Detection of arginine post-translational modifications by single-molecule protein sequencing on the Quantum-Si Platinum™ platform

Kenneth A. Skinner, David Kamber, Haidong Huang, and Brian Reed[1]

[1] Quantum-Si Incorporated, 530 Whitfield St., Guilford, CT 06437

Abstract

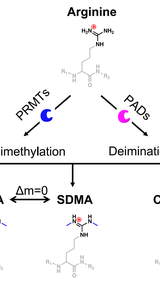
Aberrant post-translational modifications (PTMs) on arginine residues, namely methylation and citrullination, are closely linked to oncogenic processes. Detection of these PTMs is challenging with current technologies and can be hampered by low abundance and mass differences that are difficult to resolve by mass spectrometry. To overcome these hurdles, we developed methods for PTM detection using Quantum-Si's Platinum™ single-molecule protein sequencing platform.

To demonstrate the detection and differentiation of arginine PTMs, we applied our platform to distinguish between not only asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) but also between citrulline and native arginine residues. Sequencing traces revealed that arginine and ADMA bind the arginine recognizer with similar pulse duration (PD), whereas SDMA exhibited no binding. These results provide a clear kinetic difference between these isomeric arginine PTMs. Interestingly, arginine dimethylation also influenced the recognition of the upstream residue. Moreover, citrulline and arginine side chains also exhibited distinguishable kinetic signatures.

Citrullination eliminated N-terminal arginine recognition and resulted in a large increase in the median PD of preceding amino acid residues. Taken together, single-molecule protein sequencing offers an alternative approach to detection of arginine PTMs that is not based on *m/z*, but rather on the kinetic signature of binding between recognizers and N-terminal amino acids (NAAs). The ability to directly detect arginine PTMs offers potential for biomedical research. We envisage applications using Quantum-Si's platform to key areas of cancer research including biomarker development, PTM crosstalk, and drug discovery.

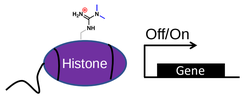
Arginine Methylation and Citrullination

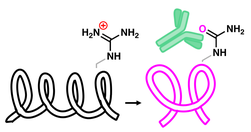
Effects of methylation:
- Increased steric interactions
- Rearranged H-bonding network
- Altered protein-DNA/RNA binding



Effects of reduced charge:
- Folding
- Function
- Half-life

Epigenetic regulation

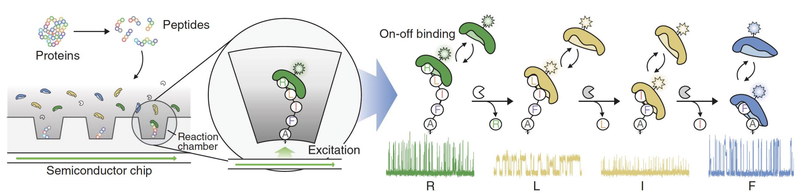




Amino acid	Moiety	Δm (Da)	pI	Charged?	H-bond Acceptors	H-bond Donors
Arginine	Guanidinium	-	11.4	Yes	0	5
Citrulline	Ureido	0.9840	5.9	No	2	3

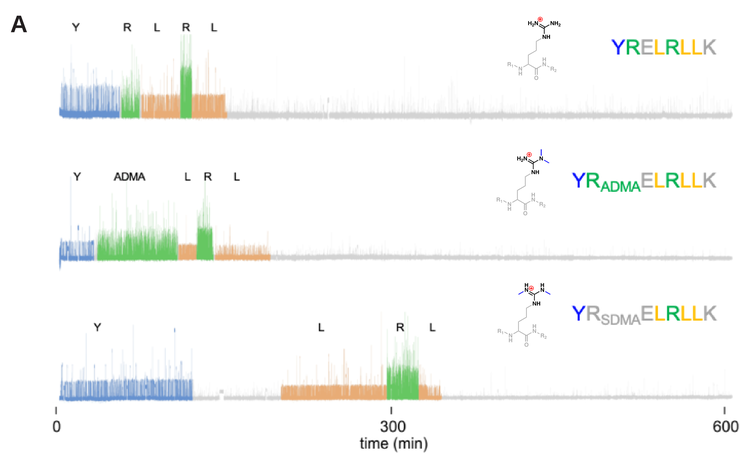
Protein Sequencing Workflow

- **Real time sequencing** of peptides immobilized in nanoscale reaction chambers
- **Recognizers** reversibly bind to the exposed NAAs.
- **Aminopeptidases** sequentially cleave one AA at a time from the N-terminus.
- **Binding kinetics** distinguish NAAs.
- **Fluorescence lifetime** differentiates dye-labeled recognizers.
- **Automated** signal processing and sequencing data analysis.



