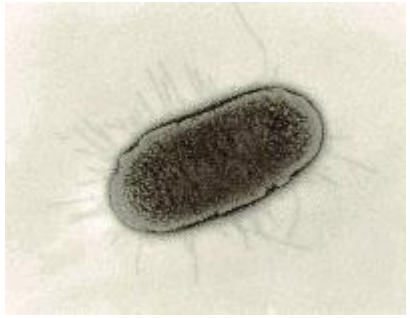
Microbial expression systems for the production of recombinant protein biopharmaceuticals : Alternative expression systems



Christopher Dale Ph.D / September 2007

Overview

- Introduction : Situation analysis ~ 1980-2007
- Dominance of *E. coli*
- Market analysis
- Emergence of *alternative* microbial expression systems
- Meeting the challenges of COG and product design
- New products for the 21st century
- Situation analysis ~ 2007 – 2017 (?)

Introduction

- Recombinant protein biopharmaceuticals produced in microbial systems account for 35-40% of market share
- Current market is segmented according to class of drug
- Segmentation is currently technology driven
- Technology is eroding technical basis for segmentation

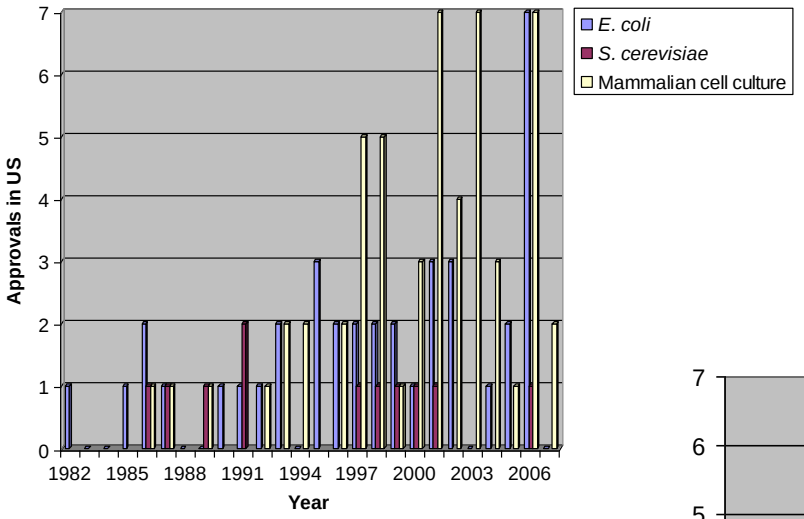
Diversity of products

Opportunity for larger market for microbial systems (total market growth)

Timing : Biopharmaceutical industry now in (young) adulthood

Comprehensive technology base for alternative microbial systems

Approval of recombinant protein biopharmaceuticals expressed in microbial systems



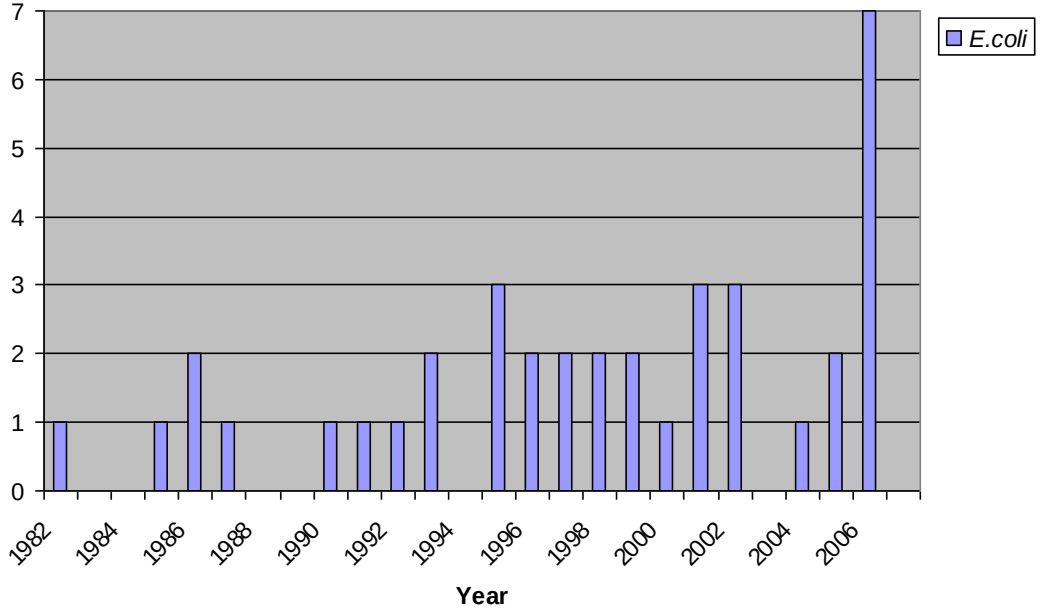
Emergence in 1982 with first FDA approval

