Dyadic International, Inc. (NASDAQ: DYAI)

"Proteins for World Health"

C1 Platform: The Urgent Need for Smarter Biomanufacturing –
A Call to Action on Avian Influenza (H5N1) & Future Pandemics

Sustainable Recombinant Proteins & Enzymes

World Vaccine Congress / Washington DC
April 23, 2025

Disclaimer

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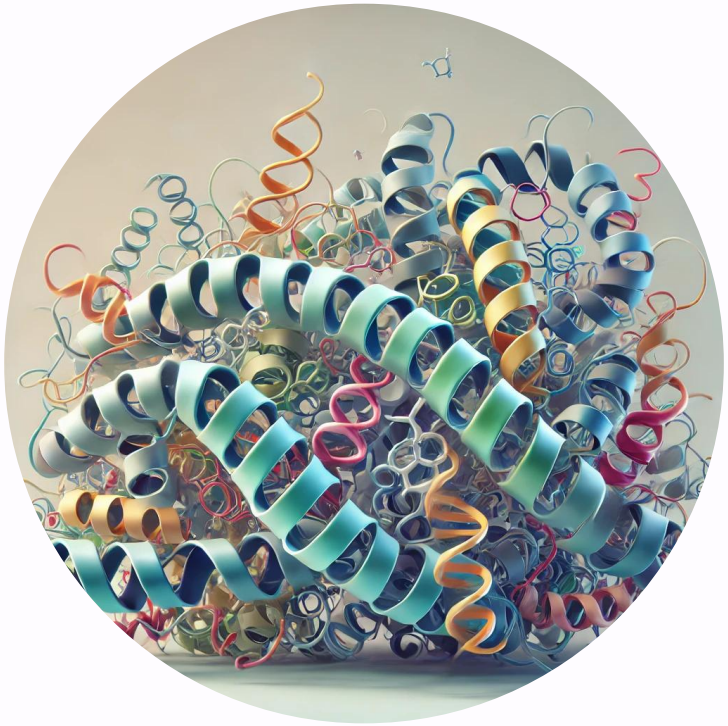
Dyadic Mission: Proteins for World Health

Quality and **efficacy**

similar to traditional platforms…



In less **time**, lower **costs**,

and higher **productivity…**

DYADIC®

TT

INSIDE®

Industrial heritage provides expertise for rapid scalability & high productivity…

The journey begins in cellulase enzymes

- Pioneered use of pumice stones in stonewashing
- Transitioned to cellulose enzymes to textile

1980

Discovery of C1 System

- R&D of the novel Thermothelomyces heterothallica filamentous fungal protein production system (C1)
- Bioindustrial applications

1990

GRAS Certification & Expanded Applications

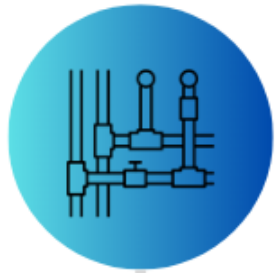
- Building toolkit and commercializing industrial
- Expanded application of industrial enzymes in biofuels, animal feed, textiles, paper, food and beverage industries

2000

Re-engineered C1 System for Biopharmaceuticals

- Sale of industrial business to DuPont.
- Glycoengineering
- Production of human and animal bioligic vaccines and therapeutic

2015



Expanded Dyadic Adoption & Pipeline

- Phase I validates platform
- Pharma partnerships
- Dapibus™ platform for food/nutrition

2022+

Filamentous Fungal Platform Market Opportunities:

Dyadic platforms positioned to deliver efficient products in key verticals

C1 Platform Readiness Level: *Commercial*

- Phase I human data for vaccines
- cGMP capability
- GRAS status
- Protease library and full complement of glycoengineering tools
- Comparable data to CHO in animal models for antigens/antibodies
- Demonstrated stability & productivity
- Wide array of infectious disease antigens and antibodies produced
- 2025 launch of first reagent: rHSA

Dapibus™ Platform Readiness Level: *Commercial*

- GRAS status
- Industrial heritage
- Demonstrated productivity
- Application testing for food grade cell culture media (albumin, transferrin)

Human Health **Animal Health**

➢ **Vaccines**

$200Bn

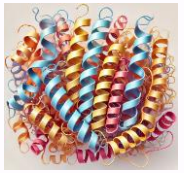
➢ **Antibodies**

$61Bn

➢ **Reagents**

$72Bn

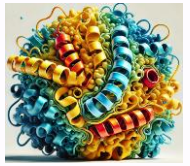
Life Sciences



➢ **Proteins**

$58Bn

➢ **Enzymes**



$7.4Bn

➢ **Biofuel**

$155Bn

Legend	
Available	<3-6 Months

Dyadic Alternative Protein product portfolio growing rapidly

- Global CCM market estimated at $4.7B with >12% CAGR
- The Good Food Institute (GFI) estimates albumin, transferrin, and growth factors make up >95% serum-free cell culture media costs Recombinant CCM products are significantly more expensive than animal derived

Cell Culture Media (Food)

Product	Total Addressable Market	Application	Status
Bovine albumin	$100-300MM	Support cell growth and protein production	C of A complete; Partnered with Proliant Health
Bovine transferrin	$100-300MM	Regulates the supply of iron to cells	C of A complete; application testing 12/2024
Bovine growth factors	$1.6B	Stimulate cellular processes like proliferation	Sampling initiated in Q4 2024

Food & Nutrition; Industrial Applications

Product	Total Addressable Market	Application	Status
Bovine Alpha-lactalbumin	$300-400MM	Food/Nutrition/Reagent	C of A complete; sampling initiated
Dairy enzyme	$110MM	Cheese production	Licensed to Inzymes; Q2 2025 launch
Hyaluronidase	$900MM	Cosmetics	Sampling initiated; testing ongoing
Cellulosic Enzymes	$1.1Bn	Biofuels	Partnered with Fermbox; 1H 2025 launch

- Bovine alpha-lactalbumin has potential markets in food and as a research grade material; current yields achieve research grade margins
- Sustainable revenue from Alternative Proteins estimated for 2025 as both product supply and licensing agreements underway

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Legend	
Available	<3-6 Months

Dyadic DNA/RNA product portfolio for rapidly growing mRNA enzyme market

Molecular Biology: DNA and RNA manipulation

Product	Total Addressable Market	Application	Status
DNASE-1 (Rnase-Free)	**$300-500MM**	Removes contaminating genomic DNA from the RNA preparation	Certificate of Analysis; ready for non-GMP production and cGMP process development
TdT terminal transferase	**$100MM**	Add nucleotides for preparing DNA templates	Stable target; initial yield and quality assessment December 2024
T4 DNA ligase	**$200MM**	Used only for template preparation (e.g., cloning)	Stable target; initial yield and quality assessment December 2024
RNase Inhibitor (murine)	**$225MM**	Protects RNA from degradation during processing	Stable target; initial yield and quality assessment December 2024
T7 RNA polymerase	**$300MM**	Core enzyme for in vitro transcription	Stable target; initial yield and quality assessment December 2024

- Global market for mRNA synthesis raw materials: ~USD 1.72 billion in 2023, CAGR of 2.85%
- Growth predicted in increase due to demand for non-COVID mRNA vaccines
- DNASE-1 (Rnase-free) analysis completed; sampling underway; multiple ongoing licensing discussions; supply agreement expected Q1 2025

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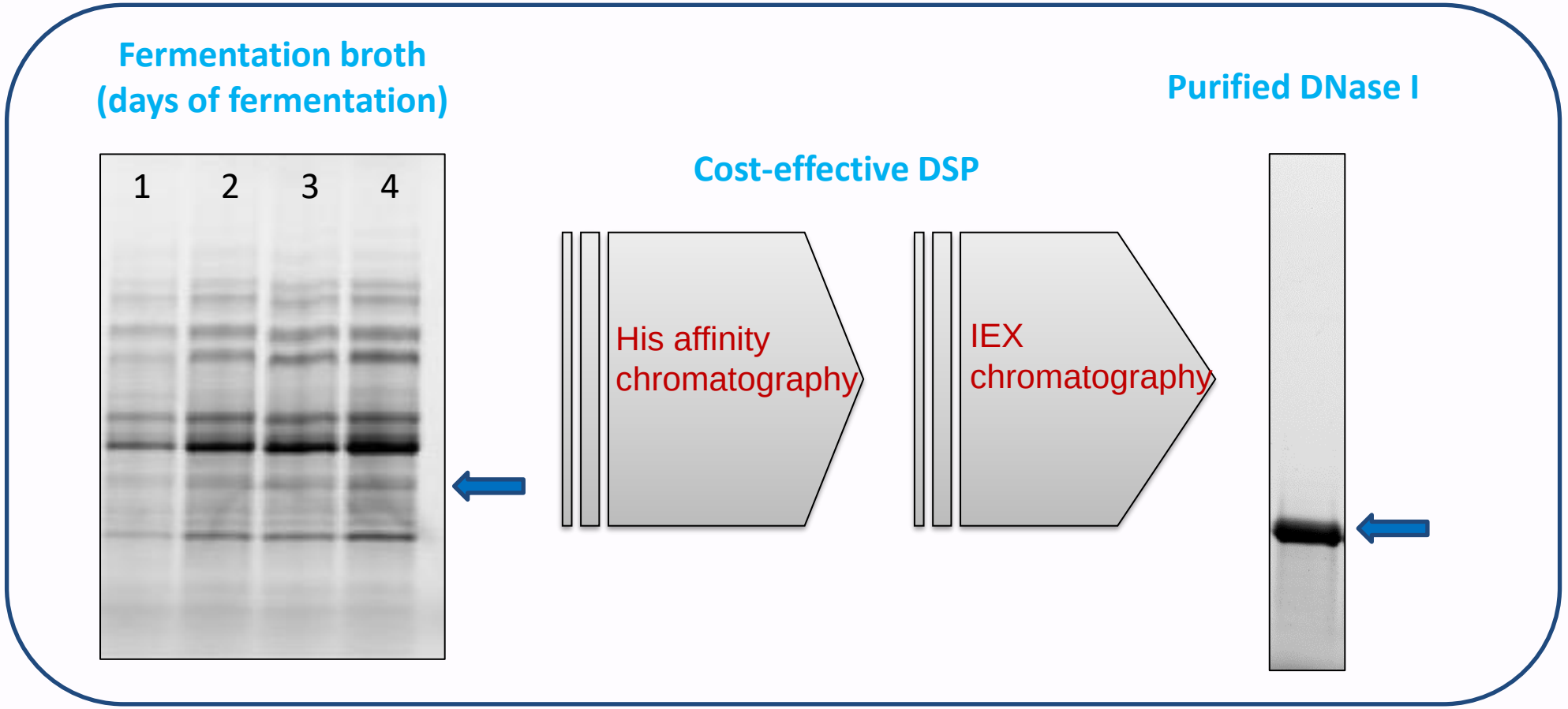
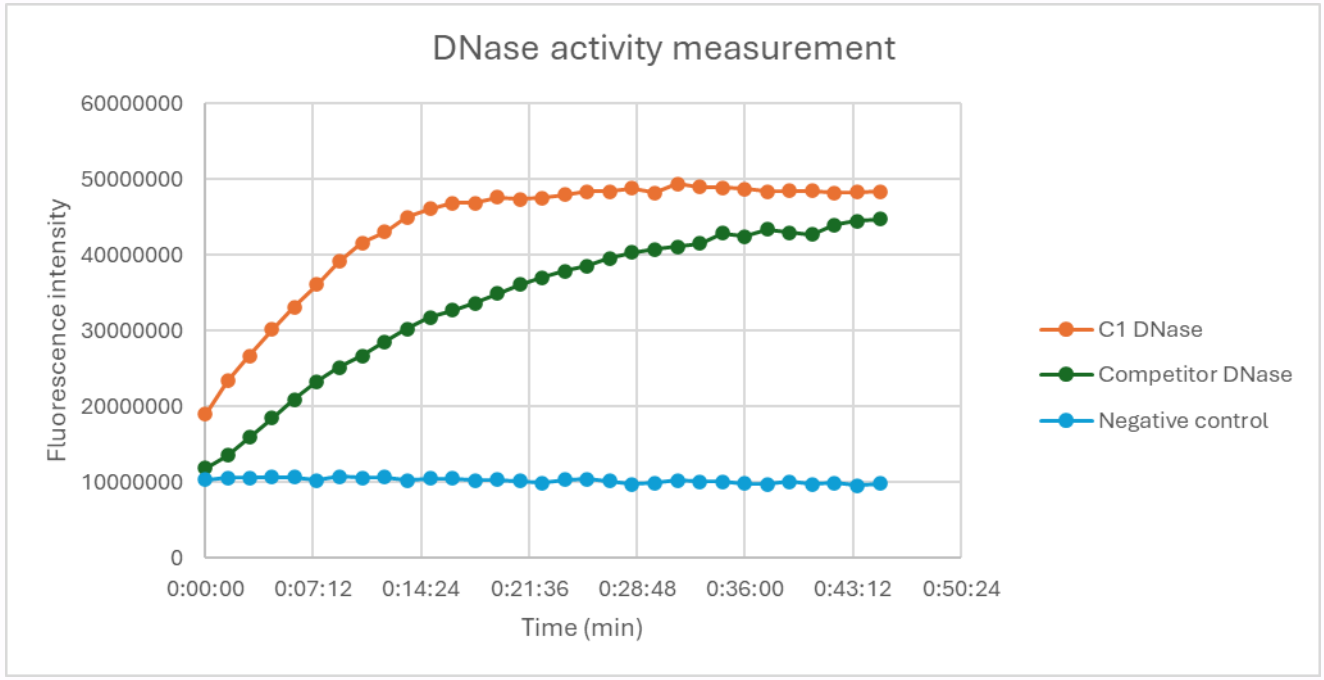
Efficient, High Yield DNase I Production Using C1 Technology

- DNase I was transformed into Dyadic's platform by standard transformation technology (site directed integration, PAnSES).
- Selected clones were fermented under standard conditions
- DNase I sample (protein concentration 1.8 mg/ml) is about 100x more concentrated compared to competitor (1 unit/ul).
- Yield reached of secreted DNase I after 4 days fermentation is **>1g/L**



Dyadic DNase I Quality Comparable to Reference Standard

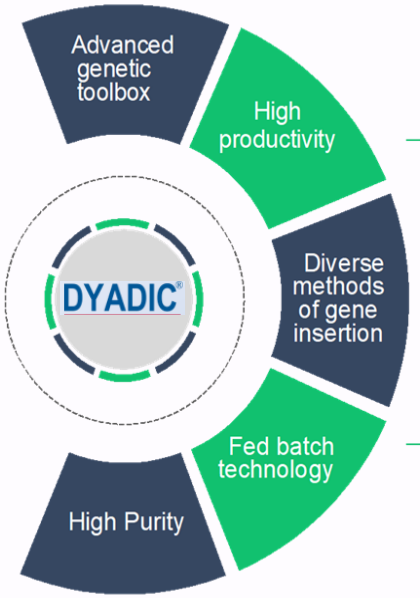


Detection kit: Jena Bioscience GmbH, DNase detection kit, Catalog number: PP-410S
Kinetic measurement for 50 min, at 37 °C, with SpectraMax i3X (495 nm / 520 nm)

- The DNase I activity/protein is comparable to reference standard.
- One unit of DNase I completely degrades 1 µg of plasmid DNA in 10 minutes at 37 °C
- One DNase I unit is equivalent to 0.3 Kunitz unit:
  - Currently used Dyadic DNase I has about 30 Kunitz/ul
  - Very efficient DNA degradation

Dyadic Platforms: Rapid, Affordable, Scalable Production of Quality Proteins

Dyadic Platform Features…



Precise and effective genetic engineering methods
Rapid methods for Knock-in and knock-out, MTP growth for screening. Marker recycling

Robust production platform for various proteins such as antigen-based vaccines, mAbs, Fabs, Fc-fusions, Bi- and Tri-specifics and enzymes

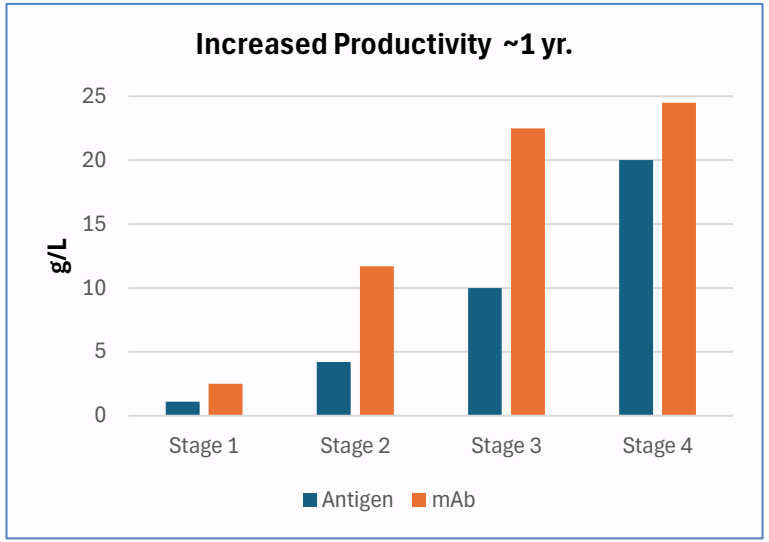
Single and multicopy integrations
Site directed integration by HR and CRISPR
Random integration by Non-HR

Define or complex media, low viscosity, tailored fermentation conditions using a wide range of Tamp. and pH

Proteins are secreted into the medium. No need for Virus clearance

Dyadic Platform Benefits…

- **Clean & Sustainable**
  No viruses or endotoxin; low carbon footprint
- **Quality & Scale**
  Phase I data w/cGMP material; production scales >100,000L
- **Rapid & Low Cost**
  Shorter processing times; high titers with inexpensive media



Protease production in C1 expression platform (53 of >100 identified)

1. Serine Proteases:
- Diagnostics: Used in clotting disorders (e.g., assays for thrombin and plasmin activity in coagulation).
- Research: Study of protein degradation and signaling pathways, including immune system responses

2. Cysteine Proteases:
- Diagnostics: Detection of diseases (e.g., cancer or inflammatory) for role in apoptosis and protein degradation.
- Research: Studying cell death mechanisms, antigen processing, and lysosomal functions.

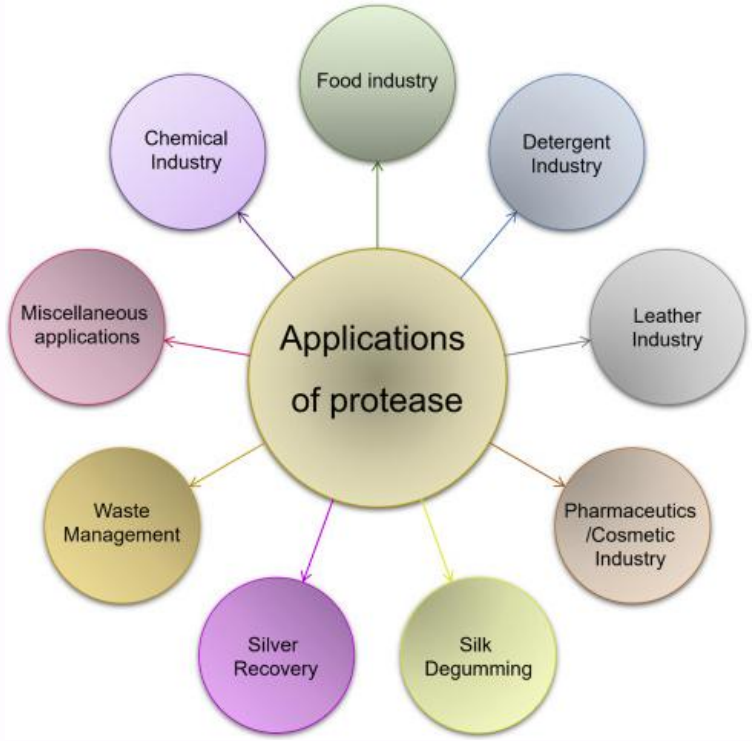
3. Aspartic Proteases:
- Diagnostics: Biomarker detection for neurodegenerative diseases (e.g., β-secretase in ALZ).
- Research: Investigating protein cleavage involved in viral replication and lysosomal degradation.

4. Glutamic Proteases:
- Diagnostics: Less common but used in specific enzymatic assays for fungal infections.
- Research: Exploring fungal biology and enzyme engineering for industrial applications.

5. Metalloproteases:
- Diagnostics: Key markers for cancer metastasis (e.g., matrix metalloproteinases).
- Research: Study of extracellular matrix remodeling, cancer progression, and angiogenesis.

6. Asparagine Peptide Lyases:
- Diagnostics: Emerging use in protein sequencing and diagnostics due to their specificity.
- Research: Protein structure analysis, particularly in mass spectrometry and proteomics.

