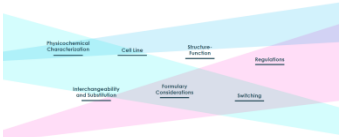


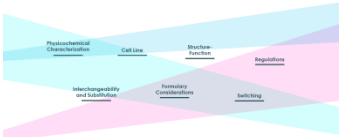
Assistant Professor Mohammad A Alsenaidy, MSc, PhD, Saudi Arabia

- Director, College of Pharmacy Research Center, King Saud University, Saudi Arabia
- Assistant Professor of Pharmaceutical Biotechnology, Department of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia

GaBI Scientific Meetings

First GCC Stakeholder Meeting on **Approval Process, Interchangeability/Substitution and Safety of Biosimilars**

20 November 2017, Holiday Inn Izdihar Riyadh, Saudi Arabia



Challenges related to physicochemical characterization and analytical comparability of biologicals/biosimilars

Assistant Professor Mohammad A Alsenaidy, MSc, PhD

20 November 2017

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

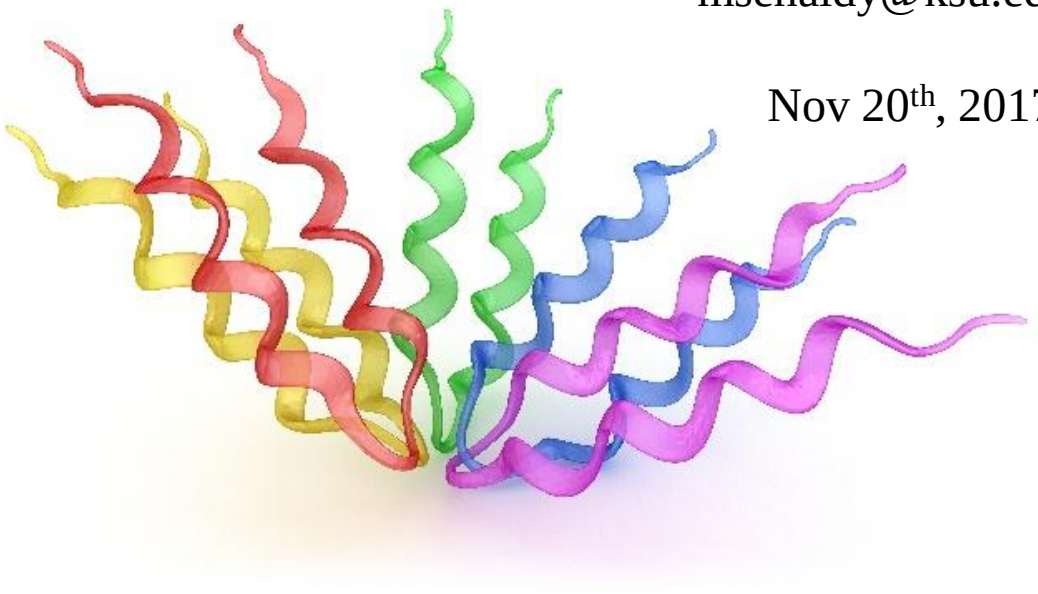
SPS Saudi Pharmaceutical Society الجمعية الصيدلية السعودية
PPCI Pro Pharma Communications International

Challenges related to physicochemical characterization and analytical comparability of biologicals and biosimilars

Mohammad A. Alsenaidy, M.Sc., Ph.D.

Assistant Professor of Pharmaceutical Biotechnology
Department of Pharmaceutics
College of Pharmacy
King Saud University
msenaidy@ksu.edu.sa

Nov 20th, 2017



Acknowledgments

Overview

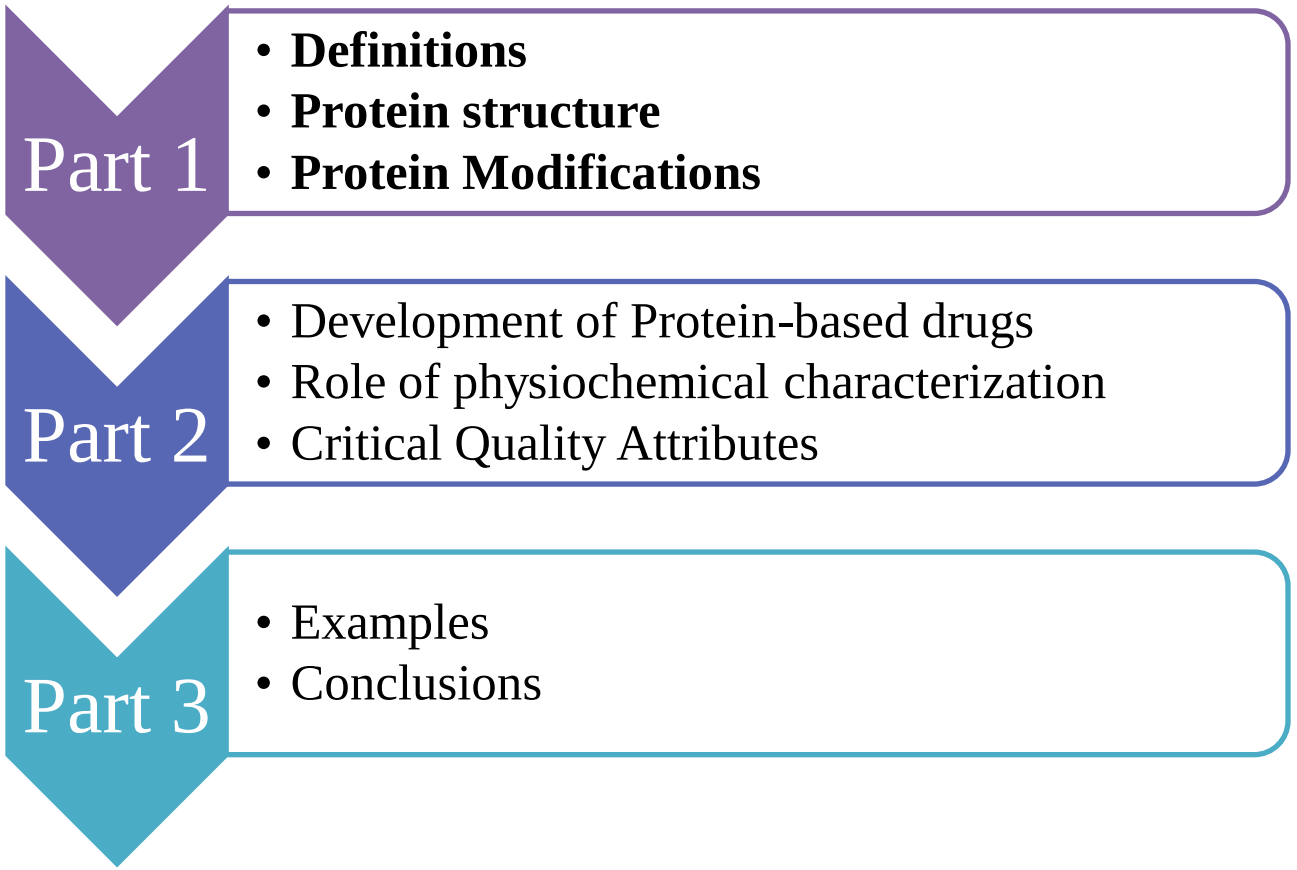
Physiochemical Characterization of Biologics and Biosimilars

Part 1
- Definitions
- Protein structure
- Protein Modifications

Part 2
- Development of Protein-based drugs
- Role of physiochemical characterization

Part 3
- Examples
- Conclusions

Overview


Part 1
• **Definitions**
• **Protein structure**
• **Protein Modifications**
Part 2
• Development of Protein-based drugs
• Role of physiochemical characterization
• Critical Quality Attributes
Part 3
• Examples
• Conclusions

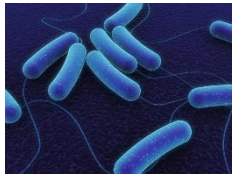
Definition

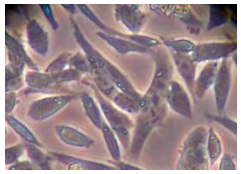
Definition of biological medicinal product

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

According to Part I of Annex I of Directive 2001/83/EC, it is a product that contains a biological substance. A biological substance is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physico-chemical-biological testing together with the production process and its control.

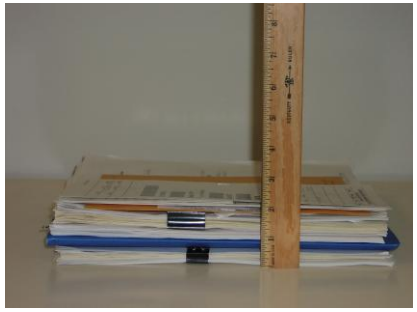
For example, recombinant proteins, monoclonal antibodies, medicinal products derived from human blood and human plasma, immunological medicinal products and advanced therapy medicinal products should be considered biological medicinal products.

