



BRIEF REPORT

REVISED Use of the informational spectrum methodology for rapid biological analysis of the novel coronavirus 2019-nCoV: prediction of potential receptor, natural reservoir, tropism and therapeutic/vaccine target [version 3; peer review: 2 approved]

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Abstract

A novel coronavirus recently identified in Wuhan, China (SARS-CoV-2) has expanded the number of highly pathogenic coronaviruses affecting humans. The SARS-CoV-2 represents a potential epidemic or pandemic threat, which requires a quick response for preparedness against this infection. The present report uses the informational spectrum methodology to identify the possible origin and natural host of the new virus, as well as putative therapeutic and vaccine targets. The performed *in silico* analysis indicates that the newly emerging SARS-CoV-2 is closely related to severe acute respiratory syndrome (SARS)-CoV and, to a lesser degree, Middle East respiratory syndrome (MERS)-CoV. Moreover, the well-known SARS-CoV receptor (ACE2) might be a putative receptor for the novel virus as well. Actin protein was also suggested as a host factor that participates in cell entry and pathogenesis of SARS-CoV-2; therefore, drugs modulating biological activity of this protein (e.g. ibuprofen) were suggested as potential candidates for treatment of this viral infection. Additional results indicated that civets and poultry are potential candidates for the natural reservoir of the SARS-CoV-2, and that domain 288-330 of S1 protein from the SARS-CoV-2 represents promising therapeutic and/or vaccine target.

Keywords

2019-nCoV, Wuhan coronavirus, SARS, MERS

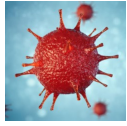
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REVISED Amendments from Version 2

The manuscript is slightly modified in accordance with the suggestions of the second Reviewer. This includes brief comments of predictions in the article which concern the SARS-CoV-2 origin and its natural reservoirs.

Any further responses from the reviewers can be found at the end of the article

Introduction

Fears are mounting worldwide over the cross-border spread of the new coronavirus (denoted as SARS-CoV-2) that originated in Wuhan, the largest city in central China, after its spread to many countries around the world. The newly emerging pathogen belongs to the same virus family as the deadly severe acute respiratory syndrome and Middle East respiratory syndrome coronaviruses (SARS-CoV and MERS-CoV, respectively). The World Health Organization (WHO) has **recently published surveillance recommendations** for a possible “large epidemic or even pandemic” (pandemic declared on March 11th, 2020) of the novel coronavirus and it has issued guidelines for hospitals across the world. However, many questions about SARS-CoV-2 remain unanswered: (i) what is the origin and/or natural reservoir of the virus? (ii) is it easily transmitted from human to human? and (iii) what are the potential diagnostic, therapeutic and vaccine targets? Currently, only nucleotide sequences of eight human SARS-CoV-2 isolates are available without any additional information about biological properties of the virus, beyond the morphological confirmation of the virion using electronic microscopy. This is likely not enough information to answer the important abovementioned questions.

The informational spectrum method (ISM), a virtual spectroscopy method for analysis of proteins, is based on the fundamental electronic properties of amino acids and requires only nucleotide sequence availability to investigate proteins¹. For this reason, ISM was previously used for analysis of novel viruses for which little or no information were available²⁻⁵. Here, the SARS-CoV-2 was analyzed with ISM to identify its possible origin and natural host, as well as putative therapeutic and vaccine targets.

Methods

Sequences

The S1 surface protein sequences from the first 8 human SARS-CoV-2, deposited in the publicly available **GISAID database** (assessed on January 19, 2020), were analyzed by ISM. The studied sequences were BetaCoV/Wuhan/IVDC-HB-04/2020, BetaCoV/Wuhan/IVDC-HB-01/2019, BetaCoV/Wuhan/IVDC-HB-05/2019, BetaCoV/Wuhan/IPBCAMS-WH-01/2019, BetaCoV/Wuhan/WIV04/2019, BetaCoV/Wuhan-Hu-1/2019, BetaCoV/Nonthaburi/61/2020, and BetaCoV/Nonthaburi/74/2020.

