Next-Gen Protein Sequencing™ (NGPS™)

Powering the Future of Cancer Research

NEXT-GEN PROTEIN SEQUENCING IN CANCER RESEARCH

NGPS enables detailed analysis of proteins in cancer pathways, uncovering biomarkers, therapeutic targets, and insights into protein interactions and modifications to advance cancer detection and treatment.

PROTEINS AND PROTEOFORMS

NGPS identifies key cancer-related proteins (e.g., PD-L1, IL-6, PTEN, p53) and their proteoforms – molecular variations influencing cancer progression and therapy resistance.

NGPS INTEGRATION WITH I2MS

Combining NGPS with individual ion mass spectrometry (I2MS) enhances protein analysis, providing deeper insights into structural and functional diversity in proteins like IL-6 and tropomyosin proteoforms.

Protein analysis is crucial in cancer research because proteins play pivotal roles in cancer development, progression, and response to treatment, serving as potential biomarkers and therapeutic targets.

Cutting-edge techniques like Next-Gen Protein Sequencing enable scientists to unravel the complexities of altered proteins and their interactions, advancing our understanding of cancer detection, prognosis, and novel therapeutic strategies.

NGPS on the Platinum® Pro instrument provides a novel tool for researchers exploring the role of proteins in cancer pathways, proteins as drug targets, and protein-protein interactions involved in immunotherapy. Here, we review a variety of NGPS applications, illustrating how this powerful tool is helping researchers unlock the mysteries of cancer biology and driving the development of next-generation cancer therapies.

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Exploring Proteins as Cancer Biomarkers

Programmed death-ligand (PD-L1) is a transmembrane protein that plays a critical role in regulating immune response in the context of cancer. Exploring the amino acid sequence of PD-L1 is essential for understanding and exploring its structure, folding, and function.

Direct protein identification and quantification of expression through NGPS can be leveraged in biomarker development with proteins like PD-L1. Here, purified PD-L1 was digested, and peptides were sequenced on Platinum, providing highly specific protein detection through thousands of unique reads with low false discovery rate (FDR).

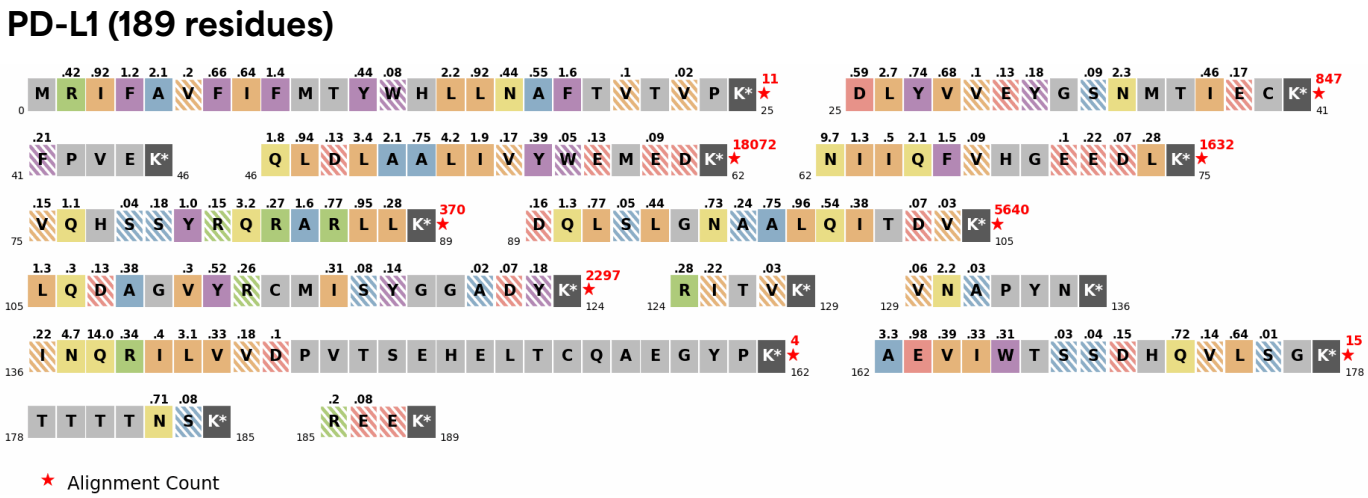


Figure 1. Graphical representation of the NGPS coverage of PD-L1 (FDR <10) from the *Peptide Alignment* workflow indicating the number of alignments recovered for each peptide. Colored amino acids represent locations within a given peptide that are available for direct recognition by an N-terminal amino acid recognizer.

