APPLICATION NOTE

Empowering Rapid and Robust Antibody Response: The Role of Next-Generation Antibody Discovery in Preparedness for Emerging Diseases and Endemic Threats

How leading researchers are revolutionizing Antibody Research during COVID response by harnessing cutting-edge technologies to eliminate epidemic threats.

In this Application Note we outline:

- How Rapid Antibody Screening with the Beacon® System Optofluidic is Revolutionizing Functional Analysis and Early Antibody Identification
- Insights from the Beacon System on Antibody Expression and Functional Analysis
- Comparative Screening Efficiency and Rapid Lead Identification
- Identifying Broadly Neutralizing Antibodies for Next Generation Therapeutic mAbs and Vaccines
- Early Identification of Potent Omicron-Neutralizing Antibodies

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Introduction

Emerging diseases and continuing mutations of endemic diseases continue to pose serious challenges. SARS-CoV-2, which has undergone multiple mutations, spreads rapidly, and has a tendency towards immune escape, presents complex challenges in the timely development of effective prevention and treatment strategies.

Conventional methods for antibody discovery can be inefficient and time-consuming, even resulting in the inadvertent discarding of promising molecules. In order to stay a step ahead of the virus, which has already claimed at least 6.9 million lives globally (World Health Organization, 2023), researchers require swift single-cell screening methods that prioritize functionality and empower them to utilize the most effective cells.

The Bruker Beacon® system, which has already helped to uncover critical insights in the global response to COVID-19, represents an evolution in antibody discovery. It was used to identify the neutralizing antibodies that went on to form AstraZeneca's Evusheld antibody cocktail, and has enabled scientists to identify candidate epitopes for use in next-generation therapeutic antibody and vaccine development programs.[1]

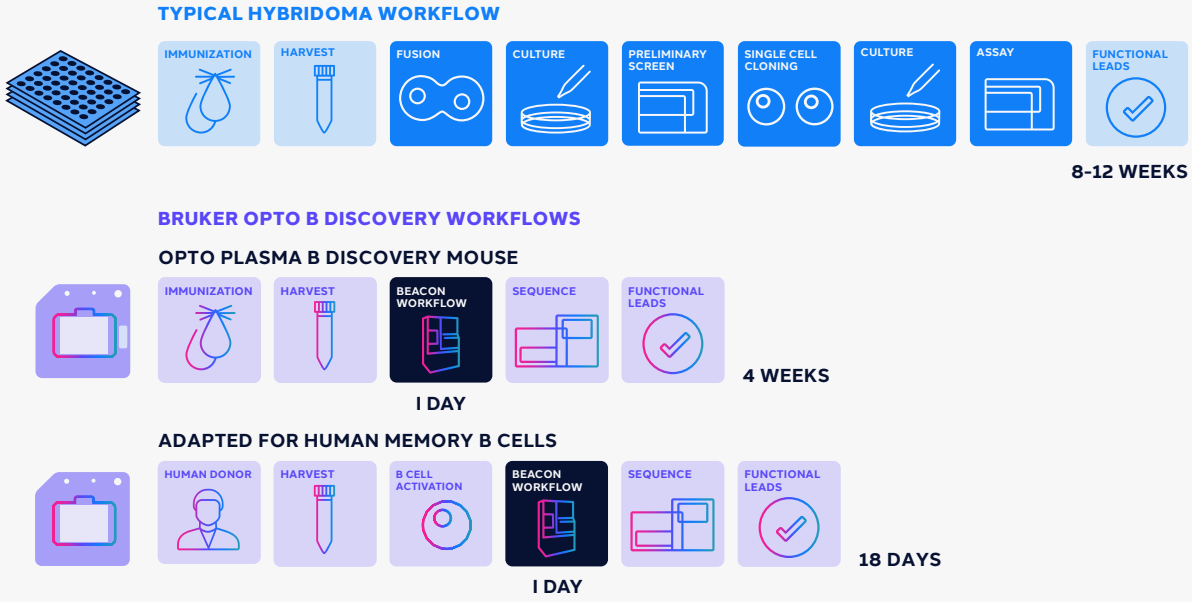
Rapid Antibody Screening with the Beacon System: Revolutionizing Functional Analysis and Early Antibody Identification

The Beacon system can screen tens of thousands of single mouse or human B cells in just one day. Upfront functional analysis of characteristics including cross-reactivity and epitope investigation allows for the early identification of high-quality antibodies, significantly slashing time from harvest to lead molecule selection when compared to traditional methods such as hybridoma.

Compatible with multiple species including mice, humans, rabbits, and alpacas, and multiple immune organs, including the spleen, lymph nodes, and bone marrow, the technology greatly increases the diversity of the resulting antibodies and antigen-antibody specificity.

