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Pioneering science delivers vital medicines™

Biopharmaceutical Manufacturing Technology: Vision for the Future

Jim Thomas, Vice President

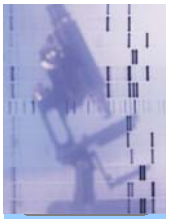
Process & Product Development

Biotech has a rich history and bright future

1950s

DNA molecule deciphered by Watson & Crick

Enzyme involved in the synthesis of nucleic acid isolated



1960s

Harris & Watkins fuse mouse and human cells

Automatic protein sequencer is perfected

1970s

Cohen & Boyer reproduce DNA in bacteria

Yeast genes are expressed in E. coli bacteria

1980s

Genentech IN BUSINESS FOR LIFE

XOMA

Applied Biosystems

biogen

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First generic markers for human disease are found

Recombinant human insulin

Commercial biotech companies formed

1990s—Today

Enbrel

Remicade

Rituxan

PROCRIT EPREX

Aranesp

EPOGEN

Neulasta

Herceptin

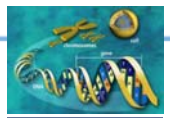
AVONEX

AVASTIN (bevacizumab)

Human Genome Project

Nearly 5 million acres of biotech crops grown

Development of leading biotech products



Tomorrow

Stem cell applications

Gene therapy

Probiotics

Efficient bioconversion of cellulose into fuel

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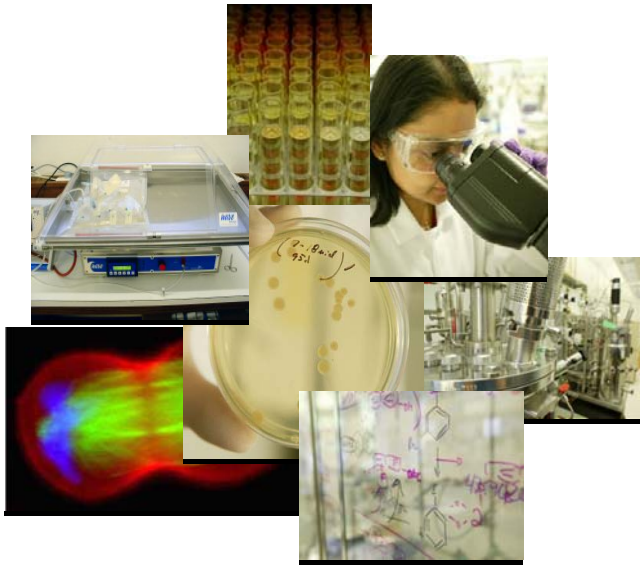
…our industry is changing rapidly

- More competitive business environment
- More challenging reimbursement environment
- More conservative regulatory environment



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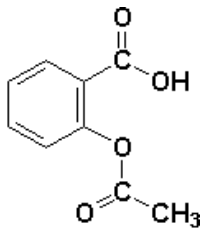
The molecules of biotechnology are complex

Small Molecules

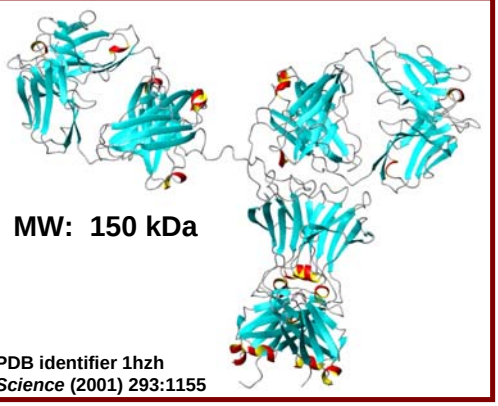
MW: 180 Da



Acetylsalicylic Acid
(Aspirin)

Organic Chemistry

Proteins
(including therapeutic antibodies)



Biochemistry
Recombinant DNA Technology

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Many patients have been served due to advances in biopharmaceutical manufacturing

…but biopharmaceutical manufacturing is a very complex and expensive business

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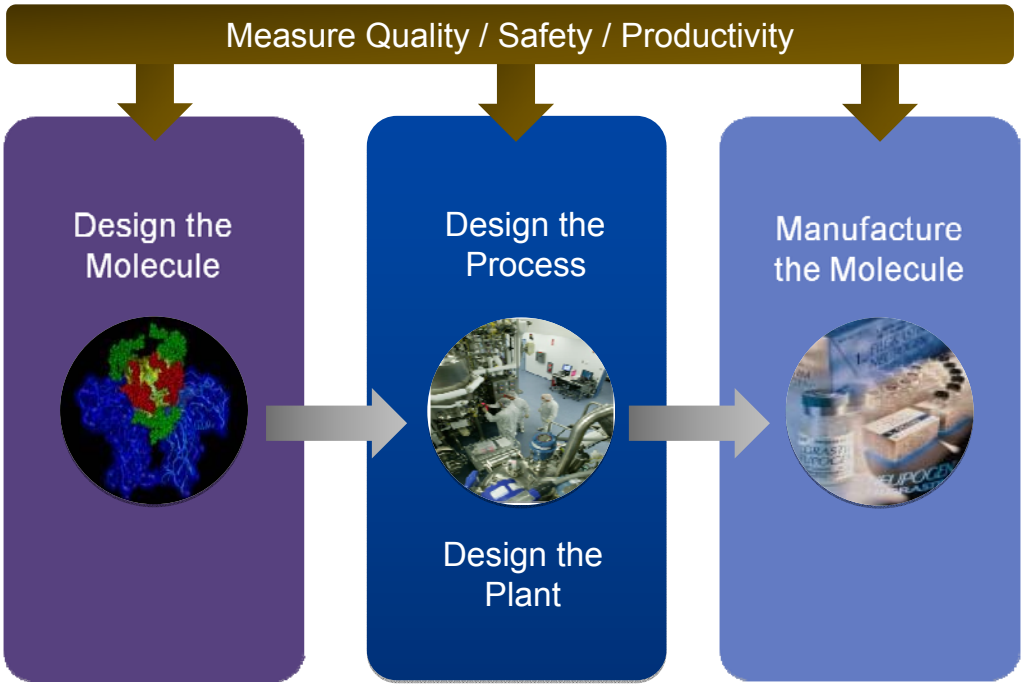
Opportunity for measurements and standards in the future manufacturing of biotherapeutics