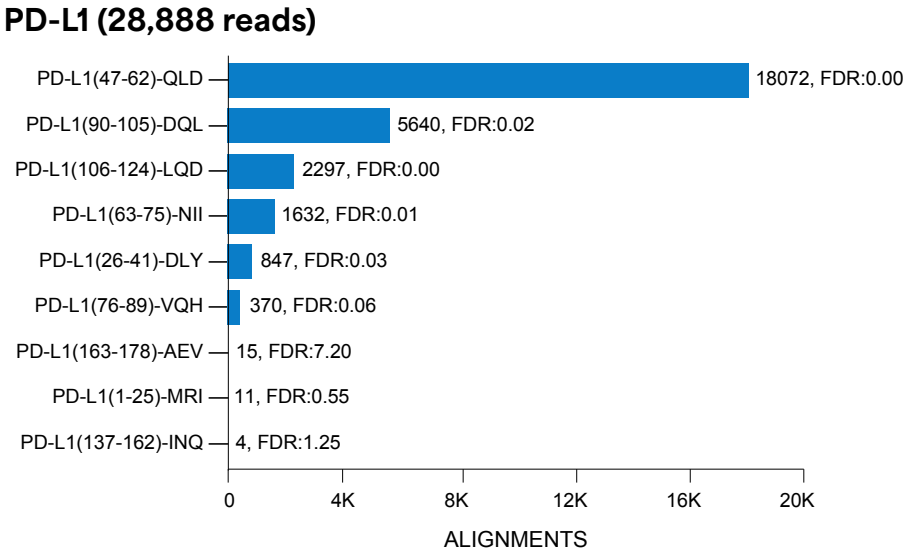
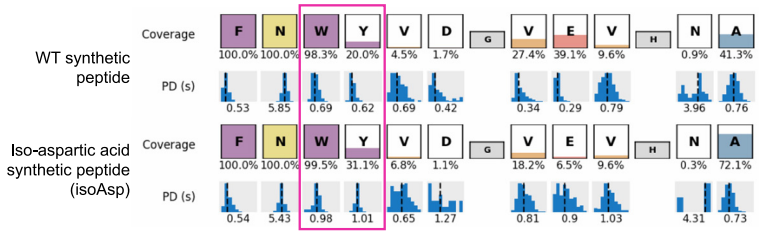


Figure 2. Bar graph demonstrating the number of aligned PD-L1 peptides. The metrics at the end of the bars represent the number of alignments and the FDR. FDR less than 10% indicates high confidence.

Characterizing Monoclonal Antibodies and Antibody-Drug Conjugates

Rituximab and trastuzumab are two widely used monoclonal antibodies in cancer therapy. Next-Gen Protein Sequencing of these antibodies revealed conserved peptides in the Fc region of both rituximab and trastuzumab. Notably, the study detected a post-translational modification (PTM) – a transformation from aspartic acid (Asp) to isoaspartic acid (isoASP) – which was detected through a shift in the kinetic signature of upstream residues. This observation suggests that the conserved Fc peptide can be used as a site for antibody-drug conjugation, allowing measurement of conjugation efficiency through changes in the kinetic signature.

Characterization of isomerization of aspartic acid (Asp) into isoaspartic acid (isoAsp) in conserved Fc peptide



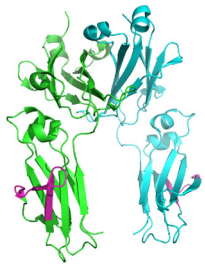


Figure: Crystal structure of the Fc region of IGG. The magenta represents the conserved Fc peptide FNWYVDGVEVHNA.

Hypothesis: Utilization of NGPS to measure drug conjugation of Fc region peptide FNWYVDGVEVHNA