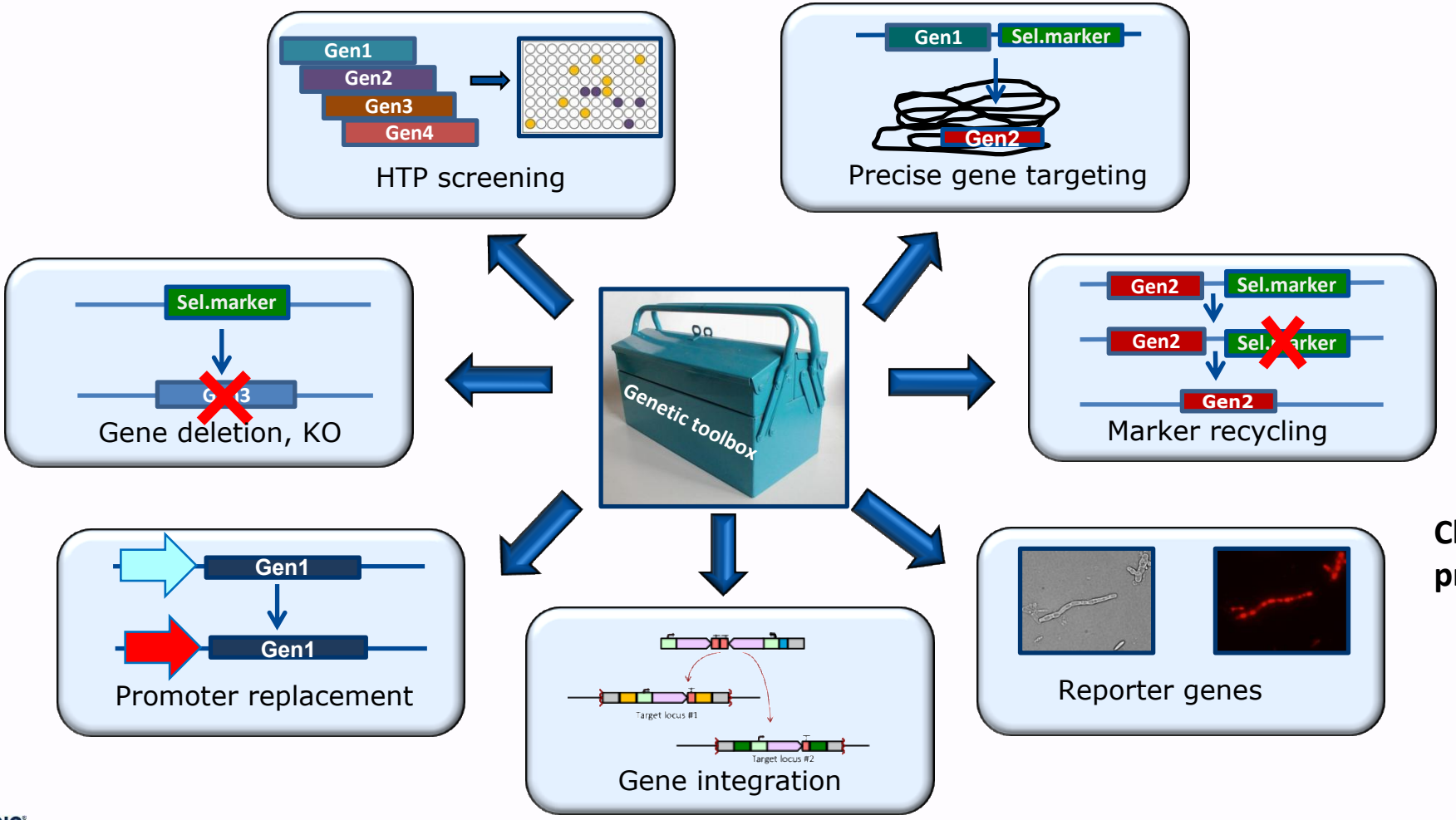
Protease Applications



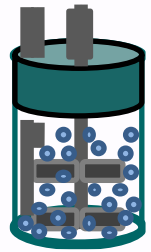
Spiking library for protease screening for C1/Dapibus

Genetic toolbox for rapid, stable, high yield C1 strain development

The advanced genetic toolkit for synthetic biology



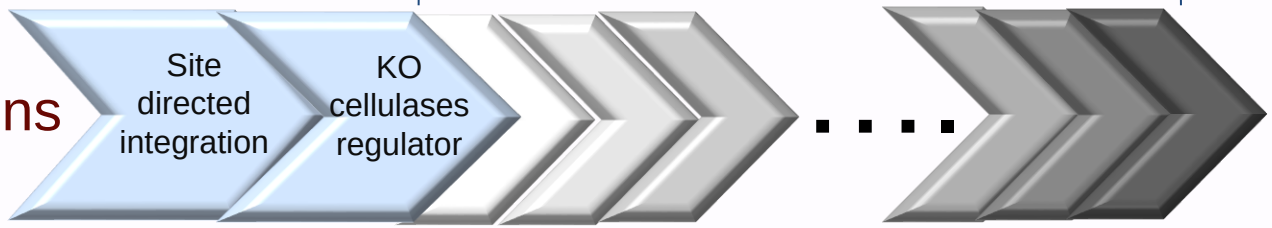
Change in morphology led to hyper-productivity and low viscosity.



Systematic deletion of active proteases in C1 host to improve stability

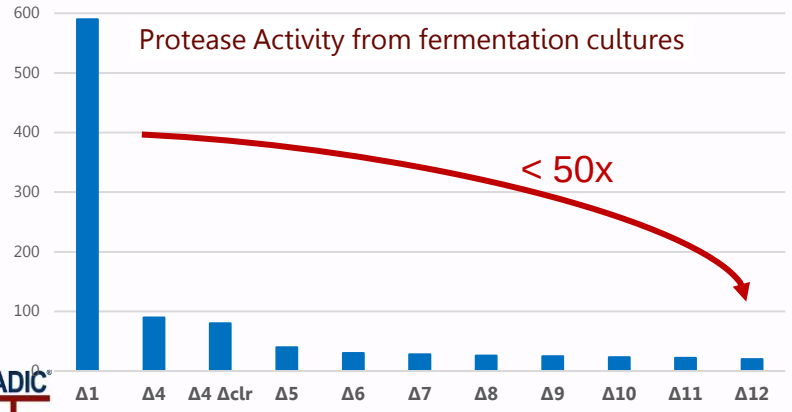
KO proteases and main secreted proteins (15 genes knocked out of C1 cell)

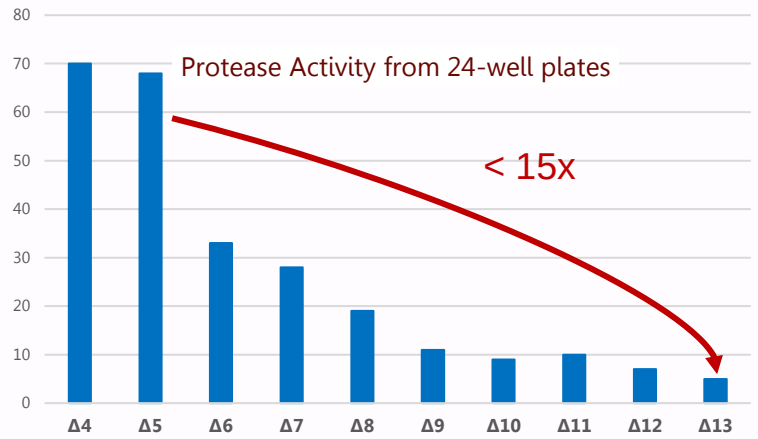
Pharma C1 strains



Systematic deletion of proteases genes based on:

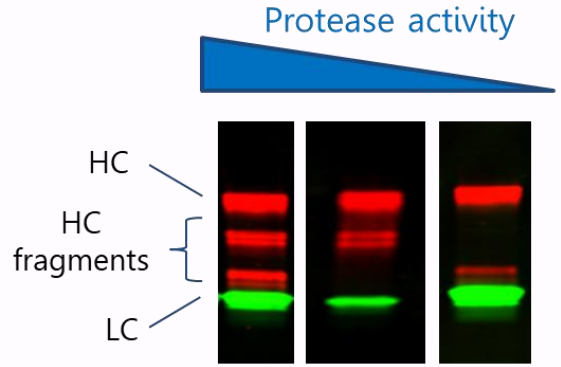
- Isolation and identification of extracellular proteases
- C1 protease library in Pichia pastoris that allows to test more than 50 proteases
- Effect of different protease inhibitors on protease activity
- mRNA sequencing data
- Protease gene annotation





*Direct fluoresence-based assay with casein substrate

mAbs stability is increased with proteases deletion

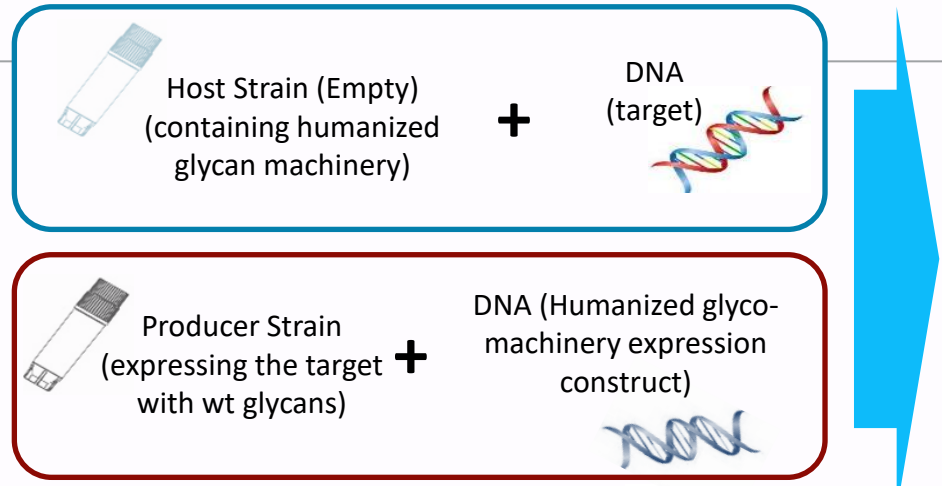


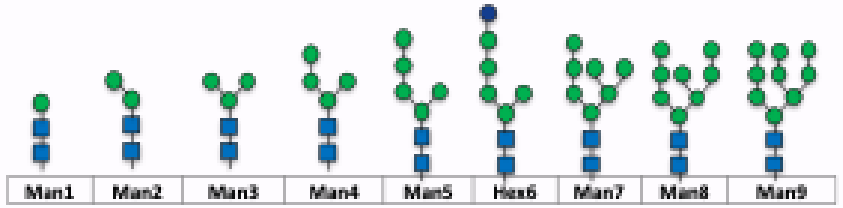
Glyco Pattern

- C1 Wild type glyco pattern targets are man3 to man9.
- Our aim has been to humanize N-glycosylation pathway and enable its use as production host for pharmaceutical proteins that require humanized glycans

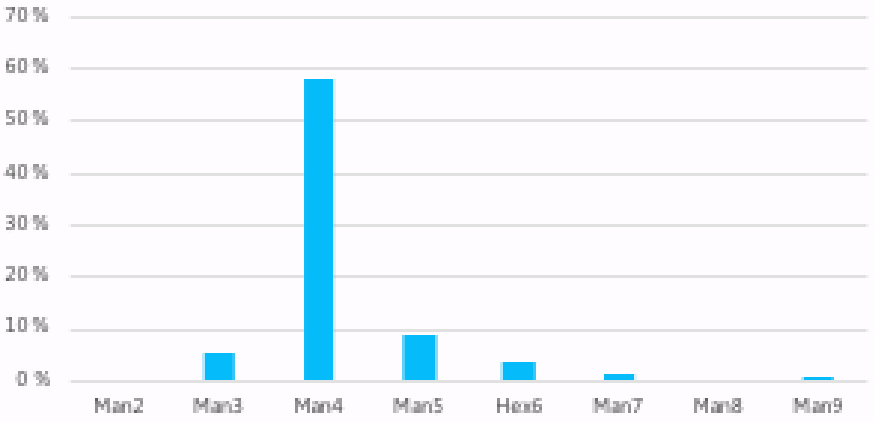
Two strategies have been sucessfully developed:

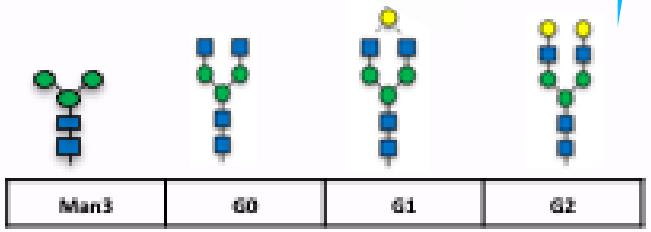
1. **Empty host strains** with humanized glyco-machinery
2. **Genetic construct** to convert a wild type glyco strain in humanized glyco strain by just one round transformation step



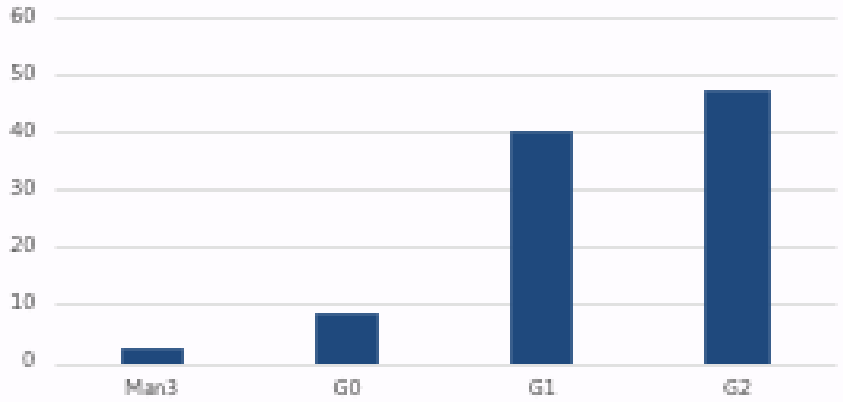


wt glyco pattern (mAb)





Humanized glyco pattern (mAb)

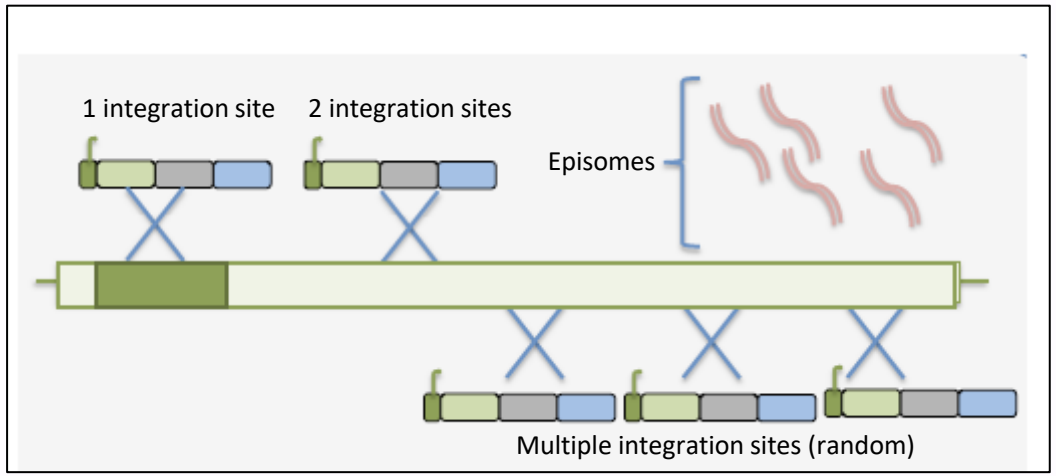


98.6% humanized glycans
92.7% Occupancy

C1 Expression Technology

Efficient transformation methods can be applied:

- •Single site directed integration
  - By standard integration: 1 or 2 genes into the same site. Two integration sites can be used
  - By Cas9 technology: site directed integration at multiple sites
- •Random integration: integration at multiple sites
- •Episomal vectors



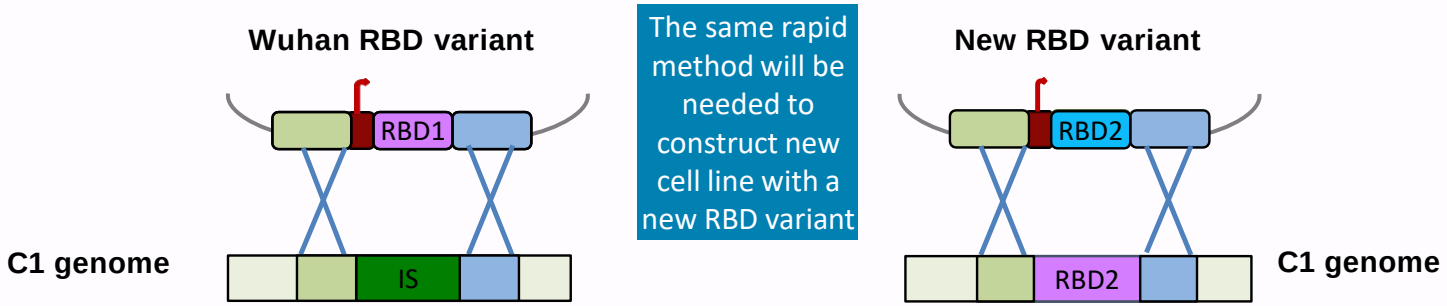
- •Transformation procedure based on chemical (PEG) method with protoplasts or electroporation
- •Frequencies for 1µg DNA:
  - **20 transformants for site specific integration**
  - **Up to 100 transformants for random integration**
  - **~13,000 transformants for telomeric vector transformation**

Productive stable cell lines of antigen variants are rapidly developed

C1 Site Directed Transformation Method leads to quick generation of stable C1 cell lines

No change in the cell line genotypes expressing the new RBD variants

Rapid Response
High Doses
Scalability
Affordability



- Set of strong promoters native and synthetic
- No need for induction
- Stable single-copy integration
- No need for transient stage

Vaccine time-line development

	1-2 weeks	1 week	3 weeks	1 week	2 weeks
Standard	Gene synthesis	Plasmid construction	Strain construction and re-isolation for monoclonality	MTP ferm., DSP and analytics & RCB generation	1-30 l scale fermentation from RCB, DSP and analytics
	1 week	**2 weeks**	**1 week**	**1 weeks**	
Emergency, Pandemic	Gene synthesis and Plasmid construction	Strain construction	Screening in Fermentors	Analytical and MCB/WCB generation	

- Rapid Development Timelines
- High Productivity – large quantities
- Purity
- Stability
- Robust Manufacturing Process
- Flexible Commercial Scales
- Low Cost

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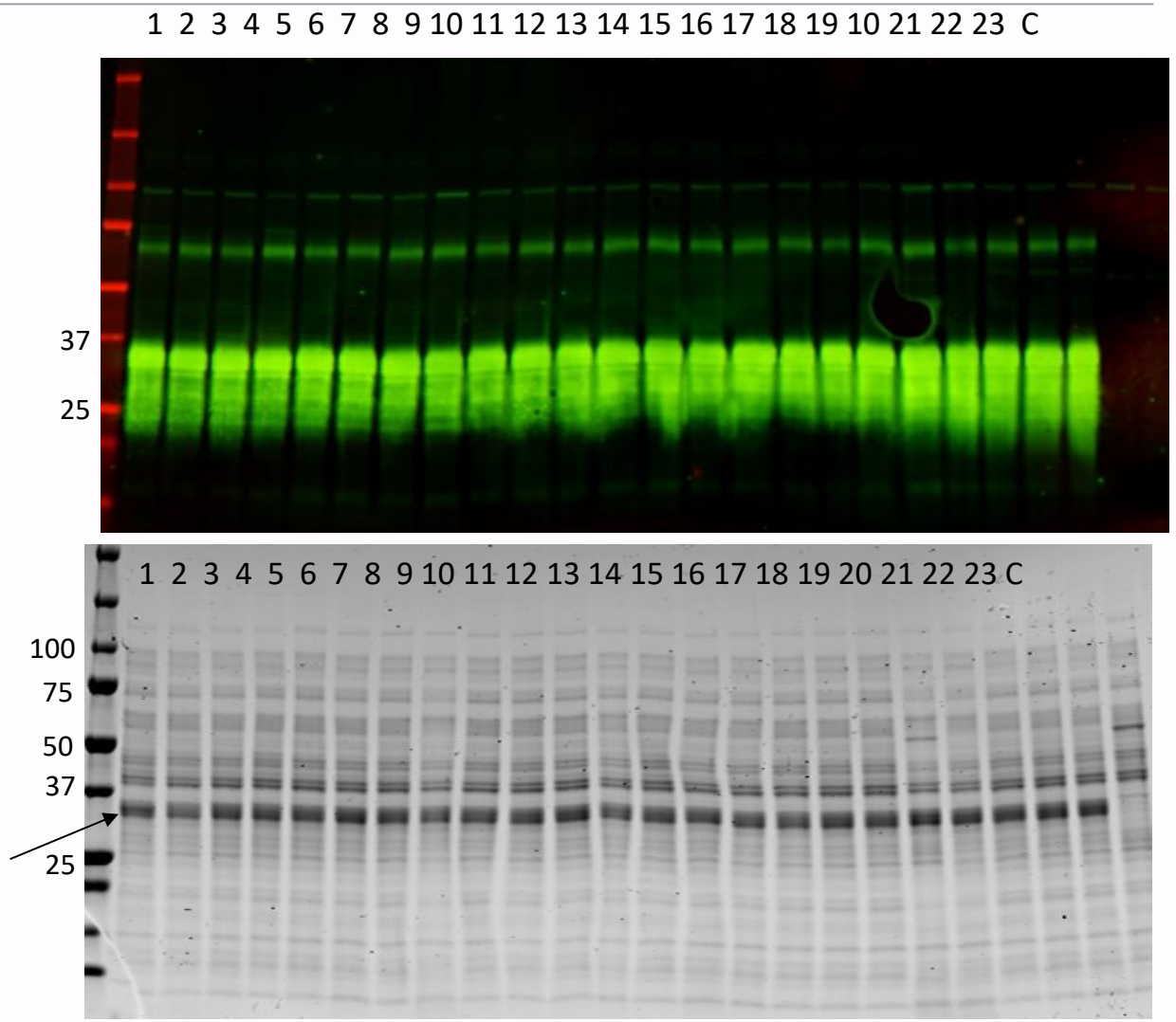
Rapid Stable Antigen C1-Cell Line Development - 21 Days

