Role of the Spike Protein in the Entry of SARS-CoV-2 into Cells and in Vaccine Design

Through the analysis of the complex structures of the receptor-binding domain of the spike protein with monoclonal antibodies and the fusion core of the spike protein, the utilization of this structural information to design potential vaccines or drugs is elucidated.

The causative agent of the COVID-19 pandemic is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), classified as a betacoronavirus. It was confirmed that human angiotensin-converting enzyme 2 (ACE2) serves as the receptor for the SARS-CoV-2 virus, utilizing the spike protein on the virus surface to infect human cells in a similar manner to SARS-CoV. The interaction between the spike protein and the ACE2 receptor enables the virus to enter cells. The spike protein is located on the surface of the virus and is composed of two subunits, S1 and S2 (**Fig. 1**). The S1 subunit contains the receptor-binding domain (RBD) that binds to ACE2, facilitating the interaction and triggering a conformational change in the spike protein. The conformational change allows the S2 subunit to utilize its fusion core, comprised of regions of heptad repeat 1 (HR1) and heptad repeat 2 (HR2), to insert into the host cell membrane.[1] According to the World Health Organization (WHO) data, as of December 2023, SARS-CoV-2 has infected over 770 million people, resulting in nearly 7 million deaths worldwide. Despite the significant challenges posed by the COVID-19 pandemic, there have been numerous global efforts to develop strategies for treatment, vaccines, and controlling the spread of the virus.

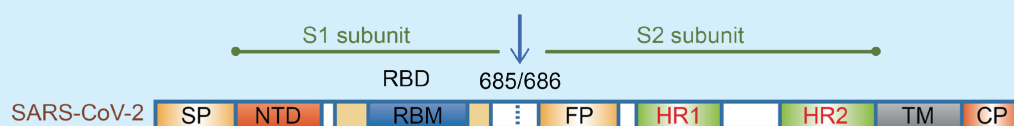

Fig. 1: Schematic illustration for SARS-CoV-2 spike protein, which can be split into two subunits, S1 and S2, at this site between 685 and 686. S1 subunit contains signal peptide (SP), N-terminal domain (NTD), and receptor-binding domain (RBD) with receptor-binding motif (RBM); S2 subunit contains fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic peptide (CP). [Reproduced from Ref. 1]

Two research teams, led by Che Ma (Academia Sinica) and Chun-Hua Hsu (National Taiwan University), collected X-ray diffraction data at **TPS 05A** and **TLS 15A1**, and **TPS 05A**, respectively. They separately solved the structures of the RBD–antibody complex and the fusion core. Using the structural information, relevant biochemical experiments were conducted. The following content briefly introduces their experimental results.

Researchers from Ma's group isolated eight neutralizing monoclonal antibodies, including EY-6A, FP-12A, IV-6D, IV-4B, IV-10C, IS-9A, IS-11B and IY-2A, from Taiwan patient samples. They all coincidentally bind to a buried region in the spike protein's RBD, thereby the antigenic determinant (epitope) was categorized as "Class 4 antibodies." Class 4 antibodies can only detect and bind to the buried site when the RBD is in an upward conformation and are therefore referred to as "cryptic antibodies." Furthermore, because this is a hidden region, not prone to mutations, it can be considered a hotspot for broadly effective antibody targeting.

The research team further analyzed the binding strengths of these eight antibodies with different variants of SARS-CoV-2. In neutralization experiments, these antibodies could broadly and effectively inhibit Omicron BA.5 and other variant strains such as Beta and Delta. In particular, the antibody IY-2A induces structural changes in the RBD and generates new neutralizing binding sites, making it the latest breakthrough discovery in current COVID-19 research (**Fig. 2**).[2]

In Ma's study, the research team described a series of antibodies identified as Class 4 antibodies, as they exhibit complete or partial competition with similar antibodies in RBD binding. Structural analysis of their binding footprints and comparison with examples in the literature allowed the identification of antigenic regions comprising multiple overlapping epitopes. One of these epitopes is newly characterized by our antibody IY-2A. These antibodies prefer binding to highly conserved epitopes and demonstrate broad effectiveness against SARS-CoV-2 variants.

