Discovery and Characterization of Spike N-Terminal Domain-Binding Aptamers for Rapid SARS-CoV-2 Detection

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Author Contributions

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Materials and Methods

Oligonucleotides. All oligonucleotides were synthesized by Integrated DNA Technologies. The ssDNA library used in the protein SELEX process was TCGCTCTTTCCGTTCTTCCGGG-N40-CGCGTGTAAGTCCGTGTCGCAA. The forward primer for amplification was FAM-TGGCTCTTTCCGTTCTTCCGGG and the reverse primer for amplification was Biotin-TTGGCCACAGGACTTACG. Oligonucleotide sequences are listed in Table S4. Modifications to oligonucleotides included: FITC (5'-FITC - sequence - 3'), biotin (5'-biotin - iSp18 - sequence - 3'), and Cy5 (5'-Cy5 - sequence - 3'). All aptamer pools and aptamers were annealed before use in SELEX, binding, characterization, and other studies. Aptamers were annealed by diluting to 1 μM in SELEX WB (see buffers below), heating at 95°C for 5 min, and snap-cooling on ice.

Buffers. The composition of SELEX wash buffer (SELEX WB) is 4.5 g/L glucose, 0.1 g/L CaCl₂, 0.2 g/L KCl, 0.2 g/L KH₂PO₄, 0.1 g/L MgCl₂·6H₂O, 8 g/L NaCl, 2.1716 g/L Na₂HPO₄, and 5mM MgCl₂. 2.25g glucose (Sigma) and 2.5 mL 1M MgCl₂ is added to 497.5 mL Dulbecco's phosphate-buffered saline (DPBS) with CaCl₂ and MgCl₂ (Corning). The buffer is filtered through a 0.22μm sterile filter and stored at 4°C. For binding buffers, yeast tRNA (Invitrogen) (tRNA) and salmon sperm DNA (Invitrogen) (SS DNA) are added to a final concentration of 0.1 mg/mL. Additionally, MACS BSA 10% stock solution (Miltenyi Biotec) is added to 0.1-5% final concentration depending on the assay.

Recombinant proteins. SARS-CoV-2 S protein S1 domain (ACROBiosystems S1N-C5255), SARS-CoV-2 S protein monomer (ACROBiosystems SPN-C52H4), SARS-CoV-2 S protein trimer (ACROBiosystems SPN-C52H8), SARS-CoV-2 S protein S1 NTD (ACROBiosystems S1D-C52H6), SARS-CoV-2 S protein RBD (ACROBiosystems SPD-C5255) were purchased in lyophilized form. SARS-CoV S protein S1 domain (ACROBiosystems S1N-S52H5) and MERS-CoV S protein S1 domain (Sino Biologics 40069-V08H) were also purchased in lyophilized form. The aforementioned proteins were reconstituted, aliquoted, and stored according to manufacturer's recommendations. SARS-CoV-2 S protein trimer “S-2P” was kindly provided by the Institute of Protein Design (IPD) at the University of Washington.

Viruses. Inactivated SARS-CoV-2 virus samples at known concentrations were kindly provided by the NIH RADx-Radical Data Coordination Center (DCC) at University of California San Diego and BEI Resources. Lentivirus was kindly provided by Prof. Drew L. Sellers. Lentivirus concentration was measured by qPCR Lentivirus Titer Kit (Applied Biological Materials).

Protein-SELEX. The protocol was based on the guidelines outlined by Wang et al.¹ and is outlined in Figure 1. Experimental conditions for 12 rounds of SELEX are summarized in Table S1. Briefly, partitioning of bound aptamers was achieved by either Dynabeads His-tag isolation & Pulldown (Novex by Life Technologies) or Nab™ protein G Spin Kit (Thermo Scientific), with the exception of round 7. In this round, the aptamer pool bound to SARS-CoV-2 S1-Fc was incubated with magnetic anti-biotin microbeads and applied onto an MS column (Miltenyi Biotec). The flow through fraction was collected and bound to protein G agarose. Aptamers were amplified with Phusion® High-Fidelity DNA Polymerase (NEB). Annealing, strand separation, and composition of buffers were described previously².

