

Rapid In-Depth Characterization of Biologics by Microchip CE-MS: mAbs, AAVs, and Nucleic Acids

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Introduction

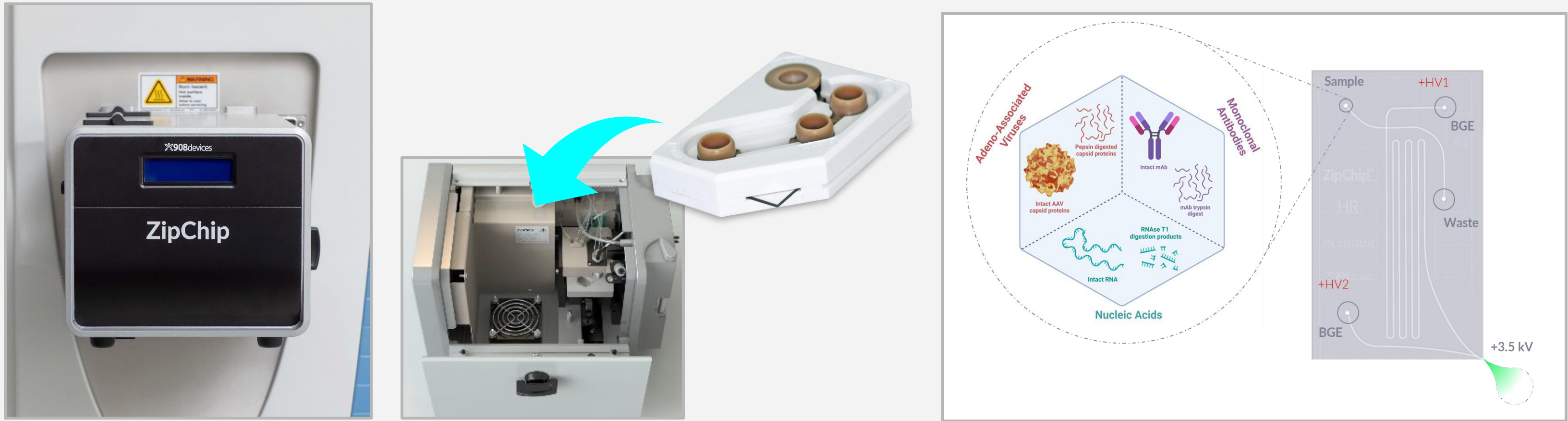
Microchip capillary electrophoresis (CE) coupled with mass spectrometry (MS) has revolutionized the characterization of therapeutic modalities in biopharmaceuticals. Here, we present the applications of an integrated workflow that couples the microfluidic CE system (PATsmart™ ZipChip® System) with MS in the characterization of biotherapeutics. The generic workflow is rapid, simple, and with minimum method development and sample prep. We will showcase 3 distinct application examples using the ZipChip System CE-MS workflow:

Multi-level characterization of monoclonal antibodies (mAbs) including charge heterogeneity, glycosylation profiles, and critical quality attributes. The combination of microchip CE and MS provides efficient analysis, enhancing understanding of mAb structure.

Characterization of adeno-associated viruses (AAVs) including intact denatured Viral Protein (VP) characterization as well as peptide mapping to further characterize PTMs of the VP. Examples of the serotype AAV6, AAV8, and AAV9 are shown in this poster

Analysis of nucleic acids covering FLP determination of Oligos as well as Oligo mapping of biological RNAs. Apart from benefits stated above, the workflow requires NO ion-pairing reagents, eliminating system contamination.

ZipChip System: A microfluidic CE-based front end for mass spectrometry



Characterization of Monoclonal Antibodies: Charge Variant Analysis¹ and Peptide Mapping²

