Ensuring the Quality of Therapeutic Proteins

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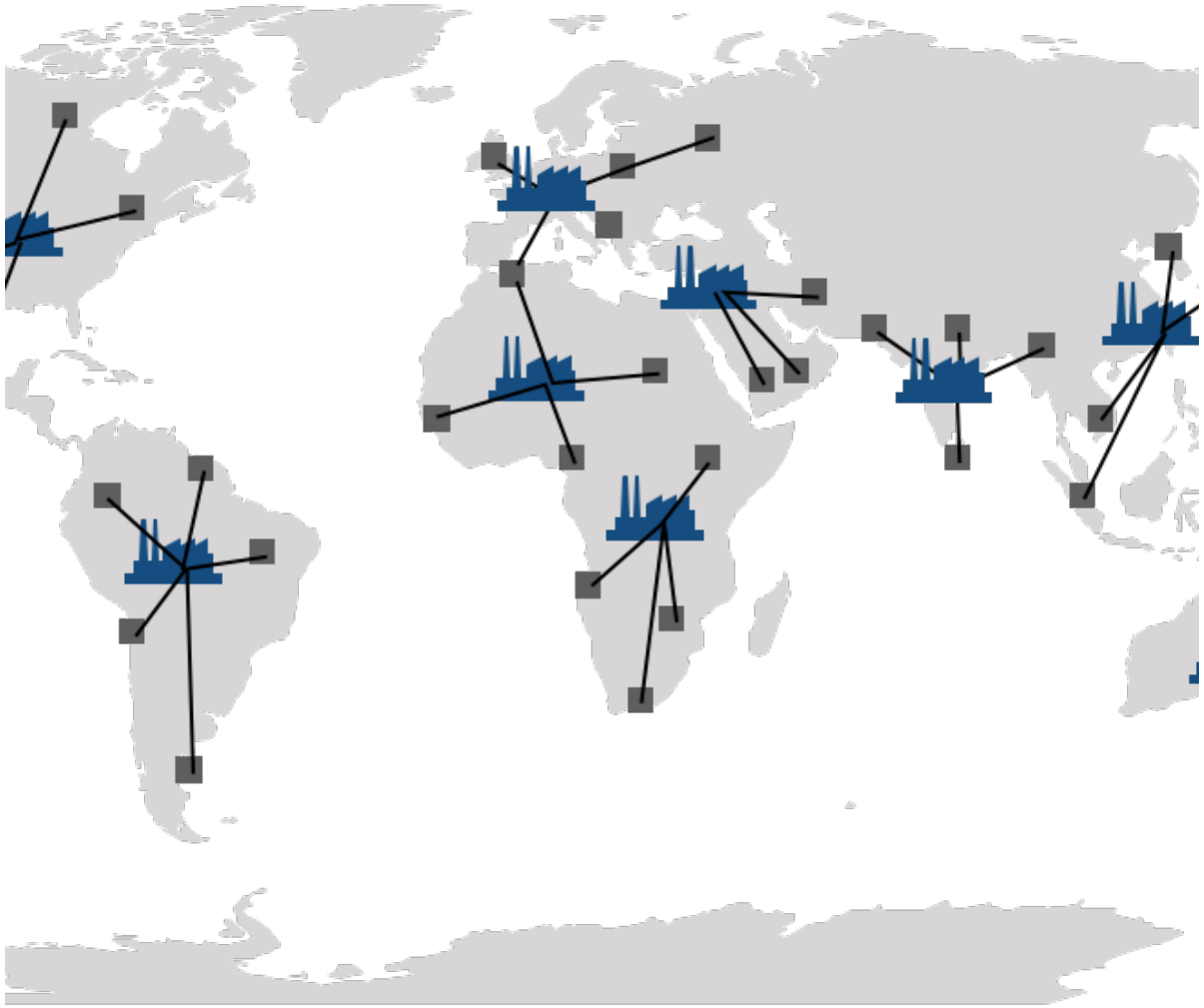
KOCH INSTITUTE
for Integrative Cancer
Research at MIT

What Are Potential Benefits to Establishing a Practical Regulatory Framework for Distributed (and Point-of-Care) Manufacturing Networks for Biologics?



Patients

- Improved diversity and accessibility to products (from small indications to big ones)
- Enhanced quality of manufactured products (coinciding with enabling technologies)

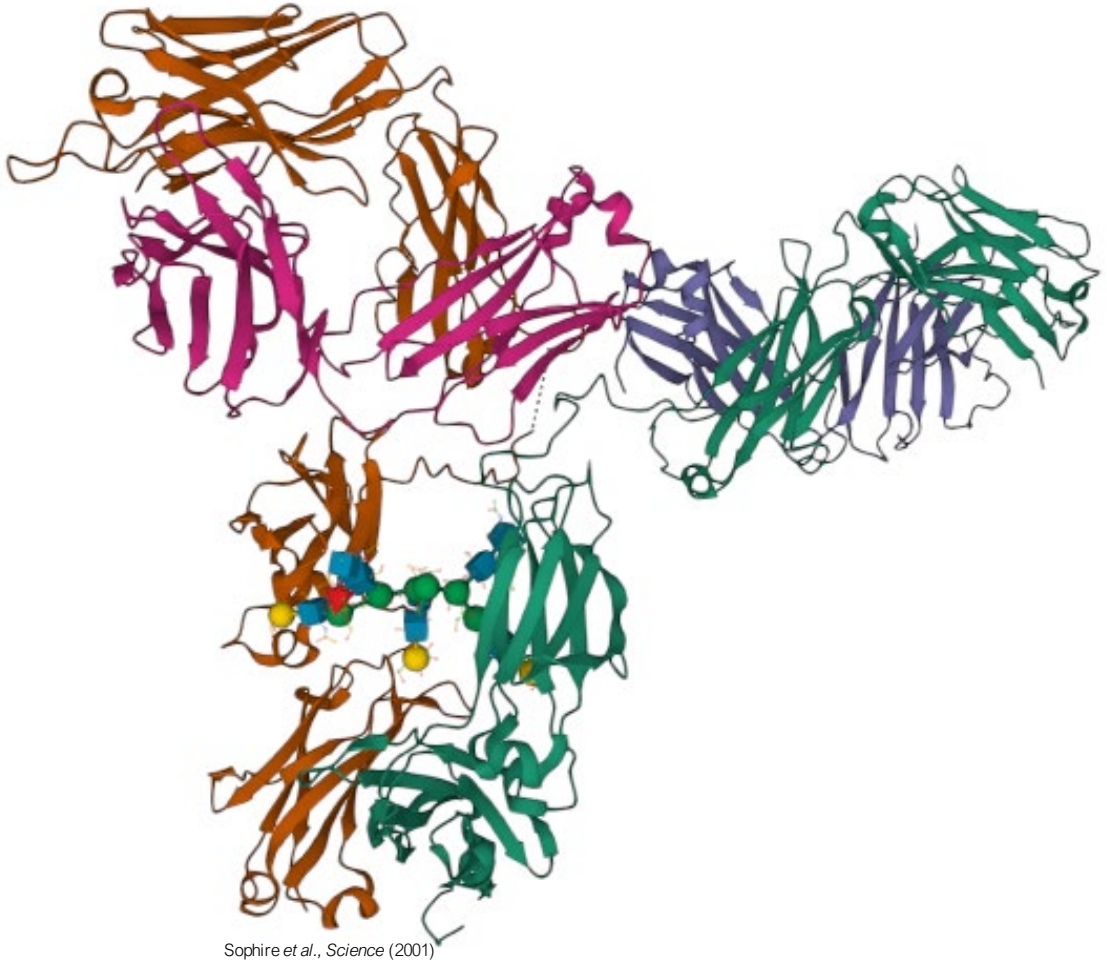
Regulatory Review

- Increased data about how manufacturing processes relate to product quality
- Expanded application of concepts for continuous verification and QbD principles