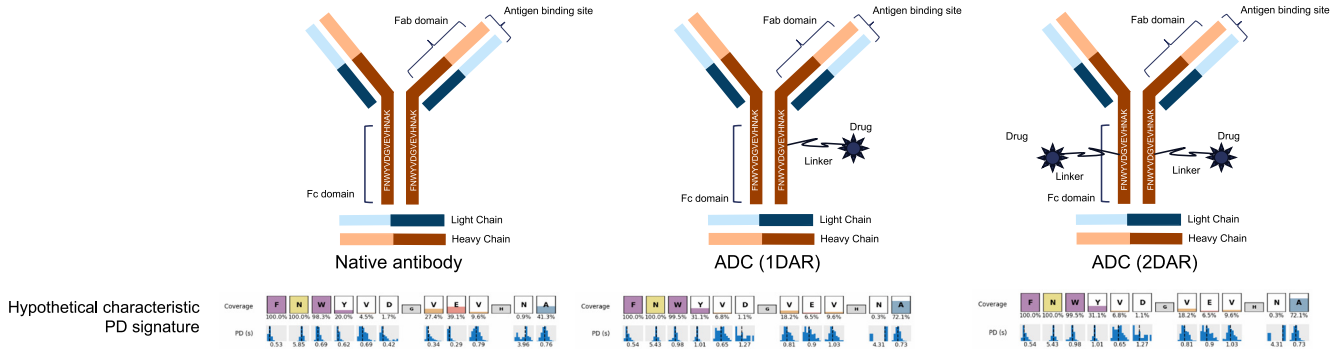


Figure 3. Top: kinetic signature of WT and isoAsp synthetic peptide from Fc region of the antibody. A change in pulse duration was observed for preceding W/Y amino acids in isoAsp mutated peptide as compared to WT peptide. Bottom: the conserved Fc peptide could be used as a site for antibody-drug conjugation, allowing measurement of conjugation efficiency through changes in the kinetic signature.

Seeking Drug Targets by Profiling Protein-Protein Interactions

Immunoprecipitation with targeted or candidate antibodies can be used to isolate proteins and protein complexes for identification with NGPS. To demonstrate an immunoprecipitation workflow, HSP90α was immunoprecipitated from HEK293 cells and subjected to in-gel digest prior to sequencing. HSP90α was identified from the NGPS data, with more than ten peptides identified with low FDR (<10%), indicating high confidence.

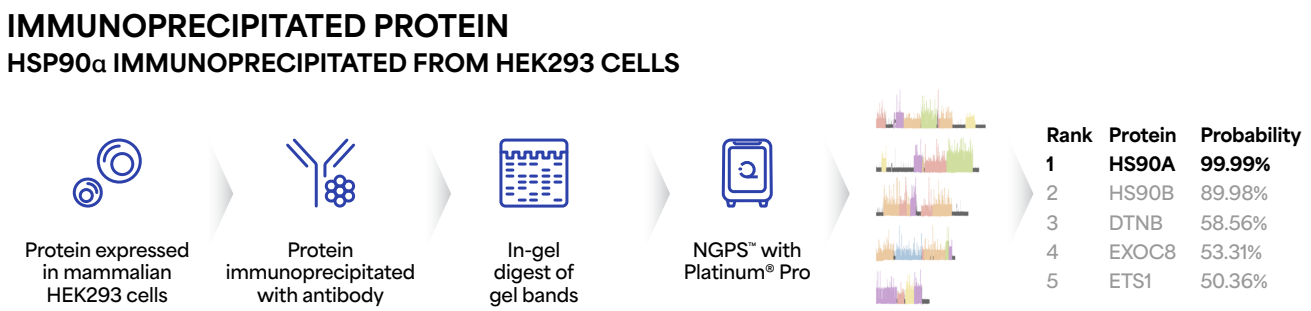


Figure 4. Representative traces, coverage, and pulse duration data for the peptides identified in HS90α immunoprecipitated from HEK293. HS90A was identified with *Protein Inference* workflow on Platinum with 99.99% probabillity.

