Introduction to Bioprocessing

Cambridge Healthtech Institute Peptalk

Palm Springs, CA

Presented by Susan Dana Jones and Sheila Magil

BioProcess Technology Consultants
www.bptc.com

BioProcess Technology Consultants

- World leader in providing strategic, technical, regulatory, and business consulting services to biopharmaceutical industry
- Founded in 1994; 20+ year proven track record
- Team of 13 consultants has provided consulting services to over 500 clients across six continents

Instructors

- **Susan Dana Jones, Ph.D.**

Vice President and Senior Consultant, BPTC

Susan is a seasoned biotechnology entrepreneur with experience in product development, outsourcing, and strategic planning. She is a subject matter expert in cell line development and characterization for biosimilar, new biopharmaceutical, and vaccine development programs. She has broad knowledge of regulatory requirements for manufacturing products for human use and has prepared CMC sections of multiple regulatory submissions.

- **Sheila Magil, Ph.D.**

Senior Consultant, BPTC

Sheila has over 20 years of experience in quality and analytical method development for biologics, peptides and small molecules. Her expertise includes quality assurance, protein and peptide biochemistry, and analytical development. Sheila has implemented quality systems and has managed external analytical and QC activities for multiple biopharmaceutical products.

Introduction to Bioprocessing Course Content

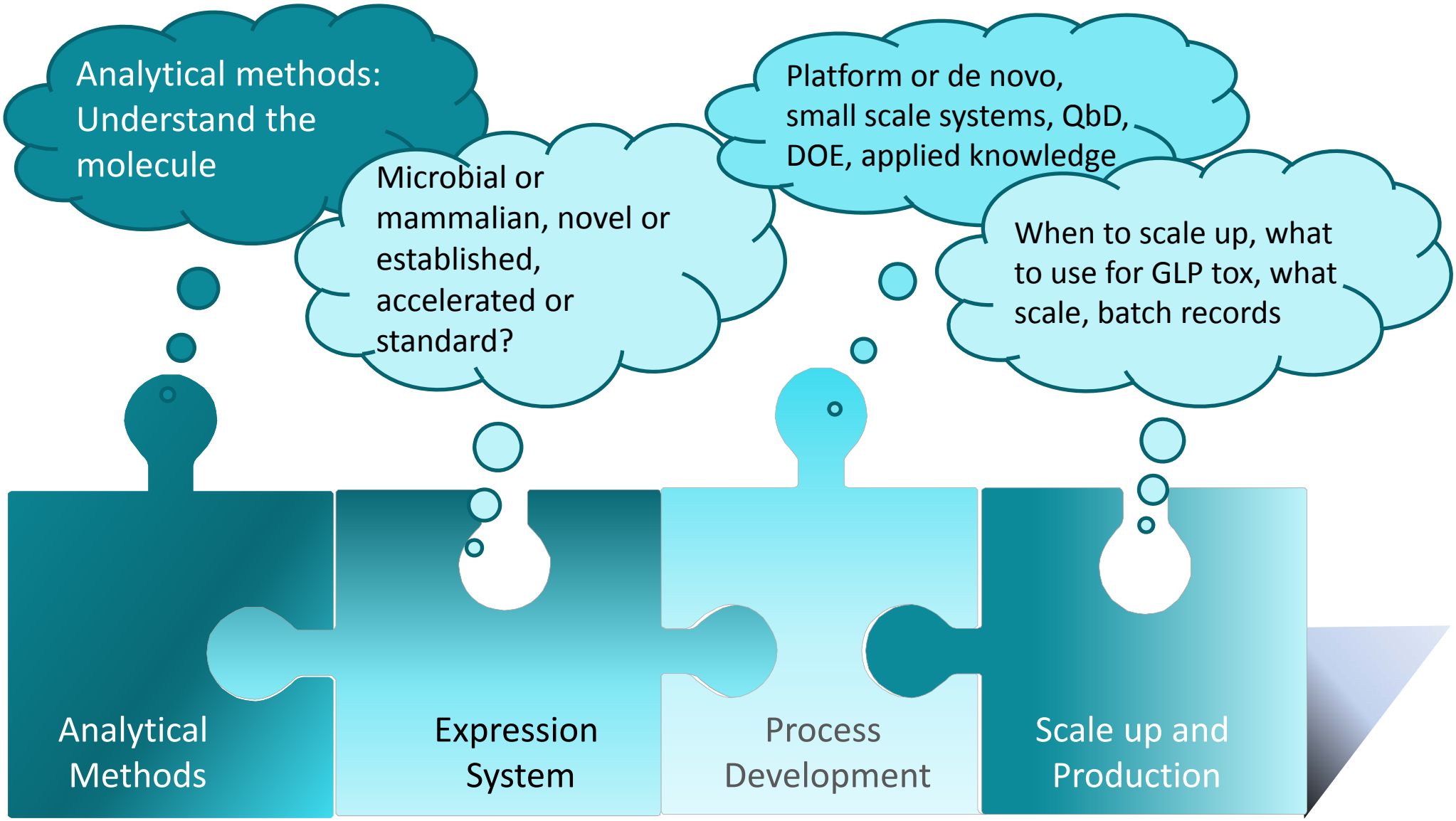
- Definition of biopharmaceutical products
- Regulatory considerations in bioprocessing
- General timelines for biopharmaceutical product development
- Critical CMC activities:
  - Project management and tracking
  - Analytical methods
  - Expression systems
  - Downstream processing and viral clearance/inactivation
  - Formulation
  - Stability
- Cost considerations
- Process qualification
- Comparability

