Lecture 5: Vibrational Spectroscopy
# Spectroscopic Techniques

<table>
<thead>
<tr>
<th></th>
<th>Gamma</th>
<th>X-Ray</th>
<th>UV/vis</th>
<th>Infrared</th>
<th>Microwave</th>
<th>Radiowave</th>
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<tr>
<td>eV</td>
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<td>8000</td>
<td>2000</td>
<td>4 - 1</td>
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<td>10^{-4} - 10^{-5}</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>10^{-6} - 10^{-7}</td>
</tr>
</tbody>
</table>

**Techniques**

- **Mössbauer**
- **XAS**
- **EXAFS**
- **ABS**
- **MCD**
- **Raman**
- **CD**
- **IR**
- **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)
  - Identification 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**
- IR Light Source
- Sample Cuvette
- Reference Cuvette
- IR Detector
- Computer
- $I_0$, $I$
- $A = -\log(I/I_0) \propto \varepsilon dc$

**Raman Experiment**
- VIS Laser
- Sample
- VIS Detector
- Computer
- $I_0, \nu$
- $I_{sc}, \nu \pm \Delta \nu$
- $I_{sc} = \propto \nu^4 I_0 c$
- Laser Frequency: Rayleigh line
- Anti-Stokes Lines
- Stokes Lines
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 H₂O) IR peaks may be directly detected (i.e. bound CO,CN⁻,NO)
II.A. Vibrational Frequencies and Normal Modes
Potential Energy Surfaces

\[
\text{"Potential Energy"} = T_{\text{el}} + V_{\text{el,nuc}} + V_{\text{el,el}} + V_{\text{nuc,nuc}}
\]

(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:
\[ V(R) = V_0 + \frac{\partial V}{\partial R} \bigg|_{R=R_0} (R - \bar{R}) + \frac{1}{2} \frac{\partial^2 V}{\partial R^2} \bigg|_{R=R_0} (R - \bar{R})^2 \]

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:

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

Reduced Mass \[ m = \frac{m_A m_B}{m_A + m_B} \]

Force Constant
The Reduced Mass and Isotope Shifts

Vibrational Frequency

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \]

Reduced Mass

\[ m = \frac{m_A m_B}{m_A + m_B} \]

Force Constant

Example: \( ^{16}\text{O}_2 \rightarrow ^{18}\text{O}_2 \)

\[ \nu(^{18}\text{O}_2) = 0.943 \nu(^{16}\text{O}_2) \]
**Force Constants**

**Units:**

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

**Practical Spectroscopy**

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

**Typical Force Constants (mdyn/Å):**

- \( \text{N}_2 : 22.41 \)
- \( \text{O}_2 : 11.41 \)
- \( \text{F}_2 : 4.45 \)
- \( \text{CO} : 18.55 \)
- \( \text{NO} : 15.48 \)
- \( \text{H}_2 : 5.20 \)

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

<table>
<thead>
<tr>
<th>( i )</th>
<th>( j )</th>
<th>( d_{ij} ) (Angström)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>0.025</td>
</tr>
<tr>
<td>H</td>
<td>1st row</td>
<td>0.335</td>
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<tr>
<td>H</td>
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<td>0.585</td>
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<td>H</td>
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<td>1st row</td>
<td>0.680</td>
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<tr>
<td>1st row</td>
<td>2nd row</td>
<td>0.900</td>
</tr>
<tr>
<td>1st row</td>
<td>3rd row</td>
<td></td>
</tr>
<tr>
<td>2nd row</td>
<td>2nd row</td>
<td>1.180</td>
</tr>
<tr>
<td>2nd row</td>
<td>3rd row</td>
<td>1.350</td>
</tr>
<tr>
<td>3rd row</td>
<td>3rd row</td>
<td></td>
</tr>
</tbody>
</table>
For a Morse potential: \[ V(R) = D_e \left[ 1 - \exp\left(-\beta R\right)\right]^2 \]

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

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: \[ \Psi_I = \Psi_{\text{Electronic}} \otimes \Psi_{\text{Vibrational}}^{I,n} \]
- „Full“ energy: \[ E = E_{\text{Electronic}}^I + E_{\text{vibrational}}^{I,n} \]
- Vibrational frequency: \[ \nu_I = \left( \frac{k}{m/4\pi^2} \right)^{1/2} \]
- Vibrational energy: \[ E_{\text{vibrational}}^{I,n} = \left( n + \frac{1}{2} \right) \hbar \nu_I \]
- Vibrational wavefunction:
  \[ \Psi_{\text{Vibrational}}^{I,n}(R) = N_n \exp\left( - \frac{R^2}{4\sigma^2} \right) H_n \left( R/\sqrt{2\sigma} \right) \]
  \[ \sigma^2 = h/(4\pi \ m \nu) \]
  **Gaussian**
  **Normalization**
  **Hermite Polynomial**
Anharmonicity and ZPE Effects