In the phylogenetic analysis, different amino acid sequences of other coronaviruses were also included: (i) S1 proteins from the following viruses: AVP78042, AVPvp78031, AY304486, AY559093, JX163927, YN2018B, KY417146, used already by other authors in the study of the phylogenetic relationship

between SARS-CoV-2 and nearest bat and SARS-like CoVs (**GISAID database**); and (ii) S1 proteins from three first isolated human MERS-CoV: AGG22542, AFS88936, AFY13307, deposited in the **GISAID database**

The ISM

Detailed description of the sequence analysis based on ISM has been published elsewhere². According to this approach, sequences (protein or DNA) are transformed into signals by assignment of numerical values of each element (amino acid or nucleotide). These values correspond to electron-ion interaction potential⁶, determining electronic properties of amino acid/nucleotides, which are essential for their intermolecular interactions. The signal obtained is then decomposed in a periodical function by the Fourier transformation. The result is a series of frequencies and their amplitudes. The obtained frequencies correspond to the distribution of structural motifs (primary structure) with defined physico-chemical characteristics responsible for the biological function of the putative protein corresponding to the analyzed sequence. When comparing proteins that share same biological or biochemical function, the technique allows detection of code/frequency pairs that are specific for their common biological properties. The method is insensitive to the location of the motifs and, therefore, does not require previous alignment of the sequences. In addition, this is the only method that allows immediate functional analysis.

Phylogenetic analysis

The phylogenetic tree of S1 proteins from coronaviruses was generated with the ISM-based phylogenetic algorithm **ISTREE**, previously described in detail elsewhere⁷. In the presented analysis, we calculated the distance matrix with the amplitude on the frequency $F(0.257)$ as the distance measure between sequences.

Results and discussion

In order to compare informational similarity between SARS-CoV-2, SARS-CoV, MERS-CoV and Bat SARS-like CoV, the cross-spectra (CS) of S1 proteins from these viruses were calculated. **Figure 1a** shows the CS of SARS-CoV-2, SARS-CoV and MERS-CoV. These CS contain only one dominant peak corresponding to the frequency $F(0.257)$. **Figure 1b** displays the CS of S1 proteins from SARS-CoV-2 and Bat SARS-like CoV. Amplitudes in these latter CS are significantly lower than in those CS presented in **Figure 1a**. These results show that (i) S1 proteins from SARS-CoV-2, SARS-CoV, MERS-CoV and Bat SARS-like CoV encode common information, which is represented with the frequency $F(0.257)$, and (ii) S1 proteins from SARS-CoV-2 are remarkable more informationally similar with S1 from SARS-CoV and MERS-CoV than with S1 from Bat SARS-like CoV. This suggests that biological properties of SARS-CoV-2 are apparently more similar to SARS-CoV and MERS-CoV than to Bat SARS-like CoV.

To confirm this conclusion, the ISM-base phylogenetic tree for S1 proteins was calculated (**Figure 2**). In this calculation the amplitude on the frequency $F(0.257)$ was used as the distance measure. As observed in **Figure 2**, all analyzed SARS-CoV-2 S1 amino acid sequences are grouped with SARS-CoV and MERS-CoV and separated from Bat SARS-like CoV. This indicates that SARS-CoV-2 are more phylogenetically