In this application note, we review three examples of how researchers have used the Beacon system in the ongoing global effort against the constantly mutating SARS-CoV-2 virus.

The Opto® Plasma B Workflow Decreases Screening and Lead Candidate Selection Time from 8–12 Weeks to Less than 4 Weeks



Antibodies can be functionally screened in a single day using both bead- and cell-based assays, enabling functional characterization during primary screening.

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Rapid Response: The First Steps to Antibody Therapeutic Development

Researchers at Vanderbilt University Medical Center (VUMC) discovered Tixagevimab and Cilgavimab, the two antibodies that went on to form AstraZeneca's Evusheld COVID-19 antibody therapy, using the Beacon system.[2-4]

The team, led by Dr. James Crowe Jr., screened thousands of neutralizing antibodies derived from SARS-CoV-2 infected patients and obtained the lead molecules in just 18 days. The single-cell sequencing platform used in this study yielded target molecules in 35 days – almost double the time.

Tixagevimab and Cilgavimab were later licensed by AstraZeneca and Evusheld was granted use authorization by the US Food and Drug Administration (FDA) in December 2021[5] and European Medicines Agency (EMA) in March 2022 to address the global COVID-19 pandemic.[6]

Evusheld is a Medicine Used to Prevent COVID-19 in Adults and Adolescents

Evusheld contains Tixagevimab and Cilgavimab, two monoclonal antibodies. A monoclonal antibody is a type of protein that has been designed to recognise and attach to a specific structure. Tixagevimab and cilgavimab have been designed to attach to the spike protein of SARS-CoV-2 (the virus that causes COVID-19) at two different sites. When the antibodies in Evusheld attach to the spike protein, the virus cannot enter the cells to multiply.

Antibody Expression and Functional Analysis: Insights from the Beacon System

The researchers expressed the recombinant spike (S) protein ectodomain prefusion trimer ($S2P_{ecto}$) and the receptor binding domain (S_{RBD}) through the 293-F cell line. Recombinant S-protein n-terminal domain (S_{NTD}) obtained from outside sources were used as antigens in antibody screening assays run on both the Beacon system and the single-cell sequencing platform.

Peripheral blood was collected from SARS-Cov-2 patients with an onset of symptoms in the previous 35 to 50 days. Fluorescence activated cell sorting (FACS) and magnetic beads were used to isolate antigen-specific memory B cells, before the team moved on to sequencing or *in vitro* amplification, differentiation, and activation. Activated plasma B cells were loaded onto the Beacon system for functional screening, or onto the Chromium. Reverse transcription, sequence analysis, cDNA gene synthesis and cloning into expression vectors were carried out in CHO trace IgG expression. Testing of the recombinant IgG constructs consisted of ELISA against the $S2P_{ecto}$ and S_{RBD} antigens, and high-throughput neutralization screening assays.

The research team used the Beacon system to identify antibodies which would bind to the SARS-CoV-2 spike protein as well as those that would specifically block interaction between the human ACE2 (hACE2) receptor and RBD. Screening on the Beacon identified 1218 cells which secreted antibodies that bound to the antigens RBD or $SP2_{ecto}$. Using a novel blocking assay, they identified 27 cells secreting antibodies that blocked the RBD/hACE2 interaction. 288 cells were exported from the Beacon system, and from those, 78 unique antigen-reactive mAbs were succesfully cloned. 10 of these blocked RBD/hACE2 interaction.

