

Biomolecular vibrational spectroscopy

Methods in Molecular Biophysics, Spring 2009

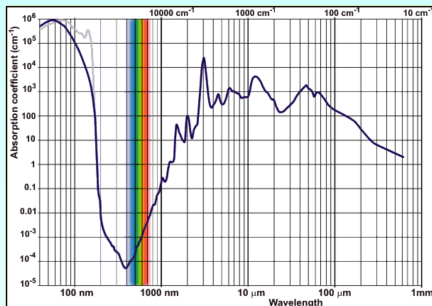
Basics of normal mode theory

Amide vibrations in proteins

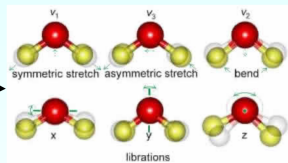
Nuclear resonance vibrational spectroscopy

Two-dimensional vibrational spectroscopy

Small molecule vibrational spectroscopy

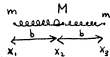


Wavelength	cm ⁻¹	Assignment	Wavelength ^a	cm ⁻¹	Assignment
0.2 mm	50	intermolecular bend?	1470 nm	6800	$av_1 + bv_3$, $a+b=2$
55 mm	183.4	intermolecular stretch	1200 nm	8330	$av_1 + v_2 + bv_3$, $a+b=2$
25 mm	395.5	L ₁ , librations	970 nm	10310	$av_1 + bv_3$, $a+b=3$
15 mm	686.3	L ₂ , librations	836 nm	11960	$av_1 + v_2 + bv_3$, $a+b=3$
6.08 mm	1645	v ₂ , bend	739 nm	13530	$av_1 + bv_3$, $a+b=4$
4.65 mm	2150	v ₂ + L ₂	660 nm	15150	$av_1 + v_2 + bv_3$, $a+b=4$
3.05 mm	3277	v ₃ , asymmetric stretch	606 nm	16500	$av_1 + bv_3$, $a+b=5$ [526]
2.87 mm	3490	v ₁ , symmetric stretch	514 nm	19460	$av_1 + bv_3$, $a+b=6$ [526]
1900 nm	5260	$av_1 + v_2 + bv_3$, $a+b=1$			Note that a and b are integers, ≠0



Normal modes of vibration

Consider a simple example, a symmetric linear triatomic molecule, with coordinates x_1, x_2, x_3 :
Assuming that the vibrations are harmonic, the potential energy is



$$V = \frac{1}{2} k (x_2 - x_1 - b)^2 + \frac{1}{2} k (x_3 - x_2 - b)^2$$

Let the equilibrium positions be x_{01}, x_{02} and x_{03} , so that

$$x_{02} - x_{01} = b = x_{03} - x_{02}$$

Define new coordinates $\eta_i = x_i - x_{0i}$ for $i = 1$ to 3 . This implies

$$V = \frac{1}{2} k (\eta_2 - \eta_1)^2 + \frac{1}{2} k (\eta_3 - \eta_2)^2 \quad (1)$$

The kinetic energy is

$$T = \frac{1}{2} m (\dot{\eta}_1^2 + \dot{\eta}_3^2) + \frac{1}{2} M \dot{\eta}_2^2 \quad (2)$$

Hence the Lagrangian function has the form

$$L = \frac{1}{2} \sum_{ij} (g_{ij} \dot{\eta}_i \dot{\eta}_j - V_{ij} \eta_i \eta_j) \quad (3)$$

where for this example

$$g_{11} = g_{33} = m ; g_{22} = M ; g_{ij} = 0 \text{ for } i \neq j$$

$$V_{11} = V_{33} = k ; V_{22} = 2k ; V_{12} = V_{21} = V_{23} = V_{32} = -k ; V_{13} = V_{31} = 0$$

Equation (3), with different values of the matrices \underline{g} and \underline{V} , is a very general one for small oscillations near equilibrium, where the potentials are approximately harmonic. Lagrange's equations of motion become:

$$\sum_j (g_{ij} \ddot{\eta}_j + V_{ij} \eta_j) = 0 \quad (\text{for all } i) \quad (4)$$

Normal modes, p. 2

Let us look for a solution of the form: $\eta_j(t) = a_j \cos(\omega t)$.

