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QM and Hybrid QM-MM Simulation of Biomolecules (Computer Simulation of Ligand Binding and Reactivity of Heme Proteins) Part III

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Computer simulation of ligand binding and reactivity of heme proteins

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Outline

In this final talk, we will discuss two complex and interesting problems:

Multiligand chemistry: the M. Tuberculosis TrHbN case

Reaction mechanism in indoleamine and tryptophan dioxygenases

Truncated hemoglobins

Truncated Hb family found in the 90´ : sequences 20-40 residues shorter than classical globins

Distributed in bacteria, unicellular eukaryotyes and higher plants (no in animals!)

Tertiary structure based on a 2-on-2 ∝ helical sandwich (trimmed modification of classical 3-on-3 fold.

Despite minimal size, they may display an inner cavity/tunnel system

TrHbN from M.T. Multiligand chemistry:

NO produced by the immune system maybe inactivated by the oxygenated Hb:

toxic $\rightarrow$ innocuous

$$NO + Fe(II)\,O_2 \rightarrow Fe(III) + [NO_3]^-$$

Relevant process in physiological (Hb, Mb) and pathological processes (it is a problem in the design of synthetic hemoglobins)

Experimental kinetic information

$Fe(II) + O_2 \rightarrow Fe(II)O_2$ $k_{on} = 25\ \mu M^{-1}s^{-1}$

Involves oxygen entry and formation of $Fe\text{-}O_2$ bond

$NO + Fe(II)\,O_2 \rightarrow Fe(III) + [NO_3]^-$

$k_{ox} = 745\ \mu M^{-1}s^{-1}$

Involves NO entry and chemical reaction

Difficult to understand

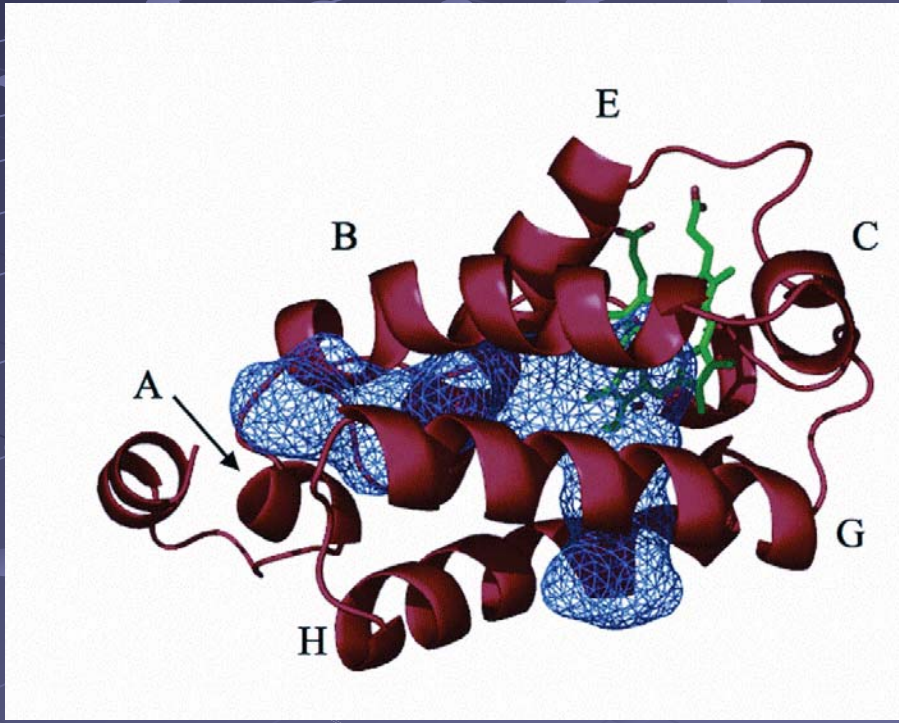
Reaction mechanism:

- O_2 migration (classical MD)
- O_2 binding (QM-MM)
- NO migration (classical MD)
- Reaction of NO with O_2 (QM-MM)
- Product release (QM-MM and classical MD)
- Protein reduction

Key reaction:

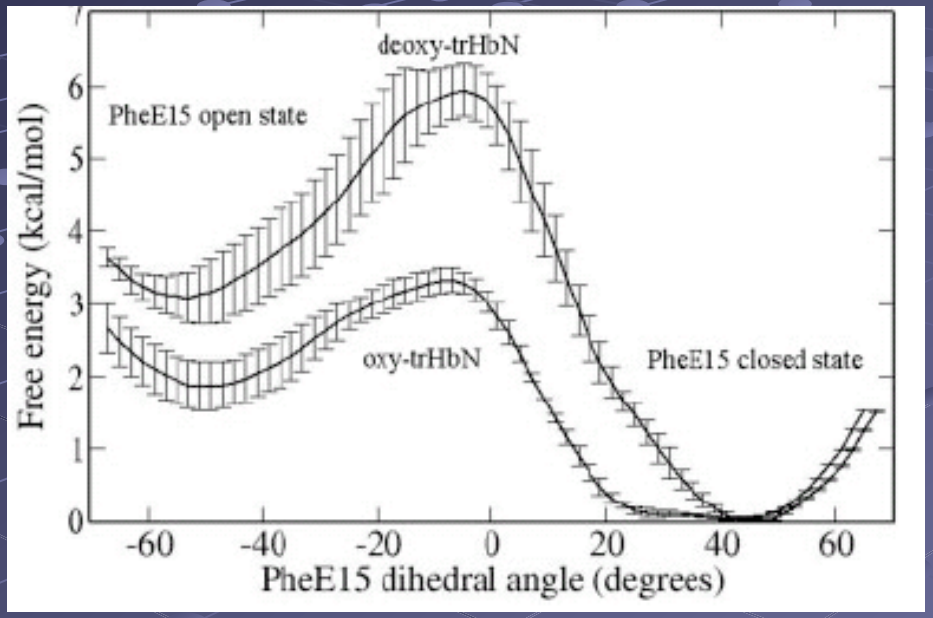
$$NO + Fe(II)\ O_2 \rightarrow Fe(III) + [NO_3]^-$$

2 channel system proposed on the basis of inspection of x-ray results (Bolognesi' s group at Milano)
But how do O_2 and NO migrate?



Long (100 ns) classical MD (Amber 9) of oxy and deoxy proteins indicate PheE15 acts as the long tunnel gate, showing two conformations (open and closed).
O_2 coordination "opens" the tunnel for NO migration

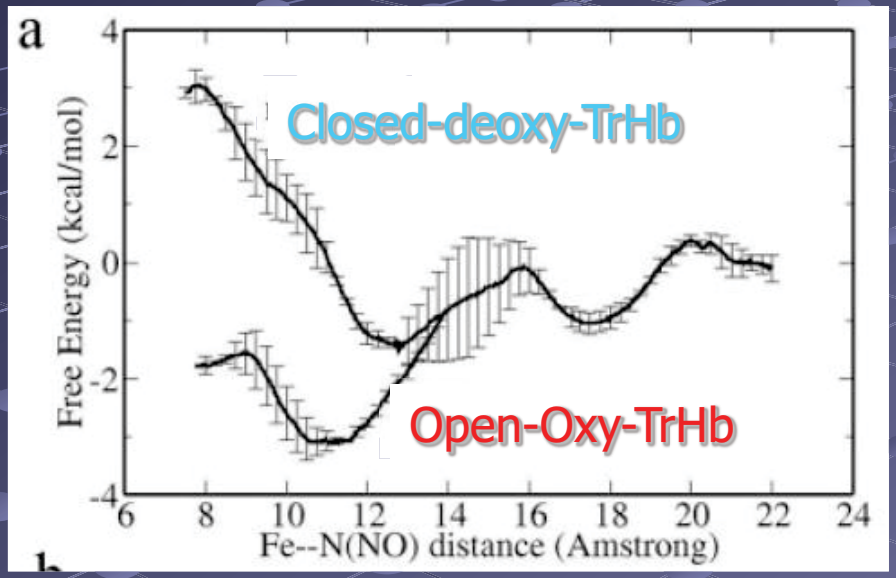
Umbrella sampling free energy profiles for channel opening



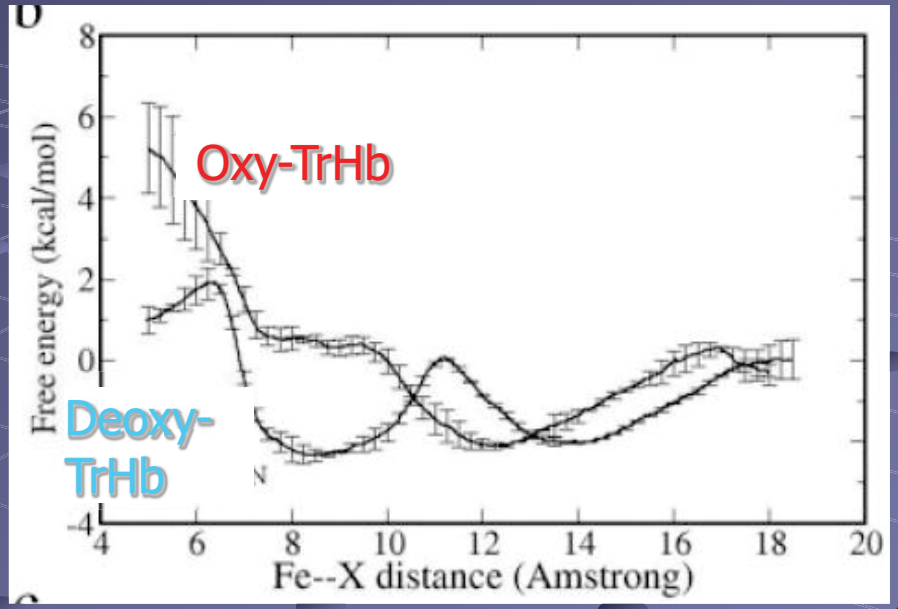
Long channel closed almost always in deoxy protein.
Oxygen binding induces channel opening
for second ligand (NO)!!