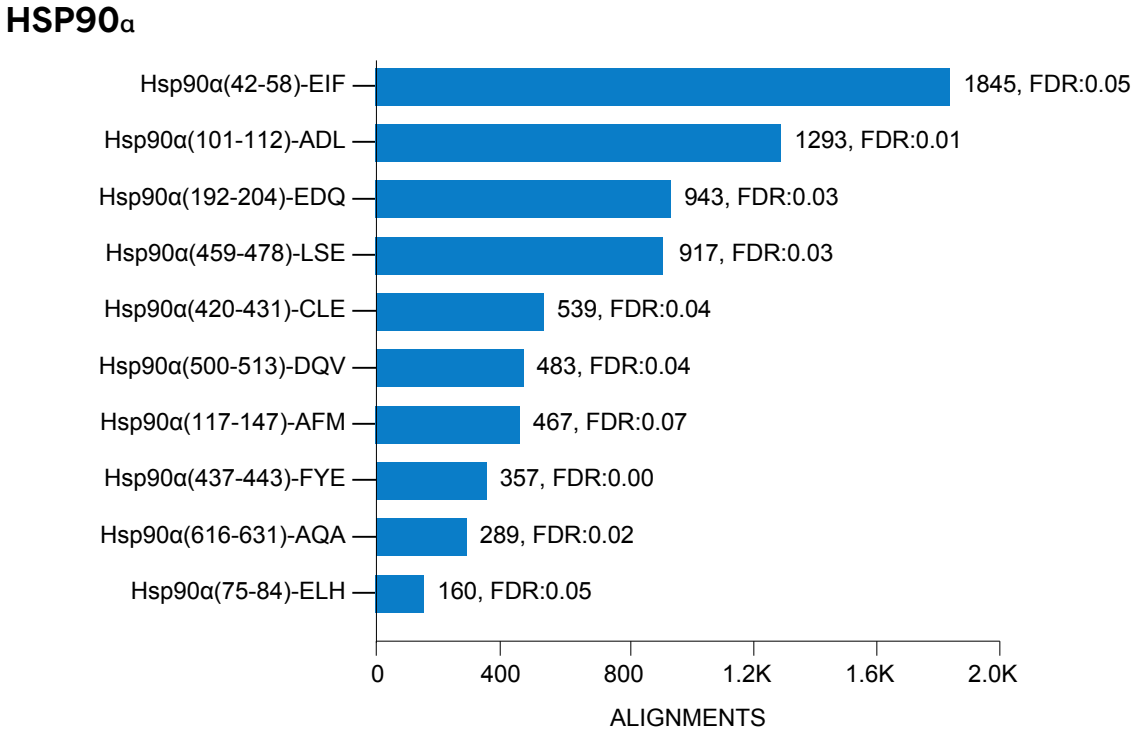


Figure 5. Bar graph demonstrating the number of aligned HSP90α peptides with FDR below 10%. The metrics at the end of the bars represent the number of alignments and the FDR..

Quantifying Protein Expression and Exploring Trafficking with Protein Barcodes

Protein barcoding with NGPS has emerged as a powerful tool for the multiplexed identification and characterization of proteins, enabling precise tracking of protein affinity, location, and direct protein expression across a tenfold dynamic range (Chinnaraj et al. 2024). In this demonstration study, five proteins were tagged with protein barcodes, expressed in *E. coli*, and quantitated via NGPS of the cleaved barcodes.

Notable cancer proteins assessed include:

- **INFγ:** cytokine, a protein secreted by immune cells that plays a crucial role in regulating immune responses
- **PTEN:** (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene preventing cells from growing and dividing too rapidly. PTEN loss or inactivation can lead to different types of cancers, including prostate, glioblastoma, endometrial, lung, and breast cancer.
- **P53:** a tumor suppressor regulating DNA damage and initiating cellular responses to repair it or induce cell death (apoptosis). Mutations in the p53 gene (TP53) are common in many types of cancer. These mutations can disrupt the tumor suppressor functions of p53, leading to uncontrolled cell growth and cancer development.

Barcode ID	Protein	UniProt ID	AA Length	MW (kDa)
BC032	IFNg	P01579	206	23.69
BC049	PTEN	P60484	443	51.43
BC051	TAU441	P10636	481	50.34
BC075	UCHL1	P09936	263	29.21
BC096	p53	P46037	433	47.90

Table 1. Summary and characteristics of the five proteins tagged with protein barcodes and expressed in *E. coli*. MW= molecular weight.

