Synthetic Ru-diamine Linked Pteridine Wires as Probes of the Pterin Site in Nitric Oxide Synthase

Edith Glazer,[a] Harry B. Gray,[b] and David B. Goodin[a]

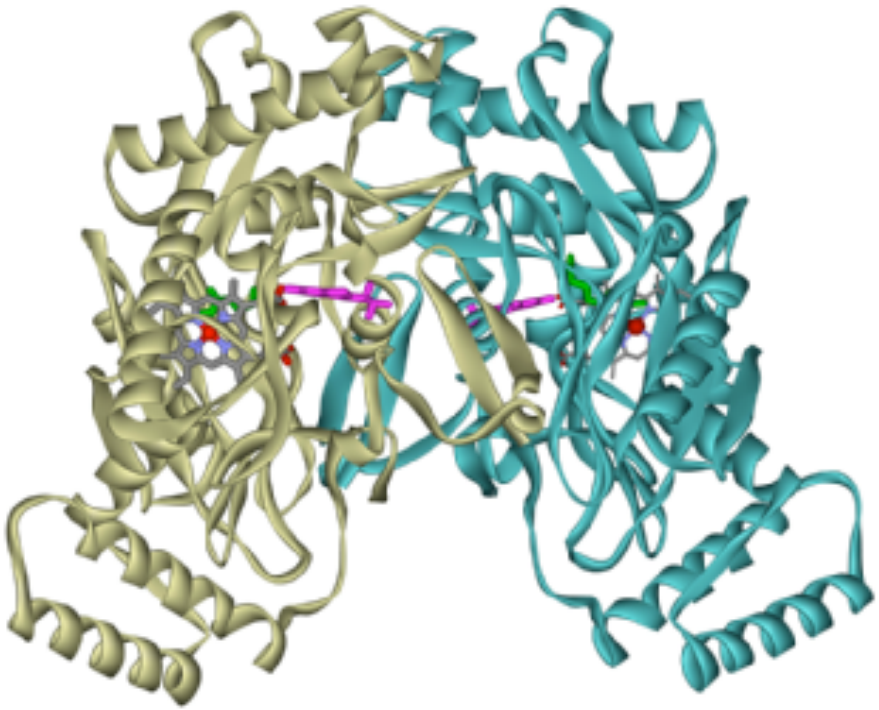
[a] Department of Molecular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; [b] Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, 91125; e-mail: dbg@scripps.edu

Nitric oxide synthase (NOS) is the enzyme responsible for producing nitric oxide (NO), a ubiquitous secondary messenger involved in a wide variety of processes implicated in human health and disease. Despite much study, significant uncertainty remains concerning the role played by essential cofactors such as 6(*R*)-tetrahydro-L-biopterin (H_4B). We have undertaken the synthesis of a series of cofactor linked Ru-diamine photosensitizers (molecular wires) that are designed to specifically target NOS at the pterin binding site. The wires were designed to allow binding of the pterin analogs at the cofactor binding site with the photosensitizer extending to the protein surface through a solvent exposed channel at the dimer interface. By targeting the pterin binding site, the enzyme active site may remain open to bind and turn over substrate, allowing the pterin site to be probed by direct charge injection/withdrawl through the wire. The pterin-based wires were designed utilizing both structural information (*1, 2*) and SAR studies of pteirn-based NOS inhibitors (*3-5*). The Ru(II)-diimine complex facilitates luminescent detection of binding by energy-transfer to the heme, and provides access to transient catalytic intermediates though photoinduced electron-transfer. The design, synthesis, binding and functional properties of these compounds to NOS heme domains will be described.

1. B. R. Crane *et al.*, *Science* **279**, 2121 (1998).
2. C. S. Raman *et al.*, *Cell* **95**, 939 (1998).
3. P. Kotsonis *et al.*, *Journal of Biological Chemistry* **276**, 49133 (2001).
4. H. Matter, P. Kotsonis, *Medicinal Research Reviews* **24**, 662 (2004).
5. H. Matter *et al.*, *Journal of Medicinal Chemistry* **45**, 2923 (2002).