Aptamer round pool binding assay. We quantified the binding of the aptamer round pools (Round 9, 10, 11, and 12) to S protein variants using an ELISA-like plate binding assay. Nunc MaxiSorp™ flat-bottom 96-well plates (Thermo Fisher Scientific) were coated with 4.2 μg/mL SARS-CoV-2 spike protein monomer (Acro Biosystems SPN-C52H4) at 4°C overnight. Then wells were washed four times with wash buffer (0.5% BSA, 0.01% Tween-20 SELEX WB.) Next, wells were incubated with blocking buffer (5% BSA, 0.1 mg/mL tRNA, 0.1 mg/mL SS DNA, 0.01% Tween-20 SELEX WB) for 1.5 hr at room temperature. After blocking buffer removal via flick and tap, 100 nM FAM-labeled aptamer pools or random sequence aptamer (RAN) in binding buffer were incubated for 30min at room temperature. Then the plate was washed four times with wash buffer and stained with HRP anti-fluorescein antibody (1:2000, Abcam cat. no. ab6656) for 1 h at room temperature. Lastly, the plate was washed four times with wash buffer. Pierce TMB substrate kit (Thermo Scientific 1854050) was mixed at 1:1 according to manufacturer’s protocol, then added to wells
and incubated at room temperature until desired blue color was developed (5-30 min.). 2M sulfuric acid was added to each well to terminate the HRP reaction. All steps use 100 µL of solution, except blocking and washing which use 200 µL. For wash steps, the plate is flicked over a sink then blotted dry. The absorbance of the solution (yellow) was measured using the Infinite 200 PRO plate reader (Tecan) at 450 nm with 550 nm as reference. The absorbance values of the groups were deducted from wells without aptamer and values normalized to the RAN negative control were plotted.

**NGS and data analysis.** The DNA pools from SELEX rounds were PCR amplified with barcoded primers described in Table S3 using the MiSeq Reagent Kit v2 (300-cycles) (Illumina) and MiSeq System (Illumina) per manufacture’s protocol. Exported FASTA files were analyzed with FASTAptamer v1.0.3. The count function was used to calculate rank and reads per million for each sequence, then the compare function was used pair-wise to compare sequences within subsequent SELEX round pools, generating the fold enrichment value. Neighbor joining trees for the top 100 sequences for rounds 9 through 12 were constructed using Simple Phylogeny (https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/), and visualized using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). In combination with the phylogenetic tree, MEME Suite v5.1.1 was used for motif prediction.

**Aptamer binding assay with magnetic spheres.** Streptavidin or Protein G Sphero™ magnetic spheres were used in binding assays. Streptavidin spheres were further modified with biotin anti-6-His epitope tag antibody (Biolegend cat. no. 906103). These spheres were used to immobilize SARS-CoV-2 S1-His and SARS-CoV-2 S1-Fc proteins, respectively. Individual Cy5 labeled aptamers were incubated with SARS-CoV-2 S1 spheres in binding buffer for 30 min at room temperature, washed twice with wash buffer (0.1% BSA, 0.01% Tween-20 SELEX WB) and analyzed by flow cytometry (Attune NxT, Invitrogen).

**Biolayer interferometry (BLI).** Studies were performed with an Octet Red96 machine (Sartorius) at 25°C and 1,000 rpm sample agitation. Pre-soaked streptavidin (SA) sensors were rinsed in 1% BSA, 0.1 mg/mL tRNA, 0.1 mg/mL SS DNA, 0.01% Tween-20 SELEX WB (“diluent”) for 100s. Next, tips were loaded with 50 nM biotinylated aptamer until reaching a 0.5 nm signal threshold. Subsequently, tips were rinsed in diluent for 100s then baselined in diluent for another 100s. After association with protein of interest diluted to the desired concentration, sensor tips were returned to the baseline diluent well for dissociation. Data was analyzed with Octet Data Analysis 9.0 (Sartorius). Kinetic values were determined from a global fit of several curves generated from serial dilutions of the protein in a 1:1 binding model. The quality of the fit was calculated with $R^2$ and $\chi^2$ values. See Table S5 for the estimated kinetics parameters from these assays.

