Can Hemoglobin binding models be used to predict toxicity of mixtures of Forever Chemicals?

Per- and poly-fluoroalkyl substances (PFAS), have become pervasive environmental contaminants across the globe. Their water and oil-repellent and heat-tolerant properties made them the key ingredient for products like Teflon, food packaging, protective clothing, among many other uses. Consequently, the prevalence of these chemicals has also increased in the environment, where both humans and wildlife can be exposed. Many PFAS are highly bio accumulative, meaning that they can build up to high concentrations in both humans and wildlife; indeed, > 99% of American adults have detectable PFAS in their blood. Concerningly, PFAS are increasingly understood to be toxic to both organisms and are associated with myriad health problems, including, but not limited to altered metabolism (especially lipid homeostasis), disruption of thyroid and reproductive systems, and even some forms of cancer, among other modes of toxicity. Compounding the problem is the sheer number of structurally diverse PFAS that have been released. There are over 14,000 known PFAS, with many more likely to be discovered as analytical capabilities advance. Because humans and other organisms can be exposed to complex mixtures of PFAS, there is a pressing need for models that can predict the effects and toxicity of PFAS singly and in the context of mixtures, testing all PFAS and possible mixtures is logistically not feasible. Because PFAS interact strongly with proteins, one method to predict toxicity of single PFAS and mixtures might be to focus on building *in silico* models that predict PFAS-protein interactions based on structure and then calibrating these models with assays testing binding affinities and toxicities of structurally diverse PFAS. Evidence has shown that PFAS can bind to hemoglobin (Hb), interfering with oxygen transport. The small non-biting midge, *Chironomus dilutus,* is the perfect model for testing PFAS/Hb binding as these aquatic insects produce an abundance of hemoglobin to keep up with oxygen demands. Midge plasma proteins are composed of roughly 98% hemoglobin, and midges are extremely sensitive to PFAS exposure, suggesting Hb-mediated PFAS toxicity. **Using human and midge hemoglobin, we aim to determine if PFAS-Hb binding is a traceable signal for predicting toxicities of single PFAS and mixtures.** To test this, we are using a combination of *in silico* models, which will be validated and refined using a combination of equilibrium dialysis experiments, dose-response experiments measuring changes in fluorescence spectra when PFAS bind to Hb's, and *in vivo* toxicity assays with the midges themselves. We will present preliminary findings for a subset of experiments conducted as a part of this large, collaborative project. Ultimately, we hope that this approach will enable prediction of how single and mixture exposures to PFAS lead to toxicity for the tens of thousands of PFAS and PFAS mixtures found in the environment and biota worldwide.