

# Benefits of Alternative Hosts in Vaccine Manufacturing: Speed, Cost, and Quality

Laura Crowell  
Director R&D, Sunflower Tx  
DCVMN Webinar  
August 13 2024



# Today's Speakers



**LAURA CROWELL**

Director,  
Research & Development

**Sunflower Tx**



**ALEXANDRA BONNYMAN**

Director,  
Engineering Operations

**Sunflower Tx**



Sunflower is an early-stage company delivering next-generation biomanufacturing solutions to global innovators servicing a >\$500B market for protein medicines, vaccines and food.

Just like anyone can grow sunflowers anywhere, our solutions are easy to use and empower anyone to efficiently make proteins anywhere.

# Conventional Biomanufacturing is Expensive, Highly Manual, and not Continuous



# Our Goal: Make Protein Manufacturing More Accessible



Accessibility

=



Easy to obtain

+



Easy to use

Host organism impacts cost and simplicity for bioprocess



## What host organism do you most commonly use?

- A. Bacteria (e.g. *E.coli*)
- B. Yeast (e.g. *P. pastoris*)
- C. Mammalian cells (e.g. *CHO*)
- D. Other organism

# Eukaryotic Microbes Enable Process Simplicity

	<b>Bacteria (<i>E. coli</i>)</b>	<b>Eukaryotic Microbes (<i>P. pastoris</i>)</b>	<b>Mammalian Cells (<i>CHO</i>)</b>
Protein Secretion		✓	✓
Initial Purity	<<50%	>70%	<50%
No Endotoxin		✓	✓
No Adventitious Agents	✓	✓	
<b>Process Complexity</b>	<b>HIGH</b>	<b>LOW</b>	<b>MED</b>

# Eukaryotic Microbes Enable Lower Cost

	<b>Bacteria (<i>E. coli</i>)</b>	<b>Eukaryotic Microbes (<i>P. pastoris</i>)</b>	<b>Mammalian Cells (<i>CHO</i>)</b>
Process Complexity	<b>HIGH</b>	<b>LOW</b>	<b>MED</b>
Doubling Time	<b>&lt;1 HR</b>	<b>2-3 HR</b>	<b>&gt;12 HR</b>
<b>Facility Utilization</b>	<b>POOR</b>	<b>GOOD</b>	<b>POOR</b>



# Eukaryotic Microbes Enable Lower Cost

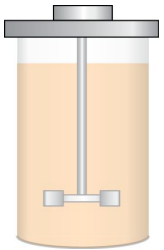
	<b>Bacteria (<i>E. coli</i>)</b>	<b>Eukaryotic Microbes (<i>P. pastoris</i>)</b>	<b>Mammalian Cells (<i>CHO</i>)</b>
Process Complexity	<b>HIGH</b>	<b>LOW</b>	<b>MED</b>
Doubling Time	<b>&lt;1 HR</b>	<b>2-3 HR</b>	<b>&gt;12 HR</b>
<b>Facility Utilization</b> +	<b>POOR</b>	<b>GOOD</b>	<b>POOR</b>
Raw Material Cost	<b>LOW</b>	<b>LOW</b>	<b>HIGH</b>

# Eukaryotic Microbes Enable Lower Cost

	Bacteria ( <i>E. coli</i> )	Eukaryotic Microbes ( <i>P. pastoris</i> )	Mammalian Cells ( <i>CHO</i> )
Process Complexity	HIGH	LOW	MED
Doubling Time	<1 HR	2-3 HR	>12 HR
Facility Utilization + Raw Material Cost	POOR LOW	GOOD LOW	POOR HIGH
<u>Cost of Goods Manufactured</u>	MED	LOW	HIGH

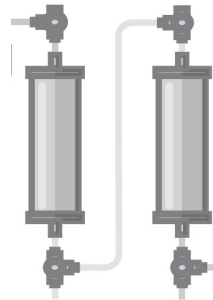
# Pichia pastoris has Many Beneficial Properties

## UPSTREAM



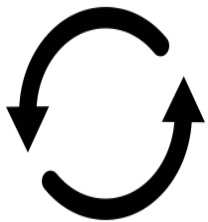
- Secretes complex proteins
- Grows like a microbe (2-3 hr doubling time)
- Reaches ultra high biomass

## DOWNSTREAM



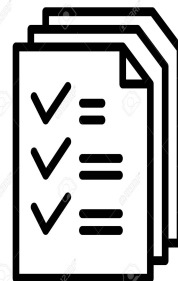
- Raw secreted proteins typically 60-80% pure
- 10X fewer secreted host cell proteins than CHO
- No adventitious viruses

## DEVELOPMENT CYCLES



- Sequence to research cell bank in 4 weeks
- Sequence to bulk drug substance in as few as 8 weeks

## REGULATORY

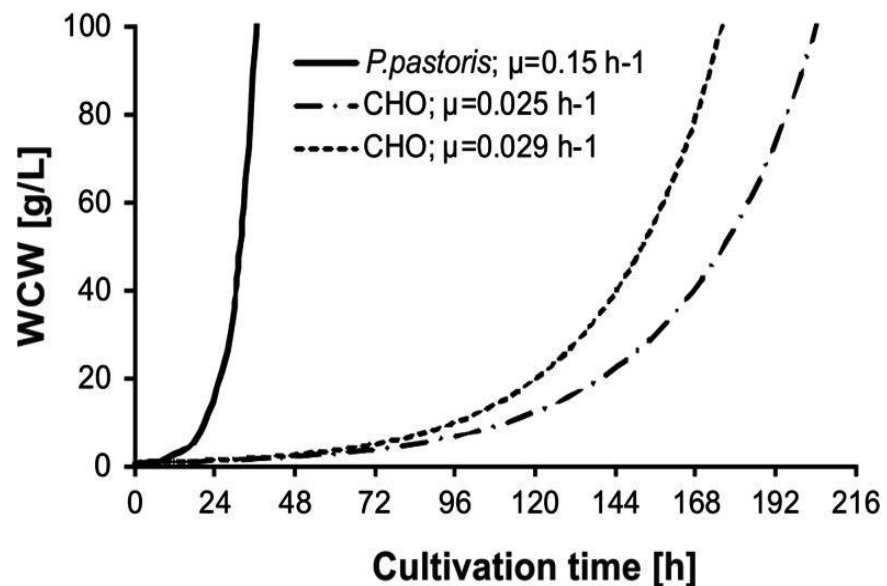


- Multiple FDA and EMA approved products
- GRAS\* organism
- No endotoxins

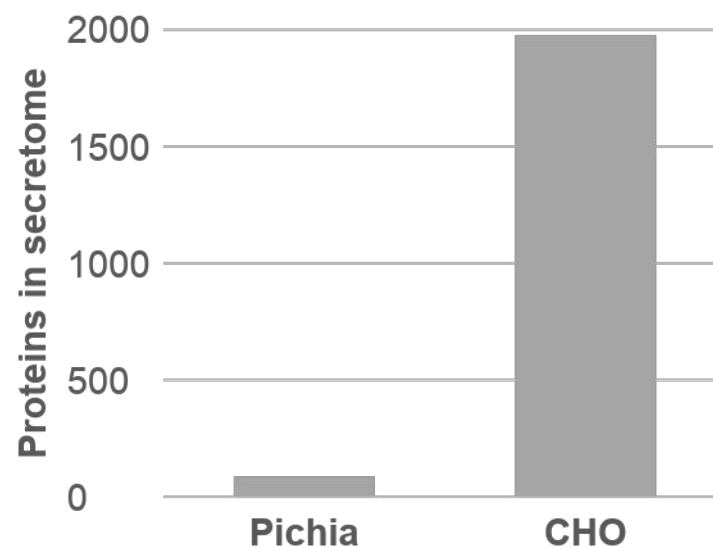
\*Generally Recognized As Safe (US FDA)

