

Analisis *Whole Genome Sequencing (WGS)* SARS-CoV-2

Rafika I. Paramita

Dept. Kimia Kedokteran;
Bioinformatics Core Facilities – IMERI
Fakultas Kedokteran
Universitas Indonesia

Email : rafikaindah@ui.ac.id

Outline

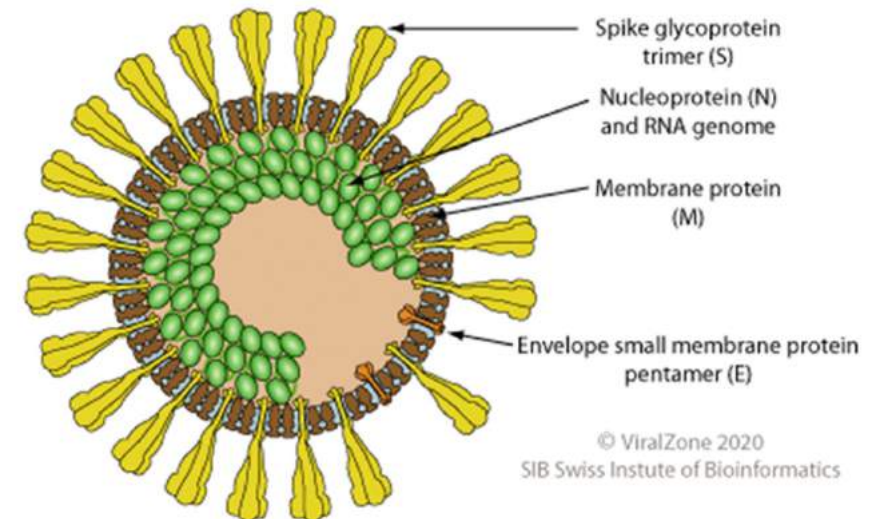
- Overview virus SARS-CoV-2
- *Whole Genome Sequencing*
- Analisis pengaruh mutasi pada protein Spike SARS-CoV-2 terhadap hACE2

SARS-CoV-2

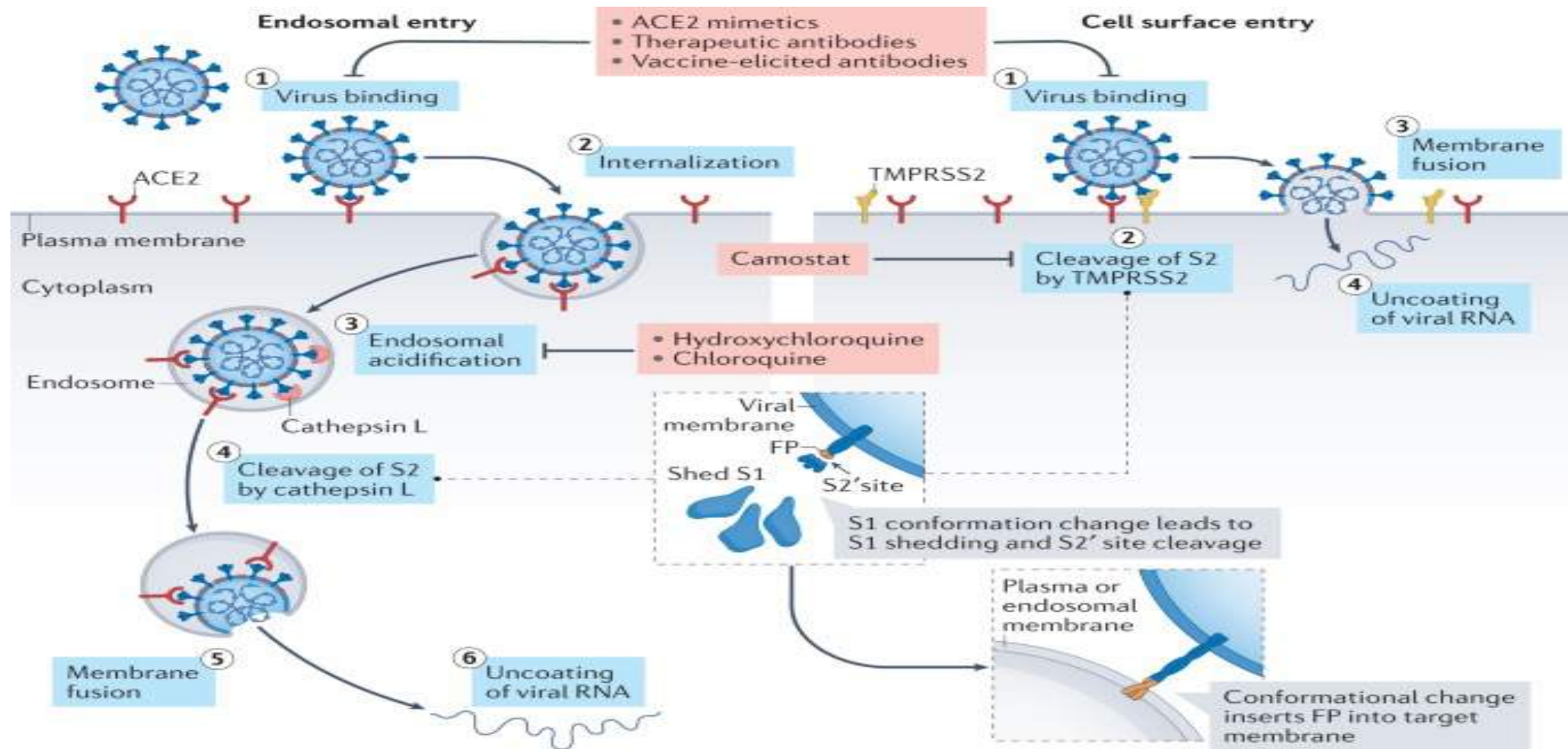
- SARS-CoV-2 merupakan virus dengan panjang ~30kb positive-sense single strand RNA yang berada dalam genus Betacoronavirus yang menyebabkan penyakit COVID-19



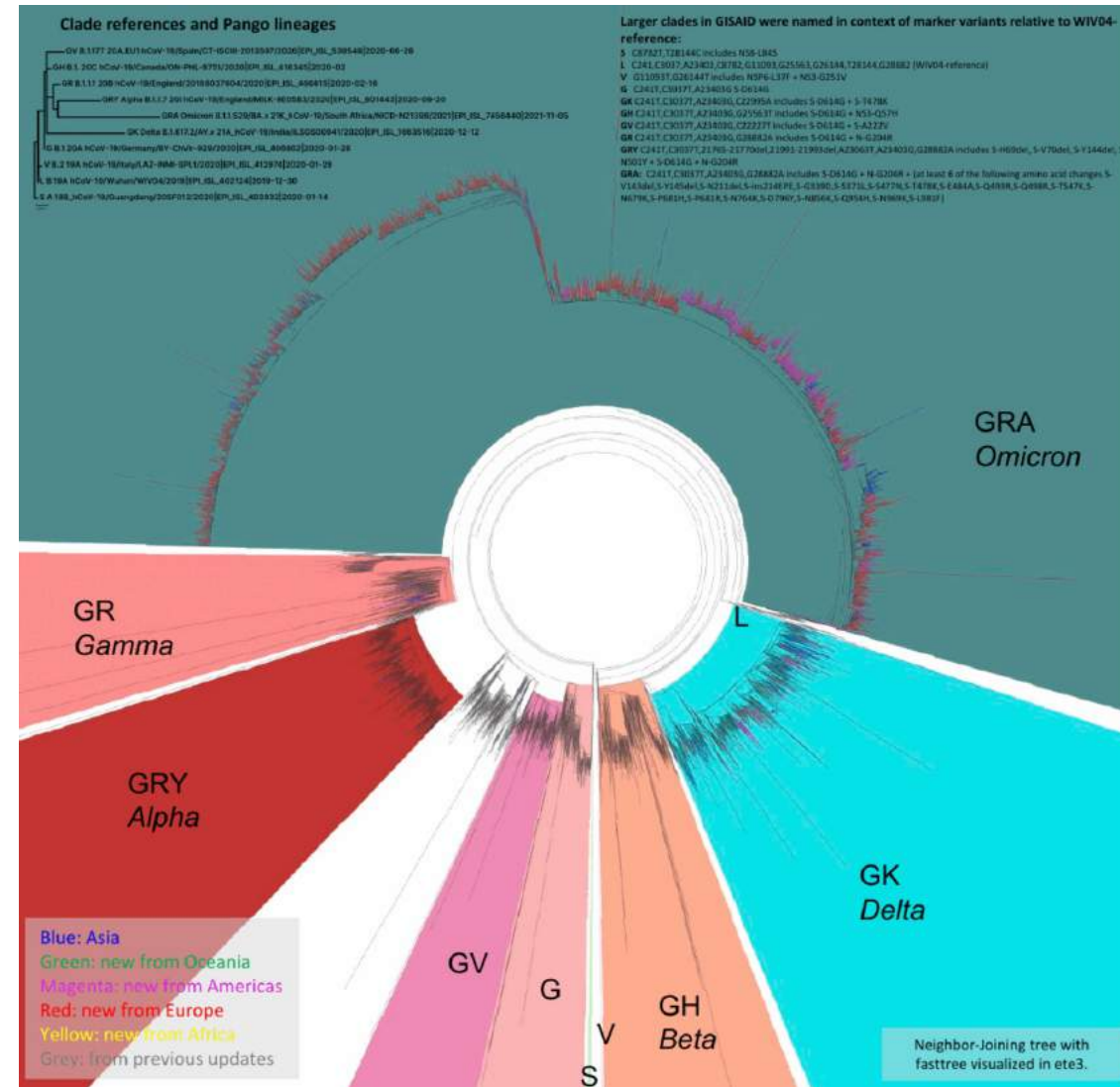
- SARS-CoV 2 tersusun atas empat protein struktural, yaitu Spike, Envelope, Membran dan Nucleocapsid



Mekanisme *cell entry* SARS-CoV-2



- Varian SARS-CoV-2 dapat diklasifikasikan menjadi:
 - Variants of interest (VOI)
 - Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda, Mu
 - Variants of Concern (VOC)
 - Alpha (B.1.1.7), Beta (B.1.351), Gamma (P1), Delta (B.1.617.2 + AY.xx), Omicron (B.1, B.1.1, B.2)



by BII/GIS. A*STAR Singapore

<https://www.epicov.org/epi3/frontend#lightbox-352698812>
<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>

Whole Genome Sequencing

Sekuensing genom

- Sekuensing genom adalah suatu metode laboratorium yang digunakan untuk menentukan susunan genetik (urutan basa nukleotida) dari organisme atau tipe sel tertentu. Metode ini dapat digunakan untuk menemukan adanya varian/mutasi pada genom.
- *Whole Genome Sequencing* → dilakukan pada keseluruhan genom organisme

Alur kerja analisis WGS SARS-CoV-2

Ekstraksi RNA

- Sample swab orofaring → PCR positif (Ct < 25)