Dominance of *E. coli* premier microbial production platform

One yeast system as sole challenger

E. coli = 38 approvals (1982-2007)

S. cerevisiae = 11 approvals

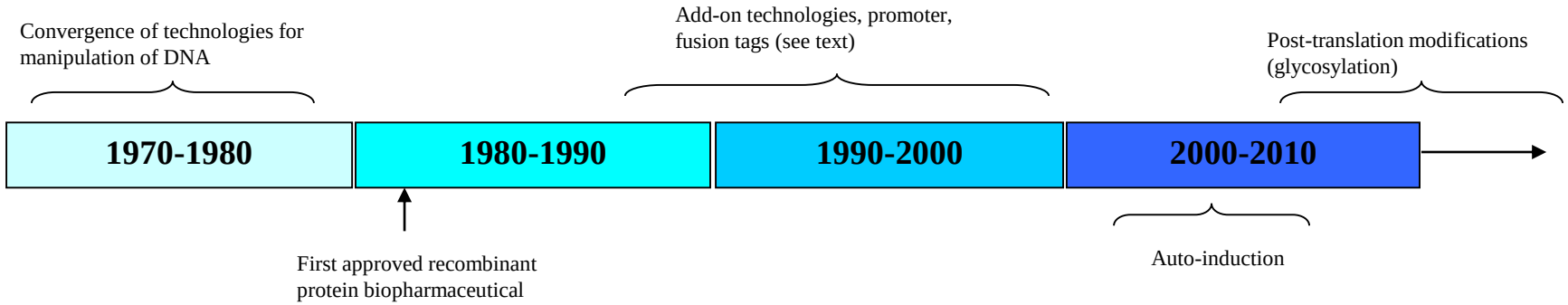


Dominance of *E.coli* as the bacterial expression system of choice

- Serendipity
- Use of *E. coli* (prokaryotic) and *S. cerevisiae* (eukaryotic) as tools for genetic research
- Emergence and convergence of technologies for manipulating DNA, transformation of bacteria and induction of expression
- First product approval in 1982
- Once established, successive regulatory approvals have ensured dominance of *E. coli*
- Regulatory familiarity, conservatism and support from technology providers have sustained dominant position.

E. coli : 1970 - 2007

- Why has E. coli sustained a dominant position ?



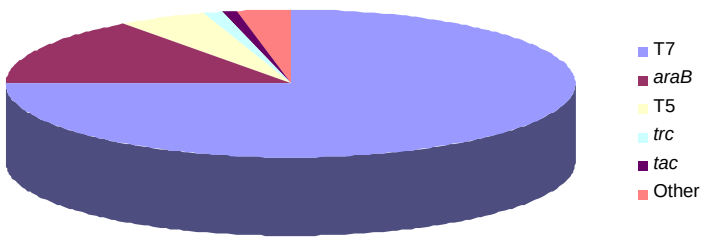
- **Technology ~ continuous improvements**

Choices

Despite > 50 strains available, production strains of *E. coli* for biopharma applications are restrcited and derived from origins in K or B strains

- HMS 174 / HMS174 (DE3)
- W3110
- BL21 / BL21 (DE3) = BLR
- DH1 or DH5 for pDNA

75% B strains
25% K strains



Frequency of promoter usage in *E. coli* systems (based on a survey of outsourced projects)

