

Structural and Dynamical Aspects of Hemeproteins from Molecular Simulations

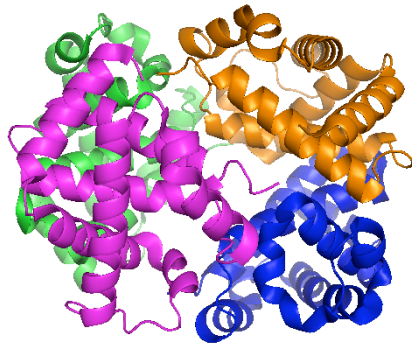
F. J. Luque

Departament de Fisicoquímica and Institut of Biomedicine
Facultat de Farmàcia
Universitat de Barcelona

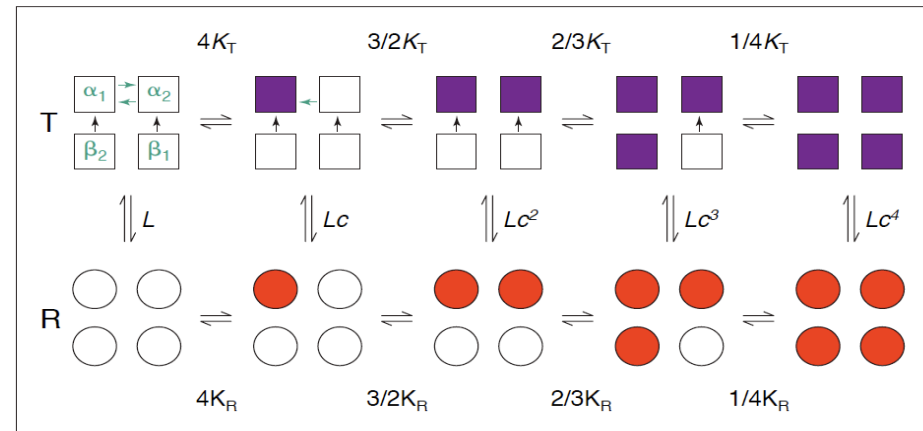
Barcelona, October 26 2010



The Globin superfamily



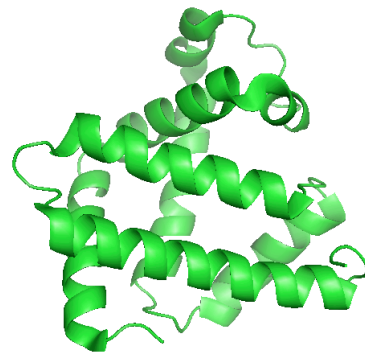
Hemoglobin (Hb)
Blood
~145 residues
O₂ storage/transport



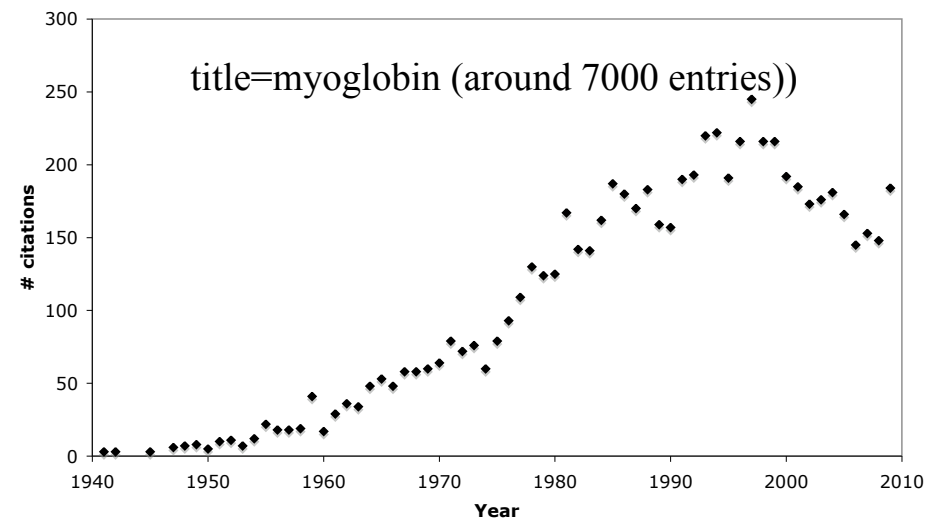
Monod-Wyman-Changeux
model of allosterism in Hb



J C Kendrew



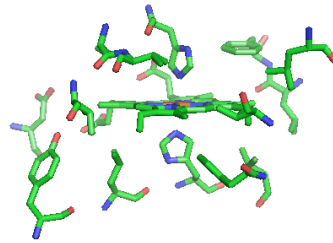
Myoglobin (Mb)
Muscle
~150 residues
O₂ storage



(first protein to have its 3D structure revealed by X-ray crystallography at 6 Å resolution ; 1957)

“Newcomers” to the Globin Superfamily

Hexacoordination of the Fe atom

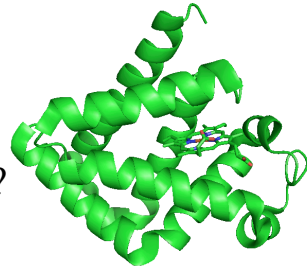


Cytoglobin (Cgb)

~all tissues

~195 residues

O₂ sensor/collagen synthesis?

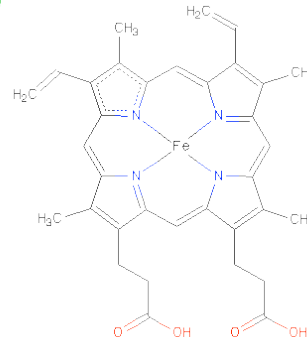
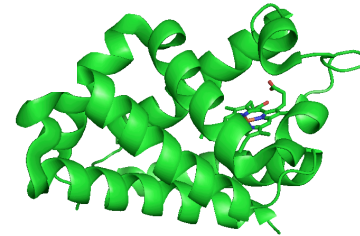


Neuroglobin (Ngb)

Nervous system

~135 residues

O₂ supply to mitochondria?

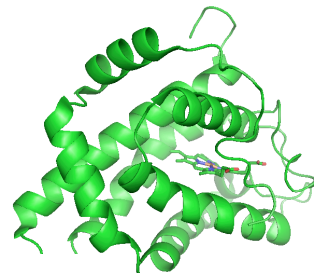


Protopoglobin (Pgb)

Methanosarcina acetovirans

~195 residues

CO sensor/supply?

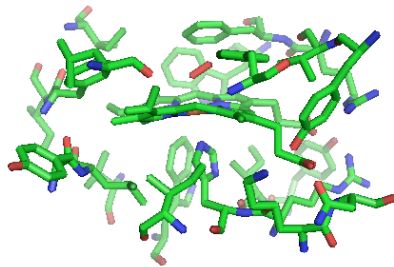
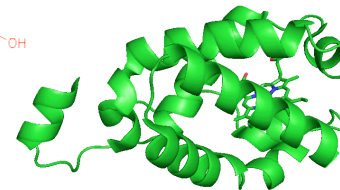


Truncated Hemoglobin (TrHb)

Mycobacterium tuberculosis

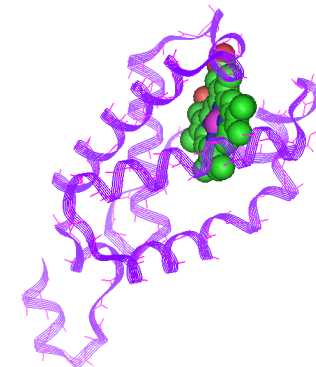
~135 residues

NO dioxygenase?



Highly distorted heme group

2-on-2 sandwich fold



Heme proteins—Diversity in structural characteristics, function, and folding

Lorna J. Smith,^{1*} Abdullah Kahraman,² and Janet M. Thornton²

Proteins 2010; 78:2349–2368.

Key questions:

Structure of the heme binding site and its chemical/catalytic reactivity properties

Role of cavities/tunnels in the protein matrix

‘Omic’ evaluation of proteins with which globins interact to exert their functions

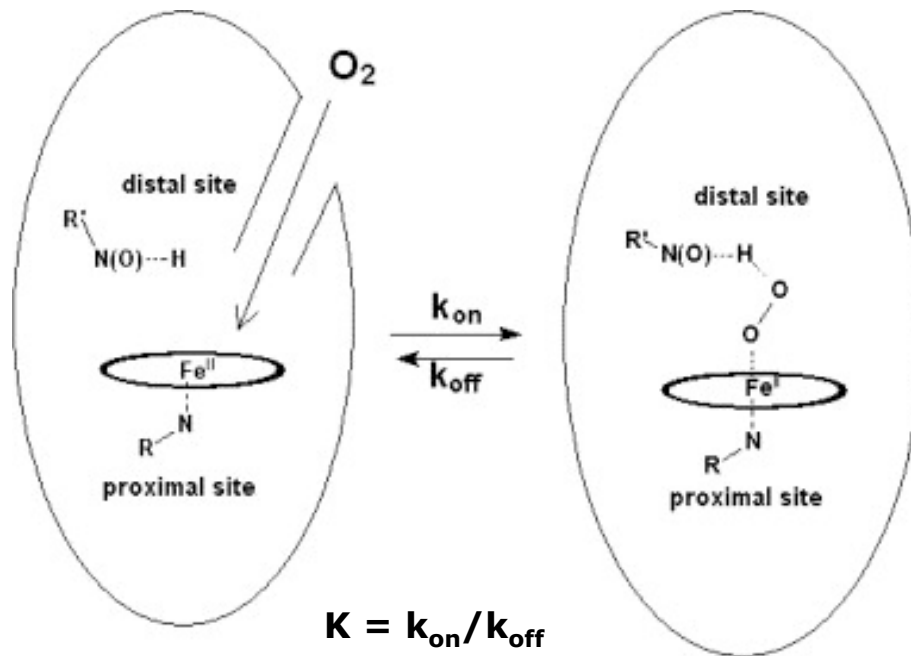
Phylogenetic analysis to understand the origin of functional differences

“Protein engineering” to design chimeras with novel functions (i.e., biosensors)

Around 2300 entries in Protein Data Bank

Number of proteins in data set	
Total	34
Electron transfer subset	6
Binds small molecules subset	6
Enzymes subset	9
Transient heme binding subset	5
Multi-heme protein subset	6
Other protein subset	2
Protein structure adopted	
CATH Class Mainly α	15
CATH Class Mainly β	7
CATH Class $\alpha\beta$	12
Number of heme groups	
Total	50
Heme <i>a</i>	2
Heme <i>b</i>	27
Heme <i>c</i>	17
Heme <i>d</i> ₁	1
Heme P460	1
Siro heme	1
Heme from myeloperoxidase	1
Axial ligands for heme group	
His	61
Met	8
Cys	6
Tyr	3
Pro	1
N-terminal amino	1
Vacant/water	18
Changes on oxidation/reduction	2

Ligand binding

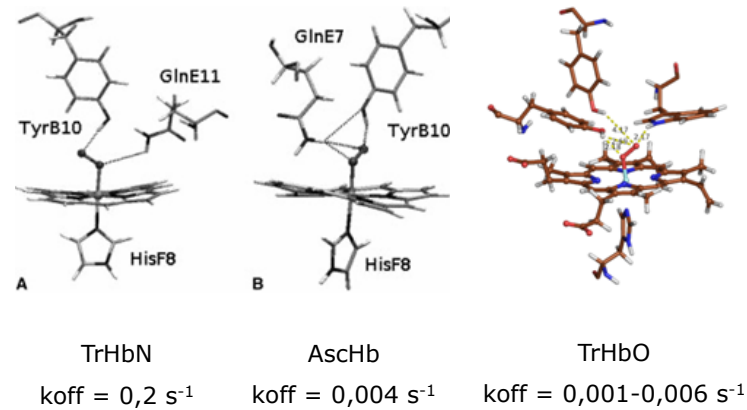


k_{on}

- accessibility to the heme cavity
- migration across protein matrix

k_{off}

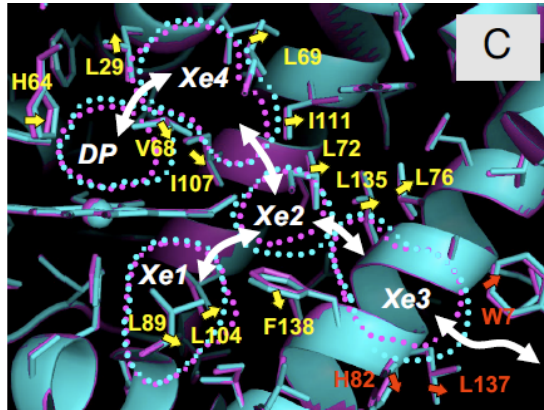
- strength of ligand-heme bond
- H-bond in the distal site



Visualizing breathing motion of internal cavities in concert with ligand migration in myoglobin

Ayana Tomita^{a,b}, Tokushi Sato^{a,b}, Kouhei Ichihara^b, Shunsuke Nozawa^b, Hirohiko Ichikawa^b, Matthieu Chollet^a, Fumihiro Kawai^c, Sam-Yong Park^c, Takayuki Tsuduki^d, Takahisa Yamato^{d,e}, Shin-ya Koshihara^{a,b}, and Shin-ichi Adachi^{b,f,1}

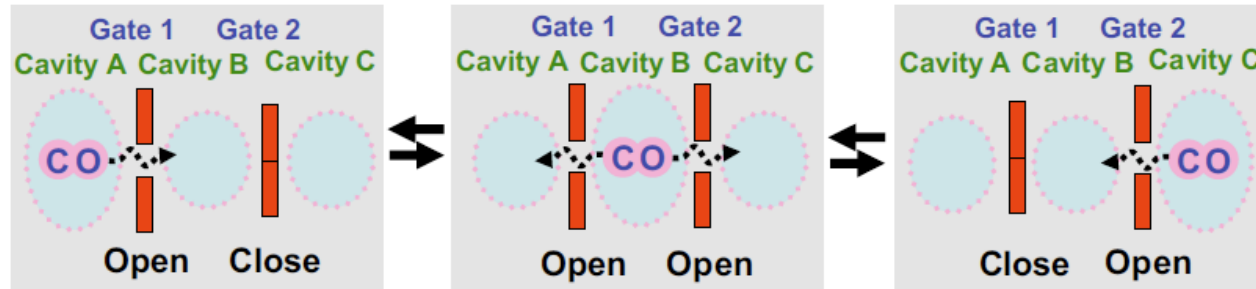
2612–2616 | PNAS | February 24, 2009 | vol. 106 | no. 8



Time-resolved X-ray crystallography

140 K

CO in Myoglobin



Molecular simulations

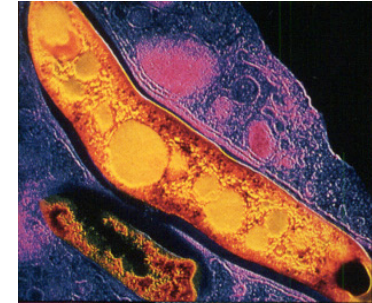
Structural and dynamical description of protein fluctuations

Plasticity of cavities/tunnels

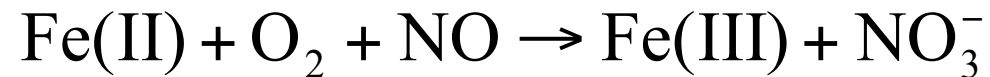
Energetic description of ligand migration

Truncated Hemoglobin N from *M. tuberculosis*

During infection, nitric oxide (NO) formed in macrophages contributes to restrict bacteria in latency. However, the toxic effects of NO can be minimized by resistance mechanisms. This is the role played by **truncated hemoglobin N**.