Substituting this into Eq. (4) yields

$$\sum_j (V_{ij} a_j - \omega^2 g_{ij} a_j) = 0 \quad (5)$$

This is a generalized eigenvalue problem that we will encounter again.

In general there will be particular eigenvalues ω_k , $k=1$ to n , that will solve Eq. (5), and associated with them will be eigenvectors which we now label by two indices; a_{jk} is the j^{th} element of the k^{th} eigenvector. It can be shown [Goldstein pp. 323-4] that the ω 's and a 's may all be chosen to be real, and we will assume this here.

Hence, one of the equations (5) may be written

$$\sum_j V_{ij} a_{jk} = \omega_k^2 \sum_j g_{ij} a_{jk} \quad (6)$$

Another is

$$\sum_j V_{ij} a_{jl} = \omega_l^2 \sum_j g_{ij} a_{jl} \quad (7)$$

Now multiply Eq. (6) by a_{il} and sum over i ; multiply Eq. (7) by a_{ik} and sum over i ; subtract the two equations, remembering that $V_{ij} = V_{ji}$. This yields

$$0 = (\omega_k^2 - \omega_l^2) \sum_{ij} g_{ij} a_{jk} a_{il}$$

We will now assume that none of the eigenvalues is degenerate, i.e. that all of them are different. (The more general case is discussed by Goldstein.) Hence, if $k \neq l$

$$\sum_{ij} g_{ij} a_{jk} a_{il} = 0 \quad (8)$$

Normal modes, p.3

Further, we are free to normalize the a 's so that, if $k=l$

$$\sum_{ij} g_{ij} a_{ik} a_{jl} = 1 \quad (9)$$

Equations (8) and (9) together imply:

$$\sum_{ij} g_{ij} a_{ik} a_{jl} = \delta_{kl} \quad (10)$$

Now, return to Eq. (7) and multiply by a_{ik} and sum over i :

$$\sum_{ij} V_{ij} a_{ik} a_{jl} = \omega_k^2 \delta_{kl} \quad (11)$$

Hence, the a_{jk} 's can transform both the kinetic energy and potential energy to diagonal form. This process of diagonalizing a matrix is very common in quantum mechanics. Note that often we get to Eq. (5), the rest of the argument only required that V and g be symmetric.

But we haven't yet solved our problem. Eq. (5) is a linear, homogeneous set of Equations that will have solutions only if:

$$\det \begin{vmatrix} V_{11} - \omega^2 g_{11} & V_{12} - \omega^2 g_{12} & \cdots \\ V_{21} - \omega^2 g_{21} & V_{22} - \omega^2 g_{22} & \cdots \\ \vdots & \vdots & \ddots \end{vmatrix} = 0 \quad (12)$$

For our example we have:

$$\det \begin{vmatrix} k - \omega^2 m & -k & 0 \\ -k & 2k - \omega^2 M & -k \\ 0 & -k & k - \omega^2 m \end{vmatrix} = 0 \quad (13)$$

Expansion of the determinant yields

$$\omega^2 (k - \omega^2 m) (k [M + 2m] - \omega^2 M m) = 0 \quad (14)$$

which has three solutions:

$$\omega_1 = 0 \quad \omega_2 = \sqrt{\frac{k}{m}} \quad \omega_3 = \sqrt{\frac{k}{m} \left(1 + \frac{2m}{M}\right)}$$

The first frequency corresponds to translation of the center of mass, i.e. it is not a vibration at all. In 3-D one would always find three such frequencies, with the same interpretation. Putting $\omega = 0$ into equation (5) yields three simultaneous equations:

$$\left. \begin{aligned} k a_{11} - k a_{21} &= 0 \\ -k a_{11} + 2k a_{21} - k a_{31} &= 0 \\ -k a_{21} + k a_{31} &= 0 \end{aligned} \right\} \quad (15)$$

While the normalization condition, eq. (9), becomes

$$m(a_{11}^2 + a_{31}^2) + M a_{21}^2 = 1 \quad (16)$$

These are easy to solve, giving

$$a_{11} = a_{21} = a_{31} = (2m + M)^{-1/2} \quad (17)$$

showing that this mode indeed corresponds to uniform motion of all three atoms. Putting $\omega = \omega_2 = \sqrt{k/m}$ into equations (5) and (9) yields

$$a_{12} = (2m)^{-1/2}; \quad a_{22} = 0; \quad a_{32} = -(2m)^{-1/2} \quad (18)$$

This is a symmetric stretch, where the center atom does not move, and the two outer atoms vibrate 180° degrees out of phase.