T7 : 75%
araB : 15%
T5 : 5%
trc : 1%
tac : 1%
Other : 3%

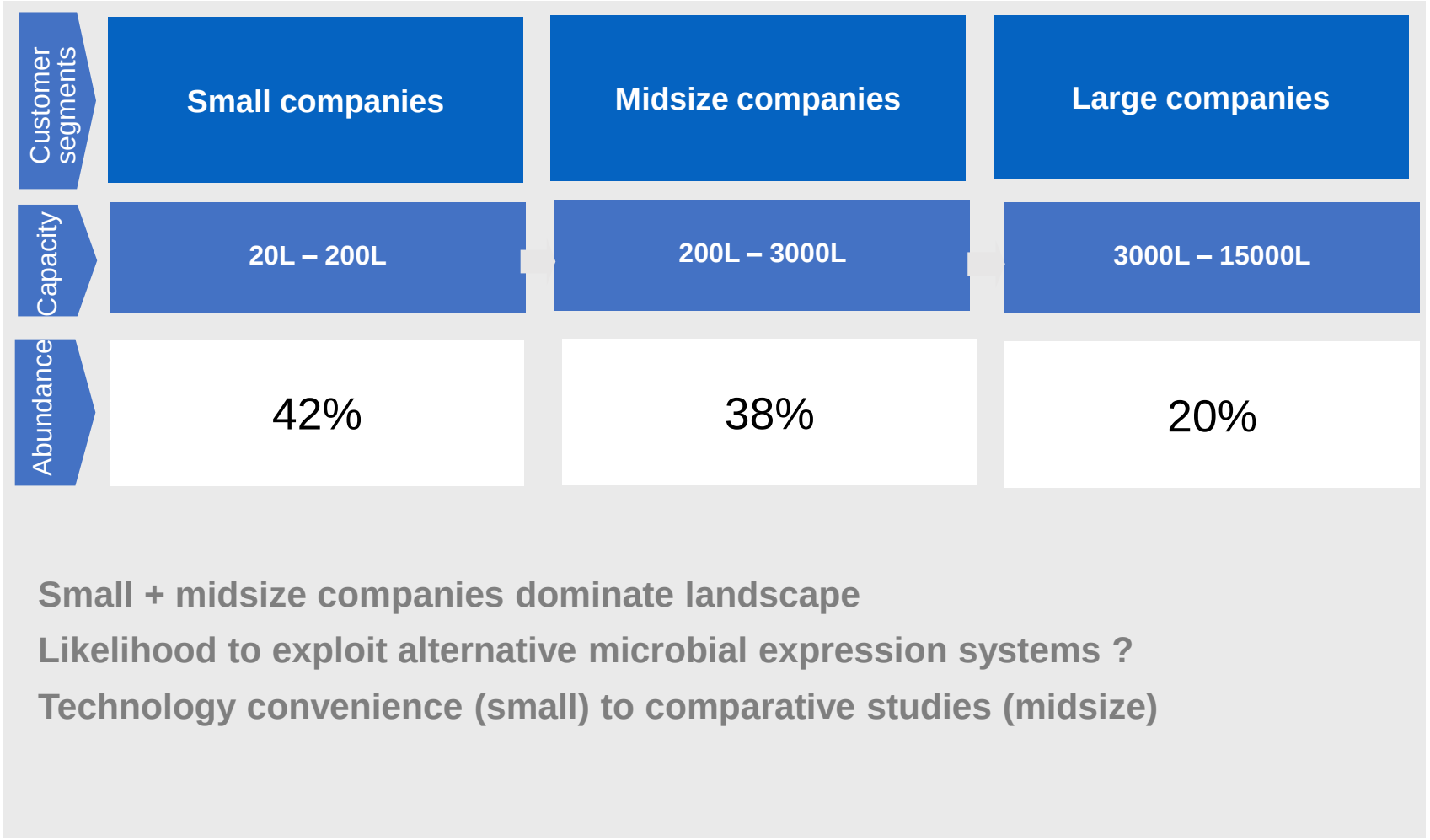
Best does not always result in success

- Exclusion based on market dominance **VHS**
- Loyal sector **Mac computers**
- Innovation seizes market dominance **Ipod**

Factors

- Technology awareness (brand concept)
- Brand support
- Timing (readiness for acceptance)
- Precedent

Biopharmaceutical company distribution (CMO perspective)



Small + midsize companies dominate landscape

Likelihood to exploit alternative microbial expression systems ?