Global Health

- Better reliability of manufacturing supply
- Promote economic development in healthcare

Establishing Quality of Protein-Based Biologics



Sophire *et al.*, *Science* (2001)

…Relies on a Holistic Control Strategy

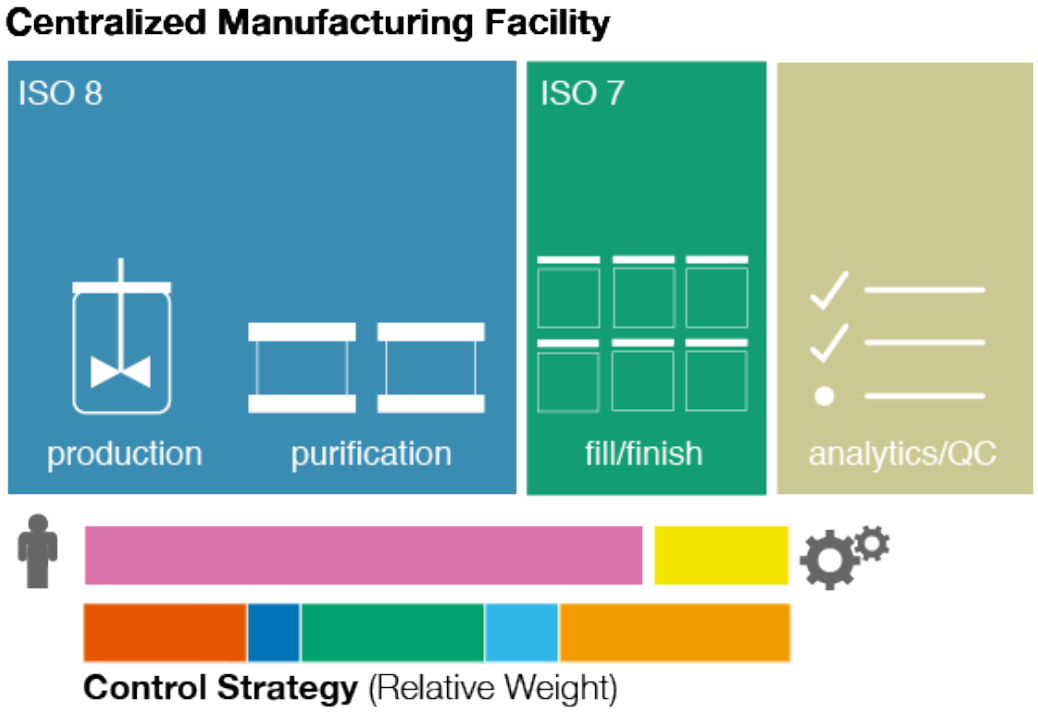
- Set of controls (procedures and analyses) that together assure "process performance and product quality" (ICH Q11)
- May include controls for each step of production, *[where appropriate (21 CFR 211)]* :
  - Raw Materials
  - Environmental / Facility Considerations
  - Limits on Manufacturing Processes (Operational parameters; Sequence of Operations)
  - In-Process Tests or Data
  - Release Testing on Drug Substance or Product or Both
- *Why is this important?*
  To enable consistent manufacturing and products

Considerations for Biopharmaceutical Quality

Elements of a Control Strategy for Enabling Good Manufacturing Practice (GMP)

Facility	Any building or buildings used should be of appropriate size, construction and location for cleaning, maintenance and operations (*21 CFR 211 Subpart A*)
Raw Materials	Appropriate for intended use and clear of biological contaminants or impurities that could affect the quality (*ICH Q6B; Q11*)
Process Design	Definition of the operations and controls that will enable manufacturing
Process Controls	Assurance of batch uniformity and integrity of process/product through manufacturing process (*ICH Q6B; 21 CFR 11 Subpart F*)
Specifications	Attributes of the product that assure identity, purity, potency and safety

Every Manufacturing Strategy Has Unique, Intrinsic Potential Risks



Facility

- Contamination of open process equipment
- Has significant operator traffic in controlled spaces

Raw Materials

- Biological ingress of adventitious agents (bacteria, mycoplasma, virus)

Process Design

- Complex requirements for recovery using many process operations
- Aseptic handling of large fluid volumes

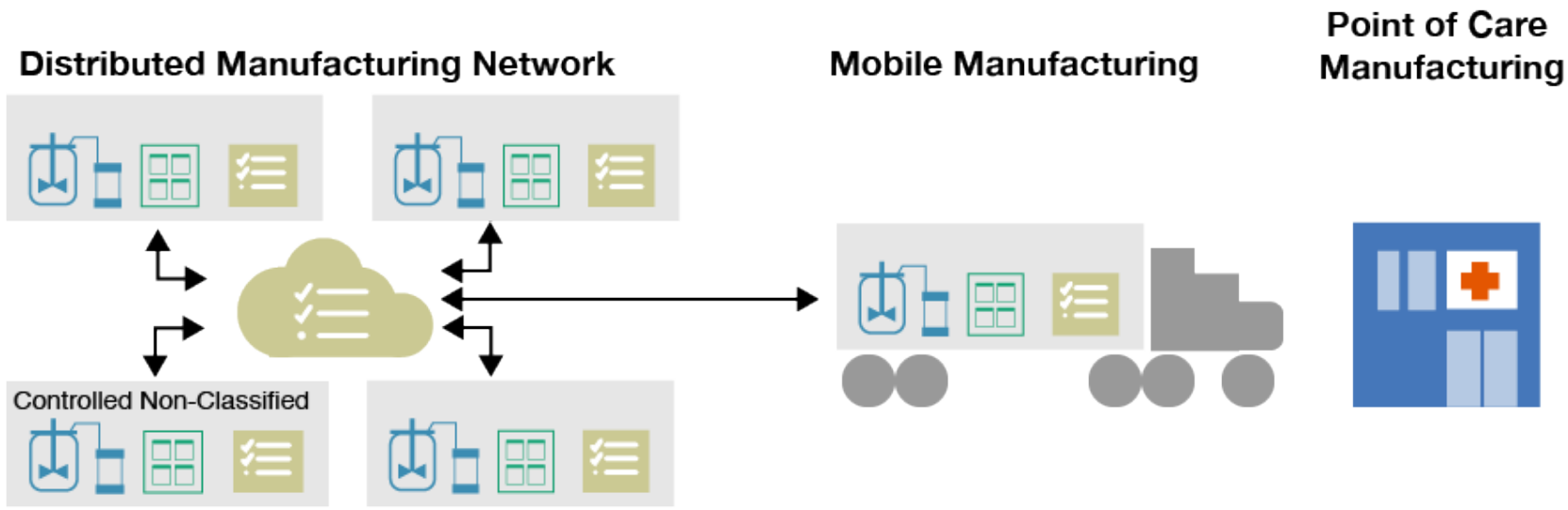
Process Controls

- Manual interventions to address process variations
- Imperfect understanding or control on biological systems

