3rd Penn State Bioinorganic Chemistry Workshop

Lecture 5: Vibrational Spectroscopy

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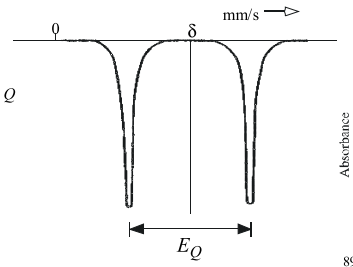
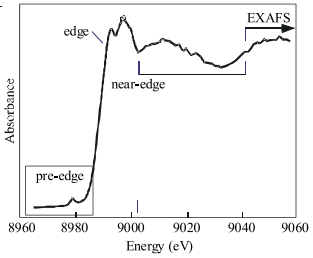
Spectroscopic Techniques

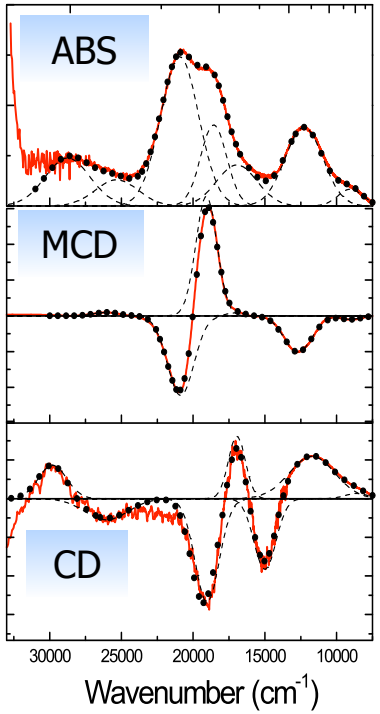
	Gamma		X-Ray		UV/vis	Infrared	Microwave	Radiowave
eV	14000	8000		2000	4 - 1	0.1-0.01	10^{-4} -10^{-5}	10^{-6} -10^{-7}

Mössbauer

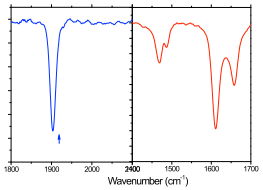


XAS EXAFS

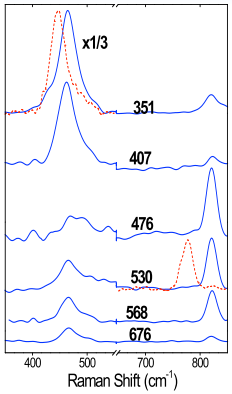




IR

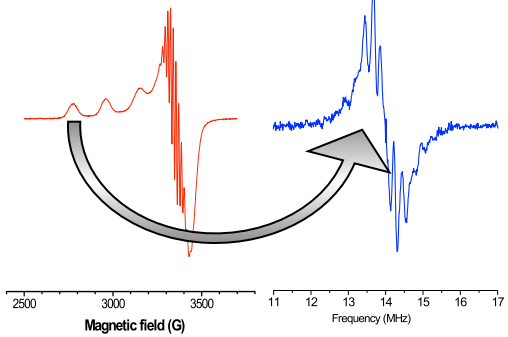


Raman



EPR

ENDOR



NMR

Outline

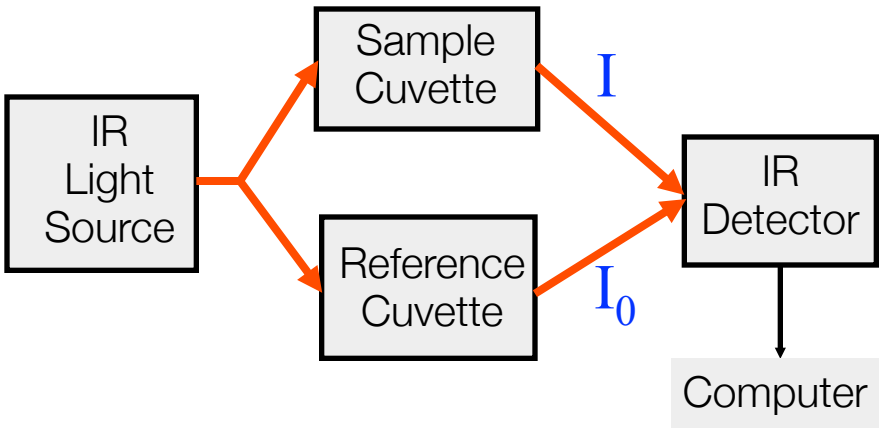
1. Introduction
2. Molecular Vibrations
   - Vibrational Frequencies and Normal Coordinates
   - Physical Origin of IR Intensities
   - Physical Origin of Raman Intensities
   - Physical Origin of Resonance Raman Intensities
3. Experimental Techniques
   - Raman Spectroscopy
   - Resonance Raman Spectroscopy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

Why Vibrational Spectroscopy ?

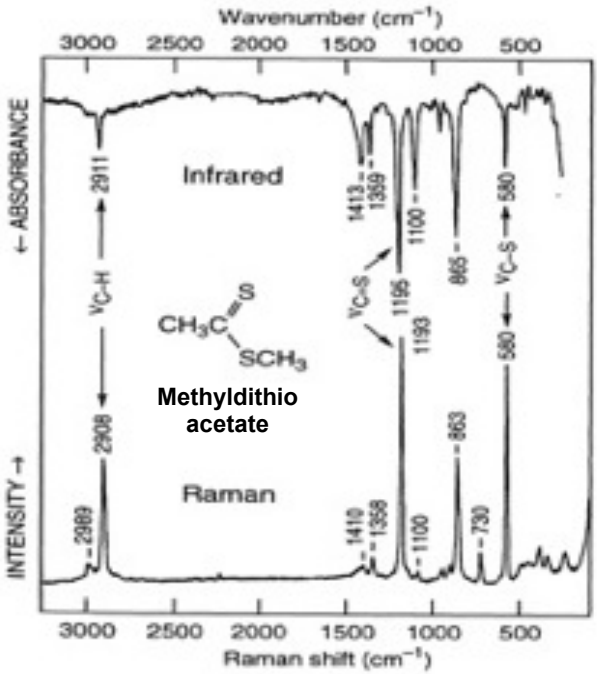
- Structural Information (IR/Raman/resonance Raman)
  - Identication of characteristic vibrations
  - Isotope shifts
  - Normal coordinate analysis
  - Detection of functional groups
- Electronic Information (resonance Raman)
  - Identification of electronic transitions
  - Excitation profiles
  - Insight 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 dependent on magnetic properties (EPR,MCD)
  - Much higher resolution than absorption and CD spectroscopy
  - Not limited to certain isotopes (Mössbauer)

Experiment: IR versus Raman Spectroscopy

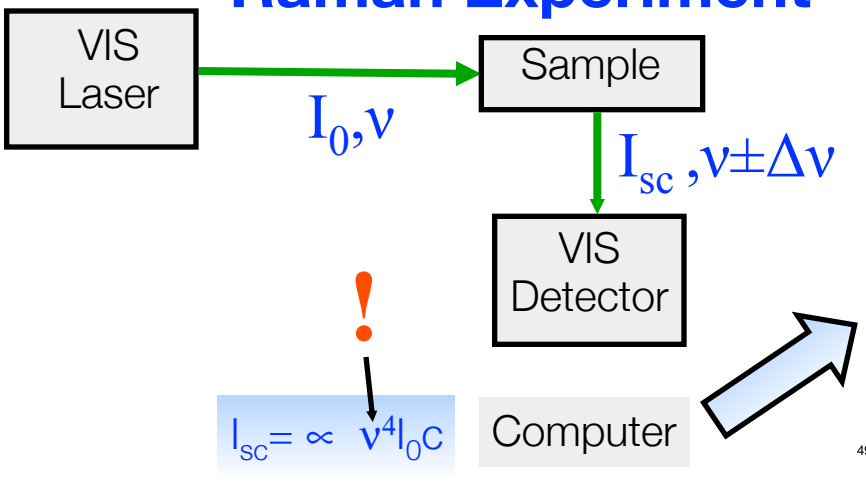
IR Experiment

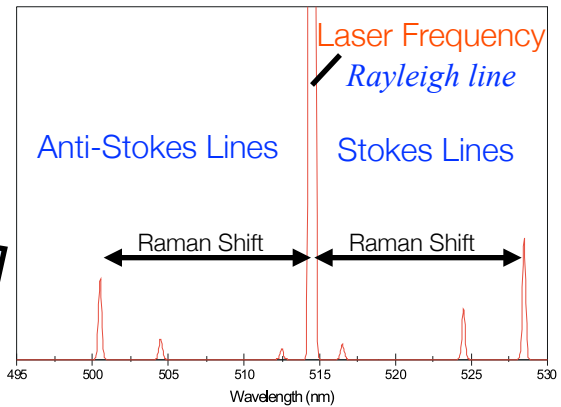


$A = -\log(I/I_0) \propto \varepsilon dc$

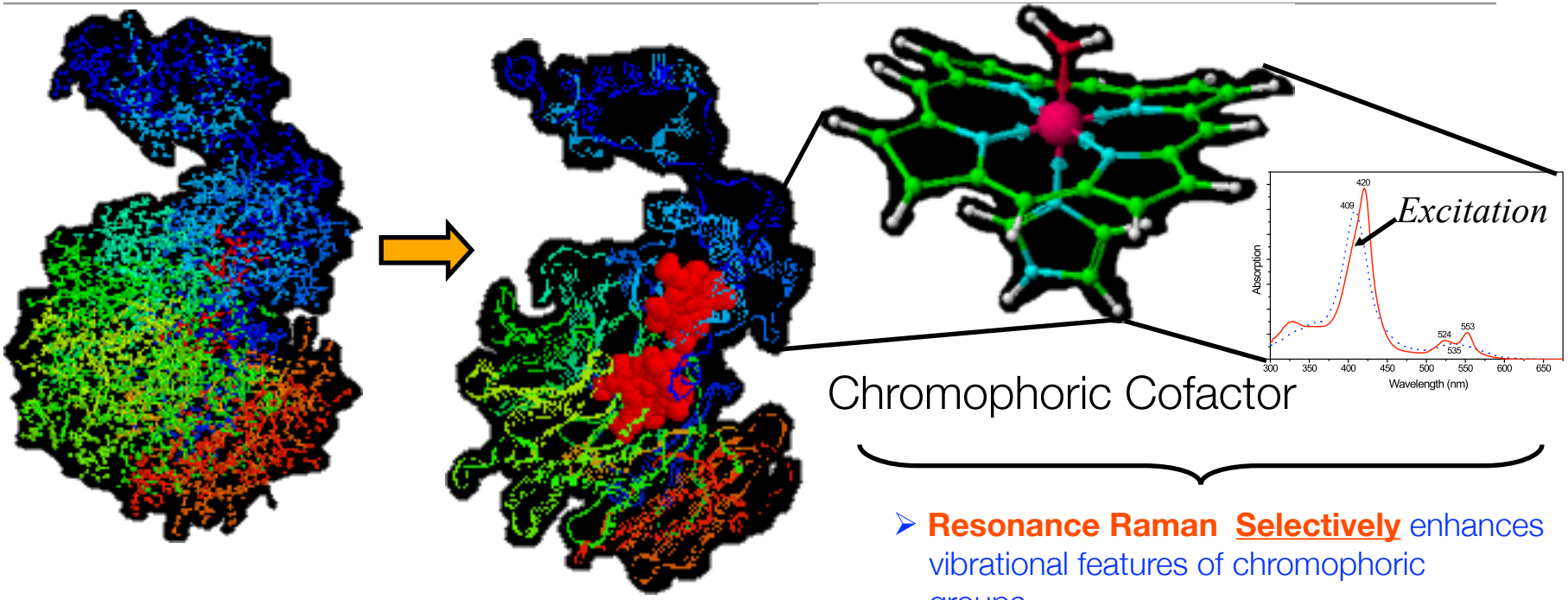


Raman Experiment





IR versus Raman Spectroscopy



Tertiary Structure

- > 1000 Atoms
- Thousands of peaks in IR+Raman spectra
- Impossible to understand in Detail

Secondary Structure

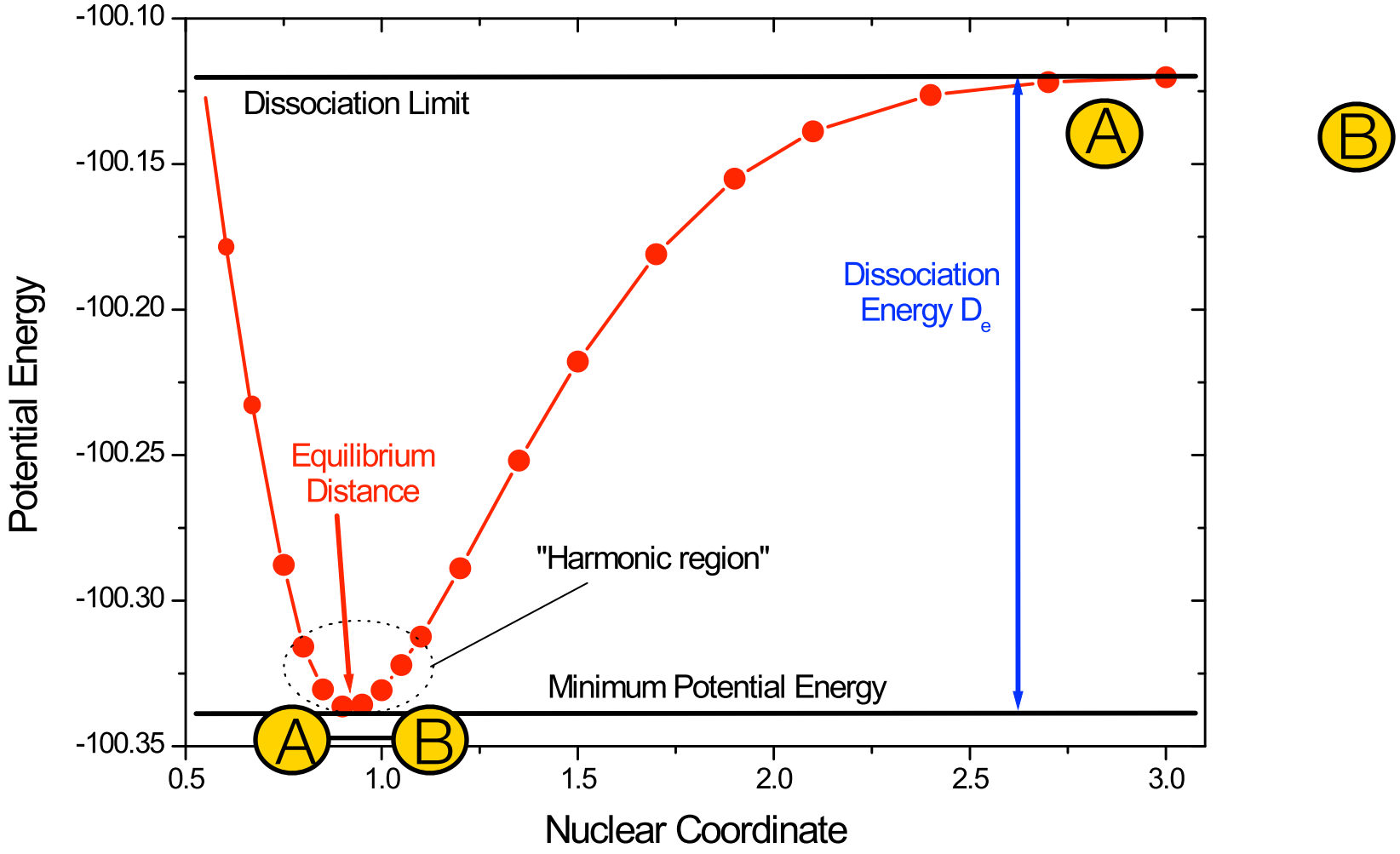
Both IR and Raman are „globally sensitive“ to Secondary structure elements (similar to CD)

Chromophoric Cofactor

- **Resonance Raman** **Selectively** 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)

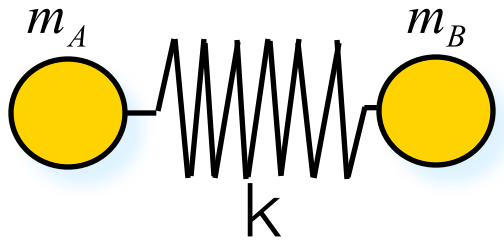
The Vibrations of a Diatomic Molecule

Newton's law:

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

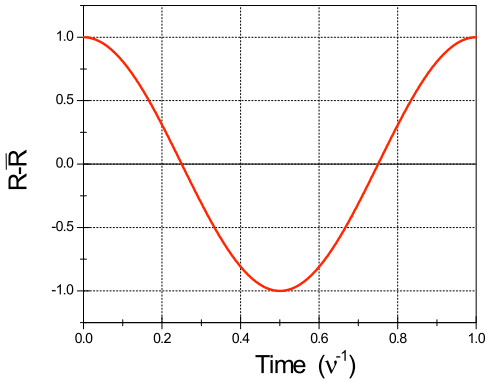
Molecule:

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



Thus:

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



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:

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

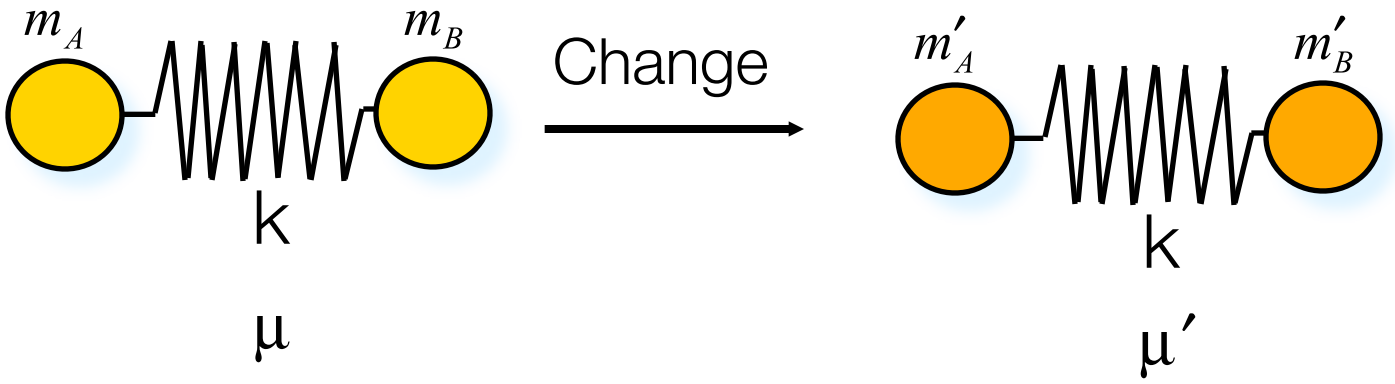
Reduced Mass $m = \frac{m_A m_B}{m_A + m_B}$

The Reduced Mass and Isotope Shifts

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

Force Constant

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



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

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 (mdyn/Å):

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

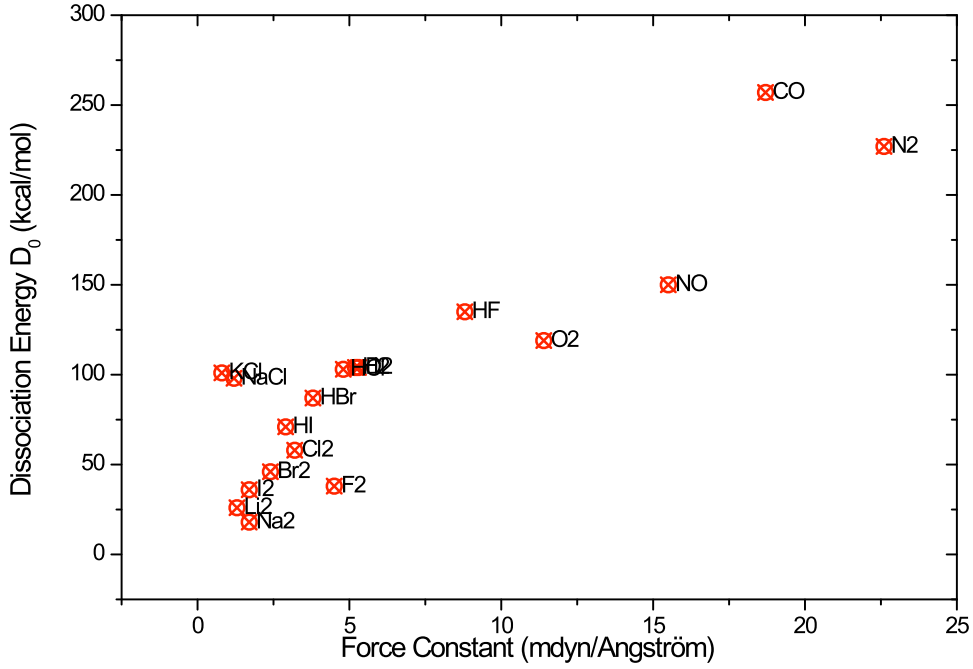
Observation (Badger, R.M. (**1934**) *J. Chem. Phys.*, 2, 128)

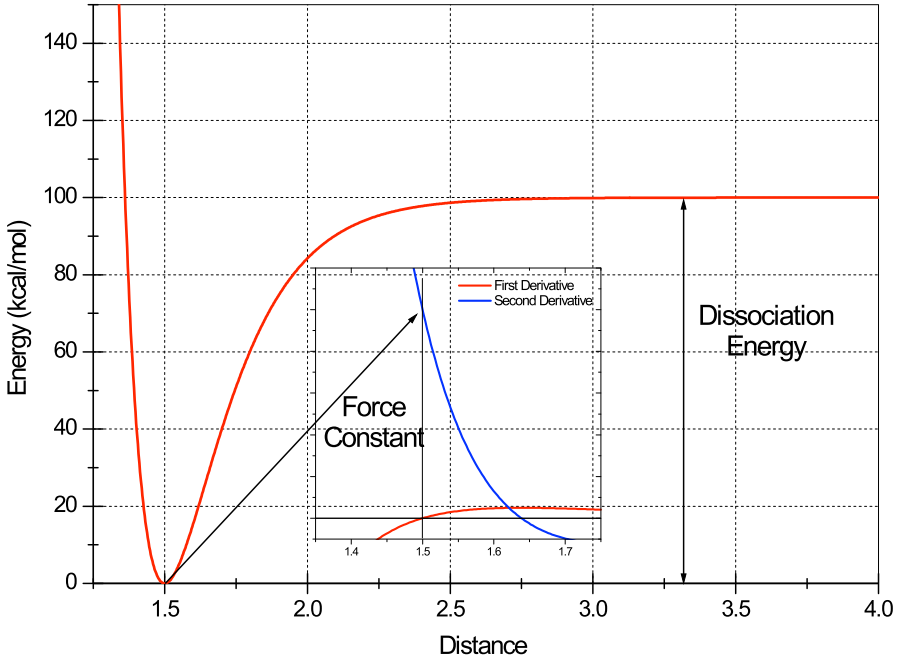
$$k_{ij} \approx 1.86\left(\bar{R} - d_{ij}\right)^{-3}$$

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



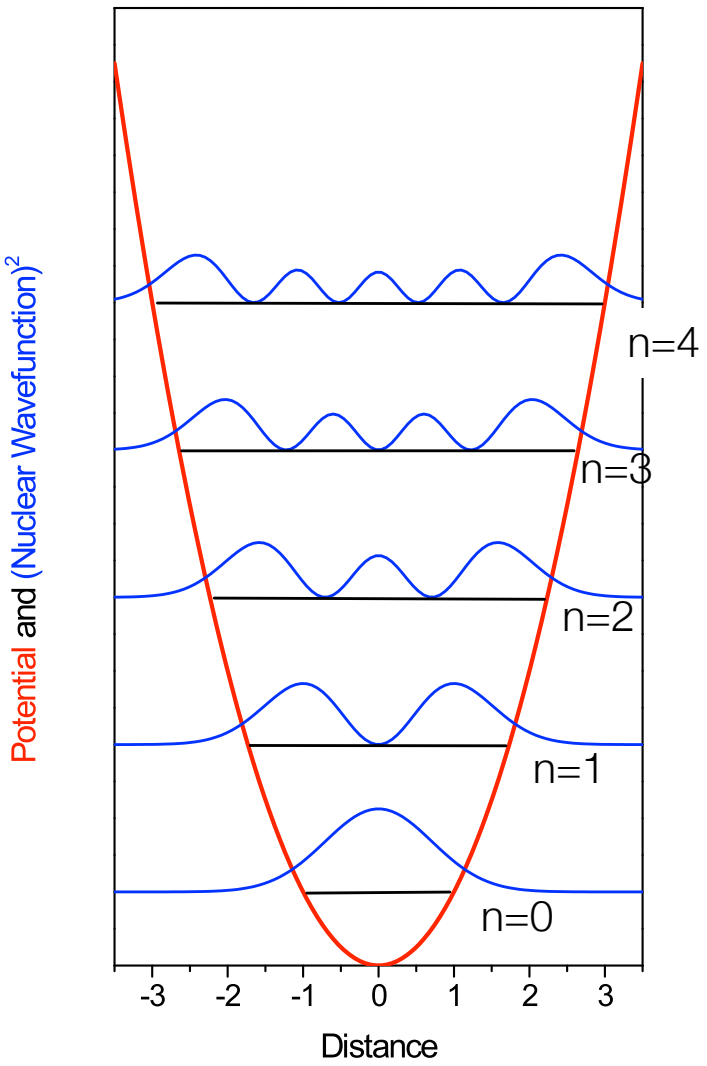


For a Morse potential: $V(R) = D_e \left[1 - \exp\left(-\beta R\right)\right]^2$

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

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:

