The Effect of Glycosylation on Peak Resolution of Antibodies on SCIEX BioPhase 8800 CE-SDS System

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CEED | API Proteins
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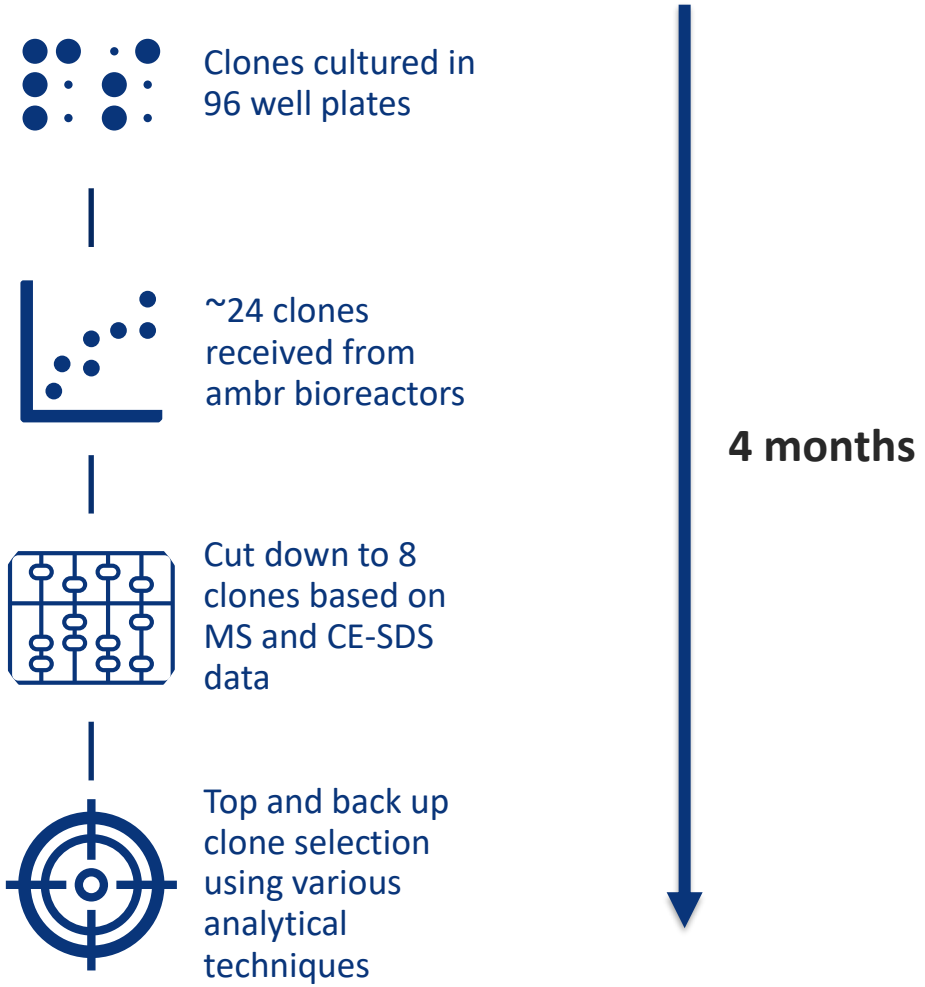
Outline

- Janssen Pharmaceuticals: Who we are
- Closing the reliability and robustness gap of CE-SDS
- Case Study
  - Labchip GXII reduced and non-reduced run
  - PA 800 reduced and non-reduced run
  - BioPhase 8800 reduced and non-reduced run
  - Degly sample runs
- Conclusions
- Acknowledgments

Who we are

- **Janssen Pharmaceuticals**
  - Cell Engineering and Early Development group in Drug Product and Development Sciences (DPDS)
- Our goal is to use orthogonal methods to develop cell lines with high titer and high product quality
  - Large molecule drug products
  - Evaluate reduced and non-reduced purity to identify low and high molecular weight product-related impurities and potential HCPs

Characterization of monoclonal material for product quality leads to clone selection for clinical drug development



Role of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) in Cell Line Selection

- Characterization of product quality in order to
  - Determine developability for clinical manufacturing
  - Determine product quality for multiple clones
  - Pick the best clone and back up clone for cell banking
- Characterization of Low molecular and high molecular weight species to determine yield and purity for downstream manufacturing
- Evaluation of new drug modalities on product quality
- **This Data is critical to the timely selection and development of new drug products for Janssen**