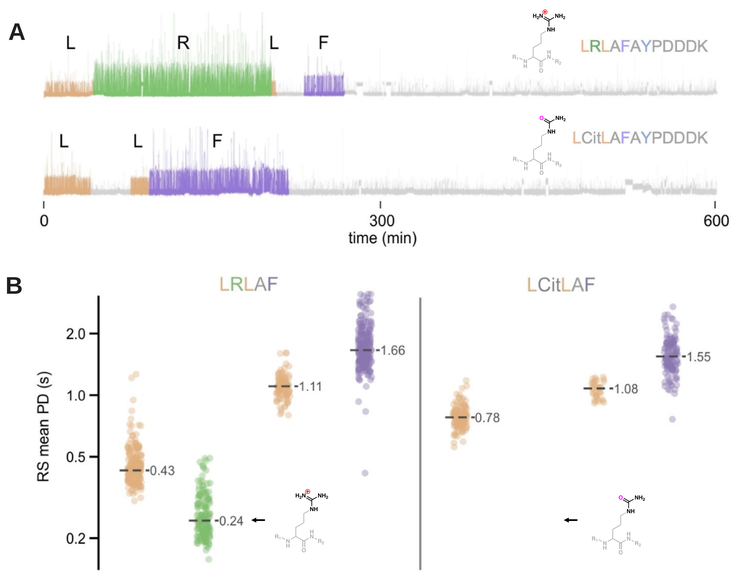
Results and Discussion

Asymmetric dimethylarginine (ADMA) increases pulse duration of preceding residue



Binding kinetics distinguish arginine residues and sites with arginine PTMs.
(A) Example sequencing traces for p38MAPKα-derived peptides (Methylation) and synthetics (Cit).
(B) Distribution of recognition segment (RS) mean PD for RSs corresponding to residues in each peptide sequence. Dashed lines indicate median values for each distribution.

Citrulline (Cit) eliminates R recognition and increases PD of preceding residue



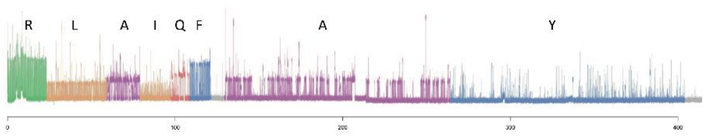
Conclusion and Outlook

Increased number of AAs detected with new recognizers

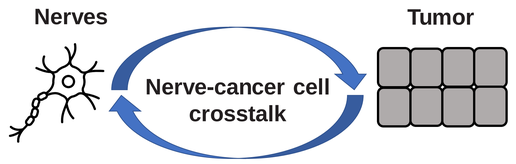
2022 I 3 recognizers I 7 AAs

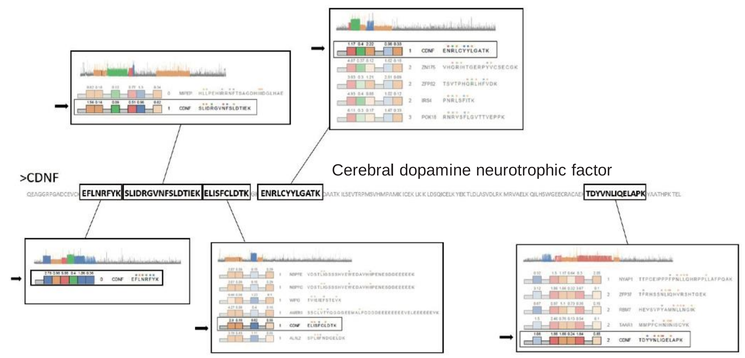
Q1 2023 I 5 recognizers I 11 AAs

Peptide: RLAIQFAYPDDD



Profile oncogenic proteins and biomarkers





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