Mycobacterium Tuberculosis (Electron micrograph)



Enzyme	O ₂ binding to ferrous trHbN			NO oxidation by oxy trHbN
	k_{on} (M ⁻¹ s ⁻¹)	k_{off} (s ⁻¹)	K _d (M)	k (M ⁻¹ s ⁻¹)
trHbN	2.5 x 10 ⁷	2.0 x 10 ⁻¹	8.0 x 10 ⁻⁹	7.5 x 10 ⁸
Myoglobin	1.4 x 10 ⁷	1.2 x 10 ¹	8.6 x 10 ⁻⁷	3.4 x 10 ⁷

NO conversion to nitrate by oxy-trHbN is faster than O₂ binding to the deoxy protein, i.e. the detoxification efficiency is determined by the ability to capture O₂ and NO.

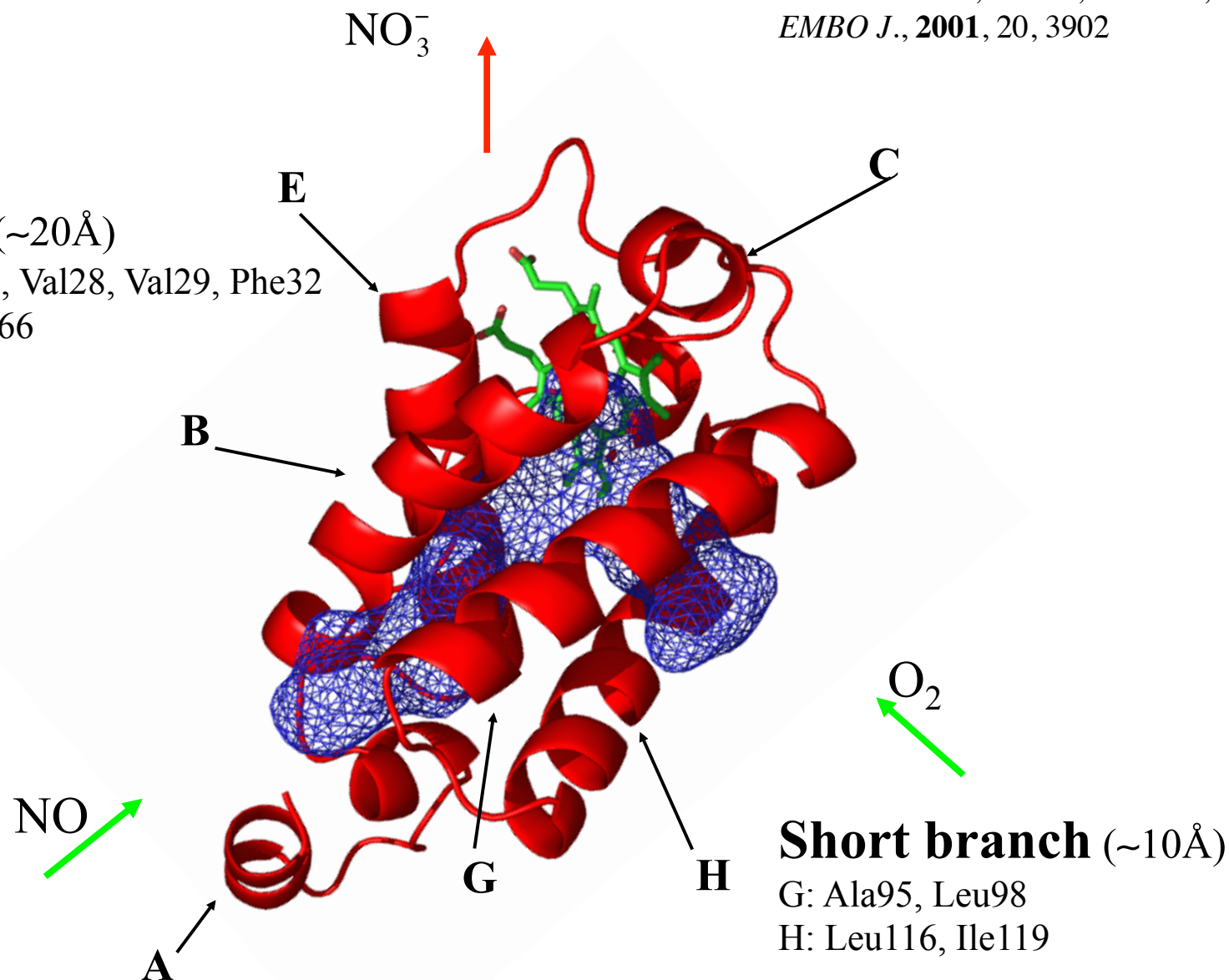
Truncated Hemoglobin N: Dual-path ligand-modulated migration mechanism

PDB ID: 1IDR, Milani, M. *et al.*,
EMBO J., **2001**, 20, 3902

Long branch (~20Å)

B: Ile19, Ala24, Ile25, Val28, Val29, Phe32

E: Phe62, Ala63, Leu66



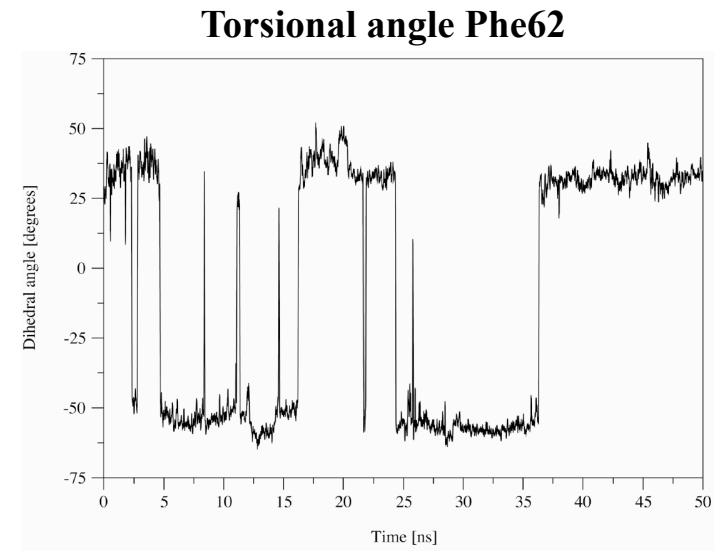
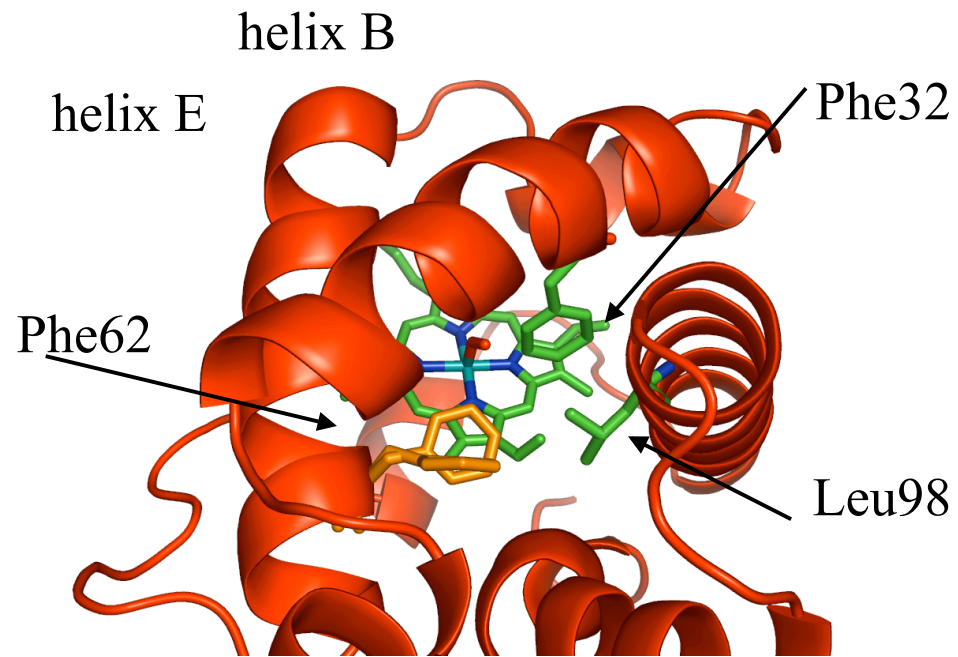
Short branch (~10Å)

G: Ala95, Leu98

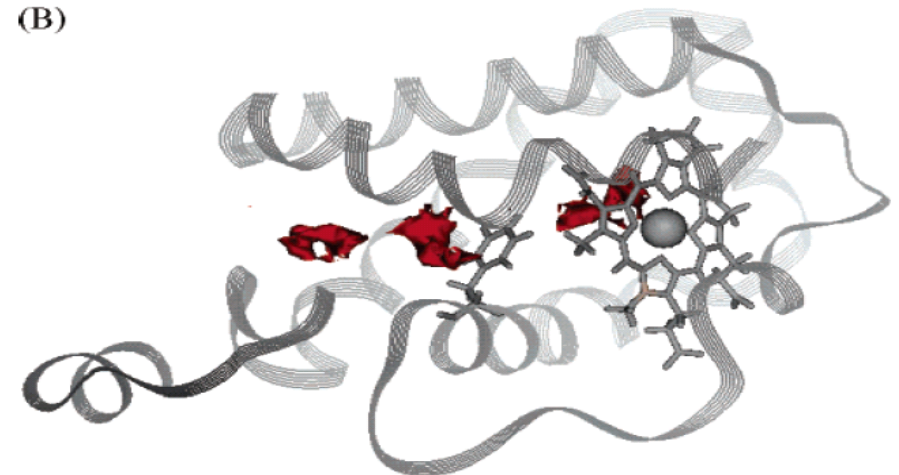
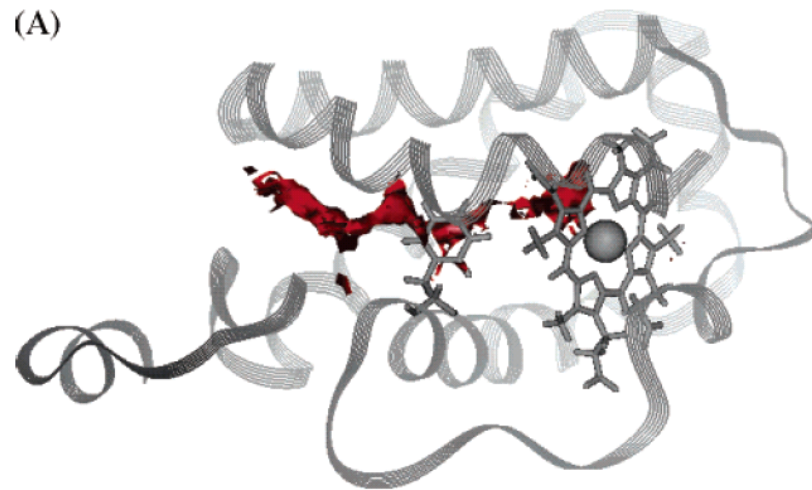
H: Leu116, Ile119