What are Biopharmaceutical Products?

- Therapeutic proteins including antibodies, nucleic acids, gene therapy viral vectors
  - Generally produced by recombinant DNA technology
- Excluded from definition are traditional biologic products
  - Non-recombinant vaccines, plasma-derived proteins
- Focus of this training course is on production of biopharmaceutical products, especially monoclonal antibodies, in mammalian cell culture

Example Biopharmaceutical Product	MW	# of Amino Acids	# of Protein Chains	Glycosylated
Insulin	~6 kDa	51	2	No
Growth Hormone	~22 kDa	191	1	No
Erythropoietin	30-34 kDa	165	1	Yes
Coagulation Factor IX	~52 kDa	461	1	Yes
Monoclonal antibody (IgG_1)	~150 kDa	~660	4	Yes
Factor VIII	~240 kDa	2133	1	Yes

Project Management: Integration of Development Activities



Protein Structure

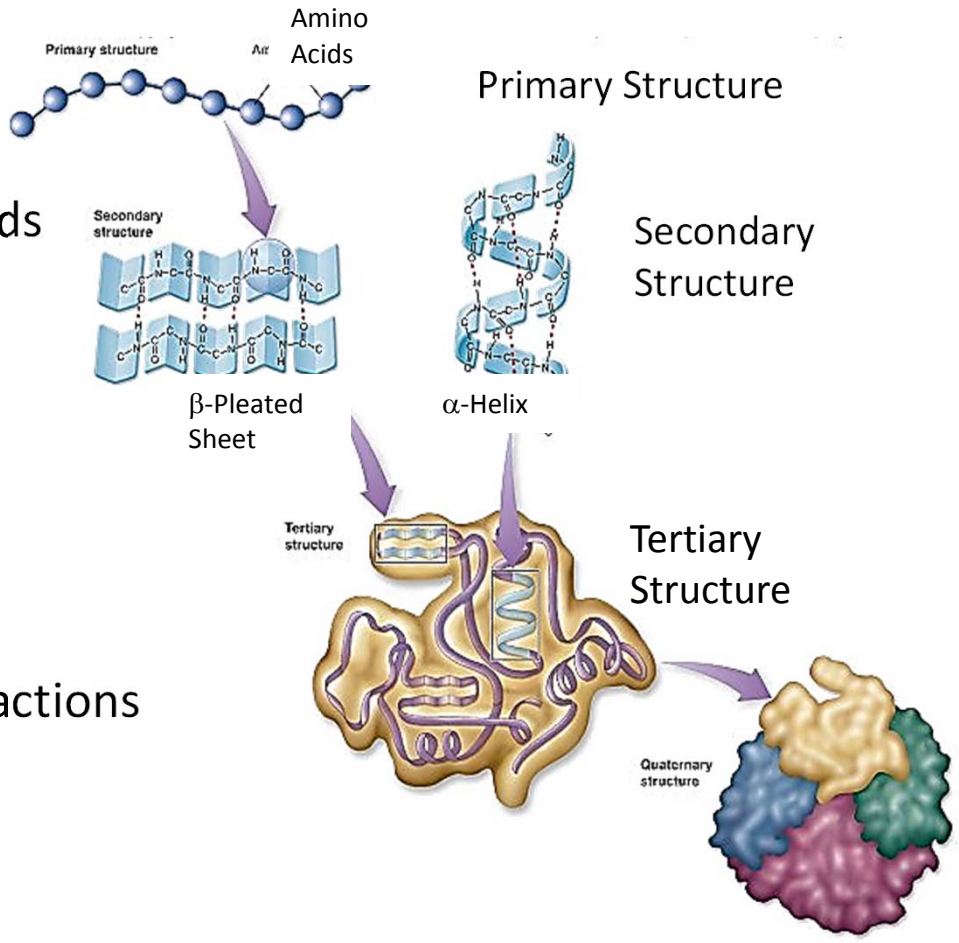
- Proteins have an inherent degree of structural heterogeneity

