

Analysis by and presented on behalf of GISAID by

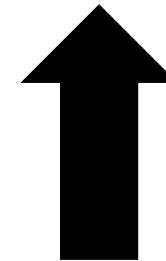
Dr Sebastian Maurer-Stroh

Deputy Executive Director (Research)



← BII/A*STAR, DBS/NUS, NPHL/NCID

GISAID

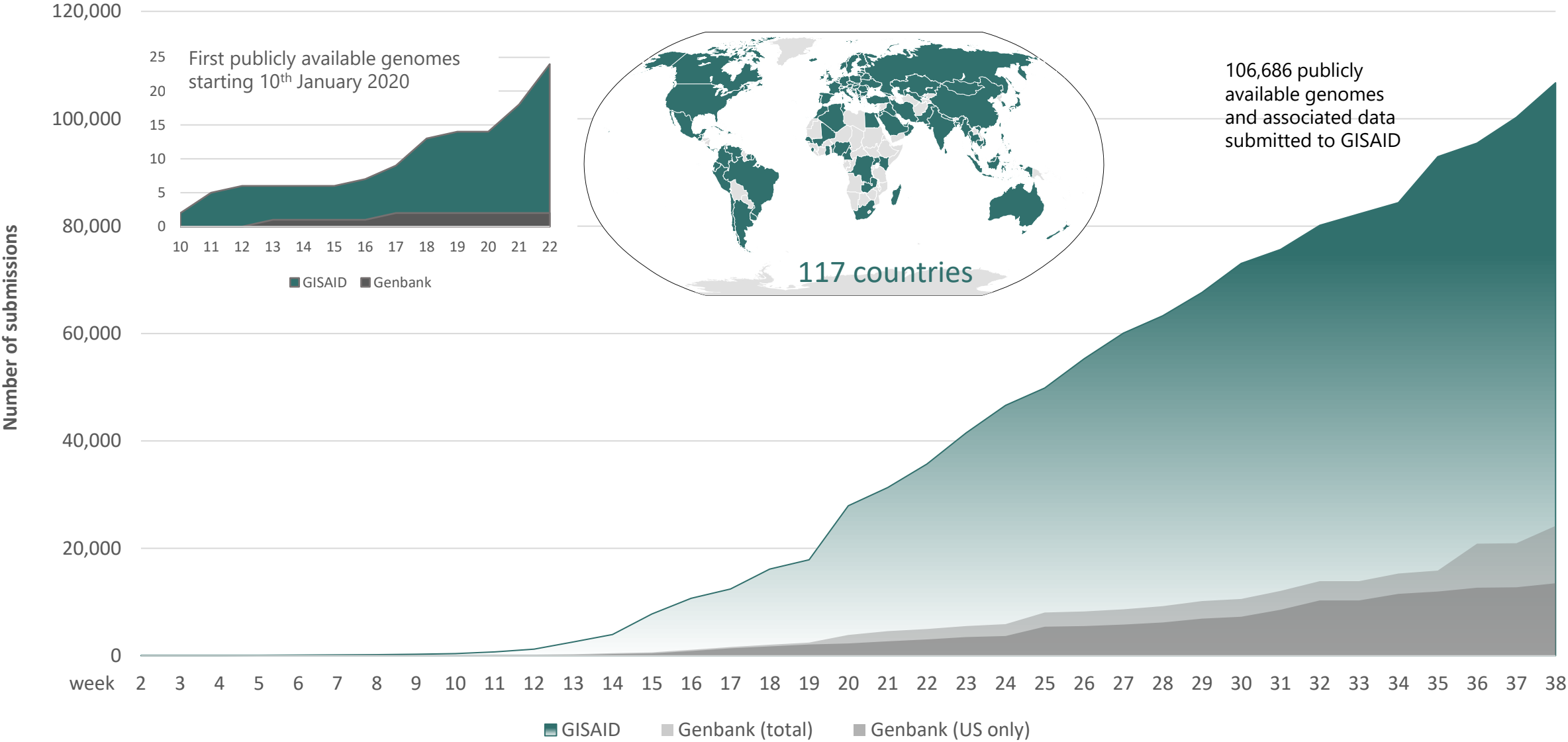


GISAID provisions for analysing Spike mutations

- Single comprehensive source of all outbreak genomes
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- Integrated mutation analysis tool CoVsurver:
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- Upcoming: live dashboard for spike mutations
- Needed in near future: serological panel data linked to sequences

Real-time data sharing is not achieved by governmental Regulations

it is incentivized by the confidence in trusted sharing mechanisms



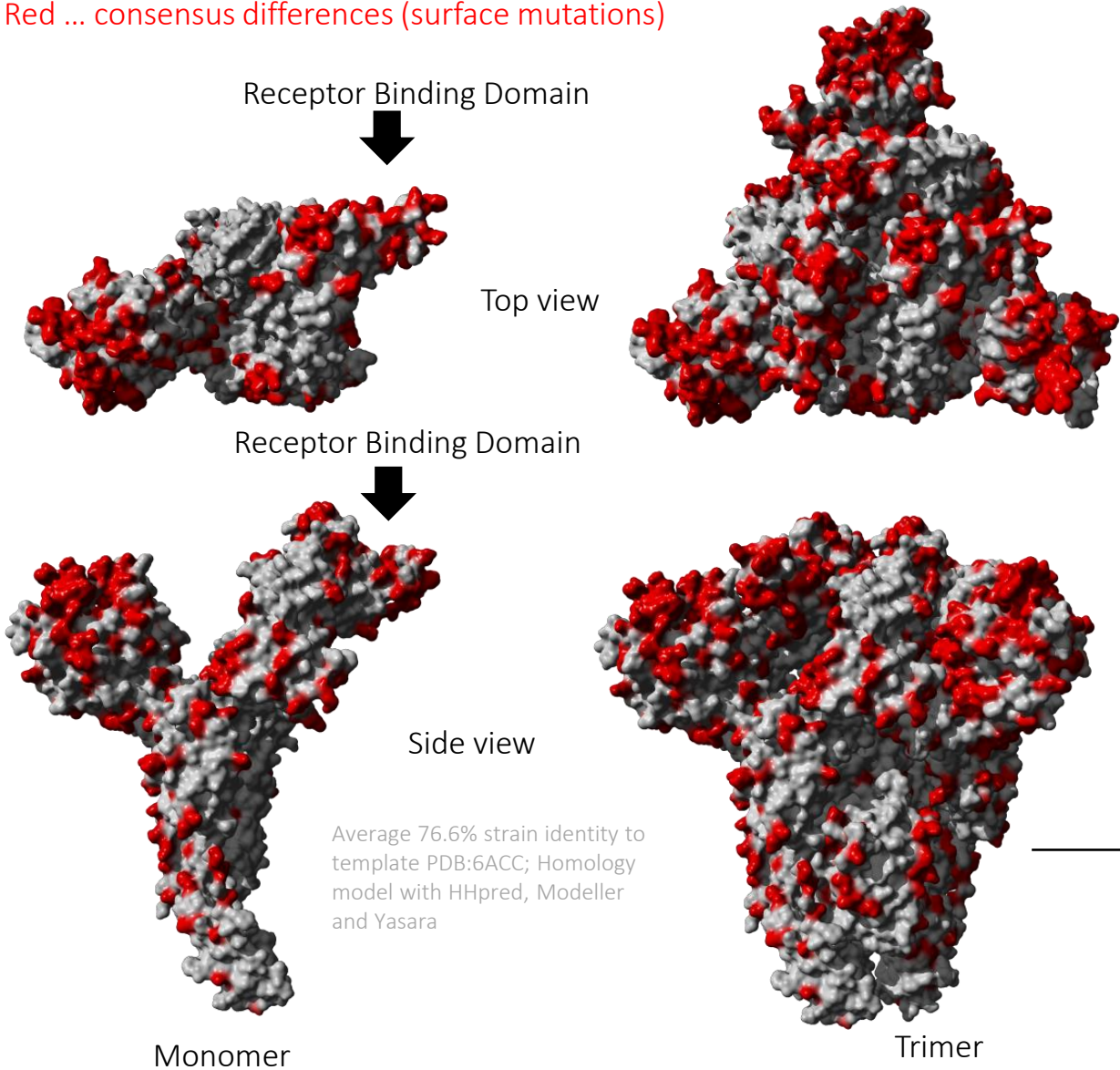
Spike mutations summary (9 months, >100k genomes)