J. Am. Chem. Soc. **2005**, 127, 4433; **2008**, 130, 1688

Phe62 acts as the gate of the long tunnel in oxy trHbN



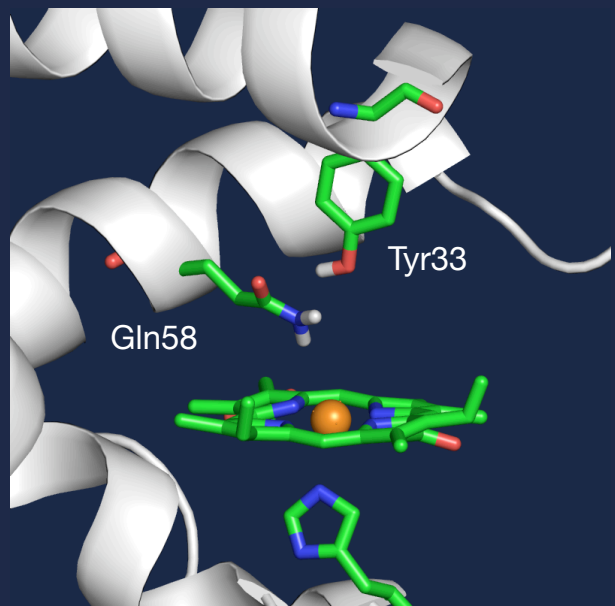
CMIP energy isocontour



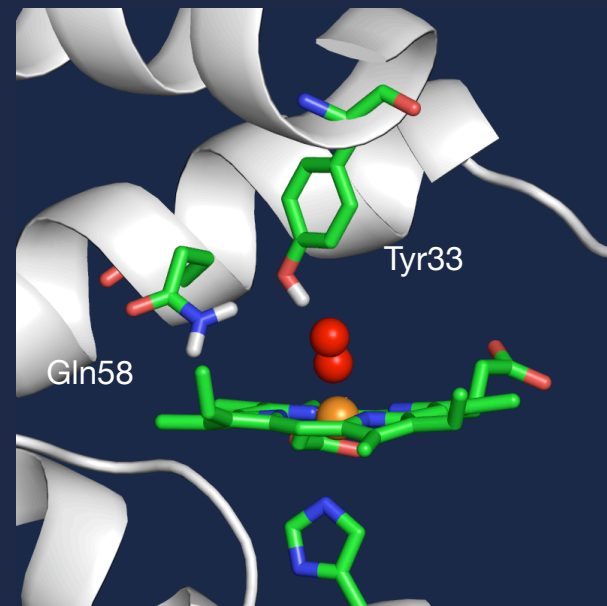
Local effects in the heme cavity

deoxy-trHbN

oxy-trHbN

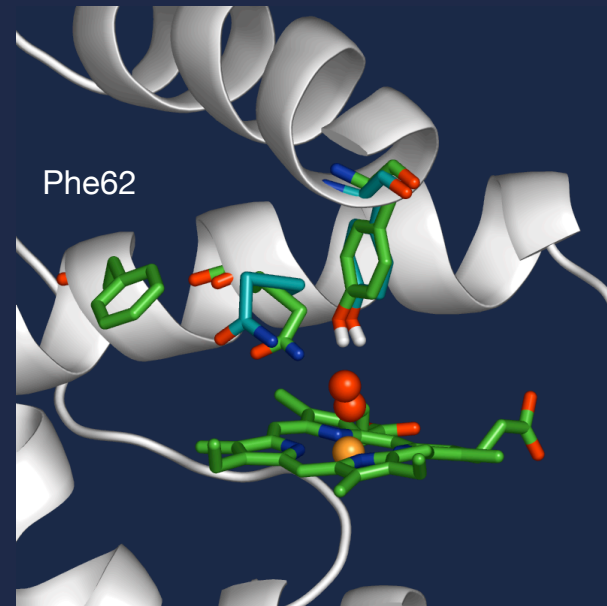
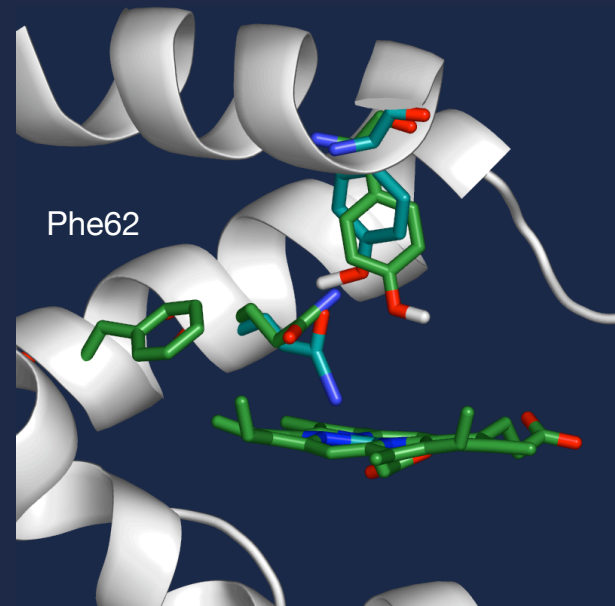


O₂



Essential Movements

(maximum displacements)



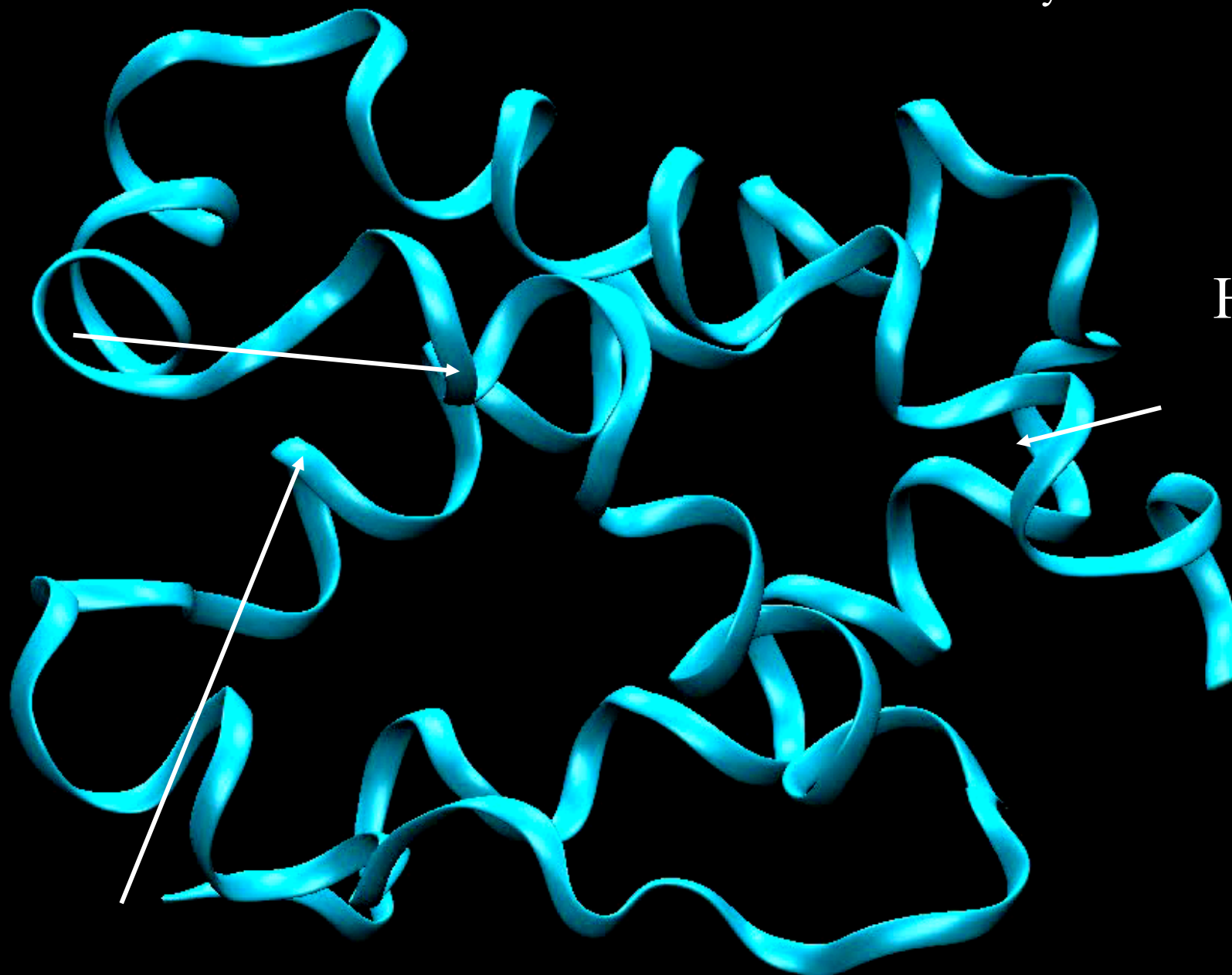
Oxy trHbN

Essential dynamics analysis

Helix
E

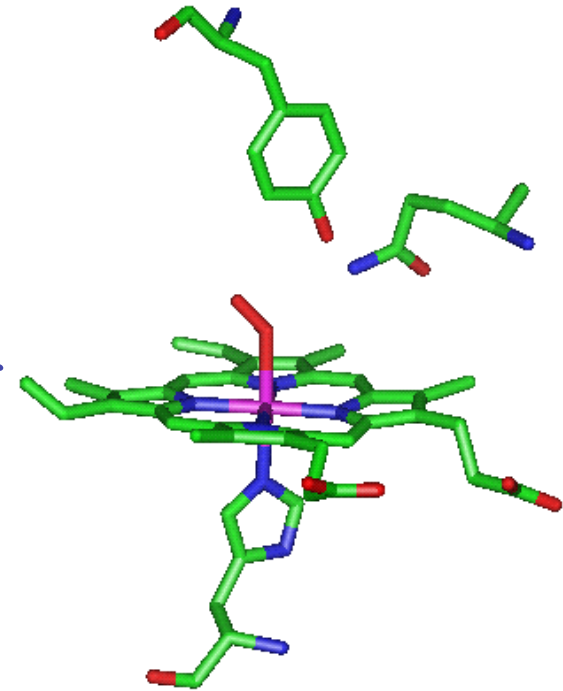
Helix
B

Heme group



The Tyr33-Gln58 pair is essential for trHbN:

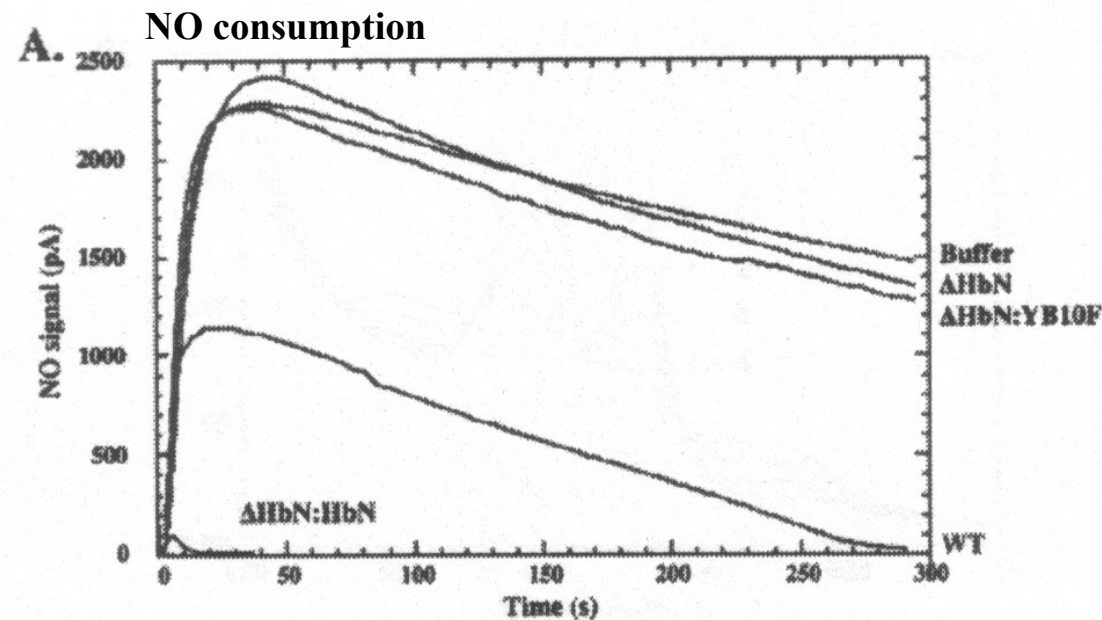
- 1) to modulate binding affinity of O₂ to the heme,
- 2) to assist anchoring of the incoming NO reactant, and
- 3) **to act as a molecular switch that regulates access of NO through the long branch of the tunnel**



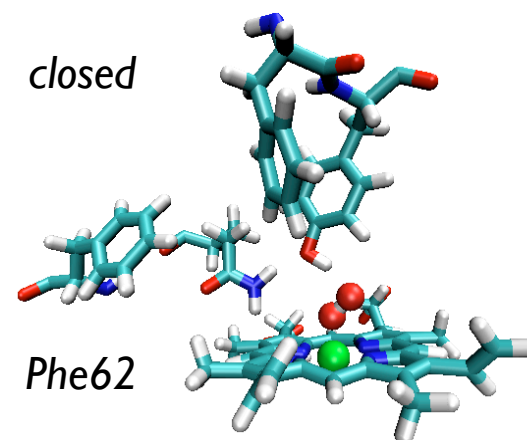
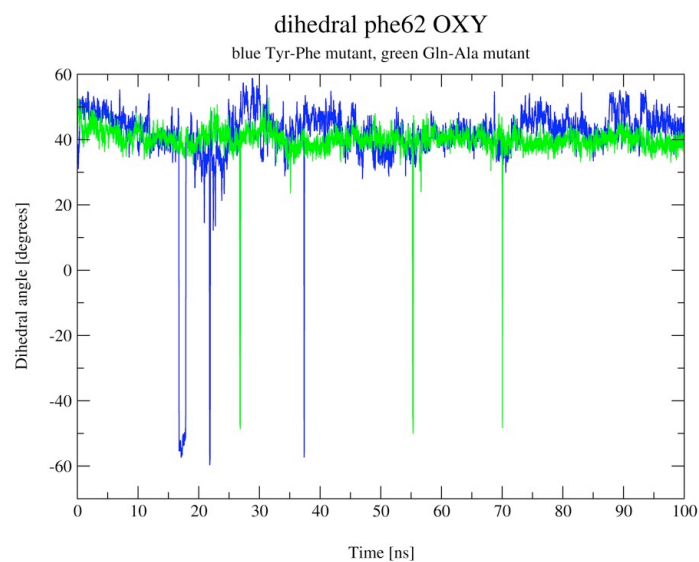
Truncated hemoglobin HbN protects *Mycobacterium bovis* from nitric oxide

Hugues Ouellet^{*†}, Yannick Ouellet^{*†}, Christian Richard[†], Marie Labarre[†], Beatrice Wittenberg[‡], Jonathan Wittenberg[‡], and Michel Guertin^{†§}

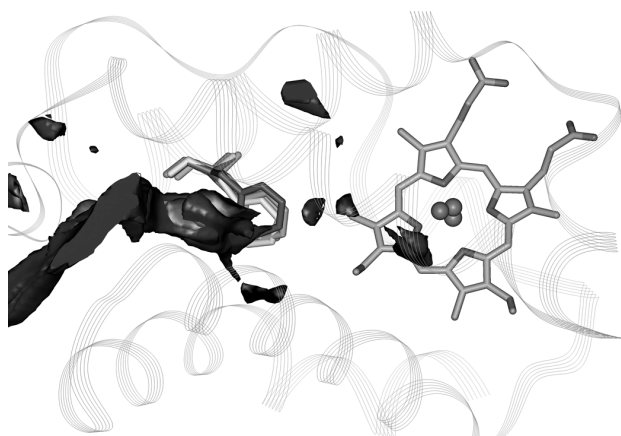
Proc Nat Acad Sci USA **2002**, 99, 5902



