QuantumSi

APP NOTE

# Enrichment and Sequencing of Albumin Protein from Urine

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## Summary

The identification and analysis of protein markers in biofluids play a crucial role in disease diagnosis. Urine, as a non-invasive sample source, enables the monitoring of disease progression through biomarker analysis. However, traditional methods for detecting biomarkers often overlook important protein variants and require complex techniques. The Quantum-Si's next-generation protein sequencing Platinum™ workflow has the potential to revolutionize biomarker analysis. In this application note, we demonstrate enriching human serum albumin (HSA) from urine and sequencing HSA on Platinum.

## Introduction

Disease-related diagnosis often requires enrichment of protein markers from biofluids. Analyzing biomarkers and variants of biomarkers, such as albumin, from biofluids is important to understand disease. Urine represents an ideal biomarker source as samples can be collected via non-invasive methods, enabling temporal monitoring of patient conditions. In addition, physiological states are often reflected in urine as homeostasis leads to the continuous removal of these potential biomarkers from blood. Human serum albumin (HSA) is the most abundant protein in circulation (0.6 mM), accounting for 60% of the total proteins in plasma. However, albumin is normally filtered in the glomeruli and reabsorbed or catabolized by the proximal tubules, leading to absent or barely detectable albumin levels in urine. Thus, kidney damage and albumin leakage resulting from ailments such as

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.

diabetes and chronic kidney disease (CKD) may manifest as albuminuria.

Standard techniques to detect albumin and other biomarkers from biofluids include antibody-based methods such as enzyme-linked immunosorbent assay (ELISA), measurement of light scattering such as nephelometry/turbidimetry, and instrumentation such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS). These techniques often require expensive reagents or sophisticated equipment to enrich albumin from sample matrices. Pulldown methods such as affinity chromatography are also widely employed to not only remove abundant albumin that may mask the isolation of serum proteins of interest but also separate albumin for downstream applications. However, these techniques may not provide critical information about the presence of biomarker variants that could impact disease. A sensitive method capable of detecting changes at the amino acid level is required to identify biomarker protein variants.

The Platinum™ next-generation protein sequencing workflow enables deep interrogation of biomarkers isolated from biofluids. With single-molecule resolution of the amino acid sequence, Platinum can distinguish changes in amino acid sequence and proteoform variants of the same protein, further enhancing our understanding of the influence of changes in biomarkers on disease. To demonstrate how biomarkers can be isolated from biofluids and sequenced on Platinum, we enriched HSA from urine with elevated protein levels and sequenced HSA on Platinum.

## Methodology & Workflow

To demonstrate a potential workflow to enrich and sequence HSA from urine with elevated protein levels, we utilized an affinity ligand-based resin to enrich HSA spiked into pooled human urine from healthy donor samples. Micromolar HSA levels in urine may indicate macroalbuminuria. Thus, commercial HSA was diluted into pooled urine at a final concentration of 10 µM to demonstrate the feasibility of our workflow and technology for enriching and sequencing physiological levels of HSA from a clinically relevant biofluid.

In this workflow, HSA reversibly binds to Cibacron Blue, a polycyclic and polysulfonate triazine adsorbent covalently coupled to cross-linked agarose. HSA is a negatively charged plasma protein, facilitating reversible binding to the solid support via electrostatic and hydrophobic interactions.

Instructions were followed based on manufacturer recommendations for Pierce Albumin Depletion Kit (Thermo Scientific). Briefly, the albumin depletion resin, supplied as a 50% slurry, was resuspended into a spin column and centrifuged to remove storage solvent. The resin was then equilibrated with binding/wash buffer consisting of 25 mM Tris, 75 mM NaCl, pH 7.5. 100 µL of HSA-containing urine was added to the conditioned resin and briefly incubated at room temperature. The sample was then centrifuged, and the flow-through was reapplied to the column to increase protein recovery. The resin was then washed to remove unbound proteins. HSA was eluted from the resin via stepwise increasing ionic strength with NaCl to disrupt HSA-ligand binding. Next, the enriched HSA was denatured with guanidine hydrochloride and subjected to library preparation using Quantum-

Si's Library Preparation Kit (Catalog Number: 910-10011-00), consisting of reduction of disulfide bonds, alkylation of cysteine residues, digestion with LysC protease, and conversion of C-terminal lysines for subsequent coupling to macromolecular linkers for immobilization and sequencing on our semiconductor chip.

The completed HSA peptide library was loaded at 10 nM in one flow cell of a semiconductor chip following the standard Quantum-Si protein sequencing protocol (Part Number: 950-100006-00) and utilizing reagents and materials from a Quantum-Si Protein Sequencing Kit (Catalog Number: 910-0003-00). Total run time was set to 12 hours and all other device and chip parameters were set to default settings. During the run, five fluorescently labeled N-terminal amino acid (NAA) recognizers with specificity for 11 NAAs (F, Y, W, L, I, V, R, A, S, N, and Q) bind on and off to exposed NAAs of the immobilized peptides (Figure 1A). Fluorescence signal, including fluorescence intensity and lifetime, is recorded by the semiconductor chip. Aminopeptidases sequentially cleave amino acids, exposing the next NAA for sequencing. Data collection then continues until the whole peptide is sequenced, after the completion of the Platinum run, data was automatically and securely uploaded to Quantum-Si's Cloud software, where trace data was mapped to the albumin protein using our standard peptide alignment workflow (view **Software Technical Note**). Reads mapping to eight different peptide fragments from a LysC digestion of HSA were identified by the software.

