Advanced Tools for Virology Research

Introduction

A virus is a simple life form comprised of genetic materials (DNA or RNA) and an outer shell constituted mainly of proteins. However, even though considered as an organism with only the "bare minimum" components for life, viruses are powerful pathogens and have caused several notorious pandemics that resulted in the loss of lives and the devastation of the regional or global economy.

Viruses require a host to survive, propagate and change for the adaptation to environmental stresses. They interact with the host cells through the binding of virus surface proteins to their corresponding receptors and then fused into the host cells. Once inside, the genetic materials of the viruses are released, processed, and virus propagation is achieved by hijacking various cellular machineries involved in gene transcription and protein translation (Figure 1).

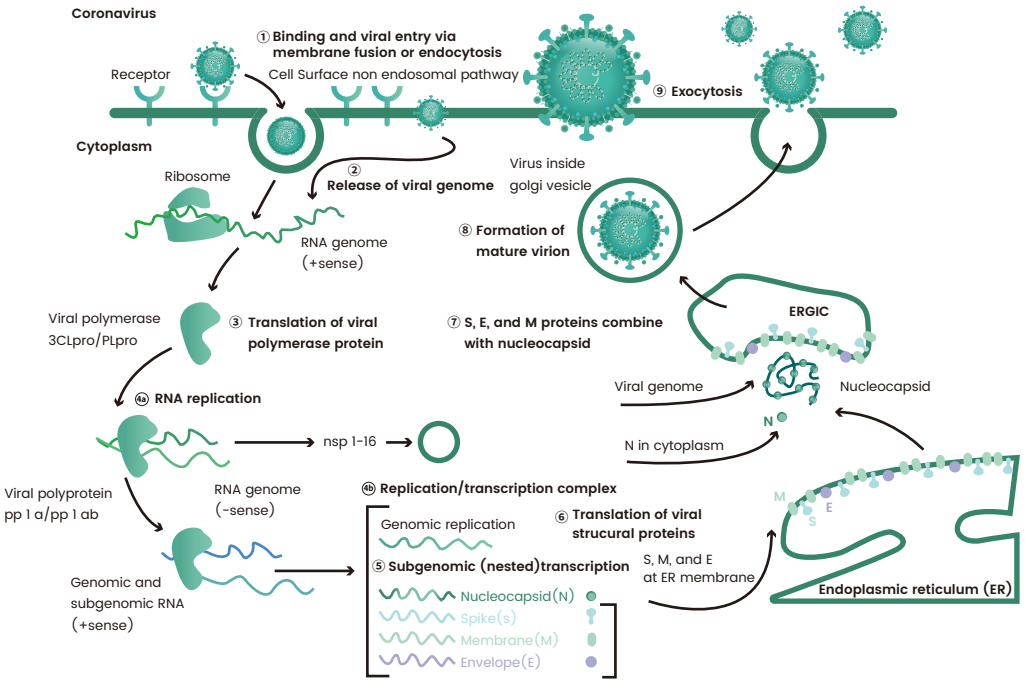

Figure 1: An example of the life cycle of a virus.

Based on their unique biochemistry, pathology, and physiology, highly specialized biochemical tools are required to gain a deep understanding into the pattern of interaction between a virus and its host and to reveal the mechanisms for virus propagation and assembly. Information derived from such researches helps pave the paths for the development of virus diagnostic tools, public health interventions, and anti-viral therapeutics. This article focuses on the development of advanced biochemical tools for virology researches. These tools include recombinant virus proteins, antibodies, and pseudovirus particles. In the meantime, it is also worth mentioning that as a novel concept in vector science, certain forms of viruses are now widely used as vehicles for gene delivery and major components in vaccines. Such applications are not discussed in this article.

Recombinant Virus Proteins

Proteins occupy 50% (w/w) of a virus particle and they function in a variety of ways. Some proteins serve as the attachment and entry points for the virus (surface proteins, usually bear heavy glycosylations) while others catalyze the synthesis of virus genome and processes of newly synthesized virus proteins (enzymes). These virus proteins hold the key to the understanding of virus physiology and also guide the generation of immunological diagnostic tools and anti-viral medications. With recombinant protein expression technology, it is now feasible to produce virus proteins in large quantities and use them as tools for a variety of aspects in virology research.