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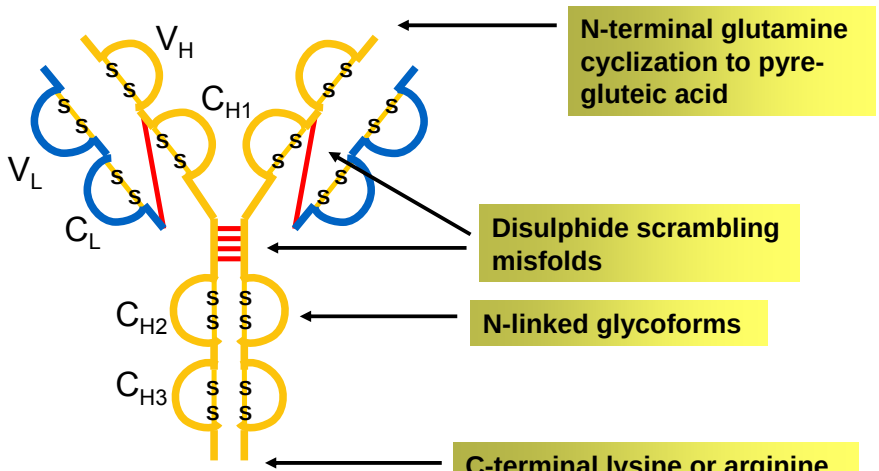
The Molecule

Challenges and Trends in Measurement

Heterogeneity can be influenced by the manufacturing process

Product variants from upstream (cell culture) processing



mAbs IgG II subclass

Challenges and trends in measuring protein therapeutics

- **Greater sensitivity, more specificity**
  - Peak profile (i.e. CEX) does not identify specific chemical changes
  - Move toward more informative assays
- **Elimination of subjectivity**
  - Visual inspection- presence of particles, color
  - Move toward automated instrument-based inspection
- **Focus on Critical Quality Attributes (CQA)**
  - Move toward knowing the specific chemical change and the site of modification
  - Move toward understanding the biological importance of chemical changes

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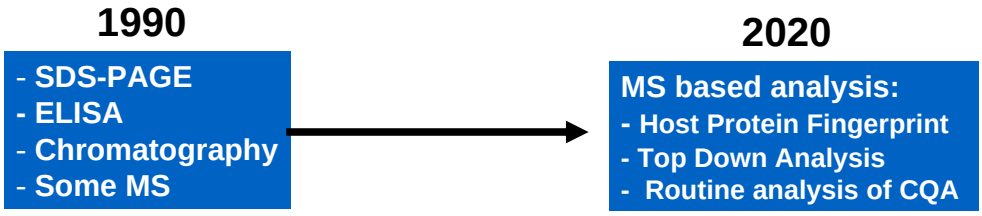
Trends in analytical tools

- **Measuring host cell protein**
- **Measuring molecule fragmentation**
- **Measuring protein microheterogeneity**



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Trends in mass spectrometer (MS) sensitivity*

Year	Detection limit of peptide (pmol)
1990	100
1993	10
1997	1
2000	0.1
2003	0.01
2005	0.001
2008	0.0001

* typical industrial laboratory

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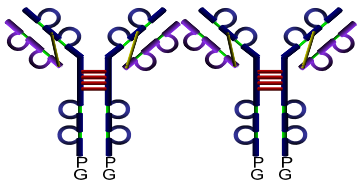
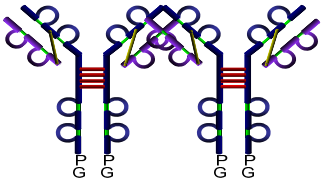
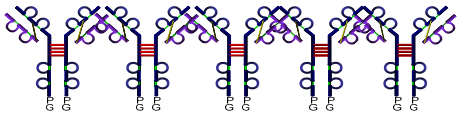
Proteins can form aggregates or particles



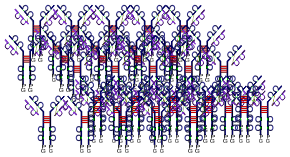
Dissociable Dimer



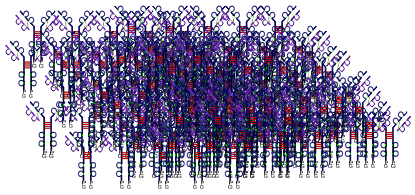
Non Dissociable Dimer



Non Dissociable Aggregate



Subvisible Particles



Visible Protein Particles

Visible Extraneous Particles

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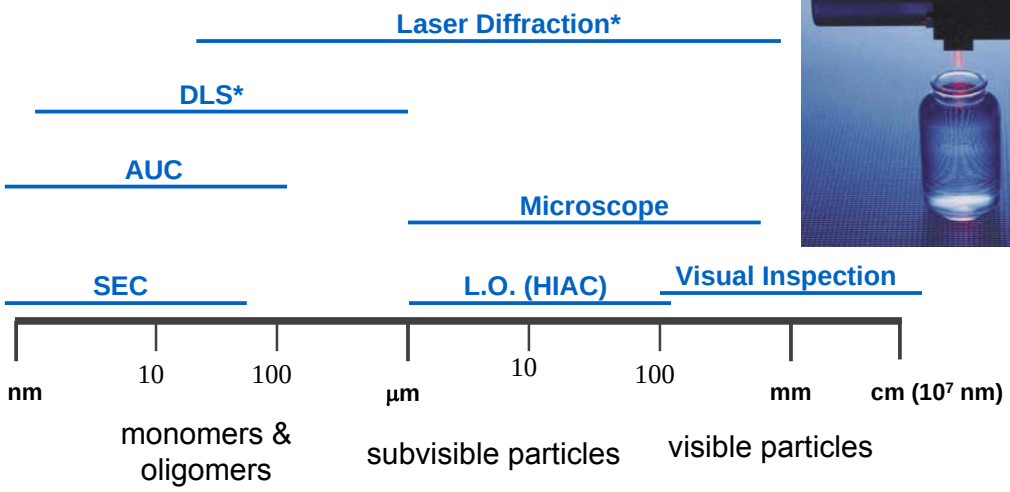
Measuring protein aggregation/particulation



