The area of our research is Molecular Enzymology - i.e. the characterization of structure, function and mechanism of enzymes with important biological and/or biotechnological roles. Our lab is essentially renowned for its contribution in the field of structure-function relationship of Nitric Oxide Synthases and related redox enzymes. NO has been widely recognized as an important signaling molecule in many physiological processes. Underproduction or overproduction of NO is linked to various pathological conditions.

NO is involved in many aspects of cell function and disease, including signal transduction in the brain, control of blood pressure and heart rate, gastric motility, oxygen delivery, immunologic destruction of tumor cells and microbes, infertility, impotence, and stroke. Cardiovascular diseases (CVDs) are the leading global cause of death. NO generated by endothelial NO synthase (eNOS) and Reactive oxygen species (ROS) play central role in CVDs and other pathological conditions. One common early feature of CVDs is suboptimal NO production by eNOS and an associated uncoupled production of ROS. A detailed understanding of how NO production is controlled at the molecular level within the eNOS enzyme is crucial for achieving a more thorough understanding of the role of NOS enzymes in cardiac function and the development of new therapeutic treatments for cardiovascular diseases. We study NO biosynthesis at the molecular level and how it is regulated, and also study the impact of NO synthesis on certain aspects of cells and tissues. Our interests include the NO synthase enzyme chemistry, understanding how the enzyme's protein structure relates to its function.

We are developing and evaluating lipid nanocarrier for eNOS delivery and its consequences on uncoupled NO synthesis and superoxide generation. Establishment of nanomaterial-based methodologies for the effective delivery of eNOS-loaded nanocarriers to the cells could provide a real-time toolkit for exploring its role in NO production and ROS production. We expect that eNOS-loaded nanocarriers have the potential to serve as an easily administered source of NO, which could act as vasodilator. A prominent NO donor nitrosyl ruthenium bound to lipid carriers are used for topical administration but they have very short half-life and are unstable during storage. Nano-carrier loaded eNOS could have longer stability and NOS-enzymatic activity could be retained for longer period. Thus, nanocarriers-loaded eNOS may facilitate the development of multifunctional systems for targeted NO delivery.

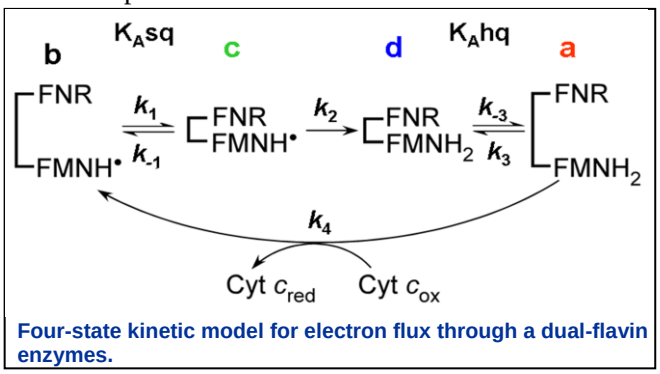
Our laboratory is interested in the general class of cofactor-binding proteins, and particularly flavoproteins and flavoheme enzymes. In addition, the scope of work extends to learning how other cellular proteins can control activity of Nitric Oxide Synthase (NOS) and other related redox enzymes like Cytochrome P450 reductase (CPR), and Methionine synthase reductase (MSR), to cite a few. We are also interested in a membrane-associated enzyme NADPH oxidase (NOX) that catalyzes the production of superoxide.

<u>Few specific contributions:</u>

- During my research in USA, I have generated a super eNOS (eNOS chimera) that has capability of NO production by 4-fold. Our findings and work were highly appreciated by our peers and resulted in publication of the same in the prestigious ***PNAS, USA (2007;104(22):9254-9)***. This super eNOS is potentially an important candidate for gene therapy for CVDS. This work provides the basis to manipulate the enzyme for treatment of disease. For this important contribution to biomedical sciences, I was awarded the *"Innovator Award"* of the year by the Cleveland Clinic, USA.
- In one of the studies we have demonstrated that the electrostatic contacts of the FMN module play a significant role in controlling electron transfer and minimizing auto-oxidation which could generate ROS, a prominent causative agent for various cancers and provides basis for how generation of ROS could be minimized in cancer patients. We also found that how sensitive NOS activity is to changes in the oxygen concentration and reveals a novel means for the FMN module or protein-protein interactions to alter nNOS activity. These studies gives us significant insights into the possible and applicable methods of clinically controlling the NOS enzyme at the catalytic level, which can potentially be used for drug development for neurodegenerative diseases

implicated with nNOS expression like Alzheimer disease, Hungtinton's disease and Parkinson's disease. Our findings resulted in several publications in ***J. Biol. Chem*** *and* ***Biochemical Journal***.

- Our other studies reveal the detailed mechanism by which calmodulin regulates electronic communication between NOSoxy and NOSred. Also we have indisputably demonstrated that the Auto Inhibitory loop significantly influence the FMN shielding and controls the Calcium sensitivity of CaM. These mechanisms of regulation could be used as a viable target for drug development. In fact, identification of such protein targets which play a critical role in the regulation of the activity of NOS are the most preferred therapeutic targets for the clinical management of diseases implicated with NOS dysfunction. We published our findings in ***J. Biol. Chem*** *and* ***FEBS Journal.***
- Using single molecule fluorescence resonance energy transfer (FRET) spectroscopy, we find that calmodulin binding to nNOSr both alters and restricts the distributions of NOSr conformational states and the conformational lifetimes, thereby revealing a physical means by which calmodulin may control electron transfer for catalysis. Nevertheless, our study provides, the first dynamics-based, single molecule demonstration that under physiologic conditions. We published work on single molecule and FRET studies of neuronal NO synthase (nNOS) in the prestigious journal PNAS (***Proc Natl Acad Sci U S A. 2015 Sep 22;112(38):11835-40***) and our work is highly appreciated by our peers.
- We utilized concepts of closed and open conformations and information from crystal structures of CPR and NOSr to create a 4-state kinetic model that describes electron flux through dual-flavin enzymes. Simulating the kinetic model provides rate estimates for conformational motions and inter-flavin electron transfer.



Four-state kinetic model for electron flux through a dual-flavin enzymes.

- To acknowledge our contribution in the field of NOS and flavin containing enzymes, we were invited by very reputed ***British Journal of Pharmacology*** to write a review article on the completion of 20 years of Nobel Prize in the field of Nitric Oxide. This *Special Invited review (*Nitric oxide synthase enzymology in the 20 years after the Nobel Prize) *for special edition on NOS enzymes was edited by Nobel Laureate Louis J. Ignarro (*recipient of the 1998 Nobel Prize in Physiology or Medicine).
- In one of our recent collaborative studies we have shown previously unidentified control mechanism of eNOS through the O-GlcNAc modification **(*Redox Biol. 2020 Sep;36:101625*)**. Our findings have broader implications for diseases where glucose metabolism/uptake is altered such as pulmonary hypertension, diabetes, and cancer. This therefore represents a new target whereby inhibition of O-GlcNAc may augment eNOS activity.
- In our recent findings, we uncovered plasminogen activator inhibitor-1 (PAI-1) as a potent negative regulator of eNOS function (knockdown or antagonism of PAI-1 increases NO production). This work identifies a nonproteolytic role for PAI-1, and antagonism of PAI-1 may serve as an approach to promote endothelial function and homeostasis (***Proc Natl Acad Sci U S A, 2020 Apr 28;117(17):9497-9507***).