



## *The Biotherapeutic Manufacturing Engine*

A company utilizing its game-changing manufacturing system to generate proprietary biologics and vaccines

# Snapshot

- Game-changing, ultra-high expression system
- Already well-validated commercially
- No engineering needed for some human therapeutics, and minor changes needed for others
- Platform will be used for internal pipeline of protein therapeutics, vaccines and therapeutic antibodies
- One contract signed w/ major Japanese pharma, several others outstanding
- Seeking Series A funding
- Many distinct exits possible, w/ low CapEx, and rapid timelines

# Profile of the Mature EnGen

- Proprietary fungal expression system with:
  - >20g/L expression
  - Human-neutral glycosylation
  - Lowest CoGs
  - Excellent downstream handling
- 1yr from lead candidate to Master Cell Bank w/ scalable process
- Vetted on high-value pipeline

# Current Ab Manufacturing Systems Have

## Limitations: Still Demand for a Better System

- CHO: Long time to select cellbank, long time to MCB, Expensive, 3wk fermenter time
- Plants: Incorrect or no glycosylation, Long time to MCB, Long scale up, DSP difficulties
- Bacteria: Aglycosylated, Expression level of IgG is low
- Yeast: Incorrect glycosylation, not known for high expression levels
- Pichia: Methanotroph, Low expression, Viscous
- Whole animals: Long development time, Long scale up

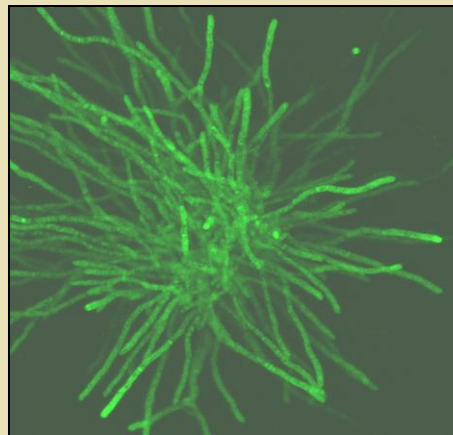
# *Chrysosporium lucknowense*: C1

**Taxonomy:** Ascomycete, filamentous fungus only distantly related to other commonly used expression hosts (*Aspergillus*, *Trichoderma*, *Pichia*, *Saccharomyces* )

**Biological Niche:** Soil-borne saprophyte, secreting cellulases and proteases

**Genomics:** Genome fully sequenced, 2006 and identified C1 as *Myceliophthora thermophila*

**Toxins:** None known



# C1: A Mature System for Manufacturing Industrial Enzymes

- Dyadic International, Inc. (15yrs of development) for industrial enzymes
- Many developed enzyme products on the market

–Textile, paper, feed, food, starch, pulp & paper related enzymes:

(Glucanases, Cellobiohydrolases,  $\beta$ -Glucosidase, Chitinase, Xylanase, Arabinofuranosidases, Arabinases, Pectinases, Esterases, Proteases, Oxidases, Phytase, Amylase)

