

# NMR I: KETO ENOL TAUTOMERIZATION

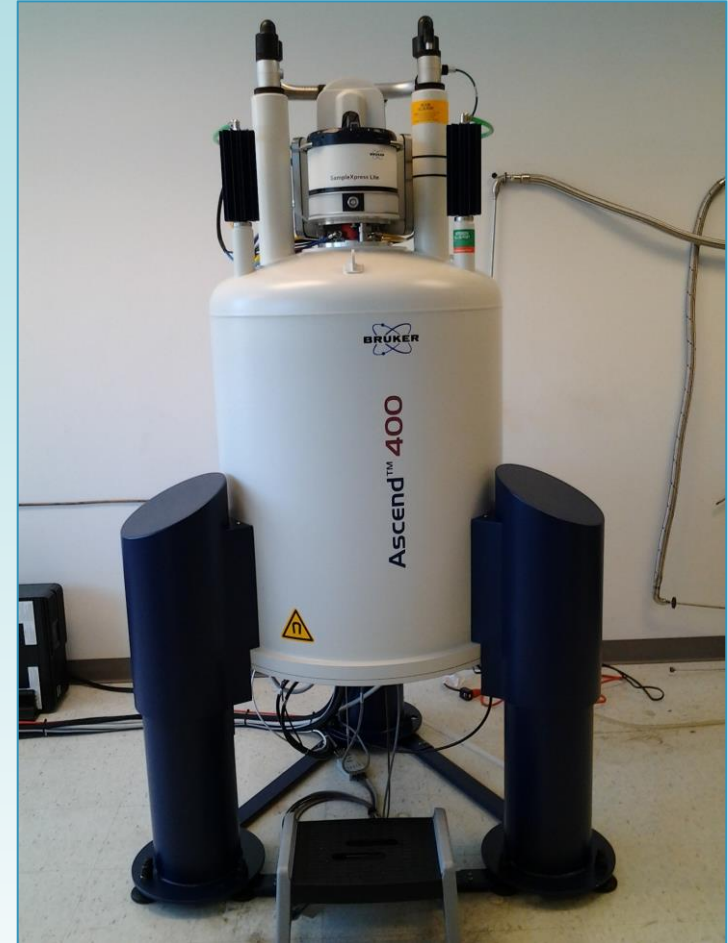
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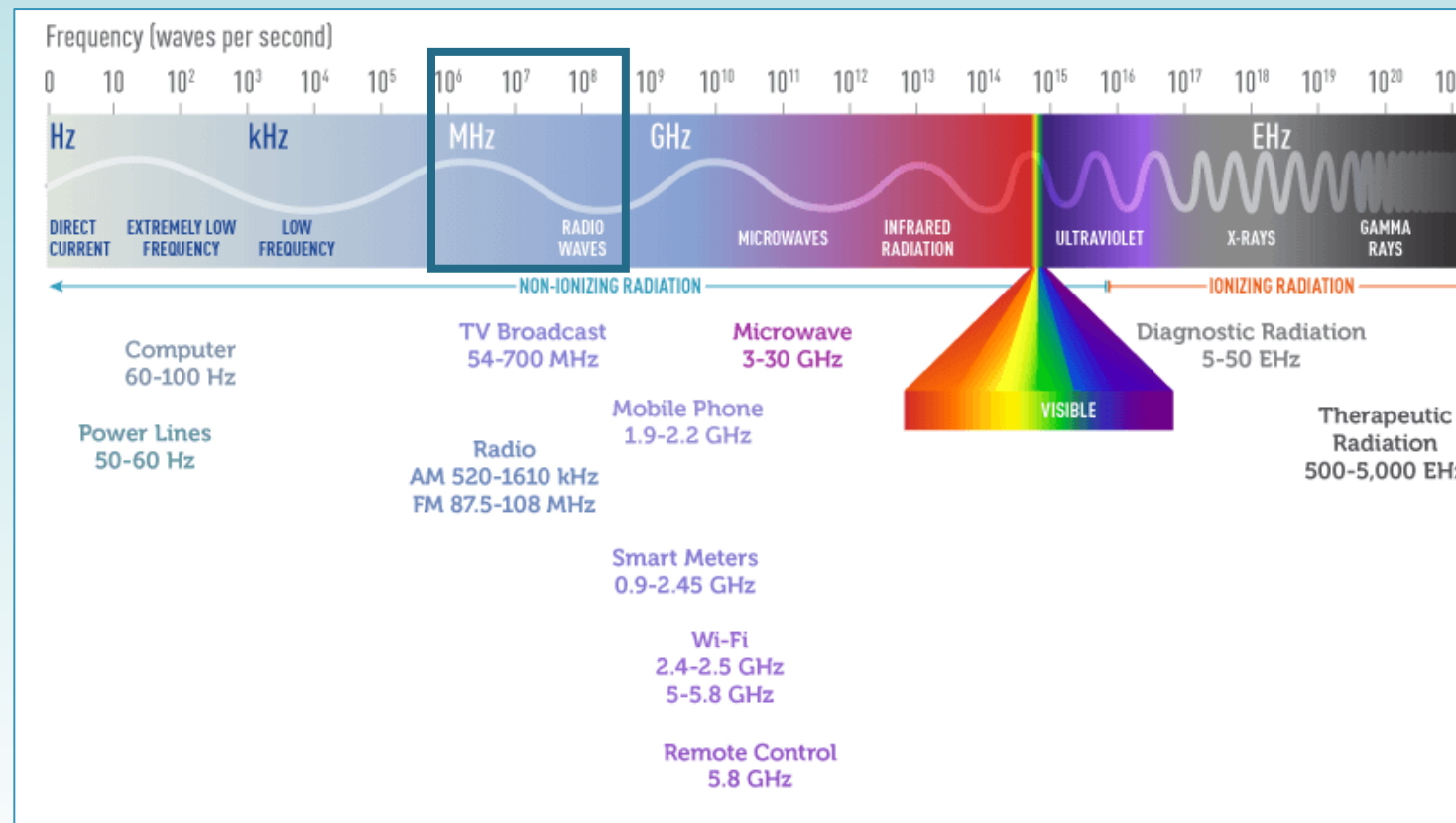
# NMR: INTRODUCTION AND THEORY

- Nuclear Magnetic Resonance (NMR)

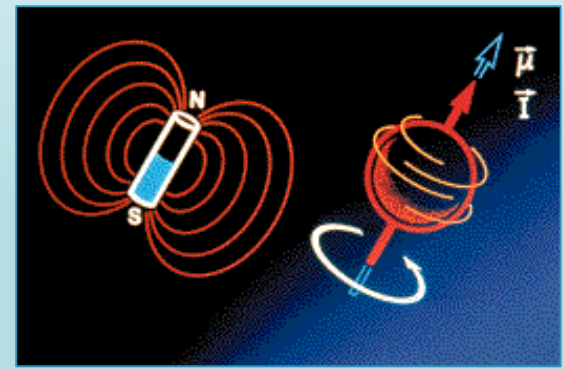


# NMR: INTRODUCTION AND THEORY

- Nuclear Magnetic Resonance (NMR)
  - Measures response of **nuclei** to radiofrequency (rf) input



# NMR: INTRODUCTION AND THEORY



- Nucleus: collective consideration of protons and neutrons
- Collective **rotation** of protons and of neutrons define the **nuclear spin**  $I$  and corresponding **nuclear spin angular momentum**  $I\hbar$
- The spinning nucleus in turn generates a **nuclear magnetic moment**  $\mu$
- The **gyromagnetic ratio**  $\gamma$  is a proportionality constant between the nuclear magnetic moment and the nuclear spin angular momentum

$$\gamma = \frac{\mu}{I\hbar}$$

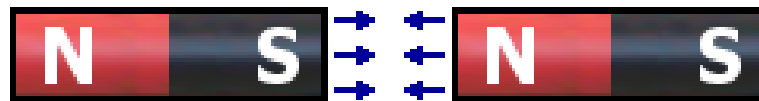


# NMR: INTRODUCTION AND THEORY

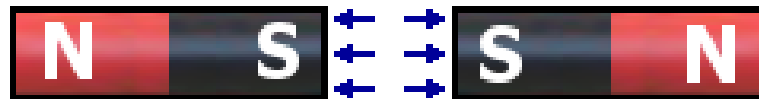
$$E = -\mu \cdot B_0$$

*Magnets interact*

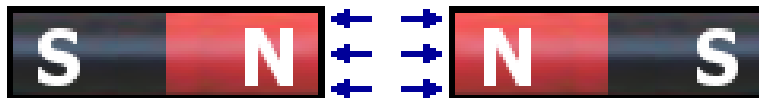
## ATTRACTION



## REPULSION

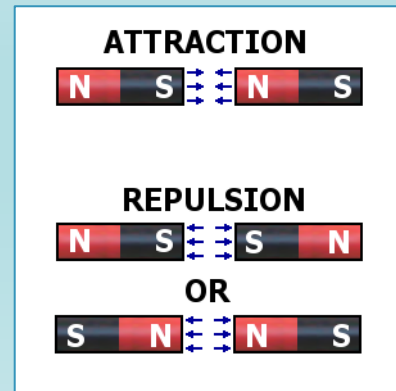


OR



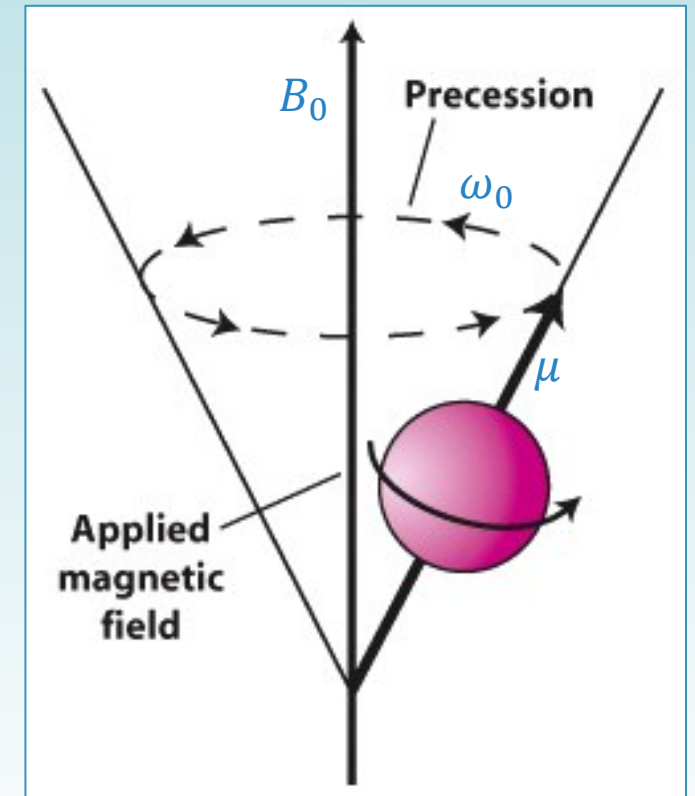
$$E = -\mu \cdot B_0$$

**Magnets interact**



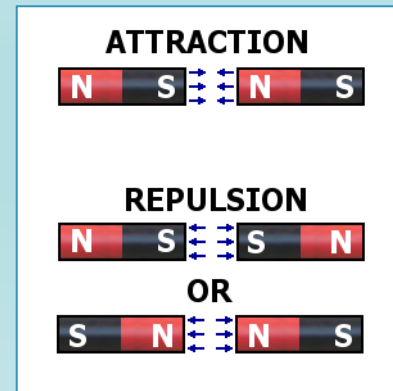
# NMR: INTRODUCTION AND THEORY

- Nuclear magnetic moment  $\mu$  interacts with static magnetic field  $B_0$ 
  - Moment  $\mu$  **precesses about** the direction of  $B_0$
  - Rate of precession (in radians/sec) is  $\omega_0 = \gamma B_0$
- Conversion to Hz (1/sec) provides the **Larmor frequency**  $\nu_0 = \frac{\omega_0}{2\pi}$



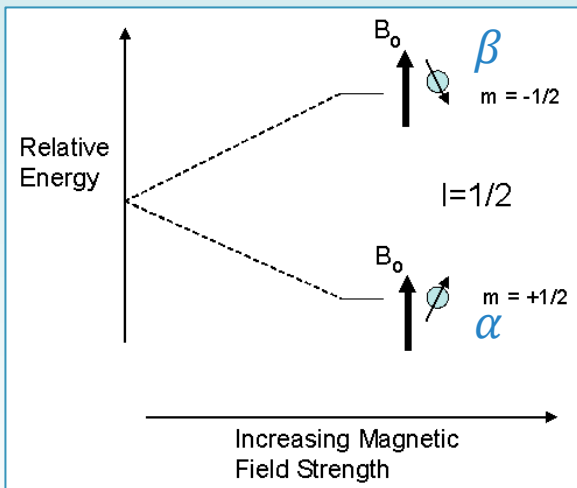
$$E = -\mu \cdot B_0$$

**Magnets interact**



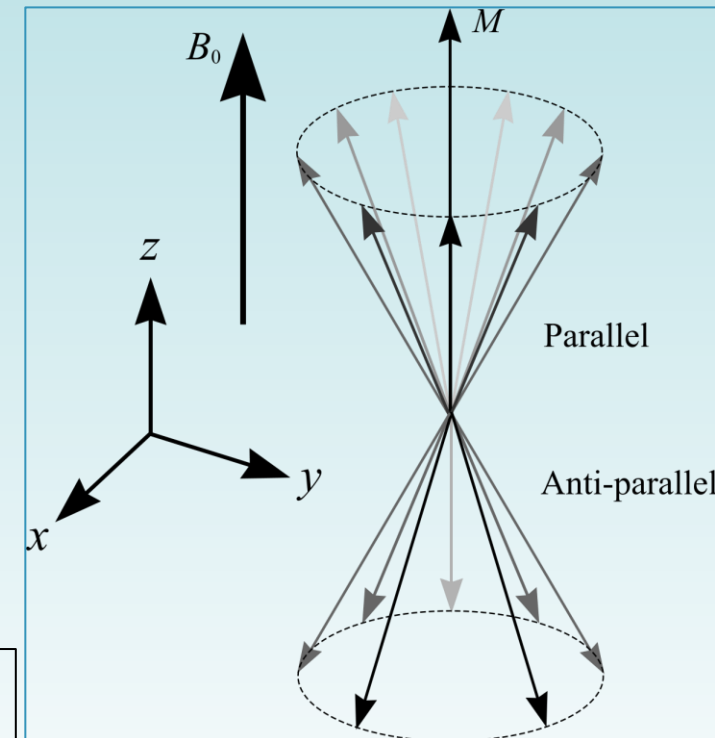
# NMR: INTRODUCTION AND THEORY

- Nuclear magnetic moment  $\mu$  interacts with static magnetic field  $B_0$ 
  - **Orientation** of  $\mu$  with respect to  $B_0$ 
    - **Number of orientations** available  $2I + 1$
    - For spin-1/2 systems ( $I = 1/2$ ), two possible orientations:
      - Lower energy orientation/state  $\alpha$  :  $\mu$  parallel to  $B_0$
      - Higher energy orientation/state  $\beta$  :  $\mu$  antiparallel to  $B_0$



