← **Post**

Justin B. Kinney @jbkinney · Aug 24

Ok, I think they mean that SC2 looks like it was constructed by taking an RaTG13-like backbone and swapping in an RBD from a CoV similar to the pangolin CoV. The “smoking gun” is that the bounds of the swapped RBD nearly match those of the minimal fragment found in the 2008 paper

3 2 18 1,036

Dr. rer. nat. Valentin Bruttel @VBruttel · Aug 25

so basically what @BiophysicsFL is describing here?
twitter.com/BiophysicsFL/s...
makes sense.
WIV wasn't making their own complete reverse genetic systems in 2008 IIRC.
and they planned to manipulate the RBD.
which almost sticks out as much as the FCS if you look at the AA seq.

growth in human cells and pathogenesis. We will test growth in primary HAE cultures and *in vivo* in hACE2 transgenic mice. We anticipate recovering ~3-5 full length genome viruses/yr. *Testing Synthetic Modifications:* We will synthesize QS with novel combinations of mutations to determine the effects of specific genetic traits and the jump potential of future and unknown recombinants. *RBD deletions:* Small deletions at specific sites in the SARSr-CoV RBD alter risk of human infection. We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of deletions will prevent virus growth in Vero cells and HAE[58]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. *S2 Proteolytic Cleavage and Glycosylation Sites:* After receptor binding, a variety of cell surface or endosomal proteases[68-71] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [72] and activating fusion-mediated entry[68,73]. We will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[74,75]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotyped particle studies and evaluate the impact of select SARSr-CoV S changes on virus replication and pathogenesis. We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain. *N-linked glycosylation:* Some glycosylation events regulate SARS-CoV particle binding DC-SIGN/L-SIGN, alternative receptors for SARS-CoV entry into macrophages or monocytes[76,77]. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and raccoon dogs[77]. While the sites are absent from civet and raccoon dog strains and clade 2 SARSr-CoV, they are present in WIV1, WIV16 and SHC014, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 disrupting residues of SARS-CoV and SHC014 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN, and in human monocytes and macrophages anticipating reduced virus growth efficiency. We will introduce the clade I mutations that result in N-linked glycosylation in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ± ectopic DC-SIGN expression[77]. *In vivo*, we will evaluate pathogenesis in transgenic hACE2 mice. *Low abundance micro-variations:* We will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD, use commercial gene blocks to introduce these changes singly and in combination into the S glycoprotein gene of the low risk, parental strain and test ACE2 receptor usage, growth in HAE and *in vivo* pathogenesis.

> **Louis R Nemzer** @BiophysicsFL · Aug 24
>
>
>
> Replying to @BiophysicsFL
>
> "a minimal insert region (amino acids 310 to 518) was found to be sufficient to convert the SL-CoV S from non-ACE2 binding to human ACE2 binding" (from 2008)
>
> ...

1 2 10 806

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technically, that divergent part is the receptor binding motive, which is only a part of the receptor binding domain, but exactly what they changed 2008.

2 2 6 863

Monali C. Rahalkar @MonaRahalkar · Aug 25

And that could be from pangolins or synthetic or modification of the bat RBD or miners virus RBD. There also could be a possibility that it was of the real 4991, which they optimised.

1 3 138

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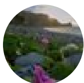
yep, good old RBM juggeling like in the 2000s. and let's put one in with amazing hACE affinity, see what happens. and while there, add an FCS that makes viruses super deadly. just 2 changes, plus maybe a glycosylation site. and why use pseudoviruses, what could go wrong...

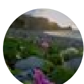
1 1 6 231

Monali C. Rahalkar @MonaRahalkar · Aug 25
What I also think is that they did not gave us the correct RBD for RaTG13/4991, and the same was added little later and proposed as banal52. The original RBD of 4991 could be better, and optimisation of 4991 or a similar virus like the miners virus could be what we as sars2.

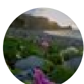
2 2 152


Zach Hensel @alchemytoday · Aug 25
This would be unbelievably silly if it weren't unbelievably offensive to your colleagues.

2 57


Zach Hensel @alchemytoday · Aug 25
Let's presuppose it's January 2020 and (1) you are at the head of an international conspiracy of virologists, (2) you know that you are responsible for the pandemic, and (3) you know that, because it's a lab leak, there will not be another one.

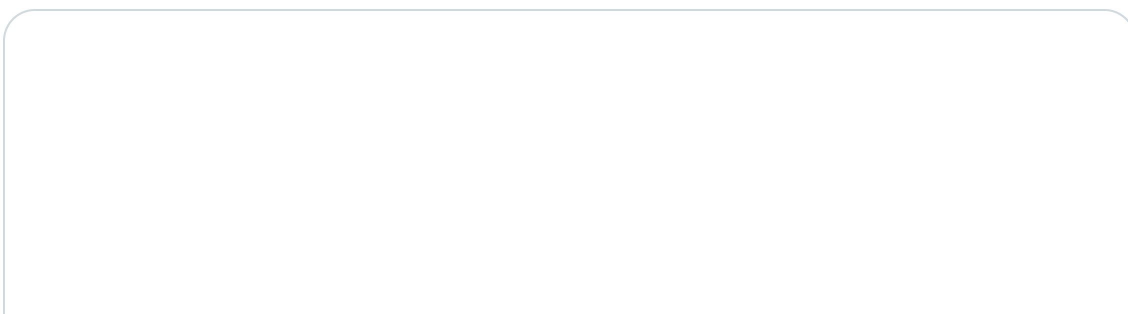
1 68


Zach Hensel @alchemytoday · Aug 25
What do you do? You just destroy whatever incriminating evidence you have and fabricate perfect evidence of origin from a susceptible animal in trade from wherever is convenient. It would be pretty easy and you'd be done by March.

