



***In Vitro* Glycoengineering (IVGE) Services**

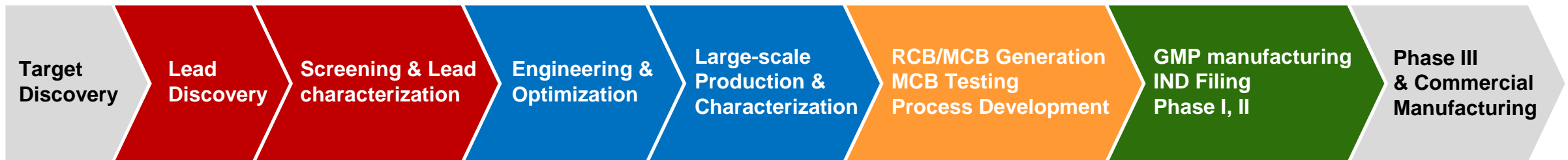
LakePharma Hayward

NR-7041.20191116



About LakePharma?

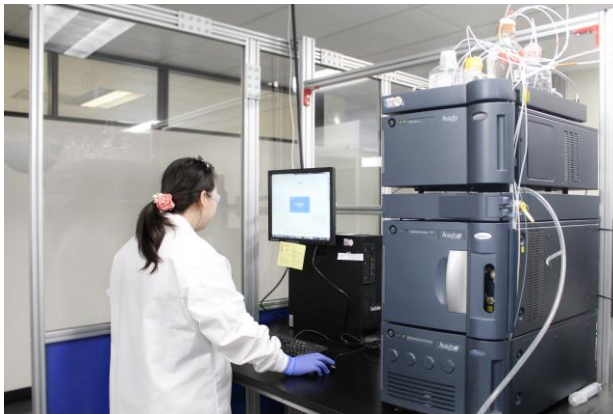
- LakePharma is a US-based Biologics CRDMO with operations in CA, MA, and TX
- Specializes in the production and evaluation of DNA vectors, viral vectors, cell lines, proteins, antibodies and conjugates, while providing integrated solutions bridging discovery, engineering, development, and GMP manufacturing. *Ask us about ISPs!*
- LakePharma has contributed to the development of 200+ therapeutic or diagnostic products and strives to do hundreds more.
- LakePharma Hayward is ISO 9001:2015 certified, and LakePharma Hopkinton is ISO 13485:2016 certified.



LakePharma Hayward

Dedicated facility for upstream and downstream process development, research cell bank (RCB) generation, and GMP manufacturing of analyte specific reagents.

- All IVGE activities are conducted at this site

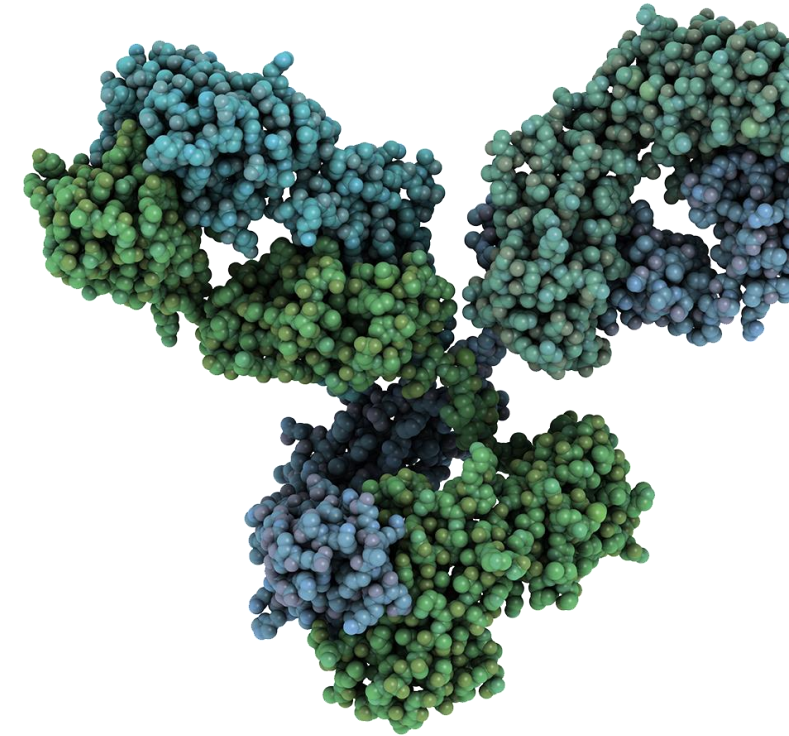


Glycosylation and the Importance of IVGE

Glycosylation is a common post-translational modification that can influence stability, biological activity, and pharmacokinetics of therapeutic antibody or protein.

