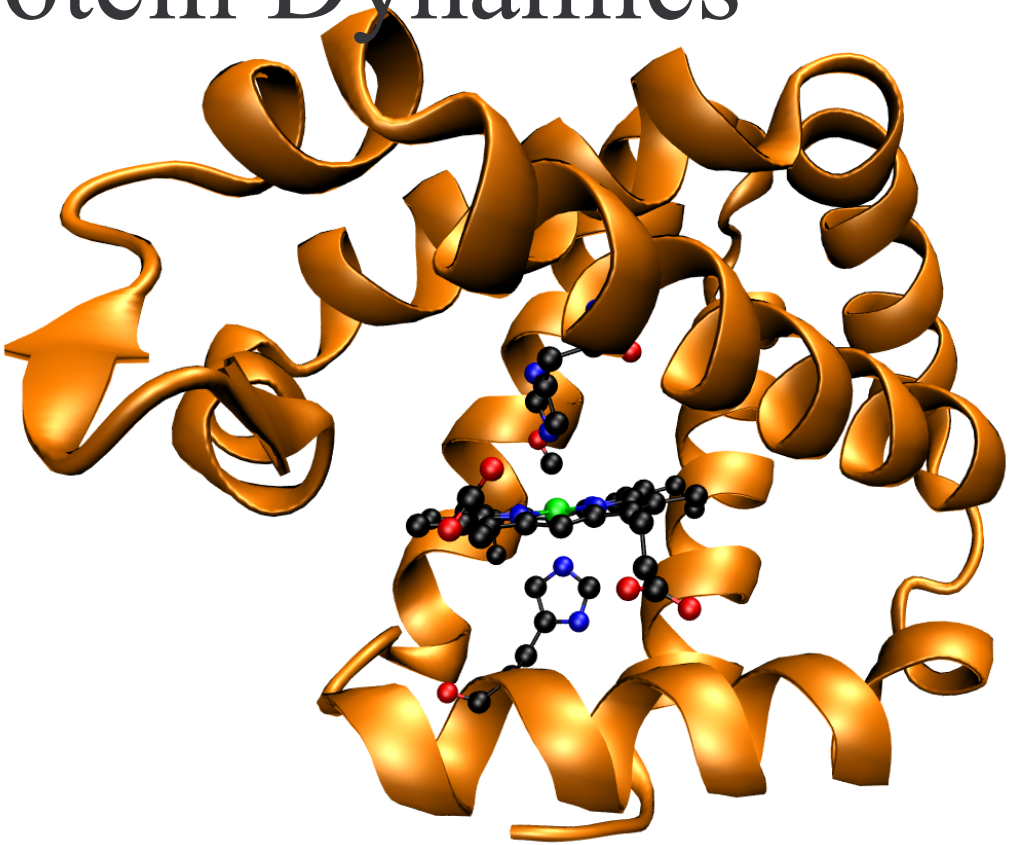
Molecular Dynamics of Neuroglobin – Ligand Binding and Protein Dynamics



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Acknowledgments

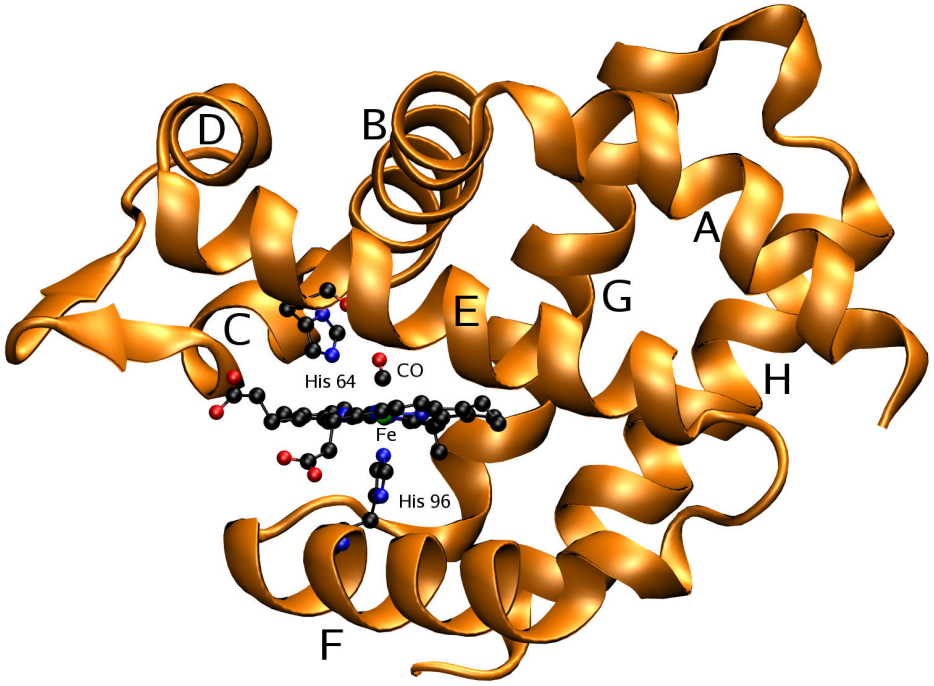
- Thanks to the Department of Biophysics at the University of Ulm
  - Prof. Dr. G. U. Nienhaus
- Thanks to the Department of Chemistry at the University of Basel
  - Prof. Dr. M. Meuwly

History of Neuroglobin

- Discovered in 2000 by Burmester et al.
- Crystal structure by Pesce et al. in 2003 and Vallone et al. in 2004
- Burmester et al. show in 2003 existence of Ngb mRNA in mouse retina and suggest as its function transport and storage of oxygen in photoreceptor cells
- In 2003 Wakasugi et al. argue it might be a sensor protein of neuronal hypoxia due to its low content and sequence homology with equivalent known signaling proteins for ischemia
- 2004 Vallone et al. show heme sliding mechanism within Ngb upon binding and rebinding, both ferrous hexa-coordination, ferrous penta-coordination and ferric hexa-coordination exist

Structure and Properties

- Globin Protein with a heme group
- 151 Amino Acids
- 94% helical fold
- Sequence Homology with Hemoglobin and Myoglobin is roughly 22%
- Capable of binding reversible CO and O_2 which are defining the ferrous (Fe^{3+}) and ferric (Fe^{2+}) states



Motivation

- Experiments on Neuroglobin suffer on the same problems as Myoglobin
  - Substates of CO after dissociation define the spectrum
    - Where is the CO?
    - How can the CO escape from the interior?
    - Which area within the protein give rise to the different CO spectra?
    - Can we see sliding of the heme plane? What effects might correlate with this?

Basics of Molecular Dynamics

- Bonds, electronic densities, steric hindrance, electrostatic interactions need to be calculated
- System sizes of ~ 6893 Atoms in Langevin dynamics up to 25000 Atoms with periodic boundary conditions
- Approximation of atomic potentials by empiric force fields
  - harmonic oscillators for bonds
  - Point charges on atoms
  - Additional situational defined repulsive term
- No dissociation and no chemistry will occur
- Limitation of the results on the used parameter set and force field

Empiric Force Field Energies

- Internal Energies
  - Bond Potential E_{Bond}
  - Bond Angle Potential E_θ
  - Dihedral Angle Potential E_ϕ
  - Improper Dihedral Potential E_ω
- External Energies
  - Electrostatic Potential E_q
  - Van-de-Waals Potential E_{VW}
- Simulation: $ma = -\nabla E$

$$E_{Bond} = \sum k_B (r - r_0)^2$$

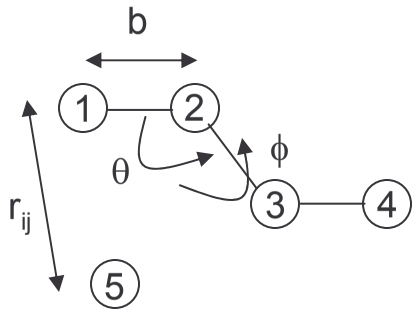
$$E_\theta = \sum k_\theta (\theta - \theta_0)^2$$

$$E_\phi = \sum |k_\phi| - k_\phi \cos(n\phi + \delta) \qquad n \in \mathrm{N}$$

$$E_\omega = \sum k_\omega (\omega - \omega_0)^2$$

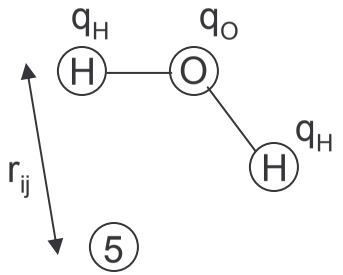
$$E_q = \sum_{\substack{i,j \\ i \neq j}} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}$$

$$E_{VW} = \sum_{\substack{i,j \\ i \neq j}} \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}$$



