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

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Introduction

- Post-translational modifications (PTMs) are critical regulators of protein function and are extremely diverse in both types and frequencies[1]
- While many PTMs can be readily identified, identification and site localization can still be challenging when the PTM introduces small mass changes or when multiple modification sites are possible
- Here, we demonstrate how N-terminal amino acid (NAA) recognizer-based sequencing, an emerging single-molecule protein sequencing technology, can detect a variety of PTMs based on changes in recognizer binding kinetics

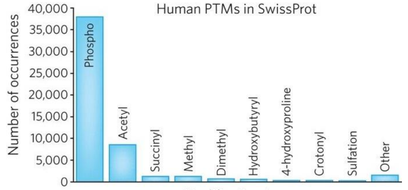
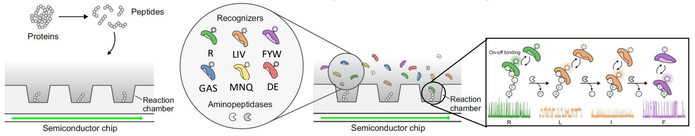


Figure from Aebersold et al. Nat Chem Biol. (2018)

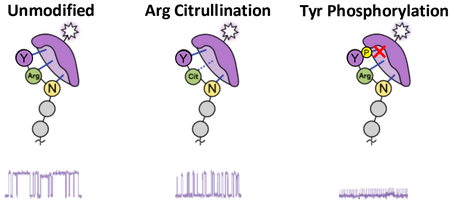
Methods

Peptide sequencing with NAA recognizers



- Transient recognizer on-off binding produces fluorescence pulses[2]
- Pulse duration (PD) can distinguish NAAs with the same recognizer

PTMs affect recognizer binding kinetics to cognate residues

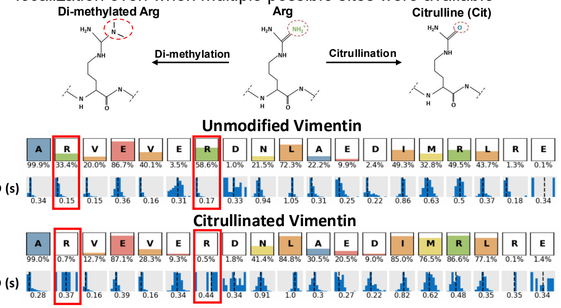


- Recognizers accommodate ~3-5 residues in their binding pockets
- PTMs can change the topology or charge of a sidechain, which could affect recognizer binding rates and affinity
- Presence of downstream PTMs may also affect binding at the NAA due to their proximity in the binding pocket

Results

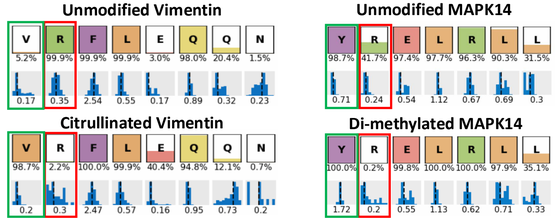
Binding kinetic shifts enabled detection of Arg citrullination events with site-specific localization

- Arg (R) can have several PTMs, including to citrullination and methylation or di-methylation.
- Citrullination, such as in vimentin, is implicated in autoimmune diseases such as rheumatoid arthritis
- Vimentin peptides were citrullinated at specific Arg residues
- Arg recognition was ablated only at Cit positions, allowing accurate localization even when multiple possible sites were available



