

## SARS-CoV-2 Spike Glycoprotein with Improved Expression and Stability

Researchers in the Sapphire lab have developed a novel pre-fusion stabilized SARS-CoV-2 Spike (S) glycoprotein termed “VFLIP” for (V) five proline, F lexibly-L inked, I nter-P rotomer spike.

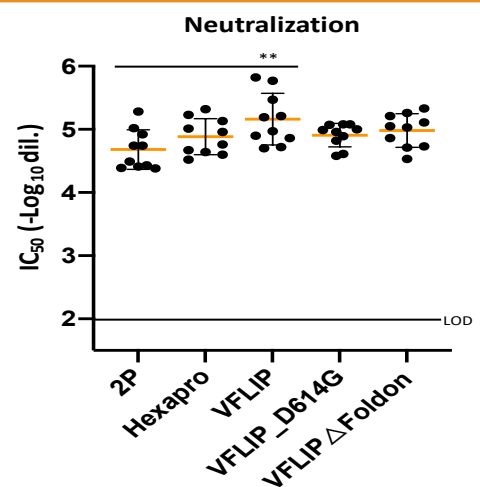
In late 2019, SARS-CoV-2 emerged in China and rapidly spread globally to infect over 120 million people and cause over 2 million deaths. The spike glycoprotein displayed on the virus surface in the “pre-fusion” metastable state is the main target of neutralizing and protective antibodies for all of the coronaviruses and thus is the main target for most CoV vaccine efforts. Most SARS-CoV-2 vaccine candidates now in use or in clinical trials employ an S-2P construct that showed initial promise but afforded few opportunities for optimization due to its rapid development. A second-generation HexaPro version offers greater yield and stability compared to S-2P but lacks the more desirable pre-fusion structure and the native glycosylation pattern of the SARS-CoV-2 S glycoprotein.

LJI researchers have designed a third-generation S glycoprotein that maintains its conformation in the desirable pre-fusion state as well as a glycosylation pattern that more accurately reflects that of the native SARS-CoV-2 S glycoprotein. Additionally, VFLIP offers ~3°C improvement in thermostability over HexaPro and retains its trimeric structure even after lyophilization, freeze/thaw cycles, and prolonged storage at room temperature, 4°C and 37°C, conditions that cause S-2P and HexaPro to separate into monomers. As such, VFLIP is an excellent vaccine candidate for further study due to its exceptional stability, high yields, and improved immunogenicity. The VFLIP technology can also be applied to SARS-CoV-2 variants as well as to other human coronaviruses.

### ADVANTAGES:

- Improved stability of S in the pre-fusion state
- Glycosylation pattern that resembles native S protein
- Retains trimeric structure due to internal disulfide linkage
- Applicable to SARS-CoV2 variants and other CoVs

*Third-generation Spike Glycoprotein is a Promising Candidate for Improved SARS-CoV-2 Vaccine Design*



*Neutralization data of VFLIP variants compared to S-2P and HexaPro*