Specifications

- Complex product attributes (e.g., folding, modifications, aggregates) may be affected by many process parameters

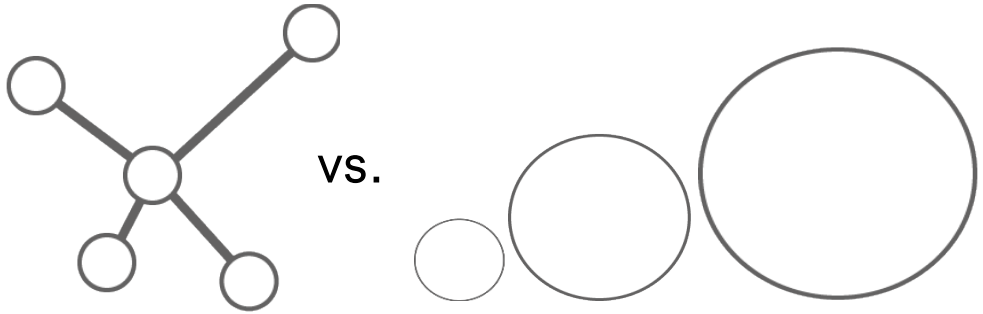
Alternative Strategies Pose Alternative Potential Risks



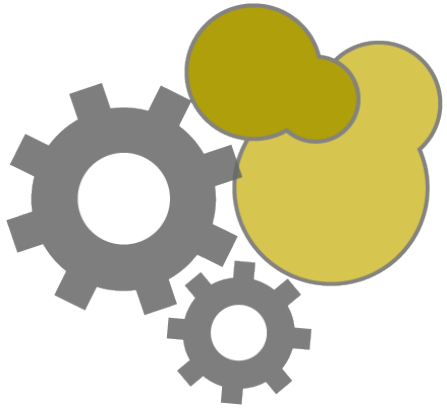
Facility	Equipment and supplies (e.g., single-use assemblies) should address most environmental controls and containment through "functional closure" of operations
Raw Materials	Additional assurance of custody and traceability on supplies may be necessary
Process Design	Integration of operations and holistic consideration of biology selected (Reduce biological risk)
Process Controls	Automated implementation with process monitoring (Reduce human error)
Specifications	Assure identity, purity, potency and safety through combination of parametric tests, process validation/verification, and product testing (e.g., endotoxin)

Could Distributed or POC Manufacturing of Protein-Based Biologics Enhance Product Quality?

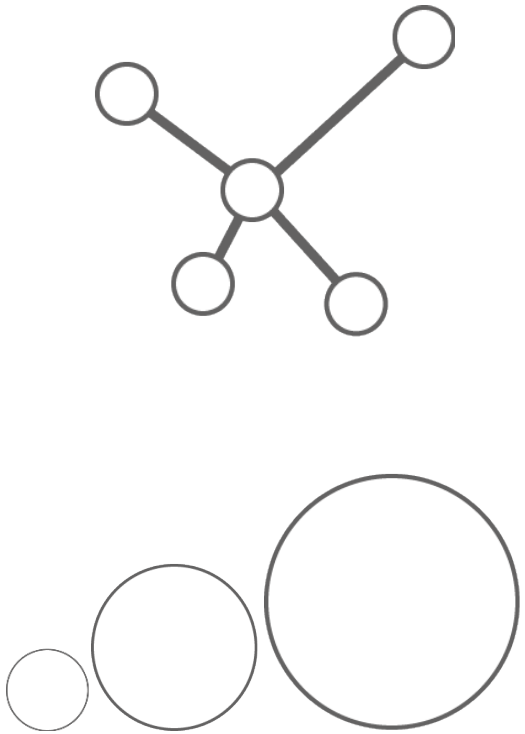
Scale-Out vs. Scale-Up Implementation



Integrated Design of Biology & Technology



How Might Scale-Out Manufacturing Benefit Quality?



Enhanced Understanding of Manufacturing Process

- At-Scale Process Development → Data at True Scale
- Many vs. Few Engineering/Qualification/Validation Campaigns
- Continuous process verification (*ICH Q11*)

Improved Predictability in Technology Transfer

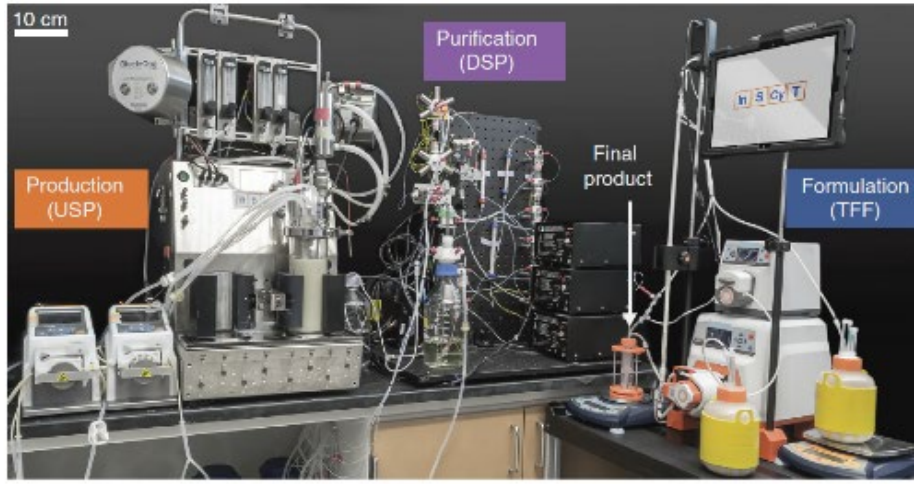
- 1-to-1 Transfer of Processes (e.g., similar scales, same automation)
- Reduced Reliance on Scaling Models for Process Operations (or…more focus on accurate models for at scale)