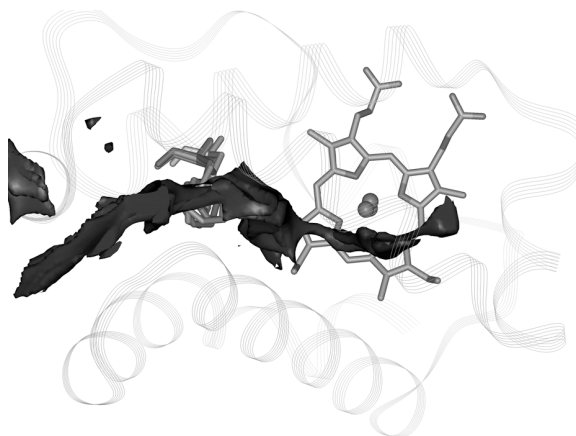
Torsional flexibility of Phe62 for the oxy state of the mutants: Tyr33→Phe and Gln58→Ala



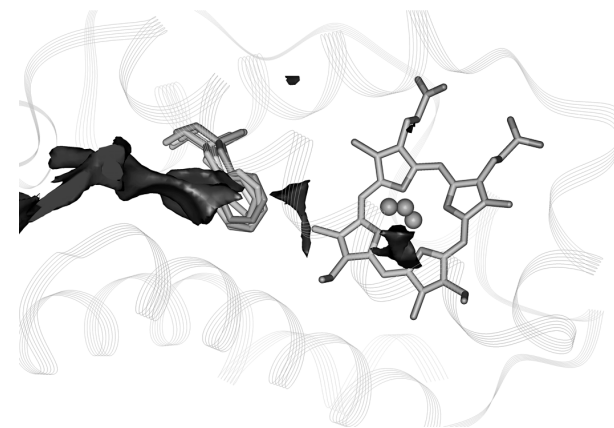
Tyr33->Phe



wt-protein

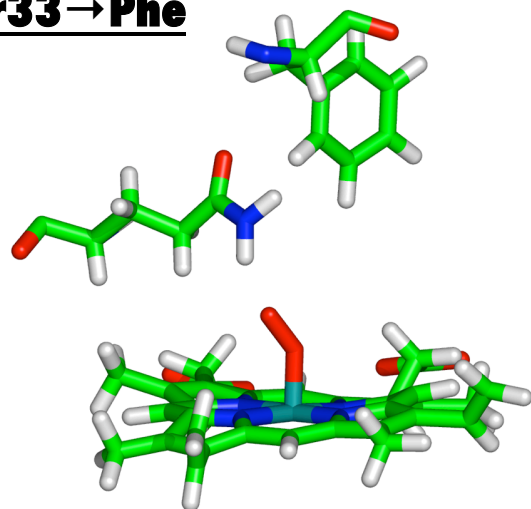


Gln58->Ala

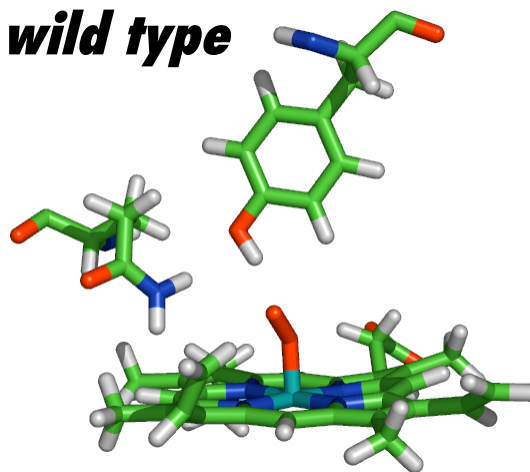


Main local conformations at the heme cavity

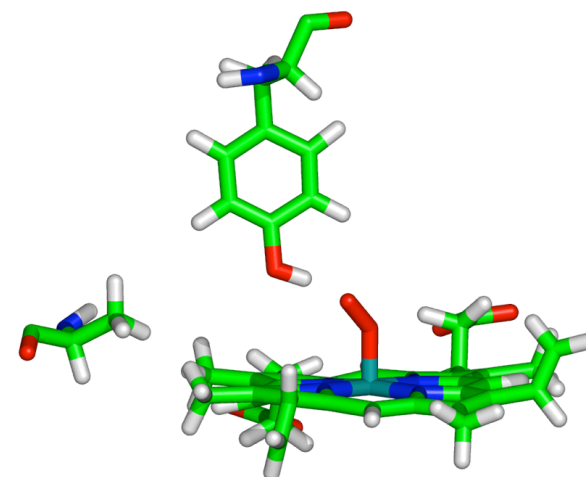
Tyr33→Phe



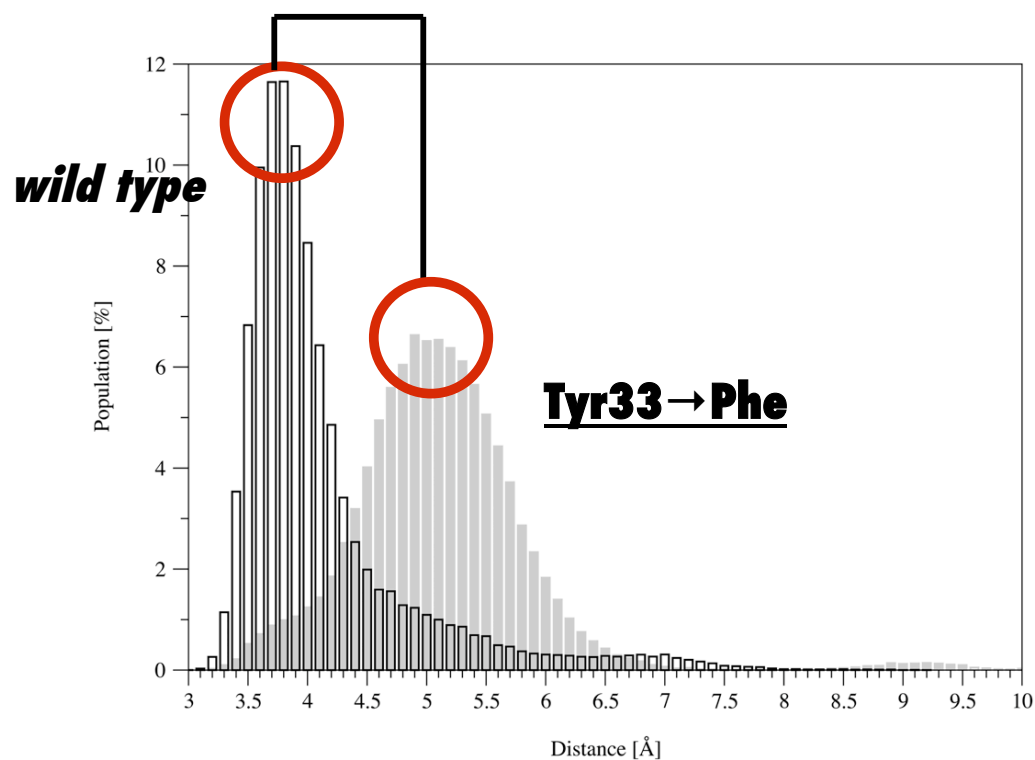
wild type



Gln58→Ala



Distribution of interresidue
distances between Gln58 and
Phe62



Validating the role of the gate Phe62(E15)

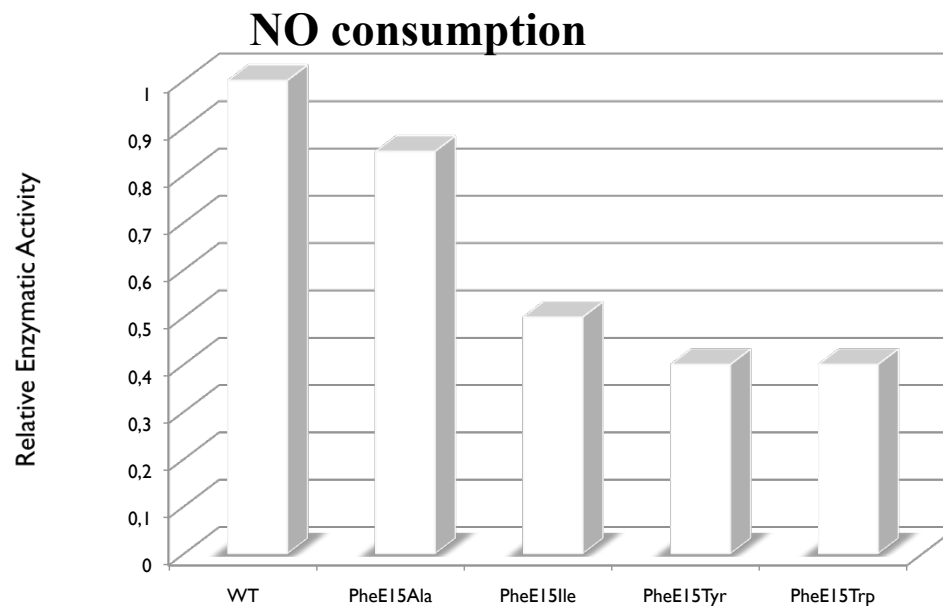
in collaboration with Prof. K. Dikshit
Institute Microbial Technology
India

Mutants

- ✱ PheE15→Ala
- ✱ PheE15→Ile
- ✱ PheE15→Tyr
- ✱ PheE15→Trp

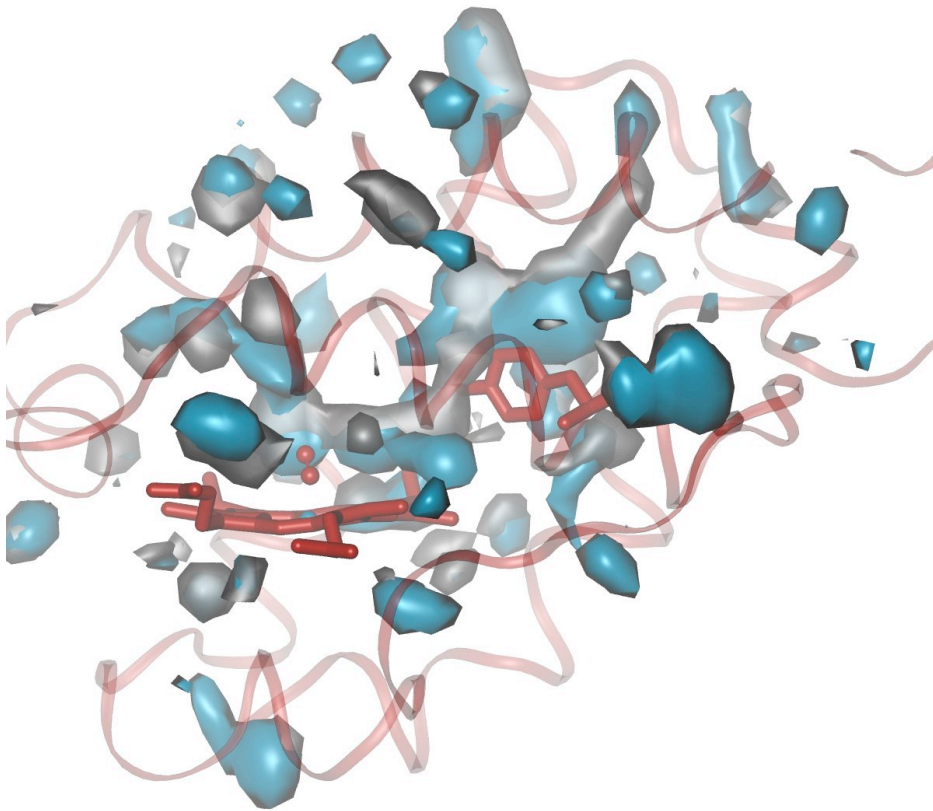
CO binding properties

Protein	$k_{on}CO$ ($M^{-1} s^{-1}$)
WT	0.66×10^7 1.08×10^7
PheE15→Ile	0.49×10^7



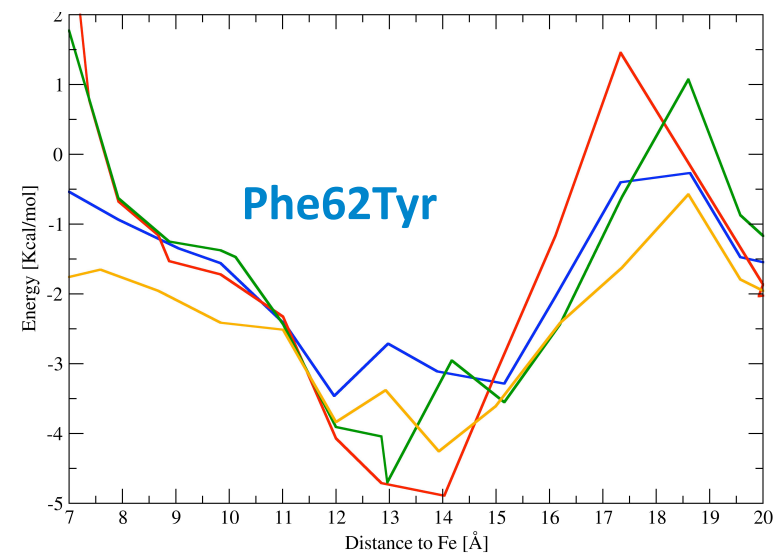
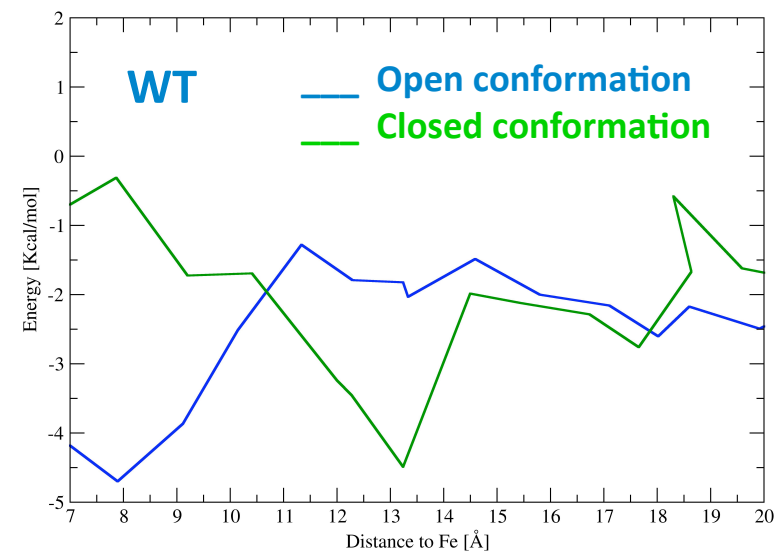
Analysis of cavities in the protein matrix