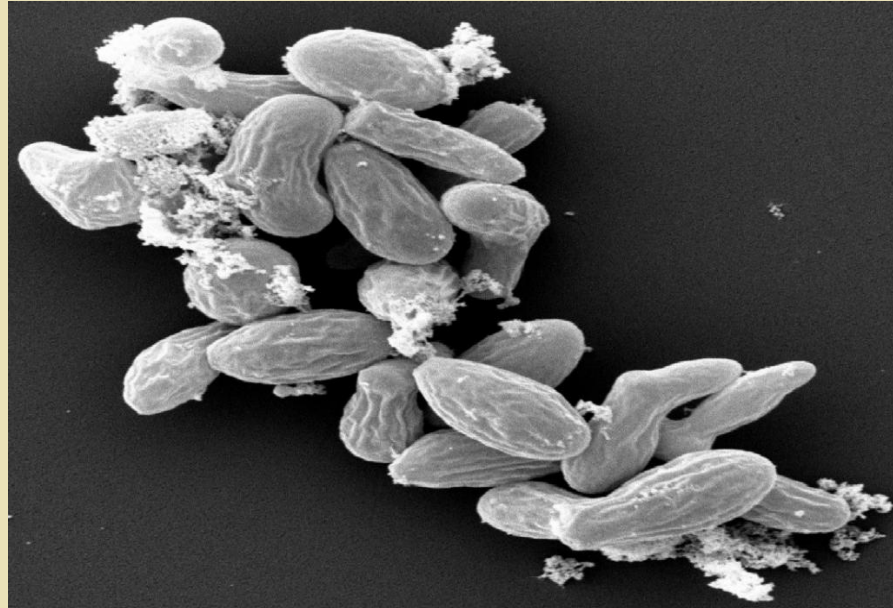
*50-100g/L Are Routine!*



# C1: A Tractable MolBio Host

- Versatile genetic tools developed
  - Transformation:  $1.3 \times 10^4$  cfus/ug; stable integration
  - Expression level correlates w/ copy number
  - Multiple, different insertions possible
  - Gene expression: efficient expression signals
  - Protein production: very efficient secretion
  - Gene disruption: efficient homologous recombination (>1% up to 90%)
- Fermentations at 150,000L scale
- Target protein is secreted

# Unique C1 Morphological Structure Solves Viscosity Problem of Filamentous Fungi



Scanning EM of C1 propagules.

Propagules instead of hyphae.

Low viscosity, low lysis, high productivity.

# Comparison of Fermentation Systems

	<u>Aspergillus</u>	<u>Trichoderma</u>	<u>Pichia</u>	<u>CHO</u>	<u>C1</u>
pH	3.0-6.0	3.0-6.0	5.5-7.5	7.0	<b>4.5-9.0</b>
Temp	30-37	25-32	25-32	37	<b>25-43</b>
Viscosity (centipoise)	1500-2000	200-1000	~1000	10	<b>&lt;10</b>
Fermentation (days)	8-9	6-7	5-6	21	<b>5-6</b>

# C1 Growth Media & Conditions

- Fully defined media of simple (inexpensive) salts
  - Rapid scale-up
  - Upfront process development, due to linear scalability
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- \$5/Kilogram protein (CoGs)
  - C1 is compatible with current fermentation equipment



# C1 Capture & DSP

- Capture:

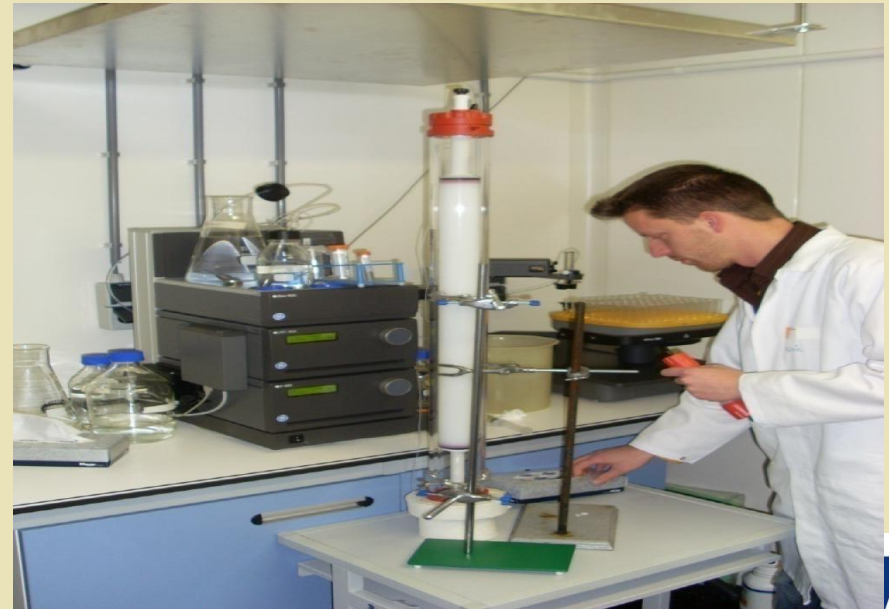
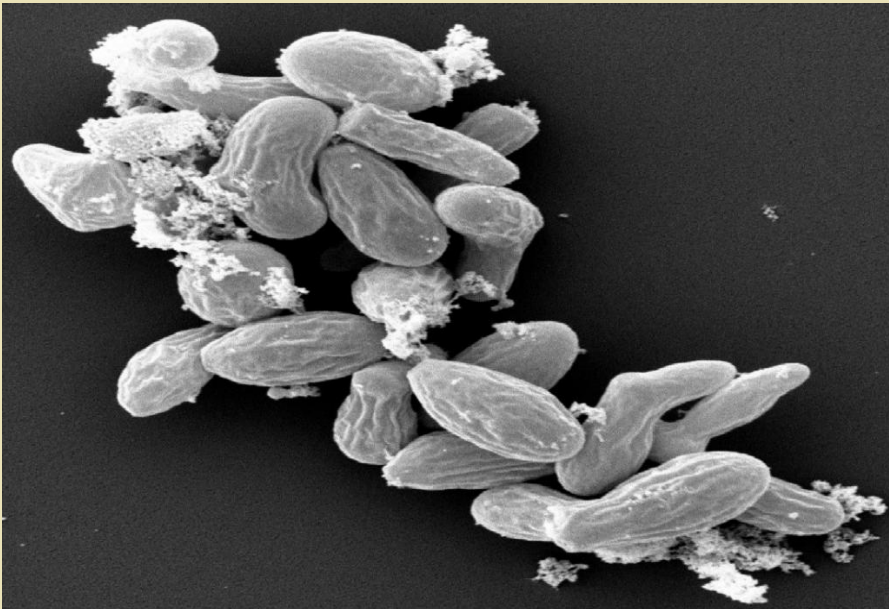
- Durable propagules, so low HCP

