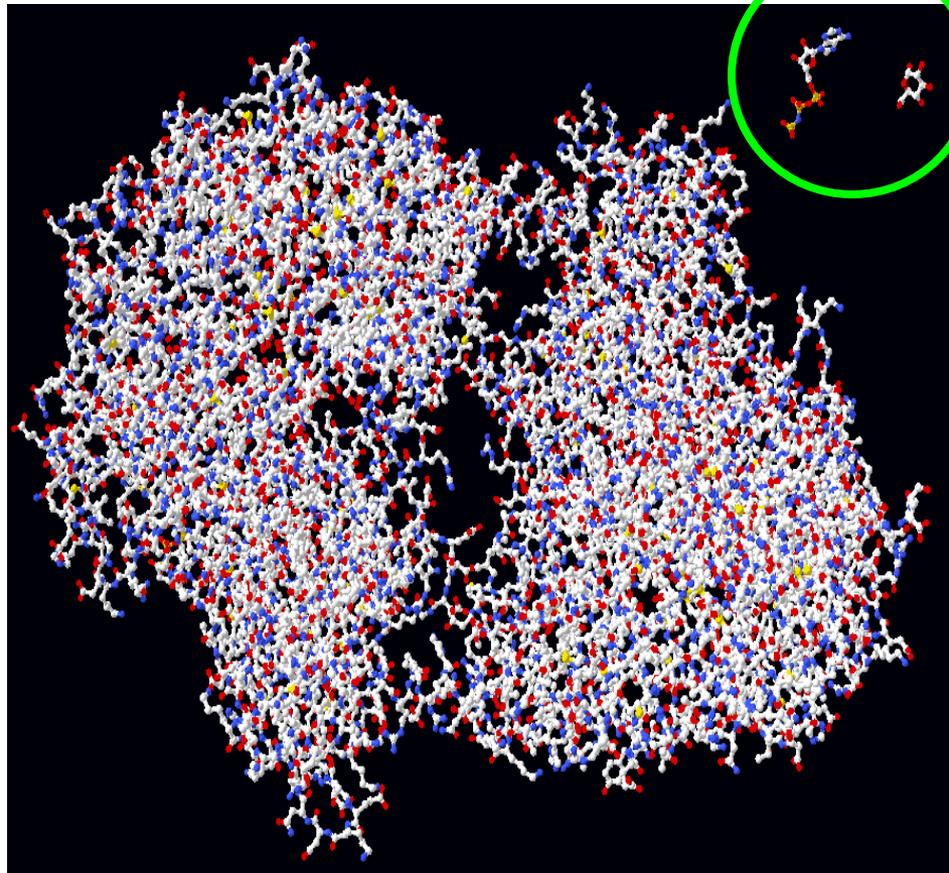




Chemistry, Manufacturing and Controls for Therapeutic Protein INDs

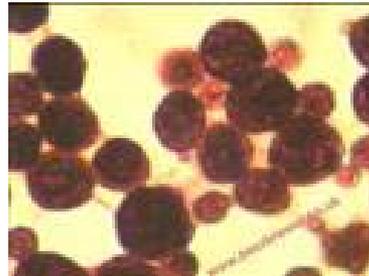
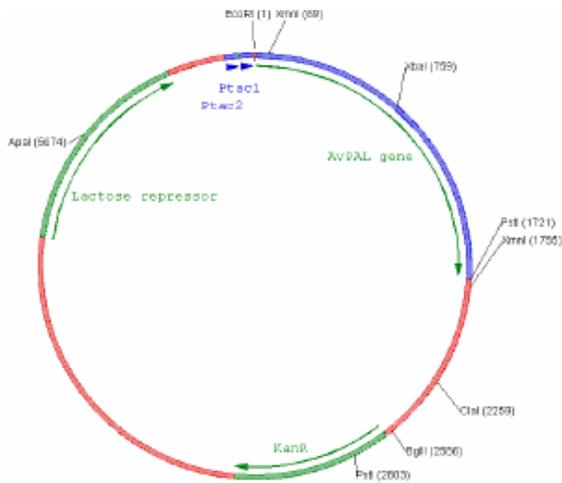
Gibbes Johnson, Ph.D.
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Therapeutic Proteins are Large and Structurally Complex Macromolecules Recombinant or Natural Sources