# *Pichia pastoris* has Many Beneficial Properties

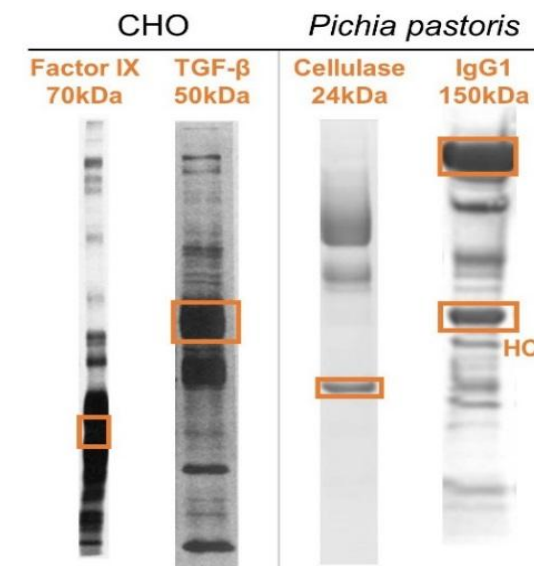
## SPEED



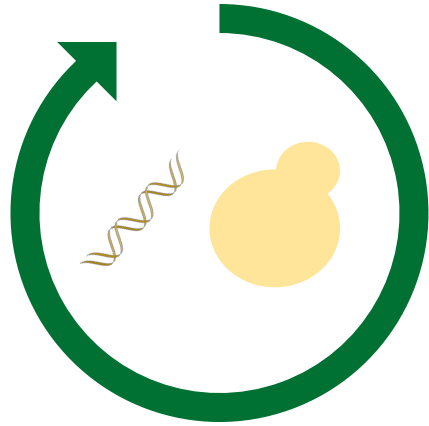
## SIMPLICITY



## PURITY



# Eukaryotic Microbes Enable Simplicity in Multiple Ways



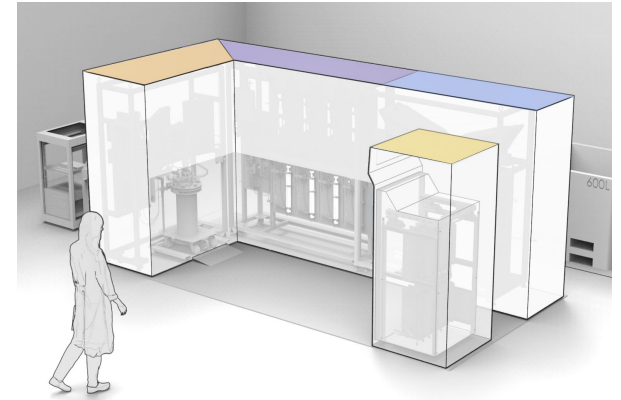
## RAPID DESIGN CYCLES

for parallel strain & sequence optimization



## PLATFORMABLE PROCESSES

using integrated and continuous techniques



## NEXT-GENERATION EQUIPMENT

flexible, scalable, and user-friendly

# Design Cycles for Yeast Strain Production are Fast

Design Cycles for Improved Expression



ATG  
GCT  
ATA

**Sequence  
selection**  
1 day



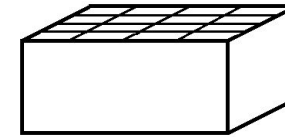
**Vector  
Design**  
1 day



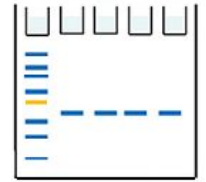
**DNA  
Synthesis**  
5 - 10 days



**Strain  
Creation**  
10 days

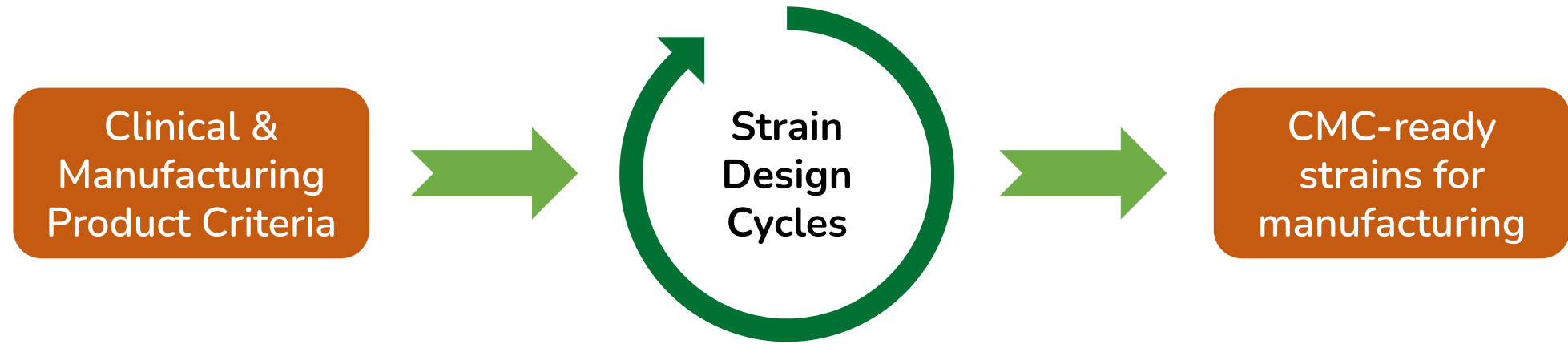


**Scout  
Cultivation**  
3-5 days



**Evaluate  
Expression**  
1-3 days

# Design Cycles Support Rapid Product Optimization For Multiple Criteria Simultaneously



## Key Levers for Optimization

### Product Sequence

- Codon usage
- PTMs
- AA swaps

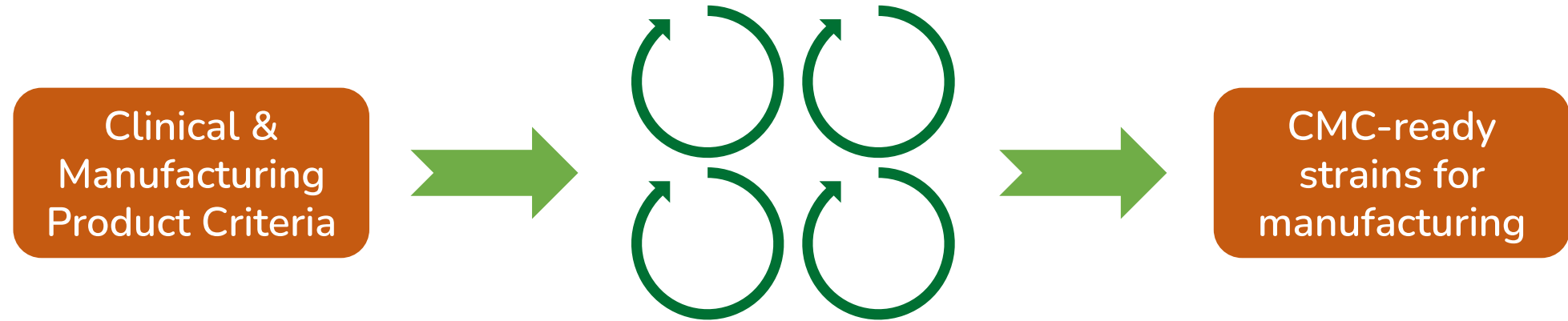
### Vector Design

- Promoter
- Locus
- Signal Sequence
- Terminator

### Strain Creation

- Strain Lineage
- Knock-outs
- Knock-ins

# Parallelization Enables Even Faster Timelines



## Key Levers for Optimization

### Product Sequence

- Codon usage
- PTMs
- AA swaps

### Vector Design

- Promoter
- Locus
- Signal Sequence
- Terminator

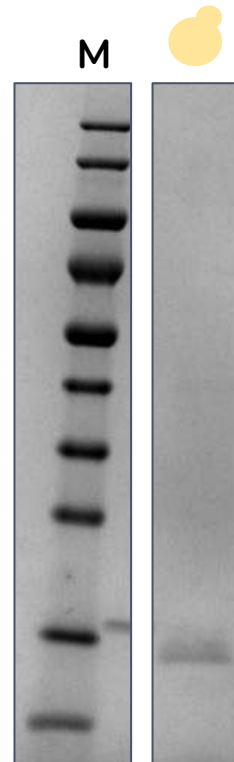
### Strain Creation

- Strain Lineage
- Knock-outs
- Knock-ins

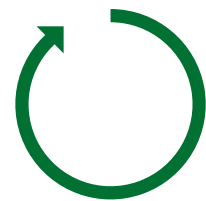


# Design Cycle Approach for a Single-Domain Antibody

## DESIGN CYCLE #1: INITIAL EXPRESSION

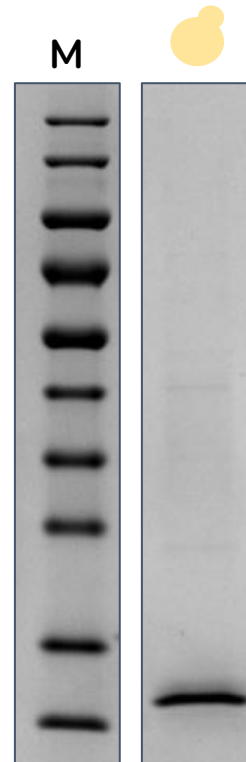


◀ Variant  
◀ Expected sdAb

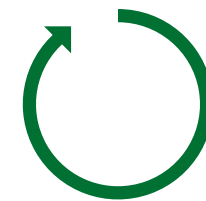


Modify sequence to  
reduce variants

## DESIGN CYCLE #2: SEQUENCE MODIFICATION

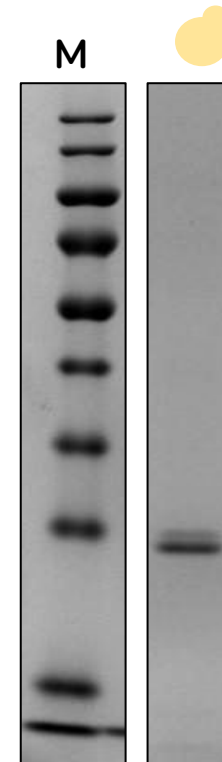


◀ Variant  
◀ Expected sdAb



Express dimer

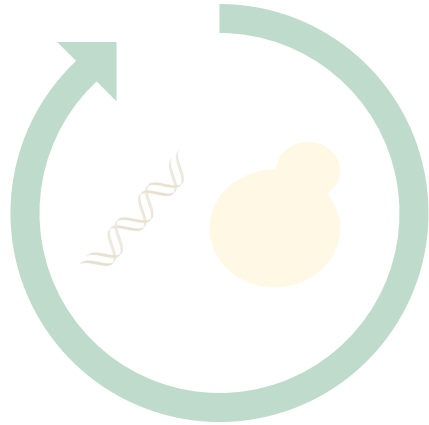
## DESIGN CYCLE #3: DIMER EXPRESSION



◀ Dimer sdAb

 *Unpurified* cultivation supernatant

# Eukaryotic Microbes Enable Simplicity in Multiple Ways



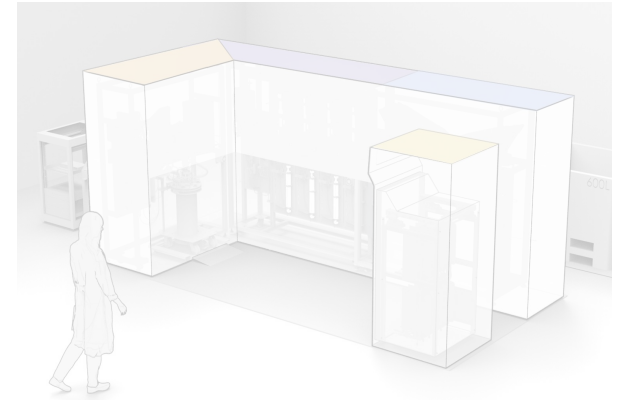
## RAPID DESIGN CYCLES

for parallel strain & sequence optimization



## PLATFORMABLE PROCESSES

using integrated and continuous techniques

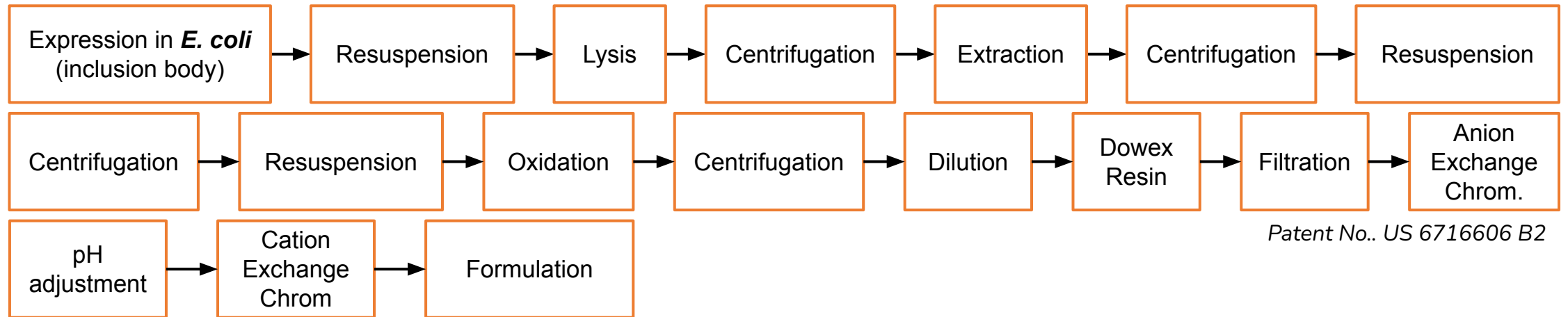


## NEXT-GENERATION EQUIPMENT

flexible, scalable, and user-friendly

# Conventional Processes Are Lengthy

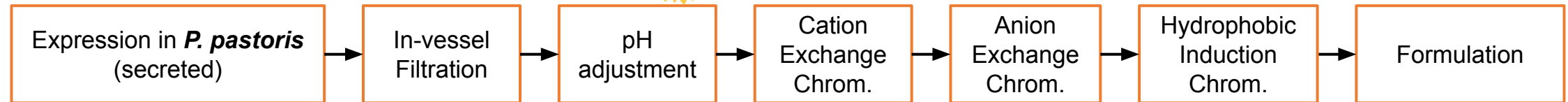
## G-CSF in *E. coli* (18 Step conventional process)



Patent No.. US 6716606 B2

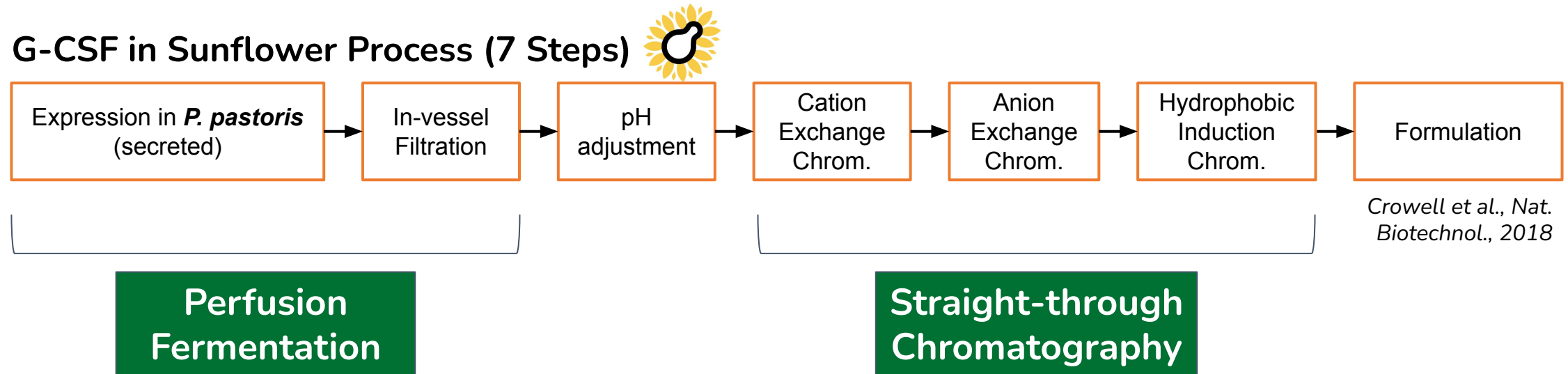
# Eukaryotic Microbes Enable Efficient Integrated Processes