- Primary Structure
  - Amino acid sequence
  - Held together by peptide (amide) bonds

- Secondary Structure
  - Held in place by hydrogen bonding

- Tertiary Structure
  - Driven by hydrophobic and ionic interactions

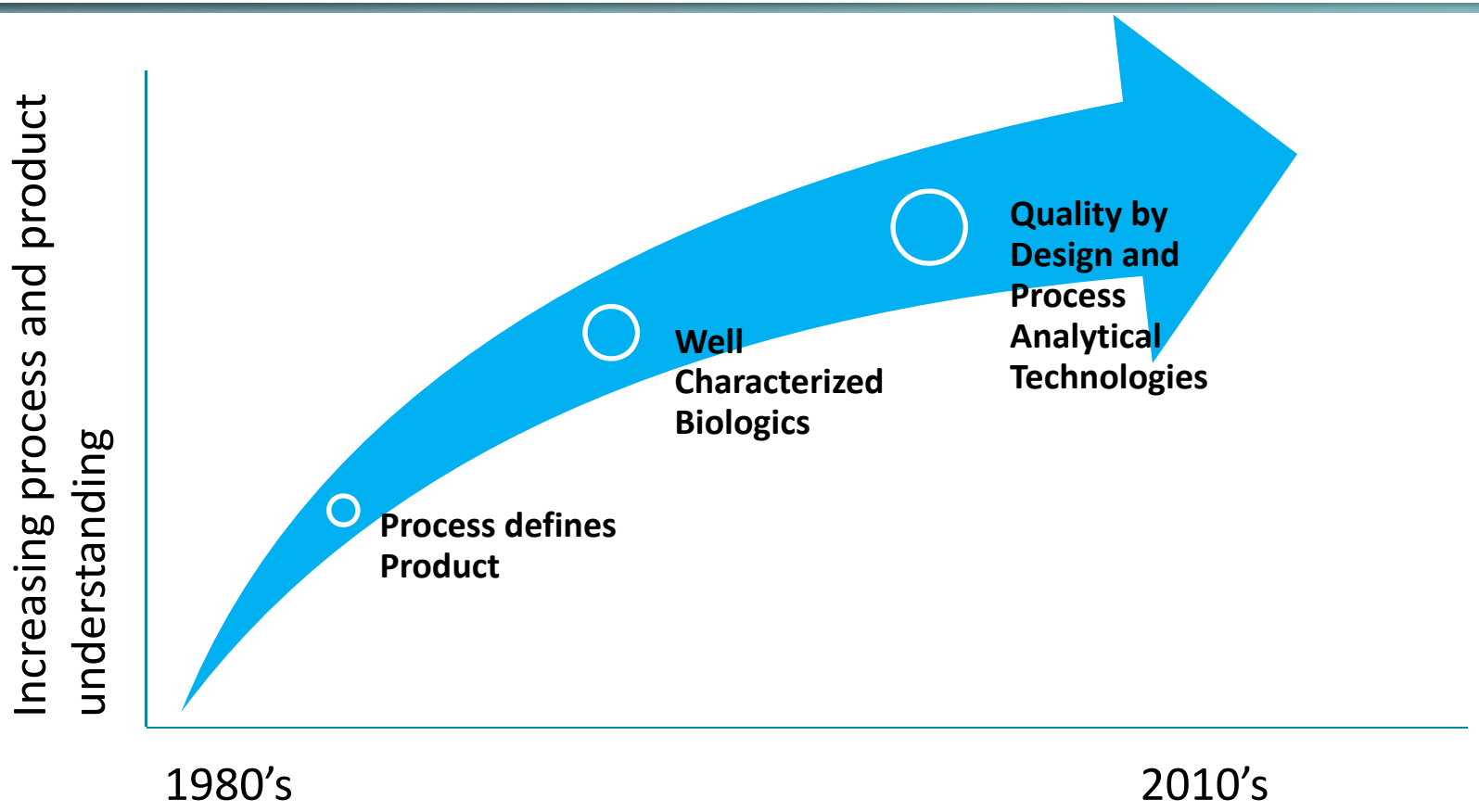
- Quaternary Structure
  - Cluster of similar or different proteins



