# What I did last 27 years

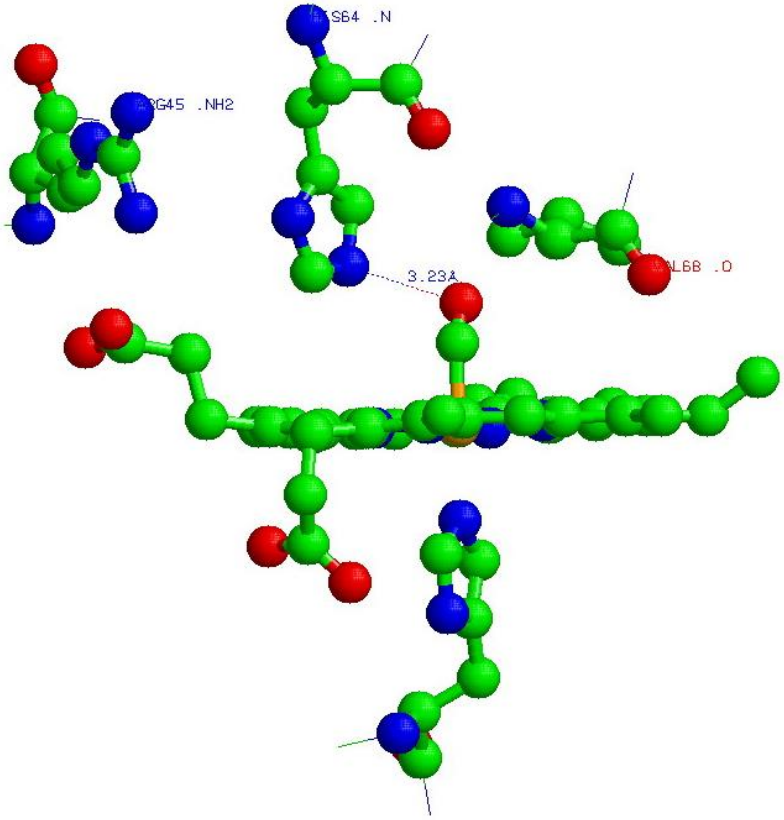

***Solomon S. Stavrov***

Sackler Institute of Molecular Medicine, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Israel