## G-CSF in Sunflower Process (7 Steps)



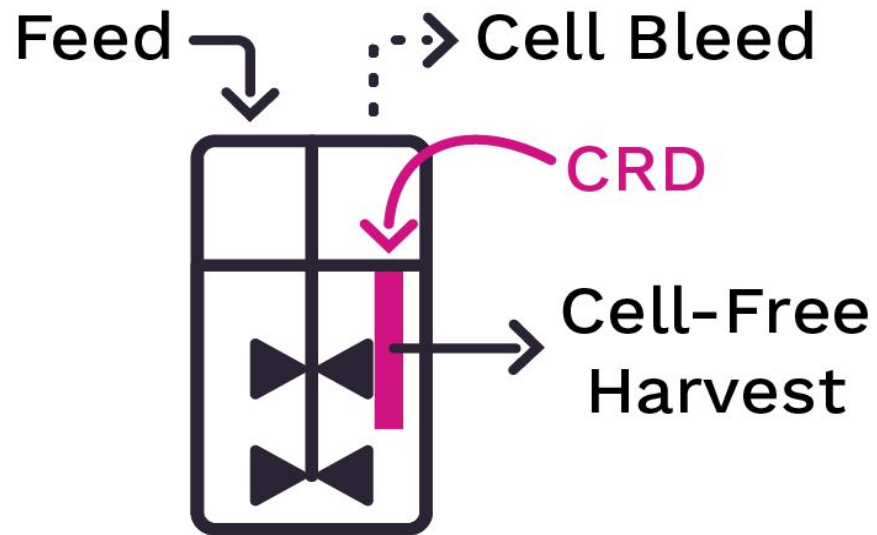
Crowell et al., Nat. Biotechnol., 2018

# Eukaryotic Microbes Enable Efficient Integrated Processes



Perfusion fermentation and straight-through Chromatography lead to a 60% reduction in the number of manufacturing steps

# Sunflower's Pioneering Technology Enables Perfusion Fermentation for Eukaryotic Microbes



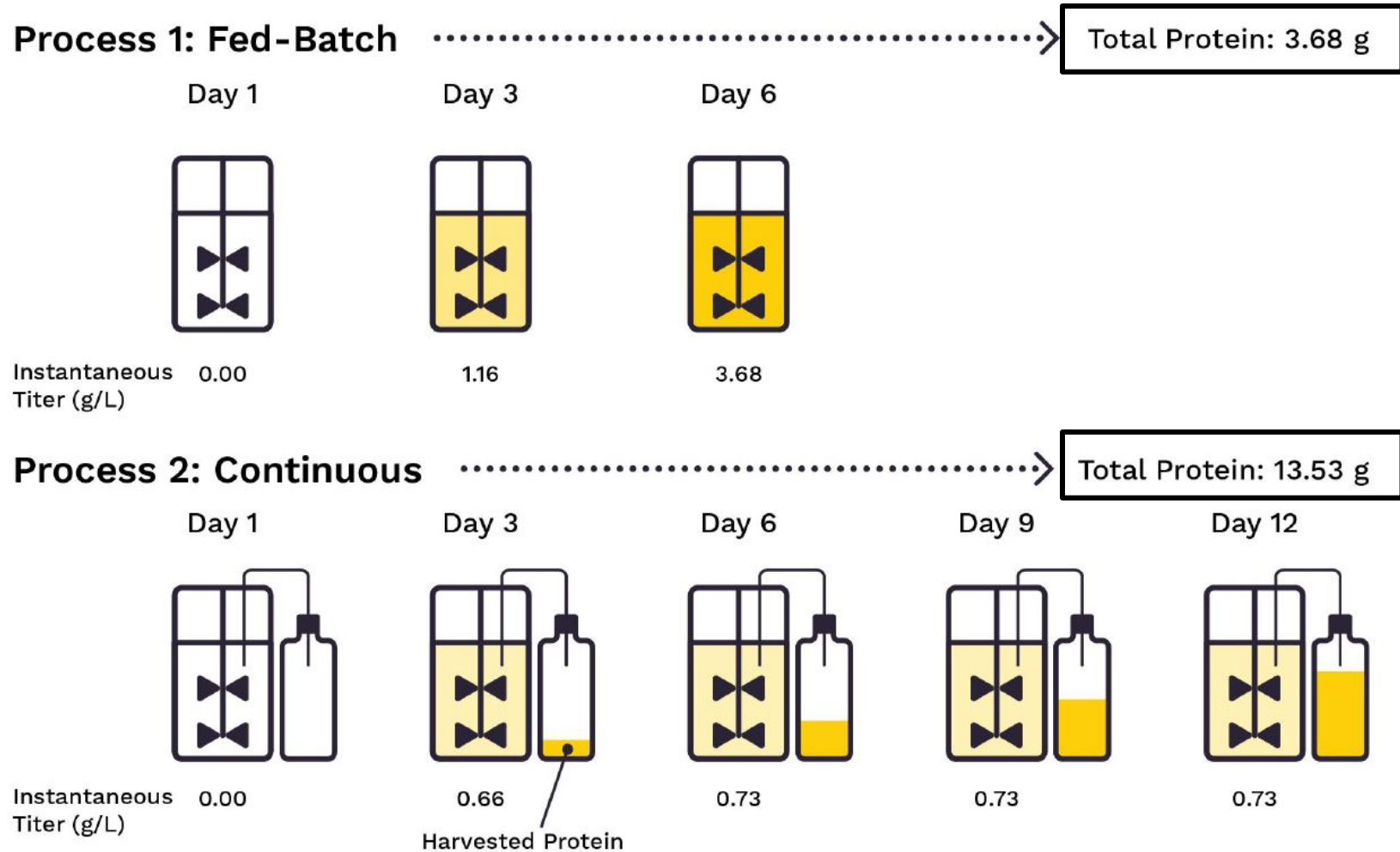
\*Cell Retention Device

## Benefits of Perfusion Fermentation

- **In-vessel cell retention device**
- Cells never removed from bioreactor environment
- Healthier, more consistent cell culture
- Longer campaigns
- Ultra high cell mass achievable and maintainable
- Continuous harvest of secreted protein

Sunflower's device and automated controls delivers the *only available perfusion capabilities* for fermentation

# Continuous Manufacturing with Eukaryotic Microbes Enables Productivity



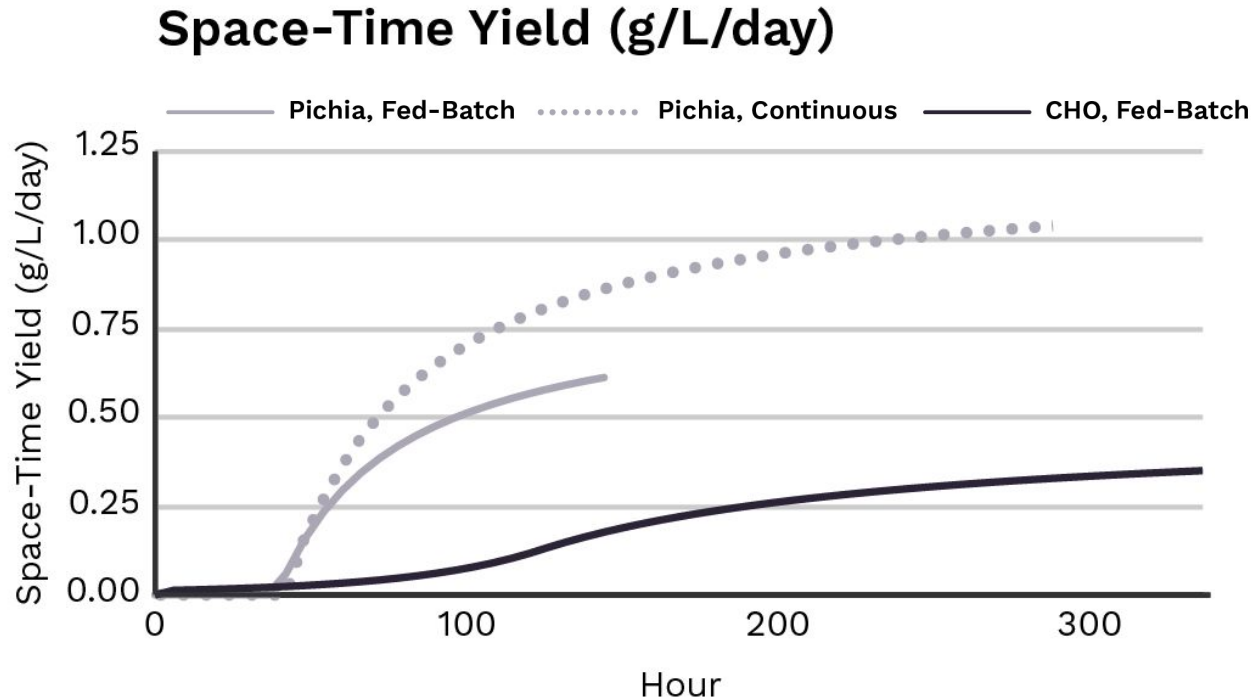


Are you familiar with the metric of space-time yield for measuring the productivity of a process?

- A. I've never heard of it!
- B. I've heard of it, but don't use it regularly.
- C. I regularly use space-time yield to evaluate my processes.



# Continuous Manufacturing with Eukaryotic Microbes Enables Productivity



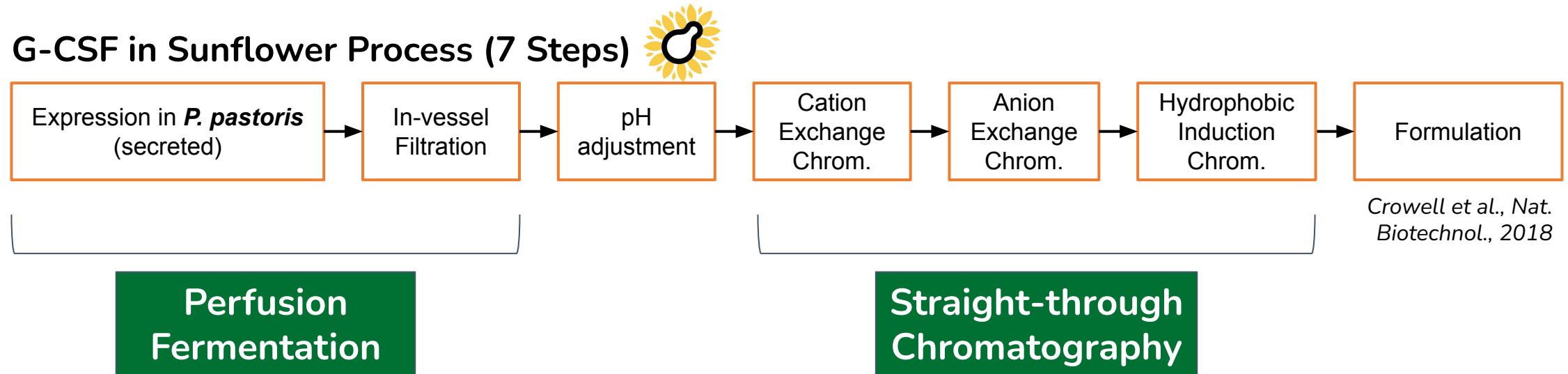
**Space-Time Yield** is a metric for normalized comparison of fermentations

*Space-Time Yield =*

**Mass of Protein per Bioreactor Volume per Cultivation Day**

Continuous manufacturing with *Pichia pastoris* enables significantly higher space-time yields

