Dynamics from Nuclear Resonance Inelastic Scattering

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Purdue University

Workshop on Emerging Areas of Biological Crystallography - July 28, 2004

Advanced Photon Source

Outline

1. General comments on dynamics
2. Physics behind NRVS
3. Do proteins really have normal modes?
4. Comparative heme “rigidity”
5. Fixing a molecular dynamics problem

Biology **is** Motion:

•Jack Johnson - time scale for capsid transformation

•Wah Chiu - local motion of scruin vs actin

•Tom Steitz - finding “where all the atoms are” after reaction

•Zbig Dauter - time-dependent damage…

•Richard Kahn - denaturation at high pressure…

•Bob Von Dreele - gradual phase transition after grinding…

•Elspeth Garman - dynamics of free radicals…

•Max Nanao - time-dependent radiation damage…

•Vukica Srajer - everything in her talk!

•Lois Pollack - everything here too!

(Maybe Richard Matyi is still too much of a materials scientist….)

How did bio-crystallography get this far?

•high brightness sources

•tunability of x-rays (MAD, SAD)

• and detectors, computers, cryocooling….

How do we see dynamics now?

•Debye-Waller factors

•Time-resolved XRD (pump-probe…)

•Flow studies with small-angle XRD

•Before/after structures

•Spectroscopies - NRVS…

Possible emerging areas:

- Diffuse scattering
- High resolution inelastic x-ray scattering
- resonant versions
- X-rays with long time coherence?
- picosecond time resolution?
- X-rays plus microspectrophotometer
  THz, FIR, IR probes
  Raman, CD, etc….

A word about spectroscopy:

1. What we all want is a fast video camera with atomic resolution. We don't have one. But we do have more and more tools for giving us real-time structural information.
2. Molecular dynamics simulations seem like the next best thing, BUT details of input parameters are suspect.
3. Instead of a real-time picture, take the Fourier transform into the frequency picture. Now normal modes matter, and these can sometimes be measured. Models can then be refined against real data.

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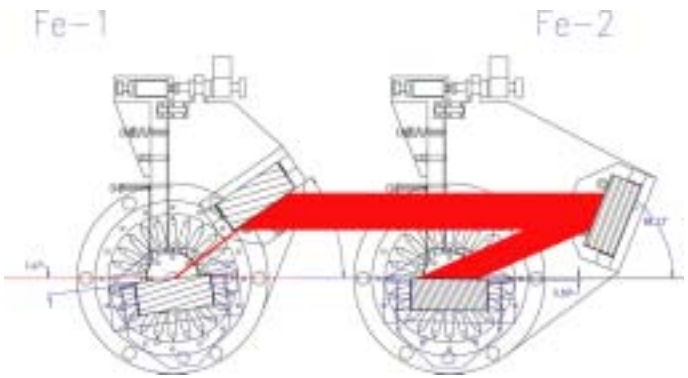
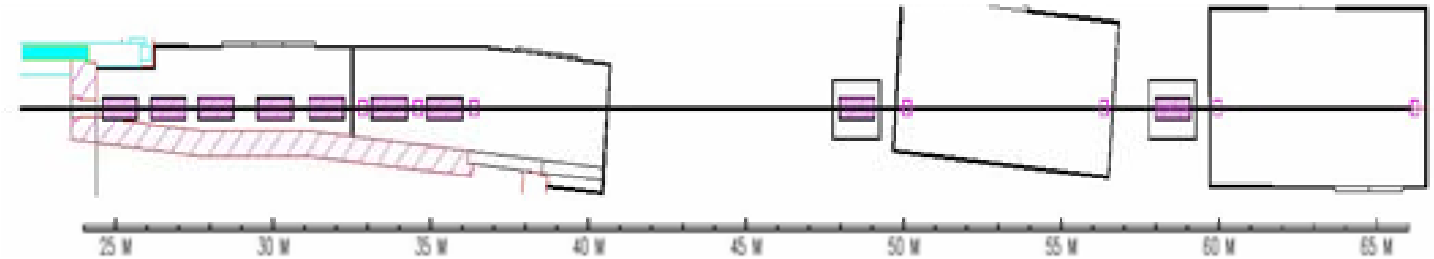
Argonne National Laboratory

High resolution monochromator

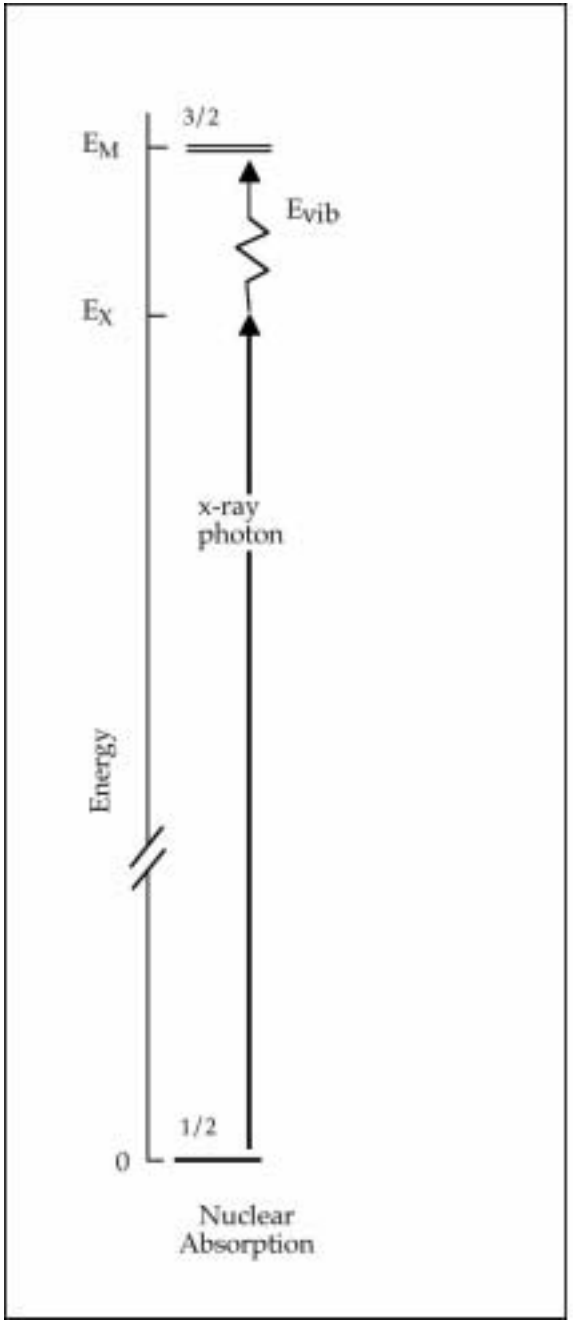
1 meV (Thomas Toellner et al.)

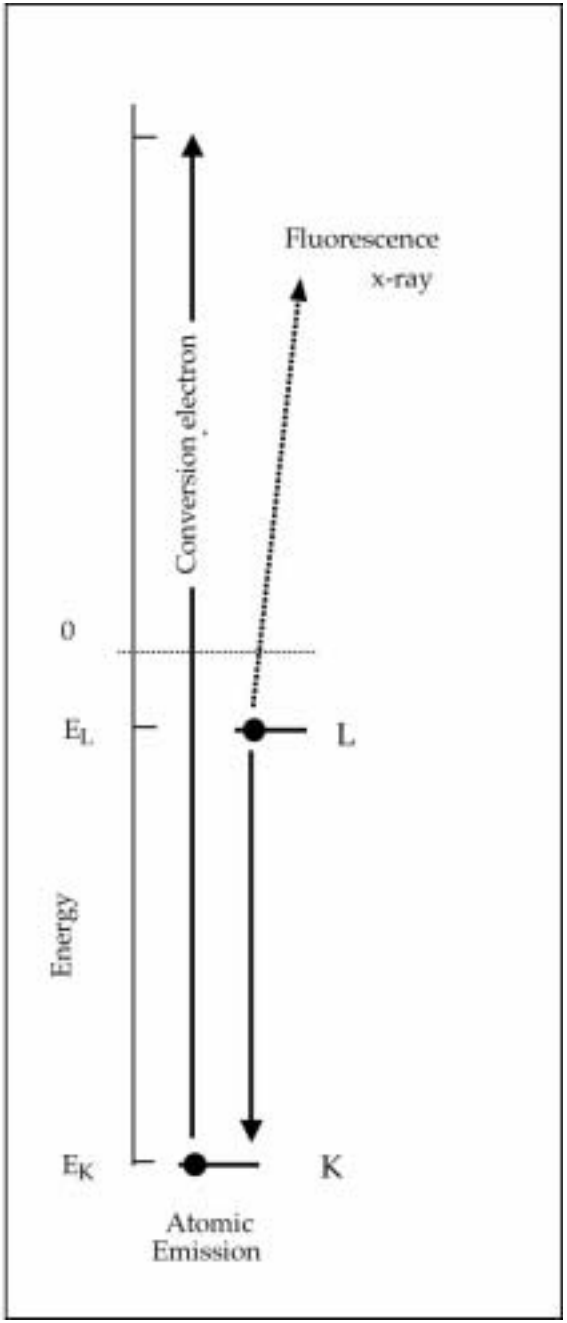


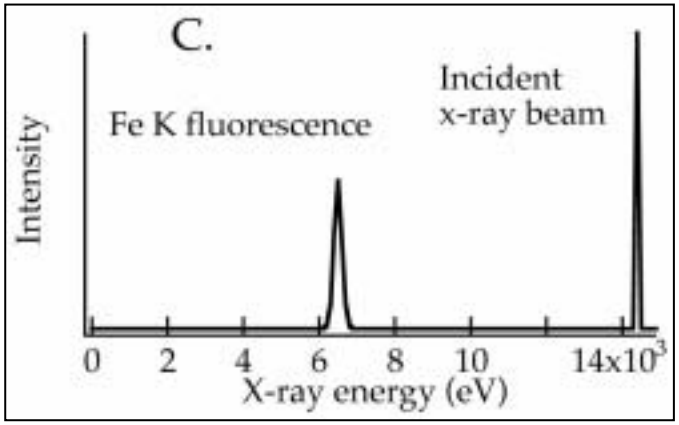


Sector 3 Undulator beam line: 1 mm beam height, 65 meters from source.

