Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
A high-affinity aptamer with base-appended base-modified DNA bound to isolated authentic SARS-CoV-2 strains wild-type and B.1.617.2 (delta variant)

Hirotaka Minagawa a, Hirofumi Sawa b, c, d, *, Tomoko Fujita a, Shintaro Kato a, Asumi Inaguma a, Miwako Hirose a, Yasuko Orba a, c, Michihito Sasaki b, Koshiro Tabata b, Naoki Nomura e, Masashi Shingai c, e, Yasuhiro Suzuki f, Katsunori Horiia, **

A high-affinity aptamer with base-appended base-modified DNA bound to isolated authentic SARS-CoV-2 strains wild-type and B.1.617.2 (delta variant).

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the novel coronavirus disease-2019 (COVID-19) pandemic, triggers acute respiratory diseases, and the COVID-19 pandemic is a global threat to public health [1]. Recently, one of the variants of concern (VoCs), B.1.617.2 (delta variant) has spread rapidly all over the world. SARS-CoV-2 mainly infects human alveolar epithelial cells by binding to angiotensin-converting enzyme 2 (ACE2) that is expressed on the surface of the host cell via the receptor-binding domain (RBD) of the spike (S) protein, a surface glycoprotein on the viral particle [2,3]. Therefore, the S protein is an important target for detecting SARS-CoV-2 as well as developing antiviral antibodies and compounds [4].

Antibodies, which bind specifically to target proteins, are widely used in research and therapeutics. For example, a lateral flow assay has been developed using antibodies against SARS-CoV-2 [5,6]. Antibodies are the gold standard for recognizing molecules; however, they have drawbacks, including instability and high costs of development and production [7].

Aptamers are single-stranded DNA (ssDNA) or RNA oligonucleotides that bind to specific molecules or cells [8,9]. Therefore, aptamers are able to regulate the functions of the targets similarly as antibodies. Additionally, aptamers have some advantages, such as easiness of both efficient production and chemical modification,
Abbreviations

Severe acute respiratory syndrome coronavirus 2 SARS-CoV-2  
systematic evolution of ligands by exponential enrichment SELEX  
base-appended base BAB  
receptor-binding domain RBD  
selection buffer SB  
forward Fw  
reverse Rv  
double-stranded DNA dsDNA  
single-stranded DNA ssDNA  
surface plasmon resonance SPR  
enzyme-linked aptamer assay ELAA  
fast string-based clustering FSBC  

and reversible folding without aggregation [10–12]. Apatamers are usually isolated from combinatorial nucleic acid libraries using an iterative selection process called systematic evolution of ligands by exponential enrichment (SELEX) [13]. The SELEX method uses a library comprising primer regions and random regions to select the sequences binding to the target [14,15]. We have developed analogs with modified bases containing other bases, i.e., base-appended bases (BABs). Using these modified bases, we have generated aptamers with extremely high binding affinities for various targets [22–25].

Aptamers for SARS-CoV-2 S protein or RBD have been selected from a DNA library using natural bases [16,17]. Also, an aptamer inhibiting SARS-CoV-2 infection has been identified [18–21]. In this study, a high-affinity artificial nucleic acid aptamer for the SARS-CoV-2 S RBD was obtained using a modified DNA library containing the following base-appended base modifications; analog guanine derivative at the fifth position of uracil (Ugu) [23]. This aptamer was shown to detect authentic SARS-CoV-2 strains belonging to lineages A and B.1.617.2.

2. Materials and methods

2.1. Target protein and virus

The recombinant RBD of the SARS-CoV-2 (2019-nCoV) S protein (YP_009724390.1, Arg319-Phe541) and the His-tagged S1+S2 ECD protein (YP_009724390.1, Val16-Pro1213) (Sino Biological, Beijing, China) were used as the selection targets.

