Analytics-driven Disruptive Changes in Bioprocessing

Analytical Applications to Enable More Affordable Biologics

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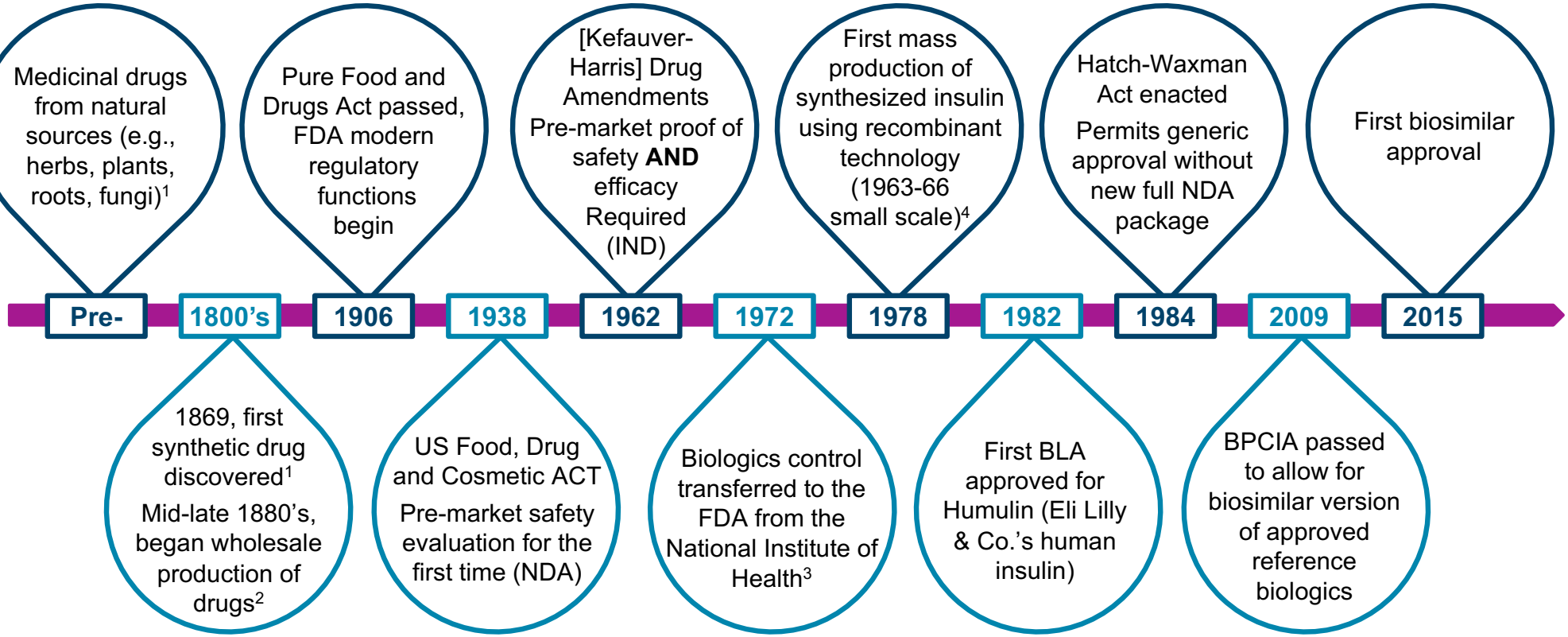
About the Presenter

- PhD in Pharmaceutical Sciences, 45+ years of teaching, research, entrepreneurship, inventorship, authorship, lectureship, and philanthropy.
- 100+ refereed papers, 40+ major books, 500+ lectures, 75+ patents, fellowships, civil awards, recognitions.
- Inventions: wine-making, whiskey-making, automobile safety, single-blade wiper, LCD camera shutter, new drugs, dosage forms, combinations, etc.
- Reinvented bioprocess technology for making a new class of drugs and vaccines—making affordable.
- Established the first and the only US-based developer and manufacturer of recombinant drugs (Chicago).
- Forbes Magazine: “Most Interesting Man.”

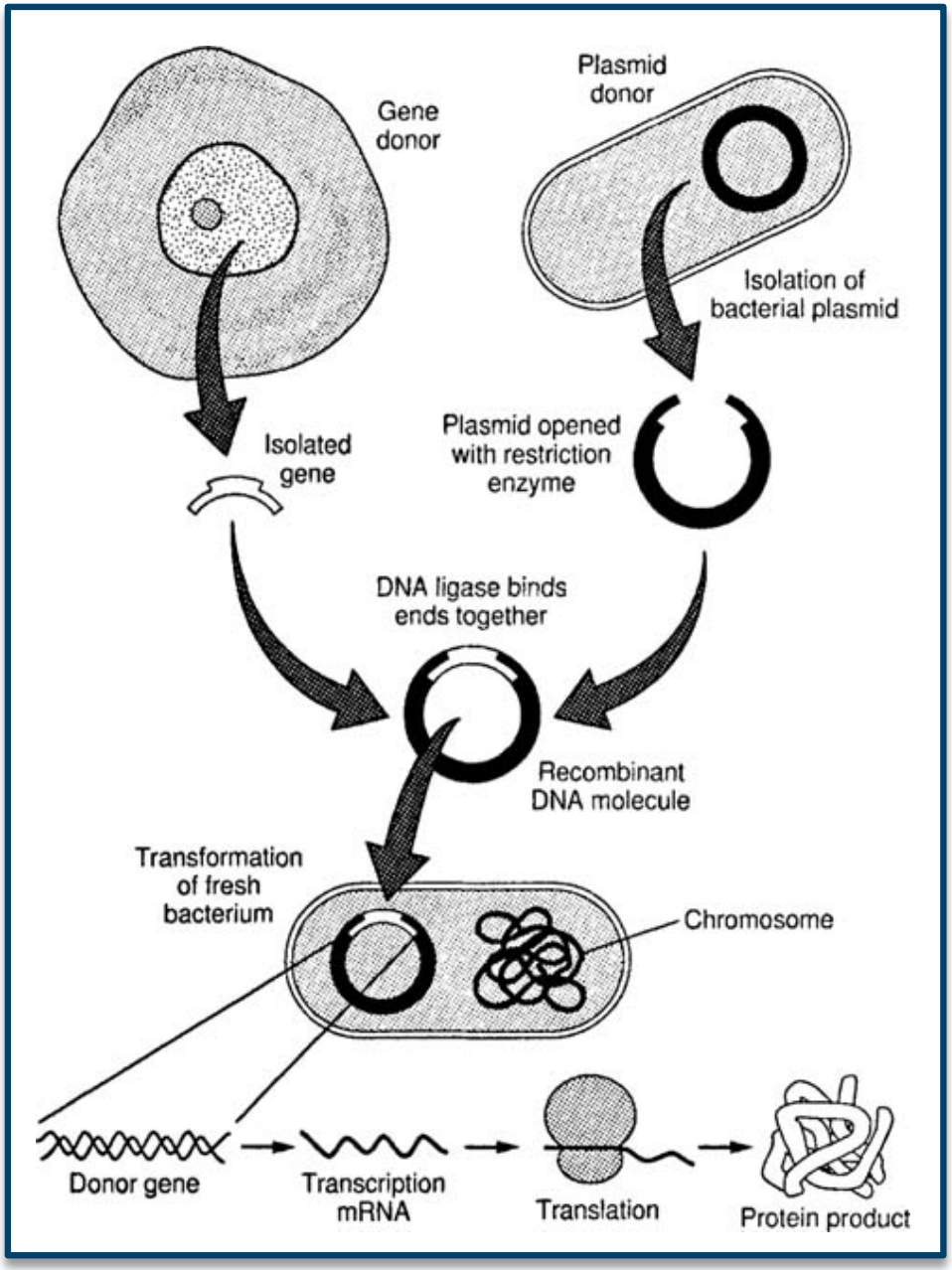
We've come a long way

- In 1900 31% of all deaths in the US were from pneumonia, tuberculosis and gastrointestinal infections[5]
- These diseases are rare and treatable/preventable today thanks to the evolution of pharmaceuticals and the introduction of vaccines in the mid 1900's