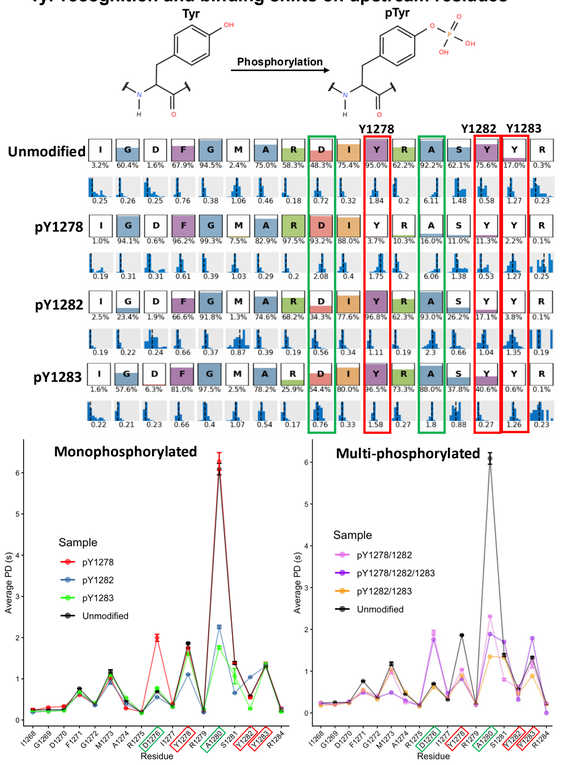
Arg citrullination and di-methylation may induce binding shifts at the neighboring upstream residue

- Arg methylation is implicated in several pathways, including cellular proliferation, differentiation, and gene expression regulation
- Peptide VRF from citrullinated vimentin protein was distinguished from WT via **ablated Arg recognition** and **increased recognition of upstream Val**
- Meanwhile, di-methylated Arg in peptide YRE from MAPK14 **ablated Arg recognition** and **increased PD of upstream Tyr**



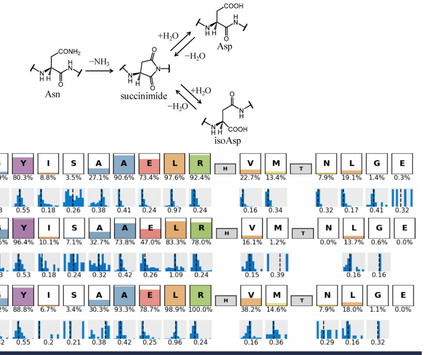
Binding shifts distinguished phosphorylated Tyr sites in peptides from ALK kinase domain

- Tyr (Y) phosphorylation is critical in many biological processes, including cell signaling, proliferation, metabolism
- Anaplastic lymphoma kinase (ALK) is a driver of several solid and hematological cancers, and is activated by phosphorylation at several residues such as Y1278, Y1282, and Y1283
- Tyr mono- and multi-phosphorylation were localized to specific sites on synthetic ALK peptides using a **combination of ablated Tyr recognition and binding shifts on upstream residues**



Asn deamidation events may be detected based on substitution to Asp or isoAsp

- Asn (N) can spontaneously deamidate, forming either Asp (D) or isoaspartate (isoD)
- This mechanism serves as a marker of molecular aging and can regulate protein stability and function
- Peptide DGN from calmodulin was synthesized as WT (N98) or its deamidated products (N98D or isoD). N98D and N98isoD substitutions were detected with the DE recognizer. However, isoD substitution significantly reduced recognition by the DE recognizer



Conclusions and future directions

- NAA recognizer-based sequencing can detect PTMs using changes in recognizer binding kinetic changes to the modified residue or its upstream residues
- NAA recognizer sequencing can be used to detect PTMs in synthetic peptides and recombinant proteins
- Future works will focus on expanding the detection to other PTM types, as well as expanding applications to modified proteins from biological samples

References and Acknowledgements

References

1. Aebersold, R. et al. How many human proteoforms are there? *Nat. Chem. Biol.* **14,** 206-214 (2018).
2. Reed, B. D. et al. Real-time dynamic single-molecule protein sequencing on an integrated semiconductor device. *Science* **378**, 186-192 (2022).

Conflict of Interest Disclosure

G.M.S. is on the scientific advisory board for Quantum-Si and is a co-founder and chief scientific officer of NeoSplice Therapeutics. K.N., A.V., J.V., and M.L.C. are employees of Quantum-Si. The other authors declare no competing interests.