- Low viscosity

- Centrifugation

- Highly concentrated supernatant minimizes processing volumes

- No viruses, mycoplasmas, mycotoxins, etc.



# Development of a Low Background Host: (White Strain)

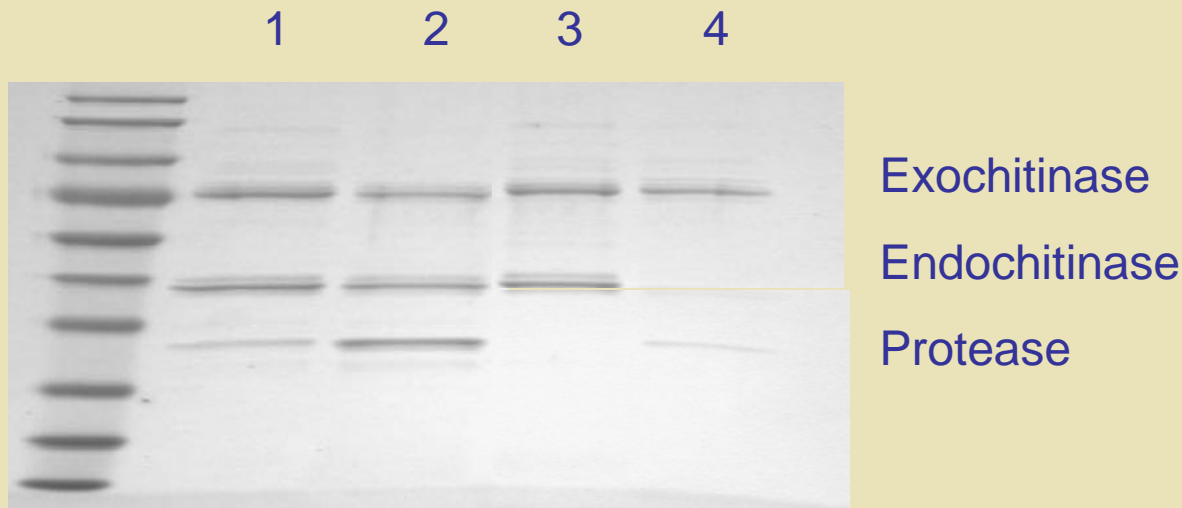
Aim: production of a strain w/ less secreted endogenous proteins.



