

Comprehensive Characterization of Disulfide Linkages in a Cysteine-Engineered Bispecific Antibody Using EThcD-on a modified Orbitrap Hybrid Mass Spectrometry

Abby J. Chiang^{1,2}, Elsa Gorre², Reiko Kiyonami³, Alexander N. Barnakov², Min Du³, Cong Wang⁴, Peter Krueger⁴, Andy Mahan², Hirsh Nanda²

(1) Protein Metrics Inc., Boston, MA (2) Johnson & Johnson Innovative Medicine, Spring House, PA (3) Thermo Fisher Scientific, Lexington, MA (4) Thermo Fisher Scientific, Bremen, Germany

Introduction

- Multispecific antibodies are the next generation of biotherapeutics. Their single-chain fragment variable (scFv) domains composed of variable light chain (VL) and heavy chain (VH) are critical and known to have a lower stability and a tendency to aggregate.
- We (mAbs. 2023) revealed a stapling strategy by introducing two engineered disulfide bonds between VL and VH domains. The stapled scFv (called spFv) technology for bispecific achieves higher thermal stability and minimal aggregation.
- Here, we demonstrate a workflow to improve the challenge of a comprehensive disulfide mapping of stapled bispecific antibodies.

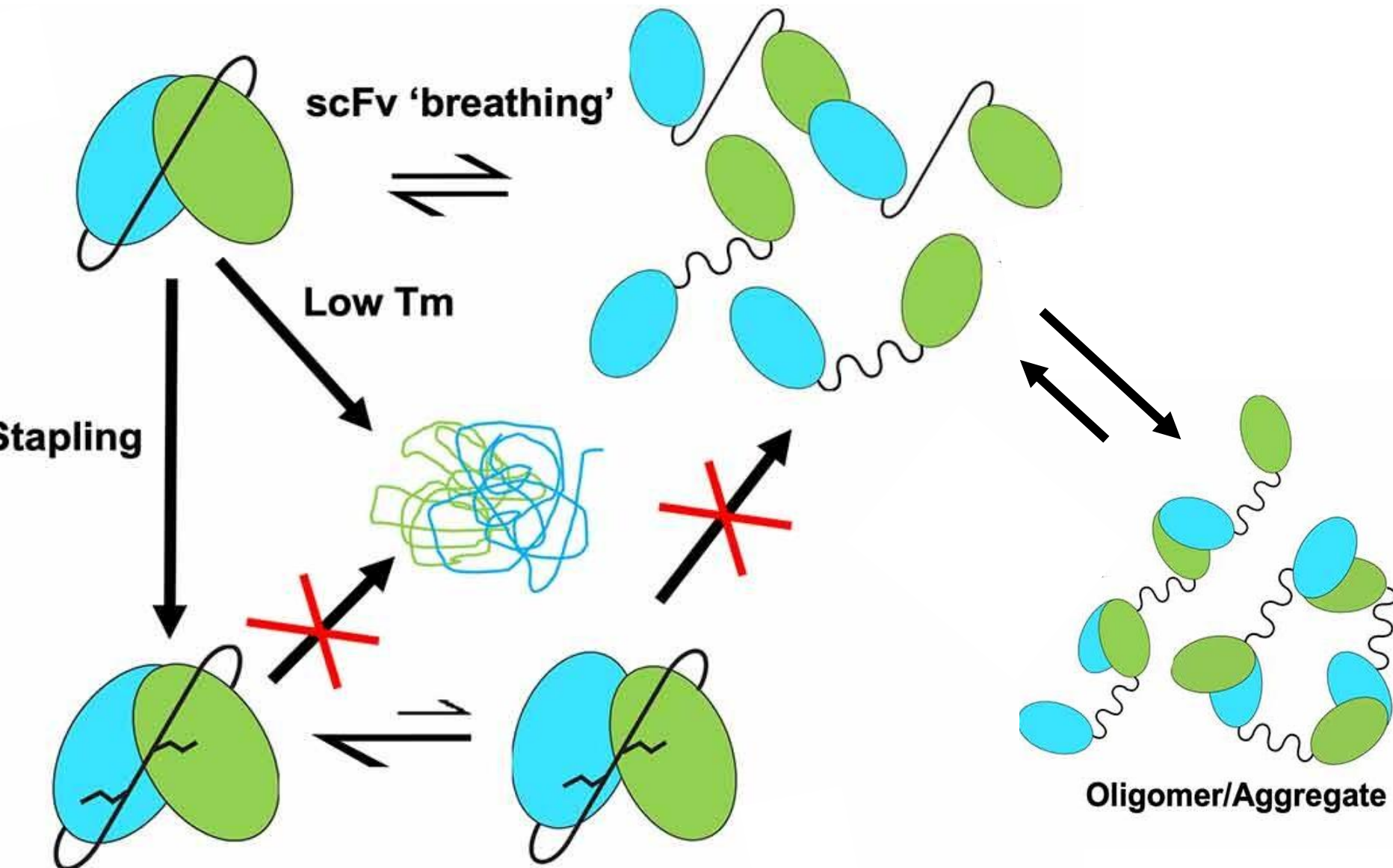
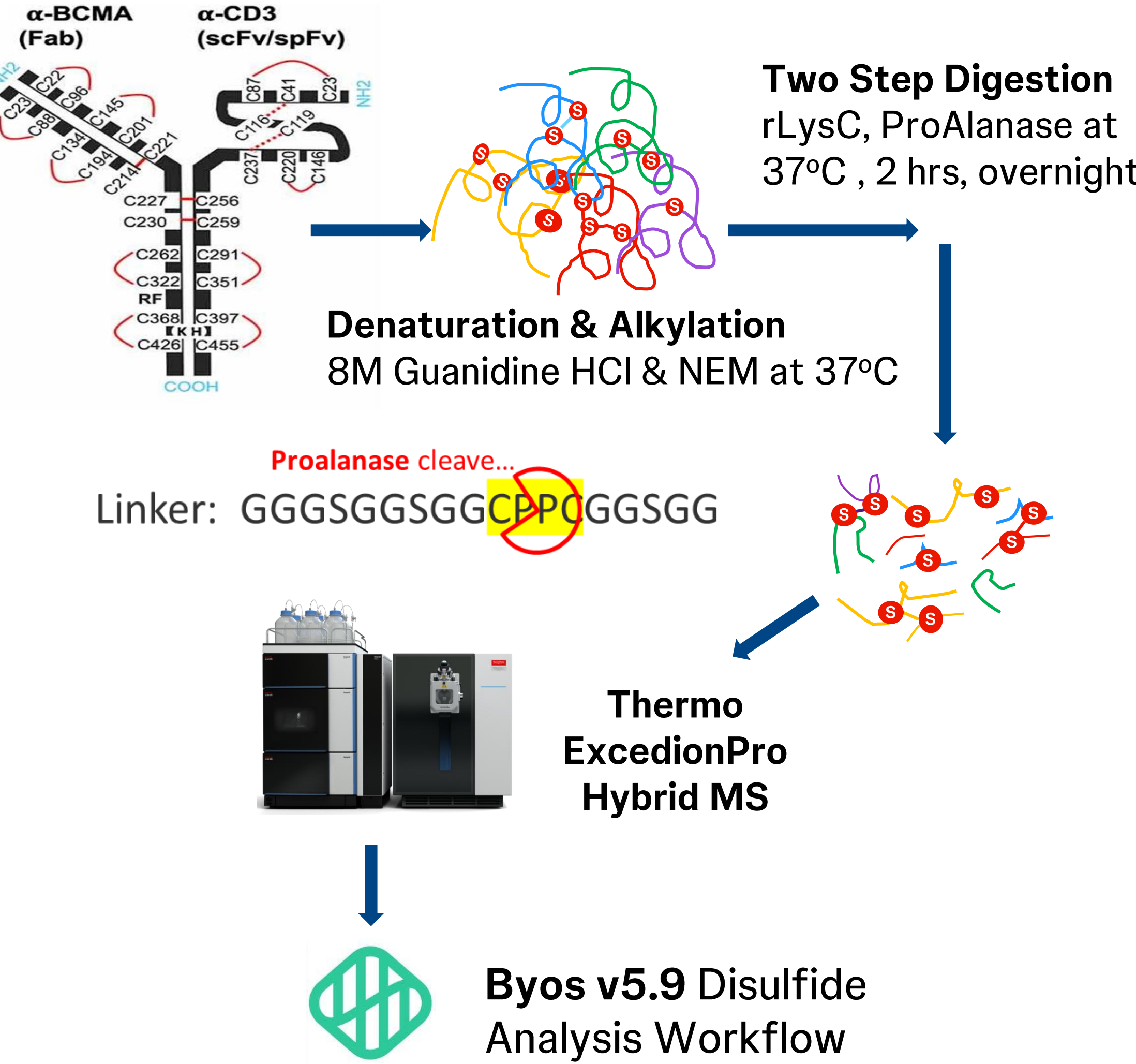


