Vibrational Spectroscopy

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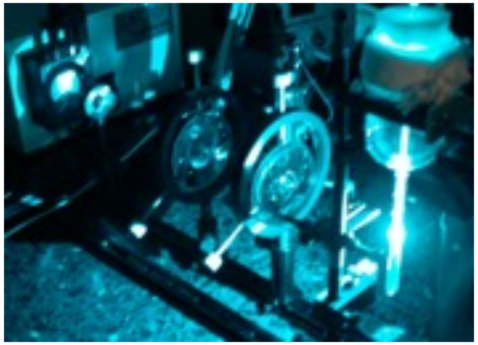
Sir
Chandrasekhara Venkata Raman
(1956)

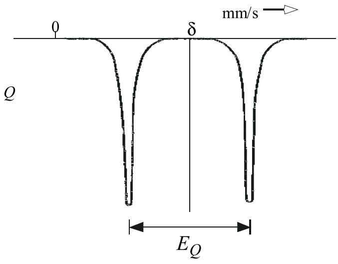
2nd Penn State Bioinorganic Chemistry
Workshop
May/June 2012



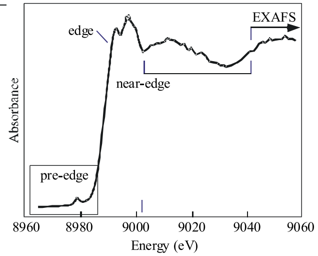
Spectroscopic Techniques

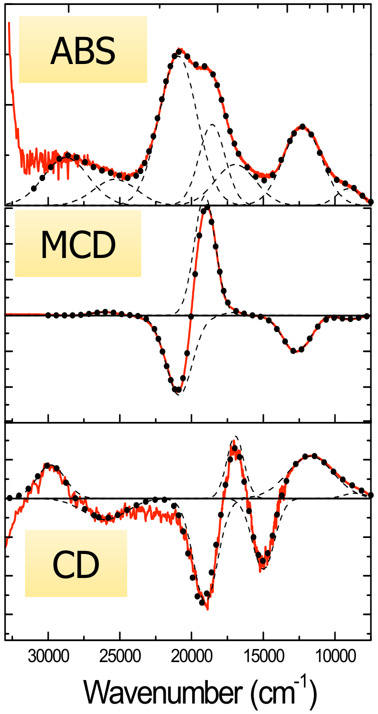
	Gamma	X-Ray	UV/vis	Infrared	Microwave	Radiowave
eV	14000	8000 2000	4 - 1	0.1-0.01	10^{-4} -10^{-5}	10^{-6} -10^{-7}

Möss-
bauer

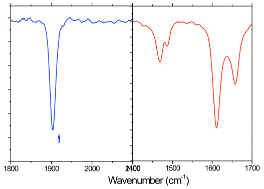


XAS
EXAFS

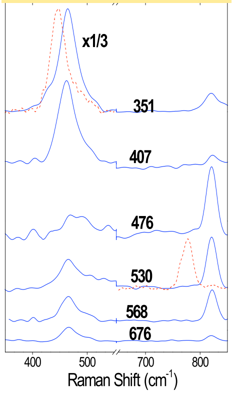




IR

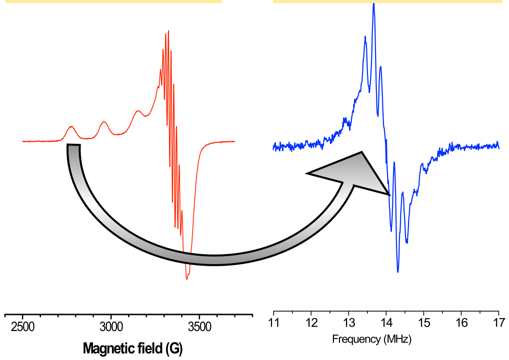


Raman



EPR

ENDOR



NMR

Outline

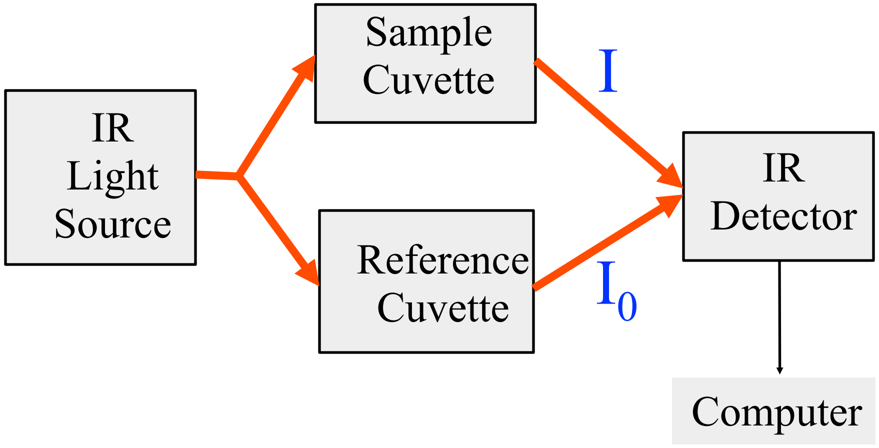
1. Introduction
2. Molecular Vibrations
    - Vibrational Frequencies and Normal Coordinates
    - Physical Origin of IR Intensities
    - Physical Origin of Raman Intensities
    - Physical Origin of Resonance Raman Intensities
3. Experimental Techniques
    - Raman Spectroscopy
    - Resonance Raman Spectroscopy
    - Infrared Spectroscopy (FT-IR)
4. Applications in Bioinorganic Chemistry
    - Hemoproteins
    - Copper Proteins
    - Metal-Radical Sites
    - Mononuclear Iron-Dioxygen Interactions

Why Vibrational Spectroscopy ?

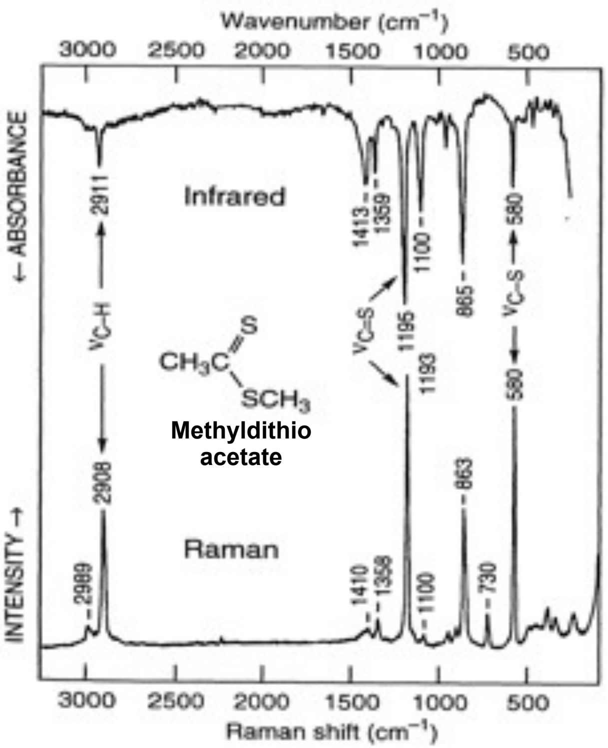
- Structural Information (IR/Raman/resonance Raman)
  - Identication of Characteristic Vibrations
  - Isotope Shifts
  - Normal Coordinate Analysis
  - Detection of functional groups
- Electronic Information (resonance Raman)
  - Identification of Electronic Transitions
  - Excitation Profiles
  - Insight into Bonding
- Mechanistic Information (IR/Raman/resonance Raman)
  - Trapping of Short Lived Intermediates
  - Freeze Quench Techniques
  - Combination with Electrochemistry, Stopped Flow, Continuous Flow,...
- Complementary to other Techniques
  - Not dependendent on Magnetic Properties (EPR,MCD)
  - Much higher Resolution than Absorption and CD Spectroscopy
  - Not Limited to Certain Isotopes (Mössbauer)

IR versus Raman Spectroscopy

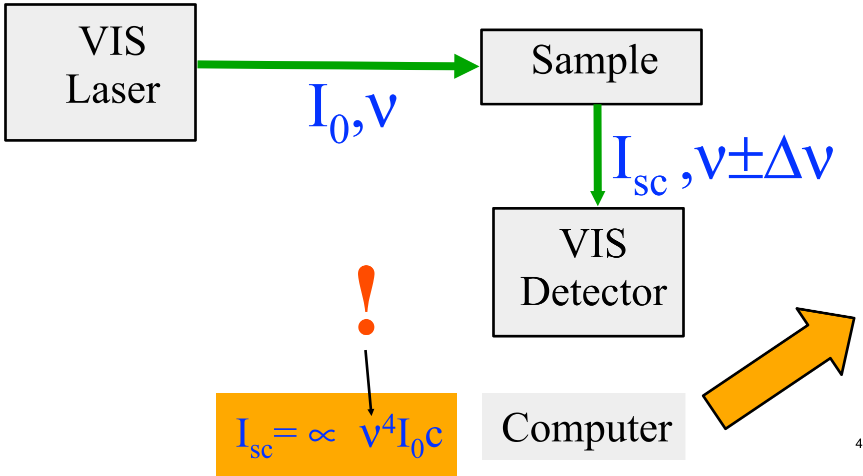
IR Experiment



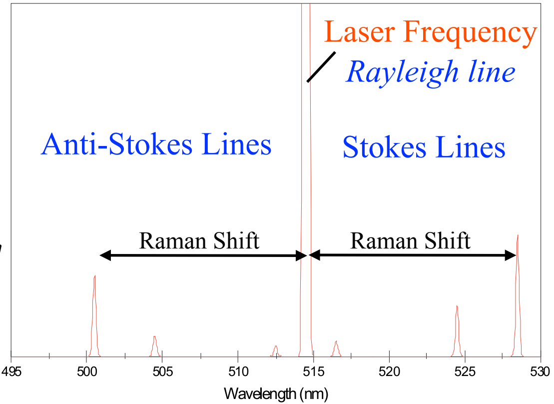
$A = -\log(I/I_0) \propto \varepsilon d c$



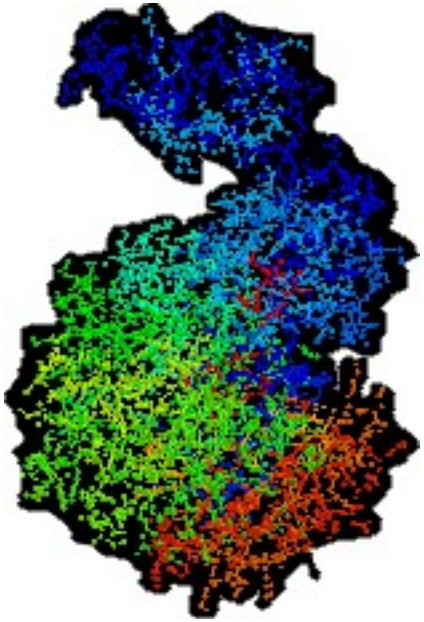
Raman Experiment



$I_{sc} = \propto \nu^4 I_0 c$

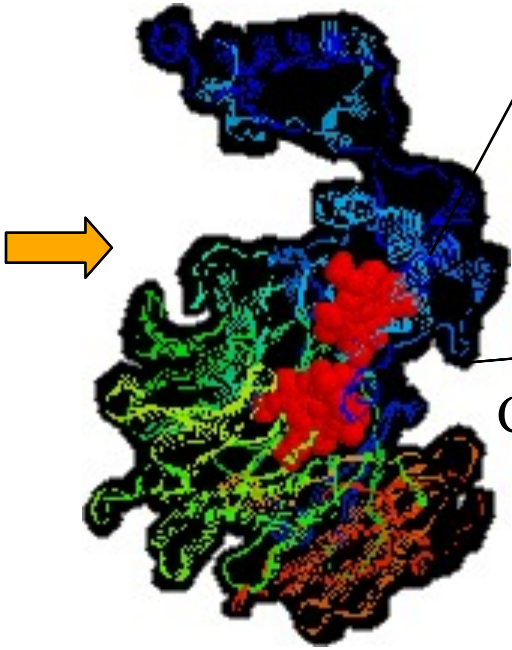


IR versus Raman Spectroscopy



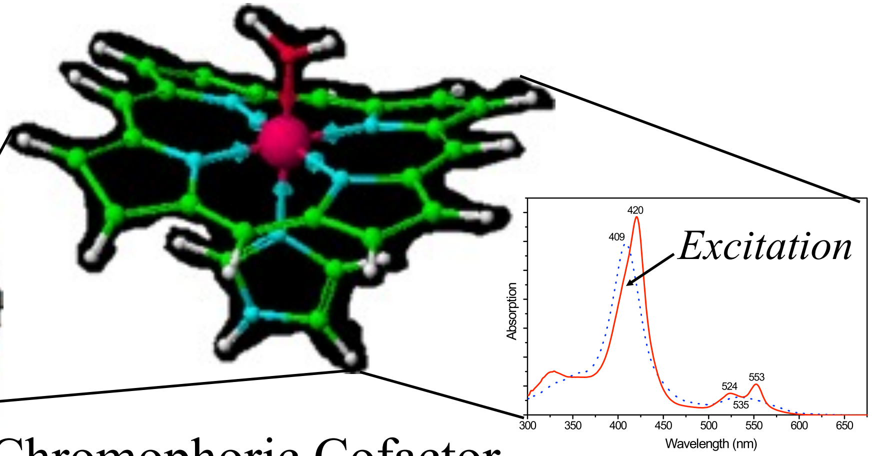
Tertiary Structure

- > 1000 Atoms
- Thousands of Peaks in IR+Raman Spectra
- Impossible to understand in Detail



Secondary Structure

Both IR and Raman are „globally sensitive“ to Secondary Structure Elements (similar to CD)

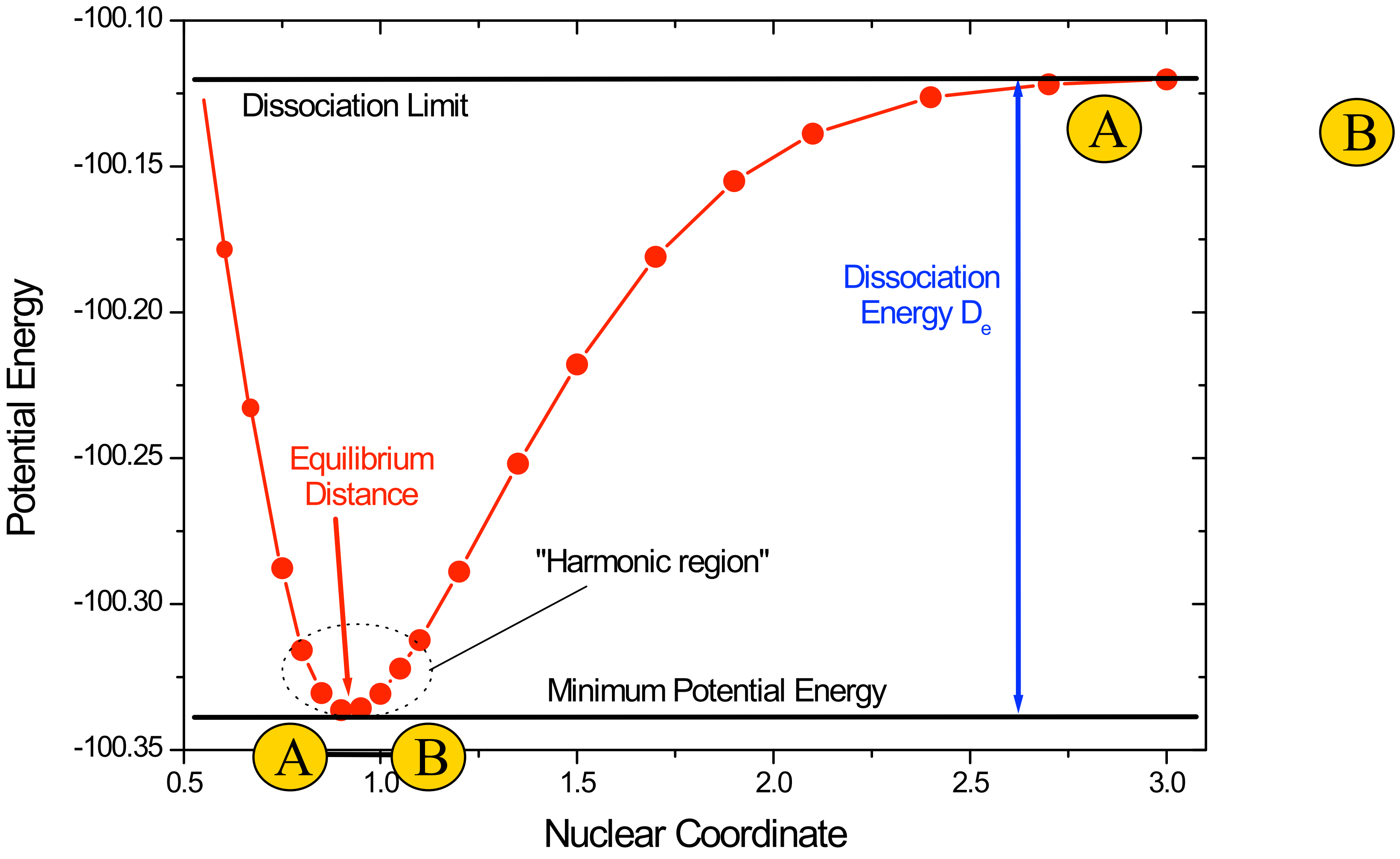


Chromophoric Cofactor

- **Resonance Raman** **<u>Selectively</u>** Enhances Vibrational Features of Chromophoric Groups
- **Resonance Raman** is Orders of Magnitude More Sensitive than off-Resonance Raman
- Highly Sensitive **Difference-FT-IR** can Provide Information about *Changes* in Parts of the Protein
- In „**Non-Crowded Regions**“ (Protein and H_2O) IR peaks may be Directly Detected (i.e .Bound CO,CN^-,NO)

II.A. Vibrational Frequencies and Normal Modes

Potential Energy Surfaces



„Potential Energy“$=T_{el}+V_{el,nuc}+V_{el,el}+V_{nuc,nuc}$

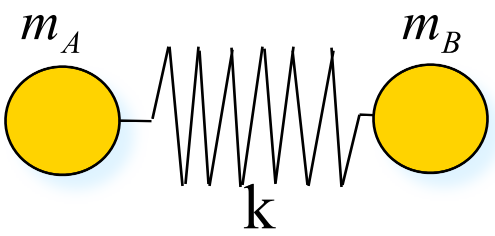
(Solution to Time Independent, Non-relativistic Schrödinger Equation)

The Vibrations of a Diatomic Molecule

Newton‘s law:

$$F = -\frac{\partial V(R)}{\partial R} = m\frac{\partial^2 R(t)}{\partial t^2}$$

Molecule:

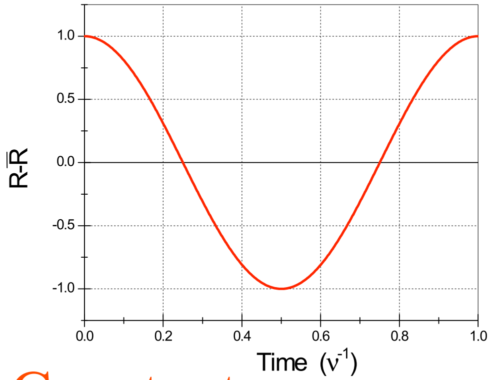


$$V(R) = V_0 + \underbrace{\left.\frac{\partial V}{\partial R}\right|_{R=\bar{R}}(R-\bar{R})}_{0} + \frac{1}{2}\underbrace{\left.\frac{\partial^2 V}{\partial R^2}\right|_{R=\bar{R}}}_{k}(R-\bar{R})^2 + \ldots$$

Thus:

$$\frac{\partial^2 R(t)}{\partial t^2} = -\frac{k}{m}(R-\bar{R})$$

Solution:



$$R(t) = \bar{R} + c_1 \sin\left(\sqrt{\frac{k}{m}}\,t\right) + c_2 \cos\left(\sqrt{\frac{k}{m}}\,t\right)$$

Characteristic Quantities:

Force Constant

Vibrational Frequency $$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

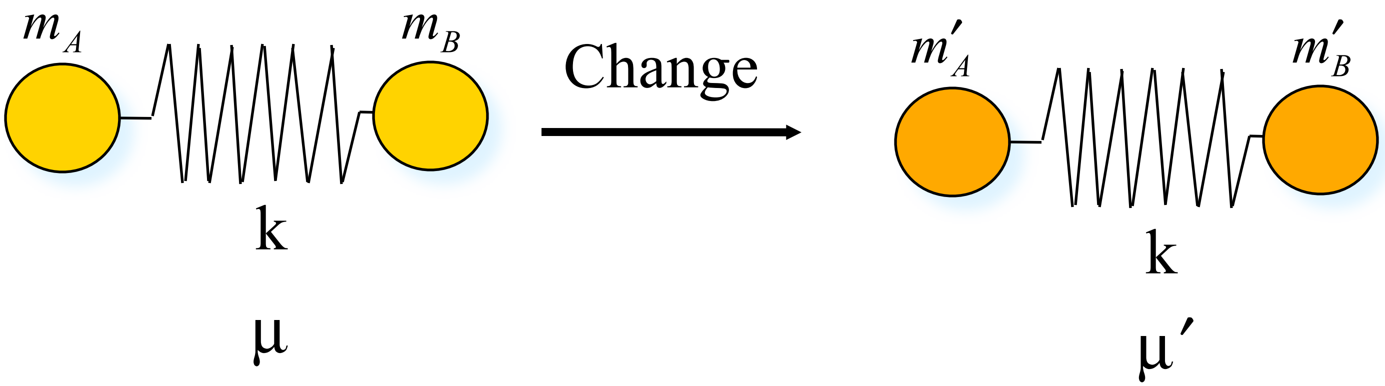
Reduced Mass $$m = \frac{m_A m_B}{m_A + m_B}$$

The Reduced Mass and Isotope Shifts

Force Constant

Vibrational Frequency $\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$

Reduced Mass $m = \frac{m_A m_B}{m_A + m_B}$



$$\boxed{\nu' = \nu\sqrt{\frac{m}{m'}} = \nu\sqrt{\left(\frac{m_A m_B}{m_A + m_B}\right)\left(\frac{m'_A + m'_B}{m'_A m'_B}\right)}}$$

Example: $^{16}O_2 \rightarrow {}^{18}O_2$

$\nu(^{18}O_2) = 0.943\ \nu(^{16}O_2)$

Force Constants

Units:

$$k = \left.\frac{\partial^2 V}{\partial R^2}\right|_{R=\bar{R}} \qquad [k] = \frac{Energy}{Area}$$

Practical Spectroscopy

$$[k] = \frac{10^{-18}\,J}{\text{Å}^2} = 10^2\,Nm^{-1} = \frac{mdyn}{\text{Å}}$$

Typical Force Constants:

N_2 : 22,41 O_2 : 11,41 F_2 : 4,45

CO : 18,55 NO : 15,48 H_2 : 5,20

Force Constants Become Large if the Bonds are Strong
(more correctly - if the bonds are *stiff*)

Observed Trends:

1. Bonds with Large Force Constants have High Dissociation Energies
2. Bonds with Large Force Constants are Short

Badgers Rule

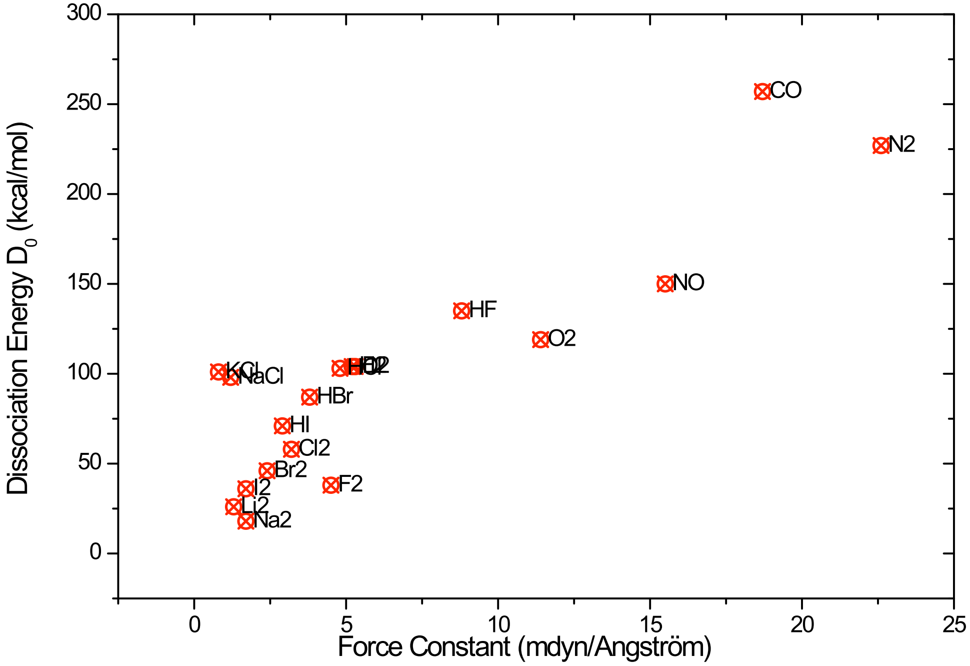
Observation (Badger, R.M. (**1934**) *J. Chem. Phys.*, 2, 128)

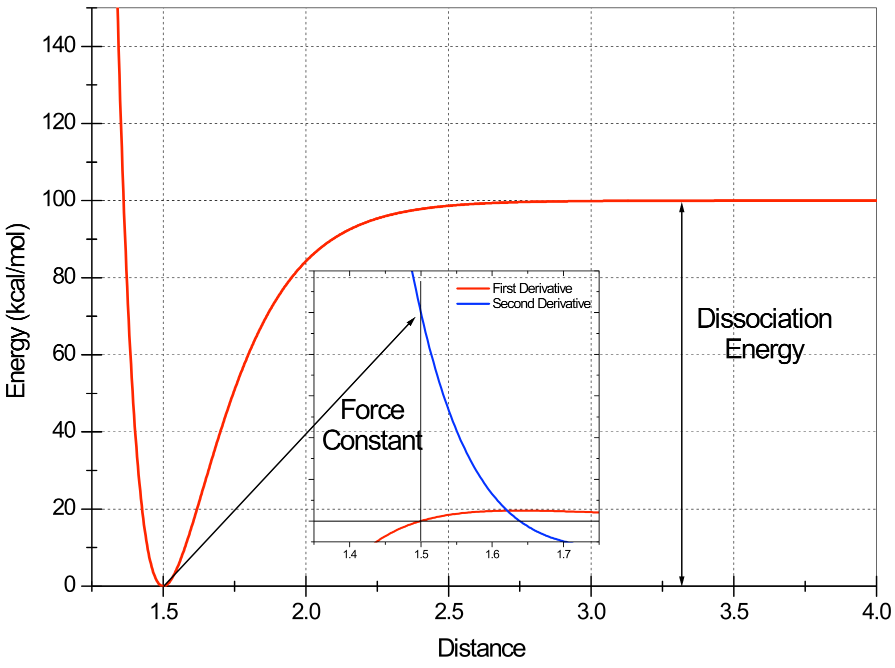
$$k \approx 1.86\left(\overline{R} - d_{ij}\right)^{-3}$$