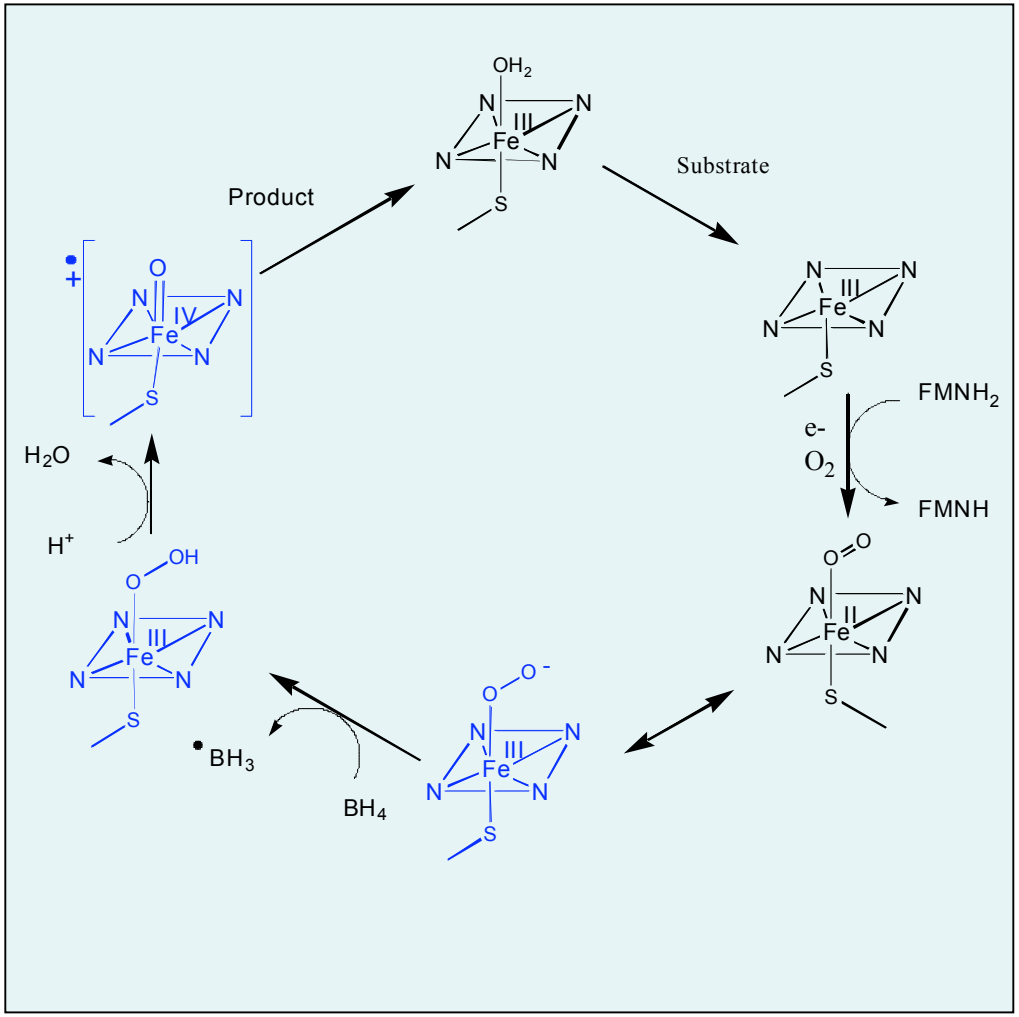
Introduction

Nitric oxide synthase (NOS) produces nitric oxide (NO), a ubiquitous second messenger. NO functions in diverse physiological processes such as blood pressure control, neurotransmission, and as a defensive cytotoxin. Despite widespread interest in this medical target, questions remain concerning the fundamental mechanism of NO production. We are using sensitizer-linked substrate (SLS) probes to directly address mechanistic questions. Future applications include development of potentially high-throughput assays for NOS inhibition and regulation.