**Cryo-EM specimen preparation and data collection.** SARS-CoV-2 S protein (Acro Biosystems: SPN-C52H8) was reconstituted according to manufacturer’s protocol and concentrated and buffer-exchanged into TBS pH 8.0 using a 30 kDa MWCO Amicon Ultra centrifugal filter (EMD Millipore). SARS-CoV-2 S protein was incubated with annealed SNAP1 with or without S2M11 Fab fragment at 1:3:2 molar ratio of Spike protomer:SNAP1:S2M11 Fab for 1 hour at room temperature. For the Spike:SNAP1 complex without S2M11, 3 µL of the complex at a concentration of 0.3 mg/mL SARS-CoV-2 S was applied onto glow-discharged C-flat, Cu 200 mesh, CF-1.2/1.3 grids. For the Spike:SNAP1:S2M11 complex, 3 µL of the complex at a concentration of 0.3 mg/mL SARS-CoV-2 S was mixed with 0.3 µL 1% octyl-glucoside (OG) immediately before application onto glow-discharged C-flat, Cu 200 mesh, CF-1.2/1.3 grids. The grids were plunge-frozen in liquid ethane and cooled with liquid nitrogen, using an FEI MK4 Vitrobot with a 6.5 or 7 s blot time. The chamber was kept at 20°C and 100% humidity during the blotting process. Data acquisition was carried out with the Leginon data collection software4 on a 200 kV FEI Glacios electron microscope and equipped with a Gatan BioQuantum energy filter (slit width of 20 eV) and a Gatan K2 Summit camera. The nominal magnification was 36,000x and the pixel size was 1.16 Å. The dose rate was adjusted to 8 counts/pixel/s and each movie was acquired in super-resolution mode fractionated in 50 frames of 200 ms each.
Cryo-EM data processing.
For the dataset of Spike in complex with SNAP1 without S2M11, Warp was used to perform motion correction, CTF estimation, particle picking and particle extraction. 25,758 extracted particles with a box size of 400 pixels were exported to cryoSPARC for reference-free 2D classification.

For the Spike:SNAP1:S2M11 dataset, movies were motion-corrected using Warp and exported to cryoSPARC for CTF estimation using CTFFIND4. A set of manually picked particles were used to generate 2D class averages that were subsequently low pass-filtered to 20 Å for Template Picker in cryoSPARC. 55,369 particles were selected and underwent local refinement in cryoSPARC. 41,071 particles were selected for ab initio reconstruction (no symmetry) and non-uniform refinement (NUR) (C3 symmetry), followed by CTF refinement and another round of NUR in cryoSPARC. The particles were exported to RELION v3.0 for Bayesian polishing and then uploaded to cryoSPARC for NUR. To improve the density around the SARS-CoV-2 S NTD/SNAP1 interface, the particles were C3-symmetry-expanded and subjected to focus 3D classification without refining angles and shifts using a soft mask encompassing SNAP1 and part of the NTD in RELION. 55,369 particles belonging to the best-resolved classes with the aptamer bound were selected and underwent local refinement in cryoSPARC. Local resolution estimation, local filtering and map sharpening were performed in cryoSPARC. Reported resolutions are based on the gold-standard Fourier shell correlation (GS-FSC) at 0.143 criterion and FSC curve was corrected for the effects of soft masking by high-resolution noise substitution. See Figure S8E for detailed data processing workflow.

Lateral flow assay (HybriDetect). HybriDetect universal lateral flow dipsticks (Milenia Biotec) were used in combination with biotinylated SNAP1.50 aptamer and FITC-labeled SNAP1.50 aptamer. 100 µL of 25 nM of both aptamers were incubated with S protein, UV-inactivated SARS-CoV-2 virus, or lentivirus in binding buffer for 20 minutes at room temperature. Then, the lateral flow strip is dipped in the solution and results were interpreted in 10-30 minutes. Images were quantified with ImageJ.

ELISA. Wells of a white streptavidin-coated 96-well plate (Thermo Fisher Scientific) were washed and blotted three times with SELEX WB then incubated with 50 nM biotinylated NS or SNAP1 aptamer at 4°C for 30 min. After three washes with wash buffer (0.1% Tween-20 2% BSA SELEX), wells were incubated with blocking buffer (5% BSA, 1:100 biotin blocking solution (Vector Laboratories), 0.1 mg/mL tRNA, 0.1 mg/mL SS DNA, 0.1% Tween-20 SELEX WB) for 1.5 hr at room temperature. Subsequently, the wells were incubated with S protein, UV-inactivated SARS-CoV-2 virus, or lentivirus in binding buffer (2% BSA, 0.1 mg/mL tRNA, 0.1 mg/mL SS DNA, 0.1% Tween-20 SELEX WB) for 30 min at room temperature. Then wells were washed four times before incubation with anti-SARS-CoV-2 antibody with HRP (Novus Biologicals cat. no. NBP2-90980H). Lastly, wells were washed six times. All steps use 100 µL of solution, except washing (200 µL of solution.) For wash steps, the plate is flicked over a sink then blotted dry. Ice-cold ELISA Femto Substrate (Thermo Fisher Scientific) was added and the plate luminescence was immediately measured by Infinite 200 PRO plate reader (Tecan) with 250 nm integration time and automatic attenuation.