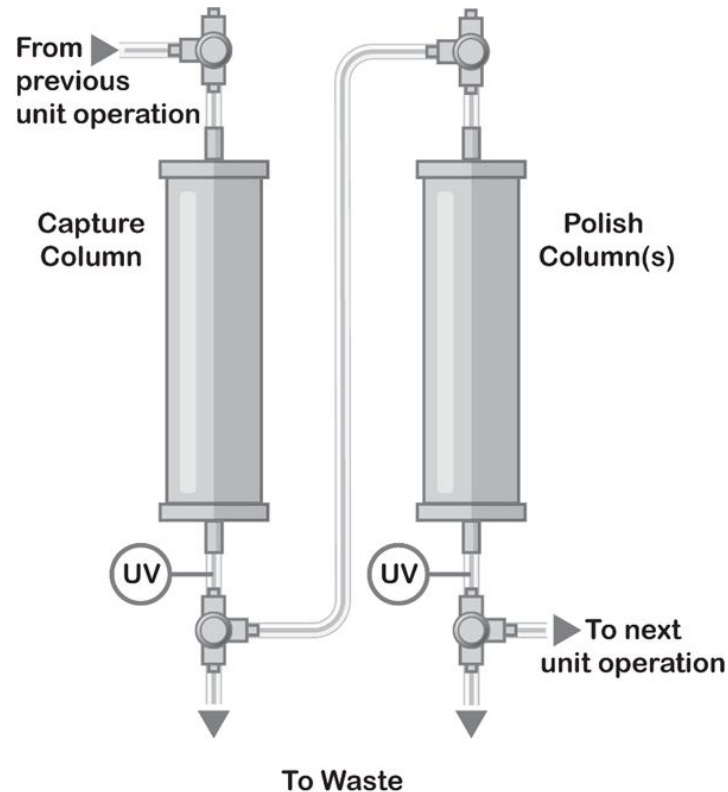
# Eukaryotic Microbes Enable Efficient Integrated Processes



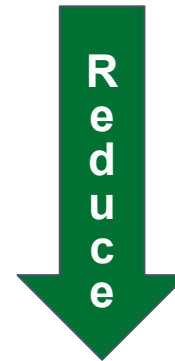
Perfusion fermentation and straight-through Chromatography lead to a 60% reduction in the number of manufacturing steps

# Integrated Chromatography Intensifies Manufacturing

## Straight-through chromatography



**Eliminate buffer conditioning  
(pH / salt changes)  
between chromatography steps**



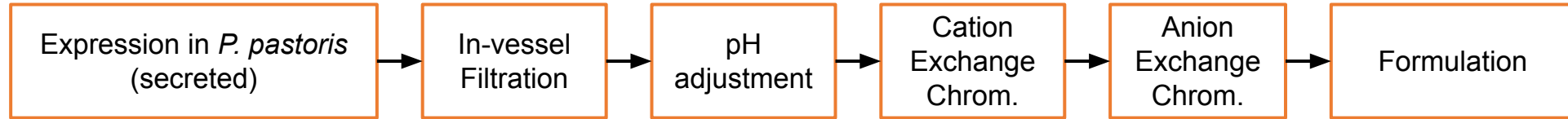
- # of hold tanks
- buffer usage
- processing time
- manufacturing footprint
- operator touch points

# Sunflower's Bioprocesses are Similar for Many Proteins



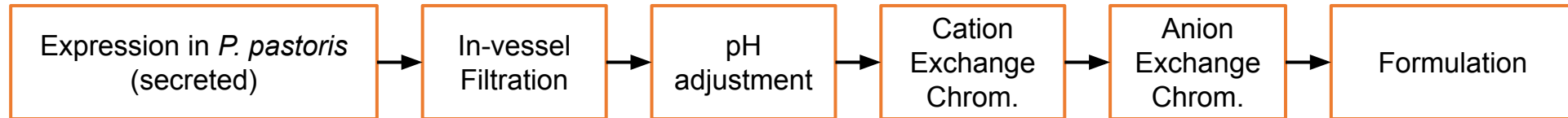
## Human growth hormone

Crowell et al., *Nat. Biotechnol.*, 2018



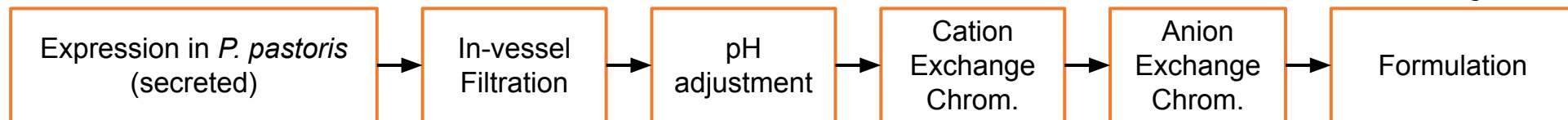
## Recombinant protein vaccines

Dalvie et al., *PNAS* 2021;  
Dalvie et al., *Microb. Cell Factories* 2021

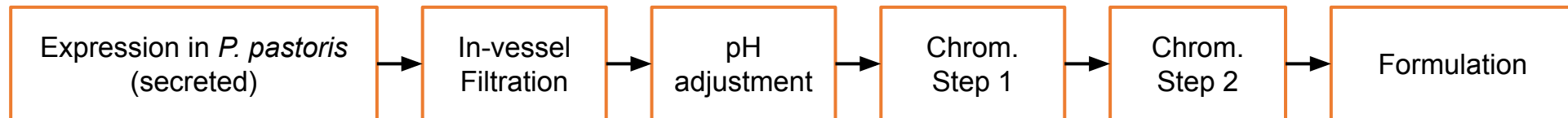


## Single-domain antibody (nanobody)

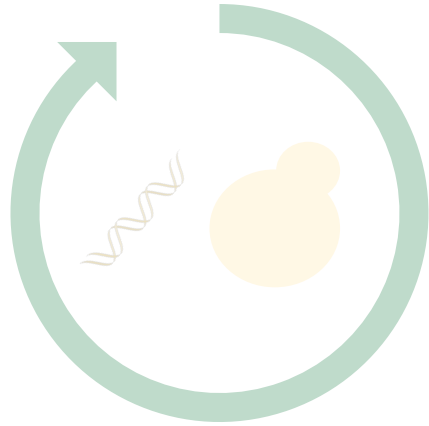
Crowell et al., *Biotechnol. Bioeng.* 2021



## Monoclonal antibody (mAb)



# Eukaryotic Microbes Enable Simplicity in Multiple Ways



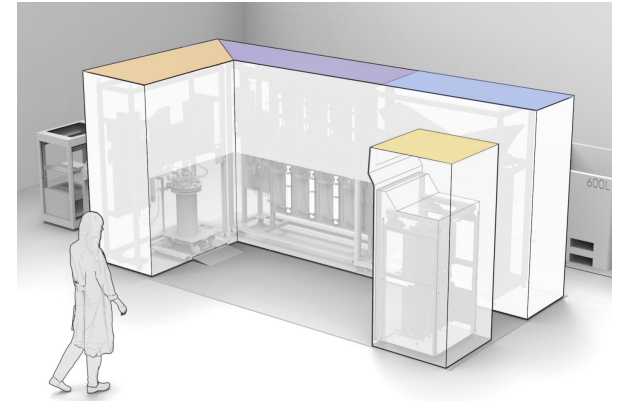
## RAPID DESIGN CYCLES

for parallel strain & sequence optimization



## PLATFORMABLE PROCESSES

using integrated and continuous techniques



## NEXT-GENERATION EQUIPMENT

flexible, scalable, and user-friendly

# Sunflower Continuous Manufacturing Systems: Automated Hardware to Enable Capacity Anywhere



## Daisy Petal™

### R&D/Discovery

Continuous Fermentation  
(up to grams per week)

*Commercially available now*

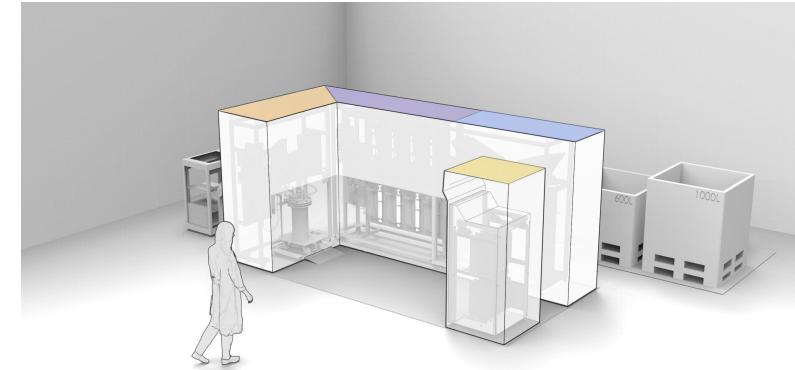


## Dahlia Petal™

### Translational Development

Continuous Fermentation  
(up to 100s of grams per week)

*Seeking early access customers*



## Dahlia™ System

### Efficient Commercial Production

Protein Bulk Production  
(up to 10 kg annually in <50m<sup>2</sup>)

*Early access launch in 2025*

**SUNFLOWER'S STANDARDIZED APPROACH FACILITATES PRODUCT TRANSITIONS**

# Daisy Petal™ Single-Use Bioreactor System: Perfusion fermentation for eukaryotic microbes



## EFFICIENT PRODUCTION IN A SMALL FOOTPRINT

- Bench-top sized but **produces like larger reactors** (up to grams protein in 1-2 weeks from 1L working vol.)
- Unique single-use structured consumable for **intuitive installation by non-expert users** and rapid **flexibility**
- Custom software enables **fully automated** operation
- **Immediately deployable** – just add cells & process fluids

## EARLY ACCESS UNITS DELIVERED SUMMER 2022

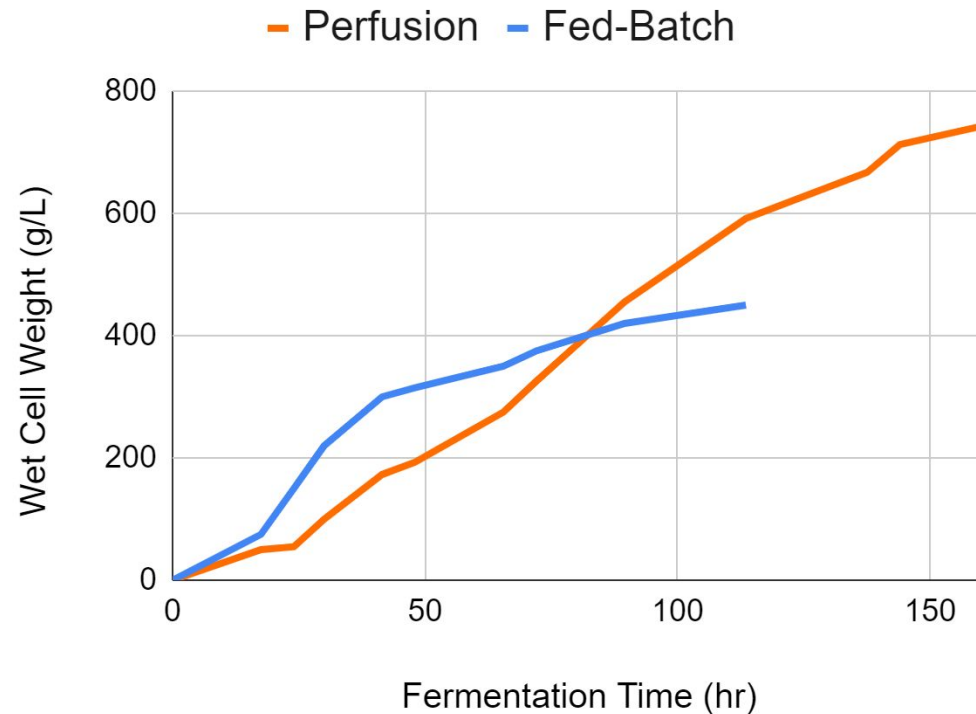
***“The Daisy Petal is really simple to use. I knew exactly how to get started with the system and consumables.”***