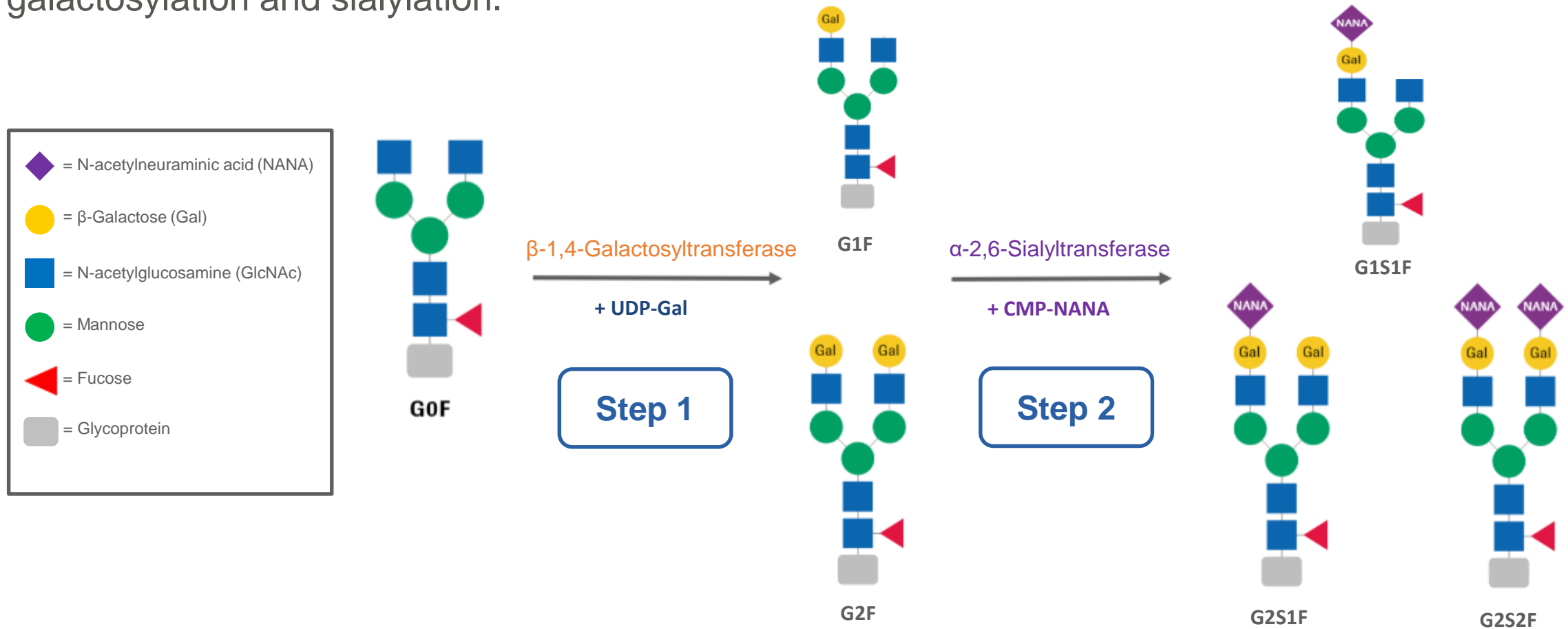
Producing therapeutic antibodies or proteins with consistent glycosylation profile remains a considerable challenge to the biopharmaceutical industry.

IVGE is a technology that can modify glycosylation in a controlled manner after proteins are produced and purified, and thus can isolate glycosylation management from cell culture manipulations.



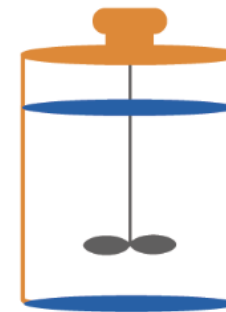
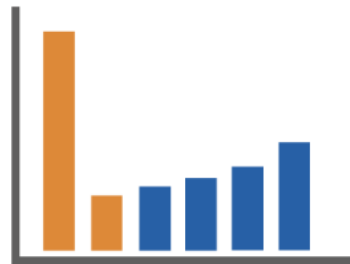
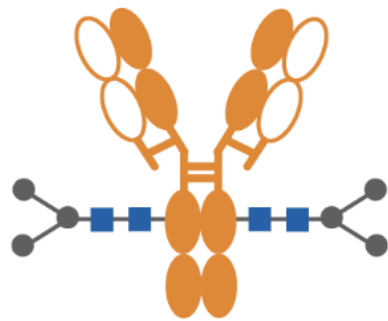
How IVGE Works

IVGE utilizes enzymatic reactions to control glycosylation. It consists of two reaction steps: galactosylation and sialylation.