1. Wang, T., Yin, W., AlShamaileh, H., Zhang, Y., Tran, P. H. L., Nguyen, T. N. G., ... & Duan, W. (2019). A detailed protein-SELEX protocol allowing visual assessments of individual steps for a high success rate. Human gene therapy methods, 30(1), 1-16.
2. Sefah, K., Shangguan, D., Xiong, X., O’donoghue, M. B., & Tan, W. (2010). Development of DNA aptamers using Cell-SELEX. Nature protocols, 5(6), 1169.
3. Alam, K. K., Chang, J. L., & Burke, D. H. (2015). FASTAptamer: a bioinformatic toolkit for high-throughput sequence analysis of combinatorial selections. Molecular Therapy-Nucleic Acids, 4, e230.
4. Suloway, C., Pulokas, J., Fellmann, D., Cheng, A., Guerra, F., Quispe, J., ... & Carragher, B. (2005). Automated molecular microscopy: the new Leginon system. *Journal of structural biology, 151*(1), 41-60.

5. Tegunov, D., & Cramer, P. (2019). Real-time cryo-electron microscopy data preprocessing with Warp. *Nature methods, 16*(11), 1146-1152.

6. Punjani, A., Rubinstein, J. L., Fleet, D. J., & Brubaker, M. A. (2017). cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods, 14*(3), 290-296.

7. Zivanov, J., Nakane, T., Forsberg, B. O., Kimanius, D., Hagen, W. J., Lindahl, E., & Scheres, S. H. (2018). New tools for automated high-resolution cryo-EM structure determination in RELION-3. *eLife, 7*, e42166.

8. Chen, S., McMullan, G., Faruqi, A. R., Murshudov, G. N., Short, J. M., Scheres, S. H., & Henderson, R. (2013). High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy, 135*, 24-35.
Table S1. SELEX conditions for 12 rounds of selection. T indicates 0.1 mg/mL yeast tRNA; S indicates 0.1 mg/mL salmon sperm DNA; HTID indicates His-Tag Isolation Dynabeads™.

| Round | Aptamer pool (nM) | Binding conditions | Negative Selection | Time (min) | Positive Selection | Time (min) |
|-------|------------------|--------------------|--------------------|------------|--------------------|------------|
| 1     | 5000             | 0.1% BSA, T        | -                  | -          | 50 nM SARS-CoV-2   | 60         |
|       |                  |                    |                    |            | S1-His; 50 µL HTID |            |
| 2     | 500              | 0.1% BSA, T        | 50 µL HTID         | 30         | 20 nM SARS-CoV-2   | 30         |
|       |                  |                    |                    |            | S1-His; 50 µL HTID |            |
| 3     | 250              | 0.5% BSA, T        | 100 µL HTID        | 30         | 5 nM SARS-CoV-2    | 30         |
|       |                  |                    |                    |            | S1-His; 10 µL HTID |            |
| 4     | 250              | 1% BSA, T          | 100 µL protein G   | 30         | 2.5 nM SARS-CoV-2  | 30         |
|       |                  |                    | agarose            |            | S1-Fc; 10 µL protein |            |
|       |                  |                    |                    |            | G agarose          |            |
| 5     | 100              | 1% BSA, T          | 100 µL protein G   | 30         | 1 nM SARS-CoV-2    | 30         |
|       |                  |                    | agarose            |            | S1-Fc; 10 µL protein |            |
|       |                  |                    |                    |            | G agarose          |            |
| 6     | 100              | 2% BSA, T, S       | 100 µL protein G   | 30         | 1 nM SARS-CoV-2    | 30         |
|       |                  |                    | agarose            |            | S1-Fc; 10 µL protein |            |
|       |                  |                    |                    |            | G agarose          |            |
| 7     | 100              | 2% BSA, T, S       | Anti-Biotin        | -          | 5 nM SARS-CoV-2    | 30         |
|       |                  |                    | magnetic beads;    |            | S1-Fc; 10 µL protein |            |
|       |                  |                    | MS column          |            | G agarose          |            |
| 8     | 100              | 2% BSA, T, S       | 100 µL HTID        | 20         | 2.5 nM SARS-CoV-2  | 20         |
|       |                  |                    |                    |            | S1-His; 10 µL HTID |            |
| 9     | 100              | 2% BSA, T, S       | 2.5 nM MERS-CoV S1;| 20         | 2.5 nM SARS-CoV-2  | 20         |
|       |                  |                    | 10 µL HTID         |            | S1-His; 10 µL HTID |            |
| 10    | 100              | 2% BSA, T, S       | 25 nM MERS-CoV S1; | 20         | 2.5 nM SARS-CoV-2  | 20         |
|       |                  |                    | 100 µL HTID        |            | S1-His; 10 µL HTID |            |
| 11    | 100              | 2% BSA, T, S       | 25 nM SARS-CoV S1; | 20         | 2.5 nM SARS-CoV-2  | 20         |
|       |                  |                    | 100 µL HTID        |            | S-His; 10 µL HTID  |            |
| 12    | 100              | 2% BSA, T, S       | 10 nM SARS-CoV S1; | 20         | 1 nM SARS-CoV-2    | 20         |
|       |                  |                    | 100 µL HTID        |            | S-His; 10 µL HTID  |            |
Table S2. Next generation sequencing primers. Barcodes are in lowercase.