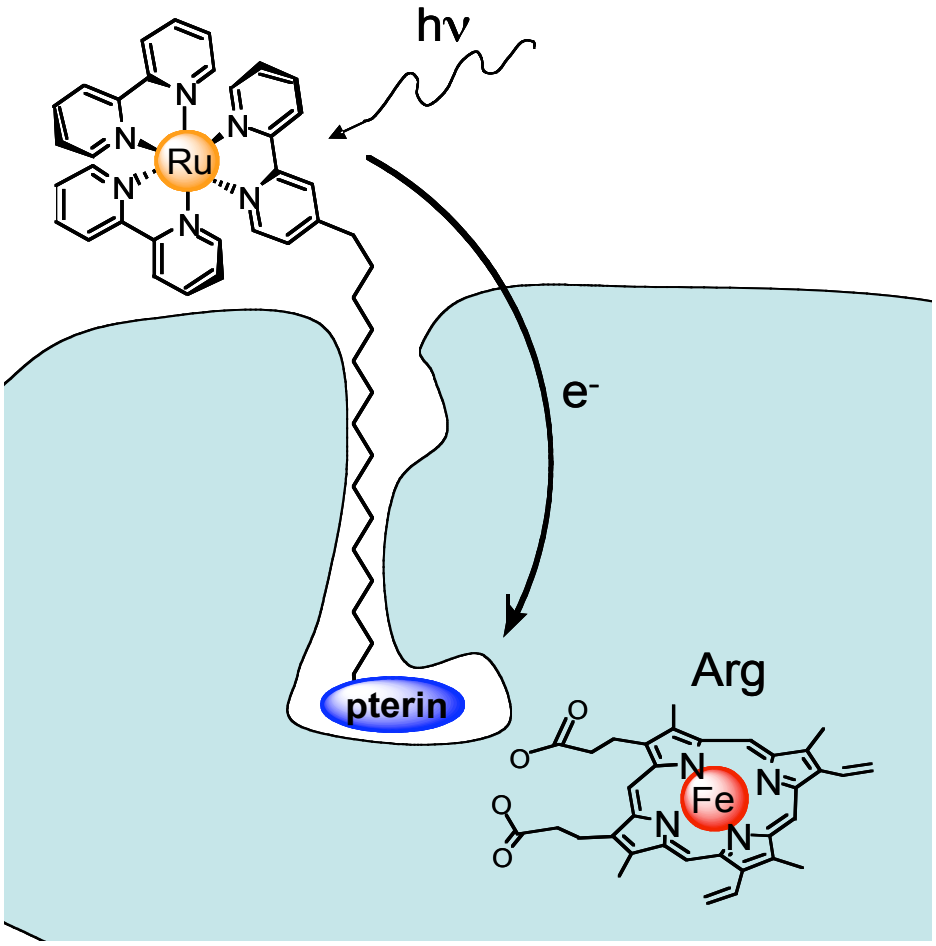
Structure of $iNOS_{ox}$

NOS Mechanism



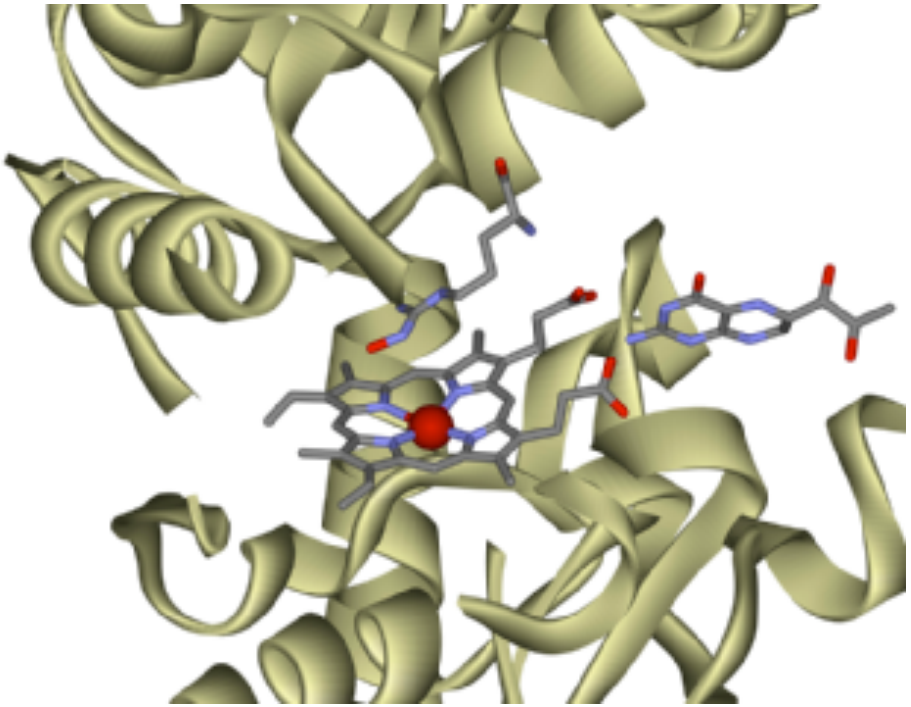
- Proposed mechanism for NOS catalysis involves multiple electron/proton transfers.
- Pterin has been implicated in the second heme reduction step.
- The nature of the pterin coupling with the heme and key catalytic intermediates following this step have yet to be characterized.

Pteridine wires may be useful tools for the study of NOS catalysis

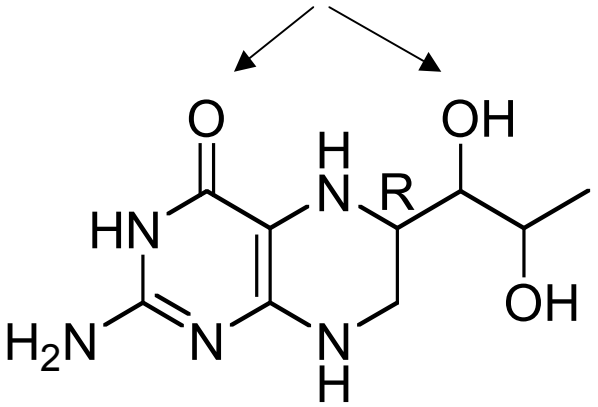


- We have synthesized a series of pterin analogs coupled to photoactive sensitizers.
- These wires were designed to bind at the pterin site at the NOS dimer interface.
- Linked Ru-diamines allow for direct photochemically induced charge injection to the deeply buried heme.
- Sensitive detection of the enzyme is also possible through energy transfer quenching via the heme.

Pterin wire design



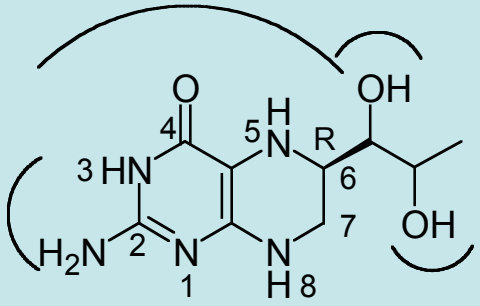
Potential sites for attachment of linker to Ru^{II}



H_4B

π-stacks with oxidase domain Trp

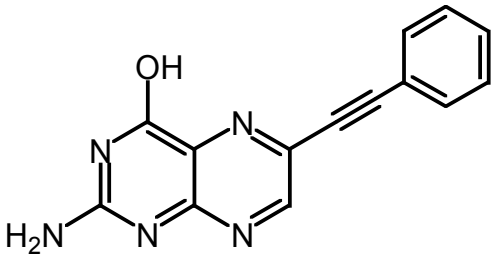
Interacts with heme carboxylate



Diol forms hydrogen bonds with neighboring residues

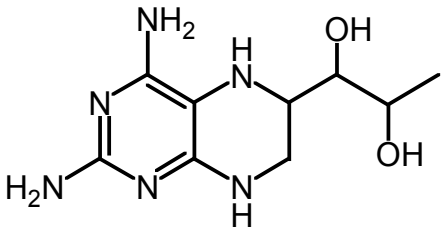
π-stacks with reductase domain Phe

Select IC_{50} values for pterin inhibitors of nNOS



1

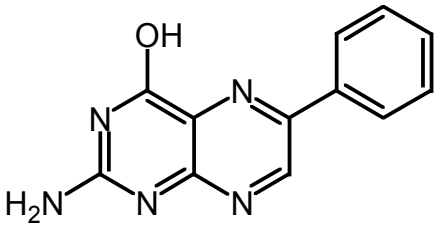
IC_{50}=21 μM



2

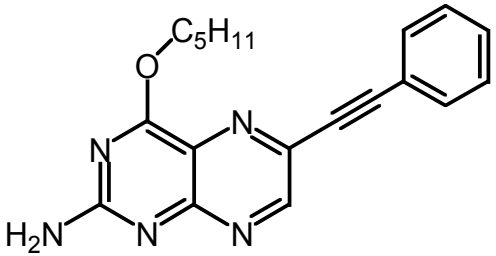
4-amino-H_4B

IC_{50}=9 μM



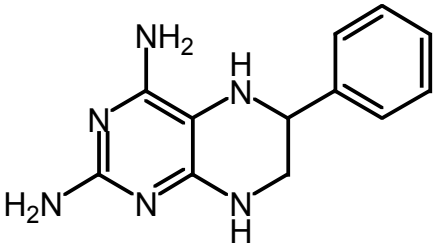
3

IC_{50}=26 μM



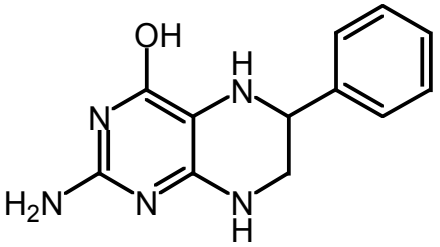
4

IC_{50}=8 μM



5

IC_{50}=6 μM

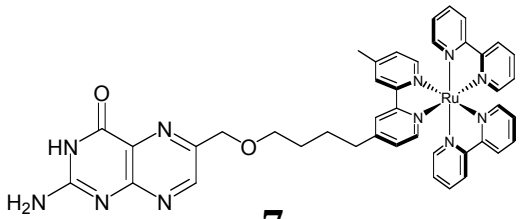


6

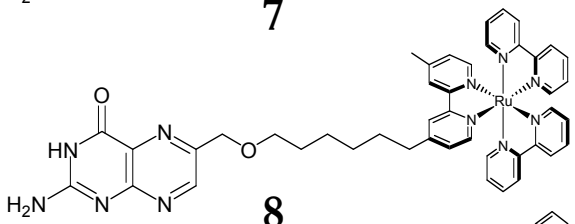
IC_{50}=7.3 μM

From Matter, et al., *J. Med. Chem.* **2002**, *45*, 2923-41.