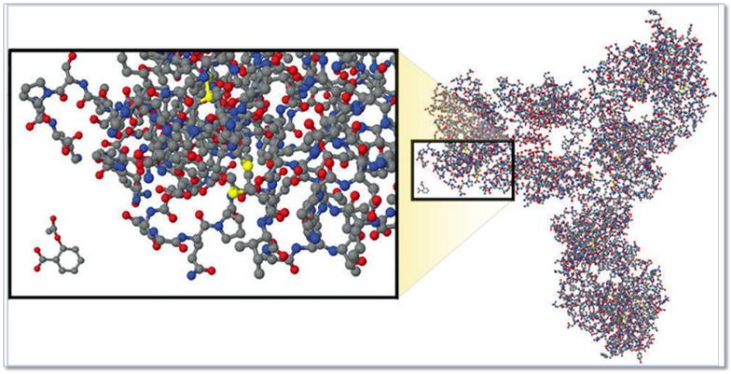




3 Components

- From a living system.
- Challenging manufacturing process.
- Complex molecule.





http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2014/10/WC500176775.pdf

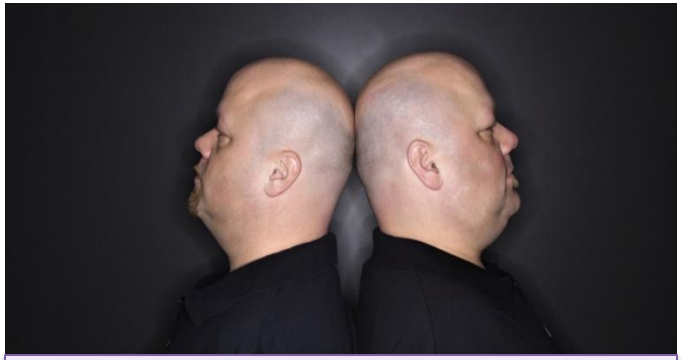
Definition: Biosimilar
(EMA, FDA, HC, WHO)

What is a biosimilar medicine?

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'). Biosimilars are not the same as generics, which have simpler chemical structures and are considered to be identical to their reference medicines.

The active substance of a biosimilar and its reference medicine is essentially the same biological substance, though there may be minor differences due to their complex nature and production methods. Like the reference medicine, the biosimilar has a degree of natural variability. When approved, its variability and any differences between it and its reference medicine will have been shown not to affect safety or effectiveness.



Highly Similar to Ref. product

But not Identical.

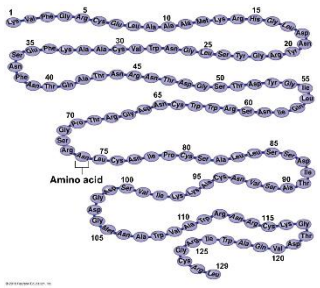
No clinically meaningful differences.

Regulatory Agency	Name
FDA	Biosimilar Biological Product (BBP)
EMA	Similar Biological Medicinal Product
WHO	Similar Biotherapeutic Product (SBP)
HC	Subsequent Entry Biologic (SEB)
Japan	Follow-On-Biologic

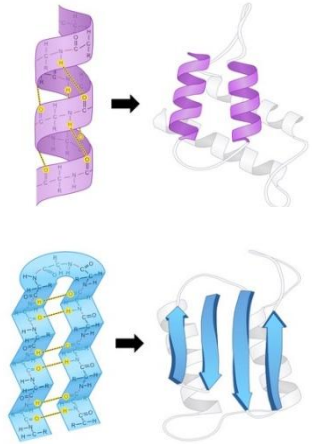
Proteins to work ,,, They have to form the correct conformation ,,,
and
maintain their conformation throughout manufacturing and storage

Three main structural levels

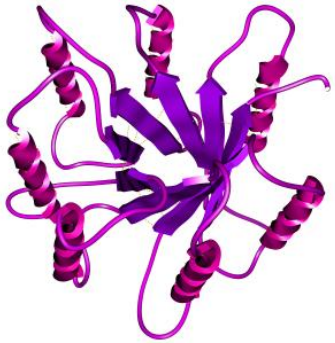
Primary Structure



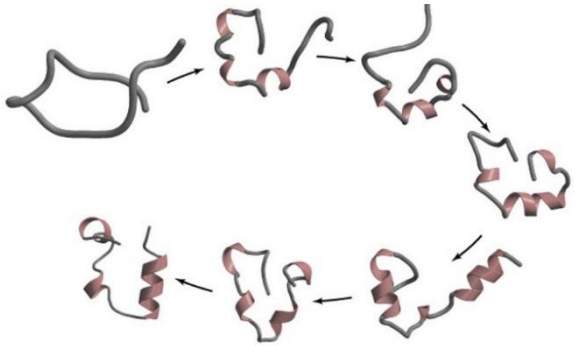
Secondary Structure



Tertiary Structure

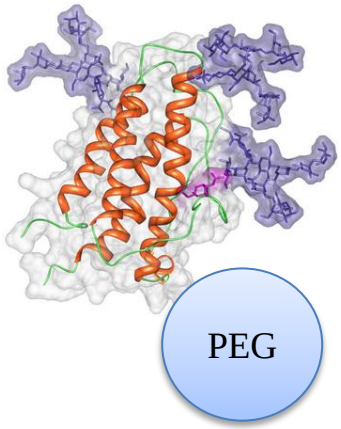


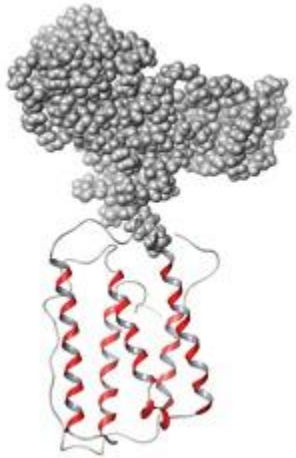
Assembly → Folding → Packing

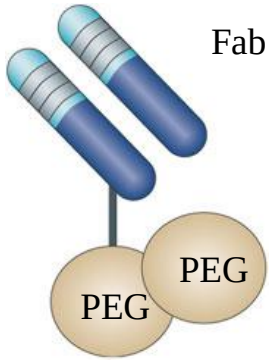


Also, in many cases, Proteins get further modifications „

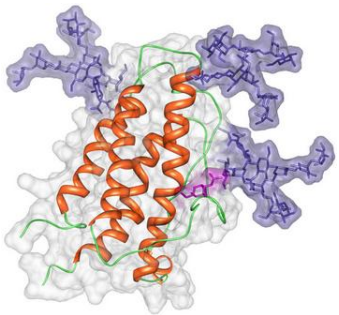
1- Chemical Modifications (e.g. Pegylation)

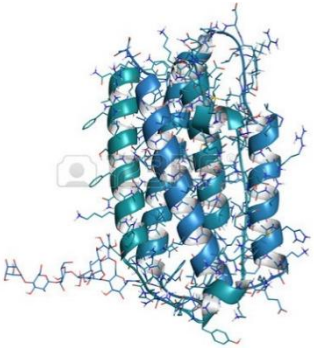


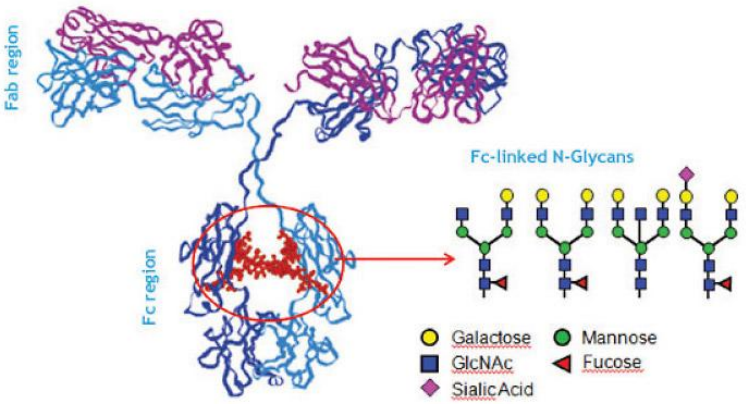




2 - Post-Translational Modifications (e.g. Glycosylation, C-terminal Lys)







Stability

Solubility

Functionality

PK/PD

Immunogenicity

Glycosylation plays an important role in functionality

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 30, Issue of July 26, pp. 26733–26740, 2002

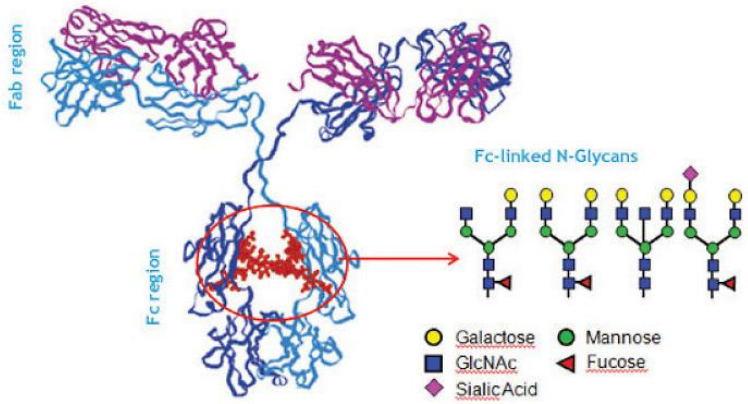
Printed in U.S.A.

