## **QUANTUMS**

# **Quantum-Si's Next-Generation Protein Sequencer<sup>™</sup> Enables Protein Detection and Peptide Characterization from Biological Samples**

#### P-III-0753

John Kudolo, Khanh D. Q. Nguyen, Kenneth Skinner, Mathivanan Chinnaraj, Abde Ali Kagalwalla, Ben Moree, and **Brian Reed** 

Quantum-Si Incorporated, 29 Business Park Drive, Branford, CT 06405, U.S.A.

#### INTRODUCTION

Studies of the proteome would greatly benefit from methods that enable direct protein detection and peptide characterization with exceptional sensitivity. In this context, we present the unique capabilities of Next-Generation Protein Sequencing<sup>™</sup> on Quantum-Si's Platinum<sup>®</sup> instrument, which utilizes singlemolecule protein sequencing to achieve detection of unknown proteins and protein variants. Individual peptides are digested from proteins, immobilized on a semiconductor chip, and probed by dye-labeled N-terminal amino acid (NAA) recognizers, followed by subsequent aminopeptidase cleavage to expose each NAA in the peptide for recognition. Through the recording and analysis of fluorescent intensity, lifetime, and binding kinetics of each NAA binding event, we can successfully identify proteins and detect PTMs.

Below, the 25-kDa band was excised, sequenced, and identified to be APOA1.



Citrullination of arginine abolished arginine recognition by the R recognizer and led to an increase in PD of the preceding L residue.



To demonstrate the power of protein sequencing, we identified proteins from serum without prior knowledge, identified pathological PTMs, and utilized protein barcodes to screen and characterize proteins. We **isolated proteins from human serum** via immunoprecipitation or SDS-PAGE and correctly identified **them** from the sequencing data with high confidence by mapping to an 8,000-protein reference panel. Additionally, we demonstrated the power of Platinum to **detect PTMs based on** kinetic changes by detecting citrullination and dimethylation of arginine-two PTMs that play key roles in disease states such as cardiovascular disease, autoimmune disease, and cancer. Finally, we showcased the detection of peptide biomarkers along with the utilization of barcoding techniques to streamline protein engineering, antibody engineering, and enzyme engineering.

These results demonstrate the transformative potential of singlemolecule protein sequencing using Platinum for comprehensive proteome analysis. Our technology enables the detection and characterization of proteins and peptides in complex mixtures and biofluids, as well as the detection of critical PTMs implicated in human pathophysiology. As proteomics continues to advance, Quantum-Si's platform offers a promising approach to drive new insights into protein research and disease mechanisms.

Similarly, the 55-kDa band was identified to be ALBU with 99.99% confidence.



Finally, the 75-kDa band was identified to be TRFE with 99.99% confidence.



Below is a detailed analysis of the TRFE dataset, showing the coverage needed to correctly identified without prior knowledge, together with the number of alignments, level of confidence, and kinetic signatures of each peptide.

When spiked into a protein sample, a similar result was observed. Citrullination abolished arginine recognition and led to the recognition of the preceding L residue due to an increase in PD.



#### **METHODS**

- Proteins are reduced, alkylated, and digested with LysC.
- Peptides are functionalized, conjugated, and immobilized on the surface of a proprietary semiconductor chip.
- Fluorescently labeled N-terminal amino acid (NAA) recognizers and
- aminopeptidases are added to the semiconductor chip.
- Fluorescent intensity and duration of each NAA binding event generates a unique kinetic signature.
- Kinetic signatures are analyzed to align reads to reference peptides and compute false discovery rate (FDR).