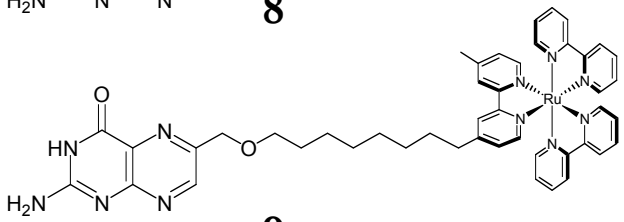
Examples of pterin wires synthesized



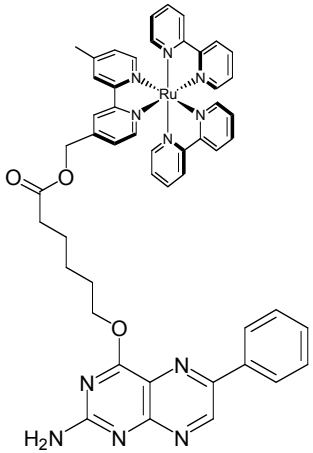
7



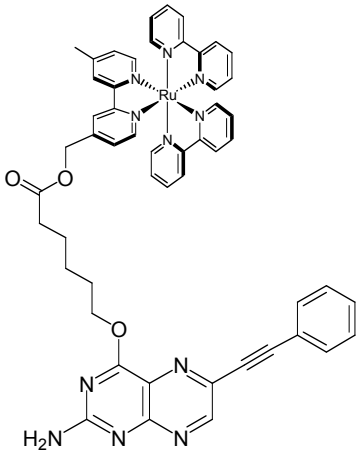
8



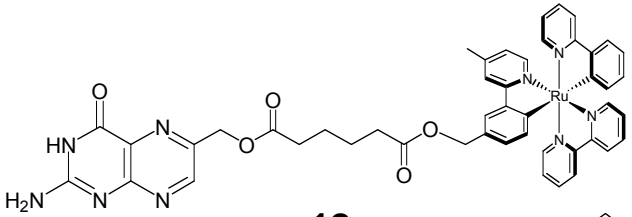
9



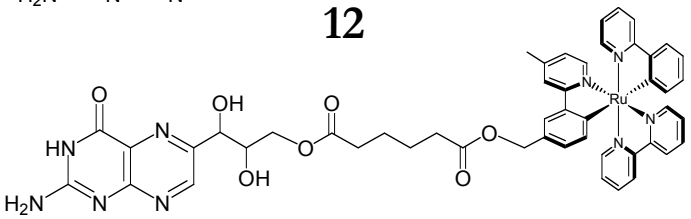
10



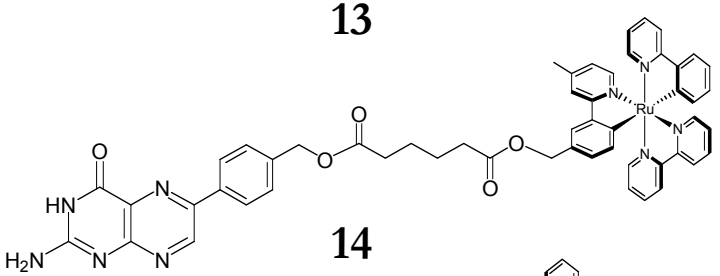
11