$$\Delta E = \hbar\omega_0 = \hbar\gamma B_0 = h\nu_0 = \frac{\mu B_0}{I}$$

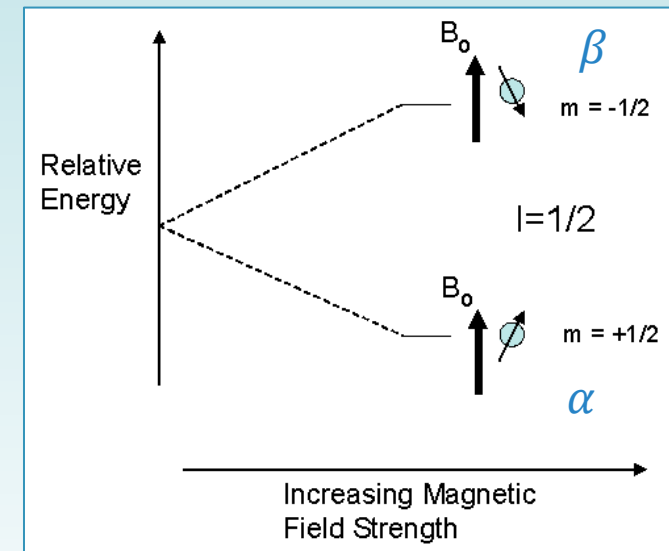
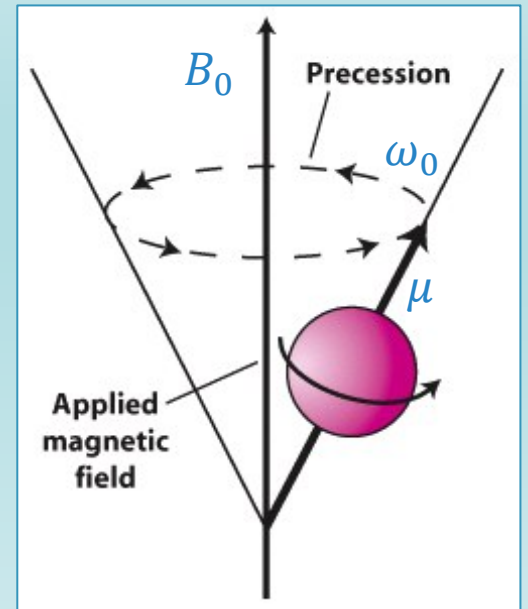
Hence, using the  $\gamma$  for the  $^1\text{H}$  nucleus, a 400 MHz NMR spectrometer has a 9.4 T magnetic field, both of which specify the energy separation between the two spin-1/2 states





# NMR: INTRODUCTION AND THEORY

- To induce a transition from a lower energy state to a higher energy state, the nuclei are hit with radiation proportional to their Larmor frequency (*rf*)
- As we expect, the nuclei will not stay in the excited state indefinitely
  - As the excited nuclei relax back down to the lower energy state, the circular motion associated with their precession produces a **free induction decay** (FID)
- The Fourier transformation of the FID gives rise to the spectral peaks that we recognize as an NMR spectrum (*more on this in NMR II*)



$$\Delta E = \hbar\omega_0 = \hbar\gamma B_0 = h\nu_0$$

# NMR: INTRODUCTION AND THEORY

- **Chemical Shift**

- Relates relative frequency of detected FID to frequency of the spectrometer
- In units of ppm
  - Numerator has units of Hz
  - Denominator has units of MHz

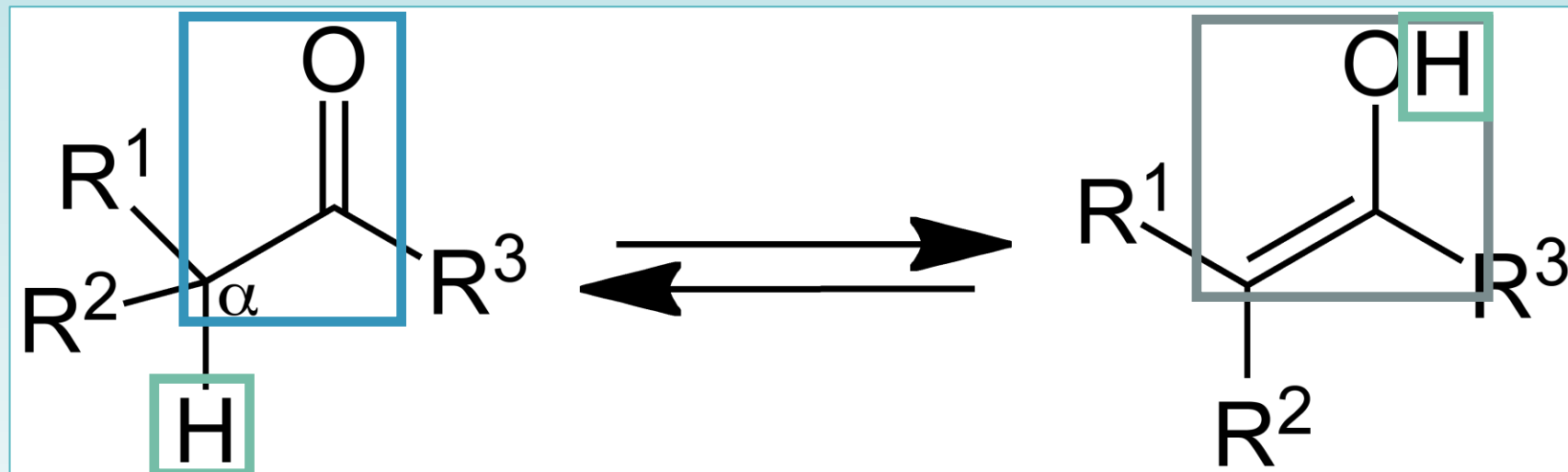
$$\delta = \frac{\nu_{observed} - \nu_{reference}}{\nu_{spectrometer}}$$

- Reference frequency is often an additive such as TMS or a peak clearly identifiable as the solvent
- Chemical shift relates information about the **chemical environment**
  - Downfield (higher shift), less shielding >> responding nucleus subjected to more of the magnetic field
  - Upfield (lower shift), more shielding >> responding nucleus subjected to less of the magnetic field

# EXPERIMENT

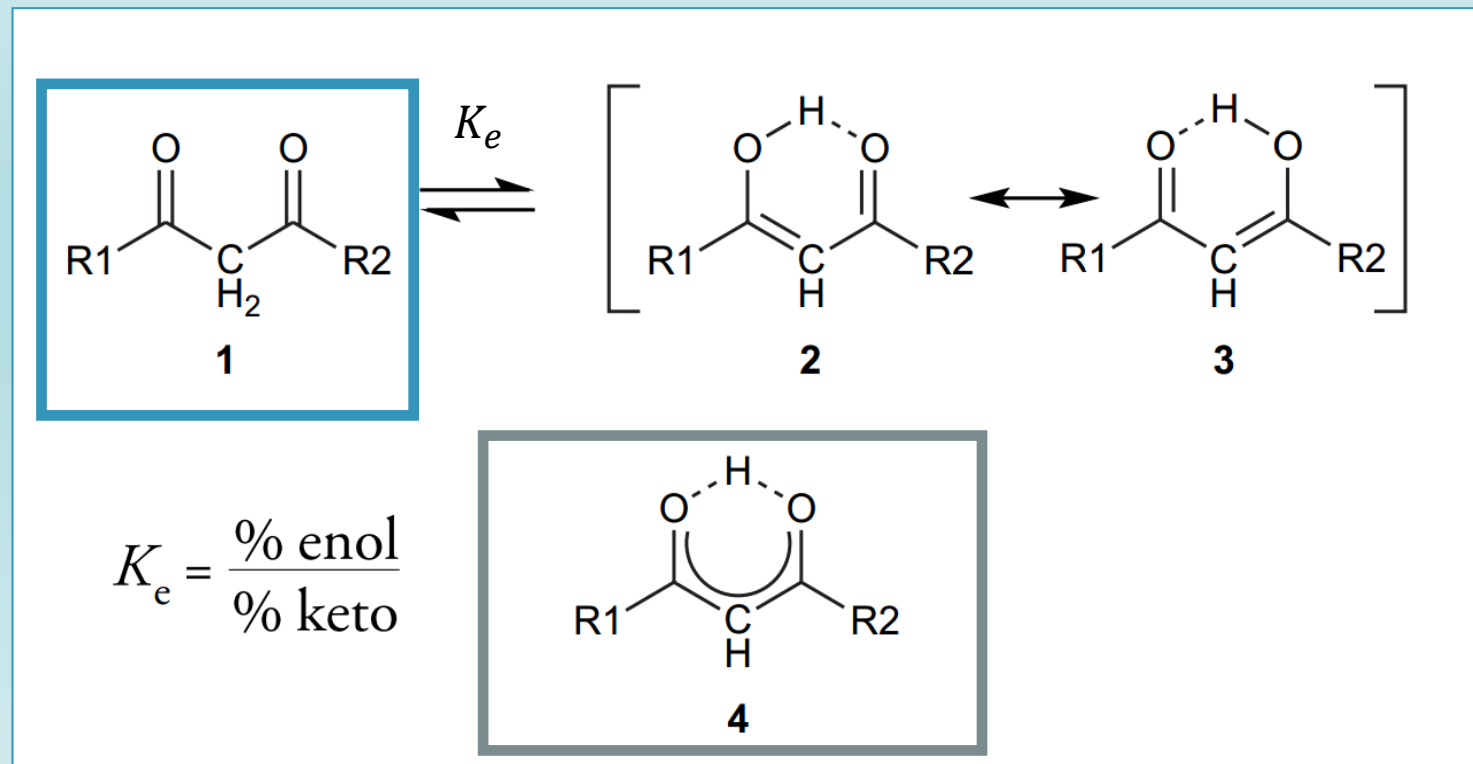
We will use  $^1\text{H}$  NMR spectroscopy to study the **kinetics of 2,4-pentanedione isomerization** from its **keto** form to its **enol** form

○ Isomerization of keto/enol forms is alternatively called **tautomerization**



# EXPERIMENT

- In our case 2,4-pentanedione has two ketone groups (**1**), and as such there exist two enol forms (**2** and **3**)



- The two enol forms **2** and **3** are averaged together into one structure **4** and we detect the peak for this structure in our experiments
- By carefully comparing the **peak integration** of **4** to **1** we can obtain the equilibrium constant  $K_e$  for tautomerization

# EXPERIMENT

- We will conduct several  $^1\text{H}$  NMR experiments while varying the three following conditions independently:
  1. *Concentration*
  2. *Solvent*
  3. *Temperature*
- For each experiment, we will determine  $K_e$
- The goal is to determine how each of the different conditions affects the tautomerization equilibrium based on our results

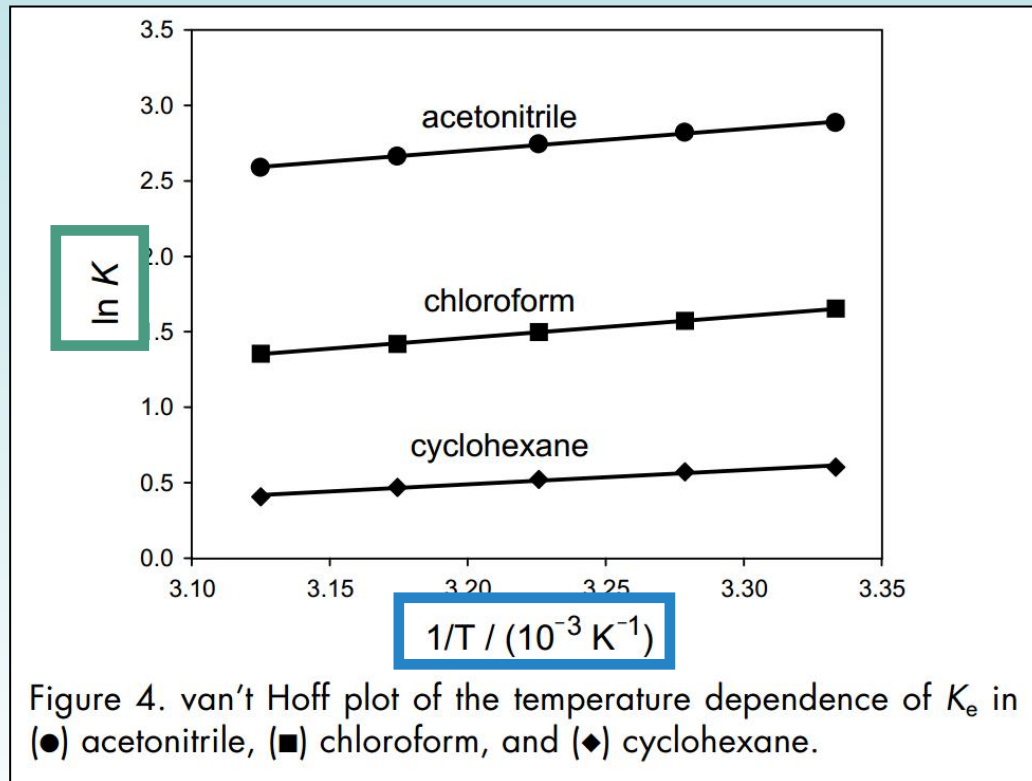
# EXPERIMENT

- The solvent studies will use the following:
  - *Chloroform-d<sub>3</sub>*
  - *Benzene-d<sub>6</sub>*
  - *Acetone-d<sub>6</sub>*
  - *Dimethylsulfoxide-d<sub>6</sub>*
  - *Acetonitrile-d<sub>3</sub>*
  - *CCl<sub>4</sub>*

# EXPERIMENT

$$\ln(K_e) = \left(\frac{\Delta H}{R}\right) \left(\frac{1}{T}\right) + \left(\frac{\Delta S}{R}\right)$$
$$y = mx + b$$

- In addition to calculating  $K_e$  for the variable temperature experiments, we will create a Van't Hoff plot by plotting  $\ln(K_e)$  by  $\frac{1}{T}$



- A linear fit to these plots will allow us to determine thermodynamic information ( $\Delta H$  and  $\Delta S$ ) about the equilibration