Classes of Analytical Methods

Classes of Methods	Specific Methods
Spectroscopy	UV-Vis, CD, FTIR, Fluorescence, Raman
Chromatography	Reverse Phase, Size Exclusion , Ion Exchange, Hydrophobic Interaction, Affinity
Electrophoretic	SDS-PAGE and Normal Phase, Reduced and Non-reduced, 2-Dimensional, Isoelectric focusing, capillary Coomassie and Silver stains
Immunophoretic	Western Blot, ELISA
Immunoassays	Bioassays, ELISA
Potency	Bioassays, Cell-based, Binding
Microbiological Analyses	Bioburden, Sterility, Viability, Microbial Contamination, Endotoxin
Chemical (Compendial)	USP/EP/BP/JP raw material testing
Other	Analytical Ultracentrifugation, Particulate Matter, pH, Visual appearance

Role of Analytics

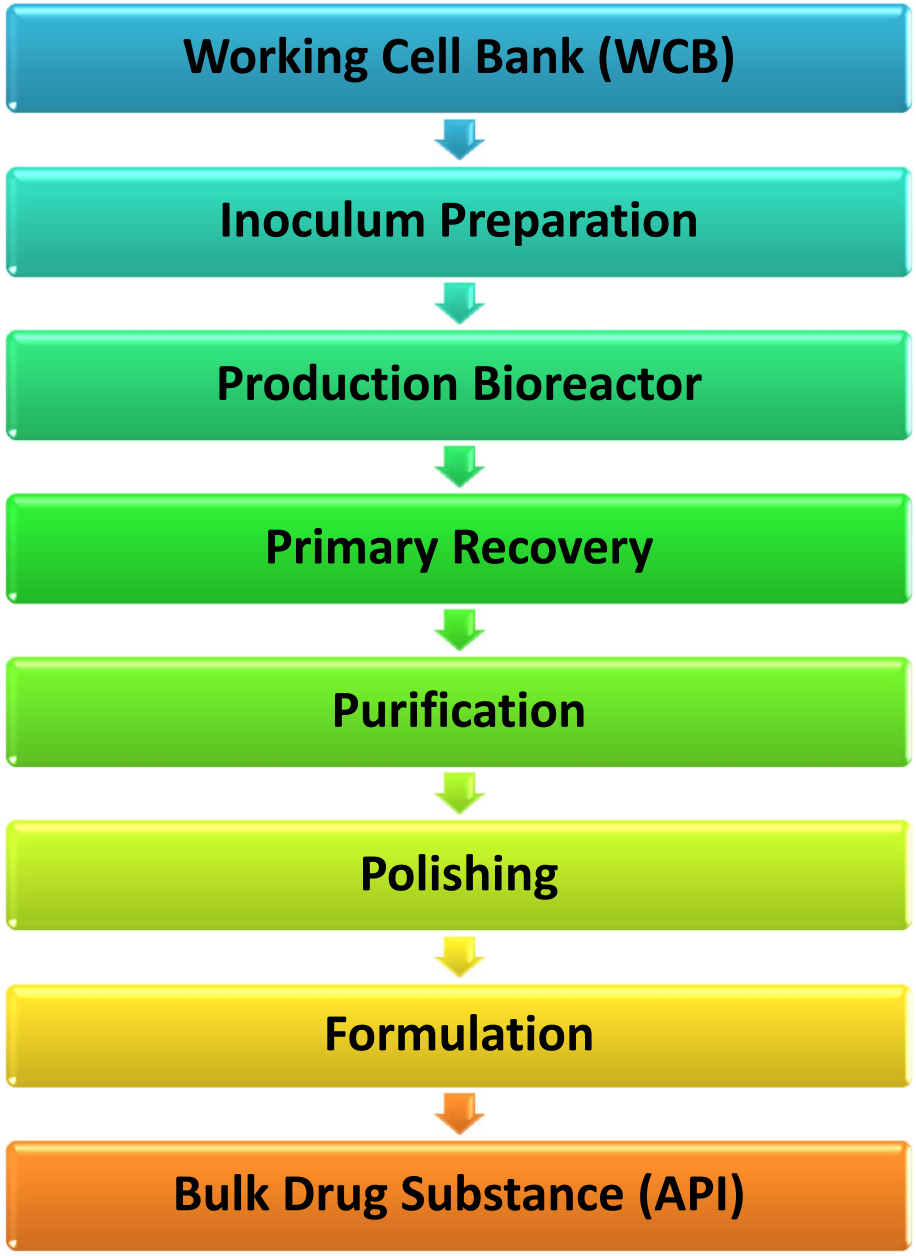


With each step, analytical methods have become increasingly important and front-end loaded

"The QbD process design starts with an intensive characterization of the product through a large array of biochemical and biophysical analyses at normal and stressed conditions…"

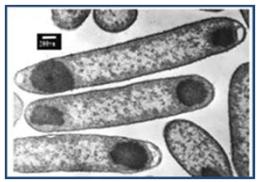
Banerjee A. BioPharm Intl 23(5): 26-40.

General Scheme for Biopharmaceutical Manufacturing

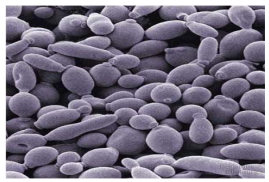


Common Production Hosts

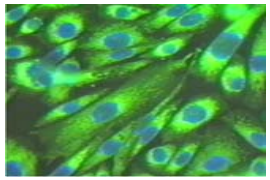
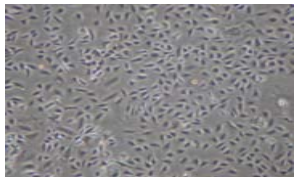
- Bacteria
  - *E. coli*
  - *Pseudomonas fluorescens*

- Yeast
  - *Saccharomyces cerevisiae*
  - *Pichia pastoris*



- Mammalian cells
  - Chinese hamster ovary (CHO)
  - BHK and HEK (specific indications)
  - Per.C6 and other human lines

Critical issues to address

- Technical
  - Overproduction may cause aggregation or degradation by host, toxicity to host cell, inaccurate or incomplete processing
  - Post translational modification (glycosylation)
- Intellectual Property Rights
- Manufacturing Capabilities for Clinical and Commercial Supplies

