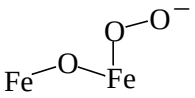
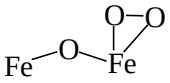
This still doesn't tell us <u>how</u>
O_2 binds to hemerythrin

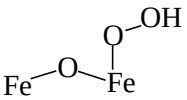


end-on

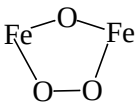


side-on

Superoxo or Peroxo ?



hydroperoxo

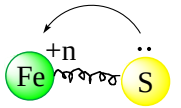


bridging

Resonance Raman experiments
helped to address this question……..

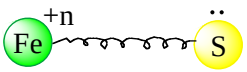
Resonance Raman

allows you to selectively probe vibrations associated with metal–ligand chromophores.



hν = visible light

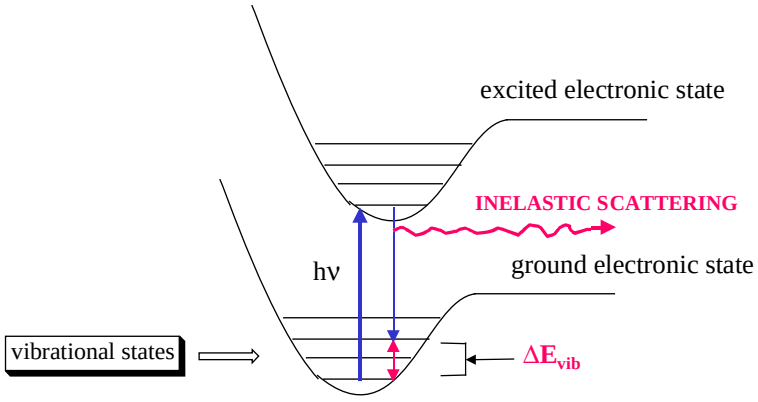
even in the presence of the all of the protein vibrations which would dominate the IR or RR



vibrationally excited state

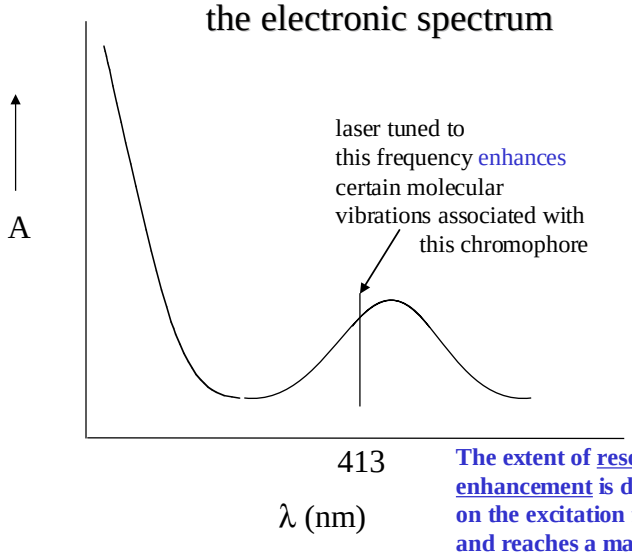
"stretched"

Resonance Raman



$E_{scattered} = h\nu - \Delta E_{vib}$

The frequency used in the Resonance Raman experiment is determined by the Laser type, and by inspecting the electronic spectrum



Kr-ion: 413.1 nm
530.9 nm
647.1 nm

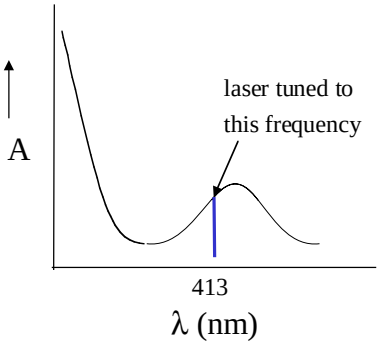
Ar-ion: 488 nm
514.5 nm

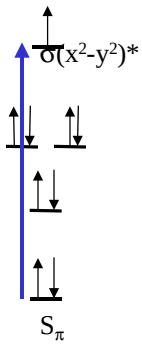
He–Ne: 632.8 nm

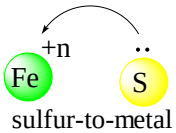
The extent of resonance enhancement is dependent on the excitation frequency, and reaches a maximum when it coincides with λ_{max}

Resonance Raman can be used to determine the nature of an electronic absorption band

If characteristic vibrations (e.g. Fe–S) are enhanced when irradiation frequencies are tuned to a particular chromophore (e.g., λ= 450(2000) nm), then this tells you that this chromophore corresponds to an electronic transition between orbitals that change the Fe–S bond.