Technology convenience (small) to comparative studies (midsize)

Bio Adaptive Index

Innovation

Adaptation

Biopharmaceutical industry is a complex mix of innovation and conservatism.

Regulatory familiarity favors conventional technologies

Innovation drives continuous improvement and economic performance

Change : Positive decision drivers

- Royalty structure applied to existing *E. Coli* systems
- Flexible terms for new system agreements
- Potential for high volumetric productivity and favorable COG
- Support from technology providers and enablers
- Product quality : Better control of desirable and undesirable features
- Market differentiator (partner, sell concept)

Change : Negative decision drivers

- Unproven technology
- First to gain regulatory approval
- Magnitude of existing literature and industry experience of high cell density *E. Coli* fermentation
- Potential for locked-in agreement with technology provider
- Long term support : Continuity ?
- Conservatism : Limited funding, risk mitigation
- Business decision : Partner or sell drug

A tale of two systems

Pichia pastoris

- No approved products in US
- Principal yeast technology for R&D in academia, small companies
- CMO : 75% of all yeast processes/opportunities
- Problems : Expression, localization, glycosylation, protease activity

Pseudomonas fluorescens

- 3 years old
- Logical alternative to *E. coli*
- Evaluation mode
- 5 year wait for first approval in US ?

Pseudomas fluorescens

- *Pfenex* = Launched by Dow Pharma in 2004
- Comprehensive business model to support technology acceptance
- Cloning, process development in house
- Qualified CMOs
- Aggressive advertising and marketing support
- Technical advantages over *E. coli* for expression of soluble recombinant proteins, fermentation physiology
- Publicised deals/collaborations with Pfizer, Iomai (2005) Cambrex, Insmed, VGX Pharmaceuticals, Viventia Biotech, Abbott (2007)
- Collaboration with Cygnus Technologies

Key requirements for emerging microbial expression systems

- A good understanding of cellular physiology in the context of high cell density fermentation (C usage, N limitation, protease activities)
- Available vectors for cloning, MCS locations, selection markers, elements for plasmid maintenance within bacterial cells
- Analytical methods for host cell protein (HCP)
- Analytical methods for endotoxin (where applicable to Gram negative bacteria)
- Full sequence of host genome (desirable)