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

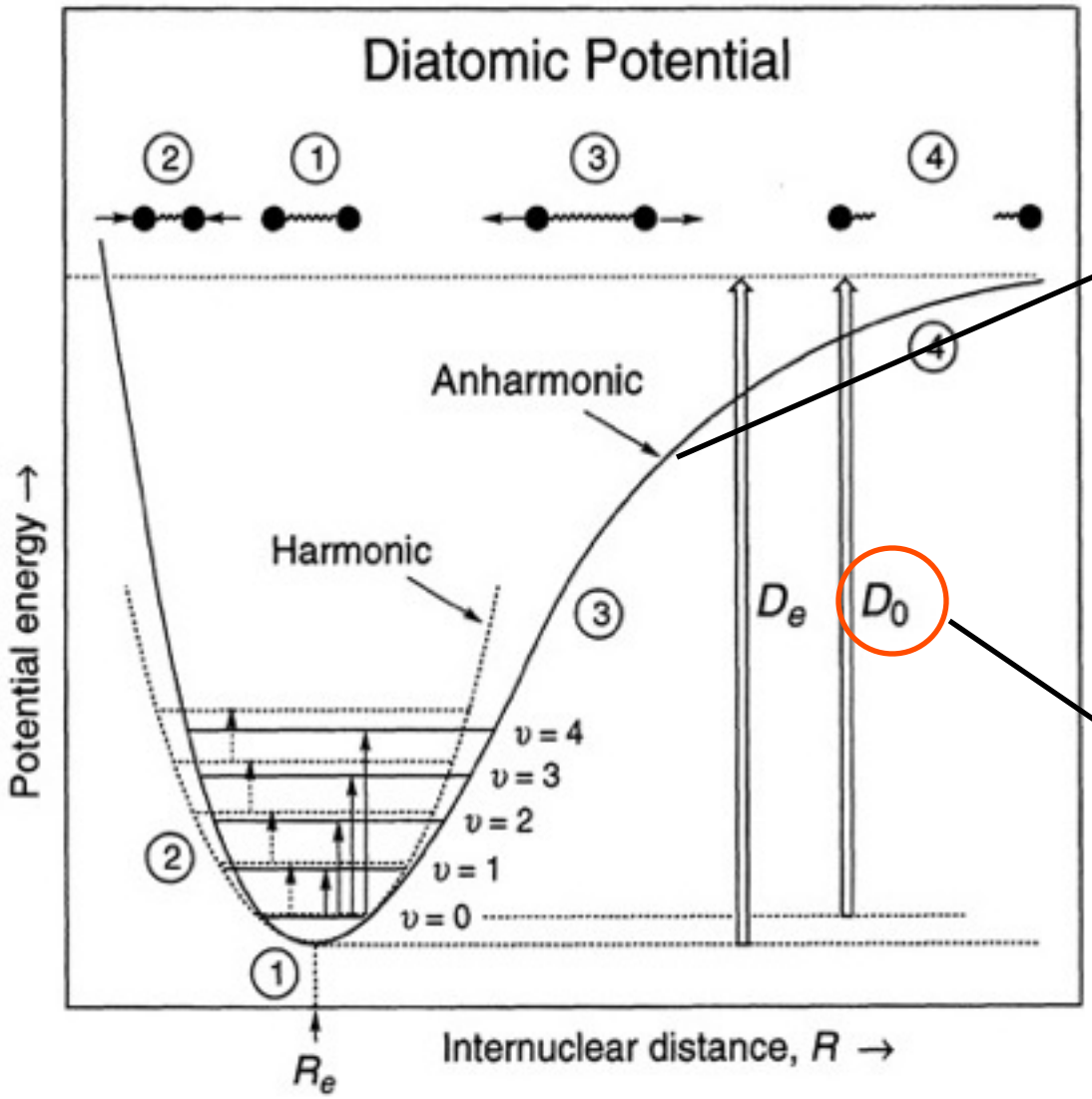
$$\sigma^2 = \hbar / (4\pi\, m\nu)$$

Gaussian

Normalization

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

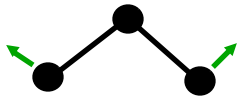
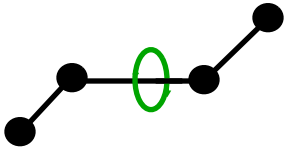
$$D_0 = D_e - h\nu$$

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:

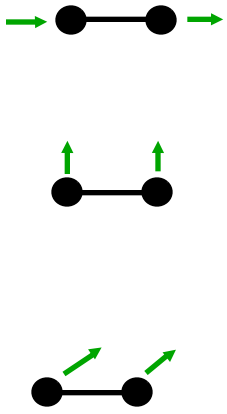
Stretches *Bends:*  *Torsions:* 

- 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**, 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***).

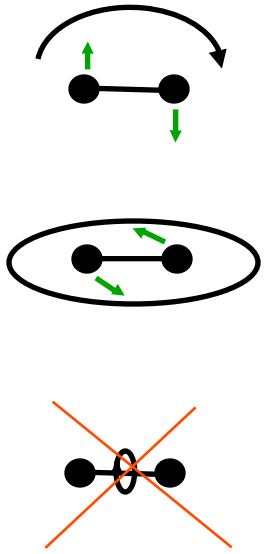
Rotations and Translations

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

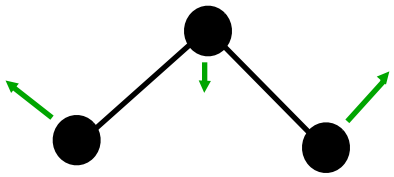


Rotations



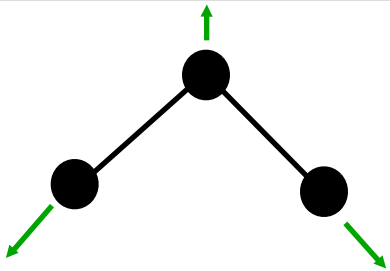
Not a Rotation!

Normal Coordinates of Water



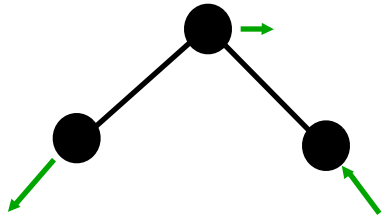
ν_1 (a_1)
Bending

1595 cm^{-1}



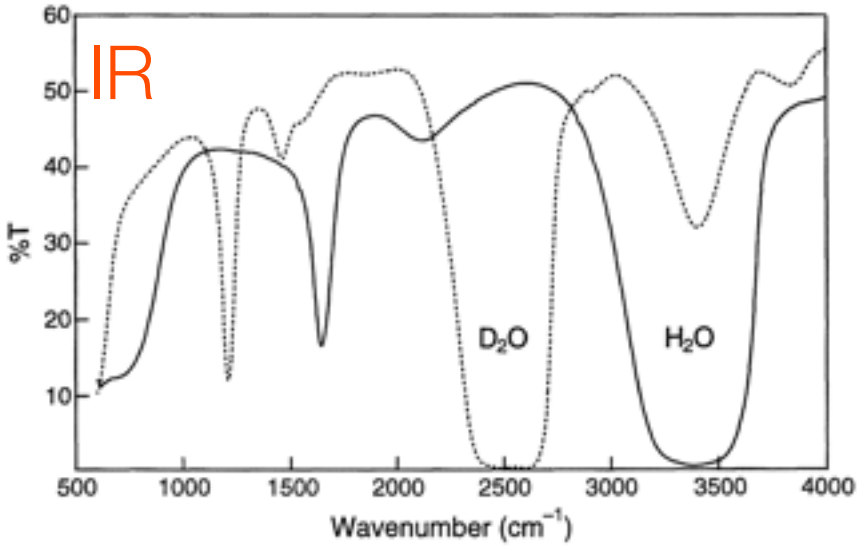
ν_2 (a_1)
Symmetric
Stretch

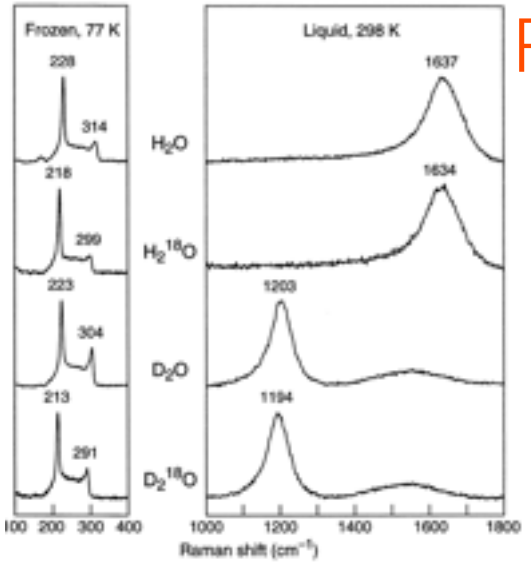
3652 cm^{-1}



ν_3 (b_1)
Asymmetric
Stretch

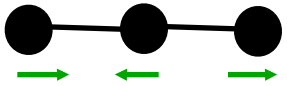
3756 cm^{-1}





Raman

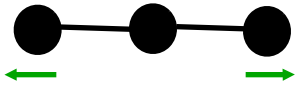
Normal Coordinates of CO_2



$\nu_3\ (\sigma_g^+)$

Antisymmetric Stretch

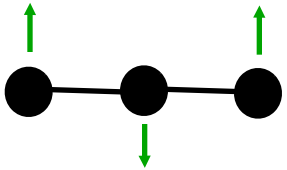
2349 cm^{-1}



$\nu_2\ (\sigma_g^+)$

Symmetric Stretch

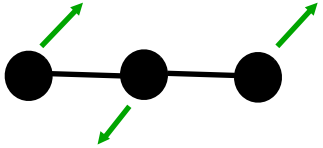
1337 cm^{-1}



$\nu_1\ (\pi_u)$

Out of plane Bending

667 cm^{-1}



$\nu_1\ (\pi_u)$

In plane Bending

667 cm^{-1}

„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
	Secondary Amines	3500
C≡N	Nitriles	2260-2240

General Normal Coordinate Analysis

In general there will be a „force constant (Hessian) matrix“ that is most simply calculated in Cartesian displacements (e.g. with ***ORCA***):

$$F_{X_A Y_B} = \frac{\partial^2 E}{\partial X_A \partial Y_B}$$

Masses

$$\tilde{F}_{X_A Y_B} = 15.57 \frac{F_{X_A Y_B}}{\sqrt{M_A M_B}} \quad \text{(in mdyn/Å)}$$

Diagonalization

$$\tilde{\mathbf{F}}\mathbf{Y}_I = x_I \mathbf{Y}_I$$

Frequencies and Modes

$$\nu_I = 1302.78\sqrt{x_I} \quad \text{(in cm}^{-1}\text{)}$$

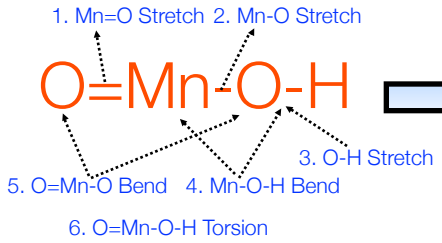
$$\mathbf{Q}_I = N^{-1/2}\left(\mathbf{M}^{-1/2}\mathbf{Y}_I\right)$$

Normalization

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*. To this end, 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.)

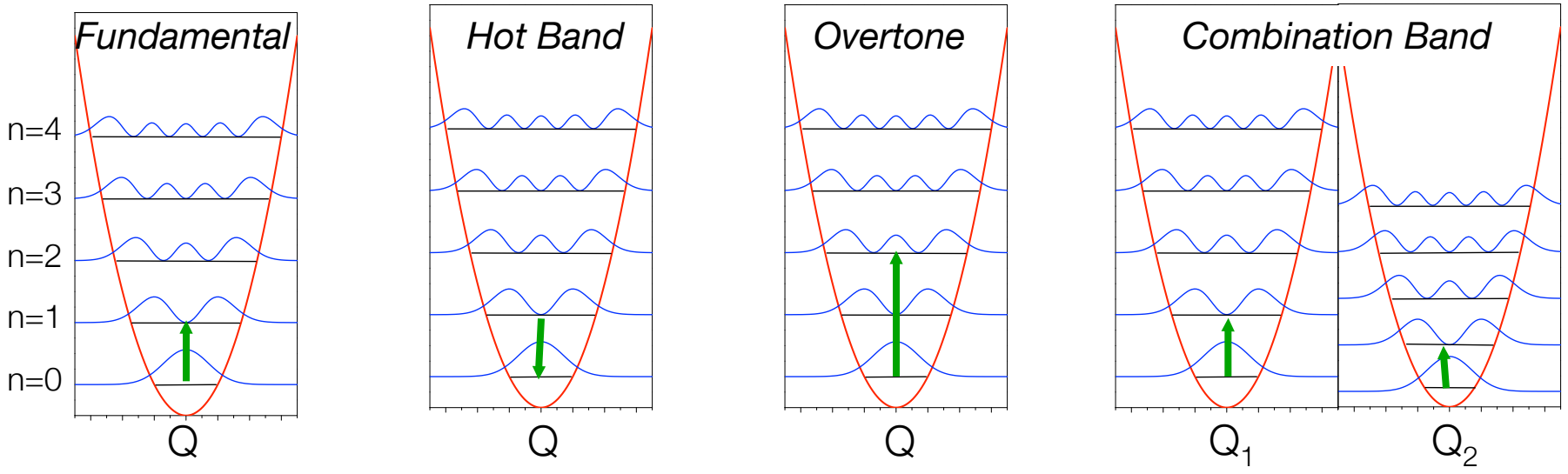
1. Normal coordinate fitting is fairly involved (→Specialists ☺)
   - Many parameters → Underdetermined equations!
   - Depends on subjective choice of model system
2. Often strong simplifications are necessary.
3. Results may apply to larger classes of compounds

II.B. Vibrational Intensities

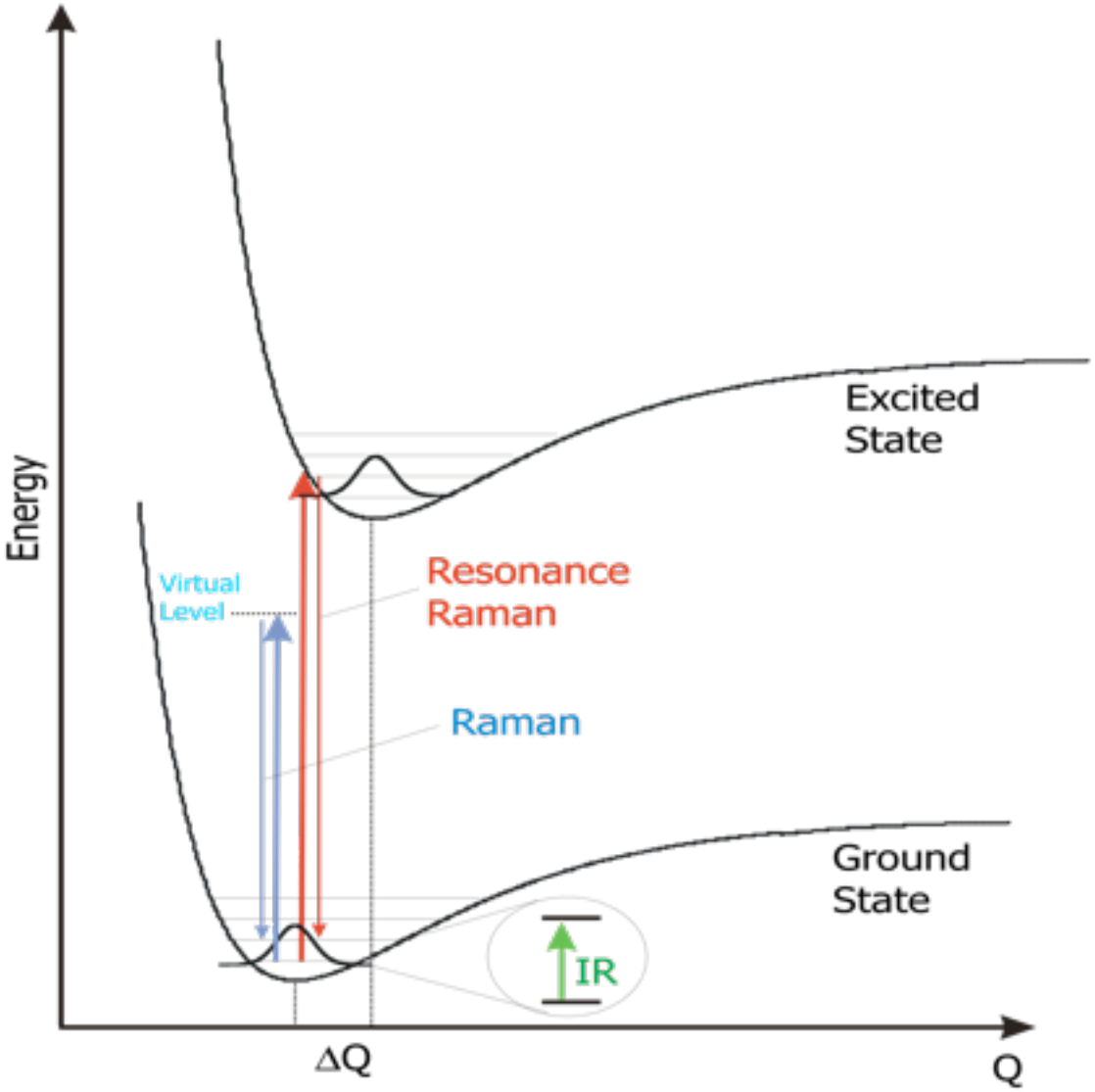
IR and Raman Transitions

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

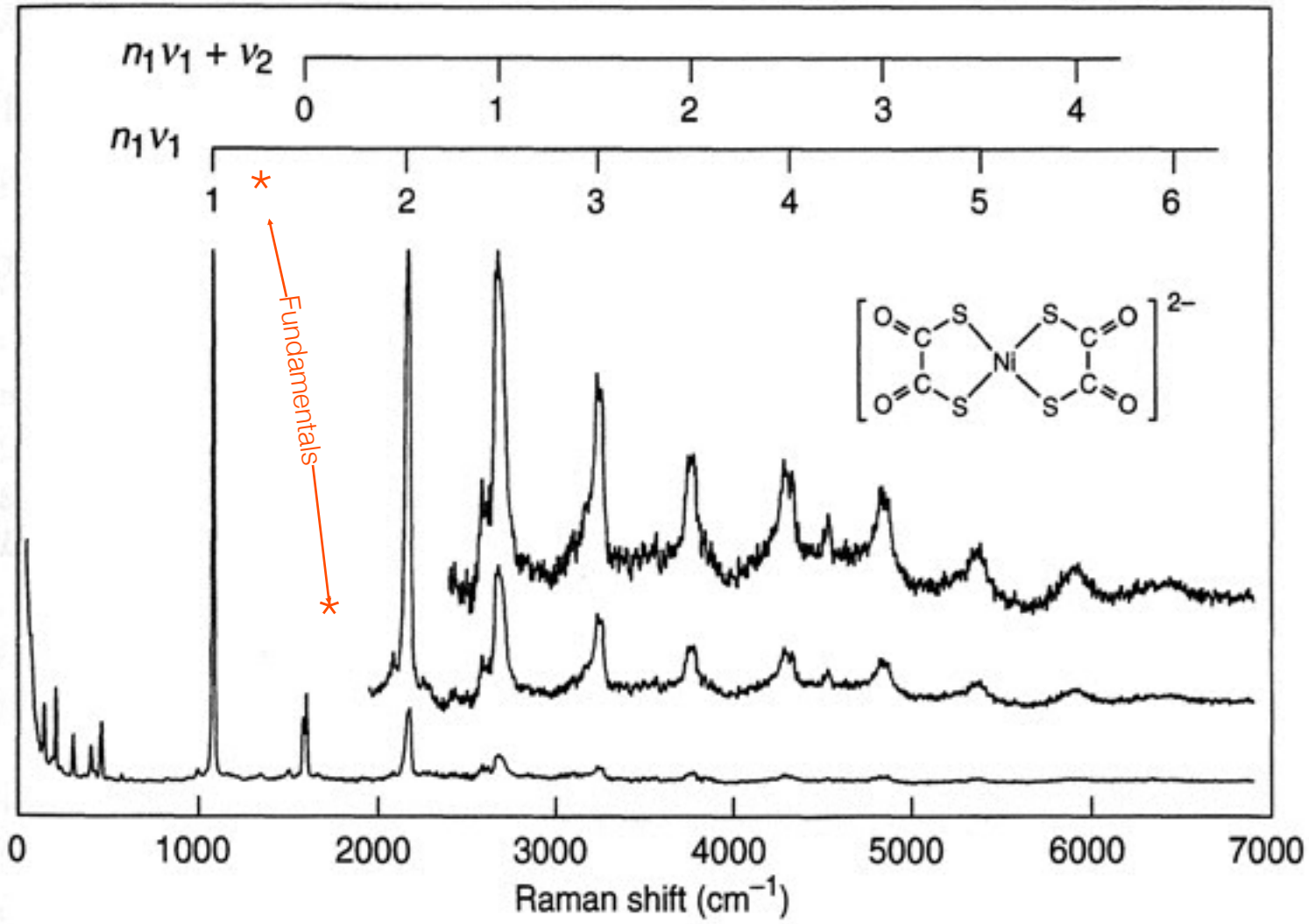
- **Fundamentals** : **n=0 → n‘=1** **ΔE=hν**
- „Hot“ Bands : n=1 → n‘=n±1 ΔE=hν
- Overtones : n=0 → n‘=2,3,... ΔE=2hν, 3hν,...
- Combination Bands: n_1=0, n_2=0 → n_1‘=1, n_2‘=1 $\Delta E=h\nu_1+h\nu_2$



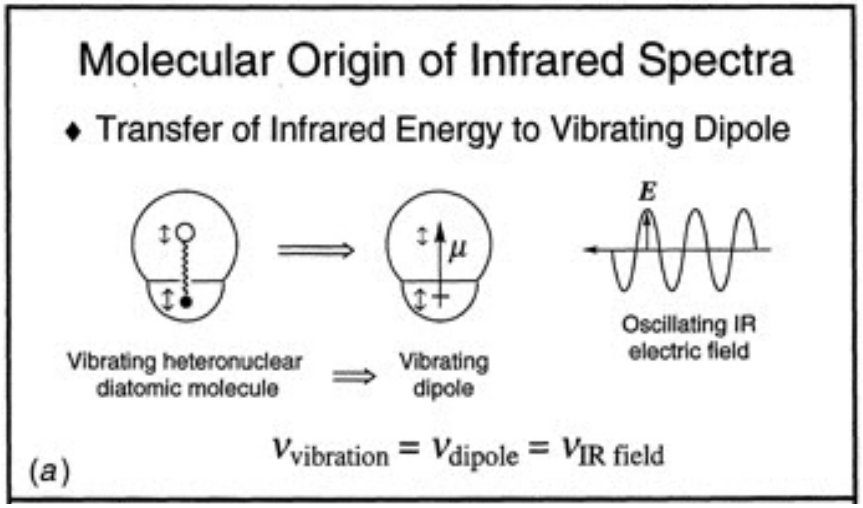
Physical Principles of IR and Raman Spectroscopy