A. Bidon Chanal et al, Proteins (2006), 64, 457-464.

Long channel



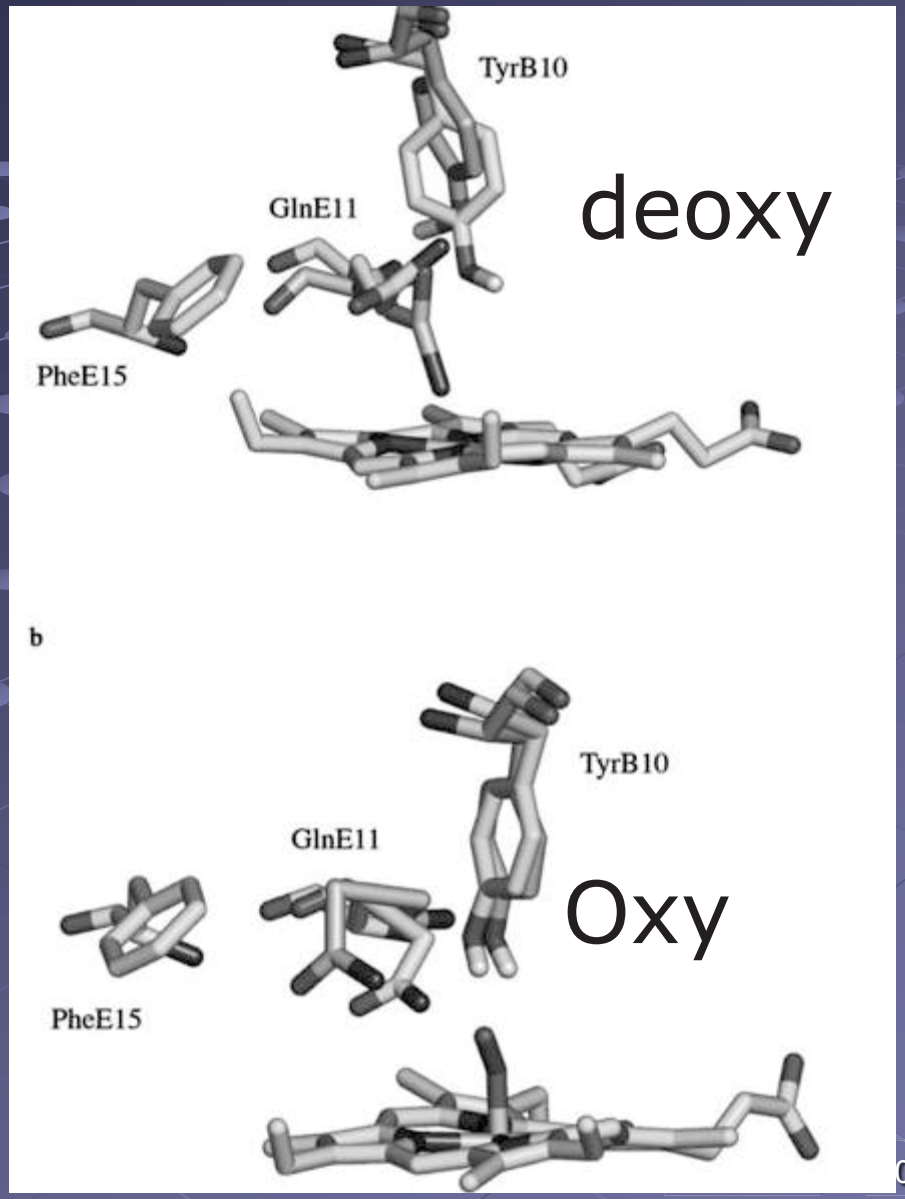
Short channel



Long channel closed (deoxy) vs open (oxy) NO entry to oxygenated protein

Short channel closed (oxy) vs open (deoxy) O_2 entry to deoxy protein.

Why does this happen?

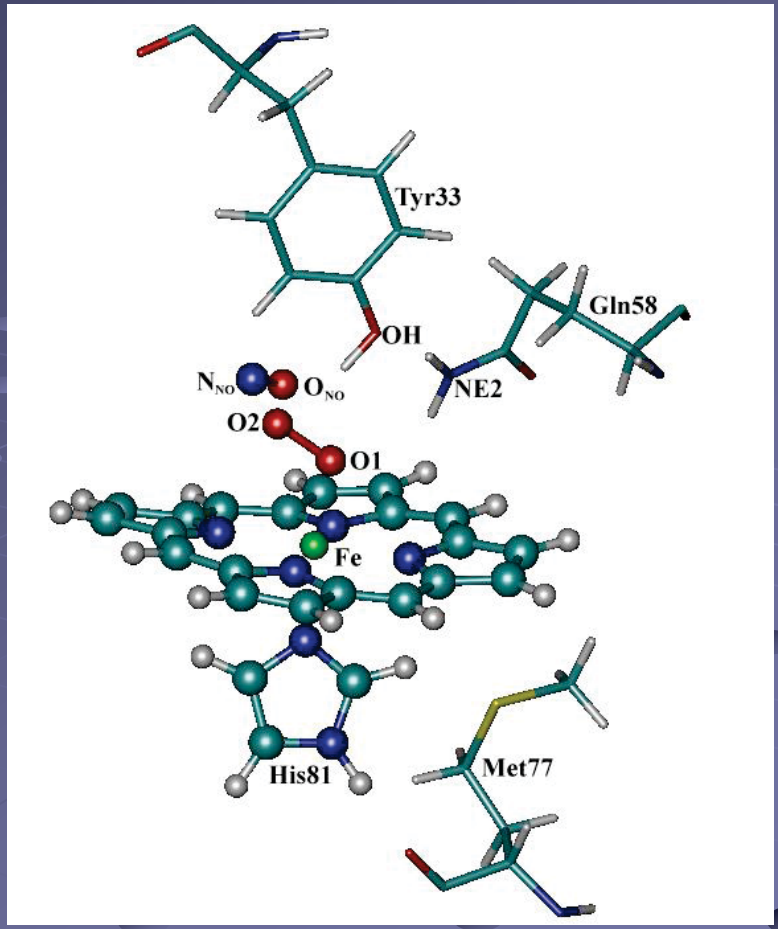


GlnE11 moves away upon O_2 binding, pushing PheE15, due to competition in H bonding of oxygen (which carries a significant negative charge)

Oxygen affinity

Hydrogen bond with OH of Tyr B10

Effect of TyrB10→PheB10 mutation on k_{off} is reproduced. Affinity is large (consistent with the detoxification role).



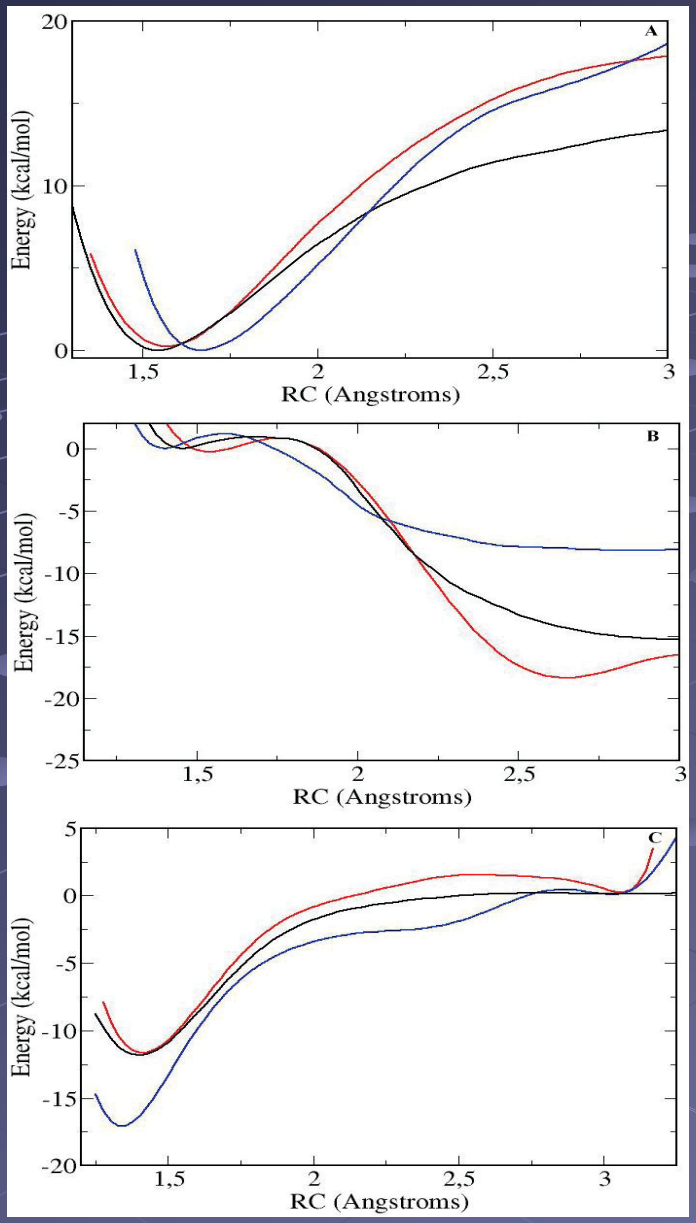
isolated	Mb	TrHb	TyrB10→PheB10
-21.4	-25.0	-37.2	-34.3 kcal/mol

Chemical reaction:

$Fe(II)\text{-}O_2 + NO \rightarrow Fe(III)[\text{-}OONO]$

$Fe(III)[\text{-}OONO] \rightarrow Fe(IV)=O + NO_2$

$Fe(IV)=O + NO_2 \rightarrow Fe(III)[\text{-}NO_3]$



Vacuum
Water
Protein

Almost barrierless reaction. Profiles in protein similar to those in water

A. Crespo et al,
J. Am. Chem. Soc. (2007), 129, 6782.

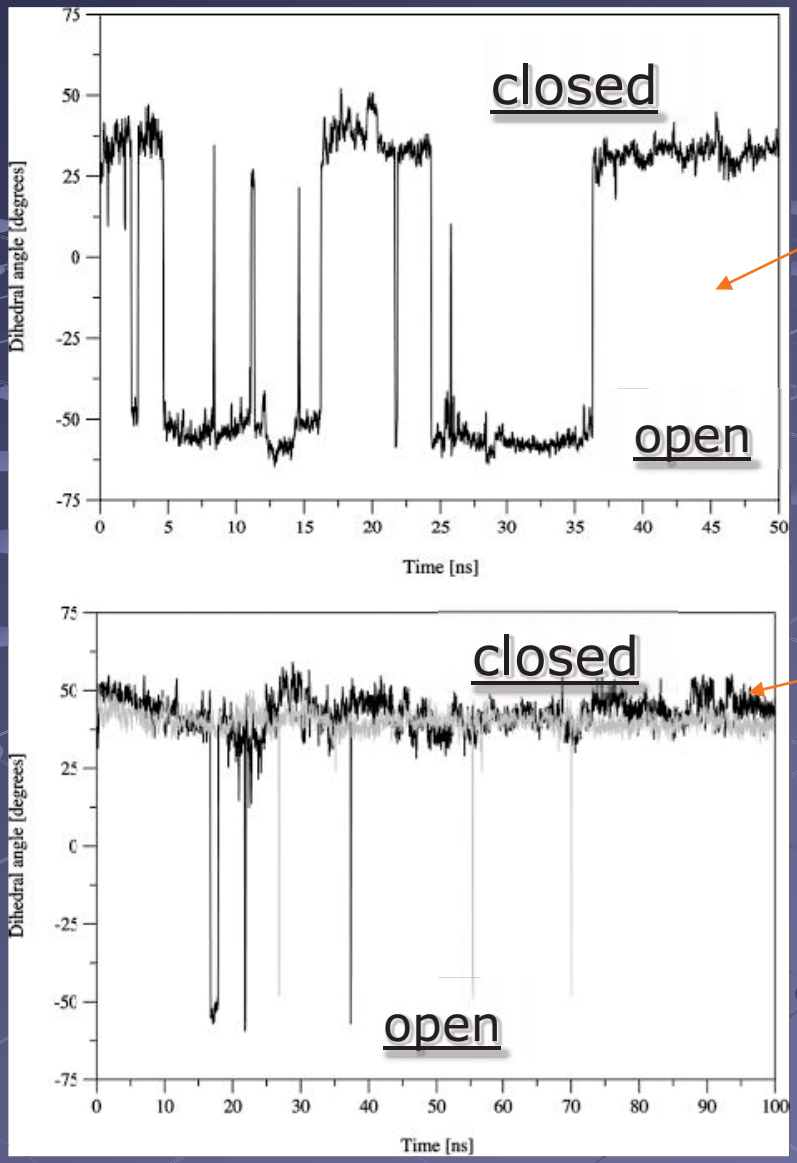
Further confirmation: *in silico* mutated proteins

- TyrB10-Phe mutant: does not detoxify efficiently . Why?

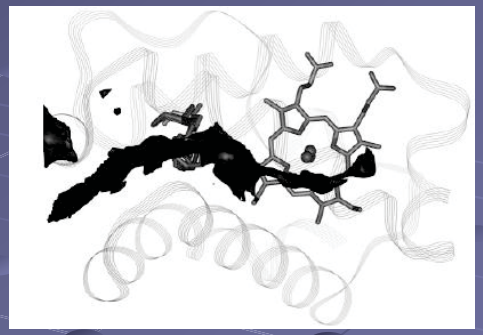
Ouellet et al, PNAS (2002), 99, 5702.

A. Bidon-Chanal et al, J. Am. Chem. Soc. (2007), 129, 6782.

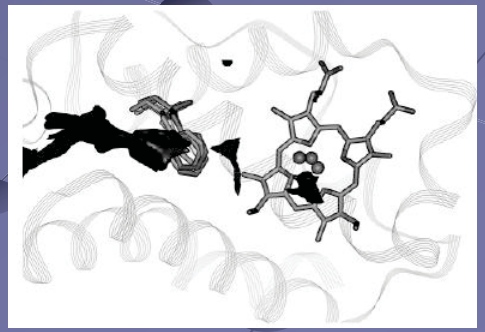
MD of oxygenated proteins



Wt



TyrB10-Phe (black)
GlnE11-Ala (grey) mutants
Always closed!!!

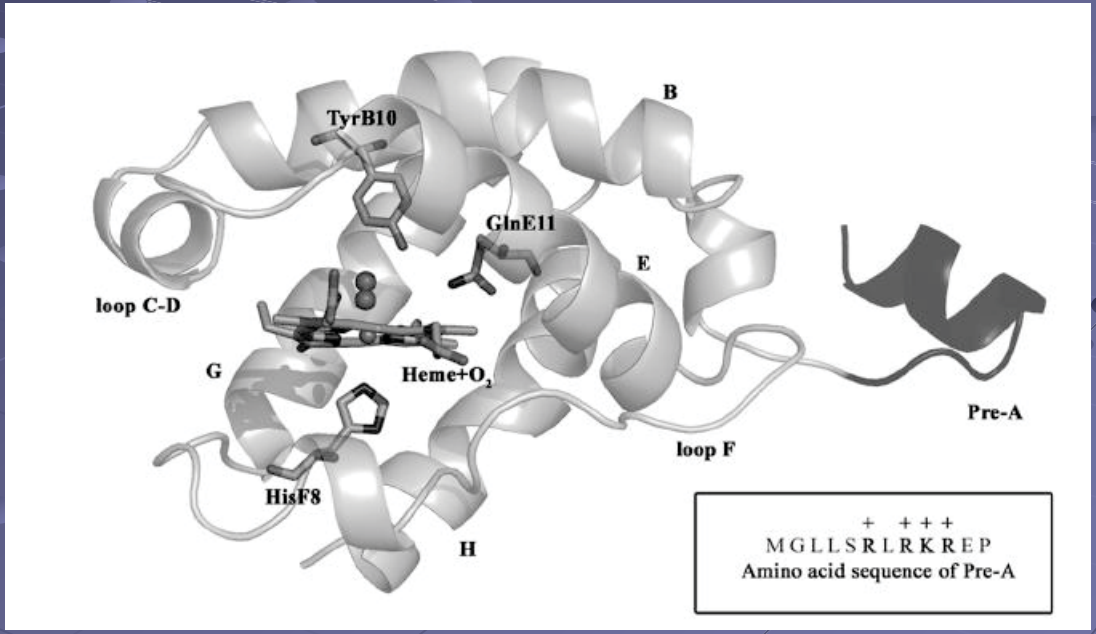


- Our data strongly support the hypothesis that the access of NO to the heme cavity is dynamically regulated by the TyrB10-GlnE11 pair, which acts as a molecular switch that controls opening of the ligand diffusion tunnel.

- Binding of O_2 to the heme group triggers local conformational changes in the TyrB10-GlnE11 pair, which favor opening of the PheE15 gate residue through global changes in the essential motions of the protein skeleton.

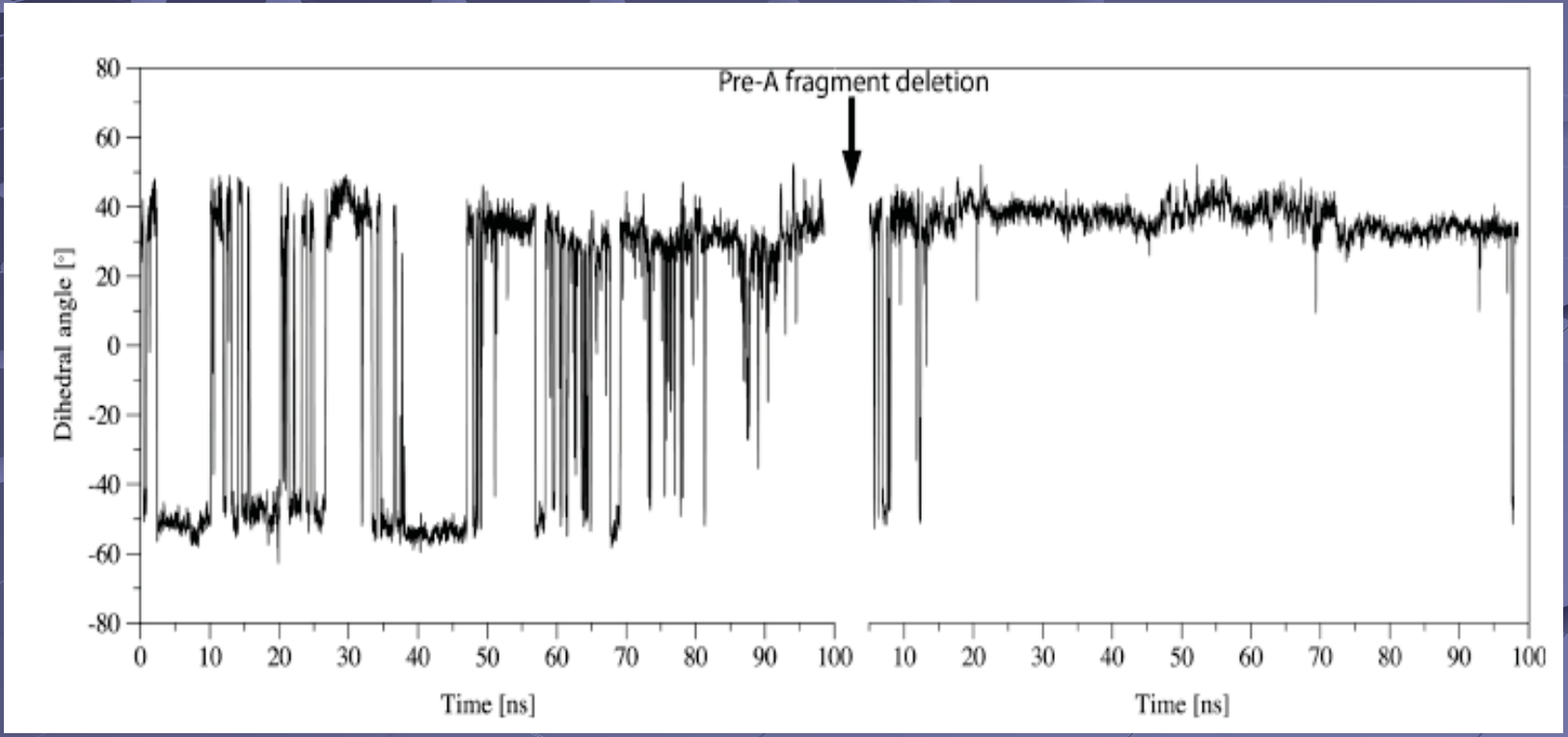
Role of the pre A helix in ligand entry modulation