Closing the reliability and robustness gap of CE-SDS

- BioPhase 8800 system parallel processing of 8 samples
- Inter- and intra-capillary robustness and reproducibility facilitates sample comparison
- Improved timeline for quicker course correction in cell development process
- **Improved resolution of normally coeluting heavy chain peaks**

Research plan-Bispecific Antibody

- Comparison of Labchip GXII (Perkin Elmer), PA800 (SCIEX) and BioPhase 8800 (SCIEX)
  - Reduced and Non-reduced samples
- Comparison of peak resolution after treatment with PNGase F

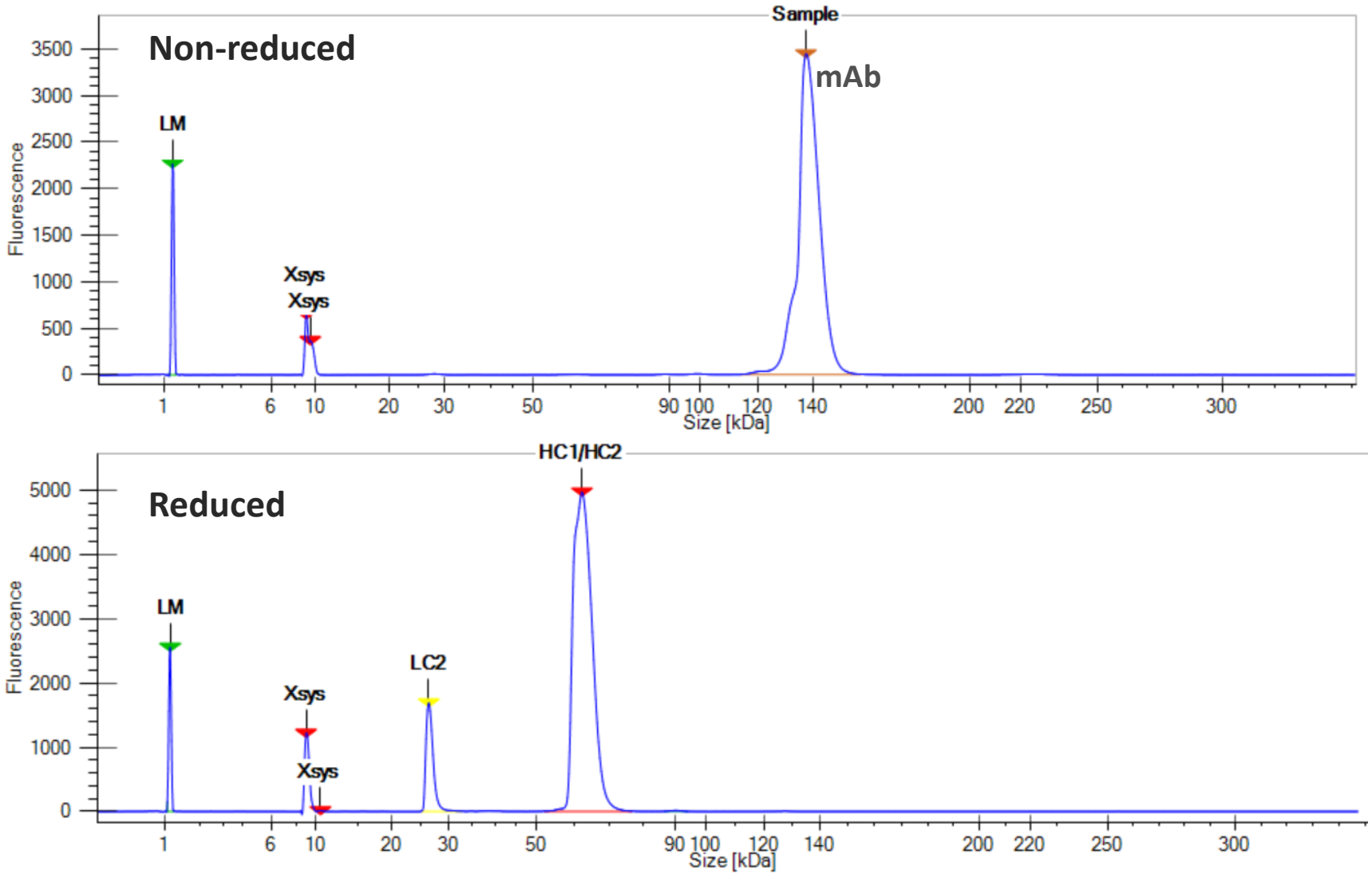
How do the CE-SDS systems compare

	Labchip GXII	PA800	BioPhase 8800
Sample Prep Time	6 hours	2-3 hours	2-3 hours
Sample Run Time	40 sec	50-60 mins	50-60 mins (34-44mins) X 8
Detection system	FLR	UV or LIF	UV and LIF
Automation	Compatible	limited	Compatible
Sample volume	5µl	24 µL/136 µL	45 µL/57 µL
Sensitivity	0.2 mg/ml	0.2 mg/ml	0.2 mg/ml

Case Study: Effect of CE-SDS systems on Peak resolution

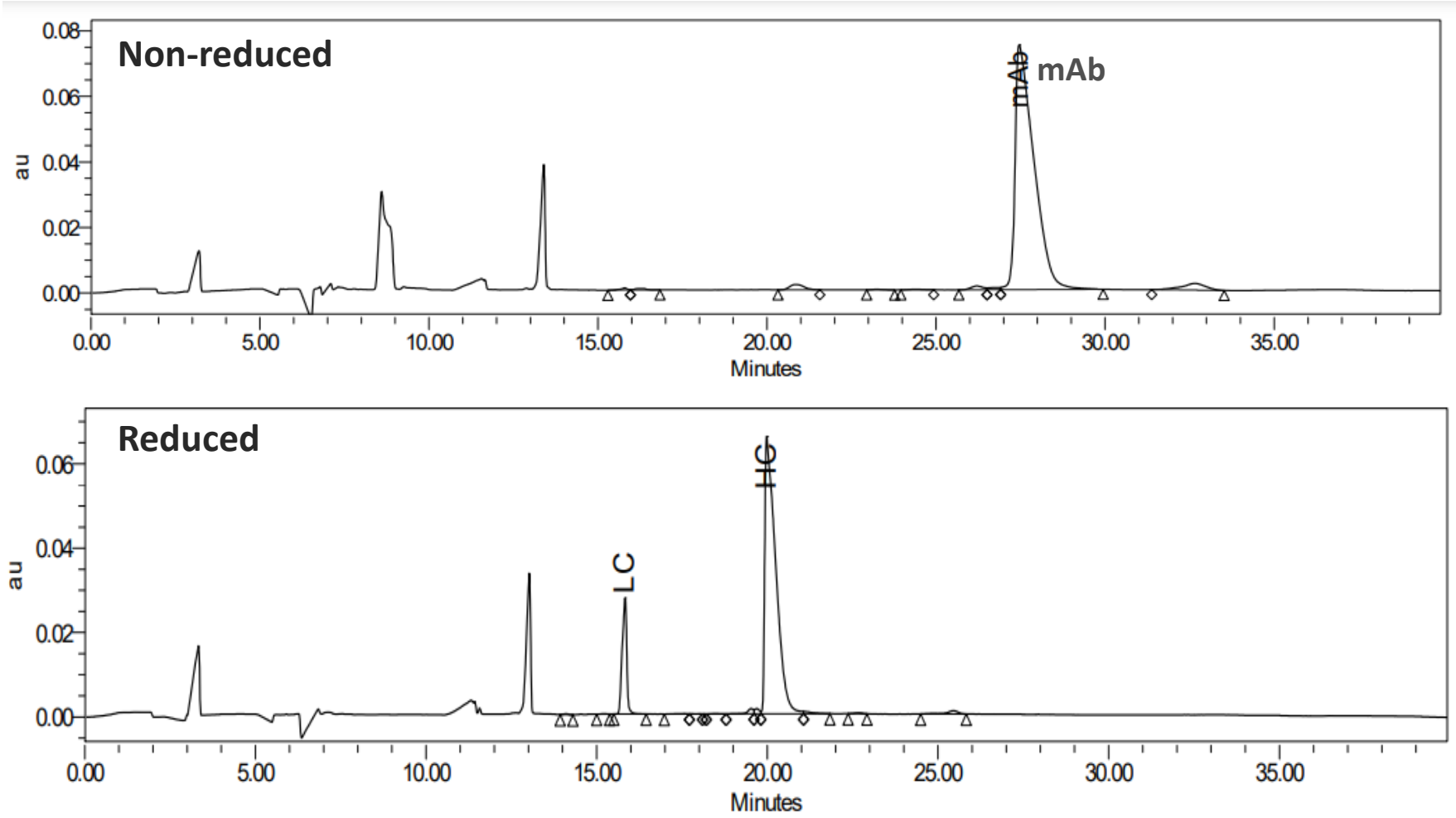
Evaluation of Peak Splitting on Bispecific antibodies