* qualitative

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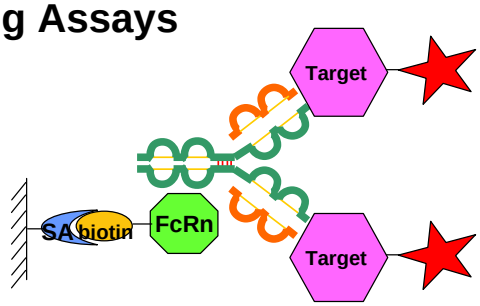
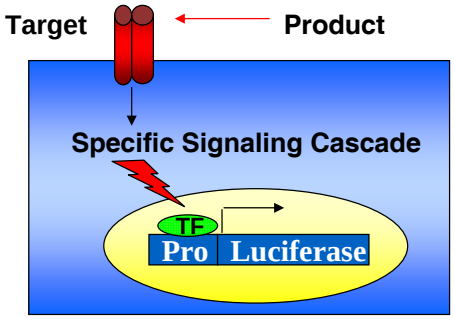
Potency measurements

- **In Vitro Binding Assays**



- **Cell Based Reporter Gene**



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Developing the Manufacturing Process

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How will biotherapeutics be manufactured in the future?

- ~~Transgenic animals~~
- ~~Transgenic plants~~
- ~~Cell free systems~~
- Cell based systems

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The Host Cell

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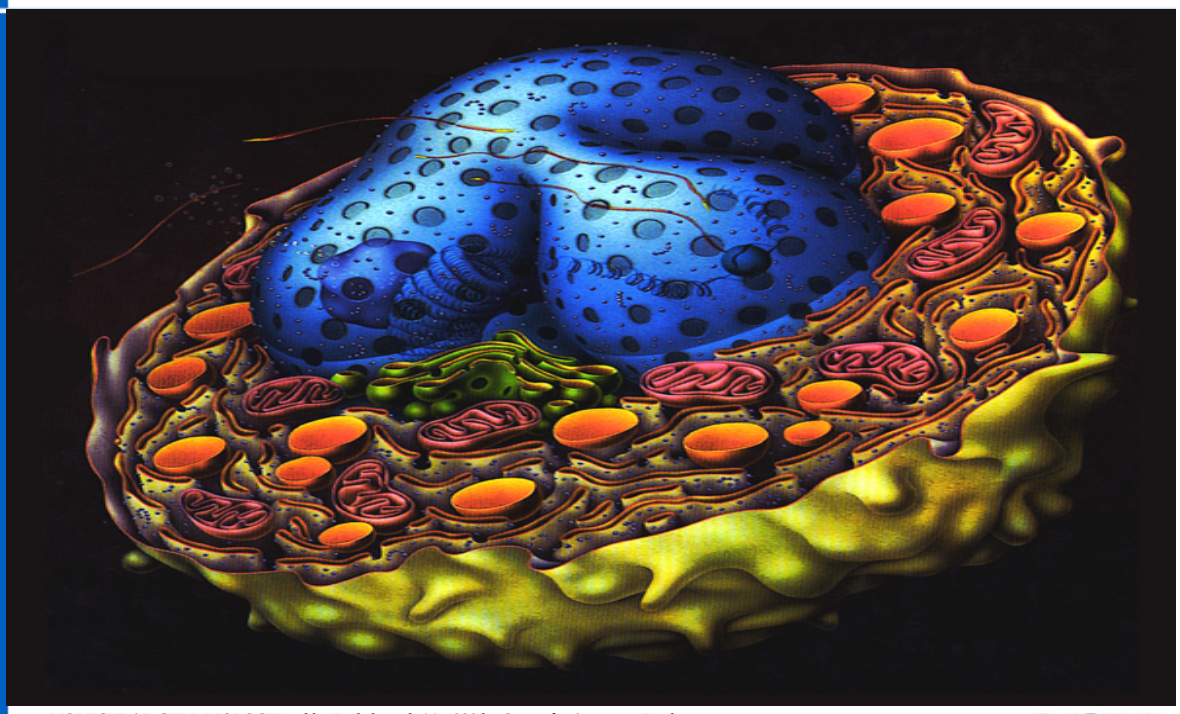
Mammalian Cells:
Core Manufacturing Technology



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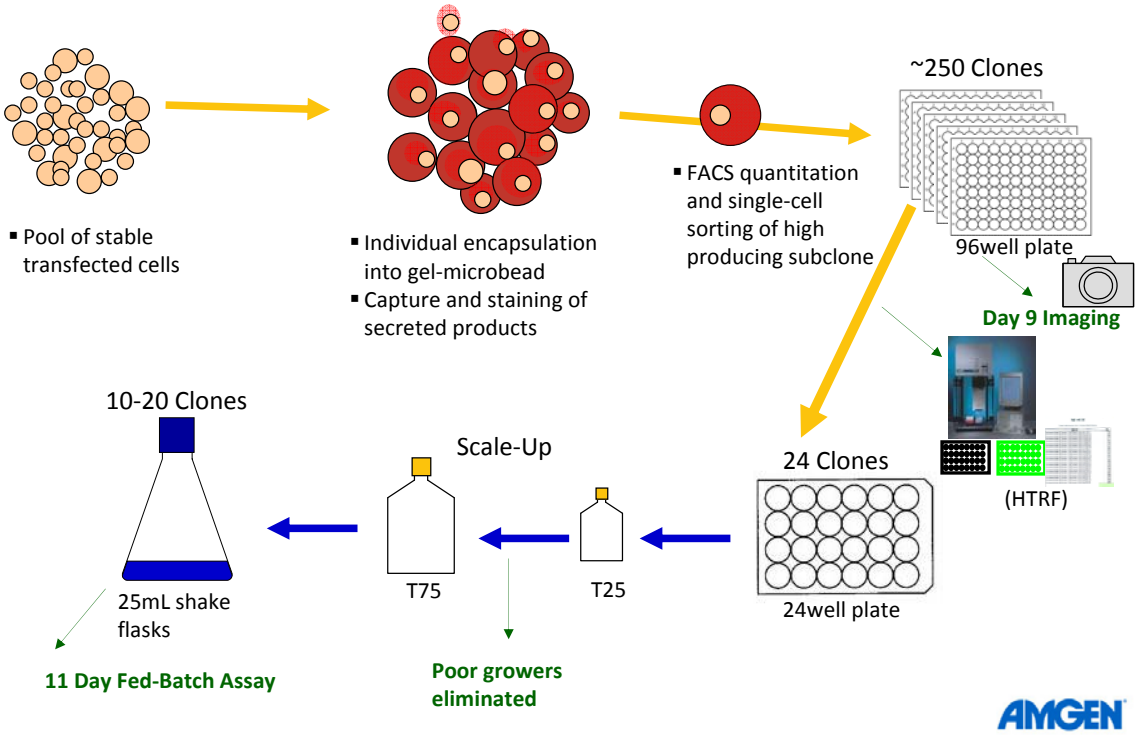
Isolating the best cell clones is challenging and time consuming