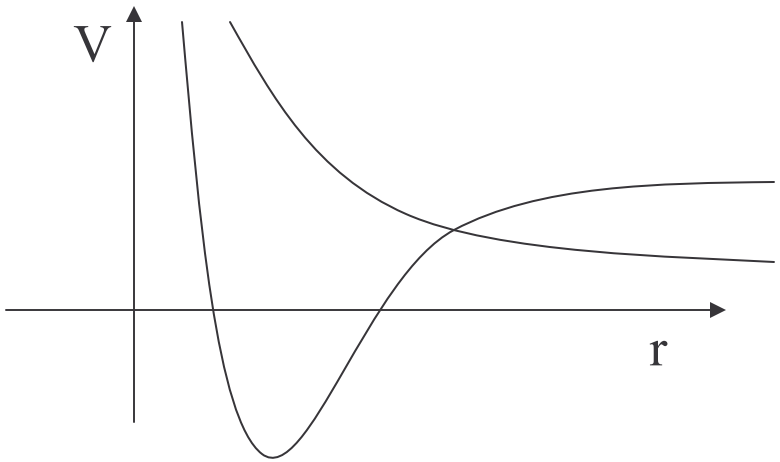
Water Model

- TIP3P (transferable intermolecular potential function: three point)
- Interaction: $$E_{mn} = \sum_i \sum_j \frac{q_i q_j e^2}{r_{ij}} + \frac{A}{r_{OO}^{12}} - \frac{C}{r_{OO}^{6}}$$
- Parameters A,C and $2q_H=q_O$ are optimised to reproduce water density, intermolecular energies, isobaric compressibility and heat of vaporization
- Errors and Problems are discussed in Jorensen et. al.



Dissociation Model

- Spin state of Fe changes
  - Force Field Parameters change
- The sudden bond break due to photo dissociation needs to be modelled

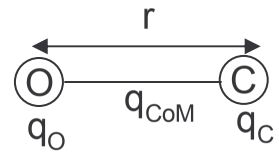


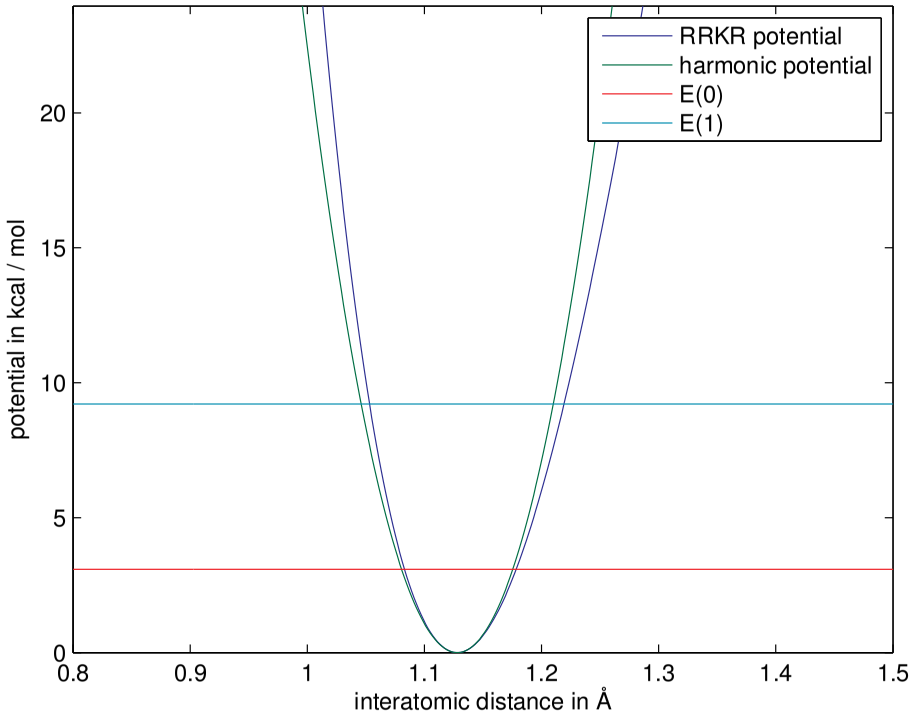
$$V = C\left(\frac{r}{r_0}\right)^{-12}$$

CO 3P Model

- Fluctuating Charge Model three point for Carbon Monoxide
- Conservation of dipole moment
- Conservation of quadrupole moment
- Bond potential is no longer harmonic but a Rotational Rydberg-Klein-Rees Potential
  - Constructed by perturbation theory of Morse potential

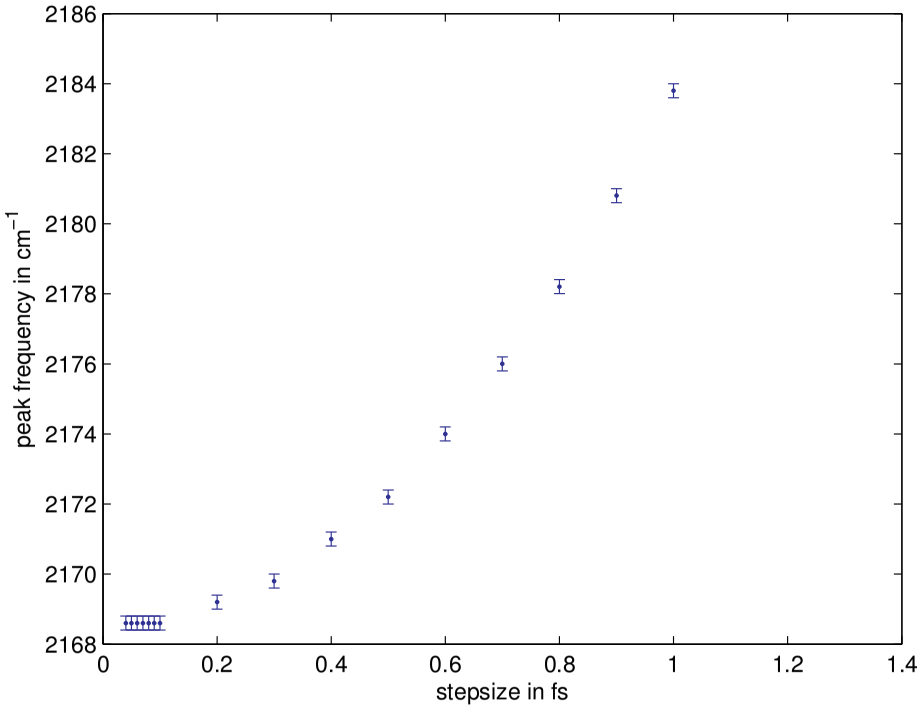
$$q = a_0 + a_1 r + a_2 r^2 + a_3 r^3$$





CO 3P Model

- Reproducing the measured term spectrum of CO up to n=20
- Coefficients fit to experimental data
- Classic approximation within the oscillator and step size dependency



$$G(n)=\bar{\nu}_e\left(n+\frac{1}{2}\right)-x_e\bar{\nu}_e\left(n+\frac{1}{2}\right)^2+y_e\bar{\nu}_e\left(n+\frac{1}{2}\right)^3-z_e\bar{\nu}_e\left(n+\frac{1}{2}\right)^4+a_e\bar{\nu}_e\left(n+\frac{1}{2}\right)^5-b_e\bar{\nu}_e\left(n+\frac{1}{2}\right)^6$$

$$\bar{\nu}_e = 2169.81801 \quad \text{cm}^{-1}$$

Spectral Properties of Fluctuating Dipole Moments

- The fluctuating charge model gives information about the dipole moment of the CO molecule every fs.
- As shown in McQuerrie et al. the lineshape is given by

$$I(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-\omega t} \langle \varepsilon \cdot M(0) \varepsilon \cdot M(t) \rangle dt$$