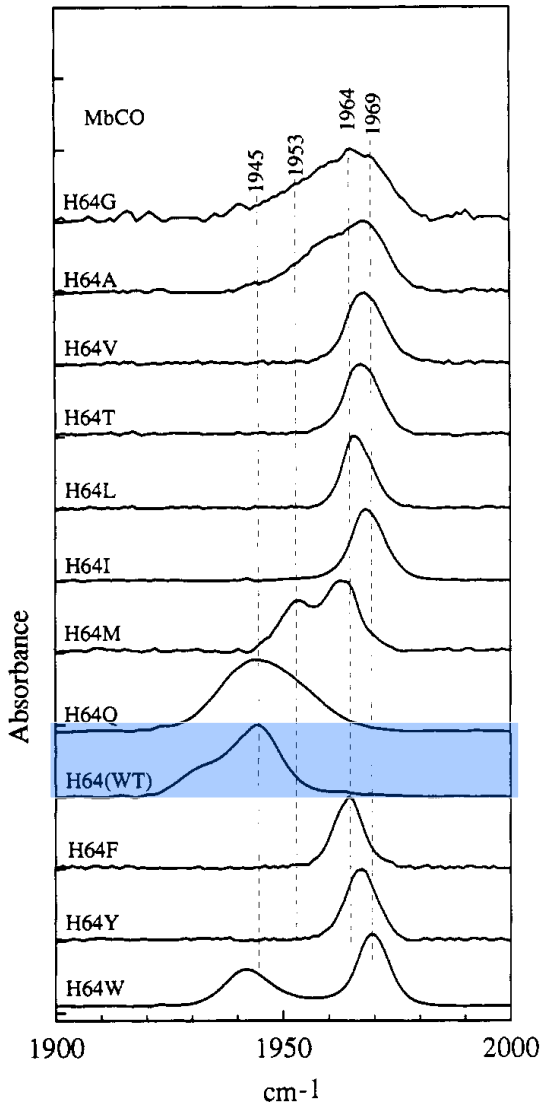
stavrov@post.tau.ac.il

# Objects

# Carbonmonoxy myoglobin

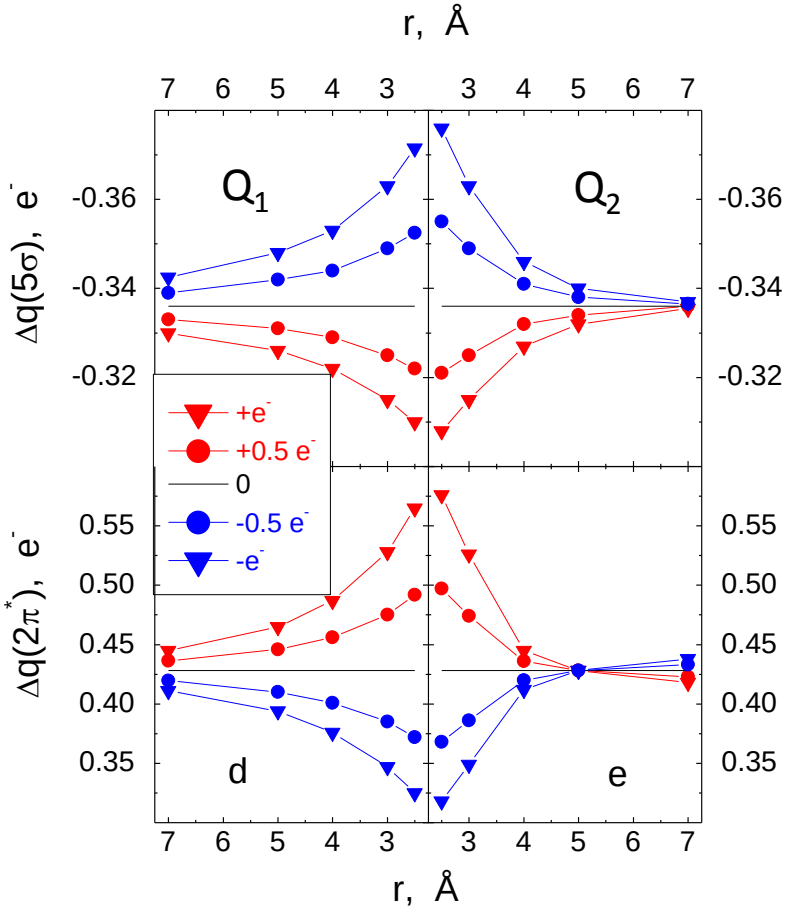


# Effect of the His64 mutations on the CO infrared absorption bands of MbCO (*Li et al., 1994*)

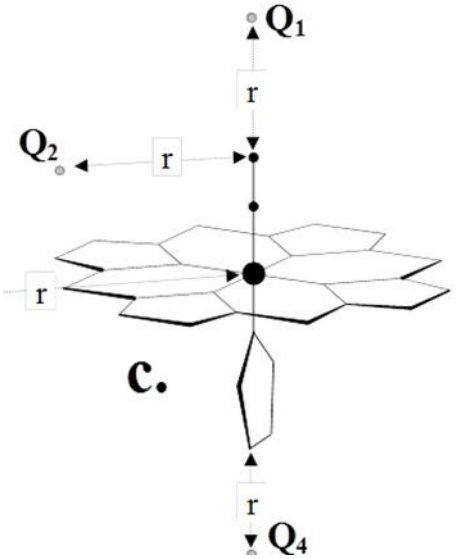


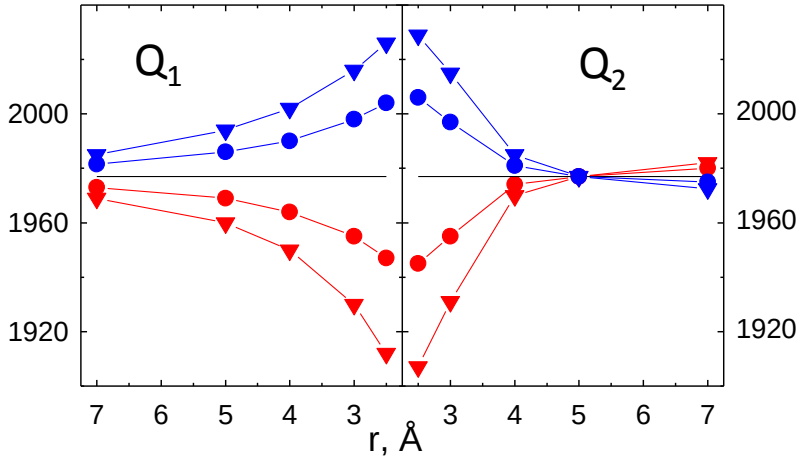

IR spectra of position 64 mutants of sperm whale CO–myoglobin measured at room temperature (~22 °C) in 0.1 M potassium phosphate/1 mM EDTA (pH 7.0). The mutant proteins are designated by a single letter abbreviation for the native residue, the position in the primary sequence, and the abbreviation for mutant amino acid, *viz.*, $His^{64} \rightarrow Gly$ is listed as H64G. WT stands for wild-type myoglobin. The spectra of the mutants are shifted to higher frequencies due to the loss of hydrogen-bonding interaction with $His^{64}$.

# Effect of the electric field on the C-O vibrational frequency




VTA

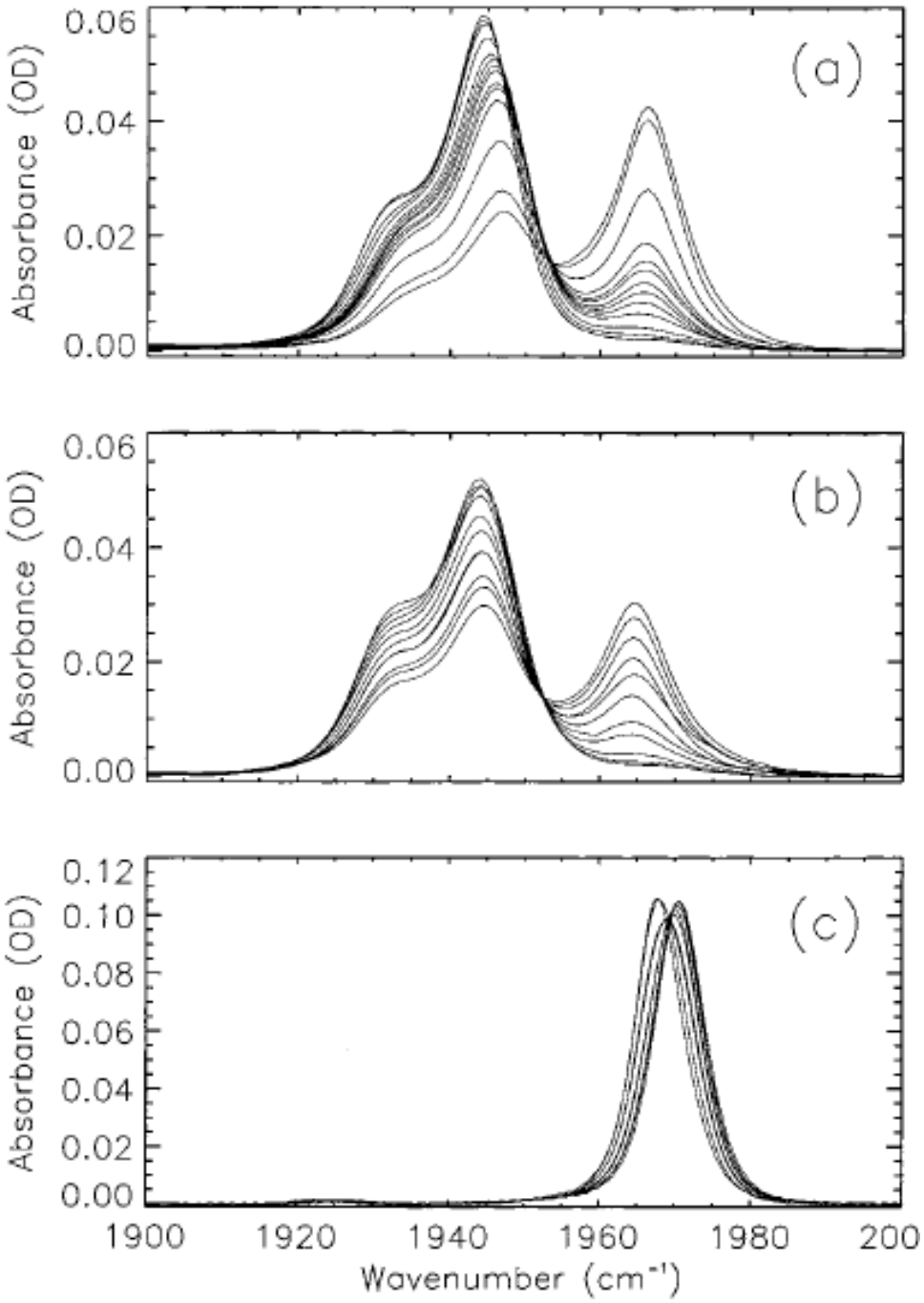


# Conformational Substates of MbCO Observed in the IR Absorption Spectra

*CO stretch infrared spectra in the pH range from 4.2 to 9.5 of (a) swMbCO, (b) proximal mutant H97F, and (c) distal mutant H64L*

