

Force Spectroscopy of the Iron Atom in Heme Proteins

J. Timothy Sage¹, Bogdan M. Leu^{1,3}, Tom H. Ching¹, Yong Zhang², John E. Straub², Wolfgang Sturhahn³, E. Ercan Alp³
¹Northeastern University, Boston, MA; ²Boston University, Boston, MA; ³Argonne National Laboratory, Argonne, IL

Abstract: Nuclear resonance vibrational spectroscopy (NRVS) selectively reveals the complete vibrational density of states (VDOS) of a Mössbauer probe nucleus within a protein. Frequency moments of the VDOS determine effective force constants for ⁵⁷Fe at the active sites of cytochrome *c* (cyt *c*) and deoxymyoglobin (Mb). The stiffness measures the force needed to displace the Fe with the other atoms fixed, and probes the nearest neighbor interactions with the Fe. The stiffness of the low spin Fe environment in cyt *c* greatly exceeds that for the high spin Fe in Mb, reflecting the shorter Fe—N bonds to the heme. Moreover, a significant stiffness decrease upon oxidation of cyt *c* tracks the longer Fe—S bond to Met 80 in the oxidized protein. Quantitative comparison with ⁵⁷Fe/⁵⁴Fe frequency shifts suggests that Fe-L vibrations contribute to the Raman signal of cyt *c* recorded in resonance with the heme Soret band. The resilience measures the force needed to displace the Fe with the surrounding atoms free to respond, and determines the magnitude of the thermal fluctuations of the Fe on a time scale determined by the experimental energy resolution (ca. 4 ps for the results reported here). Quantitative agreement with the temperature-dependent mean squared displacement determined from independent Mössbauer measurements confirms longstanding assumptions that vibrational motion dominates thermal fluctuations of the heme Fe below the well-known dynamical transition at ca. 200 K and identifies THz frequencies below 100 cm⁻¹ as the dominant contribution. The resilience increases significantly for cyt *c* with respect to Mb, which we attribute to the increased number of covalent links between heme and peptide in the former protein. Molecular dynamics simulations reproduce the increased resilience of cyt *c*, but find no significant change with oxidation state.

nuclear resonance vibrational spectroscopy (NRVS)
 ➤ yields vibrational density of states (VDOS)
 ➤ determines effective force constants for displacement of Mössbauer nucleus:

Stiffness

H. J. Lipkin *Phys. Rev. B* 52, 10073 (1995)

$$k_s = m_{Fe} \langle \omega^2 \rangle$$

$$= \frac{1}{3} m_{Fe} \int \omega^2 D(\omega) d\omega$$

- fixed neighbors
- probes nearest-neighbor interactions
- inverse correlation with bond length

Resilience

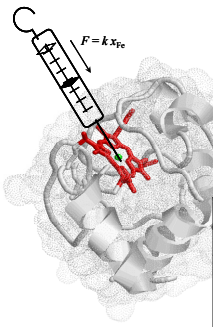
G. Zaccari *Science* 288, 1604 (2000)

$$k_r = m_{Fe} \langle \omega^{-2} \rangle^{-1}$$

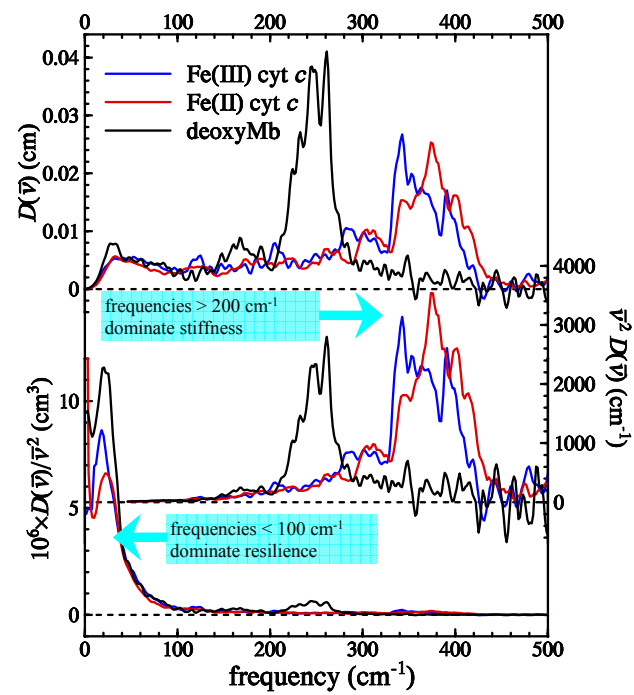
$$= \frac{m_{Fe}}{\frac{1}{3} \int \frac{D(\omega)}{\omega^2} d\omega} = \frac{k_B}{d \langle x^2 \rangle / dT}$$

- neighbors free to respond (center of mass fixed)
- probes local elasticity
- determines thermal excursions of Fe atom