1. http://www.ncbi.nlm.nih.gov/pubmed/21698778, 2. http://pubs.acs.org/cen/coverstory/83/8325/8325emergence.html
4. http://www.karger.com/ProdukteDB/Katalogteile/isbn3_8055/_83/_53/Insulin_02.pdf 5. http://www.cdc.gov/nchs/data/dvs/lead1900_98.pdf

How are biologics and biosimilars manufacturerd?



Biologic Drugs

- Created from organic origins
- Manufactured through recombinant technology
- Highly complex and difficult to characterize

Small Molecule Drugs

- Created from chemicals
- Chemically synthesized
- 100% characterization and understanding possible

Source: CliffsNotes™ Recombinant DNA and Biotechnology accessed 4/28/2014

The pharmaceutical industry continues to evolve

Pharmaceutical innovation continues today with complex biologics providing relief and sometimes a cure for diseases thought to be terminal

- Recombinant technology continues to advance
  - The first approved recombinant protein was created in a bacterial expression system
  - Now mammalian expression systems can be used to create more complex biologics e.g., monoclonal antibodies
  - There are companies who are expanding expression systems to plant, leveraging tobacco, carrot or safflower to 'grow' recombinant biologics
- There are companies solely focused on curing previously thought incurable diseases
  - Gilead's Hepatitis C treatment Sovaldi was approved in December 2013 and showed an 84-96% cure rate depending on genotype in clinical trials
- The field of proteomics leverages large scale studies of proteins and how they act and interact in order to understand cellular processes[1]
  - By having this level of understanding, treatments targeting specific biomarkers can be created
  - Targeted treatments may one day have a greater impact in the treatment of complex diseases such as cancer

1. http://proteomics.cancer.gov/whatisproteomics

Evolution of Pharmaceuticals Comes at a Price

On average, biologic drugs cost 50x more than small molecule drugs and >100x more than generics at ~$2,000-3,000 per dose[1]

Product	2010 US Sales $Billions[2]
Plavix	6,154
Lipitor	5,329
Advair	4,026
Abilify	3,606
Actos	3,582
Enbrel	3,304
Singulair	3,219
Seroquel	3,107
Remicade	3,099
Avastin	3,068

Product	2015 US Sales $Billions[2]
Humira	7,663
Harvoni	6,298
Lantus	4,824
Enbrel	4,541
Remicade	4,337
Neulasta	3,674
Rituxan	3,547
Revlimid	3,291
Tecfidera	3,082
Avastin	2,882

Product	2018 US Sales $Billions[2]
Humira	8,589
Remicade	4,732
Revlimid	4,566
Enbrel	4,401
Tecfidera	4,235
Opdivo	3,701
Harvoni	3,483
Lantus	3,389
Lyrica	3,306
Eylea	3,185

Biologic

1. http://www.amcp.org/WorkArea/DownloadAsset.aspx?id=15722 2. EvaluatePharma 2015

Price of Biologics put into Perspective

SOLIRIS® (eculizumab) Concentrated solution for intravenous infusion — **~$400,000 annual cost[1]**

Herceptin® trastuzumab — **~$54,000 annual cost[3]**

AVASTIN® bevacizumab Solution for intravenous infusion — **~$50,000 annual cost[1]**

Remicade® INFLIXIMAB — **~$24,000 annual cost[2]**

HUMIRA® adalimumab — **~$19,000 annual cost[2]**

1. http://www.forbes.com/2010/02/19/expensive-drugs-cost-business-healthcare-rare-diseases.html 2. http://www.ncbi.nlm.nih.gov/pubmed/22411424 3. http://www.nytimes.com/2012/10/02/business/current-length-of-herceptin-treatment-is-supported-by-studies.html

Biosimilars created to provide an affordable alternative

- Biosimilars have been on the market in Europe since 2006
- On average they provide a 20-30% discount from reference biologics
- Discounts have reached as high as ~70% which would represent ~$3,000 savings on one treatment cycle of filgrastim
- Savings are projected from $11-33B through 2020 in the EU alone[1]

- The FDA just approved the United States' first biosimilar
- Savings are projected from $44-250B over the next 10 years in the US[2]

1. http://ip-science.interest.thomsonreuters.com/biosimilars-report-2014 2. http://www.rand.org/content/dam/rand/pubs/perspectives/PE100/PE127/RAND_PE127.pdf
3. http://lab.express-scripts.com/insights/industry-updates/the-$250-billion-potential-of-biosimilars

What is a biosimilar?

World Health Organization

A biotherapeutic product, which is **similar** in terms of **quality, safety and efficacy** to an already licensed reference biotherapeutic product.[3]

FDA

The product is **highly similar** to the reference product notwithstanding minor differences in clinically inactive components and there are **no clinically meaningful differences** between the biological product and the reference product in terms of **safety, purity and potency** of the product.[1]

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

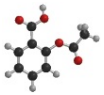
A biosimilar medicine is a biological medicine that is developed to be **similar to an existing biological medicine** (the 'reference medicine').

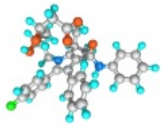
When approved, **its variability** and any differences between it and its reference medicine will have been shown **not to affect safety or effectiveness**.[2]

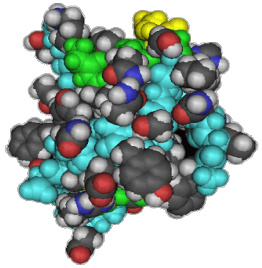
1. FDA, http://www.fda.gov/downloads/Drugs/Guidances/UCM273001.pdf, 2 EMA, *Questions and answers on biosimilar medicines (similar biological medicinal products)*. (2012, September 27), 3 WHO, *Guidelines on Evaluation of Similar Biotherapeutic Products (SBPs)*. (2009, October 23)

Where are biosimilars available?