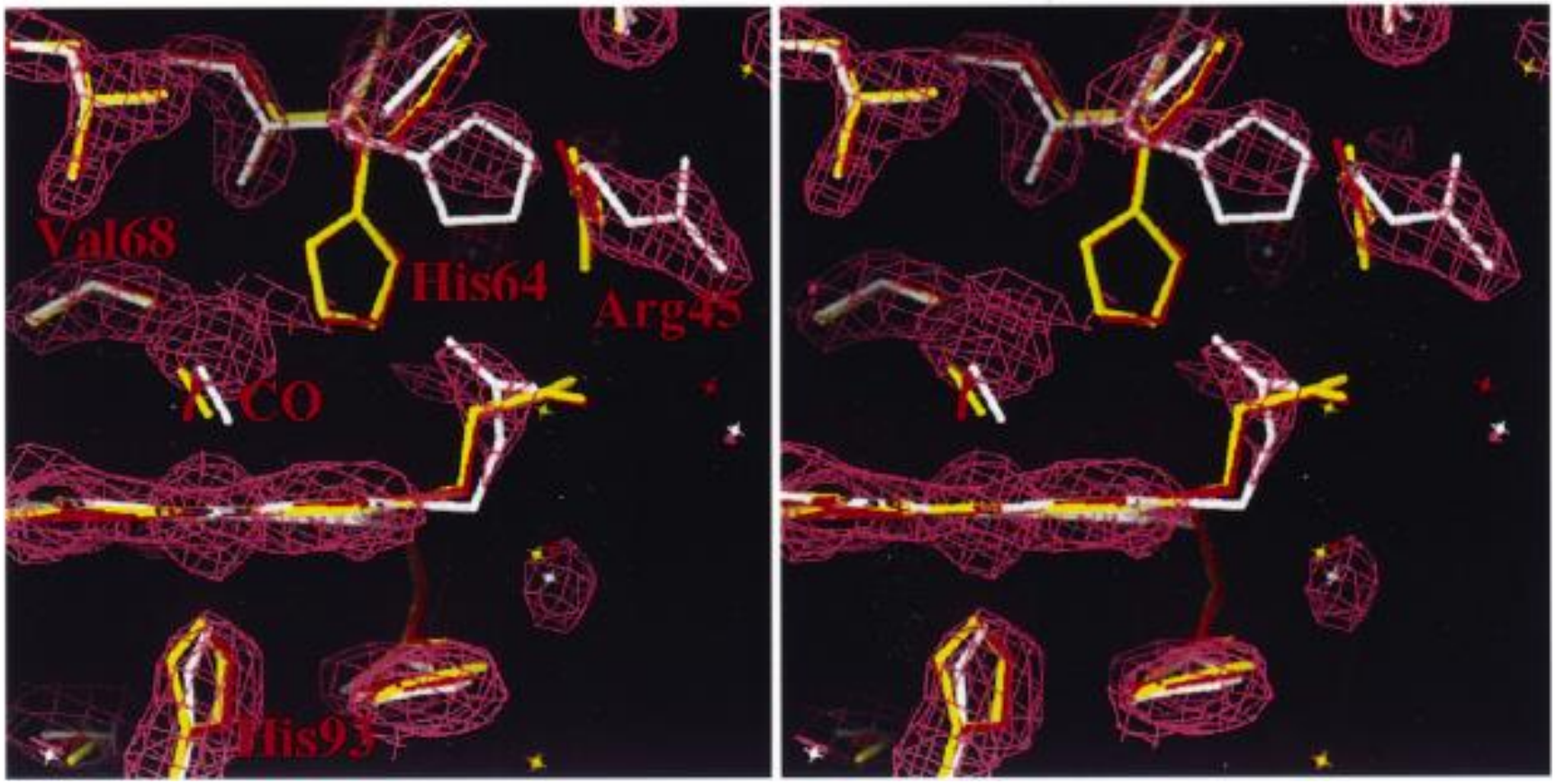
*(Muller et al. Biophysical Journal 77(2): 1036-1051)*



# Effect of pH on the Distal Histidine Position

pH 4 (white), 5 (yellow) and 6 (red)

(*Yang, F. and G. N. Phillips,* ***256****(1996) 762*)