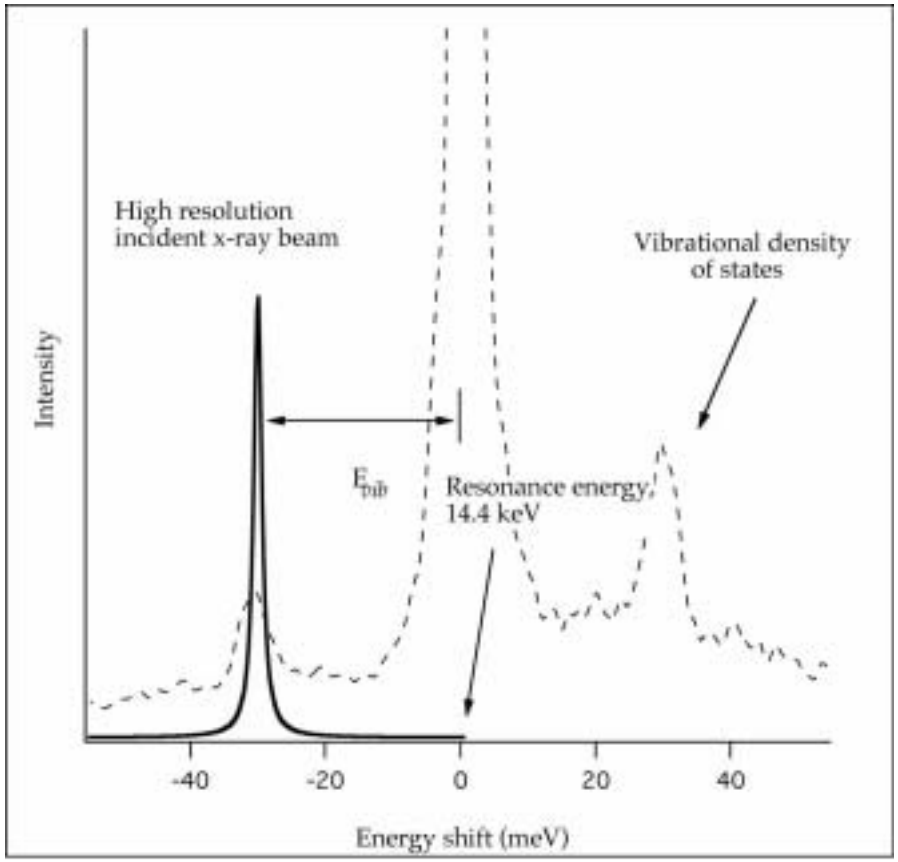
Nuclear resonance beam lines are also at Spring8 (Japan) & ESRF (Grenoble).



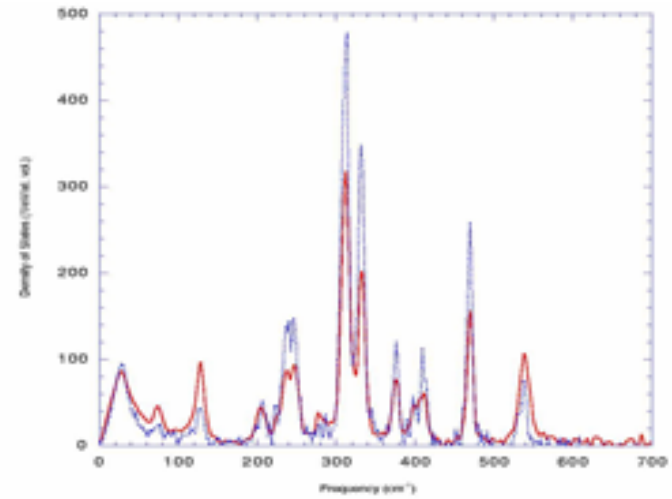


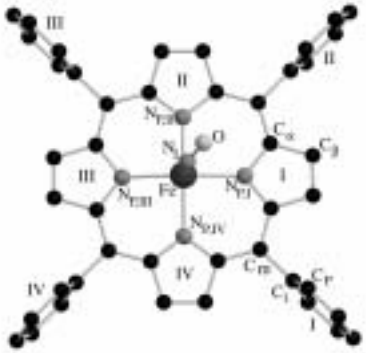


1. Incident x-ray combines with quantum of vibrational energy to produce an excited nucleus.
2. The nuclear energy is converted into electronic energy, ultimately leading to fluorescence.



Fe vibrational density of states:





