

RESEARCH ACCOMPLISHMENT - FERADICAL

My research career has provided an outstanding opportunity to understand metalloprotein structure-function relationships and the chemistry of free radicals in electron/radical transfers in biology.

► **Conceptual Contributions:**

✓ Discovery of the biological charge resonance (CR) stabilization phenomenon, which shows a characteristic spectroscopic signature in the near-infrared region (doi: [10.1073/pnas.1301544110](https://doi.org/10.1073/pnas.1301544110) & [10.1002/anie.201410247](https://doi.org/10.1002/anie.201410247)). The biological CR has later been resonated by similar findings in many other systems by other laboratories.

✓ Discovery of the metal-dependent, dioxygen-independent, non-oxidative decarboxylation is the first of its kind. It represents a new type of biological decarboxylation (doi: [10.1021/bi061031v](https://doi.org/10.1021/bi061031v) - published under "New Concepts in Biochemistry") and original work (doi: [10.1021/ja0532234](https://doi.org/10.1021/ja0532234)).

✓ Further elaborating the charge maintenance hypothesis in non-heme iron enzyme catalysis initially proposed by Lipscomb and others but left unattended for decades (doi: [10.1021/acscatal.1c04770](https://doi.org/10.1021/acscatal.1c04770)).

► **Describing a Bioinorganic Chemistry & Protein Structure-based Redox Sensing and Signaling Mechanism:**

✓ Discovery of a groundbreaking redox-sensing mechanism that initiates the immune system by an iron-containing protein Pirin, in the cell nucleus. We found that human Pirin utilizes an iron redox state-mediated structural switch to sense redox bursts in the cell nucleus and activates the NF- κ B pathways (doi: [10.1073/pnas.1221743110](https://doi.org/10.1073/pnas.1221743110)).

► **Describing Unprecedented Intermediates in Metalloenzymes:**

✓ Discovery of a *bis*-Fe(IV) intermediate in the diheme enzyme, MauG, which uncovered a novel natural strategy for storing two oxidizing equivalents (doi: [10.1073/pnas.0801643105](https://doi.org/10.1073/pnas.0801643105)). This is described as a Nature's sniper for long-range remote catalysis (doi: [10.1007/s00775-014-1123-8](https://doi.org/10.1007/s00775-014-1123-8)).

✓ Discovery of a protein-based diradical intermediate located on a tryptophan residue and an adjacent 7-hydroxyl-tryptophan residue. Not only the di-tryptophanyl radical but also the 7-OH-Trp radical is the first of its kind (doi: [10.1073/pnas.1215011110](https://doi.org/10.1073/pnas.1215011110)).

✓ Captured and structurally illustrated the first compound 0 intermediate, Fe(III)-OOH, in tyrosine hydroxylase (TyrH) with a bound substrate (doi: [10.1021/jacs.1c00175](https://doi.org/10.1021/jacs.1c00175)). All previous compound 0 intermediate structures were obtained through cryoradiolytic reduction of oxy-ferrous complexes.

✓ Discovery of the first heme-based high-spin ($S = 5/2$) compound 0 intermediate in the reaction of *Mycobacterium tuberculosis* P450 enzyme CYP121 (doi: [10.1074/jbc.M117.794099](https://doi.org/10.1074/jbc.M117.794099)) and later determined its structure (doi: [10.1021/jacs.3c04991](https://doi.org/10.1021/jacs.3c04991)). All previously described heme-based Fe-OOH species are in a low-spin ($S = 1/2$) ground state.

► **Uncovering New Metalloenzyme Activities:**

✓ Discovery of novel C-F bond cleavage reactions mediated by dioxygen- and non-heme iron-dependent enzymes (doi: [10.1038/s41589-018-0085-5](https://doi.org/10.1038/s41589-018-0085-5)) and hydrogen peroxide- and heme iron-dependent enzymes (doi: [10.1021/acscatal.9b00231](https://doi.org/10.1021/acscatal.9b00231) & [10.1021/jacs.1c00175](https://doi.org/10.1021/jacs.1c00175)).

✓ Discovery of enzyme-mediated O-demethylation in a P450 enzyme and determined its mechanism by characterizing an intermediate (doi: [10.1021/acscatal.9b04596](https://doi.org/10.1021/acscatal.9b04596)).

► **Describing New Protein-bound Cofactor and Motif:**

✓ Discovery of a novel catalytic heme cofactor; it is neither type *b* nor type *c* heme, but in between, with a single thioether bond in a cysteine-vinyl link. The cofactor has an unusual H $_x$ HxxxC motif in its protein sequence (doi: [10.1039/DoSC06369J](https://doi.org/10.1039/DoSC06369J)).

✓ Discovery of a transition metal cofactor in the kynurenine pathway decarboxylase, an enzyme that had long been thought cofactor-free before our work (doi: [10.1021/ja0532234](https://doi.org/10.1021/ja0532234)).

✓ Discovery of a C-X-X-C-G-X(n)-C-P-X-C-G rubredoxin-like metal-binding motif functioning as an iron reservoir, which is shown in over 2,000 protein structures without a known function and present in over 74,071 non-redundant protein sequences (doi: [10.1074/jbc.M115.650259](https://doi.org/10.1074/jbc.M115.650259)).