# Conformational substates of MbCO observed in the IR absorption spectra

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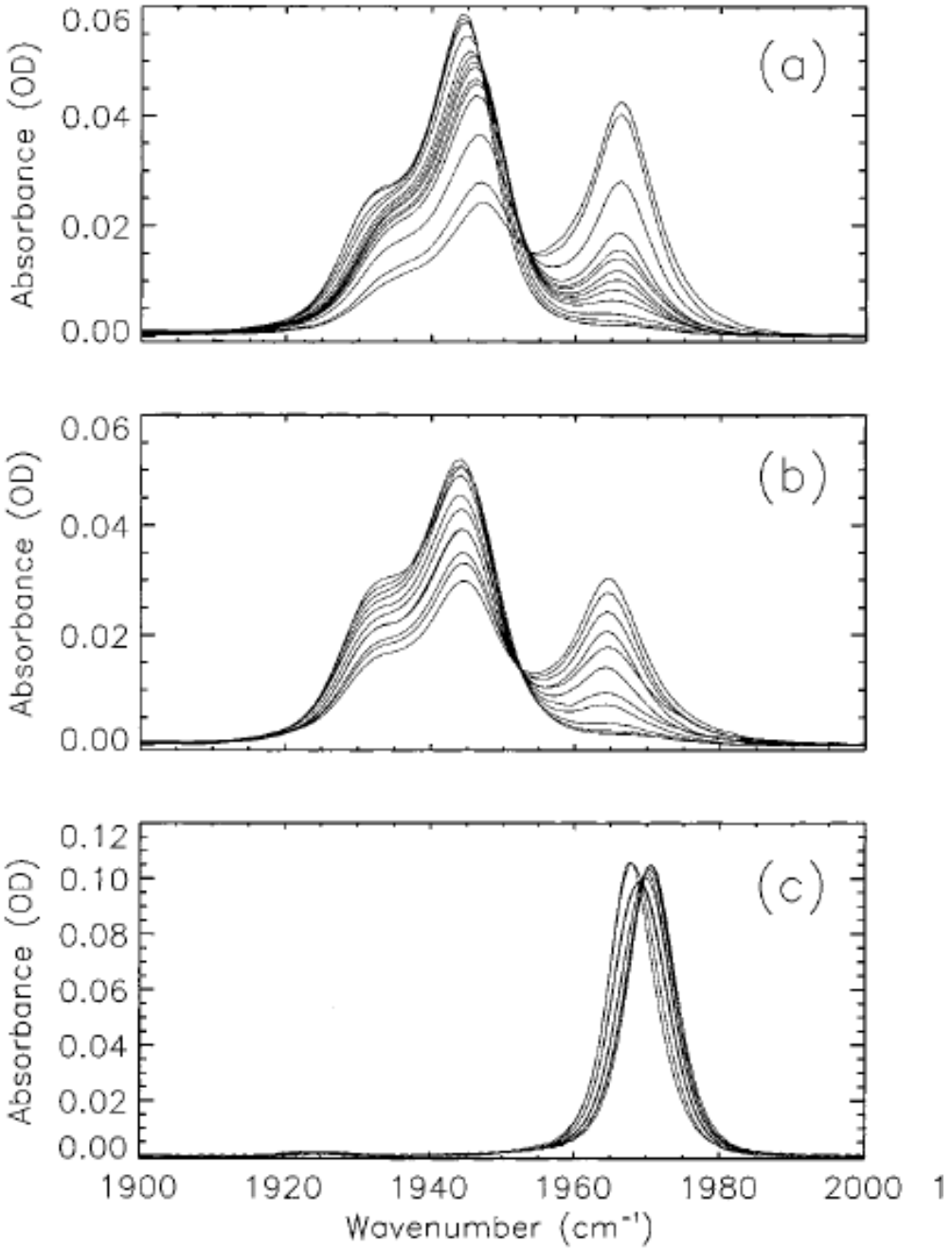
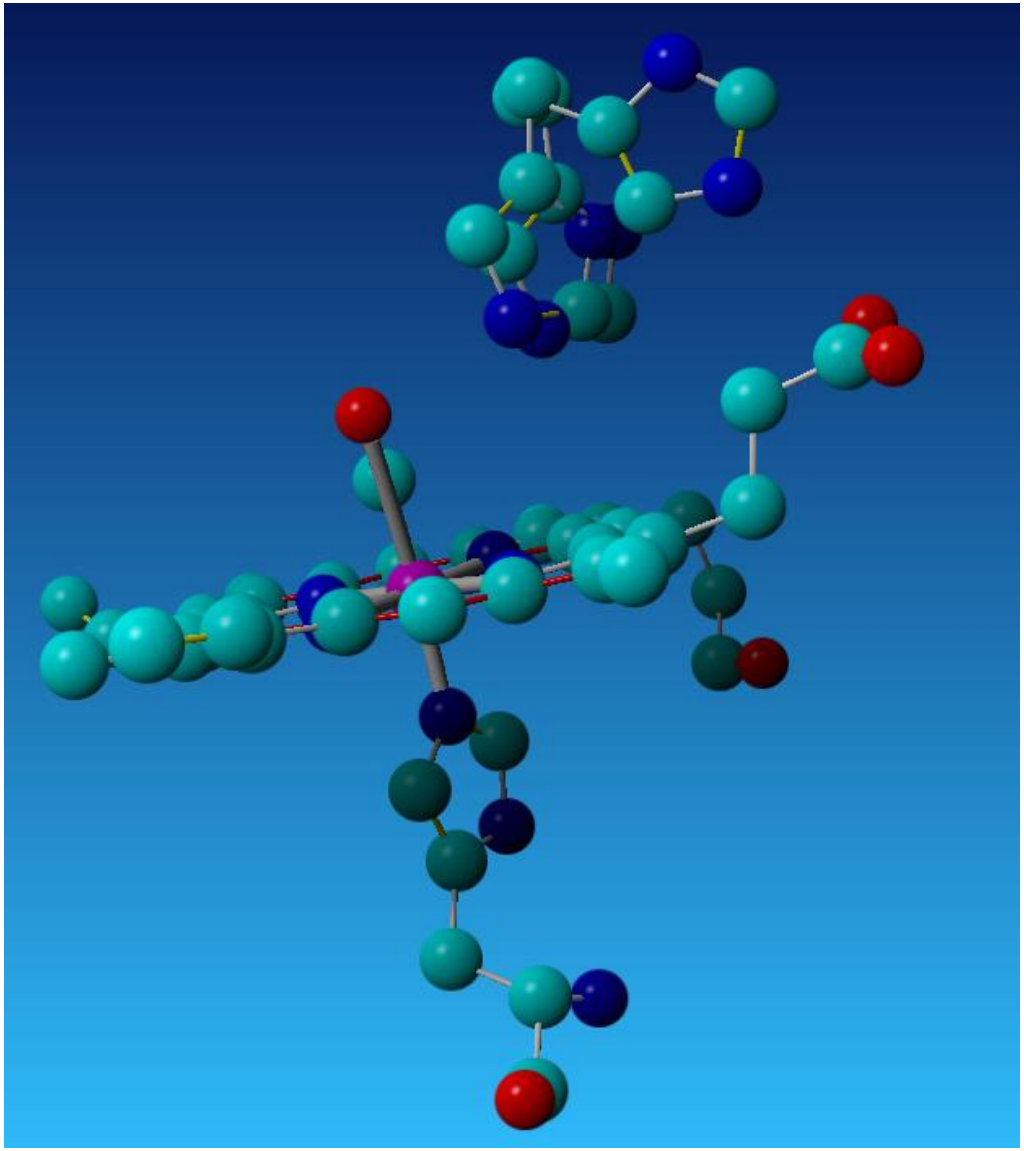