Normal modes, p. 5

Finally, for ω_3 , we have

$$a_{13} = a_{33} = \left[2m \left(1 + \frac{2m}{M} \right) \right]^{-1/2}; \quad a_{23} = -2 \left[2M \left(2 + \frac{M}{m} \right) \right]^{-1/2} \quad (19)$$

This is an asymmetric stretch, where the two outer atoms vibrate with the same amplitude, while the inner atom oscillates out of phase with them.

Now, the general solution to Lagrange's equations, Eqs. (4), will be a sum of terms of the kind we have been considering:

$$\eta_j(t) = \sum_R C_R a_{jR} \cos(\omega_R t + \delta_R) \quad (20)$$

where we have added an arbitrary amplitude C_R and phase shift δ_R , which will be determined from the initial conditions. It is now useful at this point to define normal coordinates J_j :

$$\eta_i = \sum_j a_{ij} J_j \quad (\text{definition of } J) \quad (21)$$

In terms of these new coordinates, the kinetic and potential energies become:

$$\begin{aligned} 2T &= \sum_{ij} g_{ij} \dot{\eta}_i \dot{\eta}_j = \sum_{ijkl} g_{ij} (a_{iR} \dot{J}_R) (a_{jL} \dot{J}_L) \\ &\stackrel{E_k(10)}{=} \sum_{RkL} \delta_{RkL} \dot{J}_R \dot{J}_L = \sum_R \dot{J}_R^2 \end{aligned} \quad (22)$$

$$\begin{aligned} 2V &= \sum_{ij} V_{ij} \eta_i \eta_j = \sum_{ijkl} V_{ij} (a_{iR} J_R) (a_{jL} J_L) \\ &\stackrel{E_k(11)}{=} \sum_{RkL} \omega_k^2 \delta_{RkL} J_R J_L = \sum_R \omega_R^2 J_R^2 \end{aligned} \quad (23)$$

Normal modes, p. 6

Hence, in terms of the \dot{q}_k , both the kinetic and potential energies are diagonal, i.e. they contain no cross terms. The new Lagrangian is

$$L = \frac{1}{2} \sum_k \left(\dot{q}_k^2 - \omega_k^2 q_k^2 \right) \quad (24)$$

and Lagrange's equations become

$$\ddot{q}_k + \omega_k^2 q_k = 0 \quad (25)$$

That is, each normal mode coordinate oscillates like a simple oscillator, independently of the motion of the other modes. Normal mode coordinates will be extremely useful when we discuss quantum vibrations later.

Reading : (a) Goldstein, Classical Mechanics, Chap 10, discusses this problem in more detail, showing that the eigenvalues + eigenvectors are real, and what happens with multiple eigenvalues.

(b) Kerker and Porter, Atoms and Molecules, Section 7.9 discuss in great detail vibrations of triatomic molecules.

Problems (1) Derive Eq. (4).

(2) Expand the determinant in Eq. (13) to produce Eq. (14).

(3) Derive Eq. (18).

(4) Develop an explicit expression for the normal mode coordinates, i.e. an equation (for the general problem) of the form $\dot{q}_j = ?$

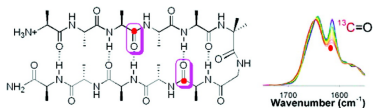
(5) Write explicitly the three normal mode coordinates for our sample problem.

Amide vibrational spectra in proteins

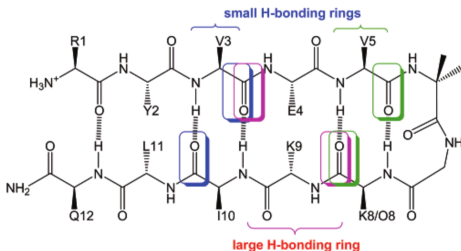
Cross-Strand Coupling of a β -Hairpin Peptide Stabilized with an Aib-Gly Turn Studied Using Isotope-Edited IR Spectroscopy

Rong Huang, Vladimir Setnic#ka, Marcus A. Etienne, Joohyun Kim, Jan Kubelka, Robert P. Hammer, and Timothy A. Keiderling