- **Spike similarity to other Coronaviruses**
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Spike similarity to other coronaviruses

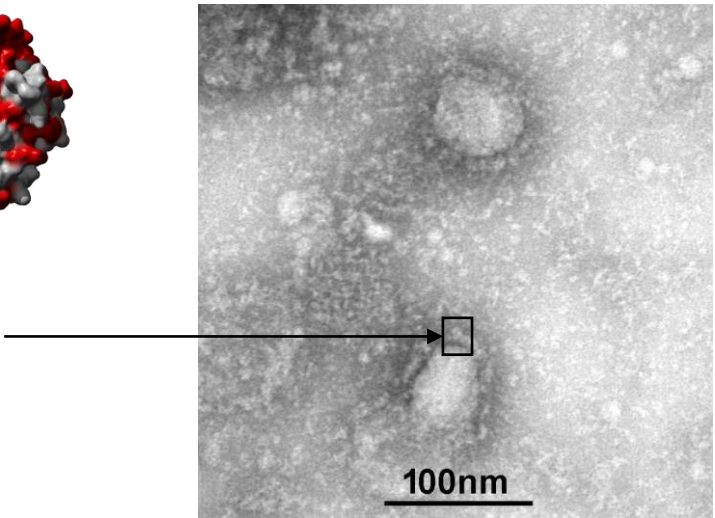
Spike glycoprotein hCoV-19 vs SARS

Red ... consensus differences (surface mutations)



Query/Reference	hCoV-19	Bat RaTG13	SARS	MERS	OC43	HKU1	229E	NL63
hCoV-19								
Bat RaTG13	97.7%							
SARS	77.8%	78.2%						
MERS	35.4%	35.4%	35.2%					
OC43	37.3%	37.1%	36.9%	39.5%				
HKU1	35.2%	35.3%	35.0%	39.0%	67.0%			
229E	41.7%	41.5%	41.8%	41.8%	43.5%	43.5%		
NL63	36.3%	36.2%	36.2%	35.4%	39.7%	37.8%	64.7%	