- C1 was transformed with an expression construct of the Mpox antigen-ferritin fusion protein
  - **Mpox C1 Ferritin Nanoparticle Antigen**
  - **Stable Cell Line Developed & Antigen Expressed in ~ 21 Days**
  - **4.5 Grams Per Liter Productivity in 7-day fermentation**
- Transformants were grown in 24-well plate cultures and the supernatants were analyzed with Western blotting with C-tag detection and with stained SDS gels
- A strong signal of expected size (32 kDa) is detected in Western
- An abundant band with correct size is detected in stained gel
- No background is coming from the negative control (C)
- Fermentation
- Antigen purification



C1 Fed-batch Fermentation – Rapid, Robust, Affordable, Scalable & No Bioburden

C1 FERMENTATION

CHEMICALLY DEFINED MEDIUM

- Low-cost medium
- Fed-batch technology w/ glucose feeding
- Wide range of operating temp and conditions
- Low viscosity culture
- ~4 - 7 day process

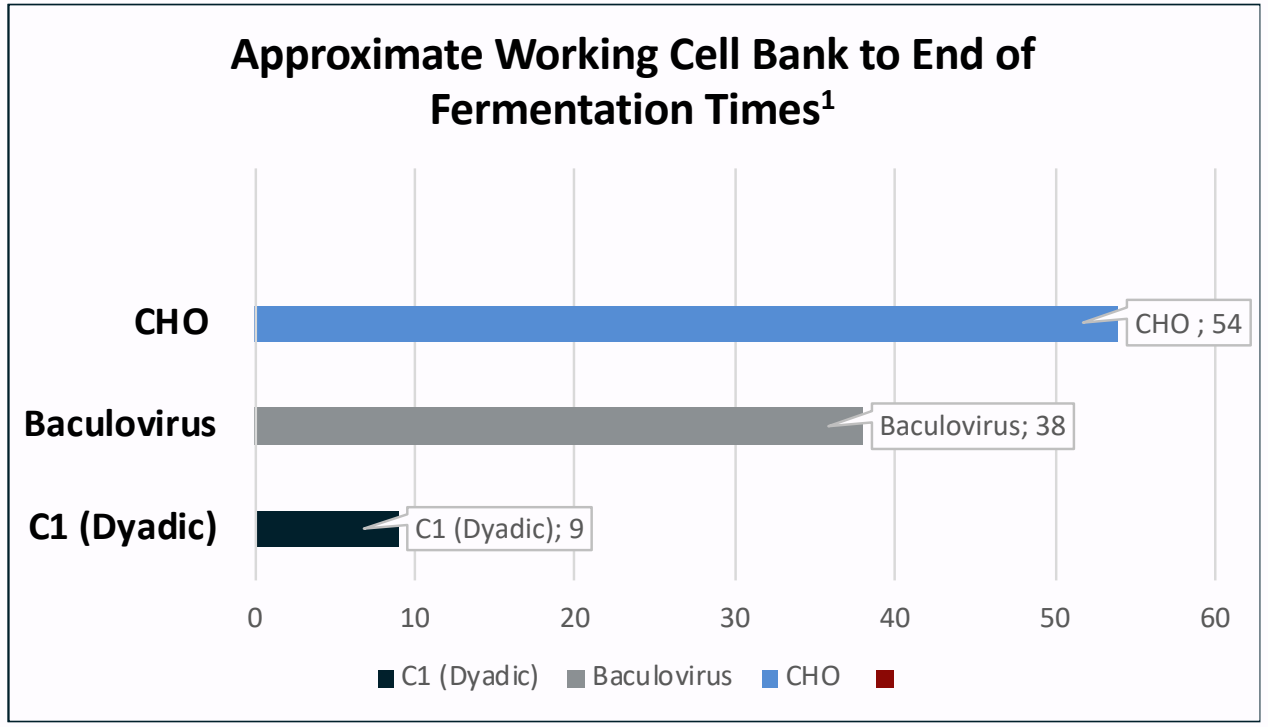
SEPARATION, PURIFICATION AND CONCENTRATION

- 1L to 500,000L fermentation scale
- Protein production requires no inducer
- Protein is (typically) secreted to the media
- Stable cells & protein in ~7 weeks
- No viruses or endotoxin
- Low carbon footprint

UPSTREAM PROCESSING | **FERMENTATION BIOPROCESS** | **DOWNSTREAM PROCESSING**

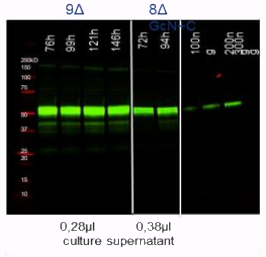
Working Cell Bank Advantage: EOF Time & Yield Favors C1 vs. Insect & CHO Cells

Less Time, No Viral Clearing Needed



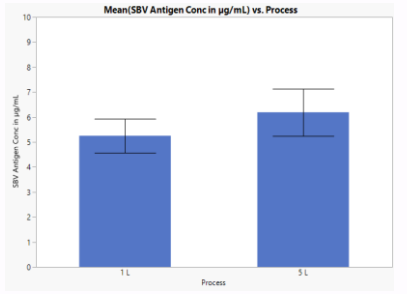
C1 Higher Productivity

C1 Fermentation



X300

Baculovirus Fermentation



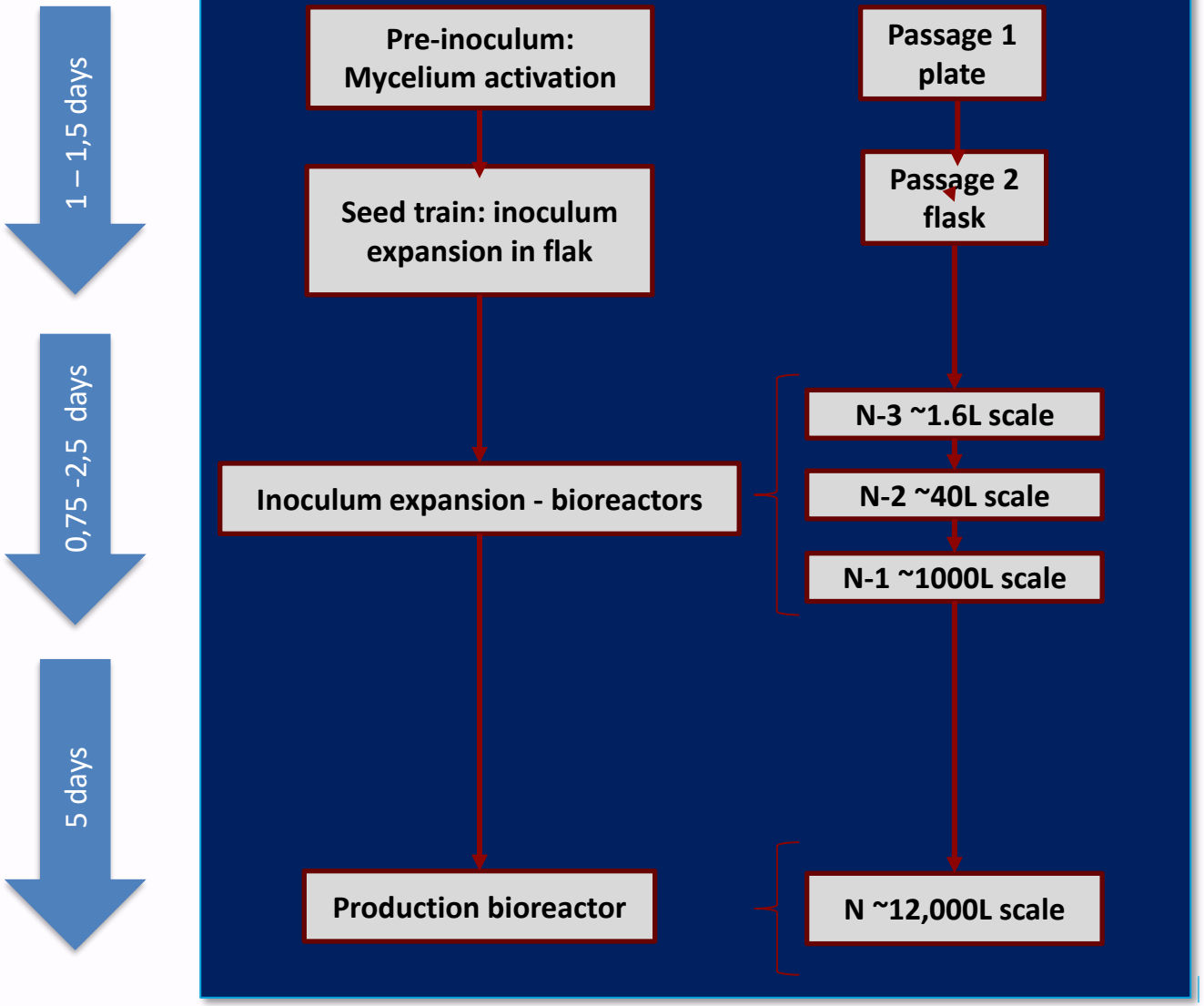
SBV yields: 1800 mg/L (time point 121h)

[1] Estimated Ranges: C1 7-9 days; Baculovirus 28-38 days; CHO 41-54 days

2 Baculovirus and C1 SBV antigens – Data from EU ZAPI Project

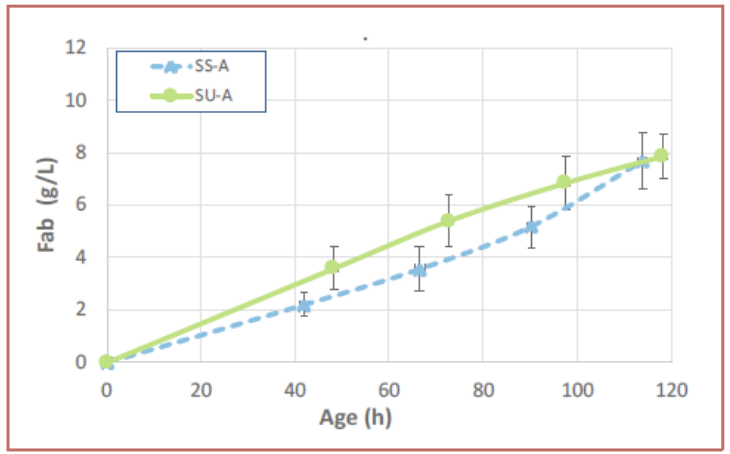
Generic process flow chart for C1 is 7 - 9 days

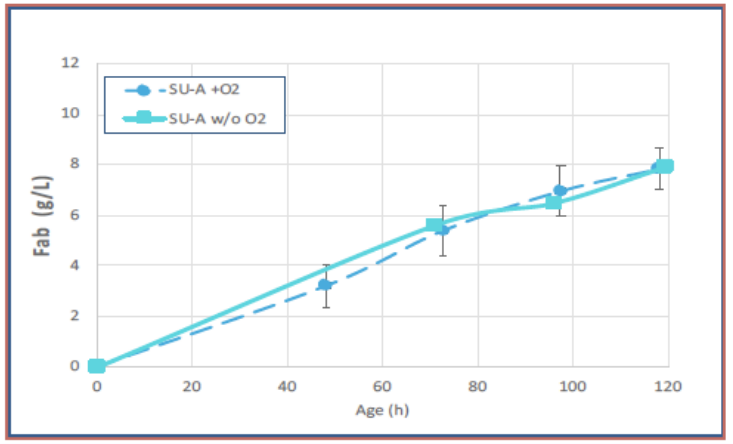
Generic Process Flow Chart for CHO is 41 – 54 days



Certolizumab production with C1 in Single use Bioreactor (SUB)

Conditions A

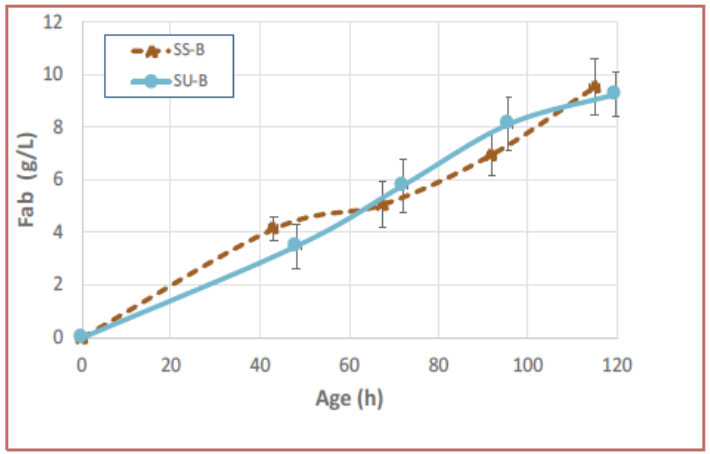


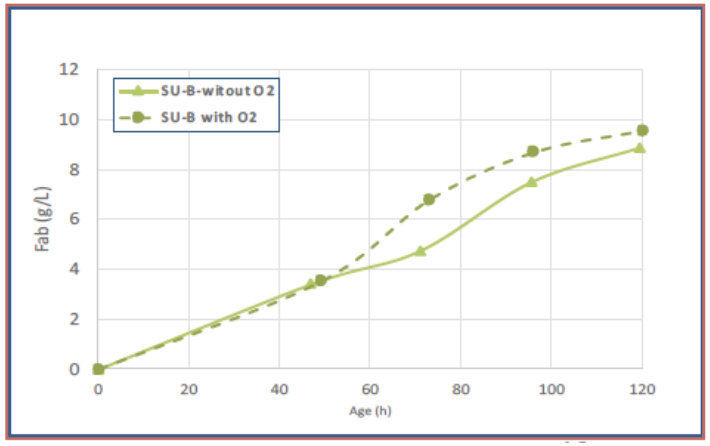


GE's Xcellerex™ XDR-50 MO

- Six batches were tested in 2 different conditions with or without O_2 supplementation.
- Conditions B have been shown to be more productive than A in both SSB and USB.
- Supplementation of O_2 slightly improve Certolizumab productivity

Conditions B



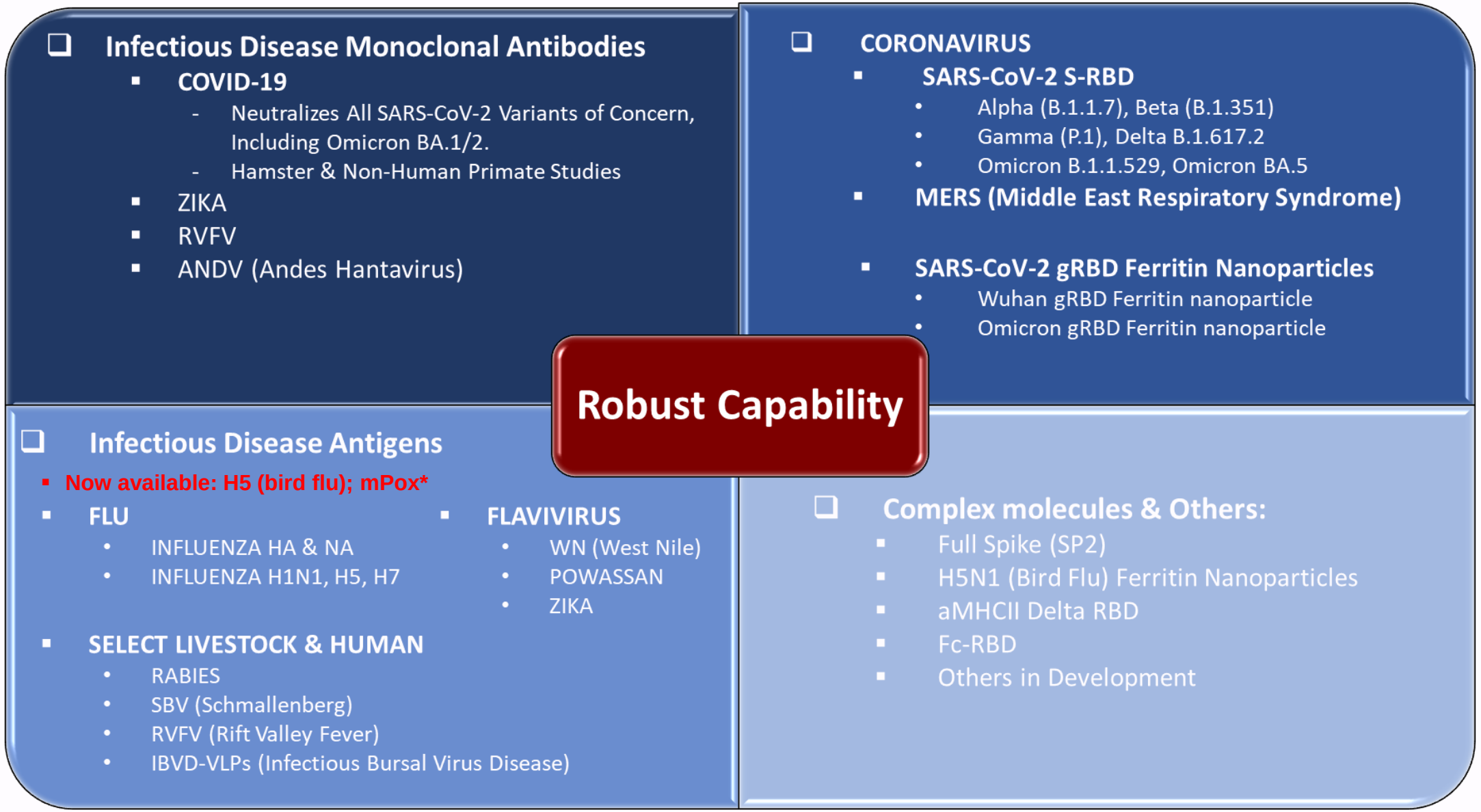


Cygnus developed the C1 HCP ELISA Kit, available to Dyadic and Cygnus customers

- Cygnus - Industry Trusted For Purification, QC & Lot Testing. Long-Term Supply Of Kits w/ Same Ab.
- C1 HCP ELISA Assay to Enhance Quality Assurance in Biomanufacturing
  - C1 HCP ELISA Kit, Item # F1025 is commercially available from Cygnus
- Solution for testing C1 HCP impurity during product lot release testing, thereby enhancing the quality assurance process in biomanufacturing.
- Collaboration with Cygnus resulted in the development of a first-in-class C1 HCP ELISA assay, making a significant step toward greater adoption of the C1 technology globally."

"We are very excited about the launch of the highly anticipated C1 HCP ELISA Assay. The assay is expected to play an important role in facilitating the broad adoption of Dyadic's C1 protein production platform which enables rapid and efficient low-cost production of antigens, monoclonal antibodies, and other therapeutic proteins. We are proud to partner with Dyadic to help accelerate the adoption of their C1 platform, ultimately aiming to enhance access and affordability of healthcare for patients in developed and developing countries." Christine Dolan, Cygnus Technologies Chief Operating Officer.

Demonstrated consistent successful expression of broad array of potent antigens & mAbs from C1-Cells



Key Limitations of Existing Recombinant Protein Production Platforms

Cell Line Limitations	Baculovirus Cells	CHO Cells	C1 Cells
Viral clearance for DS release	✓	✓	✗
Lengthy and costly USP/DSP	✓	✓	✗
Long doubling time (>20 hours)	✓	✓	✗
Narrow temp and PH range for production	✓	✓	✗
Up to 3-4x longer production cycle times	✓	✓	✗
Potential limitation of molecule size (E.g. HIV nanoparticle too large for viral clearance)	✓	✓	✗
Lower antigen productivity (i.e. Limits rapid large-scale access $ higher costs)	✓	✓	✗

C1 Cell Line Advantages

- **Faster stable cell line development**
- **Shorter drug substance production**
- **Quicker Release of significantly more doses using smaller manufacturing footprint at lower cost**

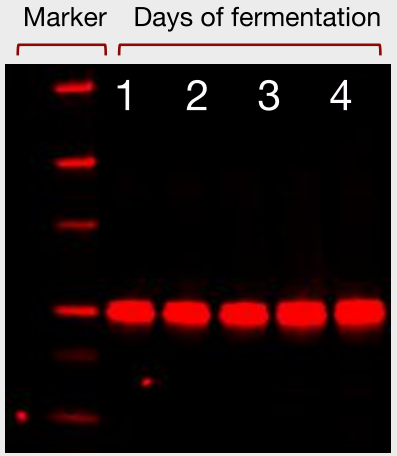
DYADIC® INSIDE®

C1, Faster, More Productive Lower Cost Antigens

Production of the RBD of Wuhan CoV-2 Variant

- **In ~2 months a C1 strain expressing the Receptor Binding Domain (23kDa) of SARS-CoV-2 spike protein**
- **Stable C1 production strain was developed that expressed the RBD originally at a level of ~ 1 g/L**
  - **Strain engineering and fermentation optimization elevated the production level to +/- 2 g/l in 4-5 days**
- **C1 fermentation is based on fed-batch technology with glucose feeding**

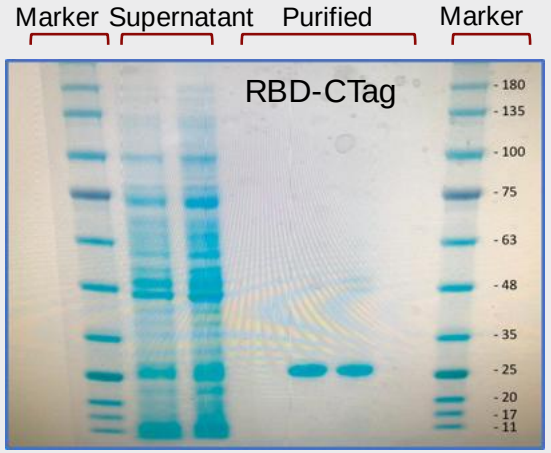
C1 cell line in Ambr250 fermenter system



C1 RBD strain was run in Ambr250 for 5 days

WB analysis off fermentation broth

C1 cell line in 1L fermenter system



C1 RBD strain was run in 5L scale fermentation

The RBD was purified twice with CaptureSelect™ C-tag 10ml column.

