Efficient discovery of SARS-CoV-2-neutralizing antibodies via B cell receptor sequencing and ligand blocking

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Although several monoclonal antibodies (mAbs) targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been approved for coronavirus disease 2019 (COVID-19) therapy, development was generally inefficient, with lead generation often requiring the production and testing of numerous antibody candidates. Here, we report that the integration of target–ligand blocking with a previously described B cell receptor-sequencing approach (linking B cell receptor to antigen specificity through sequencing (LIBRA-seq)) enables the rapid and efficient identification of multiple neutralizing mAbs that prevent the binding of SARS-CoV-2 spike (S) protein to angiotensin-converting enzyme 2 (ACE2). The combination of target–ligand blocking and high-throughput antibody sequencing promises to increase the throughput of programs aimed at discovering new neutralizing antibodies.

Technologies for developing preventive and therapeutic measures that can counteract potential pandemics are of the utmost significance for public health. The COVID-19 pandemic has emphasized the importance of rapid countermeasure development. Through pandemic preparedness initiatives, effective SARS-CoV-2-neutralizing antibodies were discovered and validated within months1–6, as were SARS-CoV-2 vaccine candidates7. However, even with such unprecedented speed of vaccine and therapeutic development, the pandemic has inflicted devastating worldwide effects. Accelerating actions by weeks or months can make an enormous difference in an exponentially evolving pandemic. Therefore, efficient methods for discovery of effective countermeasures against emerging pathogens can play a critical role in pandemic preparedness for future infectious disease outbreaks.

Antibodies are a major modality for therapy and vaccine design strategies for a wide range of diseases; however, the functional antibody discovery process can be inefficient. Typically, at the screening step, B cells are prioritized based on antigen recognition, but this often requires time-intensive subsequent mAb validation steps for discovery of functional neutralizing antibodies. This limitation was exemplified by SARS-CoV-2 antibody discovery initiatives, as testing of large numbers of antibodies (frequently hundreds to thousands) was generally required to identify a small fraction of neutralizing antibodies with a wide range of hit rates when using S protein as an antigen bait (about 2 to 23%) or when using receptor-binding domain (RBD) and/or S protein subunit 1 (S1) (about 2–55%)8,9,10,11 in various studies.

Results
To overcome this limitation, we developed LIBRA-seq with ligand blocking, a second-generation LIBRA-seq technology that incorporates a functional readout into the antibody discovery process13. LIBRA-seq uses DNA-barcoded antigens to map antibody sequence to antigen specificity using next-generation sequencing14. For LIBRA-seq with ligand blocking, a ligand and its cognate target antigen(s) are each labeled with a unique oligonucleotide barcode (Extended Data Fig. 1a), enabling the transformation of antigen–ligand interactions into sequenceable events. In these experiments, B cells that can block antigen–ligand interactions are expected to have high LIBRA-seq scores for the target antigen(s) and low LIBRA-seq scores for the ligand (Extended Data Fig. 1a). Therefore, a single high-throughput LIBRA-seq with ligand blocking experiment provides both antigen recognition and ligand-blocking information simultaneously for many B cells.

To evaluate this technology, we sought to discover SARS-CoV-2-specific antibodies from B cells from individuals with past SARS-CoV-2 infection, because antibodies that block the interactions between the SARS-CoV-2 S protein and its host receptor ACE2 are among the most potently neutralizing identified
**Fig. 1** | Antibody discovery using LIBRA-seq with ligand blocking. a, Experimental setup of three LIBRA-seq experiments: experiment 1, LIBRA-seq with ligand blocking; experiment 2, LIBRA-seq with a SARS-CoV-2 S (SARS2 S) titration; experiment 3, LIBRA-seq with a SARS-CoV-2 S titration and ligand blocking. For experiments 2 and 3, six different aliquots of S protein were added in a titration series (1–6); HIV ZM197 envelope and influenza hemagglutinin H1 NC99 were used as controls. b–d, Left, after next-generation sequencing, hundreds of B cells (dots) were recovered that had paired heavy/light chain sequencing information and antigen reactivity information for the three experiments. For experiments 1 (b), 2 (c) and 3 (d), select LIBRA-seq scores for all cells per experiment are shown as open circles ($n=828$, 829 and 957, respectively). Antibodies selected for expression and validation are highlighted and numbered in light blue. Right, LIBRA-seq scores for the selected antibodies for all antigens from each experiment are shown as a heat map from −2 (tan) to 2 (purple); scores outside of this range are shown as the minimum and maximum values. For experiments 1 and 3, antibodies with negative scores for ACE2 are shown above the dotted line, while antibodies with positive scores for ACE2 are shown below the dotted line and are controls. For experiment 2, all SARS-CoV-2-reactive antibodies are shown above the dotted line, whereas influenza-specific antibody 53181-3 is shown as a control below the dotted line.
to date. We performed three LIBRA-seq experiments with screening libraries that included the following: experiment 1, ACE2 and SARS-CoV-2 S; experiment 2, a titration series of different aliquots of SARS-CoV-2 S, each labeled with a unique barcode; and experiment 3, ACE2 and a titration series of S (Fig. 1a). The incorporation of a titration series of S antigen in the screening library for experiments 2 and 3 aimed to assess the strength of B cell receptor–antigen interactions (Extended Data Fig. 1b,c).
The application of LIBRA-seq resulted in 828, 829 and 957 antigen-specific B cells for the three experiments, respectively. We prioritized a set of B cells for mAb production and validation based on the following conditions: for experiments 1 and 3 (with ACE2 in the screening library), we selected B cells with high LIBRA-seq scores for S and low scores for ACE2; for experiment 2, we selected B cells that had positive scores for multiple aliquots of S (Fig. 1b–d). B cells with high S and high ACE2 scores were also selected as controls from experiments 1 and 3, along with an influenza-specific B cell from experiment 2 (Fig. 1b–d). We further aimed to prioritize antibodies with diverse sequence features, although some of the selected antibodies appeared to be clonally related (Extended Data Fig. 2a).