J. Am. Chem. Soc., **2007**, 129 (44), 13592-13603 • DOI: 10.1021/ja0736414 • Publication Date (Web): 11 October 2007



Scheme 1. Design Structure of Gellman A Peptide Showing Numbering of Individual Residues in Sequence and $^{13}\text{C}=\text{O}$ -Labeling Patterns (colored rectangles)



Deconvoluting complex spectra

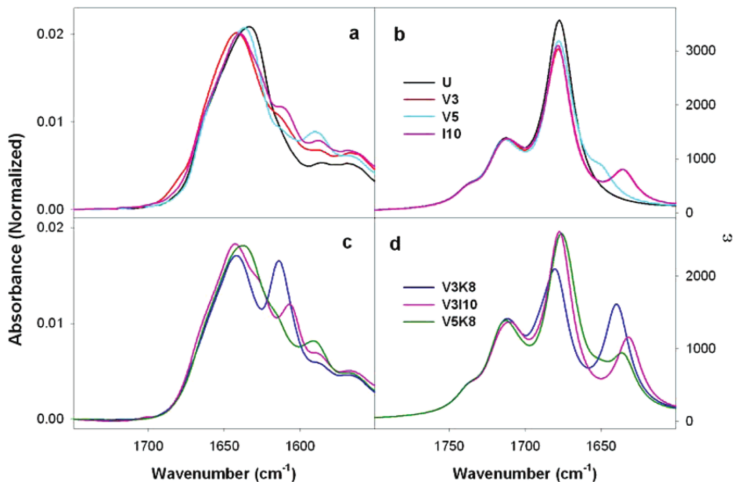


Figure 2. Experimental IR spectra of (a) the unlabeled and single-labeled hairpin peptides and (c) the double-labeled peptides. The experiments were normalized to have a peak area of 1 between 1720 and 1550 cm^{-1} . The original spectra of all peptides were measured at ~ 23 mg/mL deuterated phosphate buffer in a 100 μm path length cell. Simulated IR spectra of $\text{Ac-A}_{12}\text{-NHCH}_3$ by transfer from shorter fragments are in unlabeled peptide compared with single-labeled peptides **A3**, **A5**, and **A10** and (d) for the double-labeled peptides **A3A8**, **A3A10**, and **A5A8**.

Deconvoluting complex spectra

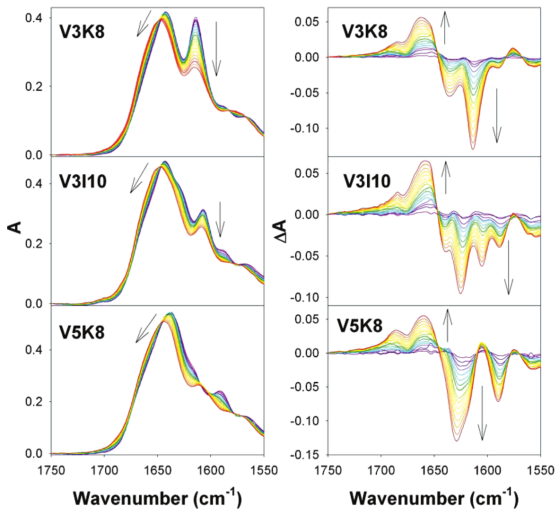


Figure 3. Original temperature-dependent IR spectra (left panels) and difference spectra (right panels) for double-labeled peptides V3K8, V3I10, and V5K8. All peptide concentrations were ~23 mg/mL in 20 mM deuterated phosphate buffer measured in a 100- μ m path length cell. The difference spectra were taken by subtracting the spectrum at 5 °C from each spectrum at higher temperatures. In all panels the spectra are color coded for temperature, from blue at the cold (5 °C) extreme to red at the hot (95 °C) end, as suggested by the arrows.

Fitting conformational preferences

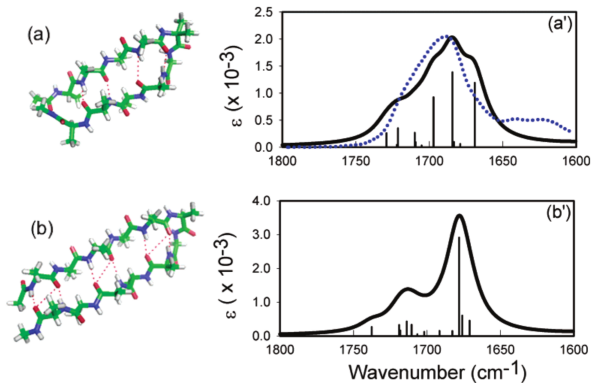


Figure 6. Amide I IR spectra for structures IV and V. Snapshot schematic of structure IV, obtained from an NMR structure, is shown in part a. Structure V, modeled from the crystal structure of IIFC is shown in part b. The corresponding spectra are in parts a' and b', respectively. The experimental spectrum (low T) of the HBG peptide is shifted and scaled as in Figure 5 and represented as the blue, dotted spectrum in part a'.