Expression and Production Systems

Expression vector

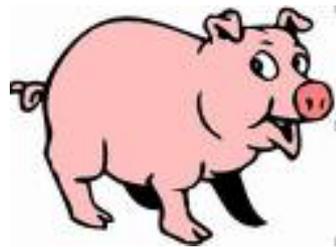


Yeast

Rabbit

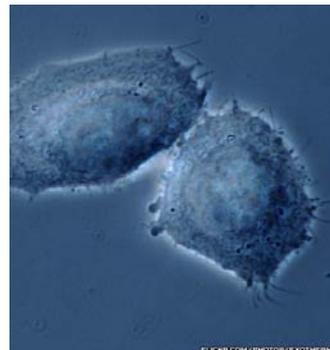


Carrot calli



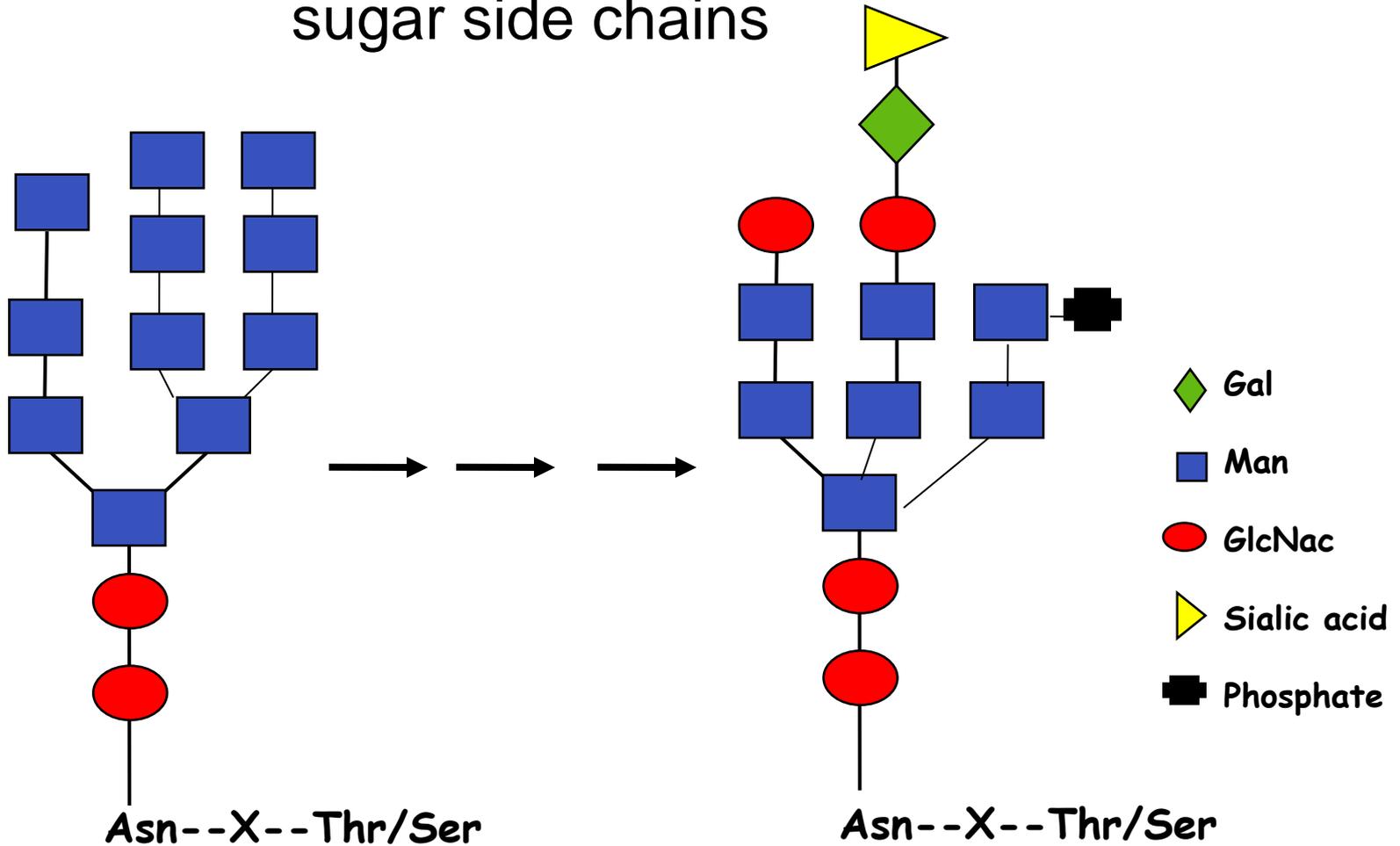
Pig

CHO cells



E. coli

Eukaryotes produce glycoproteins with complex sugar side chains



ER to Golgi → remodeling in the Golgi → mature glycoprotein

Manufacturing of Protein Therapeutics is Challenging

- Structurally Complex Proteins
- Protein Heterogeneity
- Potential Adventitious Agents
e.g. virus, mycoplasma, bacteria

Chemistry, Manufacturing and Controls

- Production system
- Fermentation and harvesting (for cell lines)
- Purification of drug substance
- Formulation of drug product
- Characterization
- Release and stability testing
- Comparability

Information Needed for INDs

- Vector
 - Gene sequence confirmation
- Cell banks
 - Identity, purity and freedom from adventitious agents
- Tissues
 - Clearance of adventitious agents

Information Needed for INDs

- Fermentation

Summary description of critical in-process controls and flow charts will suffice

- Purification of drug substance

Simple description of the column, matrices, buffer, elution conditions and process controls

- Formulation of drug product

Clear definition of final drug product (e.g. excipients)