Percent identity of spike glycoproteins from relevant coronaviruses

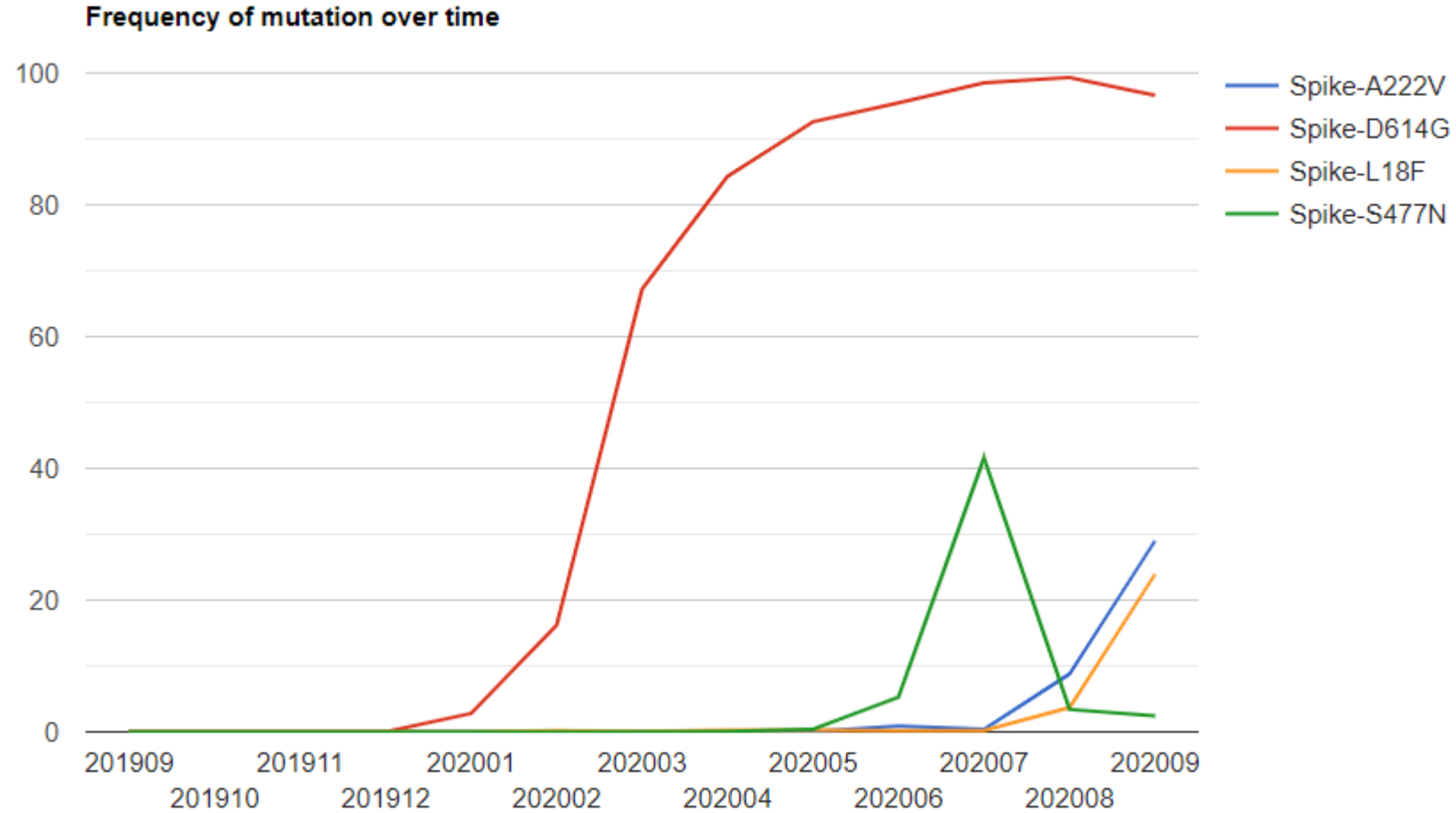


Cryo-EM image IVDC, China CDC

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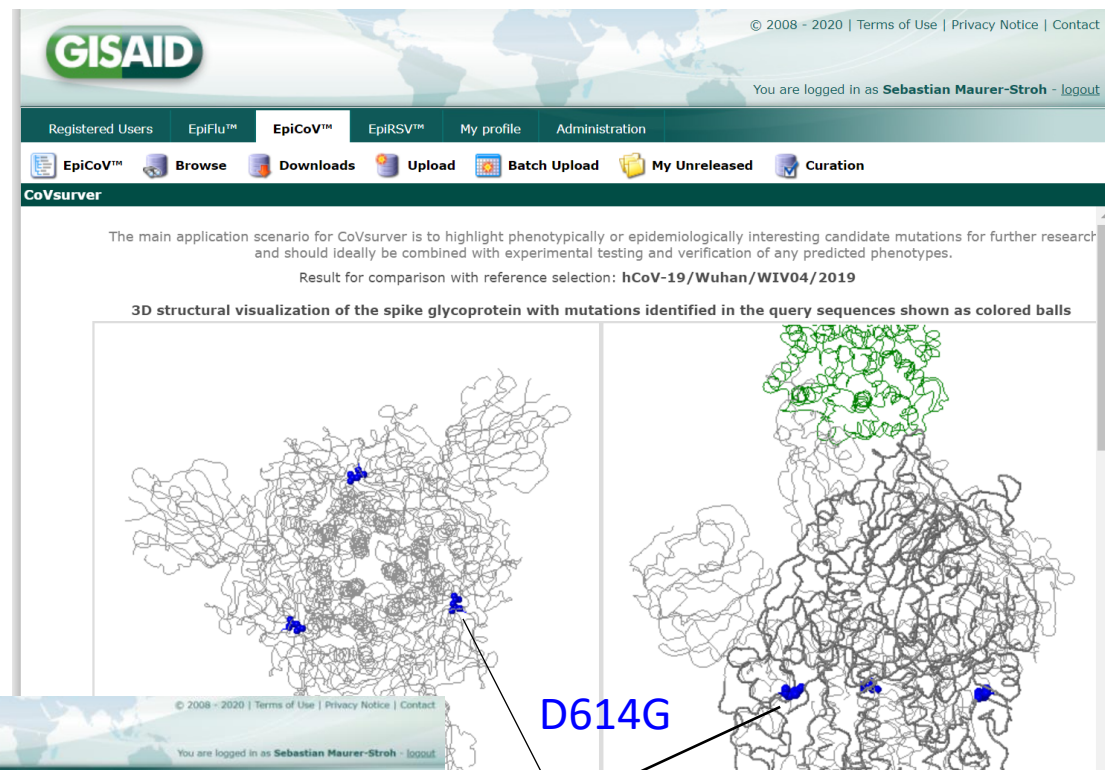
Common spike mutations within the outbreak – Sep 20 2020



CoVsurver:

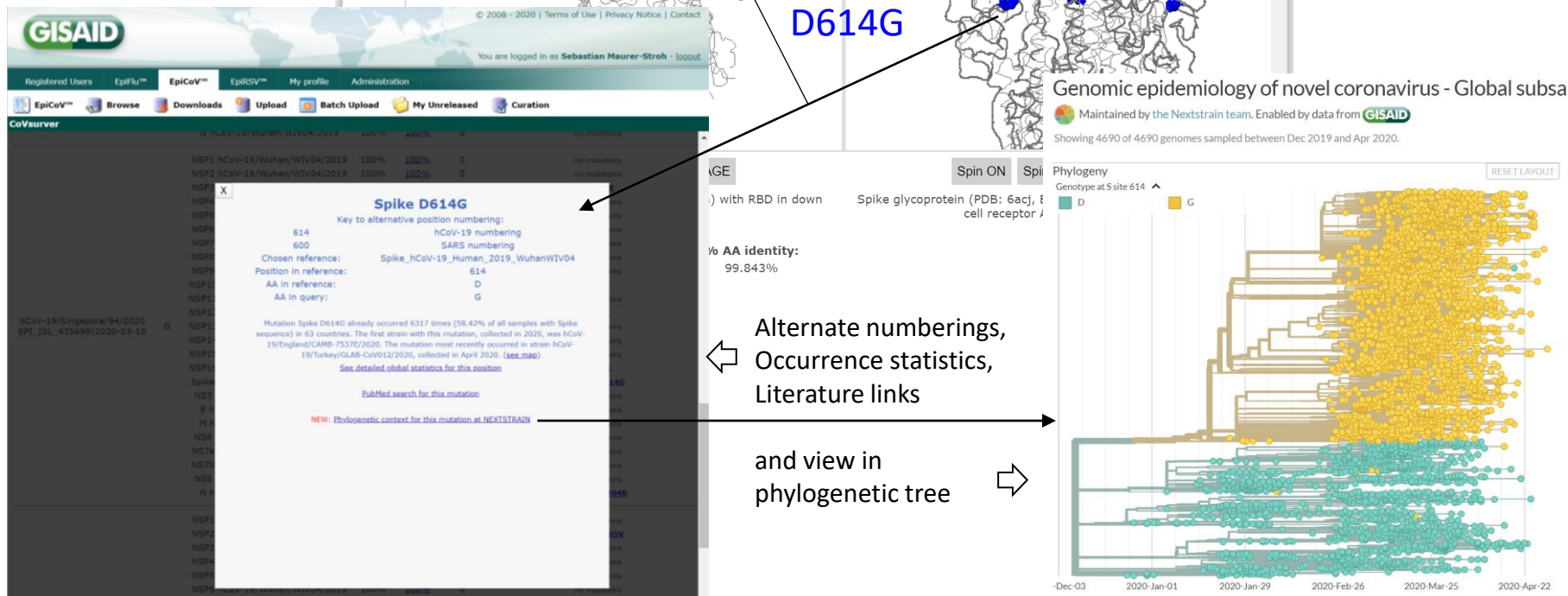
New analysis tool released on GISAID
developed by BII A*STAR

Capabilities: Identification and interpretation of mutations including 3D structure and database with curated literature links for 166 mutation reports