Fitting conformational preferences

23594 *J. Phys. Chem. B*, Vol. 110, No. 46, 2006

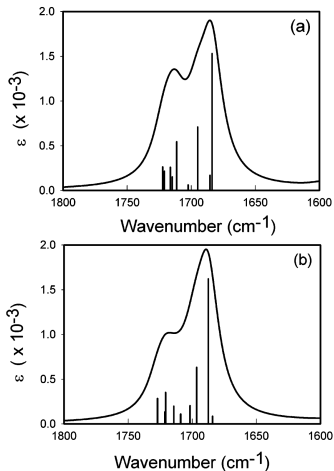
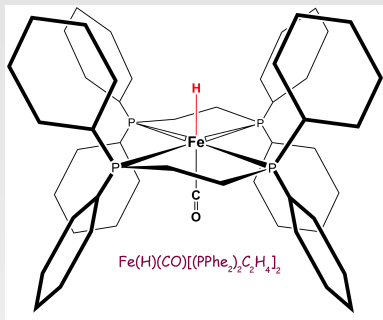
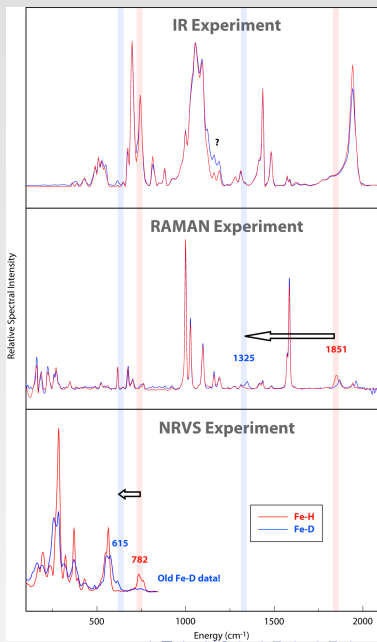


Figure 4. Amide I IR spectra of the 9-amide β -hairpin model. (a) Simulated spectra by the CSPT and (b) simulated spectra with the direct DFT.