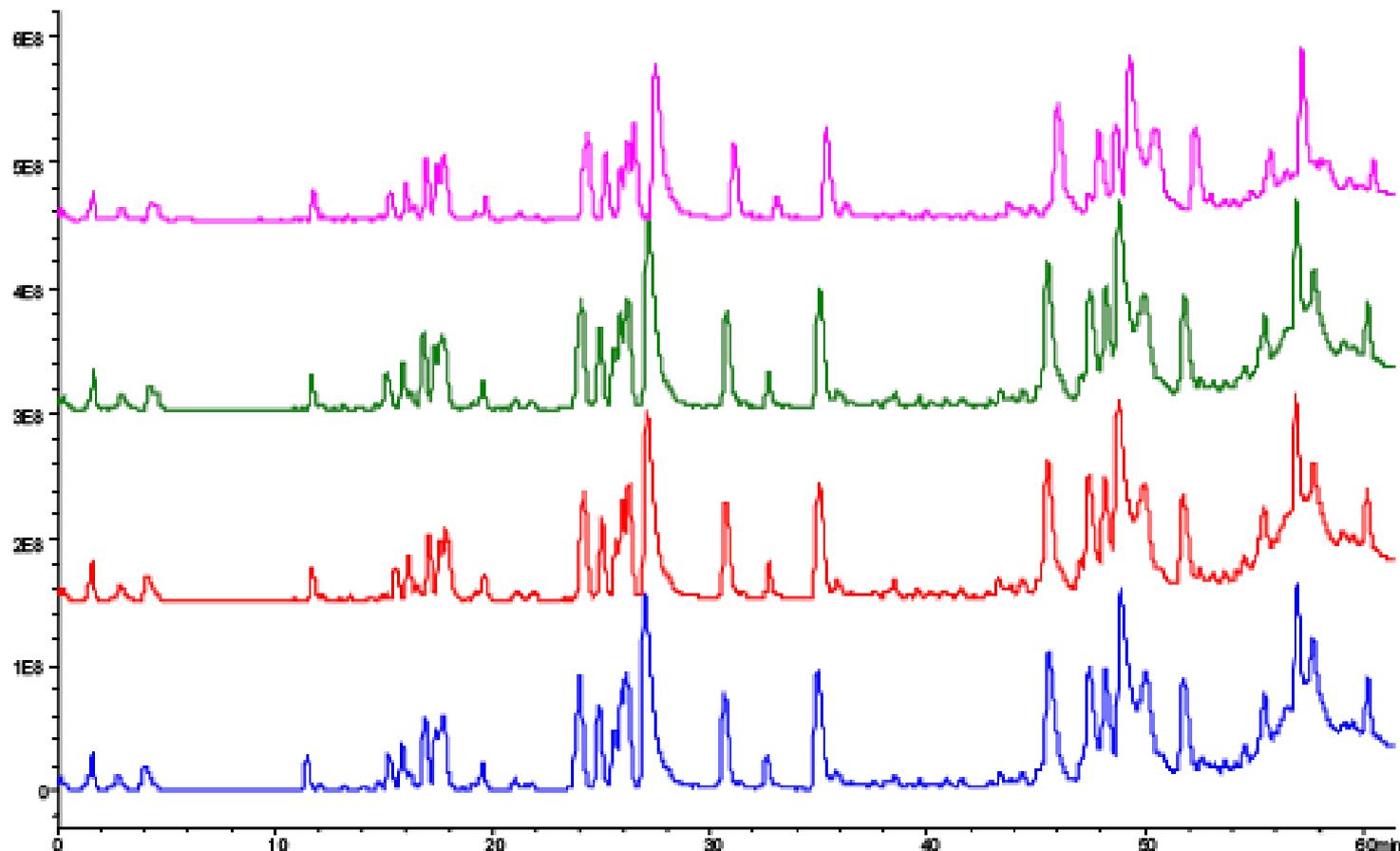
Drug Substance Characterization

Analysis of the physicochemical and biological properties of the protein, using a variety of techniques to assess:

- Identity
- Structure
- Purity
- Potency
- Post-translational modifications
- Degradation pathways

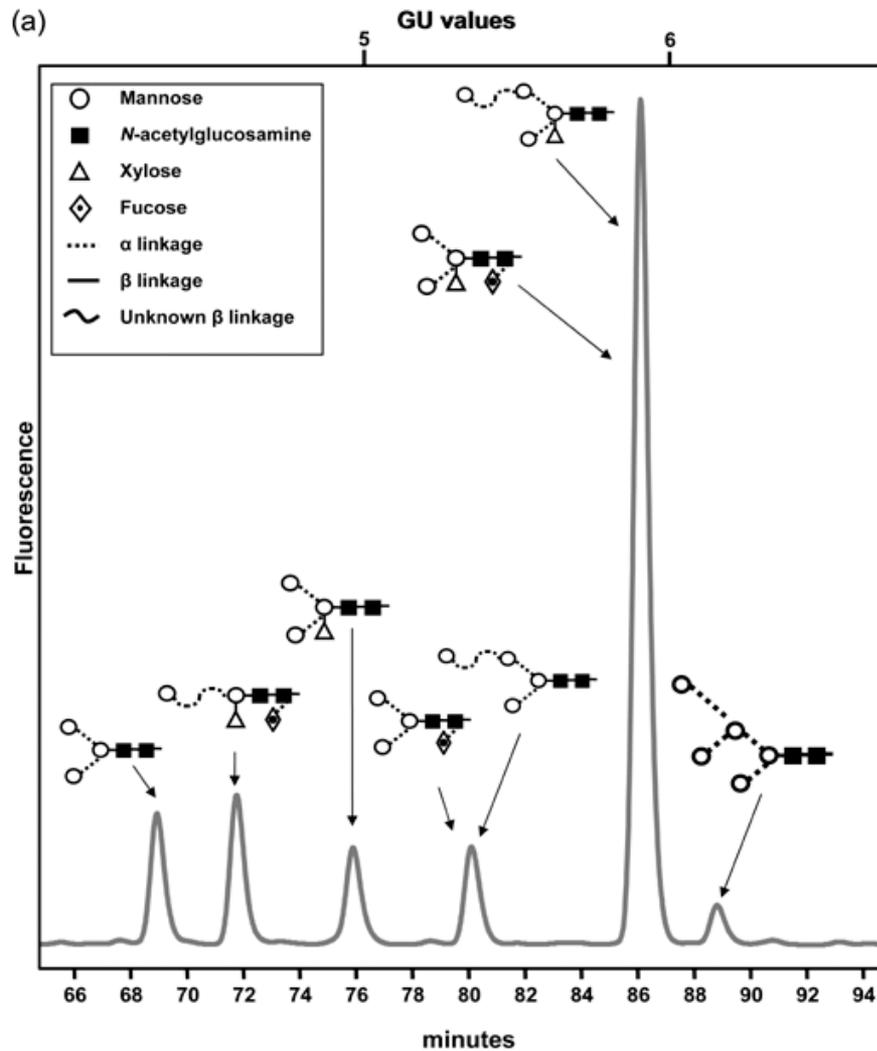
Method	Measures	Detects
Cell-based potency assays	Biological Activity	Overall integrity of the molecule
SDS-PAGE Reduced and non-reduced MALDI-TOF	Molecular Weight	Subunit molecular mass Fragmentation Covalent crosslinking
RP-HPLC, HIC-HPLC	Surface Hydrophobicity	Chemical and conformational variants
IEF, IEX-HPLC	Net Surface Charge	Charge variants
ELISA RIA Western Blot Surface Plasmon Resonance	Antibody Recognition	Specific epitopes in the protein, may function as identity tests
Peptide Map Amino Acid Composition N and C terminal sequence	Composition Post-translational modifications	Primary Structure variants Primary structure integrity Integrity of PTMs
CD (Far UV)	Optical Activity	Secondary Structure
UV Absorbance Fluorescence CD (Near UV)	Aromatic Amino Acids Side Chains	Tertiary Structure
SEC HPLC Analytical Ultracentrifugation	Hydrodynamic Radius	Molecular size Conformational changes Aggregation/dissociation
Light Scattering	Radius of Gyration	Molecular size Aggregation/dissociation
NMR X-ray Crystallography	Nuclear magnetic energy Transitions X-ray diffraction	Complete molecular structure