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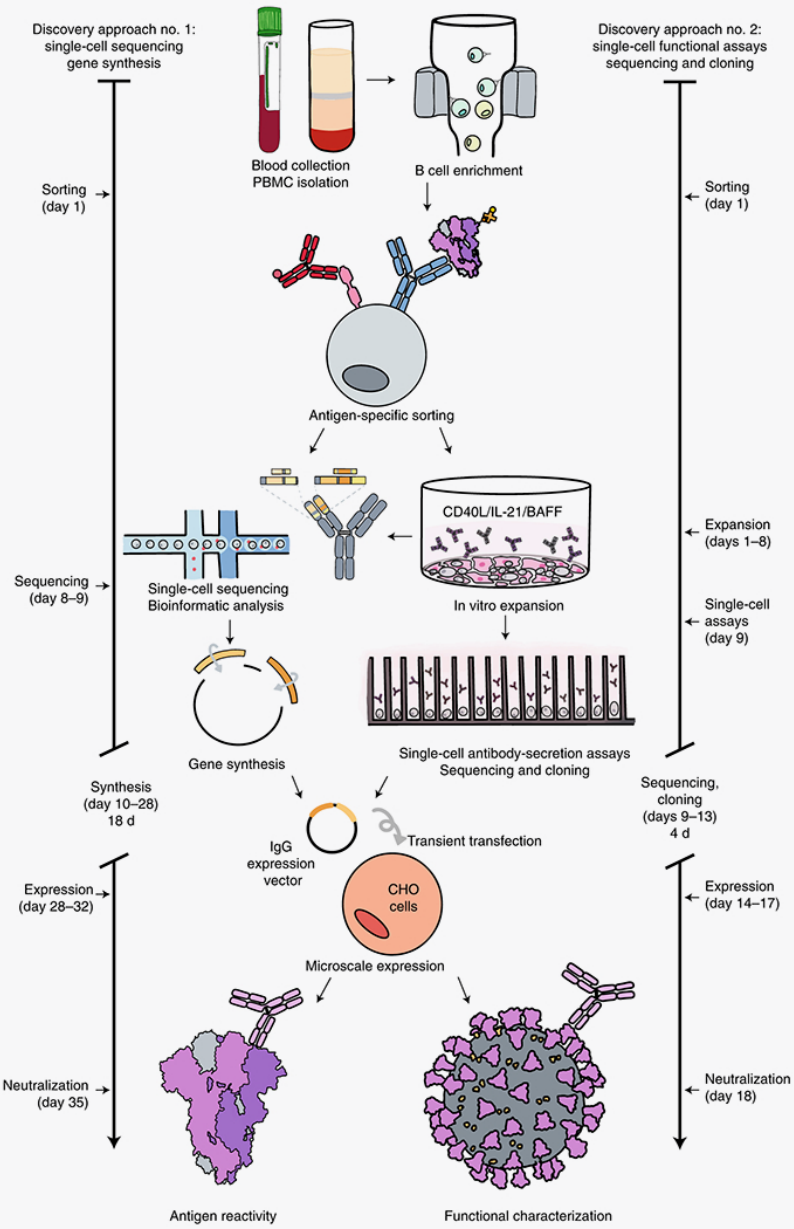
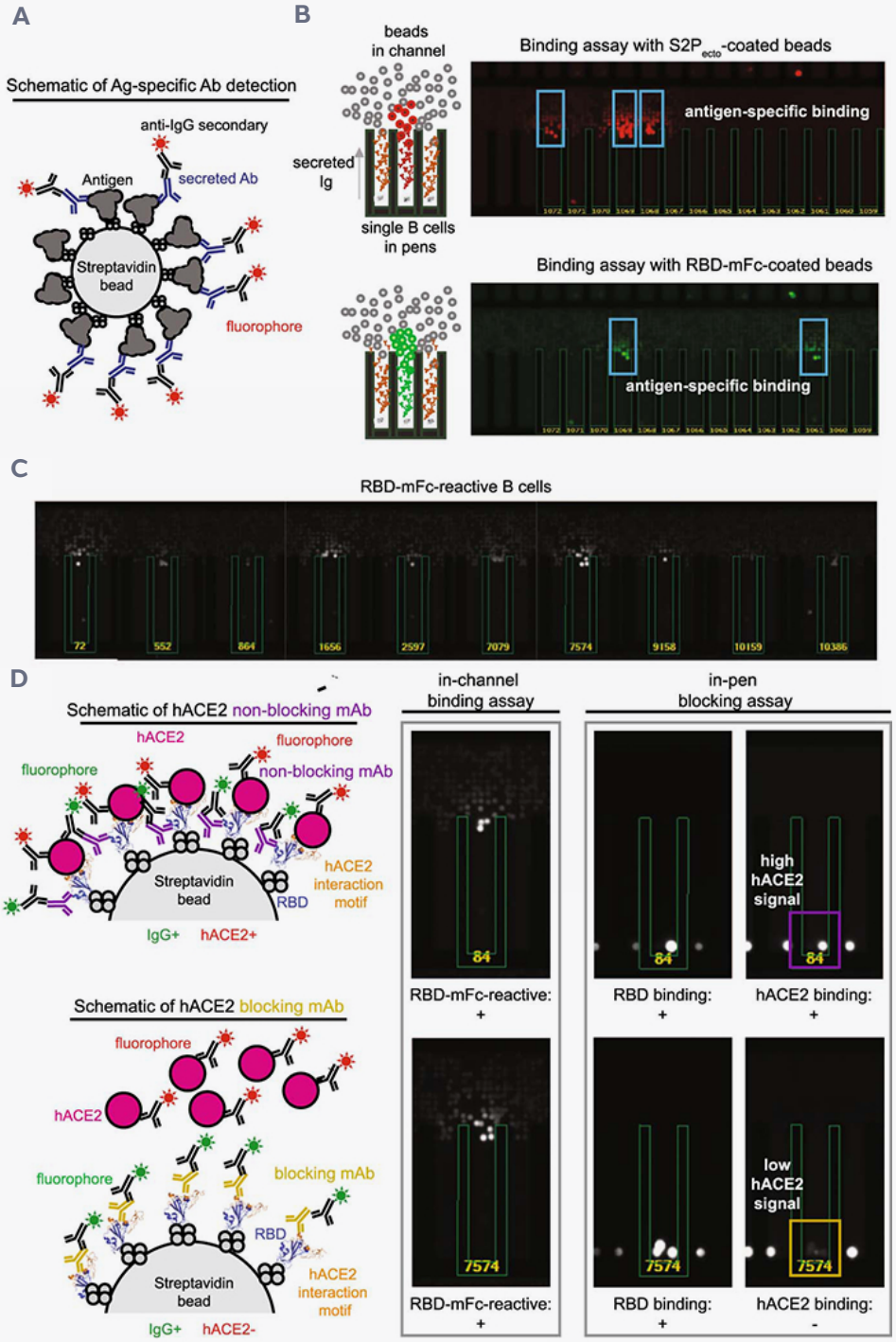
Workflows and Timelines