| Name          | Sequence                                                                 |
|---------------|--------------------------------------------------------------------------|
| Aptamer\_F\_v2 | AATGATACGCGACCACCCGAGATCTACACACAGACCGTGCTGCTCTTTCCGCTCTTCCTCG            |
| Aptamer\_R\_3 0v2 | CAAGCAGAAGACGGCATACGAGATtggtcagcaCGAGGAGATACCTTCGACACAC                  |
| Aptamer\_R\_3 1v2 | CAAGCAGAAGACGGCATACGAGATactatgcaatCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 2v2 | CAAGCAGAAGACGGCATACGAGATcgacgctacCGAGGAGATACCTTCGACACAC                 |
| Aptamer\_R\_3 3v2 | CAAGCAGAAGACGGCATACGAGATgatacggaacCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 4v2 | CAAGCAGAAGACGGCATACGAGATttatccggatCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 5v2 | CAAGCAGAAGACGGCATACGAGATtagagtaataCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 6v2 | CAAGCAGAAGACGGCATACGAGATggcgttaaggCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 7v2 | CAAGCAGAAGACGGCATACGAGATacttaaccttCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 8v2 | CAAGCAGAAGACGGCATACGAGATcaaccgctaaCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_3 9v2 | CAAGCAGAAGACGGCATACGAGATgaccttgataCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_4 0v2 | CAAGCAGAAGACGGCATACGAGATggcgttaaggCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_4 1v2 | CAAGCAGAAGACGGCATACGAGATacttaaccttCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_4 2v2 | CAAGCAGAAGACGGCATACGAGATcaaccgctaaCGAGGAGATACCTTCGACACAC                |
| Aptamer\_R\_4 3v2 | CAAGCAGAAGACGGCATACGAGATgacttgataCGAGGAGATACCTTCGACACAC                 |
| Aptamer\_R\_4 4v2 | CAAGCAGAAGACGGCATACGAGATctgatatcCGAGGAGATACCTTCGACACAC                 |
Table S3. Motif sequences enriched via SELEX in rounds 9 through 12. Hits are motifs found by MEME Suite within the top 100 sequences. Percent Representation is reads per million of the motif divided by total reads per million. Fold enrichment is the fold enrichment in % representation over previous round. Motifs 1 (purple) and 3 (gold) are underlined. The following letters represent ambiguous base identities: R = AG, Y = CT, K = GT, M = AC, S = CG, W = AT, B = CGT, D = GAT, H = ACT, V = ACG.