Lack of Fucose on Human IgG1 *N*-Linked Oligosaccharide Improves Binding to Human FcγRIII and Antibody-dependent Cellular Toxicity*

Received for publication, March 1, 2002, and in revised form, April 19, 2002
Published, JBC Papers in Press, May 1, 2002, DOI 10.1074/jbc.M202069200

Robert L. Shields‡, Jadine Lai‡, Rodney Keck§, Lori Y. O'Connell‡, Kyu Hong¶, Y. Gloria Meng¶, Stefanie H. A. Weikert‖, and Leonard G. Presta‡**

From the ‡Department of Immunology, §Department of Analytical Chemistry, ¶Department of BioAnalytical Technology, and ‖Department of Cell Culture and Fermentation, Genentech, Inc., 1 South San Francisco, California 94080



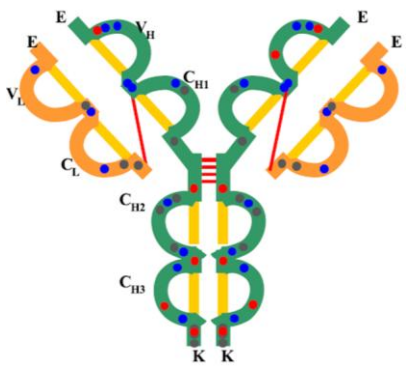
Immunoglobulin (IgG)

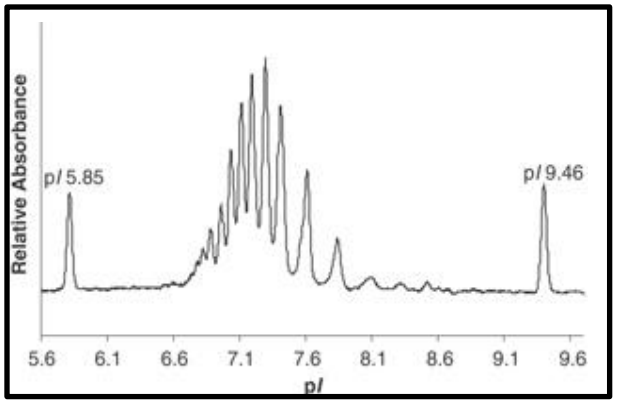
50-fold

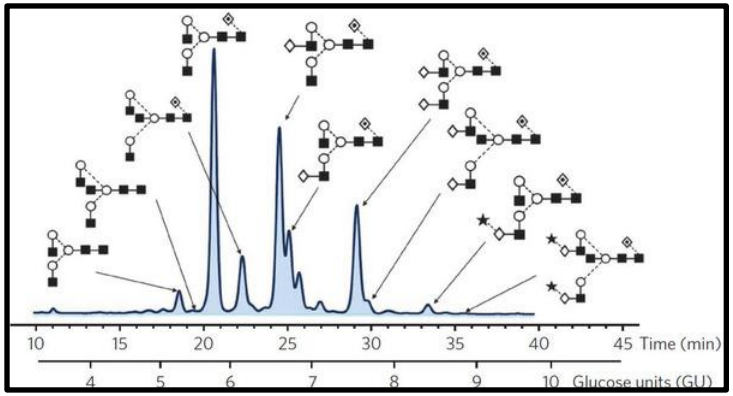
Fact: Protein are Heterogeneous in Nature !

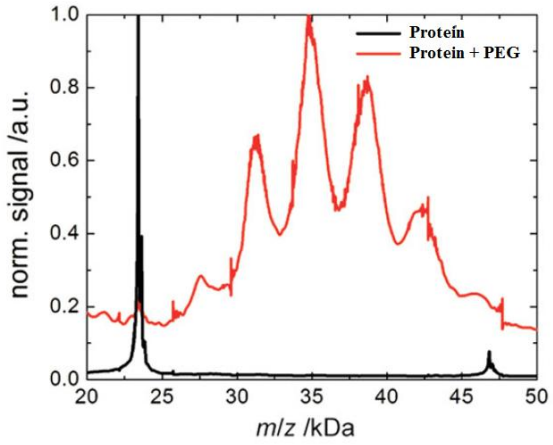
Examples of some sources of product heterogeneity:

N- and C-terminal modifications	Hydrophobicity	Charge
N-linked Glycosylation	O-linked Glycosylation	PEGylation









The overall heterogeneous mixture defines the product

The process is the product

http://www.nature.com/articles/srep280988

https://www.ncbi.nlm.nih.gov/pubmed/23616211

Overview

Part 1

- Definitions
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Part 2

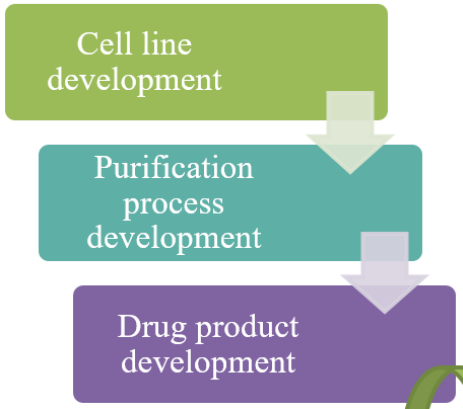
- **Development of Protein-based drugs**
- **Role of physiochemical characterization**

Part 3

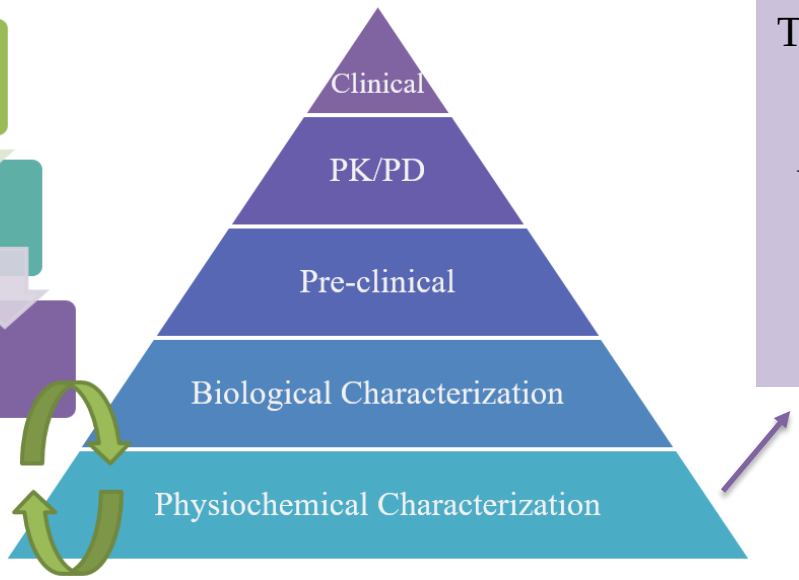
- Examples
- Conclusions

Development of Protein-based drugs

(A)



(B)



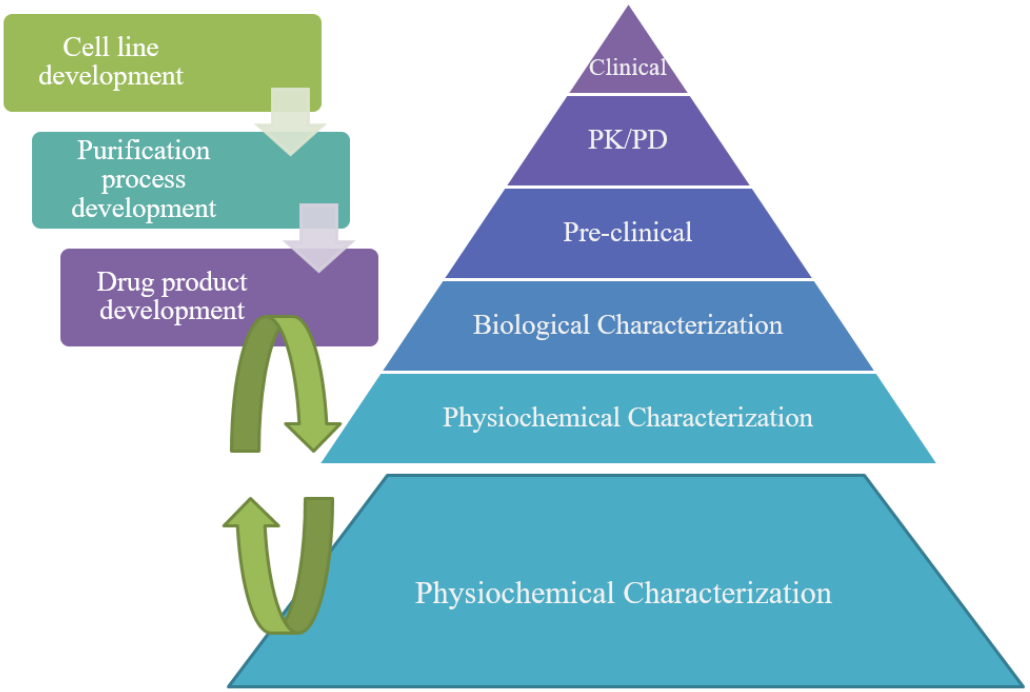
The Collective studies applied for the evaluation of biophysical and biochemical attributes for quality demonstration.