All the experiments using SARS-CoV-2 were performed under the guidelines of the Biosafety Management Committee on Pathogens and Other Hazardous Agents of Hokkaido University and the International Institute for Zoonosis Control. SARS-CoV-2 strains belonging to lineages A [26], B.1.1.7 (alpha variant), B.1.351 (beta variant), P1 (gamma variant), B.1.617.2 (delta variant), and human coronavirus OC43 (HCoV-OC43) were prepared separately. Those SARS-CoV-2 strains were isolated and provided by Drs. Saijyo and Shimojima at the National Institute of Infectious Diseases, Tokyo, Japan. First, each strains of SARS-CoV-2 was inoculated into Vero-TMPRSS2 cells [27] cultured in Dulbecco’s modified Eagle’s medium containing 2% fetal bovine serum, and the supernatants were collected upon observation of cytopathic effects. Meanwhile, HCoV-OC43 was inoculated into MRC-5 cells, and the supernatants were collected in the same manner. Then, the viruses were pelleted by ultracentrifugation at 110,000 × g for 4 h with a 20% sucrose cushion. Afterward, the pellets were resuspended in phosphate-buffered saline (PBS) and stored at −80 °C. Viral titers were measured by plaque assays [27].

2.2. SELEX

SELEX was conducted as previously reported [23]. Dynabeads MyOne Carboxylic Acid and Dynabeads MyOne SA C1 magnetic beads (Invitrogen, Waltham, MA) were used for target solidification and biotinylated DNA retrieval, respectively. The target beads were prepared by binding MyOne Carboxylic Acid to recombinant RBD according to the manufacturer’s instructions and washed with the selection buffer [SB; 40 mM HEPES (pH 7.5), 125 mM NaCl, 5 mM KCl, 1 mM MgCl2, and 0.01% Tween 20]. A double-stranded DNA (dsDNA) with inserted Ugu was prepared using a 5’-biotin-modified complementary strand (5’-GAATACAGACACCTCCTTGGTTCN30-GATTTCAGTGGCGGAGACATACC-3’), a forward (Fw) primer (5’-GTATGTCCTCCGACCTGAAAATC-3’), and KOD Dash (Toyobo, Osaka, Japan). After the dsDNA was bound to MyOne SA C1 magnetic beads, ssDNA was eluted with 0.2 M NaOH and neutralized with 0.08 M HCl to prepare the Ugu ssDNA library. The primers, random pool, and aptamer clone templates were purchased from Integrated DNA Technologies (Tokyo, Japan).

The 130 pmol library was mixed for 15 min with 250 μg of target beads at 25 °C. Then, the beads were washed with SB, and the bound ssDNA was eluted with 7 M urea and amplified by polymerase chain reaction (PCR) using the Fw primer and a reverse (Rv) primer (5’-GAATACAGACACCTCCTTGGTTCN30-GATTTCAGTGGCGGAGACATACC-3’) with modification by the 5’-biotin. Next, the amplified dsDNA was bound to MyOne SA C1 magnetic beads, and the Fw chain was eluted with 0.02 M NaOH. The ssDNA produced by this method, using the Rv chain, Fw primer, and Ugu-immobilized in magnetic beads, were used in the next round. After eight rounds of selection, PCR with the Fw and Rv primers and subsequent sequencing were conducted using a MiniSeq System (Illumina, San Diego, CA). The sequence data obtained were clustered by fast string-based clustering (FSBC), and 1 sequence each was selected from the top and another higher-ranked clusters [28].

2.3. Surface plasmon resonance analysis

All the surface plasmon resonance (SPR) measurements were performed at 25 °C using the ProteOn XPR360 (Bio-Rad, Hercules, CA) [22]. For the aptamer clones, the ligand was set by appending poly-A20 to the 3’-end and hybridizing the 5’-end to an NLC sensor chip (Bio-Rad) with biotin-modified oligo (dT20) [23]. The analytes included the RBD, S1+S2, recombinant 2019-nCoV S1 (Elabscience, Houston, TX), and recombinant 2019-nCoV S trimers (Elabscience). Bovine serum albumin (Sigma-Aldrich, St. Louis, MO) with SB was used as the running buffer. The dissociation constants between the aptamer and recombinant RBD or S protein were calculated using a simple 1:1 biomolecular interaction model, the most common kinetic fit model for SPR data analysis, according to the manufacturer’s instructions. The dissociation constant of S protein was estimated as the apparent Kd value due to its trimeric formation.

