QuantumSi

APP NOTE

Quantum-Si's Next Generation Protein Sequencing Technology Enables Rapid and Accurate Distinction of Variants of the SARS-CoV-2 Virus

SUMMARY

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Accurate and rapid identification of SARS-CoV-2 variants is crucial for effective surveillance and disease monitoring. Furthermore, patients with long COVID may still have residual spike protein in their blood. Distinguishing variants in long COVID patients via protein sequencing could lead to new understandings and treatment of the disease.[1] In this application note, we present a method utilizing Quantum-Si's protein sequencing technology on the Platinum™ instrument to distinguish the Alpha, Delta, and Omicron variants based on differences in the amino acid sequence of their spike proteins.

Introduction

Since its emergence in December 2019, the SARS-CoV-2 virus has spread rapidly across the globe, leading to millions of infections and deaths. The emergence of new variants has raised concerns about the effectiveness of current countermeasures.[2,3] Currently, three dominant variants of concern, Alpha, Delta, and Omicron, have been identified by the World Health Organization (WHO) due to their increased transmission rates and potential to cause more severe disease. These variants have different mutations in their genetic sequence, leading to differences in their characteristics and behavior.

Quantum-Si provides an accessible protein sequencing technology to identify the unique protein sequences of a virus and distinguish between different variants. The technology relies on recognition of

Q-Si Technology

Quantum-Si's benchtop Platinum™ instrument enables protein sequencing from biological samples in a simple user-friendly workflow. Our technology utilizes dye-tagged N-terminal amino acid recognizers and semiconductor chip technology to detect the binding characteristics and binding order of N-terminal amino acids, resulting in unique kinetic signatures that can be used to differentiate and identify amino acid residues and PTMs. A more detailed overview of the workflow and technology can be found in our Science Paper.

single N-terminal amino acids (NAAs) with different NAA recognizers and sequential cleavage of NAAs with aminopeptidases. The information-rich output from protein sequencing enables the detection of differences among protein variants, such as single amino acid differences in peptide sequences and the presence or absence of specific peptides during infection and in long COVID patients with residual viral proteins. This feature makes the platform highly effective in detecting the various changes at the protein level that result from genetic differences among variant viral strains.

Methodology & Workflow

The receptor-binding domain (RBD) regions of the spike proteins in the SARS-CoV-2 variants are sequenced on Quantum-Si's Platinum instrument using our library preparation and real-time sequencing workflow as previously described.[4] The method entails digesting proteins into peptide fragments then linking them to macromolecular linkers at the C-terminus. The peptides are then immobilized on Quantum-Si's semiconductor chip, exposing the N-termini for sequencing. Dye-labeled recognizers bind on and off to NAAs, resulting in pulsing patterns with characteristic kinetic properties. Individual NAAs are removed sequentially by aminopeptidases in solution, uncovering subsequent amino acids for recognition. Fluorescence lifetime, intensity, and kinetic data are collected in real time, and analyzed with Cloud-based software to identify the peptide sequence and corresponding protein.

The trace-level output from protein sequencing on Quantum-Si's Platinum instrument consists of distinct pulsing regions called recognition segments (RSs). Each RS corresponds to a time interval between aminopeptidase cleavage events during which a recognizer binds on and off to the exposed NAA. Point substitutions to a target NAA can lead to binding events from a different recognizer or other changes in recognition pattern, resulting in a characteristic change in the binding kinetics observed as pulse durations (PD). Furthermore, substitutions at lysine positions can change the digestion pattern of the protein during library preparation by removing LysC cleavage sites, leading to the absence of peptides that would otherwise be present in the library and thus detected during sequencing.

Results & Discussion

ALPHA VARIANT CAN BE DIFFERENTIATED BY THE L452R MUTATION

We first sought to demonstrate the differentiation of the Alpha variant from the Delta and Omicron variants by single-molecule protein sequencing. We focused on a key mutation L452R that occurs in the

sequences of Delta and Omicron but not Alpha.

We obtained the RBDs of the spike proteins of the SARS-CoV-2 variants Alpha (Sino Biological, Beijing, China; #40592-V02H1), Delta (Sino Biological, Beijing, China; #40592-V08H90), and Omicron (Sino Biological, Beijing, China; #40592-V08H130).

We sequenced each protein on Quantum-Si's Platinum instrument using five recognizers—PS610 (F, Y, W), PS1165 (A, S), PS1220 (R), PS1223 (L, I, V), and PS1259 (N, Q)—and analyzed data to identify RSs, determine the mean PD of each RS, and characterize the kinetic signature of the peptides of interest. For the Alpha variant, we focused on the peptide VGGNYNYLYRLFRK, while for Delta and Omicron, we investigated the peptide VGGNYNYRYRLFRK.

The two peptides displayed distinguishable patterns due to the distinct kinetic influences of the L452R mutation on recognizer binding (see example traces in Figure 1). The leucine in the Alpha variant was observed via the long PD recognition event upon binding with recognizer PS1223, whereas the arginine in the Delta and Omicron variants was recognized by PS1220 and observed as a shorter PD recognition event. This clear kinetic difference demonstrates that the L452R mutation can be detected with Quantum-Si's Platinum instrument, effectively differentiating the Alpha variant from Delta and Omicron.