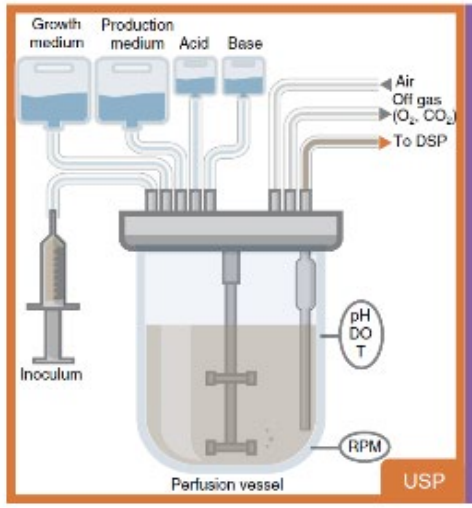
Expanded Data Linking Process Parameters & Quality Attributes

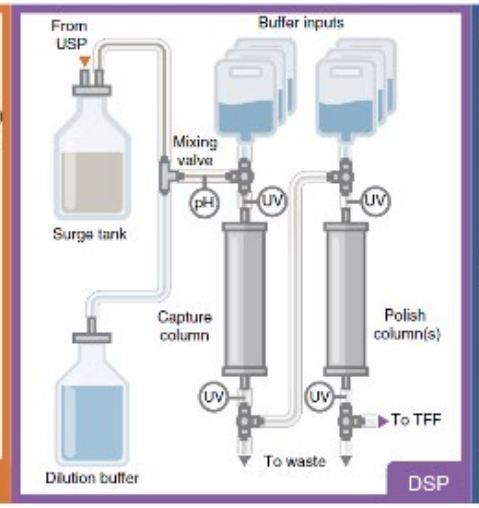
- Extensive process data to support parametric release based on validation approaches (*EMA Guideline on Real Time Release Testing*)
- Such approaches could address comparability of systems/locations

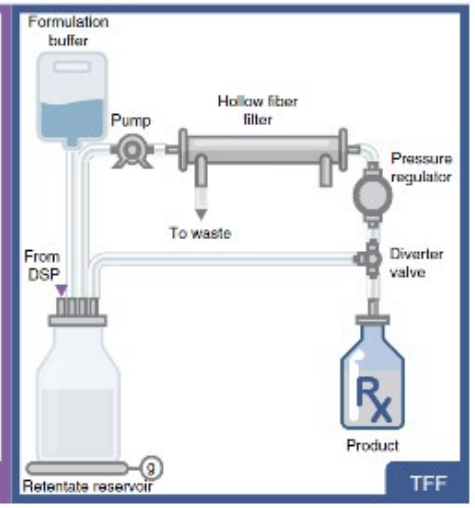
Small-Footprint Manufacturing Systems Rely on Automation

Integrated, Continuous Bench-Top System for Protein Production









Fully Automated, Hands-Free

Reduced Operational Steps
(>50% vs. standard processes)

12+

Proteins Expressed and Purified
(Many tested in vivo)

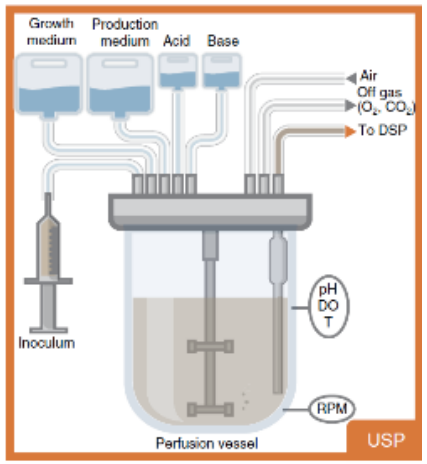
7+

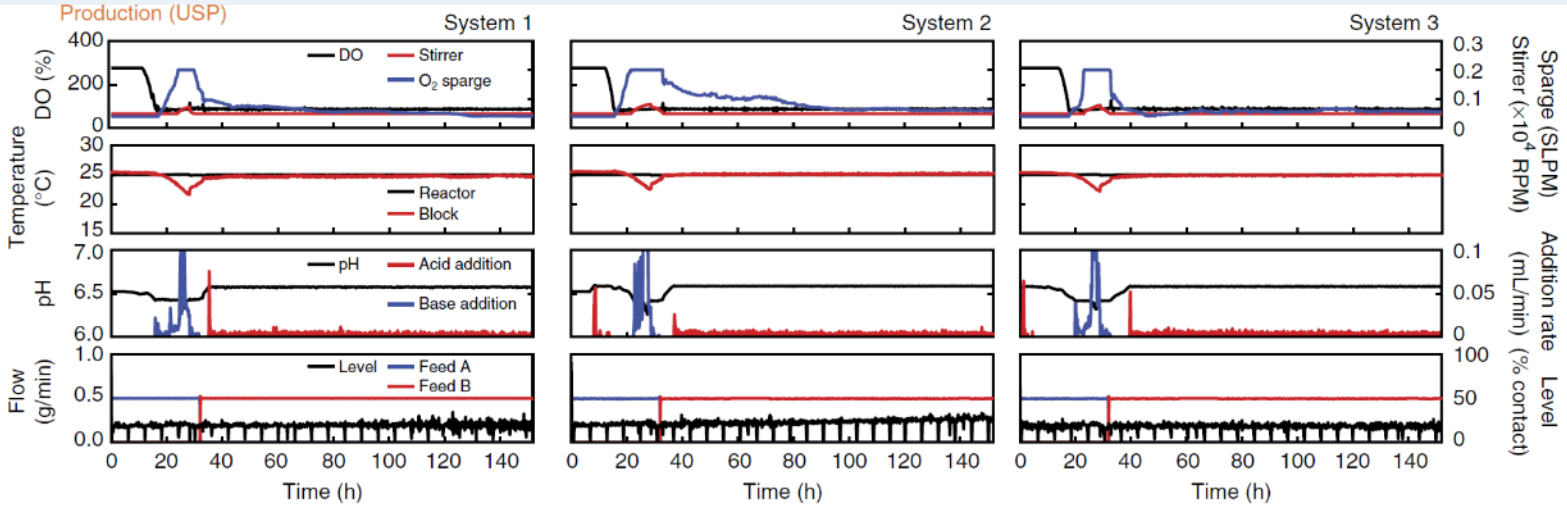
Days of
Continuous Microbial Production

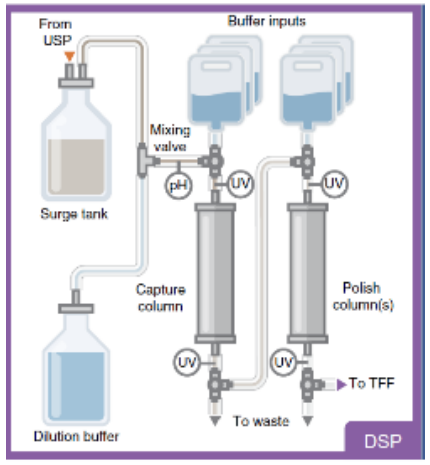
Crowell, Lu, Love, *et al.*
Nature Biotechnology, 2018