| Round and hit ranking | Sequence                                                                 | % Representation | Fold enrichment |
|-----------------------|--------------------------------------------------------------------------|------------------|----------------|
| R9 Hit 1              | CATTGTGCAYCCTGACTGACCCTAAGGTCGAACATCGC                                  | 0.09%            | -              |
| R9 Hit 2              | KCGCAAYASYARACARHARHWTGDVSTR                                             | 0.017%           | -              |
| R9 Hit 3              | SMAASMTACMGK                                                             | 0.031%           | -              |
| R10 Hit 1             | AACTYTTGATHMW                                                            | 0.14%            | -              |
| R10 Hit 2             | CATTGTGCAYCCTGACTGACCCTAAGGTCGAACATCGC                                  | 0.03%            | 0.33           |
| R10 Hit 3             | TTAGBTACAKMGYBTWHATTWCTMAARTCTWTTGTCTA                                 | 0.009%           | 0.29           |
| R11 Hit 1             | TTAGGTACATCGTCATTCTCATAAGGTACATTGTAC                                   | 0.46%            | 51             |
| R11 Hit 2             | CAKTBYCCACACTYTTGATTCAATWCTCAATTAAATAC                                 | 0.18%            | -              |
| R11 Hit 3             | CATTGTGCAYCCTGACTGACCCTAAGGTCGAACATCGC                                  | 0.13%            | 4.3            |
| R12 Hit 1             | TCATTGTGCATCTGACTGACCCTAAGGTCGAACATCGC                                  | 64.3%            | 495            |
| R12 Hit 2             | CATTGTGCATCTGACTGACCCTAAGGTCGAACATCGC                                  | 0.18%            | 0.39           |
| R12 Hit 3             | CAGTSAYCCASACTTGATWGCAWA                                               | 0.12%            | 0.67           |
Table S4. Aptamer sequences. See methods for list of modifications.

| Name   | Sequence                                                                 |
|--------|--------------------------------------------------------------------------|
| RAN    | 5'-ATCCAGAGTGACGCAGCAAATTCCAAACTCGAGTAAGCGTAGAGCCTCTCATCGCCTCAAAATGGGACACGGTGCTTAGT-3' |
| SNAP1  | 5'-TCGCTCTTTTCCGCTTTTTCGCGGGTCATTGTGCATCCTGACTGACCCTAAGGTGCGAACATCGCCCGTGTAAGTCCGTGTGTGCGAA-3' |
| SNAP3  | 5'-TCGCTCTTTTCCGCTTTTTCGCGGTATTGTACATCGTCTTCATTTCTCAAGTCATTGTCTACACCGCGTGTAAGTCCGTGTGTGCGAA-3' |
| NS     | 5'-CCAGAGTGACGCAGCAAATTCCAAACTCGAGTAAGCGTAGAGCCTCATCGCCTCAAAATGGGACACGGTGCTTAGT-3' |
| scrSNAP1 | 5'-TCGCTCTTTTCCGCTTTTTCGCGGATCGCGTGGCATCGACCAAGCGCATCTATCTTCCGAATCGCGTGTAAGTCCGTGTGTGCGAA-3' |
| SNAP1.66 | 5'-CGCTTTTCCGCGGTATTGTGCATCCTGACTGACCCTAAGGTGCGAAATCGCGCGTGTAAGTCCGTGTGTGCGAA-3' |
| SNAP1.50 | 5'-CGCGGTCATTGTGCACTCCTGACTGACCCTAAGGTGCGAATCGCGCGTGTAAGTCCGTGTGTGCGAA-3' |
| CD8    | 5'-CACAGTGACGCAGCAAACAGAGGTGTAGAAGTACACGTGAAAGTACACGCGAACAGCTTGGG-3' |
Table S5. SNAP1 and SNAP3 binding kinetic values and fit parameters.

| Aptamer | SARS-CoV-2 Protein | $K_{on}$ 1/nM•s • 10⁻⁵ | $K_{off}$ 1/s • 10⁻⁵ | $K_D$ nM | $\chi^2$ | $R^2$ |
|---------|-------------------|----------------------|---------------------|---------|---------|-------|
| SNAP1   | S1-His            | 0.71 ± 0.00          | 27.88 ± 0.03        | 39.32 ± 0.12 | 2.5844 | 0.9978 |
| SNAP1   | NTD-His           | 8.64 ± 0.22          | 521.30 ± 3.20       | 60.35 ± 1.61 | 0.0299 | 0.9740 |
| SNAP1   | S                 | 19.53 ± 0.01         | -                   | -       | 5.6620 | 0.9981 |
| SNAP1   | S-2P              | 0.16 ± 0.00          | -                   | -       | 5.4186 | 0.9914 |
| SNAP1   | S-B.1.1.7         | 4.71 ± 0.18          | -                   | -       | 0.1741 | 0.9410 |
| SNAP3   | S1-His            | 1.26 ± 0.01          | 96.77 ± 0.17        | 76.59 ± 0.45 | 0.5248 | 0.9969 |
| SNAP3   | NTD-His           | 24.44 ± 1.09         | 1302.00 ± 18.00     | 53.25 ± 2.49 | 0.0094 | 0.9509 |
| SNAP1.66| S1-His            | 0.90 ± 0.00          | 28.53 ± 0.02        | 31.70 ± 0.10 | 0.8597 | 0.9990 |
| SNAP1.66| NTD-His           | 5.01 ± 0.10          | 527.20 ± 1.90       | 105.30 ± 2.17 | 0.0452 | 0.9888 |
| SNAP1.50| S1-His            | 0.80 ± 0.00          | 28.63 ± 0.02        | 35.77 ± 0.16 | 0.5739 | 0.9986 |
| SNAP1.50| NTD-His           | 3.74 ± 0.11          | 508.10 ± 1.70       | 135.70 ± 4.10 | 0.0179 | 0.9903 |