Overview of rapid mAb discovery workflows. The overall scheme is shown, representing the several specific workflows conducted in parallel. Blood was collected and white blood cells were separated, B cells were enriched from PBMCs by negative selection using magnetic beads and antigen-specific cells were isolated by flow-cytometric sorting and then were processed for direct B cell selection and sequencing or in vitro expansion/activation. Cultured B cells were loaded on a Beacon instrument (Bruker) for functional screening (Extended Data Fig. 1) or in a single-cell sequencing platform followed by reverse transcription with PCR, sequence analysis, cDNA gene synthesis and cloning into an expression vector and microscale IgG expression in Chinese hamster ovary (CHO) cells by transient transfection. Recombinant IgG was tested by ELISA for binding to determine antigen reactivity and by a high-throughput neutralization screening assay with authentic virus in a biosafety-level-3 (BSL-3) laboratory for functional characterization.

Extended Data Fig. 1. Functional Assays from Single Antigen-Reactive B Cells.



Rapid screening of B cell clones secreting antibodies that block the binding of human ACE2 to RBD by Beacon sytem.[1]

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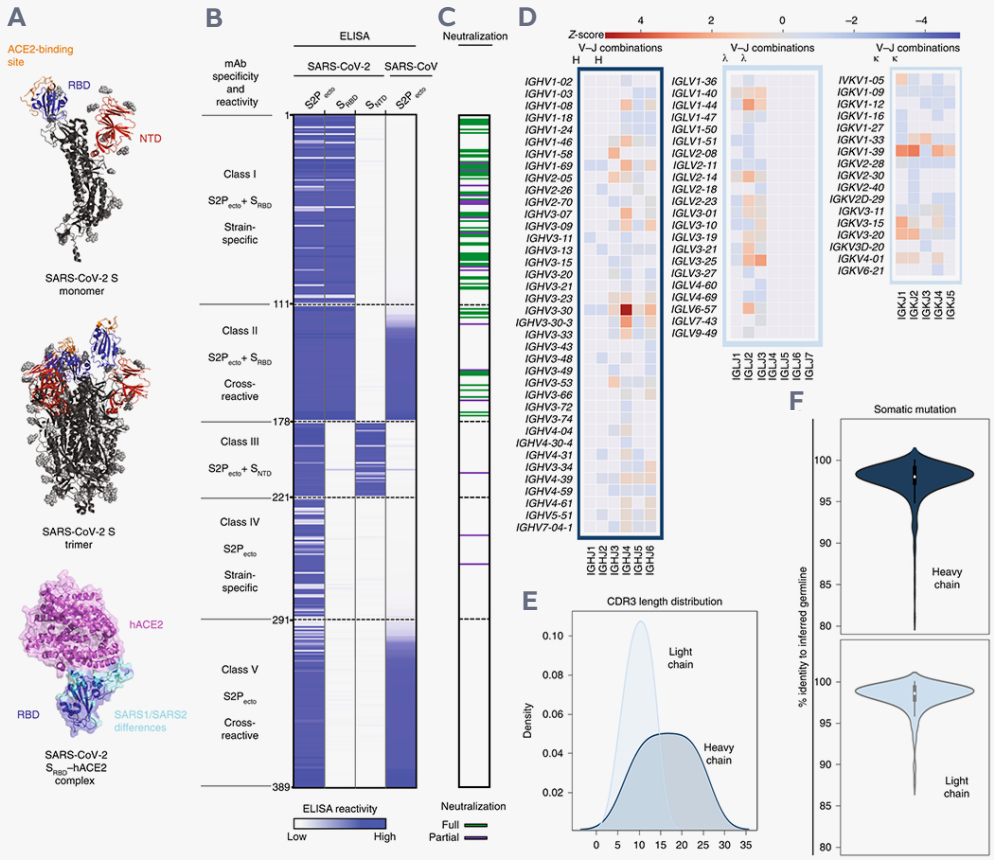
In total, the researchers analyzed the functional characteristics of 389 recombinantly expressed antibodies from both the Beacon and Chromium platforms. They found that 178 of these bound to RBD, and 43 to S_{NTD}, of which 70 were neutralizing antibodies. 67 of these mapped to RBD, suggesting it was a critical site for SARS-CoV-2 neutralization in the selected patients. 49% of the SARS-CoV-2-specfic RBD mAbs and 17% of the SARS-CoV cross-reactive mAbs exhibited neutralizing activity at several half maximal inhibitory concentration (IC50) below 100 ng ml^{-1}. This confirmed that mAbs that recognize multiple epitopes of the S protein can neutralize SARS-CoV-2 and cross-react with SARS-CoV, and that the RBD of SARS-CoV-2 was a potential target.

