Peptide Mapping of Purified Proteins
By Liquid Chromatography—Tandem Mass Spectrometry

Introduction

Peptide mapping, an identity test for proteins, is an essential analytical method for elucidating the primary amino acid structure of proteins. For recombinant protein pharmaceuticals, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), peptide mapping is used for proof of identity, primary structural characterization, and quality assurance (QA). Regulatory agencies issued ICH Q6B guidance which specifies the use of peptide mapping as a critical quality test procedure for drug characterization used to confirm desired product structure for lot release purposes. Peptide mapping involves enzymatic treatment of a protein, resulting in the formation of peptide fragments, followed by separation and identification of the resultant fragments by LC-MS/MS in a reproducible manner. It is capable of identifying single amino acid changes resulting from events such as errors in the reading of complementary DNA (cDNA) sequences or point mutations. Peptide mapping is not a general method, but involves developing specific maps for each unique protein (USP 1055, Biotechnology Derived Articles - Peptide Mapping).

Poochon developed a standardized platform for peptide mapping of purified proteins using protease digestion and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). This service package can be used for the entire protein sequence confirmation, as well as determination of post-translational modifications (PTMs) and sequence variants.

Specifications

→ **Method** – Protease digestion and LC-MS/MS

→ **Key Instruments** – Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, Orbitrap Exploris™ 240 Mass Spectrometer & 480 Mass Spectrometer

→ **Specificity** – 100% sequence coverage; mean delta mass <2 ppm

→ **Acceptable Samples** – Purified protein, in solution or dried (≥99% purity, ≥20 µg/sample)

→ **Turnaround Time** – Typically, reports are available within 10 business days of sample receipt