M. Smegmatis TrHbN is almost identical to that of M.T. but lacks a fragment (pre A helix)



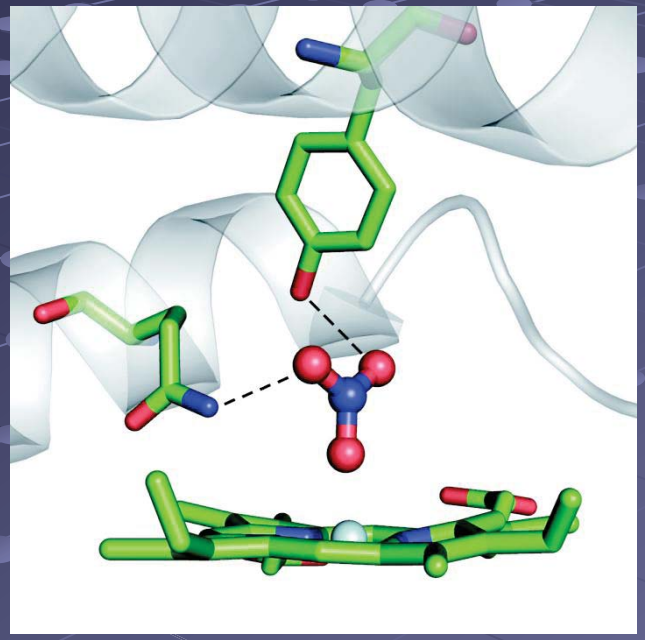
Strikingly, M. Smegmatis trHbN does not detoxify efficiently NO!

Simulation shows that deletion of pre A helix results in the trapping in the closed conformation, consistently with experimental results



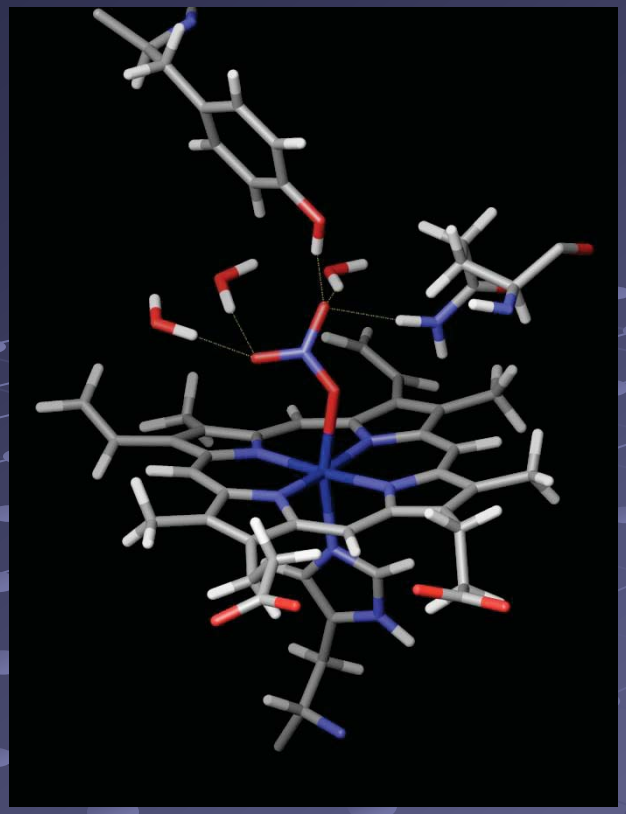
Lama et al, JBC, 284, 14457, 2009.

Product release



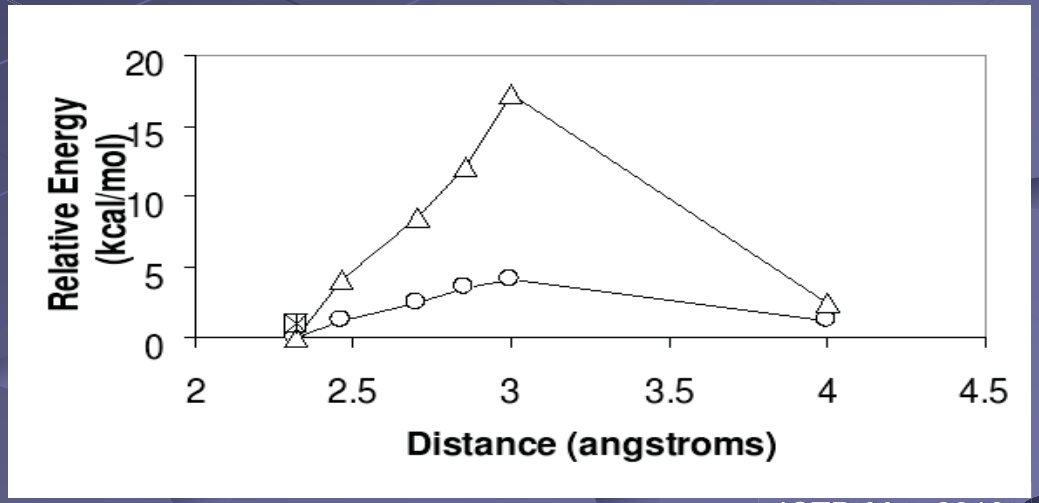
- Nitrate should be released to regenerate the enzyme.
- We have performed classical MD simulations
- QM model system and QM-MM calculations

Marti et al, JACS, 2008, 130, 1688.



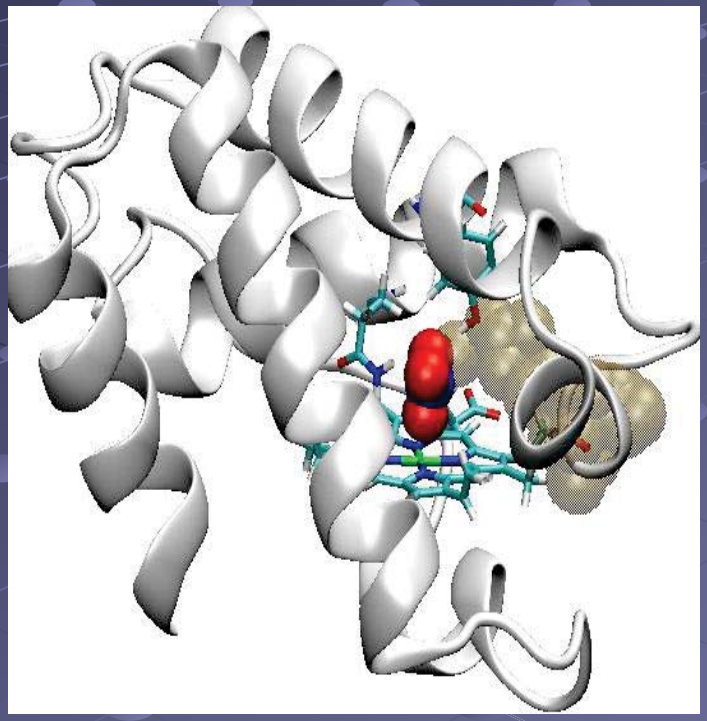
QM-MM calculations: heme+ligand+water+TyrB10+ GlnE11; just heme+ligand QM subsystems

High spin (sextet) is ground state at LACV3P*/B3LYP level for the large system

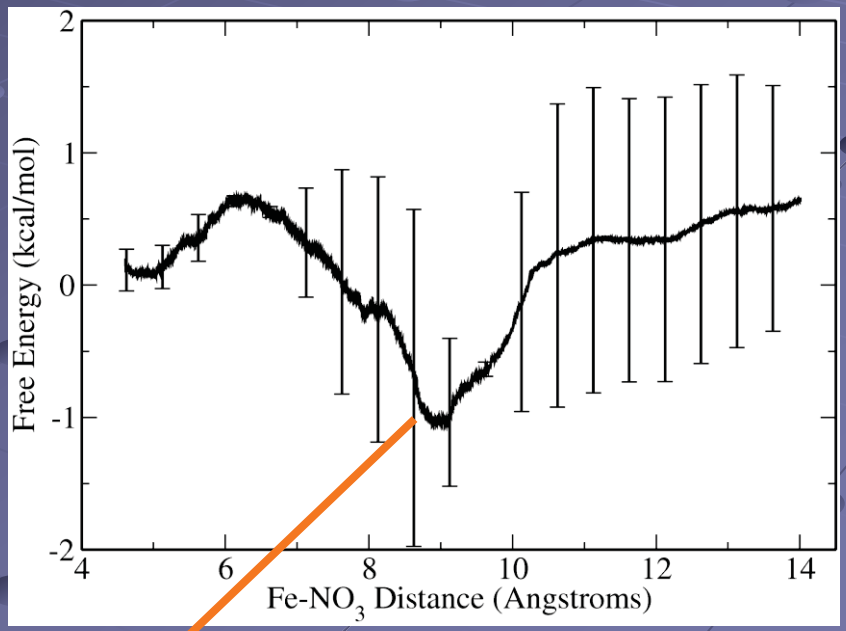


This electrostatic screening loosens the Fe-nitrate bond (barrier less than 4 kcal/mol), compared to 18 kcal/mol in the reduced model

Finally, once the bond is broken, classical MD simulations indicate that nitrate exits the protein very easily, but not along the (apolar) tunnels used for (NO, O_2) ligand entry.

