Cell Reports, Volume *38*

Supplemental information

Rapid identification of neutralizing antibodies against SARS-CoV-2 variants by mRNA display

Shiho Tanaka, C. Anders Olson, Christopher O. Barnes, Wendy Higashide, Marcos Gonzalez, Justin Taft, Ashley Richardson, Marta Martin-Fernandez, Dusan Bogunovic, Priyanthi N.P. Gnanapragasam, Pamela J. Bjorkman, Patricia Spilman, Kayvan Niazi, Shahrooz Rabizadeh, and Patrick Soon-Shiong

Supplemental Information

Rapid Identification of Neutralizing Antibodies against SARS-CoV-2 Variants by mRNA Display

Shiho Tanaka, C. Anders Olson, Christopher O. Barnes, Wendy Higashide, Marcos Gonzalez, Justin Taft, Ashley Richardson, Marta Martin-Fernandez, Dusan Bogunovic, Priyanthi N.P. Gnanapragasam, Pamela J. Bjorkman, Patricia Spilman, Kayvan Niazi, Shahrooz Rabizadeh, Patrick Soon-Shiong

Supplemental Information

Supplementary Tables

Table S1. Ten spike binding VH/VL sequences identified by mRNA display (related to Fig. 1 and mRNA display method).

Ab	CDRH1 (26-35)	CDRH2 (50-58)	CDRH3 (93-101)	CDRL3 (89-86)	
N-612-017	GFTFSSYAMH	AIWGSGSNTY	ARGRDLAAFTKTA	QQHDALPW	
N-612-056	GFTFSSYAMS	LISGSGGSTY	ARDLWGSGFFA	QQDAGTPL	RBD binder
N-612-074	GFTFSAYAMH	AIWGSGGSTY	ARDLWMAMWFG	QQRSTYPL	
N-612-004	GFTFSSYYMH	AISGSGGYTY	ARDRDHAYDWG	QQWADWPL	SD1 binder
N-612-041	GFTFSSYTMH	AISGSGGYTY	ARDRDLLWMGWA	QQYANWPL	
N-612-002	GFTFSSYTMH	AISGSGGSTY	ARDLFDWG	QQDYGFPL	NTD binder
N-612-014	GFTFSSYAMT	YISGSGGGTY	ARDRWASGWLA	QQAYAYPL	
N-612-007	GFTFSNYAMH	AISGNGGSTG	ARDRWYVKNA	QQLDGTPF	
N-612-044	GFTFSNYAMH	AISGSGGSTY	ARDLSFWLTYHLASA	QQSYSDPL	S2 binder
N-612-086	GFTFSSYAMH	AISWSGRSTY	ARDLSSNWGSG	QQSADTPF	

Table S2. BLI kinetic parameters obtained for various SARS-CoV-2 Spike domains (RBD, RBD-SD1, S1, S2)(related to Fig. 1 and BLI kinetic analysis method).

Analyte	**nAb**	**k_{on} (1/Ms)**	**k_{off} (1/s)**	**K_D (nM)**
RBD	N-612-017	6.77E+05	3.58E-03	5.29
	N-612-056	3.37E+05	1.01E-03	2.98
	N-612-074	1.33E+05	1.48E-03	11.1
RBD-SD1	N-612-017	4.56E+05	3.94E-03	8.65
	N-612-056	3.59E+05	1.04E-03	2.89
	N-612-074	1.21E+05	3.97E-03	32.9
	N-612-004	1.51E+05	9.79E-04	6.49
	N-612-041	2.10E+05	3.35E-03	16.0
S1	N-612-017	2.77E+05	2.04E-03	7.37
	N-612-056	1.51E+05	5.86E-04	3.89
	N-612-074	1.01E+05	1.46E-03	14.4
	N-612-004	9.92E+04	2.13E-04	2.15
	N-612-041	1.25E+05	1.83E-03	14.4
	N-612-002	2.68E+05	7.26E-04	2.71
	N-612-014	1.76E+05	2.18E-03	12.4
S2 (Ligand)	N-612-007 (Fab)	3.70E+05	1.40E-03	3.79
	N-612-044 (Fab)	1.70E+05	1.61E-03	9.44
	N-612-086 (Fab)	2.67E+05	0.96E-02	11.6

Table S3. BLI kinetic parameters obtained for Spike trimer using bivalent model fit (related to Fig. 1 and BLI kinetic analysis method).