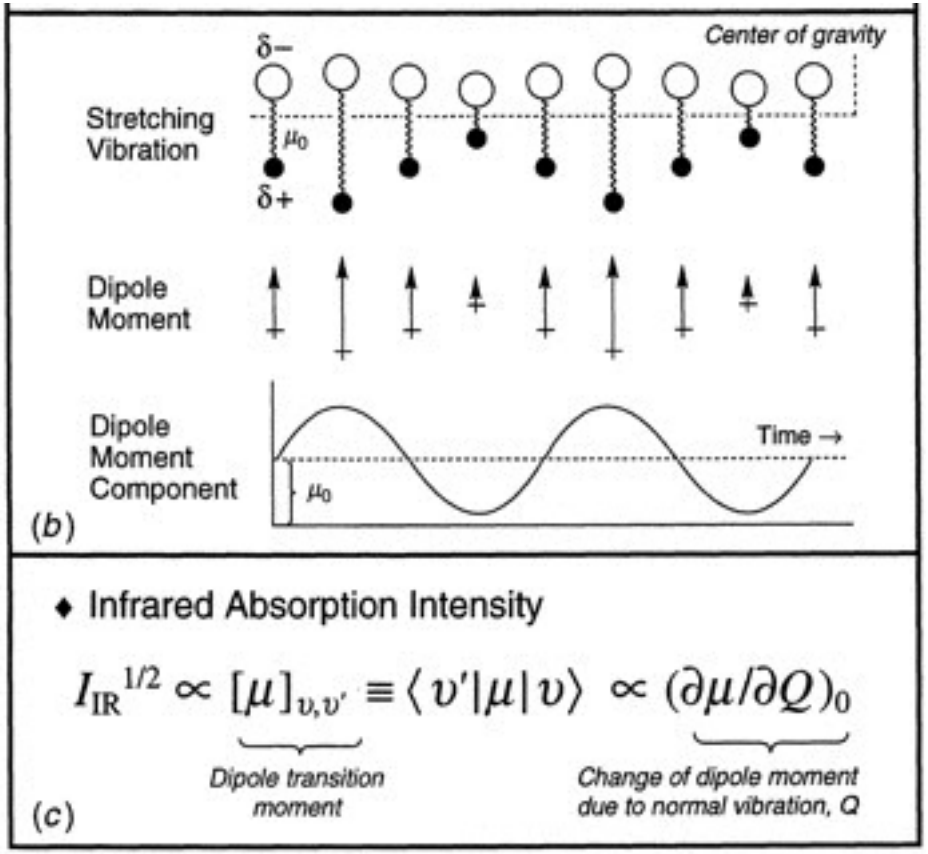


Overtone and Combination Bands

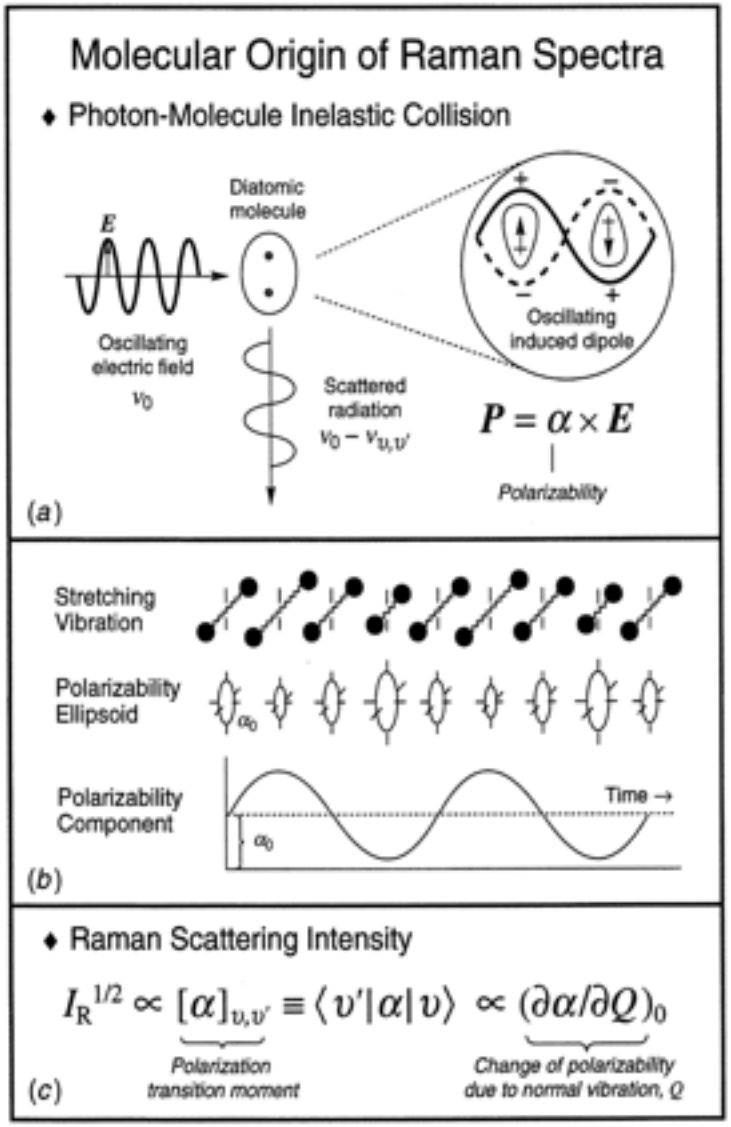


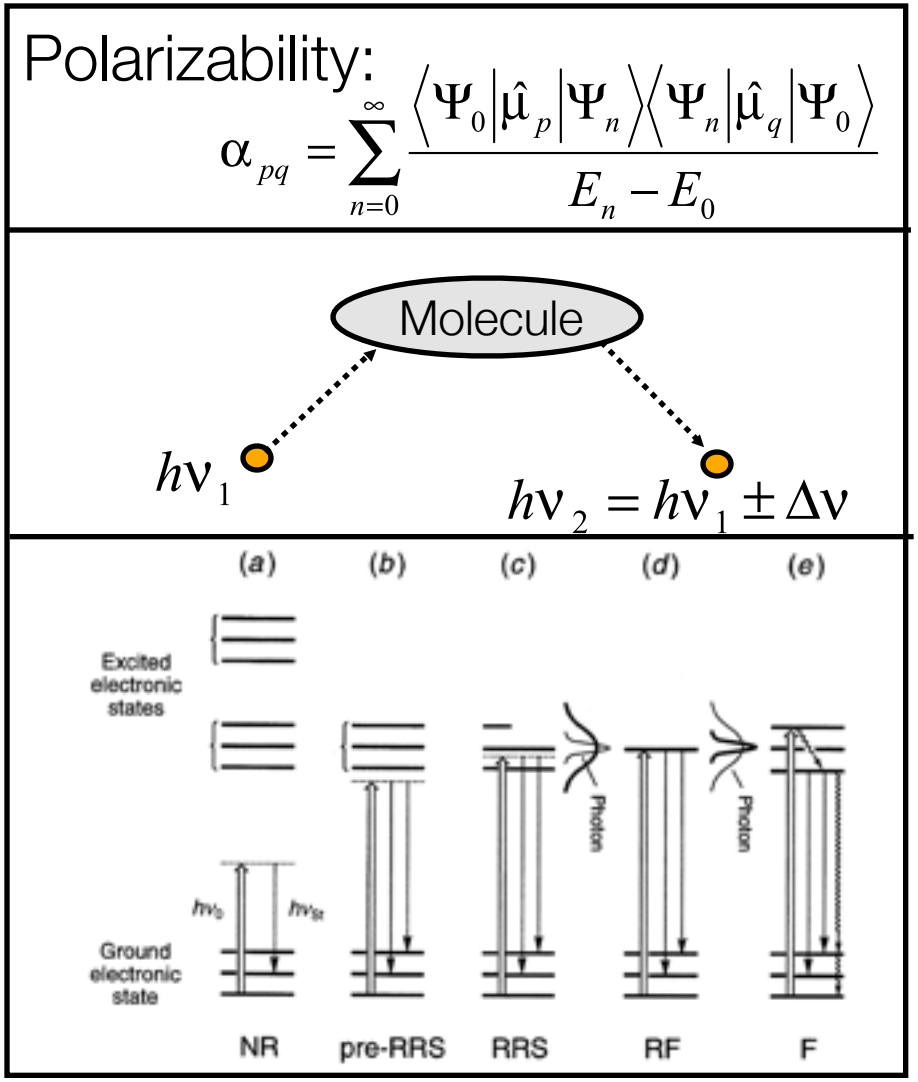
Physical Principle of IR Spectroscopy



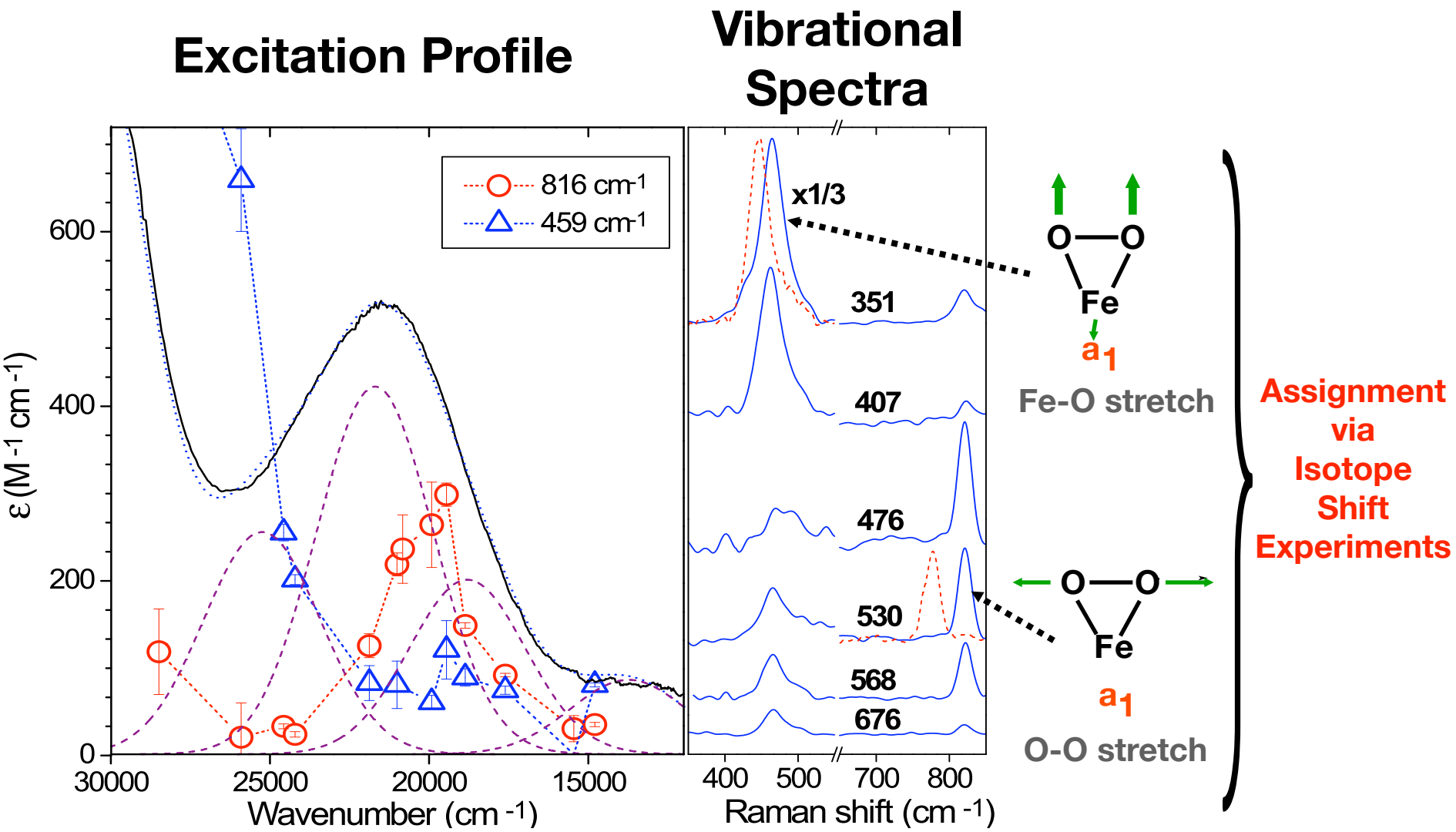


Physical Principle of Raman Spectroscopy





Dimensions in a Resonance Raman Experiment



Resonance Raman Intensity Mechanisms

- ★ In ordinary Raman spectroscopy one chooses a laser frequency far away from an excitation energy of the molecule (typically a Nd:YAG laser @ 1064 nm).
- ★ One then has to worry about the **change of polarizability** along the normal modes $\mathbf{Q}_i$

$$\alpha_{KL} = \sum_{n>0} \frac{\left\langle \Psi_n \mid \mu_{ED,K} \mid \Psi_0 \right\rangle \left\langle \Psi_0 \mid \mu_{ED,L} \mid \Psi_n \right\rangle}{E_n - E_0}$$

$$I_{Raman}\left(\omega_i\right) \propto \left(\frac{\partial \alpha}{\partial Q_i}\right)^2 \qquad \text{compare:} \qquad I_{IR}\left(\omega_i\right) \propto \left(\frac{\partial \mu_{ED}}{\partial Q_i}\right)^2$$

- ★ If the laser frequency approaches an excitation energy, it is no longer justified to use the frequency independent polarizability but instead we need the **frequency dependent polarizability**:

$$\alpha_{KL}\left(\omega\right) = \sum_{n>0} \underbrace{\frac{\left\langle \Psi_0 \mid \mu_{ED,K} \mid \Psi_n \right\rangle \left\langle \Psi_n \mid \mu_{ED,L} \mid \Psi_0 \right\rangle}{E_n - E_0 - \hbar\omega + i\Gamma}}_{resonant} + \underbrace{\frac{\left\langle \Psi_0 \mid \mu_{ED,K} \mid \Psi_n \right\rangle \left\langle \Psi_n \mid \mu_{ED,L} \mid \Psi_0 \right\rangle}{E_n - E_0 + \hbar\omega + i\Gamma}}_{non-resonant}$$

Transition Dipole Moment

Transition Energy

Photon Energy

Damping Factor (Linewidth)

Resonance Raman Intensity Mechanisms

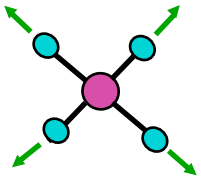
★ Thus, if the frequency of the light is such that it nearly matches an excitation energy (say for state I) the first term of the FDP strongly dominates and one has for the change of polarizability:

Transition Energy Gradient!

$$I_{rR}^{1/2}\left(Q_i\right) \propto \sum_{KL} \frac{\partial \alpha_{KL}\left(\omega\right)}{\partial Q_i} = \frac{1}{i\Gamma} \sum_{KL} \underbrace{\frac{\partial}{\partial Q_i} \left\langle \Psi_0 \mid \mu_{ED,K} \mid \Psi_I \right\rangle \left\langle \Psi_I \mid \mu_{ED,L} \mid \Psi_0 \right\rangle}_{SMALLER\ \ TERM \left(non\text{-}totally\ symmetric\ modes\right)} + \underbrace{\alpha_{KL}\left(\omega\right) \frac{\partial (E_I - E_0)}{\partial Q_i}}_{LARGER\ TERM \left(totally\ symmetric\ modes\right)}$$

➡ Most resonance Raman spectra are dominated by totally symmetric vibrations (those that do not break the symmetry of the molecule)

➡ Very good to observe stretching vibrations



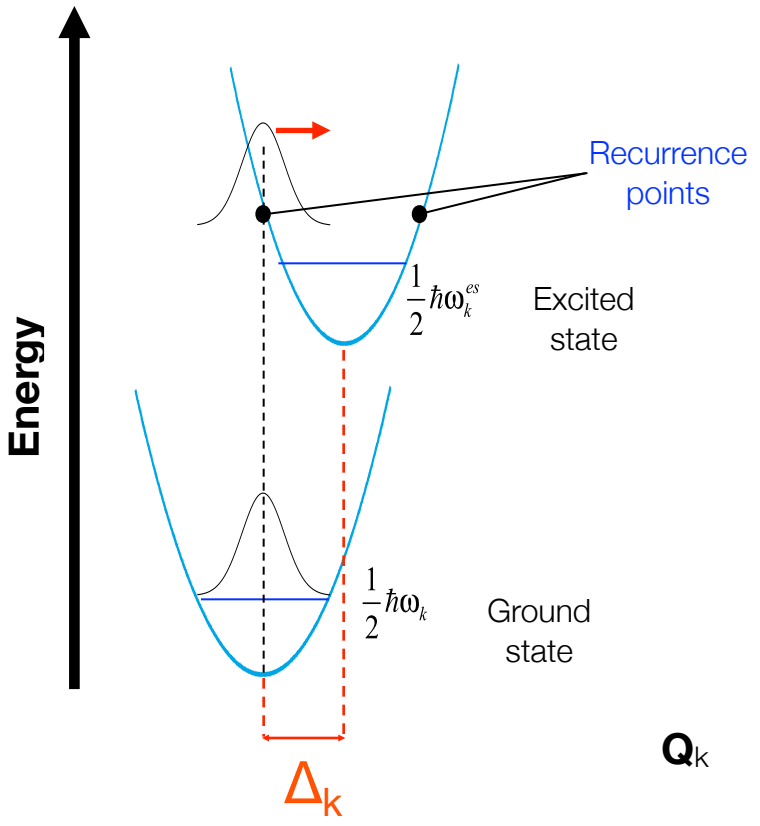
Time Dependent Picture of Resonance Raman

Heller Theory
(Time-domain wave packet propagation)
Cost ~ (Number of Modes)

$$\sigma(E_L) = \frac{4\pi e^2}{3\hbar c} M^2 \times E_L \times \mathrm{Re} \int_0^\infty \langle i | i(t) \rangle e^{i(E_L + \varepsilon_i + i\Gamma)t} \, dt$$

$$i(t) = e^{-i\hat{H}_{ex}t} | i \rangle$$

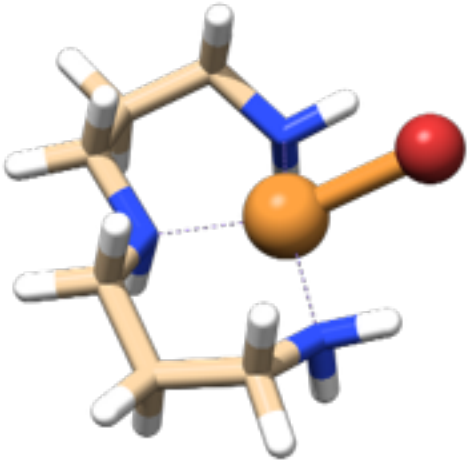
$$(\alpha_{\rho\lambda})_{I \to F} = M_\rho M_\lambda \int_0^\infty \langle f | i(t) \rangle e^{i(E_L + \varepsilon_i + i\Gamma)t} \, dt$$

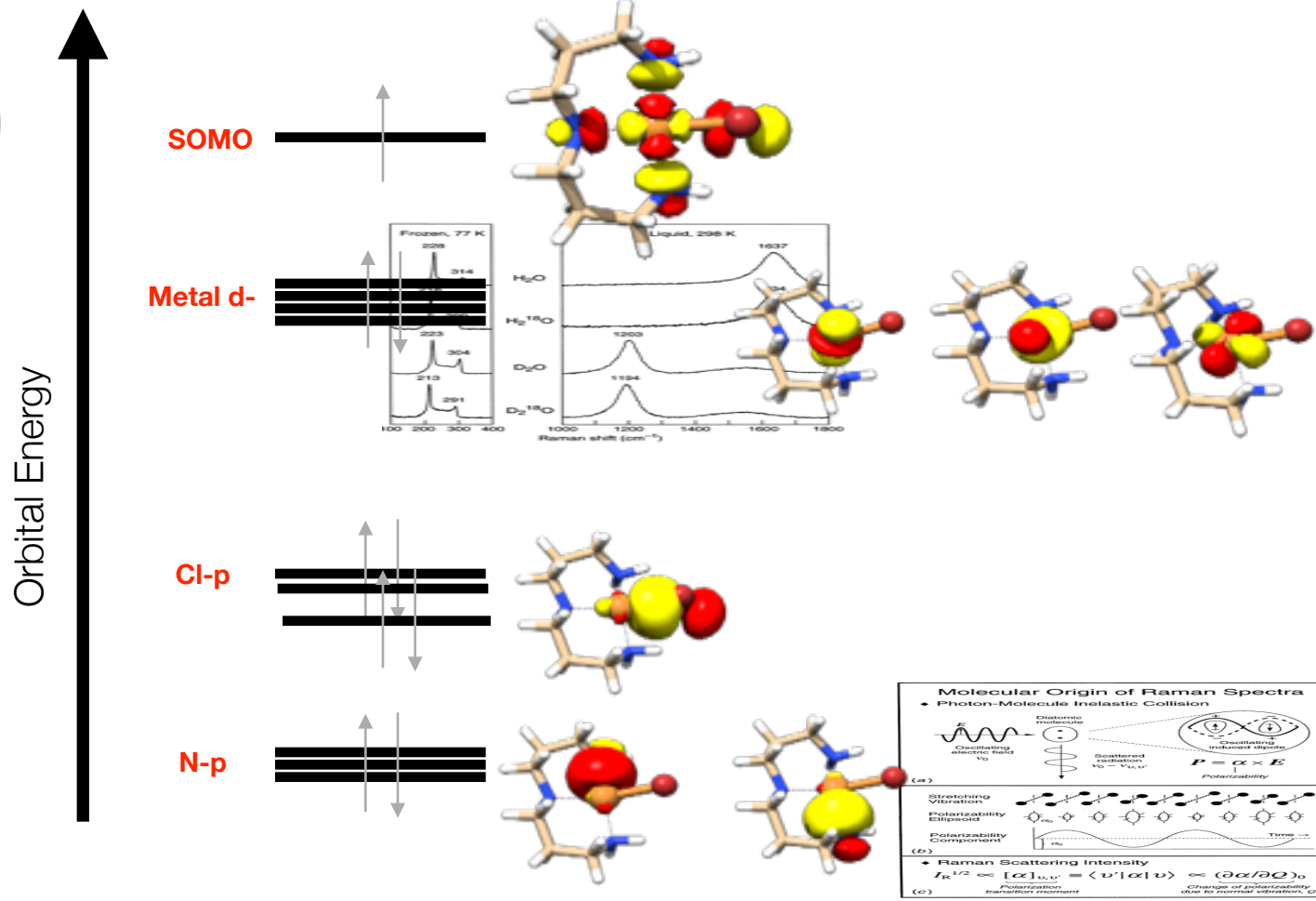


Lee, S. Y.; Heller, E. J. *J. Chem. Phys.*, **1979**, 71, 4777 Tannor, D. J.; Heller, E. J. *J. Chem. Phys*, **1982**, 77, 202
Petrenko, T.; FN *J. Chem. Phys.*, **2007** 127, 164319