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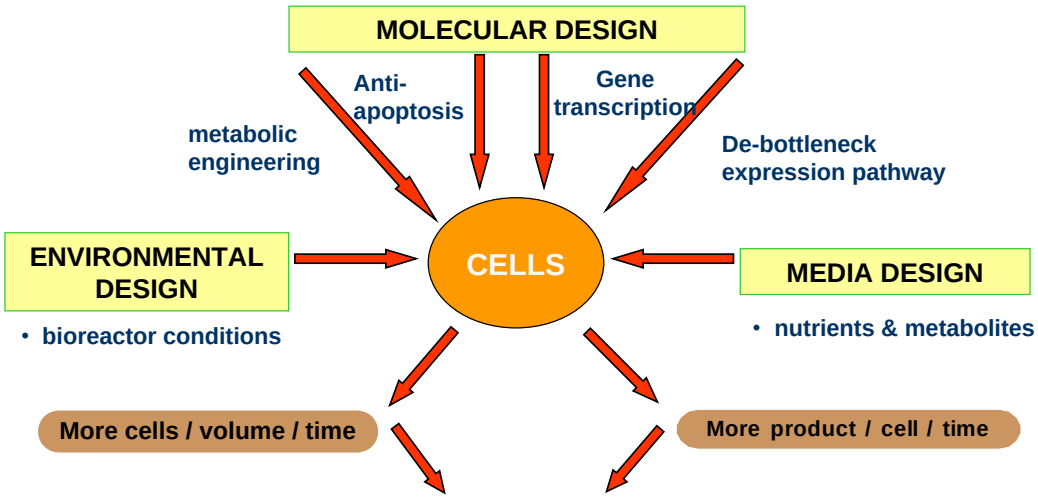
Greater productivity requires improved fundamental scientific understanding



Increased product titer

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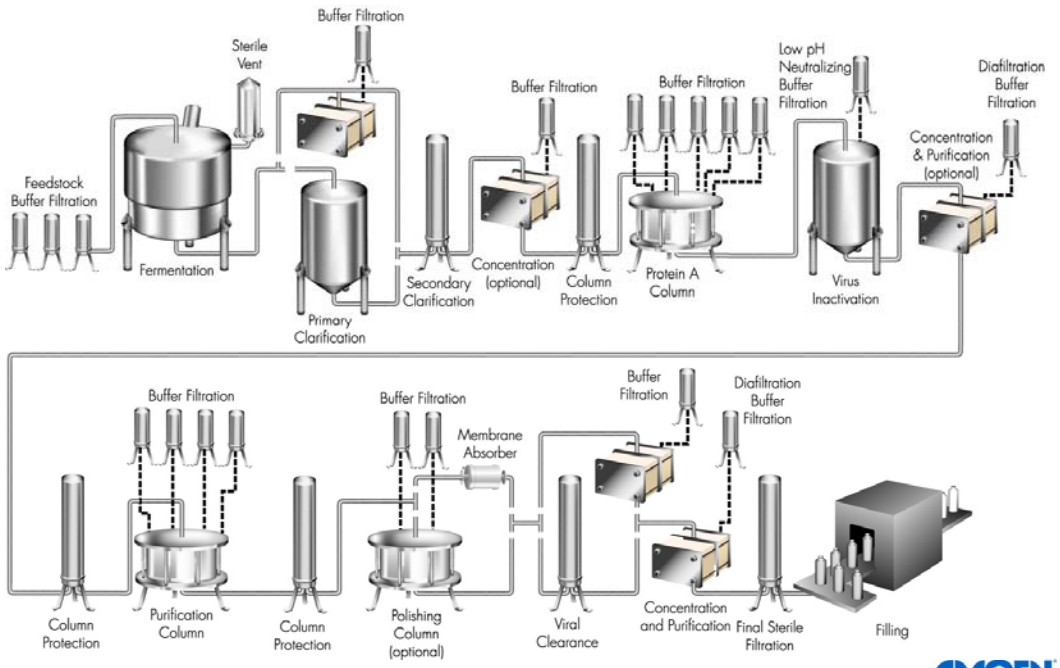
Mapping the design space of critical unit operations is a significant challenge