PheE15Tyr



Tunnel in wild type shown in gray

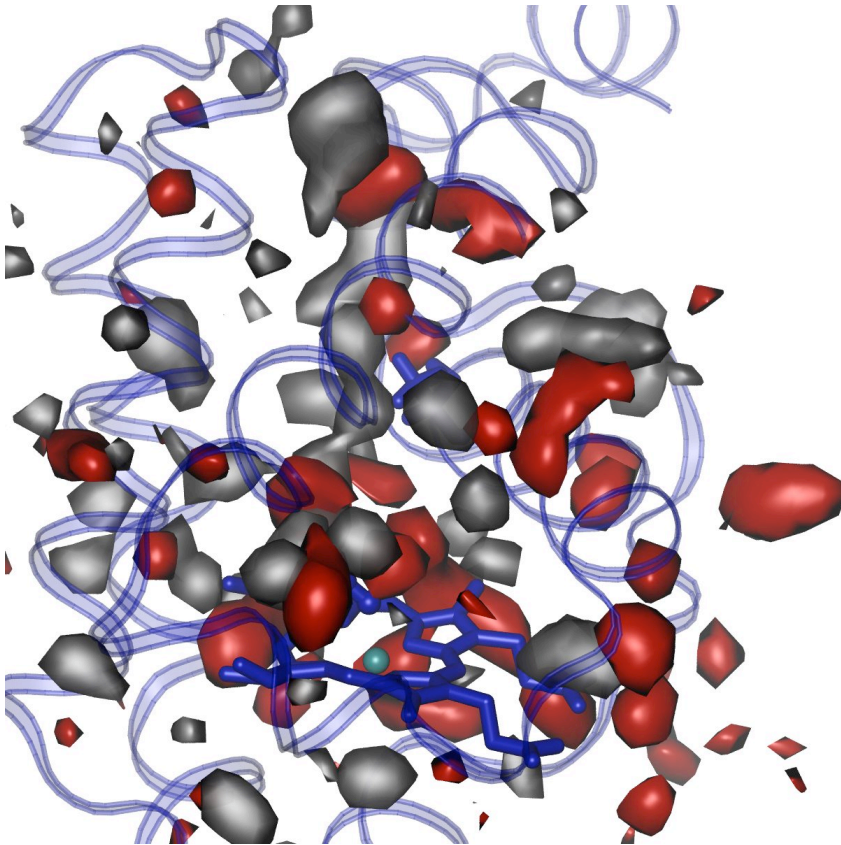
Implicit ligand sampling for ligand migration



— 30-40ns — 50-60ns
— 70-80 ns — 90-100 ns

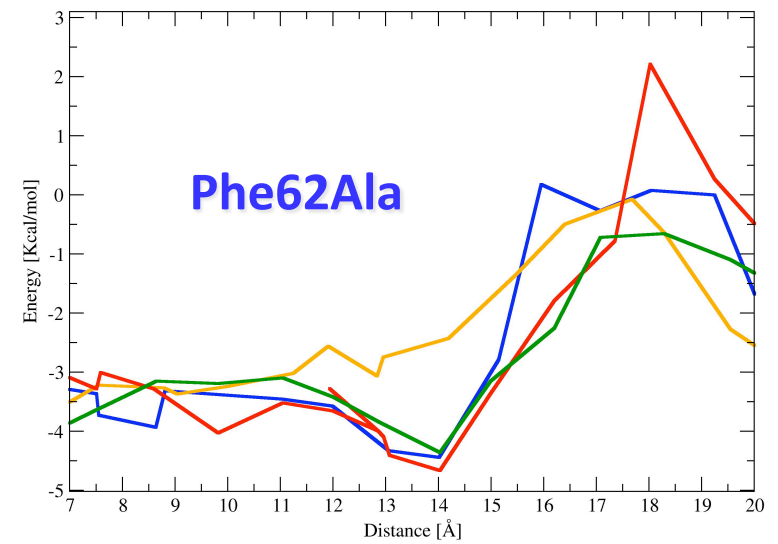
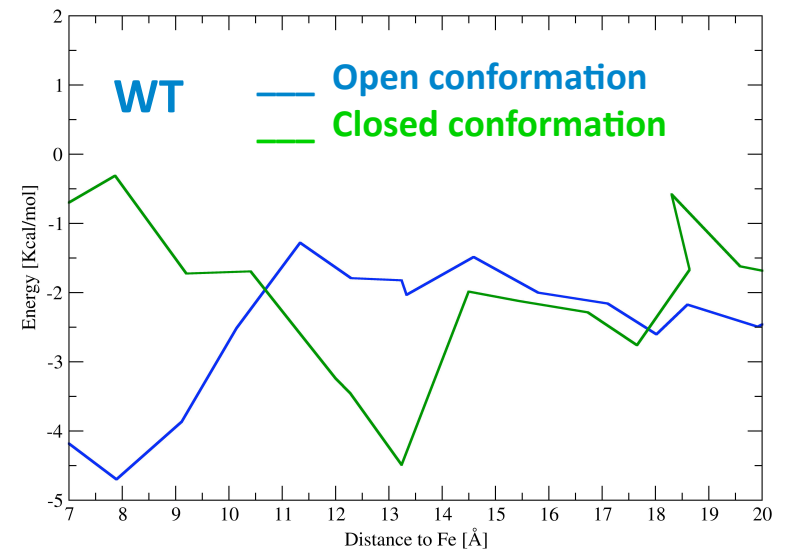
Analysis of cavities in the protein matrix

PheE15Ala



Tunnel in wild type shown in gray

Implicit ligand sampling for ligand migration



— 30-40ns — 50-60ns
— 70-80 ns — 90-100 ns

Class 1 Non-Symbiotic Hemoglobin from *A. thaliana*

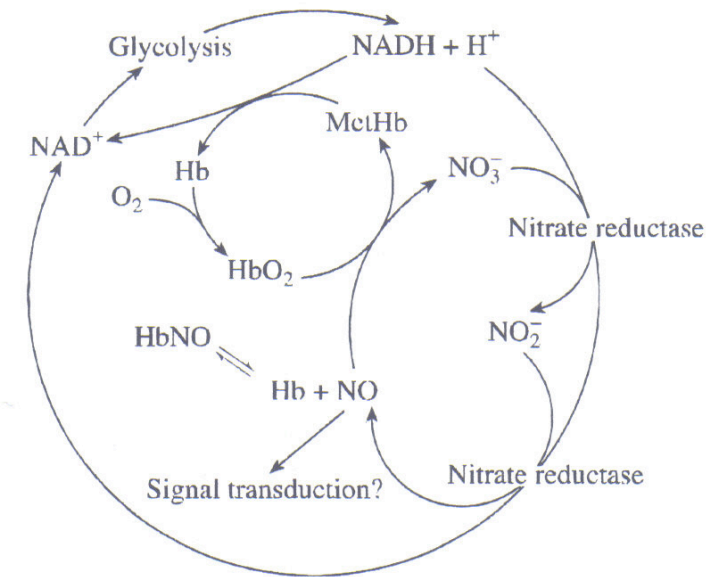
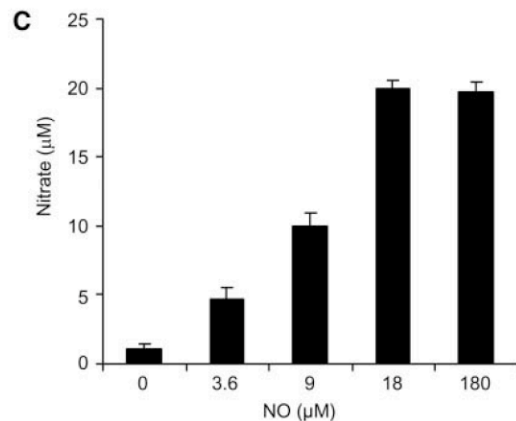


High affinity for O₂

Enzyme	O ₂ binding to ferrous form		
	k_{on} (M ⁻¹ s ⁻¹)	k_{off} (s ⁻¹)	K_{d} (M)
AHb1	7.5×10^7	0.12	1.6×10^{-9}
Myoglobin	1.4×10^7	12	8.6×10^{-7}

Expressed by hypoxic stress and high levels of nitrate

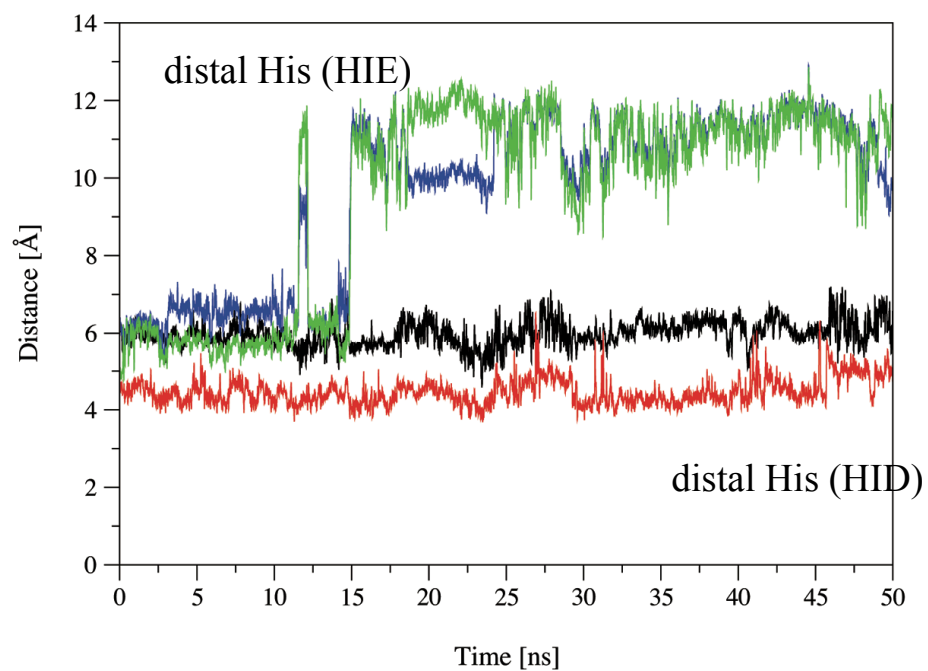
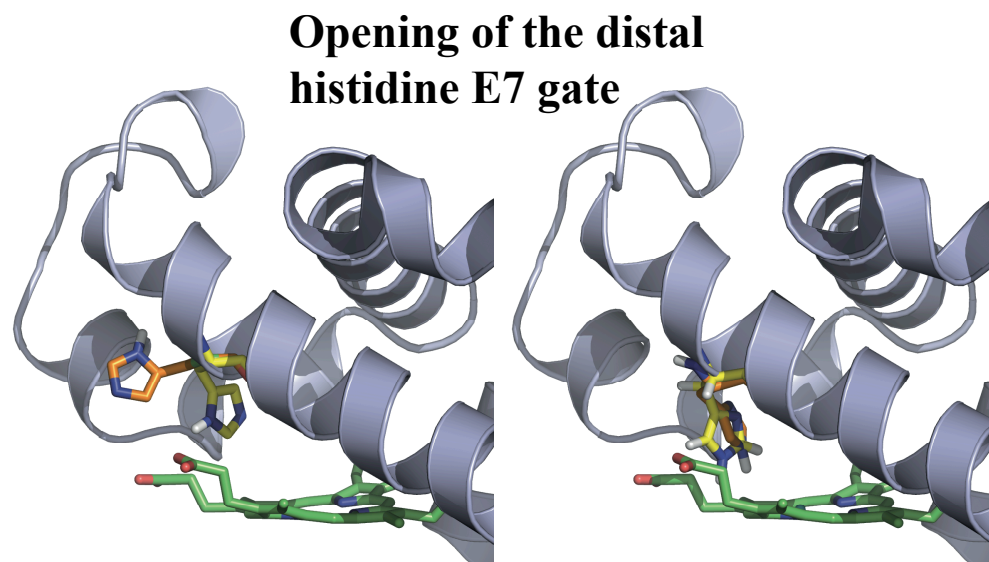
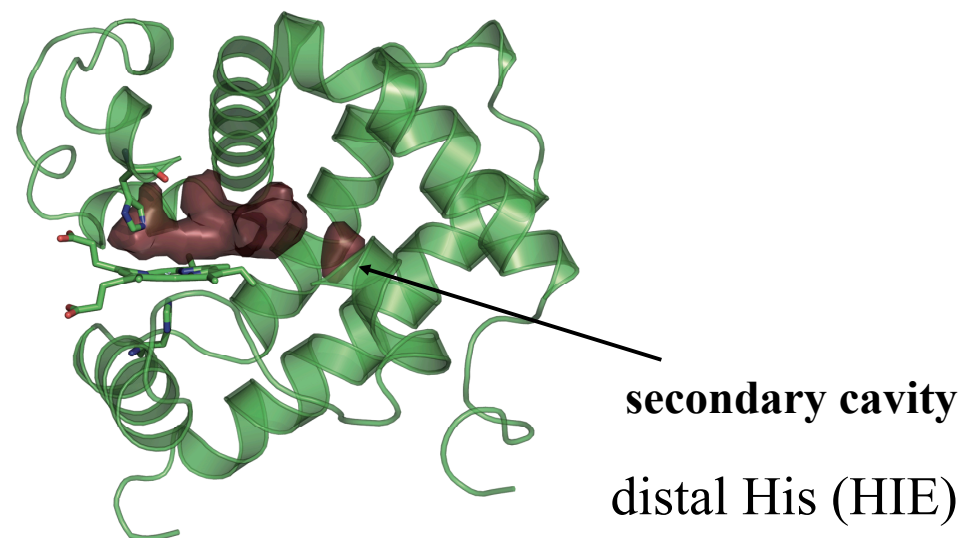
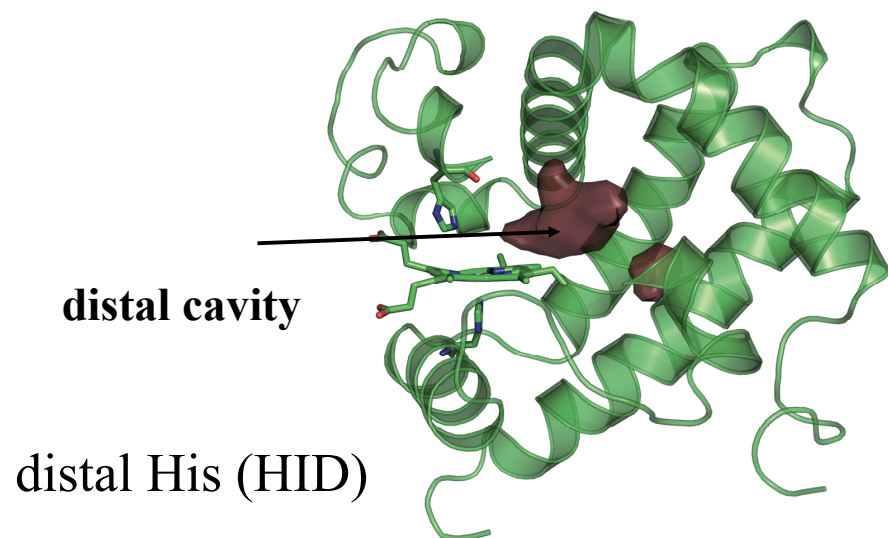
Nitrate production from
18 μM oxygenated AHb1