Fe NitrosylTetraphenylporphyrin

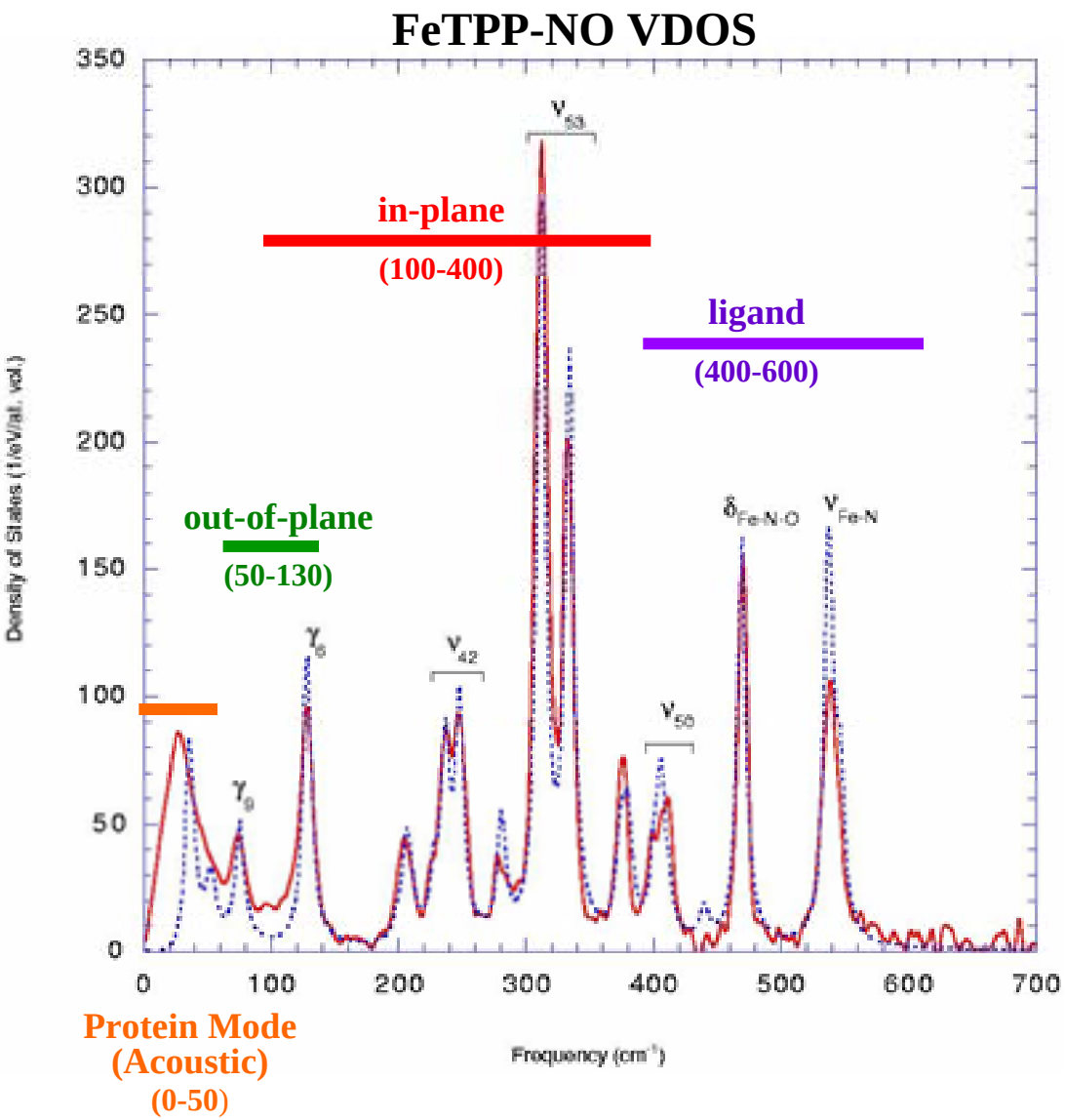
FeTPP-NO

6. Narrow incident beam is scanned in energy while absorption is monitored via fluorescence.

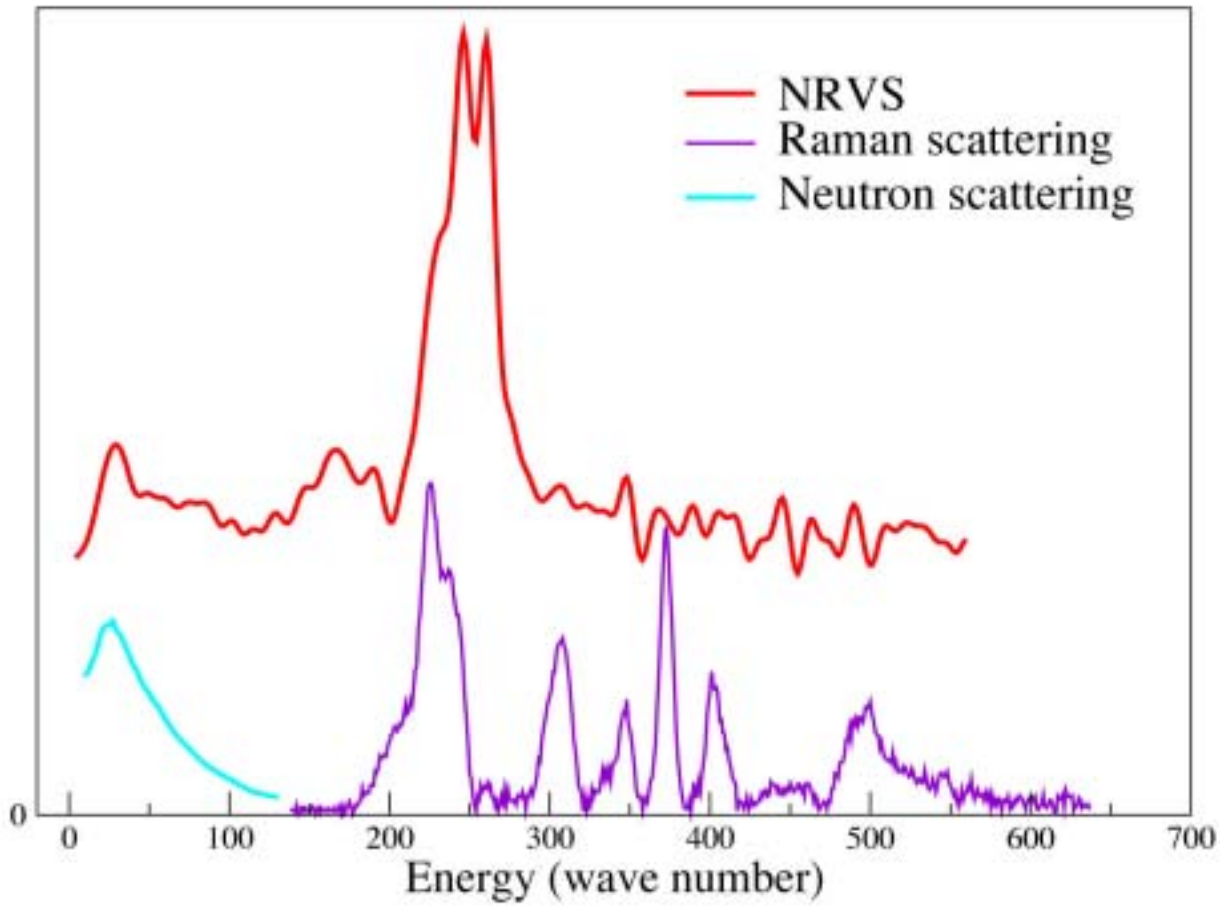
7. Observe Stokes-antiStokes vibrational spectra; determines sample temperature.

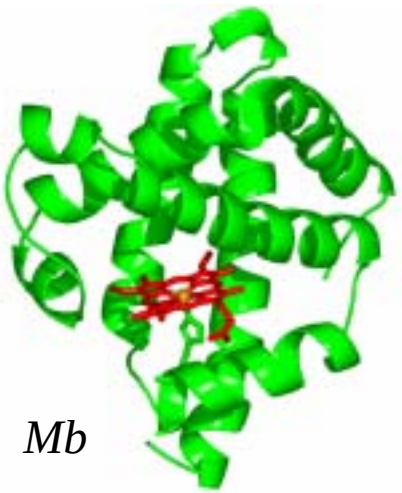
8. Lipkin sum rules and PHOENIX program converts data into quantitative VDOS. **Measures absolute magnitudes**.

Patterns seen in fits to porphyrin compounds



- sequence of acoustic, doming-like, in-plane, and ligand modes.
- core porphyrin modes, used for labeling purposes, are heavily mixed for ALL modes, and are limited utility.
- any change in structure tends to cause significant changes in spectra.
- Peripheral components have large impact on low frequency, out-of-plane modes.





1. Inelastic neutron scattering sees vibrations of the protein backbone (Cusack & Doster 1990).
2. Resonant Raman sees the entire heme molecule, not protein; difficult to determine Fe role in any of the peaks (isotopic substitutions needed).
3. NRVS sees protein peak, plus all Fe modes (no optical selection rules), quantitatively.

Do proteins have normal modes?

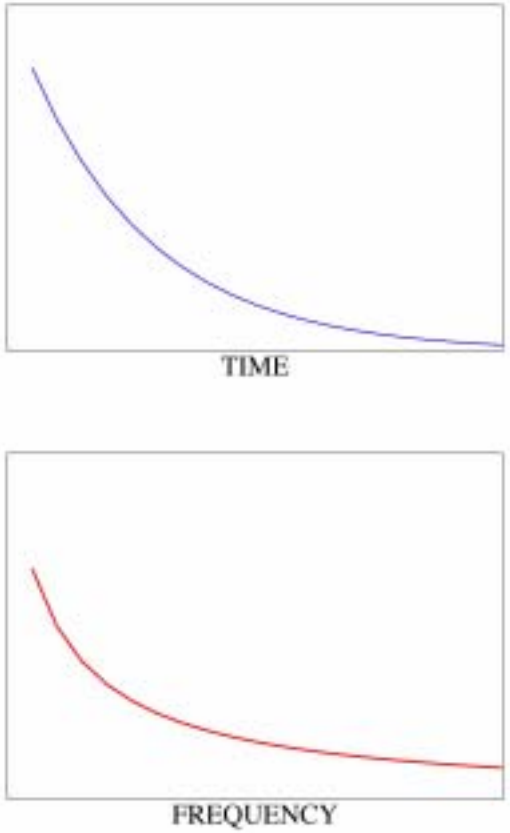
Do proteins have well-defined <u>normal modes</u> of the entire molecule?

Can they "ring" like a bell?

Mb

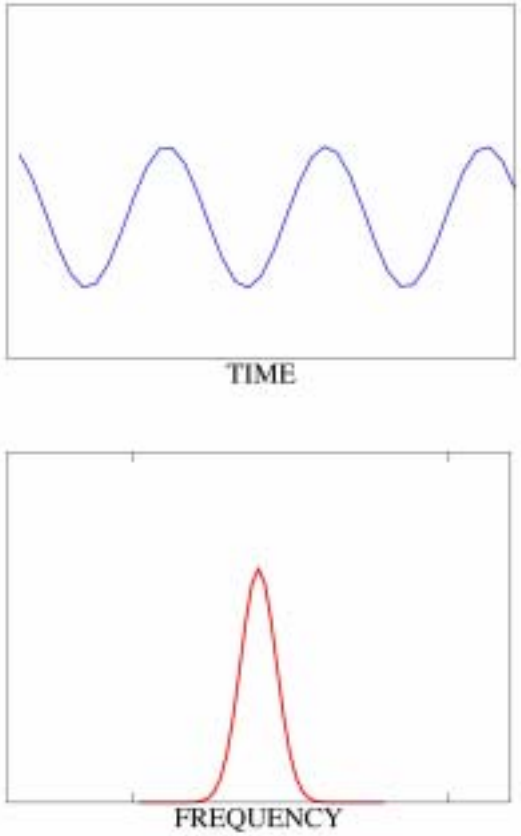
Or are they <u>dissipative</u> systems, like a high-viscosity liquid?

<u>Overdamped</u>, dissipative dynamics



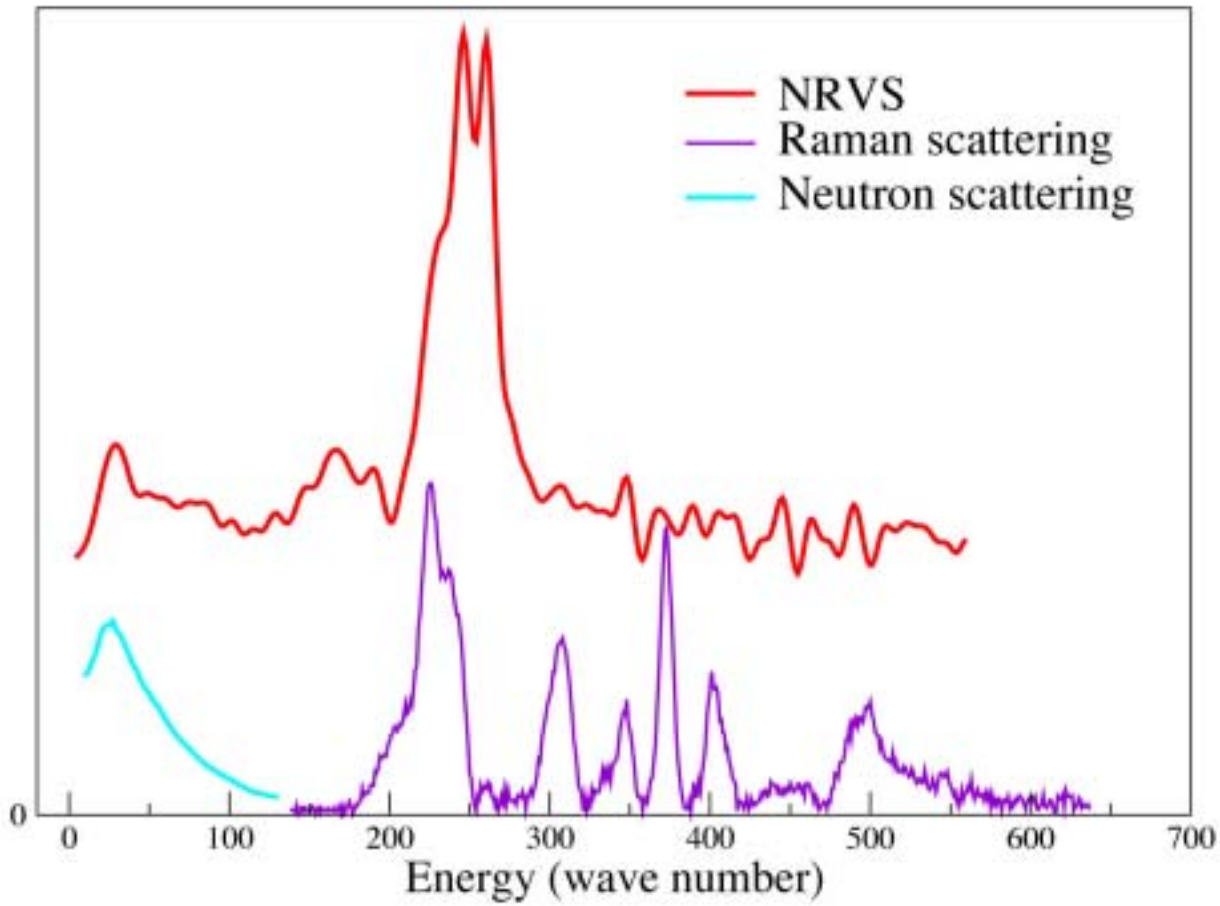
Steady decrease from peak at origin.