A. Strain UV18-25:  
Non viscous derivative of C1  
Commercial fermentation  
High cellulolytic activities  
Diverse enzyme mixture  
➤60 g/L total protein

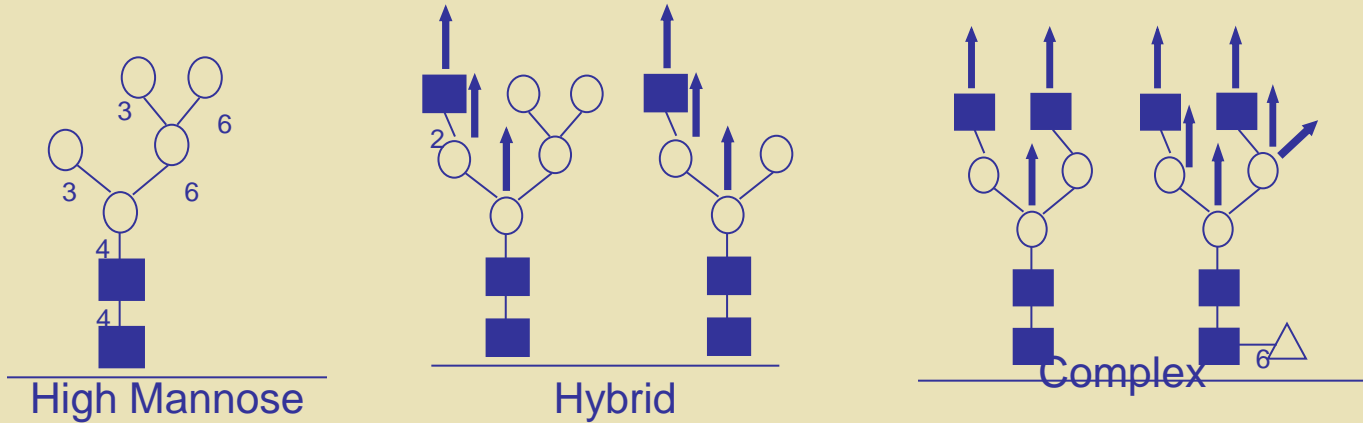
B. White C1 Strain:  
Derivative of UV18-25  
Almost no cellulolytic activities  
Lab-sized scale fermentation  
Very few endogenous secreted proteins  
~ 10-20 g/L total protein  
~1- 8 g/L target protein

# Development of a Low Background Host: Protease Deletion



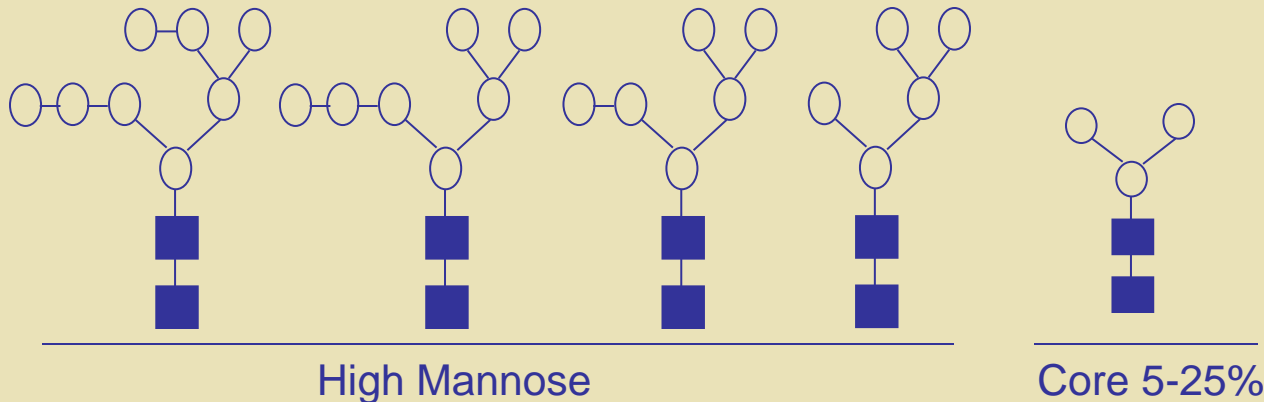
- 1 White strain
- 2 Protease overproducing white strain
- 3 Protease-deleted white strain
- 4 Chitinase deleted white strain