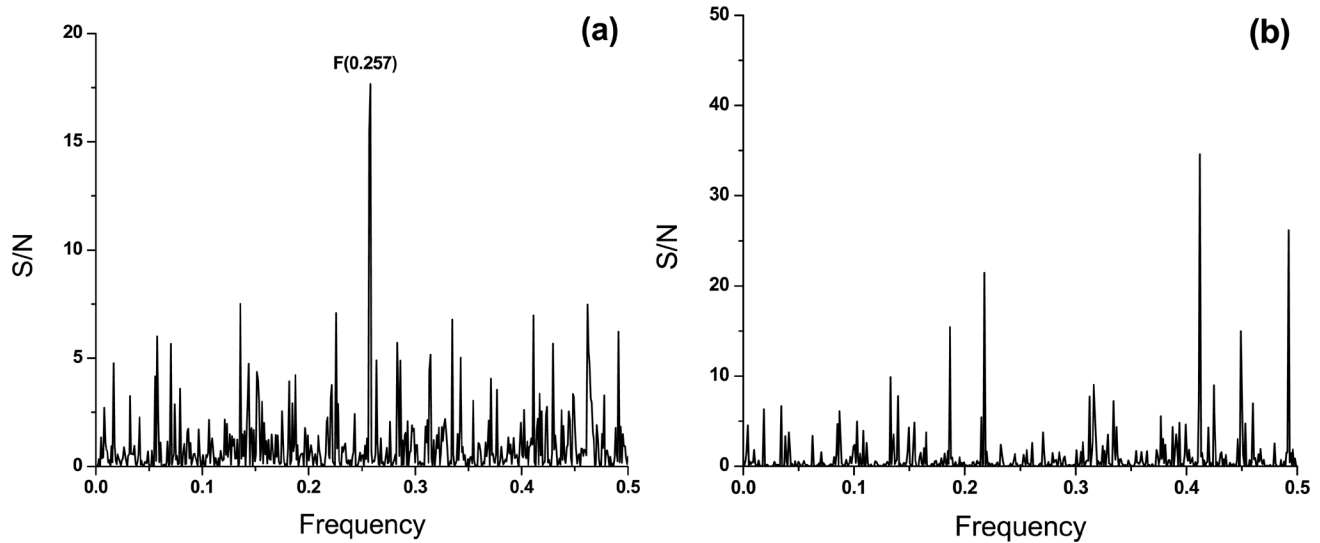


Figure 1. Cross-spectrum (CS) of S1 proteins. (a) CS of S1 from SARS-CoV, MERS-CoV and SARS-CoV-2; (b) CS of Bat SARS-like CoV and SARS-CoV-2. The abscissa represents the frequencies from the Fourier transform of the sequence of electron-ion interaction potential corresponding to the amino-acid sequence of proteins. The lowest frequency is 0.0 and the highest is 0.5. The ordinate represents the signal-to-noise ratio (the ratio between signal intensity at one particular IS frequency and the main value of the whole spectrum, S/N).

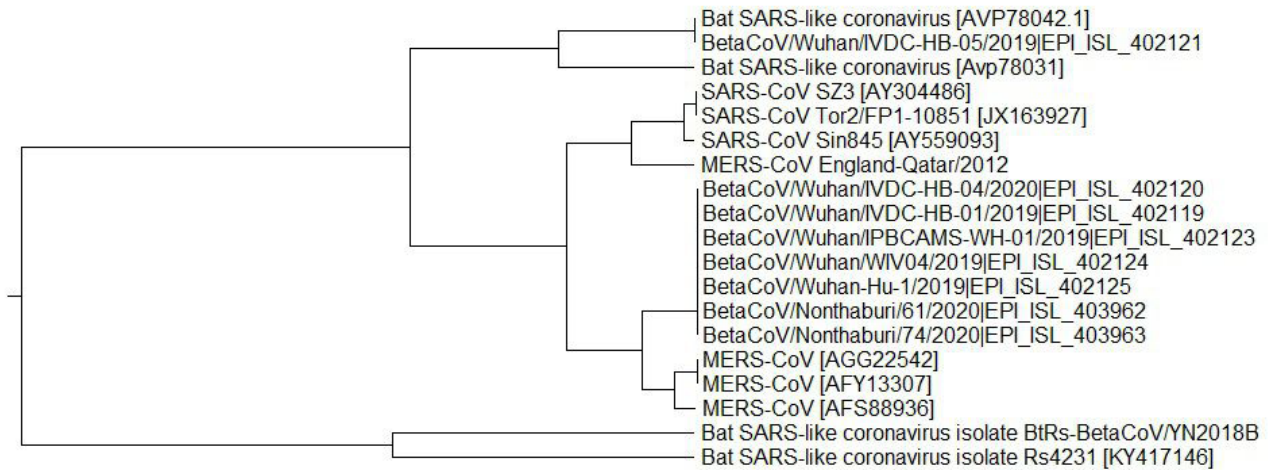


Figure 2. Informational spectrum method-based phylogenetic tree for S1 proteins from SARS-CoV, MERS-CoV, Bat SARS-like CoV and SARS-CoV-2. The frequency $F(0.257)$ as the distance measure was used.

similar to SARS-CoV and MERS-CoV than to Bat SARS-like CoV. This result differs from those obtained with the homology-based phylogenetic analysis, which showed that SARS-CoV-2 are closely related to Bat SARS-like CoV (<https://platform.gisaid.org/epi3/frontend#lightbox1296857287>).

It has been previously shown that the dominant frequency in the informational spectrum of viral envelope proteins corresponds to interaction between the virus and its receptor^{2,3,7,8}. The ISM analysis showed that the frequency component $F(0.257)$ is present in the CS of S1 SARS-CoV and its receptor angiotensin converting enzyme 2 (ACE2)⁹, but not in the CS of S1

MERS-CoV and its main receptor dipeptidyl peptidase 4 (DPP4)¹⁰. Of note is that both receptors ACE2 and DPP4 are expressed in airway epithelia. Presence of $F(0.257)$ in the informational spectrum of MERS-CoV (Figure 1) suggests also possible interaction between this virus and the ACE2. The dominant peak on the frequency $F(0.257)$ in the CS of S1 from SARS-CoV and MERS-CoV and ACE2 supports this possibility (Figure 3), although this has not been formally proved for MERS-CoV¹¹.