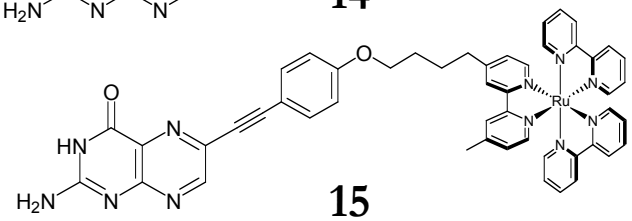
12



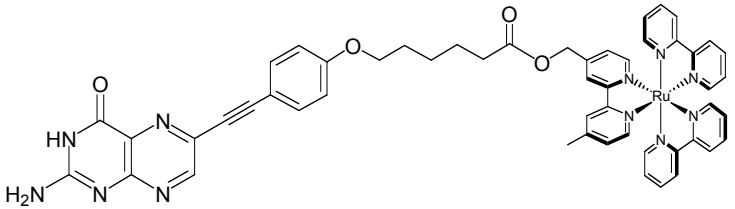
13



14

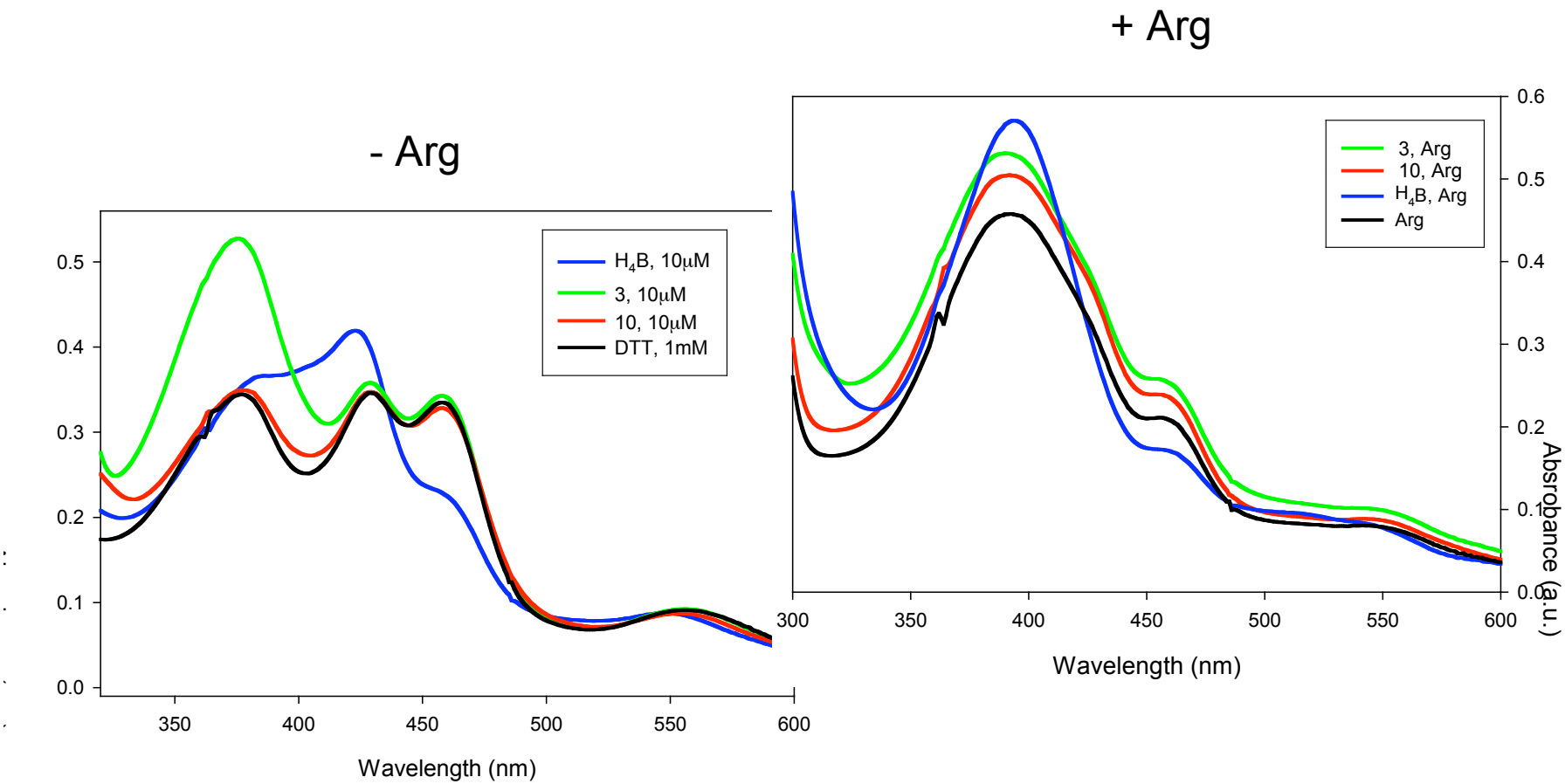


15

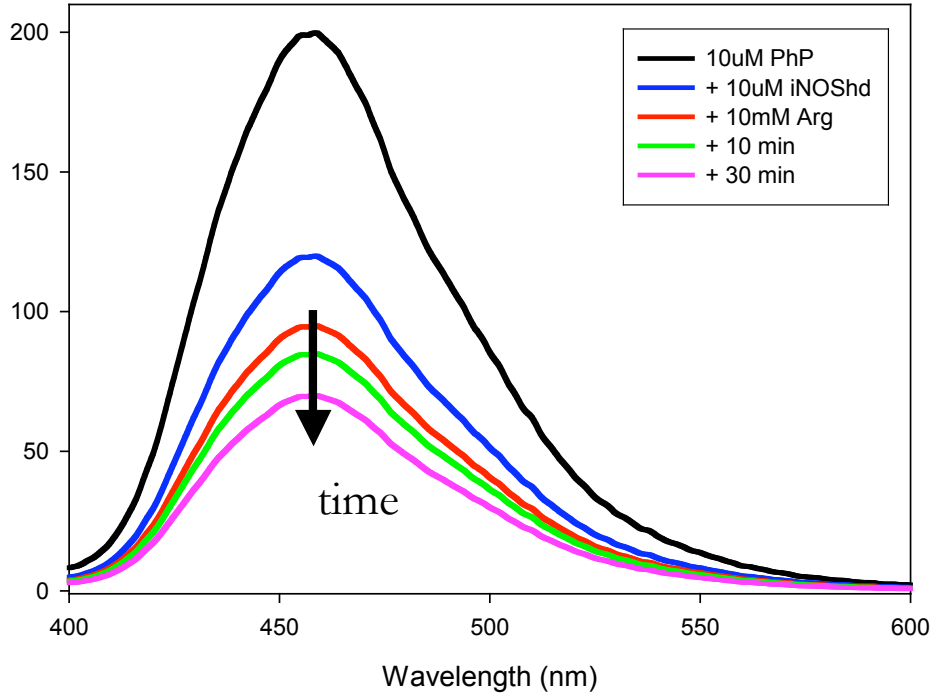


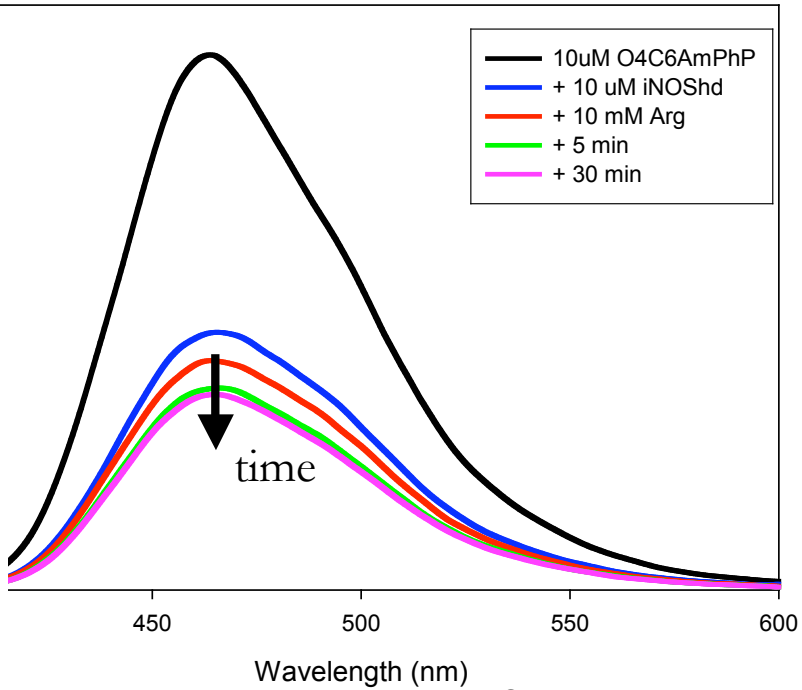
16

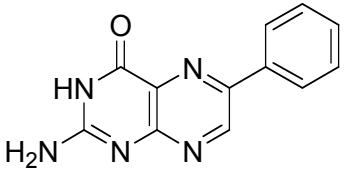
Effect of pterin and wires on UV/Vis spectra of murine iNOS heme domain