# NMR II: LINESHAPE ANALYSIS OF REVERSIBLE HYDROLYSIS REACTION

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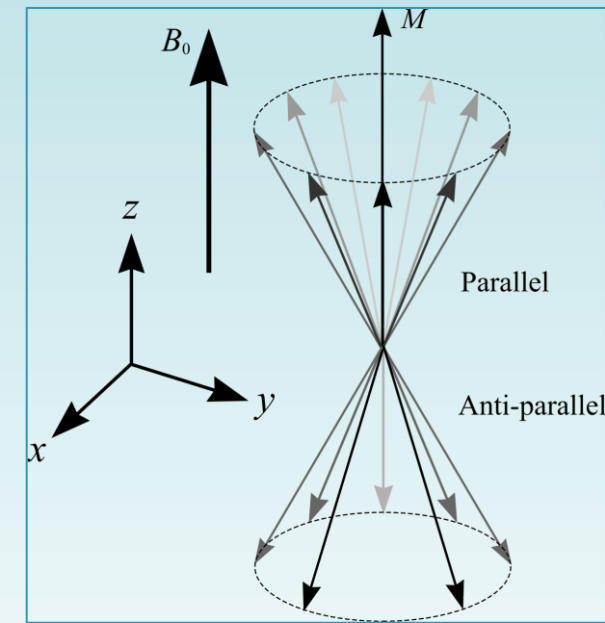
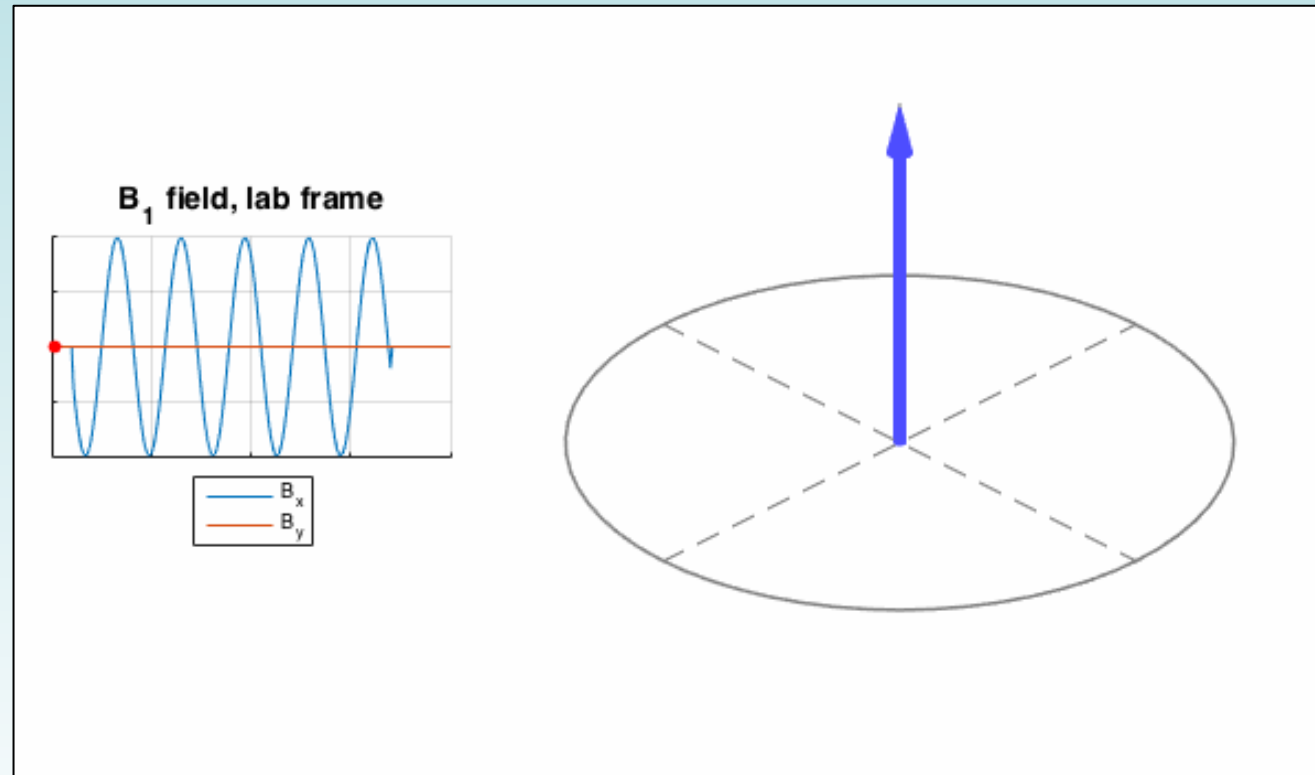
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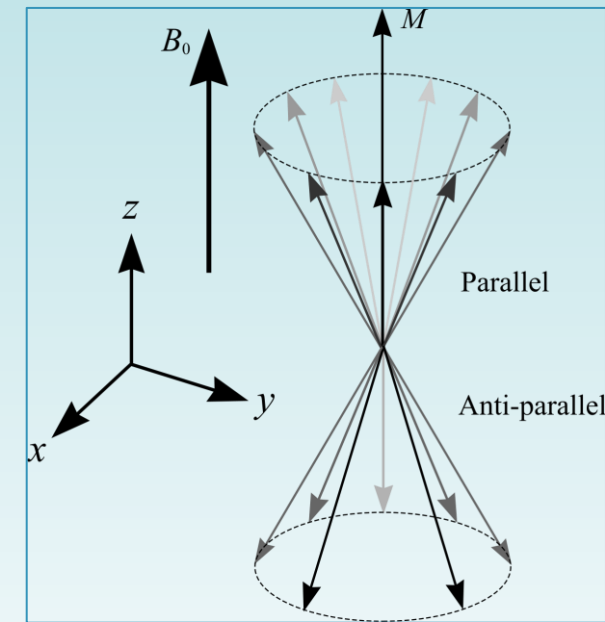
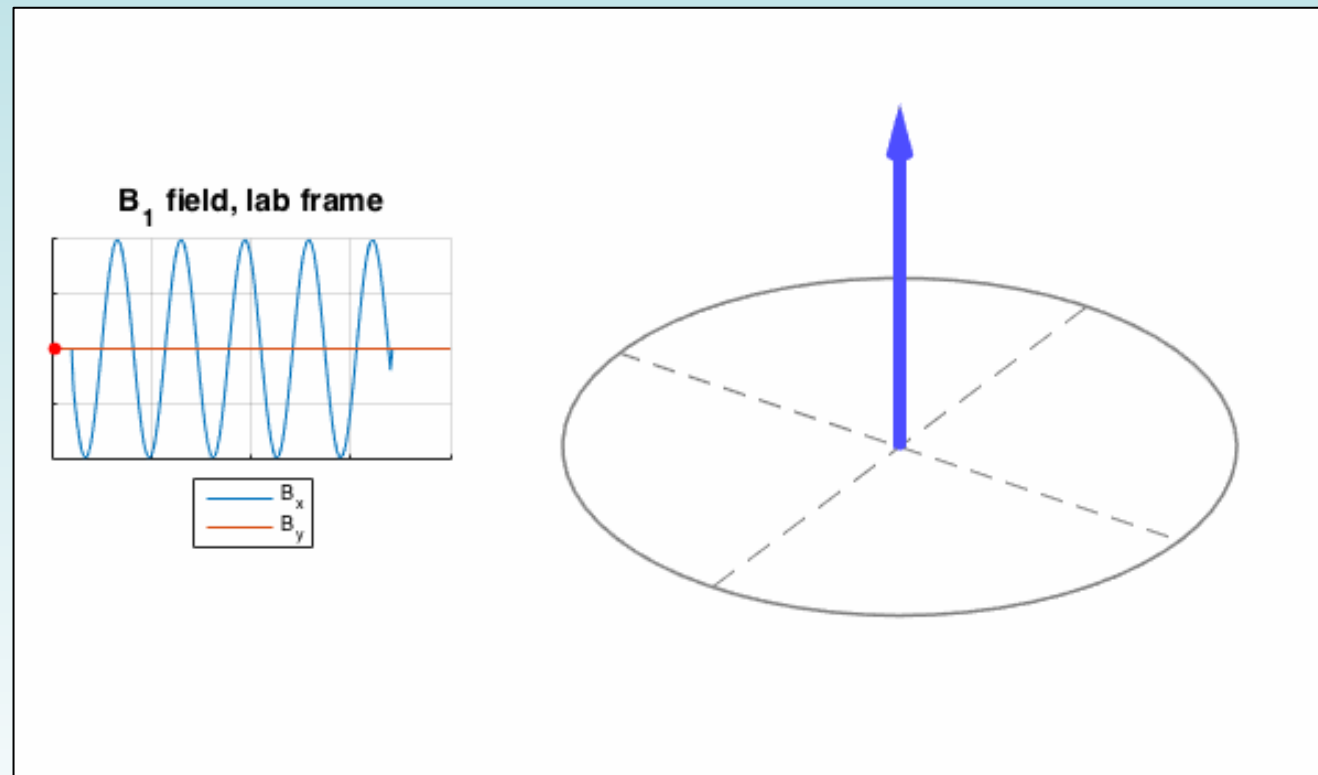
# Background: Free Induction Decay

Applying a radio frequency (*Larmor frequency*) pulse  $B_1$  disrupts equilibrium of nuclear spins (**net magnetization, often  $M_0$** )



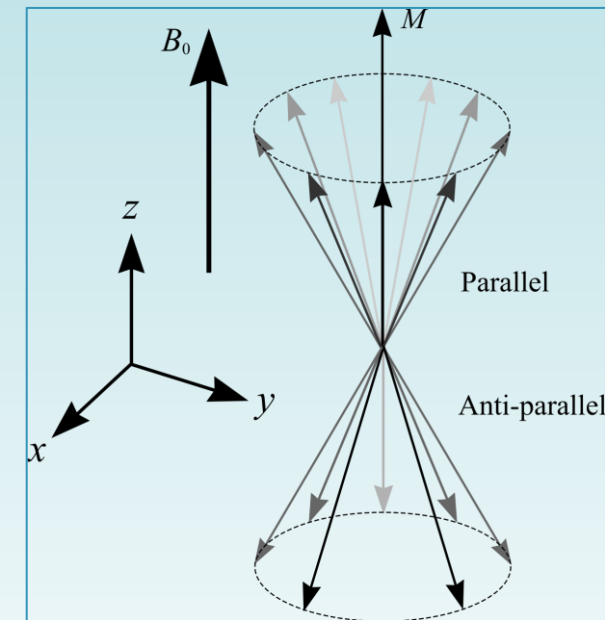
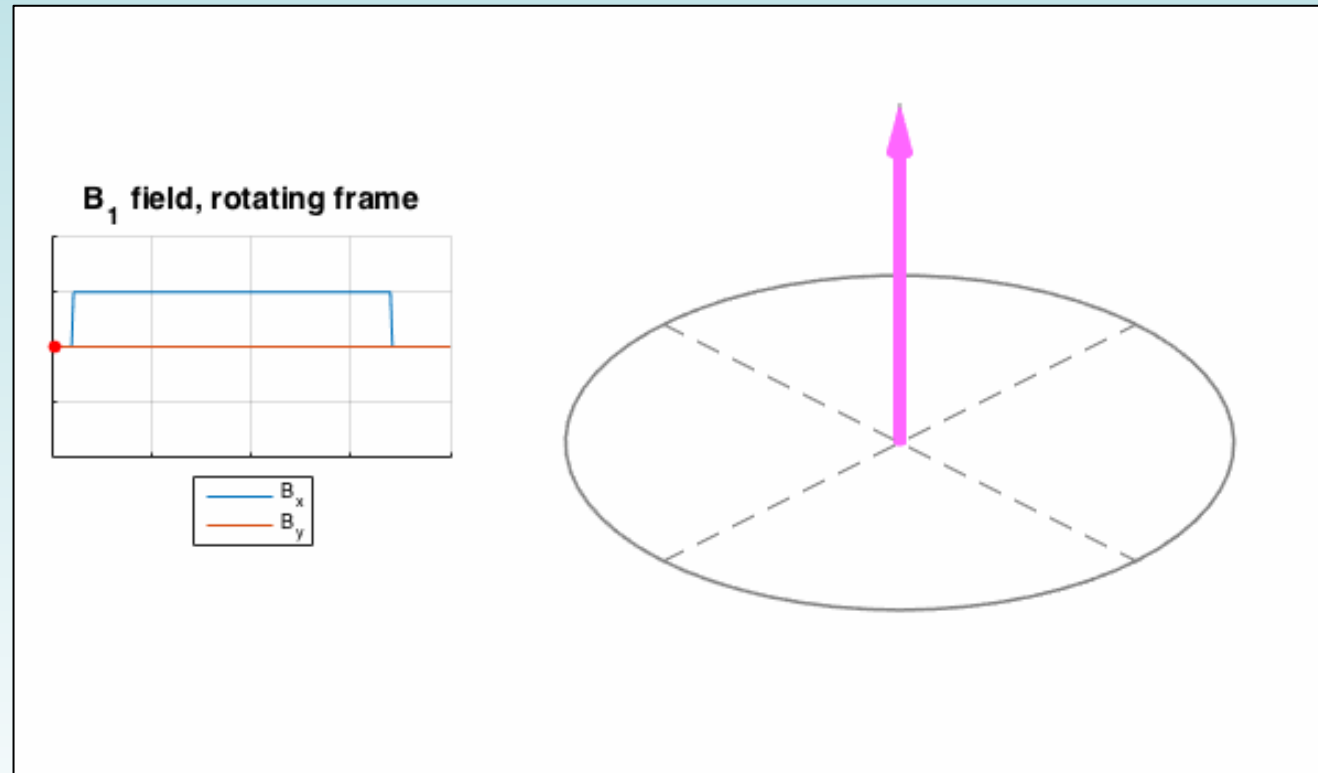
# Background: Free Induction Decay

Recall the nuclei are already rotating (*spinning*) at the **Larmor frequency** given by the **gyromagnetic ratio** and **external magnetic field  $B_0$**  ( $\omega_0 = \gamma B_0$ )



