *Californiconus californicus (California cone) (Conus californicus)* (UniProtKB: *D2Y169 - CU51C_CONCL*) protein.

```
MRFYIGLMAALMLTSVLRTDSASVGQTGTKSELAVIERVIRQRDAADVKPVARQNEGPGRDPAPCCQHPIETCCRR

EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001        551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001        601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650

EMBOSS_001        651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001        701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750

EMBOSS_001        751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800

EMBOSS_001        801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850

EMBOSS_001        851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900

EMBOSS_001        901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950
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EMBOSS_001      951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR  1000

EMBOSS_001     1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM  1050

EMBOSS_001     1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT  1100

EMBOSS_001     1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE  1150

EMBOSS_001     1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL  1200

EMBOSS_001        1 -----------------MRFYIGLMAALMLTSVLRTDSASVGQTGTKSEL    33
                                    :.|..||:|.:|:|.:|
EMBOSS_001     1201 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIML----------------  1234

EMBOSS_001       34 AVIERVIRQRDAADVKPVARQNEGPGRDPAPCCQHPIETCCRR-------    76
                                                ||   :.:||..
EMBOSS_001     1235 ------------------------------CC---MTSCCSCLKGCCSC  1250

EMBOSS_001     1251 GSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and **Alpha**-conotoxin CIB, *Conus catus (Cat cone)* (UniProtKB: *P0DPT2* - *CA1B_CONCT*) protein.

SDGRNEAANDEASDVIELALKGCCSNPVCHLEHPNACGRRR

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EMBOSS_001        1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS    50

EMBOSS_001       51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI   100

EMBOSS_001      101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK   150

EMBOSS_001      151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY   200

EMBOSS_001      201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT   250

EMBOSS_001      251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK   300

EMBOSS_001      301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV   350

EMBOSS_001      351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF   400

EMBOSS_001      401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN   450

EMBOSS_001      451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT   500

EMBOSS_001      501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG   550

EMBOSS_001      551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP   600

EMBOSS_001      601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL   650

EMBOSS_001      651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG   700

EMBOSS_001      701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS   750

EMBOSS_001      751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF   800

EMBOSS_001      801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI   850

EMBOSS_001      851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM   900

EMBOSS_001      901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD   950
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EMBOSS_001        951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000

EMBOSS_001       1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050

EMBOSS_001       1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100

EMBOSS_001       1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE   1150

EMBOSS_001          1 --------------------------------SDGRNEAA---NDEASDV     15
                                                      .|..||.|   |:...|:
EMBOSS_001       1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL   1200

EMBOSS_001         16 IEL---------------------------------------ALKGCCSN     26
                      .||                                      .||||||
EMBOSS_001       1201 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCS-   1249

EMBOSS_001         27 PVCHLEHPNACGRRR------------------     41
                            ||...
EMBOSS_001       1250 ----------CGSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and **Kunitz-type serine protease inhibitor conotoxin Cal9.1a, *Californiconus californicus (California cone) (Conus californicus)*** (UniProtKB: *D2Y488 - VKT1A_CONCL*) protein.

MTFLLLLVSVCMMATGEERTKRDVCELPFEEGPCFAAIRVYAYNAETGDCEQLTYGGCEGNGNRFATLEDCDNACARY

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EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001          1 ------------------------------MTFLLLLVSVC-----MMATG     16
                                                     .|..:|.::.|     .:.|.
EMBOSS_001        551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001         17 EERTKRDVCELPFEEGPCF---AAI---------RVYAYNAETGDCEQLT     54
                      ...|...|..| ::::..|.    .||         |||:    ||.....|
EMBOSS_001        601 GTNTSNQVAVL-YQDVNCTEVPVAIHADQLTPTWRVYS----TGSNVFQT    645

EMBOSS_001         55 YGGCEGNGNRFATLEDCD-----NACARY--------------------     78
                      ..||...........:||     ..||.|
EMBOSS_001        646 RAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAY    695

EMBOSS_001        696 TMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGD    745

EMBOSS_001        746 STECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIK    795

EMBOSS_001        796 DFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIA    845
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EMBOSS_001       846 ARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQ    895

EMBOSS_001       896 IPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASAL    945

EMBOSS_001       946 GKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDR    995

EMBOSS_001       996 LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGK   1045

EMBOSS_001      1046 GYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVF   1095

EMBOSS_001      1096 VSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPEL   1145

EMBOSS_001      1146 DSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNE   1195

EMBOSS_001      1196 SLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLK   1245

EMBOSS_001      1246 GCCSCGSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Alpha-conotoxin-like Qc1.2, *Conus quercinus (Oak cone)* (UniProtKB: *Q6PTD6 - CA12_CONQU*) protein.

MGMRMMFTVFLLVALATTVASFTLDRASNGRNAAADDKPSDWIALAIKQCCANPPCKHVNCR