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

\[ E_{vibrational}^{1,n} = h\nu (n + \frac{1}{2}) - x_e h\nu \left(n + \frac{1}{2}\right)^2 + ... \]

\[ D_0 = D_e - h\nu \]

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:

  \[ M = 3N - 6 \] (3N - 5 if the molecule is linear) vibrational frequencies \( \nu_i \) 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!
Normal Coordinates of Water

\[ \nu_1 (a_1) \]
Bending
1595 cm\(^{-1}\)

\[ \nu_2 (a_1) \]
Symmetric
Stretch
3652 cm\(^{-1}\)

\[ \nu_3 (b_1) \]
Asymmetric
Stretch
3756 cm\(^{-1}\)

IR

Raman
Normal Coordinates of CO\textsubscript{2}

\begin{tabular}{llll}
\hline
\textbf{Mode} & \textbf{Symmetry} & \textbf{Frequency} & \textbf{Description} \\
\hline
\(v_3 (\sigma_g^+)\) & Antisymmetric Stretch & 2349 cm\textsuperscript{-1} &  \\
\(v_2 (\sigma_g^+)\) & Symmetric Stretch & 1337 cm\textsuperscript{-1} &  \\
\(v_1 (\pi_u)\) & Out of plane Bending & 667 cm\textsuperscript{-1} &  \\
\(v_1 (\pi_u)\) & In plane Bending & 667 cm\textsuperscript{-1} &  \\
\hline
\end{tabular}

„Doubly Degenerate“ Vibration
## Group Frequencies

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound Class</th>
<th>Frequency Range (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H</td>
<td>Alkanes</td>
<td>2965-2850</td>
</tr>
<tr>
<td></td>
<td>-CH₃</td>
<td>1450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1380</td>
</tr>
<tr>
<td></td>
<td>-CH₂</td>
<td>1465</td>
</tr>
<tr>
<td></td>
<td>Alkenes</td>
<td>3095-3010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>700-1000</td>
</tr>
<tr>
<td></td>
<td>Alkynes</td>
<td>~3300</td>
</tr>
<tr>
<td>C-C</td>
<td>Alkanes</td>
<td>700-1200</td>
</tr>
<tr>
<td>C=C</td>
<td>Alkenes</td>
<td>1680-1620</td>
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<tr>
<td>C≡C</td>
<td>Alkynes</td>
<td>2260-2100</td>
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<td>C=O</td>
<td>Ketones</td>
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<td>Aldehydes</td>
<td>1740-1720</td>
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<td></td>
<td>Carbonic Acids</td>
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<td></td>
<td>Esters</td>
<td>1750-1730</td>
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<td></td>
<td>Amides</td>
<td>1700-1630</td>
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<td>1300-1000</td>
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<td>Alcohols, isolated</td>
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<td>Alcohols, H-bonded</td>
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<td>Carbonic Acids</td>
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<td></td>
<td>Secondary Amines</td>
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<tr>
<td>C≡N</td>
<td>Nitriles</td>
<td>2260-2240</td>
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</table>
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):

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

Diagonalization

\[
\tilde{F} Y_I = x_I Y_I
\]

Masses

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

Frequencies and Modes

\[
v_I = 1302.78 \sqrt{x_I} \quad \text{(in cm}^{-1}\text{)}
\]

\[
Q_I = N^{-1/2} \left( M^{-1/2} Y_I \right)
\]

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*. To this end, a suitable fragment is chosen:

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

• **Fundamentals** : $n=0 \rightarrow n'=1$ \hspace{1cm} $\Delta E=h\nu$
• „Hot“ Bands : $n=1 \rightarrow n'=n\pm1$ \hspace{1cm} $\Delta E=h\nu$
• Overtones : $n=0 \rightarrow n'=2,3,...$ \hspace{1cm} $\Delta E=2h\nu, 3h\nu,...$
• Combination Bands: $n_1=0, n_2=0 \rightarrow n_1'=1, n_2'=1$ \hspace{1cm} $\Delta E=h\nu_1+h\nu_2$
Physical Principles of IR and Raman Spectroscopy
Overtone and Combination Bands

[Graph showing Raman shift cm^{-1} vs. frequency with peaks and labels for fundamentals and overtone and combination bands]
Physical Principle of IR Spectroscopy

Molecular Origin of Infrared Spectra

- Transfer of Infrared Energy to Vibrating Dipole

\[ \nu_{\text{vibration}} = \nu_{\text{dipole}} = \nu_{\text{IR field}} \]

- Infrared Absorption Intensity

\[ I_{\text{IR}}^{1/2} \propto [\mu]_{\nu,\nu'} \equiv \langle \nu' | \mu | \nu \rangle \propto (\partial \mu / \partial Q)_0 \]