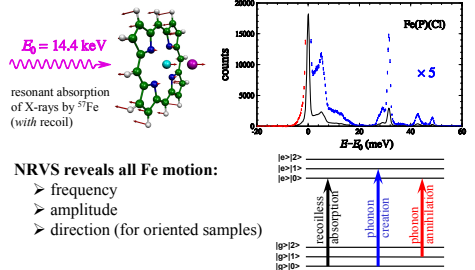
	force constants (pN/pm)		bond lengths ^c (pm)		
	stiffness ^a	resilience ^b	Fe—S _{Met}	Fe—N _{His}	Fe—N _{pyr}
Fe(II) cyt <i>c</i>	322 ± 17	32.6 ± 1.5	229 ± 2	200 ± 2	199 ± 2
Fe(III) cyt <i>c</i>	284 ± 17	28.3	233 ± 2	198 ± 2	198 ± 2
deoxyMb	190 ± 20	21.1 ± 1.3	—	220 ± 2	205 ± 2



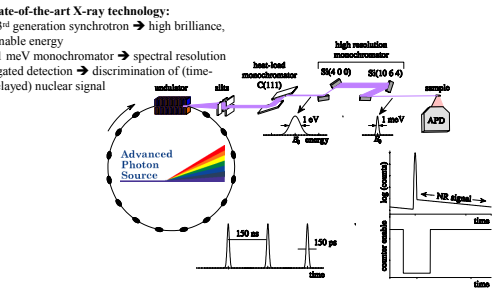
a) Leu et al. *J. Phys. Chem. B*, in press (2009) b) Leu et al. *Biophys. J.* 95, 5784 (2008) c) Cheng et al. *Inorg. Chem.* 38, 5703 (1999); Chance et al. *Biochemistry* 35, 9014 (1996)



Nuclear Resonance Vibrational Spectroscopy



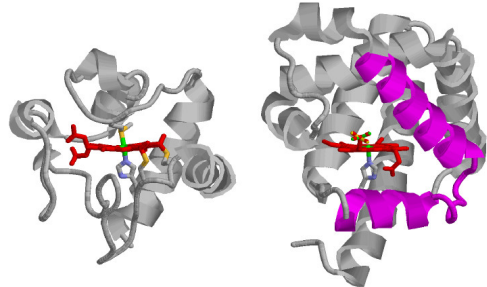
NRVS: Instrumentation Details



Other NRVS presentations at the Biophysical Society meeting:
 • 2240-Pos - Reactive Vibrational Dynamics of Iron in Heme
 • Pos-L285 - Complete Vibrational Spectrum of Fe and Protonation Issue of the Iron-oxo in Myoglobin Compound II

Experimental Comparison: cyt *c* vs. deoxyMb

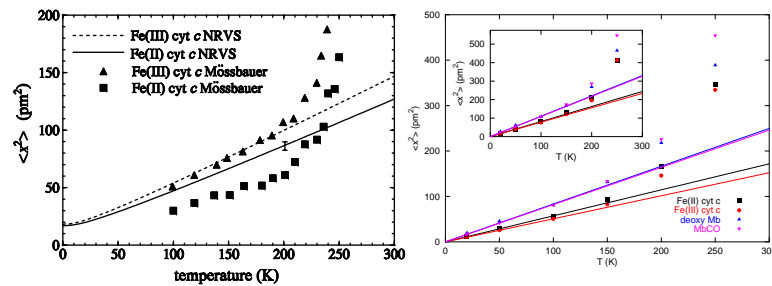
- increased Fe stiffness reflects
 - shorter Fe—N_{pyr} bonds in cyt *c* (low spin)
 - shorter Fe—S_{Met} bond in Fe(II) cyt *c*
- increased Fe resilience reflects
 - additional heme-protein connectivity in cyt *c* (four covalent links)
- low frequency vibrations determine sound velocity and compressibility (Leu et al., submitted)



Many Roads to Resilience

resilience determines temperature dependence of atomic mean square displacements (MSD):

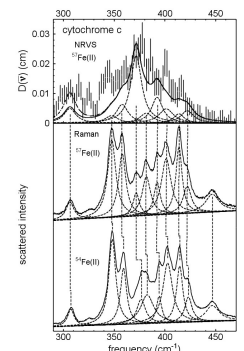
- Mössbauer: temperature-dependent observations track NRVS prediction for Fe(II) cyt *c*
- neutron spectroscopy: resilience ~3× larger for Fe sites than H sites
- MD simulations: reproduce increased resilience for cyt *c*



Taming Spectral Complexity in Cytochrome *c*

reduced symmetry increases spectral congestion (and information content)

- for cyt *c*:
 - three chemically distinct bonds to Fe
 - more than 10 vibrational modes contribute to stiffness of Fe environment
 - all Raman modes from 300—440 cm⁻¹ contribute to NRVS signal



Acknowledgments:

NSF PHY-0545787
 NSF CHE-0750309
 DOE DE-AC02-06CH11357