sulfur-to-metal charge transfer transition

Characteristic stretching frequencies
In metal oxygen and metal thiolate complexes

ν_{M-O}	600 – 200 cm^{-1}
$\nu_{O-O\bullet^-}$	1011–1146 cm^{-1}
$\nu_{O-O^{2-}}$	780 – 900 cm^{-1}
ν_{Fe-S}	368 cm^{-1}
ν_{Fe-OH}	565 cm^{-1}

In order to definitively show that a vibration assignment is correct, you need to show that the vibration shifts upon **isotopic substitution**

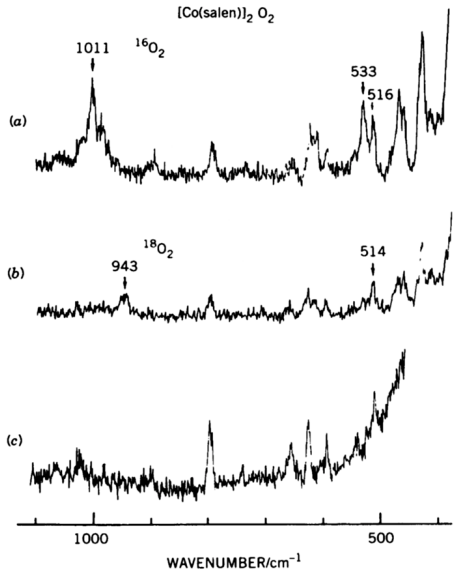
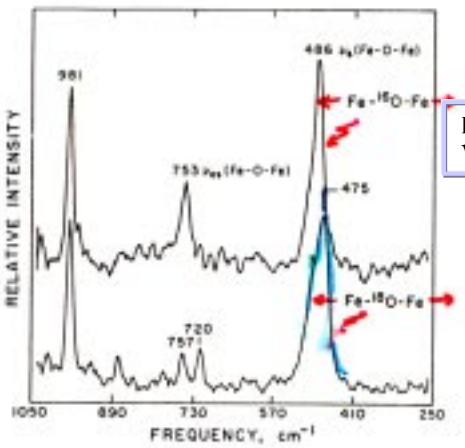


Fig. III-65. The RR spectra of (A) $[Co(salen)]_2$ $^{16}O_2$, (B) $[Co(salen)]_2$ $^{18}O_2$, and (C) Co(salen) (579 nm excitation, ~100 K).[1028]

Nakamoto "IR and Raman Spectra of Inorganic and Coordination Compounds", p. 159

Sanders–Loehr in "Clusters in Biology"

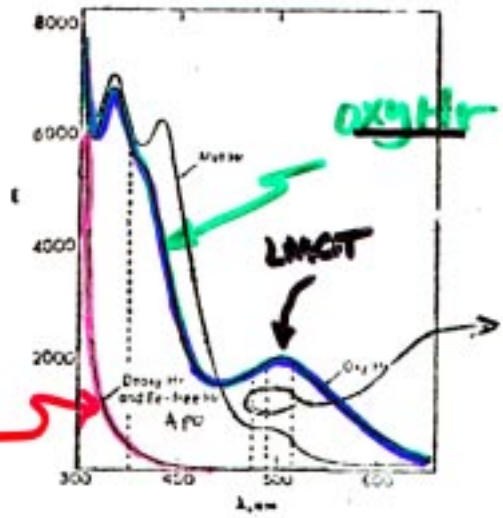
Resonance Raman spectrum of oxy–Hr



predict $\nu_{Fe-^{16}O} = 1.2\ \nu_{Fe-^{18}O}$

Figure 5. Resonance Raman spectra of oxyhemerythrin with O-16 (upper) and O-18 (lower) in the oxo bridge. Protein (1.2 mM) and Na_2SO_4 (0.3 M) maintained at 5°C in a flow cell and probed with 363.8 nm excitation. (Reproduced from Ref. 34. Copyright 1984 American Chemical Society.)