Complex molecules require complex science







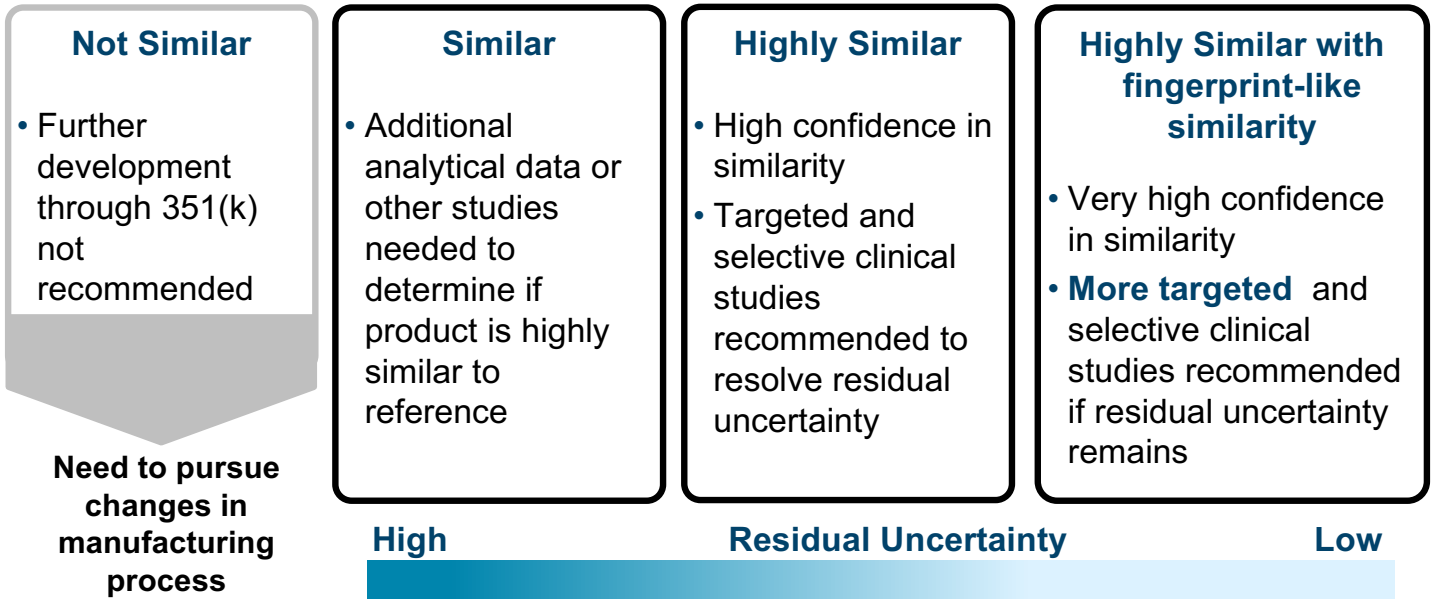
Simple Small Molecule	Small Molecule	Simple Biologic	Complex Biologic
Aspirin	**Lipitor®**	**Insulin**	**Humira®**
Molecular Weight			
180 daltons	558 daltons	5,808 daltons	~148,000 daltons

Biosimilars are complex molecules that by nature cannot be identical like chemically synthesized drugs

Source: DrugBank Accessed 3/20/2014

Biosimilars require advanced analytics

The burden of proof is on the biosimilar manufacturer to prove the purity, potency and safety of the biosimilar, which starts with determining structure

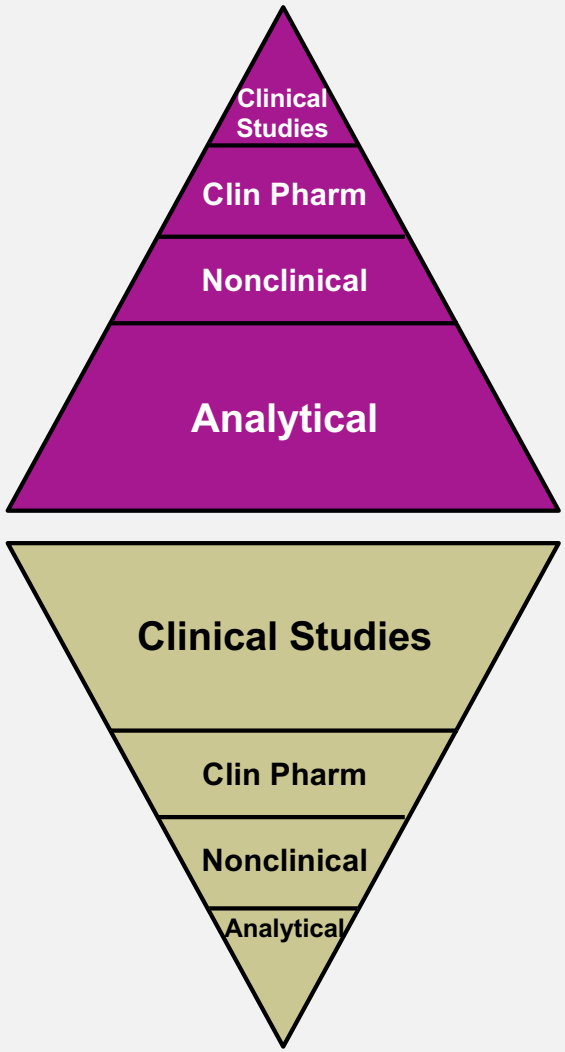


Highly similar: The results of the ***comparative analytical characterization*** permit high confidence in the analytical similarity…

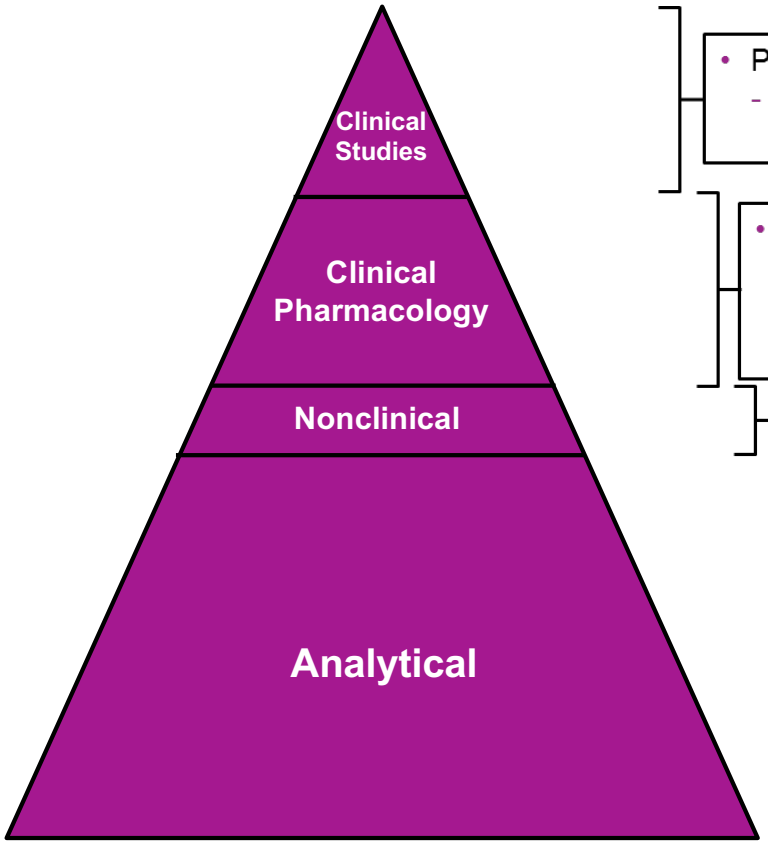
Highly similar with fingerprint-like similarity: based on ***integrated, multi-parameter approaches*** that are extremely sensitive in identifying analytical differences. The results of these ***fingerprint-like analyses*** permit a very high level of confidence in the analytical similarity…