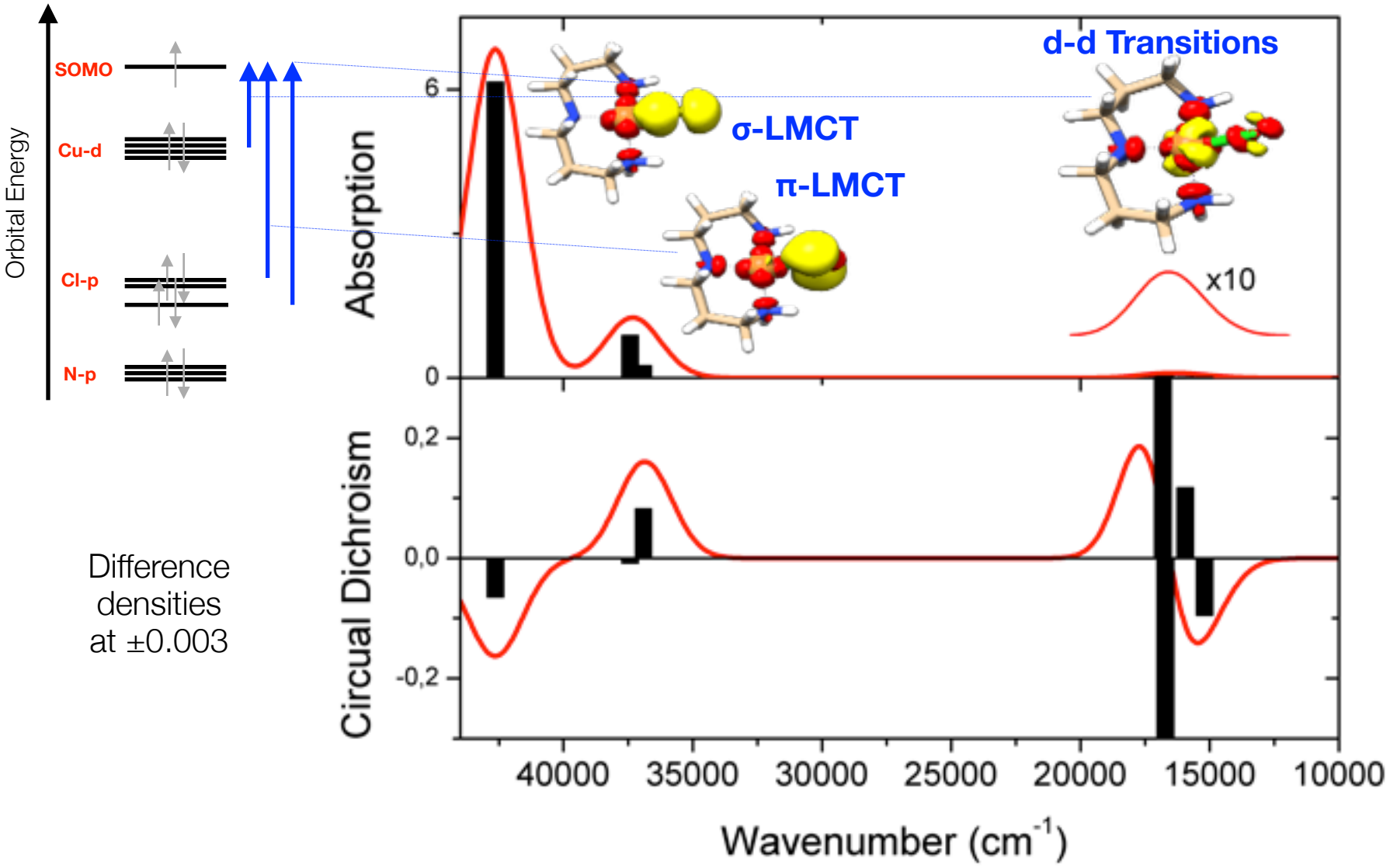
A Toy Model: Resonance Raman Intensities

In order to grasp the essence of the rR intensity mechanism, let's look at a simple hypothetical model Cu(II) d^9 complex with a coordinating Cl^- ligand





A Toy Model: Optical Spectra

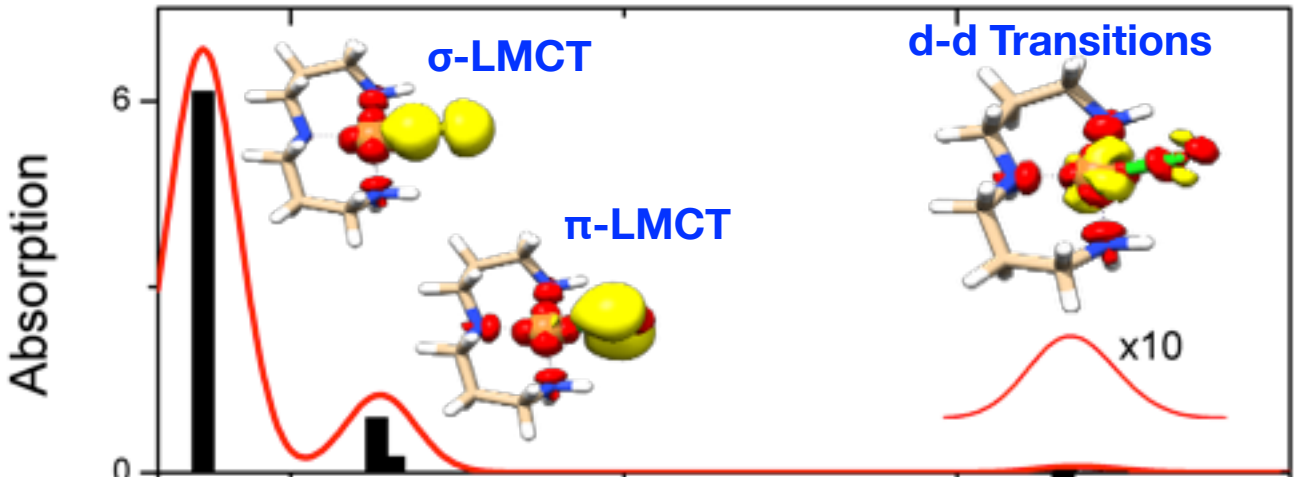


Difference densities at ±0.003

Short Digression: Difference vs Transition Density

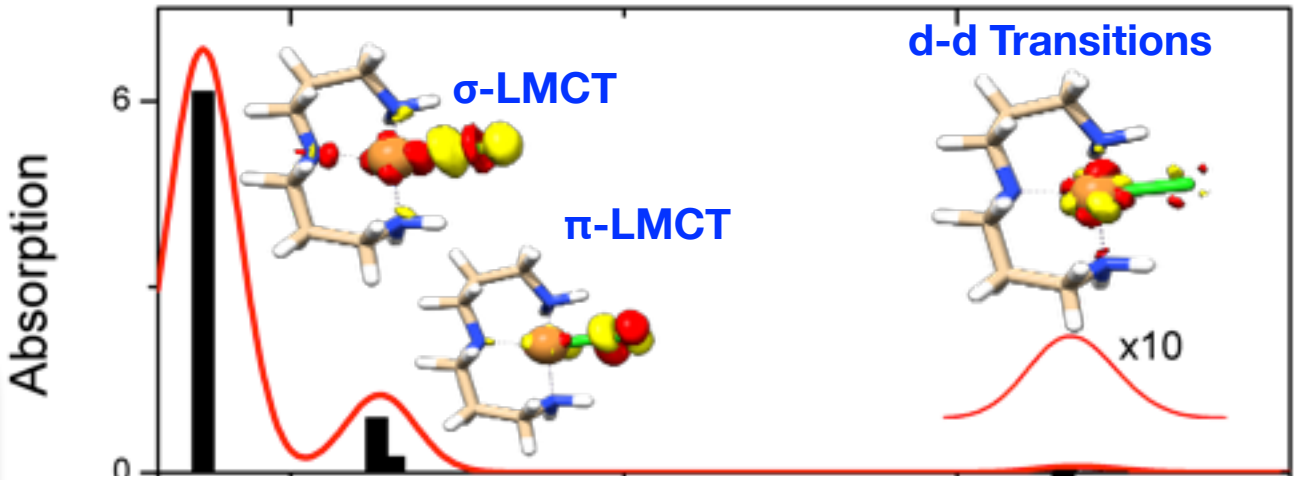
Difference densities at ±0.003

$$\Delta P \propto \psi^2_{acceptor} - \psi^2_{donor}$$



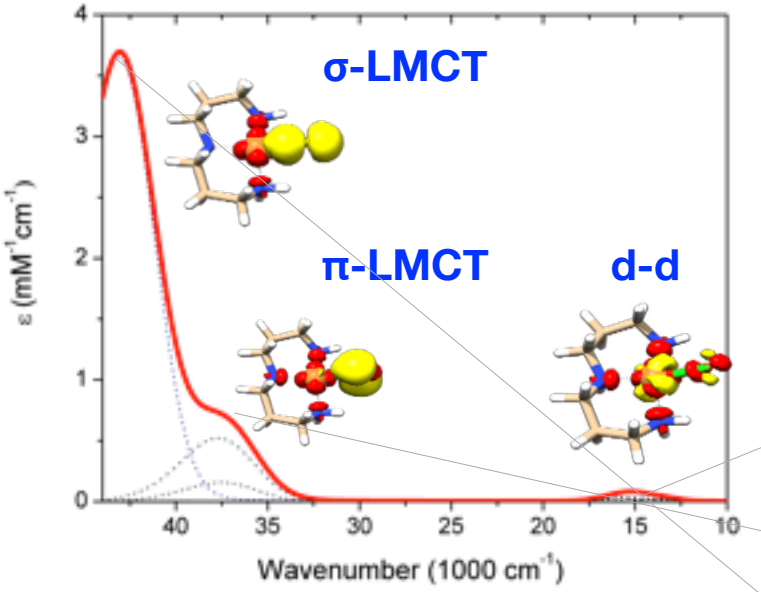
Transition densities at ±0.003

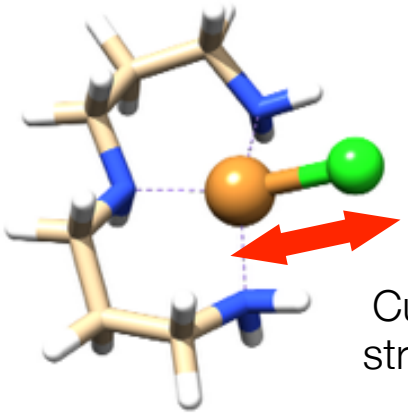
$$D \propto \psi_{acceptor}\psi_{donor}$$



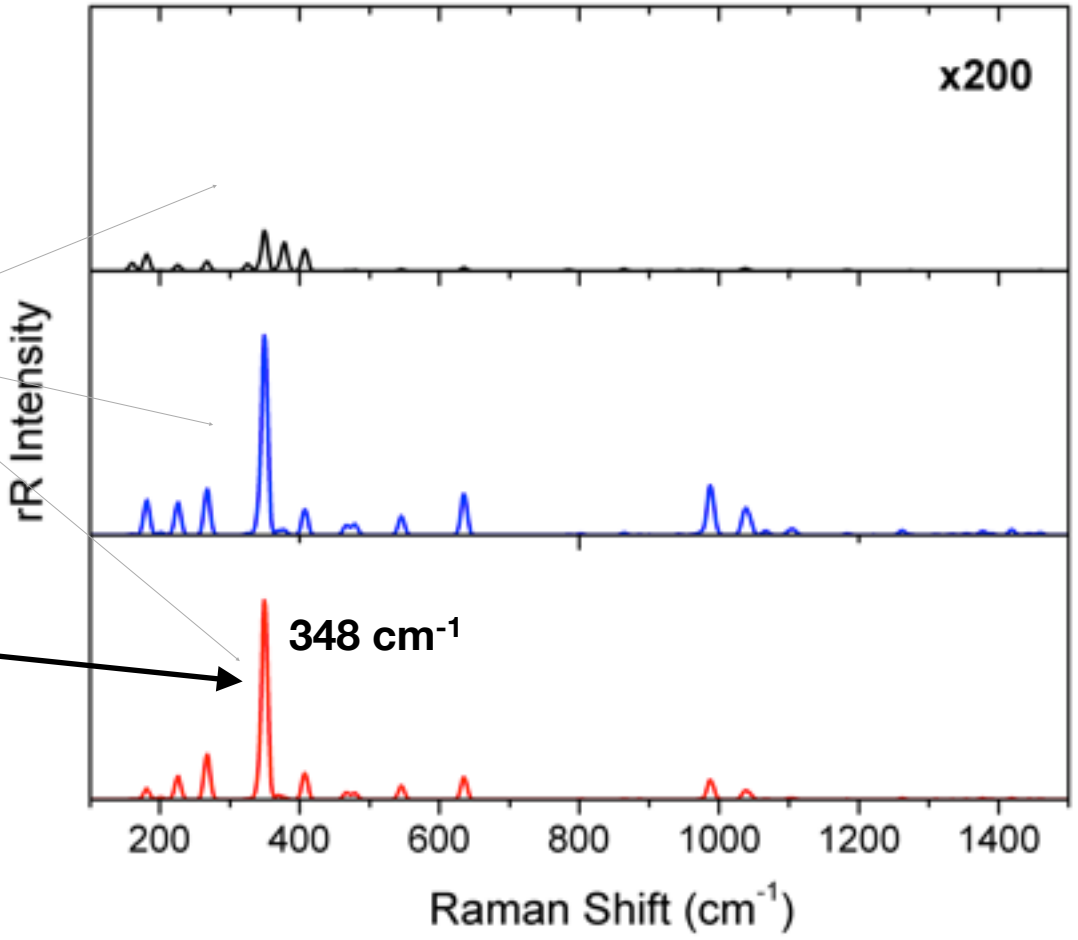
ABS Intensity ∝ D^2

A Toy Model Resonance Raman Spectra



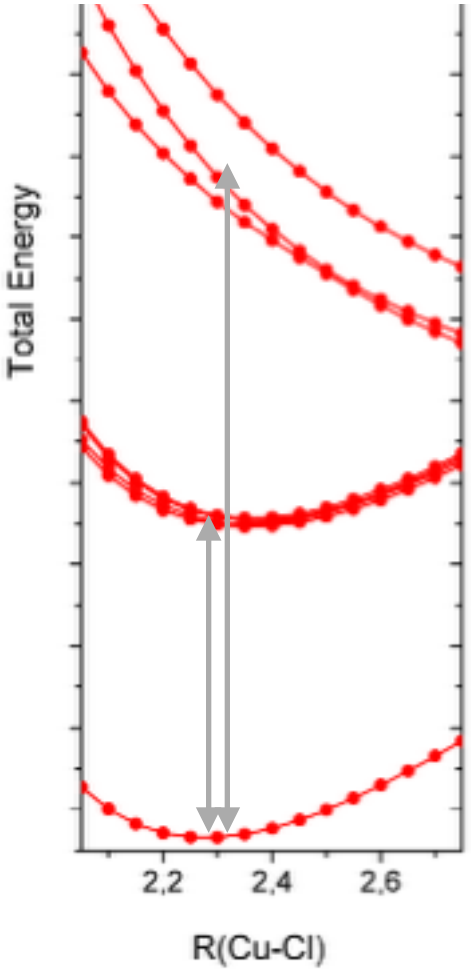


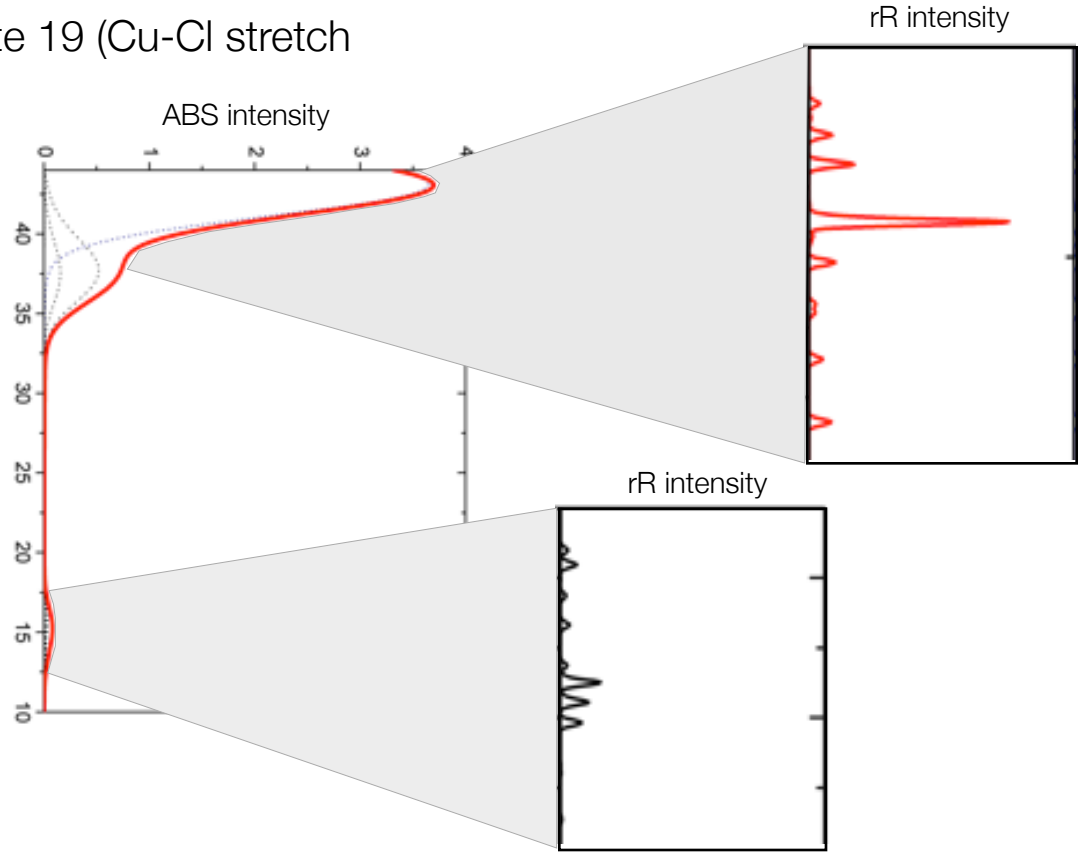
Cu-Cl stretch



Potential Energy Surfaces and Raman Intensity

Scan along the normal coordinate 19 (Cu-Cl stretch

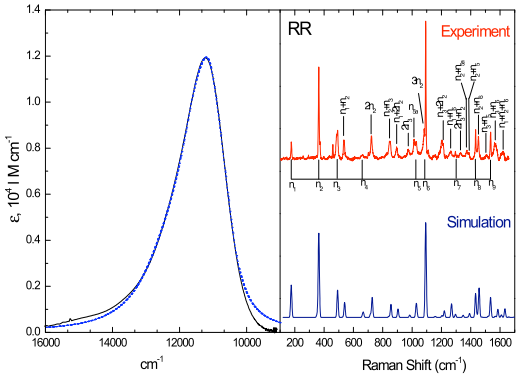




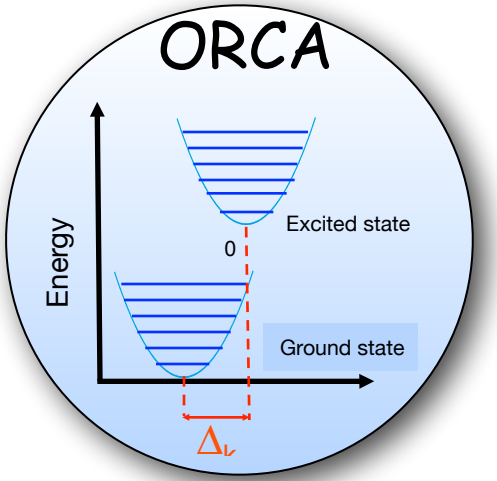
- ✓ d-d excitations have very small excited state distortions and hence very little rR intensity
- ✓ LMCT excitations have large excited state distortions and hence large rR intensities

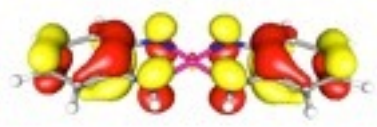
Resonance Raman: Theory and Experiment

Experiment



Quantum Chemistry





$$\Delta_k \cong \frac{\partial E}{\partial Q_k} \frac{1}{\omega_k^{es}\sqrt{\hbar\omega_k^{es}}}$$

Excited state energy gradient along normal mode k

Frequency of k-th normal mode in the excited state

Efficient Analytic TD-DFT gradient

FIT

CALC

1. Measure absorption
2. Measure RR at different wavelengths
3. Assign spectra (IR, normal Raman, isotope perturbations, calculations,...)
4. Simulate RR+ABS spectra starting from theoretically predicted displacements

1. Optimize geometry
2. Calculate ground state harmonic frequencies and normal modes
3. Obtain excited state energies and transition moments
4. Estimate excited state displacements from E.S. gradients and Hessians

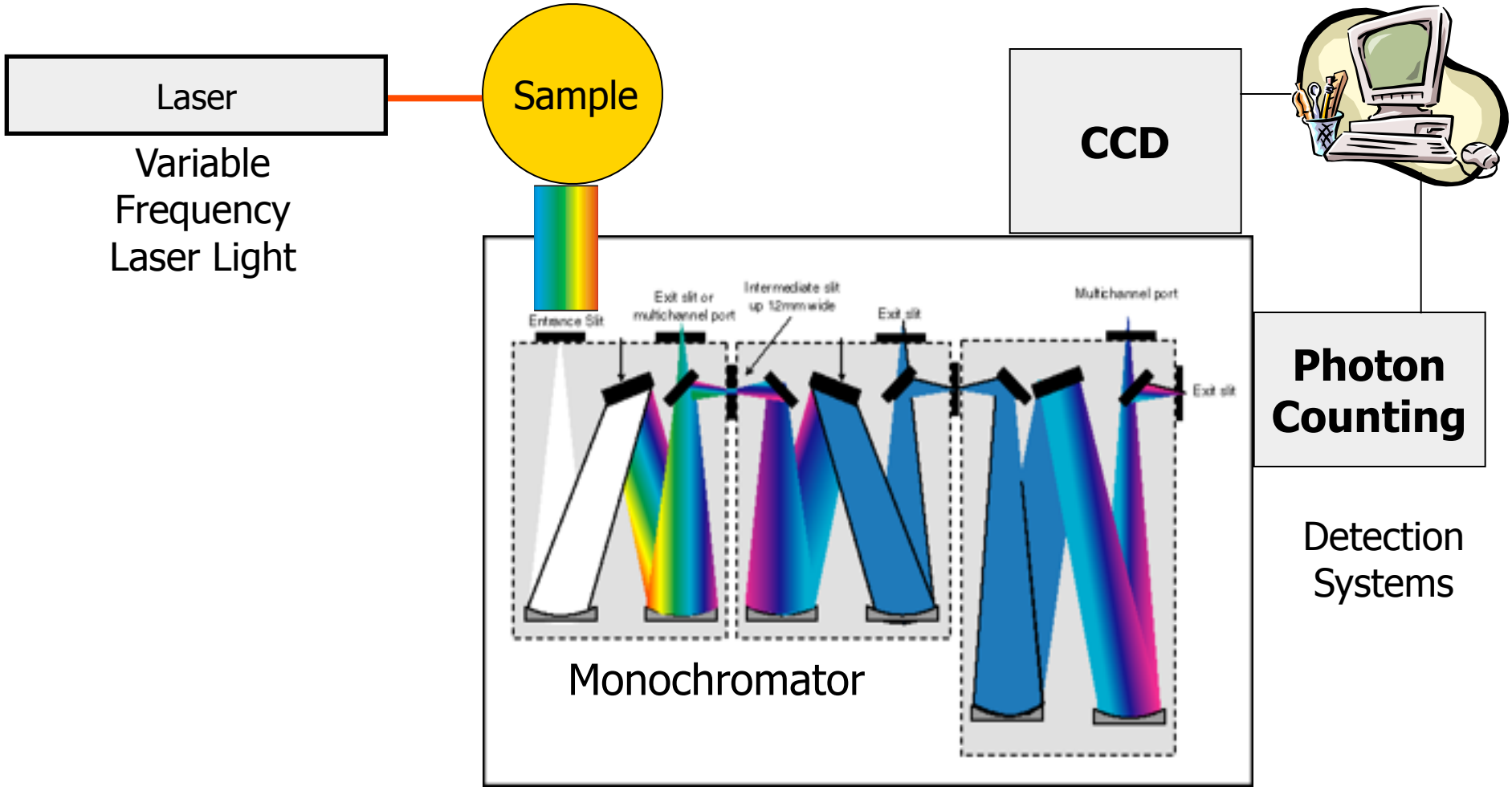
Theory: Petrenko, T.; FN *J. Chem. Phys.*, **2007** 127, 164319

Summary of Theoretical Aspects

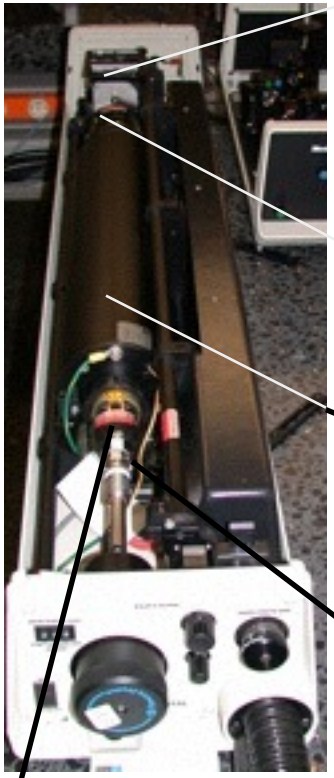
1. Force constants measure the stiffness of internal motions (stretches, bends, torsions).
2. Normal modes and vibrational frequencies describe the „Eigenvibrations“ (distortions) of the Molecule. They depend on the force constants (different for each electronic state!), the masses of the atoms and the 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).