Konversi RNA → cDNA

- RNA dengan kemurnian 1.80-2.00

Library preparation

- Menggunakan kit COVIDSeq Illumina

Sequencing

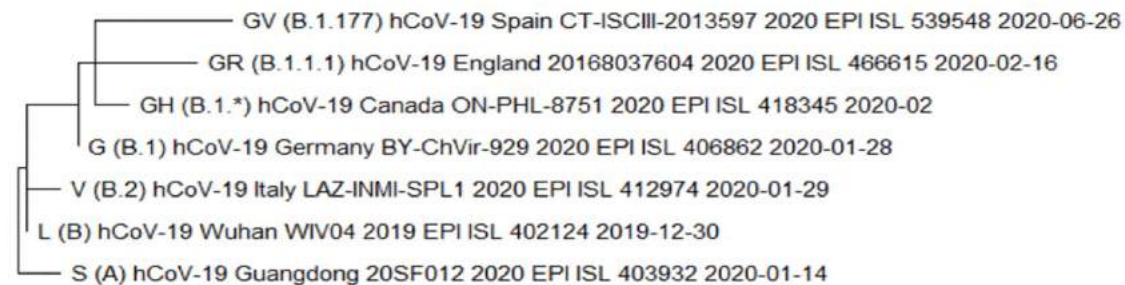
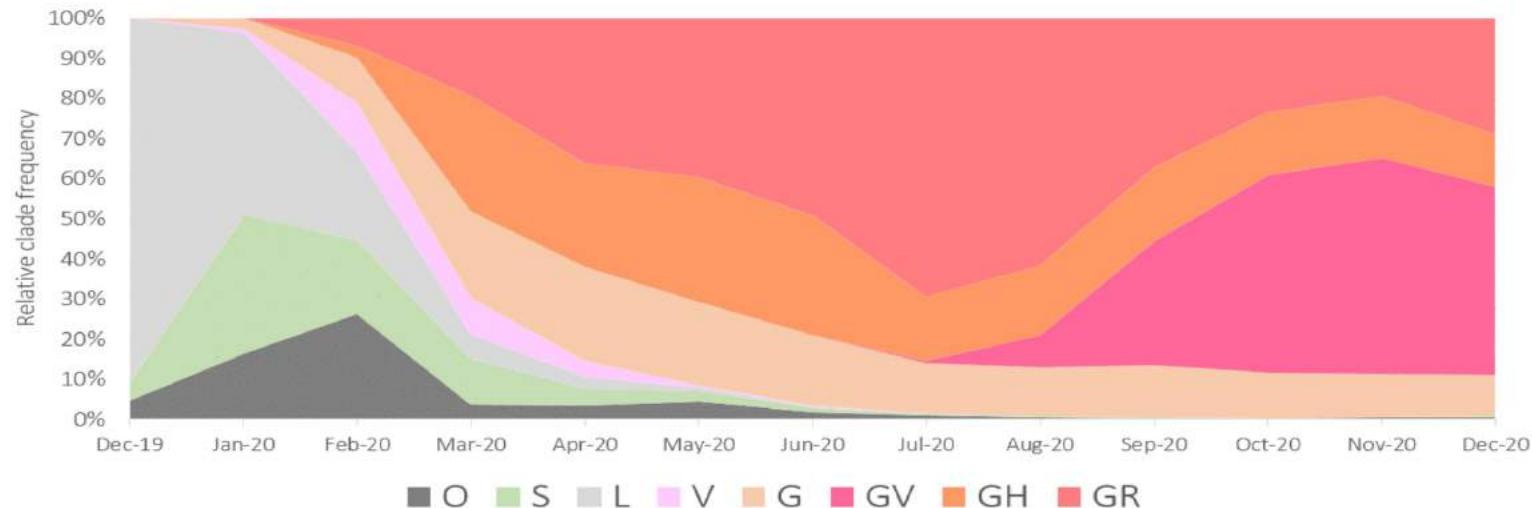
- Illumina MiSeq

Analisis Bioinformatika

- QC file fastq
- Sekuen WGS SARS-CoV-2
- Anotasi lineage

Evolusi Clade SARS-CoV-2 pada tahun 2020

Clade evolution in the first year



0.000050

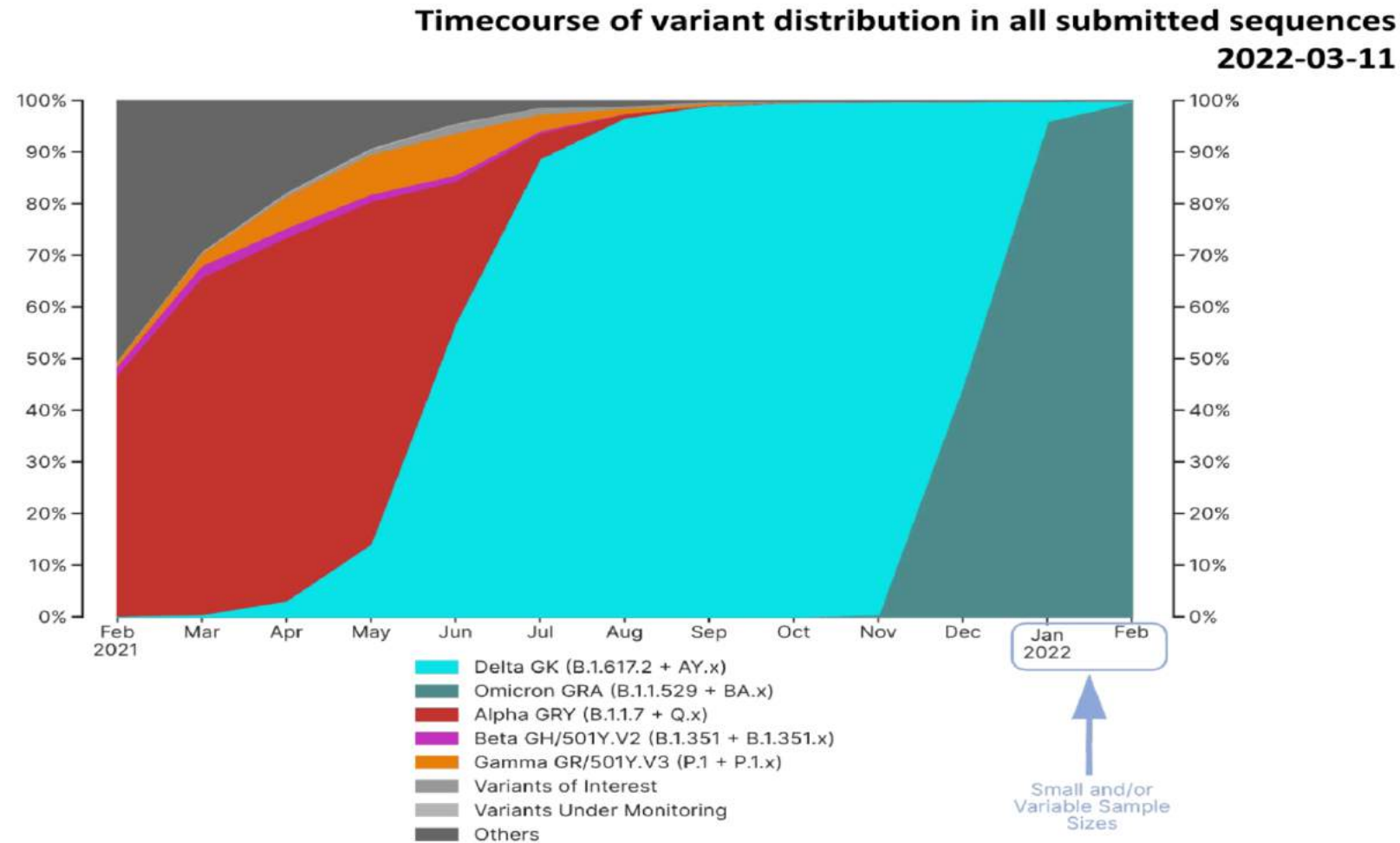
GISAID clades and
PANGO lineages

by BII/GIS, A*STAR
Singapore



<https://www.epicov.org/epi3/frontend#12b8db>

Distribusi Varian SARS-CoV-2



<https://www.epicov.org/epi3/frontend#12b8db>

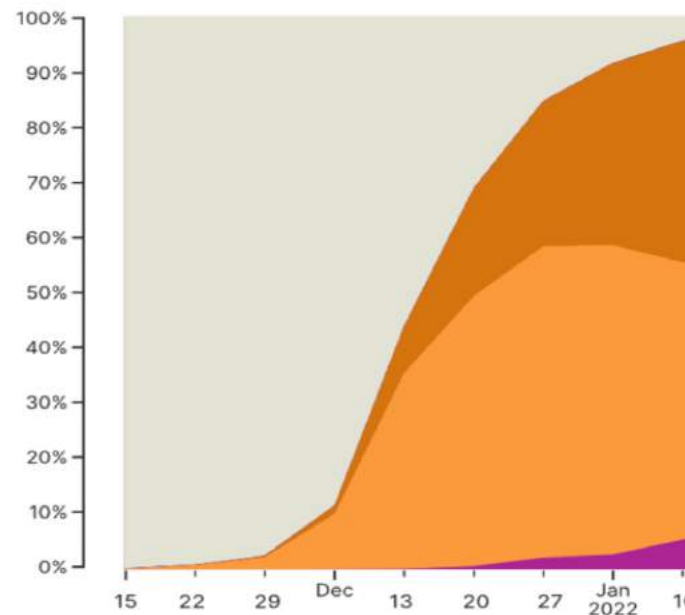
Distribusi lineage varian Omicron

Timecourse of Omicron variant sublineage distribution

2022-03-11

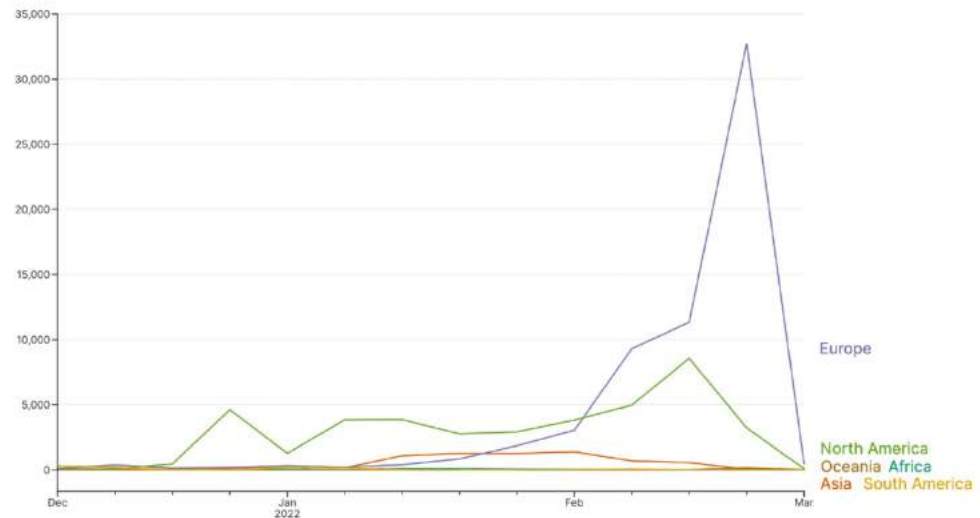
Distribution of collection dates of new sequences

2022-03-15



■ B.1.1.529
■ BA.1
■ BA.1.1
■ BA.2
■ BA.3
■ Other lineages

This slide shows NEW data in GISAID on 2022-03-15 submitted since last report 4 days ago (new by submission date, not collection date).