We confirmed the predicted antigen specificity for 26/27 (96%) antibodies and mapped the general antibody epitope regions by testing antibodies for binding to recombinant SARS-CoV-2 subdomain proteins (Fig. 2a and Extended Data Fig. 2b). The majority of antibodies from experiments 1 and 3 (but none from experiment 2) recognized the RBD (Fig. 2a and Extended Data Fig. 2b). Further, the antibodies had a wide range of affinities for RBD or N-terminal domain (NTD), including several antibodies with a dissociation constant ($K_d$) of $<1 \text{ nM}$, although we did not observe a correlation between LIBRA-seq S protein score and affinity (Fig. 2b). Next, we tested the ability of the antibodies to block ACE2 binding to S protein. For antibodies predicted to block ACE2 by LIBRA-seq, 57% from experiment 1 and 67% from experiment 3 demonstrated ACE2 blocking via enzyme-linked immunosorbent assay (ELISA), whereas no antibodies from experiment 2 blocked ACE2 binding (Fig. 2c and Extended Data Fig. 2c).

Next, we tested the antibodies in a VSV–SARS-CoV-2 chimeric virus neutralization assay (Fig. 2d and Extended Data Fig. 2d).
For antibodies predicted to block ACE2 by LIBRA-seq, 86% from experiment 1 and 67% from experiment 3 were neutralizing, while only two clonally related antibodies (29%) from experiment 2 were neutralizing (Fig. 3a,b). For the antibodies from experiments 1 and 3, the ACE2 LIBRA-seq scores were correlated with the percent reduction in ACE2 binding (Fig. 3c; Spearman $r = -0.54$, $P = 0.017$). Furthermore, several antibodies also showed potent neutralization against authentic SARS-CoV-2 virus in a plaque reduction assay and in some cases against multiple SARS-CoV-2 variants (Fig. 4). Together, these results highlight the importance of including ligand-blocking applied to cross-reactive antibody discovery.
blocking in LIBRA-seq for selectively identifying potent neutralizing antibodies.

To investigate antibody recognition of SARS-CoV-2 S, we determined a 9-Å-resolution cryo-electron microscopy (cryo-EM) structure of the antigen-binding fragments of antibodies 5317-4 and 5317-10 bound to the SARS-CoV-2 S extracellular domain (Fig. 5a). We chose 5317-4 based on its potent neutralization (IC50 value of 7.3 ng ml−1) against authentic SARS-CoV-2; Fig. 4) and ACE2 competition. The three-dimensional reconstruction revealed that 5317-4 binds to RBD in the ‘up’ and ‘down’ conformations, and its epitope partially overlaps the ACE2-binding footprint (Fig. 5a,b). When bound to the RBD in the down conformation, 5317-4 competes with ACE2 binding to the adjacent up RBD (Fig. 5b). We investigated 5317-10 because of its inconclusive epitope, as it bound to S1 but not individual RBD or NTD constructs (Fig. 2a). The map revealed that 5317-10 binds a quaternary epitope that bridges an RBD in the down position and the NTD of an adjacent protomer (Fig. 5a). This mode of recognition may prevent the RBD from transitioning into an ACE2-accessible up position, thereby preventing binding by ACE2.