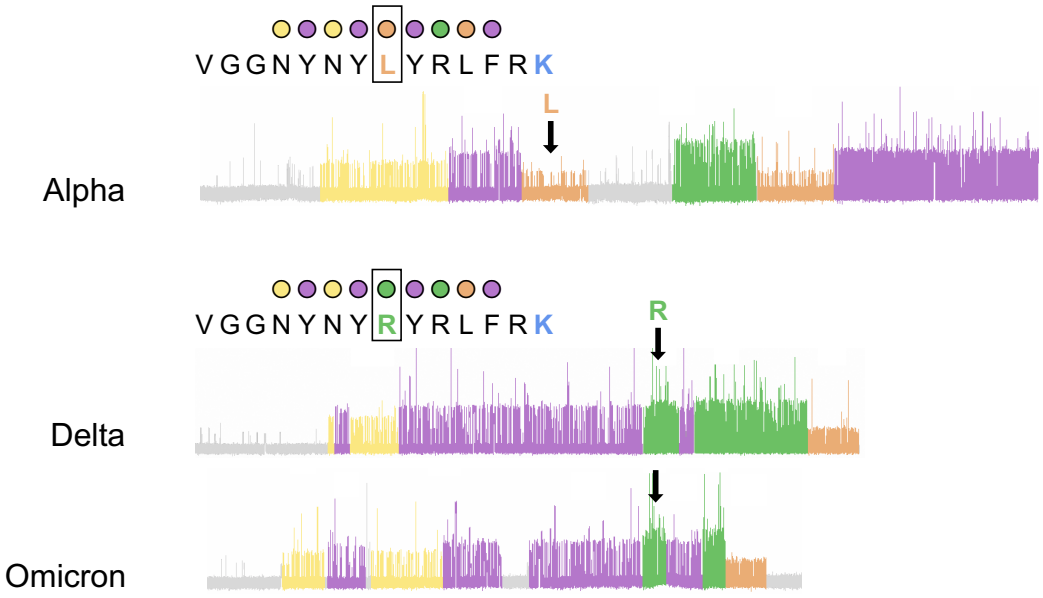

Figure 1. *Example protein sequencing traces for the peptide where the L452R mutation occurs.*

The two peptides displayed distinguishable patterns due to the distinct kinetic influences of the L452R mutation on recognizer binding (see example traces in Figure 1). The leucine in the Alpha variant exhibited was observed via the long PD recognition event upon binding with recognizer PS1223, whereas the arginine in the Delta and Omicron variants was recognized by PS1220 and observed as a shorter PD recognition event. This clear kinetic difference demonstrates that the L452R mutation can be detected with Quantum-Si's Platinum instrument, effectively differentiating the Alpha variant from Delta and Omicron.

OMICRON VARIANT CAN BE DIFFERENTIATED BY PEPTIDE I418–K424 DUE TO THE K417N MUTATION

Next, we sought to demonstrate the differentiation of the Omicron variant from the Alpha and Delta variants by focusing on the detection of peptide I418–K424 (IADYNYK). In the Alpha and Delta variants, this peptide is present due to lysC cleavage that occurs at residue K417 during library preparation. In the Omicron variant, the K417N mutation results in the loss of cleavage, leading to the absence of this peptide (Figure 2A).

We sequenced the recombinant spike RBD for each variant on Quantum-Si's Platinum instrument and analyzed the sequencing data to detect the presence of peptide I418–K424. For the Alpha and Delta variants, this peptide was readily detected and displayed a characteristic pulsing pattern (see example trace in Figure 2B). This peptide was not detected in the sequencing output from the Omicron variant. The absence of peptide I418–K424 distinguishes the Omicron variant from Alpha and Delta.

FIGURE 2A

FIGURE 2B

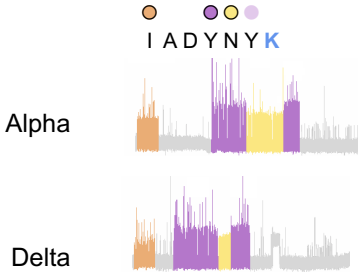

Figure 2. *A) The K417N mutation in the Omicron variant results in the loss of LysC cleavage and absense of the peptide I418–K424 (IADYNYK). B) Example protein sequencing trace for peptide I418–K424 in the Alpha and Delta variants. This peptide was not detected in the Omicron variant.*

These results indicate that the three variants Alpha, Delta, and Omicron can be distinguished using Quantum-Si's Platinum instrument. For the Alpha variant, we detected a leucine at position 452 and the presence of peptide I418–K424. For Delta, we detected the L452R mutation and peptide I418–K424. For Omicron, we detected the L452R mutation but not peptide I418–K424. These results are summarized in the table below.

	Position 452	Peptide I418–K424
Alpha	L	Detected
Delta	R	Detected
Omicron	R	Not detected

Conclusion

In this application note, we accurately distinguished three SARS-CoV-2 variants, Alpha, Delta, and Omicron using Quantum-Si's Platinum instrument. As the virus continues to mutate and new variants emerge, it is critical that Covid-19 variants be identified and tracked rapidly so that scientists can understand their potential impact on public health and develop effective treatments and vaccines. Furthermore, detecting the sequence of residual viral proteins in long COVID patients will aid in understanding the long-term effects of the disease. The ability to directly distinguish the variants of SARS-CoV-2 virus via Quantum-Si's next-generation protein sequencing platform can potentially accelerate the diagnosis and treatment of Covid-19 patients and support surveillance efforts to contain the spread of the virus.

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