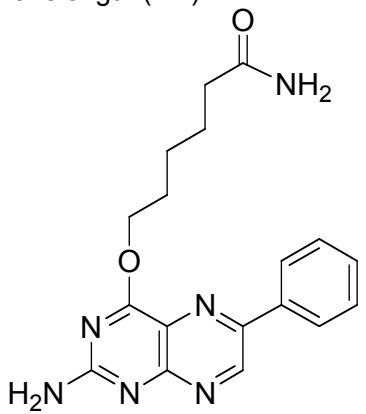


iNOS induces quenching of pterin emission. Arg addition enhances quenching



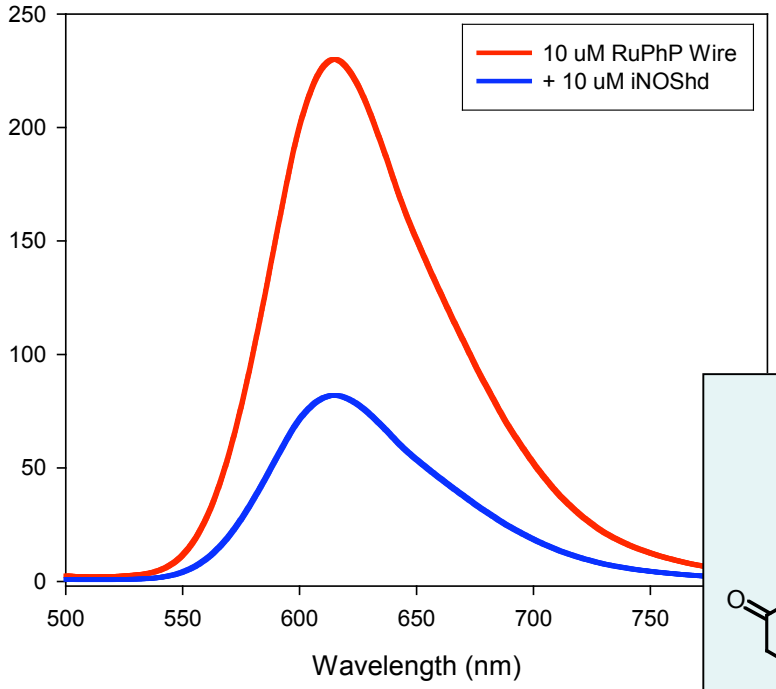




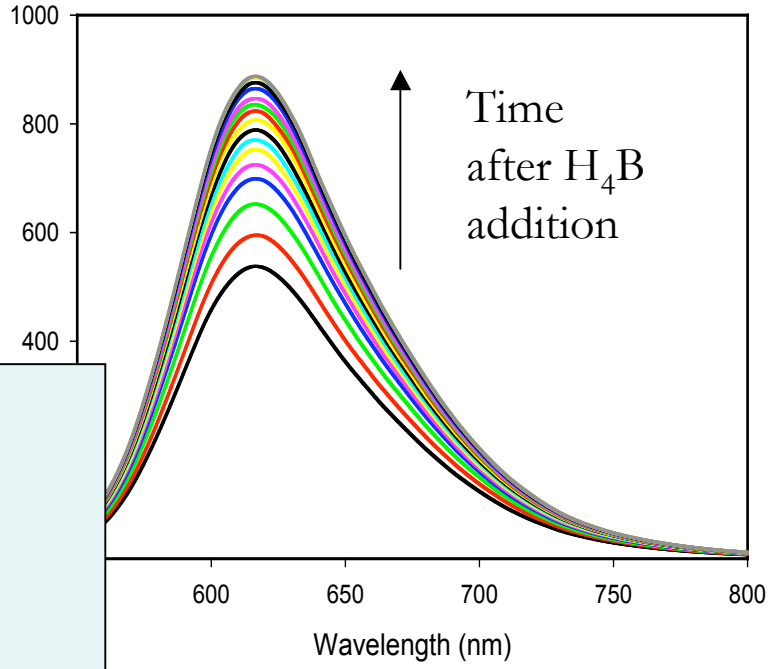


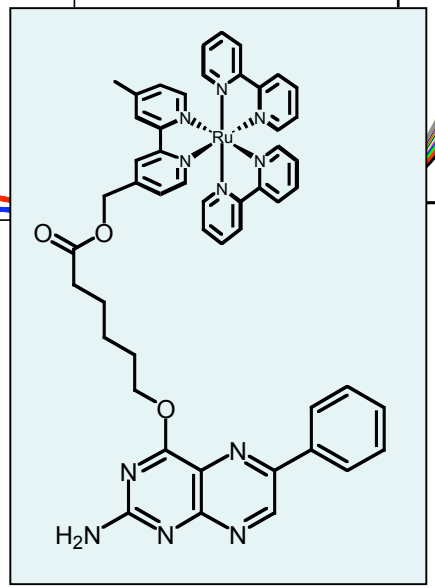
Energy transfer quenching of Ru(II) wire emission is reversed by pterin addition