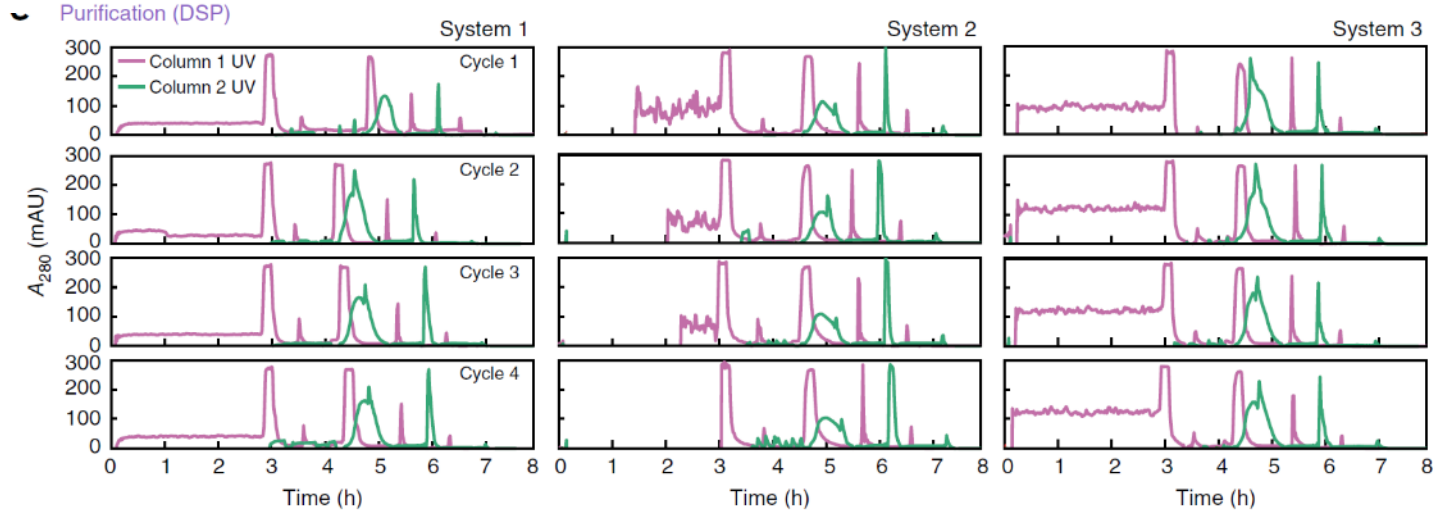
Automated Systems Can Operate Reliably and Consistently

Three Independent Systems Conducting the Same Operations



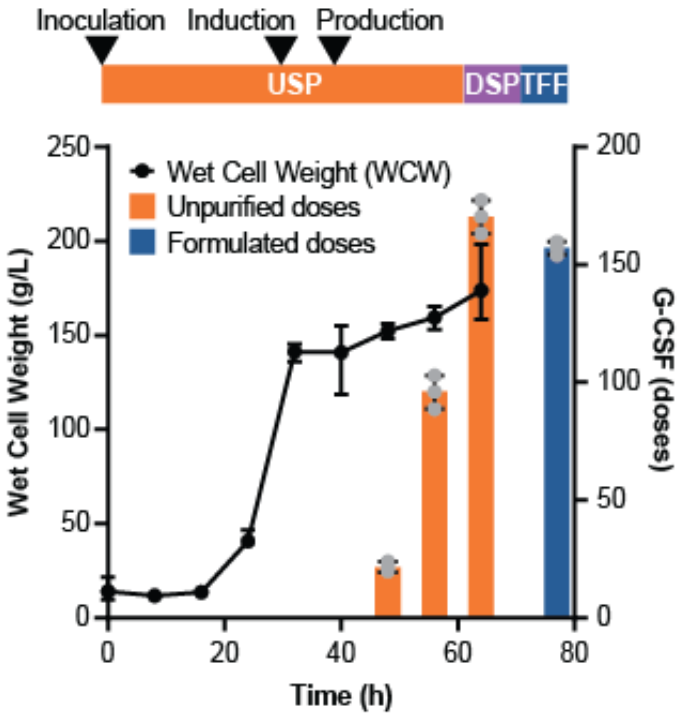






Crowell, Lu, Love, *et al.*
Nature Biotechnology, 2018

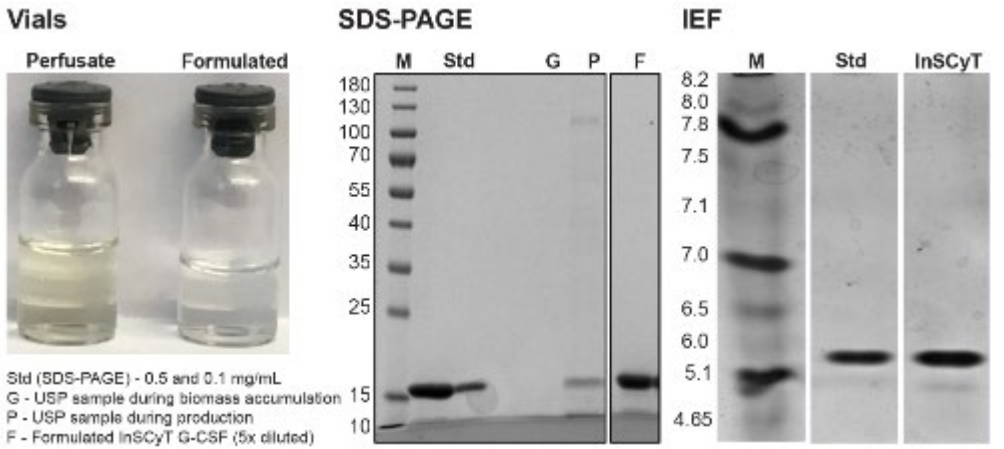
…to Produce Clinical-Grade Drug Substances

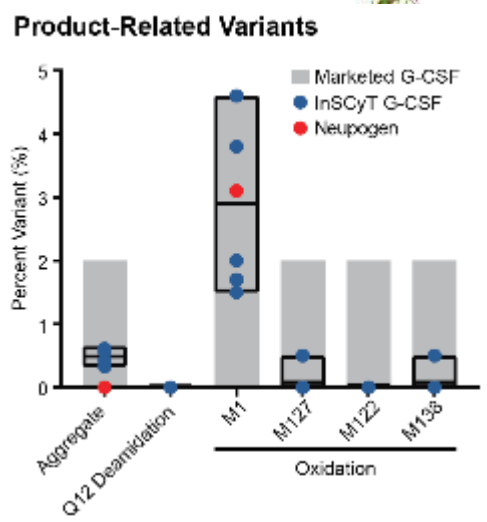


Sequence identical to licensed products (100% by mass spectrometry)