Reactivity and Functional Activity of 389 Human mAbs.



A) SARS-CoV-2 S Antigen Structures. Top: S protein monomer of SARS-CoV-2 with highlighted RBD (blue) and NTD (red) subdomains expressed as recombinant proteins. ACE2-binding site on RBD shown in orange. Middle: Trimeric SARS-CoV-2 spike with one RBD in 'head up' conformation. Bottom: Structure of SARS-CoV-2 RBD (blue) and hACE2 (pink), highlighting differences from SARS-CoV (cyan). B) mAbs Binding Heatmap. Heatmap displaying binding of 389 recombinant mAbs to four S proteins/subdomains, indicated by OD values (450 nm) – white for no binding, blue for binding, darker blue for higher OD values. C) Neutralizing Activity Screening. Testing mAbs using RTCA neutralization test, indicated by colors: green for full protection, purple for partial protection, white for no neutralizing activity. mAbs grouped into classes based on binding and neutralization properties. D) Antibody Variable-Gene Segment Usage. Heatmap showing frequency of V/J gene usage among 389 unique antibody sequences. Red denotes common usage, blue denotes less common usage. E) CDR3 Amino Acid Length Distribution. Histogram showing distribution of CDR3 amino acid lengths for heavy and light chains using kernel density estimation. F) Divergence from Germline Sequences. Violin plots illustrating mutation percentages of mAbs relative to inferred germline variable gene sequences for heavy and light chains.

Comparative Screening Efficiency and Rapid Lead Identification

While a smaller number of candidate molecules were screened by the Beacon system than by the single-cell sequencing route overall, the hit rate was ten-fold higher. In addition, time from antigen-specific B cell sorting to the transfer of antibody sequences to downstream production was just 18 days – half the 35 days achieved with the parallel single-cell sequencing process.

The Beacon system with the Opto® Plasma B workflow enabled the rapid screening of antigen binding and function at the single B cell level at the high throughput of hundreds of thousands of antibodies. It enabled the rapid identification of two lead molecules, both targeted against the surface spike protein of SARS-CoV-2, that went on to help change the trajectory of the pandemic.

Staying Ahead of Mutations: Identifying Broadly Neutralizing Antibodies for Next Generation Therapeutic mAbs and Vaccines

In the ongoing battle against the SARS-CoV-2 virus, researchers need to stay one step ahead of the next mutation. A US National Institutes of Health (NIH) team, led by Dr. Joshua Tan, has been doing just that, and has used the Beacon system to identify broadly neutralizing antibodies against the coronavirus fusion peptide, and discovering a candidate epitope for next-generation antibody therapeutic and vaccine development.[7]

The researchers used the technology to screen 23000 B cells, identifying ~60 cross-reactive mAbs to 3 coronaviruses from the optofluidic screening, and ultimately 6 of which had broad-spectrum neutralizing activity against 7 coronaviruses, including SARS (SARS-CoV-1), and Middle East respiratory syndrome virus (MERS-CoV). Of these six, two were capable of neutralizing a variety of SARS-CoV-2 mutants, such as Alpha, Beta, BA.2 and BA.4/5. Further research found that all six antibodies targeted the well-conserved fusion peptide regions of the coronaviruses.