- Customer in Petal Early Access Program with no previous bioprocess experience

# Perfusion Fermentation Enables Ultra-High Cell Mass

CONTINUOUS FERMENTATION SUPPORTS WET CELL WEIGHT OVER 700 g/L

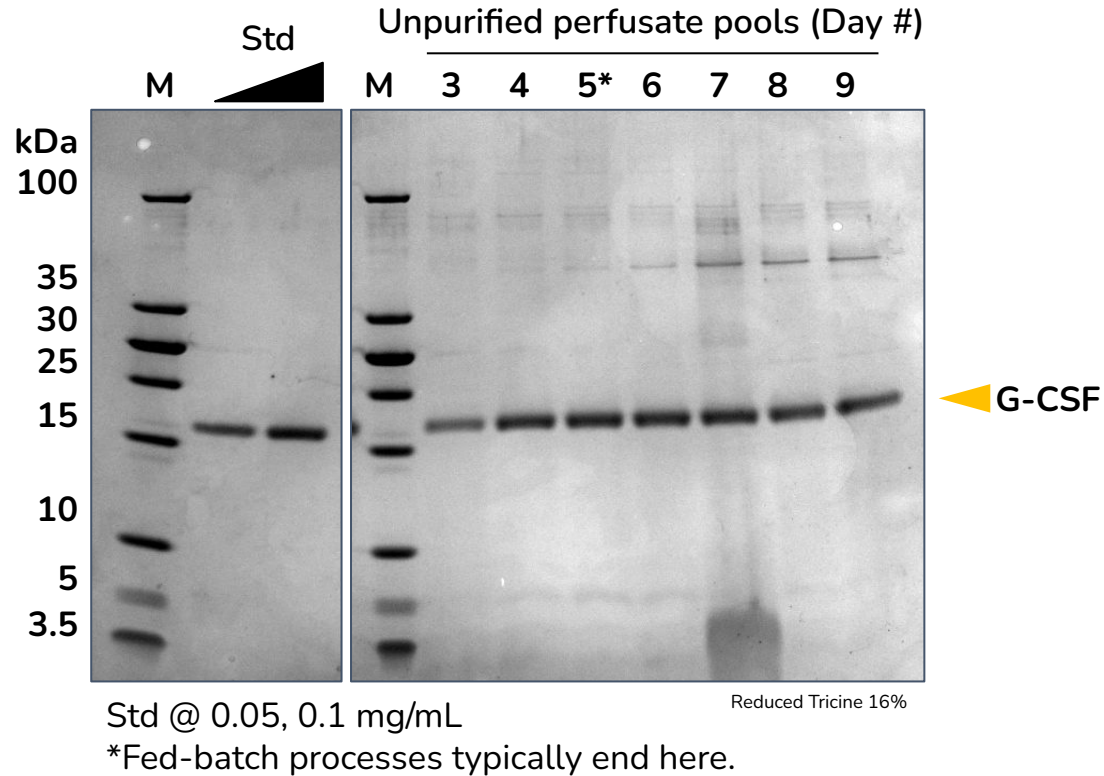
- **>700 g/L wet cell weight** achieved
- Up to 2-fold higher cell masses realized compared to fed-batch\*
- Cell mass maintained for 40% longer than fed-batch
- Perfusion fermentation enabled by proprietary in-vessel cell retention device



\*Typical fed-batch process according to [Invitrogen Pichia Fermentation Process Guidelines](#)



# Perfusion Fermentation Sustains Production of High-Quality Proteins (Cytokine Case Study)

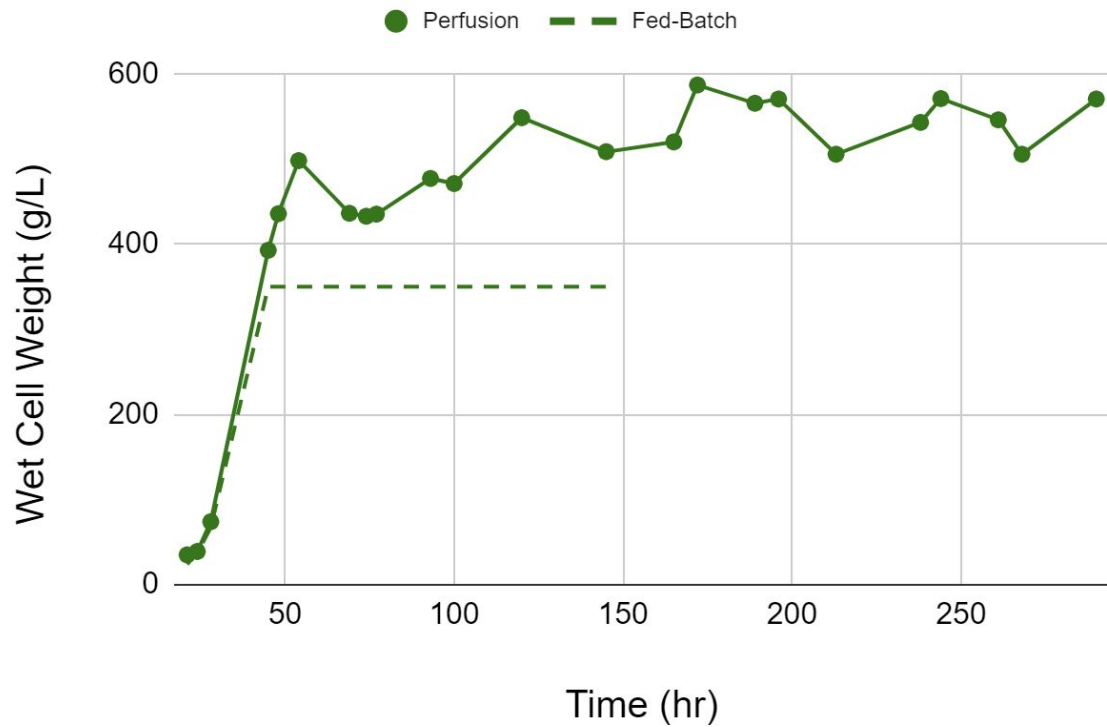


## CONTINUOUS MICROBIAL PRODUCTION IS MORE EFFICIENT THAN BATCH

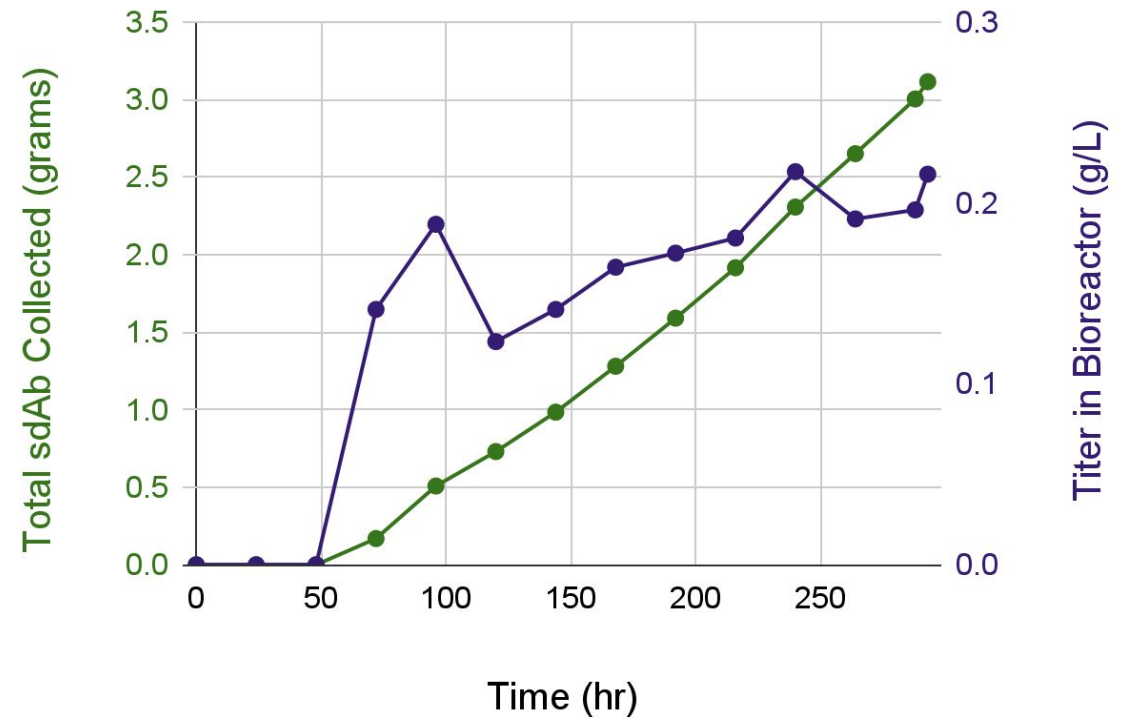
- **>1.2 grams** (4,200 dose equivalents) of unpurified G-CSF produced in **9 days** in 1 L working volume reactor
- Space-Time Yield: **>130 mg/L/day**  $\cong$  650 mg/L *Pichia* fed-batch titer  $\cong$  1.8 g/L CHO fed-batch titer
- Process ran **4 days longer** than conventional batch cultivation
- Perfusion fermentation enabled by proprietary in-vessel cell retention device

# Perfusion Fermentation Sustains Production of High-Quality Proteins (Single-domain Antibody)

MAINTAIN HIGHER CELL MASS FOR LONGER COMPARED TO FED-BATCH



COLLECT GRAMS OF PROTEIN FROM A SINGLE EXPERIMENT



# Sunflower Systems are Designed to Scale Easily

## Daisy Petal™



15 – 20L fluid output  
per 12-day campaign

Immediate  
Process  
Scale-up



## Dahlia Petal™



200 – 300L fluid output  
per 12-day campaign

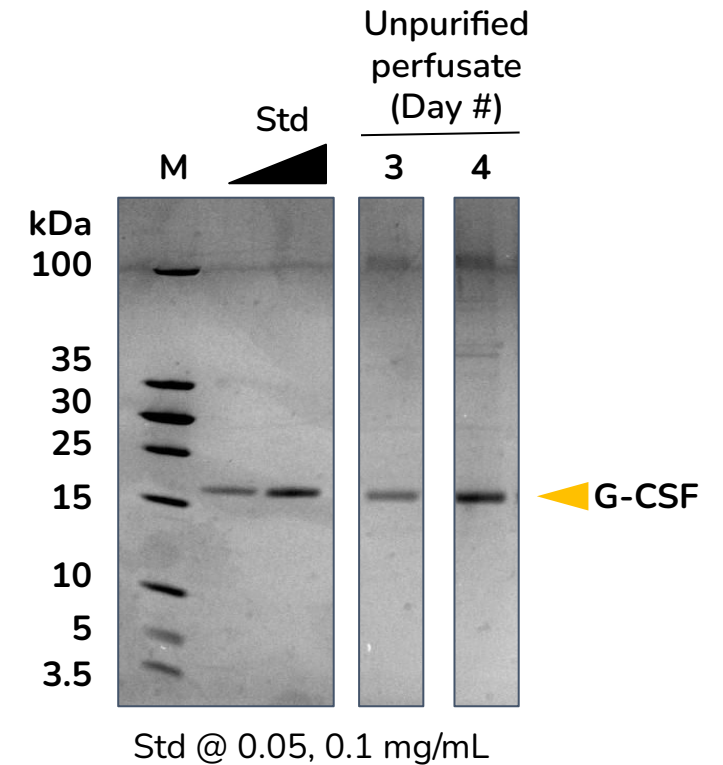
# Dahlia Petal™ is Equivalent to 200L Batch Bioreactor

## SCALE-UP TO DAHLIA PETAL™ RESULTS IN CONSISTENT SPACE-TIME YIELDS



**15L Working Volume**  
Can produce **100 grams**  
**protein per week** in **<20 m<sup>2</sup>**

- G-CSF successfully produced during hardware testing
- **>5 grams** G-CSF produced in only **4 days**
- Space-Time Yield: **>70 mg/L/day\***
- \*Space-Time Yield from Daisy Petal™ @ 4 days: 77 mg/L/day



# Projected Annual Product Output From Our Approach

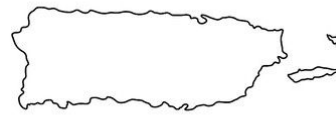
**VACCINE DOSES\***  
(50 ug / dose)

**MAB (Trastuzumab) DOSES\*\***  
(1 g / dose)

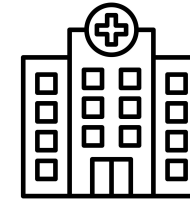


2.9  
Million

**Puerto Rico**



580



**Hospital  
or  
Medical  
Center**



40  
Million

**California**



**Argentina**

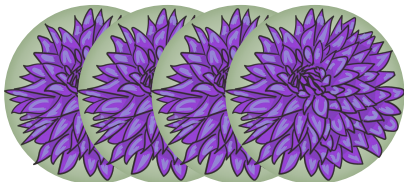


OR

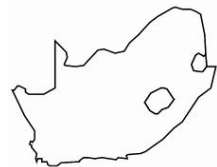
9,000



**Colombia**



160  
Million



**2.5x  
South Africa**

36,000



**Southern  
Africa**

\*Medium titer process (1 g/L);  
50 - 70% population vaccinated; 2 doses per person