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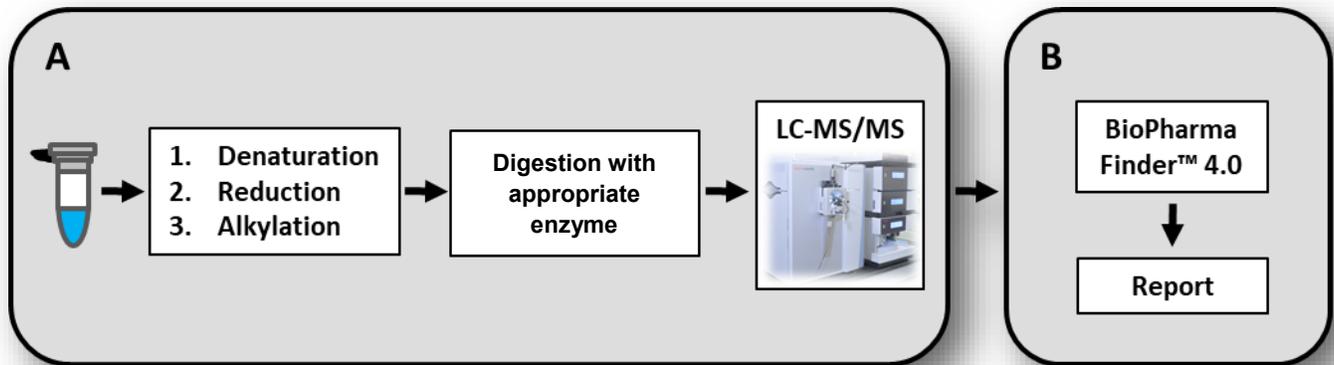
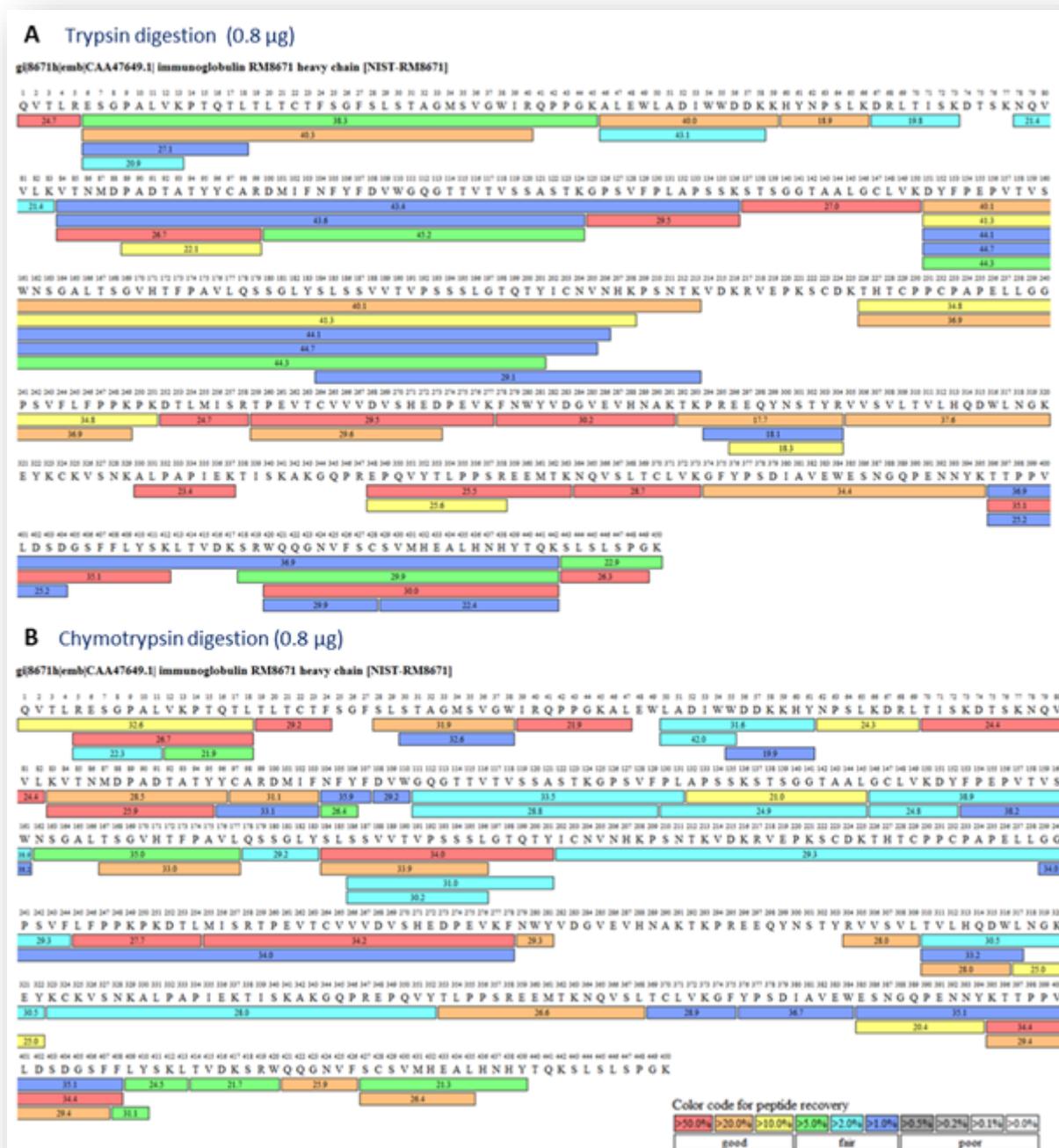## Schematic of Procedure Workflow

Figure 1:

A) Workflow for peptide mapping. Note: LC-MS/MS = liquid chromatography and tandem mass spectrometry.

B) Bioinformatic analysis approach used for analysis of MS raw data.

