Biologics Process Development Analytics

The Integration of Mass Spectrometry to the Process Development of Fusion Protein Therapeutics

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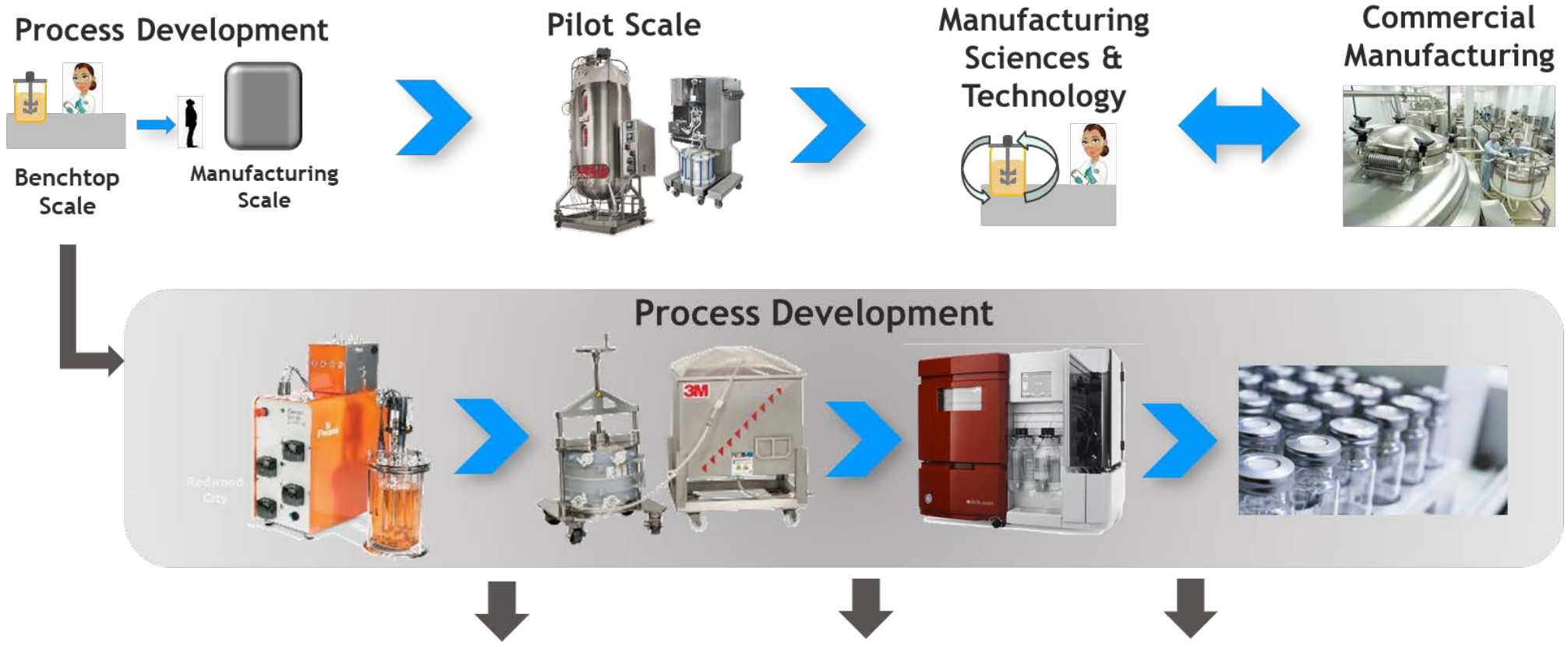
Bristol Myers Squibb™

Outline

- Introduction
- Site-Specific Glycosylation Monitoring with LC-MS
- Summary
- Acknowledgement

Bioprocess Development Analytical Support



Analytical Support

- High-throughput Platform Assays
- Sample Automation
- PAT for Real time Analytics
- **Mass Spectrometry for In-Process Support**