1 74

Dr. rer. nat. Valentin Bruttel
@VBruttel

they had to publish RaTG13 in some form.
they had already uploaded it's RdRp to NIH servers in 2018.
it was extremely similar.



Bat SARS-like coronavirus strain RaTG13_Yunnan RNA-dependent RNA polymerase (RdRp) gene, partial cds

GenBank: MH615898.1

FASTA Graphics

Go to:

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LOCUS       MH615898                2757 bp    RNA     linear   VRL 10-AUG-2022
DEFINITION  Bat SARS-like coronavirus strain RaTG13_Yunnan RNA-dependent RNA
            polymerase (RdRp) gene, partial cds.
ACCESSION   MH615898
VERSION     MH615898.1
KEYWORDS    .
SOURCE      Bat SARS-like coronavirus
  ORGANISM  Bat SARS-like coronavirus
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Nidovirales; Cornidovirineae; Coronaviridae; Orthocoronavirinae;
            Betacoronavirus; Sarbecovirus.
REFERENCE   1  (bases 1 to 2757)
  AUTHORS   Zhou,P., Yang,X.-L., Wang,X.-G., Hu,B., Zhang,L., Zhang,W.,
            Si,H.-R., Zhu,Y., Li,B., Huang,C.-L., Chen,H.-D., Chen,J., Luo,Y.,
            Guo,H., Jiang,R.-D., Liu,M.-Q., Chen,Y., Shen,X.-R., Wang,X.,
            Zheng,X.-S., Zhao,K., Chen,Q.-J., Deng,F., Liu,L.-L., Yan,B.,
            Zhan,F.-X., Wang,Y.-Y., Xiao,G.-F. and Shi,Z.-L.
  TITLE     A pneumonia outbreak associated with a new coronavirus of probable
            bat origin
  JOURNAL   Nature 579 (7798), 270-273 (2020)
  REMARK    DOI: 10.1038/s41586-020-2012-7
REFERENCE   2  (bases 1 to 2757)
  AUTHORS   Yu,P., Hu,B., Li,B., Luo,D., Zhu,G., Zhang,L., Holmes,E.C., Shi,Z.
            and Cui,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-JUL-2018) CAS Key Laboratory of Special Pathogens and
            Biosafety and Center for Emerging Infectious Diseases, Wuhan
            Institute of Virology, Chinese Academy of Sciences, No. 44 Xiao
            Hong Shan, Wuhan, Hubei 430071, China
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..2757
```

```
Ra13_RdRp      VSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFLKTNCCRFQEKDEDDNLIDSYFVVK    60
SC2_RdRp       VSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFLKTNCCRFQEKDEDDNLIDSYFVVK    60
               ************************************************************

Ra13_RdRp      RHTFSNYQHEETIYNLLKDCPAVAKHDFFKFRIDGDMVPHISRQRLTKYTMADLVYALRH    120
SC2_RdRp       RHTFSNYQHEETIYNLLKDCPAVAKHDFFKFRIDGDMVPHISRQRLTKYTMADLVYALRH    120
               ************************************************************

Ra13_RdRp      FDEGNCDTLREILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKTVQFC    180
SC2_RdRp       FDEGNCDTLKEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKTVQFC    180
               *********:**************************************************

Ra13_RdRp      DAMRDAGIVGVLTLDNQDLNGNWYDFGDFIQTTPGSGVPIVDSYYSLLMPILTLTRALTA    240
SC2_RdRp       DAMRNAGIVGVLTLDNQDLNGNWYDFGDFIQTTPGSGVPVVDSYYSLLMPILTLTRALTA    240
               ****:**********************************:********************

Ra13_RdRp      ESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWDQTYHPNCVNCLDDRCILHCANF    300
SC2_RdRp       ESHVDTDLTKPYIKWDLLKYDFTEERLKLFDRYFKYWDQTYHPNCVNCLDDRCILHCANF    300
               ************************************************************

Ra13_RdRp      NVLFSTVFPPTSFGPLVRKIFVDGVPFVVSTGYHFRELGVVHNQDVNLHSSRLSFKELLV    360
SC2_RdRp       NVLFSTVFPPTSFGPLVRKIFVDGVPFVVSTGYHFRELGVVHNQDVNLHSSRLSFKELLV    360
               ************************************************************

Ra13_RdRp      YAADPAMHAASGNLLLDKRTTCFSVAALTNNVAFQTVKPGNFNKDFYDFAVSKGFFKEGS    420
SC2_RdRp       YAADPAMHAASGNLLLDKRTTCFSVAALTNNVAFQTVKPGNFNKDFYDFAVSKGFFKEGS    420
               ************************************************************