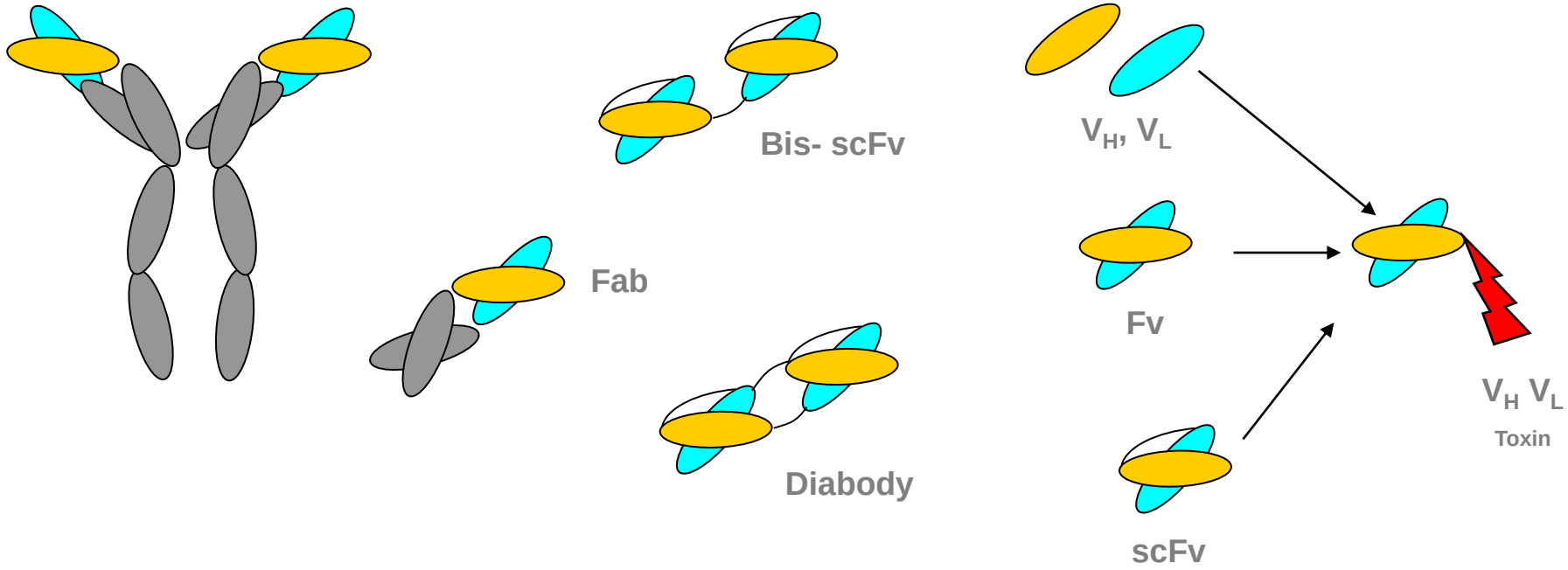
Old drugs in new systems

- Antibodies
- Aglycosylated : Yeast production, expression of heavy and light chains, assembly and export
- Humanized glycoforms (antibodies)
- Control of diversity in molecular species (glycoforms)
- Publicised success of re-enginered *Pichia pastoris* (Merck GlycoFi)
- Emerging bacterial glycosylation research

Product landscape of the future

- Products derived from mammalian cell culture will retain a significant market share
- Next generation protein therapeutics will be produced in microbial systems
- Driver = Lower COG structure
- Therapeutic molecular substructures added by bacterial cell (glycoforms) or simplified structures (for example antibody fragments)
- Highly active structures with enhanced stability (Nanobodies)

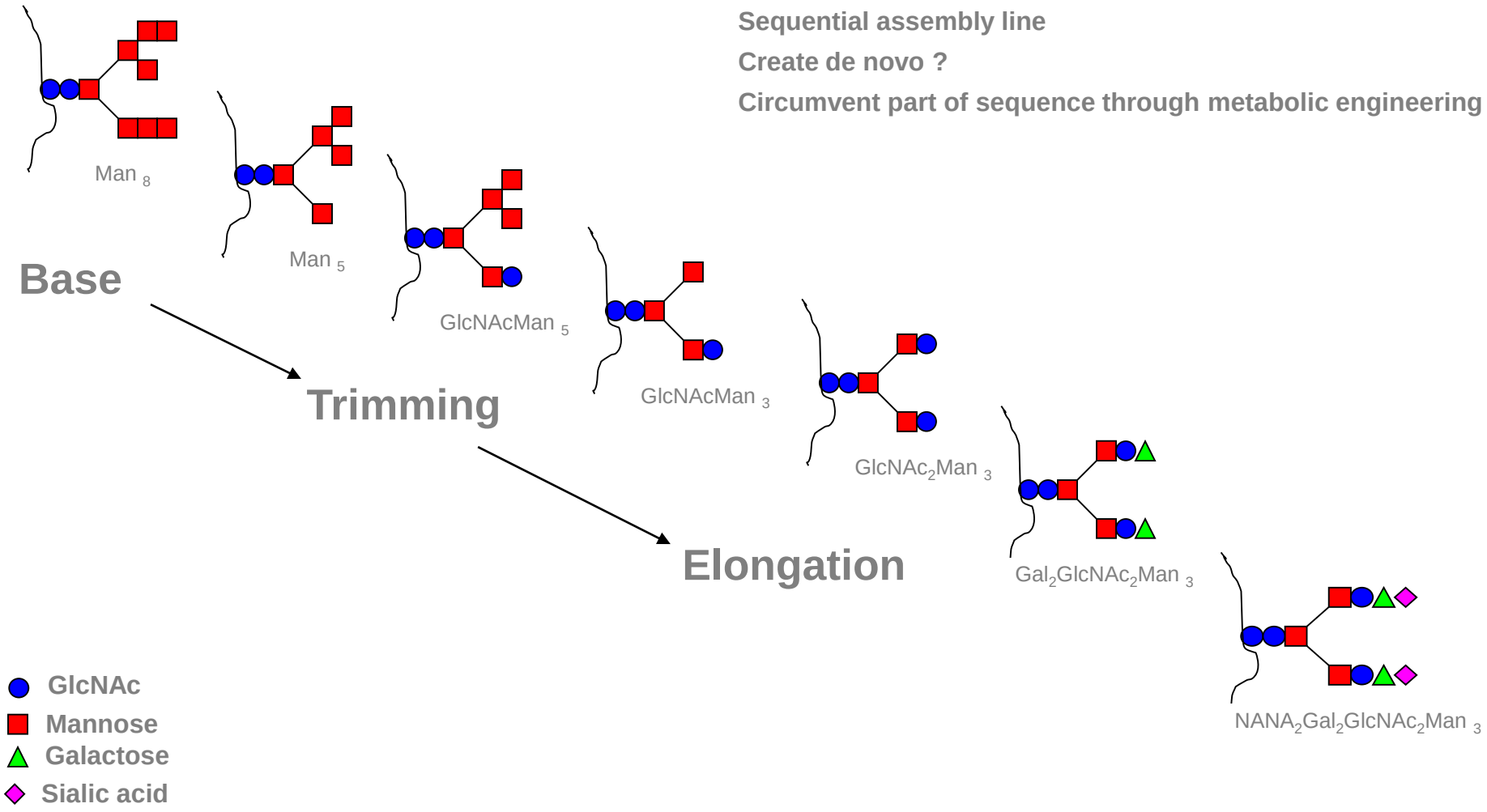
Antibodies and antibody derived molecules