Fc-fusion Protein Therapeutics

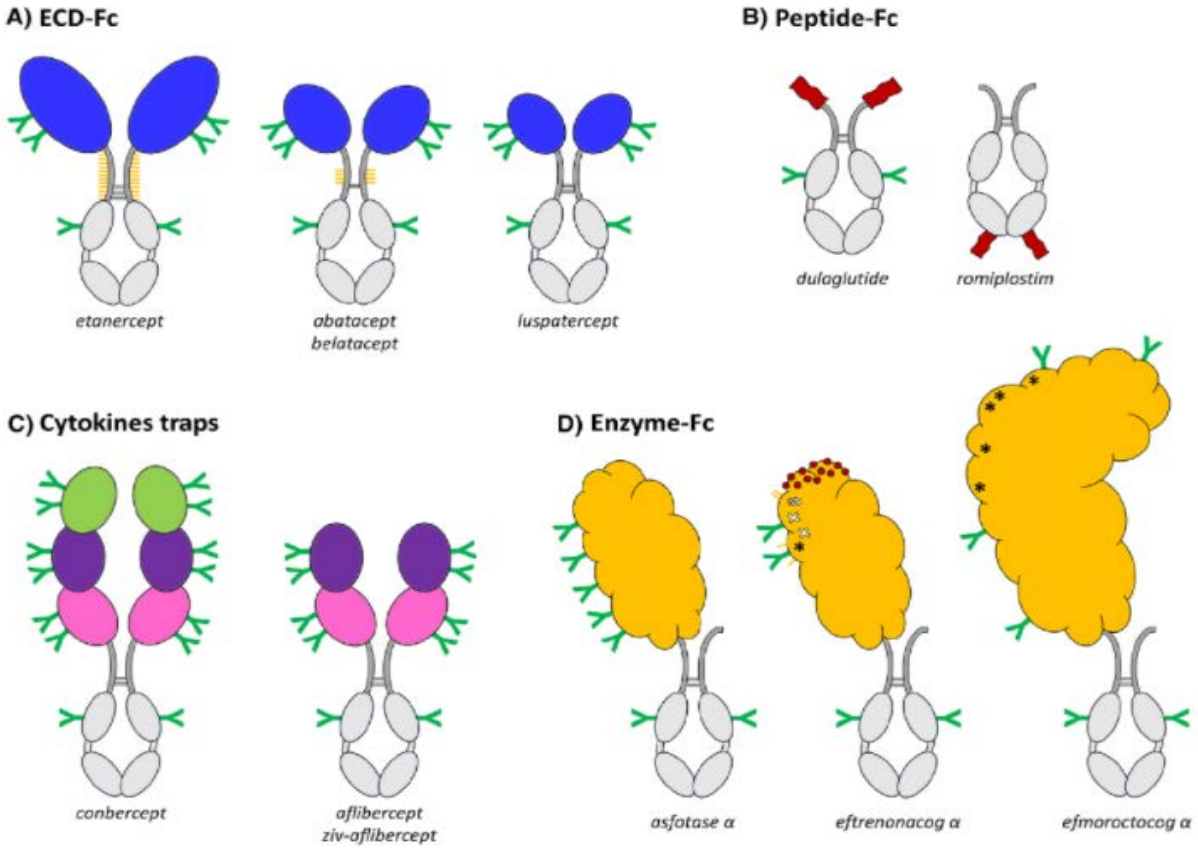
- Fc-fusion protein therapeutics are one of the most successful classes of IgG-based products;