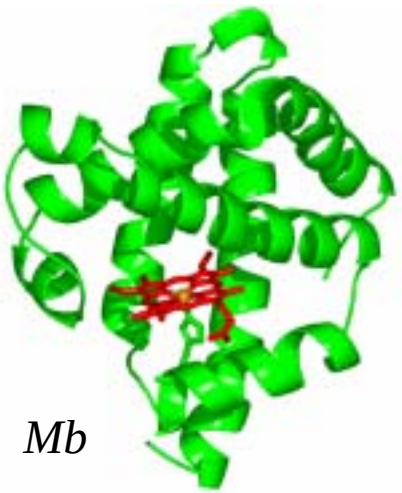
<u>Underdamped</u> normal mode dynamics:



Well-defined spectral peaks

An open question for some…





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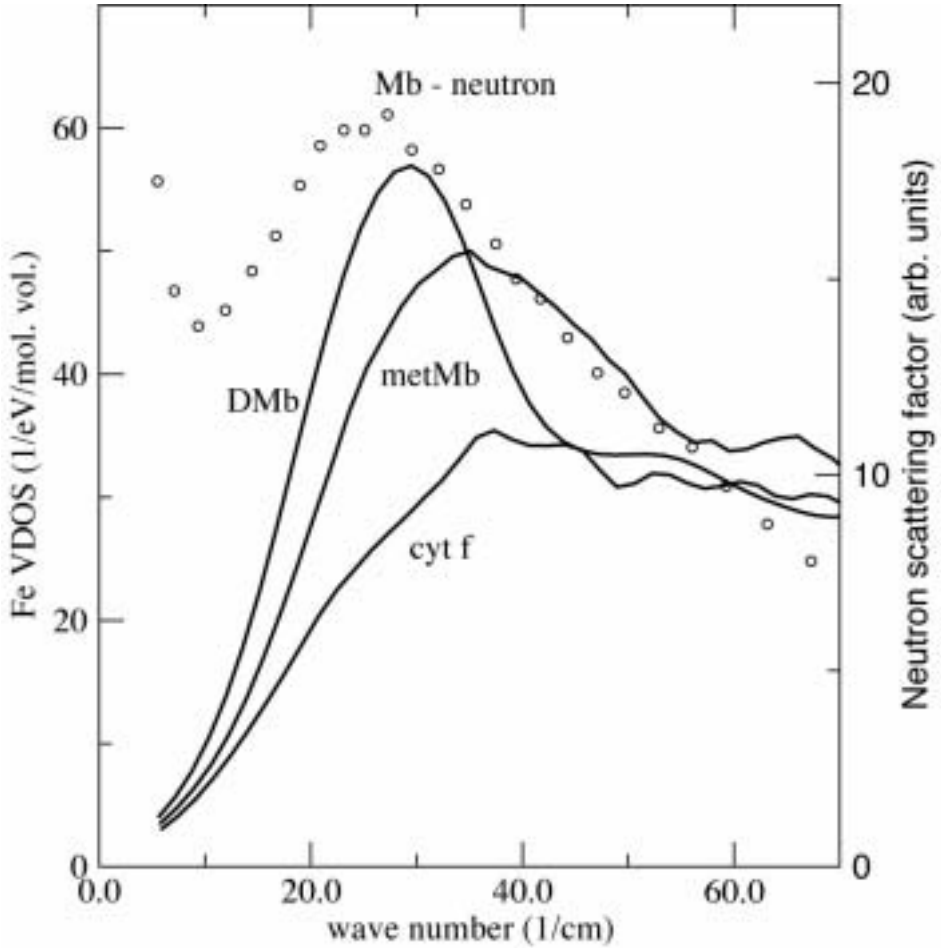
Protein Peak Comparison:

• cyt *f* has the smallest displacements at low, protein peak frequencies, despite the most direct coupling to protein vibrations (N-terminus)

• existence of protein peaks shows that protein normal modes are long-lived, underdamped, <u>not</u> dissipative.

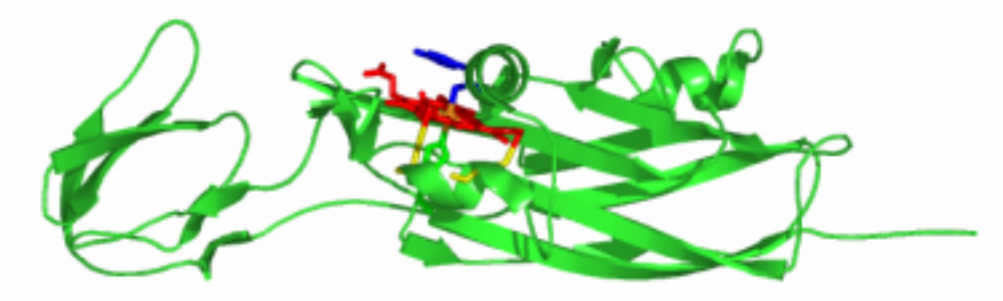


(Neutron - Cusack & Doster 1990)

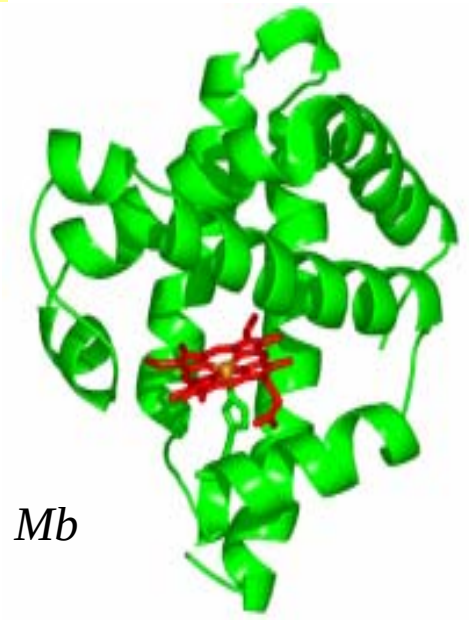
Comparative heme “rigidity”

Comparison of heme proteins -

cytochrome f *vs* myoglobin



Cytochrome f



Mb

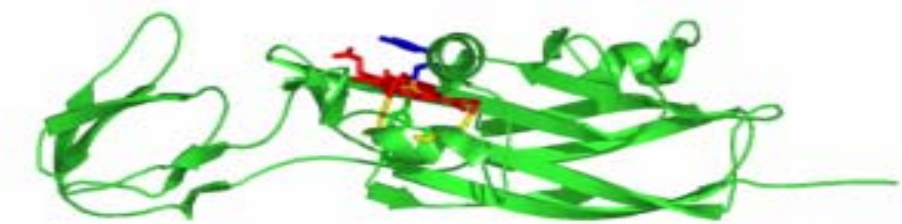
An electron-transfer membrane protein,

part of the cytochrome b_6f complex of oxygenic photosynthesis. Has unusual Fe linkage to N-terminus of protein.

Oxygen ligand-binding protein, found in muscle tissues, etc.

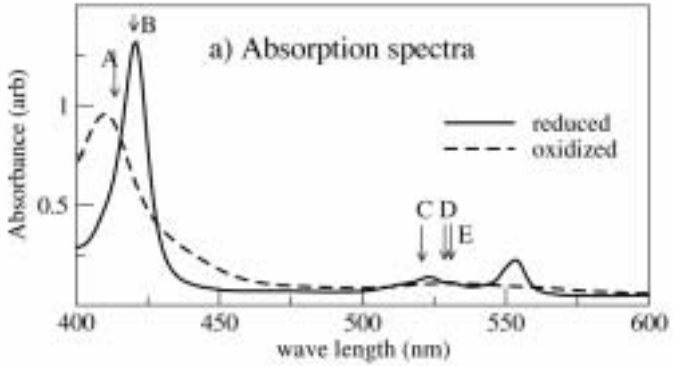
Resonant Raman on Cyt *f*:

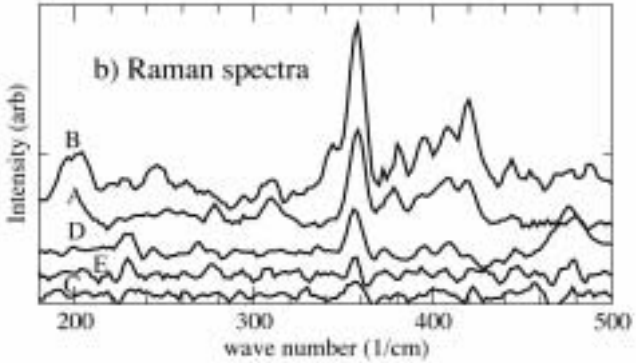
- wealth of detailed peaks found with 5 different wavelengths at Soret and Q absorption bands.
- peak intensities are <u>highly</u> variable, cannot be correlated with normal mode amplitudes.
- hard to determine role of Fe in any of the peaks.