2. SEQUENCE



(103-117): 15.548 Alianmen

P	D	К*		Т	VR	R W	С	A \	V S	E	Н	EA	Т	К*		C	Q S	F	R	D	H M	1 K*	¢	S	V	I P	S	D	G	PS	V	A	c v	<b>K</b> *		Α	S Y	L	D	C	R	Α	IA	A	NI	E A	D	A V	Т	LD	A	G	LV	Y	DA	Y	L	A P	Ν	Ν
v	v	А	E F	Y	G	S K	(*			E D	P	Q	T F	Y	Υ	A 1	V A	V	V	<b>K</b> *			DS	G	F	QI	MN	I Q	L	R	G K*			S	C	Н	T G	L	G	RS	Α	G	WN	I.	P	G	L	LY	С	DL	P	E	PR	К*			PI	E	<b>K</b> *	
																																																	197	ALIG		NTS   F	FDR = 3	3%						
V	Α	Ν	F F	S	G	s (	A	Р	С	A D	G	Т	DF	Р	Q	L	c q	L	С	P	G C	G	С	S 1	ΓL	Ν	Q	YF	G	Υ	s c	A 6	F	К*		С	L	<b>K</b> *		D	G	Α	G D	V	AF	· V	<b>K</b> *		н	S	ТІ	F	E	NL	Α	NK	*			
														-																						-														-			29	9 ALI	GNME	INTS	FDR =	2%	_	-
D	R	D	QY	E	L	L	C L	D	Ν	TR	K*			Р	V	DI	EY	K*			D	С	н	LA	A Q	v	Ρ	SF	I T	V	VA	A R	S	MG	G	<b>K</b> *			E	DI	. 1	W	EL	. L	N	A S	Q	EH	F	GK	*		E	F	QI	L F	S	S P	Н	G
																						6	6 41 10		NTS		- 5%																																	
L	L	F	К*			DS	5 A	Н	G	FL	<b>K</b> *			VF	PP	R	М	D	A K <sup>a</sup>	¢			MY	L	G	Y E	Y	V	Т	A   I	R	N	LR	E	G 1	гс	Р	E	A P	Т	D	EC	K*			P	/ K*			W	C A	L	S	H H	E	R L	<b>K</b> *			
																								102	ALIG	NME	NTS	FDR	= 13%																															
D	Е	W	s v	N	S	V	G K*			1	E	C	v s	Α	Е	ΤΊ	ГЕ	D	С	IA	A K*	¢		1	м	N	GE	A	D	AN	1 S	L	DG	i G	F	VY	1	A	GK	*		C	G	L	/ P	V	LA	Е	NY	r N	К*									
																-	_	_			_			-			_															_			79	ALIG	NMEN	ITS   F	DR = 1	13%		_	_	_	_		_	_		
D	Ν	С	E D	Т	Р	E A	A G	Y	F	A V	A	V	VK	*		S	Α	SI	D L	т	W	DI	NL	<b>K</b> *			S	СН	Т	A	VG	R	т	A G	6 W	Ν	I P	Μ	G	LI	. Y	Ν	<b>K</b> *			I N	H	C R	F	DE	F	F	SE	G	CA	I P	GS	5 K*		
																				18	7 ALI	GNN	IENTS	FD	R = 6%	6																																		
S	S	L	СК	*		1	LC	М	G	s g	i L	Ν	LC	E	Р	N	NK	k			EG	Y	Y	G ۱	ΥT	G	Α	F F	t C	L	VE	<b>K</b> *			G	D	VA	A F	V	К*			н	т	V	PO	Ν	ΤG	G	К*			N P	D	PV	N A	К*			
		-	•								-			-										-		-									0	2								< .		. ~			Ū					D						
		_			_		_							_				_	_						_	_	_										_			_				_																
L	Ν	E	К*		D	Υ	E	LL	. C	L	DO	GΤ	R	К*			Р	V	E	Υ	Α	N	СН	L	A	R A	P	Ν	ни	/ V	V	ТІ	R K <sup>a</sup>	¢		D	К*			Е	A	c v	н	K*																
						_							_										88	ALIG		NTS	FDR	= 5%									_			_				_																
L	R	Q	QQ	H	L	FG	G S	Ν	V	TC	C	S	G N	F	С	LI	FR	S	Е	TK	(*		D	L	L	FF	R D	D	Т	v	L	Α	K*			LH	D	R	N T	Y	E	<b>K</b> *			YL	G	EE	Υ	VK	*		A	V	G	L	RK	(*			

Pulse Duration (s) Example Trace	0.53       0.56       0.86       1.17       2.04       0.42       0.33         0.53       0.56       0.86       1.17       2.04       0.42       0.33         0.53       0.56       0.86       1.17       2.04       0.42       0.33         0.55       0.56       0.45       0.51       0.56       0.56       0.55       1.26       0.55         ignments       FDR = 13%)       INHCRFDEFFSEGCAPGSK (79 alignments)       FDR = 13%)       EGYYGYTGAFRCLVEK (187 alignments)       FDR = 6%)         Im       S 9%       92.2%       0.35       0.55       1.26       0.45       1.14%       Image: Second
$Example Trace$ $IMNGEADAMSLDGGFVIAGK (102 alignments   FDR = 13%)$ $Coverage \qquad 1 m n n r r r r r r r r r r r r r r r r r$	ignments   FDR = 13% $ignments   FDR = 13%$
IMNGEADAMSLDGGFVYIAGK (102 alignments   FDR = 13%) $Coverage 1 0.8% 91.2% 2.9% 45.1% 5.9% 92.2% 0.0% 9.12% 17.6% 47.1% 16.7% 9.8%$ $Pulse Duration (s) 0.44 0.56 0.26 0.53 1.37 0.28 0.28 0.78 2.26 0.32 0.68 0.42 0.39$ $IIMCRFDEFFSEGCAPGSK (79 alignments   FDR = 13%)$ $IIMCRFDEFFSEGCAPGSK (79 a$	ignments   FDR = 13%) $\begin{bmatrix} \mathbf{N} \\ \mathbf{S} \\ S$
Coverage       Im	M       S       L       D       GG       I       A       I       N       HC       T       Z       S       E       GC       A         0.28       0.78       2.26       0.32       0.68       0.42       0.39       1.28       1.04       0.27       0.34       0.51       0.46       1.59       1.59       0.33       1.27       0.84       0.44       0.46       0.58       0.61       0.33       2.64       0.42       1
Pulse Duration (s) 0.44 0.56 0.26 0.53 1.37 0.28 0.28 0.78 2.26 0.32 0.68 0.42 0.39 1.28 1.04 0.27 0.34 0.51 0.46 1.59 1.59 0.33 1.27 0.84 0.44 0.46 0.58 Example Trace	
Example Trace	
Mill of a transmit (Month) manufact (Month) manufact (Month) and a second	

