Structure, Volume 28

Supplemental Information

The Architecture of Inactivated SARS-CoV-2

with Postfusion Spikes Revealed

by Cryo-EM and Cryo-ET

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	71 MT407657.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/CHN/OS3/2020
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	MT044258.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA6/2020
	MN996530.1 Severe acute respiratory syndrome coronavirus 2 isolate WIV06
	MN996528.1 Severe acute respiratory syndrome coronavirus 2 isolate WIV04
	MT020881.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WA1-F6/2020
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	73 MN985325.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WA1/2020
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	MT106053.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA8/2020
	MN996527.1 Severe acute respiratory syndrome coronavirus 2 isolate WIV02
	— MT019531.1 Severe acute respiratory syndrome coronavirus 2 isolate BetaCoV/Wuhan/IPBCAMS-WH-03/2019
73	MT407658.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/CHN/OS2/2020
	MT072688.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS0CoV-2/61-TW/human/2020/ NPL
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	M1044257.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-IL2/2020
	MN996529.1 Severe acute respiratory syndrome coronavirus 2 isolate WIV05
	MT039888.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-MA1/2020
	MN988669.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV WHU02
	— MT019533.1 Severe acute respiratory syndrome coronavirus 2 isolate BetaCoV/Wuhan/IPBCAMS-WH-05/2020
	MN994467.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA1/2020
	MN988668.1 Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV WHU01
	MT019532.1 Severe acute respiratory syndrome coronavirus 2 isolate BetaCoV/Wuhan/IPBCAMS-WH-04/2019
	MT066176.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/NTU02/2020/TWN
	— MT093631.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/WH-09/human/2020/CHN
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86	LC522974.1 Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-501/2020
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	LC522973.1 Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-012/2020
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- F	EPI ISL 408482 hCoV-19/Shandong/IVDC-SD-001/2020
I _{EP}	ISL 408481 hCoV-19/Chongqing/IVDC-CQ-001/2020
	→ MT226610.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/CHN/KMS1/2020

0.0001

Figure S1 | Phylogenetic characteristics of BetaCoV/Shenzhen/SZTH-003/2020. This isolate was indicated with a triangle. Red triangle, this study; Black dot, WIV04; black squar, CN2; black diamond, UCC91-02. Related to Figure 1.



Figure S2 | Characterization of purified SARS-CoV-2 by negative staining EM. (A) Image of negative stained SARS-CoV-2. Nail-like spikes can be clearly seen. (B) Enlarged view of virion boxed in (A). (C) Averaged 2D projection of SARS-CoV-2 nailshaped spike (Song et al., 2018). The length (L), diameter of stem (D) and spike's head (H) are 23nm, 4nm and 7nm, respectively. Scale bars, 100 nm in A, 50 nm in B. Related to Figure 2.



Figure S3 | Fourier-shell-correlation (FSC) plot of postfusion spike subtomogram averaged density maps. The two dashed lines mark the FSC value of 0.5 and 0.143. The resolution is 20 Å at FSC of 0.5 and 10.7 Å at FSC of 0.143. Related to Figure 3.

PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	NNSYECDIPIGAGICASYQTQTNSPRRAR SVASQSIIAYTMSLGAENSVAYSNNSIAIPT DTSYECDIPIGAGICASYHTVSLLR STSQKSIVAYTMSLGADSSIAYS<mark>N</mark>NTIAIPT :.************************************	716 698
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	NFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK NFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAAEQDR **:**:***::****::*****:******.*********	776 758
P0DTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQ NTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQ **:*******:*** :* **** :* ************	836 818
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	YGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI YGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQI **:***** *****************************	896 878
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNA PFAMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNA ******	956 938
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA QALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA *****	1016 998
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFT EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQER <mark>N</mark> FT ************************************	1076 1058
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	TAPAICHDGKAHFPREGVFVS <mark>N</mark> GTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIV <mark>N</mark> NT TAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNT *******:***:********* *** **:*****:.********	1136 1118
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	VYDPLQPELDSFKEELDKYFK <mark>N</mark> HTSPDVDLGDISGI <mark>N</mark> ASVVNIQKEIDRLNEVAKNL <mark>N</mark> ES VYDPLQPELDSFKEELDKYFK <mark>NHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES</mark> ******	1196 1178
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	LIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKF LIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKF ********************	1256 1238
PODTC2 SPIKE_SARS2 P59594 SPIKE_CVHSA	DEDDSEPVLKGVKLHYT 1273 DEDDSEPVLKGVKLHYT 1255	