# The Possible Structure of the Conformational Substates

High-resolution (1.2 Å) X-ray study of MbCO showed presence of three different sub-structures of MbCO (*J. Vojtechovsky et al. Biophys. J.*, ***77****(1999, 2009) 2153-2174*)



# Horseradish peroxidase

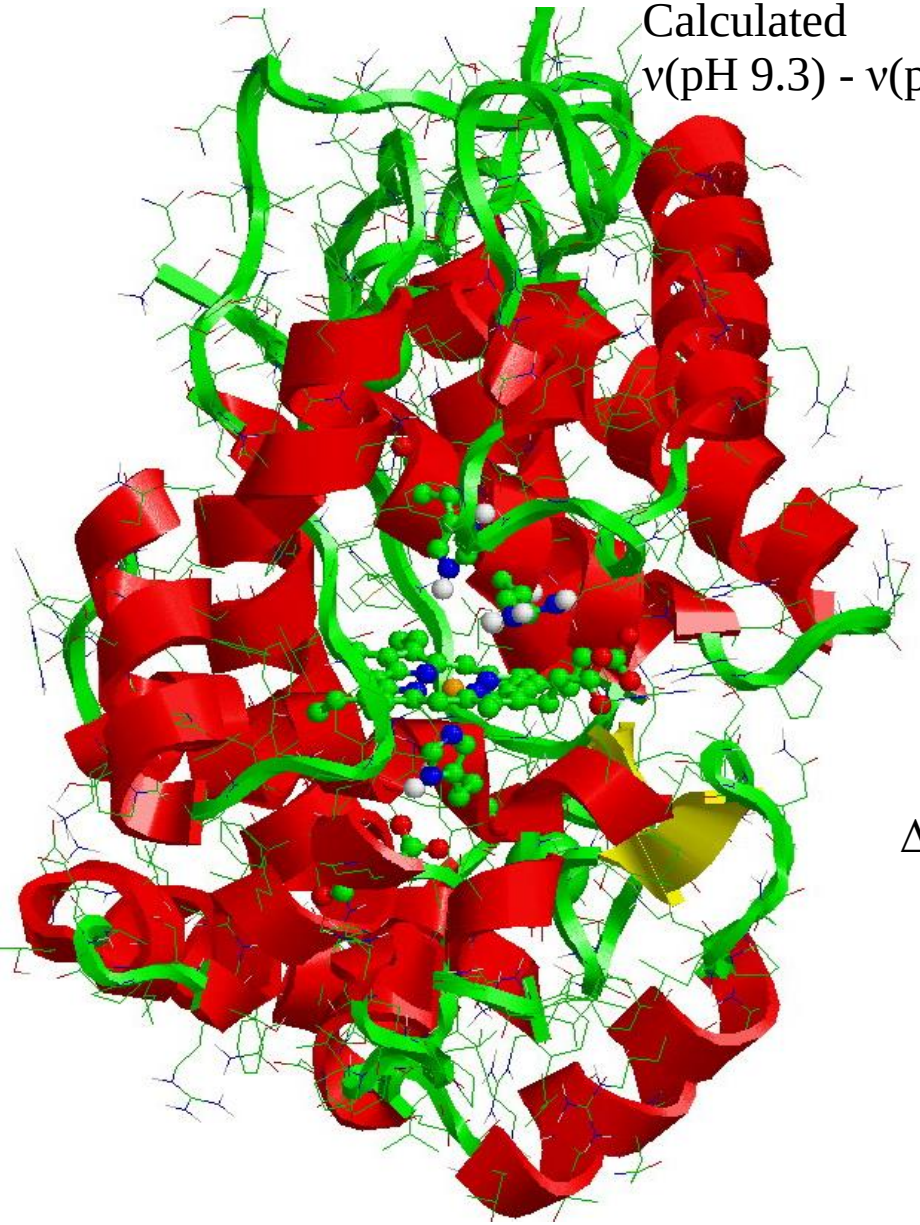
Calculated

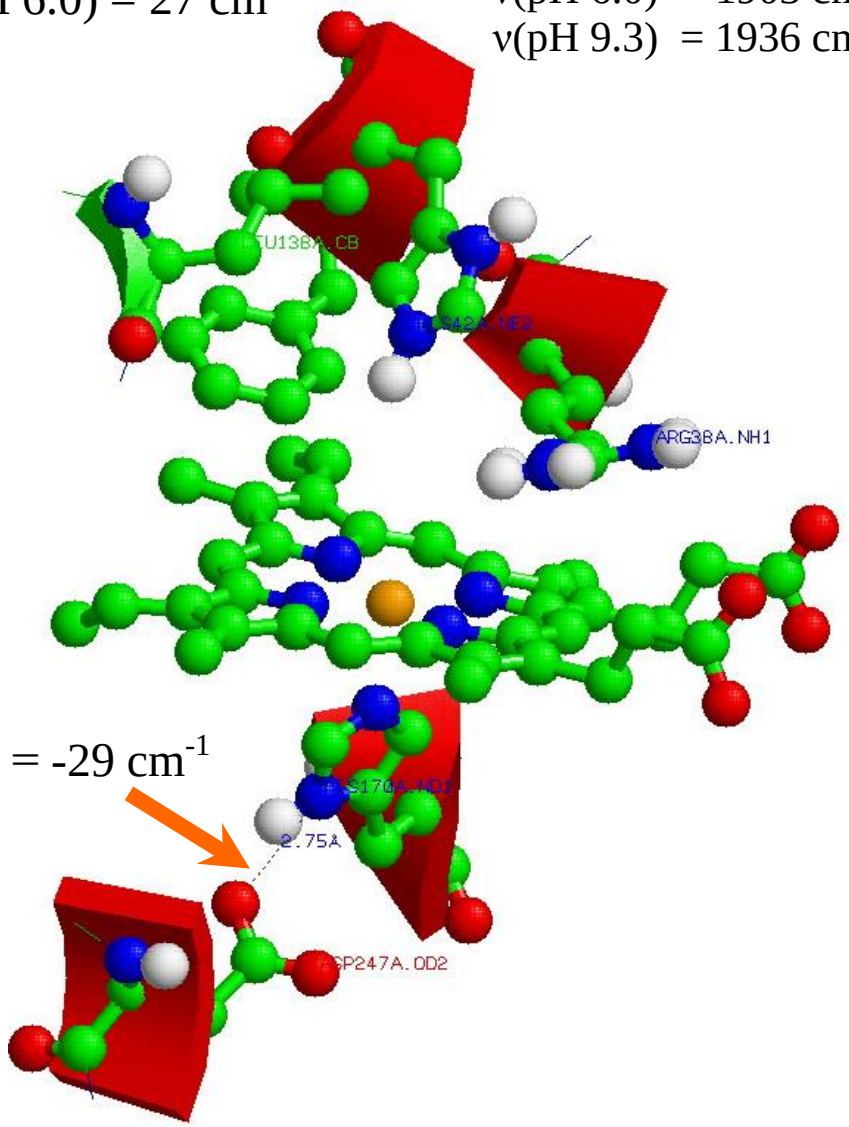
$\nu$(pH 9.3) - $\nu$(pH 6.0) = 27 $cm^{-1}$