Extensively applied for Phase-to-Phase And Batch-to-batch variability

Attributes :

- Primary Structure
- Secondary Structure
- Tertiary Structure
- Free Sulfhydryl gps
- Disulfide bridges
- Deamidation
- Oxidation
- N- and O-glycosylation
- Charged isoforms
- Aggregation
- Fragmentation
- Glycation
- N- and C-terminals

Development of Protein-based drugs



Innovator's Product

Biosimilar Product

Extensively applied for Phase-to-Phase And Batch-to-batch **And** **Innovator-to-biosimilar** variability

Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product

Guidance for Industry

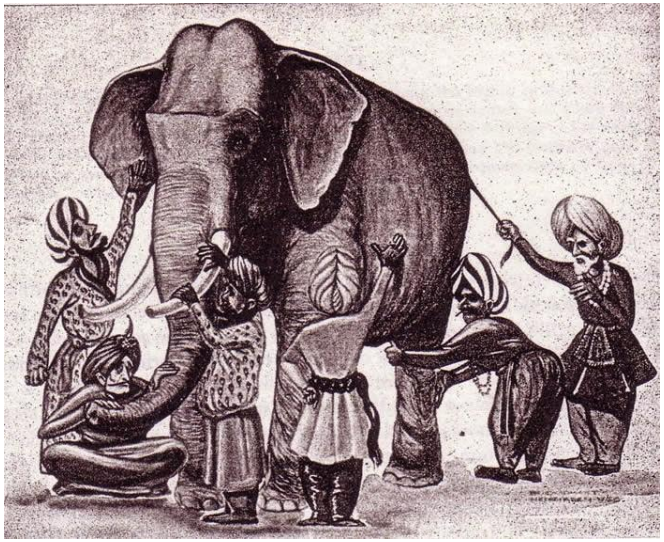
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

April 2015
Biosimilarity

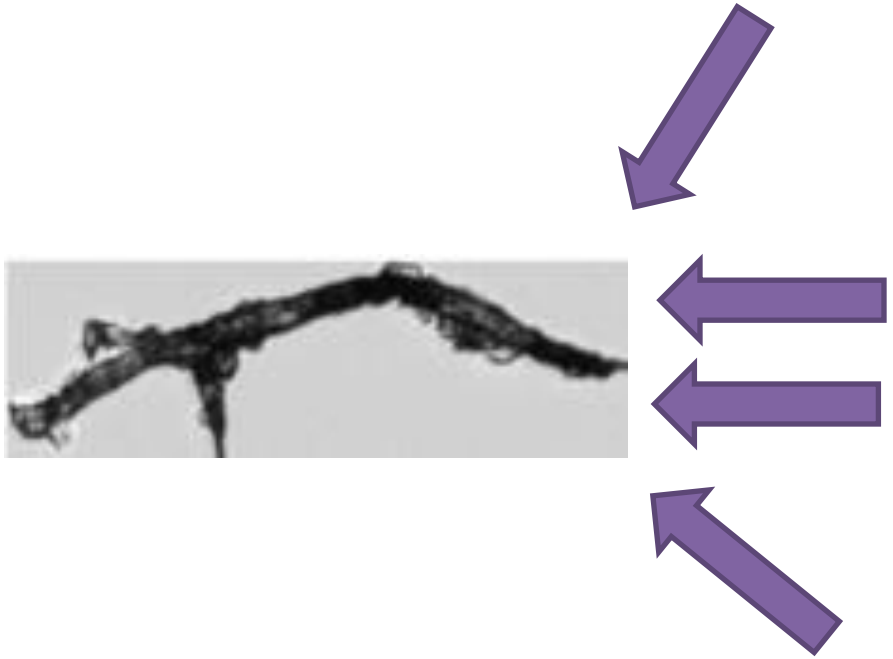
Assessment of Physicochemical Properties:
"The objective of this assessment is to maximize the potential for detecting differences in quality attributes between the proposed product and the reference product."

Fact: The use of Orthogonal techniques is essential

Due to their complexity, biopharmaceuticals require a vast array of testing using orthogonal techniques

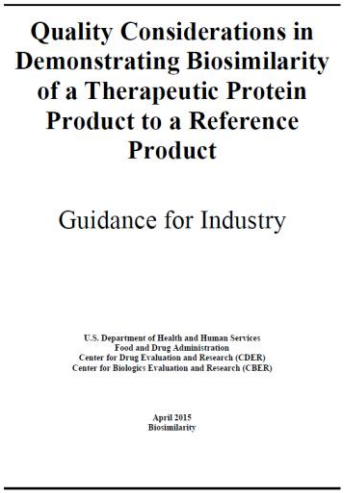


The Tale of the blind men and an elephant



That is especially true for Biosimilars development:

"Methods that use different physicochemical or biological principles to assess the same attribute are especially valuable because they provide independent data to support the quality of that attribute (e.g., orthogonal methods to assess aggregation). In addition, the use of complementary analytical techniques in series, such as peptide mapping or capillary electrophoresis combined with mass spectrometry of the separated molecules, should provide a meaningful and sensitive method for comparing products."



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- **Examples**
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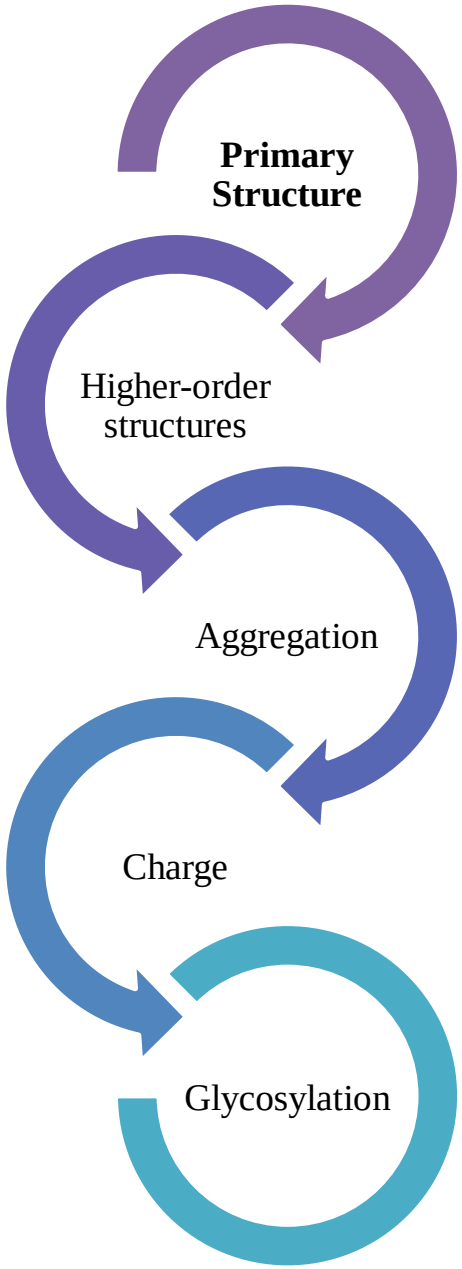
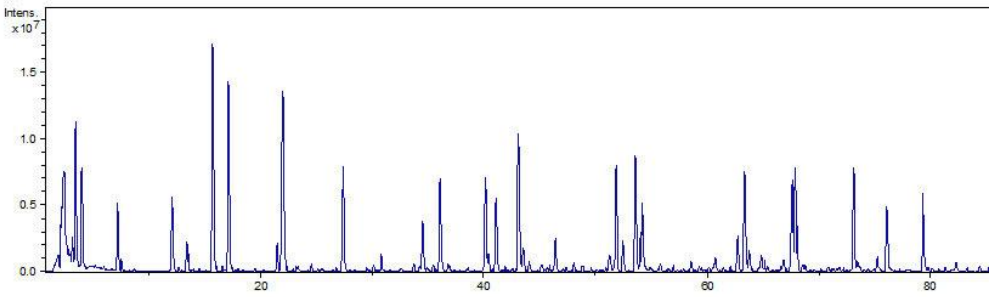
Physiochemical Characterization

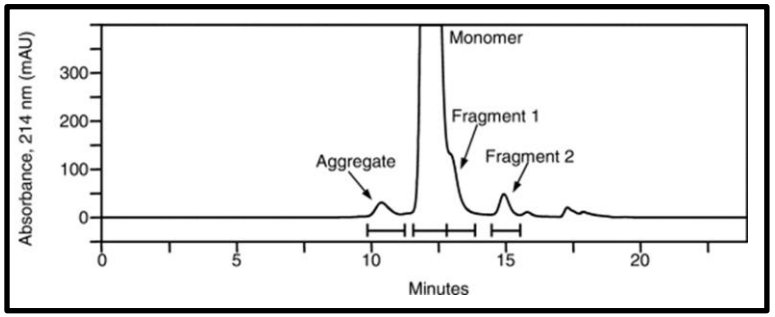
Compare to a reference standard



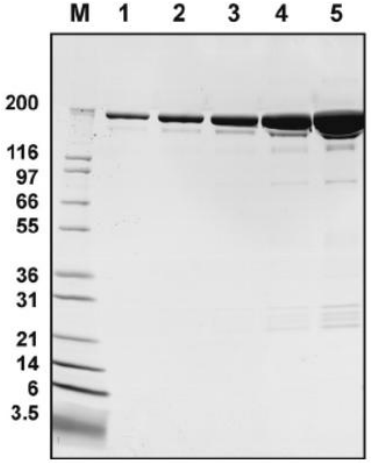


Mass spectrometry (Peptide mapping)