Ra13_RdRp      SVELKHFFFAQDGNAAISDYDYYRYNLPTMCDIRQLLFVVEVVDKYFDCYDGGCINANQV    480
SC2_RdRp       SVELKHFFFAQDGNAAISDYDYYRYNLPTMCDIRQLLFVVEVVDKYFDCYDGGCINANQV    480
               ************************************************************

Ra13_RdRp      IVNNLDKSAGFPFNKWGKARLYYDSMSYEDQDALFAYTKRNVIPTITQMNLKYAISAKNR    540
SC2_RdRp       IVNNLDKSAGFPFNKWGKARLYYDSMSYEDQDALFAYTKRNVIPTITQMNLKYAISAKNR    540
               ************************************************************

Ra13_RdRp      ARTVAGVSICSTMTNRQFHQKLLKSIAATRGATVVIGTSKFYGGWHNMLKTVYSDVENPH    600
SC2_RdRp       ARTVAGVSICSTMTNRQFHQKLLKSIAATRGATVVIGTSKFYGGWHNMLKTVYSDVENPH    600
               ************************************************************

Ra13_RdRp      LMGWDYPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANECAQVLSEMVMCGGSL    660
SC2_RdRp       LMGWDYPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANECAQVLSEMVMCGGSL    660
               ************************************************************

Ra13_RdRp      YVKPGGTSSGDATTAYANSVFNICQAVTANVNALLSTDGNKIADKHVRNLQHRLYECLYR    720
SC2_RdRp       YVKPGGTSSGDATTAYANSVFNICQAVTANVNALLSTDGNKIADKYVRNLQHRLYECLYR    720
               *********************************************:**************

Ra13_RdRp      NRDVDTDFVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVF    780
SC2_RdRp       NRDVDTDFVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVF    780
               ************************************************************

Ra13_RdRp      MSEAKCWTETDLTKGPHEFCSQHTMLVKQGDDYVYLPYPDPSRILGAGCFVDDIVKTDGT    840
SC2_RdRp       MSEAKCWTETDLTKGPHEFCSQHTMLVKQGDDYVYLPYPDPSRILGAGCFVDDIVKTDGT    840
               ************************************************************

Ra13_RdRp      LMIERFVSLAIDAYPLTKHPNQEYADVFHLYLQYIRKLHDELTGHMLDMYSVMLTNDNTS    900
SC2_RdRp       LMIERFVSLAIDAYPLTKHPNQEYADVFHLYLQYIRKLHDELTGHMLDMYSVMLTNDNTS    900
               ************************************************************

Ra13_RdRp      RYWEPEFYEAMYTPHTVLQ    919
SC2_RdRp       RYWEPEFYEAMYTPHTVLQ    919
               *******************
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Post your reply! Reply

Dr. rer. nat. Valentin Bruttel @VBruttel · Aug 25 ···

also, we are not making up anything here.
they proposed in 2018 to
- test synthetic modifications of the RBD
- insert those binding to human ACE2 into SARS related coronavirus backbones
- also insert polybasic/furin cleavage sites
- do live virus binding assays at BLS2 in Wuhan

Testing Synthetic Modifications: We will synthesize QS with novel combinations of mutations to determine the effects of specific genetic traits and the jump potential of future and unknown recombinants. *RBD deletions:* Small deletions at specific sites in the SARSr-CoV RBD alter risk of human infection. We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of deletions will prevent virus growth in Vero cells and HAE[58]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. *S2 Proteolytic Cleavage and Glycosylation Sites:* After receptor binding, a variety of cell surface or endosomal proteases[68-71] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [72] and activating fusion-mediated entry[64,73]. We will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[74,75]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we

collect viral load data from fresh fecal pellets. SARSr-CoV spike proteins will be sequenced, viral recombination events identified, and isolates used to identify strains that can replicate in human cells. The Univ. N. Carolina (UNC) team will reverse-engineer spike proteins of a large sample of high- and low-risk viruses for further characterization. This will effectively freeze the QS we analyze at t=0. These QS_0 strain viral spike glycoproteins will be synthesized, and those binding to human cell receptor ACE2 will be inserted into SARSr-CoV backbones (non-DURC, non-GoF), and inoculated into humanized mice to assess capacity to cause SARS-like disease, efficacy of monoclonal therapies, the inhibitor GS-5734[8] or vaccines against SARS-CoV[8-12].

Q: Given that coronavirus research in most places is done in BSL-2 or BSL-3 labs--and indeed, you WIV didn't even have an operational BSL-4 until recently--why would you do any coronavirus experiments under BSL-4 conditions?

A: The coronavirus research in our laboratory is conducted in BSL-2 or BSL-3 laboratories.

relevant to ACE2 binding. We will conduct *in vitro* pseudovirus binding assays, using established techniques[2], and live virus binding assays (at WIV to prevent delays and unnecessary dissemination of viral cultures) for isolated strains. Initial model predictions based on these

2 7 134

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