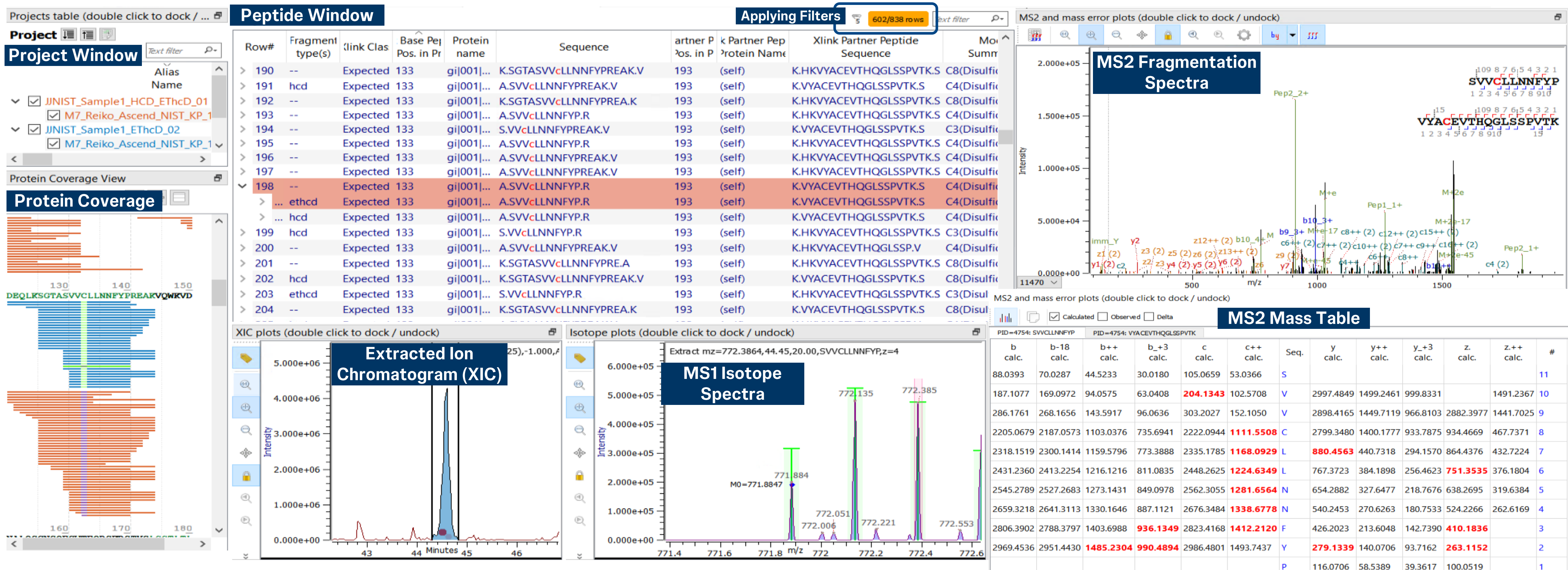
Figure 1. Stapling scFv improves stability and minimizes breathing-mediated aggregation in scFv (mAbs. 2023)