98% purity

70% recovery

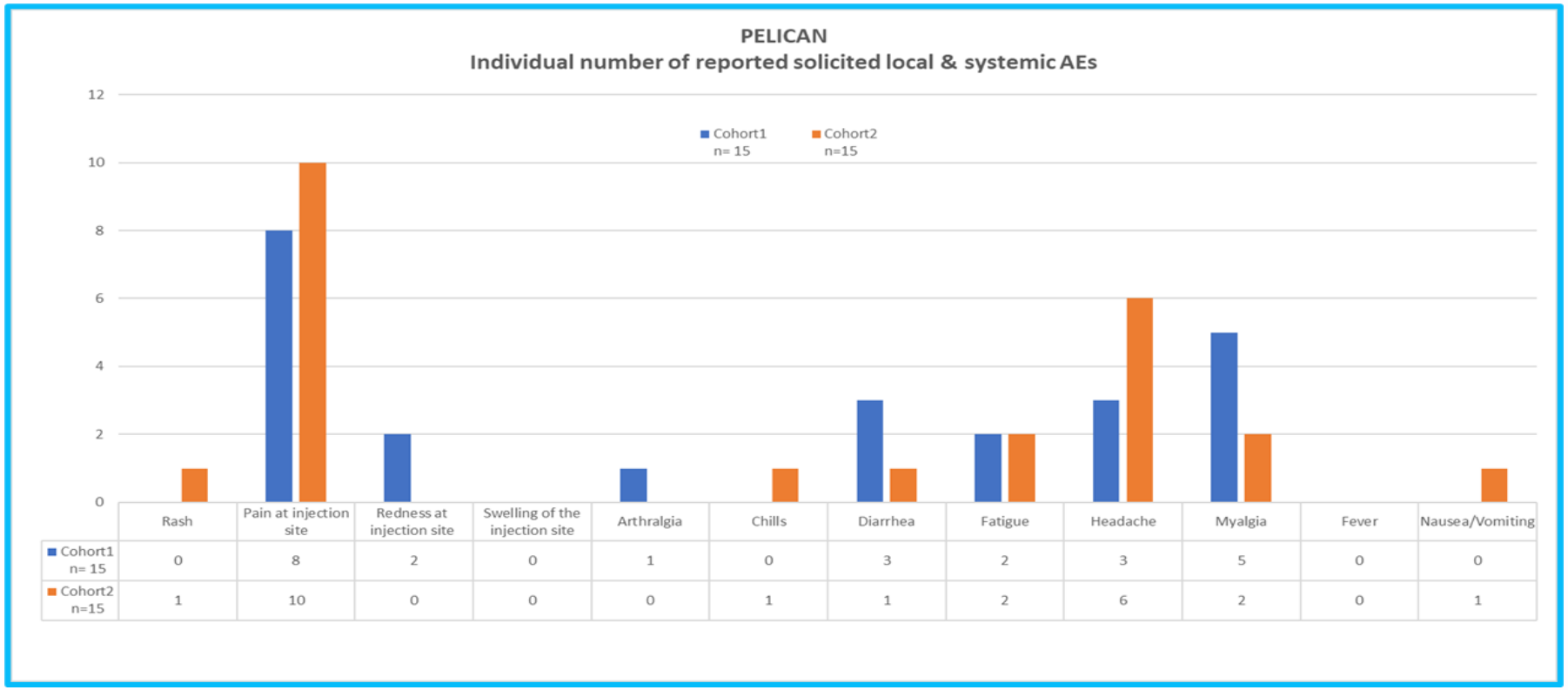
Successful DYAI-100 Phase 1 Results: Primary Endpoint Achieved

Phase 1 Study Results:

- ***Double blind placebo-controlled safety study of 30 healthy adults conducted in South Africa.***
- ***Primary endpoint of the study was to demonstrate the safety and reactogenicity of DYAI-100 as a single booster vaccine administered at two dose levels.***

Top-line safety data:

- ***Study met its primary endpoint demonstrating that both dose levels are safe and well tolerated.***
- ***DYAI-100 induced robust immune responses, including both humoral and cell-mediated responses, in vaccinated individuals.***

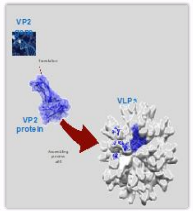


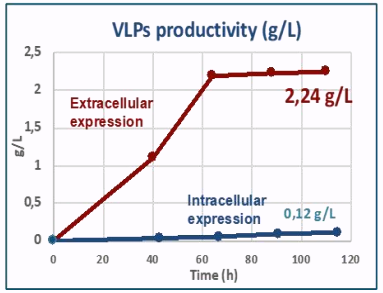
	Rash	Pain at injection site	Redness at injection site	Swelling of the injection site	Arthralgia	Chills	Diarrhea	Fatigue	Headache	Myalgia	Fever	Nausea/Vomiting
Cohort1 n= 15	0	8	2	0	1	0	3	2	3	5	0	0
Cohort2 n=15	1	10	0	0	0	1	1	2	6	2	0	1

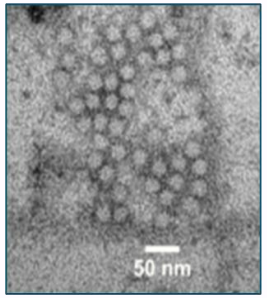
C1- Rapid Production of Complex NextGen Antigens in Larger Quantities

C1 VLP's

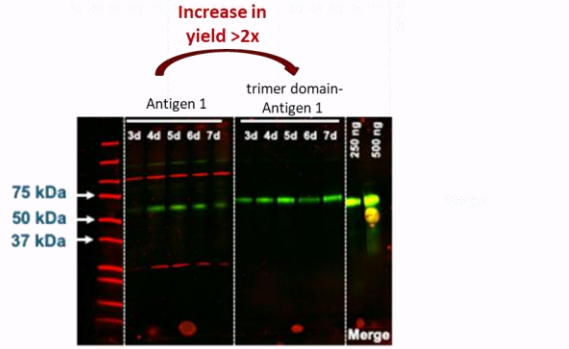
- C1 expressed secreted VLPs from Gumboro at **2.24 g/L** in the extracellular fraction (Infectious bursal disease)
  - VLP size: 3000 kDa / 25 nm diameter







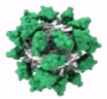
C1 Trimers

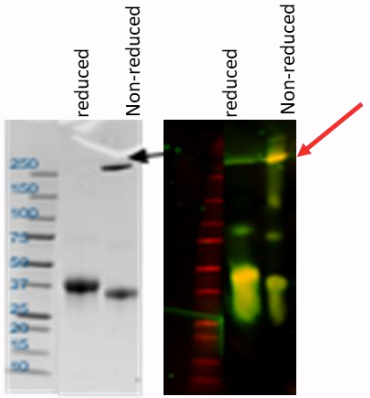


- Yield is increased by fusion of trimerization domain
- Animal studies to analyze immuno-response are ongoing

C1 Ferritin Nanoparticles *(One-Step Production)*

- All F10-RBD variants shows high molecular weight bands under non-reduced conditions indicating putative oligomerization forms

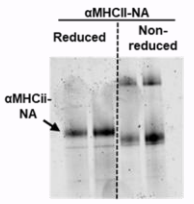


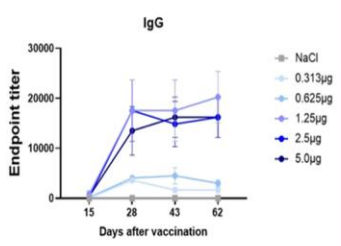


Target	Titer (5 days)*
F10-Wuhan-gRBD	3.48 g/L
F10-Omicron BA2-gRBD	0.7 g/L
F10- H5-RG71A/RG8A	2.0 g/L

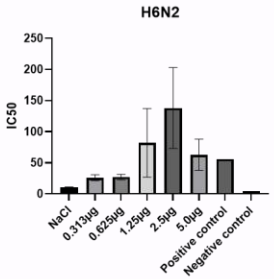
*Bioreactor cultivations (prior to optimization)

C1 aMHCII Targeting



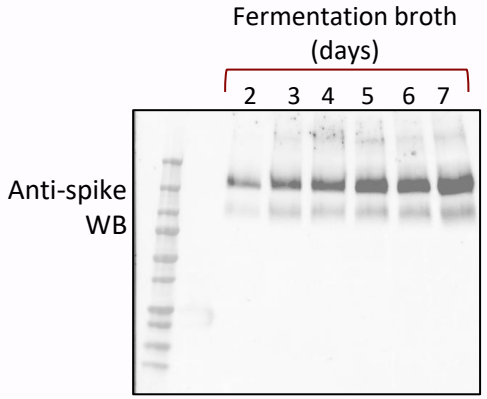


- Production of MHCII-targeted HA NA in C1 is highly efficient



SARS-CoV-2 Spike protein produced at high yield by C1-cells

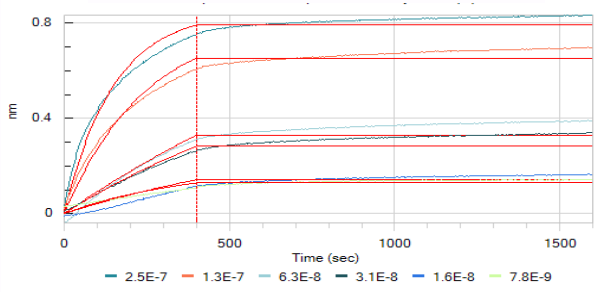
Production of SARS-CoV-2 Spike protein by C1



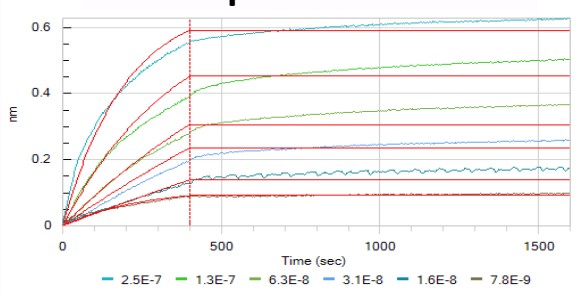
- Spike protein is properly expressed and secreted in C1 platform
- Yield: **1g/L**
- No clipping or degradation is observed
- The minor band detected by WB is also observed in the spike reference produced by CHO cells

Binding behavior of spike protein of the SARS-CoV-2 by Bio-layer interferometry (BLI)

Full Spike from C1

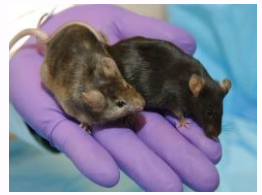


Full Spike from CHO

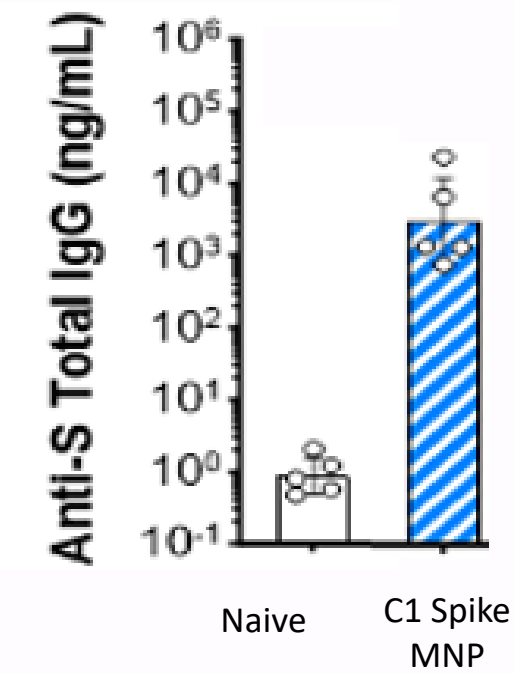


The binding curves of C1 Spike and BEI Reference CHO spike share similar features with KD of 1.2 mM

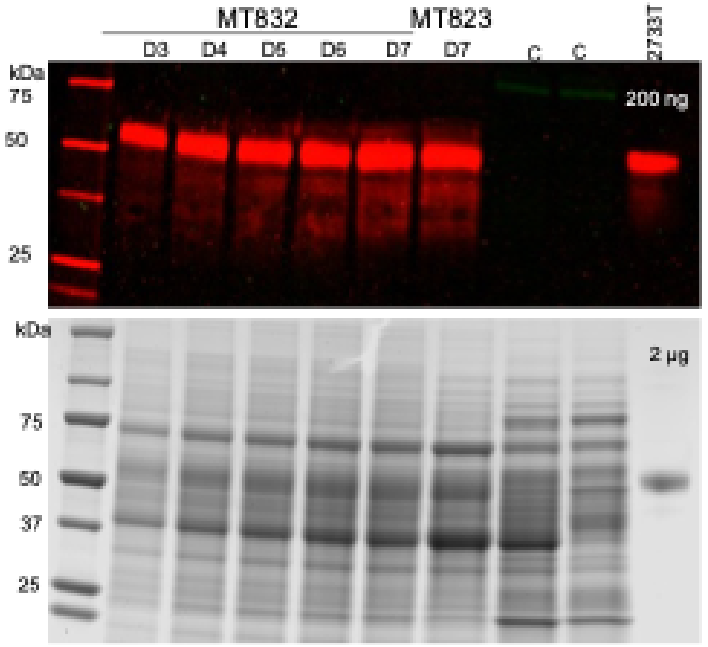
Ag-specific total IgG levels two weeks after single immunization with Full-Spike protein from C1



SARS-CoV-2 Spike protein from C1 is **very immunogenic**



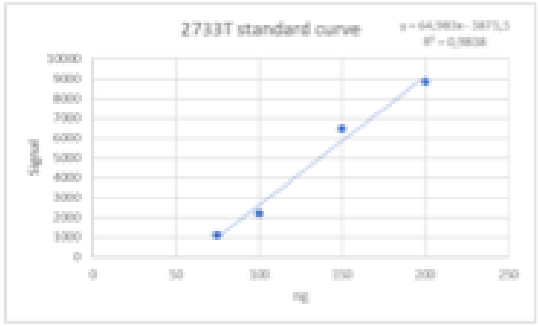
Successful High Yield Expression of RSV Trimers in C1



Sample loading: 0.5 µl s/n in WB, and 1.0 µl s/n stained

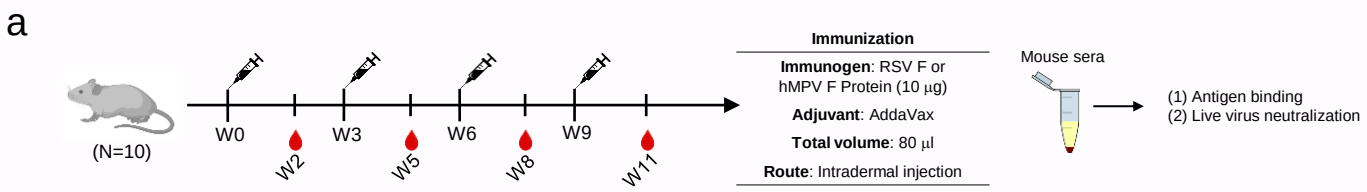
RSV (red): 1st ab: D25 antibody, 1:2000, 2nd ab: Anti-human IgG (H+L, Rockland) DyLight680, 1:10000

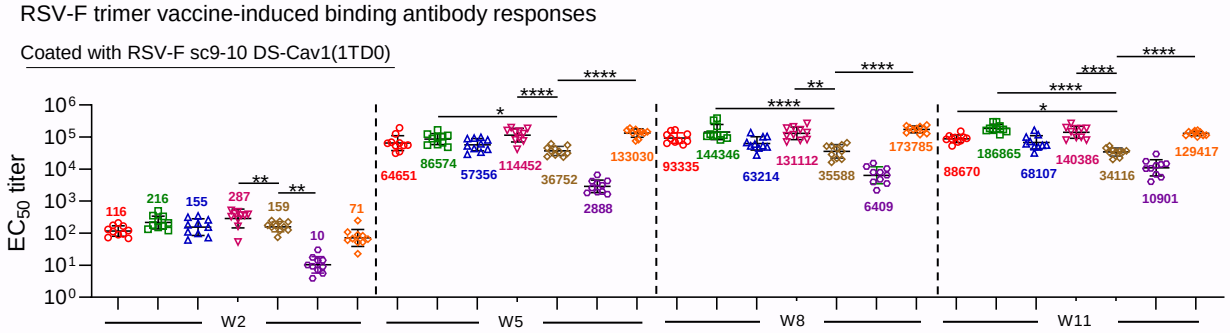
- Uvaxbio RSV strain in DNL173 (14xsrp10 + weak kex2, pyr4-)
  - MT832: clone 59-4, variant 1 (2733T)
- Fermentations: 1-litre fed-batch: 30 g/L YE batch, 15 g/L YE feed, pH 6.8, +32°C, 7 days
- Analysis of fermentation supernatants by Western blotting and stained gel
  - Production level appears to be similar with a sample from first fermentation (MT823)
  - Possible protease activity detected from samples of both fermentations
- Quantification from the 7 day samples of both fermentations (MT823 and MT832)
  - Six dilutions of the control protein (25 ng, 50 ng, 75 ng, 100 ng, 150 ng and 200 ng)
  - Five dilutions of the supernatant samples (1:60, 1:80, 1:100, 1:150 and 1:200)
  - **Yield is ~ 1.7 g/l for both MT823 and MT832 7 day time point**

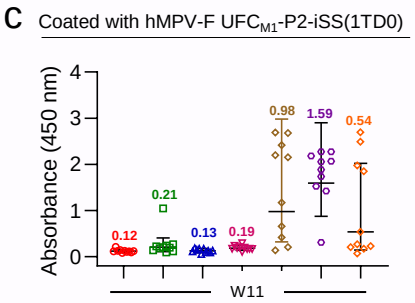


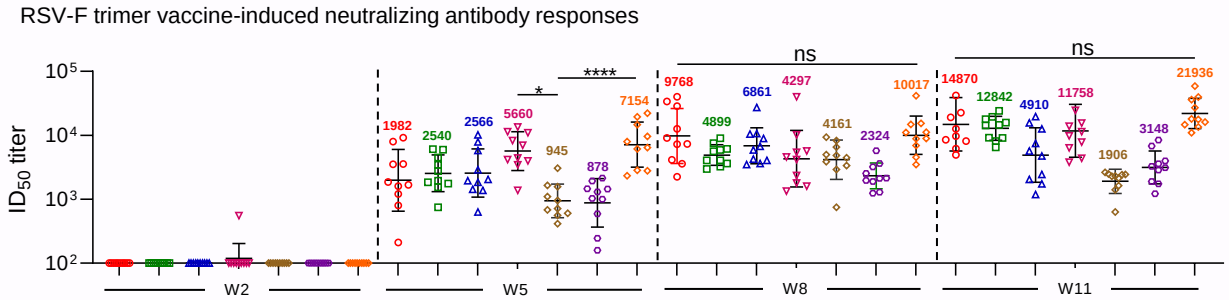
In vivo evaluation of RSV-F (and hMPV-F) constructs in mice

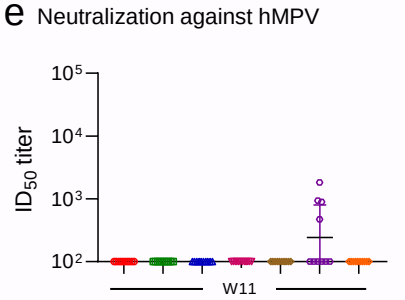
Open trimers vs. prefusion/postfusion mixed trimers vs. uncleaved, prefusion-closed (UFC) trimers

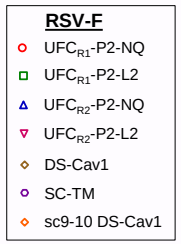


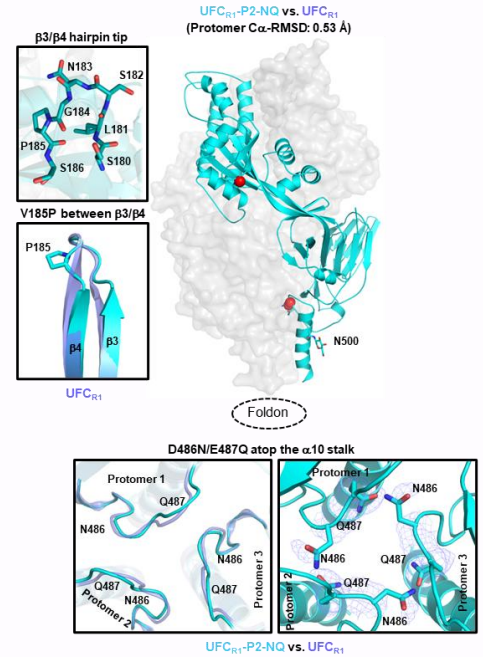












Lee, Y. Z., Han, J., Zhang, Y. N., Ward, G., Braz Gomes, K., Auclair, S., ... & Zhu, J. (2024). **Rational design of uncleaved prefusion-closed trimer vaccines for human respiratory syncytial virus and metapneumovirus**. *Nature Communications*, *15*(1), 9939.