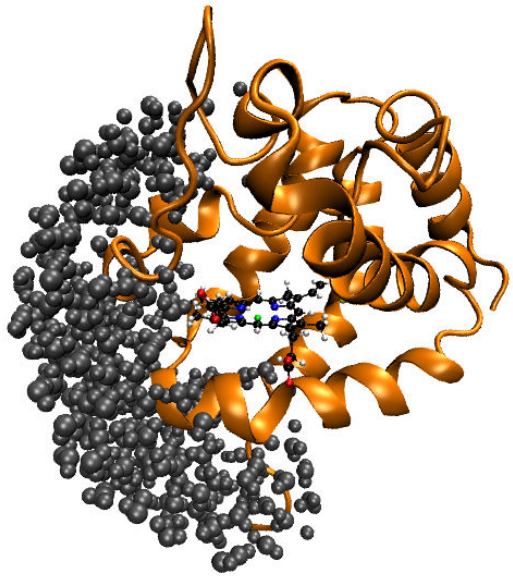
- By smoothing the discrete time series with a Blackmann-Harris Filter and applying a discrete Fourier transformation the spectrum can be obtained

Langevin Dynamics

- Equation of motion: $m_i \ddot{r}_i = -\nabla_i U(r) - \beta_i \dot{r}_i + \sigma_i \xi(t)$
- Friction coefficients applied to all heavy atoms
- Conservation of Energy because of the fluctuation dissipation theorem $\sigma_i^2 = 2k_B T \beta_i$
- Advantages:
  - Less atoms within the system
  - And free motion within the reaction center
- Disadvantages:
  - Additional frictional forces in the boundary area
  - Not the whole protein is dissolved and free to move

Methods

- Protocoll:
  - Crystall Structure
  - Adding missing Atoms
  - Solvation in a 25 A TIP3P water sphere
- Friction coefficients on all heavy atoms within the shell
- Boundary Potential for constraining the system to a sphere
- Reaction center with original Newton's Equation of Motions
- 3x Solvatisation and equilibration of water sphere



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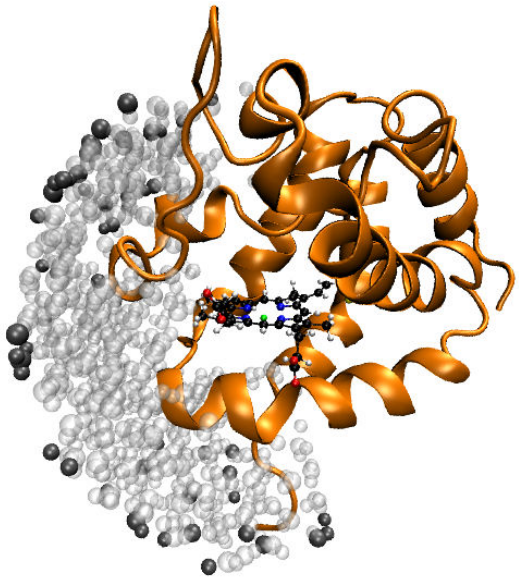

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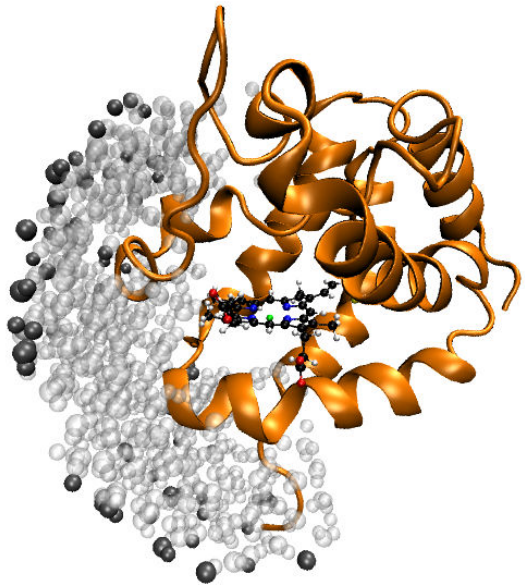

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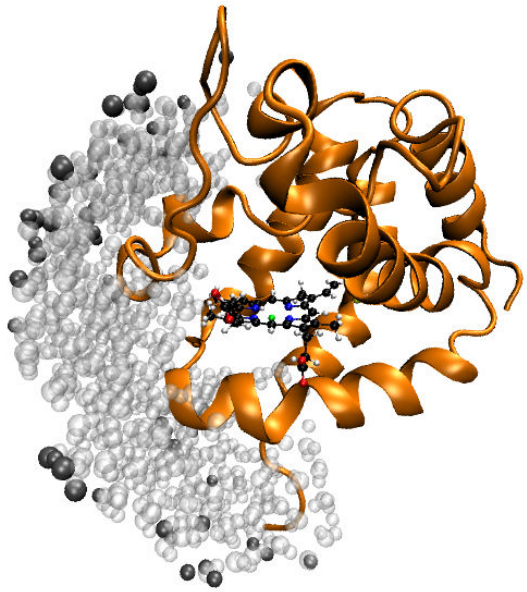

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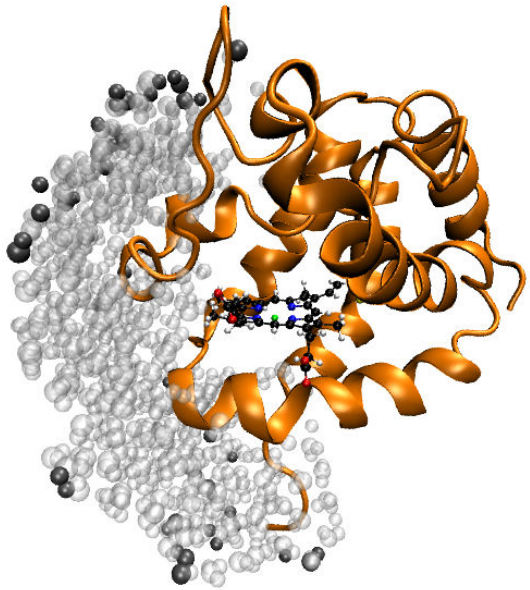

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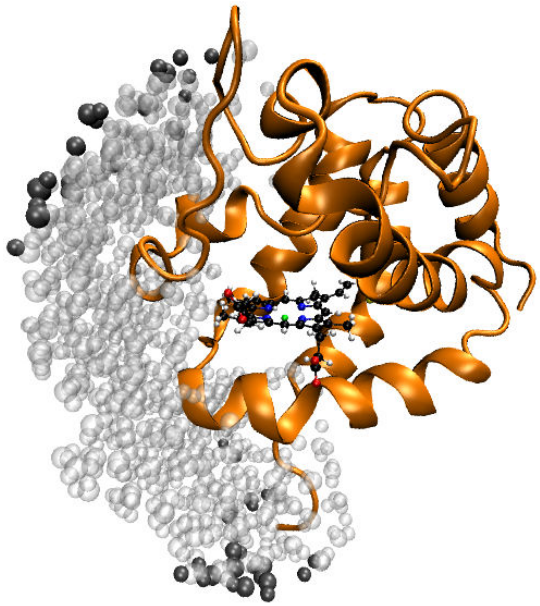