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Peptide Mapping of NIST mAb RM8671

Figure 2:

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Peptide Mapping of NIST mAb RM8671
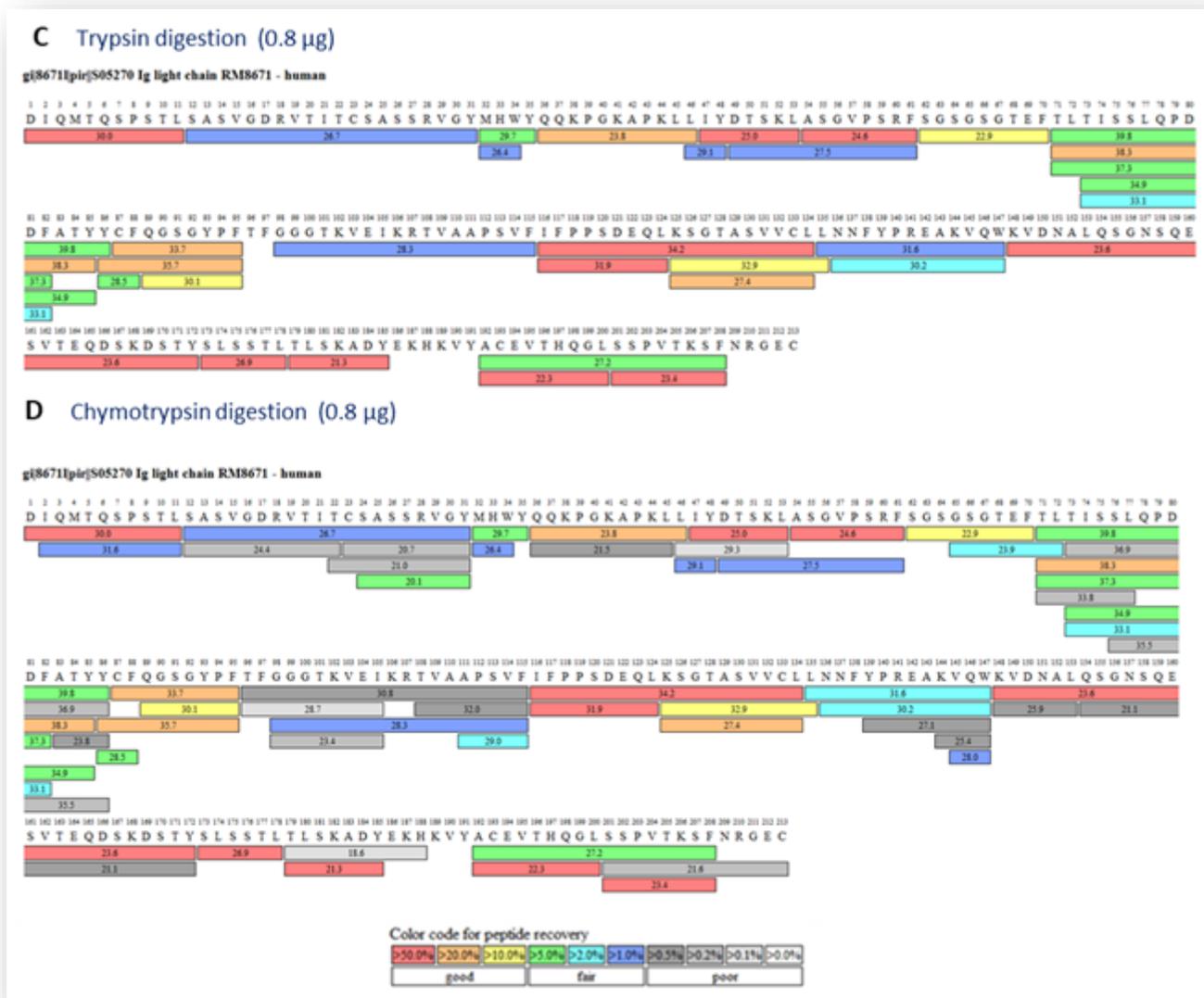
Figure 2: *(continued)*

Figure 2: NIST RM8671 peptide mapping results. Peptides from digestion of Heavy Chain by Trypsin/LysC **(A)** and by Chymotrypsin **(B)**; Peptides from digestion of Light Chain by Trypsin/LysC **(C)** and by Chymotrypsin **(D)**. Peptides were identified using BioPharma Finder™ 4.0 software, 100% sequence coverage.