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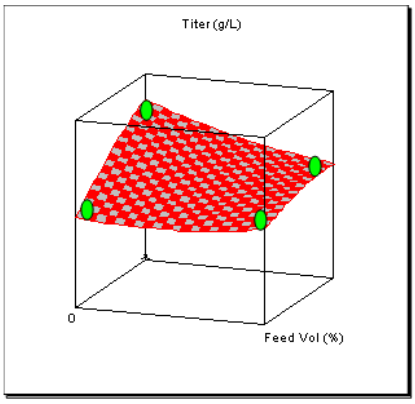
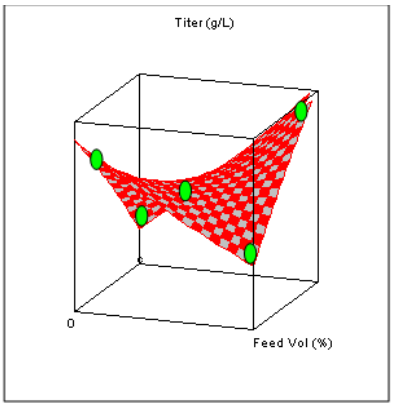
…but will be important for building quality into the manufacturing process



Media - A



Media - B

- Response surfaces represent titer
- Green dots represent conditions confirmed in 2L bioreactors

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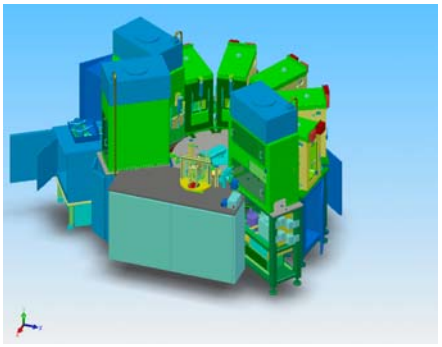
High throughput process development is needed



5 incubators
Temp. CO2, O2. Humidity, Mixing
1260 mini-reactors
8 sources for liquid addition (± 1.5ul)
pH control (< ± 0.1)
OD measurement
Integrated database
Link to other systems
TECAN, Guava etc.

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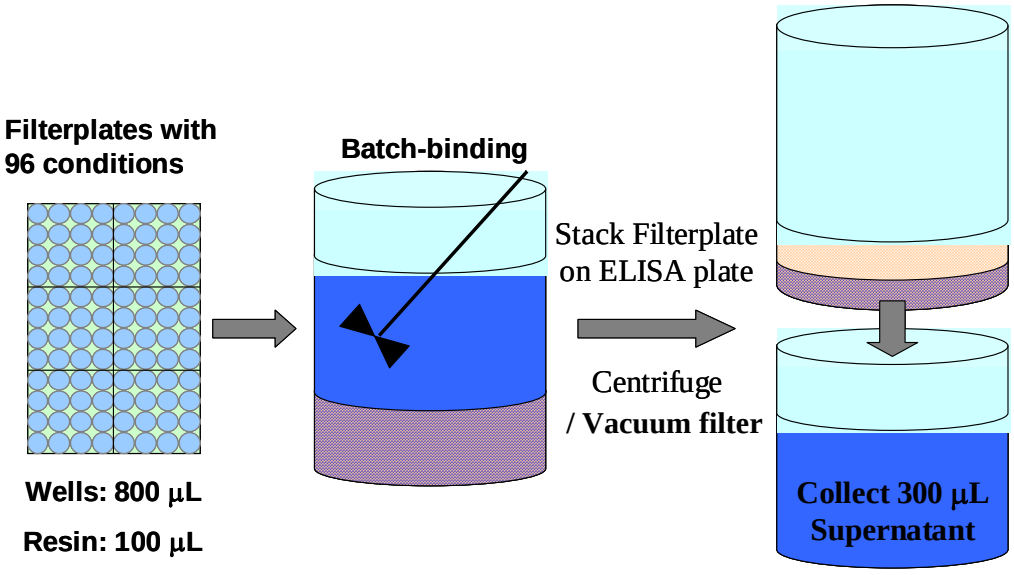
…including high throughput purification development



- HTS batch binding and elution narrows choice of conditions column runs for further optimization

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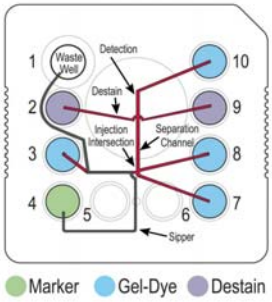
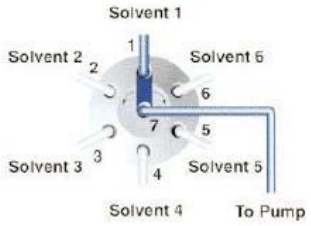
High throughput analytics are needed to support real time data acquisition and analysis





Examples of implemented HT assays:

- Titer
- Aggregate
- High mannose
- Amino acid analysis
- CHOP Elisa

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The Manufacturing Operation

Process Design
Plant Design
Measuring the Environment
Quality of Raw Materials

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Manufacturing operations will be more efficient in the future

- Higher yielding processes
- Greater plant flexibility
- Better utilization of capital
- Significant reduction in operating costs

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Holistic approach to optimization

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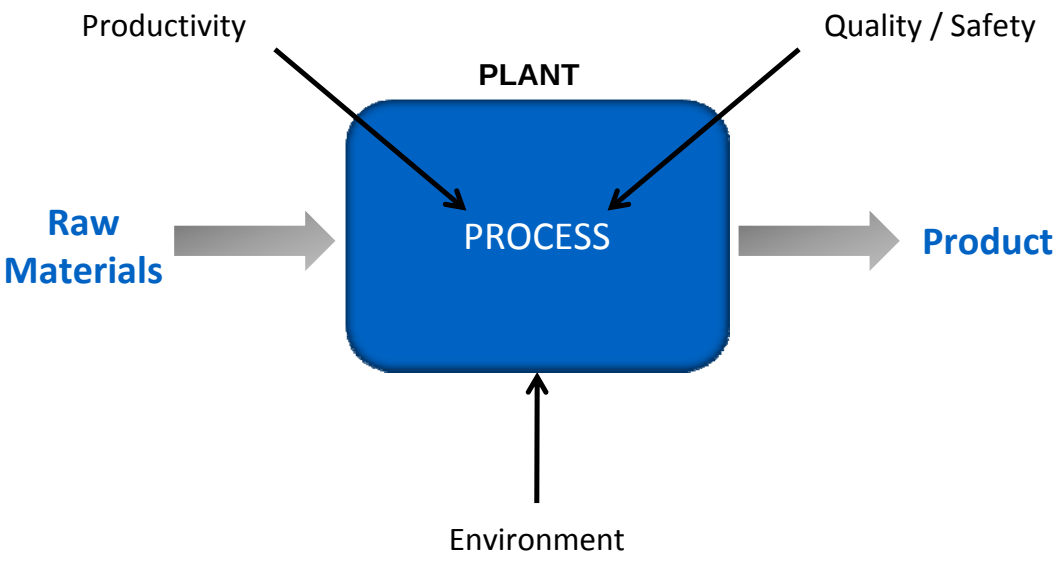
The manufacturing operation - opportunities for measurements and standards