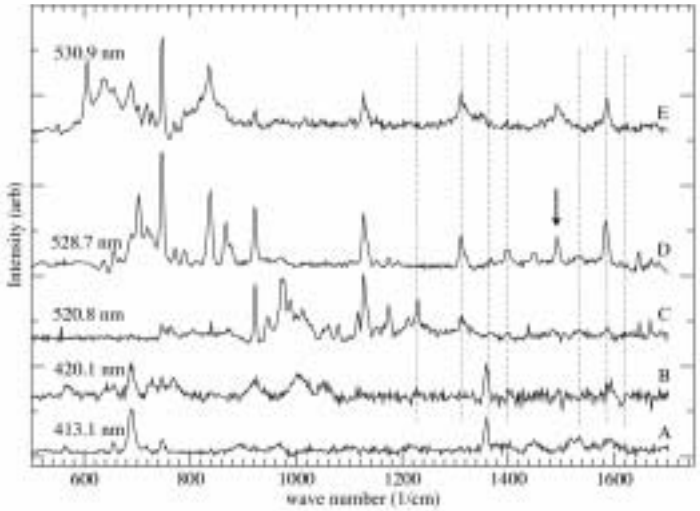


Cytochrome f

Data from S. Tsoi, A. K. Ramdas, et al.

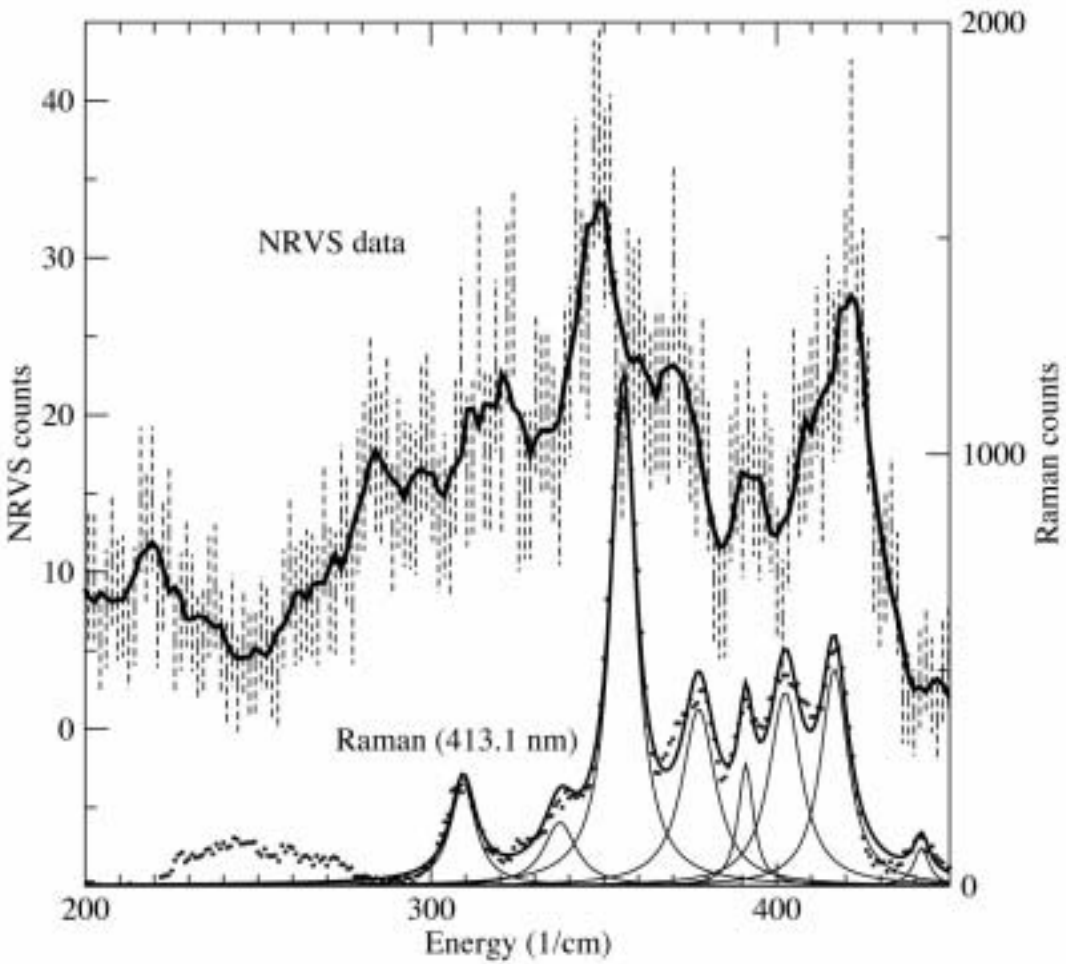




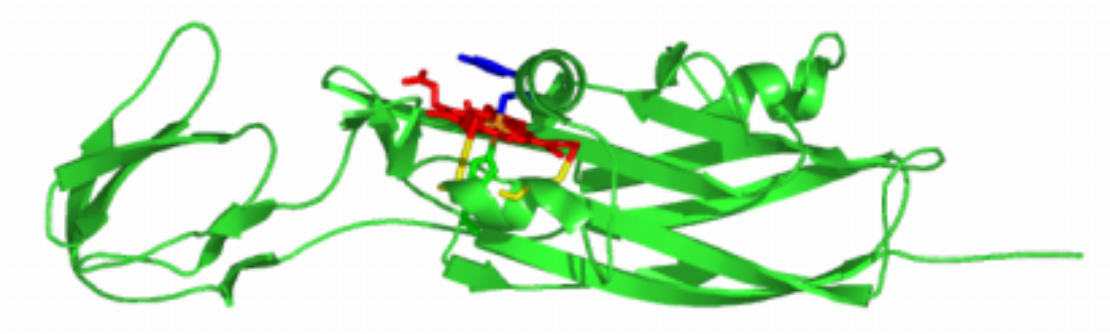


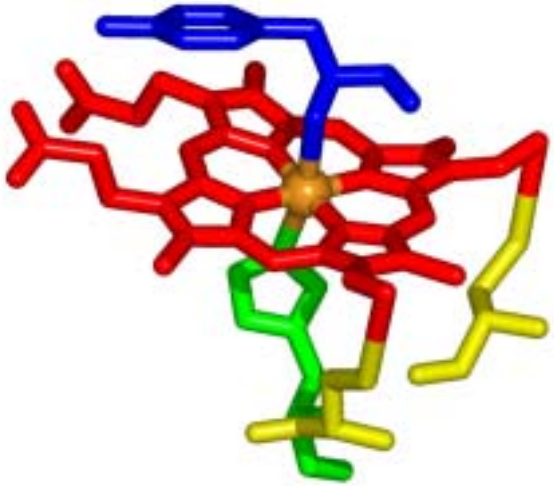
Compare with NRVS:

- All of the RR peaks below 500 cm^{-1} must have significant Fe motion.
- NRVS shows large DOS where RR does not; no optical selection rules.
- Largest RR peak was previously labeled ν_8, but that has no Fe motion by symmetry.

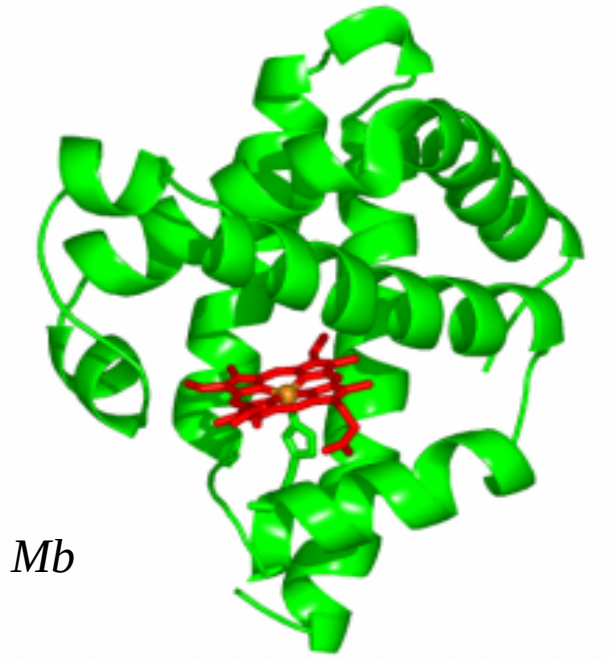


NRVS only sees ^{57}Fe atoms, and sees ALL modes, not just the Raman-allowed modes.





Cytochrome f



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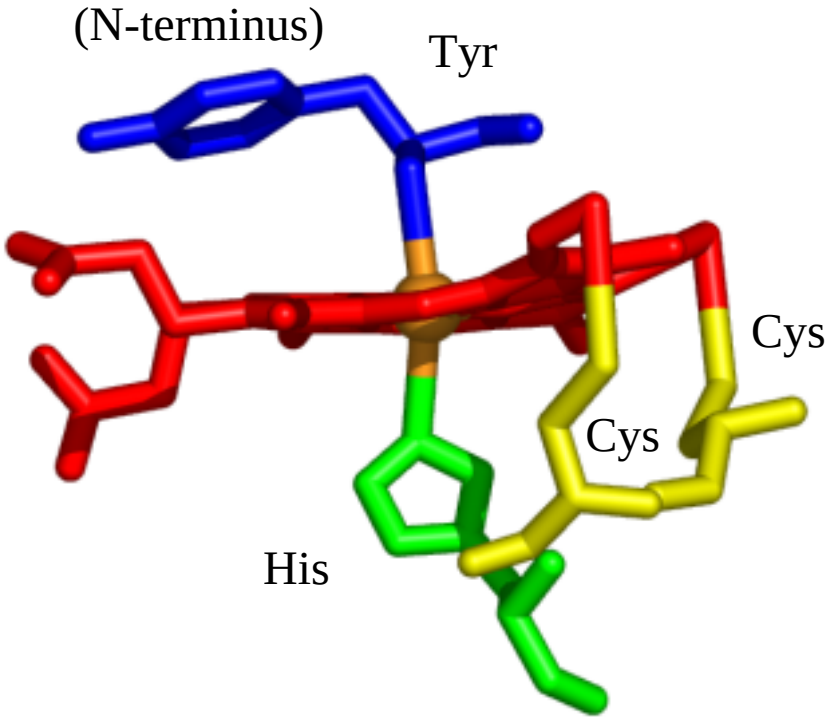
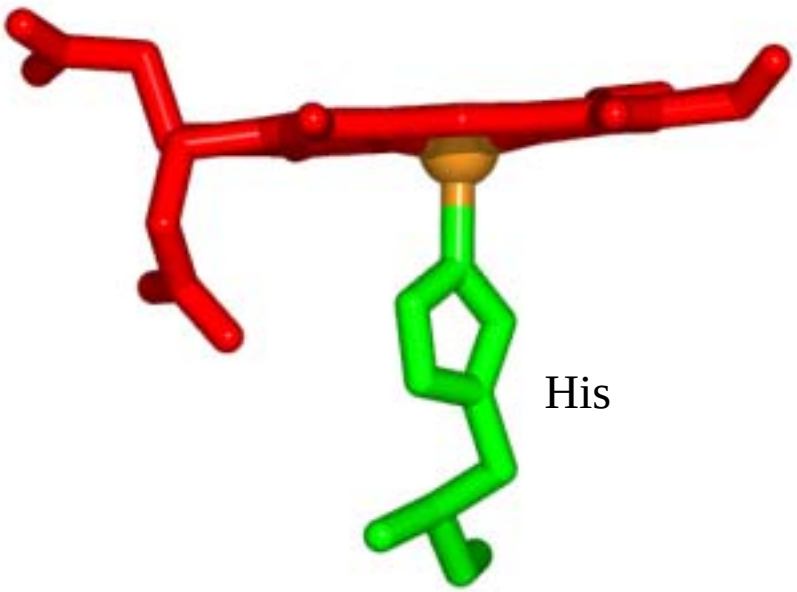
Cytochrome f