To further demonstrate the utility of LIBRA-seq with ligand blocking, we sought to identify antibodies that show cross-reactivity between SARS-CoV-2 and SARS-CoV and that are capable of blocking S–ACE2 interactions. To that end, we applied LIBRA-seq to B cells from an individual with past SARS-CoV-2 infection by using an antigen library that included SARS-CoV-2 S, SARS-CoV S and ACE2 (Fig. 6a). This resulted in 120 IgG+ B cells with high LIBRA-seq scores for both SARS-CoV-2 S and SARS-CoV S (Fig. 6b). Only 8% of these cells were associated with low LIBRA-seq scores for ACE2 (Extended Data Fig. 3a), highlighting the advantage of including ligand blocking to screen for such rare cells (although we also note that information about B cells that show cross-reactivity but are not ACE2 blocking is also retained, enabling characterization of B cells with alternative phenotypes as well). Based on LIBRA-seq antigen and ligand-blocking scores, we produced and validated a set of antibodies, including eight with high scores for both S antigens and low scores for ACE2 (Fig. 6c,d). Of these, 100% bound SARS-CoV-2 S, 88% showed the predicted SARS-CoV-2/SARS-CoV cross-reactivity and 63% demonstrated strong ACE2-blocking ability via ELISA (Fig. 6c–d and Extended Data Fig. 3b,c), confirming that LIBRA-seq with ligand blocking efficiently identified ACE2-blocking antibodies with cross-reactivity between multiple coronaviruses.

Discussion

Together, the results from the four LIBRA-seq experiments reported here showcase the advantages of including ligand blocking as part of the sequencing readout. As with most screening tools, there are limitations to the LIBRA-seq with ligand blocking approach, including the prerequisite for a defined antigen–ligand interaction as well as the potential for identifying false positives. Nevertheless, through a single high-throughput sequencing experiment, LIBRA-seq with ligand blocking identified potent SARS-CoV-2 antibodies, requiring the subsequent production and validation of less than a dozen antibodies per experiment. The observed hit rates for the discovery of potently neutralizing antibodies are an improvement over what has been reported in the literature, which also typically required the screening of hundreds to thousands of antibody candidates isolated for their reactivity to antigen alone (recombinant S, S1 or RBD)1–6,8–12. Further, unlike RBD-only discovery efforts, LIBRA-seq with ligand blocking applied to S antigens has the potential for more comprehensive coverage of antibody epitopes, as evidenced by the discovery of the RBD-NTD antibody in Fig. 5a. Overall, the application of LIBRA-seq with ligand blocking can provide critical advantages for rapid development of therapeutic and preventive countermeasures and presents a general platform with applications to virtually any area where targeting the disruption of antigen–ligand interaction is a prime therapeutic goal.

Online content

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Antigen purification. A variety of recombinant soluble protein antigens were used in the LIBRA-seq experiment and other experimental assays.

Plasmids were transiently transfected in Expi293F cells using polyethyleneimine and encoded the following: residues 1–1208 of the SARS-CoV-2 S protein with a mutated S1/S2 cleavage site, proline substitutions at positions 817, 892, 899, and a C-terminal T4-fibritin trimerization motif, an 8×His tag and a TwinStrepTag (SARS-CoV-2 S-9011); residues 1–1199 of the SARS-CoV-2 S protein with proline substitutions at positions 968 and 969 and a C-terminal T4-fibritin trimerization motif, an 8×His tag and a TwinStrepTag (SARS-CoV-2 S-2P) and residues 1–615 of human ACE2 with a C-terminal HRV3C protease cleavage site, a TwinStrepTag and an 8×His tag (ACE2). Transfected supernatants were collected 5 d after expression and purified over a StrepTactin or StrepTrap column (Cytiva Life Sciences). Both recombinant SARS-CoV-2 S HP and ACE2 were further purified to homogeneity using a Superose6 column (Cytiva Life Sciences). For LIBRA-seq, non-AviTagged antigens were biotinylated using EZ-Link Sulfo-NHS-Biotin (Thermo Fisher Scientific) using a 50:1 biotin-to-protein molar ratio.

For the HIV-1 gp140 SOSIP variant from strain ZM197 (clade C) and hemagglutin from strain A/New Caledonia/20/99 (H1N1) (GenBank accession number ACF41878), recombinant, soluble antigens contained an AviTag and were expressed in Expi293F cells using polyethyleneimine transfection reagent and cultured. Freestyle F17 expression medium supplemented with 0.1% Pluronic Acid F-68 and 20% 4 mM methyl-D-mannopyranoside. Protein elutions were buffer exchanged into PBS, concentrated and run on a Superdex 200 Increase 10/300 GL sizing column on the AKTA fast protein liquid chromatography (FPLC) system. Fractions corresponding to correctly folded protein were collected and analyzed by SDS–PAGE, and antigenicity was characterized by ELISA using known mAbs specific to each antigen. For LIBRA-seq, AviTagged antigens were biotinylated using BiaBiotin ligase (AviBi).