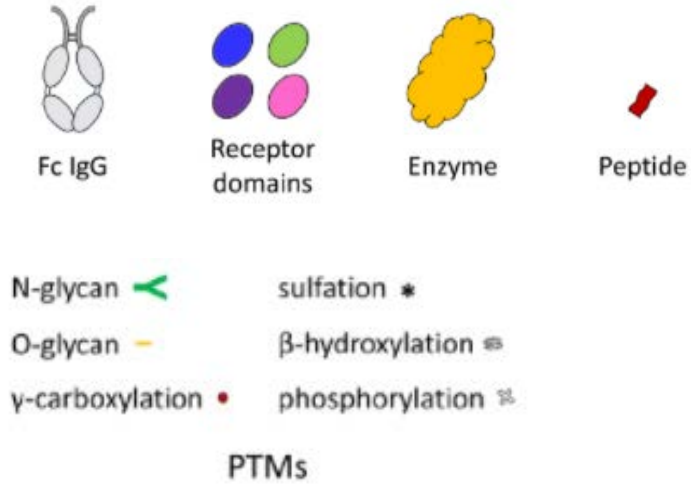
Product	Sales in 2019 (billion, USD)
Eylea	7.5
Enbrel	7.2
Trulicity	4.3
Orencia	3.2
Elocta	1.2

- Fc-fusion protein combine the pharmacological properties of biological ligands with the additional stability and inherent properties of IgG Fc domain;
- Fc-fusion protein can significantly improve the clinical potential of active protein drugs such as extend the plasma half life as well as engage immune-mediated effector functions;