SEC

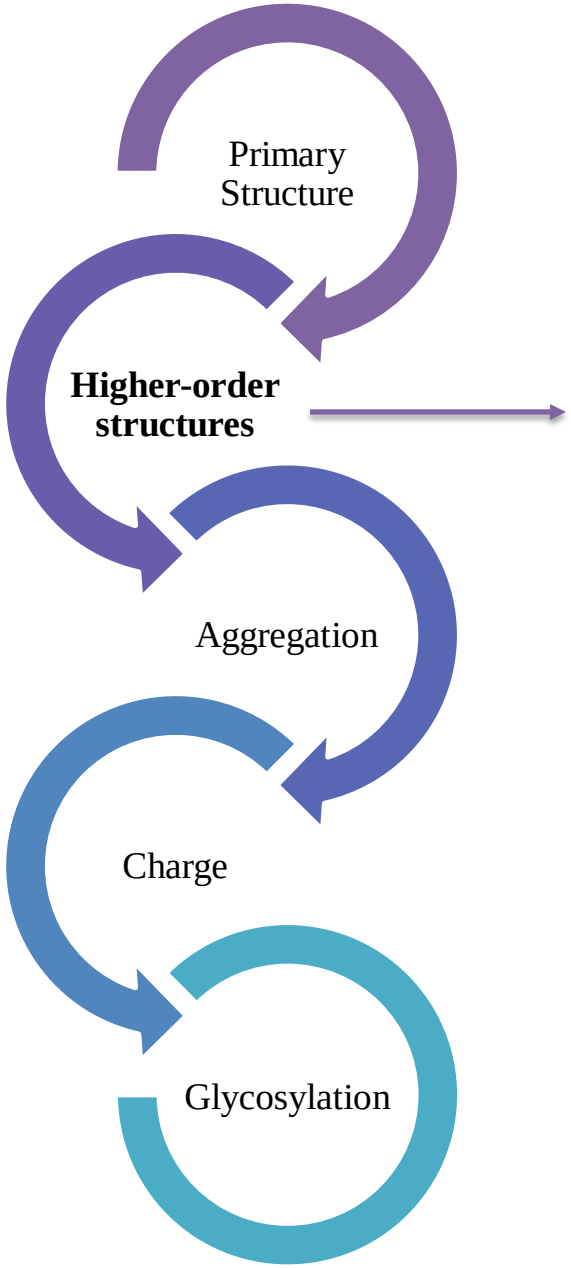


SDS-PAGE

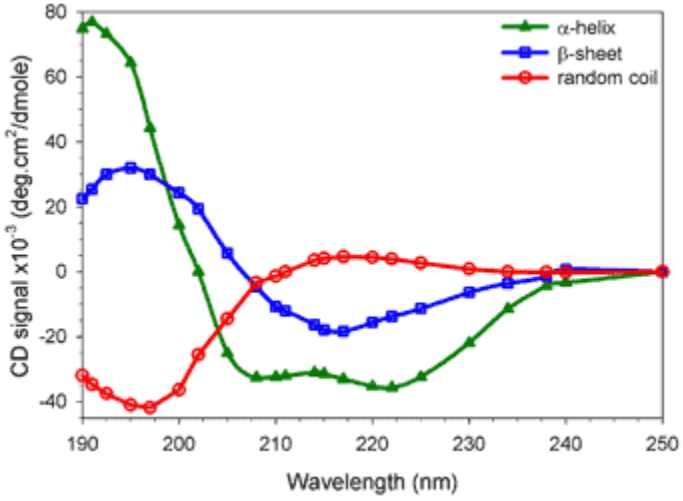


Physiochemical Characterization

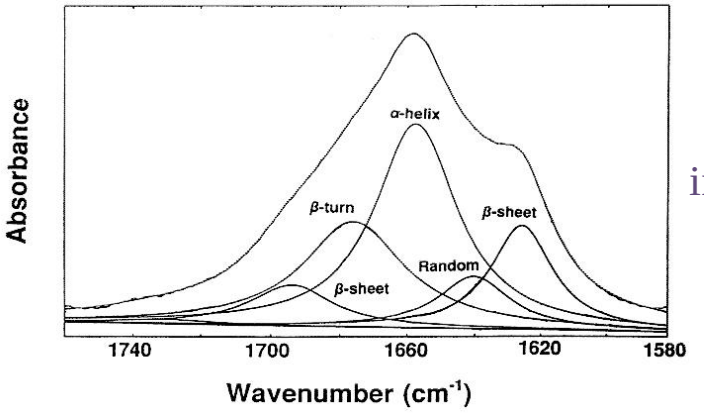
Compare to a reference standard



Secondary Structure



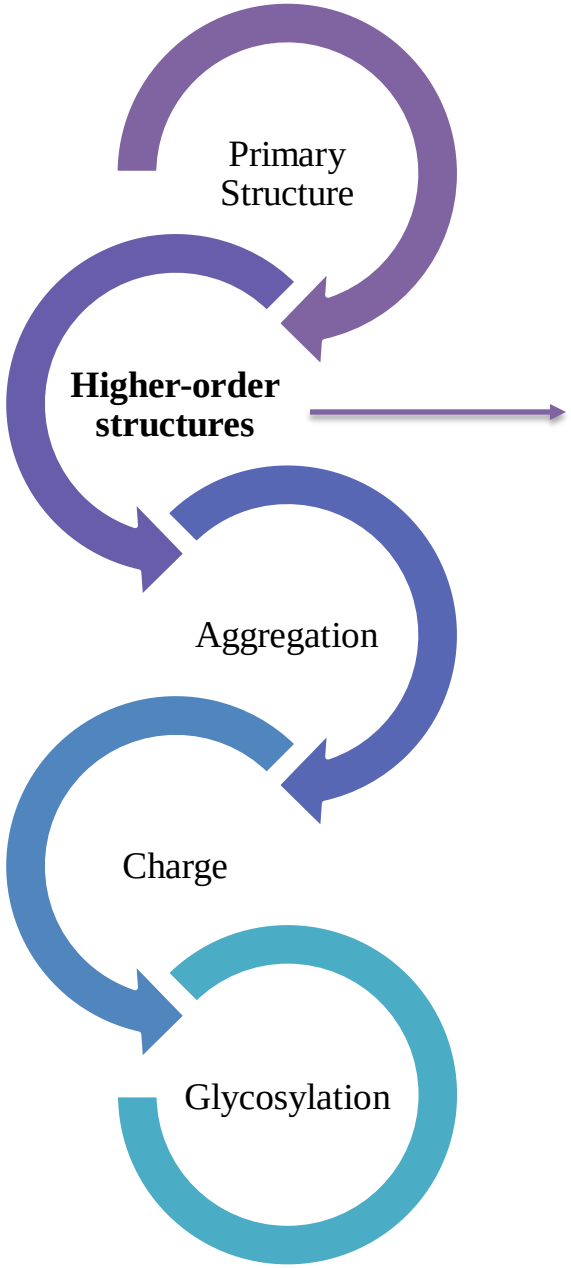
Circular Dichroism (CD)



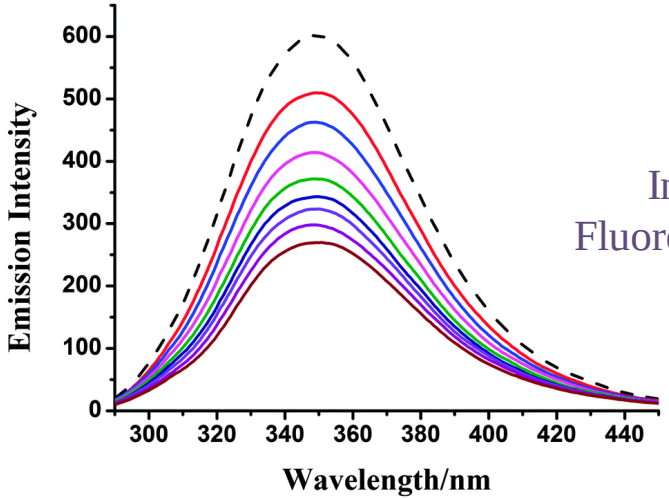
Fourier transform infrared spectroscopy (FTIR)