Strong analytical is the foundation of a biosimilar

New Biosimilar



New Biologic



- Phase III clinical trials
  - Recommended if residual uncertainty remains

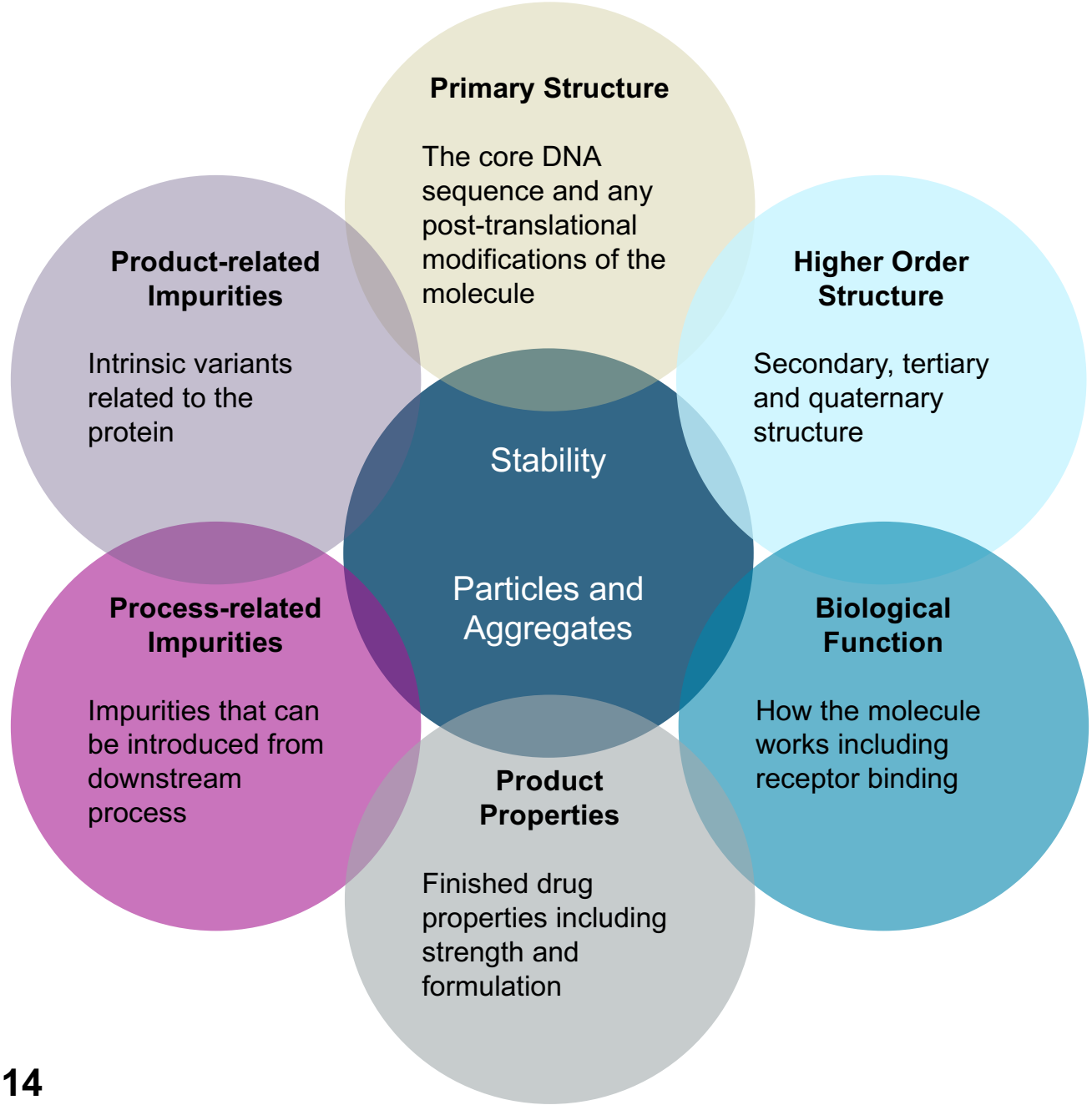
- Human Clinical Pharmacology Trials

PK	PD	Safety
AUC_{inf}	ANC	Immunogenicity
AUC_{last}	CD34+	SEAEs
C_{max}		TEAEs

- Animal studies – Toxicology, immunogenicity, etc.

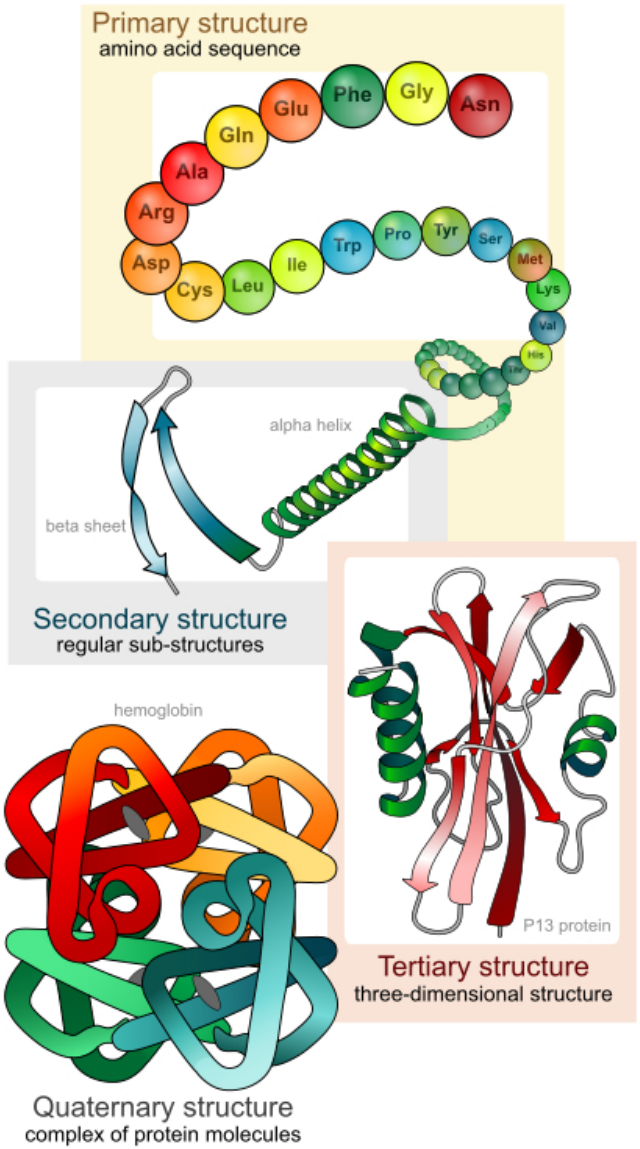
- Structural and functional characterization
  - Range from complex with high likelihood of clinical response (e.g., bioassay, peptide map, 4D) to low predictability of clinical response (e.g., appearance, intact mass, etc.
- Forms the basis for demonstration of biosimilarity