Figure S4 | Sequence alignment between SARS-CoV-2 S protein (Uniprot P0DTC2, Spike_SARS2) and SARS-CoV S protein (Uniprot P59594, Spike_CHHSA), shown in S2 region. Residues in bold indicate S2 residues. Residues in red indicates residues solved in PDB 6M3W. Residues highlighted in green are the glycosylation sites. Alignment done with CLUSTAL Omega (1.2.4). Related to Figure 3.

Characteristics	Description			
Age	62			
Gender	male			
Disease severity	severe			
Wuhan exposure history	Yes (Jan 1, 2020 – Jan 14, 2020)			
Date of symptoms onset	Jan 11, 2020			
Initial symptoms	Fever/Cough			
Date of hospitalization	Jan 15, 2020			
Co-existing chronic disease	none			
Influenza A virus	-			
Influenza B virus	-			
Respiratory syncytial virus	-			
Human Bocavirus	-			
Adenovirus	-			
Human metapneumovirus	-			
Rhinovirus	-			
HCoV-229E	-			
HCoV-OC43	-			
HCoV-HKU1	-			
HCoV-NL63	-			
MERS-CoV	<u>-</u>			
SARS-CoV	-			
Interferon atomization	Jan 16, 2020			
Ribavirin	Jan 15, 2020			
Methylprednisolone	no			
High-flow oxygen therapy	yes			
Mechanical ventilation	No			
CT finding	Bilateral pneumonia			
BALF sampling date	Jan 21, 2020			
Outcome	Discharged			

Table S1. Clinical information of the enrolled patient. Related to Figure 1.

Table S2: Summary of data acquisition and image processing statistics.

Related to Figure 2 and 3

Data acquisition					
EM equipment	Titan Krios				
Voltage (kV)	300				
Detector	Gatan K2				
Energy filter	Gatan GIF Quantum, 20 eV slit				
Super-resolution mode	Yes				
Pixel size (Å)	1.4				
Total electron dose (e ⁻ /Å ²)	123				
Dose rate (e-/Ų/s)	2.55				
Frame number	10				
Acquisition scheme	-60/60°, 3°				
Defocus range (µm)	-1.5 to -5.0				
Number of tilt-series	383				
Software	SerialEM				
Data processing					
Number of tomograms	242				
Number of subtomograms	956				
Symmetry imposed	C3				
Final resolution (Å)	20 Å at 0.5FSC, 10.7 Å at 0.143FSC				
Software	IMOD/emClarity/Dynamo				
EMDB number	EMD-11627				

Table S3. Comparison of inactivated viruses from four vaccine candidates with this study. Related to Figure 2 and 3.

Vaccine Name	Developer	Type of	Vaccine	EM images	reference
		Vaccine	strain		
PiCoVacc	Sinovac	β-	CN2	CryoEM, most	Gao et al,
		propiolactone		postfusion Spikes	Science 2020
N/A	Wuhan Institute of	β-	WIV04	NO EM image	Xia et al. JAMA
	Biological Products/	propiolactone			2020
	Sinopharm				
BBIBP-CorV	Beijing Institute of	β-	HB02	Negative stain most	Wang et al. Cell
	Biological Products/	propiolactone		prefusion Spikes	2020
	Sinopharm				
N/A	Institute of Medical	formaldehyde	UCC91-02	Negative stain, both	Chen et al.,
	Biology, Chinese			prefusion and post	BioRxiv 2020
	Academy of			fusion	
	Medical Sciences				
N/A	Institute for	β-	SZTH-003	Negative stain and	This study
	Hepatology,	propiolactone		cryoEM/ET, both	
	National Clinical			prefusion (26%) and	
	Research Center for			postfusion (74%)	
	Infectious Disease,				
	Shenzhen				