LakePharma's IVGE Service Workflow

LakePharma is teaming up with Roche CustomBiotech to offer custom IVGE service.



IVGE Pilot Scale Run

- Preliminary reaction by standard protein:enzyme ratio
- Protein purification
- Analytical testing
- Verification of glycan conversion

IVGE Process Optimization

- Perform various protein:enzyme ratio reactions
- Protein purification
- Analytical testing
- Glycan profiling
- Optimal protein:enzyme ratio evaluation

IVGE Scale-Up

- Mini to large scale
- Perform optimized IVGE reactions
- Analytical testing
- Glycan profiling
- IVGE materials delivered

Key Features about LakePharma's IVGE Service



Rapid IVGE process with high protein recovery

- IVGE reactions can be completed in 2-3 days, with projects and data completed within 1-2 weeks
- Minimal protein loss



Robust and consistent glycan conversion via IVGE

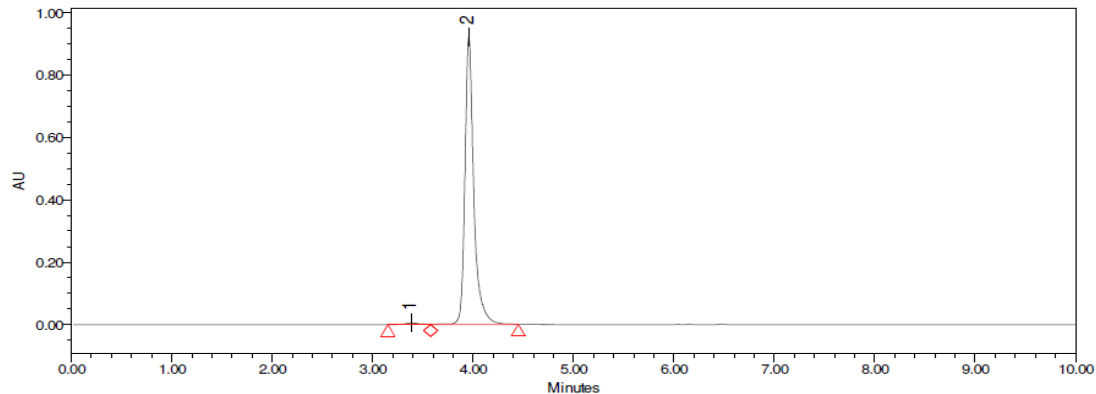
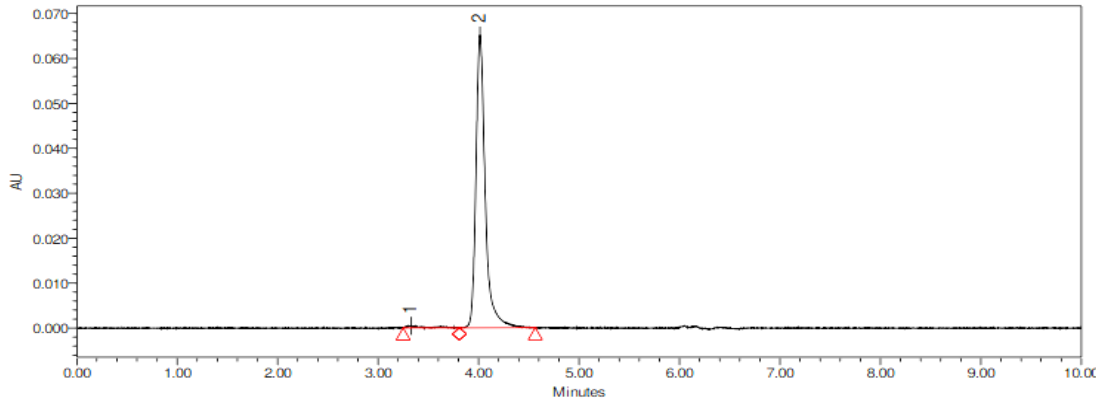
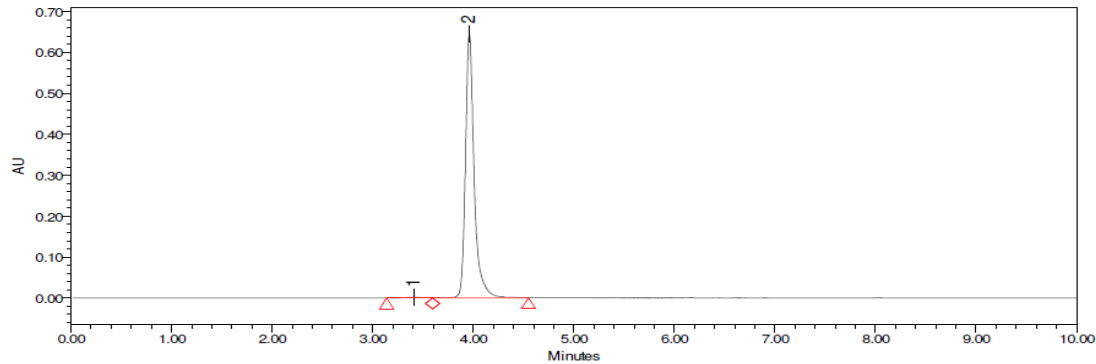
- Lot-to-lot consistency for glycan conversion and protein recovery
- Improved glycosylation and increased stability of therapeutic proteins observed after IVGE



