INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Reference-free Chemical Threat Characterization

Christopher Harrilal Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Adam Hollerbach** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Katherine Schultz** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Danielle Ciesielski** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Yehia Ibrahim** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Simone Raugei** Physical and Computational Sciences Directorate, Pacific Northwest National Laboratory **Bobbie-Jo Webb-Robertson** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory **Robert Ewing** National Security Directorate, Pacific Northwest National Laboratory **Thomas Metz** Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory

This presentation summarizes results from an application directed towards the ‘reference-free’ identification of fentanyl analogs using a novel multi-dimensional approach for high-confidence chemical threat characterization and identification that eliminates the need for standard reference materials (SRMs). This approach integrates, state-of-the-art mass spectrometry (MS)-based empirical measurements that provide a combined resolution greatly surpassing traditional measurement platforms, and accurate property predictions performed using machine learning (ML)/quantum chemical (QC) approaches. MS-based techniques are well-suited for the early detection of bio/chemical threats as they have the sensitivity and resolution required to detect and characterize various compounds in trace amounts. Chemical identification using traditional MS approaches relies on matching measured chemical signatures (Ex. chromatographic retention time, mass, and fragmentation spectra) to similar information contained in reference libraries that are generated through analysis of pure compounds. This approach, however, fails to adequately address the chemical identification challenge because: 1) the number of authentic reference compounds is limited compared to the number of potential chemical threats and 2) the number/dimensionality of chemical signatures routinely measured using traditional approaches is inadequate for resolving structurally similar chemicals or providing the necessary specificity for unambiguous identification. To overcome these limitations, the m/q Initiative at Pacific Northwest National Laboratory (PNNL) is applying a novel approach where we experimentally measure 5 chemical properties with high resolution, predict those measured properties for several input chemicals using QC and ML, and finally match between the predicted and experimental signatures for confident reference-free identification of chemical constituents.
Here, we will discuss the success and challenges of this approach from our proof-of-concept demonstration using fentanyl analogs, a class of constantly evolving synthetic opioids that continue to pose a significant public health risk. In this demonstration, a mixture of fentanyl analogs was measured using new multidimensional platforms developed at PNNL that couple Structures for Lossless Ion Manipulations (SLIM) with Orbitrap MS and SLIM coupled to cryogenic infrared (IR) ion spectroscopy and MS. The enhanced resolution of these platforms led to the observation of signatures highly indicative of the presence of fentanyl analogs. Analysts processed the full data set in a blinded fashion to evaluate the potential of the integrated multi-dimensional experimental/computational approach to identify fentanyl analogs in a reference-free manner. To address the problem of accounting for the vast chemical space of potential new fentanyl analogs, we developed software to computationally predict possible structures via combinatoric rearrangement and stored the library to aid in downstream analysis. Computationally predicted properties were generated for 90,000 analogs: CCS values were calculated using ML algorithms (SigmaCCS) and first principle-based methods (HP-Ω), while tandem mass spectra were predicted using the graph neural network tool QC-GN2oMS2. Comparison of the measured properties to computationally predicted properties resulted in a single fentanyl analog match to each set of properties, demonstrating the feasibility of high-dimensional measurements and computational predictions of chemical properties for reference-free identification of bio/chemical threats.

The authors would like to acknowledge the m/q initiative - a Laboratory Directed Research and Development Program at PNNL.