Ab	k_{on1} (1/Ms)	k_{on2} (1/Ms)	k_{off1} (1/s)	k_{off2} (1/s)	*Apparent* K_D (nM)
N-612-017	8.35E+04	1.55E+00	<1.0E-07	1.69E-02	<0.001
N-612-056	6.24E+04	1.94E+00	<1.0E-07	5.15E-02	<0.001
N-612-074	9.23E+04	8.12E-01	<1.0E-07	1.89E-01	<0.001
N-612-004	7.60E+04	4.24E+00	<1.0E-07	1.00E+00	<0.001
N-612-041	1.11E+05	4.06E-01	<1.0E-07	1.75E-01	<0.001
N-612-002	5.69E+04	6.82E-02	<1.0E-07	6.76E-02	<0.001
N-612-014	2.54E+04	3.82E-03	2.29E-07	2.82E+00	0.0033
N-612-007	5.78E+04	7.80E-02	<1.0E-07	2.92E-02	<0.001
N-612-044	7.15E+04	2.62E+00	<1.0E-07	7.14E-02	<0.001
N-612-086	6.11E+04	1.55E-01	<1.0E-07	5.53E-02	<0.001

Table S4. Developability assay summary table (related to Fig. 1 and developability assay method).

Ab	Polyreactivity MSD (Fold-over-PBS)	HIC (min)	BLI-CSI (nm)	Accelerated stability % monomer increase/day	Fab Tm (℃)
N-612-017	3	10.1	-0.06	0.09	85
N-612-056	7	11.5	0.00	0.10	74
N-612-074	31	**22.1**	-0.01	0.10	76
N-612-004	14	14.9	0.05	0.13	85
N-612-041	10	**20.4**	0.05	**0.37**	88
N-612-002	22	13.9	0.08	0.18	82
N-612-014	4	12.3	0.09	0.16	88
N-612-007	10	11.8	0.04	0.11	82
N-612-044	8	**13.4/15.4**	-0.06	0.10	88/91
N-612-086	10	13.2	0.07	0.11	88
Acceptance criteria	<50	<16	<0.2	<0.2	>65 ℃

Table S5. Cryo-EM data collection and refinement statistics (related to Figs 3 and 5).

	N-612-017 Fab SARS-CoV-2 S 6P	N-612-014 Fab SARS-CoV-2 S 6P	N-612-004 Fab SARS-CoV-2 S 6P
PDB	**7S0C**	**7S0D**	**7S0E**
EMD	**24786**	**24787**	**24788**
Data collection conditions			
Microscope	Talos Arctica	Talos Artica	Talos Arctica
Camera	Gatan K3 Summit	Gatan K3 Summit	Gatan K3 Summit
Magnification	45,000x	45,000x	45,000x
Voltage (kV)	200	200	200
Recording mode	counting	counting	counting
Dose rate (e^-/pixel/s)	13.5	13.3	13.8
Electron dose ($e^-/Å^2$)	60	60	60
Defocus range (μm)	0.7 – 2.0	0.7 – 2.0	0.7 – 2.0
Pixel size (Å)	0.8689	0.8689	0.8689
Micrographs collected	2,585	3,791	3,717
Micrographs used	2,132	3,211	2,047
Total extracted particles	282,890	505,695	595,163
Refined particles	175,986	389,223	115,068
Particles in final refinement	108,746	137,684	107,271
Symmetry imposed	C1	C1	C1
FSC 0.143 (unmasked/masked)			
unmasked	4.4 Å	6.4 Å	7.1 Å
masked	3.2 Å	3.5 Å	4.8 Å
Refinement and Validation			
Initial model used	6XKL	6XKL	6XKL
Number of atoms			
Protein	29,164	33,650	5,307
Ligand	434	873	28
MapCC (global/local)	0.81/0.78	0.79/0.76	0.87/0.69
Map sharpening B-factor	65.8	75.3	155
R.m.s. deviations			
Bond lengths (Å)	0.01	0.005	0.02
Bond angles (°)	0.9	0.89	1.5
MolProbity score	2	2.2	2.46
Clashscore (all atom)	13.4	17.7	7.9
Poor rotamers (%)	0.2	0.1	0
Ramachandran plot			
Favored (%)	94.7	93.4	92.8
Allowed (%)	5.1	6	6.3
Disallowed (%)	0.2	0.6	0.9

Table S6. X-ray crystallography data collection and refinement statistics (related to Fig. 4).