Experimental

$\nu$(pH 6.0) = 1903 $cm^{-1}$

$\nu$(pH 9.3) = 1936 $cm^{-1}$






$\Delta\nu$ = -29 $cm^{-1}$

# Horseradish peroxidase + BHA

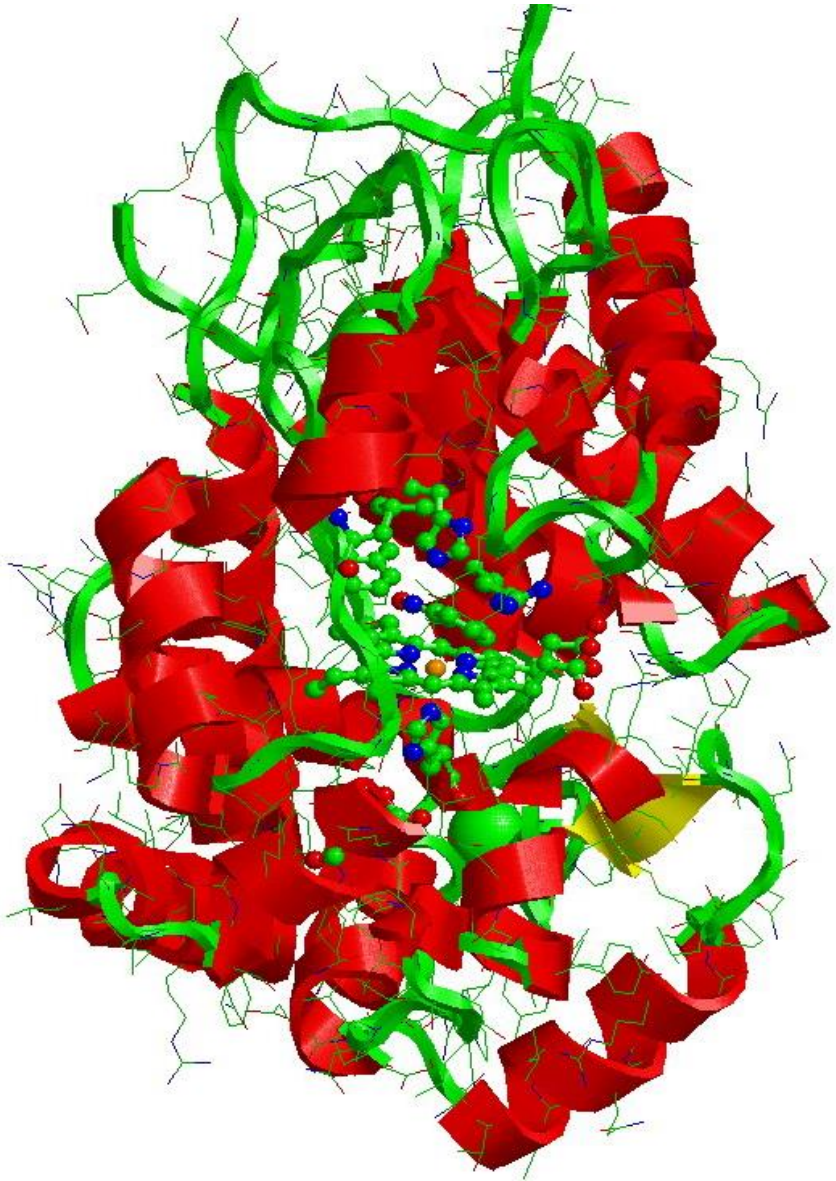
Calculated

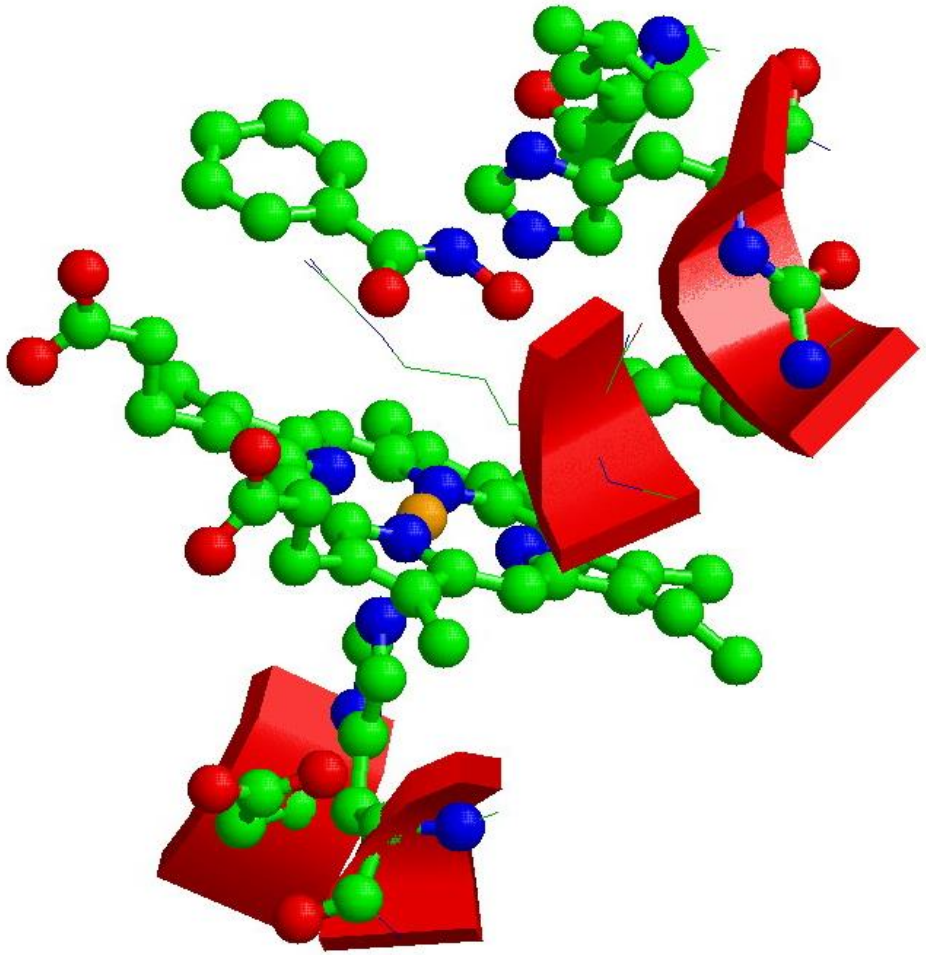
$\nu$(HRP+BHA) - $\nu$(HRP) = 6 $cm^{-1}$