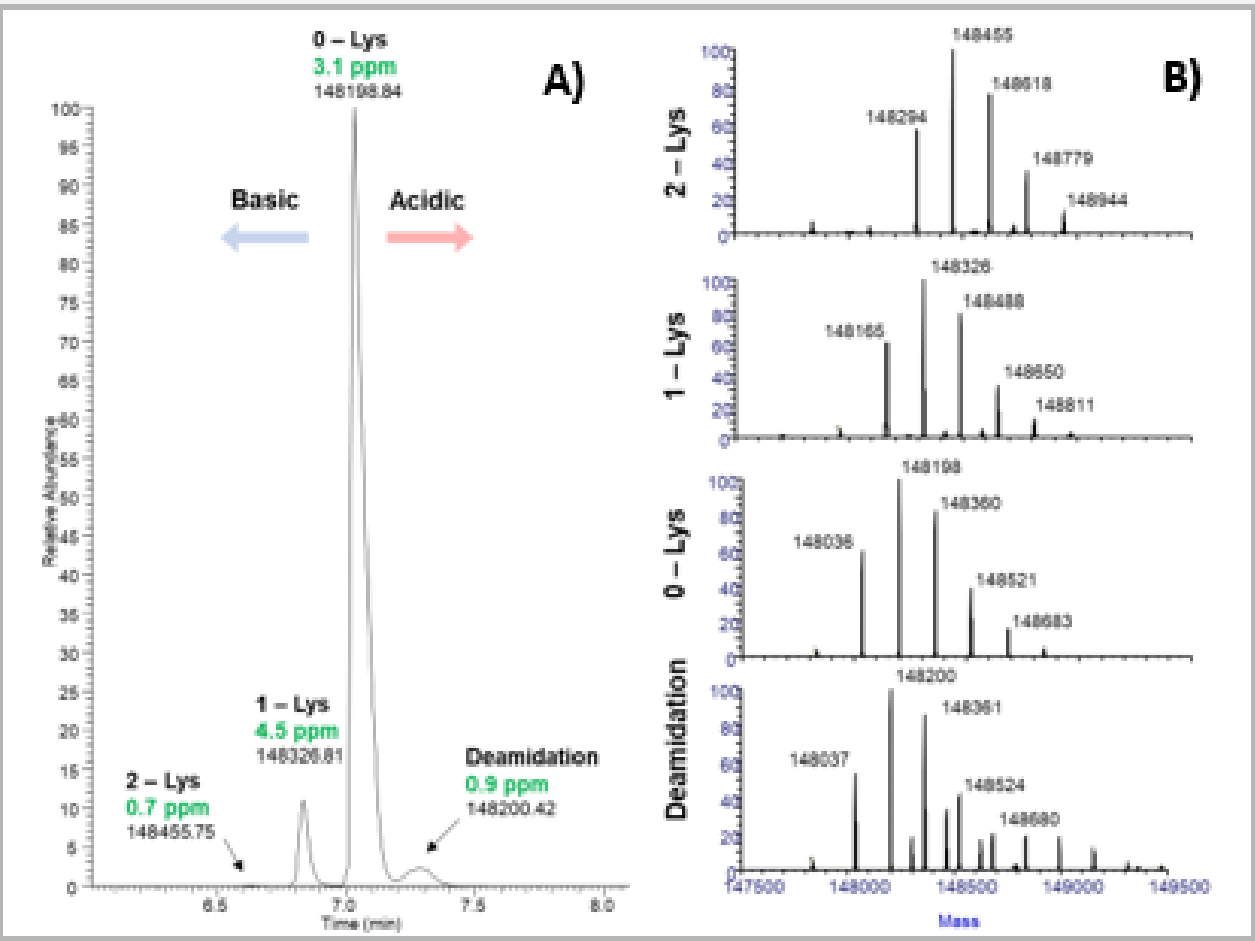


Figure 1. A) Electropherogram showing acidic, basic and main charge variants of the NIST mAb. B) Deconvoluted mass spectra of the variants showing glycoforms.

Charge variant analysis (CVA) is crucial for identifying critical quality attributes (CQAs) of mAbs. The ZipChip System provides ready-to-use consumables for a simplified CVA protocol, requiring only dilution and injection. Figure 1 shows CVA results for NIST mAb. When coupled with High Resolution MS (HRMS), this generic approach enables rapid and highly sensitive detection of charge variants and glycoforms of mAbs for a variety of mAbs and bispecific antibodies without further method development.³ Furthermore, the peak profiles align with ICIEF, allowing direct MS identification of unknown species.³ The ZipChip/MS System tolerates matrices like detergents and salts, eliminating the need for species removal through cleanup procedures.