Relationship between Bond Lengths and Vibrational Frequencies

i	j	d_{ij} (Angström)
H	H	0.025
H	1st row	0.335
H	2nd row	0.585
H	3rd row	0.650
1st row	1st row	0.680
1st row	2nd row	0.900
1st row	3rd row	
2nd row	2nd row	1.180
2nd row	3rd row	
3rd row	3rd row	1.350

Force Constants and Bond Strengths



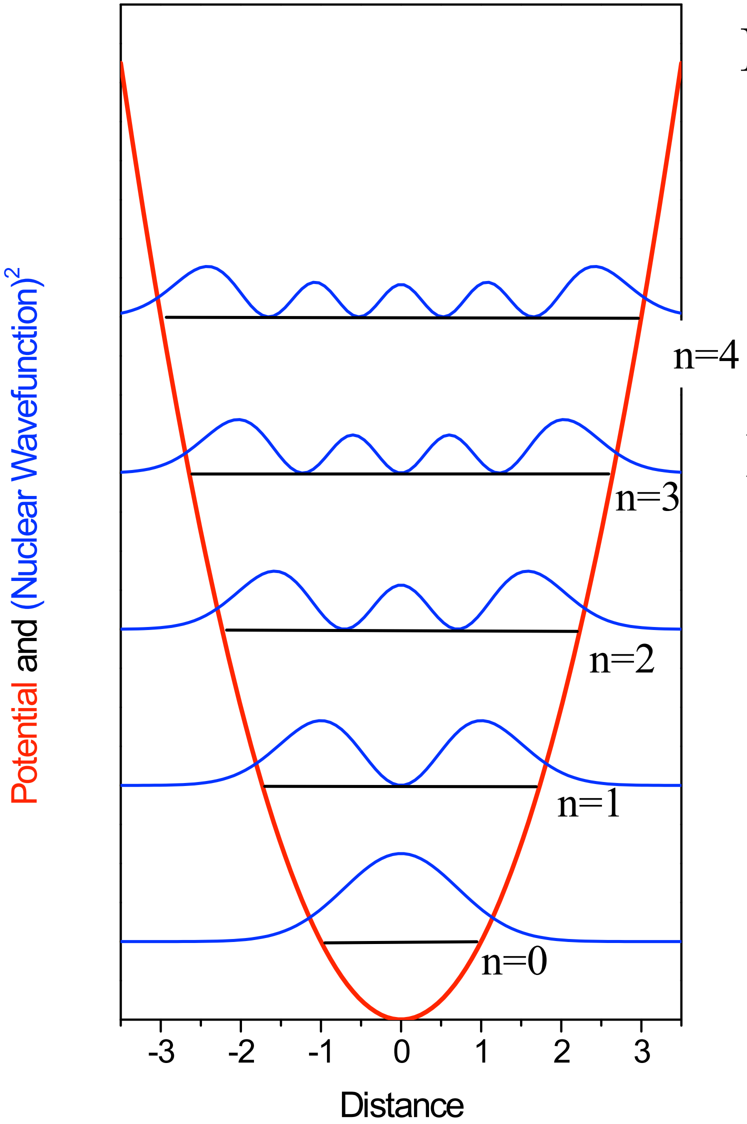


For a Morse Potential: $V(R) = D_e[1 - \exp(-\beta R)]^2$

$$\frac{\partial^2 V(R)}{\partial R^2} = 2D_e\beta^2$$

Qualitative Justification
Of the Observed Trends

Vibrational States of a Diatomic



In the Quantum Mechanical Oscillator:

- Energy is Quantized
- Can only give a Probability for Finding the Nuclei in a Certain Arrangement
- There always is a Zero-Point Energy

Key Equations:

- „Full“ Wavefunction: $\Psi^{I} = \Psi^{I}_{Electronic} \otimes \Psi^{I,n}_{Vibrational}$
- „Full“ Energy: $E = E^{I}_{Electronic} + E^{I,n}_{vibrational}$
- Vibrational Frequency: $\nu_{I} = \left(k_{I} / m / 4\pi^{2}\right)^{1/2}$
- Vibrational Energy: $E^{I,n}_{vibrational} = \left(n + \tfrac{1}{2}\right) h\nu_{I}$
- Vibrational Wavefunction:

$$\Psi^{I,n}_{Vibrational}(R) = N_{n} \exp\left(-R^{2}/4\sigma^{2}\right) H_{n}\left(R/\sqrt{2}\sigma\right)$$

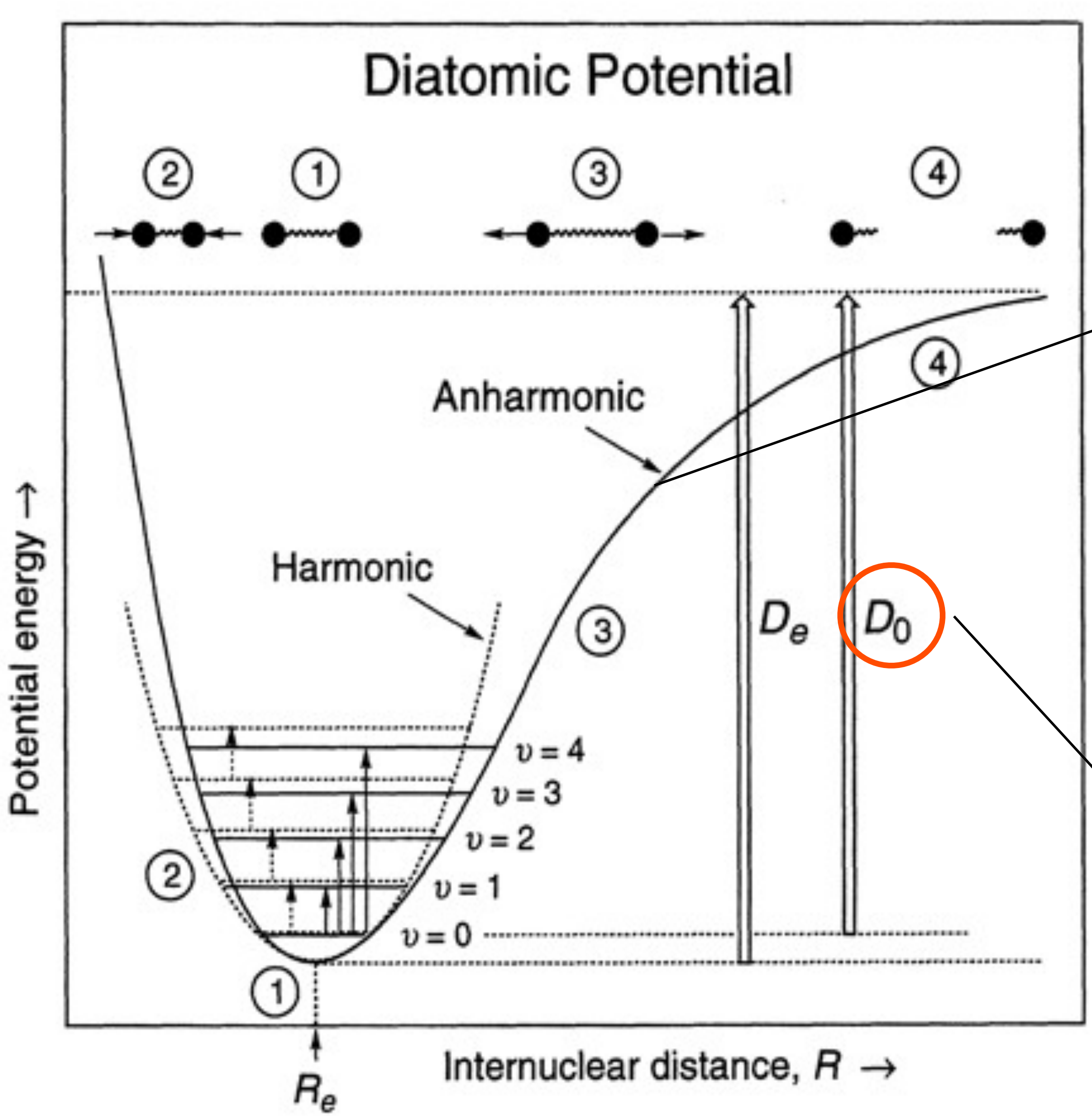
$$\sigma^{2} = \hbar/(4\pi\, m\nu)$$

Normalization

Gaussian

Hermite Polinomial

Anharmonicity and ZPE Effects



$$E^{I,n}_{vibrational} = h\nu\left(n+\tfrac{1}{2}\right) - x_e h\nu\left(n+\tfrac{1}{2}\right)^2 + \ldots$$

1. Deviations from Equal Spacing
2. Actual Frequency is Always Lower than the Harmonic one
3. Effects are Larger the Larger the Frequency
4. Overtones become Allowed

$$D_0 = D_e - h\nu$$

Observable Quantity

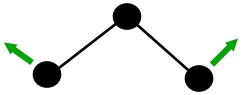
Vibrations of Polyatomic Molecules

In a Polyatomic Molecule Many Vibrations are Possible.

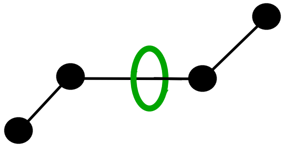
- A Potential for a Diatomic Molecule Leads to 1 Eigenfrequency and 1 Vibrational Mode (Stretching Vibration)
- In Polyatomic Molecules there are Different Forms of Movements:

Stretches

Bends:



Torsions:

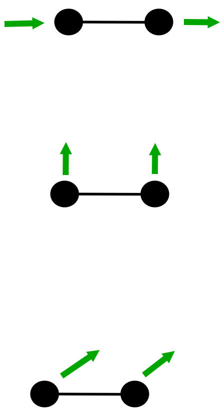


- A Potential for an *N*-Atomic Molecule Leads $M=3N-6$ ($3N-5$ if the Molecule is Linear) Vibrational Frequencies (ν_i) and also *M* Vibrational Modes (=**„Normal“ Modes**, $\mathbf{Q}_i$)
- In General *all* Atoms move in each Normal Mode which consists of Linear Combinations of „primitive“ Stretches Bends and Torsions (i.e. the Modes are *Delocalized*).

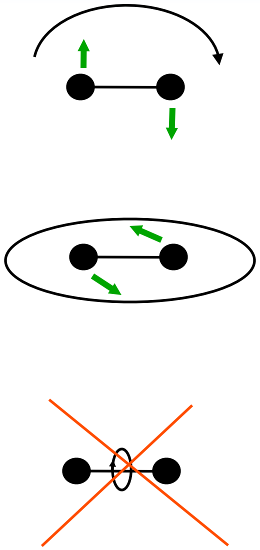
Rotations and Translations

If the Molecule Rotates or Translates as a Whole there is NO Restoring Force and therefore these Movements are Associated with the Vibrational Frequency Zero!

Translations

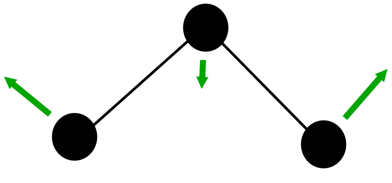


Rotations



Not a Rotation!

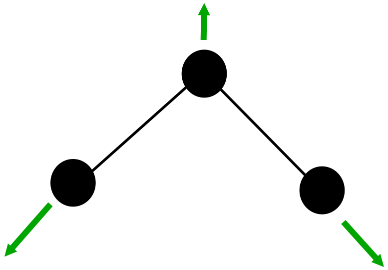
Normal Coordinates of Water



ν_1 (a_1)

Bending

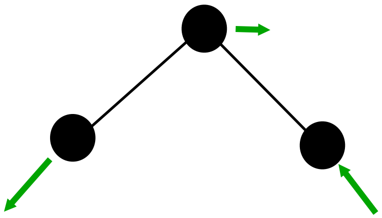
1595 cm^{-1}



ν_2 (a_1)

Symmetric Stretch

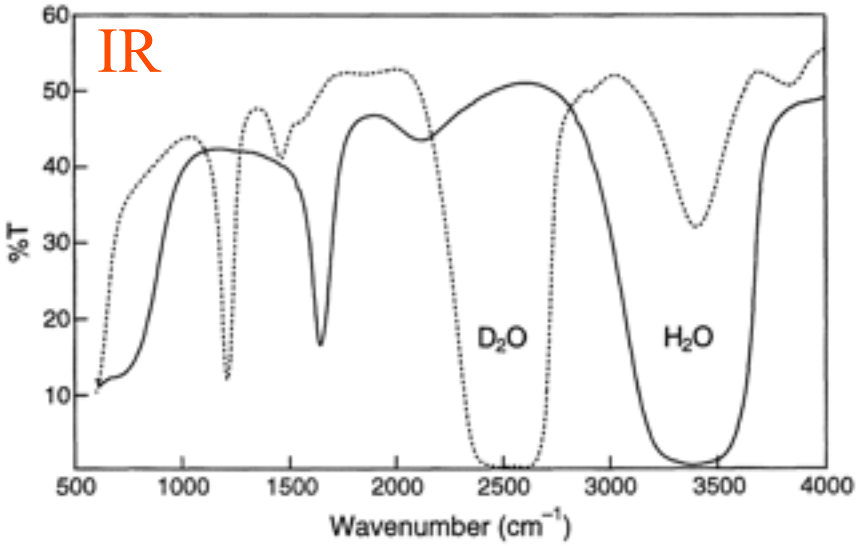
3652 cm^{-1}



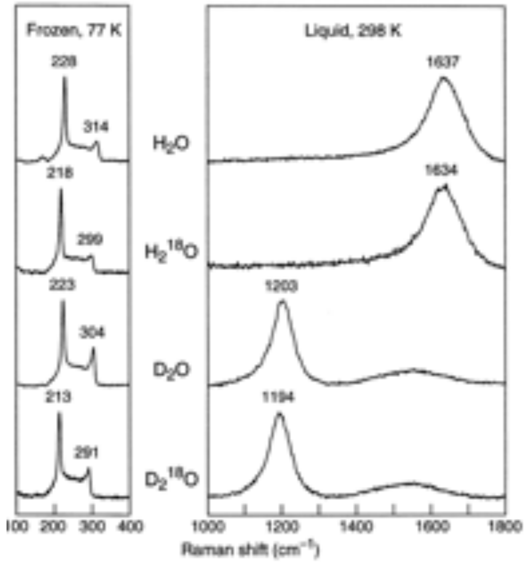
ν_3 (b_1)

Asymmetric Stretch

3756 cm^{-1}



IR



Raman

Normal Coordinates of CO_2

$\nu_3\ (\sigma_g^+)$
Antisymmetric
Stretch

2349 cm^{-1}

$\nu_2\ (\sigma_g^+)$
Symmetric
Stretch

1337 cm^{-1}

$\nu_1\ (\pi_u)$
Out of plane
Bending

667 cm^{-1}

$\nu_1\ (\pi_u)$
In plane
Bending

667 cm^{-1}

$\underbrace{\hspace{12em}}$

„Doubly Degenerate“ Vibration

Group Frequencies

Group	Compound Class	Frequency Range (cm^{-1})
C-H	Alkanes	2965-2850
	$-CH_3$	1450
		1380
	$-CH_2$	1465
	Alkenes	3095-3010
		700-1000
	Alkines	~3300
C-C	Alkanes	700-1200
C=C	Alkenes	1680-1620
C≡C	Alkines	2260-2100
C=O	Ketones	1725-1705
	Aldehydes	1740-1720
	Carbonic Acids	1725-1700
	Esters	1750-1730
	Amides	1700-1630
	Anhydrides	1850-1800
C-O	Any	1300-1000
-O-H	Alcohols, isolated	3650-3590
	Alcohols, H-bonded	3400-3200
	Carbonic Acids	3300-2500
-N-H	Primary Amines	~3500
	Secundary Amines	3500
C≡N	Nitriles	2260-2240

General Normal Coordinate Analysis

In General there will be a „Force Constant Matrix“ that is most Simply Calculated in Cartesian Displacements (e.g. with a *Quantum Chemistry Program*):

$$F_{X_AY_B} = \frac{\partial^2 E}{\partial X_A \partial Y_B} \xrightarrow[\textit{Masses}]{} \tilde{F}_{X_AY_B} = 15.57 \frac{F_{X_AY_B}}{\sqrt{M_A M_B}}$$

(in mdyn/Å)

$$\xrightarrow[\textit{Diagonalization}]{} \tilde{\mathbf{F}}\mathbf{Y}_I = x_I \mathbf{Y}_I \xrightarrow[\textit{Frequencies and Modes}]{} \nu_I = 1302.78\sqrt{x_I}$$

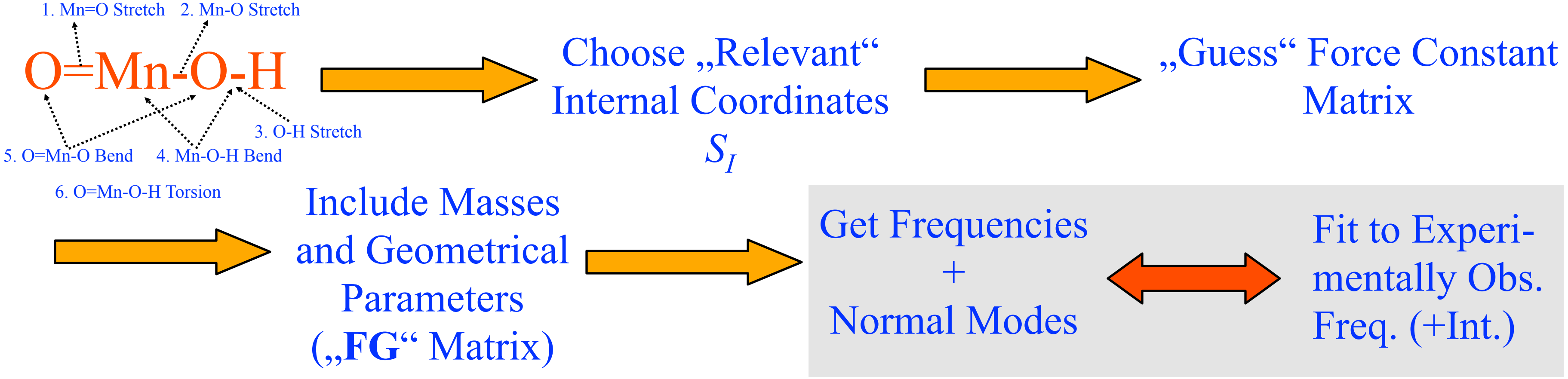
(in cm^{-1})

$$\mathbf{Q}_I = \underbrace{N^{-1/2}}_{\text{Normalization}} \left(\mathbf{M}^{-1/2}\mathbf{Y}_I\right)$$

1. Great if you have an Accurate Force Field (Good Quantum Chemical Method/Program/Theoretician)
2. Errors Reflect the Shortcomings of the Theoretical Methodology. Hard to Fix!

Normal Coordinate Fitting

In Practice one often Wants to do an *Experimentally Motivated Analysis*. For this Reason a Suitable Fragment is Chosen



Choose „Relevant“ Internal Coordinates S_I

„Guess“ Force Constant Matrix

Include Masses and Geometrical Parameters („**FG**“ Matrix)

Get Frequencies + Normal Modes

Fit to Experimentally Obs. Freq. (+Int.)

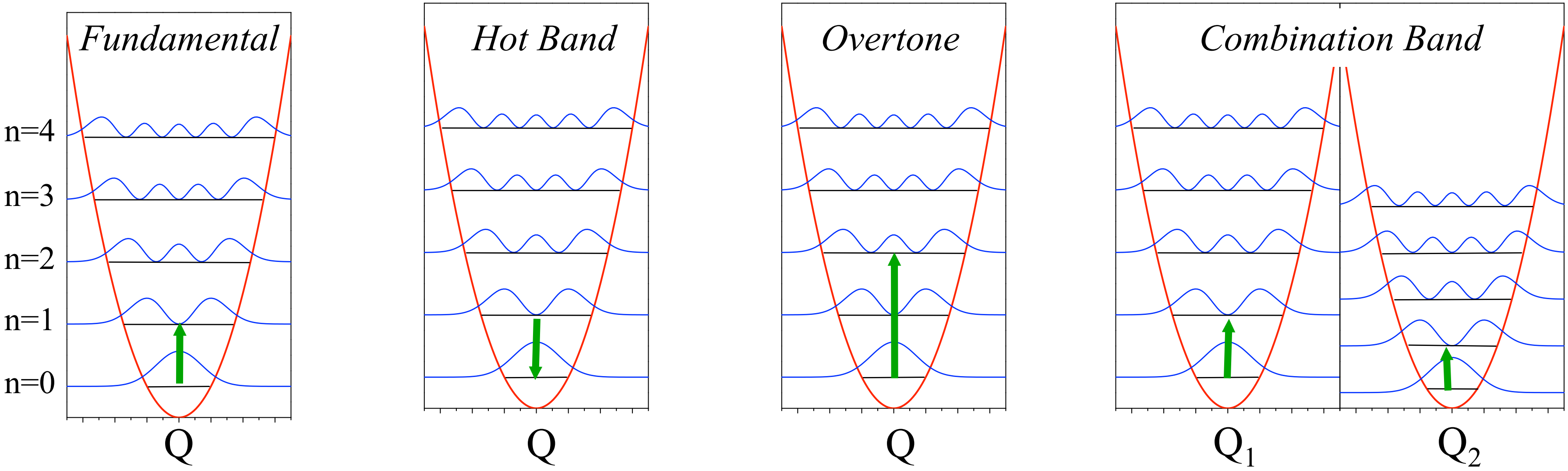
1. Normal Coordinate Fitting is Fairly Involved (→Specialists ☺)
   - Many Parameters → Underdetermined Equations!
   - Depends on Subjective Choice of Model System
2. Often Strong Simplications are Necessary.
3. Results may Apply to larger Classes of Compounds

II.B. Vibrational Intensities

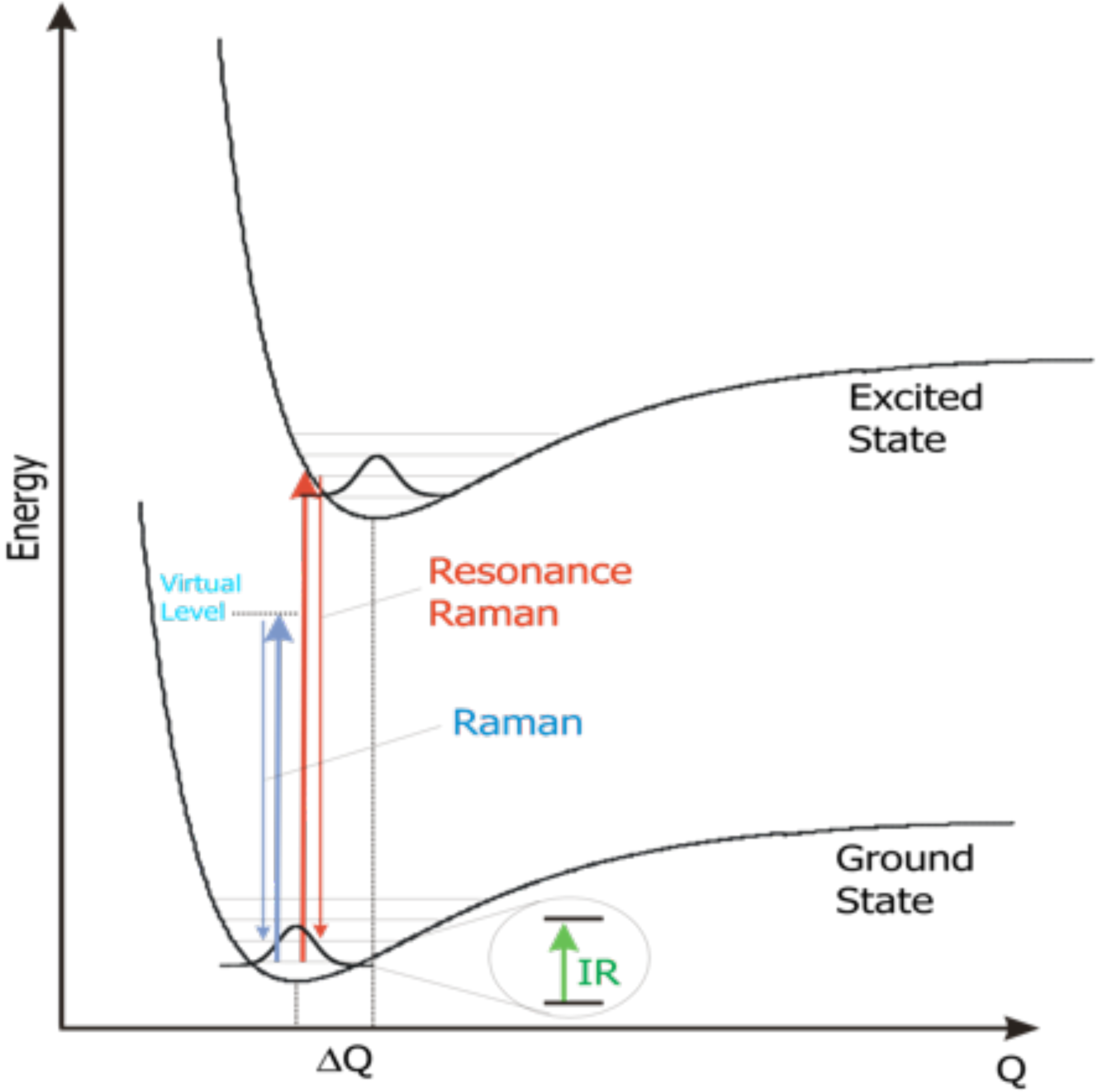
IR and Raman Transitions

IR and Raman Transitions lead from one Vibrational State on a given Electronic Potential Energy Surface to Another Vibrational States:

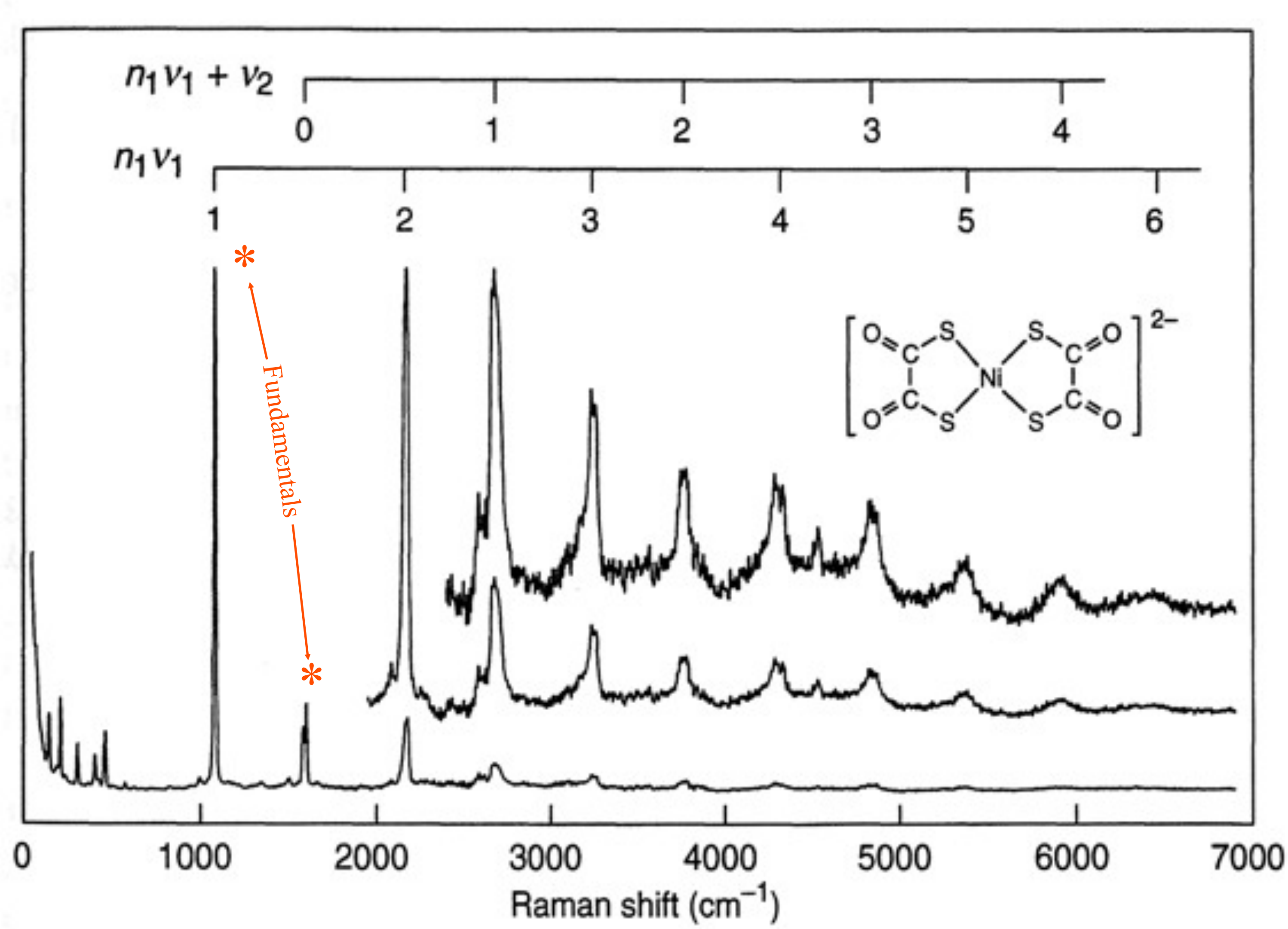
• **Fundamentals** :	**$n=0 \rightarrow n'=1$**	**$\Delta E=h\nu$**
• „Hot“ Bands :	$n=1 \rightarrow n'=n\pm1$	$\Delta E=h\nu$
• Overtones :	$n=0 \rightarrow n'=2,3,\ldots$	$\Delta E=2h\nu, 3h\nu,\ldots$
• Combination Bands :	$n_1=0, n_2=0 \rightarrow n_1'=1, n_2'=1$	$\Delta E=h\nu_1+h\nu_2$



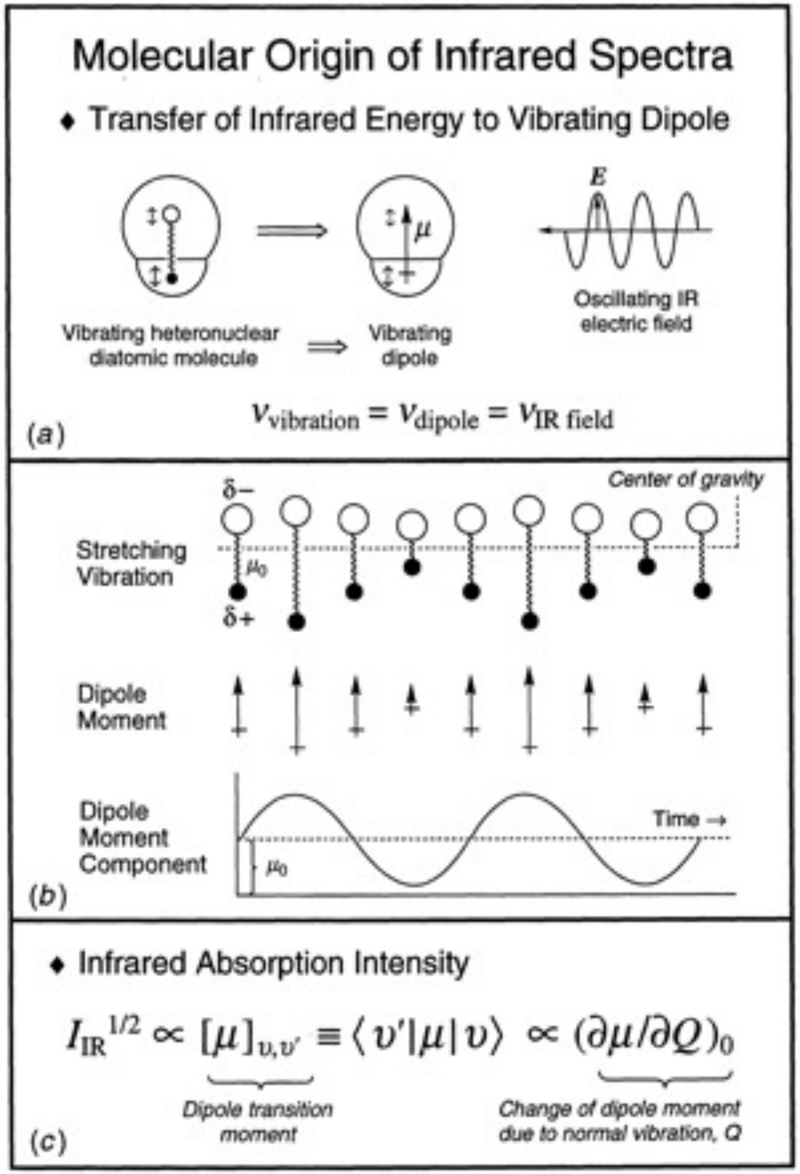
Physical Principles of IR and Raman Spectroscopy



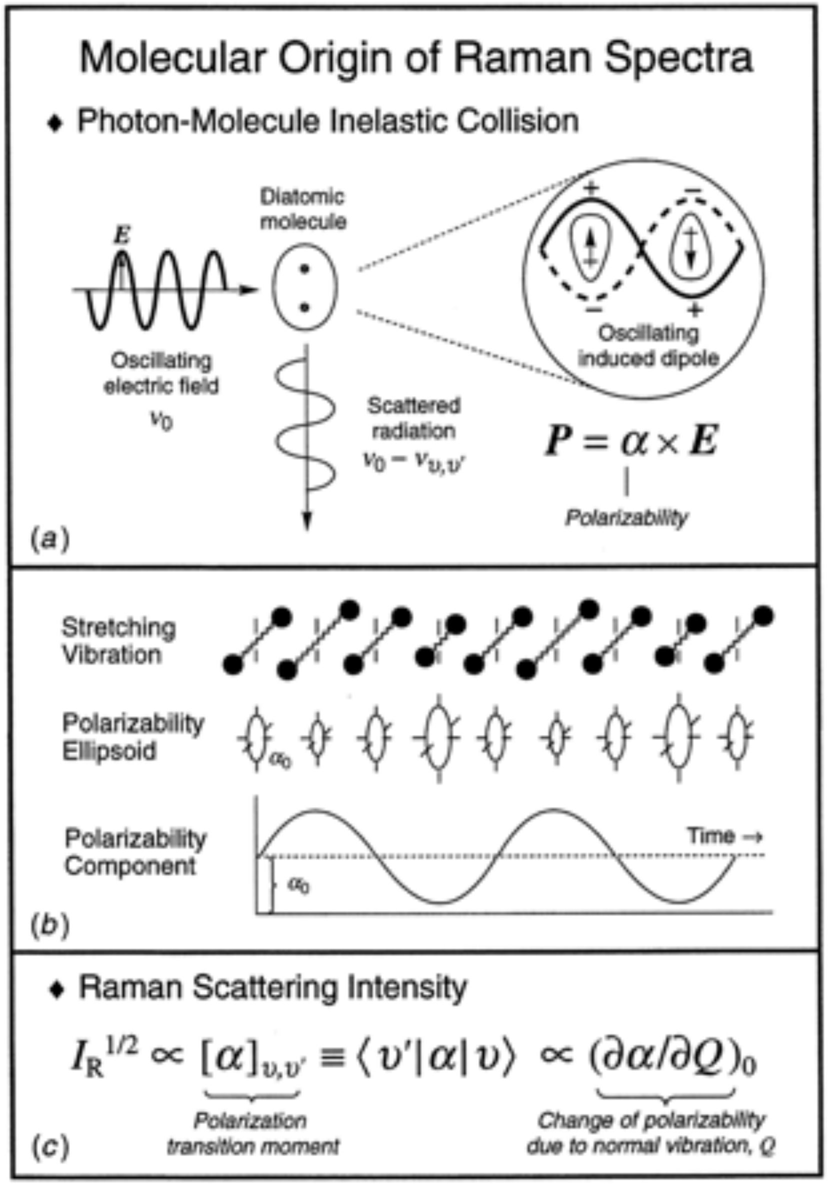
Overtone and Combination Bands



Physical Principle of IR Spectroscopy

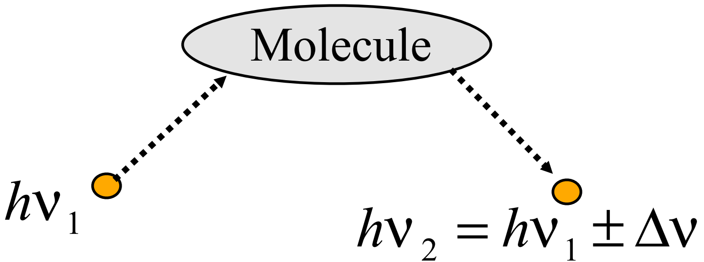


Physical Principle of Raman Spectroscopy



Polarizability:

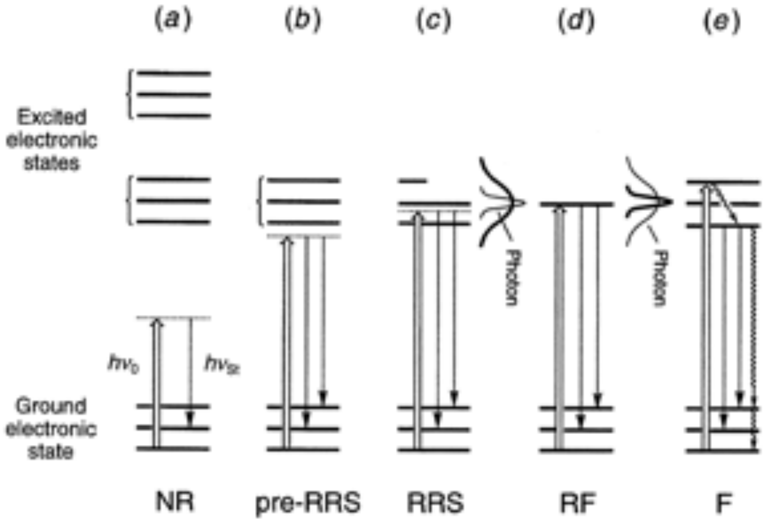
$$\alpha_{pq} = \sum_{n=0}^{\infty} \frac{\langle \Psi_0 | \hat{\mu}_p | \Psi_n \rangle \langle \Psi_n | \hat{\mu}_q | \Psi_0 \rangle}{E_n - E_0}$$



Molecule

$h\nu_1$

$h\nu_2 = h\nu_1 \pm \Delta\nu$



Raman vs. Resonance Raman Spectroscopy

If the Incident Light is Close to an Electronic Transition Energy one needs the Frequency Dependent Polarizability (FDP):

$$\alpha_{pq}(\nu) = \sum_{n=0}^{\infty} \underbrace{\frac{\langle\Psi_0|\hat{\mu}_p|\Psi_n\rangle\langle\Psi_n|\hat{\mu}_q|\Psi_0\rangle}{E_n - E_0 - h\nu + i\Gamma}}_{Resonant} + \underbrace{\frac{\langle\Psi_0|\hat{\mu}_p|\Psi_n\rangle\langle\Psi_n|\hat{\mu}_q|\Psi_0\rangle}{E_n - E_0 + h\nu + i\Gamma}}_{non-Resonant}$$

Transition Dipole Moment ($I_{ABS} \propto M_{0n}^2$)

Transition Energy

Photon Energy

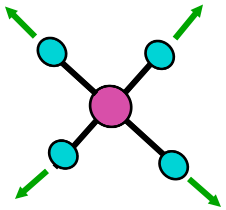
Damping Factor (Linewidth)

Thus, if : $E_I - E_0 \approx h\nu$ The *I*'th Excited State Dominates the FDP and:

Transition Energy Gradient

$$I_{Raman}^{1/2}(Q_n) \propto \sum_{p,q} \frac{\partial \alpha_{pq}(\nu)}{\partial Q_n} = \frac{1}{i\Gamma}\sum_{p,q} \underbrace{\frac{\partial}{\partial Q_n}\langle\Psi_0|\hat{\mu}_p|\Psi_I\rangle\langle\Psi_I|\hat{\mu}_q|\Psi_0\rangle}_{\substack{\text{Smaller Term} \\ \text{(Non Totally Symmetric Modes)}}} - \underbrace{\alpha_{pq}(\nu)\Big|_{Q=0} \frac{\partial}{\partial Q_n}\frac{(E_I - E_0)}{i\Gamma}}_{\substack{\text{Larger Term} \\ \text{(Totally Symmetric Modes)}}}$$

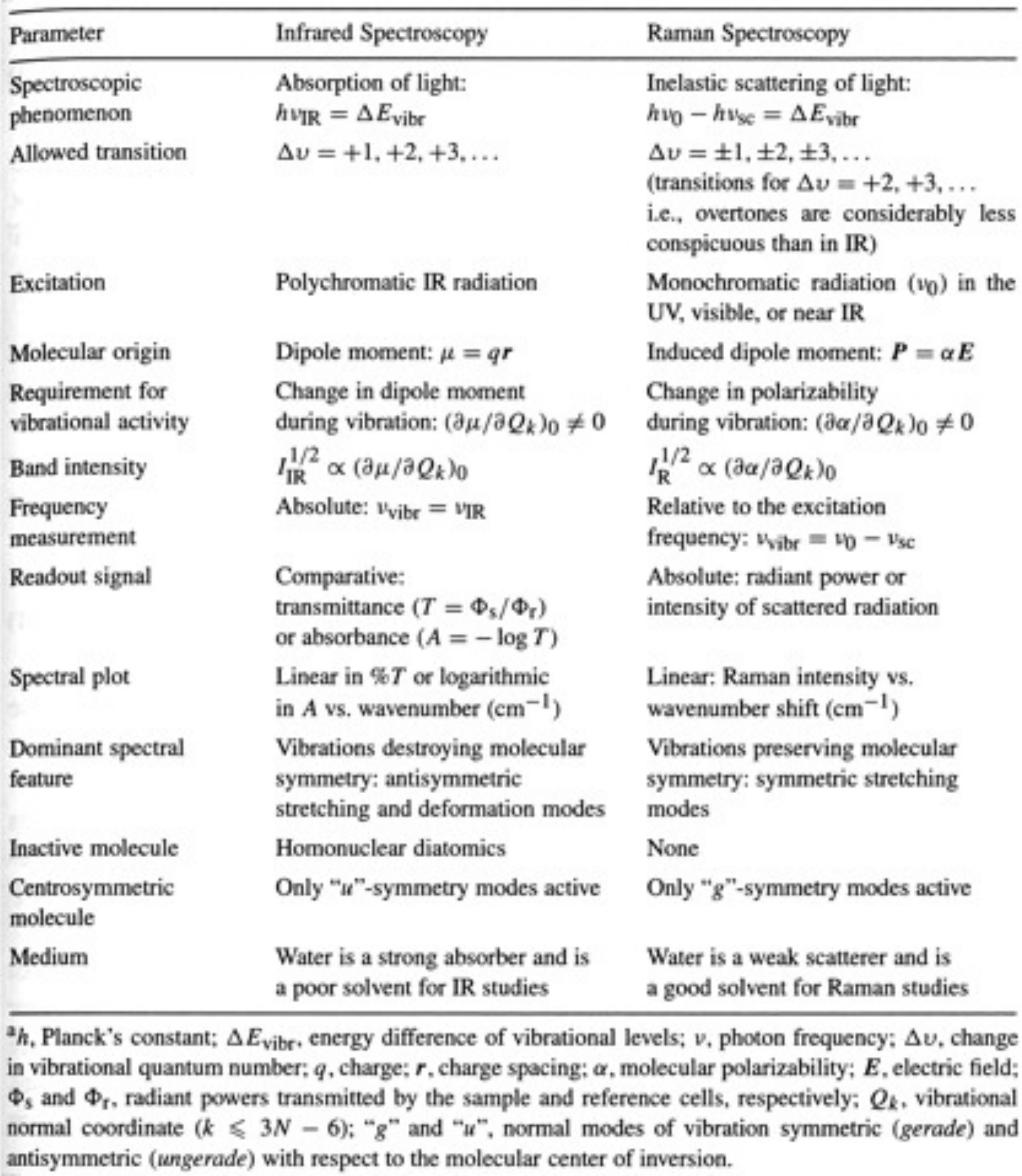
- Most Resonance Raman Spectra are Dominanted by Totally Symmetric Vibrations (Those that do not Break the Symmetry of the Molecule)
- Very good to Observe Stretching Vibrations



Summary of Theoretical Aspects

1. Force Constants Measure the Stiffness of Internal Motions (Stretches, Bends, Torsions).
2. Normal Modes and Vibrational Frequencies Describe the „Eigenvibrations“ (Distortions) of the Molecule. They Depend on the Force Constants (different for each Electronic State!), the Masses of the Atoms and the Geometry.
3. Isotope Shifts of Vibrational Frequencies Occur if the Masses of Atoms are Changed.
4. Normal Coordinate Analysis is the Combined Theoretical and Experimental Determination of the Force Field.
5. IR Intensities Depend on the Change of Dipole Moment During the Normal Vibrations.
6. Raman Intensities Depend on the Change of Polarizability During the Normal Vibrations (Infinite Summation over Excited States).
7. Resonance Raman Intensities Depend on the Distortion of the Molecule in the Electronically Excited State Reached by Laser Photon (Transition Energy Gradients) and the Transition Dipole Moments (like Absorption Spectra).

Summary of IR versus Raman

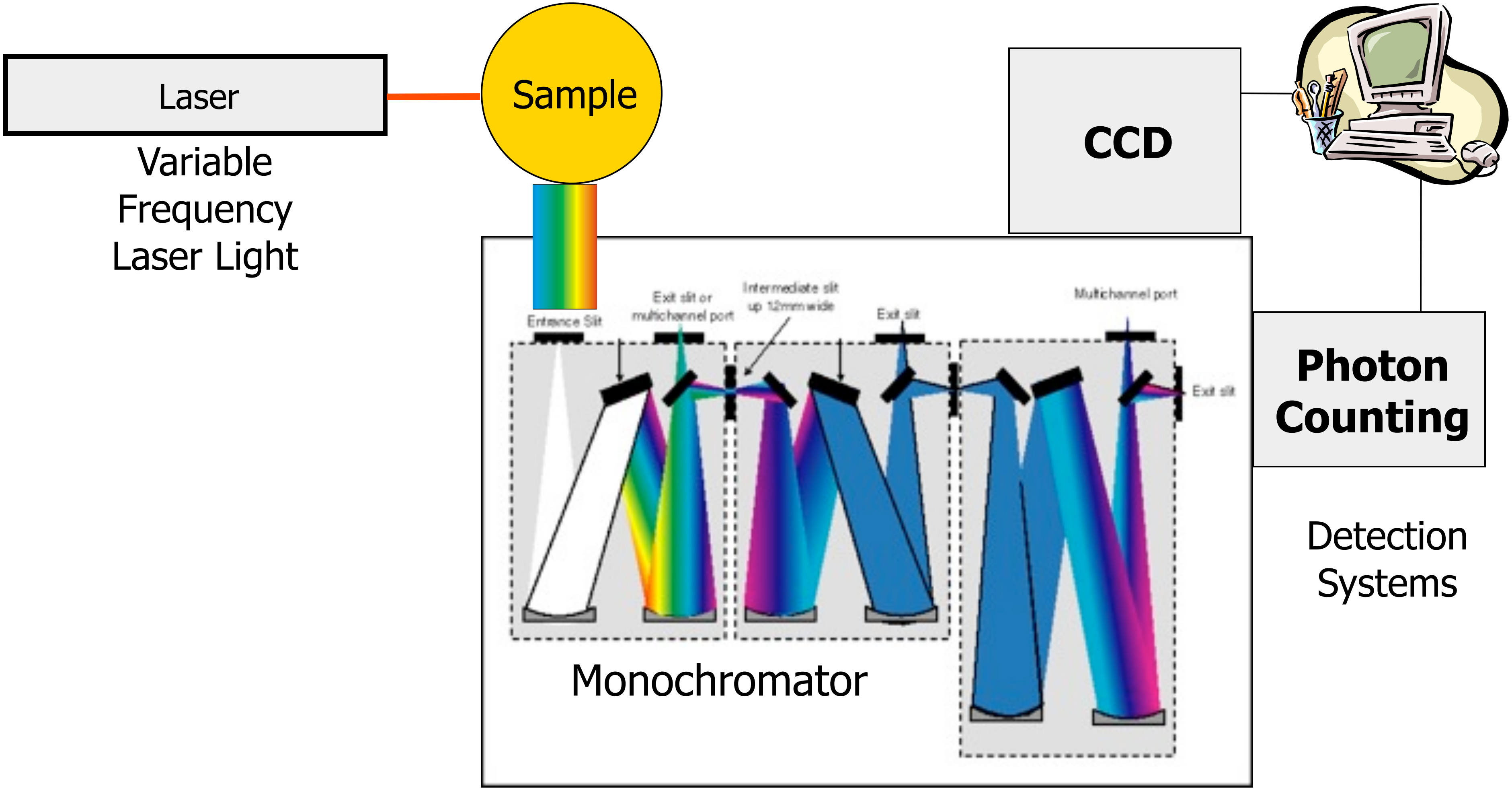


Parameter	Infrared Spectroscopy	Raman Spectroscopy
Spectroscopic phenomenon	Absorption of light: $h\nu_{\mathrm{IR}} = \Delta E_{\mathrm{vibr}}$	Inelastic scattering of light: $h\nu_0 - h\nu_{\mathrm{sc}} = \Delta E_{\mathrm{vibr}}$
Allowed transition	$\Delta\upsilon = +1, +2, +3, \ldots$	$\Delta\upsilon = \pm1, \pm2, \pm3, \ldots$ (transitions for $\Delta\upsilon = +2, +3, \ldots$ i.e., overtones are considerably less conspicuous than in IR)
Excitation	Polychromatic IR radiation	Monochromatic radiation (ν_0) in the UV, visible, or near IR
Molecular origin	Dipole moment: $\mu = q\boldsymbol{r}$	Induced dipole moment: $\boldsymbol{P} = \alpha \boldsymbol{E}$
Requirement for vibrational activity	Change in dipole moment during vibration: $(\partial\mu/\partial Q_k)_0 \neq 0$	Change in polarizability during vibration: $(\partial\alpha/\partial Q_k)_0 \neq 0$
Band intensity	$I_{\mathrm{IR}}^{1/2} \propto (\partial\mu/\partial Q_k)_0$	$I_{\mathrm{R}}^{1/2} \propto (\partial\alpha/\partial Q_k)_0$
Frequency measurement	Absolute: $\nu_{\mathrm{vibr}} = \nu_{\mathrm{IR}}$	Relative to the excitation frequency: $\nu_{\mathrm{vibr}} = \nu_0 - \nu_{\mathrm{sc}}$
Readout signal	Comparative: transmittance ($T = \Phi_s/\Phi_r$) or absorbance ($A = -\log T$)	Absolute: radiant power or intensity of scattered radiation
Spectral plot	Linear in %T or logarithmic in A vs. wavenumber (cm^{-1})	Linear: Raman intensity vs. wavenumber shift (cm^{-1})
Dominant spectral feature	Vibrations destroying molecular symmetry: antisymmetric stretching and deformation modes	Vibrations preserving molecular symmetry: symmetric stretching modes
Inactive molecule	Homonuclear diatomics	None
Centrosymmetric molecule	Only "*u*"-symmetry modes active	Only "*g*"-symmetry modes active
Medium	Water is a strong absorber and is a poor solvent for IR studies	Water is a weak scatterer and is a good solvent for Raman studies

[a] h, Planck's constant; ΔE_{vibr}, energy difference of vibrational levels; ν, photon frequency; $\Delta\upsilon$, change in vibrational quantum number; q, charge; $\boldsymbol{r}$, charge spacing; α, molecular polarizability; $\boldsymbol{E}$, electric field; Φ_s and Φ_r, radiant powers transmitted by the sample and reference cells, respectively; Q_k, vibrational normal coordinate ($k \leqslant 3N - 6$); "*g*" and "*u*", normal modes of vibration symmetric (*gerade*) and antisymmetric (*ungerade*) with respect to the molecular center of inversion.