```
EMBOSS_001         1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50

EMBOSS_001        51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100

EMBOSS_001       101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150

EMBOSS_001       151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200

EMBOSS_001       201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250

EMBOSS_001       251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300

EMBOSS_001       301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350

EMBOSS_001       351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400

EMBOSS_001       401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450

EMBOSS_001       451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500

EMBOSS_001       501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550

EMBOSS_001       551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600

EMBOSS_001       601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650

EMBOSS_001       651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700

EMBOSS_001         1 ----------MGMRMMFTV-----FLLVALATTVASFT----------     23
                           :.:...||:     .|.|::..|....|
EMBOSS_001       701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750

EMBOSS_001        24 ------------LDRASNGRNAAADDKPSDWIALAIKQCCANPPCKHVNC     61
                            |:||..| .|...||.:.:.:...:||....||.|....
EMBOSS_001       751 NLLLQYGSFCTQLNRALTG-IAVEQDKNTQEVFAQVKQIYKTPPIKDFGG    799

EMBOSS_001        62 R-------------------------------------------------     62
                     .
EMBOSS_001       800 FNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDL    849
```



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EMBOSS_001        850 ICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFA    899

EMBOSS_001        900 MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQ    949

EMBOSS_001        950 DVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITG    999

EMBOSS_001       1000 RLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHL   1049

EMBOSS_001       1050 MSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNG   1099

EMBOSS_001       1100 THWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFK   1149

EMBOSS_001       1150 EELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLID   1199

EMBOSS_001       1200 LQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCS   1249

EMBOSS_001       1250 CGSCCKFDEDDSEPVLKGVKLHYT   1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin VnMLCL-031, *Conus ventricosus (Mediterranean cone)* (UniProtKB: *Q9BP53 - CT0C5_CONVE*) protein.

MLCLPXFIILLLLASPAAPNPLQTRXQSNLIRAGPEDANIKTXKRVIISGLXXSILVPLIDAIIG

```
EMBOSS_001          1 MLCLPXFIILLLLASPAAPNPLQTRXQ-----SNLIRAG-----------      34
                        :. |::||.|.|....| |.|| |     :|....|
EMBOSS_001          1 ---MFVFLVLLPLVSSQCVN-LTTRTQLPPAYTNSFTRGVYYPDKVFRSS      46

EMBOSS_001         35 ----------PEDANIKTXKRVIISGL-----XXSILVPLIDAIIG----      65
                              |..:|:.. ..:.:||.       :.::|..|.:..
EMBOSS_001         47 VLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTE      96

EMBOSS_001         97 KSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYH     146

EMBOSS_001        147 KNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKN     196

EMBOSS_001        197 IDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHR     246

EMBOSS_001        247 SYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPL     296

EMBOSS_001        297 SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATR     346

EMBOSS_001        347 FASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVY     396

EMBOSS_001        397 ADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVG     446

EMBOSS_001        447 GNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYG     496

EMBOSS_001        497 FQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGL     546

EMBOSS_001        547 TGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS     596

EMBOSS_001        597 VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTR     646

EMBOSS_001        647 AGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYT     696

EMBOSS_001        697 MSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDS     746

EMBOSS_001        747 TECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKD     796

EMBOSS_001        797 FGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA     846

EMBOSS_001        847 RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI     896

EMBOSS_001        897 PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALG     946
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EMBOSS_001        947 KLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRL    996
EMBOSS_001        997 ITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKG   1046
EMBOSS_001       1047 YHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFV   1096
EMBOSS_001       1097 SNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELD   1146
EMBOSS_001       1147 SFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES   1196
EMBOSS_001       1197 LIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKG   1246
EMBOSS_001       1247 CCSCGSCCKFDEDDSEPVLKGVKLHYT    1273
```

Protein Sequence Alignment between SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Conotoxin Bu22, *Conus bullatus (Bubble cone)* (UniProtKB: *P0CY77 - CA122_CONBU*) protein.

SDRASDGRNAAANDRASDLVALTVRGCCTYPPCAVLSPLCD

```
EMBOSS_001          1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS     50
EMBOSS_001         51 TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNI    100
EMBOSS_001        101 IRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNK    150
EMBOSS_001        151 SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGY    200
EMBOSS_001        201 FKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT    250
EMBOSS_001        251 PGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK    300
EMBOSS_001        301 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASV    350
EMBOSS_001        351 YAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSF    400
EMBOSS_001        401 VIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYN    450
EMBOSS_001        451 YLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPT    500
EMBOSS_001        501 NGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTG    550
EMBOSS_001        551 VLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP    600
EMBOSS_001        601 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL    650
EMBOSS_001        651 IGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLG    700
EMBOSS_001        701 AENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECS    750
EMBOSS_001        751 NLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGF    800
EMBOSS_001        801 NFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI    850
EMBOSS_001        851 CAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM    900
EMBOSS_001        901 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQD    950
EMBOSS_001        951 VVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGR   1000
EMBOSS_001       1001 LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLM   1050
EMBOSS_001       1051 SFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGT   1100
```

```
EMBOSS_001     1101 HWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKE  1150

EMBOSS_001        1 ---------------------------------SDRASD-GRNAAANDRAS    17
                                                      .||.:: .:|  .|:...
EMBOSS_001     1151 ELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKN--LNESLI  1198

