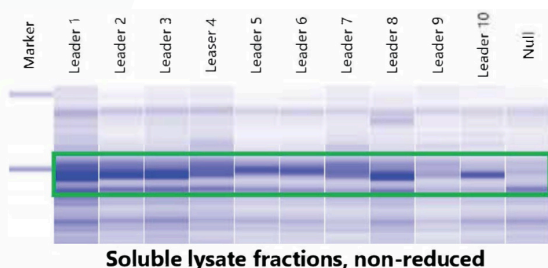## Background

- About 10% of cow antibodies have exceptionally long heavy chain CDR3 regions, including a stalk and knob region[1]
- The knob region of CDR H3 is about 4-6 kDa in size with a highly diverse sequence content and multiple disulfide bonding patterns[1]: these are known as Picobodies™
- Picobodies can be used for inhaled therapy because of their small size and stability [2]

Challenge

Because of their small size Picobodies can be independently genetically engineered and developed to therapeutically target crevices, pores, or other protein epitopes that larger antibodies cannot. However, Picobodies with complex disulfide bonding patterns can be difficult to produce.

Solution

The Pfenex Expression Technology® platform, with its extensive toolbox of genetic elements, fusion partners and modified hosts strains, was utilized to quickly identify expression strains producing high titer and high quality Picobodies for further preclinical development.

Key Results
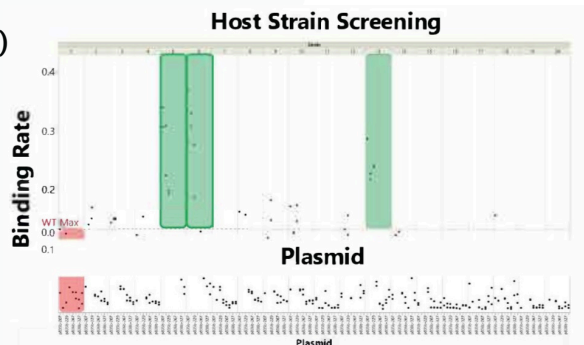

Soluble lysate fractions, non-reduced

SDS-CGE Analysis of Soluble Picobodies (90 min/plate)

SDS-capillary gel electrophoresis (SDS-CGE) used to assess expression and solubility.
SDS-CGE detected induced bands of soluble Picobody fusion proteins (green) up to ~2g/L at 96-well expression scale.
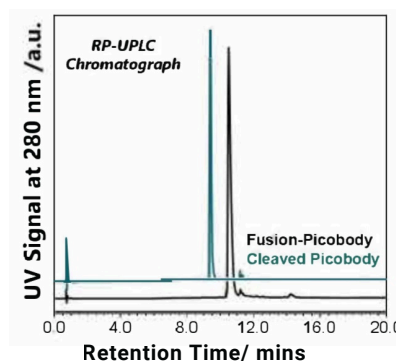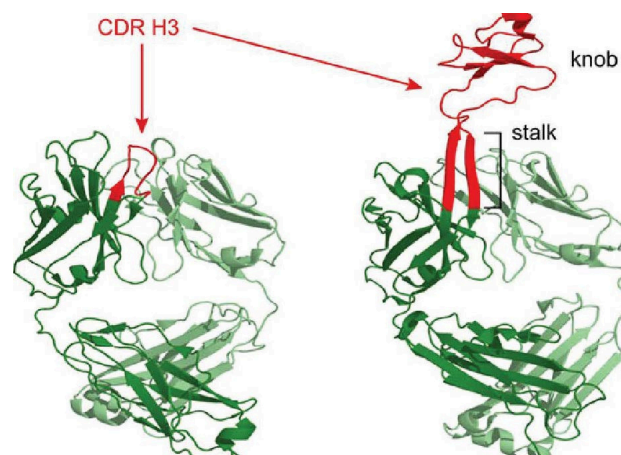
BLI Activity Analysis (30-60 min per plate)

Product quantity and quality assessed via biolayer interferometry (BLI). Strains producing highly active soluble Picobody fusion proteins (green) identified by binding to SARS CoV-2 spike protein compared to wild-type strain (red to dashed line).

Cleaved Picobody Maintains Binding to SARS CoV-2 Spike Protein

RP-UPLC and LC-MS confirm expected cleavage of Picobody from fusion protein. ELISA analysis confirmed binding to SARS CoV-2 spike protein (IC50 = 19 nM).

Intact LC-MS Analysis

Protein ID	Theoretical	Observed (Da)
Fusion-Picobody	28566	28566
Cleaved Picobody	5445	5445

ELISA Analysis
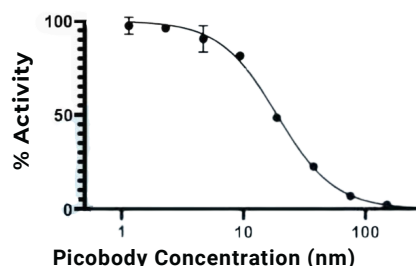
1 Reference doi: 10.3389/fimmu.2018.01262
2 Reference: Bill Harriman and Vaugn Smider, "Picobodies™ as Building Blocks for Novel Therapeutics': PEGS Boston 2021