III. Experimental Aspects

Experimental Raman Setup



Laser

Variable
Frequency
Laser Light

Sample

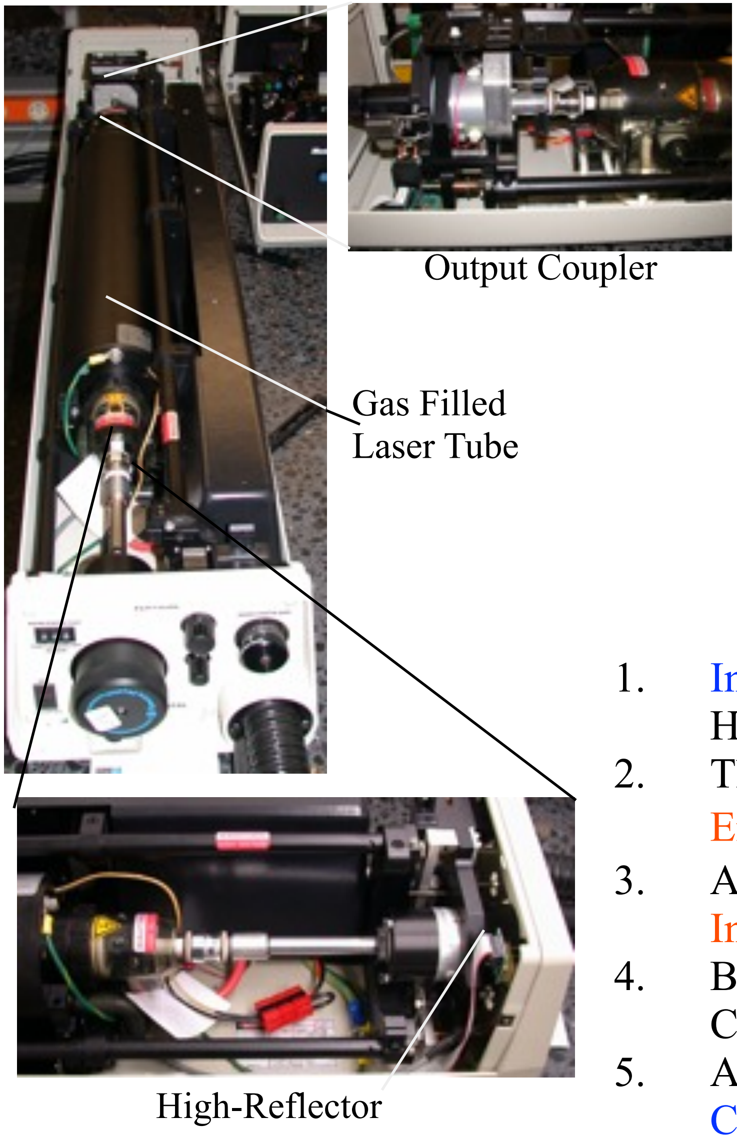
CCD

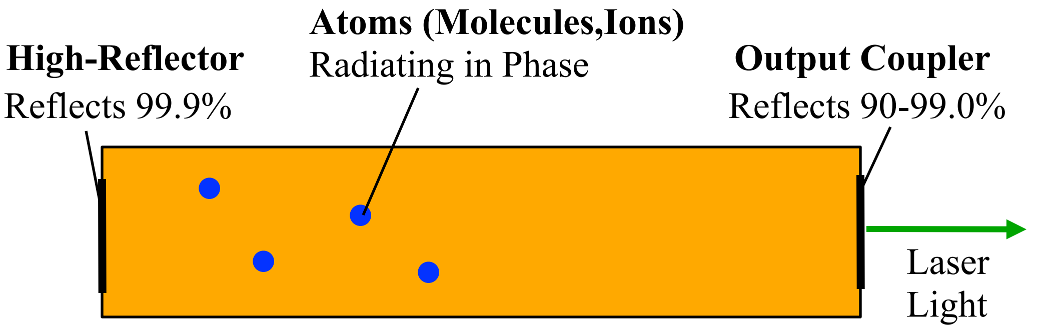
Photon Counting

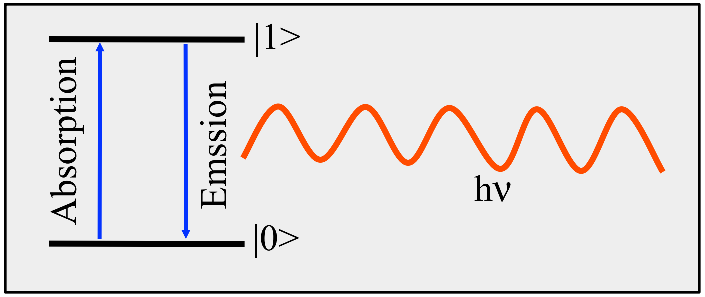
Detection
Systems

Monochromator

Lasers (Light Amplification by Stimulated Emission)

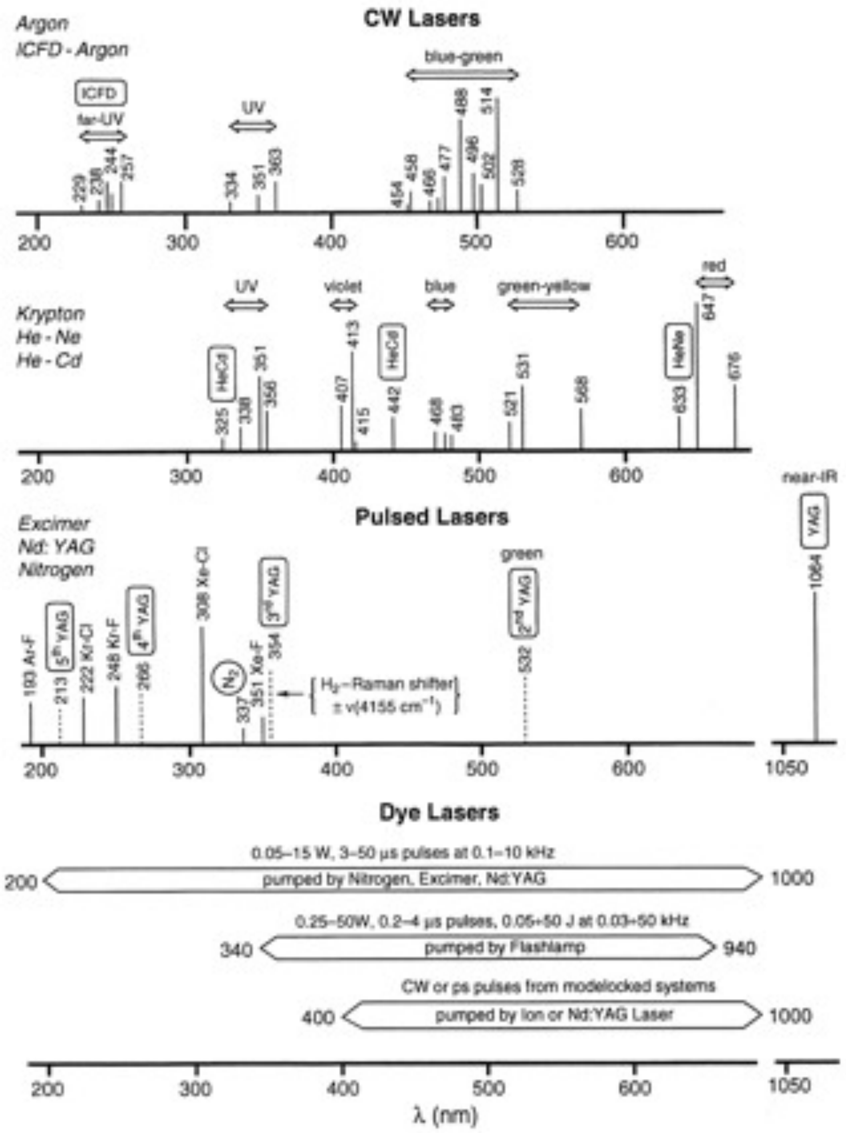






1. Initial Photons are created by Spontaneous Emission due to Heating or Electrical Discharge.
2. The emitted Photons Stimulate other Atoms (Molecules, Ions) to Emitt Photons of the Same Frequency *in Phase* ($A^*+h\nu \rightarrow A+2h\nu$)
3. As these Photons are absorbed by neighbouring Atoms Population Inversion is achieved.
4. By putting the Atoms (Molecules, Ions) between two Mirrors a Coherent Photon Avalanche is Created.
5. A Small Percentage of Photons is „Liberated“ through the Output Coupler.

Laser Sources



Ar+ pump Laser

Ar^+/Kr^+ Mixed GasLaser ~350-670 nm

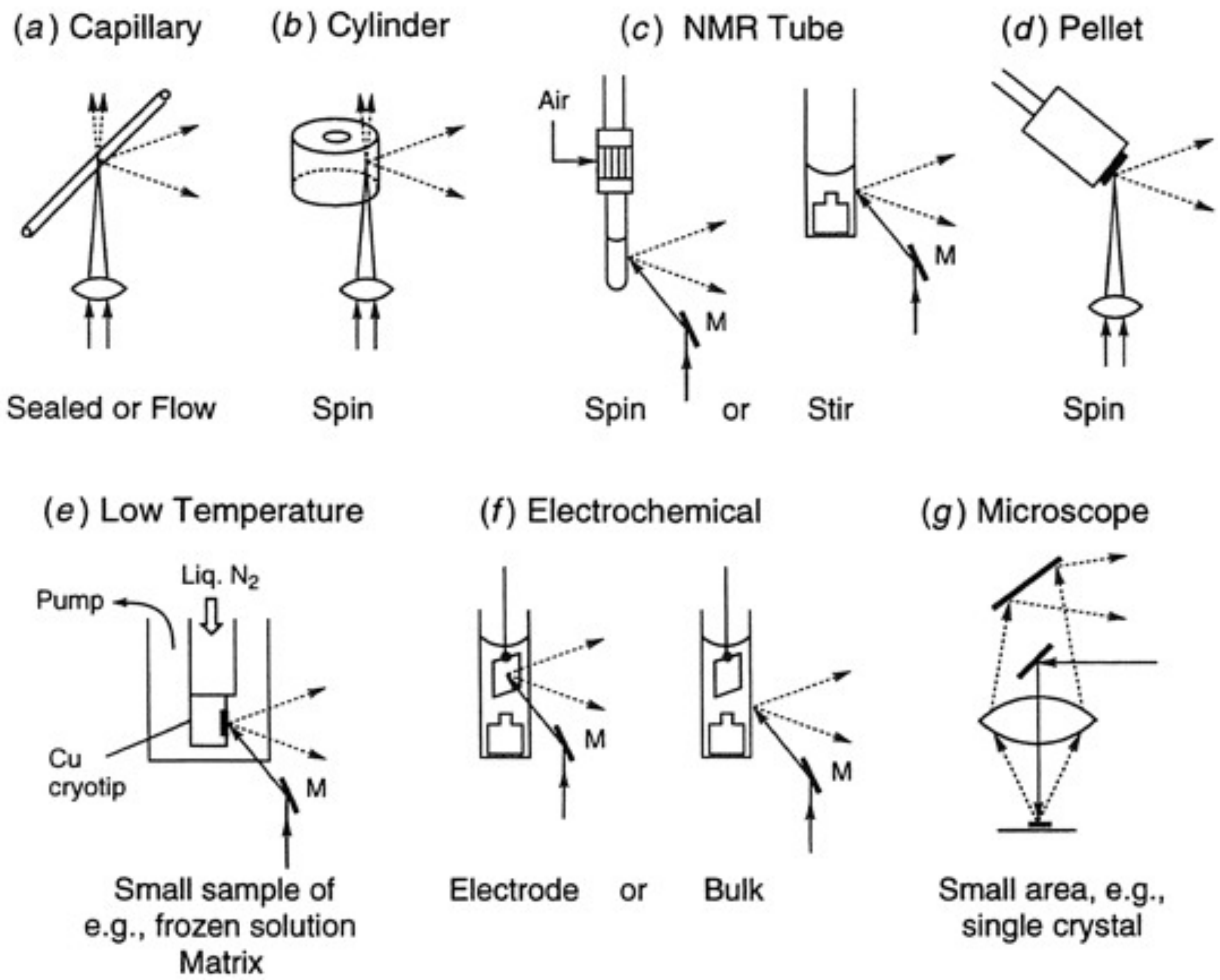
Power -meter

Ti:Sa Laser ~700-1000 nm

Bandpath Filter

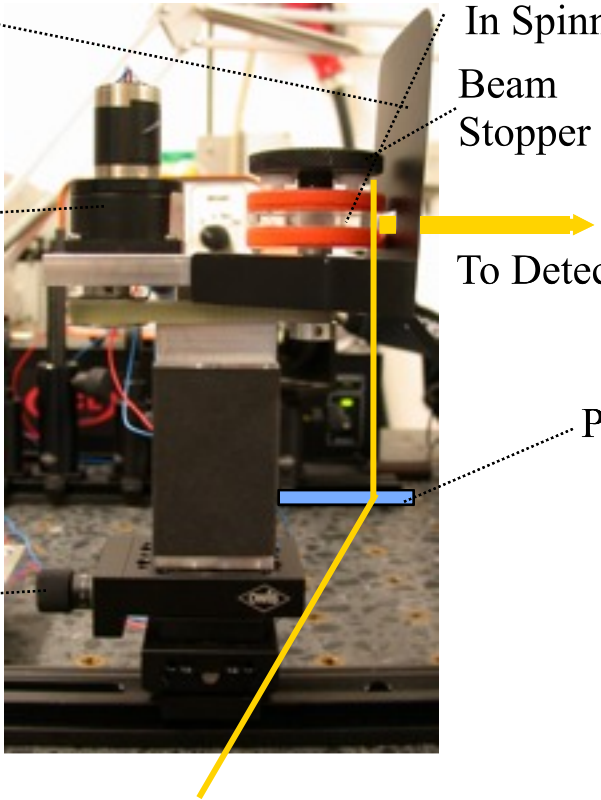
Focussing Lens

Sample Compartments



Sample Compartments

Rotating Cell



Reflection Shield

Liquid Sample In Spinning Cell

Beam Stopper

Motor

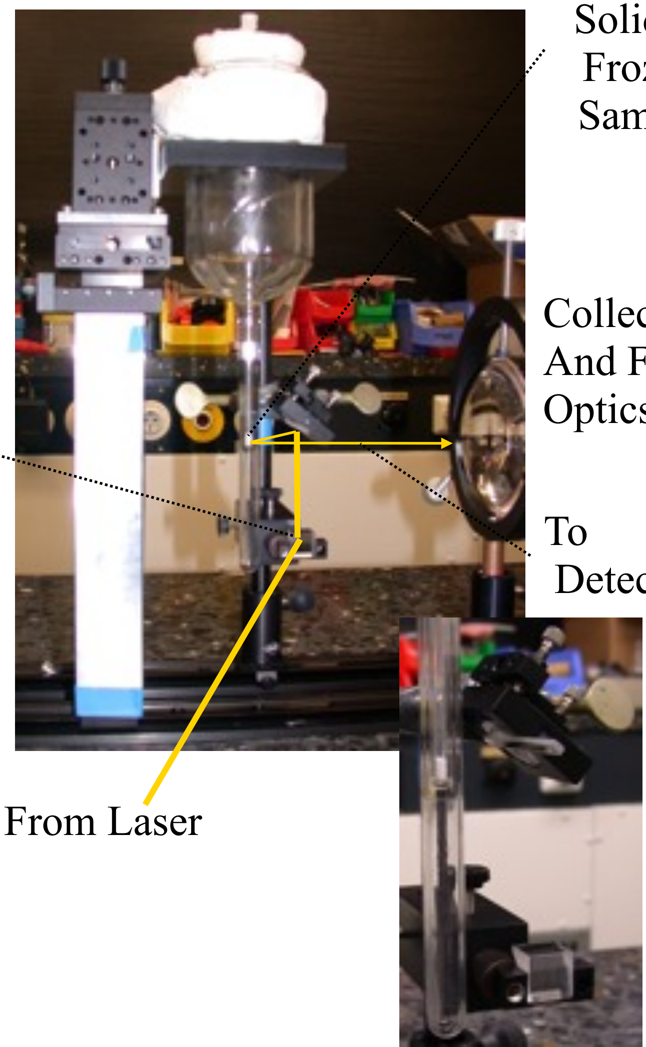
To Detector

Prism

Position Adjustment

From Laser

Liquid N_2 Dewar



Solid or Frozen Sample

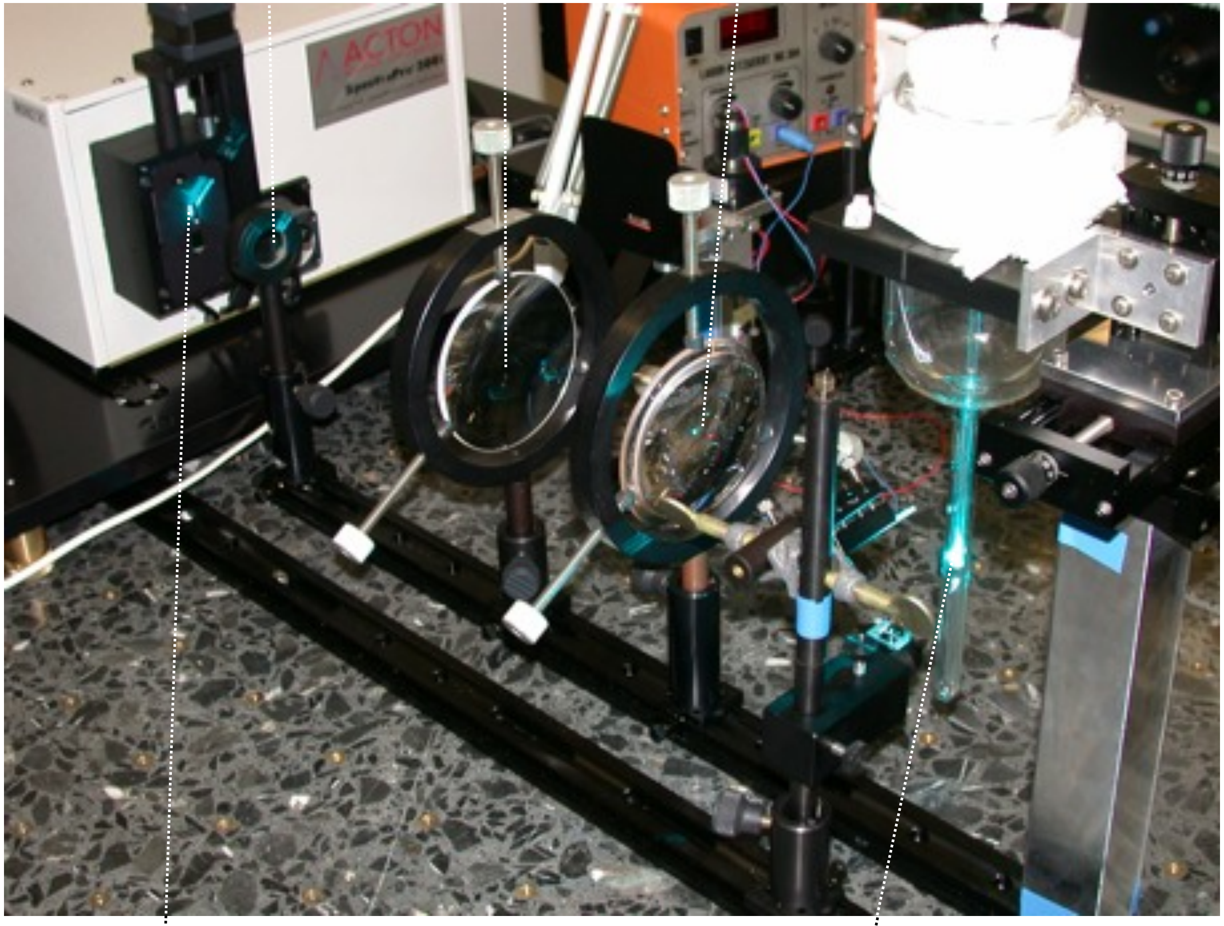
Collection And Focussing Optics

Prism

To Detector

From Laser

Collecting Optics



Depolarizer

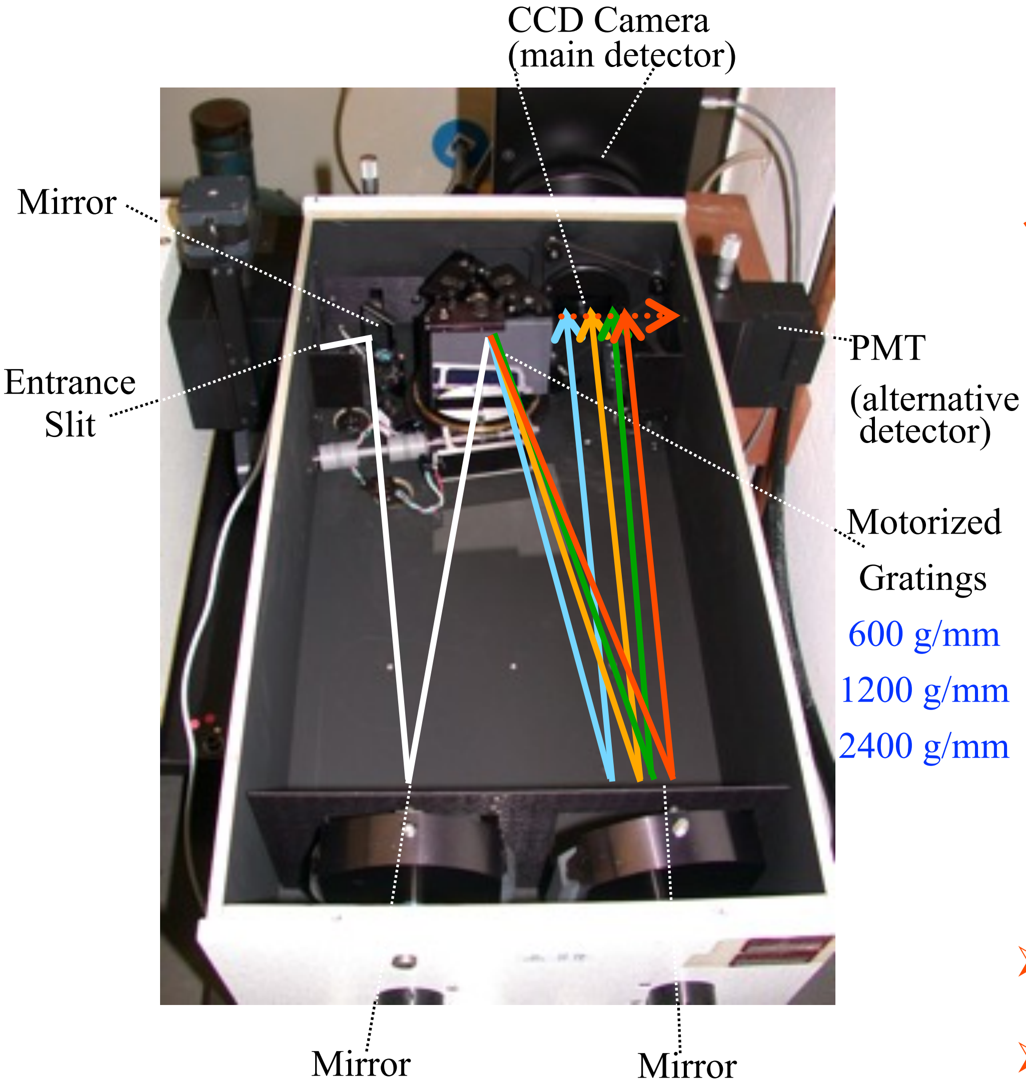
Focussing
Lens

Collecting
Lens

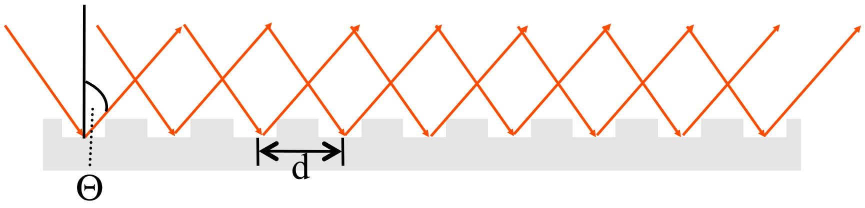
Entrance
Slit

Sample

Monochromators



A Diffraction Grating is an *Dispersive Element* used to Separate Light into Single Wavelength Contributions



Interference of Incoming and Outgoing Light Waves Produces and Interference Pattern with Maxima if:

$$d\sin(\Theta) = m\lambda$$

wavelength

Distance between Grooves

,Order' of the Maximum=0,1,...

Resolving Power of a Grating

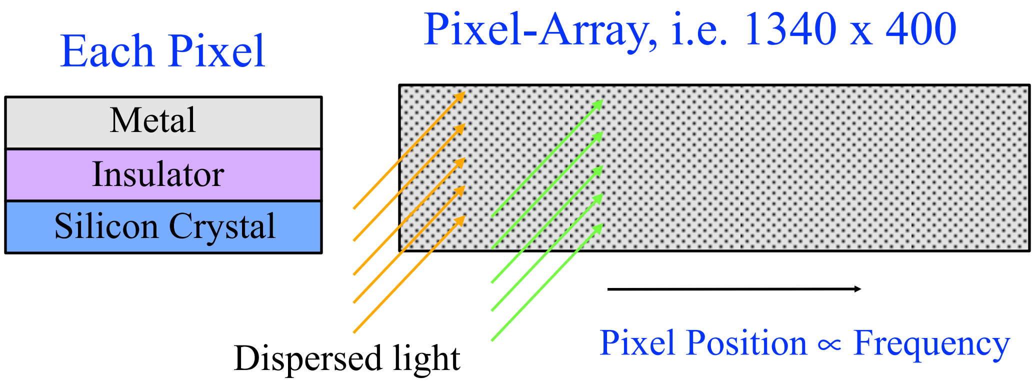
$$\frac{\lambda}{|\Delta\lambda|} = mN$$

Number of grooves

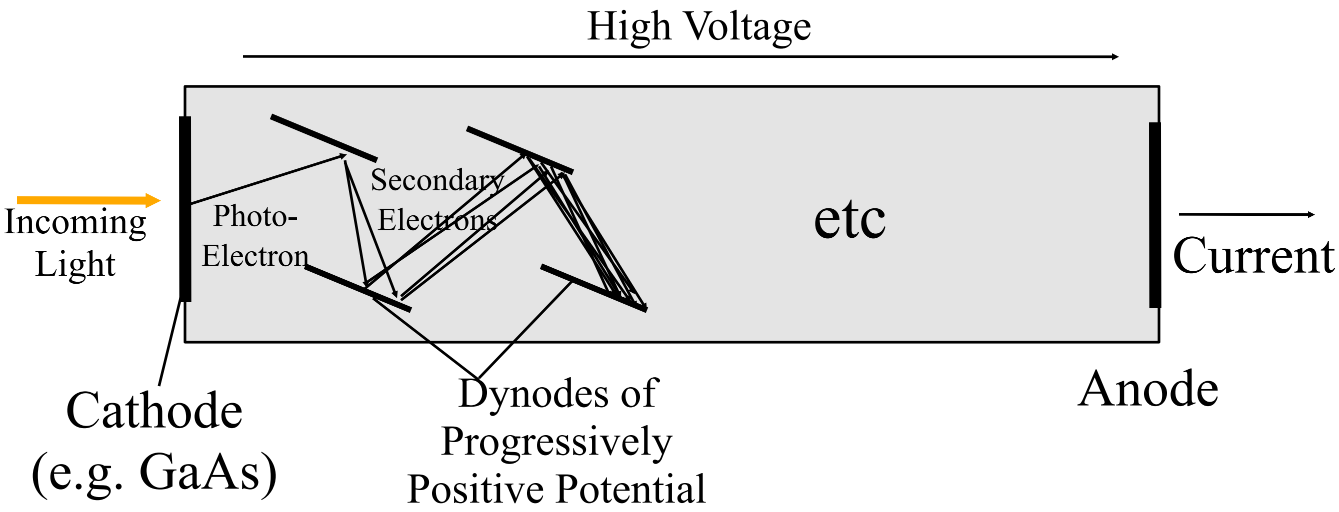
- More Grooves = More Resolution
  = Smaller Bandpath
- Longer Wavelength= Higher Resolution
  = Smaller Bandpath

→ Use Smaller Grating at Longer Wavelengths

Detection Systems (CCD, PMT)



- High Conversion Efficiency (40-60%)
- Low Noise Levels
- Simultaneous Detection of Large Parts of the Spectrum!