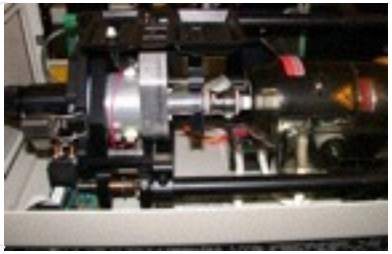
III. Experimental Aspects

Experimental Raman Setup



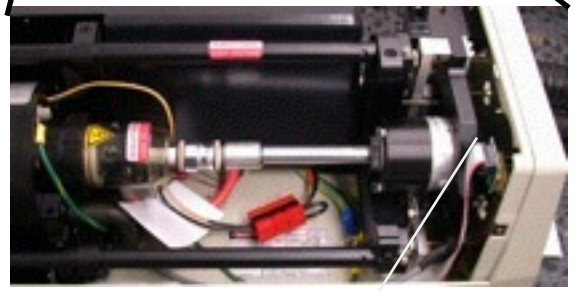
Lasers (Light Amplification by Stimulated Emission)



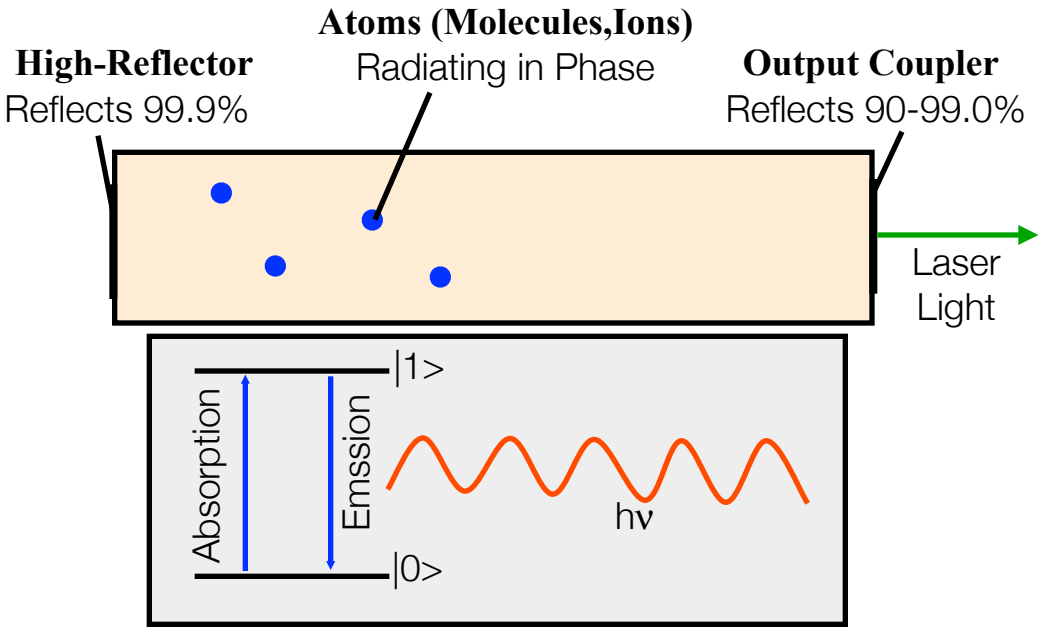


Output Coupler

Gas Filled
Laser Tube

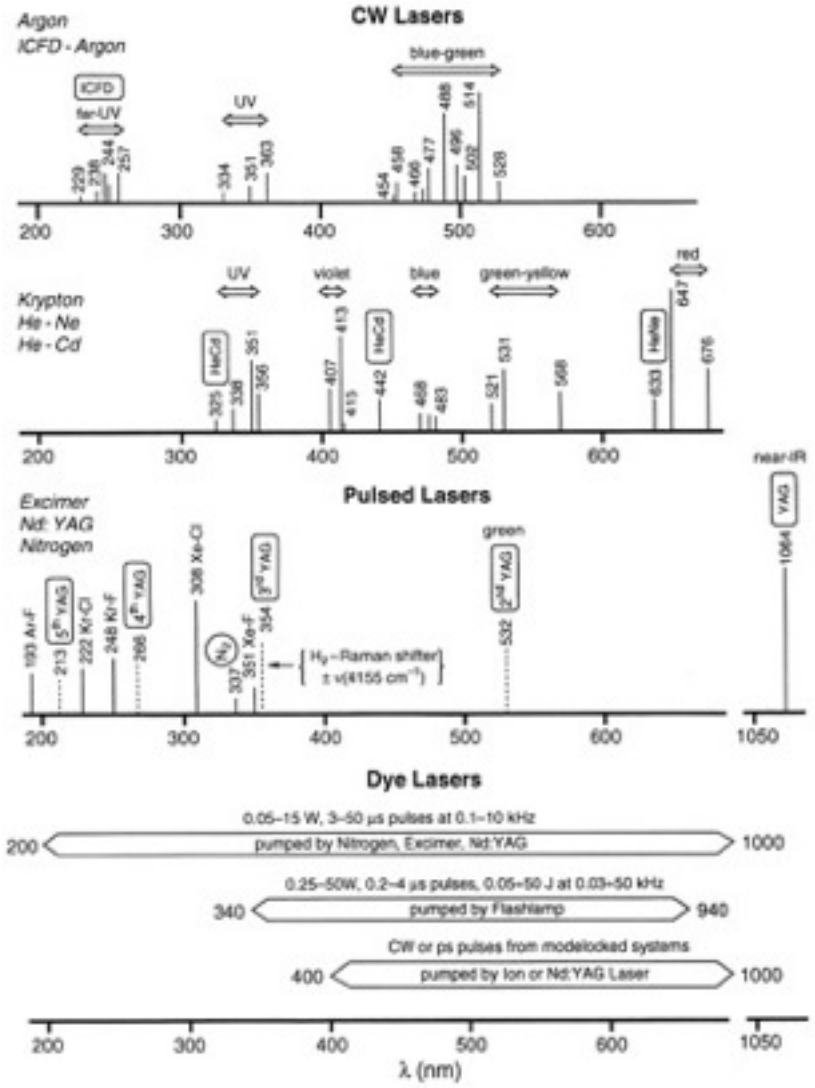


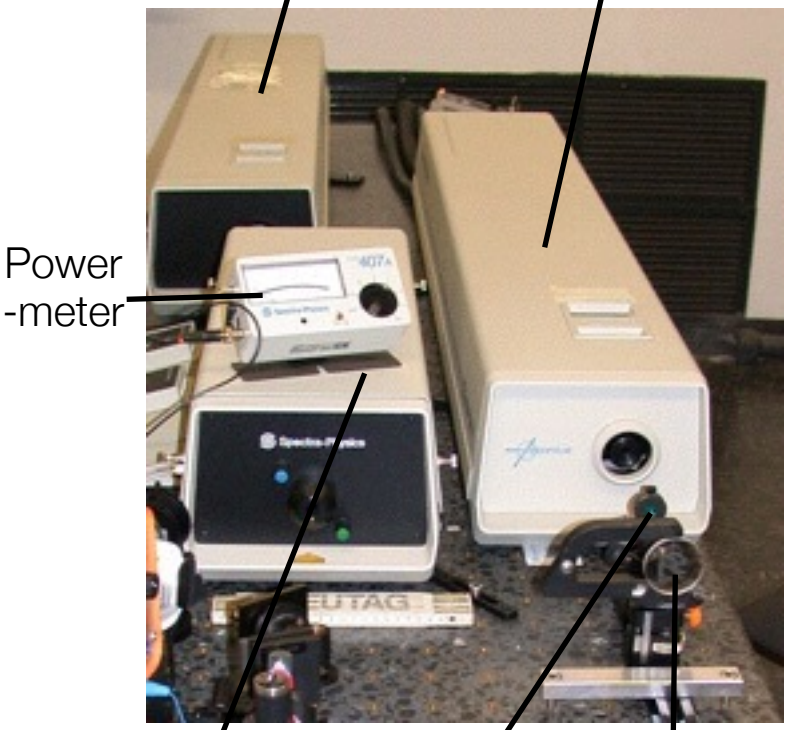
High-Reflector



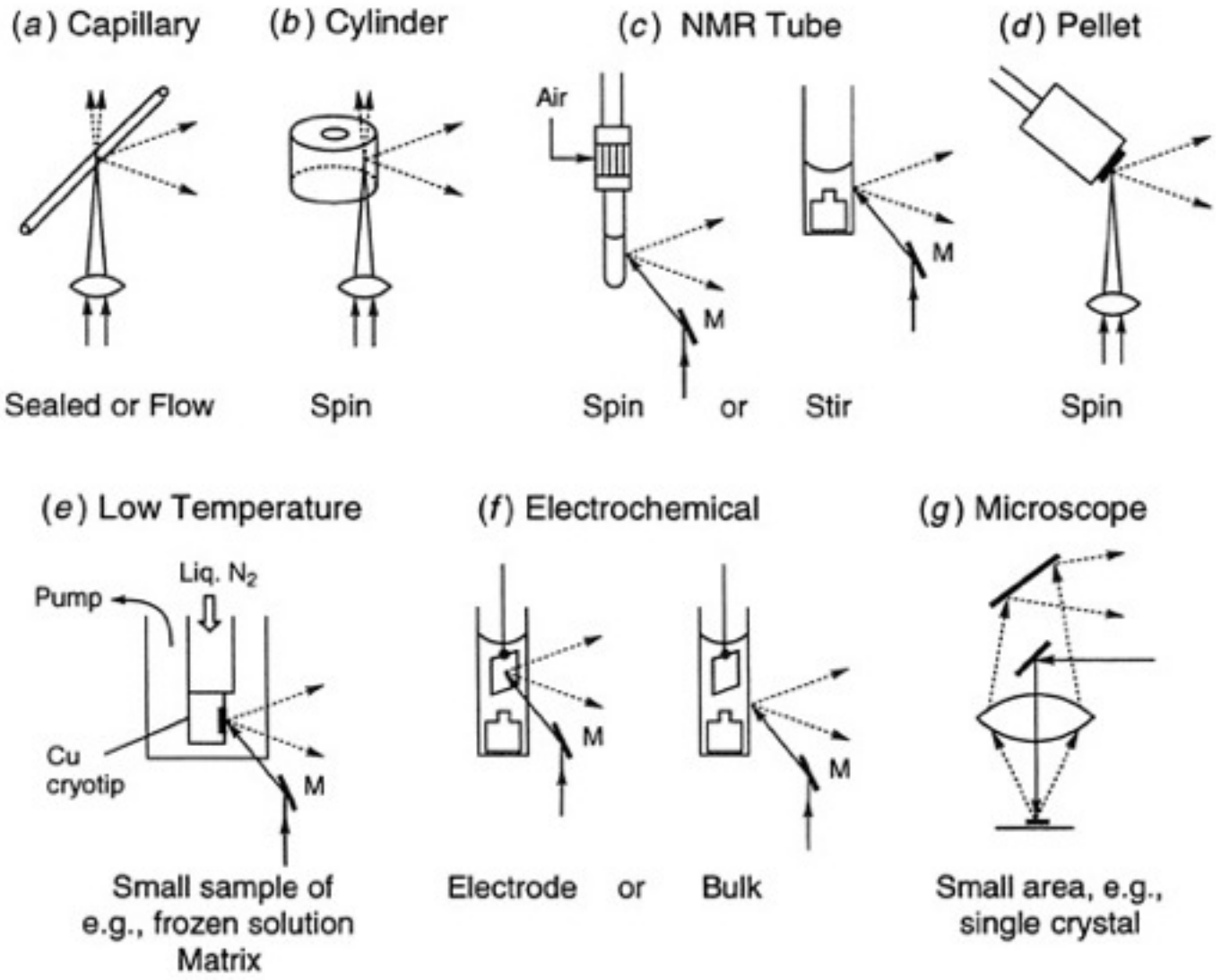
1. Initial Photons are created by spontaneous emission due to heating or electrical discharge.
2. The emitted photons stimulate other atoms (molecules, ions) to emit photons of the same frequency *in Phase* ($A^{*}+h\nu \rightarrow A+2h\nu$)
3. As these photons are absorbed by neighboring atoms population 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



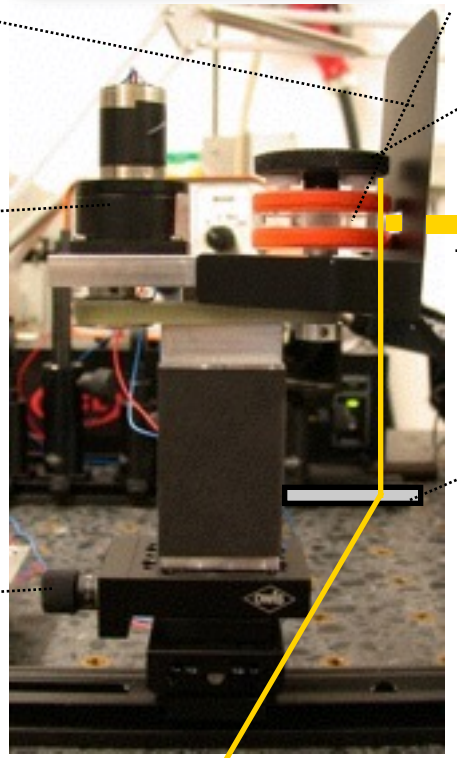


Sample Compartments

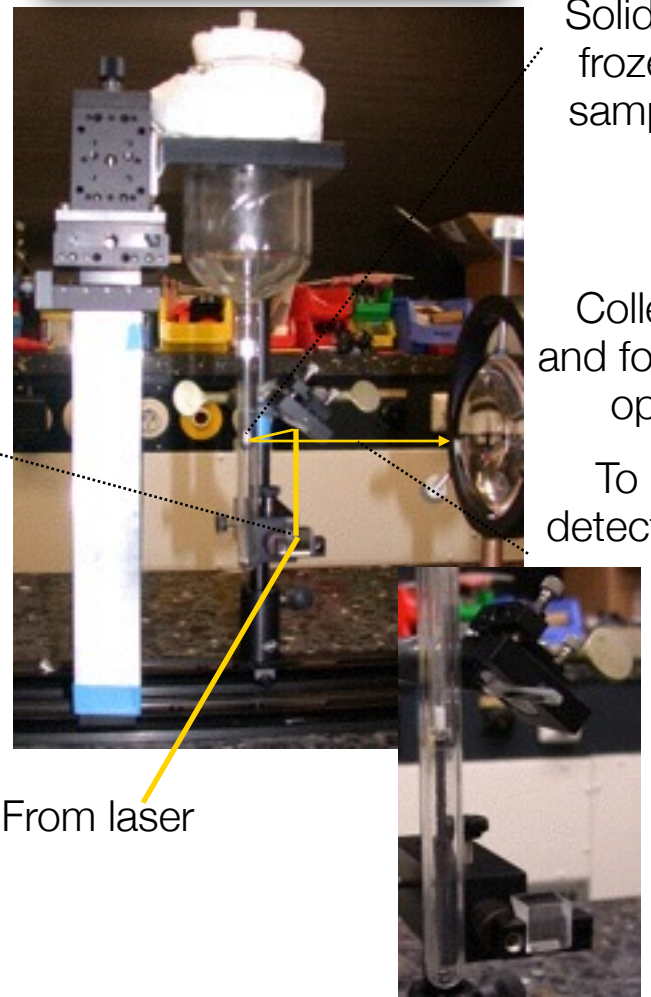


Sample Compartments

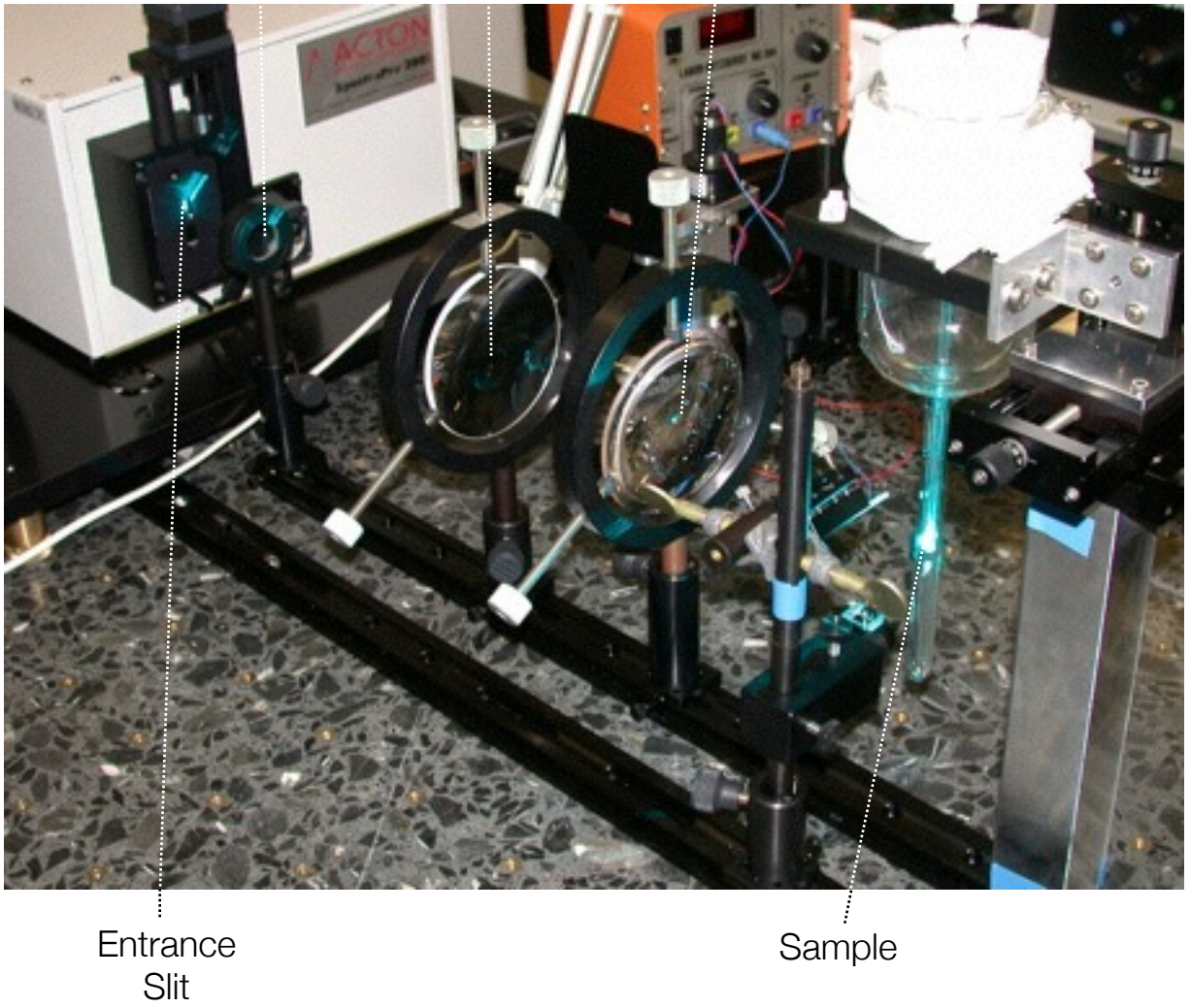
Rotating Cell



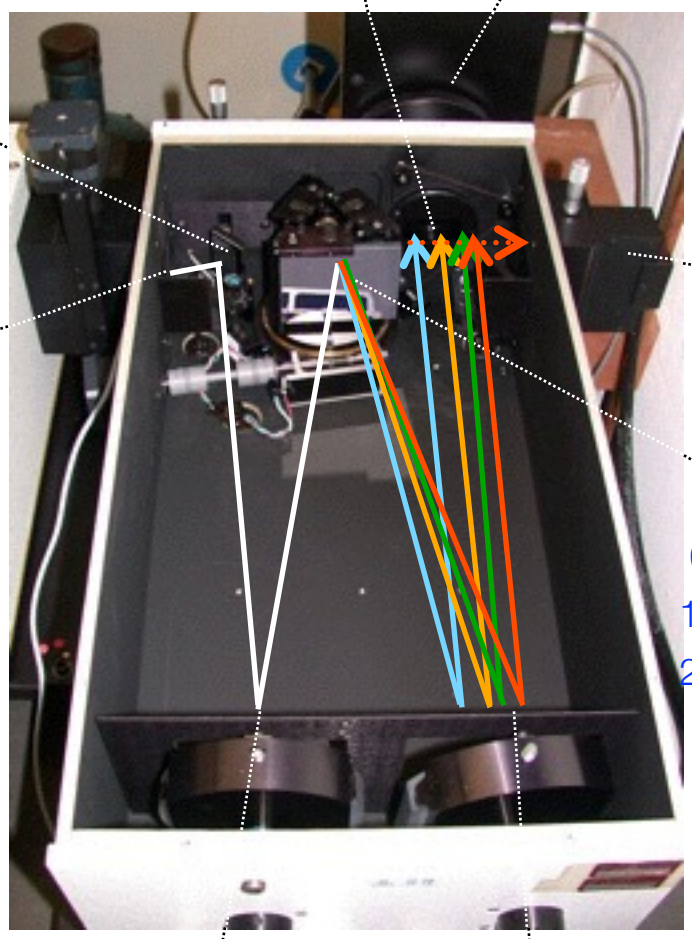
Liquid N_2 Dewar



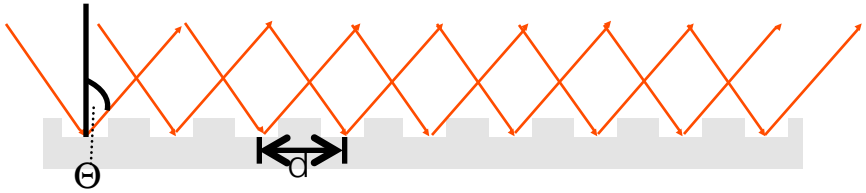
Collecting Optics



Monochromators



A diffraction grating is a ***dispersive element*** used to separate light into single wavelength contributions



Interference of incoming and outgoing light waves produces an interference pattern with maxima if:

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

d: Distance between Grooves; m: ‚Order' of the Maximum=0,1,...; λ: wavelength

Resolving power of a grating

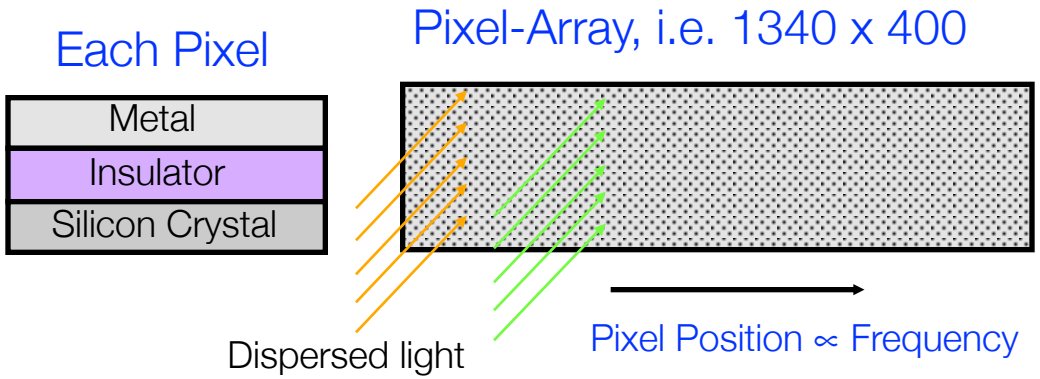
$$\frac{\lambda}{|\Delta\lambda|} = mN$$

N: Number of grooves

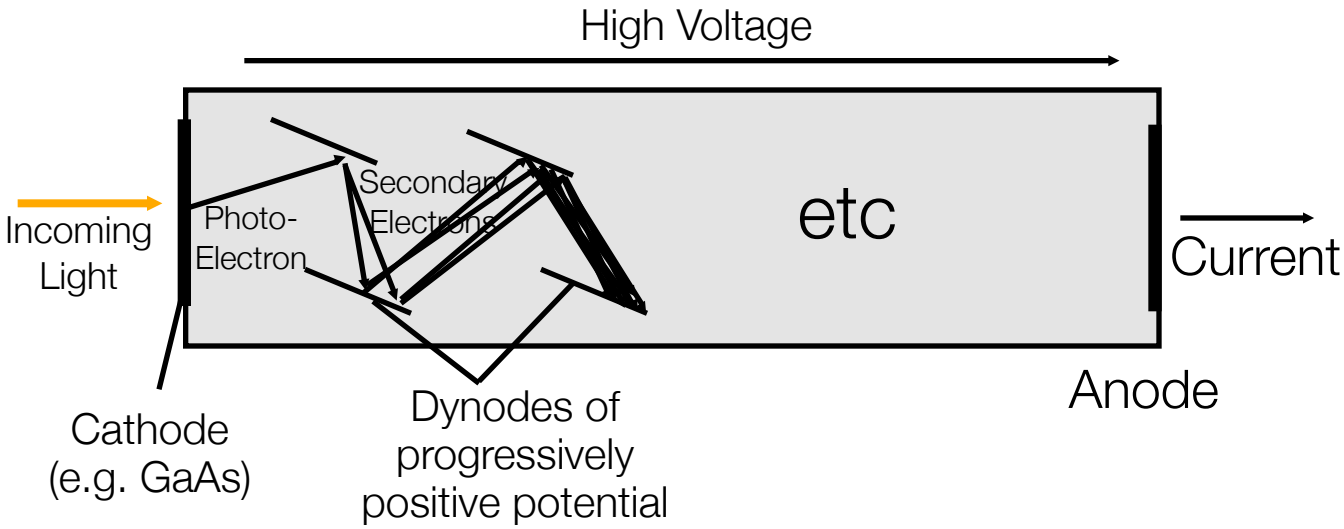
- More Grooves = More Resolution = Smaller Bandpath
- Longer Wavelength = Higher Resolution = Smaller Bandpath

→ Use smaller grating at longer wavelengths

Detection Systems (CCD, PMT)



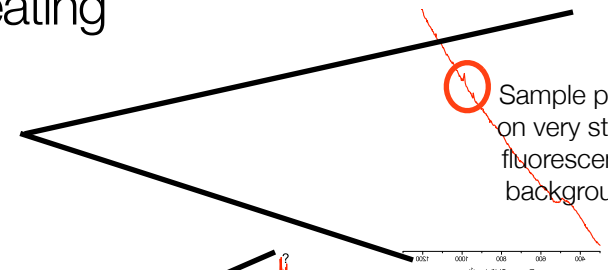
- High conversion efficiency (40-60%)
- Low noise levels
- Simultaneous detection of large parts of the spectrum!

