THREAT AGENT DEFEAT MODELING AND TESTING USING WMD SIMULANTS

Identification and Validation of Protein-chemical Threat Interactions from Activity-based Protein Profiling (ABPP) Data

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Activity-based protein profiling (ABPP) is a chemoproteomic technique that uses small molecule probes to mimic chemical structures and/or reactivities to identify their protein targets in complex proteomes, such as tissue homogenates. We previously developed 13 probes for 7 different chemicals of concern, including organophosphates (OPs) and pharmaceutical-based agents (PBAs), and applied these probes to profiling 5 tissue types across 6 mammalian species, including humans. The protein targets identified through our ABPP approach represent proteins that bind the chemical threats of interest in vitro. However, ABPP alone cannot elucidate the nature of the binding interaction, whether it activates, inhibits, or does not affect the protein's function. Therefore, to confirm the biological consequences of these protein-ligand interactions, we are performing both in silico modeling and experimental validation.

For computational modeling, we verified that our synthesized probes accurately represent the accessibility of the corresponding chemical threats, in terms of steric hindrance and energetic preferences, through molecular dynamics simulations. Additionally, we automated the generation of input files, the parallel execution, and the analysis of output data for DiffDock and AutoDock Vina. These high-throughput docking simulations provided in silico evidence that our probes accurately mimic actual ligand binding in terms of both binding site locations and binding energy rankings. For ligand bindings that involve covalent bonds, we have recently utilized a state-of-the-art diffusion deep learning program, RosettaFold All Atom (RFAA). We have also automated the retrieval of protein structures predicted by AlphaFold2 (AF2). Most of these AF2-predicted structures were comparable to those obtained from experimental methods and other protein structure prediction programs (i.e., OmegaFold2, RosettaFold2, ESMFold, and RFAA) as confirmed by our extensive benchmarks.

Because our ABPP approach generated a list of numerous target proteins, we prioritized proteins for experimental validation by incorporating observations with metadata associated with the tissue type and animal model. This included development of a scoring model that accounts for protein-ligand interactions across multiple tissue types, animal biological sex, and the observation of ABPP targets among predicted orthologs identified for the set of animal models. Our model also allows for the weighting of specific criteria within the metadata, such as applying greater weight to the observation of target proteins only within specific animal models, number of orthologs, tissues, or within one biological sex. Visualization of calculated scores has allowed the prioritization of targets, with many of these supported by previously published findings. However, some targets appear to be novel entities for additional investigation.

For experimental validation, we selected 11 proteins that bind to agents such as paraoxon, fentanyl, ketamine, and dexmedetomidine. One of our targets, histamine N-methyl transferase (HNMT), was identified through ABPP experiments and corroborated by in silico modeling. Our detailed substrate and probe titration experiments provided robust data on binding characteristics, including specificity. We are currently performing an HNMT assay to further characterize the interaction dynamics, such as competitive versus non-competitive inhibition or the potential for drugs to act as alternative substrates.

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