The remarkable discovery of nitric oxide (NO*) as a lipid permeable signaling molecule led to the recognition of the Noble Prize of Medicine in 1998.¹ NO* is an indispensable therapeutic radical molecule that acts as a first line defense against invading pathogens in the biological body. Increased NO* concentration is fatal for pathogens, helping the immune system combat pathogenesis.² Several pathogens employ iron-containing enzymes to detoxify nitric oxide to non-toxic products, thus supporting microbial pathogenesis. Flavodiiron proteins (FDPs) found in several pathogens are recognized to have dual activity for O₂ and NO* reduction.³ However, the geometric and electronic properties of the specific active site lead to a catalytic preference between the two substrates. FDPs that are active for nitric oxide reduction to nitrous oxide (N₂O) are addressed as Flavodiiron Nitric Oxide Reductases (FNORs) (Figure 1a). Interestingly, hemerythrin-like proteins (HLPs), despite having closely related active site, are inactive towards NO* reduction (Figure 1b). Instead, they catalyze NO* oxidation to nitrite (NO²⁻).⁴ The structure-activity relationship and its mechanistic implications is an exciting area of research for the strategic development towards therapeutic relevance.

Flavodiiron nitric oxide reductases (FNORs) contain a nonheme diiron site and a flavin mononucleotide (FMN) cofactor. The two iron centers are held together by bridging hydroxide and carboxylate ligands (Figure 1a). The mechanism of NO* reduction to N₂O is believed to proceed through a diiron dinitrosyl intermediate \( \{\text{FeNO}\}_2 \) (see reference 8 for details). Interestingly, other nonheme diiron enzymes like soluble methane monooxygenase (sMMO) and ribonucleotide reductase (RNR) can form \( \{\text{FeNO}\}_2 \) adducts⁸, but are still unreactive towards NO* reduction. This underlines the necessity to probe the implications of the geometric and electronic constraints on FNORs reactivity, particularly the significance of bridging ligands.
To probe the role of bridging ligands in FNORs, White and Lengel et al.\(^6,7\) developed synthetic model complexes employing a BPMP (2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol) ligand and bridging propionate group (Complex 1, Figure 2a). Additionally, they synthesized different variants by replacing the bridging ligands with monodentate ligands (Complex 2-X, Figure 2b). While complex 1 rapidly formed quantitative amount of N\(_2\)O upon a one-electron reduction, the yields were significantly diminished for the monodentate variants (Complex 2-X).

Although, the \([\text{FeNO}]^7\)_2 intermediate was successfully characterized, an increased (O)N-N(O) distance (from 2.80 Å to 3.96 Å) and distortion of the \([\text{FeNO}]^2\) core (Figure 2b) (from 5.9° to 85.1°) proved detrimental for NO\(^*\) reactivity in monodentate variants. This highlights the importance of geometric constraints on the activity of FNORs. Moreover, induced distortion facilitates the formation of a dinitrosyl iron complex (DNIC) where the presence of a second iron center, in proximity, drives unprecedented reactivity. Unlike monoiron DNICs, this binuclear DNIC (Figure 3) evades the spin forbidden mechanism for N-N coupling reaction of two NO\(^*\) ligands to release N\(_2\)O.

To understand the role of secondary coordination sphere residues in FNORs reactivity, Biswas et al.\(^10\) synthesized the Y197F variant of \textit{Thermotoga maritima} (Tm) FNOR where conserved tyrosine (Tyr) residue in wildtype Y197 was replaced by phenylanine (Phe) residue. \([\text{FeNO}]^7\)_2 formed in the Y197F variant showed no reactivity towards NO\(^*\) reduction. To understand the role of Tyr residue further, Biswas et. al\(^10\) resorted to DFT studies.

Upon the formation of a \([\text{FeNO}]^7\)_2 adduct, the highly covalent Fe-N bond reduces the coulombic repulsion between two nitrogen atoms of NO\(^*\) moieties (Figure 4a). It further enables
formation of a hyponitrite intermediate. The optimal pH range for the formation of a monoprotonated hyponitrite intermediate (Figure 4b) was found to be 6-12, which falls within the physiological pH range. The rate determining step, rotation around the Fe-N coordination axis transforming 1,2-N,N- to 1,2-O(H),N-bridging complex (Figure 4c,d), is facilitated by hydrogen bonding from the nearby tyrosine hydroxyl. Subsequently, N-OH bond cleavage takes place to release N2O, forming the resting state (Figure 4e).

Figure 4 Chain of reactions for N2O formation in Thermotoga maritima (Tm) Flavodiiron Nitric Oxide Reductase (FNOR). Adapted from Biswas et al.10

Hemerythrin-like proteins (HLPs) in mycobacteria (Mka-HLPs) are responsible for NO• oxidation to NO2−. In contrast to diiron active site of Flavodiiron nitric oxide reductases (FNORs), the active site of Mka-HLPs lack a third carboxylate ligand. Instead, they contain a distant tyrosine ligand, making the local environment electron poor. Poptic et al.5 modelled this local environment by employing Py4DMcT [2-((di(pyridin-2-yl) methyl) thio)-5-((pyridin-2-yl(pyridin-3-yl) methyl) thio)-1,3,4- thiadiazole], μ-acetate and μ-hydroxide ligands (Figure 5). Increased N-N distance and poor ligand environment led to no activity of the modelled complex towards N2O formation. Instead, upon introduction of the oxidant tris(4-bromophenyl)-aminium hexafluorophosphate [NAr₃][PF₆], nitrite (NO2−) was produced.

To conclude, electronic and geometric properties play a vital role towards selective reactivity of diiron dinitrosyl complexes. Bridging ligands in FNORs ensure coplanarity of {FeNO}²⁻ and drive NO• reduction to N₂O. Hydrogen bonding from Tyr (Y197) residue in the secondary coordination sphere in Thermotoga maritima (Tm) FNOR lowers the activation barrier for forming a 1,2-O(H),N-bridging complex (Figure 4). However, if the N-N distance between two nitrosyl moieties is increased and an electron poor local environment is accessible, the oxidation of NO• to NO2− is prevalent as found in Mka-HLPs.
References


8. In metal nitrosyl complexes, owing to the redox-active non innocent nature of NO*, Enemark-Feltham notation is employed. It is denoted as {M(NO)x}n, where x denotes number of NO*ligands bound to metal and n represents total number of valence electrons combining metal d- and π* electrons of NO*.9