# C1's Glycans Very Close to Human



- N-acetylglucosamine
- Mannose
- Fucose
- Galactose
- Sialic acid

## Major Vertebrate N-Glycans

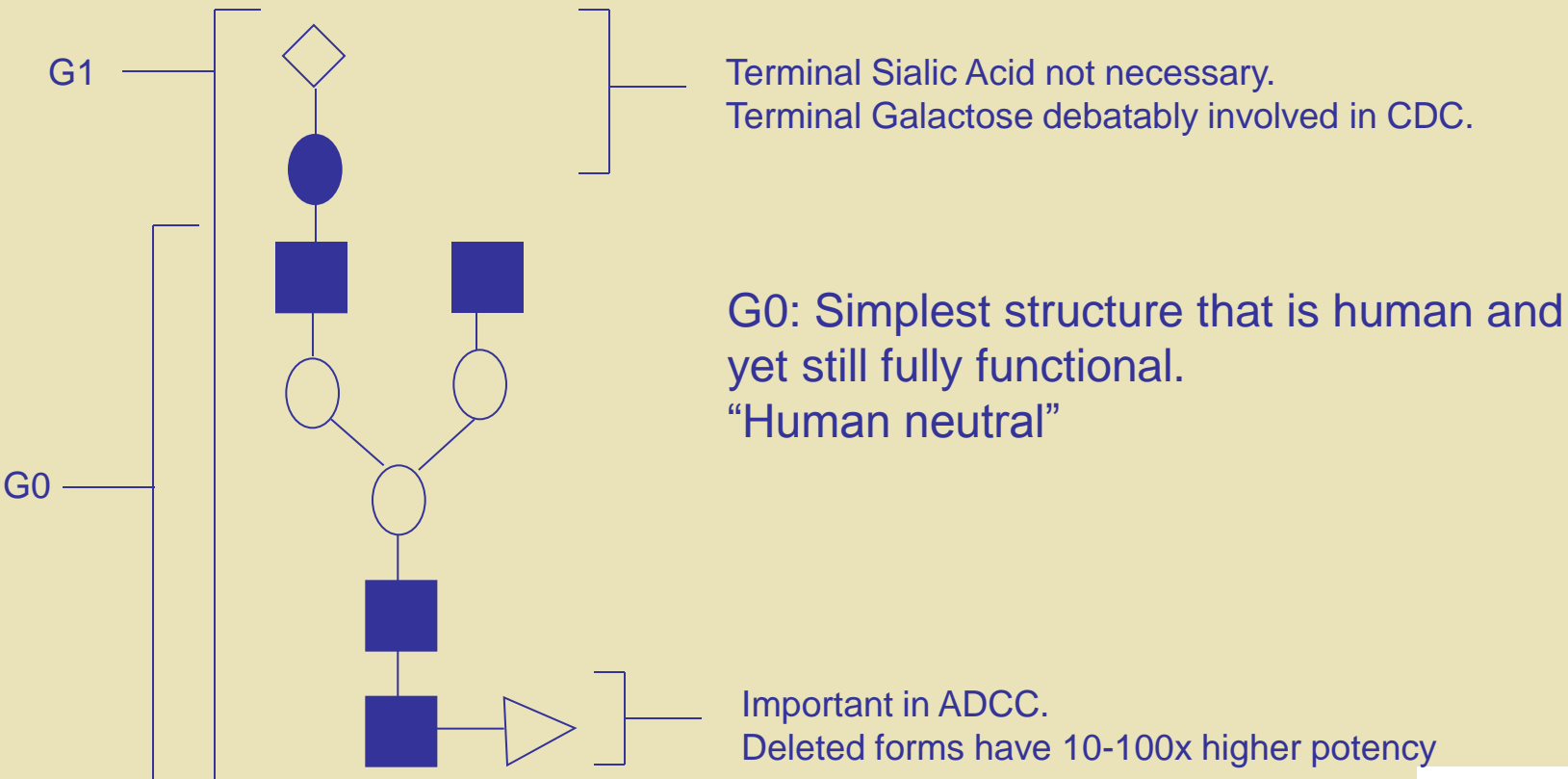


## Major C1 N-Glycans

# Minimal Engineering Required to Make G0 Structure (+/-Fucose) in C1

Correct Structures:  $T_{1/2} \sim 2-3$  Weeks

Incorrect Structures: Can shorten  $T_{1/2}$ , increase immunogenicity, decrease potency