Sample 1 on Labchip GXII



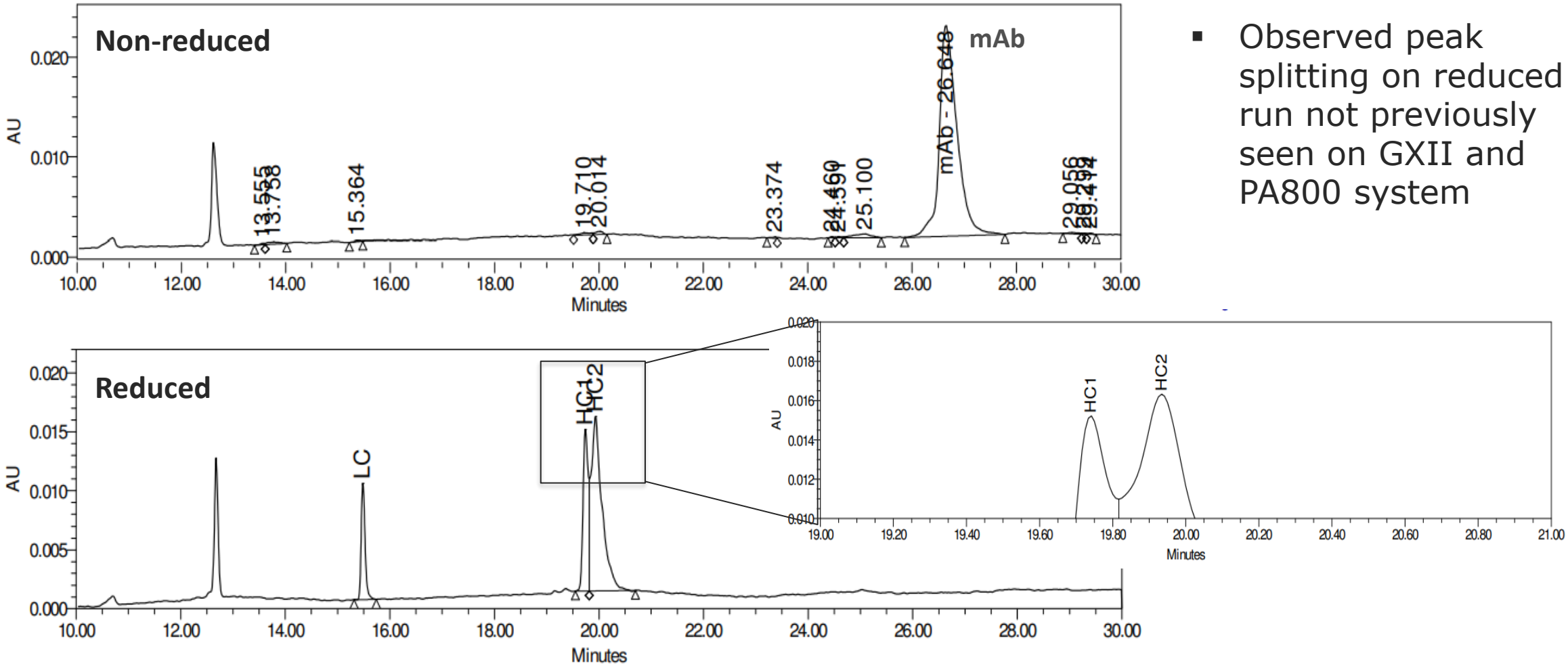
- Expected Peak of Non-reduced antibody at ~140kDa
- Coelution of HC1 and HC2 peaks

Sample 1 on PA800



- Observance of characteristic single peak on non-reduced sample and LC and HC peaks on reduced sample