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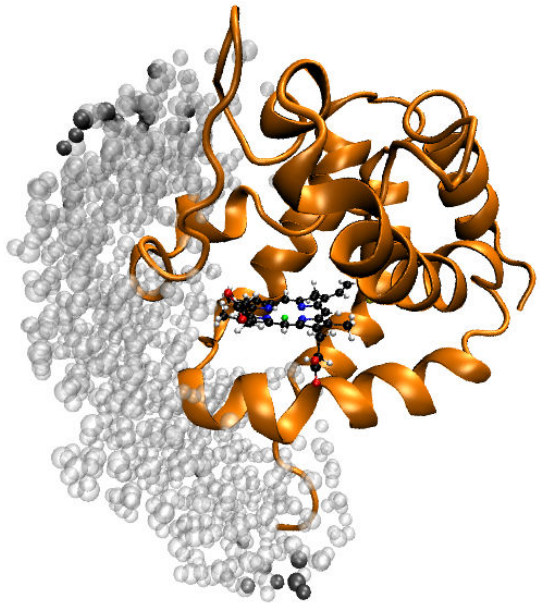
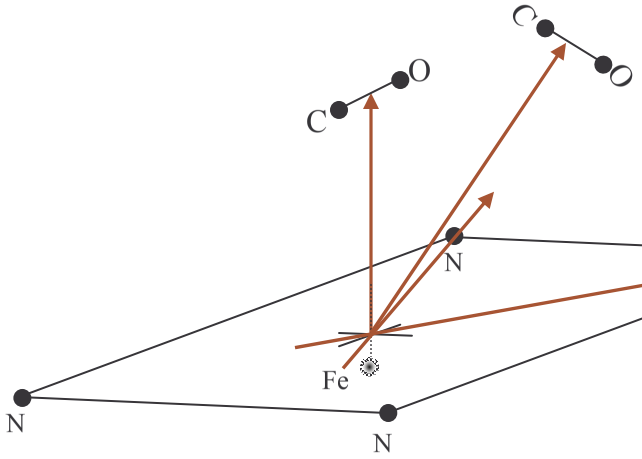
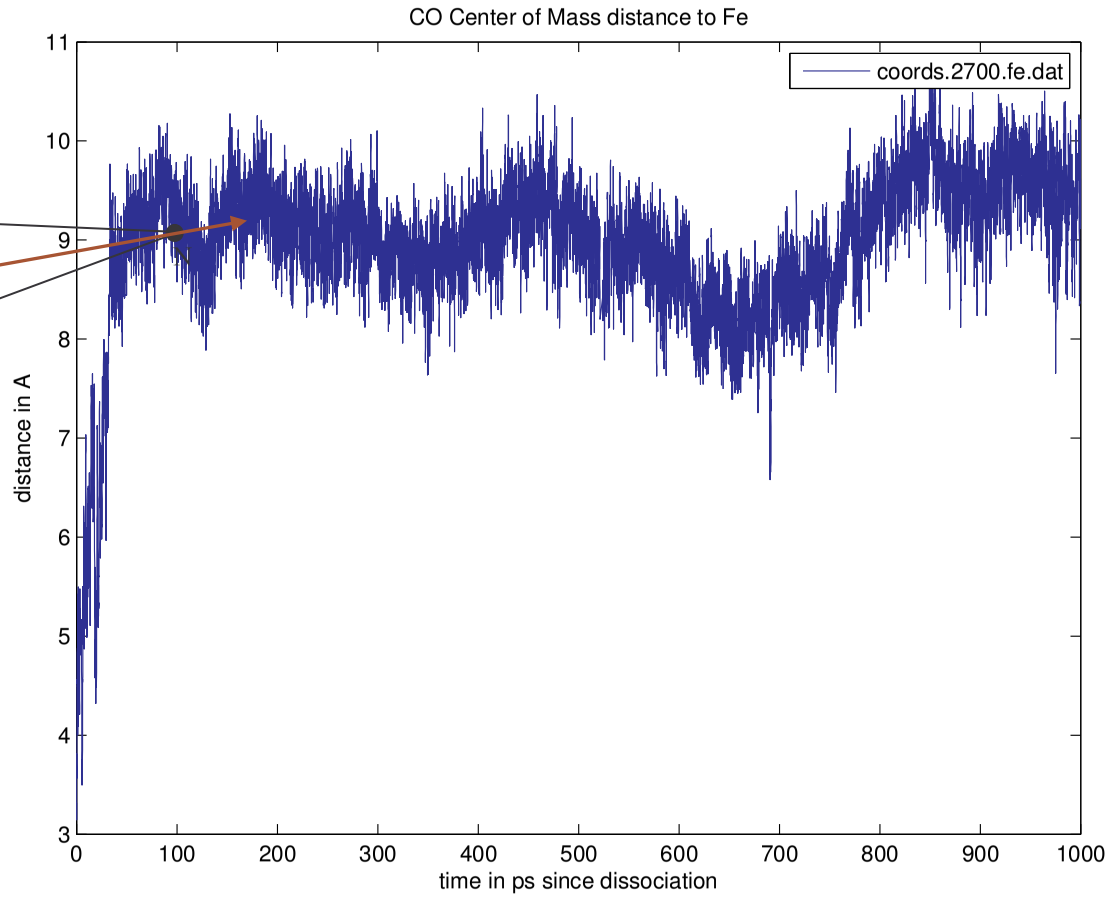
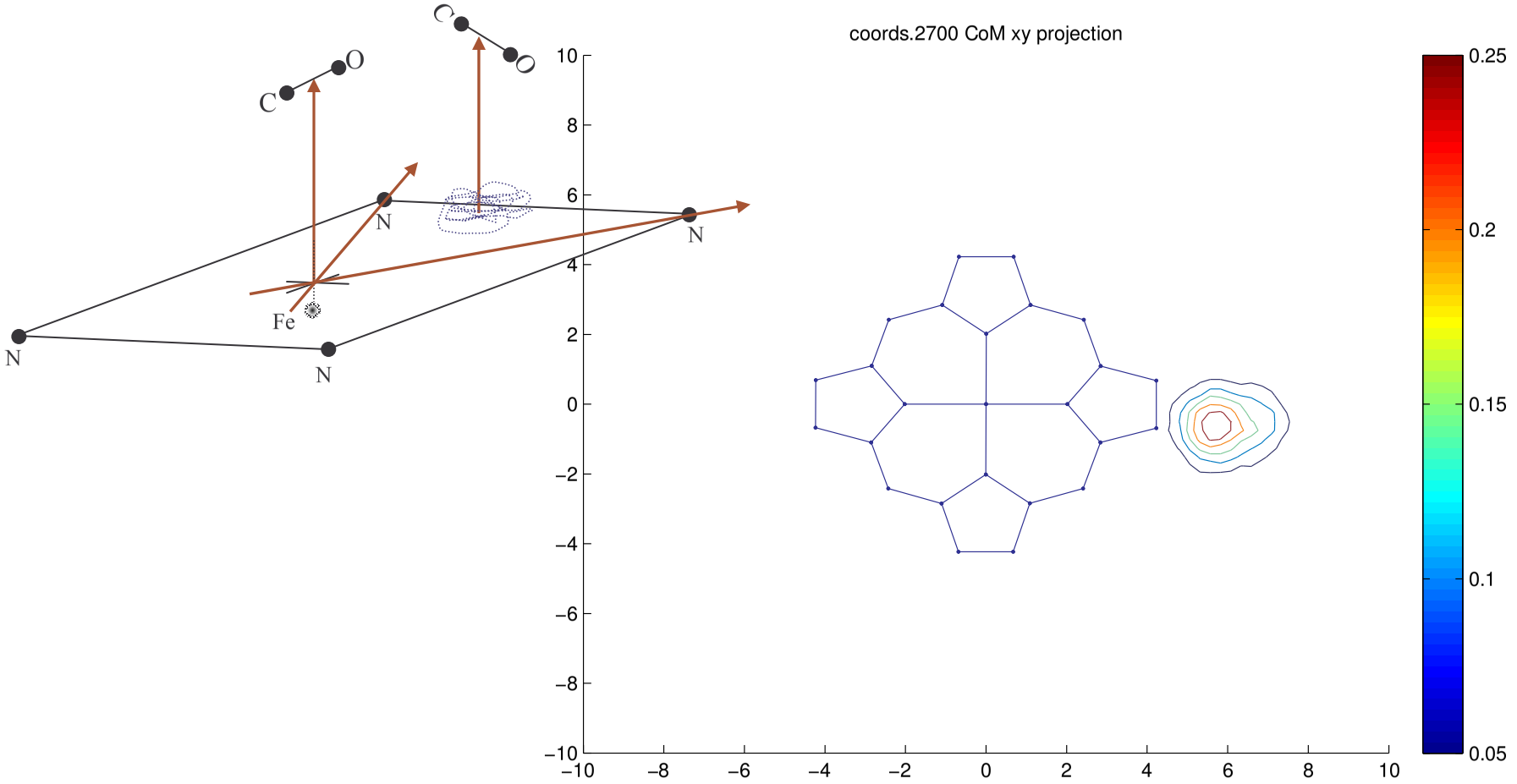