Experimental

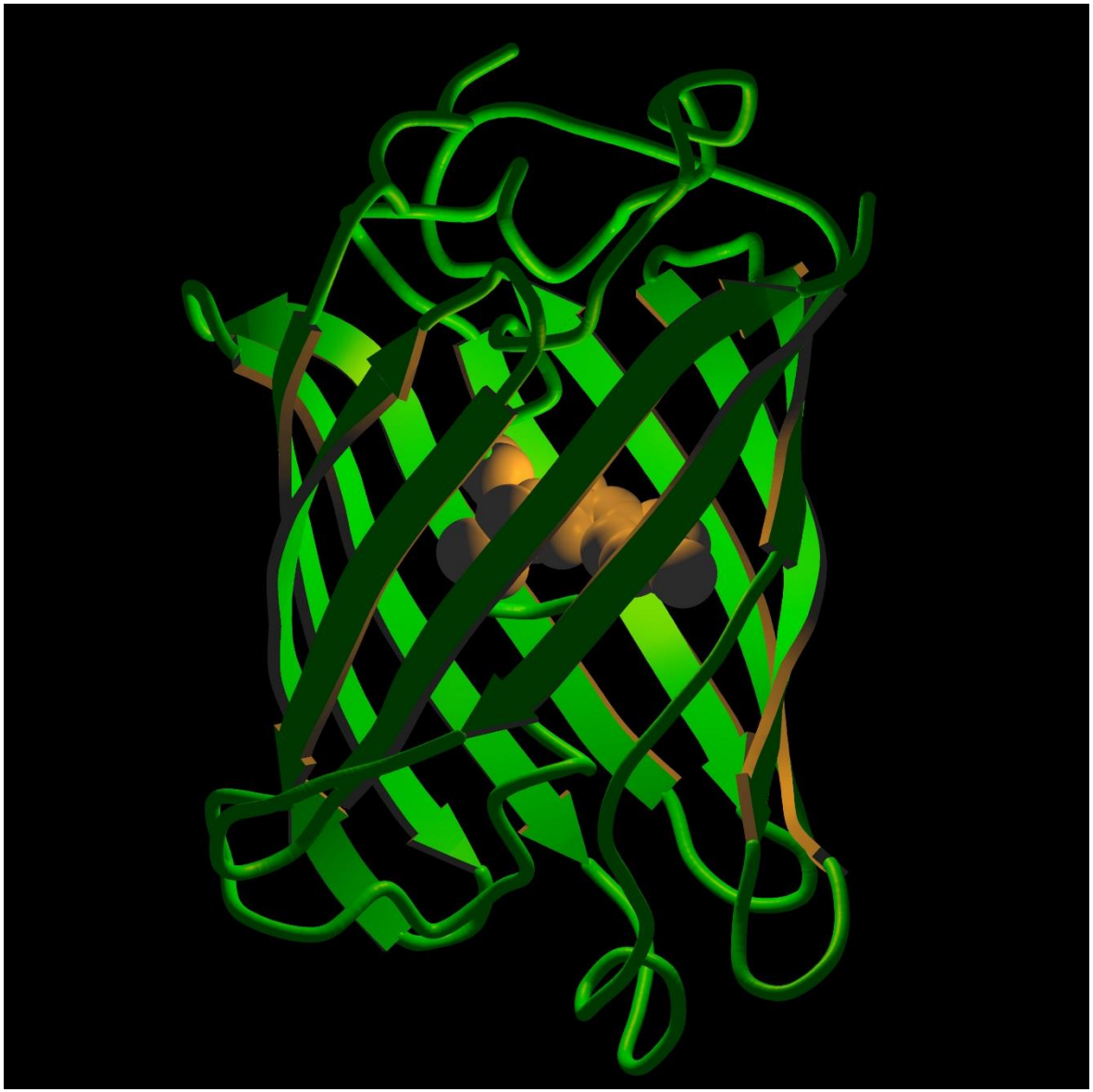
$\nu$(HRP) = 1903 $cm^{-1}$

$\nu$(HRP+BHA) = 1911 $cm^{-1}$

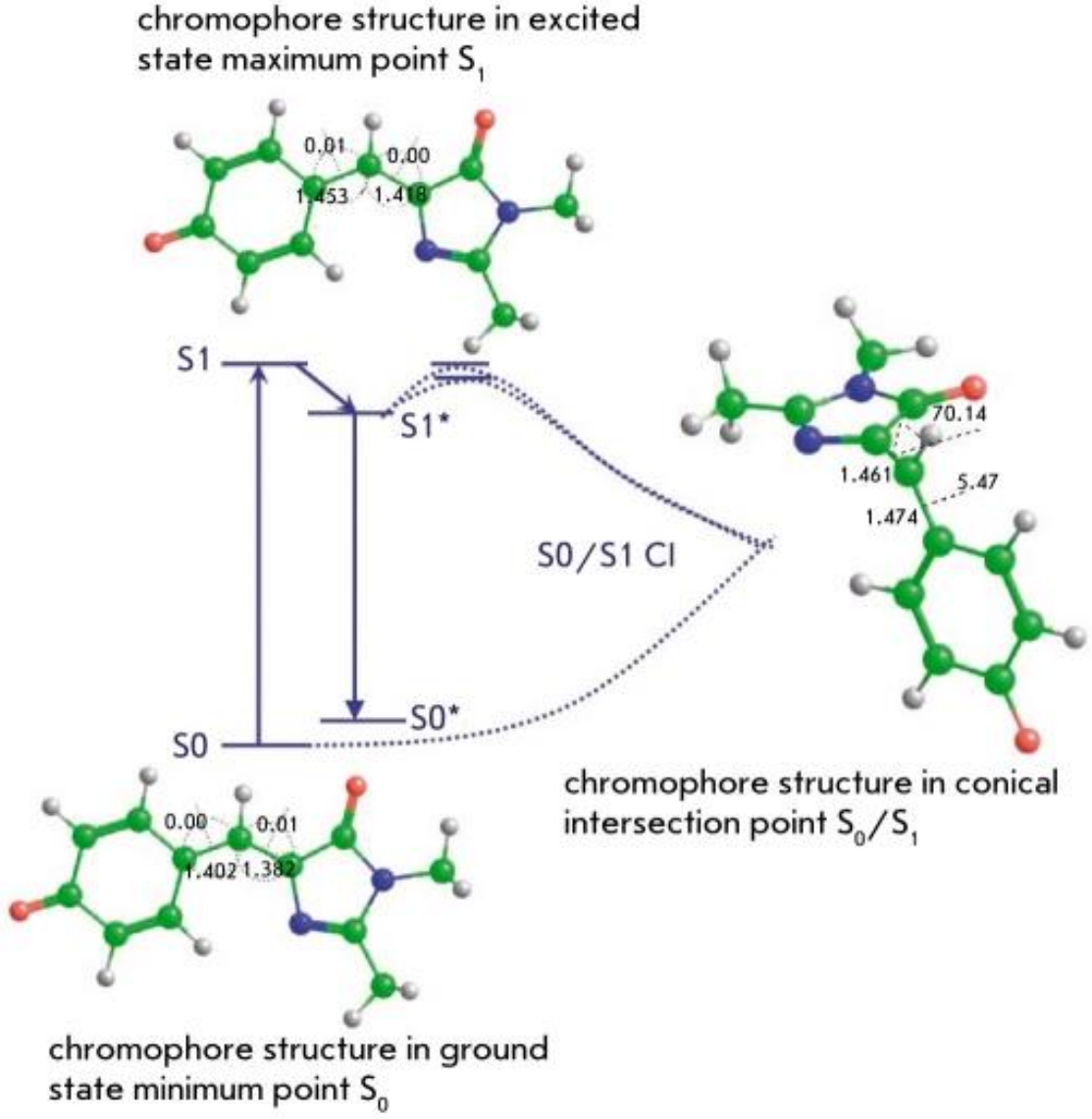




# GFP



# Ground state, excitation and decay of the excited state of the model chromophore



# Relationship between the fluorescence spectra of the chromophore and protein

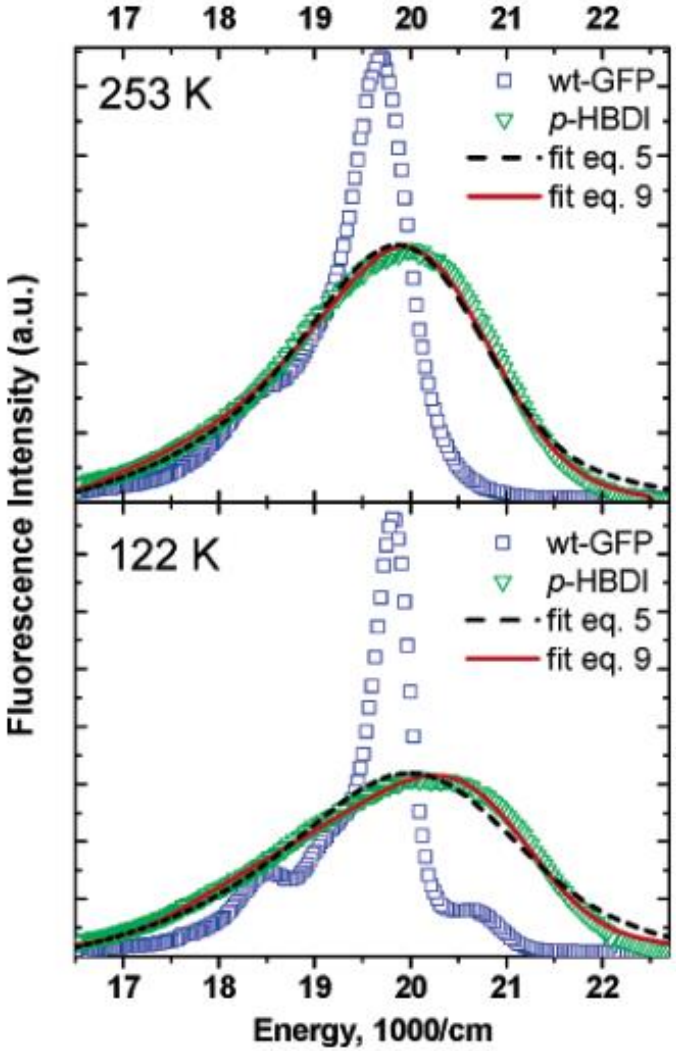
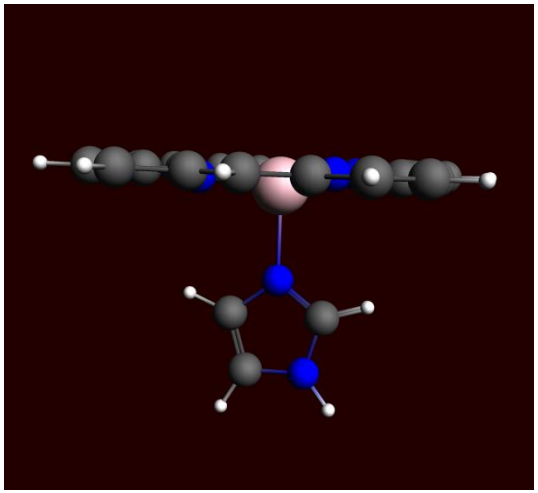