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Process Design

Productivity and Quality

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Upstream parameters for potential measurement and control

- O_2, CO_2, pH, temperature
- Nutrient composition
- Depletion of nutrients
  - Metabolic byproducts
  - Amino acids
  - Carbohydrates
- Cell mass, cell number, cell viability
- Product titre
- Product quality
  - Aggregation
  - Clipped species
  - Glycosylation

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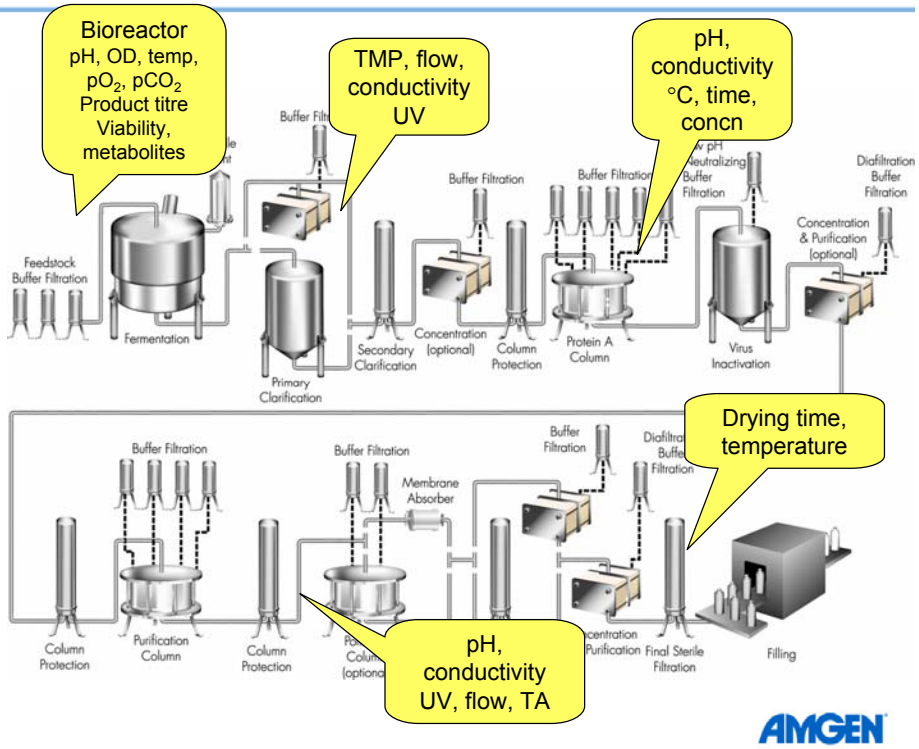
Examples of monitoring and control points

Environmental

Raw materials
Process aids

Water



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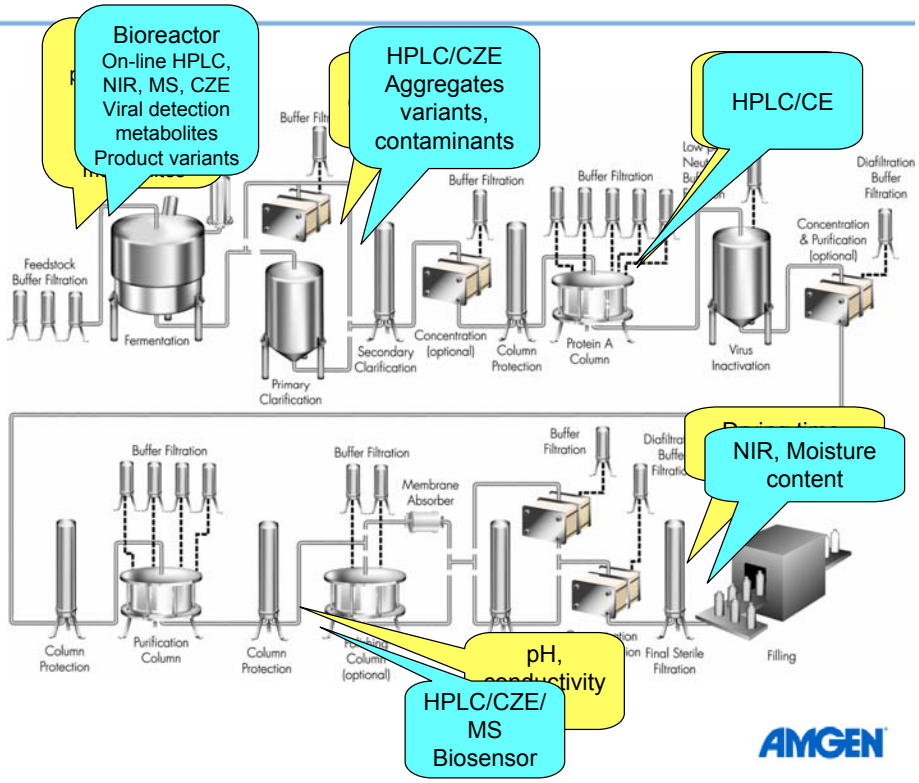
Opportunities for monitoring and control

Raw materials
Process aids

Water

Environmental



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Plant Design

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Trends in manufacturing plant design