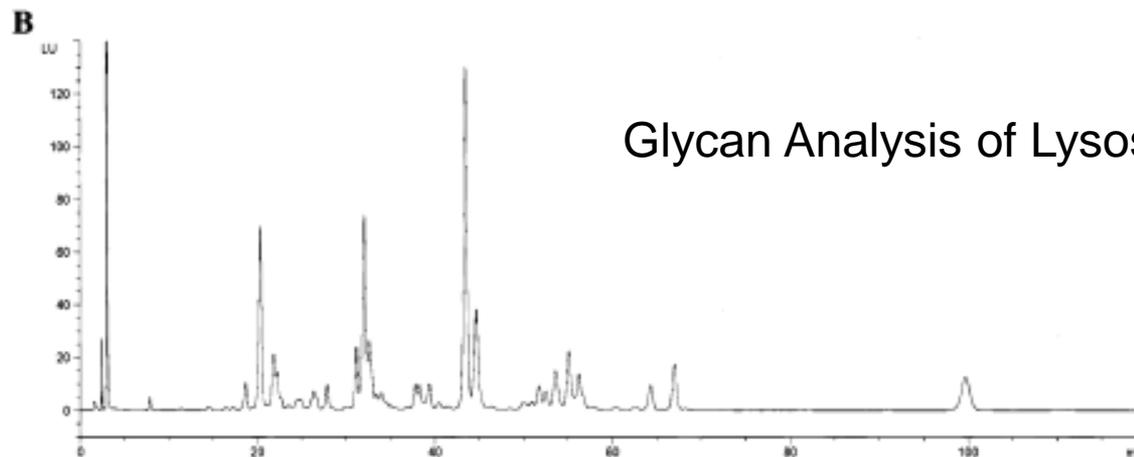
Peptide Mapping



Chromatograms recorded at UV 214 nm. Magenta trace: Lot 070111; green trace: Lot NP1603-07001; red trace: Lot NP1603-07002; blue trace: Lot AP1603-07003.