	N-612-056 - SARS2-RBD *(12-2, SSRL)*
PDB ID	**7S0B**
Data collection[a]	
Space group	$P2_12_12$
Unit cell (Å)	102.3, 153.7, 96.6
α, β, γ (°)	90, 90, 90
Wavelength (Å)	0.979
Resolution (Å)	38.9-29 (3.04-2.9)
Unique Reflections	34,420 (4,471)
Completeness (%)	99.8 (99.1)
Redundancy	6.6 (6.7)
$CC_{1/2}$ (%)	99.3 (72.8)
<I/σI>	6.4 (1.5)
Mosaicity (˚)	0.25
R_{merge} (%)	18.7 (133)
R_{pim} (%)	8.3 (59.4)
Wilson *B*-factor	56.6
Refinement and Validation	
Resolution (Å)	384 - 2.9
Number of atoms	
Protein	9,829
Ligand	28
Waters	0
R_{work}/R_{free} (%)	212/25.4
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (˚)	1.2
MolProbity score	2.47
Clashscore (all atom)	11.6
Poor rotamers (%)	5
Ramachandran plot	
Favored (%)	94.8
Allowed (%)	5.2
Disallowed (%)	0
Average *B*-factor (Å)	73.5

[a]Numbers in parentheses correspond to the highest resolution shell

Table S7. BLI kinetic analysis of N-612-017, N-612-017-01, N-612-017-03, N-612-017-5B02, and N-612-017-5B05 against RBD-WT, RBD-B.1.351 (K417N/E484K/N501Y) and RBD-L452R (related to Fig. 7 and BLI kinetic analysis method).

	RBD-WT			RBD-B.1.351			RBD-L452R		
	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
N-612-017	6.77E+05	3.58E-03	5.29	4.45E+05	1.36E-02	30.6	--	--	N.B.
N-612-017-01	5.59E+05	3.59E-04	0.64	5.31E+05	4.06E-04	0.77	2.34E+05	7.75E-03	33.1
N-612-017-03	6.48E+05	1.60E-04	0.25	6.47E+05	2.33E-04	0.36	1.18E+05	6.90E-03	58.7
N-612-017-5B02	8.24E+05	9.26E-05	0.11	7.99E+05	1.20E-04	0.15	5.74E+05	3.54E-03	6.16
N-612-017-5B05	9.62E+05	3.16E-03	3.28	9.68E+05	2.48E-03	2.56	8.01E+05	1.96E-03	2.44