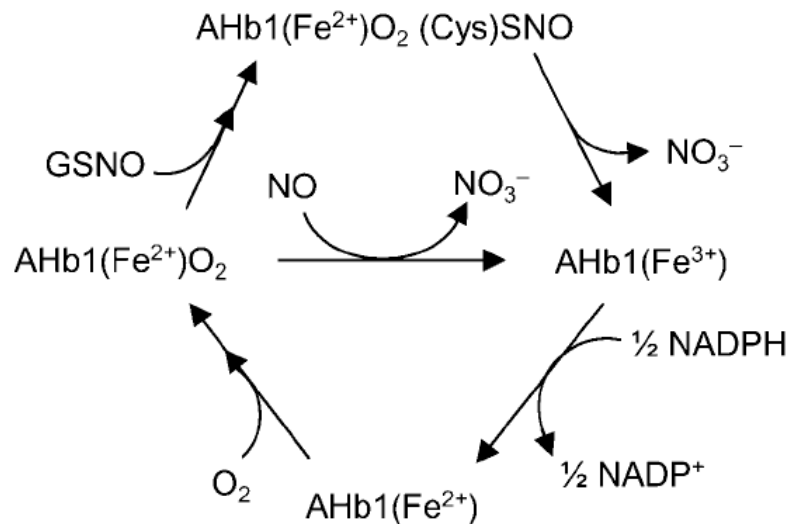
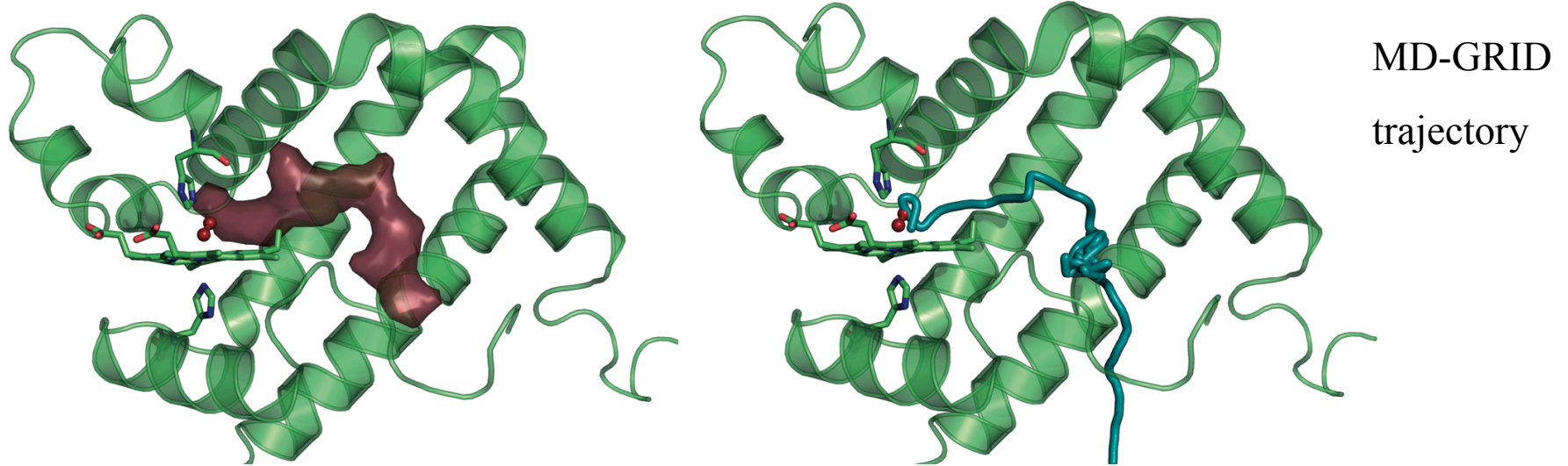
The Plant Cell, Vol. 16, 2785–2794, October 2004,

AHb1 may be crucial in short-term survival of plant root cells by regulating the levels of NO

Cavities in deoxygenated wild type AHb1 (Pentacoordinated form)



Binding of O₂ to wild type AHb1 is associated with the formation of a tunnel linking the distal cavity with the protein surface



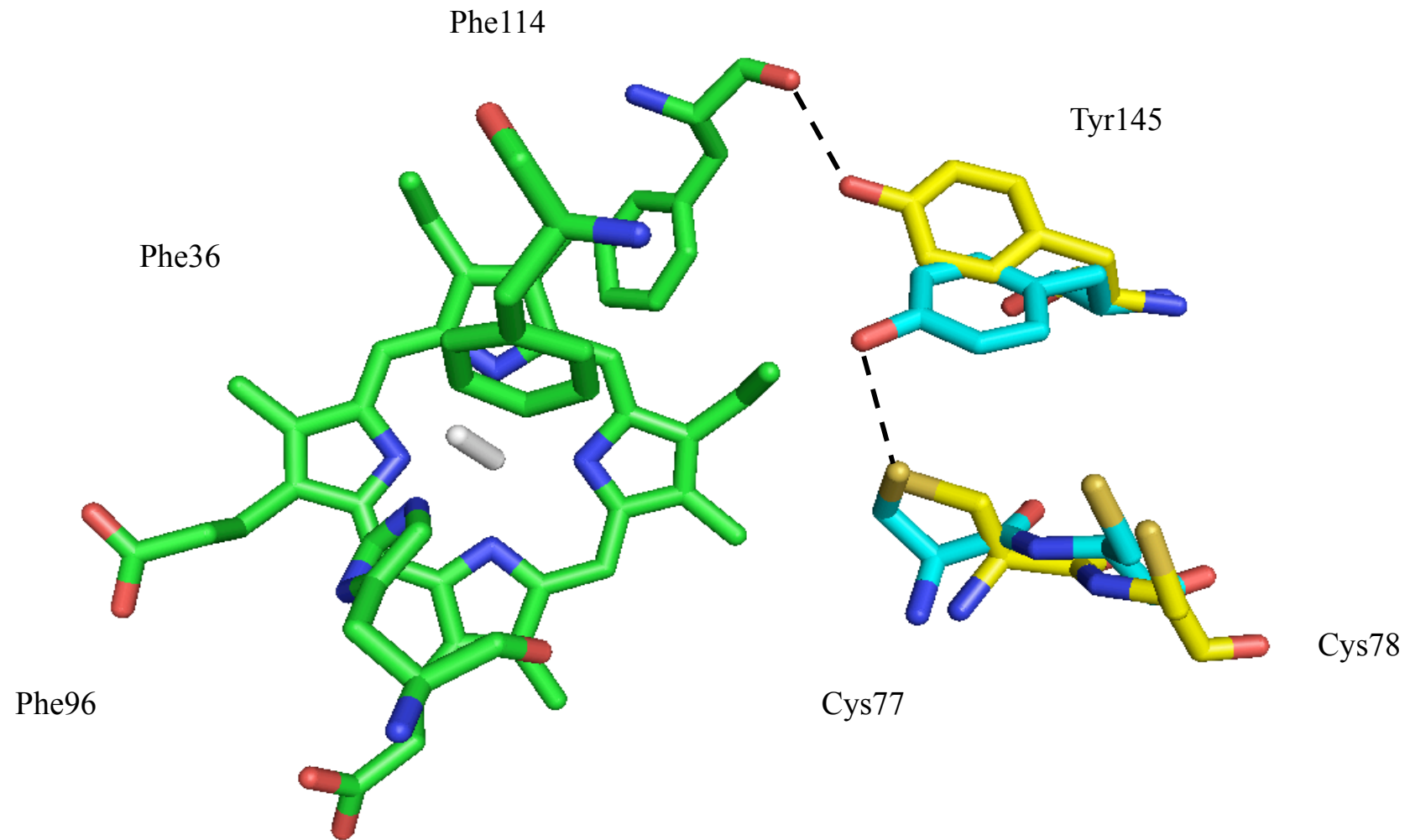
Proposed reaction cycle for NO metabolism by AHb1
(*Plant Cell* **2004**, 16, 2785)

- 1) Direct binding of NO to oxygenated AHb1
- 2) Reaction with nitrosylated glutathione (GSNO)

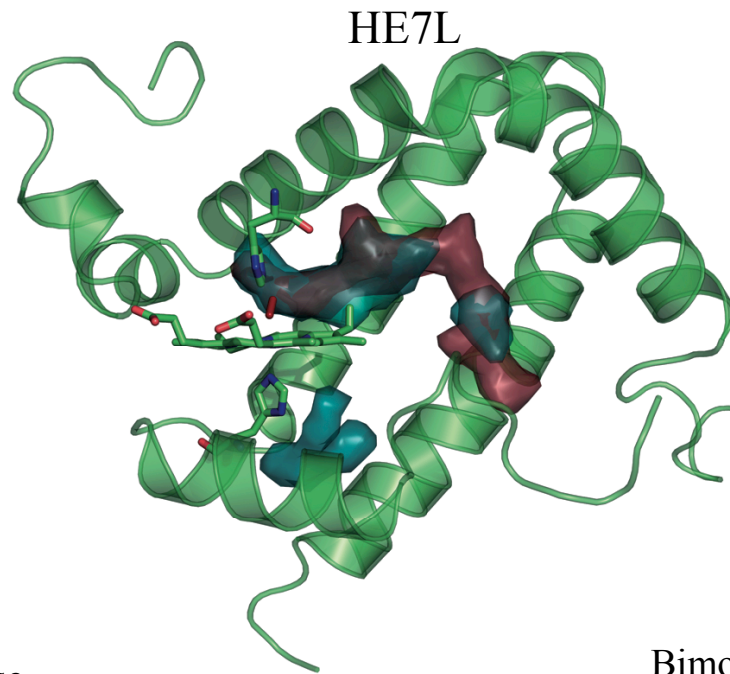
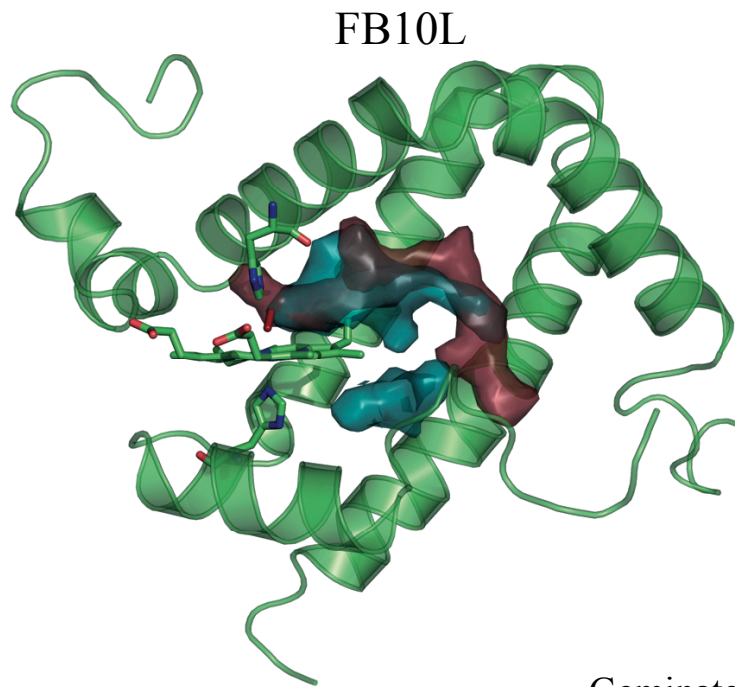
Conformational rearrangement of Tyr145 in AHb1:

a) deoxygenated (yellow)

b) oxygenated (cyan)



Cavities found in snapshots collected for oxygenated mutants



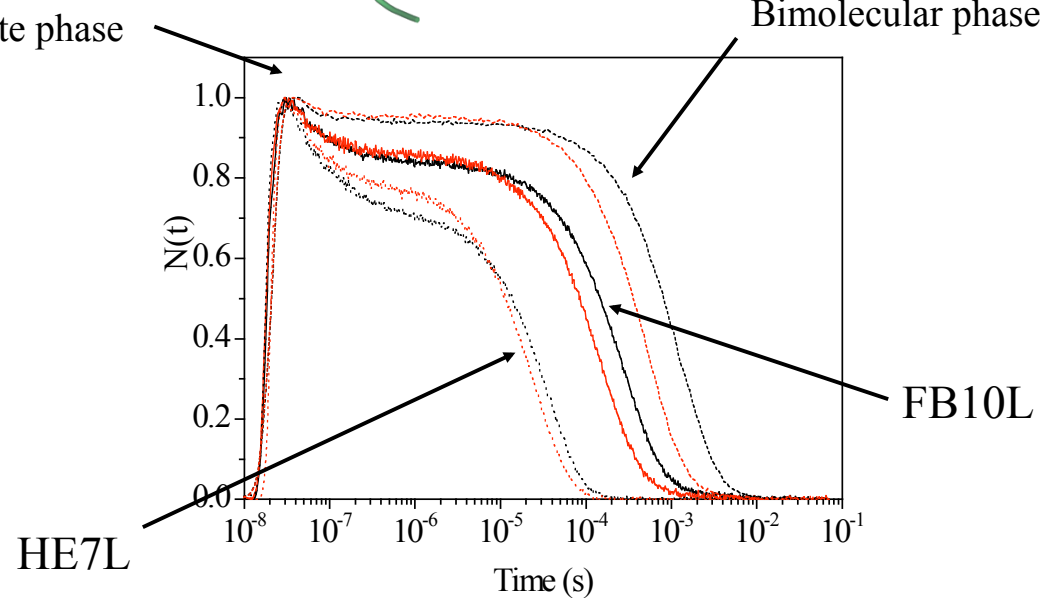
CO rebinding kinetics to wt AHb1, FB10L and HE7L.