As it is shown in Figure 1a, the frequency $F(0.257)$ is also present in the informational spectrum of the SARS-CoV-2,

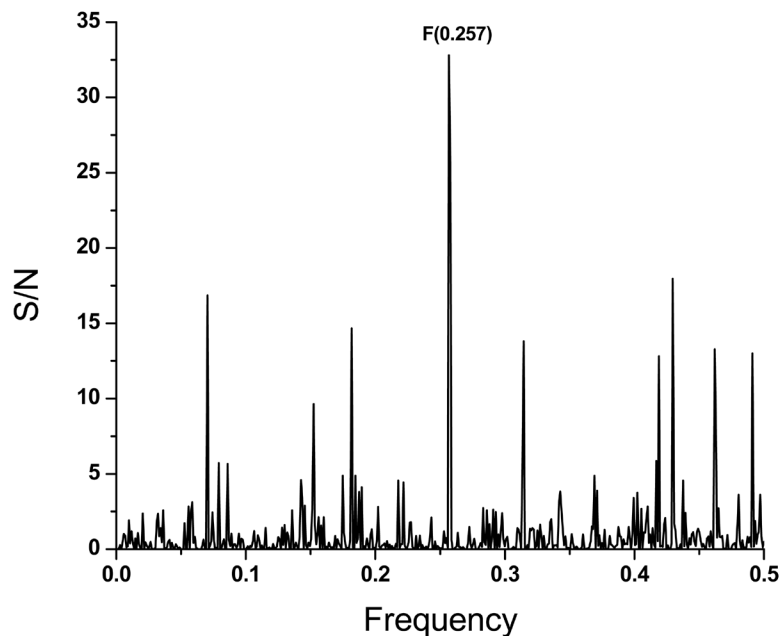


Figure 3. Cross-spectrum of ACE2 and S1 proteins from SARS-CoV and MERS-CoV. The abscissa and the ordinate are as described in Figure 1.

suggesting that ACE2 might be the receptor for this novel coronavirus too. This prediction was subsequently confirmed by functional studies *in vitro*¹². Calculation of the CS for S1 protein from the SARS-CoV-2 and all ACE2 sequences available at the UniProt database revealed that the highest amplitudes on the frequency F(0.257) correspond to ACE2 from civet and chicken. This result indicates that these species can be included as potential candidates for the natural reservoir of the SARS-CoV-2. However, it is possible that SARS-CoV-2 viruses use very different receptors in the natural host(s) and not only the ACE2 as it is the putative case in humans. An experimental study performed on chicken, however, indicated lack of susceptibility of this species to the novel virus¹³; civets so far have not been tested, but the indicated study confirmed susceptibility of domestic cat to SARS-CoV-2.

Finally, the S1 amino acid sequence from the SARS-CoV-2 was scanned to look for the domain that gives the highest contribution to the information represented by the frequency F(0.257) (Figure 4a). This analysis revealed domain 266–330 (numbering concerns the matured protein) is essential for interaction of SARS-CoV-2 with ACE2. Of note is the striking homology between these domains of S1 proteins from SARS-CoV-2 and SARS-CoV, but not from MERS-CoV for which ACE2 is not the main receptor (Figure 4b).

Further, S1 spike proteins from SARS-CoV (Table 1) and SARS-CoV-2 (Table 2) were compared. The CS of S1 proteins from SARS-CoV (Figure 5a) and SARS-CoV-2 (Figure 5b) were assessed. Principal information encoded in S1 proteins from SARS-CoV and SARS-CoV-2 is represented with two different frequencies F(0.222) and F(0.478), respectively. This

result indicates some potential difference(s) in the virus-host interaction of these two viruses although they apparently use the same receptor ACE2.

To identify the host proteins involved in the attachment and/or internalization of the SARS-CoV-2, the UniProt database (<https://www.uniprot.org>) was screened by ISM for human proteins with the dominant peak on the frequency F(0.478). The list of human proteins that have a dominant peak in IS at the frequency F(0.478) are given in Table 3. According to the IS criterion, these proteins are potential candidate interactors with the SARS-CoV-2 S1 protein. Further, literature data mining was performed to identify which proteins presented in Table 3 might be involved in the processes of infection with human coronaviruses. This analysis revealed that the actin protein plays an important role in the early entry events during human coronavirus infections¹⁴. Actin proteins were selected as the best candidate interactors for the SARS-CoV-2 among the host proteins that are characterized with frequency F(0.478). Figure 5c shows that CS of actins from different mammalian species (Table 4) contains the dominant peak on F(0.478), suggesting that actin probably encodes the conserved information important for their biological function.

The data mining of the PubMed database (www.ncbi.nlm.nih.gov/pubmed/) also showed that actin protein plays an important role in the rapid virus cell-to-cell spread and dissemination of infection¹⁵. Additionally, the actin filament reorganization is a key step in lung inflammation induced by systemic inflammatory responses caused by infectious agents¹⁶. These findings indicate that interaction between actin proteins and the S1 could be involved in the infection and pathogenesis of SARS-CoV-2.

