Structural O-Glycoform Analysis of SARS-CoV-2 Enabled by Top-down Mass Spectrometry and Trapped Ion Mobility

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) utilizes an extensively glycosylated surface spike (S) protein to mediate host cell entry, and the S protein glycosylation plays key roles in altering the viral binding/function and infectivity. However, the molecular structures and glycan heterogeneity of the new O-glycans found on the S protein regional-binding domain (S-RBD) remain cryptic because of the challenges in intact glycoform analysis by conventional bottom-up glycoproteomic approaches. Here, we report the complete structural elucidation of intact O-glycan proteoforms through a hybrid native and denaturing top-down mass spectrometry (MS) approach employing both trapped ion mobility spectrometry (TIMS) quadrupole time-of-flight and ultrahigh-resolution Fourier transform ion cyclotron resonance (FTICR)-MS. Native top-down TIMS-MS/MS separates the protein conformers of the S-RBD to reveal their gas-phase structural heterogeneity, and top-down FTICR-MS/MS provides in-depth glycoform analysis for unambiguous identification of the glycan structures and their glycosites. A total of eight O-glycoforms and their relative molecular abundance are structurally elucidated for the first time. These findings demonstrate that this hybrid top-down MS approach can provide a high-resolution proteoform-resolved mapping of diverse O-glycoforms of the S glycoprotein, which lays a strong molecular foundation to uncover the functional roles of their O-glycans.

In this Webinar, you will hear about new strategies in top-down proteomics, trapped ion mobility, and native mass spectrometry to enable the structural characterization of O-glycan microheterogeneity and complex glycoproteins. David Roberts, from the laboratories of Professors Ying Ge and Song Jin at UW-Madison, will present novel proteoform-resolved top-down mass spectrometry approaches using the Bruker timsTOF Pro and 12T solariX FTICR-MS for the comprehensive analysis of the S-RBD. This hybrid top-down mass spectrometry strategy can also be leveraged to elucidate the molecular signatures of emerging S-RBD variants of SARS-CoV-2 toward structure–function studies and can help catalyze the studies of other O-glycoproteins in general.

David is a rising 5th year PhD student in the Department of Chemistry at the University of Wisconsin (UW)-Madison. He obtained his Bachelor of Science in 2016 from the University of California, San Diego, where he double-majored in chemistry and mathematics. In 2017, David joined the labs of Professors Ying Ge and Song Jin as a joint chemistry PhD student. His graduate thesis goal is to establish new top-down mass spectrometry (MS) compatible methodologies and novel functionalized nanomaterials that can be utilized to investigate a wide range of biological questions. Focusing on addressing the major challenges of the human proteome dynamic range and the comprehensive analysis of complex proteins with post-translational modifications (PTMs), David's highly interdisciplinary research work ultimately seeks to provide new technologies, knowledge, and methods for the advancement of top-down proteomics and functional materials for investigating cardiovascular related proteins, receptor proteins, and, by extension, the human proteome.