Table S6. SARS-CoV-2 spike protein variants. Ranges are based on the SARS-CoV-2 S protein sequence (GenBank ID: QHD43416.1, PDB: P0DTC2)

| Name | Range | Furin Site Mutations | Other Mutations | Tags | Trimerization Domain | Source |
|------|-------|----------------------|-----------------|------|----------------------|--------|
| S    | Val 16 – Pro 1213 | R683A, R685A | none | His | Yes: not disclosed | ACROBiosystems |
| S-2P | Gln 14 – Lys 1230 | R682S, R683G, R685G | K986P, V987P | His | Yes: C-terminal foldon | PDB: 6VXX |
| S-B1.1.7 | Val 16 – Pro 1213 | R683A, R685A | F817P, A892P, A899P, A942P, K986P, V987P; HV69-70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H | His | Yes: T4 fibrin | ACROBiosystems |
Figure S1. Binding of aptamer round pools to SARS-CoV-2 S monomer. Fluorescently-labeled aptamer pools (100 nM) were tested for binding to SARS-CoV-2 S monomer immobilized on high protein-binding plates. Background-subtracted data were normalized to the baseline OD450 value (dotted line) and two technical replicates were graphed (black diamonds).
Figure S2. NGS of aptamer round pools reveals motifs enriched during SELEX. Each phylogenetic tree represents the top 100 sequences in each aptamer pool from round 9 to round 12. The number next to each node represents its rank in the top 100. Motif 1 (purple) and motif 3 (gold) are highlighted in each tree. Trees were generated with Simple Phylogeny (https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/) and FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and binding motifs predicted with MEME analysis (MEME-suite.org).
Figure S3. Secondary structure prediction and alignment of SNAP1 and SNAP3 aptamers. A) Secondary structure was predicted using NUPACK (http://www.nupack.org/) for aptamers SNAP1 (left) and SNAP3 (right) with the following conditions: 4°C, 0.137 M Na+, 0.0055 M Mg++. Arrows represent the 3’ end of the aptamer. Nucleotides are color-coded by their equilibrium probability (see legend on right). B) Local alignment of motif 1 (40-bp random region of SNAP1) and motif 3 (40-bp random region of SNAP3) was conducted with EMBOSS Water (https://www.ebi.ac.uk/Tools/psa/).
Figure S4. Aptamers SNAP1 and SNAP3 bind to SARS-CoV-2 S1. Protein G magnetic spheres coated with Fc-tagged SARS-CoV-2 S1 (400 nM) or human CD71 (200 nM) were incubated with 100 nM Cy5-labeled NS (gray), SNAP1 (purple), or SNAP3 (gold) aptamer. Fluorescence of spheres was measured by flow cytometry. Each histogram is one technical replicate.
Figure S5. Aptamers SNAP1 and SNAP3 do not bind to SARS-CoV S1 or MERS-CoV S1. A) Magnetic spheres coated with 200 nM His-tagged proteins MERS-CoV S1 (red), SARS-CoV S1 (orange), or SARS-CoV-2 S monomer (dark green) were incubated with 100 nM Cy5-labeled NS (left), SNAP1 (center), or SNAP3 (right) aptamer. Fluorescence of spheres was measured by flow cytometry. Each histogram is one technical replicate. B) Kinetic parameters were measured through BLI. Biotinylated NS or SNAP1 aptamer were loaded on streptavidin biosensors, associated with 200 nM S1 of MERS-CoV (red), SARS-CoV (orange), or SARS-CoV-2 (light green) between 0-4200s, and then dissociation occurred between 4,200-8,400s.
Figure S6. Aptamers SNAP1 and SNAP3 do not interfere with SARS CoV-2 S1 binding to ACE2. 