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In accordance with the "central dogma", recombinant protein expression techniques utilize a vector (namely a plasmid) that contains the target gene encoding for the protein-of-interest and introduces it into a host cell. The target gene is either inserted into the host cell genome to guide protein translation or the protein-of-interest is produced via plasmid-mediated direct gene translation (Figure 2). Various prokaryote and eukaryote host systems have been developed during four decades to meet the production of recombinant proteins of desired features in terms of post-translational modifications (PTMs), conformation, and activities (Figure 2).

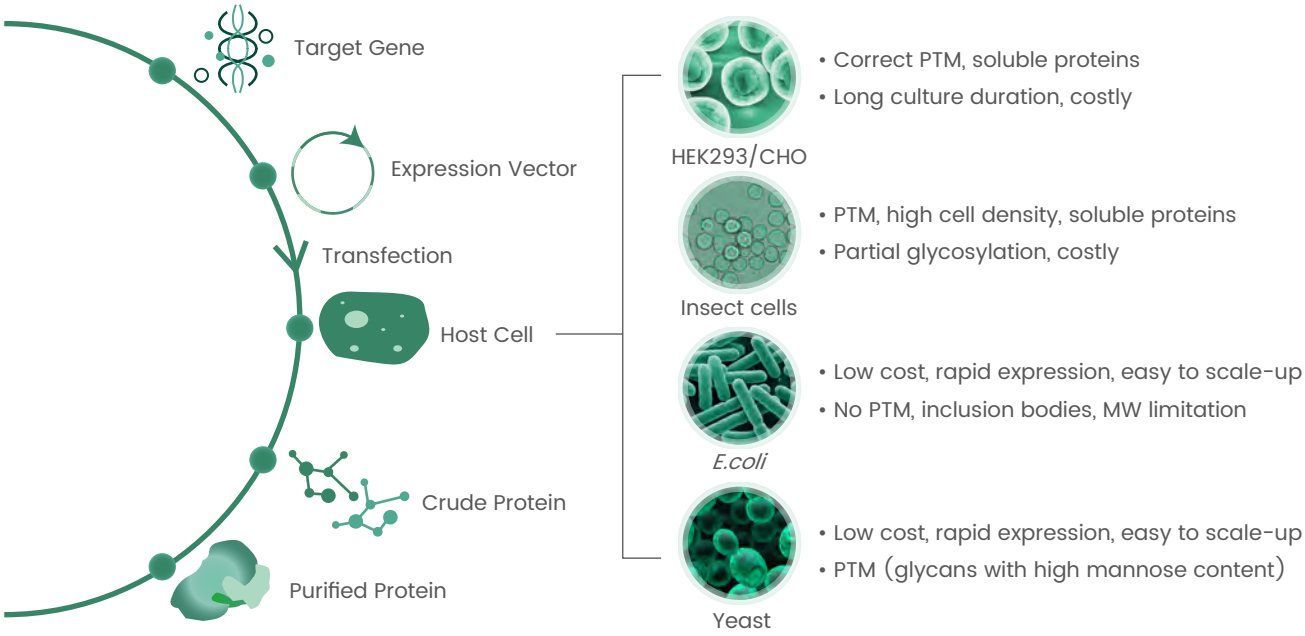
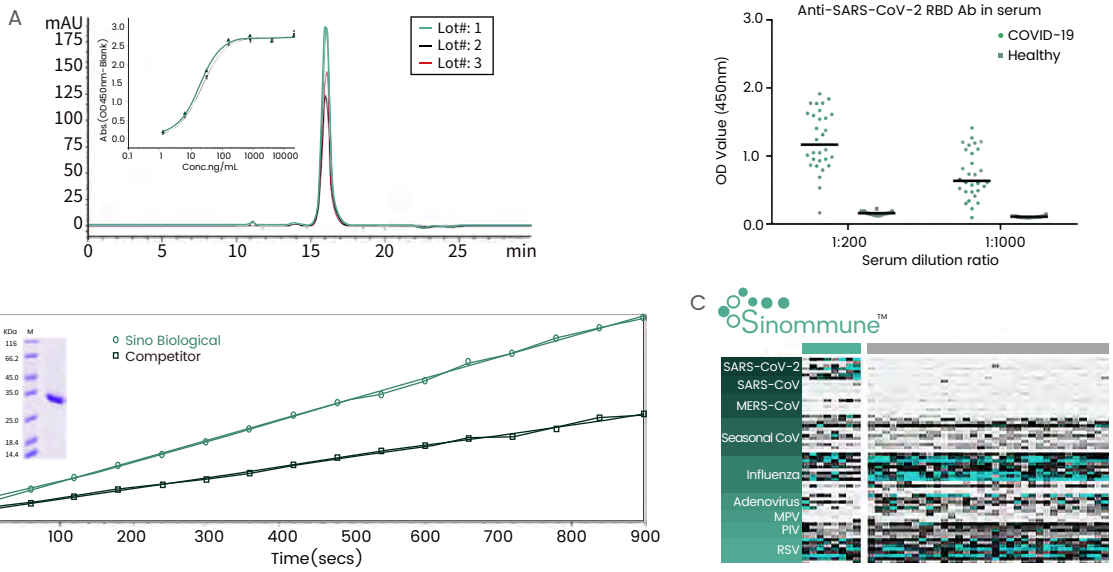