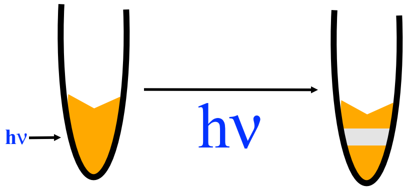


Possible Experimental Complications

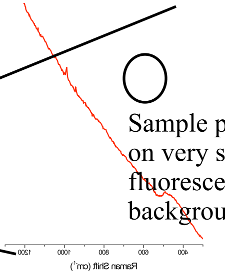
(„Dirty Laundry Slide“)

1. Decomposition due to Overheating
2. Photodissociation of Bonds
3. Fluorescence Background
4. Exceedingly Weak Signals
5. Features Obscured by Solvent Peaks
6. External Artifacts

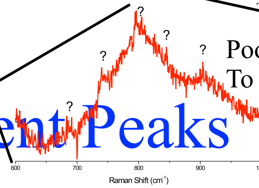
   (Cosmic Rays,Other Light Sources)
7. Plasma Lines from the Laser



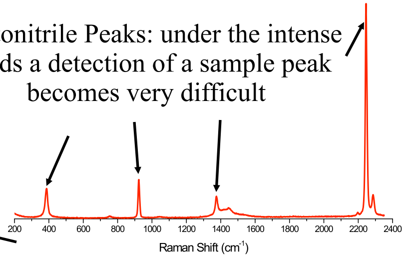
Illuminated area changes colour which indicates decomposition



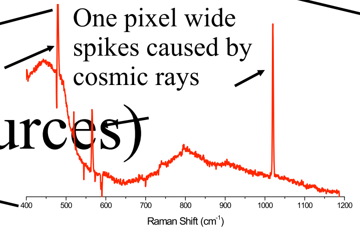
Sample peak on very steep fluorescence background



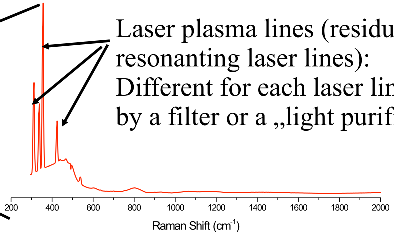
Poor Signal To Noise ratio



Acetonitrile Peaks: under the intense bands a detection of a sample peak becomes very difficult

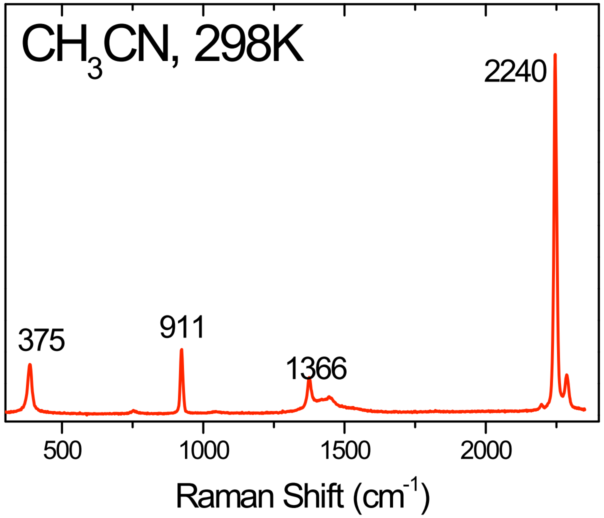


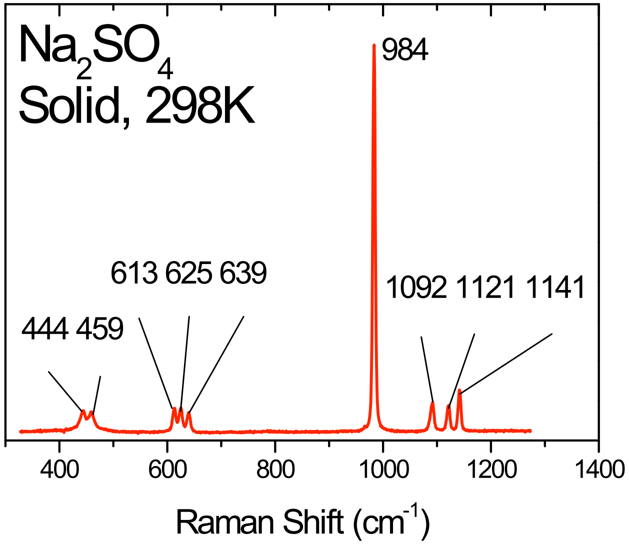
One pixel wide spikes caused by cosmic rays

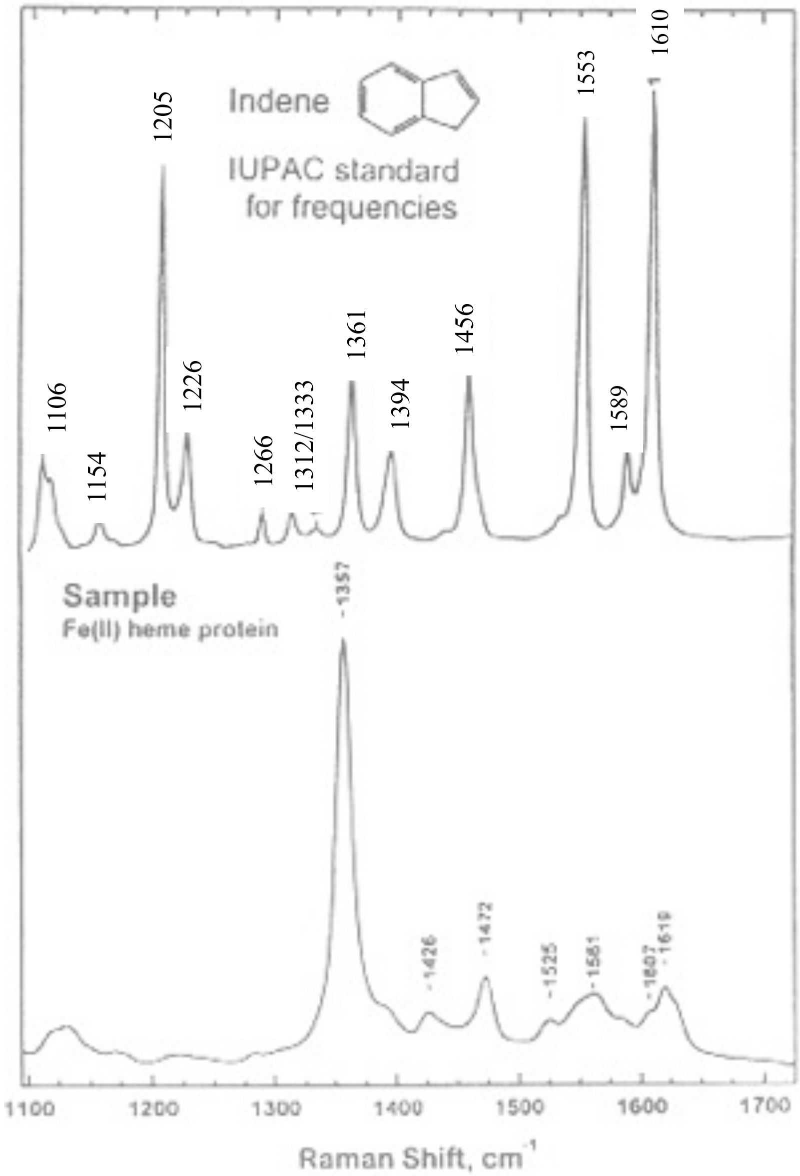


Laser plasma lines (residual intensity from non-resonanting laser lines):
Different for each laser line; sharp; maybe cut off by a filter or a „light purification device“)

Frequency Calibration



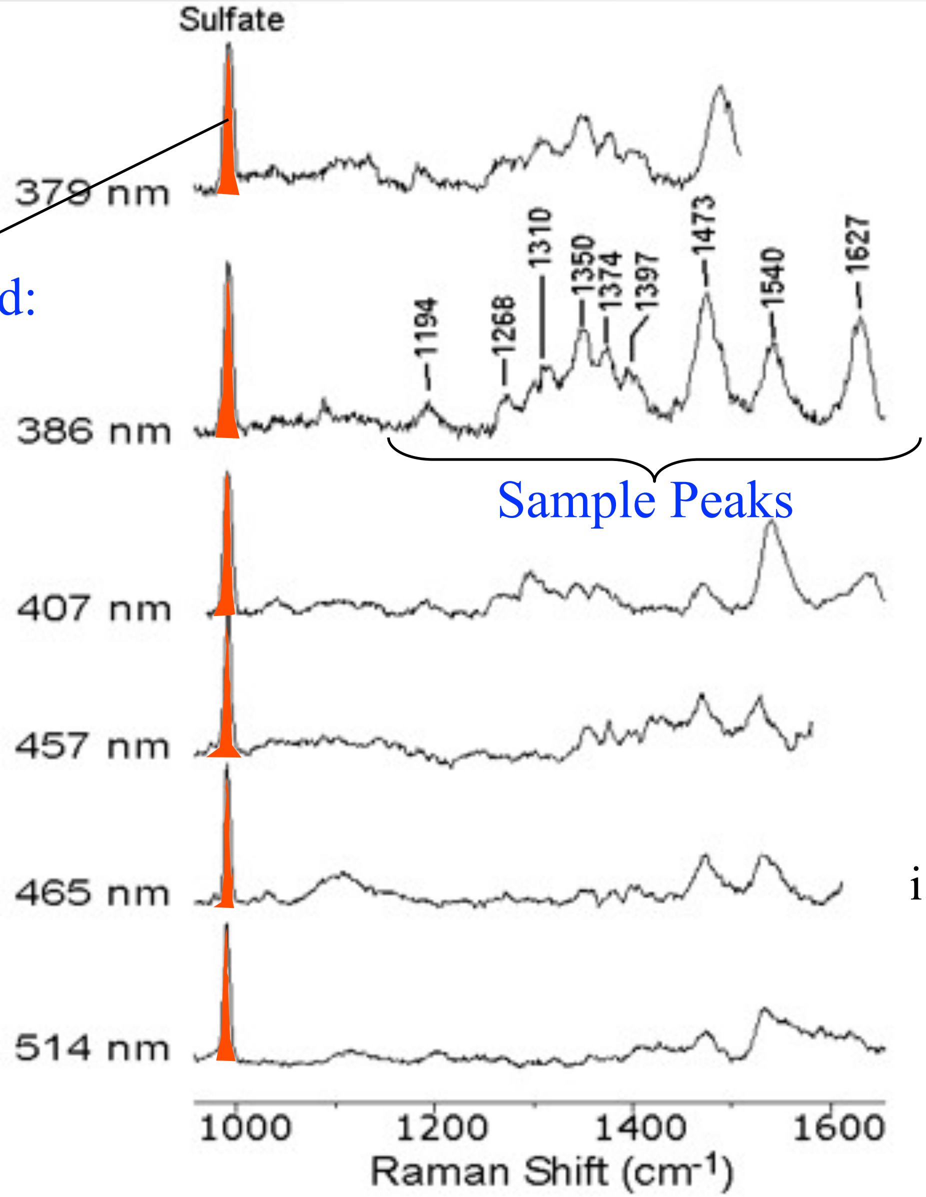




Intensity Calibration

Peak of Internal Standard:

- Frequency
- Intensity



$$\frac{I_{sample}}{I_{Standard}} \propto \frac{c_{sample}\nu^{4}I_{0}(\nu)}{c_{standard}\nu^{4}I_{0}(\nu)}\,\frac{P^{RR}_{sample}(\nu)}{P^{R}_{standard}}$$

$$P^{RR}_{sample}(\lambda) = const \times \frac{I_{sample}}{I_{Standard}}$$

Can Construct an

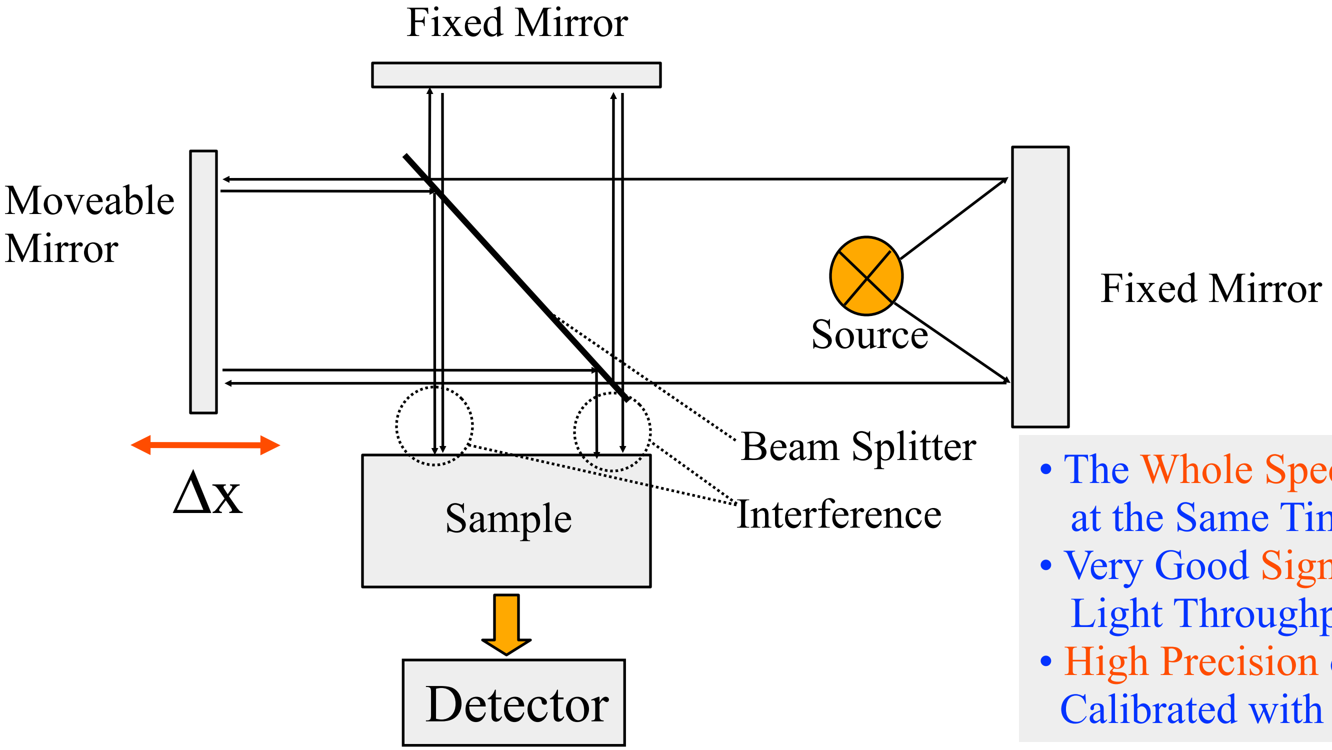
„Excitation Profile“

i.e. the Intrinsic RR Intensity

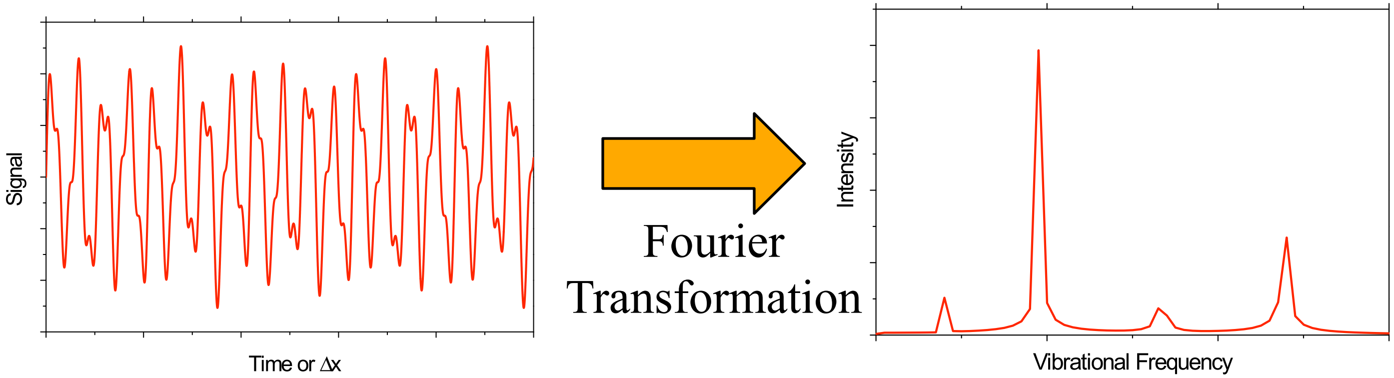
P(ν) as a Function of

Excitation Wavelength

Experimental Setup for IR Spectroscopy



- The Whole Spectrum is Measured at the Same Time
- Very Good Signal/Noise due to High Light Throughput
- High Precision of Frequencies if Calibrated with a Laser (~0.1-0.01 cm^{-1})

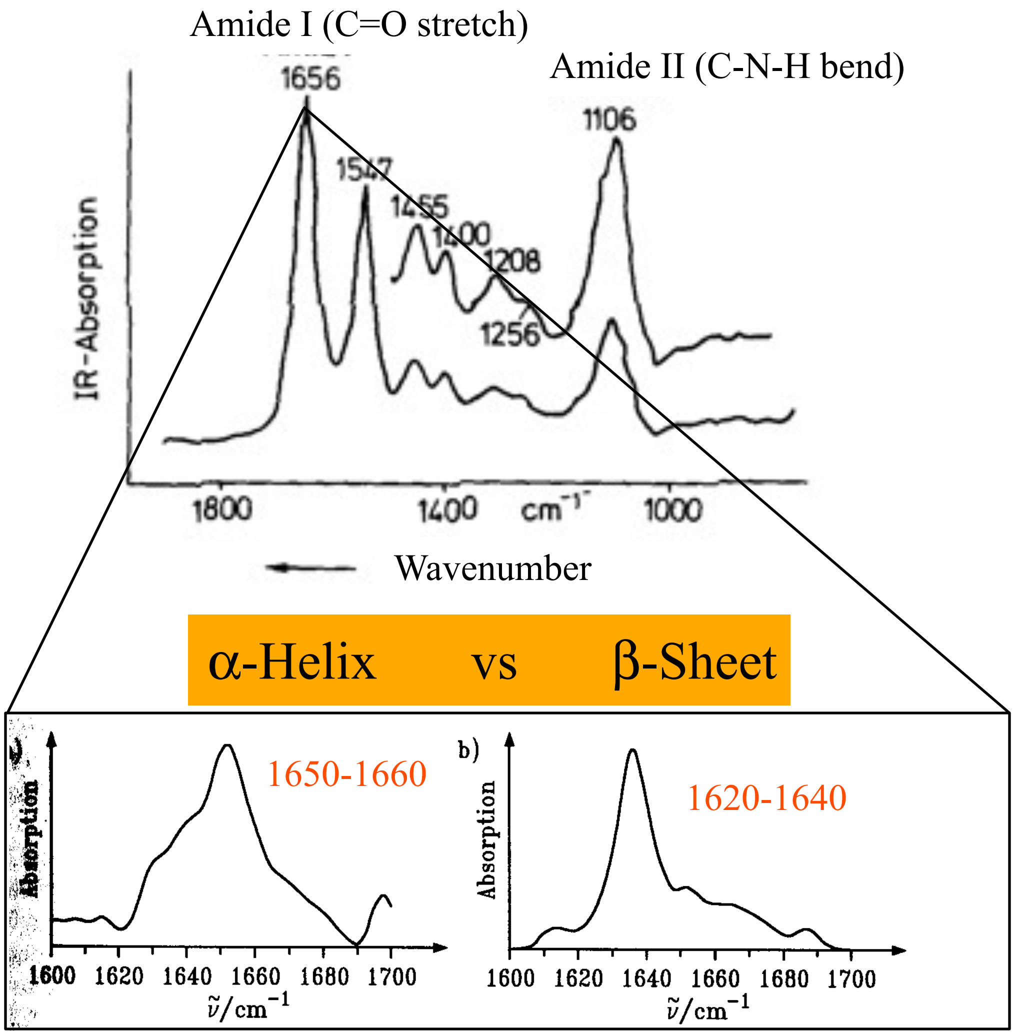


IR-Spectrum = Measurement with and without the Sample

IV. Bioinorganic Examples

Protein Secondary Structure

Protein FT-IR Spectra



Amide I (C=O stretch)

Amide II (C-N-H bend)

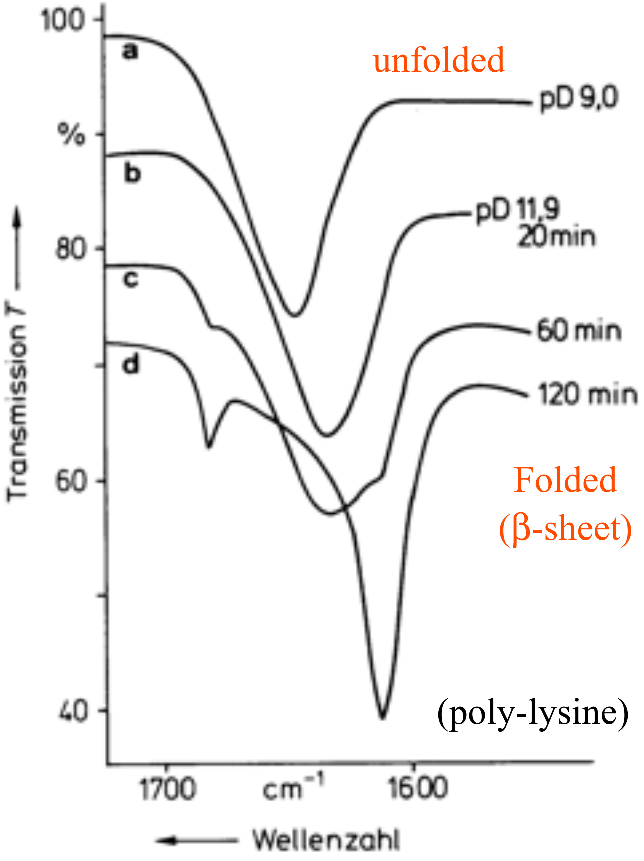
Wavenumber

α-Helix vs β-Sheet

1650-1660

1620-1640

Follow Protein Folding

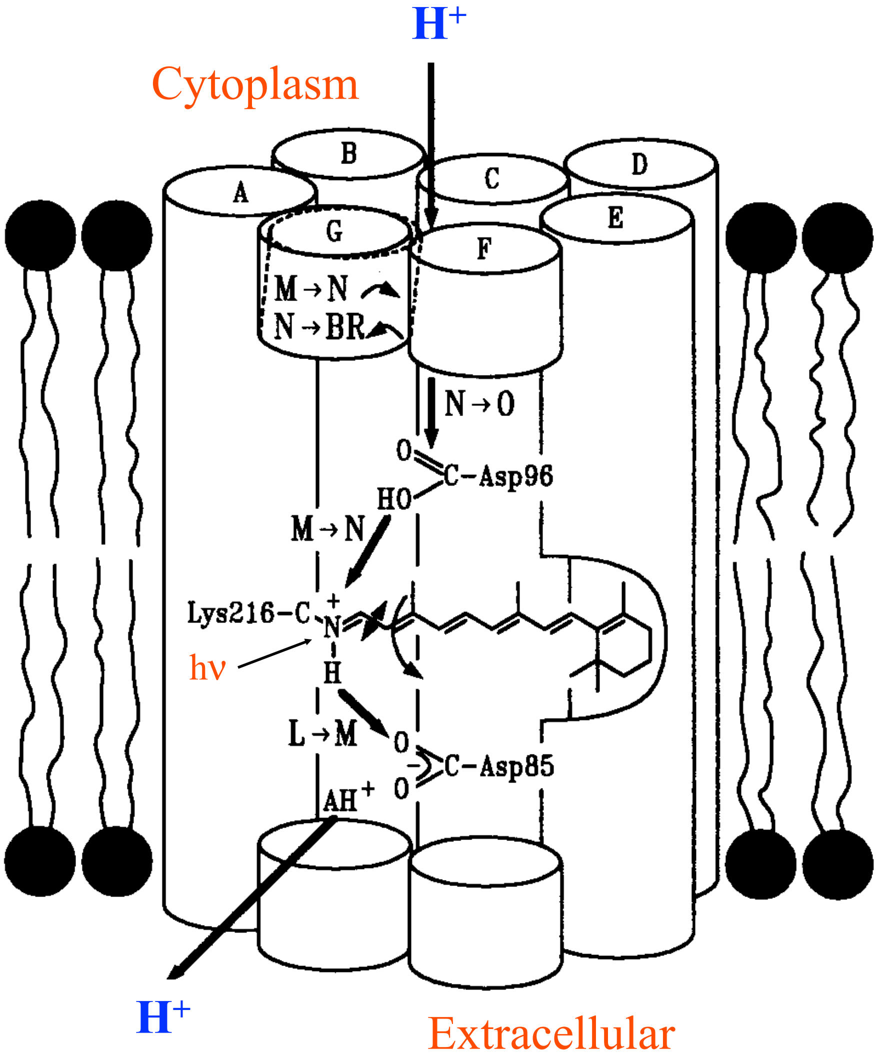


unfolded

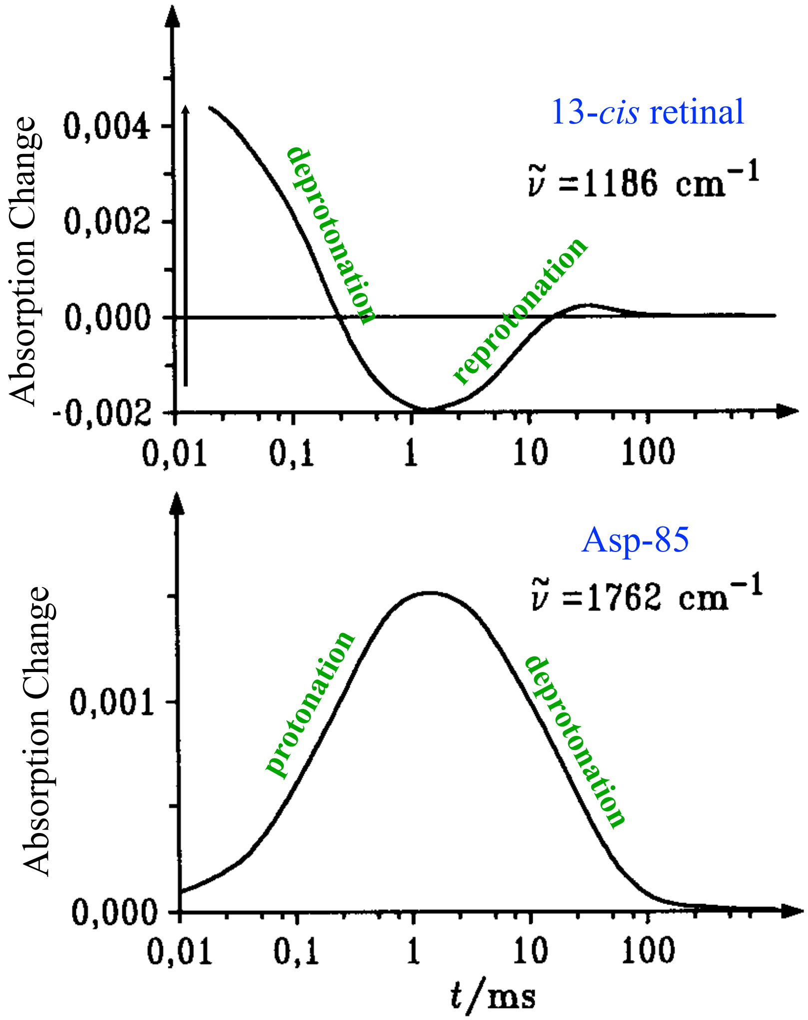
Folded (β-sheet)