Holistic approach to analytics and biosimilarity

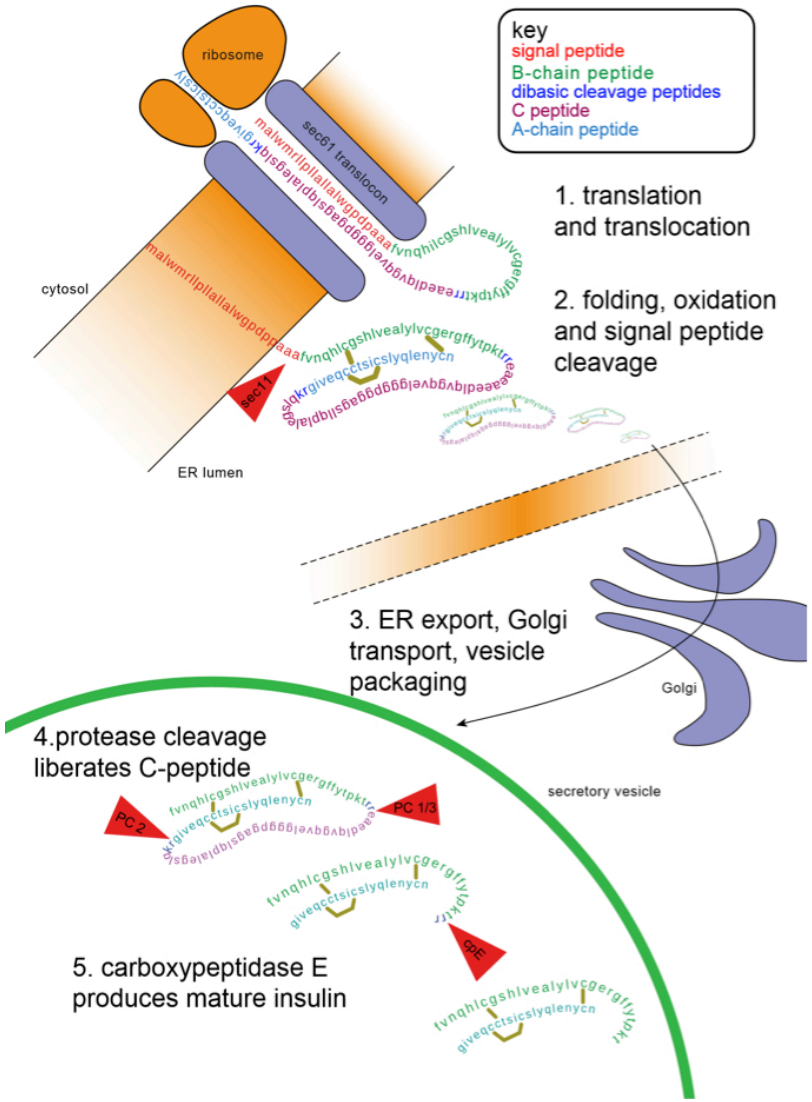


- Ability to understand and replicate new molecular entities
- Leaps and bounds in advancement of analytical methods to characterize molecules
- Understanding the mode of action of complex drugs

Biosimilarity requires characterization of layers of structure



Additionally, post translational modifications need to be accounted for



Number and sophistication of available tests has increased tremendously

The goal is to remove residual uncertainty through extensive characterization and achieve fingerprint-like similarity

Science has advanced to a stage where robust characterization and increased understanding of a protein is possible

- Neulasta® was approved in 2002, over a decade ago
- There are test methods that exist today that weren't possible in 2002

Example: mass spectrometry

- There has been a **10 million-fold increase** in detection limit for peptides since 1990[1]

Test Methods	
PH	Bioassay
N-C terminal Sequencing	CIEF
Deliverable volume or dose	SDS-Page (Purity)
Acetate	Appearance
DSC	Primary Structure- Mass Spec
HCP	Tertiary Structure-NMR
Sorbitol	Tertiary Structure- 4D
Peptide Map Primary Sequence	Antibody Binding - Elisa
Peptide Map Disulfide Bond	Western Blot
Tertiary Structure- Fluorescence	2D-SDS Page
Color	Sterility
Leachables/Extractables	Subvisible Particles
Osmolality	Visible Particles
Disulfides	Bioburden
Density	Endotoxin
Clarity	SDS-Page (ID)
AUC	CEX
Receptor Binding- Elisa	Absorbance - UV Spect
Residual DNA	SEC- aggregates
Biacore	Product Concentration
Molecular Weight	RP-HPLC Purity
Secondary Structure- CD	

Source: Mire-Sluis, T.: The Regulatory Implications of the ever increasing power of Mass Spectrometry and its role in the Analysis of Biotechnology Products – Where do we draw the line? CASSS MassSpec 2012.

Analytical Tests Ranked by Affect on Product Safety, Purity, Potency, Immunogenicity and Strength

Level of Affect	Test Name	Testing For
High	Subvisible Particles	Purity
	Visible Particles	Purity
	Bioburden	Safety
	Endotoxin	Immunogenicity
	SDS-Page (ID)	Structure
	CEX (Cation Exchange)	Structure
	Absorbance - UV Spectroscopy	Structure
	SEC- aggregates	Purity
	Product Concentration	Potency
	RP-HPLC Purity	Purity
Medium	HCP (Host cell protein)	Purity
	Sorbitol	Stability
	Peptide Map Primary Sequence	Structure
	Peptide Map Disulfide Bond	Structure
	Tertiary Structure- Fluorescence	Structure
	Color	Safety
	Leachables/Extractables	Purity
	Osmolality	Function
	Disulfides	Structure
	Density	Structure
	Clarity	Purity
	AUC	Function
	Receptor Binding- Elisa	Function
	Residual DNA	Purity
	Biacore	Immunogenicity
	Molecular Weight	Structure
	Secondary Structure- CD	Structure
	Bioassay	Function
	CIEF	Purity
	SDS-Page (Purity)	Purity
	Appearance	Structure
	Primary Structure- Mass Spec	Structure
	Tertiary Structure-NMR	Structure
	Tertiary Structure- 4D	Structure
	Antibody Binding - Elisa	Function
	Western Blot	Structure
	2D-SDS Page	Structure
	Sterility	Purity
Low	PH	Safety
	N-C terminal Sequencing	Structure
	Deliverable volume or dose	Function
	Acetate	Stability
	DSC (differential scanning calorimetry)	Function

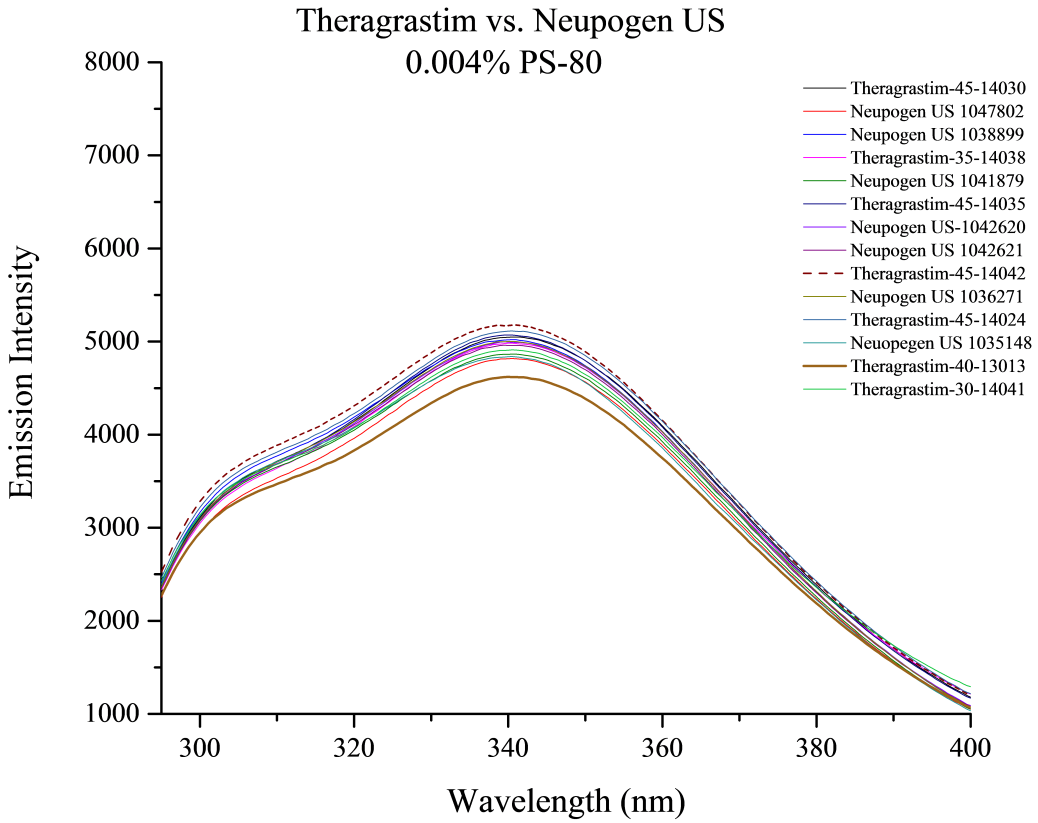
Focus

- TPI performs numerous tests on the molecule to measure biosimilarity related to many factors
- The tests that have the highest potential to affect safety, purity, potency, immunogenicity and strength are the most cricitcal