Time Series

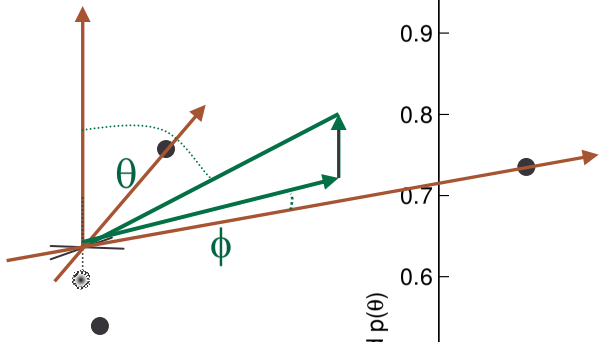

C
O
C
O
N
Fe
N
N

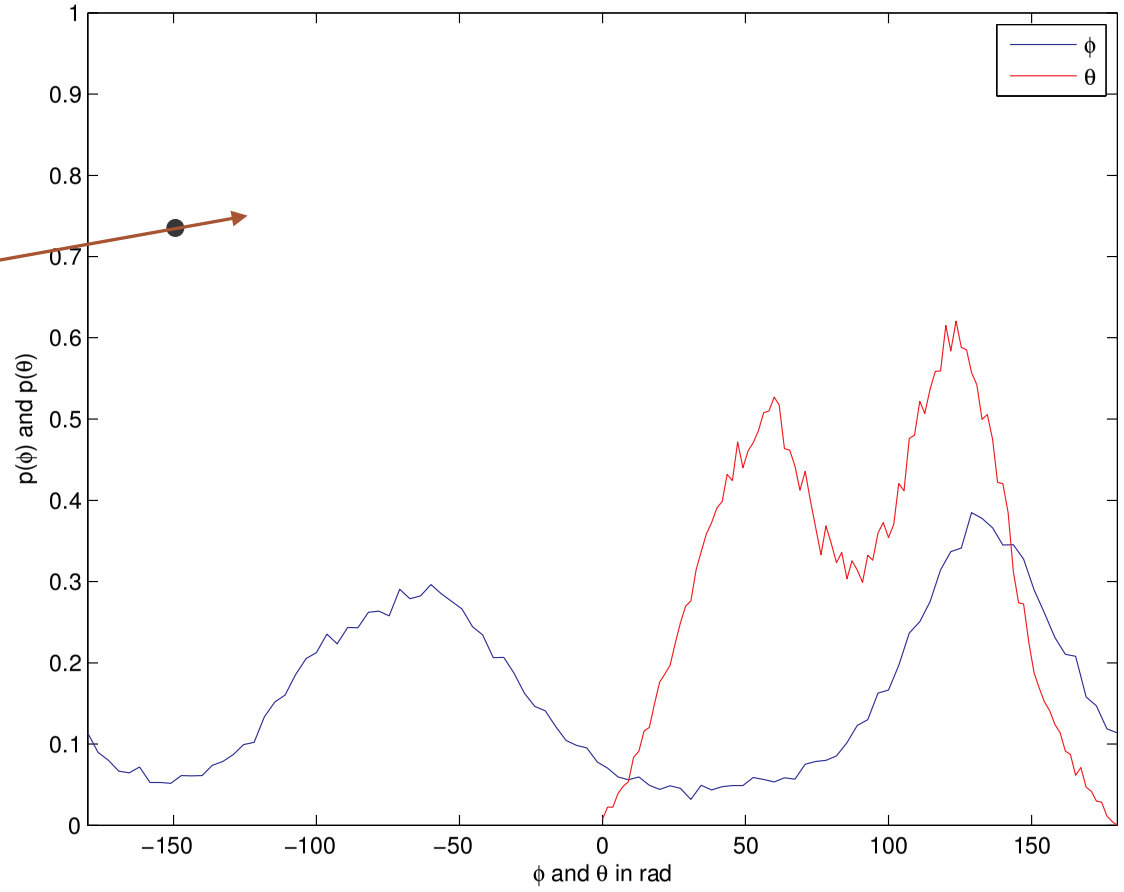

CO Center of Mass distance to Fe
coords.2700.fe.dat
distance in A
time in ps since dissociation
11
10
9
8
7
6
5
4
3
0
100
200
300
400
500
600
700
800
900
1000

Spatial Distribution

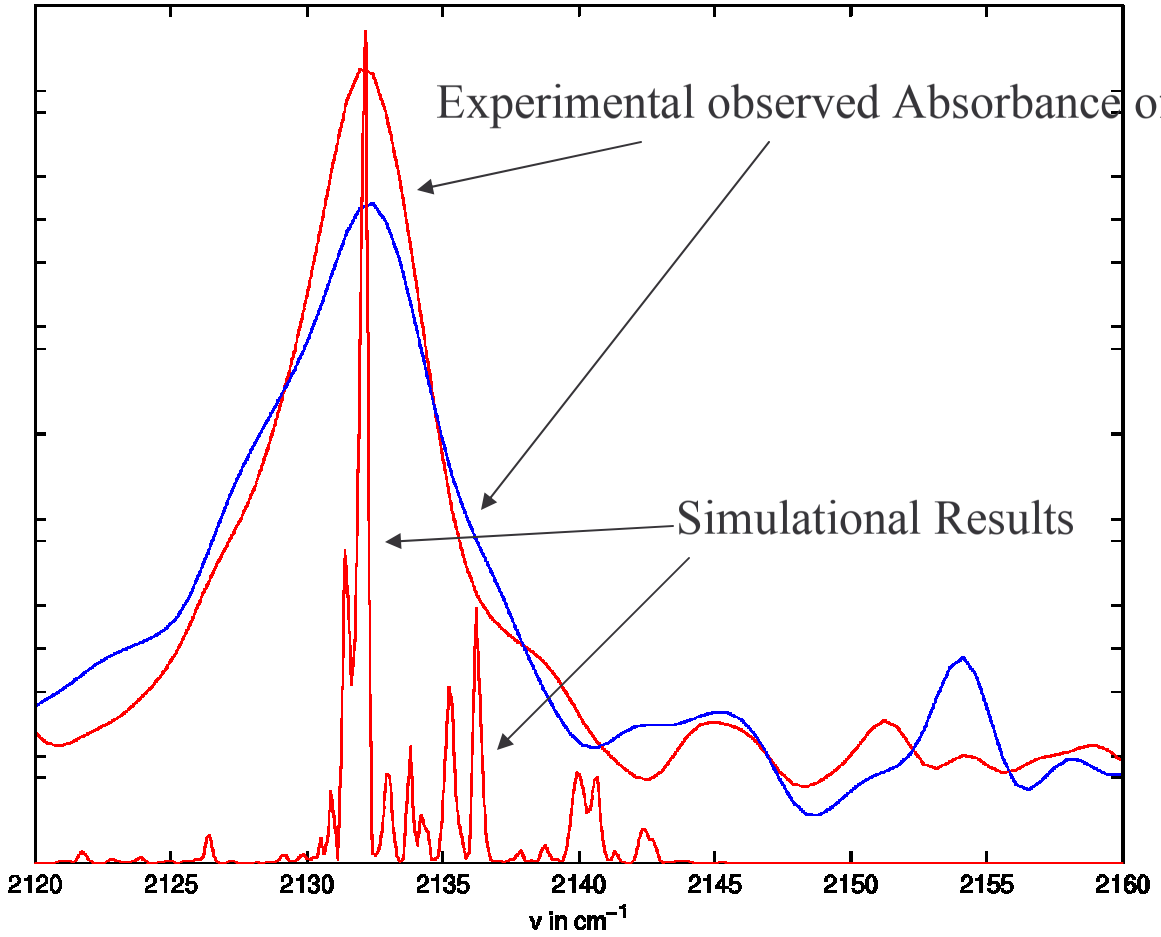


Orientational Distribution





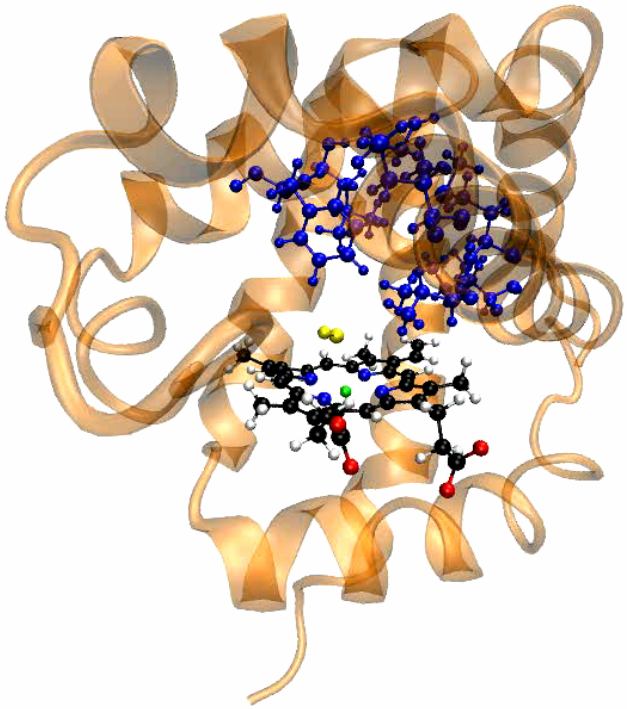
Spectrum of this particular trace



Thanks to Karin Nienhaus for the experimental data

So where is CO in this trace

- Surrounding Amino Acids:
  - GLY 24, LEU 27, PHE 28, ILE 65, MET 69, ILE 72
  - In Myoglobin this is the Xe 4Pocket
- With this evidence peak C is proposed to be the Xe 4 Pocket of Neuroglobin