Figure 2: Basics for recombinant protein expression and host systems.

The biochemistry and function of a protein dictate the selection of expression host and purification strategies. For virus surface proteins, mammalian cells are usually the preferred hosts due to their complex high-order structures and extended glycosylation profiles, while a prokaryote system (e.g. *E.coli*) is usually adequate in generating active virus enzymes for both structural and functional studies. Insect cells, on the other hand, are versatile expression hosts and are used to produce both secreted and intracellular proteins. Proteins expressed by the insect system usually exert equivalent functionalities comparing to those derived from the mammalian system but they bear a more simplified PTM profile to benefit structural studies. The insect cell system serves as an excellent alternative, especially for the production of the hard-to-express proteins. With well-established platforms and over a decade of experience in recombinant protein expression, Sino Biological has created the largest collection of virus proteins worldwide, with more than 1,000 virus proteins covering over 350 virus strains, from popular influenza virus, coronavirus, to the more deadly filoviridae such as Ebola and Marburg virus (ProVir™ Viral Antigen Bank). Some examples of virus proteins are presented in Figure 3.



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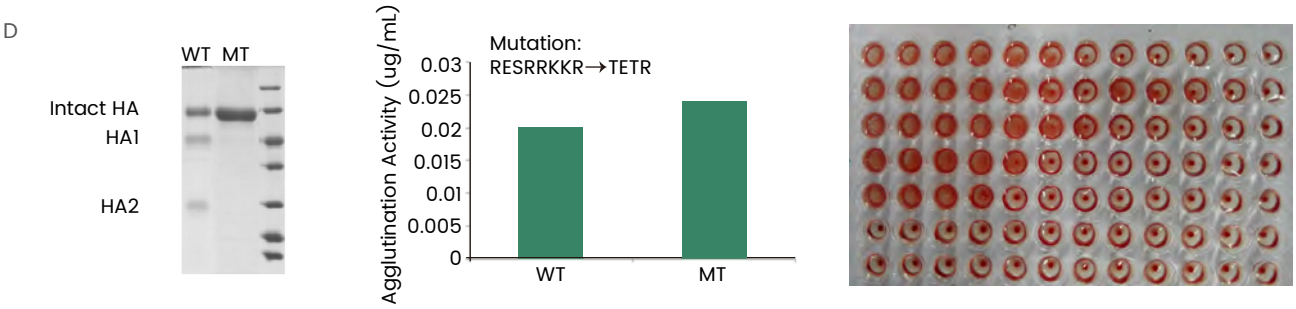