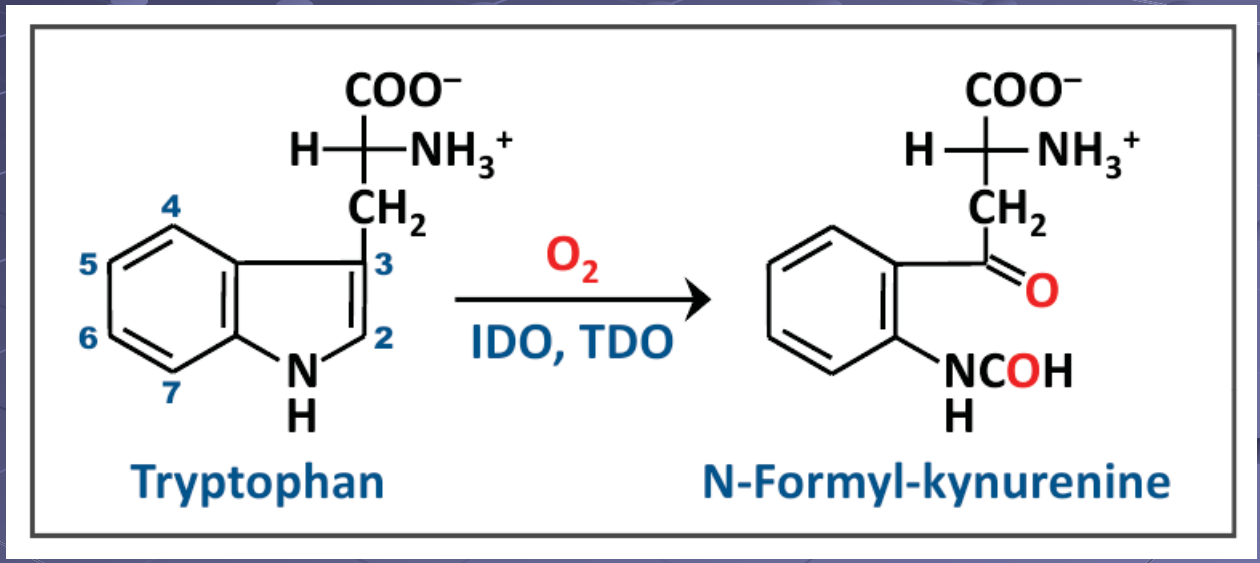


Free energy profile for ligand exit



Minimum: interaction with ThrE2, key in assisting exit

Tryptophane and Indoleamine dioxygenases (TDO, IDO)



TDO in liver, IDO widespread distribution
IDO target of anticancer drugs

Differences between IDO and TDO

1. **Localization and structure**
   - ✓ IDO is ubicuous and monomeric (PM ≈ 45 kDa)
   - ✓ TDO is tetrameric and located mainly in liver (PM≈ 167 kDa)
   - ✓ Sequence identiy <10%
2. **Catalysis:**
   - ✓ IDO reacts with *L-Trp, D-Trp* and other indoles
   - ✓ TDO is more specific for *L-Trp*
3. **Function:**
   - ✓ IDO is induced by *IFN-γ* and is related with immune response regulation
   - ✓ TDO is implied in Trp metabolism in liver

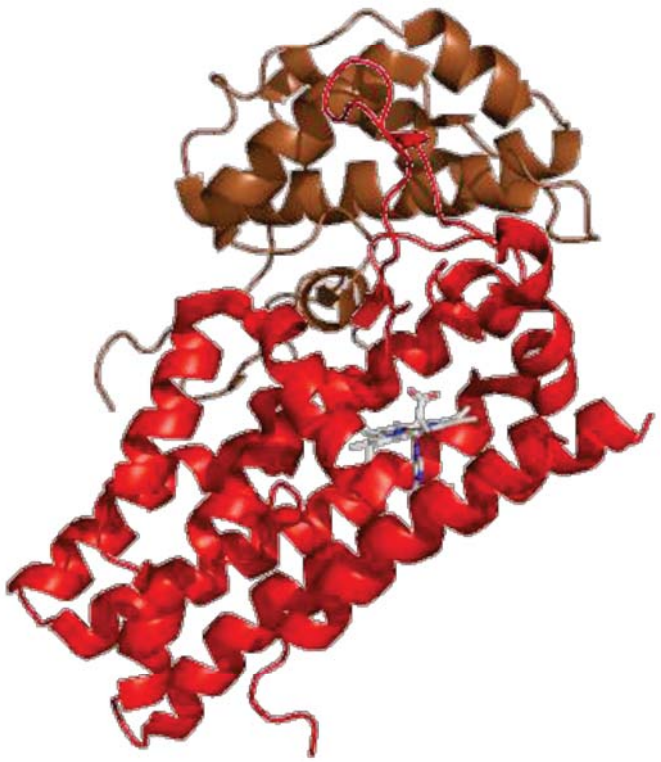
More about IDO

- **IDO is expressed in placenta and in cancer cells to reduce T cells proliferation**
- **It has been observed that its inhibition leads to a reduction in tumoral growth**

Development of selective inhibitors for IDO (which do not inhibit TDO) is of great interest

Samelson-Jones, B. *et al*. *Biochemistry*. (**2006**)
Munn, D.H. et al.. Science 281, 1191–1193 (1998).

Human IDO

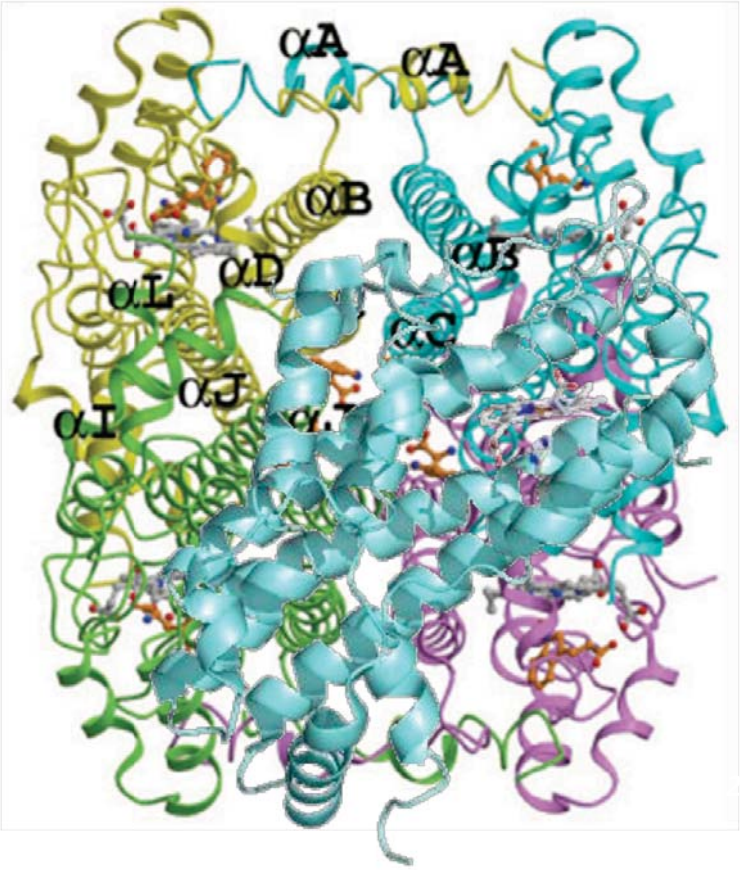


Monomer with two domains

Heme group in each monomer

High structural similarity

X. Campestris TDO



- Homotetramer
- One heme group per monomer
- Binds 8 Trp molecules per tetramer

Relevant questions associated to these proteins

Reaction mechanisms. Are there any differences?

How is substrate stabilized in the active site, and how this explains the stereoselectivity?

Which are the key residues in substrate binding and catalysis?