Glycan Mapping



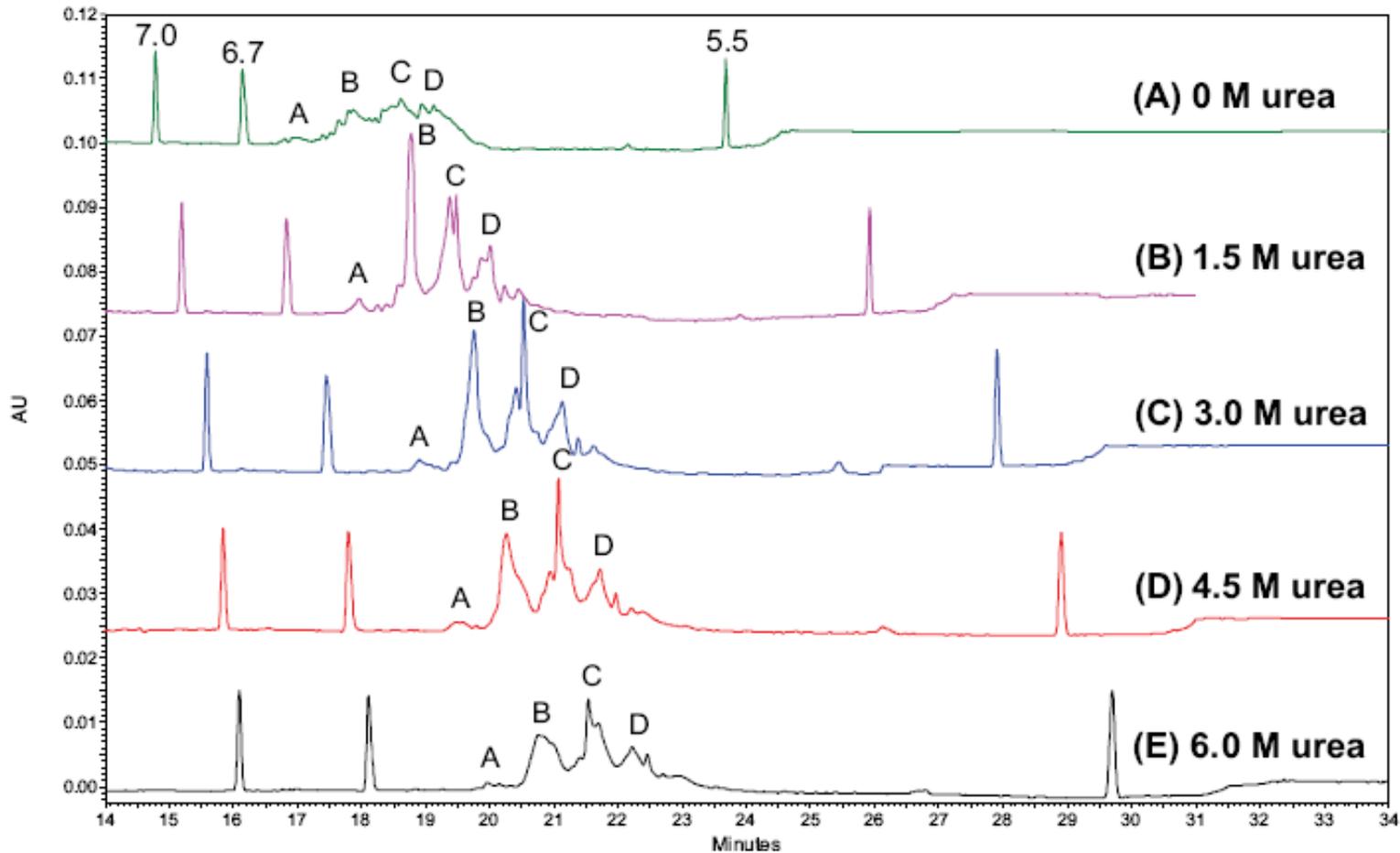


Glycan Analysis of Lysosomal Acid Lipase

Proposed structures of chLAL-derived oligosaccharides:

Retention Time	Proposed Structure (based on mass)
18.9	Man ₇ GlcNAc ₂ + Fucose
20.5	Man ₉ GlcNAc ₂ , Man ₇ GlcNAc ₂ Gal + Fucose
22.2	Man ₇ GlcNAc ₂ Gal ₁ + Fucose
22.7	Man ₇ GlcNAc ₂
25.5	Man ₇ GlcNAc ₂
26.5	Man ₈ GlcNAc ₂
28.3	Man ₈ GlcNAc ₂
31.5	Man ₇ GlcNAc ₂ Gal + Fucose + Sialic acid (NeuAc)
32.3	Man ₇ GlcNAc ₂ Gal ₂ + Fucose + Sialic acid (NeuAc), Man ₇ GlcNAc ₂ Gal + Sialic acid (NeuAc)
34.1	Man ₇ GlcNAc ₂ Gal + Fucose + Sialic acid (NeuAc), Man ₇ GlcNAc ₂ Gal ₃ + Fucose + Sialic acid (NeuAc)
43.7	Man ₇ GlcNAc ₂ Gal ₂ + Fucose + Sialic acid (NeuAc) ₂
44.9	Man ₇ GlcNAc ₂ Gal ₂ + NeuAc ₂
52.1	Man ₇ GlcNAc ₂ + PO ₄
52.6	Man ₈ GlcNAc ₂ + PO ₄
53.9	Man ₈ GlcNAc ₂ + PO ₄
55.4	Man ₇ GlcNAc ₂ Gal ₃ + Fucose + Sialic acid (NeuAc) ₂ , Man ₈ GlcNAc ₂ + PO ₄
56.4	Man ₇ GlcNAc ₂ Gal ₃ + Fucose + Sialic acid (NeuAc) ₂
67.3	Man ₈ GlcNAc ₂ Gal + Fucose + Sialic acid (NeuAc) + PO ₄
99.4	Man ₇ GlcNAc ₂ + 2PO ₄

Capillary Isoelectric Focusing of IgG1 mAb



Release and Stability Testing for INDs

- Sufficient information to ensure that the drug substance and drug product is
 - adequately controlled for purity, potency, identity and safety
 - stable for the duration of the clinical trial
- Drug product endotoxin, sterility and particulate testing are often required (e.g. parenteral drugs)
- For critical assays (i.e. potency) acceptance criteria should have an upper and lower limit

Methods for Well-Characterized Protein Products

METHOD	ATTRIBUTE	TYPICAL USE
pH (if liquid)	General quality	C, R, S
Karl Fisher (if lyophilized)	Moisture, integrity	C, R, S
Appearance	General quality	C, R, S
UV Absorbance	Concentration	C, R, S
SDS-PAGE (R/NR)	Purity, integrity	C, R, S
SEC-HPLC	Purity, integrity	C, R, S
RP-HPLC, IEX-HPLC, HIC-HPLC	Purity, integrity	C, R, S
Peptide Mapping	Identity, integrity	C, R, S
Mass Spectrometry	Identity, integrity	C, R, S
Isoelectric Focusing	Integrity	C, R, S
Capillary Electrophoresis	Integrity	C, R, S
Immunoassay/ELISA	Identity, integrity	C, R, S
Ligand Binding Assay	Identity, potency, integrity	C, R, S
In Vitro Bioassay	Identity, potency, integrity	C, R, S
N terminal Sequencing	Identity	C, R
Amino Acid Analysis	Identity, concentration	C, R
Product Residuals	Purity	C, R
Process Residuals	Purity	C, R
Monosaccharides	Identity	C, R*
Oligosaccharide	Identity	C, R*
Sialic Acid	Identity	C, R*
Circular Dichroism, FTIR	Conformation	C
AUC	Impurities (Aggregates)	C

[only in specific instances for selected glycoproteins]*

What is Comparability?

- A determination that a product is “Comparable” indicates that products are **highly similar** before and after a manufacturing change and that **no adverse impact** on the quality, safety or efficacy of the **drug product** occurred
- ICH Q5E guideline for highly purified proteins and manufacturing process changes made by a single sponsor (www.ich.org)

Physicochemical Comparability and INDs

- If there are differences in the manufacturing process of the non-clinical and clinical lot, the results of direct head-to-head comparisons of lots used in the non-clinical studies and to be used in the proposed clinical studies, with qualitative and quantitative information, must be provided in the original IND

Recommendations for Therapeutic Protein INDs

- Dosing should be based upon protein mass
- Do not change the manufacturing process between non-clinical and Phase I clinical studies
- For guidance on viral safety issues related to biotechnology products see ICH Q5A (www.ich.org)
- Request a Pre-IND meeting with FDA



Thanks to
Emanuela Lacana