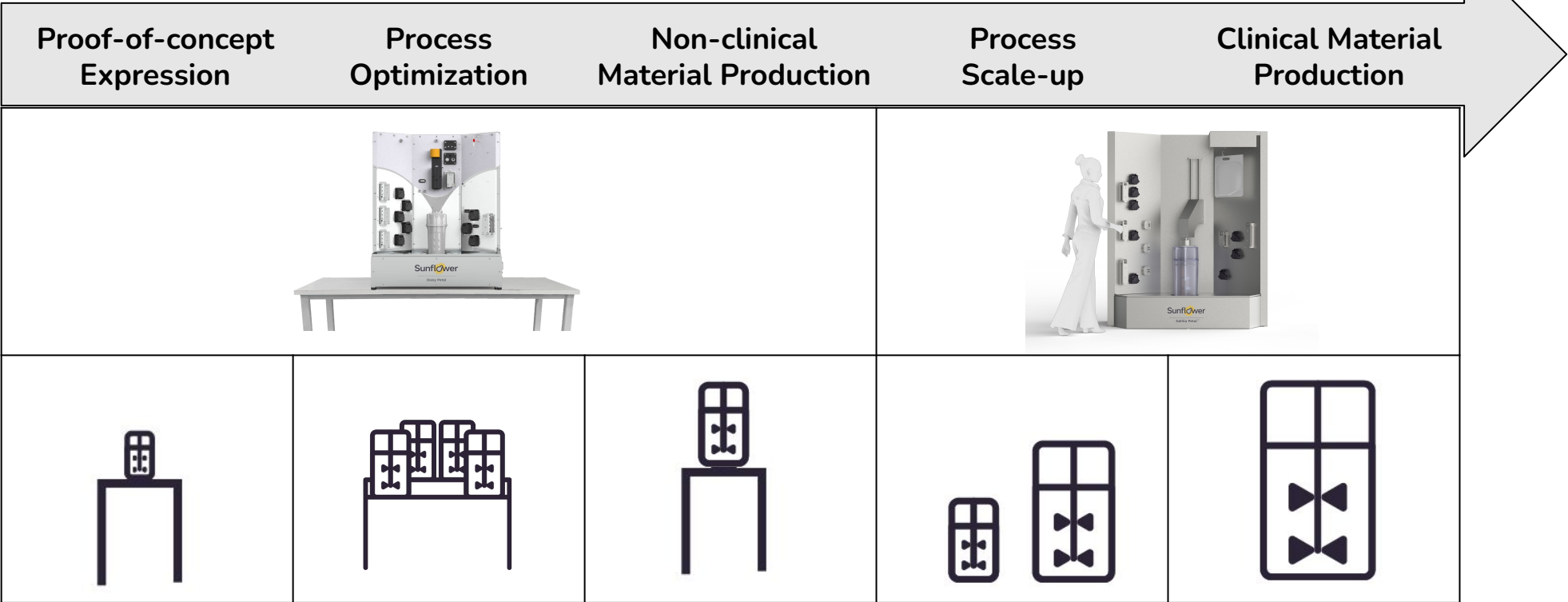
\*\*High titer process (4 g/L); HER2+ breast cancer patients;  
18 doses per person per year; 30-50% market penetration


# Sunflower's Equipment Approach Efficiently Supports Product Translational Development Activities

## KEY DEVELOPMENT ACTIVITIES FROM DISCOVERY TO THE CLINIC



LEGACY  
FED-BATCH



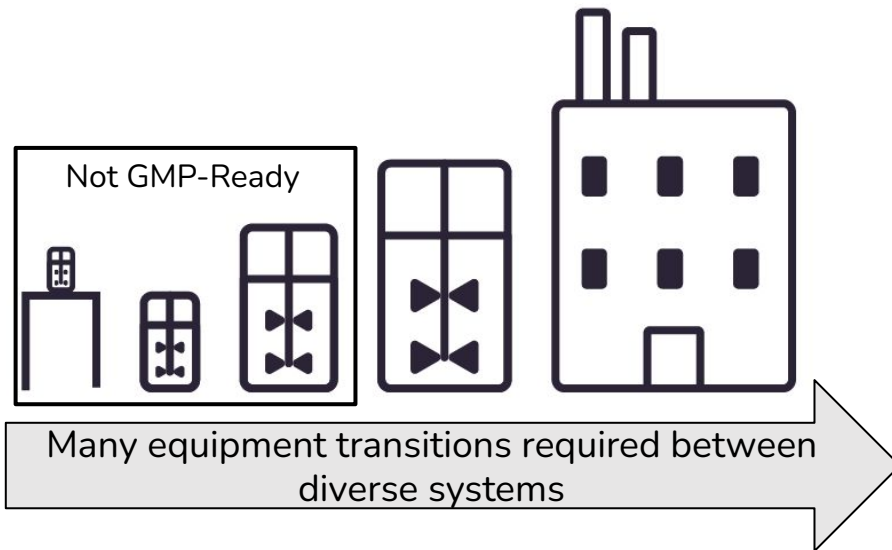


**How many pieces of bioreactor equipment do you typically use between proof-of-concept expression and clinical material production?**

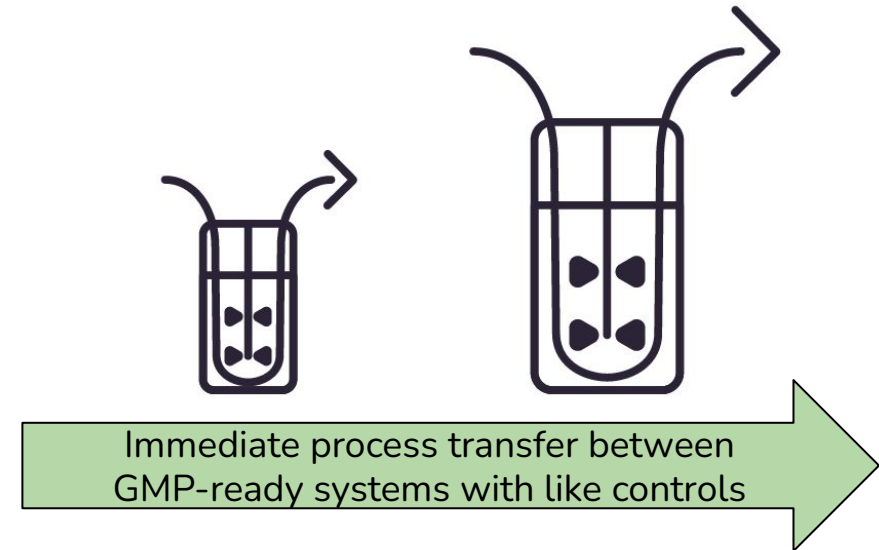
- A. 1-2**
- B. 3-4**
- C. 5-6**
- D. 7 or more**

# Sunflower's Approach is Efficient in Scaling to GMP-ready Operations & Clinical Volumes

LEGACY APPROACH IS INEFFICIENT



 **SUNFLOWER'S APPROACH IS SIMPLE**





# Dahlia™ System: Breakthrough automated small footprint commercial protein manufacturing

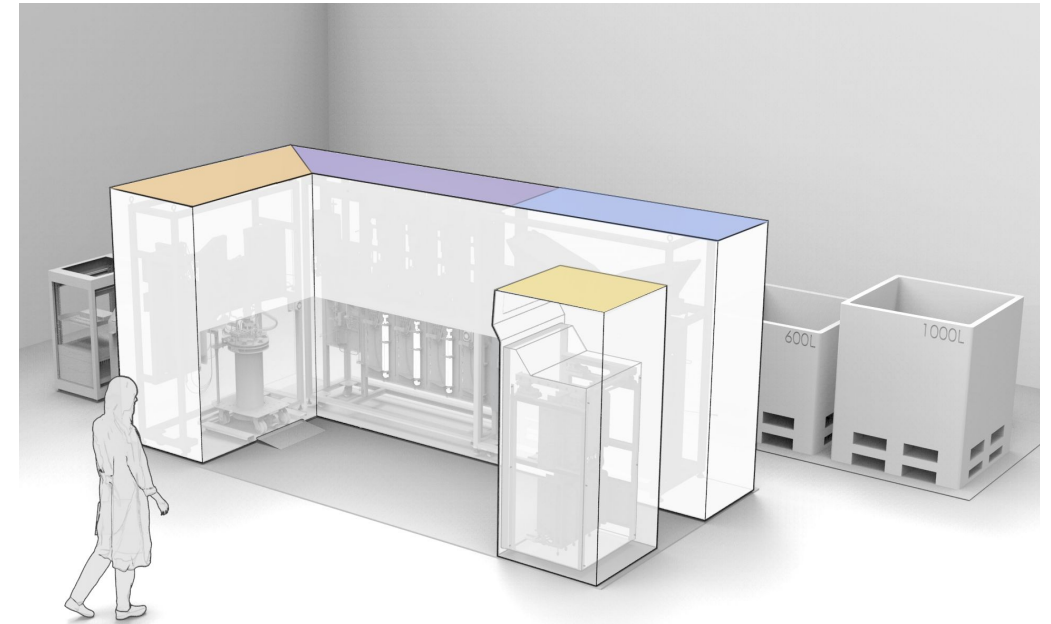
## INTEGRATED SYSTEM FOR CONTINUOUS PRODUCTION OF PROTEIN BULK

**End-To-End:** Integrated operations for expression, purification, formulation and collection

**Multi-Product:** Functionally-closed single-use design for agility and flexibility

**Fully Automated:** Add cells, push start, & collect protein bulk for a product in just days

**“Move-In” Ready:** Operates in many different types of spaces and environments



# Dahlia™ Produces High-Quality Proteins Anywhere

DEMONSTRATION RUNS\* PRODUCED CLINICAL QUALITY BULK DRUG SUBSTANCES IN SIMPLE MANUFACTURING ENVIRONMENTS

2

Different Proteins  
Made & Formulated in a  
**Uncontrolled Warehouse  
Environment**

Protein bulk produced	Dose equivalents	Host-Cell DNA (ng DNA/mL)	Host-Cell Protein	Bioburden (CFU/plate)
G-CSF	>1,000	<10	<0.1%	0
COVID-19 Vax Candidate	>50,000	<10	<0.1%	0
Clinical Benchmarks	N/A	< 10	<0.1%	0

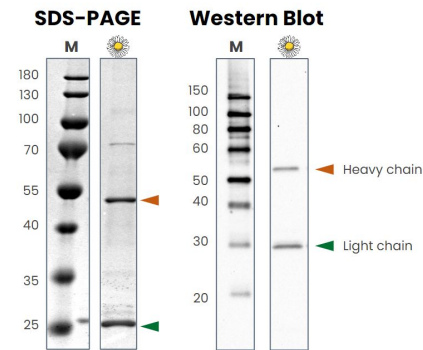
*Other quality attributes also similar to innovator products. Bioburden testing was performed by third-party contractor according to the Compendial method USP 61 microbiological examination of Non-Sterile Products (Microbial enumeration test).*

*\*Demonstrations funded by US DoD and BMGF*

# Sunflower Systems Support Diverse Products

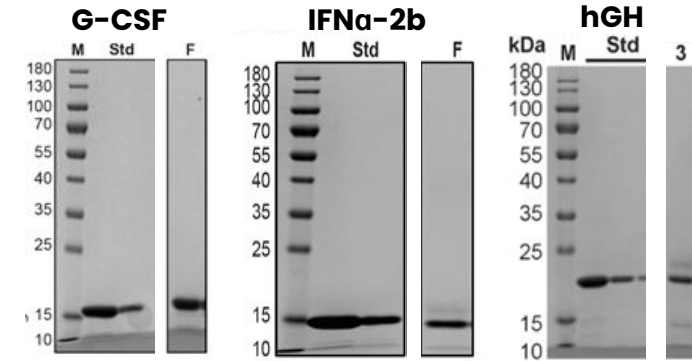
## ANTIBODIES & MAB-LIKE PRODUCTS

- Monoclonal antibodies
- Single-domain antibodies (sdAb) & multimers
- Fragment antigen-binding regions (Fabs)
- Fc-fusions