- To date, ~**37** therapeutic fusion proteins are in clinical development and **13** products have been approved by the FDA, CFDA, and EMA;
  - Enbrel (TNFR-Fc fusion, FDA approval in 1998) and Orencia (CTLA4-Fc fusion, FDA approval in 2005) for treatment of rheumatoid arthritis;

Duivelshof BL, et al. J Sep Sci. 2021. Jan; 44(1):35-62.

Major Groups of Fc-fusion Proteins





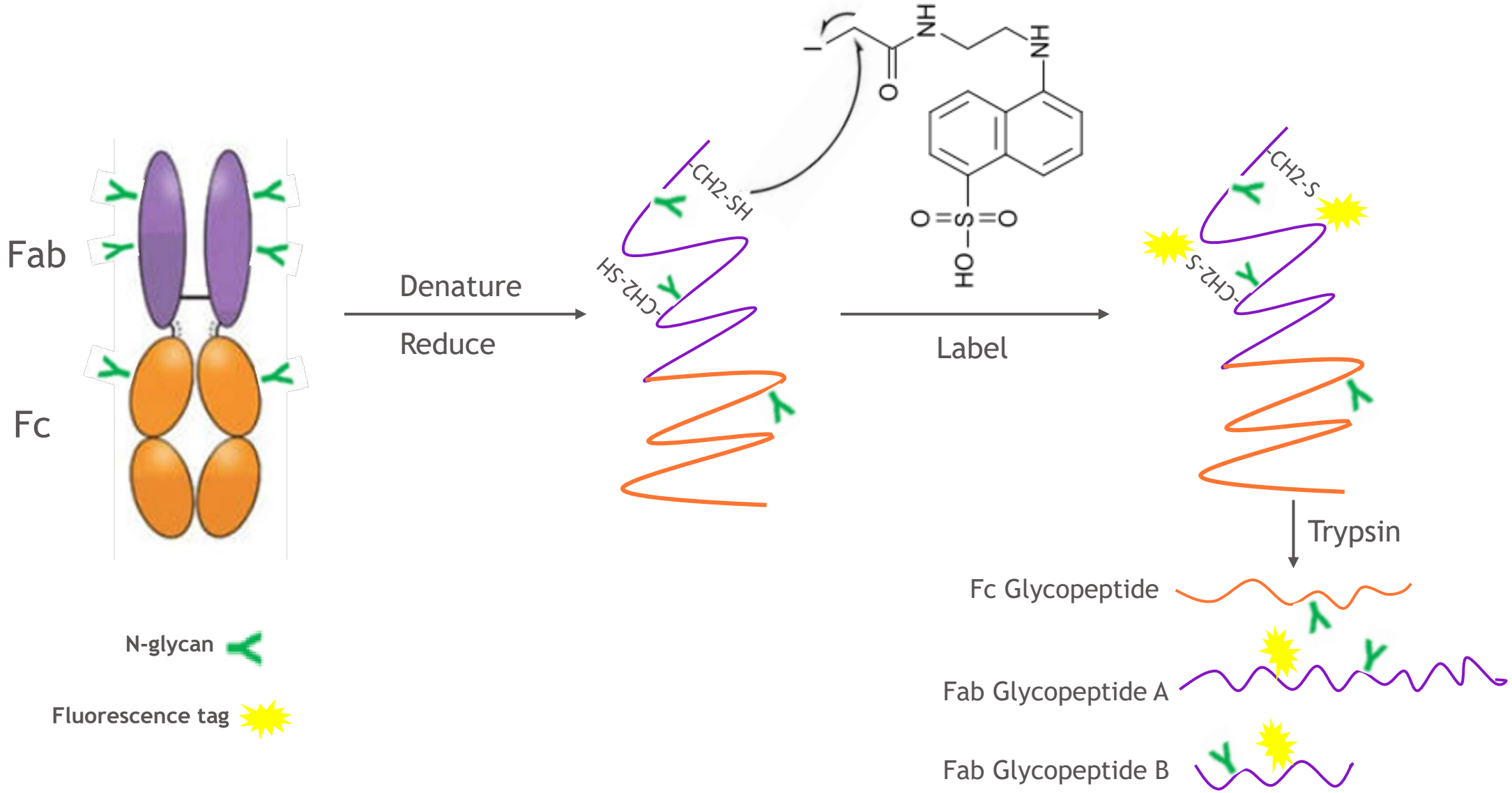
Therapeutic Fc-fusion proteins: Current analytical strategies
Duivelshof BL, et al. J Sep Sci. 2021. Jan; 44(1):35-62.