Figure 3: Examples of recombinant virus proteins from Sino Biological. (A) Receptor binding domain (RBD) of SARS-CoV-2 expressed in HEK293 cells. The protein showed good batch-to-batch consistency in terms of its purity and binding affinity against ACE2. It has been used in a serological assay to measure the serum antibody content of COVID-19 patients. (B) SARS-CoV-2 3C-like protease was expressed in insect cells with enhanced catalytic activity assayed by the cleavage of a fluorescent peptide substrate. (C) A collection of virus protein antigens for upper respiratory tract infections have been developed and formulated into the Sinommune™ chip, suitable for high-throughput and fast disease diagnostics. (D) Influenza (H5N1) hemagglutinin (HA) protein expressed in insect cells with agglutination activities. Mutations at the furin cleavage site abolished protein cleavage by host cells without compromising the agglutination activities.

As an extension of the conventional protein expression techniques, the transient protein expression method by HEK293 allows the establishment of a high-throughput protein expression platform where hundreds of constructs can be expressed simultaneously to create a recombinant protein library. This technical platform is extremely useful to cope with the high-frequency mutation rate of RNA viruses. So far, this service platform has been used to generate over 630 influenza proteins and over 170 recombinant SARS-CoV-2 variants to facilitate high-throughput screening campaigns to identify broad-spectrum neutralizing antibodies.

Virus-specific Antibodies and Pseudovirus

When faced with a novel pathogen, the host will mount immune responses to eliminate the threat; a process during which pieces of the pathogen are recognized by immune cells followed by the generation of a pool of antibodies (immunoglobulin) to specifically "flag" the pathogen for destruction by various effector cells. With high specificities and sensitivities, virus (pathogen) specific antibodies are valuable tools for virus identification in a serological assay and neutralization, if used as a therapeutic agent. Equipped with comprehensive antibody generation platforms as well as extensive pre-existing antibody libraries, Sino Biological is highly experienced in virus-specific antibody discoveries.

For the current COVID-19 pandemic, efforts were made in both pre-existing antibody library screenings and new antibody discoveries via animal immunizations. More than 60 antibodies against the SARS-CoV-2 spike or nucleocapsid (N) protein have been obtained, among which a few antibody pairs were identified to formulate ELISA assays with pg/mL sensitivities (Figure 4A). The N protein assays have been proven as mutation-proof when tested against N protein mutants from the B1.1.7 lineage, with only a slight decrease of sensitivities observed (Figure 4C). Lastly, ultra-sensitive anti-N antibodies have also been identified during the screening process to boost the detection limit of N antigen at a phantom molar level in the Simoa HD-X system (Figure 4B).

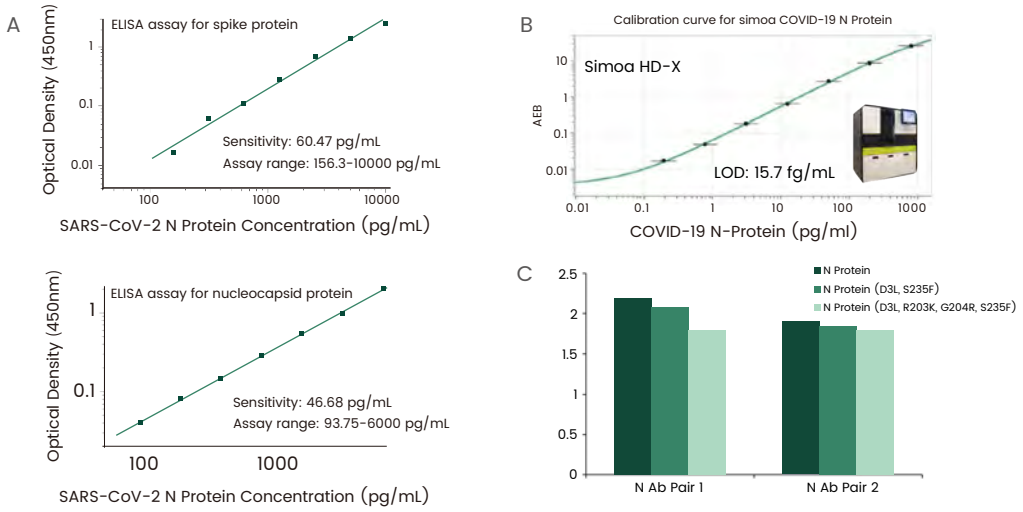

Figure 4: Highly sensitive antibody pairs for serologic assay development.