Barcoding of antigens. We used oligonucleotides that possessed a 15-base pair antigen barcode, a sequence capable of annealing to the template switch oligonucleotide that is part of the 10× head-delivered oligonucleotides and contains truncated TruSeq small RNA read 1 sequences in the following structure: 5′-CCCTGGGGCCGCAGGTTTCACCAAGNNNNNNNNNCATTAAAG A′*A′-3′, where Ns represent the antigen barcode13. For each antigen, a unique DNA barcode was directly conjugated to the antigen itself. For experiment 1, the barcodes included SARS-CoV-2 S (GACACGCGTATACTGGA), SARS-CoV-2 S aliquot 2 (TGGTTATTCCTCCTGTT), SARS-CoV-2 S aliquot 3 (GCAGCGTGATAGTCA), SARS-CoV-2 S aliquot 4 (GGTCTCTAGGGTGA), SARS-CoV-2 S aliquot 5 (AGACTAATAGCAG), SARS-CoV-2 S aliquot 6 (GGGTTCTCTGGATA), SARS-CoV-2 S aliquot 7 (GCAGCGTTAGGA), H1 N99 (TCATTTCTCCGAGT) and ZM197 (TACGCGTATAACTTG) and ACE2 (CTTACCTCGAGC). For experiment 2, the barcodes included SARS-CoV-2 S-9011 aliquot 1 (GCAAGCTGTAGCTGCA), SARS-CoV-2 S aliquot 2 (TTGTTGATTCCTGTT), SARS-CoV-2 S aliquot 3 (GCACGCTTATAGTCA), SARS-CoV-2 S aliquot 4 (GCTCTCTATAGCAG), SARS-CoV-2 S aliquot 5 (AGACTAATAGCAG), SARS-CoV-2 S aliquot 6 (GGGTTCTCTGGATA), SARS-CoV-2 S aliquot 7 (GCAGCGTTAGGA), H1 N99 (TCATTTCTCCGAGT) and ZM197 (TACGCGTATAACTTG) and ACE2 (CTTACCTCGAGC). For experiment 3, the barcodes were included as experiment 2 and also included ACE2 (CTTACCTCGAGC). For experiment 4, the barcodes included SARS-CoV-2 S (GCAAGCTGTAGCTGCA), SARS-CoV-2 S (GCTCTCTATAGCAG), SARS-CoV-2 S (AGACTAATAGCAG), SARS-CoV-2 S (GGGTTCTCTGGATA), SARS-CoV-2 S (GCAGCGTTAGGA), H1 N99 (TCATTTCTCCGAGT) and ACE2 (CTTACCTCGAGC). In particular, 5′-amino-oligonucleotides were conjugated directly to each antigen using the SoluLink Protein-Oligonucleotide Conjugation kit (Trilink, S-9011) according to manufacturer's instructions. Briefly, the oligonucleotide and protein were desalted, and the amino-oligonucleotide was modified with the 4FB cross-linker, and the biotinylated antigen protein was modified with S-Hyd. Then, the 4FB-oligonucleotide and the HyNic-antigen were mixed. This process causes a stable bond to form between the protein and the oligonucleotide. The concentration of the antigen–oligonucleotide conjugates was determined by a bicinchoninic acid (BCA) assay, and the HyNic molar substitution ratio of the antigen–oligonucleotide conjugates was analyzed using a NanoDrop according to the SoluLink protocol. AKTA fast protein liquid chromatography (FPLC) system. Fractions corresponding to correctly folded protein were collected and analyzed by SDS–PAGE, and antigenicity was characterized by ELISA using known mAbs specific to each antigen.
exchanged into PBS three times using Amicon Ultra centrifugal filter units and concentrated. Antibody plasmids were sequenced. If antibody sequences did not match expected heavy or light chain, antibody was excluded from downstream analysis.