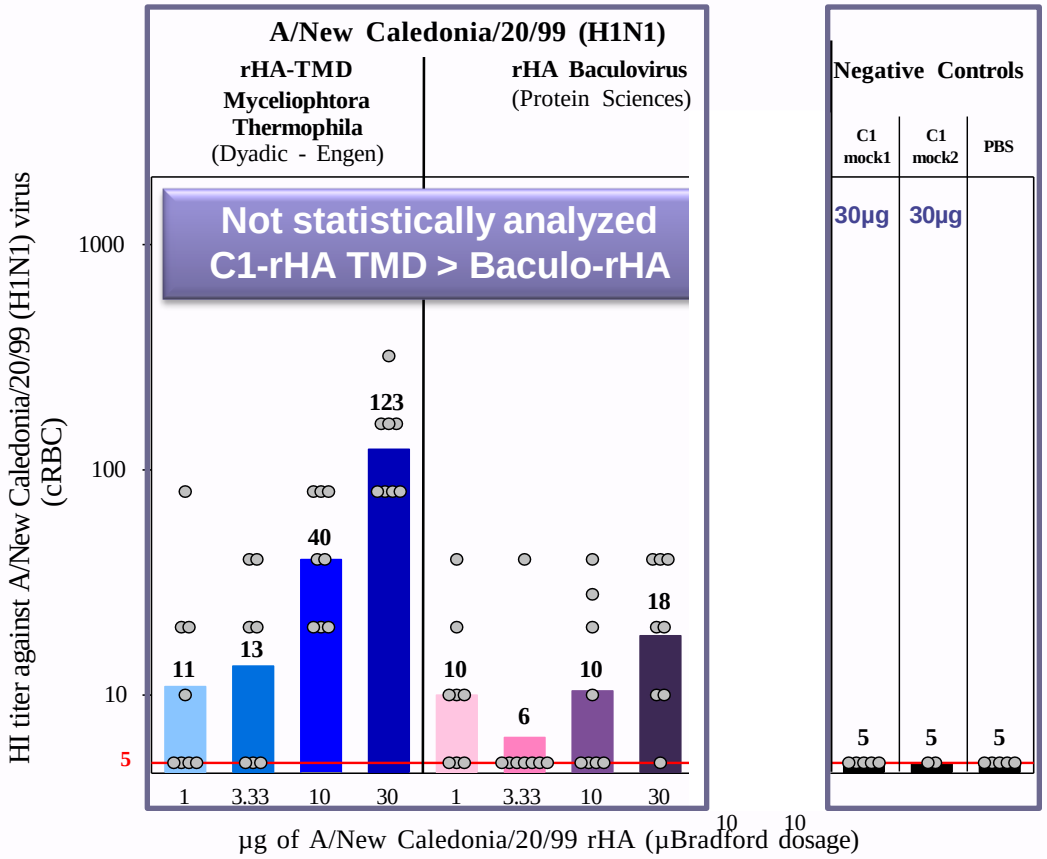
Conclusions:

1. Overall, DS-Cav1 (GSK) and SC-TM (J&J) are the least effective RSV-F trimer vaccines, sc9-10 DS-Cav1 (NIH) is the best performer with the highest ID_{50} titers across time points, our UFC_{R1}-P2-NQ ranks the second best and is close to sc9-10 DS-Cav1 at week 8, and our UFC_{R2}-P2-NQ-iSS is equivalent to sc9-10 DS-Cav1.
2. None of the tested RSV-F trimers can induce cross-NAb responses against live hMPV.

uvaxbio

Sanofi Immunogenicity evaluation of C1 rHA-TMD

Results: A/NC/20/99 HI specific response post-1 IM (D27)



HI response after **1 IM** immunization in mice:

Statistical analysis was not possible due to too much non responders

Although:

- **Clear evidence of the superiority of C1-rHA TMD over baculo-rHA (number of responders and mean titers)**

Percent of responders

Expression system	Dose of rHA 1µg	3.33µg	10µg	30µg
C1	50% (*4/8*)	57.1% (*4/7*)	**100%** (*8/8*)	**100%** (*8/8*)
Baculo	62.5% (*5/8*)	12% (*1/8*)	50% (*4/8*)	75% (*6/8*)

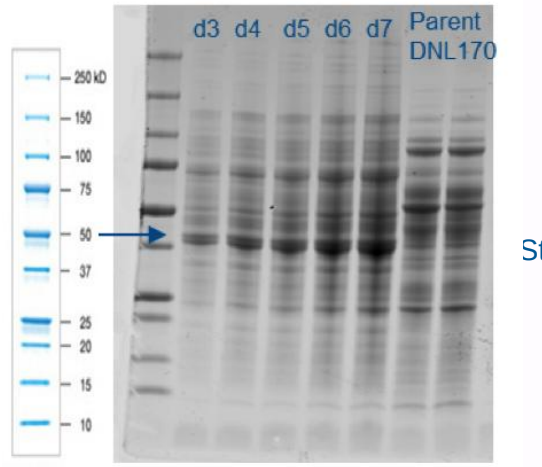
DYADIC®

Production of Cross Protective, Adjuvanted, Self-assembling Nanoparticle H5-2.3.4.4b A/Astrakhan Subunit Vaccine Candidate

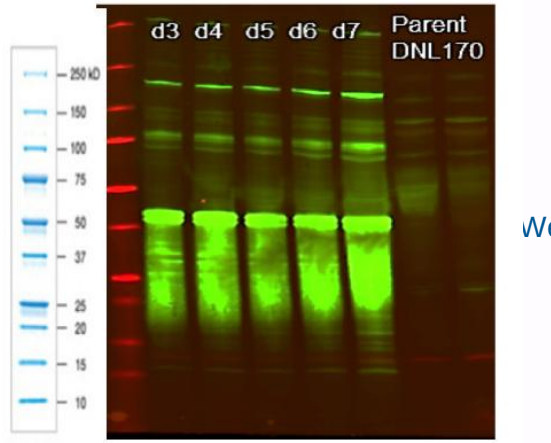
MASS PRODUCIBLE H5-2.3.4.4b FERRITIN NANOPARTICLE ANTIGEN

Production of H5 - Clade 2.3.4.4b A/Astrakhan/3213/2020 Ferritin Nanoparticle Antigen

Fermentation results



Stained

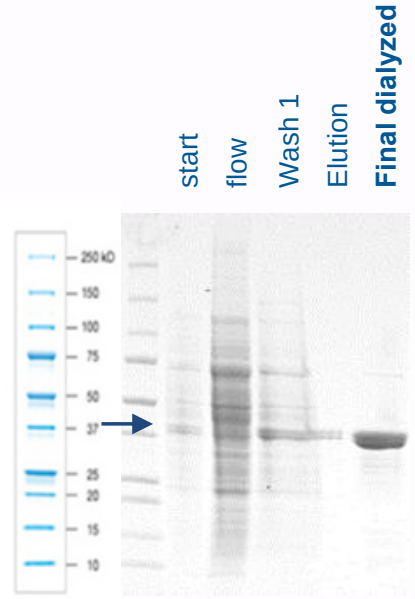


Western blot

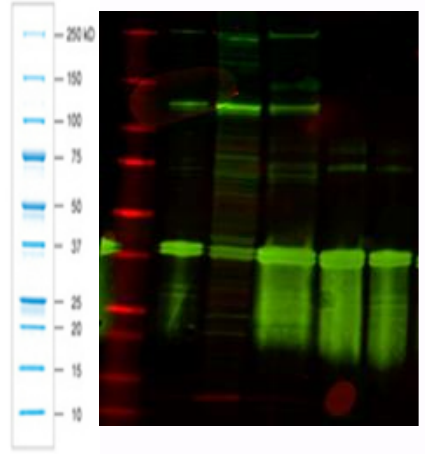
C1 Produced H5 - Clade 2.3.4.4b A/Astrakhan/3213/2020 Ferritin Nanoparticle

Time point	Concentration (g/L)
Day 6	1,57
Day 7	1,97

Larger scale protein purification

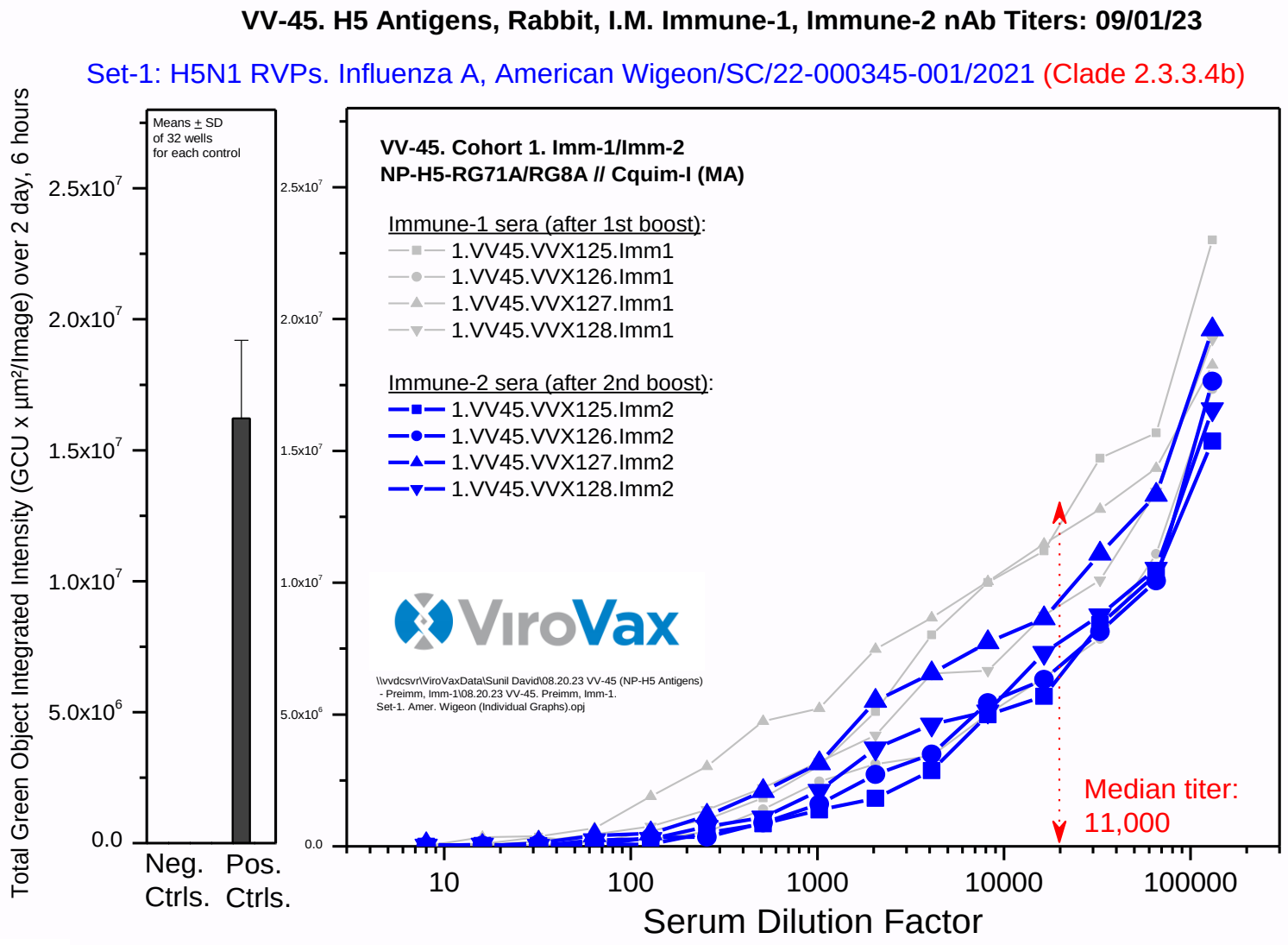


Stained



Western blot

Rabbit Intramuscular H5 Subunit Ferritin Nanoparticle Vaccine



- C1 produced H5 nanoparticles
  - **2 g/l in 7 days**
    - *** Prior to optimization**

mAb Titers: 1 & 2 Boosts

Rabbit Study:

1. Day 0: Pre-immune test bleed (Preimmune sera)
2. Day 0: Primary vaccination I.M., 0.2 mL/dose.
3. Day 15: Boost 1 vaccination. I.M., 0.2 mL/dose.
4. Day 25: Test-bleed-1 (“Immune-1” sera)

DYADIC INSIDE ViroVax

Confirmation of Neutralizing Antibody Titers: Hemagglutination-inhibition Assay

Two Independent Experiments: Recombinant H5 viruses, A/Vietnam/1203/2004 and A/Astrakhan/3213/2020 – Yoshihiro Kawaoka Lab

Virovax Raw Data -- De-randomized and Sorted by Rabbit ID and Cohort

U. Wisc. Ref & VV Randomized Index	A/Vietnam/1203/2004 HI Date: 10-19-2023	A/Vietnam/1203/2004 HI Date: 10-26-2023	A/Astrakhan/3212/2020 HI Date: 10-19-2023	A/Astrakhan/3212/2020 HI Date: 10-26-2023	U. Wisc Ref and Rabbit ID	VVX Cohort	Antigen(s) and Adjuvant used
F71/10**	<10	<10	<10	<10	Ref. 1		
F62/15		160		<10	Ref. 2		
T1240	<10	<10	160	80	Ref. 3		
158	<10	<10	160/320	320	VVX-125	Cohort 1	Cohort 1: NP-Astrakhan-2020 // Cquim-I
134	10/20*	10	640	320/640	VVX-126	Cohort 1	
179	<10	<10	80	80	VVX-127	Cohort 1	
171	<10	<10	160	160	VVX-128	Cohort 1	
140	80	80/160	<10	<10	VVX-129	Cohort 2	Cohort 2: NP-H5-VIET04 // Cquim-I
170	80	80	<10	<10	VVX-130	Cohort 2	
434	80	80	10/20	20	VVX-131	Cohort 2	
214	80	80	<10	<10	VVX-132	Cohort 2	
162	160	160	320	320/640	VVX-133	Cohort 3	Cohort 3: NP-Astrakhan-2020 + NP-VIET04 // Cquim-I
362	40	40	80	80	VVX-134	Cohort 3	
412	160	160	160/320	160/320	VVX-135	Cohort 3	
283	160	160	160	160	VVX-136	Cohort 3	
216	20	20	10/20	10/20	VVX-137	Cohort 4	Cohort 3: NP-Astrakhan-2020 + NP-VIET04 // Alhydroxiquim-II
184	<10	<10	<10	<10	VVX-138	Cohort 4	
201	20	20	40	20	VVX-139	Cohort 4	
503	20	20	10	10/20	VVX-140	Cohort 4	

*Please Note: The forward slash "/" symbol implies that the last well in that dilution series showed only partial inhibition and that the full inhibition endpoint titer appears to have occurred between the two dilutions.

**Regarding the F71/10 positive control sera: A small circular pellet formed in the first well (suggesting a minute amount of inhibition), but it did not flow so I would consider it <10. No pellet formed in the Astrakhan virus wells for this sera.

Black Fill = Not done.

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

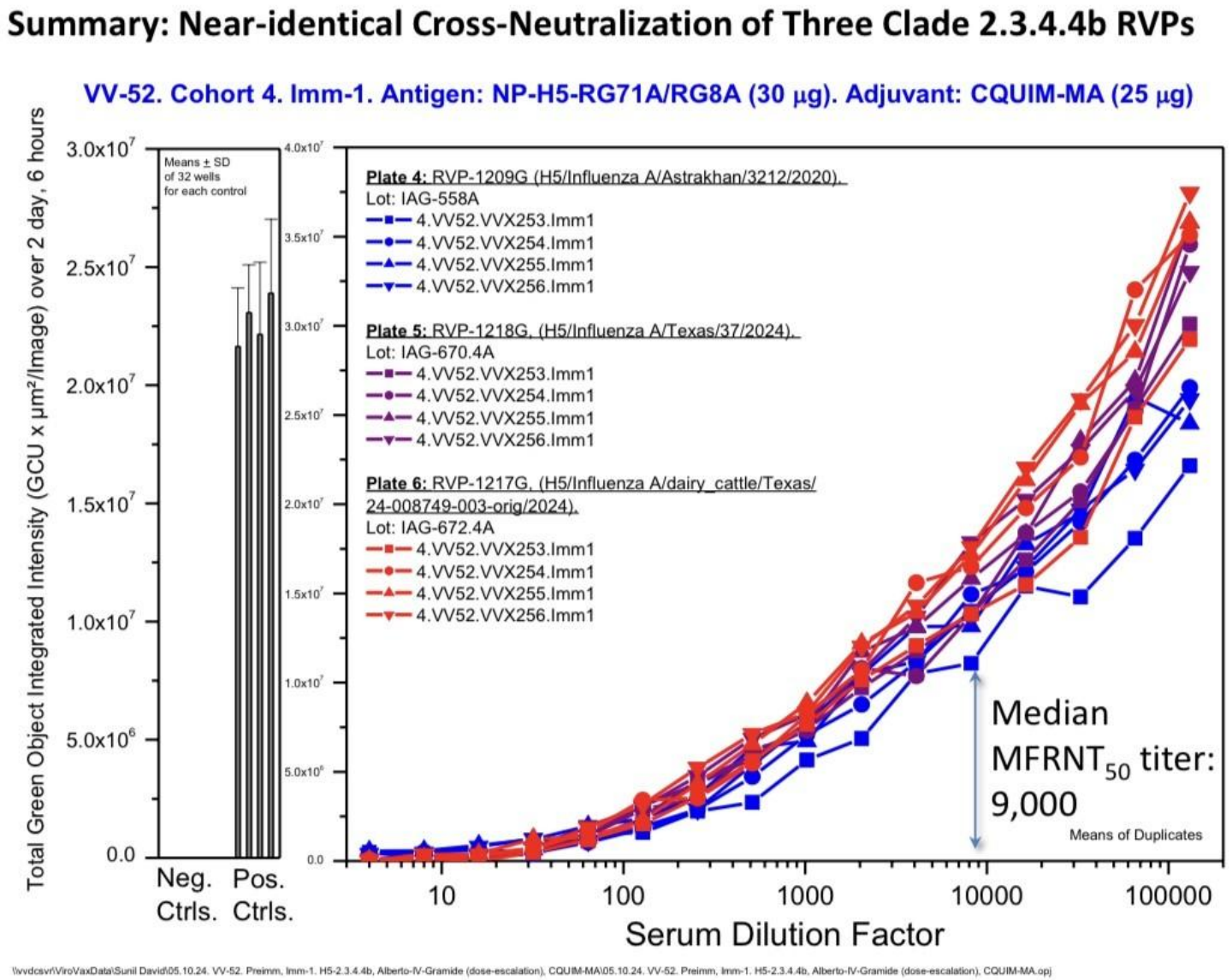
DYADIC INSIDE

ViroVax

Summary: Near-identical Cross-Neutralization of Three Clade 2.3.4.4b RVPs

- **Confirmed cross-neutralization against all three circulating strains:**
  - **(a) H5/Influenza A/Astrakhan/3212/2020,**
  - **(b) H5/Influenza A /Texas/37/2024**
  - **(c) H5/Influenza A/Dairy Cattle/Texas/24-008749-003/2024**

Cohort 4 of VV-51 rabbits



Example 1, C1 Antigens Manufactured at Flexible Commercial Scales…Affordably

Key Attribute	C1 Cells
• **Proactive Solution**: With scalability and flexibility, this C1 produced adjuvanted H5 Clade 2.3.3.4.b A/Astrakhan ferritin nanoparticle vaccine candidate to address global health needs	✓

• **Rapid, High Yield, Low Cost:** Already ~2 g/l in 7-day fermentation w/o strain and fermentation optimization
  • **E.g. Using a 2K-liter standard microbial bioreactor and a vaccine dosage of 50 mcg (animal studies used 30 mcg)**
  • Estimated **40 million doses** of a C1 produced H5 ferritin nanoparticle C-Tag antigen can be manufactured and purified in ~ 2 weeks using low-cost chemically defined media.
  • Estimated **300 million doses** with the use of one **15K-liter** microbial bioreactor.
	✓