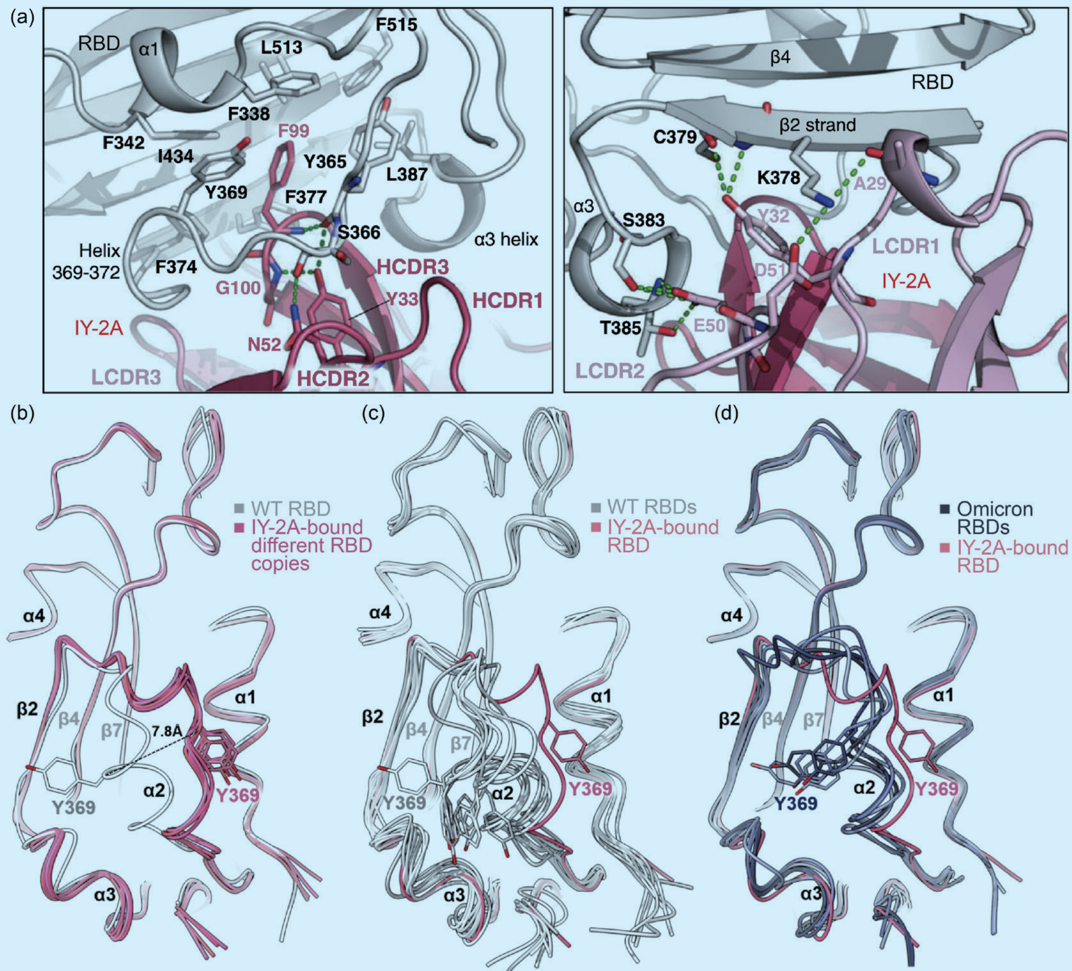

Fig. 2: Detailed interface of RBD/IY-2A and the conformational change of RBD upon IY-2A binding. (a) The IY-2A antibody (heavy chain, dark red; light chain, light red) induces a conformational change in the RBD (gray), creating new neutralizing binding sites. HCDR/LCHR refers to heavy/light chain complementarity determining region. Hydrogen bonds are illustrated with green dashed lines. (b) The superimposition of four copies of the IY-2A-bound RBD (varied pink colors) reveals a consistent conformation in this region. Y369 is depicted as sticks, showing a Cα atom displacement of 7.8 Å from the ACE2-bound WT RBD (PDB 6M0J). (c) Overlaying ten WT RBDs (PDB code: 6M0J, 6ZER, 7M7W, 7R6X, 7R6W, 6W41, 7RKU, and 7JMW, the IS-9A-bound, and the FP-12A-bound RBD structures, in light gray shades) demonstrate a conserved conformation in the 364-376 region (α2 helix and α2-β2 linker) compared to the IY-2A-bound RBD (pink), with Y369 highlighted in sticks. (d) Comparing the WT RBD (PDB 6M0J, gray) with five Omicron RBDs (BA.1: PDB 7XAZ, 7XO6; BA.2: PDB 7XB0, 7ZF7, 7XOC, in different purple shades) and the IY-2A-bound RBD (pink) unveils structural variation in this region. [Reproduced from Ref. 2]