An important trend – single use bioreactors

Xcellerex
XDR™ Bioreactor

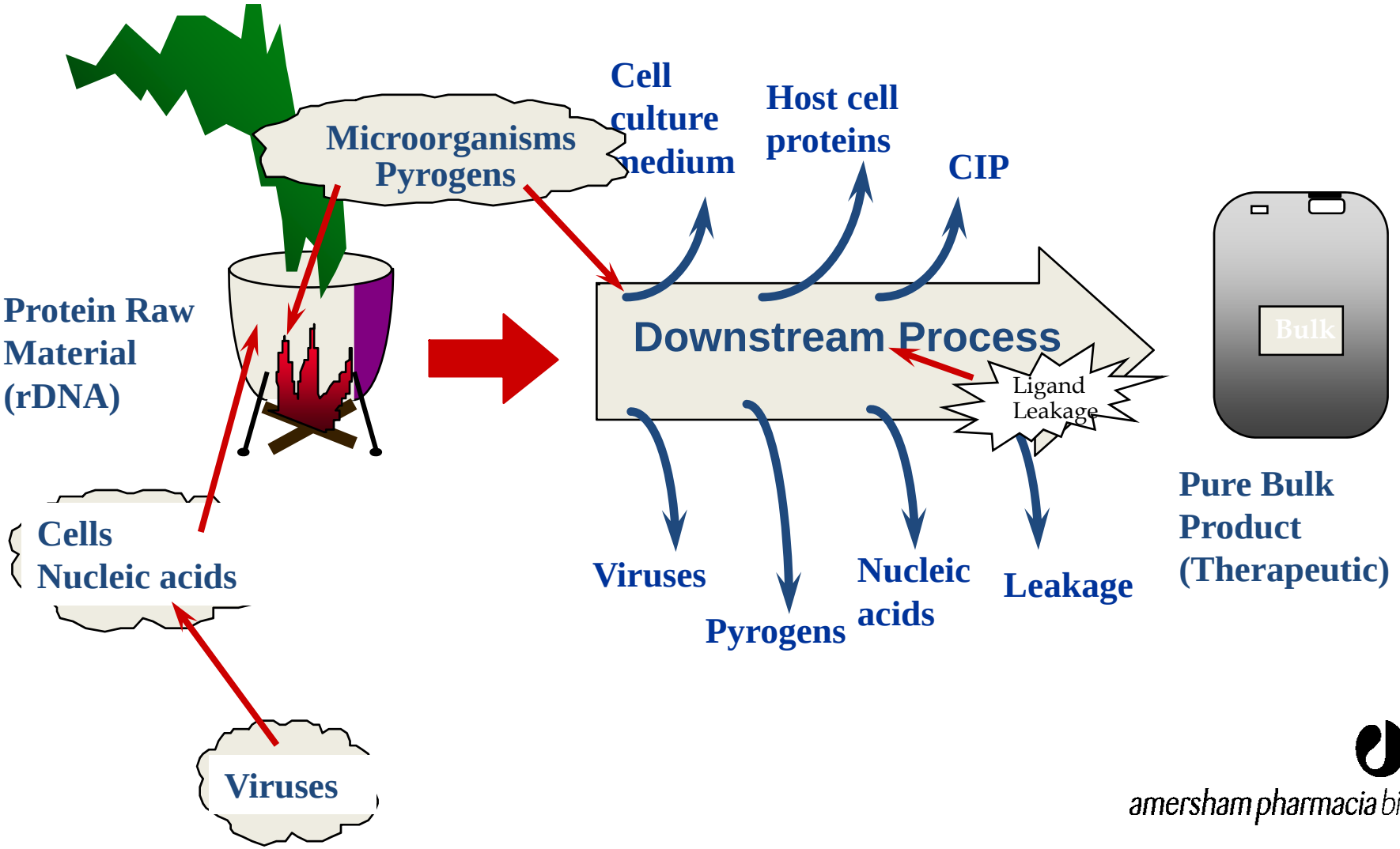
Sartorius Stedim
Biostat® Culti-bag

Thermo Fisher (Hyclone)
Single-use Bioreactor

GE Healthcare
Wave Bioreactor

ATMI
Nucleo™ Bioreactor

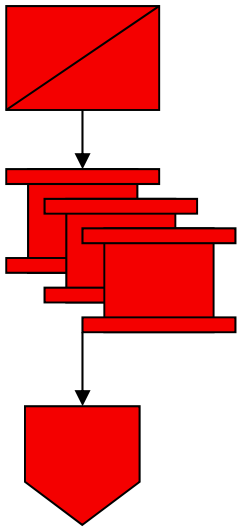
Downstream processing of biopharmaceuticals