Looking at analytical methods differently

Purpose	Method
4D proprietary test developed to characterize quarternary structure	Proprietary fluorescence spectra under thermodynamic stressed conditions in solution phase

4D TEST at 0.004% PS-80



Source: preliminary internal analysis

Examples of Other Analytical Tests

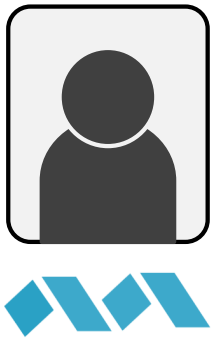
Name: RP-HPLC

Testing: Purity

Criticality: High

Test Explained:

- Reversed-phase high-performance liquid chromatography measures product related impurities
- Solution is passed through a column filled with a solid absorbent (stationary phase and mobile phase)
- Each component in the sample interacts slightly differently leading to separation
- Reverse phase HPLC has a non-polar stationary phase and a moderately polar mobile phase



Name: UV absorbance

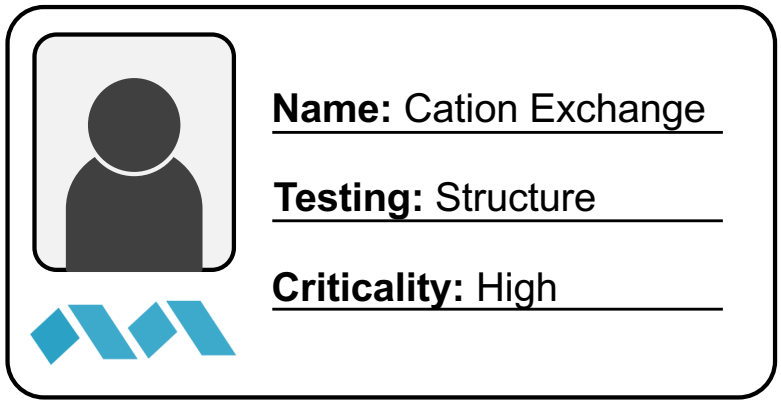
Testing: Structure

Criticality: High

Test Explained:

- Measures protein concentration
- Leverages the absorption of ultraviolet light when the molecule moves from the ground state to an excited state
- Molecules containing non-bonding electrons absorb UV light and biosimilar molecules should exhibit the same pattern
- Performed using UV absorbance spectroscopy

Examples of Other Analytical Tests



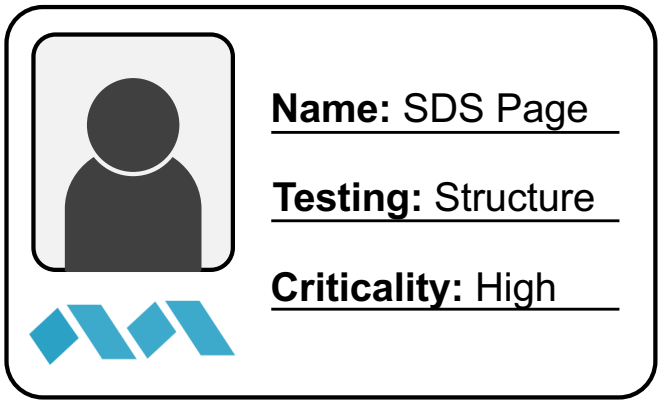
Name: Cation Exchange

Testing: Structure

Criticality: High

Test Explained:

- Separates proteins based on differences in the surface charge of the molecules
- Separation is dictated by the proteins interaction with the stationary phase
- Leverages negatively charged interactions
- Performed using ion exchange chromatography