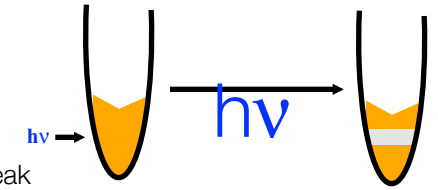


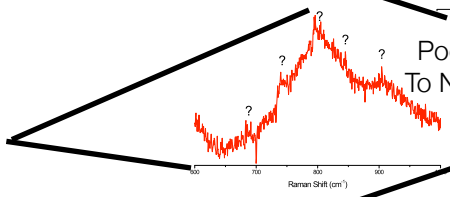
Possible Experimental Complications
(„Dirty Laundry Slide“)

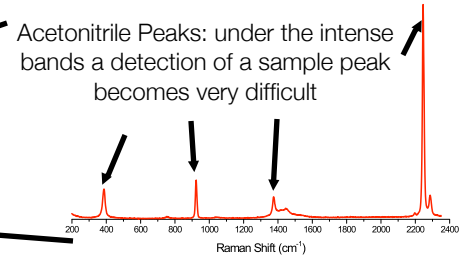
1. Decomposition due to Overheating
2. Photodissociation of Bonds
3. Fluorescence Background
4. Exceedingly Weak Signals
5. Features Obscured by Solvent Peaks
6. External Artifacts

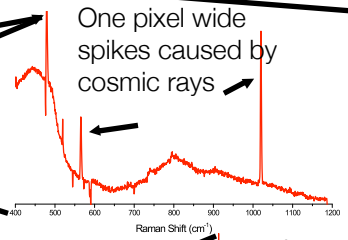
   (Cosmic Rays,Other Light Sources)
7. Plasma Lines from the Laser

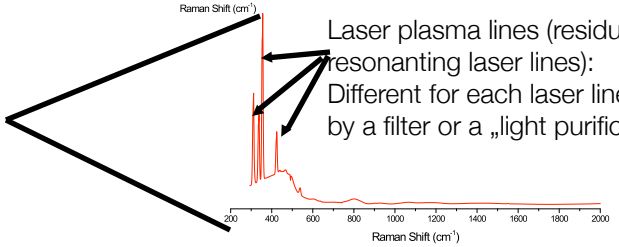




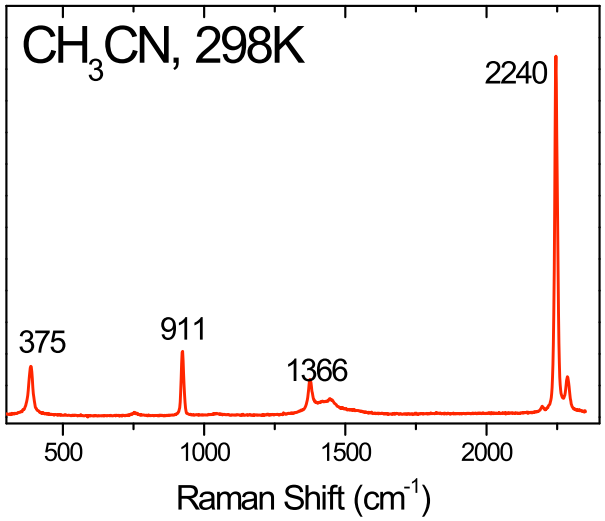


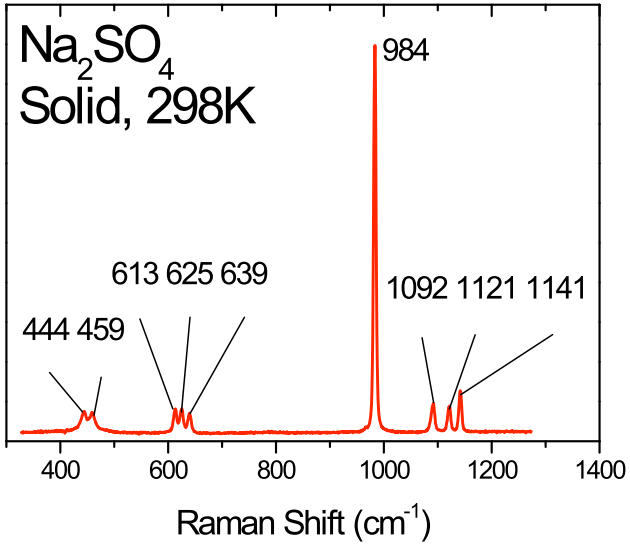


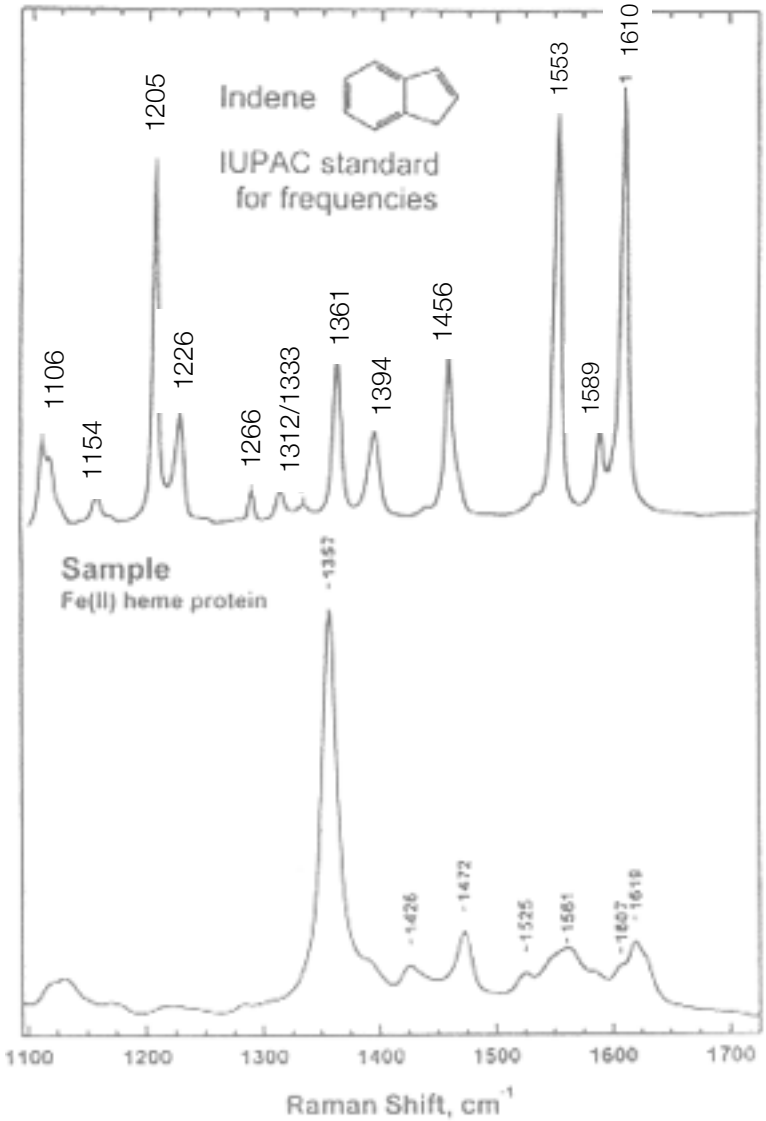




Frequency Calibration



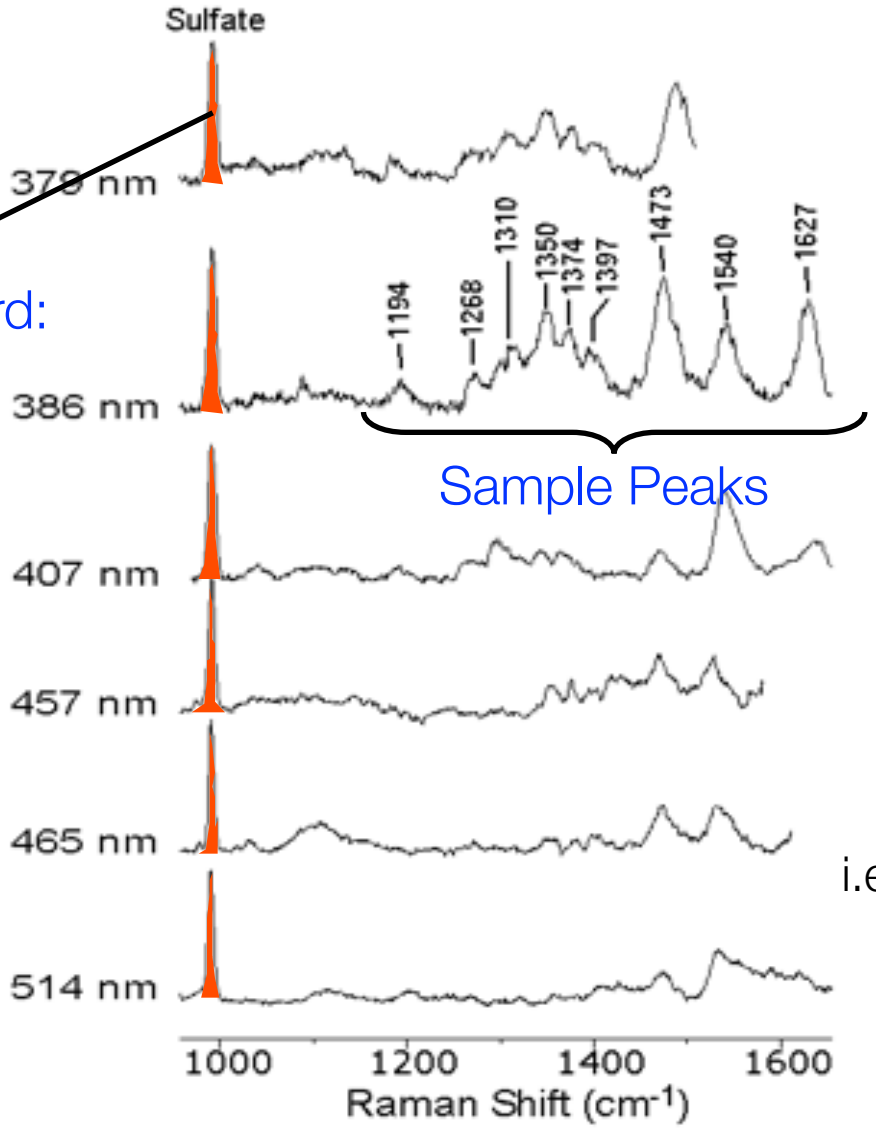




Intensity Calibration

Peak of internal standard:

- Frequency
- Intensity

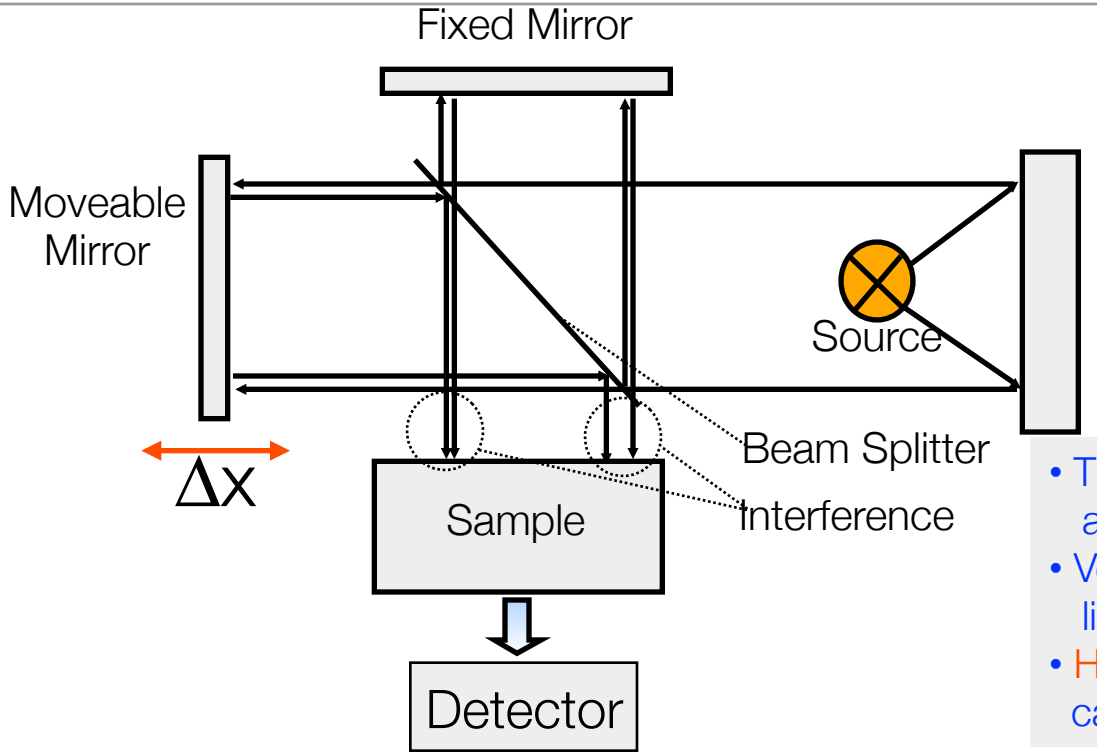


$$\frac{I_{sample}}{I_{Standard}} \propto \frac{c_{sample}\nu^4 I_0(\nu)}{c_{standard}\nu^4 I_0(\nu)} \frac{P^{RR}_{sample}(\nu)}{P^{R}_{standard}}$$

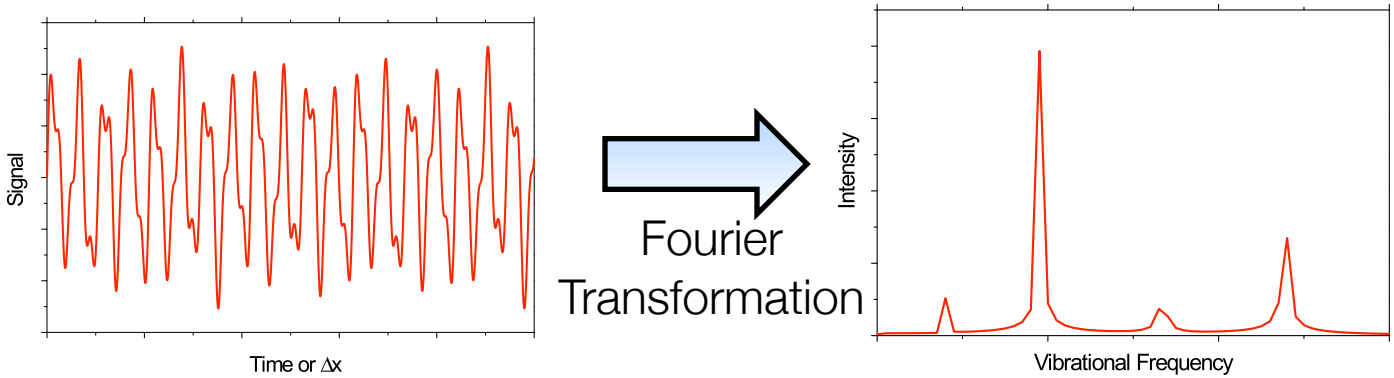
$$P^{RR}_{sample}(\lambda) = const \times \frac{I_{sample}}{I_{Standard}}$$

Can construct an „excitation profile“ i.e. the intrinsic RR intensity P(ν) as a function of excitation wavelength

Experimental Setup for IR Spectroscopy



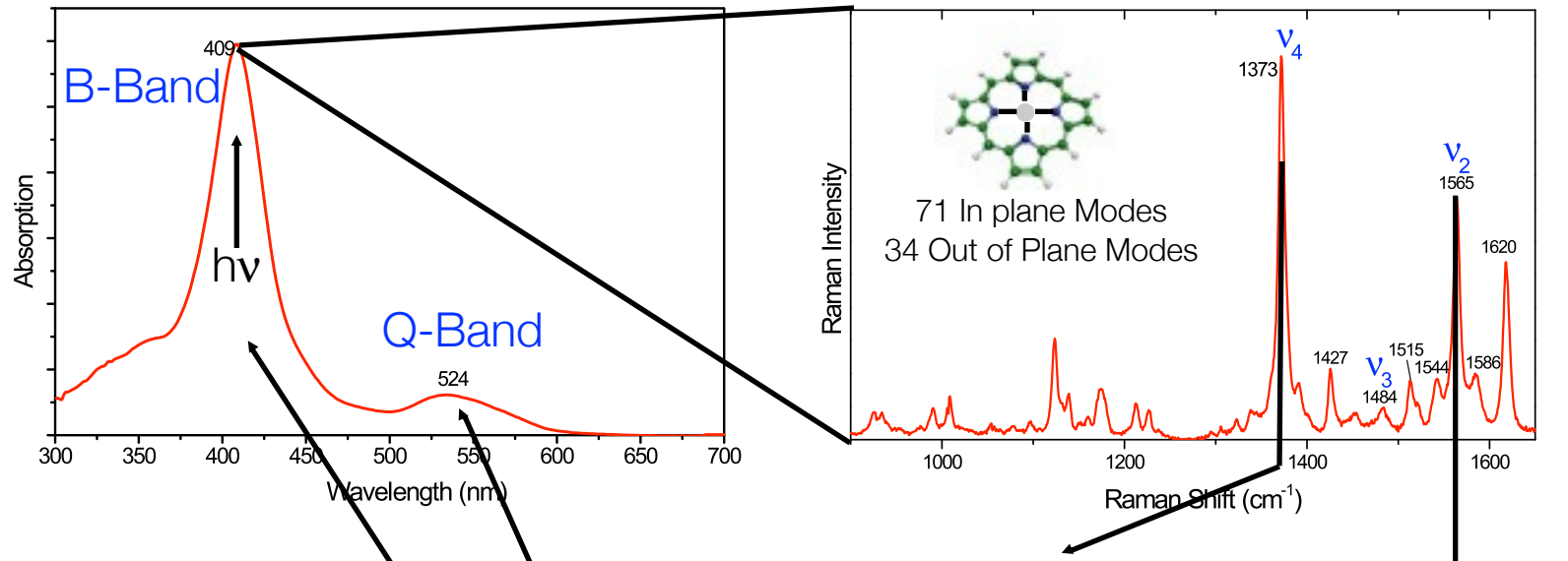
- The whole spectrum is measured at the same time
- Very good signal/noise due to high light throughput
- High precision of frequencies if calibrated with a laser (~0.1-0.01 cm^{-1})

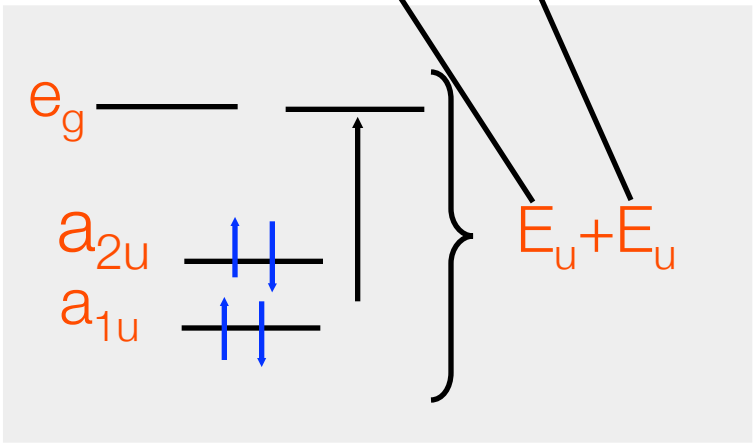


IR-Spectrum = measurement with and without the Sample

IV. Bioinorganic Examples

Hemoproteins – Spin and Oxidation State





Oxidation State Marker

- Fe(III) : ν_4 ~ 1375 cm^{-1}
- Fe(II) : ν_4 ~ 1360 cm^{-1}
- Fe(IV) : ν_4 ~ 1380 cm^{-1}

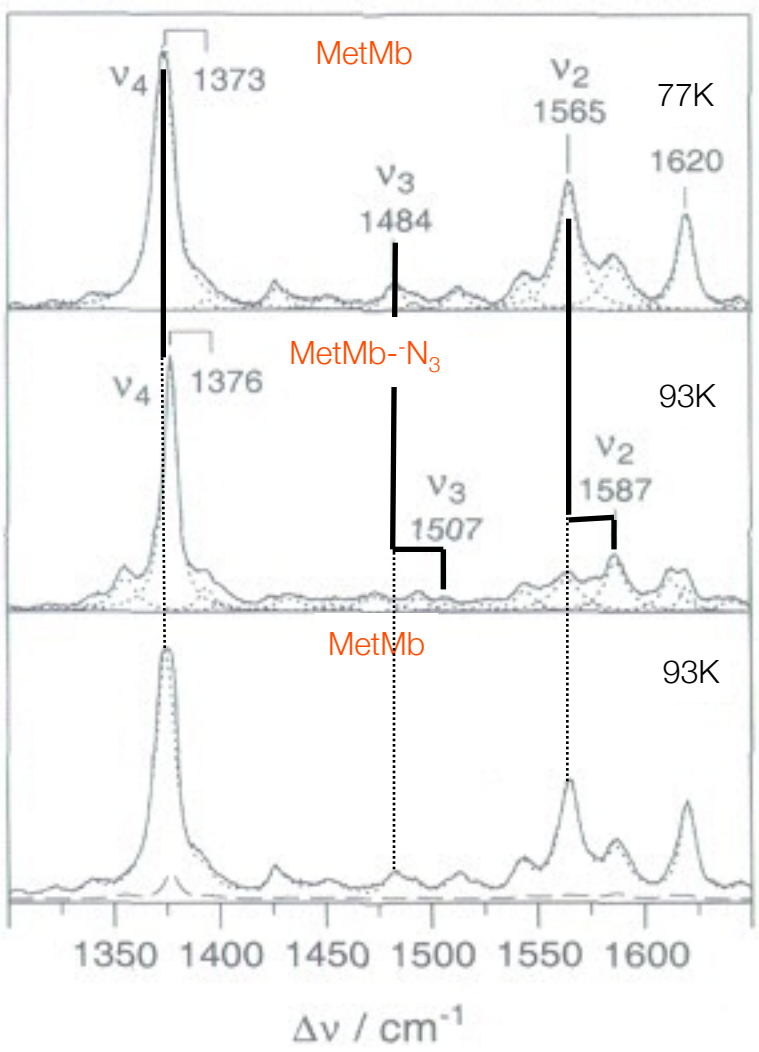
But note (Fe(II)NO,CO ν_4 ~ 1375 cm^{-1}