- Due to highly **heterogenous** structure (the presence of sialic acid, complex glycan structure, etc.), the analysis of Fc-fusion proteins is more challenging and complex than monoclonal antibodies;
- **Product-specific** methods over conventional generic or platform methods are often desirable to support process development;

Fab Site-Specific Glycosylation Monitoring

- A Fc-fusion protein with **four** *N*-glycosylation sites in Fab region and **two** *N*-glycosylation sites in the Fc region;
- A LC method was developed to monitor **Fab site-specific glycoforms** (G0F, G1F, G2F, G2FS1, and G2FS2) as the understanding about site-specific glycosylation as pCQA is continuously evolving during product development lifecycle;
- Mass spectrometry is coupled with LC for the peak identification of the complex chromatogram and the optimization of LC method parameters;
  - Mobile phase screening
  - Fluorescence tag screening
  - LC gradient optimization

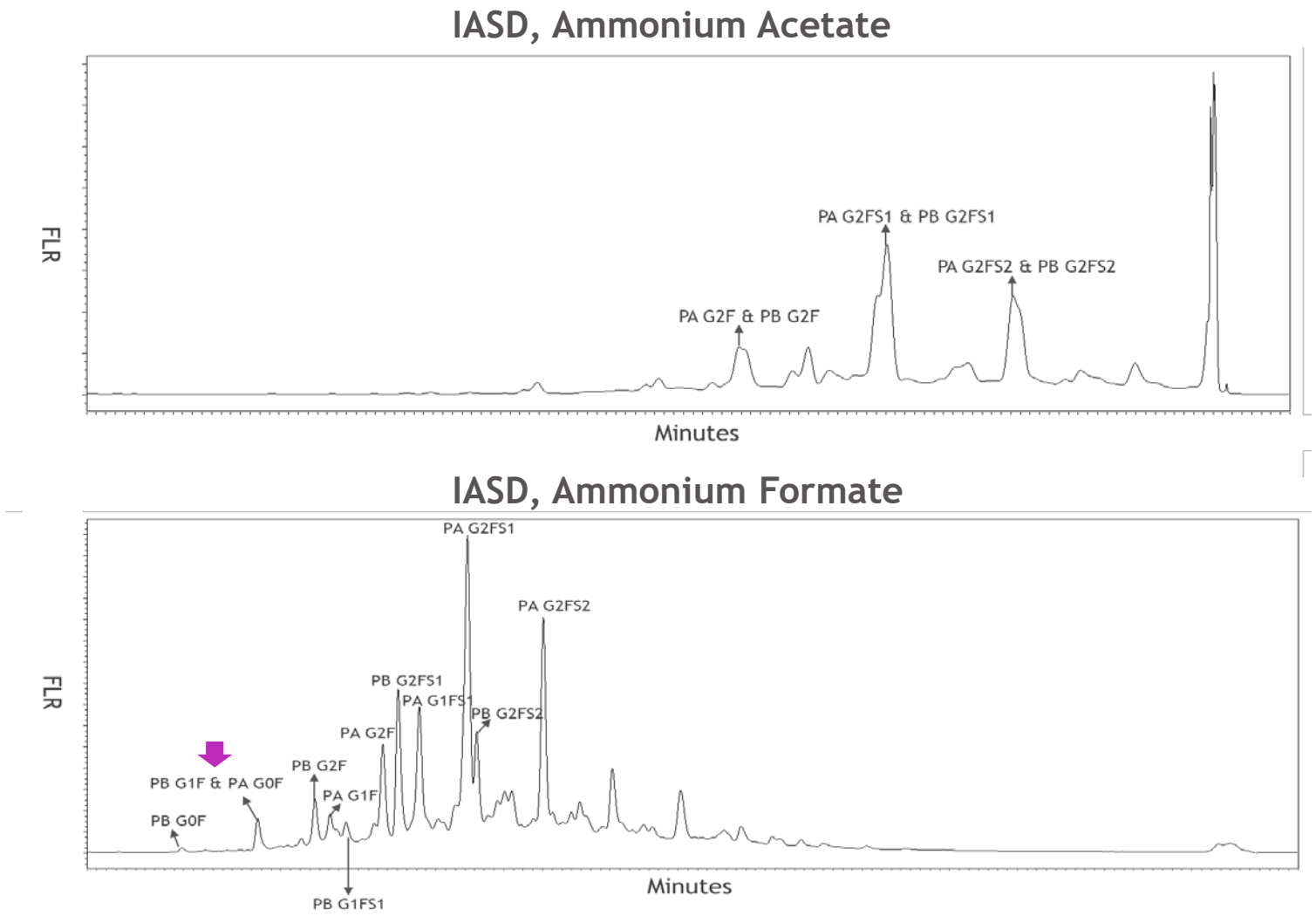
Glycopeptide Chemical Labeling



Workflow Overview

Label	• Denature • Reduce • **Label Cys with fluorescence tag (Alkylation)**
Desalt	• Sample desalt with SEC spin column
Proteolysis	• Trypsin digestion
LC Separation	• **HILIC for glycopeptide separation**
MS Identification & FLR Quantitation	• **MS for peak identification** • **FLR for Fab glycopeptide relative quantitation**