# Expression of Humira in C1: High Expression and Full Activity

- Production levels

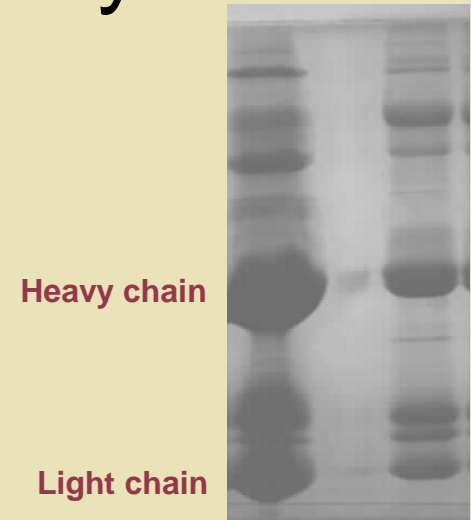
- 4 g/l levels reached  
after 3 days in fermenter

- Protein A binding:

- incomplete recovery: due to overloading
- alternative recovery possibilities

- TNFalpha-inhibition assay: (MTT assay)

- Produced protein is bioactive
- Humira reference



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# C1: A Compelling List of Advantages

*Low scientific risk: Mostly engineering tasks*

<b>Discovery/ Development</b>	<b>Expression</b>	<b>Down Stream Processing</b>	<b>Product Attributes</b>
<ul style="list-style-type: none"><li>• Mature system of industrial enzymes</li><li>• Short time to develop MCB</li><li>• Direct and single step transformation w/minimum strain selection (fast clone-to-clinic)</li><li>• Can be used for various high-value Rx proteins today</li><li>• Already proven for human antibodies</li><li>• Process validation upfront for biosimilars and streamlining first-in-class molecules</li></ul>	<ul style="list-style-type: none"><li>• High expression level, allowing for smaller reactors</li><li>• Adaptable to current reactors &amp;/or to new, single use reactors</li><li>• Low cost of media</li><li>• Defined media</li><li>• Short fermenter times</li><li>• Wide range of growth conditions</li></ul>	<ul style="list-style-type: none"><li>• Target protein secreted into media</li><li>• Secretion at high titer (no microbial inclusion bodies)</li><li>• Low host cell protein in supernatant</li><li>• No viral inactivation/removal/validation</li><li>• Low viscosity</li></ul>	<ul style="list-style-type: none"><li>• Glycoprofile needs little modification to become 'human neutral'</li><li>• Naturally afucosylated</li><li>• GRAS designation</li><li>• "Simpler" glycoprofile</li><li>• Enhanced SAR</li></ul>

*What to do with a mega-producing microbial manufacturing engine?*

# GlycoFi: A Model for Success

- *Pichia pastoris*, not known for especially high expression levels
- Re-engineered *Pichia* for human Rx
  - *Pichia* glycoprofile not very close to human
  - Knocked out and re-built entire glycosylation machinery
- 5 years from founding to completion of re-engineering
- Methanotrophic
- Fast time to MCB
- \$30M raised and 55 employees hired at maximum
- Acquired by Merck for \$400M as the cornerstone to their biotherapeutics strategy

# Merck's interest in Pichia\*

- Main value of Pichia over CHO is front-end speed
  - Faster cell line development
  - Faster process development, b/c each run is much shorter
  - Faster CMC since product is very homogeneous; hence less time and money spent on bioanalysis
  - More versatility around glycoengineering, including enhanced ADCC

Overall, time from ID of clinical candidate to first patient injected is 1 year vs. 2 years for CHO