Potential for glycosylation in microbial systems

- Recognition that three stage system for human glycosylation pathway : Initial, trim, extension
- Cellular localization of discrete enzyme functions (eukaryotic systems)
- The secretory pathway is a cellular assembly line
- Glycosylation to level of terminal sialyation is a common goal
- Post-purification derivitization (in vitro) to confer glycosylation is not the way forward
- Need to confer specificity of N glycosylation sites in prokaryotic systems ?

Challenge of the human glycosylation pathway



Bacterial glycosylation

Species	Glyco element	Linkage
Borrelia burgdorferi	GlcNAc	Asparagine
Campylobacter jejuni	Pseudaminic acid	Serine/Threonine
Escherichia coli	Heptose	Serine/Threonine
Mycobaterium tuberculosis	A(1-2)$Man_{(N)}$	Threonine
Ehrlichia spp	Glucose, galactose, xylose	Serine/Threonine
Pseudomonas aeruginosa	Complex	Serine
Neiserria meningitidis	B-Gal-(1-4)-α-Gal-(1-3)-X	Serine

- **Predominance of O-linked glycoforms**
- **Restricted to pilin, flagellin, outer membrane and outer surface proteins ?**

Bacterial glycosylation

Eukaryotes

- N-linked : Consensus sequence Ser/Thr-X-Asn
- O-linked : No consensus sequence
- Co-translational prior to full folding ?

Bacterial (prokaryotes)

- General glycosylation machinary (not human-like)
- Specific glycosyltransferases (gene adjacent to genes encoding specific proteins)
- Glycosylation of flexible sections of folded proteins ?

Strategies for bacterial glycosylation

- #1
- Post-translational and post purification
- Application of purified glycosyltransferases to purified bulk protein
- Least difficult
- Expensive
- Need source of purified glycosyltransferases

- #2
  - Full assembly line in bacteria
  - High # glycosyltransferases to be introduced in correct sequence
  - Most difficult

- #3
  - Hybrid mechanism
  - Exploit intermediate to offset complexity of human assembly line

√

- X

Drivers for change (2007-2017)

- COG
- Raise productivity to >5g/L in microbial systems
- Platform approach for purification
- High value protein drugs based on monoglycoforms (most effective glycoform to confer maximum therapeutic effect/unit mass)

Situation analysis 2017

- *E. coli* continues to receive re-engineering and add-on technologies
- Controlled glycosylation in bacterial systems established
- *Pfenex* receives first commercial approval
- *E. Coli* secures renewed future as dominant platform for pDNA production
- IB production scenarios revisited
- Location : TBD !