A) BLI experiment was conducted by loading His-tagged ACE2 onto Ni-NTA biosensors and testing association with 50 nM SARS-CoV-2 S1 alone and in the presence of 500 nM NS, SNAP1, or SNAP3 aptamer. Dotted line indicates switch from aptamer association (0-600s) to dissociation (600-1500s). Black lines indicate global fit of the kinetic data for a 1:1 binding model. 

B) Increasing concentrations of unlabeled NS, SNAP1, or SNAP3 aptamers were co-incubated with 25 nM Fc-labeled SARS-CoV-2 S1 for binding to ACE2-expressing HEK293T cells. Fluorescence of cells was measured by flow cytometry. Each point represents one technical replicate, with APC anti-Fc antibody fluorescence normalized to cells incubated only with 25 nM S1 (dotted line).
Figure S7. Aptamers SNAP1 and SNAP3 compete for the same binding site on SARS-CoV-2 S1. Protein G magnetic spheres immobilized with Fc-tagged SARS-CoV-2 S1 protein were incubated with 25 nM Cy5-labeled SNAP3 in the presence of increasing concentrations of unlabeled NS aptamer (dark gray) or SNAP1 (purple). Fluorescence of spheres was measured by flow cytometry. Each point is a mean calculated from three biological replicates normalized to no competitor (light gray), bars indicate s.d. Statistical comparison of NS to SNAP1 for each ratio was calculated by 2-way ANOVA, * denotes p < 0.05 and ** denotes p < 0.01.
Figure S8. Cryo-EM characterization of the SNAP1/S/S2M11 complex. A) Representative micrograph. Scale bar, 100 nm. B) Reference-free 2D class averages. Scale bar, 100 Å. C) Gold-standard Fourier shell correlation (GSFSC) curves of the global map and the locally refined map of the NTD-bound aptamer. D) Unsharpened maps colored by local resolution in cryoSPARC of the SNAP1/S/S2M11 complex, as well as the locally-filtered map of the locally refined reconstruction of NTD and SNAP1 (inset). E) Cryo-EM data processing flow chart. Selected groups of particles at a given step are boxed.
Figure S9. SNAP1 binds SARS-CoV-2 S trimer variants. A) Schematic that highlights important differences among S (left), S-2P (middle), and S-B.1.1.7 (right), including predicted N-terminal residue identities and mutations. B) Corresponding BLI measurements of binding kinetics. Biotinylated SNAP1 was loaded on streptavidin biosensors and associated with variants of SARS-CoV-2 S trimer. Each graph contains replicate experiments with different concentrations of proteins as denoted in the legend (bottom). Dotted lines indicate switch from analyte association to dissociation. $K_{on}$ values (mean±s.d., n=2-6) were determined from a global fit (dark gray line) of the kinetic data at various concentration of proteins for a 1:1 binding model.
Figure S10. Truncations of SNAP1 have similar binding affinities as whole SNAP1. A) Secondary structure was predicted using NUPACK (http://www.nupack.org/) for SNAP1 (left), SNAP1.66 (center), and SNAP1.50 (right) with the following conditions: 4°C, 0.137 M Na\(^+\), 0.0055 M Mg\(^{++}\). Arrows represent the 3’ end of the aptamer. Nucleotides are color-coded by their equilibrium probability (see legend on right). B) Kinetic and binding equilibrium constants were measured by BLI. Biotinylated SNAP1 (left), SNAP1.66 (center) or SNAP1.50 (right) was loaded on streptavidin biosensors and associated with SARS-CoV-2 S1 domain (upper row) or NTD (lower row). Each graph contains different concentrations of proteins as denoted in the legend (right). Dotted lines indicate switch from analyte association to dissociation. K_D values (mean±s.d., n=6) were determined from a global fit (dark gray line) of the kinetic data at various concentration of proteins for a 1:1 binding model.
Figure S11. Aptamer-based detection of SARS-CoV-2 S protein. A) HybriDetect LFA strips were dipped in solutions of S protein incubated with SNAP1.50. Left: image of developed HybriDetect LFA test strips. Right: quantification of band intensity. B) ELISA assay using NS-biotin or SNAP1-biotin as capture agents to detect various concentrations of SARS-CoV-2 using anti-S HRP antibody for detection. Bar graph shows mean (bar) of three replicates. Statistical comparison of NS to SNAP1 was determined by two-way ANOVA, * denotes p < 0.05 and n.s. denotes no significance.