Other advantages are

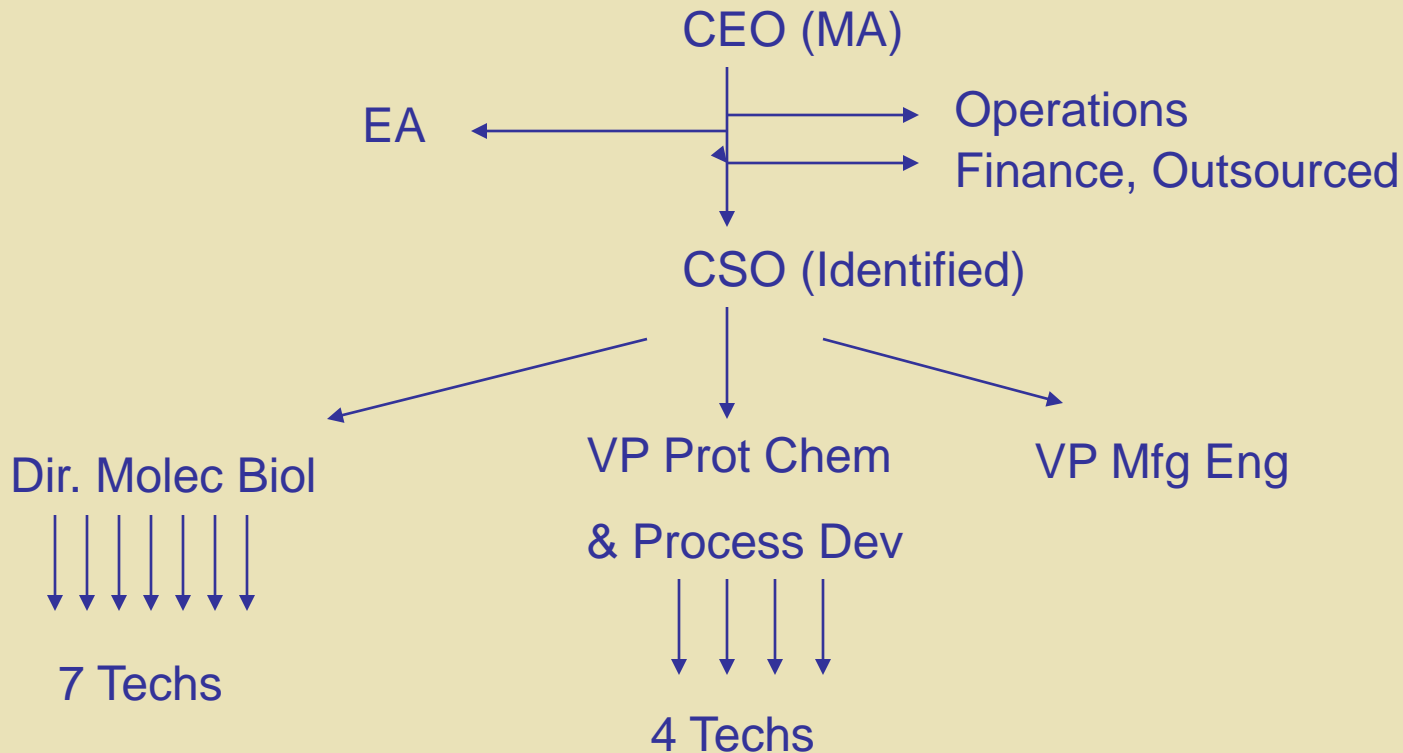
- Cost of production
- Acceptable titres, ~1g/l
- Cells spend 1/3rd the time in bioreactor, hence lowering cost
- Media is cheaper and bioreactor is simpler, ie just keep dripping in MeOH rather than constantly adjusting pH, adding nutrients
- No viral filtration or validation

# Re-engineering C1 for BioTherapeutics: *Surpassing the GlycoFi Model*

- Optimized expression levels
  - No promoter optimization yet done
  - No biomass optimization yet done
  - Target-specific media optimization
- Secreted protein deletions
  - Deletion of Cellulases/Proteases
- Glycoprofile modification
  - C1 is remarkably close to human