Sample 1 on BioPhase 8800

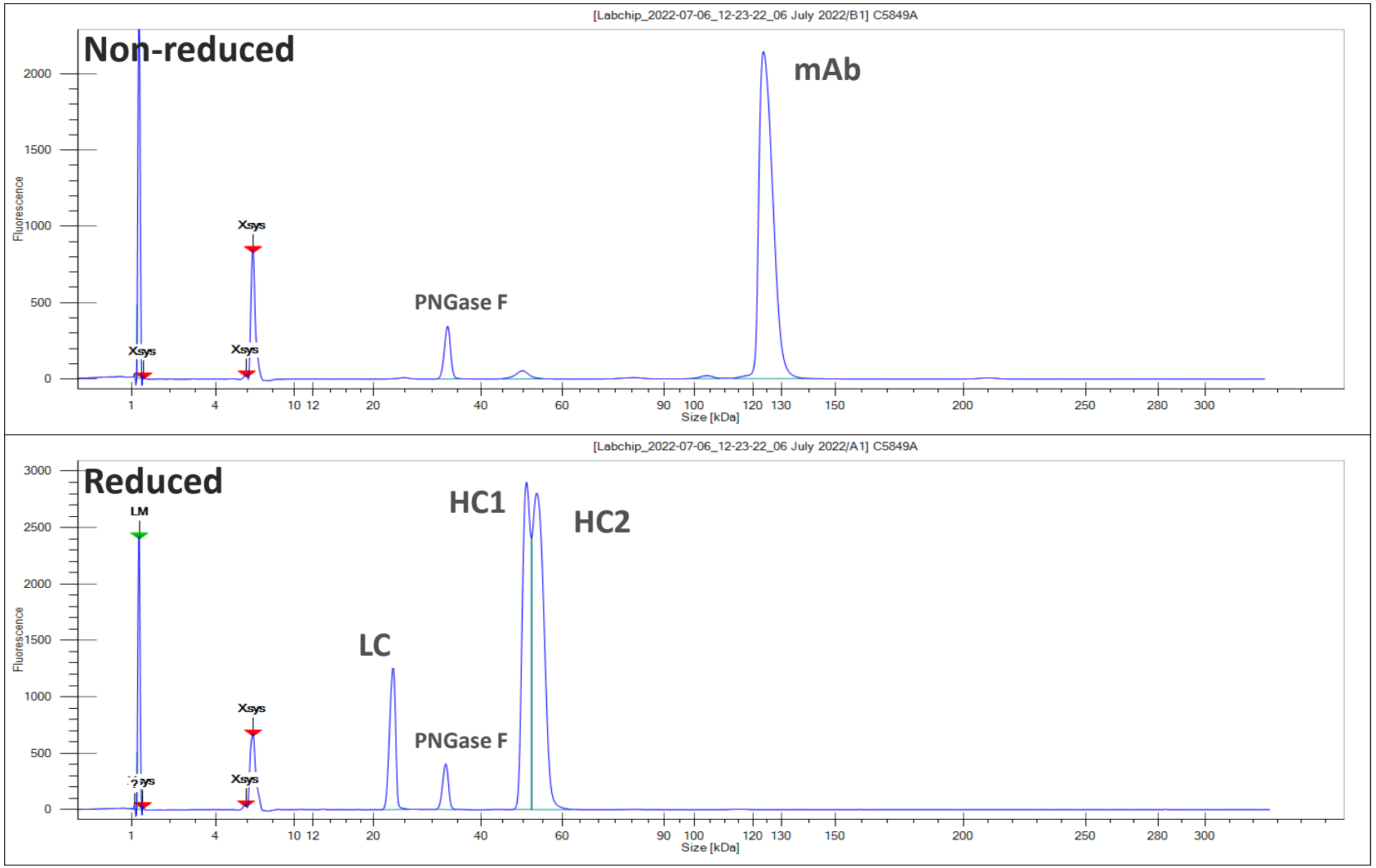


- Observed peak splitting on reduced run not previously seen on GXII and PA800 system