- Dipole transition moment
- Change of dipole moment due to normal vibration, Q
Physical Principle of Raman Spectroscopy

Polarizability:

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

\[ h\nu_1 \quad h\nu_2 = h\nu_1 \pm \Delta\nu \]
Dimensions in a Resonance Raman Experiment

Excitation Profile

Vibrational Spectra

Assignment via Isotope Shift Experiments

Fe-O stretch

O-O stretch
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 $Q_i$

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

$$I_{Raman}(\omega_i) \propto \left( \frac{\partial \alpha}{\partial Q_i} \right)^2$$

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:

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

Transition Energy
Photon Energy
Damping Factor (Linewidth)
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:

\[
I_{rR}^{1/2}(Q_i) \propto \sum_{KL} \frac{\partial \alpha_{KL}(\omega)}{\partial Q_i} = \frac{1}{i \Gamma} \sum_{KL} \frac{\partial}{\partial Q_i} \left< \Psi_0 \mid \mu_{ED,K} \mid \Psi_I \right> \left< \Psi_I \mid \mu_{ED,L} \mid \Psi_0 \right> + \alpha_{KL}(\omega) \frac{\partial (E_I - E_0)}{\partial Q_i}.
\]

- 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 \( \sim \) (Number of Modes)

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

\[
i(t) = e^{-i \hat{H}_{\text{ext}} t} |i\rangle
\]

\[
(\alpha_{p \lambda})_{I \rightarrow F} = M_p M_\lambda \int_0^\infty \langle f | i(t) \rangle e^{i(E_L + \varepsilon_i + \Gamma)t} \, dt
\]

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) d⁹ complex with a coordinating Cl⁻ ligand.
A Toy Model: Optical Spectra

Orbital Energy

SOMO
Cu-d
Cl-p
N-p

d-d Transitions

σ-LMCT
π-LMCT

Absorption

Circual Dichroism

Wavenumber (cm⁻¹)
Short Digression: Difference vs Transition Density

Difference densities at ±0.003

\[ \Delta P \propto \psi_{\text{acceptor}}^2 - \psi_{\text{donor}}^2 \]

Transition densities at ±0.003

\[ D \propto \psi_{\text{acceptor}} \psi_{\text{donor}} \]

ABS Intensity \( \propto D^2 \)
A Toy Model Resonance Raman Spectra

\[ \sigma\text{-LMCT} \]

\[ \pi\text{-LMCT} \quad d\text{-d} \]

Cu-Cl stretch

348 cm\(^{-1}\)
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

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

Quantum Chemistry

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

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

Laser
Variable Frequency Laser Light

Sample

Monochromator

CCD

Photon Counting
Detection Systems
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 emit photons of the same frequency *in Phase* \((A^*+hv\rightarrow A+2hv)\)
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

**Ar+ pump Laser**

**Ar+/Kr+ Mixed Gas Laser ~350-670 nm**

**Ti:Sa Laser ~700-1000 nm**

**Power-meter**

**Bandpass Filter**

**Focussing Lens**
Sample Compartments

(a) Capillary
Sealed or Flow

(b) Cylinder
Spin

(c) NMR Tube
Spin or Stir

(d) Pellet
Spin

(e) Low Temperature
Pump
Liq. N₂
Cu cryotip
Small sample of e.g., frozen solution Matrix

(f) Electrochemical
Electrode or Bulk

(g) Microscope
Small area, e.g., single crystal
Sample Compartments

**Rotating Cell**
- Reflection shield
- Motor
- Position adjustment
- From Laser
- Liquid sample
- In spinning cell
- Beam stopper
- Prism
- To detector

**Liquid N₂ Dewar**
- Solid or frozen sample
- Collection and focussing optics
- To detector
- From laser
Collecting Optics

- Depolarizer
- Focussing Lens
- Collecting Lens
- Entrance Slit
- Sample
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:

\[ d \sin(\Theta) = m\lambda \]

- **Resolving power** of a grating
  \[ \frac{\lambda}{|\Delta\lambda|} = mN \]
  - More Grooves = More Resolution
  - Longer Wavelength = Higher Resolution
  - Use smaller grating at longer wavelengths
Detection Systems (CCD, PMT)

- Each Pixel
  - Metal
  - Insulator
  - Silicon Crystal

Pixel-Array, i.e. 1340 x 400

- Dispersed light
- Pixel Position $\propto$ Frequency

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

High Voltage

Cathode (e.g. GaAs)

Incoming Light

Photo-Electron

Secondary Electrons

Dynodes of progressively positive potential

Anode

Current

e tc
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

**CH$_3$CN, 298K**

- 375
- 911
- 1366
- 2240

Raman Shift (cm$^{-1}$)