High-throughput antibody expression. For high-throughput production of recombinant antibodies, approaches were used that are designated as microscale. For antibody expression, microscale transfection was performed (~1 ml per antibody) with CHO cell cultures using the Gibco ExpiCHO Expression System and a protocol for deep 96-well blocks (Thermo Fisher Scientific). In brief, synthesized antibody-encoding DNA (~2 μg per transfection) was added to OptiPro serum-free medium (OptiPro SFM), incubated with ExpiFectamine CHO Reagent and added to 800 μl of ExpiCHO cell cultures in deep 96-well blocks using a ViaFlo384 liquid handler (Integra Biosciences). The plates were incubated on an orbital shaker at 1,000 rpm, with an orbital diameter of 3 mm at 37 °C and 8% CO₂. The next day after transfection, ExpiFectamine CHO Enhancer and ExpiCHO Feed reagents (Thermo Fisher Scientific) were added to the cells, followed by a 4-d incubation for a total of 5 d at 37 °C in 8% CO₂. Culture supernatants were collected after centrifuging the blocks at 450g for 5 min and were stored at 4 °C until use. For high-throughput microscale antibody purification, fritted deep-well plates were used containing 25 μl of settled Protein G resin (GE Healthcare Life Sciences) per well. Clarified culture supernatants were incubated with protein G resin for antibody capturing, washed with PBS using a 96-well plate manifold base (Qiagen) connected to the vacuum and eluted into 96-well PCR plates using 80 μl of 0.1 M glycine (pH 2.6 to 2.8) buffer to each well. After elution, antibody was captured onto Protein G and a protocol for deep 96-well blocks (Thermo Fisher Scientific). In brief, antibody was added into wells containing only buffer. Data were reference subtracted, and kinetics were assessed in four fivefold dilutions (starting from a 1:20 sample dilution), and their concentrations were not normalized. Neutralization was calculated as the percentage of maximal cell index in control wells without virus minus cell index in control (virus-only) wells that exhibited maximal CPE at 40–48 h after applying virus–antibody mixture to the plates. Antibody neutralization was classified as complete if the virus–antibody mixture was classified as partially neutralizing if it delayed but did not fully prevent CPE at the highest tested concentration, while an antibody was classified as partially neutralizing if it delayed but did not fully prevent CPE at the highest tested concentration.

RTCA neutralization assay. To determine neutralizing activity of IgG, we used an RTCA assay on an xCelligence RTCA MP analyzer (ACEA Biosciences) that measures virus-induced CPE. Briefly, 50 μl of cell culture medium (DMEM supplemented with 2% FBS) was added to each well of a 96-well E-plate using a ViaFlo384 liquid handler (Integra Biosciences) to obtain background readings. A standard procedure was followed, where at 16 h after seeding the cells, the virus–antibody mixtures were added in duplicates to the cells in 96-well E-plates. Triplicate wells containing virus only (maximal CPE in the absence of antibody) and wells containing only vero cells in medium (no CPE wells) were included as controls. Plates were measured continuously (every 15 min) for 48 h to assess virus neutralization. Normalized cellular index values at the endpoint (48 h after incubation with the virus) were determined using the RTCA software version 2.1.0 (ACEA Biosciences). Results are expressed as percent neutralization in a presence of respective antibody relative to control wells with no CPE minus cellular index values from control wells with maximum CPE. RTCA IC₅₀ values were determined by non-linear regression analysis using Prism software.

Plaque reduction neutralization test. Virus neutralization with live authentic SARS-CoV-2 virus (USA-WA1) was performed in the BSL-3 facility of the Galveston National Laboratory using vero E6 cells (ATCC, CRL-1586) following the standard procedure. Vero E6 cells were cultured in 96-well plates (104 cells per well). The next day, fourfold serial dilutions of antibodies were made using MEM supplemented with 2% FBS to get an initial concentration of 100 μg ml⁻¹. Equal volumes of diluted antibodies (60 μl) were mixed gently with original SARS-CoV-2 virus (USA-WA1) (60 μl containing 200 P.F.U.) and incubated for 1 h at 37 °C and 5% CO₂ atmosphere. The virus–serum mixture was discarded gently, and the cell monolayer was overlaid with 0.6% methylcellulose and incubated for 2 d. The overlay was removed, and the plates were fixed in 4% paraformaldehyde twice following BSL-3 protocol. The plates were stained with 1% crystal violet, and virus-induced plaques were counted. The percent neutralization and/or neutralizing antibody titer at 50% inhibition (NT₅₀) of antibody was calculated by dividing the plaques counted at each dilution with plaques of virus-only control. For antibodies, the IC₅₀ values were calculated in Prism software (GraphPad) by plotting the midpoint interval between the upper and lower plateau of the neutralization curve among dilutions. The Alpha variant virus was incorporated into the following substitutions: deletion 69–70, deletion 144, E484K, N501Y, A570D, D614G, P681H, T716I, S982A and D1187H. The Beta variant incorporates the following substitutions: deletion 24, deletion 242–243, D80A, D215G, K417N, E484K, N501Y, D614G, H665Y and T1027I. The Delta variant incorporates the following substitutions: T19R, G142D, deletion 156–157, R138G, L452R, D734G, D614G, P681R, deletion 689–691 and D950N; the deletion at positions 689–691 has not been observed in nature, and it was identified following one passage of the virus.