• **Sustainable & Affordable Alternative to mRNA:** Using Dyadic's C1-cells to develop, manufacture and distribute antigens (stable at 2-8°C), antibodies and other therapeutic treatments could be a game-changer in the fight against infectious and other diseases with less restrictive manufacturing and storage conditions.	✓

Adjuvanted & Unadjuvanted
C1-Produced H5N1 Microneedle Vaccine Candidate

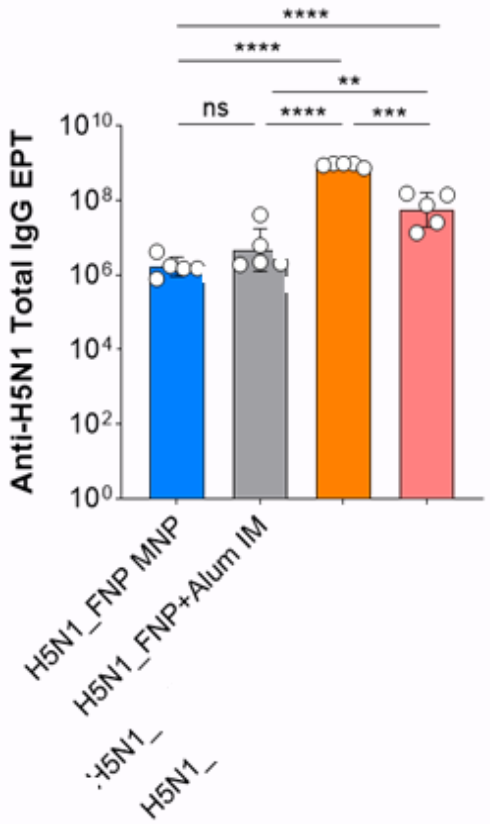
Groups

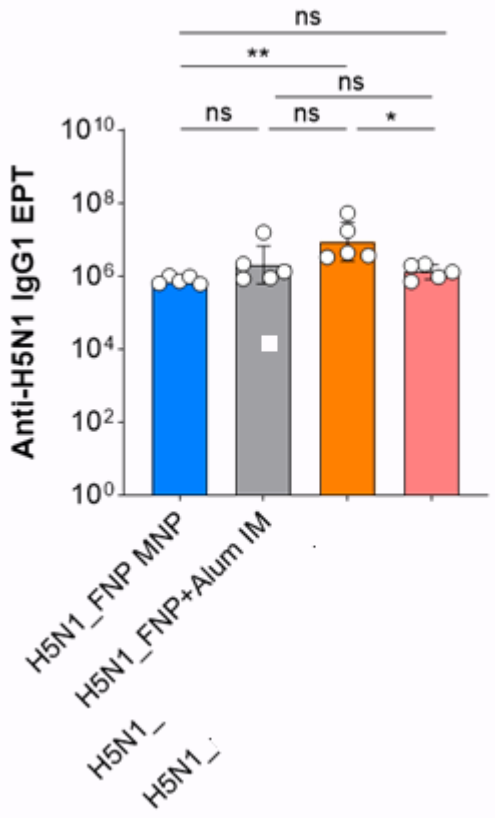
Naïve
NP-H5-RG71A/RG8A (1 µg) MNP – No Adjuvant
NP-H5-RG71A/RG8A (1 µg) + Alum (50 ug) IM
NP-H5-RG71A/RG8A (1 µg) + Novel Adjuvant (5 ug)
NP-H5-RG71A/RG8A (1 µg) + QS-21 (1 ug) + Novel Adjuvant (1 ug)

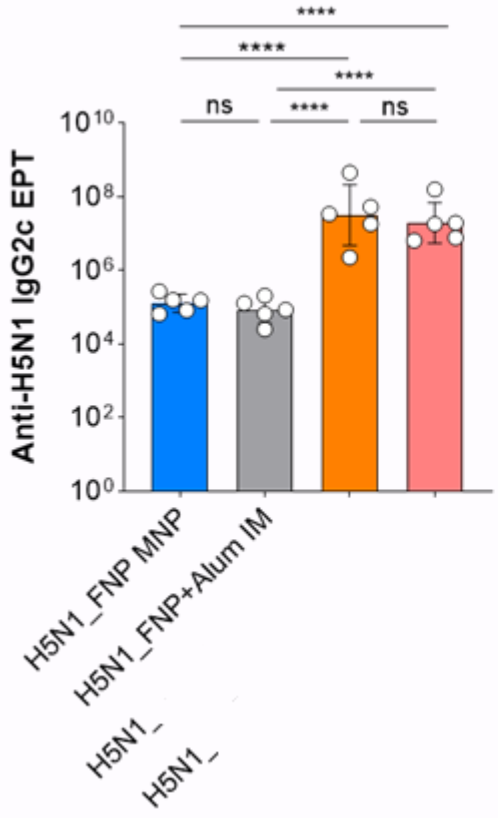
Experimental Schedule

C57BL/6 mice
n = 5

Week 0 — Immunize
3 — Immunize
5 — Bleed (ELISA)







Example 2 : C1 Antigens Manufactured at Flexible Commercial Scales…Affordably

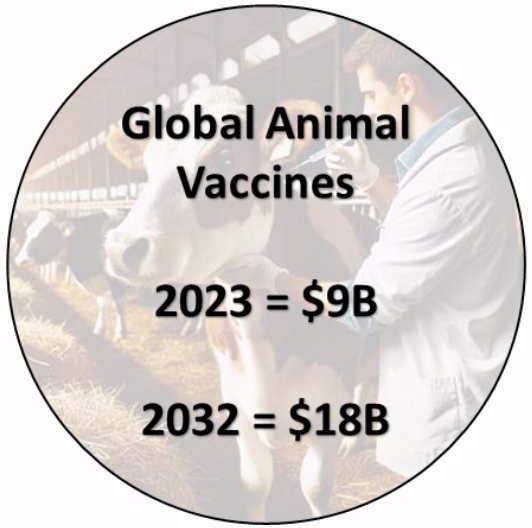
Key Attribute	C1 Cells
• **Proactive Solution**: With scalability and flexibility, this C1 produced adjuvanted H5 Clade 2.3.3.4.b A/Astrakhan ferritin nanoparticle vaccine candidate to address global health needs	✓

• **Rapid, High Yield, Low Cost:** Already ~2 g/l in 7-day fermentation w/o strain and fermentation optimization
  • **E.g. Using a 2K-liter standard microbial bioreactor and a vaccine dosage of 5 mcg (animal studies used 1 mcg)**
  • Estimated **400 million doses** of a C1 produced H5 ferritin nanoparticle C-Tag antigen can be manufactured and purified in ~ 2 weeks using low-cost chemically defined media.
  • Estimated **3 billion doses** with the use of one **15K-liter** microbial bioreactor.
	✓

• **Sustainable & Affordable Alternative to mRNA:** Using Dyadic's C1-cells to develop, manufacture and distribute antigens (stable at 2-8°C), antibodies and other therapeutic treatments could be a game-changer in the fight against infectious and other diseases with less restrictive manufacturing and storage conditions.	✓

C1: Animal Health Vaccines & mAbs

Dyadic C1 advantages provide opportunity within Animal Health

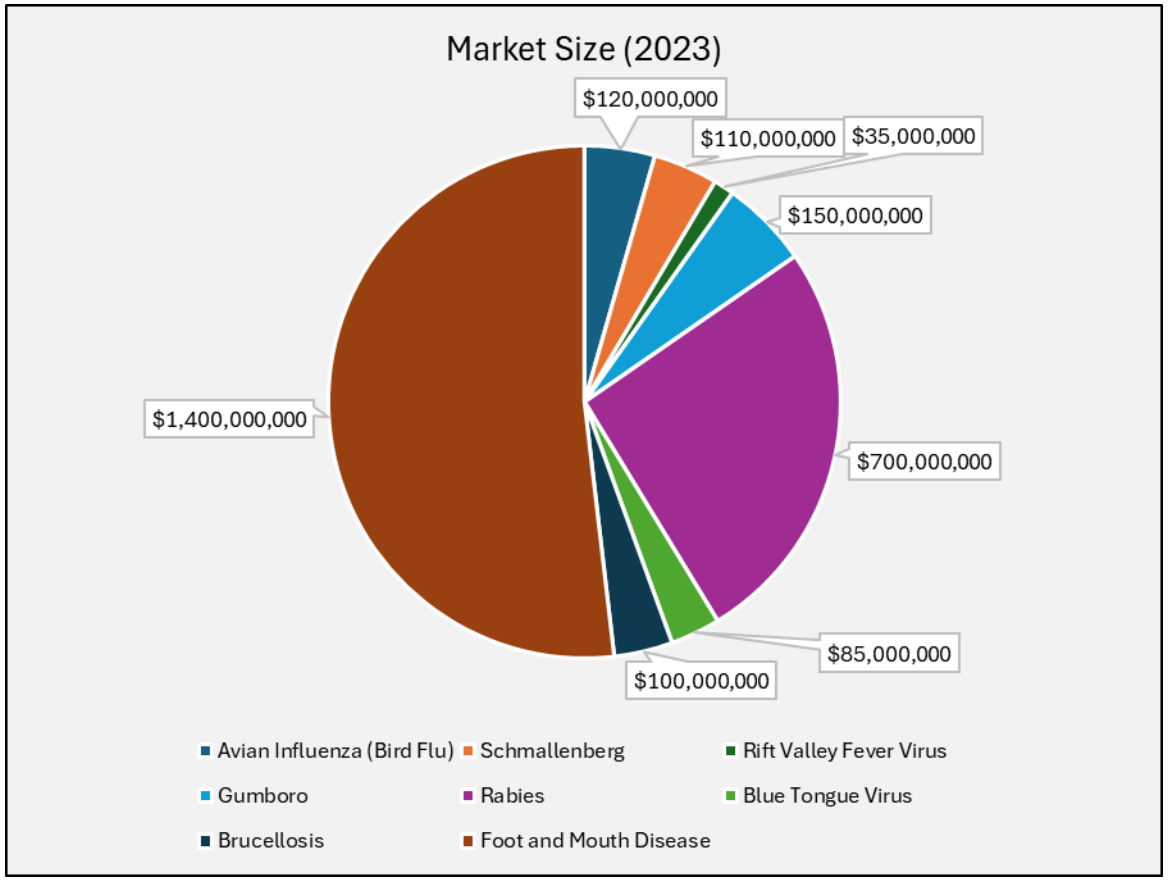


TAM of ~$3.4B for Recombinant Vaccine Segment (2030)

Higher yields with lower overall costs

Protein-base Antigens	Total Yield	Yield Per Day
Avian Livestock -1	20.0 g/L	2.85g/L/d
Avian Livestock -2	1.9 g/L	0.27g/L/d
Rift Valley Fever Virus (RVFV)	1.6 g/L	0.32 g/L/d
H5 Influenza A/Astrakhan/2020 (Ferritin)	1970 mg/L	281 mg/L/d
Schmallenberg Virus (SBV)	1.8 g/L	0.3 g/L/d

Potential Animal Health Vaccines

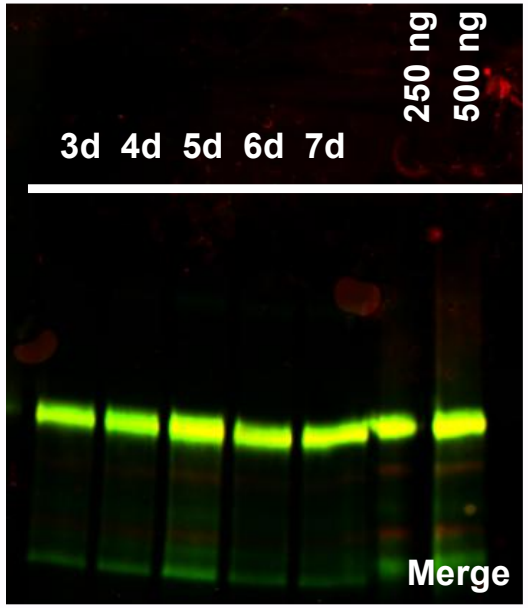


Record Optimization of Expression of an Antigen for Livestock Viral Disease

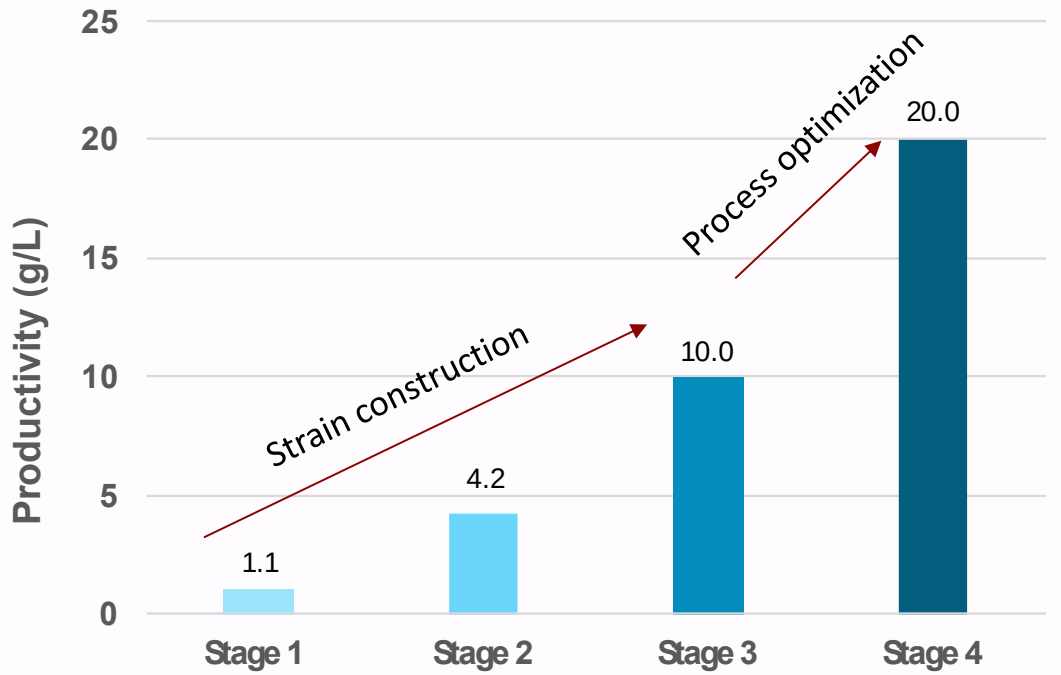
- *Record production of an antigen has been successfully expressed and purified rapidly from C1 cells*
- *Successful animal study demonstrated high Immunogenicity response*

Expression in C1

Antigen	Yield in C1
Antigen X	20.0 g/L/7d



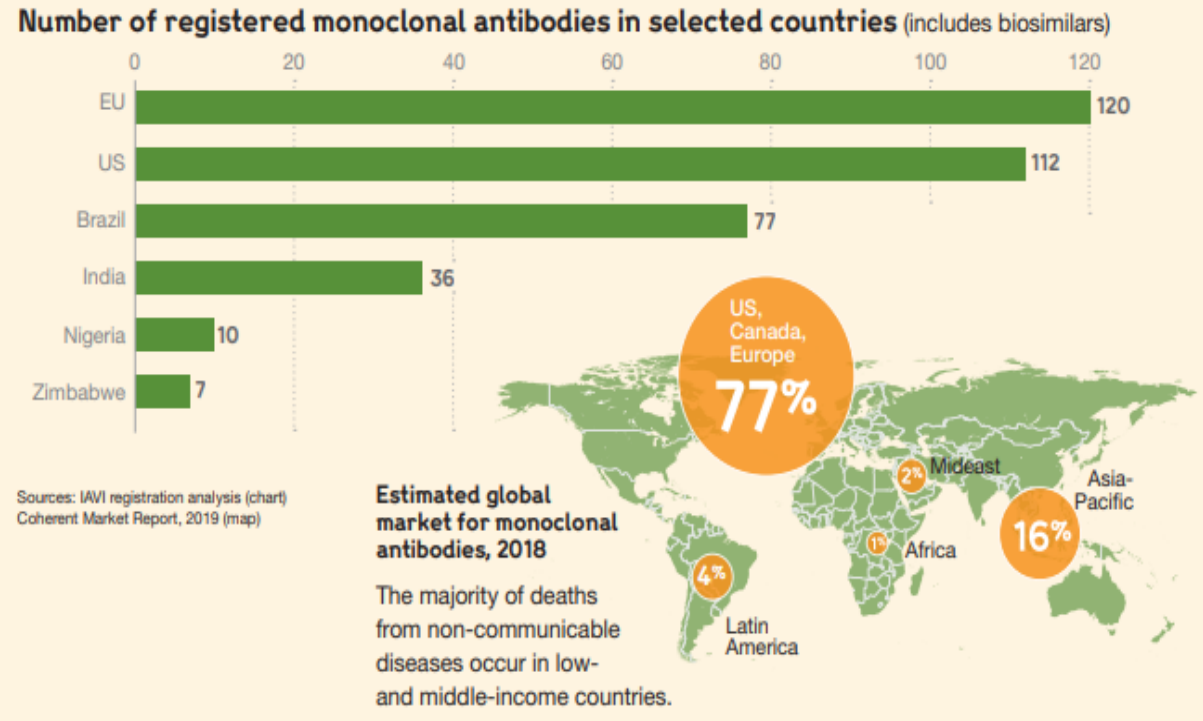
Optimization of Antigen Production



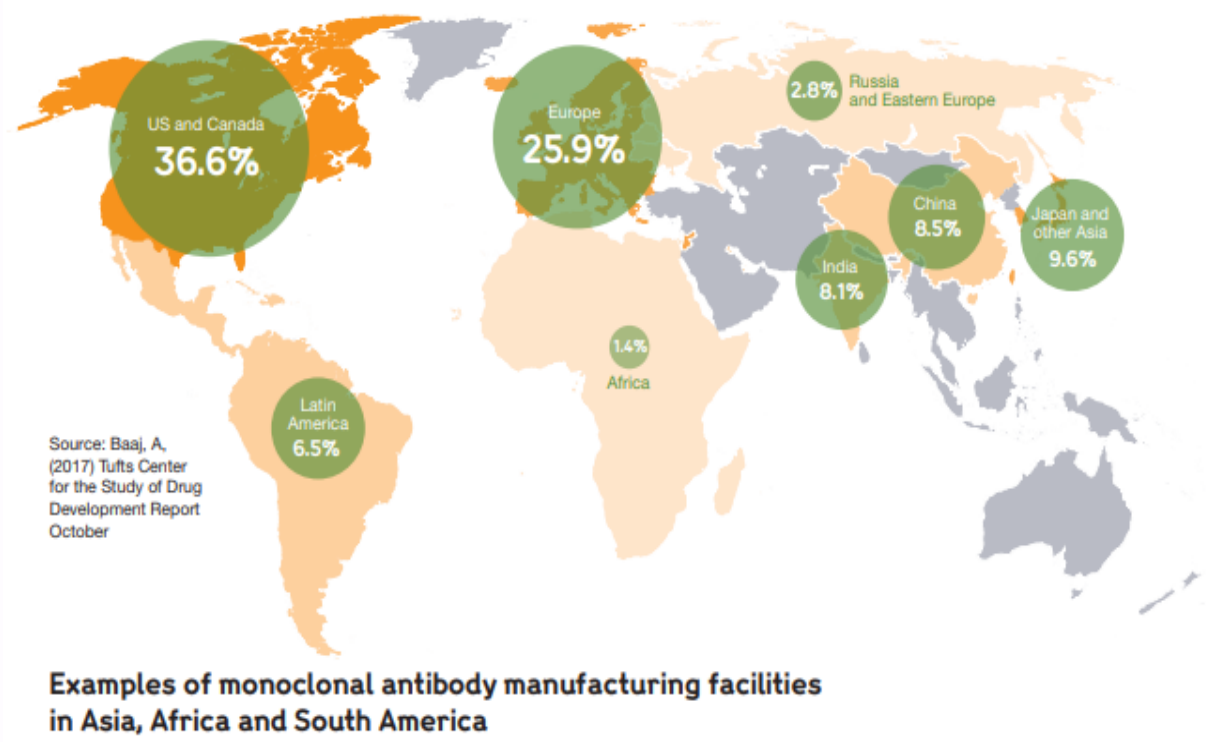
C1, Faster, More Productive Lower Cost mAbs

Current costs of producing mAbs are simply too great to support independent production in LMICs

Access Challenges



Affordability Challenges



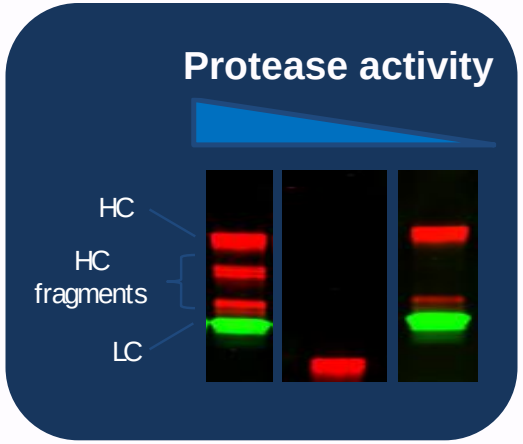
C1 mAb Proof of Concept: Nivolumab

Nivolumab strain development for high production level, high stability and Human glycan structure