Dedicated analytical tools and equipment for evaluation of IVGE success and data verification

- SE-UPLC via Waters ACQUITY UPLC
- cIEF via ProteinSimple iCE3
- Post-translational modification analysis via mass spec (Xevo G2-XS, Waters)

Case Study 1: IVGE on IgG1



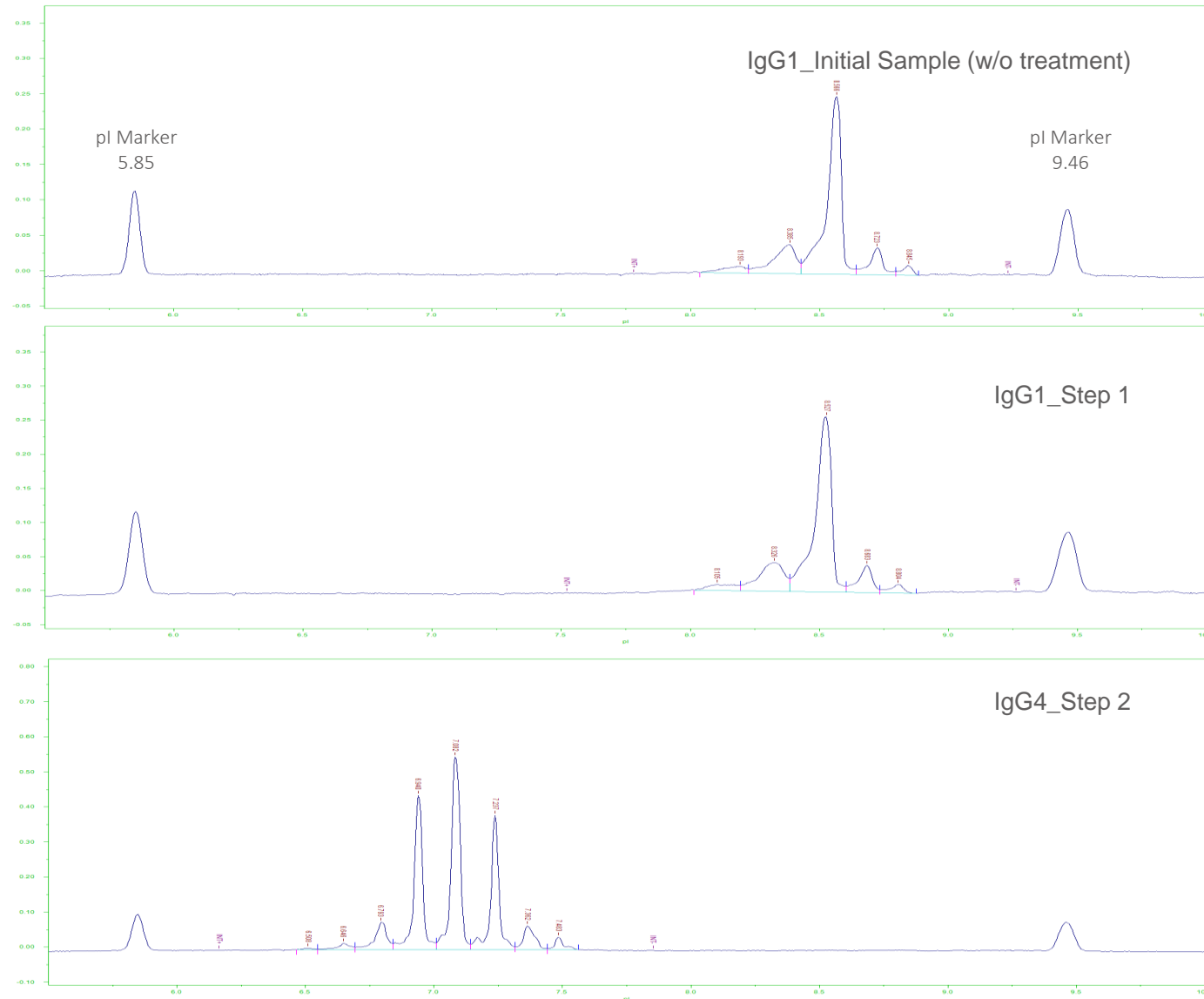
SE-UPLC (Aggregation Analysis)

