# Computational Studies of Solvation Shell Influence on Heme Ruffling in Nitrophorin 4


PURDUE UNIVERSITY

Samuel S. Egel[1,2] and Jorge H. Rodríguez[2]*

[1]Department of Chemistry

[2]Theoretical & Computational Biomolecular Physics Group, Department of Physics, Purdue University



## ABSTRACT

Nitrophorins are a class of heme containing enzymes used by certain blood sucking insects to transport a heme-bound nitric oxide (NO) molecule into a victim's bloodstream. The nitric oxide is released from the nitrophorin on entry into the host's blood due to an increase in pH and dilution. Nitric oxide serves as a signaling molecule that induces vasodilation in a victim to provide a better blood meal for the insect. The heme group found in some nitrophorins is not planar as the heme groups in most other heme proteins. It has been hypothesized that the ruffled nature of this heme prevents reduction of the ferric iron center, allowing reversible binding of the nitric oxide and enabling rapid release of the ligand. The close packing of leucine 123 and 133 methyls to the heme has been proposed as a source of van der Waals interactions that give rise to heme ruffling [1]. The present study seeks to determine which physico-chemical interactions between the heme active site and surrounding protein residues give rise to the heme's ruffled conformation. A variety of computational approaches, including semiempirical and density functional (DFT) quantum mechanical methods, will be used to quantify the interaction energies between the iron porphyrin of nitrophorins and their surrounding residues to elucidate the physico-chemical origin of the unusual ruffled structures.

Above are two friendly insects that utilize nitrophorins. At left is Rhodnius prolixus (Kissing Bug), and shown at right is Cimex lectularius (common Bed Bug).

## INTRODUCTION

Although seven nitrophorin enzymes are known to exist and perform similar functions, the present study is directed only at nitrophorin 4. The nitrophorin 4 enzyme is precisely engineered to deliver NO to a victim's bloodstream. A general survey of topical literature indicates that the NO molecule is reversibly coordinated to the heme's iron center and enclosed in a tightly wrapped solvation shell when the nitrophorin is in a low pH solution, such as the approximate pH 5 of relevant insects' saliva. When the nitrophorin system is diluted and introduced to a higher pH, the body's normal pH is around 7, long protein arms unfold and allow the NO to be emitted from the heme pocket and induce vasodilation in the victim.

The key to the nitrophorin's ability to rapidly release NO is postulated to be the reversible binding between NO and the ferric iron center of the heme. A correlation between the degree of heme ruffling and the coupled ligand species has shown that the largest degree of heme ruffling occurs when NO is paired with ferric iron [2]. Ferric iron also has a much weaker bond with nitric oxide compared to ferrous iron (Kd = $10^{-12}$-$10^{-15}$ for $Fe^{2+}$ but Kd = $10^{-6}$-$10^{-9}$ for $Fe^{3+}$) [3]. The difference in bond strength probably originates from ferrous iron's ability to contribute electron density to π anti-bonding orbitals in the nitric oxide for an increased bond strength from the NO molecule to iron at the expense of bond strength between nitrogen and oxygen in a process called π backbonding.

## HEME RUFFLING

The large extent of heme folding or ruffling has been cited as the method used by nitrophorins to maintain the necessary oxidation state of iron. The carbons in a ferric centered NP4 heme bound to NO display a total deviation from planarity of -.81 Å. This is much greater than the next highest degree of heme ruffling of any experimentally determined ligand – heme system, -.69 Å, which occurs when NO binds ferrous iron hemes [2].

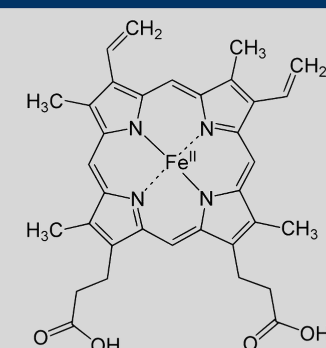



**Figure 1:**
Fig. 1 is a two dimensional portrayal of a generalized heme structure. Most heme structures exist in a conformation where the carbons in the ring system share the same plane, but NP4 carbons deviate sharply.

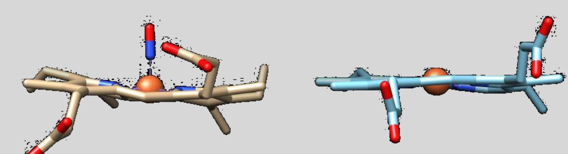

**Figure 2:**
Fig.2 shows a head-on view of an NP4 heme (carbons in tan) on the left and a heme from cytochrome P450 (carbons in blue) on the right. The degree of heme carbon distortion from planar in NP4 can easily be noted from this visual comparison.

## METHODS

To investigate protein interactions with the heme moiety, X-ray crystallographic structures of nitrophorin 4 bound to nitric oxide were obtained from the RCSB Protein Data Bank [8]. A system composed of the heme, histidine 59, nitric oxide, leucines 123 and 133, along with lysines 125 and 88 was chosen as a starting point for basic iron center-protein interaction energy calculations. LEU 123 and LEU 133 are within van der Waal contact of the heme; respectively 3.35 Å and 3.47 Å [1]. Lysines were added because electrostatic interactions between their positively charged terminal amino groups and the nearby propionate arms of the heme seemed probable. The closest points found between the heme and LYS 125 was 2.216 Å and LYS 88 were ≈4.980 Å.