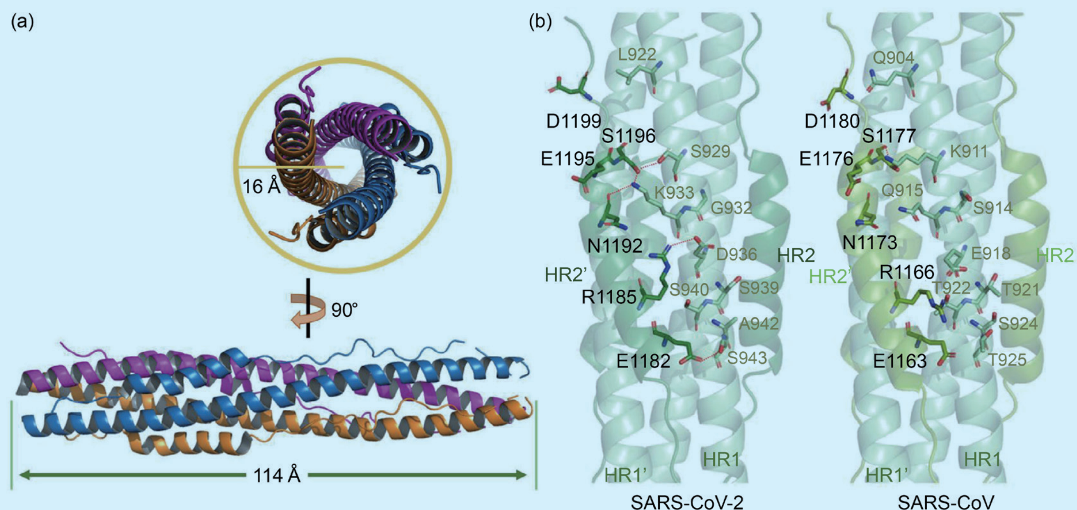

Fig. 3: Overall structure and interaction between HR1 and HR2 of SARS-CoV-2. (a) The three HR1–L6–HR2 molecules are presented in the asymmetric unit. (b) The hydrophobic interaction between HR1 and HR2 of SARS-CoV-2 and SARS-CoV. It shows that the two complex structures have similar architecture. Residue differences in fusion cores are shown as stick models. [Reproduced from Ref. 1]

On the other hand, Hsu's team constructed a single chain incorporating the HR1 and HR2 peptide segments connected through an L6 linker (referred to as HR1–L6–HR2) and conducted a series of experiments to investigate its structure and stability. They observed the spontaneous assembly of this protein into a highly stable trimeric complex. Crystallographic analysis revealed a trimeric coiled-coil structure in the fusion core (**Fig. 3(a)**),[1] with specific residues involved in binding. The research team suggested that the fusion core of SARS-CoV-2 shares a similar conformation with those of other class I viruses, remarkably resembling the fusion core of SARS-CoV spike protein (**Fig. 3(b)**).[1] This indicates the possibility of employing similar methods to identify inhibitors effective against SARS-CoV-2 infection. Additionally, this structure provides crucial detailed information and target sites for structure-based drug design.

In summary, both studies elucidated the structural characteristics and biophysical properties of the RBD and fusion core. They noted that these features are essential in developing vaccines and therapeutic approaches for SARS-CoV-2 and other sarbecoviruses within the Coronaviridae family. (Reported by Chun-Hsiang Huang)

This report features the work of Che Ma, Chun-Hua Hsu and their co-workers published in Nat. Commun. ***14****, 311 (2023) and J. Chin. Chem. Soc.* ***70****, 1208 (2023).*

TPS 05A Protein Microcrystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography
- XPS, Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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Secrets of the Deadly Honeybee-Infecting Virus Unveiled

The atomic-resolution capsid structure of the honeybee-infecting virus could furnish valuable insights into the mechanisms governing viral capsid assembly, function, and infection with dynamic motions. This information can be harnessed for the development of natural drugs for honeybee colony protection.

Taiwan's warm temperatures, humid climate, and diverse array of nectar sources create a favorable environment for fostering honeybee habitats and promoting their propagation. Honeybees, in turn, play a crucial role in pollinating wild plants and agricultural crops. The two major honeybees, *Apis cerana Fabricius* (*A. cerana*) (Eastern honeybee) and *Apis mellifera Linnaeus* (*A. mellifera*) (Western honeybee) both play a crucial role in contributing to commercially valuable honey harvests and are both major honeybee species in the global beekeeping industry.[1] Colony collapse disorder (CCD) is a noteworthy phenomenon characterized by the sudden disappearance of as much as 90% of beehives in apiaries. Pathogens, including viruses, bacteria, fungi, mites, and environmental factors, such as chemical exposure and forage availability, have been identified as major contributors to disease in managed honeybee populations and are often associated with CCD.[2] Although the precise mechanisms of CCD remain elusive, evidence suggests that a newly discovered honeybee-infecting virus, Lake Sinai virus (LSV), plays a key role in this devastating disorder. The primary strains of LSV encompass two principal types, namely LSV1 and LSV2, along with other variants. LSV comprises a characteristic $T = 4$ quasi-equivalence nonenveloped capsid for packaging the single-stranded positive-sense RNA genome. The RNA genome size is ~5.6 kb and it encodes three major genes: Orf1 with unknown function, RNA-dependent RNA polymerase responsible for viral RNA replication, and the capsid protein (CP) for host recognition and viral capsid assembly. This work aims to explore the structures of LSV virus-like particles (VLPs), characterize all domain functions, and understand capsid assembly and RNA packaging during viral infection.

Although mature LSV virions are generally considered to exist solely in $T = 4$ assemblies, cryo-electron microscopy (cryo-EM) analyses of LSV2 and delta-N48 LSV1 VLPs uncovered structural polymorphism. By examining