GISAID
by BII/GIS, A*STAR Singapore

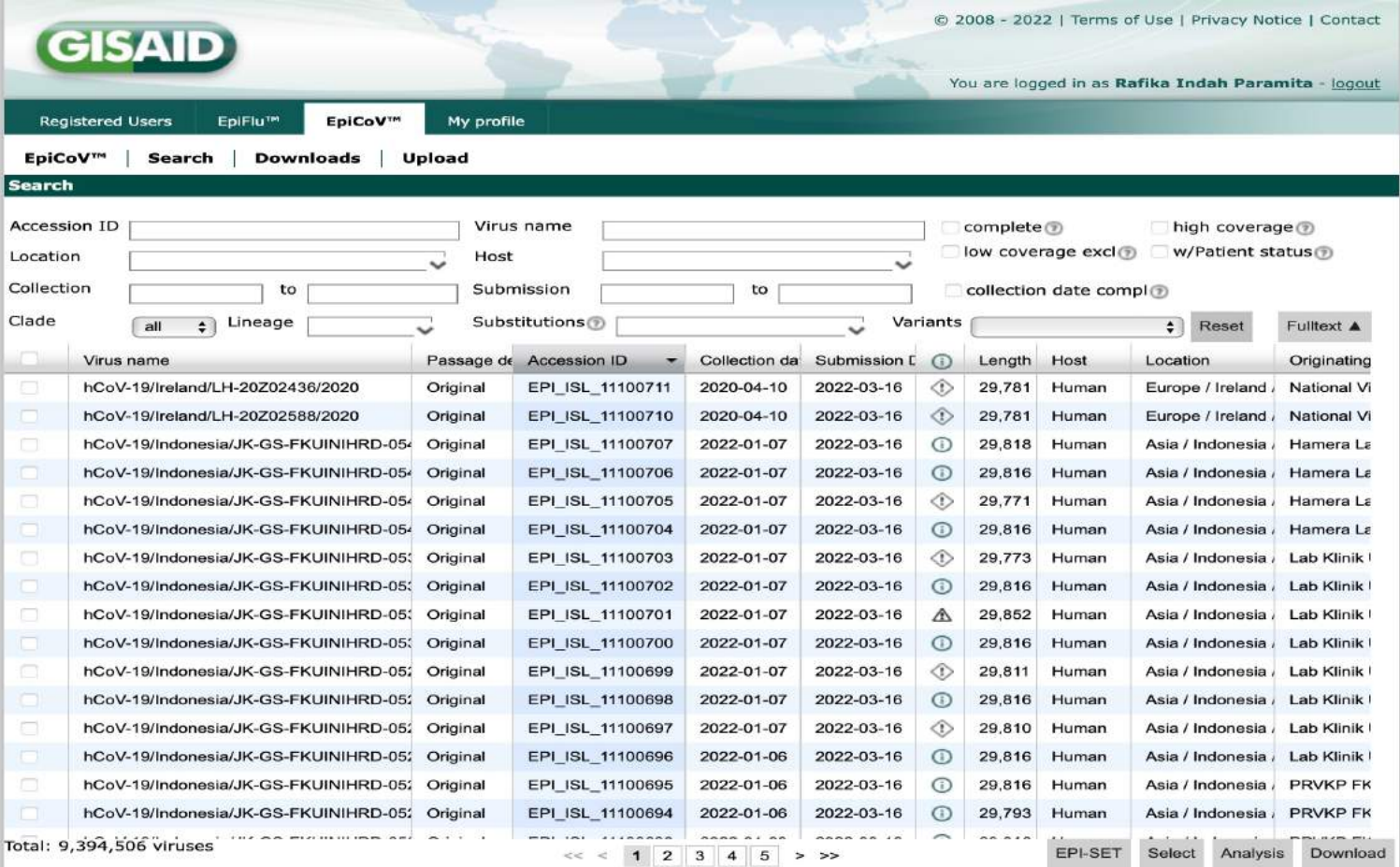
*We gratefully acknowledge
the Authors from Originating
and Submitting laboratories
of sequence data on which the
analysis is based.*

GISAID
by BII/GIS, A*STAR Singapore

See <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/> for variant information and definitions

<https://www.epicov.org/epi3/frontend#12b8db>

Analisis WGS di FKUI



GISAID

© 2008 - 2022 | Terms of Use | Privacy Notice | Contact

You are logged in as **Rafika Indah Paramita** - [logout](#)

Registered Users | EpiFlu™ | **EpiCoV™** | My profile

EpiCoV™ | Search | Downloads | Upload

Search

Accession ID Virus name ☐ complete ☐ high coverage ☐
 Location Host ☐ low coverage excl ☐ w/Patient status ☐
 Collection to Submission to ☐ collection date compl ☐
 Clade Lineage Substitutions Variants

☐	Virus name	Passage date	Accession ID	Collection date	Submission date	Length	Host	Location	Originating
☐	hCoV-19/Ireland/LH-20202436/2020	Original	EPI_ISL_11100711	2020-04-10	2022-03-16	29,781	Human	Europe / Ireland	National Vi
☐	hCoV-19/Ireland/LH-20202588/2020	Original	EPI_ISL_11100710	2020-04-10	2022-03-16	29,781	Human	Europe / Ireland	National Vi
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100707	2022-01-07	2022-03-16	29,818	Human	Asia / Indonesia	Hamera La
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100706	2022-01-07	2022-03-16	29,816	Human	Asia / Indonesia	Hamera La
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100705	2022-01-07	2022-03-16	29,771	Human	Asia / Indonesia	Hamera La
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100704	2022-01-07	2022-03-16	29,816	Human	Asia / Indonesia	Hamera La
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100703	2022-01-07	2022-03-16	29,773	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100702	2022-01-07	2022-03-16	29,816	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100701	2022-01-07	2022-03-16	29,852	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100700	2022-01-07	2022-03-16	29,816	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100699	2022-01-07	2022-03-16	29,811	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100698	2022-01-07	2022-03-16	29,816	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100697	2022-01-07	2022-03-16	29,810	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100696	2022-01-06	2022-03-16	29,816	Human	Asia / Indonesia	Lab Klinik I
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100695	2022-01-06	2022-03-16	29,816	Human	Asia / Indonesia	PRVKP FK
☐	hCoV-19/Indonesia/JK-GS-FKUIIHRD-055	Original	EPI_ISL_11100694	2022-01-06	2022-03-16	29,793	Human	Asia / Indonesia	PRVKP FK

Total: 9,394,506 viruses

Important note: In the GISAID EpiFlu™ Database Access Agreement, you have accepted certain terms and conditions for viewing and using data regarding influenza viruses. To the extent the Database contains data relating to non-influenza viruses, the viewing and use of these data is subject to the same terms and conditions, and by viewing or using such data you agree to be bound by the terms of the GISAID EpiFlu™ Database Access Agreement in respect of such data in the same manner as if they were data relating to influenza viruses.

<https://www.epicov.org/epi3/frontend#34365a>

Virus detail	
Virus name:	hCoV-19/Indonesia/JK-GS-FKUINIHRD-0511/2022
Accession ID:	EPI_ISL_11100683
Type:	betacoronavirus
Clade:	GRA
Pango Lineage:	BA.1 (Pango v.3.1.20 2022-02-28), Omicron (BA.1-like) (Scorpio)
AA Substitutions:	Spike A67V, Spike D614G, Spike D796Y, Spike E484A, Spike G142D, Spike G339D, Spike G446S, Spike G496S, Spike H69del, Spike H655Y, Spike ins214EPE, Spike K417N, Spike L212I, Spike L981F, Spike N211del, Spike N440K, Spike N501Y, Spike N679K, Spike N764K, Spike N856K, Spike N969K, Spike P681H, Spike Q493R, Spike Q498R, Spike Q954H, Spike S371L, Spike S373P, Spike S375F, Spike S477N, Spike T95I, Spike T478K, Spike V70del, Spike V143del, Spike Y144del, Spike Y145del, Spike Y505H, E T9I, M A63T, M Q19E, N E31del, N G204R, N P13L, N R32del, N R203K, N S33del, NSP3 A1892T, NSP3 K38R, NSP3 L1266I, NSP3 S1265del, NSP4 T492I, NSP5 P132H, NSP6 G107del, NSP6 I189V, NSP6 L105del, NSP6 S106del, NSP9 T34I, NSP12 P323L, NSP14 I42V
Variant:	VOC Omicron GRA (B.1.1.529+BA.*) first detected in Botswana/Hong Kong/South Africa
Passage details/history:	Original

Sample information	
Collection date:	2022-01-05
Location:	Asia / Indonesia / Jakarta
Host:	Human
Additional location information:	
Gender:	Female
Patient age:	37
Patient status:	unknown
Specimen source:	Oropharyngeal swab
Additional host information:	
Sampling strategy:	
Outbreak:	
Last vaccinated:	
Treatment:	
Sequencing technology:	Illumina MiSeq
Assembly method:	BWA + iVar
Coverage:	3.120x
Comment:	📍 Insertion of 6 nucleotides when compared to the reference WIV04 sequence. Gap of 36 nucleotides when compared to the reference WIV04 sequence.

Institute information	
Originating lab:	Smartco Laboratorium
Address:	Jl. Hang Jebat II No.1, RW.4, Gunung, Kec. Kby. Baru, Daerah Khusus Ibukota Jakarta 12138
Sample ID given by the originating laboratory:	
Submitting lab:	Faculty of Medicine, Universitas Indonesia
Address:	Salemba Raya 6, Jakarta
Sample ID given by the submitting laboratory:	
Authors:	Fera Ibrahim, Fadilah, Rela Febriani, Indri Nurzahra, Andi Yasmon, Rafika Indah Paramita, Khaerunissa Anbar Istiadi, Linda Erlina, Ajeng Megawati Fajrin, Wahyu Dian Utari, Fithriyah, Beti Ernawati, Pratiwi Sudarmono, Budi Wiweko, Badriul Hegar, Anis Karuniawati, Ari Fahrial Syam
Submitter information	
Submitter:	Syam, Ari Fahrial
Submission Date:	2022-03-16
Address:	Jl. Salemba Raya No. 6 10430 Jakarta Pusat Indonesia
FASTA	

>hCoV-19/Indonesia/JK-GS-FKUINIHRD-0511/2022|EPI_ISL_11100683|2022-01-05
TACCTTCCTAGGTAACAACCAACCAACTTTCGATCTCTTGATAGATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGC
TGTCACCTCGGCTGCATGCTTAGTGCACCTCAGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAAC
CGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCTGTTGTCAGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTG
ACCGAAAGGTAAAGATGGAGAGCCTTGTCCTGGTTTCAACGAGAAAACACACGTCCAACCTCAGTTTGCCTGTTTTACAGG
TTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGC
ACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTTCATCAACCGTTCGGATGC
TCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTTCAGTACGGTCGTAGTGGTGAGA
CACTTGGTGTCTTGTCCCTCATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAA
GGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTAGGCGACGAGCTTGGCACTGATCCTTATGAAGA
TTTTCAAGAAAACCTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACA
CTCGCTATGTCGATAACAACCTCTGTGGCCCTGATGGCTACCCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGT
AAAGCTTCATGCACCTTTGTCCGAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCA
TGAAATTGCTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAATTAATTTGGCAAGAAAT
TTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCATAATCAAGACTATTCAACCAAGGGTTGAA
AAGAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAATGCAACCAAATGTG
CCTTTCACACTCTCATGAAGTGTGATCATTTGTGGTGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTTCGAAT
TTTGTGGCACTGAGAATTTGACTAAAGAAGGTGCCACTACTTGTGGTTACTTACCCCAAAATGCTGTTGTTAAAAATTTAT
TGCCAGCATGTCACAATTGAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATTAATGAATCTGGCTTGAAAACCAT
TCTTCGTAAGGGTGGTCGCACTATTGCCTTTGAGGCTGTGTGTTCTCTTATGTTGGTTGCCATAACAAGTGTGCCATT
GGGTTCCACGTGCTAGCGCTAACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAAC
CTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATATTGTTGGTGACTTTAAACTTAATGAAGAGATCGCCATTAT
TTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAACGTGTGAAAGGTTTGGATTATAAAGCATTCAACAAATTTG
TTGAATCCTGTGTTAATTTTAAAGTTACAAAAGGAAAGCTAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATA



UNIVERSITAS
INDONESIA

Veritas, Probatas, Iustitia

FAKULTAS

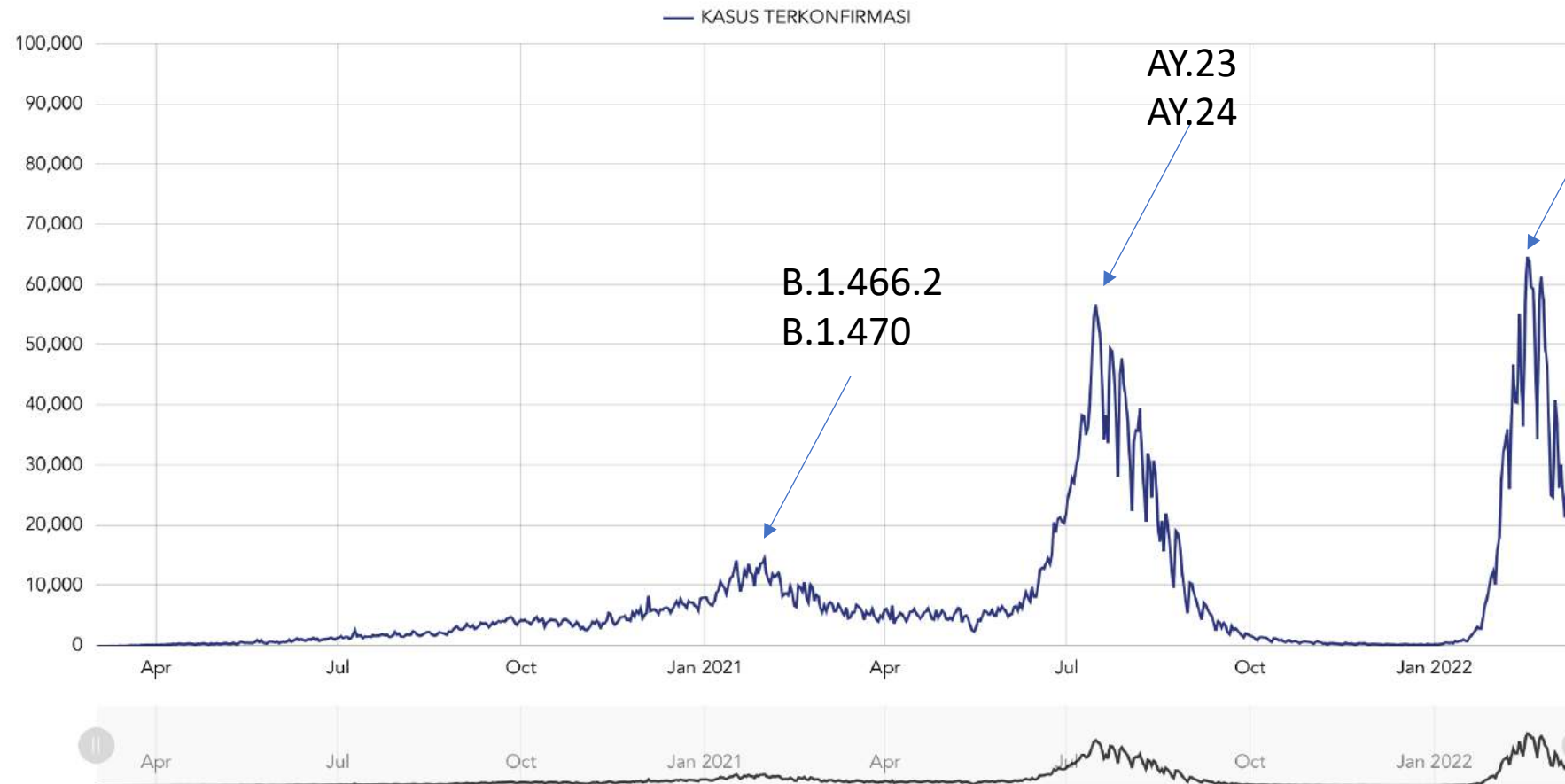
KEDOKTERAN

Analisis pengaruh mutasi pada protein Spike SARS-CoV-2 terhadap hACE2

Kasus COVID-19 di Indonesia

Perkembangan Kasus Terkonfirmasi Positif Covid-19 Per-Hari

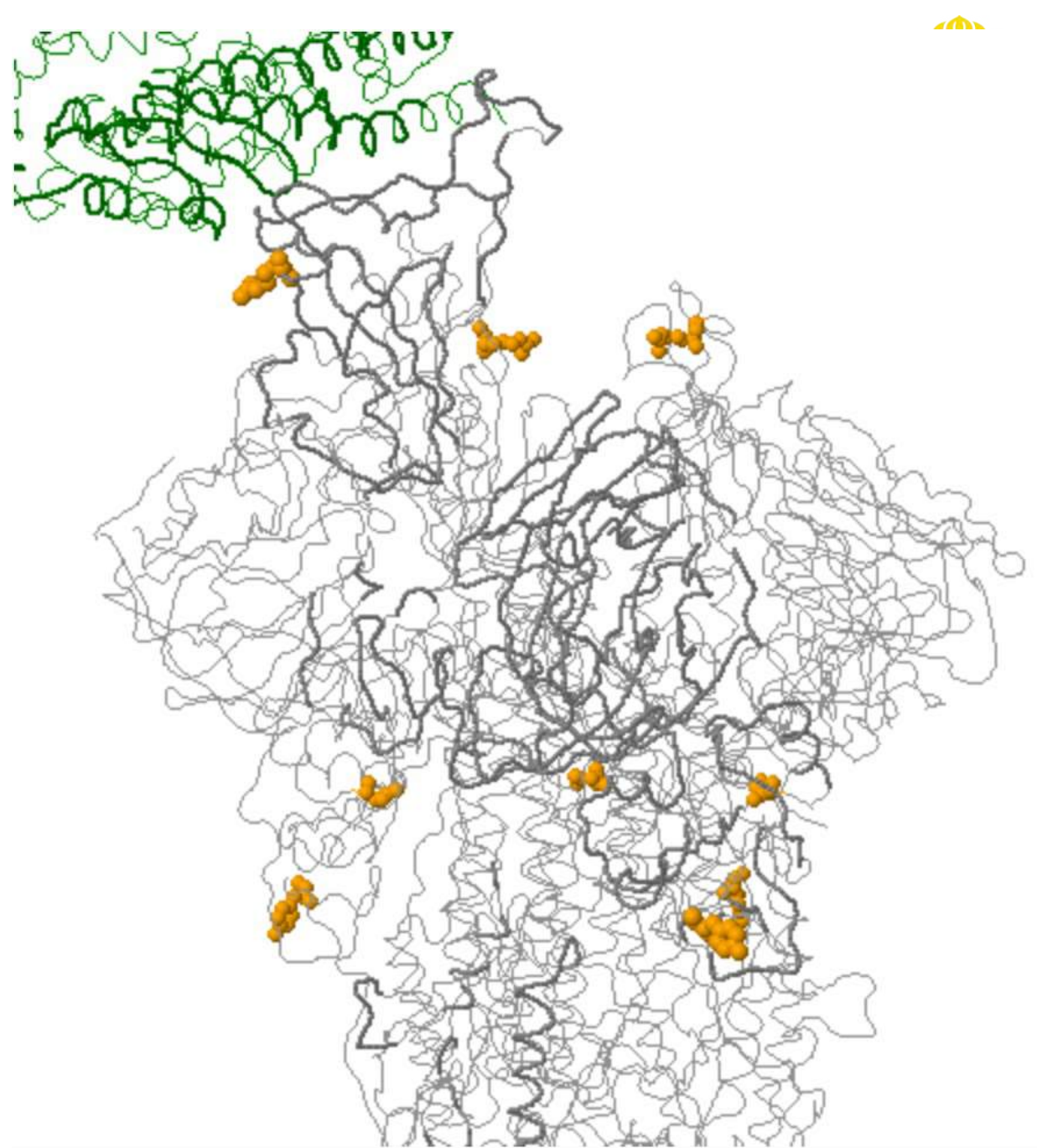
NASIONAL



<https://covid19.go.id/peta-sebaran>

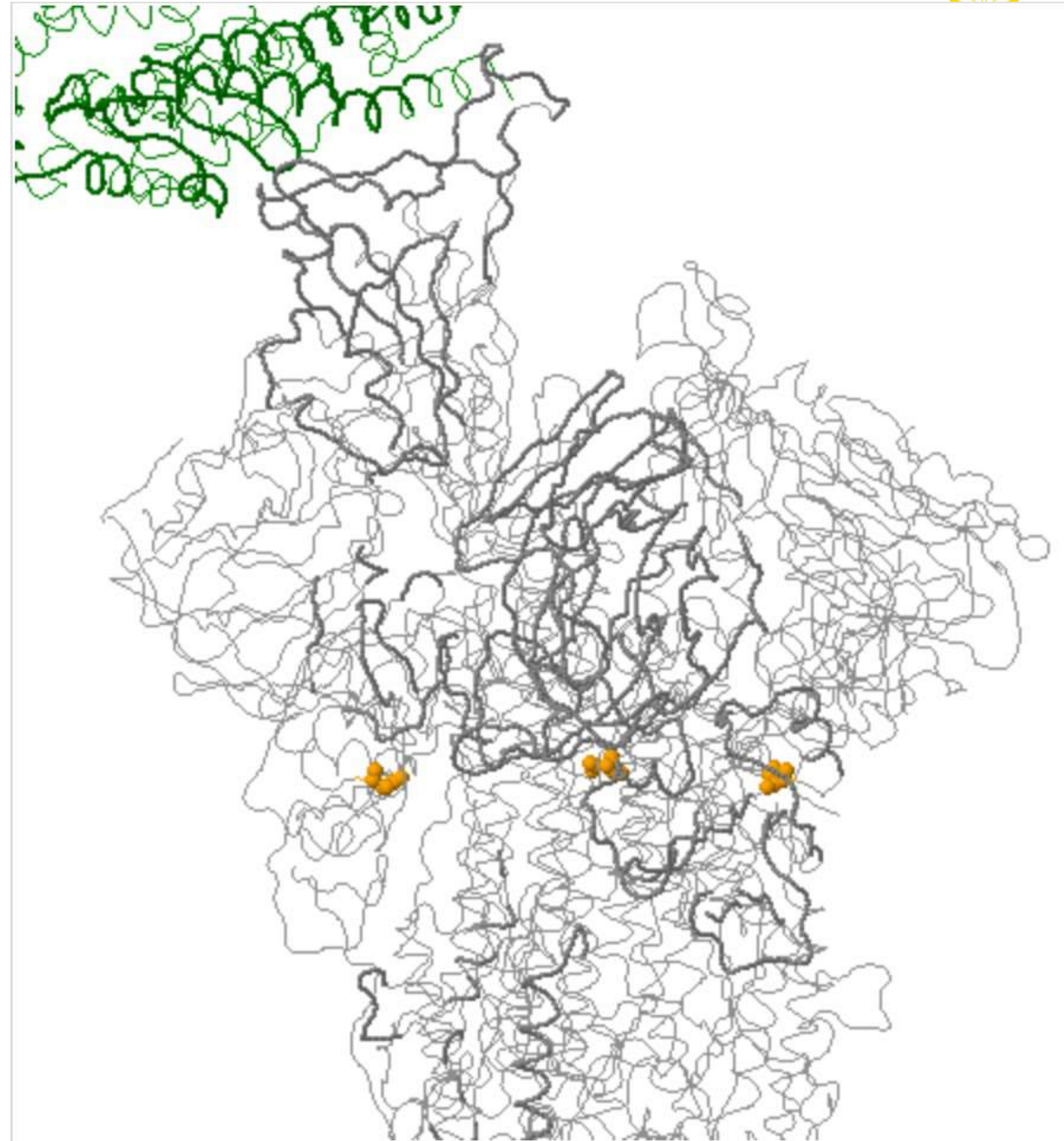
B.1.466.2

- Pada spike protein varian B.1.466.2, terjadi perubahan asam amino **N439K**, **D614G**, **P681R**



