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The NADPH oxidase family is comprised of seven proteins: DUOX1, DUOX2, and NOX1-5. They serve as integral membrane proteins that produce reactive oxygen species (ROS) when electrons are transferred across membranes from NADPH to FAD via integral membrane proteins. This production of ROS is a critical culprit for cardiovascular failures. While the NOX enzymes all share multifarious conserved features such as having six transmembrane domains, four heme binding histidine residues, and more, NOX4, in particular, proves to be unique because it produces the ROS, hydrogen peroxide, while the remaining six NOX proteins generate superoxide. In addition, unlike the other NOX proteins, NOX4 is constitutively active and found in the interior membranes of cells in vasculature instead of the cell surface membranes (see Figure 1). NOX4 plays a key role in the development of cardiovascular hypertrophy through ROS generation, but plays a vasoprotective role during inflammatory stress or ischemia. Developing further insights on NOX4 mechanisms and its maturation process can serve as a gateway for investigations into treatments and therapies for cardiovascular disorders.

The aim was to track how the process of heme insertion into NOX4 can be influenced by various cellular factors. Because heme insertion is substantial for the maturation of NOX enzymes, developing a thorough understanding of the heme insertion process and the cellular factors involved can provide insights for cardiac disorder therapies. The Stuehr lab previously demonstrated that NO and Hsp90 can have an impact in the process of heme insertion into proteins such as soluble guanylate cyclase. The Fulton Lab showed that NOX1-3 and NOX5 had reduced their activity upon exposure to NO while Hsp90 acts to regulate NOX1-3 and NOX5. In addition, it was noted that NOX4 is not affected by either NO nor Hsp90. In order to fulfill this aim, a transfection was performed on cos-7 cells using NOX4 vectors and empty vectors as the control. A western blot was then performed in order to determine that the transfection worked effectively, and the NOX4 protein was shown to be expressed only in the NOX4 transfected cells and not in empty vector transfected cells. Then, AmplexRed Assays were performed in order to determine whether factors such as NO, CO, Hsp90, heme, and Grp94 will have an effect on NOX4 cells that were not heme deficient. The assays indicated that introducing NOX4 to heme had the most substantial effect in increasing hydrogen peroxide production which can suggest that NOX4 may not be fully bound to heme in resting conditions.