Productivity

Best productivity 22.3 g/L in 7 days fermentation[1]

Stability



N-Glycan

Glycan	Amount (%)
M3	0,35
M3B	0,45
G0	32,54
G1	17,21
G1_2	29,51
G2	19,94
Sum w/o IS	100
Response w/o IS	101843184
All human-like	99,2

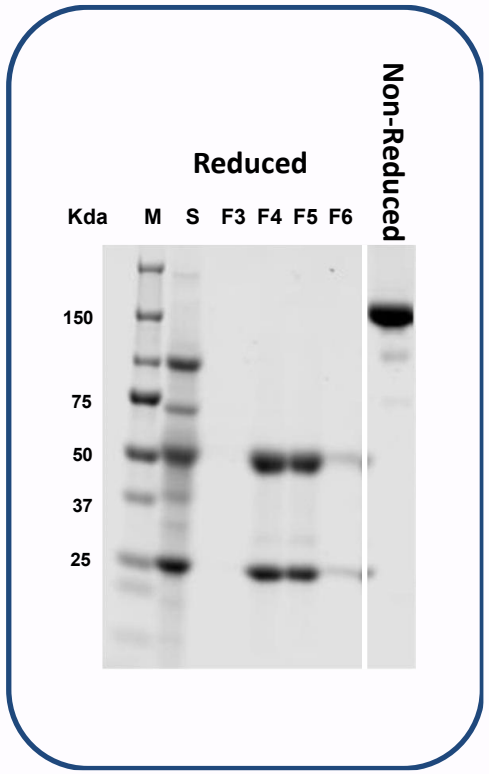
Constructing Commercial Strains:

Developing highly productive (>20g/L), stable mAb with human type of N-Glycan structure

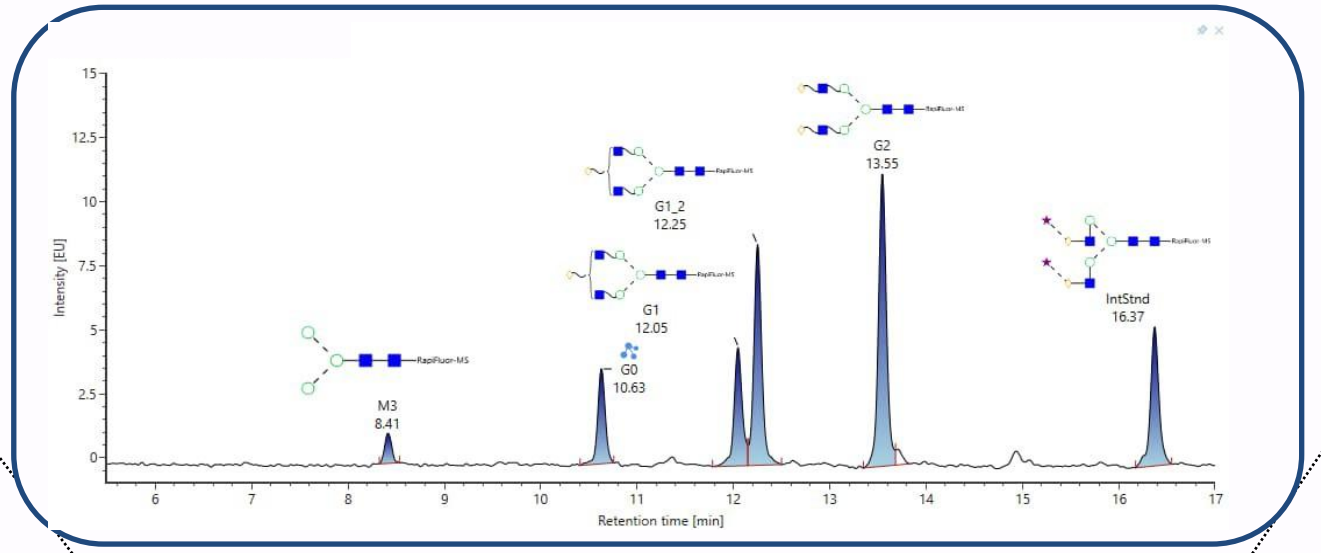
1. Data from non-glycoengineered C1-cell using engineered protease deficient C1-cell

Engineering Nivolumab C1 G2/G1 strain

The use of library of folding factors enhanced the productivity of the Nivolumab G2/G1 strain
New production record obtained: <u>9.2 g/l</u> Good product quality with excellent N-glycan profile



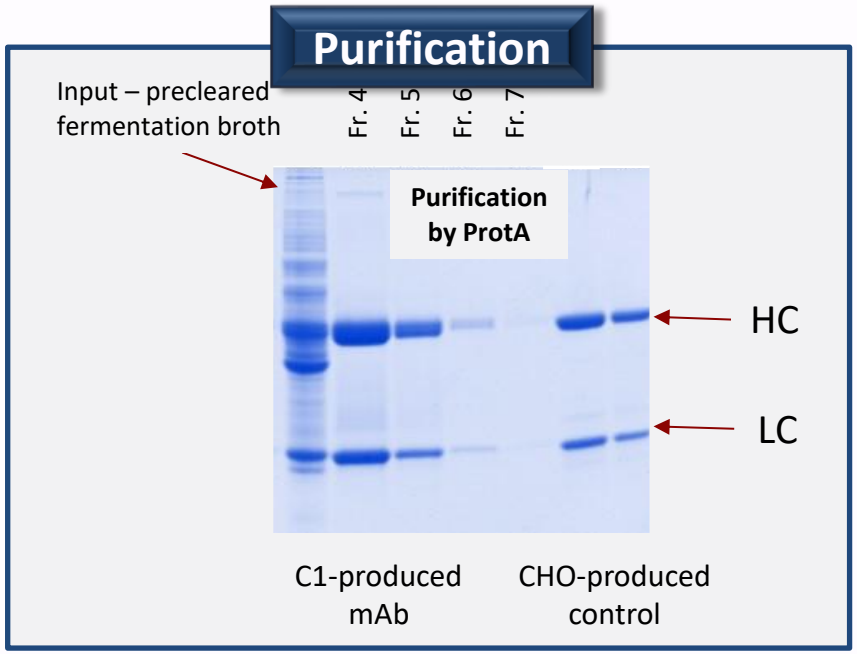
Single-step purification using 1 ml Eshmuno A (Protein A) column

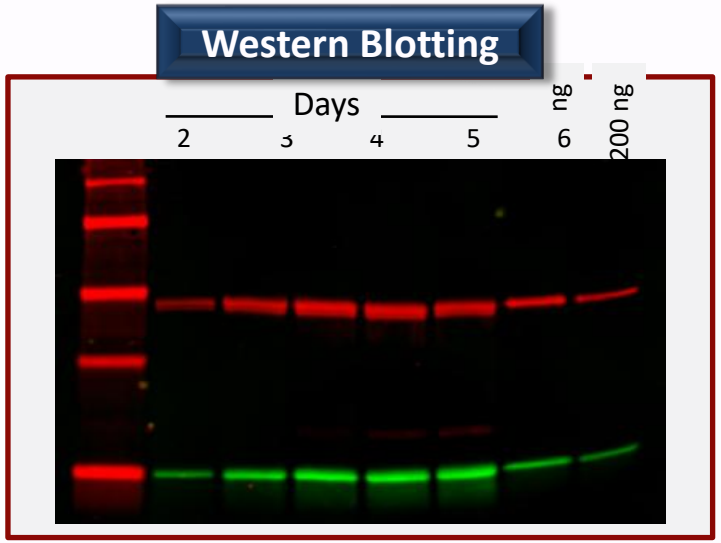


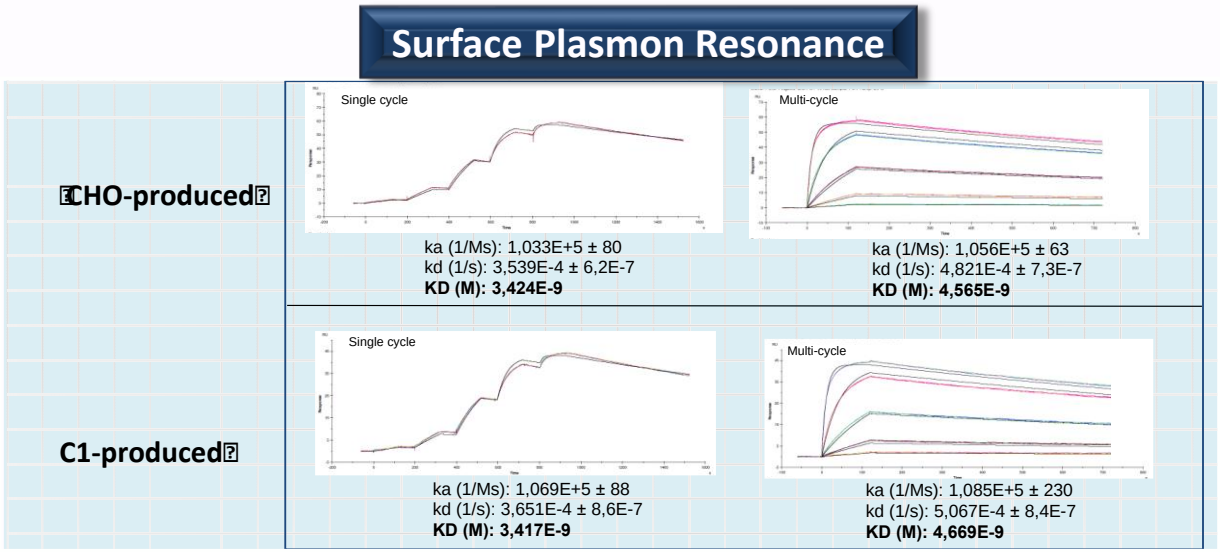
		MT771-6
		M7184
Glycan	**Obs. RT (min)**	**Amount, %**
Man2	6,22	-
Man3	8,42	3,51
G0	10,63	12,01
G1	12,05	16,29
G1_2	12,25	29,20
G2	13,55	38,99
% Humanised N-glycans		96,49
Total response		137,9
Nivolumab, g/l		9,23

C1 manufactured monoclonal antibodies are stable and correctly folded

- **Typically, stable mAbs production levels are between**
  - **5 – 10 g/L in 5-7 days**
- **Best productivity at Ambr system:**
  - **24.5 g/L in 7 days (3.5 g/l/day)**
- **Best productivity so far at 30L scale:**
  - **20.6 g/L at 157 h (3.1 g/L/day)**

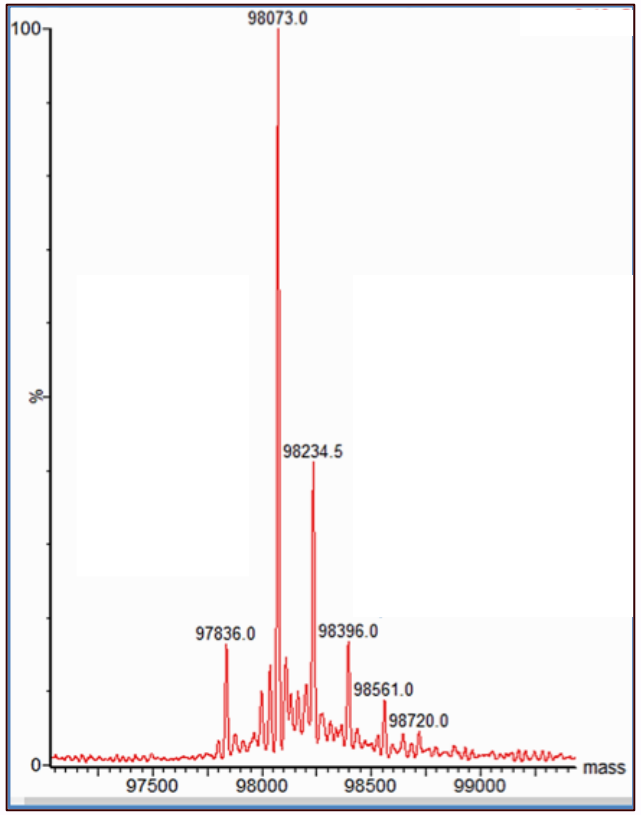




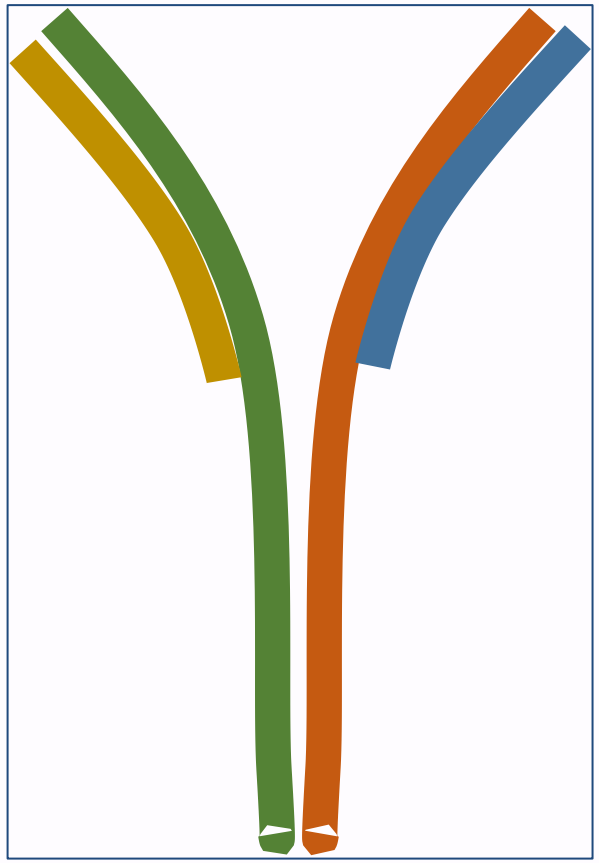


C1 has capability to express bispecific antibodies, nanobodies, other therapeutic proteins

- The best production levels so far are: **Bispecific X at 7.62 g/L in 7 days (unoptimized)**
- Mass spectrometry analysis indicates that vast majority of Bispecific X is correctly assembled



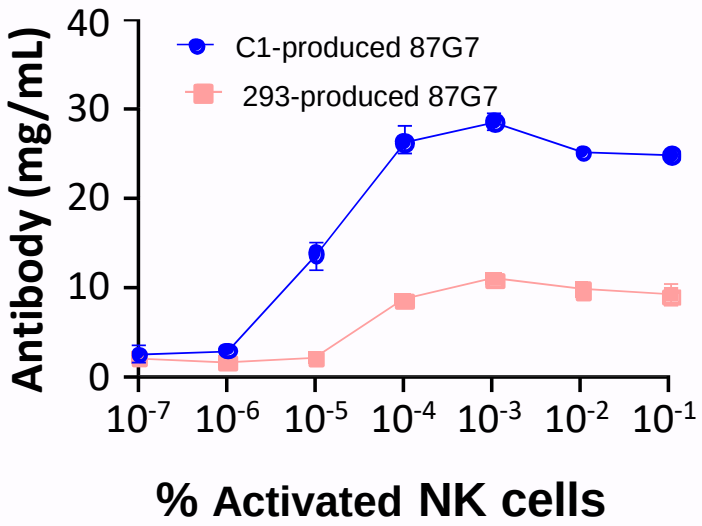
- MS Deconvoluted spectrum analysis
- Followed the expected spectrum



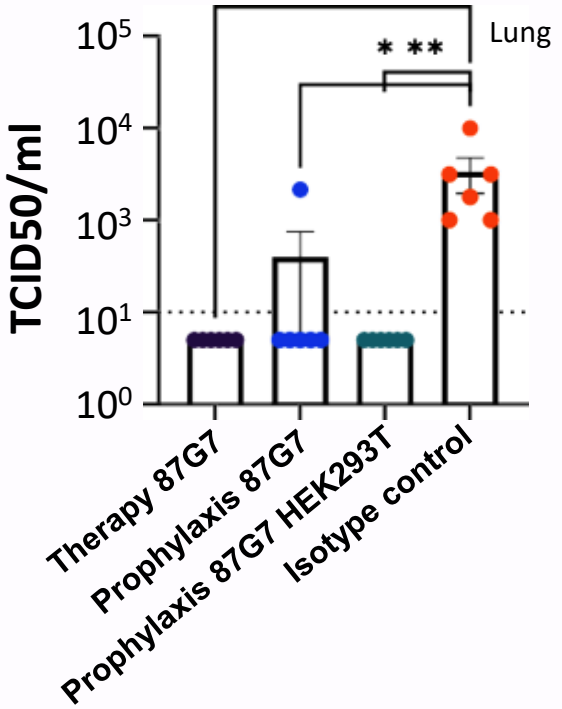
Pre-clinical studies with C1 Produced 87G7 Monoclonal Antibody

Nature Communications Study - C1-produced human monoclonal antibody provides protection against SARS-CoV-2 in hamster and non- human primate models

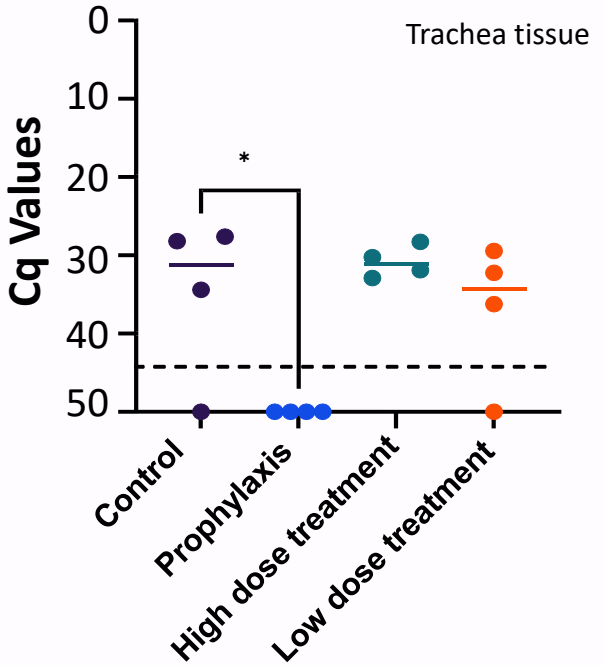
In vitro assay



Hamster study



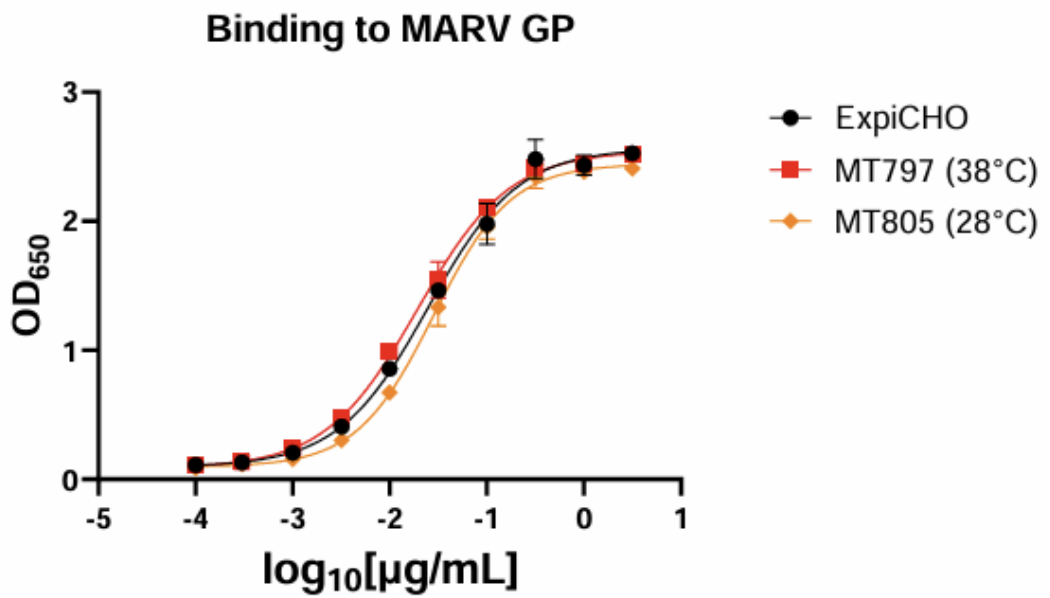
Non-human primates



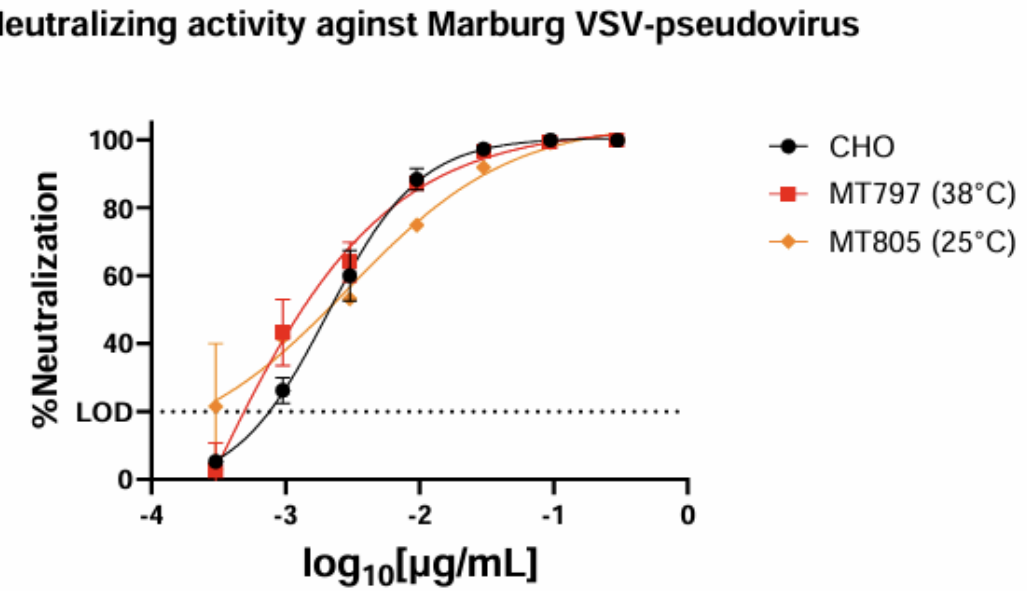
HuMabs expressed in genetically engineered C1 filamentous fungus have the potential to supersede mammalian cell-produced HuMabs for the prevention and treatment acute respiratory virus infections. As was previously shown for C1-produced proteins as vaccine candidates