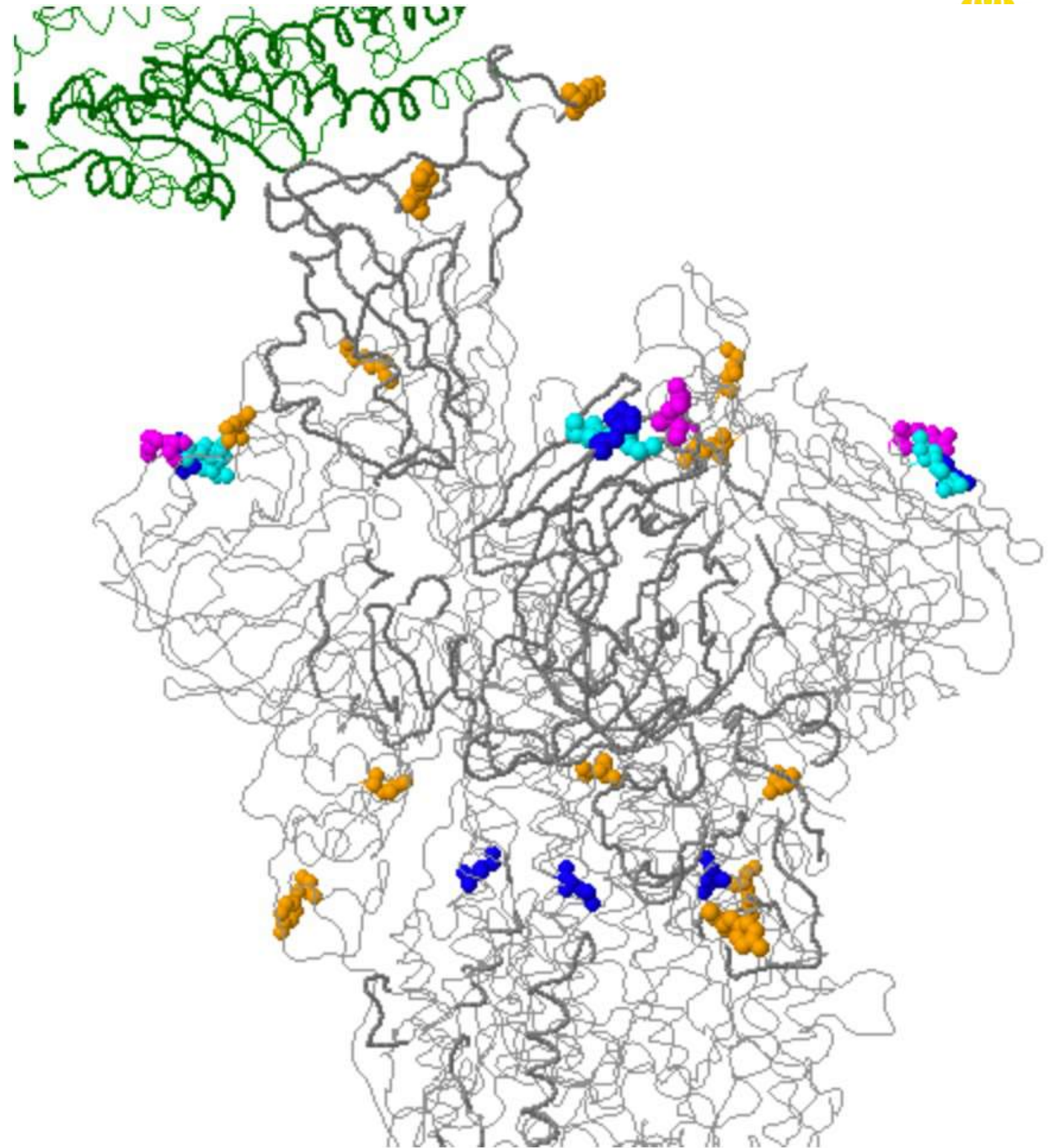
B.1.470

- Pada spike protein varian B.1.4670, terjadi perubahan asam amino **D614G**



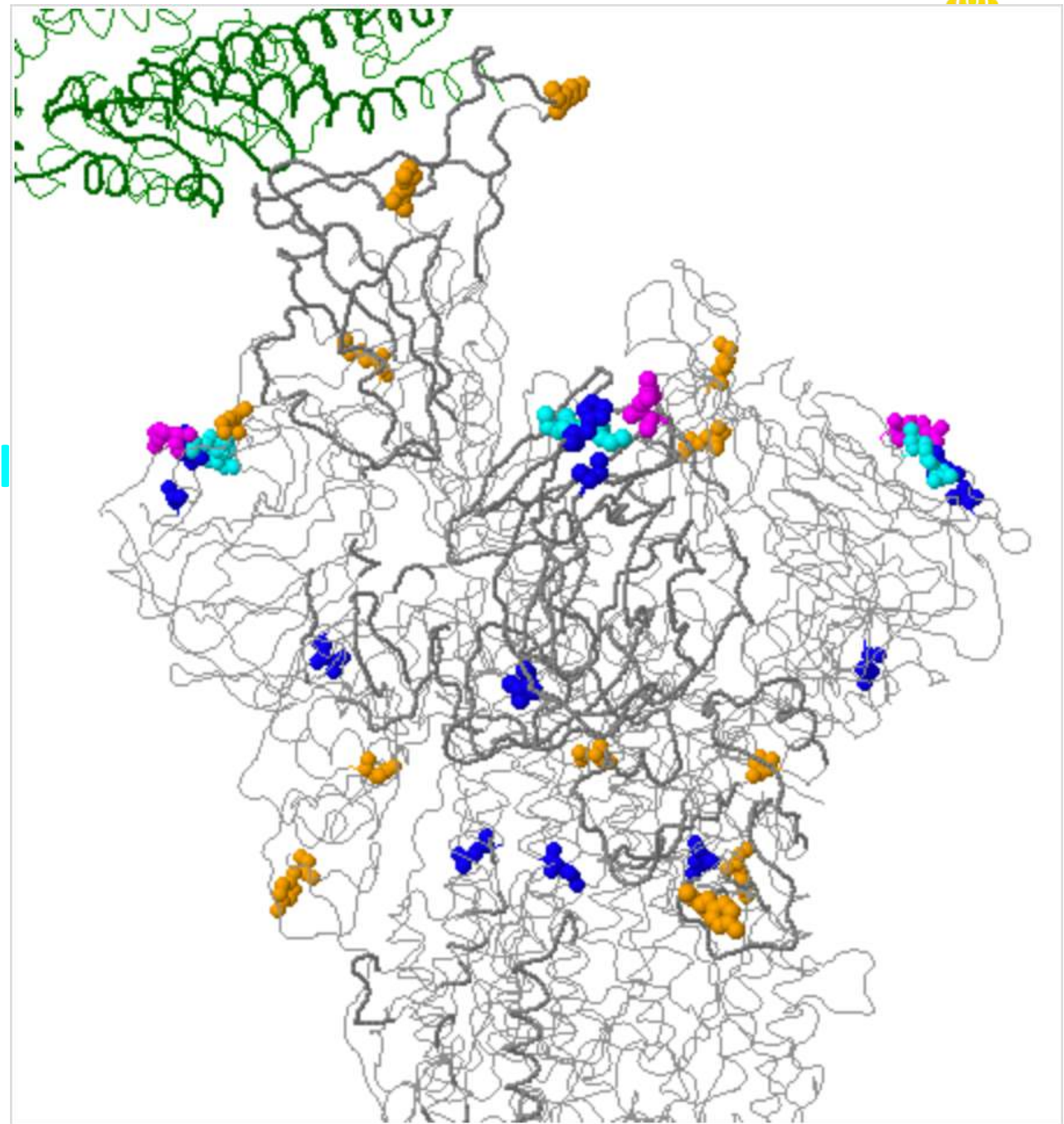
AY.23

- Pada spike protein varian AY.23, terjadi perubahan asam amino **T19R**, **E156G**, **F157del**, **R158del**, **L452R**, **T478K**, **D614G**, **P681R**, **D950N**



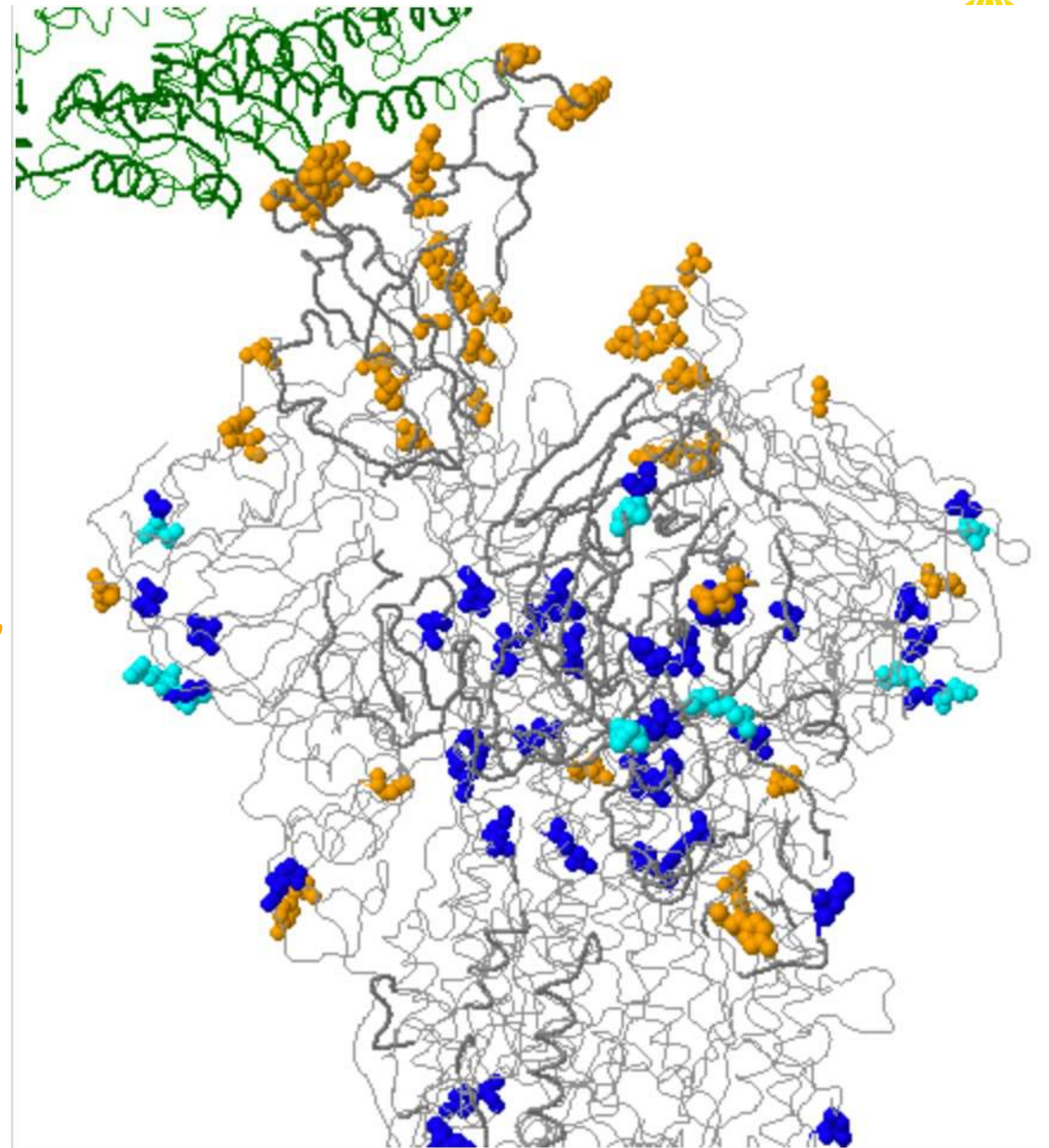
AY.24

- Pada spike protein varian AY.24, terjadi perubahan asam amino **T19R**, **G142D**, **E156G**, **F157del**, **R158del**, **A222V**, **L452R**, **T478K**, **D614G**, **P681R**, **D950N**