Fab preparation. To generate Fabs, IgGs were incubated with Lys-C at 1:4,000 (wt/ wt) overnight at 37 °C. EDTA-free protease inhibitor (Roche) was dissolved to 25X and added to the sample at a final 1X concentration. The sample was passed over a Protein A column. The flow-through was collected and run on a Superdex 200 Increase 10/300 GL sizing column on the AKTA FPLC system. Fabs were visualized by SDS–PAGE.
Electron microscopy sample preparation and data collection. Purified SARS-CoV-2 S 5′ NTA ctodomain \(^{17}\) and Fab 5317-4 and 5317-10 were combined at a final complex concentration of 0.4 mg ml\(^{-1}\). Fab 5317-10 was added to S and incubated on ice for 30 min before the addition of Fab 5317-4 immediately before grid deposition and freezing. The complex was deposited on Au-300 1.2/1.3 grids that had been plasma cleaned for 4 min in a Solarus 950 plasma cleaner (Gatan) with a 4:1 ratio of O\(_2\)/H\(_2\). Excess liquid was blotted for 3s with a force of ~4 using a Vitrobot Mark IV (Thermo Fisher) and plunge-frozen into liquid ethane. A total of 2,655 micrographs were collected from a single grid with the stage at a 30° tilt using a Titan Krios (Thermo Fisher) equipped with a K3 detector (Gatan). Movies were collected using SerialEM \(^{20}\) at x29,000 magnification with a corresponding calibrated pixel size of 0.81 Å per pixel.

Cryo-EM. Motion correction, contrast transfer function estimation, particle picking and two-dimensional classification were performed using cryoSPARC v.3.2.0 (ref. 18). The final iteration of two-dimensional class averaging distributed 17,710 particles into 50 classes using an uncertainty factor of 3. From that, 13,232 particles were selected, and an ab initio reconstruction was performed with four classes followed by heterogeneous refinement of those four classes. A total of 6,803 particles from the highest-quality class were used for homogenous refinement of the best volume without imposed symmetry. The resulting volume was used for an additional round of homogenous refinement. To filter out additional junk particles, an ab initio reconstruction was performed with three classes followed by heterogeneous refinement of those three classes. A total of 5,171 particles from the highest-quality class were used for homogenous refinement by heterogeneous refinement of those three classes. A total of 5,171 particles from the highest-quality class were used for homogenous refinement of the best volume without imposed symmetry, resulting in a final 9 Å map.

Quantification and statistical analysis. ELISA error bars (s.e.m.) were calculated without imposed symmetry, resulting in a final 9-Å map. Spearman \(r\) quantification and statistical analysis. Using GraphPad Prism version 8.0. Spearman \(r\) correlation was performed using GraphPad Prism 8.0. Analysis of variance was performed for neutralization potency comparisons using GraphPad Prism version 8.0.0.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All unique reagents generated in this study are available from the corresponding author with a completed material transfer agreement (a template can be found in the manuscript files). Sequences for antibodies identified and characterized in this study have been deposited to GenBank (MZ517191–MZ517250, OM001674–OM001699). Raw sequencing data has been deposited to Sequence Read Archive (PRJNA744567, SANN24369247). Cryo-EM maps for the 3D reconstruction of trimeric SARS-CoV-2 S bound to Fabs 5713-4 and 5713-10 have been deposited with the Electron Microscopy Data Bank under accession code EMD-26064.

Supplementary information
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Author contributions
A.R.S., I.S. and I.S.G. developed the methodology. A.R.S., K.J.K., S.C.W., N.S., D.W., S.P., K.A.P., N.R., N.E.S., L.M.W., J.E.C., A.B., R.H.C., J.E.C., A.R.S. and K.J.K. developed software. A.R.S. and K.J.K. performed validations. A.R.S. and I.S.G. wrote the original draft. All authors reviewed and edited the manuscript. I.S.G., J.E.C., A.B., R.H.C., I.S.G. and A.R.S. and K.J.K. acquired funding. I.S.G., J.E.C., A.B., R.H.C. and J.S.M. provided resources. I.S.G. supervised the work.