2.4. Enzyme-linked aptamer assays

Enzyme-linked aptamer assays (ELAAs) were conducted as previously described [29]. Briefly, recombinant RBD protein and purified SARS-CoV-2 were diluted with 50 mM carbonate buffer (pH 9.6), added to MaxiSorp plates (Thermo Scientific, Waltham, MA), at 1 μg/well and 1.0 × 108 PFU/well, respectively, and solidified at 4 °C overnight. Recombinant His-tagged hemagglutinin (HA) protein of influenza A/California/04/2009H1N1 (Sino Biological, Beijing, China) was used as a negative control. Then, the wells were washed once with SB and blocked with Tris-buffered saline.
Blocking Buffer (Pierce Biotechnology, Rockford, IN) at 25 °C for 1 h. Then, a 5′ biotinylated aptamer at 1 μM was prepared in SB, de-natured by heating at 95 °C for 5 min, and cooled. Next, the aptamer was added and incubated at room temperature for 1 h. After three washes with SB, streptavidin–horseradish peroxidase (HRP) (1:1,000 diluted; Citiva, New York, NY) was added, and the plates were incubated for 30 min at room temperature to detect the bound biotinylated aptamers. After three washes with SB, a 3,3′,5,5′-Tetramethylbenzidine (TMB) solution (Thermo Fisher Scientific) for RBD detection and 1-step™ Ultra TMB-ELISA substrate solution (Thermo Fisher Scientific) for SARS-CoV-2 detection was added, and the plates were incubated at room temperature for 10 min. The reaction was stopped by adding 0.5 N sulfuric acid, and the absorbance was measured at 450, 490 and 620 nm.

2.5. Statistical analysis

All statistical analyses were performed using R version 4.0.4. For analyses between two groups, a one-tailed Student’s unpaired T-test was applied. For comparisons among more than two groups, one-way ANOVA with Tukey’s multiple comparisons was used. The methods of statistical analysis are described in the figure legends for each experiment.

3. Results and discussion

For the detection of SARS-CoV-2, the S protein-binding aptamers were screened using a Ugu-modified nucleic acid library. Four S protein-binding aptamer candidates [28], two for the RBD and two for the S1+S2 protein (Table S1), were selected from sequence analysis. Four analytes, S1 and S trimers in addition to RBD and S1+S2, were used for SPR analysis. The two RBD-binding aptamers, RBD-Ugu1 and RBD-Ugu2, bound all four analytes. However, the two S1+S2-binding aptamers, S1S2-Ugu1 and S1S2-Ugu2, failed to bind to the RBD (Fig. S1).

The RBD-binding ability of a previously reported aptamer, CoV2-RBD-1 [16], was compared with that of RBD-Ugu1 and RBD-Ugu2 using SPR analysis. The dissociation constants of RBD-Ugu1 and RBD-Ugu2 were 1.2 and 1.7 nM, respectively, demonstrating more strong binding affinities than CoV2-RBD-1 (Fig. 1a). In a solution containing dextran sulfate, a polyanion suppressing nonspecific binding to nucleic acids [30,31], CoV2-RBD-1 significantly lost its binding activity. Conversely, the dissociation constants for RBD-Ugu1 and RBD-Ugu2 were 3.2 and 3.5 nM, respectively, indicating that their binding activities remained largely unaffected (Fig. 1b).