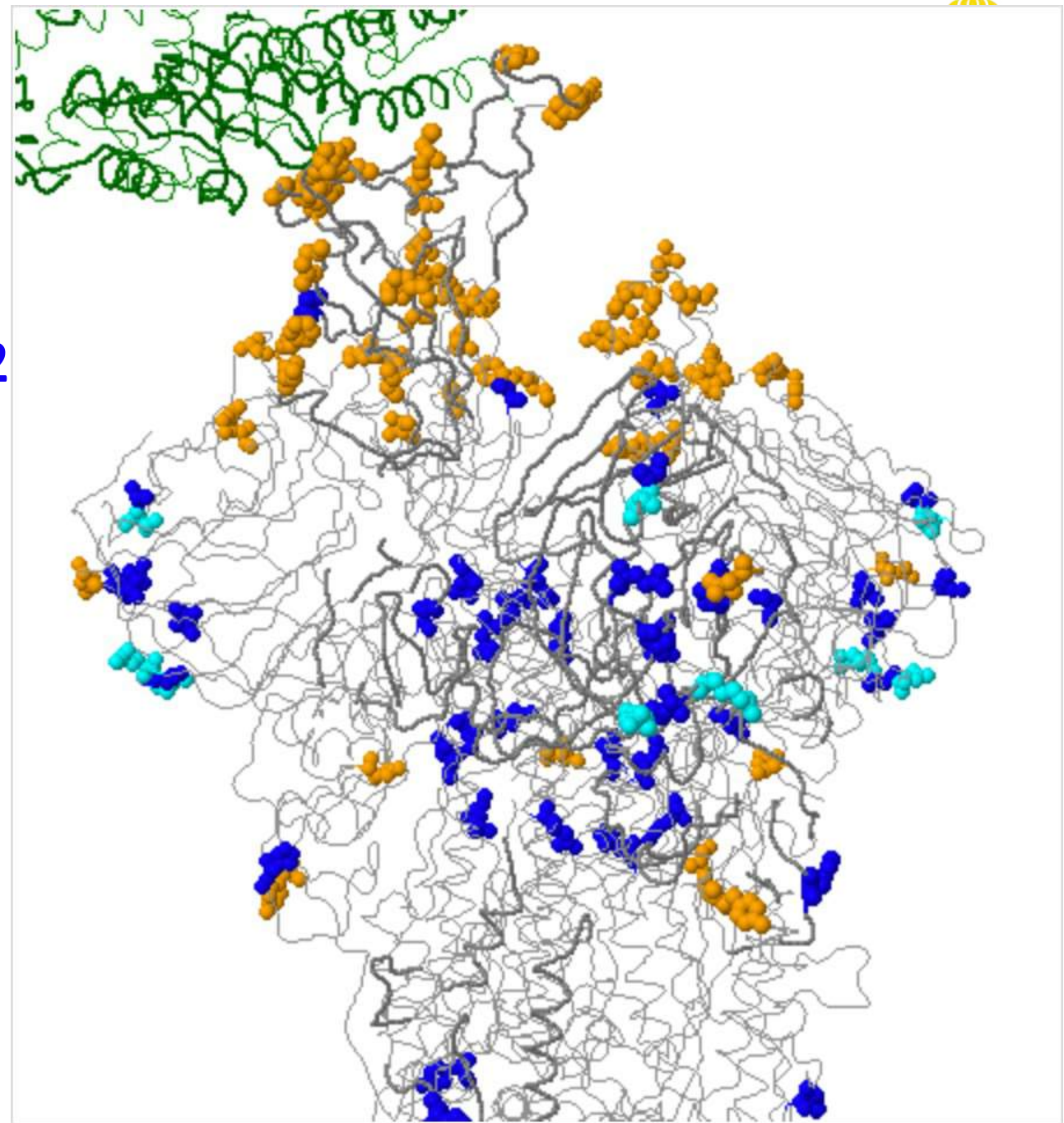
BA.1

- Pada spike protein varian BA.1, terjadi perubahan asam amino **A67V**, **H69del**, **V70del**, **T95I**, **G142D**, **V143del**, **Y144del**, **Y145del**, **N211del**, **L212I**, **ins214EPE**, **G339D**, **K417N**, **G446S**, **S477N**, **T478K**, **E484A**, **Q493R**, **G496S**, **Q498R**, **N501Y**, **Y505H**, **T547K**, **D614G**, **H655Y**, **N679K**, **P681H**, **N764K**, **D796Y**, **N856K**, **Q954H**, **N969K**, **L981F**



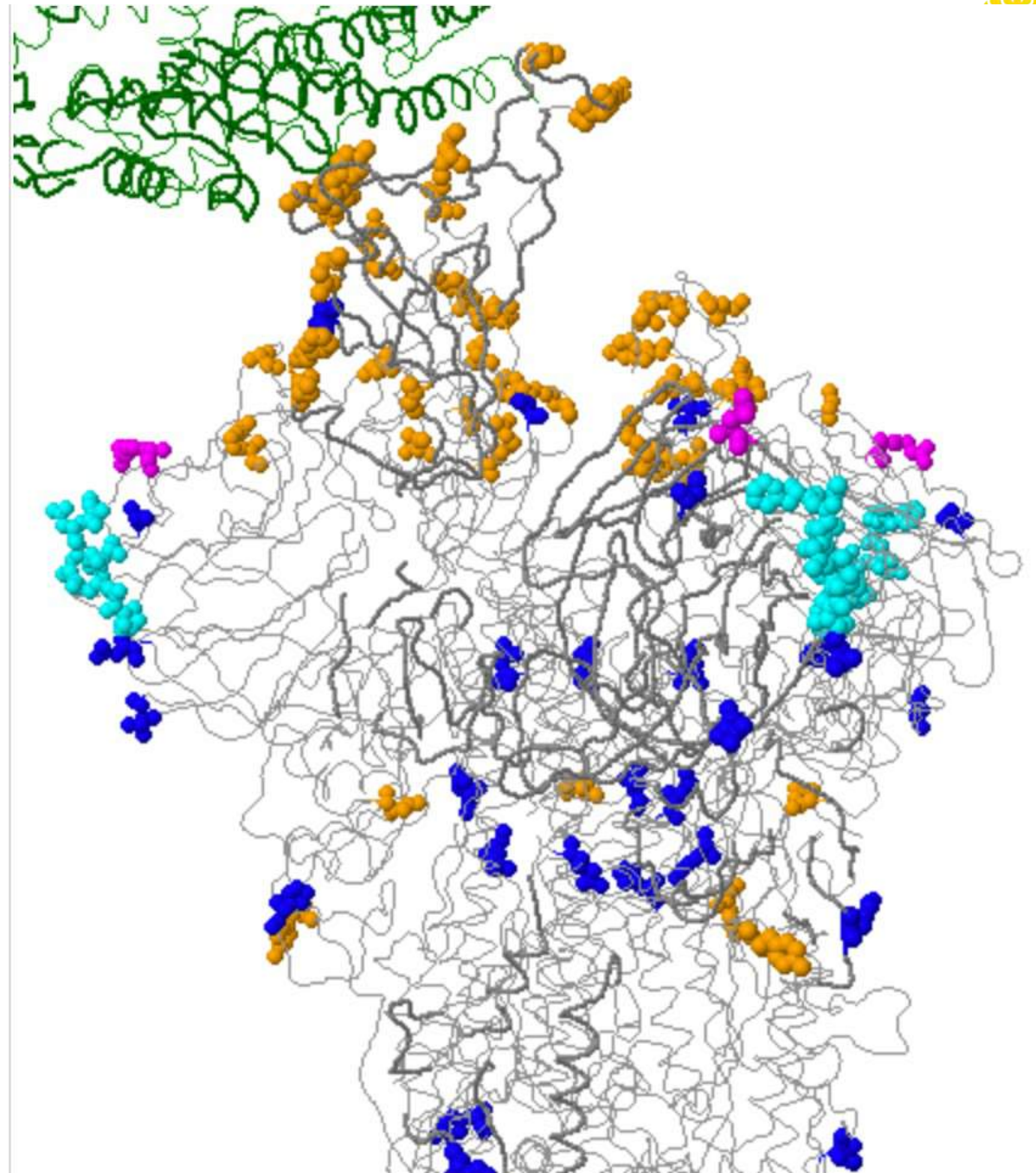
BA.1.1

- Pada spike protein varian BA.1.1, terjadi perubahan asam amino **A67V**, **H69del**, **V70del**, **T95I**, **G142D**, **V143del**, **Y144del**, **Y145del**, **G181V**, **N211del**, **L212I**, **ins214EPE**, **G339D**, **R346K**, **S371L**, **S373P**, **S375F**, **K417N**, **N440K**, **G446S**, **S477N**, **T478K**, **E484A**, **Q493R**, **G496S**, **Q498R**, **N501Y**, **Y505H**, **T547K**, **D614G**, **H655Y**, **N679K**, **P681H**, **N764K**, **D796Y**, **N856K**, **Q954H**, **N969K**, **L981F**



BA.2

- Pada spike protein varian BA.2, terjadi perubahan asam amino **T19I**, **L24del**, **P25del**, **P26del**, **A27S**, **G142D**, **V213G**, **G339D**, **S371F**, **S373P**, **S375F**, **R408S**, **K417N**, **N440K**, **S477N**, **T478K**, **E484A**, **Q493R**, **Q498R**, **N501Y**, **Y505H**, **D614G**, **H655Y**, **N679K**, **P681H**, **N764K**, **D796Y**, **Q954H**, **N969K**

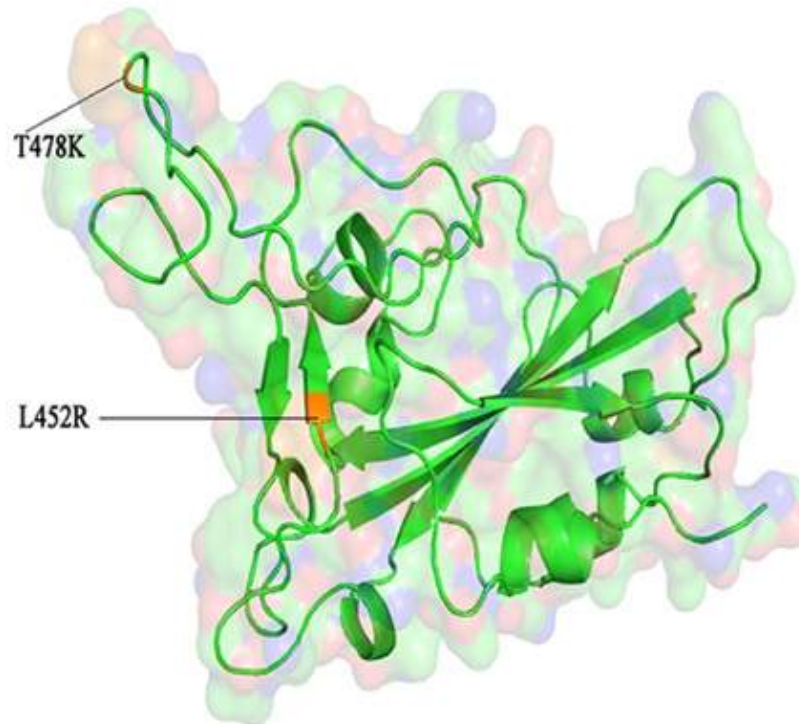


Perubahan AA	B.1.466.2	B.1.4670	AY.23	AY.24	BA.1	BA.1.1	BA.2
T19R			V	V			V
L24del							V
P25del							V
P26del							V
A27S							V
A67V					V	V	
H69del					V	V	
V70del					V	V	
T95I					V	V	
G142D				V	V	V	V
V143del					V	V	
Y144del					V	V	
Y145del					V	V	
E156G			V	V			
F157del			V	V			
R158de			V	V			
G181V						V	
N211del					V	V	
V213G							V
L212I					V		
Ins214EPE					V	V	
A222V				V			
G339D					V	V	V
R346K						V	
S371L						V	V
S373P						V	V
S375F						V	V

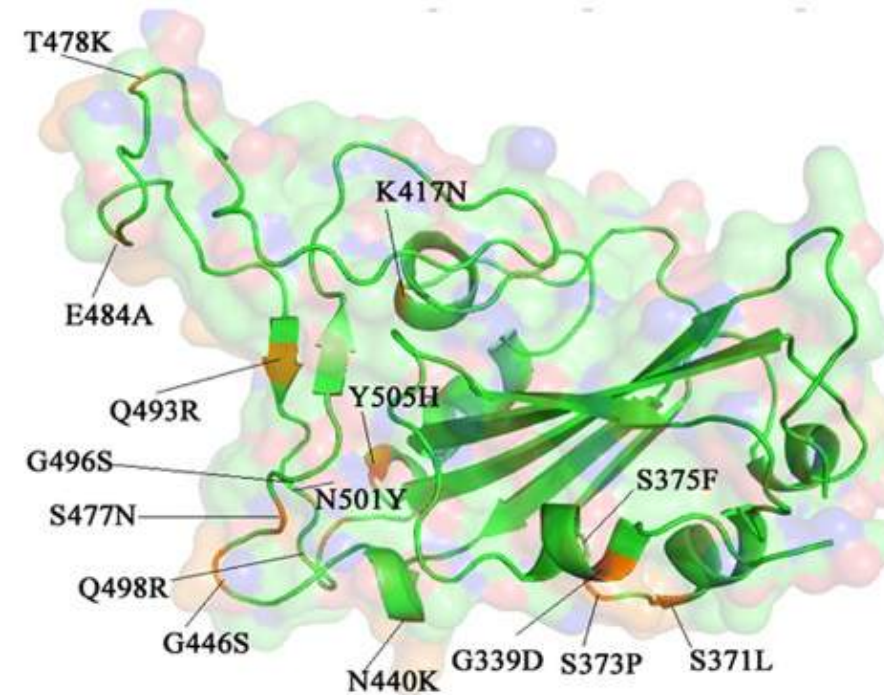
Perubahan AA	B.1.466.2	B.1.4670	AY.23	AY.24	BA.1	BA.1.1	BA.2
R408S							V
K417N					V	V	V
N439K							
N440K						V	V
G446S					V	V	
L452R			V	V			
S477N					V	V	V
T478K			V	V	V	V	V
E484A					V	V	V
Q493R	V				V	V	V
G496S					V	V	
Q498R					V	V	V
N501Y					V	V	V
Y505H					V	V	V
T547K					V	V	
D614G	V	V	V	V	V	V	V
H655Y					V	V	V
N679K					V	V	V
P681R	V		V	V	V	V	V
N764K					V	V	V
D796Y					V	V	V
N856K					V	V	
D950N			V	V			
Q954H					V	V	V
N969K					V	V	V
L981F					V	V	