(poly-lysine)

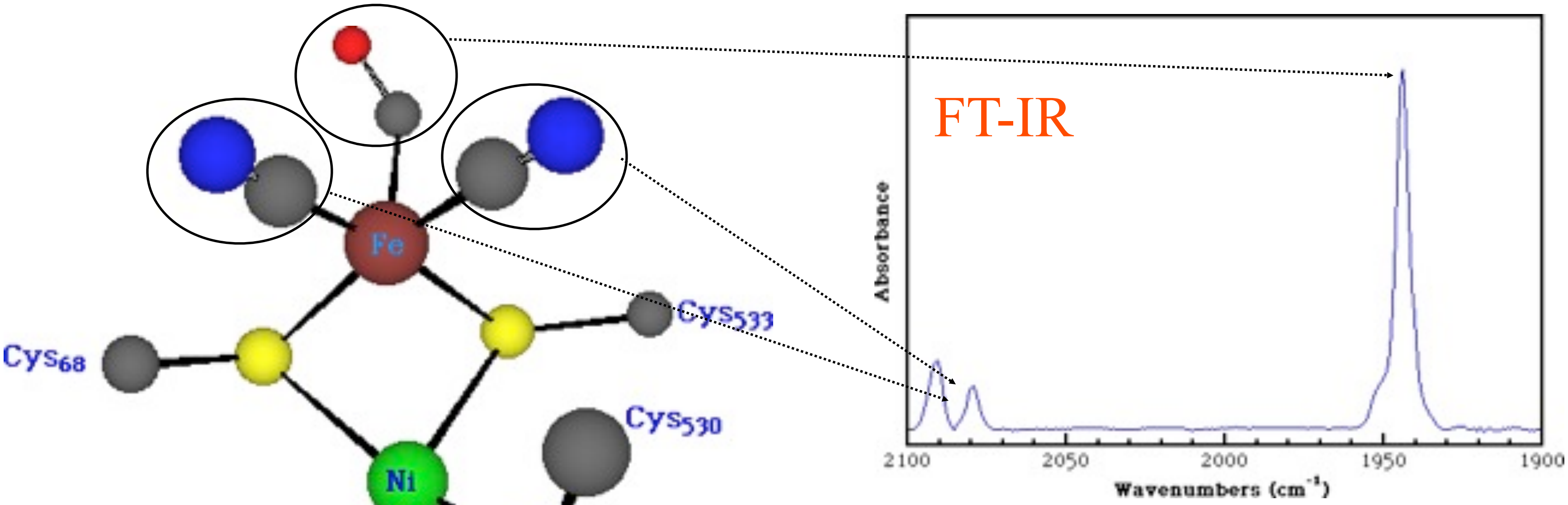
IR Kinetics - Bacteriorhodopsin



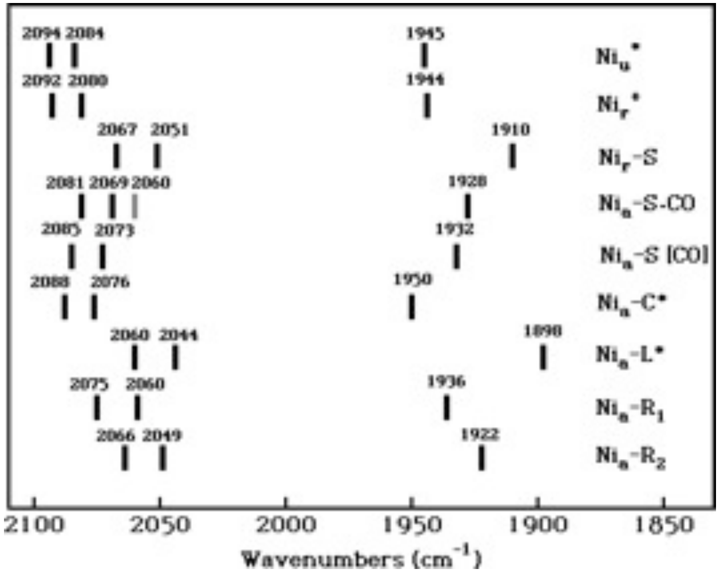
Time Resolved FT-IR



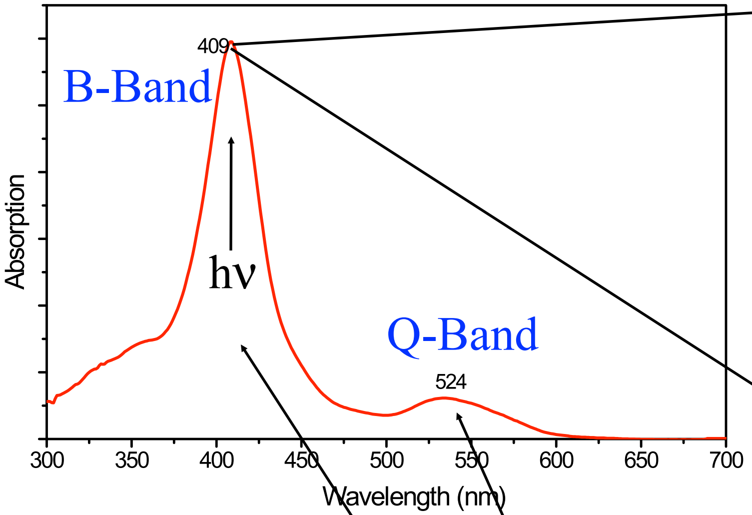
Hydrogenase

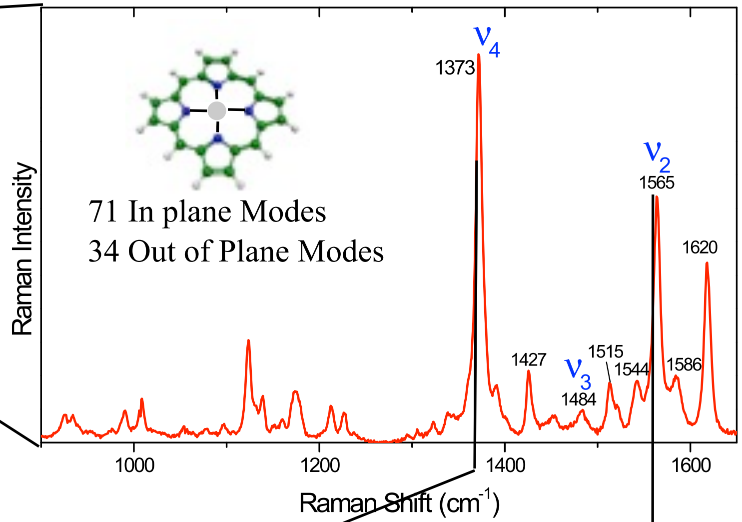


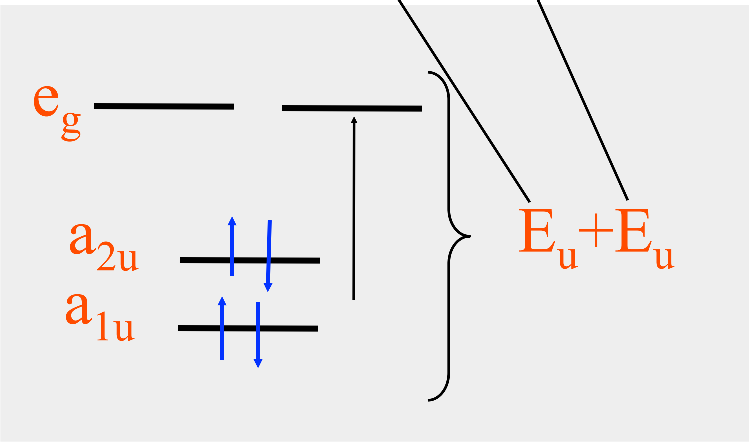
$$H_2 \longrightarrow 2H^+ + 2e^-$$



Hemoproteins – Spin and Oxidation State







Oxidation State Marker

- Fe(III) : $\nu_4 \sim 1375$ cm^{-1}
- Fe(II) : $\nu_4 \sim 1360$ cm^{-1}
- Fe(IV) : $\nu_4 \sim 1380$ cm^{-1}

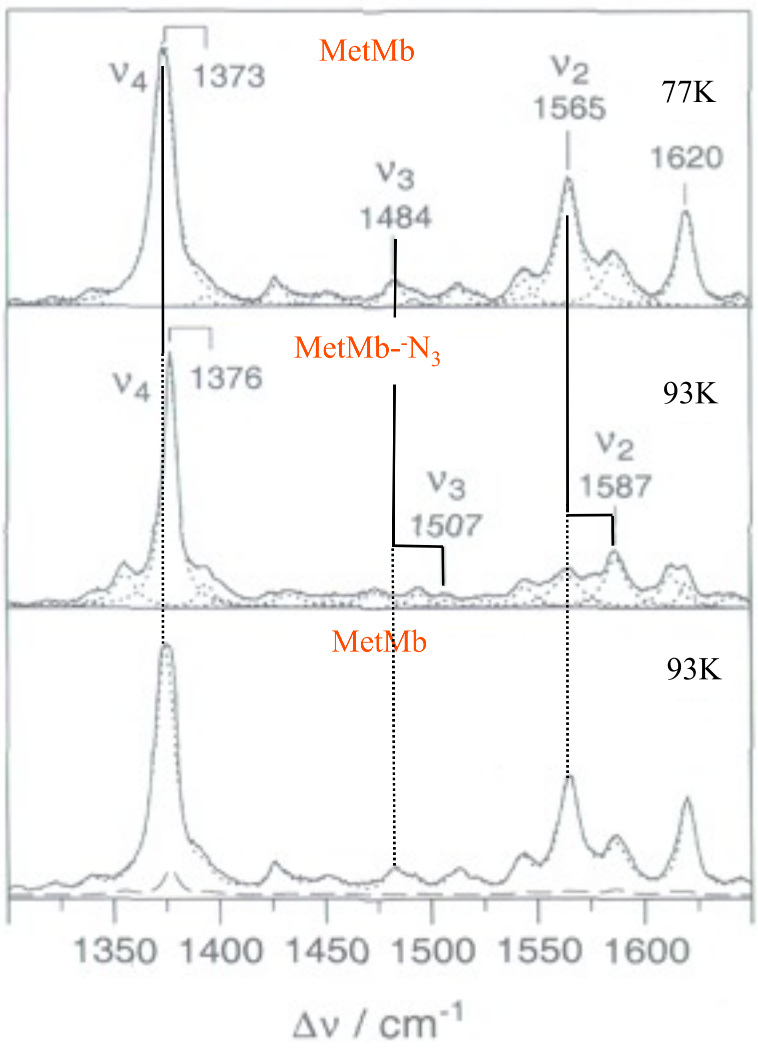
But note (Fe(II)NO,CO $\nu_4 \sim 1375$ cm^{-1}

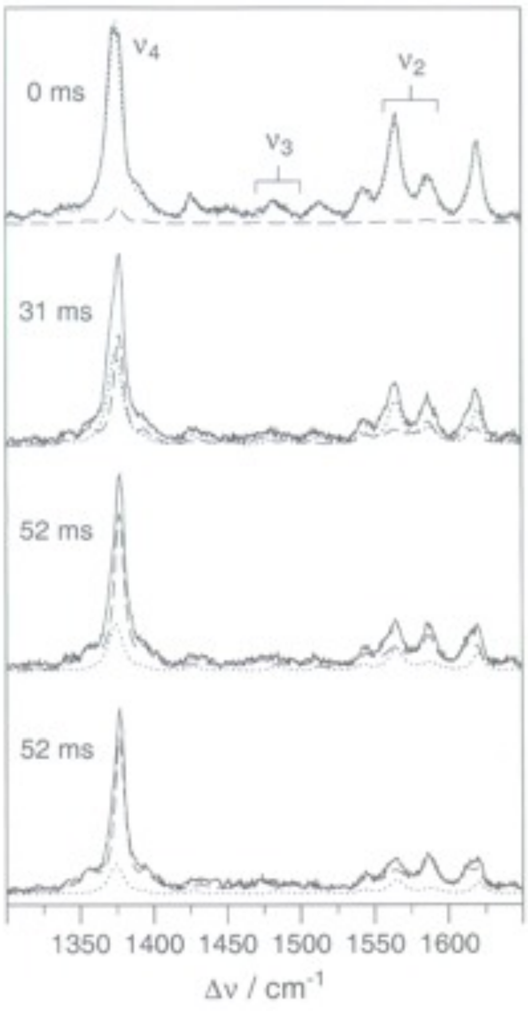
Spin State and Core-Size Marker

- Low-Spin: $\nu_2 \sim 1580\text{-}1590$ cm^{-1}

Freeze Quenching

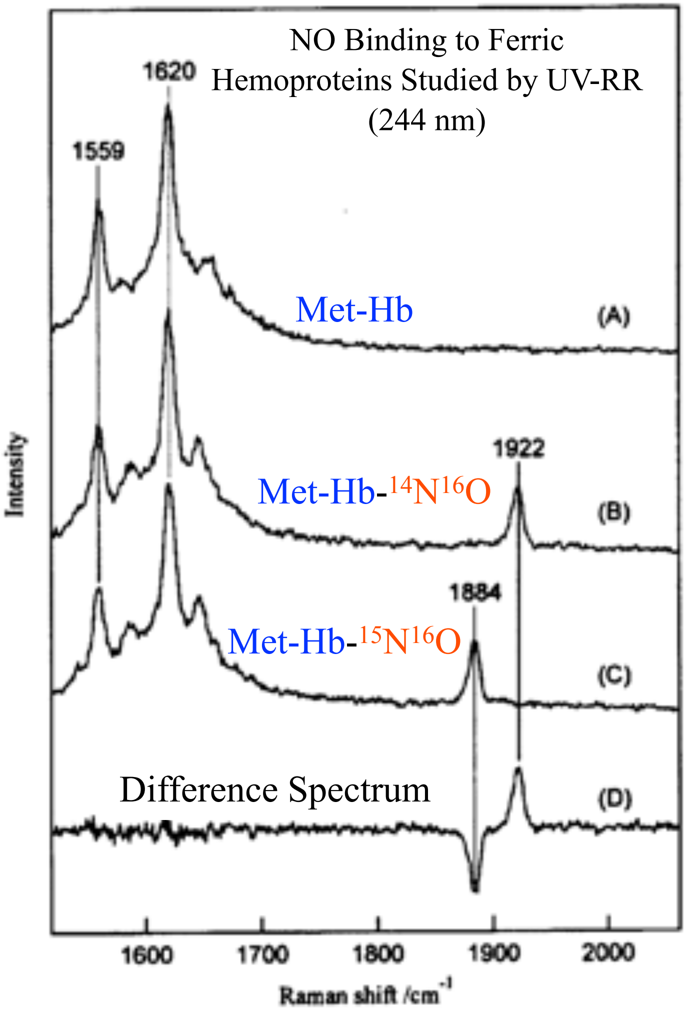
- N_3^- Binding to Myoglobin -





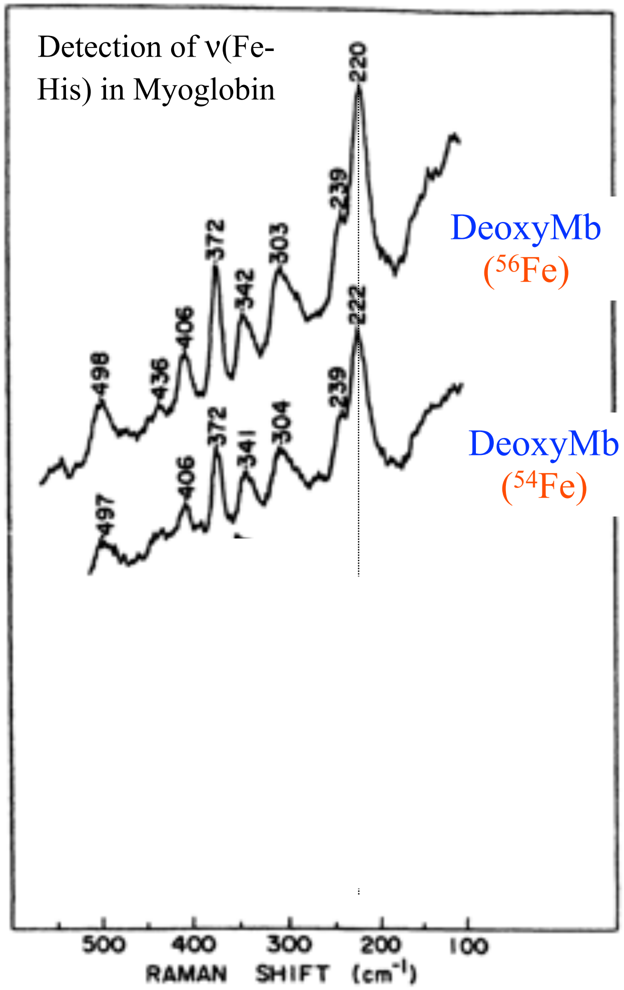
Hemoproteins – Axial Ligands

Exogenous Ligands



NO Binding to Ferric Hemoproteins Studied by UV-RR (244 nm)

Endogenous Ligands

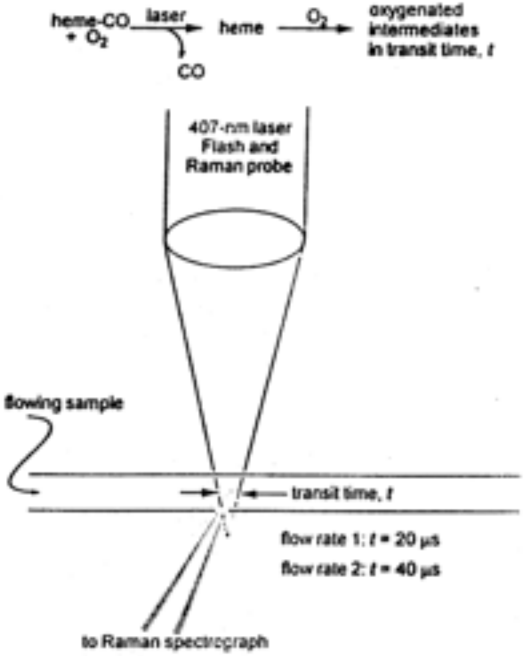


Detection of ν(Fe-His) in Myoglobin

Hemoproteins – Reaction Intermediates

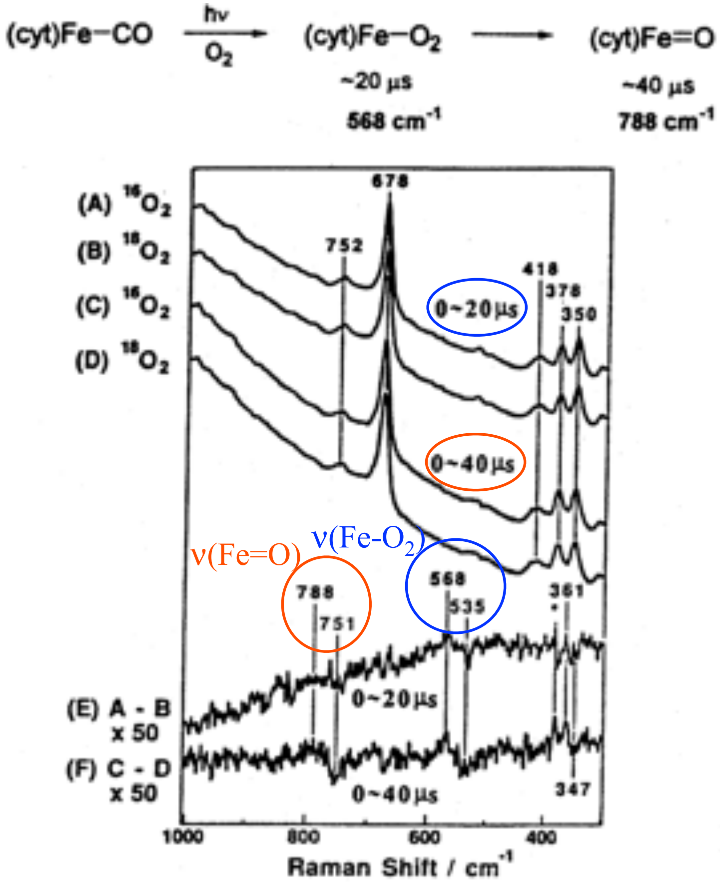
Cytochrome c Oxidase

Experimental Setup

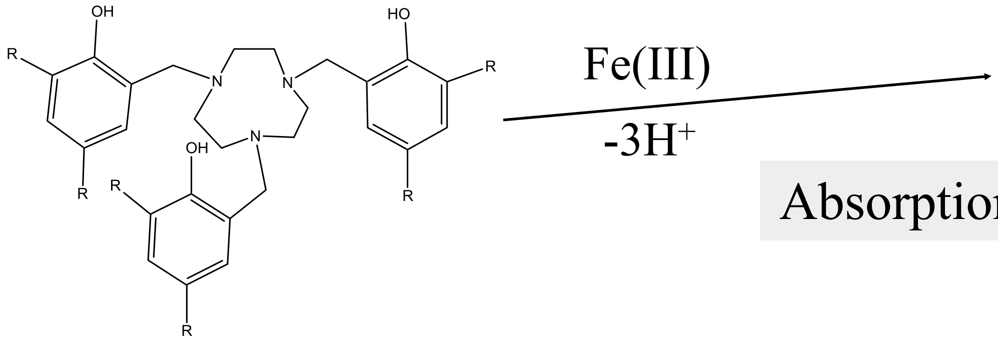


Expected Intermediates

Experimental Results



Phenolates and Phenoxyl Radicals



Fe(III)