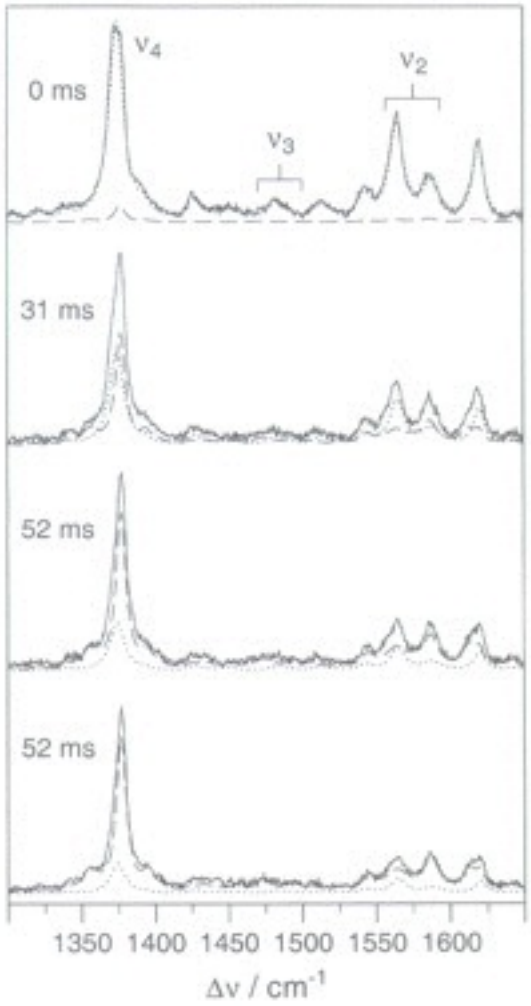
Spin State and Core-Size Marker

- Low-Spin: ν_2 ~ 1580-1590 cm^{-1}
- High-Spin: ν_2 ~ 1550-1565 cm^{-1}

Freeze Quenching

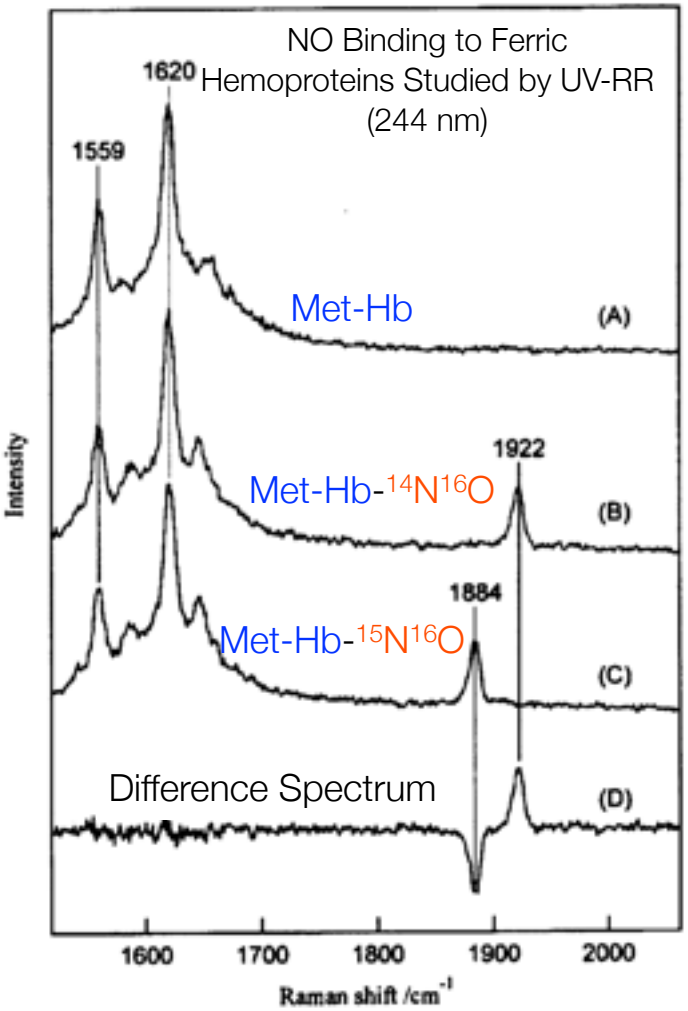
- N_3^- Binding to Myoglobin -



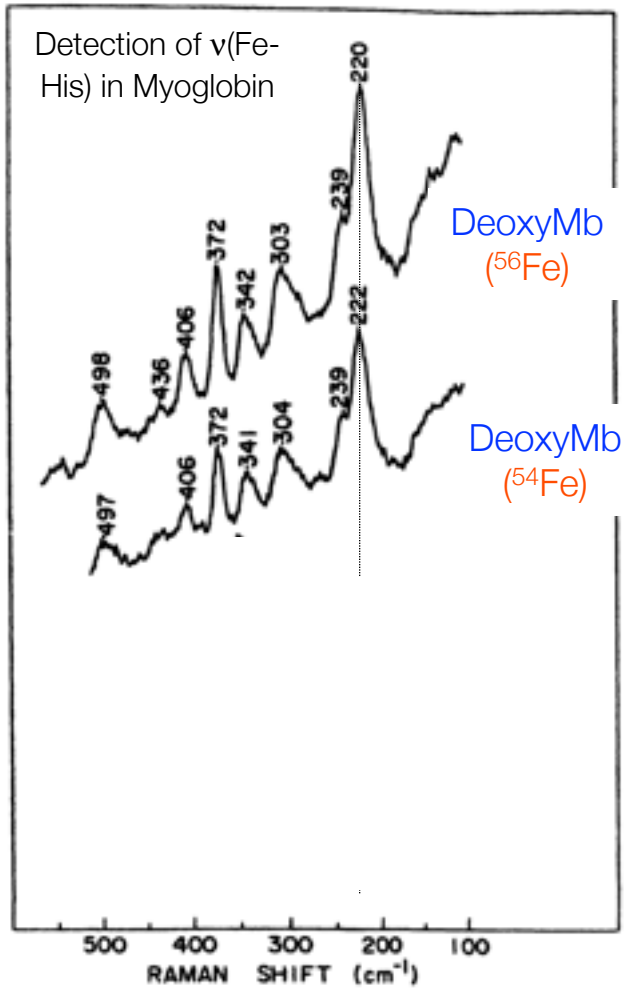


Hemoproteins – Axial Ligands

Exogenous Ligands



Endogenous Ligands

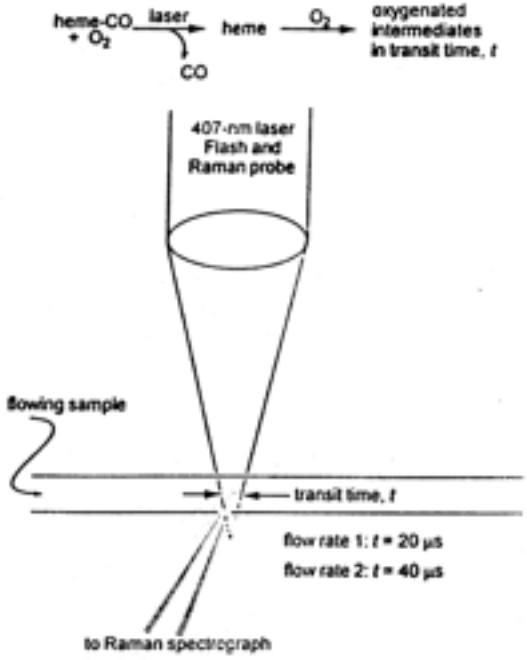


Hemoproteins – Reaction Intermediates

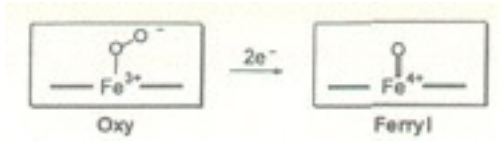
Cytochrome c Oxidase

O_2 → H_2O

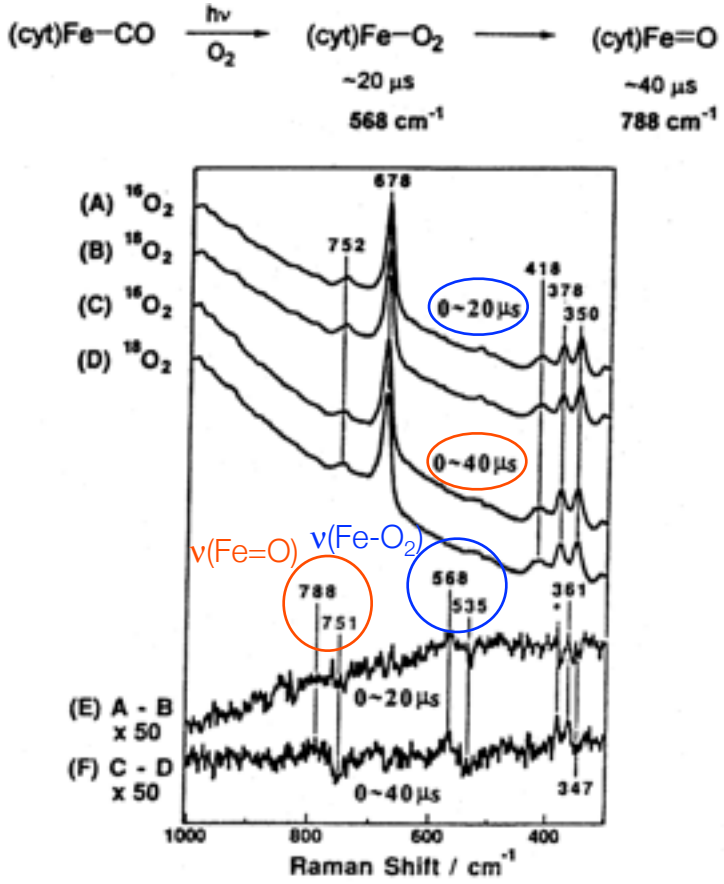
Experimental Setup



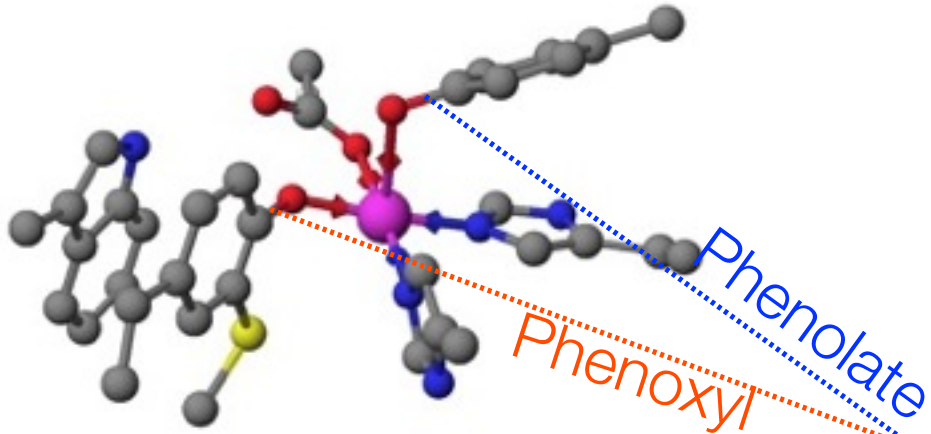
Expected Intermediates

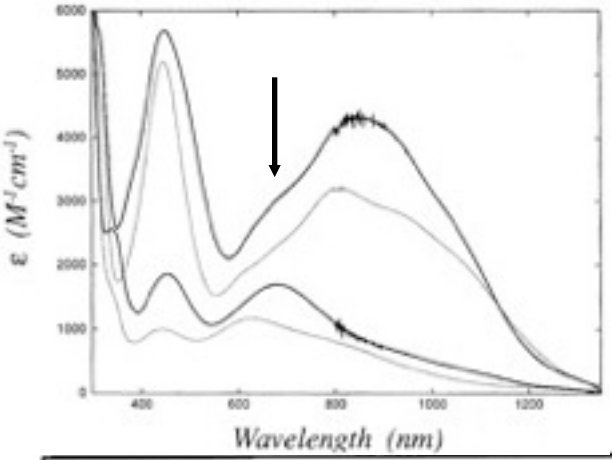


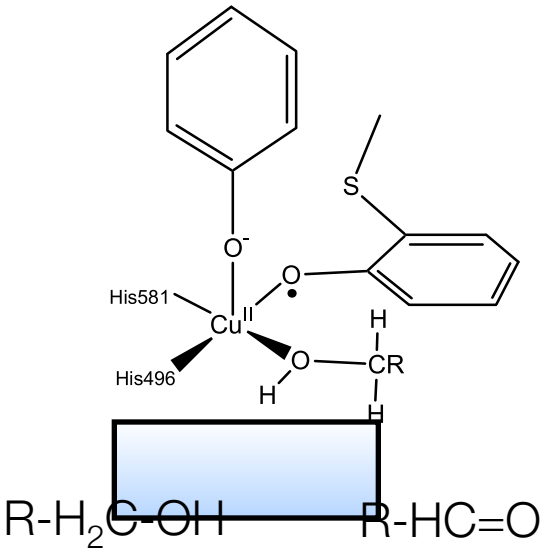
Experimental Results

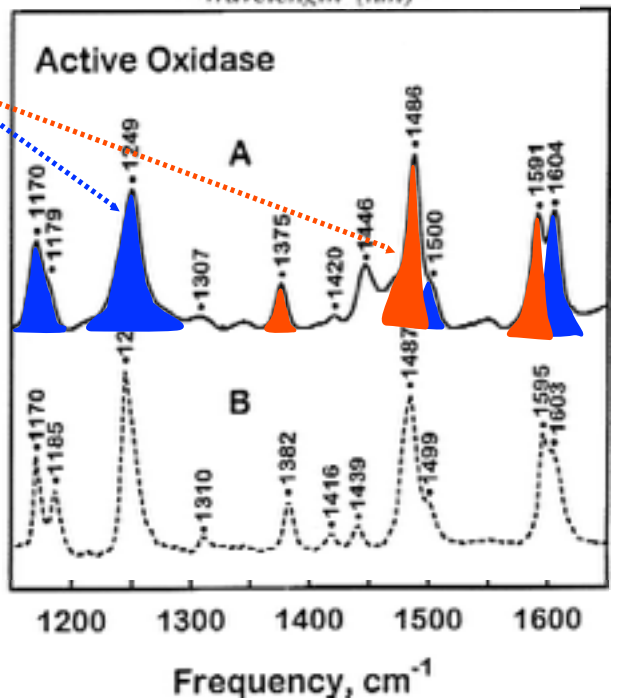


Galactose Oxidase – Detecting Radicals

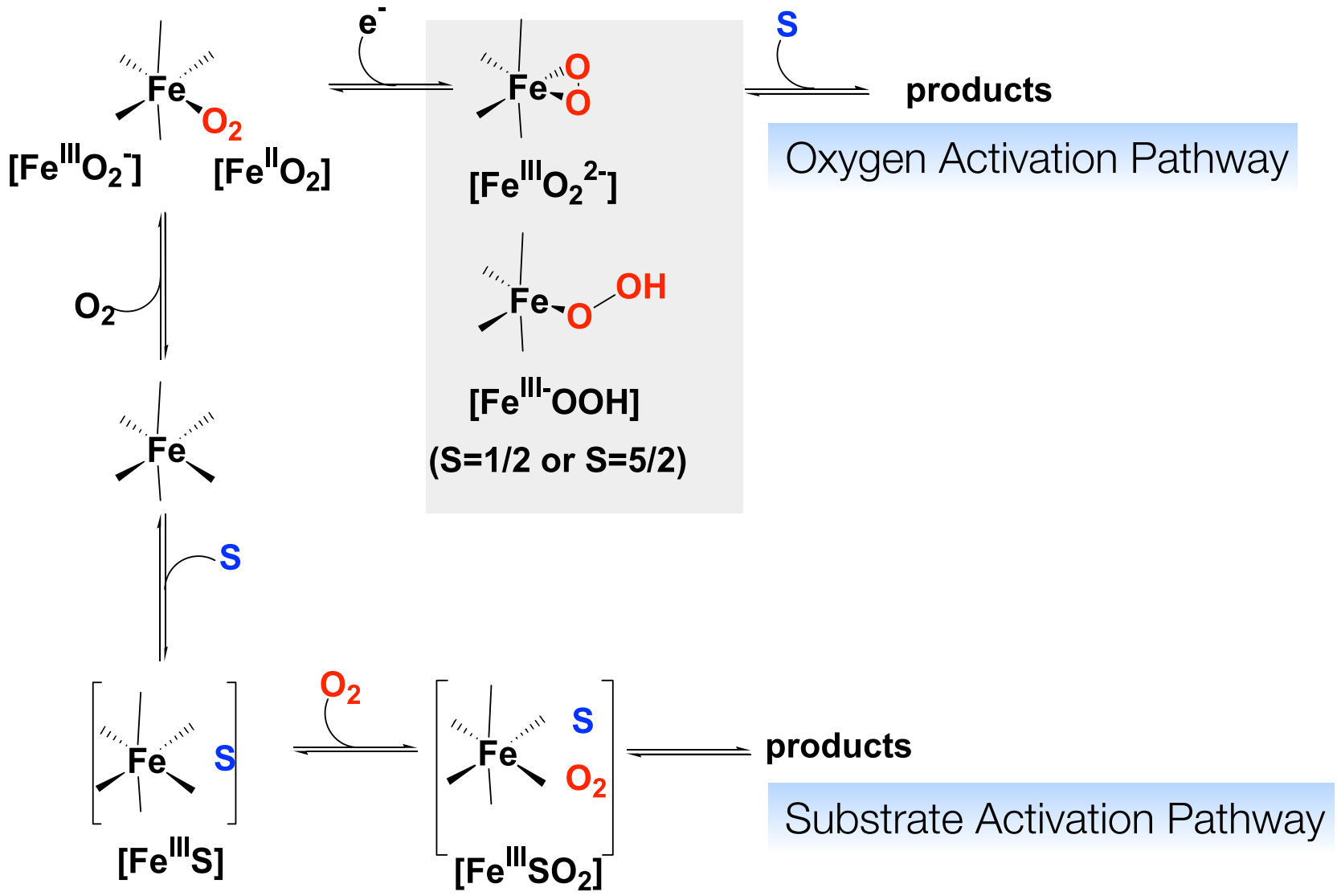




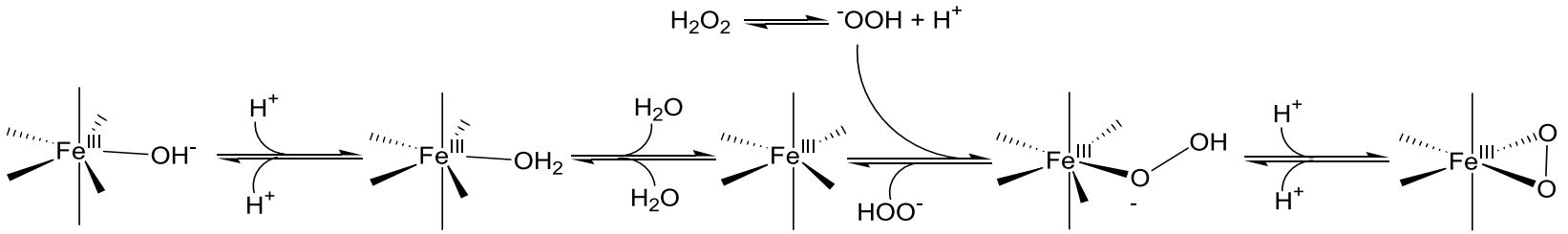




Oxygen Activation in Mononuclear Iron Proteins

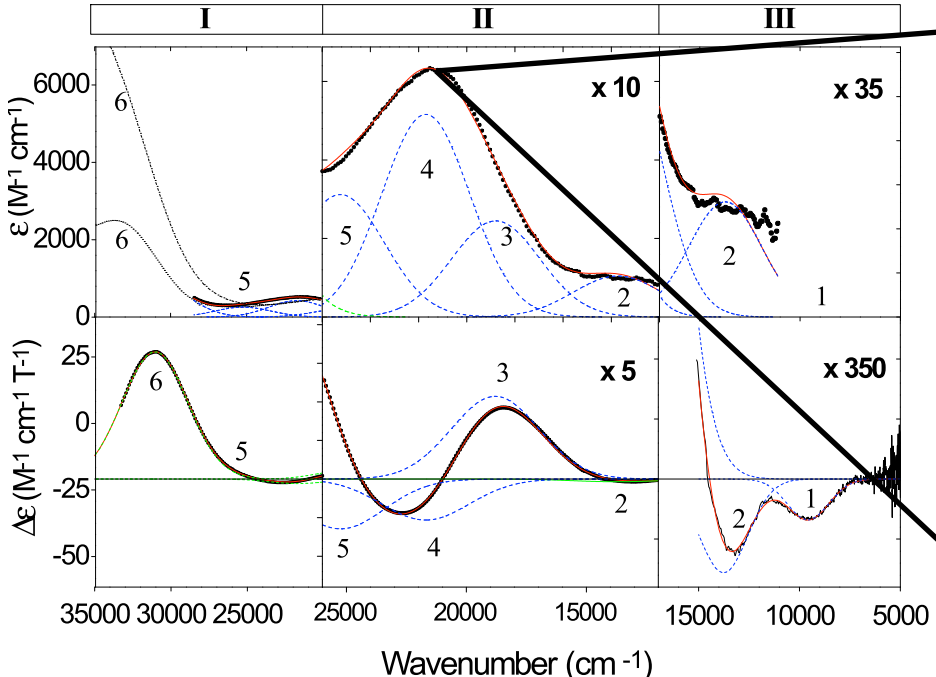


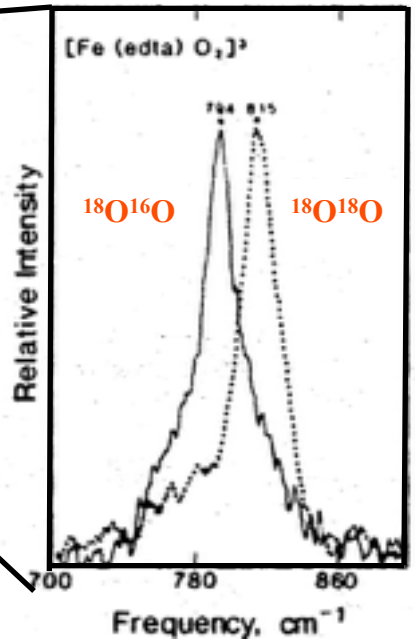
Electronic Structure of the Side-On Fe(III) Peroxo Bond