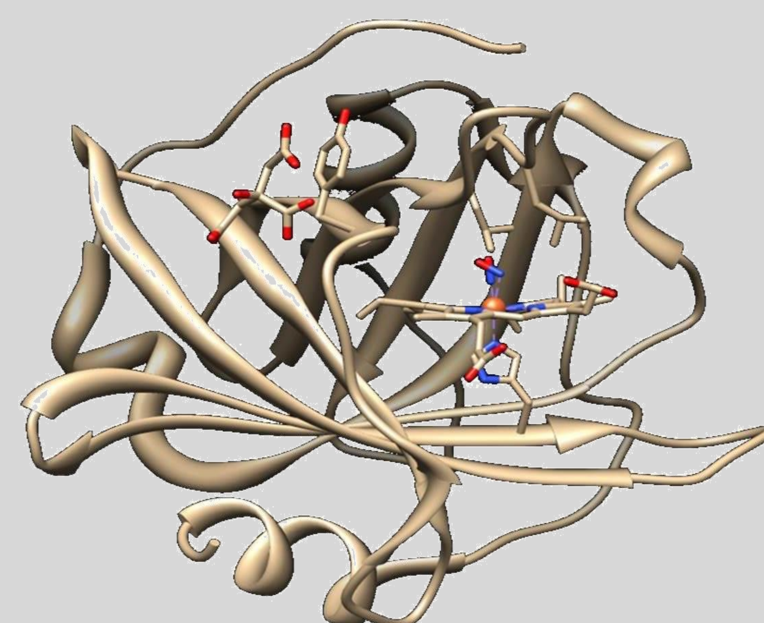

**Figure3.** Ribbon diagram of the entire NP4 enzyme prior to extraction of the system chosen as the heme active site.

The appropriate protein residues were selected in BallView [4] and hydrogens were added using the IDATM [5] algorithm implemented in Chimera [6]. The hydrogen coordinates were optimized to a local minima of energy using UPM6 as implemented in Gaussian [7]. Following hydrogen optimization, residues were extracted from the complete model and internal energies were calculated.

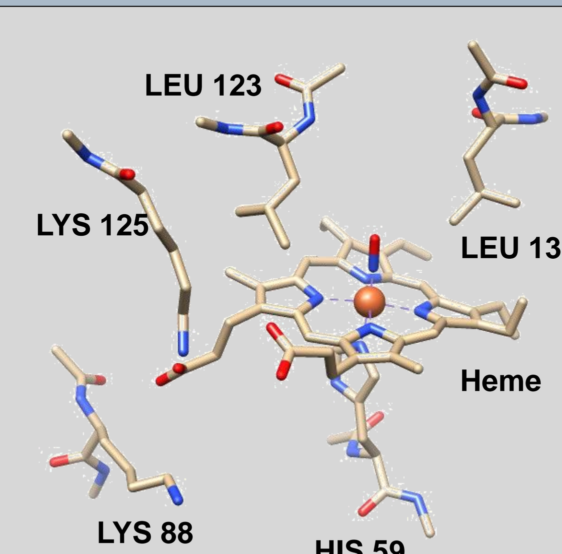



**Figure. 4.** Model used for energy computations. Hydrogens are omitted for visual clarity, carbon is shown in tan, nitrogens in blue, oxygens in red, and iron is the very middle bronze colored atom. Abbreviations are LEU for leucine, LYS for lysine, HIS for histidine. The model consists of both LEU 123 & 133 as recommended by literature for creating van der Waals interactions with the heme plane and two lysines chosen for potential electrostatic interactions. The distal histidine is coordinated to the heme to anchor it in the enzyme.

Many heme proteins contain a planar heme anchored in place by a distal histidine. Therefore, HIS 59 with the heme and NO was chosen as a base system and its energy was calculated as a single chemical unit. The internal energies of the solvation shell protein residues were first computed separately and then with the base model included. Interaction energies between the [Heme•NO + HIS59] base model and individual residues were tabulated as the difference between the energy of the *supermolecule* [Heme•NO + HIS59 + Residue] minus the energy of its individual fragments:

$$E_{Int} = E_{Supermolecule} - E_{[Heme \bullet NO+HIS59]} - E_{Residue} \quad (1)$$

The computational procedure was repeated for the U-PM6 and U-B3LYP methods where U stands for spin unrestricted. PM6 is a semiempirical method for solving, in a self-consistent field (SCF) fashion, a molecular Hamiltonian. The latter is an operator that represents the total internal energy of a molecule. PM6 is a useful starting point, but it neglects rigorous computation of important physical interactions, such as correlation effects between electrons, in favor of less expensive computation. B3LYP provides better approximations to the energy of a system at somewhat greater computational cost. B3LYP is a hybrid density functional theory (DFT) method where a system's internal energy includes, to a good approximation, electron-electron exchange and correlation energies based on SCF calculations.