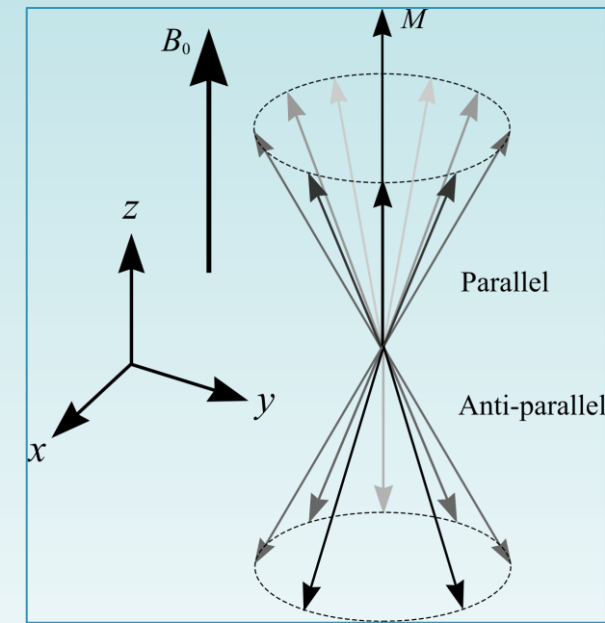
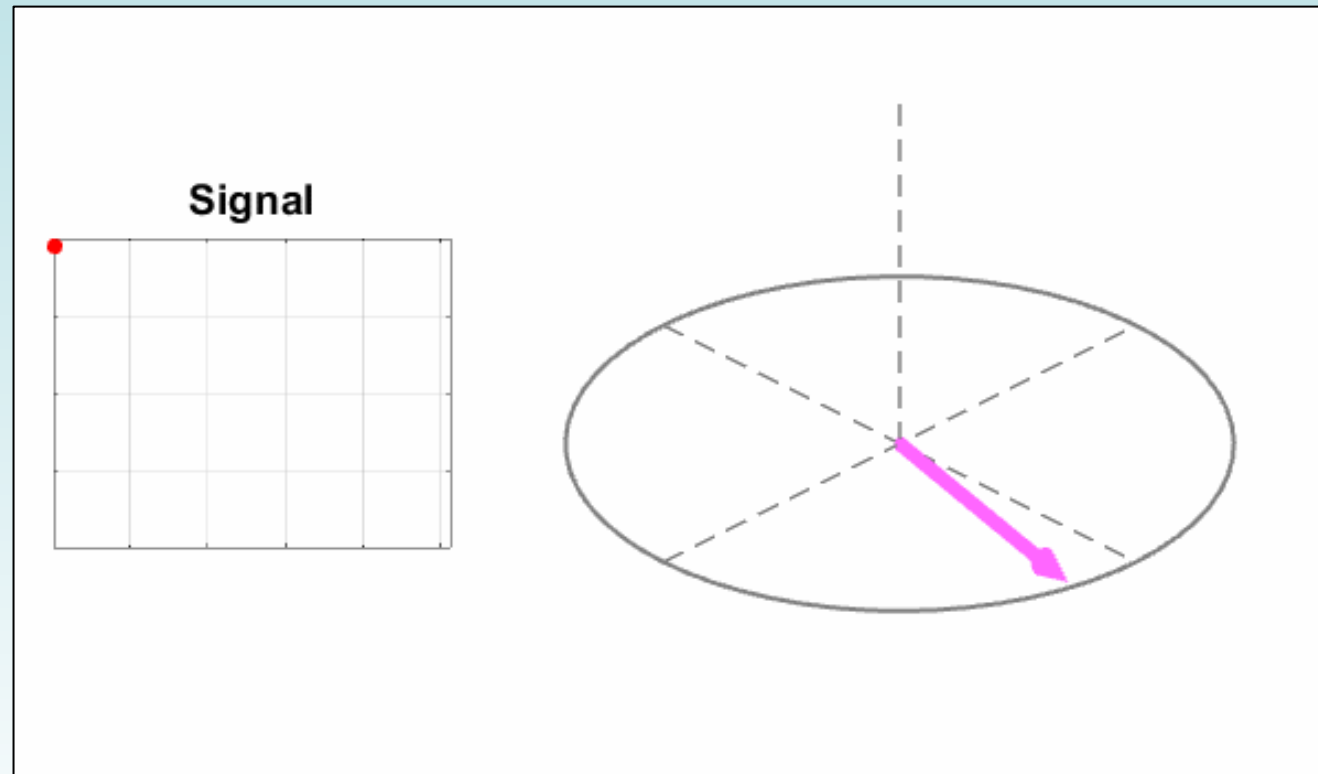
# Background: Free Induction Decay

Defining a new coordinate frame (**rotating frame**) in which the  $x$ ,  $y$ , and  $z$  axes are *rotating at the Larmor frequency* simplifies our analysis



# Background: Free Induction Decay

After the  $B_1$  pulse, the net magnetization returns to equilibrium, generating the NMR signal (**FID**) in the process, shown here in the **rotating frame**



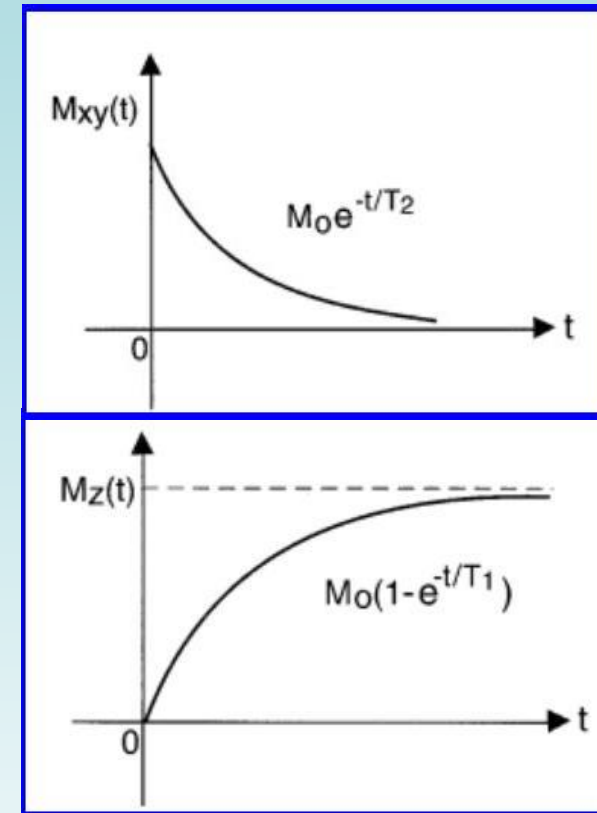
# Background: Free Induction Decay

The **FID** is a *decaying exponential*  $e^{-t/T_2}$

- It is the rate of decay of nuclear spins from an excited state to thermal equilibrium
- The *envelope* of the FID provides the  $T_2^*$  *relaxation*
  - $T_2^*$  denotes decaying signal due to factors other than the chemical system itself (e.g., inhomogeneity of the magnetic field)
- $T_2$  is called the *transverse relaxation* or *spin-spin relaxation*
  - $T_2$  characterizes how the net magnetization “leaves” the x-y or transverse plane to return to equilibrium along z. ***It is a product of the chemical environment.***

Additionally  $T_1$  relaxation occurs

- $T_1$  is called the *longitudinal relaxation* or *spin-lattice relaxation*
  - $T_1$  characterizes how the net magnetization “returns” to orientation along the z-axis or the longitudinal plane, in line with the **external magnetic field  $B_0$** ; ***it is also a product of the chemical environment.***
- Obtained by a different **pulse sequence** than that shown previously



# Background: Fourier Transformation

*Fourier Transformation of the **FID** (time-domain) provides the familiar NMR spectrum (frequency-domain)*

Signal in the frequency domain (NMR spectrum) →  $f(\nu) = \int_{-\infty}^{+\infty} e^{-i2\pi\nu t} s(t) dt$  ← Signal in the time domain (FID)

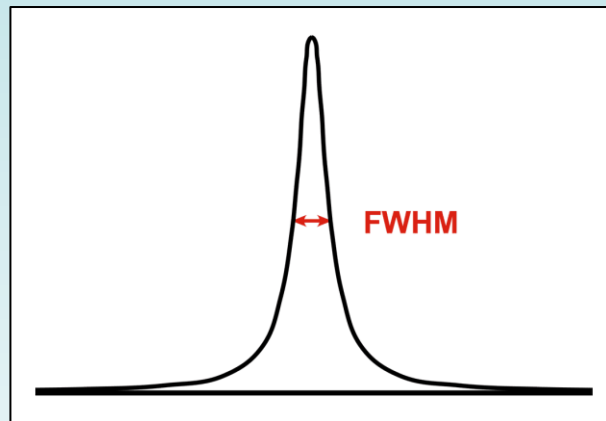
- **Different representations of the same information**
  - Extracts the frequency components  $f(\nu)$  of the oscillating exponential decay  $f(t)$
- Fourier Transformations have various important properties, obey several rules

# Background: Fourier Transformation

$$f(v) = \int_{-\infty}^{+\infty} e^{-i2\pi vt} s(t) dt$$

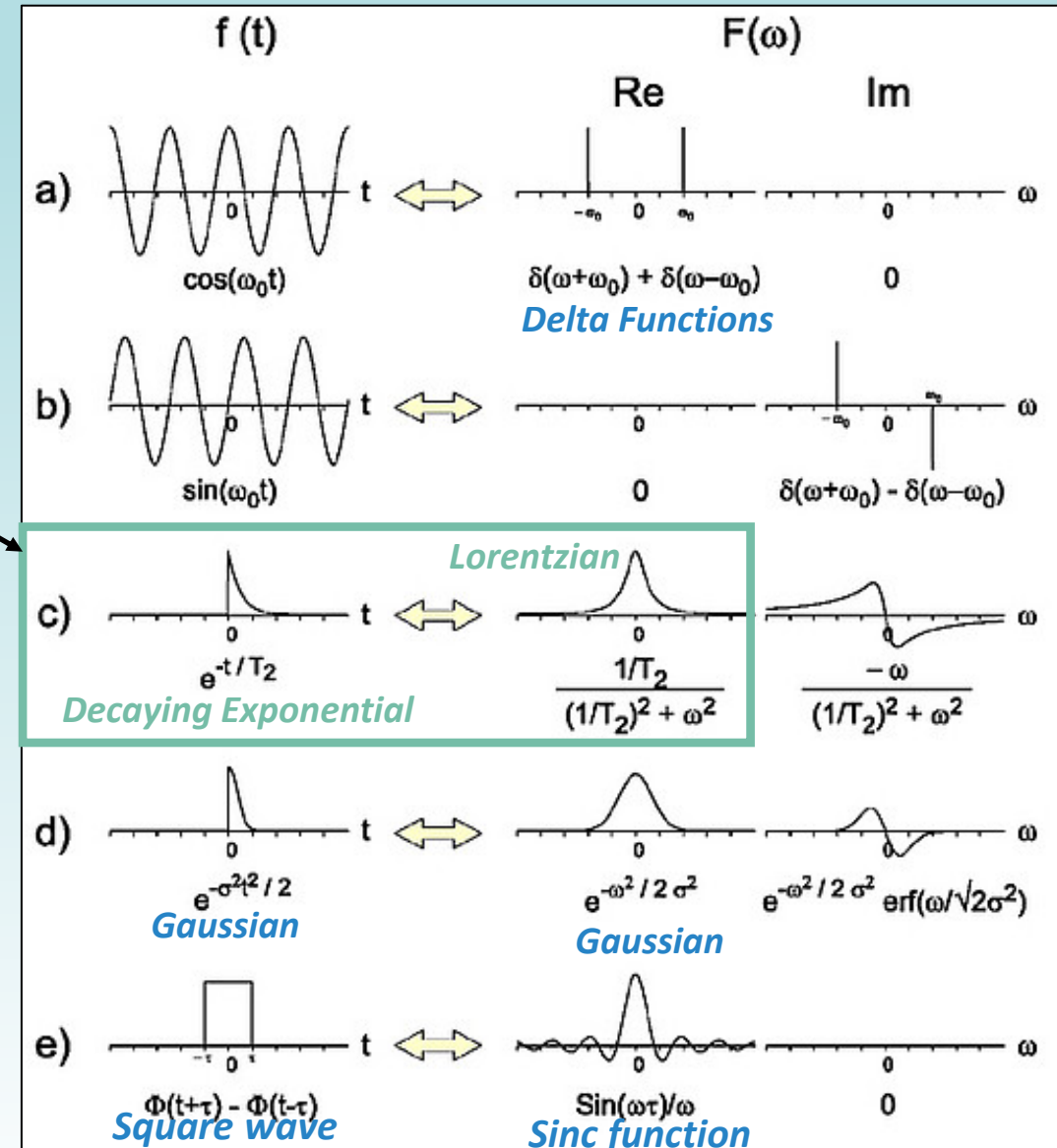
There exist several Fourier Transform pairs

- We are concerned with (c)
- A **Lorentzian** is the lineshape associated with peaks in an NMR spectrum

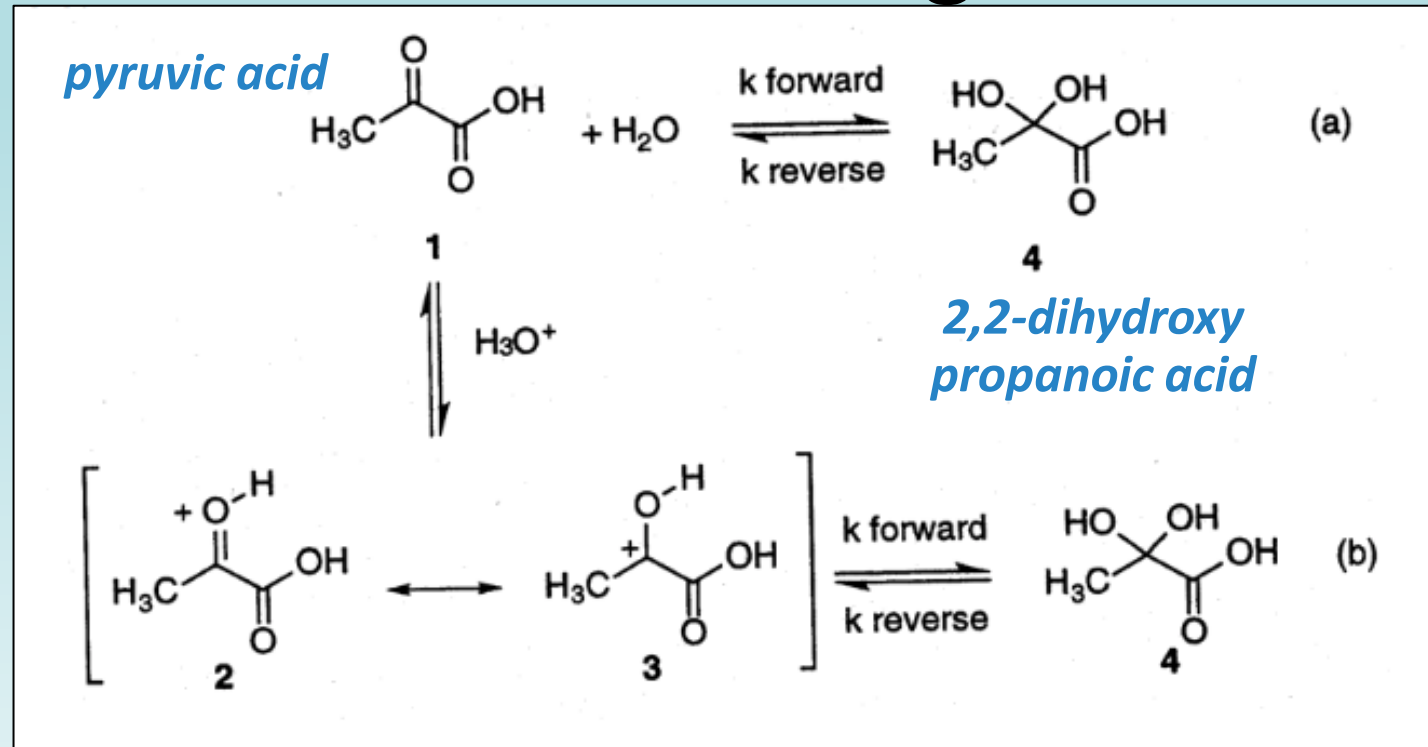


The width of a Lorentzian line, often called the **full width half maximum (FWHM)** or in your handout simply **W**, is determined by  $T_2^*$

$$W = \frac{1}{\pi T_2^*}$$



# Experiment: Proton Exchange



- We can use NMR to analyze chemical system using either of 2 main principles:
  - (1) Relative area of Lorentzian peaks provides relative concentrations of those nuclei (previous lab)
  - (2) Linewidth of Lorentzian peaks provides information on nuclei lifetimes when **exchange** occurs
- We will be studying the proton exchange of pyruvic acid hydrolysis both in the **absence of acid catalyst (a)** and in the **presence of acid catalyst (b)**



# Experiment: Proton Exchange

- In the presence of proton exchange, we express  $T_2$  as  $T_2'$  because it contains additional information ( $\frac{1}{\tau}$ )

$$\frac{1}{T_2'} = \frac{1}{T_2^*} + \frac{1}{\tau}$$

$T_2$  in presence of exchange  $\rightarrow$   $T_2'$  =  $T_2^*$  +  $\tau$   $\leftarrow$  Mean lifetime of exchange