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High-frequency mutagenesis is a feature of most RNA viruses. Viruses evolve through those randomized mutations to adapt to the new immune landscape while the mutations could be critical when occurred at key epitopes and compromise antibody efficacy significantly. The creation of a mutant protein library is useful in assessing the impact of mutation on antibody efficacy but the fluctuation of binding affinity does not directly correlate to the overall behavior of the virus.

That being said, pseudovirus is a useful tool to compensate for such shortcomings and enhance the biological relevance in the assessments of the biology of mutations. Taken advantage of current viral vector manipulation techniques, the protein-of-interest can be engineered onto the surface of the virus particles while a reporter gene is encapsulated and activated once the pseudovirus is incorporated into the host cells. The readouts of such assays are usually in the form of fluorescent intensities and the results provide useful information to assert the virulence of a virus and assist in the investigation of the impact of mutations. One such example is presented in Figure 5.

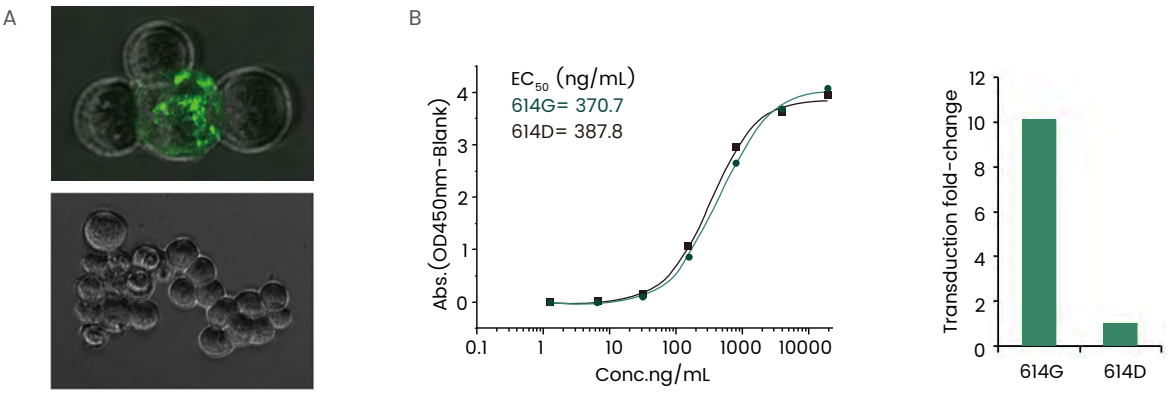

Figure 5: Application of SARS-CoV-2 pseudovirus in assessing the impact of D614G mutation. (A) A proof of concept experiment showed that the SARS-CoV-2 pseudovirus is able to bind and internalize into the host cell. Top: SARS-CoV-2 pseudovirus infected ACE2 over-expression HEK293 cell line; bottom: negative control. (B) The D614G mutation did not cause a significant alteration in ACE2 protein binding affinity judging by ELSA (top) while a SARS-CoV-2 pseudovirus containing such mutation resulted in a ~5-fold increase in the transduction fold-change, indicating a higher infectious potency given by the D614G mutation.

Conclusion Remarks

With the constant expansion of industrialization and human habitat, zoonotic viruses are lurking in the shadows, ready to emerge, and future pandemics are imminent. Recombinant virus protein, virus-specific antibodies and pseudovirus are powerful tools to help understand the biology of these viruses and facilitate the efforts of pandemic control. However, the key of pandemic prevention still lies in the rational planning of urbanization, establishment of optimized resource distribution and exploitation of new alternative technologies to achieve a long-term and continuous development of the human society.

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