Supplemental Figures

Figure S1

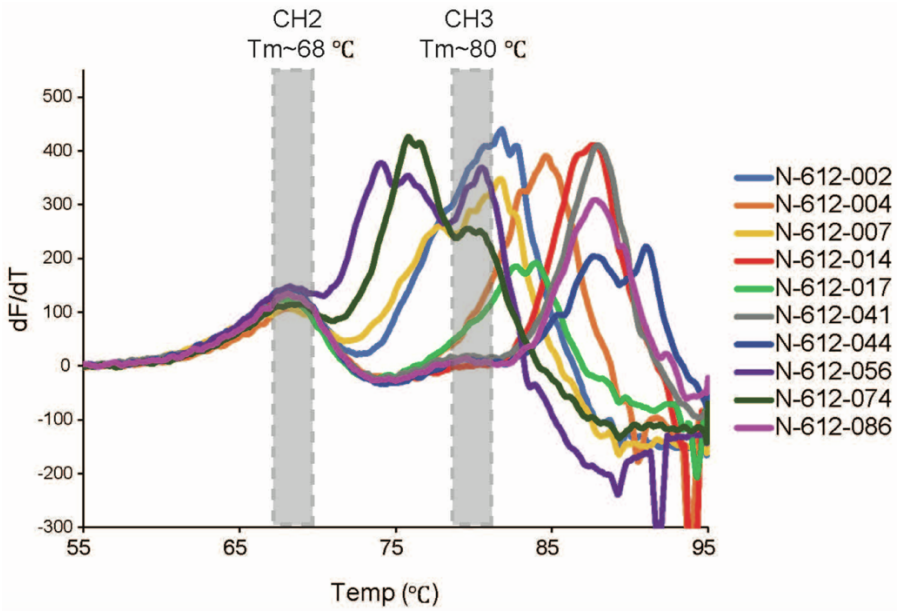


Figure S1. Tm thermograms of 10 Abs identified from mRNA library display (related to Fig. 1 and developability assay method).

Figure S2

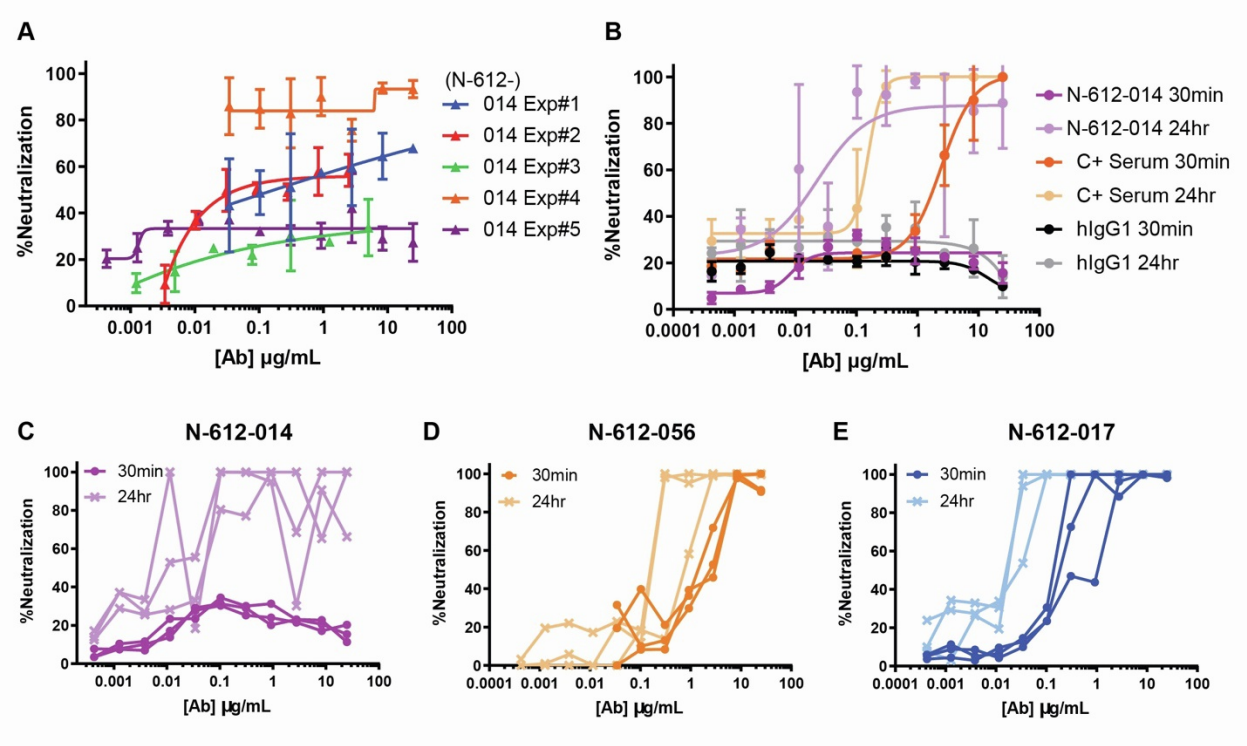


Figure S2. Vero E6 live virus neutralization assay (related to Fig. 2 and Vero E6 neutralization assay method). (A) Neutralization activity of N-612-014 in 5 separate experiments with 30 min virus-antibody incubation. (B) Comparison of neutralization activity of N-612-014 and positive control convalescent serum (C+) with 30 min. vs 24 hr virus-antibody incubation. Comparison of 30 min and 24 hr virus-antibody incubation for: (C) N-612-014, (D) N-612-056, and (E) N-612-017.