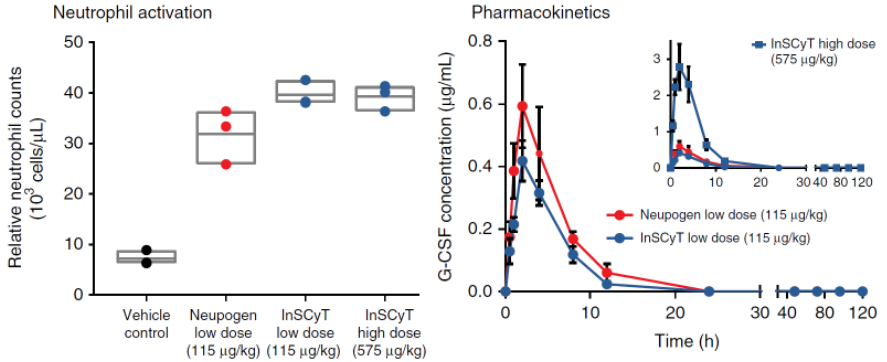
Product related variants:

- **N-terminal truncation variant**
- **O-linked di-mannose (T134)**





Similar *in vivo* Activity as Neupogen® (Amgen)



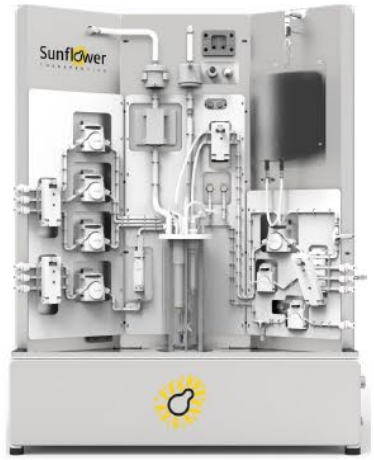
Toxicology

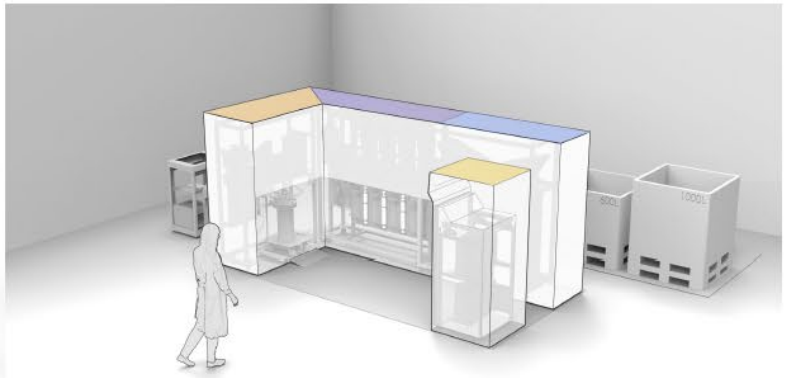
	Vehicle control	InSCyT G-CSF	Neupogen
Hematology			
Neutrophils	9.15*	32.65**	39.61**
Lymphocytes	86.15*	59.78†	52.74
Monocytes	1.93*	4.42**	4.17**
Clinical chemistry (M)			
ALP	155.2*	289.4**	384.6**
Clinical chemistry (F)			
ALP	65.6*	193.8**	217.8**
Chloride	102.10*	98.96**	99.42†
Creatinine	0.458*	0.420	0.364**

One-way ANOVA
*1% significance (all groups)
**1% signifcance (compared to vehicle control)
†5% significance (compared to vehicle control)

Crowell, Lu, Love, *et al.* *Nature Biotechnology*, 2018

Sunflower Manufacturing Systems:
Simple hardware to enable capacity anywhere





DAISY Petal™

Early Stage R&D
Continuous Fermentation
Accepting early access customers

DAISY™ System

R&D/Process Development
Automated Protein Bulk Production
(up to grams per week)

DAHLIA™ System

Efficient Commercial Production
Automated Protein Bulk Production
(up to 10 kg annually in <50m^2)

SUNFLOWER GROWS CAPACITY INCREMENTALLY BASED ON NEEDS & RESOURCES

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

Small but Efficient: Up to 10 kilos per year in less than 50 m^2

"Move-In" Ready: Operates in many different types of spaces and environments

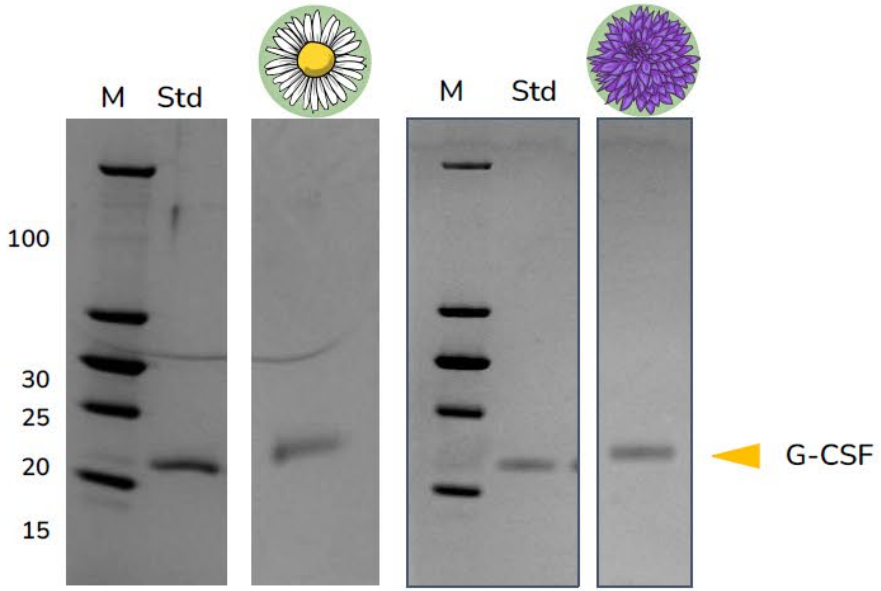
Sunflower THERAPEUTICS

Shared with J. Christopher Love, MIT - October 28, 2022
for use in FDA PQRI Workshop