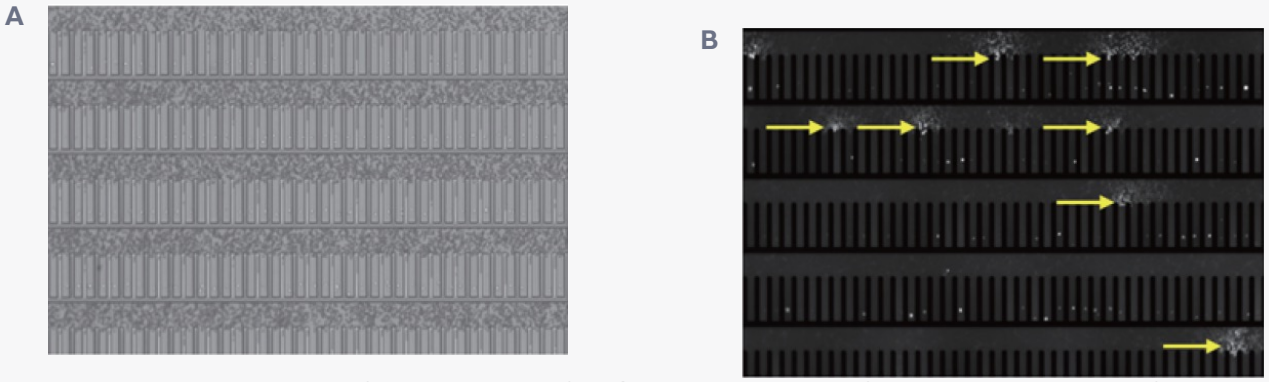
Characterization and Potential of Crossreactive Antibodies: A Study on Multiple Coronaviruses

Dr. Tan's team studied the effect of the plasma immunoglobulin G (IgG) of 142 people who had recovered from COVID-19 on seven human coronaviruses: SARS-CoV-2 (Wuhan Hu-1), SARS-CoV-1, MERS-CoV, HCoVHKU1, HCoV-OC43, HCoV-NL63 and HCoV-229E. Their focus was the reactivity of the spike glycoprotein.

Monoclonal antibodies from the samples of 19 donors were isolated and characterized on the Beacon system:

- Memory B cells were isolated from PBMCs and co-cultured with 3T3-CD40L feeder cells for 10 days to prompt proliferation and in vitro activation
- Pre-screened B cells were loaded onto the system, where the OEP® technology captured each individual cell and escorted them to individual NanoPen® chambers
- Two binding detection assays were performed, one using MERS-CoV and HCoV-OC43 spike protein-coated microbeads and one using SARS-CoV-2 spike protein-coated microbeads.

COVID-19 Convalescent Donor Screening and Isolation of Broadly Neutralizing mAbs.



Representative images show optofluidic screening of MBC secreted antibodies for cross-reactivity. The left panel shows individual MBCs sorted into nanopens and the right panel shows the fluorescent signal of antigen-specific antibodies binding to coronavirus spike-coated beads (yellow arrows).[4]