This observation suggests that charge has little influence on the ability of RBD-Ugu1 and RBD-Ugu2 to bind RBD, even though the binding of aptamers to the target is usually influencing by charge. These data indicate that RBD-Ugu1 and RBD-Ugu2 exhibit little nonspecific binding. Additionally, the evaluation of the binding of RBD-Ugu1 and RBD-Ugu2 to the S trimer using SPR revealed a dissociation constant of less than 1 nM for both aptamers, indicating exceptionally strong binding (Fig. S2). The binding sites on RBD for RBD-Ugu1 and RBD-Ugu2 are unclear. Since BAB clones have different structures from natural-base clones with corresponding sequences (in which dUx is dT) [22], the more complex structures of RBD-Ugu1 and RBD-Ugu2 may result in stronger RBD binding.

Generally, a longer sequence results in the instability of an aptamer’s conformation at the interface of the target [32]. Hence, the length of aptamers should be minimized to enhance the

**Fig. 1.** SPR response curves of the interaction between the SARS-CoV-2 S RBD and the aptamer candidates, RBD-Ugu1, RBD-Ugu2, and CoV2-RBD-1 (a–b) Different concentrations of RBD (25–400 nM) were injected over the respective aptamer-immobilized sensor chips for 120 s at a flow rate of 50 μL/min, and the measurements were performed using multicycle kinetics. Black dot line and red line represent the measured and fitting curves, respectively. The average of the squared differences between the data points and the corresponding fitted values is represented as $\chi^2$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
aptamers’ binding ability and reduce their cost of production [33]. We designed a truncated aptamer sequence (RBD-Ugu1-1) with the region in RBD-Ugu1 most strongly related to binding (5’-GGAATT-CATG-3’), which was estimated with FSBC, considering the maintenance of the secondary structure of the binding region. RBD-Ugu1-1 was a 42-mer with the sequence of 5’-CCACTGAAATCCGtGCC-tAAtCtCACCCCACGGAAttCAtGG-3’, with t indicating Ugu. The secondary structure of RBD-Ugu1-1 was predicted with RNAfold of ViennaRNA package [34] (Fig. 2a). The SPR response curves of the interaction between SARS-CoV-2 S RBD protein and RBD-Ugu1-1. Black dot line and red line represent the measured and fitting curves, respectively. The average of the squared differences between the measured data points and the corresponding fitted values is represented as $\chi^2$. In (b), SB was used as a running buffer; in (c), SB with 0.1% dextran sulfate was used. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 2. Properties of the truncated RBD-Ugu1-1 aptamer
(a) The predicted secondary structure of RBD-Ugu1-1 which was estimated with RNAfold of ViennaRNA package [34] was displayed using Forna [37].
(b–c) The SPR response curves of the interaction between SARS-CoV-2 S RBD protein and RBD-Ugu1-1. Black dot line and red line represent the measured and fitting curves, respectively. The average of the squared differences between the measured data points and the corresponding fitted values is represented as $\chi^2$. In (b), SB was used as a running buffer; in (c), SB with 0.1% dextran sulfate was used. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Binding capacities of RBD-Ugu1, RBD-Ugu1-1, and S1S2-Ugu3 to the RBD of the spike protein and HA protein derived from influenza H1N1 virus as measured by direct ELAA
The amount of the 5’ biotinylated aptamer captured by immobilized RBD and HA was determined the absorbance at 490 nm. The experiment was performed in triplicates. Error bars represent standard deviation for 6 data points. Buffer was used Selection Buffer [40 mM HEPES (pH 7.5), 125 mM NaCl, 5 mM KCl, 1 mM MgCl2, and 0.01% Tween 20]. One-tailed unpaired T-test was applied to compare the mean of ELAA signals of the RBD with that of HA protein or buffer for each aptamer. The symbol of two asterisks stands for $P$ value less than 0.01.