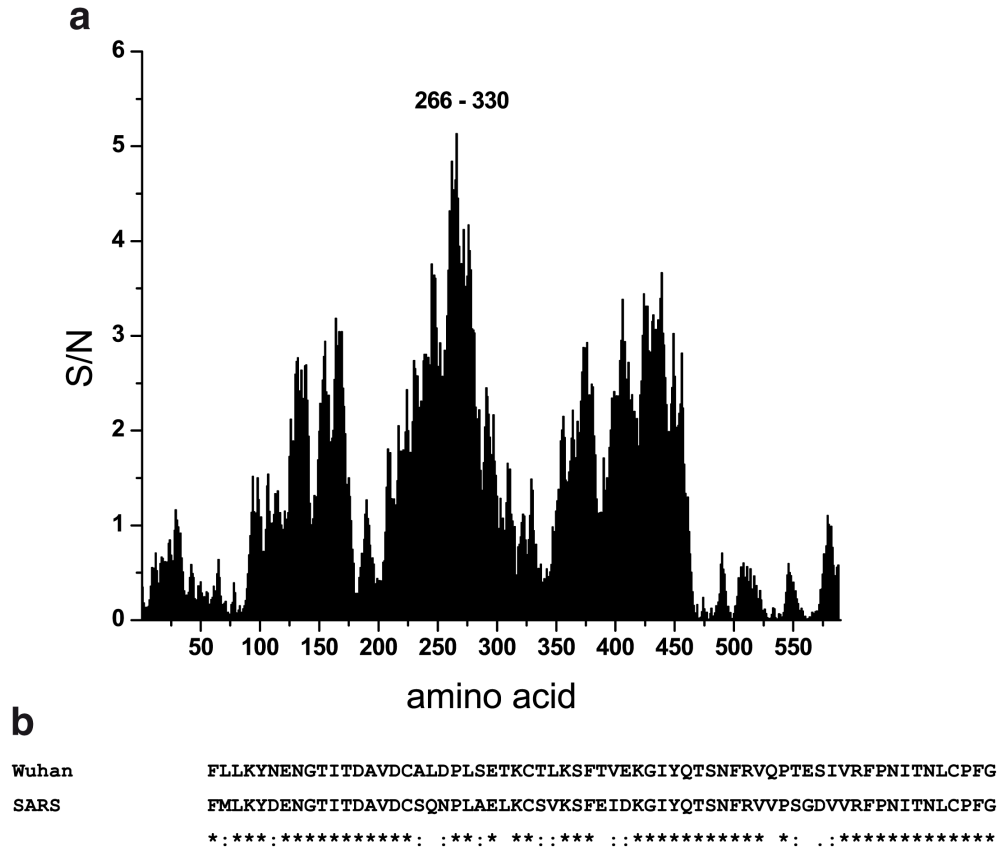


Figure 4. Domain of S1 protein which is important for SARS-CoV-2/ACE2 interaction. (a) Mapping of the domain of S1 protein from SARS-CoV-2 (BetaCoV/Wuhan/IVDC-HB-01/2019) which gives the dominant contribution to the information represented with the frequency F(0.257). **(b)** Sequence homology between domains of S1 proteins from SARS-CoV and SARS-CoV-2 with essential contribution to the information corresponding to the frequency F(0.257).

Table 1. S1 proteins from SARS_CoV (uniprot.org).

SPIKE_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q19QX0_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
A7J8L4_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
J9SFL2_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
J9TDZ0_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202F4_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202E5_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202E9_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202F5_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202E6_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202H5_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202F2_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202F9_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202G8_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202G3_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202H8_CVHSA	Spike glycoprotein OS=Human SARS coronavirus
Q202G5_CVHSA	Spike glycoprotein OS=Human SARS coronavirus

Table 2. S1 proteins from SARS-CoV-2 (GISAID).

BetaCoV/Wuhan/IVDC-HB-04/2020
BetaCoV/Wuhan/IVDC-HB-01/2019
BetaCoV/Wuhan/IVDC-HB-05/2019
BetaCoV/Wuhan/IPBCAMS-WH-01/2019
BetaCoV/Wuhan/WIV04/2019
BetaCoV/Wuhan-Hu-1/2019
BetaCoV/Nonthaburi/61/2020
BetaCoV/Nonthaburi/74/2020
BetaCoV/Wuhan/WIV07/2019
BetaCoV/Wuhan/WIV06/2019
BetaCoV/Wuhan/WIV05/2019
BetaCoV/Wuhan/WIV02/2019
BetaCoV/Wuhan/HBCDC-HB-01/2019
BetaCoV/Zhejiang/WZ-01/2020

In consequence, the possibility to interfere on this interaction might represent a valid hypothesis for development of promising prevention and therapeutic strategies.