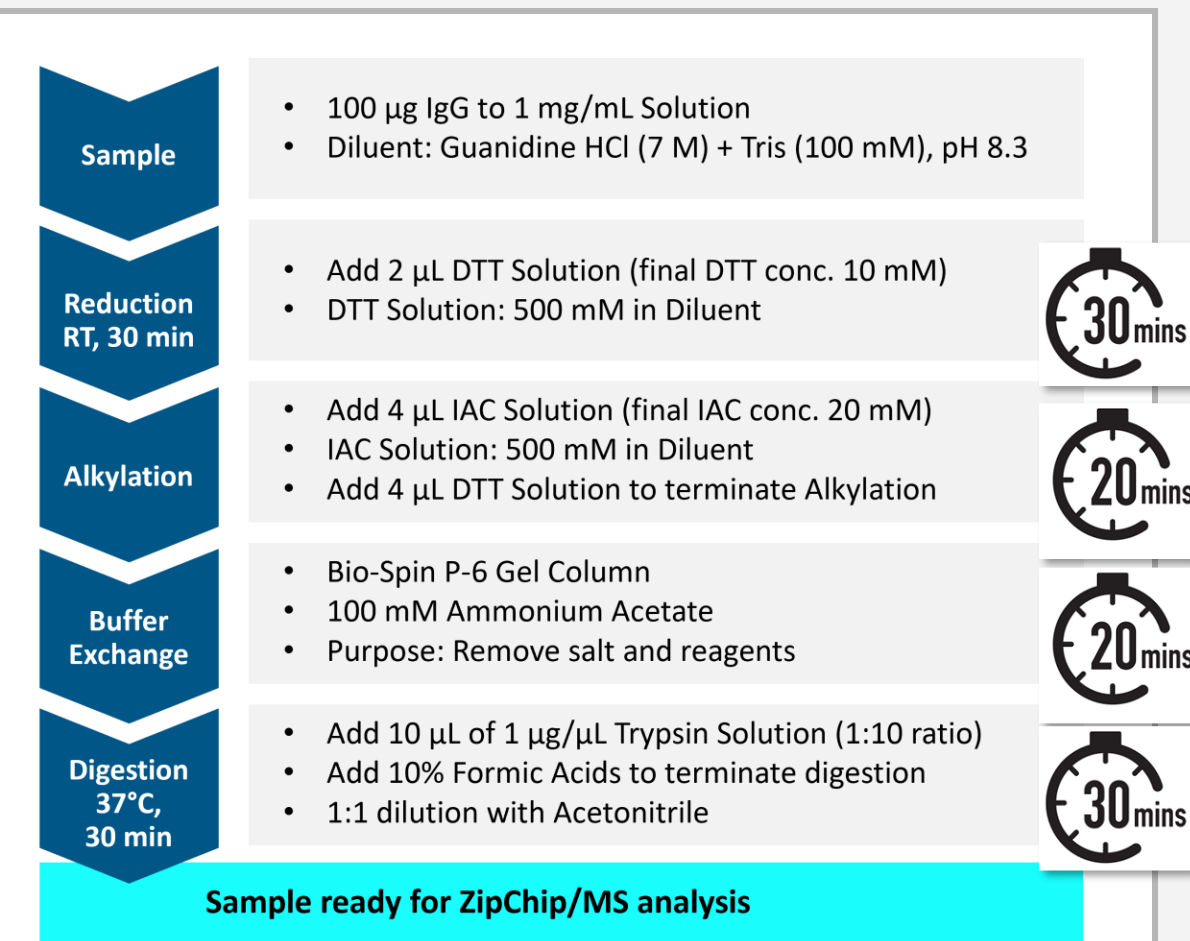


Figure 2. Workflow showing the steps involved in sample preparation procedure for peptide mapping of a mAb for ZipChip System analysis.

The **Peptide Mapping** sample preparation protocol is shown in Figure 2. Ammonium acetate replaces Tris buffer for the trypsin digestion, making it directly compatible with ZipChip/MS analysis with no further sample prep steps. When compared with LCMS protocol using TRIS buffer, this workflow demonstrated similar performance for CQAs identified with only 8 nL of 1 mg/mL of material needed per injection. Figure 3 shows the result of NIST mAb peptide mapping showing excellent sequence coverage and accurate identification of main PTMs within less than 20 minutes per run. The workflow was applied to different biopharmaceutical formats (IgG4, IgG1, Fc fusion proteins) with great results.⁴