# Personnel & Facilities

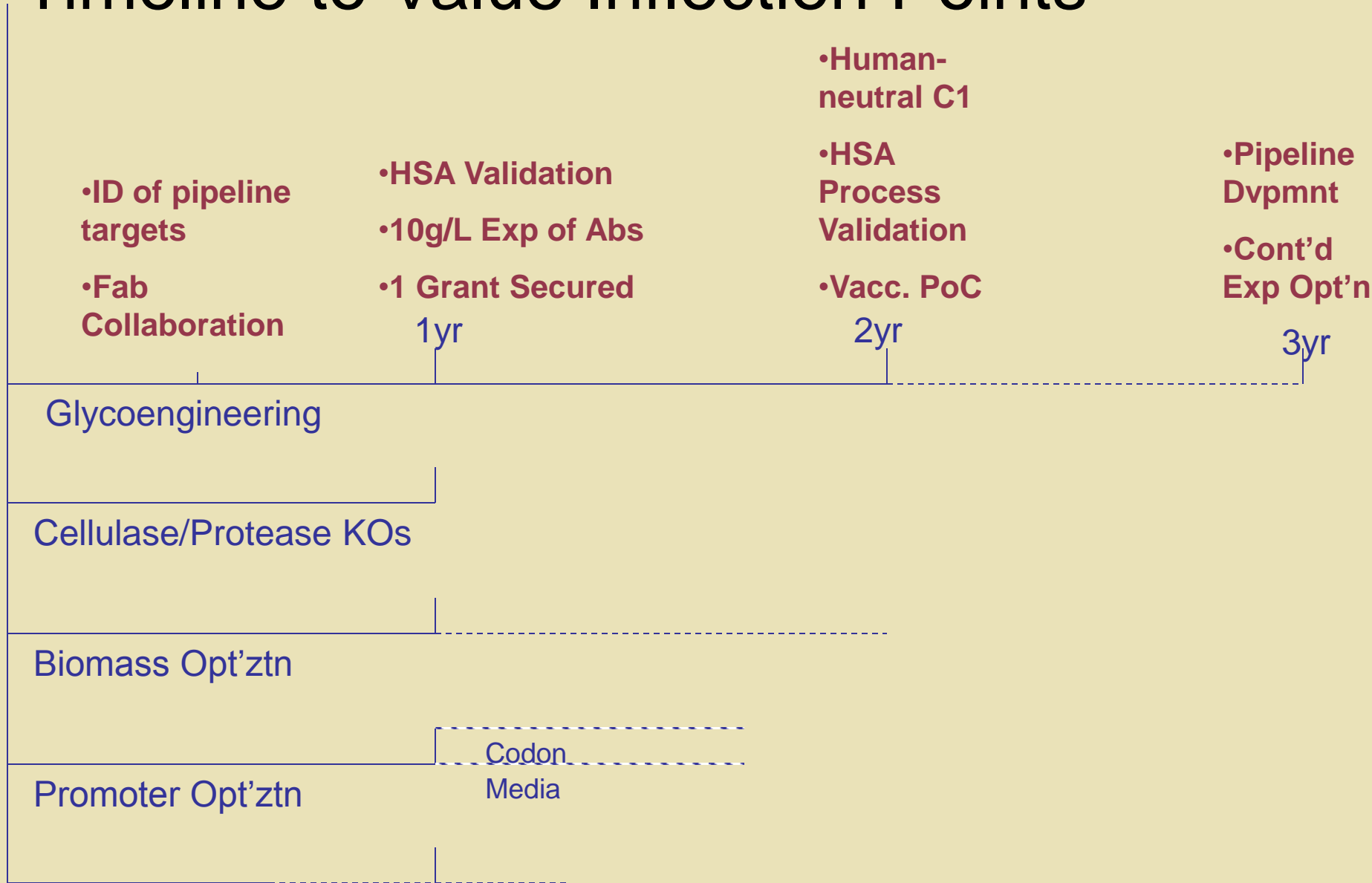
Easy to build remainder of team. Facility already identified and contract negotiated



18 person maximum headcount

~5-7000 sq ft, mixed wet lab/ office space

# Timeline to Value Inflection Points



# Three Year Pro Forma with Initial \$15M\*

	<u>Year 1</u>	<u>Year 2</u>	<u>Year 3</u>
Balance	15M	10.05M	5.1M
Grants	---	---	---
Contracts	---	---	---
G&A	(1.1M)	(1.1M)	(1.1M)
Glycoengineering	(1.65M)	(1.65M)	(1.65M)
Molecular Opt'ztn (In house & CRO)	(0.95M)	(0.95M)	(1.1M)
Prot. & Proc. Opt'ztn (In house & CRO)	(0.95M)	(0.95M)	(1.1M)
Legal	<u>(0.3M)</u>	<u>(0.3M)</u>	<u>(0.3M)</u>
	(10.05M)	(5.1M)	(-0.15M)

\*Assuming \$275K FTE cost

# Conclusions

- Mature biologics production system w/ advantages over all other systems (including CHO, Per.C6 and Pichia)
- Low scientific risk, quantifiable engineering tasks to complete
- Rapid timelines to completion
- High value system, as a platform alone
- Government funding readily available
- Strategic and funded collaborations possible early
- High value pipeline identified to create added value