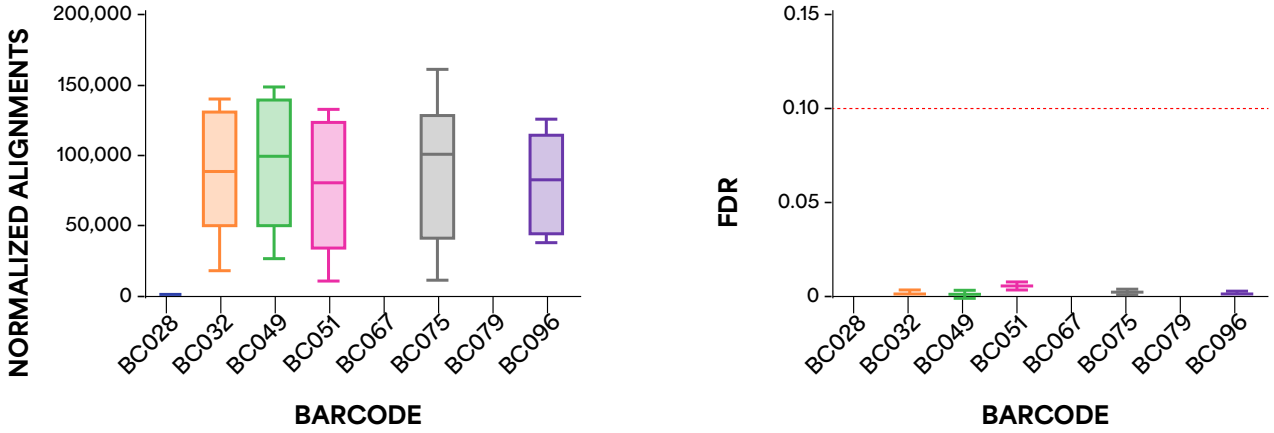


Figure 6. Normalized alignments recovered across eight sequencing runs containing the five proteins mixed at equimolar concentrations and the FDR across the eight runs; red dotted line indicates 10% FDR cutoff.

Detection and Characterization of Protein Variants and Proteoforms

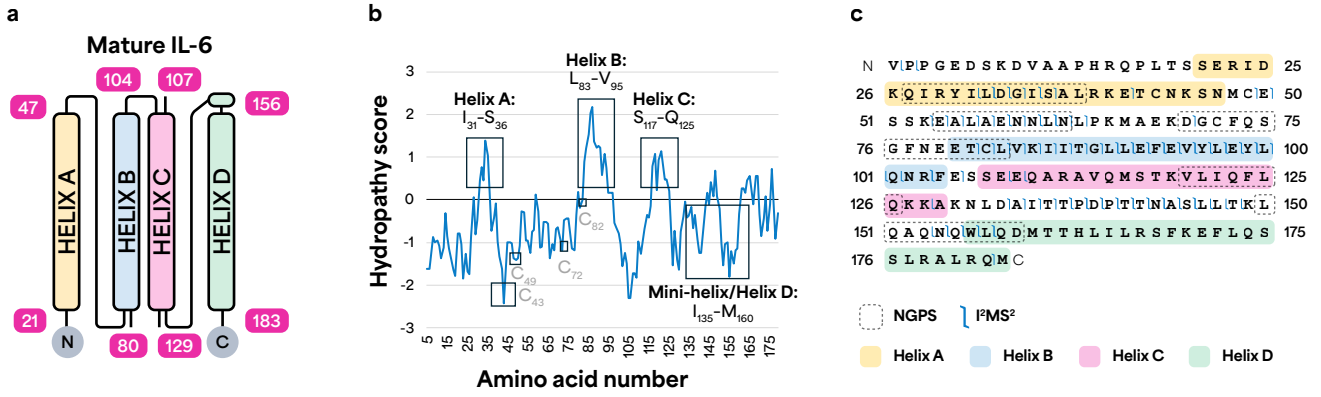
Proteoforms – different molecular versions of a protein resulting from genetic, splicing, or post-translational changes – play a crucial role in cancer. These variations can alter protein function, stability, and interactions, enabling cancer cells to evade immune detection, resist cell death, and promote growth. Cancer-specific proteoforms can disrupt key signaling pathways like PI3K/Akt or MAPK, and influence the tumor microenvironment, aiding in processes like angiogenesis and immune suppression. As valuable biomarkers, proteoforms offer potential for early cancer detection, prognosis, and targeted therapies.

Two recent studies illustrate the ability and potential of NGPS to detect and characterize proteoforms by complementing traditional proteomics methods like mass spectrometry (MS).

Increasing Coverage of IL-6 by Combining NGPS and I2MS

In a recent preprint publication (Skinner et al. 2025), researchers from Northwestern University evaluated the complementarity of NGPS and top-down mass spectrometry (individual ion mass spectrometry, or I2MS) – for analyzing recombinant human IL-6. Interleukin-6 is a cytokine that plays a crucial role in cancer development and progression, promoting the formation of new blood vessels within tumors, providing them with oxygen and nutrients. IL-6 also enhances the ability of cancer cells to spread to other parts of the body suppressing the immune system, which allows cancer cells to evade detection and destruction.

The researchers found that NGPS achieves single-amino acid resolution across multiple regions of IL-6, including key peptides within helices A and C that impact IL-6 function. Meanwhile, I2MS provides significant sequence coverage in crucial regions, including helices B and D, which are involved in IL-6 signaling. The integration of NGPS and I2MS provides 52% total sequence coverage, offering a more comprehensive view of IL-6's structural and functional diversity.



