TITLE: **Using Fluorescence Spectroscopy to Study PFAS Mixtures: A Case Study with Hemoglobins**

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There is a need for the development of novel approaches for studying the toxicity of per- and polyfluoroalkyl substances (PFAS) mixtures. This is a critical gap as > 12,000 different PFAS have been reported and it would be impossible to test all PFAS and their combinations using standard toxicity approaches. The overall goal of our work, is to determine whether PFAS mixture toxicity deviates from additivity. We take advantage of PFAS’ ability to bind to proteins with different affinities to answer this question. We will present data on PFAS-Hb interactions including emission spectra, fluorescence intensity, and fluorescence lifetime data from human Hb incubated with 11 PFAS ligands (PFNA, GenX, PFOS, PFDA, PFOA, PFDS, PFBA, PFHxS, PFBS, PFHxA, and PFHpA) both singly and in binary combinations. Binding affinity constants will be compared across PFAS and between single and binary mixtures. This is the first study using this approach for evaluating PFAS mixture toxicity. This data will be used to inform and validate computational in silico models being developed to predict PFAS toxicity in vivo and result in concentration addition.