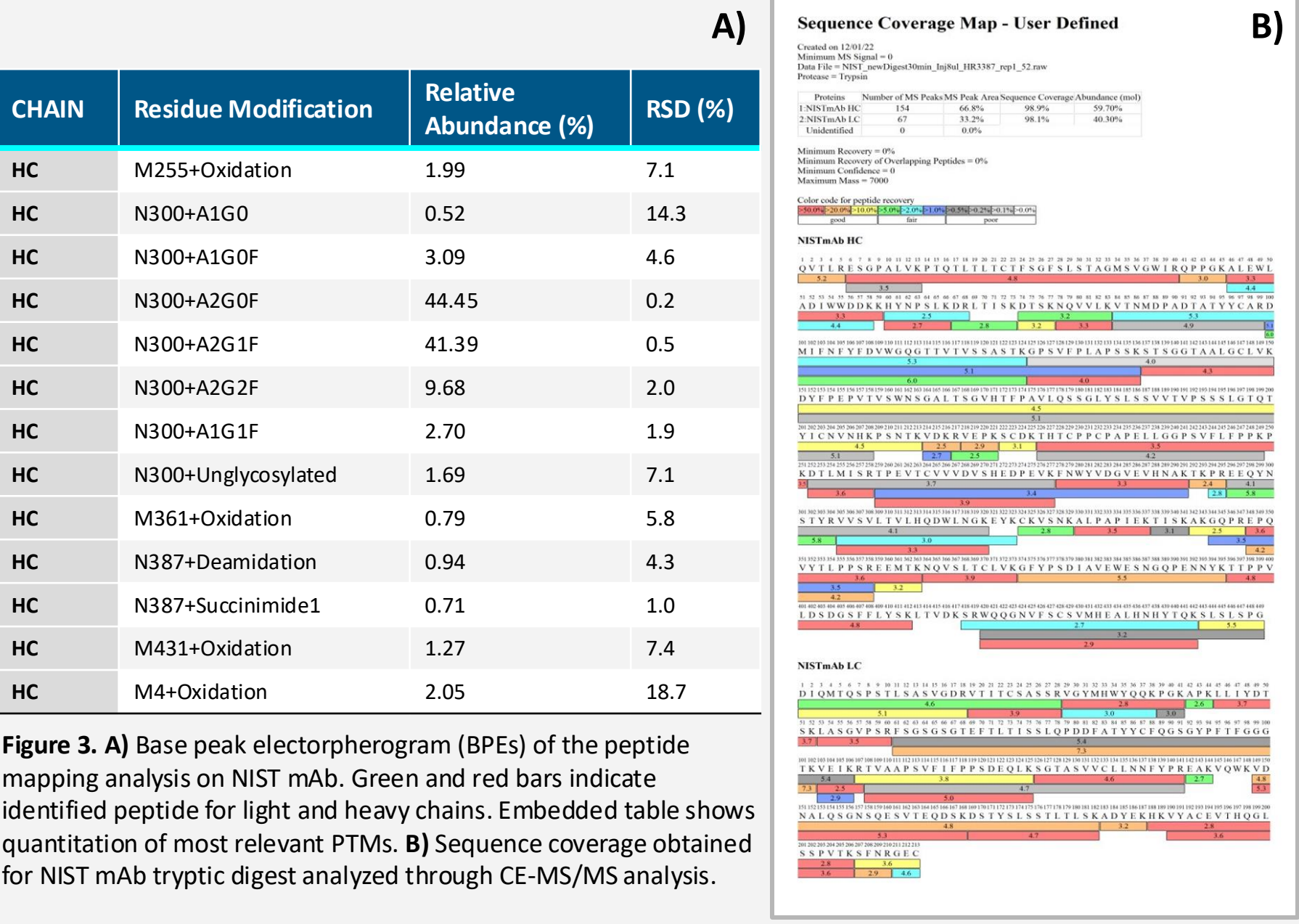


Figure 3. A) Base peak electropherogram (BPEs) of the peptide mapping analysis on NIST mAb. Green and red bars indicate identified peptide for light and heavy chains. Embedded table shows quantitation of most relevant PTMs. B) Sequence coverage obtained for NIST mAb tryptic digest analyzed through CE-MS/MS analysis.

Characterization of Viral Capsid Proteins (VP) for AAVs: Intact Denatured and Peptide Mapping⁵