Evaluating impact on resolution due to glycosylation

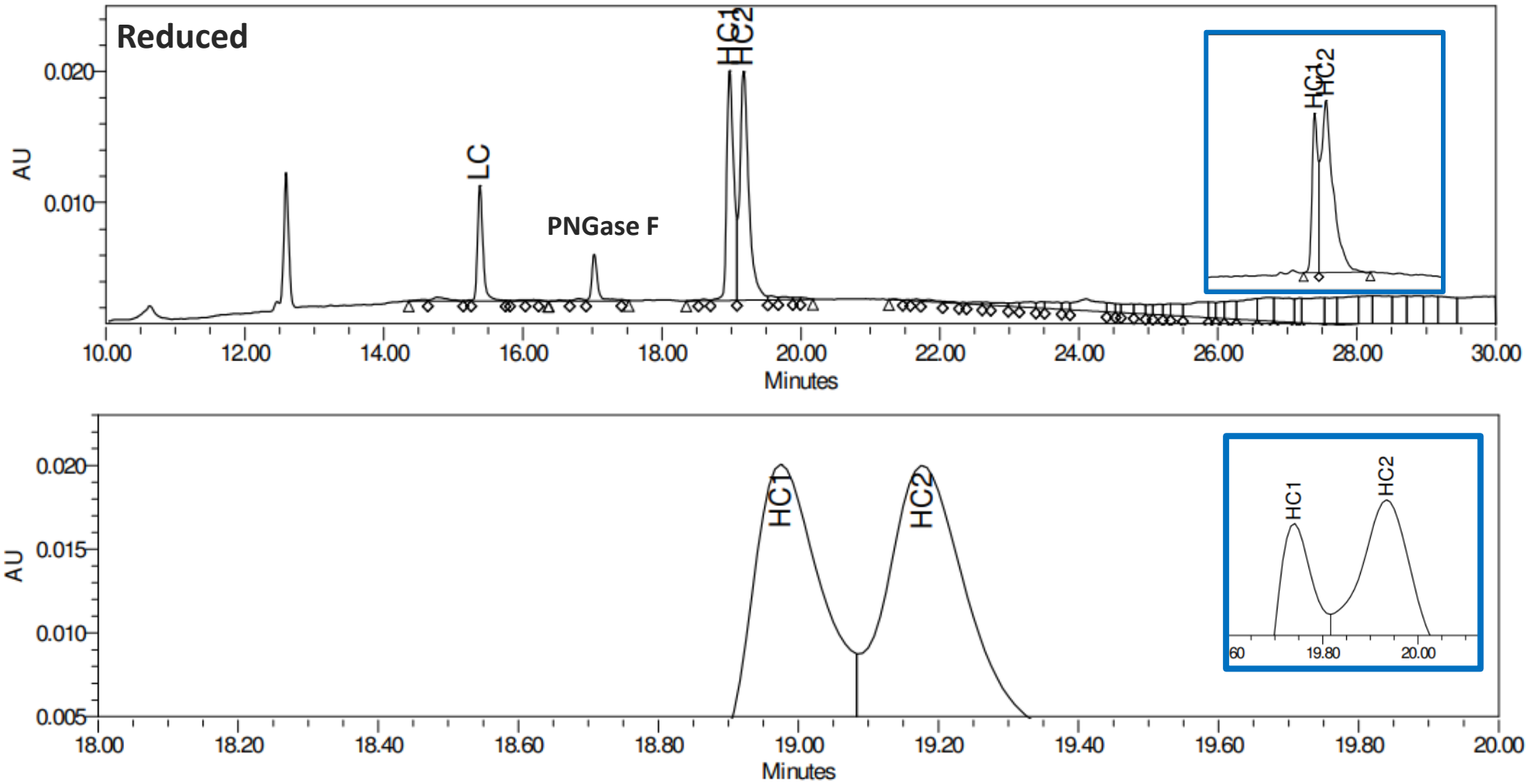
- Samples treatment with PNGase F
  - PNGase F- cleaves N-linked High mannose oligosaccharides (glycans) off asparagine residues on glycoproteins
  - MW of 35.5kDa
  - Evaluate the effect of Deglycosylation on CE-SDS separation
- Re-evaluated on BioPhase and GXII systems

Sample 1 on GXII with PNGase F treatment



- Observed peak splitting on reduced sample only after treatment with PNGase F

Sample 1 on BioPhase 8800 with PNGase F treatment



- Observed better peak resolution and an expected shift to lower kDa for heavy chains after treatment with PNGase F

Conclusions

- The BioPhase allows for better peak resolution than other CE-SDS
  - BioPhase 8800 showed an increased ability to resolve the HC1 and HC2 peaks of a bispecific antibody without any additional treatment
  - We observed that BioPhase can separate glycoproteins that are only 1200Da difference (HC1, HC2
  - We observed an increased resolution after treatment with PNGase F induced deglycosylation
  - The increased resolution assists with better identification of known and unknown species
- We are collaborating with SCIEX to better understand the new detection system and the ability to resolve the heavy chain peaks

Pushing the envelope of CE-SDS - traditional vs lightning methods