Determinig structure of DHP in complex with larger, polycyclic substrates utilizing micoED/3DED

As a model for probing the protein structure-function correlation in the hemoprotein superfamily in humans, our work has focused on the enzyme dehaloperoxidase (DHP), the coelomic hemoglobin from the marine worm *Amphitrite ornata*. The enzyme employs several forms of enzymatic activity and we have established that the binding of the substrate itself is determinant for the form of DHP activity that the enzyme will carry out in the process of modification/degradation of that particular substrate. Exhibiting the variety of enzymatic activities such as peroxidase, peroxygenase, oxidase and oxygenase, this enzyme has been shown to play a role of a detoxifying agent for *A. ornata* to survive harsh environments by performing the oxidation of marine pollutants that are produced by other organisms in its living environment. We have also shown (1,2) that great many of existing as well as emerging pollutants of anthropogenic origin, such as bi- and bisphenols that have been linked to adverse health effects in humans and are commonly monitored in the environment, have turned out to be substrates for DHP. Therefore, it is particularly important to determine their mode of interaction with DHP and their subsequent modification by the one of the activities of this enzyme. For illustration, the reaction of BPA with DHP in presence of the co-substrate H_2O_2 is shown in Figure 1, also showing products of this reaction.

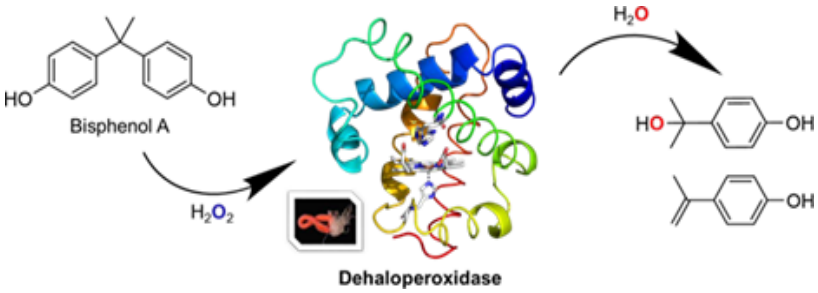

Figure 1. The reaction of BPA with DHP, shown as its 3D structure, in presence of H_2O_2 leads to its oxidation products generated by DHP's peroxidase activity (*Amphitrite ornata* is also shown).

In general, we have been able to determine structures of DHP in complex with many of its substrates using X-ray crystallography. However, many larger polycyclic substrates have been recalcitrant to both co-crystallization and crystal soaking and although we are able to determine the substrate binding affinities (using UV-Vis spectroscopy) we were not able to determine structures of very important DHP-substrate complexes, such as BPA (Figure 1) utilizing X-ray crystallography. Therefore, we want to use the cryo-EM method microED since we will be able to use sub micrometer size crystals to increase the likelihood of diffusion of these larger substrates into the crystal.

For the microED structure determination that we want to explore when the substrates are too large for easy diffusion into a preformed crystal, we have optimized the preparation of small, micron to submicron size crystals by varying volume ratio of protein (30 mg/ml) and mother liquor containing 40% PEG 4000 as the crystalizing component using 0.6 ml Eppendorf tubes and incubating one or two days at 4 °C (Figure 2A and 2B)

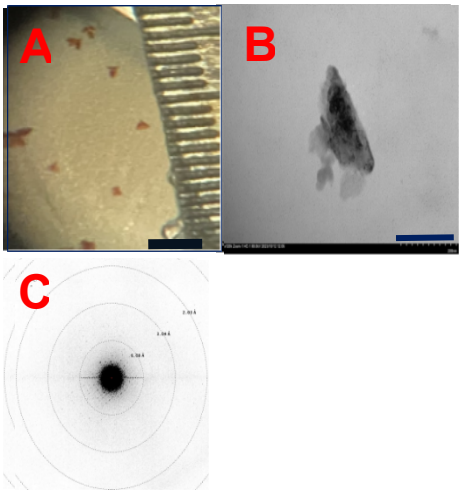

Figure 2. A) Sizes of crystals grown for microED experiments in batch crystallization mode as described (scale bar 100 µ), B) size of crystal on TEM grid selected for a microED experiment (scale bar 200 nm), C) diffraction pattern recorded in the initial trials of collecting electron diffraction of DHPb crystal in the cryo-EM core facility at UNC-CH: recorded on K3 camera in counting mode using Thalos Arctica electron microscope operating at 80 K and acceleration voltage of 200 kV.

According to the initial experiments I was able to observe the electron diffraction of DHP crystals, but I am still working on the improvement of the crystal electron diffraction, trying to reduce viscosity of the crystallization solution in order to further reduce the thickness of ice in which the crystals are embedded. In the latest developments I was able to prepare grids using Leica GP2 cryo plunger at NIEHS cryo-core facility, from which it is easier to observe crystals in the cryo-TEM screening, shown in figure 3, although to identify crystals with more precision I still need to perform diffraction screening (not yet available at NIEHS facility) on selected areas in order to eventually be able to collect the whole, publishable electron diffraction data sets, and process them using established macromolecular X-ray crystallography

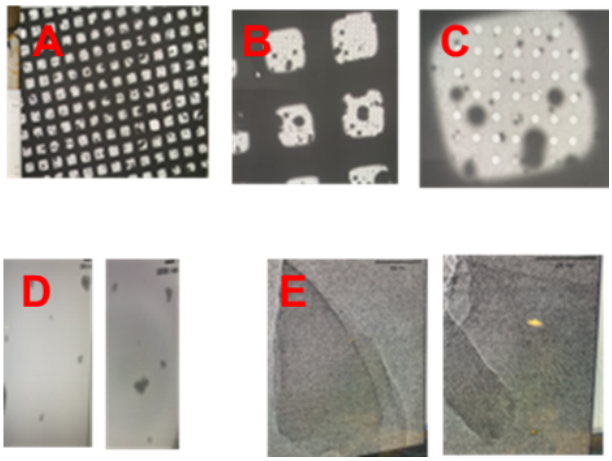

Figure 3. A) Grid atlas at 65.5 X magnification, B) atlas zoomed in, C) grid square at 1250 X magnification, D) grid square zoomed in showing probable crystal, E) crystals at 36000 X magnification.

References

1. Yun, D. et al. J. Inorg. Biochem. 238, 112020, 2022
2. Aktar, M.S. et al. J. Inorg. Biochem. 247, 112332, 2023