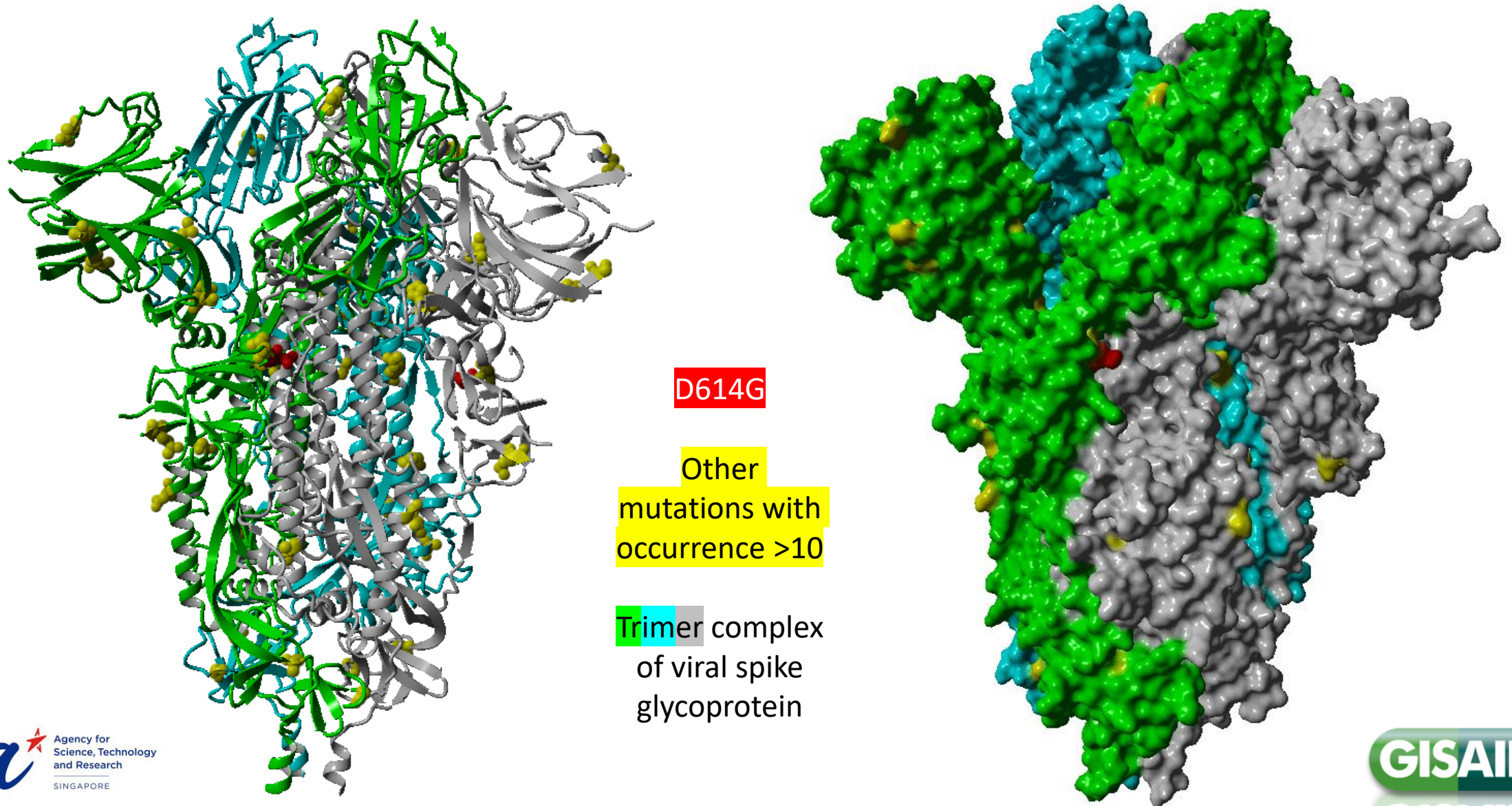


Example D614G:

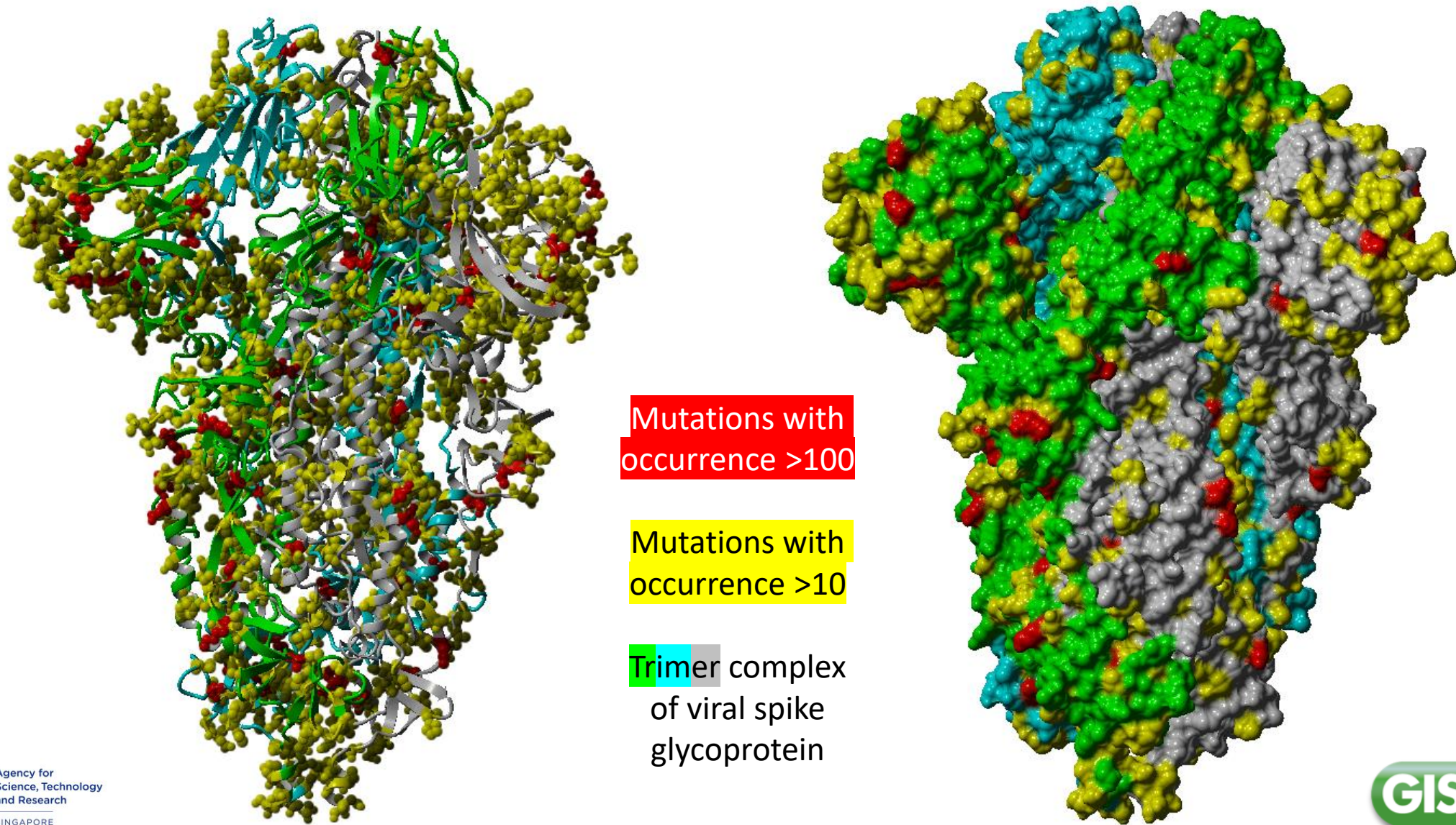
- No prior history of site for phenotypic relevance in related viruses
- Structural position not near receptor binding site, not in classical RBD antigenic sites, **at oligomer interface (can indirectly affect stability of complex)**
- Phylogenetic distribution points to founder-like effect and is accompanied with multiple other mutations



Common spike mutations within the outbreak – May 5 2020



Common spike mutations within the outbreak – Sep 20 2020



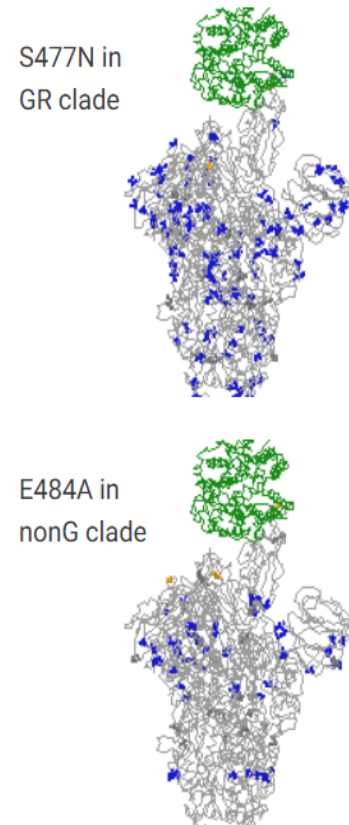
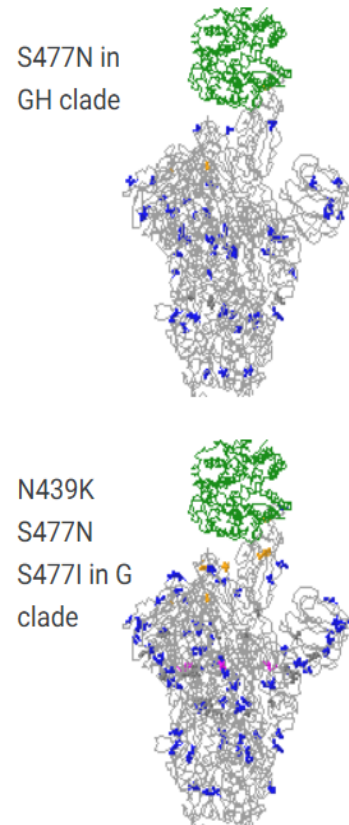
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Receptor binding surveillance for complete genomes 2020-09-22