Figure S3

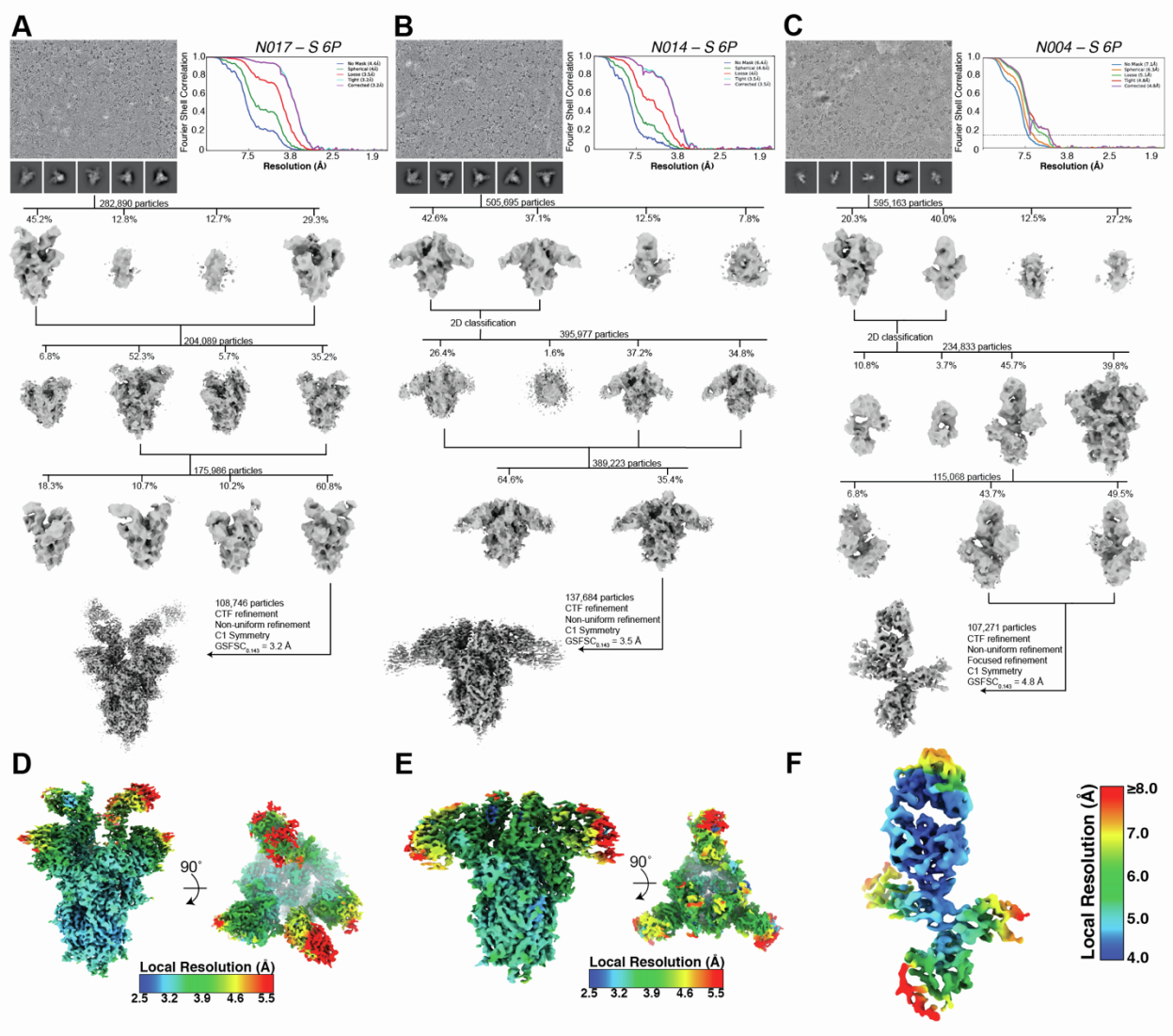


Figure S3. Cryo-EM data processing and validation (related to Figs 3 and 5). (A-C) Representative micrograph, 2D class averages, data processing workflow, and Gold Standard FSC plots for the final reconstructions of (A) N-612-017 – S 6P, (B) N-612-014 – S 6P, and (C) N-612-056 – S 6P complexes. (D-F) Local resolution estimates calculated in cryoSPARC v3.1 for (D) N-612-017 – S 6P, (E) N-612-014 – S 6P, and (F) N-612-056 – S 6P complexes.