Flexibility
- for optimizing plant capacity

Capital Cost
- engineering construction
- materials

Operating Cost
- utilities
- maintenance
- environmental control/monitoring

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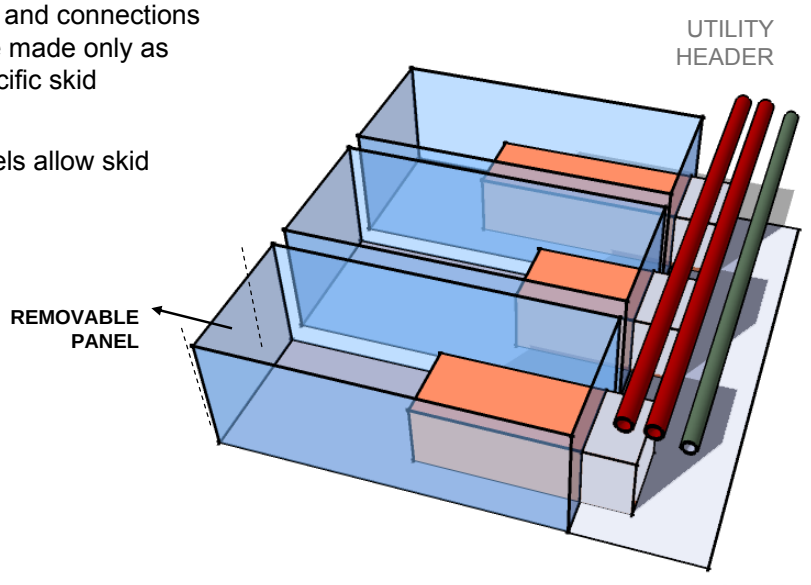
Plug ‘n Play concept assembly

Assembly

1. Taps off the headers and connections to the skids would be made only as needed for each specific skid requirement
2. Removable wall panels allow skid replacement



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Modular pre-fabrication approaches

Degree of Integration

- **Comprehensive Prefab**
  - integrated prefab structure / facility / process
- **"Box-in-a-Box"**
  - integrated pre-fab facility within a stick-built structure
- **Partial Prefab**
  - prefab assemblies for insertion in stick-built structure and facility

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Disposable technology could improve plant utilization and decrease utility cost

- Top mounted magnetic-drive agitator
- Disposable optical pH and DO sensors
- Sparge, overlay, and exhaust filter lines
- Weldable media addition and sample lines
- Fully customizable bag design
- Touch screen PLC controller
- Movable platform skid

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Measuring the Environment

Mycoplasma
Bacteria
Viruses

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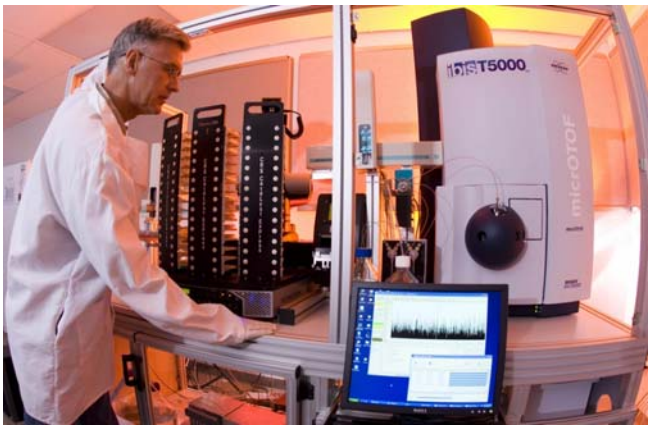
High throughput methods for detecting microbes will improve environmental control

- **Detects all infectious threat agents**
  - **Bacteria, mycoplasma, viruses, fungi, protozoa**
- **Resolves mixtures of organisms**
- **Quantitative**
- **Sub-species resolution (strain genotyping)**



Universal Pathogen Detector

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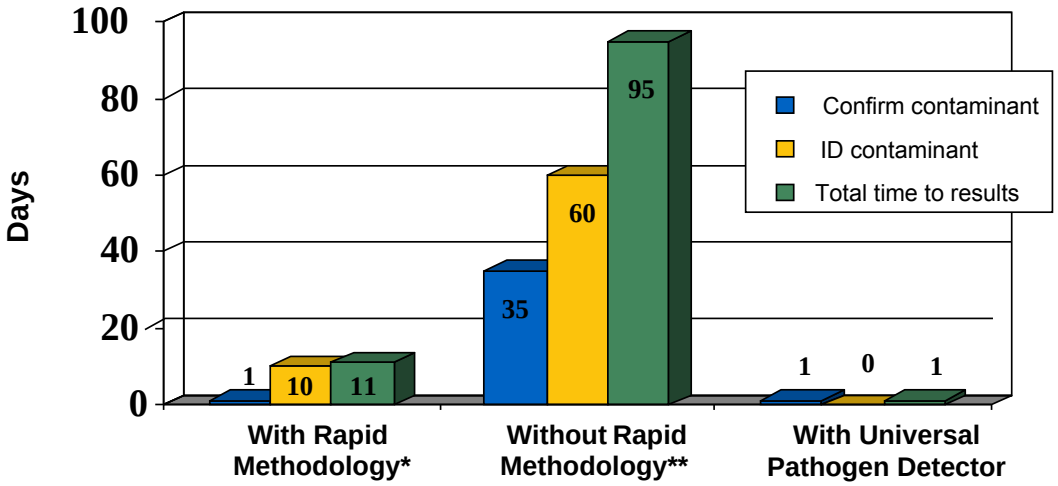
Contamination turn-around time



***** Assumes a detection assay exists for the contaminant

**Best case scenario. Some reports indicate over a year's time investigation without definitive identification (finally identified using the universal pathogen detector)