Electronic Absorption Spectrum of Hr



Deoxy Hr

Excitation frequencies

Resonance Raman spectrum of oxy–Hr

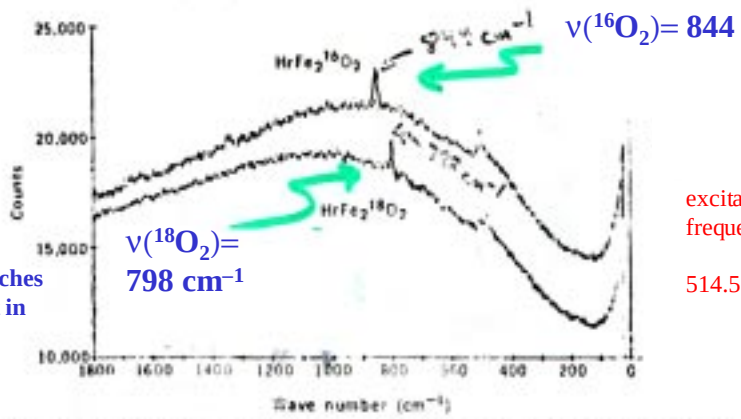


Fig. 9. Resonance Raman spectrum of oxyhemerythrin in solution, using 514.5 nm excitation.

$\nu(^{16}O_2)$= **844 cm^{-1}**

$\nu(^{18}O_2)$= **798 cm^{-1}**

these stretches are absent in deoxy–Hr

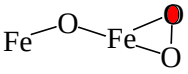
excitation frequency=

514.5 nm

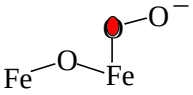
Conclude: dioxygen is reduced to **peroxide** upon binding to the binuclear Hr iron site

This <u>still</u> doesn't tell us anything about the O_2 binding mode

terminal

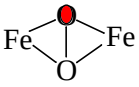


side–on

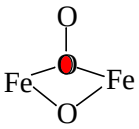


end–on

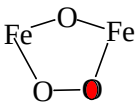
bridging



side–on

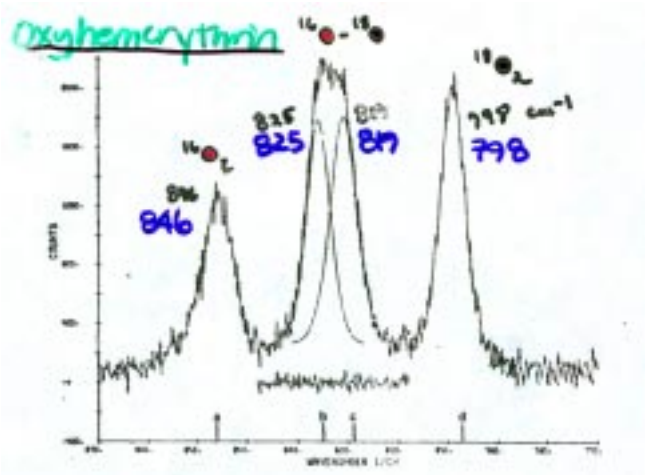


end–on

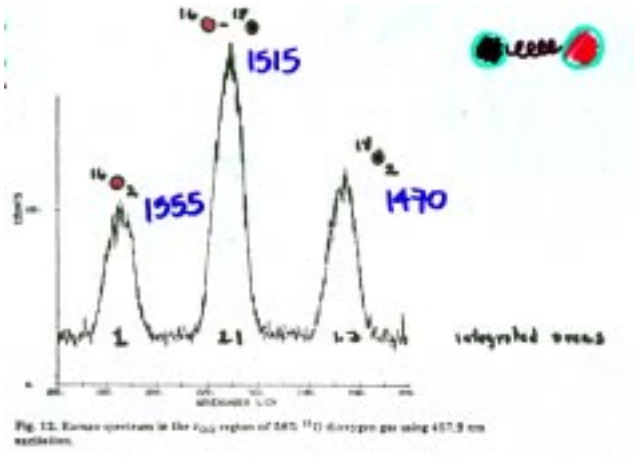


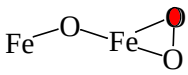
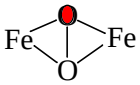
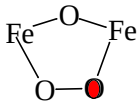
end–on

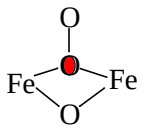
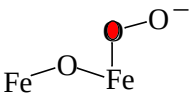
Resonance Raman spectrum of Hr exposed to an isotopic mixture: $^{16}O_2$, ^{16}O–^{18}O, $^{18}O_2$



Resonance Raman spectrum of an isotopic mixture of $^{16}O_2$, ^{16}O–^{18}O, $^{18}O_2$



Binding mode	number of stretches expected for ^{16}O–^{18}O
 side–on	one stretch
 side–on	one stretch
 end–on	one stretch

Binding mode	number of stretches expected for $^{16}O–^{18}O$
 **end–on**	two stretches
 **end–on**	two stretches

Conclude: dioxygen binds end–on as a peroxide