aptamers’ binding ability and reduce their cost of production [33]. We designed a truncated aptamer sequence (RBD-Ugu1-1) with the region in RBD-Ugu1 most strongly related to binding (5’-GGAATT-CATG-3’), which was estimated with FSBC, considering the maintenance of the secondary structure of the binding region. RBD-Ugu1-1 was a 42-mer with the sequence of 5’-CCACTGAAATCCGtGCC-tAAtCtCACCCCACGGAAttCAtGG-3’, with t indicating Ugu. The secondary structure of RBD-Ugu1-1 was predicted with RNAfold of ViennaRNA package [34] (Fig. 2a). The dissociation constant of RBD-Ugu1-1 for RBD was 2 nM in SB (Fig. 2b) and 5.3 nM in dextran sulfate (Fig. 2c), exhibiting a binding ability comparable with that of the original sequence. Next, a direct ELAA was conducted using the 5’-terminal biotinylated aptamers to the recombinant RBD proteins and various SARS-CoV-2 strains immobilized on 96-well plates. RBD-Ugu1 and RBD-Ugu1-1 bound equally well to the RBD. However, S1S2-Ugu3 did not bind to the RBD (Fig. 3). These results were consistent with those of the SPR binding assays. No binding signal of aptamers to influenza virus HA protein was detected, suggesting that aptamers specifically recognize spike proteins of SARS-CoV-2. Then, the ability of RBD-Ugu1-1 to detect authentic SARS-CoV-2 was evaluated with SARS-CoV-2 lineages A, B.1.617.2, B.1.1.7, B.1.351 and P.1 using direct ELAA (Fig. 4). As a control, a commercially available anti-SARS-CoV-2-RBD antibody conjugated with HRP (Abcam, Cambridge, UK) was used with 1:1,000 dilution. The aptamer was bound to authentic SARS-CoV-2 wild-type and B.1.617.2 immobilized on a plate (Fig. 4a). The binding signals of the
Tukey’s multiple comparisons was used. H. Minagawa, H. Sawa, T. Fujita et al. Biochemical and Biophysical Research Communications 614 (2022) 207-212

(a) The amount of the 5′-biotinylated aptamer and an anti-SARS-CoV-2 RBD antibody captured by immobilized SARS-CoV-2 strains lineage A (wild-type) and B.1.617.2 (delta variant) was determined using SA-HRP and TMB substrate. The absorbance was determined by subtracting the absorbance at 620 nm from that at 450 nm. The experiment was repeated twice with each data point measured in triplicates. Error bars represent standard error. Selection Buffer ([40 mM HEPES (pH 7.5), 125 mM NaCl, 5 mM KCl, 1 mM MgCl2, and 0.01% Tween 20] was used as a negative control.

(b) The amount of the 5′-biotinylated aptamer captured by immobilized SARS-CoV-2 strains (10^3-10^7 PFU), including lineage A (wild-type), B.1.1.7 (alpha variant), P1 (gamma variant), B.1.617.2 (delta variant) of SARS-CoV2 and HCoV-OC43 was determined in the SB containing 0.1% dextran sulfate using SA-HRP and TMB substrate. The absorbance was measured at 450-620 nm. The experiment was repeated twice with each data point measured in triplicates. Error bars represent standard error. One-tailed unpaired t-test was applied to compare the mean of ELAA signals between each strain and buffer [SB; 40 mM HEPES (pH 7.5), 125 mM NaCl, 5 mM KCl, 1 mM MgCl2, and 0.01% Tween 20]. Double asterisks (**) indicate p < 0.01; single asterisk (*) indicates p < 0.05. For multiple comparisons between SARS-CoV-2 strains, one-way ANOVA with Tukey’s multiple comparisons was used.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbr.2022.04.071.