Competing interests
A.R.S. and I.S.G. are cofounders of AbSeek Bio. I.S.G., A.R.S. and K.J.K. are listed as inventors on antibodies described herein. I.S.G., A.R.S. and I.S. are listed as inventors on patent applications for the LIBRA-seq technology. J.E.C. has served as a consultant for Luna Biologics, is a member of the Scientific Advisory Board Meisa Vaccines and is Founder of IDBiologics. The Crowe laboratory has received funding support in sponsored research agreements from AstraZeneca, IDBiologics and Takeda. The Georgiev laboratory at VUMC has received unrelated funding from Takeda Pharmaceuticals. The remaining authors declare no competing interests.

Additional information
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**Extended Data Fig. 1** | Schematic representation of LIBRA-seq experiments.

**a.** An antigen screening library of oligonucleotide-labeled antigens was generated. This library consisted of SARS-CoV-2 spike antigens and negative controls. Additionally, oligo-labeled ACE2 (the SARS-CoV-2 spike host cell receptor) was included. The antigen screening library was mixed with donor PBMCs. This approach allowed for assessment of B cell ligand blocking functionality from the sequencing experiment.

**b.** An antigen screening library containing an antigen titration was generated, with a goal of identifying high affinity antibodies from LIBRA-seq. In this experiment, six different amounts of oligo-labeled SARS-CoV-2 S protein, each labeled with a different barcode, were included in a screening library.

**c.** Schematic of LIBRA-seq with S titrations and ACE2 included for ligand blocking.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Characterization of LIBRA-seq-identified antibodies. a. Genetic characteristics for monoclonal antibodies prioritized for expression and validation. \(V_{\text{H}}\), \(J_{\text{H}}\), \(V_{\text{L}}\), \(J_{\text{L}}\) inferred gene segment identity is shown at the nucleotide level. CDRH3 and CDRL3 amino acid sequence and length are also shown. b. ELISA binding of antibodies to SARS-CoV-2 spike, SARS-CoV-2 S1, SARS-CoV-2 RBD, SARS-CoV-2 NTD, SARS-CoV-2 S2 and influenza hemagglutinin H1 NC99. Data are represented as mean ± SEM of technical duplicates and represent one of at least two independent experiments (n = 2). c. ACE2 blocking ELISA. Antibodies were added to spike, and recombinant ACE2 was added and detected. Antibodies that block ACE2 binding show a reduction in absorbance compared to ACE2 binding without competitor (dotted line). ELISAs were performed at one antibody concentration, and data are represented as mean ± SEM of technical triplicates and represent one of at least two independent experiments (n = 2). d. Antibodies were tested in a VSV SARS-CoV-2 real time cell analysis (RTCA) neutralization assay. Neutralization curves and IC50 values are shown. Data are represented as mean ± S.D. of technical triplicates, and represent one of two independent experiments (n = 2).
Extended Data Fig. 3 | Characterization of selected cross-reactive antibodies. a. For the IgGs that showed high LIBRA-seq scores (>1) for both SARS-CoV-2 and SARS-CoV, the percent of cells with low ACE2 scores (<-1) is shown. b. ELISA binding of antibodies to SARS-CoV-2 spike, SARS-CoV spike, influenza hemagglutinin H1 NC99, SARS-CoV-2 S1, SARS-CoV-2 RBD, and SARS-CoV-2 S2. Data are represented as mean ± SEM of technical duplicates and represent one of at least two independent experiments (n = 2). c. ACE2 blocking ELISA. ACE2 binding without competitor is shown as a dotted line. ELISAs were performed at one antibody concentration, and data are represented as mean ± SEM of technical triplicates and represent one of at least two independent experiments (n = 2).
Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection  BCR contigs were processed using Cell Ranger 3.1.0 (10X Genomics) using GRCh38 as reference. Antigen barcode libraries were also processed using Cell Ranger 3.1.0 (10X Genomics).

Data analysis  For sequencing analysis, we used our previously described pipeline to use paired-end FASTQ files of oligo libraries as input, process and annotate reads for cell barcode, UMI, and antigen barcode, and generate a cell barcode - antigen barcode UMI count matrix [Setliff et al. Cell, 2019][Shakolas et al. Cell Reports Medicine, 2021]. We also utilized High V-Quest (Alamyar et al. Methods Mol Biol, 2012) and ChangeO (Gupta et al. Bioinformatics, 2015) for further sequence processing.

Additional data in the study was analyzed using GraphPad Prism 8.0.0.

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All unique reagents generated in this study are available from the corresponding author with a completed Materials Transfer Agreement. Sequences for antibodies identified and characterized in this study have been deposited to GenBank (MZ517191-MZ517250, OM001674)
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Life sciences study design

All samples must disclose on these points even when the disclosure is negative.