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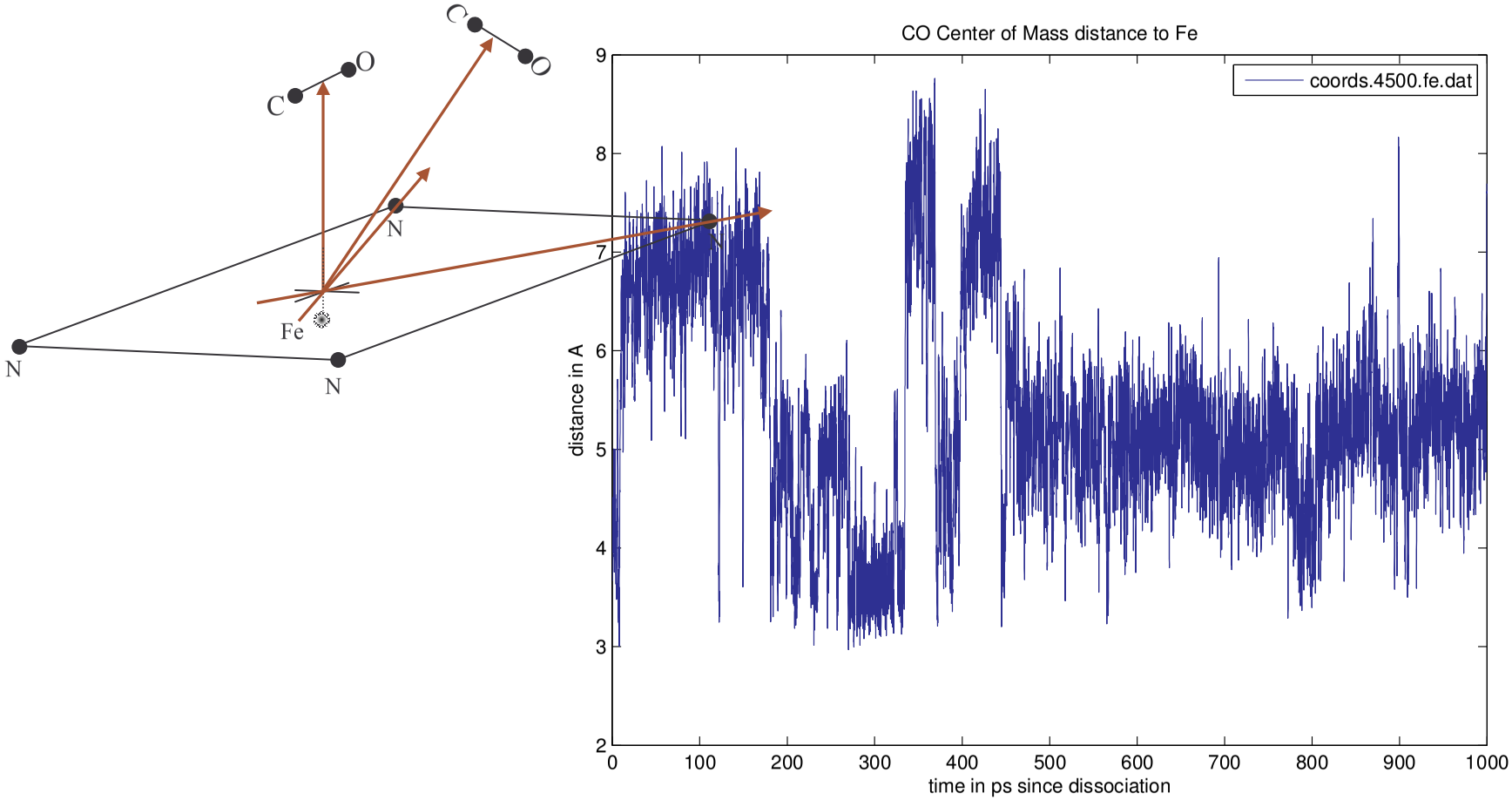
C
O
C
O
N
Fe
N
N
CO Center of Mass distance to Fe
coords.4500.fe.dat
distance in A
time in ps since dissociation
2
3
4
5
6
7
8
9
0
100
200
300
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500
600
700
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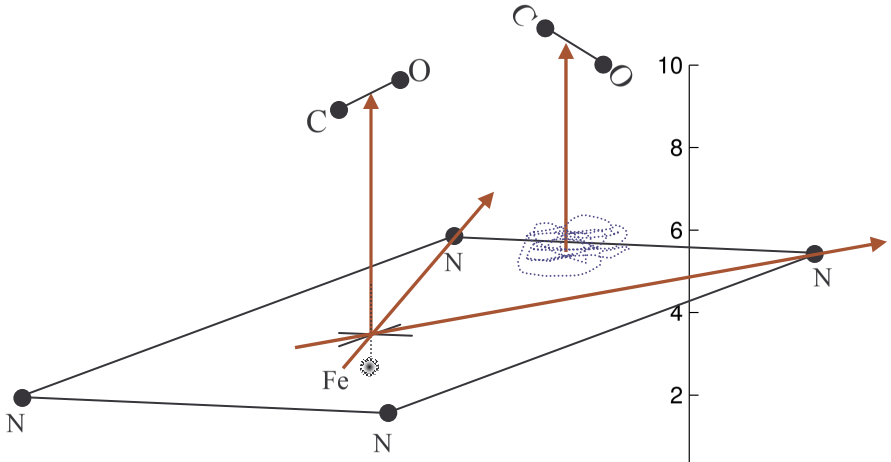
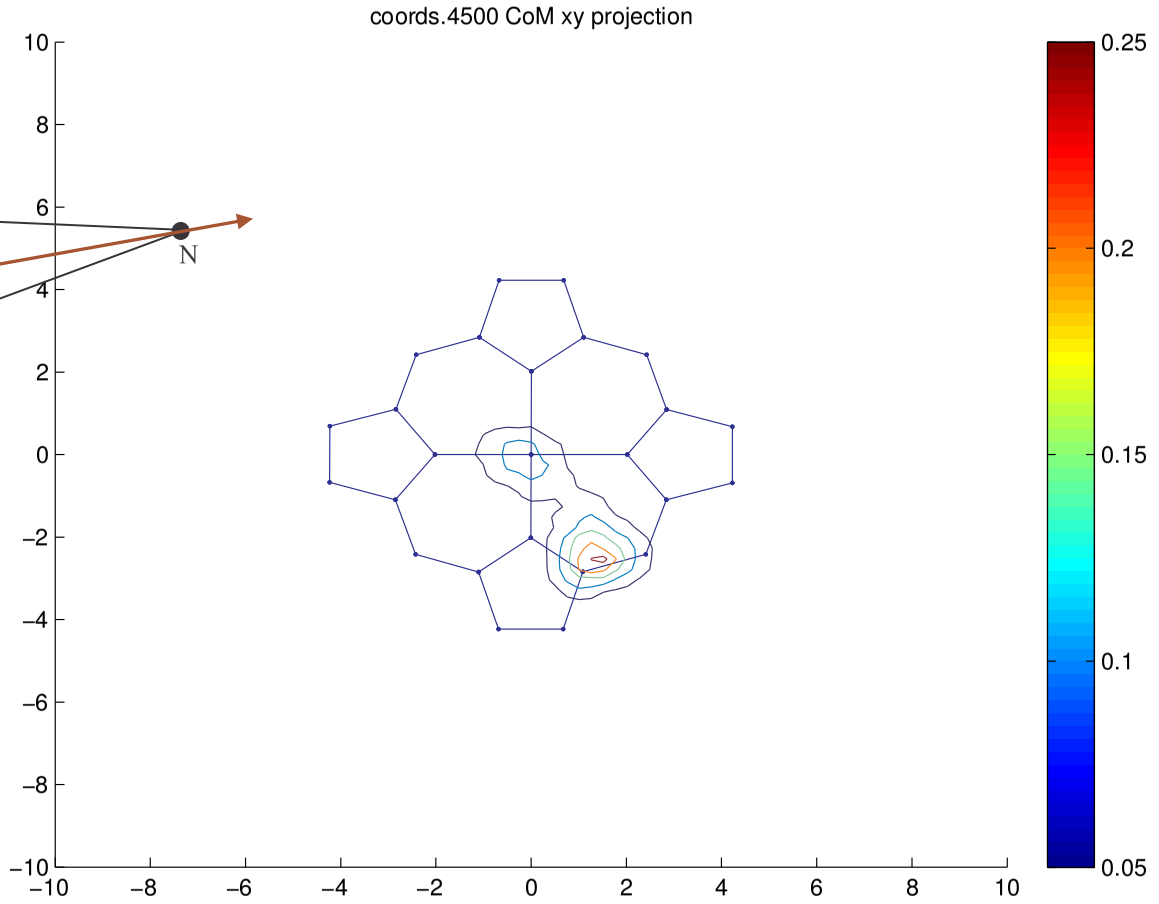
900
1000