High-Quality Protein Generated on Both Systems

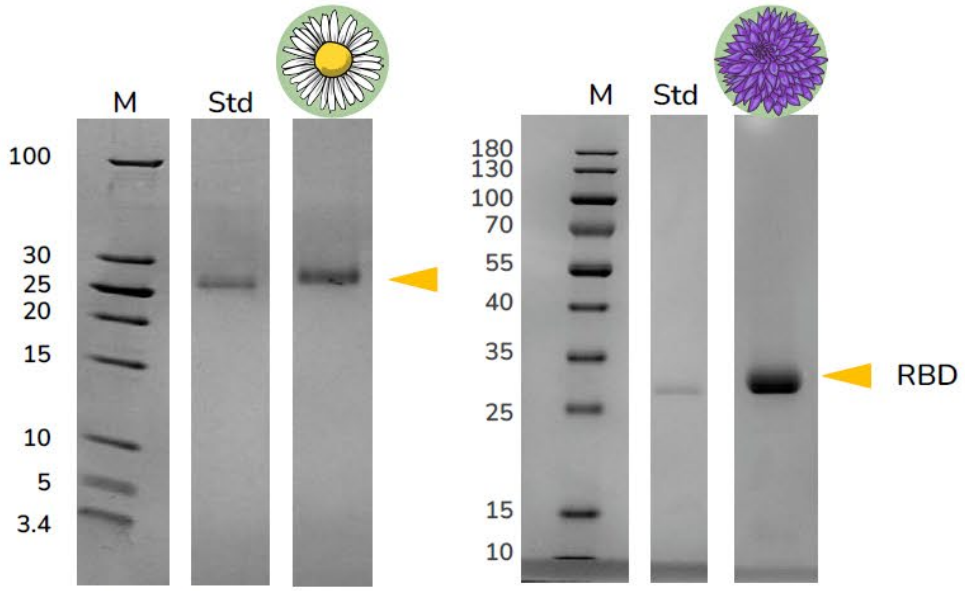
DAISY & DAHLIA SYSTEMS DEMONSTRATE MULTI-PRODUCT CAPABILITIES USING PROCESSES TRANSFERRED FROM ONE TO THE OTHER

G-CSF (filgrastim)



G-CSF Std @ 0.05 mg/mL

SARS-CoV-2 RBD (subunit vaccine)



RBD Std @ 0.05mg/mL

**Processes deployed for both molecules were not optimized for yield (Drafted for equipment demonstrations only)*

Bulk Drug Substances Produced on Systems are Similar

DAISY & DAHLIA SYSTEMS PRODUCED CLINICAL QUALITY BULK DRUG SUBSTANCES IN SIMPLE MANUFACTURING ENVIRONMENTS IN < 1 WEEK

Protein	System Used	Dose Equivalents	Host-cell DNA (ng DNA/mL)	Host-cell Protein	Bioburden* (CFU/plate)
G-CSF		~100	<10	< 0.1%	0
		>1,000	<10	< 0.1%	0
COVID-19 Vax Subunit		>2,000	<10	< 0.1%	0
		>50,000	<10	< 0.1%	0

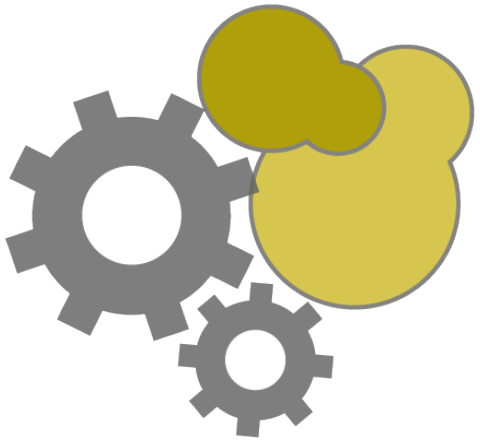
*Performed by third-party contractor according to the Compendial method USP 61 microbiological examination of Non-Sterile Products (Microbial enumeration test)

One Conceptual Analytic Approach to Address Comparability of Drug Substance Produced in Distributed Manufacturing Networks

Attribute	Routine Tests during Process Development	On-Site Tests (~ 5 instruments – Small Lab)
Identity – comparable primary and secondary protein structure	SDS-PAGE, Western blot LC-MS, intact mass (ESI, MALDI) RP-HPLC, Size-excl. chrom. (SEC), Ion-exchange chrom. (IEX) Circular dichroism (CD)	RP-HPLC, SEC, IEX (Product-specific capture chromatography)
Purity – comparable physicochemical properties (protein structure and product-related variants)	RP-HPLC, SEC, IEX Isoelectric focusing (IEF)	RP-HPLC, SEC, IEX IEF
Efficacy – comparable biological properties	ELISA In-vitro cell-based activity assays	ELISA/SPR (Octet)
Safety – comparable level of impurities (process-related contaminants and aggregates)	SEC Host-cell-specific ELISAs (HCPs) LC-MS (HCPs) Picogreen/qPCR (HC DNA)	ENDO-safe (Rapid enzymatic test for endotoxin, pyrogens) Particle imaging (e.g.—FlowCAM for microbial bioburden, foreign particles, visible aggregates)

...PLUS Extensive Process Data on Operations Connecting Process Performance with Quality Attributes

How Can Holistic Integration of Biology and Technology Enhance Assurance of Quality?



Reduce Risk for Patients with "Fit-For-Purpose" Biology

- Address potential risks (e.g., adventitious virus) by choice of host
- Engineer host to support target molecular attributes (e.g., glycosylation)
- Design molecules to make safe, effective, & manufacturable drugs

Simplify Process Designs for Robust Operations/Automation

- No viral contamination risk? No viral inactivation needed in process
- Simple host – Simpler Impurities – Simpler Recovery (No Protein A?)
- Reduced Operations → Less Interfaces → Improved Automation

EMA/CHMP/74766/2013 Assessment Report – Ocriplasmin (Jetrea®)

*"Ocriplasmin drug substance is produced by a genetically modified yeast expression system, Pichia pastoris followed by separation of the active substance and purification through various chromatographic steps. **Yeast fermentation does not support the propagation of viruses and therefore it is considered that there is no risk of contamination with viral adventitious agents** as a result of the manufacturing process.*

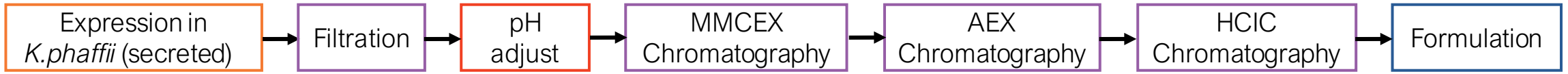
*Furthermore, there is **no need to test the cell banks or the drug substance for contamination with mycoplasma species**."*

The Biology Selected for Manufacturing Strongly Shapes the Control Strategy and Residual Risks

Example: Simple Host → Simple Process
Yeast Perfusion → Multi-Product Platform

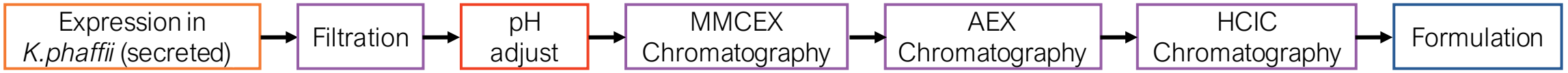
G-CSF

>50% Reduction



Interferon α2b

>50% Reduction

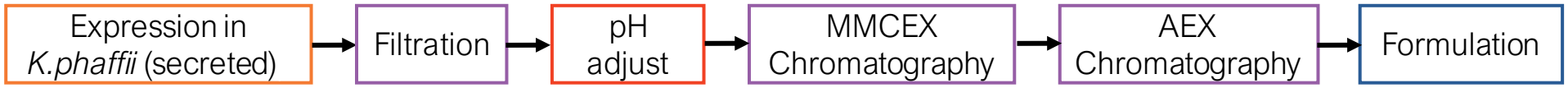


Recombinant protein vaccines (Rotavirus & SARS-CoV-2)