Perbandingan RBD Spike varian Delta dengan Omicron

(A) DELTA-RBD



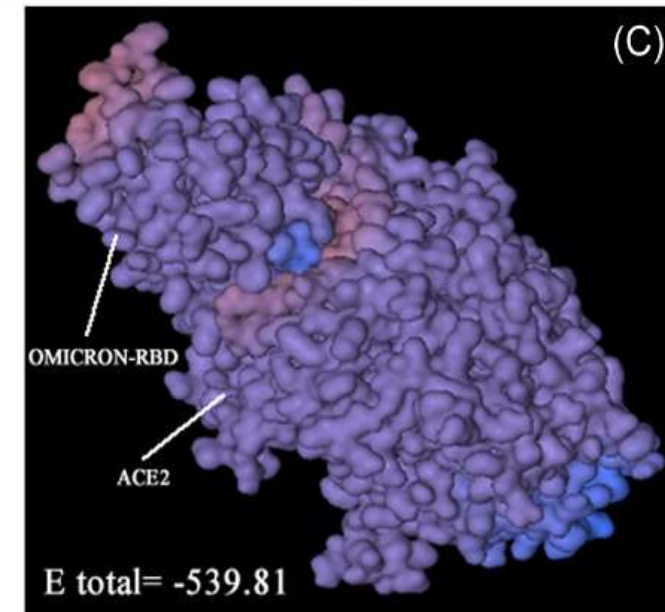
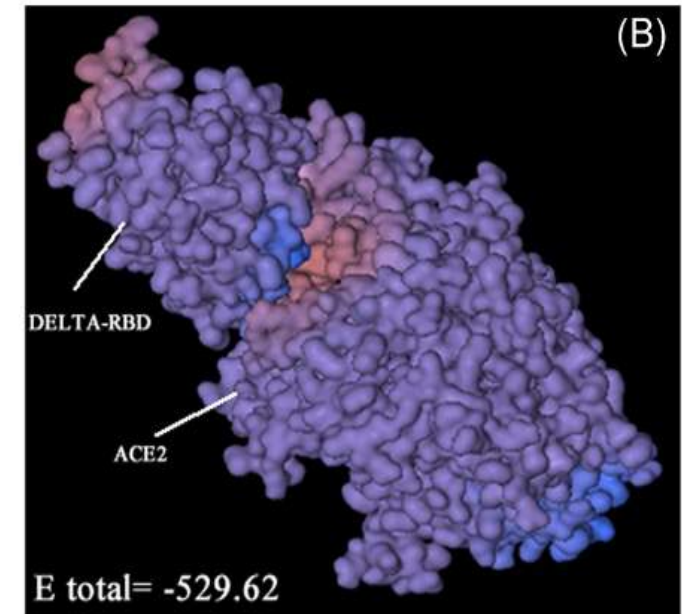
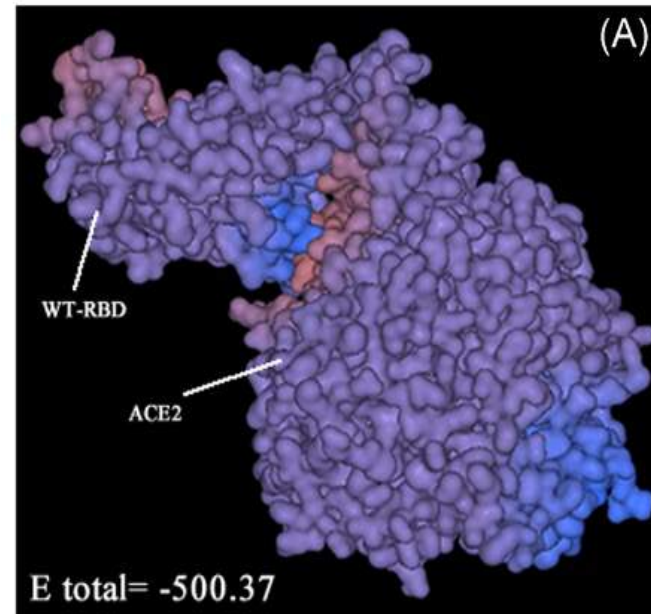
(B)OMICRON-RBD



Perbandingan RBD Spike varian Delta dengan Omicron

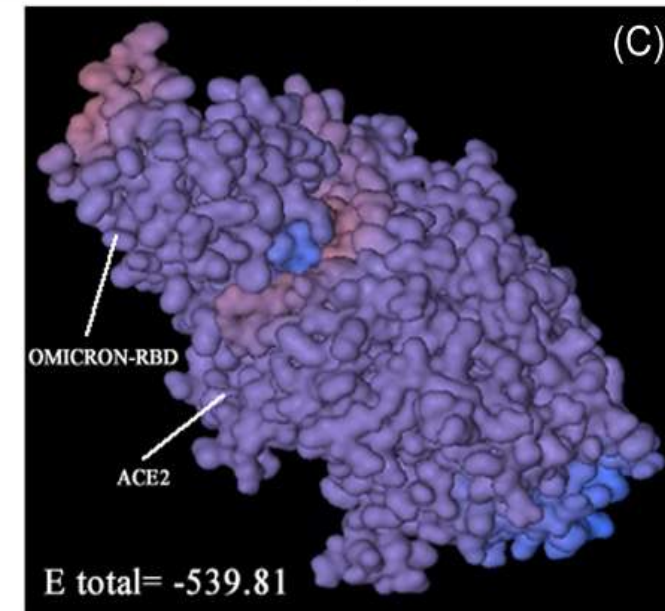
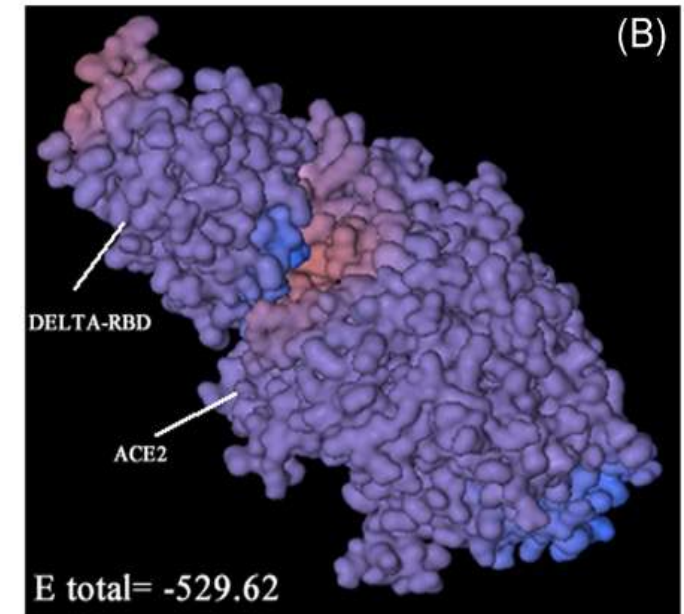
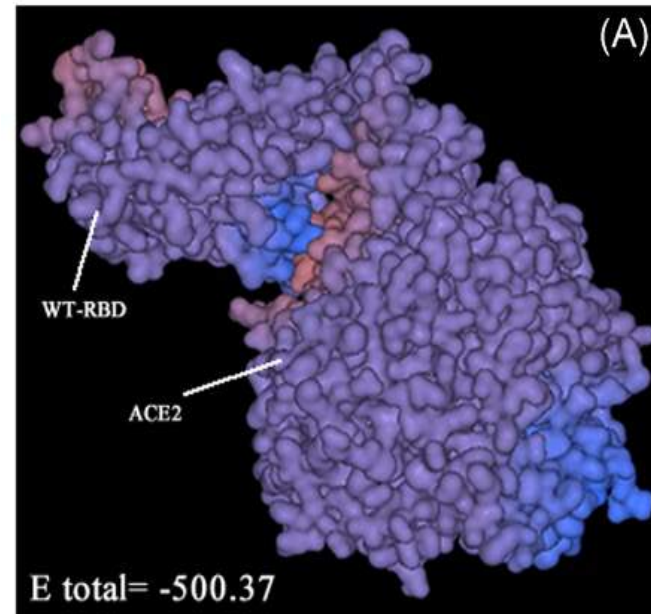
- Hasil docking antara hACE2 dengan Receptor Binding Protein (RBD) Spike: A. Wild-type; B. Delta, C. Omicron (B.1.1) menunjukkan:

RBD Omicron memiliki afinitas ikatan paling tinggi terhadap hACE2 dibandingkan dengan wild-type dan varian Delta → ikatan RBD Omicron dengan hACE2 lebih stabil



Perbandingan RBD Spike varian Delta dengan Omicron

- Lebih baiknya afinitas ikatan RBD Omicron disebabkan oleh kontribusi asam amino: Q493R, N501Y, S371L, S373P, S375F, Q498R, dan T478