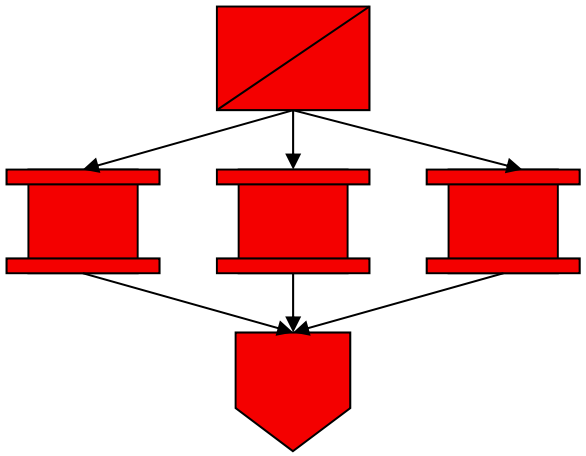
amersham pharmacia biotech

Column sizing and optimization

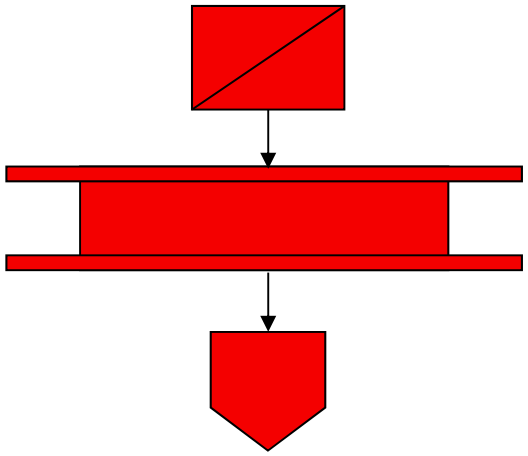
- Balance cost of production (COP), production rate (g/hr) and productivity (g/hr/L support)
  - Trade-off between capacity, throughput, and cost
  - Multiple cycles vs. a larger column?
  - How frequently should media be replaced?
  - Support equipment sizing and cost



