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Revealing Functional Proteoforms by Native Top-Down Proteomics

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Native mass spectrometry (nMS) measures proteins and complexes that are functionally relevant to biology. Top-down proteomics (TDP) reveals identification, sequence, and proteoform information. The combination of these platforms, native top-down proteomics (nTDP), could be ideal for understanding how proteins interact with other proteins (and ligands and cofactors), identifying unknown proteoforms, and gain information on their function at near physiological conditions. We are developing a nTDP workflow based on data independent acquisition (DIA) without on-line chromatographic separation to address two microbial-based projects. Understanding the host-pathogen interface is key to combating antimicrobial resistance. For unfractionated secretomes of model Gram+ pathogenic bacteria, Corynebacterium diphtheriae, a single direct infusion revealed more than 370 unique masses. We identify more than 70 proteoforms, including novel virulence factors and complexoforms reaching 300 kDa. A functional proteomics platform based on slow mixing mode (SLOMO) and DIA-proton charge reduction (PTCR)/higher-energy collisional dissociation (HCD) was developed to determine how they acquire iron during infection. For the second project, we apply nTDP to elucidate the structure and composition of the cellulosome, a massive (0.5-2 MDa), self-assembling, multi-enzyme complex with potential implications to the carbon cycle and sustainable biofuel production. Its function is to break down lignocellulose and other biopolymers. To date, little is known about the specific structure and composition of intact cellulosomes. Preliminary nMS of putative Clostridium thermocellum cellulosomes reveals highly complex spectra. By applying electron-capture charge reduction (ECCR), masses in the 100-300 kDa range were deconvolved. For a 184 kDa complex detected, HCD-based nTDP identified it as a homohexamer enoyl hydratase (29 kDa monomer) complex.

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yes

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