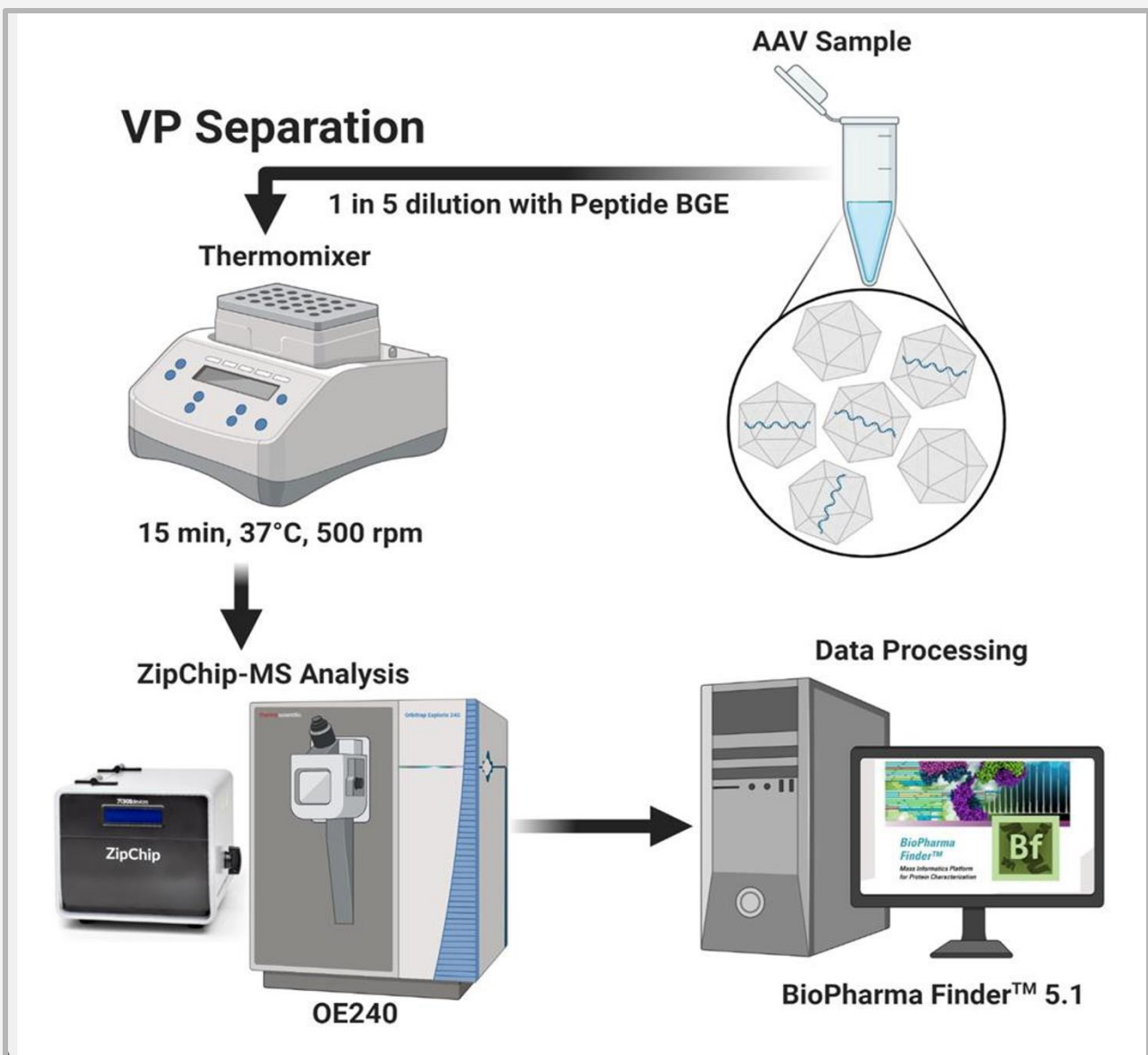


Figure 4. Schematic showing the workflow for analysis of AAV viral capsid proteins.

Intact Denatured Analysis is performed using a ZipChip System (Repligen) coupled to an Orbitrap Exploris™ 240 MS (Thermo Scientific). The analysis time was 5 minute using a standard High Resolution (HR) chip (Repligen) along with a Background Electrolyte (BGE) containing 4% DMSO. The workflow is shown in Figure 4.