**Na$_2$SO$_4$ Solid, 298K**

- 444 459
- 613
- 625
- 639
- 984
- 1092
- 1121
- 1141

Raman Shift (cm$^{-1}$)

**Indene IUPAC standard for frequencies**

**Sample Fe(II) heme protein**

Raman Shift, cm$^{-1}$
Intensity Calibration

Peak of internal standard:
- Frequency
- Intensity

Sample Peaks

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

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

**Spin State and Core-Size Marker**
- Low-Spin: $\nu_2 \approx 1580-1590$ cm$^{-1}$
- High-Spin: $\nu_2 \approx 1550-1565$ cm$^{-1}$
Freeze Quenching
- $\text{N}_3^-$ Binding to Myoglobin -
Hemoproteins – Axial Ligands

Exogenous Ligands

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

Met-Hb

Met-Hb

Met-Hb

Met-Hb

Met-Hb

Met-Hb

Difference Spectrum

Endogenous Ligands

Detection of ν(Fe-His) in Myoglobin

DeoxyMb

DeoxyMb

DeoxyMb

DeoxyMb
Hemoproteins – Reaction Intermediates

Cytochrome c Oxidase

Experimental Setup

- Expected Intermediates
  - $\nu(\text{Fe}=\text{O})$
  - $\nu(\text{Fe}-\text{O}_2)$

Experimental Results

- (cyt)Fe=CO $\rightarrow$ (cyt)Fe$^+$-CO
- (cyt)Fe=O

- 568 cm$^{-1}$
- 788 cm$^{-1}$

- Raman Shift / cm$^{-1}$

- Flow rate 1: $t = 20 \mu s$
- Flow rate 2: $t = 40 \mu s$

- Transformed spectra with 407-nm laser flash and Raman probe
Galactose Oxidase – Detecting Radicals
Oxygen Activation in Mononucleular Iron Proteins

Oxygen Activation Pathway

Substrate Activation Pathway

\[
\begin{align*}
\text{Fe}^{III}O_2^- & \quad \text{Fe}^{II}O_2^- \\
& \quad \text{O}_2 \\
\text{Fe}^{III}O_2^2^- & \quad \text{Fe}^{III}OHH \\
& \quad \text{S} \\
\text{Fe}^{III}S & \quad \text{Fe}^{III}SO_2^- \\
\end{align*}
\]

(products)

Oxygeen Activation Pathway

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

[Fe(EDTA)(OH)]^{2-} \quad [\text{Fe(EDTA)}]\text{-} \quad [\text{Fe(EDTA)(O)}_{2}]^{3-}

Absorption+MCD \quad \text{Raman} \quad \text{Orbitals}

\begin{align*}
\text{H}_2\text{O}_2 & \rightarrow \ \text{`OOH} + \text{H}^{+} \\
\text{H}^{+} & \rightarrow \ \text{Fe(III) Peroxo Bond}
\end{align*}
Resonance Raman Spectra of [Fe(EDTA)(O_2)]^{3-}

![Graph showing resonance Raman spectra with wavenumber and Raman shift axes, and molecular structures indicating Fe-O-O bonds with a_1 and b_1 vibrations.]
Excitation Profile Analysis for [Fe(EDTA)(O₂)]⁻

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 $[\text{Fe(EDTA)}(\text{O}_2)]^-_3$
Assignment of Absorption Bands of [Fe(EDTA)(O_2)]^{3-}
Electronic Structure Insights: \([\text{Fe(EDTA)(O}_2\text{)}]^{3-}\)
Fe(III) Side On Peroxo versus End-On Hydroperoxo

<table>
<thead>
<tr>
<th></th>
<th>Fe-O&lt;sub&gt;π&lt;/sub&gt;</th>
<th>Fe-O&lt;sub&gt;σ&lt;/sub&gt;</th>
<th>O-O</th>
<th>Reactivity</th>
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<td>strong</td>
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<td>strong</td>
<td>unreactive</td>
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<tr>
<td>strong</td>
<td>weak</td>
<td>strong</td>
<td>moderate</td>
<td>Fe(II) + HOO•</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>weak</td>
<td>[FeO]&lt;sup&gt;2+&lt;/sup&gt; + HO•</td>
</tr>
</tbody>
</table>

\[ \text{Fe(II)} + \text{HOO•} \rightarrow \text{Fe(III)} \]

\[ \text{[FeO]}^2+ + \text{HO•} \]

\[ \nu(\text{Fe-O}) \]
\[ 459 \]
\[ 816 \]
\[ 469 \]
\[ 842/876 \]
\[ 626 \]
\[ 789 \]

\[ \nu(\text{O-O}) \]

\[ \nu(\text{Fe-O}) \]

\[ \nu(\text{O-O}) \]
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


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


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


*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.*


*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*