## CYTOKINES & HORMONES

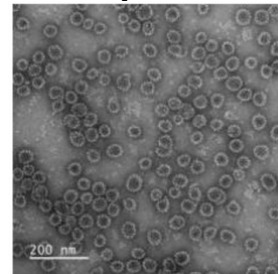
- G-CSF
- IFN $\alpha$ -2b
- hGH
- Single-chain insulins



## VACCINE ANTIGENS

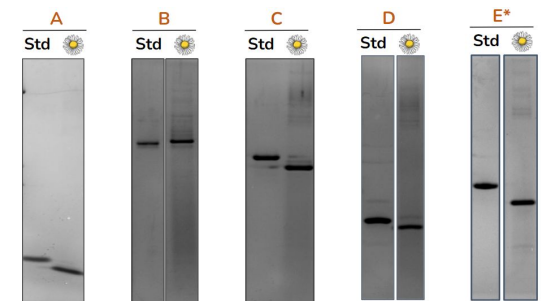
- COVID subunit vaccine candidates
- Rotavirus subunit vaccine candidates
- Virus-like particles (VLPs)
- Additional viral & bacterial antigens

VLPs expressed in *P. pastoris*



## ENZYMES & OTHER PROTEINS

- DNA / RNA editing enzymes
- Bacterial toxins
- Membrane proteins
- Small binding proteins
- Peptides



Crowell et. al. Nat. Biotechnol. 2018; Dalvie et. al. Microb. Cell Fact. 2021; Dalvie et. al. PNAS 2021; Crowell et. al. Biotechnol. Bioeng. 2021

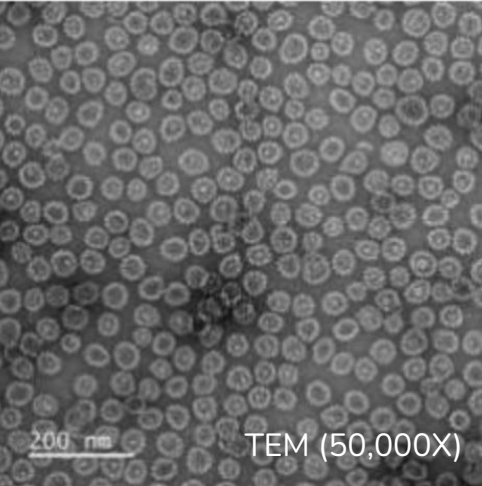


## Case Study: Manufacturing VLPs in *Pichia pastoris*

# Yeast-produced material forms VLPs and shows similar efficacy to insect cell material

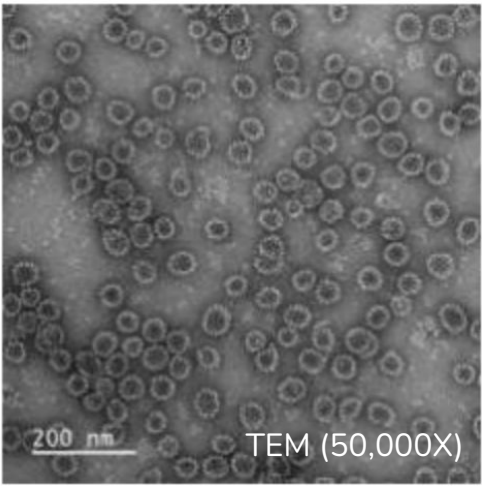
## VLP Formation (assessed by TEM)

from Insect Cells



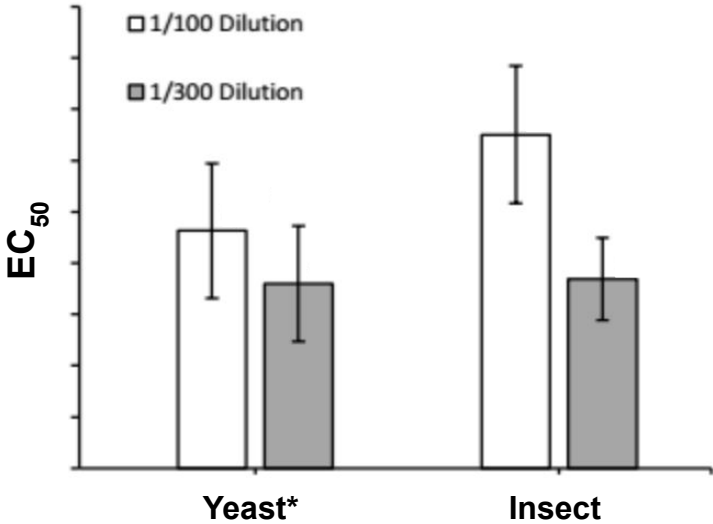
Z-Ave: 58.1 nm (SD: 0.5)

from Yeast\*



Z-Ave: 52.2 nm (SD: 0.4)

## VLP Efficacy (assessed by ELISA on mouse serum)

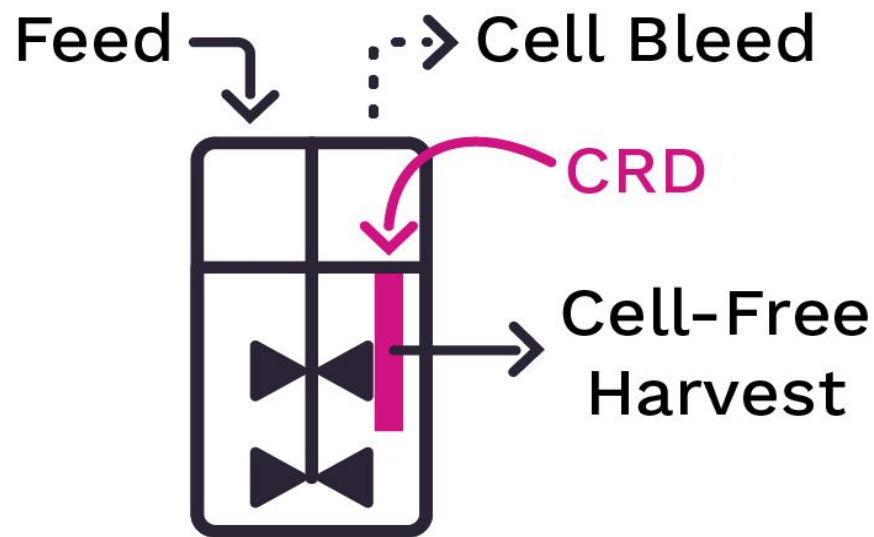


**VLPs produced in yeast from unoptimized purification process shows similar quality and efficacy to material generated in optimized insect cell process.**

\*Yeast material cultivated in shake flask and purified using minimally developed method; Insect cell material created using optimized process

# Utilizing Daisy Petal™ for perfusion fermentation

Sunflower's Daisy Petal™ single-use bioreactor system was used to cultivate VLPs (intracellularly) by perfusion fermentation



\*Cell Retention Device

## **Benefits of perfusion fermentation for intracellular expression**

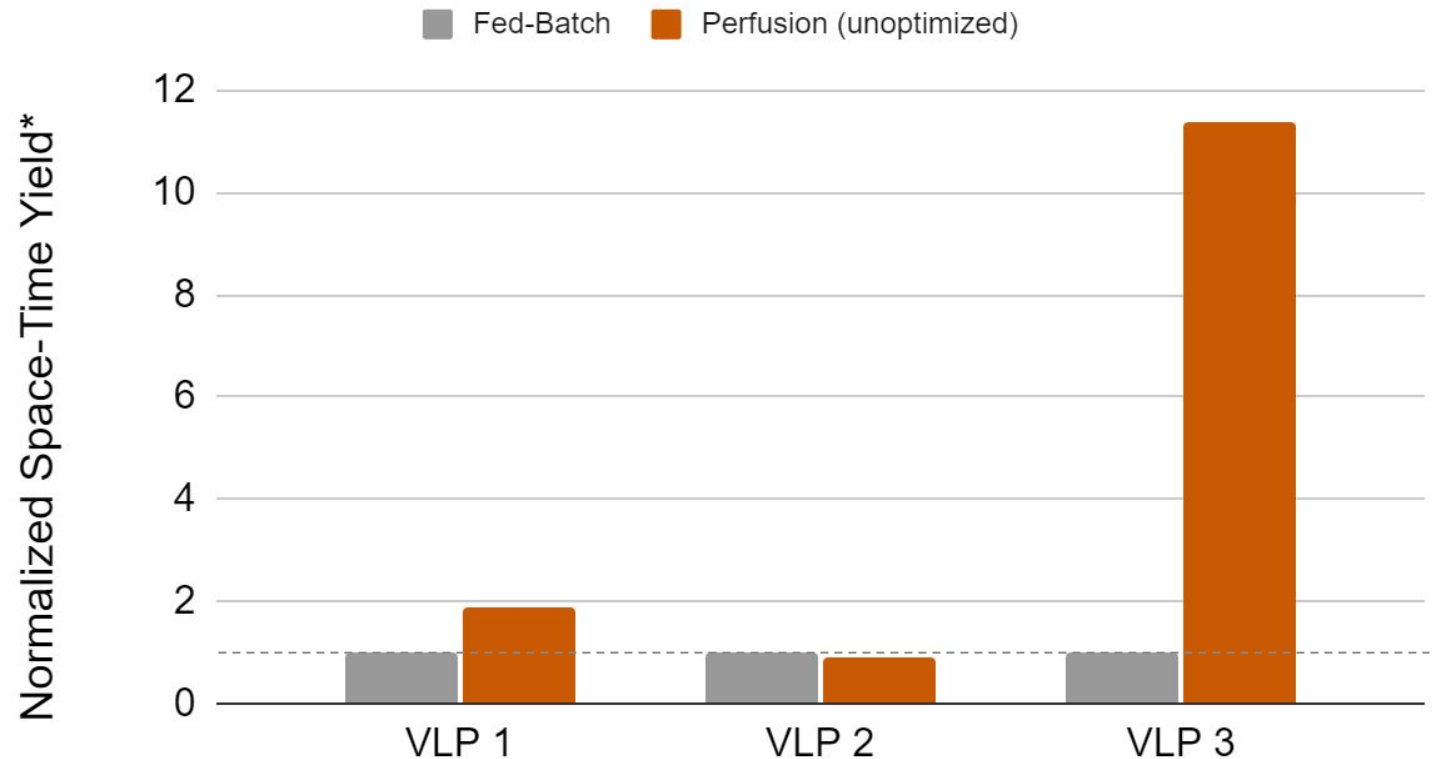
- Cells are harvested continuously through the cell bleed line and at the end of the cultivation
- Continuous feeding and continuous removal of waste products leads to
  - Higher, healthier biomass
  - Longer production campaigns

# Comparison of Space-Time Yield in Fed-Batch vs Perfusion

- Space-Time Yield = **Total protein collected** per **cultivation volume** per **cultivation length**
- Total protein collected measured by serotype-specific ELISA following cell lysis

**Unoptimized perfusion fermentation shows similar or higher space-time yield compared to fed-batch fermentation**

Fed Batch vs. Perfusion



\*Normalized to Fed-Batch space-time yield



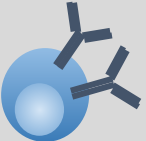
# Eukaryotic Microbes Offer Key Opportunities for Protein Development and Manufacturing



# Key Opportunity: Rapid Product Development with Yeast

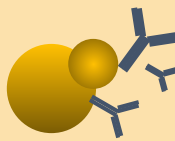
## Discovery (5-7 days)