Spatial Distribution





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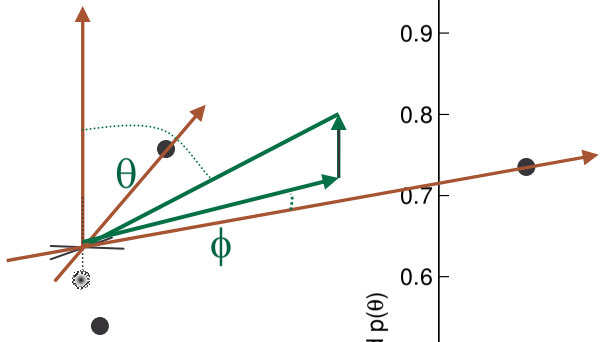
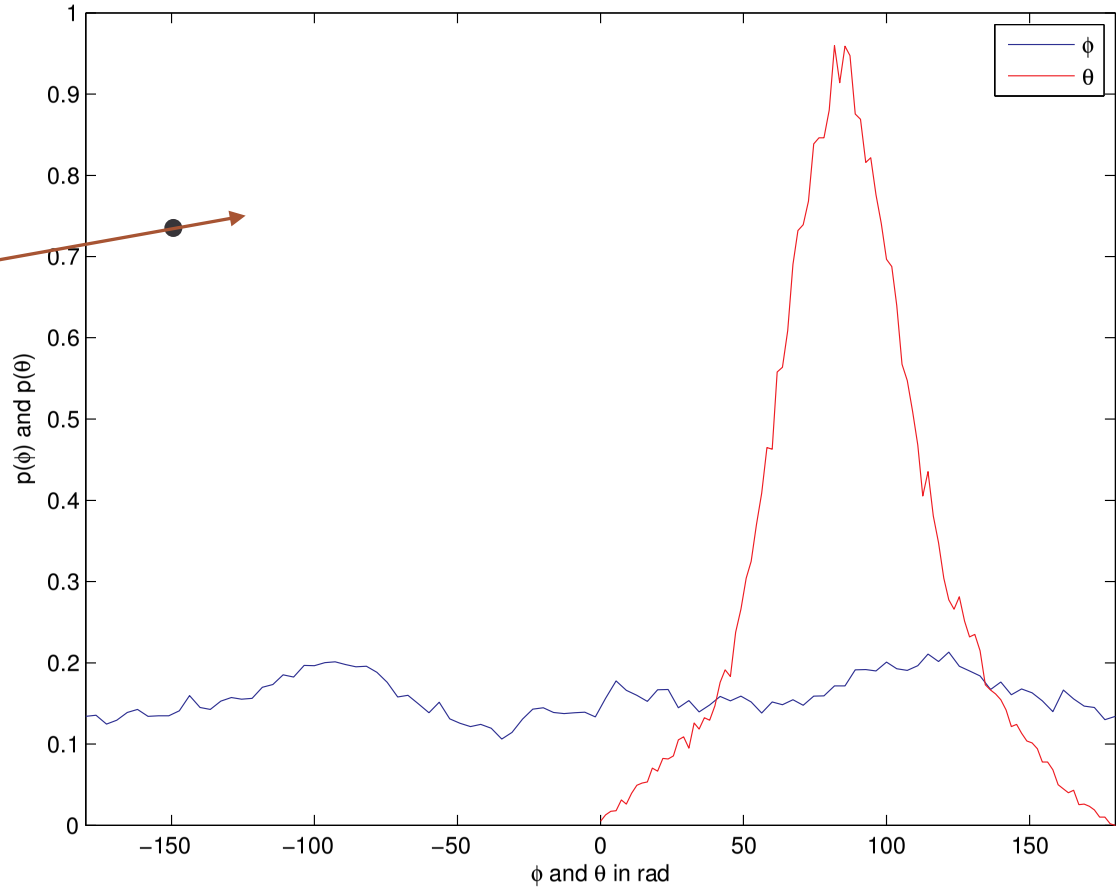
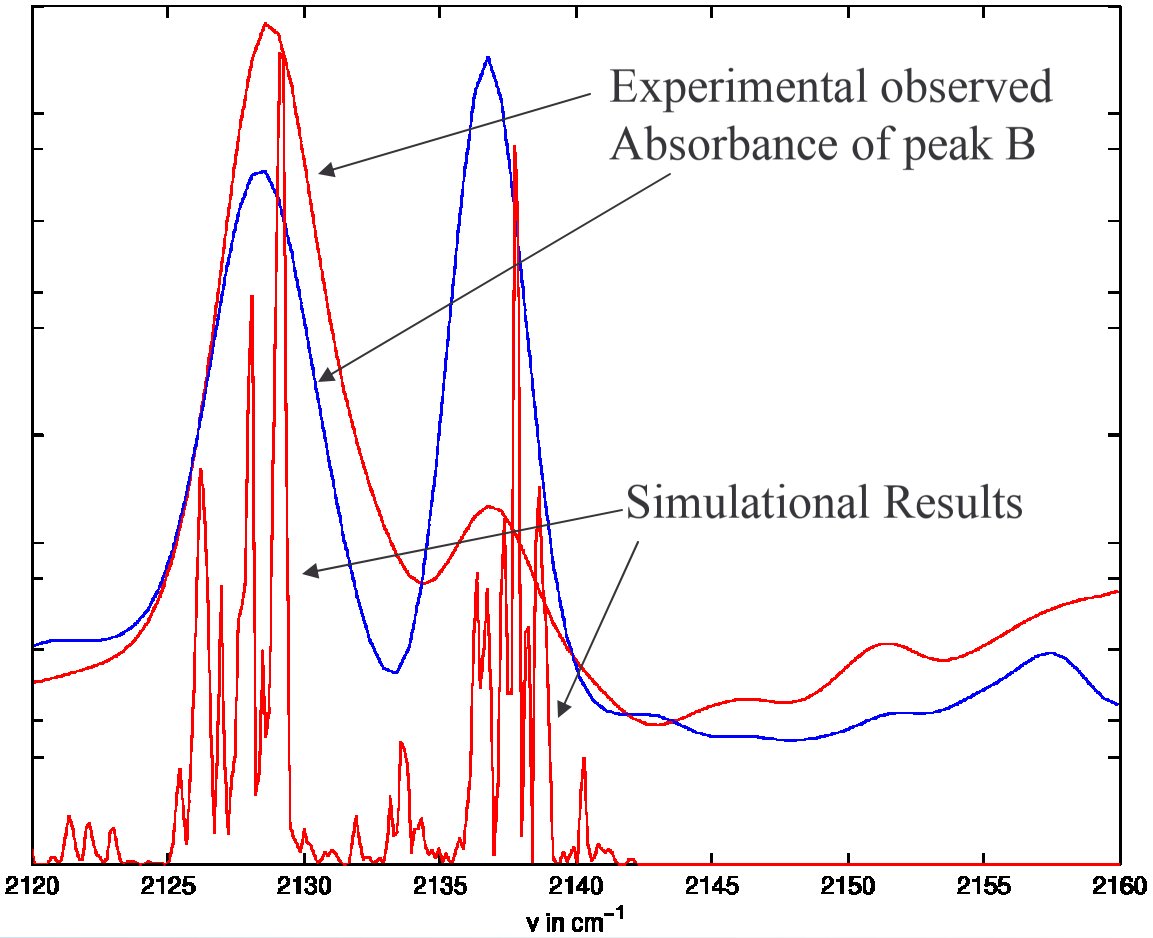


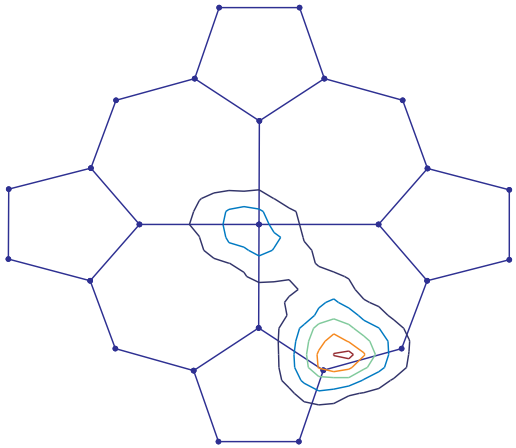


B-Site? And something else?



