
Supplementary information

Converting non-neutralizing SARS-CoV-2 antibodies into broad-spectrum inhibitors

In the format provided by the authors and unedited

1 Supplementary Information:

2

3 Converting non-neutralizing SARS-CoV-2 antibodies into broad-spectrum
4 inhibitors

5 Payton A.-B. Weidenbacher^{1,2}, Eric Waltari³, Izumi de los Rios Kobara⁴, Benjamin N. Bell^{1,5},

6 Mary Kate Morris⁶, Ya-Chen Cheng^{1,7}, Carl Hanson⁶, John E. Pak³, Peter S. Kim^{1,3,7}

7 ¹Stanford ChEM-H, Stanford University, Stanford, CA 94305, ² Department of Chemistry, Stanford University, Stanford, CA 94305,

8 ³ Chan Zuckerberg Biohub, San Francisco, CA 94158, ⁴ Stanford Immunology Program, Stanford University School of Medicine,

9 Stanford, CA 94305, ⁵ Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford,

10 California 94305, ⁶ California Department of Public Health, Richmond, California, USA, ⁷ Department of Biochemistry, School of

11 Medicine, Stanford University, Stanford, CA 94305

12

13 Supplementary note:

14 **Signal Peptide**

15 *Hexa-His tag*

16 **TEV Site**

17 ACE2

18

19 **CV10-ACE2 Fusion scFv**

20

21 **MGWSCILFLVATATGVHS**QVQLQESGPGLVKPSSETLSLTCNVSGGSISSYYWSWIRQP

22 PGKGLEWIGYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGFD

23 YWGQGTLLVTVSSASGGGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLSCRASQSVS

1 SIYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ
2 YAGSPWTFGQGTKVEIKGGSGSHHHHHHASTGGGSGGSPSQAGAAASEENLYFQGS LF
3 VSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQN
4 MNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQONGSSVLSEDKSKRLNTI
5 LNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEV GKQLR
6 PLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIK
7 PLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPGQKPNID
8 VTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTA
9 WDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAV
10 GEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQALTIVGTL PFTYMLEKWRWMVF
11 KGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQ
12 FOFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLAENNVGAKNMN
13 VRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

14

15 **CV27-ACE2 Fusion scFv**

16

17 MGWSCILFLVATATGVHSQVQLVESGGGVVQPGRSLRLS CAASGFTFSSYAMHWVR
18 QAPGKGLEWVALISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC
19 ARSFGGSYYYGMDVWGQGTTVTASGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSIT
20 ISCTGTSSDVGGYNYVSWYQQHPGKAPKLMYDVS NRPSGVSNRFSGSKSGNTASLTIS
21 GLQAEDEADYYCSSYTSSSTPYVFGTGTKVGGSGSHHHHHHASTGGGSGGSPSQAGAA
22 ASEENLYFQGS LFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWN
23 YNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQONGSSV

1 LSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAW
2 SWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQL
3 IEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSL
4 TVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGN
5 VQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRN
6 GANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQALTIVGTLPTFTYM
7 LEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYS
8 FIRYYTRTLYQFQFQEALCQAAKHGEPHKKCDISNSTEAGQKLFNMLRLGKSEPWTLAL
9 ENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
10
11 **COVA2-14-ACE2 Fusion scFv**
12
13 MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGSSVKVCKASGGTFSSYAIIVWRQ
14 APGQGLEWMGGIPIFGTANYAQKFQGRVTITTDSTAYMELSSLRSEDVAVYYCAR
15 VRYDSSGGYEDYWGQGTLVTVSSASGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGER
16 ATLSCRASQSVSSYLAWYQQEPGQAPRLLIYDASNRAIGIPARFSGSGGTDFLTISLE
17 PEDFAVYYCQRSNWPPMYTFGQGTKVEIKGGSGSHHHHHHASTGGGSGGSPGQAGA
18 AASEENLYFQGSFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASW
19 NYNTNITEENVQNMNAGDKWSAFLKEOSTLAQMYPLQEIQNLTVKLQLQALQONGSS
20 VLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAW
21 ESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQ
22 LIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYS
23 LTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPG

1 NVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLR
2 NGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQALTIVGTLPFTY
3 MLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDY
4 SFIRYYTRTLYQFOFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLA
5 LENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
6
7 **COV2-2449-ACE2 Fusion scFv**
8
9 MGWSCILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCATSGFTFSSFALHWVRQ
10 APGKGLEWVTVISDDGNNKYYVDSVKGRFTISRDN SKNTLFLQMNSLRVEDTAIYYCA
11 RASYN SNWSIGEYFRDWGQGT LVTVSSASGGGGSGGGGSGGGGSDIVMTQSPDSLAVS
12 LGERATINCKSSQSLLYTSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
13 GTDFTLTISSLQAEDVAVYYCQQYYSPPWTFGQGTKVEIKGGSGSHHHHHHASTGGGS
14 GGPSGQAGAAASEENLYFQGS L FVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAED
15 LFYQSSLASWNYNTNITEENVQNMNAGDKWSAFLKEOSTLAQMYPLQEIQNLTVKLO
16 LQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSL
17 DYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNG
18 VDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM
19 WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGF
20 WENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDM
21 AYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQA
22 LTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDP

1 ASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLR
2 LGKSEPWTLAENVVGAKNMNV RPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
3
4 **COV2-2143-ACE2 Fusion scFv**
5
6 MGWSCILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCAASGFTVSSNYMSWVR
7 QAPGKGLEWVSVIYSAGSTYYADSVKGRFSISRDKSKNTLYLQMNSLRAEDTAVYYCA
8 KEGGSGSLRYYYYGMDVWGQGTTVTVSSASGGGGSGGGGSQSVVTQPPSASG
9 TPGQRTVITCSGSSSNIGYNIVN WYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSAS
10 LSISGLQSEDEADYYCAA WDDSLNGYVFGTGTKVTVLGGSGSHHHHHHASTGGGSGGP
11 SGQAGAAASEENLYFQGS LFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFY
12 QSSLASWNYNTNITEENVQNMNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLO
13 ALQONGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDY
14 NERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVD
15 GVDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWG
16 RFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWE
17 NSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAY
18 AAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQALTI
19 VGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPAS
20 LFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLG
21 KSEPWTLAENVVGAKNMNV RPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
22

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Signal Peptide

Hexa-His tag

TEV Site

ACE2

COV2-2449-LC-ACE2 Fusion

MGWSCILFLVATATGVHSDIVMTQSPDSLAVSLGERATINCKSSQSLLYTSNNKNYLA
WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYYS
PPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GECGGSGSHHHHHHASTGGGSGGSPGQAGAAASEENLYFQGSLFVSNHAYGGSGGEA
RVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGDKWSAFLK
EQSTLAQMYPLQEIQNLTVKLQLOALQONGSSVLSEDKSKRLNTILNTMSTIYSTGKVC
NPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEM
ARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAK
LMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDA
QRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCT
KVTMDDFLTAHHEMIGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLK
SIGLLSPDFQEDNETEINFLKQALTIVGTLPTFTYMLEKWRWMVFKGEIPKDQWMKKW
WEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHE

1 GPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTW

2 LKDQNKNSFVGWSTDWSPYAD

3

4 **Signal Peptide**

5 **Knob/Hole Mutations**

6 CrossMAb Swaps

7

8 **COV2-2449-HC-Knob**

9

10 **MGWSCILFLVATATGVHS**QVQLVESGGGVVQPGRSLRLSCATSGFTFSSFALHWVRQ

11 APGKGLEWVTVISDDGNNKYYVDSVKGRFTISRDNKNTLFLQMNSLRVEDTAIYYCA

12 RASYNSNWSIGEYFRDWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY

13 FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT

14 KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH

15 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN

16 KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLICLVKGFYPSDIAVEWESNG

17 QPENNYKTTTPVLDSGSSFLYSKLTVDKSRWQQGNVDFSCSVMHEALHNHYTQKSLSL

18 SPGK

19

20 **CV10-LC-CrossMAb**

21

22 **MGWSCILFLVATATGVHSE**IVLTQSPGTLSPGERATLSCRASQSVSSIYLAWEYQQK

23 PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAGSPWTFGQ

1 GTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
2 TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
3
4 **CV10-HC-CrossMAb-Hole**
5
6 MGWSCIILFLVATATGVHSQVQLQESGPGLVKPSSETLSLTCNVSGGSISSYYWSWIRQP
7 PGKGLEWIGYIYYSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGFD
8 YWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
9 LQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
10 ECDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
11 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
12 SKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTP
13 PVLDSGDGSFFLLSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK
14

1 Tables:

2

3 Supplementary Table 1 – live virus neutralization

Inhibitor	Wuhan-1 50% CPE nM	BA.1 50% CPE nM	BA.2 50% CPE nM
scFv-based ReconnAbs			
CV10	8.1	29	56
CV27	3.3	306	211
2449	10	101	57
IgG-based ReconnAb			
CrossMab	21	171	106
CrossMab + TEV	375	346	>500.00
Scale			
<200nM	>200nM		

4

5 Supplementary Table 1 – IC₅₀ values from a limiting dilution live SARS-CoV-2 neutralization assay shows ReconnAbs
 6 neutralize live viruses as expected. Cytopathic effect (CPE) was scored as binary, either infection or no infection and
 7 IC₅₀ was calculated using the Spearman-Kärber method. Data presented is the average of 3 assays, if one assay was
 8 above the limit of detection, the average was computed assuming the limit of detection was the value.