## Results & Discussion

Sequencing data analysis using Quantum-Si's Cloud software pipeline resulted in 8 unique peptides identified and properly mapped to the albumin protein (Figure 1B). Peptide identification and mapping was based on the identification of traces containing multiple high-confidence recognition segments (RSs) with the correct recognizer order and average pulse durations (PD) corresponding to predictions from Quantum-Si's kinetic prediction model. Example traces are displayed for each HSA peptide that was identified by the software analysis pipeline in Figure 1C.

These results show that protein sequencing on Platinum has the power to identify important proteins isolated from biological fluids. We applied an affinity resin that offers affordability, operational simplicity, scalability, and high binding capacity for HSA to our streamlined library prep workflow and automated software analysis to produce sequencing alignments that map to HSA. We envisage that this workflow can be used to not only enrich and sequence HSA from complex sample matrices but also identify interacting partners of HSA, a carrier for proteins and drugs, and distinguish variants of other biomarkers found in biofluids.

A.

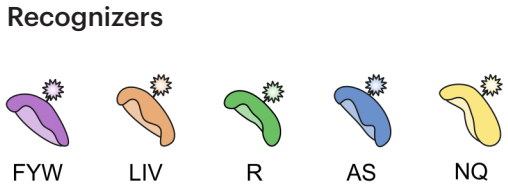


***Figure 1A.*** *The Five NAA Recognizers Used in This Study. The target NAAs for each recognizer are indicated.*

B.


***Figure 1B.*** *The Protein Sequence of HSA (Official Name ALBU) Was Digested with LysC In Silico and Run Through Quantum-Si's Kinetic Prediction Software. Amino acid positions with high predicted average pulse duration (> 0.3 s) are indicated by circles with bold shading. Positions with low predicted average pulse duration (0.1–0.3 s) are indicated with light shading. Boxes indicate HSA peptides that were identified in the sequencing output by the software analysis pipeline.*

C.

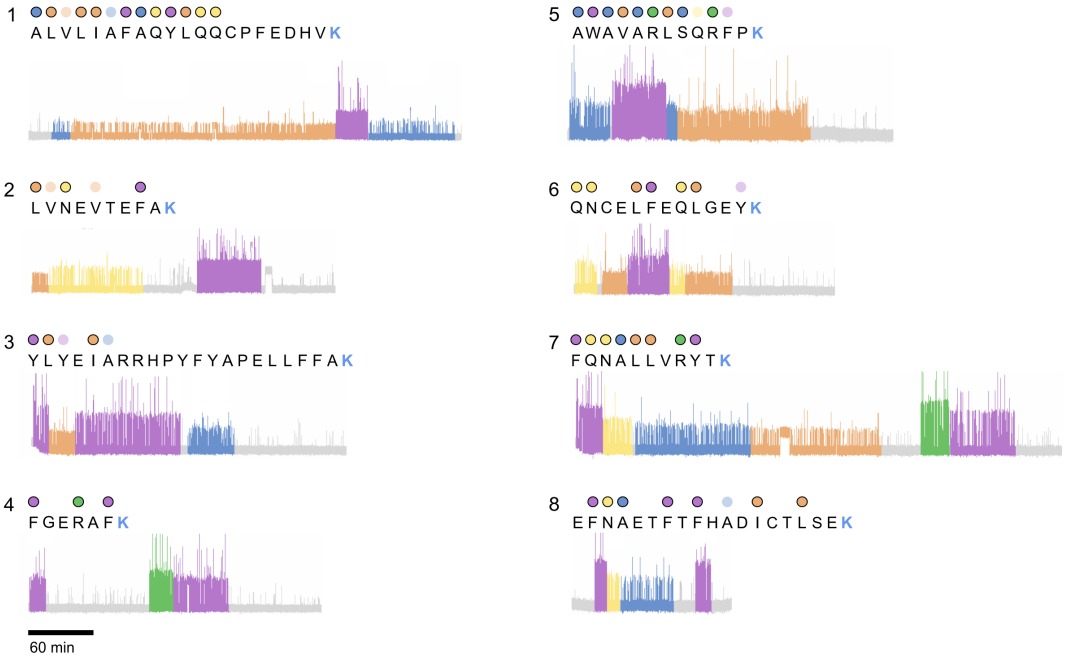


***Figure 1C.*** *Example traces are displayed for each HSA peptide that was identified by the software analysis pipeline.*

## REFERENCES

Software technical note Quantum-Si Cloud: A Scalable Informatics Platform for Protein Sequence Data Analysis