iNOS addition quenches Ru(II) wire emission

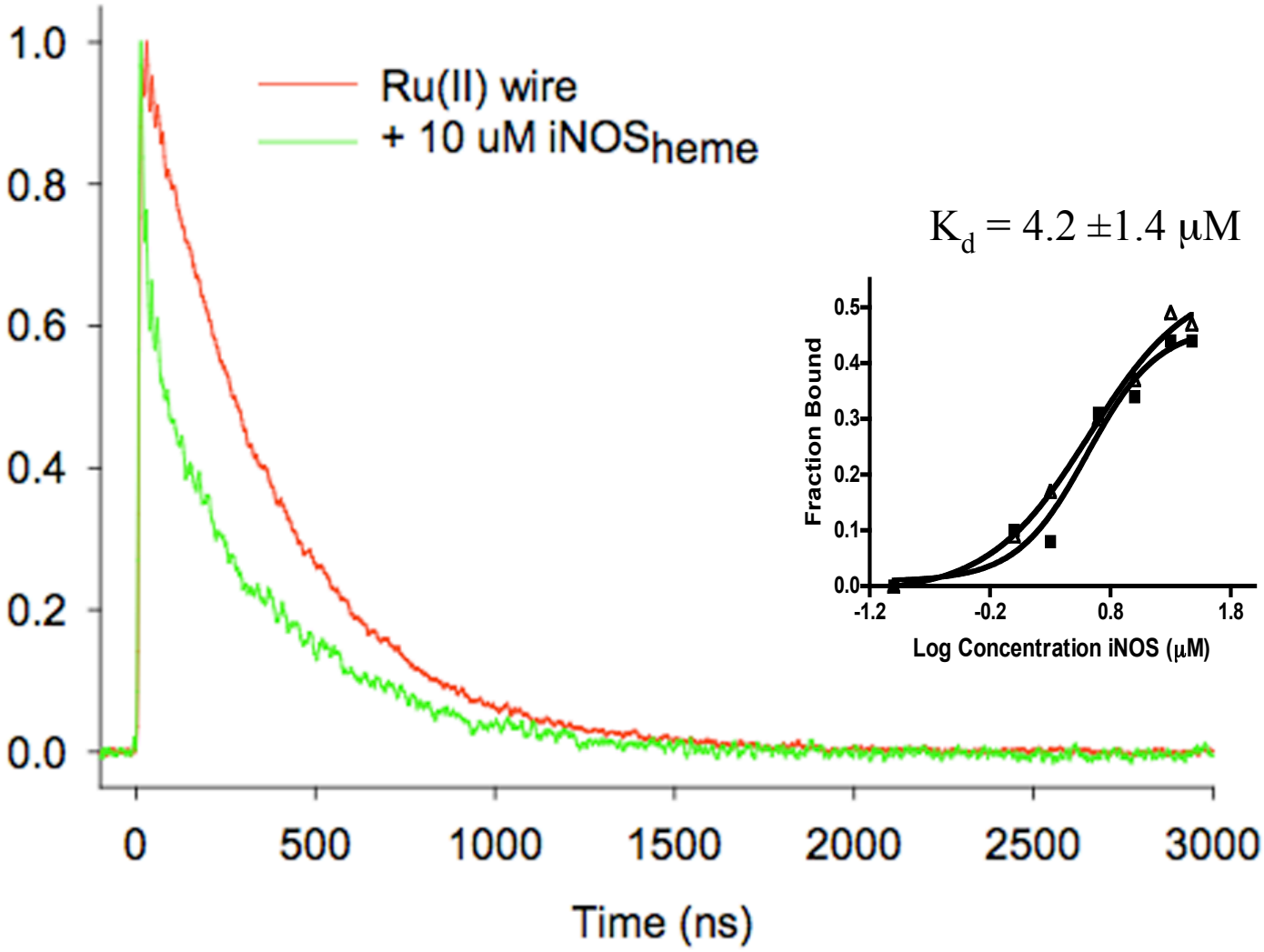


H_4B induces recovery of of Ru(II) emission





iNOS induces bi-exponential ruthenium emission decay



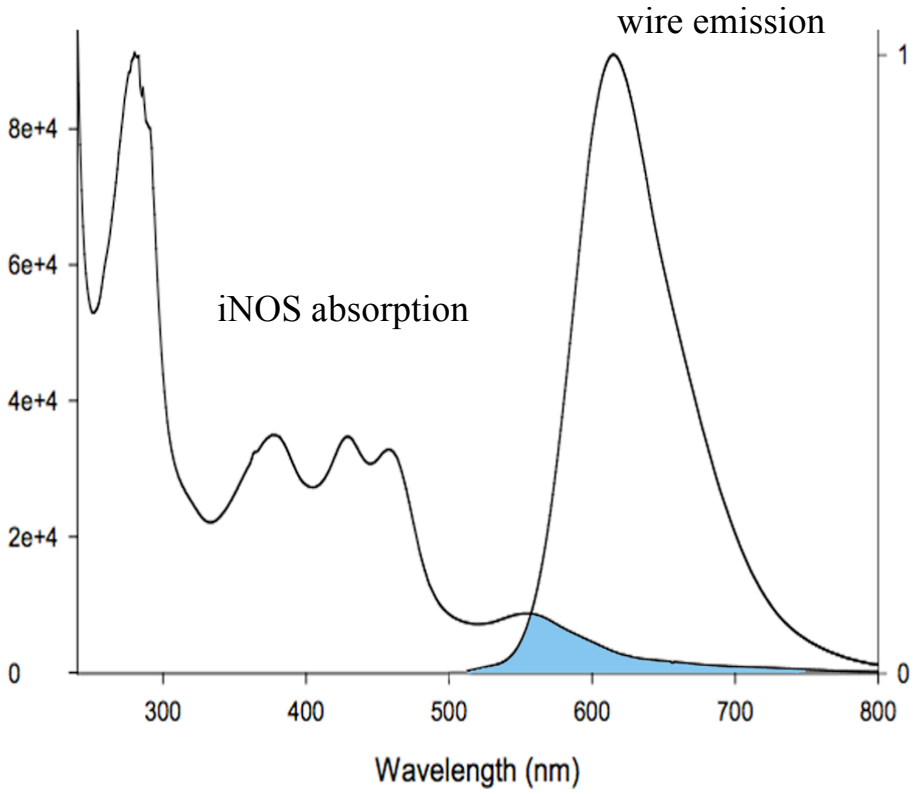
Förster energy transfer quenching of ruthenium wire emission by NOS