Interestingly, further data mining revealed that ibuprofen (FDA approved drug with excellent safety record) attenuates interleukin- 1β -induced inflammation as well as actin reorganization¹⁷. Actin was also found to be the primary component by which ibuprofen can bind to the tissue in different organs¹⁸. This suggests that ibuprofen might impact the SARS-CoV-2-induced disease by indirect interaction with actin proteins. Previously, ibuprofen was predicted as a candidate entry inhibitor for Ebola virus using the same *in silico* approach¹⁹, and this prediction was confirmed experimentally at a later time point^{20,21}. These results prompt the possibility to experimentally test the effects of ibuprofen on SARS-CoV-2 infection under *in vitro* and *in vivo* conditions.

In silico methods are considered very important tools to generate first hypotheses and identify first drug candidates against newly discovered agents, like in the case of SARS-CoV-2,

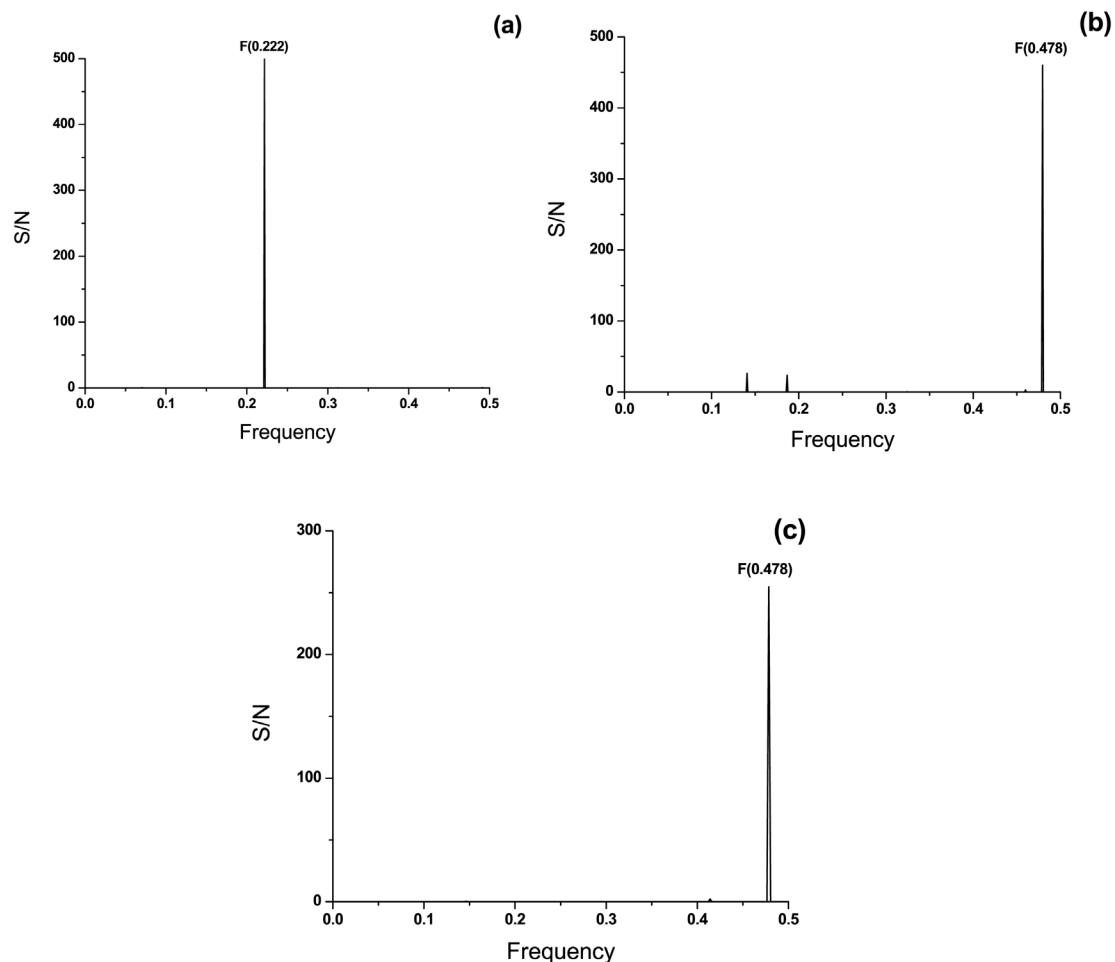


Figure 5. CS of S1 proteins from SARS-CoV and SARS-CoV-2 and actin proteins. (a) CS of S1 proteins from human SARS-CoV; (b) CS of S1 proteins from SARS-CoV-2; (c) CS of mammalian actin proteins. The abscissa and the ordinate are as described in Figure 1.