EMBOSS_001       18 DL----------------------------VALTVRGCCTYPPCAVLSPLC    40
                    ||                             |.:|:..||....|:.|...|
EMBOSS_001     1199 DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCC  1248

EMBOSS_001       41 D-------------------------     41
                    .
EMBOSS_001     1249 SCGSCCKFDEDDSEPVLKGVKLHYT   1273
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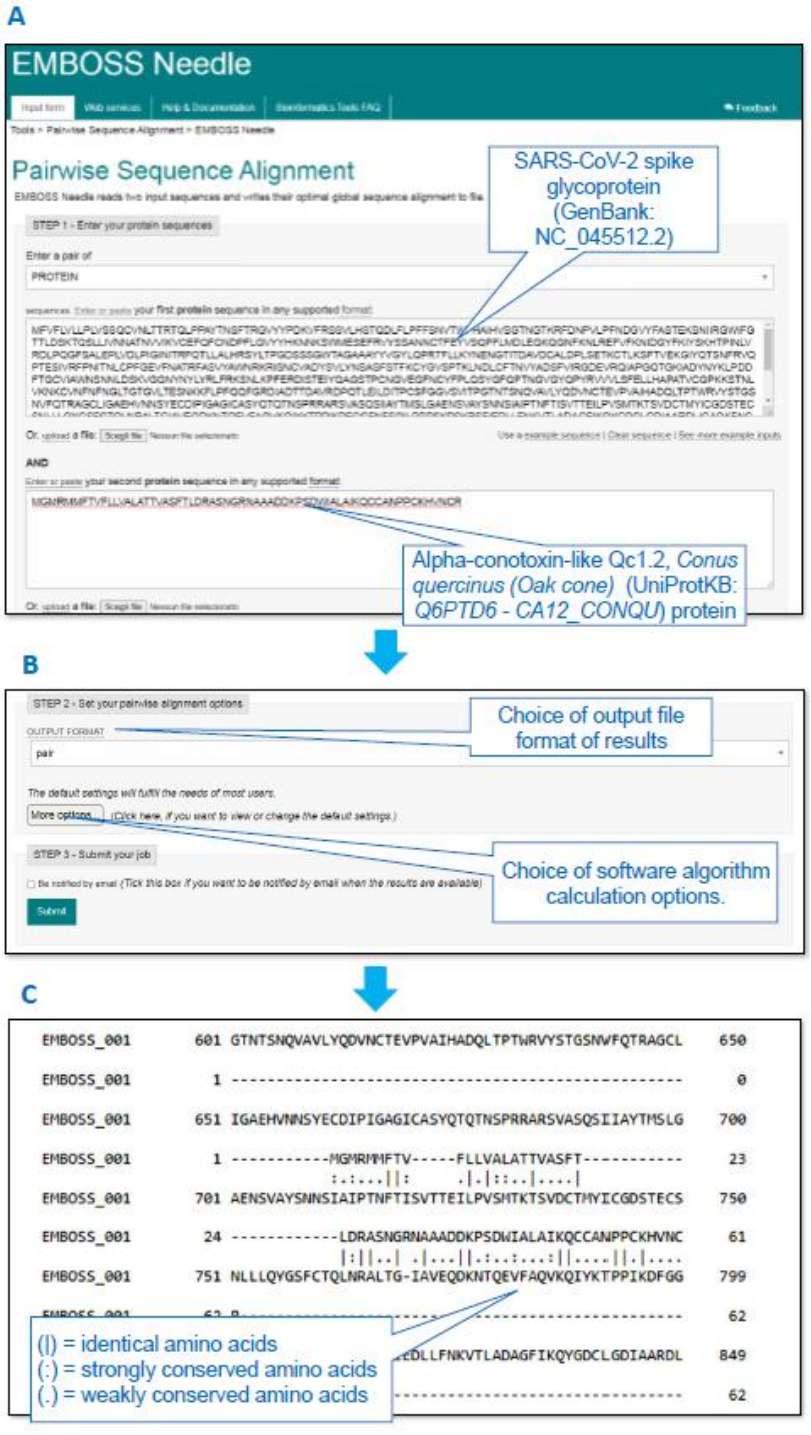

Supplementary Figure 1. Simplified schematic representation of the use of the EMBOSS Needle web tool (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) for protein sequence alignment and comparison. A: Step 1 - Input Sequences. In this step, the software requests the input of the two protein sequence sequences to be compared represented by a free (raw) text list of a block of characters retrieved from the Protein Sequence Databases. In this case, the sequence of SARS-CoV-2 spike glycoprotein (GenBank: NC_045512.2) and Alpha-conotoxin-like Qc1.2, Conus quercinus (Oak cone) (UniProtKB: Q6PTD6 - CA12_CONQU) protein are compared. B: Step 2 - Set alignment and output file options and submit. In this step it is possible to set some calculation options to the algorithm according to the needs of the bioinformatics analysis to be performed. It is also possible to choose a specific output format of the results file according to the needs of the study. C: Output file with the results of the comparison of the analyzed protein sequences. Identical amino acids are connected with a "|" symbol, strongly similar amino acids are connected with a ":" symbol and weakly similar ones are indicated with ".".