Established Research Centers  
& Commercial Services



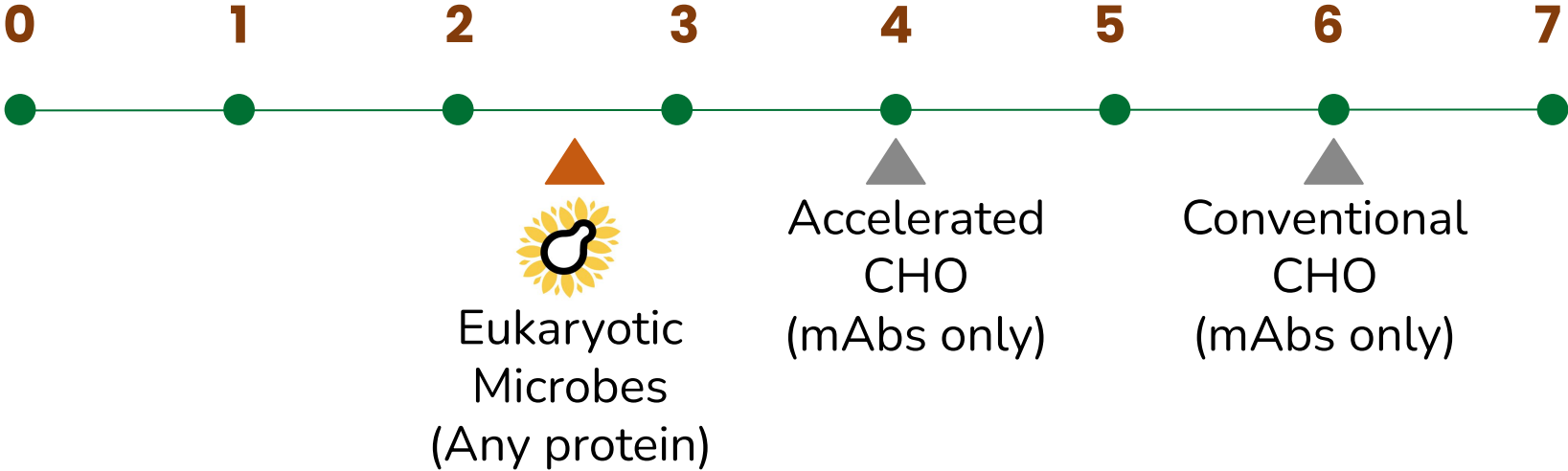
## Translation (28-42 days)

Strain Preparation, Process  
Development & Execution



## Production (7-10 days)

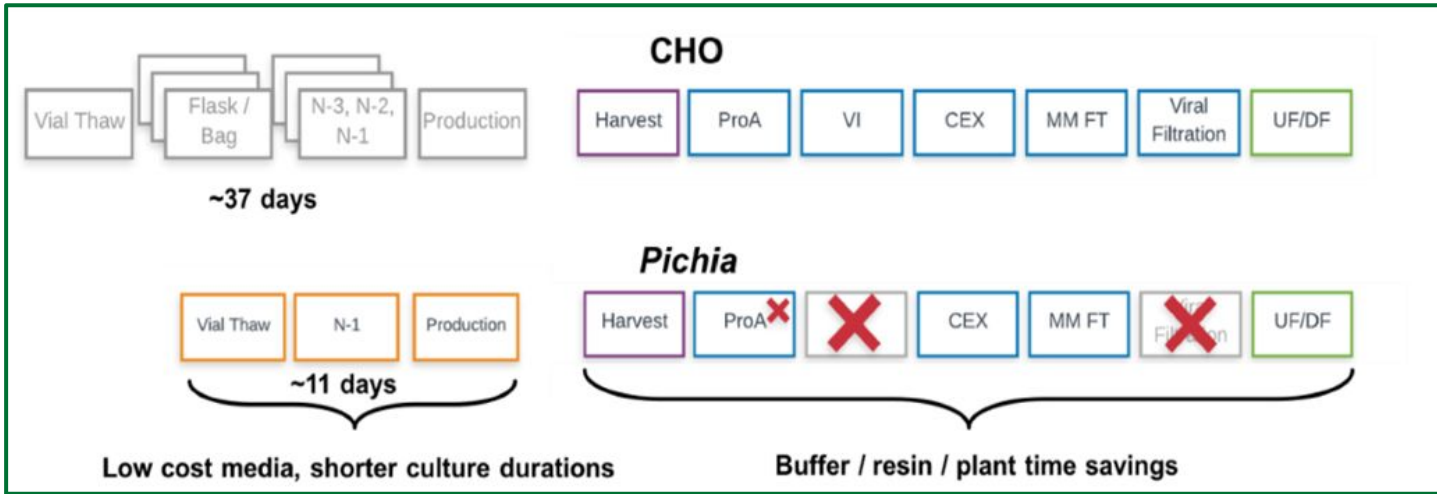
Stand-Alone or Parallel  
Manufacturing for IND Drug Substance



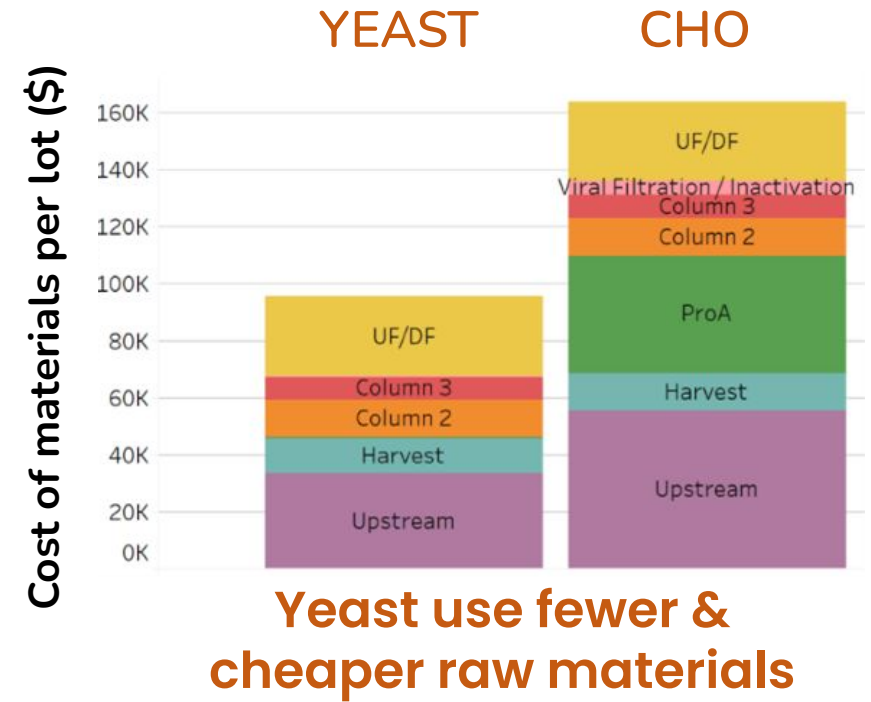
**Months to IND-ready  
Drug Substance  
based on platform  
processing**

*Timelines based on Brady and Love, Nature Biotechnol. 2021;  
Kelley, Nature Biotechnol. 2020*

# Key Opportunity: Cost Effective Manufacturing with Yeast



Yeast-based processing is faster and simpler

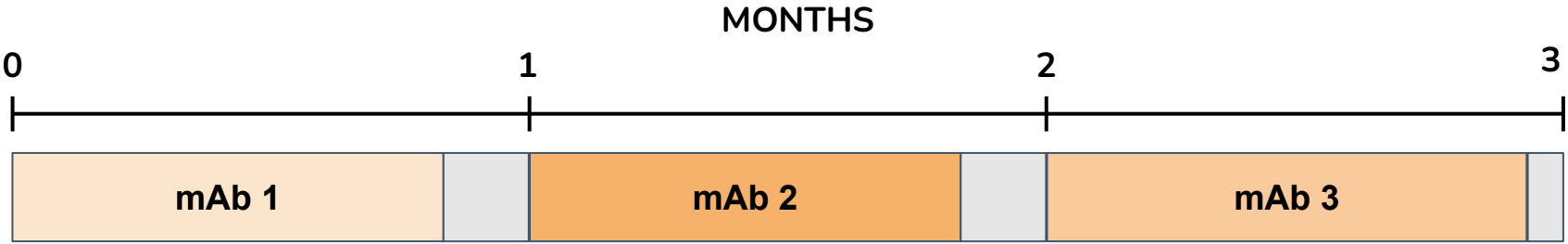


Yeast could achieve similar costs to CHO at ~50% titers

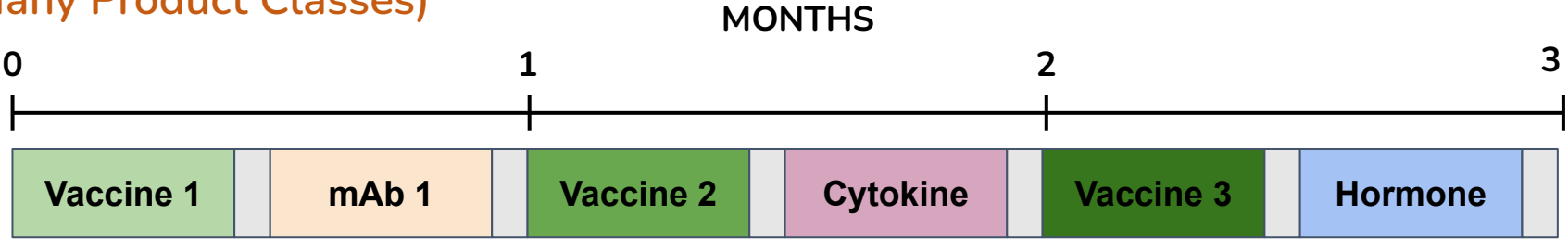
E. Coleman, MIT/Amgen; LGO Master's thesis 2020

# Key Opportunity: Improved Agility with Yeast

STATE-OF-THE-ART SINGLE-USE PILOT PLANT

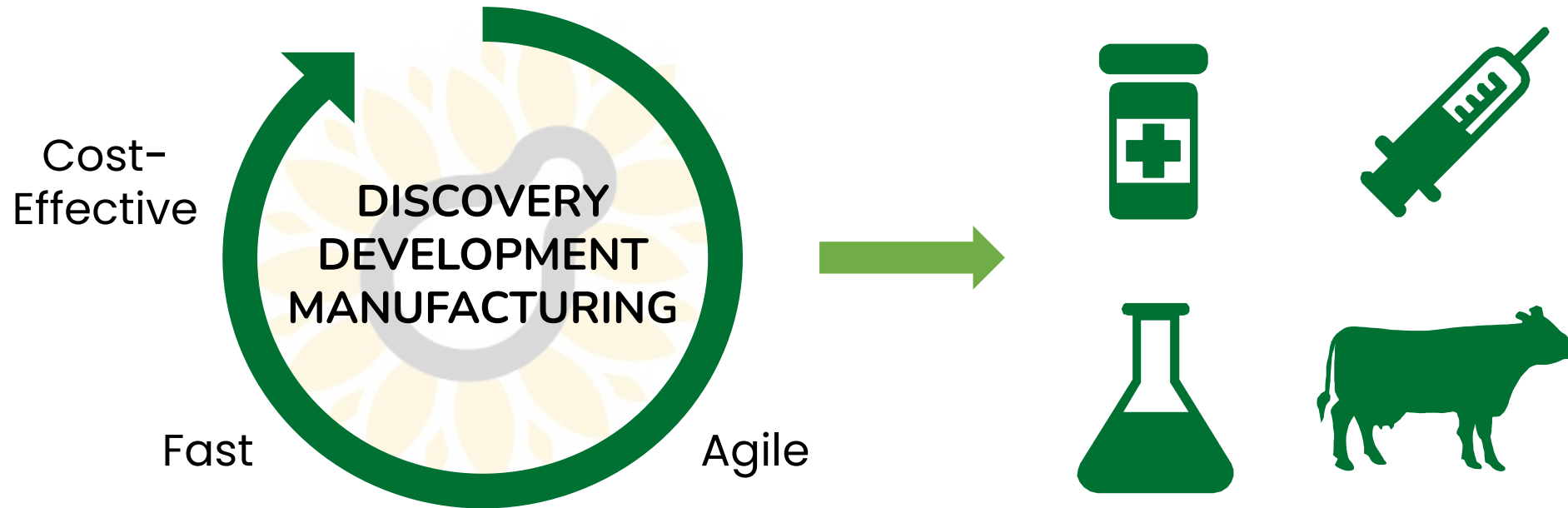


SUNFLOWER (Many Product Classes)



**Multi-product capability enables agility and improved facility utilization**

# Sunflower's Approach Supports Product Translation



Iterative development enabled by Sunflower's technologies can reduce translational timelines and development costs

# Acknowledgements



## Sunflower Team

**Alexandra Bonnyman** Kerry Love  
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Sophie Lee Mary Kate Tracey  
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