Table 3. Human proteins (uniprot.org) with the dominant peak on the frequency F(0.478) in the informational spectrum.

ABC8_HUMAN ATP-binding cassette sub-family B member 8, (Human)
ACTB_HUMAN Actin, cytoplasmic 1 (Human)
ACTC_HUMAN Actin, alpha cardiac muscle 1 (Human)
ACTK_HUMAN Kappa-actin (Human)
ACTS_HUMAN Actin, alpha skeletal muscle (Human)
ATL3_HUMAN ADAMTS-like protein 3 (Human)
AUP1_HUMAN Ancient ubiquitous protein 1 (Human)
CA064_HUMAN Putative uncharacterized protein C1orf64 (Human)
CETN2_HUMAN Centrin-2 (Human)
CPNE1_HUMAN Copine-1 (Human)
CR034_HUMAN Uncharacterized protein C18orf34 (Human)
CSEN_HUMAN Calsenilin (Human)
EXOC4_HUMAN Exocyst complex component 4 (Human)
F108B_HUMAN Abhydrolase domain-containing protein FAM108B1 (Human)
FGF13_HUMAN Fibroblast growth factor 13 (Human)
FRMD1_HUMAN FERM domain-containing protein 1 (Human)
GCDH_HUMAN Glutaryl-CoA dehydrogenase, mitochondrial (Human)
GKN2_HUMAN Gastroskin-2 (Human)
GPDA_HUMAN Glycerol-3-phosphate dehydrogenase [NAD+] (Human)
HPS1_HUMAN Hermansky-Pudlak syndrome 1 protein (Human)
HXK3_HUMAN Hexokinase-3 (Human)
IL23R_HUMAN Interleukin-23 receptor (Human)
KAD3_HUMAN GTP:AMP phosphotransferase mitochondrial (Human)
KRA71_HUMAN Keratin-associated protein 7-1 (Human)
LGMN_HUMAN Legumain (Human)
MYOG_HUMAN Myogenin (Human)
NTR2_HUMAN Neurotensin receptor type 2 (Human)
RD3_HUMAN Protein RD3 (Human)
S2543_HUMAN Solute carrier family 25 member 43 (Human)
SMDF_HUMAN Neuregulin-1, sensory and motor neuron-derived factor (Human)
SOX17_HUMAN Transcription factor SOX-17 (Human)
THOC4_HUMAN THO complex subunit 4 (Human)
TXND1_HUMAN Thioredoxin domain-containing protein 1 (Human)
VATE2_HUMAN Vacuolar ATP synthase subunit E 2 (Human)
ZN516_HUMAN Zinc finger protein 516 (Human)

especially in the short-term. ISM, a technology based on electronic biology, allowed identifying potential importance of human actin proteins for viral infection/dissemination as well as one FDA approved drug that may have an indirect antiviral activity within weeks of the initial outbreak. However,

additional experiments are required to confirm our initial findings.

In conclusion, results of the presented *in silico* analysis suggest the following: (i) the newly emerging SARS-CoV-2 is

Table 4. Mammalian actin proteins (uniprot.org).

ACTB_HUMAN Actin cytoplasmic 1 OS=Homo sapiens
ACTB_MOUSE Actin cytoplasmic 1 OS=Mus musculus
ACTC_HUMAN Actin alpha cardiac muscle 1 OS=Homo sapiens
ACTB_CAVPO Actin cytoplasmic 1 OS=Cavia porcellus
ACTS_MOUSE Actin alpha skeletal muscle OS=Mus musculus
ACTB_PONAB Actin cytoplasmic 1 OS=Pongo abelii
ACTA_MOUSE Actin aortic smooth muscle OS=Mus musculus
ACTH_MOUSE Actin gamma-enteric smooth muscle OS=Mus musculus
ACTG_MOUSE Actin cytoplasmic 2 OS=Mus musculus
ACTB_MESAU Actin cytoplasmic 1 OS=Mesocricetus auratus
ACTG_HUMAN Actin cytoplasmic 2 OS=Homo sapiens
ACTS_RABIT Actin alpha skeletal muscle OS=Oryctolagus cuniculus
ACTH_HUMAN Actin gamma-enteric smooth muscle OS=Homo sapiens
ACTC_MOUSE Actin alpha cardiac muscle 1 OS=Mus musculus
ACTC_RAT Actin alpha cardiac muscle 1 OS=Rattus norvegicus
ACTA_HUMAN Actin aortic smooth muscle OS=Homo sapiens
ACTG_RAT Actin cytoplasmic 2 OS=Rattus norvegicus
ACTB_RAT Actin cytoplasmic 1 OS=Rattus norvegicus
ACTB_BOVIN Actin cytoplasmic 1 OS=Bos taurus
ACTS_HUMAN Actin alpha skeletal muscle OS=Homo sapiens