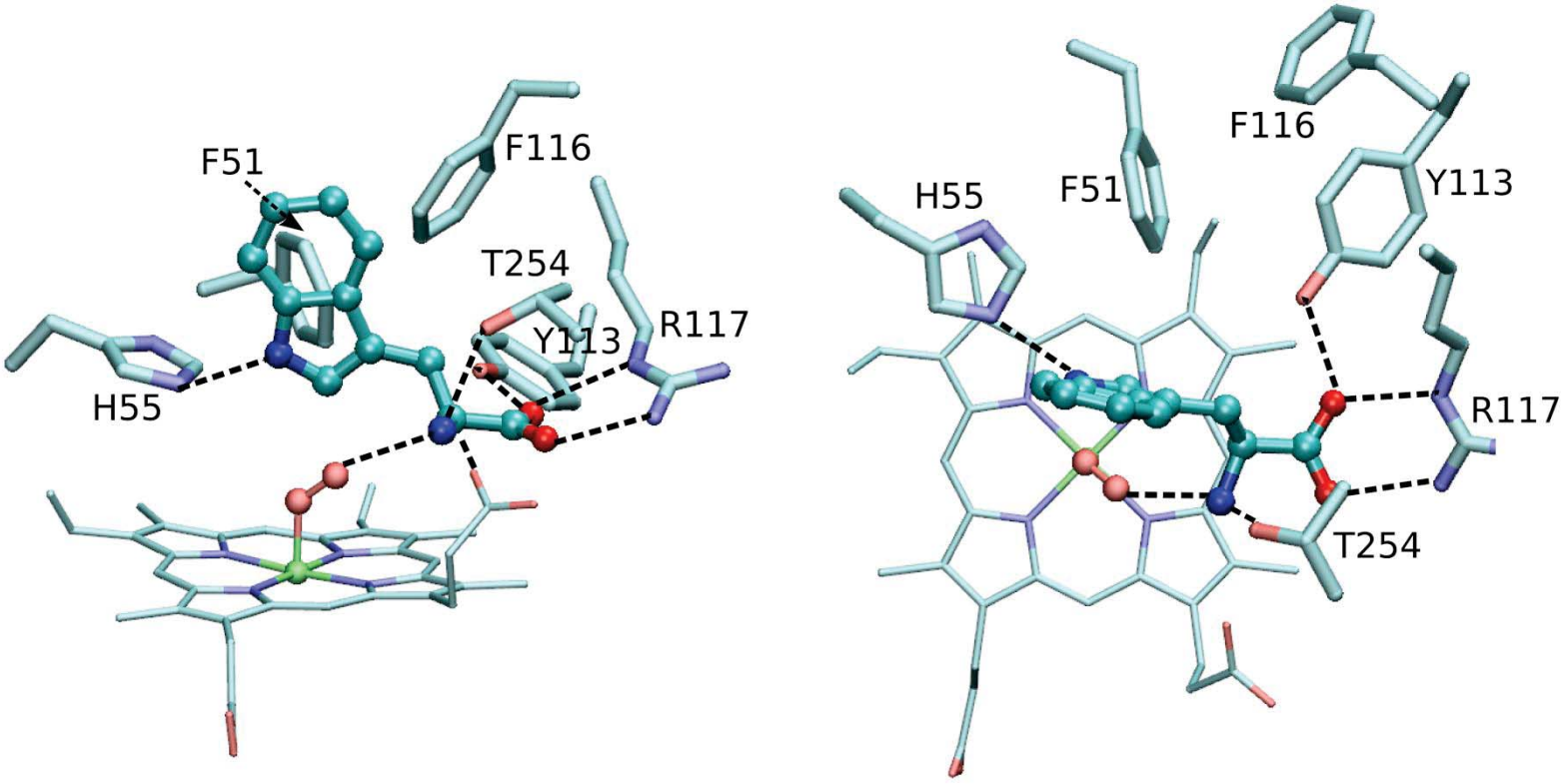
How can we answer these questions?

- Flexible **Docking** (*Autodock*) to obtain enzyme-substrate initial structures
- Classical MD simulations (*Amber)* to analyze

Interactions due to ligand binding

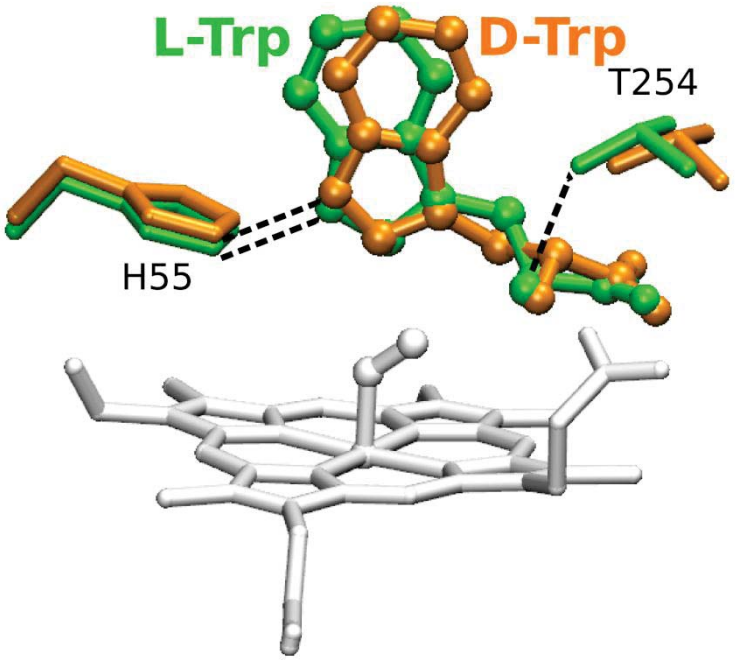
- Free energy binding calculations using continuum solvent methods (*MMGBSA).*
- **QM-MM** (*Hybrid – SIESTA*) calculations for the reaction mechanism and oxygen affinity