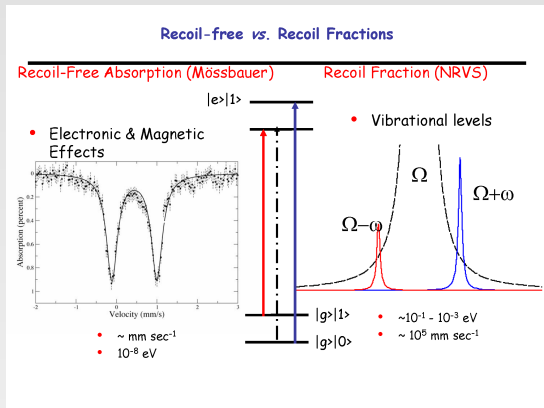
Complementary vibrational probes



cf. Guo, et al. *Inorg. Chem.* **47**:
3969, 2008

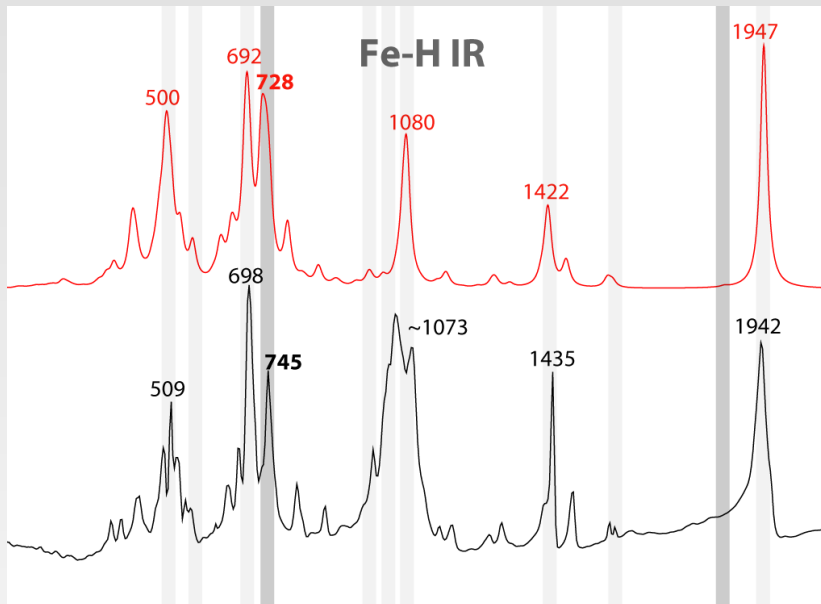


Nuclear resonance vibrational spectroscopy

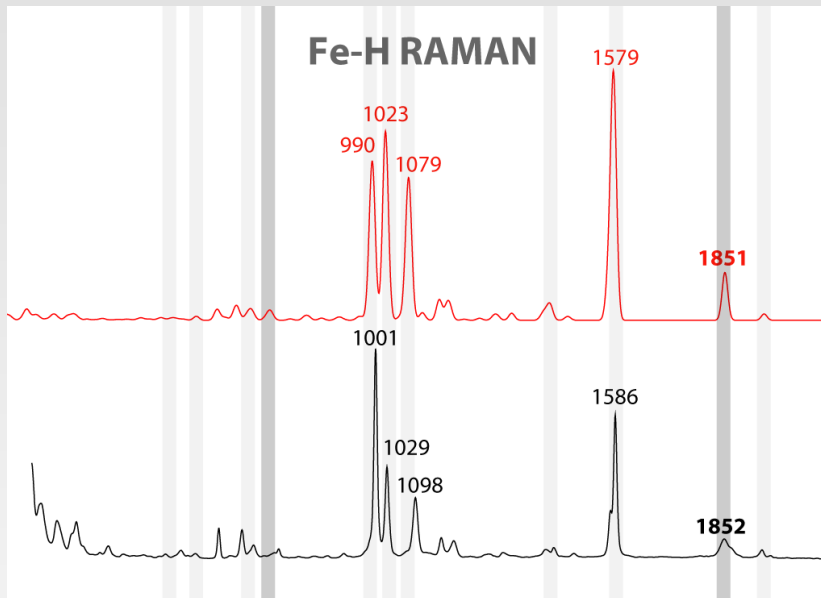


- “vibrational Mössbauer”
- only ^{57}Fe contributes (no background)
- intensity is proportion to KE in mode due to iron
- “acoustic” librational modes visible (in principle)