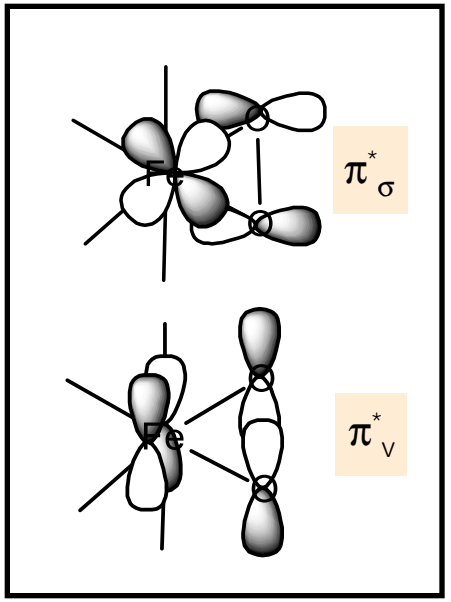


$[Fe(EDTA)(OH)]^{2-}$ $[Fe(EDTA)]^{-}$ $[Fe(EDTA)(O_2)]^{3-}$

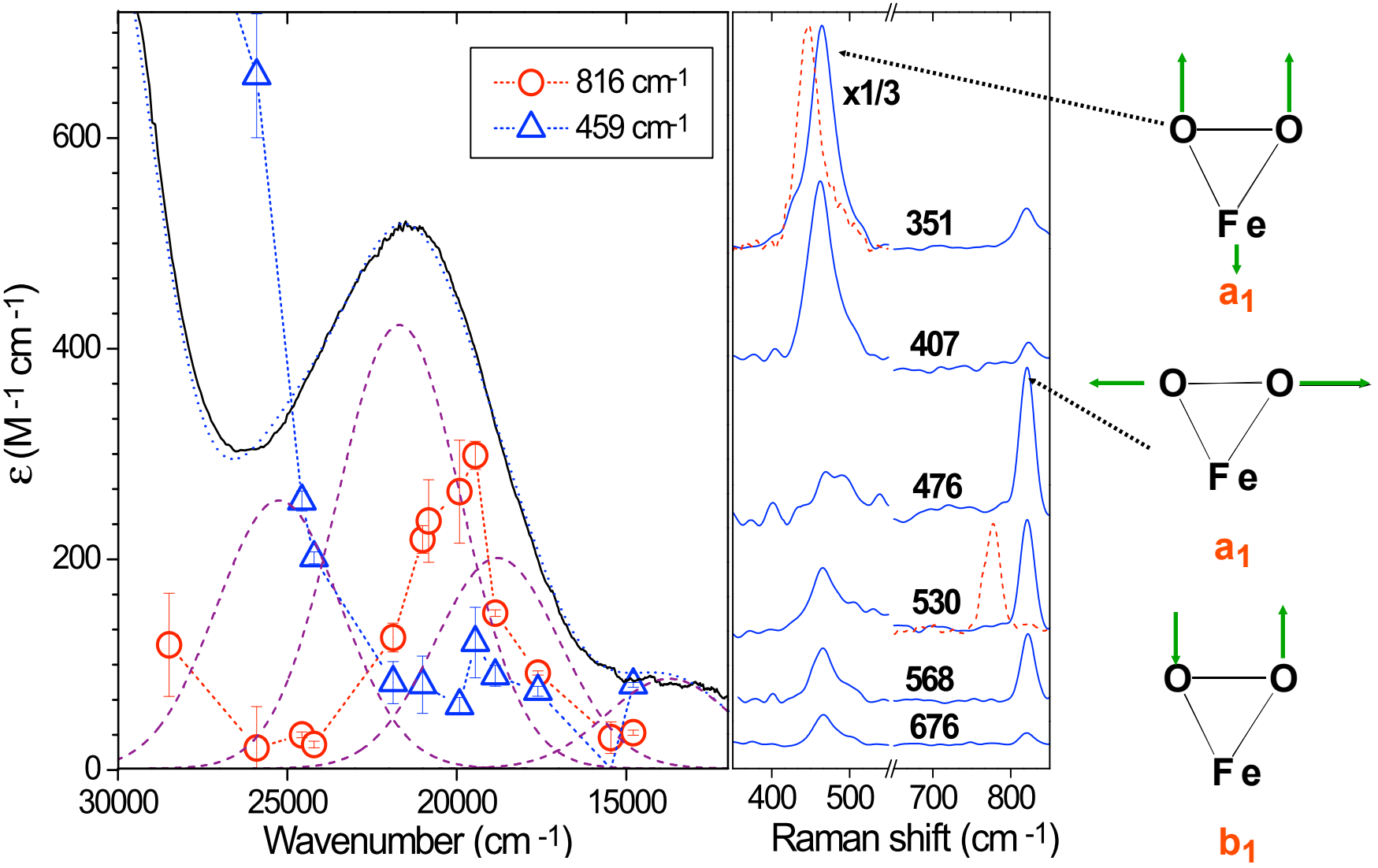
Absorption+MCD Raman Orbitals



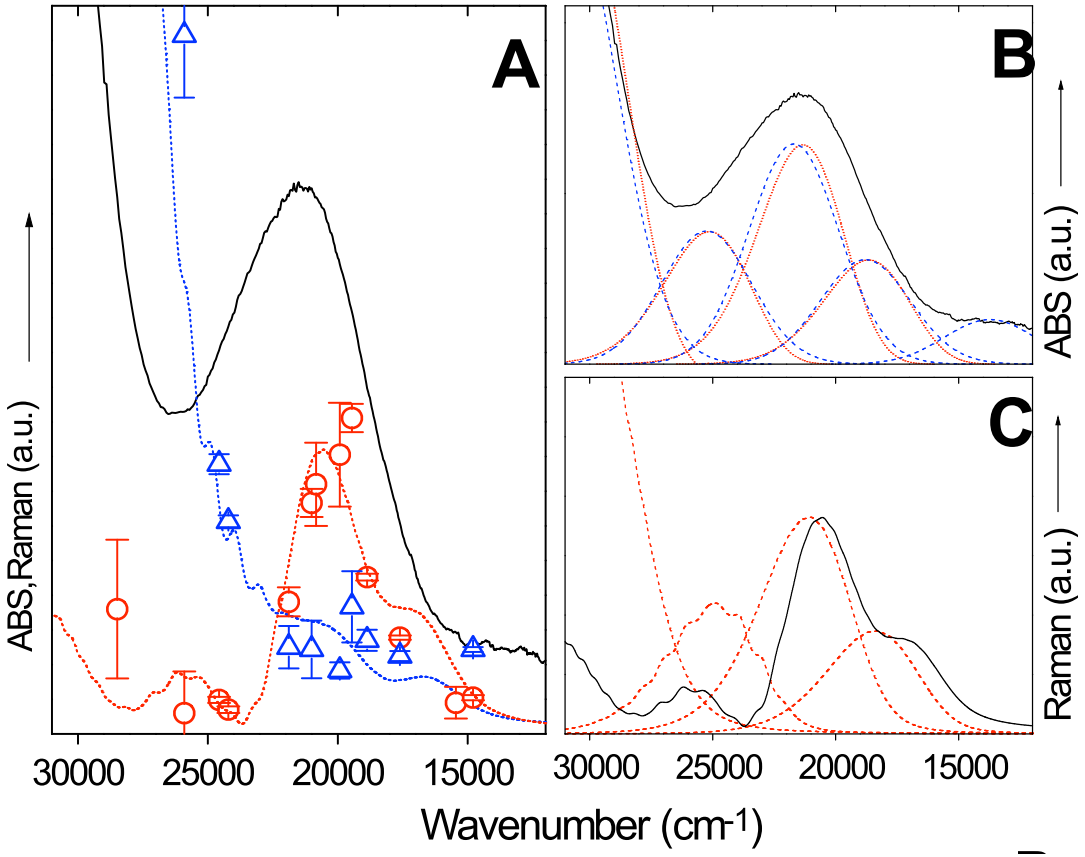


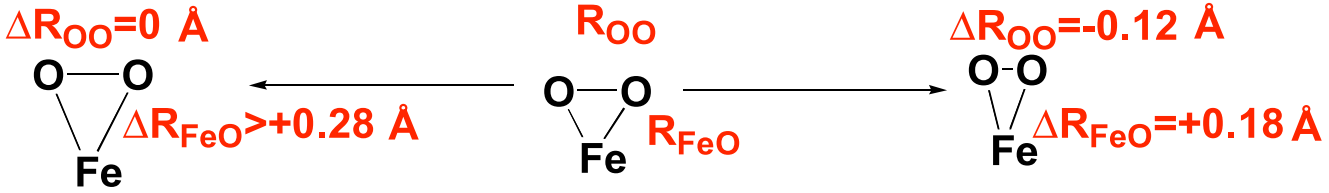


Resonance Raman Spectra of $[Fe(EDTA)(O_2)]^{3-}$

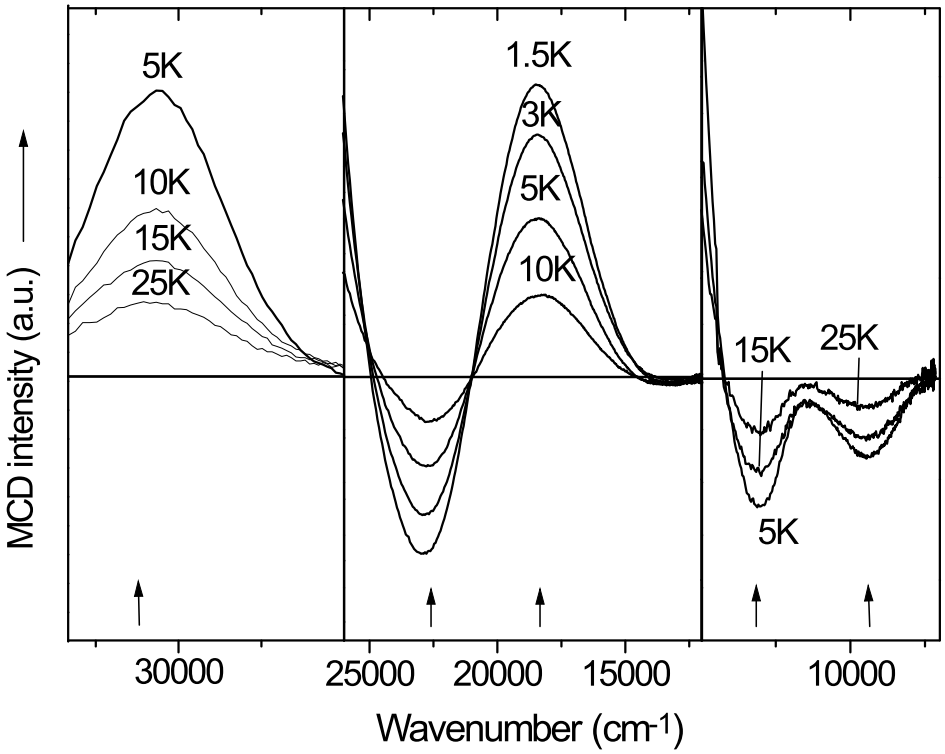


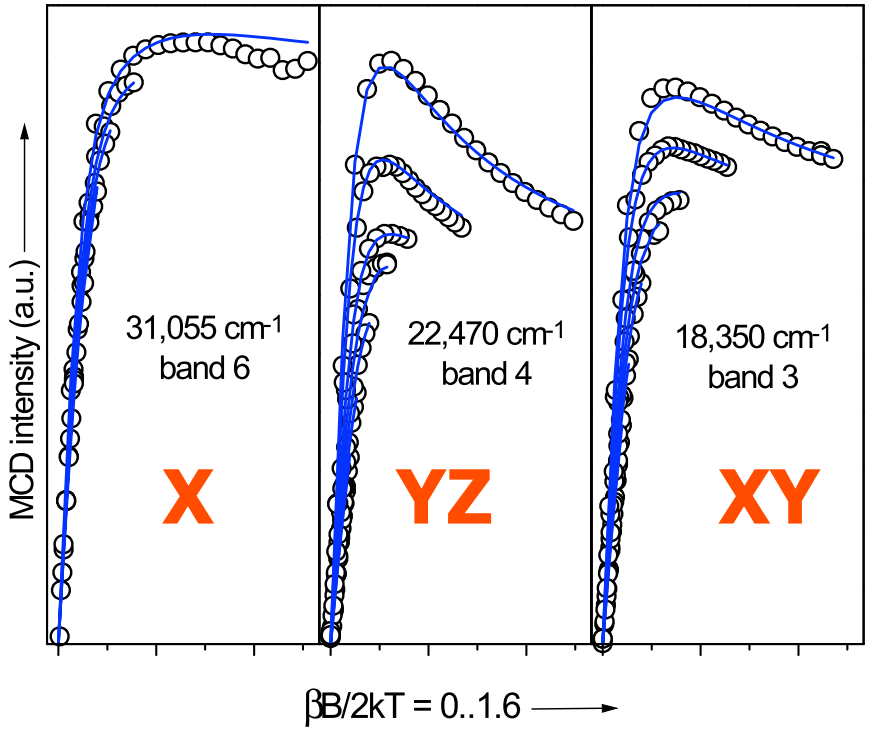
Excitation Profile Analysis for $[Fe(EDTA)(O_2)]^{3-}$



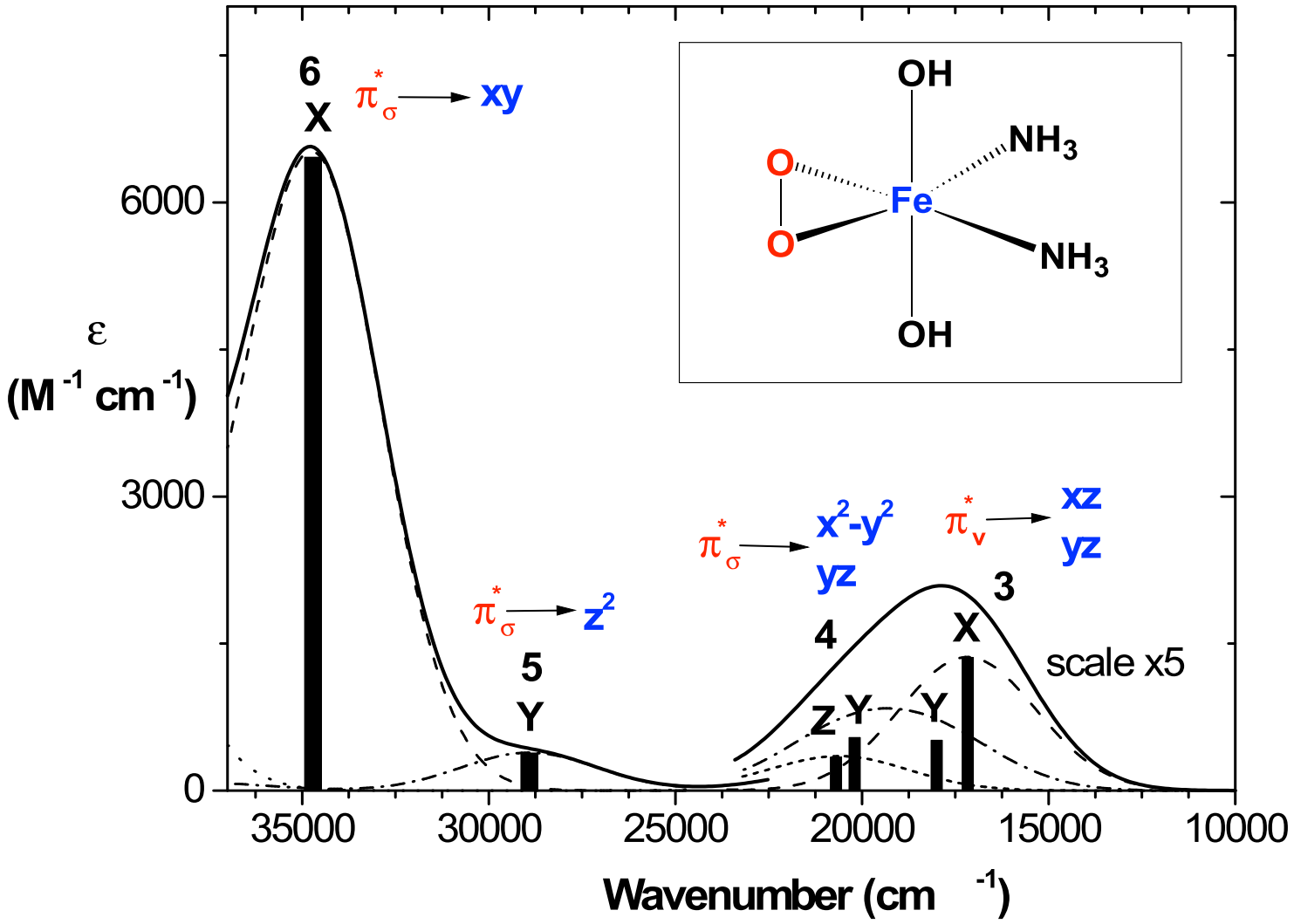


MCD Spectra of $[Fe(EDTA)(O_2)]^{3-}$

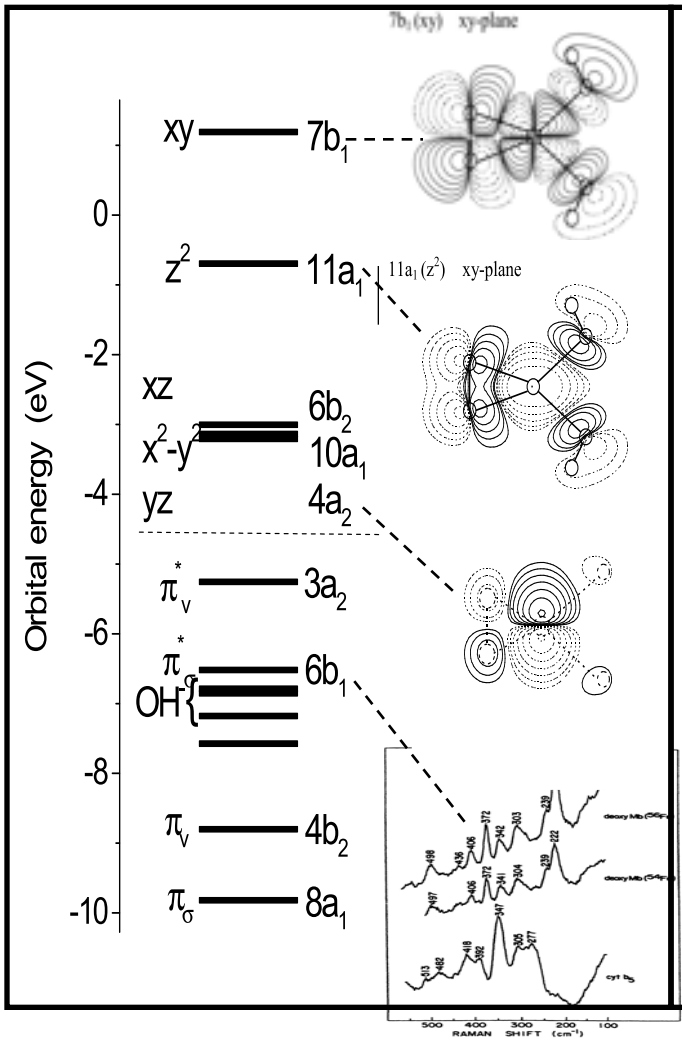


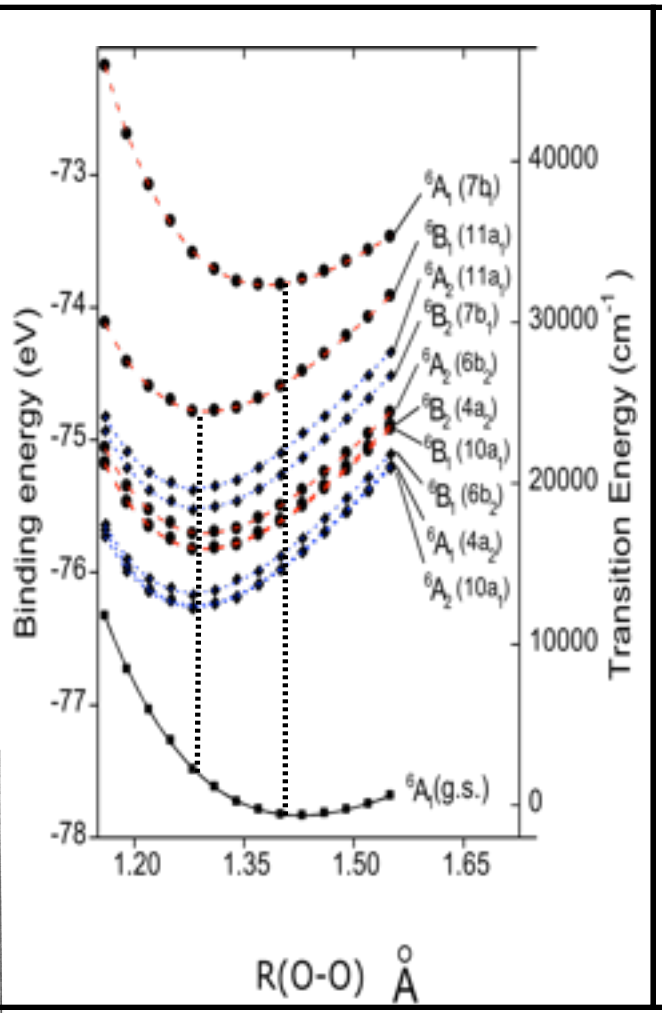


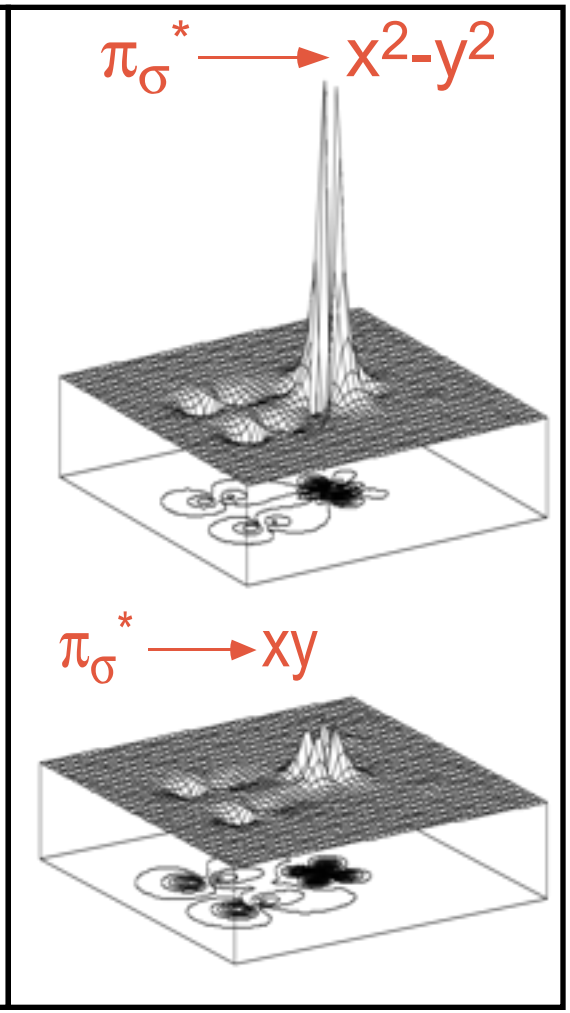
Assignment of Absorption Bands of $[Fe(EDTA)(O_2)]^{3-}$



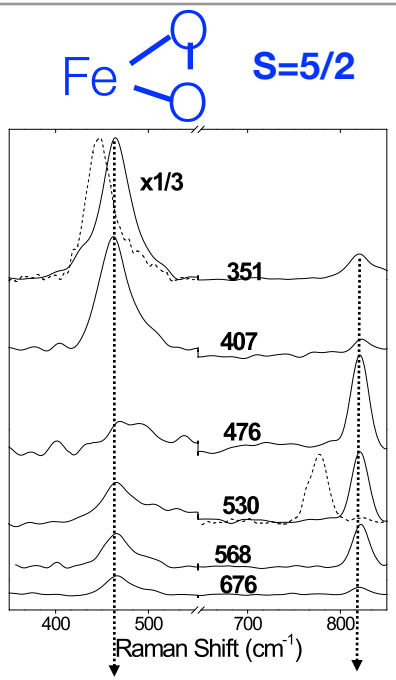
Electronic Structure Insights: $[Fe(EDTA)(O_2)]^{3-}$



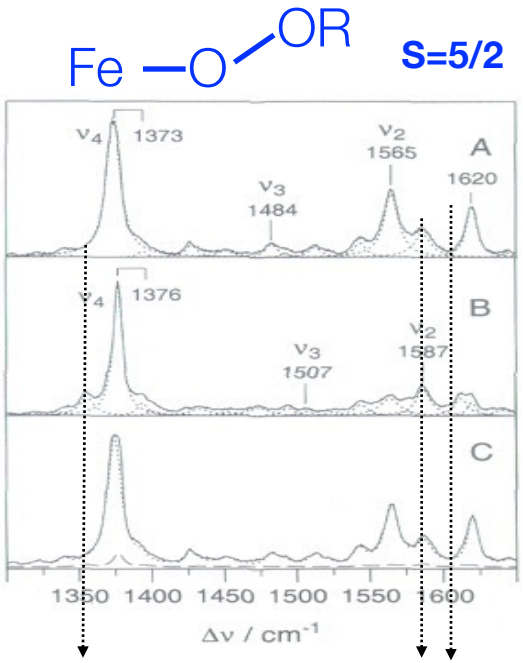




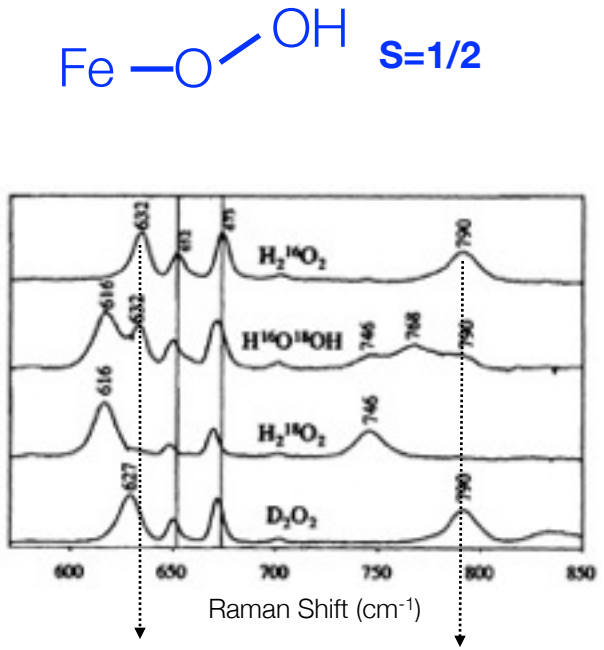
Fe(III) Side On Peroxo versus End-On Hydroperoxo



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

$Fe\text{-}O_\pi$	weak	strong	strong
$Fe\text{-}O_\sigma$	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