$T_2$  in absence of exchange  $\leftarrow$   $T_2^*$

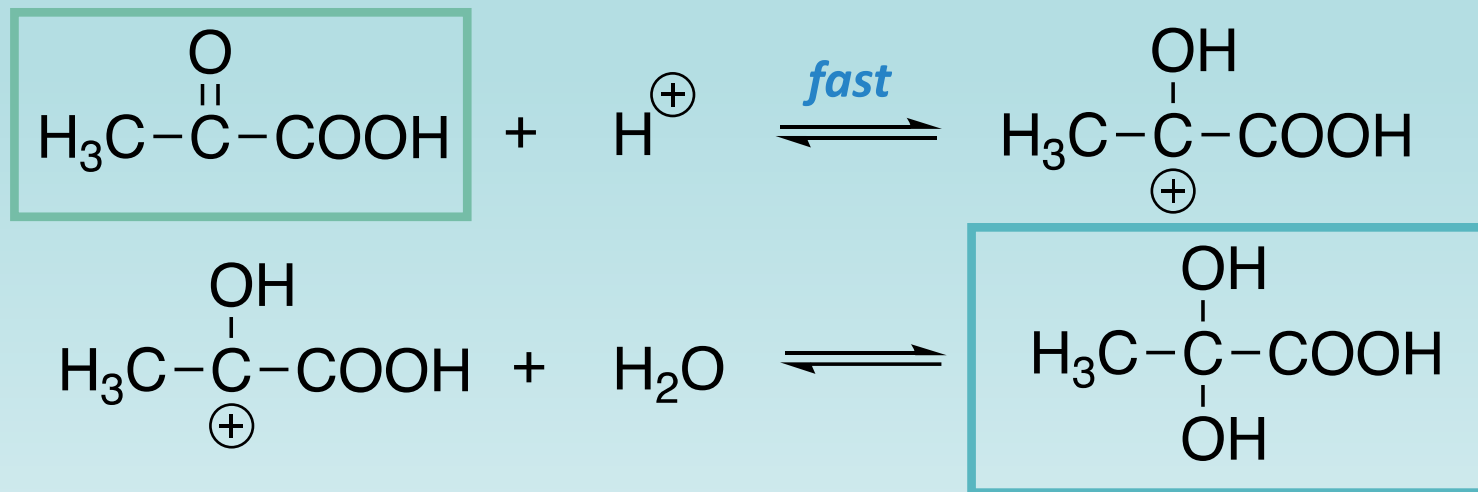
- From kinetic analysis described in handout, the mean lifetime of exchange is related to the uncatalyzed and catalyzed rate constants

$$\frac{1}{T_2'} = \frac{1}{T_2^*} + k_0 + k_{H^+} [H^+]$$

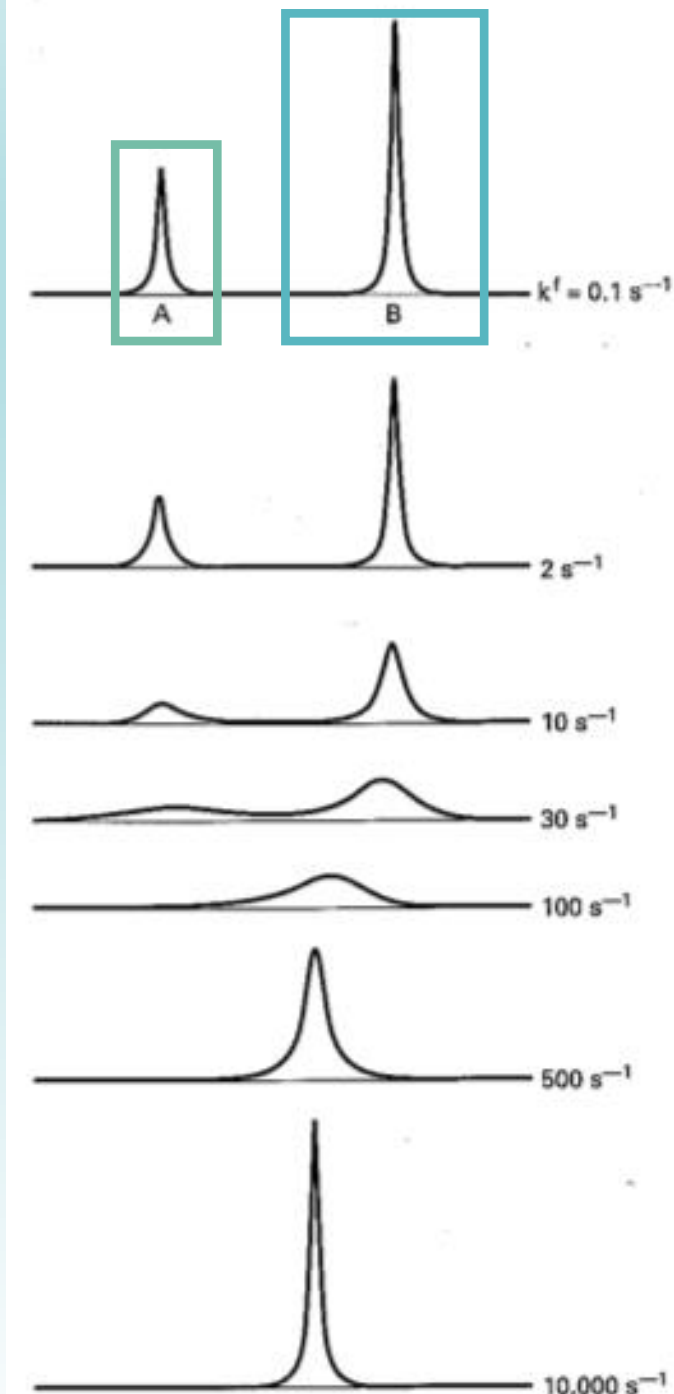
Rate constant for uncatalyzed exchange  $\leftarrow$   $k_0$   $\leftarrow$   $k_{H^+} [H^+]$   $\leftarrow$  Concentration of  $H^+$  catalyst

Rate constant for catalyzed exchange  $\leftarrow$   $k_{H^+} [H^+]$

# Experiment: Proton Exchange



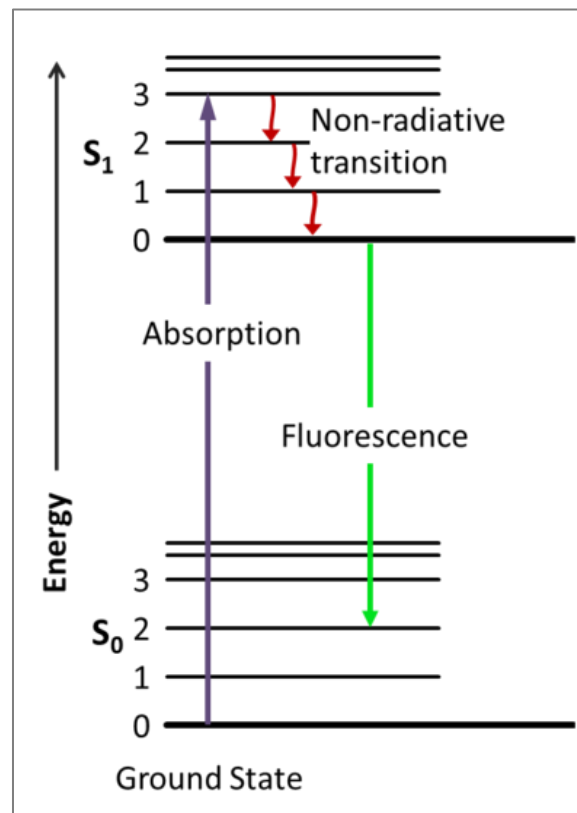
- We will observe 3 proton resonant peaks
- Two peaks **A** and **B** (*right*) correspond to the pyruvic acid and hydrated pyruvic acid, respectively
- The third peak (not shown) is an averaged singlet due to rapid exchange of proton between **A**, **B**, and **water**
- *As the rate of exchange between **A** and **B** increases, the resonance broadens until exchange timescale exceeds the timescale of  $T_2$*



# Experiment: Proton Exchange

- We will study the effect of  $[H^+]$  on the hydrolysis equilibrium:
  - Collect NMR spectra at varied concentrations of acid
  - Fit the resonance peaks to Lorentzian line to determine the linewidth (FWHM)
  - ***Excel sheet to assist in the fitting, FWHM determination***
  - By plotting linewidth as a function of concentration, rate constants can be determined (for forward and reverse reactions)
  - Equilibrium constant can be calculated
- Additional considerations:
  - Assess the linear fit of FWHM vs  $[H^+]$
  - Chemical shift variations as function of  $[H^+]$

# Fluorescence and Absorption of Polycyclic Aromatic Hydrocarbons



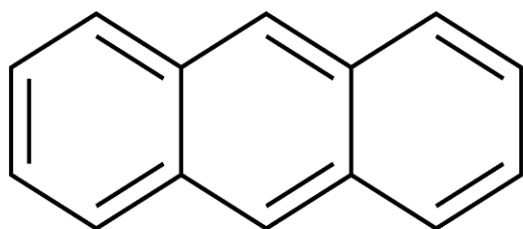
TA: ALEC BEATON

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ADAPTED FROM EK (2017), PM(2015-16) & I. B. BERLEMAN, HANDBOOK OF FLUORESCENCE SPECTRA OF AROMATIC MOLECULES (SECOND EDITION, ACADEMIC PRESS, NEW YORK, 1971).

# Polycyclic Aromatic Hydrocarbons

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**Figure 1.** Anthracene emits a ~400-500 nm (blue) fluorescence under UV radiation

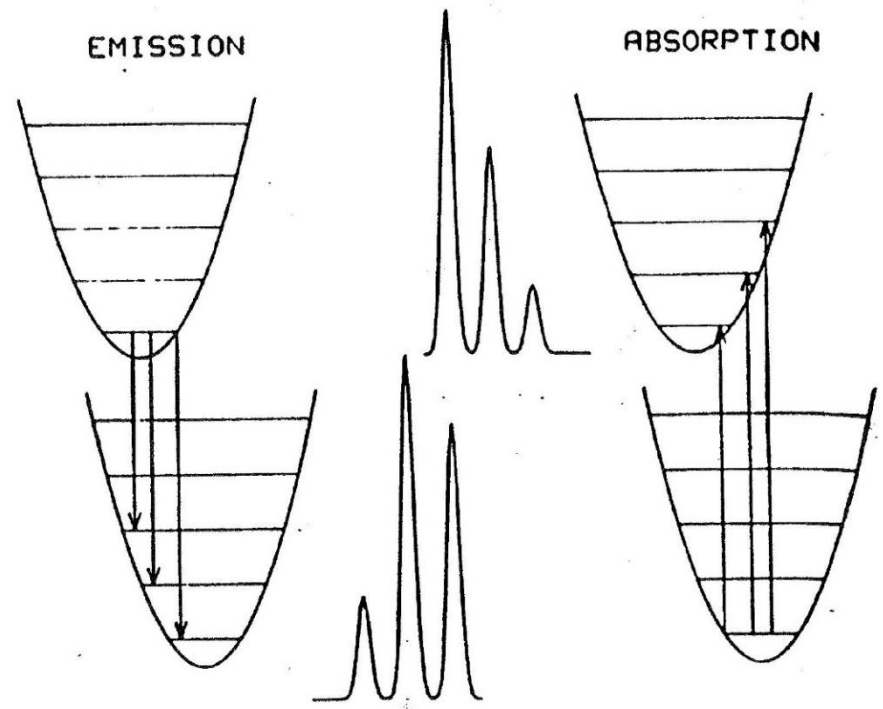
- Organic byproducts of various industrial processes (fossil fuel burning, biomass burning, cigarette smoke)[1]
- Environmental pollutants
- Adverse effects on health
- Network of  $\pi$ -electrons readily enables spectroscopic analysis
- As structural rigidity increases, fluorescence increases
- As rigidity decreases, vibrations increases and fluorescence weakens

[1] Rivera-Figueroa, A. M et al. Fluorescence, Absorption, and Excitation Spectra of Polycyclic Aromatic Hydrocarbons as a Tool for Quantitative Analysis. *J. Chem. Educ.* **2004**, *81* (2), 242.

# Electronic Absorption and Fluorescence Fundamentals

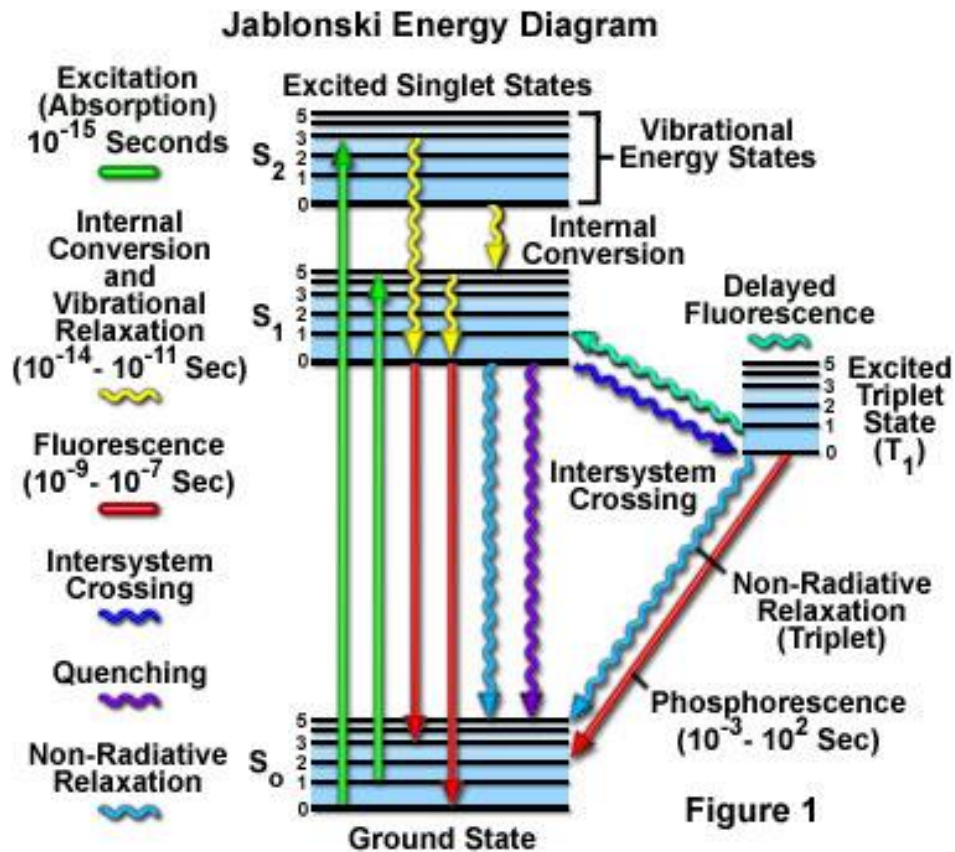
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- Quantized **electronic**, **vibrational**, and **rotational** levels
- Electronic - optical or UV
- Vibrational – IR
- Rotational – microwave