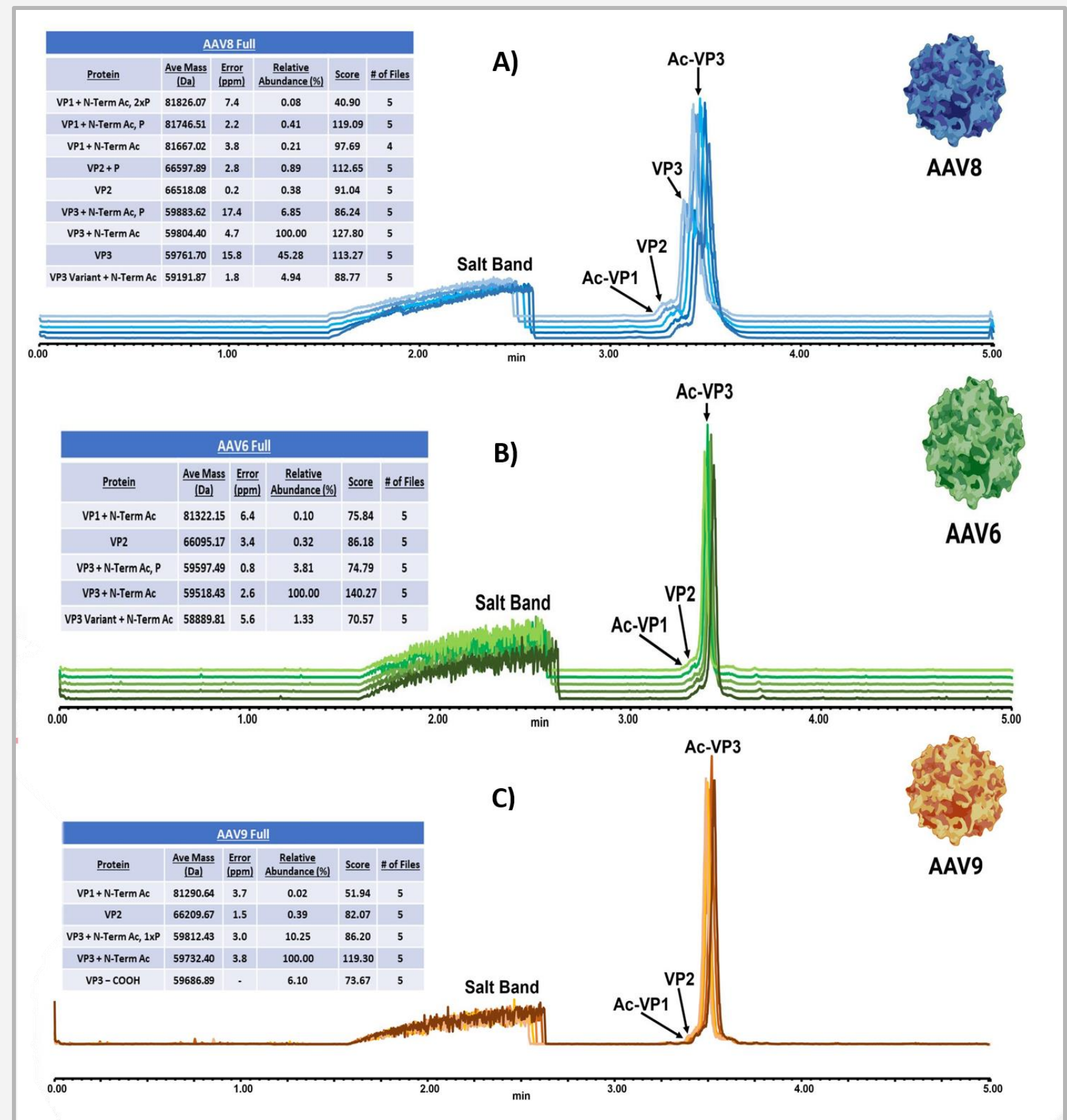


Figure 5. BPEs of the 5 replicates analysis (overlay) performed for AAV8 (3A), AAV6 (3B) and AAV9 (3C). Data show separation of the three VPs during the analysis with capability to detect multiple proteoforms for each VP, including phosphorylation and acetylation. LOD testing showed that all identified proteoforms were detected at concentrations 8.80E6 viral particles, with at least one proteoform of each VP detected at concentrations as low as 2.64E6 viral particles.

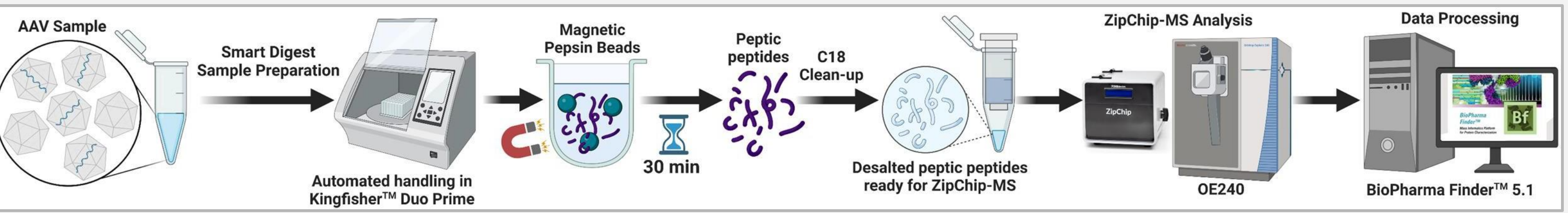


Figure 6. Schematic showing the sample preparation strategy along with analysis followed by data processing for digested AAV capsid proteins.

Peptide Mapping Analysis for AAVs is performed using the ZipChip/Exploris 240 workflow demonstrated on Figure 6. 10 µg of AAV (determined using NanoOrange protein assay) was digested with SMART Digest pepsin magnetic beads on a Kingfisher™ DuoPrime. Desalting is performed using a C18 spin column and then reduced to dryness in a speed-vac. The analysis was performed by reconstitute digested samples in 10 µL of the Peptide BGE and loaded directly into an HR chip primed with Peptide BGE. Each run was 20 minutes, and 2 injections were performed for each sample. Results of the peptide mapping analysis for AAV is shown in Figure 7.

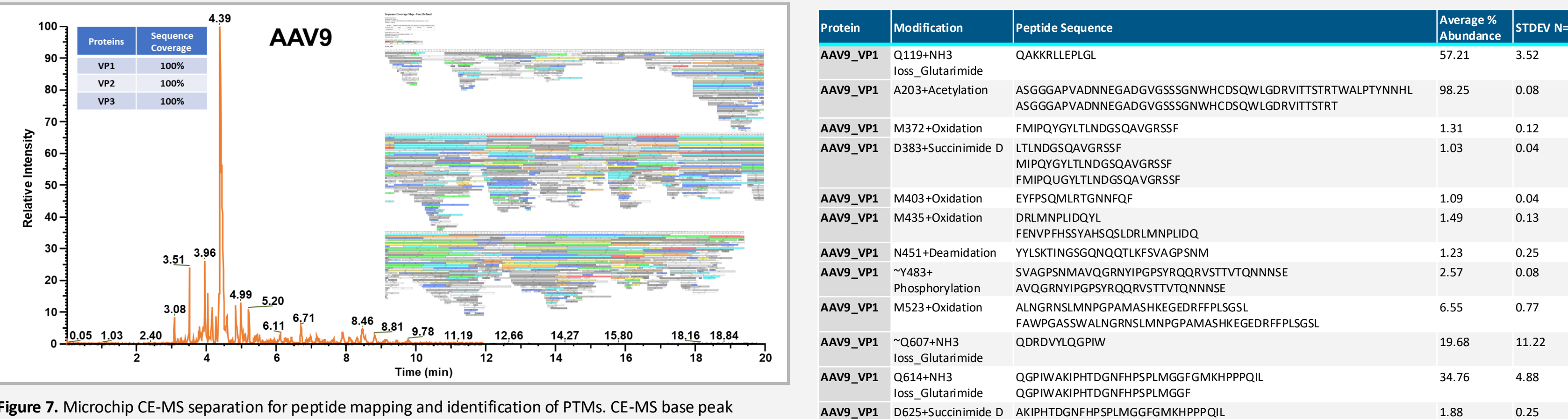


Figure 7. Microchip CE-MS separation for peptide mapping and identification of PTMs. CE-MS base peak electropherograms of AAV9 serotype (left) illustrating high sequence coverage. On the left the table of identified PTMs.