► **Defining New Protein Families or Redefining and Expanding Existing Superfamilies:**

✓ Discovery of a new protein subfamily within the amidohydrolase superfamily, which helped to annotate correctly over 700 genes previously misannotated (doi: [10.1021/bio60108c](https://doi.org/10.1021/bio60108c)). The subfamily enzymes (now over 3,500) are decarboxylases and hydratases distinct from the rest of the hydrolase enzymes. This subfamily with new catalytic functions is now widely recognized as amidohydrolase-2 in various protein databases.

✓ Discovery of a heme-dependent aromatic oxygenase (HDAO) superfamily that utilizes a histidyl-ligated heme to mediate oxygenation of aromatic substrates (doi: [10.1073/pnas.2106561118](https://doi.org/10.1073/pnas.2106561118)).

► **Kynurenine Pathway for Tryptophan Catabolism - Structure, Mechanism, Regulation:**

✓ Discovery of the missing gene for the dehydrogenase of the kynurenine pathway (which is the primary route for tryptophan catabolism). This human dehydrogenase was incorrectly assigned by others to a retinal dehydrogenase, causing missing enzymes for the dehydrogenation and the following steps in an important metabolic pathway (doi: [10.1074/jbc.RA118.003320](https://doi.org/10.1074/jbc.RA118.003320)).

✓ Making the kynurenine pathway's non-heme Fe dioxygenase the best-understood oxygen activation enzyme by capturing and structurally and spectroscopically defining seven catalytic intermediates, five of which are after the arrival of the dioxygen substrate at the iron center (doi: [10.1073/pnas.2005327117](https://doi.org/10.1073/pnas.2005327117)).

✓ Making the kynurenine pathway's NAD⁺-dependent dehydrogenase the best-understood dehydrogenase through trapping and structurally characterizing the first thiohemiacetal intermediate along with its first structure, binary and ternary enzyme-substrate complex structures and a subsequent thioacyl intermediate of the enzymatic reaction (doi: [10.1038/ncomms6935](https://doi.org/10.1038/ncomms6935)).

✓ Discovery of a genetic disorder, hypertryptophanemia, and defined its molecular rationale, and provided a novel strategy to target tryptophan dioxygenase that cancer cells overexpress for immune escaping (doi: [10.1016/j.ymgme.2017.02.009](https://doi.org/10.1016/j.ymgme.2017.02.009)).

✓ Discovery of a natural strategy by which an enzyme employs loop dynamics to accommodate two substrates with disparate polarities (doi: [10.1074/jbc.RA118.002698](https://doi.org/10.1074/jbc.RA118.002698)).

✓ Describing of a pitcher-and-catcher isomerization mechanism in dehydrogenase to prepare substrate in the correct conformation for oxidation (doi: [10.1074/jbc.M116.759712](https://doi.org/10.1074/jbc.M116.759712)).

✓ Discovery of a substrate-induced ferrous enzyme reactivation mechanism from its catalytically primed but dormant state in two independent cases with heme (doi: [10.1074/jbc.M111.253237](https://doi.org/10.1074/jbc.M111.253237)) and non-heme Fe enzymes (doi: [10.1074/jbc.RA120.013915](https://doi.org/10.1074/jbc.RA120.013915)). The former solved an over 80-year mystery.

✓ Experimentally observing protein quaternary structure to regulate enzyme catalytic activities (doi: [10.1074/jbc.M113.496869](https://doi.org/10.1074/jbc.M113.496869) & [10.1074/jbc.RA119.009035](https://doi.org/10.1074/jbc.RA119.009035)). We demonstrated that a tightly associated protein dimer could dynamically dissociate and reassociate. This was achieved in a decarboxylase with two catalytically critical Arg residues, with one from its neighboring subunit. We found that the mixture of two inactive Arg single mutants is catalytically active. We determined the crystal structure of the mixture and demonstrated the formation of a heterodimer, with one subunit having an intact active site but the other having a double mutation. Before this work, protein subunit association/dissociation had never been demonstrated by crystallography.

► **Determining the *De Novo* or the First Crystal Structure of Enzymes:**

✓ Determination of the *de novo* crystal structure of 3-methyl-L-tyrosine hydroxylase (doi: [10.1039/Do5Co6369j](https://doi.org/10.1039/Do5Co6369j)) L-DOPA dioxygenase (doi: [10.1021/acs.biochem.9b00396](https://doi.org/10.1021/acs.biochem.9b00396)), α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase (doi: [10.1021/bio60903q](https://doi.org/10.1021/bio60903q)) by the multiwavelength anomalous dispersion (MAD) phasing method and the first crystal structure of 2-aminomuconate-6-semialdehyde dehydrogenase by molecular replacement method (doi: [10.1038/ncomms6935](https://doi.org/10.1038/ncomms6935)).

► **Method Development in Studying Metalloenzyme and Protein-Derived Cofactors:**

✓ We were the first group to introduce the existing genetic code expansion technology to study the protein-derived cofactors by site-specifically substituting the cofactor-bearing residue(s) to non-canonical amino acid(s) (doi: [10.1038/s41589-018-0085-5](https://doi.org/10.1038/s41589-018-0085-5) & [10.1002/anie.201803907](https://doi.org/10.1002/anie.201803907)). The unnatural amino acid approach has proven powerful in studying amino acid crosslinking mechanisms and cofactor biological functions (doi: [10.1021/jacs.0c08992](https://doi.org/10.1021/jacs.0c08992) & [10.1021/acs.biochem.9b00006](https://doi.org/10.1021/acs.biochem.9b00006)).

✓ We used equivalent multiple single crystals to conduct single-crystal EPR for enzymes and reactive intermediates in multiple pieces of enzymatic mechanism studies).