High-resolution detection of post-translational modifications using single-molecule protein sequencing

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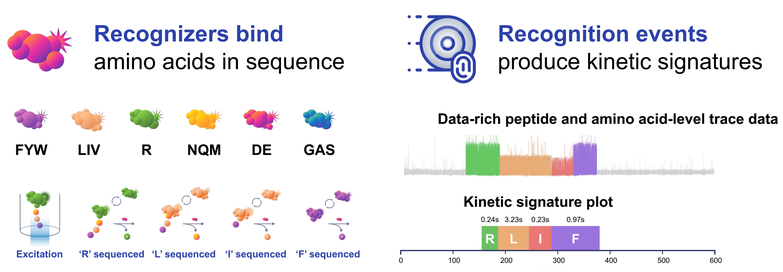
Summary

Post-translational modifications (PTMs) play a critical role in regulating protein function and cellular processes. However, due to the diversity and complexity of the proteome, methods to detect and quantify PTMs have lagged behind advances in DNA sequencing. While conventional mass spectrometry and antibody-based methods provide valuable information, they are limited in their capacity to resolve and quantify PTMs at the amino acid level, particularly for PTMs with minimal differences in mass and without available antibodies. The Quantum-Si Platinum® and Platinum® Pro sequencers are benchtop instruments designed for high-resolution, single-molecule protein sequencing. These instruments use engineered N-terminal amino acid recognizers to accurately determine the sequence composition of proteins, enabling the detection of PTMs and making advanced proteomic analysis accessible in a standard laboratory setting. In this study, we report the application of this next-gen protein sequencing (NGPS) method to detect and quantify multiple PTMs across diverse protein types. We demonstrate the detection of phosphorylation at several sites within the anaplastic lymphoma kinase (ALK) protein, citrullination events on vimentin, asparagine deamidation as a marker of molecular aging, and the identification and differentiation of aspartic acid stereoisomers in calmodulin sequences. This is the first study demonstrating the ability to resolve and quantify multiple PTMs with NGPS, enabling differentiation of chemical changes and stereochemical variants that, while subtle, can nevertheless have significant biological impacts. We expect this technology to democratize advanced protein characterization, making the high-resolution detection of PTMs available to a broader scientific community.

Introduction to next-gen protein sequencing (NGPS)

- **Simple sample prep process:** Proteins are digested, functionalized, conjugated, and immobilized on the surface of a semiconductor chip.
- **Unique *kinetic signature* mechanism:** Fluorescently labeled N-terminal amino acid (NAA) recognizers and aminopeptidases are added to the semiconductor chip.
- **Single-molecule level data:** Fluorescent intensity and duration of each NAA binding event generate a unique kinetic signature.
- **Automated analysis:** Kinetic signatures are automatically analyzed to align reads to reference peptides and compute false discovery rate (FDR).

The Platinum Pro benchtop instrument enables single-molecule protein sequencing



Kinetic detection of PTMs with NGPS

Kinetic response to PTMs is a universal feature of NAA recognizers — when the chemical makeup of the peptide changes, the kinetics change. No extra reagents or processing steps are needed.

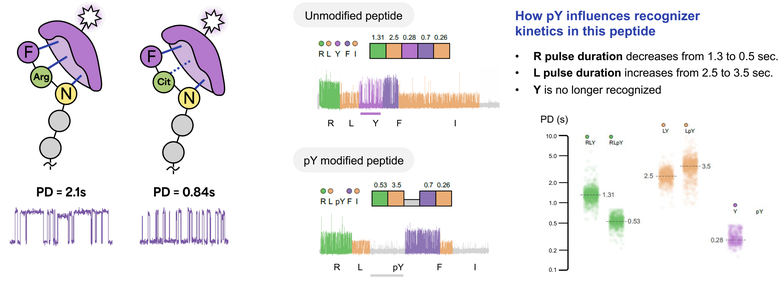


Figure 1: The presence of a PTM affects the binding kinetics of each NAA recognizer at both the site of the modification and multiple amino acids upstream.

Detection of phosphorylation in ALK, an important cancer driver

- Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) and a driver of several solid and hematological cancers.
- ALK is known to be phosphorylated at several sites during activation, including Y1278, Y1282, and Y1283.

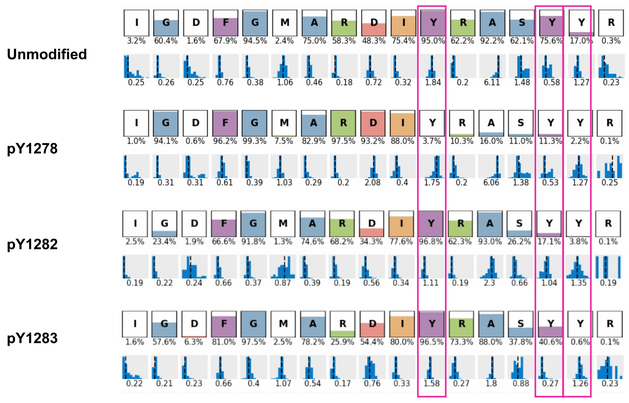


Figure 2: NGPS of multiple phosphorylated peptides from ALK. Changes in in the kinetic signatures are observed both at the phosphorylation site and at upstream residues.