Cycling small column in series

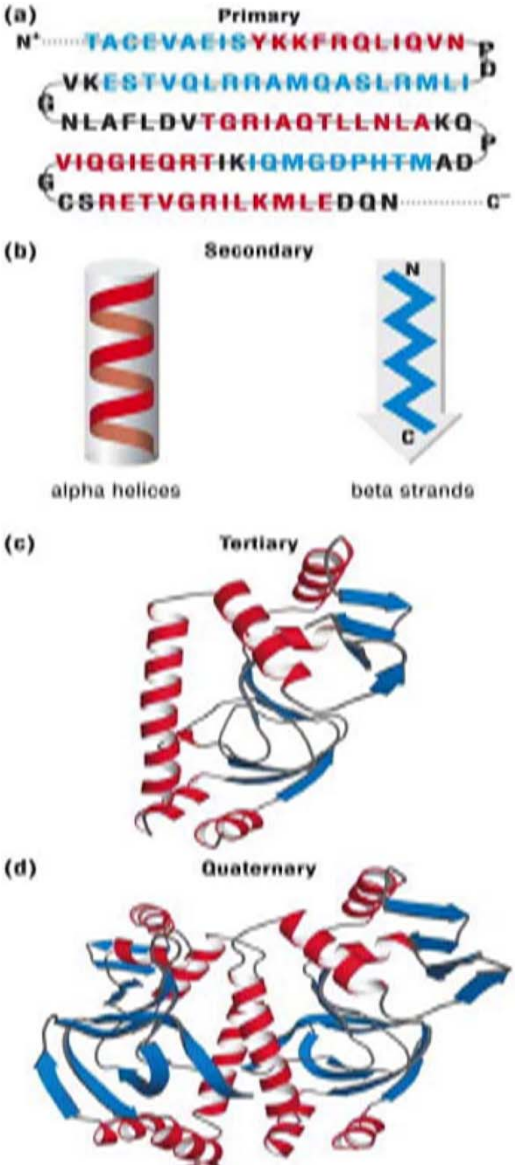


Multiple small columns in parallel



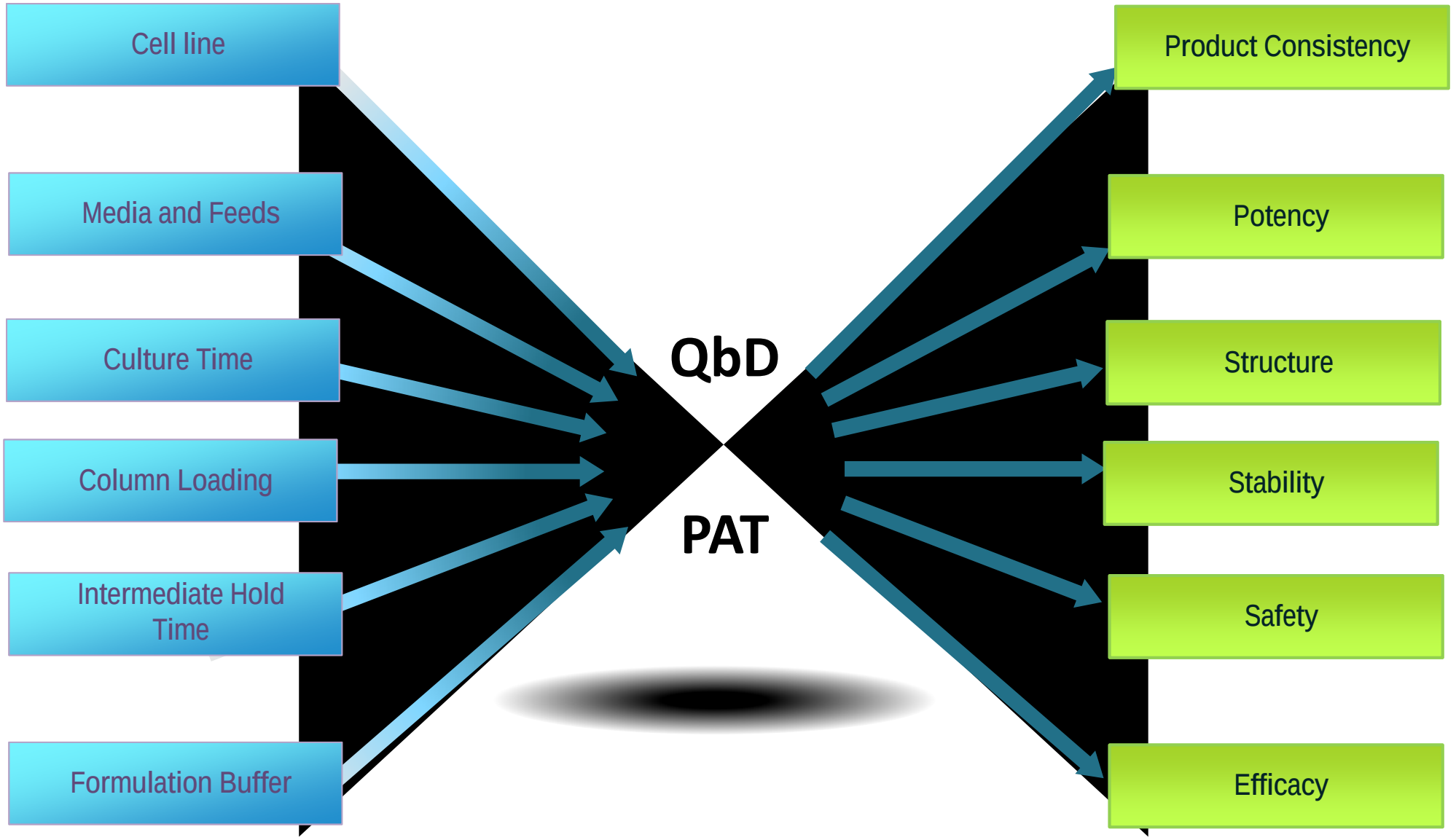
Single large column

Product Comparability



- Primary structure
  - Amino acid sequence
- Secondary structure
  - Three-dimensional structure of the protein (alpha helices, beta sheets, loops/turns)
- **Tertiary structure**
  - **3-D structure of protein through interaction of the secondary structures**
  - **Increased regulatory emphasis on this aspect of structure**
- Quaternary structure
  - Describes the three-dimensional arrangement of protein subunits

Product Control Through Process Control



Thank You!

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