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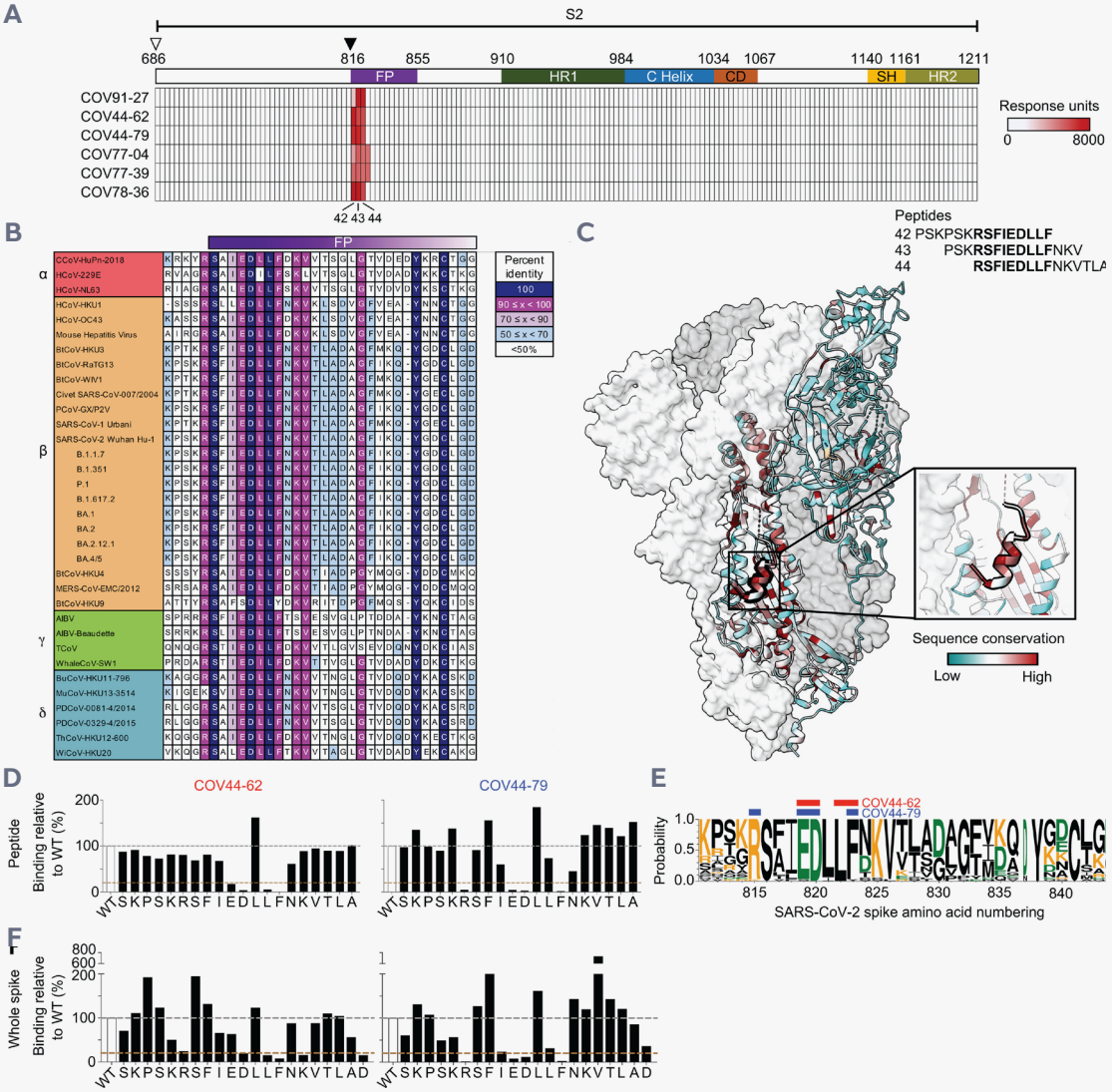
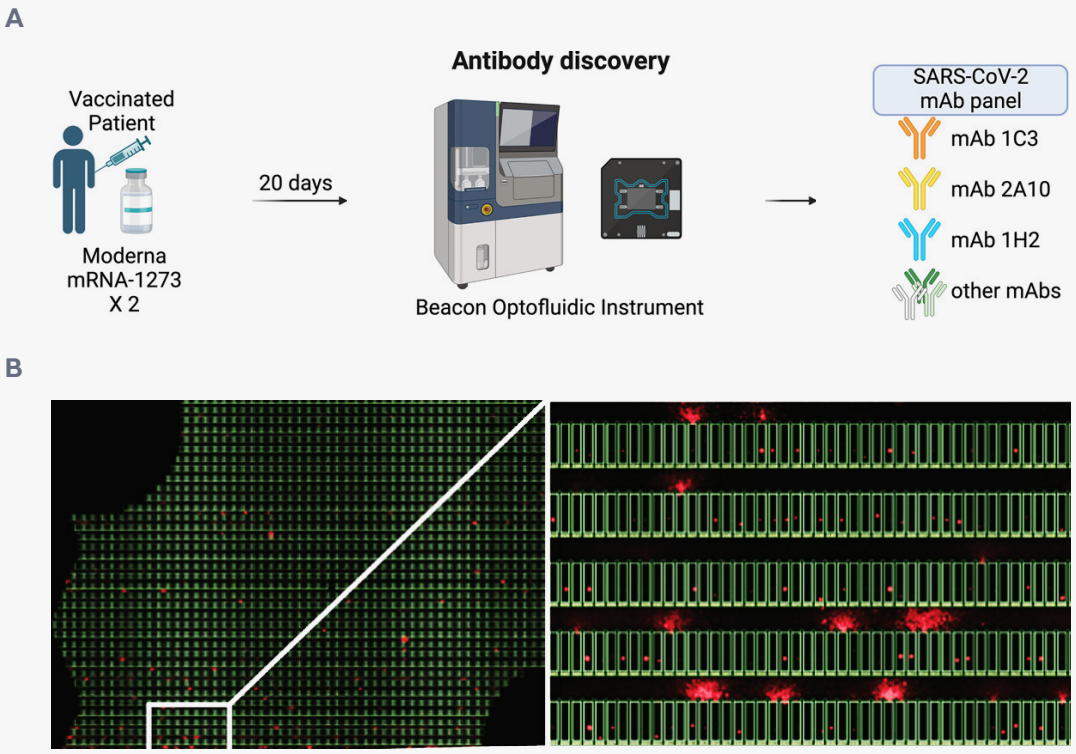
A total of 60 mAbs found to be crossreactive to all three coronaviruses (MERS-CoV, HCoV-OC43, and SARS-CoV-2) were identified, exported, and sequenced. The process took just one day. In a series of follow-up assays, Dr. Tan's team found six of the mAbs were able to bind to the spike protein of the seven selected coronaviruses capable of infecting humans. All targeted the conserved fusion peptide region adjacent to the S2' cleavage site.