References
[1] Coronavirus Study Group of the International Committee on Taxonomy of Viruses, The species severe acute respiratory syndrome-related coronavirus: classifying, nCoV and naming it SARS-CoV-2, Nat. Microbiol. 4 (2020) 536–544, 2019.
[2] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, Cell 181 (2020) 281–292, https://doi.org/10.1016/j.cell.2020.02.058, e6.
[3] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, Q. Zhou, Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science 367 (2020) 1444–1448, https://doi.org/10.1126/science.abb2762.
[4] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.L. Hsieh, O. Abiona, B.S. Graham, J.S. Mcelhan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (2020) 1260–1263, https://doi.org/10.1126/science.abb2507.
[5] B.D. Grant, C.E. Anderson, J.R. Wilflord, F.L. Alonzo, V.A. Gluhkova, D.S. Boyle, B.H. Weidj, K.P. Nichols, SARS-CoV-2 coronavirus nucleocapsid antigen-detecting half-strip lateral flow assay toward the development of point of care tests using commercially available reagents, Anal. Chem. 92 (2020) 11305–11309, https://doi.org/10.1021/acs.analchem.0c01075.
[6] L. Azzi, A. Baj, T. Alberno, M. Lualdi, G. Veronesi, G. Carcano, W. Ageno, C. Gambarrini, L. Maffioli, S.D. Saverio, D.D. Gasperina, A.P. Genoni, E. Premi, S. Donati, C. Azzolini, A.M. Grandi, F. Dentali, F. Tettamanzi, L. Tettamanzi, C. Iacovelli, L. Vigezzi, E. Monti, V. Iori, D. Iovino, G. Letto., ASST dei Sette Laghi Rapid Salivary Test Nurse staff Research Group, P.A. Grossi, A. Tagliaclue, M. Fasano, Rapid Salivary Test suitable for a mass screening program to detect SARS-CoV-2: a diagnostic accuracy study, J. Infect. 81 (2020) e78, https://doi.org/10.1016/j.jinf.2020.05.042.
[7] C. Ellis, J. N Blake, Electrochemical biosensors for pathogen detection, Biosens. Bioelectron. 159 (2020) 112234.
[8] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822, https://doi.org/10.1038/346818a0.
[9] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505–510, https://doi.org/10.1126/science.2206121.
[10] F. Odeh, H. Nsairat, W. Alshaer, M.A. Ismail, E. Esawi, B. Qaqish, A.A. Bawab, S.J. Ismail, Aptamers chemistry: chemical modifications and conjugation strategies, Molecules 25 (2019) 3, https://doi.org/10.3390/molecules25010003.
A highlight of recent advances in aptamer technology and its application, Molecules 20 (2015) 11959–11980, https://doi.org/10.3390/molecules200711959.

Y. Song, J. Song, X. Wei, M. Huang, M. Sun, L. Zhu, B. Lin, H. Shen, Z. Zhu, C. Yang, Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein, Anal. Chem. 92 (2020) 9895–9900, https://doi.org/10.1021/acs.analchem.0c01354.

L. Jiaxing, Z. Zijie, G. Jimmy, S. Hannah D. A. Jani C. C. Alfredo, D.M.F. Carlos, M. Karen, L. B. Cynthia, S. Bruno J. Y. Deborah, S. Leyla, M. Matthew S. B. John D, L. Yingfu, Diverse high-affinity DNA aptamers for wild-type and B.1.1.7 SARS-CoV-2 spike proteins from a pre-structured DNA library, Nucleic Acids Res. 49 (2021) 7287–7279, https://doi.org/10.1093/nar/gkbz574.

M. Sun, S. Liu, X. Wei, S. Wan, M. Huang, T. Song, Y. Lu, X. Weng, Z. Lin, H. Chen, Y. Song, C. Yang, Aptamer blocking strategy inhibits SARS-CoV-2 virus infection, Angew. Chem., Int. Ed. Engl. 60 (2021) 10266–10272, https://doi.org/10.1002/anie.202100225.

X. Liu, Y. Wang, J. Wu, J. Qi, Z. Zeng, Q. Wan, Z. Chen, P. Manandhar, V. Cavener, N. Boyle, X. Fu, E. Salazar, S. Kuchipudi, V. Kapur, X. Zhang, M. Umetani, M. Sen, R. Willson, S. Chen, Y. Zu, Neutralizing aptamers block S/RBD-ACE2 interactions and prevent host cell infection, Angew. Chem., Int. Ed. Engl. 60 (2021) 10273–10278, https://doi.org/10.1002/anie.202100345.