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Deletions/substitutions of amino acids and fragments impact IL-6	Peptides detected by NGPS with single-amino acid resolution	Fragments detected by I²MS² with single-amino acid resolution
Q_{27}IRYILDG (Refs. 57, 59)	Helix A: Q_{27}IRYILDGISALRK	Helix A: D_{32}G
L_{56}AEN (Ref. 60)	AB loop: E_{54}ALAENNLNPK	AB loop: L_{56}AENNLNL
Q_{74}SGF (Refs. 60, 61)	AB loop: D_{70}GCFQSGFNEETCLVK	–
G_{76}FNEETCLVKIITGLLE (Ref. 62)	–	Helix B: C_{82}LVKIITGLLEFEVYLEYLQN
IL-6Δ4 isoform del. E_{79} – A_{129} (Ref. 38)	Helix C: V_{120}LIQFLQK	–
W_{156}LQDMTTHLILRSFK (Refs. 53, 54, 63-65)	Helix D: L_{150}QAQNQNQWLQDMTTHLILRSFK	Mini-helix: I_{125}TTPDPTTNASLLTK Mini-helix/Helix D: L_{150}QAQNQWLQDM Helix D: T_{161}THLILRSFK

Figure 7. NGPS (Platinum) and I2MS provide broad coverage of IL-6, combining to resolve 52% of single amino acids.

Detecting Unique Peptides that Discriminate Tropomyosin Proteoforms

Tropomyosin (TPM), an actin-binding protein, plays a role in cancer development and progression, influencing processes like cell proliferation, migration, and invasion, and can be used as a potential biomarker or therapeutic target. Some studies suggest that certain tropomyosin isoforms, like TPM1, can act as tumor suppressors, while others, like TPM3, may promote cancer progression.

In a recent preprint manuscript (Sittipongpittaya et al. 2024), researchers at University of Virginia evaluated whether NGPS can be used to detect proteoform-specific peptides representing key variation in tropomyosin proteoforms. In this study, NGPS detected all three major types of variation: genetic (paralogs), transcriptional/post-transcriptional (tissue-specific proteoforms and alternative splicing), and PTMs. Some of these variants can be challenging to differentiate with orthogonal technologies (e.g., isobaric peptides on MS). This foundational study illustrates the capacity of NGPS to detect proteoform variation at the single-amino acid level, including variants associated with disease phenotypes.

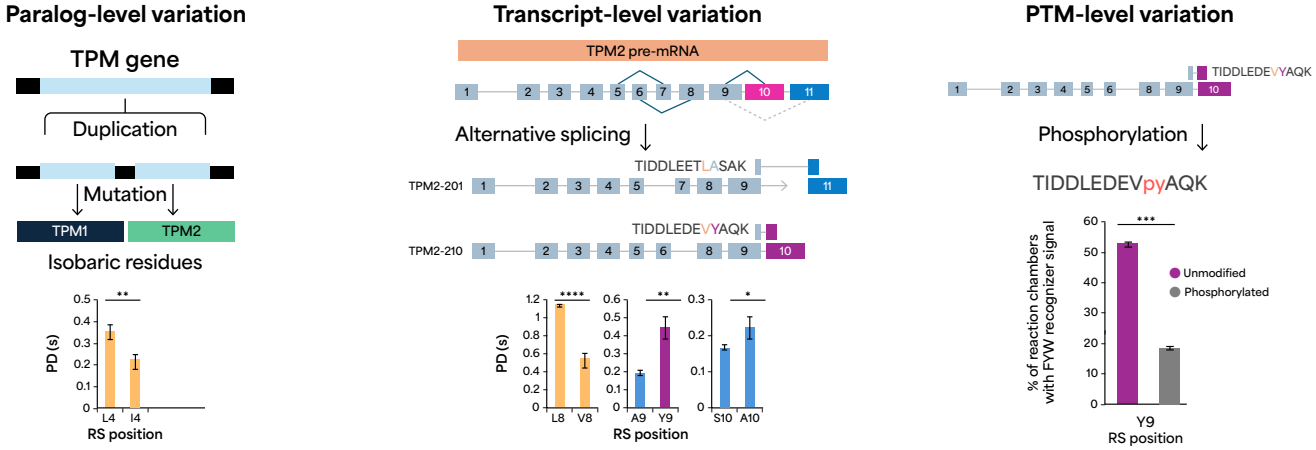

Figure 8. Evaluation of NGPS with Platinum's ability to distinguish three main types of proteoform variation.

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