## RESULTS AND DISCUSSION

| Energies from UPM6 & B3LYP Computations | | |
|---|---|---|
| Model Name | U-PM6 (A.U.) | U-B3LYP (A.U.) |
| [HEME·NO+HIS59] | -1.40E-002 | -3.98E+003 |
| LEU133 | -1.67E-001 | -6.06E+002 |
| LEU123 | -1.71E-001 | -6.06E+002 |
| LYS125 | 1.47E-001 | -4.56E+002 |
| LYS88 | 9.16E-002 | -6.61E+002 |
| HEME+LEU133 | -1.82E-001 | NA |
| HEME+LEU123 | -1.89E-001 | -4.58E+003 |
| HEME+LYS125 | -7.05E-002 | -4.43E+003 |
| HEME+LYS88 | -4.52E-002 | -4.64E+003 |

**Table 1**. Values for energies of the base model, individual residues and base model combined with each of the individual residues. Computations were carried out for U-PM6 and U-B3LYP.

Results from these computations indicate that a stronger interaction energy exists between the lysines and the heme histidine system than either leucine and the heme histidine system. Although both mathematical approaches seem to hint at this trend, the LEU 133 model was not able to be converged for the 6-31G* basis set. The output log and input com files will be examined for sources of error. The energy values that have been obtained appear very different between the two models, but agree more closely in the final tabulation of interaction energies seen in the following table.

| Interaction Energy Between [HEME + HIS 59] and Given Residue | | |
|---|---|---|
| Protein Residue | Interaction Energy [A.U.] | Interaction Energy [A.U.] |
| LEU 133 | -9.87E-004 | NA |
| LEU123 | -4.14E-003 | -1.13E-003 |
| LYS 125 | -2.03E-001 | -1.97E-001 |
| LYS88 | -1.23E-001 | -1.22E-001 |

**Table 2**. Interaction energies between individual residues and the base model computed according to Eq. (1).

The energy differences in Table 2 agree somewhat more closely between methods than in the total energy data due to cancellation of error through the subtraction operation. However, differences in these numbers is to be expected due to the difference in predictive ability of these two approaches (PM6 *versus* B3LYP). One important conclusion is that the trend of LYS 125 having the greatest magnitude of interaction energy followed by LYS 88 and then LEU 123 is consistent for both techniques.

| Interaction Energy Ratios Between Each Model | | | | |
|---|---|---|---|---|
| | LEU 133 | LEU 123 | LYS 125 | LYS 88 |
| LEU 133 | | NA | NA | NA |
| LEU 123 | 4 | | 174 | 108 |
| LYS 125 | 206 | 49 | | 2 |
| LYS 88 | 125 | 30 | 2 | |

**Table 3.** Ratio of the larger to the smaller of each combination of interaction energies. The grey boxes in the lower half of the table are from U-PM6 and the un-shaded upper half of the table corresponds to U-B3LYP.

## CONCLUSIONS

The energy values derived in this study provide evidence for concluding that the interaction energies between the heme and LEUs 123 and 133 are unlikely to provide the greatest contribution toward heme ruffling. This result was expected due to the larger energy in electrostatic interactions, such as between the LYS 125 nitrogen and heme oxygens, than in dispersion forces, which are the only possible interaction between the leucines and the porphyrin ring. However, B3LYP does not properly compute dispersion so further anlysis and calculations are needed. It is also not surprising that LYS 125 seems to have a stronger interaction with the heme than LYS 88 due to LYS 125's closer proximity. This study does not fully explain the origin of heme ruffling. However, we identified the stronger interactions between the heme and certain residues.

## REFERENCES


[1] Roberts, S. A.; Weichsel, A.; Qiu, Y.; Shelnutt, J. A.; Walker, F.A.; Montfort, W. R. Biochemistry 2001, 40, 11327.
[2] Estelle, M. M.; Roberts, S.A.; Weichsel, A.; Montfort, W.R. Biochemistry 2005, 44, 12690
[3] Andersen, J.F.; Ding, S.D.; Balfour, C.; Shokhiereva, T.K.; Champagne, D.E.; Walker, F.A.; Montfort, W.R.; Biochemistry 2000, 39, 10118.
[4] Moll A, Hildebrandt A, Lenhof HP, Kohlbacher O. BALLView: A tool for research and education in molecular modeling. Bioinformatics (2006), 22(3):365-366
[5] Meng, E.C.; Lewis, R.A.; Journal of Computational Chemistry (1991), 12(7), 891.
[6] Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. "UCSF Chimera - A Visualization System for Exploratory Research and Analysis." J. Comput. Chem. 25:1605-1612 (2004).
[7] GaussView, Version 5, Dennington, R.; Keith, T.; Millam, J. Semichem Inc., Shawnee Mission KS, 2009.
[8] PDB ID: 1ERX Weichsel, A.; Andersen, J.F.; Roberts, S.A.; Montfort, W.R.; Nat. Struc. Biol. (2000), 7: 551-554


## ACKNOWLEDGEMENTS


Thanks to Dr. Jorge Rodríguez for his advise and for teaching me the value of using the Linux operating system (SSE).



www.PosterPresentations.com