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Quality of Raw Materials

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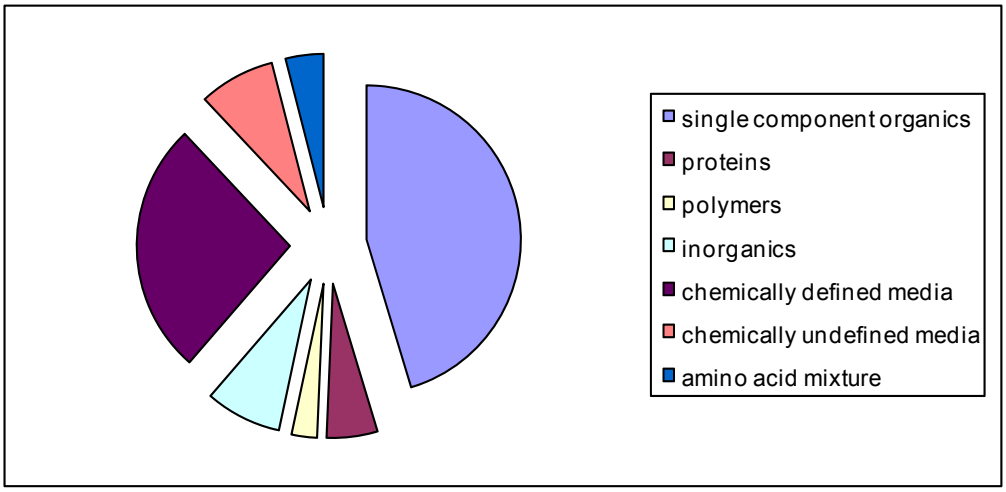
Distribution of chemical classes for raw materials in upstream cell culture



^{1}H and ^{13}C NMR cover all chemical classes except inorganics

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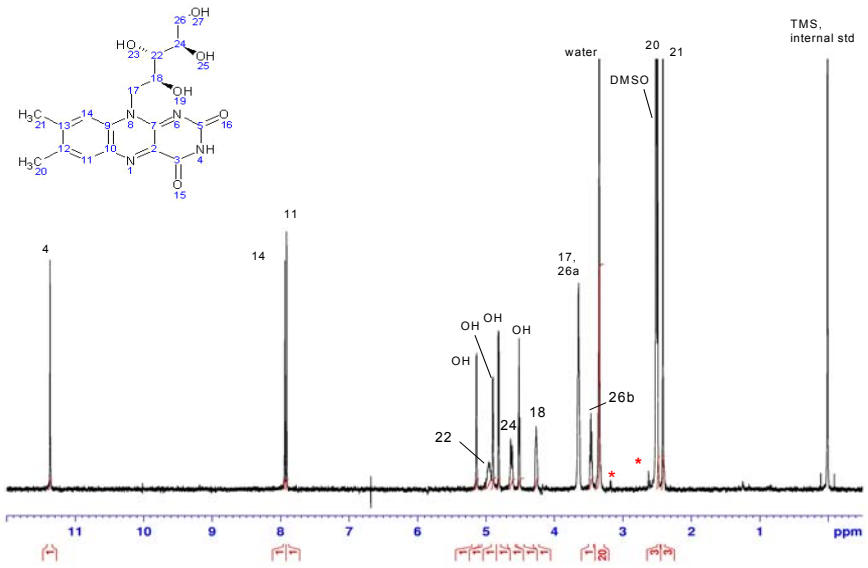
Riboflavin

- **^{1}H NMR spectrum of riboflavin lot # 0010011629. Asterisks denote impurity peaks.**



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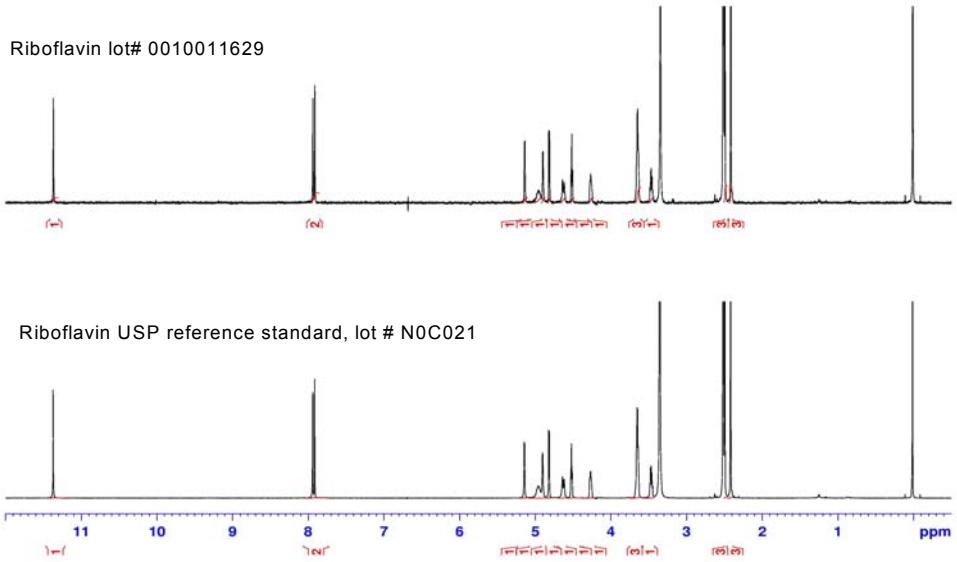
Riboflavin

- ^{1}H NMR spectral comparison between riboflavin lot # 0010011629 and USP reference standard lot# N0C021



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Technological opportunities for improving the future of biotherapeutic manufacturing

- Quick assessment of chemical modifications and their biological relevance
- Merging of characterization, release and online product quality assays to deliver PAT
- Improve measurements associated with creating and quickly selecting high expressing clones
- High throughput tools for process development and formulation development that function as scale down models
- More continuous upstream and downstream processing with associated measurements and controls
- Standard high throughput methods for measuring microbes
- Powerful analytical tools and shared standards for assuring the quality of raw materials.

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Acknowledgments

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Drew Kelner *Analytical & Formulation Sciences*
Brent Kendrick *Analytical Sciences*
Duncan Low *Process Development*
Jeff McGrew *Cell Sciences & Technology*
Linda Narhi *Formulation & Analytical Resources*
Timothy Osslund *Analytical & Formulation Sciences*
Suresh Vunuum *Purification Process Development*
Siowfong Wee *Analytical & Formulation Sciences*
Zhongqi Zhang *Formulation & Analytical Resources*

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