deoxy-Myoglobin



Four strong bonds to protein

Only one bond to protein

NRVS Results:

- DMb is softest
- metMb causes slight hardening (high to low spin, planar Fe site, smaller bond length)
- Cyt *f* shows significant hardening, despite nominal similarity with metMb

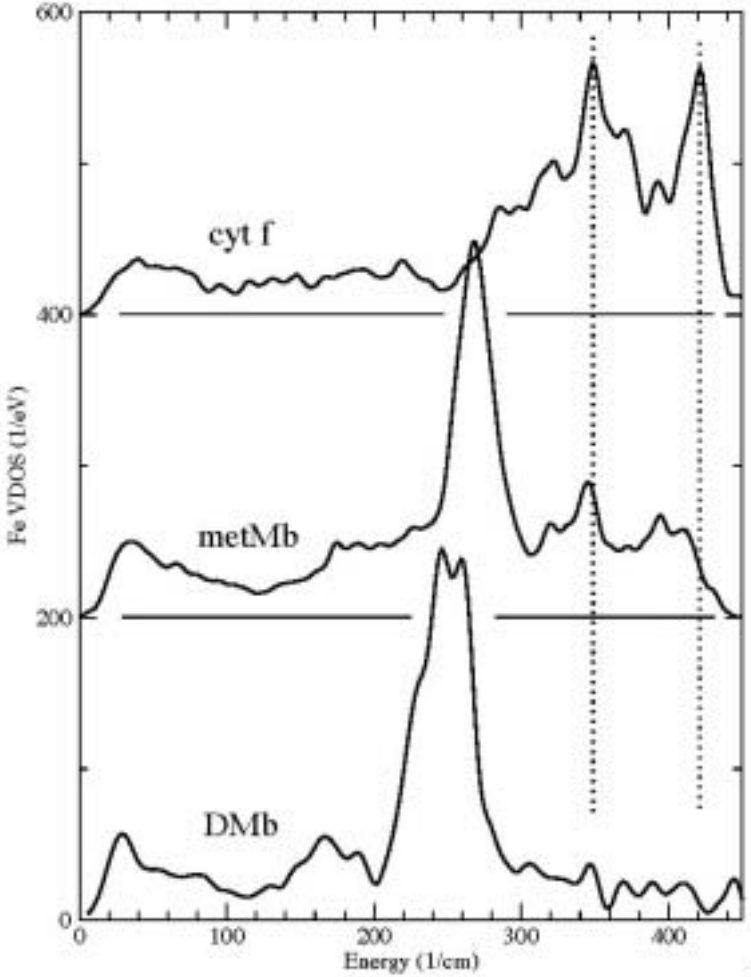
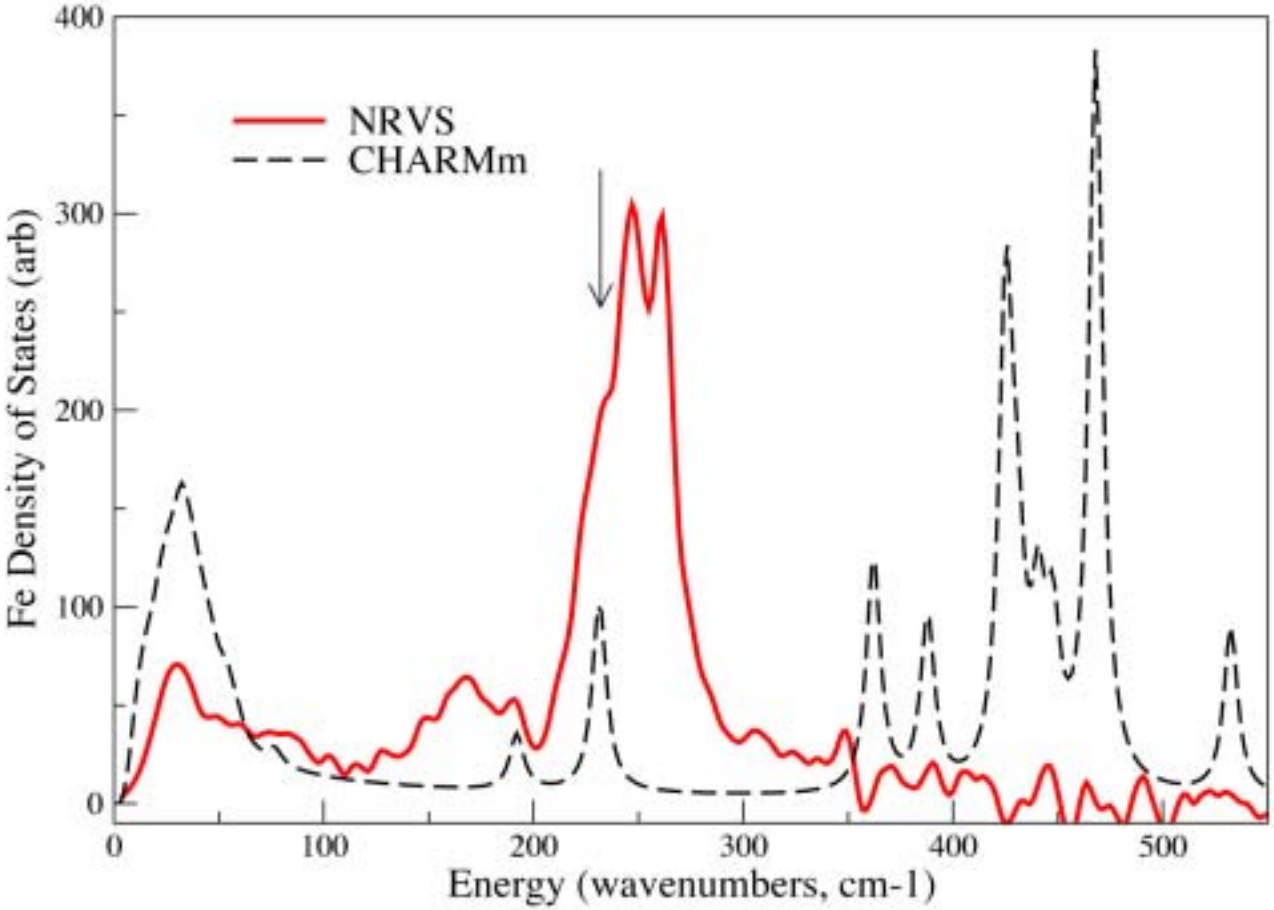