Name: SDS Page

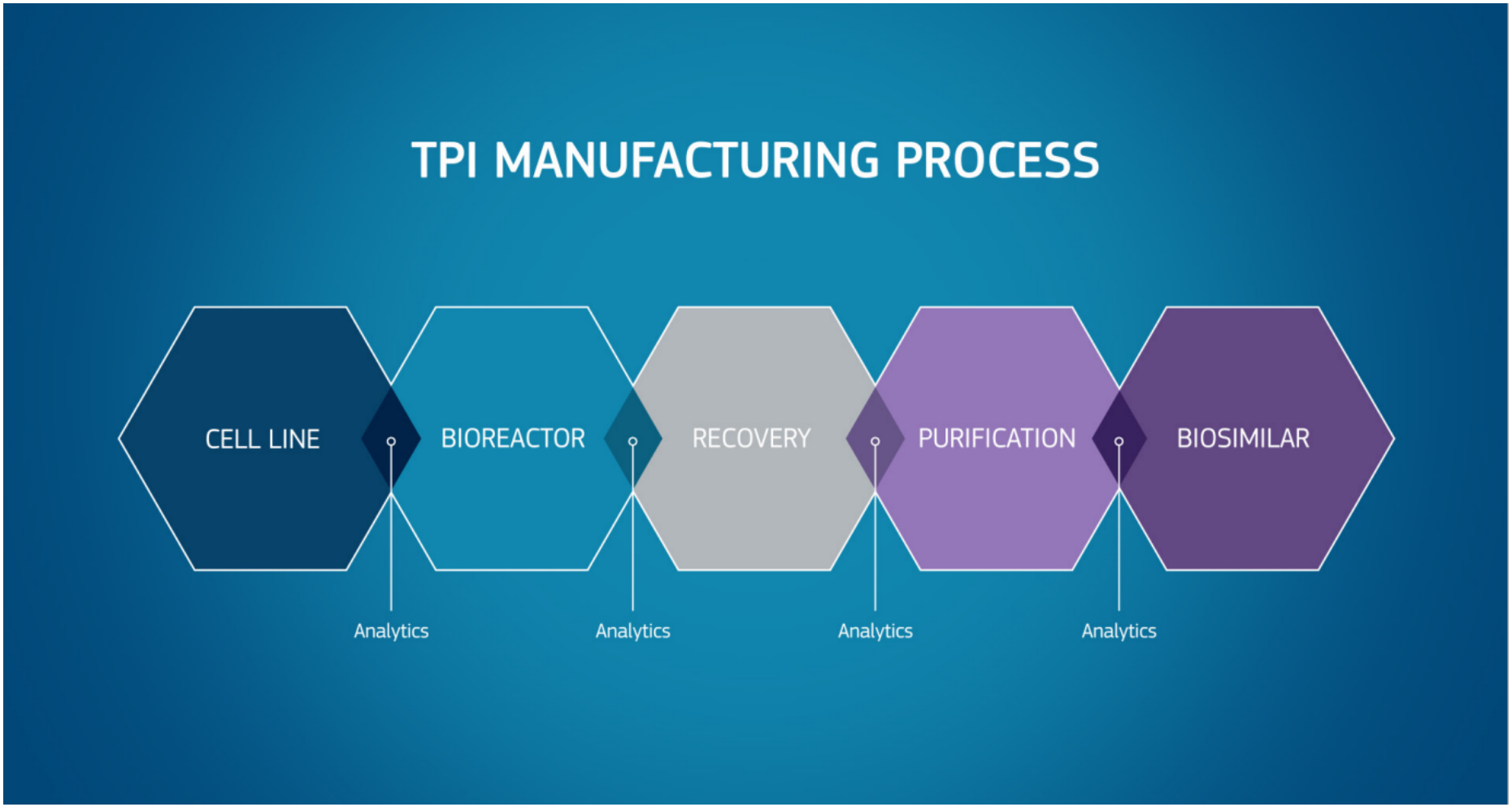
Testing: Structure

Criticality: High

Test Explained:

- Separates the proteins based on their ability to move within an electrical current
- Differences are based on the length of the proteins' polypeptide chains or molecular weight
- Biosimilar proteins should exhibit the same separations

Also looking at the process differently



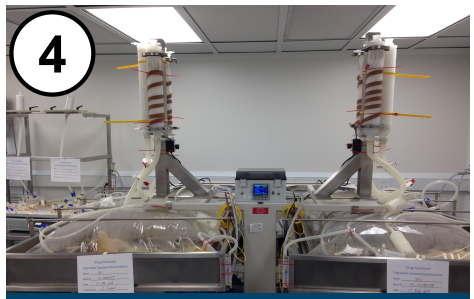
How can we simplify the process while reducing the cost and time to market?

Disrupting a 7000 Year Old Technology

Roller Bottle System

Deep Tank System

Single-use Bioreactor



TPI Single-use Bag

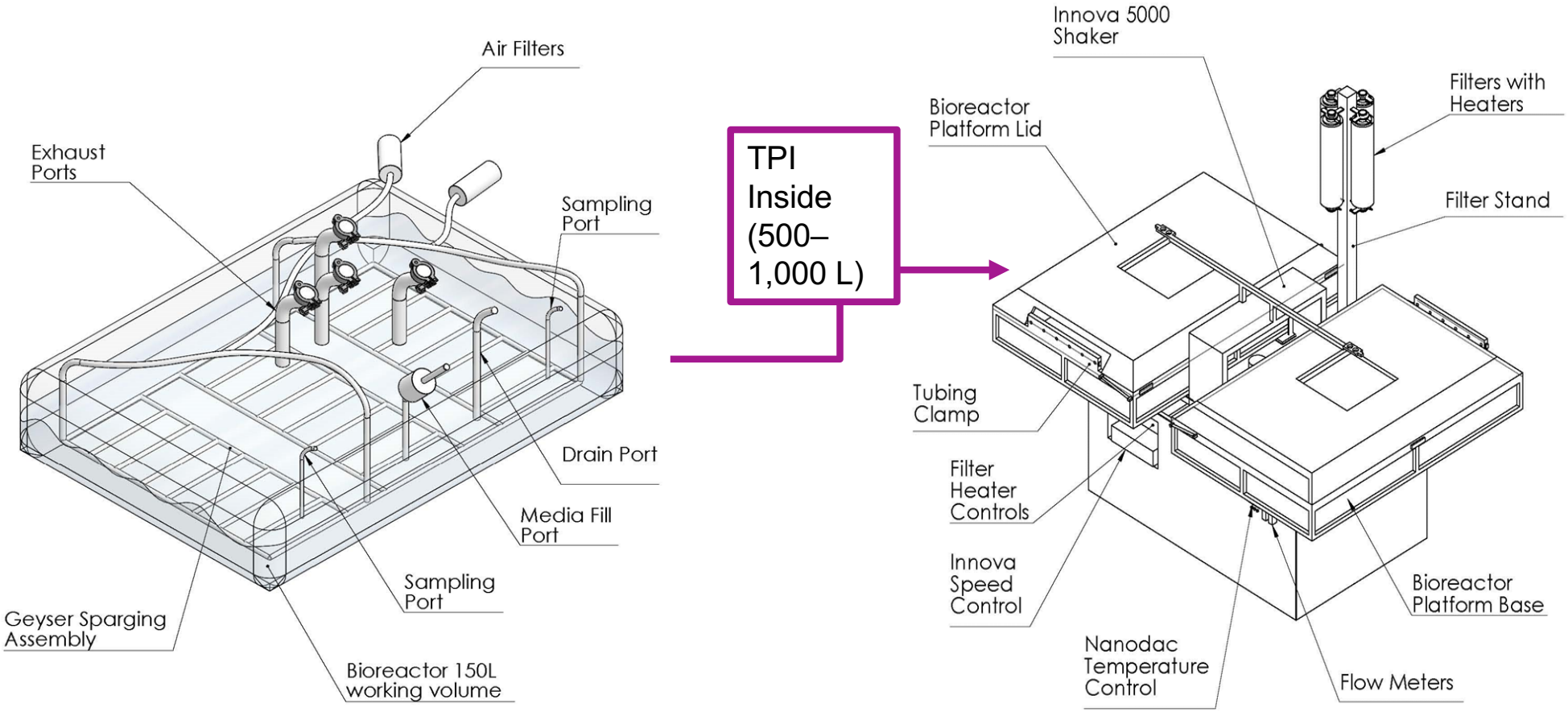
TPI Proprietary Approach

- 45 patents filed in U.S. and other regulated markets between 2009 and 2014.

Patent Category	Number
Bioreactors	15
Harvesting/Purification	16
Harvesting	1
Purification	3
Facility	2
Characterization	4
Formulation	2
Media Growth	2

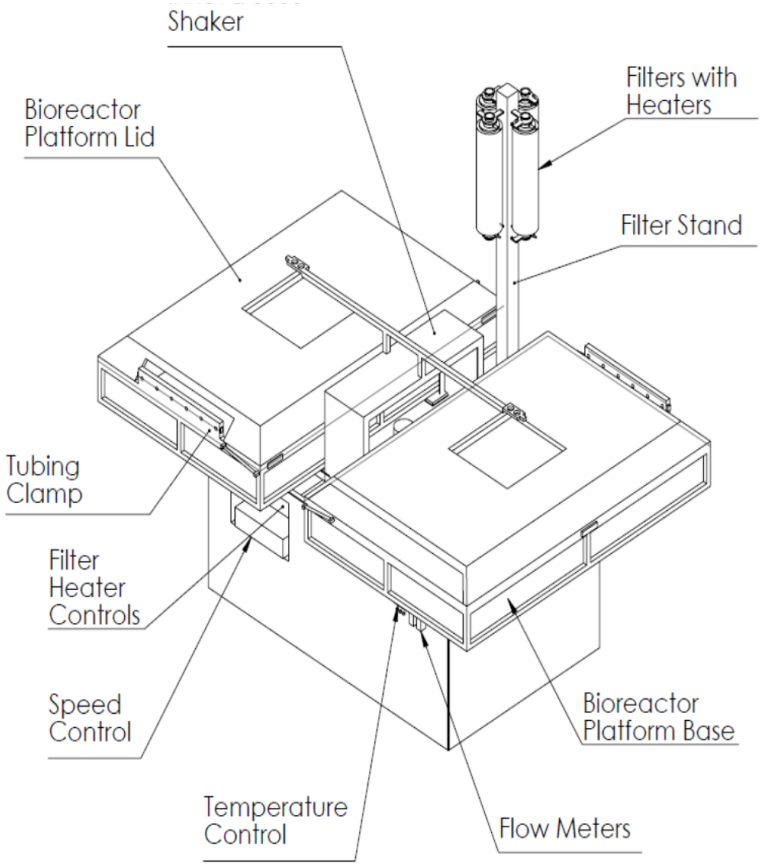
Roller Bottle Credit: REUTERS/V. RICHARD HARO/WIREPIX Source: http://www.reuters.com/article/2007/08/16/us-amgen-idUSN8F26518720070816 (accessed June 2014)