Again Localization within the Protein

- In the central orientation above the heme iron, CO is surrounded by PHE 28, PHE 42, PHE 61, His 64, ILE 65 which defines a hut where the CO pops into after dissociation.
- There is an opening below PHE 28 which leads the CO into the other pocket
- defined by PHE 28, LEU 31, PHE 32, VAL 109



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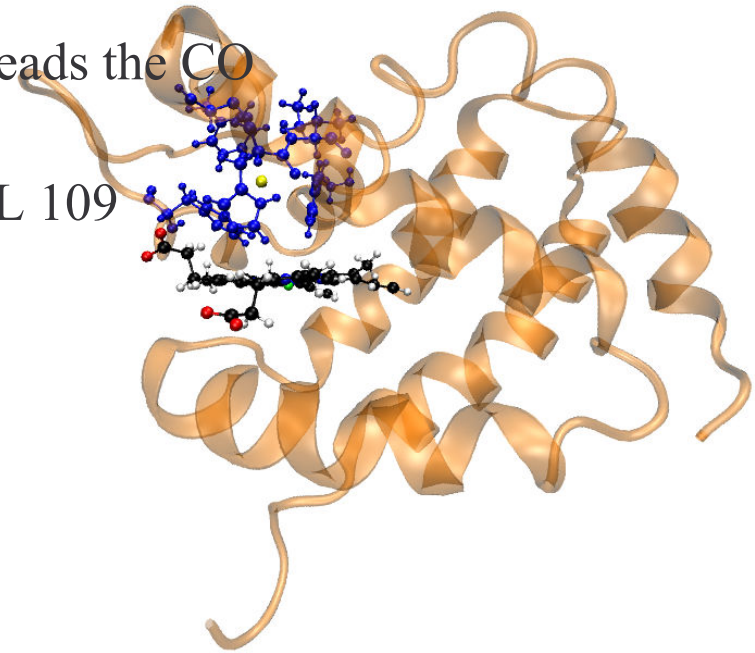

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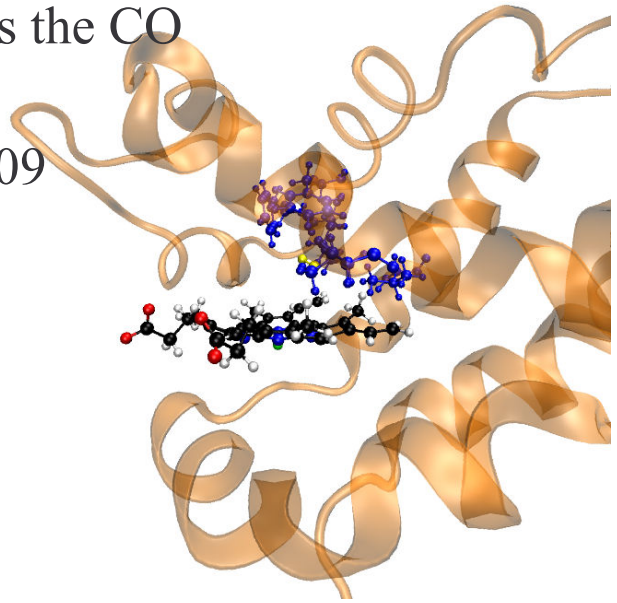
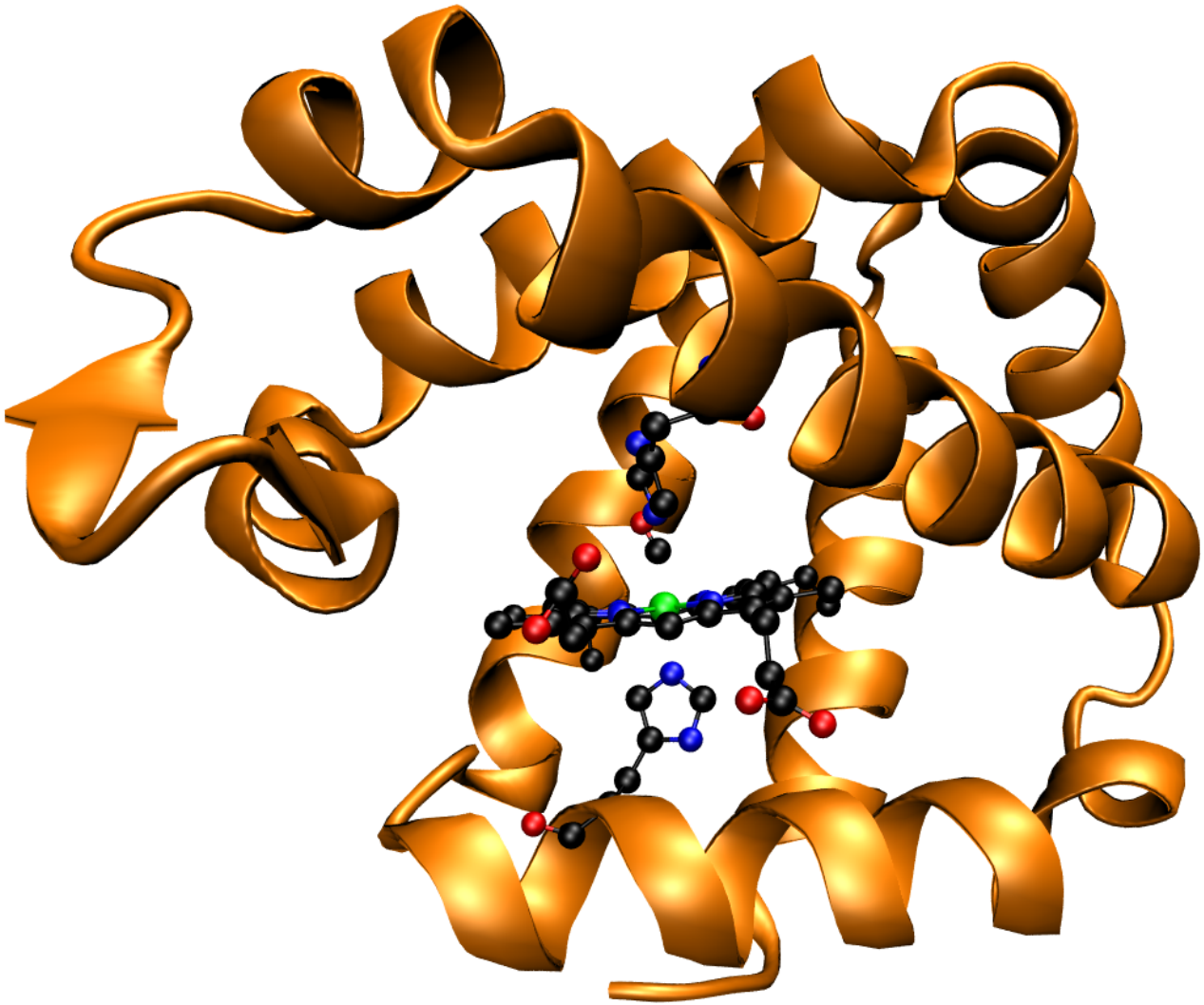


Summary

- Localized the spatial substates of CO within the protein
- Reproduced the measured CO spectra
- Found several other possible regions for further investigations in the not shown data



Thank you for your attention!

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