New occurrence of receptor binding mutations 25x S477N in Switzerland, 3x S477N in Germany, 1x S477N in Australia/NSW, 4x S477I in England, 2x N439K in Switzerland, 1x N439K in Germany, 1x E484A in Spain

Total: 37 different rare variants near the binding interface not known to be linked to severity with >1x occurrence. 4157x **S477N** (3709 Australia/VIC, 235 Australia/NSW, 106 England, 27 Switzerland, 21 Australia/QLD, 13 Scotland, 11 Northern Ireland, 8 Sweden, 8 Australia/SAP, 5 Australia/ACT, 4 USA/FL, 3 Germany, 2 Australia/NT, 1 Lebanon, 1 USA/MA, 1 Australia/TAS, 1 USA/WA, 1 Australia/WA), 737x **N439K** (548 Scotland, 95 Ireland, 38 England, 26 Northern Ireland, 12 Wales, 10 Switzerland, 3 USA/IL, 2 Norway, 1 Germany, 1 Romania, 1 USA/WI), 108x **T478I** (107 England, 1 Spain), 40x **N501Y** (36 Australia/VIC, 1 USA/OR, 1 Wales, 1 Brazil, 1 USA/NY), 38x **G485R** (37 Australia/VIC, 1 Anhui), 30x **S494P** (17 England, 3 USA/MI, 2 Sweden, 2 Scotland, 2 India, 1 Singapore, 1 Wales, 1 Nigeria, 1 Spain), 20x **G476S** (9 USA/WA, 4 England, 1 USA/OR, 1 Singapore, 1 India, 1 Suriname, 1 USA/WI, 1 Belgium, 1 United Arab Emirates), 19x **E484Q** (12 Wales, 3 India, 2 Spain, 1 South Africa, 1 USA/CA), 18x **G446V** (3 England, 3 Australia/VIC, 2 USA/VA, 2 South Korea, 1 USA/MN, 1 Wales, 1 USA/CA, 1 Scotland, 1 USA/WA, 1 Finland, 1 Israel, 1 Portugal), 15x **S477I** (7 England, 2 Scotland, 1 Singapore, 1 Indonesia, 1 USA/SC, 1 Colombia, 1 Luxembourg, 1 Australia/VIC), 14x **L455F** (5 England, 2 South Africa, 2 South Korea, 1 Italy, 1 USA/CA, 1 Scotland, 1 USA/MO, 1 Australia/VIC), 13x **R403K** (11 USA/VA, 2 Australia/VIC), 13x **A475V** (3 USA/OR, 2 England, 2 USA/AZ, 2 Australia/VIC, 1 Italy, 1 USA/NY, 1 USA/FL, 1 Australia/NSW), 11x **E484K** (3 England, 2 Sweden, 2 Spain, 1 Wales, 1 Switzerland, 1 USA/IL, 1 USA/CA), 10x **Y453F** (5 Netherlands, 3 Switzerland, 2 South Africa), 8x **F490S** (8 England), 6x **Q493L** (3 USA/WI, 3 USA/MD), 5x **F490L** (2 Singapore, 1 USA/LA, 1 Australia/VIC, 1 USA/CA), 5x **K417N** (2 Germany, 1 Scotland, 1 England, 1 Northern Ireland), 4x **S494L** (2 England, 1 Australia/VIC, 1 Switzerland), 4x **S477R** (2 Switzerland, 1 Egypt, 1 England), 3x **V503F** (1 Switzerland, 1 Netherlands, 1 USA/NY), 3x **E484A** (2 Spain, 1 Northern Ireland), 3x **V445A** (1 Israel, 1 Australia/VIC, 1 Mexico), 3x **T478K** (2 Scotland, 1 England), 3x **S477G** (1 Austria, 1 Spain, 1 Japan), 2x **S477T** (1 England, 1 USA/UT), 2x **Y505W** (1 USA/WA, 1 USA/UN), 2x **G504D** (1 Australia/VIC, 1 Spain), 2x **V503I** (1 England, 1 Scotland), 2x **G446S** (2 England), 2x **N501S** (2 England), 2x **F456L** (1 USA/TX, 1 South Korea), 2x **F486L** (2 Netherlands), 2x **V445I** (2 England), 2x **E484D** (1 Germany, 1 Thailand), 2x **G476A** (1 England, 1 USA/MI)



Mutations in the spike glycoprotein for the 1519 new complete genomes.

We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence data on which the analysis is based.

Green ... ACE2 human host receptor

Gray ... CoV spike glycoprotein trimer

Gray balls ... Spike glycoprotein variation occurring once (in EpiCoV)

Blue balls ... Spike glycoprotein variation occurring more than once (in EpiCoV)

Red balls ... Spike glycoprotein variation near host receptor with effect history

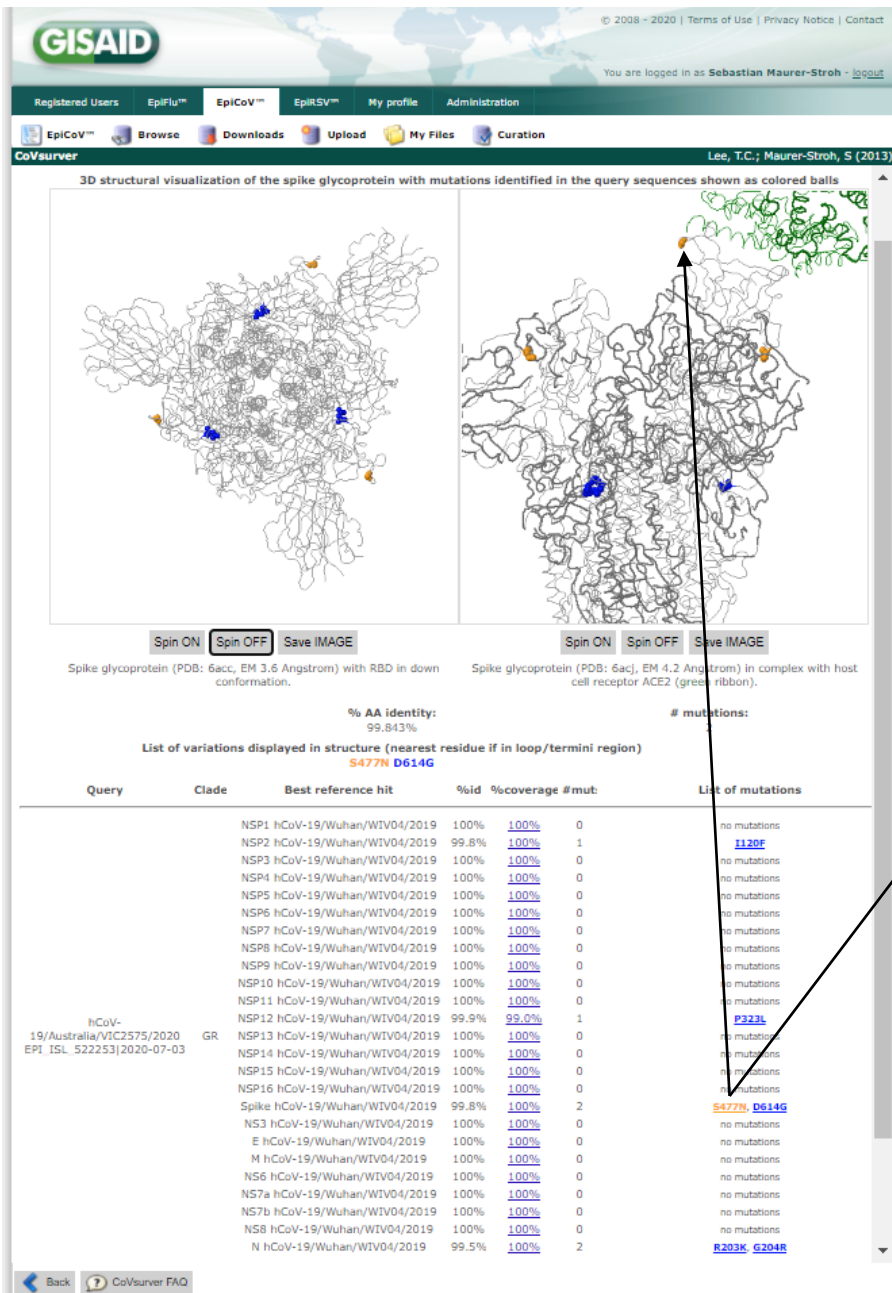
Orange balls ... Spike glycoprotein variation near host receptor, or other functional annotation