Oxy – L-Trp adduct in TDO



Arg 117, Tyr 113, His55 y Phe 51 stabilize Trp in active site

L-Trp vs D-Trp in TDO

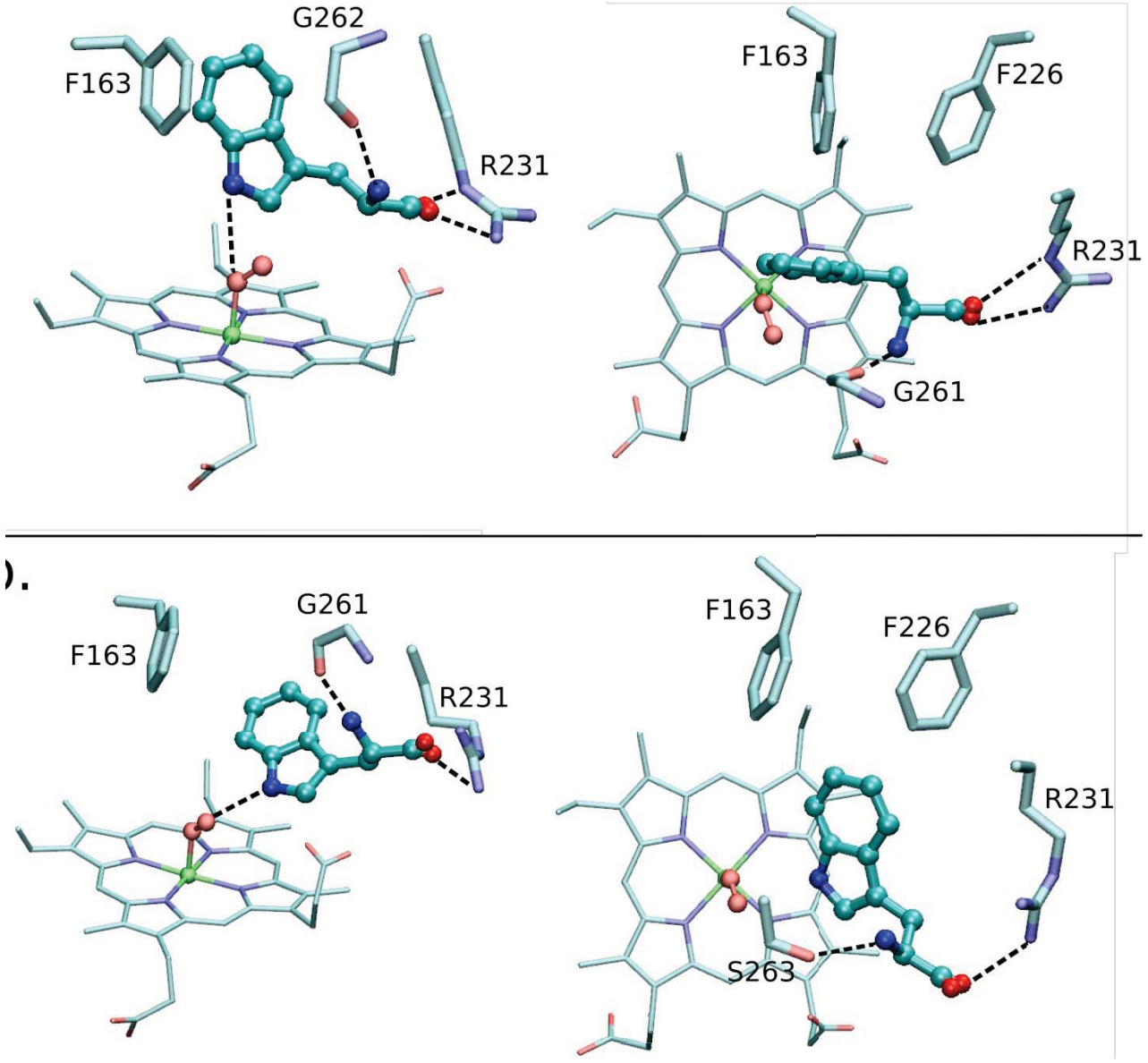


$xcTDO_{wt}$-O_2/D,L-Trp

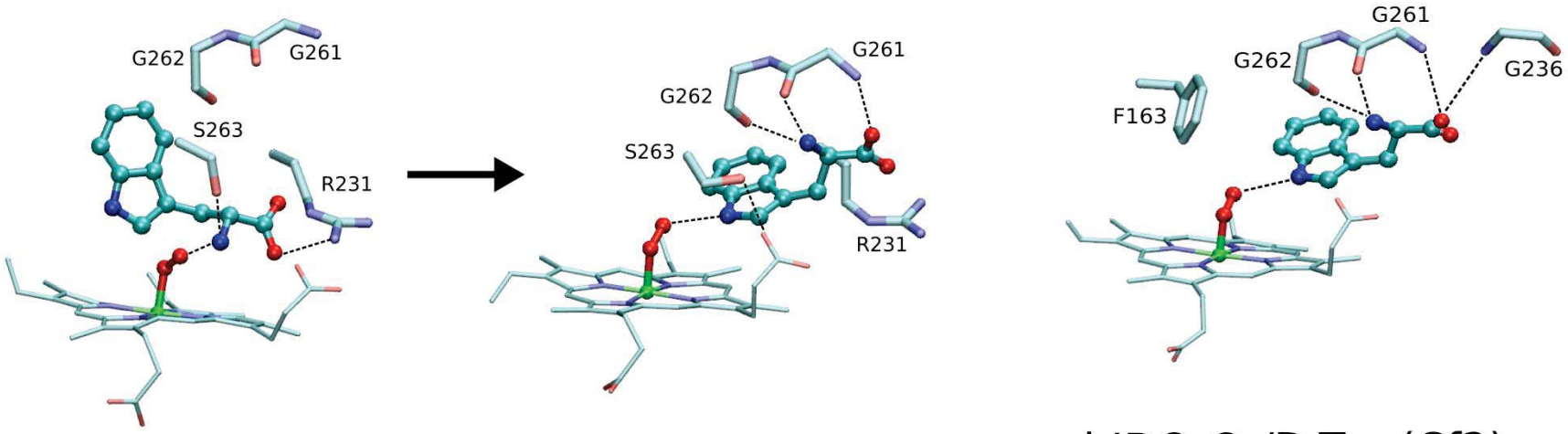
	xC-TDO	
Donor-Acceptor Pairs	**L-Trp**	**D-Trp**
Trp O – R 117 Nε	100	100
Trp OXT – R117NH1	100	100
Trp O – Y113 OH	100	100
H55 Nε2 - Trp Nε1	100	95
Trp OXT - T254 NH	100	85
T254 OH - Trp NH_3^+	**98**	**14**
Binding energy (kcal/mol)	**-51.7**	**-46.1**
Km (mM)	**0.114**	**16**

Oxy – L-Trp adducts in IDO:

Two conformations for L-Trp in active site



D-Trp in IDO

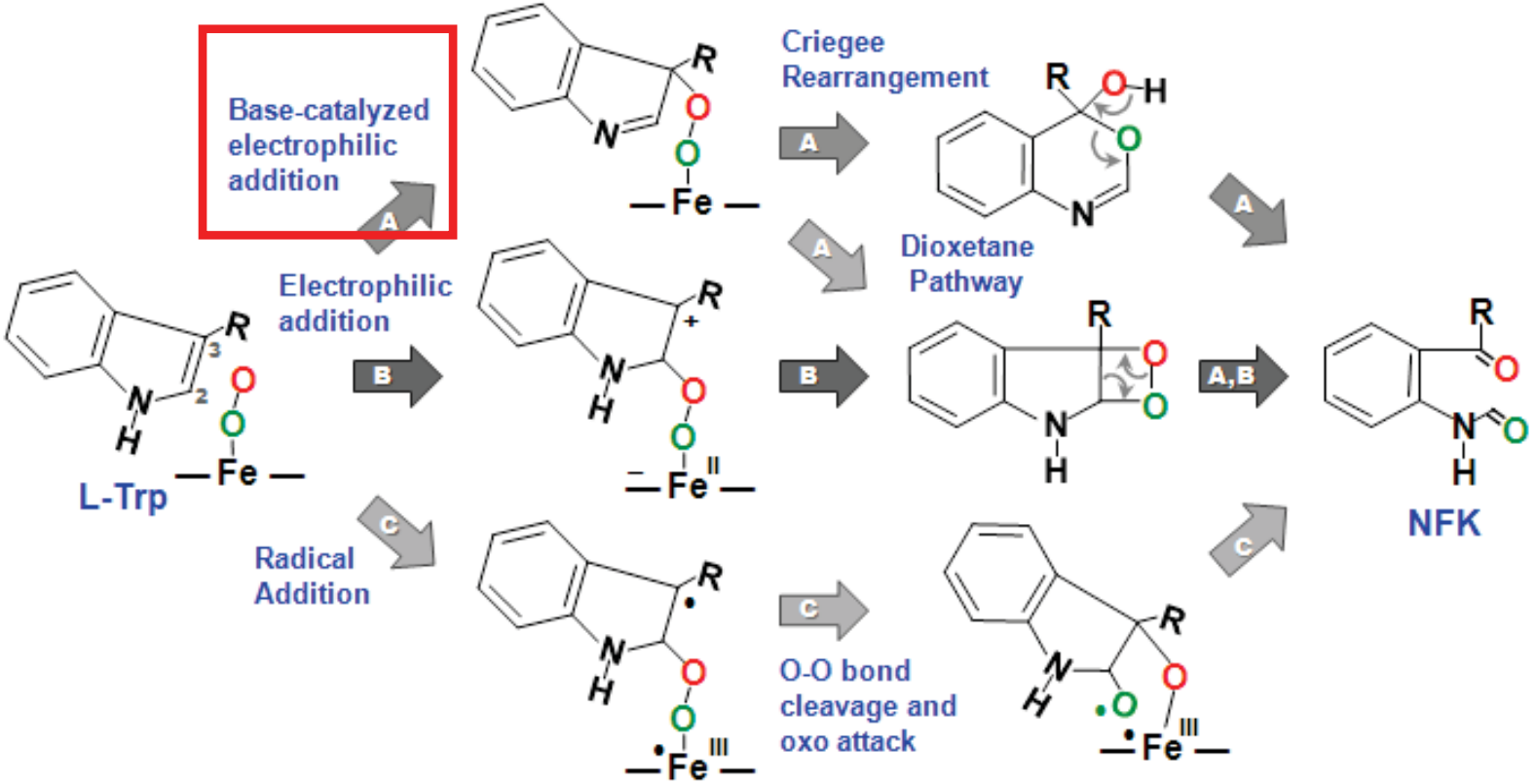


hIDO-O_2/D-Trp (Cf1)

hIDO-O_2/D-Trp (Cf2)

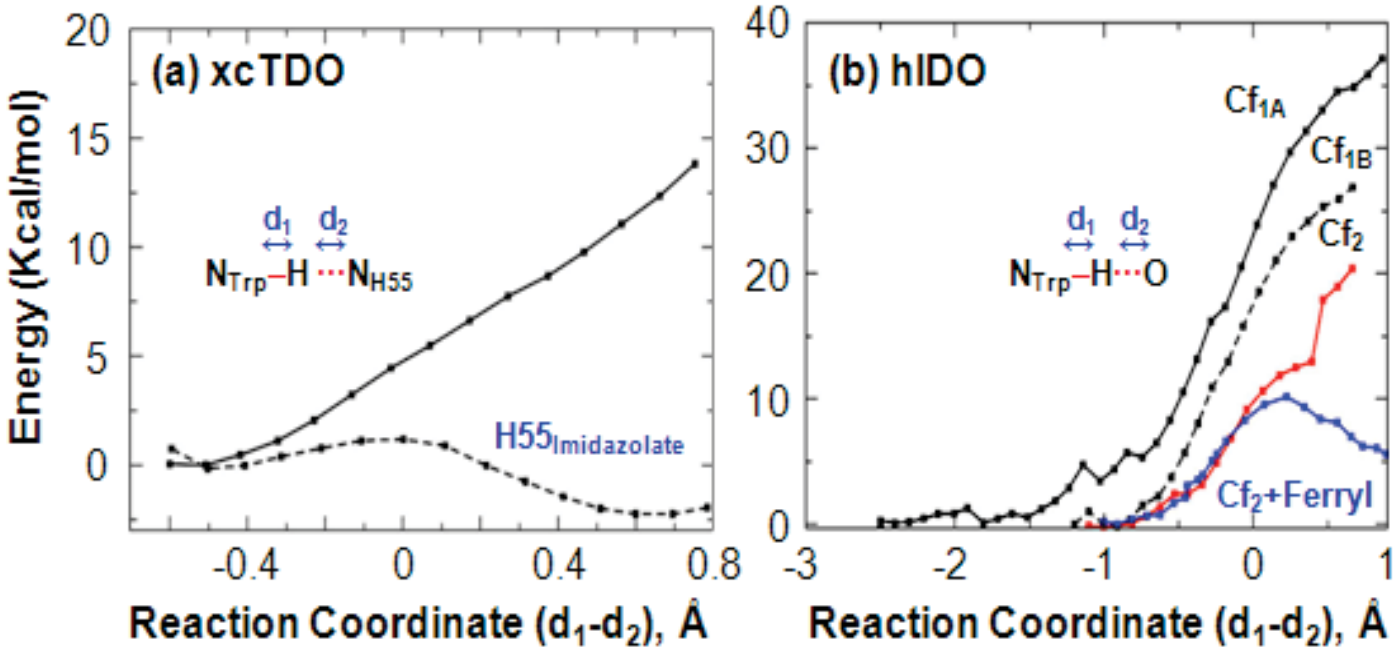
- Just one conformation (conf 2) was stable
- D-Trp, the structure is similar to that found for L-Trp Cf_2 , but lacks the interaction with R231.