>50% Reduction

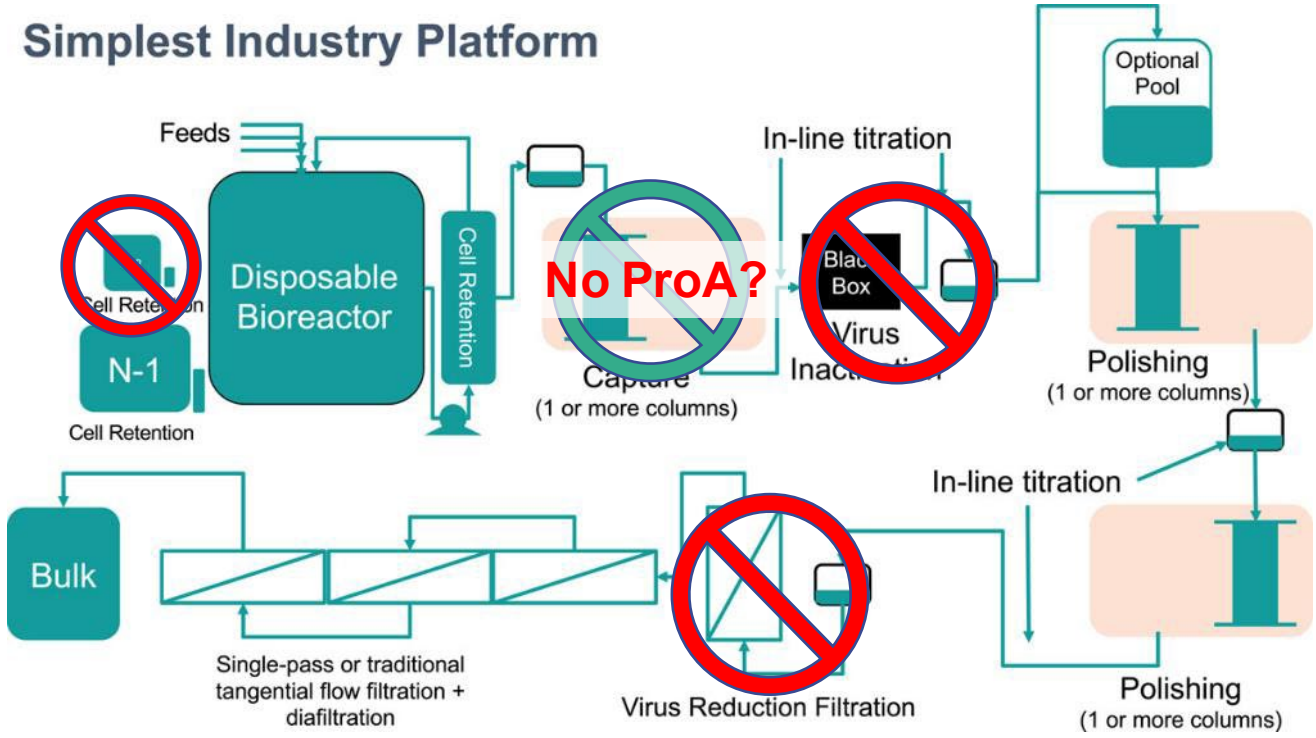
Single domain antibody (VHH)

25% Reduction



L. Crowell *et al. Nature Biotech*. 2018
N. Dalvie, J. Brady *et al. Microbial Cell Factories*, 2021
L. Crowell *et al. Biotech. Bioeng*. 2021a
L. Crowell *et al. Biotech. Bioeng*. 2021b
Dalvie, Aponte Rodriguez, *et al. Proc. Natl. Acad. Sci*. 2021

Distributed/POC Solutions May Rely on Reduced Process Complexity By Design to Enhance Robustness and Quality

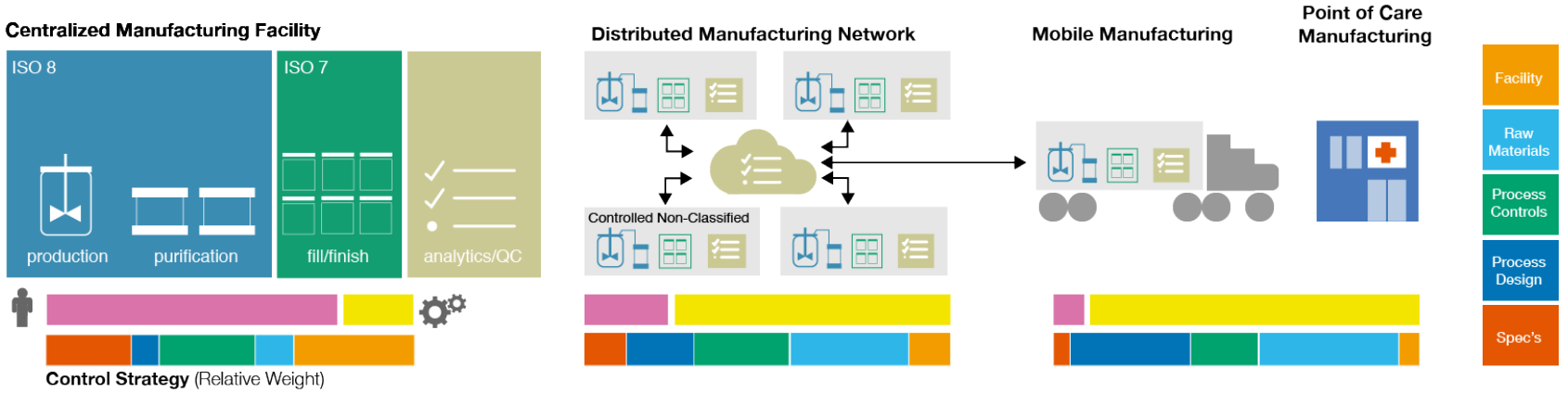


Biotechnology and Bioengineering, First published: 22 January 2021, DOI: (10.1002/bit.27688)

Simple Processes Inherently Reduce Potential for Variation & Enhance Understanding Related to Quality

Based on analysis by E. Coleman, MIT LGO master thesis, 2020 (available on MIT Dspace)

Summarized Thoughts for Contemplation and Dialogue



Establishing Quality of Protein-Based Drugs in a Distributed/POC Manufacturing Model

- May invoke alternative, model-appropriate, & risk-based control strategies distinct from traditional centralized models
- Will benefit from additional reliance on automation and continuous process verification and 'fit-for-purpose' biology for simplified processes to address potential risks
- May need holistic data packages of parametric data and limited release testing to address comparability (and allow bioequivalence waivers)

Partnered Development of a Practical Framework for Enabling New Manufacturing Models

- Will require pragmatic dialogue and collaboration among stakeholders to find a balance between realizing potential benefits for patients and health care with realistic use cases and staged deployment