Cyan ... Insertion/deletion

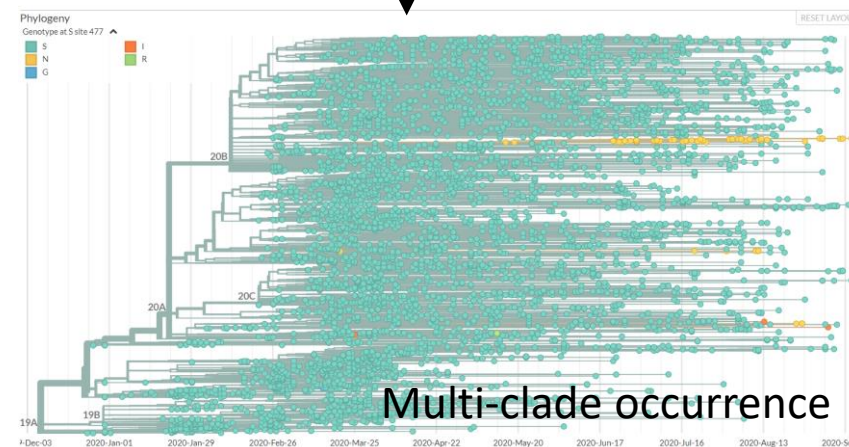
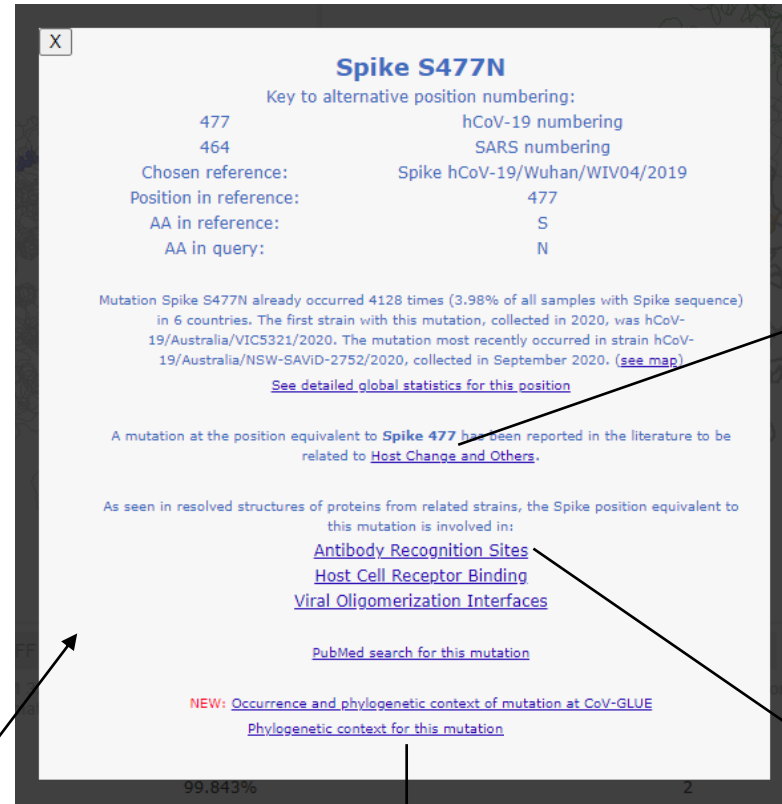
Magenta balls ... Spike glycoprotein variation altering potential N-glycosylation sites

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CoVsurver tool to analyse mutations – example Spike S477N



Protein: Spike
Coronavirus type: Yeast SARS-CoV-2 (2019)
Mutation (as in paper): S477N
neutral AA: S
neg. eff. AA: **N**
Effect: Host Change

Receptor binding

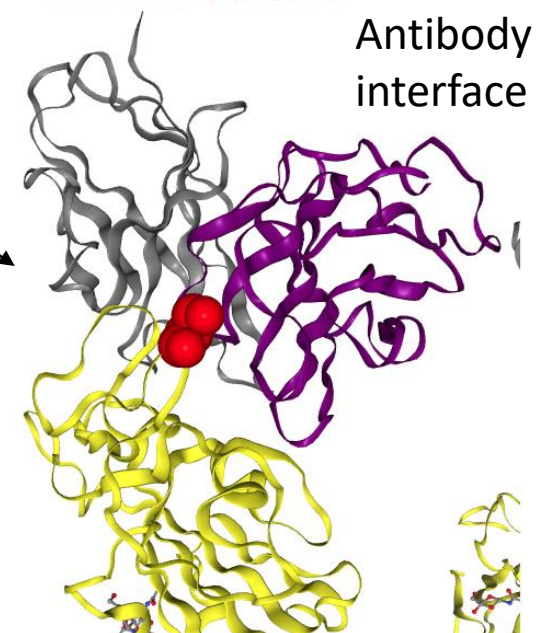
Comment:

In a deep mutational scanning experiment that expresses Spike RBD in a yeast-display platform, S477N mildly increases the binding to ACE2 (apparent dissociation constant delta-log10 value: 0.06)

Literature reference

(Mutation S477N in the paper is at an equivalent position of the mutation in your query)

Antibody interaction: The mutation position (red atoms) corresponds to position 477 on viral chain E (yellow backbone) of protein entry 6xcn, originating from Severe acute respiratory syndrome coronavirus 2 and with a label of Spike glycoprotein. The mutation is within 6 Å from antibody chain F (purple backbone).