Physiochemical Characterization

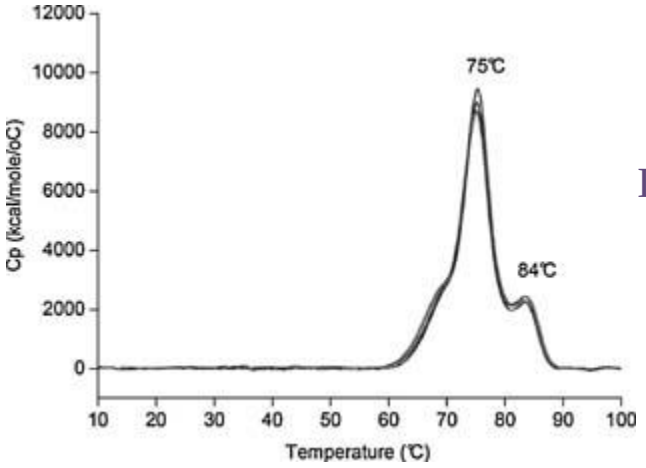
Compare to a reference standard



Tertiary Structure

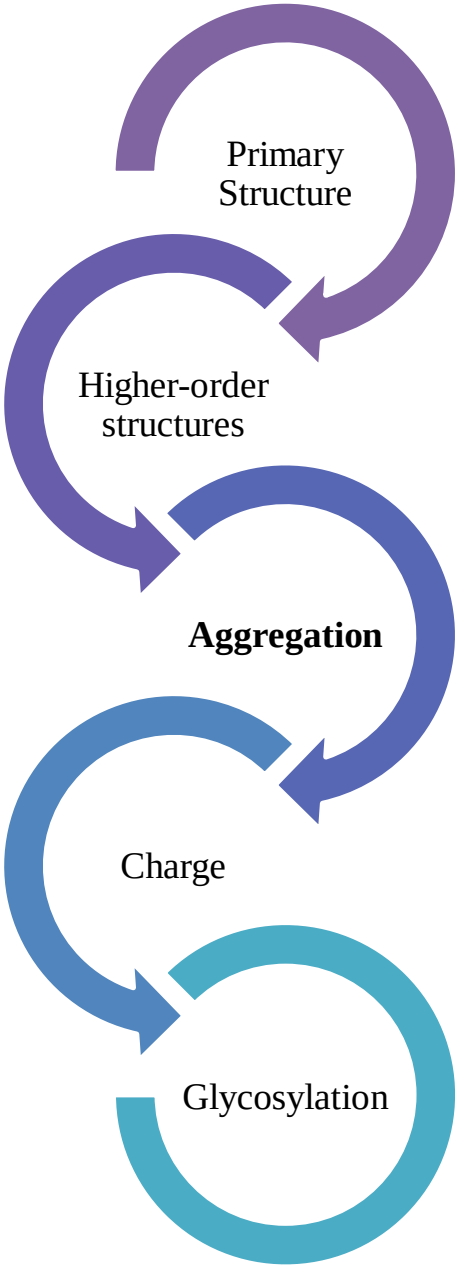


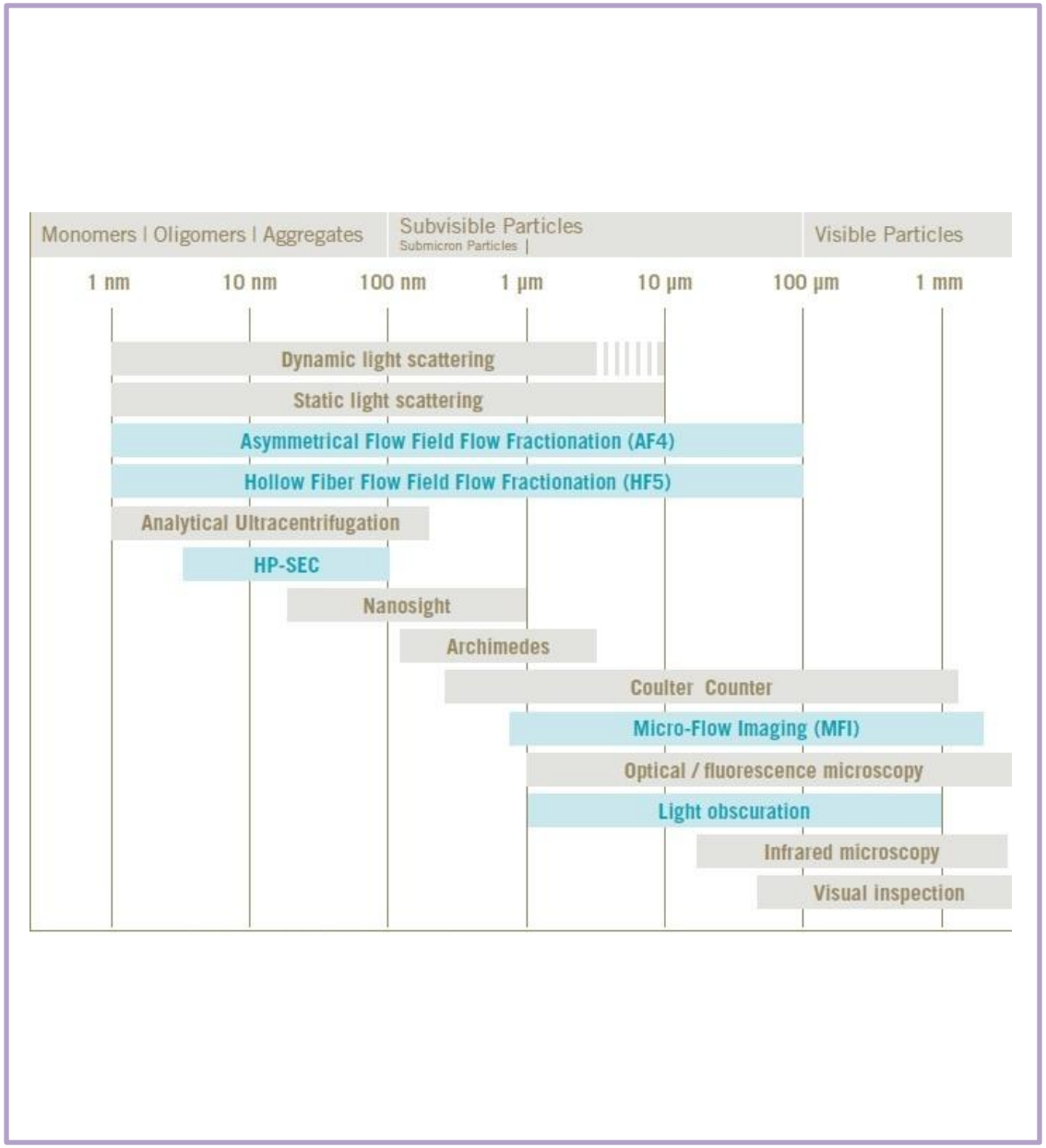
Intrinsic/Extrinsic Fluorescence Spectroscopy



Differential Scanning Calorimetry (DSC)