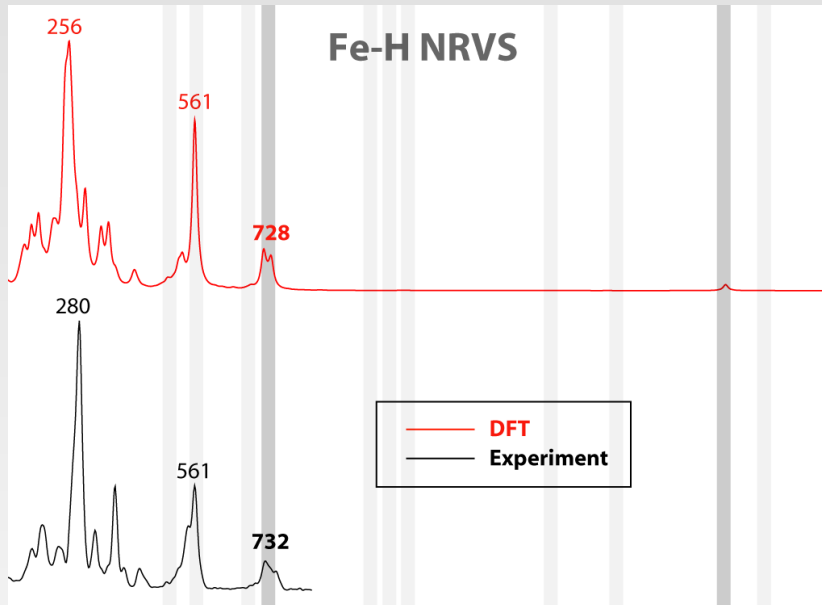
Model compound IR spectra



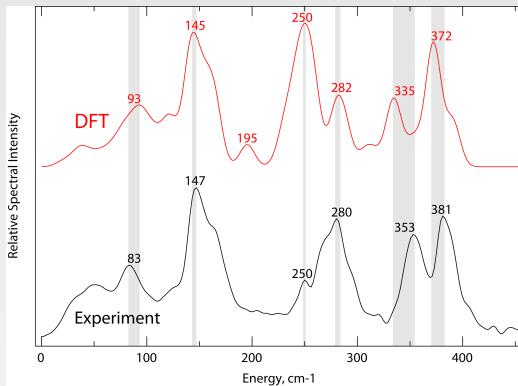
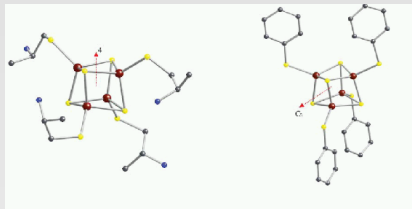
Model compound Raman spectra



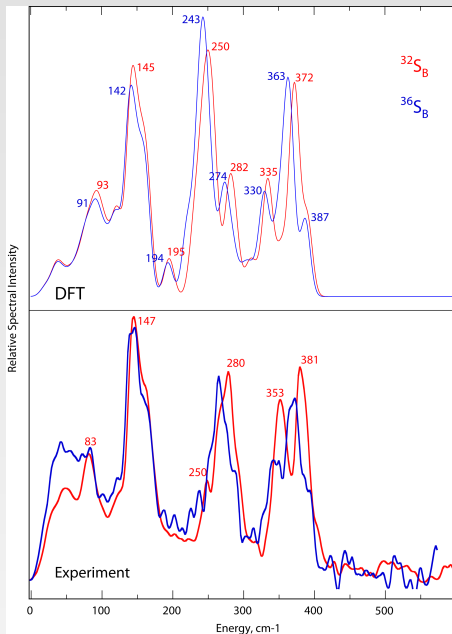
Model compound NRVS spectra



Moving to a ferredoxin protein



Isotope shifts in the protein environment



The interaction of (infrared) radiation with matter

In free space, in Gaussian units, Maxwell's equations become

$$-\nabla^2 \underline{E} - \frac{1}{c^2} \frac{\partial^2 \underline{E}}{\partial t^2} = 0 \quad ; \quad \nabla^2 \underline{H} - \frac{1}{c^2} \frac{\partial^2 \underline{H}}{\partial t^2} = 0 \quad (1)$$

Separating variables, and writing our solutions as

$$\underline{E}(\underline{r}, t) = \omega g(t) \underline{E}'(\underline{r}) \quad ; \quad \underline{H}(\underline{r}, t) = p(t) \underline{H}'(\underline{r}) \quad (2)$$

Substituting (2) into (1):

$$\frac{\nabla^2 \underline{E}'}{\underline{E}'} = \frac{1}{c^2} \frac{\ddot{g}(t)}{g(t)} \equiv -k^2, \quad \text{a constant of separation.}$$

$$\nabla^2 \underline{E}' + k^2 \underline{E}' = 0 \quad ; \quad \nabla^2 \underline{H}' + k^2 \underline{H}' = 0 \quad (3)$$

Let us normalize the solutions to this wave equation so that

$$\int |\underline{E}'|^2 dV = \int |\underline{H}'|^2 dV = 4\pi \quad (4)$$

Now the energy in the field is

$$H_{\text{rad}} = \frac{1}{8\pi} \int [|\underline{H}'|^2 + |\underline{E}'|^2] dV = \frac{1}{2} [P^2 + \omega^2 g^2] \quad (5)$$

Now this looks like the classical Hamilton's function for a harmonic oscillator. But, so far $p(t)$ and $g(t)$ are just arbitrary definitions.