highly related to SARS-CoV and, to a lesser degree, MERS-CoV, and ACE2 is a likely receptor of it; (ii) civets and poultry are potential candidates for the natural reservoir of the SARS-CoV-2, (iii) human actin proteins possibly participate in attachment/internalisation of SARS-CoV-2, (iv) drugs which interact with actin proteins (e.g. ibuprofen) should be investigated as possible therapeutics for treatment of SARS-CoV-2 infection, and (v) domain 266-330 of S1 protein from the SARS-CoV-2 represents promising therapeutic and/or vaccine target. Further research on these issues are needed, including the development of reverse genetics and animal models to study the biology of SARS-CoV-2. Due to the fast evolving of scientific knowledge on SARS-CoV-2, the first prediction has been already confirmed, while the chicken as potential

candidate as intermediate host has not been supported. Importantly, link between ibuprofen/actin interactions and viral entry remains an exciting path for future therapeutic investigations.

Data availability

Underlying data

Sequence data of the viruses were obtained from the [GISAID EpiFlu™ Database](#). To access the database each individual user should complete the “[Registration Form For Individual Users](#)”, which is available alongside detailed instructions. After submission of the Registration form, the user will receive a password. There are not any other restrictions for the access to GISAID. Conditions of access to, and use of, the GISAID EpiFlu™ Database and Data are defined by the [Terms of Use](#).

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Version 3

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Maria Salvato 

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² STARBIOS2 (EU-funded consortium), Baltimore, USA

The authors have made the minor corrections requested and the manuscript looks like a valuable contribution to the literature.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, viral pathogenesis, viral genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 16 April 2020

<https://doi.org/10.5256/f1000research.24526.r61263>

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This manuscript presents a machine-learning analysis of the published sequences of the novel 2019-nCoV. The authors use the Informational Spectrum Method (ISM), a virtual spectroscopy method for protein analysis based on the electronic properties of each amino acid. Their goal is to identify sites on the virus most likely to interact with other molecules like drugs, antibodies or viral receptors.

Due to the rapidity of this field and the time elapsed since the manuscript was submitted (Jan 27), most of their conclusions are no longer new: nCoV is most related to SARS-CoV and less to MERS-CoV, ACE2 is a likely receptor, the natural reservoir might be civets and poultry, human actin proteins participate in internalization, ibuprofen that interacts with actin proteins should be investigated as a therapeutic, and finally that domain 266-330 of the S1 protein should be targeted by drugs or vaccines.

It is a nice piece of work. The conclusions could be updated, for example they could say that the first of these predictions are supported by recent publications, that bat CoVs now appear to be the most closely related and bats are more likely to be the natural reservoir, and the link between ibuprofen/actin interactions and viral entry remains an exciting path for future therapeutics.

Minor corrections

- In the first sentence of "Update" the authors refer to 'these proteins'...do they mean Actin and SARS-CoV proteins? If they only mean actin they should say so, and then the sentence would read: "...actin protein is suggested as a host factor that participates in infection and pathogenesis of 2019-nCoV. Drugs modulating the biological activity of actin (e.g., ibuprofen) were suggested as candidates that should be investigated for the treatment of 2019-nCoV infection."
- In the UPDATE, last sentences should say "...which are presented in Figure 5."
- Introduction pg 3 "Fears are mounting worldwide over the cross-border spread of the new strain of coronavirus (denoted as 2019-nCoV) originated in Wuhan,...." Instead say "...that originated in Wuhan..."
- Introduction pg 3 "...eight human 2019-nCoV isolates are available without any additional information about biological properties of the virus, beyond the morphological confirmation..." Replace "morphology" with "morphological", it is an adjective.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, viral pathogenesis, viral genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 March 2020

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Possibly the ISM method invented by the authors has high potentiality. Here, apparently, the method is applied to answer urgent questions in relation to sequence analysis of the novel coronavirus 2019-nCoV. It is interesting to verify that different papers at the moment seem to have reached the same conclusions, although in my opinion a comparison with other methods which are already published would add to the paper. On top, R. Yan *et al.*,¹ *Science* 2020 4 March, beautifully detailed the putative region of the virus/ACE2 interaction. I recommend the authors quote this finding as well and if possible add the reference. This would help in validating a quite interesting method of sequence analysis.

References

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Computational Biology, Structural bioinformatics, Functional annotation, Machine and deep learning

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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