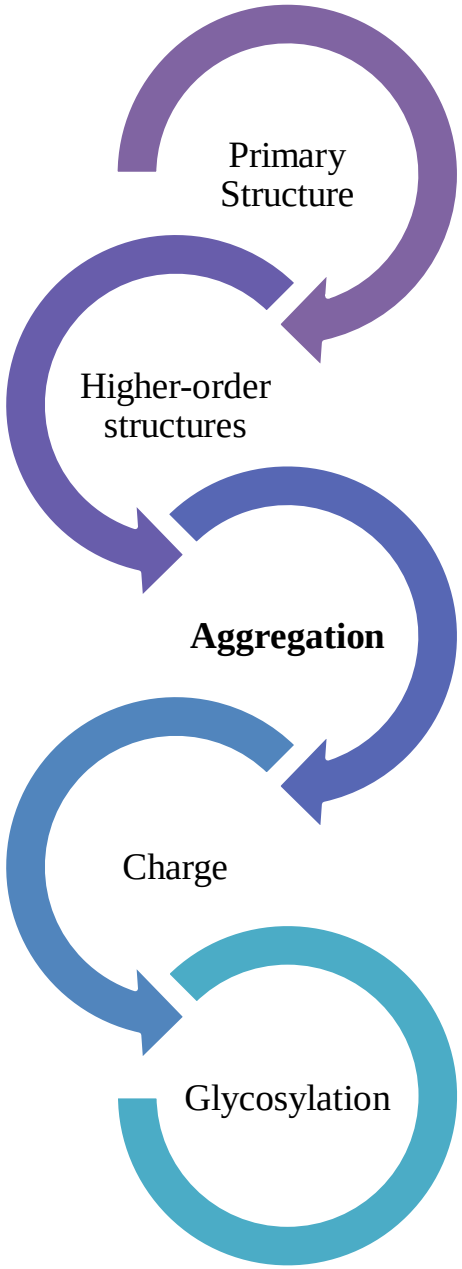
Physiochemical Characterization

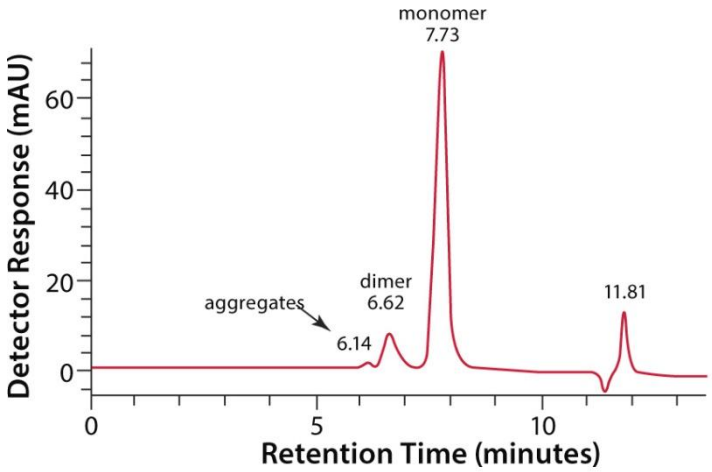




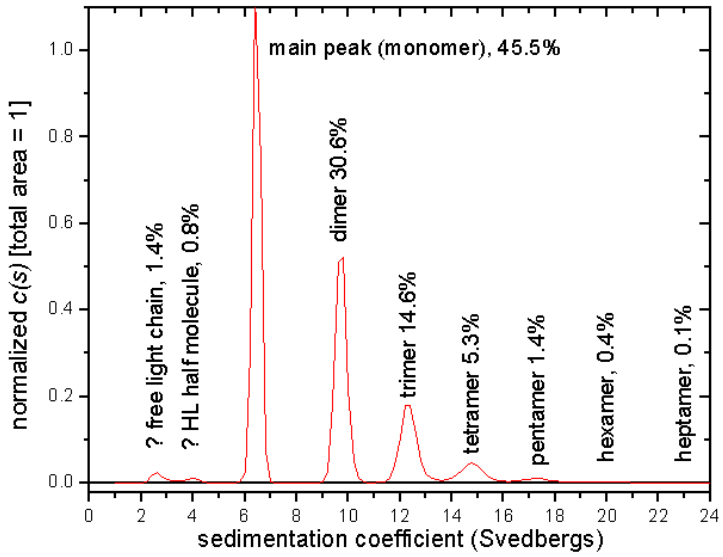
Physiochemical Characterization

Compare to a reference standard





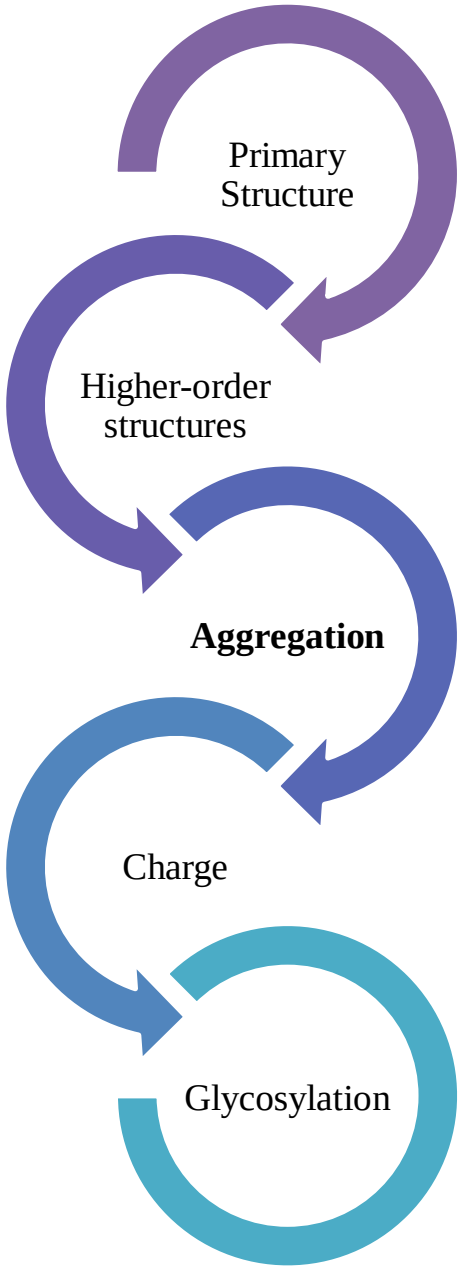
Size Exclusion Chromatography (SEC)

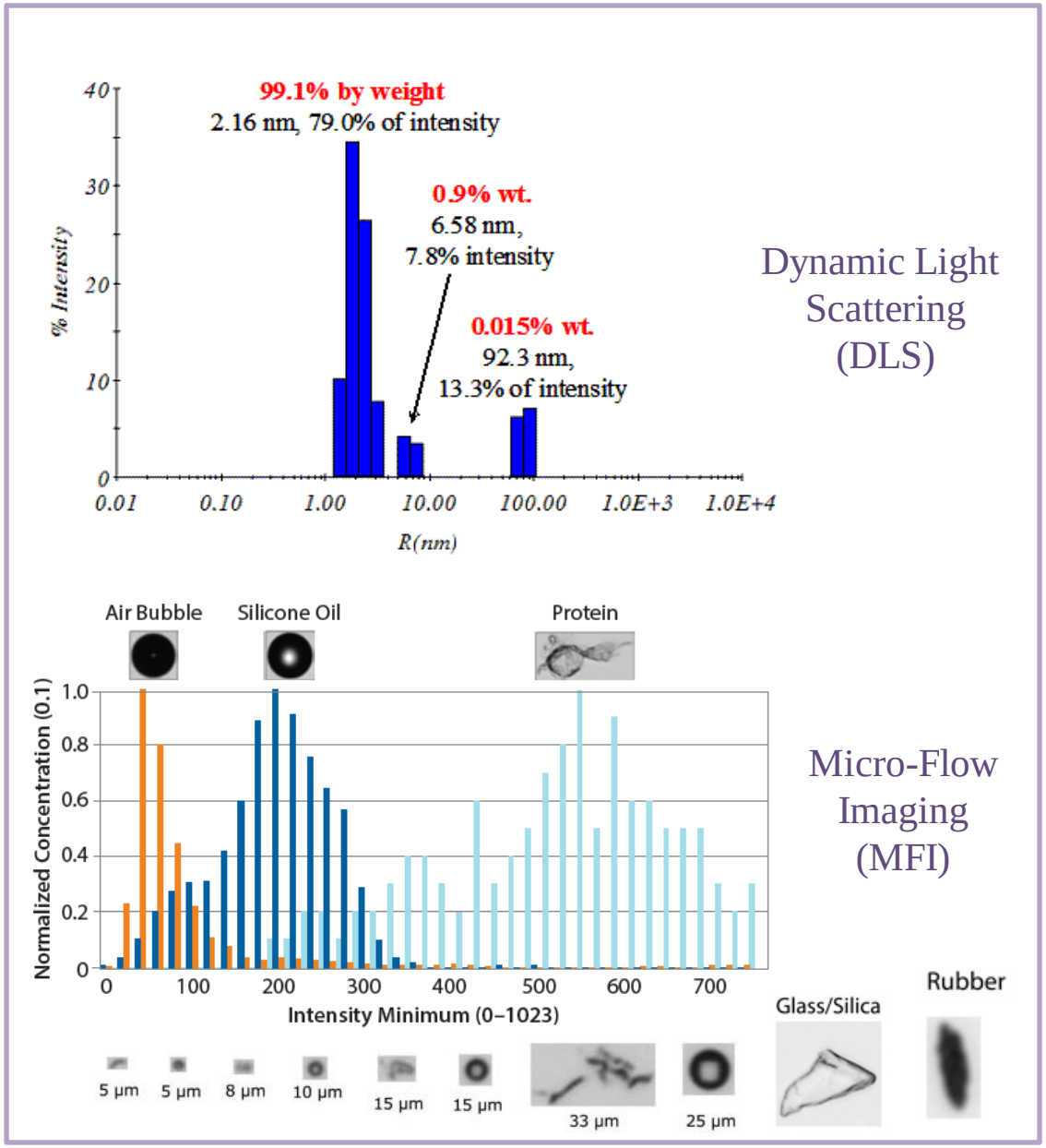


Analytical Ultra-Centrifugation (AUC)