Analysis of asparagine deamidation, a marker of molecular aging

- Asparagine and glutamine deamidation are spontaneous post-translational modifications that serve as a molecular clock, offering insights into protein aging and stability. Age- and stress-related accumulation of deamidated calmodulin may contribute to cellular dysfunction and disease.
- Distinguishing IsoAsp and Asp following from Asn deamidation can be challenging by mass spectrometry due to their identical mass.

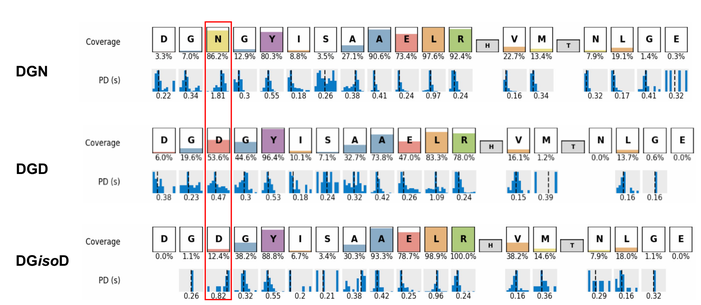


Figure 3: NGPS of peptides representing a deamidation site in human calmodulin (CALM1). Distinct kinetic signatures are observed for the N, D, and isoD variants.

Detection of arginine citrullination in vimentin, a key antigen in rheumatoid arthritis

- Arginine (Arg or R) can be converted into citrulline through citrullination, which is carried out by a family of peptidyl arginine deaminases. Arg citrullination is implicated in inducing immune responses, and aberrant citrullination has been linked to various autoimmune diseases.
- Arg citrullination results in a loss of positive charge and an increase of <1 Da in molecular weight (~0.98 Da), requiring high-resolution MS or Cit-specific antibodies for detection.

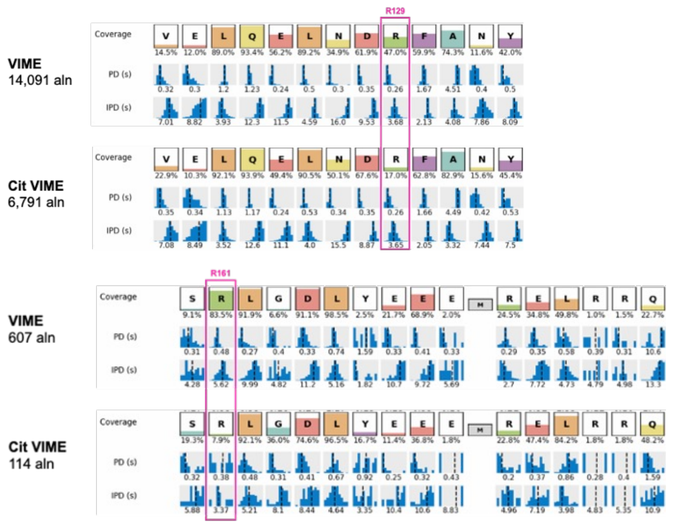


Figure 4: NGPS of recombinant citrullinated vimentin protein reveals multiple sites with kinetic signature changes when citrullinated arginine is present.

Conclusions and future directions

- NGPS can be used to detect post-translational modifications (PTMs) at the single-molecule, single-amino-acid level by leveraging the kinetic signature data produced during sequencing.
- Use of NGPS to measure phosphorylation, deamidation, and citrullination has been demonstrated in both peptides and recombinant proteins.
- Future work will focus on expanding the number of PTMs tested and analyzing PTMs from biological samples.
- The upcoming launch of the Proteus instrument at the end of 2026 is expected to significantly increase throughput (80M wells, 4 samples per run) and amino acid coverage, enabling more detailed detection and quantification of PTMs and analysis of complex samples.

The Proteus instrument will feature automated sequencing, higher throughput, and analysis of up to 4 samples

References

1. Reed et al. (2022). Real-time dynamic single-molecule protein sequencing on an integrated semiconductor device. *Science*, 378(6619), 186–192
2. Sittipongpittaya et al. (2025). Protein Sequencing with Single Amino Acid Resolution Discerns Peptides That Discriminate Tropomyosin Proteoforms. *Journal of Proteome Research,* 24(8), 3798-3807

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