We now want to show that they are indeed related by Hamilton's equation:

$$\dot{P} = -\frac{\partial H}{\partial g} = -\omega^2 g \quad ; \quad \dot{g} = \frac{\partial H}{\partial P} = P \quad (6)$$

Proof of Eq. 6

first, a lemma: Eq. (3) can be satisfied by
$$\begin{cases} \mathcal{L} \underline{E}' = \nabla \times \underline{H}' \\ \mathcal{L} \underline{H}' = \nabla \times \underline{E}' \end{cases} \quad (7)$$

proof: note (by a vector identity)

$$\nabla^2 \underline{E}' = \nabla (\nabla \cdot \underline{E}') - \nabla \times (\nabla \times \underline{E}') = -\nabla \times (\nabla \times \underline{E}')$$

then

$$\begin{aligned} \nabla^2 \underline{E}' + \mathcal{L}^2 \underline{E}' &\stackrel{?}{=} \frac{1}{\mathcal{L}} \nabla \times (\nabla \times (\nabla \times \underline{H}')) + \mathcal{L} (\nabla \times \underline{H}') \\ &\stackrel{?}{=} \frac{1}{\mathcal{L}} \nabla \times (\nabla^2 \underline{H}') + \mathcal{L} (\nabla \times \underline{H}') \stackrel{?}{=} \mathcal{L}^{-1} \nabla \times \underbrace{[\nabla^2 \underline{H}' + \mathcal{L}^2 \underline{H}']}_{=0 \text{ by Eq. (3)}} \end{aligned}$$

An analogous argument proves the second half of Eq. (7). End of lemma.

Now, substitute Eq. (2) into Maxwell's equations:

$$\nabla \times \underline{E} + \frac{1}{c} \frac{\partial \underline{H}}{\partial t} = 0 \quad ; \quad \nabla \times \underline{H} - \frac{\partial \underline{E}}{\partial t} = 0$$

$$\omega \mathcal{L} \underbrace{[\nabla \times \underline{E}']}_{\mathcal{L} \underline{H}'} + \frac{1}{c} \dot{p}(t) \underline{H}' = 0 \quad ; \quad p(t) \underbrace{[\nabla \times \underline{H}']}_{\mathcal{L} \underline{E}'} - \frac{1}{c} \omega \dot{q}(t) \underline{E}' = 0$$

use Eq. (7):

$$\dot{p}(t) = -\omega^2 q(t)$$

$$\dot{q}(t) = p(t)$$

qed.

Hence we can treat the radiation field as arising from the oscillator-like Hamiltonian Eq. (5). At this point one could quantize the radiation field by replacing q and p with operators. However, in keeping with the emphasis of this course, we shall keep everything classical.

Now in the electric dipole approximation, the interaction between a molecule and radiation is proportional to $-\underline{\mu} \cdot \underline{E}(0)$ where the field is evaluated at the molecular center. In a cavity of volume V , the average rms value of \underline{E} is (see Eq. (4)) $\sqrt{\frac{4\pi}{V}}$. Hence the interaction Hamiltonian between matter and radiation is

$$H_I = -\left(\frac{4\pi\omega^2}{V}\right)^{1/2} \underline{\mu}(\underline{x}) g(t) \quad (8)$$

where we have indicated that the dipole moment depends upon coordinates \underline{x} that specify the location of our molecule. The total (classical) Hamiltonian is thus

$$\begin{aligned} H &= H_{\text{matter}} + H_{\text{radiation}} + H_{\text{interaction}} \\ &= \frac{(\underline{p}_m)^2}{2m} + V(\underline{x}) + \frac{1}{2} p^2 + \frac{1}{2} \omega^2 q^2 - \left(\frac{4\pi\omega^2}{V}\right)^{1/2} \underline{\mu}(\underline{x}) g(t) \end{aligned} \quad (9)$$

This classical dynamical system may be thought of as the original molecular system with one extra degree of freedom; the potential surface for the composite system is:

$$V(\underline{x}, q) = V(\underline{x}) + \frac{1}{2} \omega^2 q^2 - \left(\frac{4\pi\omega^2}{V}\right)^{1/2} \underline{\mu}(\underline{x}) g \quad (10)$$

and one can integrate classically the equations of motion for this system, just like any other.

Miller [JCP 69, 2188 (1978)] shows how to eliminate the field variables p and q , to get a closed equation for \underline{x} and \underline{p} ; this is just the Zwanzig - Adelman, Doll - Schatz procedure all over again. The g equation is

$$\ddot{g}(t) + \omega^2 g(t) = \left(\frac{4\pi\omega^2}{V}\right)^{1/2} \underline{\mu}(\underline{x}(t)) \quad (11)$$