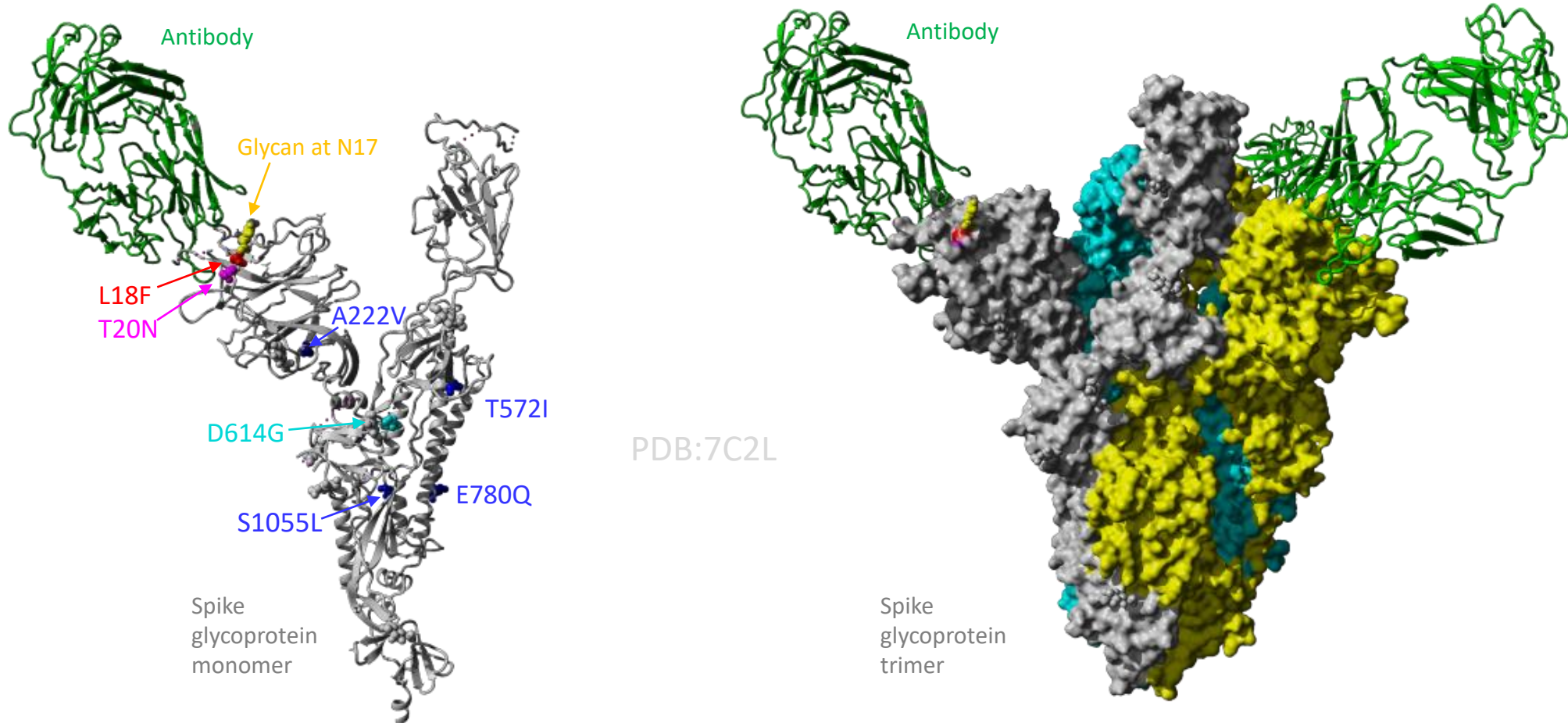
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Summary of AA differences in 4 reinfection cases

Strainname	Accession	Collection Date	Clade (Lineage)	Spike AA mutations	Other AA mutations
hCoV-19/Hong Kong/HKU-200823-001/2020	EPI_ISL_516798	2020-03-26	V (B.1.79)	E780Q	NSP3_L1304F, NSP3_P1103L, NSP5_K61R, NSP6_L37F, NS3_G251V, NS8_E64stop
hCoV-19/Hong Kong/HKU-200823-002/2020	EPI_ISL_516799	2020-08-17	G (B.2)	L18F, A222V, D614G	NSP6_L142F, NSP12_P323L, N_A220V
hCoV-19/Belgium/regi-0309752/2020	EPI_ISL_522349	2020-03-09	GR (B.1.1)	D614G	NSP6_F228L, NSP12_P323L, NS8_L84S, N_G204R, N_R203K
hCoV-19/Belgium/regi-0710751/2020	EPI_ISL_522350	2020-06-10	S (A)	S1055L	NS8_L84S
hCoV-19/USA/NV-NSPHL-A0110/2020	EPI_ISL_514673	2020-04-18	GH (B.1)	D614G	NSP1_L92F, NSP2_T85I, NSP3_A465V, NSP12_P323L, NSP13_V169F, NS3_Q57H
hCoV-19/USA/NV-NSPHL-A0207/2020	EPI_ISL_514674	2020-06-05	GH (B.1)	D614G	NSP2_T85I, NSP6_P44S, NSP12_P323F, NS3_Q57H, N_A398V
hCoV-19/Netherlands/un-EMC-751/2020	EPI_ISL_523507	2020-04-06	L (B)		NSP2_D268del, NSP13_R392C
hCoV-19/Netherlands/un-EMC-754/2020	EPI_ISL_523510	2020-06-08	L (B)	T20N, T572I	NSP2_D268del, NSP3_S100P, NSP12_T806A, NSP3_T1288I, NSP3_D821N, NSP4_H313Y, NSP13_R392C, M_S4F

4 reinfection cases spike glycoprotein comparison



2 of 4 reinfection cases have mutations possibly interfering with the structural conformation of glycosylation sites in a region that is also broadly recognized by antibodies which would provide a hypothetical mechanism for immune escape potentially contributing to permitting second infection. However, this doesn't apply to all cases and many other factors could play a role too. Importantly, these mutations are rare and occur sporadically without causing large clusters so far.

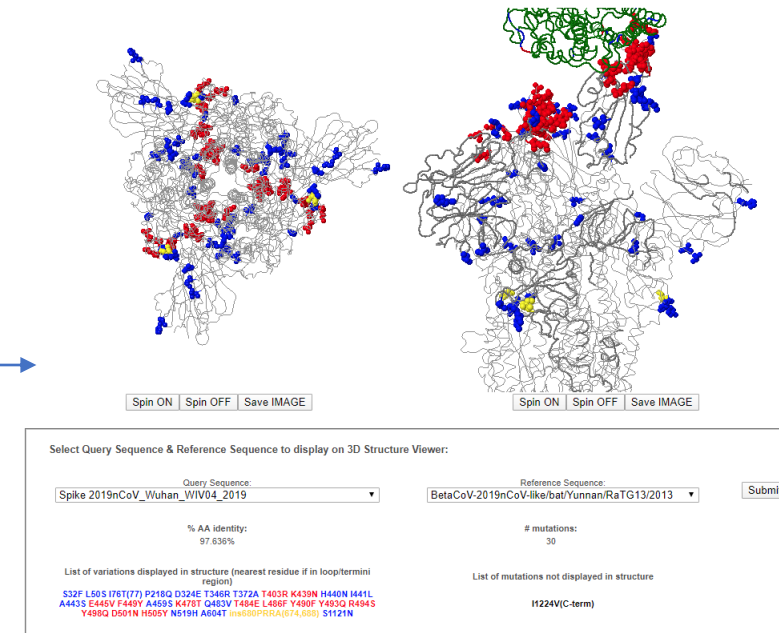
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Annex