- Rapid microchip CE-MS can be utilized for the in-depth characterization of AAVs including viral capsid protein analysis and peptide mapping.
- VP separation is performed in as little as 5 minutes while detecting multiple proteoforms of each viral capsid protein.

Characterization of Nucleic Acids: Oligonucleotide Analysis⁶ and Bottom-up Oligo Mapping of RNAs⁷

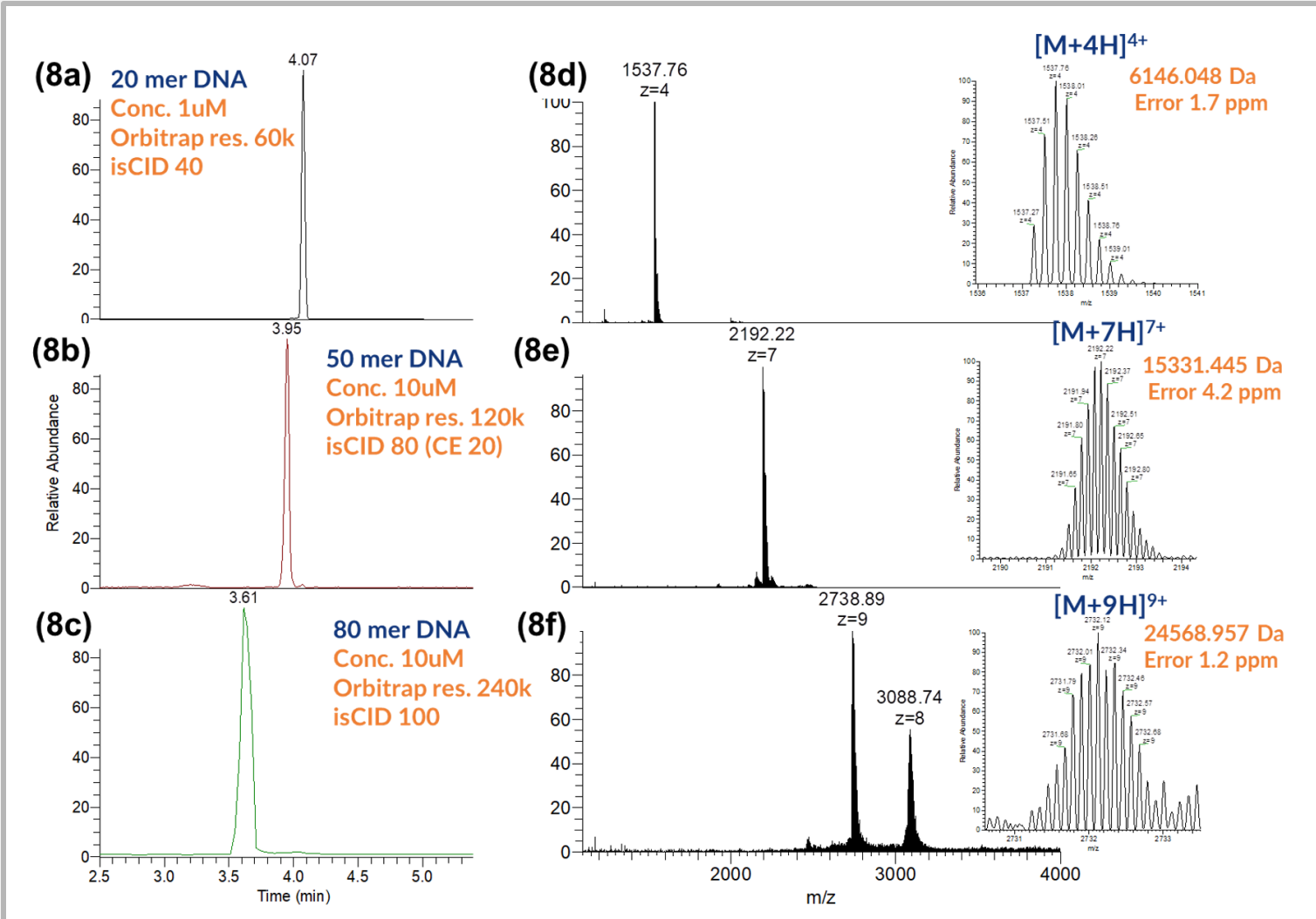


Figure 8. Extracted Ion Electropherograms and raw mass spectra of DNAs 20mer, 50mer, 80mer (8a-8c), mass spectra generated by averaging the peaks of interest (8d-8f) inserts show M+H adduct in the dominant charge state for each of the Oligo.