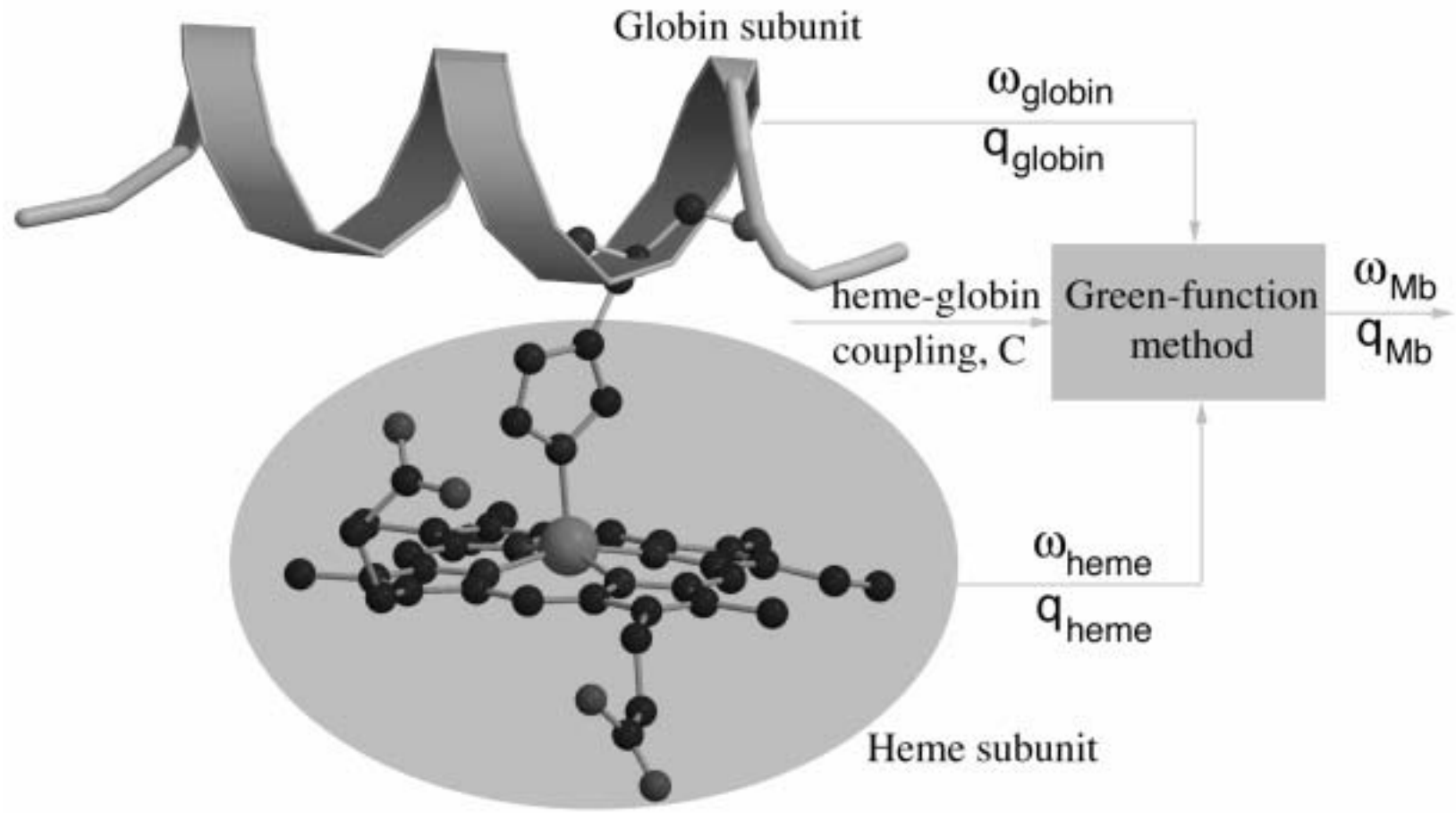
FIGURE 6

Conclusion: the cytochrome arrangement with 4 strong bonds constrains the heme into a much more rigid environment.

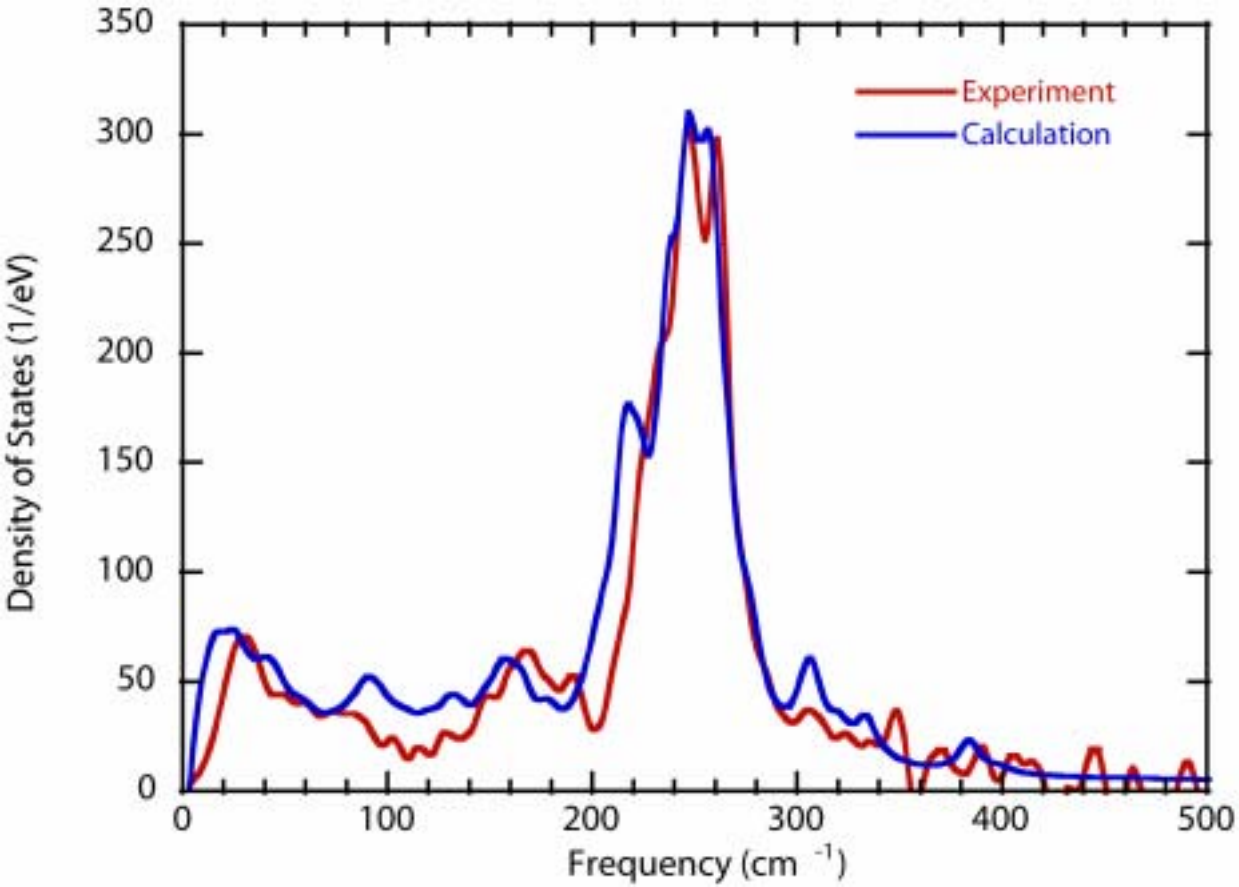
Fixing a molecular dynamics problem

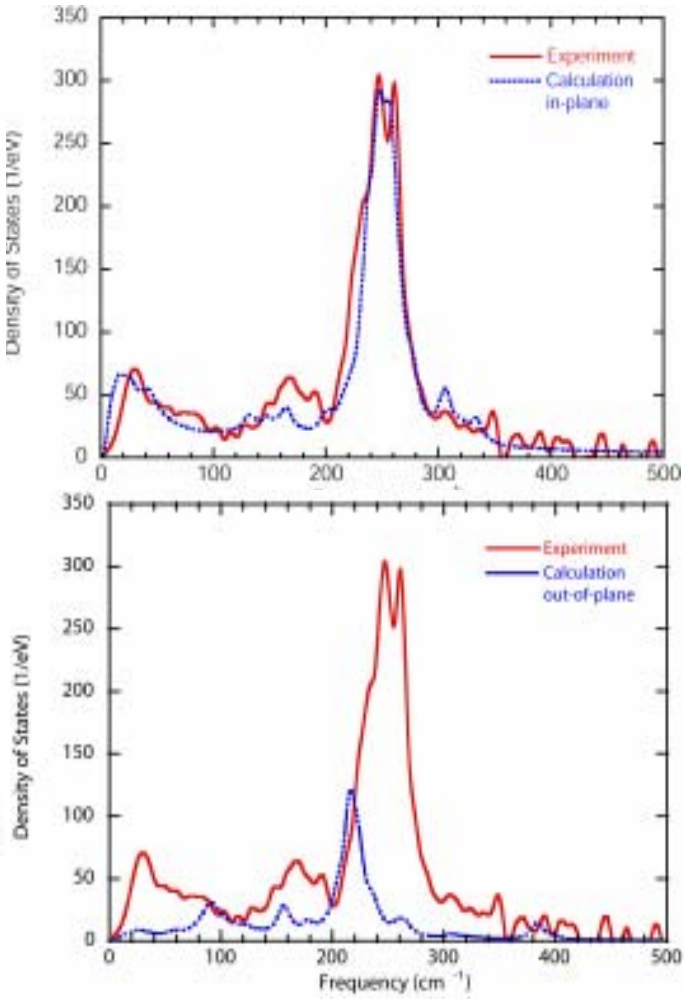


Failure of CHARM to match NRVS data….



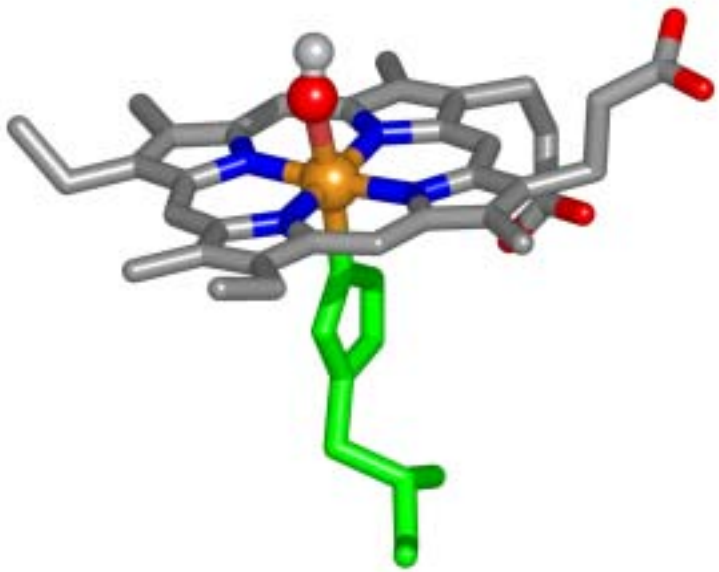
New computational technique: calculate normal modes of heme from previous NRVS force fields, obtain globin normal modes from CHARM, combine with user-defined couplings to fit to data.