***Figure 5.*** Reconstruction of the model spectra (▽) by utilizing the protein spectra

# Mossbauer spectroscopy

# Effect of the distortions and model electric fields

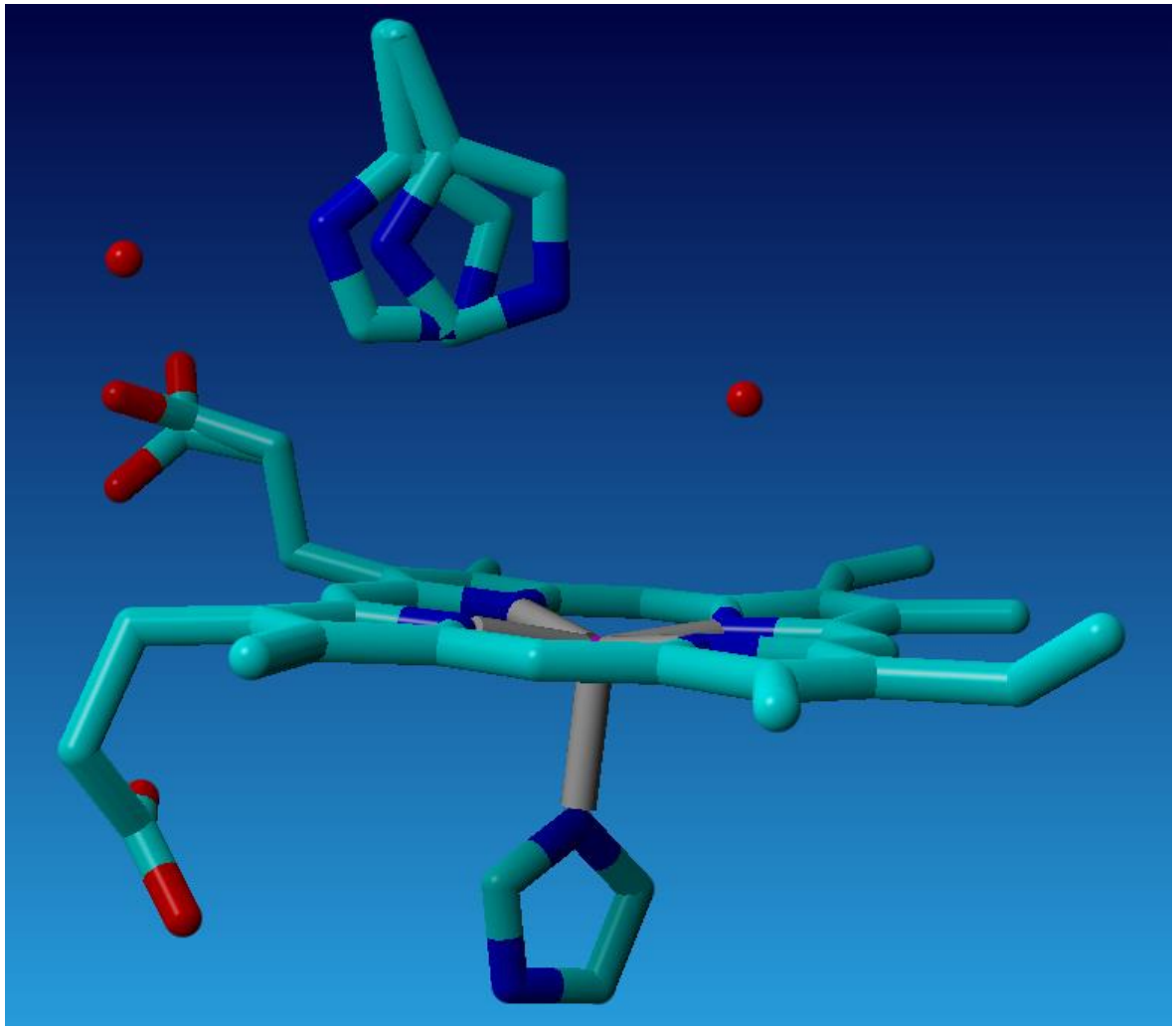


| R – Fe-$N_{Im}$; r – Fe-Ct | $\Delta E_Q$, mm/s | η |
|---|---|---|
| r = 0.32 Å, R = 2.12 Å<br>E = 0 | -2.06 | 0.77 |
| r = 0.42 Å, R = 2.12 Å<br>E = 0 | -2.13 | 0.97 |
| r = 0.32 Å, R= 2.27 Å<br>E = 0 | -2.18 | 0.48 |
| r = 0.32 Å, R = 2.12 Å<br>$E^{\parallel}$ = 0.01 a. u. | -2.06 | 0.76 |
| r = 0.32 Å, R = 2.12 Å<br>$E^{\perp}$ = 0.01 a. u. | -1.94 | 0.91 |
| r = 0.32 Å, R = 2.12 Å<br>$E^{\perp}$ = -0.01 a. u. | -2.11 | 0.64 |

# Conformational substates of deoxymyoglobin

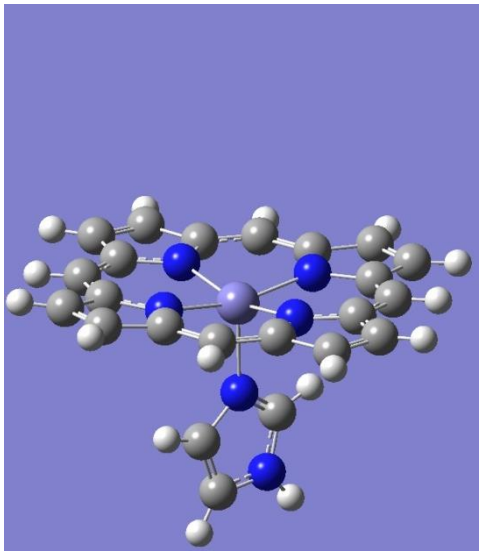
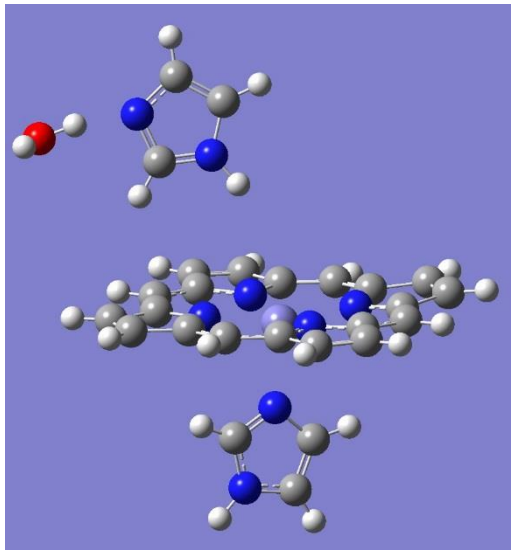
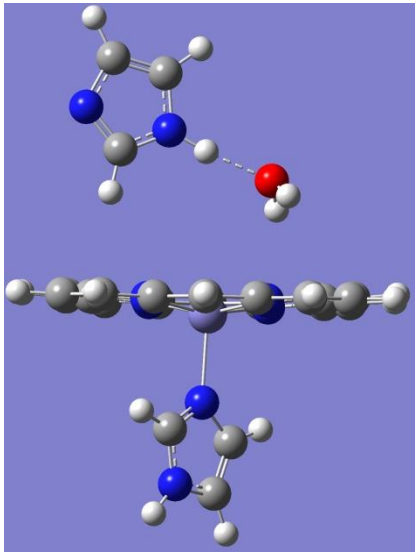
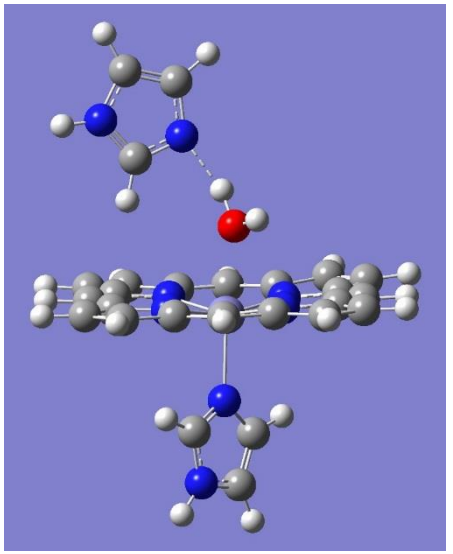
*Resolution **1.15 Å*** at room temperature

(*Kachalova et al., Science 284, 1999, 473)*



# Effect of the distal imidazole electric field

*Resolution **1.15** Å* at room temperature (*Kachalova et al., Science 284, 1999, 473*)

**$\Delta E_Q$ (mm/s) =** -2.08 -2.09 -2.18 -2.05

**$\Delta E_{Q,exp}$(Mb) = -2.16 – -2.22 (mm/s)**

(Kent, T. A. et al. (1979), BBA, 580(2): 245-258.)

# Professional activities

**Theoretical interpretation of virtually any spectroscopic studies of molecules, including biomolecules**