Previously proposed reaction mechanisn



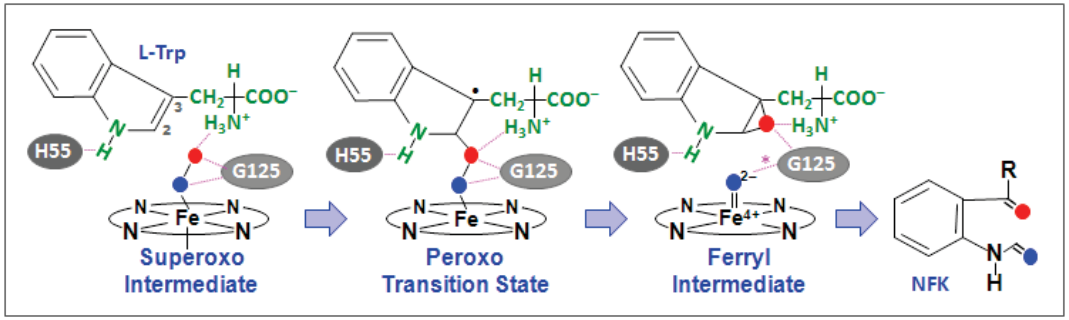
- Based on isolated Trp O_2 reactivity
- Replacing the NH moiety for S or O, inhibitors for IDO and TDO are found
- 1(N)-Methyl-Trp is a TDO inhibitor but reacts with IDO.
- H55A and H55S TDO present a lower activity towards Trp

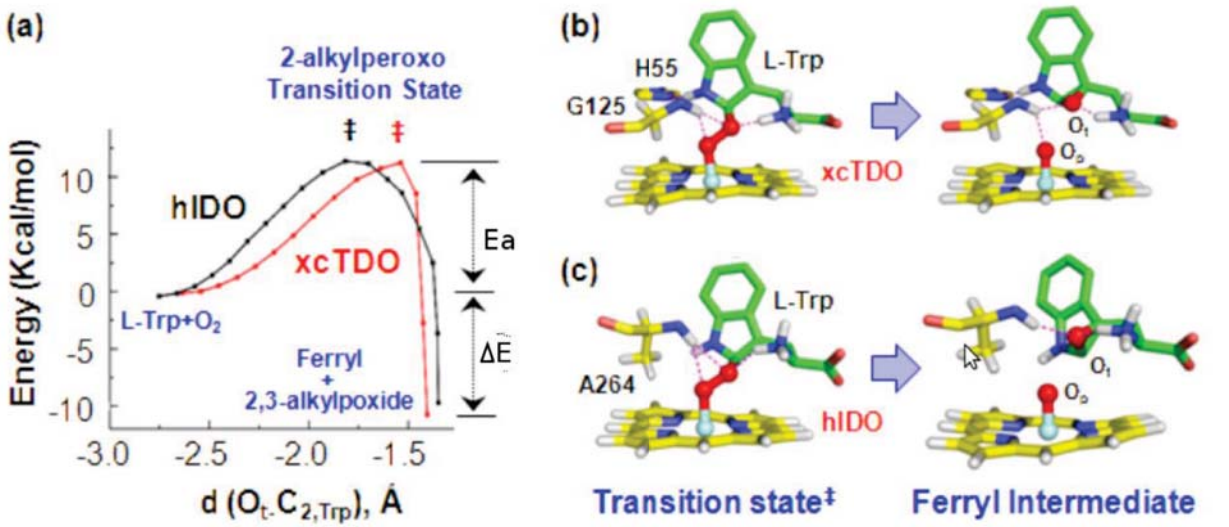
Proton transfer is not the first step!!



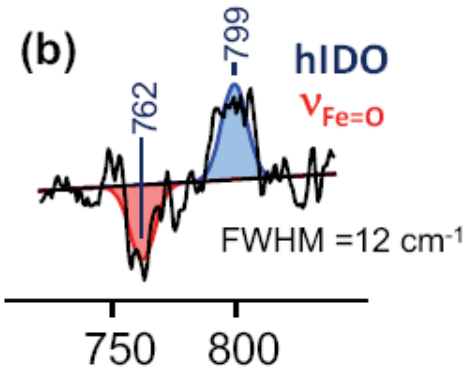
Model system	Proton affinity
$TrpN^- \Rightarrow TrpNH$	268.0
$His \Rightarrow HisH^+_{N\varepsilon}$	228.0
$Heme\text{-}O_2 \Rightarrow Heme\text{-}O_pO_tH^+$	234.4
$Heme\text{-}O_2 \Rightarrow Heme\text{-}(O_pH^+)\text{-}O_t$	214.4

Reaction starts with a direct attack to C2 to yield an epoxide and a ferryl intermediate

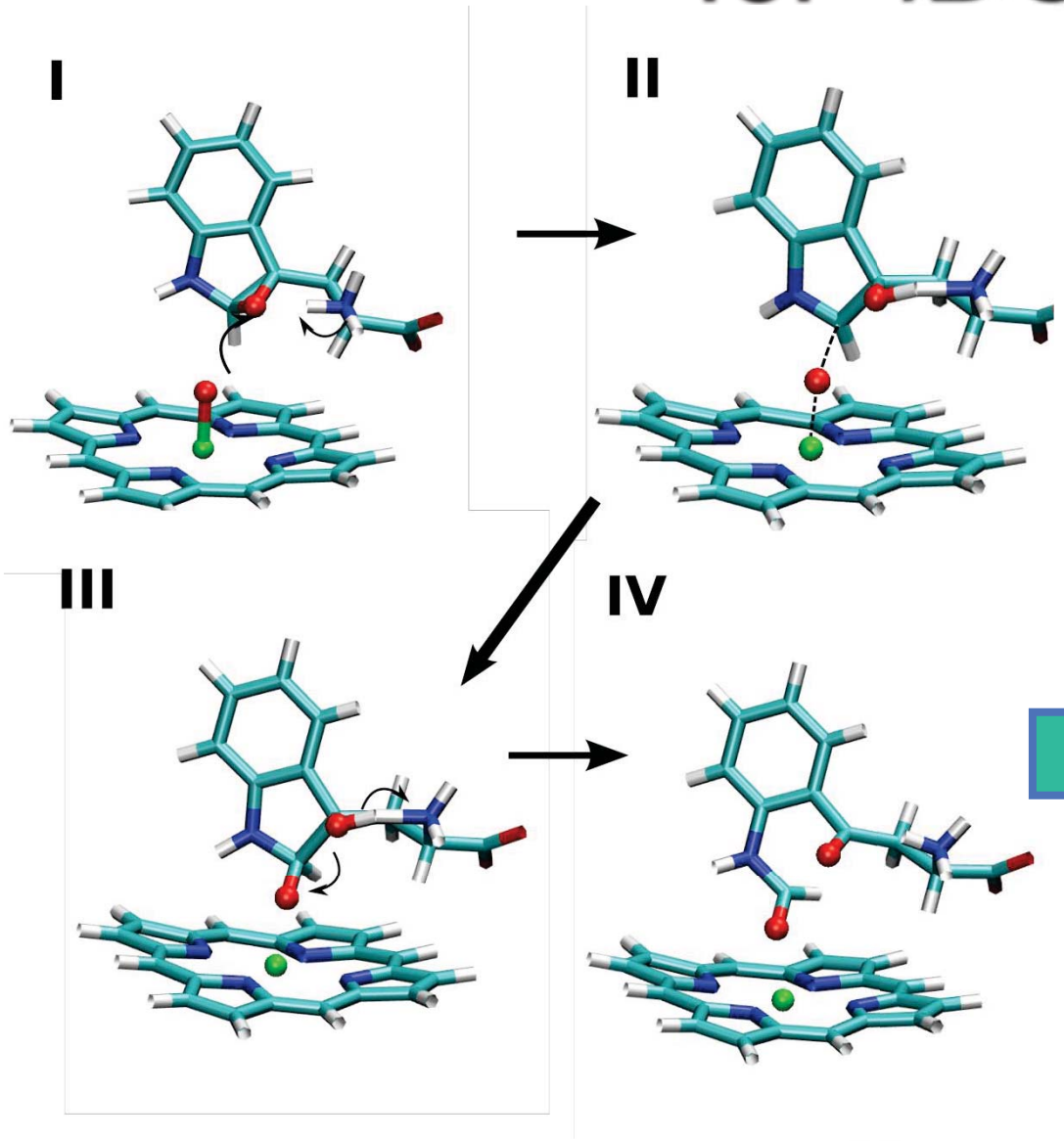


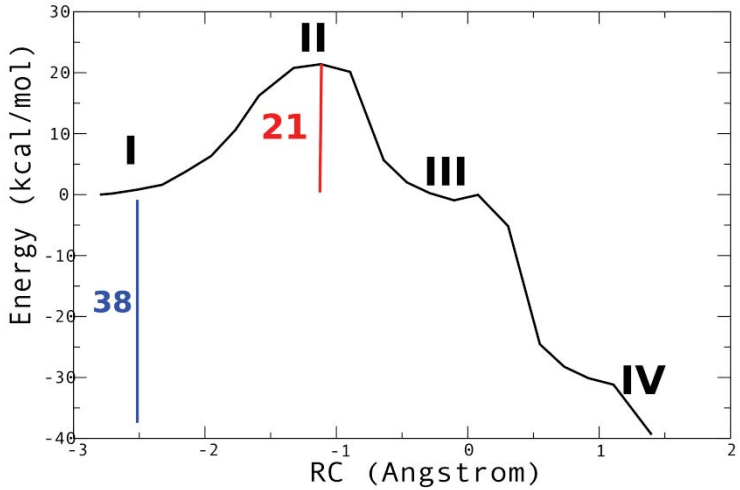


Experimental evidence



How does the reaction proceed for IDO?





$RC=d\,(C_2\text{-}C_3) + d\,(O_F\text{-}C_2)+d(C_2\text{-}O_P)$

Conclusions

- TDO and IDO active sites present significant differences regarding size and flexibility
- IDO presents two conformations for L-Trp binding, while in TDO just one conformation is found
- The first step consists on a direct attack to C2 Trp to yield an epoxide and a ferryl intermediate
- Reaction in IDO probably continues with an attack of the ferryl to C2, epoxide opening, assisted by Trp-NH_3^+.

Take Home Message:

Complex problems regarding chemical reactivity and catalysis in proteins requiere of a smart and careful combination of purely quantum, classical, and QM-MM strategies

Both careful consideration of the chosen model (level of theory) and sampling are necessary to obtain meaningful results

Choosing adequately benchamarks and comparison cases is essential to validate results

A deep interplay of computer simulation and experiment is an excellent way of getting the best possible insight into a given problem

Thanks to:

College Organizers

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