# Jablonski Energy Diagrams

- For visualizing transitions, understanding spectra
- Electronic state manifolds populated with vibrational states
- **Fluorescence:**  
Excitation( $S_0-S_1$ ) → Relaxation( $S_1$ )  
→ Fluorescence( $S_1-S_0$ )
- **Phosphorescence:**  
Excitation( $S_0-S_1$ ) → Relaxation( $S_1-T_1$ )  
→ Phosphorescence( $T_1-S_0$ )
- Phosphorescence has longer lifetime than fluorescence



Link to Interactive Jablonski diagram:

<https://micro.magnet.fsu.edu/primer/lightandcolor/fluoroexcitation.html>

# Absorption & Fluorescence

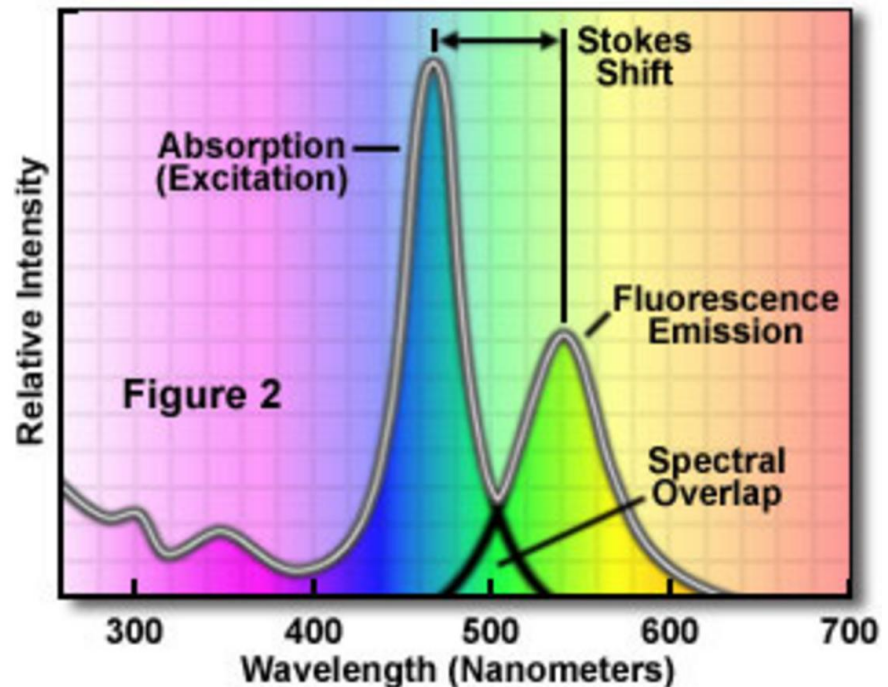
- **Excitation**

- Starts from lowest vibrational energy level of the ground electronic state, then excited to some higher electronic energy level

- **Emission**

- Relaxes from excited state and emits light, the energy of the emitted photon is lower than that of the incident photons

**Excitation and Emission Spectral Profiles**



<https://micro.magnet.fsu.edu/primer/lightandcolor/fluoroexcitation.html>



# Beer-Lambert law relates light absorbed by sample to the concentration

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$$A = \epsilon cb$$

$$-\log\left(\frac{I}{I_o}\right) = A$$

$$I = I_o 10^{-\epsilon cb}$$

**A Absorbance** (unitless)

**$\epsilon$  Extinction coefficient** ( $\text{M}^{-1} \text{cm}^{-1}$ )

**c Concentration** (M)

**b Path length** (cm)

**I Intensity of detected light**

**$I_o$  Intensity of incident light**

# Fluorescence Intensities

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- Fluorescence intensity ( $F$ ) at a particular excitation ( $\lambda_x$ ) and emission wavelength ( $\lambda_m$ ) depends on the light absorbed ( $I_A$ ) and quantum yield ( $\phi$ ) :

$$F(\lambda_x, \lambda_m) = I_A(\lambda_x)\phi(\lambda_m)$$

# Quantum Yield

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The fluorescence intensity ( $F$ ) at a particular excitation ( $\lambda_x$ ) and emission wavelength ( $\lambda_m$ ) for a dilute solution containing a fluorophore is:

$$F(\lambda_x, \lambda_m) = I_o 2.303 \varepsilon(\lambda_x) CL \phi(\lambda_m)$$

$I_o$  – incident light intensity

$\phi$  – quantum yield

$C$  – concentration

$\varepsilon$  – molar extinction coefficient

$L$  – path length

# Quantum Yield

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In the idealized case of a fluorescence quantum yield ( $Q_f$ ) of 1, the rate of radiative emission ( $k_e$ ) is the inverse of fluorescence lifetime ( $\tau_f$ )

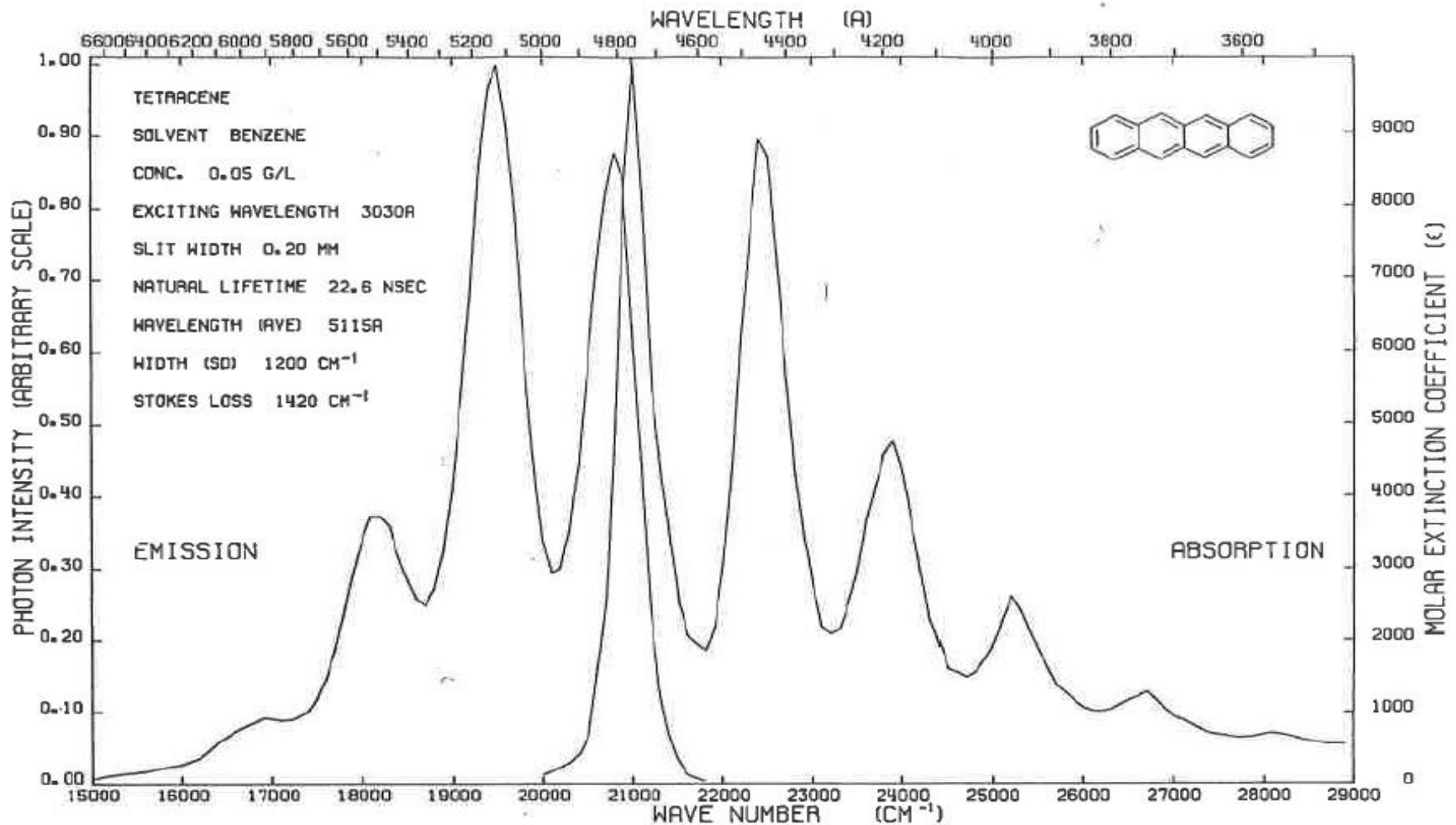
$$k_e = \frac{Q_f}{\tau_f} = \frac{1}{\tau_f}$$

In reality, there are competing non-radiative relaxation processes which will reduce the fluorescence quantum yield.

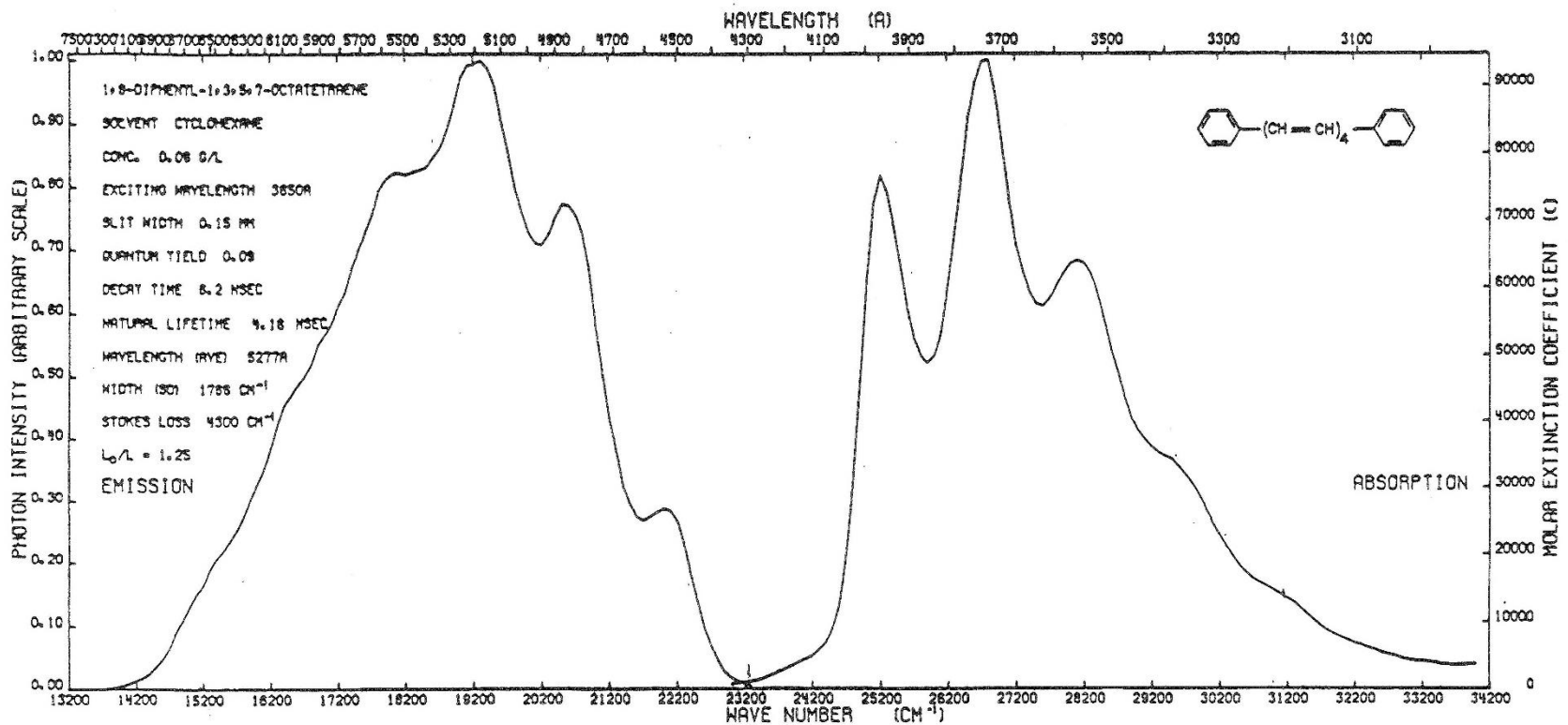
The observed fluorescence quantum yield is therefore described by the rate of radiative emission and the rate of non-radiative emission ( $k_{nr}$ ).

$$Q_f = \frac{k_e}{k_e + k_{nr}}$$

# Emission and Excitation Graph



# Unique Emission and Excitation Graph



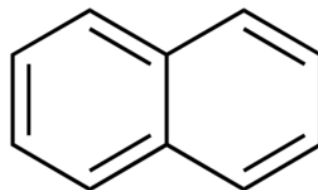
Energy



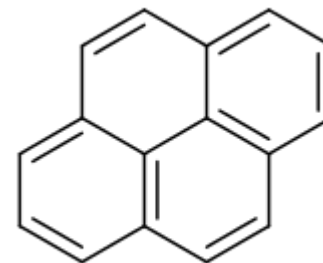
# In Lab

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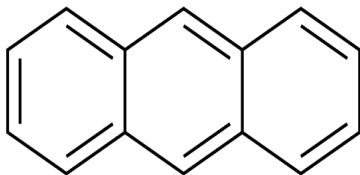
- Experimental
  - Dilute and concentrated samples of each
  - Excitation and emission scans
- Gaussian Calculations
  - Zindo & TD-DFT
- Anthracene
  - Looking at the vibrational structure of the absorption