Physiochemical Characterization

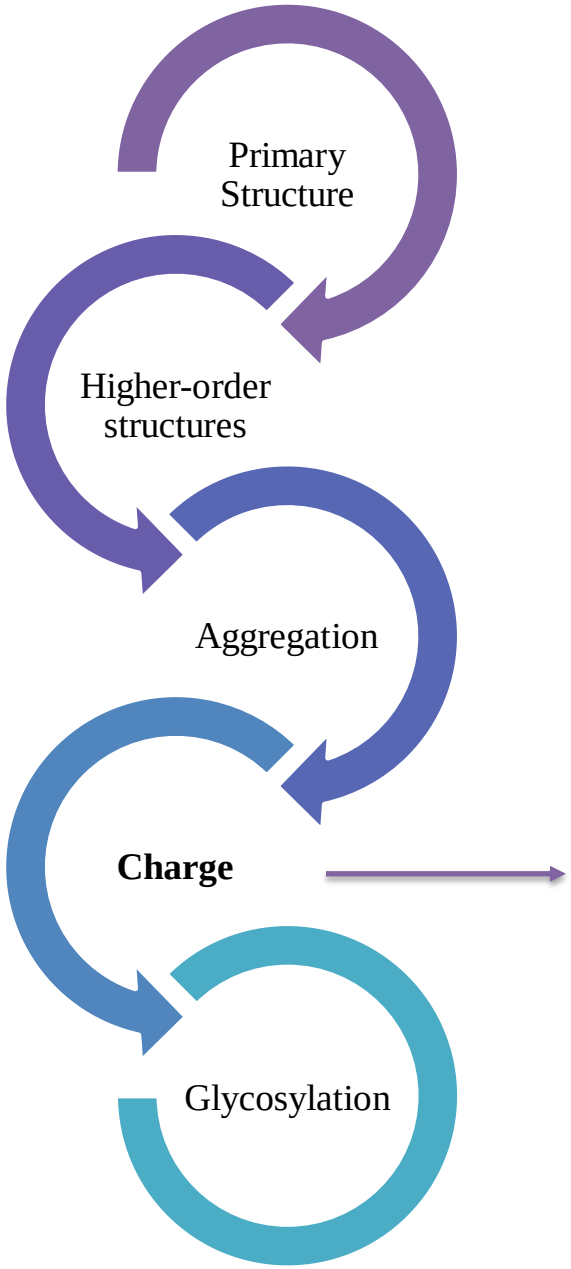
Compare to a reference standard

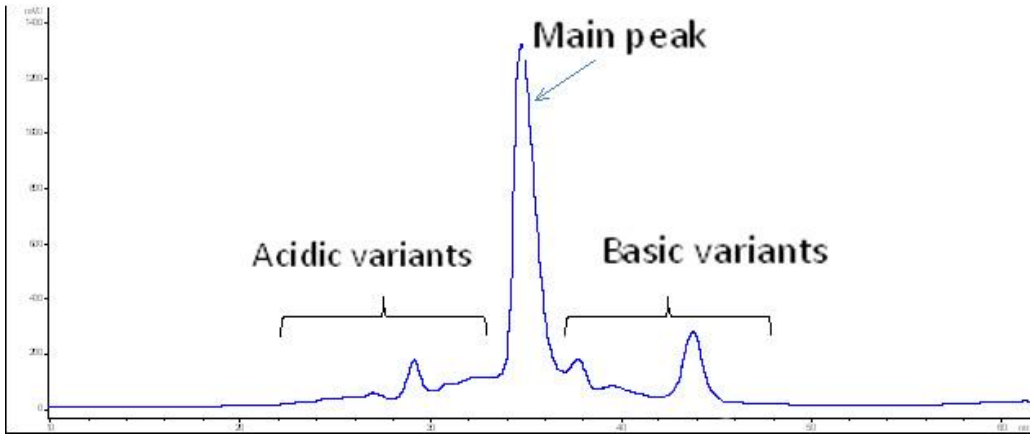




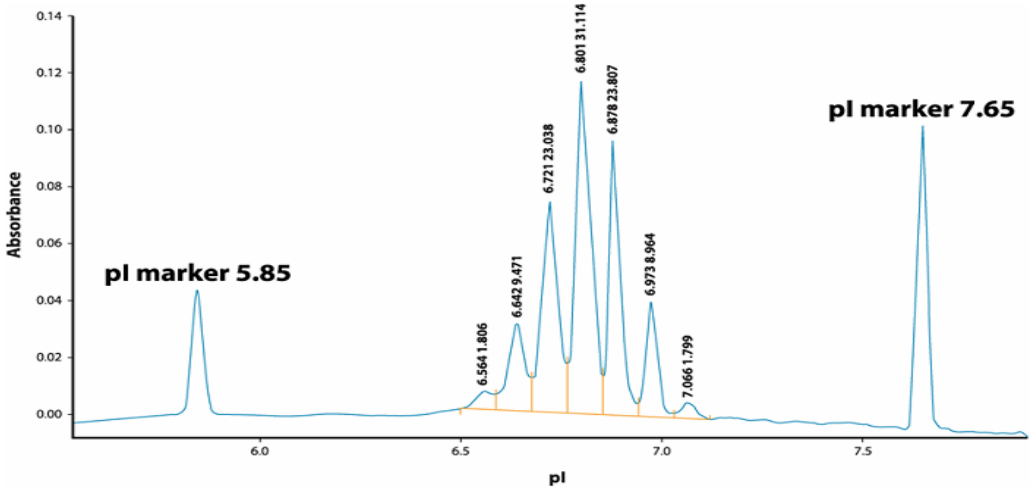
Physiochemical Characterization

Compare to a reference standard





Cation exchange chromatography (CEX-HPLC)

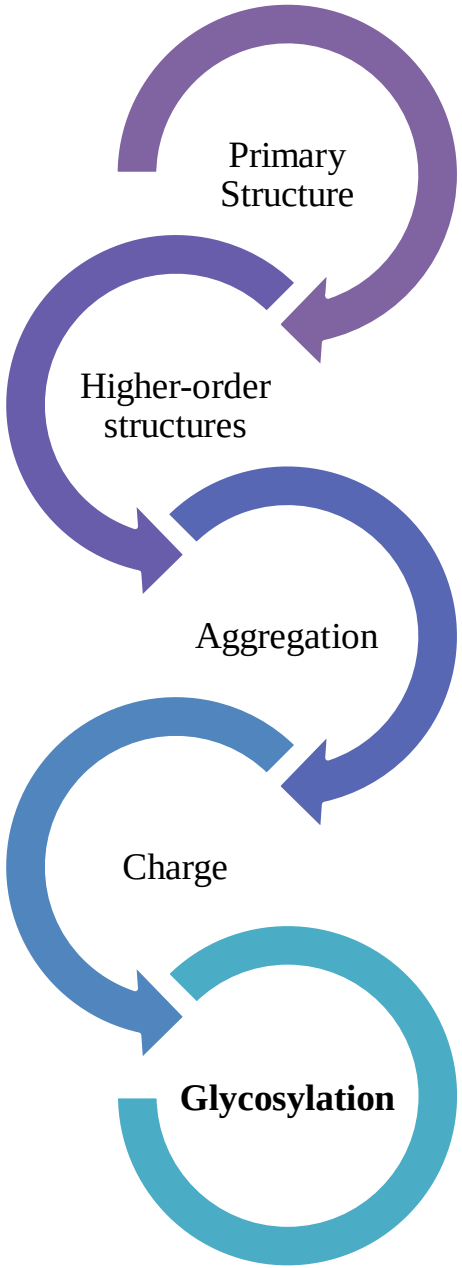


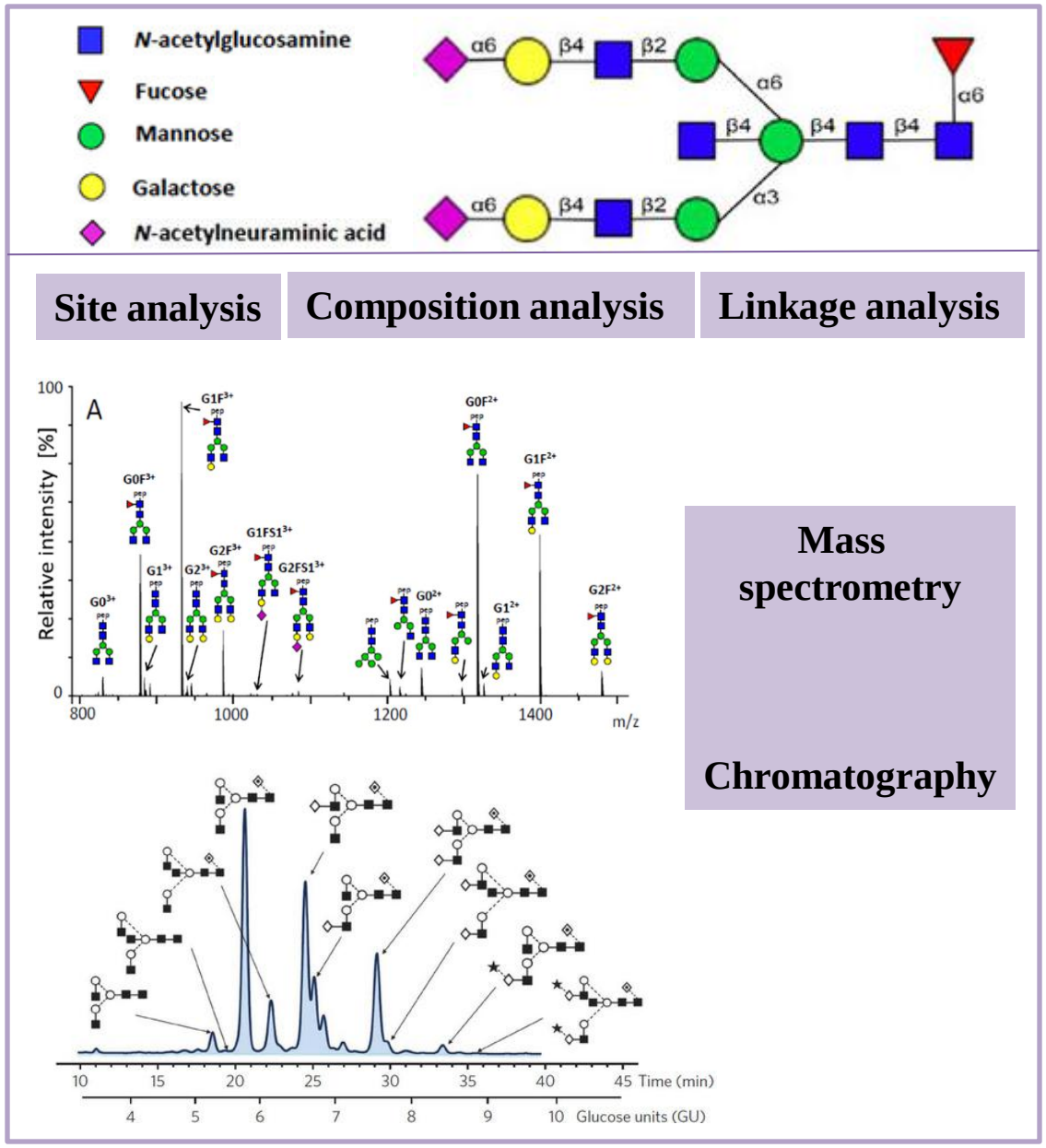
Imaged isoelectric focusing (cIEF)

http://mabprex.com/charge-variation-analysis-by-iex-hplc-ion-exchange-chromatography/

Physiochemical Characterization

Compare to a reference standard





http://www.nature.com/nchembio/journal/v6/n10/full/nchembio.437.html

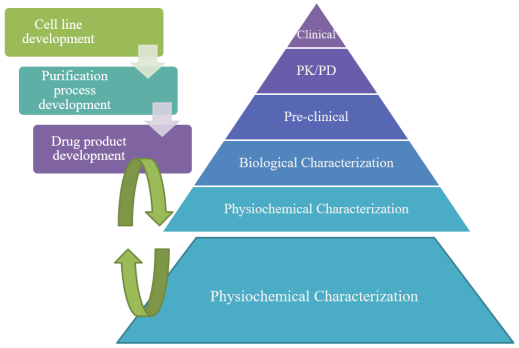
http://www.nature.com/articles/srep28098

Concluding Remarks

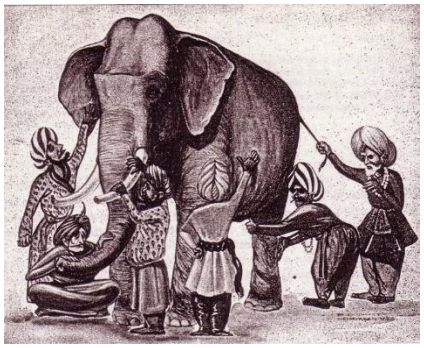
- The process of making proteins yields a **heterogeneous** protein product

“The process is The product”

- **Physiochemical characterization** studies are **corner stone** for the development of biologics and are especially important for determination of biosimilarity



- Due to their complexity, biopharmaceuticals require a vast array of testing using **orthogonal techniques**



Thank you for Your attention

Questions?