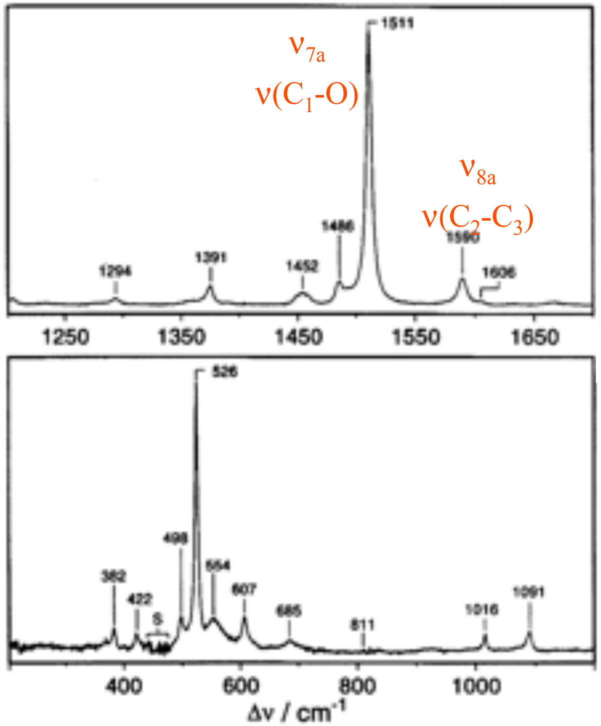
$-3H^+$

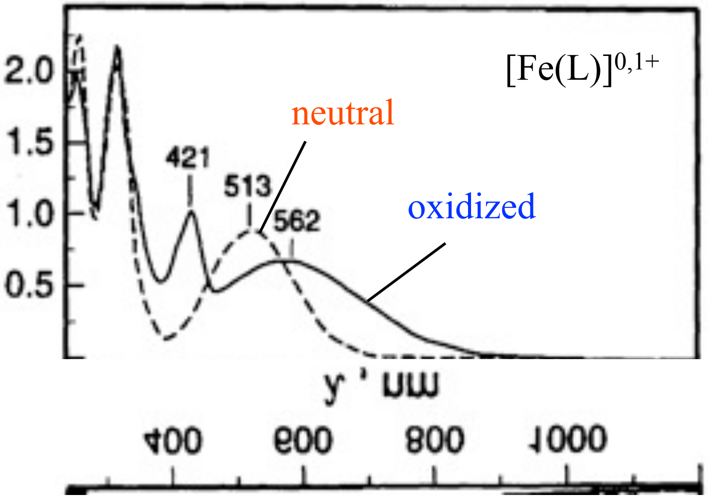
$[Fe(L)]^{0,1+,2+,3+}$

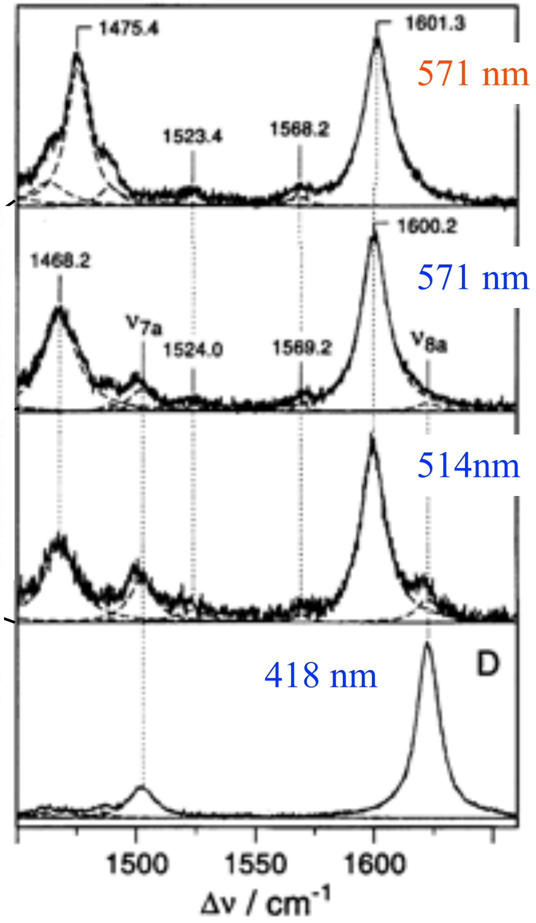
Absorption

Resonance Raman

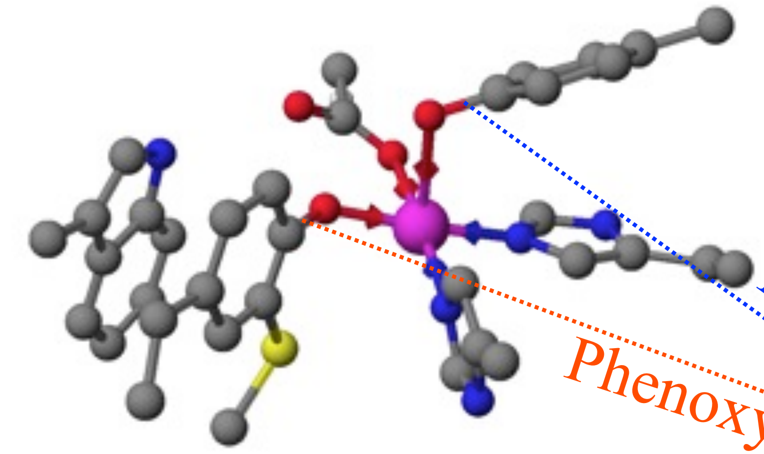
Free Ligand Radical





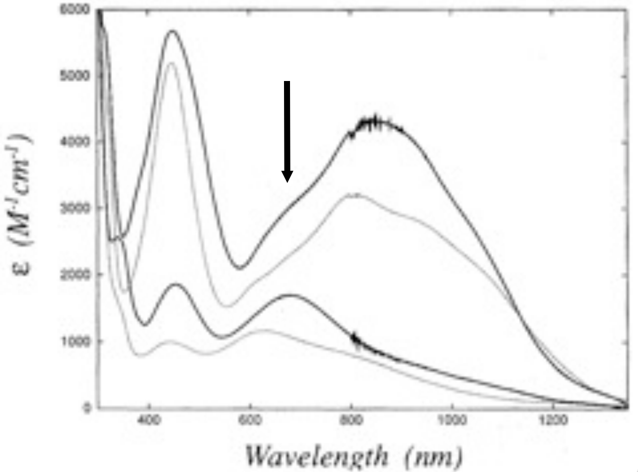


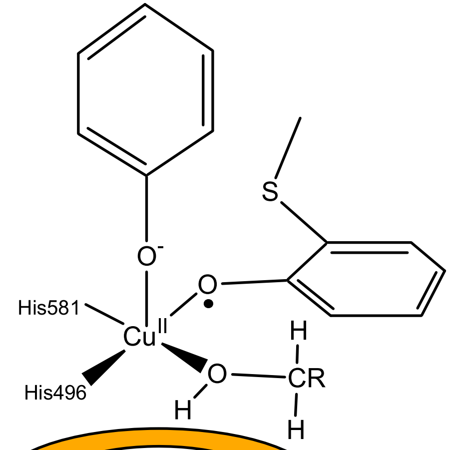
Galactose Oxidase – Detecting Radicals



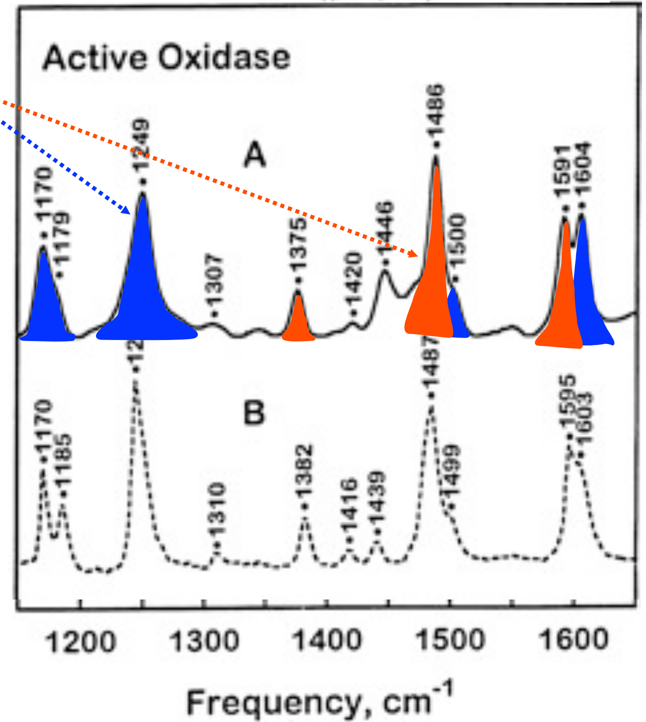
Phenolate

Phenoxyl

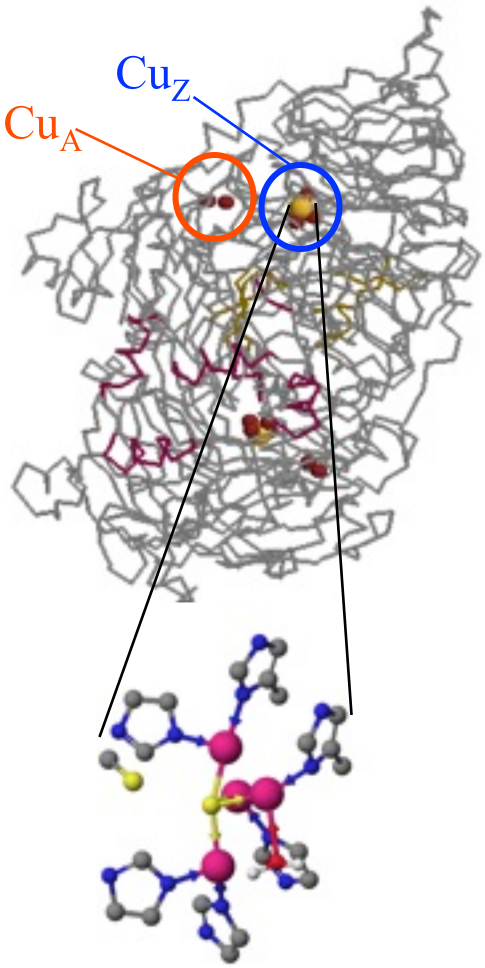




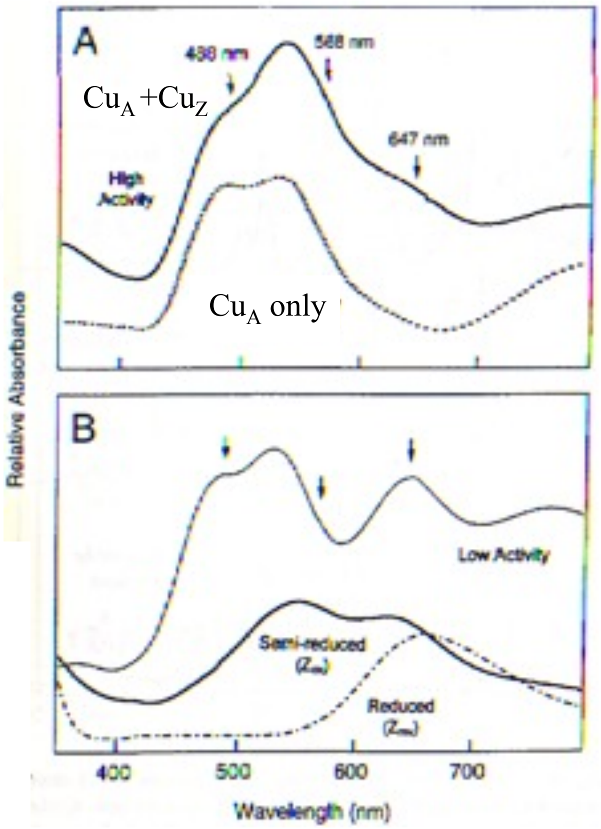
$R\text{-}H_2C\text{-}OH$ $\quad$ $R\text{-}HC{=}O$

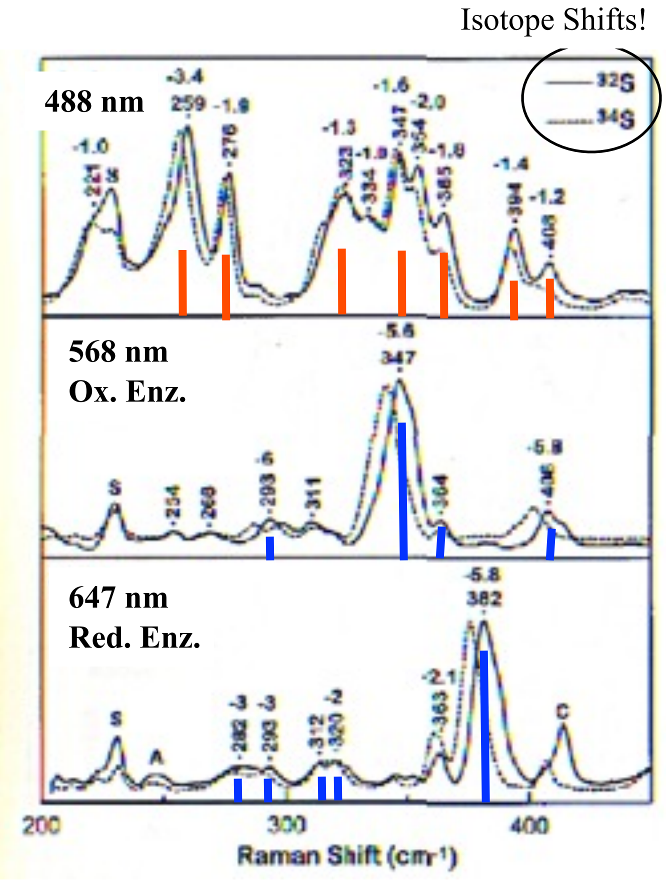


Copper Proteins - The Cu_Z Site



$N_2O \rightarrow N_2 + H_2O$

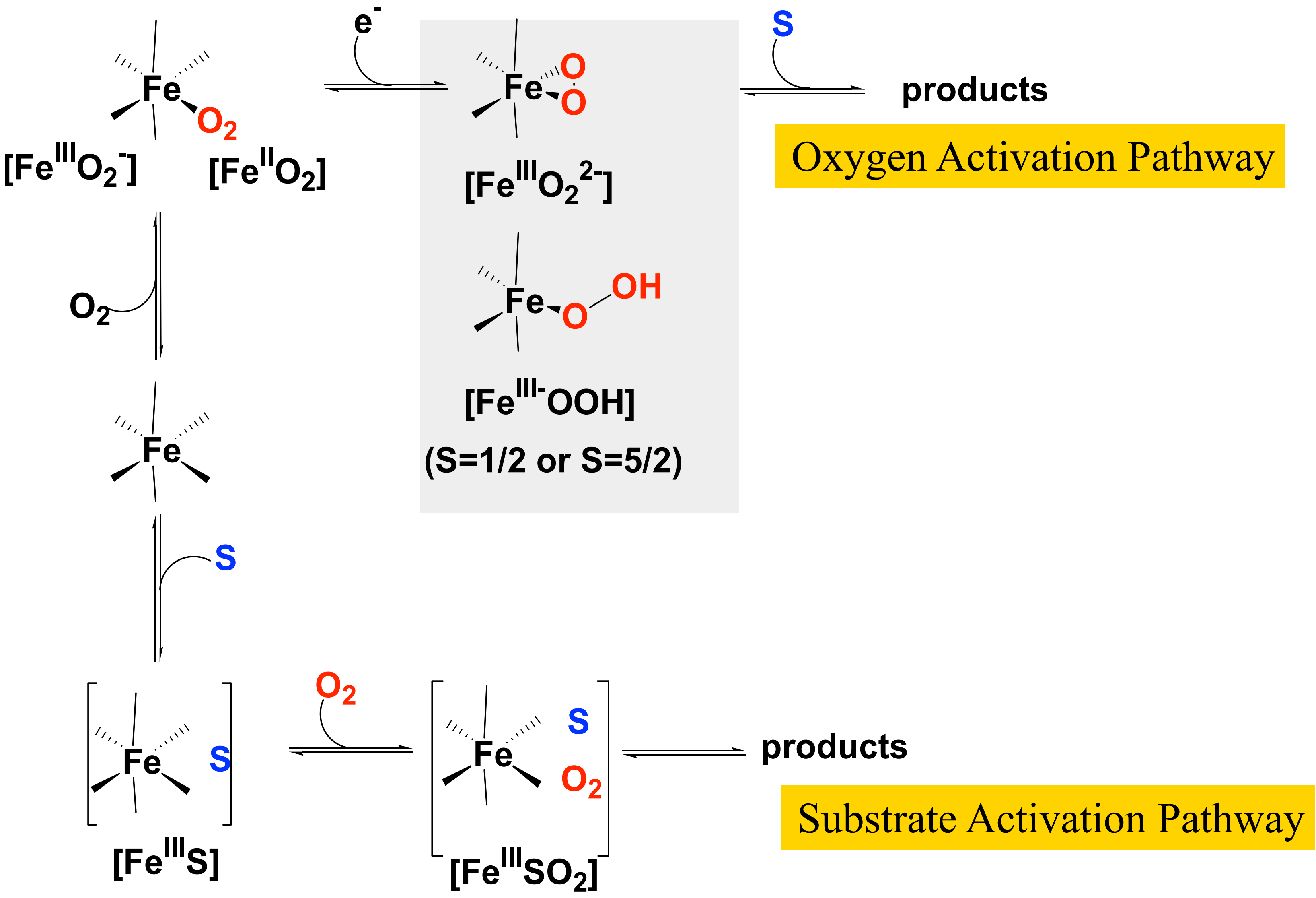




Proves Cu-S Involvement in Cu_Z

(note that in this case this had been proven before by MCD and Biochemistry in Norwich)

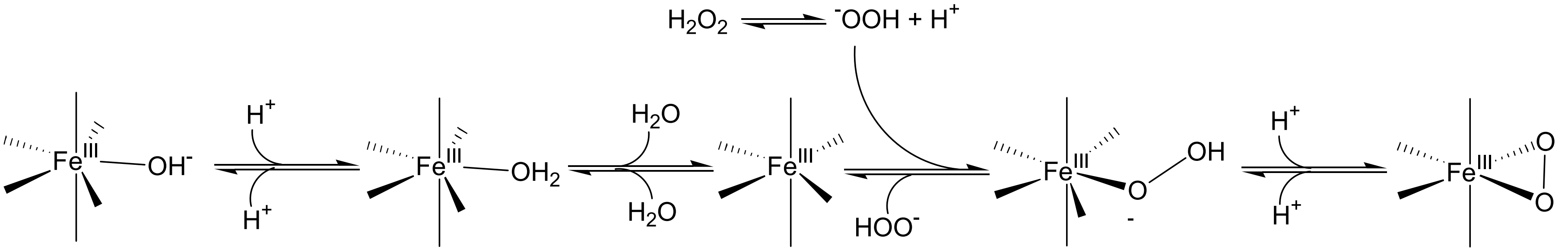
Oxygen Activation in Mononuculear Iron Proteins



Oxygen Activation Pathway

Substrate Activation Pathway

Electronic Structure of the Side-On Fe(III) Peroxo Bond

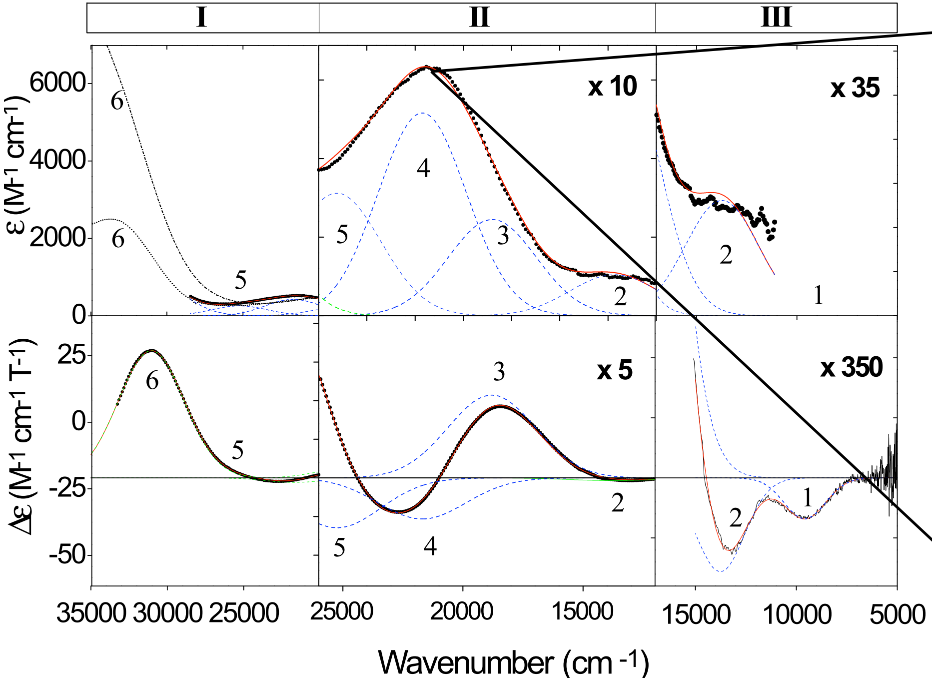


$[Fe(EDTA)(OH)]^{2-}$

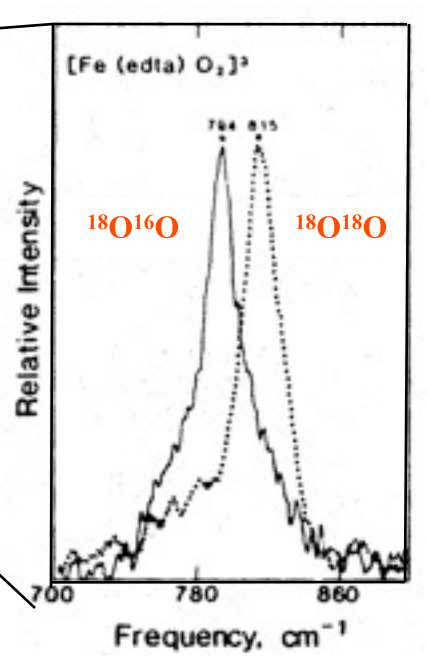
$[Fe(EDTA)]^-$

$[Fe(EDTA)(O_2)]^{3-}$

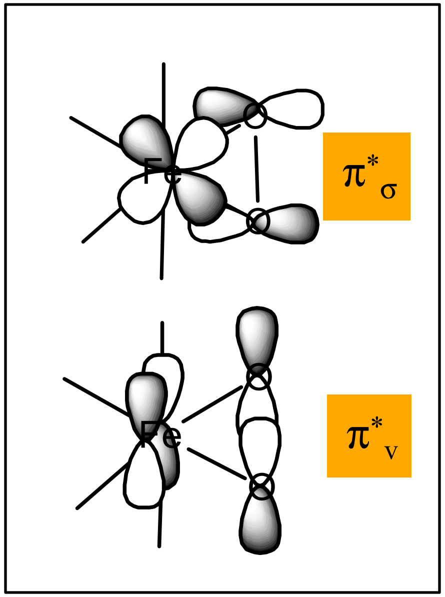
Absorption+MCD



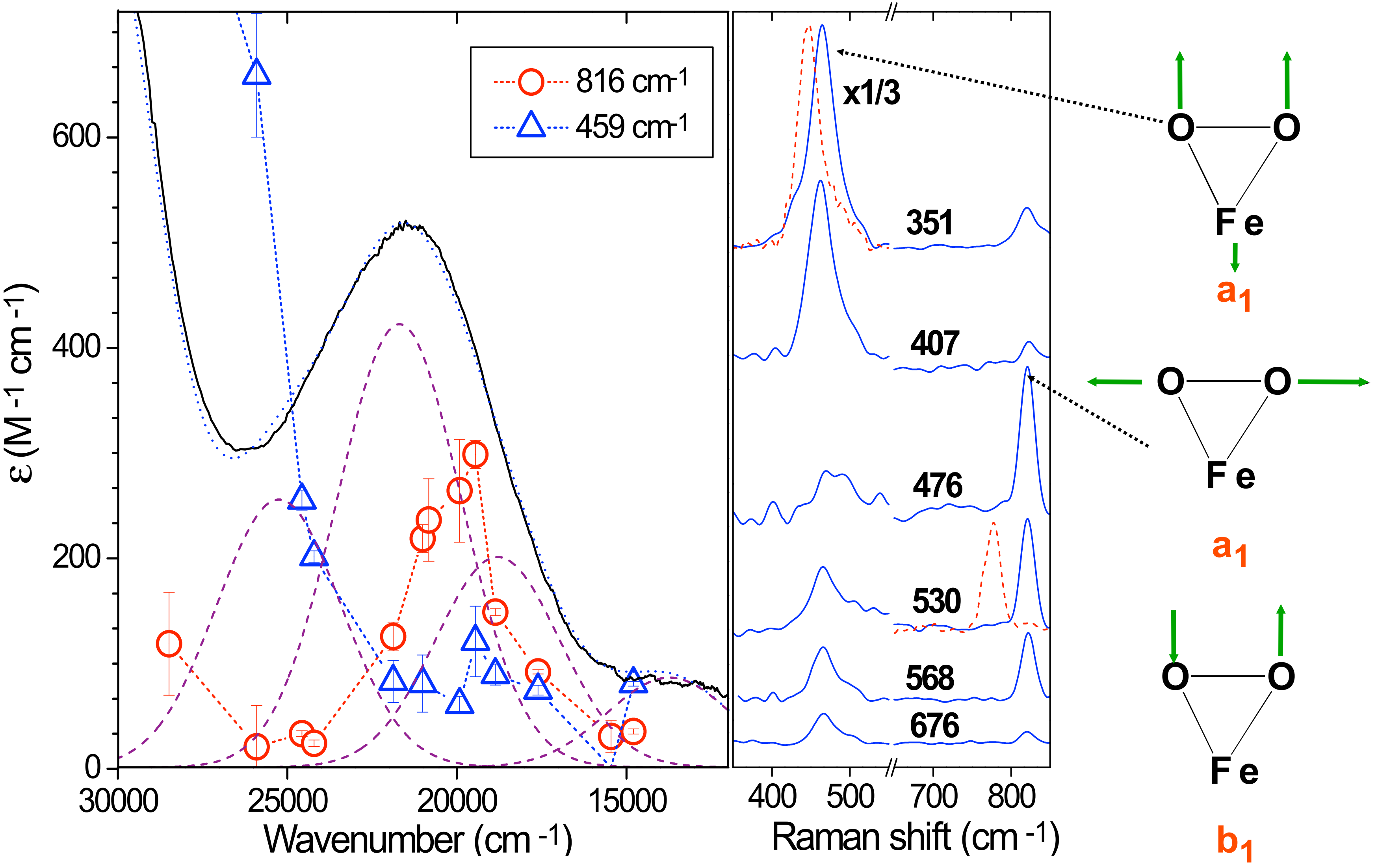
Raman



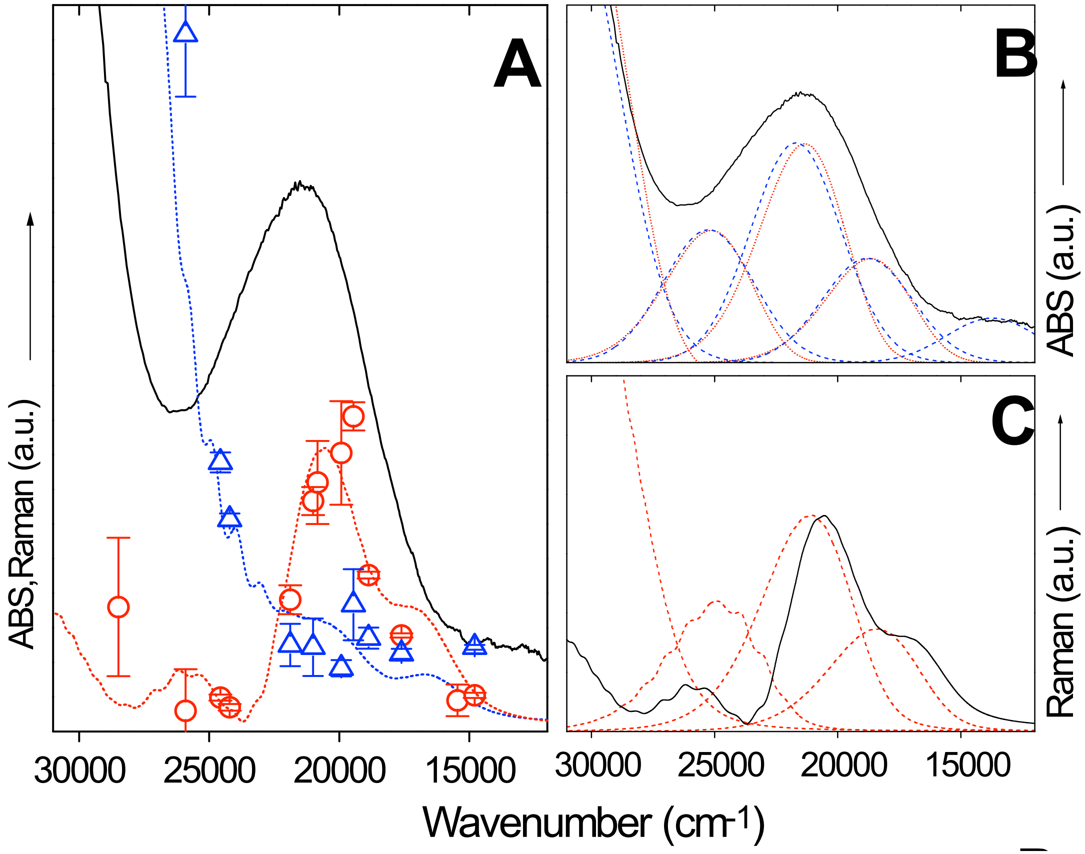
Orbitals



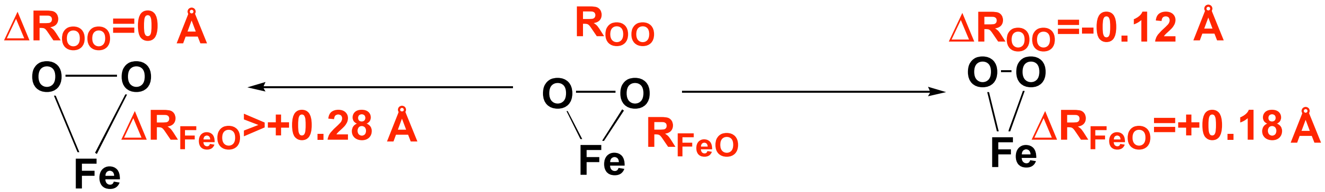
Resonance Raman Spectra of $[Fe(EDTA)(O_2)]^{3-}$