**Sample size**
No sample size calculation was performed. This study sought to highlight that potently neutralizing antibodies could be identified using a small number of antibody hits from a single next-generation sequencing experiment. Hundreds of cells were recovered from each of three experiments, and from each, less than a dozen antibodies were selected for expression and validation (as shown in the manuscript).

**Data exclusions**
Prioritized, expressed antibodies [shown in Figure 1] were excluded from characterization analysis if heavy/light chain plasmid sequencing did not match expected sequence or if ELISA binding repeats were not consistent.

**Replication**
All ELISA binding data and ACE2 blocking data were repeated at least twice in technical duplicate. Data are shown as mean +/- SEM. Additionally, replicates for neutralization data are shown as mean +/- SD.

**Randomization**
Randomization is not relevant to our study.

**Blinding**
Blinding is not relevant to our study.

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| Materials & experimental systems | Methods |
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| n/a                             | Involved in the study |
| □ ✗ Antibodies                  | ✗ ChiP-seq |
| □ ✗ Eukaryotic cell lines       | □ Flow cytometry |
| □ Palaeontology and archaeology | ✗ MRI-based neuroimaging |
| □ Animals and other organisms   |         |
| □ Human research participants   |         |
| □ Clinical data                 |         |
| □ Dual use research of concern  |         |

**Antibodies**

Antibodies were used for fluorescence activated cell sorting (FACS): APC-Cy™7 Mouse Anti-Human CD14 (BD, Cat#561709; RRID: RRID:AB_10893806), FITC Anti-Human CD3 (Tonbo Biosciences, Cat#35-0037; RRID:AB_2621662), BV711 Mouse Anti-Human CD19 (BD, Cat#563036; RRID: AB_2737968), and PE-Cy™5 Mouse Anti-Human IgG (BD, Cat#551497; RRID: AB_394220).

Antibodies were used in binding and neutralization experiments: CR3022 [Yuan et al. Science 2020], 3602-1707 [Setliff et al. Cell 2019], 46472-4 (S2 ab, Skafalis et al. Cell Reports Medicine 2021, GenBank: MZ126647, MZ1266621), 46472-6 (NTD ab, Skafalis et al. Cell Reports Medicine 2021, GenBank: MZ126649, MZ126664), 1F8 (Tang et al. Proceedings of the National Academy of Sciences 2014), DENV-2D22 (James Crowe Jr.), and COV2-2130 [Zost et al., Nature 2020].

**Validation**

BD and Tonbo Biosciences perform QC testing and validation on fluorescently labeled antibodies. Citations for other antibodies used are provided above. Additionally, antibodies used in binding and neutralization tests were tested for binding and/or neutralization of previously confirmed/known antigens or viral strains.

Eukaryotic cell lines

**Cell line source(s)**

Freestyle 293F cells (ThermoFisher Scientific, Cat#A14528)
Exp293F cells (ThermoFisher Scientific, Cat#A14527)
Authentication
ExplicHO cells were authenticated using ATCC’s Cell Line Authentication Service.

Mycoplasma contamination
ExplicHO cell line was routinely tested and were negative for mycoplasma contamination. Vero E6 cells were negative for mycoplasma contamination. Mycoplasma testing of cell lines was performed using a PCR-based mycoplasma detection kit (ATCC, 30-1012K) or a mycoplasma detection colorimetry kit.

Commonly misidentified lines
(See [CLAC register](#))
None.

Flow Cytometry

Plots
Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
PBMC samples were purchased from Cellero and stained for fluorescence activated cell sorting.

Instrument
A 4-laser FACS Aria III and a 5-laser FACS Aria III were used for FACS experiments.

Software
Flow cytometry data were analyzed using FlowJo. The sorting data are not shown in the manuscript. Summary data from the post-sorting, single cell sequencing antigen reactivity read out are shown in Figure 1.

Cell population abundance
After sorting, for experiment 1 there were 23,162 antigen+/IgG+ cells. For experiment 2, there were 65,578 antigen+/IgG+ cells. For experiment 3, there were 19,133 antigen+/IgG+ cells. These cells were then used for single cell processing and sequencing.

Gating strategy
Cells were gated on SSC-A and FSC-A. Then, singlets were isolated by gating on SSC-W/SSC-H and FSC-W/FSC-H. Next, cells were gated for negativity to APC-Cy7 (CD14 and LiveDead marker). These Live/CD14- cells were gated for CD3-FITC negative/CD19-BV711 positive. Lastly, the cells were gated for antigen-PE positivity and IgG-PE-Cy5 positivity. An FMO control without antigen-PE was also used for gating.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.