### **Kinetic Signatures Enabled the Detection of Citrullination and Dimethylation of Arginine**

Asymmetric dimethylation of arginine (ADMA) led to an increase in pulse duration (PD) of the preceding Y residue. Symmetric dimethylation of arginine (SDMA) abolished arginine recognition by the R recognizer.



0.8

0.2

0.8

0.2

0 0.1

0 0.1

1.92

Pulse Duration (s)

Pulse Duration (s)

1.93

<sup>5</sup> 10

#### **Protein Barcodes Were Detected at Sub-Picomolar** Concentration

Protein barcodes are short peptides that are highly visible and distinguishable with NGPS on Platinum. When expressed with proteins of interest, they can facilitate multiplexing of functional expression of proteins.

#### These barcodes can be used for:

- Directly correlate multiple protein functions to sequence at once to increase throughput.
- Study protein trafficking of proteins from various organelles.
- Identify and characterize protein-protein interactions.
- Screen and characterize proteins with different properties and functions (e.g. mRNA vaccines)



#### **Mix of Engineered Proteins**



3. ANALYZE	
IL4   126 residues   22,160 Reads	E
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Coverage <b>E A</b> 97.1% 38.8
N T T E K* 42 42 E T F C R A A T V L R Q F Y S H H E K* 61 61 D T R C L G A T A Q Q F H R H K* 77	PD (s) 0.33 0.1
Q       L       I       R       L       D       R       L       D       R       L       W       G       L       A       G       L       N       S       C       P       V       K*       1405       E       A       N       Q       S       T       L       E       R       L       E       R       L       E       R       L       E       R       L       E       R       L       K*       15548         77       84       84       84       84       84       84       84       8	IPD (s) 11.8 15.
T I M R E K* Y S K*	ROI Start (m) 2.86 31.
* Alignment Count	ROI Duration (m) 17.8 34.

#### RESULTS

Unknown Proteins from Human Serum Were Correctly **Identified with Platinum** 

Human serum was run on SDS-PAGE, and individual bands were excised, followed by in-gel digestion, library preparation, and sequencing on Platinum. Three proteins, alipoprotein A-I, albumin, and transferrin were correctly identified without prior knowledge using the Protein Inference analysis workflow. The results were validated with Western blot.





In this experiment, we demonstrated the detection of these barcodes in a mixture of a wide dynamic range from 1,800–0.18 pmol. All barcodes were detected with false discovery rates (FDR) of < 2%.

Peptide	Ratio	Amount at Preparation	ARL - 1x				1083	89   FDR 0%				
ARLAFAYPDDDK	1×	1800 pmol	RLA - 0.1x	30	5793   FDF	R 0%						
RLAIQFAYPDDDK	0.1×	180 pmol	FQR - 0.01x	9619   FDR	0%							
FQRIALNFAKDGYPDDDK	0.01×	18 pmol	LRY - 0.001x	241   FDR 2%								
LRYAFAYPDDDK	0.001×	1.8 pmol	EFL - 0.0001x	202   FDR 1%								
EFLNRFYK	0.0001×	0.18 pmol		0 50	0000	1000	000	150000				
				Number of Alignments								

#### REFERENCES

Brian D. Reed et al, *Science* 2022, 378 (6166) 186-192.

All trademarks are the property of Quantum-Si, Inc. or their respective owners. For specific trademark information, see www.quantum-si.com/privacy-policy.

Research use only. Not for use in diagnostic procedures.