Excitation Profile Analysis for $[Fe(EDTA)(O_2)]^{3-}$



351 nm excitation **530 nm excitation**


ΔR_{OO}=0 Å

ΔR_{FeO}>+0.28 Å

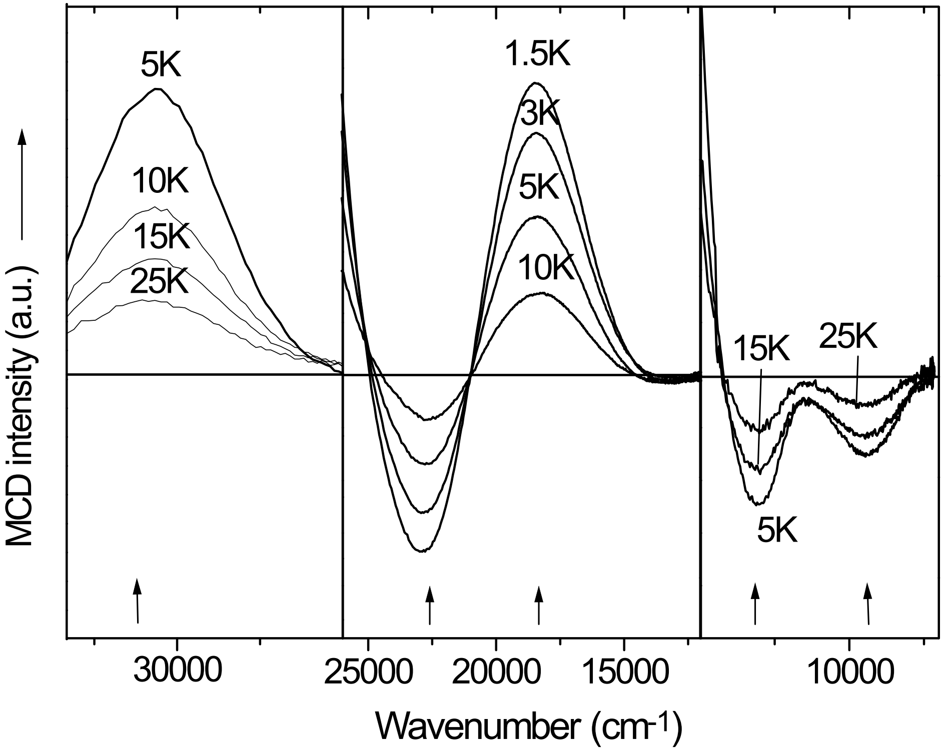
R_{OO}

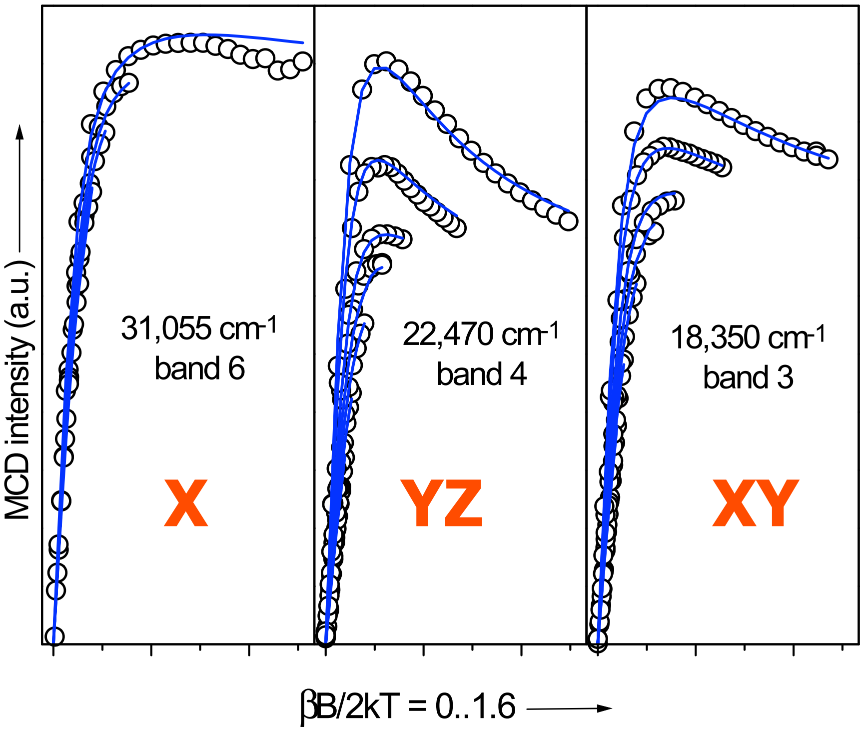
R_{FeO}

ΔR_{OO}=-0.12 Å

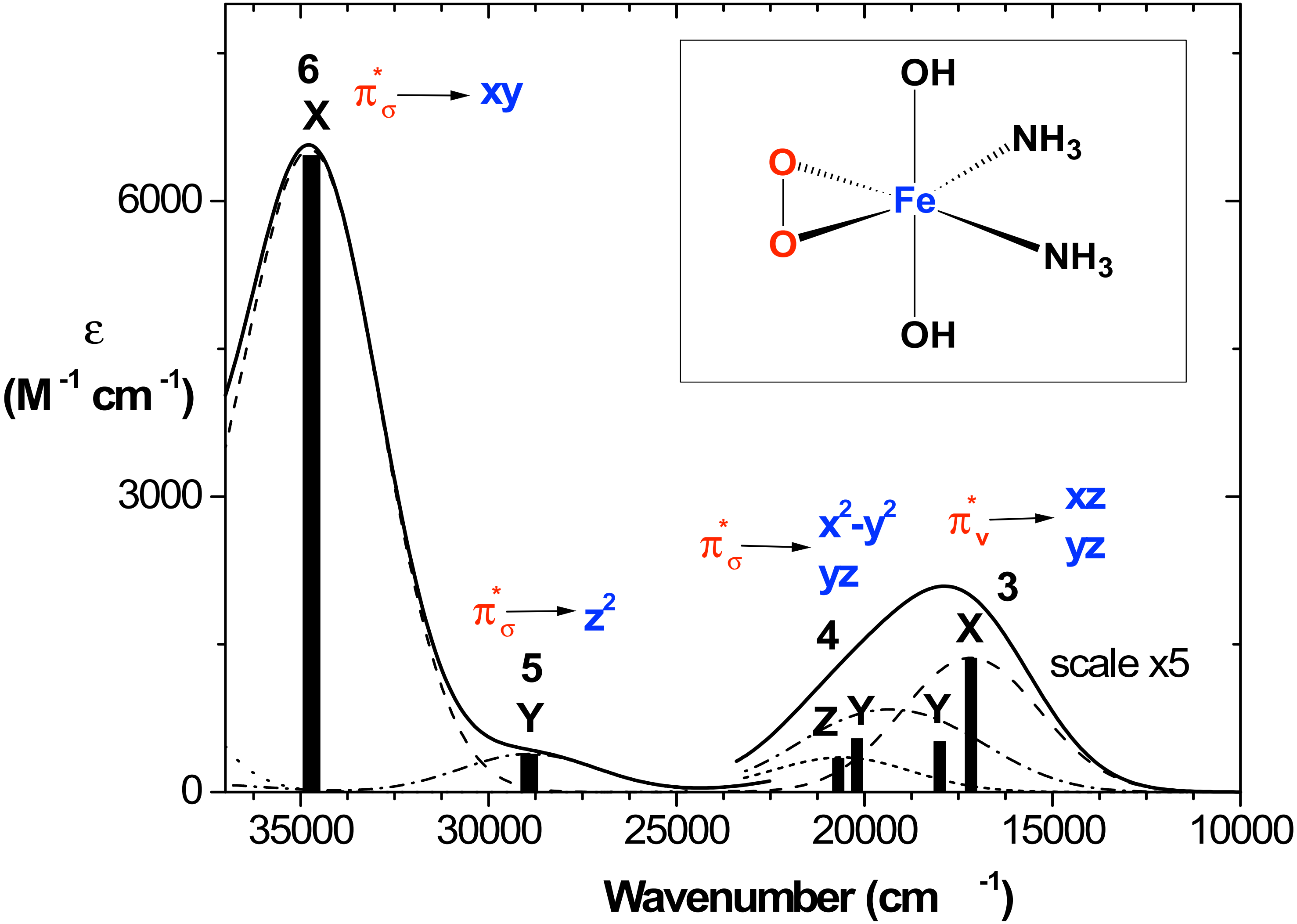
ΔR_{FeO}=+0.18 Å

MCD Spectra of $[Fe(EDTA)(O_2)]^{3-}$

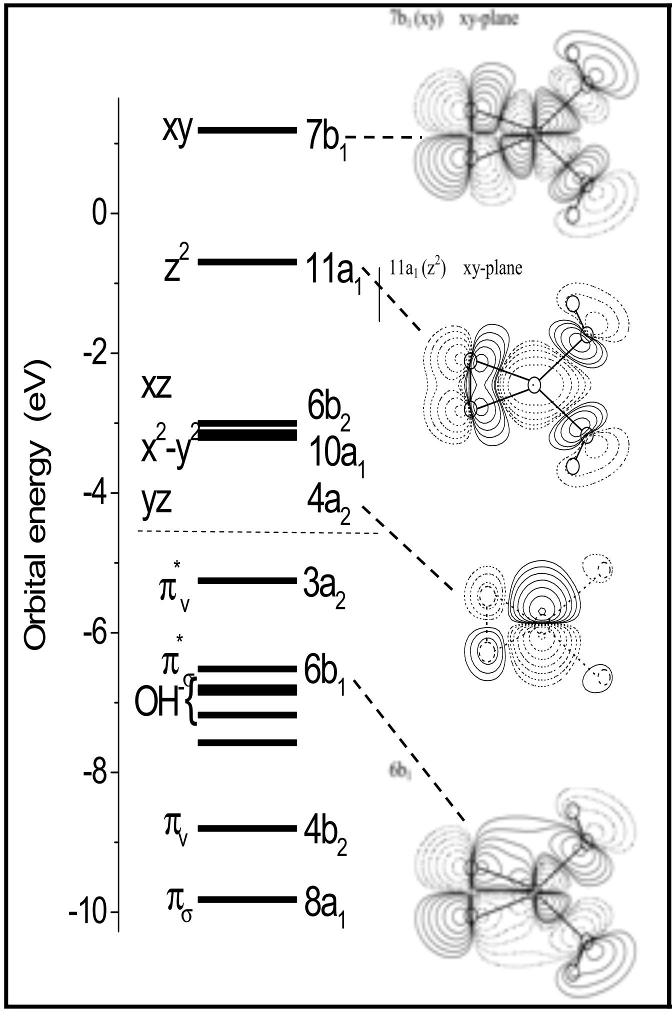


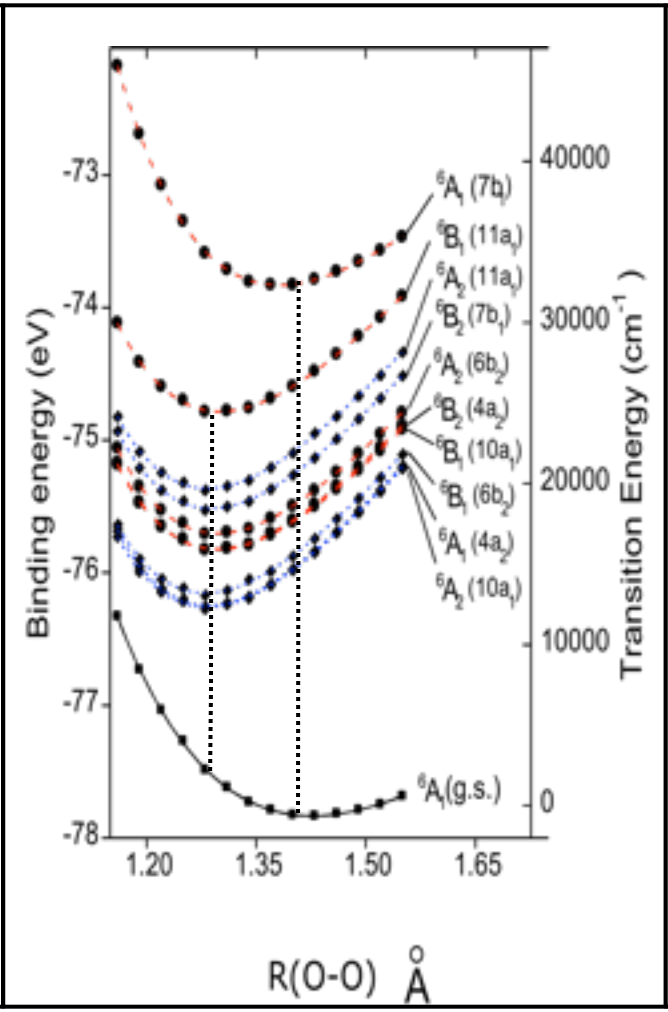


Assignment of Absorption Bands of $[Fe(EDTA)(O_2)]^{3-}$

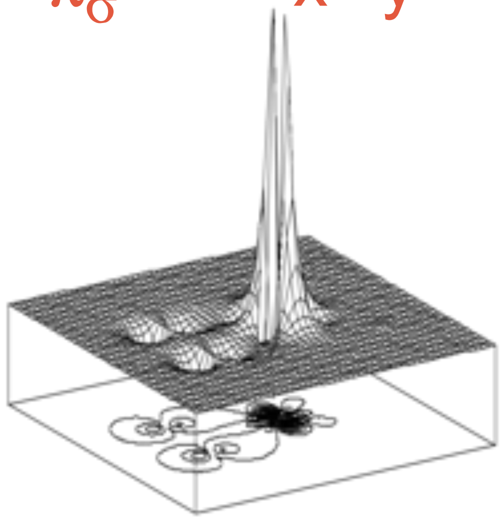


Electronic Structure Insights: $[Fe(EDTA)(O_2)]^{3-}$

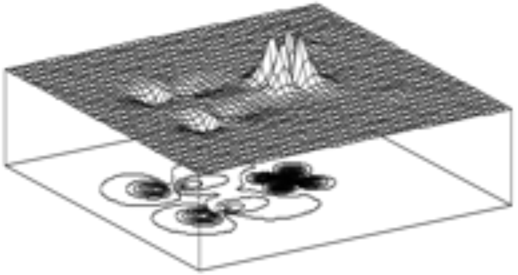




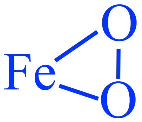
$\pi_\sigma^* \longrightarrow x^2\text{-}y^2$



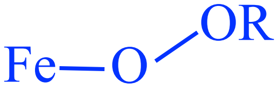
$\pi_\sigma^* \longrightarrow xy$



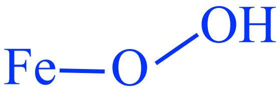
Fe(III) Side On Peroxo versus End-On Hydroperoxo



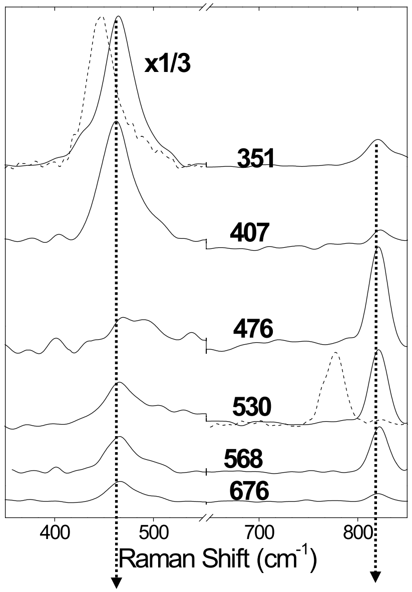
S=5/2

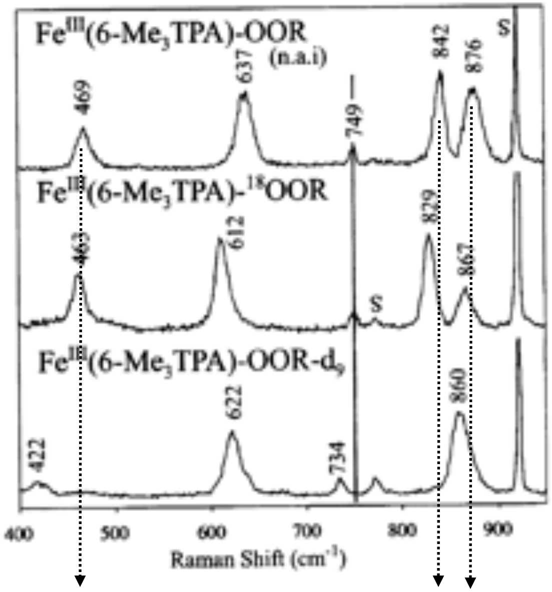


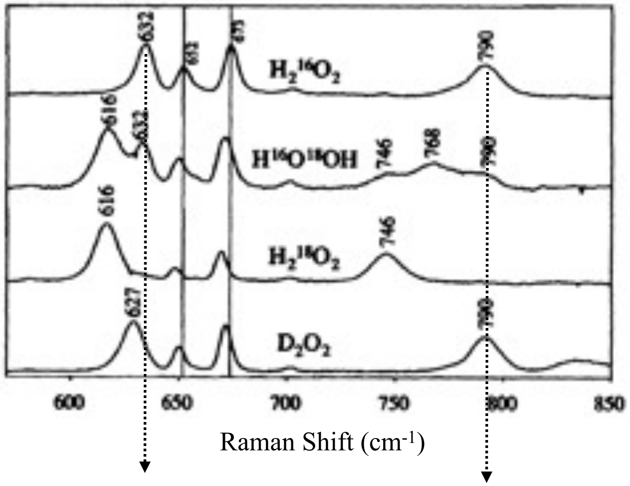
S=5/2



S=1/2







ν(Fe-O) **459** ν(O-O) **816**

ν(Fe-O) **469** ν(O-O) **842/876**

ν(Fe-O) **626** ν(O-O) **789**

Fe-O_π	weak	strong	strong
Fe-O_σ	strong	weak	moderate
O-O	strong	strong	weak
Reac- tivity	unreactive	Fe(II) + HOO•	$[FeO]^{2+}$ + HO•

Summary and Conclusions

1. FT-IR and Raman are Useful for Studying Protein Secondary Structure
2. FT-IR is Highly Suitable for Time Resolved Measurements
3. FT-IR is sensitive for Difference Spectroscopy and Fingerprinting in regions where the protein does not Strongly Absorb
4. RR is extremely Sensitive and is highly Specific in Enhancing *only Vibrations Coupled to the Chromophore*
5. RR Provides very Powerful Fingerprints
6. RR yields Detailed Electronic and Structural Information
7. RR can be Combined with Freeze-Quench or Flash-Flow Techniques to Study Kinetics
8. RR can be Combined with Electrochemistry (i.e. Surface Enhanced Resonance Raman Spectroscopy, SERR)

Literature

- Cernuszewicz, R.S.; Spiro, T.G. In: Solomon, E.I.; Lever, A.B.P. (Eds) *Inorganic Electronic Structure and Spectroscopy*. Wiley, New York, **1999**, pp. 353
- Spiro, T.G.; Cernuszewicz, R.S. In: Que, L. Jr. (ed.) *Physical Methods in Bioinorganic Chemistry*, University Science Books, **2000**, pp. 59
- Loehr, T.M. In: Solomon, E.I.; Hodgson, K.O. (Eds.) *Spectroscopic Methods in Bioinorganic Chemistry,* ACS Symp. Ser. 692*,* American Chemical Society*,* Washington DC*,* **1998,** pp. 136

These are three very good and highly pedagogical reviews written by leading experts in the field

- Wilson, E. B. Jr.; Decius, J.C.; Cross, P. C. *Molecular Vibrations*, Dover Publications Inc., New York, **1955**

The classic text on vibrational spectroscopy at an introductory level. Describes Normal coordinate analysis in detail.

- Harris, D.C.; Bertolucci, M.D. *Symmetry and Spectroscopy. An Introduction to Vibrational and Electronic Spectroscopy*. Dover Publications, New York, 1978

This is one of the many good introductory texts in vibrational spectroscopy and group theory.

- Spiro, T.G. (Ed.) Biological Applications of Raman Spectroscopy. Wiley Interscience, Volumes 1-3, **1988**

This series of books is highly recommended and gives many very detailed reviews that describe the application of Raman Spectroscopy in biochemistry.

- Myers, A. B.; Mathies, R. A. in: *Biological Applications of Raman Spectroscopy*; Spiro, T. G. (ed.) Wiley, New York, **1987**, Vol 2., pp. 1
- Zink, J. I.; Shin, K. S. K. *Adv. Photochem.*, **1991**, 16, pp. 119

These two references describe the application of the so-called „time-dependent“ theory of resonance Raman spectroscopy which is very useful for the analysis of excitation profiles

Time Dependent Theory of RR Spectroscopy

Resonance Raman Intensity (E. Heller):

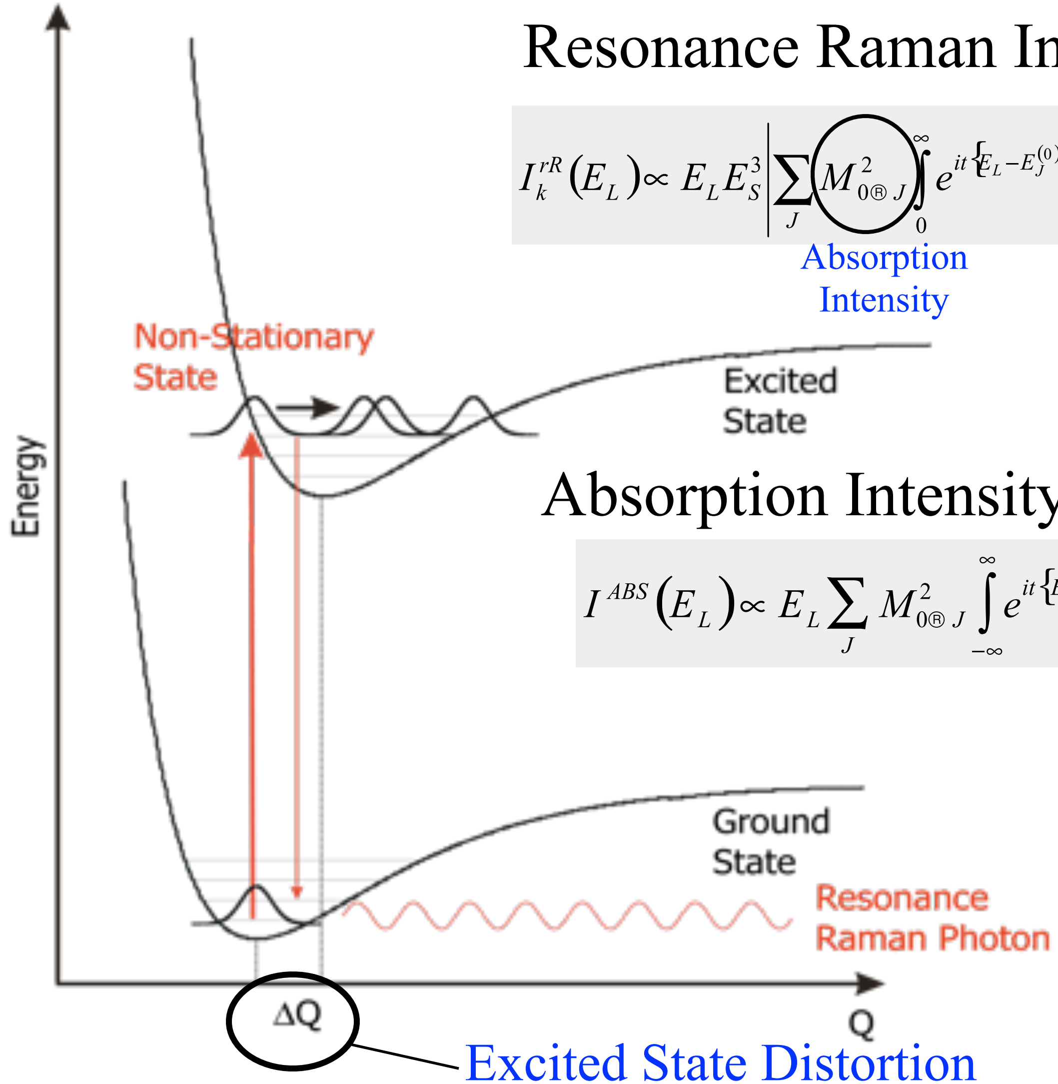
$$I_k^{rR}(E_L) \propto E_L E_S^3 \left| \sum_J \boxed{M_{0\text{®}J}^2} \int_0^\infty e^{it\{E_L - E_J^{(0)}\}/\hbar - \Gamma_J |t|/\hbar} \boxed{\left(\tfrac{1}{4}\Delta_k^J\right)} \left(e^{i\omega_k t} - 1\right) \prod_p e^{-\frac{1}{2}(\Delta_p^J)^2 \{1 - \exp(-i\omega_p t)\}} dt \right|^2$$

Absorption Intensity

Excited State Distortion

Absorption Intensity:

$$I^{ABS}(E_L) \propto E_L \sum_J M_{0\text{®}J}^2 \int_{-\infty}^{\infty} e^{it\{E_L - E_J^{(0)}\}/\hbar - \Gamma_J |t|/\hbar} \prod_p e^{-\frac{1}{2}(\Delta_p^J)^2 \{1 - \exp(-i\omega_p t)\}} dt$$



Excited State Distortion