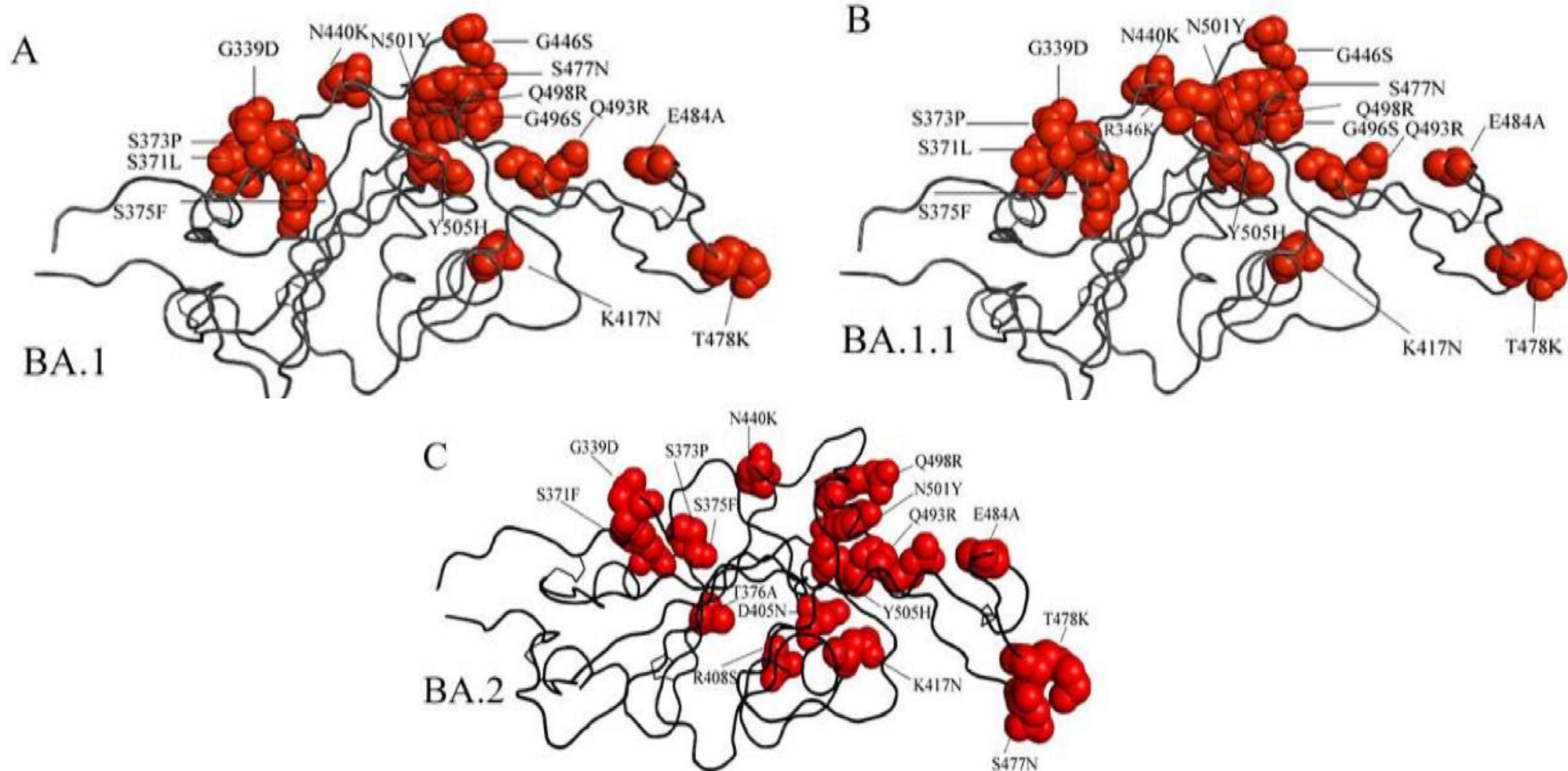


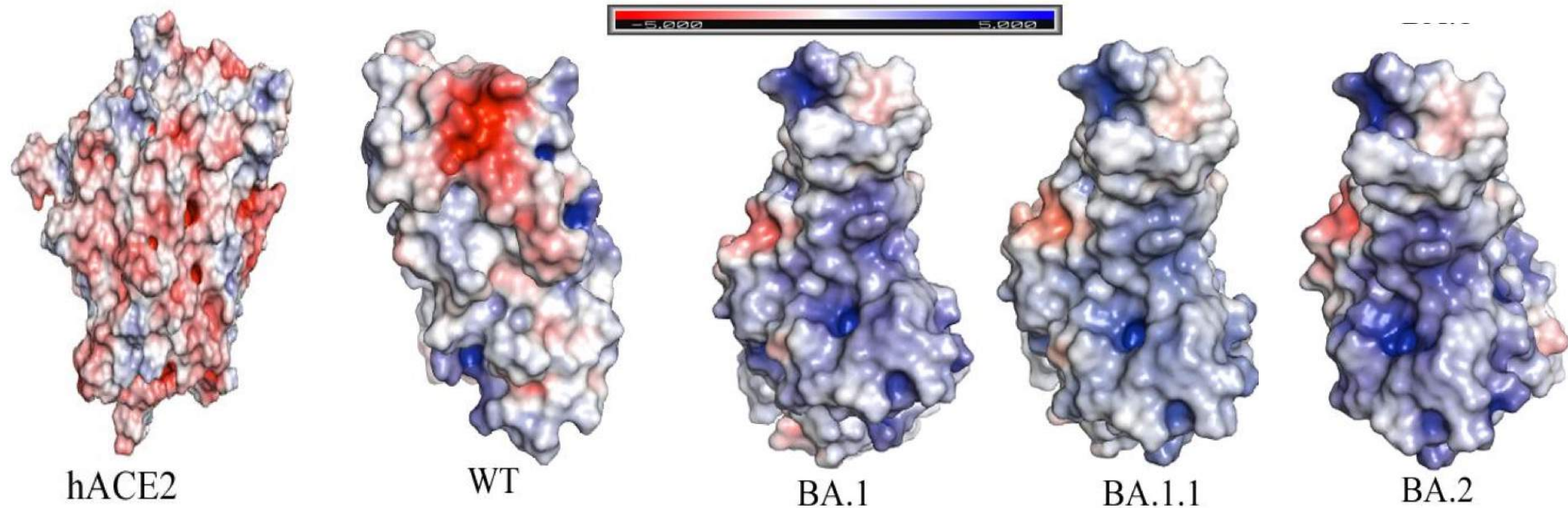


Perubahan AA	B.1.466.2	B.1.4670	AY.23	AY.24	BA.1	BA.1.1	BA.2
T19R			V	V			V
L24del							V
P25del							V
P26del							V
A27S							V
A67V					V	V	
H69del					V	V	
V70del					V	V	
T95I					V	V	
G142D				V	V	V	V
V143del					V	V	
Y144del					V	V	
Y145del					V	V	
E156G			V	V			
F157del			V	V			
R158de			V	V			
G181V						V	
N211del					V	V	
V213G							V
L212I					V		
ins214EPE					V	V	
A222V				V			
G339D					V	V	V
R346K						V	
S371L						V	V
S373P						V	V
S375F						V	V

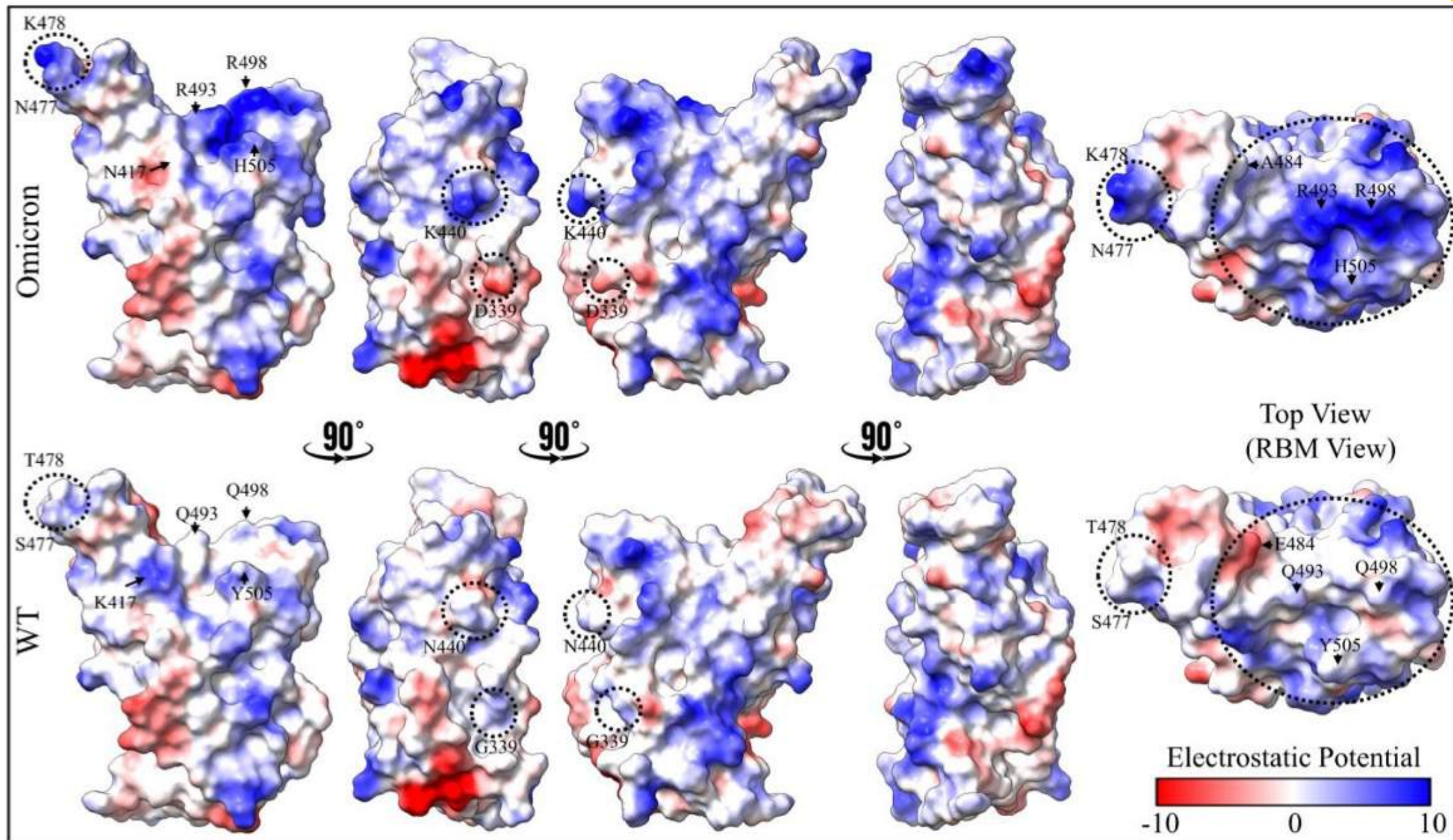
Perubahan AA	B.1.466.2	B.1.4670	AY.23	AY.24	BA.1	BA.1.1	BA.2
R408S							V
K417N					V	V	V
N439K							
N440K						V	V
G446S					V	V	
L452R			V	V			
S477N					V	V	V
T478K			V	V	V	V	V
E484A					V	V	V
Q493R	V				V	V	V
G496S					V	V	
Q498R					V	V	V
N501Y					V	V	V
Y505H					V	V	V
T547K					V	V	
D614G	V	V	V	V	V	V	V
H655Y					V	V	V
N679K					V	V	V
P681R	V		V	V	V	V	V
N764K					V	V	V
D796Y					V	V	V
N856K					V	V	
D950N			V	V			
Q954H					V	V	V
N969K					V	V	V
L981F					V	V	

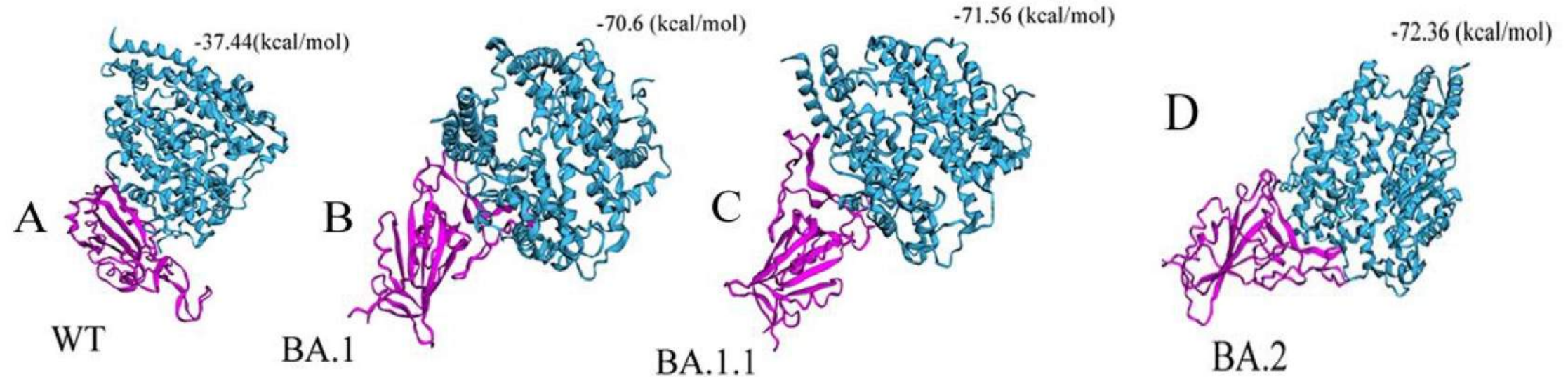
Perbandingan RBD Spike subvarian Omicron





- Berikut merupakan perbandingan *protein surface* antara hACE2, wild-type SARS-CoV-2, dan varian BA.1, BA.1.1, dan BA.2
- *Protein surface* menunjukkan potensi elektrostatis dari masing-masing protein, dengan skala -5 kT/e (merah) hingga +5 kT/e (biru)
- hACE2 menunjukkan potensi elektronegatif, sedangkan pada varian omicron menunjukkan peningkatan elektropositif dibandingkan dengan wild-type





- Berdasarkan hasil docking antara hACE2 dengan RBD, pada varian BA.1, BA.1.1, dan BA.2 menunjukkan adanya peningkatan afinitas ikatan dibandingkan dengan wild-type.
- BA.2 menunjukkan afinitas ikatan tertinggi diantara subvarian Omicron.

Summary

- Berdasarkan hasil docking, RBD Omicron memiliki afinitas ikatan paling tinggi terhadap hACE2 dibandingkan dengan wild-type dan varian Delta
- BA.2 menunjukkan afinitas ikatan tertinggi dan paling elektropositif diantara subvarian Omicron (BA.1 dan BA.1.1)
- Peningkatan afinitas ikatan dan perubahan elektrostatis RBD Spike SARS-CoV-2 wild type dengan varian delta dan omicron terhadap hACE2, memungkinkan terjadinya peningkatan masuknya virus kedalam sel host (manusia).
- Hasil analisis dilakukan dengan metode *in silico*, sehingga tetap dibutuhkan pembuktian lebih lanjut