- O4C6Ph pterin wire emission lifetime is reduced by energy transfer to the iNOS heme domain
- Calculated distance:

$$\frac{R^6}{R_0^6} = \frac{\tau_{da}}{\tau_d - \tau_{da}}$$

$R_{calc} = 15.7 \pm 1.4$ Å

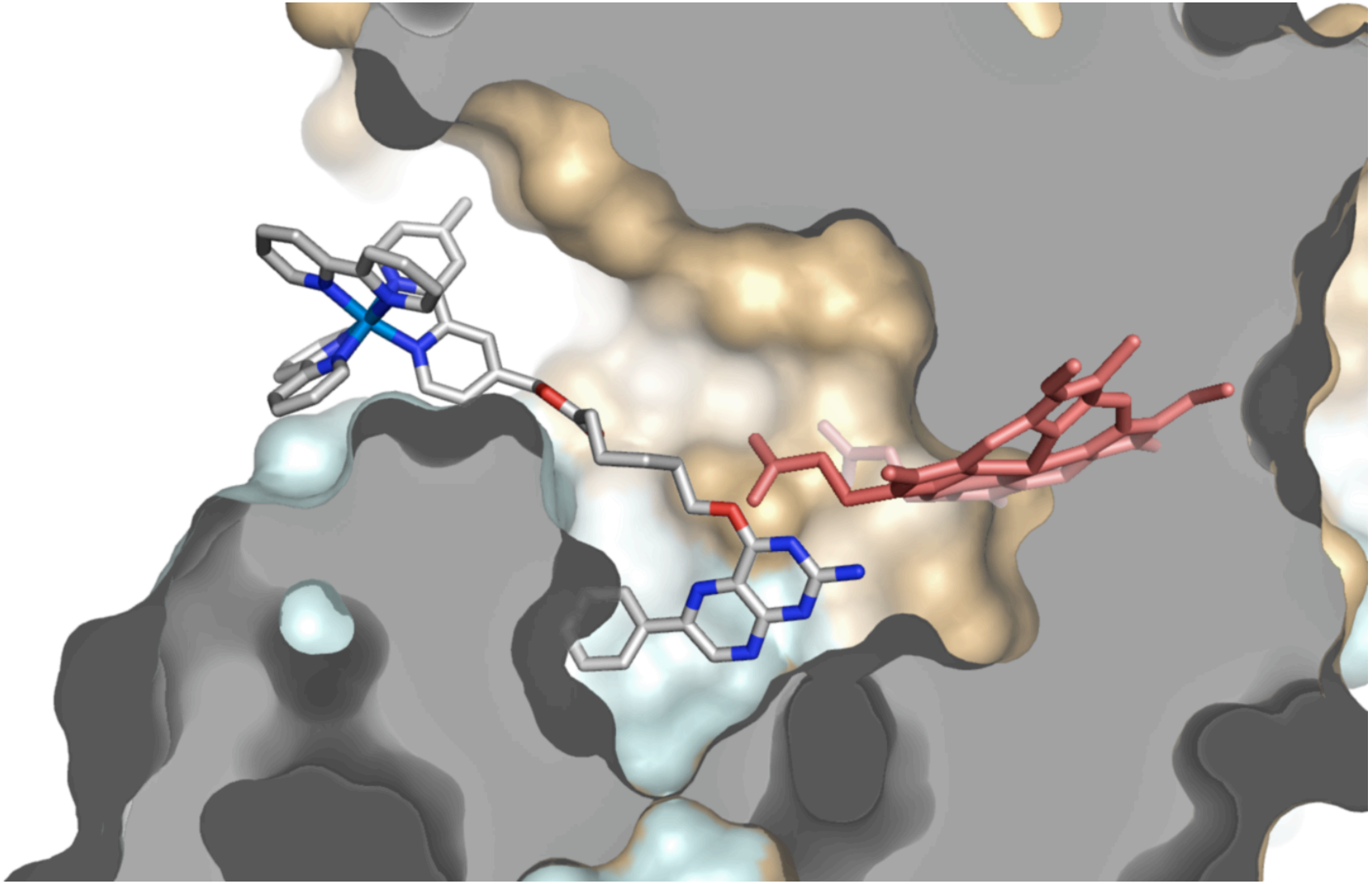
$R_{model} = 17$ Å



$$R_0 = 9.79 \times 10^3 (\kappa^2 n^{-4} \phi_d J)^{1/6}$$

$$J = \int_0^\infty F_d(\lambda) \varepsilon_a(\lambda) \lambda^4 d\lambda$$

Model of O4C6PhP wire bound at the pterin site of iNOS



Conclusions

- We have developed a synthetic route to an array of pteridine based molecular wires that will be used to probe the pterin binding site and function in NOS

- Wires with the linker extending from the O4 position of pterin appear to be accommodated in the murine iNOS heme domain dimer.

  – Arginine enhanced quenching of the pterin fluorescence by the heme

  – Pterin induced recovery of Ru-diamine fluorescence suggests competitive release

Acknowledgements

Goodin Lab
Stefan Vetter
Richard F. Wilson

Gray Lab
Wendy Belliston-Bittner
Yen Hoang Le Nguyen
Andrew K. Udit

Funding
NIH GM 070868 (to DBG and HBG)
NIH GM074406 (to EG)