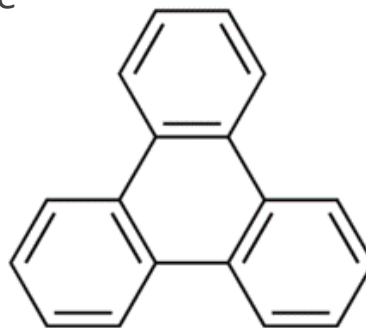
naphthalene



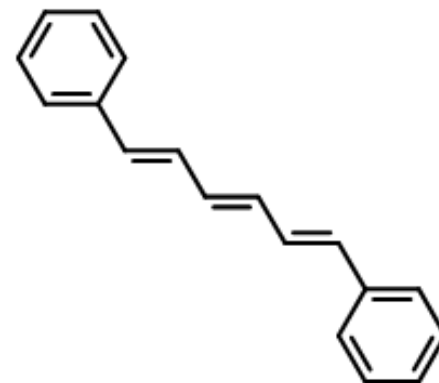
pyrene



anthracene



triphenylene



diphenylhexatriene

# Raman and Infrared of Mono-Substituted Cyclohexane Derivatives

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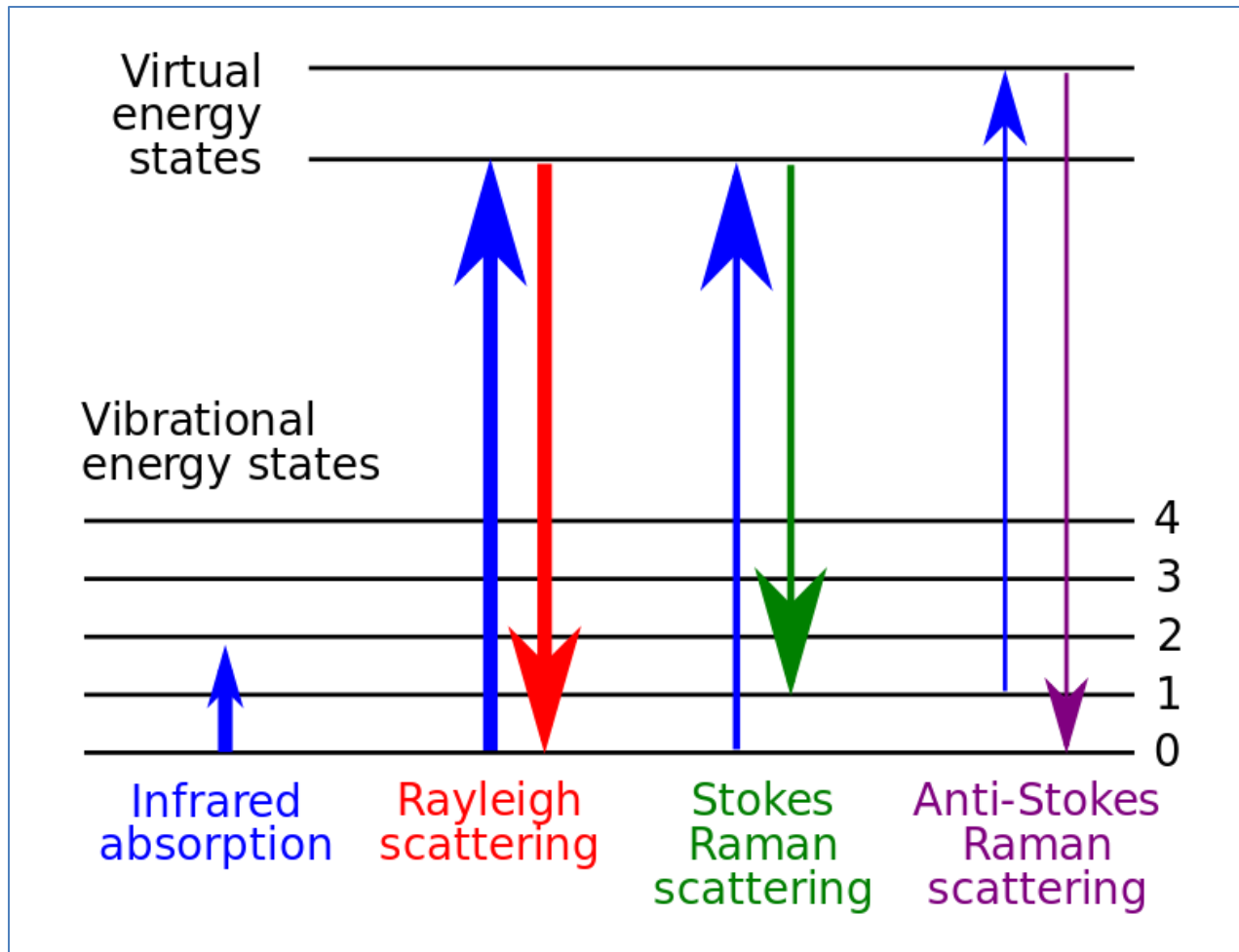
Alec Beaton

(Adpated from: Peter McLaughlin)

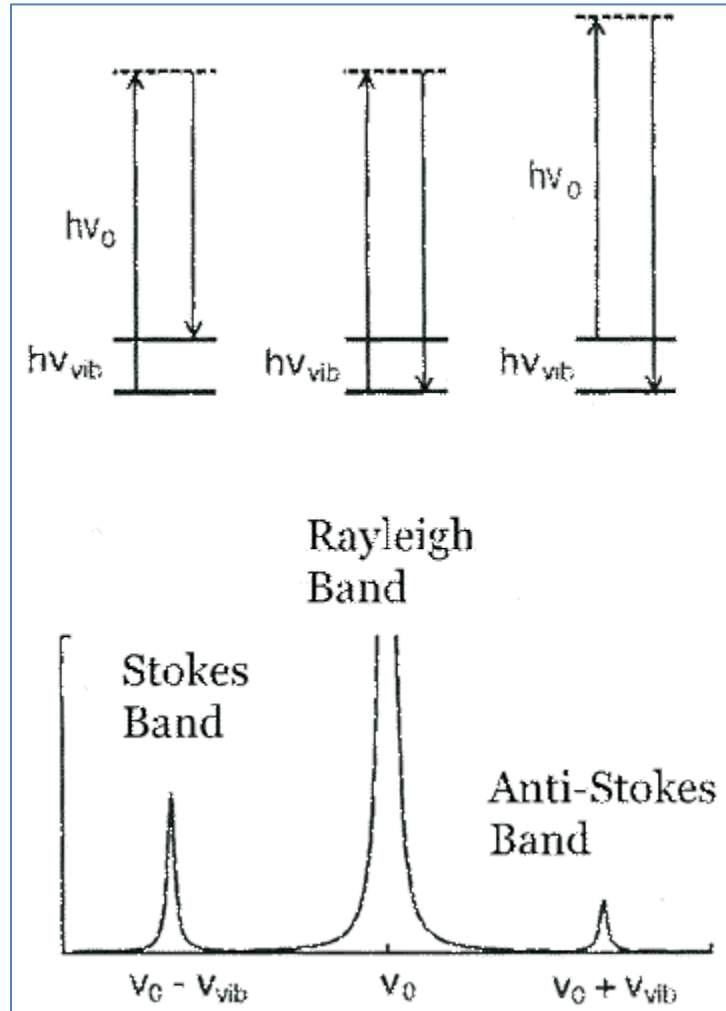


# Raman vs. Absorbance (IR)

- Raman utilizes 'virtual states'



# Raman Bands



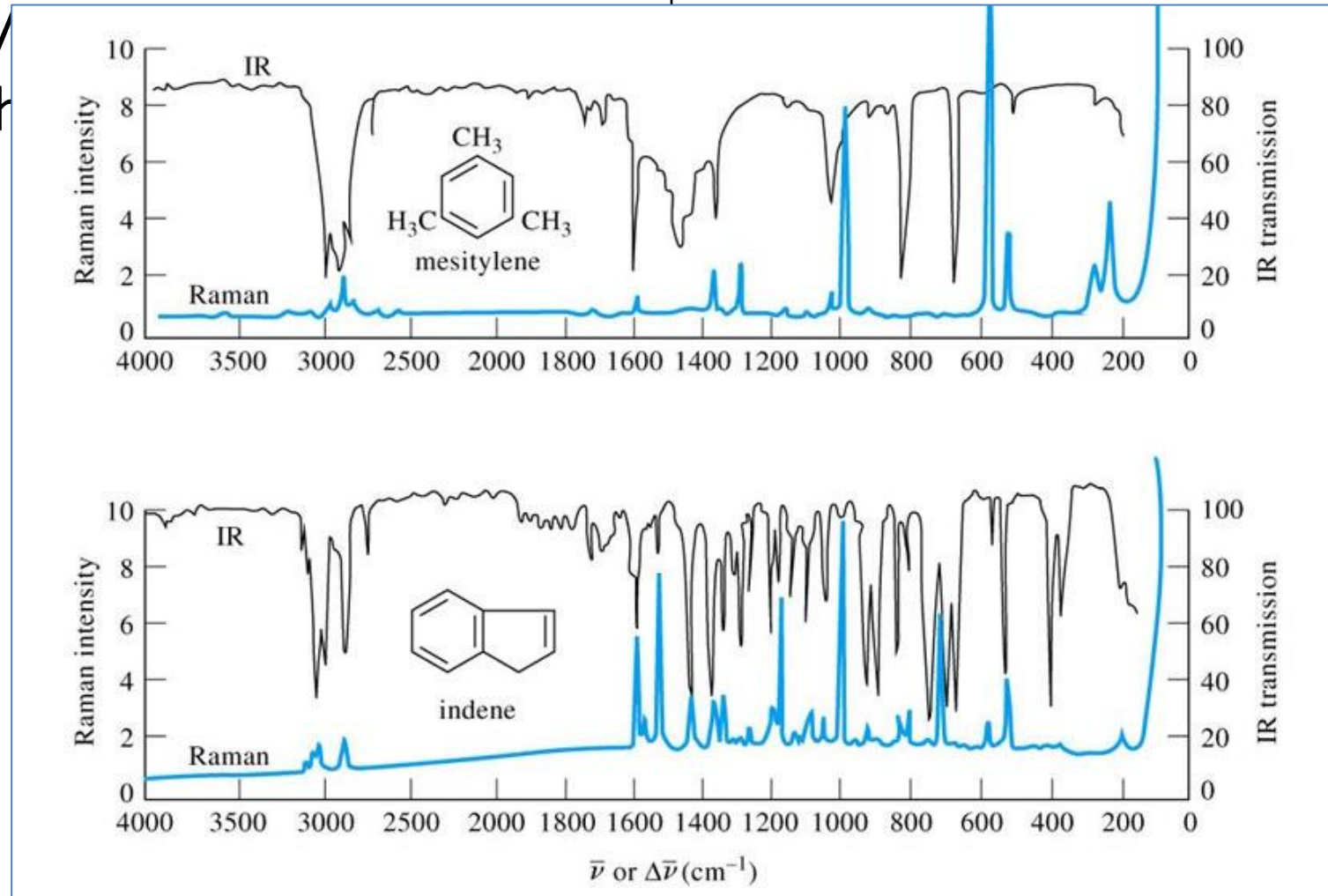
# Raman vs. Infrared

- Change in polarizability
  - Momentary distortion of the electrons
- Change in dipole moment
  - Change in charge distribution during vibration

# Raman vs. Infrared

- Change in polarizability
- Change in dipole moment

• M  
th



# Point Group Tables

**TABLE 27.5** The Character Table for the  $D_{2h}$  Point Group

	$E$	$C_2(z)$	$C_2(y)$	$C_2(x)$	$i$	$\sigma(xy)$	$\sigma(xz)$	$\sigma(yz)$	
$A_g$	1	1	1	1	1	1	1	1	$x^2, y^2, z^2$
$B_{1g}$	1	1	-1	-1	1	1	-1	-1	$R_z$ $xy$
$B_{2g}$	1	-1	1	-1	1	-1	1	-1	$R_y$ $xz$
$B_{3g}$	1	-1	-1	1	1	-1	-1	1	$R_x$ $yz$
$A_u$	1	1	1	1	-1	-1	-1	-1	
$B_{1u}$	1	1	-1	-1	-1	-1	1	1	$z$
$B_{2u}$	1	-1	1	-1	-1	1	-1	1	$y$
$B_{3u}$	1	-1	-1	1	-1	1	1	-1	$x$

Raman Active Modes

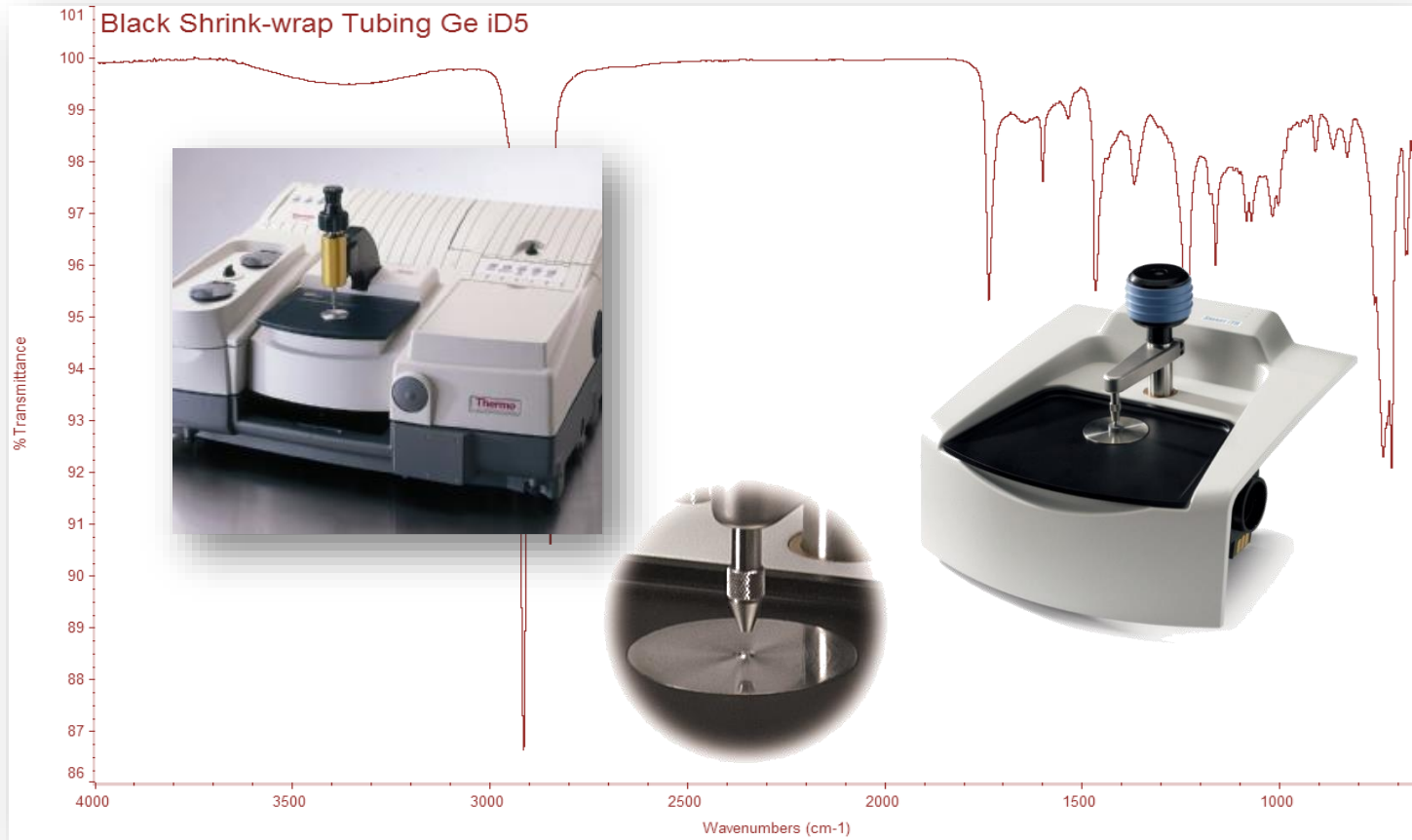
IR Active Modes

Point Group Tables are easily accessible, like in Appendix C of the textbook.

- In absorbance, a photon is **ABSORBED** by a molecule, and then re-emitted (usually) after some time period
- In Raman, a photon **INTERACTS** with a molecule, and either transfers some of its energy to the molecule (Stokes) or the molecule transfers some energy to the photon (Anti-Stokes).
  - Or there is no interaction and the photon just bounces off (Rayleigh Scattering)
- Thus, you can think of Raman as an ‘instant’ process, while emission after absorbance has a time period or a *lifetime*.