MayaBio® Bioreactors



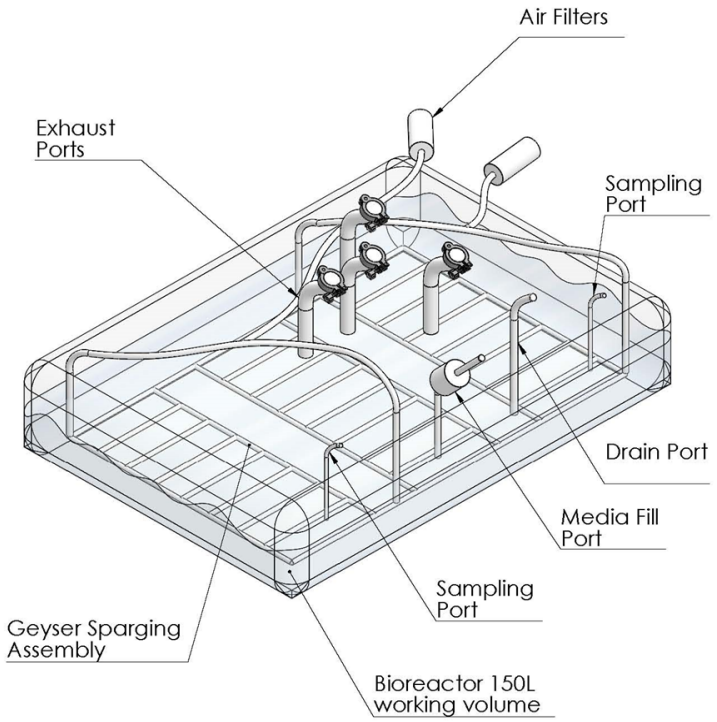
Platform Detail

- Process platform consist of bioreactor bag and orbital shaker
- The orbital shaker provides:
  - Enough energy without increasing the shear stress on cells
  - Means to control pH and DO for the culture
  - Temperature control for the culture
  - Support and protection to bioreactor bag
- TPI's process platform can support up to 650L working volume



Platform Detail

- TPI's Single-use flexible container:
  - A pillow bag with no retaining walls containing a proprietary sparging system and gamma sterilized for final use
  - All ports fully protected for preventing area contamination
  - Cross-contamination resistant - probes and filters are disposable and installed into bag prior to sterilization
  - Reduced variability in protein structure by reduced physical stress
  - Effective delivery of oxygen into the bioreactor culture by a patented design of integrated sparge rods located within the bioreactor bag
  - Modular, scalable without scale-up validation: one size of flexible bag is validated and then multiple bags are operated simultaneously to create different batch sizes
  - Animal free and Class VI Components
- In combination with orbital shaker can support:
  - Excellent temperature distribution (0.01ºC from set point)
  - Mixing times of ~15 seconds @80 RPM
  - Mass transfer efficiency and modularity

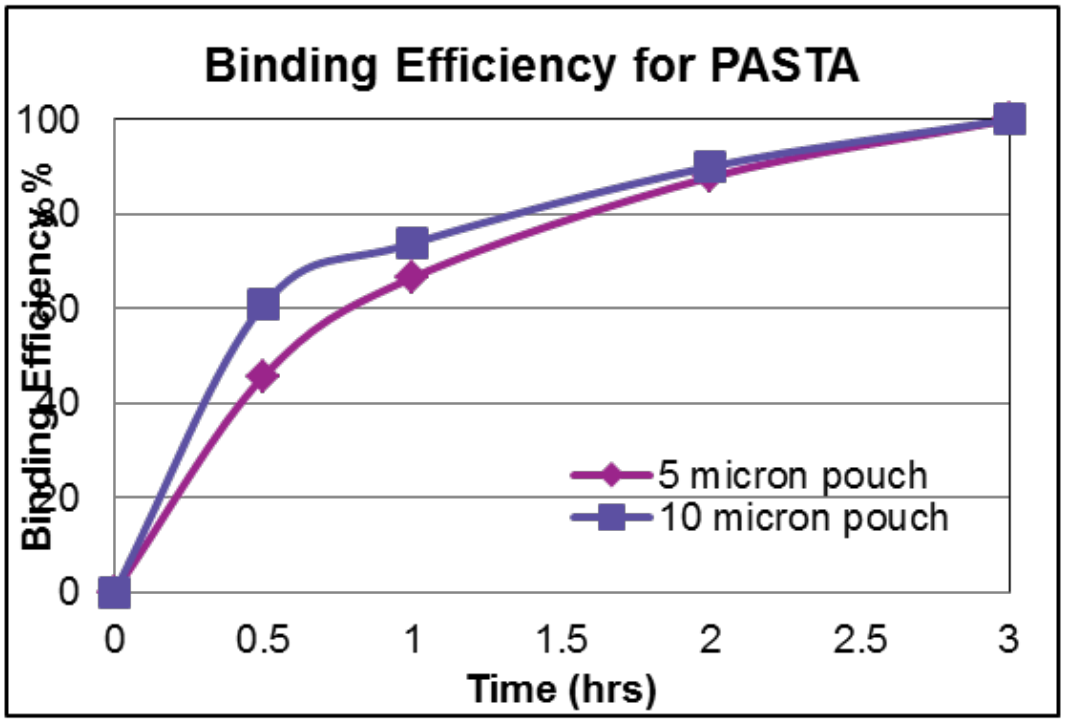


Platform Advantages

1. **High quality**
   - Shorter, gentle process on the protein and potential for increased consistency in structure
2. **Accelerated set-up and switchover**
   - Single-use technology
   - Fully portable
   - No time or expense associated with switching between products
3. **Cross-contamination Resistant**
   - Fully contained and sterile system
   - No need for clean room
4. **Fast development and scale up**
   - Allows for development of multiple products simultaneously
   - Provides flexibility in meeting market demand
5. **Cost Effective**
   - Smaller footprint relative to fixed reactor technologies

Patents beyond bioreactor

PASTA



A study showing the binding efficiency of Protein A resin in a 5µ and10µ pouch.

Source: Internal Team Analysis – preliminary

Potential future benefits of evolved technology

- Optimized platform for shorter timelines in drug discovery
- Empower research organizations to produce clinical supplies
- The majority of new drugs will be biologics, how will developing world manage these when the traditional methods require an extensive capital investment?
- Flexibility in response to challenges e.g., bioterrorism, vaccine production, etc. based on clean room requirements
- Potential to eliminate steps of the process, further reducing cost e.g., protein capture within the bioreactor

Questions?