A. Schmitz, A. Weber, M. Bayin, S. Breuers, V. Fieberg, M. Famulok, G. Mayer, M. Sun, S. Liu, X. Wei, S. Wan, M. Huang, T. Song, Y. Lu, X. Weng, Z. Lin, H. Chen, Y. Song, C. Yang, Aptamer blocking strategy inhibits SARS-CoV-2 virus infection, Angew. Chem., Int. Ed. Engl. 60 (2021) 10266–10272, https://doi.org/10.1002/anie.202100225.

I. Shiratori, J. Akitomi, D.A. Boltz, K. Horii, M. Furuichi, I. Waga, Selection of aptamer pairs for diagnostic sandwich assays, Biotechniques 56 (2014) 133–139, https://doi.org/10.2144/000114134, eCollection 2014.

S. Strauss, P.C. Nickels, M.T. Strauss, V.J. Sabinina, J. Ellenberg, J.D. Carter, S. Gupta, N. Janjic, R. Jungmann, Modified aptamers enable quantitative sub-10-nm cellular DNA-PAINT imaging, Nat. Methods 15 (2018) 685–688, https://doi.org/10.1038/s41592-018-0120-2.

D. Shangguan, Z. Tang, P. Mallikaratchy, Z. Xiao, W. Tan, Optimization and modifications of aptamers selected from live cancer cell lines, ChemBioChem 8 (2007) 603–606, https://doi.org/10.1002/cbic.200600532.

X. He, L. Guo, J. He, X. Hu, J. Xie, Stepping library-based post-SELEX strategy approaching to the minimized aptamer in SPR. Anal. Chem. 89 (2017) 6559–6566, https://doi.org/10.1021/acs.analchem.7b00700.

R. Lorenz, S.H. Bernhart, C.H.Z. Siederdissen, H. Tafer, C. Flamm, P.F. Stadler, L.L. Hofacker, ViennaRNA package 2.0, Algorithm Mol. Biol. 6 (2011), https://doi.org/10.1186/1748-7188-6-26.

J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature 581 (2020) 215–220, https://doi.org/10.1038/s41586-020-2180-5.

V. Tragni, F. Preziusi, L. Laera, A. Onofrio, S. Todisco, M. Volpicella, A.D. Grassi, C.L. Pierri, A modular molecular framework for quickly estimating the binding activity of mycophenolic acid and IMD-0354 against SARS-CoV-2, Microbiol. Immunol. 64 (9) (2020) 635–639, https://doi.org/10.1111/1348-0421.12828. Epub 2020 July 25.

M. Sasaki, K. Uemura, A. Sato, T. Toba, T. Sanaki, K. Kaenaka et al., SARS-CoV-2 variants with mutations at the S1/S2 cleavage site are generated in vitro during propagation in TMPRSS2-deficient cells, PLoS Pathog. 17 (1) (2021), e1009233, https://doi.org/10.1371/journal.ppat.1009233. Epub 2021/01/21.

S. Kato, T. Ono, H. Minagawa, K. Horii, I. Shiratori, I. Waga, K. Ito, T. Aoki, T. Aoki, FBSC: fast string-based clustering for HT-SELEX data, BMC Bioinf. 21 (2020) 263, https://doi.org/10.1186/s12859-020-03507-1.

I. Shiratori, J. Akitomi, D.A. Boltz, K. Horii, M. Furuichi, I. Waga, Selection of DNA aptamers that bind to influenza A viruses with high affinity and broad subtype specificity, Biochem. Biophys. Res. Commun. 443 (2014) 37–41, https://doi.org/10.1016/j.bbrc.2013.11.041.

O. Urs A. G. Louis, S. G. Larry, J. Nbeojis, Systematic selection of modified aptamer pairs for diagnostic sandwich assays, Biotechniques 56 (2014) 125–133, https://doi.org/10.2144/000114134, eCollection 2014.

P. Kerpedjiev, S. Hammer, I.L. Hofacker, ViennaRNA package 2.0, Algorithm Mol. Biol. 6 (2011), https://doi.org/10.1101/2021.05.26.445422. May 26.