We initiated the studies of multiprotein P450 complexes using TEM as the primary tool four years ago. We have since published three peer-reviewed articles on this subject in collaboration with Drs. Yoichi Osawa and Min Su. (1-3).

**Preliminary Data**

Using cryo-EM we reconstructed a 3D map at 6.2 Å of a soluble form of P450 variant enzyme referred to as CYP102A1 (2). CYP102A1 is a natural fusion protein containing both P450 and POR domains, and has served as a paradigm for studying electron transfer within the P450 complexes. However, unlike human P450 complexes, CYP102A1 lacks transmembrane regions, a fundamental difference that makes it unfit to model drug metabolism and pharmacokinetics. Nonetheless, the experience we gained from studies of CYP102A1 led us to initiate the studies of membrane-bound P450 complexes using cryo-EM.

*1). Preparation of membrane-bound P450:POR complexes in amphipols*

As we recently reported (1), we successfully prepared a functional tetrameric complex of P450:POR in amphipols. The stoichiometry of the complex is 1:1 for P450:POR. Using negative stain EM we showed the complex was homogenous and revealed its architecture.

*2) Preparation of the complex of P450:POR in vitrified ice*

To obtain high resolution structures, we extensively optimized the conditions for vitrification of the complex and screened for intact single particles. We succeeded in vitrifying the complex for cryo-EM studies using Chameleon. As shown in Figure S1, single particles of the vitrified complex are visible. Based on a small dataset of ~1000 micrographs, we obtained 2D images of the complexes as shown in Figure S2.

*3) Experimental approach*

Our preliminary results from negative stain EM and cryo-EM show the complexes of P450:POR are suited for further cryo-EM studies to obtain high resolution structures. We propose to obtain larger datasets in the range of ~6000-10,000 micrographs to reconstruct a 3D map of the complexes to achieve a resolution of ~4 Å or better. We will prepare the vitrified complexes with Chameleon and screen the grids on a Thermo Scientific Glacios microscope at the UM cryo-EM core facility. Selected grids will be shipped to NCCAT in liquid nitrogen. We propose to collect images on Titan Krios microscope (300 kV) with a K3 detector. If initial dataset are promising we would also like to take advantage of the energy filter available at NCCAT to improve resolution. Access to your facilities is critical for us to make progresses on our NIH funded projects.

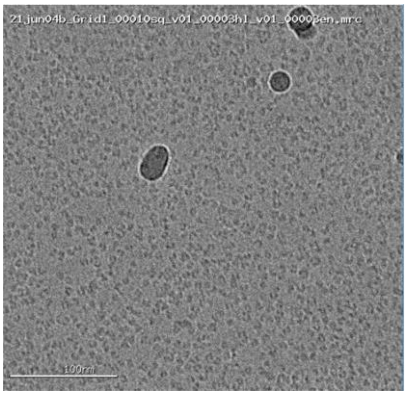

**Figure S1.** A representative micrograph of vitrified complexes of P450:POR. The complex was vitrified with Chameleon.

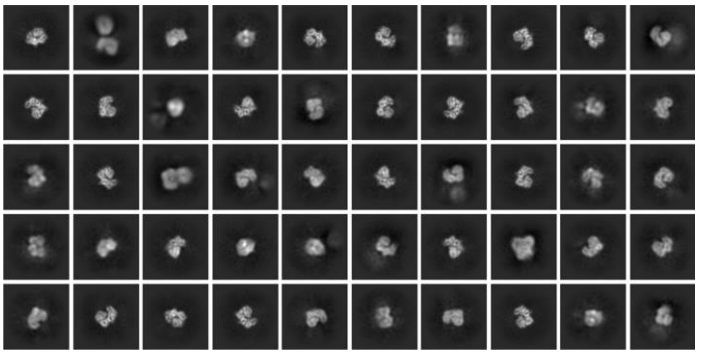

**Figure S2.** Reference-free 2D classification of single particles of the vitrified complexes of P450:POR. A small dataset of ~1000 micrographs as shown in Fig. S1 were processed with Cryosparc 3.2 to 50 classes. Micrographs were obtained on a Thermo Scientific Glacios microscope with a K2 detector.

**References**: 1). Cheng, S., et al., J Biol Chem, 2021: p. 100645. 2). Su, M., et al., J Biol Chem, 2020. 295(6): p. 1637-1645. 3). Zhang, H., et al., J Biol Chem, 2018. 293(20): p. 7727-7736.