

Glycan Heterogeneity and Microheterogeneity Analysis — Hands-on

Understand how glycan heterogeneity and microheterogeneity impact protein function, potency and regulatory acceptance. This module focuses on experimental and data analysis strategies to profile site occupancy and glycoform distributions, compare batches and conditions, and generate clear QC summaries for biologics, biosimilars and glycan biomarkers.

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Session 1

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Concepts of Glycan Heterogeneity

Macroheterogeneity vs microheterogeneity definitions

[site occupancy vs glycoform diversity](#) [protein and site level views](#) [link to function and stability](#)

Sources of glycan heterogeneity in bioprocesses

[cell line and clone differences](#) [media, feeds and culture conditions](#) [downstream processing impacts](#)

Regulatory and QC perspective on heterogeneity

critical quality attributes (CQAs) **comparability and**
lot release **biosimilar similarity expectations**

Session 2

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Experimental Strategies for Heterogeneity Profiling

Released glycan, glycopeptide and intact approaches

when to choose each strategy **depth vs throughput**
trade offs **sample and buffer constraints**

Chromatography and MS methods for heterogeneity

HILIC and RP profiling of glycoforms **intact mass and**
subunit analysis **DDA and targeted MS/MS for key**
sites

Exoglycosidase arrays and orthogonal readouts

stepwise exoglycosidase digestion **shifts in LC or MS**
patterns **confirming structural assignments**

Session 3

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Quantitative Analysis & Visualization of Glycoforms

Peak integration and relative abundance calculations

area based quantitation strategies **normalization**
within and across runs **handling low abundance**
glycoforms

Site occupancy and microheterogeneity metrics

occupied vs unoccupied site estimates **G0, G1, G2**
and afucosylation ratios **heterogeneity indices and**
summaries

Visualization and reporting of glycan profiles

stacked bar and heatmap displays **comparability**
plots across batches **templates for QC ready reports**

Session 4

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Mini Capstone: Biotherapeutic Heterogeneity Case Study

Designing a heterogeneity profile for a monoclonal antibody

Theory plus Practical

From LC–MS data to site level glycoform tables

structuring input and metadata **calculating key**
heterogeneity metrics **flagging out of specification**
patterns

Deliverables: heterogeneity summary and comparability report

CSV or XLSX glycoform tables **plots for batch**
comparison **short PDF or PPTX interpretation**