Methods

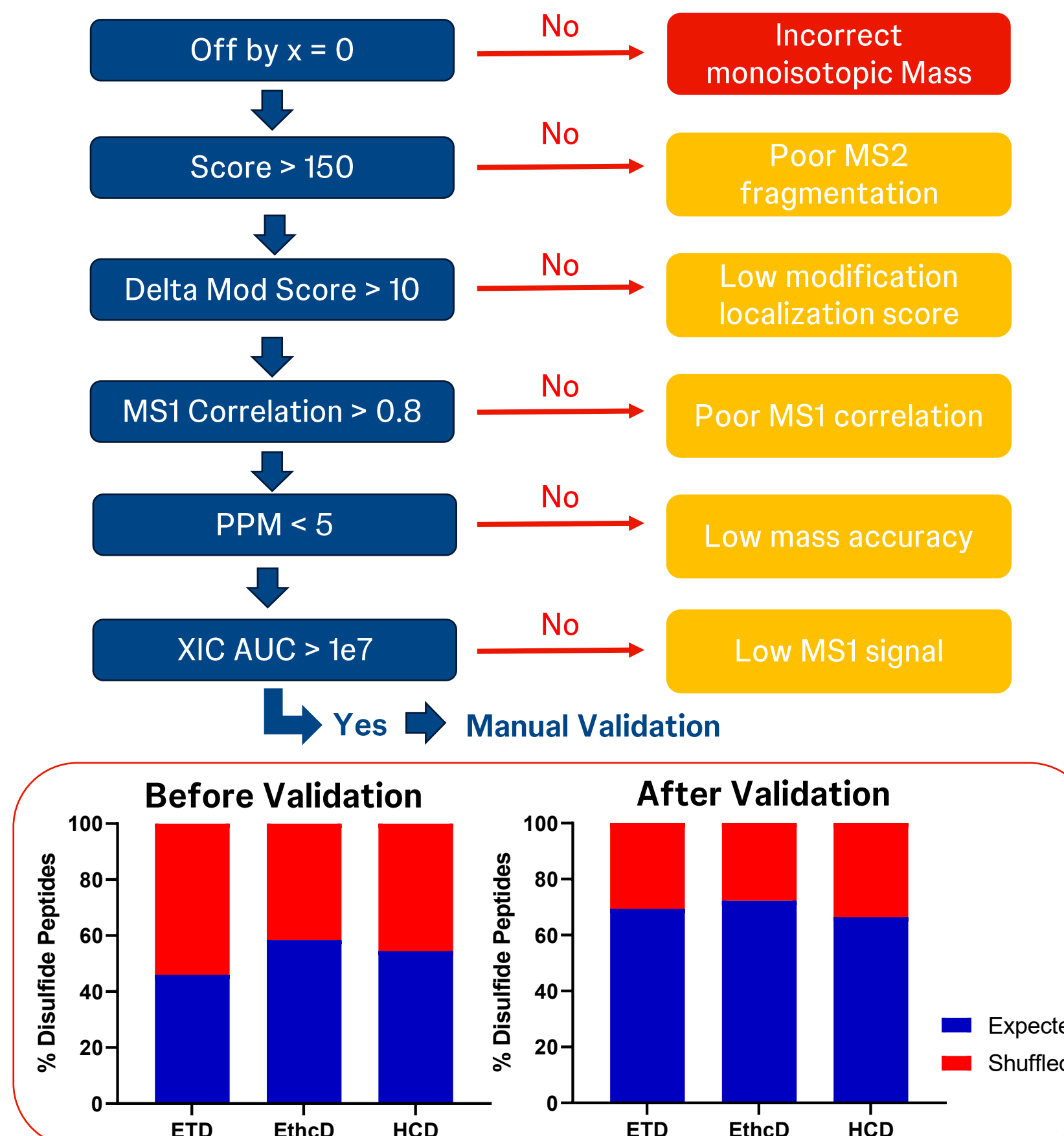


Results & Discussion

Byos Results and Inspection



Disulfide Validation



Digestion Specificity

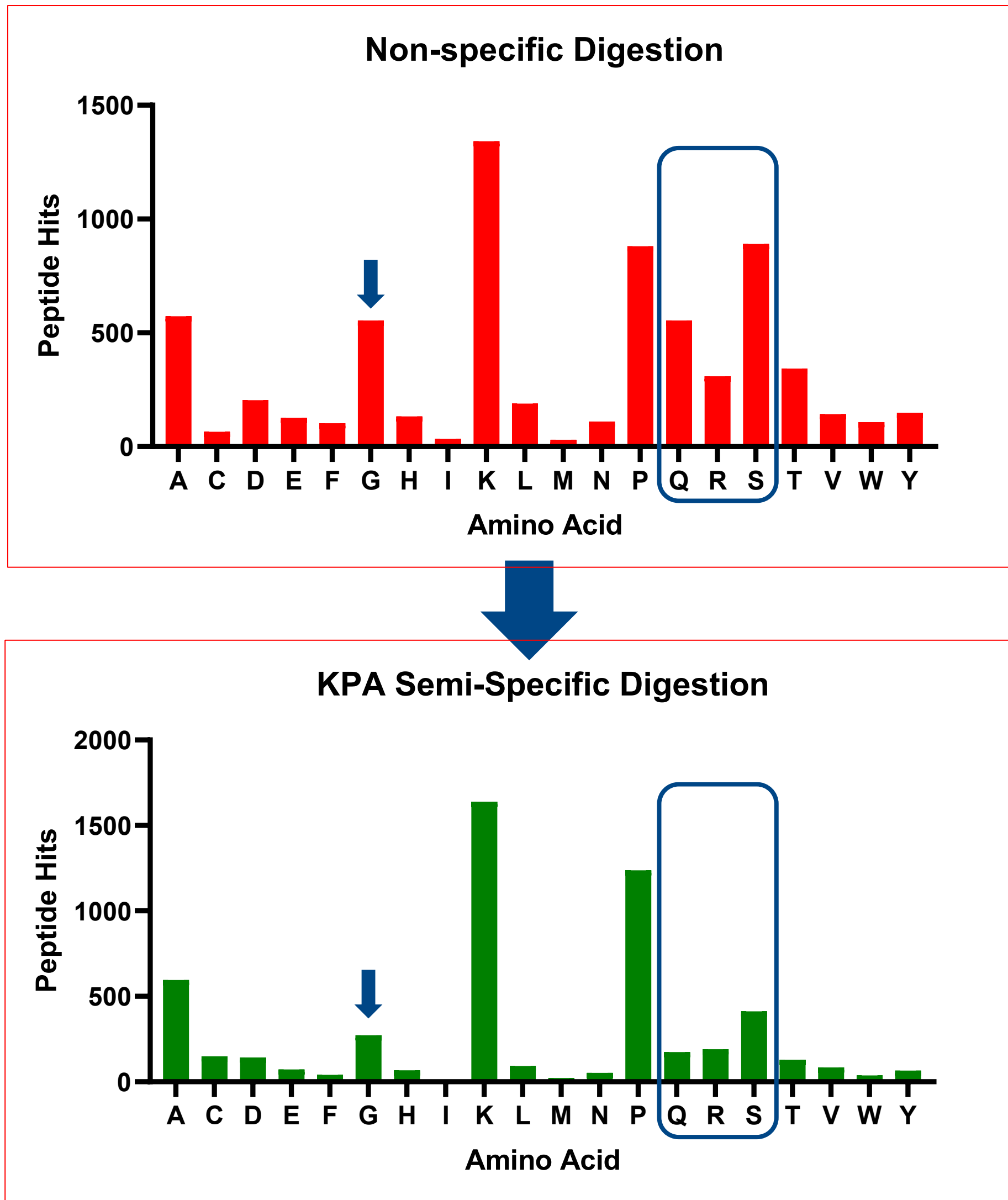
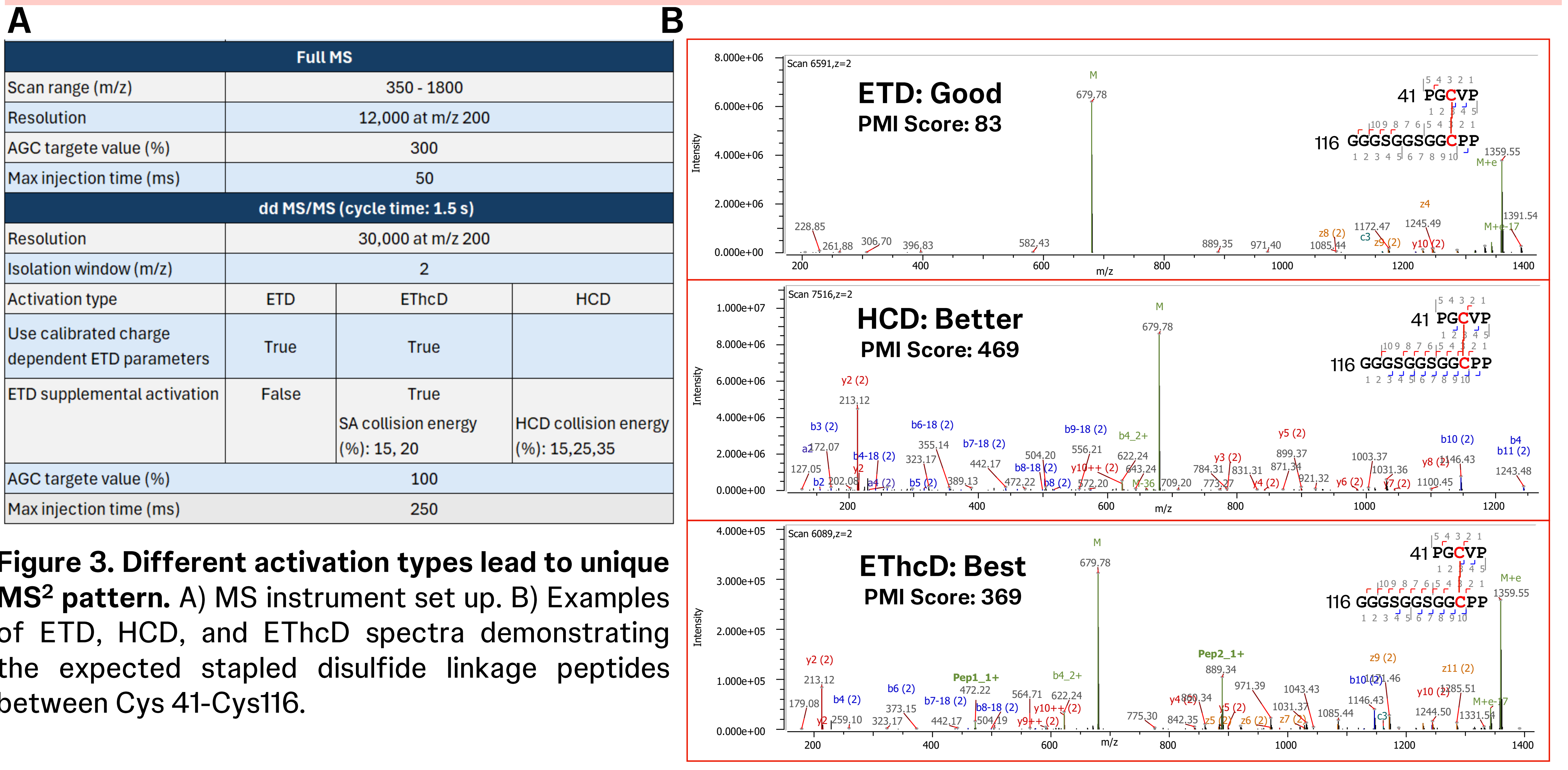


Figure 2. Digestion specificity based on cleavage site. The data were first analyzed using a “non-specific digestion” approach, which identified multiple cleavage sites by ProAla, demonstrating its non-specificity. Subsequent analysis with a semi-specific KPA digestion showed improved enzyme specificity results.

MS Acquisition/ Stapled scFv (spFv) Detection



Conclusions

- A robust Disulfide Mapping Assay was needed to confirm proper disulfide linkages and assess any scrambling occurring. To achieve this, optimization was done at multiple stages such as:
- Sample prep: introduced 2-enzyme digestion (LysC+ProAla)
 - Instrumentation: evaluated the new Orbitrap ExcedionPro BioPharma Hybrid MS
 - Data analysis: using Protein Metrics disulfide workflow improved the identification of the expected stapled disulfides in the stapled bispecifics

Boucher, L. E., Prinslow, E. G., Feldkamp, M., Yi, F., Nanjunda, R., Wu, S. J., ... Luo, J. (2023). “Stapling” scFv for multispecific biotherapeutics of superior properties. *mAbs*, 15(1).

The authors declare no competing financial interest.