Figure S4

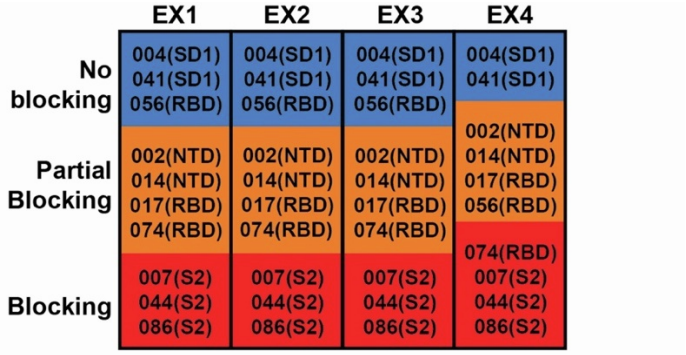
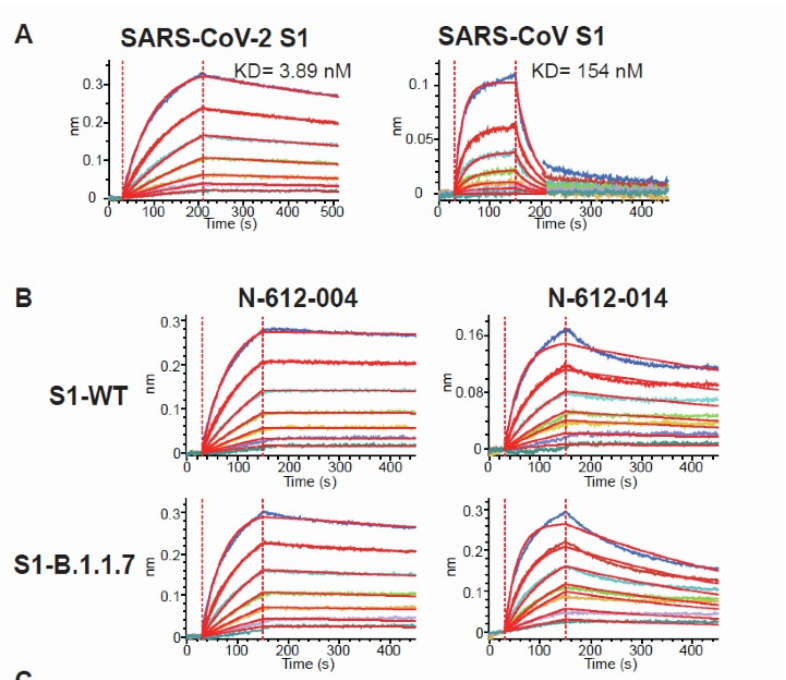


Figure S4. Convalescent plasma blocking of 10 mAbs binding to SARS-CoV-2 Spike (related to Figs 1 and 2 and convalescent plasma blocking assay method).

Figure S5



C

nAb	N-612-004 kon (1/Ms)	N-612-004 koff (1/s)	N-612-004 KD (nM)	N-612-014 kon (1/Ms)	N-612-014 koff (1/s)	N-612-014 KD (nM)
S1 wild-type	9.92E+04	1.31E-04	1.32	1.40E+05	1.79E-03	12.8
S1 B.1.1.7	1.13E+05	4.78E-04	4.24	1.68E+05	2.48E-03	14.8

Figure S5: Cross-reactivity of N-612-056 against SARS-CoV (related to Figs 1 and 7 and BLI kinetics analysis method). (A) BLI kinetic analysis of SARS-CoV-2 and SARS-CoV S1 domain binding to N-612-056. (B, C) BLI kinetic analysis of N-612-004 and N-612-014 against S1 domain from WT and B.1.1.7 variant of SARS-CoV-2 Spike protein.