Black: 10 °C

Red: 30 °C

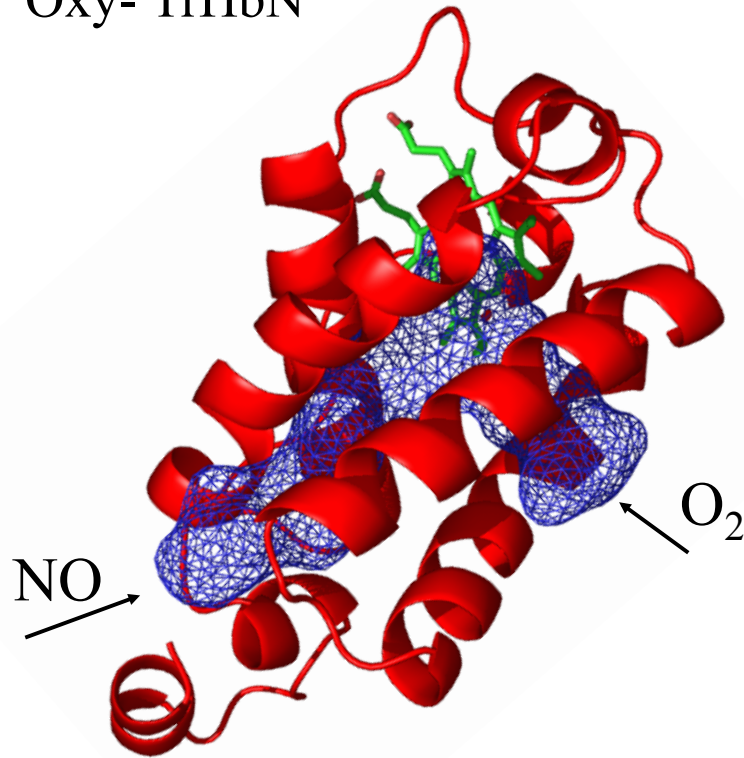
Geminate phase

Bimolecular phase

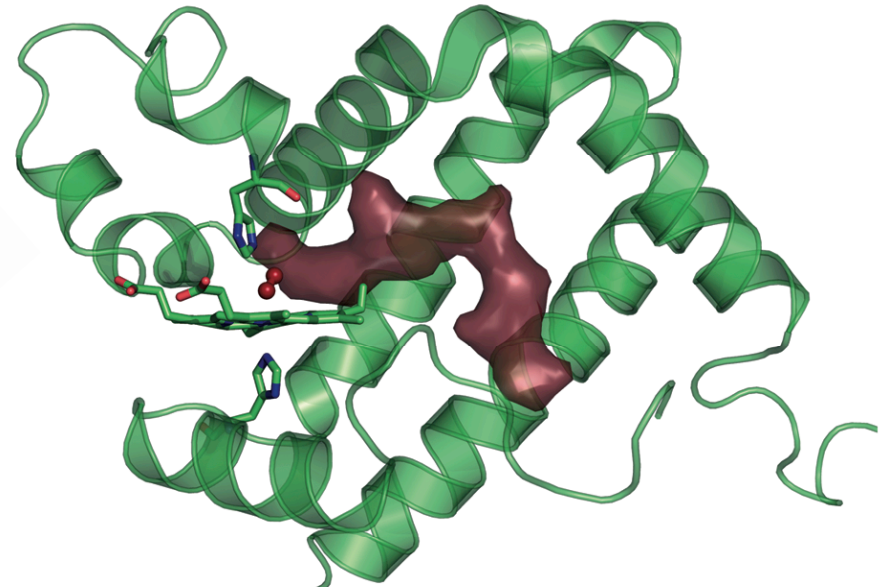


A similar functional mechanism for a common “stress” problem

Oxy- TrHbN



Oxy-AHb1



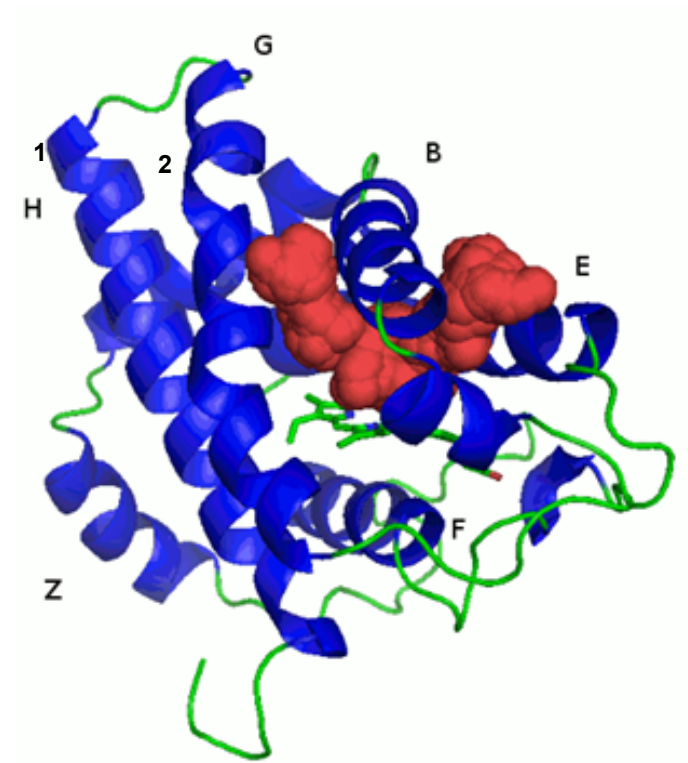
Despite the structural differences, it can be hypothesized that stress-induced hemoglobins have evolved a similar functional strategy designed to facilitate access of NO only after previous loading of O₂.

Protoglobin: the *puzzling* ancestor

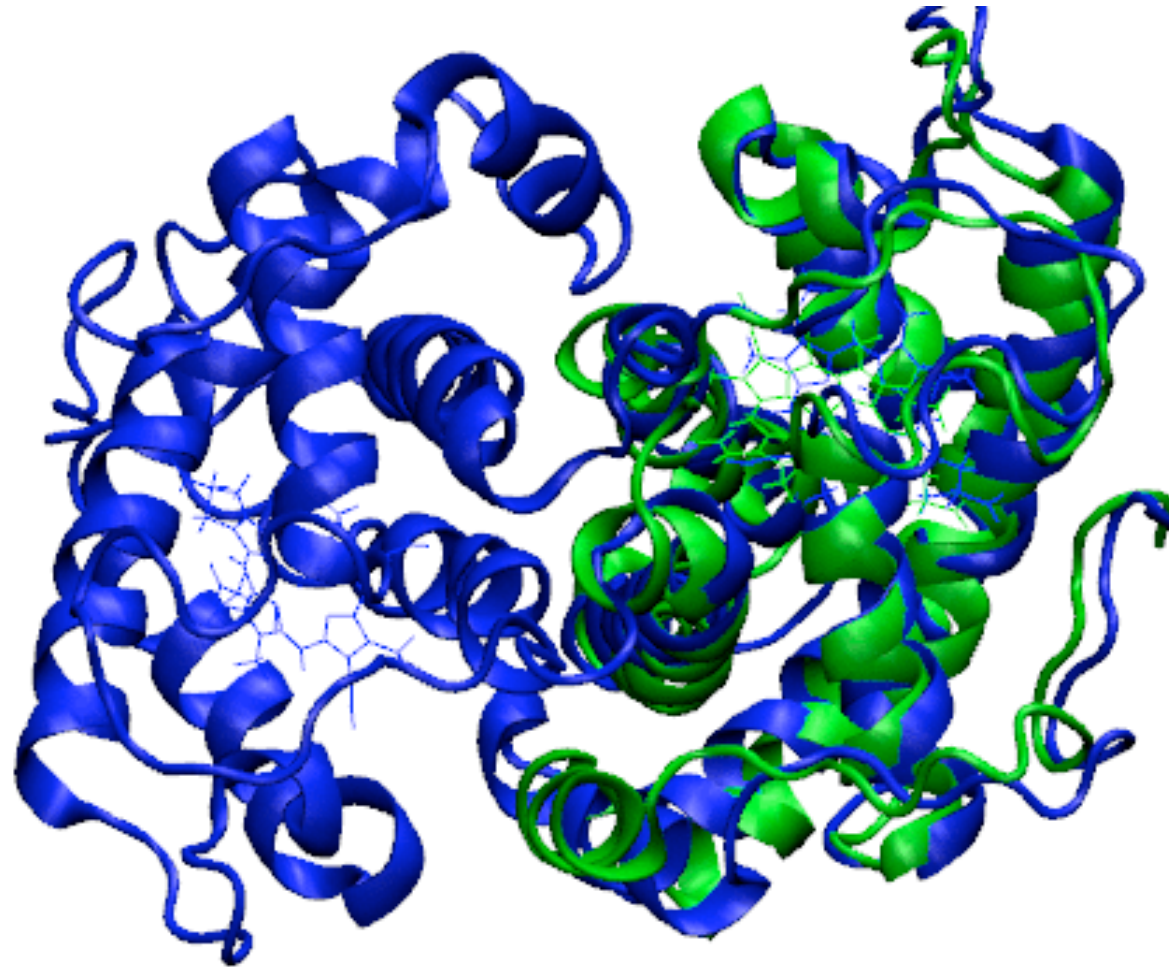
- Found in *Methanosarcina acetivorans*, which is an strict anaerobic archaea
- Unknown biological function
- Perhaps the last universal common ancestor of globins (?)

Unusual O₂ affinity ($k_{\text{off}} = 0.09\text{-}0.009 \text{ s}^{-1}$)

Protein	#Distal HB	$k_{\text{off}} (\text{s}^{-1})$
Pgb	0	$9.4 \times 10^{-3} - 9.2 \times 10^{-2}$
Mb	1	12
Mb HE7V	0	1600
trHbN	2	0.2
trHbN YB10A	1	45
AscHb	2	0.4×10^{-2}

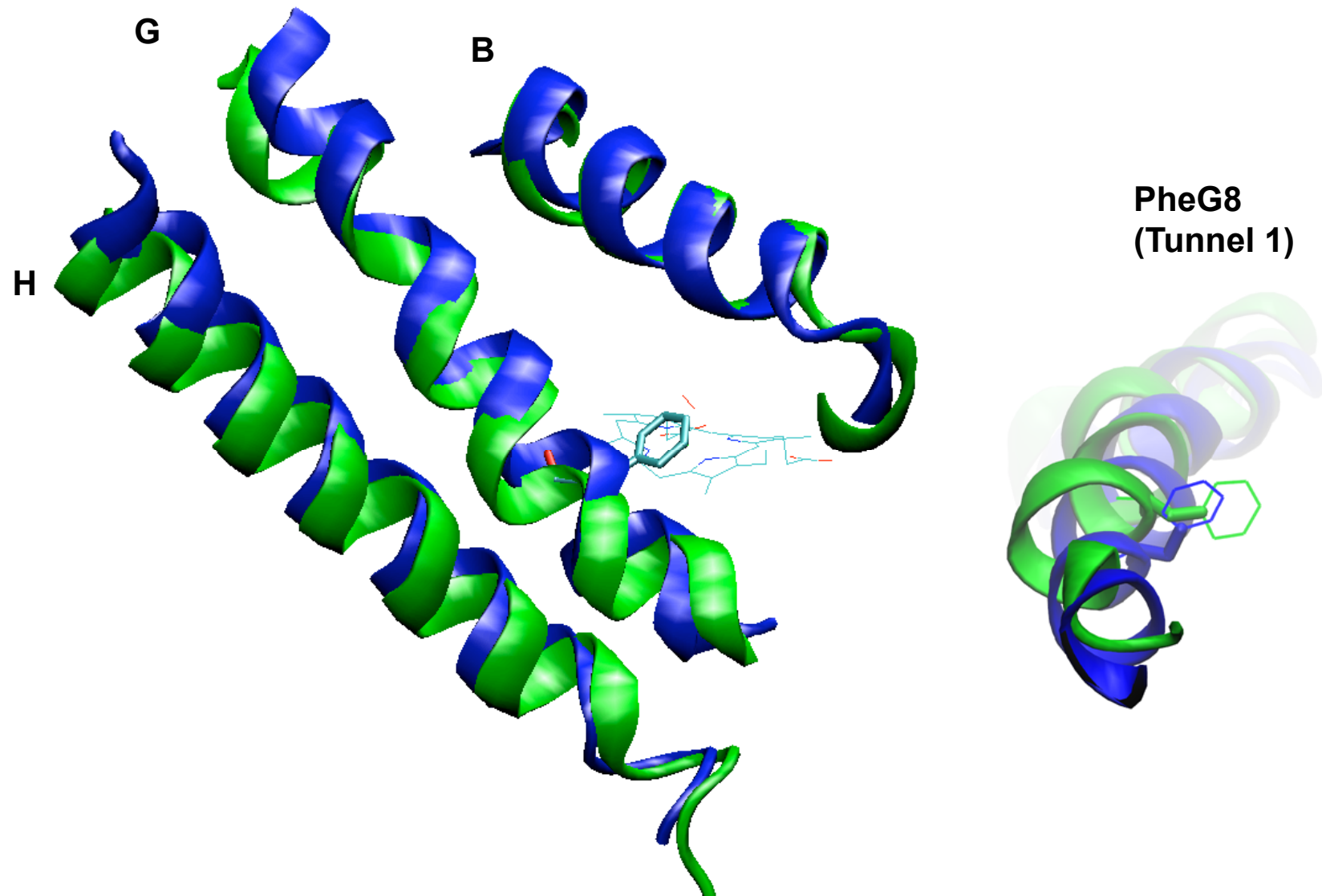


Is the dimer biologically relevant?