Broadly Neutralizing Antibodies Target the Conserved Fusion Peptide



A) S2 Peptide Array: SPR binding responses assessed via 15-mer peptide array on S2 subunit, indicating S1/S2 and S2' cleavage sites. B) Fusion Peptide Alignment: MAFFT used for fusion peptide sequence alignment from 34 coronaviruses. C) SARS-CoV-2 Fusion Conservation: Sequence conservation of fusion peptide (aa 816-843) in pre-fusion SARS-CoV-2 spike protein, with magnified inset. D) Alanine Scan: COV44-62 and COV44-79 binding to SARS-CoV-2 fusion peptide studied through alanine scan, key binding residues noted. E) Fusion Peptide Diversity: Sequence logo showing fusion peptide diversity across 34 coronaviruses, highlighting key mAbs COV44-62 and COV44-79 binding residues. F) Alanine Mutagenesis: COV44-62 and COV44-79 binding residues identified via alanine mutagenesis, compared to wild-type spike and control mAb binding.

Beacon System Used to Identify Potent Omicron-Neutralizing Antibodies 6 Months Prior to the Emergence of the Omicron Variant



A) The team at La Jolla Institute for Immunology identified antibodies from a vaccinated subject that show broad activity against multiple Omicron lineages. B) Activated B cells were screened on the Beacon system. Secreted antibodies were screened for reactivity to Delta-Spike antigen captured on streptavidin coated polystyrene beads. Antigen-sequestered IgG was detected with fluorescent anti-human IgG secondary antibody.

Early Identification of Potent Omicron-Neutralizing Antibodies

Variants of concern (VOCs) have mutations in the spike protein that can enhance the virus' transmissibility and impact the efficacy of vaccines. The Omicron variant, Omicron BA.1, which surfaced in South Africa in 2021, harbored over 30 mutations in the spike protein, posing a significant threat to vaccine efficacy. As we now know, the epitope landscape of this Omicron variant presented a significant challenge to antibody treatments. Even highly effective FDA-authorized antibodies showed reduced efficacy against one or more Omicron lineages, revealing the magnitude of this threat.

A recent study published in *Cell Reports* has revealed a panel of vaccine-derived antibodies that possess the ability to fight off various threats.[8] The findings highlight the power and limitations of SARS-CoV-2 antibodies, providing critical guidance for the advancement of broad-spectrum therapeutic treatments.

Researchers at La Jolla Institute for Immunology set out to investigate which antibodies from a previously vaccinated patient exhibit broad activity against multiple Omicron lineages. Bruker's Beacon instrument and antibody discovery protocol enabled the isolation of individual B cells, allowing for the screening of secreted antibodies for reactivity to Delta-Spike antigens. The Beacon system facilitated the rapid identification of potent Omicron-neutralizing antibodies six months prior to the emergence of the Omicron variant, showcasing the potential for accelerated vaccine development and therapeutic interventions. The findings of this study provide compelling evidence that vaccination can elicit antibodies that effectively combat emerging variants such as Omicron.

Key findings included:

- COV44-62 and COV44-79 broadly neutralized alpha and beta coronaviruses, including SARS-CoV-2 Omicron subvariants BA.2 and BA.4/5
- Co-coding fragment antigen-binding regions (Fab) of COV44-62 and COV44-79 with SARS-CoV-2 fusion peptides crystal structure analysis revealed that the fusion peptide epitope has a helical structure, and contains an S2' cleavage site that is involved with antibody recognition and potentially the conformational dynamics during S protein binding and infection.
- Animal model experiments showed that COV44-79 inhibits pathogenesis in a Syrian hamster model of SARS-CoV-2 infection

Together, these findings highlight the potential of fusion peptides as candidate epitopes in the development of next-generation COVID-19 therapeutic mAbs and vaccines.

Beacon System: A Superhero in the Fight Against Emerging and Endemic Diseases

As these studies show, the Beacon system has been – and will continue to be – an invaluable tool in the global fight against emerging and endemic diseases, especially COVID-19.

By identifying and screening tens of thousands of B cells in just one day, the Beacon system can produce a much higher quantity of quality hits more quickly than traditional methods. The automated process enables high-throughput multiplex detection and allows for the evaluation of the binding activity of multiple genera of viruses in as little as one day.

To find out how the Beacon system could set your antibody discovery processes free, visit www.brukercellularanalysis.com

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