Ammonium Acetate *vs* Ammonium Formate as Mobile Phase

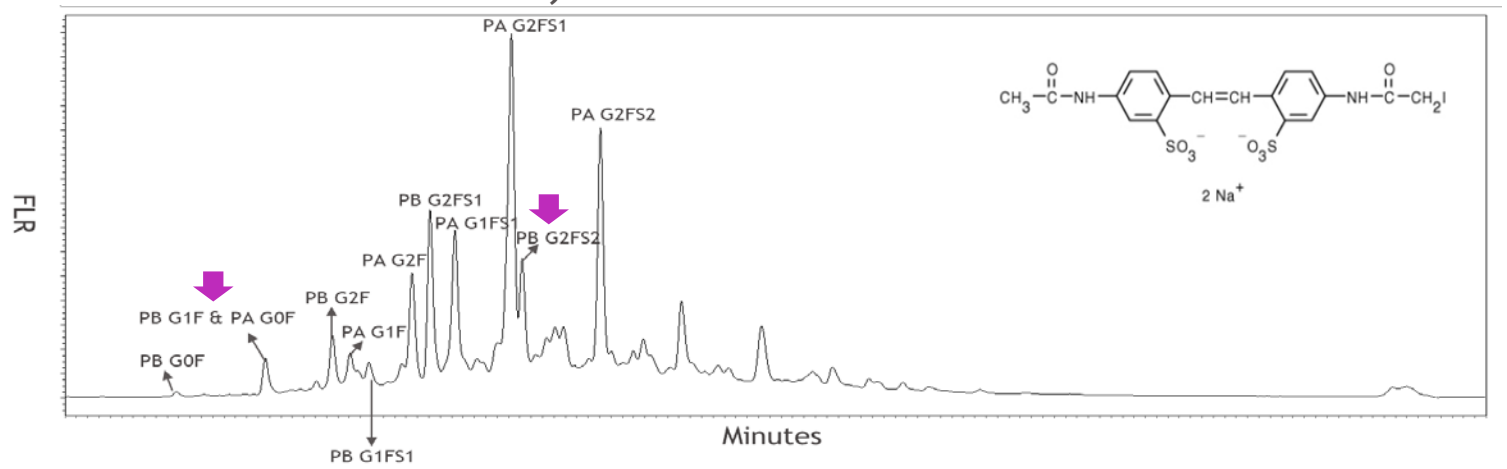


- ❑ Glycopeptide A and B coelution
- ❑ Only Glycopeptide A + G0F and Glycopeptide B + G1F coelutes

- Significant improvement in separation resolution with **ammonium formate** for IASD FLR tag;