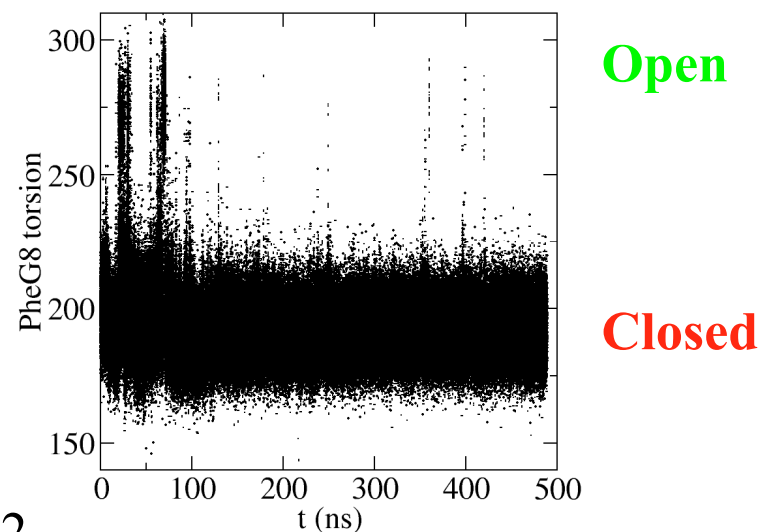
Interface:
around 2100 Å²
mainly formed by G and H helices

Molecular dynamics of monomer and dimer shows a structural rearrangement of helices G and H



Monomer: Accessibility through tunnel 1 (defined by helices G and B)

Conformational flexibility of PheG8



Free energy profiles through tunnels 1 and 2

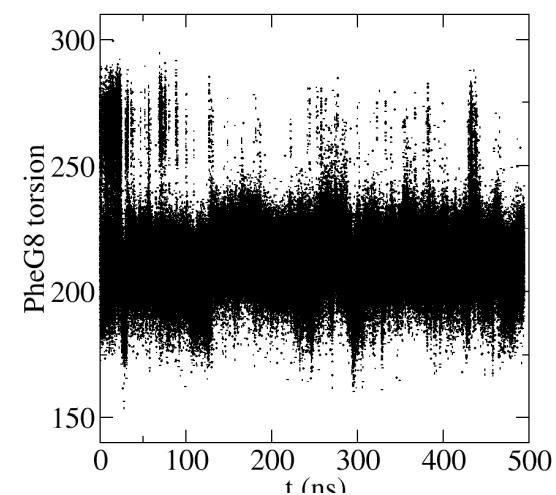
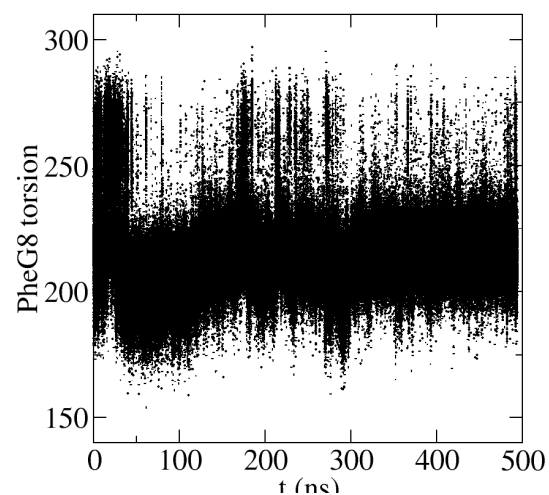


Dimer: Accessibility through tunnel 1 (defined by helices G and B)

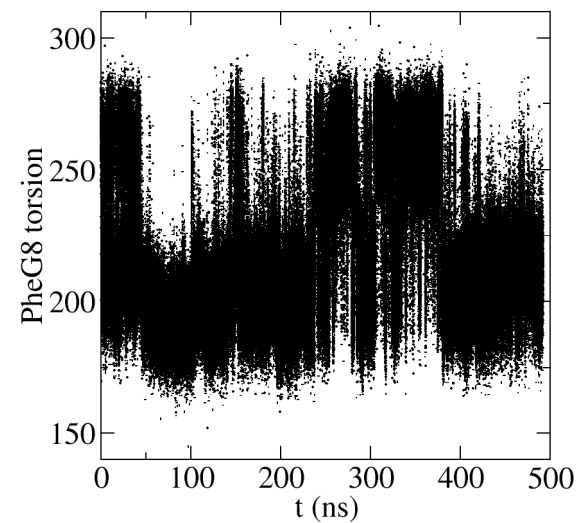
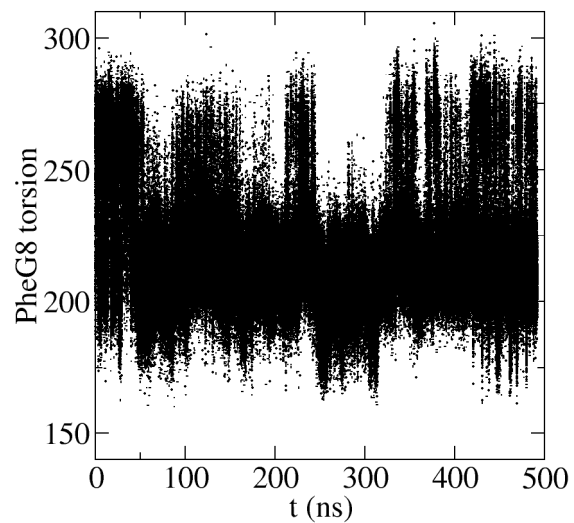
Subunit A

Subunit B

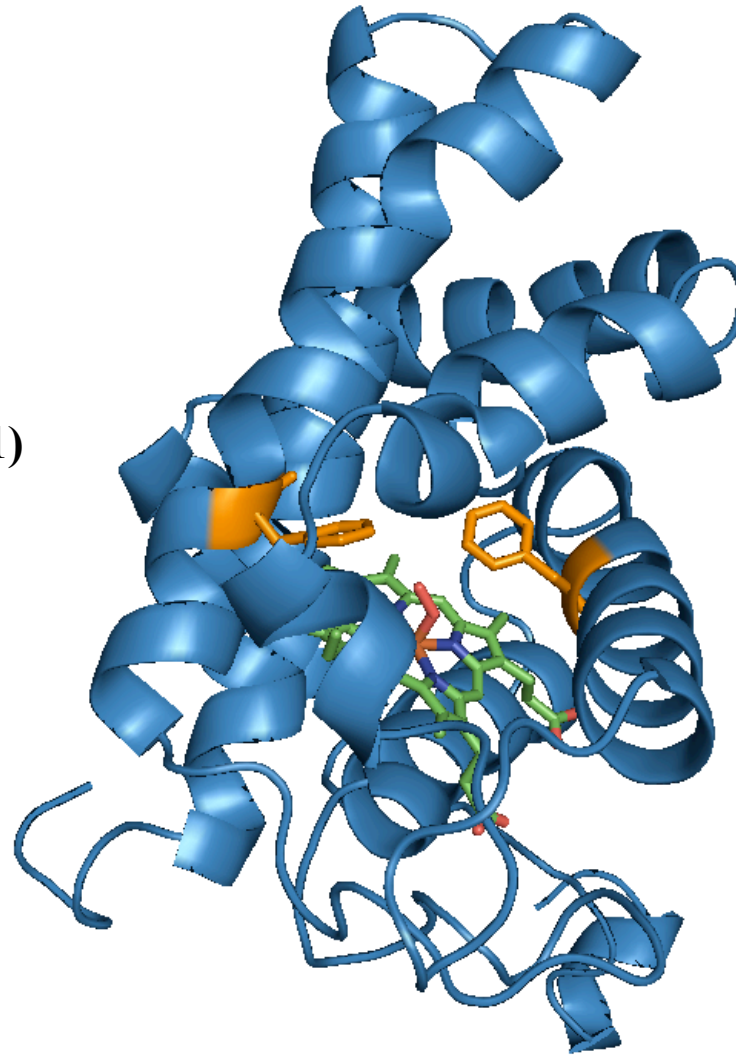
Deoxygenated dimer



Oxygenated dimer



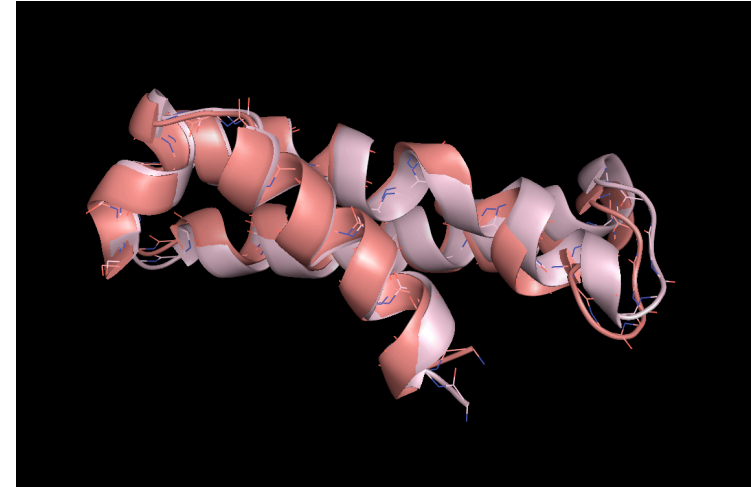
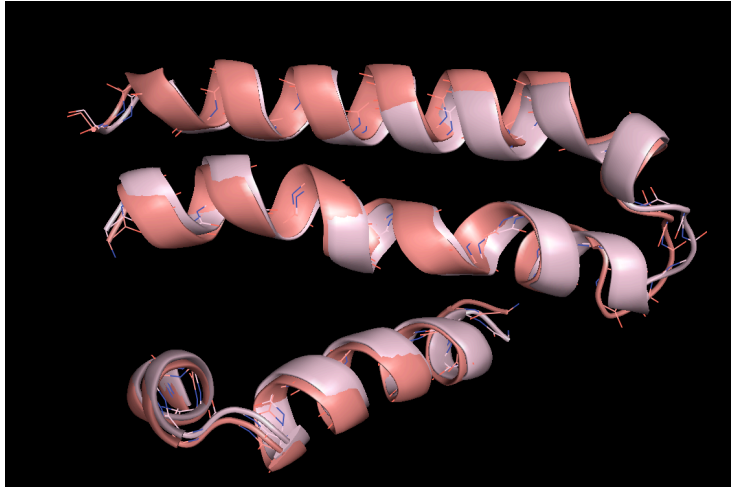
**PheG8
(Tunnel 1)**



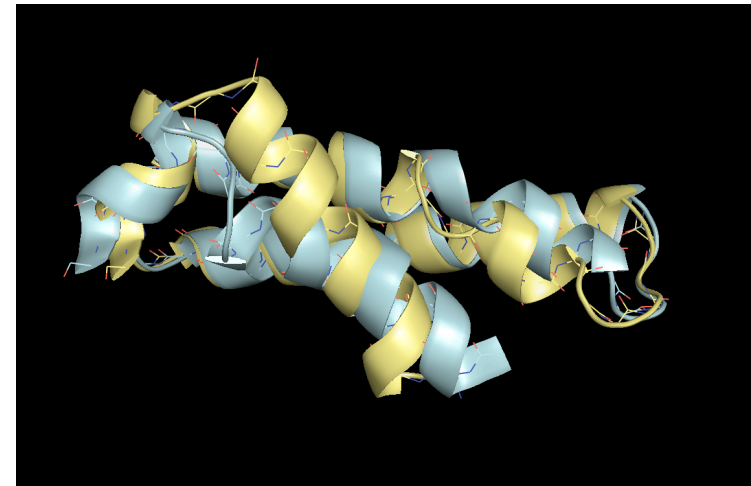
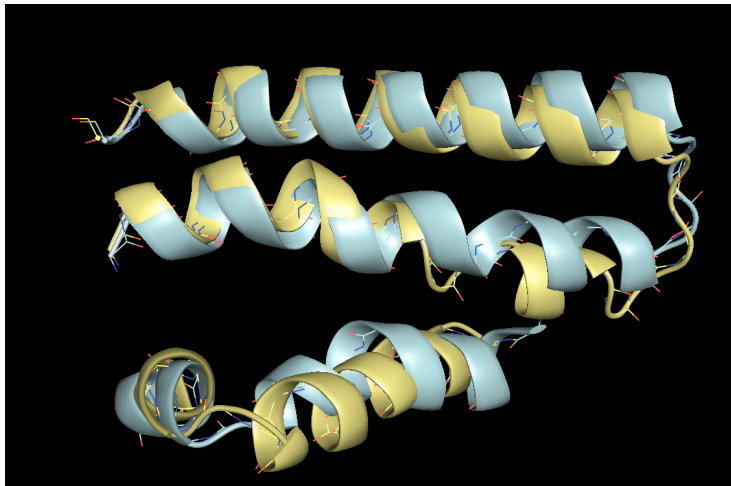
**PheE7
(Tunnel 2)**

PheE7 “senses” the bound O₂ and affects the spatial arrangement of helix B

Monomer

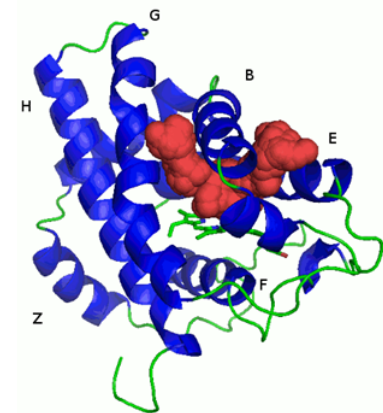
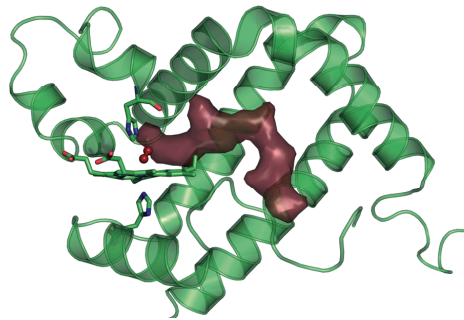


Dimer



Ligand migration is inherently linked to the structural plasticity of proteins at both local and global levels.

The shape and size of internal pockets and tunnels are dynamically delineated by conformational fluctuations, which reflect both the intrinsic flexibility of structural elements and the “sensing” properties toward incoming molecules.



3D models are important to infer relationships between structural features and biological function. However, a comprehensive insight into the molecular basis of the functional role cannot be gained without accounting for dynamics

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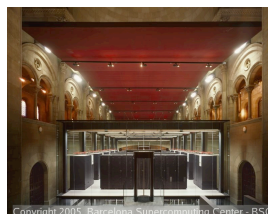
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Thank you !