Main Peak (%)	
Initial Sample	99.57
Step 1	98.32
Step 2	99.14

Background: Target IgG1 has 1 glycosylation site and sialic acid level is very low.

Results: The IVGE process does not increase aggregation of the sample.

Case Study 1: IVGE on IgG1



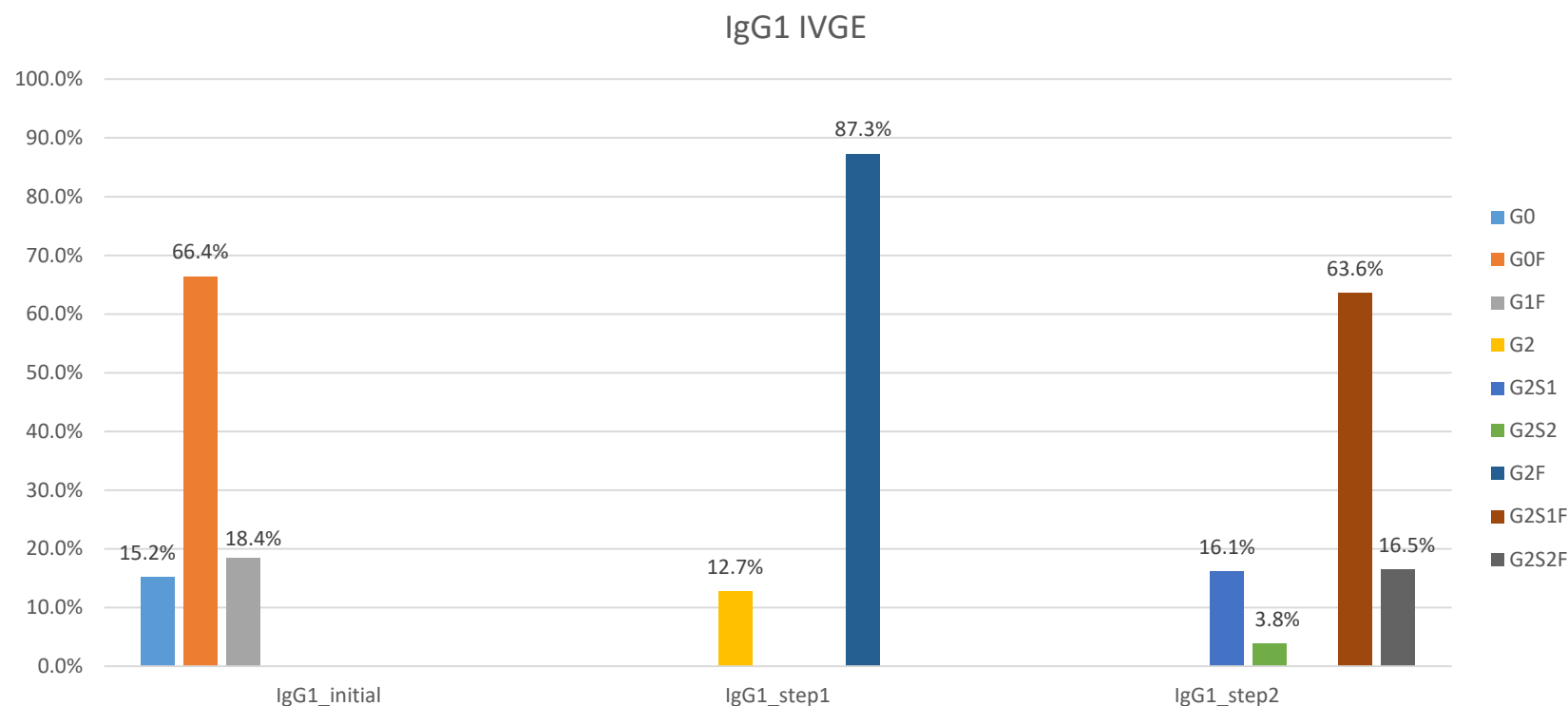
icIEF – Charge variants profiling

	Acidic Peak (%)
Initial Sample	21.35
Step 1	21.77
Step 2	35.52

Results: The charge variant profile shows a shift of peaks toward a lower pH, indicating the protein is successfully sialylated.

Case Study 1: IVGE on IgG1

Post-translational modification – Glycoprofiling



Results: After step 1, all glycans were converted to G2 and G2F. After step 2, all were sialylated.

Note: incubation with GalT1 for 12 hours, followed by ST6 for 24 hours



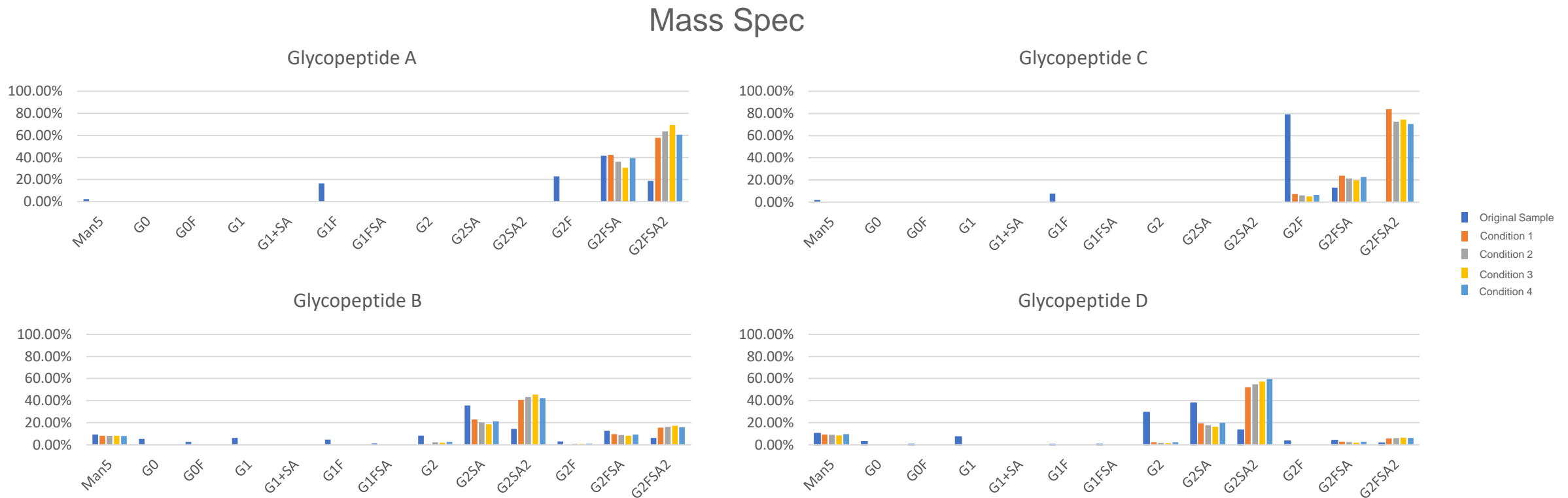
IVGE of Non-Antibody Proteins

- How well does IVGE work on different residues on different proteins?
- How does that impact process and activities?

Case Study 2: IVGE on Non-Antibody Protein

Background: Target protein has multiple glycosylation sites and sialic acid level is very low.

Results: 4 different enzyme ratio conditions were tested at small scale (~2 mg) and the final products were analyzed by mass spec. Data showed that the reacted products had significantly higher levels of galactosylated and sialylated glycans when compared to the original.



Working with LakePharma

- Comprehensive technology platform
- Technical consultation with experts in antibody discovery, protein production, and GMP manufacturing
- Online data system for 24-hour access to project information (timelines, data, team communications)
- Strong project management with regular project updates (email and teleconferences)

For more information, please contact Inquiries@LakePharma.com