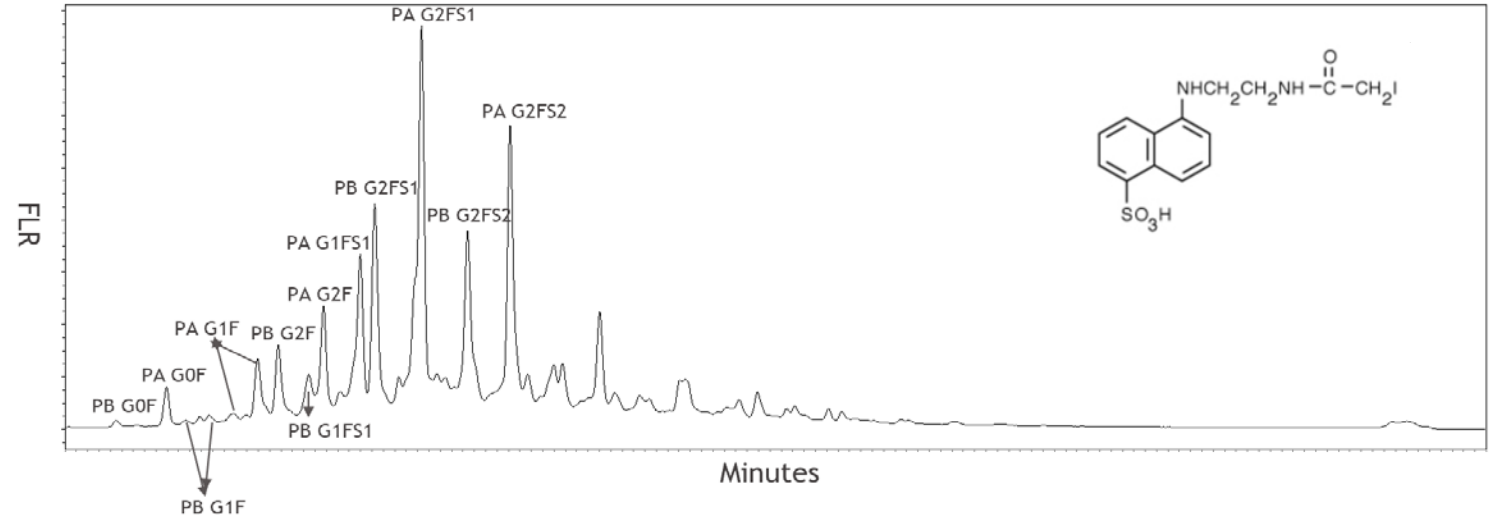
IASD *vs* IAEDANS as Fluorescence Tag

IASD, Ammonium Formate



- Only Glycopeptide A + G0F and Glycopeptide B + G1F coelutes

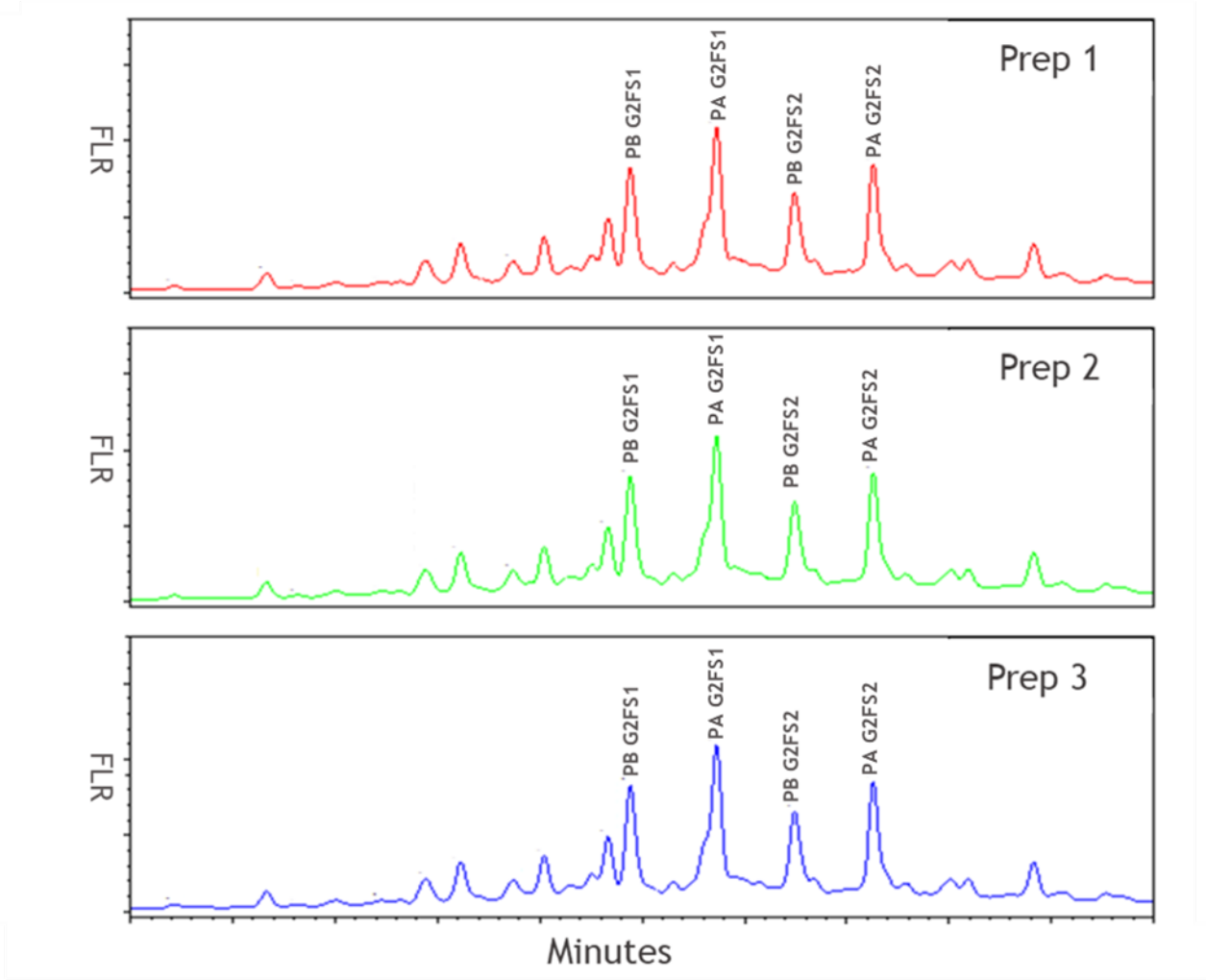
IAEDANS, Ammonium Formate

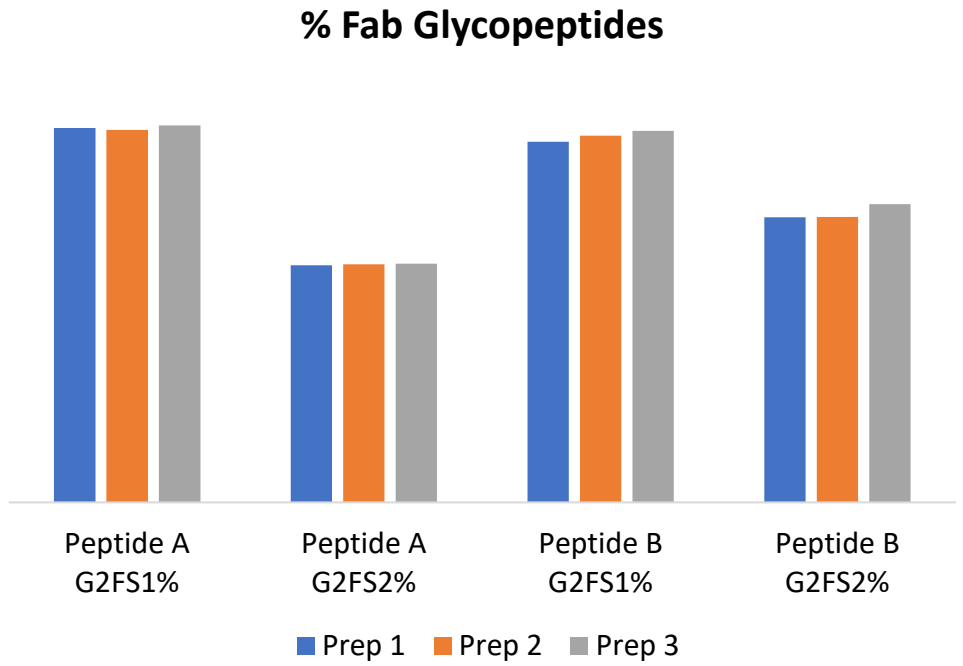


- Glycopeptide A + G0F and Glycopeptide B + G1F were separated
- Better resolution between Glycopeptide A+ G2FS1 and Glycopeptide B+ G2FS2

- Different glycoforms of Fab glycopeptide A and B are fully resolved with **IAEDANS** FLR tag;

Quantitation of Fab Site-Specific Glycopeptides through Fluorescence Peak Intensity





- **Reproducible** quantitation for glycopeptides;

Summary

- A HILIC LC-FLR-MS method was established to separate glycopeptides and quantify Fab site-specific glycosylation for a Fc-fusion protein;
- Molecule specific methods are often required to support the development of complex protein therapeutics;
- Creativity and collaboration cross functional areas is essential to develop innovative methodology for problem solving;

Acknowledgement

Partnership among Process Development Analytics, Upstream, and Downstream

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Thank You for Your Attention!


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