- Thus Raman is useful for a few reasons
  - Can use virtually any incident light source
    - However must be careful, if you use too high of an energy light source you can have competition with absorption
- Can gain information about a molecule's vibrational levels
- Can give complementary information to IR spectroscopy.
- INSTANT! So conformations that would average out in NMR are apparent in Raman

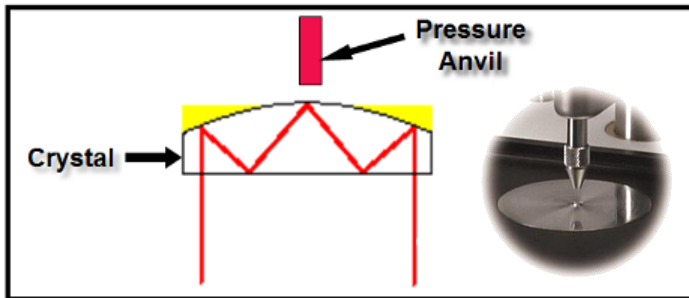
# Attenuated Total Reflectance - ATR





# Attenuated Total Reflection

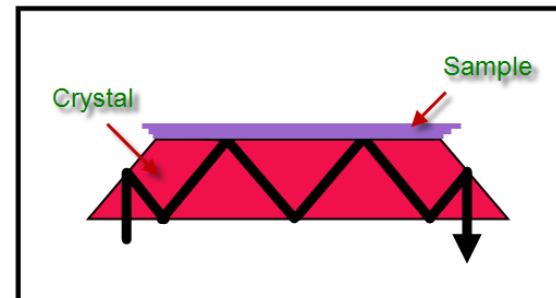
Single Bounce



- Small sampling area
- Use for strong absorbers
  - Black rubber, neat solvents
- Solid or liquid samples
  - Powders, droplets, flakes

vs.

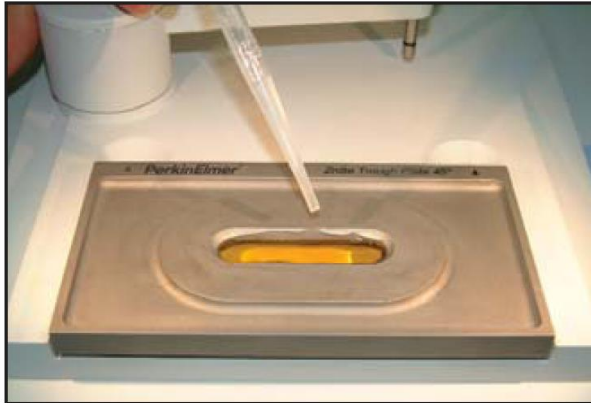
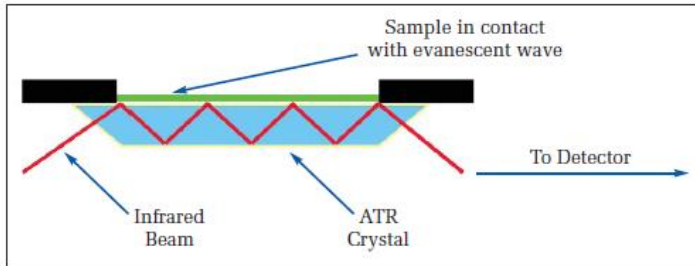
Multi-bounce



- Broad sampling area
- Larger contact with the sample
- Use for weak absorbers or dilute solutions

# FT-IR Spectroscopy

## Attenuated Total Reflectance (ATR)








*Figure 5.* Using a pipette to add a liquid sample to a ZnSe HATR trough plate.



*Figure 7.* Applying pressure to a solid sample on the Universal diamond ATR top-plate.

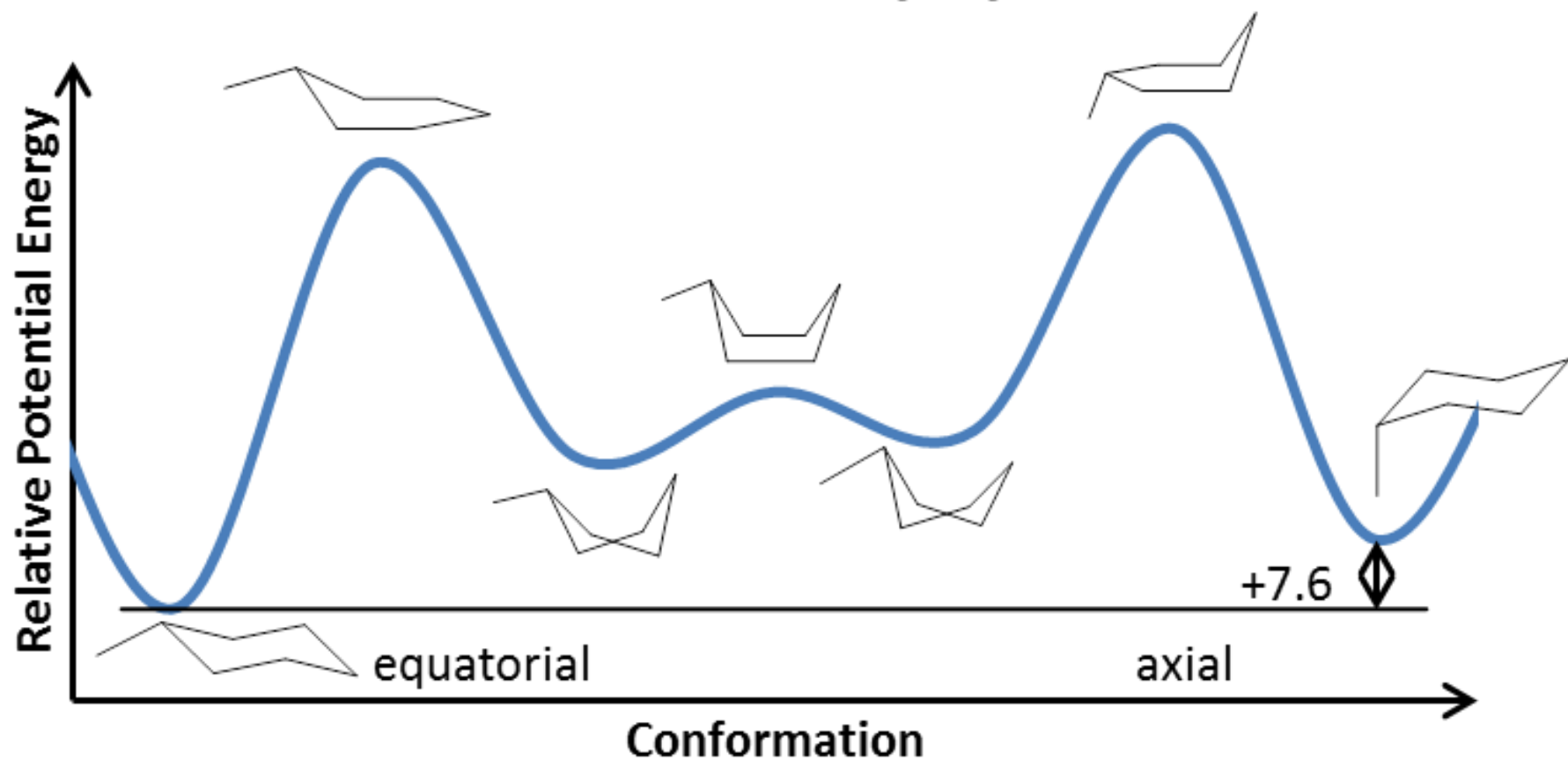
## Properties of Some Common ATR Crystals

Material	Range (cm <sup>-1</sup> )	Index	Penetration (μ) (at 45° & 1000 cm <sup>-1</sup> )	Uses
Germanium	5,500 - 675	4	 0.66	strongly absorbing samples
Silicon	8,900 - 1,500 & 360-120	3.4	 0.85	resistant to base
AMTIR	11,000 - 725	2.5	 1.77	resistant to acids
ZnSe	15,000 - 650	2.4	 2.01	general use
Diamond	25,000 - 100	2.4	 2.01	good for most samples. Needed for caustic or hard samples

# Cyclohexane and the Experiment

- 1-chlorocyclohexane can exist in in two conformers
  - The chlorine can be in the axial or equatorial position
    - What one do you think is more predominant?
- You will use Gaussian to perform an Opt&Freq calculation on both conformers
  - B3LYP functional, 6-311G(2d,2p) basis set
- You will then compare your calculated spectra to the measured spectra

# Conformations of Methylcyclohexane

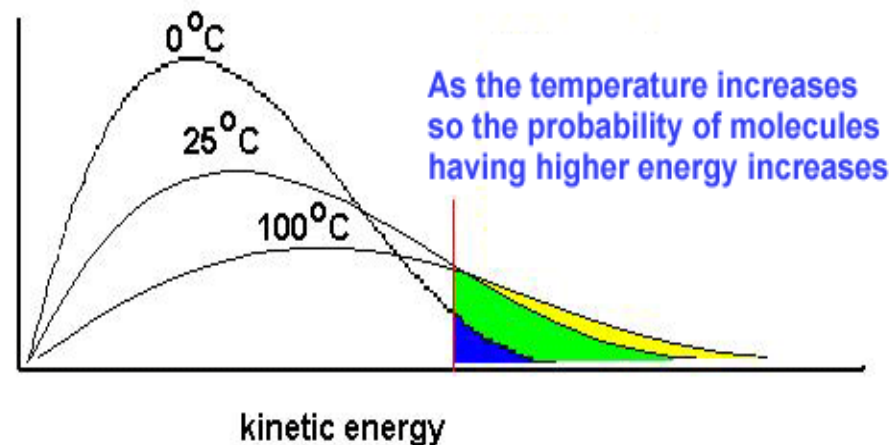
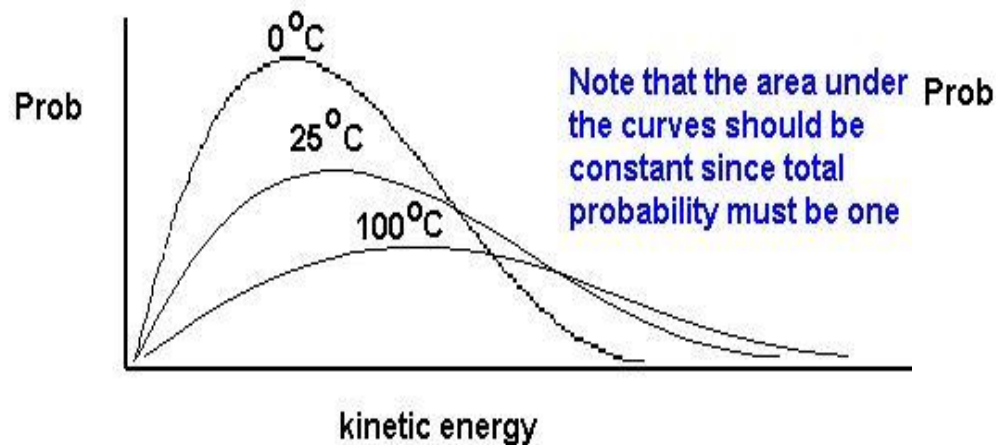


# Assigning Peaks

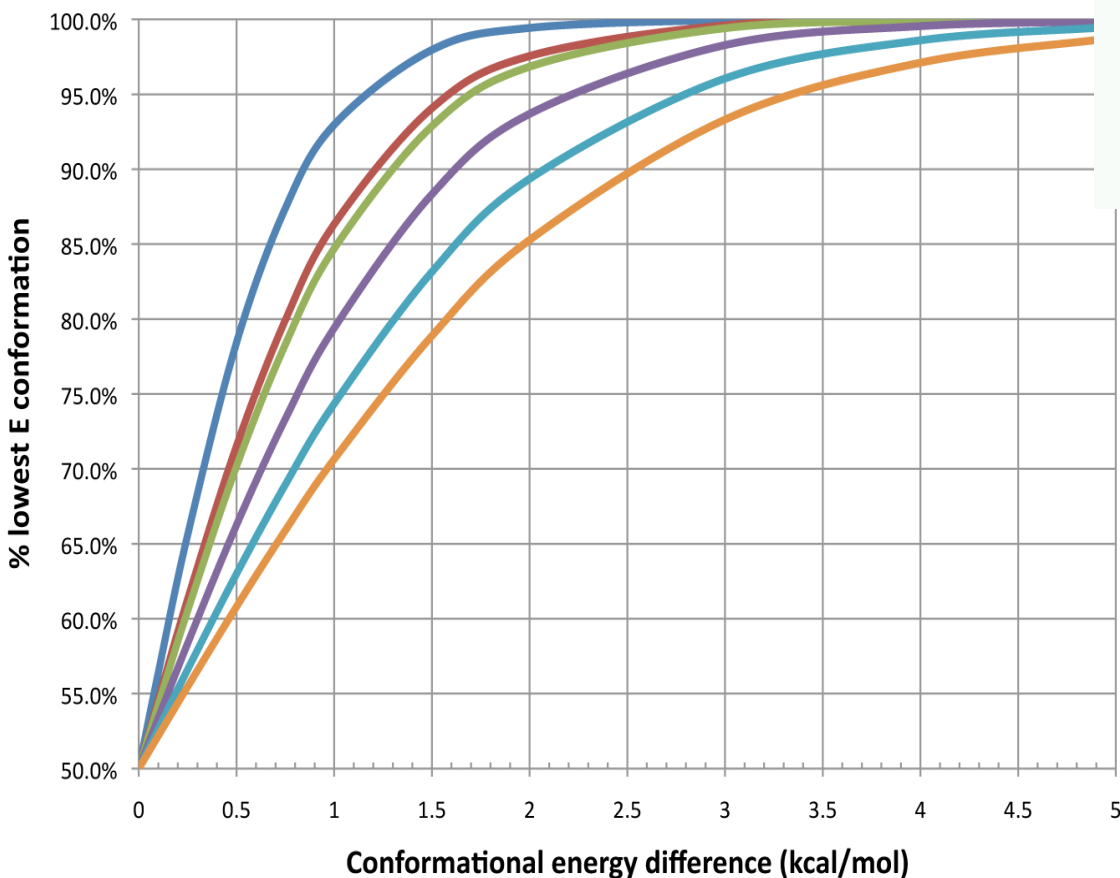
- Once you have run both calculations and you have a Raman spectra, you will assign the observed peaks to each conformation
- If there is a peak that does not agree with either calculations, you should then make arguments for where the peak came from (overtones, impurities, etc)

# Free Energies

- From the Gaussian output you should also compare the free energies, and show that one conformation is more likely to dominate.
- Provide a value for the ratio of the two forms
  - Use  $kT$  and Boltzmann's equation
- The primary contribution to free energy is the enthalpy difference, however the vibrational calculations include an estimate of the vibrational contribution to the free energy difference



### 2 Conformation Equilibrium Boltzmann Distribution



$$\frac{N_b}{N_a} = \left(\frac{g_b}{g_a}\right) \left(e^{-(E_b - E_a)/kT}\right)$$

- Left side – ratio of particles in different states
- $E_b$  = Energy of  $N_b$  level
- $E_a$  = Energy of  $N_a$  level
- 'g' terms are the degeneracy of the level



# Recap

- Perform High resolution Raman and ATR-FTIR spectra of:
  - Chloro-
  - Bromo-
  - Iodo-
  - Amino-
  - Methyl-
- Perform theoretical calculations on the axial and equatorial placement of the derivative using Gaussian
- Compare the calculated spectra with the experimental spectra, and account for any inconsistencies