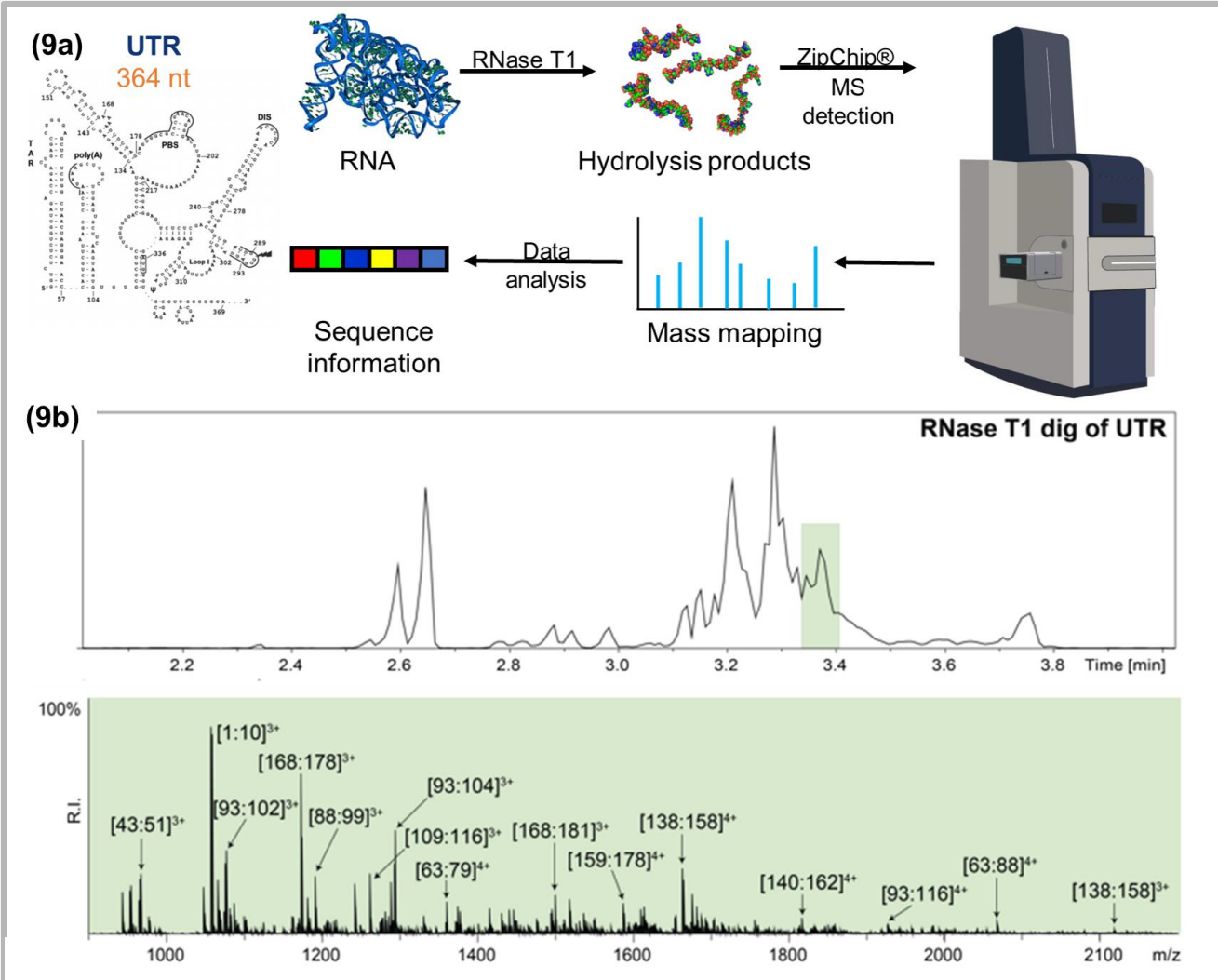


Figure 9. Protocol for digestion and analysis workflow (9a); Bottom-up analysis of UTR on ZipChip System-timsTOF (9b)

The **Intact Oligos Analysis** can be easily done by the ZipChip CE/MS platform. The method is rapid with NO need for ion-pairing reagents. In this protocol, the ready to use consumables are a High-Resolution Bare Glass (HRB) chip and the ZipChip System Oligos BGE. A generic protocol can be sued for the analysis of oligos full length product (FLP) 10–80mers. Figure 8 presents the example electropherograms and corresponding mass spectra for 20mer, 50mer, and 80mer DNA sample.

Oligo mapping of a larger biological RNA by a partial digestion was also performed using similar CE-MS protocol (Figure 9a) with analysis time to be 4 minutes. The sample used was HIV-1 5'-UTR (untranslated region) RNA (UTR), with 364 nucleotides and was partially digested using RNase T1. Figure 9b shows the electropherogram and an example mass spectrum for one of the peaks of interest. 100% sequence coverage of UTR was achieved.

Conclusion

- ZipChip CE/MS System is a versatile platform for analysis of a variety of therapeutic and pharmaceutically important molecules such as mAbs, AAVs and nucleic acids and both intact and partially digested levels.
- This microfluidic technology offers a rapid method of analysis for all formats; ready-to-use consumable kits eliminate the need for method development.
- Due to its mass spec friendly BGE solutions and lack of ion-pairing reagents, switching between methods is extremely simple, thereby reducing critical bottlenecks in analytical workflows in a biopharmaceutical mass spectrometry laboratory.

References

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