CHO-like neutralization and binding, but faster and more affordable

Marburg VIRUS



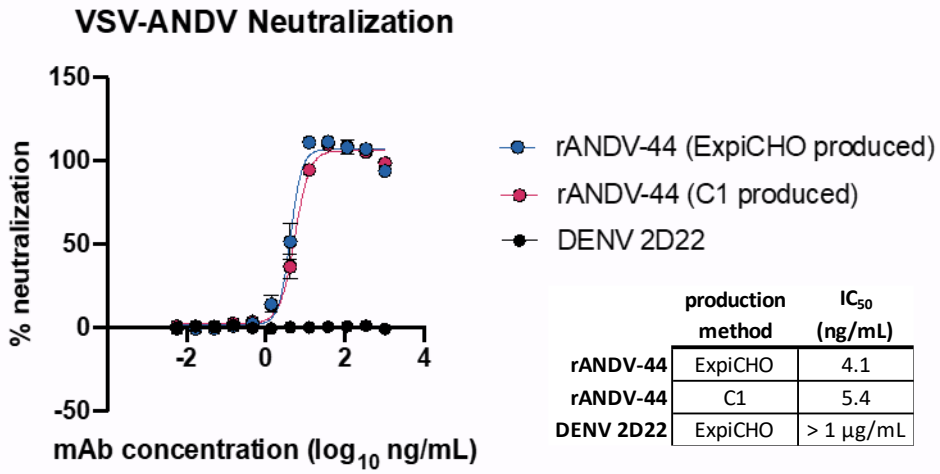
Binding of CHO produced and C1-produced (2 clones) of anti-MARV mAb ABV-04 to Marburg glycoprotein



Neutralizing activity of CHO produced and C1- produced (2 clones) of anti-MARV mAb ABV-04 using a MARV GP-VSV pseudotype Virus

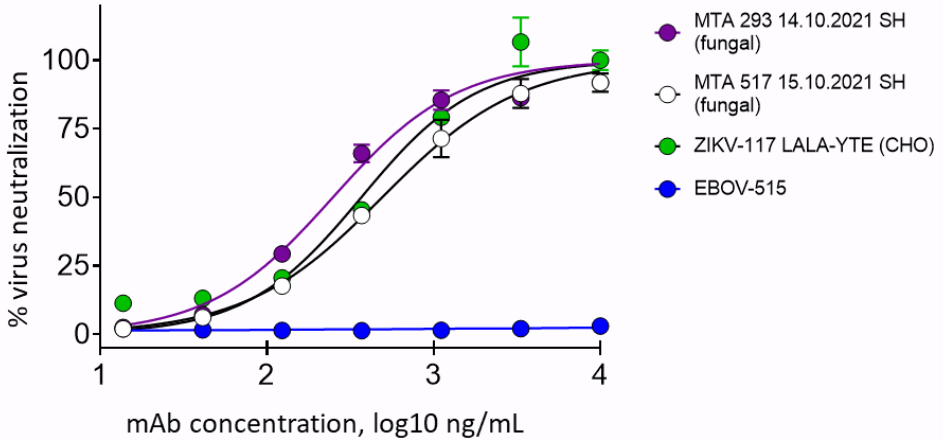
CHO-like neutralization and binding, but faster and more affordable

ANDES VIRUS

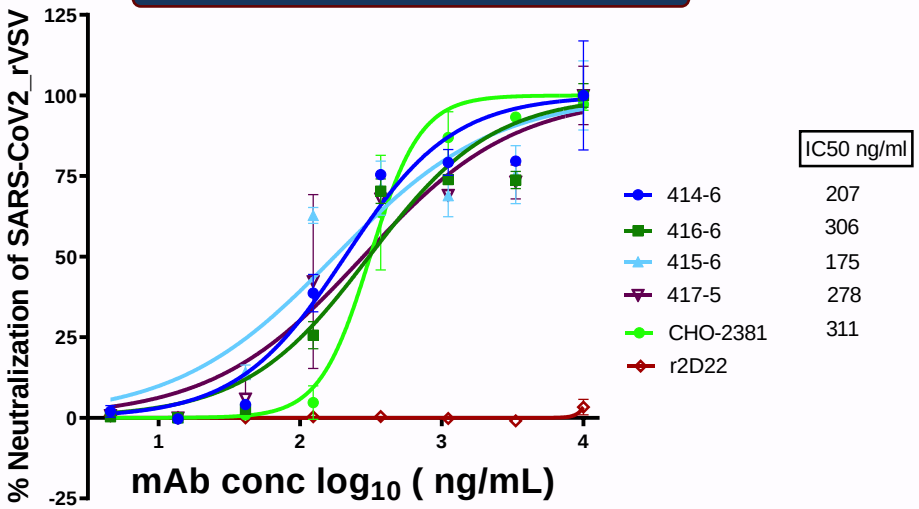


	production method	IC_{50} (ng/mL)
rANDV-44	ExpiCHO	4.1
rANDV-44	C1	5.4
DENV 2D22	ExpiCHO	> 1 µg/mL

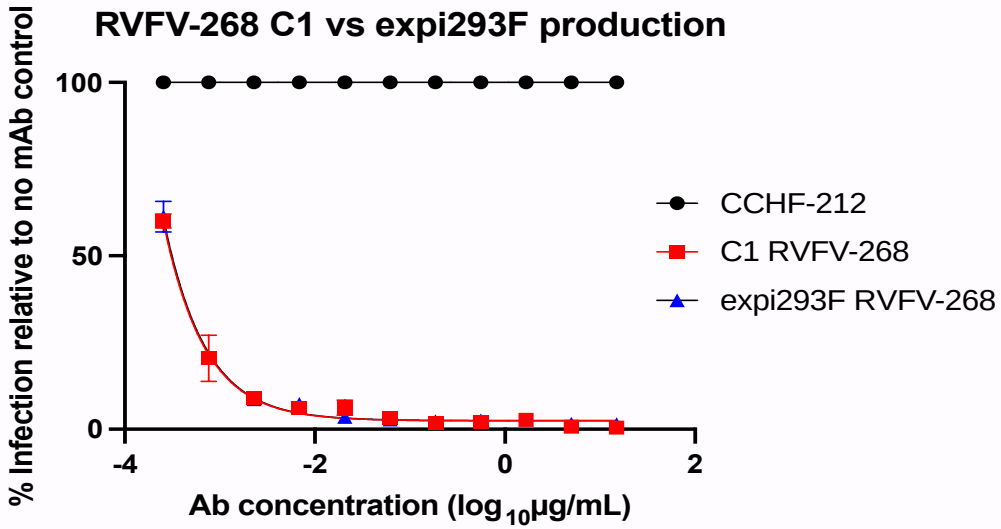
ZIKA



SARS-COV2



RIFT VALLEY



Quality of mAb Confirmed by Independent Experts

UC DAVIS
UNIVERSITY OF CALIFORNIA

Production of mAbs for use in COVID-19 diagnostic testing using C1, a filamentous fungi production platform

UC DAVIS Justin H. Wong[1], Yakir Ophir[1], Katherine Haddad[1], Varun Gore[1], Anindya Karmaker[1], Mark Emalfarb[2], Karen A. McDonald[1,3], and Somen Nandi[1,3]
McDonald/Nandi Lab Plant Bioprocessing
[1]Department of Chemical Engineering, University of California, Davis, CA, USA [2]Dyadic International, Inc., Jupiter, FL, USA
[3]Global Health Share Initiative, University of California, Davis, CA, USA

Introduction

The COVID-19 pandemic has revealed the need to develop technologies that are capable of rapidly responding to emerging biological threats.

- Monoclonal antibodies (mAbs) can be used as both a therapeutic and diagnostic tool to address SARS-CoV-2 infections
- Monoclonal antibody production in Chinese Hamster Ovary (CHO) cell cultures can be costly, time consuming, and susceptible to viral contamination
- Filamentous fungi such as (Thermothelomyces heterothallica (C1)) are emerging as a platform to produce therapeutic proteins that can address the disadvant

Figure 1. Structure of an antibody. **FC:** fragment crystallizable region; **Fab:** fragment antigen binding

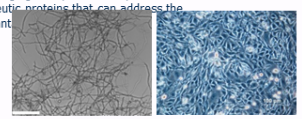


Figure 2. Microscopy image of filamentous fungi (left) and CHO cells (right). Scale bar represents 100µm

Hypothesis: We can use C1 as an alternative production system to produce mAbs that are used in both the treatment and detection of SARS-CoV-2 infection

Fermentation

Fermentation was carried out in a BioFlo-3000 bioreactor with a 5L glass vessel.

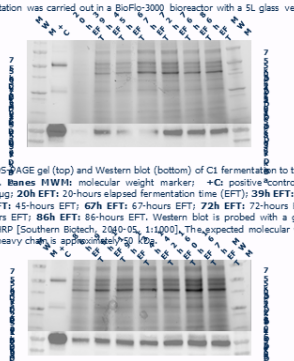


Figure 4. SDS-PAGE gel (top) and Western blot (bottom) of C1 fermentation to track mAb accumulation. **Lanes MWM:** molecular weight marker; **+C:** positive control, human serum IgG, 3µg; **20h EFT:** 20-hours elapsed fermentation time (EFT); **39h EFT:** 39-hours EFT; **45h EFT:** 45-hours EFT; **67h EFT:** 67-hours EFT; **72h EFT:** 72-hours EFT; **76h EFT:** 76-hours EFT; **86h EFT:** 86-hours EFT. Western blot is probed with a goat anti-human IgG-HRP [Southern Biotech, 2040-05, 1:1000]. The expected molecular weight of an antibody heavy chain is approximately 50 kDa.

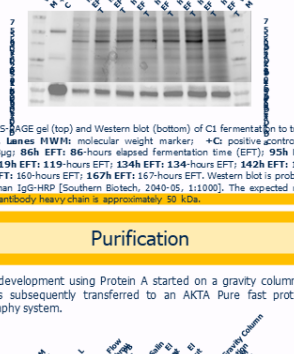


Figure 5. SDS-PAGE gel (top) and Western blot (bottom) of C1 fermentation to track mAb accumulation. **Lanes MWM:** molecular weight marker; **+C:** positive control, human serum IgG, 3µg; **86h EFT: 86**-hours elapsed fermentation time (EFT); **95h EFT: 95**-hours EFT; **119h EFT: 119**-hours EFT; **134h EFT: 134**-hours EFT; **142h EFT:** 142-hours EFT; **160h EFT:** 160-hours EFT; **167h EFT:** 167-hours EFT. Western blot is probed with a goat anti-human IgG-HRP [Southern Biotech, 2040-05, 1:1000]. The expected molecular weight of an antibody heavy chain is approximately 50 kDa.

Functional ELISA

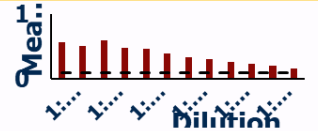


Figure 7. Titer of mAb produced as measured using an indirect ELISA method. Titer of purified mAbs with an initial concentration of 0.4 mg/mL was determined to be 1:512000. This titer corresponds to 39 pg of mAb per well. Dashed line represents the limit of detection.

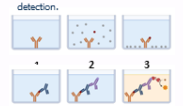


Figure 8. Sandwich ELISA setup. **1:** capture antibody binds to plate; **2:** sample added to well; **3:** antigen binds to capture antibody; **4:** detection antibody binds antigen; **5:** labeled secondary antibody binds to detection; **6:** substrate reaction produces color change.

Table 1. Concentration of RBD and Spike produced in various recombinant expression hosts as determined by a sandwich ELISA using recombinantly produced mAb as the capture antibody, Rabbit Anti-Spike (eEnzyme, SCV2-S-100) as the detection antibody, and Goat Anti-Human, HRP (Southern Biotech, 2050-05) as the secondary antibody.

Antigen	Organism
Spike (Purified)	CHO
Receptor Binding Domain (RBD, Crude)	*Nicotiana benthamiana*
Spike (Crude)	C1

Production Process

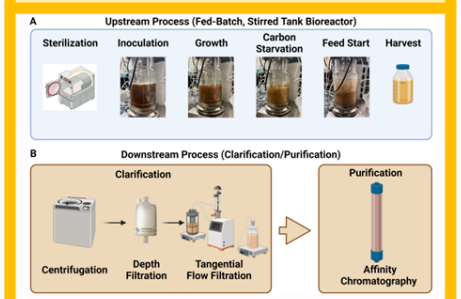


Figure 3. Bioprocess flow chart of C1 mAb production. **A.** Graphical representation of the upstream process to produce mAbs in a fed-batch process. **B.** Graphical representation of the downstream process to purify mAbs. Clarification was carried out using centrifugation, depth filtration, and tangential flow filtration, before purification by Protein A chromatography.

Purification

Purification development using Protein A started on a gravity column and the method was subsequently transferred to an AKTA Pure fast protein liquid chromatography system.

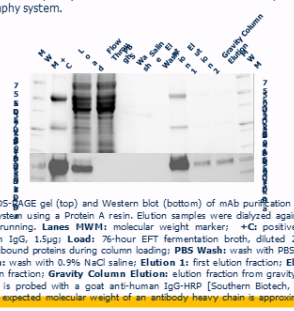


Figure 6. SDS-PAGE gel (top) and Western blot (bottom) of mAb purification from the AKTA Pure system using a Protein A resin. Elution samples were dialyzed against 0.9% NaCl before running. **Lanes MWM:** molecular weight marker; **+C:** positive control, human serum IgG, 1.5µg; **Load:** 76-hour EFT fermentation broth, diluted 2x; **Flow Through:** unbound proteins during column loading; **PBS Wash:** wash with PBS, pH 7.2; **Saline Wash:** wash with 0.9% NaCl saline; **Elution 1:** first elution fraction; **Elution 2:** second elution fraction; **Gravity Column Elution:** elution fraction from gravity column. Western blot is probed with a goat anti-human IgG-HRP [Southern Biotech, 2040-05, 1:1000]. The expected molecular weight of an antibody heavy chain is approximately 50 kDa.

Conclusions / Future Directions

- In the current unoptimized production, a single 3 L harvest would provide enough purified material to conduct 120 million ELISA tests
- Test mAbs using biolayer interferometry to determine binding affinity to various mutants of RBD
- Test mAbs in lateral flow assay
- Fermentation and purification optimization to improve productivity and recovery

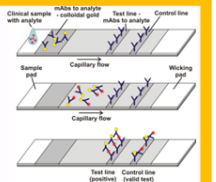


Figure 9. Lateral flow assay schematic

Acknowledgements

This work is supported by NIST/BioMADE: Rapid Assistance (for) Coronavirus Economic Response (RACER) award 70NANB22H017 from U.S. Department of Commerce (DOC), National Institute of Standards and Technology. Images created with BioRender.com. Strains developed by VTT and provided by Dyadic. Any opinions, findings, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the University of California, Davis, Dyadic International, Inc, or VTT.

Contact Information: juhwong@ucdavis.edu, kamcdonald@ucdavis.edu, snandi@ucdavis.edu

NIST BioMADE DYADIC VTT

DYADIC INSIDE

C1 Technology for the production of both antigen-Based Vaccines and neutralizing antibodies

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Key Words: Next Generation Gene Expression Platform / Antigens / mAbs / Biotherapeutics

Dyadic project APR-29
NIIMBL Future Recommendations

The APR-29 project demonstrated that *Thermothelomyces heterothallica* C1 production system has great potential to contribute to the response to emerging pandemics in the future. Production cell lines can be constructed fast (in less than 50 days), the productivities for antibodies are good (up to 9 g/l in 5-7 days fermentation) and the antibody quality provided by the system is suitable for human use.

These features make C1 an outstanding production platform as compared with other recombinant protein production systems such as yeast or mammalian cell platforms.

C1 produced mAbs have virtually identical structure, properties and activities to CHO produced mAbs

C1 platform produces comparable therapeutic proteins as CHO while overcoming key production limitations

C1 produced mAbs have virtually identical properties and activities to CHO produced mAbs

Neutralization

C1 mAbs neutralize viruses virtually identical to CHO produced mAbs

Binding

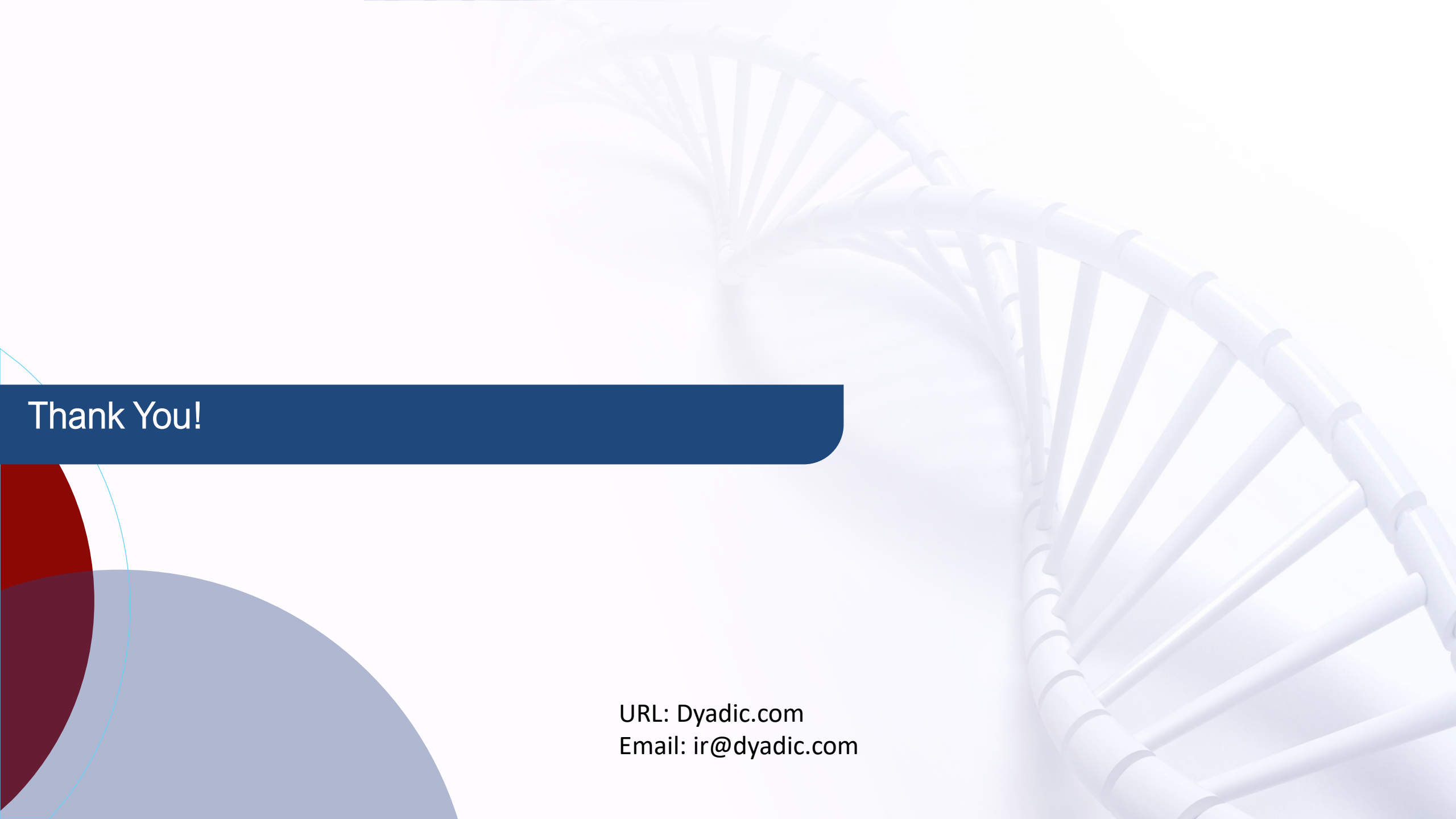
C1 mAbs are correctly folded, bind virtually identical to CHO produced mAbs

Glycan Structure

C1 native glycans are more human than yeast

C1 cells have been glycoengineered to express mAbs with human like glycan structures (~99%)

Thank You!



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