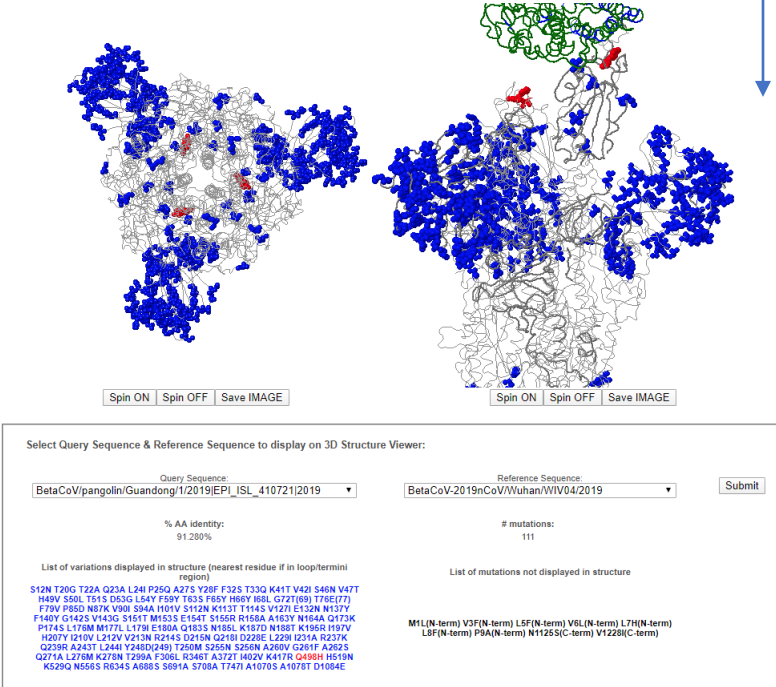
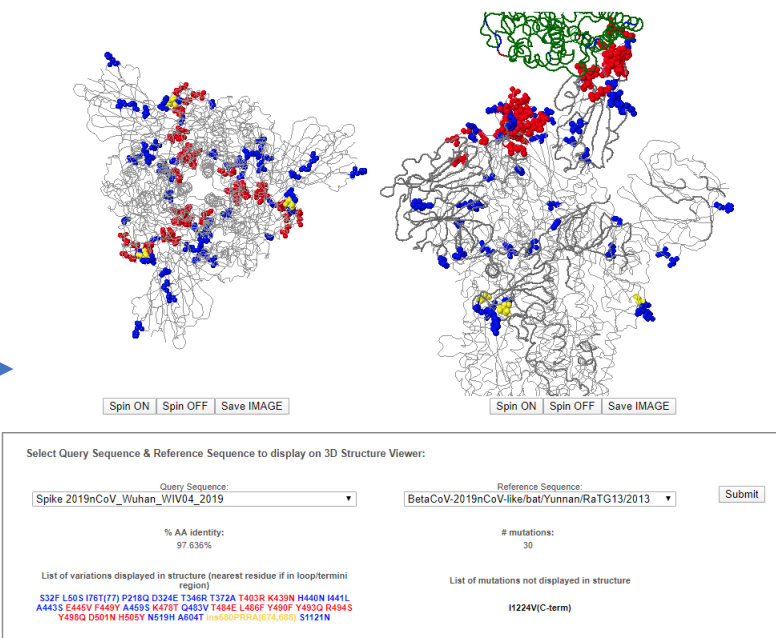
Spike host receptor changes for nearest bat and nearest pangolin sequences

Strain 1	Strain 2	Spike overall identity	Interface mutations
Human Wuhan	Bat Yunnan	98%	13
Pangolin Guangdong	Bat Yunnan	90%	13
Pangolin Guangdong	Human Wuhan	91%	1



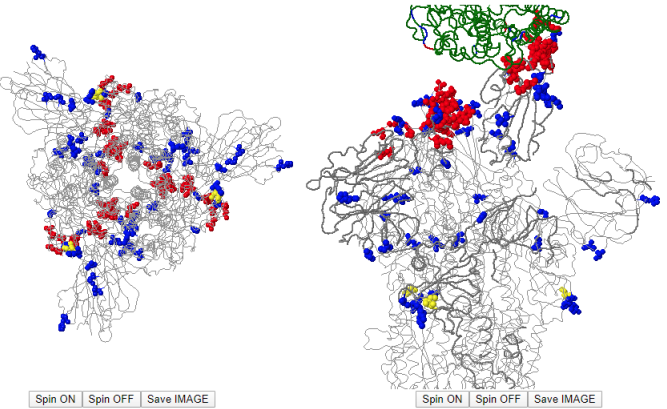
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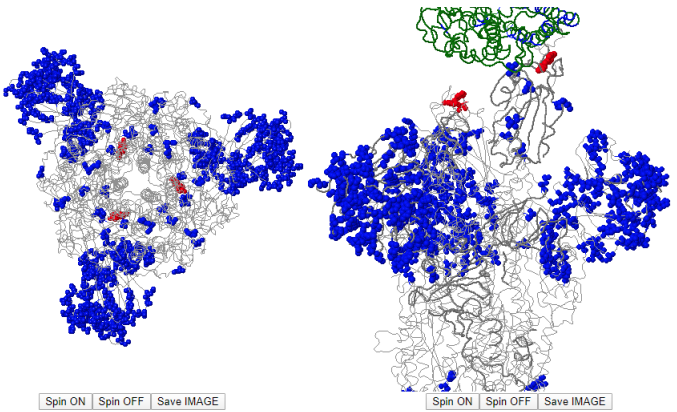
Select Query Sequence & Reference Sequence to display on 3D Structure Viewer:

Query Sequence: Spike 2019nCoV_Wuhan_WIV04_2019
Reference Sequence: BetaCoV-2019nCoV-like/bat/Yunnan/RaTG13/2013

% AA identity: 97.636%
mutations: 30

List of variations displayed in structure (nearest residue if in loop/termini region):
S32P L50S I76T(77) P218Q D324E T346R T372A T403R K430N H440N H41L A443S E445V F447Y A455S K478T Q483V T484E L486P Y489F Y490Q R494S Y498Q D501N H505Y N519H A604T H660P(P674A)(674,685) S1121N

List of mutations not displayed in structure:
H224V(C-term)



Select Query Sequence & Reference Sequence to display on 3D Structure Viewer:

Query Sequence: BetaCoV/pangolin/Guangdong/1/2019[EP]_ISL_410721/2019
Reference Sequence: BetaCoV-2019nCoV/Wuhan/WIV04/2019

% AA identity: 91.280%
mutations: 111

List of variations displayed in structure (nearest residue if in loop/termini region):
S12N T20G T22A Q23A L24I P25Q A273 Y28F F32S T33Q K41T V42I S46N V47T H49V S50L T51S D53G L54Y F59Y T63S F65Y H68Y I68L G72T(68) T78E(77) F79V P85D N87K V90I S94A H01V S112N K113T T114S V127I E132N N137Y F140Y Q142S V143G S151T M153S E164T S165R R168A A1637Y N166A Q173K P174S L176M M177L L179I E180A Q183S N186L K187D N188T K196R H197V H207Y I210V L212V V213N R214S D215N Q218I D228E L229I I231A R237K Q239R A241T L244I Y248D(D249) T260M S265N S266N A269V Q281F A282S Q271A L270M K273N T290A F306L D346E I402V T403L K417R K430N H440N H41L A443S E445V F447Y A455S K478T Q483V T484E L486P Y489F Y490Q R494S Y498Q D501N H505Y K529Q N556S R554S A585S S591A S708A T747I A1070S A1078T D1084E

List of mutations not displayed in structure:
M1(L(N-term) V3(F(N-term) L5(F(N-term) V6(L(N-term) L7(H(N-term) L8(F(N-term) P9(A(N-term) N112S(C-term) V1220(C-term)

Select Query Sequence & Reference Sequence to display on 3D Structure Viewer:

Query Sequence: BetaCoV/pangolin/Guangdong/1/2019[EP]_ISL_410721/2019
Reference Sequence: BetaCoV-2019nCoV-like/bat/Yunnan/RaTG13/2013

% AA identity: 90.307%
mutations: 123

List of variations displayed in structure (nearest residue if in loop/termini region):
S12N T20G T22A Q23A L24I P25Q A273 Y28F T33Q K41T V42I S46N V47T H49V T51S D53G L54Y F59Y T63S F65Y H68Y I68L G72T(68) T78E(77) F79V P85D N87K V90I S94A H01V S112N K113T T114S V127I E132N N137Y F140Y Q142S V143G S151T M153S E164T S165R R168A A1637Y N166A Q173K P174S L176M M177L L179I E180A Q183S N186L K187D N188T K196R H197V H207Y I210V L212V V213N R214S D215N Q218I D228E L229I I231A R237K Q239R A241T L244I Y248D(D249) T260M S265N S266N A269V Q281F A282S Q271A L270M K273N T290A F306L D346E I402V T403L K417R K430N H440N H41L A443S E445V F447Y A455S K478T Q483V T484E L486P Y489F Y490Q R494S Y498Q D501N H505Y K529Q N556S A604T R634S A684S S687A S704A T743I A1086S A1074T D1080E

List of mutations not displayed in structure:
M1(L(N-term) V3(F(N-term) L5(F(N-term) V6(L(N-term) L7(H(N-term) L8(F(N-term) P9(A(N-term)