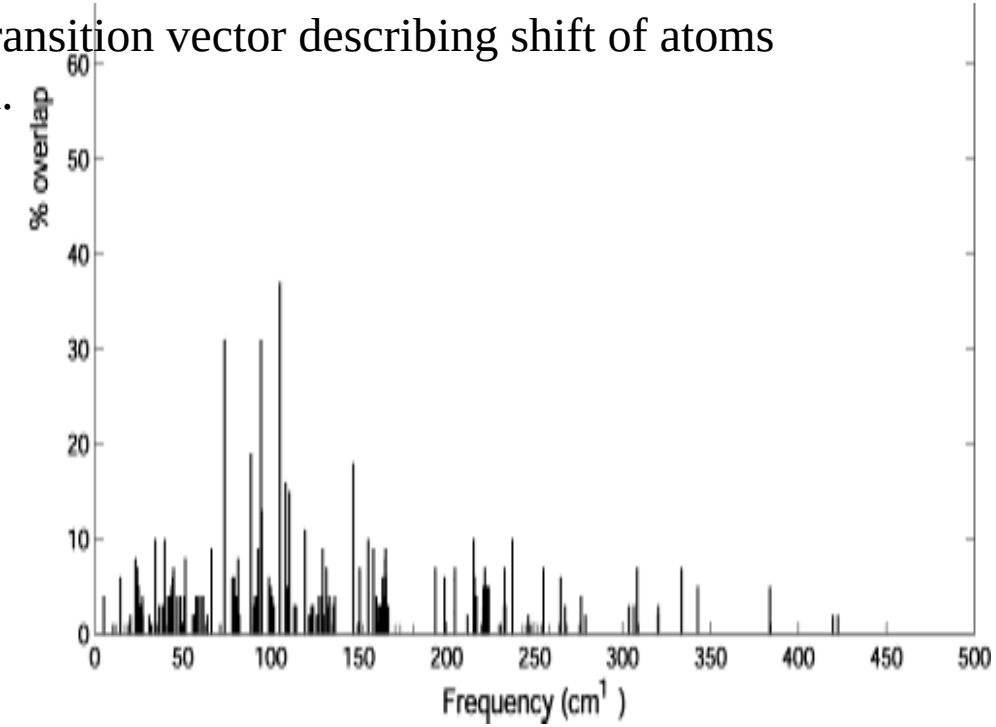




Excellent fits to data are obtained in this way. Permits one to identify particular coupling mechanisms as most important…

Can calculate projection of modes onto transition vector describing shift of atoms going from DMb to MbCO configuration.





% overlap = Mb PPIX coordinate difference of deoxy and CO conformations times deoxyMb VDOS

Result: broad range of modes contribute to transition, with greatest effect at mid-frequencies. Don’t see single “doming” mode, and not dominated by lowest frequencies (as predicted by some).

Collaborators:

Purdue (Physics):

Kristl Adams, Chenfeng Zhang

Brajesh K. Rai, Timo Budarz, Earl Prohofsky

Stanislav Tsoi, Anant K. Ramdas

Purdue (Biology):

Jiusheng Yan, William Cramer

Advanced Photon Source (Argonne):

Wolfgang Sturhahn, Ercan Alp,…

Notre Dame University (Chemistry):

Mary Ellison, Graeme Wyllie, W. Robert Scheidt

Knox College (Physics):

Charles Schulz

Northeastern University (Physics):

J. Timothy Sage & students…

Funding: NSF PHY-9988763 (Durbin)

DOE W-31-109-Eng-38 (Advanced Photon Source)

Important numbers:

X-rays in the 1.0 meV bandpass 10^9 Hz

X-rays within nuclear absorption width 5000 Hz

Typical sample requirements:

at least 10^{17} Fe atoms (10^{18} preferred)

e.g. 15 mg cytochrome *f*

concentration ~230 mg/ml

volume 4 x 2 x 8 mm; 0.064 ml

Data collection time **3 hours - 30 hours**

A. Heme protein dynamics - why should we care?

B. Results from porphyrin model compounds

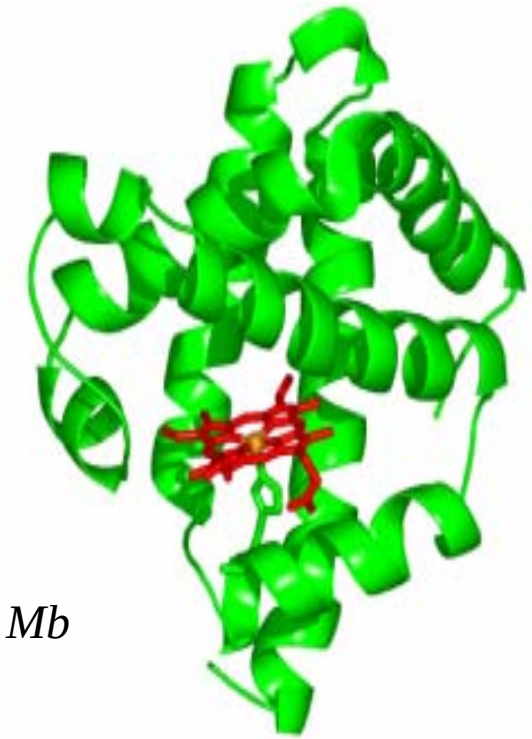
C. Comparison of heme proteins - cytochrome f *vs* myoglobin

D. Joining heme dynamics with globin normal modes for myoglobin (Green function analysis)

A. Heme protein dynamics - why should we care?

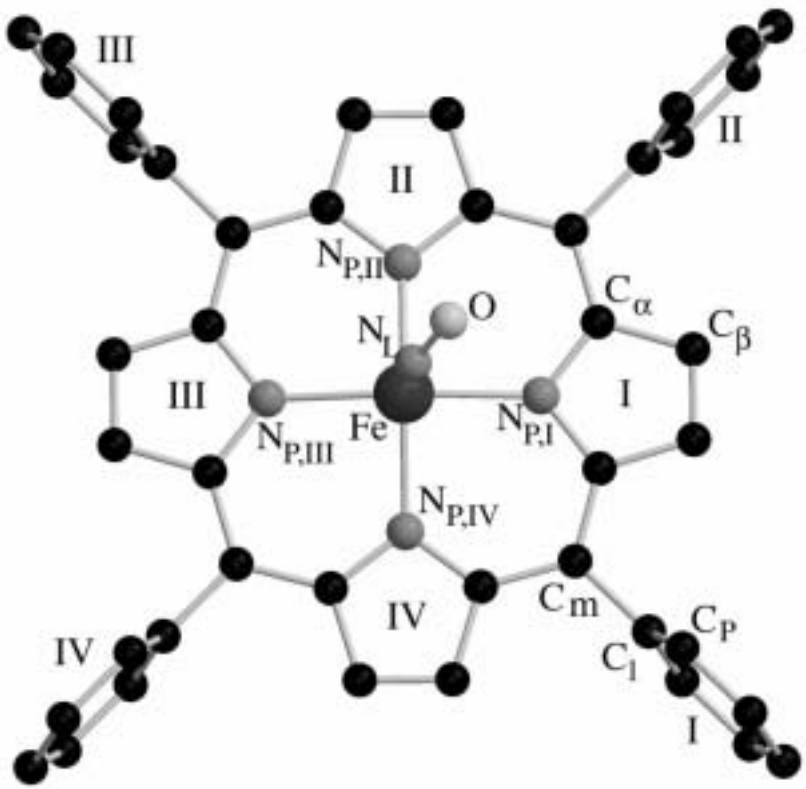
Textbook functions of proteins:

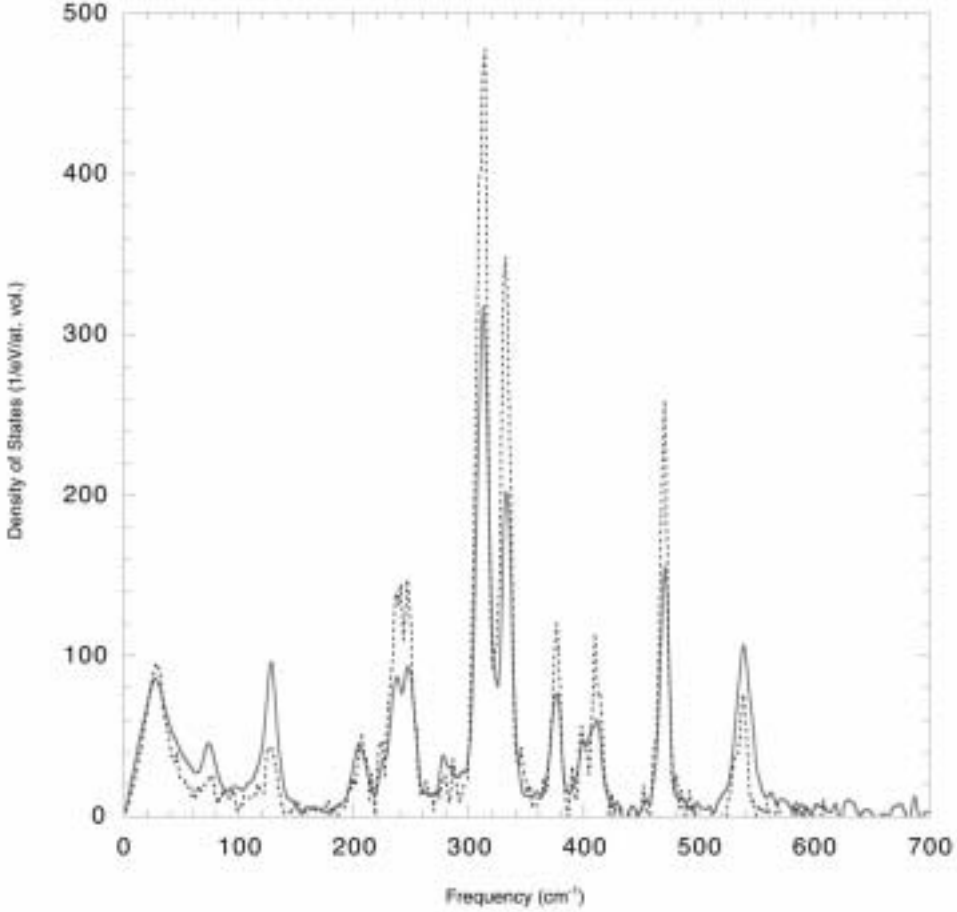
1. binding
2. catalysis
3. switching
4. structural elements



Mb

Dynamics can play an important role in some of these…





Fe TPP-NO

Rai et al., Biophys. J. **82** 2951 2002